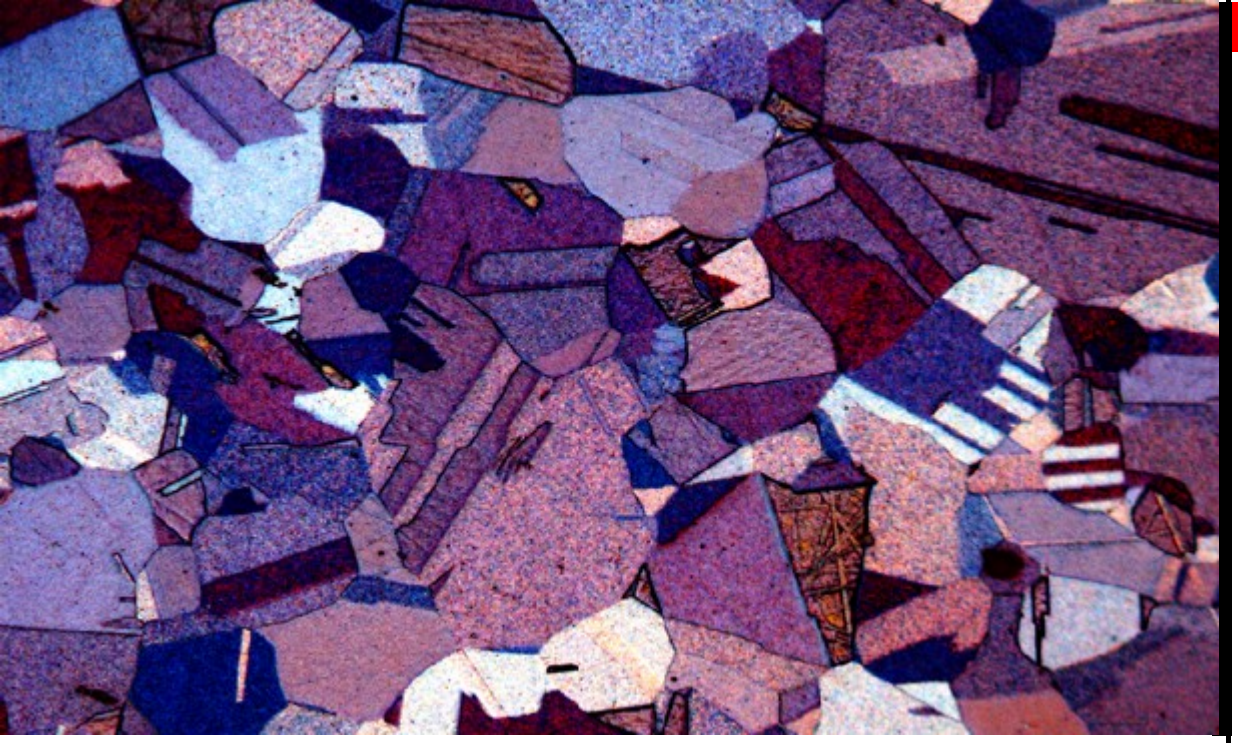

Leica Application Suite

LAS User Manual

Living up to Life

Leica
MICROSYSTEMS

LAS User Manual

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Date: April 2011

Software Version: LAS V3.8

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Contents

Leica Application Suite Overview:

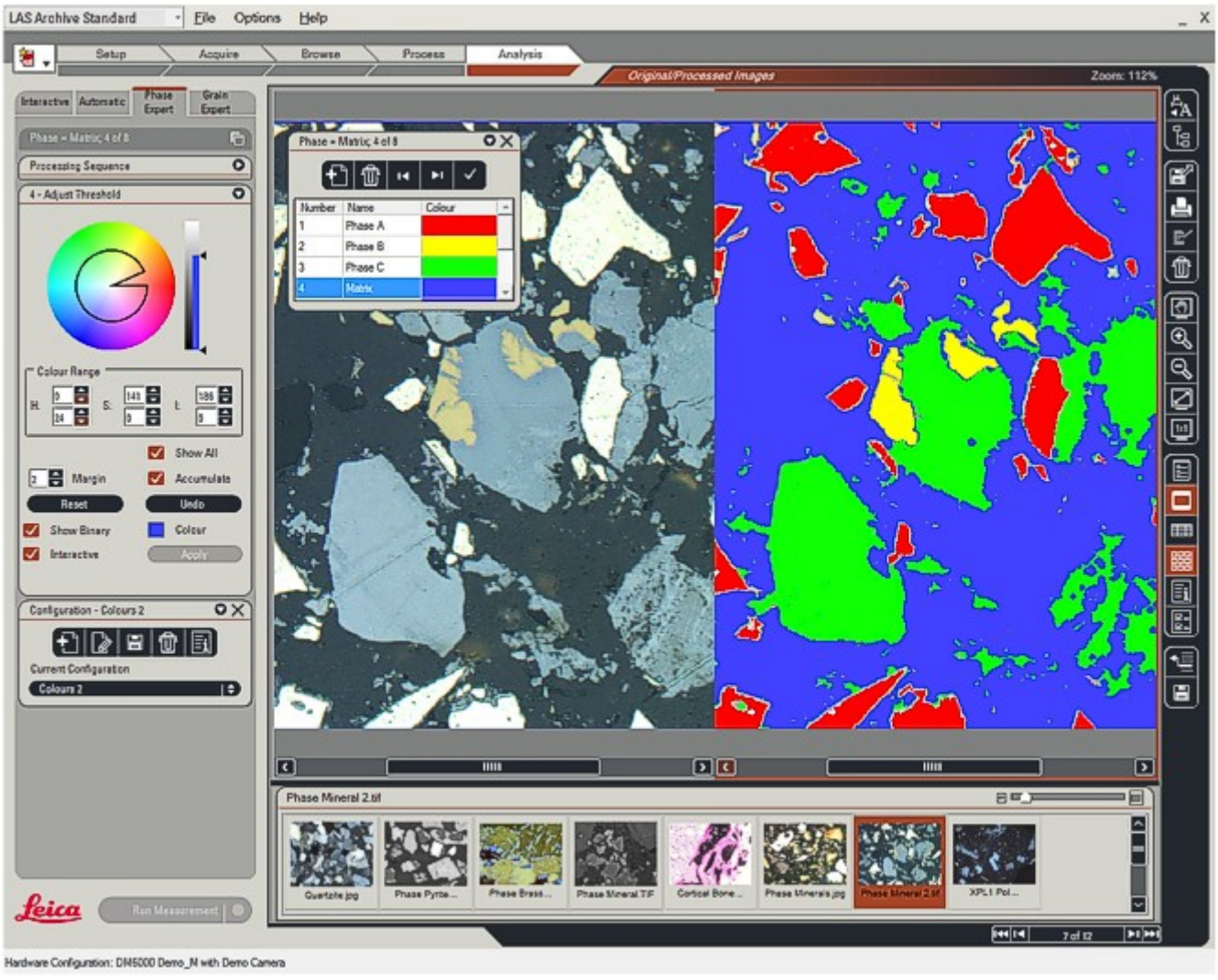
The *Leica Application Suite* (LAS) comprises:

- *The Core*: The basic part of the Suite which includes the *Framework* and the *Setup, Acquire, Browse* and *Process Workflows.* These are the essential tools needed to refine, capture and present images from the microscope. The *Core* utilities are always available and do not have to be licensed.
- *Optional Modules:* Powerful, specialised programs that perform specific functions to enhance, augment and extend the *Core*. *Optional Modules* are provided free for an evaluation period of 60 days. After that, they have to be purchased and licensed to continue to work.

When the Leica Application Suite is first installed on the computer, all of the *Optional Modules* are also installed but not enabled. Selecting *Demo* mode will allow each one to be enabled ready for evaluation.

Some of the modules may not be appropriate to the tasks the microscope will be required to perform, so they need not be enabled initially. Instead, they can be turned on at a later date to start the 60 day evaluation. However, once evaluation is started the 60 days will start to run and cannot be turned off, even if the module itself is disabled.

LAS installation is described separately in the *Installation Guide.pdf* on the LAS DVD.

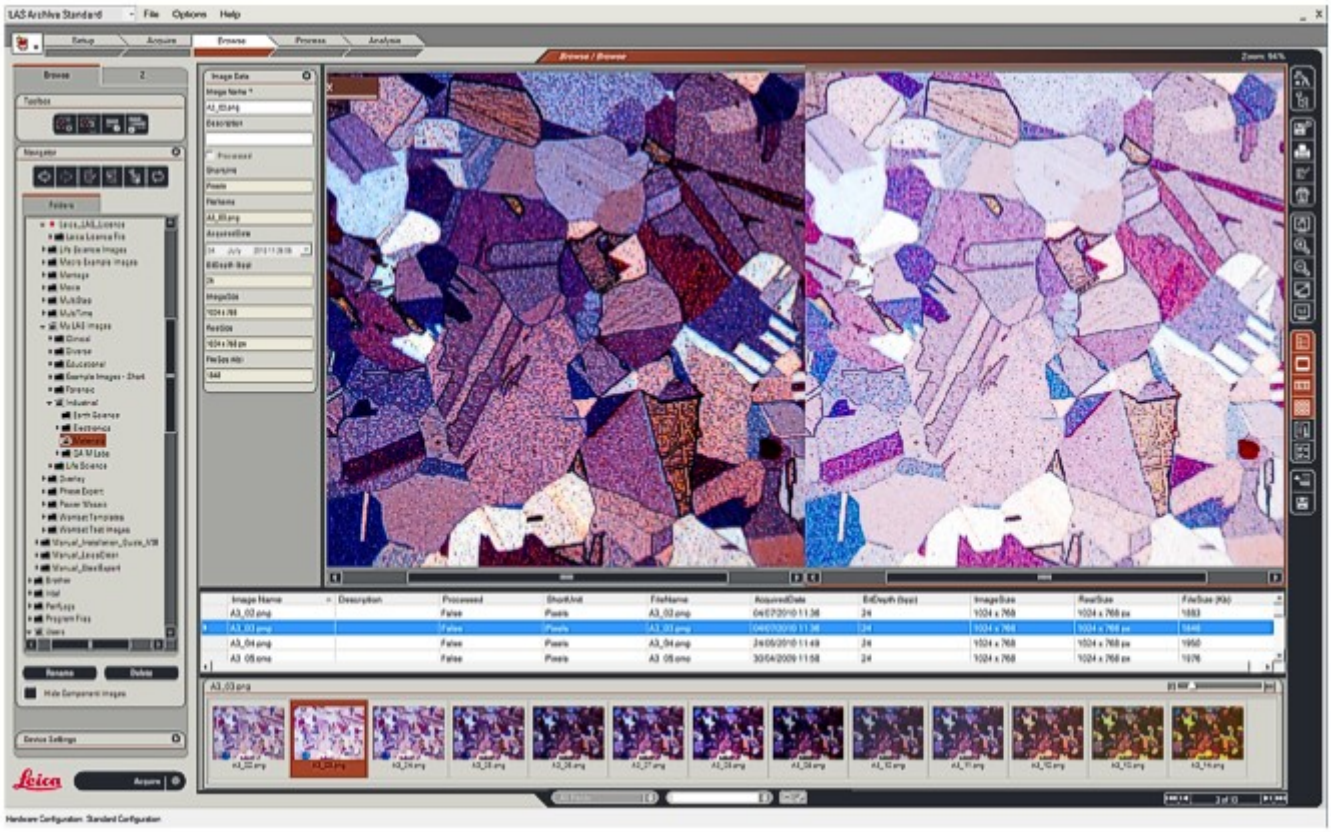


The Core:

The *Core* provides the basic software for configuration and control of the selected microscope as well as for the acquisition, analysis and processing of high quality digital images. The *Core* components include:

- *Microscope* and *Digital Camera* configuration and control - all fully integrated.
- *Auto* and *Manual Exposure* adjustments to allow optimised imaging.
- *Image Calibration* based on data read directly from Leica microscopes and cameras.
- *Scale Bar* displayed on the live image to indicate image size.
- *Digital Image Acquisition* into the familiar *Image Explorer* tree and folder structure. There is an *Optional Module* to include database *Archive*.
- *Thumbnail Gallery* of acquired images for quick and easy review.
- *Text, Scale Bar* and *Distance Tools* for straightforward image annotation.

Link to the Core: 36



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Optional Modules:

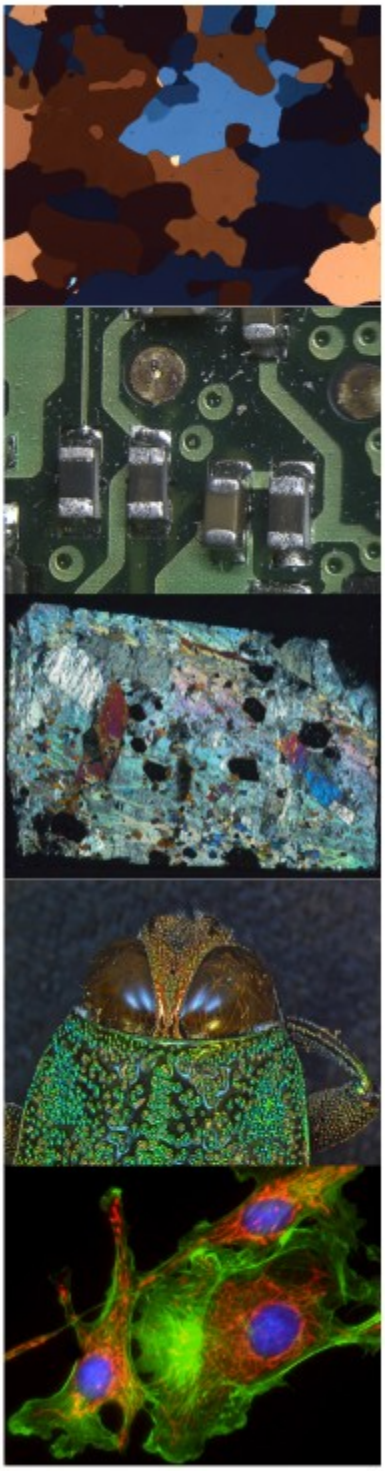
The powerful features of the *Core* can be enhanced with a range of advanced *Optional Modules*, each providing the flexibility to tailor a system to suit individual needs. The range of *Optional Modules* includes:

- LAS Archives: Basic and Standard.
- Image Analysis
- Extended Depth of Focus
- Movie Recording
- Image Measurements
- Autofocus
- Image Overlay
- Power Mosaic...

..and more.

Link to Optional Modules: 368

Link to installing the 60-day *Demo* evaluation licence and enabling the *Optional Modules:* 369



Getting Started

The following topics describe how to make effective use of LAS when you first start to use the software after installation. The following topics are covered:

Documentation *Go there...* 5
Please read the Release Notes for details of new features and restrictions in use. Check you have supported hardware and PC specification in the System Requirements

Configure the hardware *Go there...* 8
When LAS is first used, you must specify the microscope and camera hardware being used

Starting LAS *Go there...* 18
Once your system is configured, the usual starting procedure follows these notes.

The User Interface *Go there...* 19
Get familiar with the concept and terminology of the user interface

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These help to improve your productivity

Dual Monitors *Go there...* 29
If you have dual-monitors, this topic shows how to make best use of them

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Documentation

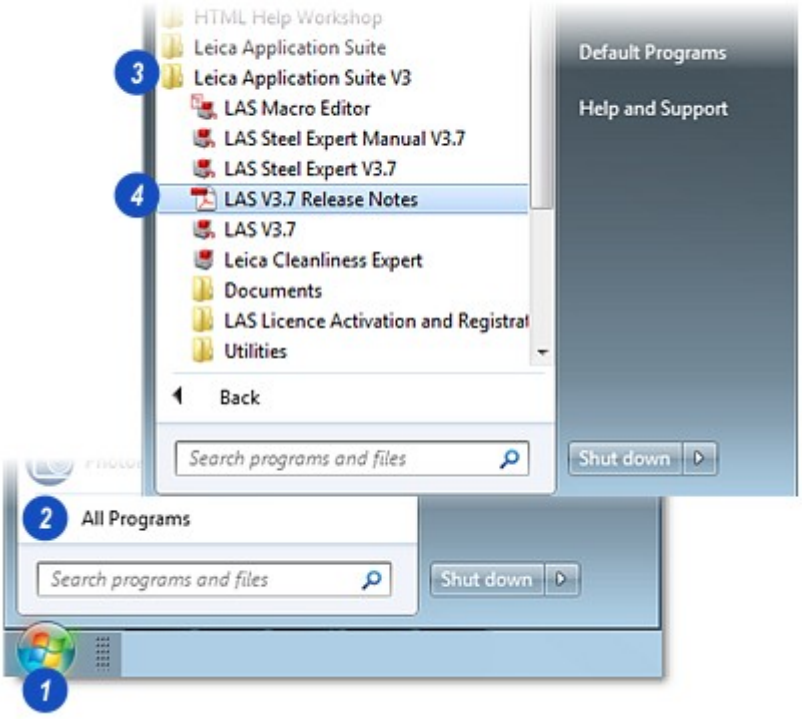
Documentation comprises *LAS User Help and Manual* and related documents concerning the installation, operation and restrictions on the use of Leica Application Suite. Please consult them before using the software.

Install Guide
A detailed description of the installation procedure for Leica Application Suite is in the *LAS Install Guide.pdf.*

Release Notes
Recent information about the version of LAS is described in the document *LAS Release Notes.pdf.* This describes features of the software that have recently changed, operational limitations and other technical information.

System Requirements
The supported hardware - the microscopes, macroscopes and cameras that can be used with LAS - is described in the document *System Requirements.pdf.* This document also describes the appropriate computer specification. Please ensure that your computer specification corresponds to the recommendations made. Other factors that influence the performance of LAS are also noted in the same document.

For Leica DM microscopes, refer to the operator's manual supplied with the microscope for detailed guidance on configuring and operating the microscope.



The help documents are found on the LAS DVD or after installation as follows:

1: Click on Windows *Start.*

2: Click on the *All Programs* arrow...

3: ...and on *Leica Application Suite V3*.

4: Click on the *Release Notes* option.

Continued... 6

The *Release Notes* contain information about changes and revisions that came too late to be included in this documentation. The Notes may be found on the original installation DVD or on the computer hard drive.

Release Notes on the DVD:
Load the DVD to the drive on the computer. In most cases it should start up automatically. If it does not:

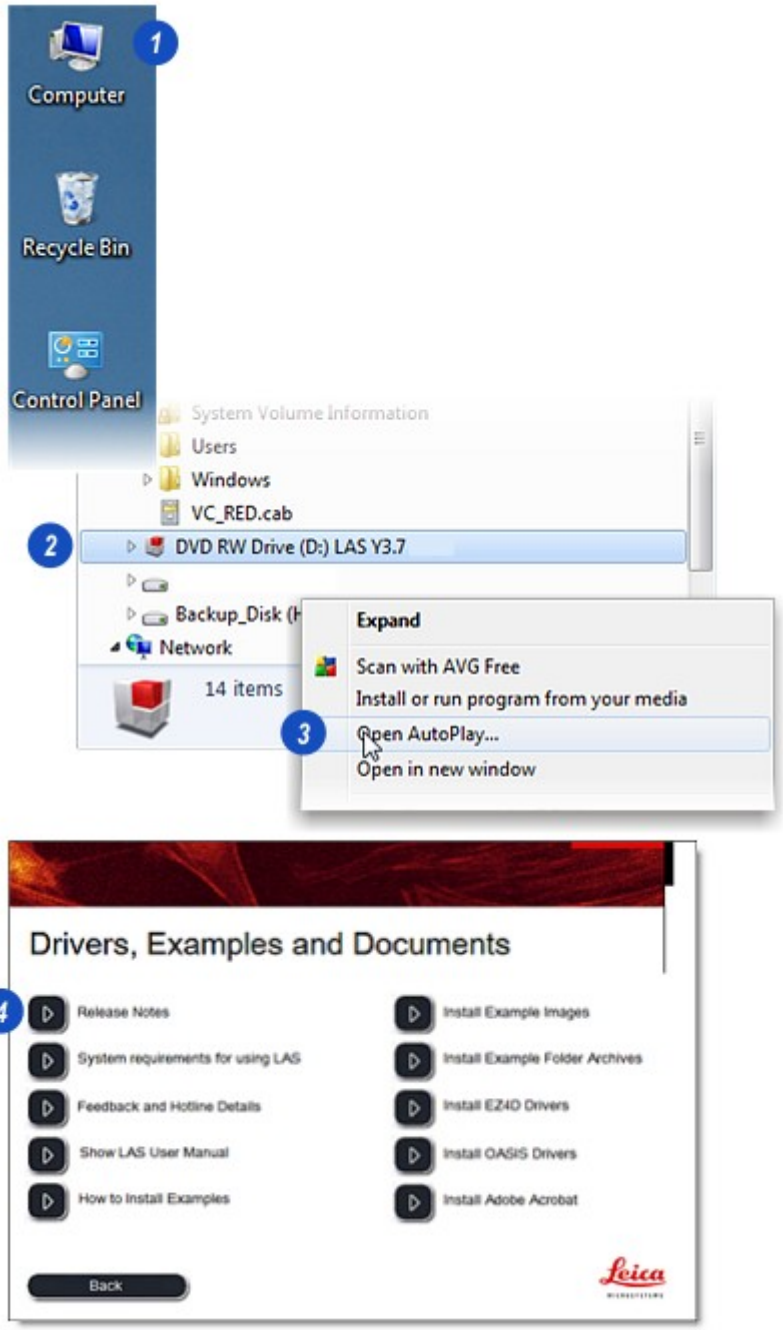
1: Click on the *My Computer icon* on the computer desktop.

2: From the dialog, right click on the *DVD drive*. The disk name will reflect the LAS Software Version and may not be the same as the illustration.

3: From the popup menu select *AutoPlay* and the DVD should start to run.

4: Click on the *Release Notes* button.

Continued... 7



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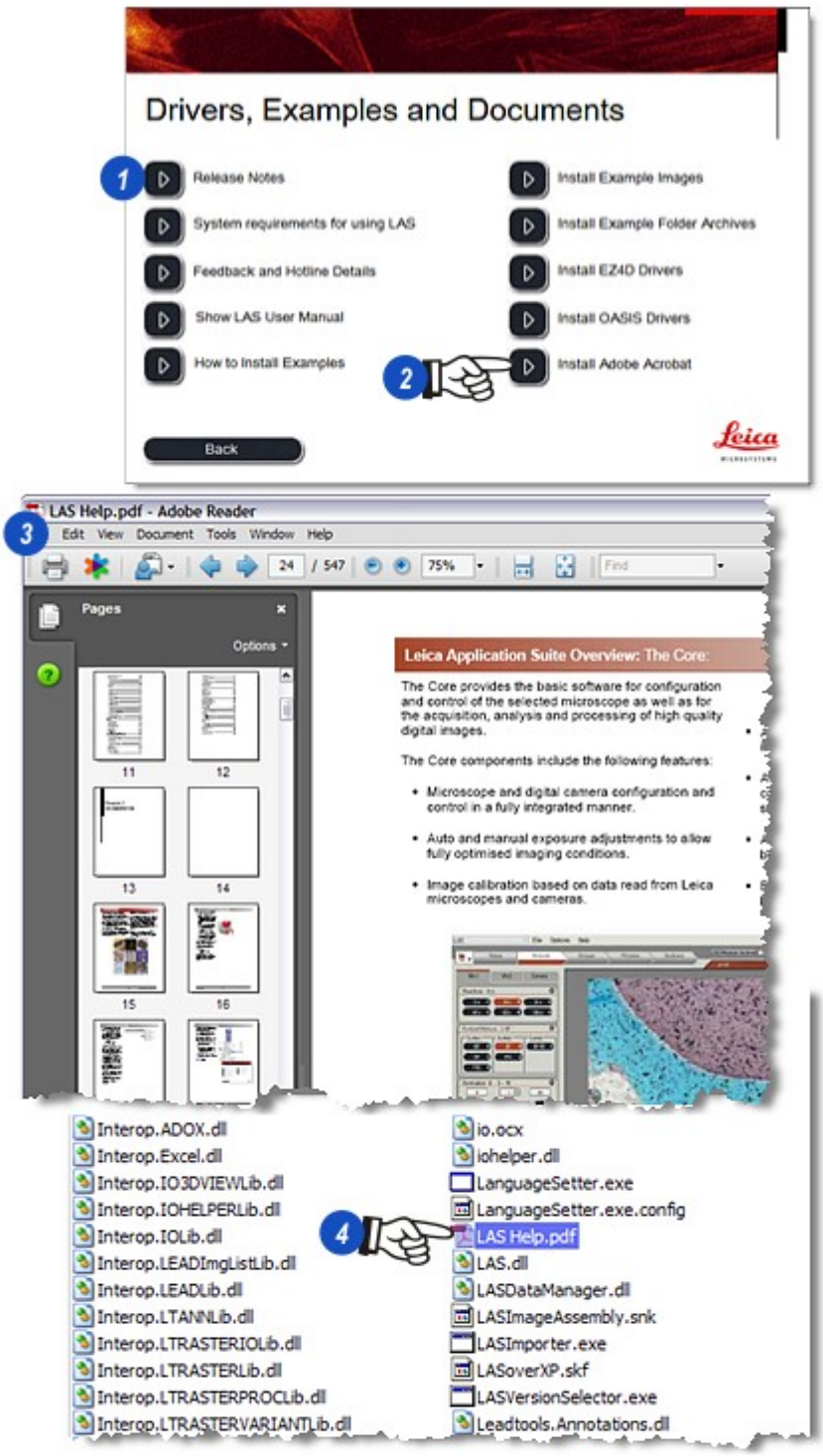
Leica Application Suite is supplied on a single DVD.

1: Click on the *Release Notes* button.

2: The *Release Notes* require that *Adobe Acrobat* reader is installed on the machine. If it is not, the program is also available on the DVD. Click on the *Install Adobe Acrobat* button. After the program has installed, return to the DVD and once again click the *Release Notes* button to display the notes **(3).**

4: If the program DVD is not available, the same *Release Notes* file may be accessed from the hard drive. Launch *Microsoft Word* and open:

C:\Program Files
Leica Microsystems
Leica Application Suite
Release Notes.pdf



Hardware Setup:

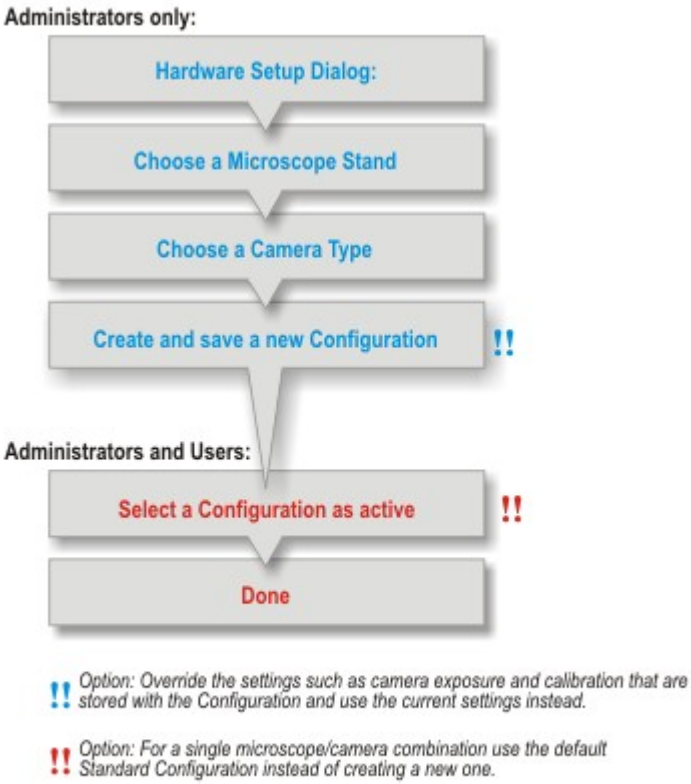
The *Hardware Setup* facility allows Administrators to create configurations for either a single microscope and camera combination or multiple combinations that are sharing the same personal computer.

Although several microscope/camera combinations can be plugged into the computer, only one can be active and connected to Leica Application Suite. *Hardware Configurations* allow a specific combination to be selected and connected.

As well as the microscope and camera combination, the Configuration saves the last used camera settings and the calibration values so when the Configuration is recalled all of the settings are automatically applied. An overwrite is available that will apply the settings established by the Administrator rather than recall those last used by the current User.

Only computer Administrators can create *Hardware Setup Configurations*, but both Administrators and Users can select and use a *Configuration*.

Continued... 9



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A: *Administrators with User Rights turned OFF:*

1: Press and hold down the keyboard *Shift* key.

2: Double-left click on the LAS *desktop icon.* The *Application Suite Framework* loads ready to open the *Hardware Setup* dialog: *Go there…* 10

B: *Administrators with User Rights turned ON:*

3: Right-click on the LAS *desktop icon*.

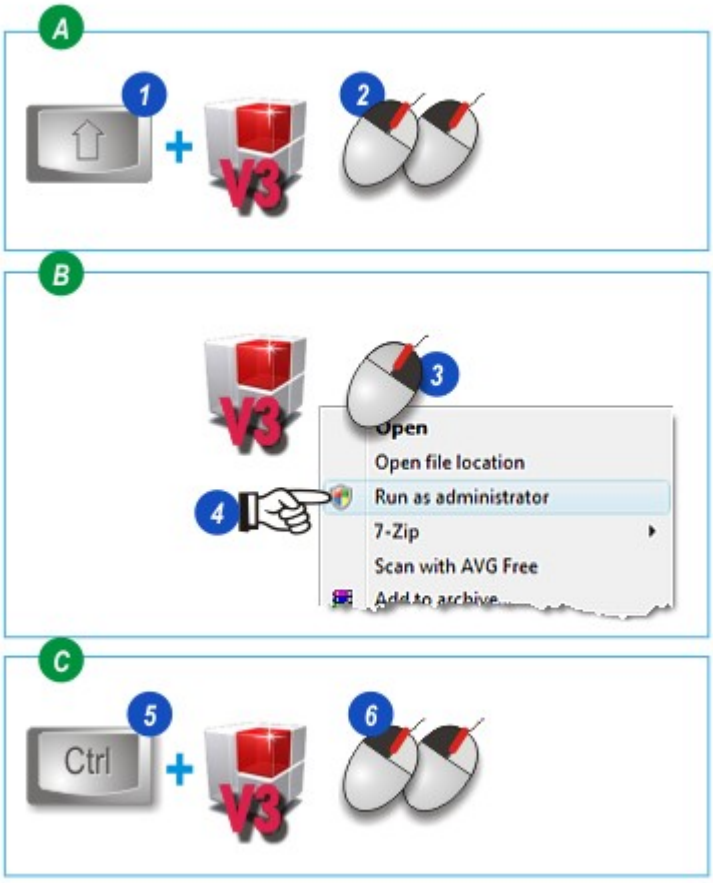
4: From the drop-down menu left-click on *Run as administrator*. The *Application Suite Framework* loads ready to open the *Hardware Setup* dialog: *Go there…* 10

Selecting a Configuration to connect to LAS:

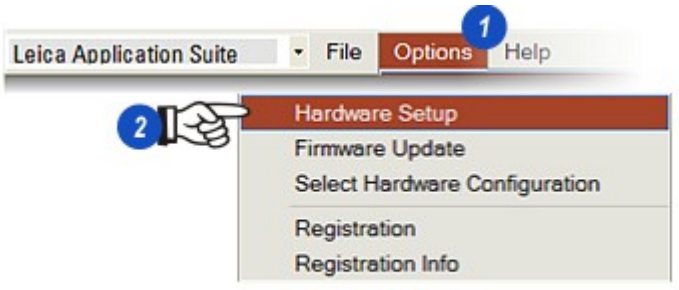
C: *Administrators and Users:*

5: Press and hold down the *Ctrl* key.

6: Double-click the LAS *desktop icon*. The *Application Suite Framework* loads with the *Configuration Selection* dialog displayed: *Go there…* 17



For Administrators:
Computers that have just one microscope attached must also have the hardware set up and saved as a configuration - usually, for convenience the default *Standard Configuration* name is used although any appropriately named configuration can be saved



1: Click on *Options* on the main header.

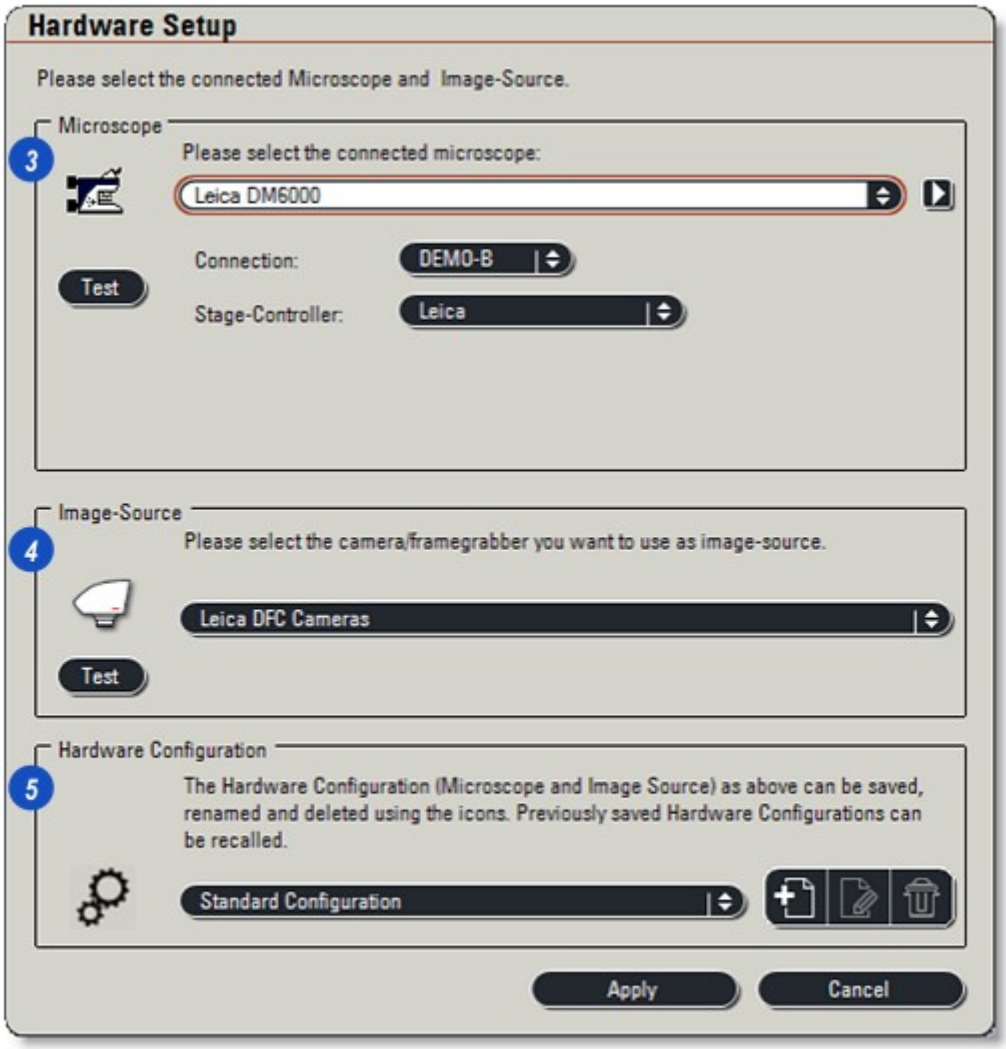
2: From the drop-down menu click to select *Hardware Setup.* The *Setup* dialog appears. It is divided into three sections:

3: *Microscope* in which a microscope stand can be selected. Additional controls appear depending upon the microscope chosen.

4: *Image Source* provides for camera selection.

5: *Hardware Configuration* provides the facility for storing the selected microscope and camera combination as a Configuration file with an appropriate name. This can be retrieved by any Administrator or User at a later date and the microscope/camera combination 'connected' to LAS.

Continued… 17



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To select the microscope stand:

1: Click on the small arrows to the right of the *Microscope* header and…

2: …from the drop down list click to select the stand model.

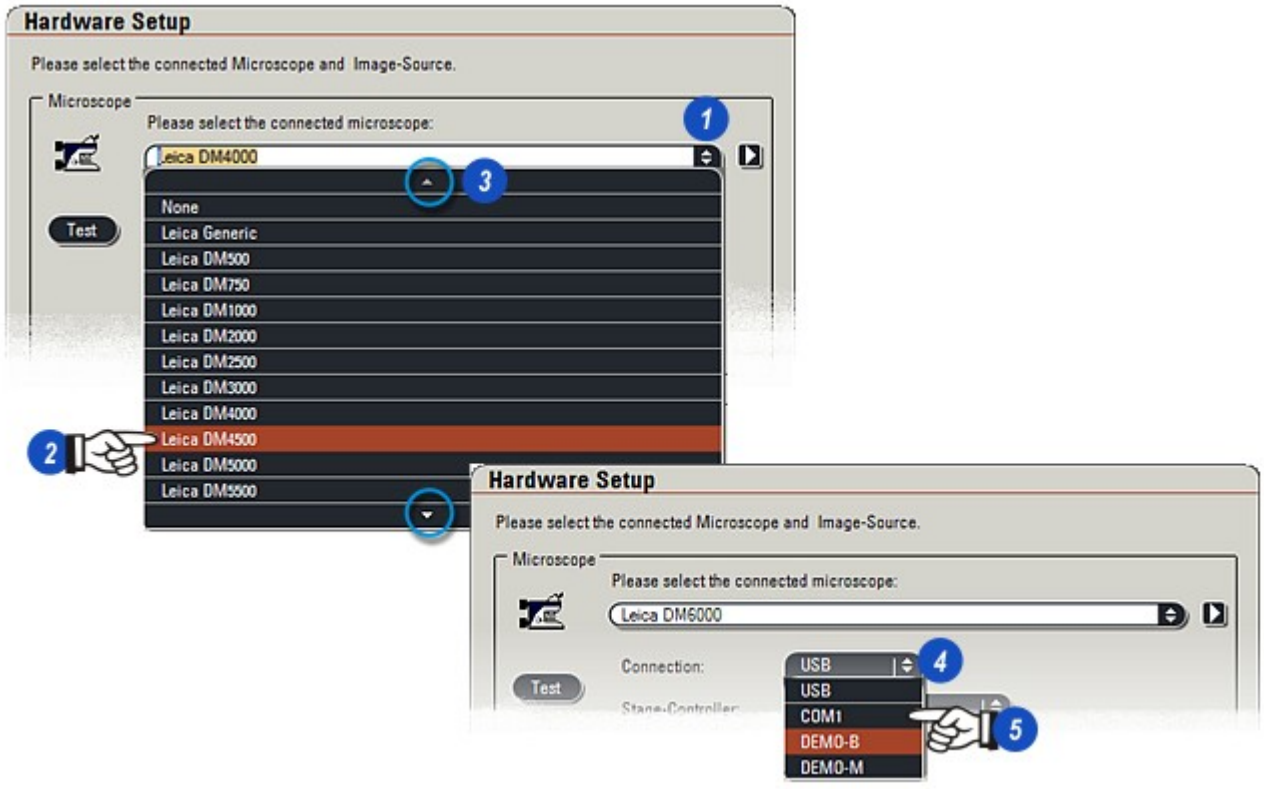
3: If necessary, use the up/down arrows to scroll the list.

Select the Connection type:

4: For microscopes that have a connection option, click on the small arrows to the right of the *Connection* header and…

5: …click to select the *Connection* type. Several microscopes have a 'virtual' or *Demo* mode – a microscope stand is not actually present but the *Leica Application Suite* software can emulate limited microscope behaviour. *Demo* modes are ideal for training and evaluation.

Continued… 12



Stage Controller setup:

1: Click on the small arrows to the right of the *Stage Controller* header and…

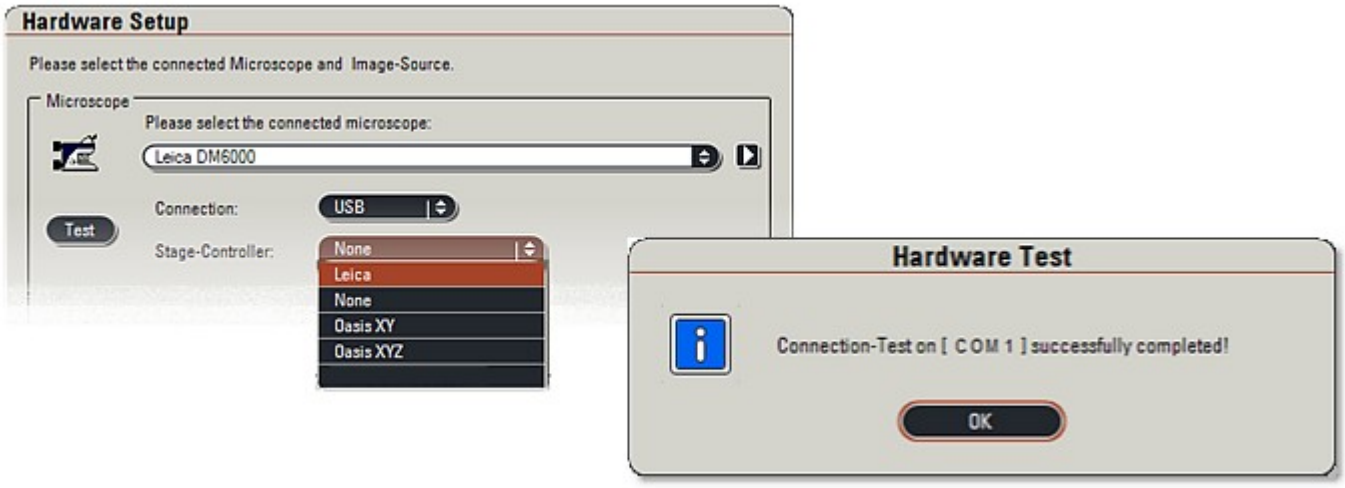
2: …from the drop down list click to select the installed controller. If the microscope does not have a motorised stage, click to select the *None* option.

Microscope Connection Test:

3: To test the connections to the microscope and any other associated hardware (excluding the *Image Source*), click on the *Test* button.

4: Click the *OK* button if the *Connection Test* is successful, otherwise check all connecting plugs and power connectors and re-try.

Continued… 13



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It is possible to keep a log of all the communications between the microscope and *Leica Application Suite* which could be of use to a service engineer. Enable communications traffic by:

1: Click on the small arrow to the right of the *Microscope* menu header.

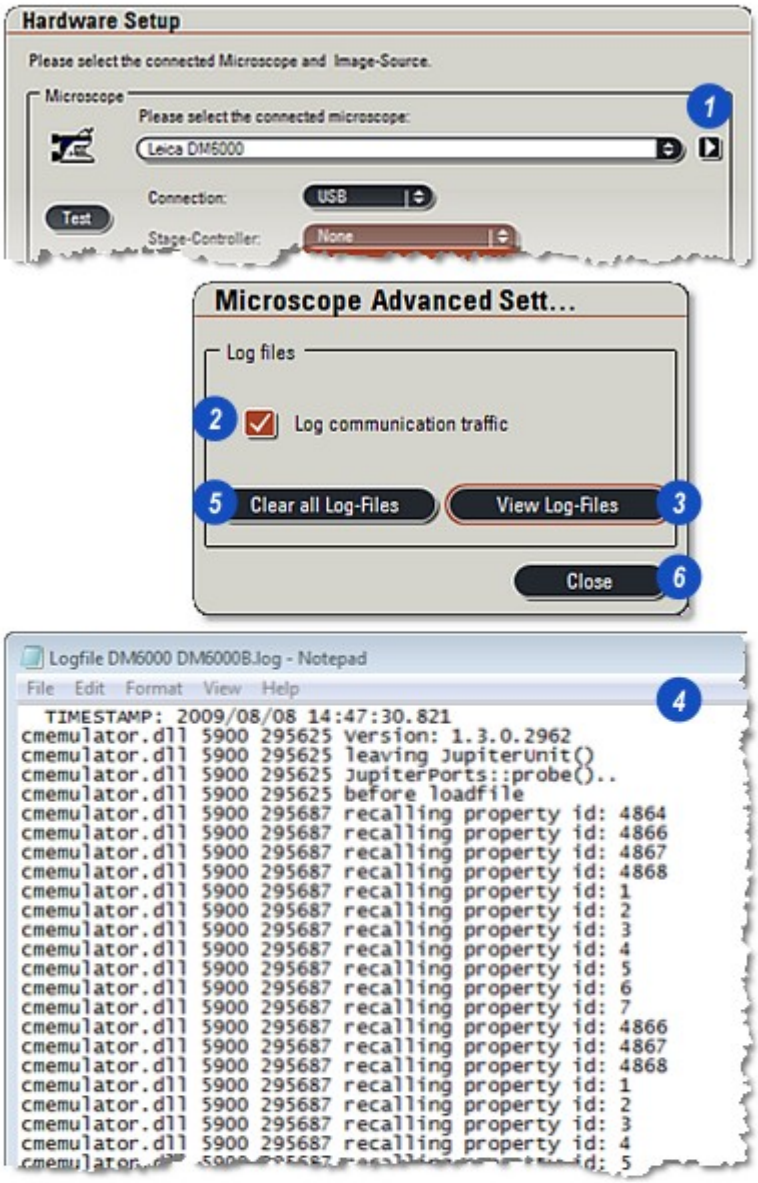
2: On the *Microscope Advanced Settings* dialog, click to enable the traffic logging check box. A small tick mark should appear.

3: Click on the *View Log Files* button to display the log data (usually in *Notepad*: **4**).

5: If required, clear the log data by clicking the *Clear all log files* button.

6: Click the *Close* button.

Continued... 15



Choose a camera by:

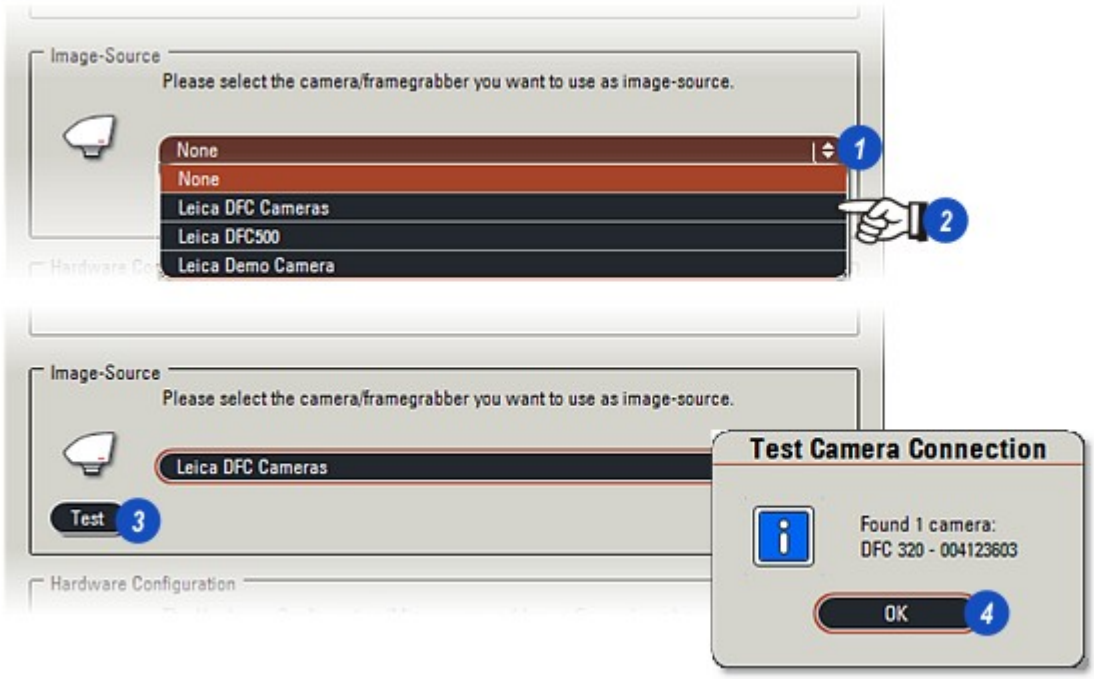
1: Click on the small arrows to the right of the *Image Source* drop down header.

2: Click to select the image capture device from the drop down menu. *Leica Demo Camera* uses an image installed with the *Application Suite* and is ideal for evaluating some of the optional modules without a camera or frame grabber attached to the microscope.

3: Click on the *Test* button (does not apply to *Demo Camera*) to check that the device selected is communicating with the software.

4: If the device is found it will be confirmed on the *Test Camera Connection* dialog. Click *OK*.

Continued… 16



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If the default *Standard Configuration* is to be used (usually for a single microscope/camera attached to the computer), skip to Saving the Configuration: *Go there...* 16

Creating a New Configuration:

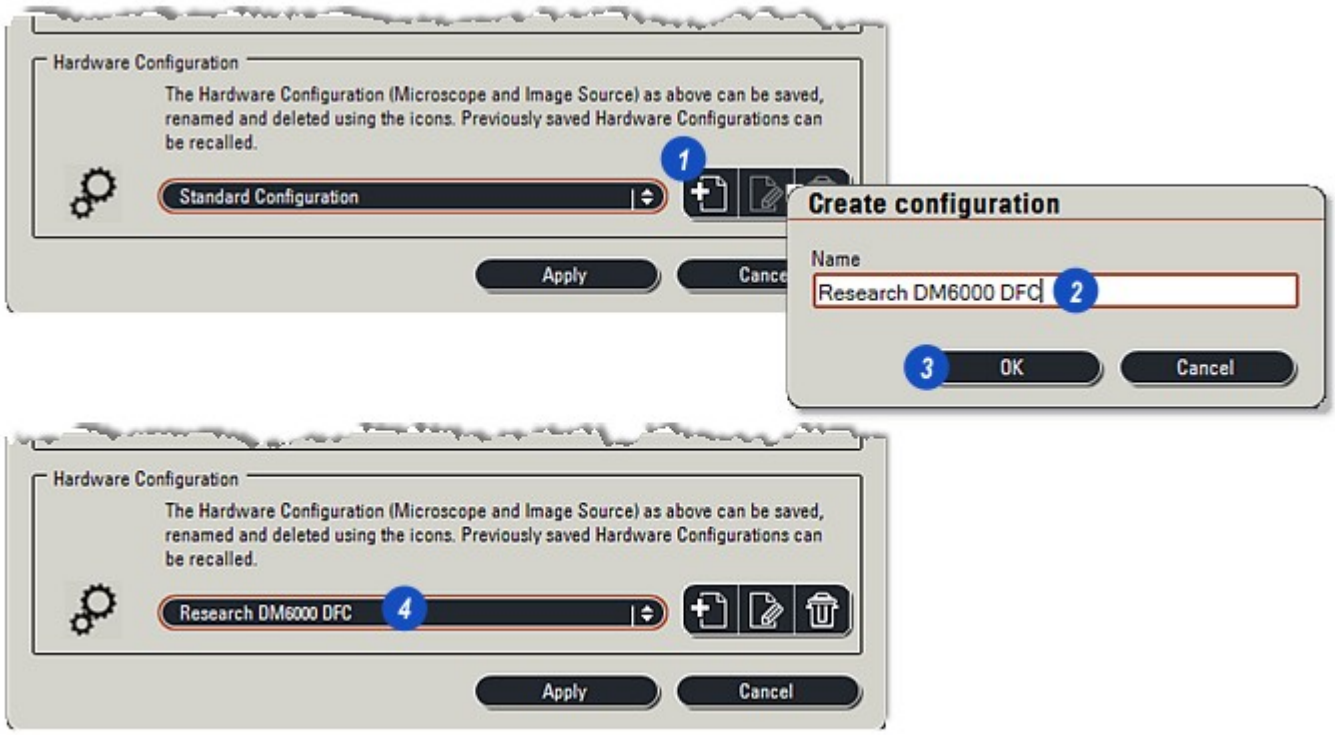
1: Click on the *Create Configuration* button.

2: On the dialog, click inside the text box and type a new, unique name for the configuration.

3: Click *OK.*

4: The new configuration name appears in the *Hardware Configuration* window.

Continued… 11



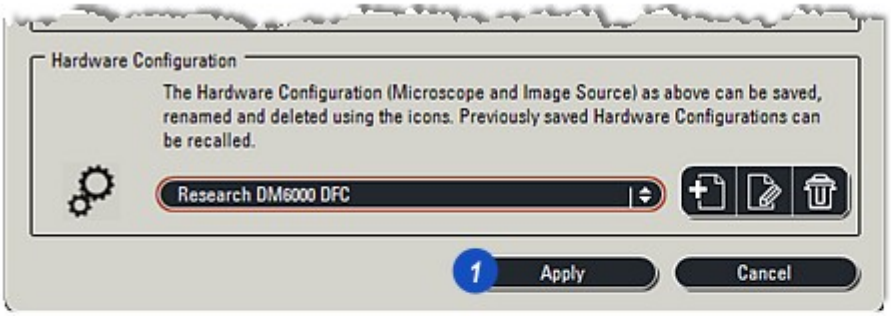
With the microscope and image source selected:

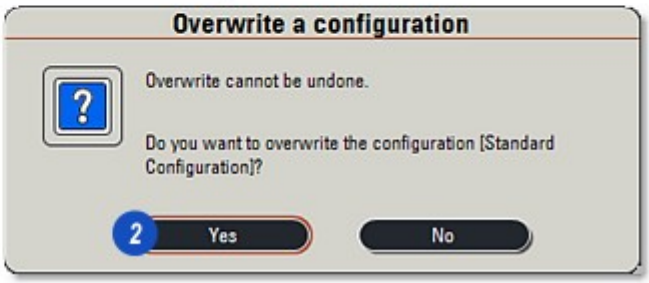
1: Click on the *Apply* button.

2: The *Overwrite* dialog appears warning that the current hardware setup will overwrite any existing values in the *Configuration* file. This safety warning appears even if the named *Configuration* is newly created and does not yet contain data. Click *Yes* to save the *Hardware Configuration.*

The process of creating and saving a configuration does not automatically apply it. Configurations have to be selected and loaded specifically. See the following page.

Continued… 17





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A: *Administrators already working in Hardware Setup and the Framework is open:*

1: Click on *Options* on the main header.

2: Click on the Select Hardware Configuration option.
Go to item **(7)** below.

B: *Administrators and Users from the Desktop starting with the Shift + Icon double-click:*

3: Click on *Options* on the main header.

4: The drop-down menu has fewer options compared to the *Administrator* only menu above. Click on the *Select Hardware Configuration* option.

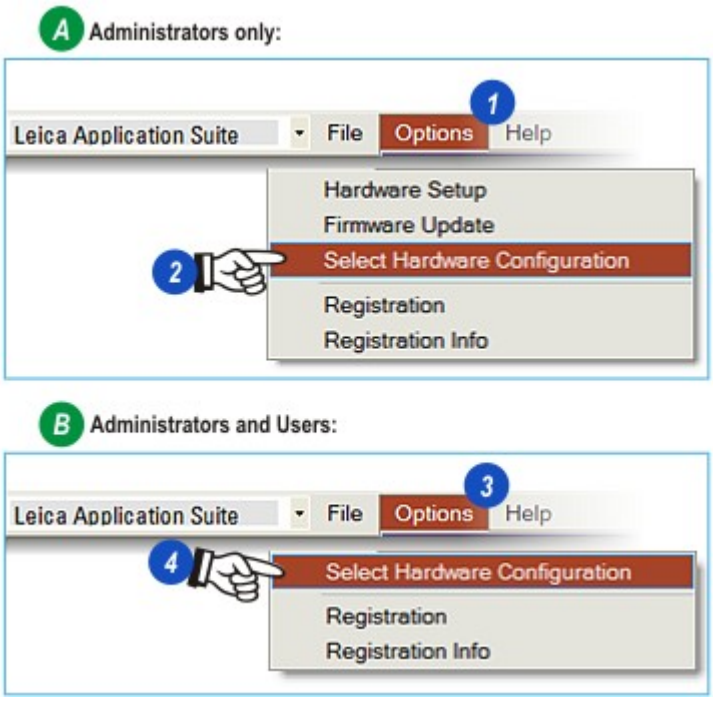
5: The *Administrators* dialog has an addition button – *Hardware Setup* **(11)** that will launch the *Hardware Setup* sequence described on previous pages.

6: The *Users* dialog.

To select a Hardware Configuration:

7: Click on the small arrows to the right of the configurations header and…

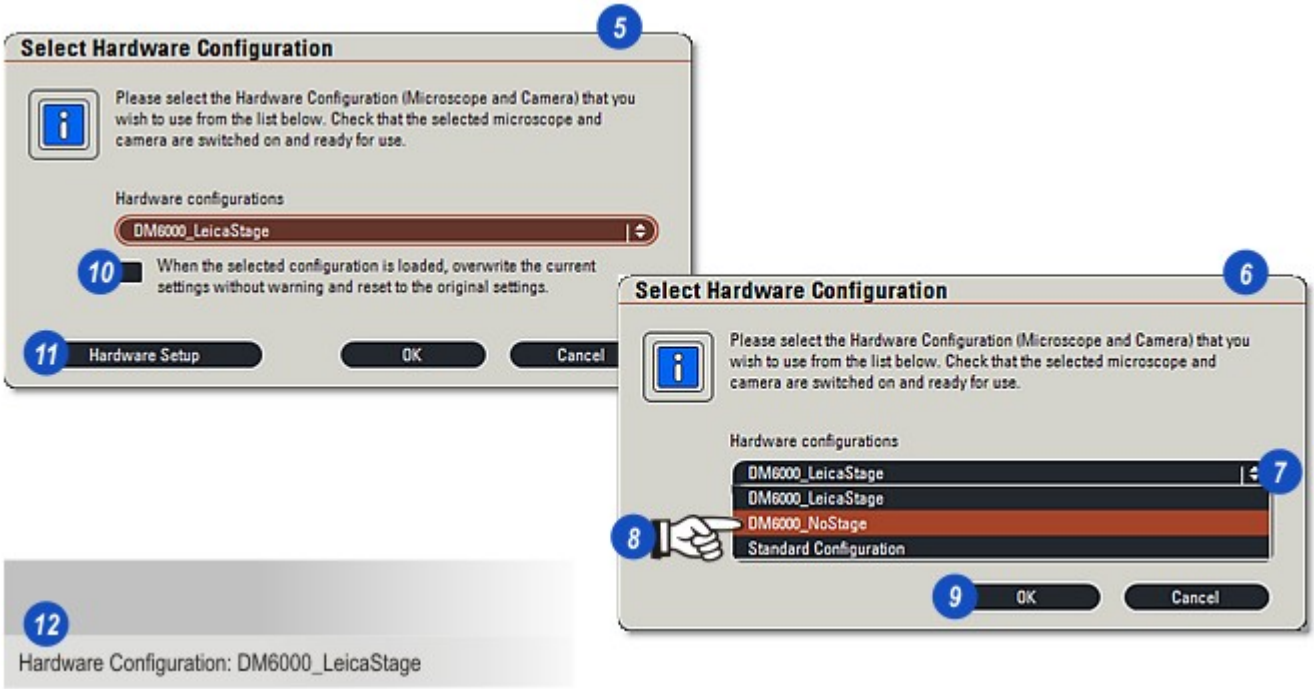
8: …click to select the required configuration.



9: Click *OK*.

10: To automatically load the prevailing camera settings – exposure, live and capture types etc., - click to enable the *Overwrite Current Settings* check box. If left unchecked the camera settings stored with the Configuration will be loaded.

12: The selected Hardware Configuration is displayed bottom left of the LAS interface.



Launching Leica Application Suite:
In most cases the Application Suite can be launched by double-clicking on the desktop icon:

To start in Administrator mode, right-click on the icon, and select 'Run as Administrator'.

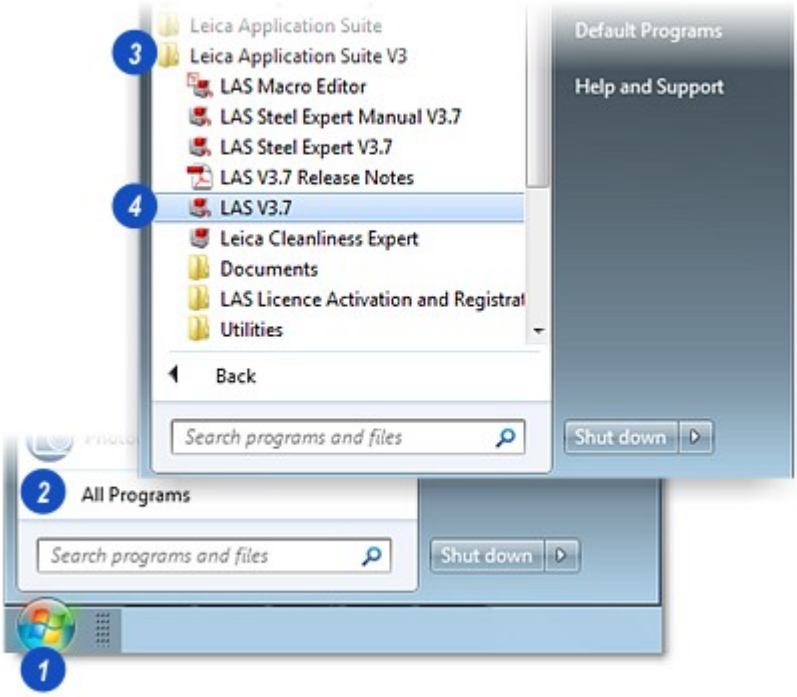
Alternatively:

1: Click the Start button on the Windows Task Bar (bottom left).

2: From the popup click on *All Programs.*

3: Locate and click *Leica Application Suite* on the *All Programs* list and...

4: Click on *Leica Application Suite* from the dropdown. LAS should load and run.



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The User Interface:

The illustration is a graphical representation of the LAS display showing the principal features:

1: *Main Tool Bar*: Come here for File, Options and Help menus.

2: *Workflows*: Select Setup, Acquire, Browse, Process and Analysis here.

3: *Module Launcher:* Click to reveal the installed modules and launch them if they are enabled.

4: *Control Panels:* All of the power tools for the selected *Workflow*.

5: *Tabbed Control Panels:* Click tabs to select additional tools for the *Workflow* and running module.

6: *Image Data Form:* Displays and edits selected data for the current image.

7: *The Image Viewer:* Display and working area for the current image: Press keyboard F5 to show full screen.

8: *Side Tool Bar:* Common working tools control all aspects of the display and tasks.

9: *The Grid:* Displays the current folder image data in a scrollable grid format. Available only with LAS *Archives*.

10: *The Gallery:* Displays a thumbnail of all the images in the selected folder.

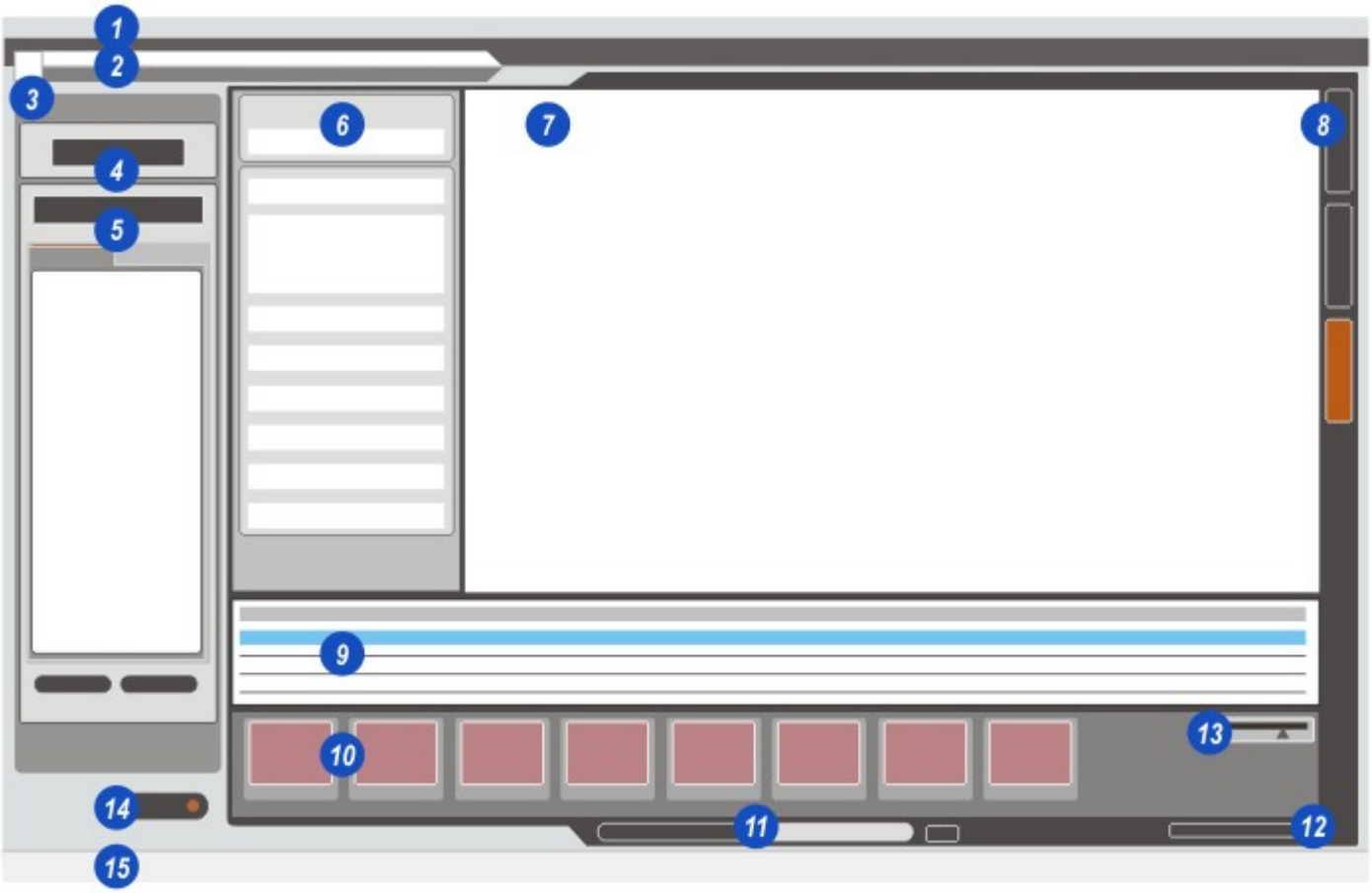
11: *Fast Search Controls*: Create filters and fast search for images. (*Archive* Option dependant).

12: Gallery *Browser*: Locate rapidly thumbnails and display in the Viewer.

13: *Gallery Thumbnail Scaler*: Click and drag to re-size the thumbnails.

14: *Acquire*: Universal capture button.

15: *Status Bar:* Displays *Hardware Configuration, RGB Intensity, Stage Position* and *Magnification* data.



The Leica Application Suite *User Interface* is divided into 5 main areas.

1: *Module Launcher:* Click here to open the Launcher and click to select the required module.

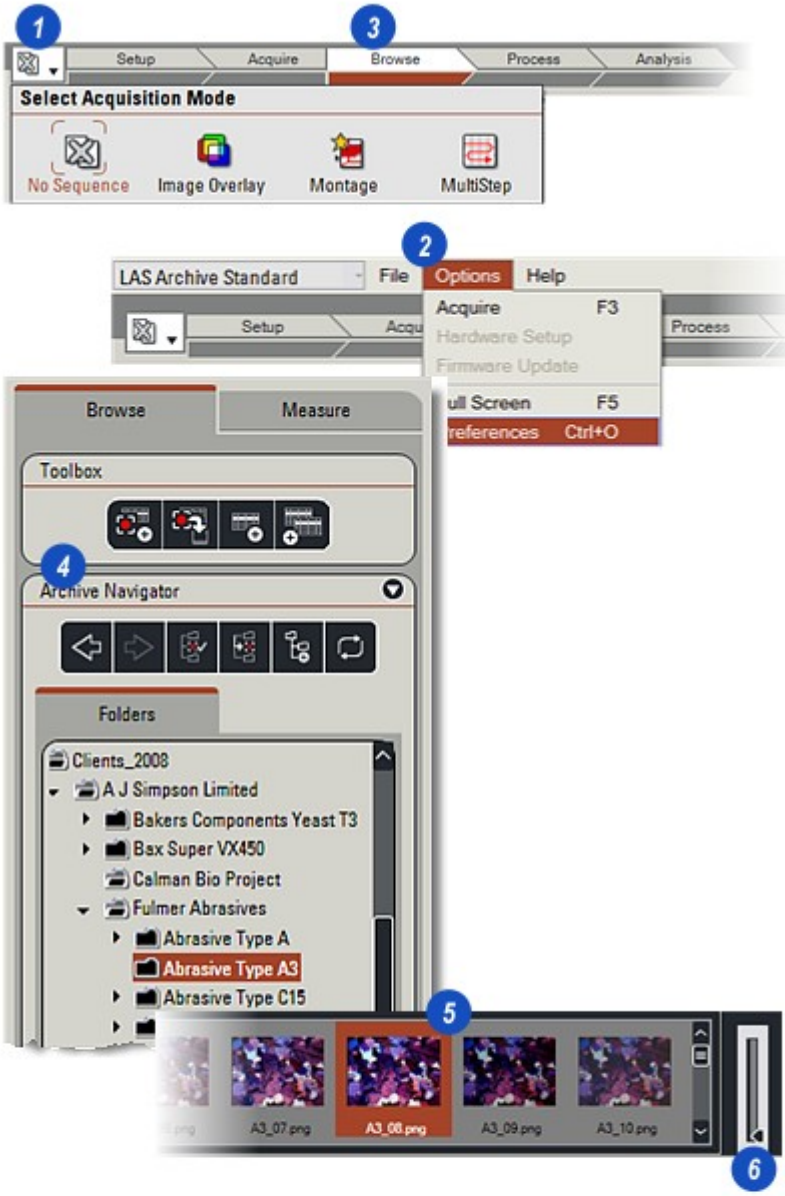
2: *Menu bar:* Select items here to access administrative options and Preferences

3: *Workflow bar*: The *Workflow* creates the appropriate panels and controls for the selected application. Click on a *Workflow* to open it.

4: *Control Panels:* The programme controls are displayed on tabbed panels. Click on the tab to display the panel.

5: *Image Viewer* and *Gallery:* The remaining part of the screen application is devoted to the *Image Viewer* , *Grid* and *Gallery.* The thumbnail images in the *Gallery* can be re-sized by clicking on the slider **(6)** and dragging it up to increase the size or down to decrease it.

.



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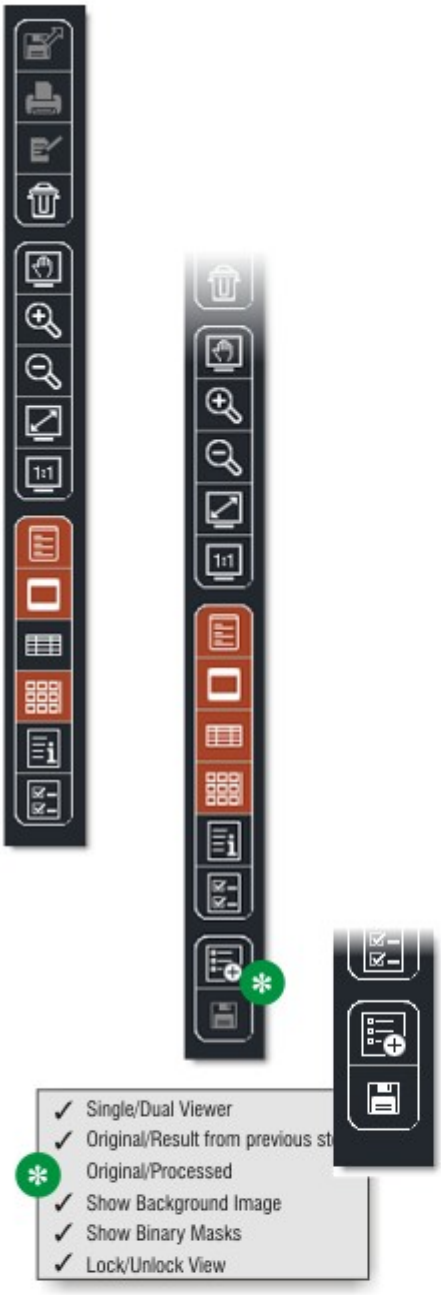
The *Side Tool Bar* is situated on the right-hand edge of the *Viewer* and provides the essential working tools for many of the tasks concerned with managing images - *Export, Delete* and *Print* - and customising the environment - *Hide* and *Reveal* display features, *Fit to Screen* - and so on.

If optional modules are installed, where appropriate the *Side Tool Bar* changes to reflect the additional functions that the modules provide (*). In the illustration the additional tools are loaded with module *Image Analysis.*



Leica Application Suite has a wide range of Keyboard Keys and Mouse combinations to simplify actions for the user and so speed throughput and improve productivity. Some shortcuts are applicable only to particular optional modules and if those modules are not installed the shortcuts will not function.

Shift + Left click LAS desktop icon: Start LAS framework only so hardware configuration and firmware download, licensing can be used. *Note: Requires administrator privileges.*

Control + Left click LAS desktop icon: Show Hardware Configuration dialog.

Show help files for the module currently active.

Acquire single image / current mode image sequence.

Show image full screen size - live and browse image.

Return from image full screen to normal LAS layout.

With Dual Monitor mode enabled - switch to second monitor to show image.

With Dual Monitor mode enabled - revert to single monitor to show image.

Left click and drag on live image: Used to set Region of Interest for spot exposure, zoom focus, etc.

Show camera refresh rate at top right hand of image.

Show actual image size.

Show Preferences dialog.

Mouse wheel on image: Image zoom in and out.

Slider control is selected: Mouse wheel adjusts slider is single steps - for example in Exposure.

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If Measurements is licensed: On/off - show a zoom region around the mouse position. For example, when setting calibration end points or drawing in Live Measurements.

When the Gallery or Grid have focus: Selects all images in Gallery.

When the Gallery or Grid have focus: Copy the selected image and its metadata to the clipboard

Paste the selected image and its metadata to the folders indicated in the navigator. Note the folder must be a folder that the user can access and also not a sequence folder.

Control plus left click on an image in the Gallery or Grid entry: Add this image to the selected images.

Shift plus left click on an image in Gallery or Grid entry: Select all the images between a previously selected image and the clicked image.

Right click on an image in Gallery or Grid entry: Show pop-up context menu with Open, Open with, Send to and Export options.

Right click on an image in Browse: Show pop-up context menu with Open, Open with, Send to, Export, Print, Copy, Zoom and Pan window options.

Select all data in the Record Details table.

Select and individual record in the Record Details table.

Select all the records between a previously selected record in the Record Details table and the clicked one.

Copy all of the records in the Record Details table to the clipboard. They can then be pasted into another application, *Word* for example.

Shortcut Keys: Live and Interactive Measurements Shortcuts:

Alt Gr held down + draw measurement: Hide the labels so that it is easier to see where the measurement is drawn. **Also** Ctrl + Alt for keyboards that do not have the AltGr key.

Shift held down: Show a zoom region around the mouse position.

Space bar held down: Temporarily switch to Edit mode after drawing.

With the Select tool ‘on’, click and drag rectangle around measurements: Selected measurements in the rectangle can be grouped. Or edited collectively.

Double click on Parameter name in Grid: Label all measurements with this Parameter if possible. *Note: Some measurements don’t have all parameters.*

Click on Grid header near small arrow: Re-orders the results in increasing or decreasing values.

Selects all of the measurements in the Grid.

Selects an individual measurement in the Grid.

Selects all of the measurements between and including the currently selected measurement in the Grid and this one.

Copies the selected measurements in the Grid so they can be pasted into another application such as *Word* or *Notepad*.

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Shortcut Keys: Annotation Shortcuts:

Start a new line in a Text Box or Rectangle.

If Measurements is licensed, toggles On/Off a zoom region around the mouse position.

With an annotation selected, copy the annotation to the clipboard.

Paste the contents of the clipboard into a *Word* document.

Select all of the annotations in the Grid.

Select an individual annotation in the Grid.

Selects all of the annotations between and including the currently selected annotation in the Grid and this one.

Copy the selected annotations to the clipboard so that they can be pasted into another application such as *Word* or *Notepad*.

Shortcut Keys: Image Analysis Shortcuts:

Double click on Parameter name in Grid: Label features with this parameter where possible.

Click on Grid header near small arrow: Re-orders the results in increasing or decreasing values

Shift held down: Show a zoom region around the mouse pointer whilst in the Binary Edit step.

Click on a feature in the Measurement step: Select the feature and move it to the top of the Grid.

Click on a row on the Grid: Selects and highlights the feature on the image.

Whilst in Measurement > Edit, deletes the select feature.

Selects all measurements.

Selects an individual measurement.

Selects a range of measurements between and including the currently selected measurement and this one.

Copies the selected measurement(s) to the clipboard so that they can be pasted into another application such as *Word* or *Notepad*.

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Shortcut Keys: Montage 3D Viewer Shortcuts:

Drag the model to a different viewing angle: Rotate and tilt.

Drag the model to a new location: Pan.

Zoom the model.

Mouse wheel click and drag: Select a region of the model to zoom.

Scale the model height.

Shortcut Keys: Power Mosaic Shortcuts:

Mouse Interactive (on side tool bar) enabled: Double click Mouse Wheel: Return directly from a zoom to show mosaic in 'Fit to Window' mode.

Move Pattern (on side tool bar) enabled: Hold down Control: Click and drag Mouse on the mosaic Pattern to rotate it.

Move Pattern (on side tool bar) enabled: Hold down Shift: Halt mosaic Pattern rotation.

Mouse Interactive (on side tool bar) enabled: Right Mouse click on Map to display Map Properties dialog.

Power Mosaic Plus Shortcuts:

Mouse Interactive (on side tool bar) enabled: Right click on Workspace to display Workspace properties.

Move Pattern (on side tool bar) and Pattern Navigator enabled: Click and drag Workspace or Pattern.

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Dual Monitors:

With the necessary *Dual Screen Video* card and software installed (*), Leica Application Suite can be configured quickly and easily to show all of the usual controls - including optional modules - on one monitor (*Primary*) whilst using the entire viewable area of the other (*Secondary*) for the image - live or captured.

The extra-large image area provides greater precision and ease of working for capture, analysis and measurement, whilst the greater area given to *Gallery Thumbnails* means many more can be displayed at once or individuals enlarged to examine fine detail.

The *Side Tool Bar* is automatically displayed on the appropriate monitor and *Dual Monitor Mode* can be enabled or disabled with a single keystroke.

(*) Use of dual monitors requires that the graphics card in the PC supports this option. Some graphics cards lack performance that slows down the movement of the mouse cursor. If this occurs, the drivers of the graphics card may need updating or it may not have the necessary performance.

The conventional layout for dual monitors is primary monitor (displaying Windows controls) in front of the user, and the secondary monitor to the right. This is initially setup in the video driver software, but can be changed in Windows so that the secondary monitor is moved to the left without affecting the smooth transition of the cursor from one screen to the other.

- LAS users may find swapping the monitors a convenient way of reducing the distance the cursor has to be moved to place it on the image.

1: Right-click on the *Desktop*.

2: On the menu, for Windows XP users, click on *Properties*. For Vista users click on *Personalise*.

3: On the dialog, Windows XP users click on the *Settings* tab and Vista users the *Display Settings* option.

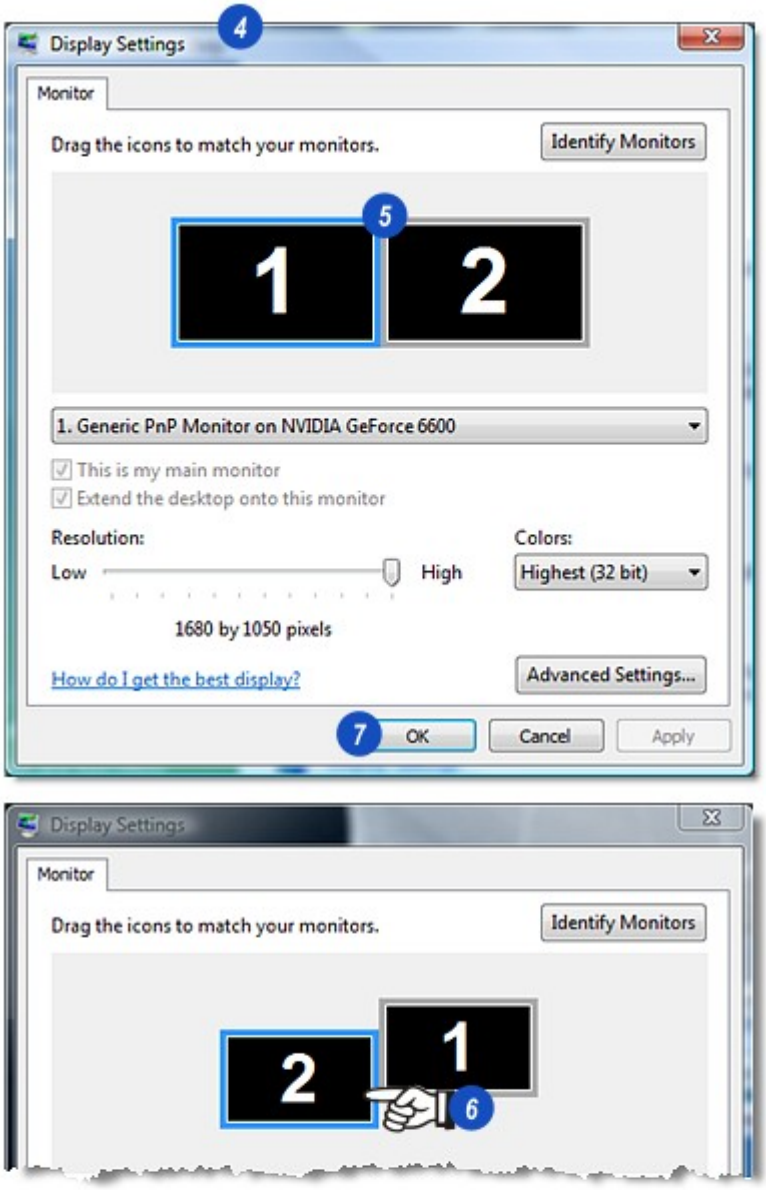
4: The illustration shows the Vista dialog but the Windows XP version is very similar.

5: The two monitors are shown as icons – *1* being the primary display and *2* the secondary.

6: Click and drag the secondary display to the left of the primary display and release the mouse button.

7: Click *OK*.

Continued... 31



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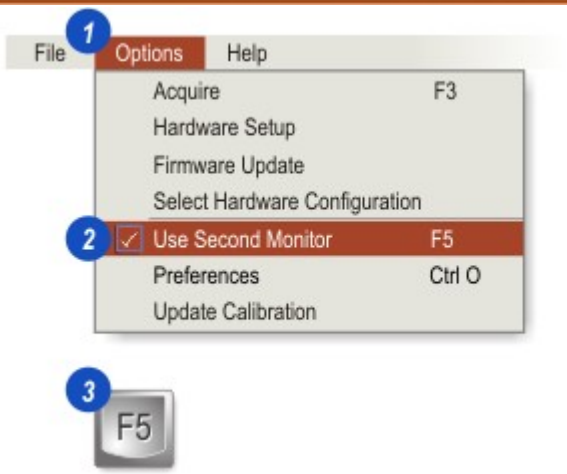
Dual Monitors: Enabling and Disabling:

The secondary monitor is turned on or off either from the *Options* menu or by pressing keyboard function key *F5.*



1: Click on *Options* on the main tool bar.

2: On the drop down menu, click to enable the check box to the left of the *Use Second Monitor* option. Clicking the checkbox again will disable the second monitor and move the *Image Viewer* back to the primary monitor – the usual LAS layout.

3: Function key *F5* performs the same operation with fewer mouse clicks. Press *F5* to enable the second monitor and press again to disable it.

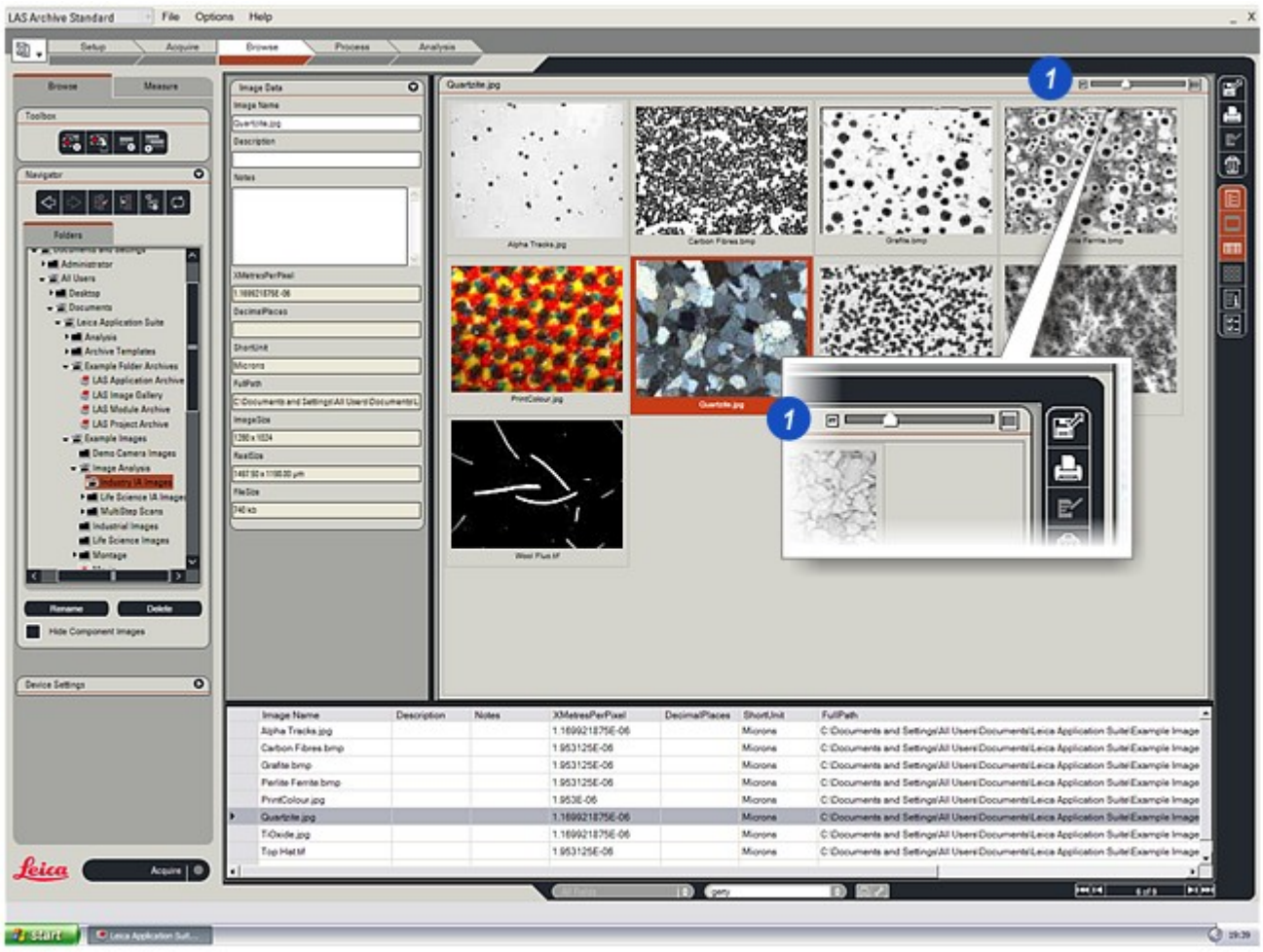
Continued… 32

With the *Viewer* moved to the *Secondary Monitor*, all of the *Primary Viewer* area can be occupied by the *Gallery Thumbnails* which means they can be larger and clearer.

The smooth *Thumbnail Sizing* slider has been moved to the top edge - click and drag to the left for smaller, more-on-view *Thumbnails*, or to the right for larger, fewer images.

All of the tools (except image zoom and sizing) are available on the *Side Bar* to show and hide the *Record Form, Record Information, Data Grid* and *Data Items* to *Display.*

Export, Print, Reporting (with some images) and *Delete* are also present. *See Side Bar Tools:* *Go there...* 34



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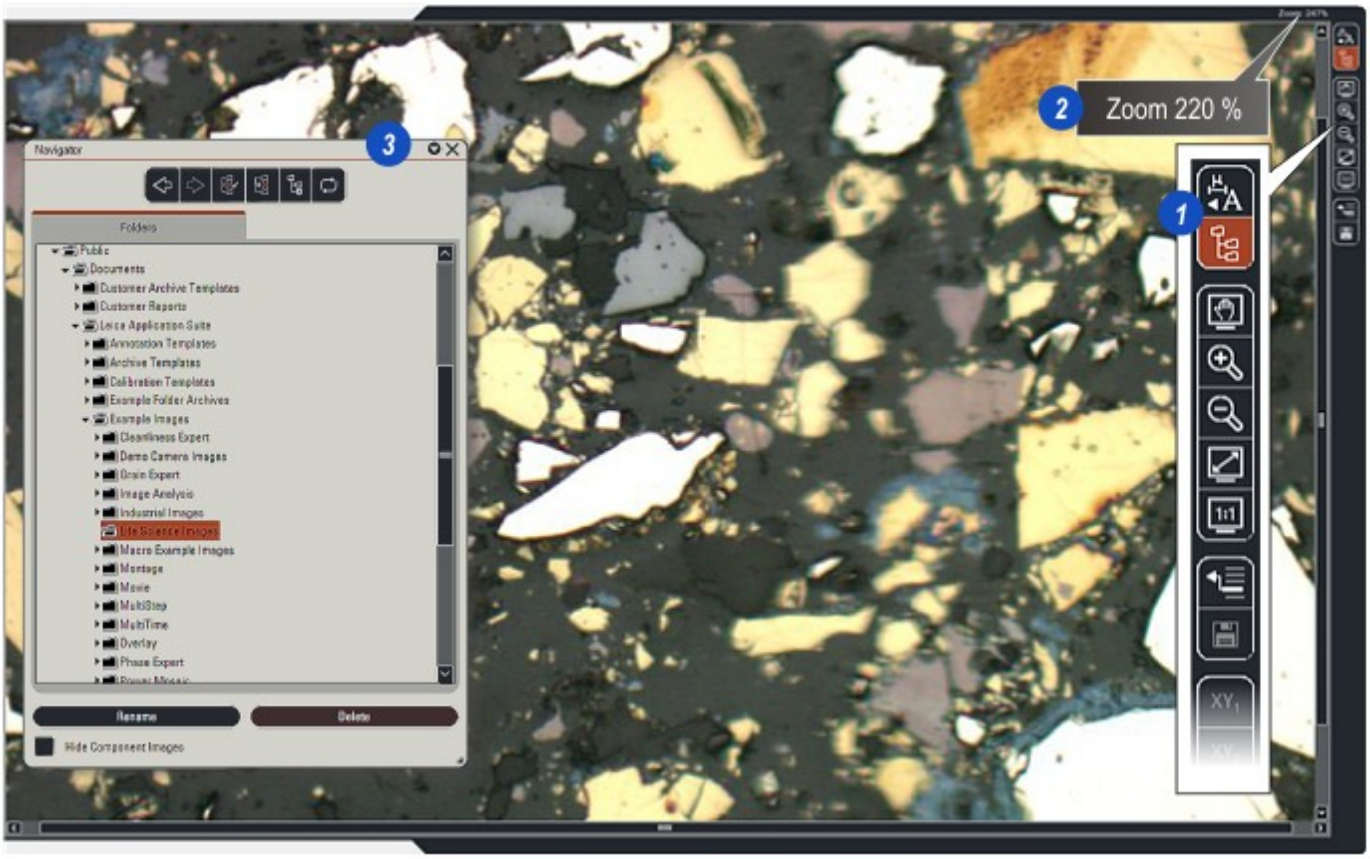
The *Secondary Monitor* is dedicated totally to the *Viewer* so that images can fill the entire screen. Taking measurements or analysing the image is easy and precise with such a large working area.

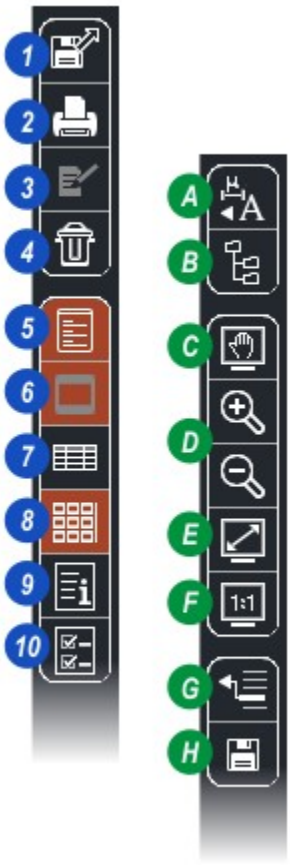
1: The *Image Control Tools* - detailed on the following page - are automatically moved to the *Side Tool Bar*.

2: A *Zoom Level* readout on the *Viewer* top edge.

3: The monitor can also display the *Floating Navigator* which allows users to move between folders without having to return to the *Browse Workflow.*



With the second monitor enabled and displaying the image, the right-hand *Tool Bar* is divided appropriately between the two displays.



On the *primary* monitor displaying the LAS controls:

1: Export image.

2: Print image.

3: Prepare Report depending upon the image type.

4: Delete image (Trash Can).

5: Show/hide the Record form.

6: Show/Hide the image, disabled in Dual Monitor mode.

7: Show/hide the Grid.

8: Show/hide the Gallery Thumbnails, disabled in Dual Monitor mode.

9: Show Record Details.

10: Show Select Visible Fields dialog.

On the *secondary* monitor displaying the Image Viewer:

A: Show the Annotations Options.

B: Open/Close the Floating Navigator (Browse).

C: Pan the Image. The Pan Window appears on the primary monitor.

D: Zoom Into/Out of image.

E: Image to Fill Viewer.

F: Display Image at Actual Size.

G: Viewer Options (Dual Viewer etc).

H: Save the Output Image.

Continued… 35

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A wide range of options is available by right-clicking the image in the *Viewer* or its *Thumbnail* in the *Gallery*. The options vary depending upon which item was clicked, the operating system and the software installed on the computer.

1: Right-click the *Thumbnail* for the context menu of basic options and...

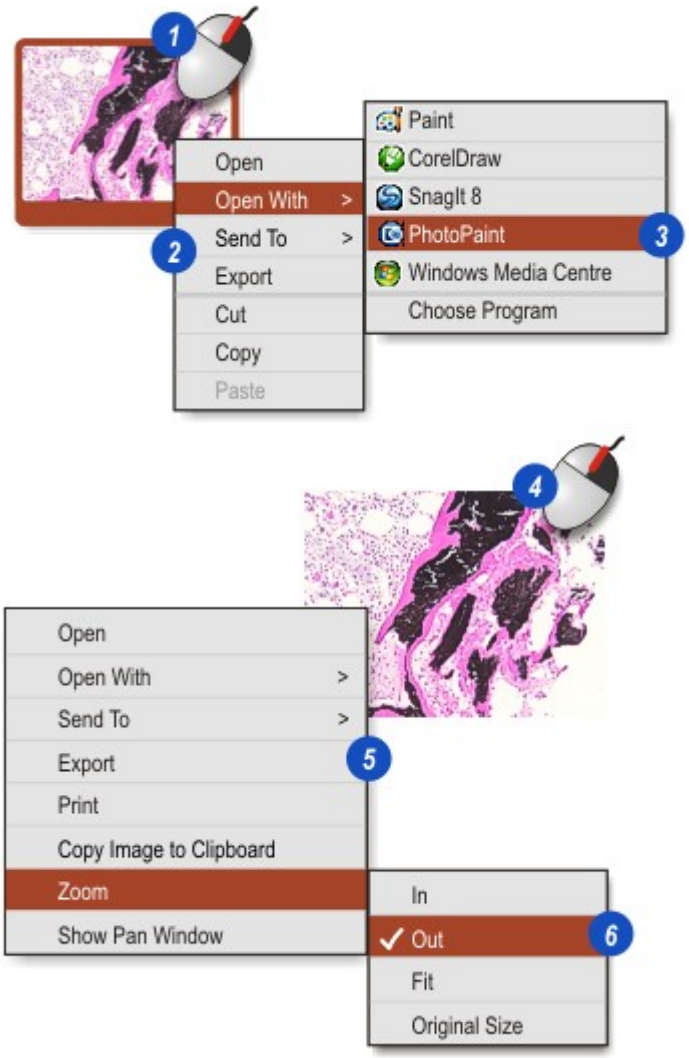
2: ...click it to select.

3: Some options have additional possibilities displayed as a sub-menu.

4: Right-clicking the image in the *Viewer* displays a different context menu.

5: Additional functions are available some of which will also have sub-menus **(6)**.

For detailed help on the available options, see the *Browse Workflow*: *Go there...* 298



This chapter describes the *Workflow* organization of the unique Leica Application Suite user interface and its basic capabilities.

LAS *Workflow* describes the order and grouping of tasks for image documentation and analysis. While the *Workflow* suggests an order for the tasks, versatility is retained so that the operation of the software is not constrained to fixed steps. Grouping tasks into related operations makes working with LAS intuitive and easy.

Click on (↗) to link to a *Workflow:*

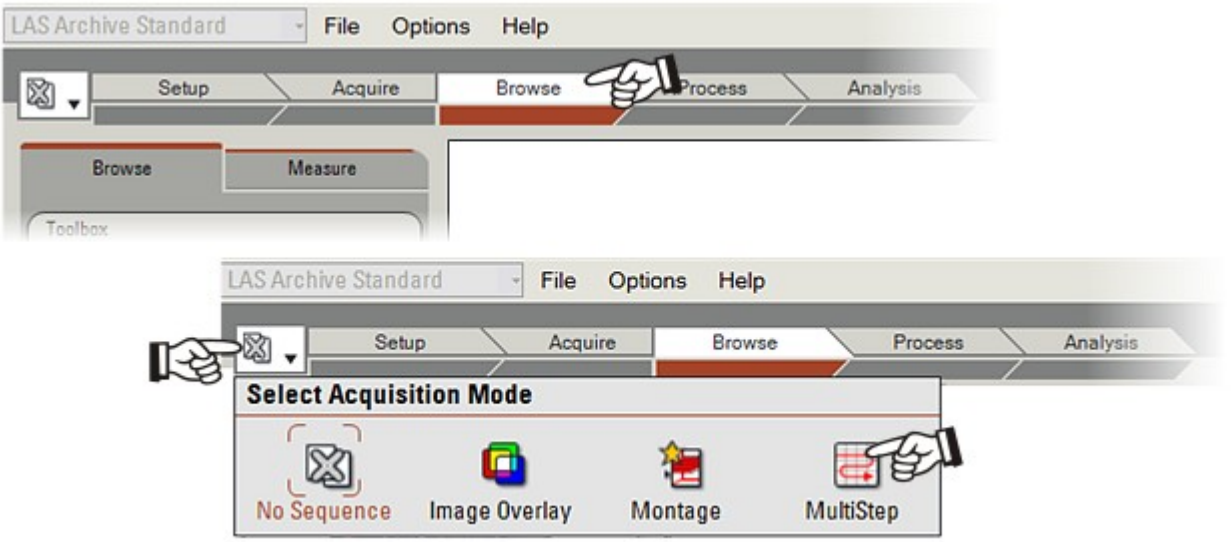
- Setup ↗ 104
- Acquire ↗ 156
- Browse ↗ 298
- Process ↗ 346 and optionally...
- Analysis ↗ 367

Each is selected by clicking on the *Workflow* bar.

Selecting a *Workflow* displays the appropriate controls arranged on one or more panels that allow the user to perform the selected action.

Because the *Workflow* arrangement is so versatile, in contrast to many Windows programs, LAS does not employ a menu bar for the main operation of the software.

Installed and enabled *Optional Modules* are listed on a menu revealed by clicking the *Select Acquisition Mode* icon to the left of the *Workflows*.



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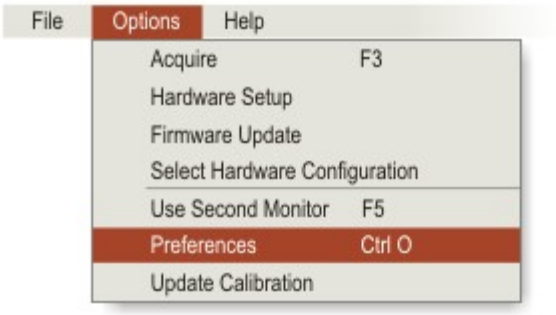
Functions Widely Available:

There are functions within Leica Application Suite that apply to several *Workflows* or have a more global application. These are available from a drop-down menu that appears when *Options* on the main menu bar is clicked.



Clicking the *Acquire* option captures an image and is the equivalent to pressing the function key *F3* on the keyboard or clicking on the *Acquire* button on the *Acquire* or *Browse Workflows.*

Use Second Monitor, or function key *F5* displays the image on the second monitor (if fitted). Click again to return to single screen. Works on all *Workflows.* If only a single monitor is in use, pressing *F5* displays the image full screen with a *'Return to normal LAS layout'* button

Additionally the *Scale Bar* can be accessed from nearly all steps and the *Export Images* feature is also widely available so both are included in this section:

- *Setting Preferences*: 38
- *Scale Bar*: 61
- *Update Calibration:* 69
- *Image Comparison:* 79
- *Export Images:* 84
- *Printing:* 91
- *Gallery Docking:* 103

Preferences:

The *Preferences* dialog allows the user to select preferred options for Leica Application Suite and most of the *Optional Modules* to reflect the fastest and most convenient working methods.

As *Optional Modules* are added to Leica Application Suite, tabs on the *Preferences* dialog are also added to include settings that are specific to the modules.

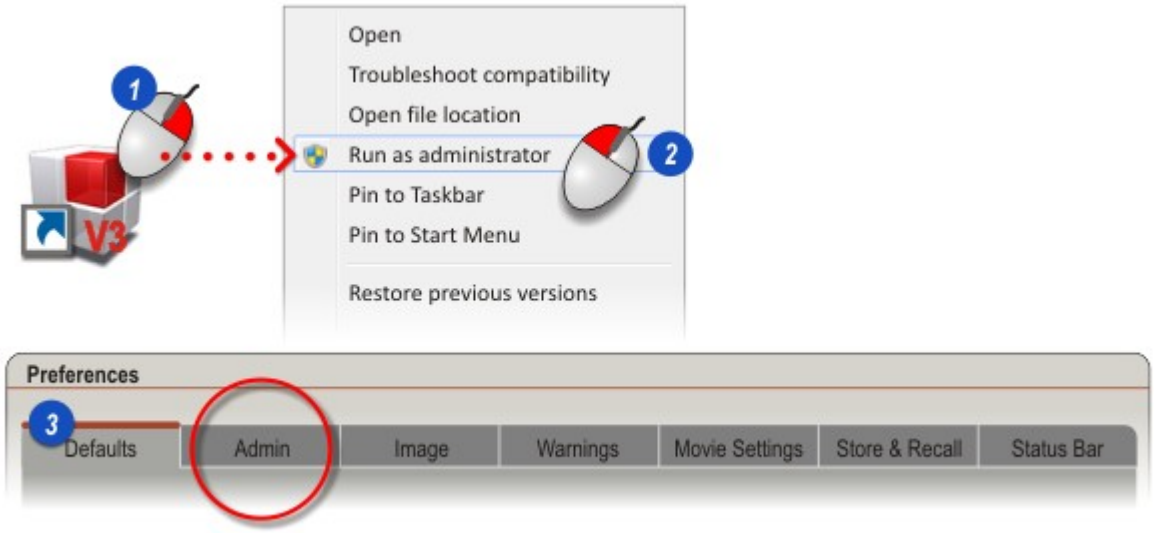
Preferences may be altered at any time – even while a module is running.

When LAS is launched by an administrator, the tabs will vary to include options that only administrators are permitted to set:

1: Administrators launch LAS by right-clicking the desktop icon and...

2: ...left-clicking the *Run as administrator* option on the context menu.

3: When LAS starts and the *Preferences* dialog is opened, the *Admin* tab is present. This is not available to other users.



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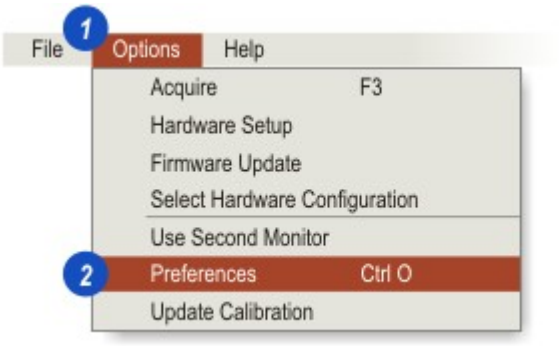
Launching Preferences:

Preferences may be altered at any time – even while a module is running.

1: On the *Main Toolbar*, click on the *Options* label.

2: From the drop down options, click to select *Preferences.*

3: The *Preferences* dialog appears. Click on a tab along the top of the dialog to reveal the options and settings required.



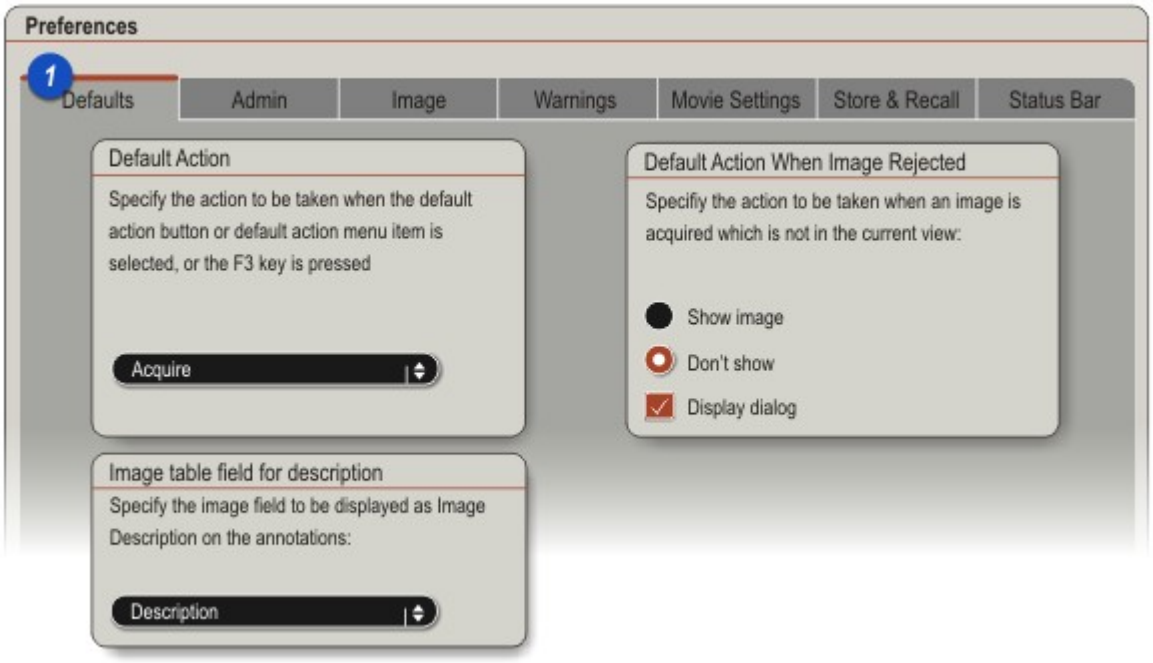
4: When changes have been made, click on the *OK* button to save them or…

5: …click on the *Cancel* button to keep existing settings.

Quick links to the Preferences tabs:

- *Defaults:* What happens after image capture.
- *Admin:* Settings that only administrators can change.
- *Image:* Formats, captions and other image settings.
- *Warnings:* Turns individual warnings on or off.
- *Movie Settings:* Controls the size of movies.
- *Store & Recall:* Save and retrieve image data.
- *Status Bar:* Display helpful information and calibrate monitors.

Click a tab on the illustration for more information.



The *Defaults* tab has three panels that specify the actions that will occur in various situations. The *Default* actions are user defined:

1: Click on the *Defaults* tab to reveal the panels.

- *Default Action*: What should happen when an image is captured.
- *Image Table Field for Description*: Specify the data field to display as the annotation on the image.
- *Default Action When Image Rejected*: The capture location is not currently active so specify the options.

Click on a panel on the illustration for more information:

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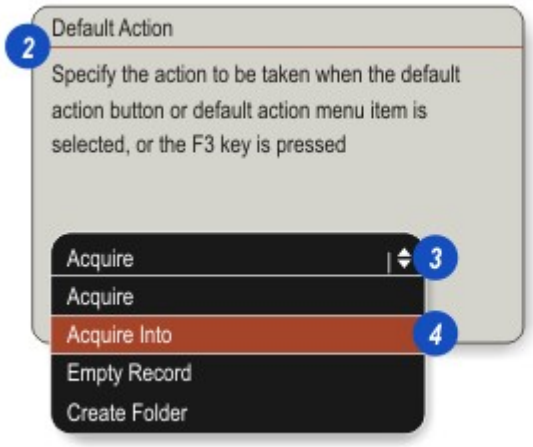
1: Click on the *Defaults* tab to reveal the panels.

2: *Default Action* that determines the action when the shortcut key *F3* is pressed with an archive selected.

3: Click on the arrows to the right of the *Default Action* drop-down header.

4: Click to select the required option that will automatically occur when key *F3* is pressed.



1: Click on the *Defaults* tab to reveal the panels.

2: An archive field from the *Image Table* can be used as part of the image description.

3: Click on the small arrows to the right of the header and...

4: ...from the drop-down menu click to select the field to use with the image. Use the scroll slider to reveal the entire list.

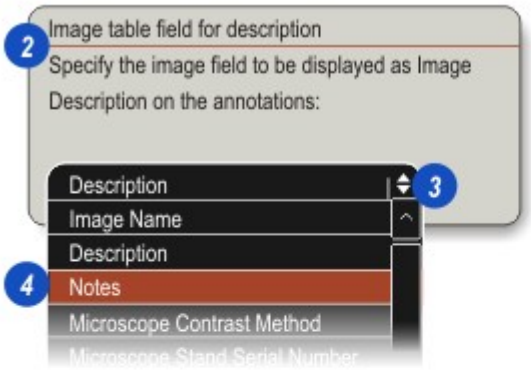
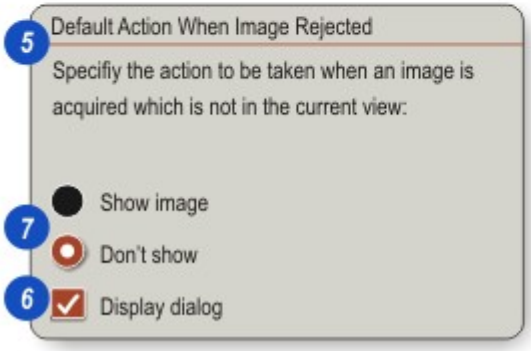


Image Rejected:

5: If an image cannot be saved in the current folder - usually because it has not been nominated as the fixed capture location - chose a default action as follows:

6: Clicking to enable the *Display dialog* check box (Tick mark visible) displays a *Capture Options* dialog to the user. It is recommended that this check box is enabled.

7: Choose to either *Show* or *Not Show* the image by clicking the appropriate radio button.



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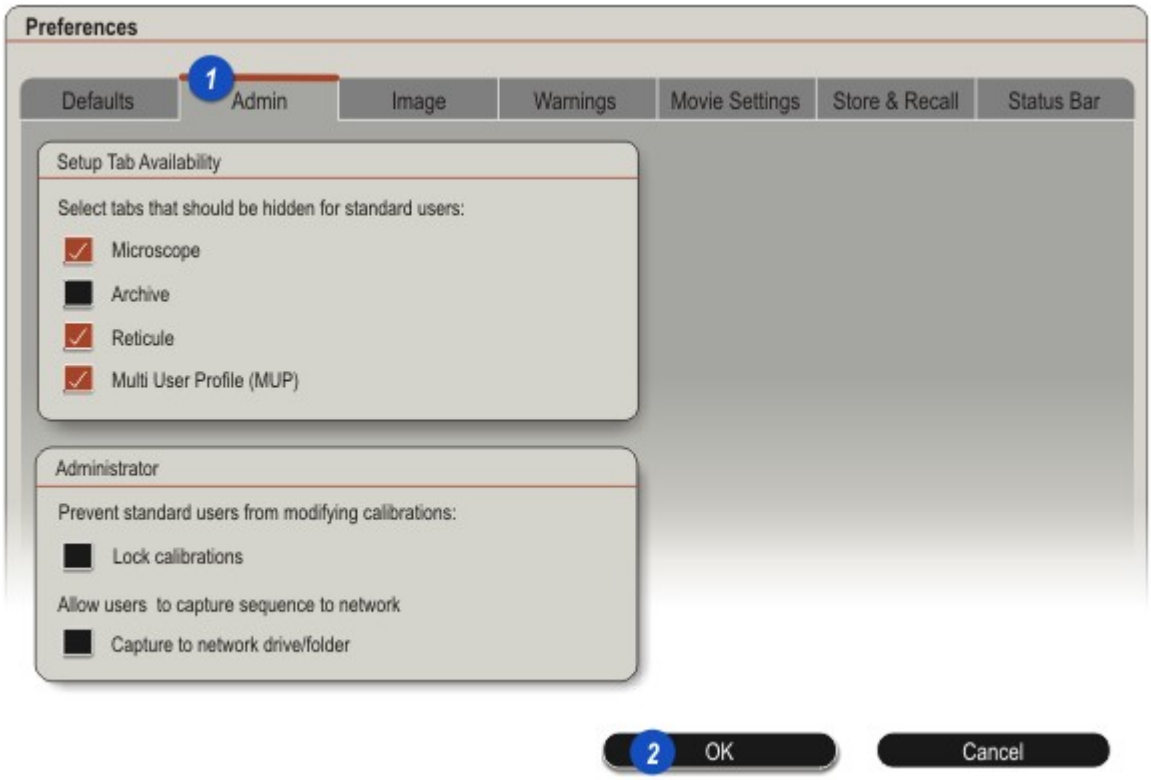

- Administrators can restrict access to panels on the *Setup Workflow* to prevent unauthorised changes being made to essential settings by standard users.
- Once a microscope has been calibrated, administrators can prevent further changes by standard users can be prevented by locking the calibrations.
- Users can be allowed to save images to a network drive as well as the local computer.

1: Click on the *Admin* tab to reveal the *Setup Tab Availability* and *Administrator panels.*

2: When changes have been made click the *OK* button.

Click on a panel for more information.

Administrators can restrict access to panels on the *Setup Workflow* to prevent unauthorised changes being made to essential settings by Standard Users.

Each of the available tabs on *Setup* can be hidden by checking the check box and so prevent display and access.

1: Click on the *Admin* tab on the *Preferences* dialog.

2: On the *Setup (Workflow) Tab Availability* dialog...

3: ...click the required check box to hide (checked) or reveal (un-checked) a tab. In the example only the *Archive* tab will be displayed in the *Setup Workflow; Microscope, Reticule* and *MUP* will be hidden and their panels inaccessible.

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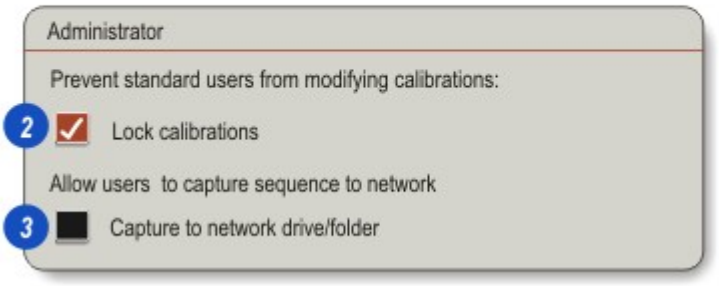
The Admin Tab: Lock calibrations& Capture to Network:

1: Click on the *Admin* tab to reveal the *Administrator* panel.

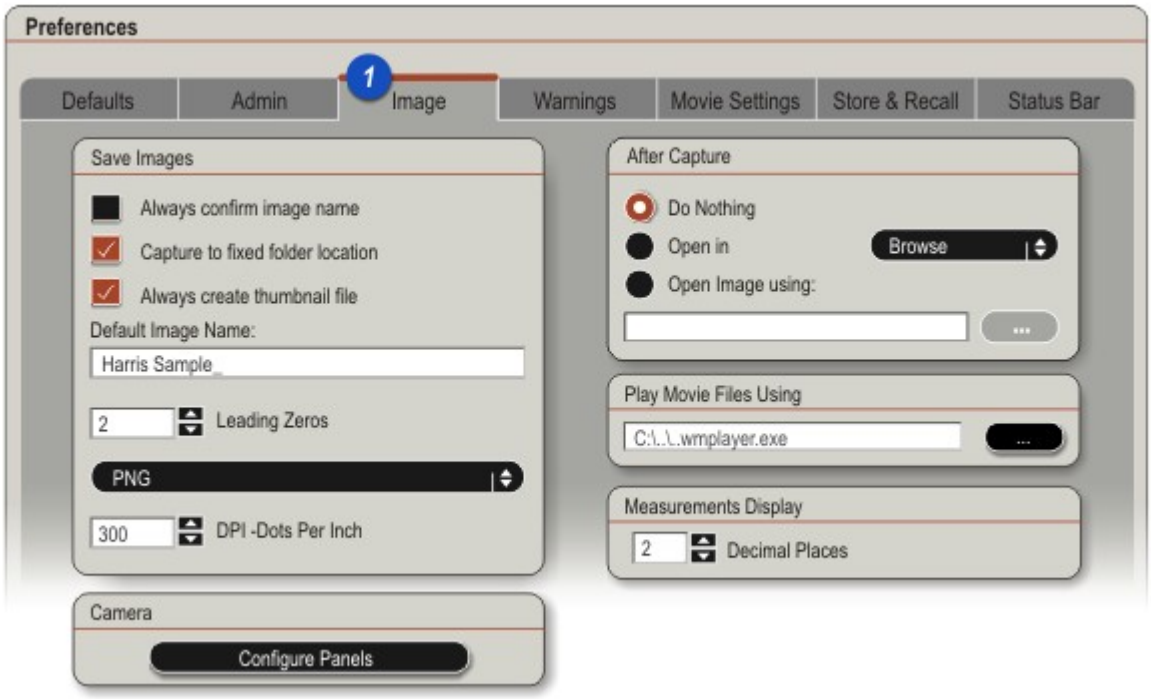
2: Once a microscope has been calibrated, administrators can prevent further changes by standard users can be prevented by checking (tick mark visible) the *Lock calibrations* check box.



3: Allow standard users to save images to a networked drive or folder as well as a local computer by clicking to enable the *Capture to network drive/folder* check box.

Points to consider before enabling *Capture to network...*

- Is the network accessible.
- Is it fast enough to capture extensive sequences like time-lapse.
- Movies cannot be saved using the network.
- A slow network will slow down LAS startup.



The controls on the *Image* tab determine the details of the image capture and what should happen after capture.

Administrators can also set up the *Camera* panels both to avoid modification and avoid clutter.

Click on the *Image* tab **(1)** to reveal the dialog. The tab has 5 individual panels:

- *Save Images* which determines the names and format of saved images.
- Application to launch *After Image Capture.*
- The application to launch for the *Movie File* player.
- *Measurement Displays* sets the number of decimal places for some measurement labels.
- *Camera* panel configuration. Determines those controls that are displayed or hidden on the *Acquire* > *Camera* panel.

Click on a panel on the illustration for more information.

Click *OK* to save the settings and close the *Preferences* dialog.

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1: Click to check and get a prompt for an image name before saving a captured image.

2: Check the *Fixed Capture Location* checkbox to have images saved in the folder selected as 'fixed' on the Browse Workflow.

3: With the *Always Create Thumbnail File* checkbox enabled, when the image is captured a separate thumbnail file with the extension *.thb* will also be created. Whilst this does occupy additional disk space, thumbnails are loaded to the *Gallery* far quicker. Applicable to all folders.

4: The *Default Image Name* is a prefix for each image saved. Click and swipe on the text box and type a new name appropriate to the work in hand.

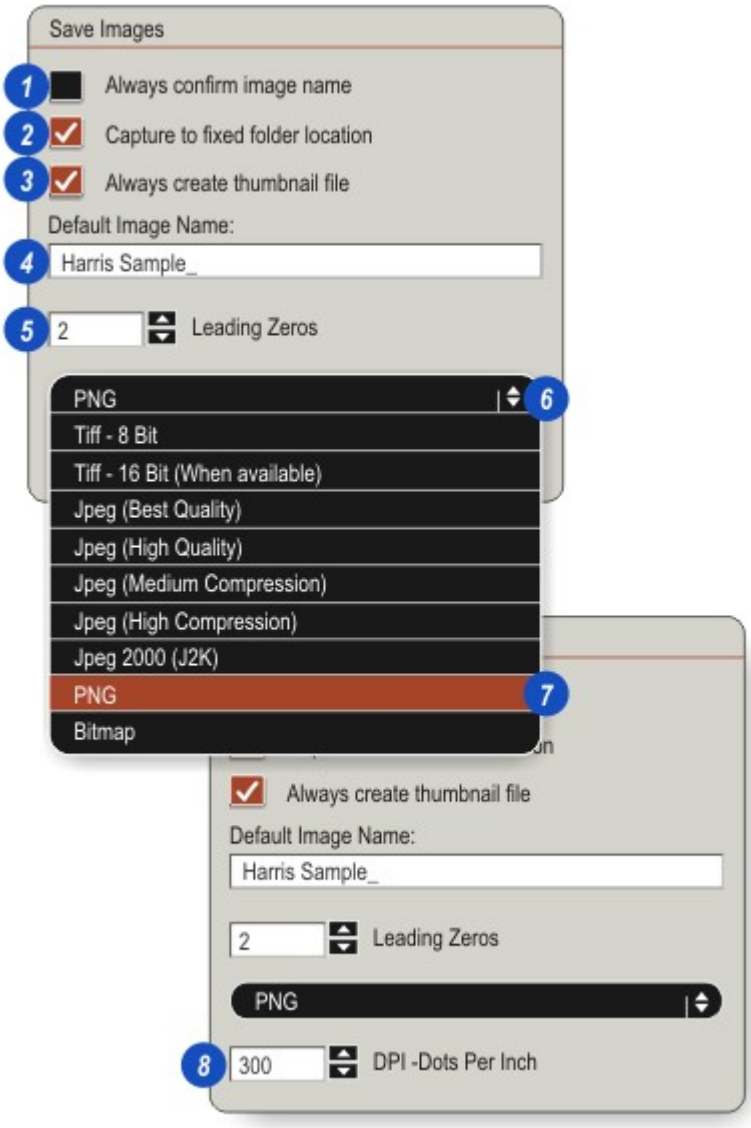
5: *Leading Zeroes* automatically pads the *Image Name* sequence with zeroes so that all image names are the same length. Use the up/down arrows to the right of the *Leading Zeroes* to set the value.

6: To select the *Image Format* and compression, click on the arrows to the right of the *In this Format* header and...

7: ...from the drop down menu click to select the format required.

8: Image resolution measured in *Dots Per Inch (DPI)*, can be set using the up/down arrows to the right of the *DPI* box. This function is particularly useful to ensure that 3rd party applications such as *Word* or *PowerPoint®,* display images correctly.

The *DPI* setting does not affect the capture format or the capture resolution set up in *Acquire* > *Camera*.



The *Acquire* > *Camera* panel has a wealth of tools and controls but for some users who do not require all of the available facilities, it can become 'cluttered' especially if *Optional Modules* are added. The number of panels on display can be reduced by setting them as hidden on the *Configure* camera *Panels* dialog. The controls are not lost only hidden.

Any user can hide or reveal a panel but only an Administrator or member of the LAS Administrator Group can lock or unlock it. If a panel is locked it cannot be hidden or revealed - its state when the lock was applied remains in force until the lock is released.

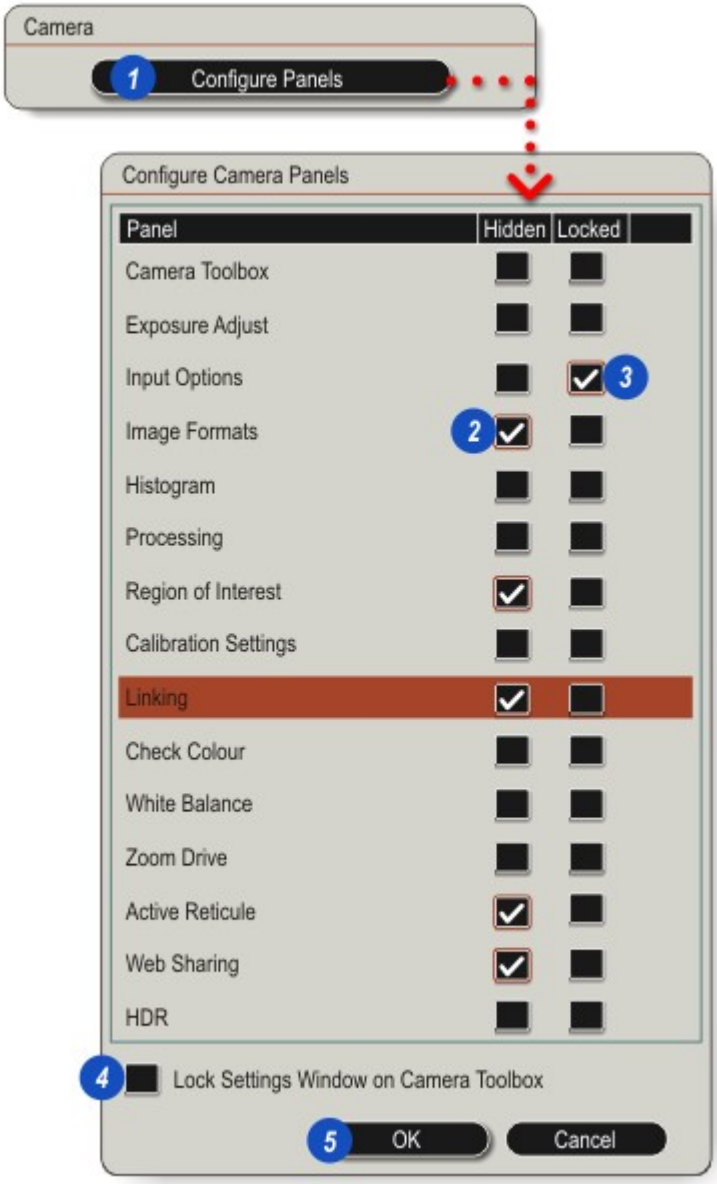
1: Click on the *Camera* > *Configure Panels* button.

2: Control panels on the *Camera* tab can be *Hidden* - the selected panel will not appear - and...

3: ...also *Locked* which locks the selected panel in position on the panel sequence. Click on a check box to the right of the selected panel to enable hiding and locking.

4: Enabling the *Lock Settings Window* will prevent the camera setting dialog from being displayed or altered.

5: Click *OK* to save the settings and close the dialog.



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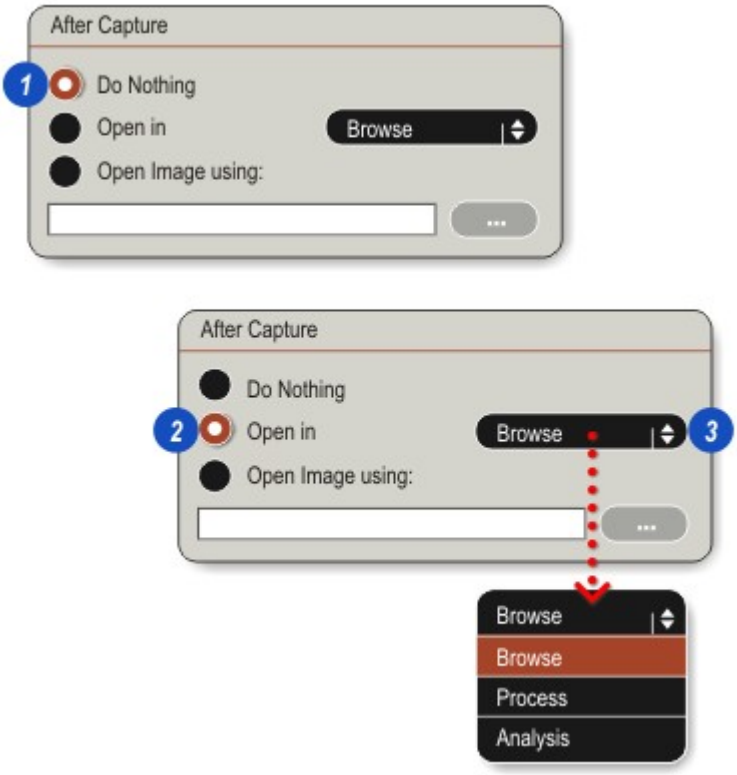
After an image is captured there are three possible automatic options that can aid speed and efficiency especially if the image required post-capture work:

1: *Do Nothing*. The image and data will be saved and the current *Workflow* will remain unaltered. Click on the radio button if a number of images need to be captured in quick succession without any intervening editing process.

2: *Open In* will automatically divert to the selected *Workflow*:

3: Click on the arrows to the right of the window and from the drop down click to select the required *Workflow*.

Continued: 50

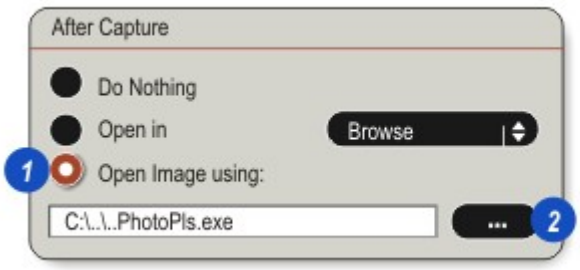


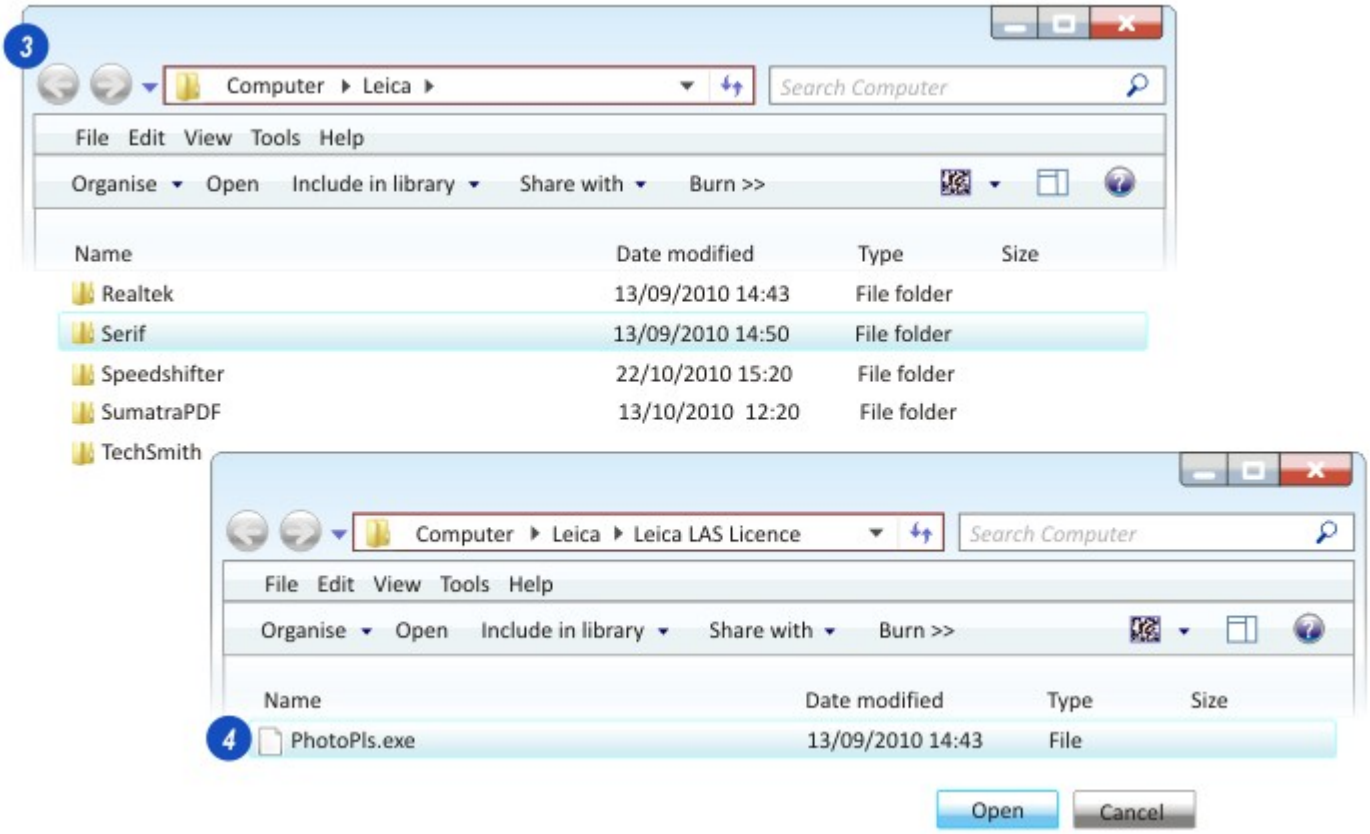
1: *Open Image Using* allows a (usually) third-party application to be launched to display and/or edit the image. Click on the radio button and then...

2: ... on the *Browse* button to reveal…

3: …the *Windows Navigator*.

4: Navigate to and select the application required and click *Open*. The selected application will appear in the *Open Image Using* text box.





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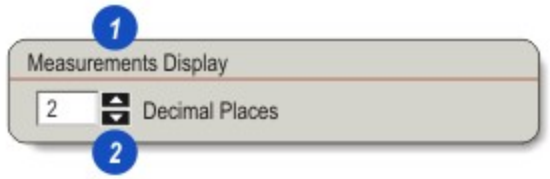
1: *Measurement Display* sets the number of decimal places that will be displayed on some of the *Workflows* and *Optional Modules:*



2: Click on the *Up/Down* arrows to the right of the *Measurements Display* widow to increase/decrease the number of decimal places.

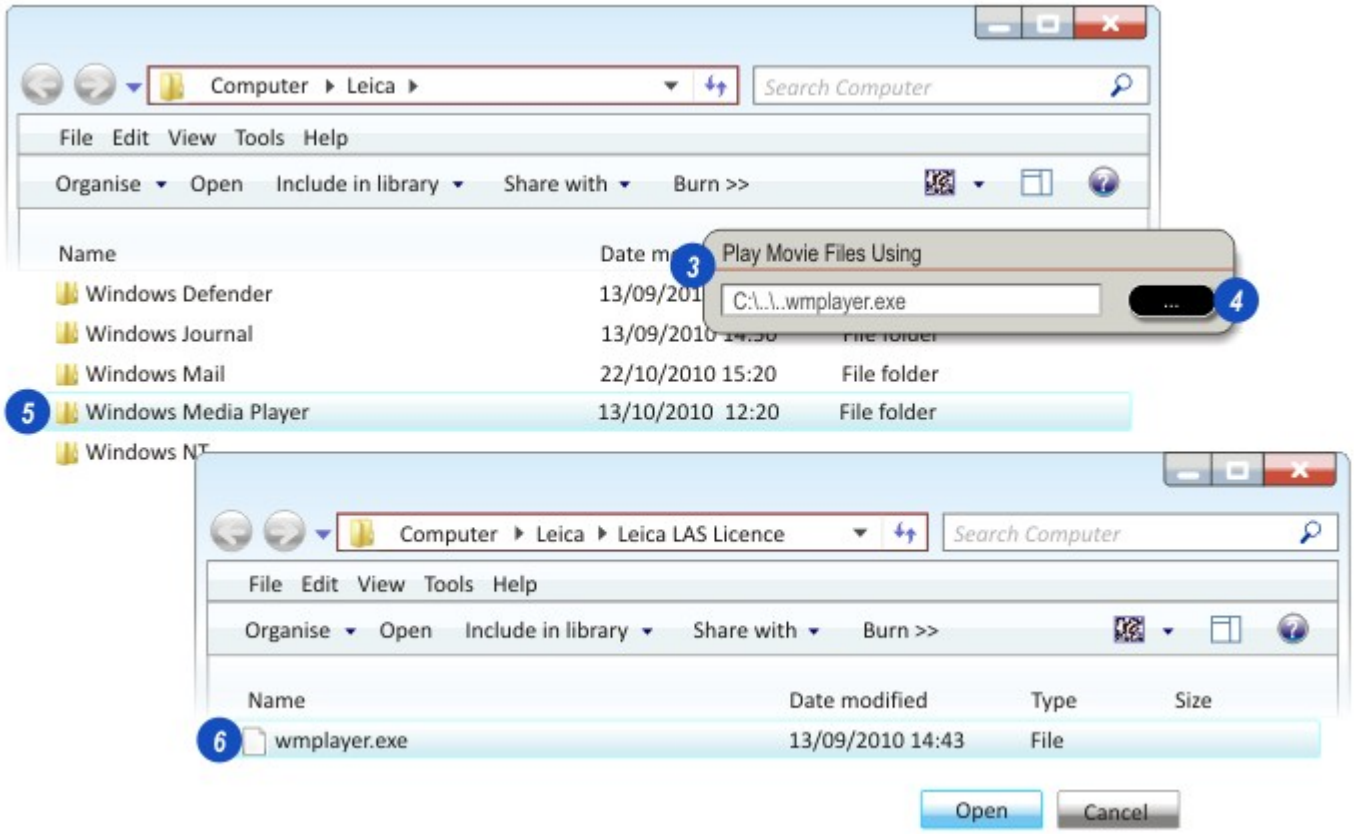
Play Movie Using...

3: After a movie has been created, it may be played using a nominated application.

4: Click on the *Browse* button to the right of the Play Movie Files text box and...

5: ... from the *Windows Navigator* navigate to...

6: .. and select the application required. Click on the *Open* button and the application name will appear in the *Play Movie Files Using* text box.



The major confirmation and warning messages can be turned on or off on this tab.

1: Click on the *Warnings* tab to reveal the options panel.

2: Click to check (message on) or un-check (message off *)* the check box to the left of each warning.

3: The *Set All* button will check all of the messages and...

4: ...the *Set None* button will clear them all.

5: Click *OK*.

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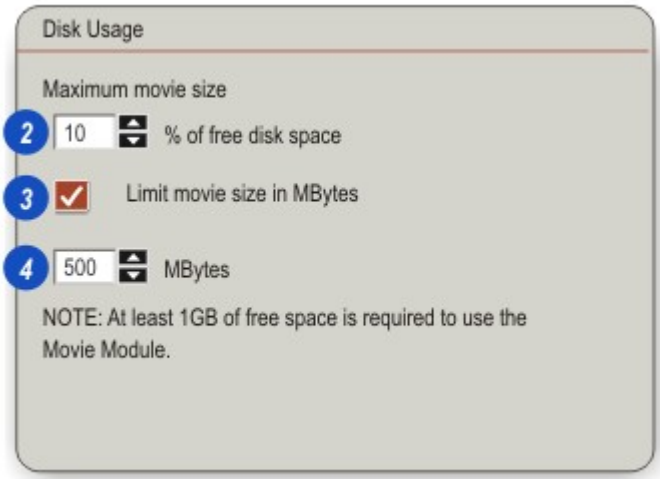
Because movies can be disk-space 'hungry', the *Movie Settings* tab provides two ways of limiting movie size:

- *Maximum Movie Size* limits file size in terms of free disk space whereas:
- *Limit Movie Size* prevents files exceeding a physical size measured in Megabytes (MBytes).

If *Limit Movie Size* is enabled, both features will run together to control movie size.

1: Click on the *Movie Settings* tab to reveal the *Disk Usage* panel.



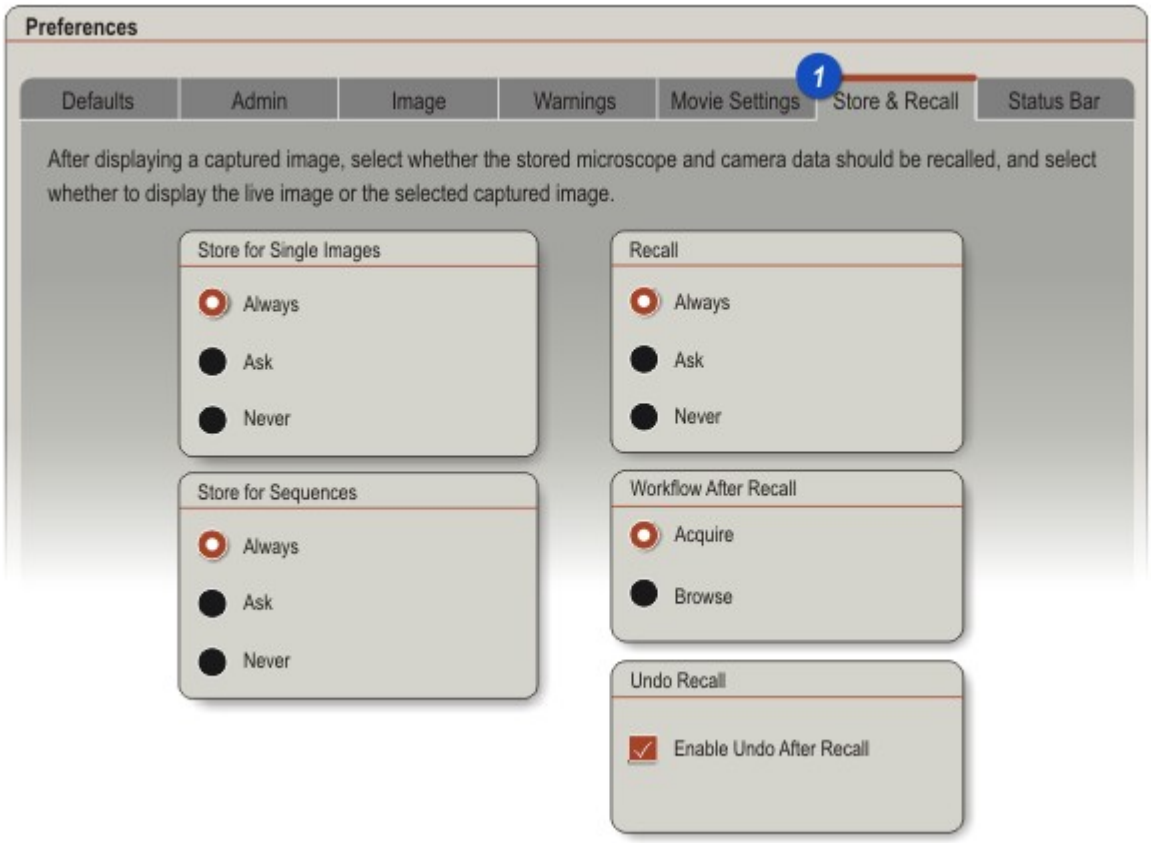
Limit the size of movies as disk space:

2: Click on the up/down arrows to the right of the *Maximum Movie Size* window to increase/decrease the percentage of disk space that can be allocated to movies.
Note that at least 1 Gigabyte (GByte) of free disk space is required simply to run the movie application.

Limit movie files to a specific size:

3: Click on the *Limit Movie Size* checkbox to enable size limiting. The checkbox will become red with a white tick.

4: Click on the up/down arrows to the right of the *Limit Movie Size* window to increase/decrease the maximum file size. Each click is a 1MByte step.



The *Store and Recall* feature allows the microscope and camera setting to be stored with an image so that precisely the same conditions may be repeated at a later date. It can also provide consistency across a range of different specimens.

Fully automated microscopes can automatically adjust to the settings; The settings display can be used to adjust manual models.

Click on a panel on the illustration for more information.

Of the five functions available:

- *Store Single Images*,
- *Store Sequences,*
- *Recall and*
- *Workflow After Recall...*

...each have options selected by clicking a button. The buttons are mutually exclusive - only one may be active within a function.

- *Undo Recall*....

...is switched on or off by clicking a checkbox. This is a toggle action.

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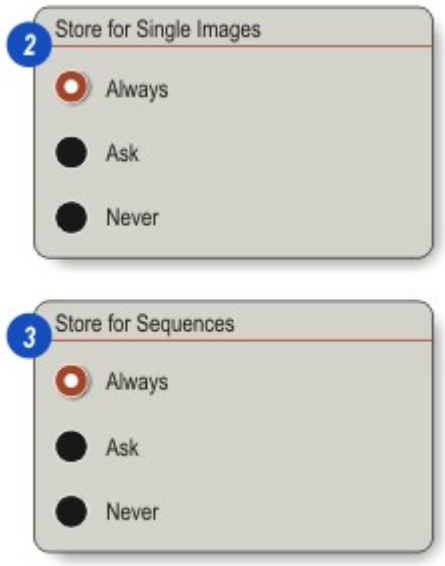
1: To reveal the *Store and Recall* options, click on the tab.

2: There is a settings panel for storing *Single Images* and...

3: ...another for storing *Sequences*.



Both panels have the same options:

- *Always* will always store the settings.
- *Ask* will prompt you to store or not when the image is saved.
- *Never* switches off settings store. The store facility adds a very short delay to storing images and the files are longer.

Click a button to select the option.

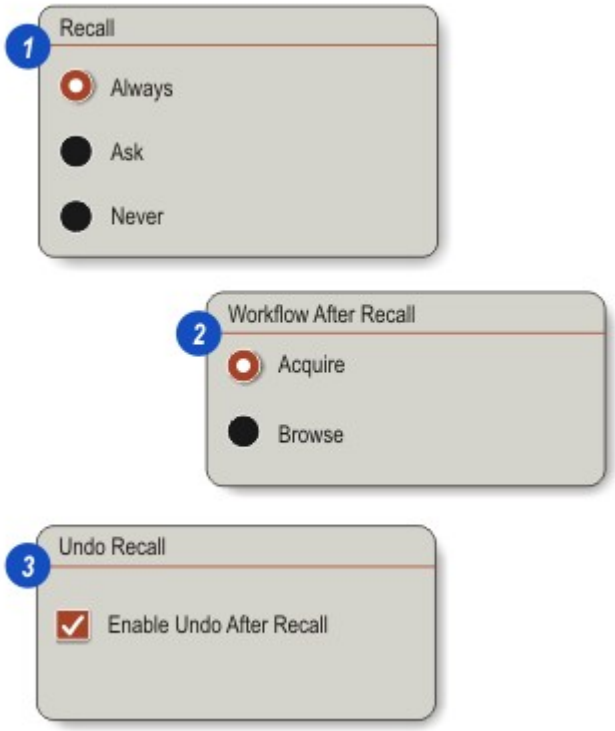
1: *Recall* determines if stored settings are also recalled when an image is retrieved from disk.

- *Always* will always recall any settings stored with the image. Automated microscopes will adopt the settings.
- *Ask* prompts if the settings should be retrieved or not.
- *Never* switches off settings recall.

2: When a stored image is selected two options are available on the *Workflow After Recall* panel:

- *Acquire* will switch to the Acquire Workflow and display the live image from the microscope, whilst...
- *Browse* will remain in the Browse Workflow and display the selected image in the *Viewer*.

3: If *Undo Recall* > *Enable Undo After Recall* is checked, the current microscope and camera settings are saved before any recalled settings are applied to the microscope and camera. Reverting to the current settings is then possible.



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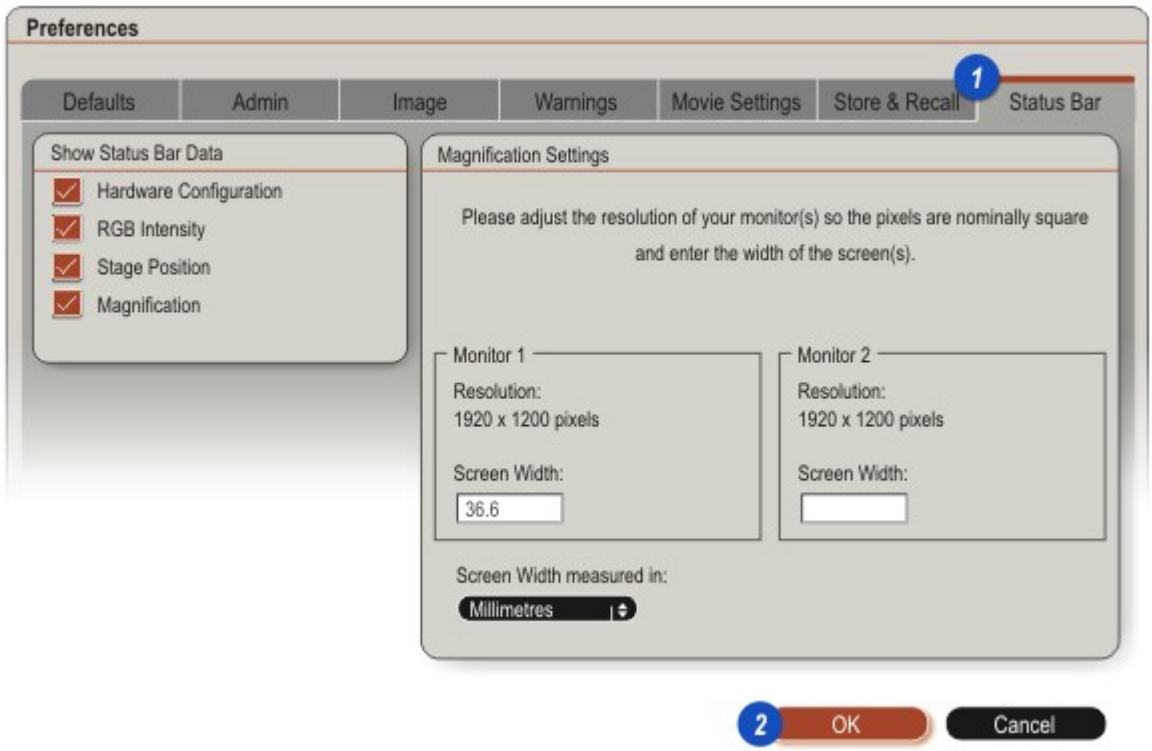


The *Status Bar* is located along the bottom edge of the *Viewer* and displays data relating to:

- The current *Hardware Configuration*.
- The intensity of the image *RGB* (Red, Green, Blue) components beneath the mouse.
- *Stage Position* is the *X/Y* co-ordinates when the image was captured.
- *Viewer Magnification*.

The *Status Bar* panel also allows the user to set up the resolution both for the PC monitor and a second monitor allowing the software to calculate the magnification.

To reveal the *Status Bar* panels:

1: Click on the *Status Bar* tab.

2: Save settings by clicking the *OK* button.

Click on a panel on the illustration for more information.

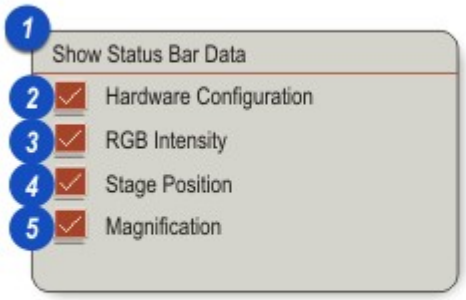
1: The *Show Status Bar Data* panel determines the items to be displayed along the *Status Bar.*

2 to 5: Enable a data item by clicking the associated check box. A tick mark indicates the item display is enabled. Click again to turn off the display.

2: *Hardware Configuration* shows the currently selected and active configuration.

3: *RGB Intensity* displays the *Red(R), Green(G)* and *Blue (B)* values of the image pixel directly below the mouse. The mouse position is indicated by the X/Y co-ordinates. Greyscale images are represented as a single value - Intensity.

4: *Stage Position* is the *X, Y* and *Z* stage co-ordinates when the selected image was captured or current values for the live image. The values are stored with the image when it is captured.



5: *Magnification* represents the monitor display magnification - not the microscope magnification. As the user clicks the *Zoom* buttons on the side tool bar the magnification value changes. The value will only display if the monitor has been set up: 59

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The microscope magnification can be read from the microscope or from *Leica Application Suite*, but the magnification on the computer monitor(s) is different because *LAS* scales the image to the user's demands and the monitor's capabilities.

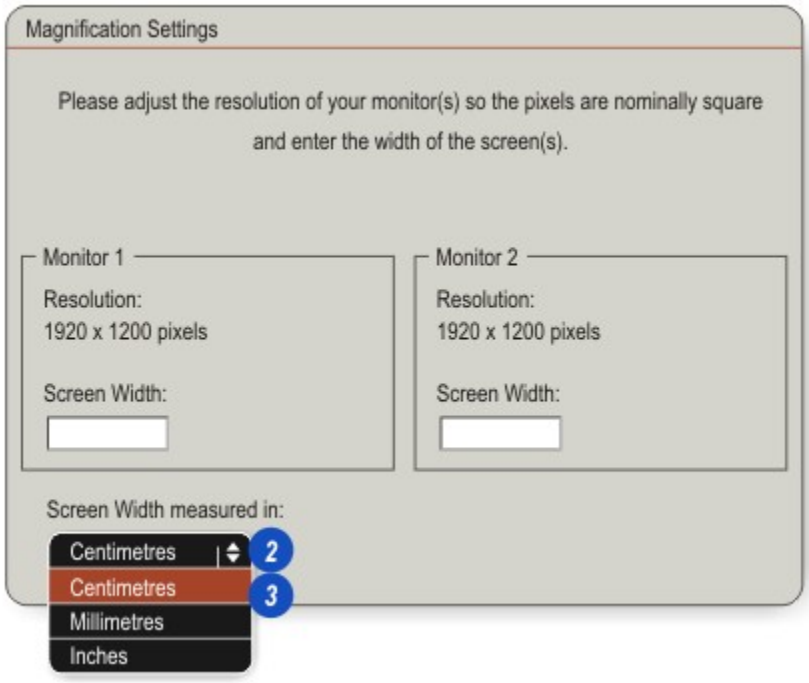
LAS can indicate the image magnification by calculation from the live or acquired image pixel size and that of the monitor. The image calibration is established as described in the section *Camera* > *Calibration*.

1: Click on the *Status Bar* tab.

2: If necessary, change the measurement type – *millimetres, centimetres* or *inches* – by clicking on the arrows to the right of the *Screen Width* header and...

3: ...click to select the measurement type required

Continued: 60

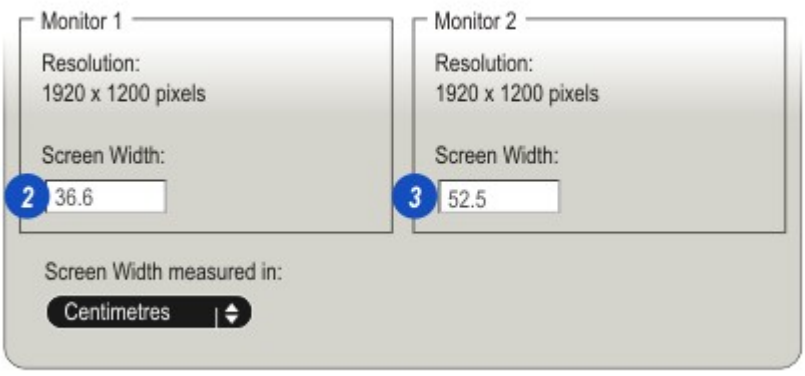


1: Measure the monitor(s) horizontally across the entire viewable area of the screen (not just the displayed image) with a ruler using the measurement type selected.

2: Click in the *Monitor 1* text box and type the monitor width.

3: Repeat the process if a second monitor is fitted, in the *Monitor 2* text box. Click *OK*.

The magnification factor is shown bottom right on the screen. It will change as the microscope objective zoom or the image zoom - either on the *Side Tool Bar* or mouse wheel - is changed. It will also update if the *Viewer* available display area is resized by opening, closing or re-sizing the *Grid* or *Gallery*.



- The magnification value is not displayed if a monitor size has not been entered or if the *Viewer* is in *Dual View* mode.

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The *Scale Bar* is available on *Acquire, Browse, Process* and *Analysis* Workflows.

1: Clicking the *Show Annotations* button on the *Side Tool Bar...*

2: ...displays the *Annotations and Scale Bar Quick Launch* menu.

3: Click to select the *Scale Bar* option.

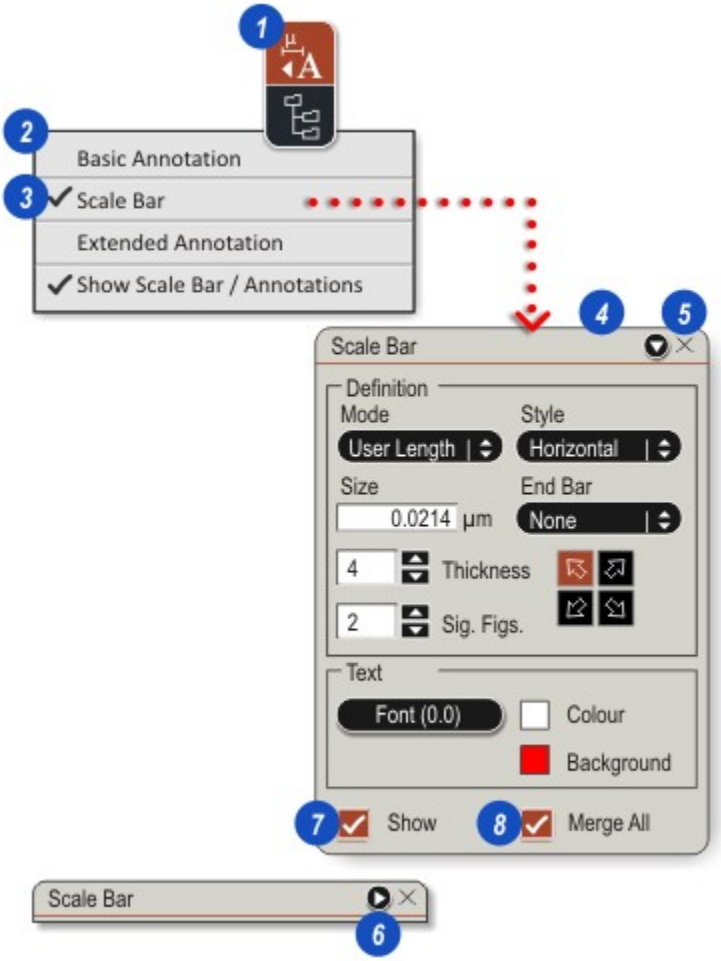
4: The *Scale Bar* dialog can be dragged by the header and 'parked' on any part of the *Viewer*.

5: Close the dialog and return it to the Workflow by clicking the 'X' on the dialog caption.

6: If the dialog is obscuring the image or controls, collapse it by clicking on the small arrow to the right of the dialog caption. Expand it by clicking the arrow again.

7: The *Scale Bar* is revealed or hidden by clicking the *Show* check box...

8: ... and can be *Merged* so that it is a permanent part of a captured image.



Scale Bar Features: Click the link (↗) for more information:

- *Modes Selection*: ↗ 62.
- *Style Selection*: ↗ 63
- *End Bars, Thickness*, *Digits*: ↗ 64
- *Placement and Font Change*: ↗ 65
- *Scale Bar and Font Colour*: ↗ 66
- *Background Colour*: ↗ 67
- *Merging*: ↗ 68

! The *Scale Bar* dialog will be active only when the Show check box is enabled.

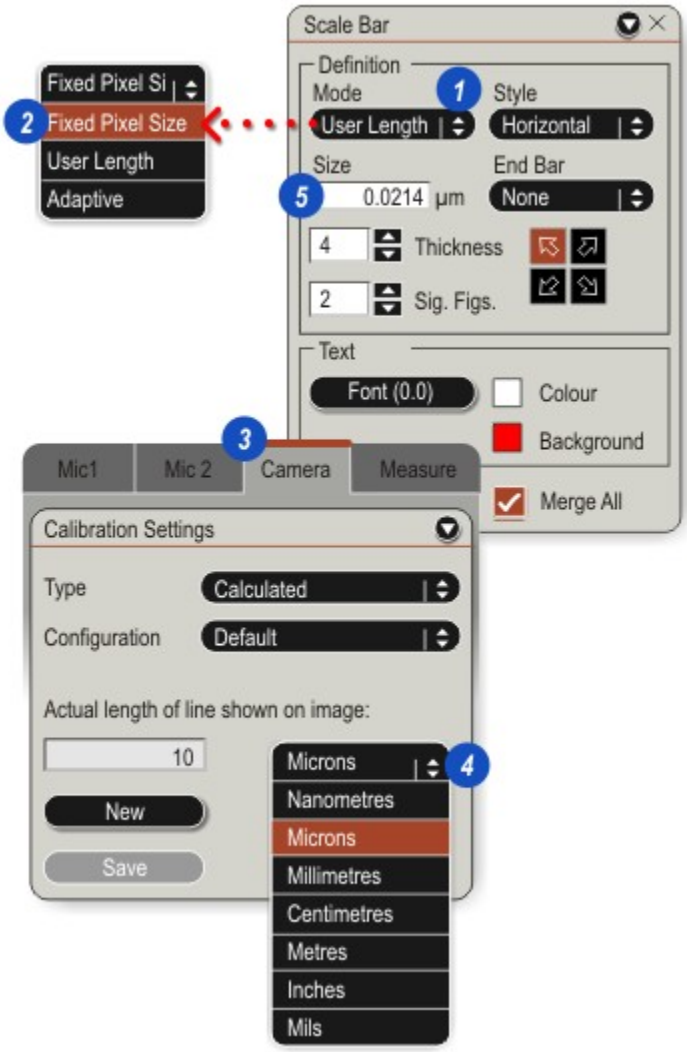
Continued ↗ 62

Three *Scale Bar* Modes are available to determine how it behaves on the image.

Fixed Pixel Size: Acts as a known distance 'ruler'. As the image is zoomed the *Scale Bar* length remains constant whilst the distance value changes to reflect the zoom level.

User Length: In contrast to the *Fixed Pixel*, the numeric value of the *User Length* option remains unchanged as the zoom is changed, but the line length on the display is adjusted. Values typed in to the *Size* text box will change to '*Undersize*' if too small or revert to the overall image width/height if too large.

Adaptive: Displays a 'true' calibrated *Scale Bar* at about 20% of the image width or height depending upon the bar orientation and as the zoom is changed, the numeric distance and the pixel size of the scale bar both change to maintain a 'reasonable' size on the image.



1: Click on the small arrow to the right of the *Mode* header and…

2: …click to select the required option.

Change the measurement units for *Fixed Pixel Size* and *User Length* modes*:*

3: On the *Camera* tab...

4: ... in *Calibration Settings*.

The *Adaptive* mode 'inherits' the last measurement units used.

5: To enter a distance in either *Fixed Pixel Size* or *User Length* modes, click in the *Size* text box and type a value.

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The orientation and shape of the *Scale Bar* is set on the *Style* menu.

1: Click on the small arrows to the right of the *Style* header and…

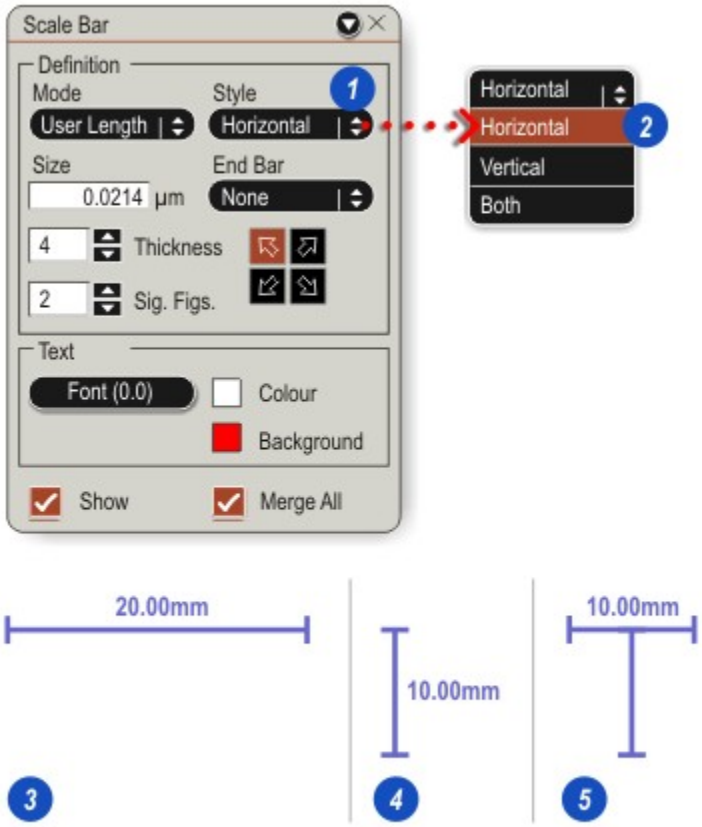
2: …click to choose the required style from the drop down list.

3: Conventional *Horizontal* style.

4: *Vertical* style.

5: Combined *Horizontal* and *Vertical* styles (*Both*). Although only one dimension is shown both 'legs' represent the same distance.

Continued 64



There are four options for the type of *Scale Bar* ends:

1: Click on the small arrows to the right of the *End Bar* header and…

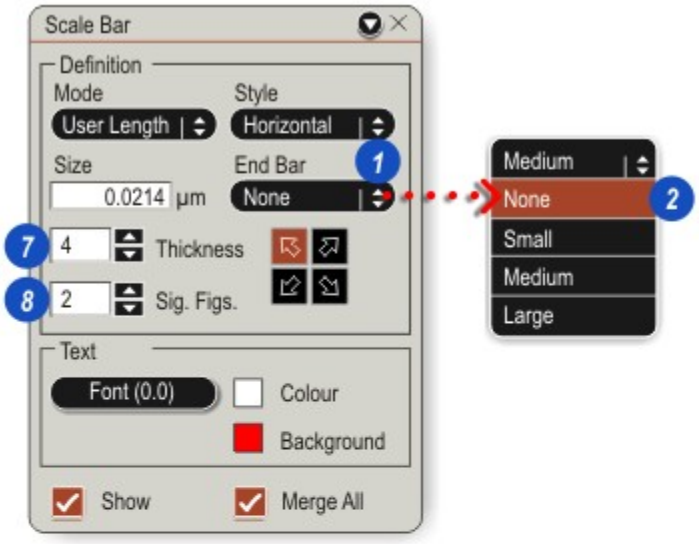
2: …from the drop down list click to select the type required:

3: *None*,

4: *Small*,

5: *Medium* or

6: *Large*.



Scale Bar Thickness:

7: Click on the *Up/Down* arrows to the right of the *Thickness* text box to increase/ decrease the Bar thickness. The End Bars are not affected.

Significant Digits:

8: Increase or decrease the number of digits displayed by clicking the *Up/Down* arrows to the right of the *Sig Fig* text box. The value determines the total number of digits displayed not just those after the decimal point. So, in Figure **(6)** the *Sig Fig* value would be 5. Whole numbers before the decimal point are not affected.

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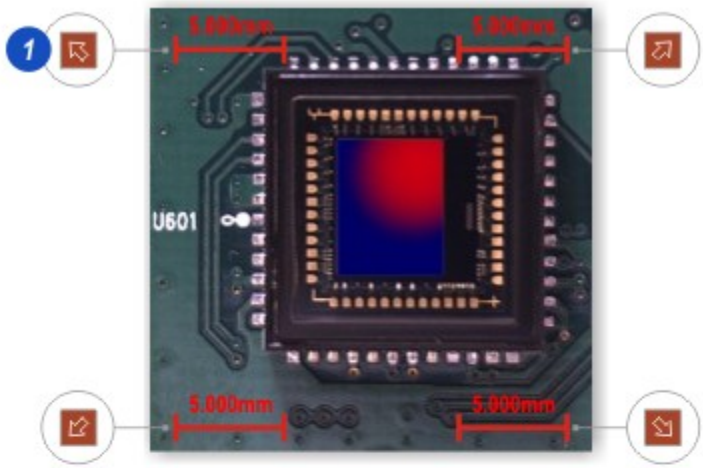
The *Scale Bar* can be placed quickly and precisely at the four corners of the image by using the *Placement* buttons.

1: The *Placement* buttons will position the *Scale Bar* at top-left, top-right, bottom-left or bottom-right of the image, live or captured, accurately and precisely simply by clicking the appropriate button. The *Scale Bar* can still be clicked and dragged if needed.



Changing the Font:

2: Click on the *Font* button.

3: On the *Font* dialog select the *Font*, *Font Style* and *Size* as required and…

4: …click *OK*.

Continued 66

Change the *Scale Bar* and *Font* colour by:

1: Click on the *Colour* button and…

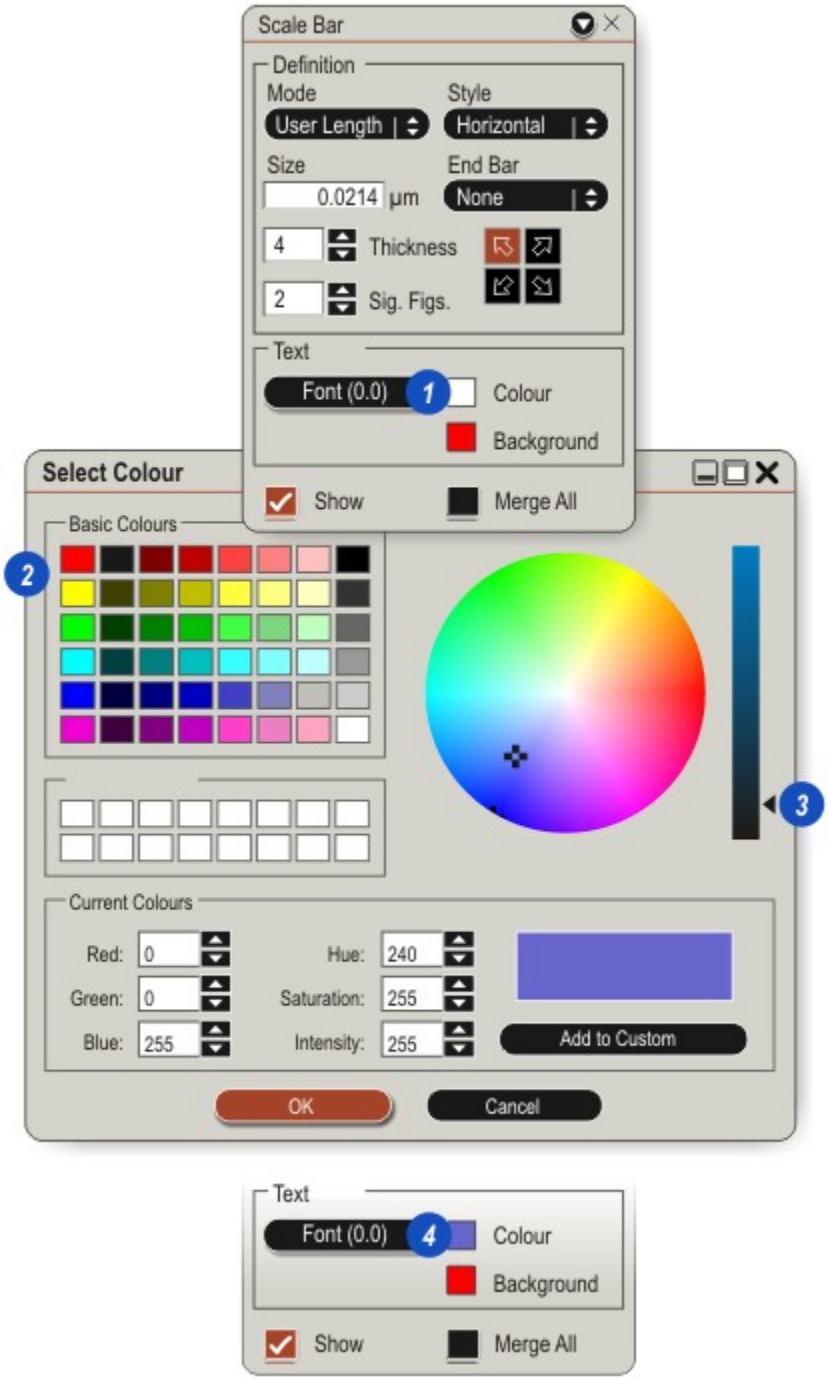
2: …on the *Select Colour* dialog choose a new colour from the swatches or from the colour wheel. Alternatively, click in the *Current Colours* text boxes and type the *Red*, *Green* and *Blue* values.

3: Adjust the colour intensity by clicking and dragging the slider on the *Intensity* bar.

4: Click *OK*.

5: The new colour is shown on the *Colour* button.

Continued 67



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The *Scale Bar* is displayed against a small panel the colour and transparency of which may be changed to suit the image:

1: On the *Scale Bar* dialog click on the *Background* button. The *Select Colour* dialog appears with additional controls to change the transparency of the background.

2: Choose a new background colour by clicking a *Basic Colour Swatch* or…

3: …clicking and dragging the 'target' on the *Colour Wheel*.

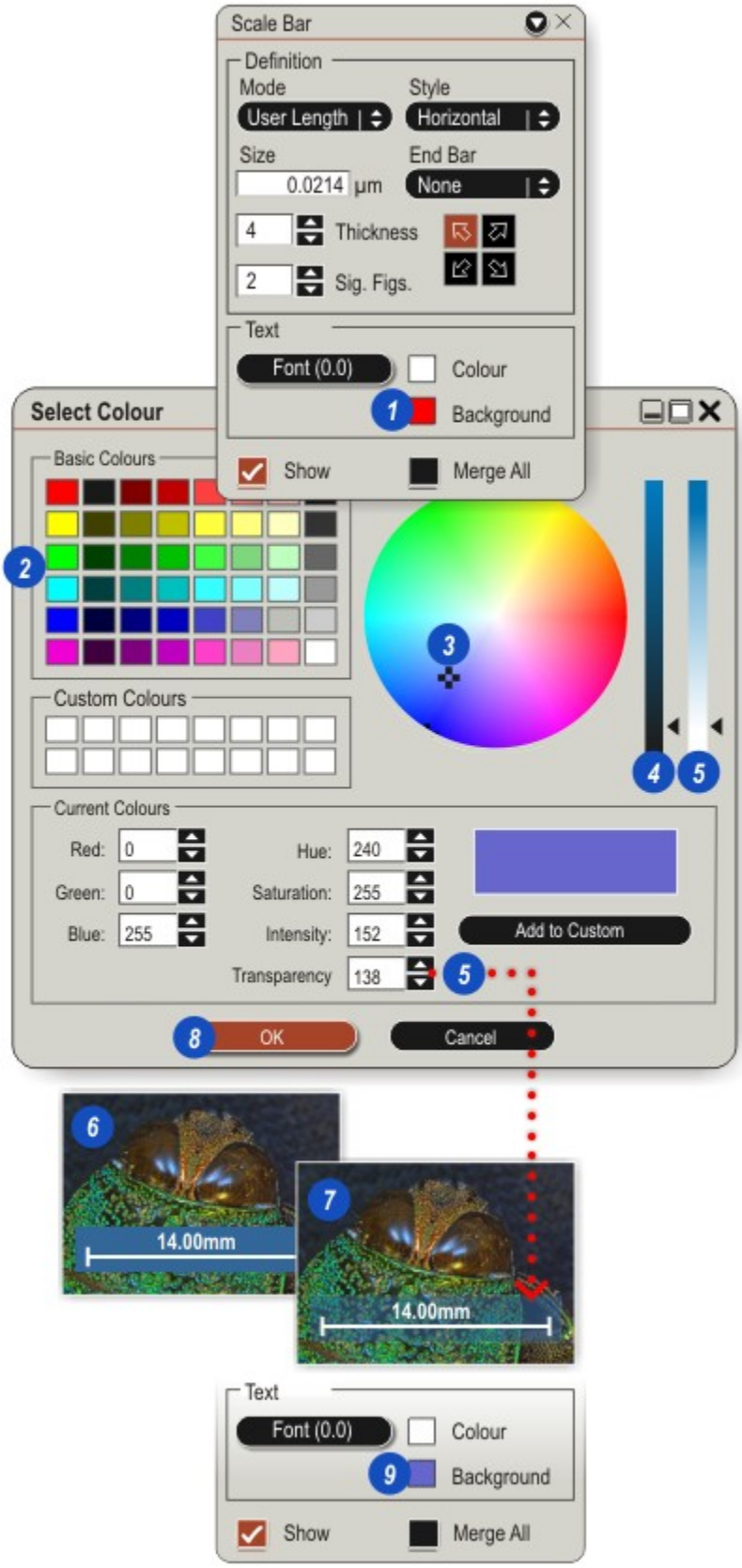
4: Adjust the colour intensity by clicking and dragging the slider on the *Colour Bar* or typing new values for *Red, Green* and *Blue* or *Hue, Saturation* and *Intensity* in the appropriate text boxes.

Background Transparency:

5: The *Scale Bar* background transparency can be altered by clicking and dragging the slider or typing a value in the *Transparency* text box. A value of 255 results in a solid colour **(6)** and a value of 0 makes the background panel disappear. The illustration **(7)** shows the result of a value of 138.

8: Click *OK*. The selected colour appears in the *Background* check box **(9).**

Continued 68



Merging is the process of combining the *Scale Bar* and its caption with the saved image.

Once merged, the image will always appear with the *Scale Bar* that cannot be altered.

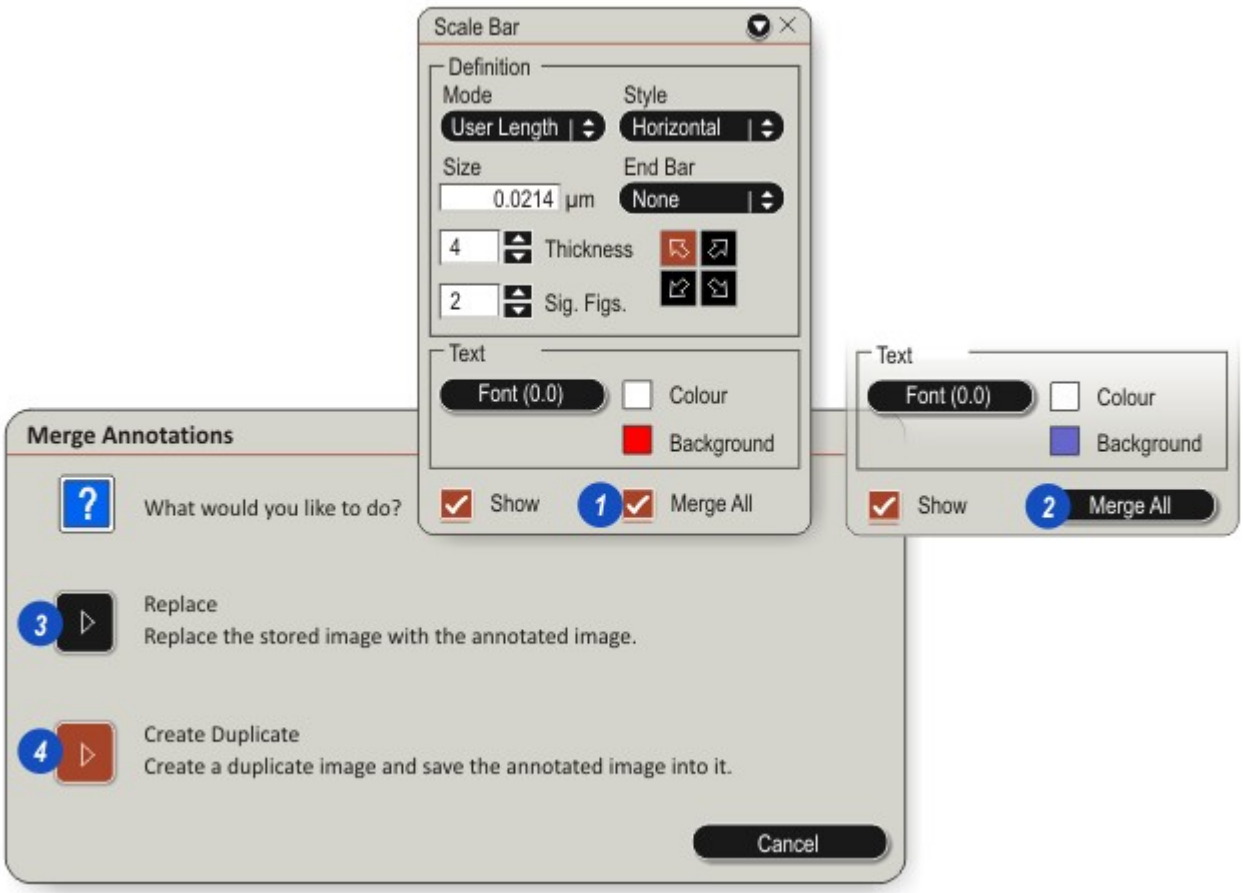
1: For live images *Merge* is a check box – click to enable merging when the image is captured.

2: For previously captured images *Merge* is a conventional LAS button – click to merge the *Scale Bar* with the image. The *Merge Annotations* dialog appears.

3: Two options are available: *Replace* the captured image including the *Scale Bar* with it, or …

4: *…Duplicate* which makes a copy of the existing captured image and merges the *Scale Bar with the copy.*

Click the required option button.



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Update Calibration:

If the system calibration has changed or images are being used that do not reflect the current calibration values, *Update Calibration* provides a simple and quick way to bring images up-to-date.

Four options for the calibration source are available:

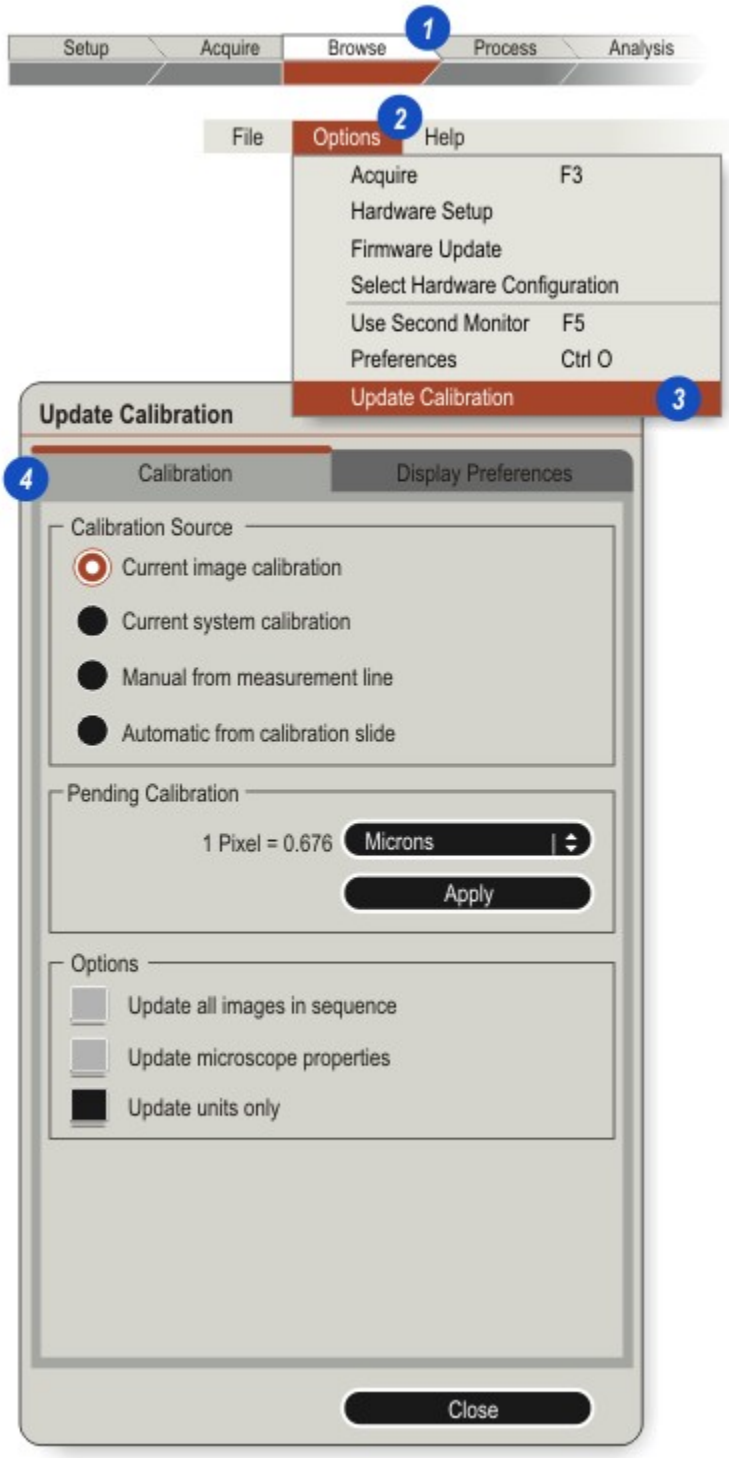
- *Use the current image:* 71 71 Uses the calibration values of the selected image..
- *Use system calibration:* 72 72 Uses the prevailing calibration settings.
- *Manual from the measurement line:* 73 73 Allows new calibration values to set up directly from a known distance on the displayed image.
- *Automatic from Calibration Slide:* 74 74 The calibration is calculated automatically from an image of a calibration slide captured at the same time as those to be updated.

Update Calibration is used on captured images including those that have a *Scale Bar* 'burnt in' (merged) but in those cases the merged displayed value will not change.

1: Click to select the *Browse Workflow.*

2: Click on *Options* on the main tool bar and from the drop-down menu,...

3: ... click to select *Update Calibration.* The *Update Calibration* dialog **(4)** appears.



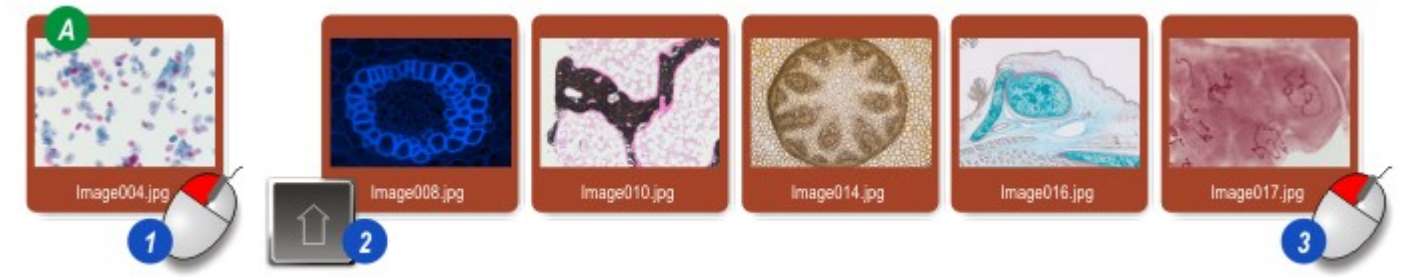
There are several ways to select images that will updated:

A: Range of images:

1: Click on the first image to be selected.

2: Press and hold down the *Shift* key.

3: Click on the image from which the calibration will be copied. All of the images between the two will also be selected.

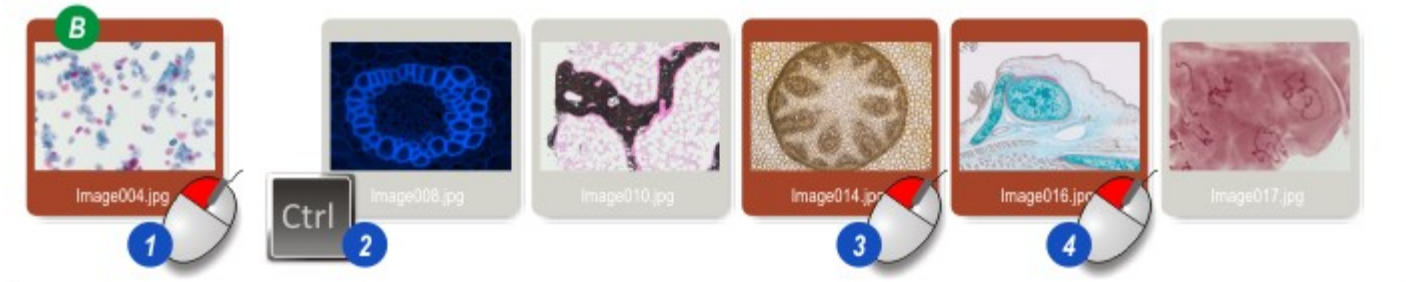


B: Individual Images:

1: Click on the first image to be selected.

2: Press and hold down the *Ctrl* key.

3: Click individually on all the other images to be included in the selection. The last image will be used as the calibration source.

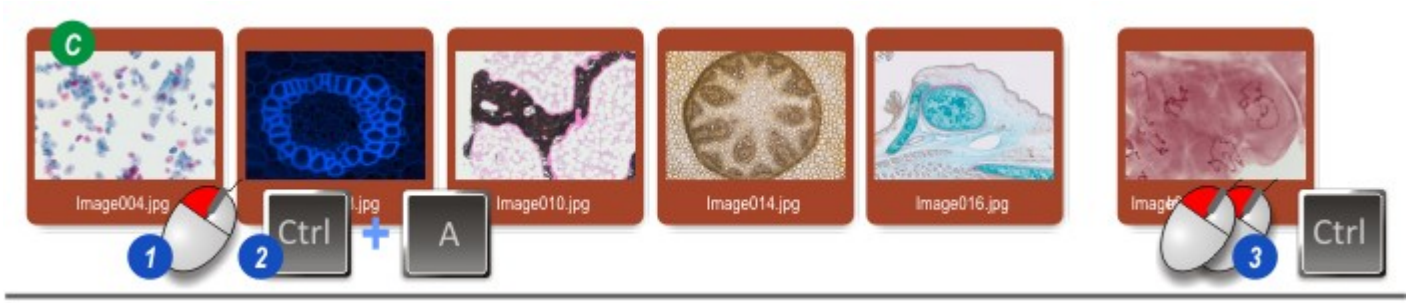


C: All of the images in the Gallery:

1: Click on the first image.

2: Press and hold down the *Ctrl* key and then press and release the *'A'* key (All).

3: To select the calibration source, still holding down the *Ctrl* key double-click the source image.



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To use an image as the calibration source for all of the other selected images, choose the images to be updated making sure that the calibration source is the last to be selected. More information 70

1: Click the *Current image calibration* button.

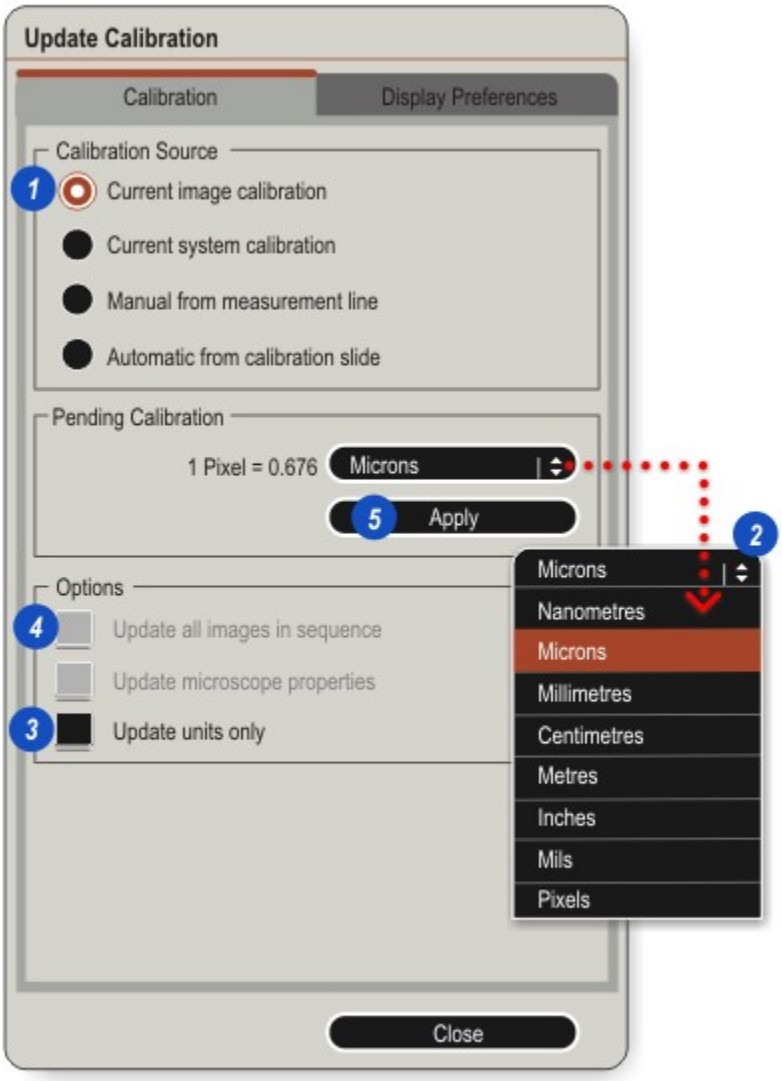
2: If necessary, change the *measurement units* by clicking the small arrows to the right of the units header and from the drop-down list, clicking to select the units.

! In the *Pending Calibration* panel the 1 *Pixel* = nnn value refers to the calibration of the selected source image. The reference to Pixel represents the unit used to store the image not the *Camera* or the *Monitor* pixels.

3: To update the *measurement units* for the selected image but **not** to change their calibration values, click to enable (tick mark visible) the *Update units only* check box.

4: If the selected images are part of a sequence the *Update all images in sequence* check box becomes available. Click to enable it and update all of the sequence images.

5: Click the *Apply* button.
Click the *Close* button to exit *Update Calibration*.



Apply the current System Calibration settings to an image or range of images by selecting the images to be updated. More information 70

1: Click to select *Current system calibration.*

2: If necessary, change the *measurement units* by clicking the small arrows to the right of the units header and from the drop-down list, clicking to select the units.

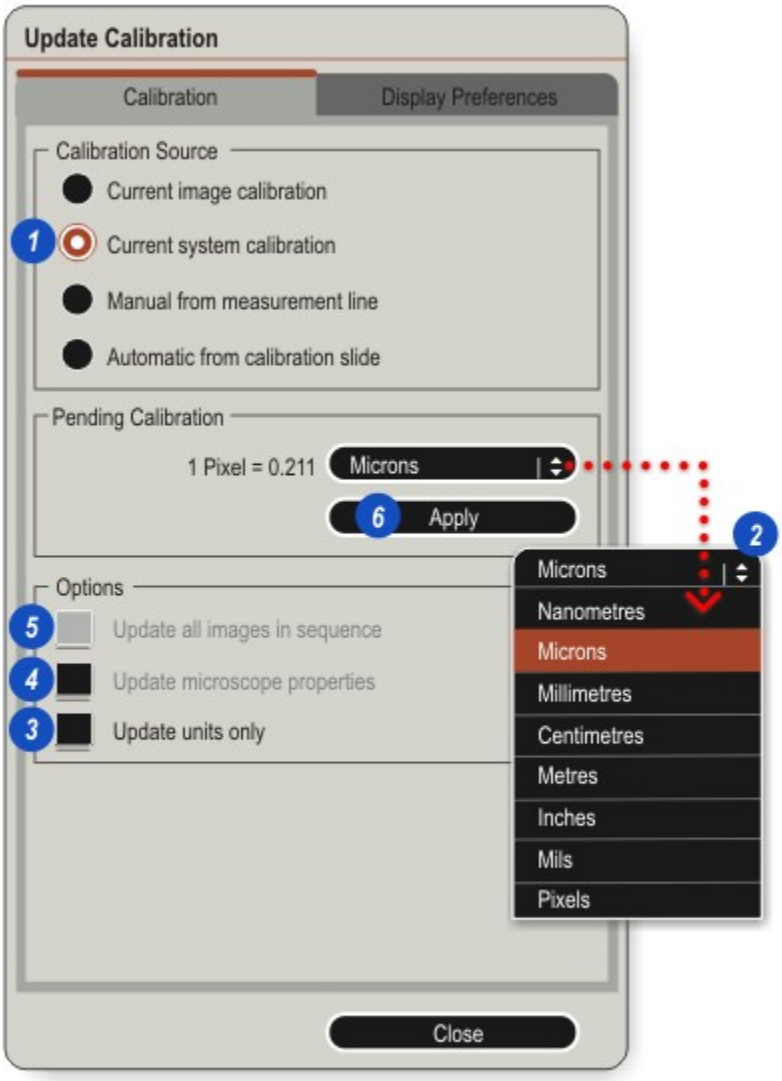
! In the *Pending Calibration* panel the 1 *Pixel* = nnn value refers to the system calibration. The reference to *Pixel* represents the unit used to store the image not the *Camera* or the *Monitor* pixels.

3: To update the *measurement units* for the selected image but **not** to change their calibration values, click to enable (tick mark visible) the *Update units only* check box.

4: Update the image microscope properties to the current system settings by clicking to enable the check box.

5: If the selected images are part of a sequence the *Update all images in sequence* check box becomes available. Click to enable it and update all of the sequence images.

6: Click the *Apply* button.
Click the *Close* button to exit *Update Calibration*



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A new calibration value can be set by using a line extended across a feature with a known dimension on the image. Best accuracy is achieved by using a Calibration Slide.

Select the images to be updated. More information 70

1: Place a specimen with a known and precise dimension on the stage. A clean *Calibration Slide* is the best option. Focus the specimen or slide.

2: If necessary, change the *measurement units* by clicking the small arrows to the right of the units header and from the drop-down list, clicking to select the units.

! In the *Pending Calibration* panel the 1 *Pixel* = nnn value refers to the system calibration. The reference to *Pixel* represents the unit used to store the image not the *Camera* or the *Monitor* pixels.

3: Click to select the *Manual from measurement line* option.

4: The measurement line appears. Click on the centre of the line and drag it so that the left end stroke aligns with the edge of the specimen or slide.

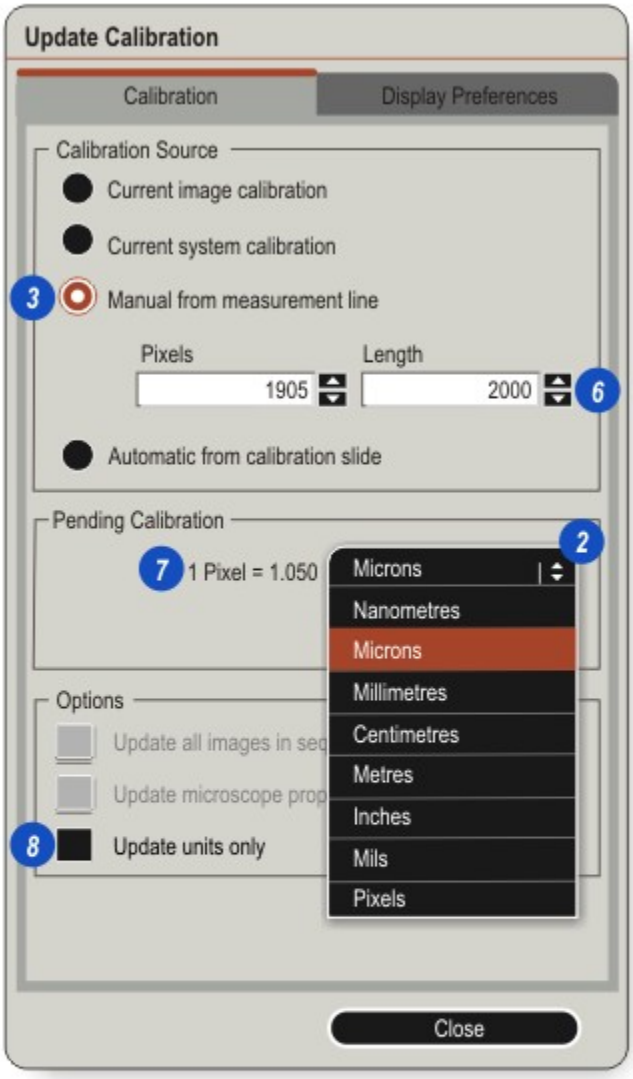
- Hold down the *Shift* key to display the magnifier, moving it with the mouse to assist alignment.

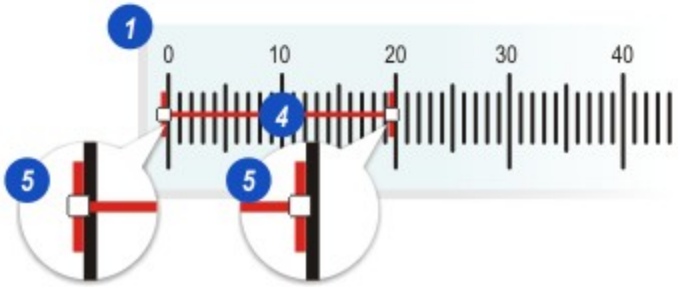
5: Aim to place the end stroke on the outside of the left edge.
Click and drag the right 'handle' so that the right stroke aligns with the inside of the right edge.

6: Click inside the *Length* text box and type the known length of the line in the chosen measurement units.

7: The calibration value is automatically calculated.

8: Enable the Update units only check box to apply changes in the measurement units only.





Click *Apply* to update the selected images.

Automatic calibration offers a fast method of applying precise calibration to selected images. It uses an image of a calibration slide that was captured using the same microscope settings as the images to be updated.

The software can accurately and automatically detect the slide image and calculate the calibration from a known interval between the divisions, providing the image is sharp and the division lines clearly defined.

Select the images to be updated making sure that the calibration slide image is the last selected and is displayed in the *Viewer.* More information 70

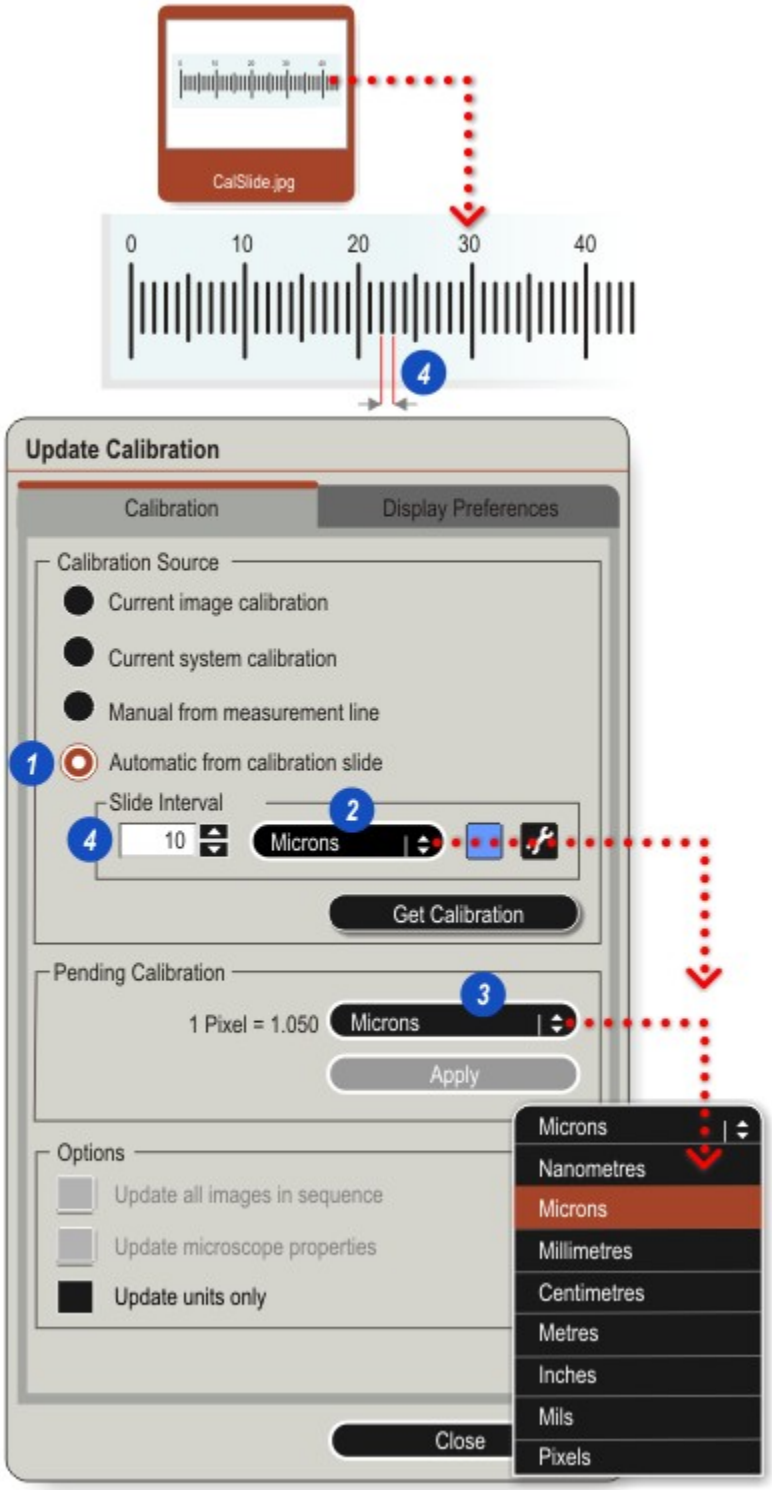
1: Click to select *Automatic from calibration slide.*

2 & 3: If necessary, change the measurement units for both the calibration slide and the calibration value by clicking the arrows to the right of the headers and selecting from the drop-down list.

- The measurement units do not have to match.
- The calibration slide image does **not** have to be perfectly horizontal or vertical, but the closer it is then the faster the detection.

4: Click inside the *Slide Interval* text box and type the interval value of the calibration slide - that is the distance between two adjacent divisions in the selected measurement units.

Continued 75



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The software will now try to find a calibration slide and verify that it is precise and suitable as a calibration source:

1: Click on the *Get Calibration* button.

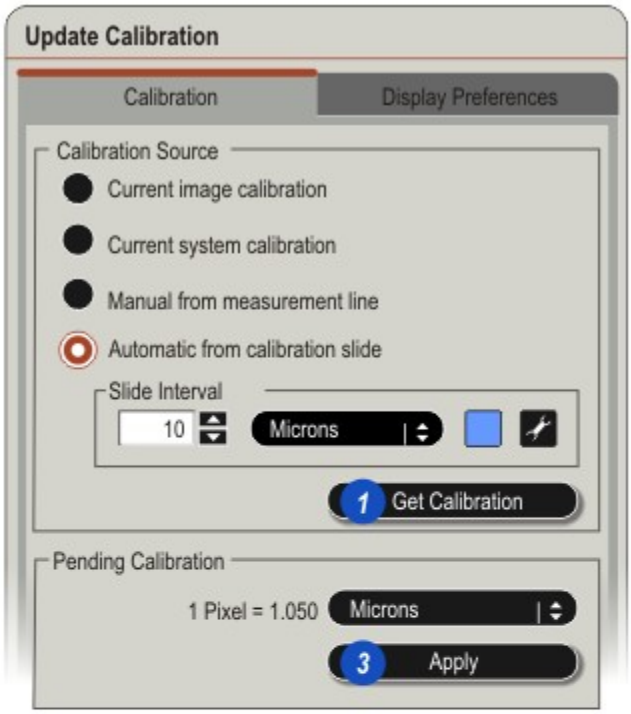
2: If a calibration scale is found and verified, the colour of the division strokes on the scale will change, a new calibration value is automatically calculated and...

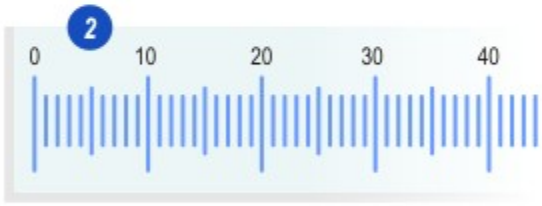
3: ...the *Apply* button becomes active. Click it to apply the new calibration value to the selected images.

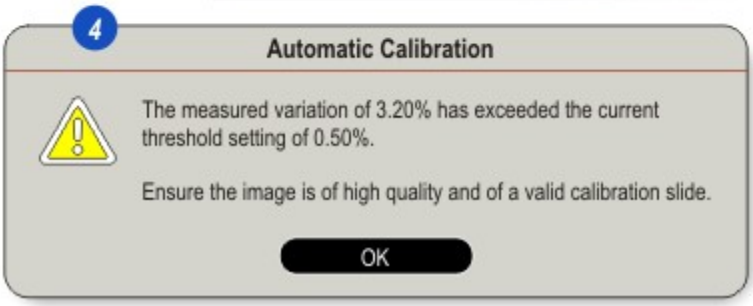
4: If a scale is not detected or does not conform to the verification parameters, an error message appears. The message changes to reflect the error.

- Calibration slide scale verification parameters can be changed: 76.
- The calibration scale detected colour can be changed:

Continued 76

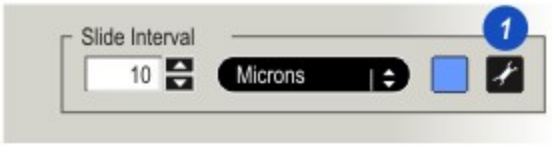


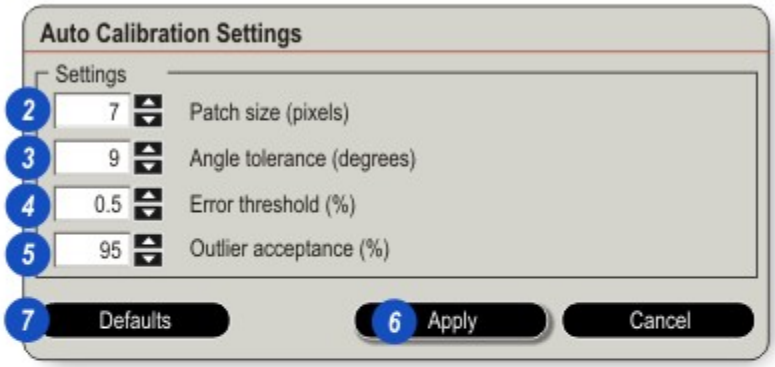


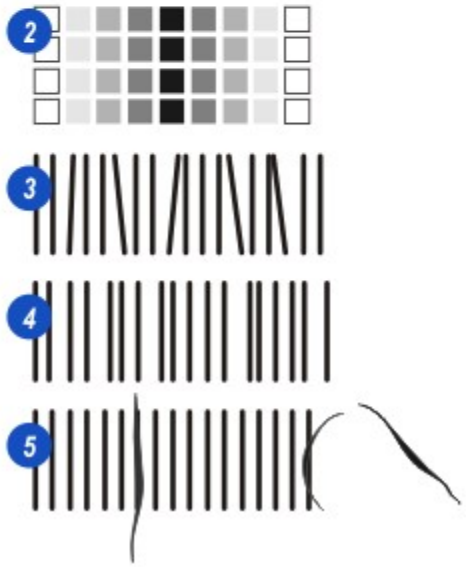


The verification parameters check that a calibration slide image is sufficiently accurate to be used as a calibration source. For example, random fibres on an calibration slide image could be interpreted as part of the scale and have to be 'filtered' out.

Users can change the settings but should be aware that significant changes can result in compromised calibration accuracy. If in doubt revert to the factory optimised defaults.







1: Click on the 'spanner' icon to reveal the *Auto Calibration Settings* dialog.

2: The *Patch size* refers to the spread of pixels leading to a discernible edge. In the illustration there are several pixels ranging from white to dark grey before the black central 'edge' appears. Increasing the patch size could 'create' spurious edges. Keep the patch size as small as possible.

3: *Angle tolerance* determines the how much the angle between two adjacent scale strokes can vary from the parallel. The software is looking for a series of parallel strokes at a consistent 'pitch' or interval.

4: The interval of the scale strokes must be close to the value entered by the user. The *Error threshold* determines how much it can be allowed to vary.

5: Outliers are scratches and debris that may be present on the image and could be interpreted as part of the scale. The *Outlier acceptance* sets the % level at which the interval *mean* (a central 'average' for all of the detected intervals) can vary. Strokes falling below the *Outlier acceptance* are removed.

6: If changes are made to the settings click the *Apply* button to save them.

7: To restore the factory settings click the *Defaults* button.

Continued 77

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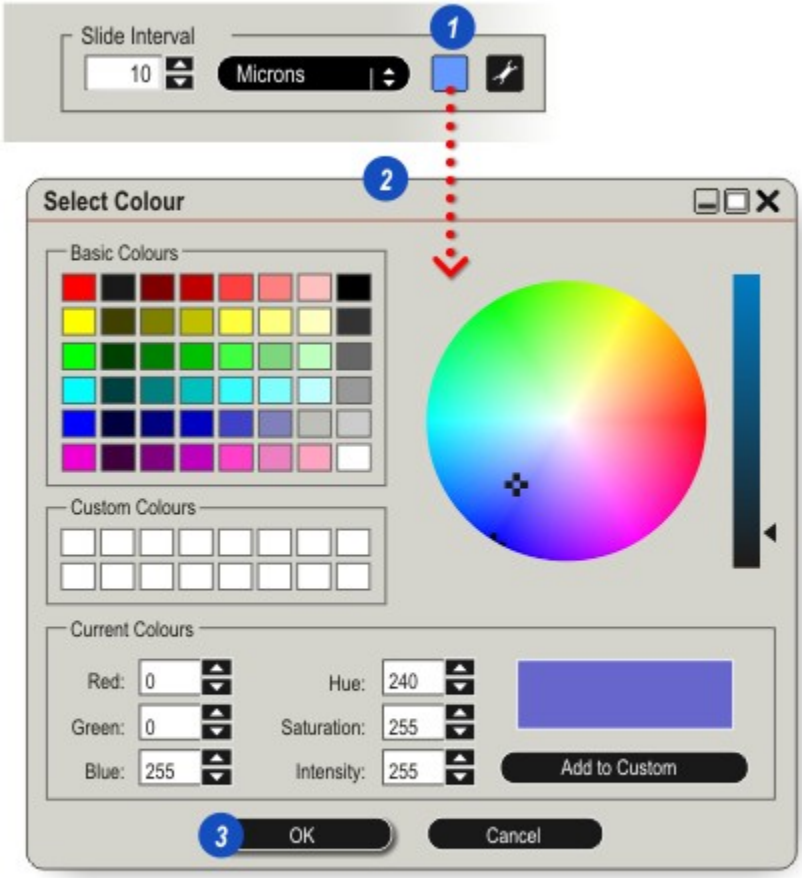
Change the colour of a detected calibration slide image by:

1: Click on the *Colour* button on the *Slide Interval* panel.

2: From the *Windows Colour* dialog choose a colour by clicking a swatch, dragging the crosshairs on the wheel or typing Red, Green and Blue (RGB) values.

3: Click *OK*.



The number of decimal places displayed in *Interactive Measurements* and *Image Analysis*, can be set by:

1: Click on the *Display Preferences* tab.

2: Using the Up/Down arrows, increase or decrease the number of digits to be displayed after the decimal place.

3: Click *Apply*.

Return to the calibration options by clicking the *Calibration* tab.

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Image Comparison - Dual Viewer:

The *Dual Viewer* option is available in the *Acquire, Browse* and *Process Workflows*. The *Viewer* area can be split to show two images simultaneously:

- In *Acquire* the current live image usually appears in the left pane) with a previously captured image selected from the *Gallery* in the right pan).
- In *Browse* and *Process* two previously captured images selected from the *Gallery* appear left and right.

1: On the *Side Tool Bar* click the *Viewer Options* button.

2: Click to enable (tick mark visible to the left) the *Dual Viewer* option. The *Viewer* will then divide into two panes.

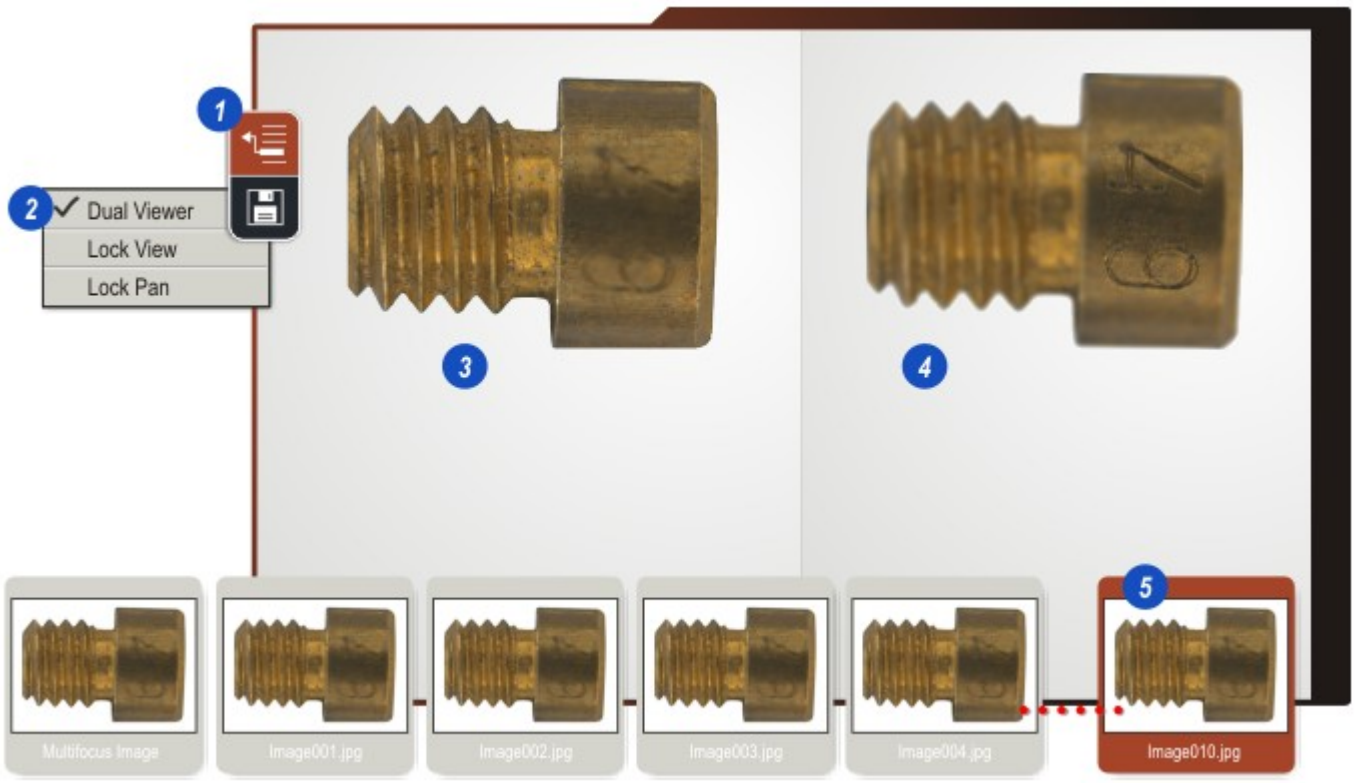
3: In Acquire the image currently being viewed will usually appear in the left-hand pane.

4: ..To display a captured image in either pane, click anywhere in the pane and then...

5: ...click a thumbnail in the *Gallery*.

Dual Viewer features and quick links (↗):

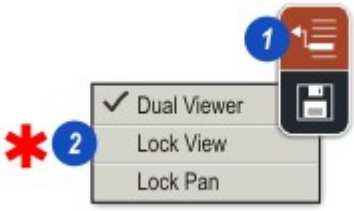
- Unlock and Lock views: ↗ 80.
- Pan Window: ↗ 81
- Comparing Features: ↗ 83



Lock View Disabled:
With the *Lock View* option disabled – no tick mark against it, both images can be scaled independently:

1: Click on the *View Options* button on the *Side Tool Bar* and…

2: …from the context menu click to disable *Lock View* – no tick mark.



3/4: Click to select the image in either pane.

5: Use the *Side Tool Bar* buttons to *Zoom In* or *Zoom Out,*

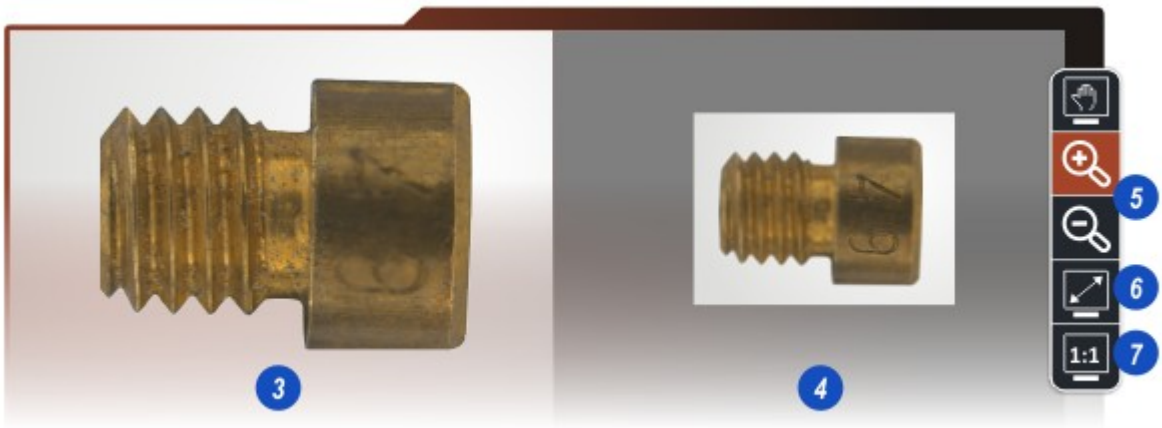
6: …*Fit to Screen* or…

7: …display at *Original Size.*

Lock View enabled:
Follow steps **(1)** and **(2)** above but click to *enable* the *Lock View* – tick mark visible.

Both images automatically scale to the smaller view.No need to click on an image because both are now synchronised – all of the *Side Tool Bar* options affect both images simultaneously.

Continued 81



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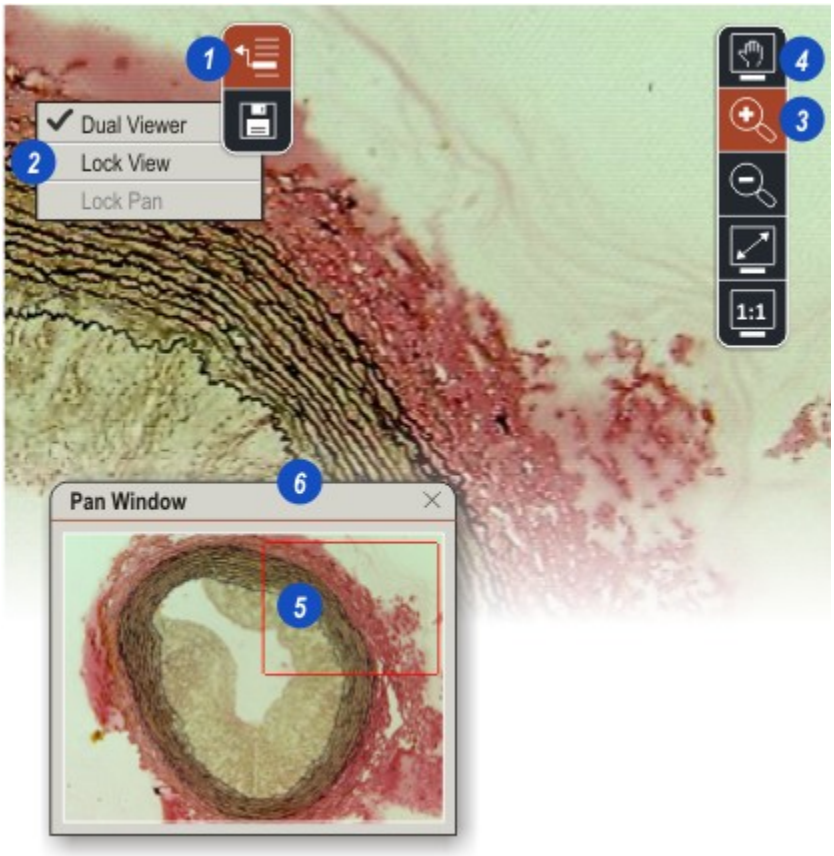
The *Pan* tool allows detailed areas of an image that exceeds the visible area of the *Viewer* to be examined. It will not work if *Fit to Viewer* is enabled because all of the image is being displayed.

Dual Viewer will work in either locked or unlocked modes when *Pan* is being used.



Pan with Lock View disabled: Only one of the images is panned

1: Click the *View Options* button on the *Side Tool Bar*.

2: From the context menu click to disable *Lock View* – no tick mark visible. The images are now independent.

3: Click on the image to pan and, using the *Zoom In* button enlarge it.

4: Click on the *Pan Window* button and the window appears with...

5: ... a red-outlined *Pan Area*. Click inside the *Pan Area* and drag it to the required position. The selected image tracks the movement

.

6: The *Pan Window* can be moved to any convenient position in the *Viewer* by clicking and dragging its header bar. Either image can be displayed in the *Pan Window* simply by clicking on it.

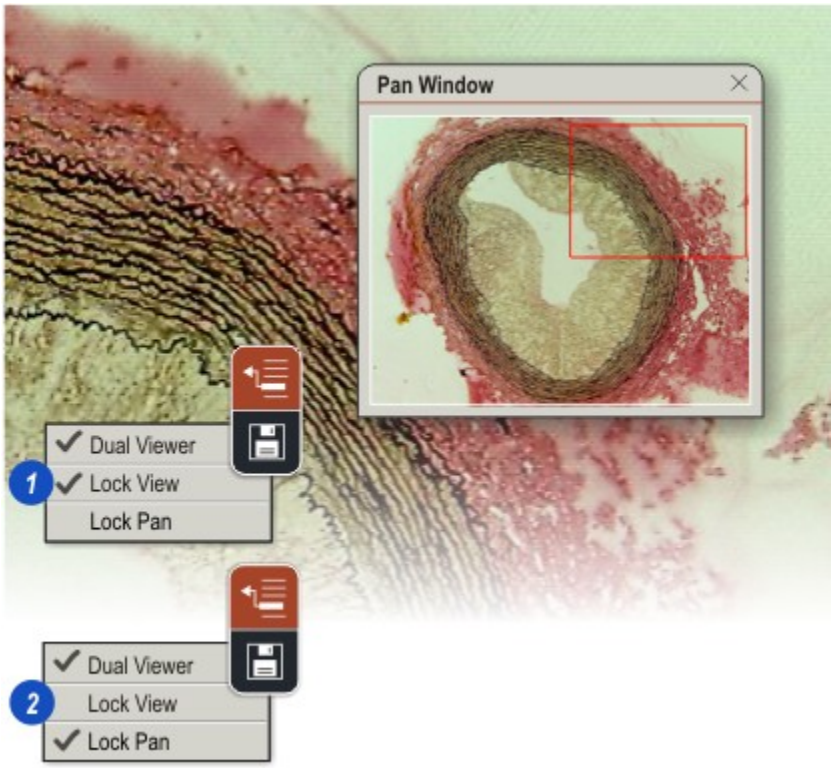
Continued: 82

Pan with Lock View enabled:

1: Follow the sequence on the previous page but click to select *Lock View* - tick mark visible. Both images are automatically scaled to the same size and both pan in unison.

Pan with Lock Pan enabled:

2: Follow the steps on the previous page and click to enable *Lock Pan* - tick mark visible. The images can be displayed at different sizes but will pan in unison as the *Pan Area* is dragged.



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To compare both live and captured images:

1: Click on the *View Options* button on the *Side Tool Bar* and…

2: …from the menu click to *enable* the *Lock View* option – tick mark visible.

3: Enlarge the images (now scaling in unison) to the desired size. In the illustration the two bolt heads are being compared and so the images were enlarged to view them easily.

4: Click on the *View Options* button again **(1)** and this time click to *disable* the *Lock View.*

5 & 6: Use the *Scroll Bars* to independently move the images so that the features are close enough to be compared.

Export Images:

The *Export* function copies a selected image or multiple images to a location of the users choice.

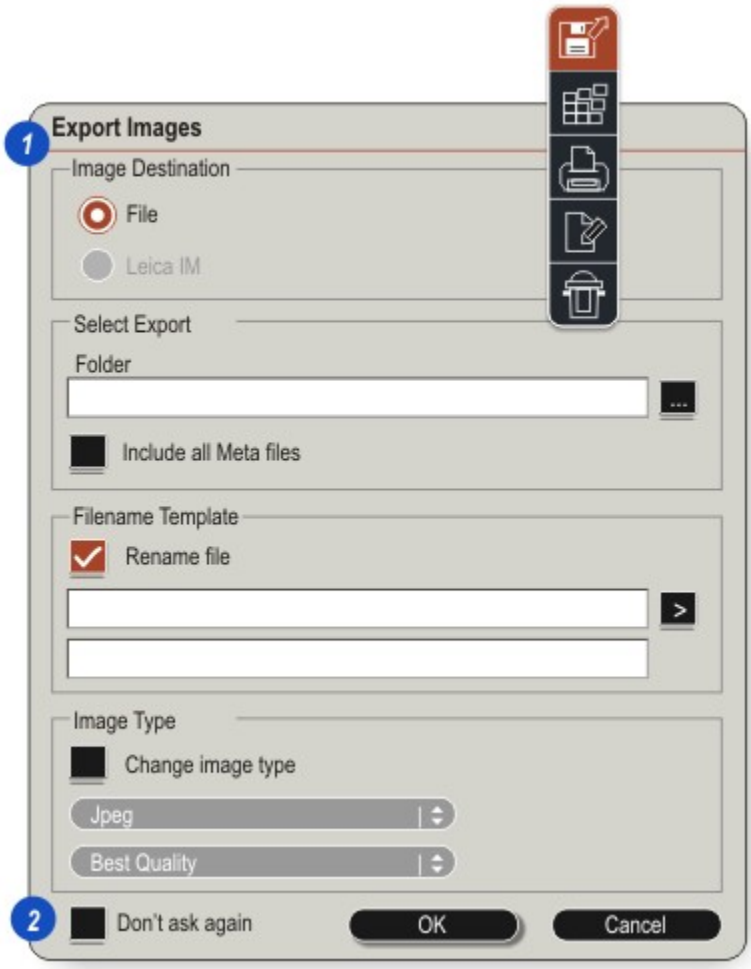
Options allow complete meta data files or individual fields of data to be attached to, and exported with the image(s). Meta data contains the settings for the *Scale Bar* and annotations so is useful only for other LAS installations.

Additionally, the image type - *jpg, png* etc - can be changed during the export process. Image names can be changed as well or image sequences exported with the same name but with an automatically applied incremental suffix.

1: On the *Export Images* dialog, there is a...

2: ...*Don't ask again* checkbox which, when enabled (a tick mark visible) will skip the dialog options. This is very useful if a large number of images are to be exported and continually completing the dialog becomes a chore.

Enabling the checkbox clears the *Show Export Options* in *Preferences,* so to display the *Export* dialog again the option has to be re-enabled in *Preferences* 85.



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Enabling the *'Don't ask again'* checkbox clears the *Show Export Options* in *Preferences*, so to display the *Export* dialog again:

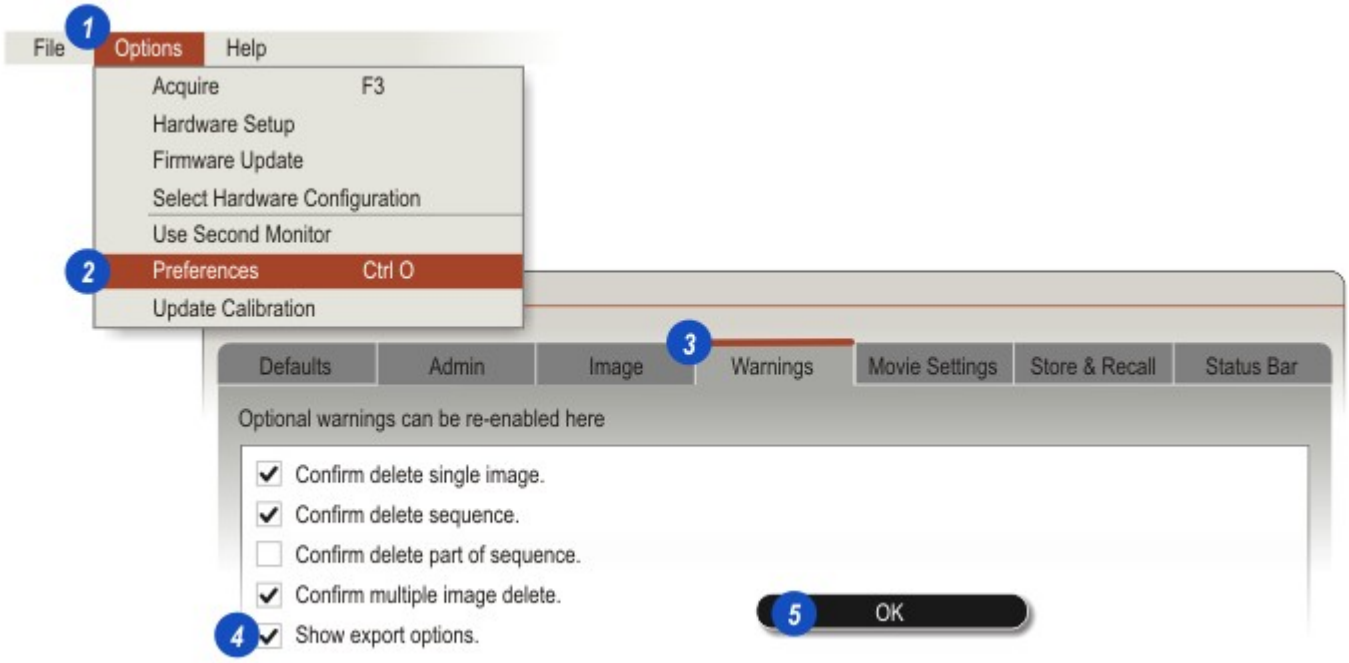
1: Click on *Options* on the *Main Menu.*

2: Click to select *Preferences*.

3: On the *Preferences* panel click the *Warnings* tab and...

4: ...click to enable the *Show Export Options* check box.

5: Click *OK* and the next time *Export* is invoked the dialog will re-appear.



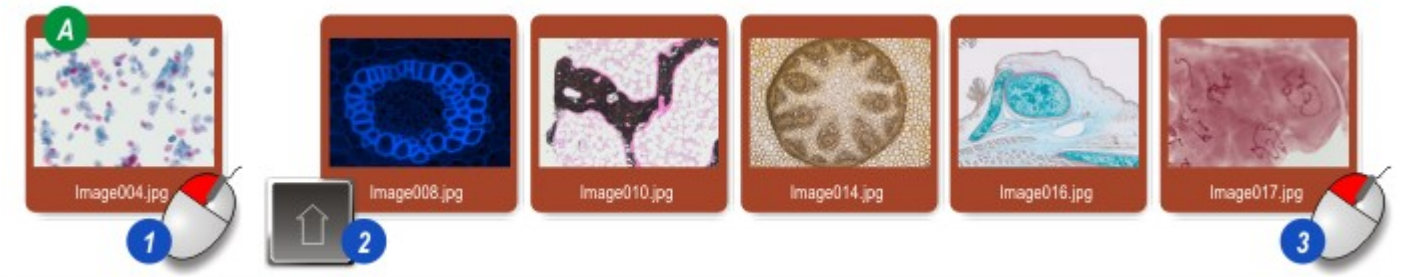
There are several ways to select images that will exported:

A: Range of images:

1: Click on the first image to be selected.

2: Press and hold down the *Shift* key.

3: Click on the image from which the calibration will be copied. All of the images between the two will also be selected.



B: Individual Images:

1: Click on the first image to be selected.

2: Press and hold down the *Ctrl* key.

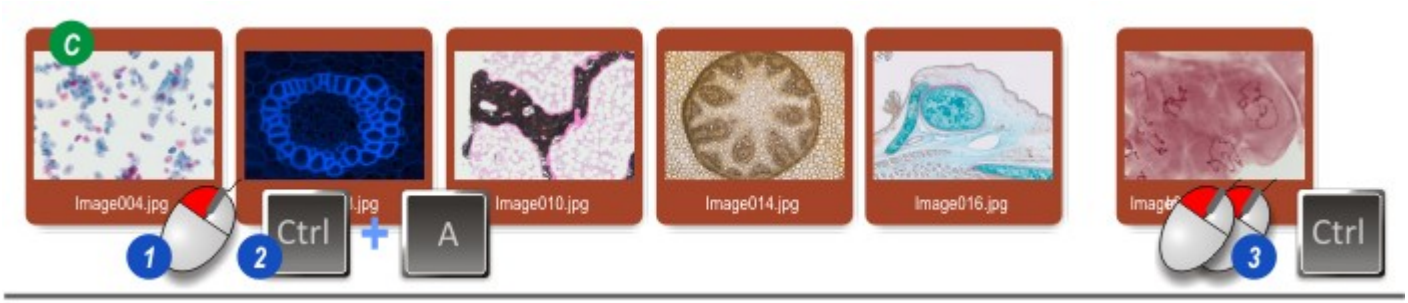
3: Click individually on all the other images to be included in the selection. The last image will be used as the calibration source.

C: All of the images in the Gallery:

1: Click on the first image.

2: Press and hold down the *Ctrl* key and then press and release the *'A'* key (All).

3: To select the calibration source, still holding down the *Ctrl* key double-click the source image.



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1: If necessary, click to select the *File* option on the *Image Destination* panel.

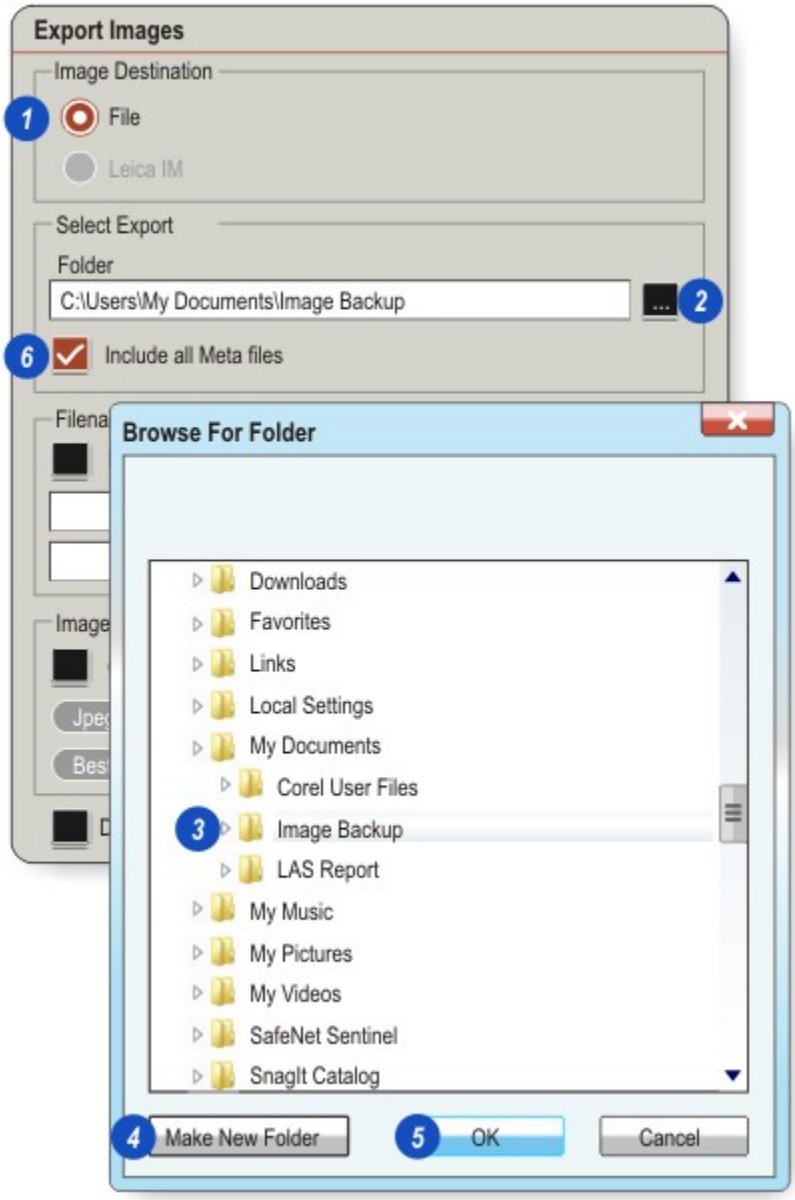
2: To change the destination folder, click on the *Browse for Folder* button and...

3: ...navigate to the destination folder. Create a new folder if required **(4).**

5: Click the *OK* button.

6: To include all of the *Meta D*ata with the image, click to enable the *Include all meta files* check box.

Continued 88



The images can be renamed before they are stored in the destination folder:

1: Click to enable the *Rename File* check box.

2: Click in the text box and type a new name for the image(s).

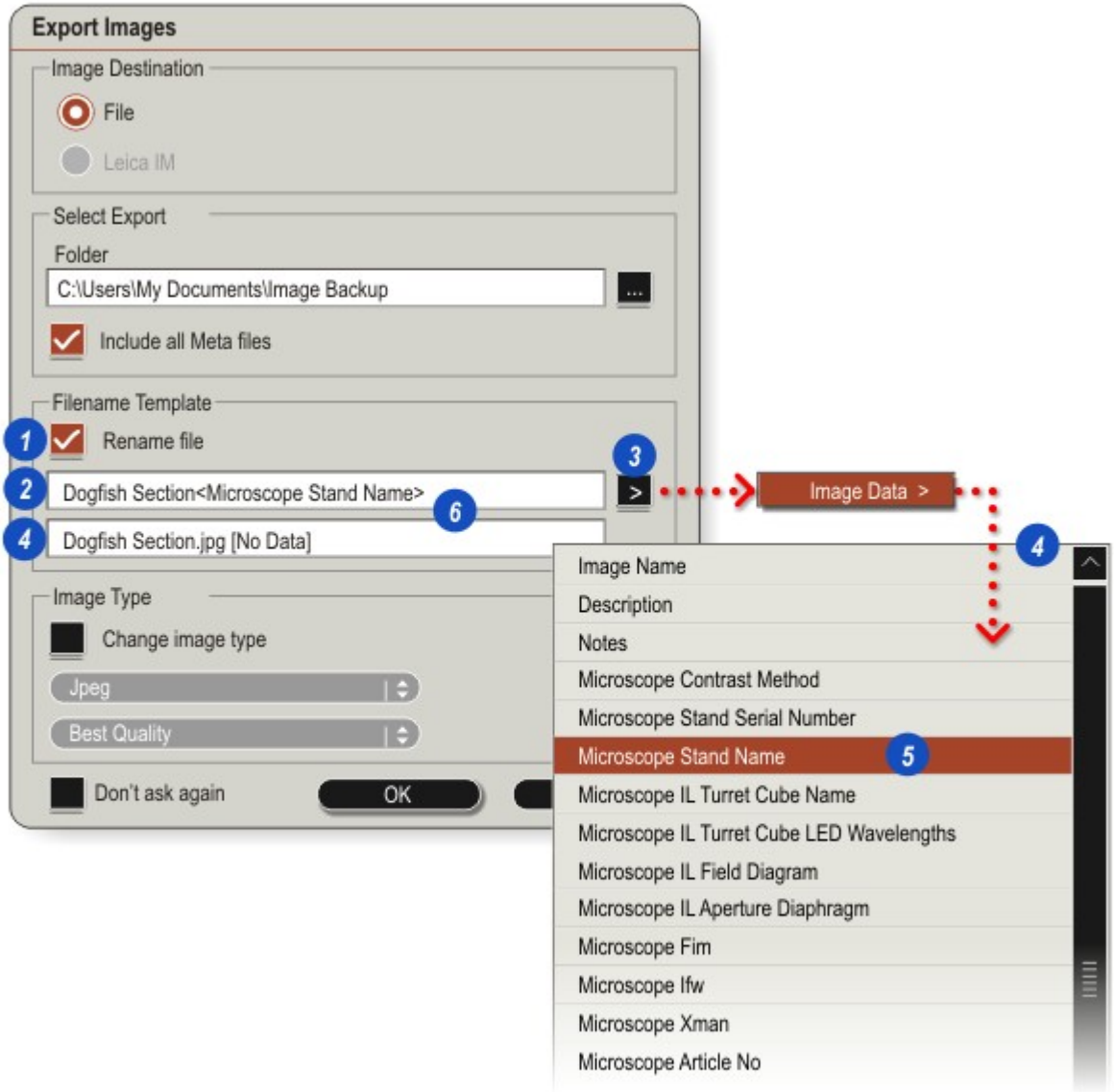
3: To include selected *Data Fields* with the image, click on the right-facing arrow and on the *Image Data* prompt.

4: The new name is displayed in the lower text box and the *Image Data* panel opens on the right.

5: To include a field and its data, click to select the *Field Name.*

6: The *Field Name* is added to the image name in the upper text box contained in tag marks <>, and relevant data to the lower text box within brackets [].

Continued 89



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The image type can be changed before it is saved:

1: Click to enable the *Change image type* check box.

2: Select the new type by clicking on the small arrows to the right of the *Image Type* header and from the drop down menu...

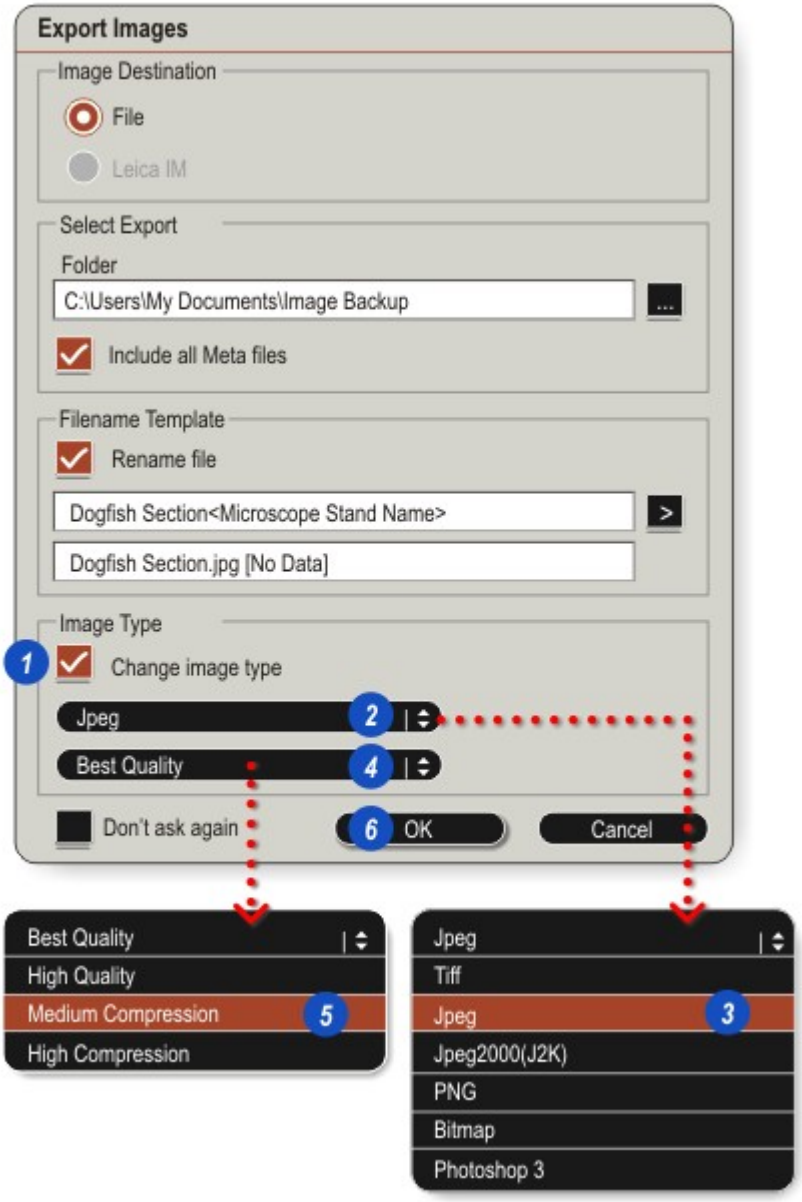
3: ...click to select the *Image Type* required.

4: When the *jpeg* option is selected the quality menu is enabled. Click on the small arrows to the right of the header and...

5: ...click to select the required quality or compression.

6: Click the *OK* button.

Continued 90



If an image with the same name already exists in the destination folder, a warning will appear. There a three options:

1: Overwrite the existing with the new export. The existing image is lost.

2: Abort the export - *Don't copy.*

3: The *Copy but keep both files* option exports the image but adds a numeric suffix to the new copy to distinguish it. This is the option to use if multiple images are being exported and a common name has been chosen for them.

4: Click to enable the *Do this...* check box to automatically employ the chosen option in the future.

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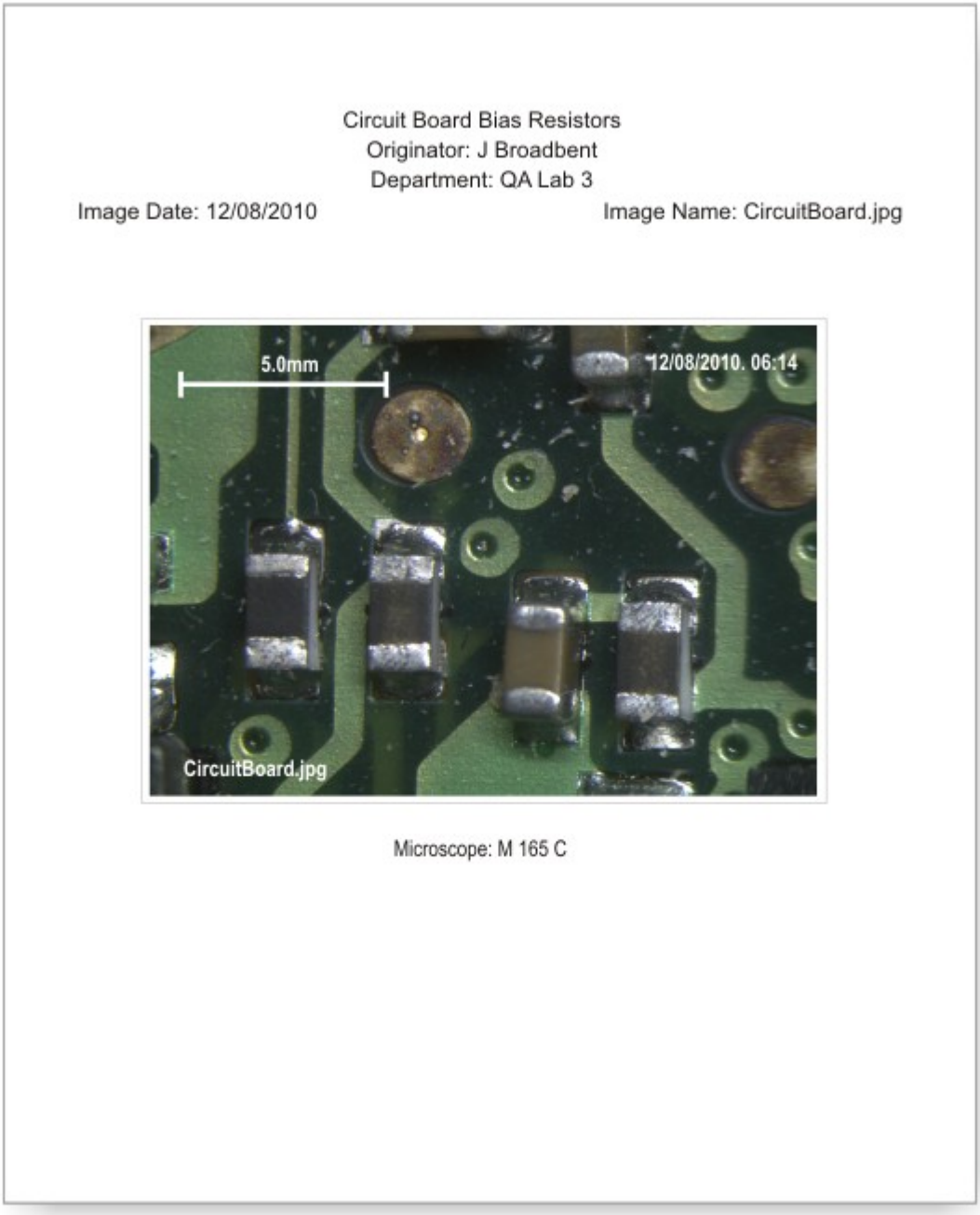
Printing:

The Printing facility allows the user to print individual images quickly and simply. The built-in formatting provides flexible styling resulting in professional-looking documents.

Printing's most powerful feature is direct access to the image data, including all of the microscope settings, that can be included with just a mouse click to display alongside the users own text.

Printing features and quick links (↗):

- Print LAS images in high resolution colour..
- Include *Annotations* and *Scale Bar* on the image: ↗ 101
- Include *Measurement Drawings* on the image.
- Include multiple *Header* and *Footer* comments: ↗ 96.
- Print landscape for maximum image size or portrait for extra comment space: ↗ 95
- Automatically insert image information - no need to type it in: ↗ 98
- Choose the *Font, Style* and *Size* to personalise the report: ↗ 96
- Comments can be re-used with other images - no need to re-type.
- See a full-screen preview before printing: ↗ 101



To print a single image together with a wide range of data:

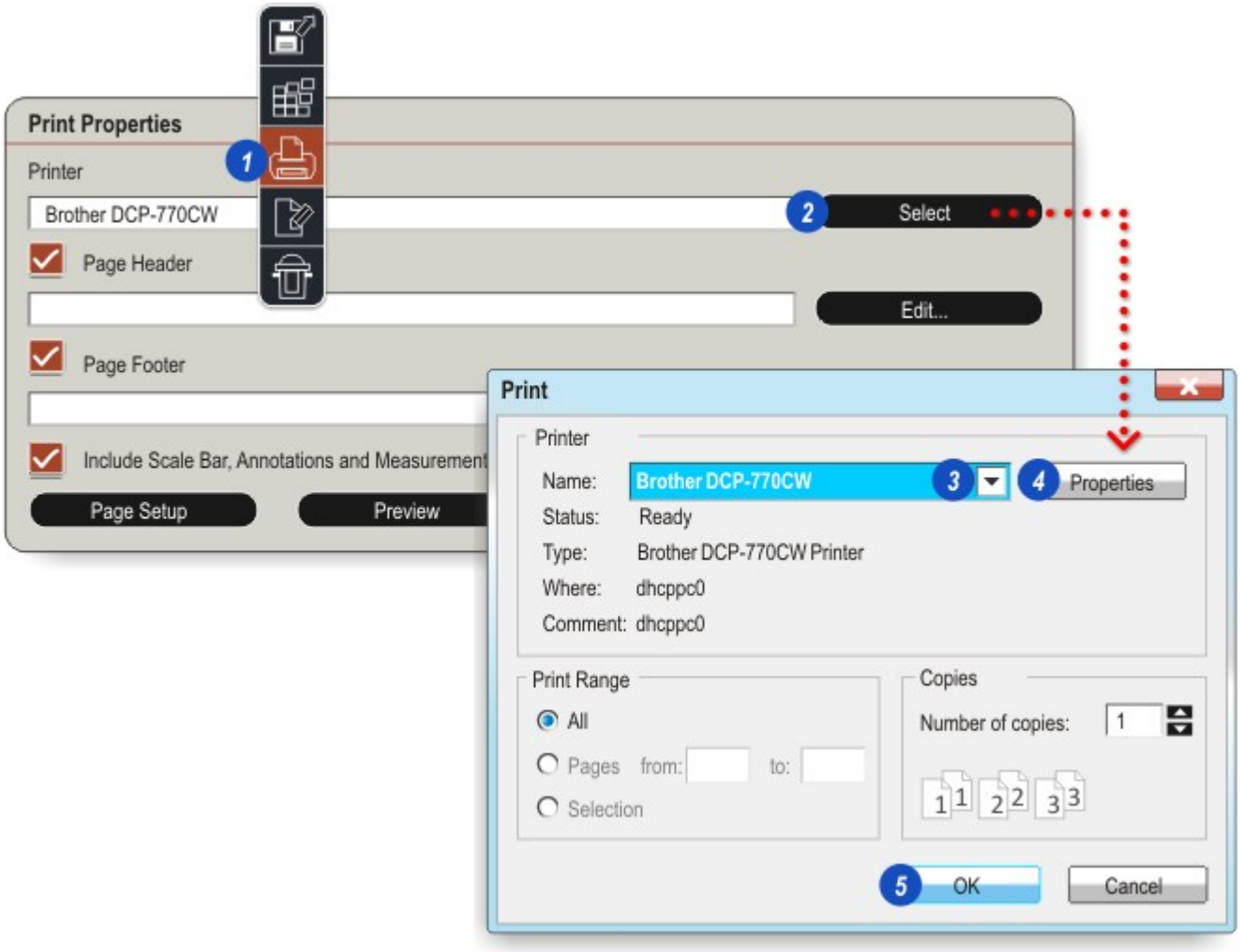
1: Click on the *Print* icon. The *Print Properties* dialog appears.

2: The currently selected printer (if there is more than one connected to the machine or network) is displayed in the *Printer* window. To change the printer, click on the *Select* button and the *Windows Printer* dialog appears.

3: Click on the arrow to the right of the *Name* text box and from the drop down list click to select and alternative printer.

4: Change the print properties by clicking on the *Properties* button.

5: Click *OK* to apply the new printer and its properties.



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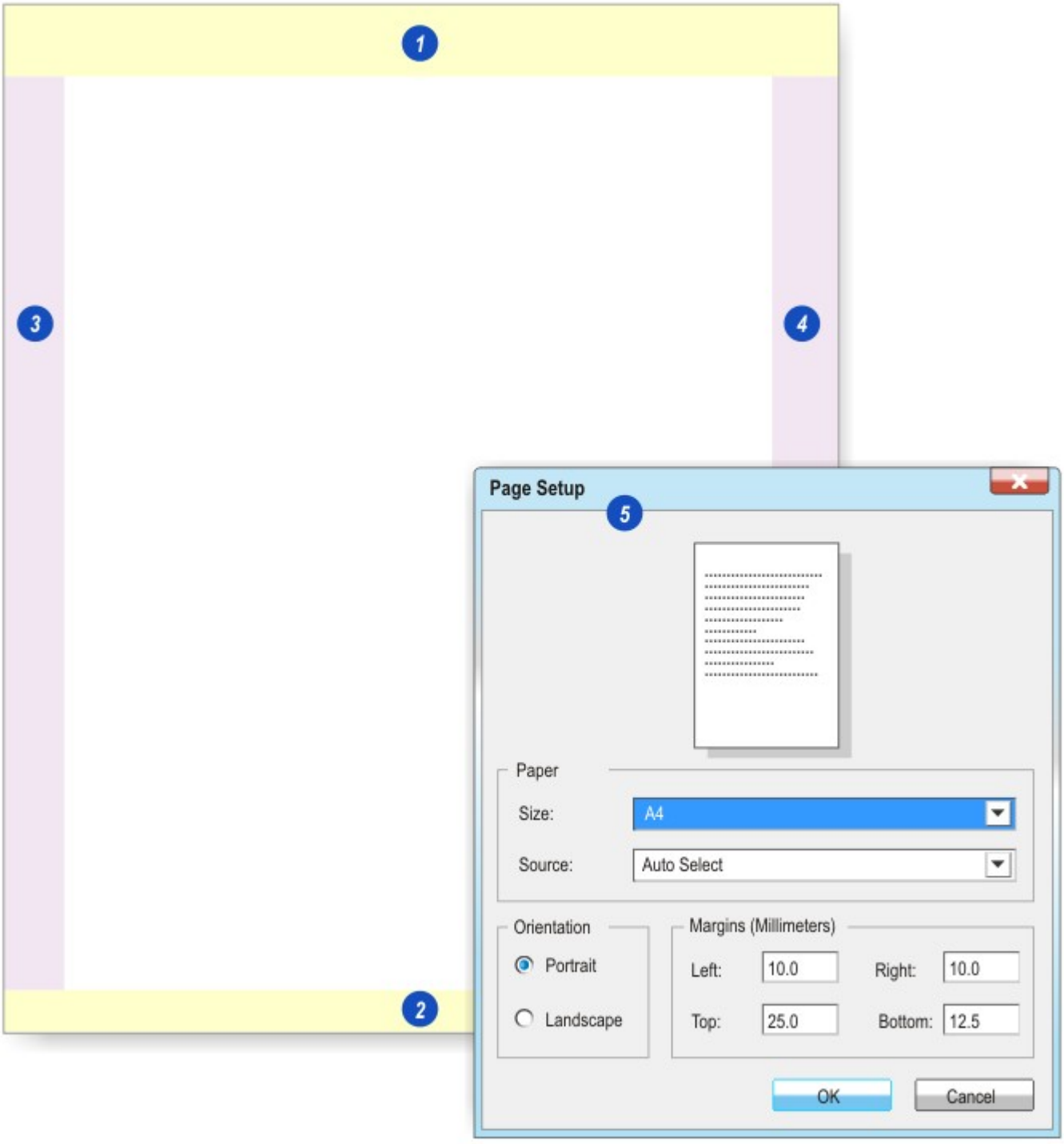
The print page is structured to provide the user with a quick and simple way of adding text and data to an image. The layout has been designed to provide maximum flexibility and a professional presentation.

There are 4 margins that can be setup by the user to accommodate different printers and bindings:

1, 2, 3 & 4: Top, bottom, left and right margins can all be set independently using the *Page Setup* dialog **(5).**

The dialog also allows the page *Size* and *Orientation* to be set.



The printable area inside the margins is divided into 3 columns each of which can contain justified text:

1: The left-hand column contains left-aligned text.

2: The centre column displays centred text, and...

3: ...the right-hand column right-aligned text.

The columns are further divided:

A: Text appearing above the image called *Header Text*, and...

B: ...text below the image referred to as *Footer Text*.

The fonts are selected and the text entered using two dialogs - *Header Text* and *Footer Text* setups **(4)**.

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To change the page size, margins and the orientation:

1: From the *Print Properties* dialog click on the *Page Setup* button.

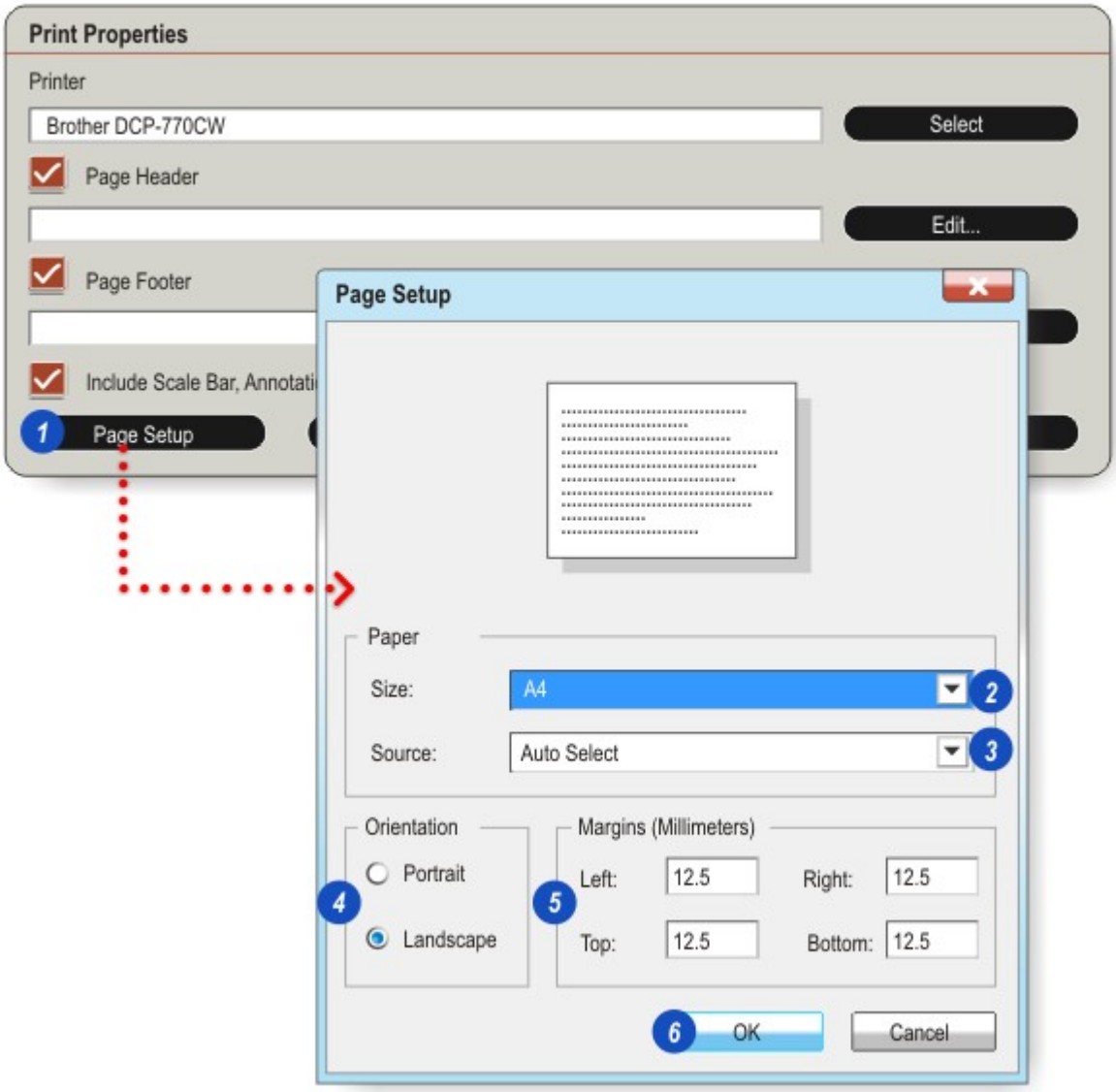
2: On the *Page Setup* dialog, if required change the page *Size* by clicking the arrow to the right of the *Size* header and selecting from the drop-down menu.

3: If the printer software allows, the printer *Paper Source* can be set by clicking the arrow to the right of the header and selecting from the menu.

4: Click to select the page *Orientation* - portrait or landscape.

5: Set the margins by clicking inside the appropriate text box and typing a value.

6: Click *OK.*



Different *Fonts, Style* and *Type Size* can be used for *Header* and *Footer* text but cannot be mixed across the columns.

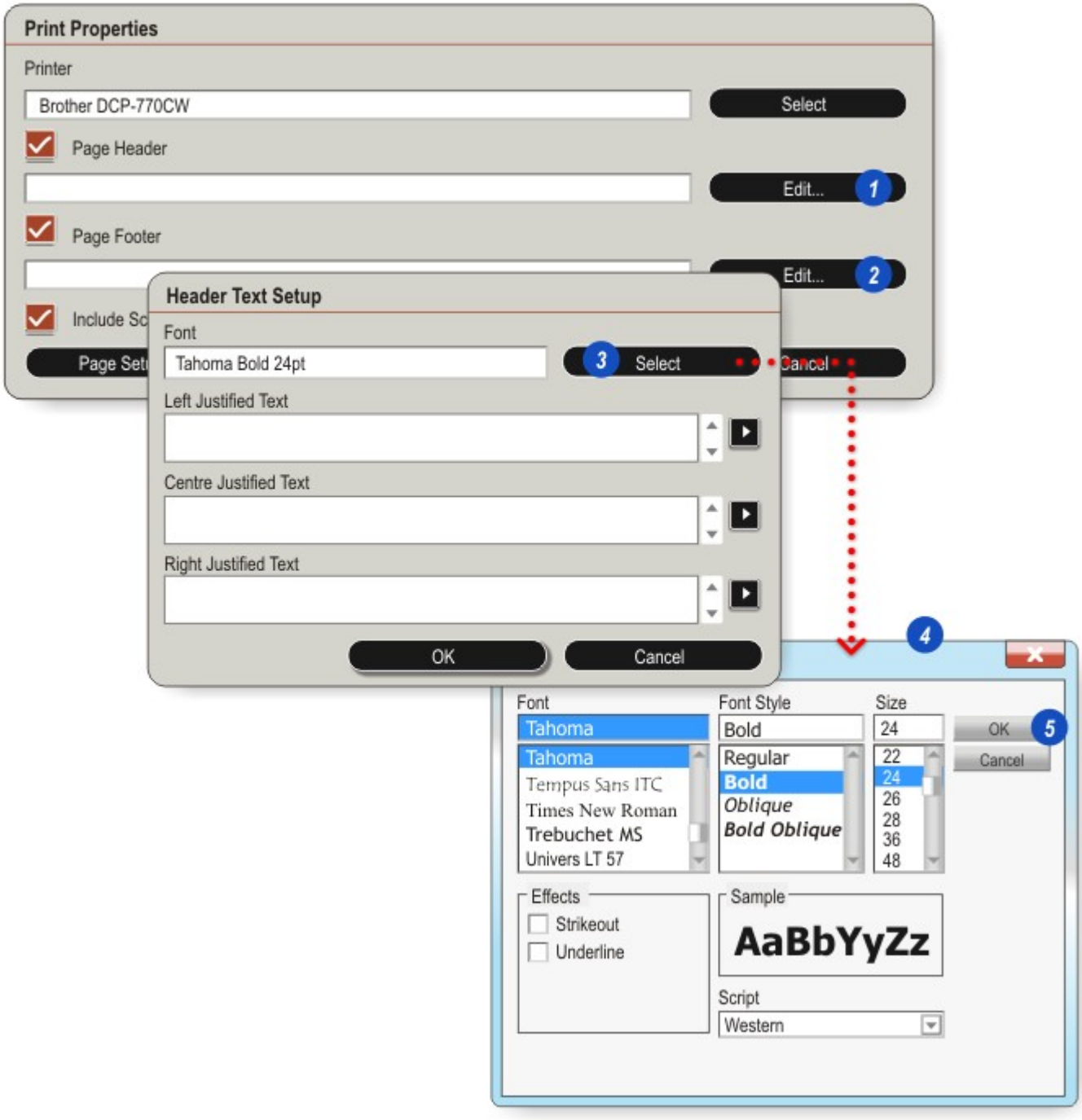
To select a *Font, Style* and *Size*:

1 & 2: On the *Print Properties* dialog click the appropriate *Edit* button - either *Header* or *Footer* text.

3: On the *Header* or *Footer Text* dialog, click the *Select* button to the right of the *Font* text box.

4: From the *Windows Font* dialog, select the *Font, Style* and *Size* from the lists.

5: Click *OK*.



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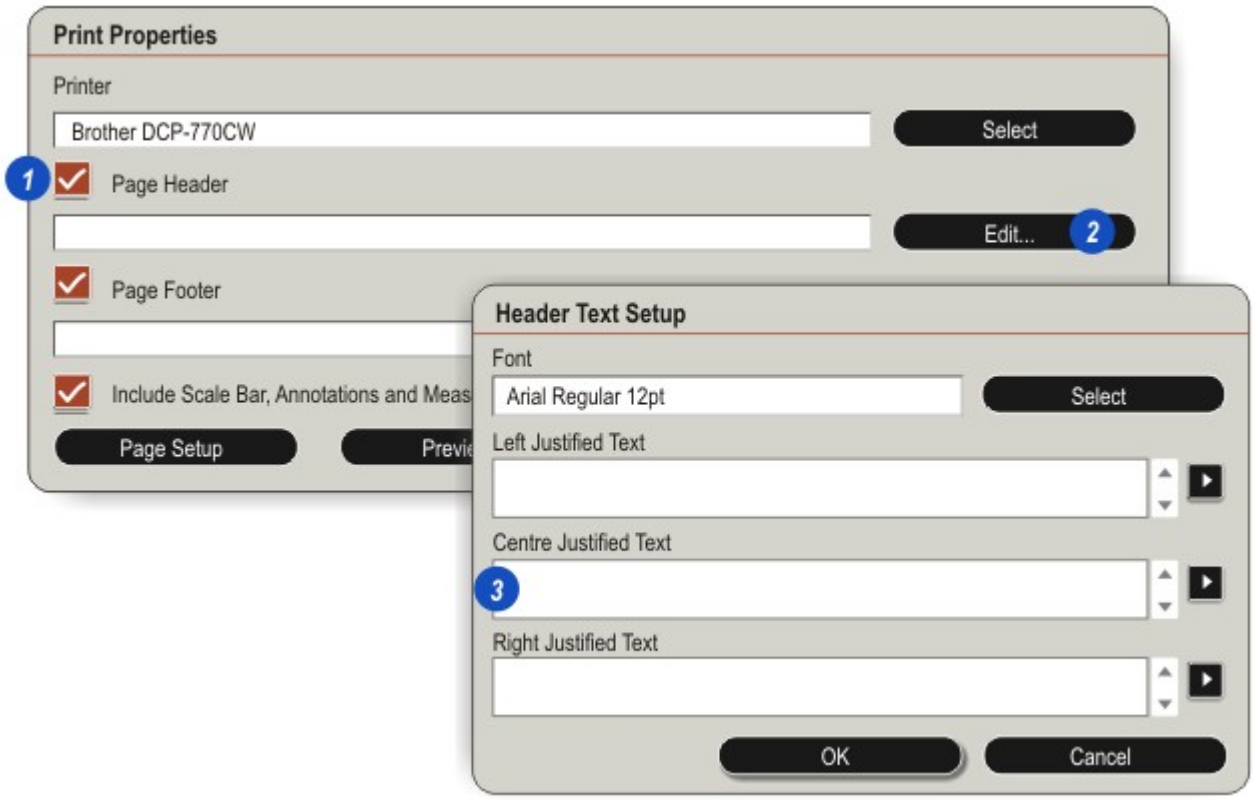


In this example the document title, the originator and the department are to be displayed in the centre column above the image. It will look like this:

1: On the *Print Properties* dialog click the *Page Header* check box to display a tick mark. This will ensure the header text prints.

2: Click the *Page Header Edit* button.

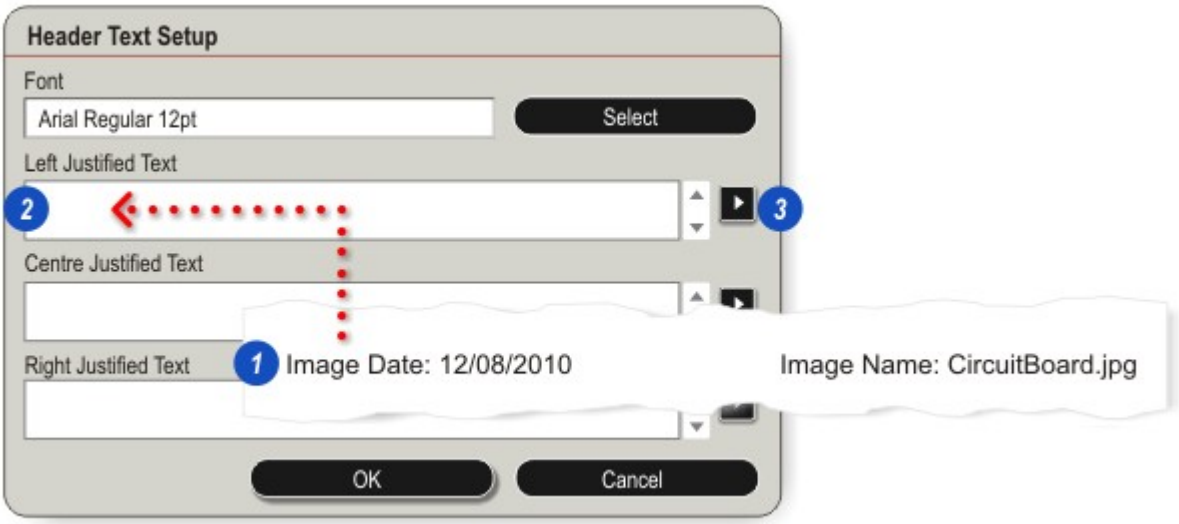
3: On the *Header Text Setup* dialog click inside the *Centre Justified Text* box.

4: The document title is typed...

5: ...followed by the key combination *Ctrl* + *Enter*: Press and hold down the *Ctrl* key and then press the *Enter* key. This will insert a new line break.

6: The originator text is added, again followed by the new line break combination *Ctrl* + *Enter.*

7: Finally, the department text is added in the same way.



LAS stores a wealth of data about an image. Individual data items can be selected from a list and added automatically to the page.

The date of the image capture and the image name are to be added to the left and right *Header Text* respectively **(1)** . The captions will be typed into the 'Justified' text boxes but the actual values are imported from the image data.

To add the image capture date:

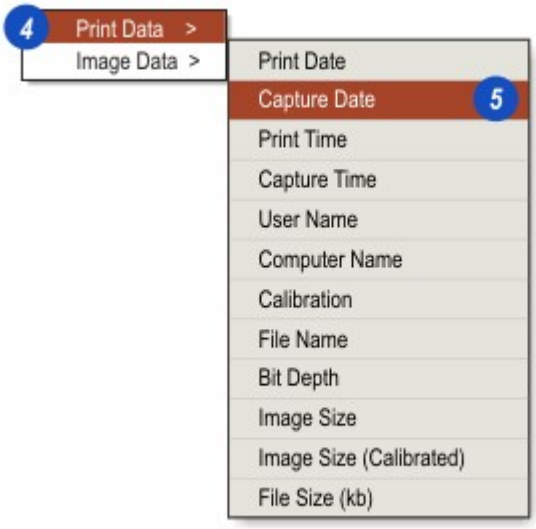
2: On the *Header Text Setup* dialog click inside the *Left Justified Text* box. In this example the text *'Image Date: '* is typed. Do not add a new line break.

3: The image *Capture Date* is going to be added to this text. Click on the arrow to the right of the *Left Justified Text* box.

4: Click to select *Print* from the data menu, and...

5: ...from the drop-down list, click to select the data item required - in this case *Capture Date*. Data items are represented in the text box enclosed in tags:

Capture Date: <Capture Date>



The *Image Name* is displayed in the same way but using the *Right Justified Text* box instead and selecting *File Name* from the list. It will appear as:

Image Name: <File Name>

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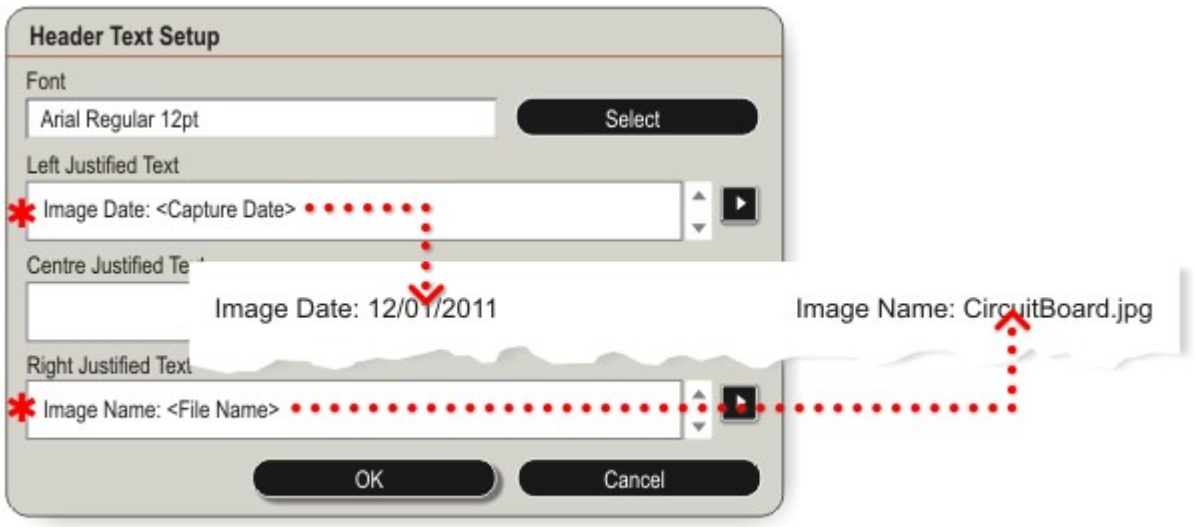
The illustration shows the *Header Text Setup* dialog as it would appear from the example on the previous page.

When the page is printed the data inside the tags (<>) is retrieved and formatted. When this image was captured it was given the name *CircuitBoard.jpg.*



Footers - text appearing below the image - is entered in the same way as described for the *Headers* with left, centre and right text boxes.

Image data - information about the hardware, exposure and illumination - is also included using the same method as for print data.

In the example the final piece of information is the microscope name which will be centred below the image. On the *Print Properties* dialog click the *Footer Edit* button and...

1: ...the *Footer Text Setup* dialog appears.

2: For centred text click inside the *Centre Justified Text* box and type - in this example the word *'Microscope*: '.

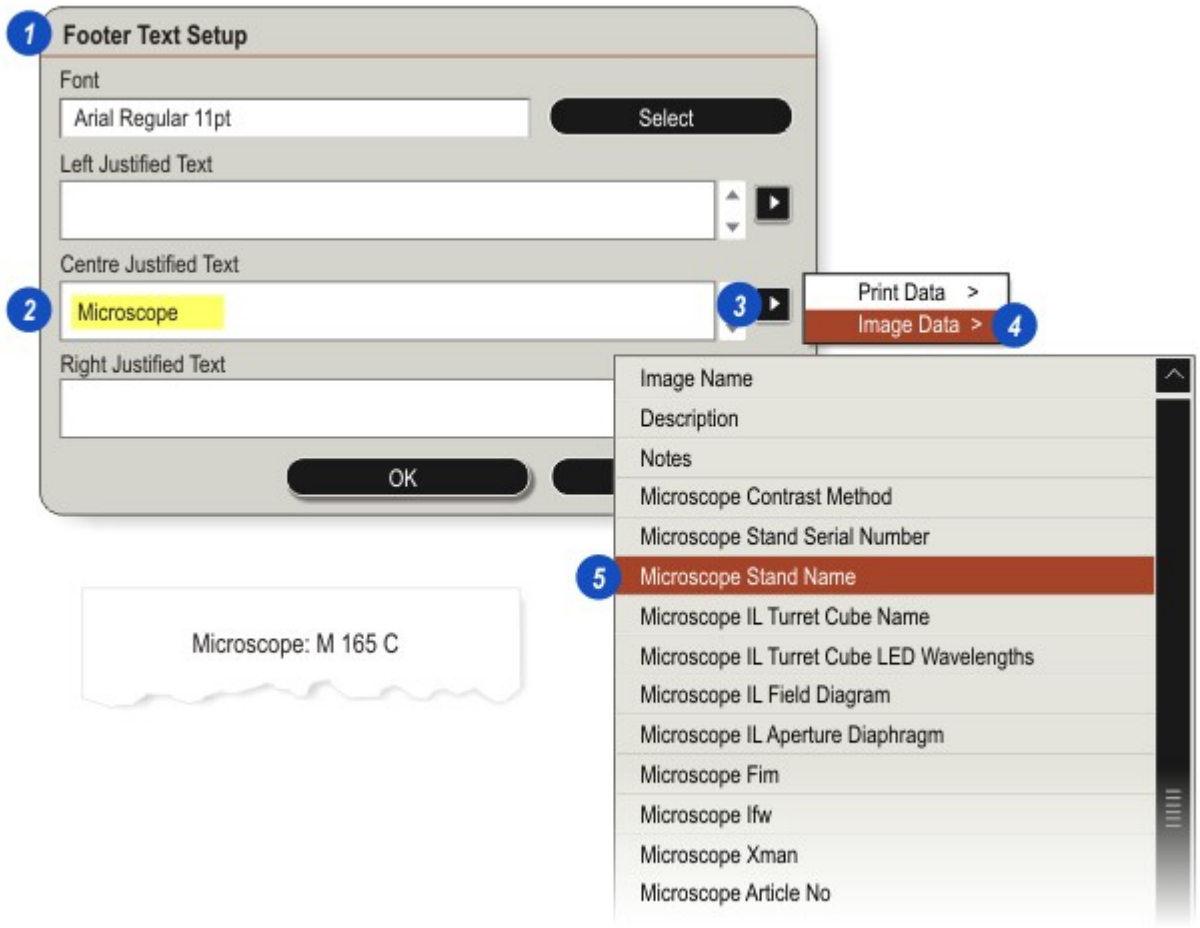
3: To add an *Image Data* item, click on the arrow to the right of the text box and...

4: ...select *Image Data* from the options.

5: Click to select the required item from the drop-down list. In this example the text would appear as:

Microscope: <Image Data.Microscope Stand Name>

Any number of data items can be added to a printout providing space allows.



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1: To preview how the page will look when printed, on the *Print Properties* dialog click the *Preview* button.

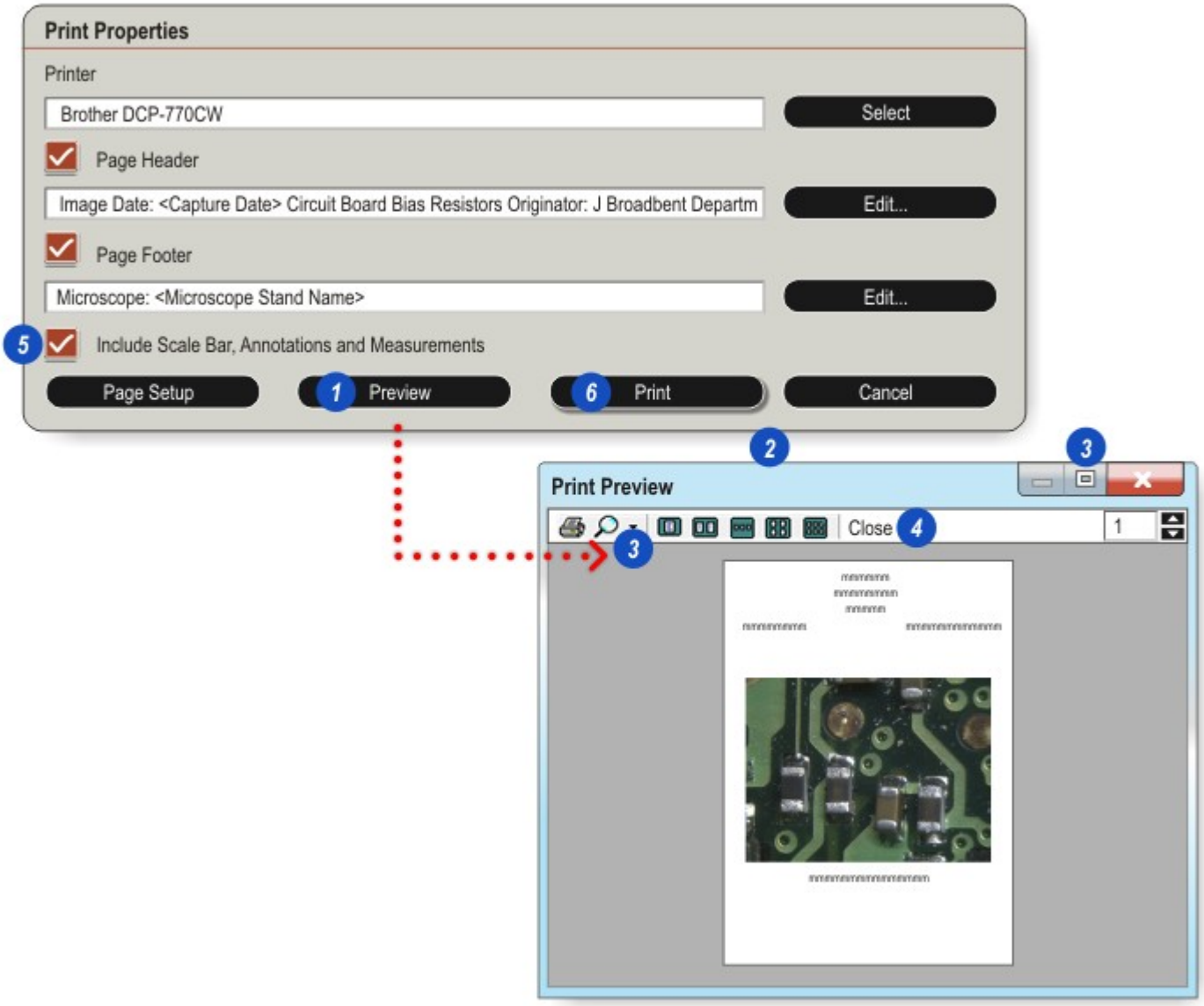
2: The *Print Preview* window appears with a scaled down representation of the page with the *Header*, *Footer* and image in relative positions.

3: Use the *Preview Zoom* to enlarge or reduce the preview or display it full-screen.

4: Close the *Preview* by clicking the *Close button.*

5: If a *Scale Bar* and *Annotations* have been added to the image but not merged, they can be included on the printout by clicking to enable the *Include Scale Bar...* check box.

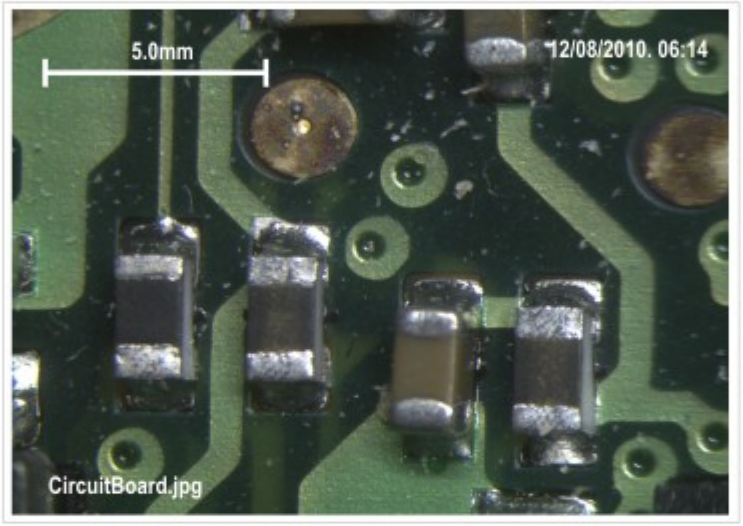
6: Click the *Print* button on the *Print Properties* dialog to print the page(s).



This is the printed result of the example described in the text.

Circuit Board Bias Resistors
Originator: J Broadbent
Department: QA Lab 3

Image Date: 12/01/2011 Image Name: CircuitBoard.jpg



Microscope: M 165 C

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Gallery Docking:

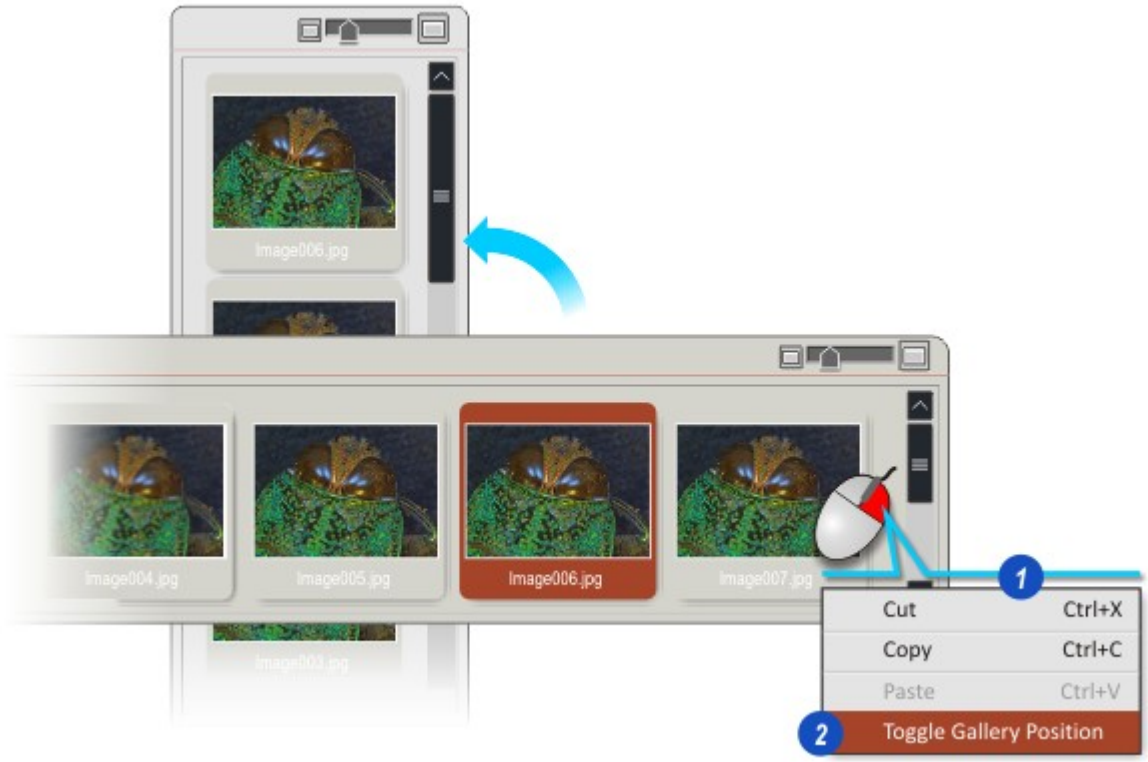
Available in *Acquire, Browse* and *the Process* Workflows, the thumbnail *Gallery* can be 'docked' either horizontally - along the bottom edge of the *Viewer* - or vertically - along the left-hand edge of the *Viewer* - to suit the user.

1: Right-click on a thumbnail or on the spaces around the thumbnails and...

2: ...from the drop-down menu, left-click to select the *Toggle Gallery Position* option.

The action toggles between horizontal and vertical docking.

Scroll bars, if required, are placed automatically.



The Setup Workflow:

The *Setup Workflow* provides the method of specifying the Leica microscope components connected to Leica Application Suite and, when the optional LAS Archive modules are used, creating archives to capture images and data.

Components such as objective types and filter descriptions can be readily selected, saved and subsequently recalled.

In addition, ‘fine tuning’ of some of the microscope features can be performed such as parfocality, setting the focus step size for each lens.

When leaving the *Workflow*, the new items/settings are permanently stored.

The major types of Leica microscope that are used with LAS are described in separate sections.

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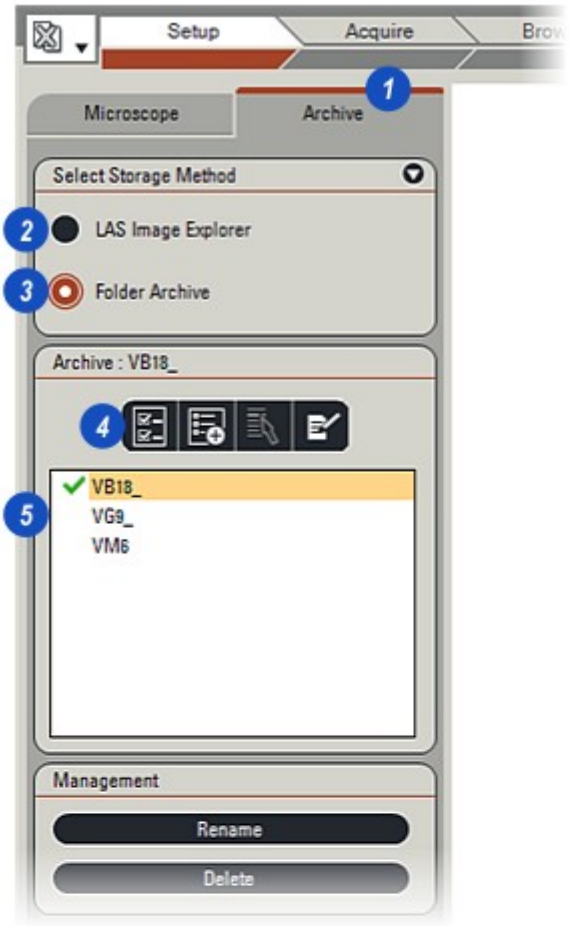
If you are an LAS Core user with no optional LAS Archive modules installed, please ignore this topic.

If LAS optional module *Archive* – either *Basic* or *Standard* – is installed, the *Archive* tab appears on the *Setup Workflow.*



1: If necessary click on the tab to reveal the control panels.

2: Because two image storage methods are available – *Image Explorer* and *LAS Archive* – the *Select Storage Method* facility allows either to be selected. The buttons are mutually exclusive so it is not possible to use both methods at the same time. Click *LAS Image Explorer* to use tree-and-folders navigation or…

3: …click *Folder Archive* to use archives.

4: The *Archive Toolbar* and…

5: …the *Archive List* window are active only when Archive is selected.

Using *Image Explorer*: *Go there...* 298

Using LAS Archives: *Go there...* 385

Stereo Microscope Setup

Stereo Microscope setup and configuration is a fast and simple exercise due to the intuitive interface and concise option lists.

The setup program is part of Leica Application Suite that is able to 'read' data from the microscope and sort and display setup options that are available and appropriate.

Each setup parameter is displayed as an icon on the *Microscope Configuration* panel **(1)** - clicking it reveals the options **(2)** and clicking an entry on the list **(3)** assigns all of the chosen attributes to the parameter.

The *Stereo Microscope Setup* dialog is reached by clicking on the *Setup Workflow* **(4)** and then on the *Microscope* tab **(5)**.

Continued... 107

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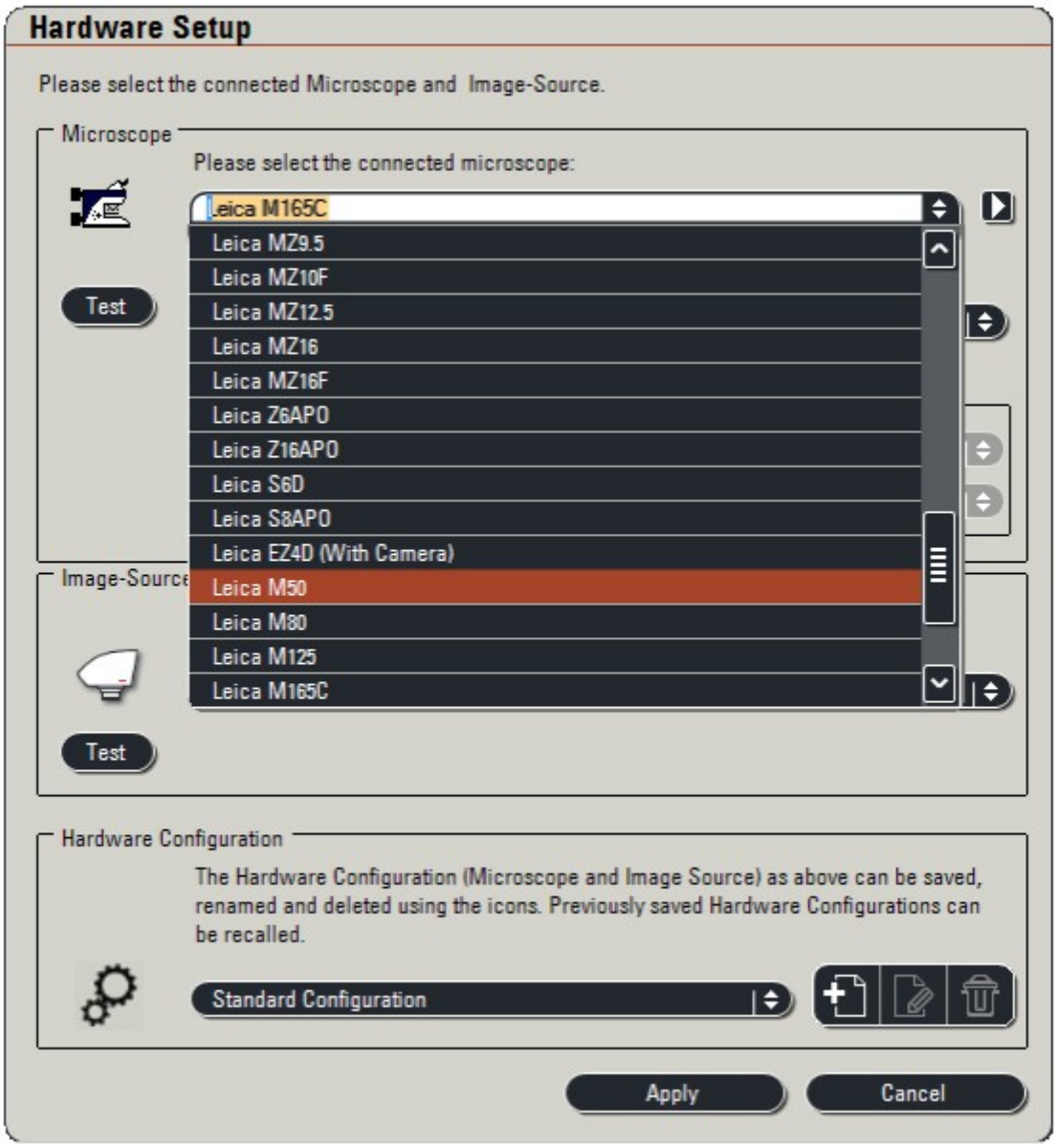
Before starting the *Stereo Microscope Setup*, the stand and camera have to be configured for use with Leica Application Suite and optionally, a new *Hardware Configuration* created.
Go there... 8



There are two selection panel styles in the Stereo Microscope Setup:

Single panel in which all of the options are displayed as a list and simply clicking on the highlighted entry selects it, and

Dual panel in which a range of controls are shown on the upper panel (**A** on the illustration) and depending upon the selection, a list of options is displayed in the lower panel (**B** on the illustration.).

The example shows the setup screen for the SmartTouch. The controls - keys, touch screen and Dual Rotary Actuator are listed in the upper panel with references to the image on the right - and the various functions that can be applied to the selected control are shown in the lower panel.

1: Click a control to select and highlight it.

2: As the mouse moves across the items in the list they are highlighted in dark brown.

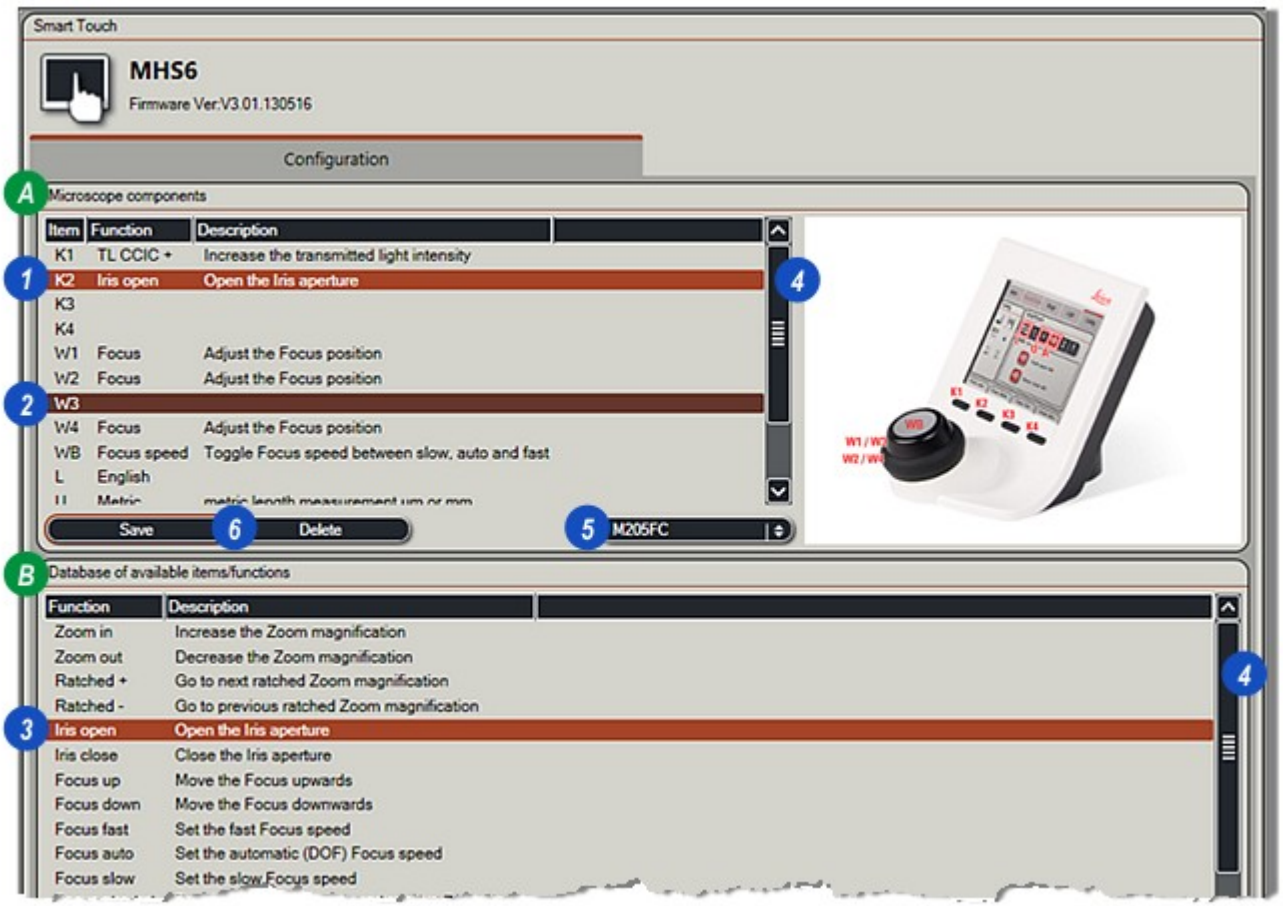
3: Clicking on an option in the lower panel assigns the function to the control in the upper panel. In the illustration, *Hardware Key K2* is selected in the upper panel and when pressed, it will *Open the Iris*. It could have been assigned any of the options in the lower panel to suit the user.

4: Long option or control lists automatically display a *Scroll Button*. Click on the button and drag it downward to view more list entries.

5: Different users will have different preferences for the way in which controls operate. Each user can store his preferences in a *Configuration* that can be recalled and loaded every time he uses the equipment.

6: Configurations are saved and if necessary, deleted using the buttons.

Continued... 109



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The available options for some microscope features are grouped as *preferred* - those recommended as better suited to the setup - and a wide range of additional options that will not be preferred.

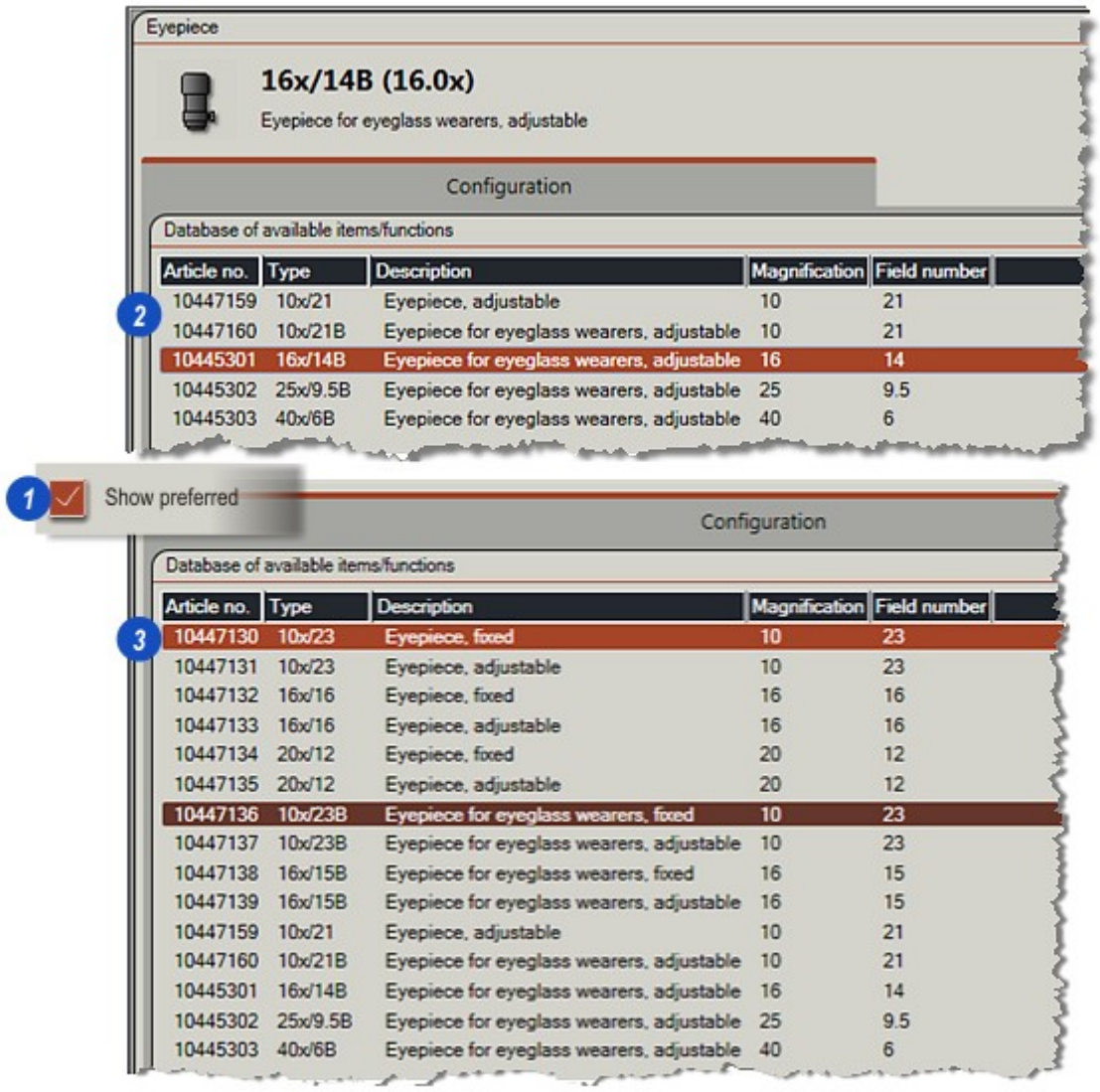
1: A checkbox appears bottom-left of the options panel...

2: ...toggles between displaying only the *preferred* options - checkbox enabled - and...

3: ...all of the additional options - checkbox disabled.

Click on the checkbox to toggle enable/disable.

Continued... 110



In most cases, the Optics Carrier type is detected by the software, highlighted on the database list but cannot be changed.

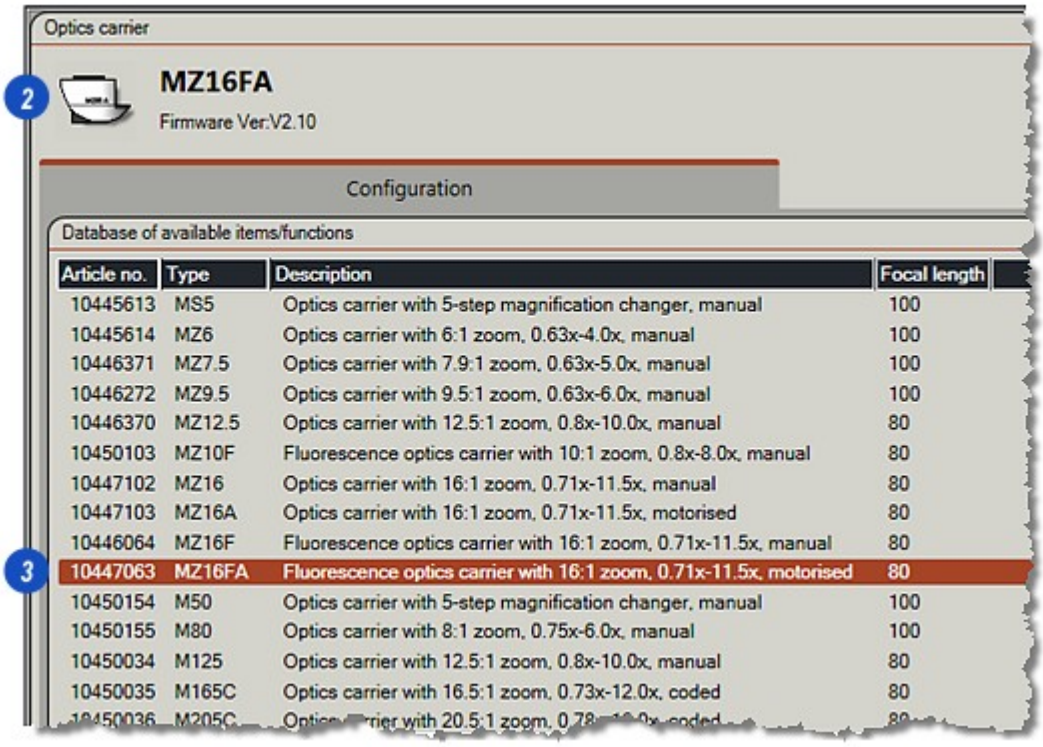
For microscopes on which assignment is possible:

1: Click on the *Optics Carrier* icon on the configuration panel.

2: The current setting is displayed on the header.

3: Click to select a new setting from the list.

Continued... 111



Article no.	Type	Description	Focal length
10445613	MS5	Optics carrier with 5-step magnification changer, manual	100
10445614	MZ6	Optics carrier with 6:1 zoom, 0.63x-4.0x, manual	100
10446371	MZ7.5	Optics carrier with 7.9:1 zoom, 0.63x-5.0x, manual	100
10446272	MZ9.5	Optics carrier with 9.5:1 zoom, 0.63x-6.0x, manual	100
10446370	MZ12.5	Optics carrier with 12.5:1 zoom, 0.8x-10.0x, manual	80
10450103	MZ10F	Fluorescence optics carrier with 10:1 zoom, 0.8x-8.0x, manual	80
10447102	MZ16	Optics carrier with 16:1 zoom, 0.71x-11.5x, manual	80
10447103	MZ16A	Optics carrier with 16:1 zoom, 0.71x-11.5x, motorised	80
10446064	MZ16F	Fluorescence optics carrier with 16:1 zoom, 0.71x-11.5x, manual	80
10447063	MZ16FA	Fluorescence optics carrier with 16:1 zoom, 0.71x-11.5x, motorised	80
10450154	M50	Optics carrier with 5-step magnification changer, manual	100
10450155	M80	Optics carrier with 8:1 zoom, 0.75x-6.0x, manual	100
10450034	M125	Optics carrier with 12.5:1 zoom, 0.8x-10.0x, manual	80
10450035	M165C	Optics carrier with 16.5:1 zoom, 0.73x-12.0x, coded	80
10450036	M205C	Optics carrier with 20.5:1 zoom, 0.78	80

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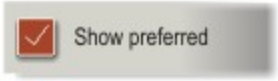
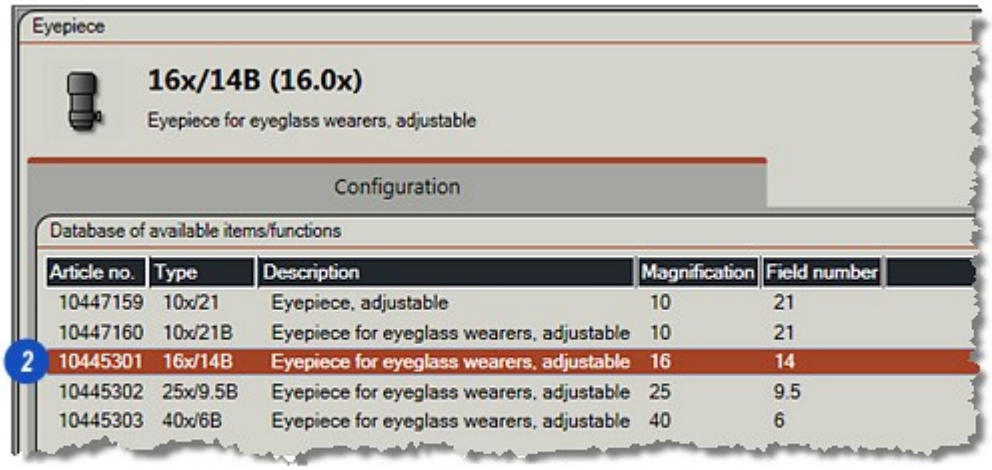
Eyepiece selection has a single list of options that will show either the preferred (checked) or additional options (unchecked):



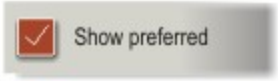
1: Click on the *Eyepiece* icon on the configuration panel.

2: Scroll to the required setting and click to select it.

Continued... 112



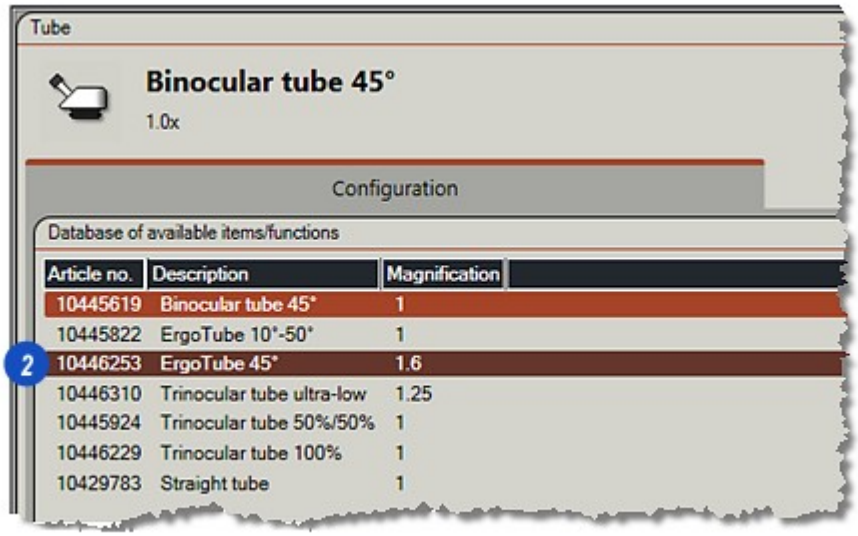
Tube Selection has a single list of options that will show either the preferred (checked) or additional options (unchecked):



1: Click on the *Tube* icon on the configuration panel.

2: Scroll to the required setting and click to select it.

Continued... 113



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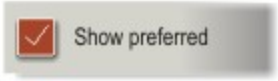
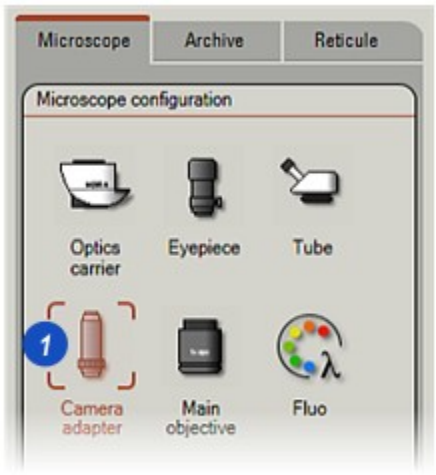
The *Camera Adapter* has a single list of options that will show either the preferred (checked) or additional options (unchecked):

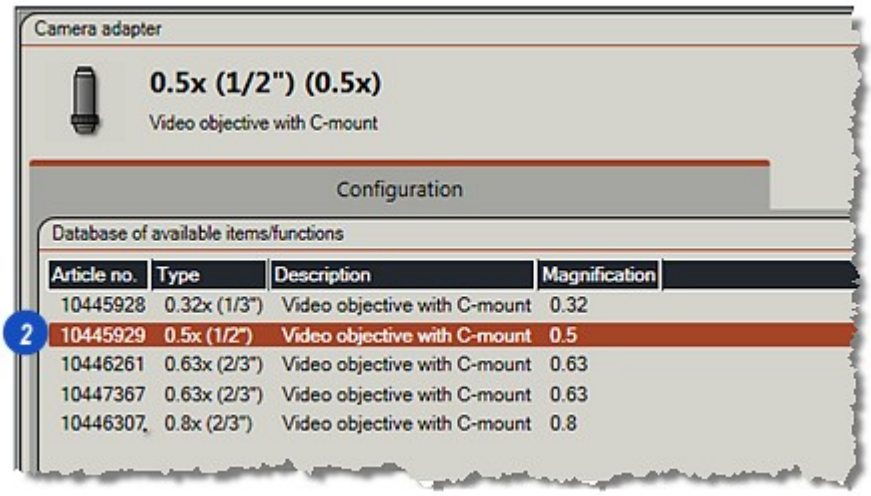


1: Click on the *Camera Adapter* icon on the configuration panel.

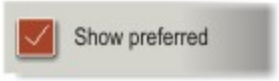
2: Scroll to the required setting and click to select it.

Continued... 114





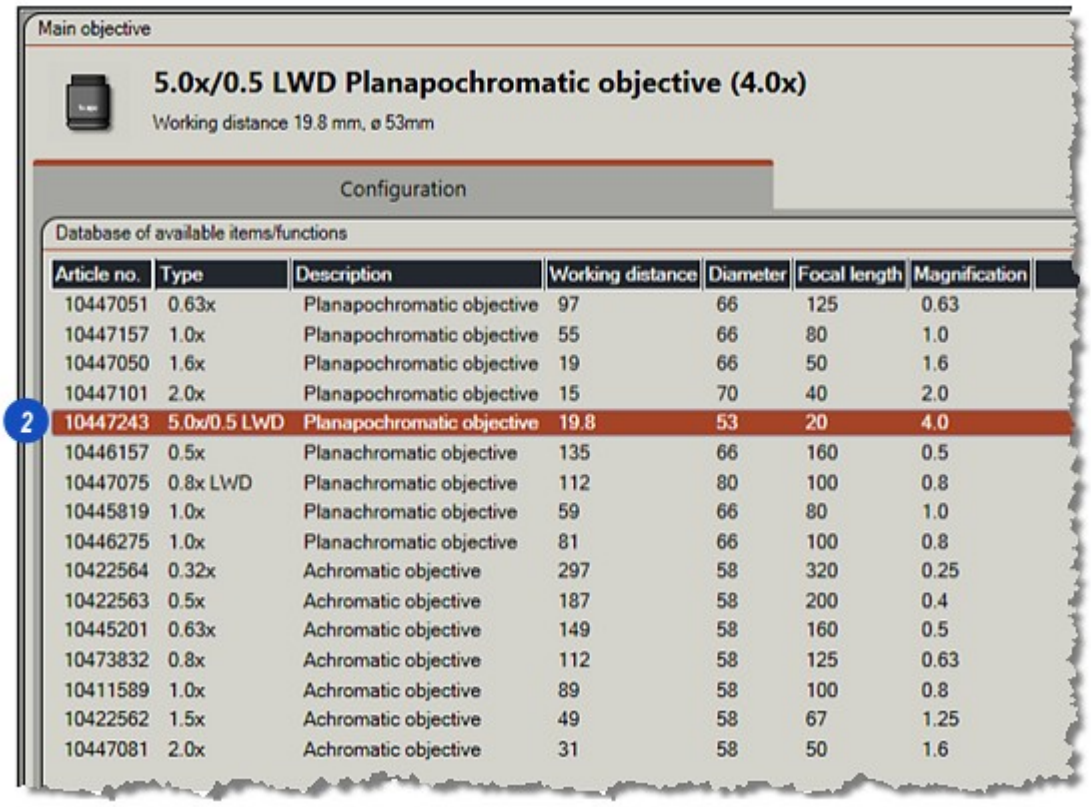
The *Main Objective* has a single list of options that will show either the preferred (checked) or additional options (unchecked):



1: Click on the *Main Objective* icon on the configuration panel.

2: Scroll to the required setting and click to select it.

Continued... 115



Article no.	Type	Description	Working distance	Diameter	Focal length	Magnification
10447051	0.63x	Planapochromatic objective	97	66	125	0.63
10447157	1.0x	Planapochromatic objective	55	66	80	1.0
10447050	1.6x	Planapochromatic objective	19	66	50	1.6
10447101	2.0x	Planapochromatic objective	15	70	40	2.0
10447243	5.0x/0.5 LWD	Planapochromatic objective	19.8	53	20	4.0
10446157	0.5x	Planachromatic objective	135	66	160	0.5
10447075	0.8x LWD	Planachromatic objective	112	80	100	0.8
10445819	1.0x	Planachromatic objective	59	66	80	1.0
10446275	1.0x	Planachromatic objective	81	66	100	0.8
10422564	0.32x	Achromatic objective	297	58	320	0.25
10422563	0.5x	Achromatic objective	187	58	200	0.4
10445201	0.63x	Achromatic objective	149	58	160	0.5
10473832	0.8x	Achromatic objective	112	58	125	0.63
10411589	1.0x	Achromatic objective	89	58	100	0.8
10422562	1.5x	Achromatic objective	49	58	67	1.25
10447081	2.0x	Achromatic objective	31	58	50	1.6

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Leica Application Suite can read settings from the microscope and will detect if a turret is fitted. If there is, then all objectives have to be selected and assigned:

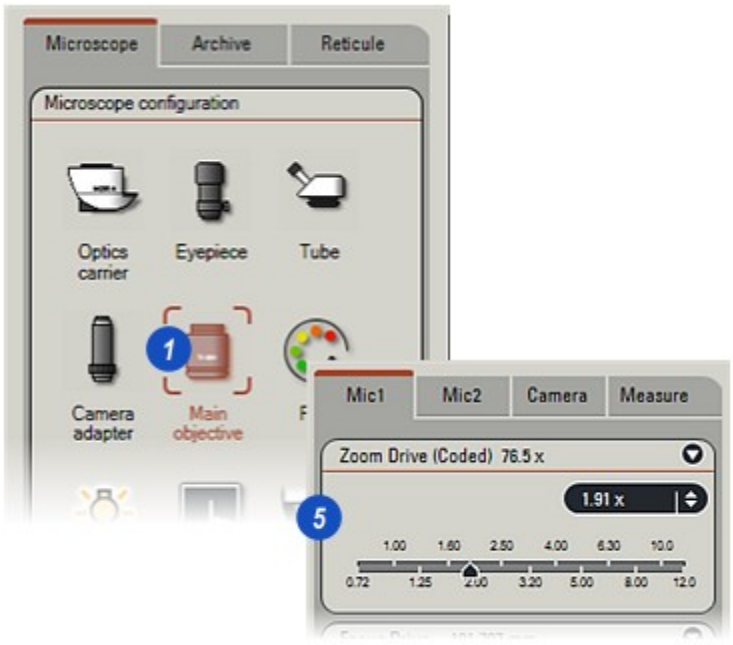
1: Click on the *Main Objective* icon on the configuration panel.

2 & 3: The upper panel lists the objectives to be selected. Click on an entry to select it and...

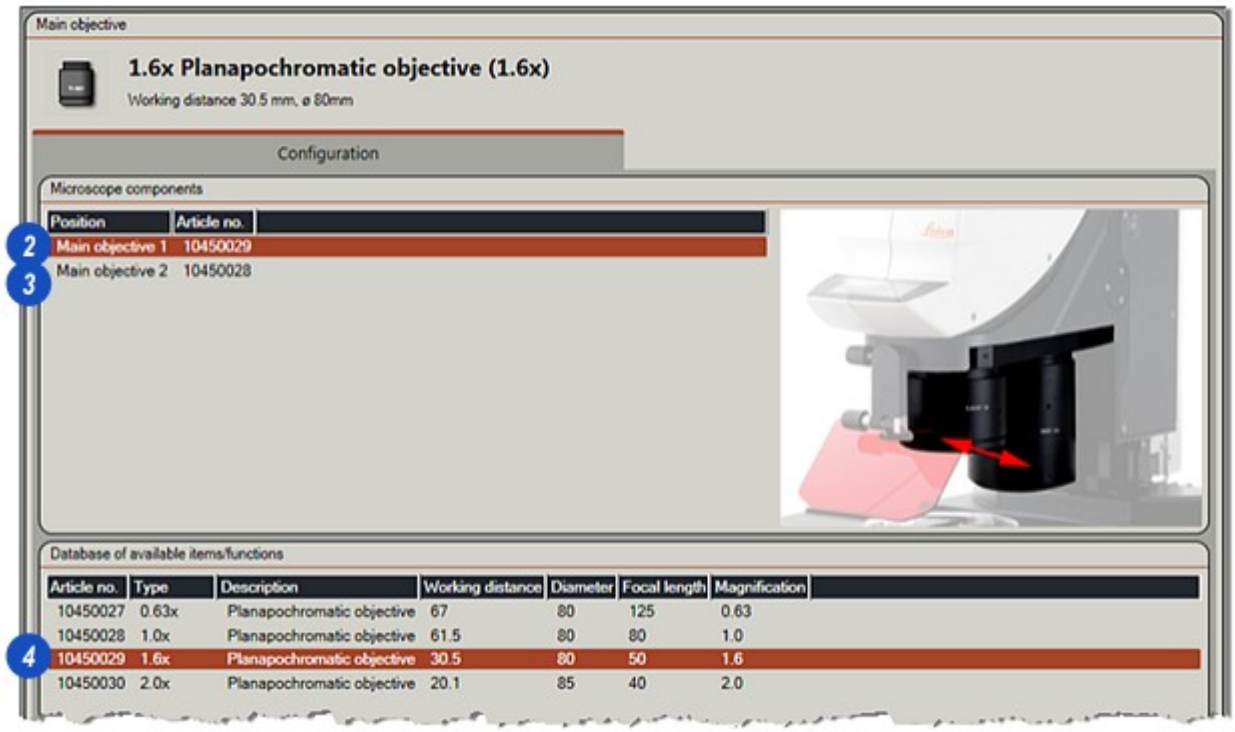
4: ...click to assign the objective type from the lower panel. Repeat for the other objective.

Users with coded objective revolvers will need to manually rotate the revolver to both positions before selecting the assignment.

5: The objective magnification is displayed on the *Zoom* panel in the *Acquire Workflow*.



Continued... 116

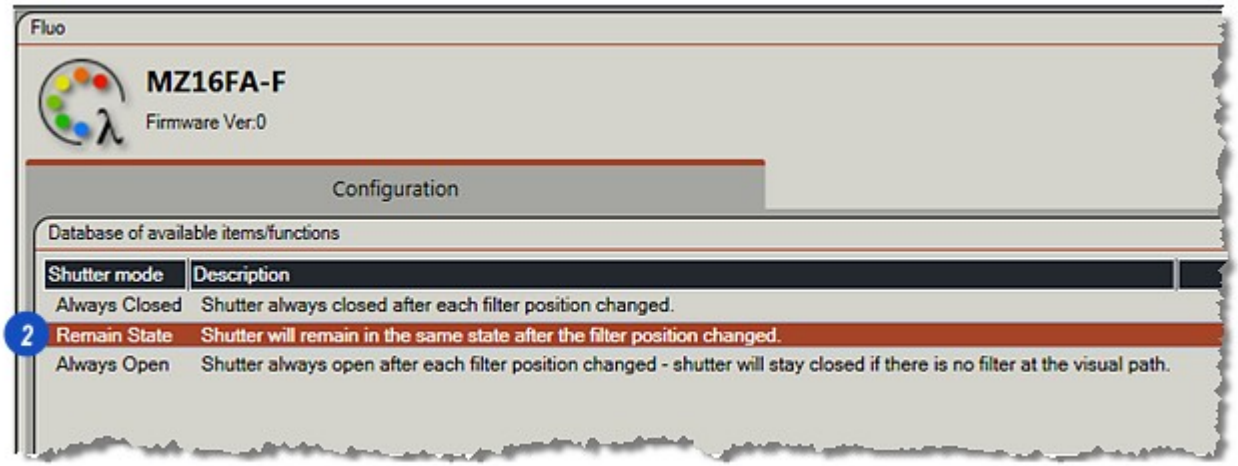


The *Fluo* setting determines how the fluo shutter will behave as filters are changed:

1: Click on the *Fluo* icon on the configuration panel.

2: Click to select the required behaviour.

Continued... 117



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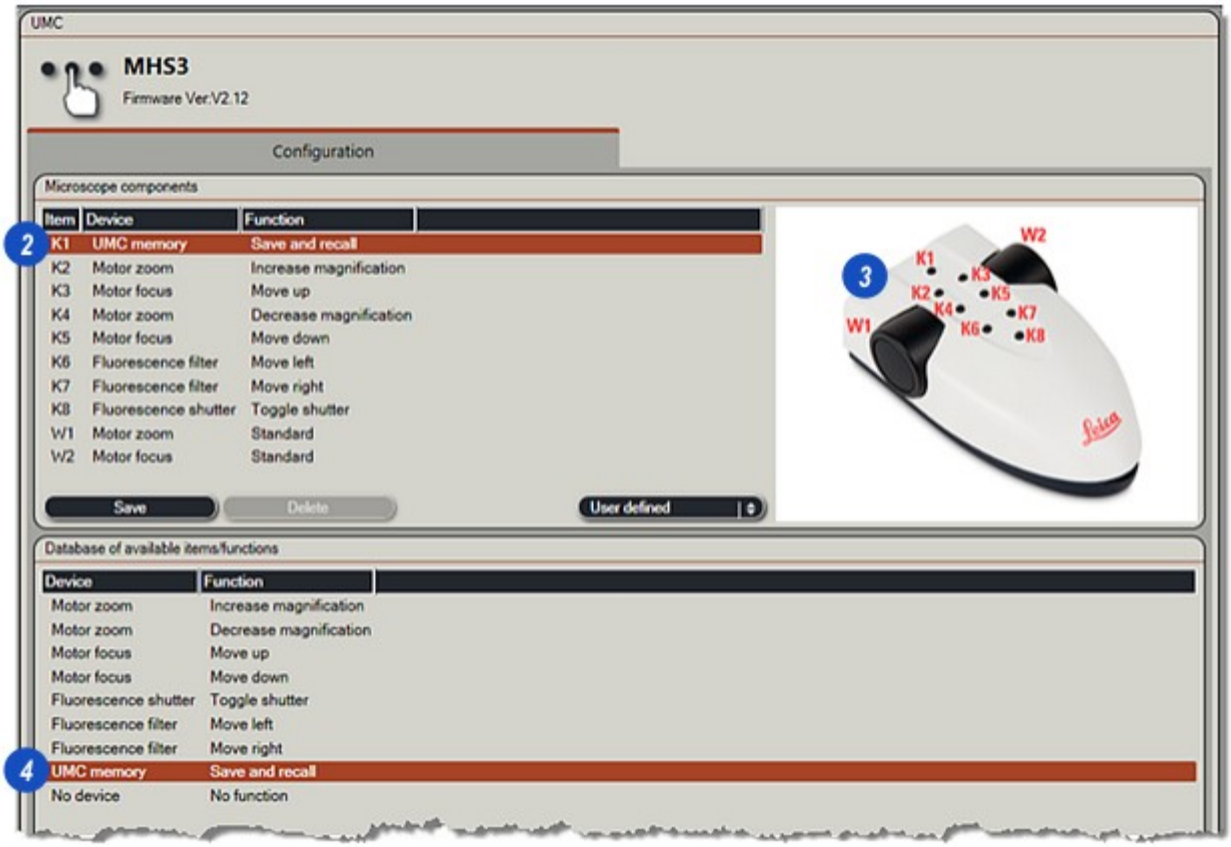
The button and wheel functions on the *Universal Manual Control (UMC)* can be changed to suit individual users and saved as configurations that can be recalled whenever a user operates the microscope.

1: Click on the *UMC* icon on the configuration panel. The icon appears only if a *UMC* is connected.

2: On the upper panel click to select the control - button or wheel - that is to be assigned a function. The image of the *UMC* **(3)** is captioned with the control references. Functions applicable to the selected control are listed on the lower panel.

4: Scroll through and click to select the function to assign to the control.

Continued... 118



To save the *UMC* setup as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header.

2: From the drop-down list, click to select *User Defined.*

3: Click on the *Save* button and the *Save UMC Configuration* dialog appears.

4: Click inside the *UMC Configuration* text box and type a unique name for the new *Configuration*.

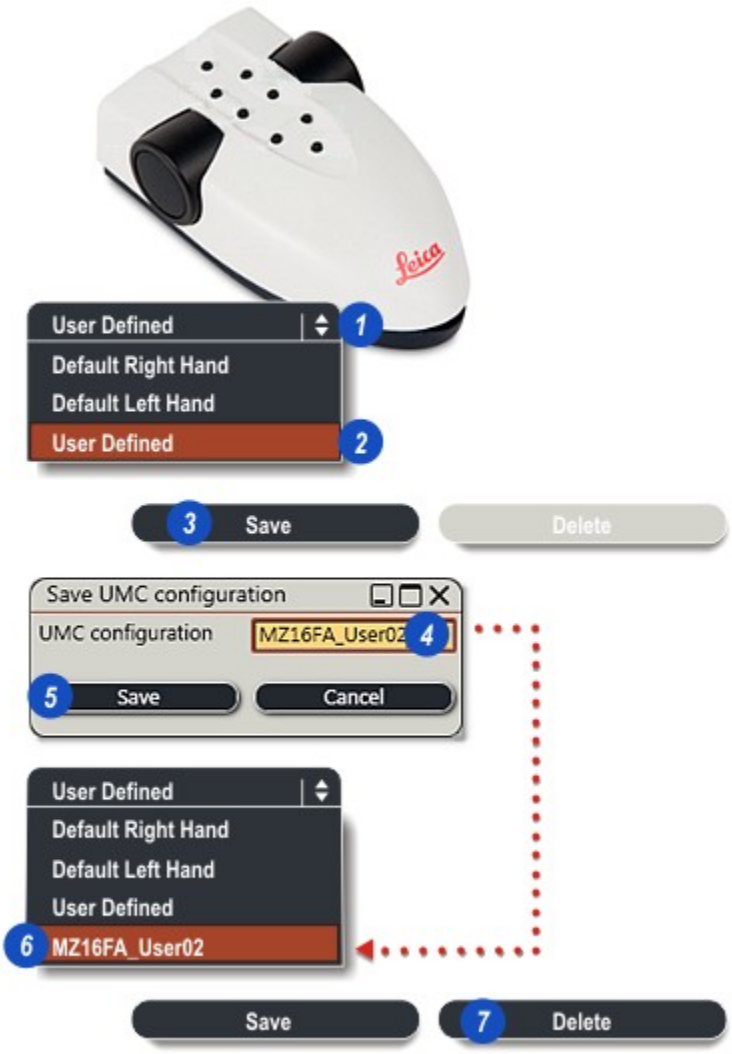
5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

Two *Default* settings - *Right-handed* and *Left-handed* users - that revert to the factory settings, can be loaded by clicking on the entry on the drop-down list.

Continued... 119



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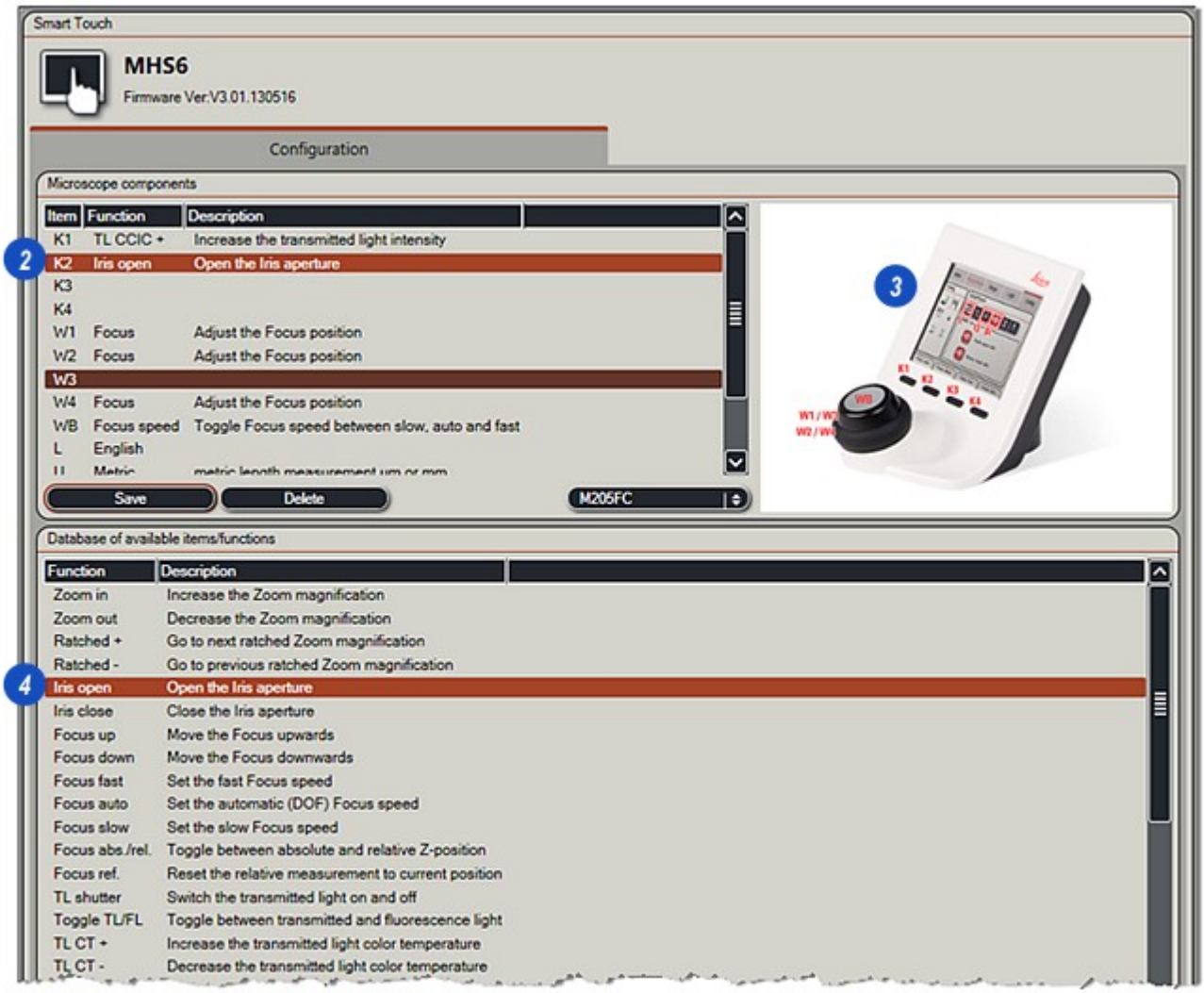
The *Hardware Keys, Double Rotary Actuator, Toggle Button* and *Touch Screen* functions on the *SmartTouch* control can be changed to suit individual users and saved as configurations that can be recalled whenever a user operates the microscope.

1: Click on the *SmartTouch* icon on the configuration panel. The icon appears only if a *SmartTouch* is connected.

2: On the upper panel click to select the control - *Hardware Key, Rotary Actuator, Toggle Button* or *Touch Screen* icon - that is to be assigned a function. The image of a *SmartTouch* **(3)** is captioned with the control references. Functions applicable to the selected control are listed on the lower panel.

4: Scroll through and click to select the function to assign to the control.

Continued... 120



To save the *SmartTouch* setup as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header.

2: From the drop-down list, click to select *User Defined.*

3: Click on the *Save* button and the *Save Smart Touch Configuration* dialog appears.

4: Click inside the *Smart Touch* text box and type a unique name for the new *Configuration*.

5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

Clicking to select the *Default* option from the drop-down list, will revert to the factory settings.

Continued... 121

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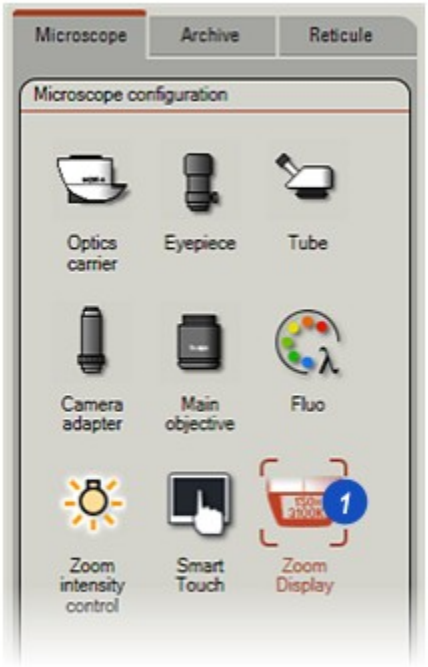
The items displayed in the Zoom Display window on the front of the microscope, can be changed to suit the user and then saved as a configuration that can be recalled whenever the user operates the microscope.

1: Click on the *Zoom Display* icon on the configuration panel.

2: On the upper panel click to select the display element that is to be assigned a feature. The image of the *Zoom Display* window **(3)** is captioned with the element references.

4: Scroll through the list in the lower panel and click to select the feature to assign to the element.

Continued... 122



To save the *Zoom Display* settings as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header.

2: From the drop-down list, click to select *User Defined.*

3: Click on the *Save* button and the *Save Zoom Display Configuration* dialog appears.

4: Click inside the *Zoom Display* text box and type a unique name for the new *Configuration*.

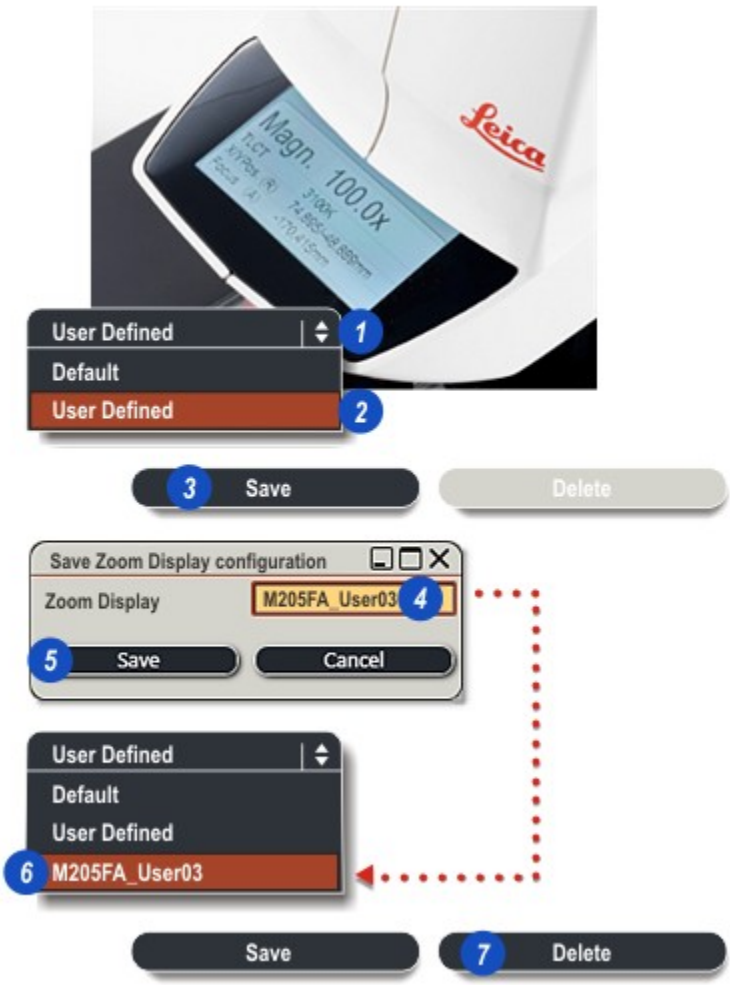
5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

Clicking to select the *Default* option from the drop-down list, will revert to the factory settings for the *Zoom Display*.

Continued...



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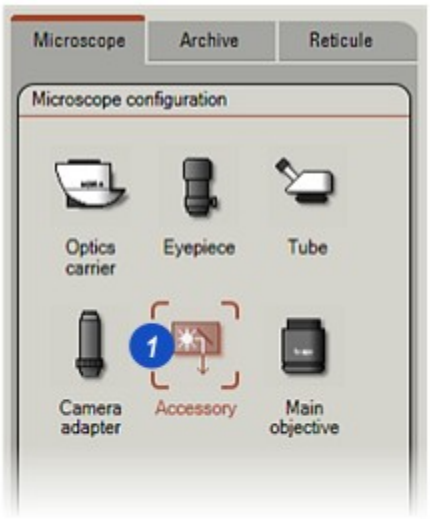
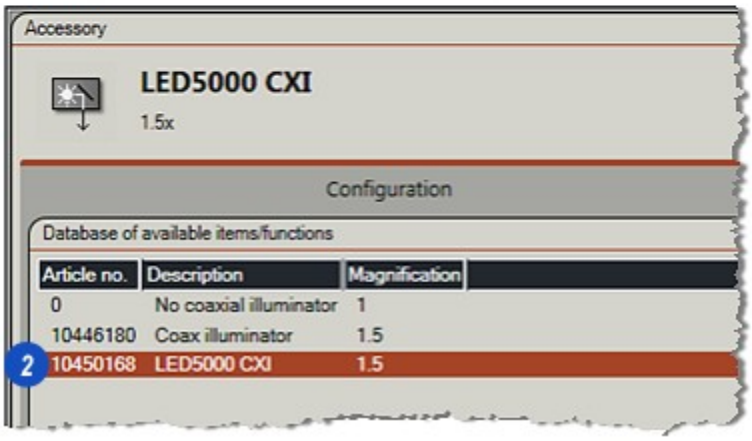
Accessories available for the current microscope are shown in a single list. When an accessory is fitted:

1: Click on the *Accessory* icon on the configuration panel. The icon is always available whether an accessory is fitted or not.

2: From the list, click to select the fitted accessory and enable its function and features.

Continued... 124





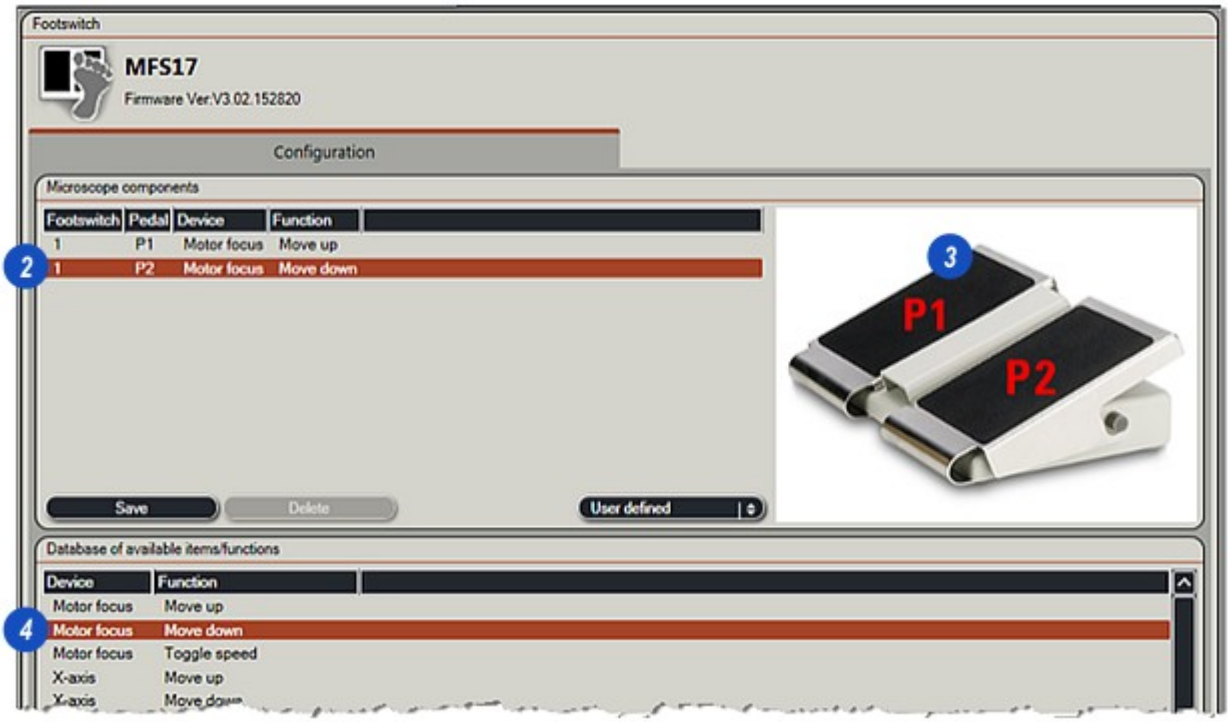
The *Footswitch* actions can be configured to suit the user and then saved as a configuration that can be recalled whenever the user operates the microscope.

1: Click on the *Footswitch* icon on the configuration panel.

2: On the upper panel click to select the pedal that is to be assigned a feature. The image in the *Footswitch* window **(3)** is captioned with the pedal 1 *(P1)* and pedal 2 *(P2).*

4: Scroll through the list in the lower panel and click to select the feature to assign to the selected pedal.

Continued... 125



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To save the *Footswitch* setup as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header and...

2: ...click to select *User Defined.*

3: Click on the *Save* button and the *Save Footswitch Configuration* dialog appears.

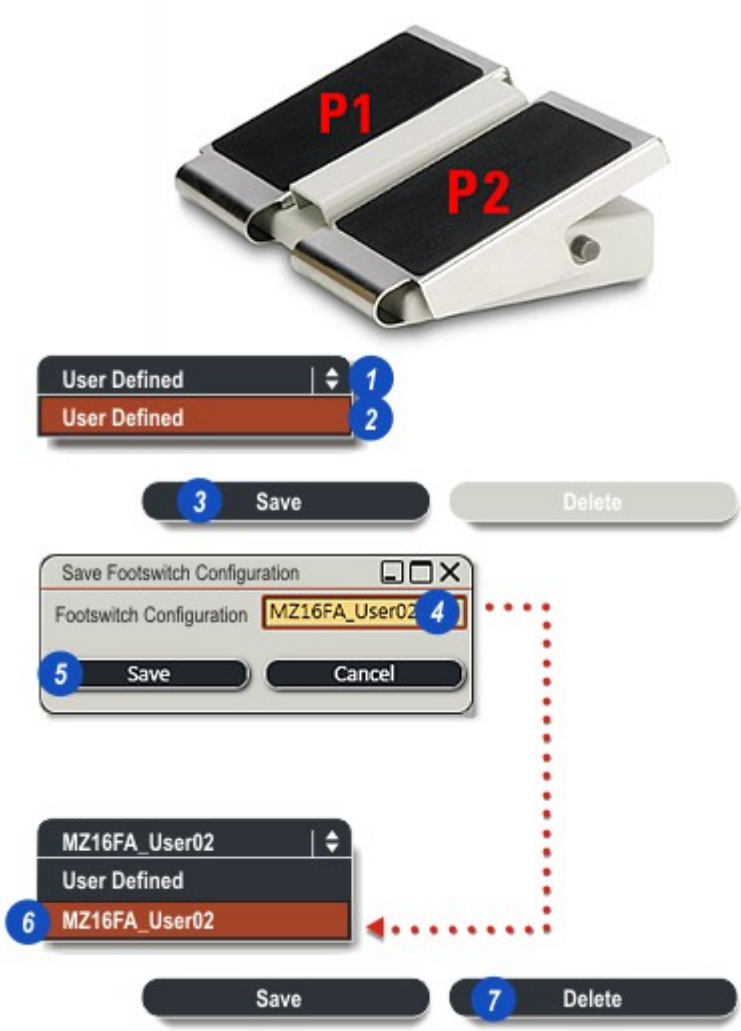
4: Click inside the *Footswitch Configuration* text box and type a unique name for the new *Configuration*.

5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

Continued... 126



The *Transmitted Light Reflectivity Control* (TLRC) can be enabled and disabled on the *Configuration* tab of the *TL CCIC* panel:

1: Click on the *Zoom Intensity Contro*l icon on the *Configuration* panel.

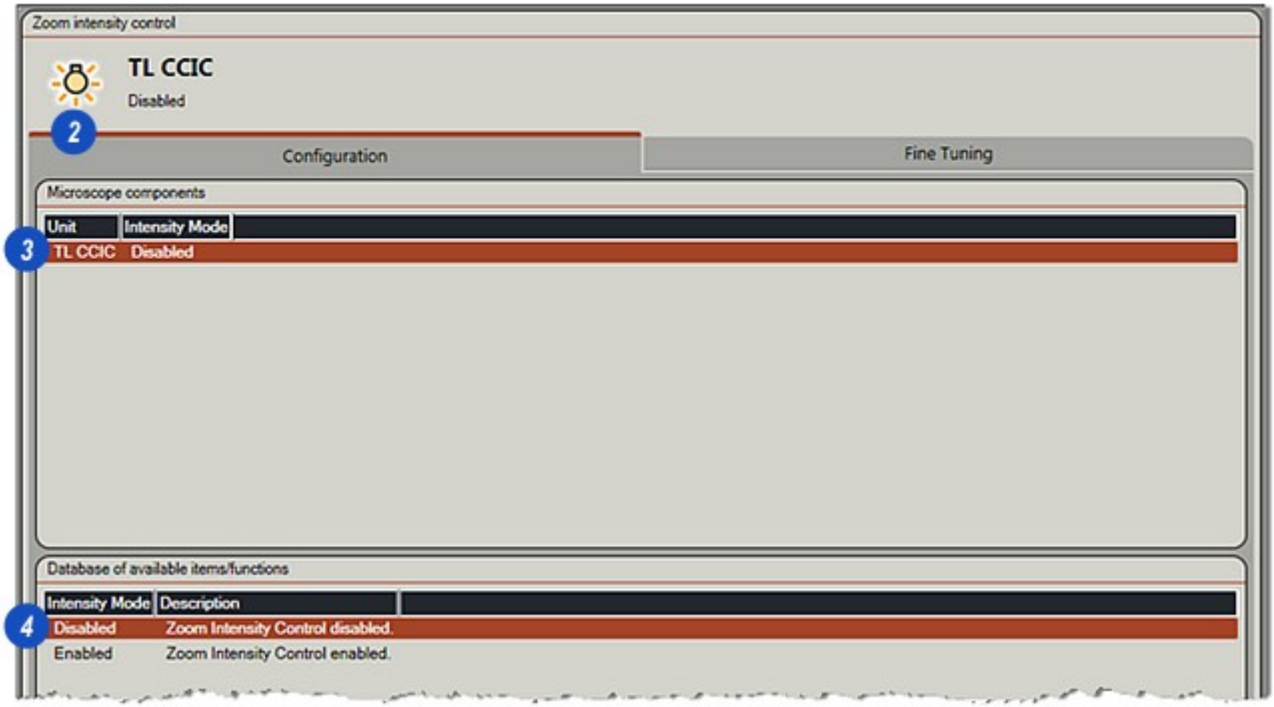
2: On the *TL CCIC* panel *Configuration* tab...

3: ...click the *TL CCIC* entry.

4: Toggle between enabled and disabled by clicking on the required option on the Database panel.

5: If the control has not been calibrated, the warning appears.

Continued... 127



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1: Click on the *Fine Tuning* tab.

2: Follow the list of setup instructions on screen.

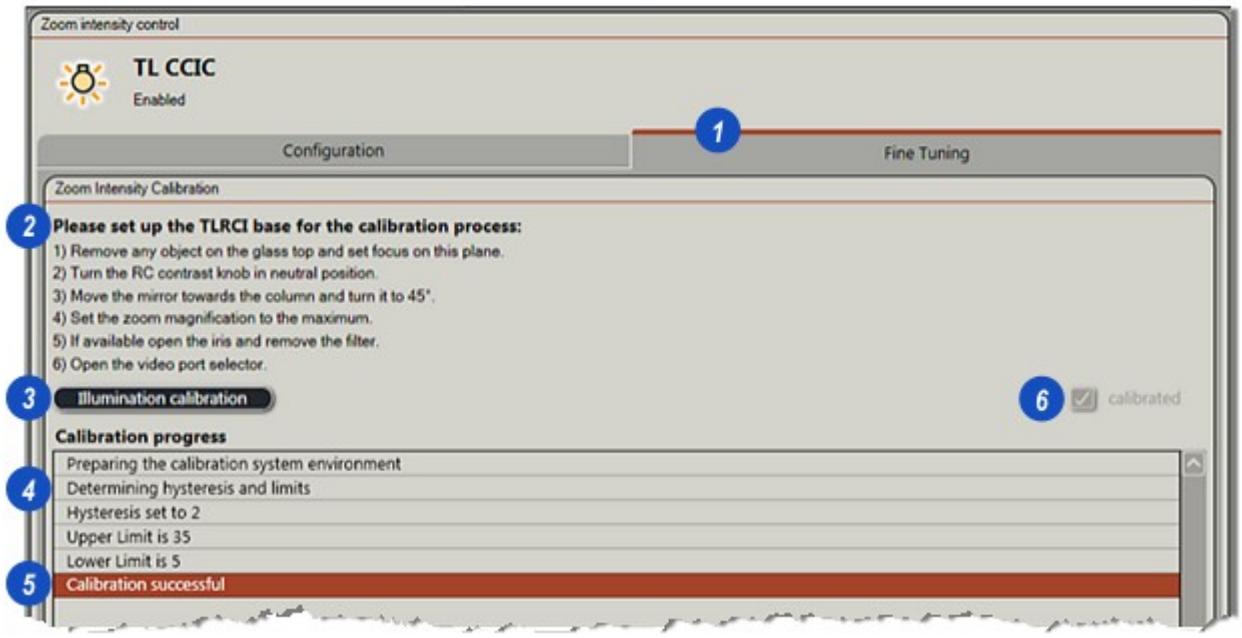
3: Click the *Illumination Calibration* button.

4: The calibration process begins with the results from each step displayed...

5: ...until the *Calibration successful* message appears...

6: ...and the *Calibrated* check box is enabled.

Return to the previous page (*Go there...* 126) to enable the TL CCIC.



DM Microscope Setup

The DM Series Microscope Setup has been arranged so that configuring the microscope is both fast and simple.

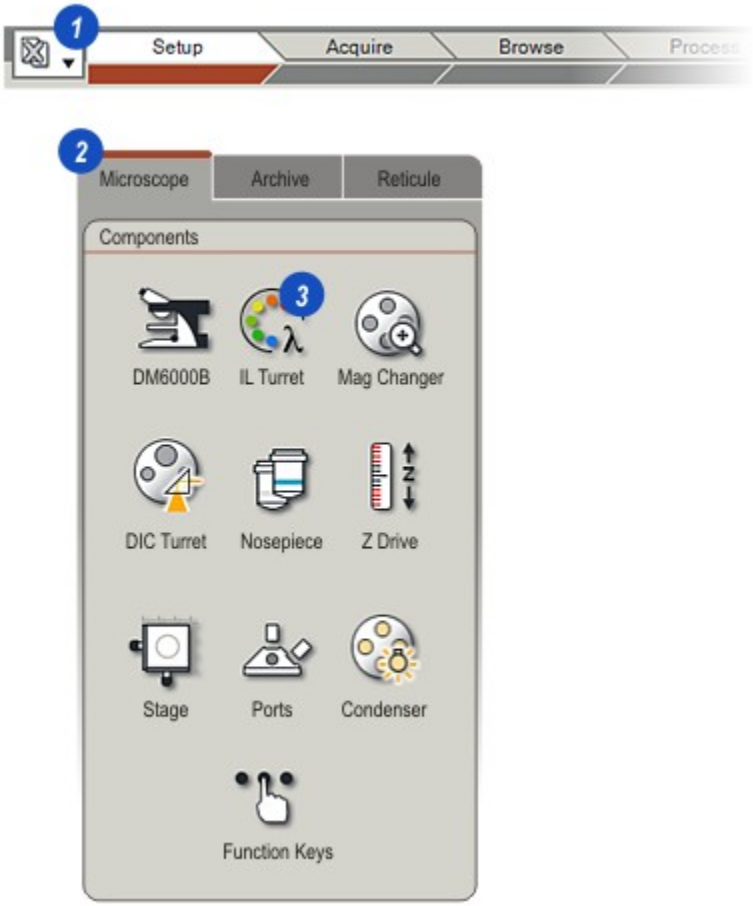
Each setup feature is displayed on the Components panel - clicking an icon will take the user directly to the appropriate setup dialogs. The feature icons displayed will depend upon the microscope model and fitted accessories.

Generally, all of the setup options for each feature are listed in a database and all that is required is a double-click on the entry to assign it to the selected feature.

1: Click on the Setup Workflow.

2: Click on the Microscope tab.

3: Click on a feature icon as described on the following pages.



Continued... 129

C-mount selection... 148

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Many of the dialogs have features designed to help user navigation. For example:

1: Click on the border around either the top or bottom panels and drag it up or down to re-size it. This can be used to display more Database items.

2: To change the width of the Database columns and so reveal more of the item descriptions, click on the vertical bar between two columns and drag to the left or right.

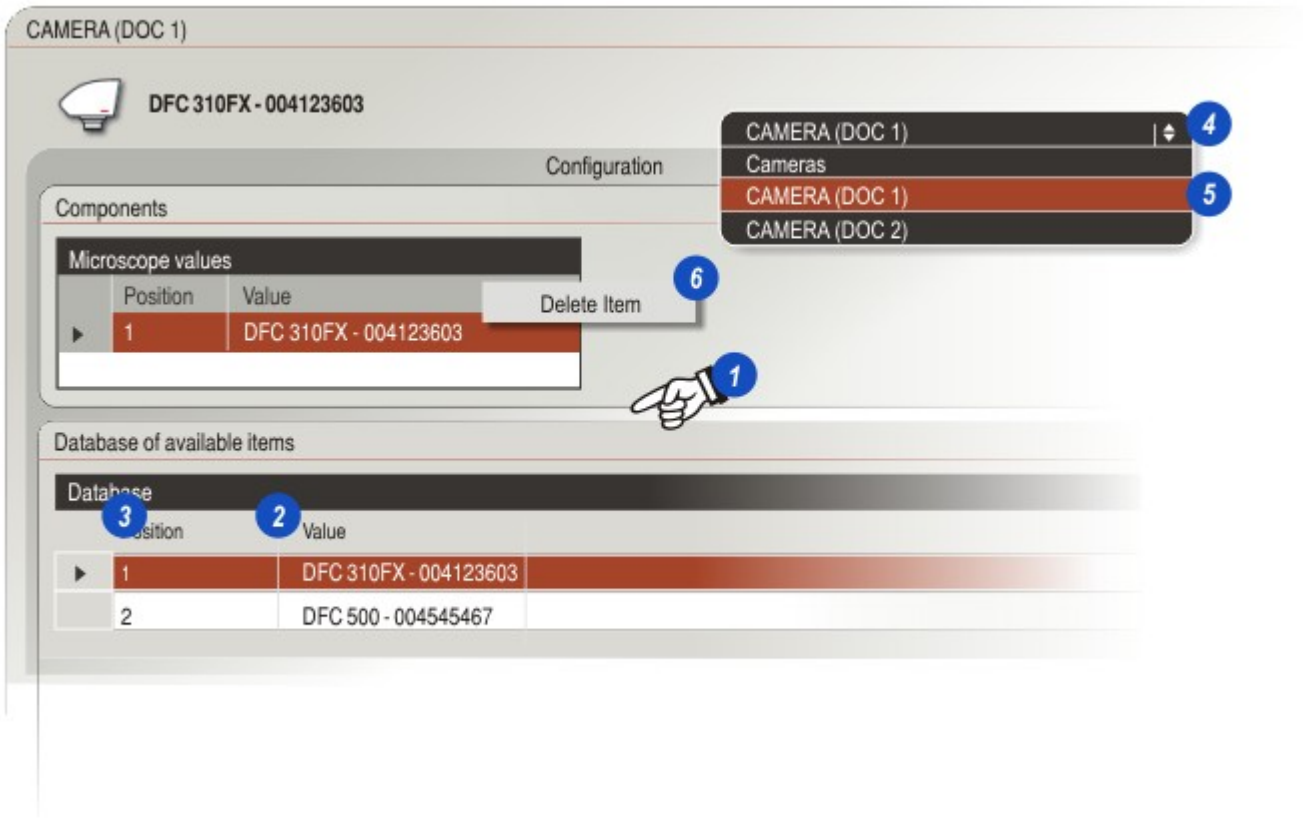
3: By default the Database items are listed in ascending order - lowest numeric value at the top of the list. Change to descending order by double-clicking on the column header.

4: Some dialogs have drop-down menus and, depending on the selection the Database listings will change. To reveal the drop-down options click on the small arrows to the right of the menu header and...

5: ...click to select the required option.

6: To remove a selected Database item from the Microscope Values list, right-click on the item and left-click on the pop-up Delete Item button.

Continued... 130



To view the microscope data:

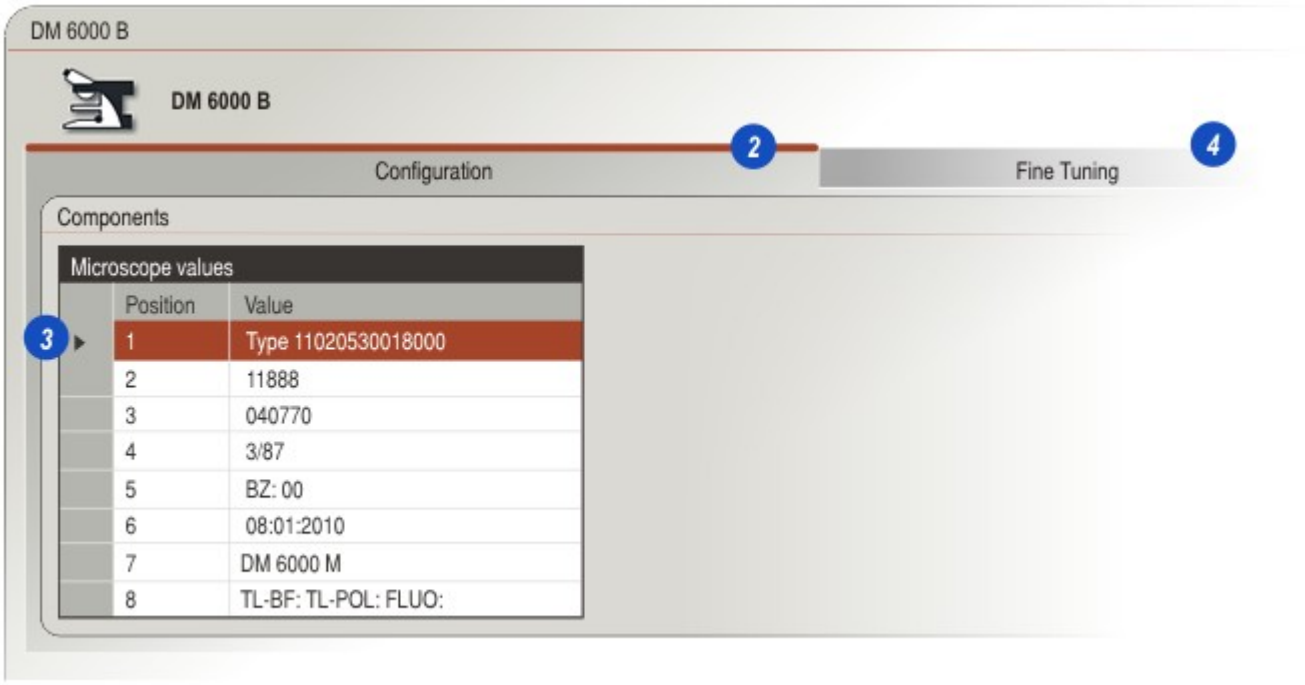
1: Click on the microscope icon on the Microscope > Components panel. The microscope stand type is retrieved from the Hardware Configuration and displayed beneath the icon.

2: Click on the Configuration tab and...

3: ...the data is displayed.

4: Click on the Fine Tuning tab to display all of the setup features in a single list. The setup can be achieved from here or by going to the required section:

- IL Turret Selection: Go there... 131.
- Mag Changer Setup: Go there... 132
- DIC Turret Setup: Go there... 133
- Nosepiece Setup and Fine Tuning: Go there... 134
- Z-Drive Setup: Go there... 152
- Stage Selection & Initialisation: Go there... 150
- Port Selection: Go there... 146
- Camera Selection: Go there.. 147. 147
- Condenser Selection: Go there... 154
- Function Key Assignment: Go there... 155



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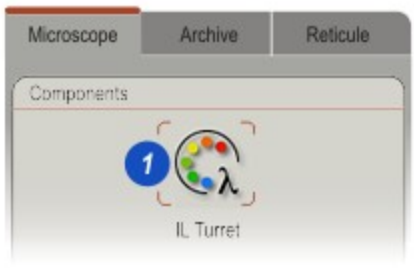
To set up the IL Turret Filter Cubes:

1: Click on the IL Turret icon on the Microscope > Components panel.



2: The IL Turret dialog appears with the number of turret positions indicated as dark circles on the graphic.

3: Click on the turret position to select it.

4: Double-click on the required database item to assign it to the turret position.

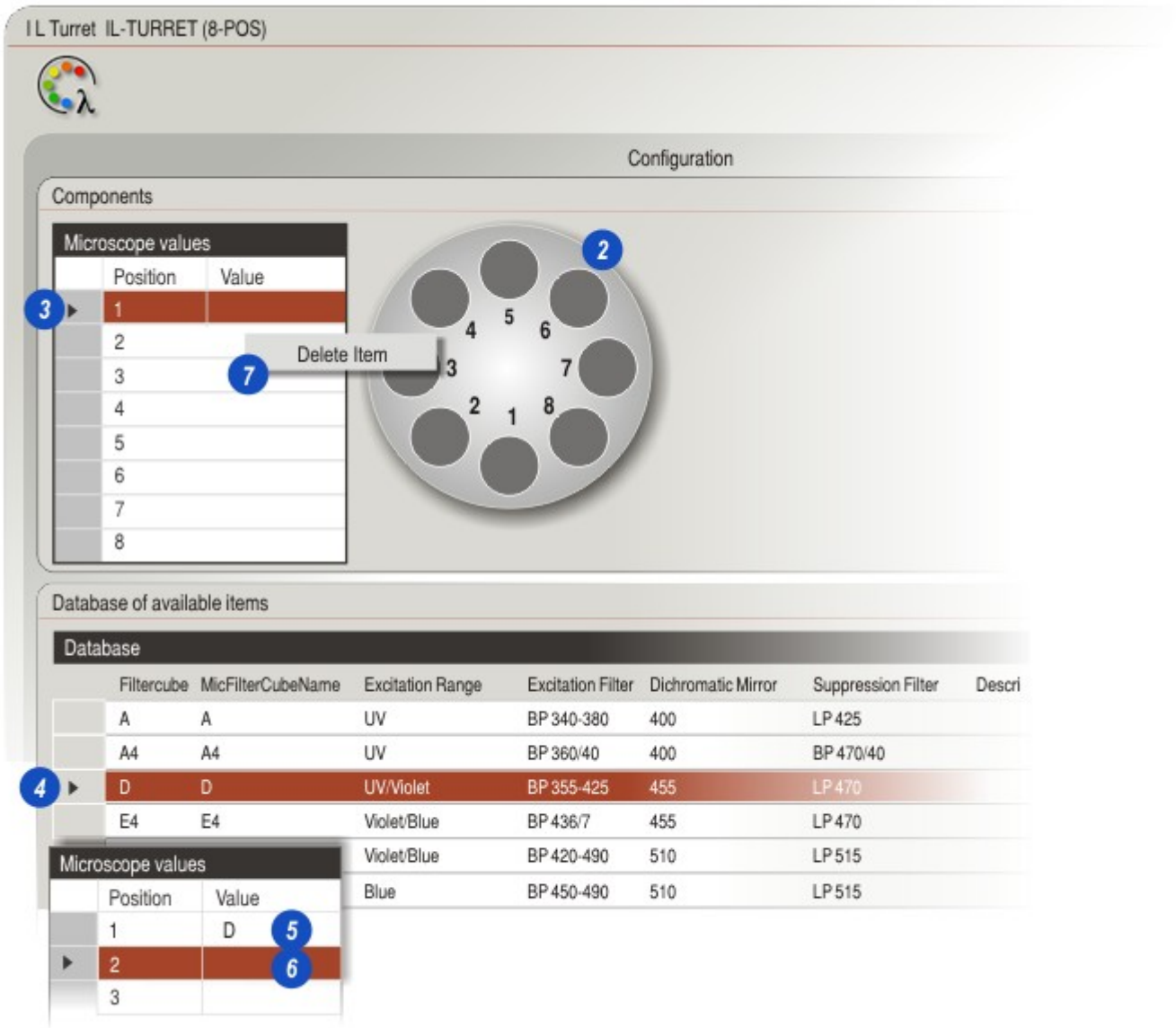
5: The database item details are displayed in the turret position and...

6: ...if there is another position to be filled, it is automatically selected.

7: To remove an item from the turret list, right-click the entry and left-click the Delete Item pop-up button.

NB: At least one position of the fluo turret has to be learned in as “EMPTY” to perform bright field contrast (BF) in transmitted light axis. EMP-BF has to be selected in case the stand is capable of a fully motorized IC contrast in transmitted light axis. If the microscope is capable of a partial manual IC contrast the EMP-DIC has to be selected.

Alternatively in case an A cube is used: select A-TL to perform contrast methods of the transmitted light axis with this fluo cube.



To set up the Mag Changer:

1: Click on the Magnification Changer icon on the Microscope > Components panel.

2: The Mag Changer dialog appears. There is a single position value that assigns the Mag Changer component.

3: Click on the position to select it.

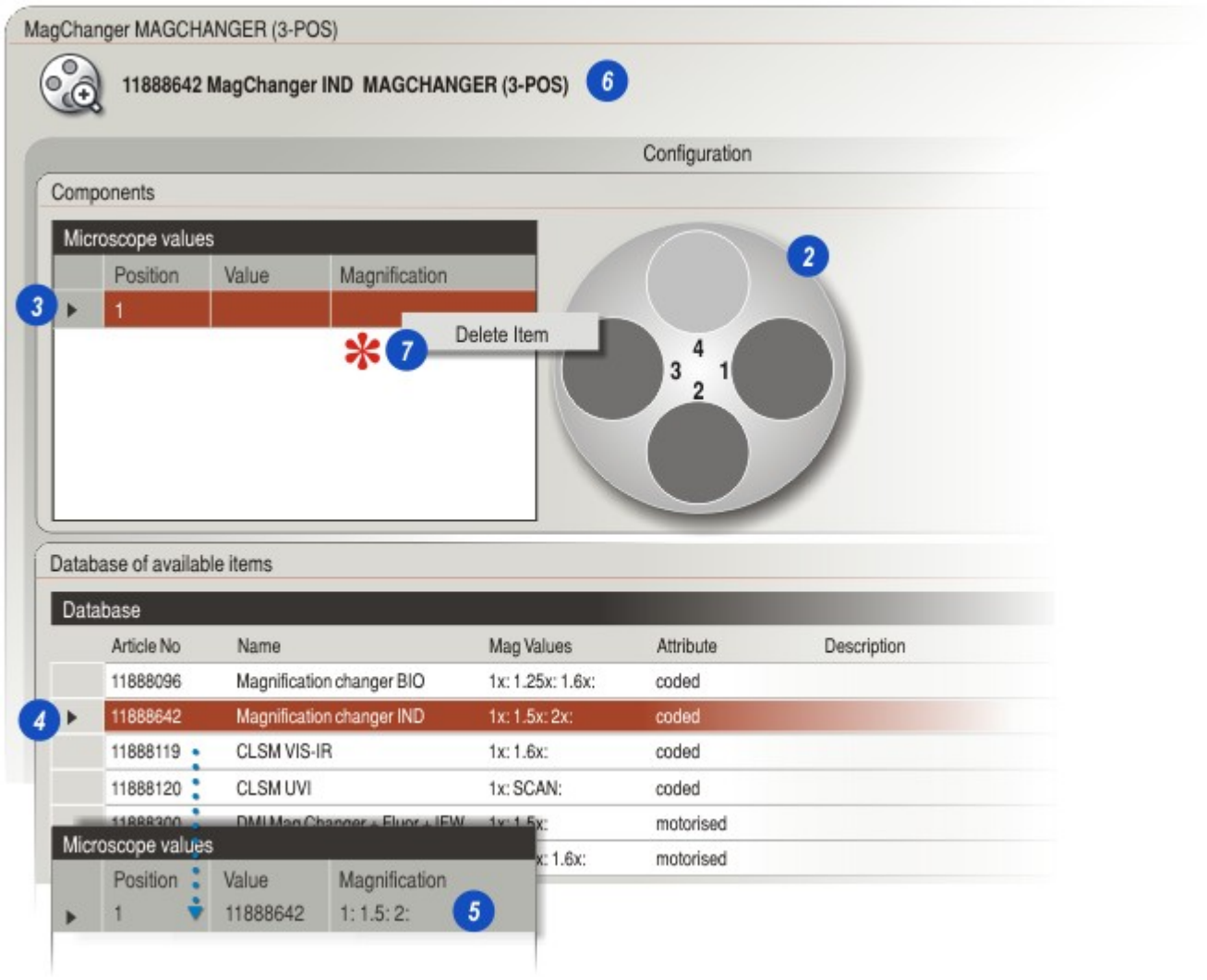
4: Double-click on the required database item to assign it to the Mag Changer.

5: The database item details are displayed in the changer position and...

6: ... on the header.

No Mag Changer Setup: *

7: If a No Mag Changer setting is needed, right-click the position entry and left-click the Delete Item pop-up button. The Microscope Value will appear as 1,1,1 which indicates No Changer.



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To set up the DIC (Differential Interference Contrast) Turret components:

1: Click on the DIC Turret icon on the Microscope > Components panel.

2: The DIC Turret dialog appears with the number of positions indicated as dark circles on the graphic.

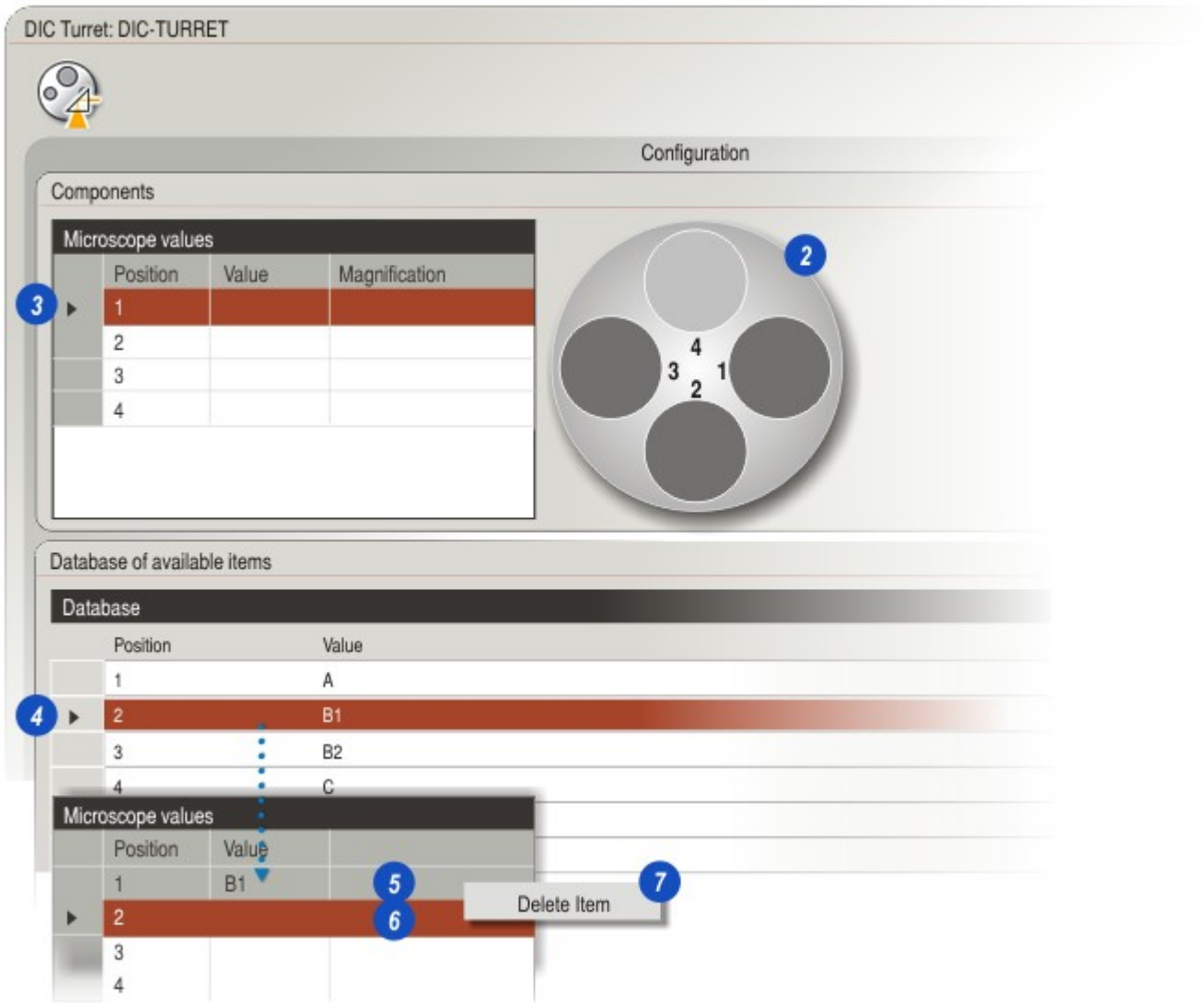
3: Click on the turret position to select it.

4: Double-click on the required database item to assign it to the turret position.

5: The database item details are displayed in the turret position and...

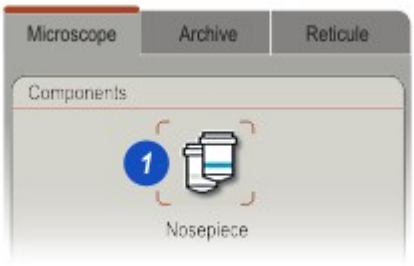
6: ...if there is another position to be filled, it is automatically selected.

7: To remove an item from the turret list, right-click the entry and left-click the Delete Item pop-up button.



Setting up the Nosepiece is a two-step process:

- Select and assign an Objective to the Nosepiece positions.
- Fine-tune the Objective setup.

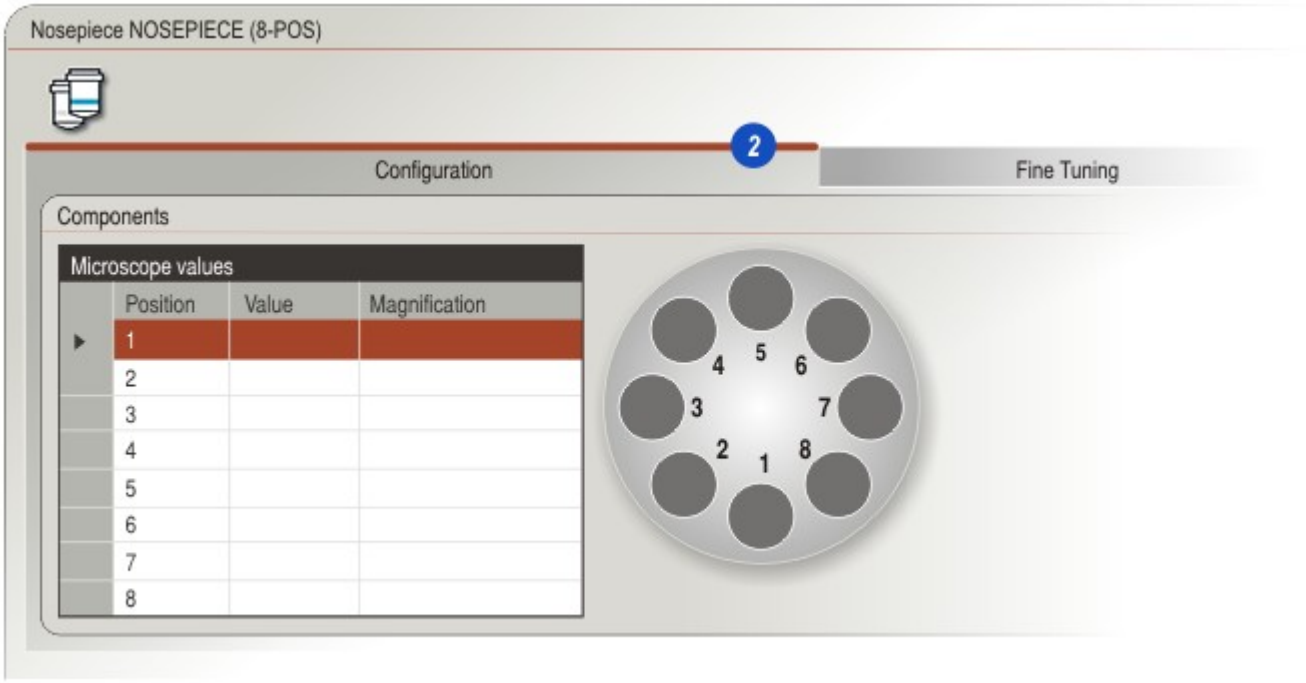


To assign an Objective to a Nosepiece position:

1: Click on the Nosepiece icon on the Control Panel.

2: Click on the Configuration tab on the Nosepiece dialog.

Continued... 135



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1: Click on the Nosepiece position.

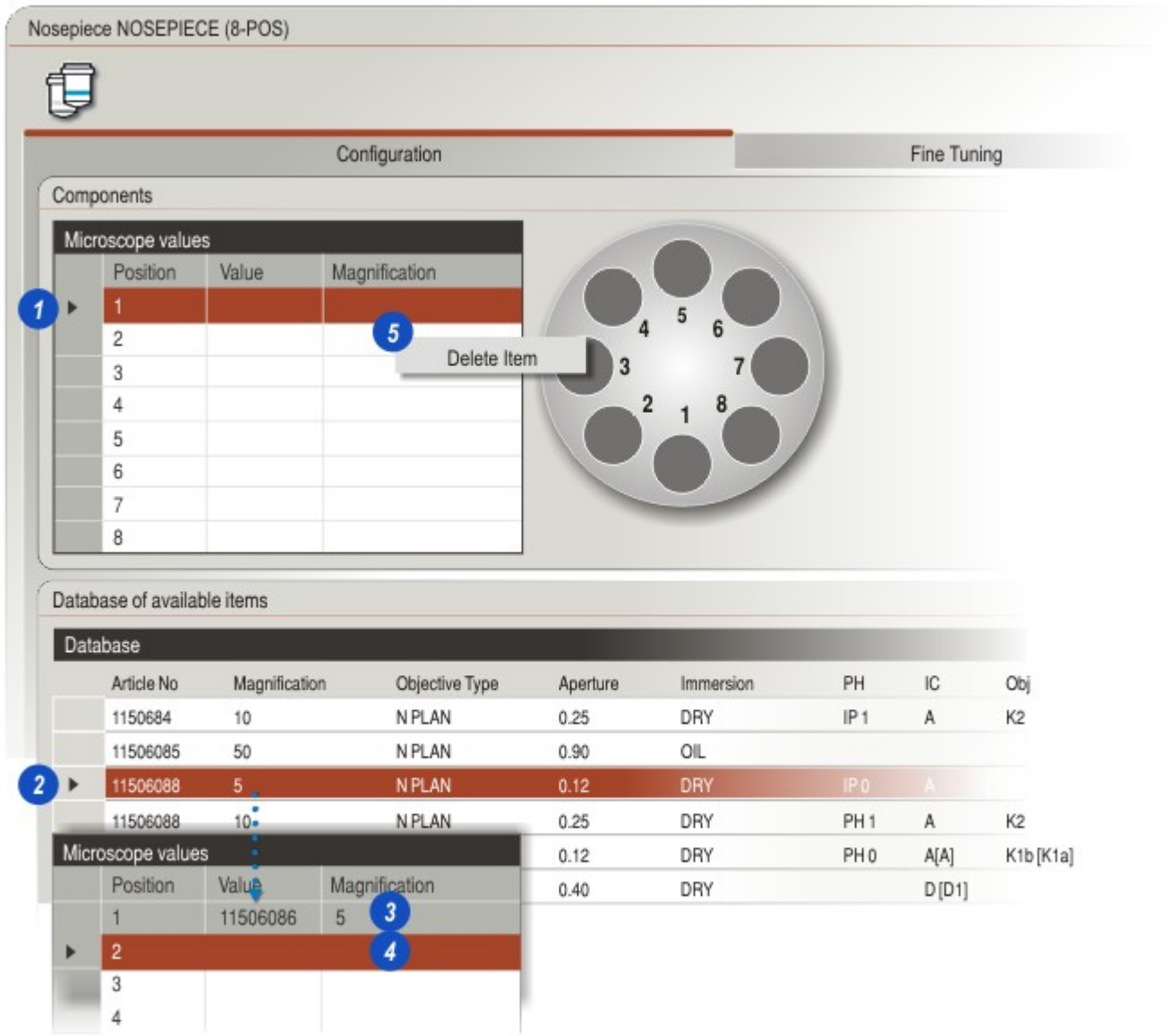
2: In the database, double-click on the required Objective.

3: The Objective details are displayed in the Nosepiece position and...

4: ...if another position remains to be assigned, it is automatically selected. Repeat Step **(3)**.

5: To remove an Objective from a position, right-click on the Nosepiece position and left-click on the Delete Item pop-up button.

Continued... 136

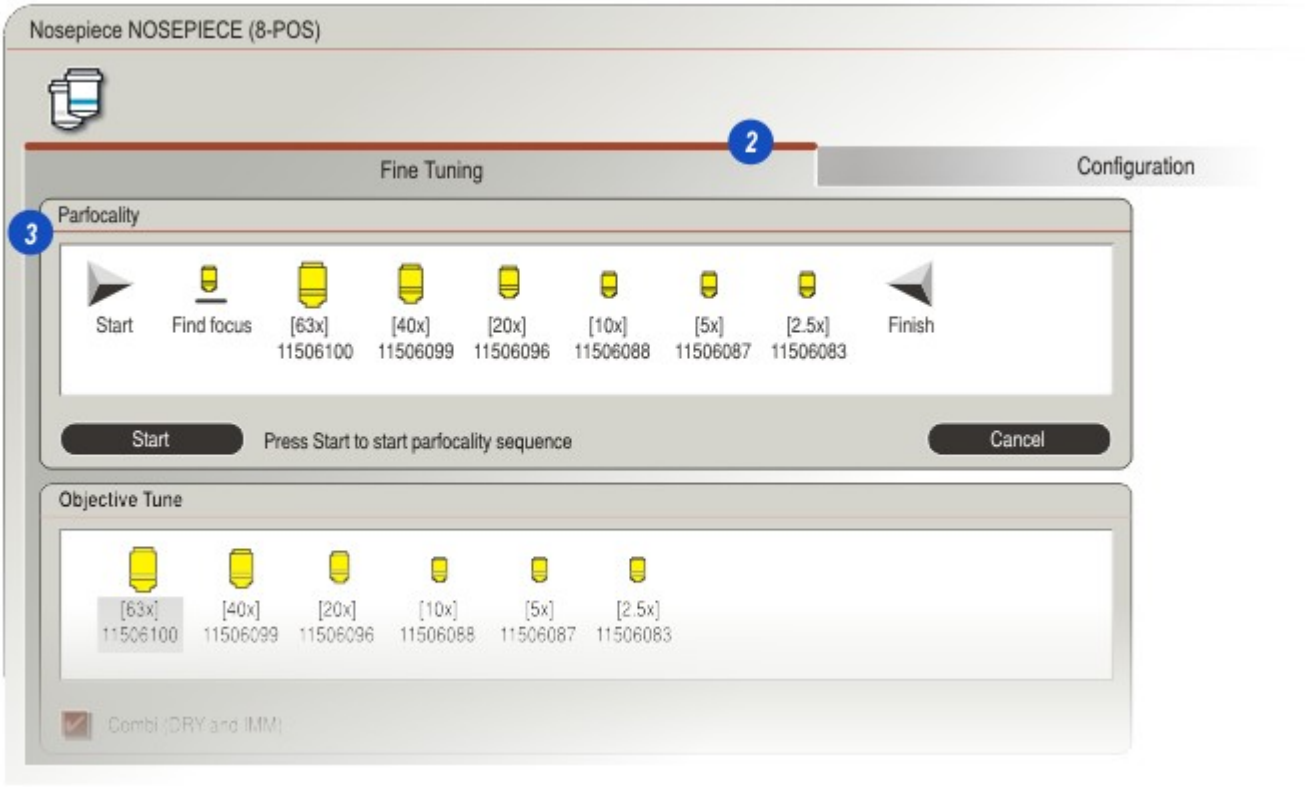


1: Click on the Nosepiece icon on the Control panel.

2: Click on the Fine Tuning tab.

3: There are individual dialogs for each of the Objective fine-tuning features:

- Parfocality: Go there... 137
- Objective Combination tag: Go there... 139
- Stage and Z-Stepsize: Go there... 140
- Auto Stage posititon: Go there... 141
- Parcentricity enable: Go there... 144
- Parcentricity adjustment: Go there... 143
- Nosepiece rotation: Go there.. 145



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Even with closely matched objective sets, Parfocality - maintaining sharp focus when an objective is changed - may need to be fine-tuned. A Leica motorised focus axis must be fitted.

The process involves initially focussing on a specimen using the objective with the greatest magnification, and then checking each of the remaining objectives in turn and, where necessary adjusting the focus to maintain a clear, sharp image.

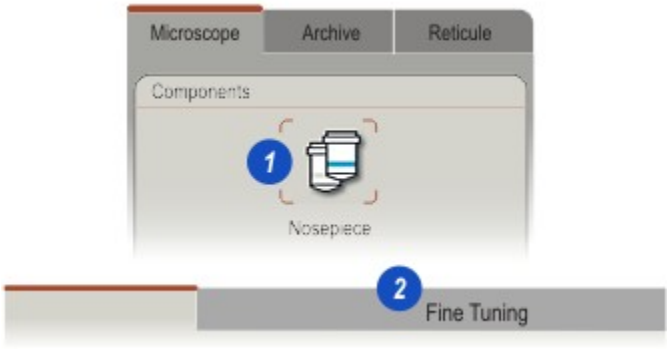
A focus factor is created for each objective, stored and retrieved when the objective is selected to drive the stage to the precise position to maintain focus.

There are two options for setting the Parfocality:

- From this setup dialog with focus being checked through the eyepieces - the monitor Viewer is not available here - and...
- From the Acquire Workflow with the specimen displayed on the Viewer.

For both options the dialogs are the same but are accessed differently:

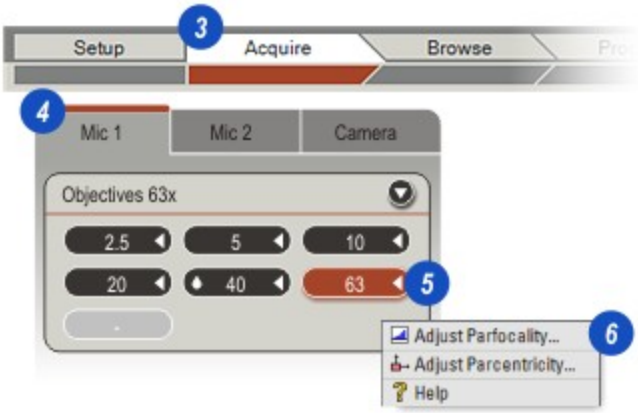
Set Parfocality from DM Setup (Here):



1: Click on the Nosepiece icon on the Microscope > Component panel.

2: Click on the Fine Tuning tab and then click to select the Parfocality dialog. Continued... 138

Set Parfocality from Acquire Workflow:



3: Click on the Acquire Workflow and then...

4: ...on the Mic 1 tab.

5: Right-click on one of the objective select buttons and...

6: ...left click to select Adjust Parfocality on the pop-up menu. Continued... 138

1: Click on the Parfocality dialog and...

2: ... the Start button. The Find Focus option is selected. Navigate the stage to and adjust for the sharpest focus on the specimen.

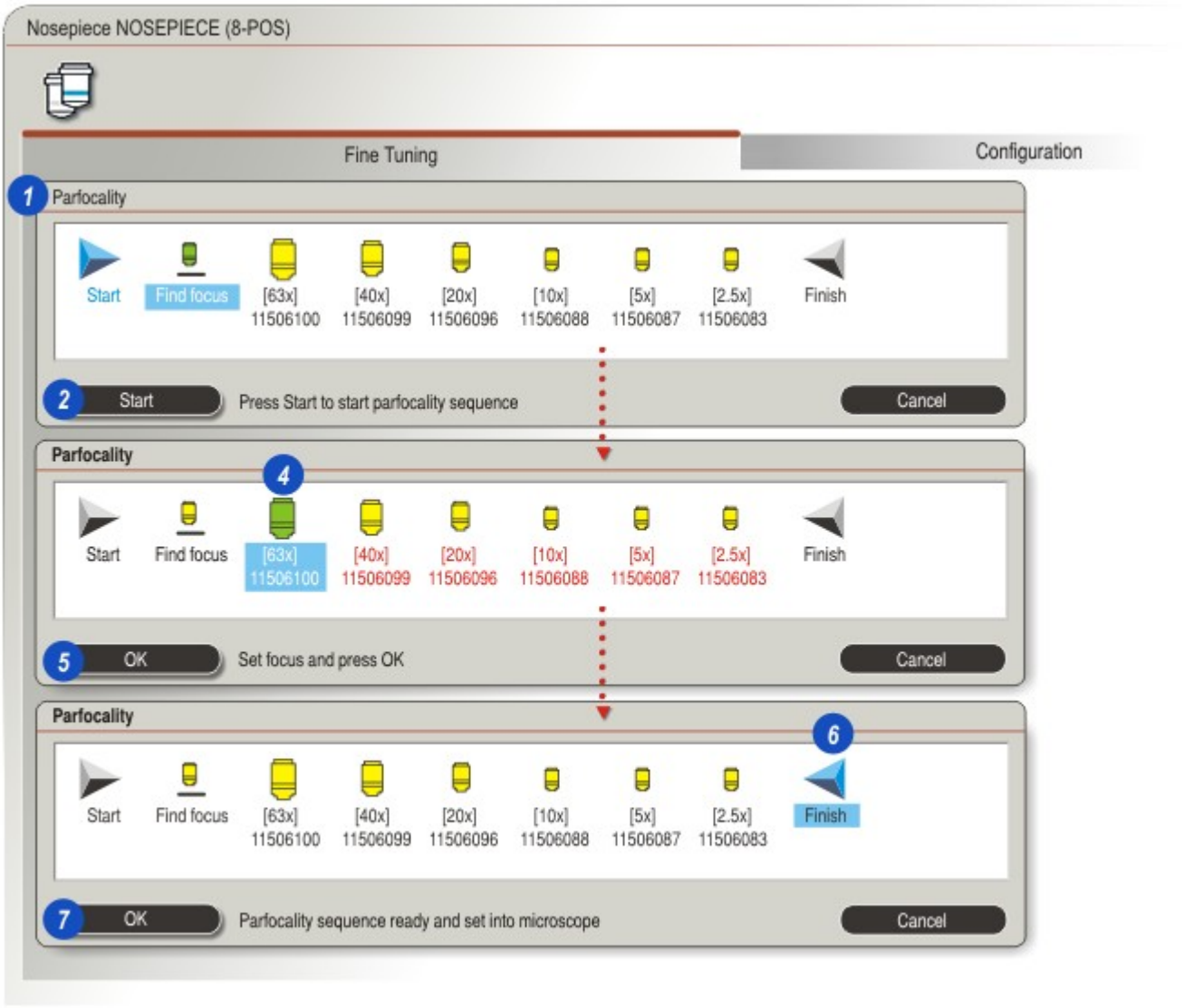
3: Click OK.

4: The objective with the greatest magnification is automatically chosen first. Focus the specimen and...

5: ...click OK. The sequence will advance to the next objective.

6: When all objectives have been fine-tuned the Finish icon is highlighted.

7: Click OK.



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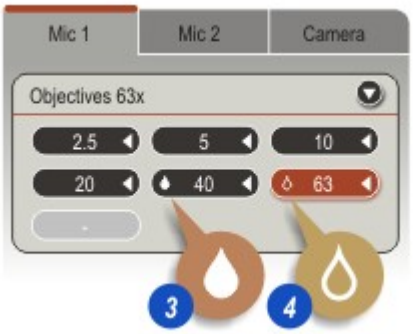
Some objectives can be used both in air and also immersed - in water or oil. These so-called Combi (Combination) objectives can be tagged so that when they are selected the user is given a warning that immersion can be an option.

1: Click on the Objective Icon to select it.

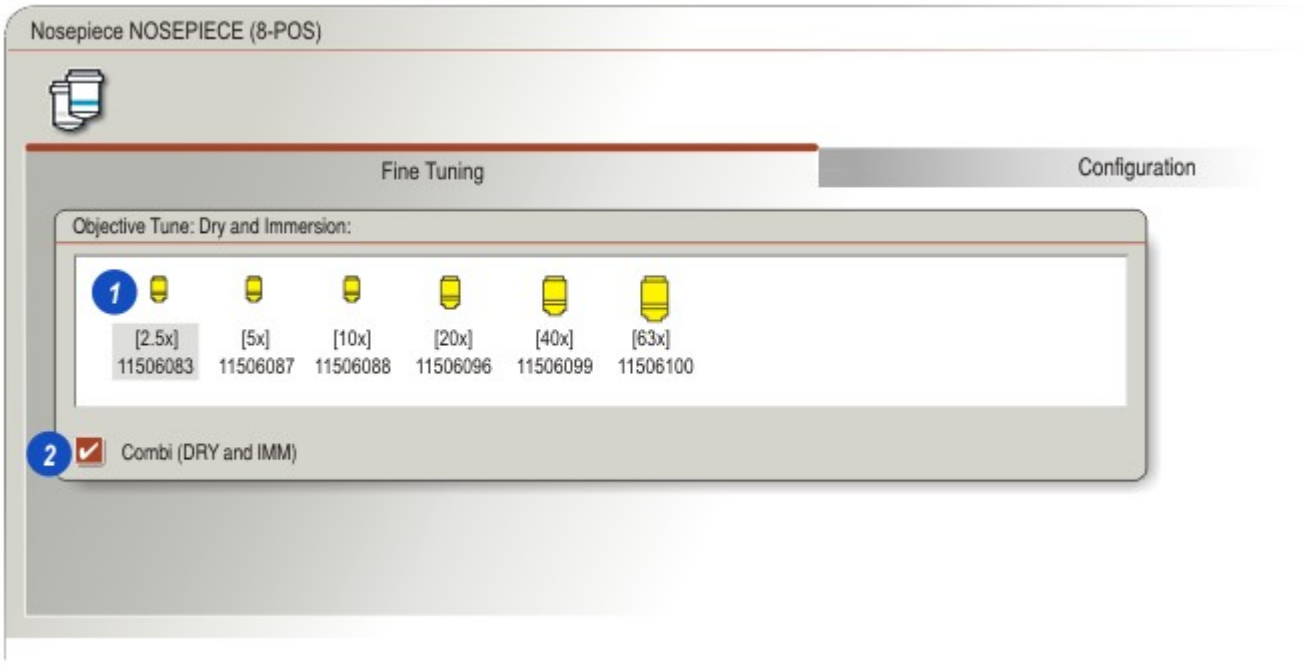
2: Click the check box to enable (ticked) or disable the Combi tag.

If either a tagged Combi or Immersion only objective is selected on the Acquire > Mic 1 tab, the button will flash to warn the user.



3: Immersion only objectives are marked with a 'filled' teardrop icon whereas...

4: ...Combi objectives are marked with an outlined teardrop.



Only available when a Leica Stage Controller is fitted, it allows a stage speed - X, Y and Z - to be matched to an objective based upon its magnification. Optimising the stage speed improves efficiency.

Higher stage speeds are used with low magnification objectives and low stage speeds with high magnification objectives both with the aim of maintaining the best navigation speed with 'losing' the image region of interest.

There are five step speeds ranging from SC - fast and coarse - to S0 - slow and fine - any one of which can be assigned to an objective so that when it is selected the appropriate stage speed is engaged automatically.

1: Click on the Objective Icon to select it.

2: Click on the small arrows to the right of the Stepsize menu header and...

3: ...click to assign the appropriate Stage speed.

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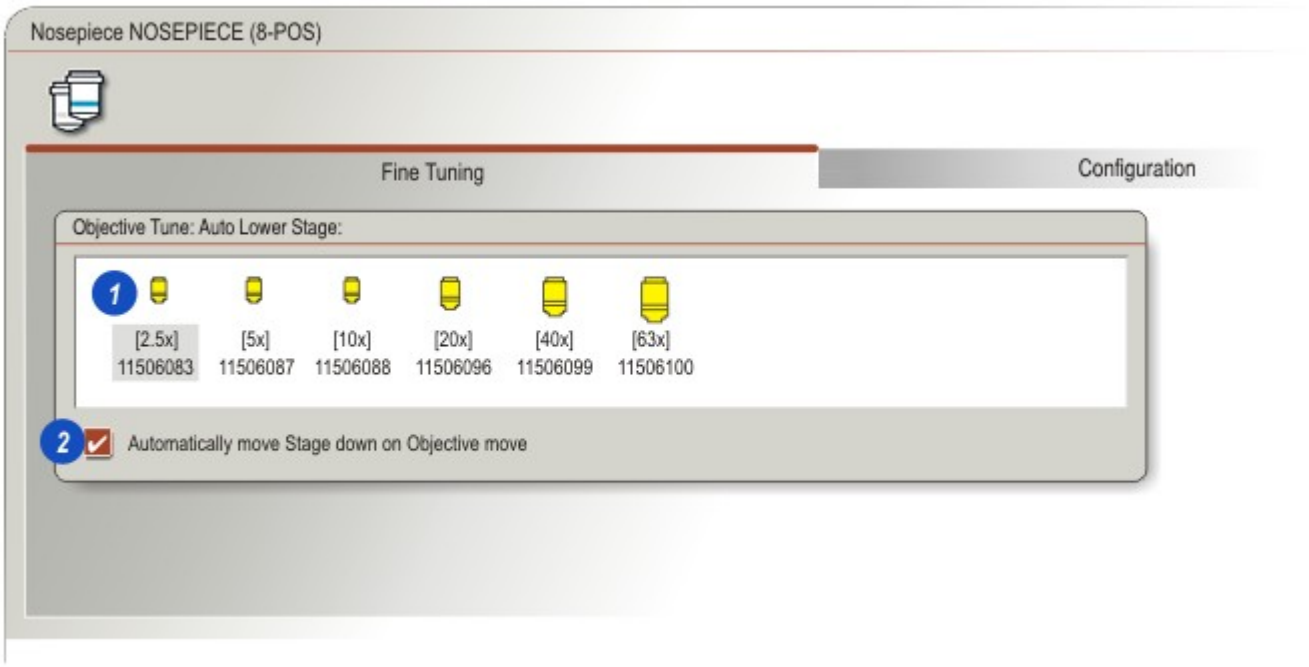
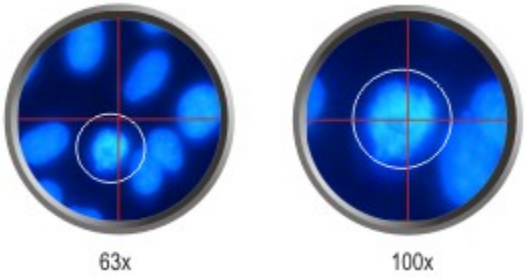
To avoid an objective colliding with the specimen, the Stage can be automatically lowered whenever the objective is selected. To assign Automatic Stage lowering:

1: Click on the Objective Icon to select it.

2: Click on the check box to enable (ticked) or disable Stage lowering for that objective.



Adjusting Parcentricity aims to maintain the specimen feature of interest as close to the centre of the field of view regardless of the objective magnification.



The process is straightforward. A feature on a specimen is focussed and moved as close as possible to the centre of the field of view using the stage navigation controls. The stage X and Y positions are stored as an 'offset factor' for the selected objective.

The objective is then changed and the stage adjusted so that the same feature is again close to the centre and the X/Y offset stored. The process is repeated for all of the objectives. Parcentricity improves if the feature position is finely adjusted for each objective.

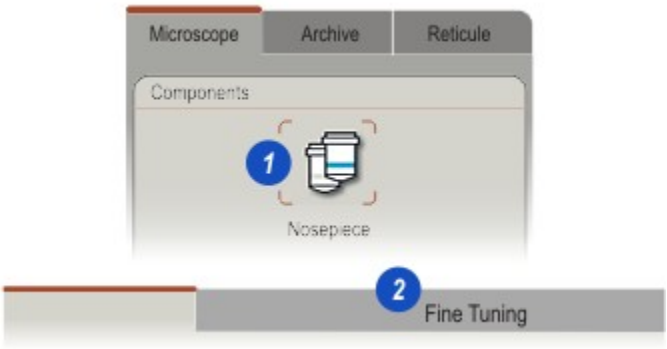
In the future when an objective is selected, the offset factor is automatically applied to the stage drivers to maintain Parcentricity.

There are two options for adjusting the Parcentricity:

- From this setup dialog with position being checked through the eyepieces - the monitor Viewer is not available here - and...
- From the Acquire Workflow with the specimen feature displayed on the Viewer.

For both options the dialogs are the same but are accessed differently:

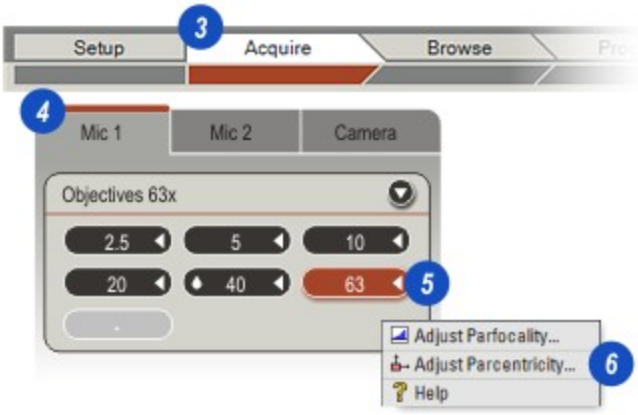
Set Parcentricity from DM Setup (Here):



1: Click on the Nosepiece icon on the Microscope > Component panel.

2: Click on the Fine Tuning tab and then click to select the Parcentricity dialog. Continued... 143

Set Parcentricity from Acquire Workflow:



3: Click on the Acquire Workflow and then...

4: ...on the Mic 1 tab.

5: Right-click on one of the objective select buttons and...

6: ...left click to select Adjust Parcentricity on the pop-up menu. Continued... 143

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1: Click on the Parcentricity dialog and...

2: ... the Start button. The Find Focus option is selected. Navigate the stage to and adjust for the sharpest focus on a recognisable feature on the specimen.

3: Click OK.

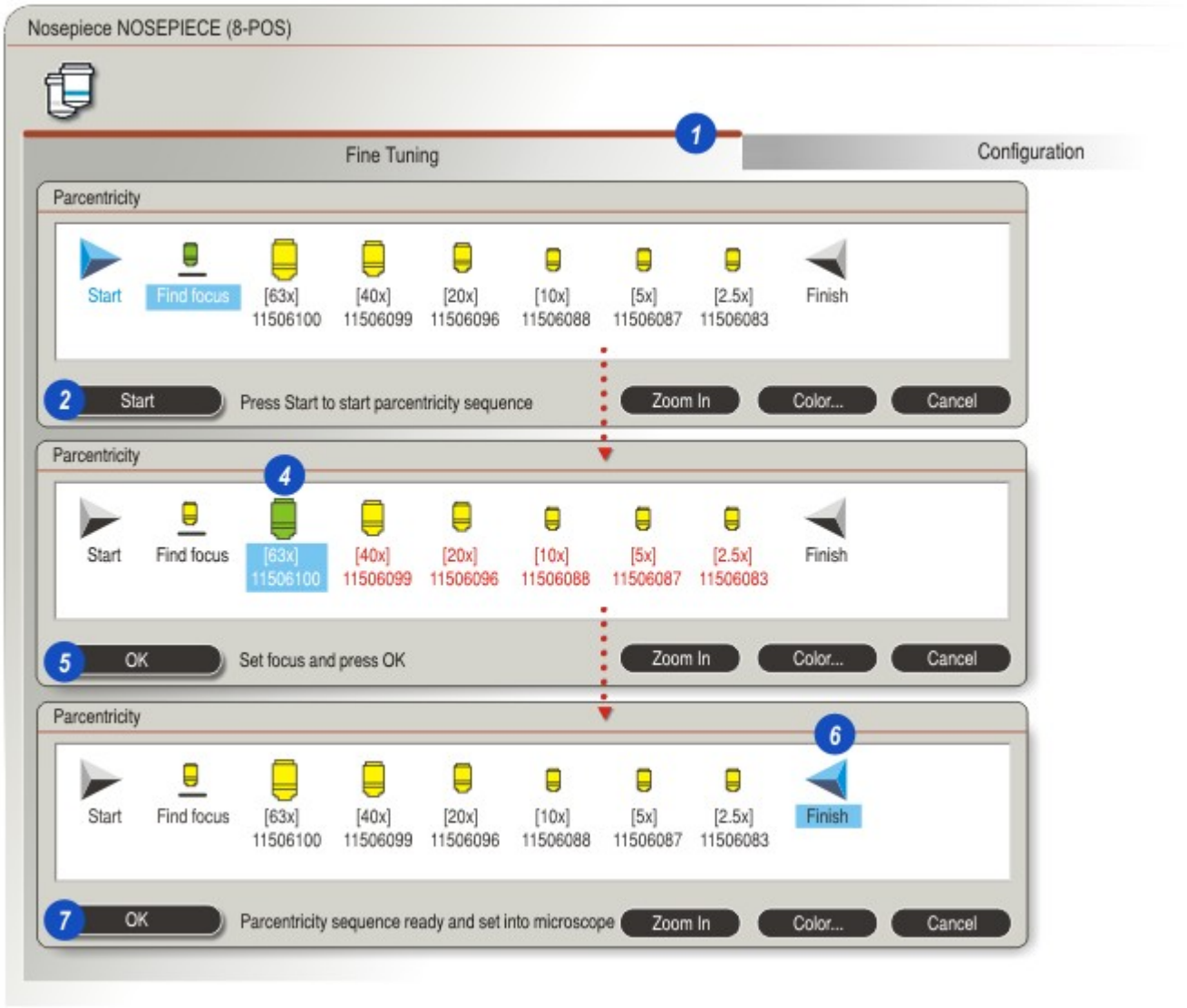
4: The objective with the greatest magnification is automatically chosen first. If necessary, focus the specimen, move the stage so that the feature is in the centre of the field of view and...

5: ...click OK. The sequence will advance to the next objective.

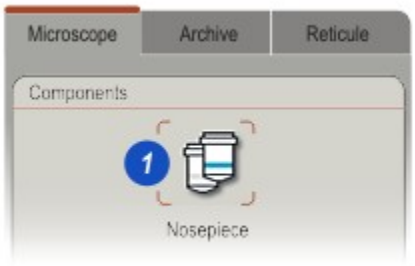
Re-focus if necessary and again centralise the feature in the field. Repeat the process for all of the objectives.

6: When all objectives have been fine-tuned the Finish icon is highlighted.

7: Click OK.



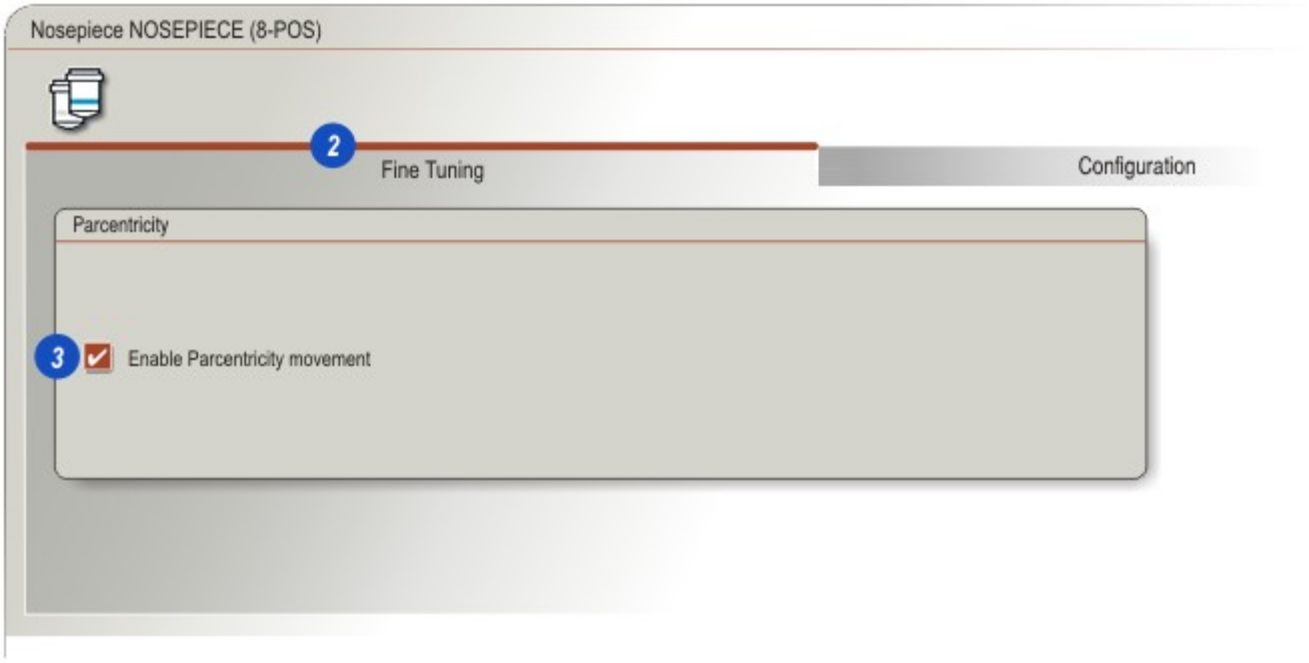
Automatic Parcentricity adjustment can be turned on and off on the Enable panel.



1: Click on the Nosepiece icon on the Microscope > Components panel.

2: Click on the Fine Tuning tab.

3: On the Parcentricity Enable panel, click the check box to enable (ticked) or disable automatic Parcentricity.



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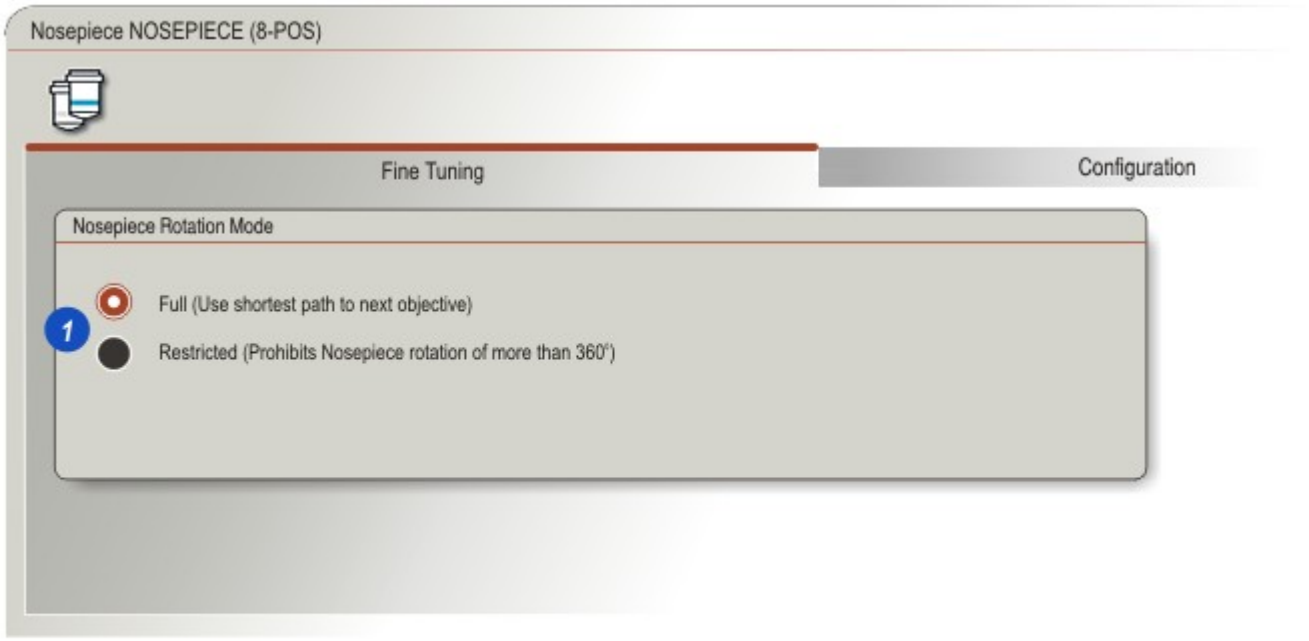
There are two options for determining how the Nosepiece behaves when an objective is selected:

1: Click to select the required mode:
Full allows the software to determine the shortest path - clockwise or counter-clockwise between the current and selected objective, or

Restricted allows the software to prevent the Nosepiece from ever rotating more than 360^0 even if that means taking the longest path between the current and selected objectives.



Select the tube Eyepiece as follows:

1: Click on the Ports icon on the Microscope > Components panel.

2: Click on the small arrows to the right of the Port Options drop-down menu header.

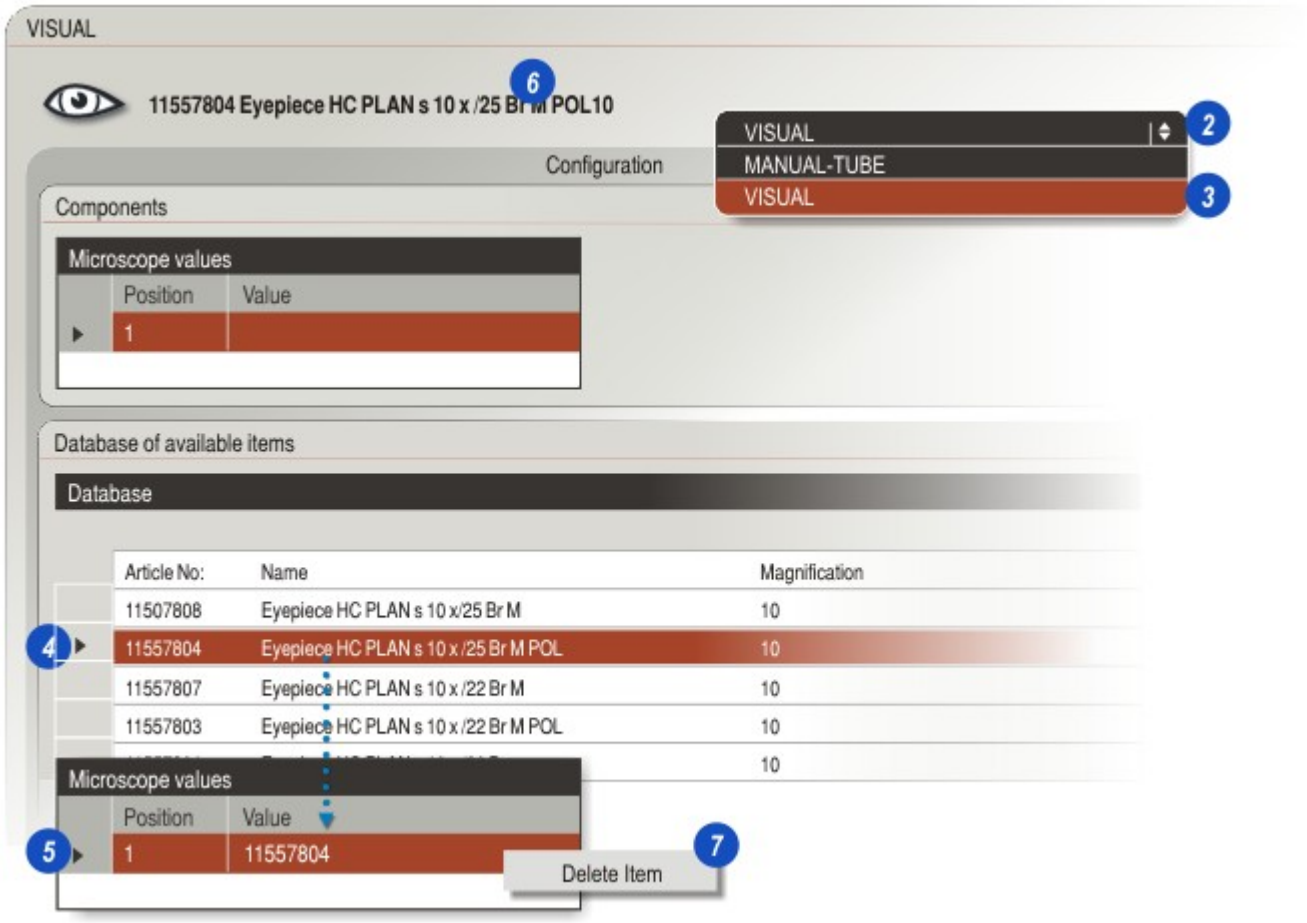
3: From the drop-down list, **c**lick to select Visual tube. The list of Eyepiece options appears on the Database panel.

4: Double-click on the Database entry to select it.

5: The item number appears in the Microscope Values list with...

6: ...the number and name displayed in the header.

7: Change a selection by right-clicking the value and then left-clicking the Delete Item pop-up button to remove the selection. Then proceed as describe to make a new selection.



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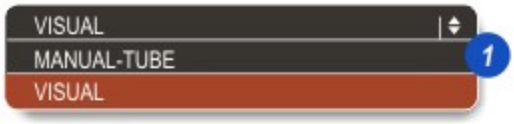
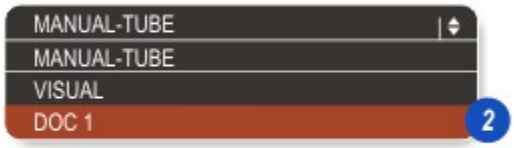
The number of Camera (DOC) Ports is determined by the fitted hardware and the configuration selected here in setup.

There a three possible setups:

1: No Camera Port - the DOC option is missing.



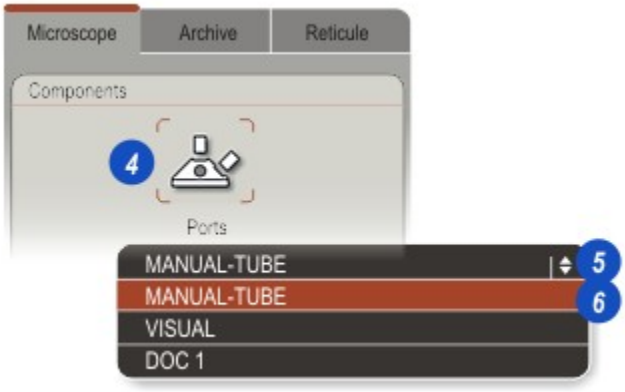
2: A single Camera Port - DOC 1 and...



3: ...dual Camera Port - DOC 1 and DOC 2.

The Camera Port(s) must be configured camera(s) can be assigned by:

4: Clicking on the Ports icon on the Microscope > Components panel.



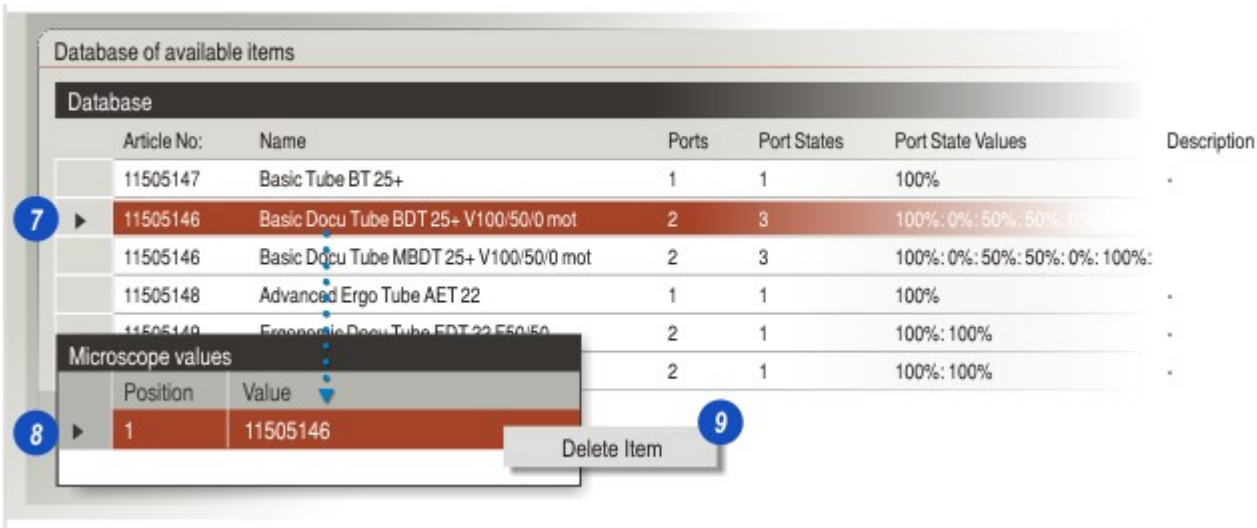
5: Click on the small arrows to the right of the Port Options header.

6: Click on the Manual-Tube option.

7: On the Database panel scroll to the required configuration and double-click to...

8: ...save it as the port setting. Repeat Step **(5)** and check that the required number of Camera Ports is available from the drop-down options.

9: Remove the configuration by right-clicking it in the Microscope Values panel and then left-clicking the pop-up Delete Item button.



With the Camera Ports configuration selected, the Camera Adapter has to be selected for each port.

1: Click on the Ports icon on the Microscope > Components panel.

2: Click on the small arrows to the right of the Port Options header and from the drop-down...

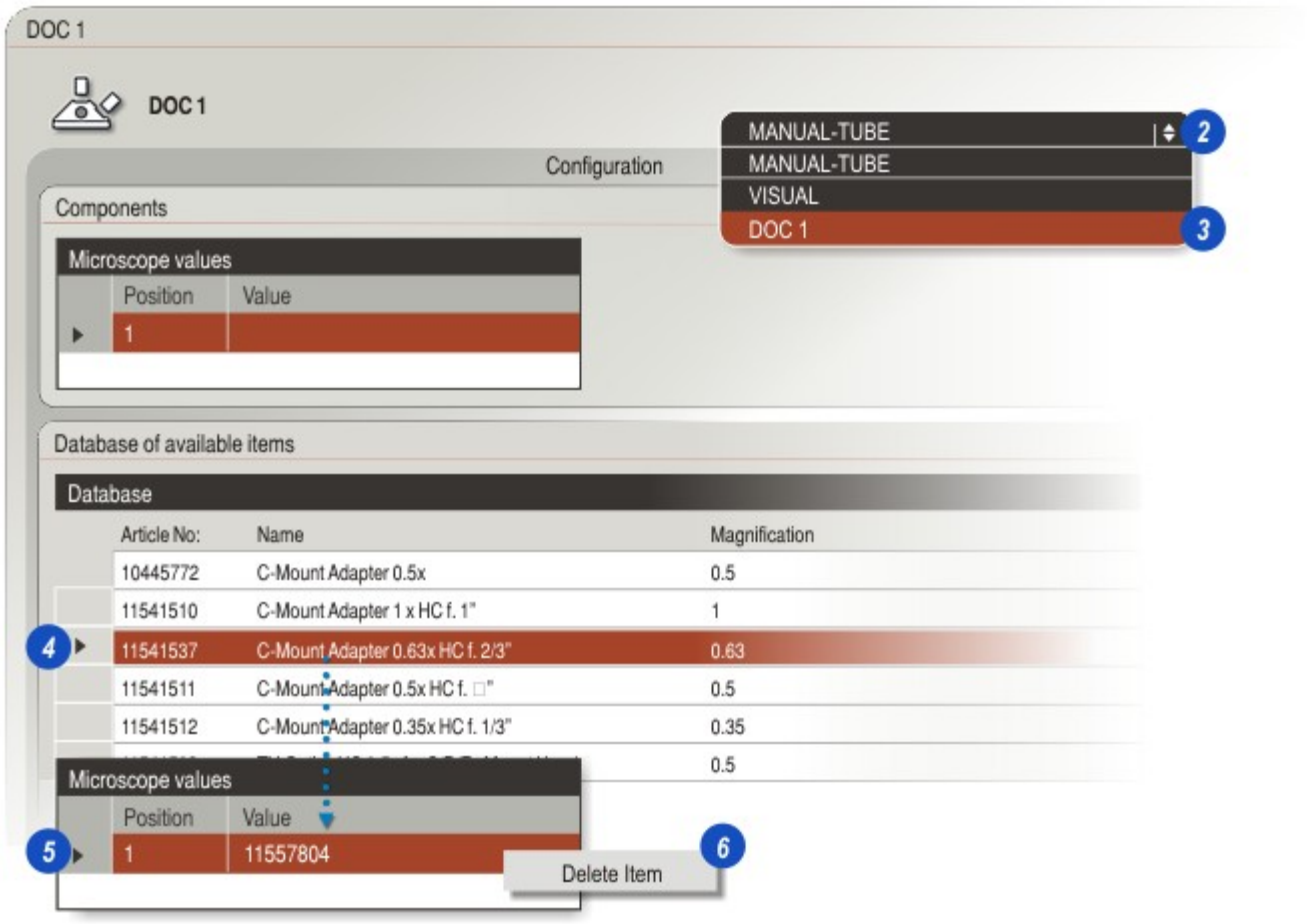
3: ... click to select the Camera Port to which the adapter will be assigned - in the example DOC 1.

4: On the Database, double-click to select the name of the fitted Adapter.

5: The selection appears in the Microscope Values panel.

6: To remove a selection, right-click on the Microscope Values entry and left-click the Delete Item pop-up button.

Repeat the process for the other Camera Port if fitted.



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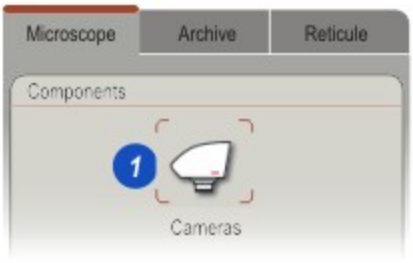
The Camera Ports and Mounts must be set up before the camera(s) can be assigned to them: Go there... 147

Fit and connect the Camera(s) so that they can be detected by the software.

1: Click on the Camera icon on the Microscope > Components panel.



2: Click on the small arrows to the right of the Camera Options header.

3: From the list, click to select Camera (DOC 1). This is Camera Port DOC 1 to which a camera is about to be assigned.

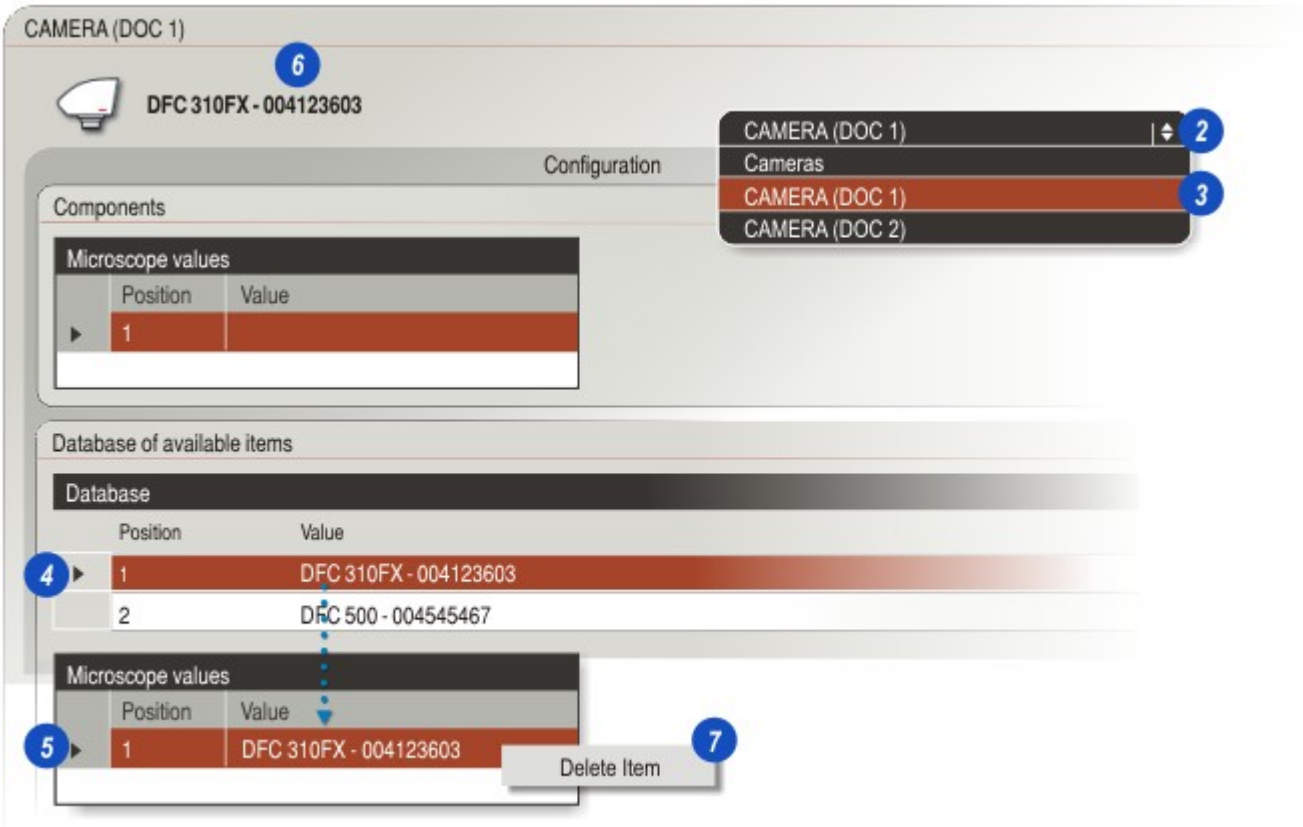
4: The Database lists the fitted cameras - for the majority of systems there will be just one Camera Port and therefore one Camera type in the list. Double-click on the Database entry and...

5: ...the Camera type is assigned to the DOC 1 port and appears in the Microscope Values list.

6: The Camera and its serial number are also displayed on the header.

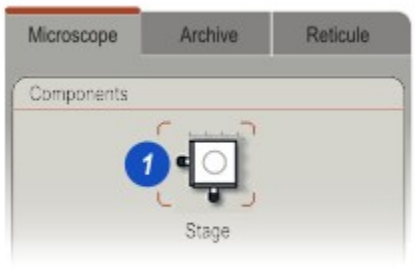
7: To remove a Camera from the Microscope Value, right-click on the entry and then left-click the pop-up Delete Item button.

Repeat the process if a second camera is fitted selecting the DOC 2 port.

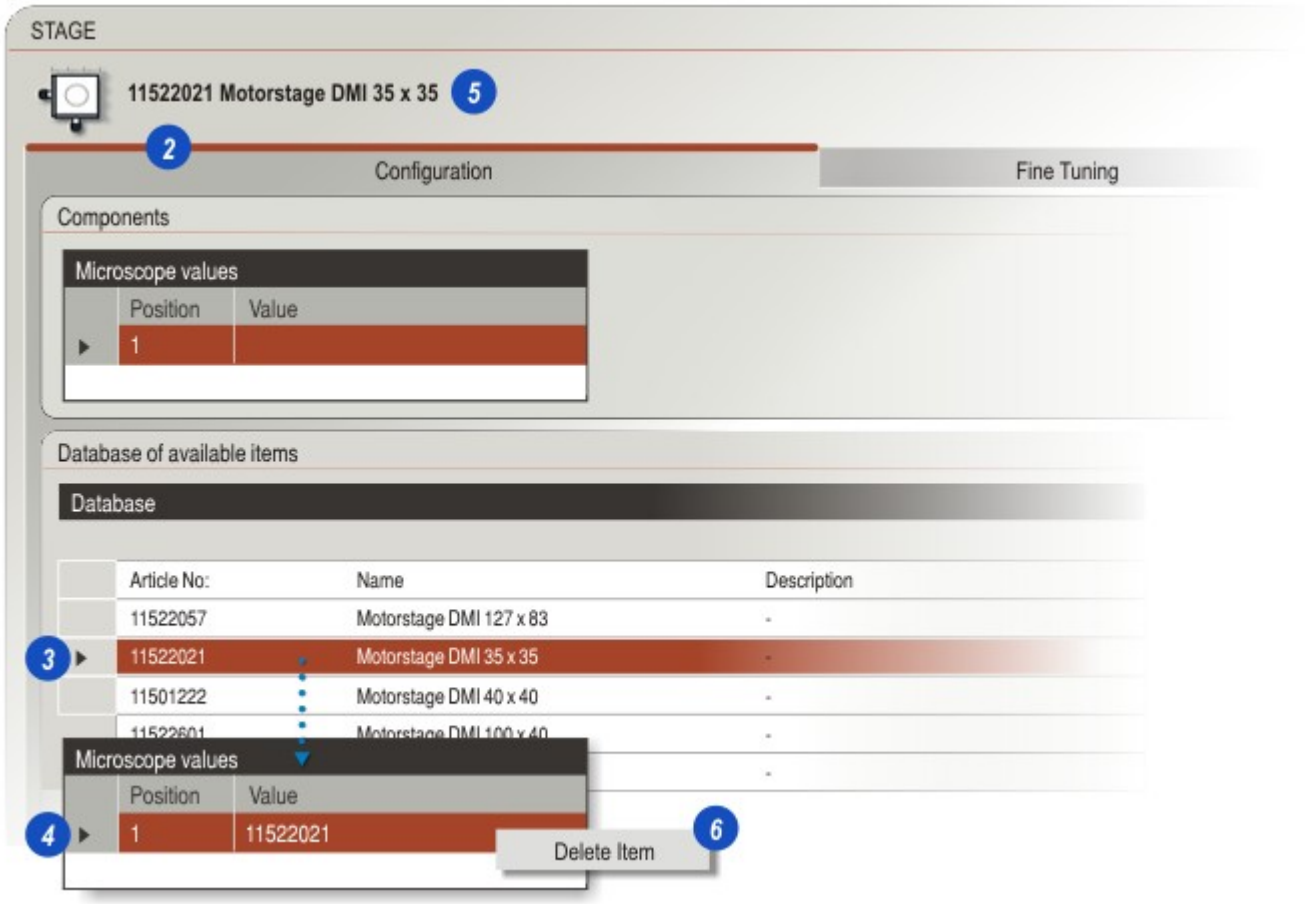


Stage Selection and Initialisation are both carried out from the same panel.

1: Click on the Stage icon on the Microscope > Components panel.



2: To select the stage type click on the Configuration tab.



3: Move to the fitted stage name and type on the Database and double-click it.

4: The stage Article Number appears in the value list with...

5: ...the description displayed on the header.

6: To change the selection, right-click on the selection in the values list and then left-click the Delete Item pop-up button. Repeat the process described above to make a new selection.

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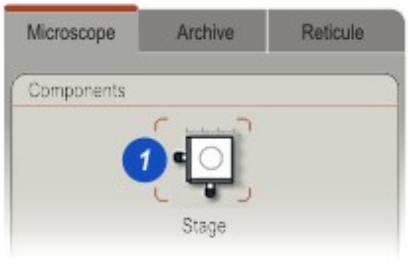
Stage Selection and Initialisation are both carried out from the same panel.

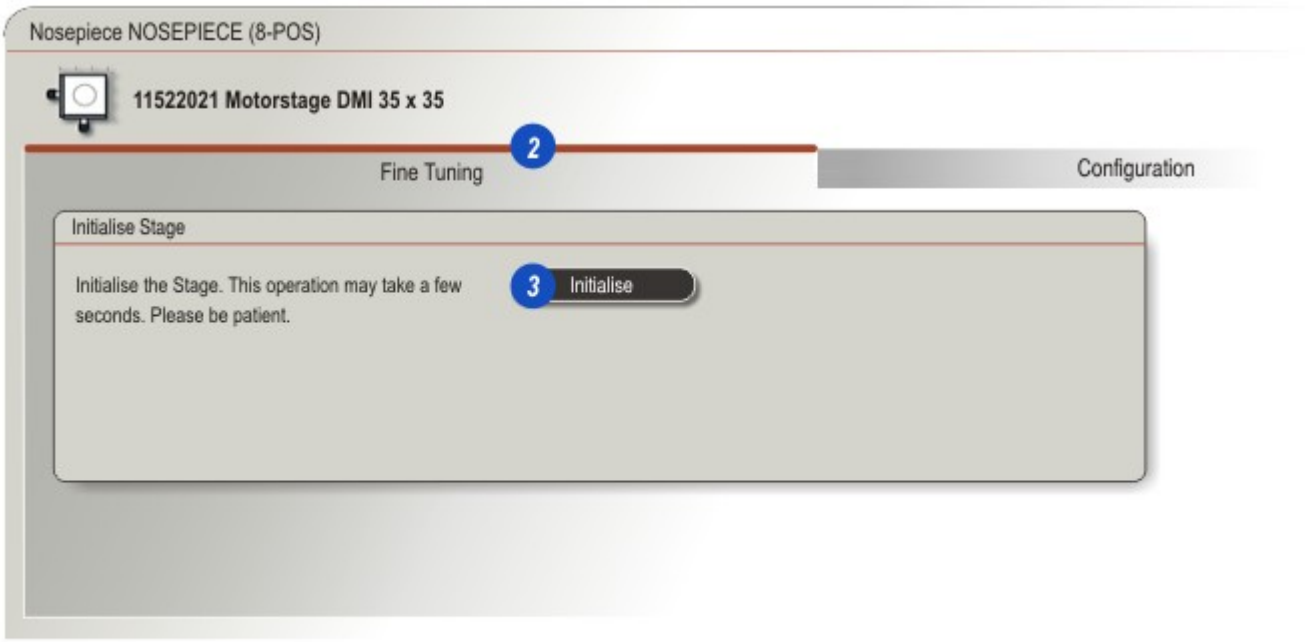
1: Click on the Stage icon on the Microscope > Components panel. For initialisation the stage must be lowered sufficiently to avoid collision with specimens or objectives.



2: To initialise the stage, click on the Fine Tuning tab and...

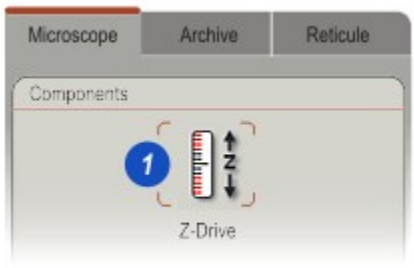
3: ...click the Initialise button to drive the stage to its limits and set the starting position to top-left (X = 0/ Y = 0).

During initialisation the stage can move very quickly and the drive mechanism is powerful enough to severely damage specimens and objectives. Also ensure that there are no cables that are likely to become entangled.



The Z-Drive dialog is divided into 2 panels - Focus Position and Initialisation.

1: Click on the Z-Drive icon on the Microscope > Components panel.



2: Set the preferred initial Handwheel Speed by clicking either Fine or Coarse. The selection is highlighted.

3: Drive immediately to the Lower Limit Switch or...

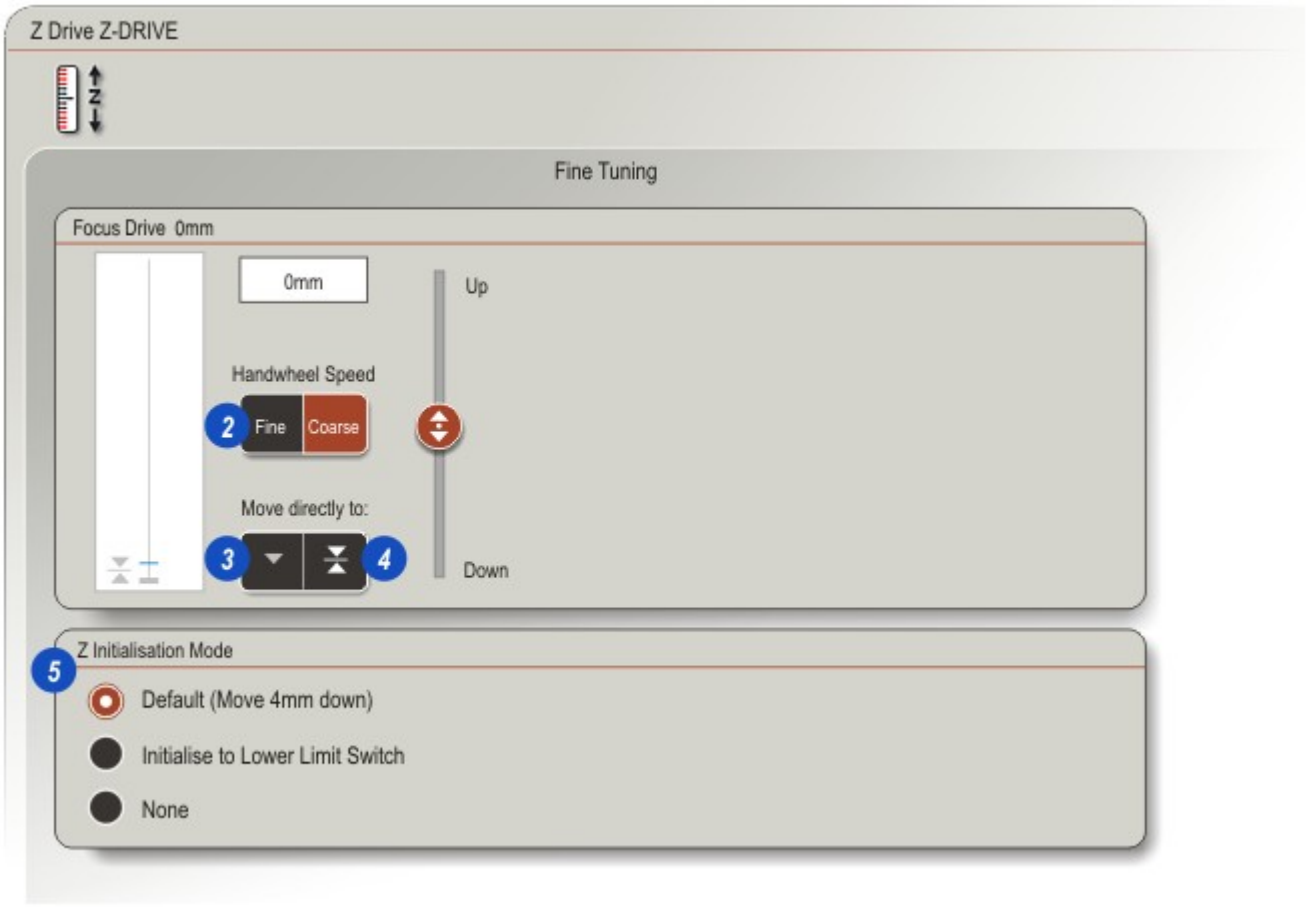
4: ...to the selected Lower Threshold (Please see the following page: Go there... 153)

5: The Initialisation Mode (Please see also Stage Initialisation: Go there... 151) can be set to either:

- Default: Factory setting for 4mm lowering.
- Lower Limit Switch: Drives the stage to its lowest position.
- None: The stage remains in the focus position last set.

Click the appropriate buttton.

Continued... 153



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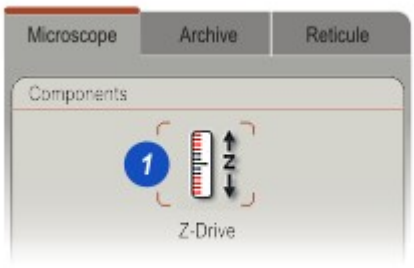
The current Z position can be saved either as a repeatable focus or as the lower threshold for initialisation.

1: Click on the Z-Drive icon on the Microscope > Components panel.



2: To set the focus or lower threshold position, click and hold down the Focus Drive Slider and move it to the required position. The distance is displayed in the window.

3: Right-click on the Slider to reveal the Settings and Help options. Click on Settings and...

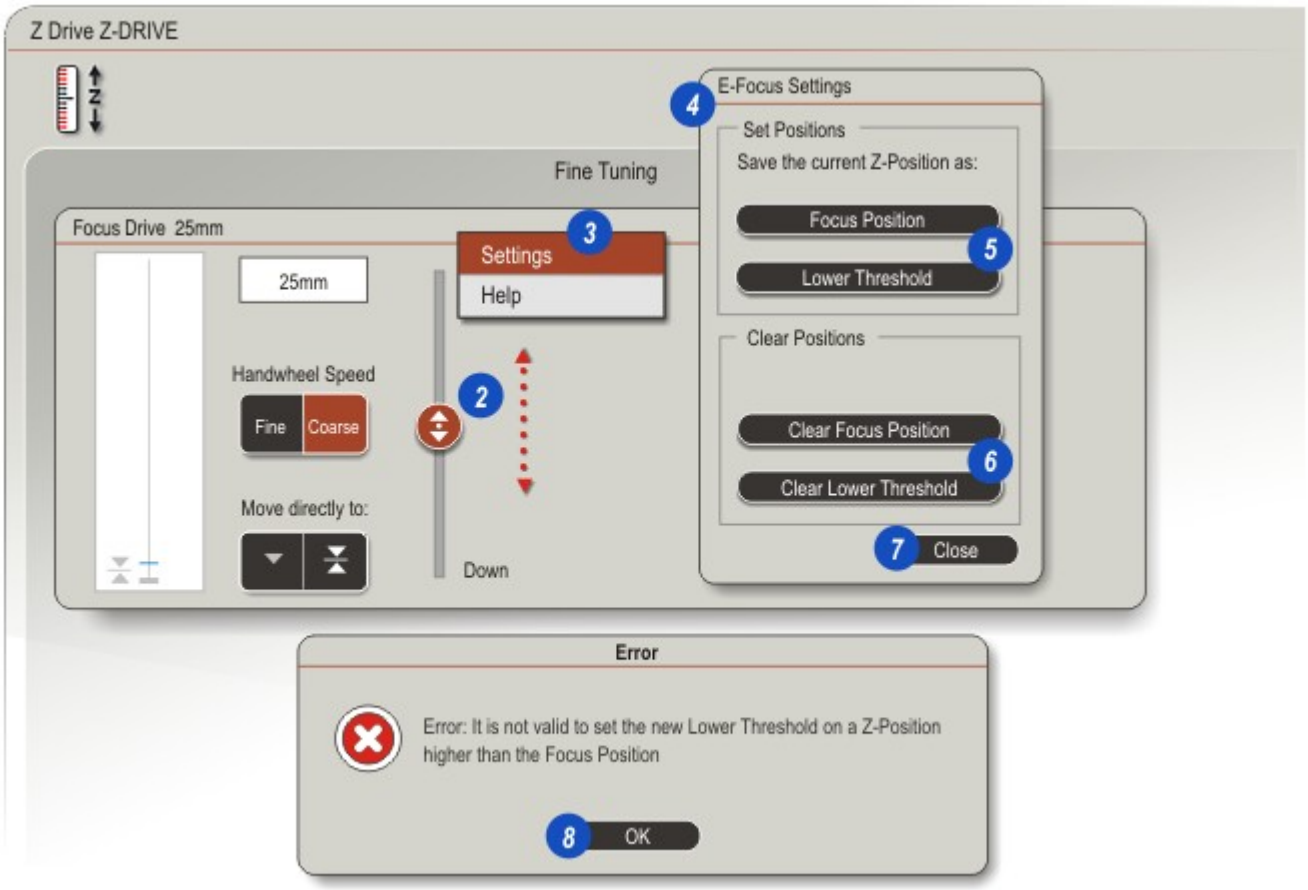
4: ...and the Focus Settings dialog appears.

5: Save the current Z position as either the repeatable Focus or the Lower Threshold by clicking the appropriate button.

6: Clear either setting by clicking the appropriate clear button and...

7: ...click OK.

8: It is not possible to save a Focus Position that is lower than the Lower Threshold - the warning message appears.



To select a single condenser or assign different condensers to turret positions:

1: Click on the Condenser icon on the Microscope > Components panel.

2: Click on the small arrows to the right of the Condenser options drop-down menu and...

3: ...click to select either a single condenser or the turret.

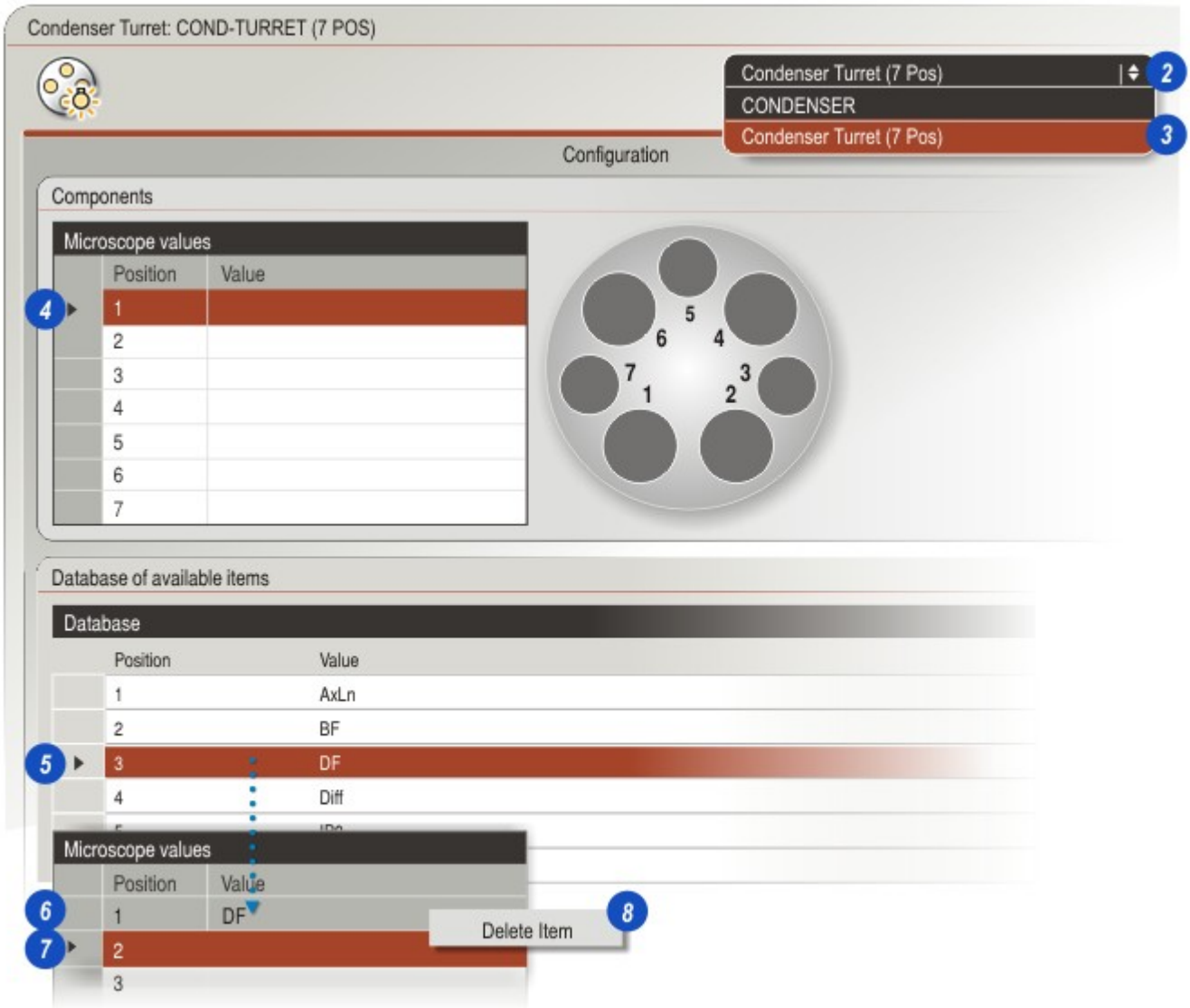
4: Either a single position or the number of turret positions is listed under Microscope Values. Click on the position to be assigned.

5: From the Database, double-click the type to be assigned to the position.

6: The selected type is displayed in the chosen position and...

7: ...the next position (if the turret option is selected) is automatically highlighted.

8: The remove an assigned condenser type, right-click on the position and left-click the Delete Item pop-up button,



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The microscope push-button controls can be assigned to functions that suit the user.

1: Click on the Function Keys icon on the Microscope > Components panel.

2: The Configuration dialog appears with the push-buttons displayed as a graphic 'map', each with a number.

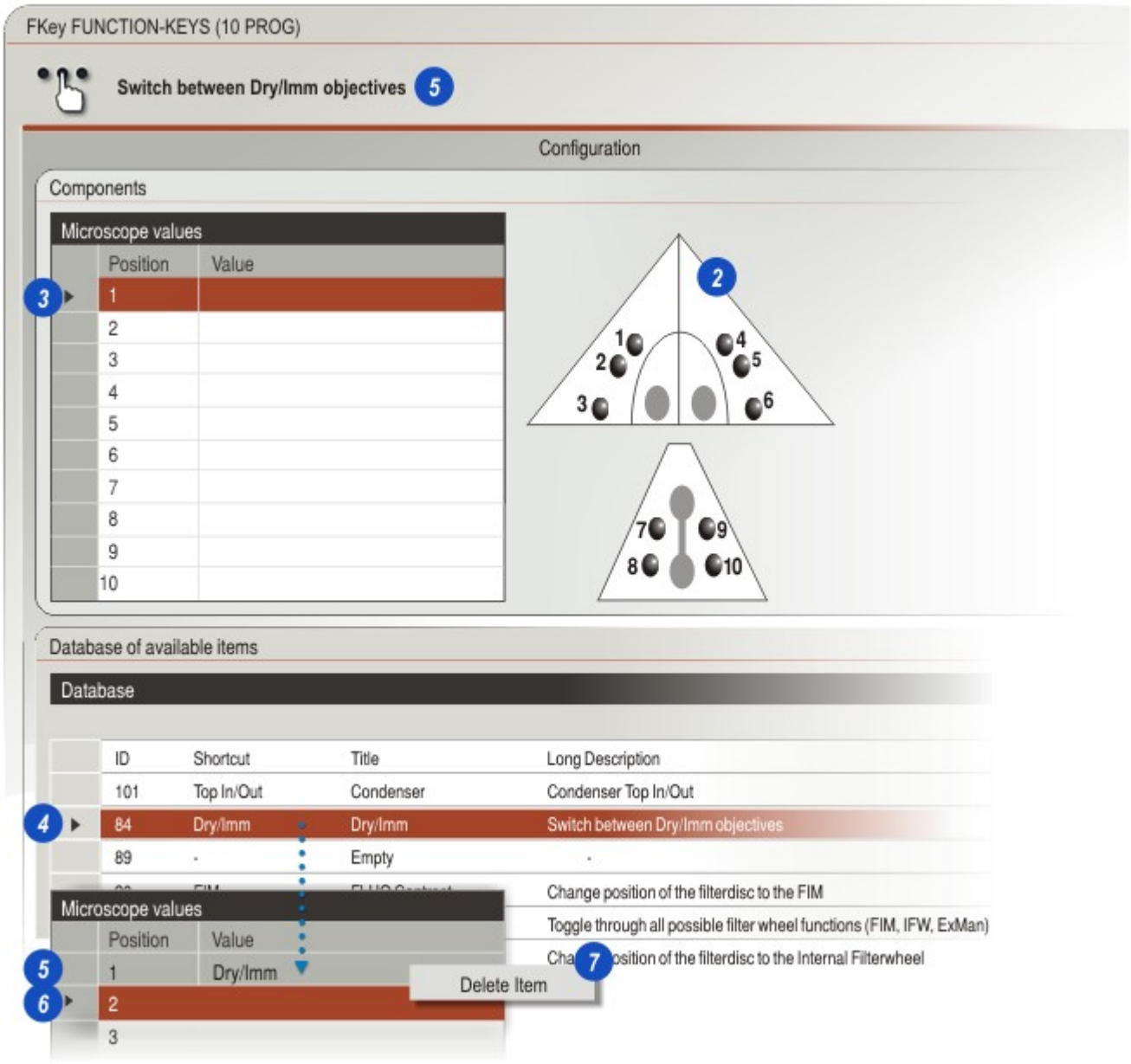
3: The push-button numbers are listed under the Microscope Values. Click a position to select the push-button.

4: Double-click the function on the Database list to assign it to the button.

5: The function is displayed on the Microscope Values list and on the header.

6: The next push-button is automatically selected.

7: Right-click on an entry and then left-click the Delete Item pop-up button to clear an assignment.



The Acquire Workflow:

The *Acquire Workflow* provides control for the attached microscope and camera and also displays a live image on the *Viewer*.

Images are acquired, stored and displayed in the *Gallery* as thumbnails.

Within *Acquire* all microscope and camera controls can be set to individual requirements ranging from objective magnification, contrast method, exposure, gain and gamma.

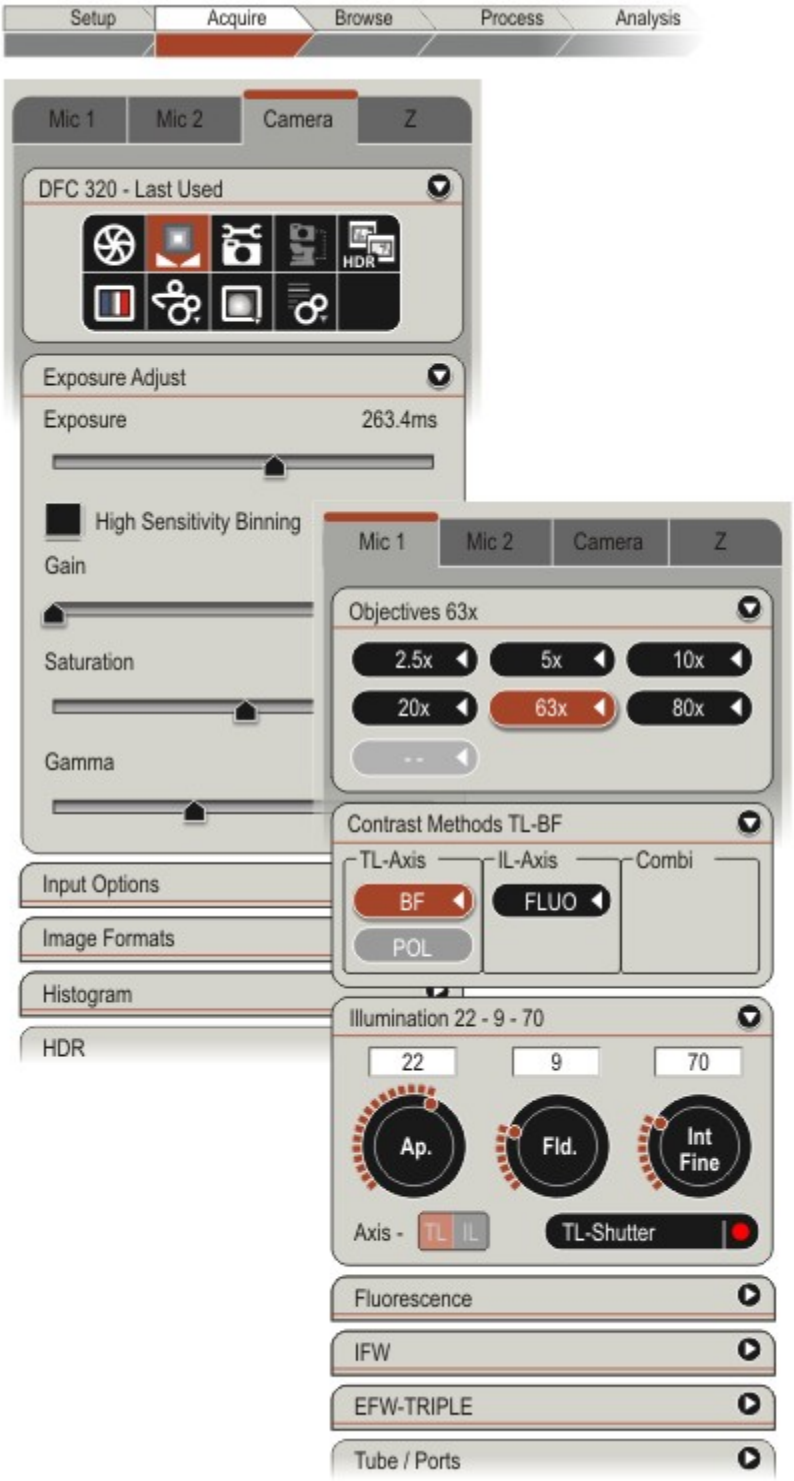
Optional Modules such as:

- Montage MultiFocus.
- Measurements
- Image Overlay
- MultiStep
- MultiTime Timelapse.
- Image Analysis
- Power Mosaic and
- Phase Expert...

...provide more specialised acquisition options

Leica Application Suite has *LAS Archive* as an optional module to provide all of the speed and power of a database for storing images and data. It is available as:

- *Archive Basic:* Essential *Archive* tools with versatile structuring and storage options, and...
- *Archive Standard:* Taking *Archive* to the very highest levels of reporting and display.



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The Acquire User Interface:

The illustration is a graphical representation of the LAS display and Acquire user interface showing the principal features and quick links (Jump to the link by clicking ↗):

1: *Workflows* ↗158 Click the *Acquire Workflow* to launch the microscope and camera controls.

2: *Tabs:* Select microscope, camera or specialised acquisition functions.

3: *Tools* ↗221 Toolbox when *Camera* is selected.

4: Control Panels for:

- ↗173 Stereo Microscope.
- ↗189 DM Microscope
- ↗215 FS Microscope
- ↗221 Camera

5: *Image Data Form* ↗170 Displays and edits selected data for the current image.

6: *The Grid* ↗169 Displays data for all of the images in the selected folder.

7: *The Gallery* ↗167 Displays thumbnails of all the images in the selected folder.

8: *Acquire Button* ↗171 Click to grab and image from the microscope camera.

9: *Side Toolbar* ↗159 Working tools for image sizing, printing and deleting as well as switches for the *Gallery* and *Grid*.

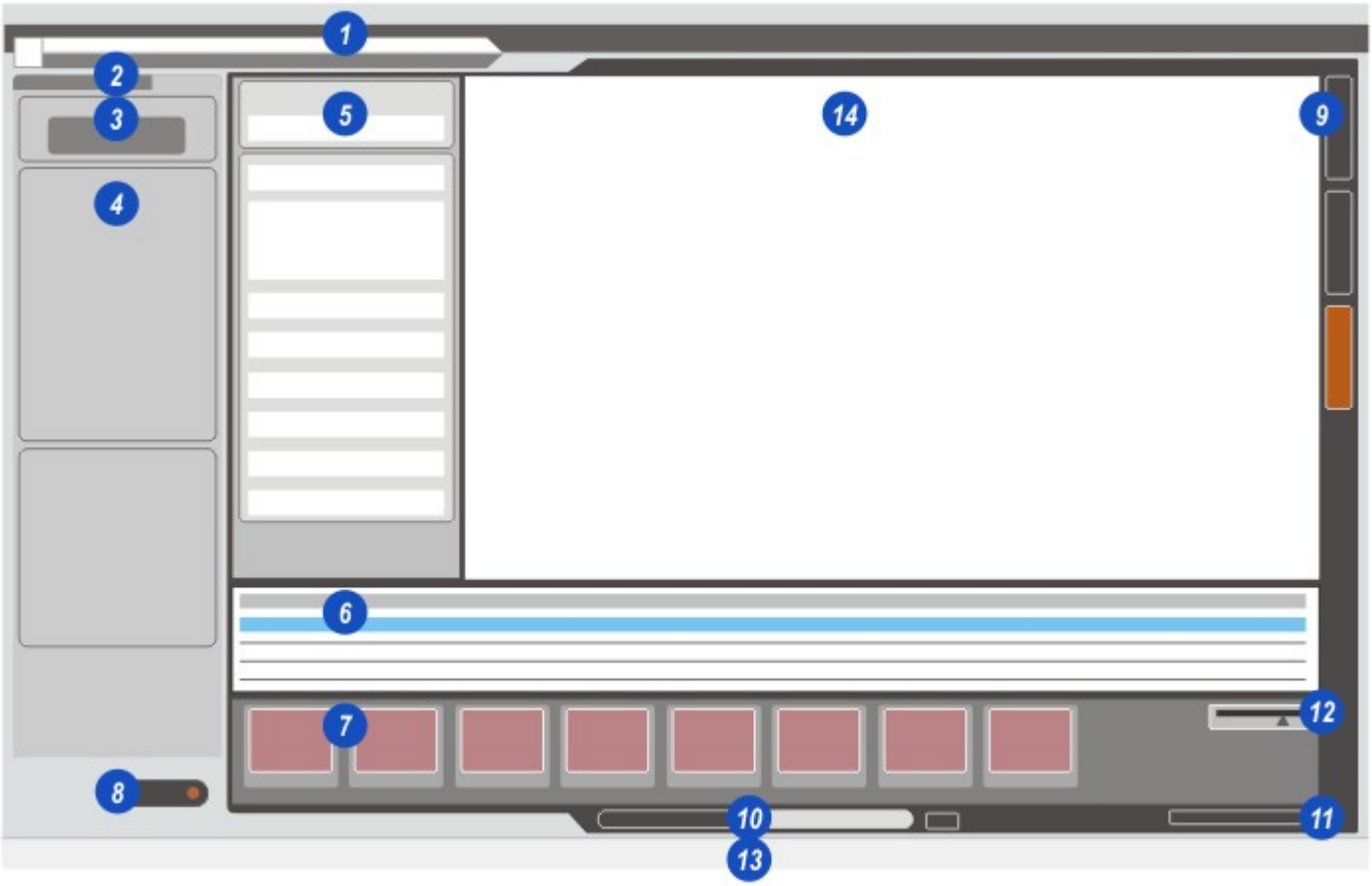
10: *The Search Controls* ↗410 Available only with *LAS Archives.*

11: *Gallery Navigation Browser* ↗167 Rapidly find thumbnails in the *Gallery*.

12: *Thumbnail Scaler* ↗167 Slider adjusts the size of the thumbnails in the *Gallery*.

13: *Status Bar* ↗ Displays Hardware Configuration, RGB Intensity, Stage Position and Magnification data.

14: *The Image Viewer* ↗166 Display and working area for the current image: Press keyboard *F5* to show full screen.



Launching Acquire:

1: Click on the *Acquire Workflow* and...

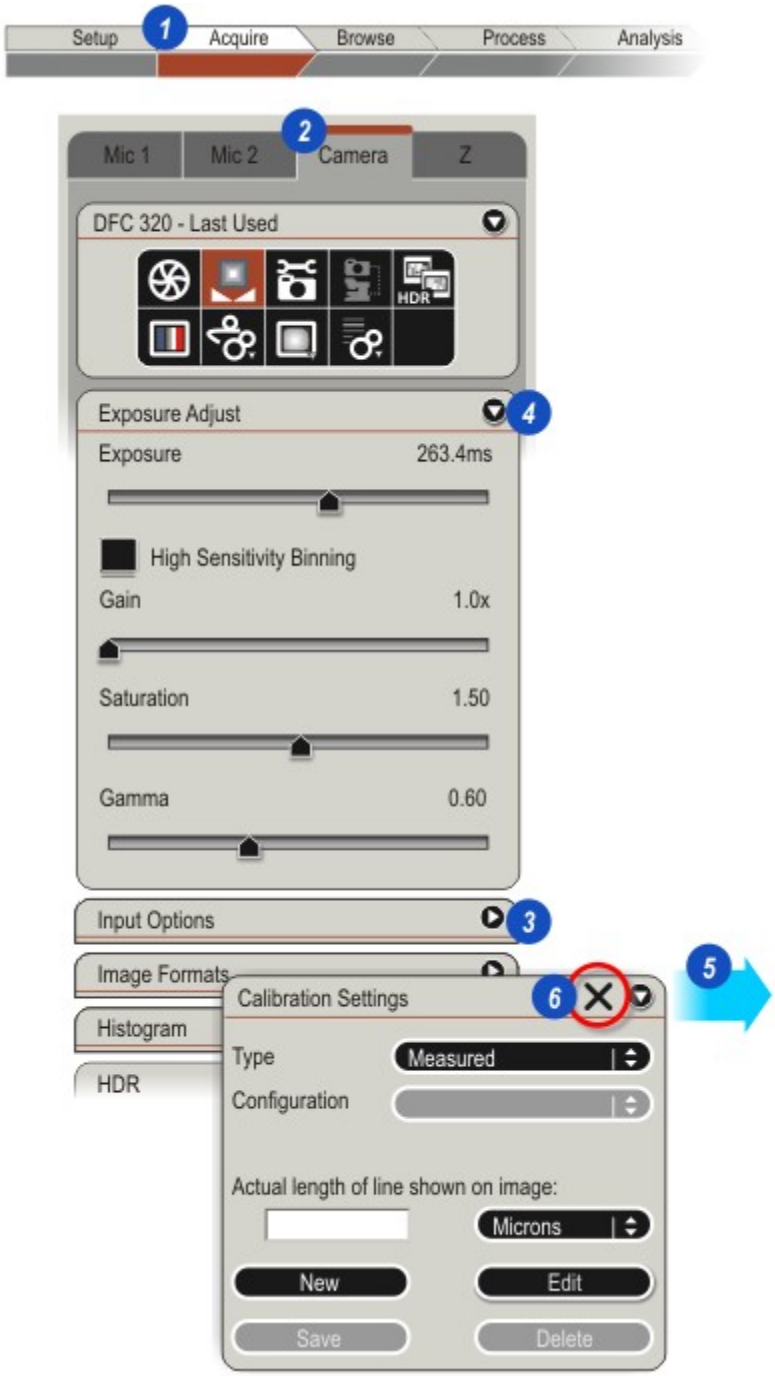
2: **..**..click on a tab to select microscope, camera, the selected sequence module or *Live Measurement.*

3: Some control panels are collapsed to avoid cluttering the working area. Expand them by...

4: ...clicking on the arrow to the right of the panel header.

5: Most panels can be moved to any part of the *Viewer* and docked by clicking on the panel header and, holding down the mouse button, dragging it to the preferred location.

6: When a panel is moved a 'snap back' symbol (X) appears on the header. Click it to return the panel to its usual position on the tab.



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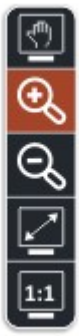
The Side Tool Bar:

This section describes the *Side Tool Bar* tools that are common to most of the Acquire features although the tool range may change with a selected feature.
Click on a *Tool Bar* button for more information:

Scale Bar and Annotations: Run Annotations and Scale Bar without leaving Browse.

Floating Navigator: Click to enable the *Floating Navigator* and click again to 'park' it.



Panning: Examine areas of images that extend beyond the *Viewer* edges into the display area.

Zoom in and...

Zoom Out.

Fit the image to the Viewer area.

Display at Original Size: Displays he image at its captured size:

Hide and Reveal the Record Panels.

Hide and Reveal the Viewer.

Hide and Reveal the Data Grid: Only available with LAS Archives.

Hide and Reveal the Thumbnail Gallery.

View the image Record Details: Not available in Acquire.

Select the Form Details to display: Not available in Acquire.

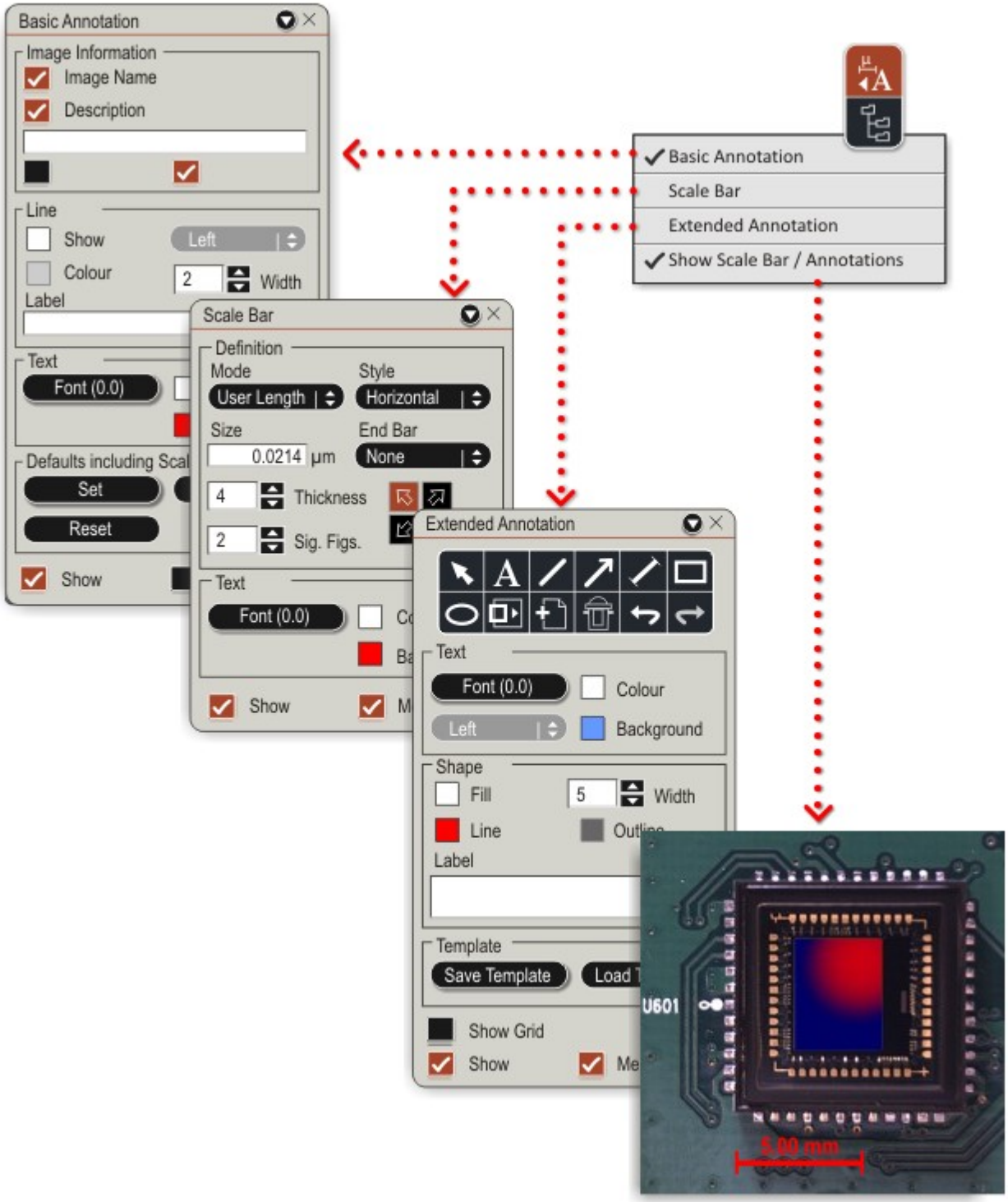
Viewer Options: Select Dual Viewer, Lock the Views and *Lock the Pan View.*

Copy Output Image: Not available in Acquire.

Clicking the *Show Annotations* button displays the *Annotations* and *Scale Bar Quick Launch* menu.

On the live image *Basic Annotation, Extended Annotation* and the *Scale Bar* setup can be launched without leaving *Acquire* by checking (a tick mark is displayed) the required function. All of the function tools are available.

The *Scale Bar* display can be turned on and off by clicking the *Show Scale Bar / Annotations* option. Turned on and the annotation tools are available.



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The Floating Navigator:

The *Browse Navigator* panel can be displayed and docked on the *Acquire Viewer* by:

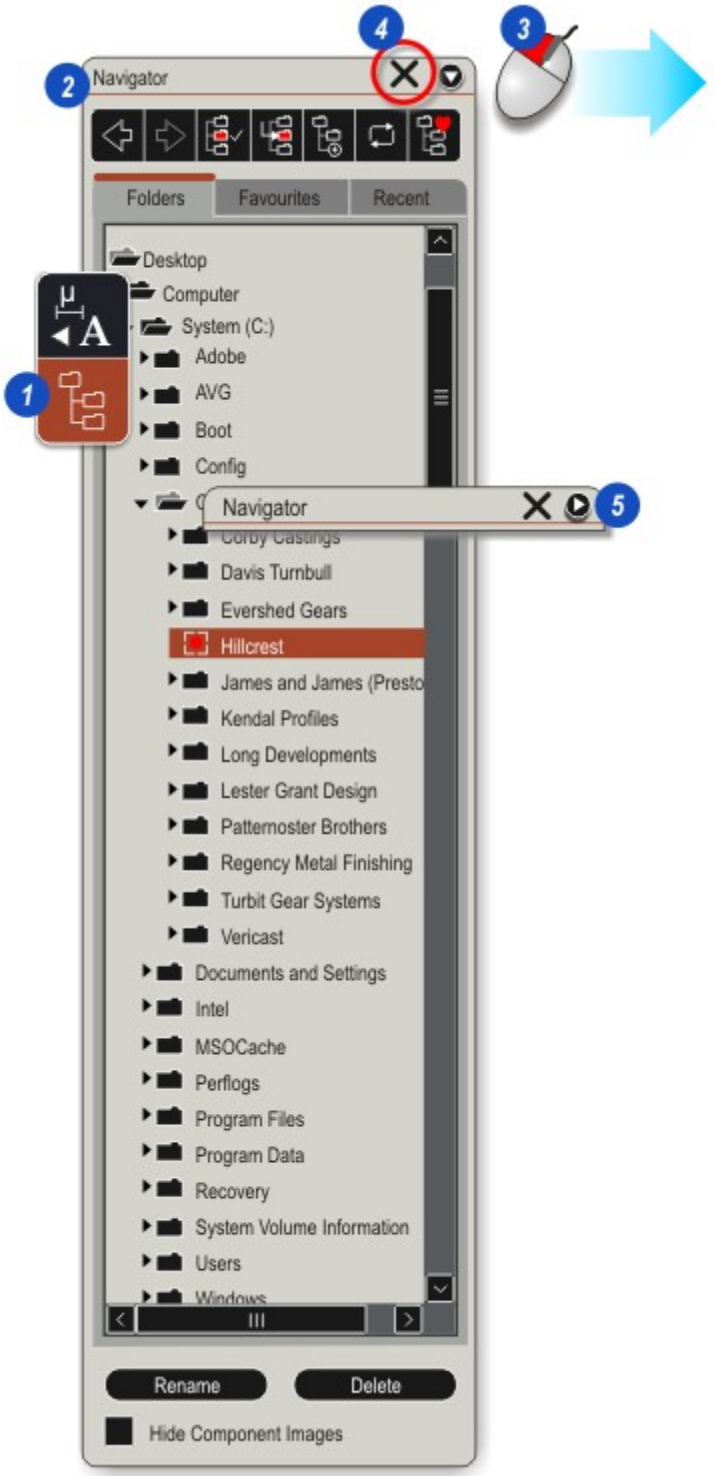
1: Click on the *Floating Navigator* button on the *Side Tool Bar.*

2: The *Navigator* panel appears and can be used in the same way as it would be from the *Browse Workflow* except the *Toolbox* is not available.

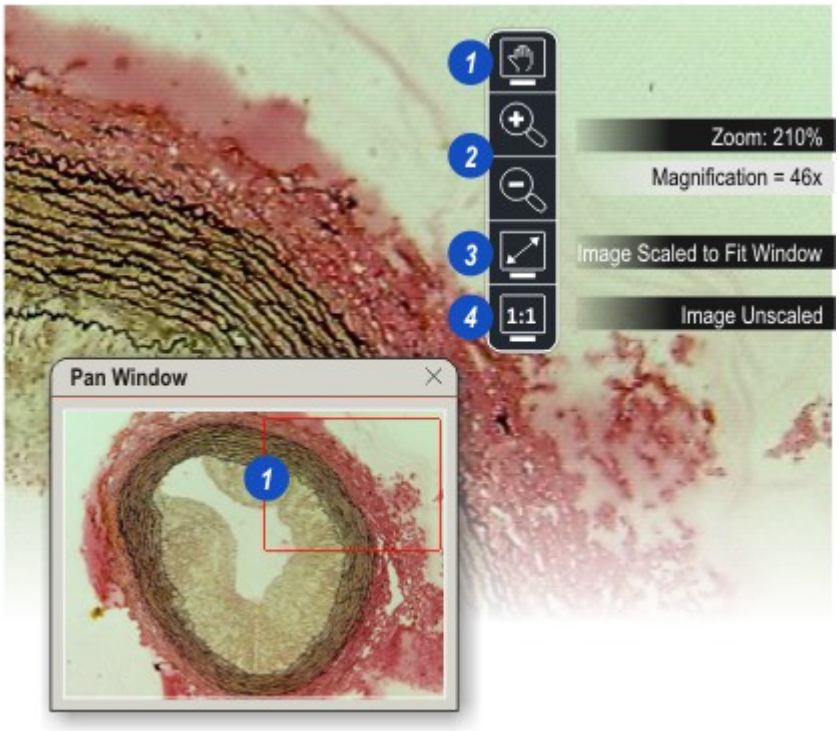
3: Move and dock the *Navigator* by clicking on the header bar and, holding down the mouse button, drag the panel to a new position.

4: Click on the 'snap-back' button (X) to close the *Navigator*.

5: Collapse the *Navigator* without closing it completely by clicking on the arrow to the right of the header. Click again to restore the panel.



Panning and Zoom:



1: *Panning:* The *Pan* tool allows detailed areas of an image that exceeds the visible area of the *Viewer* to be examined. It will not work if *Fit to Viewer* is enabled because all of the image is being displayed.

On the *Pan Window* viewer, click and hold in the outlined rectangle and drag it to the area to be examined. The selected area is displayed in the main *Viewer*.

To move the *Pan Window* away from the main *Viewer*, click and hold the header bar and drag it to another part of the screen.

Click the *Pan* tool to close the *Window*.

2: *Zoom:* Click on the (+) to zoom in to the image or (-) to zoom out. The zoom level as a percentage, is displayed top right of the the *Viewer* border.

If the monitor *Magnification Settings* have been set in *Preferences*, the image *Magnification* value appears bottom right of the *Viewer* border.

3: *Fit to Viewer:* Click to fit the image to the available *Viewer* area regardless of the original size of the image. The *Image Scaled to Fit Window* message appears top right of the *Viewer* border.

4: *Display at Original Size:* Click to display the image at its original size. The image may appear smaller or larger than the *Viewer* area. The *Image Unscaled* message appears top right of the *Viewer* border.

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Hide and Reveal:

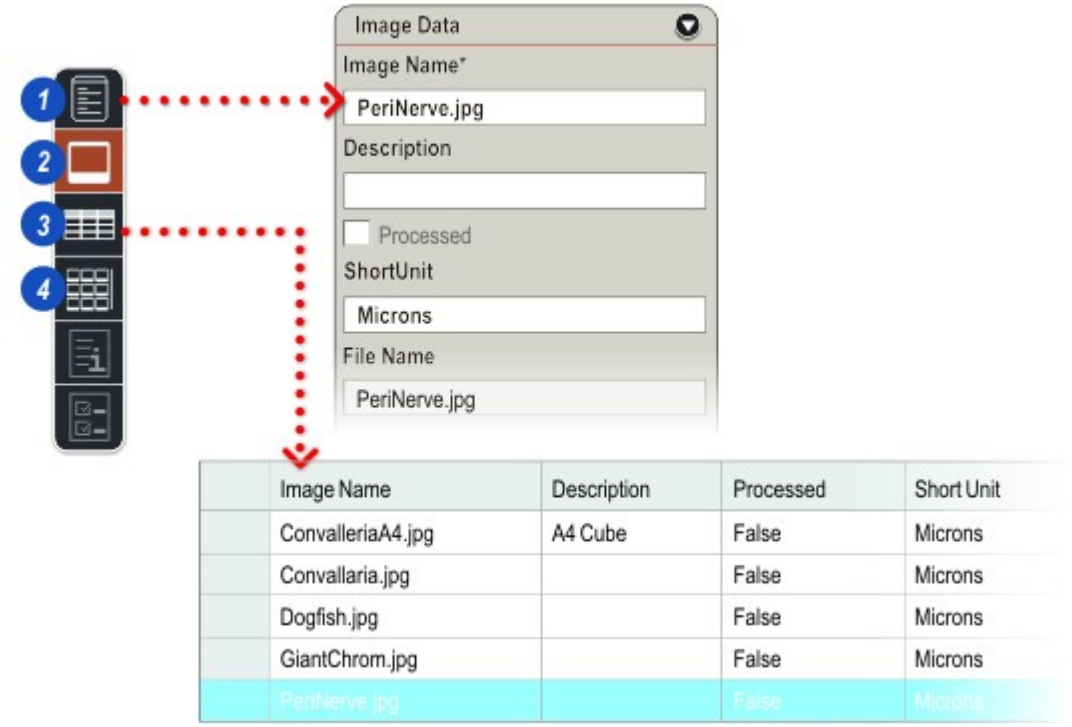
The various screen areas - *Viewer, Gallery, Report* and *Grid* (where applicable), may be revealed or hidden to create the best working environment for the user. Some tools are toggles – click once to reveal the area, click again to hide it:

1: *Hide/reveal the Record panels:* The *Image Viewer* expands to fill the vacant space.

2: *Hide/reveal the Viewer:* The *Record* panels expand to occupy the *Viewer* width.

3: *Hide/reveal the Data Grid:* The *Viewer* will expand to cover some of the vacated space. The *Grid* is only available if an *LAS Archive* is installed.

4: *Hide/reveal the Thumbnail Gallery:* The *Gallery* is hidden and the *Viewer* expands to include the *Gallery* space.



Dual Viewer:

The *Viewer* area can be split to show two images simultaneously.

1: Click the *Viewer Options* button.

2: Click to enable (tick mark visible to the left) the *Dual Viewer* option. The *Viewer* will then divide into two panes.

3: The live image currently being viewed will appear in the left-hand pane.

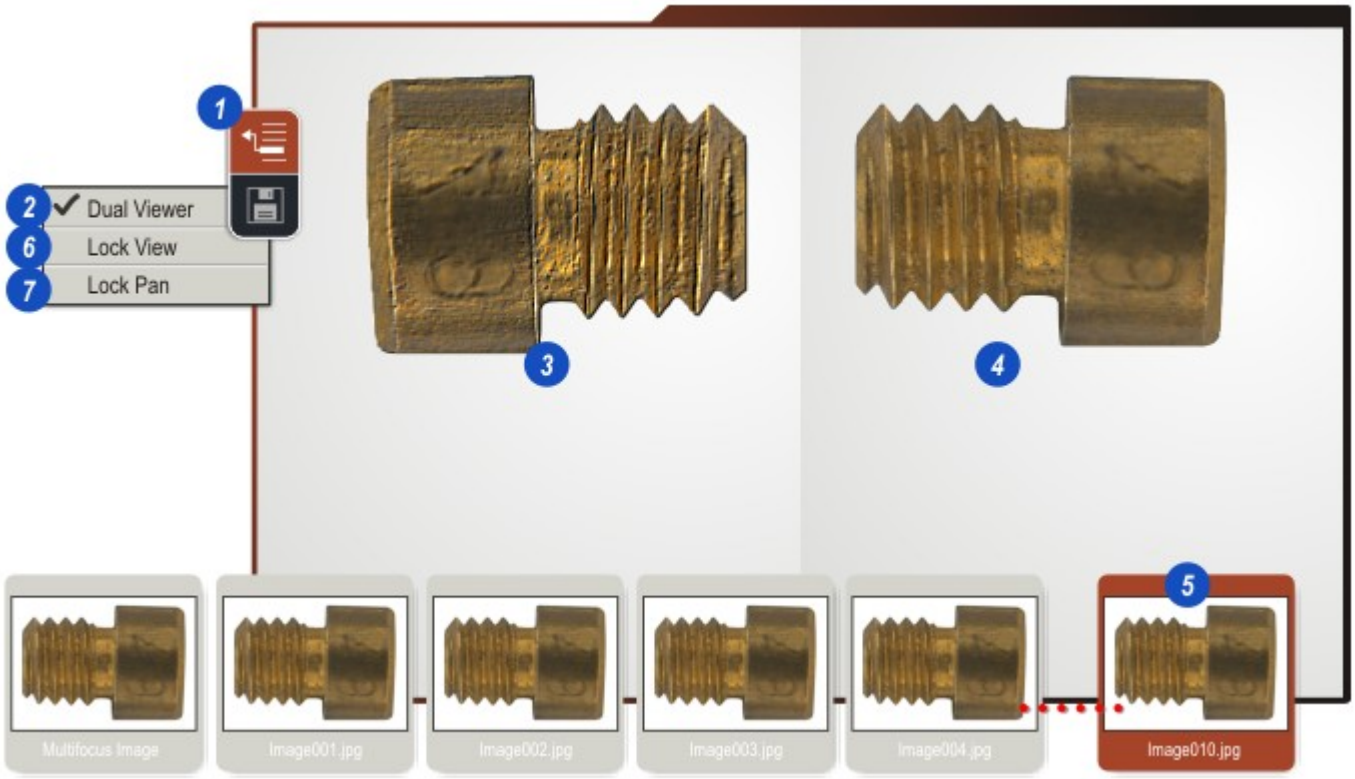
4: Display an image in the right-hand pane by clicking the pane and...

5: ...the thumbnail of the required image.

6: To synchronise the panes and enlarge or reduce the images as the zoom and fit tools are used, click to enable the *Lock View* option.

7: Enabling *Lock Pan* will synchronise the images as the *Pan* tool is used. Click the pane to pan and then on the *Panning* tool. Both images will automatically move to and display the image segment shown in the *Pan* window.

Dual Viewer more information: 79



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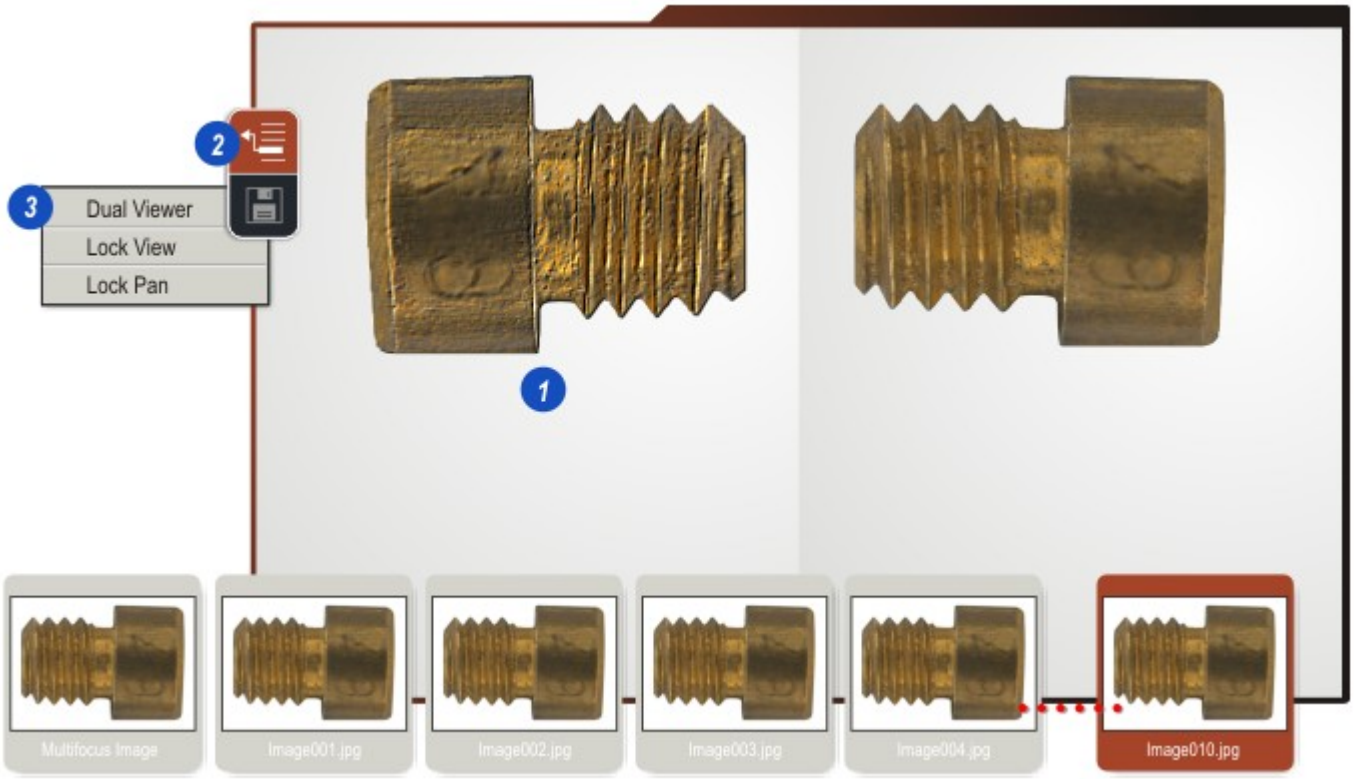
Turn Off Dual Viewer:

To revert to the live image occupying the entire *Viewer*:

1: Click on the left-hand *Viewer* pane - the current live image.

2: Click on the *Dual Viewer* button.

3: Click to disable *Dual Viewer* - the tick mark disappears - and the live image expands to fill the *Viewer*.



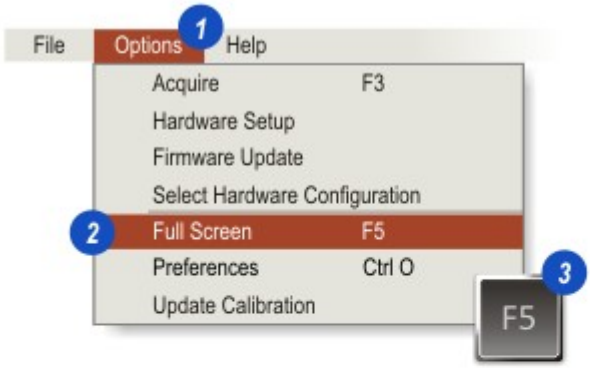
Full Screen Mode & Second Monitor:

Full Screen Mode: Single Monitor:

1: The *Viewer* area can be expanded to fill almost the entire screen by either clicking *Options* on the main menu and...

2: ...selecting *Full Screen* from the drop down menu, or...

3: Pressing *Key F5*. Press *F5* again to return to the normal display.



Second Monitor:

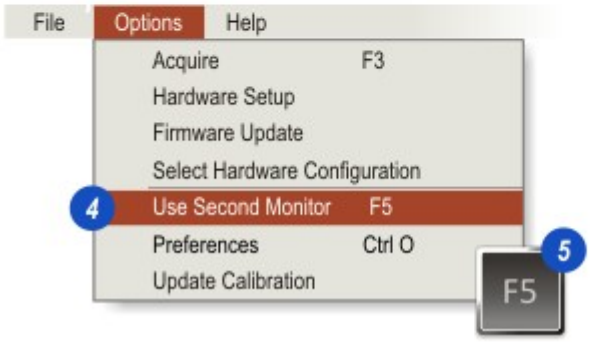
The software detects a second monitor and changes the *Options* drop-down menu to:

4: ... *Use Second Monitor*. Click the option to use both monitors.

The *Viewer* and image occupy all of the second monitor whilst the *Gallery* and *Thumbnails* together with the controls remain on the primary monitor.

The *Side Tool Bar* buttons are appropriately shared between the two monitors.

5: Alternatively, press *Key F5* to move between using both monitors and returning to single monitor.



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The Gallery:

The *Gallery* is a thumbnail display of images in the current folder in both *Image Explorer* or *LAS Archive.*

The *Gallery* can be hidden or revealed using the *Side Tool Bar* tools.

1: Clicking on a thumbnail will immediately display the full-sized image in the *Viewer* and the data associated with it in the *Record* and *Grid* (If LAS Archives is installed).

2: A *Slider* is automatically displayed for multiple rows of thumbnails - click and drag it to scroll the *Gallery*.

3: The *Navigation Bar* (bottom right of the screen) provides a way of moving through the thumbnails quickly and is especially useful with large galleries of thumbnails. Click on the arrows to move a single image **(3)** ...

4: ...or go to the extreme ends of the *Gallery*.

5: The thumbnails can be re-sized by clicking and dragging the *Scaling Slider* - slide left to reduce the thumbnail size and right to increase it.

6: Move the mouse over a thumbnail to reveal basic data bout the image.

7: Right-click a thumbnail to show the *Context Menu*. Left-click to select an option.

- *Gallery Docking Position* 168 168

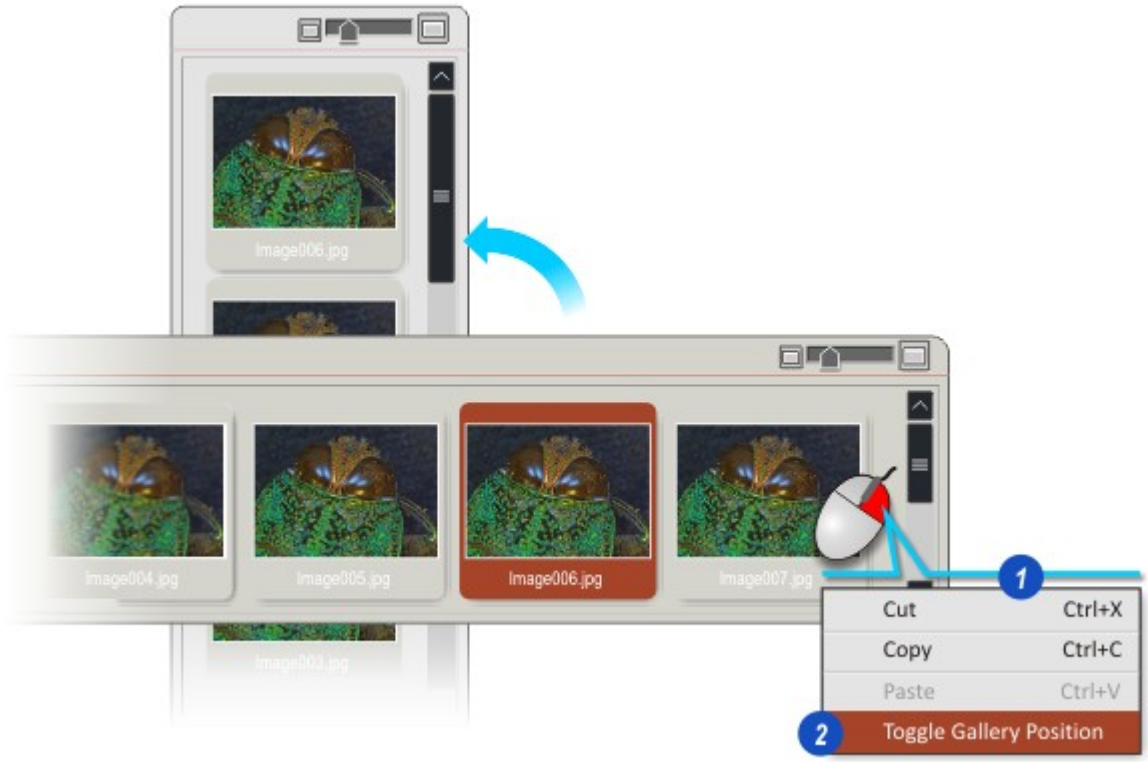
Available in *Acquire* and the *Process* Workflows as well as *Browse*, the thumbnail *Gallery* can be 'docked' either horizontally - along the bottom edge of the *Viewer* - or vertically - along the left-hand edge of the *Viewer* - to suit the user.

1: Right-click on a thumbnail or on the spaces around the thumbnails and...

2: ...from the drop-down menu, left-click to select the *Toggle Gallery Position* option.

The action toggles between horizontal and vertical docking.

Scroll bars, if required, are placed automatically.



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The *Grid* displays data for all of the captured images in a folder in a tabular structure. The image names are listed on the left and the data items as headers across the top.

In Acquire captured images are displayed in the right-hand pane when the Dual Viewer is enabled æ:

1: The *Grid* is revealed and hidden by clicking on the *Side Tool Bar* button. This is a toggle – click to reveal, click again to hide.

2: Clicking on an entry in the *Grid* will immediately display that image in the *Viewer* right-hand pane and also highlight the thumbnail.

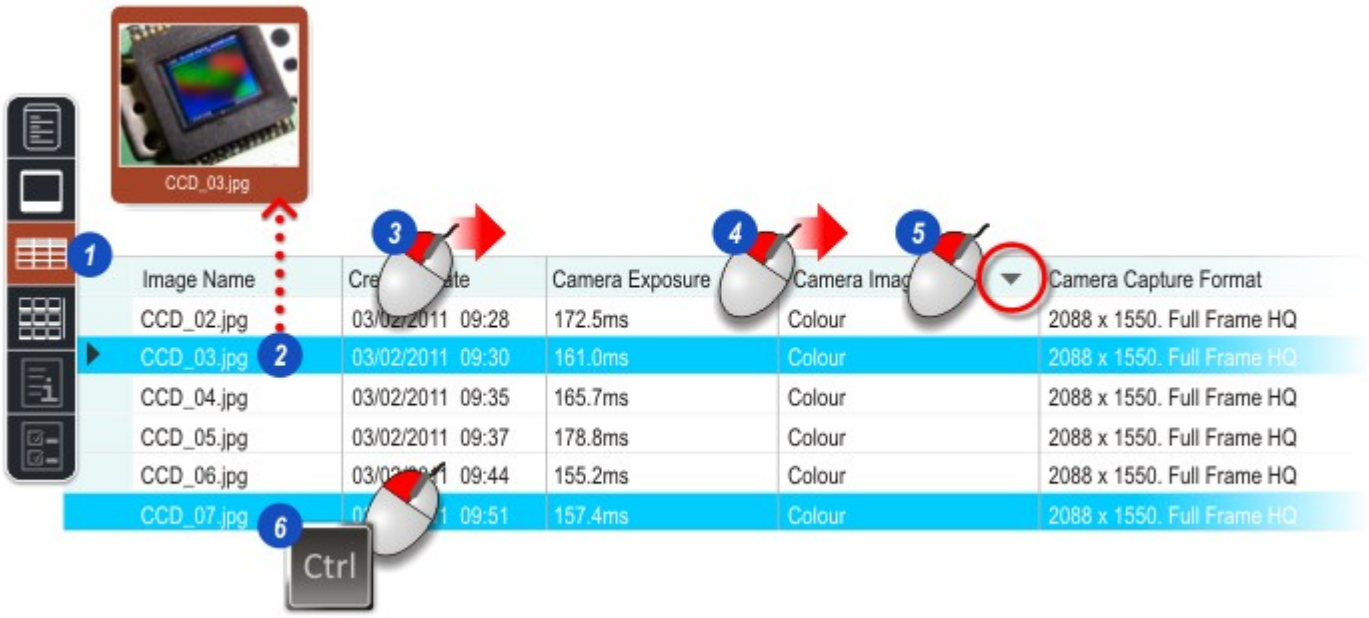
3: Header positions can be changed by clicking and holding the left mouse button on the header to be moved, dragging it to the new position and releasing the mouse button.

4: Column widths can be changed by clicking and dragging the vertical bars that separate the columns.

5: A small arrow is revealed when a header is clicked. This allows the image data to be sorted – high-to-low or low-to-high – by successive clicks on it.

6: To make multiple selections prior to cutting or exporting, hold down the keyboard *Ctrl* key whilst clicking individual thumbnails.

Keyboard combination *Ctrl + A* will select all of the image data.
Ctrl + C will copy all the selected image data to the clipboard.
Ctrl + V will paste into another application.

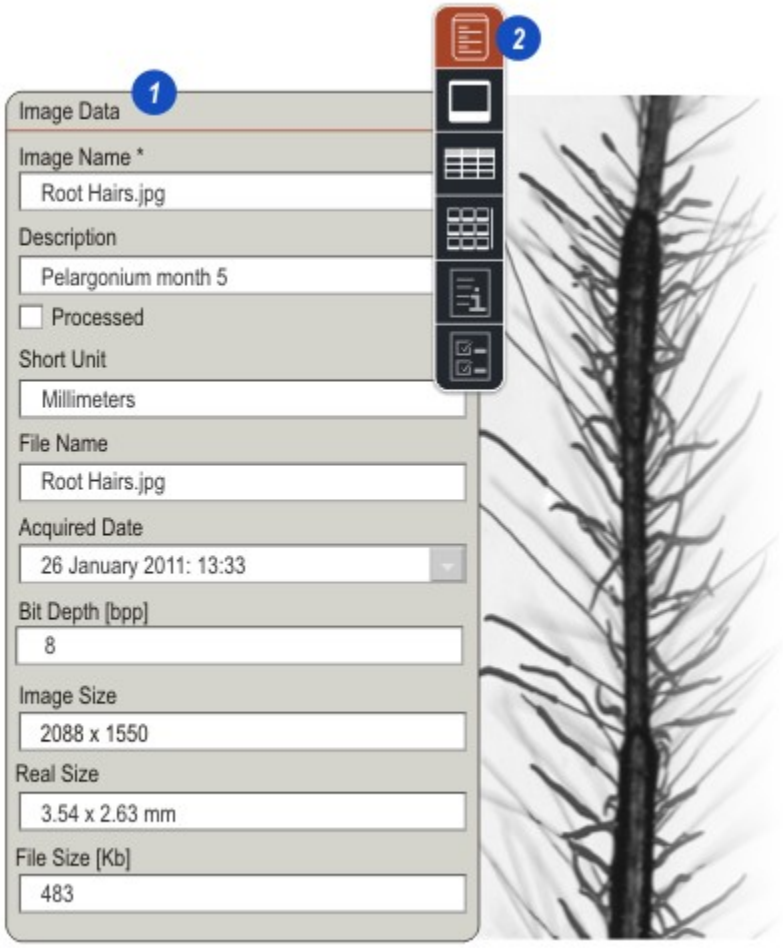


The Data Form:

1: The *Data Form* displays selected data associated with the image.

All of the data about the camera, microscope, exposure, creation date and so on, are actually stored and all can be displayed, but the Form can be configured to display only the more pertinent items.

2: The *Data Form* can be hidden by clicking the *Form* button on the *Side Tool Bar*. This is a toggle action - click again to reveal the *Form*.



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Acquire Image Controls:

The are three methods for starting image capture:

1: Click on the *Acquire Image* button at the bottom of the *Acquire Workflow*.

2: Press the keyboard *F3* function key.

3: Click on *Options* on the *Main Menu* and click the *Acquire Image* option **(4)**.

Stereo- and Macroscope Systems (SMS) is available on Leica Application Suite if an automated micro- or macroscope is connected to the computer.

SMS provides control of the micro- or macroscope from the computer and, if a digital camera is also fitted to the microscope will be especially helpful in optimising specimen images.

SMS can control:

- Motorised focus, 179
- Motorised zoom, 177
- X and Y stage positioning, 184
- Filter wheel, 176
- Zoom iris aperture, 181
- Zoom objective changer. 178
- Internal light source (TL), 183
- External light source (IL) and 182
- CCIC and Fluorescence shutters. 175

Five memory locations allow settings to be saved and recalled, precisely replicating the microscope setup.

Before using SMS, please check that the motorised focus cable is fitted, that the security clamp is properly fitted and in a position to prevent collision with the specimen and that all cables have sufficient slack to allow the carrier to travel to the top of the stand.

The following motorised/coded microscope devices are currently supported by LAS:

Schott KL 2500LCD (31 250 200 & 31 250 201)
Photonic CLS 150XD and CLS 150LS External light sources (30 111 480 & 30 110 481)
Motorised focus 300mm and 500mm (10 446 176 & 10 447 041)
M 165 C and M 205 C
MZ 16A microscope (10 447 103)
MZ 16FA microscope (10 447 063)
Z 6 APOA microscope (10 446 368)
EZ 4 D
M 205A, M 205FA, M 165FC
MST 51/MST 59 Motor Focus
TL RCI Internal light source (10 446 352)
UMC, Universal Manual Control (10 447 080)
Foot switch (10 447 398)
X/Y Motorised stage (10 447 305)

The following functions are automatically detected and can be controlled from SMS:

Internal and external light source intensity, On and Off
CCIC shutter Open and Close
Zoom magnification (Objective changer)
Motorised focus position
Fine focus position
Filter wheel position
Fluorescence shutter Open and Close
Zoom iris aperture.

The following manual microscopes and devices have reduced support in SMS:

MZ 16F (10 447 064)
MZ 16 (10 447102)
MZ 12.5 (10 446 370)
MZ 9.5 (10 446 272)
MZ 7.5 (10 446 371)
MZ 6 (10 445 614)
MS 5 (10 445 613)
M 125
S6D (10 446 297)
S8 APO (10 810 038)
Z16 APO (10 447 173)
Z6 APO (10 447 174)
Macrofluo
Fluocombi

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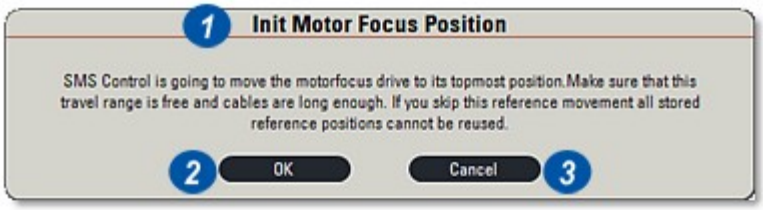
If, at start-up, LAS detects a motorised focus, it will ask to initialise the focus position by displaying the message (**1**).

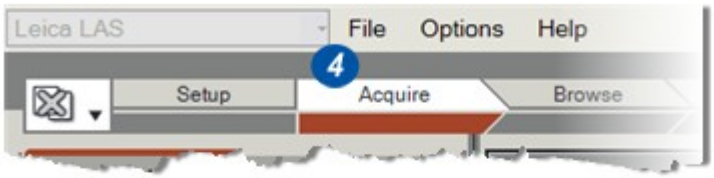
2: Click *OK* to send the carrier to the top of the stand and automatically initialise the focus position.

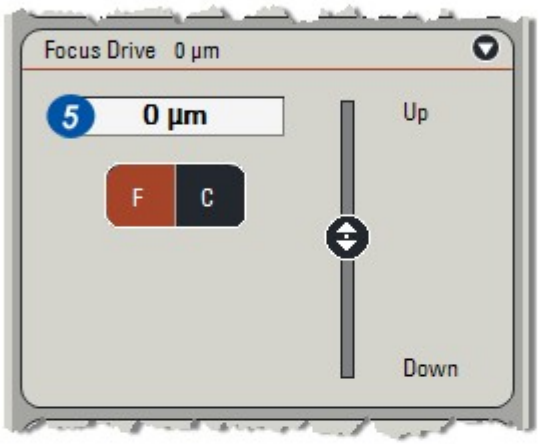
3: Click *Cancel* to skip the initialisation. If initialisation is skipped the exact focus position may not be reliable and will not be saved with other settings.

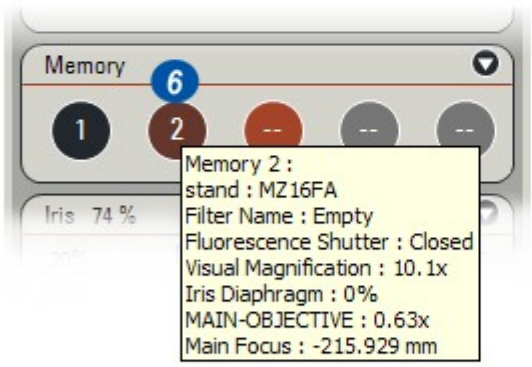
4: Click the *Acquire Workflow* to reveal the SMS control panels.
If focus was initialised the Focus Drive position (**5)** will display the current focus position.
If focus was NOT initialised, the value will be '0'.
Previous settings that were saved may be recalled and loaded by clicking the appropriate *Memory* button (**6**).

Continued... 174









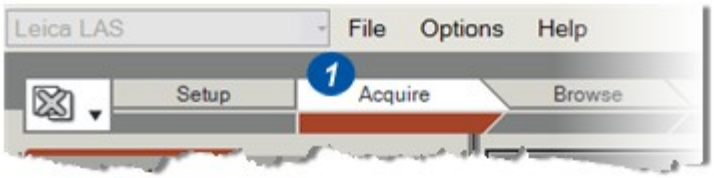
Revealing the SMS controls:
The Stereo- and Macroscope controls in the Leica Application Suite are revealed by:

1: Click on the *Acquire Workflow.*

2: Click on the *Mic1* tab. Depending upon the microscope and the functions available, the controls may be displayed on additional tabs named 'n' sequence - *Mic2* or *Mic3.*

The components of the Stereo- and Macroscopes must be setup in the Setup Workflow before the Acquire:Mic tabs can be used.

Continued... 175



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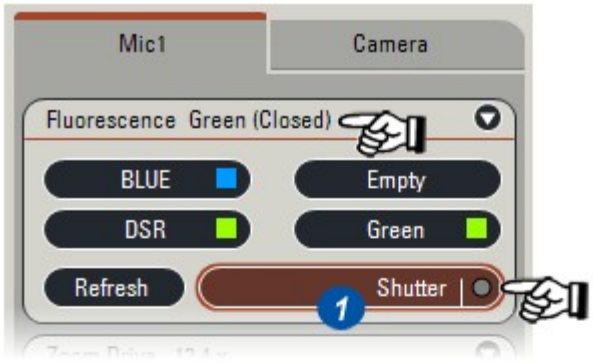
The Shutter button on the fluorescence panel opens and closes the shutter. Close the shutter when the microscope is not in use to protect delicate specimens.

1: Click on the *Shutter* button. This is a toggle action, the Shutter opening and closing on successive clicks.
The Shutter status (Opened or Closed) is shown on the Fluorescence panel header bar and when it is open a red dot appears on the button.

2: If an *Empty* filter wheel position is selected, the Shutter control is not available(**3**) and remains closed.

Continued... 176



1: Place the mouse cursor over a *Filter* button to reveal its specification (**2**). Click the button and the *Filter Wheel* will rotate to select the filter.

To change a filter:

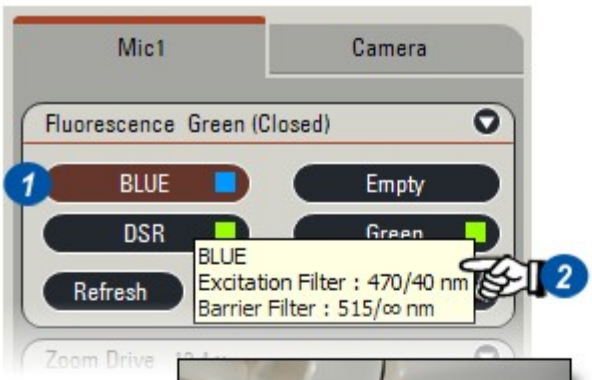
3: Manually turn the *Filter Wheel* to the required position. Each filter has an identifying tag on its rim. Carefully slide out the filter and insert the new one.

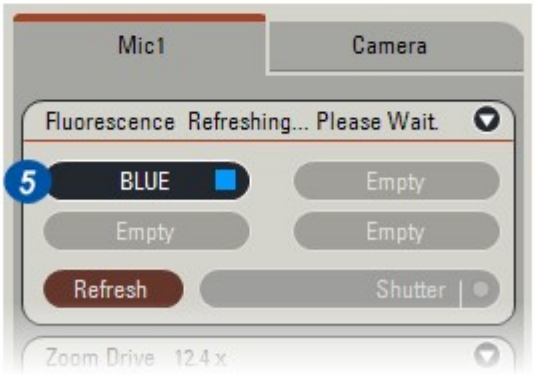
After filters have been changed:

4: Click on the *Refresh* button. All of the Filter button labels will clear, the Filter Wheel will turn and the fitted filters identified.

5: The button labels will be displayed with the correct filter data.

Continued... 177





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There are five control options for the Motorised Zoom:

Drag and drop:

1: Click and hold the *Scale Indicator* and drag it to the required zoom position. The shadow indicator (**2**) remains in the starting position until the mouse button is released.

Click on the Scale:

3: Click on the *Scale Bar* at the desired zoom position. The *Scale Indicator* will move to the selected position.

Type a value:

4: Click in the *Zoom Drive* text box and type the required zoom position. Values larger or smaller than the zoom limits will be ignored.

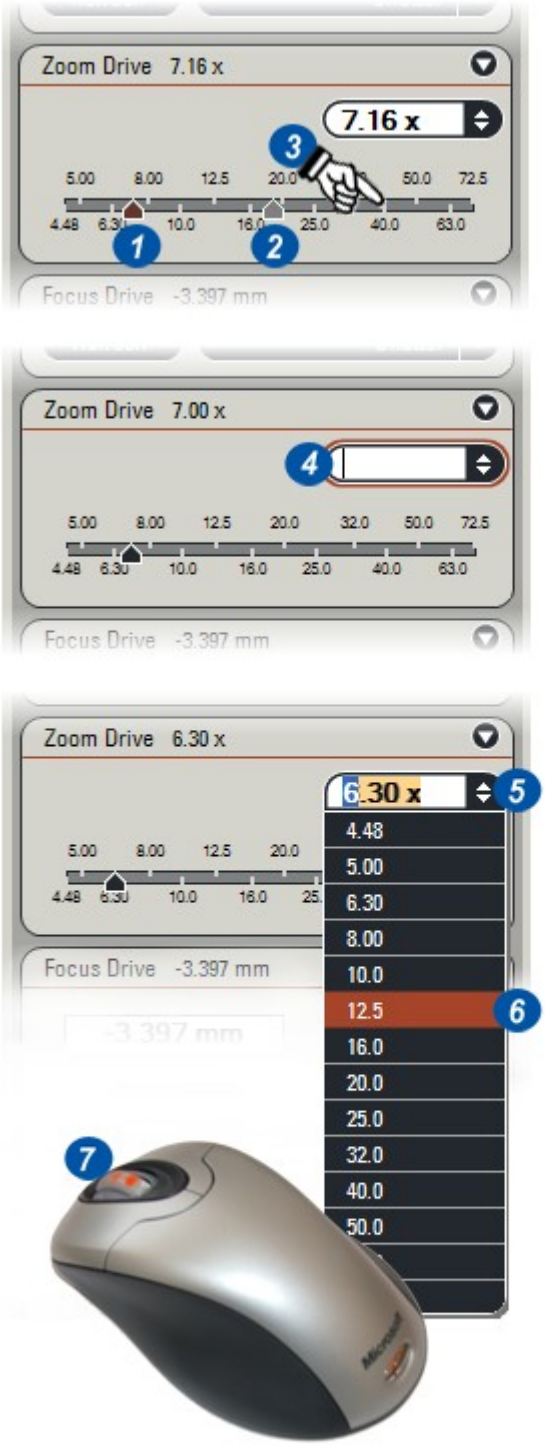
Preset positions:

5: Click on the arrows to the right of the *Zoom Drive* text box and from the drop down list click to select a preset position (**6**).

Fine adjustment:

7: Use the *mouse wheel* (if fitted) to move the zoom in small steps.

Continued... 178



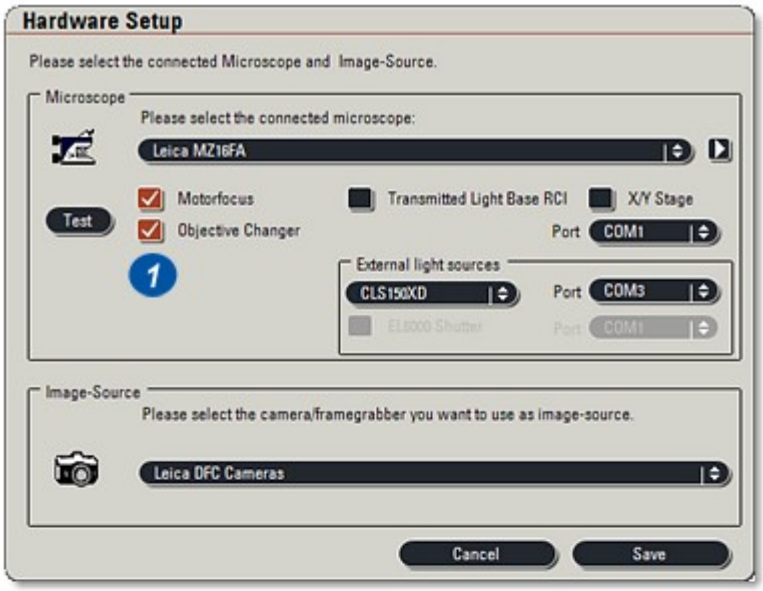
1: If the *Objective Changer* feature is fitted and is enabled on the Hardware Setup panel, High and Low magnification levels are available.

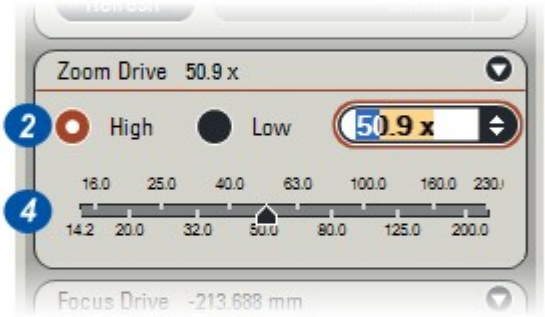
2: Click to enable *High* magnification or…

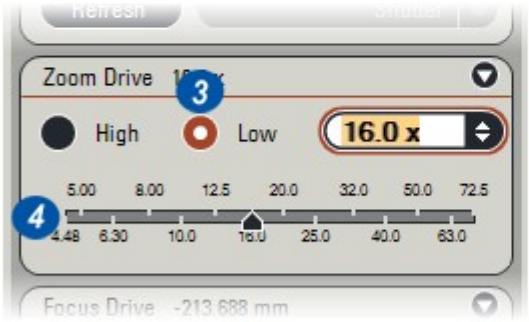
3: Click the *Low* magnification option.

4: The *Zoom Drive Scale* changes to reflect the magnification level selected.

See: Installation and Licensing Help for selecting hardware and the Hardware Setup panel.







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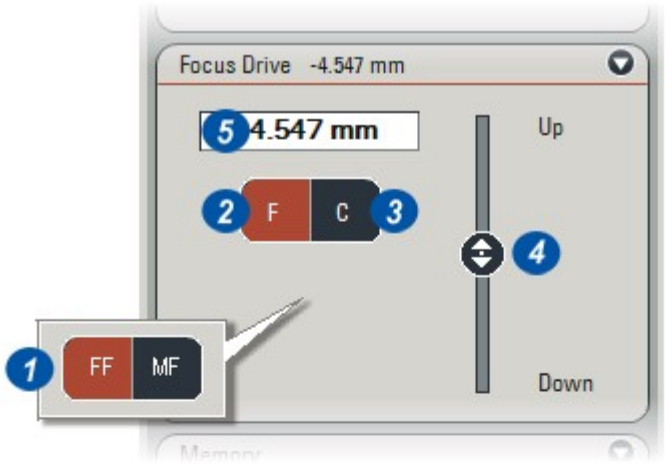
There are three options for driving the Motorised Focus:



Focus Control:

1: Fine focus (FF) and motor focus (MF) buttons are available with MF designated microscopes and Z6APO (A) and Z16APO(A) macroscopes.

2: Click on fine (F or FF) to move the focus in small increments.

3: Click on coarse (C or MF) to move the focus in large increments.

4: Click, hold and drag the *Focus Control* up or down. Release the mouse button when the desired focus is reached. Generally, start with coarse (C or MF) selected to get close to optimum focus and then select fine (F or FF) to 'tune' the focus position.

Type a position:

5: Click in the *Focus Position* text box and press the keyboard Delete key to clear the existing entry. Type a new value and press the keyboard *Enter* key.
The default units are micrometers (µm) but to move in millimeters type 'mm' after the value.

Mouse Wheel:

4: Click on the *Focus Control.*

6: Rotate the *mouse wheel* to move the motorised focus up or down. The focus step for each indent of the mouse wheel depends on the depth of focus and the zoom magnification.

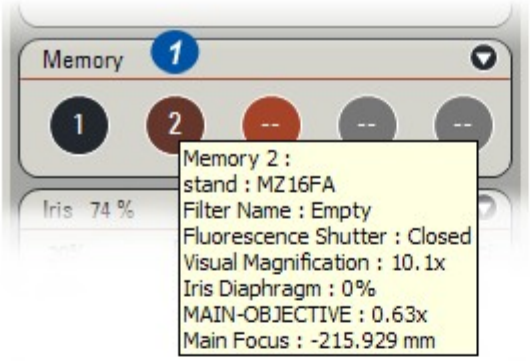
Continued... 180

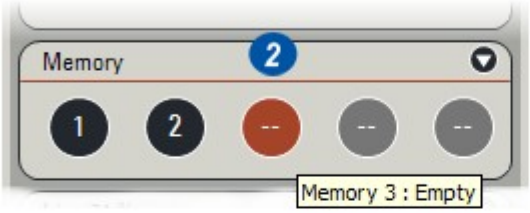
Current microscope settings may be saved in any one of five separate memory locations represented by a button and numbered 1 to 5.

1: Place the *mouse cursor* over a numbered memory button to reveal the microscope settings:
Motorised focus position,
Zoom position,
Filter selected,
Iris diaphragm setting,
Internal and external light source settings and
Motorised stage X and Y values.

Left click on the button to drive the microscope to the memorised values.

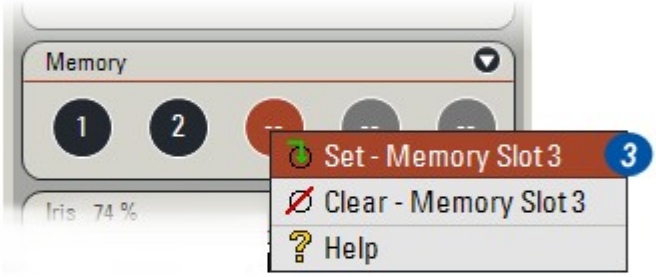
2: Empty *memory locations* are denoted by (--) on the button.



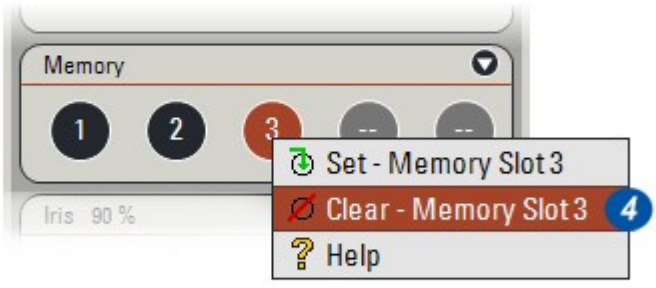


To save the current microscope settings:

3: Select a memory location - either Set or Clear - and right-click the button. From the drop down menu, click on *Set Memory*. The button will display the location number to indicate that the settings have been saved.



4: To clear a memory location, right-click on the button and from the drop down menu, click to select *Clear Memory.* The button number will be replaced with the empty (--) symbol.



Continued... 181

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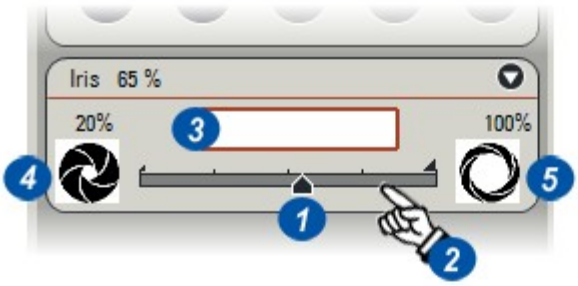
There are three methods for changing the Zoom Iris aperture:



1: Click, hold and drag the *Slider* to the left to close the iris, and right to open it. The aperture value is shown as a percentage (%) of fully open on the header bar. The minimum value is limited to 20%.

2: Click on the *Slider Bar* and the Slider will track to the selected position.

3: Click in the *Iris Setting* text box to select the existing value and press the keyboard *Delete* key to clear it: Type a new aperture value and press the *Enter* key on the keyboard. The maximum value is 100 and the minimum value 20.

4: Click on the *'closed iris'* icon to close the iris to 20% of full, or...

5: Click on the *'open iris'* to fully open the aperture.

Continued... 182

The CLS 150XD and 150LS external light sources may be controlled remotely from SMS:

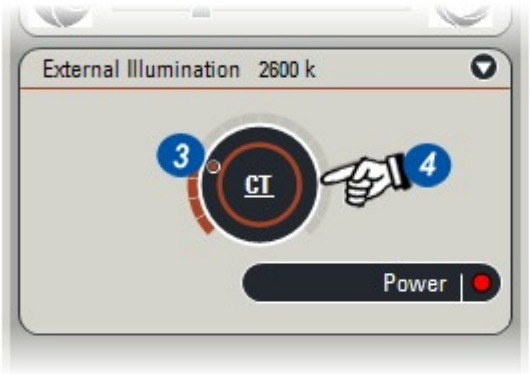
1: The *Brightness Control* may be rotated to increase or decrease brightness.

2: The light source may be turned on or off by clicking the *Power* button. A red dot indicates that the source is on.

3: Click, hold and drag the red 'handle' on the rotary control, clockwise to increase brightness, or anti-clockwise to decrease it. The light output value is displayed on the header bar.

4: Alternatively, click on the *outer rim* of the Brightness Control which will rotate to the selected position.

Continued... 183



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There are three controls for the Internal Transmitted Light Base:

- *CT (Colour Temperature)* which controls the lamp average voltage and therefore its brightness. This also affects the light colour.
- *CCIC (Constant Colour Intensity Control)* which acts like a window blind, reducing the amount of light reaching the specimen without affecting the colour.
- *Shutter* is a toggle action button which stops light reaching the specimen altogether.

The CT (1) and CCIC (2) controls work in combination so for some procedures it will be necessary to adjust the light colour using the CT control, and then adjust the brightness with the CCI control.

Both controls are adjusted in the same way:

1: Click and hold on the *red dot* on the periphery of the control. Drag either clockwise or anticlockwise to the desired position and release the mouse button. Or...

2: Click on the *outline of the control* and it will rotate to the selected position. The CT light colour (k) value and the CCIC opening (%) are displayed on the panel header.

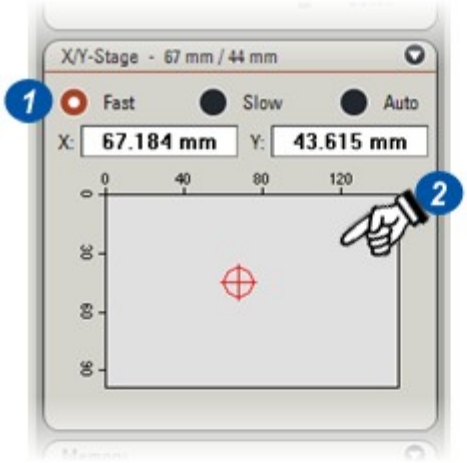
3: Click on the *Shutter* to open it as indicated by the red dot, or…

4: Click again to close it and the red dot disappears.

The Motorised Stage is represented on the control panel as a rectangle with rulers along the top and left side. Actual X and Y co-ordinates are displayed in two text boxes, and the stage initial traverse speed is selected using one of the three buttons:

- ***Fast***
- ***Slow***
- ***Auto*** for precise positioning within the field of view.

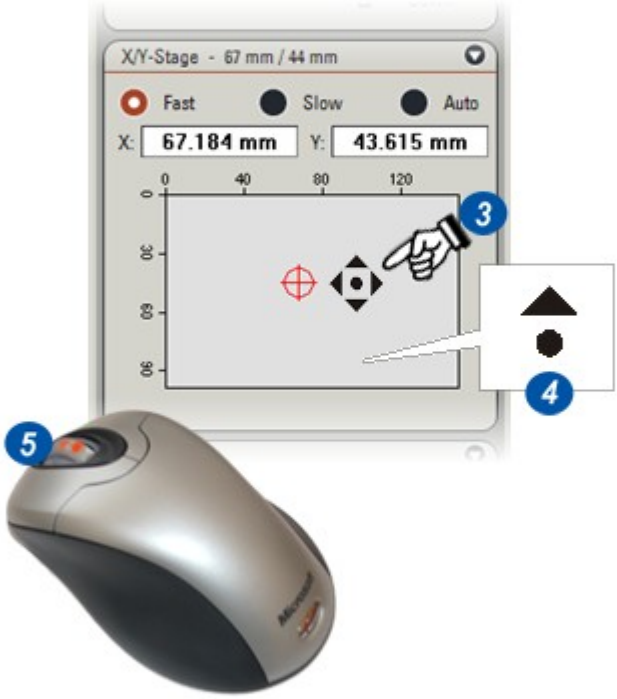
The traverse speed changes automatically as the stage nears the required position.



To move the stage to target:

1: Select the desired speed by clicking on the appropriate button. Choose *Fast* for longer distances.

2: Double-click *within the rectangle* approximately on the X/Y position required.
The *Target Marker* will move to the selected position and the stage will start to track toward it. As the stage approaches the Target the traverse speed will drop to *Slow* and within the Target, *Auto* (precise) will be selected.
Click in either the X or Y text box and then use the *Mouse Wheel* (5) to 'fine tune' the position in 2µm increments.



To move the stage interactively:

3: Click and hold *within the stage rectangle.* The *4-Way Arrow* appears.

4: Drag in the required direction. The Arrow changes to reflect the direction. The stage will follow the mouse position until the button is released. Stage traverse speed automatically slows as the mouse position is approached.

5: Click in either the X or Y text box and then use the *Mouse Wheel* to 'fine tune' the position in 2ìm increments.

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To move the stage to entered co-ordinates:
To be used when actual X/Y co-ordinate values are known. Both co-ordinates are entered in the same way:

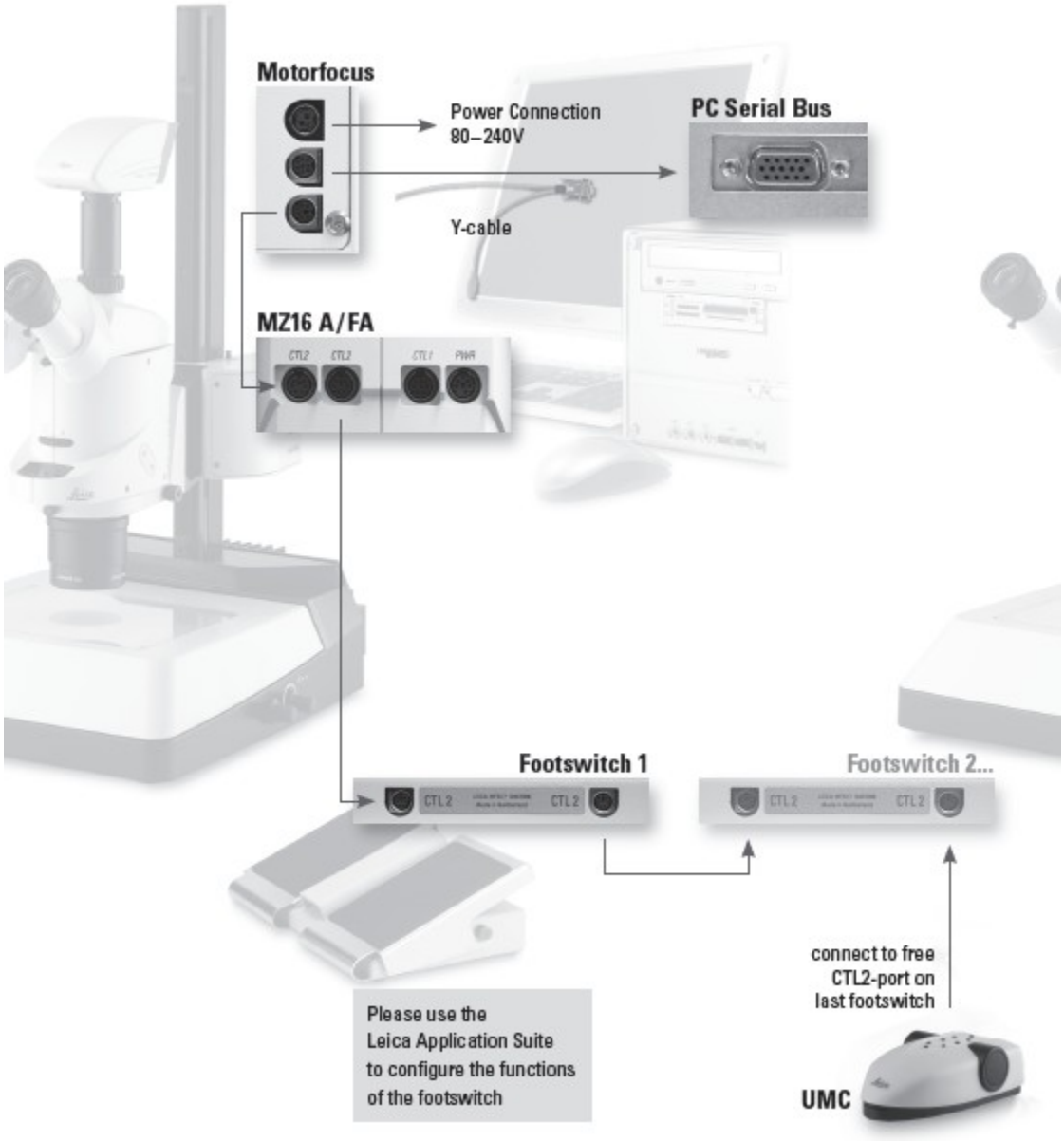
1: Click on the *X* or *Y* text box and type a new value.

2: For positions measured in *millimetres,* type '*mm*' after the value otherwise the units will default to µm.

3: Press the *Enter* key on the keyboard. The stage will go to the entered positions.

4: If necessary 'fine tune' the position in 2µm increments with the Mouse Wheel after clicking in either the X or Y text boxes.

5: Optional accessories such as the Joystick, SmartMove or UMC controls may be used together with the SMS controls.



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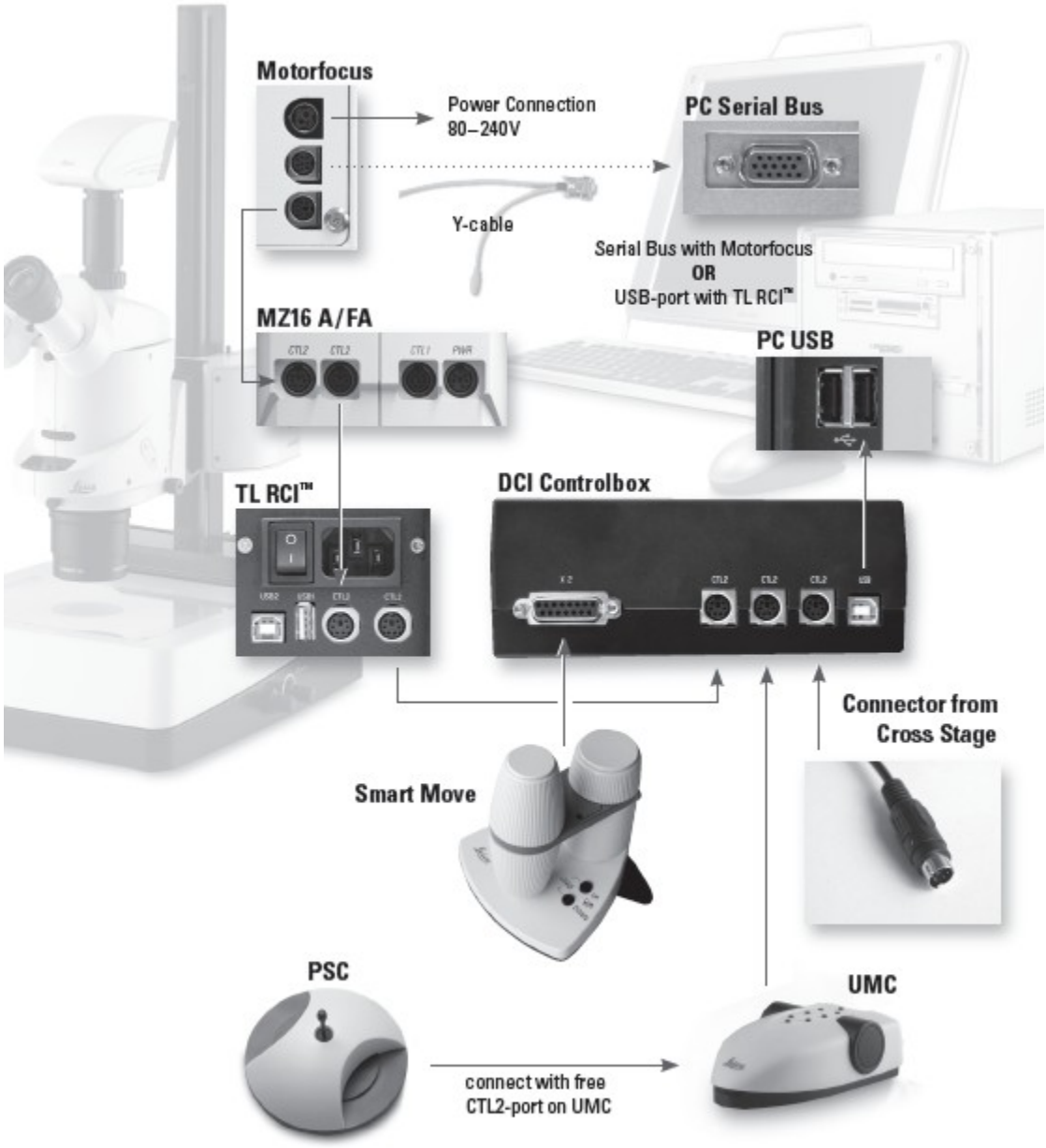
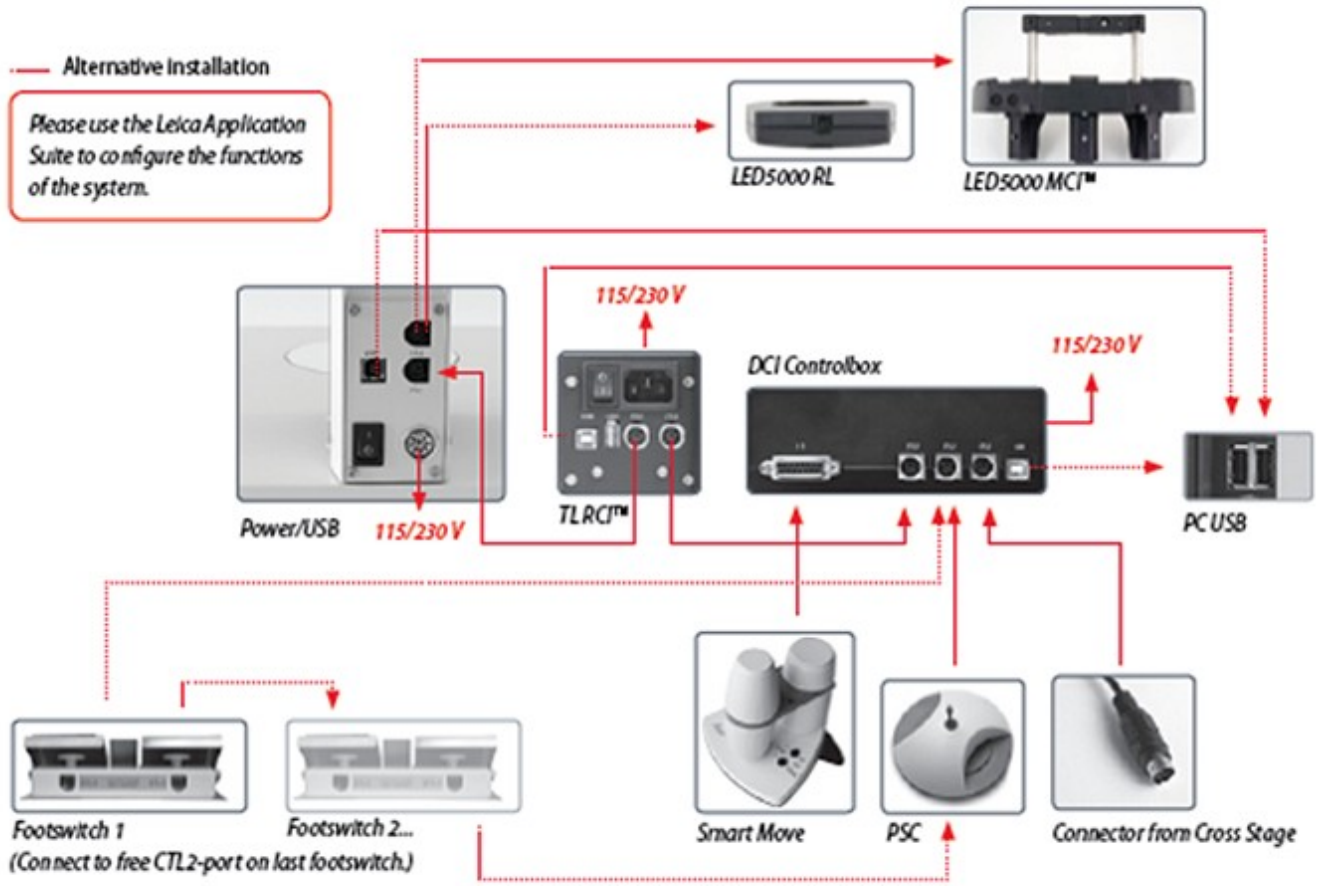

Motorfocus
Power Connection
80–240V
PC Serial Bus
Y-cable
Serial Bus with Motorfocus
OR
USB-port with TL RCI™
MZ16 A/FA
PC USB
TL RCI™
DCI Controlbox
Connector from
Cross Stage
Smart Move
PSC
UMC
connect with free
CTL2-port on UMC

Cables: Diagram



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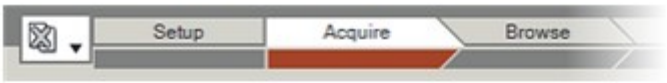
DM Control provides remote control for all motorized functions of the Leica DM microscope series.

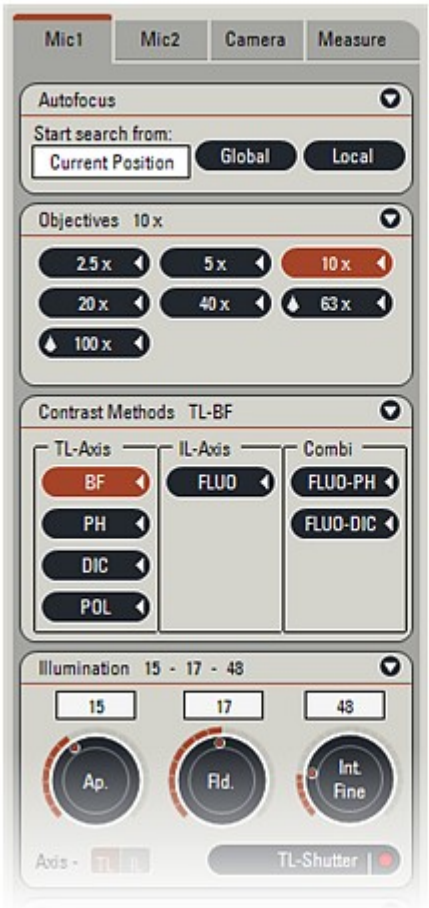
If a camera is attached to the microscope, the camera can be controlled at the same time.

DM Control consists of the following control control windows depending on the connected microscope:

- *Contrast Methods Control* 190
- *Fluorescence Control* 191
- *Illumination Control* 193
- *Objective Nosepiece Control* 194
- *Magnification-Changer Control* 195
- *Focusdrive Control* 196
- *Stage Control* 200
- *Motorised Tube Control* 197
- *Autofocus Control (optional module)* 199

The Multi-User Package has been designed especially for DM microscope users. It allows users to create profiles that store all of the hardware settings for a specific task, retrieve them later and automatically re-configure the microscope. *More about the Multi-User Package:* *Go there...*

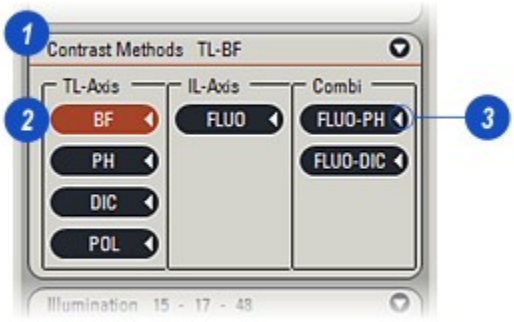




1: All available contrast methods for transmitted and incident light axis are displayed in the control window.

2: The current contrast method is highlighted on the control. Each contrast method can be selected by a left mouse click.

3: Appropriate contrast methods for the current objective in the light path are marked with a triangle ◀. If the selected contrast method is not valid 'Pseudo Bright Field' is applied.



Light rings, dark stops, DIC prisms, polarizer (mot.) and analyzer (mot.) are addressed automatically if necessary. Mechanical polarizer and analyser have to be inserted manually.

Note: For Combi-Contrast FLUO-DIC a manual analyser has to be inserted in the appropriate slot on the upper left side of the stand.

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1: All learned in *Fluo-Cubes* are listed in the control window.

2: The current fluo cube in the light path is highlighted on the control. *Fluo-Cubes* can be selected with a left mouse click.

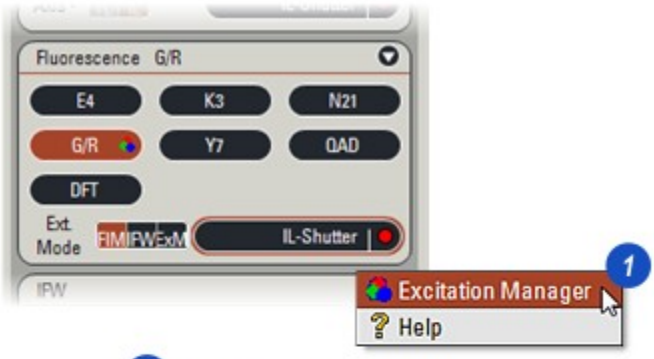
3: The *IL-Shutter* can be closed to protect the sample from bleaching.

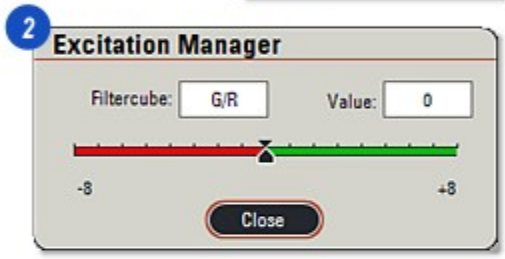
To learn in a new *Fluo-Cube*, please go to the *Setup Workflow.*

Motorized Excitation Manager (ExMan):
Allows the balancing of different fluorochrome intensities. Suitable for the following Leica dual and triple filter cube systems: *G/R; BFP/GFP; CFP/YFP; B/G/R, C/Y/R*

1: For access to the balancing slider select/ activate the appropriate dual or triple cube in *'Acquire'* and use right mouse key to open the *Excitation Manager C*ontrol Window **(2).**

Note: This function is only available if the microscope is equipped with the appropriate fluo axis.

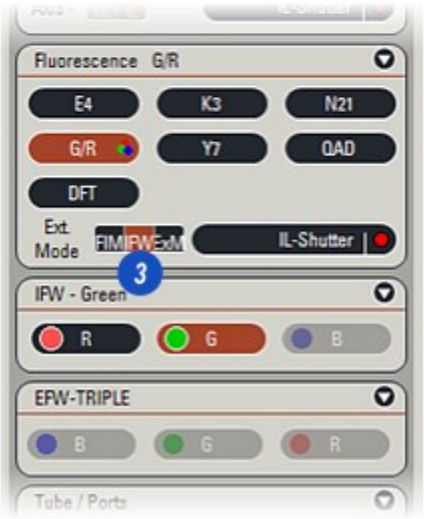




Internal Fast Filterwheel (IFW):
For fast excitation in red, blue, or green. Suitable for the following Leica dual and triple filter cube systems: *G/R; BFP/GFP; CFP/YFP; B/G/R, C/Y/R*

3: For access select/ activate the appropriate dual or triple cube in *'Acquire'*. The filter wheel positions are then directly accessible.

Note:This function is only available if the microscope is equipped with the appropriate fluo axis.



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1: The current settings for the active light-axis are displayed in the control.

2: Depending on which light-axis is activated *(TL or IL)*, light settings can be modified.

3: Use left mouse button, the mouse wheel or the cursor buttons to change the values for light *Intensity (Int)*, *Field Diaphragm (Fld)*, and *Aperture Diaphragm (Ap)* respectively. For precise adjustment of the light intensity, the Fine mode is automatically selected.

4: FIM (Fluorescence Intensity Manager) in *FLUO* mode: the intensity of the excitation light can be reduced in 5 steps to protect the sample from bleaching.

For *COMBI* mode *(FLUO/DIC or FLUO/Phase,* : use the tabs to switch between the control panel of *TL* and *IL* axis.

Mirrorhouse
To change the fluorescence illumination with the Mirrorhouse, move the mouse into the Illumination Window of LAS 'Acquire':

Press: Right Mouse Key.
Select: Mirrorhouse
Select: Light Path

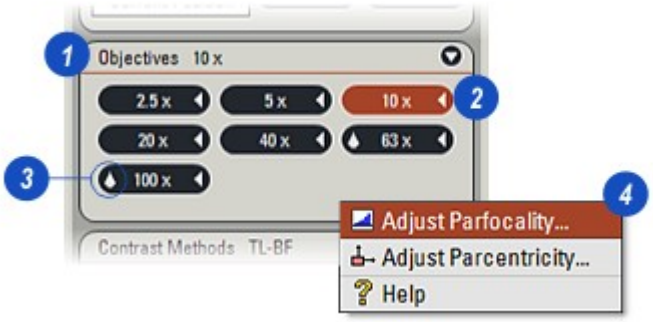
Note: Modifications in the light settings are stored and automatically recalled when the microscope in turned on.

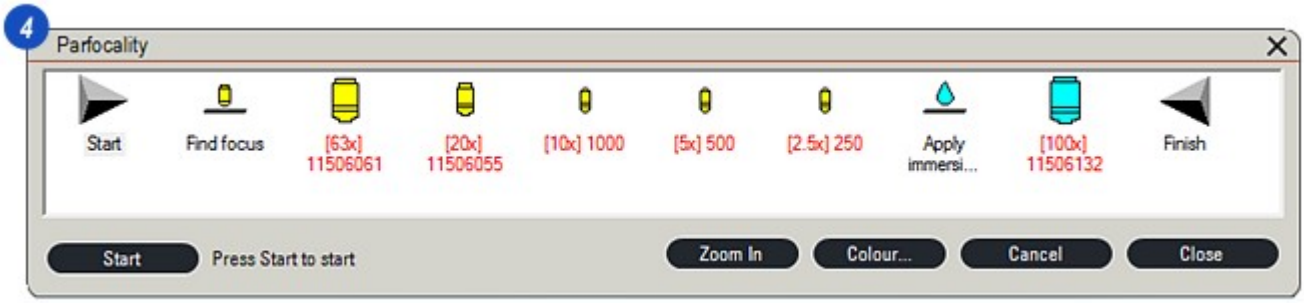
1: All learned in objectives are displayed in the control window.

2: The current objective in the light path is highlighted on the control.

3: Objectives which are valid for the selected contrast method are marked with a triangle.
Immersion objectives are marked with a black drop.

Objectives which have been learned in as combi-objectives (module 'Fine tuning') are marked with a clear drop.





The selected objective blinks if you are changing the mode from *DRY* to *IMMersion* and vice versa. The stage is lowered and you have to confirm the change of mode with an additional mouse-click.

4: *Parfocality* can be adjusted using the context menu of the *right* mouse button. This will start the *Parfocality* wizard **(5)**. It is recommended that the *Parfocality* of all listed objectives is adjusted if new objectives are learned in.

To learn in new objectives please go to the *Workflow Setup.*

Each objective button shows small status icons:

Marks an objective, if it is valid for the currently selected contrast-method.

Marks Immersion-Objectives (Oil, Water, Glycerine).

Marks Combi-Objectives (for use in both modes, Immersion- and Dry-Mode).

Starts the Parfocality Wizard

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1: The current value of *Magnification Changer* is highlighted in the control window.

Since the *Magnification-Changer* is not motorized remote control is not possible

Note: The values of the Magnification-Changer are used for the calculation of the total magnification (see LeicaScreen).

1: The current position of the Z-Focus is displayed in the control window.

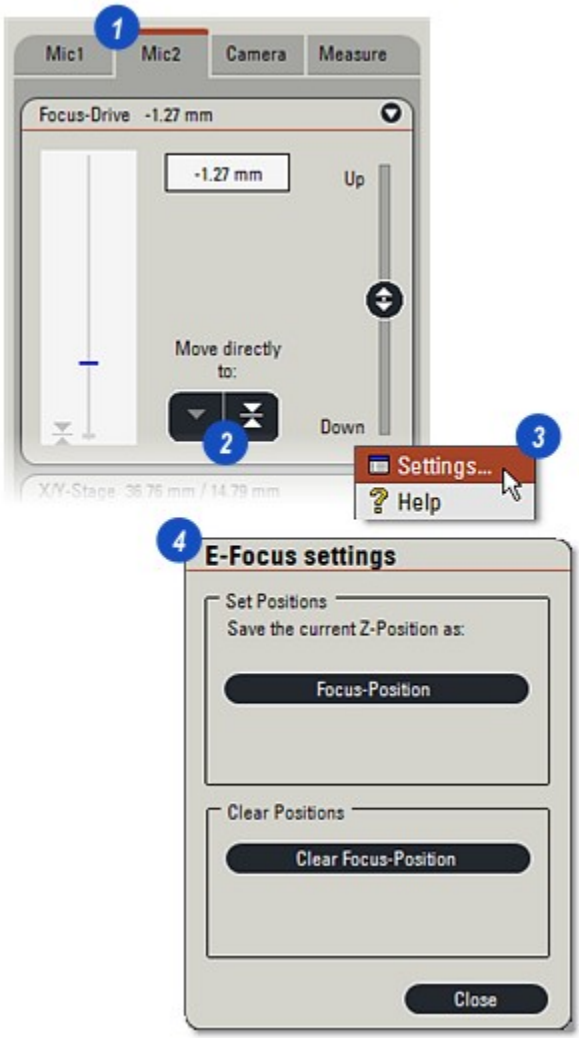
2: Lower threshold and focus position are displayed as icons in the control window and can be recalled. Press the appropriate button until the threshold or focus point is reached.
The control window uses the following status icons:

Focus-Position
Lower-Threshold

3: Please use the right mouse-button to get a context-menu for additional functions:

Advanced Settings, define/clear the lower threshold and the focus-position

4: Lower threshold and focus position can be deleted or set in the *Advanced Settings*.



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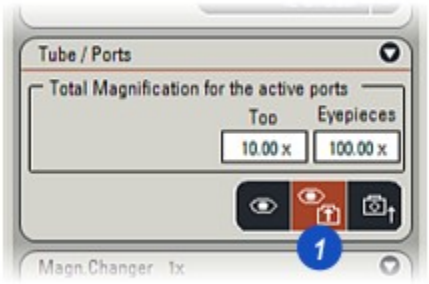
1: The current beam-ratio is displayed is highlighted in the control window. The beam-ratio for a motorized tube can be selected.



Each button on the control shows a small status icon:

 *The light path is set to 100 % visual exit (eyepieces).*

 *The light path is set to 100 % camera port.*

 *The light path is set to 50% / 50%.*

Note: After turning on the microscope the light path is automatically set to 100% visual exit (eyepieces).

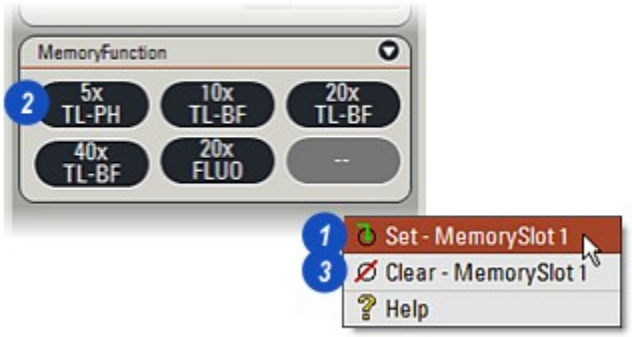
Only available for DM6000.

Allows to store the current combination of objective and contrasting method (e.g. 20x /D).

1: Using the *right* mouse key ('settings') the current combination can be stored...

2: ...available combinations recalled from the memory.

3: Clear the data on the selected memory position.



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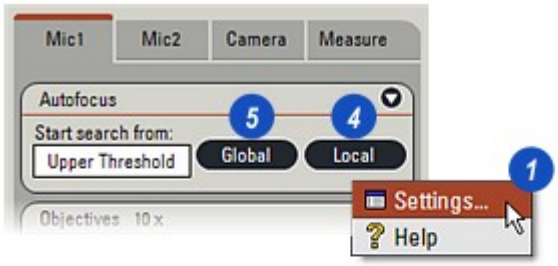
This is an optional module and needs to be licensed before use.

The cameras supported by digital *Autofocus* are listed in the document 'Systems Requirements'.

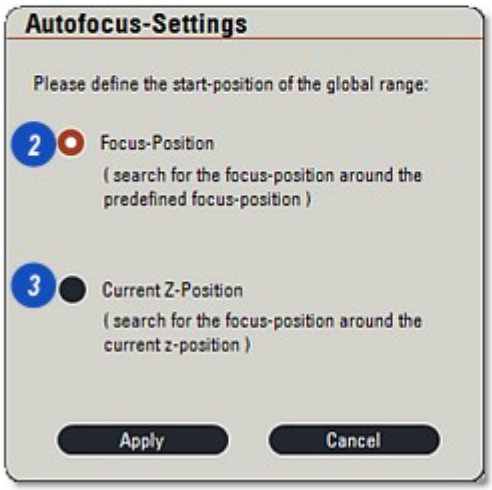
By calculation the digital *Autofocus* finds a reasonable focus plane and adjusts the Z-focus level automatically. For proper functionality please adjust the *Parfocality* values for each objective.



1: Depending on the samples two appropriate modes can be selected:

2: Search starts from pre-selected focus: suitable for sample with approximately the same thickness. Adjustment of *Parfocality* is a prerequisite for this mode.

3: Search starts from the current Z-position: suitable for samples with variable thickness.



Within each mode the span of search can be selected:

4: Near: Search of focus position considers only the near proximity.

5: Global: Range of search is extended.

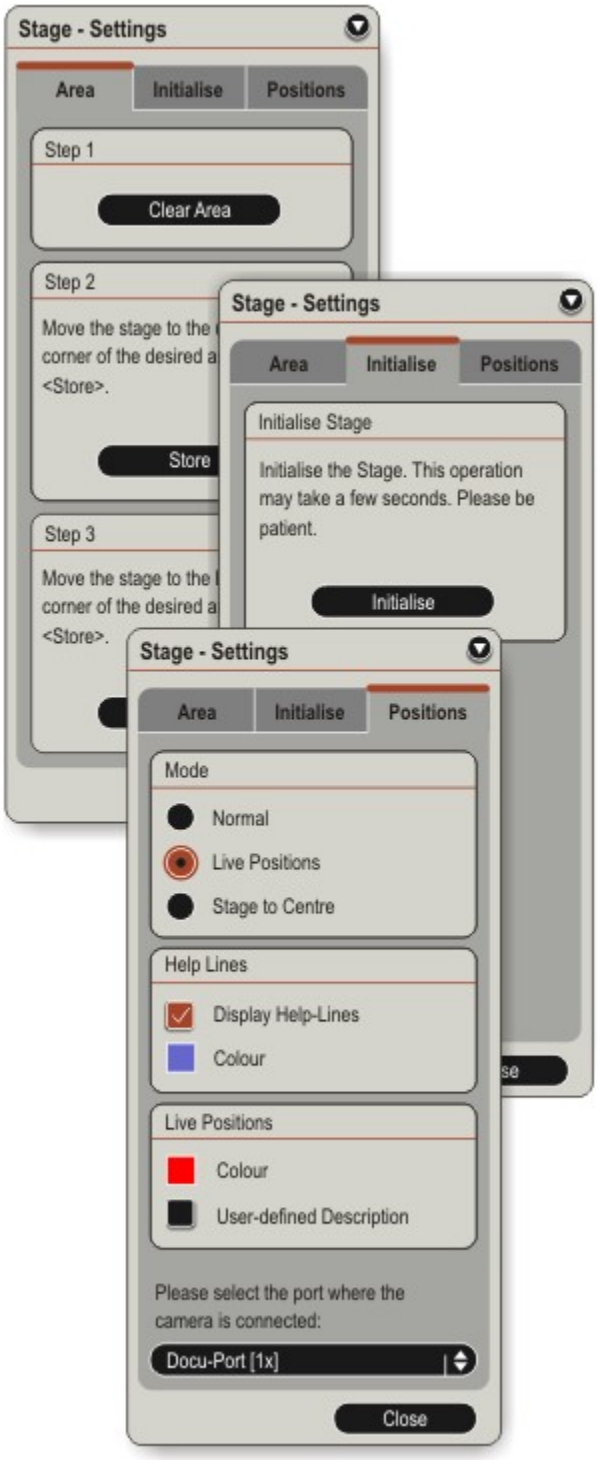
Note: For correct operation please install the newest camera driver. It is highly recommended to adjust the exposure time of the camera to values < 10 msec to ensure fast response time.

The Stage Settings panel contains three tabbed dialogs that allow the user to initialise and configure the stage working features.

The tabbed panels are:

- Initialise: Sets the top-left (X = 0 and Y = 0) and bottom-right co-ordinates of the stage limits. It will not drive beyond these co-ordinates and all of the other positioning features are measured from them. *Go there.* 201.. 201
- Area: Allows the user to setup a defined working area within the stage limits. The stage will not be driven beyond the boundaries of the user *Area*. *Go there.* 202..
- Positions: Provides the *Mode* - *Live* and *Stage to Centre* - settings that define how the stage will be driven to specific points; Controls the colour and visibility of the *Help Lines* - guides that show the stage centre; If *Live Positions* will be displayed with a number or a user description and their colour, and the nominated camera port for microscopes with several port options. *Go there.* 204..

Continued... 202



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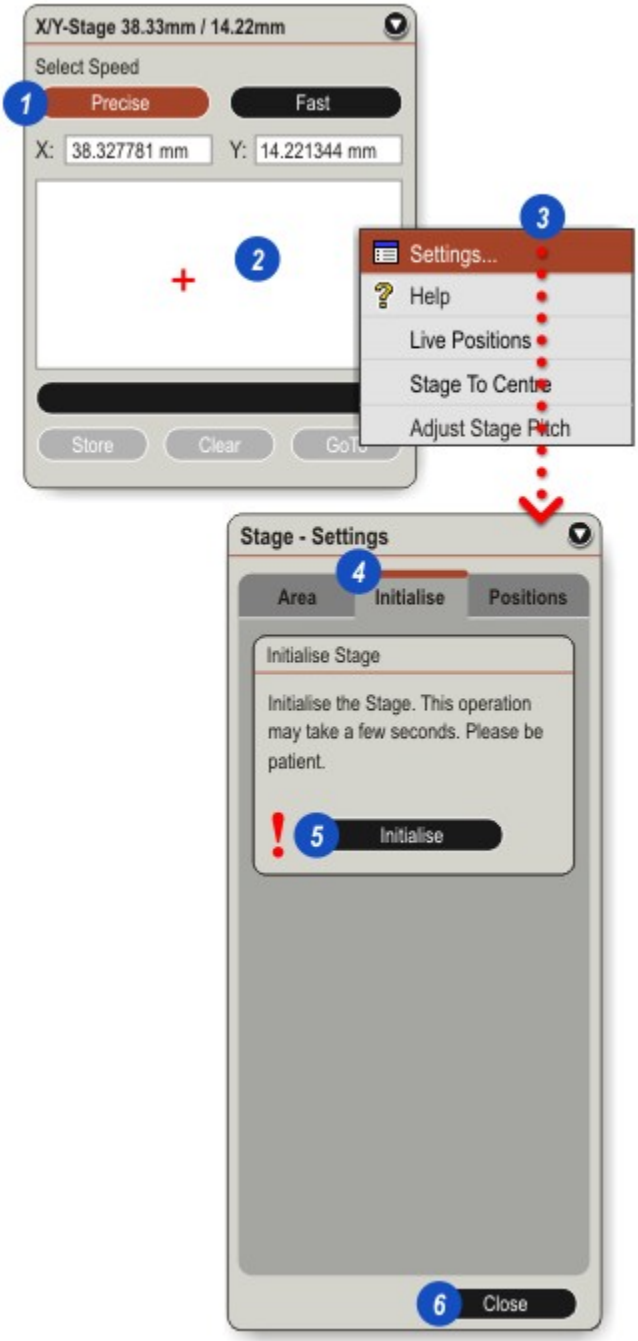
1: The speed of the stage can be modified by clicking the control buttons *'Fast'* or *'Precise'*. The *Fast* option uses the settings for pitch entered in:

Setup Workflow > Stage > Fine Tuning Tab

The *Precise* option drives the stage more slowly to avoid 'overshoot' and maintain positioning accuracy.

2: The Stage Settings are accessed by right-clicking the stage area and...

3: ...clicking to select the *Settings* option on the context menu.



Initialise the Stage:

4: Click to select the *Initialise* tab.

! *Check that the stage is clear of specimens and slides. Make sure the objectives or magnifiers will not collide with the moving stage and that the connecting cables have sufficient slack to allow the stage unrestricted movement.*

5: Click the *Initialise* button. The stage will drive to the top-left corner limit switches to establish *X = 0* and *Y = 0*. It will then travel to the bottom-right to set *X Max* and *Y Max* travel.

6: Click *Close*.

Continued... 202

1: The *Stage Settings* are accessed by right-clicking the stage area and...

2: ...clicking to select the *Settings* option on the context menu.

Area:
Within the extreme limits of the stage movement, an area that (usually) contains the image, can be user-defined - the stage will not drive beyond the boundaries of the setup area. To create a user area:

3: Click to select the *Area* tab.

4: Click the *Clear Area* button to clear any existing user settings.

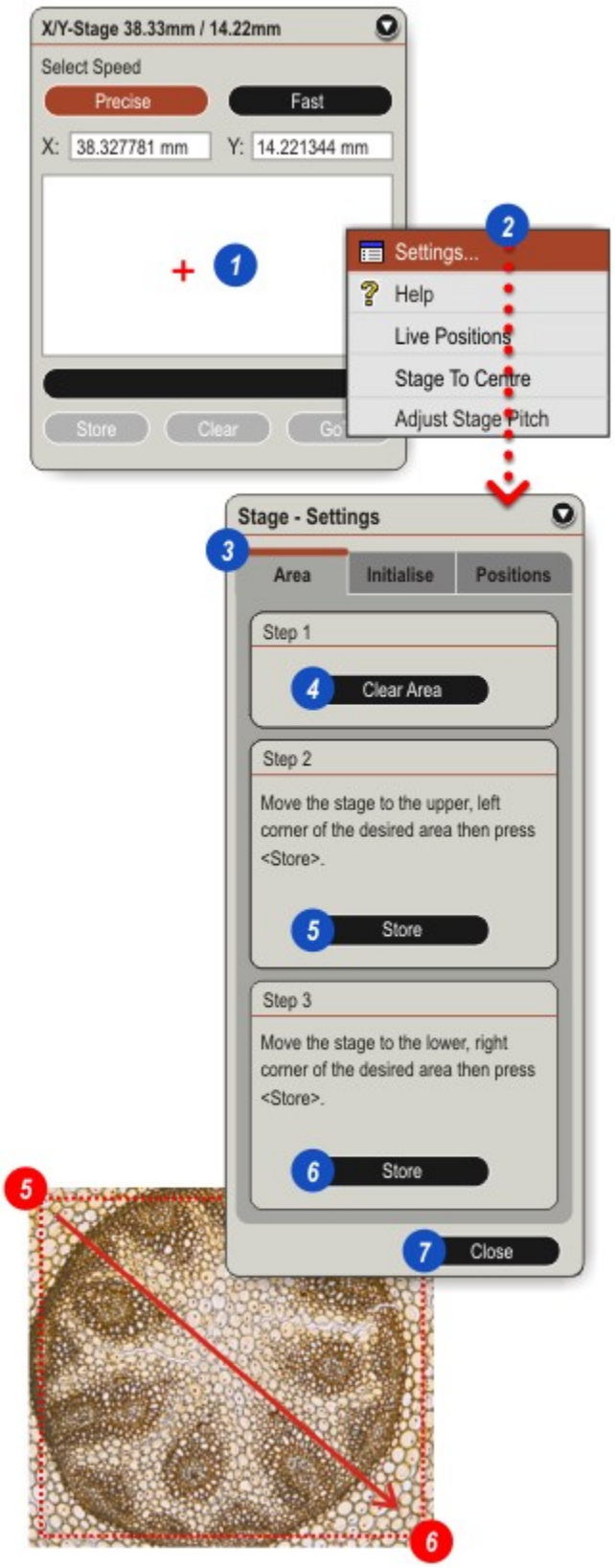
5: Drive the stage using the stage manual controls or *SmartMove*, to the *upper-left corner* of the user area. This is usually set with reference to the image. Click on the *Step 2* > *Store* button to save the X/Y position in memory.

6: Repeat the process but this time driving the stage to the *bottom-right corner* of the user area and click *Step 3* > *Store*.

7: Click the *Close* button.

Continued... 203

Note: The Area boundary co-ordinates are not stored permanently and are lost when the microscope is switched off.



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1: The *Stage Settings* are accessed by right-clicking the stage area and...

2: ...clicking to select the *Settings* option on the context menu.

Positions:
The *Positions* tab displays the controls for setting up the *Help Lines (Crosshairs)* and *Live Position* markers as well as selecting the current mode:

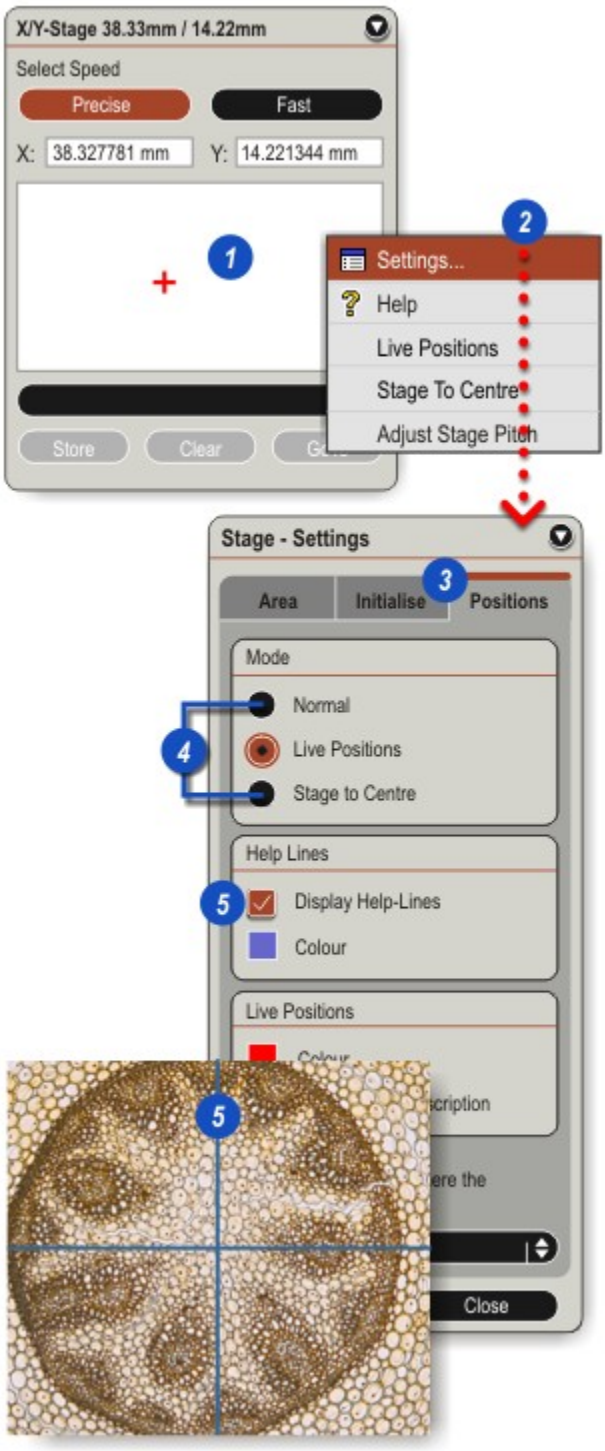
3: Click to select the *Positions* tab.

4: There are three *Mode* options selected by clicking the appropriate button:

- Normal: Disables both *Live Positions* and *Stage To Centre* and hides the *Help Lines.* This is normal stage operation.
- Live Positions: Allows up to 15 individually selected positions to be marked on the image. The stage can be driven directly to a selected position. The *Help Lines* are displayed if they are enabled.
- Stage To Centre: Drives the stage centre directly to a point indicated on the image by a double-left click. If *Help Lines* are enabled they are displayed and positioned above the point.

Live Positions and *Stage To Centre* are mutually exclusive - only one can be enabled and active. They can also be selected from the context menu **(2)** by clicking the required option.

5: The *Help Lines* - crosshairs that indicate the centre of the stage - can be turned on or off by clicking the checkbox. This is a toggle action with a tick mark displayed when the lines are enabled and visible.



Continued... 204

1: The colour of the *Help Lines* and...

2: ...that of the *Live Position* markers can be changed to suit the user by clicking on the *Colour* box and...

3: ...from the *Select Colour* dialog choosing a colour from the *Basic Colours* or...

4: ...dragging the 'target' to the required position on the colour wheel.

5: Adjust the selected colour intensity by dragging the slider along the graduated bar.

6: Click *OK* to save the colour.

7: To display an appropriate description next to a *Live Position* marker, click to enable the *User-defined Description* check box. This is a toggle action - click again to turn off the description display.

Continued... 205

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The active port option is for microscope models that have more than one port and allows the user to select the port that has the active camera attached.

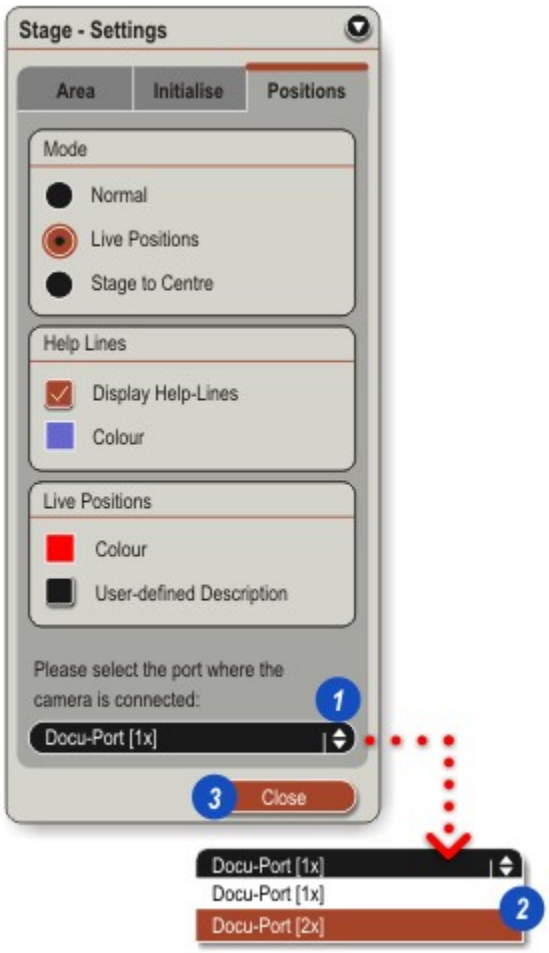
To select an active port:

1: Click on the small arrows to the right of the *Port* header.

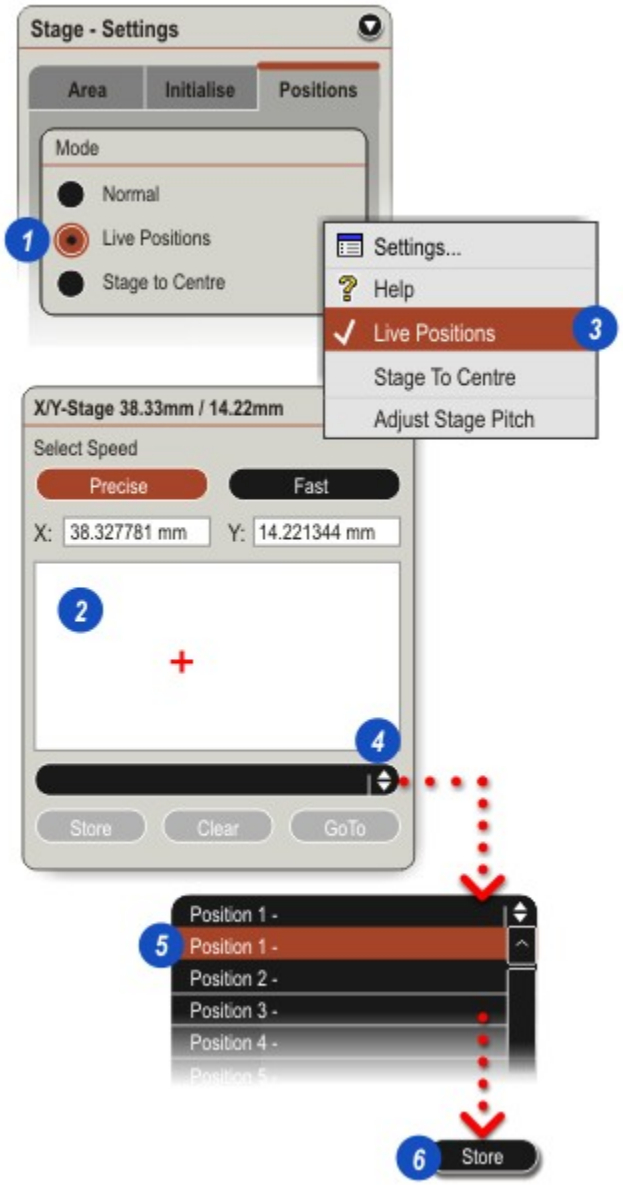
2: From the drop-down menu click to select the port that has the active camera attached.

3: Click the *Close* button to close the *Settings* dialog.

Continued... 206



The *Live Positions* feature allows the user to select up to 15 individual points on the image storing each either as a set of *X/Y* co-ordinates or as a *User Description* on a *Position List*. The *Description* option is enabled under *Stage Settings* (*Go there...* 204). The stage can then be driven directly to a point by selecting it on the *Position List* and clicking the *GoTo* button.



1: If the *Live Positions* option is not already selected on the *Stage Settings* > *Position* > *Mode* panel,

2: ...right-click inside the stage panel and...

3: ...from the context menu click to select *Live Positions*. A tick mark will appear along side the option.

4: Click on the small arrows to the right of the *Positions* header.

5: On the drop-down list, click to select an empty position. If necessary use the scroll bars to extend the list. Positions cannot be overwritten - they have to be cleared first (*Go there...* 210).

6: The *Store* button becomes enabled. Do not click it yet.

Continued... 208

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1: If *User-defined Description* has been enabled on the *Stage Settings* dialog *(Go there..* 204*)...*

2: ...move the stage so that the point of interest on the image is directly beneath the *Help Lines* intersection*. (The green square on the illustration is for indication only and does not appear in use).*

3: Click the *Store* button.

4: The *Position Description* entry dialog appears. Click in the text box and type a unique name for the for the point.

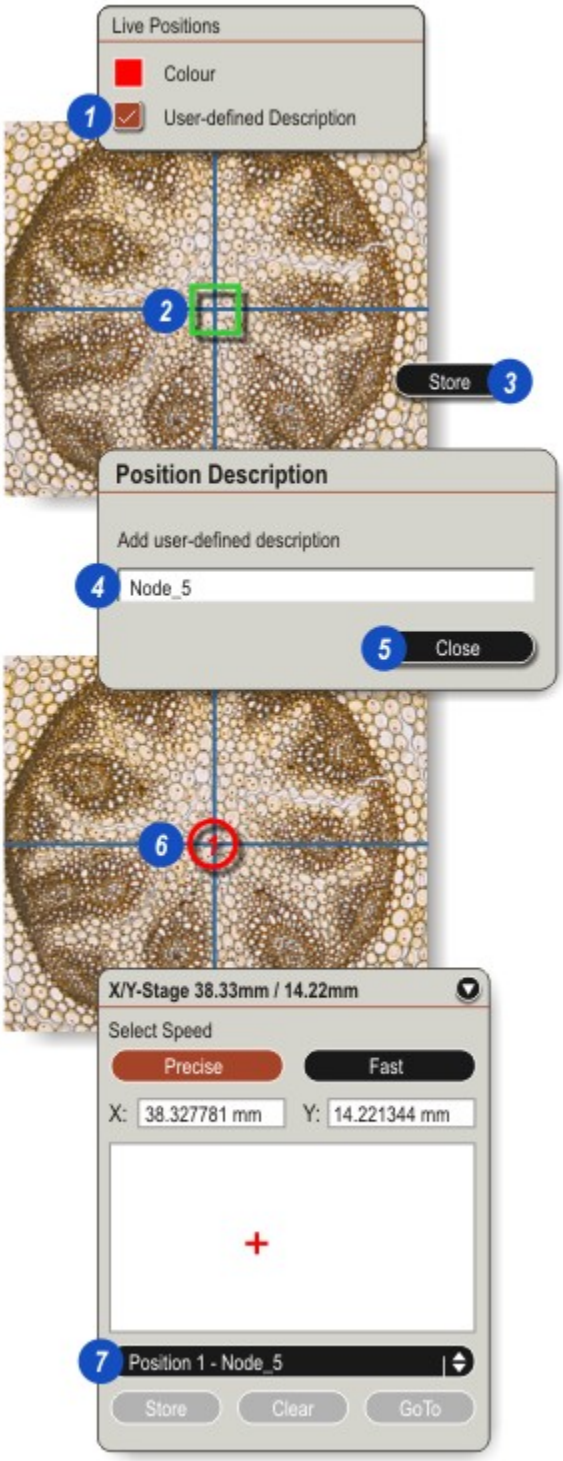
5: Click the *Close* button and...

6: ...the *Point Number* appears over the *Help Lines* and therefore, the point of interest, and...

7: ...the *Position Description* appears in the *Position List.*

Continued... 208

Note: The positions are not stored permanently and are lost when the microscope is switched off.



1: This sequence applies when the *User-defined Description* is disabled.
Two options are available:

- *Navigate to and store a position, and...*
- *Double-click to store a position.*

Navigate and Store:

2: Drive the stage to the point of interest so that it lies directly beneath the *Help Lines* - the centre of the stage.

3: Single-click the *Store* button. The marker is placed over the *Help Line* intersection and...

4: ... the position is stored as co-ordinates on the *List.*

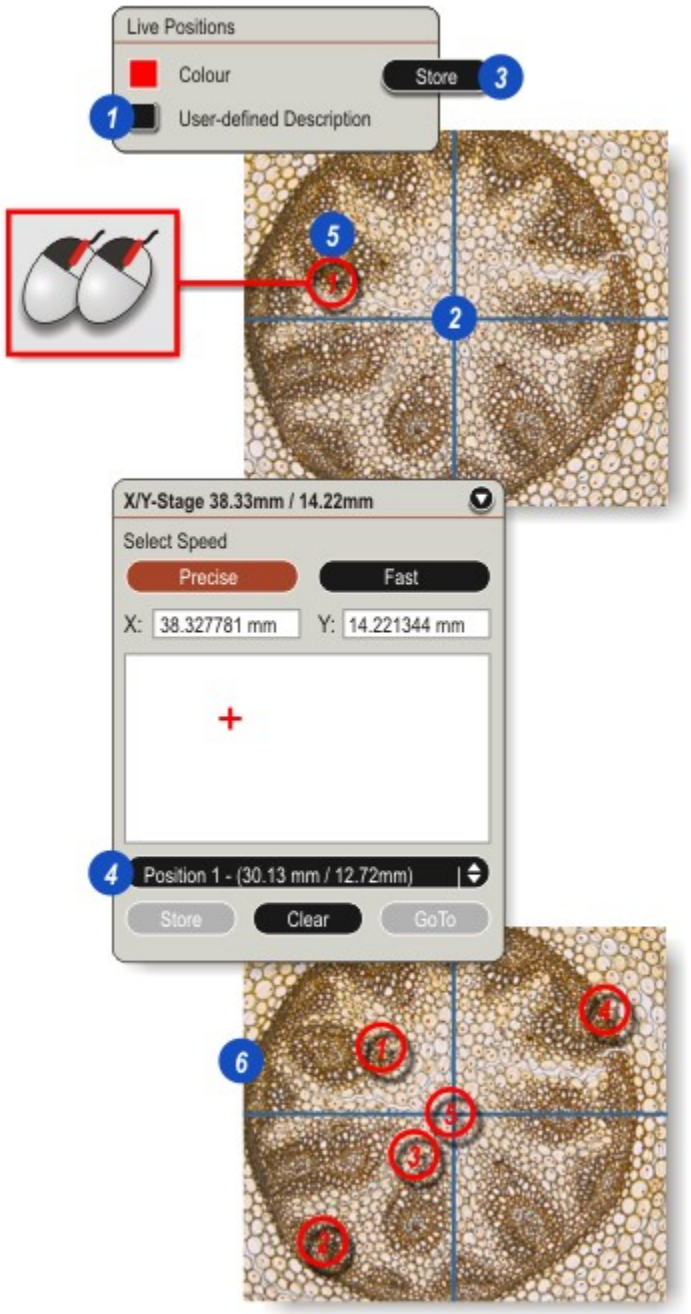
Double-click to Store:

5: Double-left click on the point of interest on the image - no need to drive the stage to the *Help Line* intersection. A marker with the position number is placed over the point and its co-ordinates are displayed on the *Position List* ***(4)***. Empty list positions can be selected and filled automatically by double-clicking again over other points on the image.

6: Up to 15 separate points can be selected on an image with each being stored on the *Position List.*

Continued... 209

Note: The positions are not stored permanently and are lost when the microscope is switched off.



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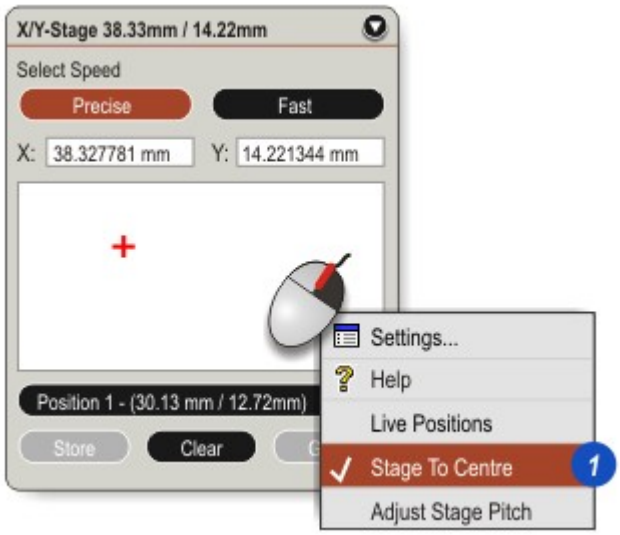
Stage to Centre is a convenient method for examining selected points of interest on the specimen quickly. It drives the stage immediately to a point indicated by a double-left click on the image so that it moves to the centre of the field of view:

1: Right-click inside the stage area and from the context menu click to select and enable the *Stage To Centre* option.



2: Double-left click a point of interest on the image...

3: ...and the stage will move immediately to the centre of the field of view.

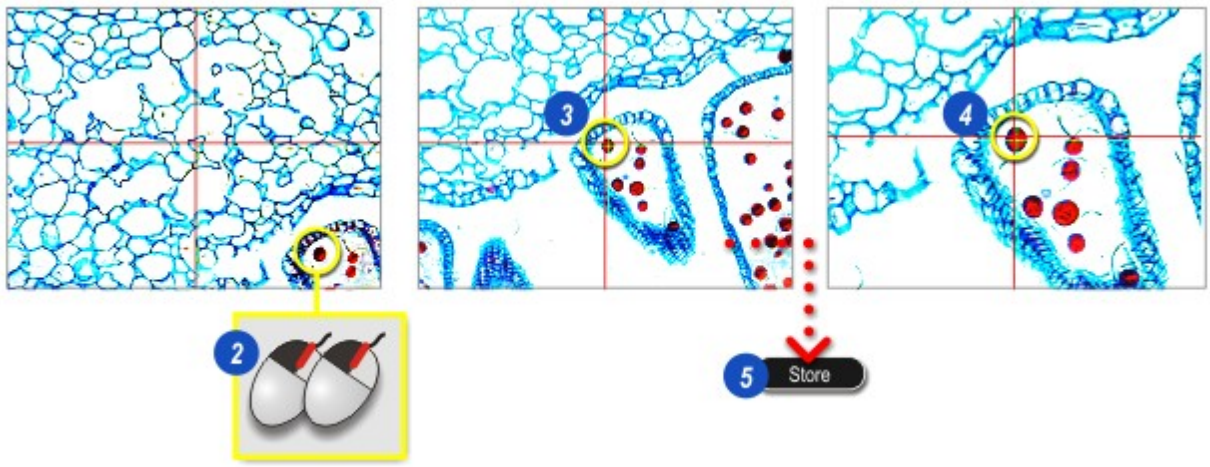
4: If required, higher magnification can be selected to examine the point of interest in greater detail which will stay in focus.

Continue to do this whilst *Stage To Centre* is enabled to effectively 'browse' the image.

5: If an empty position on the list has been selected, the *Store* button becomes available. Click it to store the position co-ordinates and return to it later using the *GoTo* feature.

To change *Stage to Centre* settings: *Go There..* 203.

Continued... 210



GoTo:
The stage can be driven directly to any of the set positions by:

1: Click on the small arrows to the right of the *Position List* header.

2: From the drop-down *Position List*, click to select the one required.

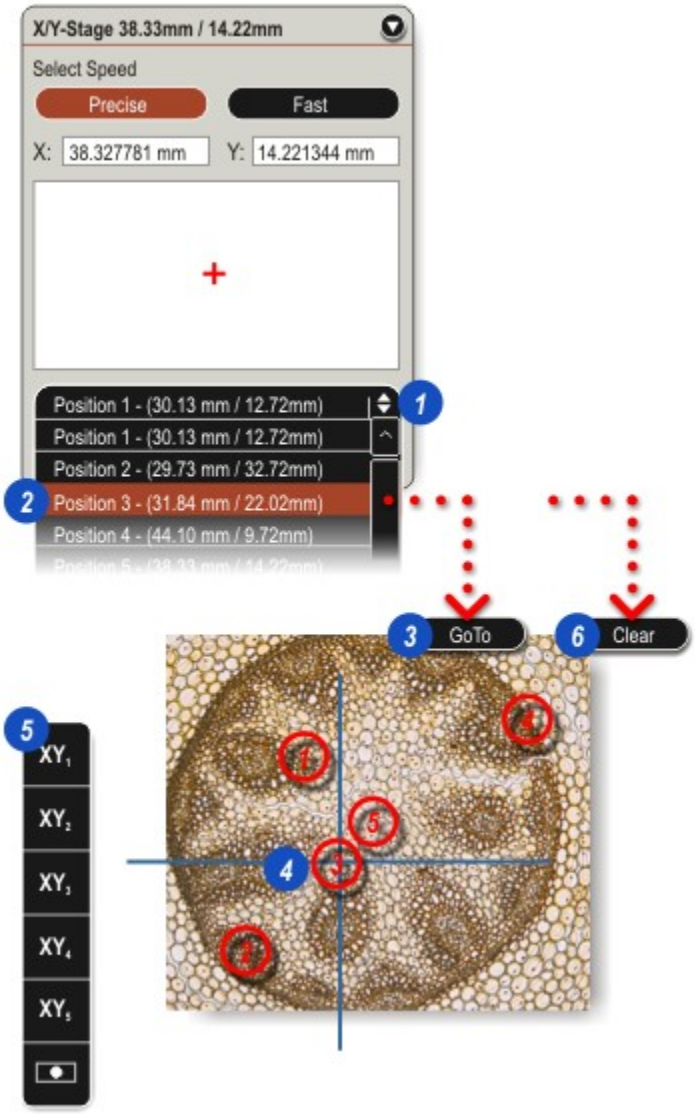
3: Click the *GoTo* button.

4: The stage drives to the position locating it beneath the *Help Line* intersection - stage centre.

5: A fast alternative to driving to a set position is to click one of the *Side Tool Bar* buttons. However, these only respond to the first 5 positions.

Clear:
With a position selected from the drop-down *Position List:*

6: Click on the *Clear* button to remove the position co-ordinates or point name. The image marker is also removed. A list entry must be cleared before it can be used for another position.



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Stage movement and accurate positioning relies upon the *Leica Application Suite* software 'knowing' just how far the stage will travel for a single revolution of a lead screw or gear train. If tiny manufacturing tolerances make the travel distance slightly larger or smaller, then precise alignment of individual montage images can be compromised.

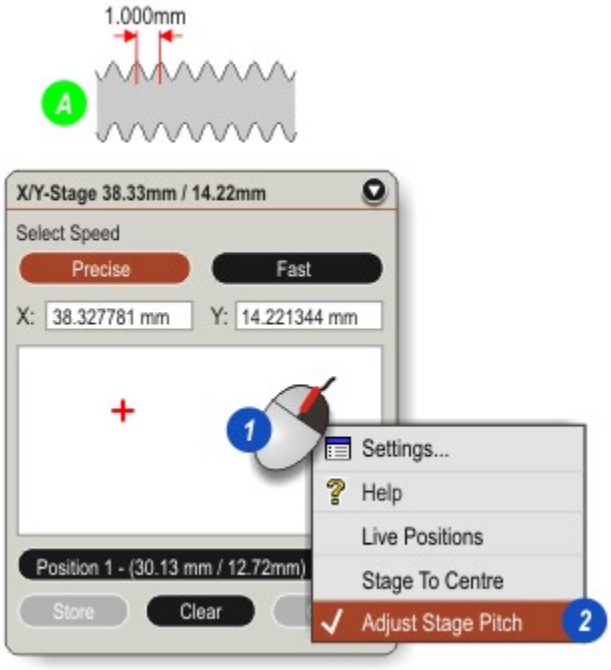
The *Adjust Stage Pitch* feature allows the users to manually position the stage at the ends of a known distance - usually a calibration slide - and from the measurement determine exactly how far the stage travels for a single revolution of the drive. The value is stored and used for subsequent stage positioning.

It is a two-step process - first for the *X* axis and then for the *Y* axis. The measurements are specific to the fitted stage and must be repeated if it is changed or swapped.

1: Right-click within the graphic stage area and...

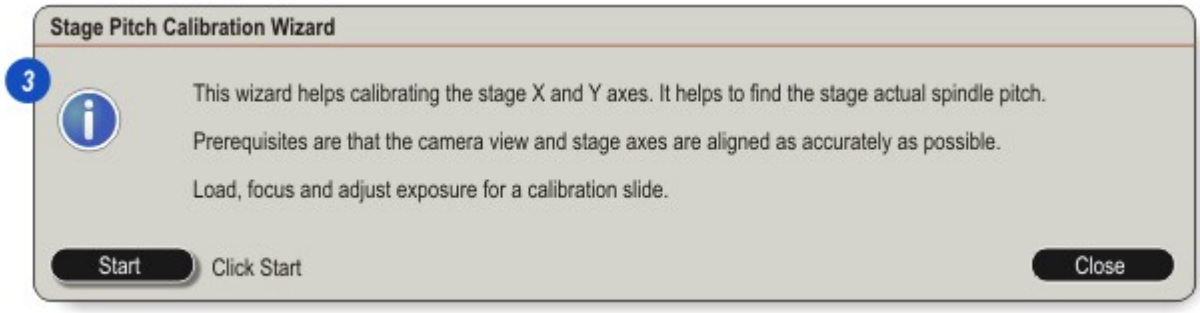
2: ...click to select the *Adjust Stage Pitch* option from the drop-down menu.

3: The *Stage Pitch Calibration Wizard* appears.



A: *Section through a stage lead screw. The manufacturer states a 1.000mm screw pitch - the stage will move 1mm for each turn of the screw. If the tolerance is ± 0.002mm then for 25 turns the stage may have moved between 24.95 and 25.05mm and perhaps not the expected 25.00mm.*

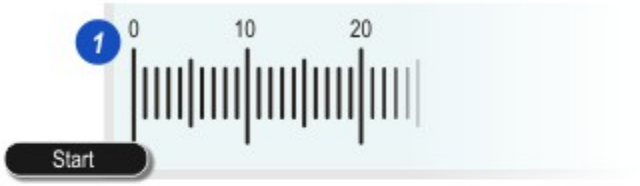
Continued... 212

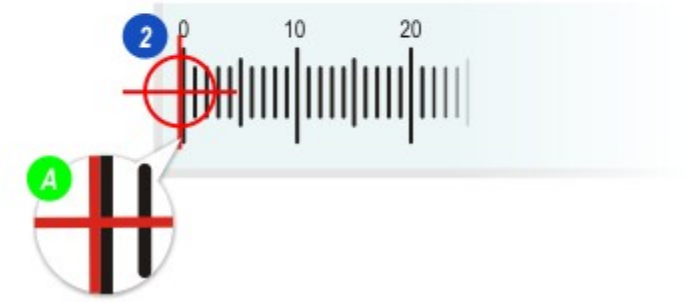


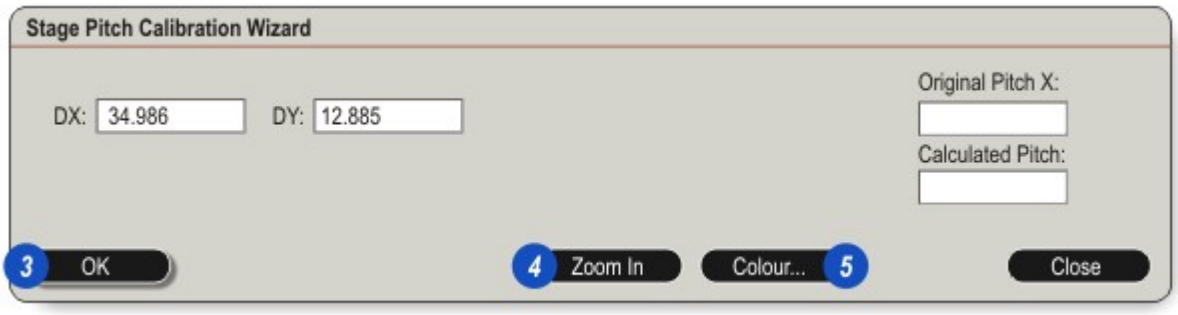
1: Place a calibration slide on the stage with the '0' mark to the left and bring it into sharp focus. Click the Wizard *Start* button. A 'sight' mark appears in the field of view.

2: Drive the stage so that the '0' mark outer edge perfectly aligns with the sight mark inner edge **(A)**.
To aid alignment, click the *Zoom In* button **(4)** for a magnified view. Change the sight mark colour to improve contrast by clicking the *Colour...* button **(5)** and selecting a preferred colour from the dialog.

3: Click the *OK* button to capture the stage position.



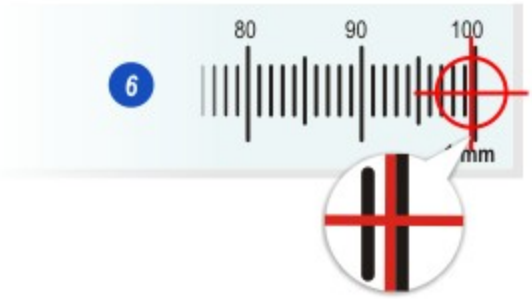




6: Drive the stage to the extreme right-hand end of the calibration slide this time positioning the inner edge of the calibration mark with the outer edge of the calibration mark.

7: Click the *OK* button.

Continued... 213



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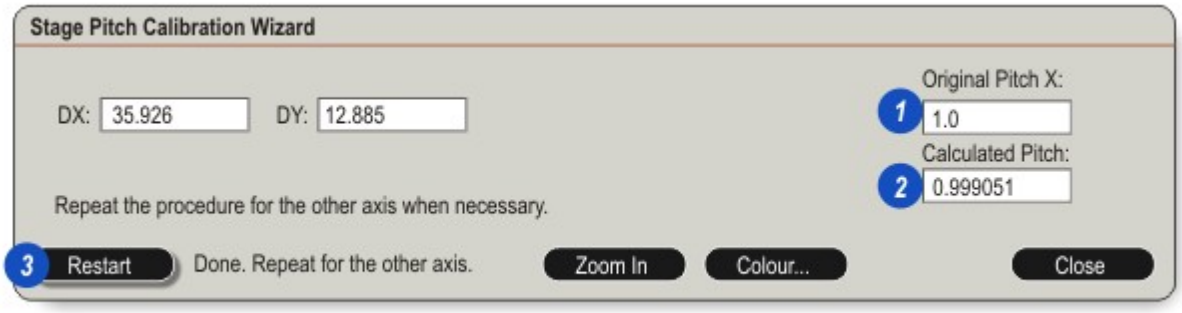
1: Click inside the *Original Pitch X* text box and type the calibration slide distance - in the example 1.0mm.

2: The *X* axis calibration is calculated and stored.

3: The *'OK'* button has been replaced with the *Restart* button ready to calibrate the *Y* axis.

Continued... 214



Follow the same procedure to calibrate the *Y* axis drive train:

1: Turn the calibration slide through 90º and check for position and focus.

2: Click the *Restart* button.

3: Drive the stage to the calibration slide *'0'* position carefully aligning it with the sight mark.

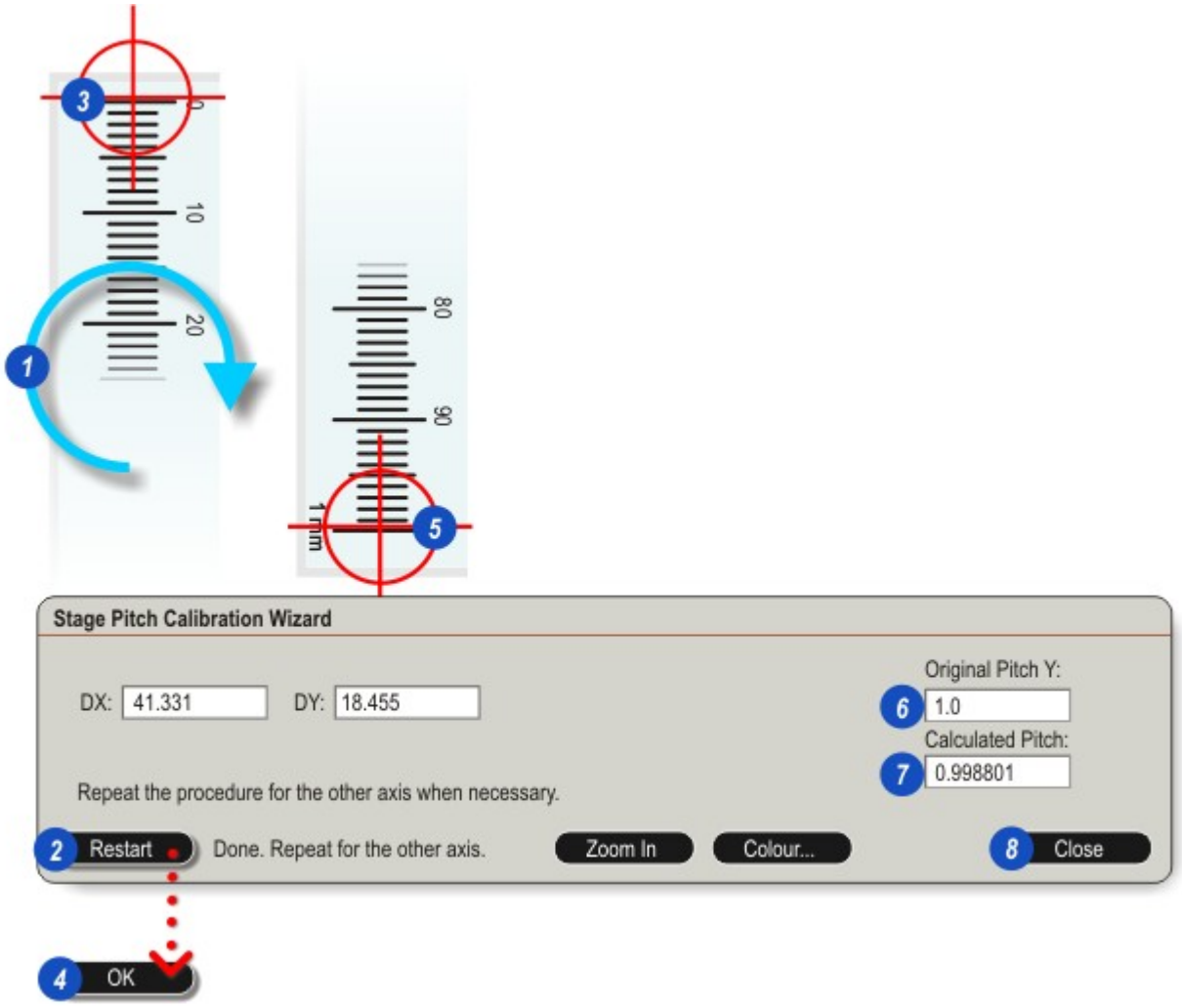
4: Click *OK*.

5: Drive the stage to the end of the calibration slide and again, carefully align it with the sight mark. Click *OK* **(4).**

6: Click inside the *Original Pitch Y* text box and type the calibration slide distance - in the example 1.0mm.

7: The *Y* axis calibration is calculated and stored.

8: Click *Close*.



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This module serves as remote control and status display of all motorized functions of the FS C and FS CB .
If a camera is attached to the microscope, it can be controlled simultaneously.
The module can consist of the following control plug-in windows: (depends on the connected hardware (FS C or FS Comparison Bridge only):

X/Y Stage
Focus Drive

Objective Magnifications:

- This window shows the status of the objectives available and teached-in for both the left and right hand revolver
- turret of the FSC. Switching over between left and right takes place by clicking the corresponding button L / R.
- The currently used objective magnification is indicated in red. This is a display function only.
- The turret of the FSC is coded but not motorized!

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Comparison Bridge Control:
This module enables the direct control of the bridge modes as well as the position and width of the dividing line. It displays the current status for the a.m. functions and indicates whether the bridge is in the calibrated (LED = green) or Zoom-mode (LED = red). Four direct control keys are available that switch the comparison bridge into one of the following modes:

- L = full left image (right side = not used)
- R = full right image (left side = not used)
- LR = Split center image. The dividing line will be positioned to its default position and default width previously
- teached-in in DM-control. Later adjustments of either line-position and or line-width result in the switch-off of
- the LED in the LR-key. This mode is then called “split image” and no longer “split center”.
- MIX = Superimposed image of both, the full left and full right image mode.

The rotary button “Pos.” controls the dividing line position that is used to introduce more or less of the left and right half-image. In its minimum and maximum settings a full left or full right image and all intermediate positions can be achieved.
The rotary button “Size” controls the width of the dividing line and can be set from a very thin (almost invisible) line to a full superimposed image by using the full potential of this function knob. This line or superimposed strip, can be moved across the image or positioned to any location in the image with the “Pos” key.
The LED “Magnification calibration” indicates two conditions of the comparison bridge:

- LED = green, the comparison bridge has identical magnifications left and right taking all optics and objectives
- into account. The specified accuracy is less than 1 per mille.
- LED = red, the comparison bridge can have different magnifications of the right and left imaging paths by as much as +/-5%.

Illumination Intensity of the cold light sources:
This window allows for the control of two independent cold light sources and displays the illumination intensity in degree Kelvin. The allocation of the light sources (L & R) depends on the connectors on the rear of the FSC.

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Magnification Changer:
The Mag. Changer window allows for the direct control of the 1.5x additional magnification factor to be introduced at any time with any objective magnification. It acts on both, the eyepieces and the photo port. After clicking on the desired factor (1x or 1.5x) the current status is indicated in red. It will further be correlated into the total magnification with the auto-calibration function.

Tube & Photo port:
This is a display only function. The beam splitter has a fixed factor of 50%/50%.

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Camera:

The *Camera* panel provides convenient control over the functions of a Leica DFC digital camera ranging from colour balance to histogram black and white levels.

- Images can be acquired in a variety of sizes, colour depths and file formats to provide even more flexibility. Setting the sharpening option and shading reference acquires images of the highest quality so that further processing requirements are minimal.
- Leica Application Suite also allows a focusing region to be defined on a live image so that areas of significance can be easily identified and focused more rapidly.
- All parameters and configurations can be saved and recalled at a later date.
- All Leica DFC cameras are controlled from the same interface although the features available do depend on the camera type.

Reveal the Camera controls by:

1: Click on the *Acquire Workflow* and...

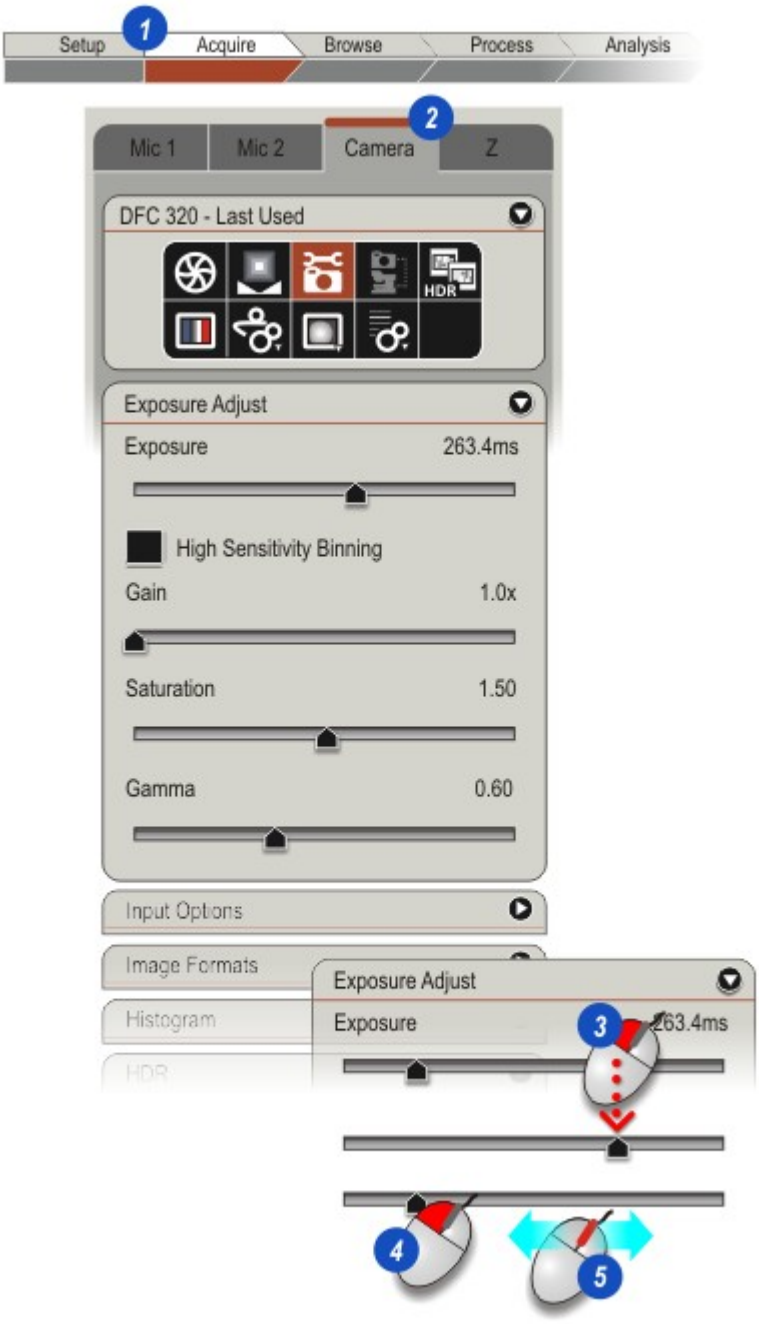
2: ...if necessary on the *Camera* tab.

Using the Slider Controls:

3: Drive a slider control to an approximate position by clicking on the slider track - the pointer moves there - and then using the mouse wheel (if fitted) to fine-tune the control value.

4: Move a slider in small increments by clicking and dragging on the slider pointer.

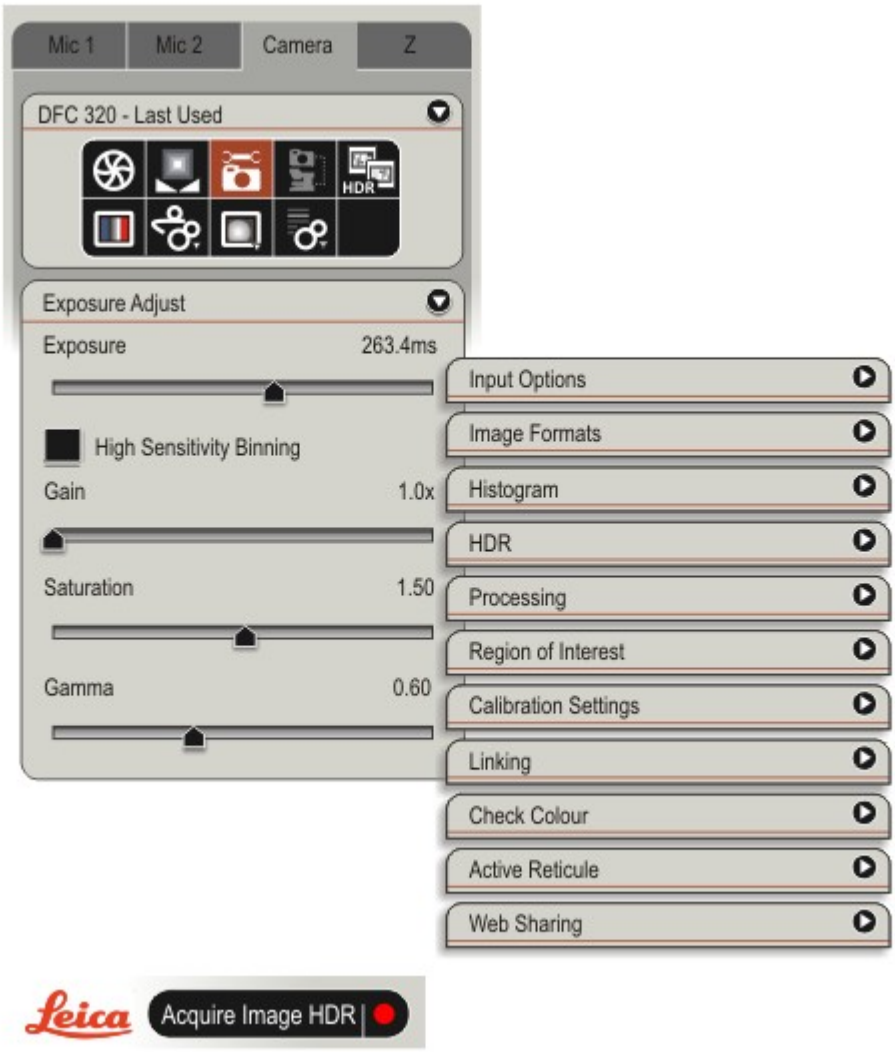
5: Once the slider is selected the mouse wheel (if fitted) can be used to fine-tune the control value.



Quick Links:

Click on a panel to go directly to the topic:

The panels to be displayed or concealed can be set up in *Preferences*.



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The *Camera Toolbox* comprises 9 buttons each of which is the link to an image control or camera feature.

Click a button for more information:


Automatic and Manual Exposure


Automatic White Balance


DFC Twain tools


Camera and Microscope Linking

High Dynamic Range / Averaging enable


Show Under/Over Exposure


Camera Configurations


Shading Configurations


Pre-defined Camera Setups

Often a very acceptable image can be achieved in seconds by making basic settings to the exposure using *Auto Exposure*, and then fine-tuning the result with white balance.

The following applies to a typical brightfield, colour image.

Make the basic exposure settings:

1: Set the *Saturation* to 1.75.

2: Set the *Gamma* to 0.60.

On the Histogram:

3: Set the black level to 0 and…

4: …the white level to *255* by clicking on and dragging the sliders.

Run Auto Exposure:

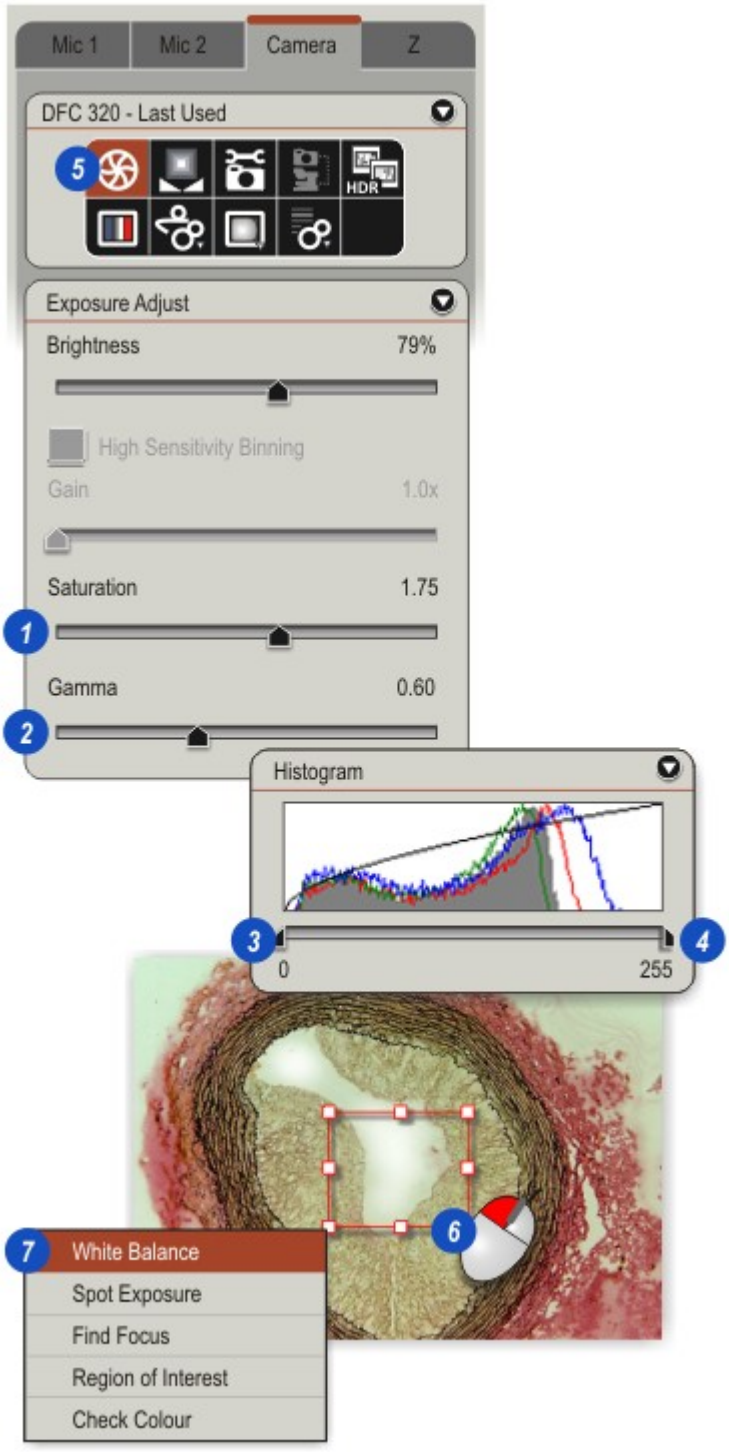
5: Click the *Auto Exposure* button and then click again to turn off *Auto Exposure*.

Set the white balance:

6: Click and drag a *Region of Interest* around a white area.

7: Select *White Balance* from the menu…

…and that is it. Done!



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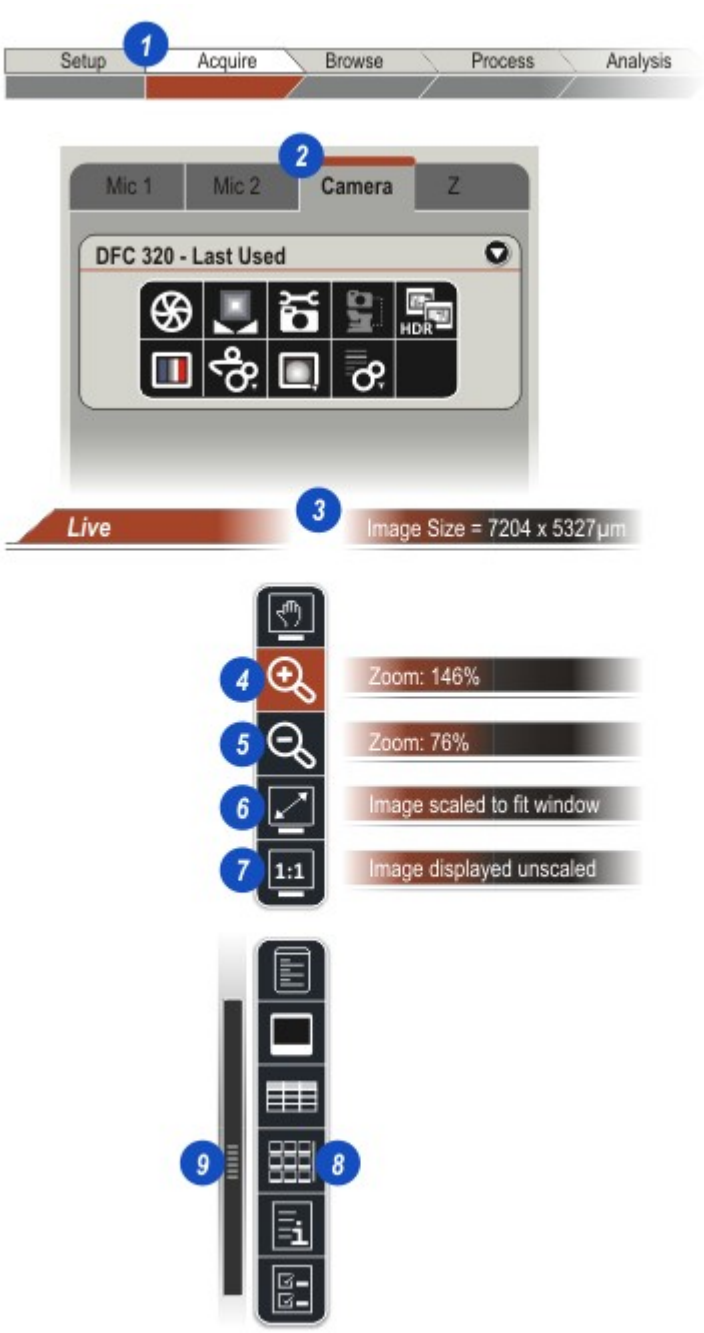
Live Image Display Controls:

Allowing closer and more detailed examination of the live image before deciding to capture it, *Live Image Display Controls* also include an extended *Side Tool Bar* and *Zoom Level* display.



1: Click on the *Acquire Workflow .*

2: If necessary click on the *Camera* tab to reveal the controls.

3: Image information is displayed in the *Live Bar* that appears along the top edge of the *Viewer*. When *Acquire* is selected the size of the *Live Image* is displayed.

4: On the *Side Tool Bar*, clicking the **(+)** button will zoom into the image.

5: Zoom out by clicking **(-).**

6: *Zoom to Fit* displays the image fitted into the *Viewer* window. The image is scaled so the largest dimension will be fitted to the *Viewer*.

7: *Same Size* displays the image at its actual size - one camera pixel is represented by one display pixel. For high resolution images only part will be displayed and for low resolution it could appear small and centred in the *Viewer*.

8: The *Gallery* thumbnails are hidden or revealed in a toggle action if images have been captured to the current folder.

9: Images zoomed beyond the boundaries of the *Viewer* are automatically displayed with *scroll bars* top and bottom as necessary. See also *Pan Live Image* 226

Pan Live Image:

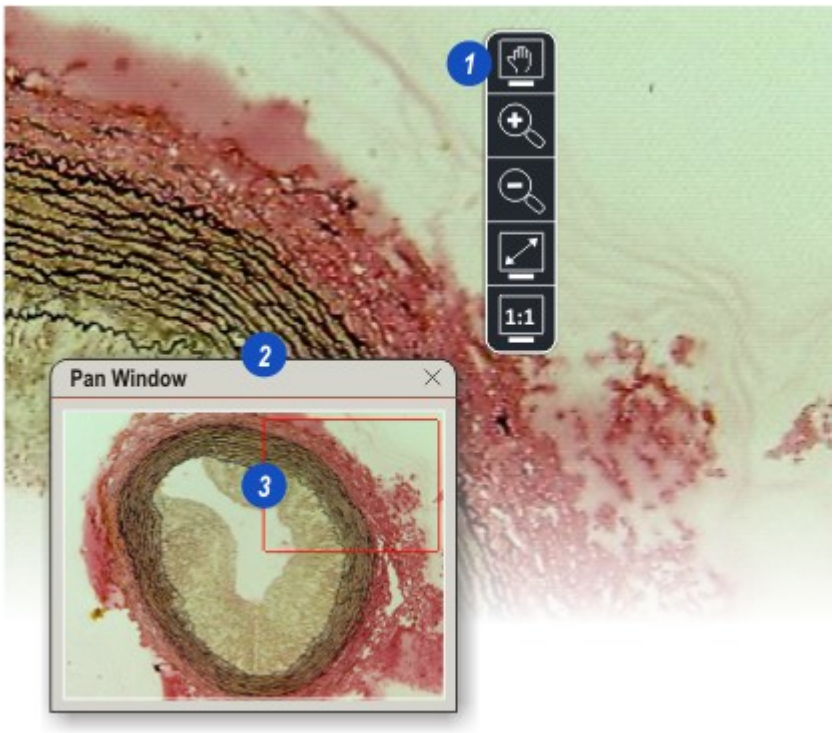
The *Pan* feature has also been included in the *Live Image Display Controls*. It works at any display level that leaves part of the image concealed beyond the boundaries of the *Viewer* . The 'hidden' area can be brought into the *Viewer* by manipulating the *Pan* window.

1: Click on the *Pan* button.

2: The *Pan Window* appears. Click on the header bar and drag the window to any convenient area on the display.

3: A *red outline* shows the part of the image currently displayed on the *Viewer*. Click inside the outline and drag it to another location in the window and the *Viewer* display will change to reflect the new position.

Click on the *Pan* button **(1)** again to hide the *Pan Window.*



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Acquire Image controls:

The are three methods for starting image capture:

1: Click on the *Acquire Image* button at the bottom of the *Acquire Workflow*.

2: Press the keyboard *F3* function key.

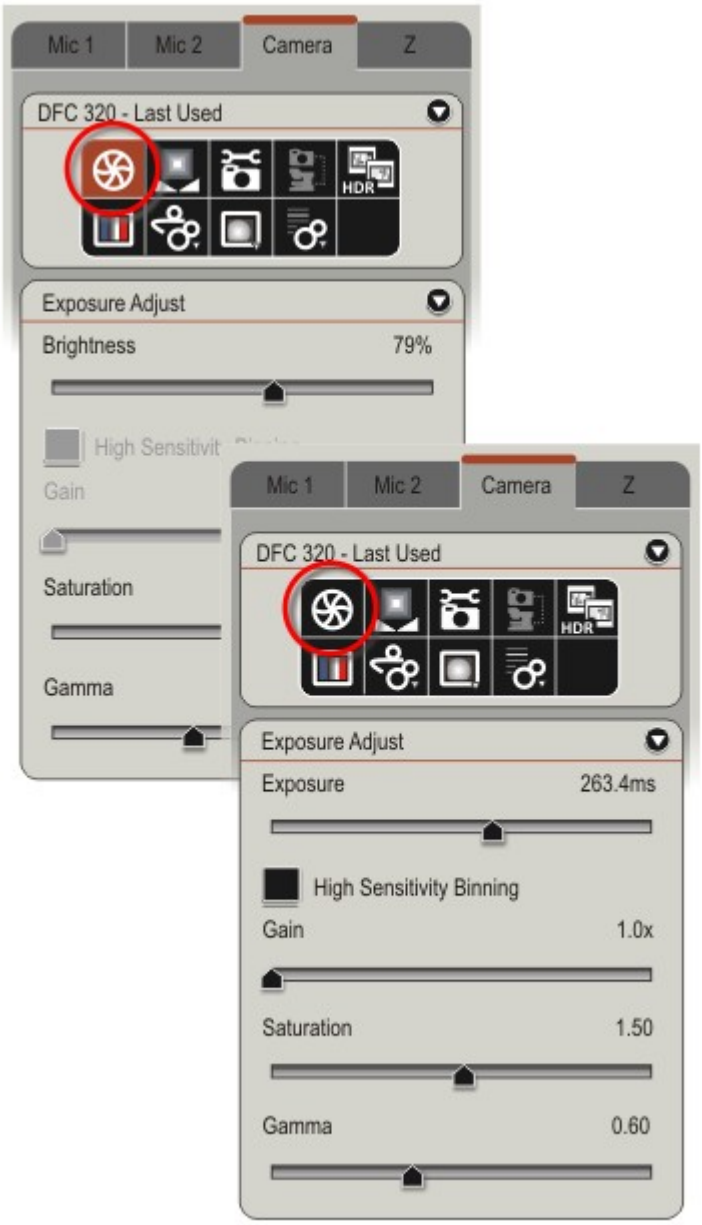
3: Click on *Options* on the *Main Menu* and click the *Acquire Image* option **(5)**.

Exposure Adjust:

There are two options for adjusting the exposure:

- *Automatic* with some fine-tuning, and.
  ..
- *Manual* with a range of precision controls.

Before starting work on a live image, using *Automatic Exposure* is a good option because combined with *Automatic White Balance* it could produce a perfectly acceptable image very quickly.



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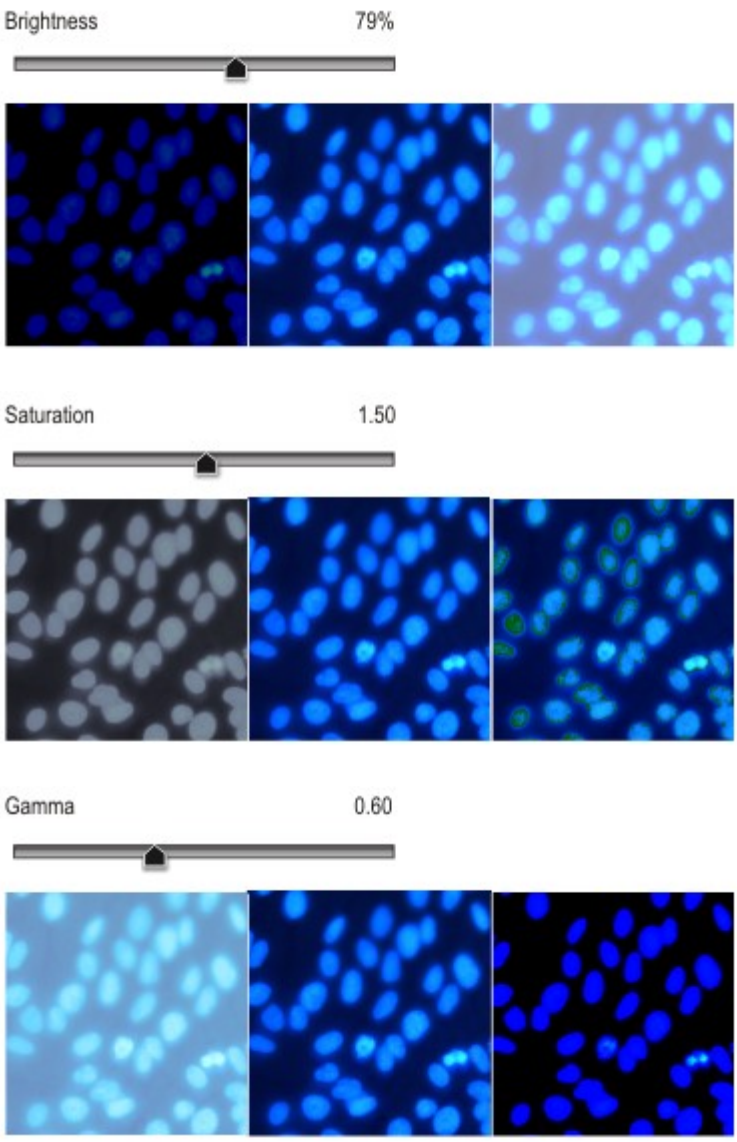
The software uses current light levels to establish best values for *Brightness*, *Saturation* and *Gamma*:

Brightness: A measure of how light or how dark each colour in the image is. It can swing from solid black to solid white.
Use small increases in brightness to help differentiate between colours; too much and detail begins to disappear.

Saturation: Determines the amount of each colour that is present. At the highest setting, each colour will be at its most vibrant - right hand image - and the colours cannot be more prominent without combining to make white.
Use *Saturation* to achieve colour subtlety in the image.
Reducing *Saturation* is a convenient way of turning a colour image into a monochrome image - essentially just shades of grey - without losing detail or becoming a black solid.

Gamma: A value applied to colour levels to compensate for different ways in which the image is viewed. Liquid crystal displays (LCDs) have a specific *Gamma* setting, monitors will have another and printers yet another.
Changes in Gamma are applied automatically so for example, when an image is printed the printer software will make adjustments before the printing.
Very small changes in *Gamma* can have dramatic effects; the examples show a range of *0.35* to *1.50* with the original in the centre. Use *Gamma* to achieve a contrast 'match' to the specimen



Automatic Exposure and White Balance:

To apply *Automatic Exposure:*

1: If necessary reveal the *Exposure Adjust* panel by clicking on the arrow right of the header bar.

2: Click on the *Automatic Exposure* button.

3: Adjust the *Brightness, Saturation and Gamma* controls 229 as necessary to achieve the required image.

Automatic White Balance:

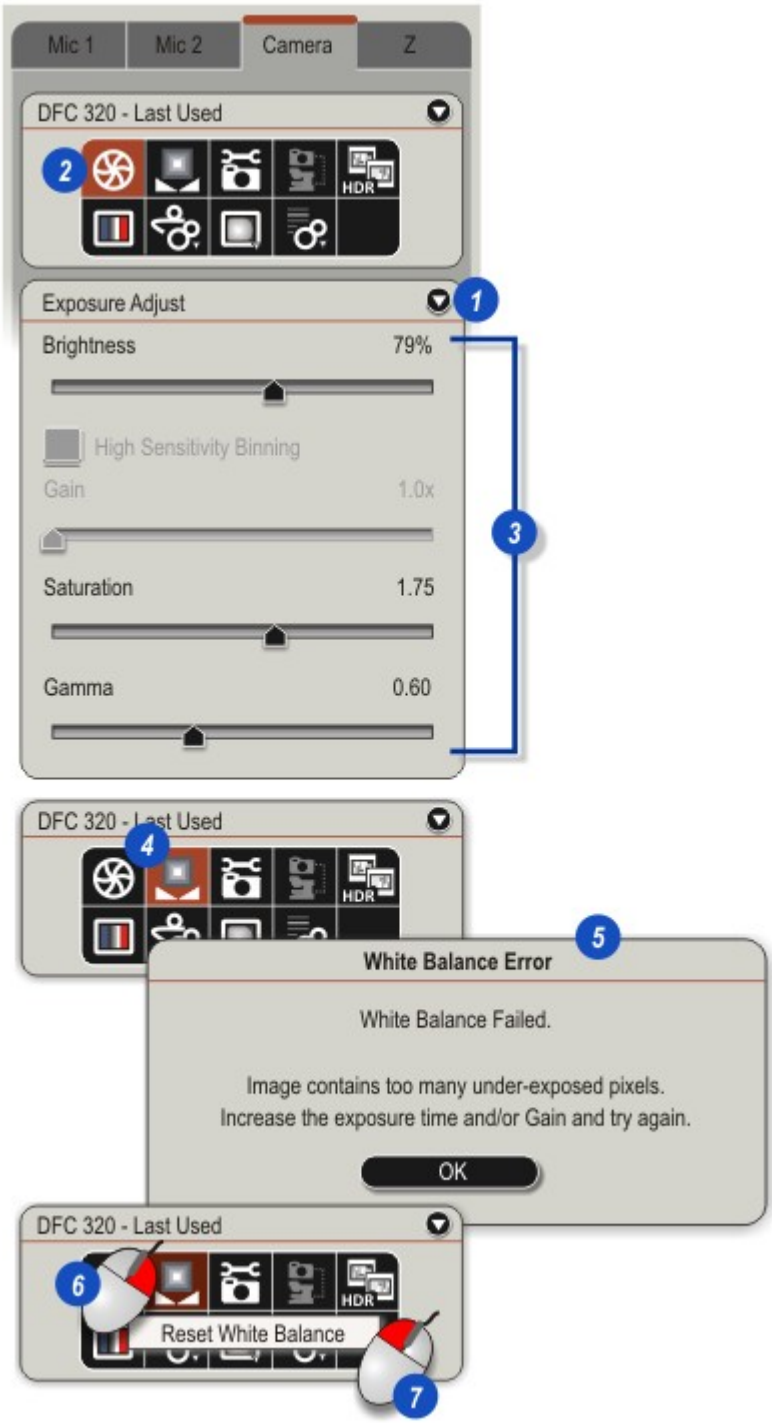
When *Automatic White Balance* is applied, all of the neutral tones - white through grey to black - are adjusted to remove any 'colour' content to maintain a clean, well-defined image.

4: Click on the *Automatic White Balance* button. *White Balance* is applied to the entire image.

5: If the image is too dark or too light *Automatic White Balance* may fail and an error message displayed. It may be possible to lighten or darken the image with the *Exposure Adjust* controls or change the lighting conditions at the microscope.

6: To undo *Automatic White Balance,* right click on the *White Balance* button and…

7: ...left click the *Reset White Balance* label.



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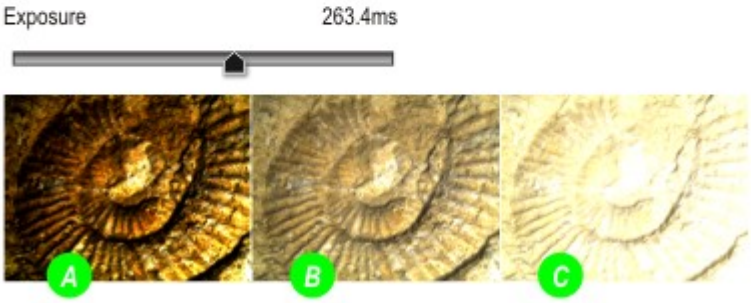
Manual Exposure:

The image controls change when *Exposure Adjust* is in manual mode - *Brightness* is replaced by *Exposure*: *Saturation* and *Gamma* remain but another control - *Gain* - is added.

If necessary, click the *Automatic Exposure* button to ensure it is disabled.



Exposure: Controls the time that the camera sensing elements are exposed to the specimen. It is sometimes called the scanning rate.

At the start of the exposure time period, all of the camera sensing elements are reset - they have no usable image information at all. Then they are exposed to the specimen and each begins to 'charge' to a value that numerically represents the light falling upon it. Individual elements are designed to respond to one of the three colours - Red, Green or Blue (RGB).

At the end of the exposure period, each element is 'read' and its value used in combination to create a pixel on the *Viewer*.

For any image, there will be an optimum period of exposure for the elements to reach values that truly represents the image **(B)**. Too short an exposure and the elements will not have sufficient time to reach the proper value - the image will be dark and muddy, under-exposed **(A)**. Too long and the image will be 'washed out' and lacking detail, over-exposed **(C)**.

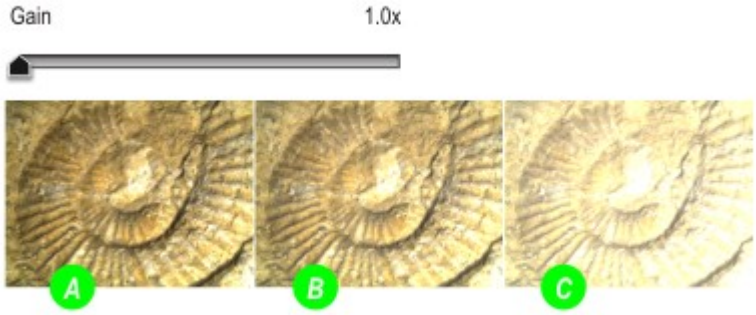
The time scale for exposure will depend upon the camera and may be measured in either microseconds(µs), or milliseconds (ms).

Gain: A function for changing the brilliance of an image without changing the exposure. The examples shown are:

Gain = 1.0 ***(A)****,*

Gain = 1.5 ***(B)*** and

Gain = 3.0 ***(C****).*



High levels of *Gain* can make an image look noisy and badly defined, so as a general rule leave the setting at 1.0 unless light levels are particularly low.

Start with a *Gain* value of 1.0 and gradually increase the value. Too high a *Gain* setting will 'bleach' the image, cause a loss of fine detail and may introduce 'noise'.

Saturation and Gamma information: 229

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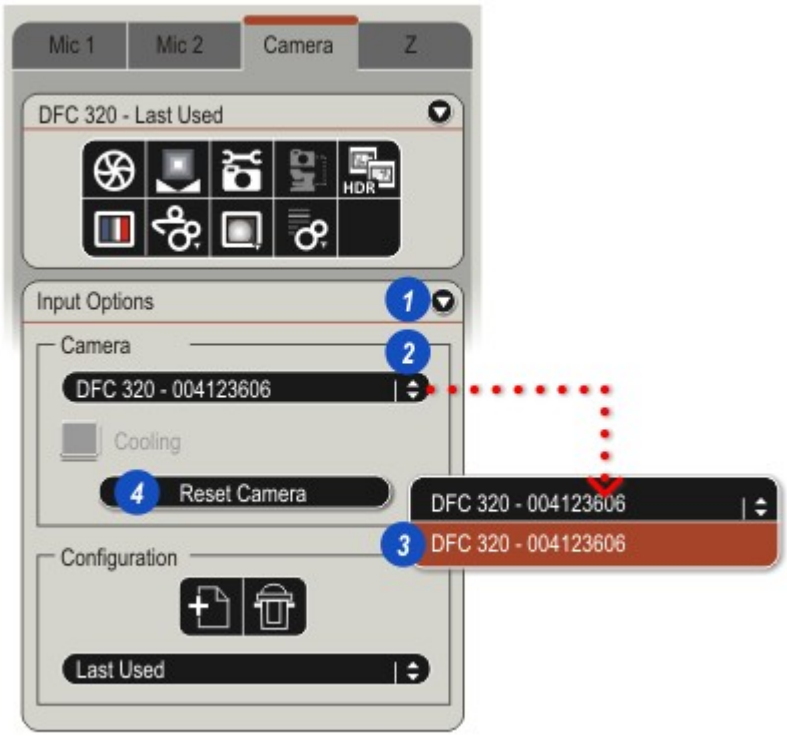
Input settings cover the camera setup. The settings are established using several of the *Camera* control panels and *Preferences*.

Users can:

- 233 Select the Active Camera.
- 234 Choose and load a Pre-defined Camera Configuration.
- 235 Save a User Camera Configuration.
- 236 Load a User Camera Configuration.
- 237 Access the Twain User Interface.



Select the Active Camera:

Occasionally, more than one camera each with its own *FireWire* connection may be fitted to the computer. They appear as a drop-down menu with model names and serial numbers. The current active camera appears at the top of the menu list.

To select a camera:

1: Click on the arrow to the right of the *Input Options* header to reveal the panel.

2: Click on the small arrows to the right of the *Camera* drop down and...

3: ...from the drop down menu click to select the camera required.

4: To restore a temporarily lost camera connection, click on the *Reset Camera* button.

Pre-defined Camera Setups:

Pre-defined Camera Setups are settings for the most common microscope contrast methods that a user can quickly select and use.

Pre-defined Setups automatically configure LAS for image exposure and processing corresponding to the selected technique.

Select the required technique by:

1: Click on the *Pre-defined Setups* button and from the drop down list...

2: ...click to select and apply the required configuration.

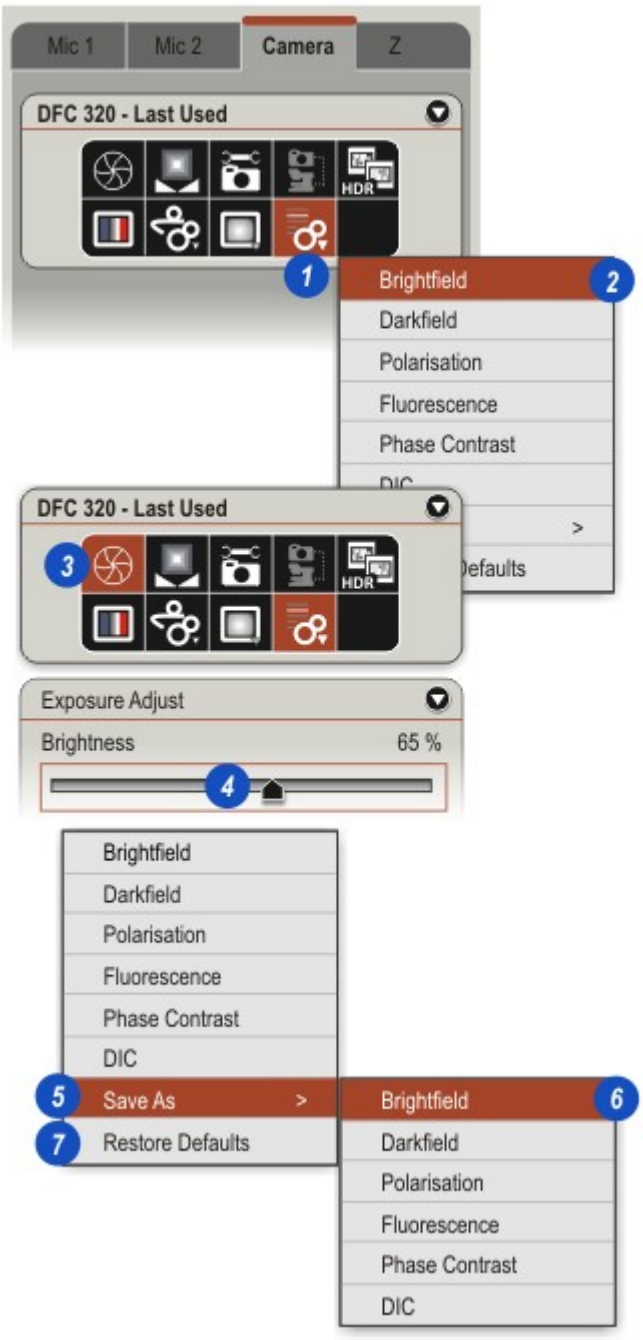
3: *Automatic Exposure* is automatically launched to allow the user to make adjustments if necessary.

4: If adjustments are made to a *Pre-defined Setup* it can be saved as an update by...

5: ...clicking on the *Save As* option and from the sub-menu...

6: ...clicking on the original setup.

7: To restore *Setups* to their original settings, click on the *Restore Defaults* options.



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Save and Delete a User Camera Configuration:

With the camera setup complete, the settings can be saved and used on another occasion to perfectly replicate current values.

1: Click on the small arrow to the right of the *Input Options* header to reveal the panel.

2: Click on the *New Configuration* button.

3: On the *Save Current Configuration* dialog, click inside the text box and type a new, unique name for the configuration.

4: Click *OK.*

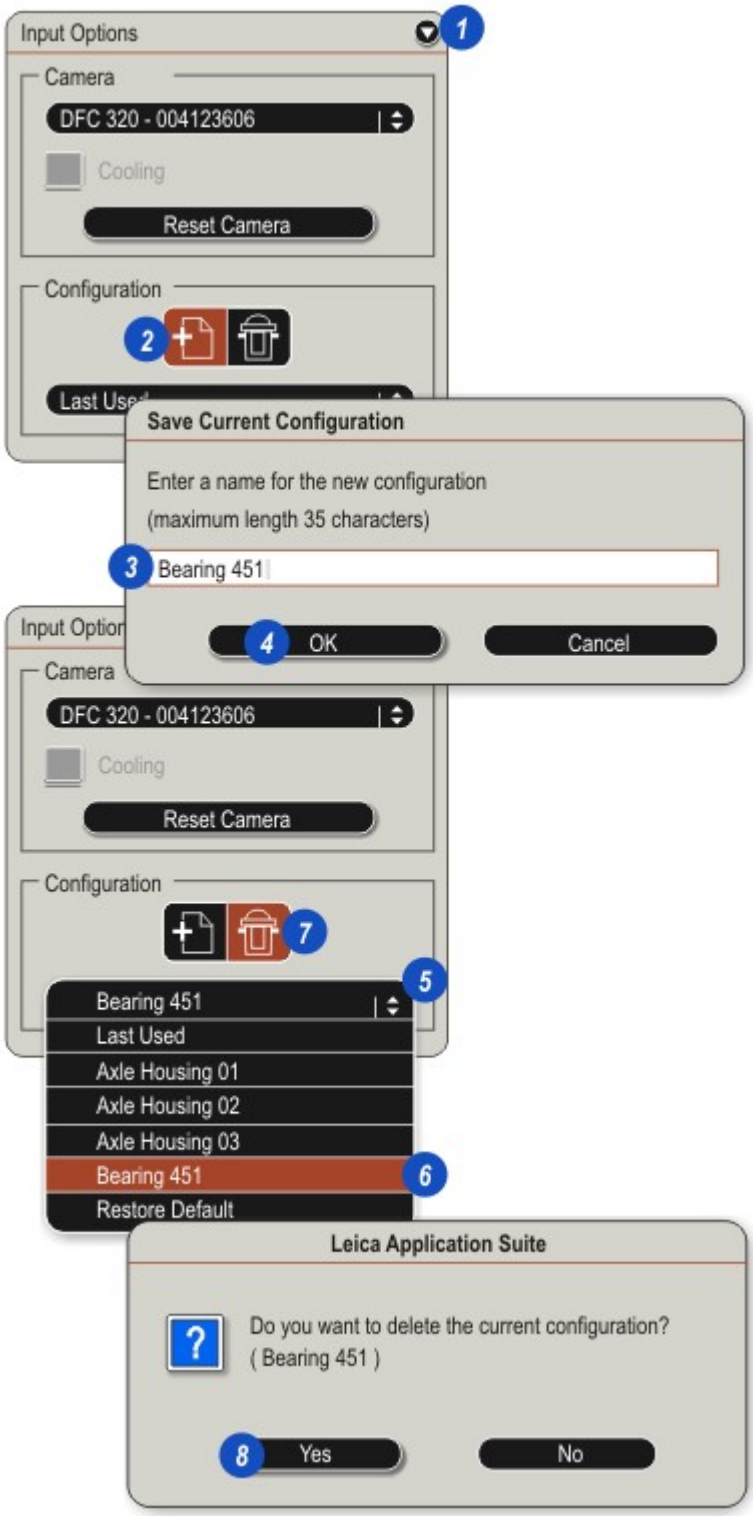
To delete the current Configuration:

5: Click on the arrows to the right of the of the *Configuration* header bar.

6: From the drop down menu click to select the configuration to be deleted.

7: Click on the *Trash Can* (Delete) button and...

8: ...confirm the deletion on the message panel by clicking *Yes.*



Load a User a Camera Configuration:

Input settings can be saved as a *Configuration* and retrieved later to quickly and precisely replicate at camera setup.

When the *Viewer* opens, the configuration defaults to the *Last Used* settings.

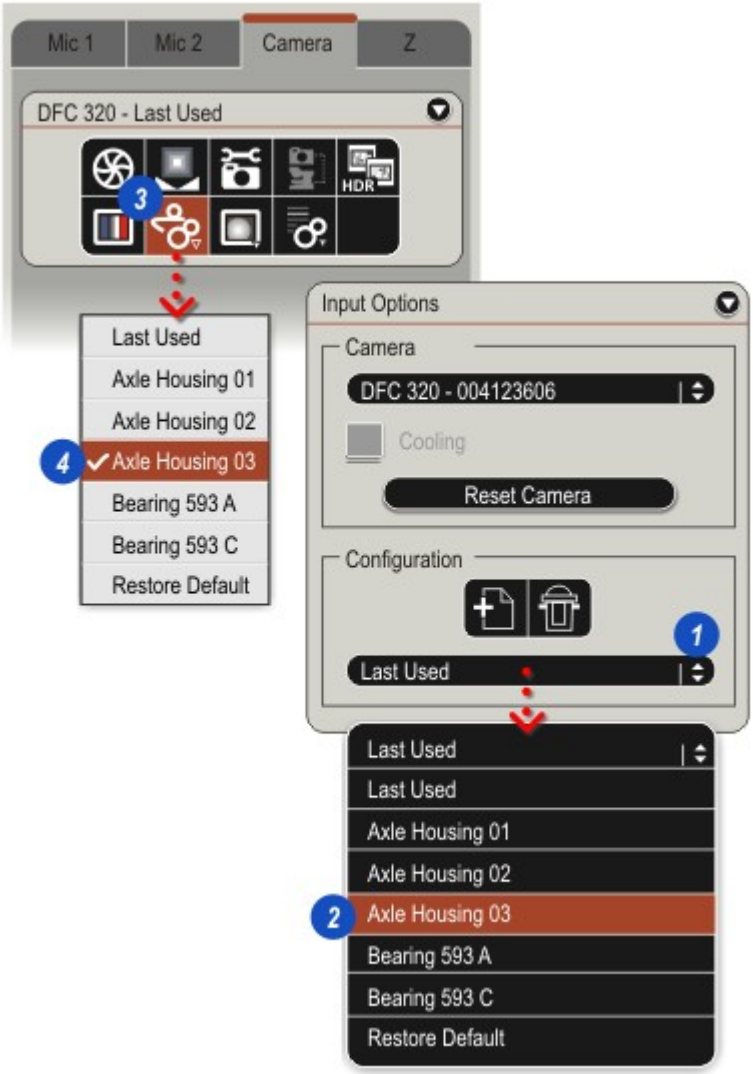
To Select and Load a previously saved User Camera Configuration:

1: Click on the arrows to the right of the *Configuration* window and...

2: ... from the drop down menu click to select a saved configuration or...

3: Click on the *Select camera configuration* button in the toolbox and...

4: ...from the drop down list click to select and load a saved configuration.



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The Twain Interface:

The *Twain* User Interface displays settings and tools associated with the active camera. It is the software underlying the camera and acquisition functions found in Leica Application Suite - many of the LAS features can be adjusted or turned on and off here.

Use the *Twain* Interface to view the camera data and make basic exposure settings on a single, compact display.

1: Click on the *Camera Settings* button on the toolbox.

2: The *Twain* User Interface appears.

For details about Twain and how to use it 238

For a detailed description of *Twain* and how it is used refer to the *Leica DFC Guides* as follows:

1: Click on the Windows *Start* button and then on *All Programs.*

2: From the menu choose *Leica DFC Twain* and...

3: ...double-click Image *Acquisition* or *Quick Start Guide.* The *Release Notes* document contains latest updates too late to include in the guides.

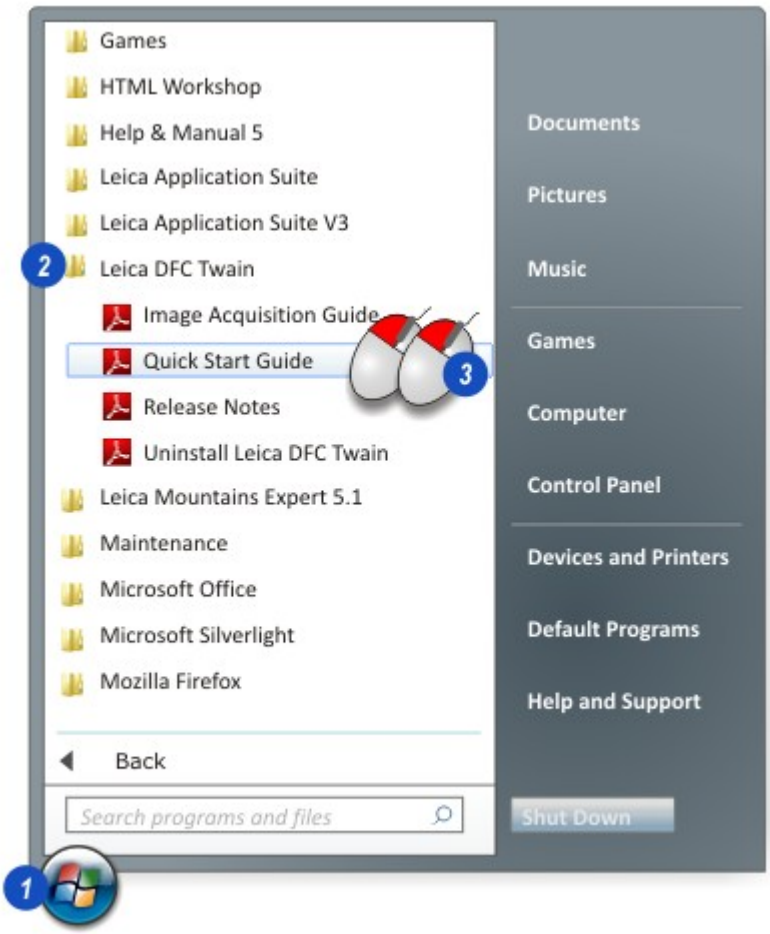

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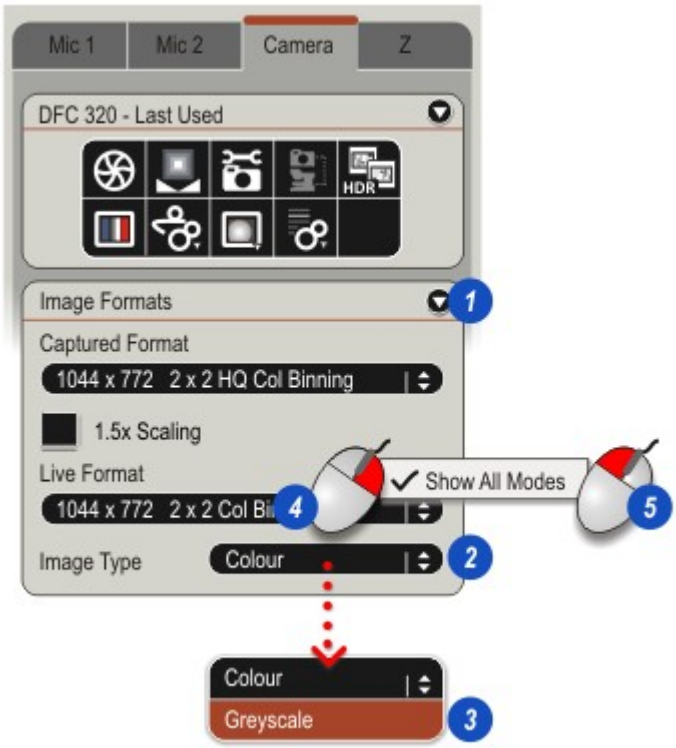
Image Formats:

On the *Image Formats* panel the user can:

- 239 Select a Colour or Grayscale image.
- 241 Select Live and Captured image formats.

Also in this section, how to:

- 242 Choose the Bitdepth.
- 243 Enable/disable High Sensitivity Binning



If a colour camera is active, both colour and greyscale (monochrome) options are available.

1: Click on the arrow to the right of the *Image Formats* header to reveal the panel.

2: Click on the arrows to the right of the *Image Type* header bar...

3: ...and from the drop down menu select the type required.

Live Format:

Live Format determines the quality and resolution of the displayed image in the*Viewer*. The active camera will determine the extent of the format options available.

To ensure that all of the possible options are available to the user:

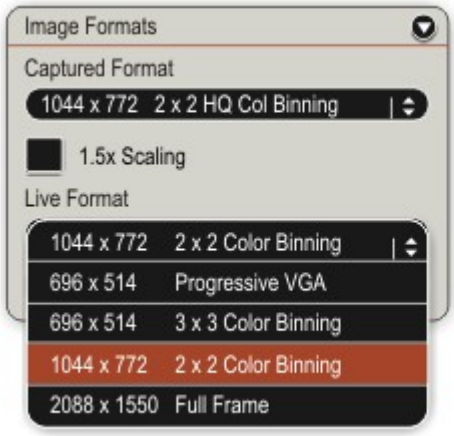
4: Right click on the *Live Format* text box and...

5: ...left click on the *Show All Modes* label to check it.

Depending upon the active camera, there will be a range of formats available for both live and captured images.

- *Progressive VGA* produces the lowest resolution images but is suitable for very fast exposure rates.
- *Colour Binning* is a process of grouping adjacent camera element pixels to create 'super' pixels. Each group is then used to 'drive' a single display pixel. The feature improves overall sensitivity and speed.
- Available binning options depend upon the camera being used; Three formats - *2 x 2, 3 x 3* and *4 x 4* - are available, each with a HQ (High Quality) option. The format numbers describe how the pixel values are grouped: *2 x 2* = 4 pixel group: *3 x 3* = 9 pixel group and *4 x 4* = 16 pixel group.

  As a guide and depending upon the camera model, the *2 x 2 Colour Binning 1044 x 772* format is a good choice for most situations.
- *Full Frame* options display each of the camera element pixels individually. Resolution and quality is very high especially if a *HQ* (High Quality) option is selected.
- *Progressive Red, Blue or Green* use only the value of the selected colour. The *Viewer* displays a grayscale image representing the intensity of the chosen colour. Even if the *Image Type* is set to colour, the image will appear monochrome.



Avoid saving images using the 16-bit format. They will be slow to expose and process and if captured and saved in the same format may be unusable in third-party image processing applications.

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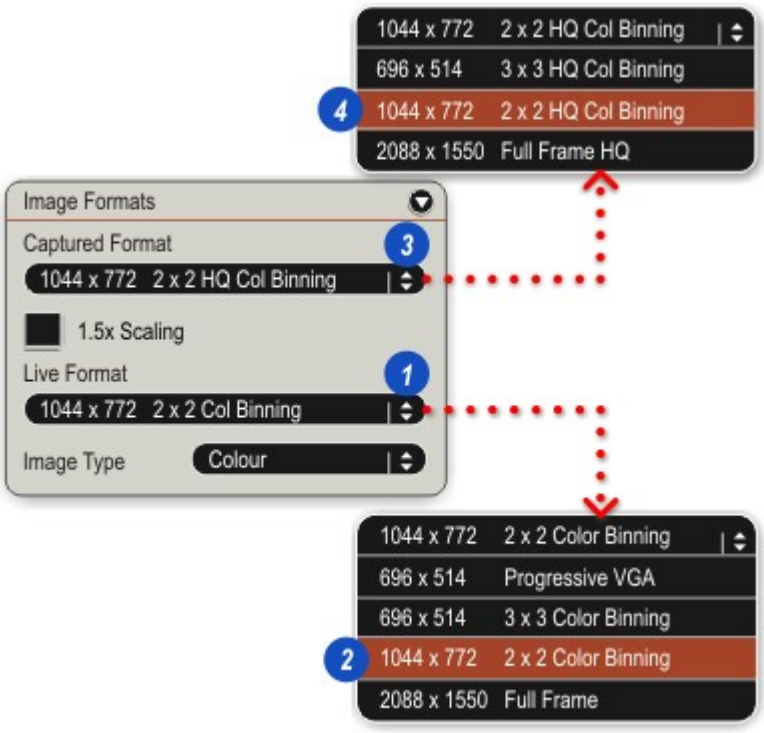
Selecting Live and Captured Formats:

Select the Live Image Format:

1: Click on the arrows to the right of the *Live Format* header bar and...

2: ... from the menu click to select a format. If the camera supports a wide range of formats, small *Scrolling Arrows* will appear at the bottom of the drop down list. Click to scroll down.



Selecting the Captured Format:

Captured Format determines how the image is finally captured and saved. In many cases, the *Captured Format* will be the same as the *Live Format* so, providing the image is saved as a bitmap on a bit-by-bit basis without compression, when the image is retrieved it will be identical to the original.

3: Click on the arrows to the right of the *Captured Format* header bar.

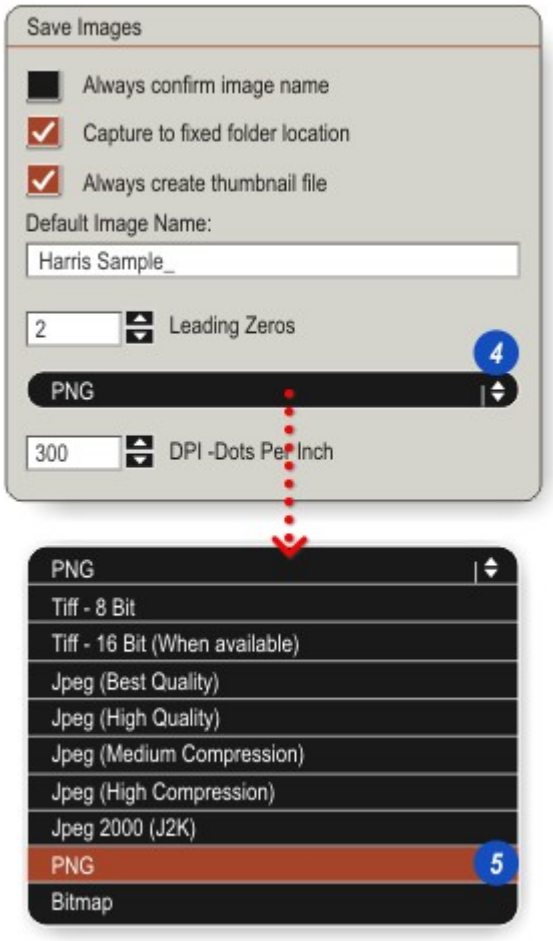
4: From the drop down menu click on the option required.
To save an image with 16-bit colour depth, a *HQ (High Quality)* option must be selected.

The Bitdepth:

The *Bitdepth* is a digital value which determines the colour range and precision of the saved image. A value of 8 bits provides a spectrum of 256 separate colours whereas 16 bits yields 65536 colours.

Greyscale images are captured and saved as either 8 or 16 bits per pixel. Colour images require three times the number of bits per pixel to store the three primary colours - *red, green* and *blue*. So, colour images are stored either as *3 x 8* bits or *3 x 16* bits (High Quality).

The *Bitdepth* setting has a considerable effect on the disc file size so generally 8 bit should be chosen unless more colour subtlety and variation are needed. The 16 bit option is not always compatible with some third-party software.



The Bitdepth setting in Preferences:

The *Image Format* setting made in *Preferences* > *Save Images* sets the image compression and, in some case the *Bitdepth*. They override those made here in *Camera*.

To check or select the Preferences settings:

1: Click on *Options* on the *Main Header* and...

2: ...click to select *Preferences*.

3: On the *Preferences* dialog, click the *Image* tab.

4: Click on the arrows to the right of the *In this format* header and...

5: ...click to select the *Compression* type and associated *Bitdepth*. Click *OK.*

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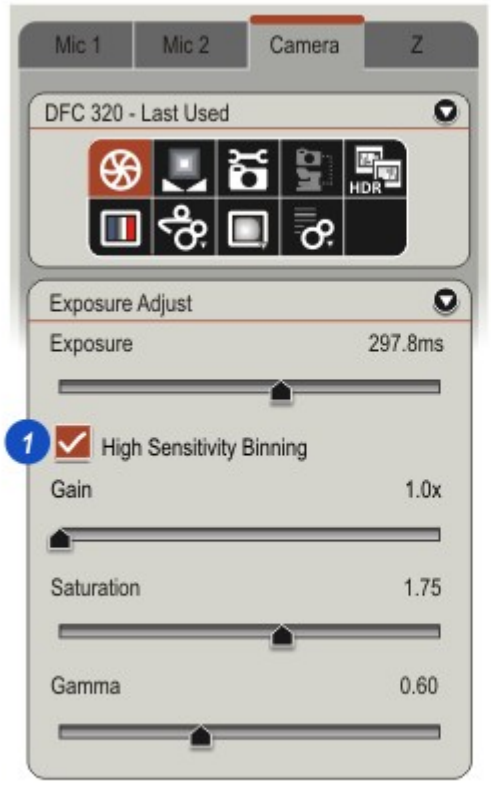
High Sensitivity Binning:

If binning is used for *Live Format* but not for the *Captured Format*, when the image is retrieved it will appear darker than expected.

To maintain binned live image brightness on the captured image:

1: Click to check (tick mark visible) the *High Sensitivity Binning* check box on the *Exposure Adjust* panel.



Histogram:

The *Histogram* is a graphical display of the colour values in the image represented as 256 points ranging from 0 (Black) through to 255 (White).

In this section:

The display detail can be set by the user by:

1: Click on the small arrow to the right of the *Histogram* header to reveal the *Histogram* panel.

2: Right click on the histogram display window.

3: From the drop down menu, left click on *Histogram Options* and...

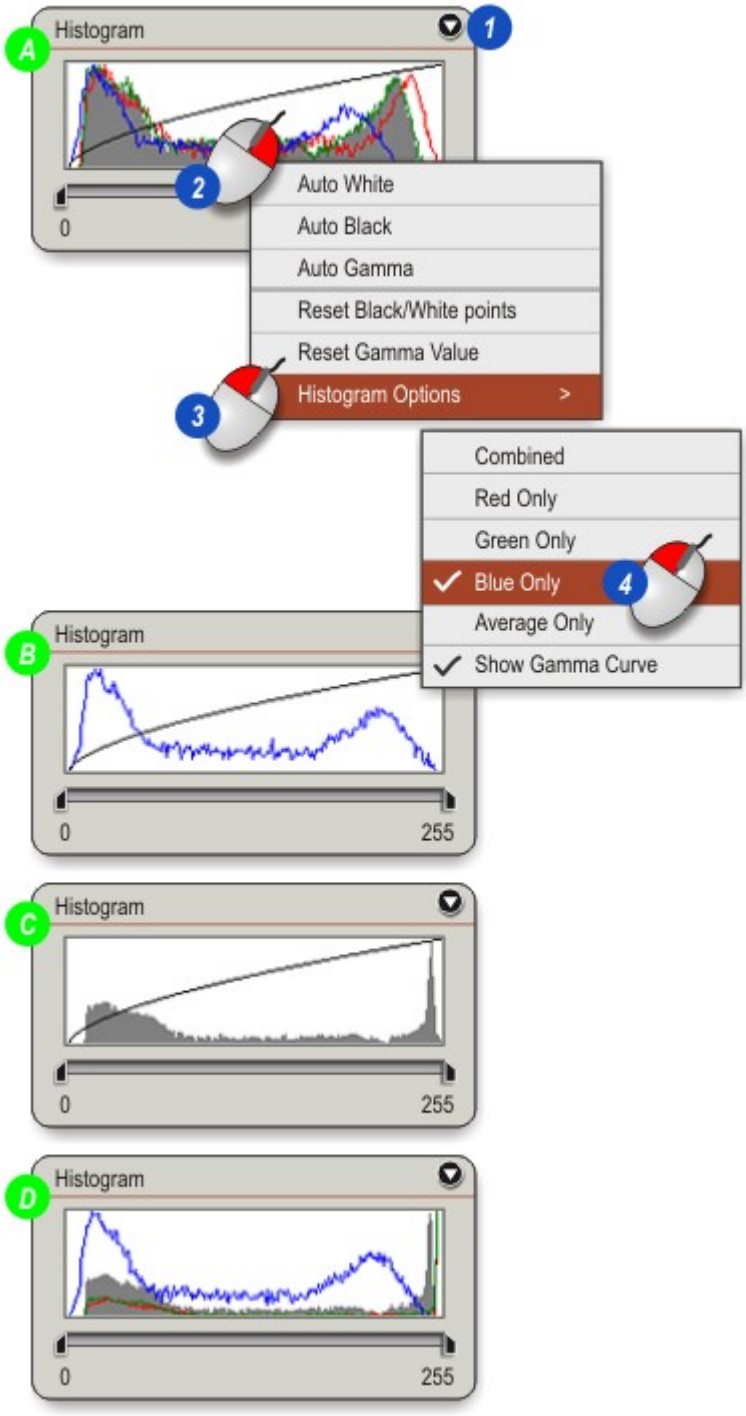
4: ...from the display options, click on the option required:

Combined ***(A)****:* Shows all of the colours and the average.

Red, Blue or Green ***(B)****):* Show that colour level only.

Average ***(C)****:* Displays the average of all of the Red, Green and Blue (RGB) values.

Show Gamma Curve ***(D)****:* Click to display or hide the *Gamma Curve*.



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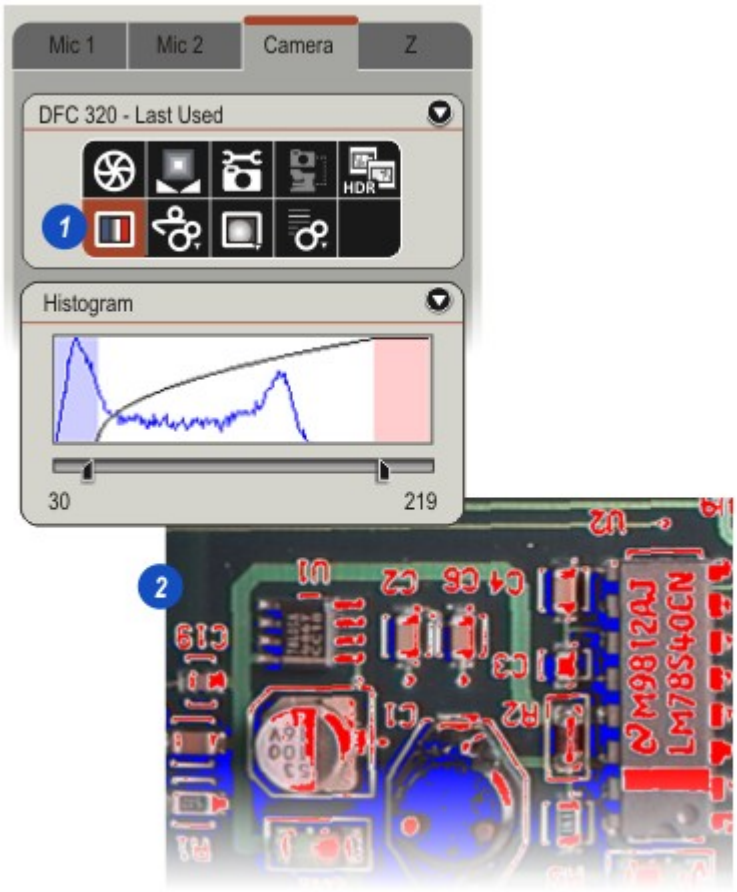
Over and Under-Exposure:

The 'one-click' *Show Under/Over Exposure* feature is a fast indication of those areas of the image that are not exposed properly - and probably not adding to the image quality.

1: To check over- and under- exposure, click on the *Show Under/Over Exposed* button.

On the *Histogram* widow two coloured panels appear at the extreme ends of the display. The blue panel indicates under-exposure at the 0 (Black) end, and the red panel over-exposure at the 255 (White) end.
The graphics are also applied to the live image so...

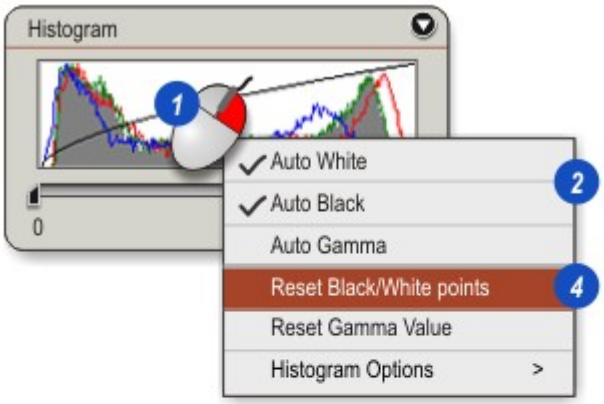
2: ...this image shows blue as under exposed areas and red as over exposed areas.

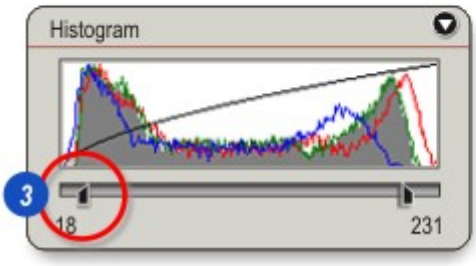


Auto Black and Auto White:

Because under- and over exposed areas of the image do not usually contribute to its quality, those levels may be ignored without detriment.

Auto White and *Auto Black* automatically ‘crops’ either or both under- and over exposed levels.





1: Right click on the *Histogram* window.

2: From the drop down menu select the *Auto White* or *Auto Black* option. The selection becomes checked and active. Click again to uncheck and disable the option.

3: Beneath the *Histogram* window, the slider corresponding to the white or black option moves to reflect the ignored light levels.

4: To reset the black and white values, right click on the *Histogram* window and from the drop down left click on the *Reset Black/White Points* option.

Auto White and -*Black* are normally switched off.

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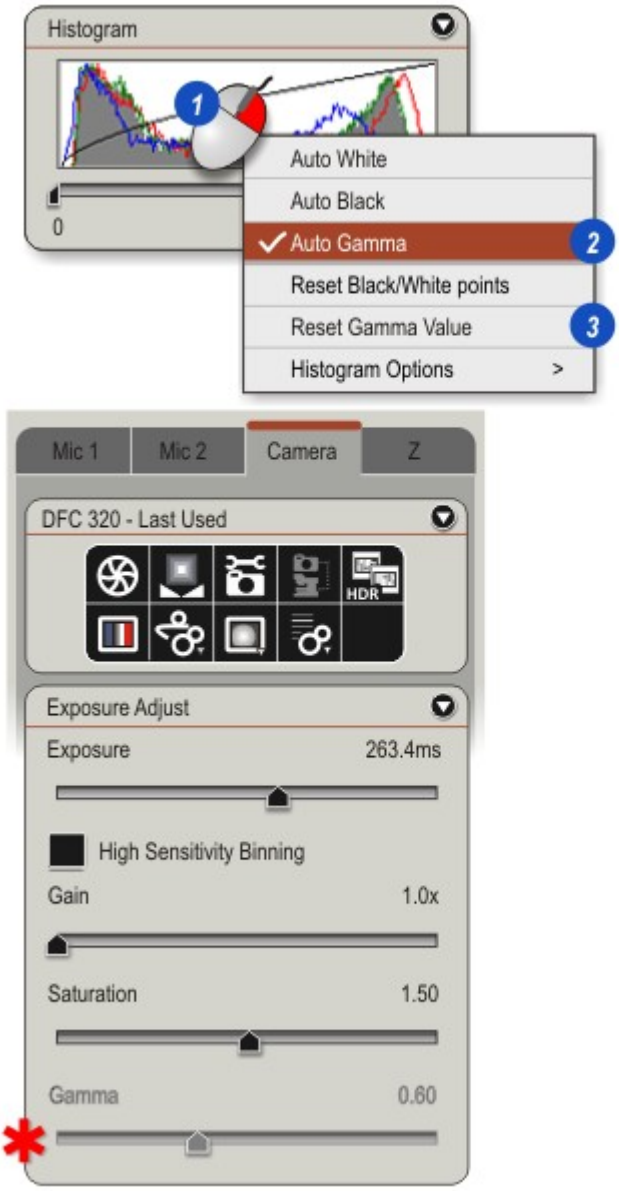
Auto Gamma:

The *Auto Gamma* option sets the gamma level based upon the active light levels. Under- and over exposed levels are not included if they have been 'cropped' either manually or automatically.

1: Right click on the *Histogram* window.

2: From the drop down menu select the *Auto Gamma* option. The selection becomes checked and active. Select again to uncheck and disable the option.

3: To reset the gamma value, right click on the *Histogram* window and from the drop down left click on the *Reset Gamma Value* option.



Whilst *Auto Gamma* is enabled, the *Gamma* control on the *Exposure Adjust* panel is disabled.

Auto Gamma is normally switched off.

HDR & Averaging:

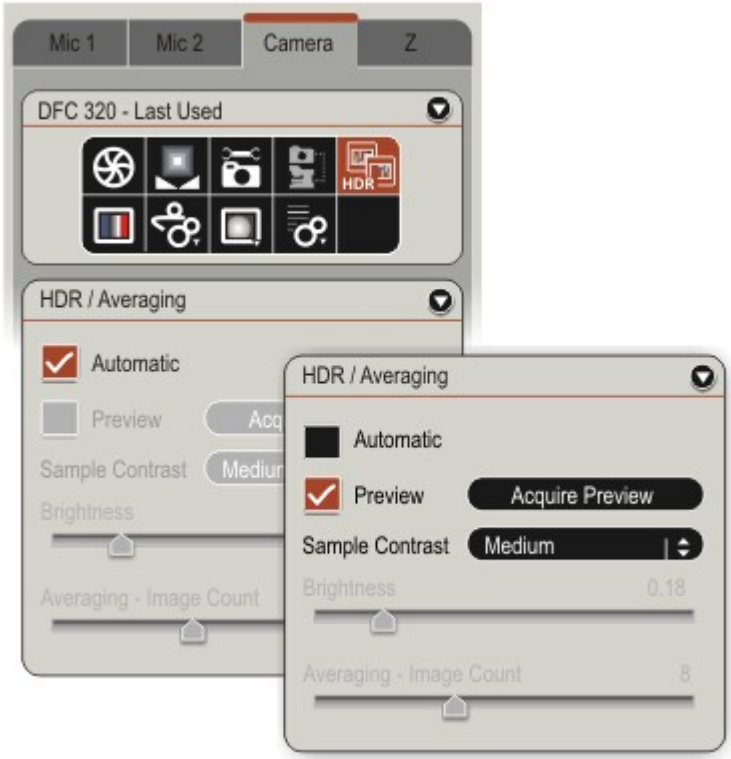
Images that exhibit high contrast between the light and dark areas, have posed an on-going problem to the microscopist - capture to maintain the dark detail and the light information is badly exposed and probably lost. Expose for the whites and the dark detail is compromised.

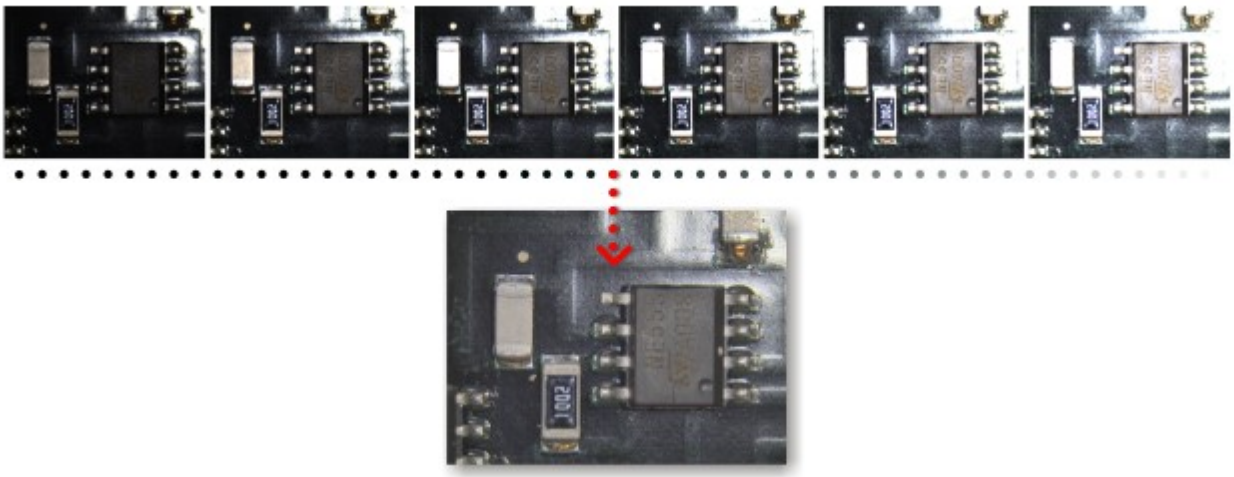
Leica High Dynamic Range (HDR) provides a fast solution by automatically capturing a number of images each at a different exposure, and then combining them digitally into a single image that balances the contrast range. Detail across the entire image is retained and clear.

Users can choose between automatic *HDR* that handles the processing and saving in a simple, fast single step, or a manual operation that allows some fine-tuning before the image is saved.

Additionally, an *Averaging* feature is available that can reduce the amount of noise in an image. Reducing noise can enhance the final image either making it look better or making it more suitable for analysis.

Continued 249





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Setup for HDR and Averaging:

Before using *HDR* or *Averaging*:

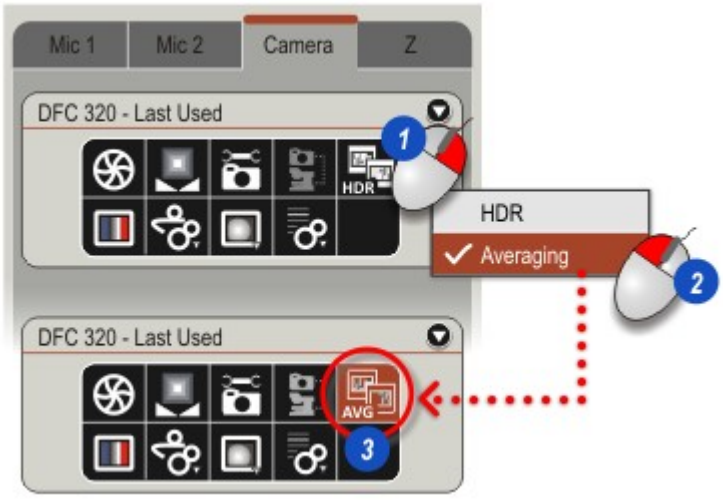
- Set the capture details in *Preferences*.
- Select a capture folder in *Browse* 299.
- Check that required *HDR* function is enabled. If not:

  1: Right-click on the *HDR* button.

  2: From the drop-down menu left-click to select either *HDR* or *Averaging*.

  3: The button caption displays the selected function - *HDR* or *AVG (Averaging)*.

- Adjust the specimen illumination so that it is even, reduces high-lights, reaches into crevices and does not cast shadows.
- Use the *Fast Track Exposure* sequence 224 and aim for a sharp and reasonably exposed image.
-



250 Link to *Automatic HDR.*

251 Link to *Manual HDR* with *Preview.*

254 Link to *Averaging*.

Automatic HDR:

The *Automatic HDR* feature captures the range of images to memory, creates the *HDR* image and saves it to the hard drive in the set capture folder.

All of the settings are controlled by the software - users do not have the facility to select the *Sample Contrast* or adjust the *Brightness* before the *HDR* image is saved, but can use the *Enhance* controls on the *Process Workflow* to make adjustments to it after capture.

Previous settings made using the *Preview* function are ignored.

1: If necessary click to disable *Automatic Exposure* and...

2: ...click on the *HDR* button.

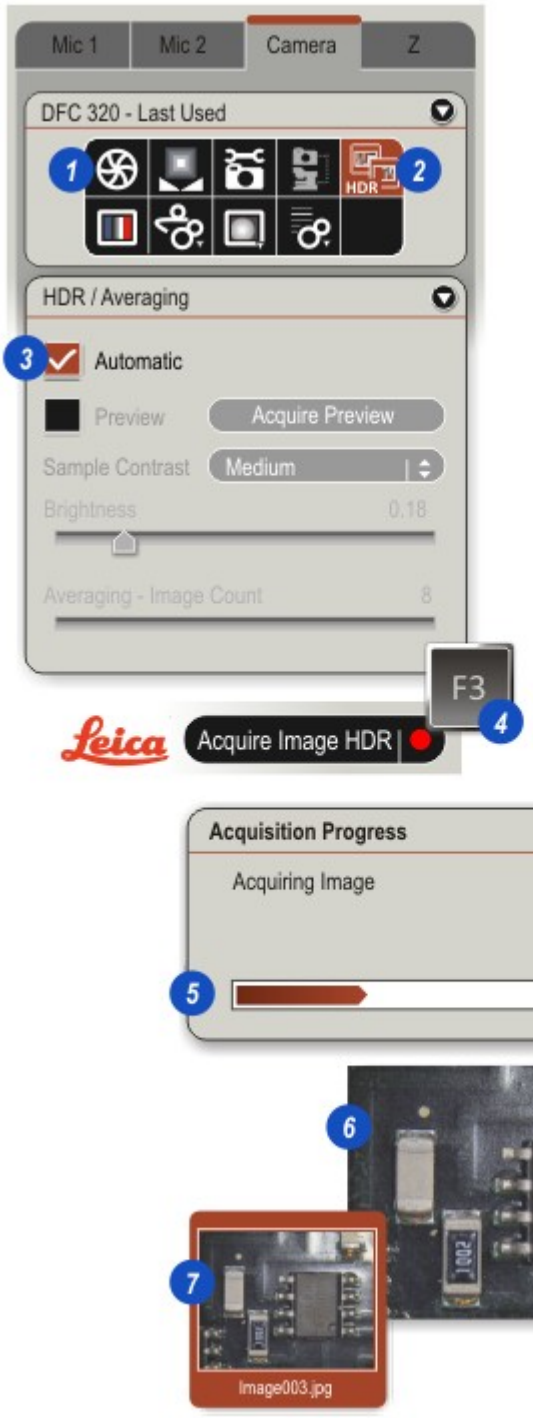
3: On the *HDR / Averaging* control panel, click to enable (tick mark visible) the *Automatic* check box.

4: Click the *Acquire Image HDR* button or function key *F3*.

5: The *Acquisition Progress* bar appears whilst the images are being captured to memory and the *HDR* image created.

6: The final *HDR* image is stored in the capture folder on the hard drive and...

7: ...a thumbnail is created in the *Gallery*.



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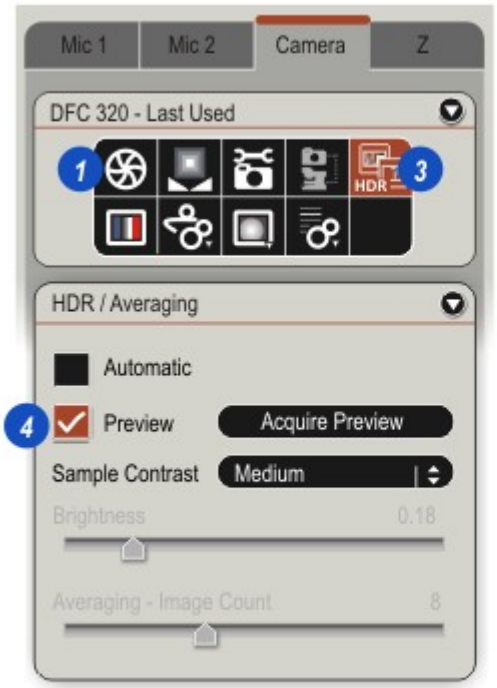
Manual HDR:

In manual mode, the Preview facility is used to optimise the appearance of the image by interactively adjusting the contrast range and brightness of the HDR image. These settings are then used for actual image acquisition while HDR is in manual mode.



1: If necessary, click to disable *Automatic Exposure* and...

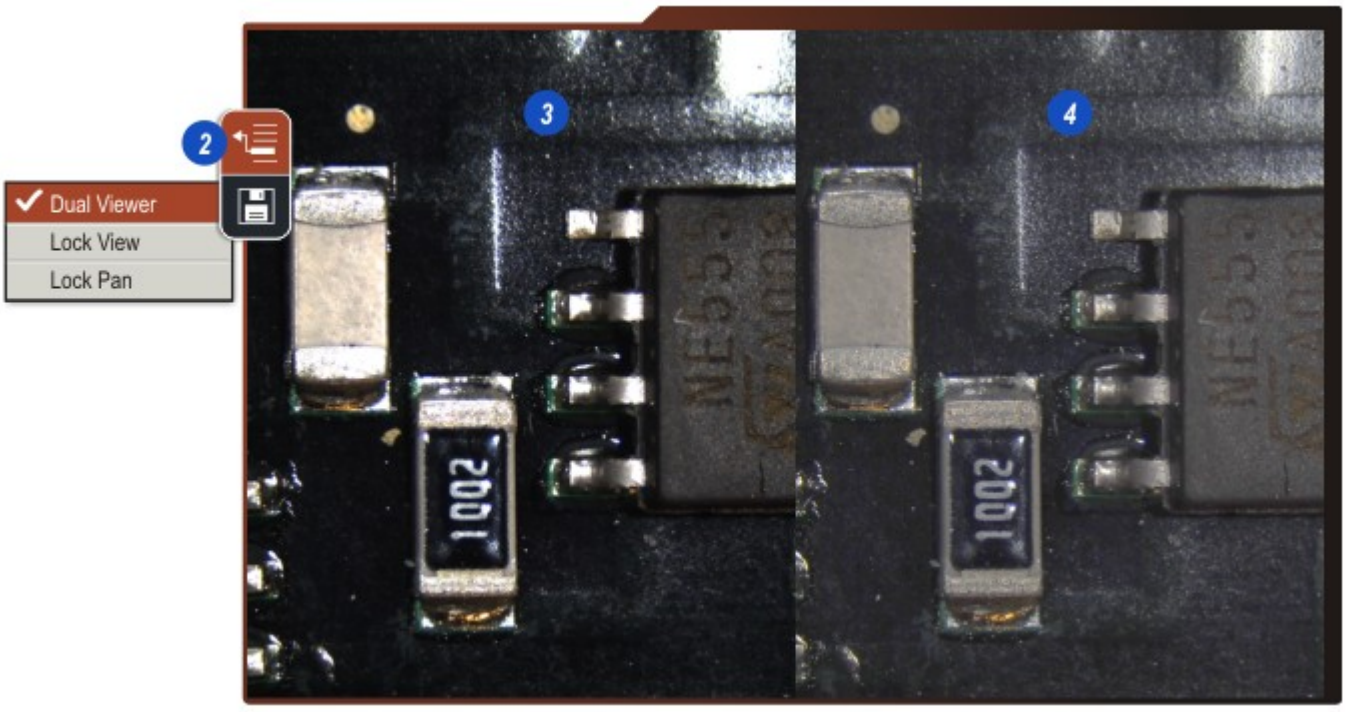
2: On the *Side Tool Bar* turn on *Dual Viewer* so that the live image can be compared with the *HDR* processed image. The live image is on the left and the *Preview* image on the right.

Users will find it convenient to select the *After Capture Do Nothing* option in *Preferences* 49 to remain on the *Acquire Workflow* after an image is captured.

3: Click on the *HDR* button.

4: On the *HDR / Averaging* control panel, click to enable (tick mark visible) the *Preview* check box.

Continued 252



Sample Contrast allows the user to select the exposure values either side of a fixed exposure point.

The additional exposure times are determined by the *Sample Contrast* setting - *Low*, *Medium*, *High* and *Very High*. Each option applies different factors as follows:

Low Contrast: -1 | Centre | 1

Medium Contrast: -1.5 | Centre | 1.5

High Contrast: -2 | Centre | 2

Very High Contrast: -3 | -1.5 | Centre | 1.5 | 3

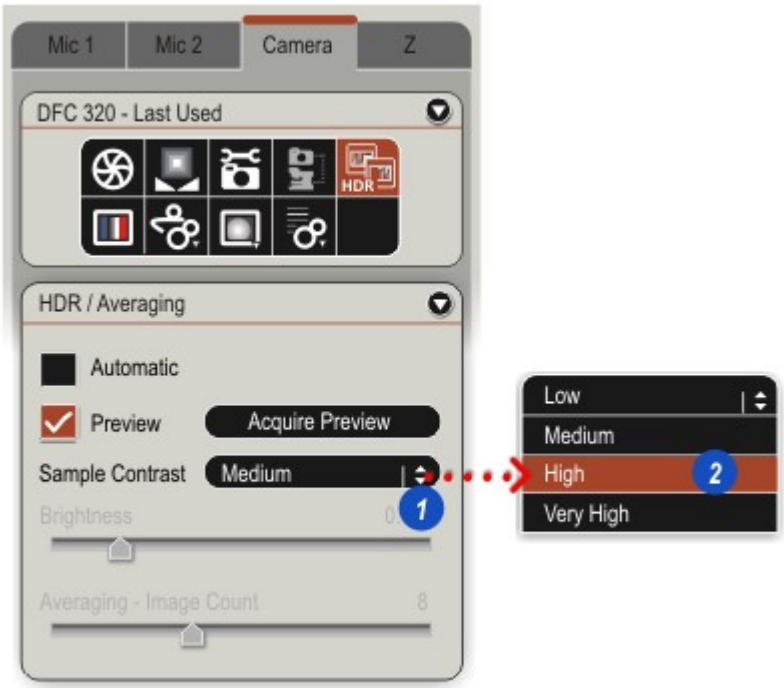
The *Very High Contrast* option captures 2 additional images either side of the centre exposure.

Users should experiment to find the best level for the image.

1: Click on the arrows to the right of the *Sample Contrast* header and...

2: ...click to select the required contrast level.

Continued 253



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Continued from the previous page 251

1: Click the *Acquire Preview* button.

2: The *Acquisition Progress* bar appears. A range of images at different exposures is being created in memory and then combined to produce...

3: ...the *HDR* image with balanced contrast displayed in the *Viewer* right-hand pane.

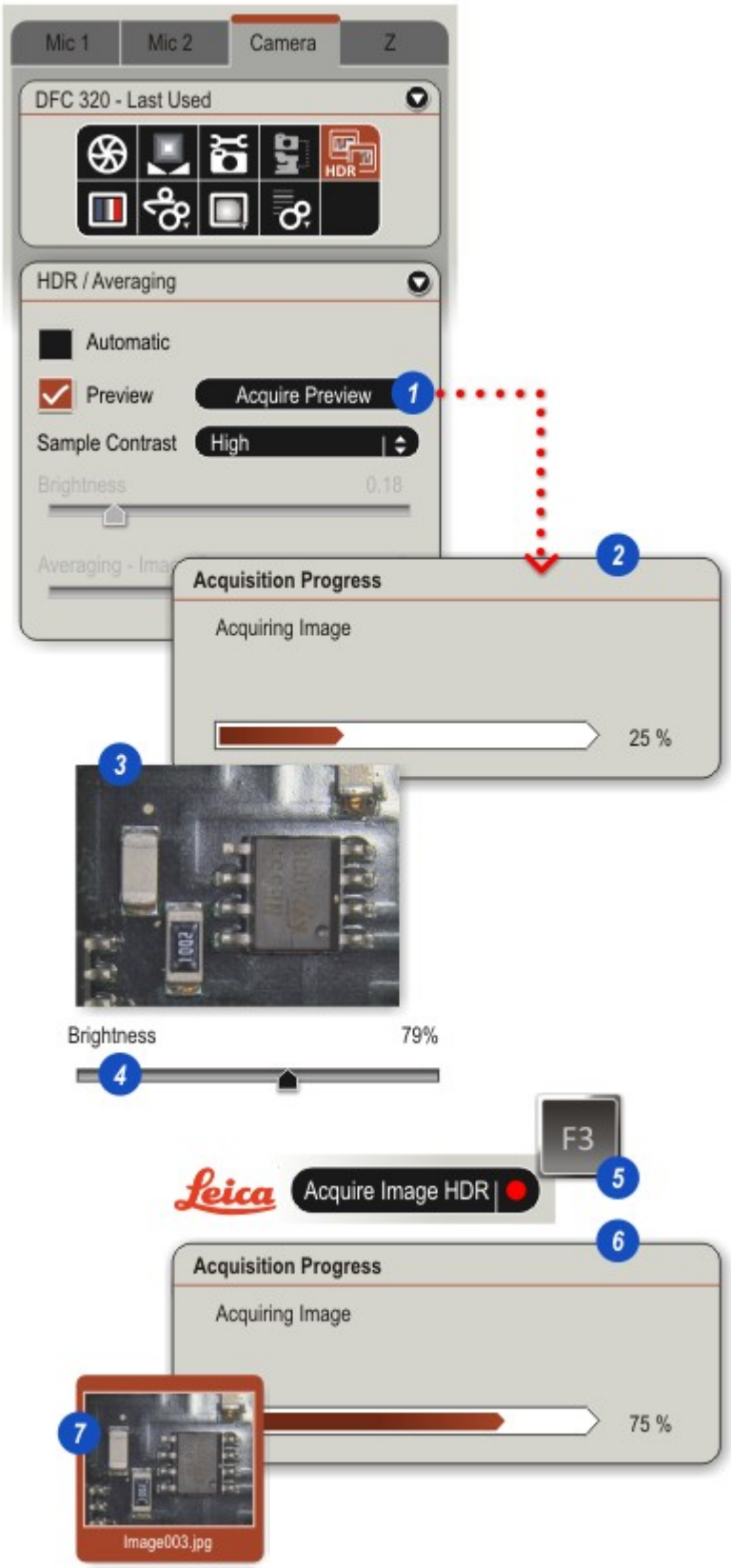
4: If necessary finely adjust the image brightness.

5: To save the image which is currently only memory, click on the *Acquire Image (HDR*) button or the *F3* function key.

6: The progress bar appears again and the *HDR* image is saved to the capture folder with...

7: ...a thumbnail in the *Gallery*.

The *HDR* settings derived from *Preview* remain active for any further captures unless changes are made to *Sample Contrast* or *Brightness.*



Averaging:

Noise can compromise the level of detail in digital images, particularly in regions where the amount of light is low or a higher gain setting has been used. Reducing noise can enhance the final image either making it look better or making it more suitable for analysis. Some techniques to reduce or remove noise soften the image as well.

The *HDR* panel includes a technique that averages multiple exposures to reduce noise. Averaging can reduce noise without reducing detail, because it actually increases the Signal-to-Noise Ratio (SNR) of the image and may retain detail by increasing the bit-depth of the image. Images captured using *Averaging* tend to take a little longer to acquire.

Image averaging works on the assumption that the noise in the image is truly random and fluctuations above and below actual image data will gradually even out as more and more images are averaged.

1: If necessary, click to disable *Automatic Exposure.*

2: Click the *AVG* button. To select the *AVG* function 249.

3: On the *HDR / Averaging* control panel all of the controls are disabled except for the *Averaging Image Count* slider.

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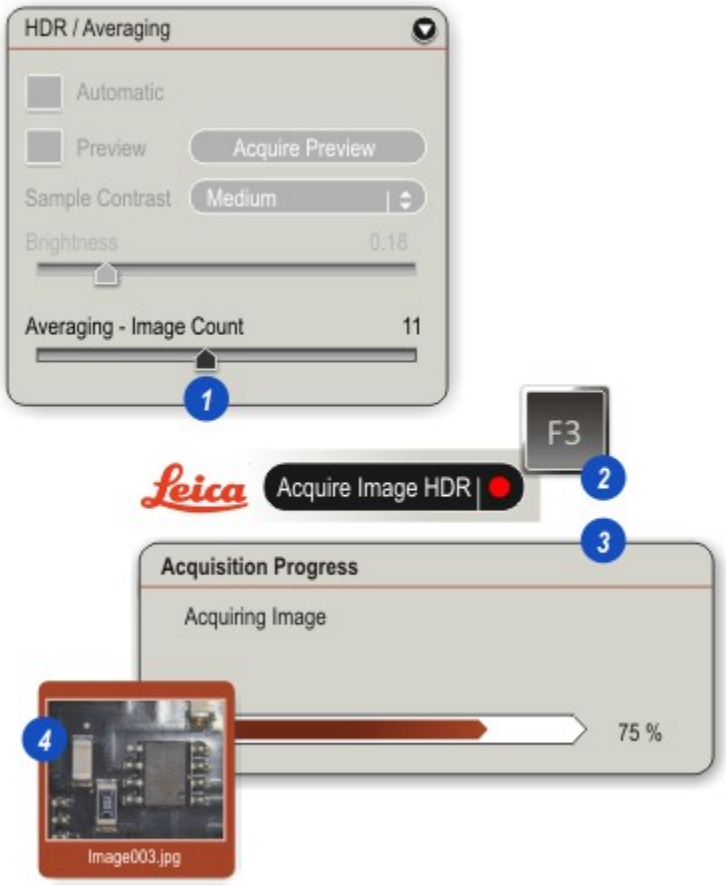
Continued from the previous page 254

1: Click and drag the *Average - Image Count* slider to the left (reduce) and the right (increase) the number of exposures to be tested.

2: Click on the *Acquire Image HDR* button or the *F3* function key.

3: The *Acquisition Progress* bar appears as the range of exposures is checked.

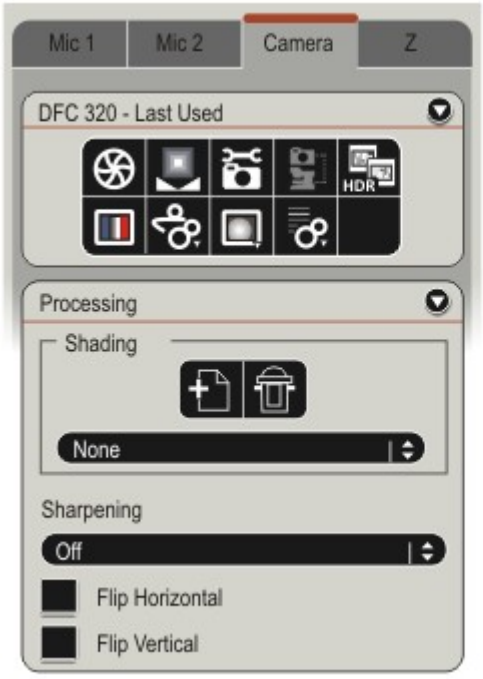
4: The exposure with the best signal-to-noise ratio - least noise - is saved to the capture folder and a thumbnail appears in the *Gallery*.



Processing:

The *Processing* panel provides tools to improve quality and orientation primarily for a captured image but may be used for live images as well.

- 257 *Shading*: Corrects light level variations that often occur due to bright spots caused by the microscope light source and the optics.
- 260 *Sharpening*: Enhances the edges of indistinct features on the image making is clearer and crisper.
- 261 *Flip Horizontal* and *Flip Vertical:* Re-orientates the image, top to bottom or side to side.



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Shading is the name given to variations in the background light level across an image.

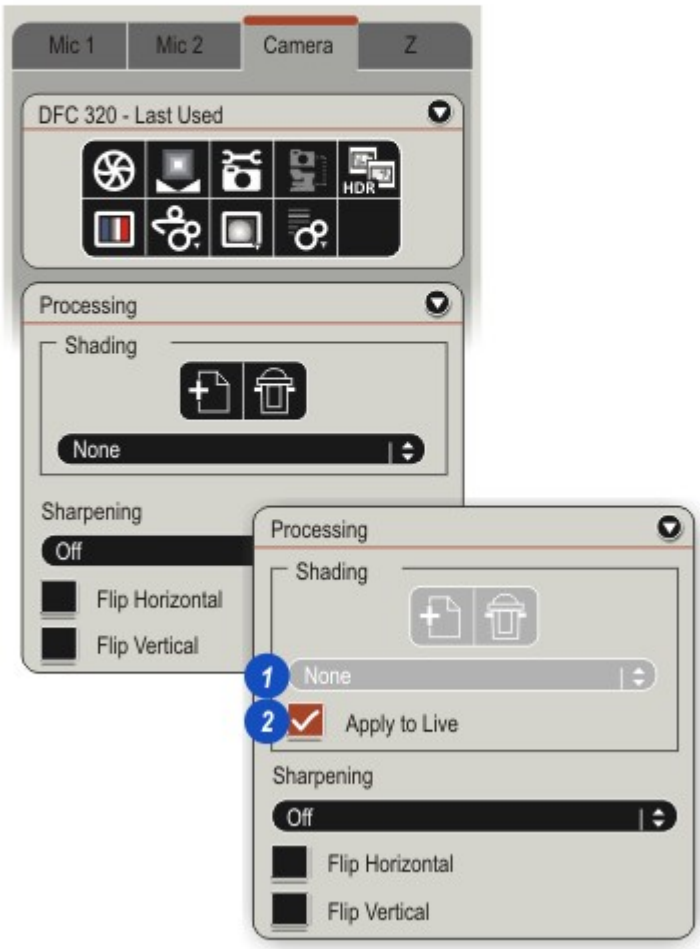
The examples show transmitted light through a microscope. On the left, the light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

Even 'illumination' on live images can be achieved in software by applying a 'blank area' value to the entire image area. The effect is shown in the right image.

The light source and any of the optical elements will produce different shading levels, so each microscope element combination should have its own shading setting.

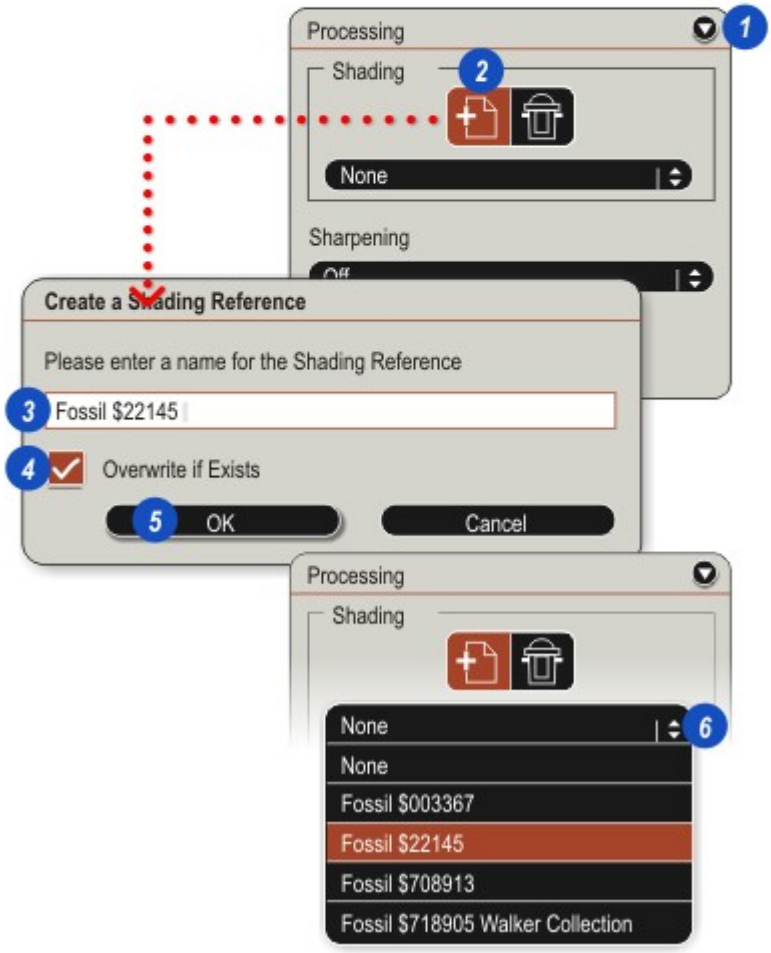
1: If *Linking* > *Shading* is enabled it takes precedence and *Processing* > *Shading* is disabled - greyed out on the *Processing* panel - and...

2: ...the *Apply to Live* check box is available to allow current settings to be applied to the live image.



Creating a Shading Reference:

- Make sure the image is sharp and well lit.
- Move the stage to a clear, unblemished area of the image – introduce a very small amount of de-focus if the chosen area is not completely clear.
- Over-exposing slightly can also help to ‘remove’ blemishes.
- Use the *Show Over/Under Exposed* control **(A)** on the toolbox and adjust the *Exposure* **(B)** until just a few red pixels are visible or...
- Replace the specimen slide with a plain slide (and cover slip if used) of the same type.



1: Expand the *Processing* panel if necessary by clicking on the arrows to the right of the *Processing* header bar.

2: Click on the *New Shading* button.

3: Type a unique name for the shading reference in the text box.

4: Enable the *Overwrite if Exists* button to replace an existing shading reference.

5: Click *OK.*

6: Click on the arrows to the right to the *Shading* header and the new shading reference is included in the drop-down list.

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Select and Load a Shading Reference:

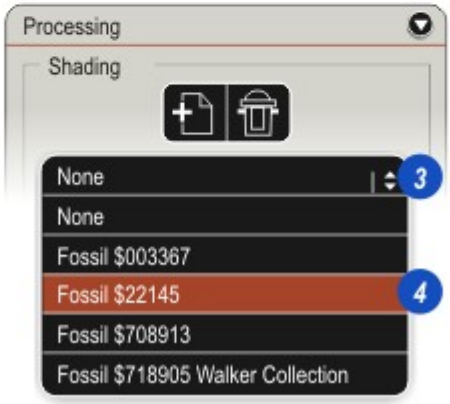
There are two methods for selecting and loading a *Shading Reference:*

1: Click on the *Toolbox Shading* button and...

2: ...click to select and load a reference from the drop-down list. It will be indicated by a tick mark to the left.

3: On the *Processing* panel, click on the arrows to the right of the *Shading* header. The drop-down list of references appears.

4: Click to select and load a reference.



Sharpening:

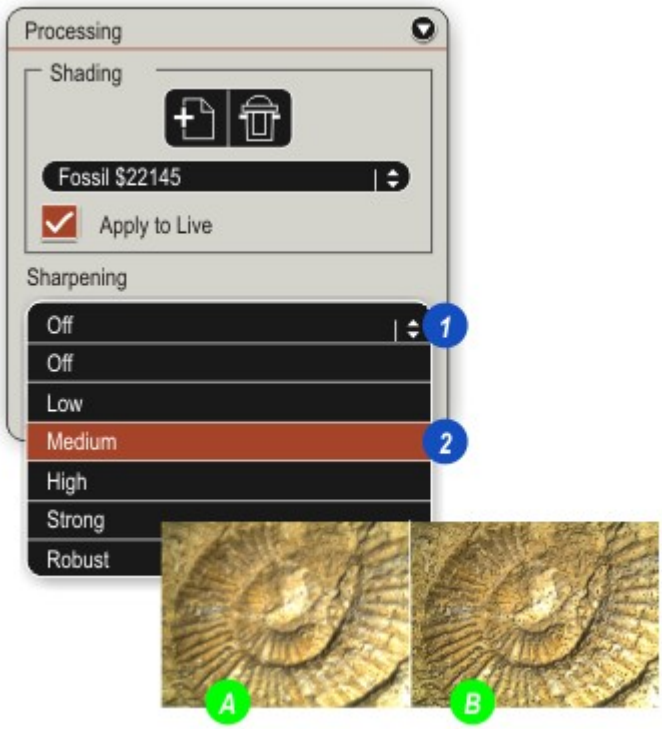
Sharpening is a software function in which the boundaries between tonal values are enhanced. Use it to improve the clarity and crispness of indistinct detail on an image.

The level of enhancement is selectable between *Low* and *Robust* (very high) but too much sharpening can make the image appear grainy and speckled. It is a fast process so the best approach is to start with the *Low* setting and work toward *Robust* step-by-step.

1: Click on the arrows to the right of the *Sharpening* header.

2: From the drop down menu select either *Off* to turn off sharpening, or the level required.
Repeat the process choosing another level if the result is not suitable.

The illustrations show the original image **(A)** and *Medium* sharpening **(B)**.



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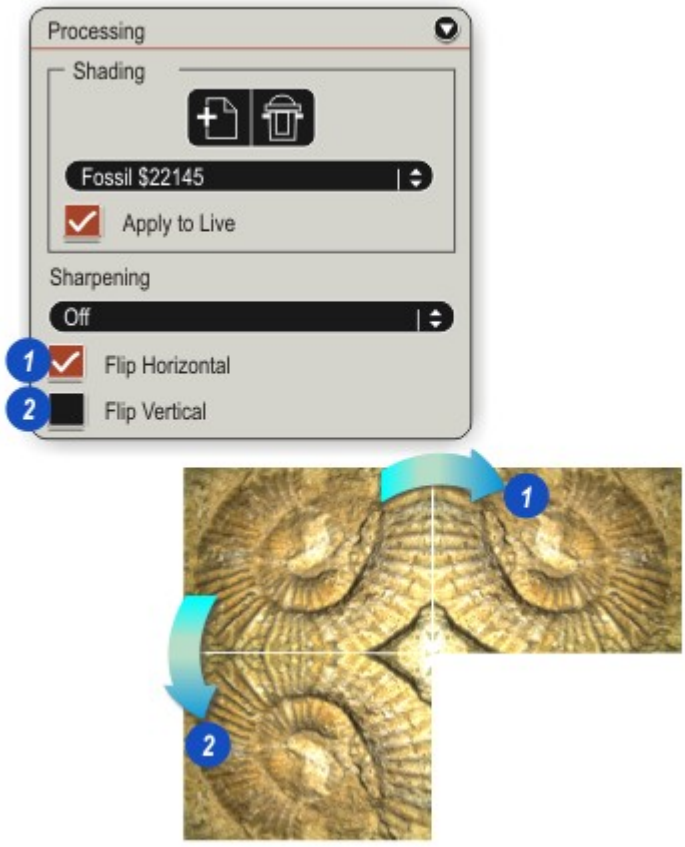
These two options - Flip Horizontal and Flip Vertical - re-orientate the image. Flipping is often used to emulate the view through the eyepieces.



To flip the image from side-to-side:

1: Click on the *Flip Horizontal* check box. Click again to return it to its original position.

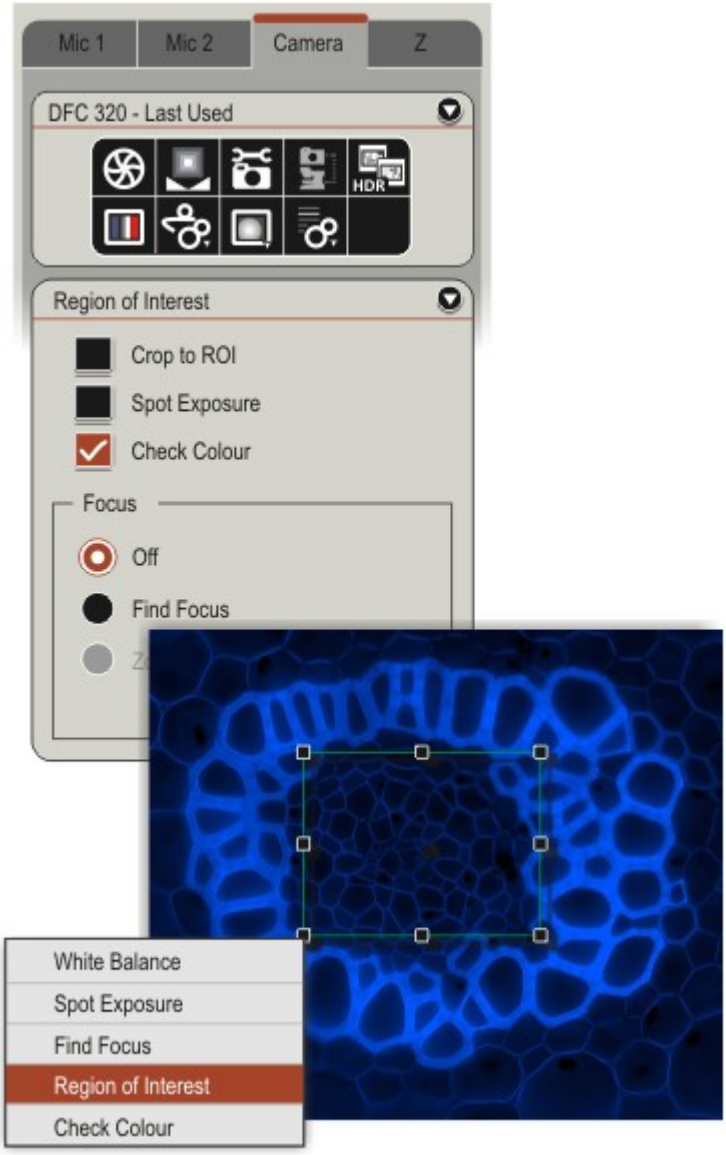
To flip the image from top-to-bottom:

2: Click on the *Flip Vertical* check box. Click again to return it to its original position.

A *Region of Interest* (RoI) is created by drawing a rectangle on an image. The area within the rectangle - the *Region of Interest* - is then the target of several special functions, the image outside the region being ignored.

Five special functions may be applied to a *Region of Interest:*

- 262 *White Balance*: Displays all of the neutral tones as shades of black and white but only within the *Region of Interest.*
- 265 *Spot Exposure:* Automatic exposure is applied to the entire image but using only the values contained within the *RoI*.
- 266 *Find Focus*: Defines a moveable *Region of Interest* in which to focus.
- 264 *Region of Interest:* When the image is acquired, only the part within the *RoI* is captured - the rest is discarded.
- 267 *Check Colour*: Used to adjust the image overall colour balance by using a small region as the reference for both *Hue* and *Saturation.*



There can be a different region for each of the five functions and with the exception of *White Balance* they are stored and can be recalled by clicking to enable the check boxes on the *Region of Interest* control panel. Each *RoI* has a different identifying outline colour.

Create a new region for any of the options at any time to overwrite the existing one.

White Balance has an immediate effect as soon as it is selected. A new region has to be created each time it is used.

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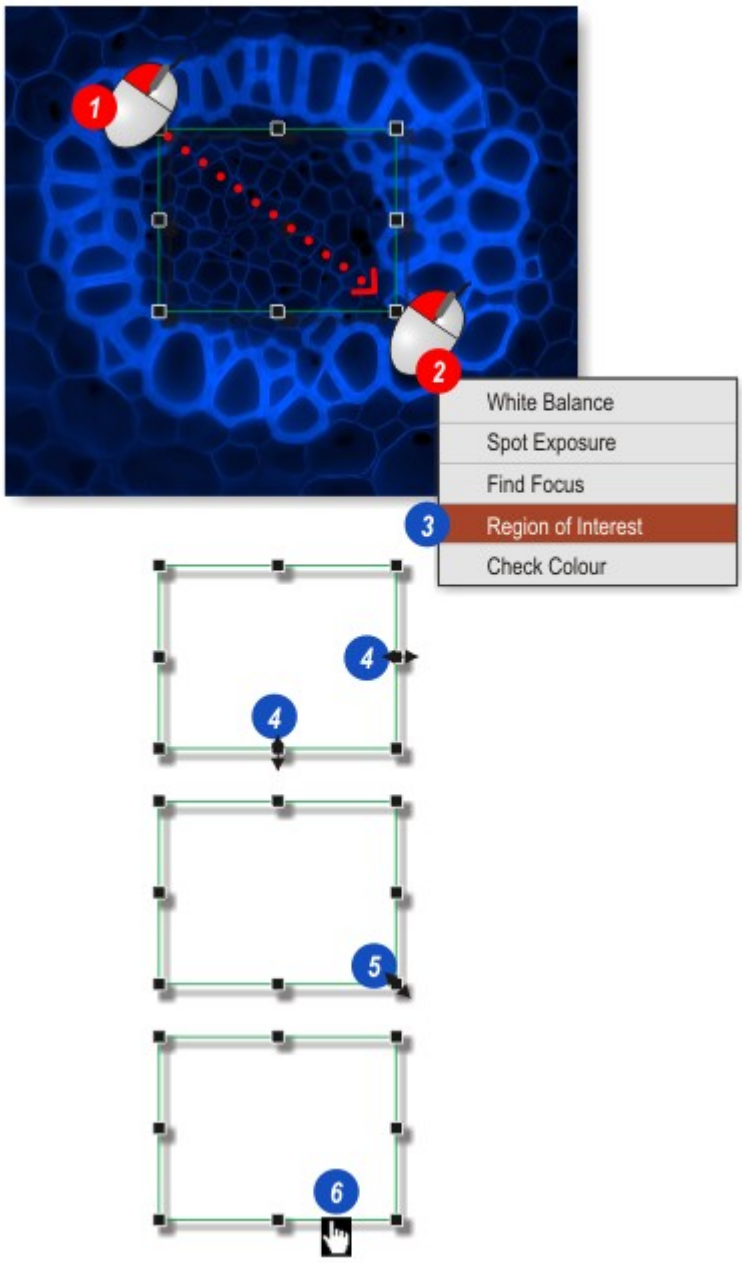
Creating a Region of Interest:

Create a *Region of Interest* by:



1: On the image, click on a corner of the region and...

2: ...holding down the mouse button drag to the opposite corner. The region is drawn on the image with small scaling 'handles' at each corner and mid-way along each side. It is not necessary to be precise because the region can be re-sized and moved later.

3: Release the mouse button and the contextual menu appears listing the five *RoI* options. The new *RoI* can be either:

- *White Balance*,
- *Spot Exposure,*
- *Find Focus,*
- *Cropping Area (Region of Interest) or*
- *Check Colour.*

  Click to select.

  If none of the options is chosen the region will default to the *Region of Interest* which represents the capture area.

  A separate *RoI* of differing dimensions and position can be created for each of the options. The location and dimensions are stored.

As the mouse cursor approaches the region, the scaling handles re-appear:

4: To stretch the region either vertically or horizontally, click on a handle. The cursor changes to a double-ended arrow. Holding down the mouse button drag the handle to re-size the region.

5: Scale the region proportionally by clicking on a corner handle - the cursor changes to a double-ended arrow - and holding down the button dragging it diagonally.

6: Move the entire region by positioning the mouse over an edge. The cursor changes to a hand. Click and holding down the cursor button drag the region to a new position.

Crop to Region of Interest:

This feature will capture only that part of the image within the *Region of Interest.* The rest of the image is ignored.

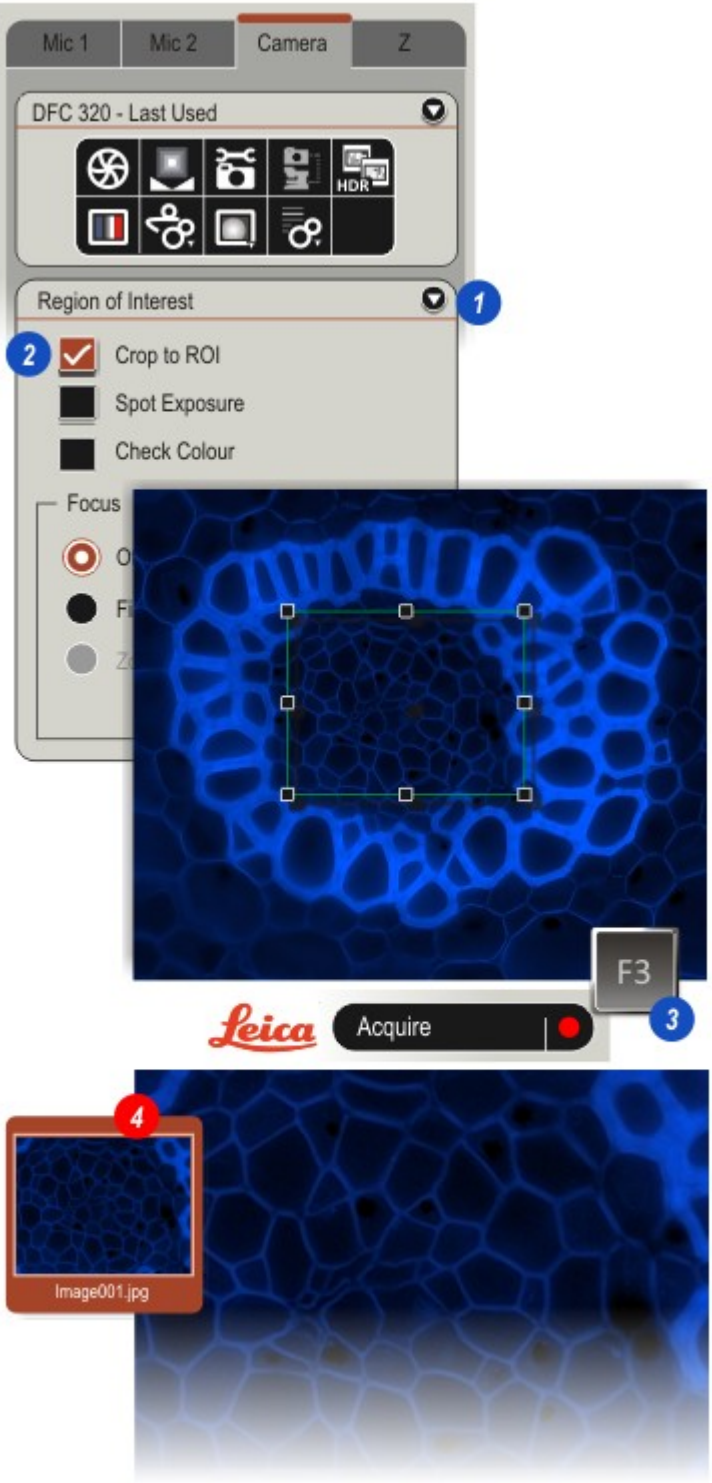
1: Click on the small arrow to the right of the *Region of Interest* header to expand the control panel.

2: If necessary, click to enable the *Crop to RoI* check box - a tick mark visible. The *Region of Interest* appears green on the image and can be re-sized and positioned as described on the previous page.

3: Click on the *Acquire Image* button or press the keyboard *F3* key.

4: Only the area of the image within the *RoI* is captured and scaled to suit the *Viewer*.

A thumbnail is displayed in the *Gallery*. Depending upon the After Capture setting in *Preferences* the program will move to another *Workflow* or remain where it is.



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Spot Exposure:

Spot Exposure will automatically adjust the exposure of the entire image using only the area with the *Region of Interest.*

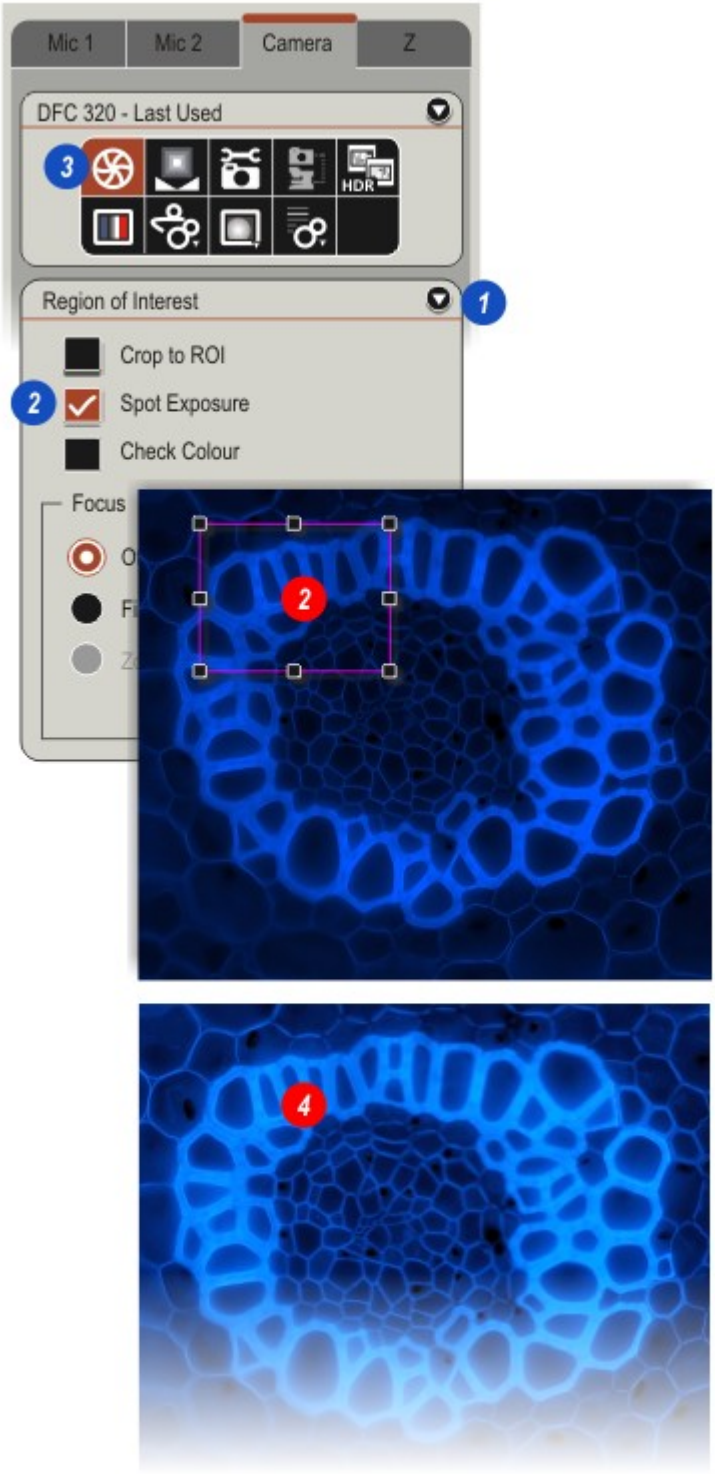
The *RoI* can be moved around the image to compare different exposure results.

1: Click the arrow to the right of the Region of Interest header to expand the control panel.

2: Click to enable the *Spot Exposure* check box. A *Region of Interest* is drawn with a magenta outline. Re-size or move the region as described previously.

3: The Toolbox *Auto Exposure* button is automatically enabled and...

4: ...the image exposure is adjusted using the values in the *RoI.*



Find Focus:

This feature displays the focus precision for the area of the live image contained within the *Region of Interest*.

The region may be moved around the image to compare different areas with a focus bar indicating the 'best' level achieved.

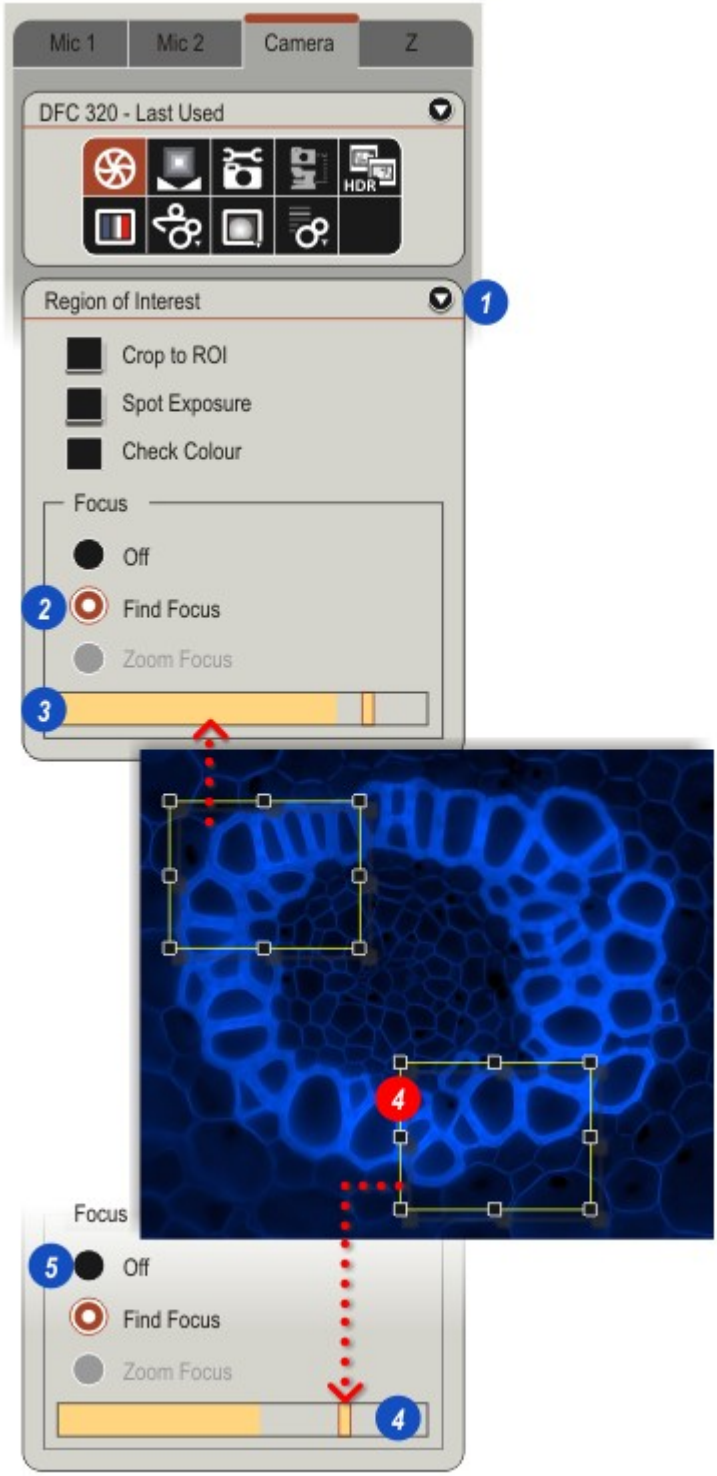
1: Click the arrow to the right of the *Region of Interest* header to expand the control panel.

2: Click to enable the *Find Focus* radio button. A *Region of Interest* is drawn with a yellow outline. Re-size or move the region as described previously.

3: The level bar moves left to right to indicate the focus precision. The further the bar is to the right, the better the focus.

4: To check the focus at another point on the image, click and hold on the yellow outline (not a 'handle'), and drag the *RoI* a new position.

5: Turn off *Find Focus* by clicking the *Off* button. Click the *Find Focus* button **(2)** to reveal the *Region of Interest* at its last size and position.



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Check Colour:

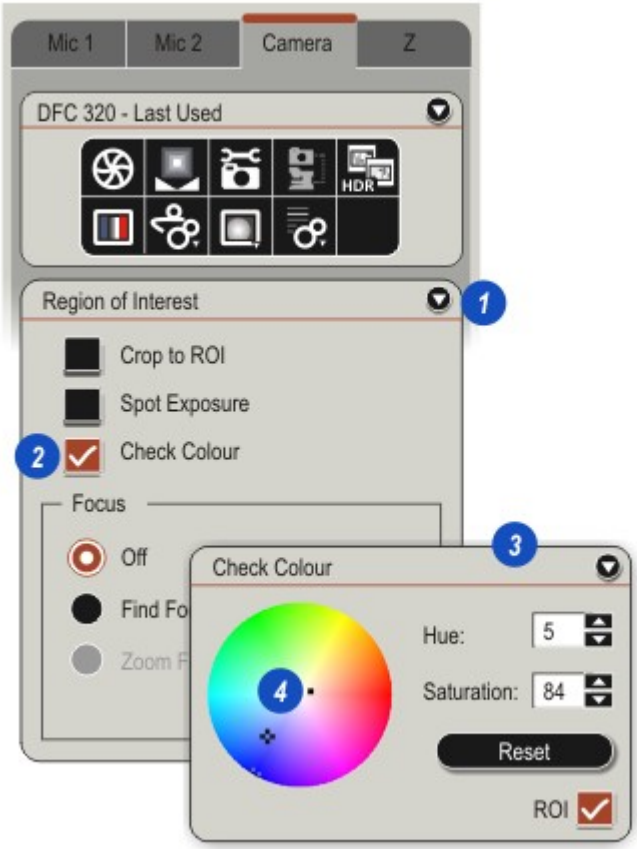
The *Check Colour* function on the *Region of Interest* panel is a convenient control for creating a region that can be used with the *Check Colour* feature.

1: Click the arrow to the right of the *Region of Interest* header to expand the control panel.

2: Click to enable the *Check Colour* check box and create a *Region of Interest* as previously described. A region is drawn with a blue outline. Re-size or move the region as necessary.

3: With *Check Colour* enabled and the *Check Colour* feature launched 296, the region is represented by...

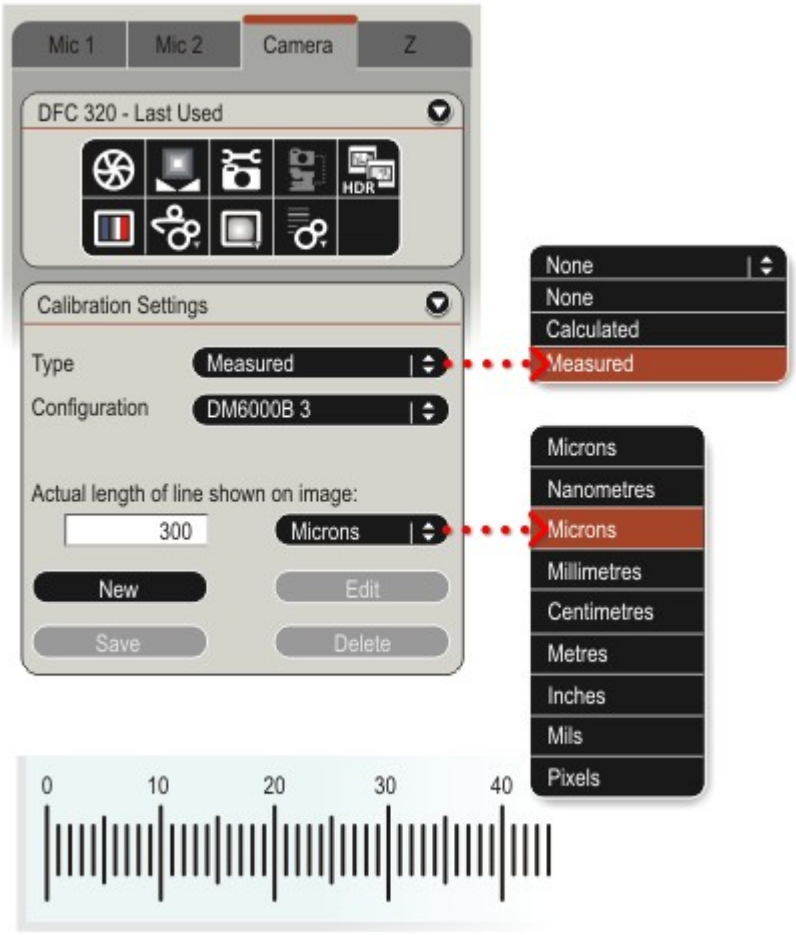
4: ...a small dot on the colour wheel which represents the *Hue* and *Saturation* levels inside it.



Calibration ensures that measurements indicated by the software are given in real world units taking into account the selected optical magnification of the microscope and the size of pixels of the digital camera.

Five calibration options are available depending upon the level of precision required:

- 269 *None:* Calibration not required - distances are measured in pixels..
- 270 *Calculated Default Configuration:* Based upon the software's 'knowledge' of the microscope optical components and the pixel size of the digital camera being used. This is the quickest way of establishing a reasonable but approximate calibration as it does not make any checks against a calibration slide.
- 271 *Calculated User Configuration:* Using a calibration slide on the stage the calibration of a single, greatest magnification objective is measured and from this all the other objective/mag changer combinations are derived. This is more accurate than simply using the *Configuration Default*.
- 274 *Measured Calibration:* Again, a calibration slide placed on the stage is used, but all objectives in combination with all Mag changer settings are measured. A 'Wizard' helps to speed the process and the result can be a high level of calibration precision.
- 279 *Automatic Calibration:* Speeds the *Measured Calibration* process by using the software to detect and measure the calibration slide. The user does not have to make any measurements.



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No Calibration:

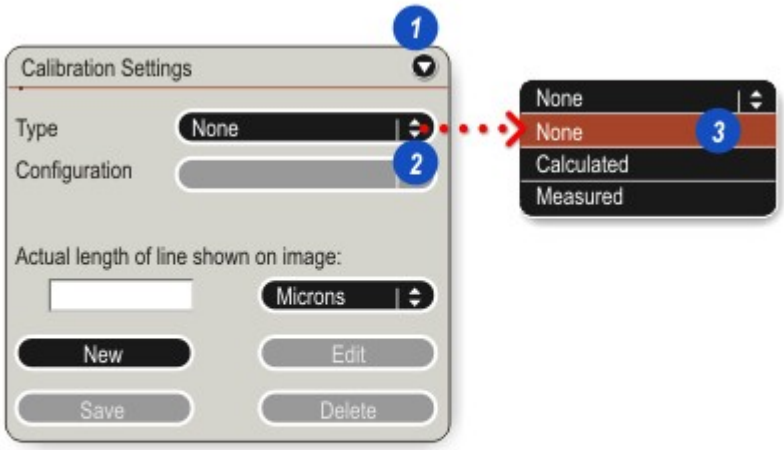
If calibration is not required then one display pixel = one camera pixel.

All measurements are reported in pixels:

1: Click on the arrow to the right of the *Calibration Settings* header to reveal the *Calibration* panel.

2: Click on the arrows to the right of the calibration *Type* header and from the drop down menu…

3: …click on the *None* option.



Calculated Calibration with the *Default Configuration* uses the microscope optical data and camera pixel size to create a calibration value. This is used to return measurements in the type selected – *Microns, Millimetres* etc. It is a very fast and moderately accurate method of setting the calibration.

1: Click on the arrows to the right of the *Type* header.

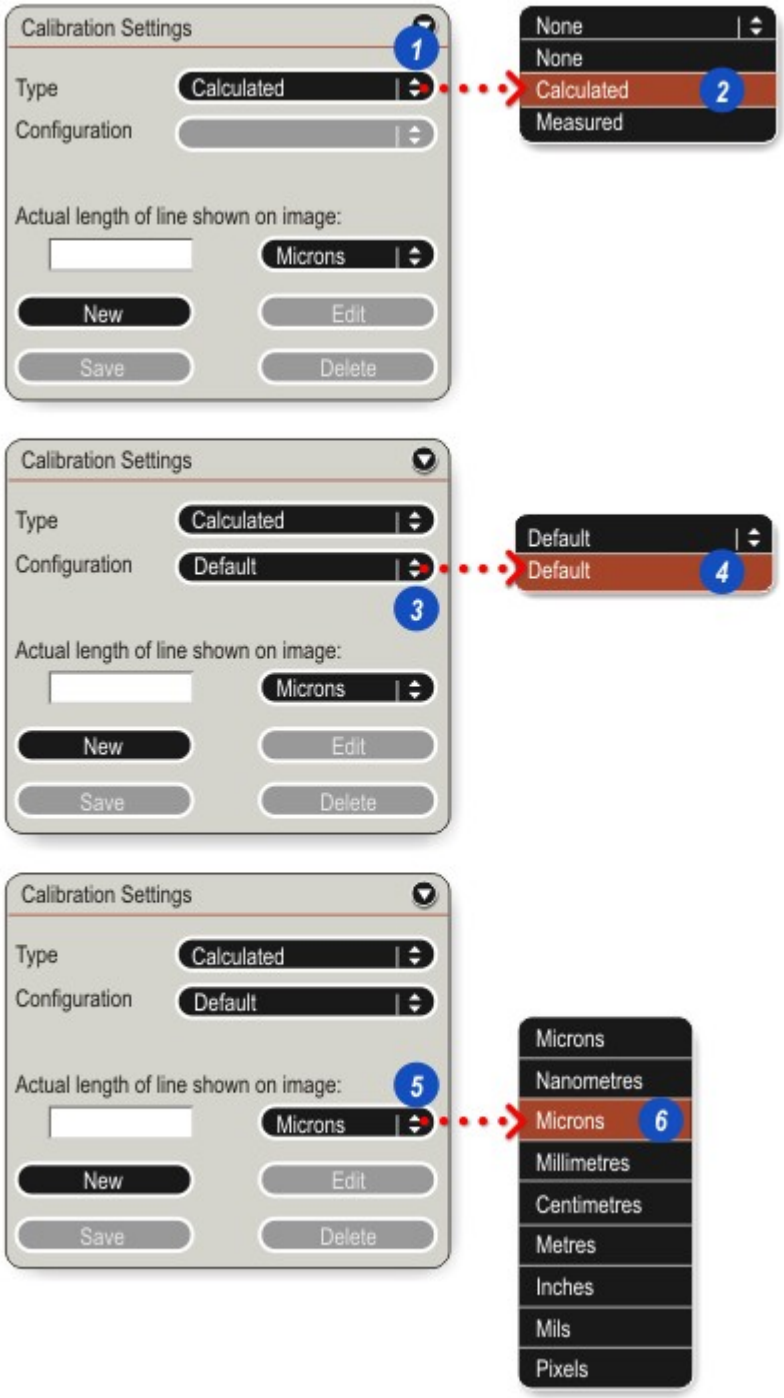
2: From the drop down menu click to select the *Calculated* option.

3: On the *Configuration* header click to the arrows to the right and…

4: …click on the *Default* option.

5: Select the measurement type by clicking on the arrows to the right of the header.

6: Click to select the measurement units required.



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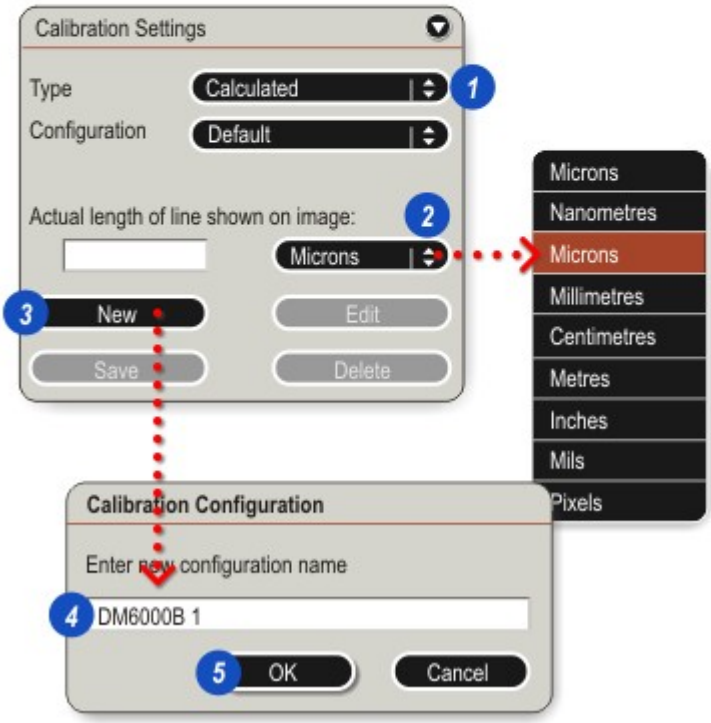
User Calibration calculates calibration values by making an on screen measurement across a *Calibration Slide* placed on the microscope stage.

Only one calibration is taken with a single objective/mag combination, the resulting value is than applied automatically to all other combinations using the microscope optical data.

- The objective with the greatest magnification must be selected and Mag changer set at 1.
- For Stereo- and Macroscopes ensure that that the zoom position is selected at one of the click stops listed in the zoom magnification values.
- Make sure the *Calibration Slide* is properly focused and corresponds to the measurement units selected in Step **(2)**.



1: Click on the arrows to the right of the *Type* header and select *Calculated* from the list.

2: Click on the arrows to the right of the measurement type header and from the drop down list click to select the *Calibration Slide* measurement units.

3: Click the *New* button.

4: On the *Calibration Configuration* dialog, click in the text box and type an appropriate name for the calibration.

5: Click *OK*.

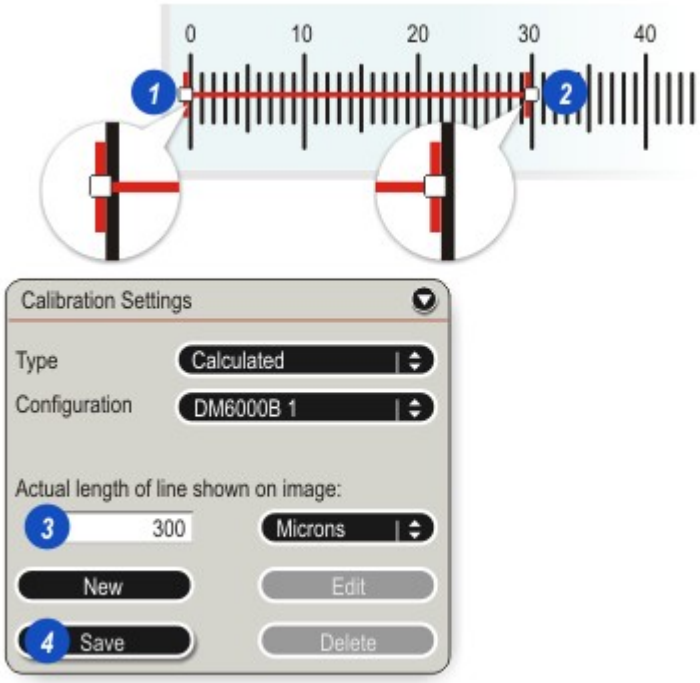
1 & 2: Click on the red measurement line that appears in the *Viewer* and drag it to sit over the calibration slide.

Click on the 'handles' at either end of the measurement line and drag them so that they precisely align with selected marks on the slide.

Greater accuracy is achieved if both of the line end strokes are aligned either to the left or right of the marks. In the illustration the measurement line represents a distance of 300 microns.

3: Click inside the *Actual length of line* text box type the length of the measurement line – in this case 300 microns.

4: Click *Save*.



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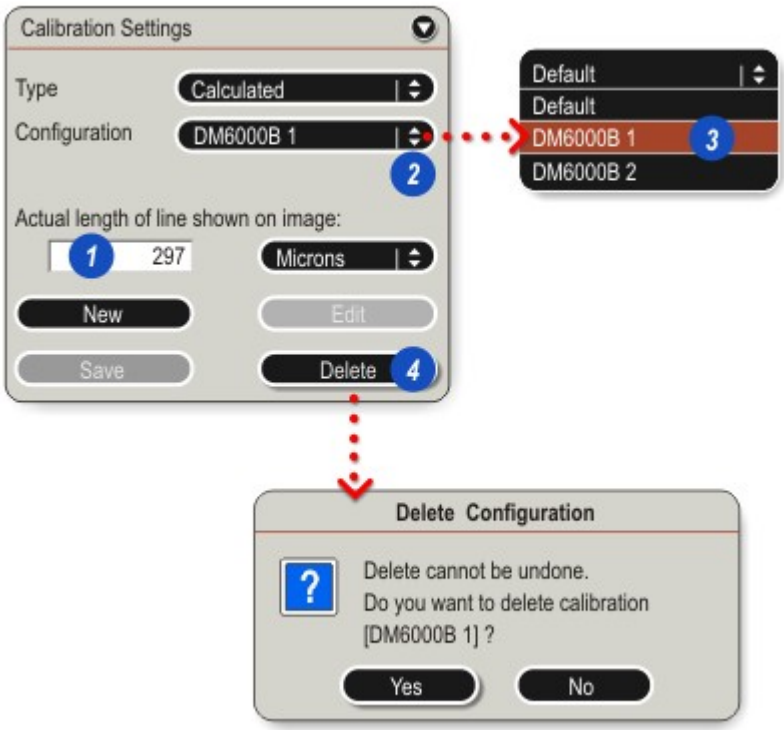
1: When the *Save* button is clicked the value in the *Actual length of line* text box may change to reflect settings made on the *Acquire* > *Scale Bar* panel. This does not affect the calibration.

2: Calibration settings can be retrieved and applied by clicking on the arrows to right of the *Configuration* header and from the drop down list…

3: …clicking to select the *Configuration Name.*

4: Delete a configuration by selecting it (as above) and then clicking the *Delete* button. On the *Delete Configuration* dialog confirm the deletion by clicking *Yes*.



Measured Calibration:

Measured Calibration provides the most precise results because every objective/Mag combination is individually calibrated using a calibration slide. A *Wizard* automatically steps through the combinations.

Place a calibration slide on the stage. It is important that the slide is clean and correctly focussed.

1: Click on the arrows to the right of the *Type* header and...

2: ... from the drop down list click to select the *Measured* option.

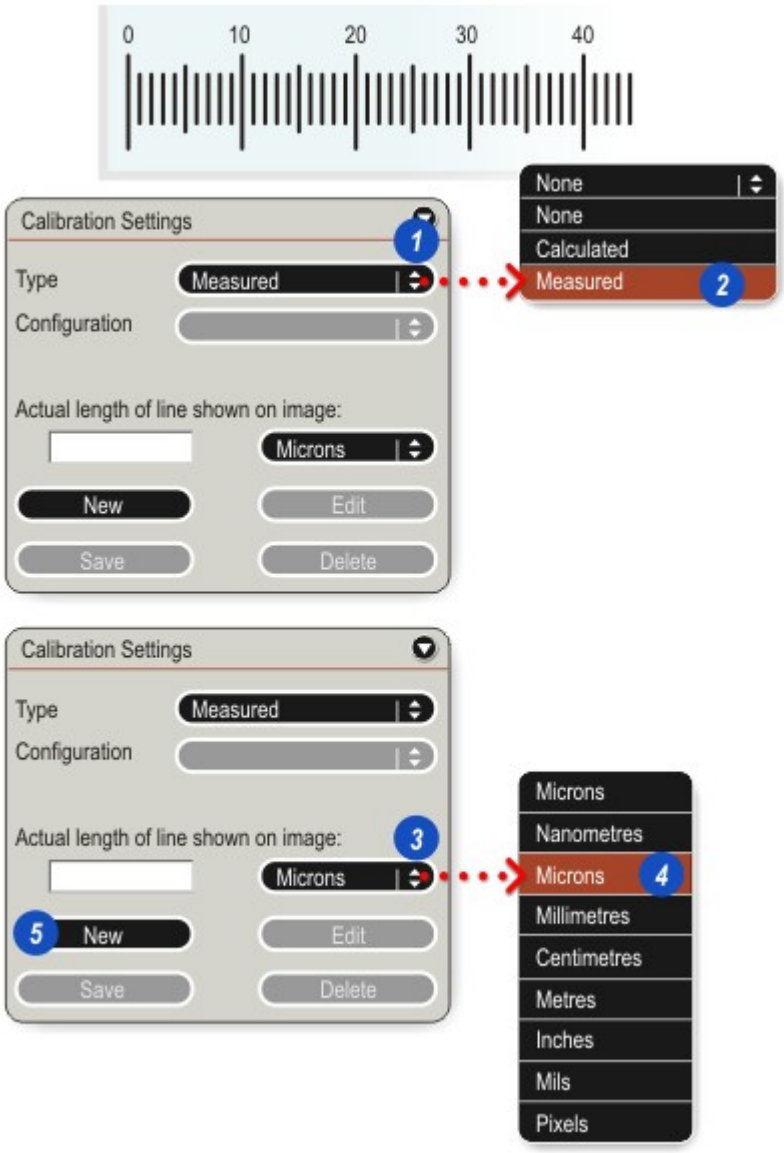
3: Select the *measurement units* by clicking on the arrow to the right of the header and…

4: …clicking to select the measurement type. This must correspond with the calibration slide that is going to be used.

5: Click the *New* button.

On the *Calibration Wizard* dialog two options are available:

- *Manual*: The user is responsible for placing a measurement line across a known distance on a calibration slide..
- *Automatic*: The software detects and measures the *Calibration Slide* and automatically applies the calibration values.



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Manual Measured Calibration:

On the *Measured Calibration Wizard* dialog:

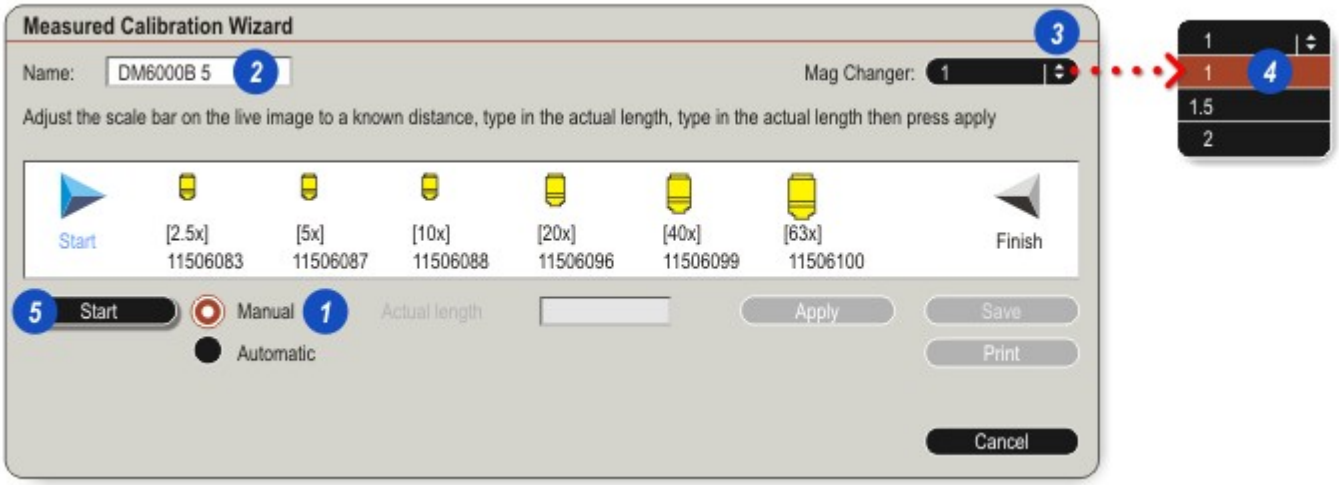
1: Click to select the *Manual* option.

2: Click in the *Name* text box and type an appropriate name for the calibration. The calibration values will be stored in a separate file using this name.

3: Click on the arrows to the right of the *Mag Changer* header and…

4: …from the drop down menu click to select the *1* option. For the first pass, every objective will be calibrated using the *1 Mag Changer*. Subsequent passes use the next incremental *Mag Changer* value with all of the objectives.

5: Click on the *Start* button.



Apply Measurement:

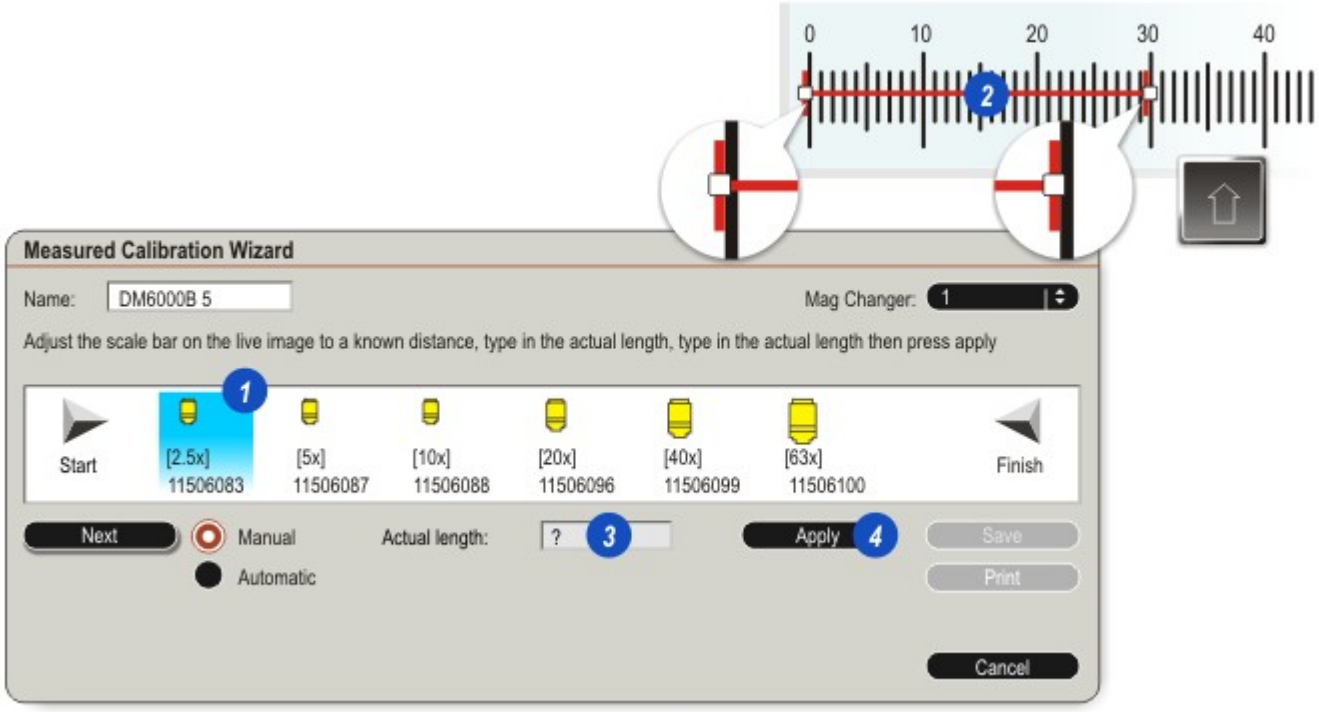
On the *Measured Calibration Wizard* dialog:

1: The first objective is automatically selected.

2: A measurement line is displayed on the *Viewer*: Click, hold and drag it so that it is positioned over the calibration slide.
Click and drag the small handles at each end of the line so that it extends to the distance required – in this case 300 microns. Position both the end stokes to either the left or right of the division marks – easier than trying to align exactly with the centre of the mark. Press and hold down the *Shift* key to display the *Magnifier*. Drag it over the calibration slide to help with the alignment.

3: Click inside the *Actual length* text box and type the distance measured – in the example 300 microns.

4: Click on the *Apply* button.



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Next Measurement:

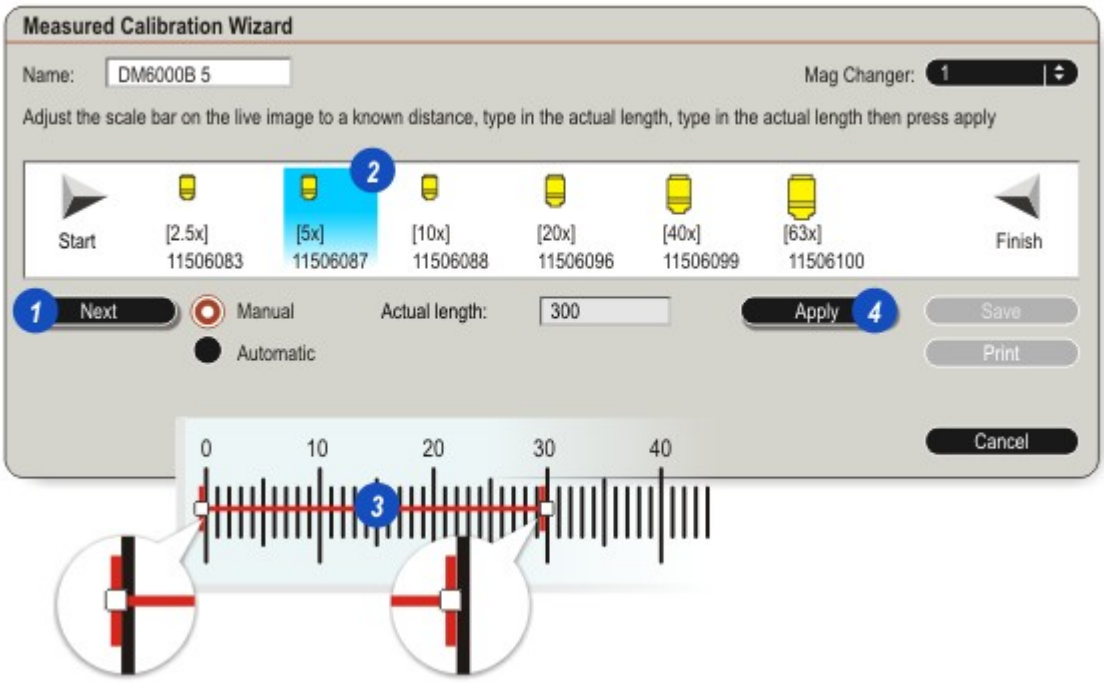
1: Click on the *Next* button.

2: The next objective is selected.

3: Repeat the measurement line procedure described on the previous page.

4: Click *Apply*.

Repeat this procedure for each of the objectives.



Next Mag:

With the last objective in combination with the *Mag 1* complete, click the *Next* button.

1: Click on the arrows to the right of the *Mag Changer* header and…

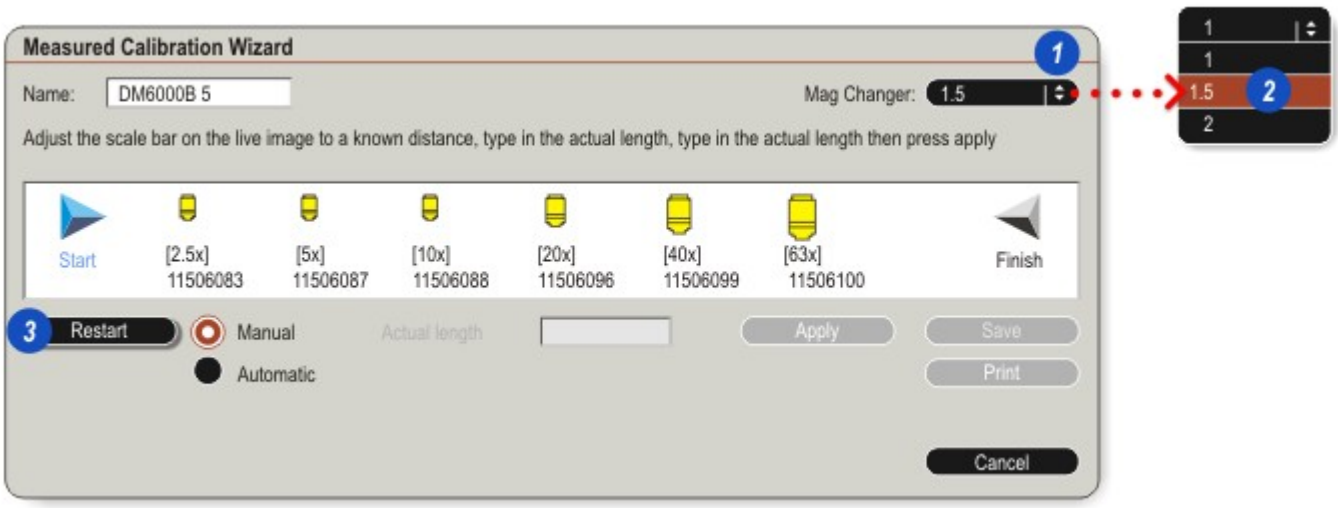
2: …click to select the next *Mag Changer* value – in the example *1.5.*

3: Click *Restart* and the first objective will be selected again.

Repeat the calibration sequence described on the previous pages.

Move through all of the *Mag Changer* values in the same way until all the combinations have been calibrated.

The configuration and calibration settings must now be saved 285.



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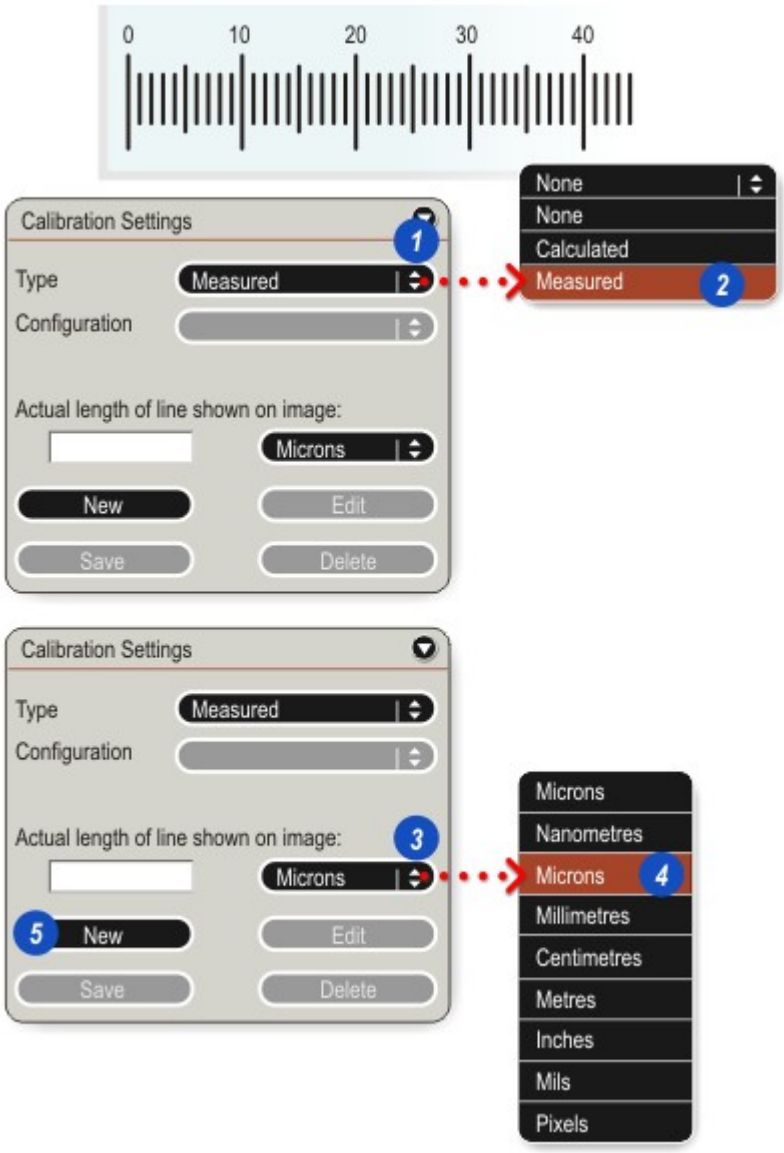
Automatic Measured Calibration offers a fast, precise method using a calibration slide and allowing the software to detect it and apply the values. The user is not involved in any measurements but an active and licensed option measurement module must be installed.

The software can accurately and automatically detect the slide image and calculate the calibration from a known interval between the divisions, providing the image is sharp and the division lines clearly defined.

Place a calibration slide on the stage. Is is important the slide is sharply focussed with distinct divisions visible, clean and clear of debris for the calibration process to be successful.

- Select high resolution for the camera capture format: 240.
- Choose an objectives greater than x2.5 on a compound microscope if using a 10µm/division calibration slide.



1: Click on the arrows to the right of the *Type* header and...

2: ... from the drop down list click to select the *Measured* option.

3: Select the *measurement units* by clicking on the arrow to the right of the header and…

4: …clicking to select the measurement type. This must correspond with the calibration slide that is going to be used.

5: Click the *New* button.

On the *Measured Calibration Wizard:*

1: Click to select *Automatic.*

2: If necessary, change the measurement units for the calibration slide by clicking the arrows to the right of the header and selecting from the drop-down list.

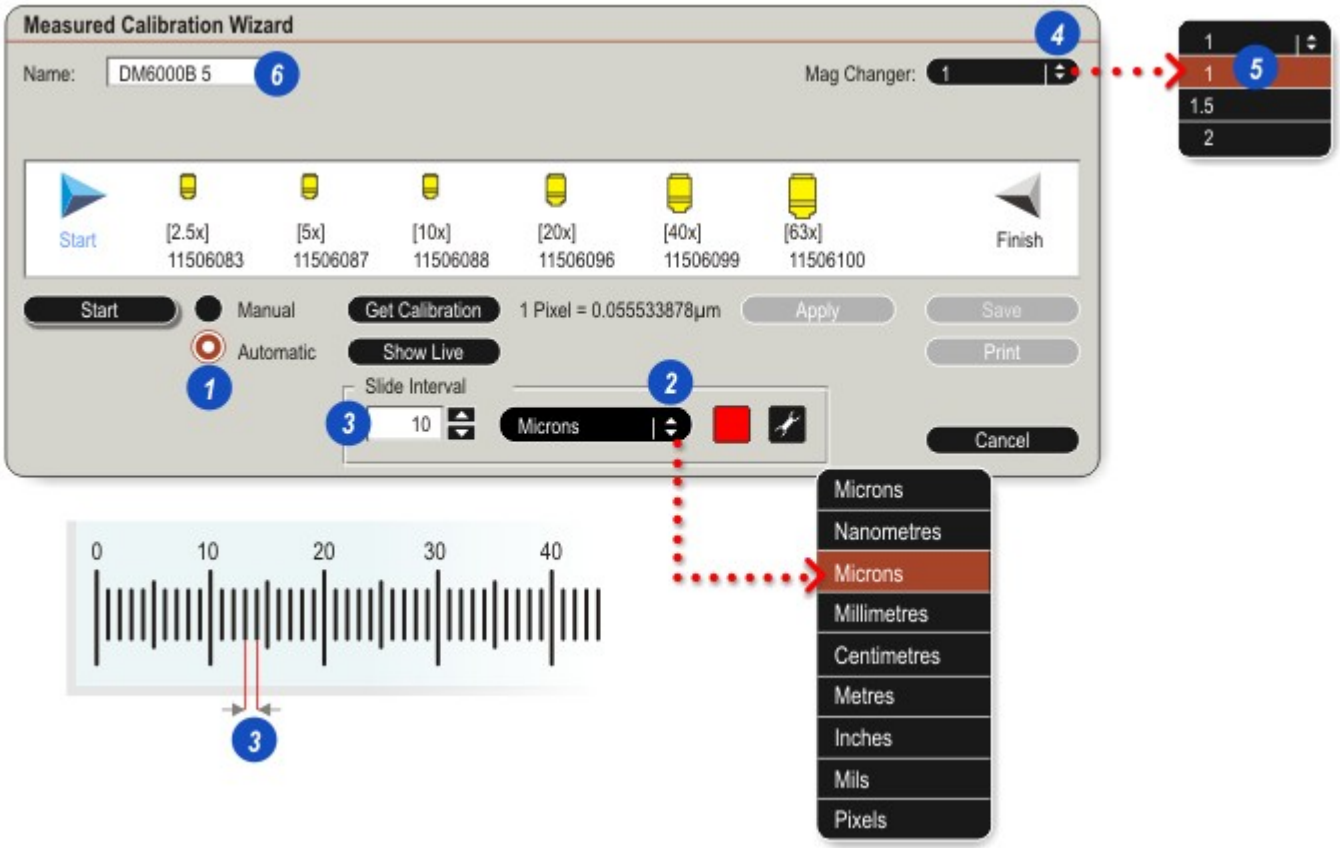
- The measurement units for the slide and calibration do not have to match.
- The calibration slide does **not** have to be perfectly horizontal or vertical, but the closer it is then the faster the detection.

3: Click inside the *Slide Interval* text box and type the interval value of the calibration slide - that is the distance between two adjacent divisions in the selected measurement units. Typically, this will be 10µm for a compound slide and 100µm for a stereo calibration slide.

4: Click on the arrows to the right of the *Mag Changer* header and...

5: ... select *1* from the drop-down list.

6: Click inside the *Name* text box and type a unique name for the calibration configuration.



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1: Click the *Start* button and...

2: ...the first objective is selected.

3: Click th *Get Calibration* button.

The software will now try to find a calibration slide and verify that it is precise and suitable as a calibration source:

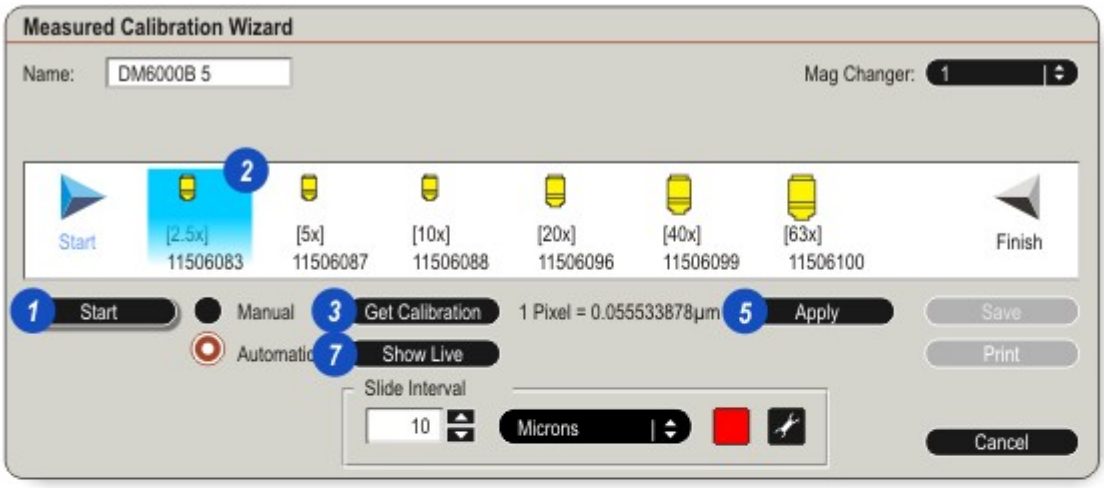
4: If a calibration scale is found and verified, a coloured overlay is applied to the division strokes on the scale, a new calibration value is automatically calculated and...

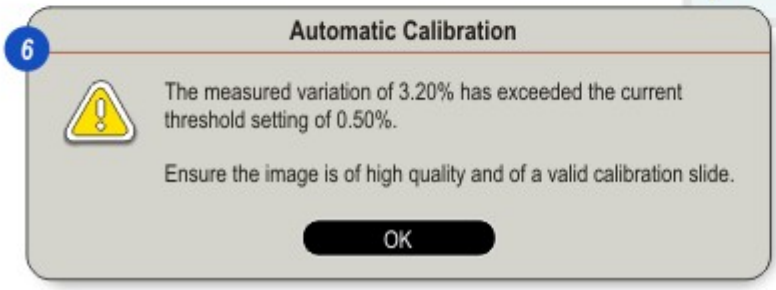
5: ...the *Apply* button becomes active. Check that the calibration intervals have been properly detected and click the *Apply* button to apply the new calibration value for the mag/objective combination.

6: If a scale is not detected or does not conform to the verification parameters, an error message appears. The message changes to reflect the error.

7: Sometimes detection can be improved by re-focussing. Click on the *Show Live* button to view the live image and adjust the focus.

- Calibration slide scale verification parameters can be changed: 283.
- Change the calibration scale detected colour: 284





With the calibration complete for the selected mag/objective combination:

1: Click the *Next* button.

2: The next objective is selected.

3: Click *Get Calibration* and the detection process will begin again.

4: Click the *Apply* button if the detection is successful.

Continue this way until all of the objectives have been calibrated and then:

5: Click on the arrows to the right of the *Mag Changer* header and from the drop-down select the next magnification.

6: Click the *Restart* button and the process will begin again for the new mag value.

7: When all of the mag/objective combinations have been calibrated, save the configuration and values: 285

- A calibration configuration can be edited to adjust individual combinations: 286 .
- The Mag/objective combinations together with the calibration settings can be printed: 287

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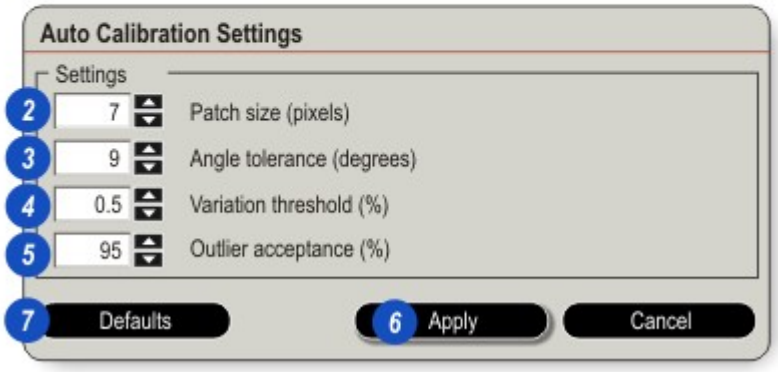
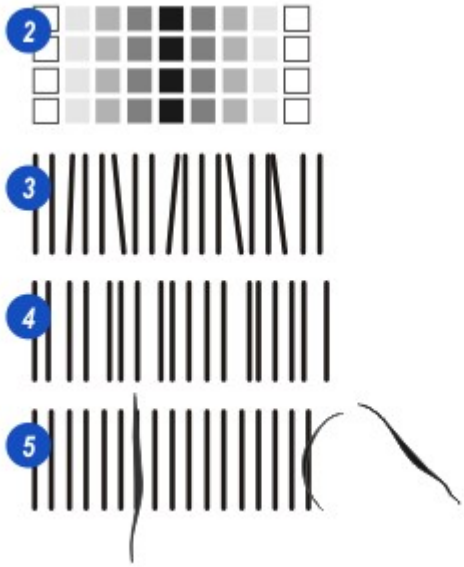
Calibration Settings:

The verification parameters check that a calibration slide image is sufficiently accurate to be used as a calibration source. For example, random fibres on an calibration slide image could be interpreted as part of the scale and have to be 'filtered' out.

Users can change the settings but should be aware that significant changes can result in compromised calibration accuracy. If in doubt revert to the factory optimised defaults.





1: On the *Measured Calibration Wizard* click on the 'spanner' icon to reveal the *Auto Calibration Settings* dialog.

2: The *Patch size* refers to the spread of pixels leading to a discernible edge. In the illustration there are several pixels ranging from white to dark grey before the black central 'edge' appears. Increasing the patch size could 'create' spurious edges. Keep the patch size as small as possible.

3: *Angle tolerance* determines how much the angle between two adjacent scale strokes can vary from the parallel. The software is looking for a series of parallel strokes at a consistent 'pitch' or interval.

4: The interval of the scale strokes must be close to the value entered by the user. The *Variation threshold* determines how much it can be allowed to vary.

5: Outliers are scratches and debris that may be present on the image and could be interpreted as part of the scale. The *Outlier acceptance* sets the % level at which the interval *mean* (a central 'average' for all of the detected intervals) can vary. Strokes falling below the *Outlier acceptance* are removed.

6: If changes are made to the settings click the *Apply* button to save them.

7: To restore the factory settings click the *Defaults* button.

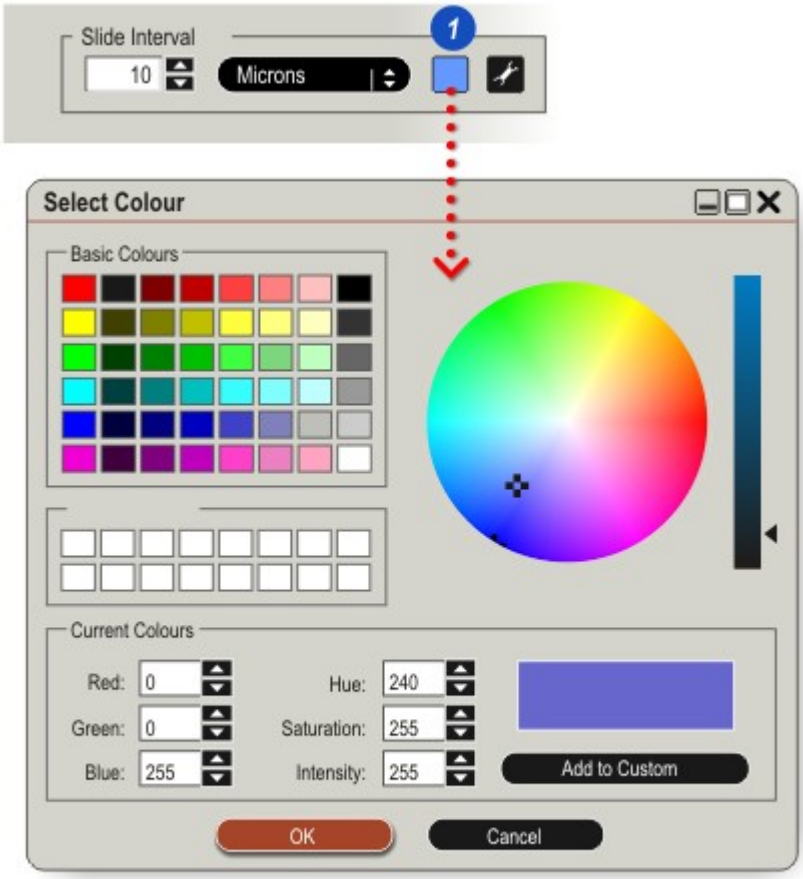
Calibration Colour:

Change the colour of a detected calibration slide image by:

1: Click on the *Colour* button on the *Slide Interval* panel.

2: From the *Windows Colour* dialog choose a colour by clicking a swatch, dragging the crosshairs on the wheel or typing Red, Green and Blue (RGB) values.

3: Click *OK*.



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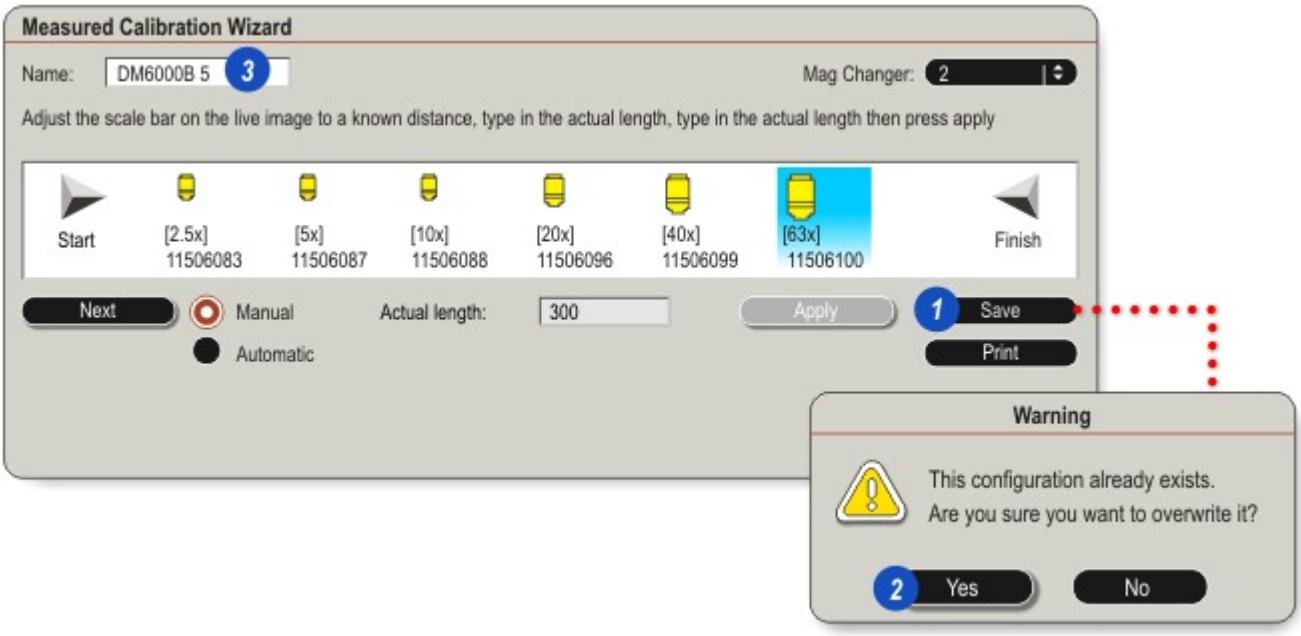
Save Calibrations:

1: When all objective/Mag combination calibrations are complete, click the *Save* button to store the calibrations.

2: If the file already exists confirm overwriting the existing calibration file by clicking the *Yes* button on the *Warning* message.

3: Alternatively, click inside the *Name* text box and type a new, unique name and click *Save* again.



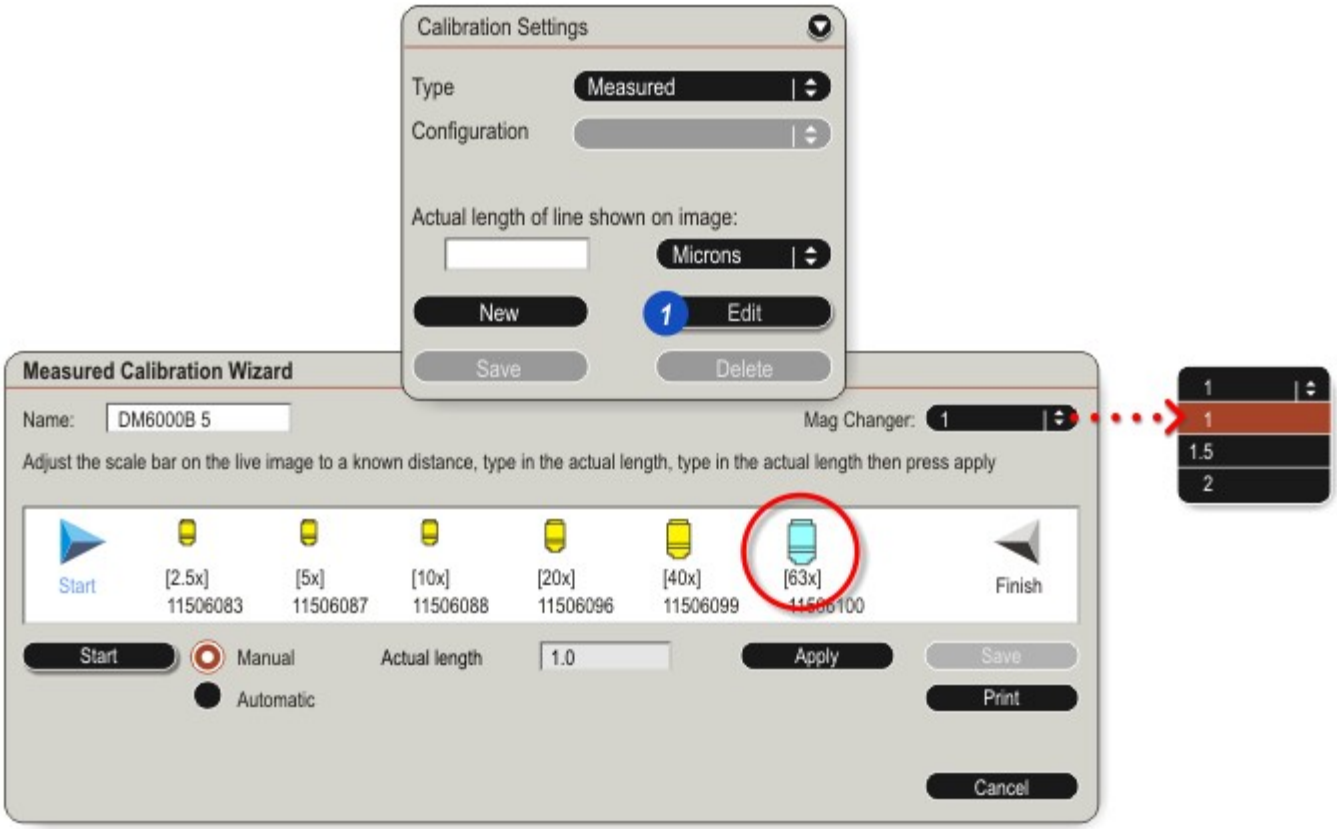
Edit:

The *Edit* feature allows selected Mag/objective combinations to be calibrated without having to go through the entire Wizard sequence, especially useful if an objective is changed.

1: Click on the *Edit* button and the *Measured Calibration Wizard* appears with un-calibrated objective(s) highlighted and the *Actual length* value reset to 1.00.

To calibrate a Mag/objective combination, use the procedure already described:

- Select a *Mag Changer* value..
- Click to select an objective.
- Extend the measurement line over the *Calibration Slide* or allow the software to detect it (Automatic calibration only).
- Type the actual value.
- Click *Apply*.
- Save and exit the calibration Wizard at any time.



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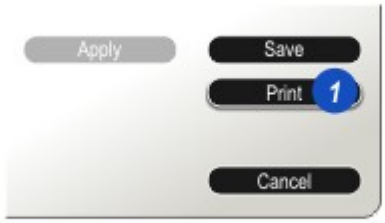
Printing Calibration Settings:

A complete list of all objective/mag combination calibrations can be displayed and printed by:

1: Click on the *Print* button. The *Calibration Sheet* is displayed on the computer's default browser.

Use the browser's print button to make a hard copy.



Leica Digital Microscopes

Calibration Sheet

Mag Changer	Objective	Calibration State	Calibration Value	Adjustment
1	1.25	Measured	0.5747 µm	0.1041
1	2	Measured	0.5747 µm	0.1666
1	4	Measured	0.5747 µm	0.3332
1	5	Measured	0.5747 µm	0.4165
1	10	Measured	0.5747 µm	0.8329
2	5	Measured	0.5747 µm	0.8329
2	10	Measured	0.5747 µm	1.6658
2	16	Measured	0.5747 µm	2.6653
2	20	Measured	0.5747 µm	3.3317

Linking:

Exposure Linking associates a microscope setup with a specific camera setup, whereas *Shading Linking* associates a microscope setup with a specific image shading level.

- 288 Exposure Linking.
- 291 Shading Linking.

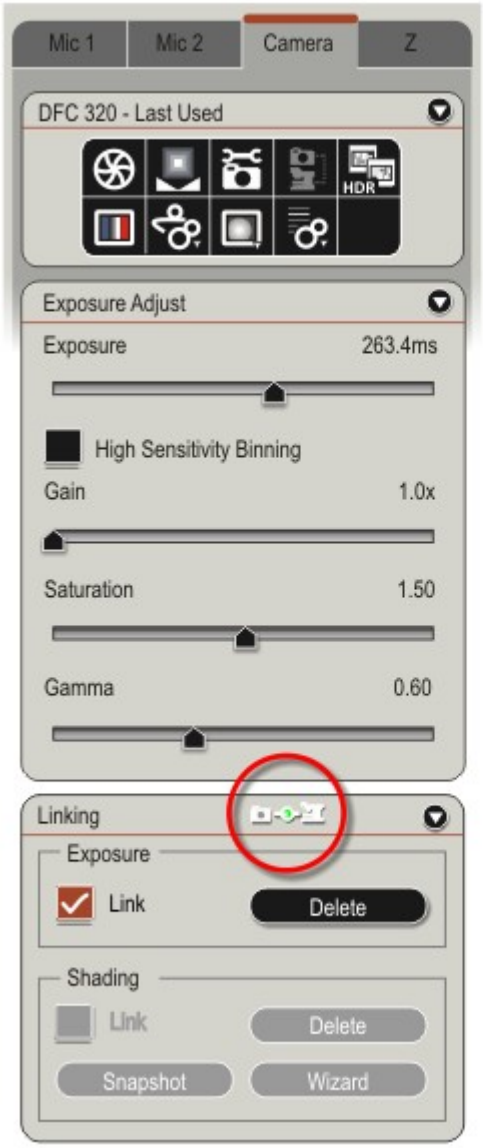
With *Exposure Linking* enabled, the Leica Application Suite automatically checks the major microscope settings against a previously stored *Linking list* and, if there is a match will retrieve and load the camera settings associated with it.

The *Linking* list is created by the user - it is not a preset part of the Leica Application Suite.

The microscope settings checked are:

- Objective or zoom level for stereo microscopes,
- Mag changer,
- Camera and port,
- Filter and...
- Contrast method.

A *Link* may be created for all of these items in any combination making it a really powerful tool for precise repetition.



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To create an *Exposure Link* the microscope must be setup for the image required.

1: Click to enable the *Link* check box.

2: The *Status Display* on the header bar will show RED.

This indicates there is no stored camera information for the current microscope settings.

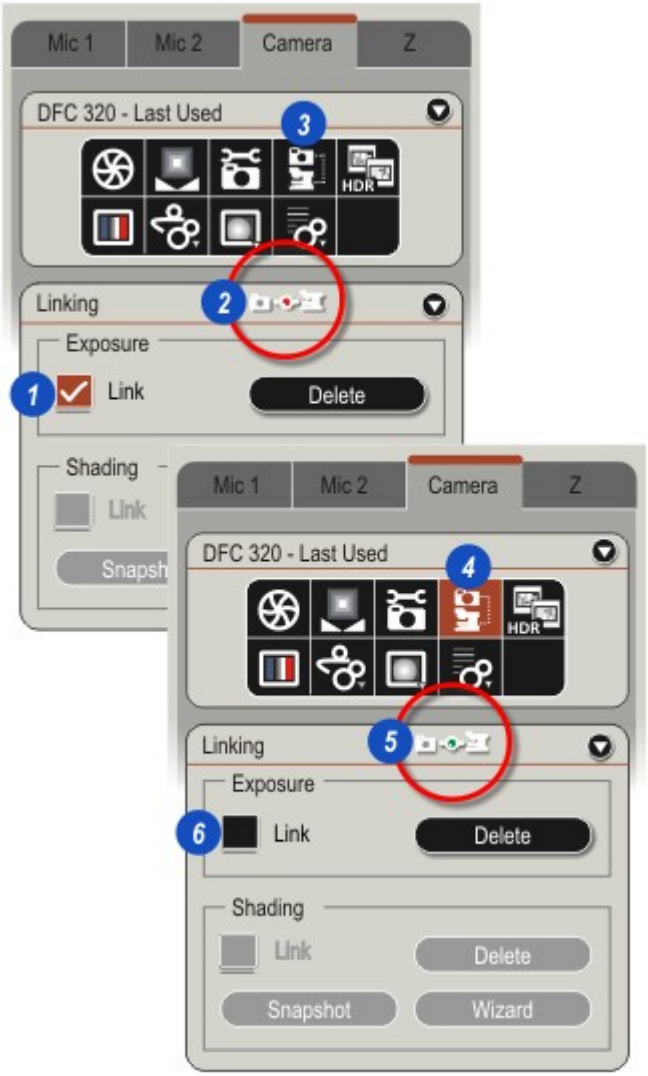
3: The *Linking* button on the*Toolbox* will be enabled - white against a black ground.

4: To create a link, click on the *Linking* button.

5: The *Status Display* will turn GREEN to indicate an established link.

To turn *Exposure Linking* on or off:

6: Click to disable (no tick mark visible) the *Link* check box.



Link Update or Delete:

If changes are made to the camera settings - in the example the *Exposure* value has been reduced -

1: ...the *Status Display* will change colour to YELLOW.

2: The *Linking* button also becomes enabled again. Click it to update the link.

If the *Exposure* setting is changed but before the link is updated, the original exposure can be retrieved by:

3: Right-clicking the *Linking* icon and then left- clicking on the *Revert* tag.

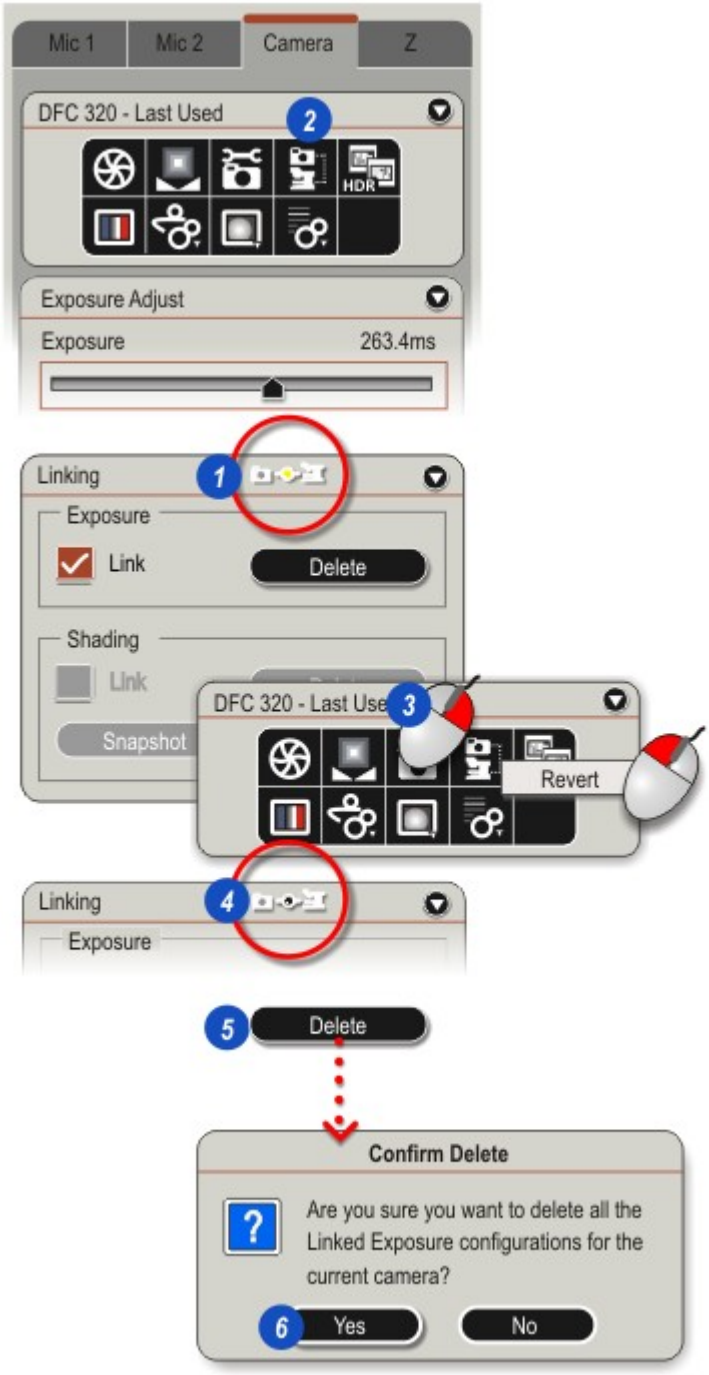
On stereo microscopes an additional warning may occur - the *Status Display* on the header bar changes to BLACK **(4)** indicating that the zoom level has changed but is being ignored. All other settings are correct. Update the link to include the revised zoom by clicking the *Linking* button **(2).**

Deleting Links:

5: All of the links for the current camera can be removed by clicking the *Delete* button and...

6: ...confirming the deletion.

This is not reversible: Use with care.



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Shading:

Shading Linking associates a specific microscope setup with a specific shading level.

Shading is the name given to variations in the background light level across an image. In the example, the left-hand image represents transmitted light through a microscope. The light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

Even ‘illumination’ can be achieved by electronically applying a blank area value to the entire image area. The effect is shown in the right-hand image.

The light source together with any of the optical elements will produce different shading levels, so each microscope element combination should have its own shading setting.

Shading Linking when enabled, checks the microscope:

- Objective or zoom level (for stereo microscopes),
- Mag changer,
- Camera and port and
- Contrast method.

...and automatically tries to find and apply a matching *Shading Link*. If a match is found the status icon on the *Linking* header bar will be GREEN. Without a match it will be RED and no shading settings applied.

Shading Links are not supplied as presets - they have to be created by the user.

Shading Snapshot:

To create an immediate *Shading Link* if one does not already exist (the icon on the Linking header bar is RED):

1: Click to enable the *Shading Link* check box - tick mark visible.

2: Click the *Snapshot* button.

3: When the *Linked Shading* prompt appears, make sure the microscope and camera exposure settings are suitable and then:

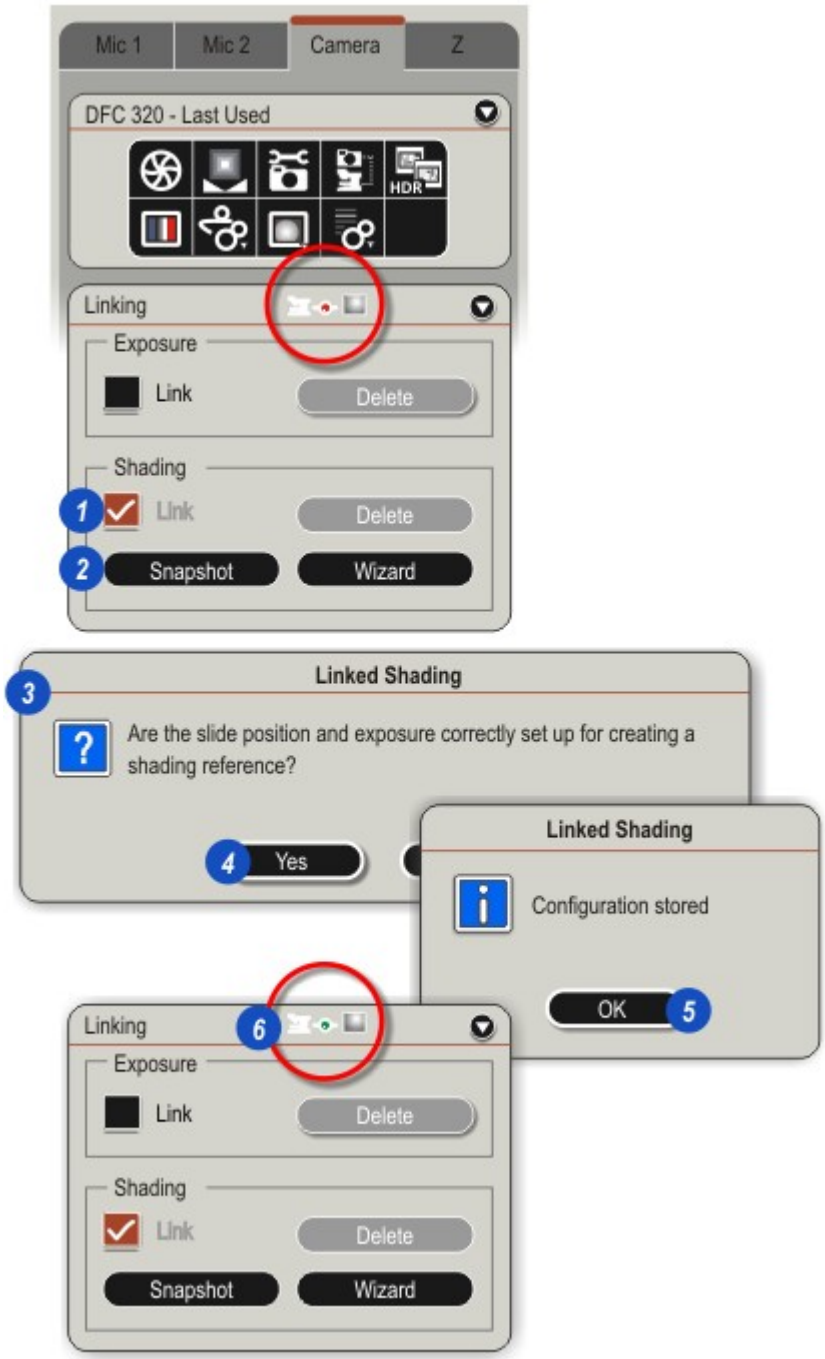
- Either move the stage to view an empty field on the specimen, or...
- Remove the specimen slide and replace it with a slide (and cover slip if necessary) of the same type and quality.
- A very small amount of microscope de-focus may be helpful to prevent contaminants affecting the *Shading* reference.

4: On the *Linked Shading* prompt click Yes.

5: A shading link will be created, and when complete the *Configuration Stored* message will appear.

Click *OK and...*

6: ...the header bar icon becomes GREEN.



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Shading Wizard:

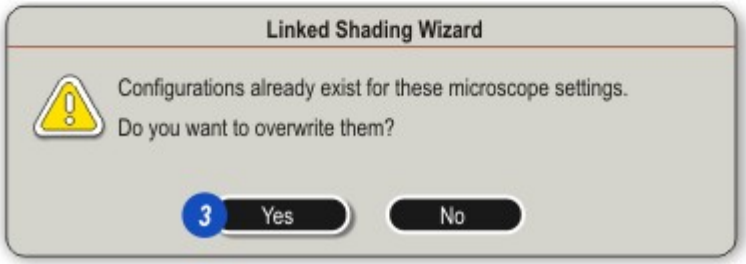
The *Shading Wizard* creates a *Shading Link* for each microscope objective in turn starting with the one selected. Having completed one, it automatically moves to the next. The Mag changer, camera and contrast method remain the same for each link.

Because it can be stopped after completing an objective, groups rather than all the objectives can be processed.

For a single objective use *Snapshot*:

Before starting the *Wizard*, the specimen image must be properly exposed and focused.

- Either move the stage to view an empty field on the specimen, or...
- Remove the specimen slide and replace it with a slide (and cover slip if necessary) of the same type and quality.
- A very small amount of microscope de-focus may be helpful to prevent contaminants affecting the *Shading* reference.



1: Click to enable the *Shading Link* check box - tick mark visible.

2: Click the *Wizard* button.

3: If shading links have already been created for the current microscope settings, a warning will appear.
Click *Yes* to overwrite the existing link or *No* to cancel the *Wizard*.

If necessary, carefully adjust *Exposure* and *Gain* on the empty field or blank specimen slide to achieve a very small amount of over-exposure. Refer to *Histogram* for details of how to turn on over/under-exposure indication: 244

On the Wizard dialog:

1: Click on the *Start* button.

2: Click on the objective to be processed. It becomes high-lighted.

The first objective is selected automatically for motorised microscopes, but can be changed by clicking on another.

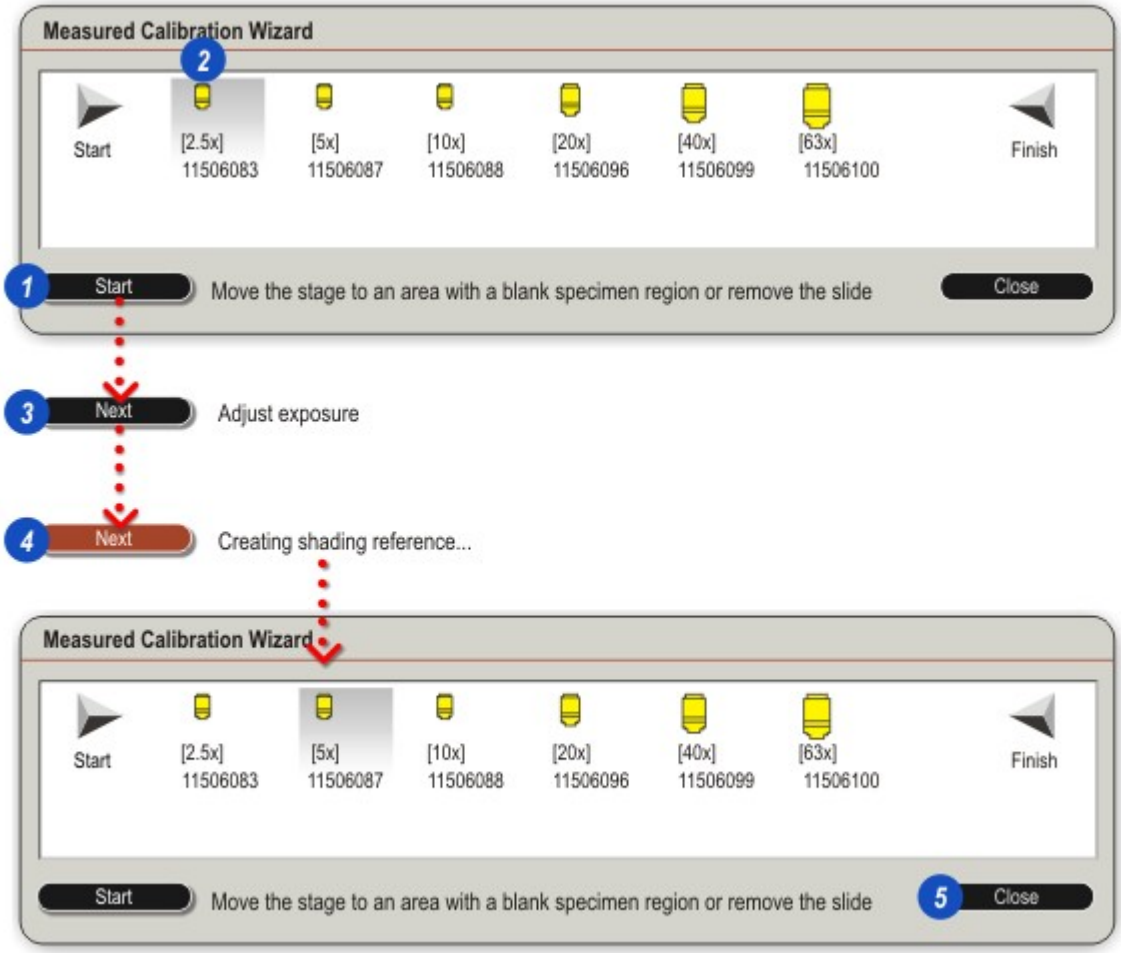
To prevent contamination affecting the shading, a very small amount of de-focus may be used.

3: Click the *Next* button.

4: The *Wizard* will create the *Shading Link* for the current objective.

When complete, it will automatically select the next objective (change the objective manually for non-auto microscopes) and return to Step **(2)**.

5: When a *Shading Link* for all of the required objectives has been created, click the *Close* button.



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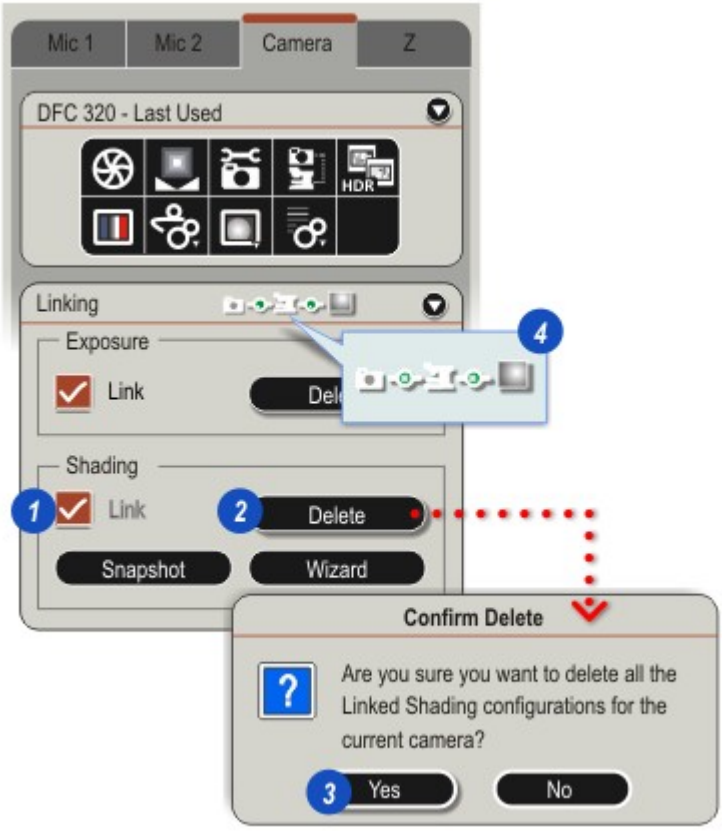
1: Turn *Shading Linking* on or off by clicking the *Link* button.

2: *Shading* links can be deleted by clicking on the *Delete* button and...

3: ...confirming the deletion by clicking the *Yes* button.

Use with care: This will delete ALL of the *Shading Links*.

4: Both *Exposure* and *Shading Linking* can be enabled together. In this case the status icons on the *Linking* header bar will appear combined.



Check Colour:

This facility is useful for adjusting the overall image colour sometimes necessary to compensate for colour shift due to small variations in the specimen lighting and camera characteristics.

Use it mainly for colour ‘fine-tuning’ after carrying out a *White Balance.*

1: Click on the arrow to the right of the *Check Colour* header to expand the panel.

2: Left click on the image and drag a rectangle around the required area to create a *Region of Interest (RoI).*

3: When the mouse button is released a context menu appears. Click to select the *Check Colour* option.

4: A small dot on the *Colour Wheel* represents the *Hue* and *Saturation* values within the region.

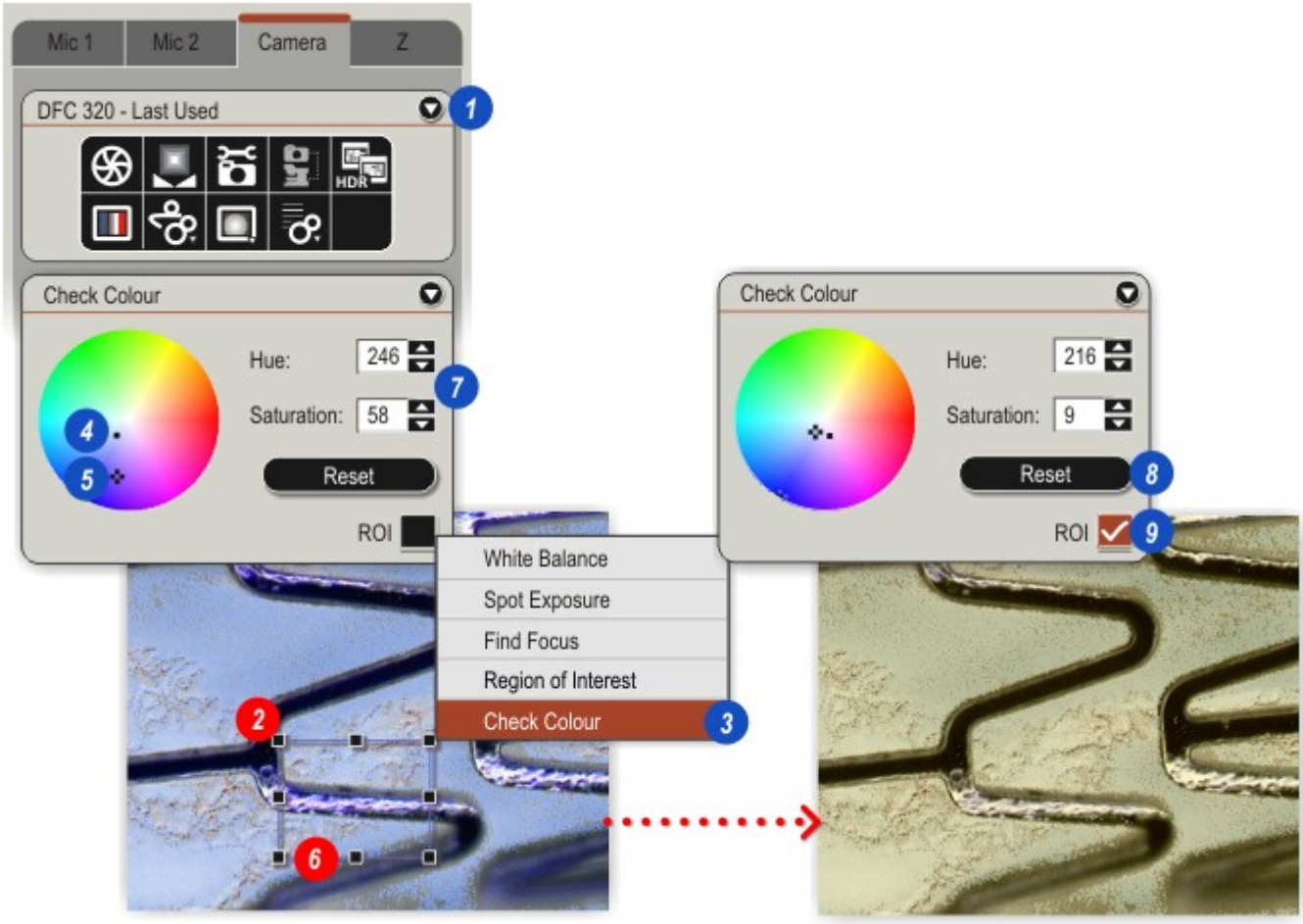
5: To apply the *Hue* and *Saturation* values to the entire image, click on the 'target' mark and drag it toward the dot.

6: To select another area of the image by clicking on the region outline (not the handles) and drag it to the new location.

7: The *Hue* and *Saturation* windows show the values at the current target location. Fine tune the values by either clicking the up and down arrows to the right of the window or clicking in the widow and typing a new value.

8: Hide or reveal the *Region of Interest* by clicking to disable the *ROI* checkbox.

9: Reset any adjustments by clicking the *Reset* button.



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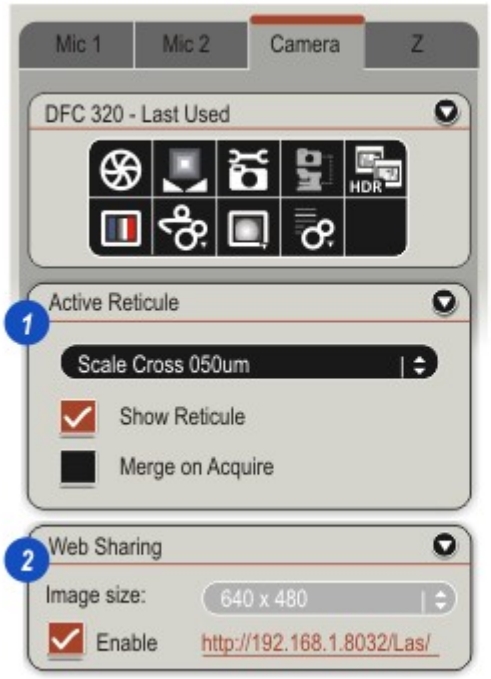
Reticule and Web Sharing:

Reticule and *Web Sharing* are optional modules that have to be installed and licensed. If either or both are installed then a control panel will appear in *Camera*.

Click on ↗ to display the help:

- ↗ 973 *Reticule*
- ↗ 984 *Web Sharing*



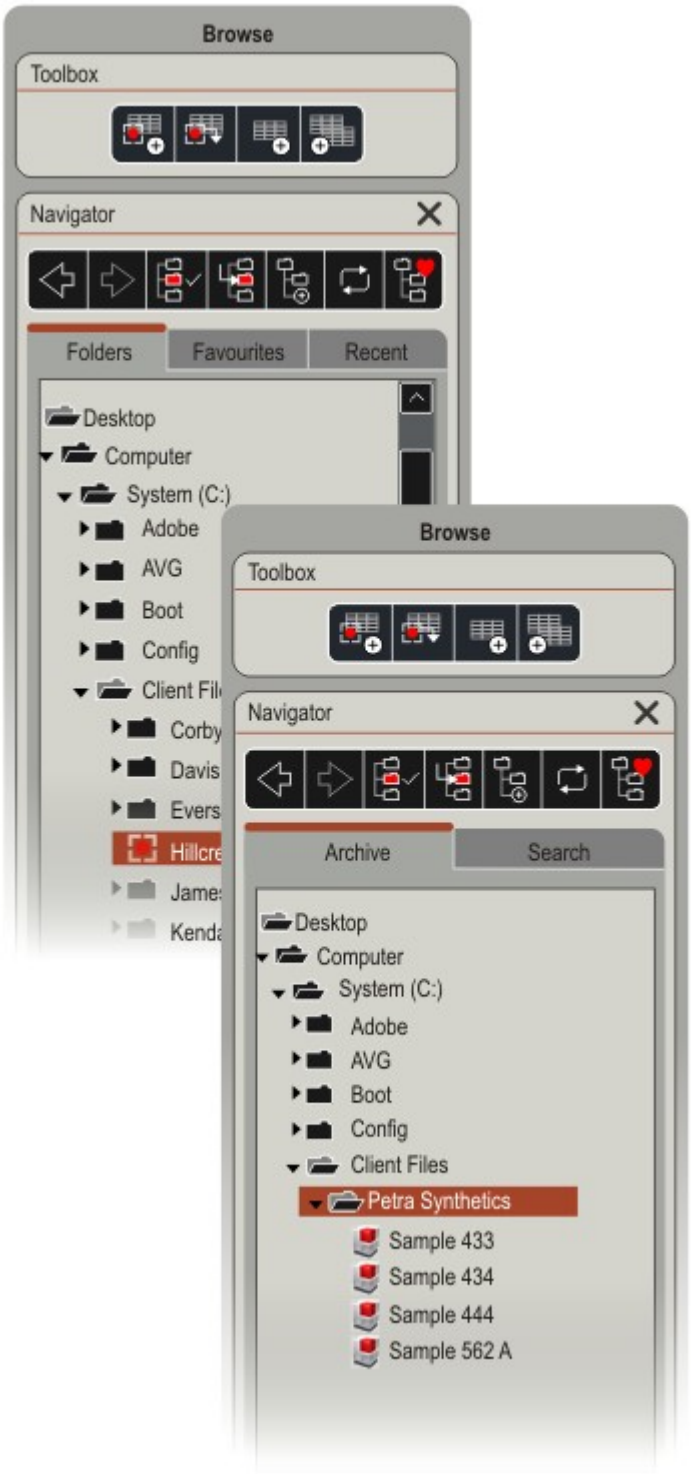
Browse uses the fast *Image Explorer* developed especially for Leica Application Suite and based upon the familiar tree and folder structure of *Microsoft Windows.* It provides access to stored images and to their relevant data such as the time of acquisition, the bit depth and the calibration.

As well as *Image Explorer* an optional module *LAS Archive* can be installed on the same computer. Users can switch between the two storage methods seamlessly.

Images - single or collections - captured before LAS V3.3 can be imported into *LAS Archive.* More about *LAS Archive* 371

Browse features and quick links (Click):

- 309 *Quick navigation* between folders with a single click.
- 310 *Create new folders* without having to leave LAS.
- 307 *Set a folder as the capture location* and immediately begin to grab images.
- 304 *Naming images automatically* with meaningful names and auto-incremented sequence numbers.
- 340 *Microscope and camera data* saved with the image: Users can add their own comments and observations
- 338 A scalable Thumbnail Gallery to show all of the images in a folder and, with a single click select and display an individual.
- 303 *A wide range of tools* for image fast storage and access.



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The illustration is a graphical representation of the LAS display and *Browse* interface showing the principal features and quick links (Click):

1: *Workflows* 300 Click the *Browse Workflow* to open the navigator.

2: *Toolbox* 303 Common tools for both *Image Explorer* and *LAS Archives.*

3: *Navigator Window* 301 Tab shows *Folders* for *Image Explorer* or *Archive* and *Search for Archives*.

4: *Image Data Form* 340 Displays and edits selected data for the current image.

5: *The Image Viewer* 336 Display and working area for the current image: Press keyboard *F5* to show full screen.

6: *The Grid* 344 Displays data for all of the images in the selected folder.

7: *The Gallery* 338 Displays thumbnails of all the images in the selected folder.

8: *Acquire Button* 304 Click to grab and image from the microscope camera.

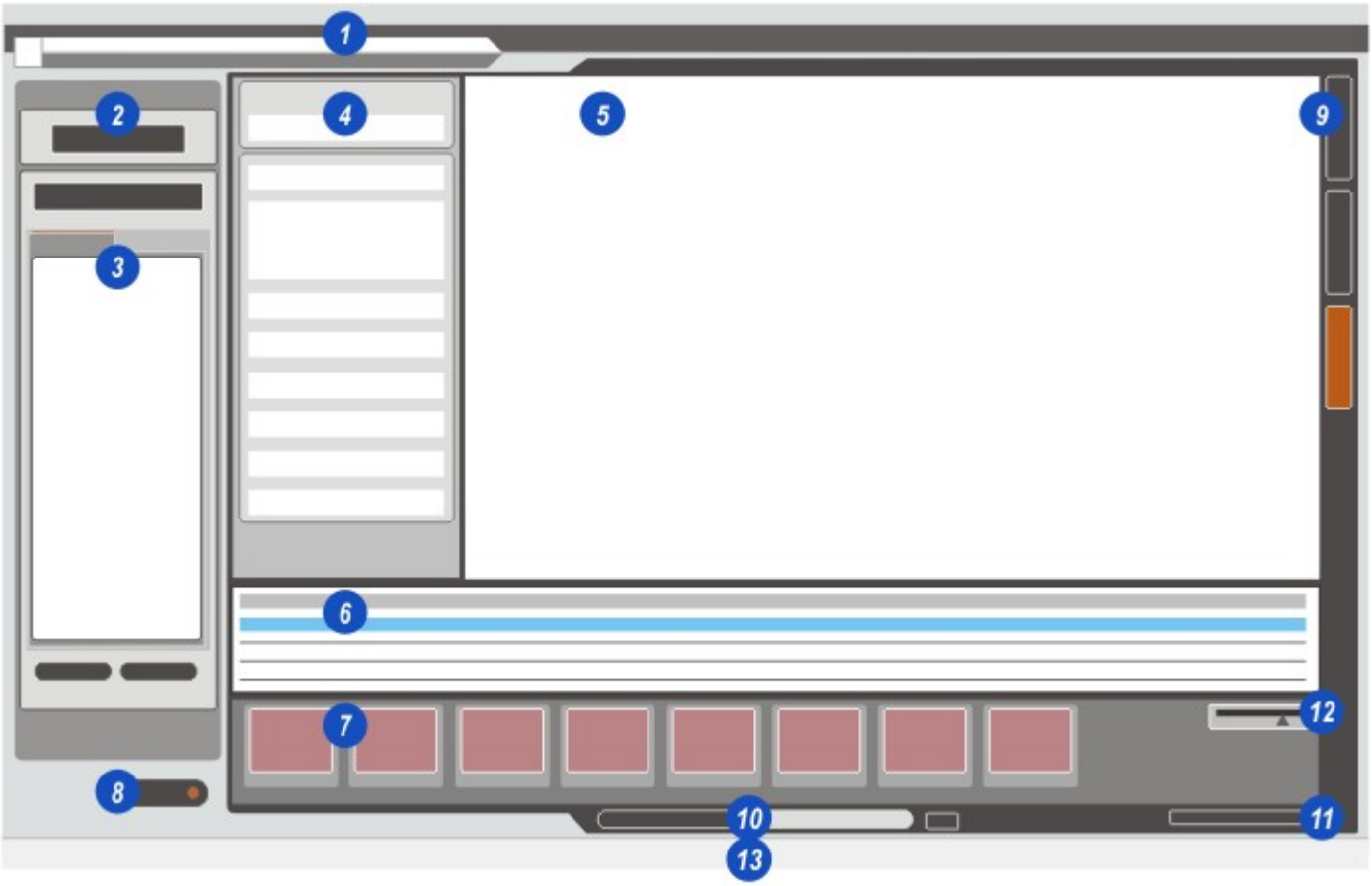
9: *Side Toolbar* 317 Working tools for image sizing, printing and deleting as well as switches for the *Gallery* and *Grid*.

10: *The Search Controls* 410 Available only with *LAS Archives.*

11: *Gallery Navigation Browser* 338 Rapidly find thumbnails in the *Gallery*.

12: *Thumbnail Scaler* 338 Slider adjusts the size of the thumbnails in the *Gallery*.

13: *Status Bar* Displays Hardware Configuration, RGB Intensity, Stage Position and Magnification data.



1: Click on the *Browse Workflow* and if necessary…

2: …on the *Browse* tab to reveal the main panel.

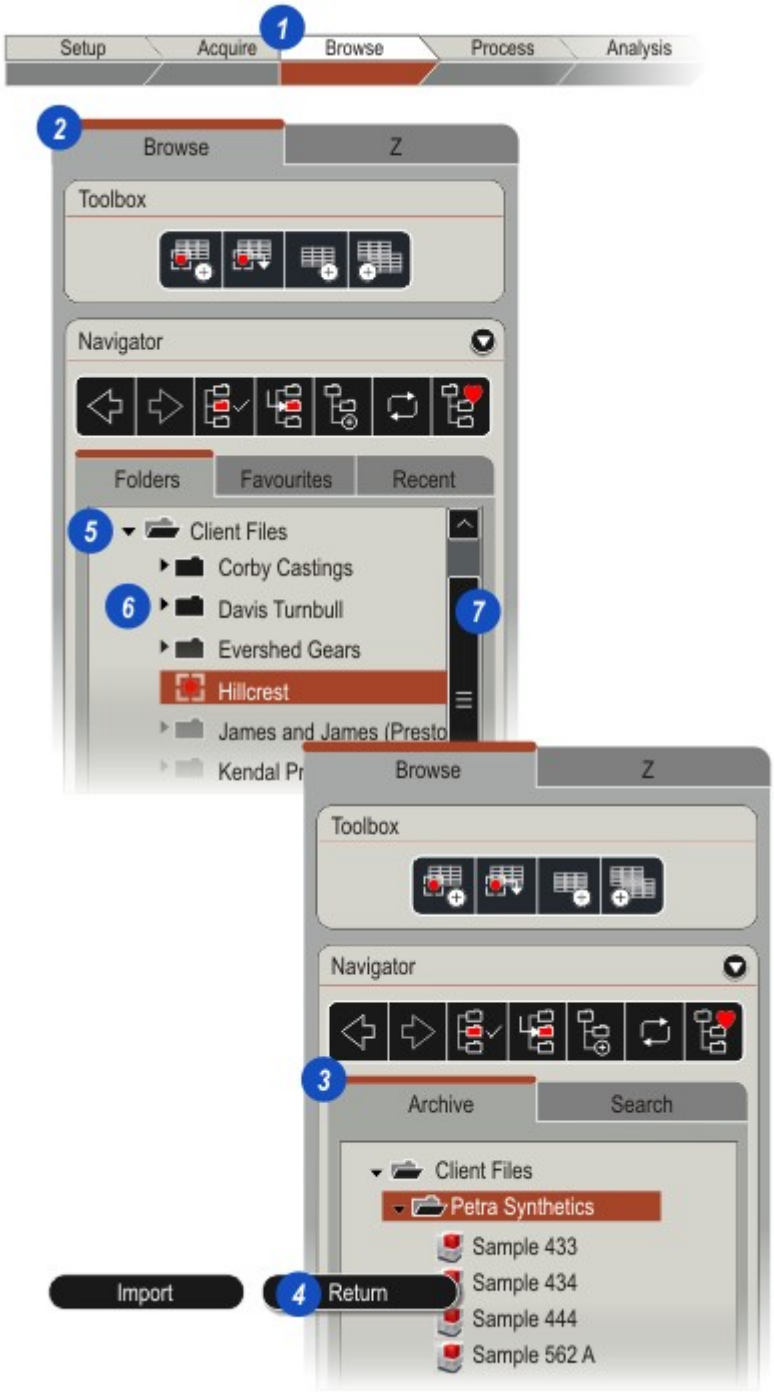
3: If *LAS Archive* is installed and has been previously selected...

4: ...click on the *Return* button to switch to *Image Explorer*.

5: Click on the small arrows to the left of the folders to expand them.

6: Click on a folder to reveal the contents.

7: The *Scrollbars* to the right and bottom of the navigation window are displayed automatically if necessary. Click and drag a *Scrollbar* to move the explorer tree within the window.



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The main panel layout and controls changes depending upon the storage system being used - either *Image Explorer* or *LAS Archives.*

Image Explorer is the default navigator supplied as part of *LAS Core*.

LAS Archives are optional modules designed especially for those users who need a fast database for image storage. More about *LAS Archives* 390

The illustration shows a typical panel with *Image Explorer* active. The control tab label displays the word *Folders*.

The principle features and quick links (Click)

- 302 *Floating Navigator:* Drag and drop the *Navigator* to a location that suits the user, even on to a second monitor..
- 303 *Toolbox:* Image grabbing and record creation.
- 307 *Navigation Buttons:* Move between folders and set the capture folder. *Favourites* creates a fast navigation list.
- 310 *Create a new Folder.*
- 313 *Rename a Folder.*
- 314 *Delete a Folder.*
- 325 *Image Sequence Move and Copy.*
- 315 *Hide Component Images.*

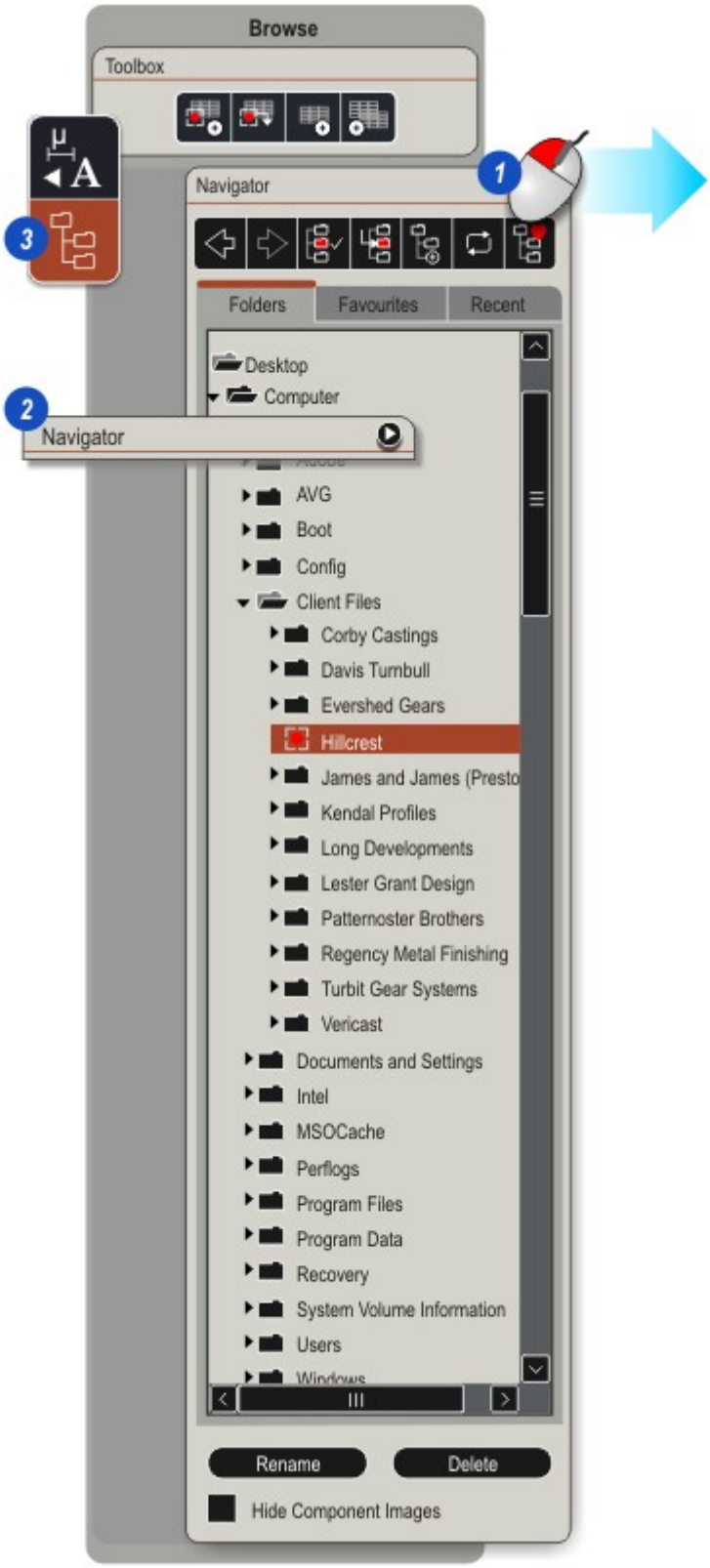
The *Navigator* panel can be detached from its 'dock' and parked anywhere on the *Viewer* - or even on another monitor - to provide access to folders and archives immediately from any workflow or Optional Module without returning to *Browse*.

To move the *Navigator*:

1: On the *Browse Workflow*, click on the *Navigator* header and, holding down the mouse button drag the panel to the required docking position. All of the *Navigator* features are available in the new position...

2: ... including the minimise button that will close the *Navigator* leaving only the header on display. Click the minimise button to reveal the *Navigator* again.

3: The *Show Navigator* button works as a toggle. If the *Floating Navigator* has been moved the first click will return it to the side panel. The second click will move it back to the user's docking position.



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The Toolbox:

The *Toolbox* buttons are grouped on a small panel at the top of the *Browse* tab **(1)**. Click a *Toolbox* button for more information.

Acquire Image: click to grab an image: Has the same function as the *Acquire* button at the bottom of the screen.

Acquire in the Current Image: Grabs an image and overwrites the current selected image.

Create an Empty Record: Click to create a record without an image – an image can be captured into it at a later date.

Duplicate Current Record: Duplicates the current record and image.

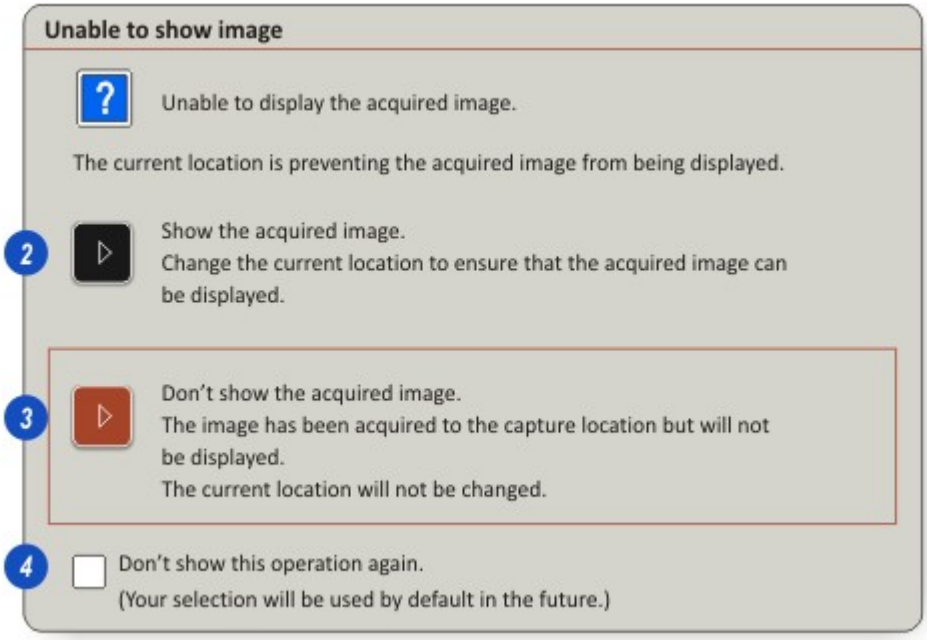
1: Click the *Acquire New Image* button. If *Capture to Fixed Location* is enabled in *Preferences*, and the folder (indicated with a red dot) is selected in the Navigator window, the image will be captured to that folder.
The *Acquire* button at the bottom of the screen performs the same function.

If a folder other than that chosen as a 'fixed location' is selected, the *Unable to Show Image* dialog appears. The options are:

2: Show the acquired image and move to the *Fixed Capture Location* to display it or...

3: Don't show the acquired image but do save it to the *Fixed Capture Location* anyway.

4: To make your choice the future default action, click to enable the *Don't show this operation again* check box



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1: *Acquire into the Current Record* replaces the selected image with a new one. Where necessary, the data is updated but the *Image ID* and *Image Name* remain the same.

2: *Create an Empty Record* does not capture an image and stores only essential microscope data, but it does give the record and *Image ID* and an *Image Name.*

The image and data may then be captured later by clicking to select the empty record and then using the *Acquire into Current Record (above).* This provides a convenient method of 'loading' images later.

3: The *Empty Record* is represented as a Leica Cube in the *Gallery* thumbnails.

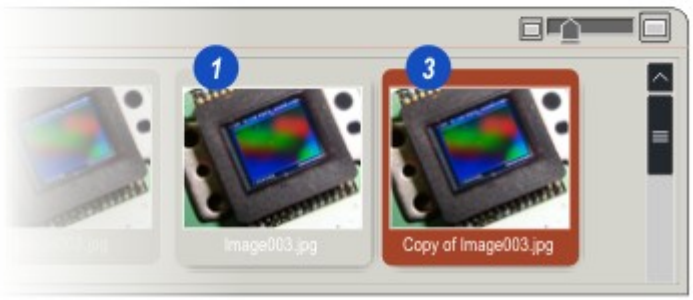
Duplicate Current Record is a simple way of copying an image and its data.

Some data such as notes and descriptions may then be changed to avoid having to type in the description again, the image and the microscope data remain the same.

1: In the *Gallery*, click on the thumbnail of the image to be duplicated.

2: In the *Toolbox* click the *Duplicate Current Record* button.

3: The duplicate appears as a thumbnail in the *Gallery* with the name *Copy of…* followed by the original *Image Name*. Change data on the *Form* as required.



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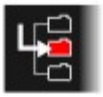
Navigation Buttons:

The *Browse Navigation Buttons* allow the user to move back and forth between folders and files, nominate a folder into which images should be captured, create a child of an existing folder and to store 'favourite' folder locations for speedy retrieval.

Click a *Navigator* button for more information:

Step Back: Click to move to the previous folder (left arrow) and return (right arrow).

Set Capture Folder: Makes the selected folder the capture target – grabbed images will be stored here.



Move to Capture Folder: Returns to the *Capture Folder* from anywhere on the tree.

Create a New Folder: Creates a folder as a child of the currently selected folder.

Refresh the Current Folder: Re-displays the *Gallery* and selected image in the *Viewer.*

Favourites: Stores and links to frequently used folders and files.

There are a further three controls below the *Navigator* window:

2: *Rename* 313 Allows the user to rename a folder.

3: *Delete* 314 Deletes the selected folder and all of its contents.

4: *Hide Component Images* 315 When enabled displays only the 'analytical' images in the *Gallery.*

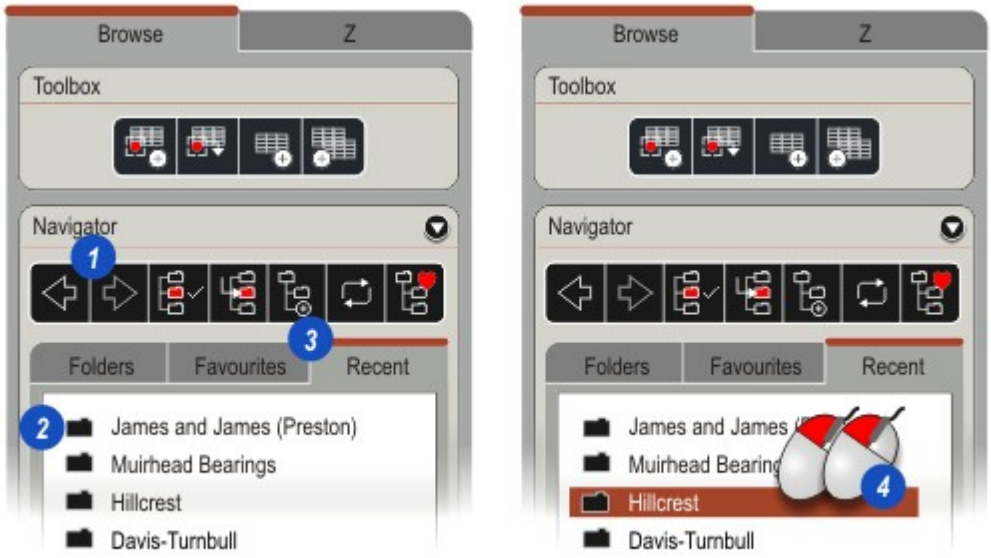
The software keeps track of the navigation by the step buttons **(1)** and allows the user to go directly to a folder without having to make multiple clicks on the step buttons.

The *Recent* feature is only available in *Folders*.

2: The list of visited folders is displayed when...

3: ...the *Recent* tab is clicked.

4: Return directly to a folder by double-clicking on it in the list.



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Users can nominate a folder into which captured images will be automatically saved. The nominated folder can be changed at any time:

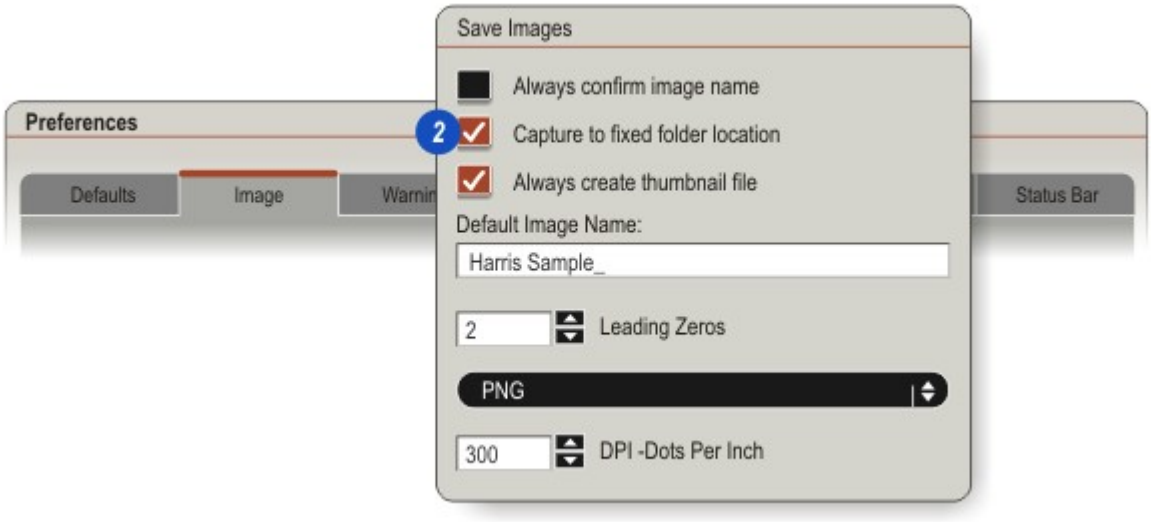
1: Click to select the *Capture Folder*.

2: Providing that the *Capture to fixed folder location* option is enabled in *Preferences* 47, clicking the *Set Capture Location* button will make the selected folder the saved image location.

3: The *Capture Folder* is indicated by the red dot to the left.

To Move to the Capture Folder:

4: If, having navigated away from the *Capture Folder* a user needs to return to it, clicking the *Move to Capture Folder* button will go directly there.



A new folder is created as a child of the selected folder that does not have to be the *Current Capture* folder.

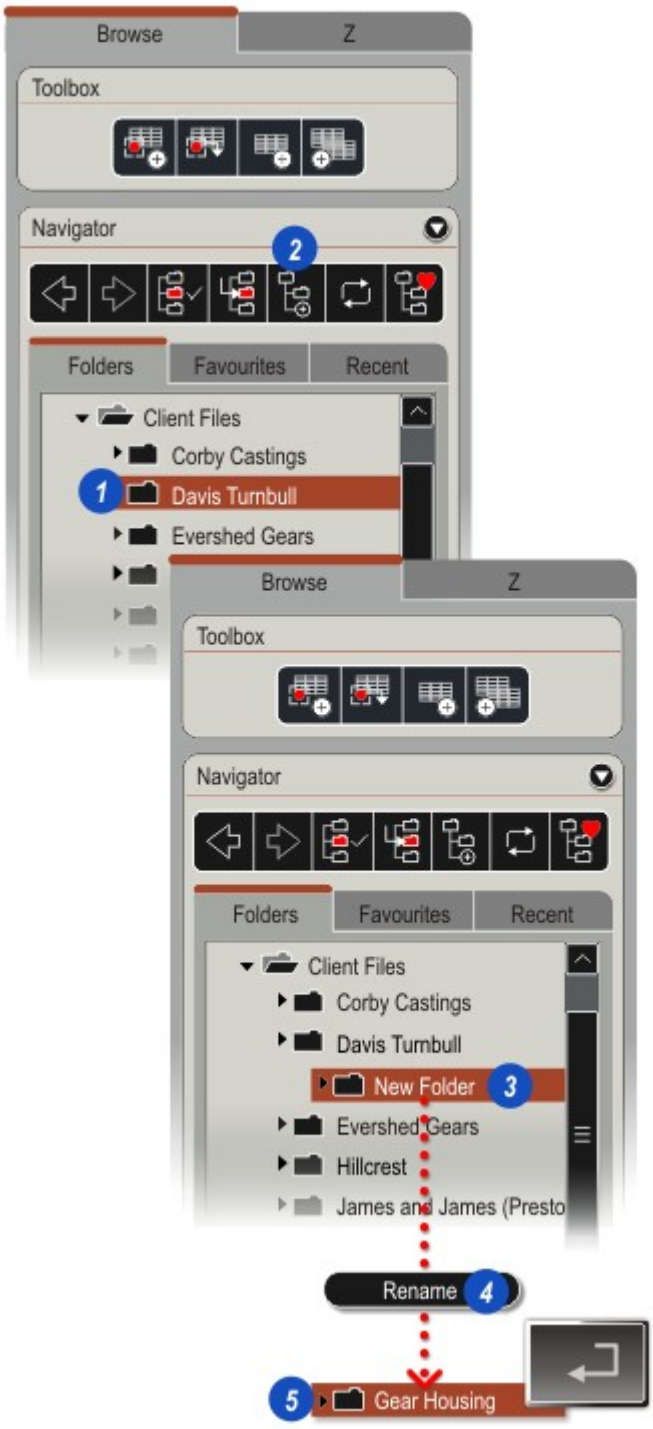
1: Click on the folder that is to be the parent of the new folder.

2: Click on the *Create New Folder* button.

3: The new folder appears with the default name *New Folder*.

4: With the new folder selected and highlighted, click on the *Rename* button and...

5: ...type a new name. Press the *Enter* key on the keyboard and the new folder has the new name.



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Users can store links to folders that they often use with the *Favourites* button. They can then return directly to a listed folder simply by double-clicking its link. This is a very useful feature for 'trees' with many folders and saves a considerable amount of time. The *Favourites* feature is only available in *Folders*.

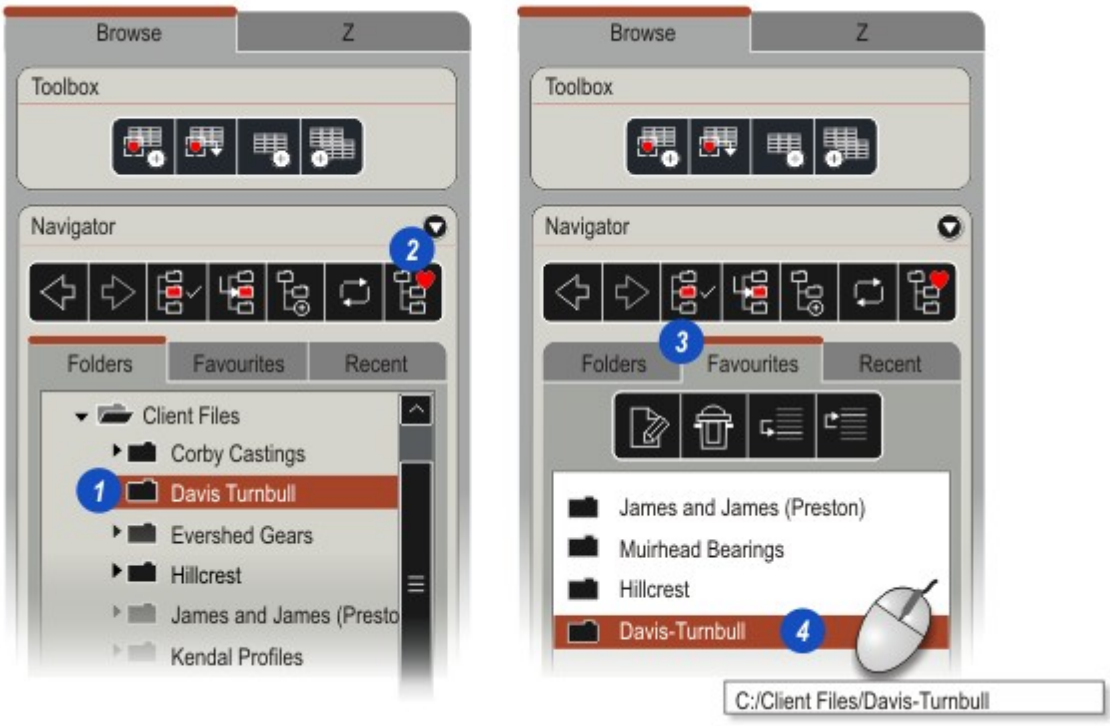
1: Click to select the folder to add to *Favourites*.

2: Click the *Add Favourite* button to create a link to the folder.

3: The list of links can be seen by clicking to *Favourites* tab...

4: ...with the newly created link highlighted. Move the cursor over the link to reveal the complete path.

Continued 312

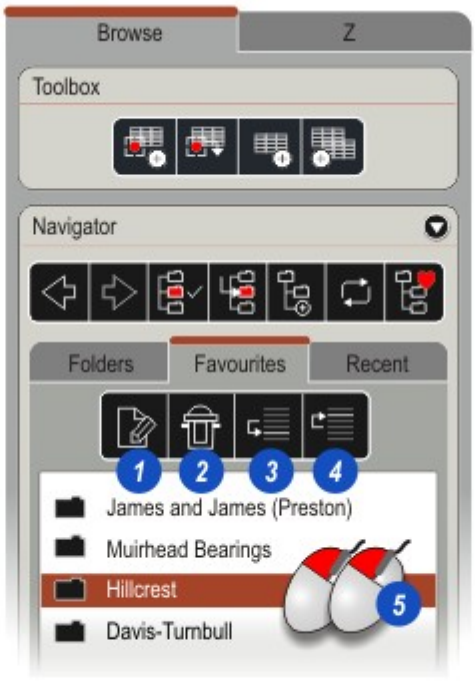


1: To change the name of a link click the *Edit* button and enter the new name.

2: Delete a link from the list by clicking to select the link and then clicking the *Delete (Trash Can)* button.

3 & 4: Move a link up or down in the list by clicking to select the link and then either the *Move Up* or *Move Down* button.

5: Return to a chosen folder by double-clicking its link. The *Folders* tab opens automatically and the selected folder is highlighted.



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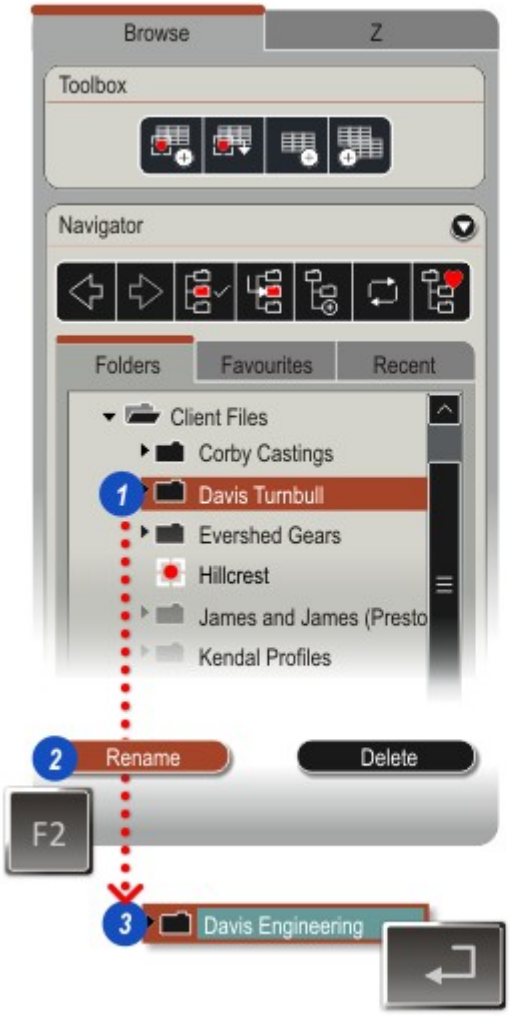
1: Click on the folder to be re-named.

2: Click on the *Rename* button or on the *F2* function key.

3: Click in the folder text box and type the new name finishing by pressing the *Enter* key on the keyboard.



Use with caution: *Folder Delete* cannot be undone.

Trying to delete a folder that contains images results in the *Unable to Delete* warning **(1)**. All of the images must be removed by:

2: Click on the thumbnail of the first image and...

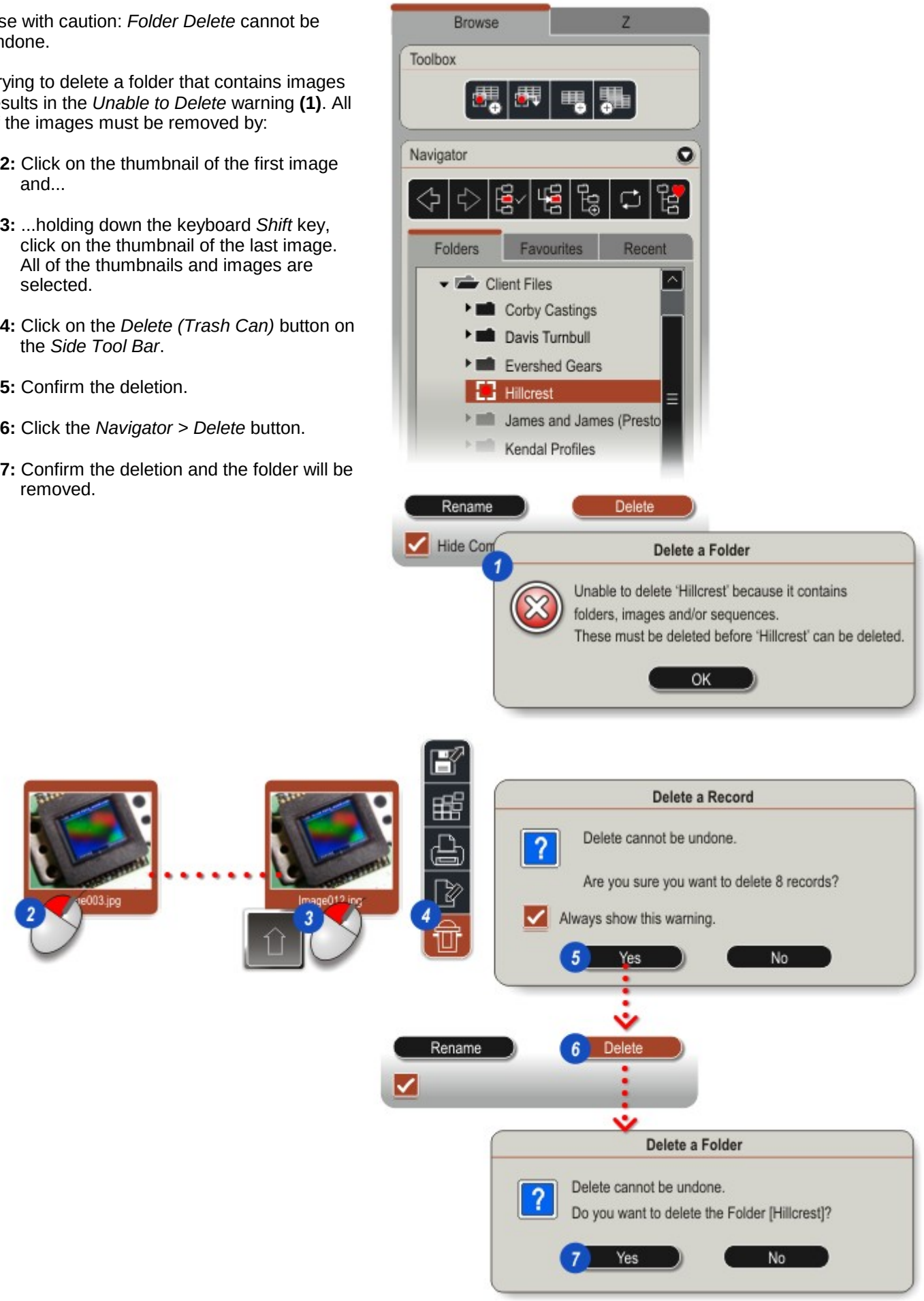
3: ...holding down the keyboard *Shift* key, click on the thumbnail of the last image. All of the thumbnails and images are selected.

4: Click on the *Delete (Trash Can)* button on the *Side Tool Bar*.

5: Confirm the deletion.

6: Click the *Navigator* > *Delete* button.

7: Confirm the deletion and the folder will be removed.



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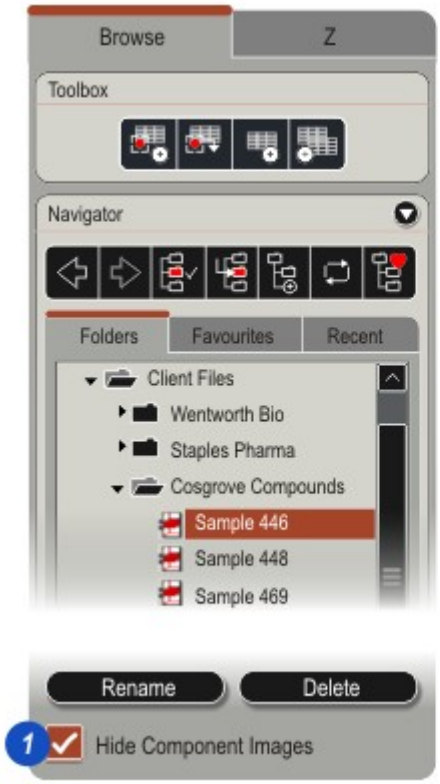
The *Gallery* when displaying sequences **(A)** can become very cluttered with the individual component images - the important results may be scattered and time-consuming to find.

The component images can be easily hidden leaving only the 'analytical' images on view in the *Gallery* **(B)** by enabling (tick mark displayed) the *Hide Component Images* check box **(1)**.

The individual images are not deleted, only hidden and can be revealed by disabling *Hide Component Images* by clicking it again.



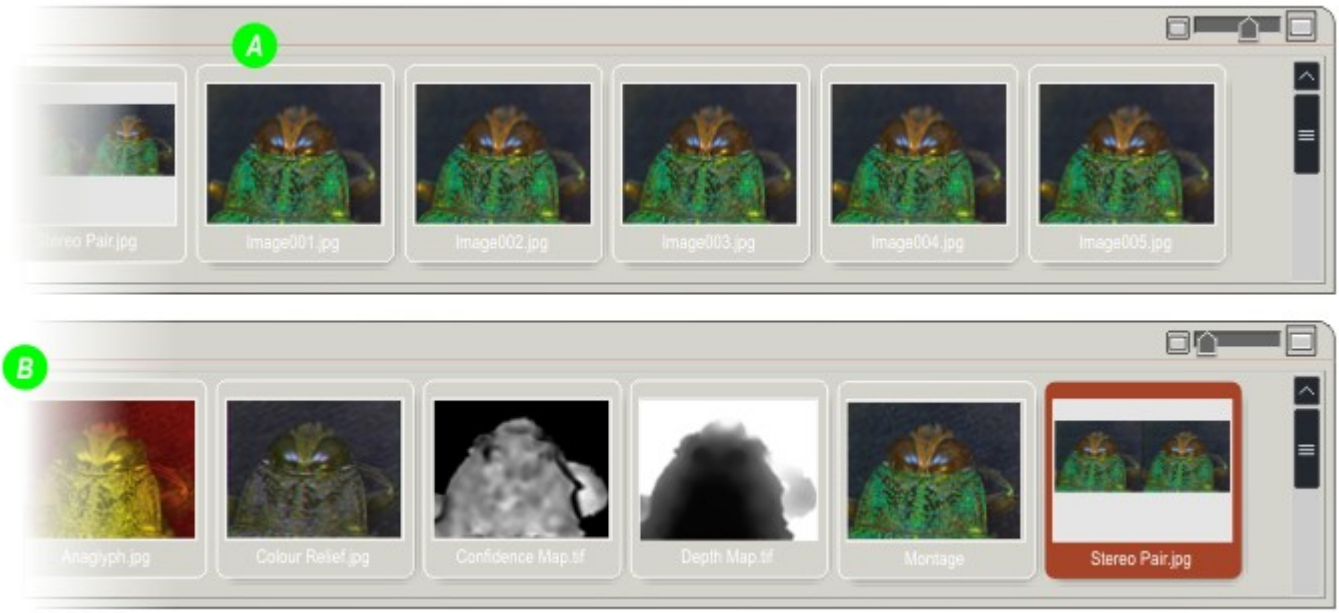


Image sequences - montage and multistep for example - can be copied or moved in a single step using mouse drag and drop:

To move a sequence:

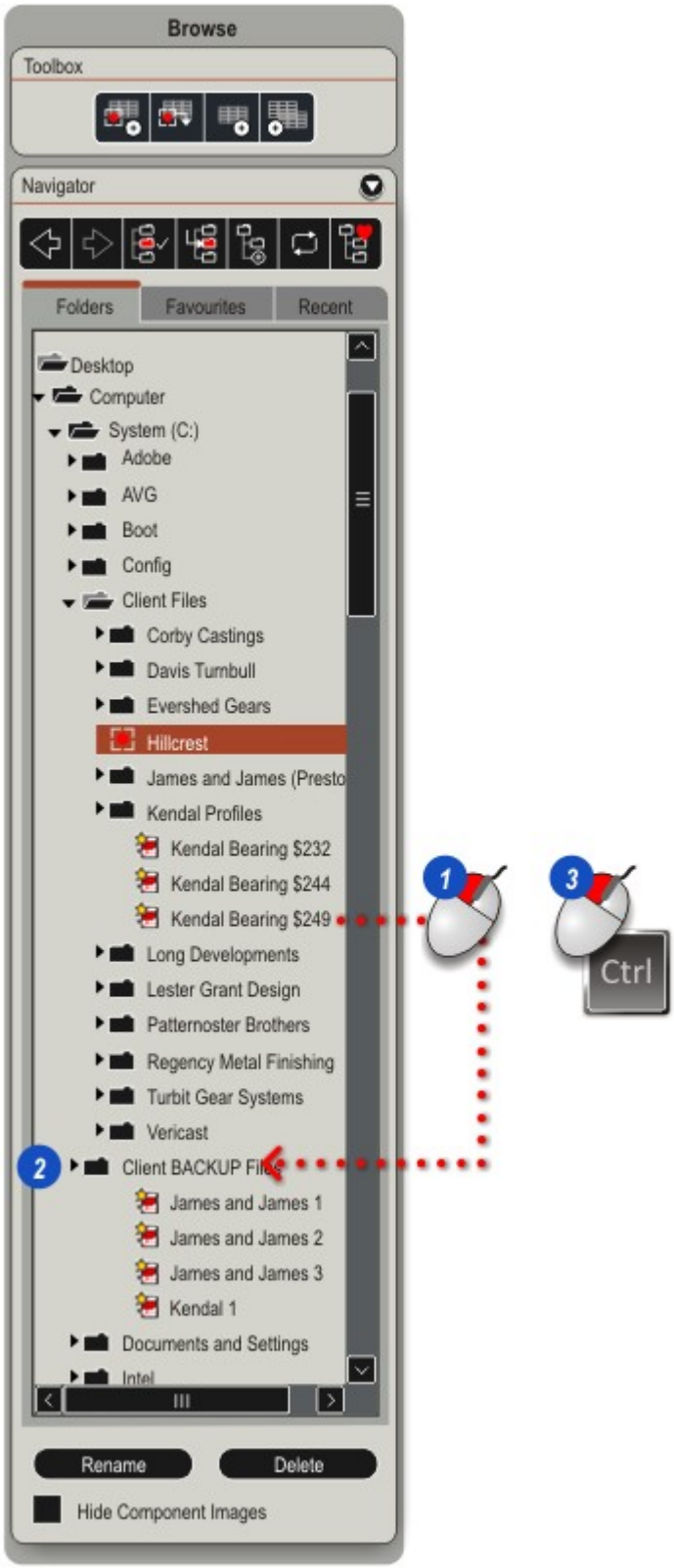
1: Left click on the sequence name and, holding down the mouse button...

2: ...drag to the target folder and release the mouse button. The sequence is moved from the source folder to the target folder.

To copy a sequence:

3: Press and hold down the keyboard *Ctrl* key, left-click and also hold down the mouse button on the sequence to be copied. Drag the mouse to the target folder and release the mouse. Release the *Ctrl* key.

The sequence is duplicated in the target folder.



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The Side Tool Bar:

This section describes the *Side Tool Bar* tools that are common to both *Image Explorer* and *LAS Archive.* Click on a *Tool Bar* button for more information:

Scale Bar and Annotations: Run Annotations and Scale Bar without leaving Browse.

Floating Navigator: Click to enable the *Floating Navigator* and click again to 'park' it.

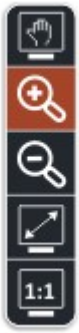
Export: Export the current image or image selection to a location of the users choice.

Image Stitching: Combines a set of overlapping images into a single, aligned overview image.

Print: Print the selected image together with headers, footers and a wide range of formatted data.

Create Report: Produces a printable report. Appears only when an archive is being displayed.

Delete the selected Image(s): Deletes the images and their associated data.



Panning: Examine areas of images that extend beyond the *Viewer* edges into the display area.

Zoom in and...

Zoom Out.

Fit the image to the Viewer area.

Display at Original Size: Displays he image at its captured size:

Hide and Reveal the Record Panels.

Hide and Reveal the Viewer.

Hide and Reveal the Data Grid: Only available with LAS Archives.

Hide and Reveal the Thumbnail Gallery.

View the image Record Details.

Select the Form Details to display: Allows the user to add or remove image details from the Form.

Viewer Options: Select Dual Viewer, Lock the Views and *Lock the Pan View.*

Save the Output File: Click to save an image of the output file currently displayed in the *Dual Viewer.*

Clicking the *Show Annotations* button displays the *Annotations* and *Scale Bar Quick Launch* menu.

Basic Annotation, Extended Annotation and the *Scale Bar* setup can be launched without leaving *Browse* by checking (a tick mark is displayed) the required function. All of the function tools are available.

Additionally, any annotations applied to the current image as well as the *Scale Bar* can be displayed.

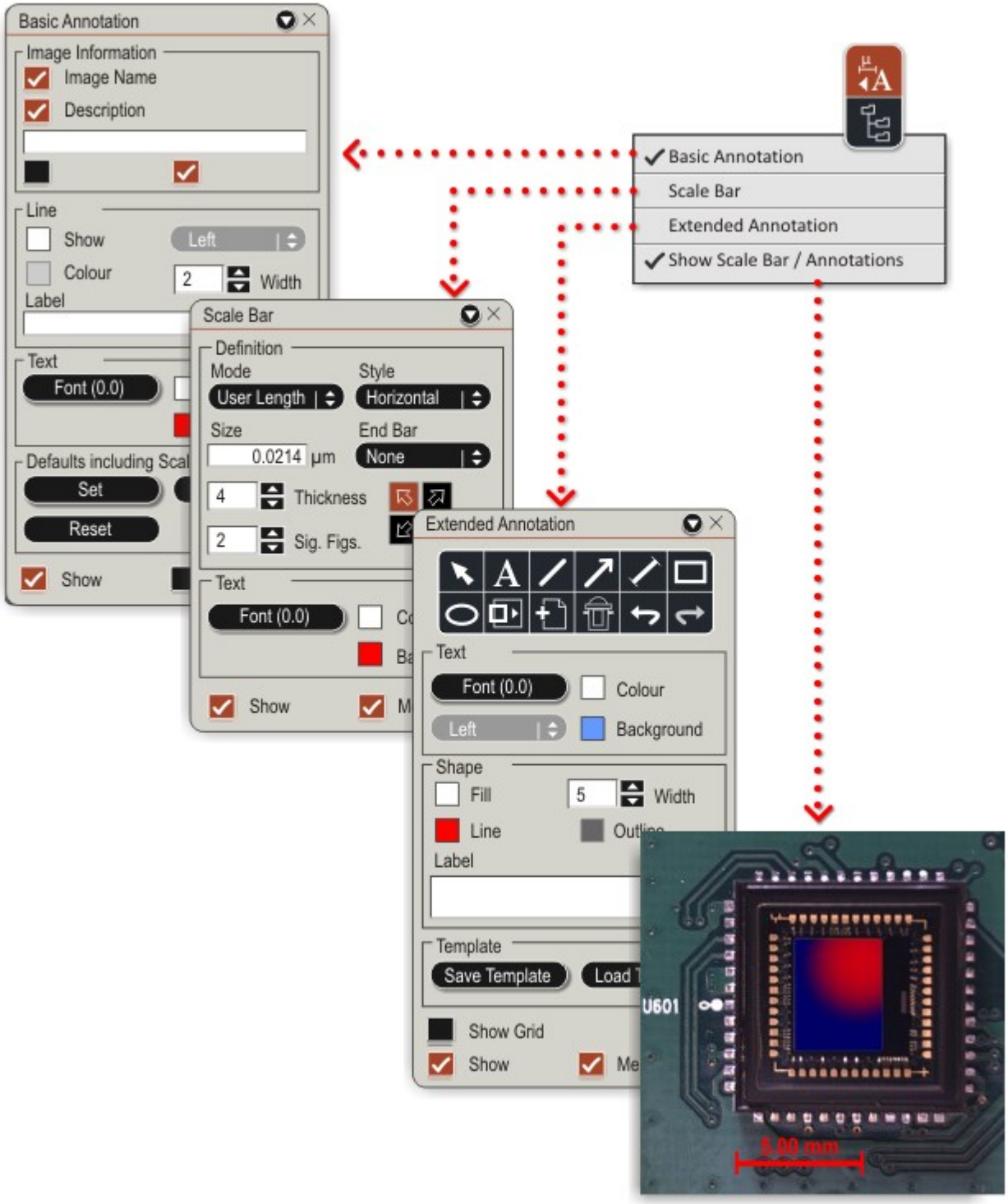


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Image Stitching:

LAS *Image Stitching* software has been designed to create a composite image of a large specimen from a sequence of individual 'tiles' captured on a manual stage.

LAS *Image Stitching* is ideal for small to moderate tile counts. It is fast and flexible and is part of the LAS Core and so available to all users.

Features:

- Designed especially for image capture using manual stages - demanding precision is not required.
- Suitable for a wide range of specimens - colour or greyscale.
- Areas of interest can be chosen from a tile sequence - there is no need to stitch an entire sequence.
- The number of tiles and image size are not fixed but the recommendation is for no more than 50 tiles or an image no larger 500MB.
- Background colour is user selectable.
- Smooth blending feature helps to correct uneven lighting effects or incorrect shading.
- Automatic image scaling to reduce the final image size.
- Users to set result image names with automatic suffix increments.

Using Image Stitching:

- 320 Tile Capture Guide.
- 326 Advanced Settings - Blend and Background Colour.
- 327 Image Reduction Factor.
- 325 Select and Stitch all Tiles in a Sequence.
- 330 Stitch Selected Tiles.

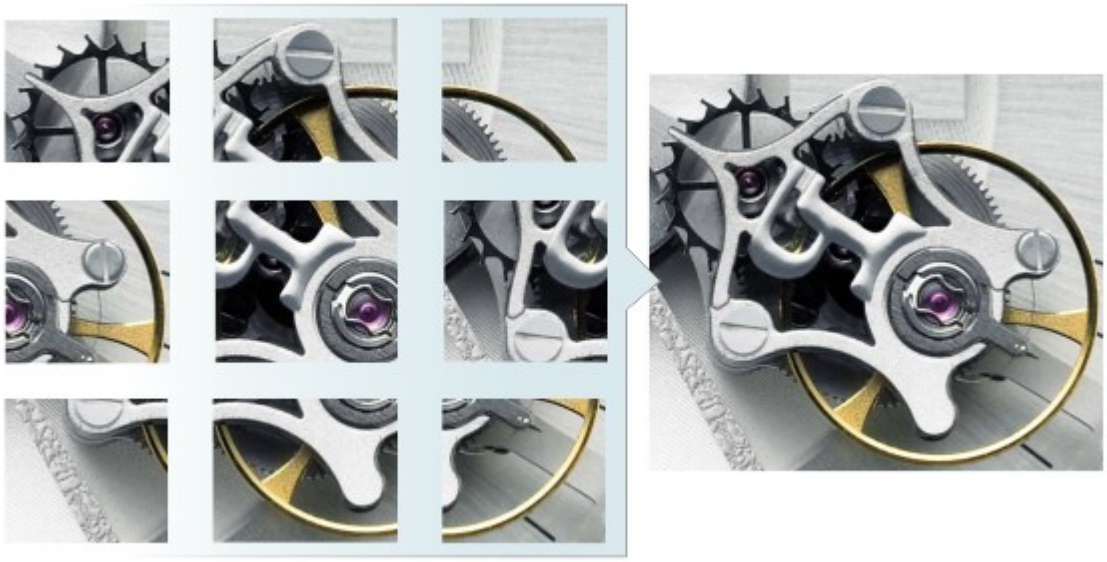

Image and Tile Requirements:

- *Image Stitching* works best with images that have complex, random detail. Avoid repetitive, grid-like detail.
- Tile overlap is essential 321 Detailed images do not require as much overlap as images with sparse features.
- All tiles must have the same magnification and be as sharp as possible. The minimum tile size is 512 x 512 pixels.
- Tiles must have the same *Capture Format* - jpg etc 322.
- All tiles must have same *Calibration* value 323 and must not be rotated with respect to each other.
- *Shading* correction 324 is not essential but is recommended to avoid 'striping' in the result image.
- The image can be colour or greyscale.

Continued 321

This is an image of a watch escapement magnified x5:

The field of view extends to just one corner...

...which means that at least 9 individual tiles are required to represent the entire image:

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Because the individual tiles are captured using a manual stage, the edges are unlikely to represent precise fits to those of their neighbours. Therefore, the stitching process compares each tile with all of the others in the selection looking for matches in pixel groups that will constitute an edge and to achieve this there must be a *guaranteed* overlap between adjacent tiles.

As a guide, users should aim for about 25% tile area overlap although for images with random, non-repetitive detail this can be lower.

The illustration shows by using different colours, how the nine tiles overlap on all internal edges.

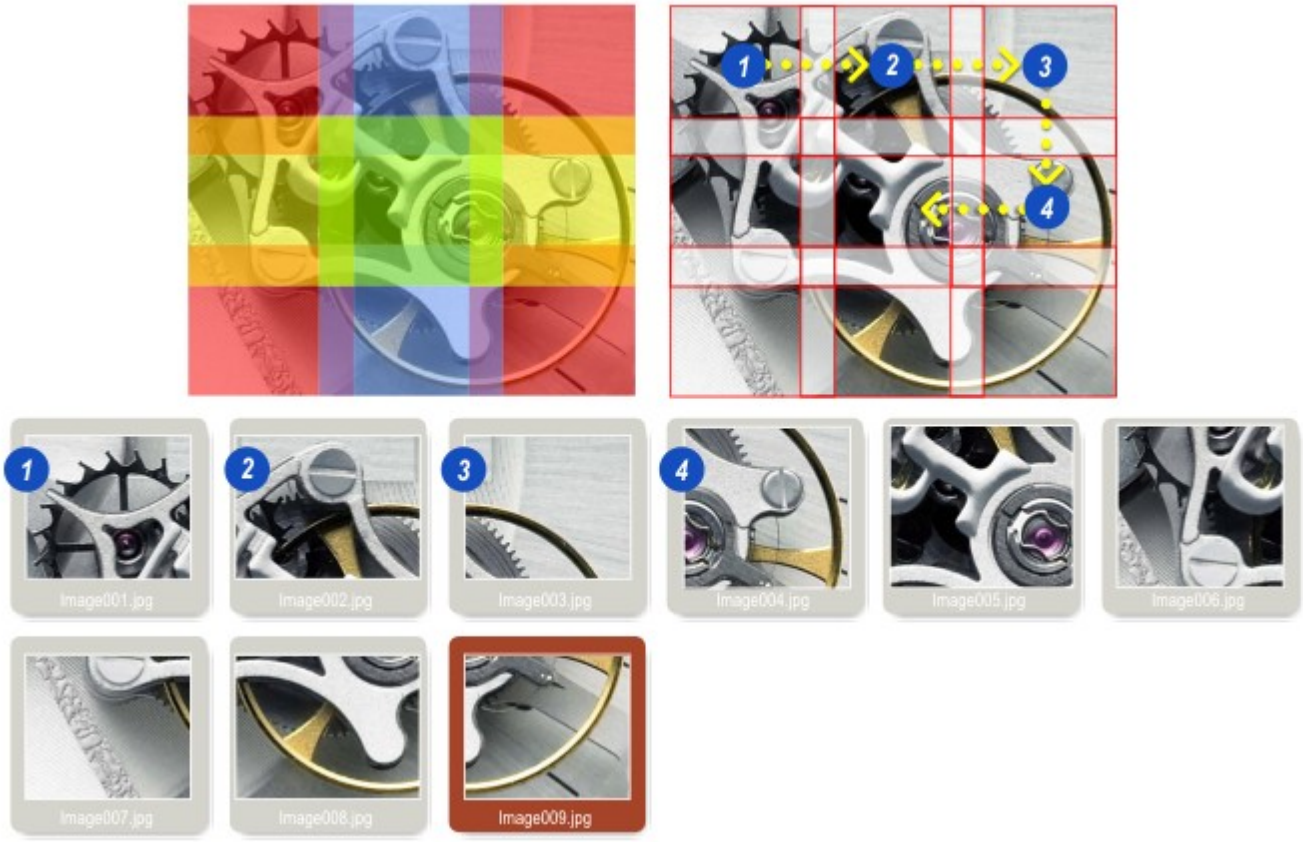
Users who have stages fitted with a Vernier scale can make some simple calculations to make sure there is sufficient overlap and use the scale to position the stage rather than just relying upon a visual estimate.

1: Capture commenced at the top-left corner.

2: The stage was them moved along the X axis to the second tile making sure there was a good overlap with the first tile.

3: This was repeated for the third tile, again ensuring a good overlap with the second.

4: Then the stage was moved along the Y axis to start the second row, checking there was adequate row-to-row overlap - and so on until the entire image had been captured.



The specimen capture settings and made in both *Preferences* > *Image* and *Acquire* > *Camera.*

1: Set the *Image Format* and compression by launching *Preferences* (↗) and clicking the Image tab.

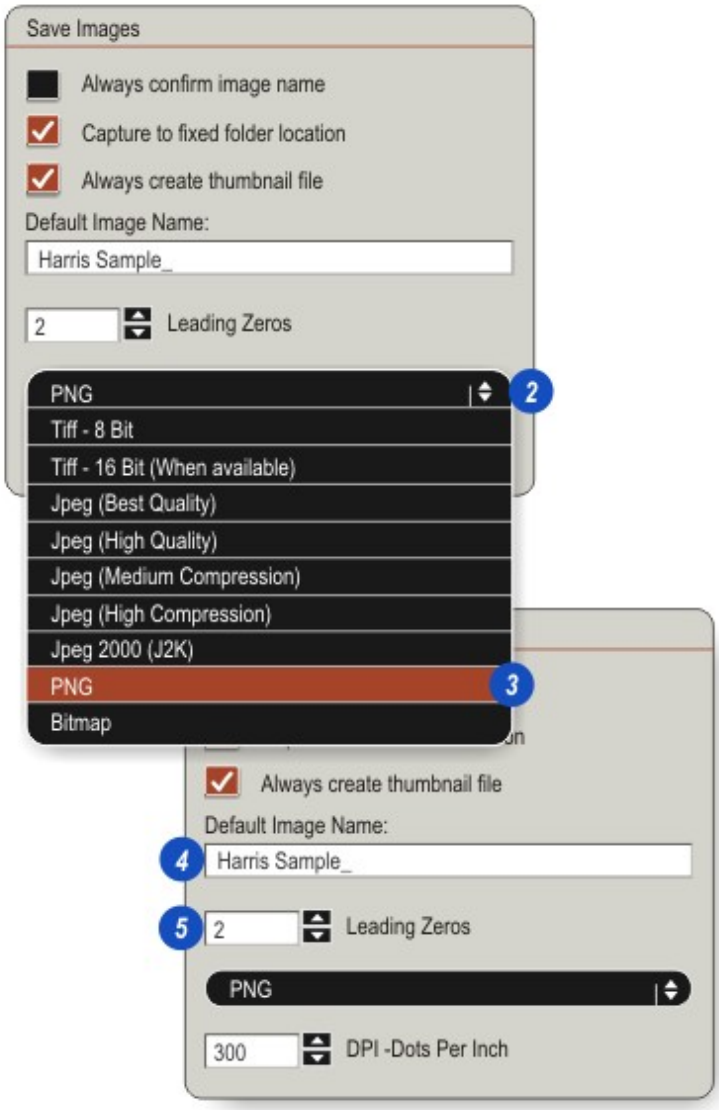
2: On the *Save Images* panel, click on the arrows to the right of the *Image Format* header and...

3: ...click to select the required format.

! Jpeg 2000 *(J2K)* is not suitable for *Image Stitching*.

4: Whilst on the *Save Images* panel, users may like to give the tiles an appropriate name by clicking inside the *Default Image Name* text box and typing a new name, and...

5: ...setting the number of zeros to be displayed in the suffix following the image name. In the example the individual tiles would be named: Harris Sample_00, 01, 02...10, 11 and so on.



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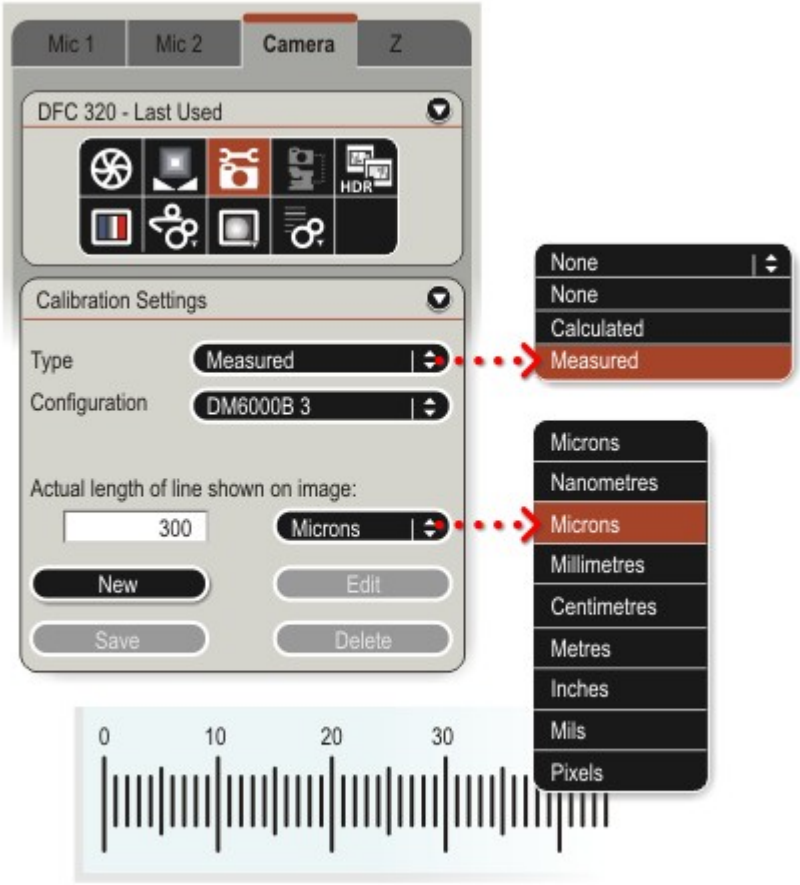
Calibration ensures that measurements displayed by the software are given in 'real world' - millimetres, inches etc - units taking into account the selected optical magnification of the microscope and the size of the camera pixels.

For initial system calibration users should refer to:

- *Acquire > Camera > Calibration* 268.

If system calibration has changed and the tiles pre-dates the change, they can be updated to ensure they are all consistent by using *Update Calibration* found in:

- *Options and Preferences > Update Calibration* .



Shading is the name given to variations in the background light level across an image.

In the example, the image on the left shows the effects of shading - the light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

A more evenly 'illuminated' image can be achieved by applying a digital 'correction' based upon the brighter part of the image. This is shown on the image on the right.

Because shading is caused by variations in the optics, each objective has to have the level of 'correction' applied individually.

1: Shading is described in detail and applied in *Acquire* > *Camera* > *Shading* 257.

2: With the *Shading* facility turned off, the stitched image has a 'corrugated' look caused by the dark edges.

Check the *Blend* option in *Advanced Settings* to help minimise the effects of uneven lighting 326.

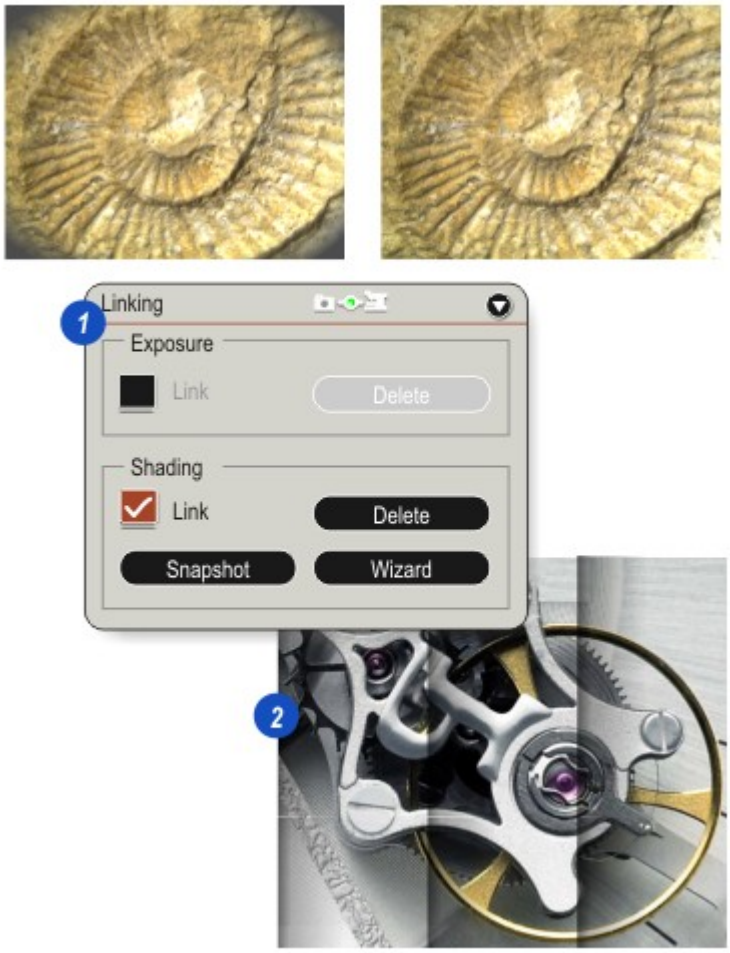


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Image Stitching: Select and Stitch all Tiles:

Start *Image Stitching* by selecting the tiles to be used - in this example all of them:

1: In the *Gallery*, left click on the first image to select it and...

2: ...holding down the keyboard *Shift* key, left-click the last tile in the collection. All of the tiles between the two selections are automatically selected - the thumbnail frame is coloured brown.

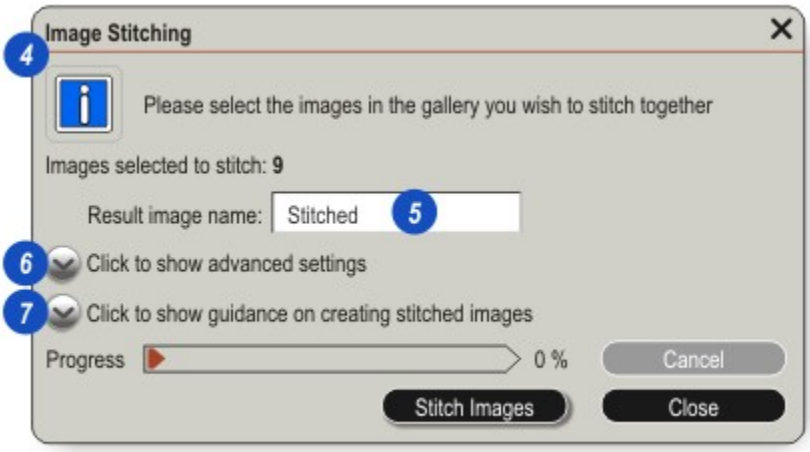
3: On the *Side Tool Bar* click the Image *Stitching* button.

4: The *Image Stitching* dialog appears showing the number of tiles that have been selected.

5: The stitched image is given a name preferred by the user. Click inside the *Result image name* text box and type a name.

6: Click the *Show advanced settings* button to set the *Blend, Background Colour* and *Reduction Factor*: 326

7: The *Further Information* button 328 when clicked, provides a reminder of the image requirements.



Continued 326

1: After *Image Stitching* is launched and the *Advanced Settings* button is clicked, the dialog expands to reveal the *Blend, Background Colour* and *Reduction Factor* options:

Blend images where they overlap:

2: The *Blend* option smoothes the tonal transition between adjacent tile edges. It especially useful if *Shading* was not enabled or incorrectly set when the tiles were captured.

Click the check box to enable (tick mark visible) the *Blend* option. Click again to turn it off. The default is enabled.

Result image background colour:

3: If unimportant tiles are not selected 330 and therefore not included in the stitching, there will be a gap in the composite image. Users can select a colour to appear in the gap(s).

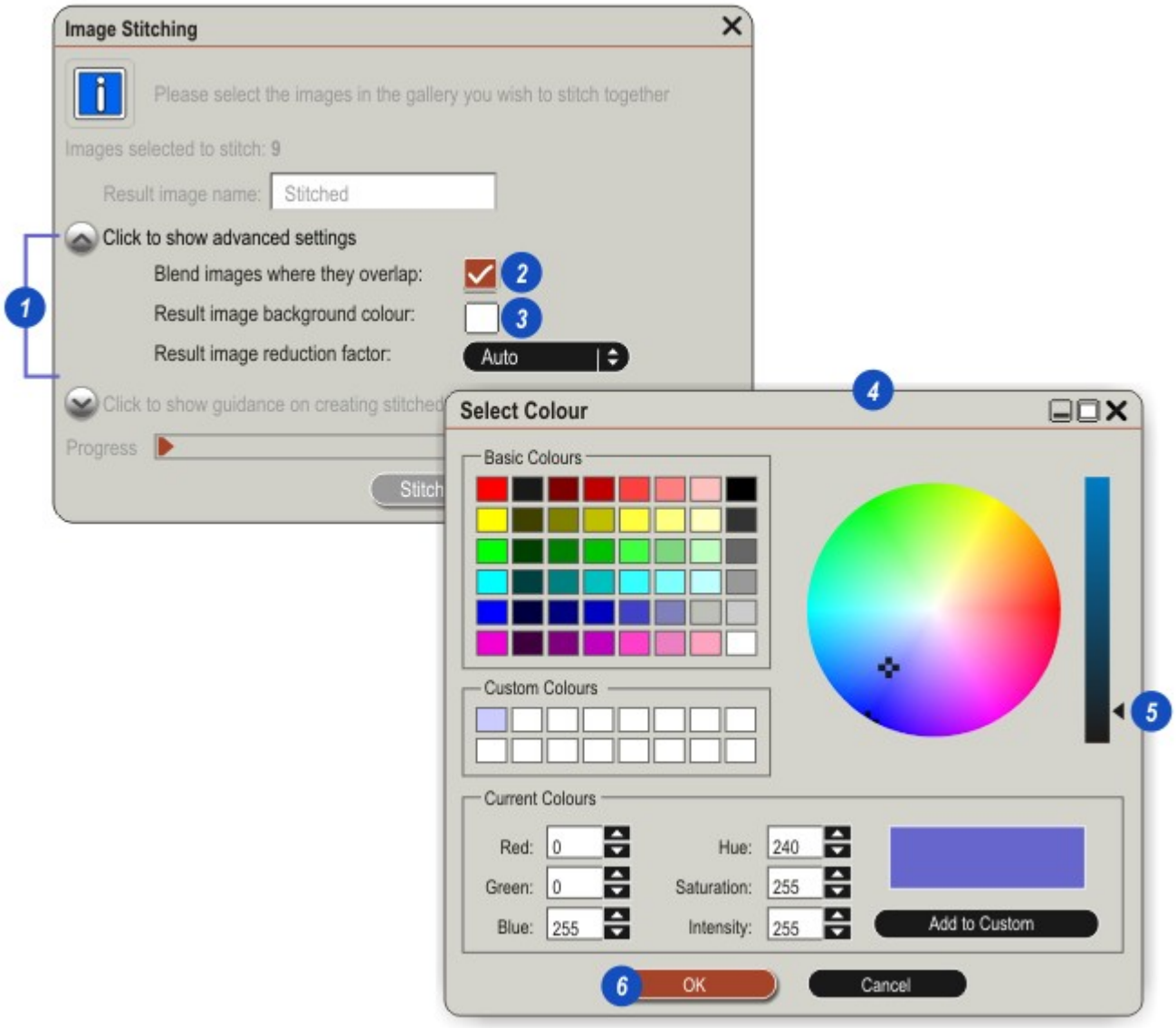
Click in the *Background colour* box and...

4: ...on the *Select Colour* dialog choose a background colour from the swatches, by dragging the 'target' on the wheel or typing values for red, green and blue (RGB) in the text boxes.

5: Adjust the colour intensity by clicking and dragging the slider.

6: Click *OK.* The selected colour appears in the box.

Continued 327



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Some stitched images can be large and unwieldy making them difficult to include in reports or share digitally. The *Image reduction factor* automatically scales the result image to reduce its size.

1: Click on the small arrows to the right of the *Result image reduction factor* and...

2: ...from the drop-down menu click to select the reduction factor required.

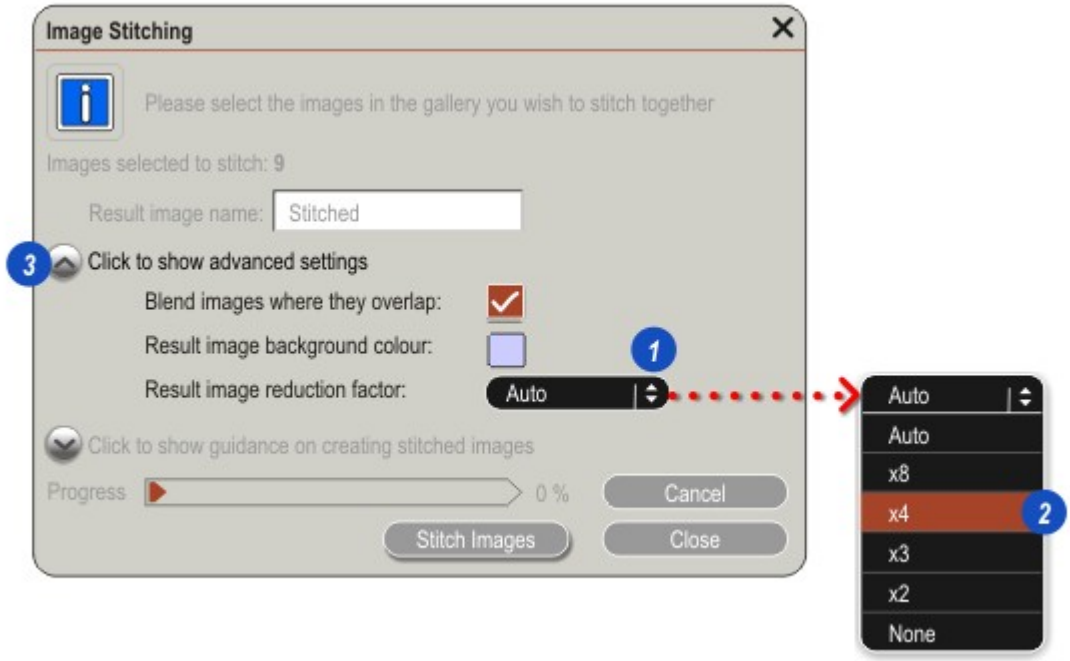
Larger values: Result in a smaller result image.

Auto: The reduction factor is based upon the pixel count of the individual tiles.

None: The result image is not scaled at all.

3: Click on the *Advanced Settings* button to close the settings.

Continued 328



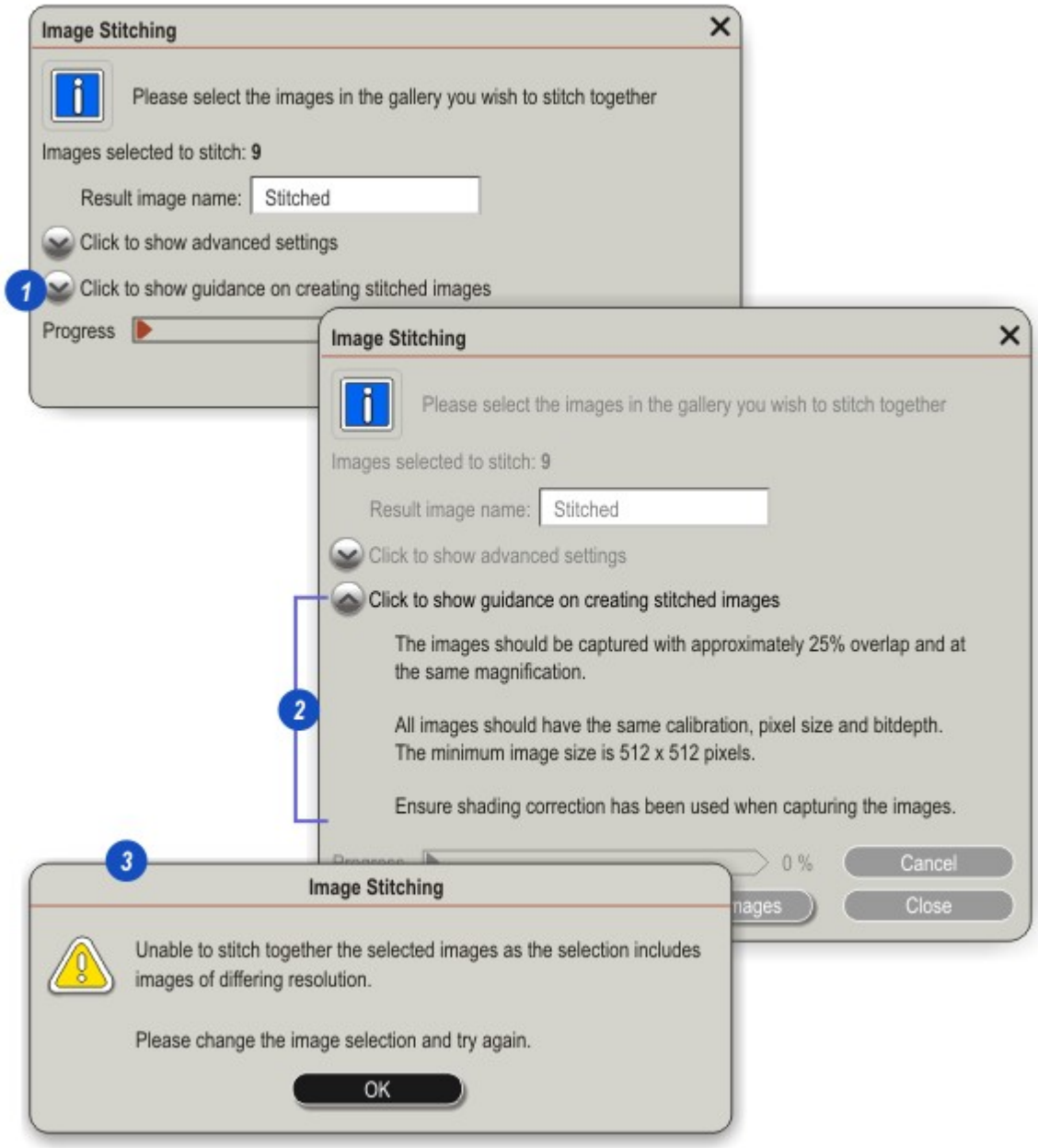
1: Click the *Show guidance...* button to...

2: ...display a reminder regarding the essential parameters for tile capture.

Click the button again to hide the dialog.

3: The *Image Stitching* program provides a range of warnings to help the user capture and select tiles that will help ensure the best possible result.

Continued 329



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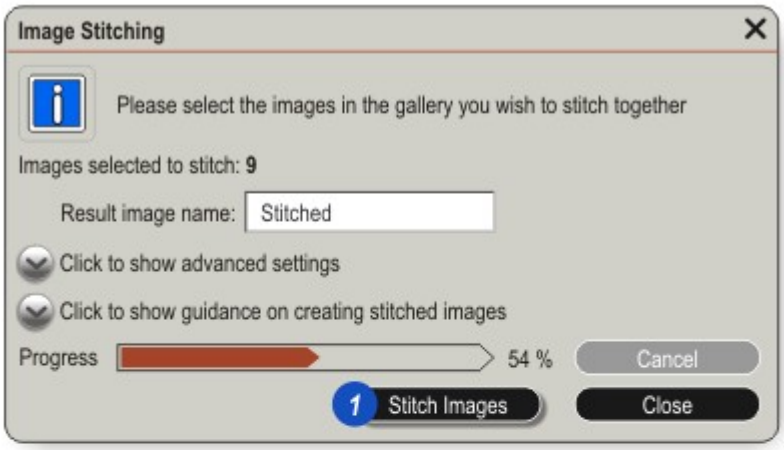
1: Start the stitching process by clicking the *Stitch Images* button.

2: The completed composite image appears in the *Viewer* together with...

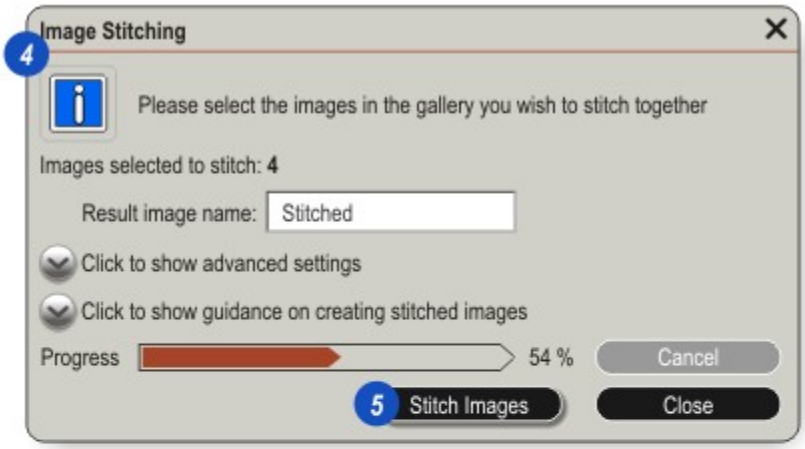
3: ...a thumbnail in the *Gallery*. The caption comprises the users preferred name together with an automatically incremented numeric suffix.



The tile selection prior to stitching does not have to include the entire sequence - tiles that represent an area of the image that is not important or needed can be omitted from the selection provided the remainder represent a contiguous area.

However, if the selected tiles do not stitch properly, include some of the omitted tiles and run *Image Stitching* again.

In this example only the main bearing is of interest and so only those tiles that are part of it are selected:



1: In the *Gallery*, left click on the first image to select it and...

2: ...holding down the keyboard *Ctrl* key, left-click the other tiles required individually. The illustration shows just the tiles that together make up the bearing.

3: On the *Side Tool Bar* click the Image *Stitching* button.

4: The *Image Stitching* dialog appears showing the number of tiles that have been selected.

5: Click the *Stitch Images* button.

6: The finished composite image with all of the tiles stitched together...

7: ...represented by a thumbnail in the *Gallery* . The final image can now be used like any other.

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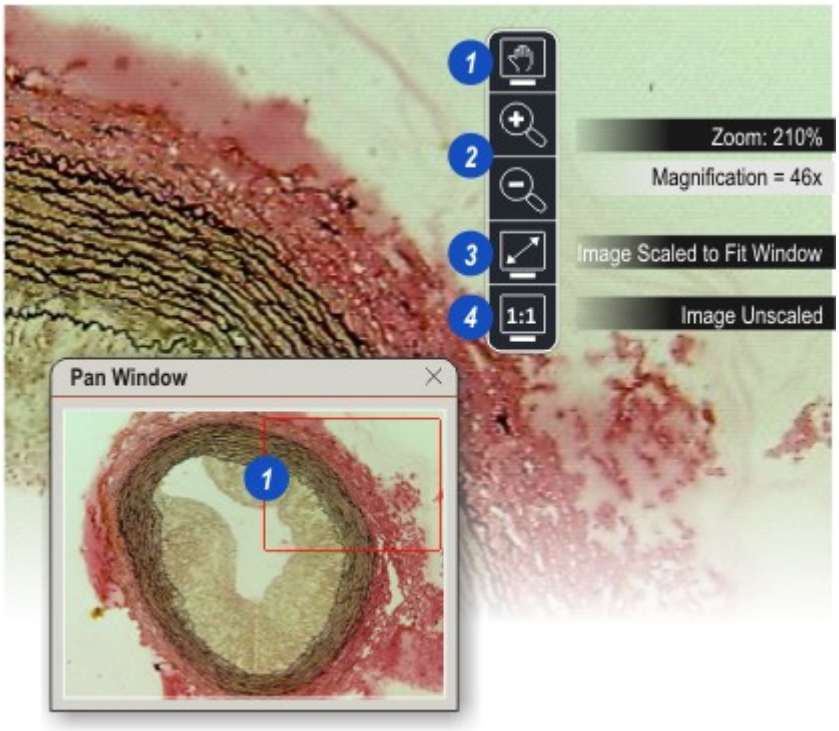

1: *Panning:* The *Pan* tool allows detailed areas of an image that exceeds the visible area of the *Viewer* to be examined. It will not work if *Fit to Viewer* is enabled because all of the image is being displayed.

On the *Pan Window* viewer, click and hold in the outlined rectangle and drag it to the area to be examined. The selected area is displayed in the main *Viewer*.

To move the *Pan Window* away from the main *Viewer*, click and hold the header bar and drag it to another part of the screen.

Click the *Pan* tool to close the *Window*.

2: *Zoom:* Click on the (+) to zoom in to the image or (-) to zoom out. The zoom level as a percentage, is displayed top right of the the *Viewer* border.

If the monitor *Magnification Settings* have been set in *Preferences*, the image *Magnification* value appears bottom right of the *Viewer* border.

3: *Fit to Viewer:* Click to fit the image to the available *Viewer* area regardless of the original size of the image. The *Image Scaled to Fit Window* message appears top right of the *Viewer* border.

4: *Display at Original Size:* Click to display the image at its original size. The image may appear smaller or larger than the *Viewer* area. The *Image Unscaled* message appears top right of the *Viewer* border.

The various screen areas - *Viewer, Gallery, Report* and *Grid* (where applicable), may be revealed or hidden to create the best working environment for the user. Some tools are toggles – click once to reveal the area, click again to hide it:

1: *Hide/reveal the Record panels:* The *Image Viewer* expands to fill the vacant space.

2: *Hide/reveal the Viewer:* The *Record* panels expand to occupy the *Viewer* width.

3: *Hide/reveal the Data Grid:* The *Viewer* will expand to cover some of the vacated space. The *Grid* is only available if an *LAS Archive* is installed.

4: *Hide/reveal the Thumbnail Gallery:* The *Gallery* is hidden and the *Viewer* expands to include the *Gallery* space.

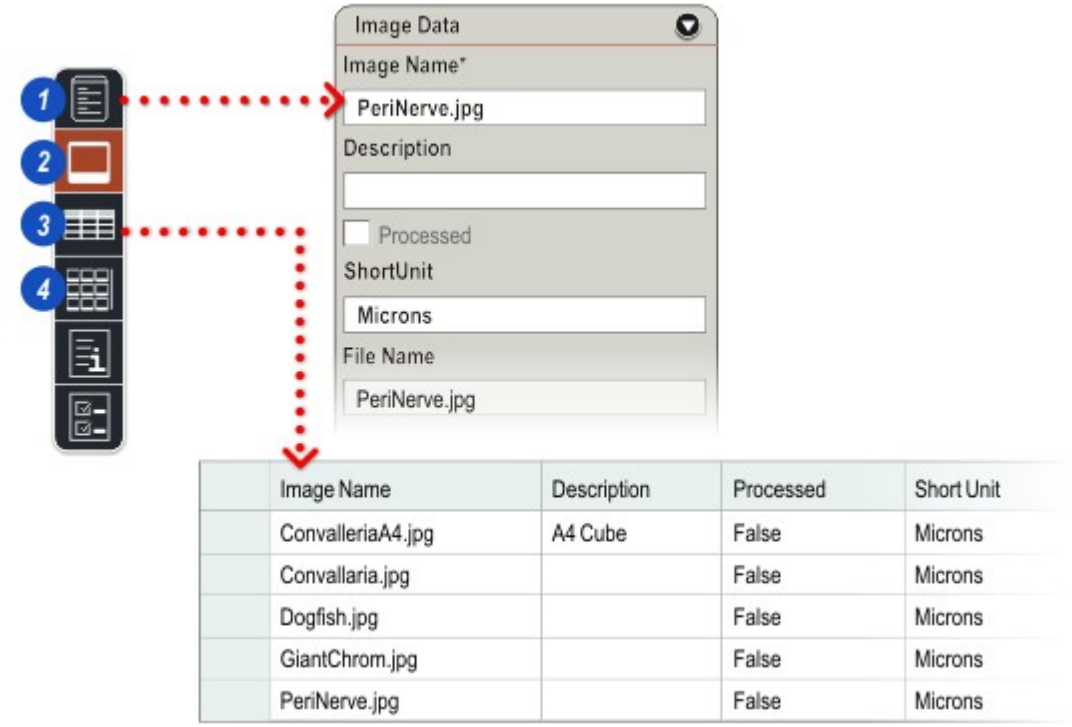

Image Name	Description	Processed	Short Unit
ConvalleriaA4.jpg	A4 Cube	False	Microns
Convallaria.jpg		False	Microns
Dogfish.jpg		False	Microns
GiantChrom.jpg		False	Microns
PeriNerve.jpg		False	Microns

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Delete a single image by clicking its thumbnail and then the *Delete (Trash Can)* button.

Alternatively, delete a group of images by:

1: Hold down the *Crtl* key and click on a thumbnail. Do this for all of the images to be deleted, or...

2: ...hold down the *Shift* key and click on the first thumbnail. Do the same for the last thumbnail in the group to be deleted and all of the images between are selected.

3: Click the *Delete (Trash Can)* button.

4: Confirm that the images are to be deleted and cannot be recovered and the images and their data will be removed from the folder.

The *Viewer* area can be split to show two captured images simultaneously.

1: Click the *Viewer Options* button.

2: Click to enable (tick mark visible to the left) the *Dual Viewer* option. The *Viewer* will then divide into two panes.

3: The image currently being viewed will usually appear in the left-hand pane. If it does not or the image needs to be changed, click on the left-hand pane and...

4: ...click a thumbnail in the *Gallery*.

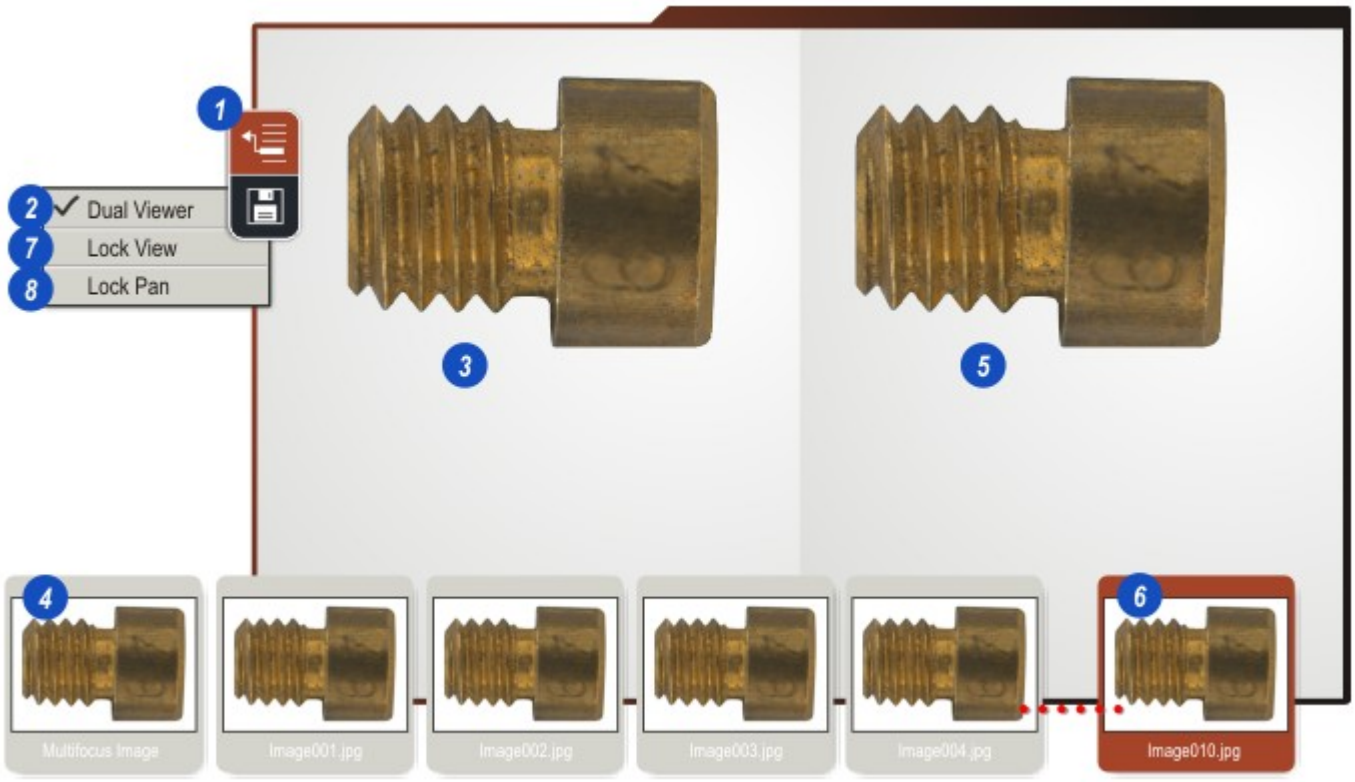
5: Display an image in the right-hand pane by clicking the pane and...

6: ...the thumbnail of the required image.

7: To synchronise the panes and enlarge or reduce the images as the zoom and fit tools are used, click to enable the *Lock View* option.

8: Enabling *Lock Pan* will synchronise the images as the *Pan* tool is used. Click the pane to pan and then on the *Panning* tool. Both images will automatically move to and display the image segment shown in the *Pan* window.

Dual Viewer more information: 79



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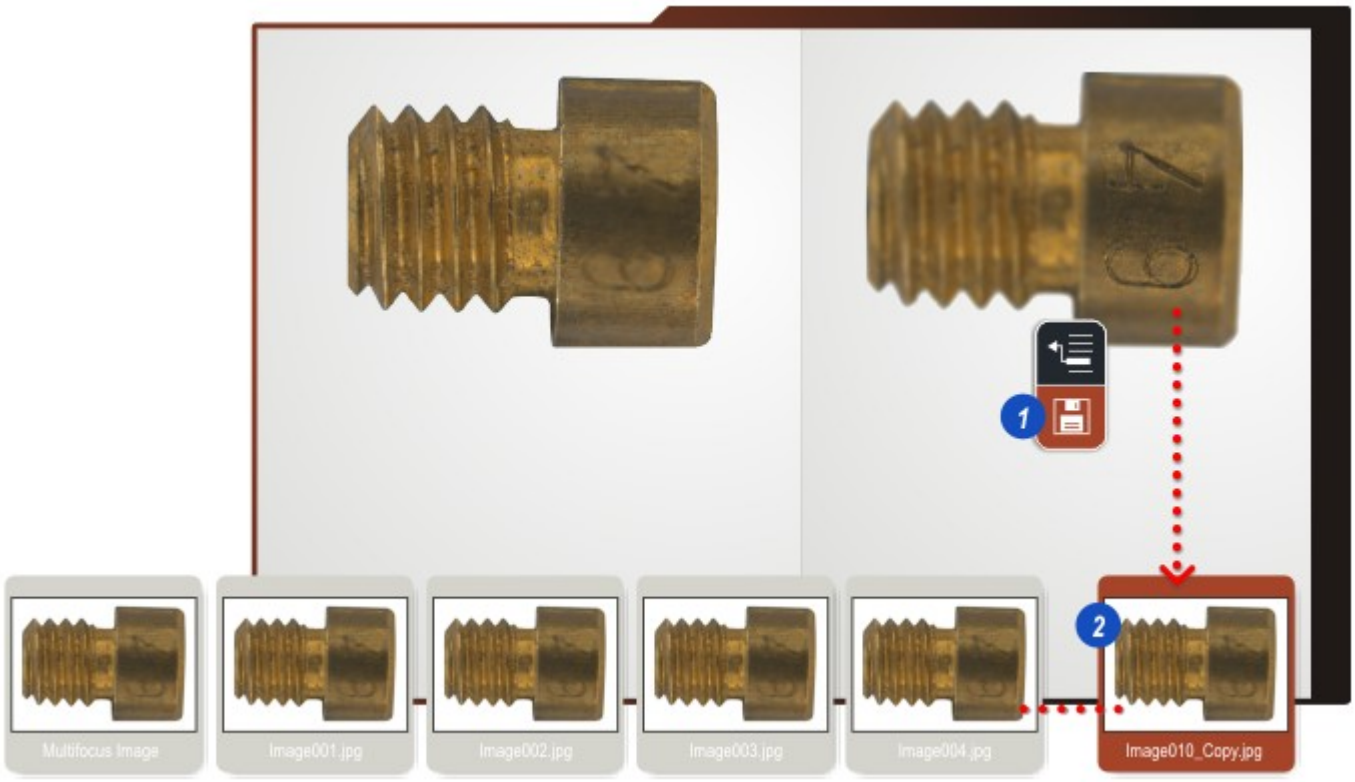
The output image can be copied (duplicated) by:

1: Click the *Copy Output Image* button.

2: The image is copied to the current folder with the output image name plus the suffix '_Copy'.



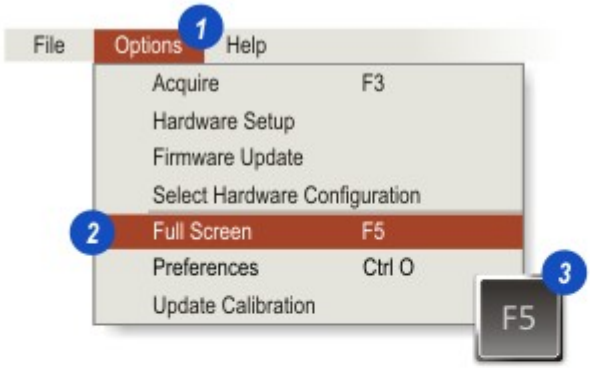
Full Screen Mode & Second Monitor:

Full Screen Mode: Single Monitor:

1: The *Viewer* area can be expanded to fill almost the entire screen by either cl icking *Options* on the main menu and...

2: ...selecting *Full Screen* from the drop down menu, or...

3: Pressing *Key F5*. Press *F5* again to return to the normal display.



Second Monitor:

The software detects a second monitor and changes the *Options* drop-down menu to:

4: ... *Use Second Monitor*. Click the option to use both monitors.

The *Viewer* and image occupy all of the second monitor whilst the *Gallery* and *Thumbnails* together with the controls remain on the primary monitor.

The *Side Tool Bar* buttons are appropriately shared between the two monitors.

5: Alternatively, press *Key F5* to move between using both monitors and returning to single monitor.

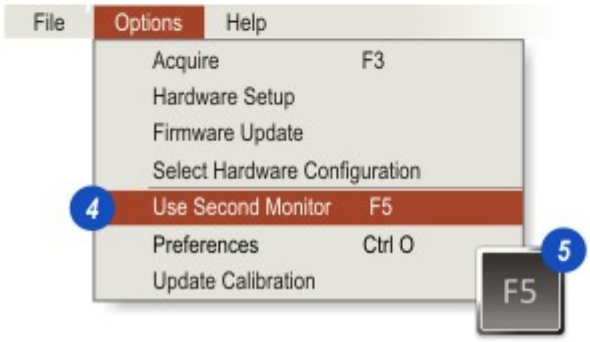


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Image Options:

A wide range of options is available by right-clicking the image in the *Viewer* or its *Thumbnail* in the *Gallery*. The options vary depending upon which item was clicked, the operating system and the software installed on the computer.

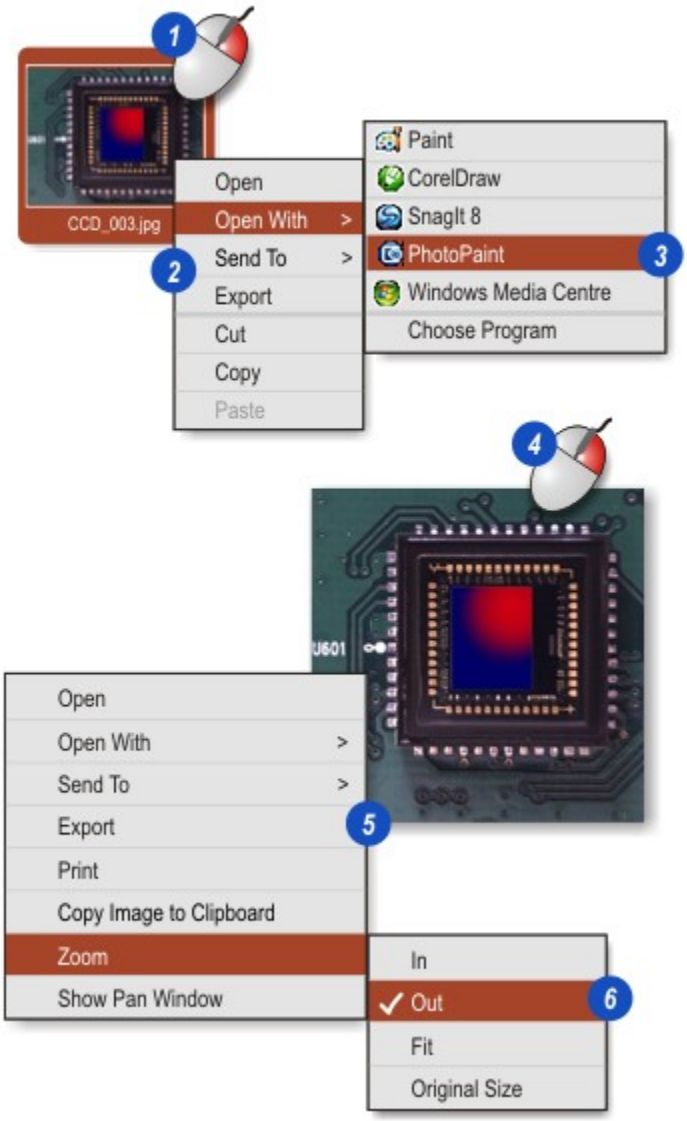
1: Right-click the *Thumbnail* for the context menu of basic options and...

2: ...click it to select.

3: Some options have additional possibilities displayed as a sub-menu.

4: Right-clicking the image in the *Viewer* displays a different context menu.

5: Additional functions are available some of which will also have sub-menus **(6)**.



The *Gallery* is a thumbnail display of images in the current folder in both *Image Explorer* or *LAS Archive.*

The *Gallery* can be hidden or revealed using the *Side Tool Bar* tools.

1: Clicking on a thumbnail will immediately display the full-sized image in the *Viewer* and the data associated with it in the *Record* and *Grid* (If LAS Archives is installed).

2: A *Slider* is automatically displayed for multiple rows of thumbnails - click and drag it to scroll the *Gallery*.

3: The *Navigation Bar* (bottom right of the screen) provides a way of moving through the thumbnails quickly and is especially useful with large galleries of thumbnails. Click on the arrows to move a single image **(3)** ...

4: ...or go to the extreme ends of the *Gallery*.

5: The thumbnails can be re-sized by clicking and dragging the *Scaling Slider* - slide left to reduce the thumbnail size and right to increase it.

6: Move the mouse over a thumbnail to reveal basic data bout the image.

7: Right-click a thumbnail to show the *Context Menu*. Left-click to select an option.

- *Gallery Docking Position* 339

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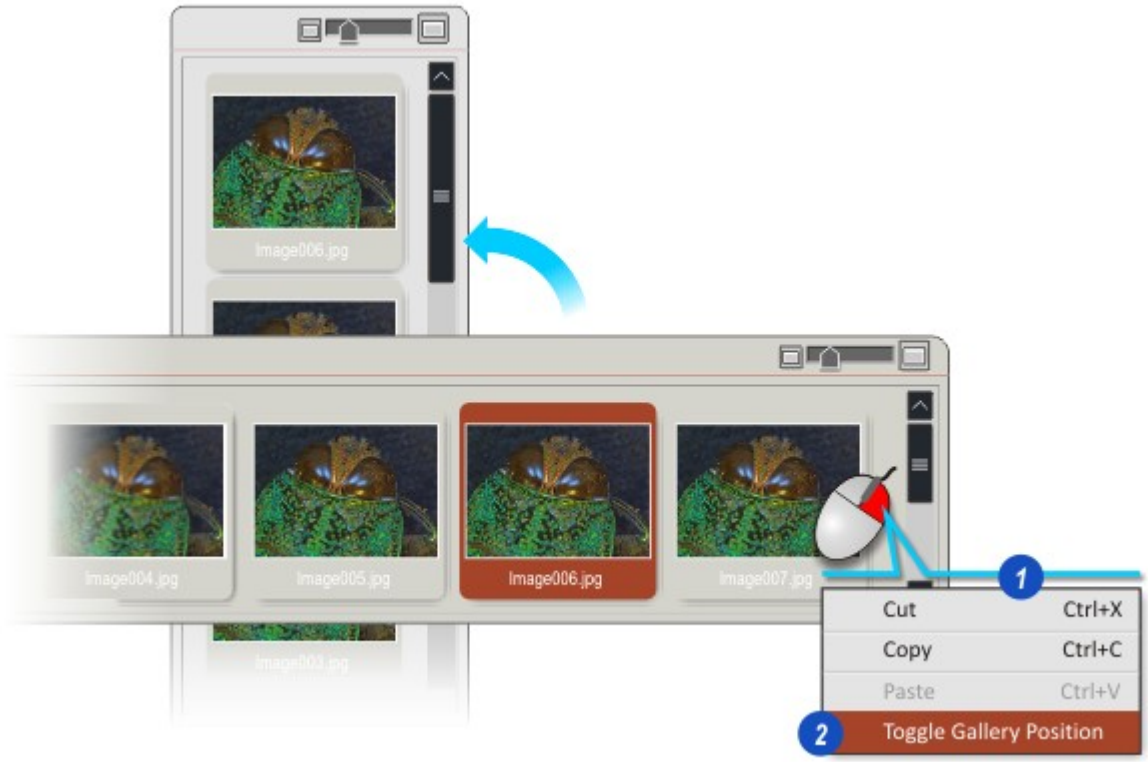
Available in *Acquire* and the *Process* Workflows as well as *Browse*, the thumbnail *Gallery* can be 'docked' either horizontally - along the bottom edge of the *Viewer* - or vertically - along the left-hand edge of the *Viewer* - to suit the user.

1: Right-click on a thumbnail or on the spaces around the thumbnails and...

2: ...from the drop-down menu, left-click to select the *Toggle Gallery Position* option.

The action toggles between horizontal and vertical docking.

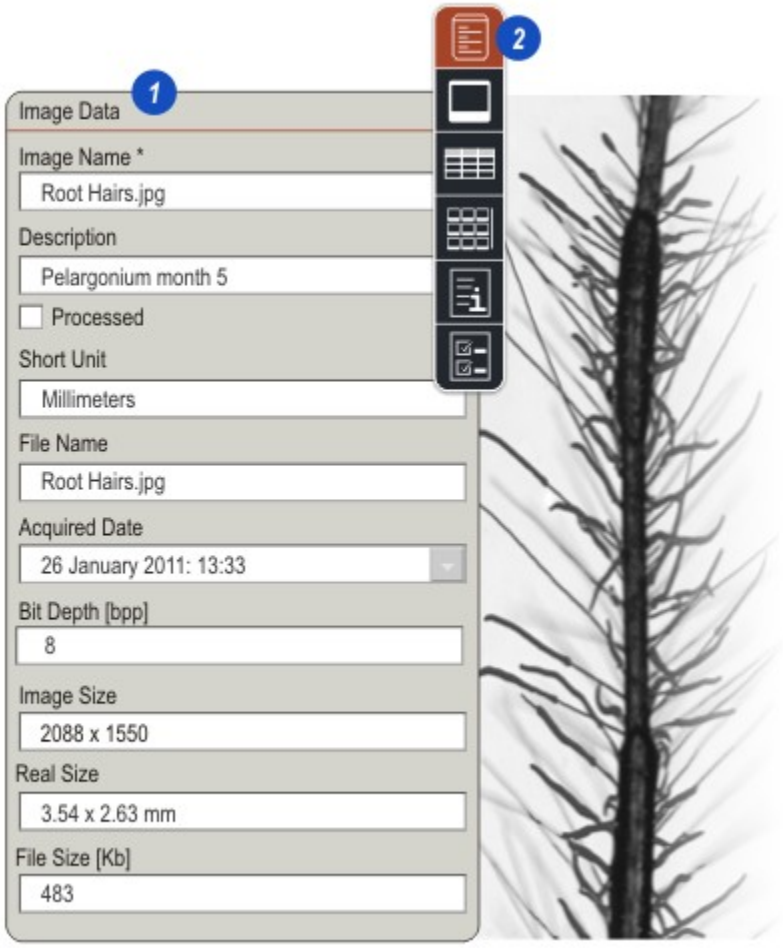
Scroll bars, if required, are placed automatically.



1: The *Data Form* displays selected data associated with the image. All of the data about the camera, microscope, exposure, creation date and so on, are actually stored and all can be displayed, but the *Form* can be configured to display only the more pertinent items.

2: The *Data Form* can be hidden by clicking the *Form* button on the *Side Tool Bar*. This is a toggle action - click again to reveal the *Form*.

- *Select Visible Fields* 341.
- *Configure Grid Columns* 342
- *Record Details* 343



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To select the data (Fields) to be displayed on both the *Data Form* and on the *Grid (*if an *LAS Archive* is installed):

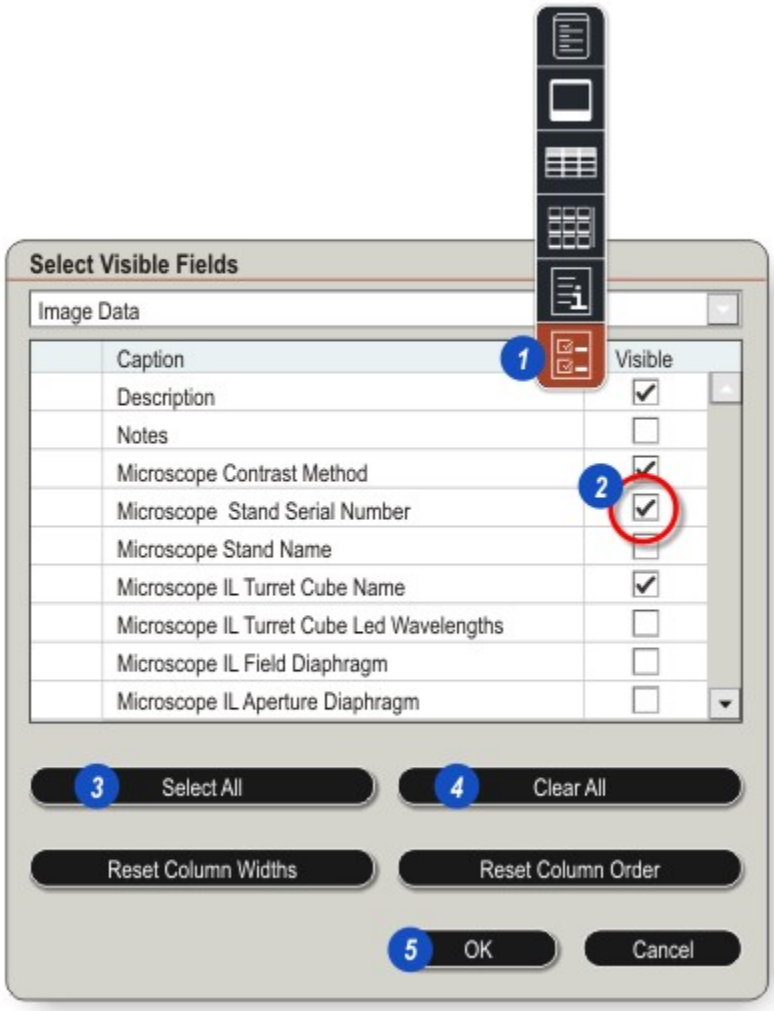
1: Click on the *Visible Fields* button on the *Side Tool Bar.*

2: To display a data field, click in the check box to the right of the field name. This is a toggle action - click again to clear the check box and hide the field.

3: Display all of the data fields on the *Data Form* by clicking the *Select All* button. Because there will now be too many items to display simultaneously, a *Scroll Bar* is automatically positioned to the right of the *Data Form.*

4: The *Clear All* button will hide all of the data fields and is useful before making a new selection range.

5: Click *OK* to close the *Select Visible Fields* dialog. The chosen data fields are immediately displayed on the *Data Form*.



1: The data fields displayed on the *Data Form* are also displayed on the *Grid* (if an LAS Archive is installed) - click the *Grid* button on the *Side Tool Bar* to reveal it.

The order of columns on the *Grid* can be changed by clicking on the column heading to be moved, holding down the mouse button and dragging the column to a new location.

In a similar manner, column widths can be changed by dragging the column *Dividing Bar* to the required width.

Reset the column widths by:

2: Click on the *Visible Fields* button on the *Side Tool Bar.*

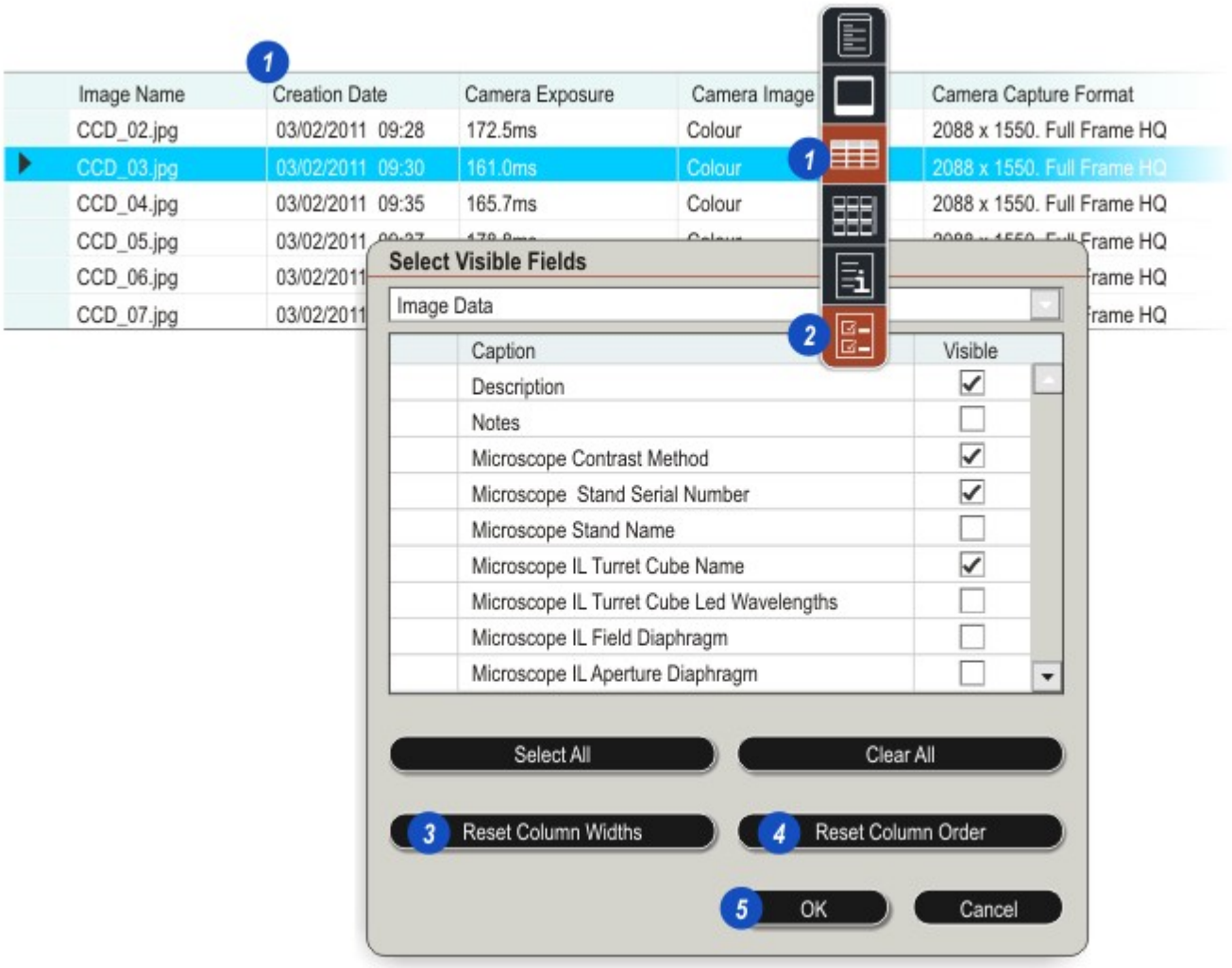
3: Click on the *Reset Column Widths* button.

Reset the column order to match the *Data Form* by:

2: Click on the *Visible Fields* button on the *Side Tool Bar.*

4: Click on the *Reset Column Order* button.

5: Click *OK*.



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To view all of the available data and if LAS Archive is installed, select them for use in a report:

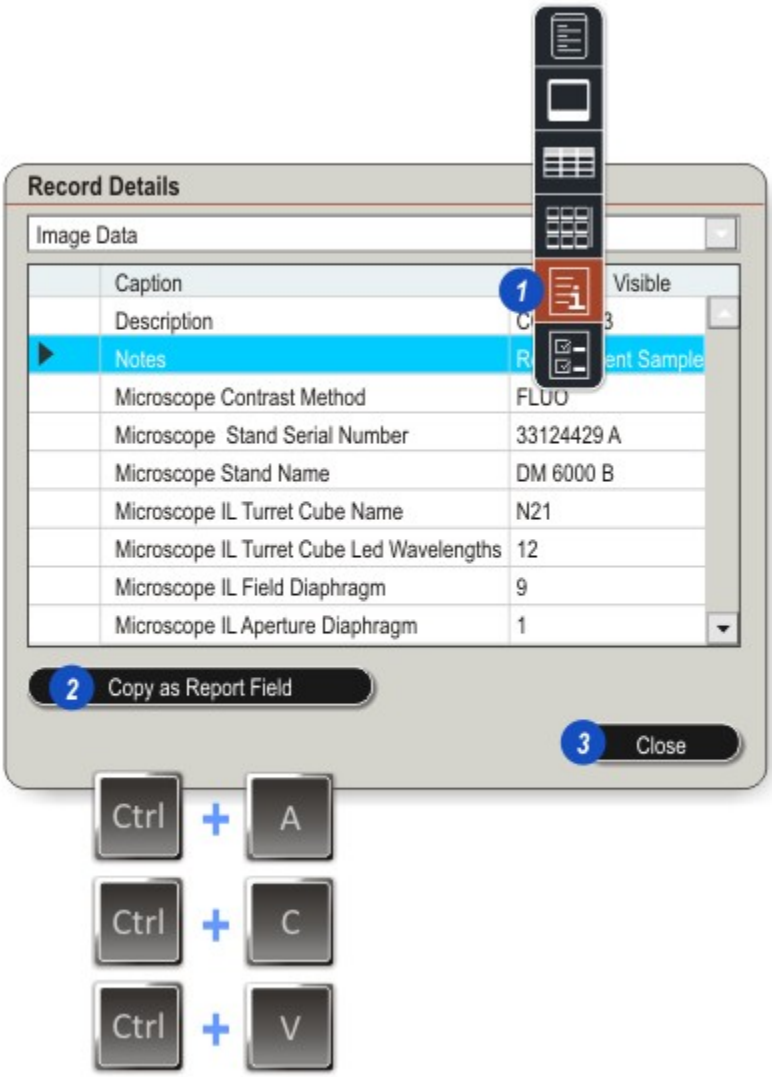
1: Click on the *View All Details* button on the *Side Tool Bar.*

The *Record Details* dialog displays all of the data fields available for the chosen image and the values that have been captured for them. Use the *Scroll Bar* on the right of the window to scroll through the list.

2: To use a field in a report, click on the required item to highlight it and then on the *Copy as Report Field* button. The field description is copied and pasted into the report template.

Use the keyboard shortcut keys to:
Select all fields: Ctrl + A
Copy all fields: Ctrl + C
Paste copied fields: Ctrl + V

3: Click the *Close* button to close the dialog.



The *Grid* displays data for all of the images in a folder in a tabular structure. The image names are listed on the left and the data items as headers across the top.

1: The *Grid* is revealed and hidden by clicking on the *Side Tool Bar* button. This is a toggle – click to reveal, click again to hide.

2: Clicking on an entry in the *Grid* will immediately display that image in the *Viewer* and also highlight the thumbnail.

3: Header positions can be changed by clicking and holding the left mouse button on the header to be moved, dragging it to the new position and releasing the mouse button.

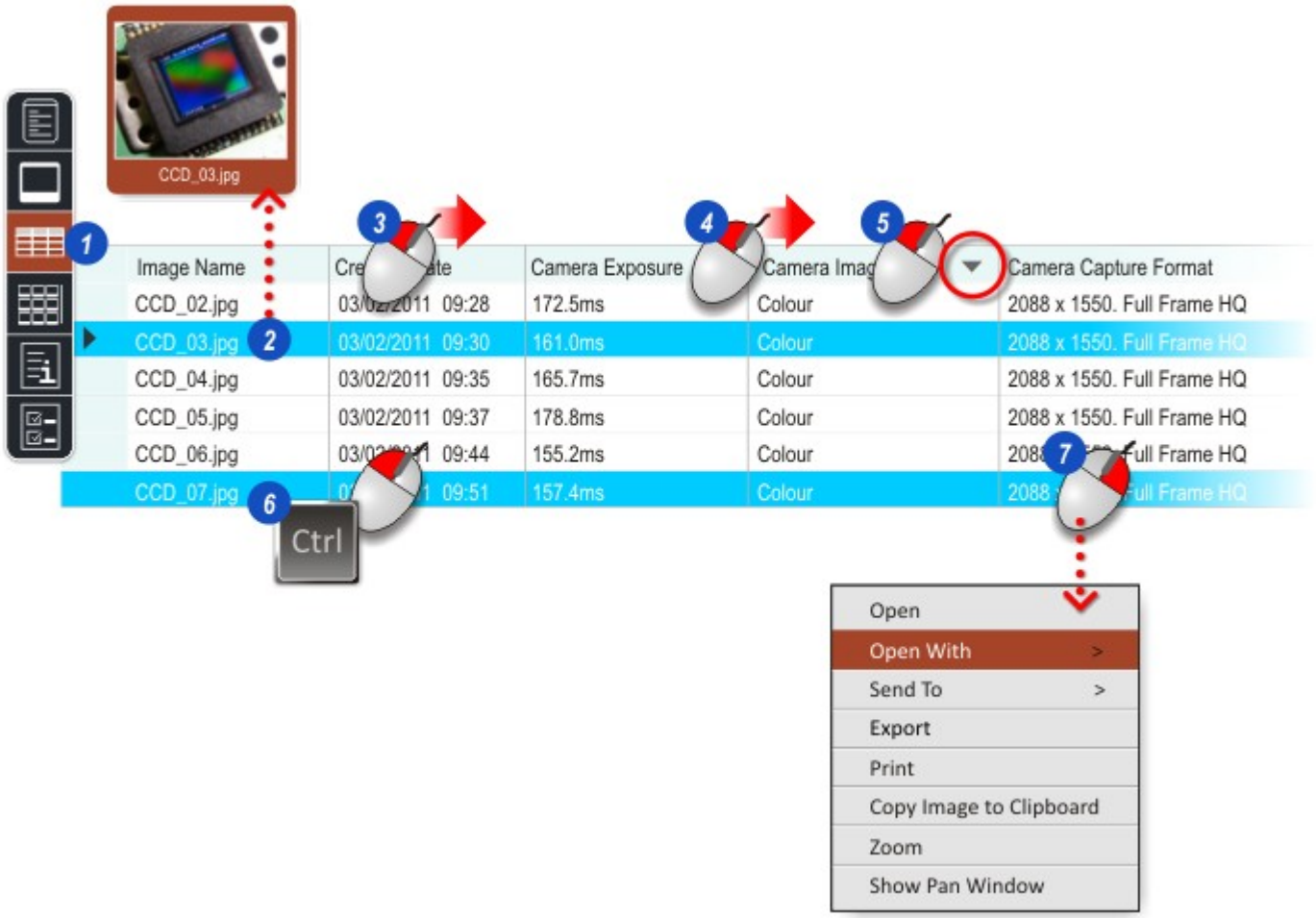
4: Column widths can be changed by clicking and dragging the vertical bars that separate the columns.

5: A small arrow is revealed when a header is clicked. This allows the image data to be sorted – high-to-low or low-to-high – by successive clicks on it.

6: To make multiple selections prior to deleting or exporting, hold down the keyboard *Ctrl* key whilst clicking individual thumbnails.

Keyboard combination *Ctrl* + *A* will select all of the image data.
Ctrl + *C* will copy all the selected image data to the clipboard.
Ctrl + *V* will paste into another application.

7: The *Grid* data can be exported to a range of other applications by right-clicking on the *Grid* then navigating to and clicking to select an application.



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Providing the *Store and Recall* module is registered and enabled, the *Store and Recall Settings* panel details the microscope data - camera and microscope setup - relevant to the displayed image.

1: Click on the expand/collapse arrow to the right of the header bar to display the settings.

2: Click the *Recall* button and...

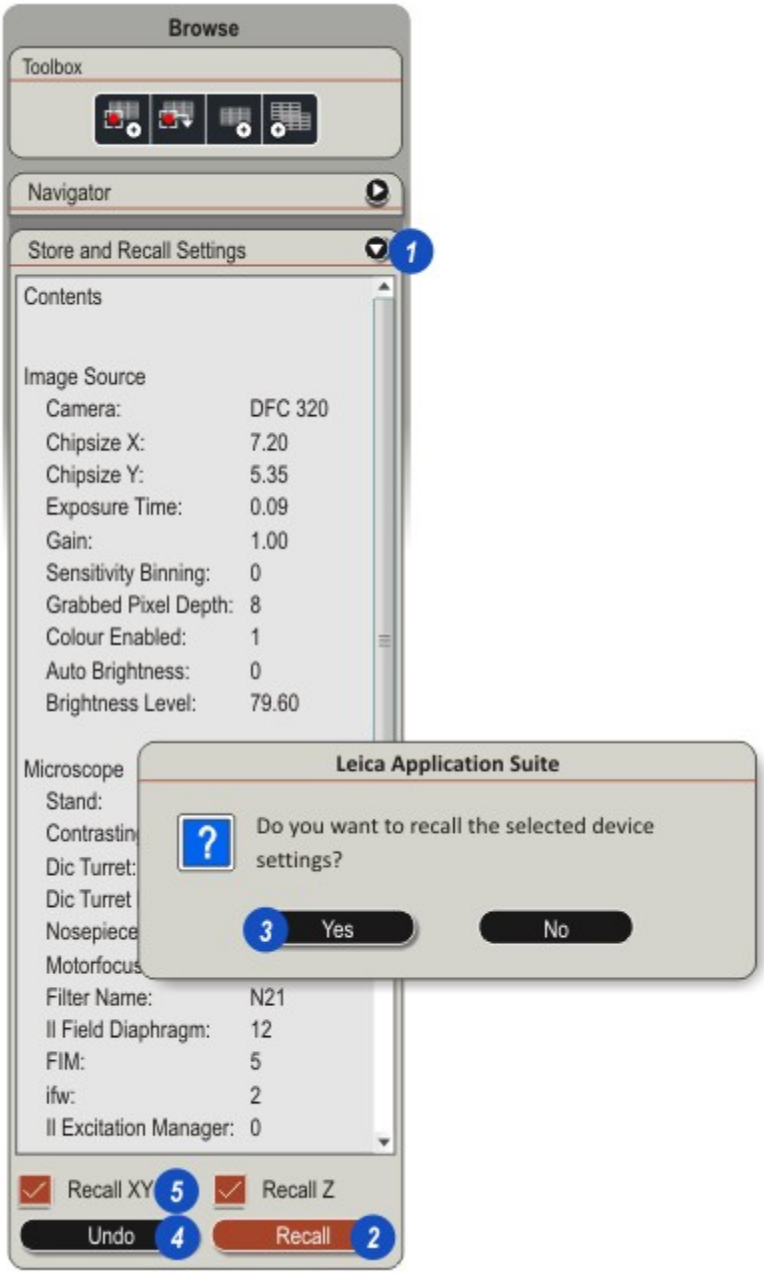
3: ...the *Recall Confirm* dialog appears. Click *Yes* to confirm.

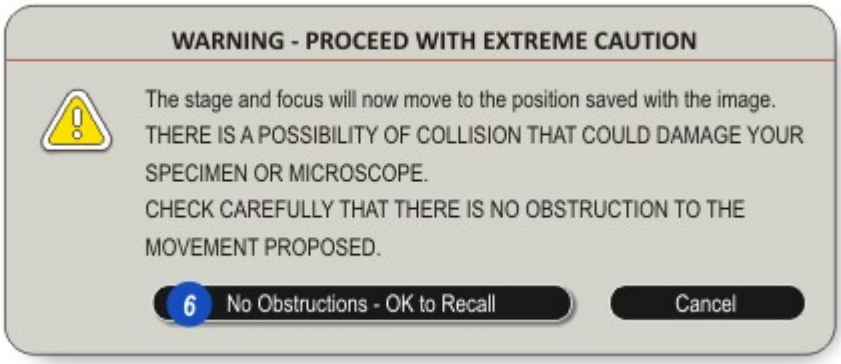
If the system has an automated microscope attached, it will automatically return the microscope and camera to the settings that were used to acquire the image.

4: To cancel the restore and return to the previous settings, click the *Undo* button.

5: If the image *XY* and/or *Z Stage* positions are required, click to enable (tick mark visible) the appropriate check box and...

6: ...when the Recall button is clicked the stage movement warning appears. Check that the stage and objective are clear of any possible obstructions and click the *No Obstructions - OK to Recall* button and the stage will move to the original capture positions.

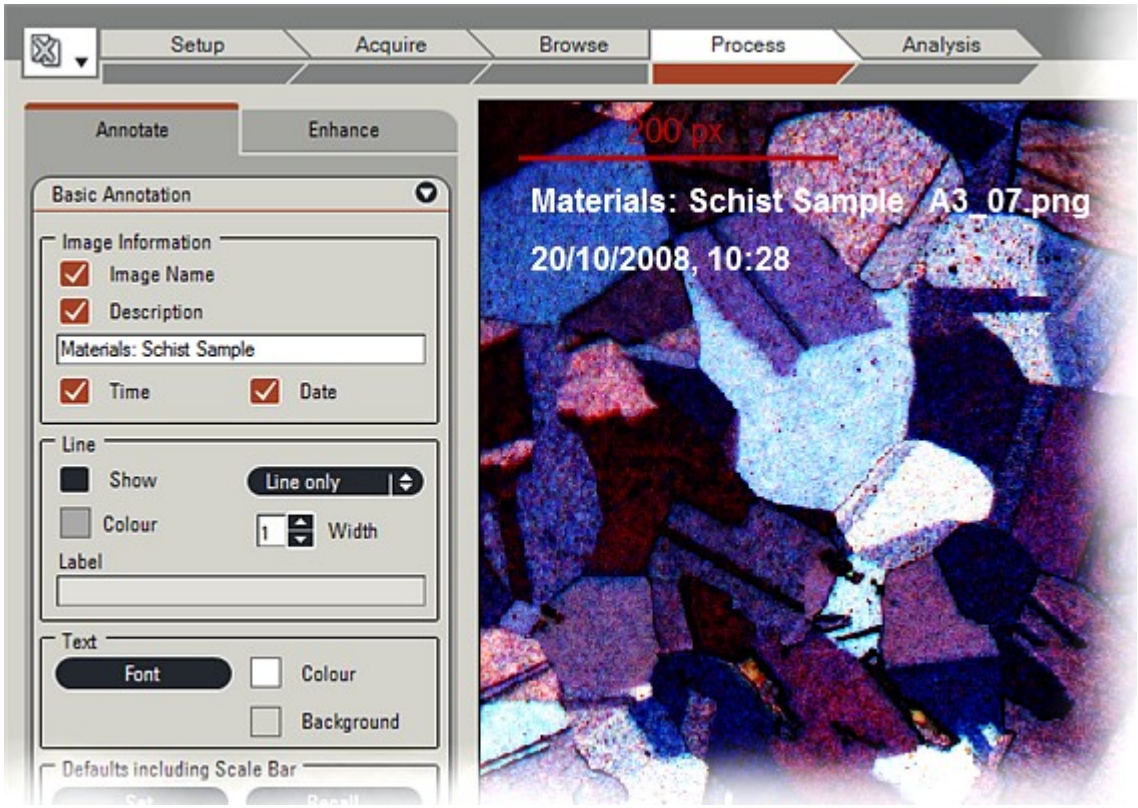




By using the *Process Workflow* with its *Basic Annotations* and *Enhance* features, images can be used to convey a wealth of added information and detail.

Annotations can either be saved with the image so that they can be edited at any time, or when the user is satisfied with the results merged with it so that the data is still visible when the image is exported.

- Basic Annotations to add simple pointers, labels and captions to your image.
- Enhance has the tools to brighten, adjust saturation, gamma and contrast as well as rotate and crop an image.



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Digital images can be annotated with graphics and text to provide information or to indicate features of interest.

The annotations are stored as a file with the image but separate from it and recalled whenever the image is displayed. They can be positioned or modified if required. However, they can also be merged with the image to produce a single, integrated image that can be exported to other applications with the annotations intact.

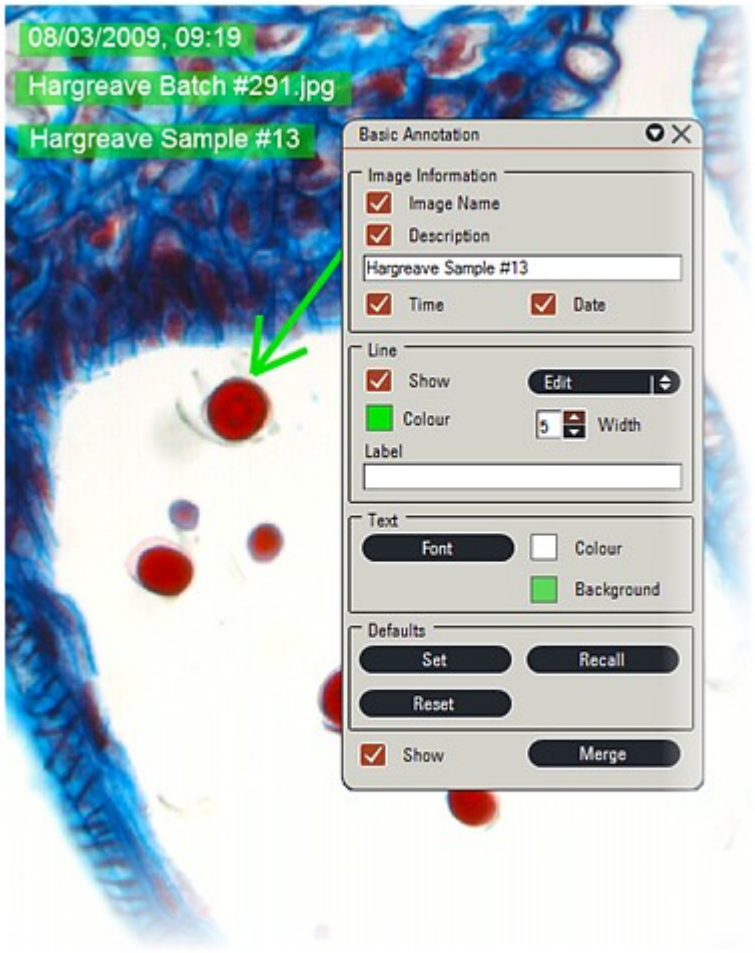
The essential tools allow automatic annotation with an Image Name, Description, Time and Date simply by enabling appropriate check boxes. A line can be drawn at any angle formatted as a Arrow with a label, a simple Line or to indicate a point-to-point Distance.

Annotations appear in colour overlaid on an image regardless of its colour depth - colour or monochrome.

Where images are larger than the window and it is possible to scroll the image, the annotations scroll to retain their positions. If the image is zoomed or reduced the annotations are scaled accordingly but limited to a readable size.

Basic Annotations normally reside on the Process Workflow but can be invoked from all of the Workflows (except Setup) using the Side Tool Bar icon.

The Annotation tools are designed to be quick and easy to use; For greater power and functionality the optional module Extended Annotation module can be added to the suite.



Basic Annotation features:

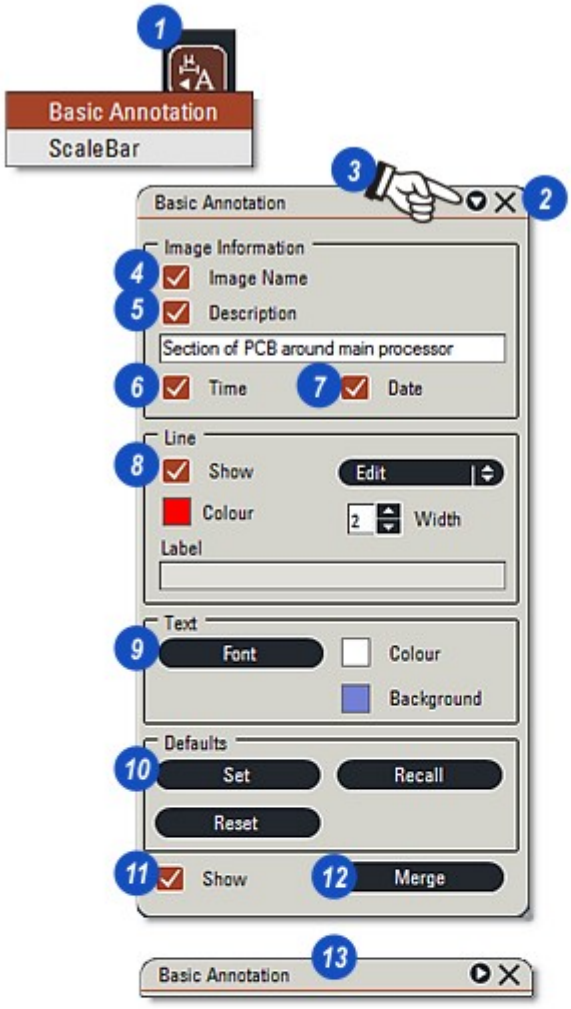
- The Control Panel: *Go there...* 348
- Display and Image Name and Description: *Go there...* 349
- Display the Time and Date: *Go there...* 350
- Draw a Line with a Caption Label: *Go there...* 351
- Selecting the Font: *Go there...* 352
- Changing the Font and Background colours: *Go there...* 354
- Defaults and Merging: *Go there...* 355

To launch the Basic Annotation Control Panel:

1: Click on the *Basic Annotation* icon on the *Tool Side Bar* and select the *Basic Annotation* option.

2: The Control Panel can be closed and re-docked in the *Process Workflow* by clicking the '*X*' to the right of the dialog caption.

3: Collapse the panel if it is obscuring the *Viewer* or other controls by clicking the small arrow to the right of the dialog caption **(13)**. Click and drag the Control Panel dialog caption to move it to another part of the screen.



The Basic Annotation features are:

4: Display Image Name: *Go there…*

5: Display a Description: *Go there…*

6: Show the Time and…

7: …the Date: *Go there…*

8: Draw an Annotation Line: *Go there…*

9: Set the Font and Colour: *Go there…*

10: Set and Reset the Defaults: *Go there…*

11: Show/Hide the Control Panel: *Go there…*

12: Merge: *Go there…*

Continued… 349

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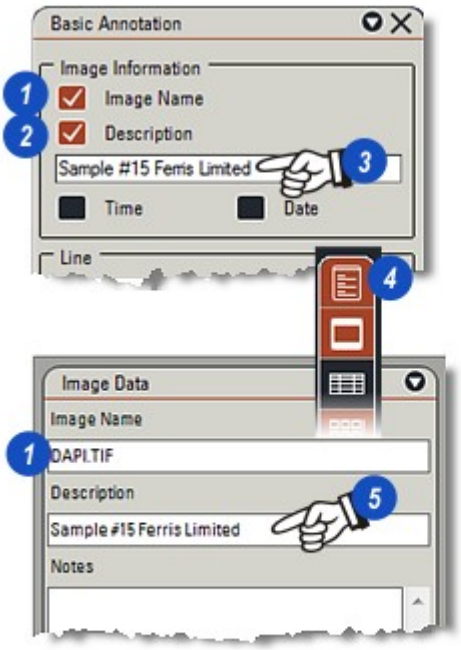
1: Click to enable the *Image Name* check box and the name by which the image was saved appears bottom left of the image **(6).** Click and drag it to another position if required. Whilst working on a live image the *Image Name* is not yet available but will be displayed after the image is captured.

2: To display a description on the image, click to enable the *Description* check box. The text is displayed bottom right of the image **(7)** but can be moved to another location by clicking and dragging.

3: Description text can be typed in the text box beneath the *Description* check box or, for a captured image…

4: …clicking the *Form* icon to display the Image *Data Form* and…

5: …clicking in the *Description* text box and typing a description.



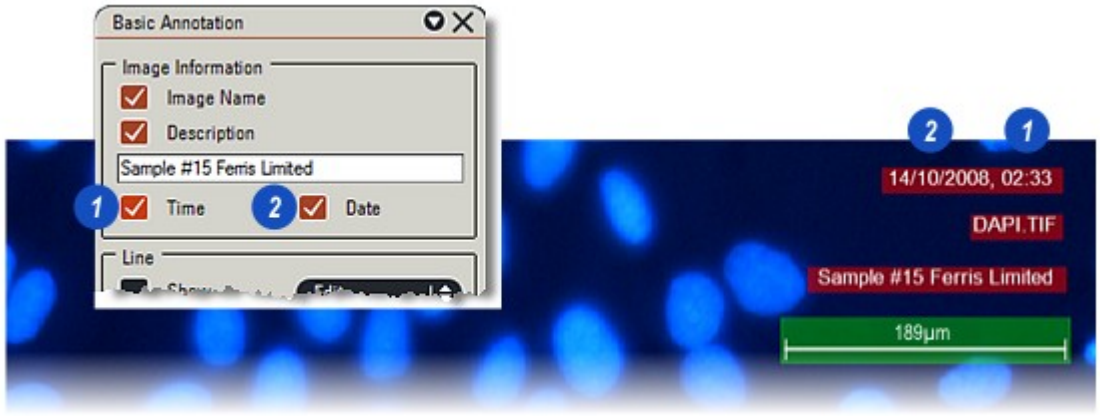
Continued… 350

1: Click the *Time* check box to display the current time in the top right corner of the image. It can be clicked and dragged to another location. The time format (12 or 24 hour) is determined by the computer settings.

2: To display the date in the regional format set in the computer, click to enable the *Date* check box. If both *Time* and *Date* are enabled they are displayed as a single line.

The illustration shows *Date* and *Time* together with the *Image Name* and *Description* dragged to a new location.

Continued… 351



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1: Click the *Show* check box to enable line drawing.

2: Click on the Up/Down arrows to the right of the *Size* text box to Increase/ Decrease the width of the line. The size of distance line ends and arrow heads scales with the line thickness.

3: Click on the *Colour* button and…

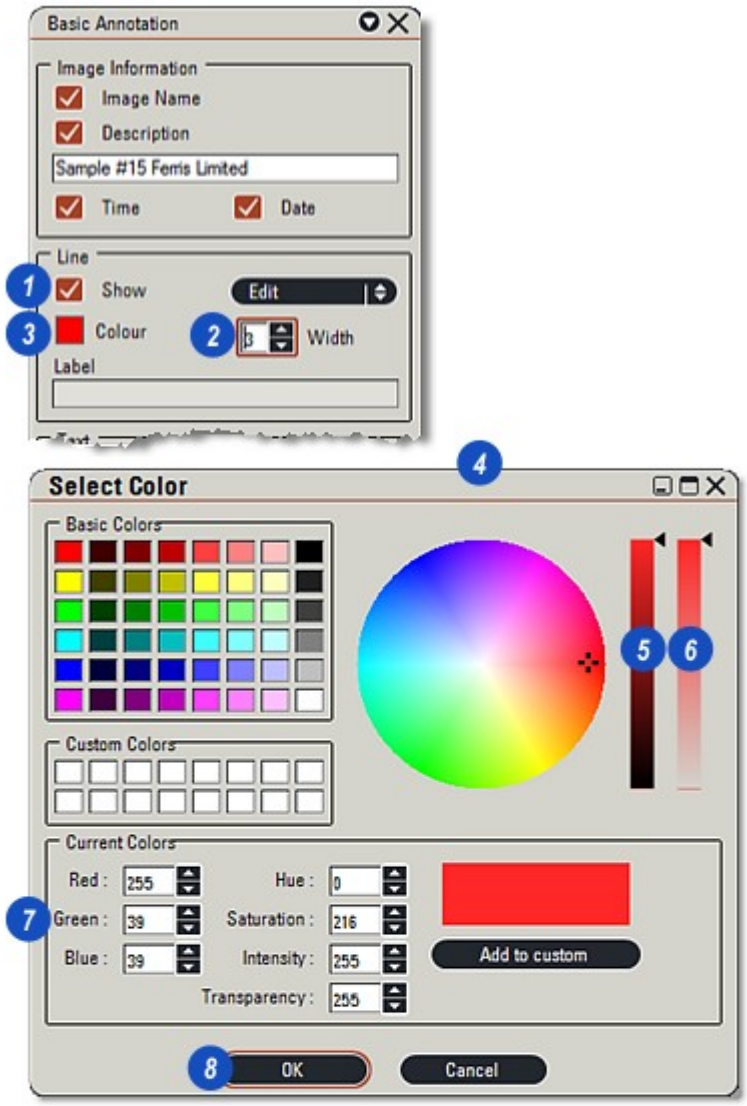
4: …on the *Select Colour* dialog choose a new colour from the swatches or from the colour wheel. Alternatively, click in the *Current Colours* text boxes **(7)** and type the Red, Green and Blue values.

5: Adjust the colour by clicking and dragging the *Colour Shade* slider.

6: Adjust the colour transparency if required by clicking and dragging the slider on the *Transparency* bar.

8: Click *OK*. The new colour is shown on the *Colour* button.

Continued… 352



1: Choose the *Line Style* by clicking on the small arrows to the right of the *Style* drop down menu and…

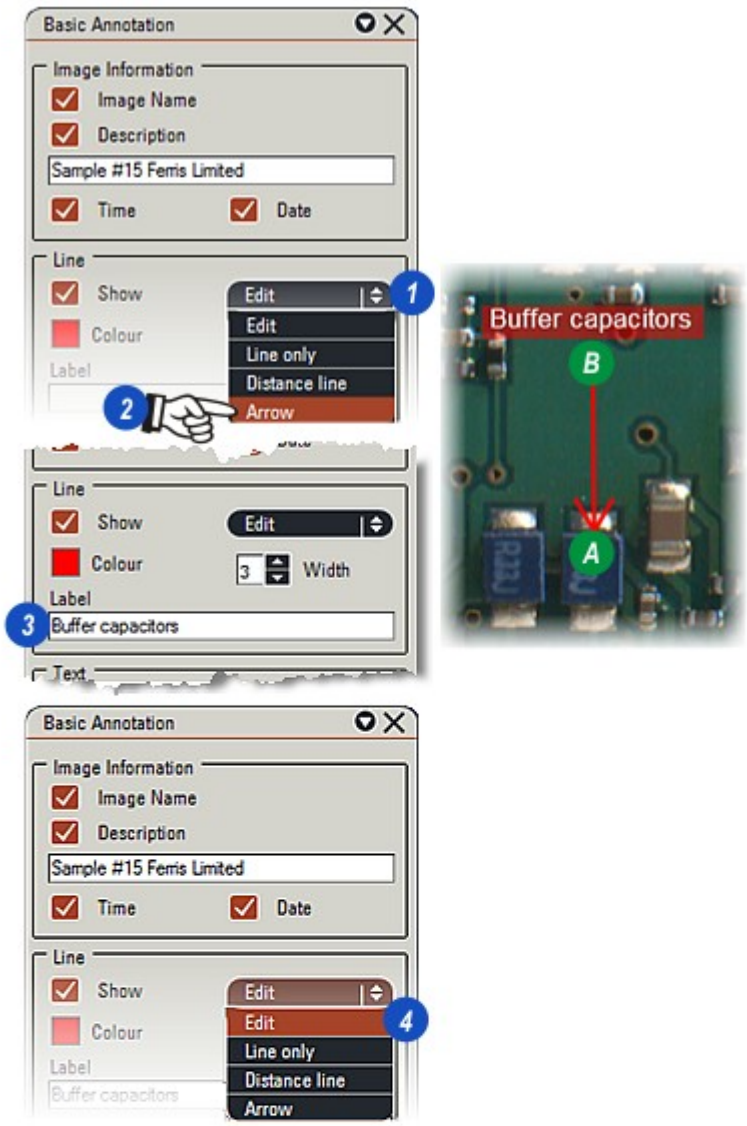
2: …click to select the required style. The *Line Only* option draws a plain line; *Distance Line* draws a line with bars at each end; *Arrow* draws a line with an arrow head.

A: To draw the line, click and hold down the left mouse button at the start point, drag to the end point **(B)** and release the mouse button. Whilst drawing, the line can be dragged in any direction and at any angle.

3: To add a *Caption Label*, click in the *Label* text box and type the caption. It can be clicked and dragged to re-position it on the image.

4: Line Width, Colour, Style and the *Label* can be changed at any time (before merging) by selecting the *Edit* option from the *Style* menu, clicking on the item to be changed and altering the settings as detailed above.

Continued 353...



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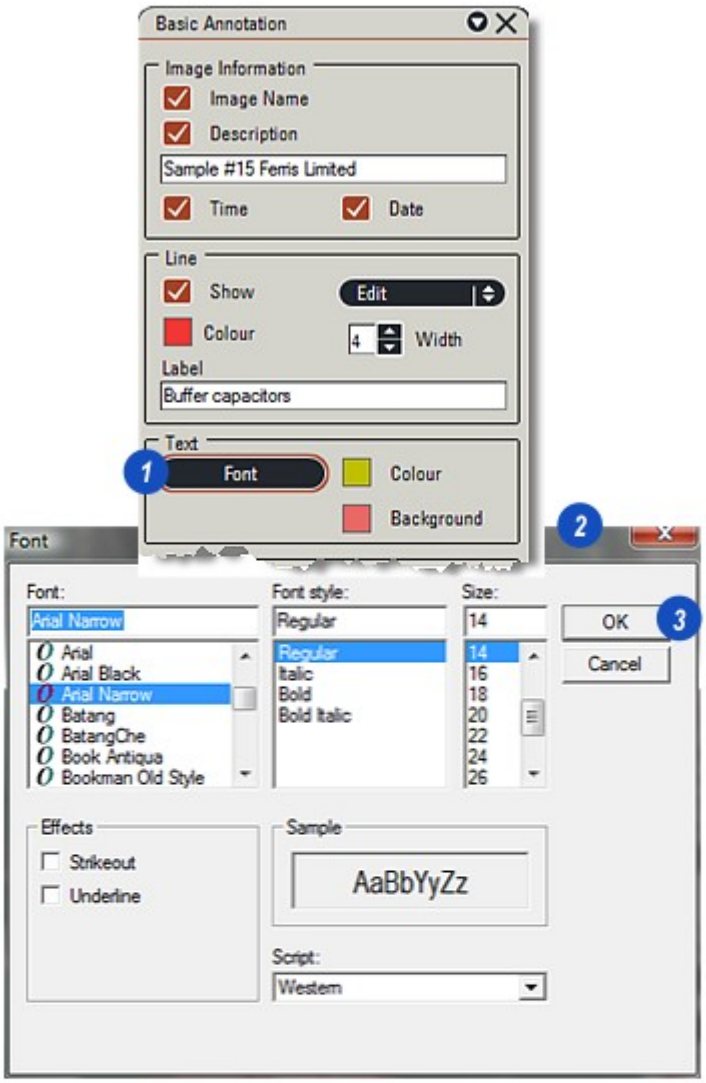
To change the Font settings for all of the Basic Annotation labels:

1: Click on the *Font* button.

2: On the *Font* dialog, select the *Font Type Face*, *Style* and *Size* as required and…

3: …click *OK*.

Continued… 354



The *Font* colour and the *Label Background* colours are both selected in the same way:

1: Click on the (Font) *Colour* button or …

2: …the *Background* button.

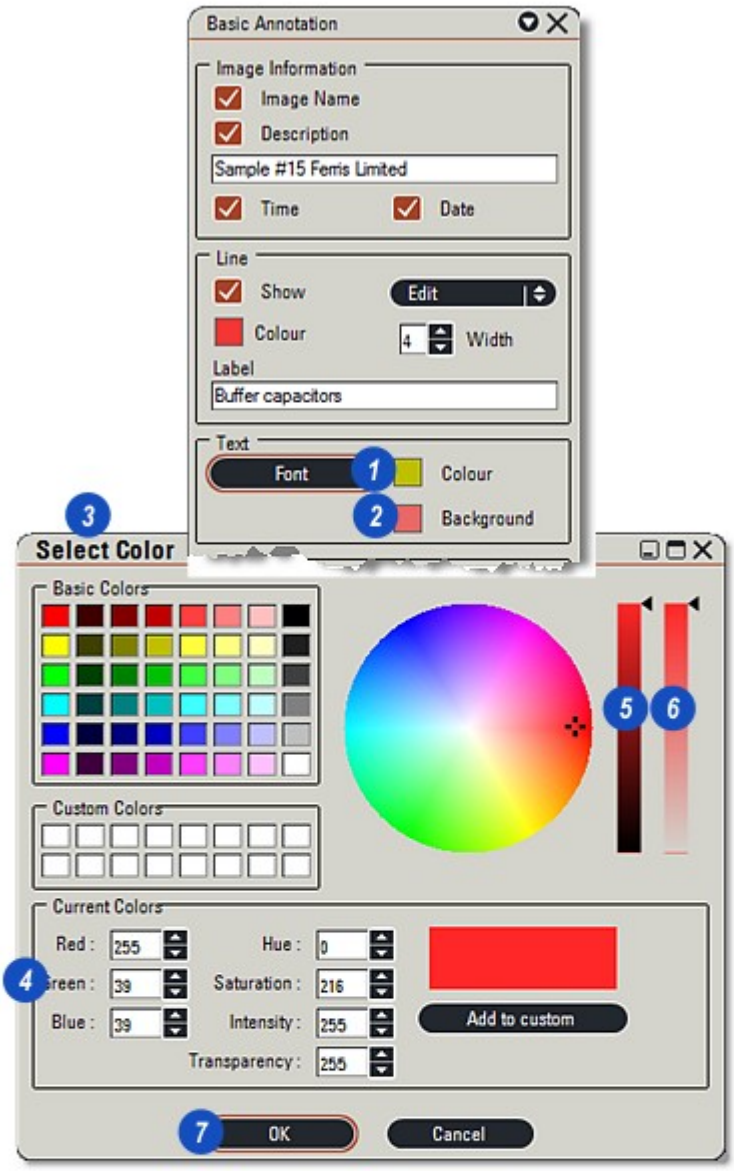
3: On the *Select Colour* dialog choose a new colour from the swatches or from the colour wheel. Alternatively, click in the *Current Colours* text boxes **(4)** and type the Red, Green and Blue values.

5: Adjust the colour value by clicking and dragging the *Colour Shade* slider.

6: If required, adjust the colour transparency by clicking and dragging the slider on the *Transparency* bar.

7: Click *OK*. The new colour is shown on the appropriate button.

Continued… 355



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1: Clicking the *Set* button will save all of the current Basic Annotation settings for future image captures. However, any of the settings can be adjusted on individual images before merging.

2: Click the *Reset* button to return the settings to the original installation defaults.

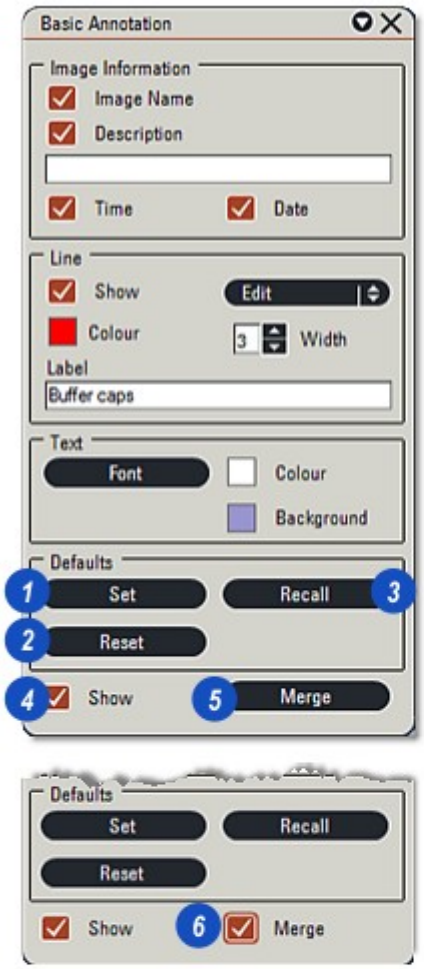
3: *Recall*.

4: The *Show* check box when enabled displays the *Basic Annotation* control panel and activates the tools.

Merging:
When *Merging* is enabled all of the Labels, Captions and Lines drawn on the image will be permanently included as part of the image and cannot be altered.

5: Merge on a captured image is a 'one shot' button and only merges annotations with the current image.

6: Merge on live images is a check box setting and whilst it remains enabled will merge annotations with all captured images.



Enhance provides a range of easy to use yet powerful controls for image enhancement.

The controls work post-capture to change Brightness, Contrast, Gamma, Hue, Saturation and Intensity. The image can be Flipped, Rotated or Cropped and saved as a new image or to replace the original.

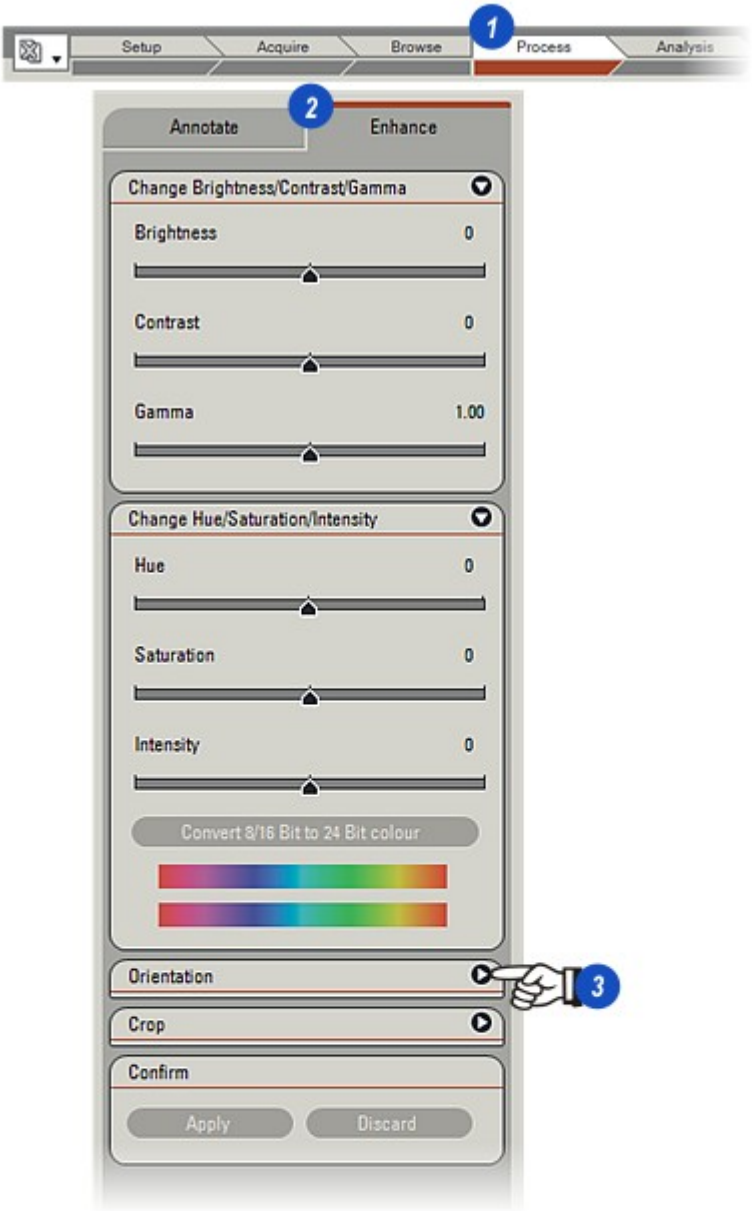
To reach the Enhance control Panels:

1: Click on the *Process Workflow.*

2: Click on the *Enhance* tab.

3: Some *Control Panels* are minimised - expand them by clicking on the small arrow to the right of the panel.

Continued 357...



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Cropping is the process of removing unwanted parts of the image leaving only the area of interest.

1: This step is not mandatory but strongly recommended. Right click on the image. The *Viewer* menu appears.

2: Select *Send To* and then select a drive to save a copy of the image - just in case the cropping or colour adjustment go wrong, this is a pristine backup.

3: Click the *Fit To Screen* icon on the *Side Bar* to display the entire image.

4: Click on the *Enhance* tab.

5: Click on the arrow to the right of the *Crop* header to reveal the panel.

6: Click *Start* on the *Crop* panel.

7: Click-and-hold on a point on the image where the top left- hand corner of the crop mask will be. Drag to the right and down. The mask appears as an outlined rectangle. When the area of interest is enclosed by the mask, release the mouse button.
The position and size of the mask (in pixels) is displayed in the *Crop* panel as the mask is being drawn.

8: Either click the *Finish* button if the mask is satisfactory, or click *Cancel* to clear the mask and start again.

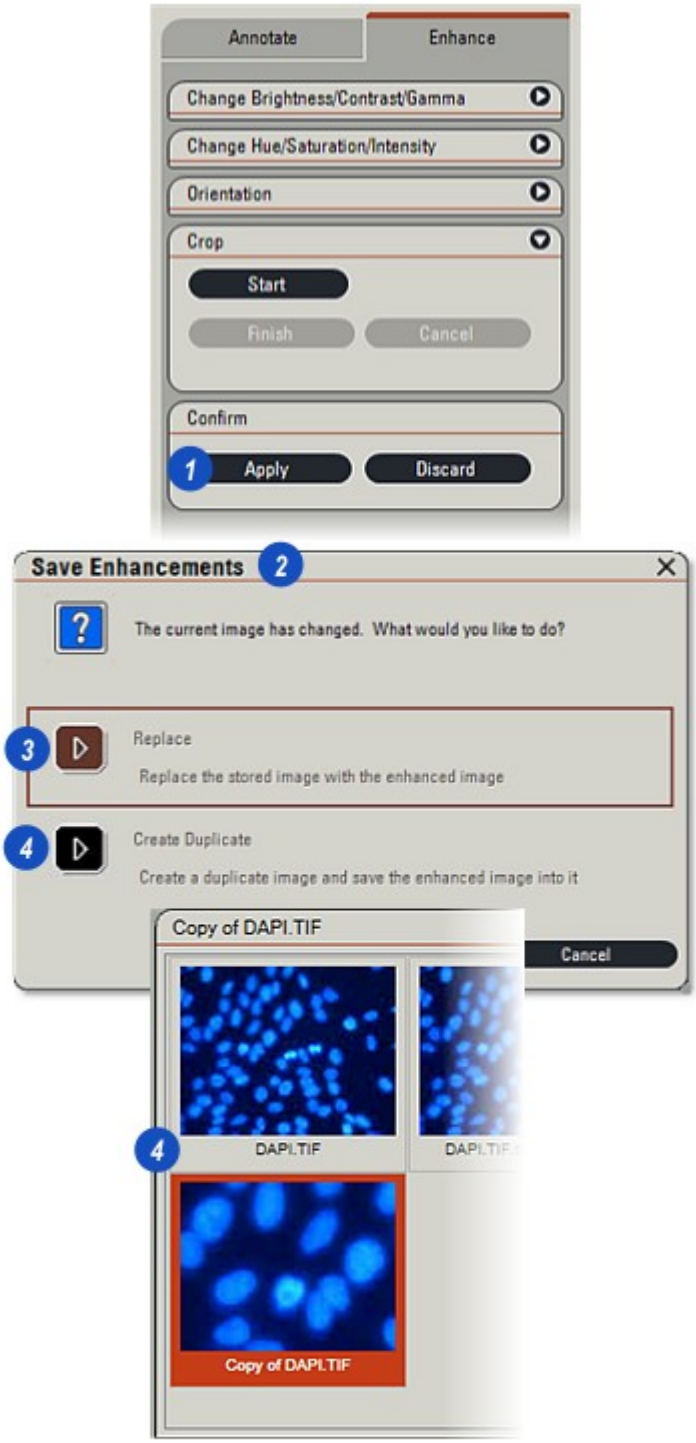
Continued... 358

1: To keep the masked area, click *Apply* on the *Confirm* panel or click *Discard* to start again.

2: If *Apply* is clicked the *Save Changes* prompt appears:

3: Click *Replace* to replace the original image with the cropped area.

4: Click *Create Duplicate* to keep the original image intact with the same image name and also create an new image of just the cropped area with the same image name prefixed by *Copy of...*



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The image may be flipped - top to bottom or side to side and rotated about its central axis.

1: Click on the arrow to the right of the Orientation bar to reveal it.

To flip the image top to bottom:

2: Click on the *Flip Vertical* button.

To flip the image side to side:

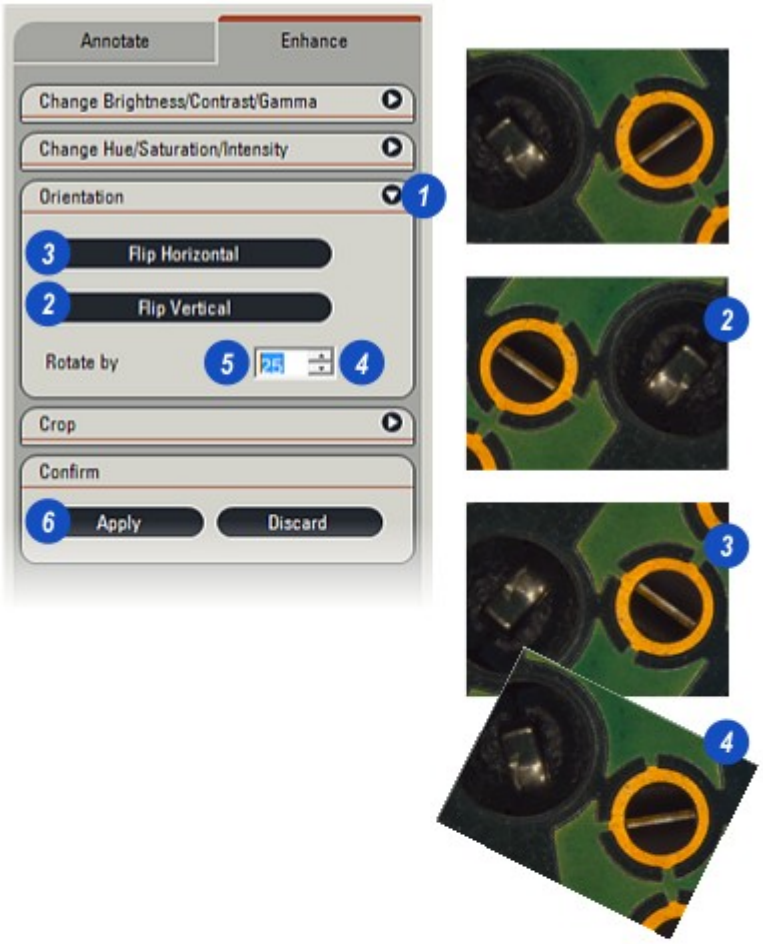
3: Click on the Flip Horizontal button.

To rotate image about its central axis:

4: Click on the *Rotate Up* arrow to rotate clockwise by one degree increments or on the *Rotate Down* arrow to rotate anti-clockwise by one degree increments, or...

5: Double-click the *Rotate* by window to highlight the existing value. Type the number of degrees to rotate and press the *Enter* key on the keyboard. The image will rotate to the required position clockwise.
To rotate anti-clockwise, precede the number with the negative (-) sign.

6: On the *Confirm* panel, click *Apply* to keep the new orientation or *Discard* to start again.



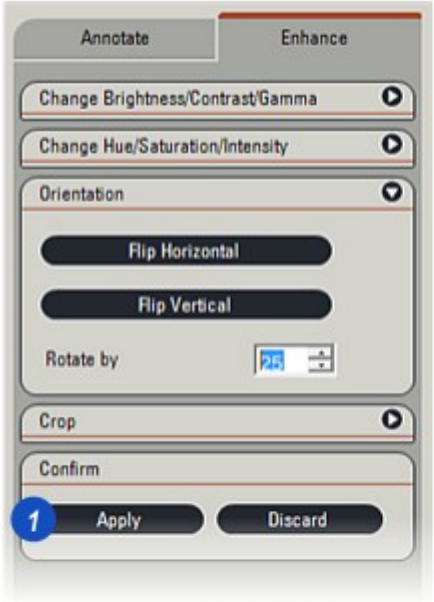
Continued... 360

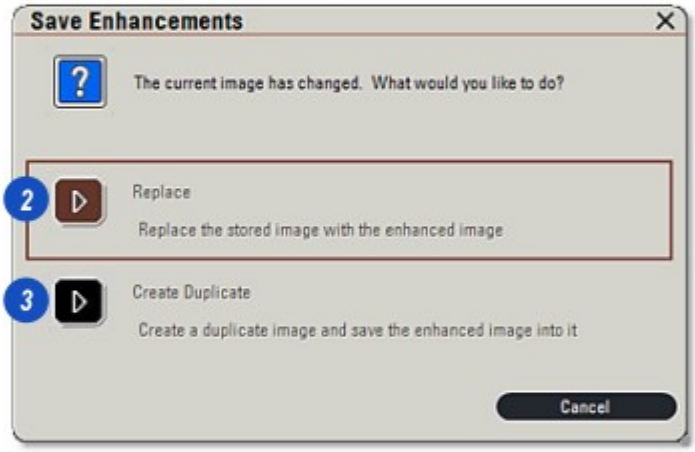
1: On the *Confirm* panel, click *Apply* to keep the new orientation or *Discard* to start again.

2: If *Apply* is clicked the *Save Enhances* prompt appear. Click *Replace* to save the cropped image overwriting the original.

3: *Create Duplicate* to save the cropped image with the original name prefixed with *Copy of...* The original image remains intact. Click *Cancel* to restore the image and start again.

Before proceeding to colour adjustment, it is good practice to make a copy of the re-orientated image as a backup.





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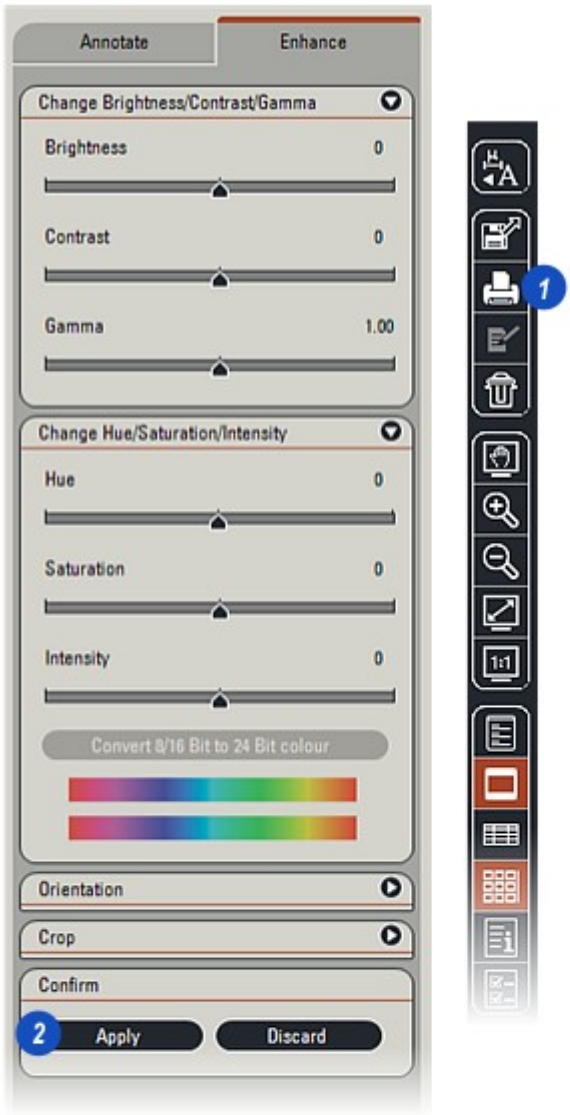
Two panels provide a range of powerful tools to adjust and modify the image colours - *Brightness: Contrast: Gamma (BCG)* and *Hue: Saturation: Intensity (HSI).* To reveal the panels click on the arrow to the right of the bar.

While the panels use complex mathematics to manipulate the image, ultimately colour is simply a matter of perception - what our eyes and our brains 'see'- and if what we see suits our purpose, then the colour is 'correct'.

Before adjusting the image colour, consider how it is going to be presented. For archiving or electronic transmission, perhaps very little adjustment will be necessary; for projection - in a Powerpoint presentation for example - *Saturation* may need to be increased to maintain colour vibrancy on the screen; for paper printing, *Gamma* and *Intensity* may need increasing to keep colours 'pure' and clean.

1: Print it to check how it looks and...

2: ...if the image is satisfactory, only then *Apply* and keep it.

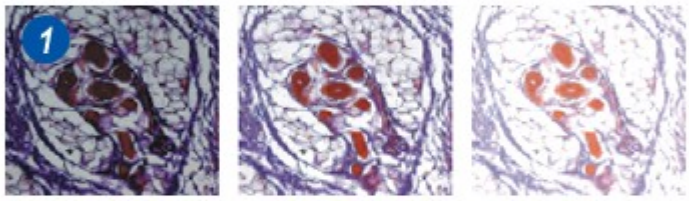


The image in the *Viewer* comprises tiny, individual ‘dots’ called pixels. Each pixel is a mixture of three primary colours - red, green and blue (RGB) and each colour is represented by a value. For 8 bit colour, the values are in the range 0 to 255.

All of the colour controls manipulate the values of the three colours to produce a certain effect.

If all three colour values are set to ‘0’(0:0:0) then black is the result. If they are all set to ‘255’(255:255:255) white is the result.

On both the *Brightness/ Contrast/ Gamma* and *Hue/ Saturation/ Intensity* colour panels, click and hold the slider and move it to the left to decrease or right to increase values. The displayed numbers are not a reflection of the colour byte values but rather a scale associated with the parameter being changed.



1: *Brightness:* increases or decreases the value of all three colours simultaneously. The illustrations show (left to right) the result to the image of a swing of -300 to +300 with the middle image representing the original captured value - ‘0’on the brightness scale. A maximum negative value will produce a black image and a maximum positive value a white image. *See: Process: Enhance, Contrast and Gamma.* *Go there...* 363

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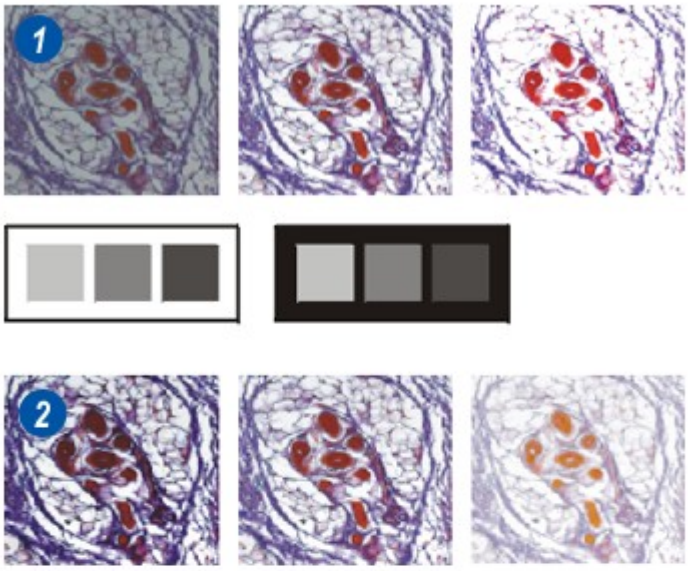
1: *Contrast* increases or decreases the colour values individually both with respect to each other and also white levels. It is a proportional adjustment. The three illustrations represent a swing of 1000 in both directions with the original image in the centre.
The perception of contrast depends upon the ambient light levels. The three small squares opposite are identical in both illustrations and yet those surrounded by black are perceived as having a lower contrast - they look closer to each other in terms of colour - than those surrounded by white. If your image is to be projected in dim lighting conditions, consider increasing the contrast to compensate.



2: *Gamma* is a value applied to colour levels to compensate for different ways in which the image is viewed. Liquid crystal displays (LCDs) have a specific Gamma setting, cathode ray tube (CRT) monitors will have another and printers yet another. Changes in Gamma are applied automatically so, when an image is printed for example, the printer software will make adjustments before the printing takes place.
Very small changes in Gamma can have dramatic effects; the examples show a range of 0.35 to 1.50 with the original in the centre. Generally, avoid altering the Gamma settings unless really necessary.

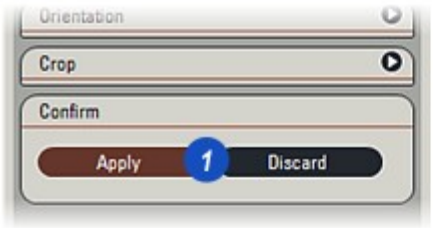
Continued... 364

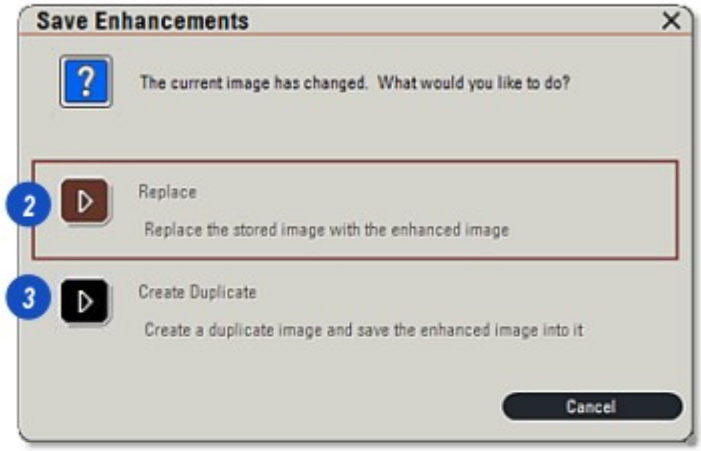
1: All three controls may be applied to the image simultaneously.
To reset the changed values and start with the original image, click *Discard* on the *Confirm* panel. To keep the changes, click *Apply*.

2: If *Apply* is clicked the *Save Enhances* prompt appear. Click *Replace* to save the cropped image overwriting the original.

3: *Create Duplicate* to save the cropped image with the original name prefixed with Copy of... The original image remains intact. Click *Cancel* to restore the image and start again.

See: Brightness. 362
See: Hue and Saturation 365.





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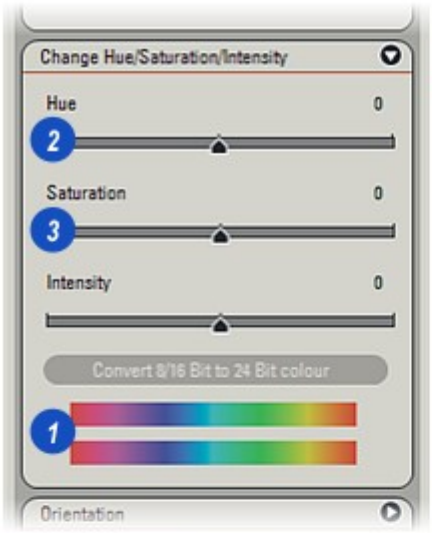
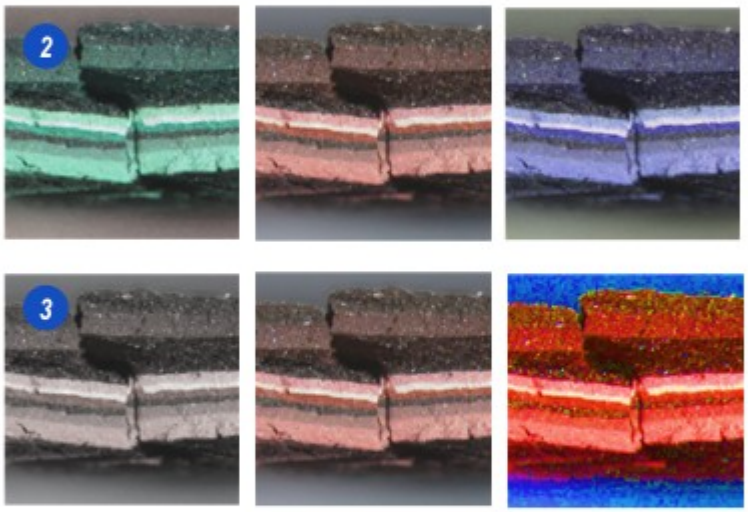
Hue, Saturation and *Intensity* control the actual colours, the amount of colour and the vibrancy. As each is adjusted, the lower of two *Spectrum Bars* **(1)** changes as a comparison to the static spectrum above it.

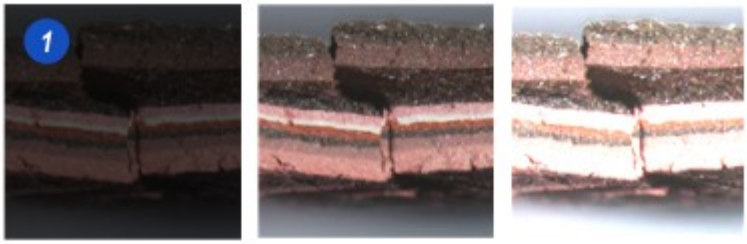


2: *Hue* is another word for colour. As the slider is moved, the colours shift from the dominant red in the middle illustration which is the original, toward green on the left or blue on the right. The *Spectrum Bar* shifts to reflect the change in dominance. Use *Hue* to correct any perceived colour imbalance, especially on printed images.

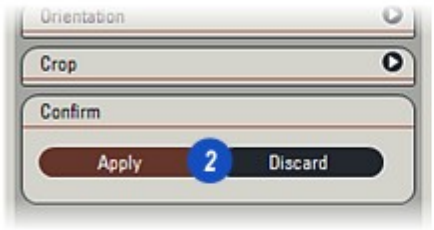
3: *Saturation* determines the amount of each colour that is present. At the highest setting, each colour will be at its most vibrant. The right hand illustration is the high setting and the colours cannot be more prominent without combining to make white. Use *Saturation* to make powerful (if a little bit 'unnatural') images. Reducing *Saturation* is a convenient way of turning a colour image into a monochrome image - essentially just shades of grey - without losing detail or becoming a black solid.



1: *Intensity* is close to *Brightness* in the way it affects the image. It is a measure of the 'strength' of each colour swinging from solid black to solid white. Use small increases in Intensity to help differentiate between colours; too much and detail begins to disappear.

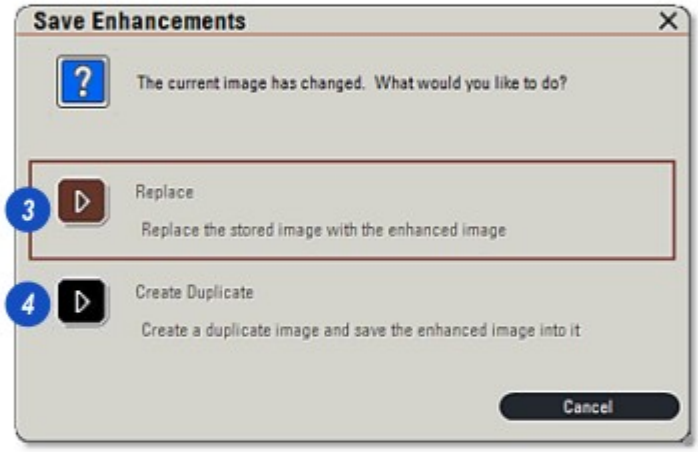


All three controls – *Hue, Saturation* and *Intensity* - may be used together to achieve a desired effect and they may be combined with *Brightness, Contrast* and *Gamma* to 'fine tune' an image.



2: *Discard*, return to the original, or *Apply* changes on the Confirm panel.

3: If *Apply* is clicked the *Save Enhances* prompt appear. Click *Replace* to save the cropped image overwriting the original.



4: *Create Duplicate* to save the cropped image with the original name prefixed with Copy of... The original image remains intact. Click *Cancel* to restore the image and start again.

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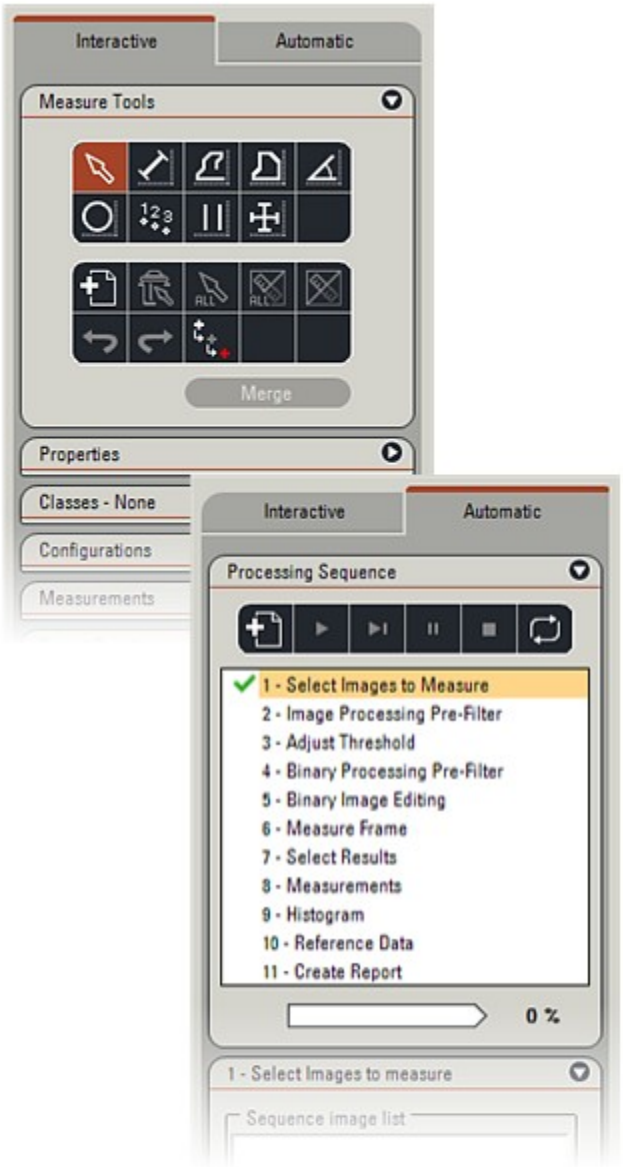
The *Analysis Workflow* appears when the optional 'Analysis' modules are installed.
In this version of the Leica Application Suite, the *Interactive Measurements* or *Automatic Measurements* modules may appear here:

Interactive Measurements: Go there... 709

Automatic Measurements: Go there... 781



Optional Modules:

The underlying capabilities of the *Core* functions can be enhanced with a range of advanced modules and applications. Each LAS module provides the flexibility to tailor a system solution to fulfill individual needs with upgrade options available for future requirements.

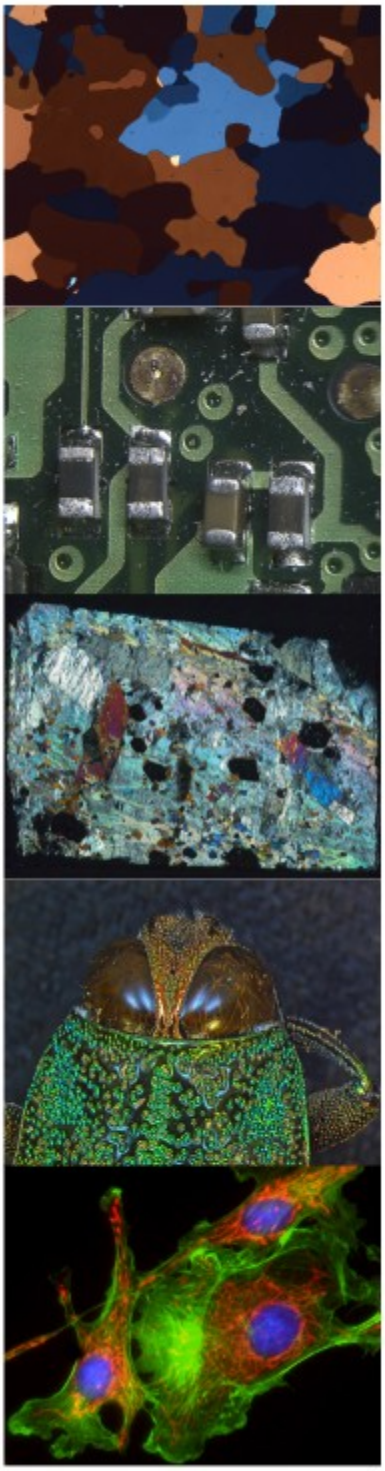
Installing the *Demo Licence* and *Enabling Optional Modules*: *Go there...* 369

Optional Modules include:

- LAS Archive Basic.
- LAS Archive Standard
- Extended depth of focus
- Movie recording
- Image measurements
- Macro Programming
- Image Overlay
- Power Mosaic
- Image Analysis
- Web sharing of images
- Grain Expert
- Phase Expert
- LAS Macro

...and many others, described in this manual and *Help*.

Use of the optional *LAS Macro* capability is described the *LAS Macro Editor* help file. *Go there...* 368



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Optional Modules, once installed have to be licensed. Users who intend to apply online for a licence or those who already have a licence file or dongle should consult the *Installation Guide* for the next steps.

This section is for users who want to install the 60-day *Demo* evaluation licence.

The Demo Licence dialog is reached through the Framework - the part of LAS that loads first and acts as a 'container' for all of the other Core features and modules.

The Framework cannot be reached through Leica Application Suite - if LAS is running it must be closed down using the normal exit procedure.

The way in which Framework is launched depends upon the user rights:

A: Administrators with User Rights OFF:

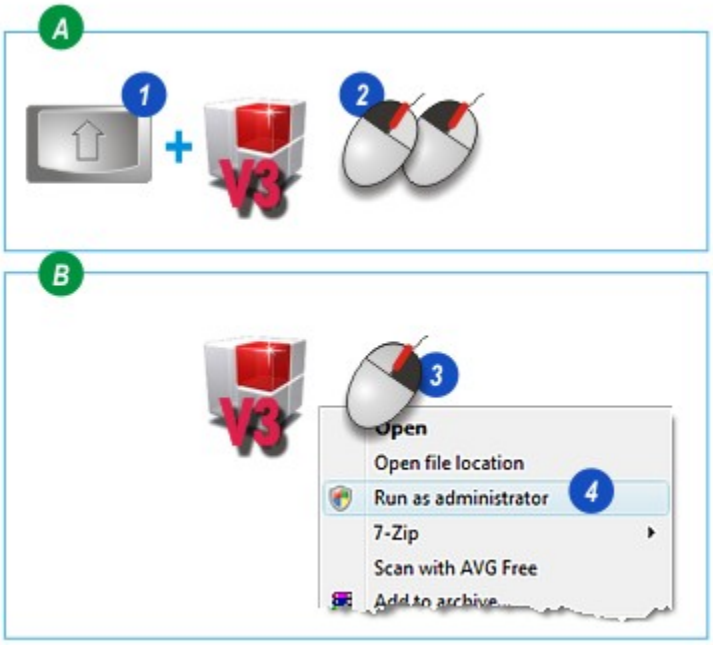
1: Press and hold down the keyboard *Shift* key.

2: Double-left click on the LAS desktop icon. The Application Suite Framework loads.

B: Administrators with User Rights ON:

3: Right-click on the LAS desktop icon.

4: From the drop-down menu left-click on *Run as Administrator*. The Application Suite Framework loads.



To reach the Registration dialogs:
With the Framework loaded:

5: Click on *Options* and from the drop-down menu...

6: ...click to select *Registration*.The *Registration* dialog appears.

Continued... 370

1: Click on the *Demo* tab on the *Licence Registration* dialog.

2: Click on the *Install* button to install the *Demo* licence.

3: When the licence is installed the *Start* button becomes active: Click it to display the *Status of Modules* dialog.

4: To enable a module, click on the associated *Demo* button. Once a module is enabled its evaluation 'clock' will start running and cannot be turned off even if the module is later disabled.

5: Click the *Start All* button to simultaneously enable all of the modules.

6 & 7: The *Enable All* and *Disable All* work as a 'switch' pair to simultaneously enable and disable all of the modules.

8: Click *Close*.

9: The status of all the modules can be displayed by clicking the *Show Licence State* button and selecting the required option from the drop-down menu.

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LAS operates in 2 distinct modes depending how the user wishes to manage the acquired images. These are:

LAS Image Explorer and
LAS Folder Archive.

LAS Image Explorer is used when images are stored to the hard drive in an informal manner. The user can save the images anywhere on the hard drive in any folder and organizes the images as convenient. While this method has the benefit of simplicity, there is a chance that over time the images will become harder to find.

LAS Folder Archives are used where a more disciplined approach to the organization of data is demanded. This might occur where it is mandatory to save additional user defined fields with the images or where several users need to add data to images in a systematic manner. This also means that images are saved in locations where other users will be able to find them. In this case it is a distinct advantage to restrict the locations that the images are saved. We call these '*Folder Archives*' because the images are still saved to the Windows file system but only in predefined locations.

Implementation Overview
Images are combined with text and numeric data, microscope information and camera parameters in individual records of a database that you can tailor to the specific needs of the application.

The content of a record is defined by use of the Archive Design tool on the Setup Workflow. The Archive Designer allows you define hierarchical levels by which data is grouped (*e.g. Lab Name >Procedure >Customer Name > Experiment >Specimen Number >Result*). There is virtually no limit to the number of different fields or the volume of information stored. The original high-resolution images are held external to the database. New fields can be added to an existing database or redundant fields removed without difficulty. System information, including operator name, data and time are added automatically, while all the microscope and camera parameters are included by default for both Basic and Standard editions.

Continued... 372

LAS keeps the metadata in a file associated with the image and in the same location although this file is normally hidden. If you do make changes to these files you do so at your peril! For the purposes of managing your disk space, you can choose where the database and associated files are kept if you want to Because the archive uses the normal Windows file system, you can use standard back-up tools.

Once the Archive is created, it remains in the location on the hard drive that was originally specified. When the Archive is created, the location of the images and metadata is also specified. However, the images can easily take up many Gb of space and may fill your hard drive. If this happens, the entire archive can be moved using normal Windows tools to an alternative location. This location can be on a remote drive but please be aware that this will affect the performance of recall for large images and be dependent on the network speed.

When more than one user accesses the same data, there is inevitably some security needed. This may be a matter of preventing less experienced users from accidentally damaging or destroying valuable data. LAS supports the Windows user log-on so that if you wish you can create databases with restricted access.

Each image in the database has an image name. Software in LAS ensures that image names are unique to a folder by appending an incrementing number where possible and by providing numbered sequences for the sequence modules.
Clearly it is vital that you have easy access to the images that are in the database and LAS provides several feature to expedite this.

If you need to export image files directly to external media or to a personal file space, you should use the export tool. This will ensure that a copy of the image is made and you will not then be likely to damage or destroy vital data in the database. The exported images from a sequence will each use the image name as part of the file name. This ensures that sequence of images will be readily recognizable by you once exported.

Summary:
In order to achieve a fast, reliable and scalable tool for microscope data capture and processing, it is important to use modern database technology and to enforce some simple rules about how the user interacts with that data. This may impose some limitations on the ways in which you work with your data and may mean that you have to change some of the procedures you operate in your work. In creating LAS Archive, we have taken great trouble, however, to provide new features that will allow you to find new working practices to achieve your workload and in a way that will allow traceability, scalability and security for the future.

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Every single image or collection of images is associated with a Record. It is a self-contained entity storing not only the actual image data but a wealth of information associated with it. Images may be:

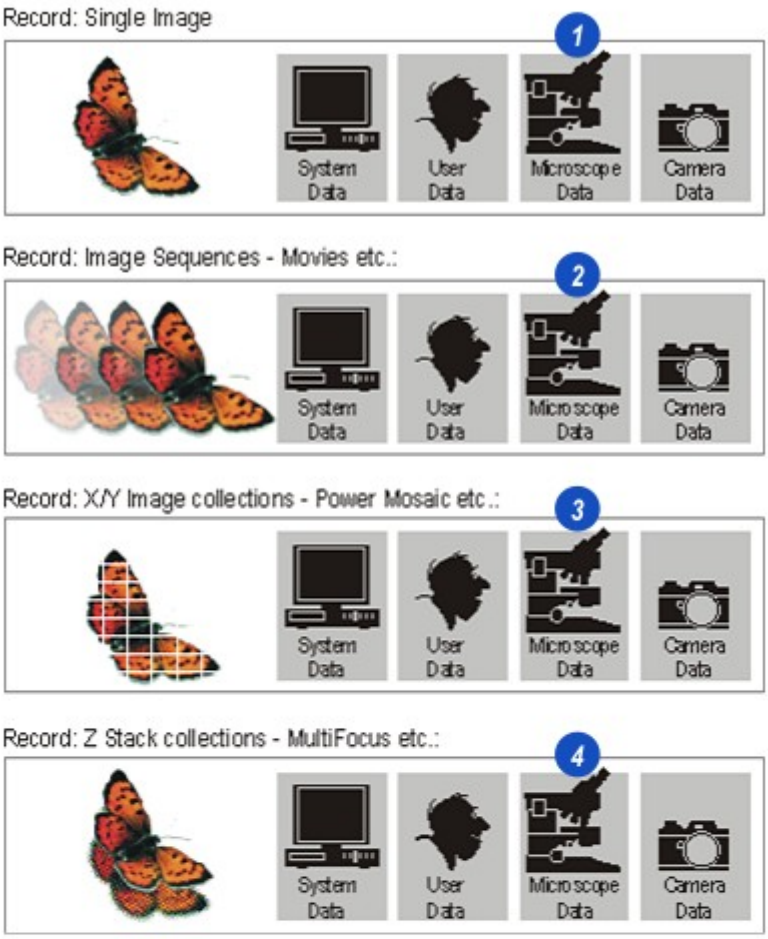
1: Single images.

2: Image sequences such as time lapse, size only being constrained by the hardware and preferences.

3: Multiple images like those from Montage.

4: Image groups produced as Z-Stacks…
…or any created and modified within Leica Application Suite.

Continued... 374



Depending upon the hardware, each image has data automatically stored with it:

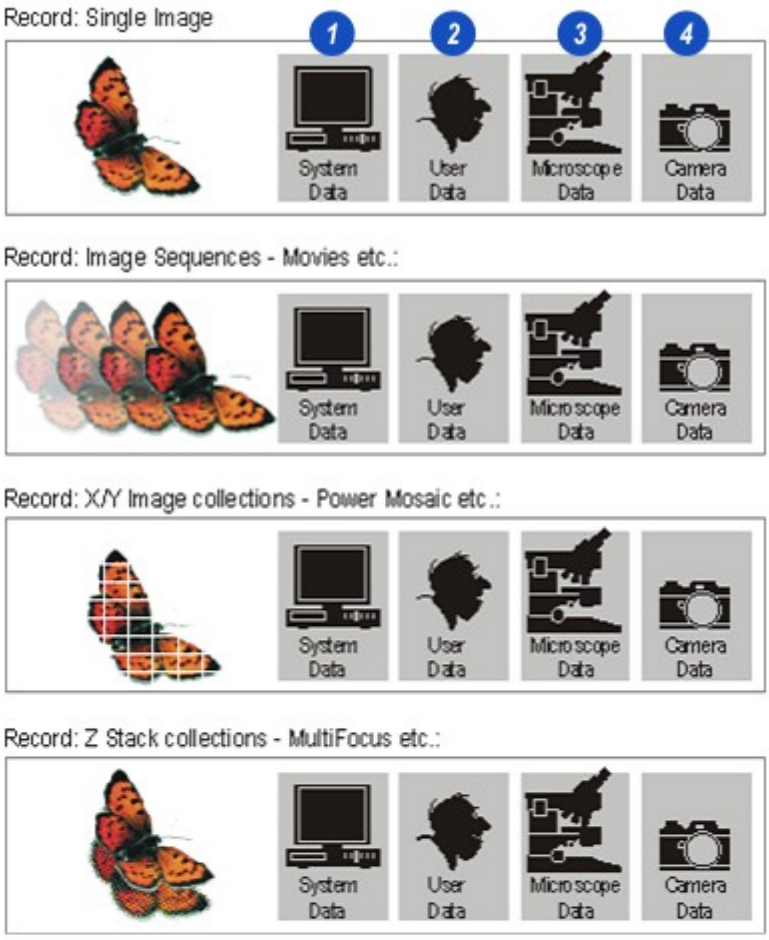
1: *System Data* such as image bit depth, size and sequence.

2: *User Data* like image name, creation date and time.

3: *Microscope Data* that will allow the conditions to be quickly replicated – model name, mag-changer, nosepiece and so on.

4: *Camera Data* relating to exposure, gamma, brightness and all of the essential settings.

The data is stored in *Fields* within the *Record.* All of the essential Fields appropriate to the hardware setup have been pre-defined by Leica – it is only necessary to click *Acquire Image* – but for more versatility users can add their own Fields and definitions with the LAS Basic and Standard Editions.



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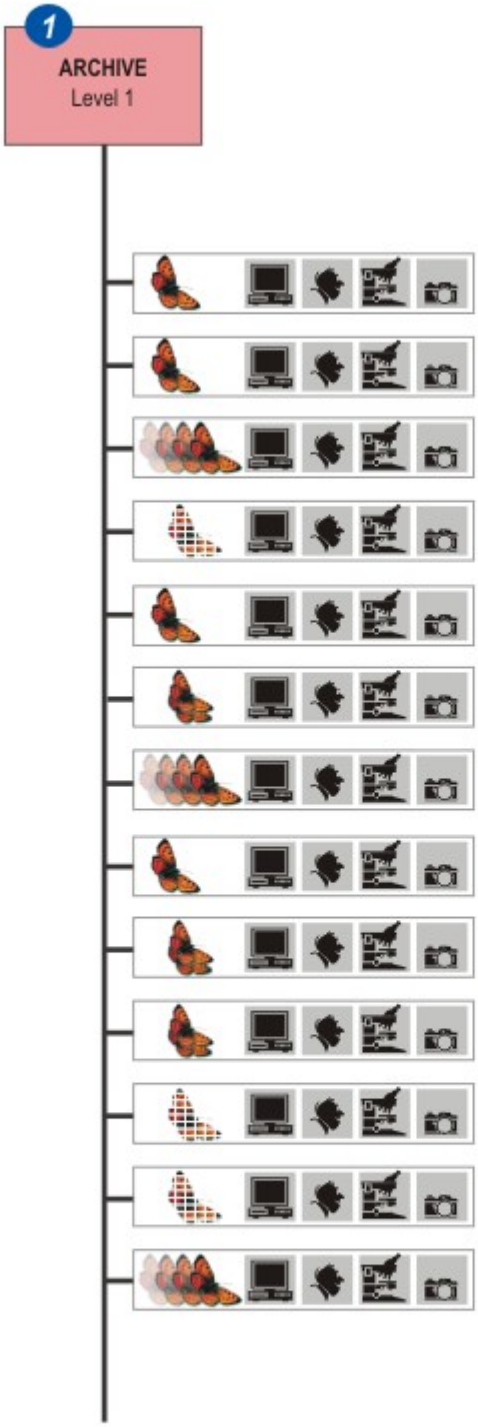
LAS Archive has a pre-defined and pre-loaded archive called *Example Archive,* which is an archive structure only; it does not contain data. This is what you see immediately after *LAS Archive Basic Edition* is installed and started for the first time.

Example Archive is a Single-Level Archive **(1)**. This means that all of the records are directly associated with *Example Archive* – or whatever a structural copy is named – it cannot have Level 2 record groups.

Example Archive is ready to use immediately. It is the fastest way - just a few minutes - of getting into production, especially for a single-station user.



There are two editions of *LAS Archive* both purchased as optional modules:

- *Basic Edition:* Essential functionality and tools for LAS Archives.
- *Standard Edition:* Builds upon the facilities available in the *Basic Edition* and additionally allows the creation of multi-level archives with many field types in addition to text fields.

Basic Edition:
Provides for 1 or 2-Level archives. This means that image records can be associated directly with the *Primary Archive* **(1)** or indirectly by grouping shown on the illustration as **(2)** and **(3)** – *Record Group A* and *Record Group B.*

The *Primary Archive* is Level 1 and the *Record Groups* are Level 2. With *Basic Edition* as many *Primary Archives* as required can be created, each with a different name. In turn, they can contain as many *Record Groups* as needed also with unique names. However, *Record Groups* can have the same name providing they reside in different *Primary Archives*.

Basic Edition also features:

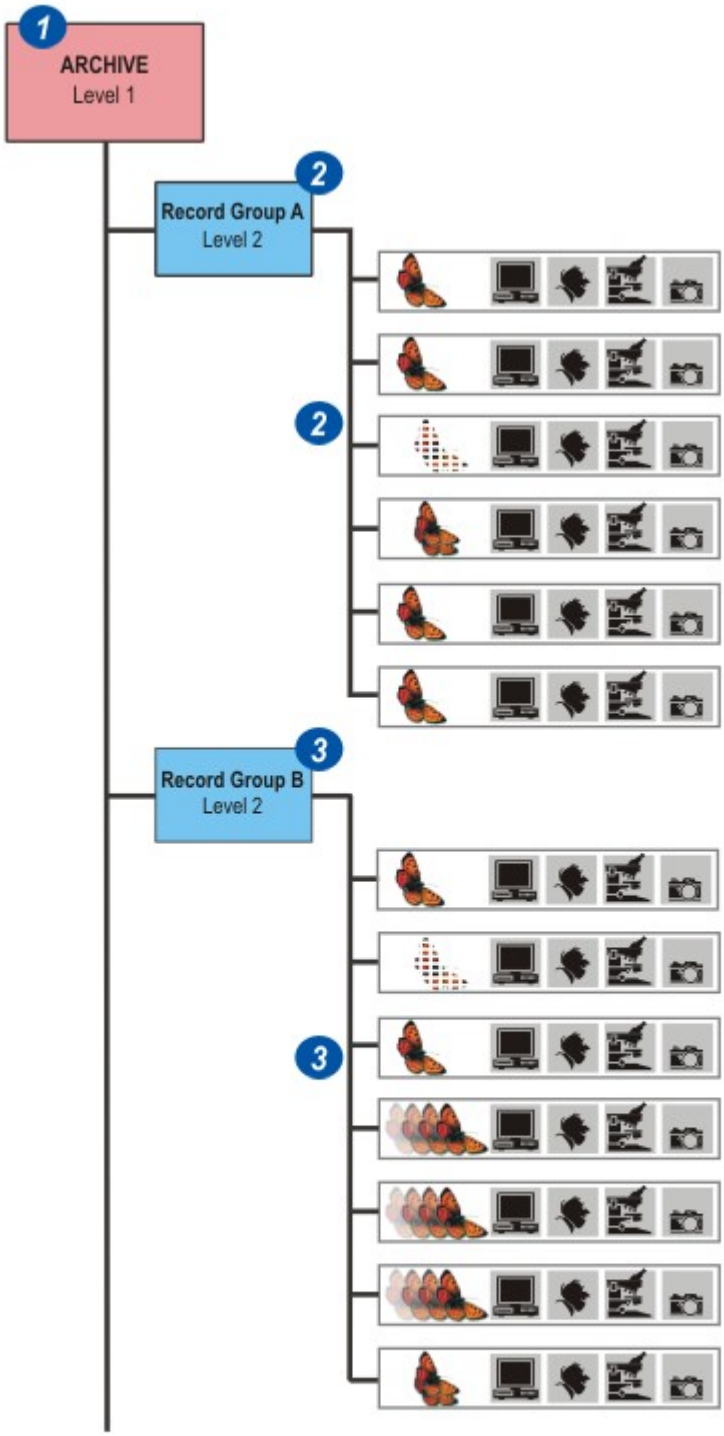
Microscope and camera data captured automatically with the image.

Fast Archive Search with detailed filtering to locate specific images and data.

User Field Selection for Form display.

Attaching documents to image in any format - not just text.

Audio recording inclusion with image. Add Multiple Text Boxes to the Archive.



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Optional module *Standard Edition Archive* extends the power and flexibility of the Basic Edition to provide:

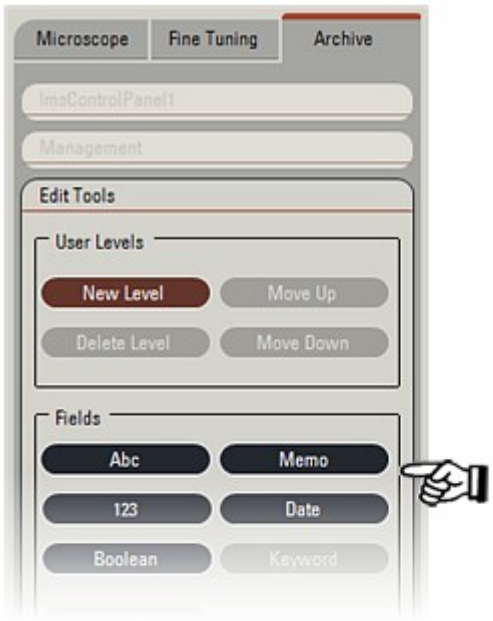
Multiple archive levels.

Wide choice of Named Archive Fields - Memo, Boolean, Numeric, Date and Keywords.

Form Layout design and control.

Highly Detailed Reports to include scaled images. Export reports to Adobe Acrobat (pdf) and Browser (html) facility.

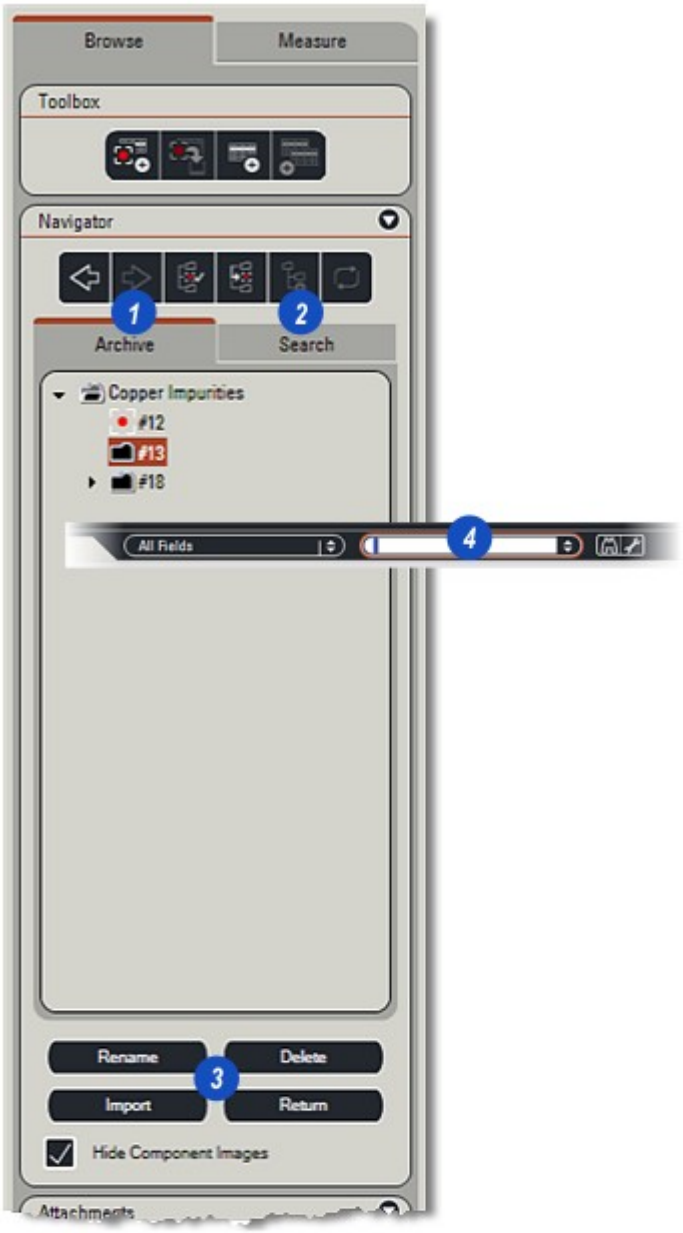
Increased flexibility and security by its use of specialised fields - fields into which only pre-determined data can be loaded.



The illustration shows a typical *Browse Navigator Panel* with an archive selected. *LAS Archives* are optional modules that can run on the same computer as *Image Explorer* although not at the same time. *See Selecting the Storage method: Go there…* 371

The panel layout with *LAS Archive* running displays the *Archive* tab **(1)** and the *Search* tab **(2)**. The rapid *Search* facility **(4)** is also available.

LAS Archives has addition function buttons **(3)** compared to *Image Explorer.*



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The image storage method – Image *Explorer* or *LAS Archive* – is selected on the *Setup Workflow.*

1: Click on the *Setup Workflow.*

2: If necessary, open the *Archive* panel by clicking the tab.

3: To select and use *Image Explorer*, click the *LAS Image Explorer* button. *Image Explorer* and *LAS Archive* cannot run simultaneously so clicking a button cancels the other. Return immediately to *Browse* by…

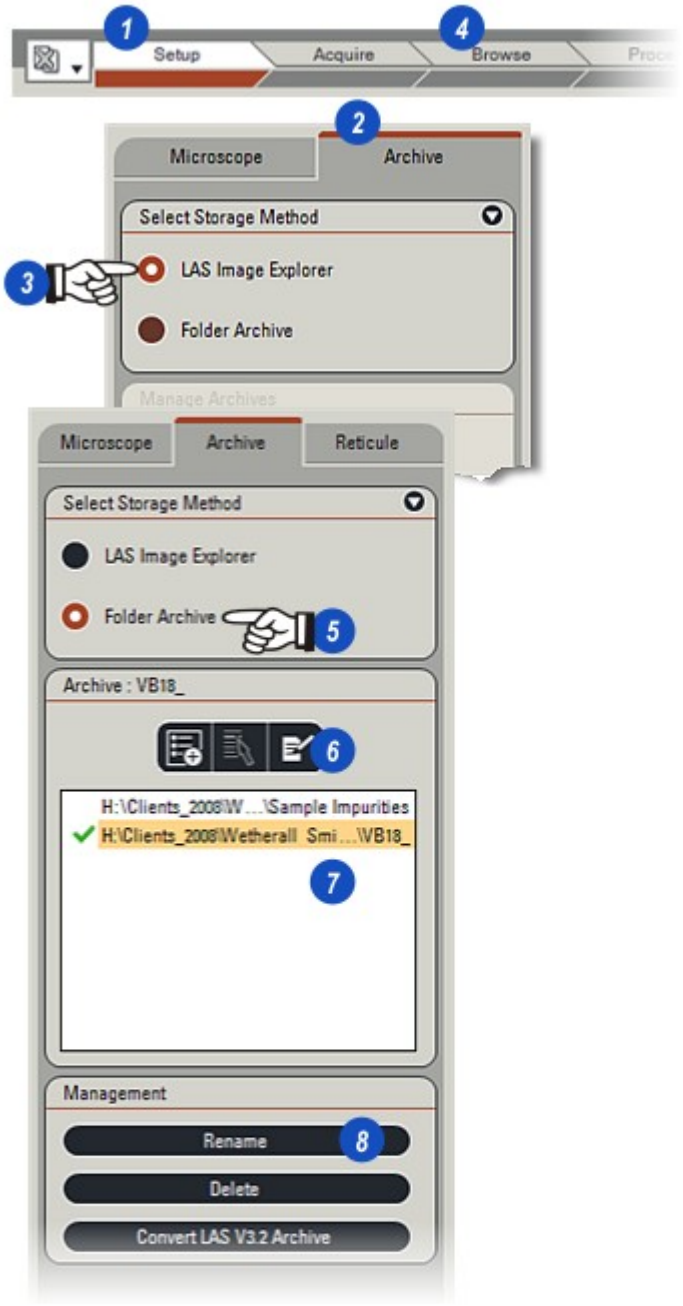
4: …clicking the *Workflow*.

5: Select *LAS Archive* by clicking the button.

6: The *Archive* toolbar,…

7: …the *Archive List Window* and…

8: …the *Management* controls immediately become active.

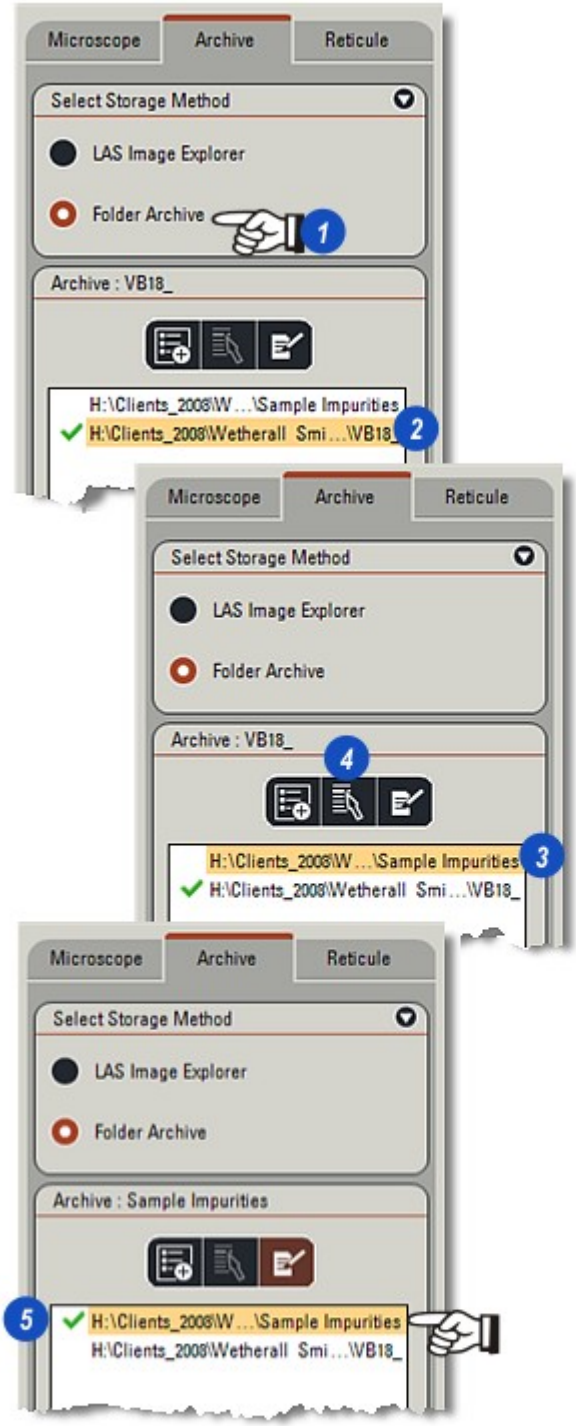


1: With *Archive* selected…

2: …a previously selected archive (if any) will be highlighted and checked – a green tick mark to the left – will be displayed in the *Archive List Window*.

3: To change to another archive either double-click it or click it once and then click the *Set As Current* (Active) Archive button **(4)**.

5: The selected archive is activated and the green tick mark appears to the left. Return to *Browse* by clicking on the *Workflow*.



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To rename an existing archive, ensure that *LAS Folder Archive* is selected and...

1: Click on the *Rename* button.

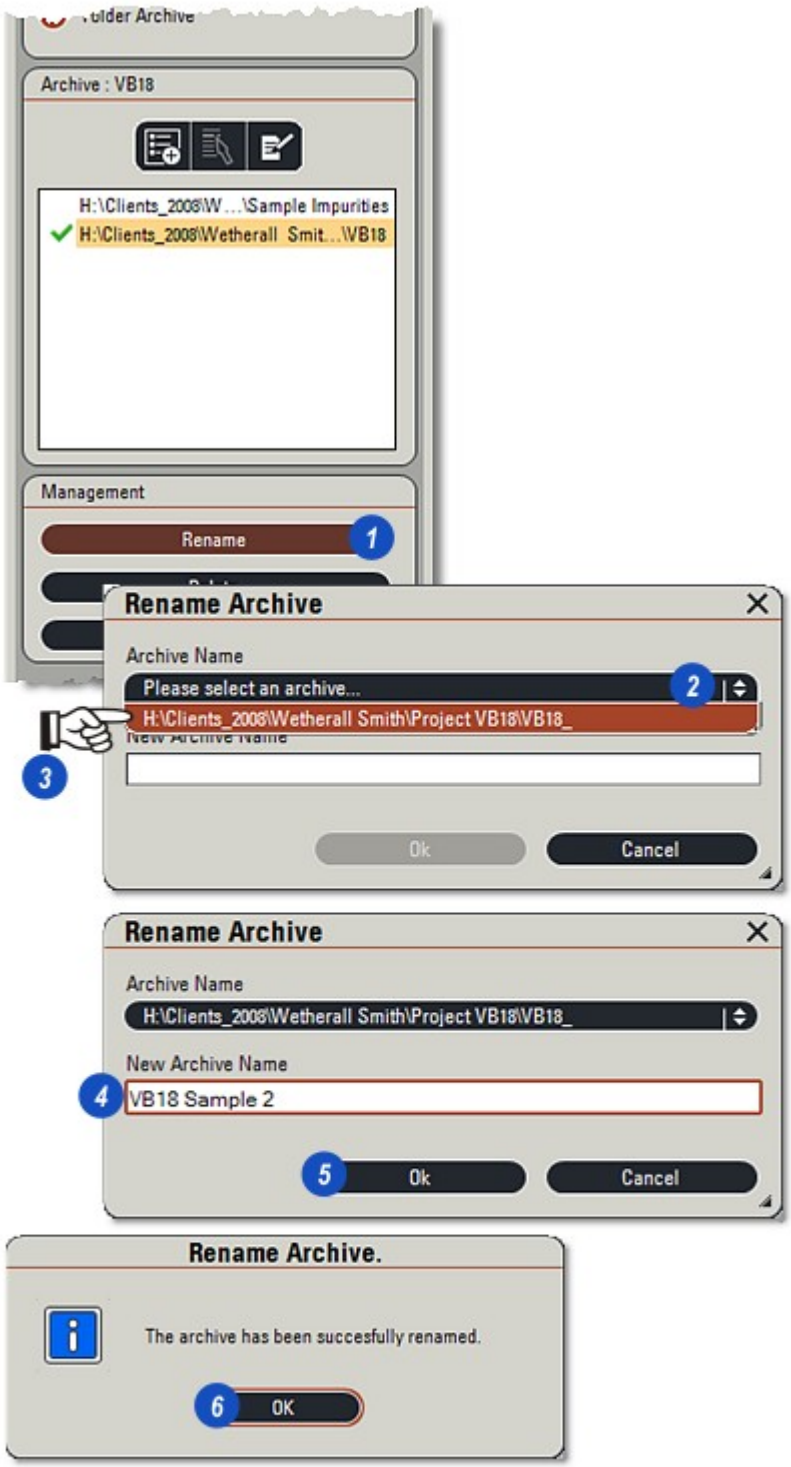
2: On the *Rename* dialog, click on the small arrows to the right of the *Archive Name* header and from the list of archives...

3: ...click to select the one to be renamed.

4: Click in the *New Archive Name* text box and type a new name.

5: Click *OK*.

6: Click *OK* on the *Rename Archive Confirmed* dialog.



Use with caution as a deletion cannot be reversed.

1: An archive cannot be deleted if it is currently active.

2: …click on the *Delete* button.

3: On the *Delete Archive dialog* click on the arrows to the right of the *Archive Name* header and from the drop down list...

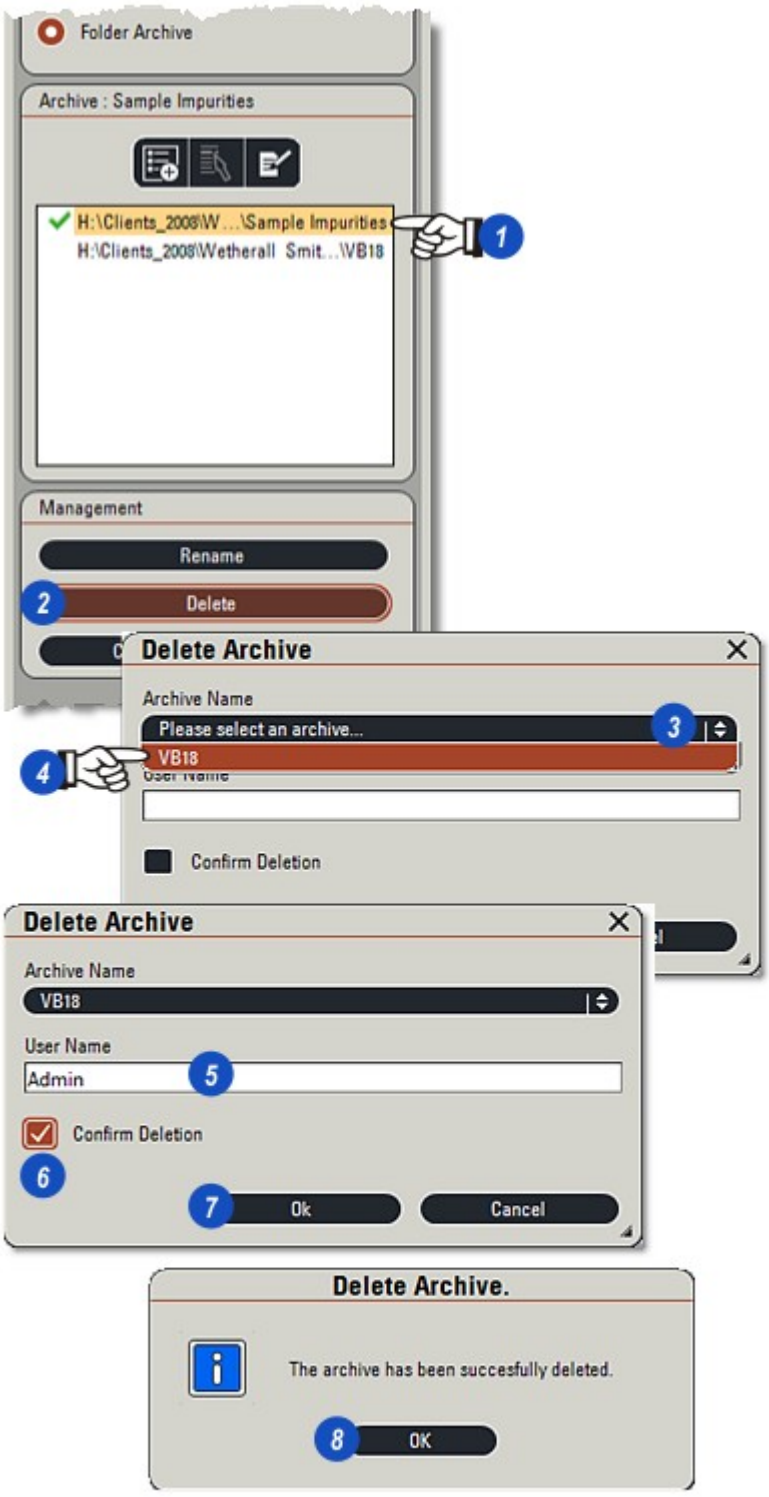
4: ... click to select the archive name.

5: Type the name of the user that installed Leica Application Suite in the *User Name* text box. Most often this will be *Admin.*

6: Click to check the *Confirm Deletion* check box.

7: Click *OK*.

8: On the confirmation dialog click *OK* and the archive is deleted.



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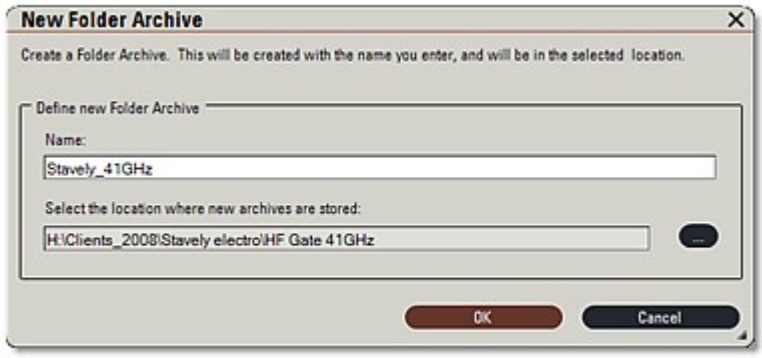
Concise details for creating different types of archive are described in *Optional Modules > LAS Archive > Basic Edition:* *Go there...* 409



The speed and efficiency improvements made by Microsoft in their latest SQL Database Engine are included from *LAS Archive Version 3.3* to keep it in the forefront of data processing technology. So that archives created with software earlier than V3,3 are not lost, a *Convert Archive* tool has been included in *Archive Management.*

To convert an archive:

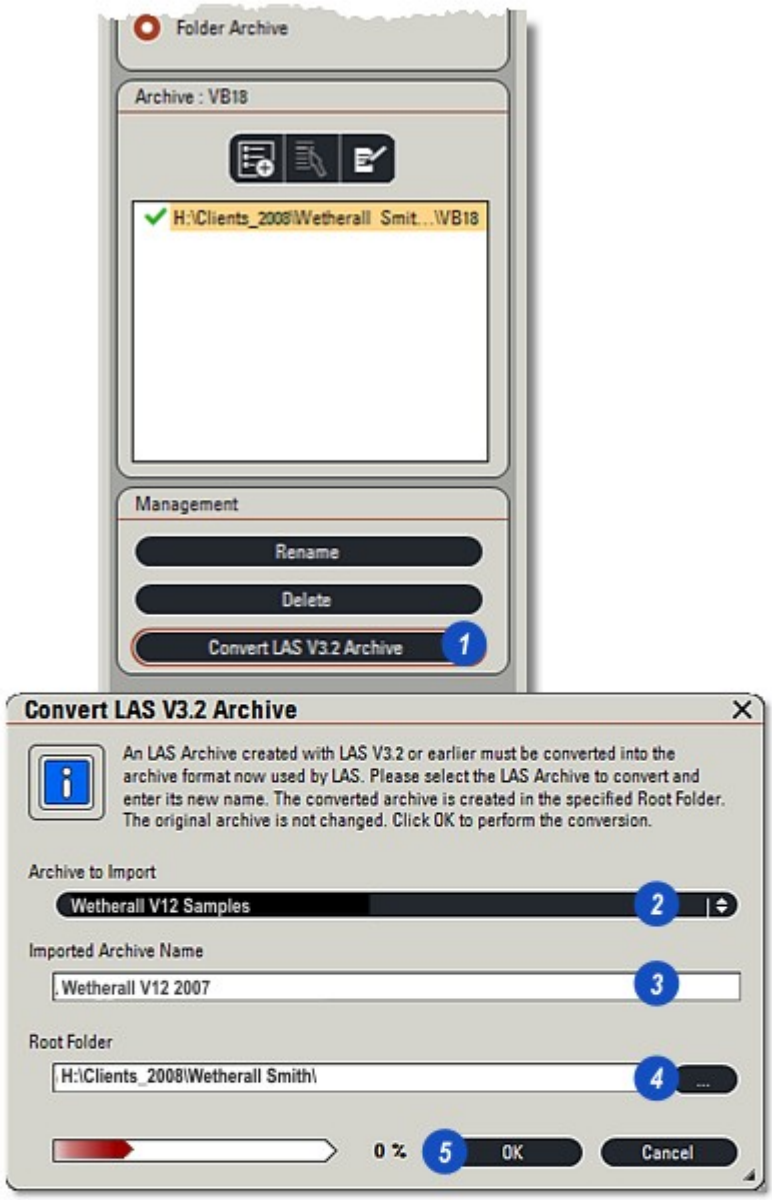
1: Ensure that *Folder Archive* is selected and click on the *Convert LAS V3.2 Archive* button.

2: On the *Convert* dialog, click on the arrows to the right of the Archive to Import header and from the list of available archives, click to select the one to import.

3: Click inside the *Imported Archive Name* text box and type a new, appropriate name for the imported archive.

4: Click on the browse button to the right of the *Root Folder* text box and navigate to the location that the imported archive will be stored. On the navigation dialog it is possible to create a new folder.

5: Click *OK*. The existing archive structure is copied - the archive remains intact - to the new location with necessary upgrades to the structure. None of the images or their associated data will be lost or corrupted.



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A complete list of available archives is displayed by:

1: Clicking on the *Setup Workflow.*

2: Clicking to select *LAS Folder Archive.*

3: The list appears in the *Archive* window with the currently selected archive (if any) having a green tick mark to the left. An archive can be made active by double-clicking on it.

4: With an archive selected and active in Setup, when the *Browse Workflow* is selected...

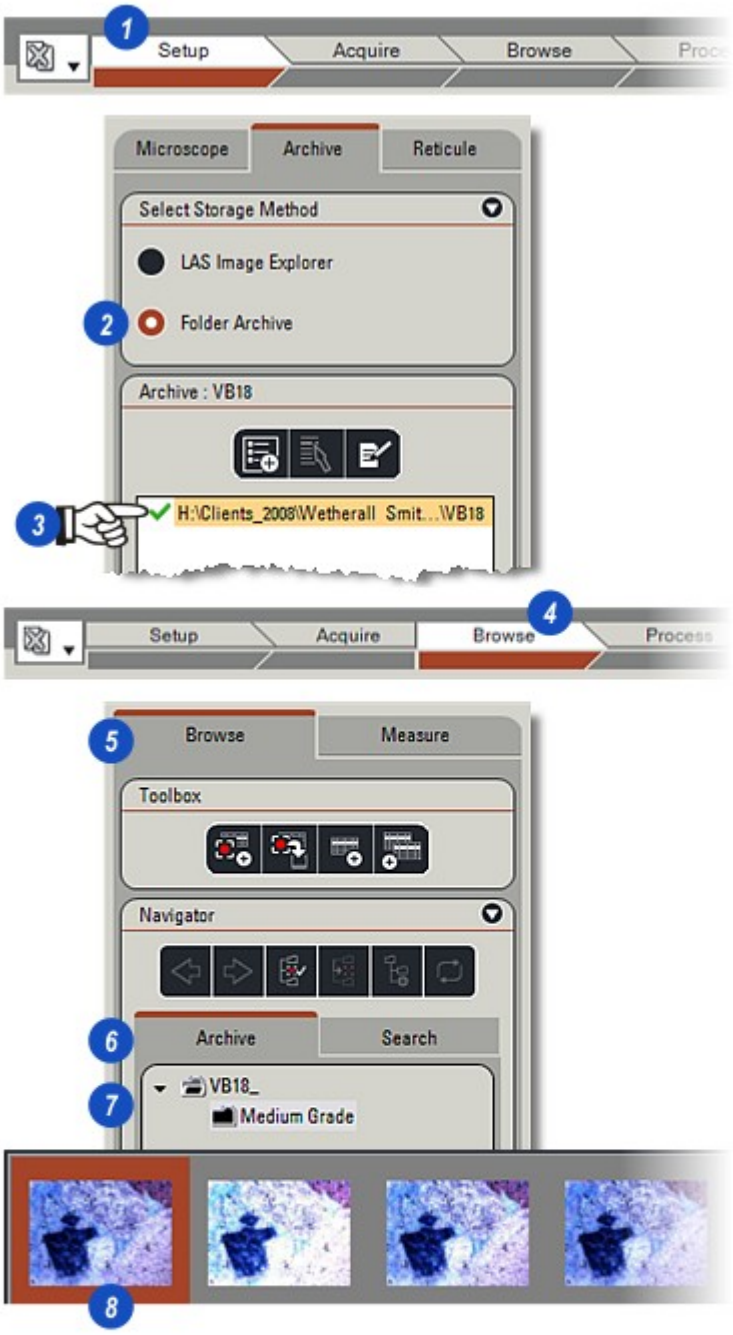
5: ...and the *Browse* tab visible...

6: ...the *Archive* and *Search* tabs are revealed and...

7: ...the active archive displayed in the *Navigator* window.

8: If the *Gallery* is enabled the archive thumbnails will be displayed and the first image and its data present on the *Viewer*.

Continued... 386



Swapping in Navigator between the conventional folders of Image Explorer and LAS Archive is as simple as a mouse click.

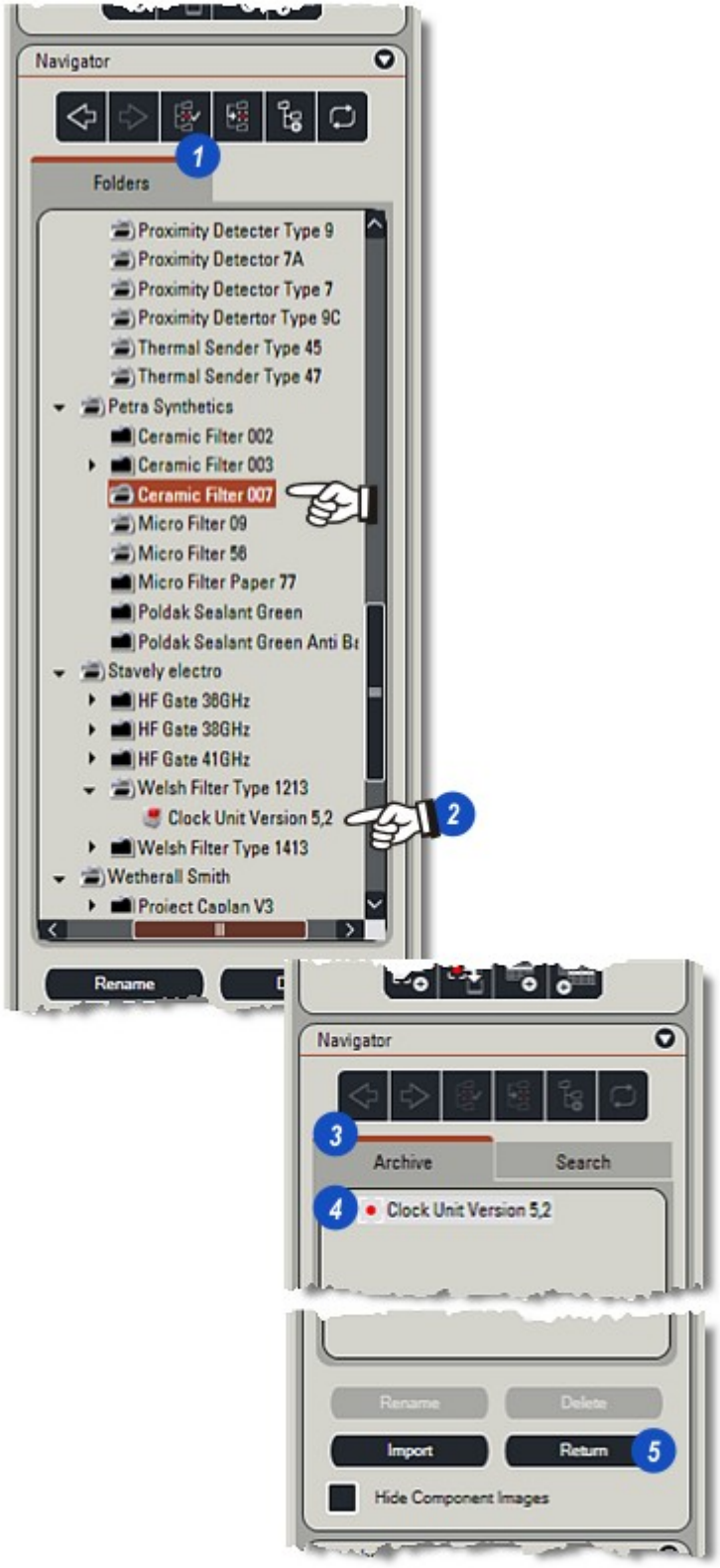
In the illustration, an Image *Explorer* folder is currently selected - the *Folder* tab **(1)** is visible and the selected folder is highlighted.

An archive name is displayed further down the tree **(2)** and is indicated by the Leica Cube icon to the left of the name.

Simply double-clicking on the archive name will change the storage mode from *Image Explorer to LAS Archive* - the *Archive* tab **(3)** appears...

...and opens and loads the images *(4)*.

Go back to browsing in *Image Explorer Folder* mode by clicking on the *Return* button **(5).**



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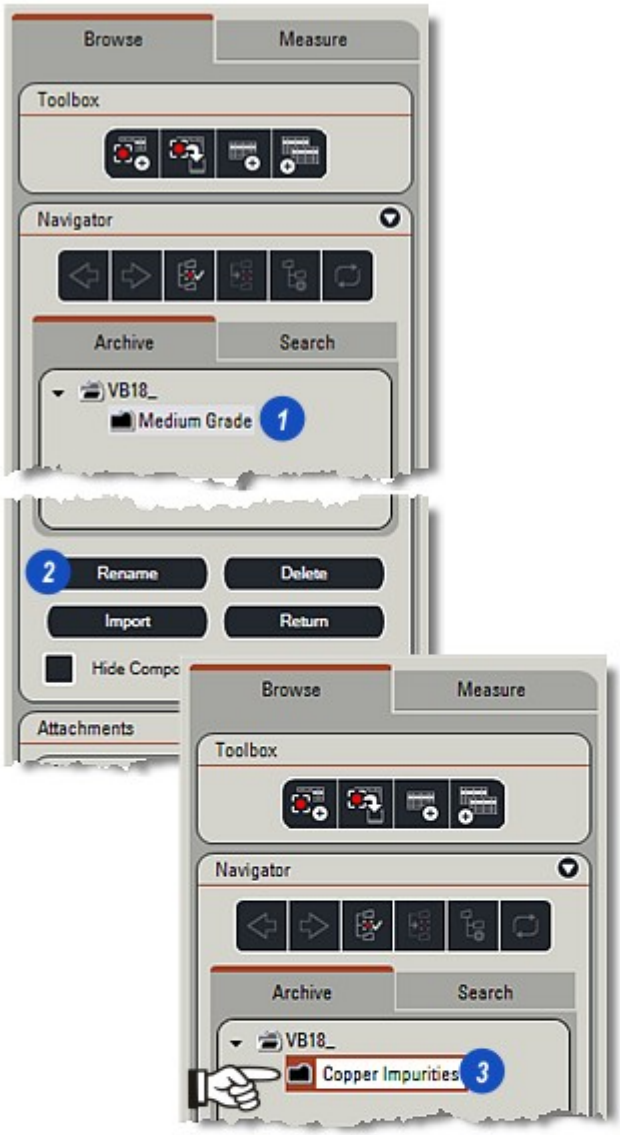
Rename an Archive by:

1: Click on the *Archive* to select it.

2: Click on the *Rename* button.

3: The *Archive Name* in the *Navigator* window changes to a highlighted outline. Type the new name and press *Enter* on the keyboard.

The Archive now appears under its new name in the Navigator.



To Delete an Archive:

1: Click on the archive to be deleted.

2: Click on the *Delete* button.

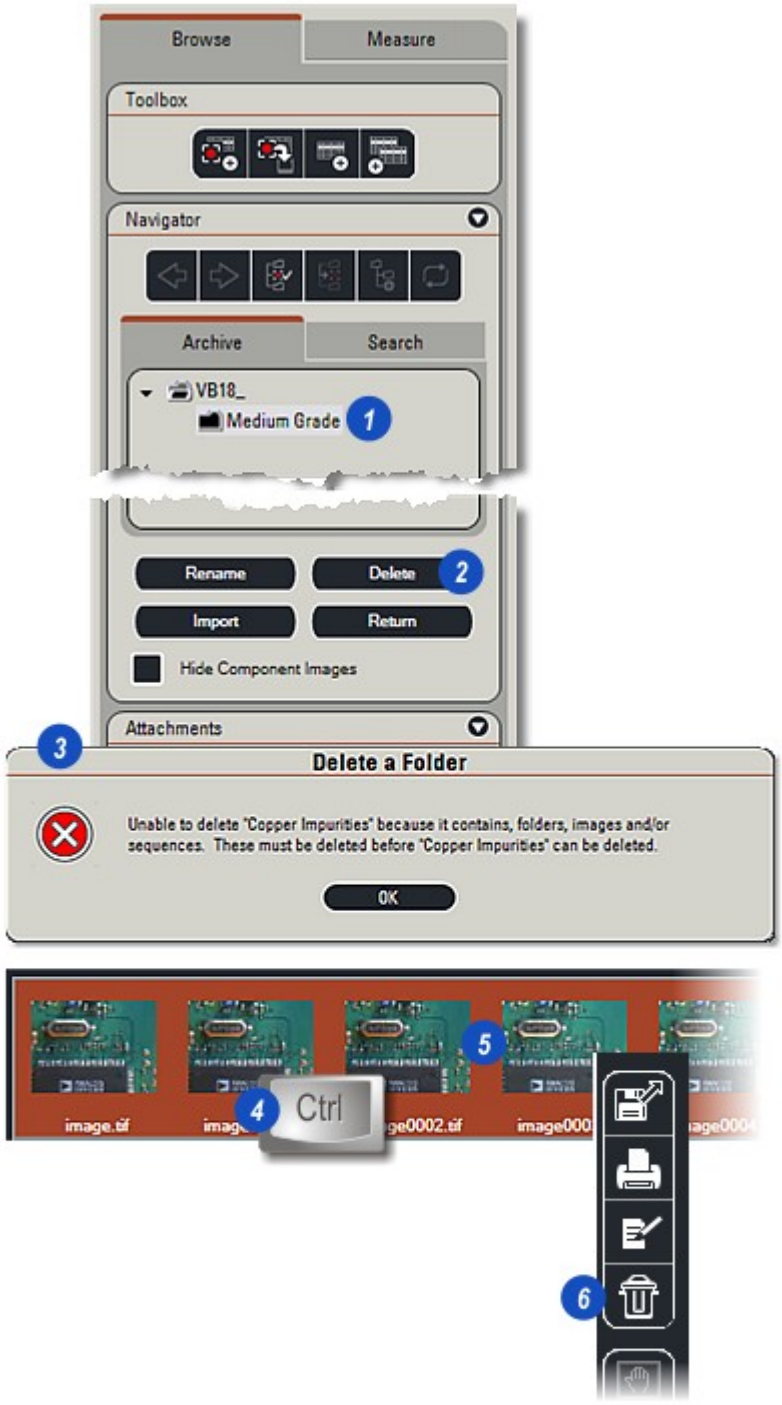
3: For security reasons, an archive that contains images and data cannot be deleted - they have to be removed first by...

4: ...holding down the keyboard *Ctrl* key and...

5: ...clicking on the individual *thumbnails* in the *Gallery*.

6: Click on the *Delete* (Trash Can) button on the side tool bar.

Continued... 389



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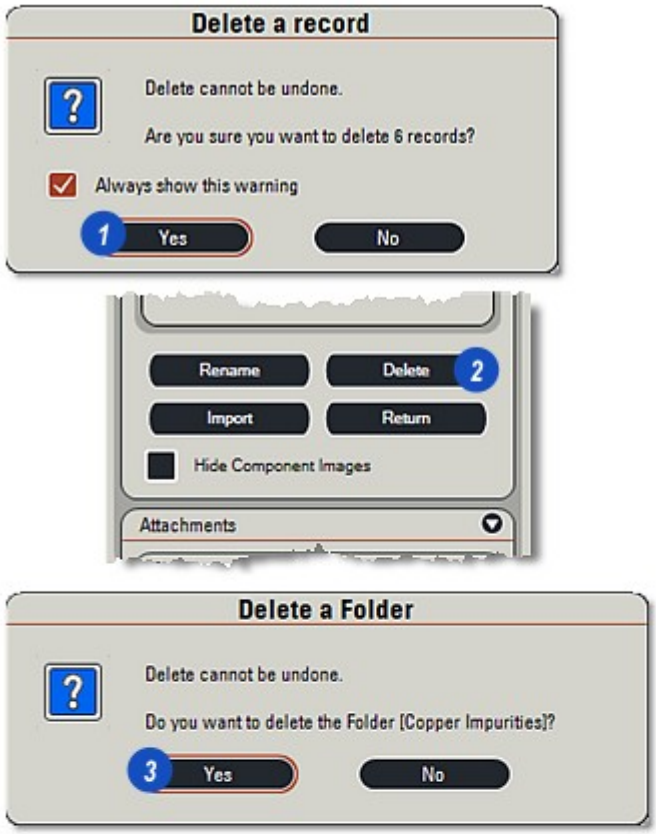
Continued from previous page:

1: Confirm that the images and data (Records) should be deleted by clicking the *Yes* button.

2: To delete the empty archive click on the *Delete* button and...

3: ...confirm the deletion on the *Delete a Folder* dialog by clicking the *Yes* button.



LAS Archive Basic Edition

With the optional Basic Edition Archive installed, all of the features and tools needed to produce powerful and versatile Archives are immediately available to create:

- User designed Single Level Archives.
- User designed 2-Level Archives with almost unlimited Record Groups (Folders).
- Archives using existing structures either from User designs or the comprehensive library of templates supplied as part of the module.
- As well as User defined data fields, microscope and camera data is captured automatically with the image.
- Elegant and detailed Form display for every image.
- Forms can be configured to display the data that the User requires and hide everything else.

Basic Edition Archive also includes features to bring even more sophistication to archiving:

- Fast Archive Search with detailed filtering to locate specific images and data.
- Attaching documents of any format - not just text.
- Audio recording.
- Adding Multiple Text Boxes to the archive.

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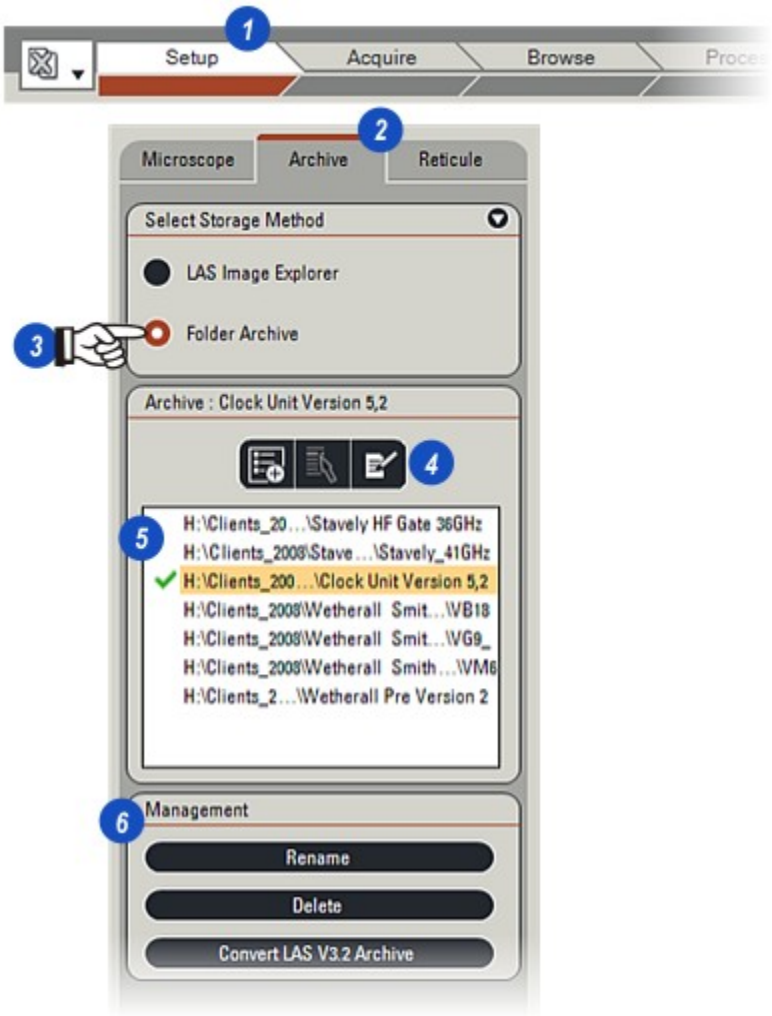
This section describes setting up both a simple single level archive and a comprehensive 2-Level archive.



Three options are available when creating a new archive:

> *From New*: Starting from a completely blank structure and configuring precisely to the user requirements.
>
> *From Template:* Creating an archive based upon a template that can be either supplied in the Leica Template Library or one created by the user previously.
>
> *From Archive:* Copies the structure and fields of an existing archive.

Archives are created in the Setup Workflow:

1: Click on the *Setup Workflow* and if necessary...

2: ...click on the *Archive* tab.

3: On the *Storage Method* panel click *Folder Archive.*

4: The *Archive Toolbar* and...

5: ...the *Archive* window together with...

6: ...the *Management* tools become active.

Continued... 392

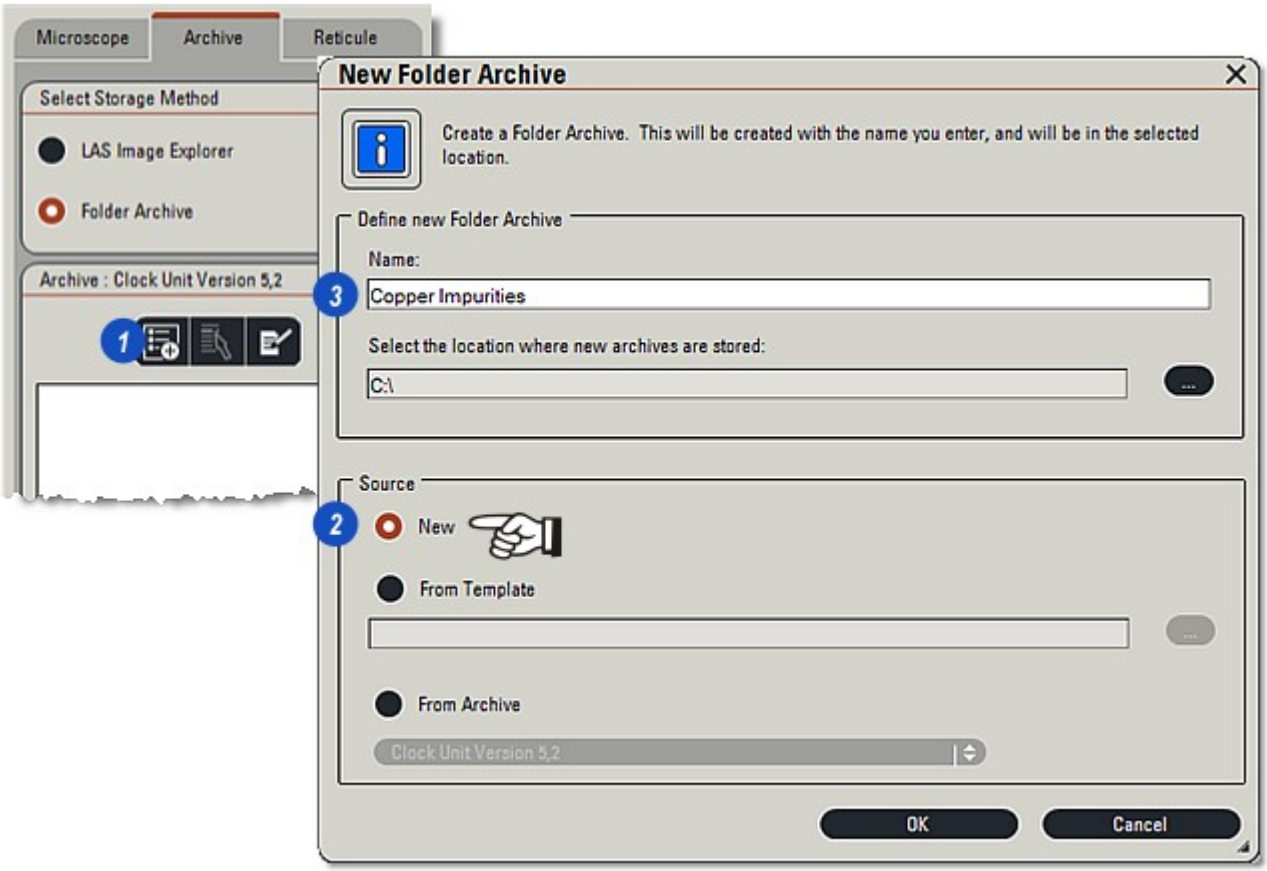
To create a completely new archive that can be structured precisely to the users needs:

1: Click on the *Create Archive* button on the toolbar. The *New Folder Archive* dialog appears.

2: On the *Source* panel click the *New* button. The three Source options are mutually exclusive - clicking one automatically de-selects any other.

3: On the *Define Folder Archive* panel click in the Name text box and type a unique name for the new archive.

Continued... 393



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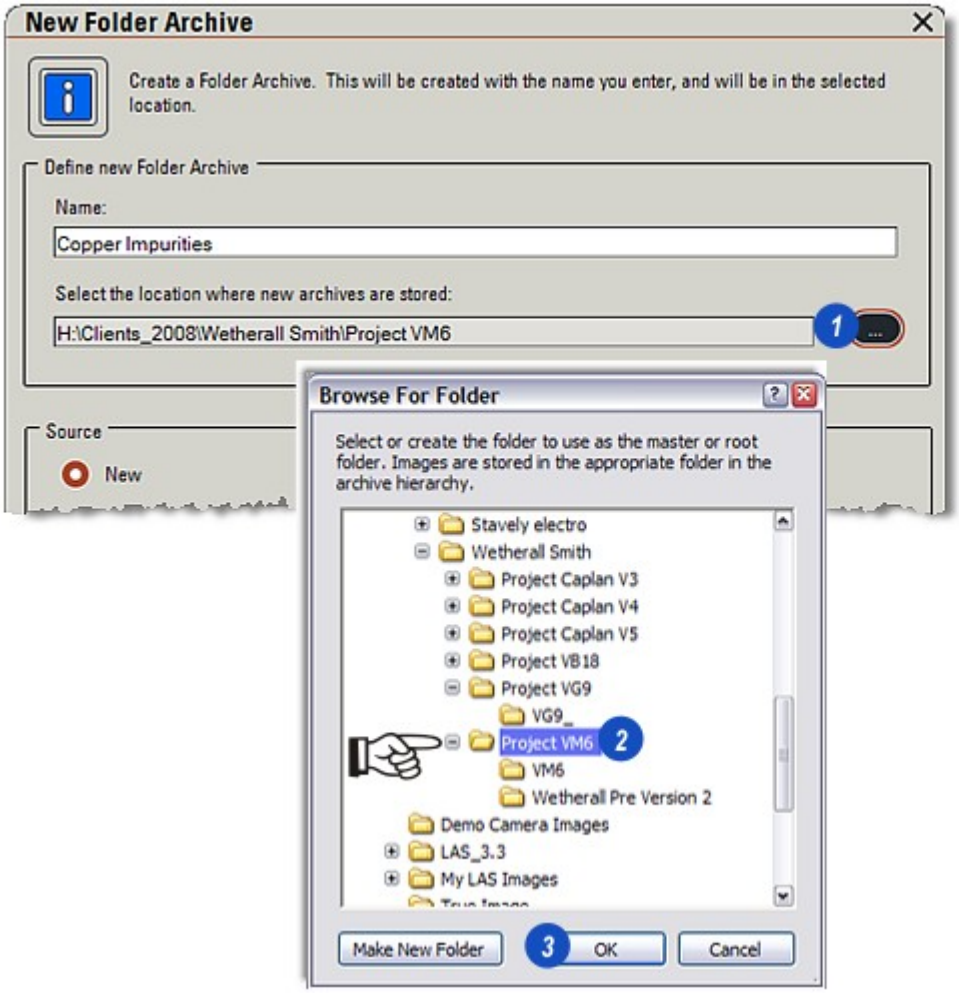
1: Click on the browse button to the right of the *Select Location* text box.

2: On the *Windows Navigation Browse for Folder* dialog, navigate to the location where the new archive is to be stored.

3: Click *OK*. The new storage location appears in the *Select Location* text box.

Continued... 394

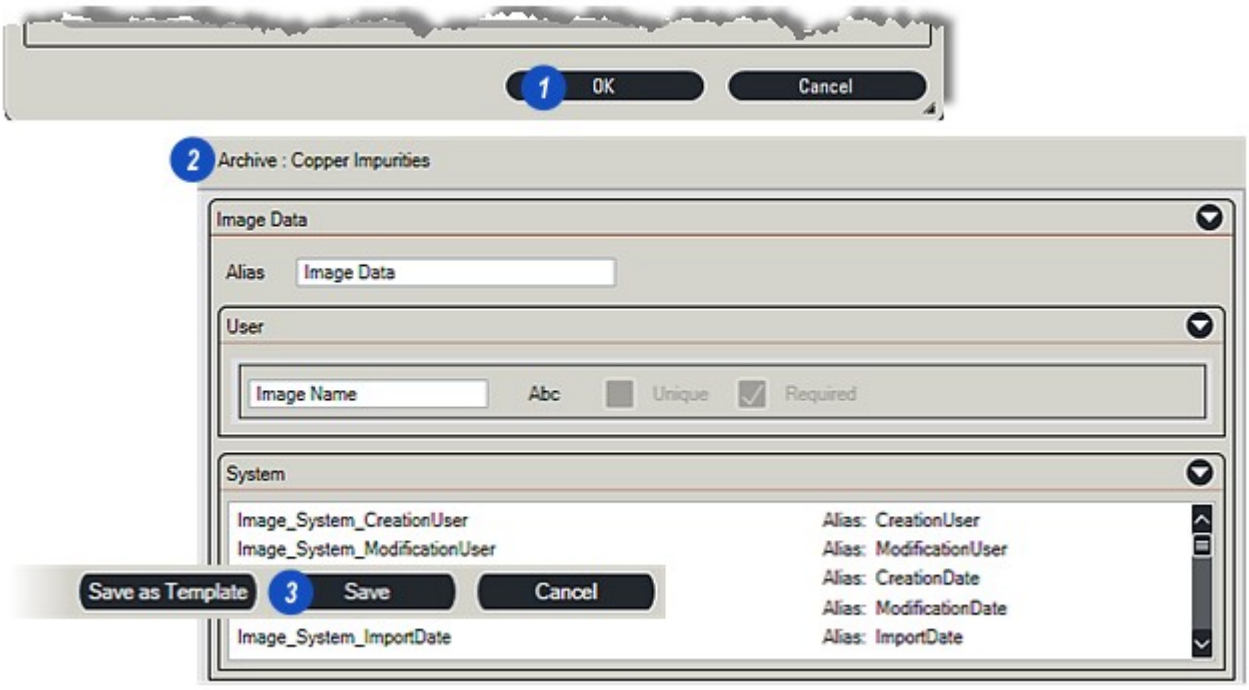


1: Click on the *OK* button.

2: The *Single Level Archive Structure* appears. This will be the basis for the Record Form for each captured image. To create a 2-Level Archive: Go there...

3: Users that require just a simple archive should just click the *Save* button (Bottom right-hand of the Viewer). There is no need to change any of the data field names.

Continued 395*...To start capturing into a single level archive.*



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With the new archive saved:

1: The new archive name appears in the *Archive List* and is set as current and active.

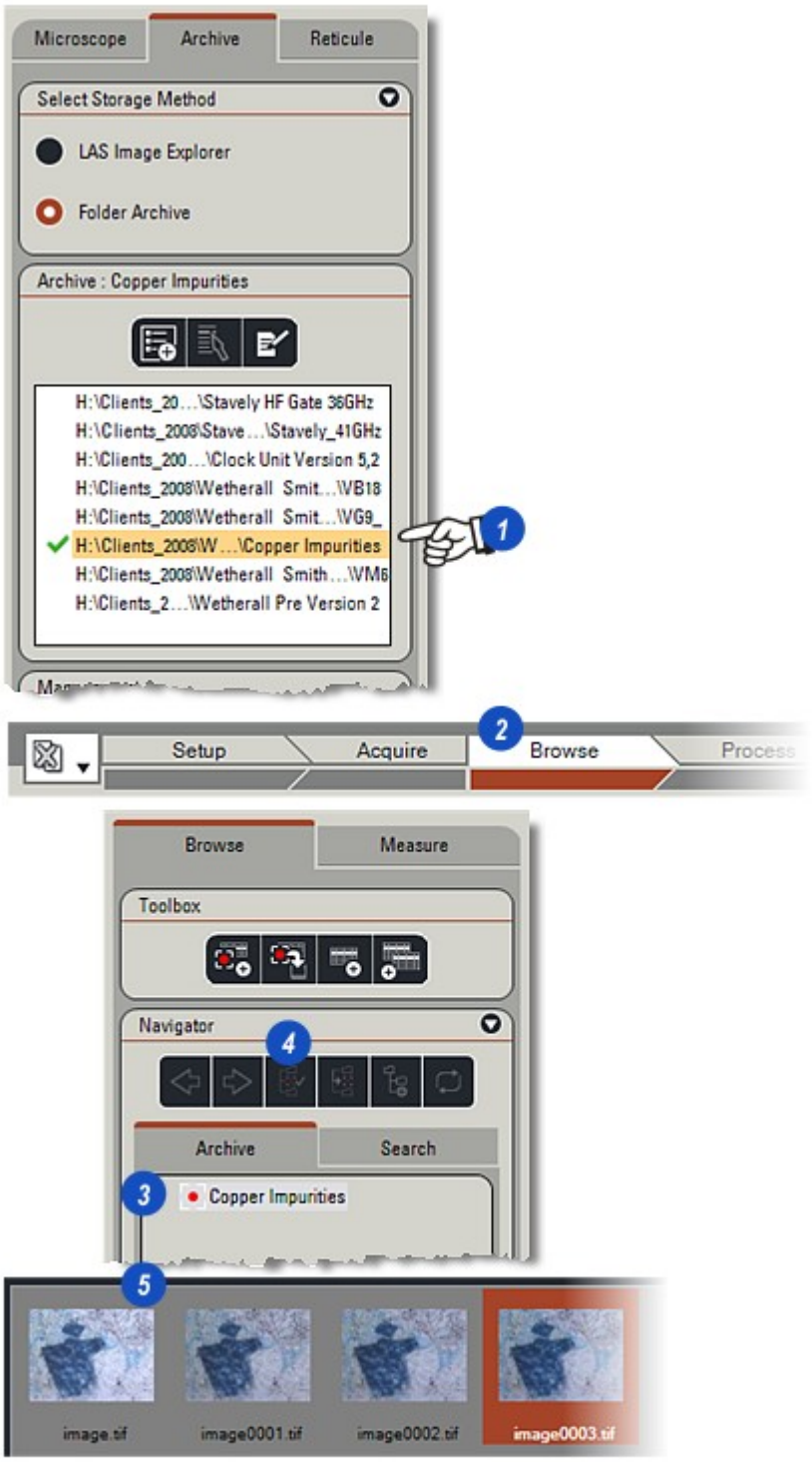
2: To start capturing images click on the *Browse Workflow* and...

3: ...click on the new archive.

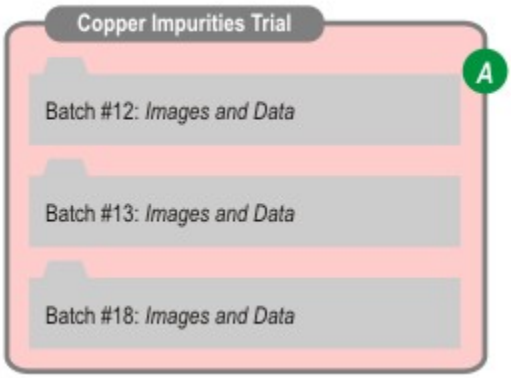
4: Click on the *Set as Capture Location* button to ensure that all images are saved in the new archive and...

5: ...start capturing images either in *Browse* or in the *Acquire Workflow*.

Acquire: Camera setup and image capture: *Go there...* 221



1: A new archive called Copper Impurities has been created as previously described. This is intended to be a 2-Level archive which means that within the archive separate collections of images will be captured and stored as Record Groups, each having a unique name.
There will be three Record Groups in the archive - *Batch #12, Batch #13* and *Batch #18* - chosen to reflect the job in hand. Figure **(A)** illustrates the structure.

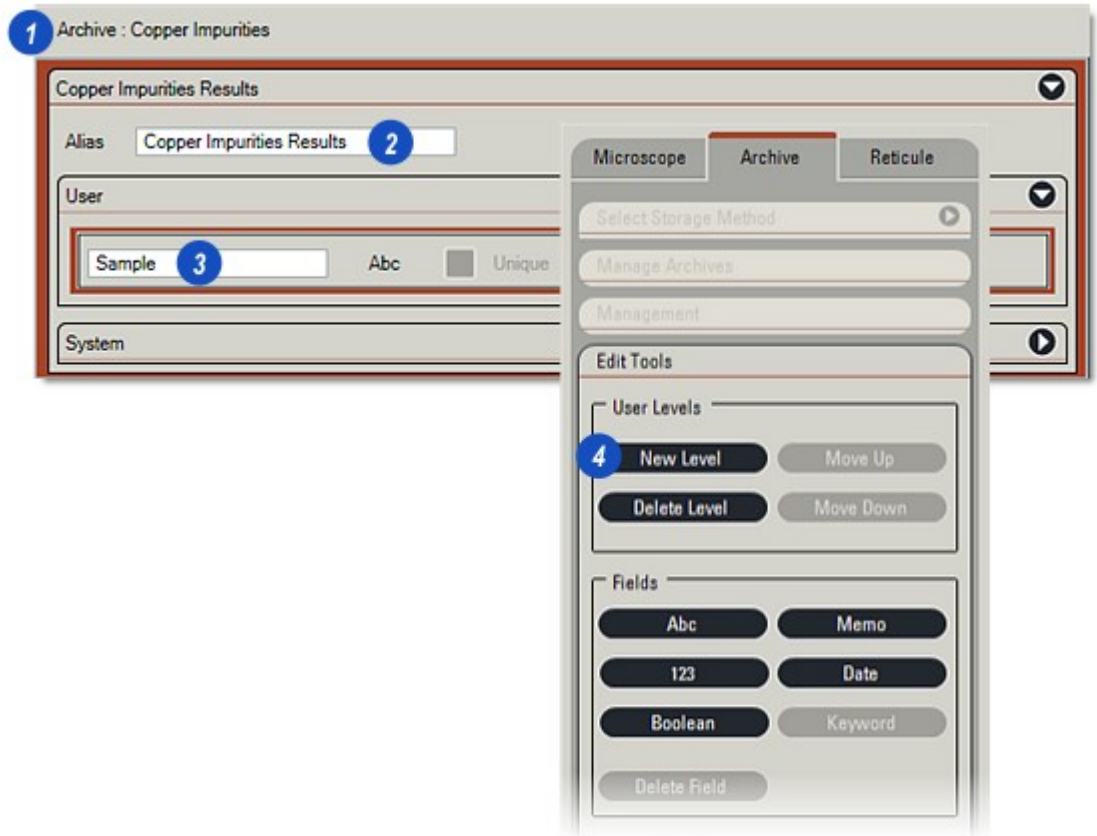


2: Every image within the archive, no matter what Record Group it is stored in will have all of the System Data Items - capture date, exposure settings, microscope stand and so on - saved with it. This data is displayed as Fields on the Form associated with each image. The default name for the Form is *Image Data* but can be changed to something more appropriate. Click in the *Alias* text box and type a new name. In the example, the new Form Name is Copper Impurities Results.

3: The default reference for the image is Image Name but this can also be changed by clicking in the User text box and type a new, more appropriate name, in this example is is called Sample. The *Abc* to the right of the text box indicates that the new name can comprise letters and numbers.

4: The next step is to create the Record Groups. Click on the *New Level* button.

Continued... 392



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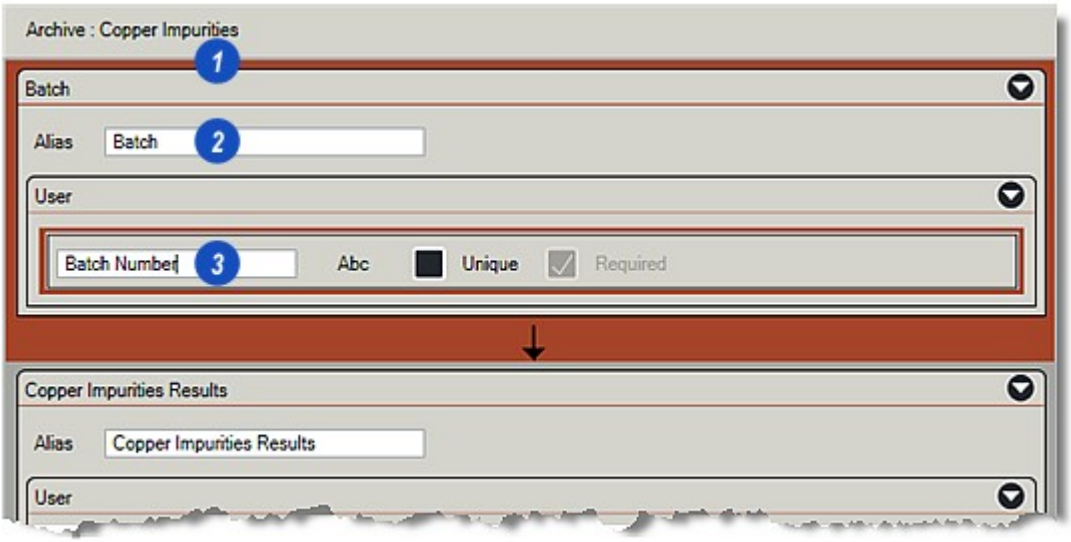
Continued from the previous page:

1: After the *New Level* button is clicked a dialog appears. This represents the structure of any Record Groups added to the archive.

2: The three Record Groups that re going to be added will be called *Batch #12, Batch #13* and *Batch #18* so in this example the structure will be given the name Batch. Click in the *Alias* text box (this is pre-loaded with the example text *New Folder*) and type an appropriate name - Batch in the example.

3: In this example each batch will be given a reference number - *#12, #13* and *#18* - and that will appear as the first User Field (pre-loaded with the example text Folder Name). Click in the first *User Field* text box and type an appropriate name - Batch Number in the example.

Continued... 398



Continued from the previous page:

1: In this project each Record Group is to have an additional field to identify the source of the sample batch. A new field to contain the Source Reference is added by clicking the appropriate Fields button - in this example it is a numeric code so the *123* (Numbers only allowed in this field) button is clicked.

2: The new field is added to the Record Group with the selected field type to the right - in this case *123*. Click in the *Field* text box and type an appropriate name - in this example Source Reference.

3: The Source Reference is a vital and required piece of information so the *Required* check box is enabled which means that an image can only be acquired if this field is complete.
Almost any number of User Specific Fields can be added in this way; This example requires only two described so...

4: ...click the *Save* button.

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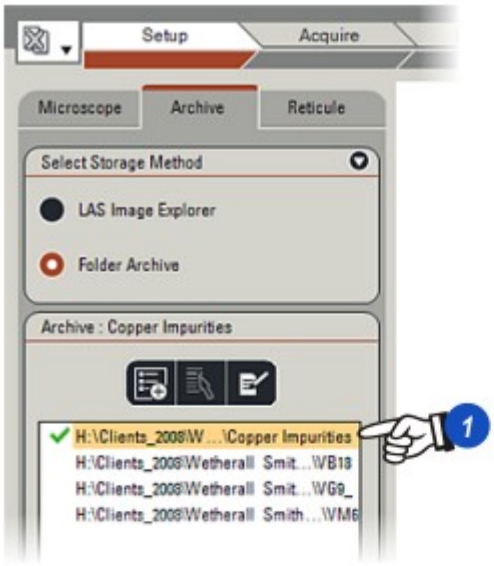
1: The new 2-Level archive now appears in the *Setup Workflow* > *Archive* window and is selected and ready to be used.

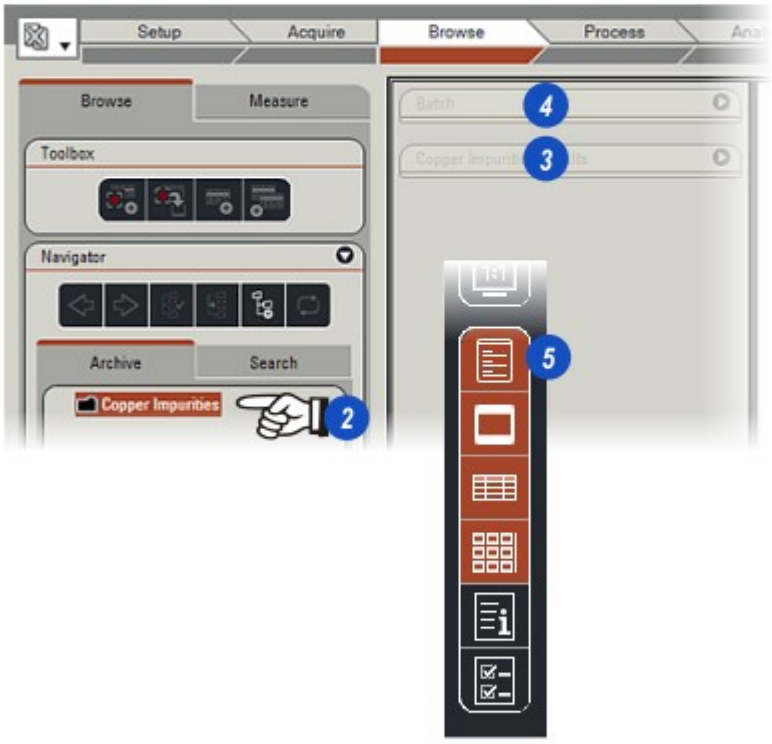
2: In the *Browse Workflow* it appears on the *Archive* tab with...

3: ...the *Form Name (Copper Impurities Results)* and ...

4: the *Record Group (Batch)* both ghosted in the *Viewer*. These will only be displayed if the *Hide/Show Form* button on the *Side Tool Bar* is enabled **(5).**

Continued.. 400





1: Click on the archive to select it.

2: Click on the *Create New (Batch)* button.

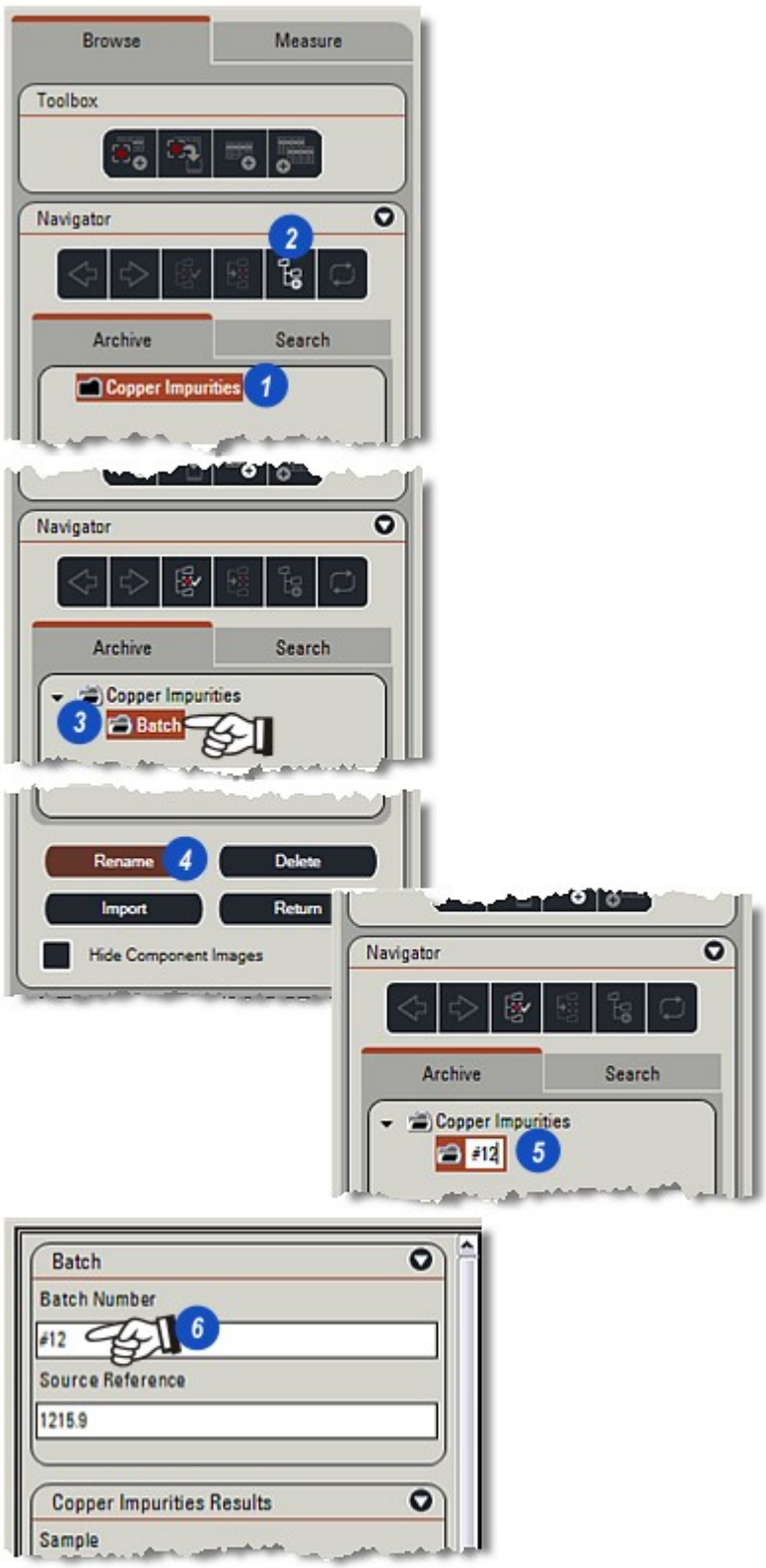
3: A new folder icon appears to represent the *Record Group* with the (*Batch*) name against it.

4: Rename the new *Record Group* - in this example it will be called *#12* - by clicking on the *Rename* button.

5: Type a new name for the *Record Group* and press *Enter* on the keyboard.

6: The new name appears on the *Form*.

Continued... 393



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1: Before images can be captured to the correct *Record Group*, it has to be set as the *Capture Location*. Click on the *Record Group* and...

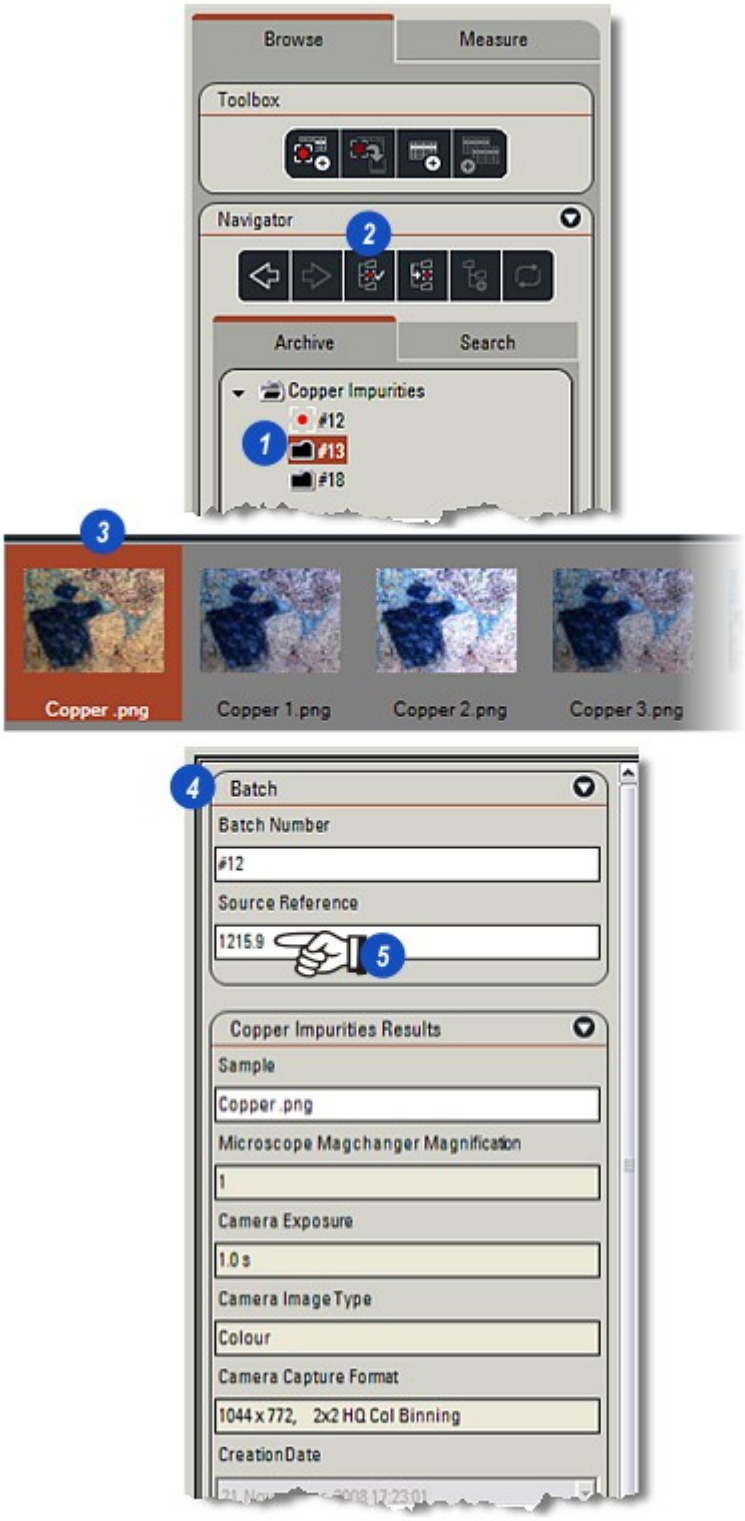
2: ...click on the *Set Capture Location* button. A red dot appears to the left of the *Record Group* to indicate it is set.

3: Capture images in *Browse* or in *Acquire*.

4: As images are captured the *System Data* is displayed on the *Form*.

5: Because in this example *Source Reference* is a required field, it must be completed before an image can be saved.

For details of Deleting and Importing an archive, see Browse: Go there... 421



It is a simple and very fast matter to copy an archive structure - all of the pre-defined fields but no data - give it a meaningful name that reflects the tasks in hand and then start capturing images and data into it.

1: Click on the *Setup Workflow.*

2: Click on the *Create Archive* tab and the *New Folder Archive* dialog appears.

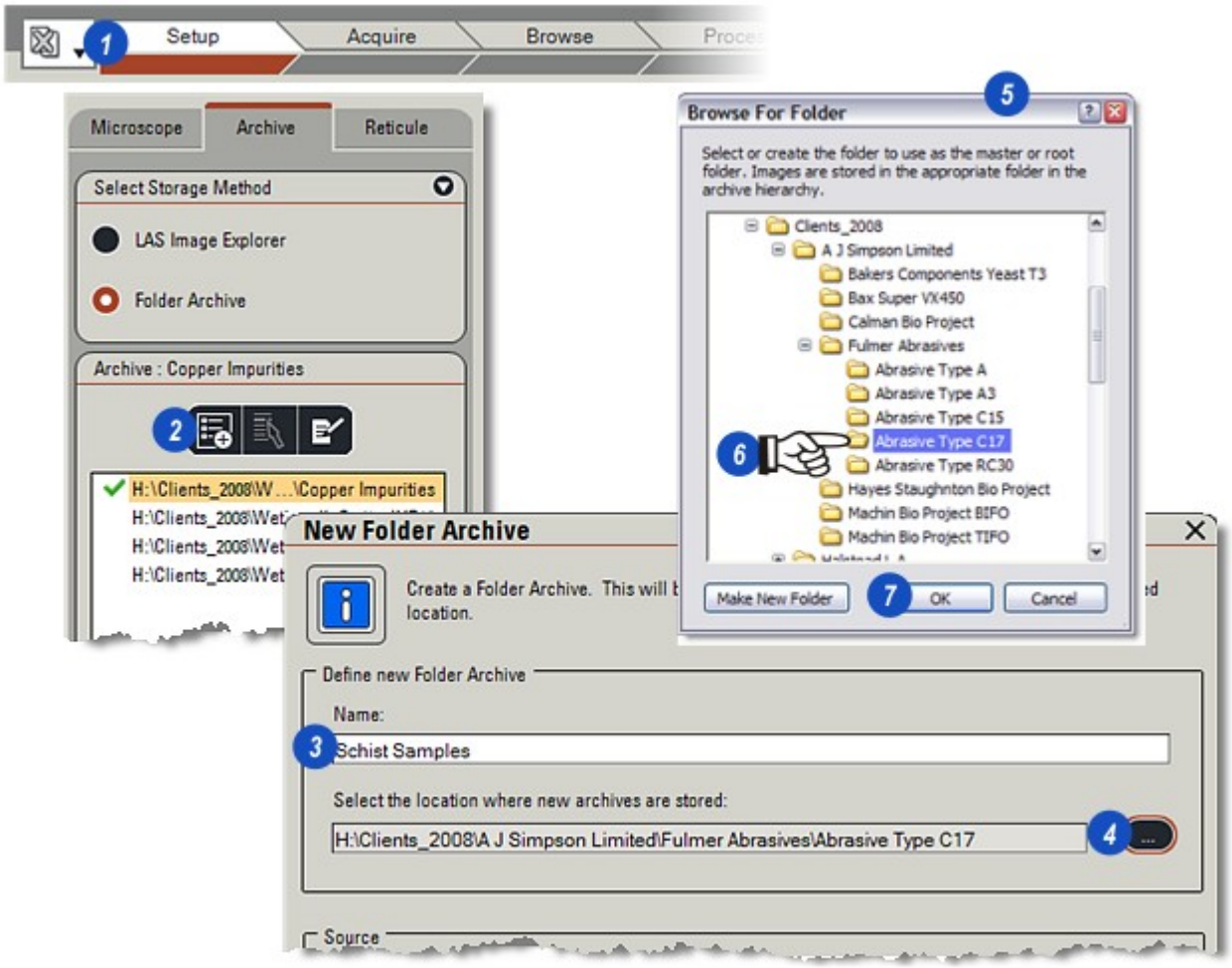
3: Click in the *Name* text box and type a name for the new archive.

4: Click on the *Browse* button to the right of the *Select Location* text box and...

5: ...on the *Browse for Folder* dialog, navigate to the folder **(6)** in which to save the new archive and...

7: Click *OK*. The path of the new location appears in the *Select Location* text box.

Continued... 404



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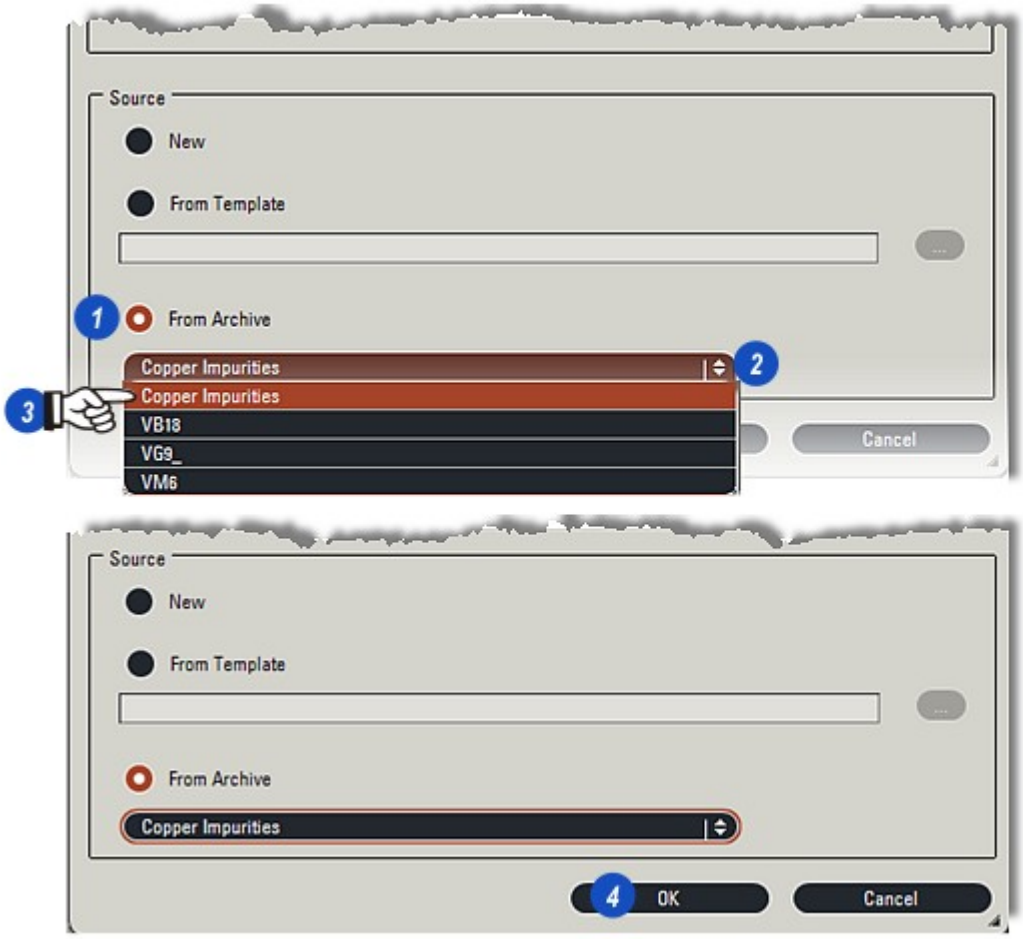
1: Click on the *From Archive* button.

2: Click on the arrows to the right of the *From Archive* header to reveal a list of archives available for copying and...

3: ...click to select the required archive

4: Click *OK*.

Continued... 403

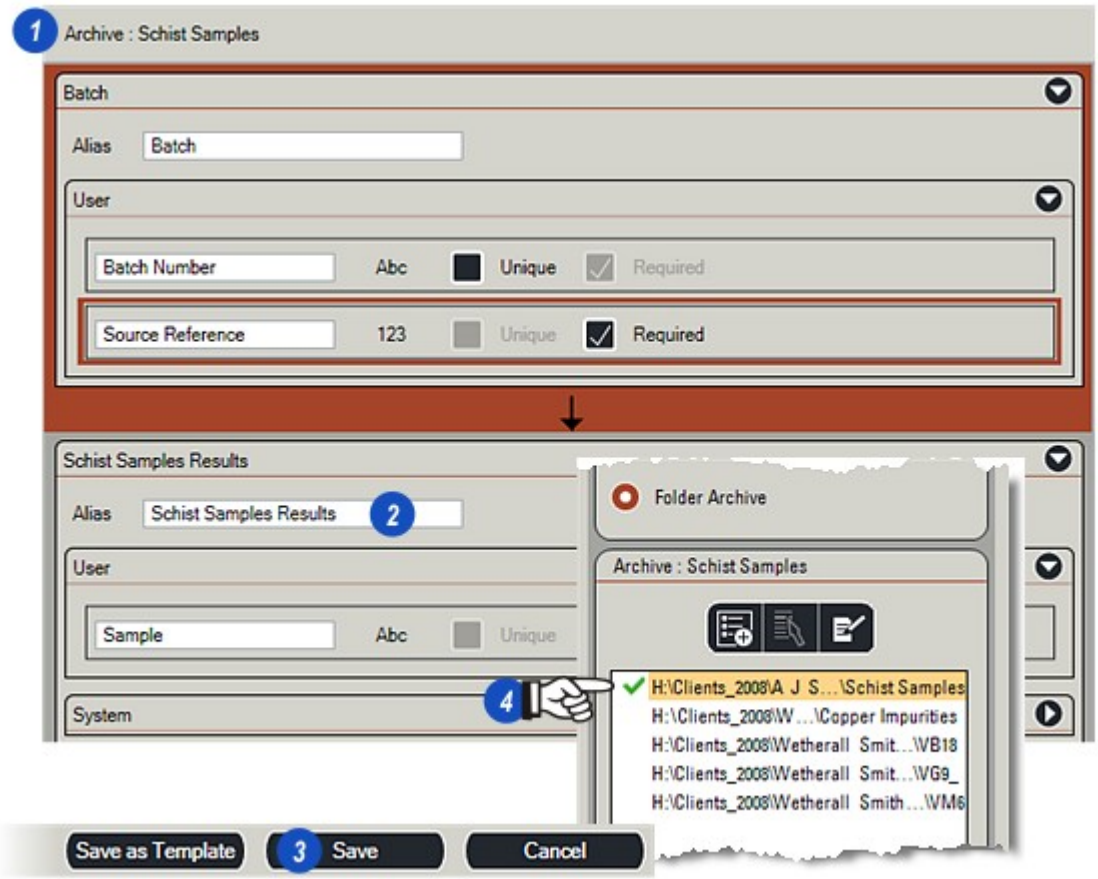


1: A new archive with a new name appears with all of the fields and their properties based upon the original archive.

2: The Field Names can be changed as required by clicking in the text boxes and typing a new, appropriate name. The field type cannot be changed.

3: Click *Save* to save the new archive.

4: It appears in the Archive List, is selected and active and ready to be used just like its 'parent'.



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Any archive may be used as a template for future archive creation, especially useful if a range of archives have to share the same corporate or end-user style.

Only the structure and field names of the source archive are replicated – not the actual data or images.

1: Click to select the Setup Workflow.

2: Click to select the archive to be used as a source for the template.

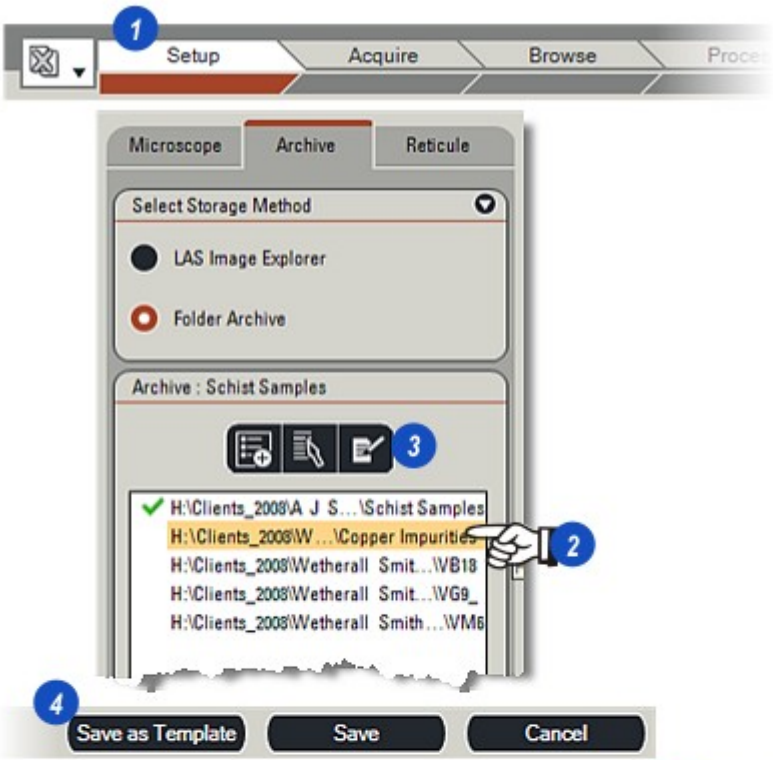
3: Click the *Edit* button. The archive fields will appear.

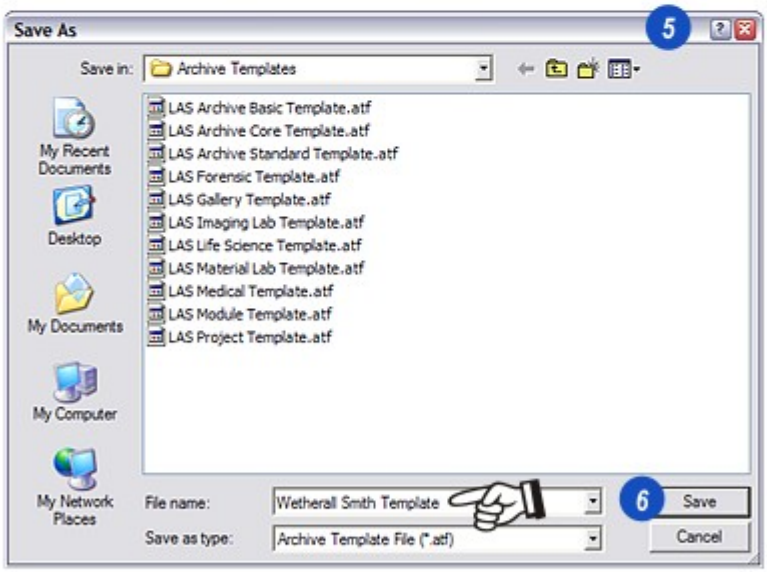
4: Click on the *Save as Template* button.

5: On the Windows dialog navigate to the folder in which to save the template.
Leica Application Suite has a default folder in which to store templates at:
C:\Documents and Settings\All Users\Documents\Leica Application Suite\Archive Templates
…and it is recommended that this be used wherever possible.
Give the template a new and unique name.

6: Click *Save* and the template will be created.

Continued... 406





A new archive can be created quickly and easily using either a template saved by the user or from the range of pre-configured templates provided by Leica and designed to suite a wide range of applications and disciplines.

1: Click to select the *Setup Workflow.*

2: Click the *Create New* button.

3: On the *New Folder Archive* dialog, click in the *Name* text box and type a name for the new archive.

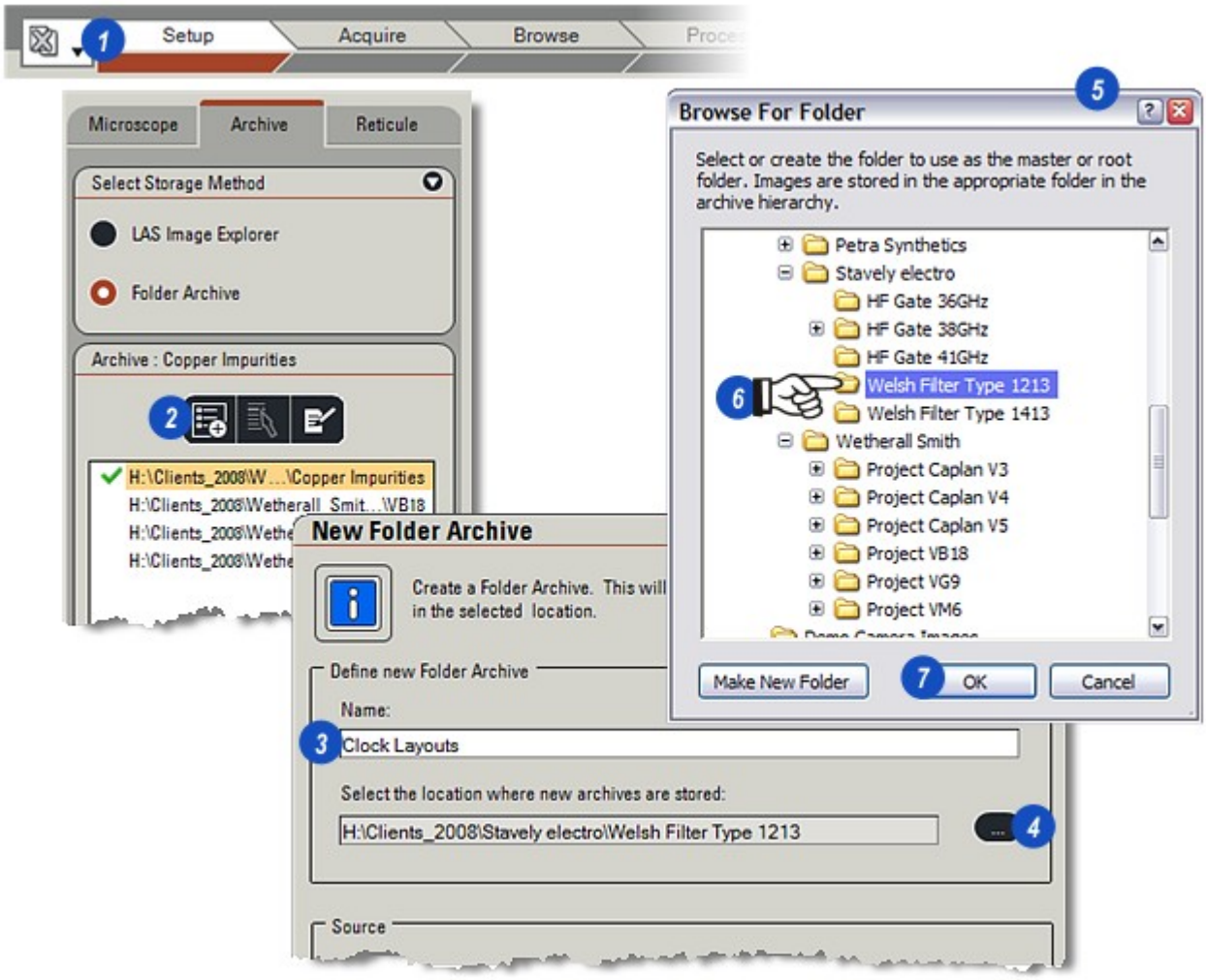
4: Click on the *Browse* button to the right of the *Select Location* text box and...

5: ...on the *Browse for Folder* dialog...

6: ...select the folder in which the new archive will be saved.

7: Click *OK*.

Continued... 407



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1: On the *New Folder Archive* dialog click the *From Template* button.

2: Click on the *Browse* button to the right of the *From Template* text box.

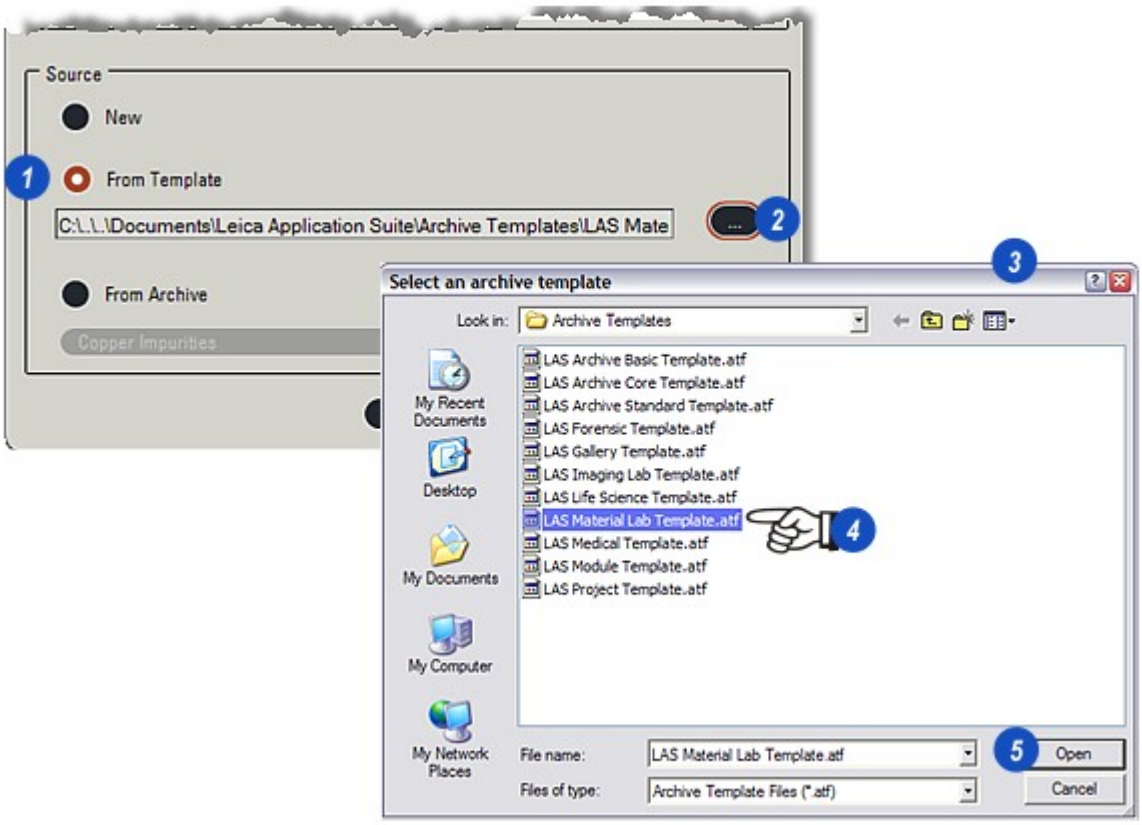
3: The pre-configured templates supplied by Leica are stored in a reserved location:

C:\Documents and Settings\All Users\Documents\Leica Application Suite\Archive Templates

4: Click to select the template style required. The name appears in the *File name* text box.

5: Click *Open*.

Continued... 408

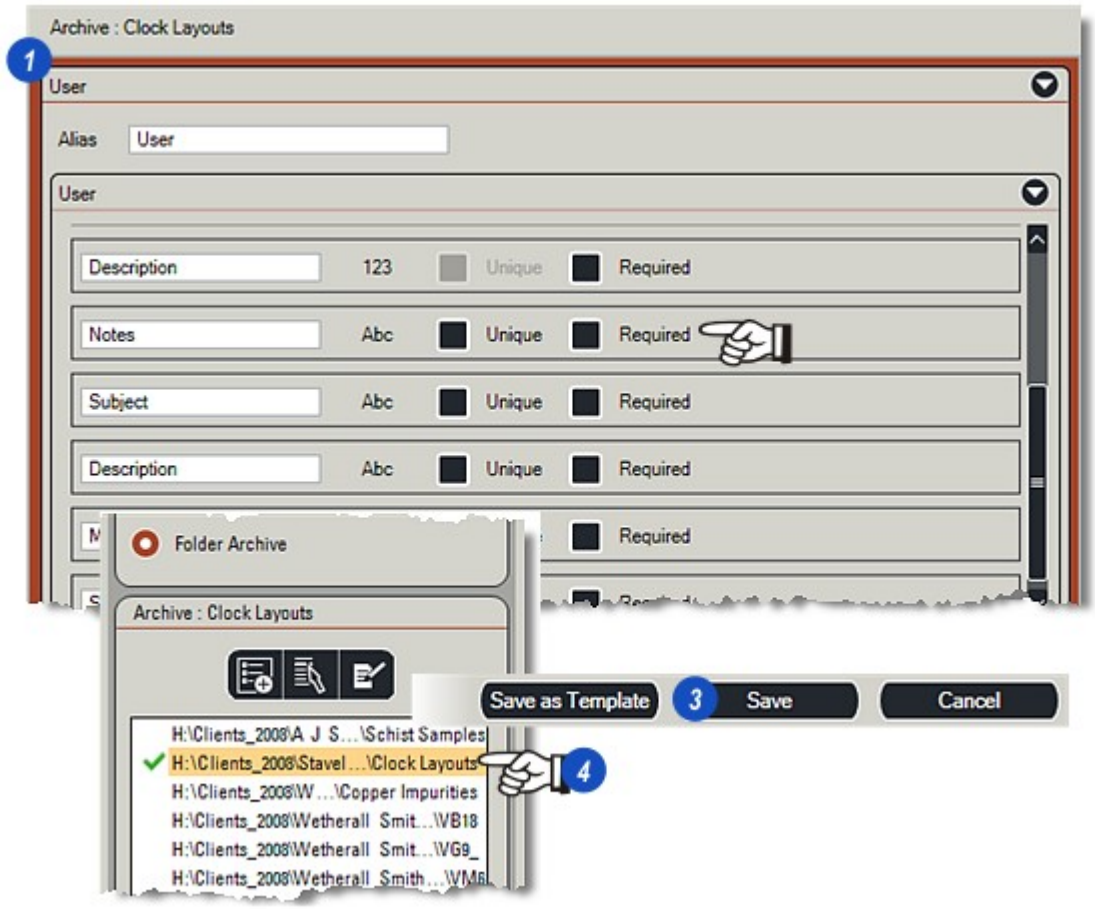


1: The new archive created from the template appears with all of its pre-defined fields.

2: Field names can be changed by clicking in the Field text box and typing an appropriate name. The Field Properties can be set or cleared but the Field style cannot.
The *Unique* property when set means the value in the field cannot be the same as any other.
The *Required* property when enabled means that the image will not be saved until there is some valid data entered in the field.
To set/clear (enable/disable) a property click on the check box to the left.

3: Click on the *Save* button to save the new archive.

4: The new archive appears in the *Setup* > *Archive* window,



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The *Grid* displays data for all of the images in a folder in a tabular structure. The image names are listed on the left and the data items as headers across the top.

1: The *Grid* is revealed and hidden by clicking on the *Side Tool Bar* button. This is a toggle – click to reveal, click again to hide.

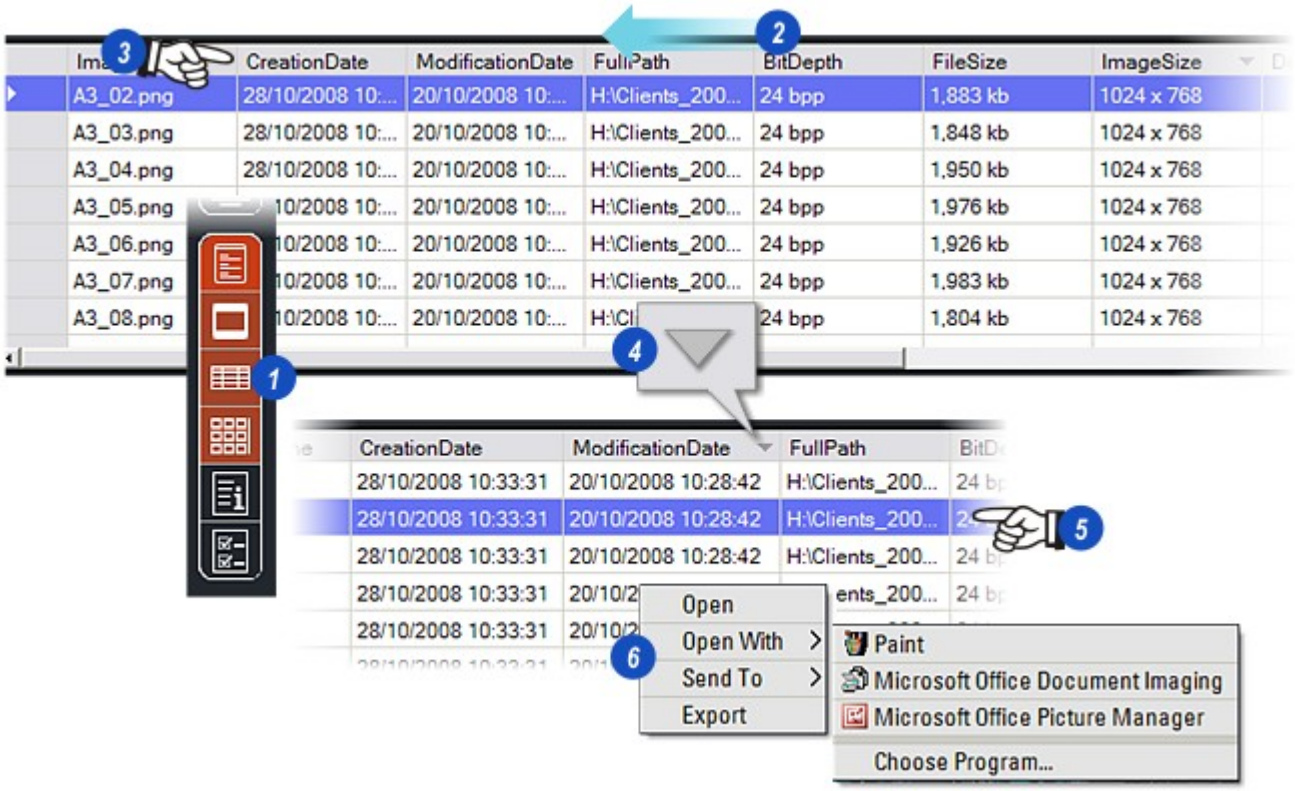
2: The header positions can be changed by clicking and holding the left mouse button on the header to be moved, dragging it to the new position and releasing the mouse button.

3: The column widths can be changed by clicking and dragging the vertical bars that separate the columns.

4: Clicking on an entry in the *Grid* will immediately display that image in the *Viewer* and also highlight the thumbnail.
Hold down the *keyboard Ctrl key* whilst clicking to make multiple selections prior to deleting or exporting.
Keyboard combination *Ctrl + A* will select all of the image data: *Ctrl + C* will copy all the selected image data to the clipboard and *Ctrl + V* will copy into another application.

5: A small arrow is revealed when a header is clicked. This allows the image data to be sorted – high-to-low or low-to-high – by successive clicks on it.

6: The *Grid* data can be exported to a range of other applications by right-clicking on the *Grid* then navigating to and clicking to select an application.



The *Search* option provided with optional *Basic* and *Standard* editions, is both fast and flexible. It is possible to search on all fields within a record group or on specific selected fields. An editor is available to create search configurations which can be used at any time simply by clicking them.

Rapid Search:
Locating specific items which have known names or text strings, is achieved quickly by:

1: Select *All Fields* in the Search Toolbar field selector - bottom right of the Viewer.

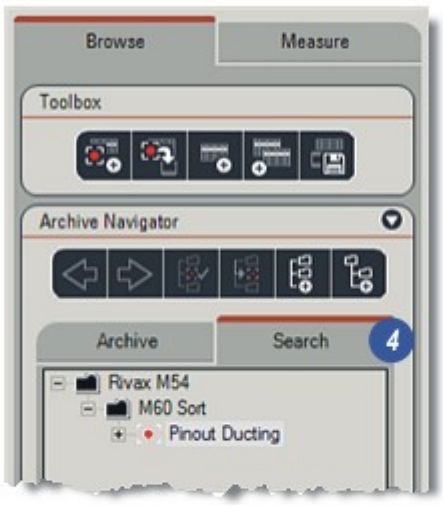
2: Type the name or text string into the *Search For* window.

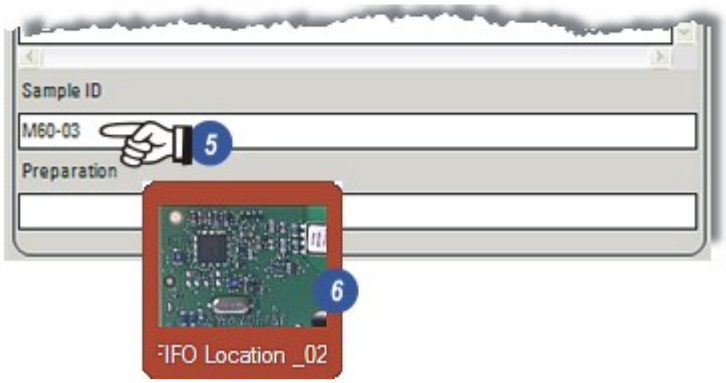
3: Click on the *Search* button.

4: If a match is found the *Search* tab is automatically selected showing the Archives in which the search was made, and...

5: ...the appropriate record is populated - if there are more than one that satisfies the search criteria, the first is selected.

6: The image *Thumbnail(s*) appear in the *Gallery.*





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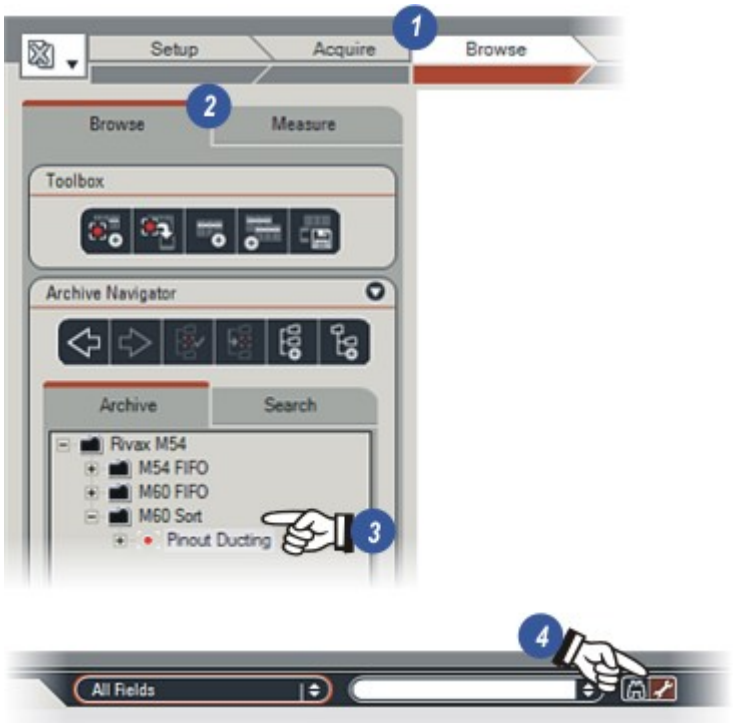
A *Search Configuration* is a file that contains all of the parameters - such as fields and search strings - needed to carry out a fast, repetitive search.

Search Configurations are stored under unique names and can be retrieved and used for fast searching, or modified to reflect changing search requirements - for example the search fields could be extended and search strings altered.

Mixing field types in a single configuration has made LAS Archive Search even more powerful. Now, up to 10 fields of different types - text, Boolean, date and so on, - can be mixed and each setup for different search criteria. And Boolean limits can be set to ensure that the search is narrow and very precise; Use the AND command to ensure that only those images that conform to every field search string are returned; Alternatively, the OR command will only retrieve images that fulfill at least one search parameter.



Create a New Search Configuration:

1: Click on the *Browse Workflow*.

2: Click to select the *Browse* tab and...

3: ...check that the correct archive is displayed in the *Archive* window.

4: Click on the *Configuration Editor* launch button on the *Search Toolbar*.

1: On the *Search Configuration Editor* dialog, click the *New* button to create a new configuration.

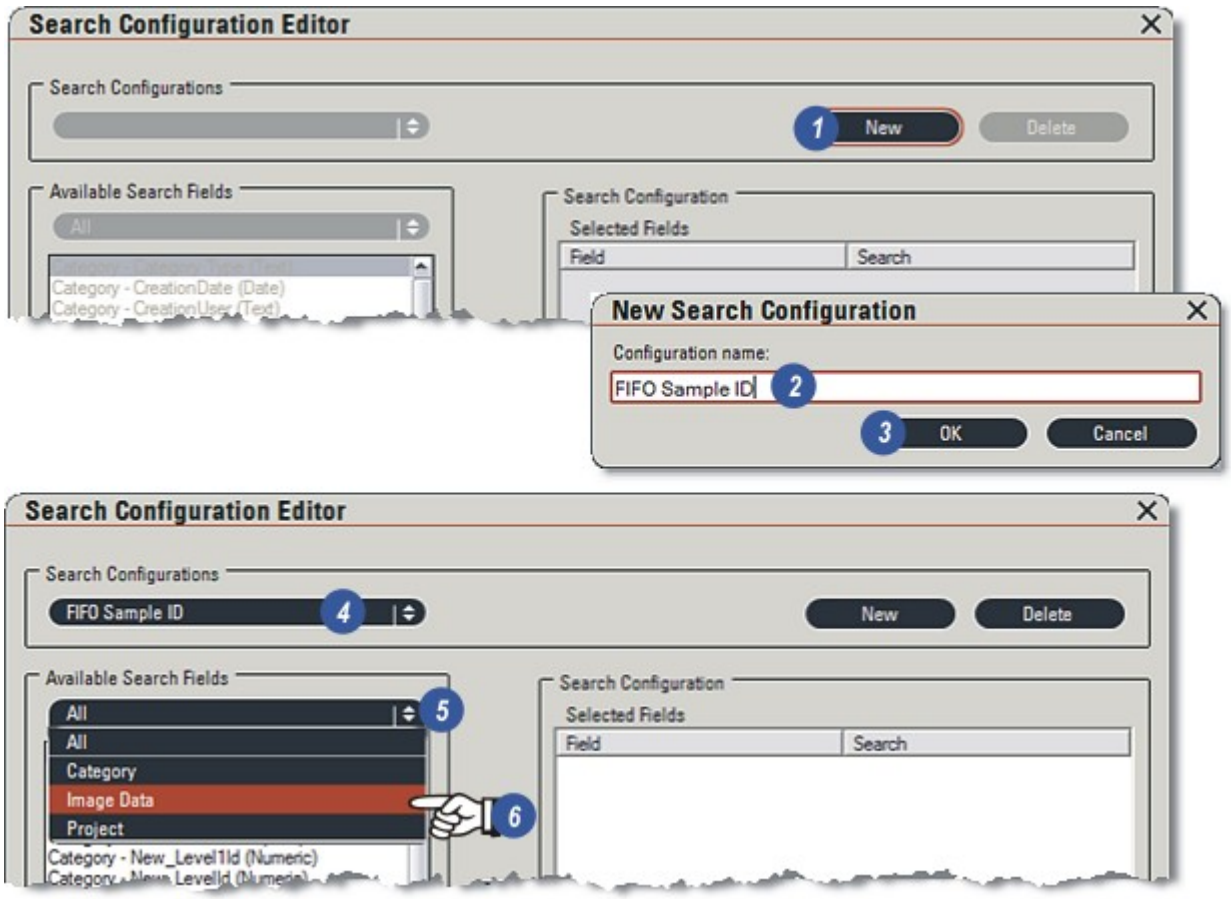
2: Give the new configuration a unique name by clicking in the *Configuration Name* text box and typing.

3: Click *OK.*

4: The new name appears in the *Search Configuration* list.

5: The fields to search can be set to *All* - the entire record set - or narrowed to include only specific groups by clicking on the arrows to the right of the header and...

6: ...clicking to select the group to be searched.



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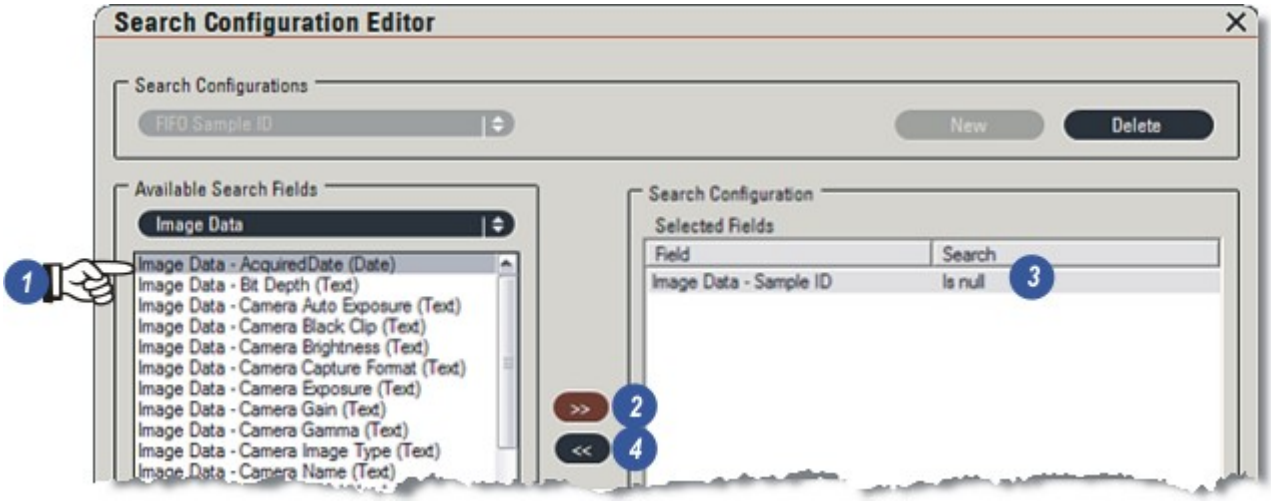
All of the available fields are shown on the left pane of the Search Configuration Editor. To include a field in the search:

1: Click on the field to select it.

2: Click on the *Select* button and...

3: ...the field name appears in the *Selected Fields* pane. Any number or any type of field can be selected.

4: To remove a field from the configuration, click to select the field in the *Selected Fields* pane and click on the *De-select* button.



The search format for a field depends upon the field type - Text and Memo fields will be searched for character strings, Numeric for numbers - and so on. The appropriate format options are automatically displayed for the selected field and are explained on the following pages (Go there...). The illustrations show a text field called 'Sample ID'.

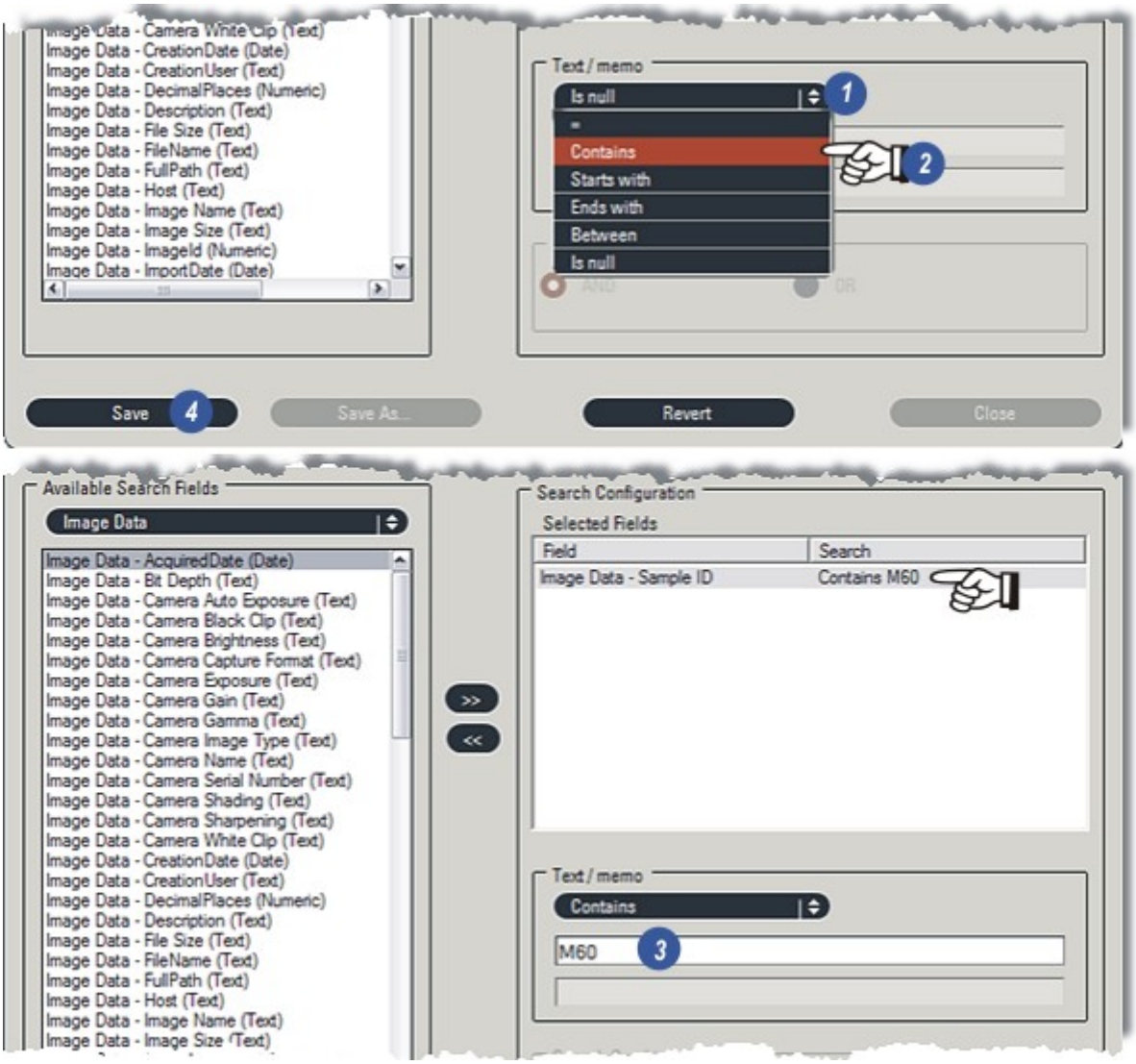
The search format is selected by:

1: Click on the arrows to the right of the format header.

2: From the drop down menu click to select the desired format. In the illustration the '*Contains'* format has been chosen.

3: Now the actual search string has to be entered. Click in the window and type the required string to search for. In the example ***'M60'*** has been entered so any image with a Sample ID field containing the characters ***'M60'*** will be returned as fulfilling the search criteria. The *'Contains'* option means that text such as *'Sample **M60'*** or *'Batch **M60** Local'* will satisfy the criteria.
The search string appears in the *Selected Fields* pane to the right of the *Field* name.

4: Click the *Save* button to save the configuration.



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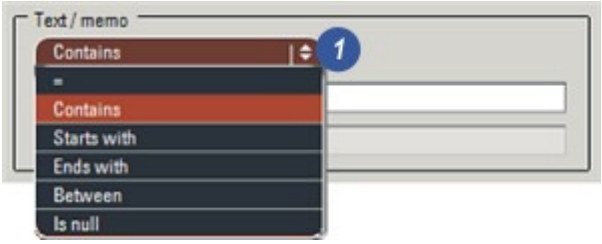
Each field type has a range of search string options associated with it which are displayed automatically. The types and options are:



Text and Memo options: Accepts numbers and characters in the search string:

1: Click on the arrows to the right of the header and click to select from the drop down.

=: The field data must match exactly the search string.

Contains: The search string can appear anywhere within the field data.

Starts with: The field data must begin with the search string.

Ends with: The field data must end with the search string.

Between: Two search strings are entered and each computed as an ASCII string value - converted to a number. The field data, also converted to an ASCII value, must lay between the two.

Is Null: The field must be empty (nothing).

Date options:

2: Selected from the drop down with additional options available if the down arrow **(6)** is clicked.

The *Date Picker* provides a simple way of moving between Years and Months (click the arrows (4), and by clicking on the required day (5).

On: Date in the field corresponds exactly with the date selected.

Before: The field date must occur before the selected date.

After: The field date must occur after the chosen date.

Between: Two date windows **(3)** open and a date is selected for both. The field date must occur between the two.

Is Null: No date in the field: Nothing.

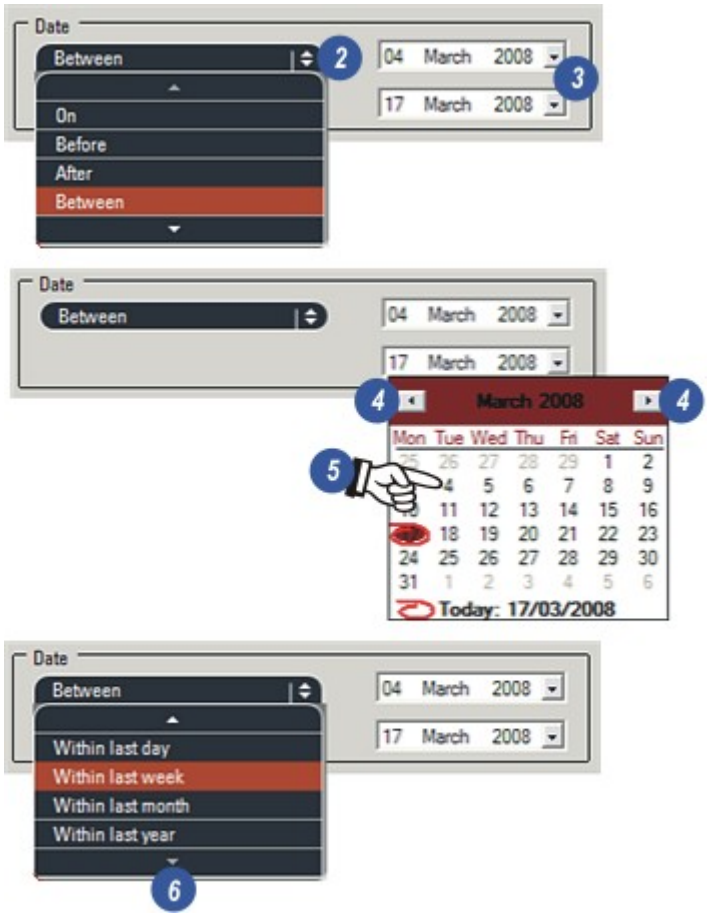
Within options:

Last Day: During the last 24 hours.

Last Week: During the last 7 days. If it is 10am on Tuesday all records that have a date and time equal or later that 10am on the previous Tuesday will be found.

Last Month: If the current date is 17 March then all records from and including 17 February of the same year are found. Leap years are automatically accommodated but the time of day is ignored.

Last Year: All images with a date on or later than the same date during the past year will be found. Leap years are accommodated automatically but the time of day is ignored.



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Numeric options:

1: Click on the arrows to the right of the header.

2: Values are entered in the text boxes. The option chosen will determine if 1 or 2 text boxes open.

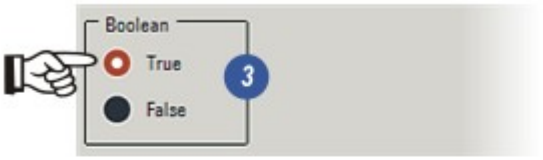
=: The field value must match the search value exactly.

<: The field value must be smaller than the search value.

>: The field value must be greater than the search value.

Between: Two values are entered for the search value limits and the field value must lay between the two.

Is Null: No value in the field: Nothing, not even zero (0).



Boolean options:

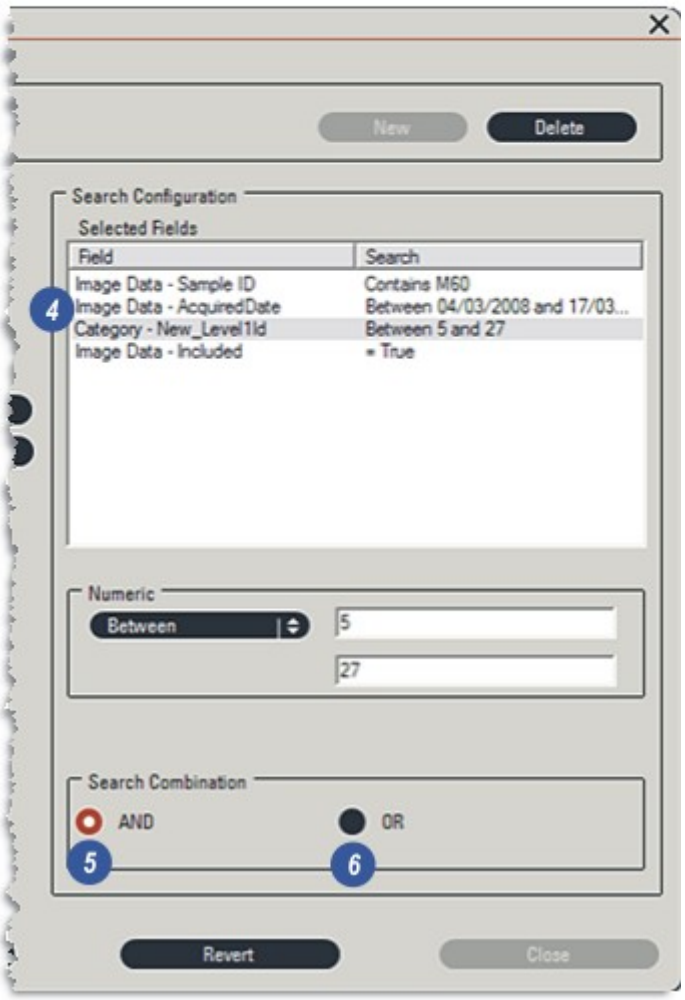
3: Select either *True* or *False*. The field setting must match.

Multiple Field Search:

4: Up to 10 fields of any type 'mix' of can be selected for a search and in this case the *Search Combination* is enabled. It is based upon two Boolean parameters...

5: ...*AND* determines that ***all*** fields must conform to the search strings to return an image.

6: *OR* means that ***one or more*** fields must satisfy the search conditions to return the image.



Delete a Search Configuration:

1: Click on the arrows to the right of the *Search Configurations* menu and click to select the configuration to be deleted.

2: Click on the *Delete* button.

3: Confirm the deletion. Deleted configurations cannot be recovered.

Save a Configuration As...

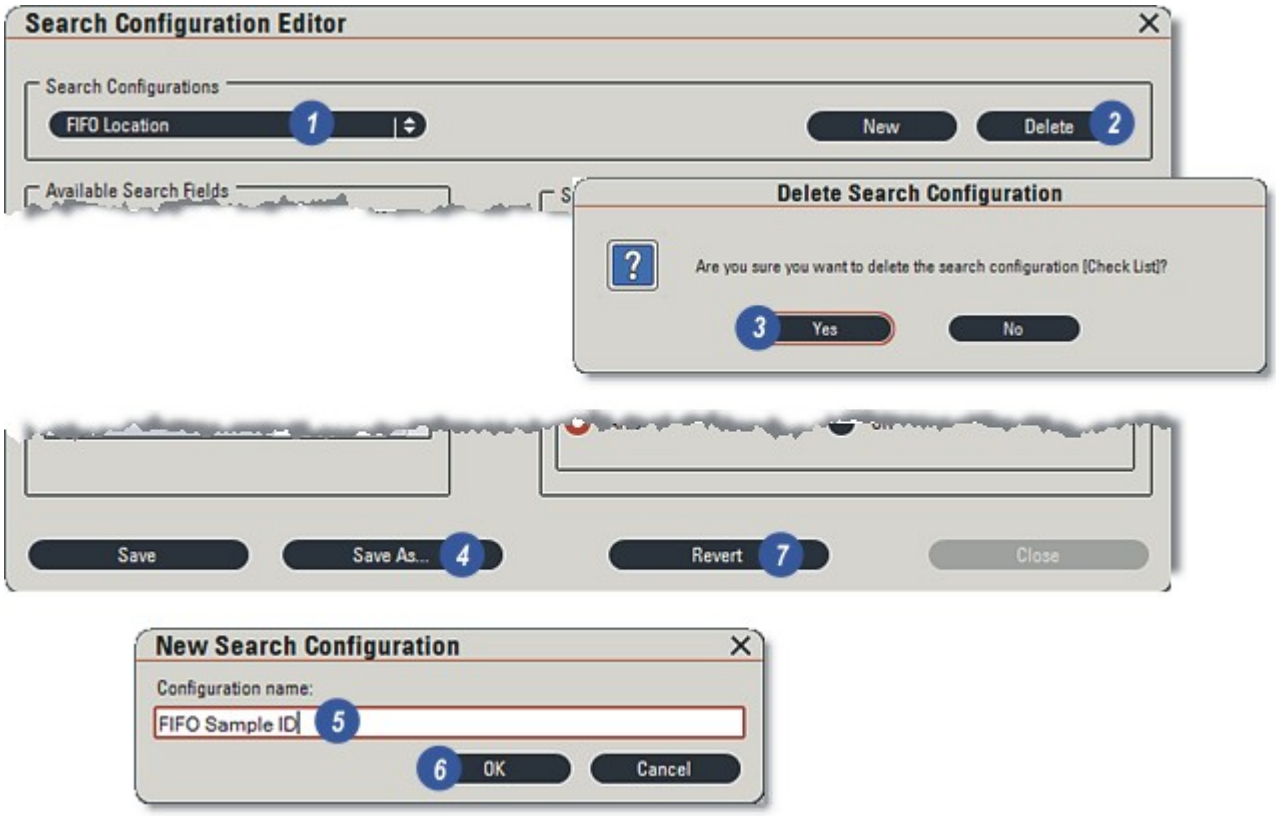
4: Clicking the *Save As* button will...

5: ...reveal the *Search Configuration* dialog. Click in the text box and type a new name for the configuration.

6: Click *OK* to save the search configuration.

Revert:

7: The *Revert* button will set the configuration to the last one saved. Any changes made since will be lost.



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If a new configuration has just been created, all that is necessary is to click on the *Search* button **(5)** to run the search.

Search using an existing Configuration:
To run a search using a previously created configuration:

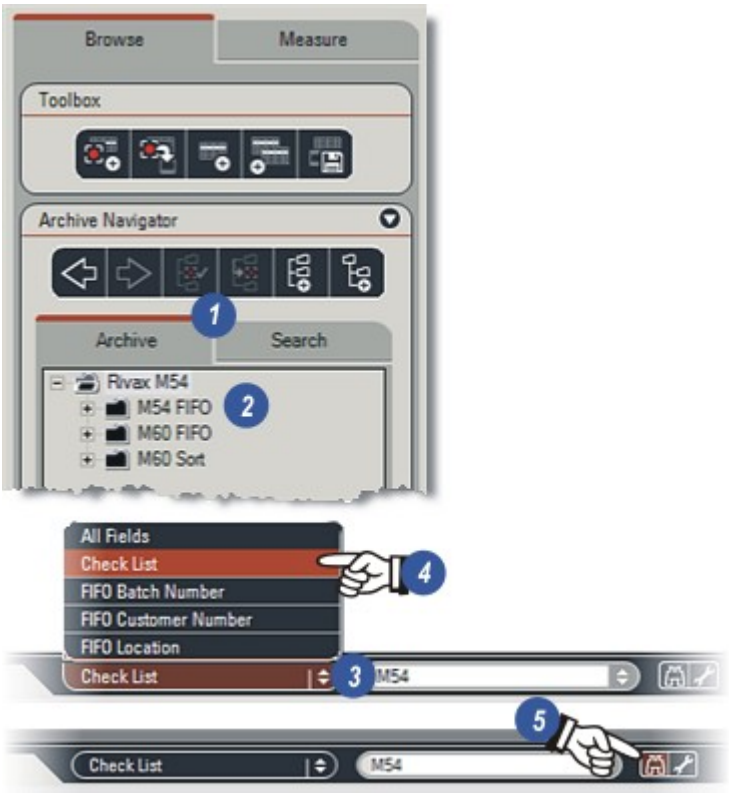
1: Click on the *Archive* tab and...

2: ...check that the required archive is selected and active.

3: Click on the arrows to the right of the *Search Configurations* header on the *Search* bar.

4: From the pop-up menu, click to select the configuration required.

5: Click on the *Search* button.

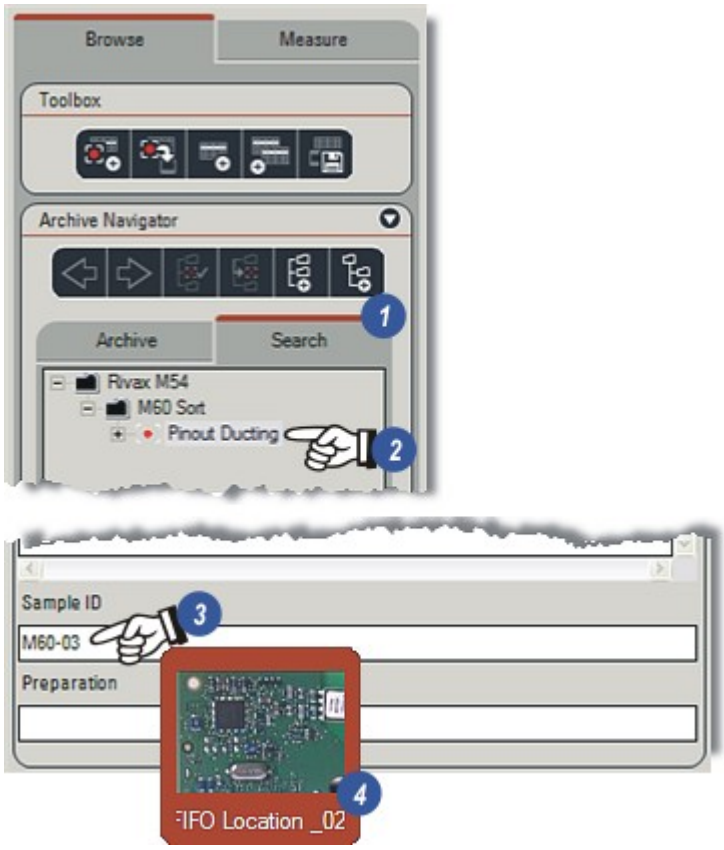


1: If a match is found the *Search* tab is automatically selected...

2: ...showing the Archives in which the search was made, and...

3: ...the appropriate record is populated - if there are more than one that satisfies the search criteria, the first is selected.

4: The image *Thumbnail(s*) appear in the *Gallery.*



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Any other file, including Audio and Text can be attached to an archive using the Attachments panel in the Browse Workflow. This feature is only available with Basic and Standard Editions.

Audio, either speech or music is limited to a running time of 2 minutes, and text files should be no greater that 32 MB.

1: Click on the arrow to the right of the *Attachments* header to reveal the Audio panel **(2)** and Text File panel **(3)** .

To attach, play and erase an Audio file:
Audio input can come from either the computer microphone input or the line input for an external CD or tape player.

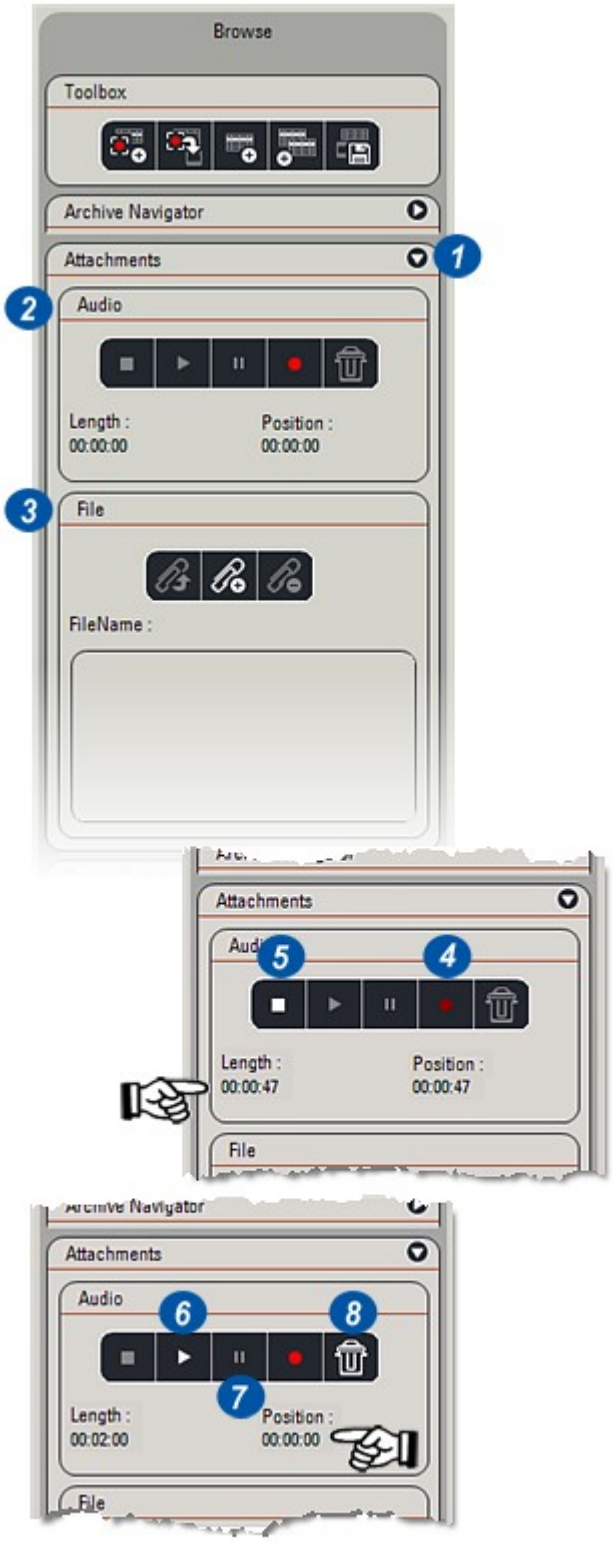
4: Click on the *Record* button to start the recording.

5: Click the *Stop* button to stop recording which will automatically stop at 2 minutes duration. The length of the recording in seconds is displayed beneath the Stop button.

6: To play the recording click on the *Play* button.

7: Halt the playback with the *Pause* button and click it again to resume playback. The recoding Length and current position as shown on the panel.

8: Erase the recording by clicking the Trash button.



1: Click on the *Attach* button.

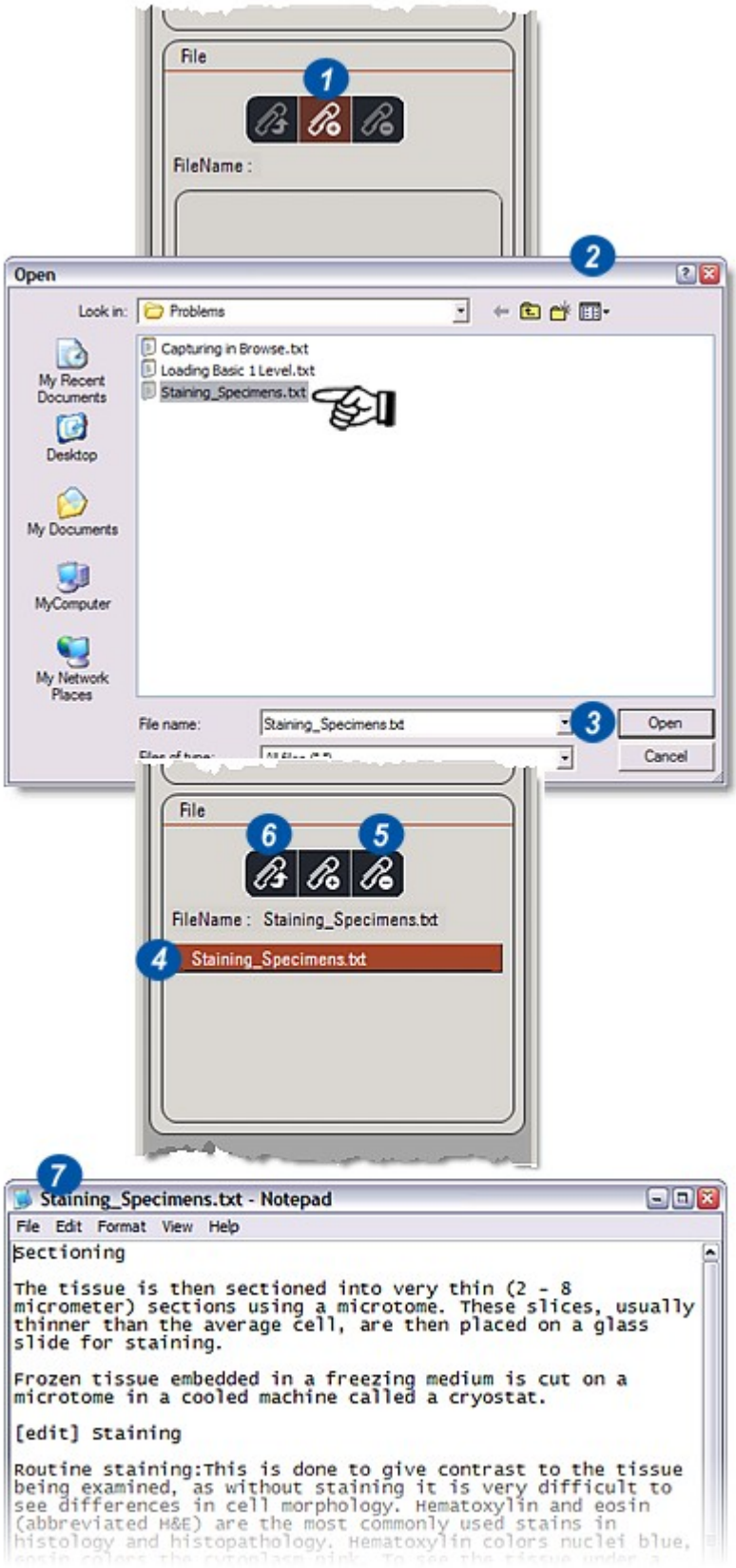
2: On the Windows Open dialog, navigate to the file to be attached, click to select it and…

3: …click *Open*. The file is attached to the database and…

4: …it is listed in the Filename window. In principle any number of text files may be attached to a database limited only by an individual file size of no more that 32 MB and an overall database size of 4GB.

5: To detach a file, click on the file to be deleted in the Filename window and then on the *Detach* button.

6: Click on the *Launch* button to display the selected file in Windows Notepad **(7).**



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LAS Archives Standard Edition

The Standard Edition Archive extends the power and flexibility of the Basic Editions to provide:

- Multiple archive levels.
- Wider choice of Named Archive Fields - Memo, Boolean, Numeric, Date.
- The Keywords feature establishes preferred field descriptions.
- Highly Detailed Reports to include scaled images.
- Export Report to Word, Adobe Acrobat (pdf) and Browser (html) facility.

The *Standard Archive* option provides a further 4 field types:

1: *Numeric*: 32-bit floating point numbers.

2: *Memo:* Alpha-numeric with over 32,000 characters. Line returns are added automatically as the text is entered.

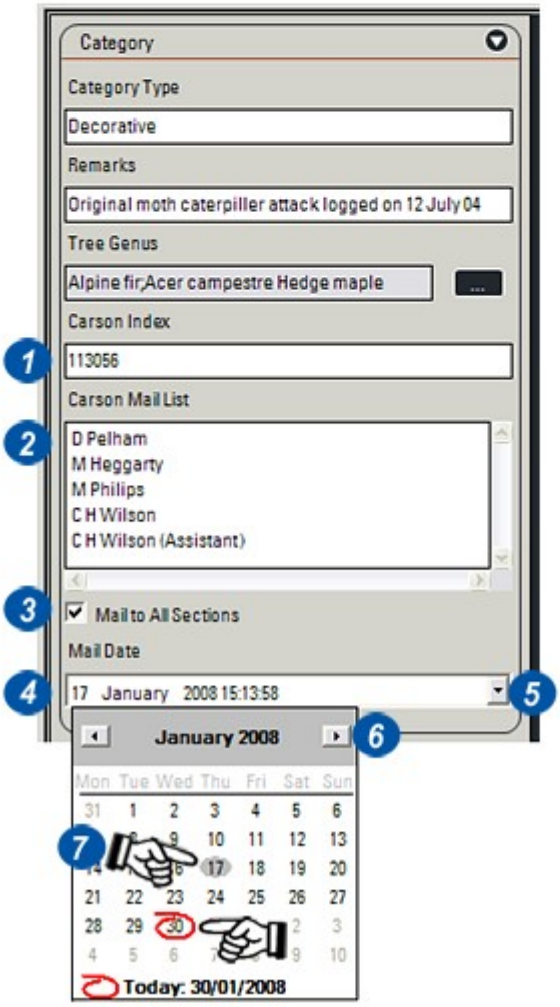
3: *Boolean:* Yes/No: True/False. On the Report this appears as a check box which, when enabled equates to true (Yes).

4: *Date:* On the report this is selected using the *Date Picker* by...

5: Clicking on the arrow to the right of the date text box.

6: Using the left and right pointing arrows to scroll through months and years, either forward or backward.

7: Clicking to select the date required. Today's date is circled in red.



To add a new field to an archive:
With the archive active:

- Select Edit mode.
- Click to select either User or Image field groups.
- Click on the required field button and the new field will appear.
- The name can be altered by clicking in the field window and typing a new name.
- Click the Save button to save the amended archive.

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A report contains an image or collection of images together with associated data extracted from the database and presented as a document in *Microsoft Word, Adobe Acrobat Pdf* or *Browser Html* format.

Reports are formatted according to predefined templates that are created in *Microsoft Word (2003 or later)* to create report templates.

Although the information and images comes from and is generated by the *Database*, the report is stored using Windows navigation and folders which allows other applications to use it.

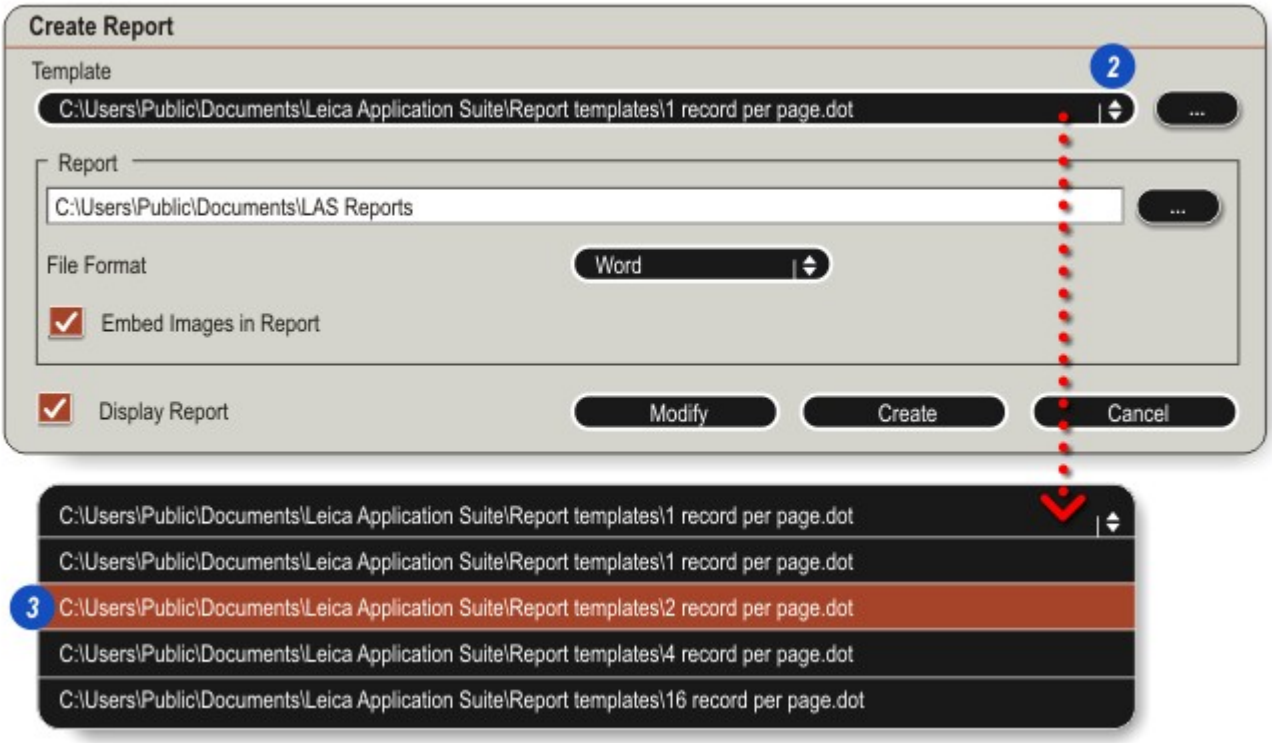
Standard Templates determine how many images may be displayed on a page. Images and text are automatically scaled to fit the page.

1: Click on the *Create a Report* icon.

2: To use a *Standard Template* supplied with LAS Archives, click on the arrows to the right of the window and from the drop-down template list...

3: …click to select the option required.

Continued...



Create a Report: Select a User Template:

To select a template created by a user:

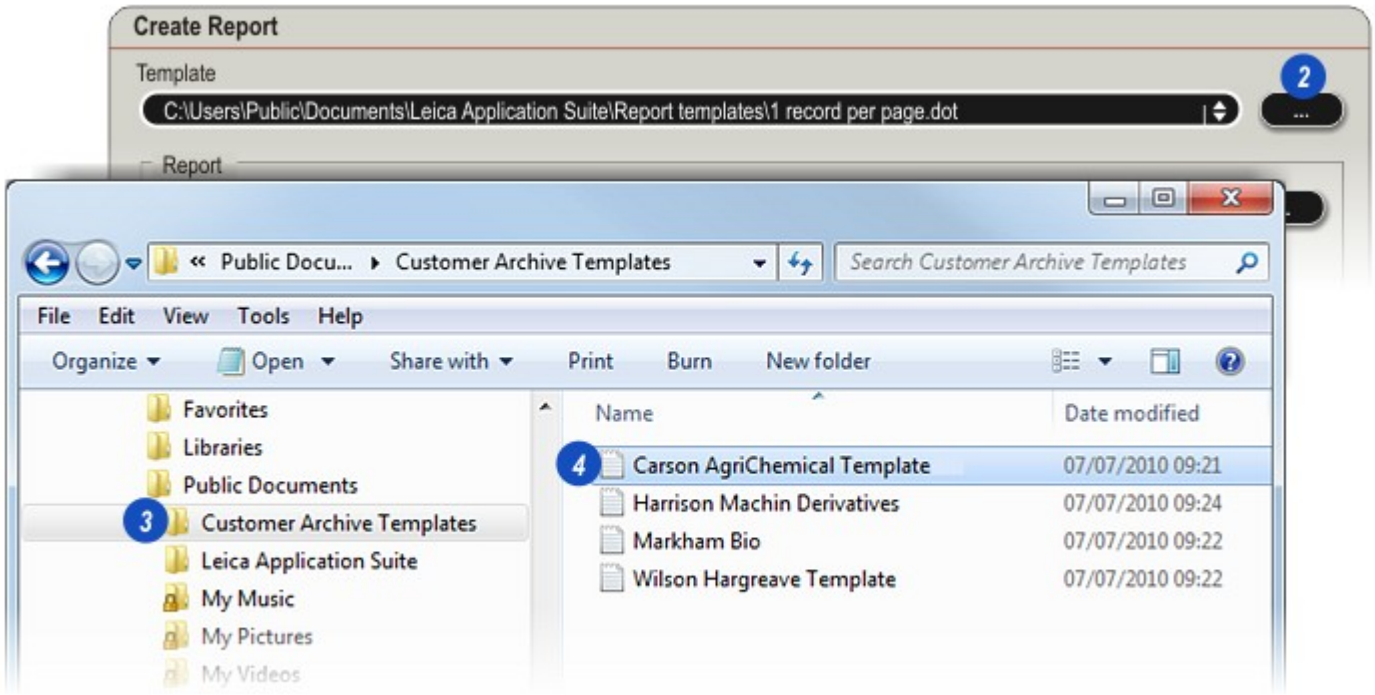
1: Click on the *Create a Report* icon.

2: On the *Create Report* dialog, click on the browse button.

3: On the Windows dialog, navigate to the folder in which the user templates are stored and...

4: ...click to select the template.

Creating a User Template: *Go there...* 432



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If multiple images are to be shown on the report all must be selected:

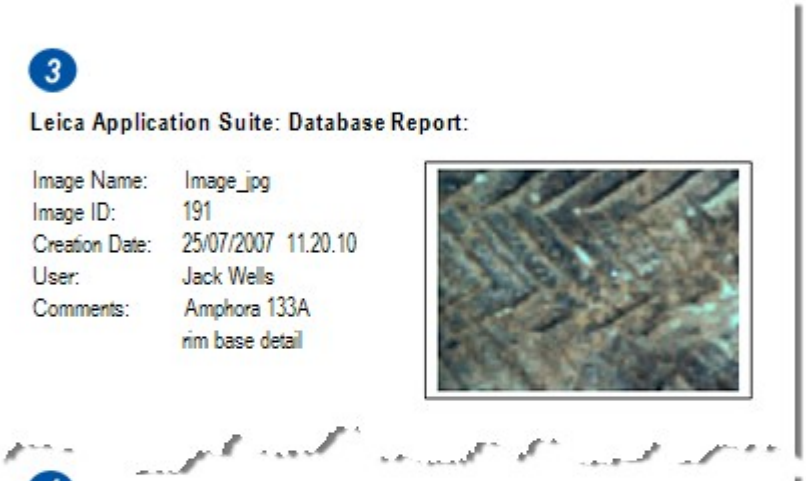
1: On the keyboard, press and hold down the *Shift* key and click on each of the thumbnails of the required images or…

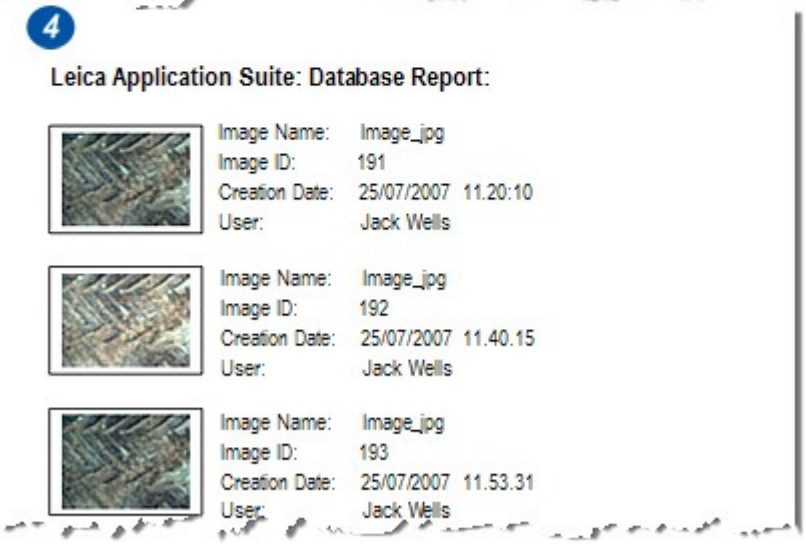
2: Press and hold down the *Shift* key and click on the image entry on the *Grid*.

3: The examples opposite show the effects of choosing a template which will display 4 records and...

4: ...another to display 16 records.

Images and text are automatically scaled to fit the text box.





Reports created using *Microsoft Word* can include images in two ways:

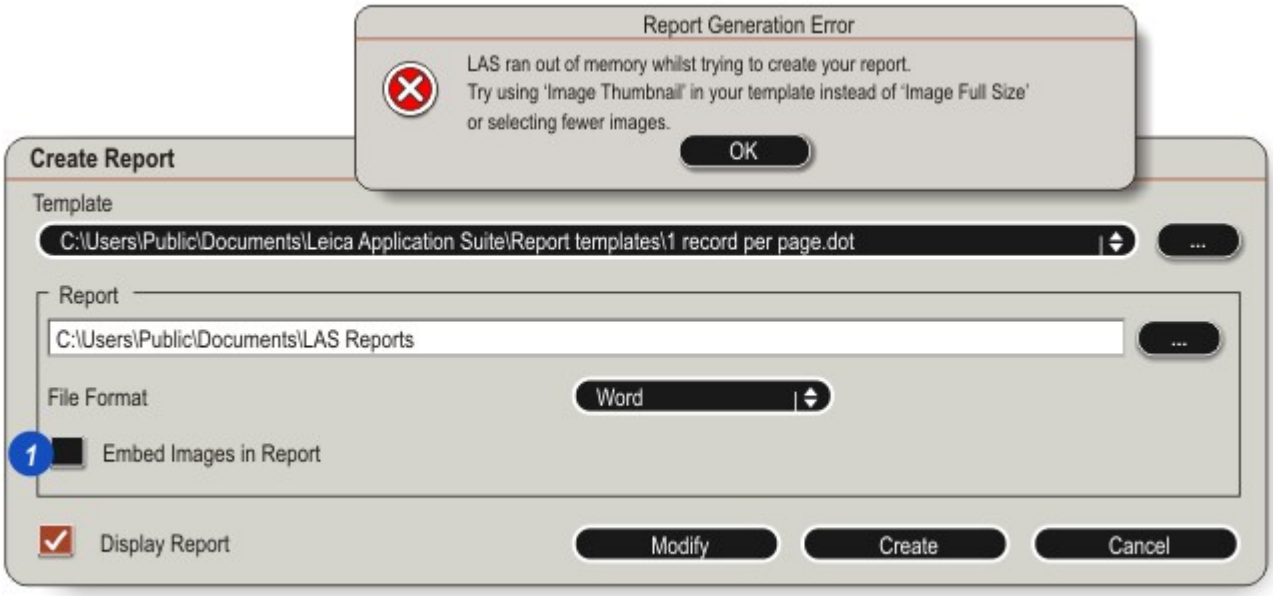
- Image Embedding: Copies the image into the report so that it becomes an intrinsic part of it. Embedding can result in very large files but they are always integrated - text and images cannot be separated.
- Image Linking: A path is established between the report and the image source and the image is included only when the report is displayed, printed or exported - by e-mail for example. Linking creates small, compact files but if the image source is moved or changed the link is lost and the images will not appear.

1: Click to choose *Embed Images* (check box ticked) or *Link to the Image* source (check box not ticked).

Images can be very large and occasionally – especially in reports that have many images – there is insufficient machine memory and a *Report Generation Error* occurs.

To cure the problem either:

- Reduce the number of images.
- Acquire smaller images by using a more compressed file format and lower resolution.
- Resample the images to a lower resolution.
- Use a report template that allows the use of LAS high quality thumbnails rather than full-size images.



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Create a Report: Select a File Format:

Select the format in which the file should be created by:

1: Click on the small arrows to the right of the *File Format* header.

2: From the drop-down list, click to select the required format.

To display the report after it has been created:

3: Click to enable (ticked) the *Display Report* check box.

To create the report:

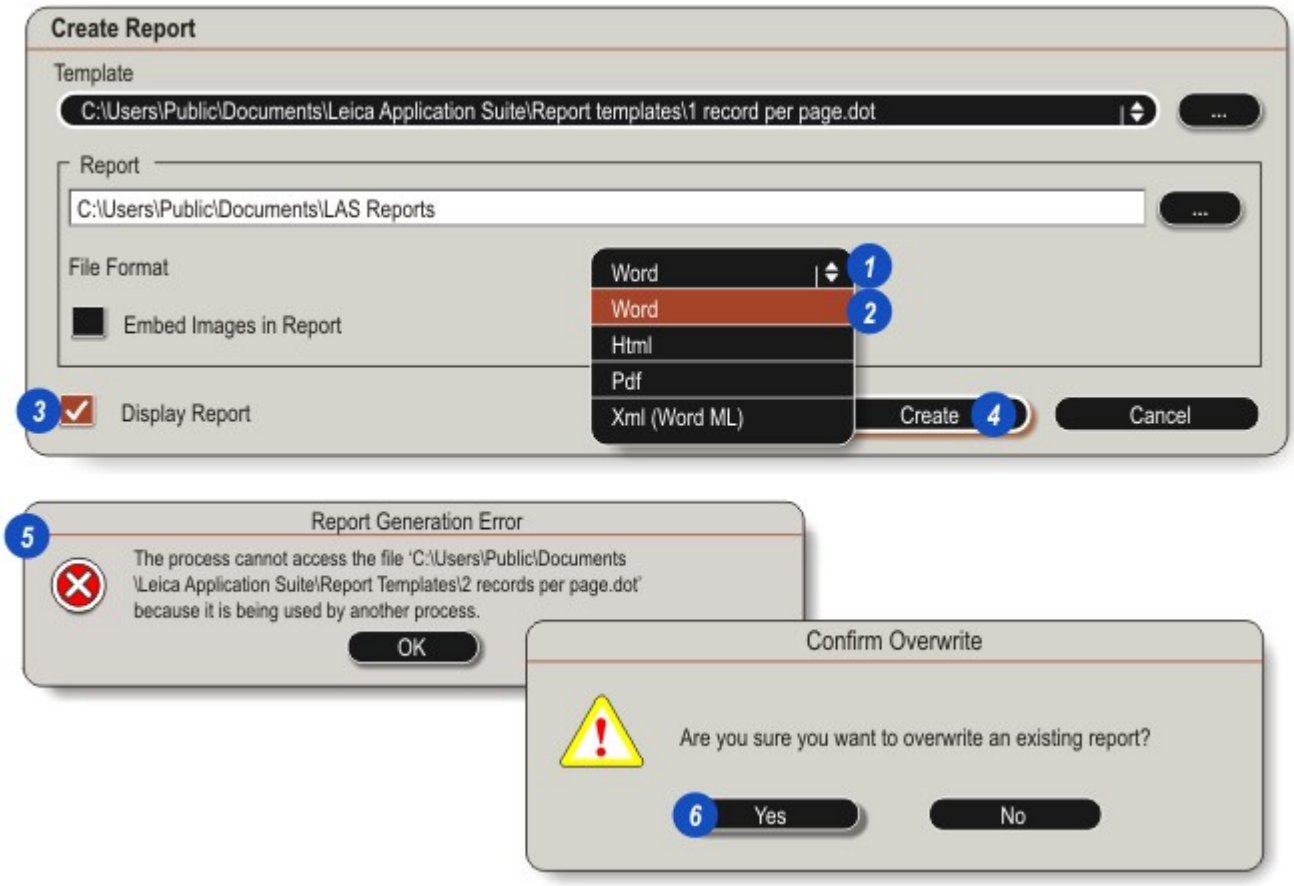
4: Click the *Create* button.

If the template is already open in another application - perhaps in the process of being modified:

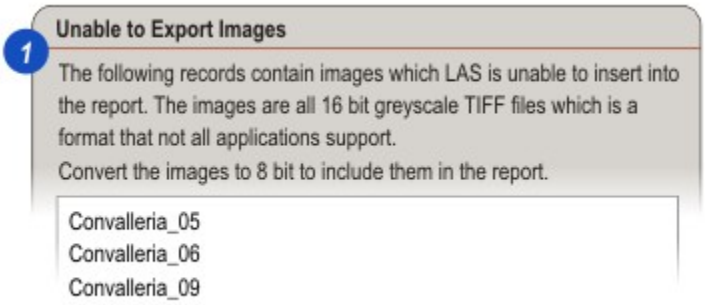
5: The *Report Generation Error* occurs. Close the other application and click the *Create* button again.

The Output File already exists:

6: The *Confirm Overwrite* warning appears. Confirm the overwrite or click *No* and give the file another name.

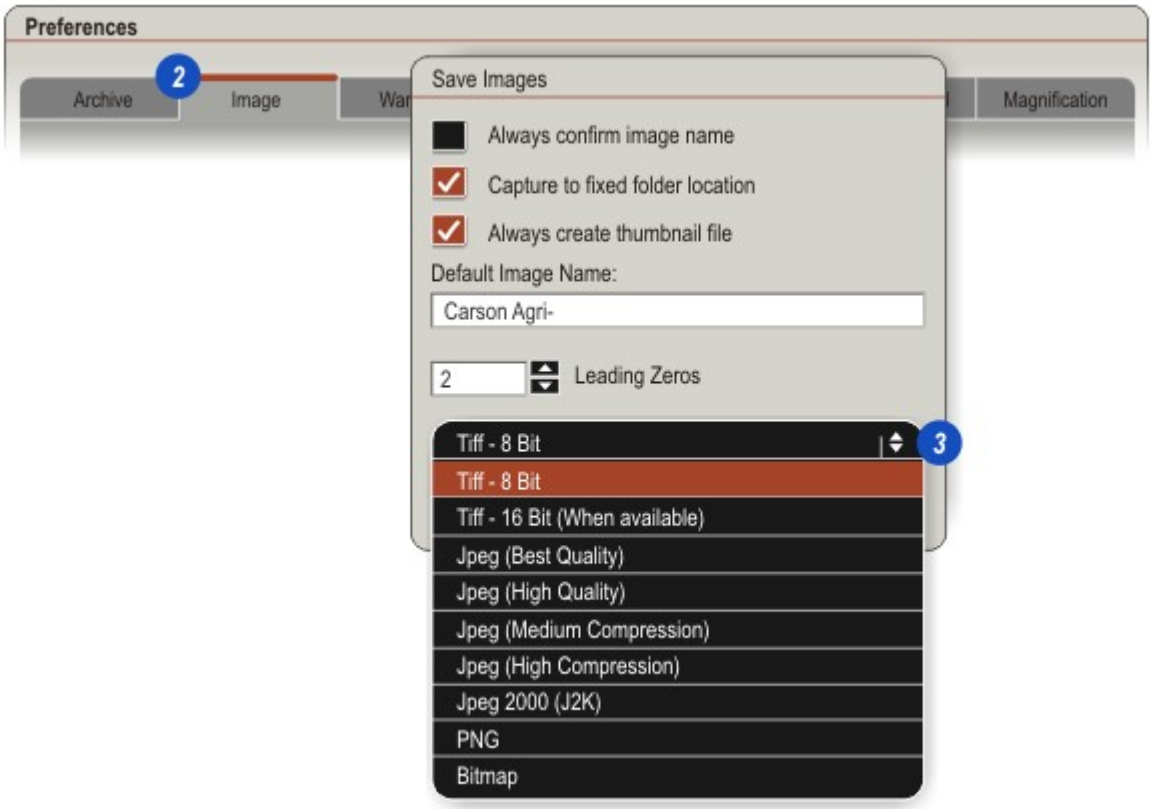


Earlier versions of *Microsoft Office Word* could not accept 16 bit images; Trying to insert a 16 bit image causes the *Unable to Export* Images warning **(1).**



The user's options are:

- Resample the images to 8-bit in LAS.
- Re-capture the images with an 8 bit format by clicking the *Image* tab in *Preferences* **(2)**, clicking on the small arrows to the right of the *Format* header **(3)** and choosing an appropriate format from the list.
- Use a template that allows thumbnails (8-bit jpeg by default) to be included.



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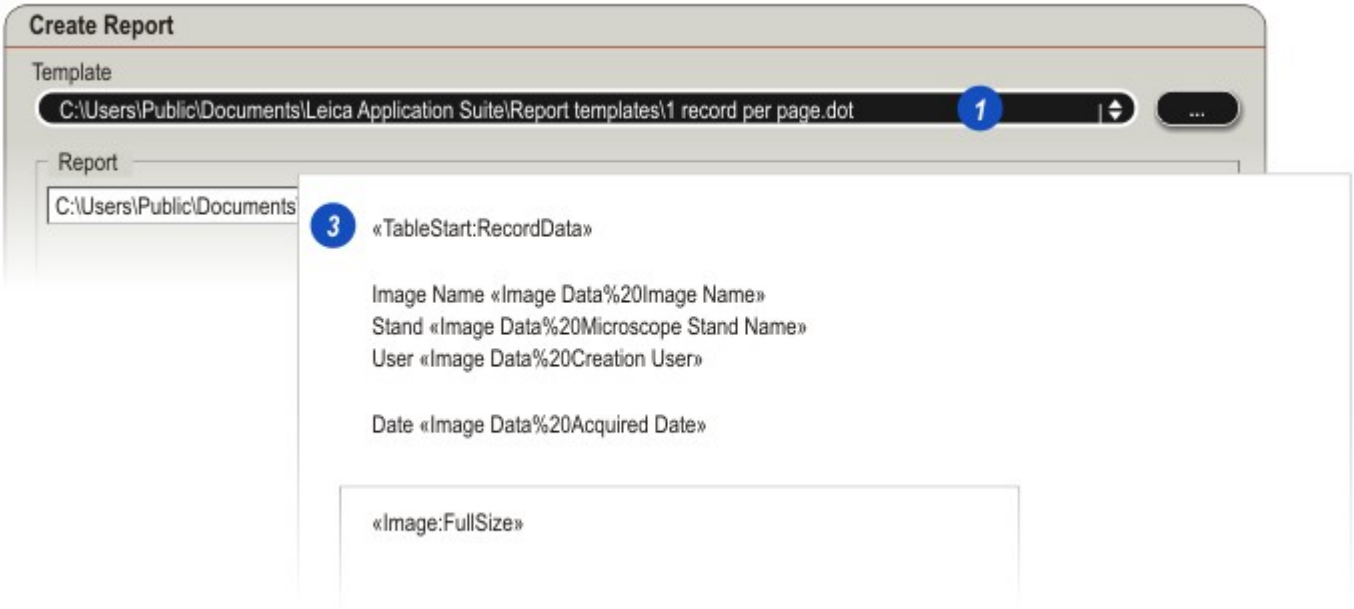
The existing Standard Templates supplied with LAS can be modified to suit the user. The page layout - margins and indents - can be altered, as well as new image data fields added and fonts changed.

1: On the *Create Report* dialog select the template to be changed and...

2: ...click the *Modify* button.

3: Word is launched and the selected template opened. Refer to the steps shown below in *Create a New Template* to make the changes.

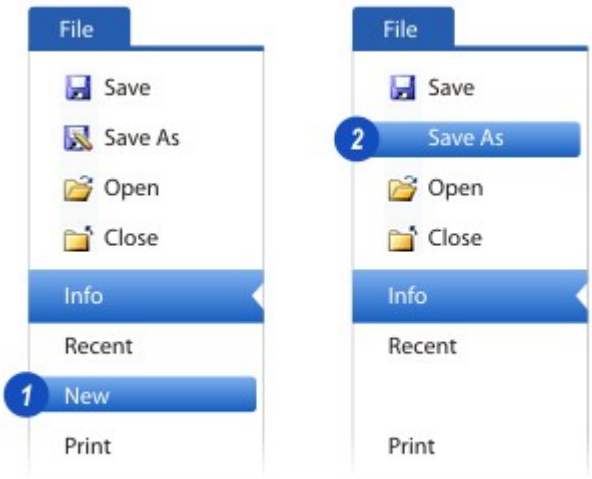
! Users should consider copying a Standard Template, saving it under a different name and making changes to the copy to preserve the original.



Create a New Report Template:

LAS Standard Report Templates can be modified to either remove fields or include others, but there are occasions when a new, bespoke report is a more appropriate solution.

Both LAS and Word must be active. The screen images included here have been created using Word 2010; Earlier versions may have slightly different dialogs but the process is the same.



1: Open *Word* and create a new document.

2: Click *Save As* and...

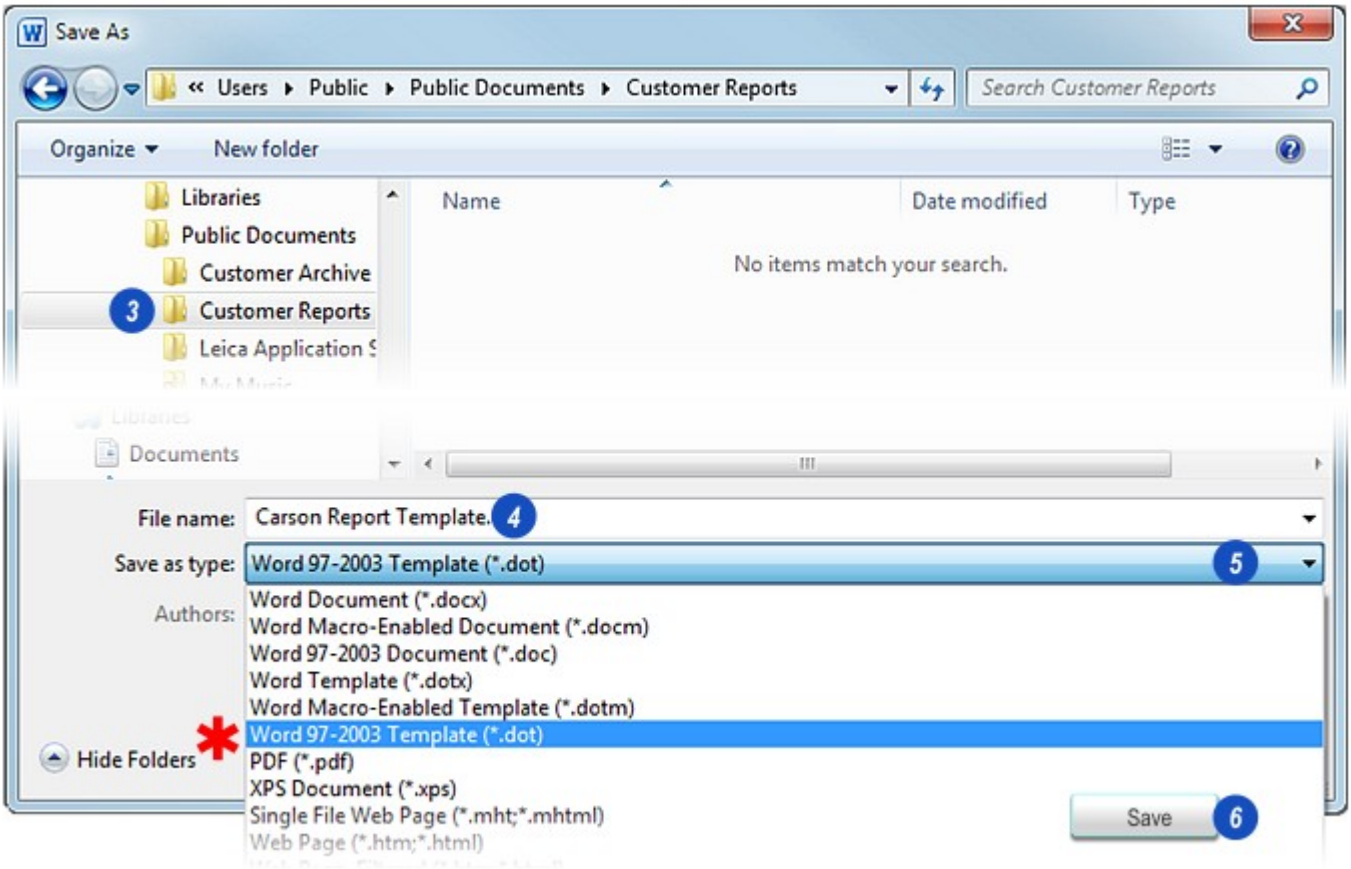
3: ...navigate to a folder of choice,...

4: ...give the document an appropriate name and...

5: ...from the *Save as type* drop-down menu, click to select *Word 97-2003 Template (*.dot).* This ensures compatibility across different versions of Word.

6: Click *Save*.

Continued... 458



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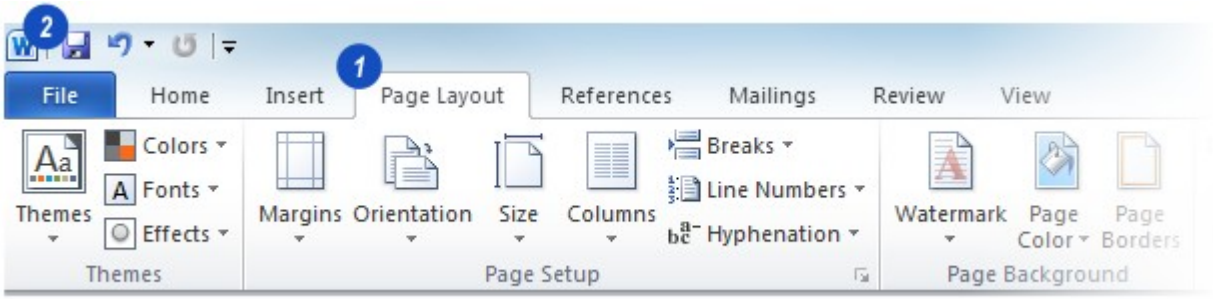

1: Click on the Word *Page Layout* tab and:

- Select a Margin: Use standard layout or set up new ones.
- Choose Portrait or Landscape: From the page *Orientation* options.
- Select the page Size. Format A4 is widely used but regions will have their own requirements.
- Select the number of Columns: The new template can use columns which is useful if there are a number of small images that need to fitted on to a page.
- *The Watermark, Page Colour and Border* features found in the *Page Background* section can be used in a template.

2: When the page format is complete, click the *Save* button.

The report template can have as many lines of 'static' text - headings, corporate disclaimers, terms of business etc. - as required. These are items that remain unchanged unless specifically altered before the template is used to create a report.

In the example a heading and sub-heading have been added to the template.

Simply type a heading or any other line(s) of text and if required, format it with the usual Word facilities. In the example the font size has been increased to 11 point, the style changed to bold and the text filled with blue.

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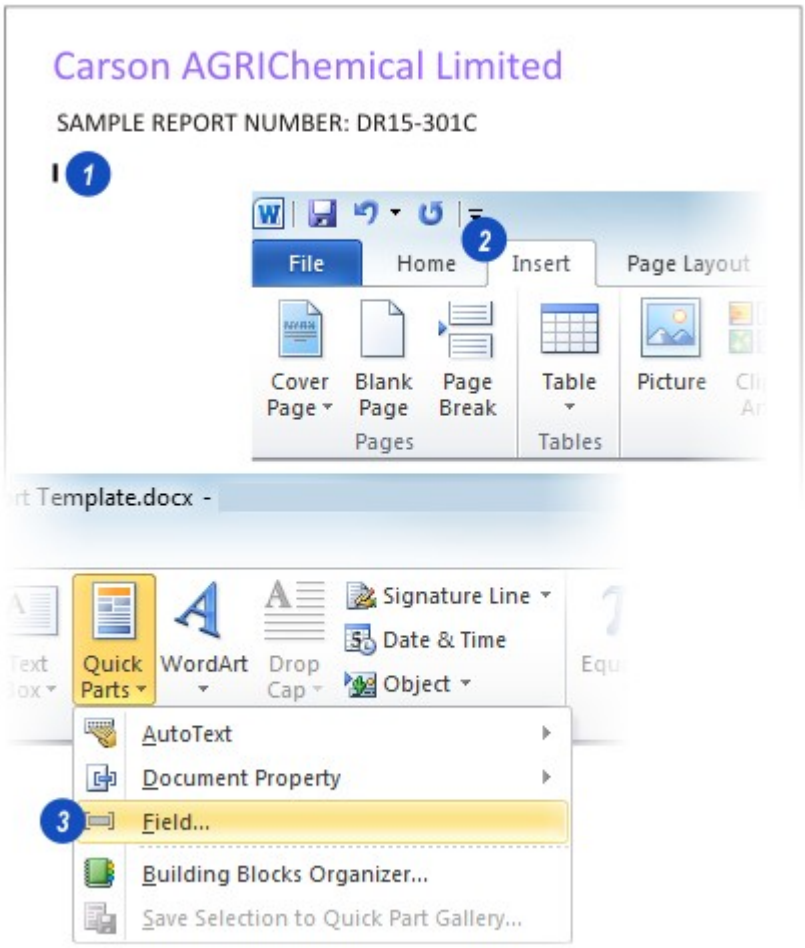
The template is a link between Leica Application Suite and the report itself. Special template fields - easily recognised by the « and » characters that enclose them - mark where image data from LAS is to be inserted into the report. Any of the LAS data items can be used.

The part of the template that contains the fields is marked with a top and a bottom 'boundary' fields; They are called *TableStart* and *TableEnd* respectively - no space between the words. The *TableStart* field must appear before any other fields and data fields cannot occur after *TableEnd*.



Like all other fields, *TableStart* and *TableEnd* are enclosed with the « and » markers and are placed on the template using the Word *Field Insert* procedure as follows:

1: On the new template move the cursor to where the data field is to be inserted.

2: Click on the Word *Insert* tab

3: Locate the *Field Insert* feature - it varies with the Word version - and select it.

Continued... 436

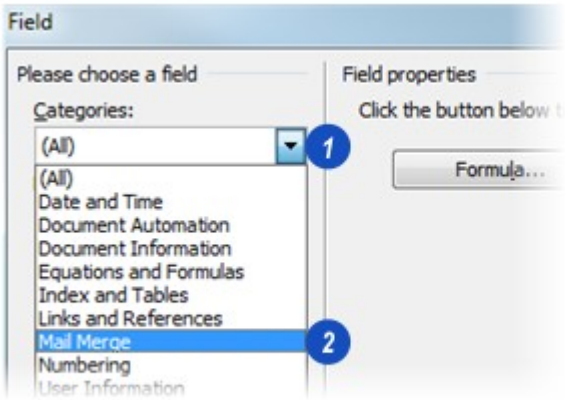
Field Structure: Select Mail Merge:

This step sets up the *Field Insert* feature to use the *Mail Merge* option. This setting is retained whenever *Field Insert* is selected unless changed by the user:

1: On the *Field* dialog, click on the small arrow to the right of the *Categories* header.

2: From the drop-down menu click to select the *Mail Merge* option.

Continued... 437



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When the Word *Field* dialog appears:

1: On the *Mail Merge* menu, click to select *Merge Field*.

2: Click inside the *Field properties* text box and type:

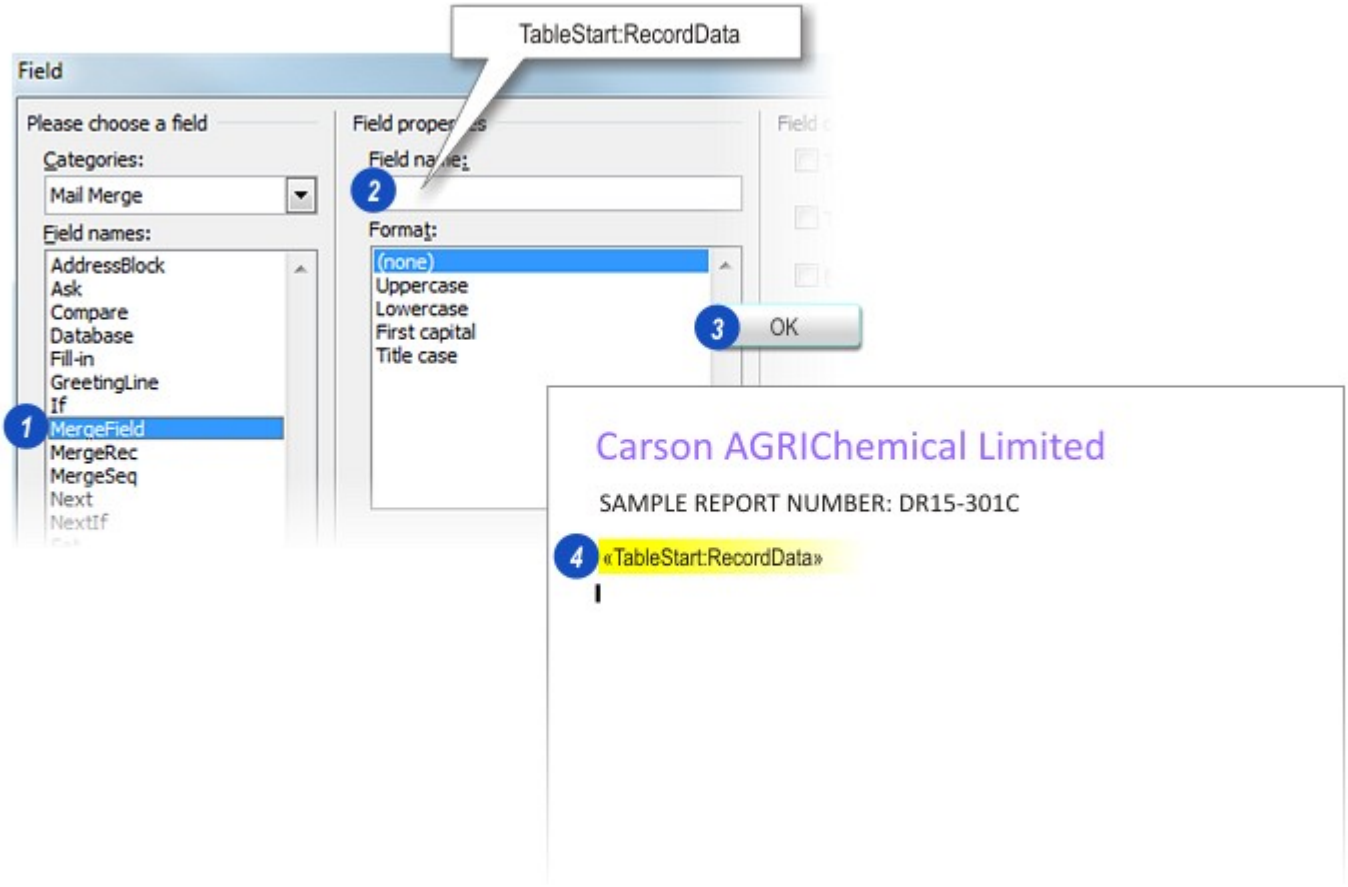
TableStart:RecordData

- There are no spaces between the words,
- A colon between *TableStart* and *RecordData* and
- No need to type the « and » markers.

3: Click *OK*.

4: The *TableStart* field appears at the cursor on the template with the markers inserted.

Continued... 438



Again, the Word *Field Insert* facility is used to create fields that will display the data imported from an *LAS Archive.*

It is a 2-step process:

- *Copy* the field name from LAS.
- *Create* a field on the template and link it to the LAS Archive field by pasting the LAS field name into it.

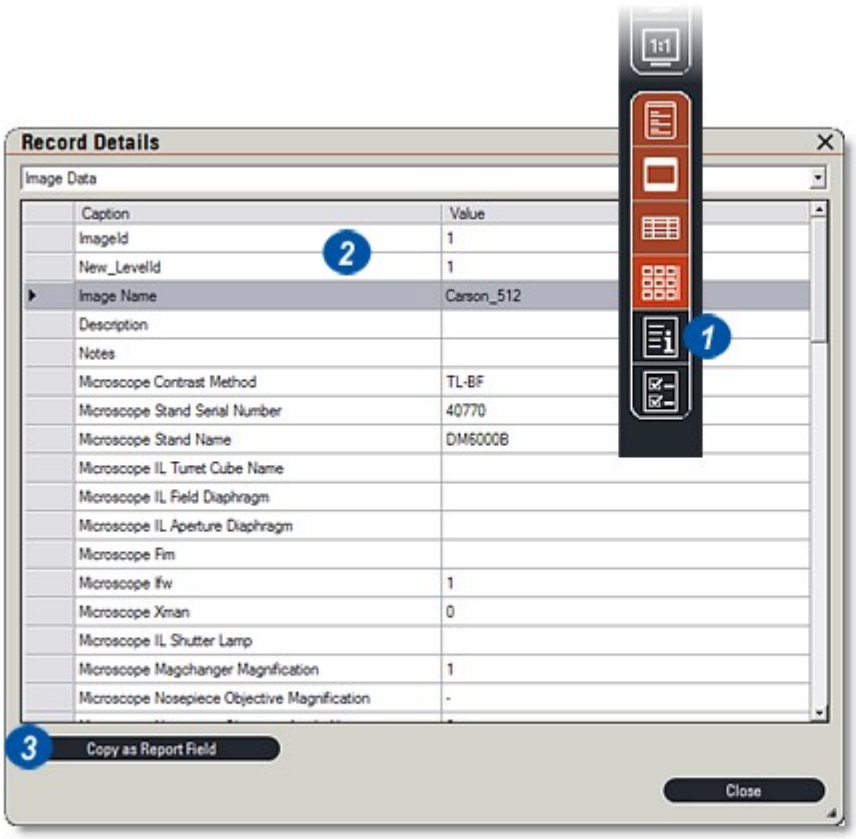
LAS must be running in the *Browse Workflow* with the required archive selected.

1: On *LAS* > *Browse* click on the *Field Information* button on the *Side Toolbar.*

2: The *Record Details* dialog appears which lists all of the fields available for that archive. In the example the Image Name field is selected to be copied to the new template in Word.

3: *LAS Field Names* are not the same as the captions displayed on the *Record Details* dialog. – they can only be retrieved by using the *Copy as Report Field* function which copies the real field name to the *Windows Clipboard*. From there it is available to Word.

Continued... 439



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This step copies the LAS Field Data link from the clipboard and places it on the new template:

1: Position the cursor on the new template where the filed data is to appear.

2: Click to select the *Merge Field* option on the *Field Names* menu.

3: Click inside the *Field name* text box and...

- Press and hold down the Ctrl key.
- Press the V key

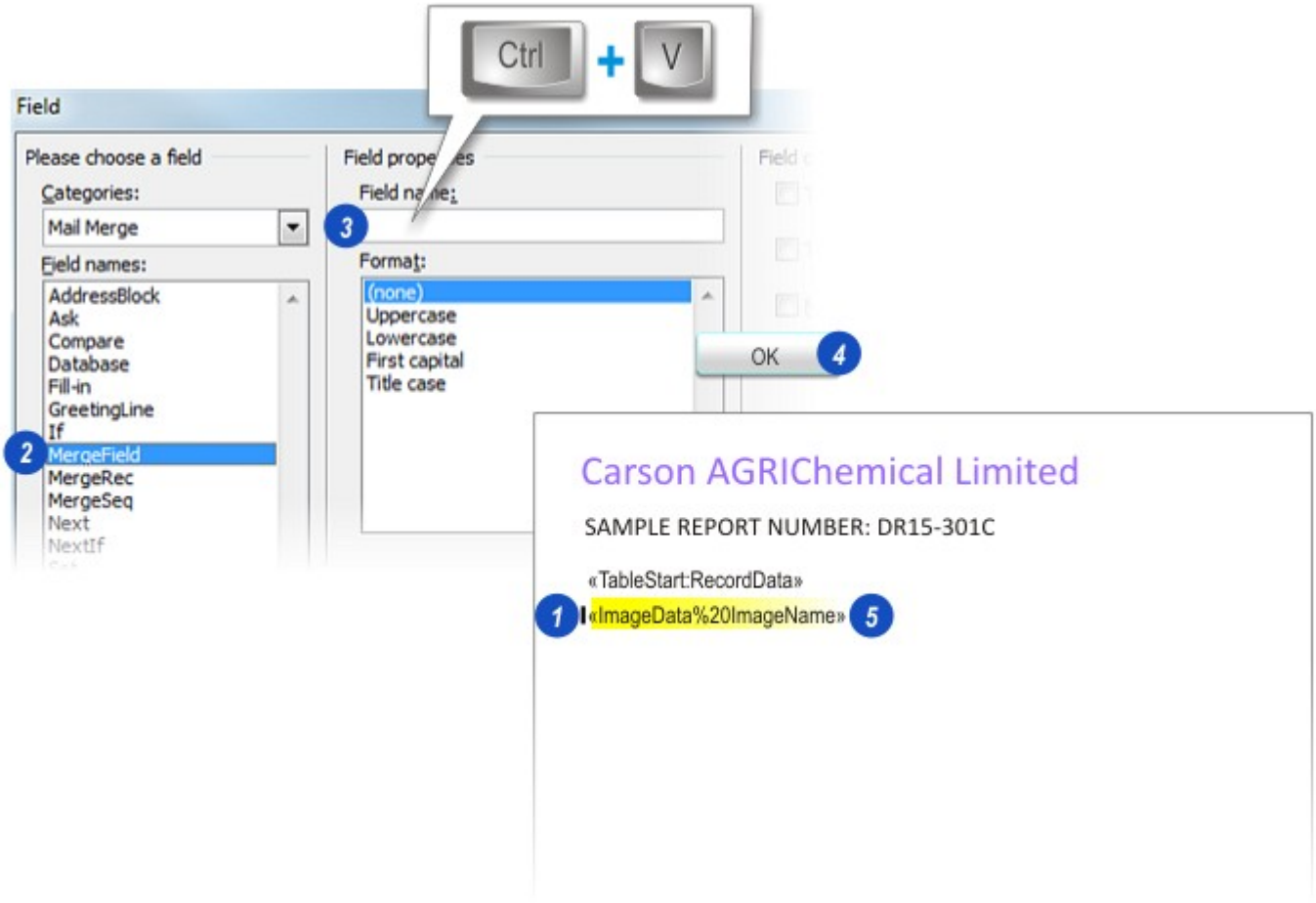
Some versions of Word may have a paste button available that can be used instead of the Ctrl+V combination.

The LAS Field Data link appears in the text box.

4: Click *OK*.

5: The LAS Field Data link complete with markers appears on the new template.

Continued... 440



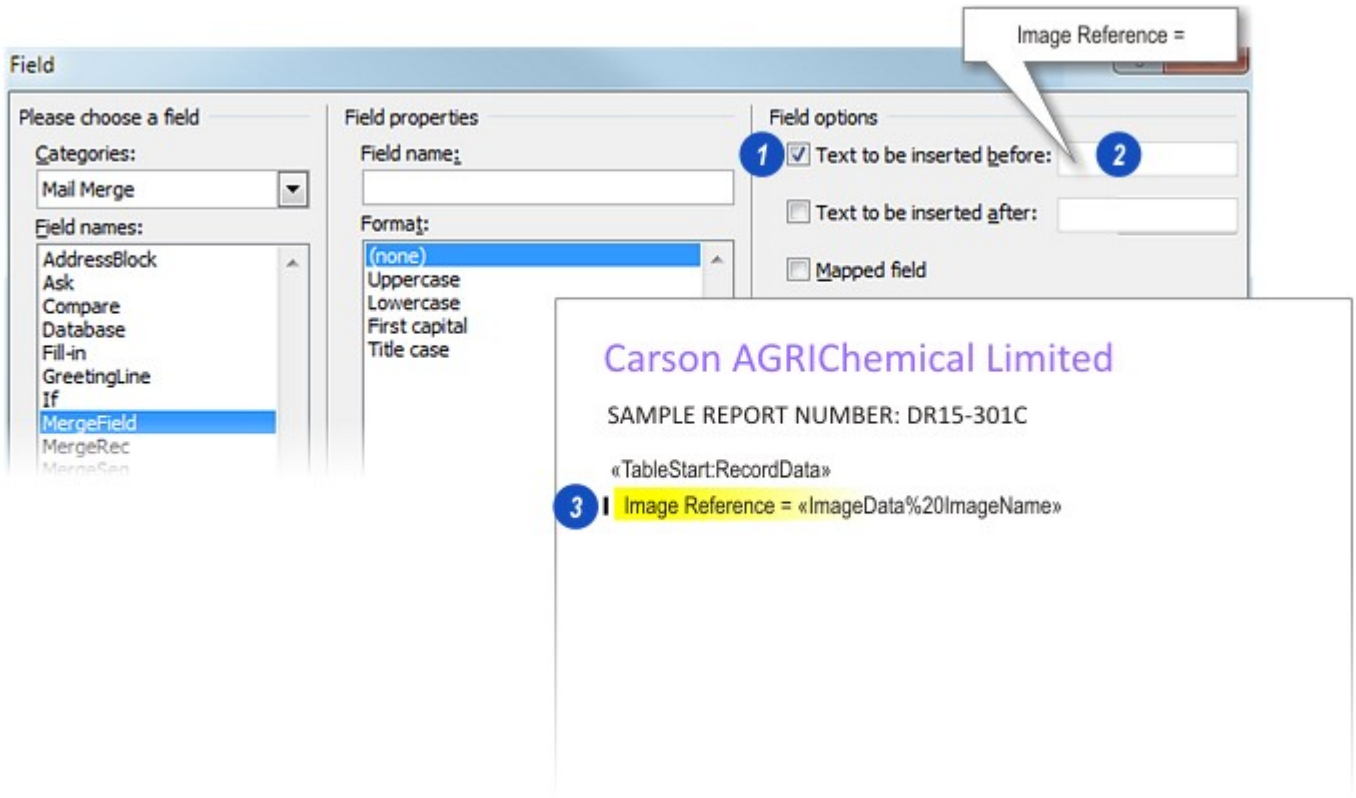
A prefix can be added before the Field Data as a descriptor that will appear on the display and printout:

1: Click to check the *Field Options* > *Text* to be inserted check box.

2: Click inside the text box and type the prefix - in this case the words *Image Reference* =.

3: When the *Field Data* is inserted into the template the prefix appears before the data.

Continued... 441



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The Word *Date Field* is dynamic - the date automatically displays the date today when the report is printed or displayed:

On the *Insert* > *Field* dialog:

1: Click the small arrow to the right of the *Categories* header and...

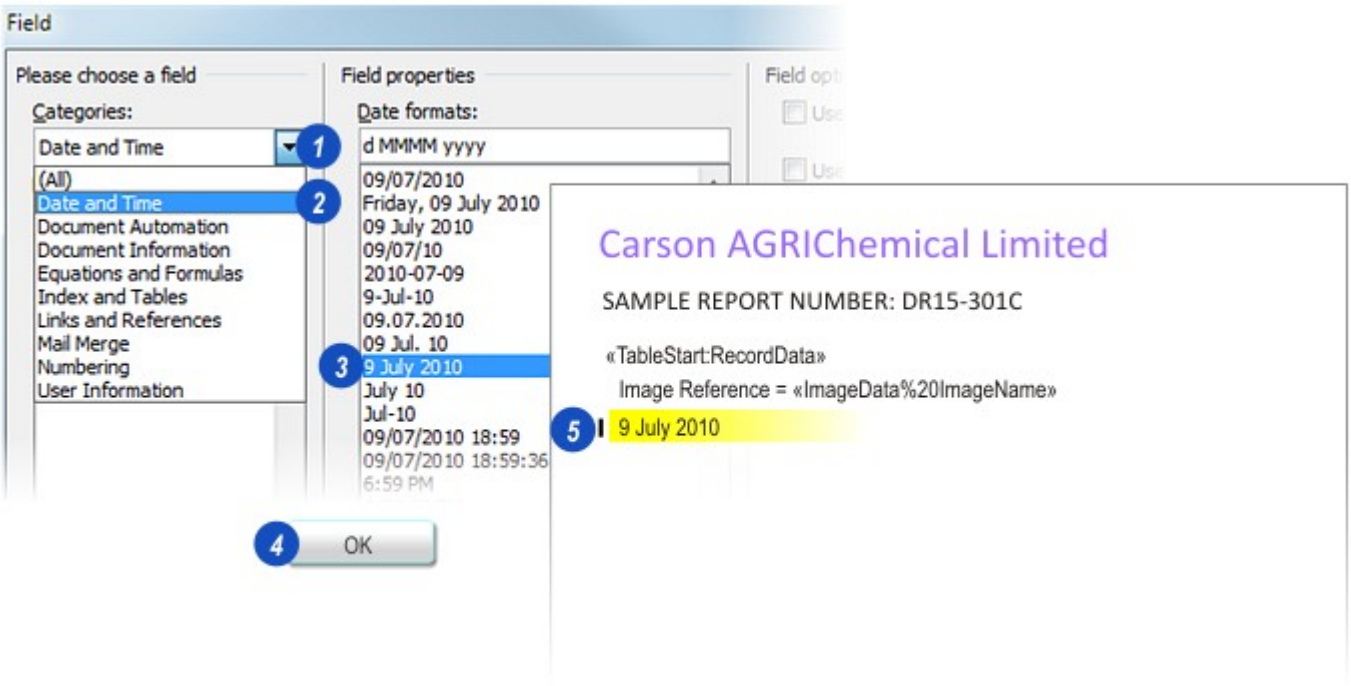
2: ...from the drop-down menu click to select *Date and time.*

3: The *Date formats* menu appears; Click to select the format required.

4: Click *OK*.

5: The date appears on the template.

Continued... 442



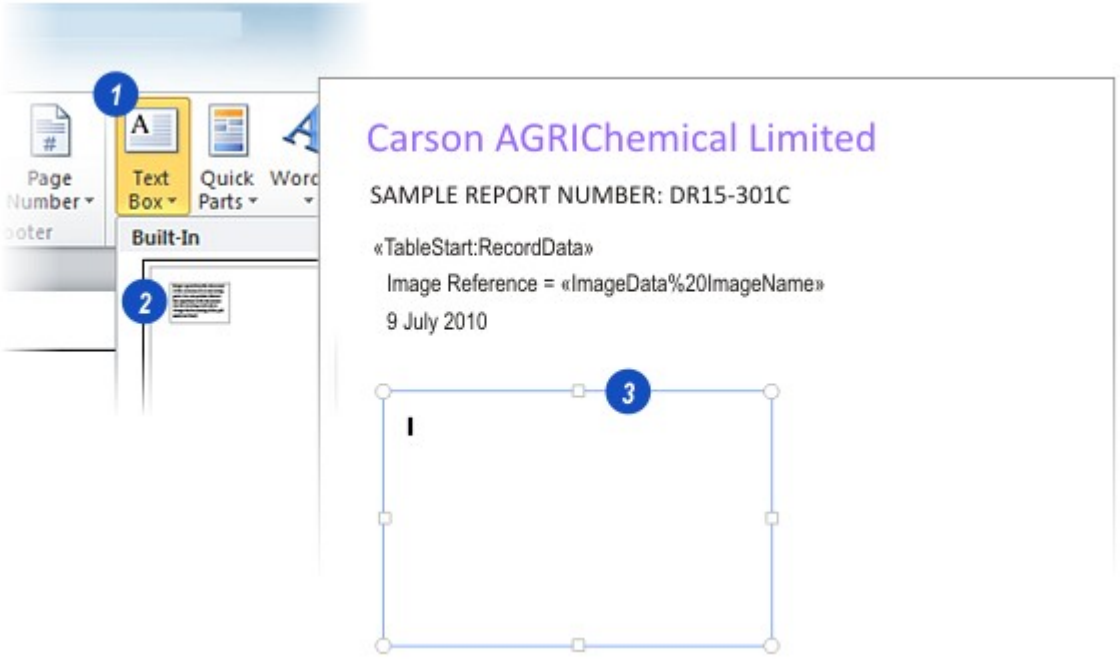
Report images are contained with Word *Text Boxes*. The first step is to create a text box:

1: On the Word *Insert* tab, click to select *Text Box.*

2: If text box styles are available in the Word version, click to select *Simple Text Box.*

3: Using the handles that surround the text box, re-position and re-shape the text box to contain and limit the extent of the image. Images are automatically scaled to fit inside the text box.

Continued... 443



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Next, a field that draws the image is created within the *Text Box*.

1: Click inside the *Text Box* and on the *Insert* > *Field* dialog...

2: ...click to select *MergeField* from the *Field names* list.

3: Click inside the *Field name* text box and type:

Image:FullSize

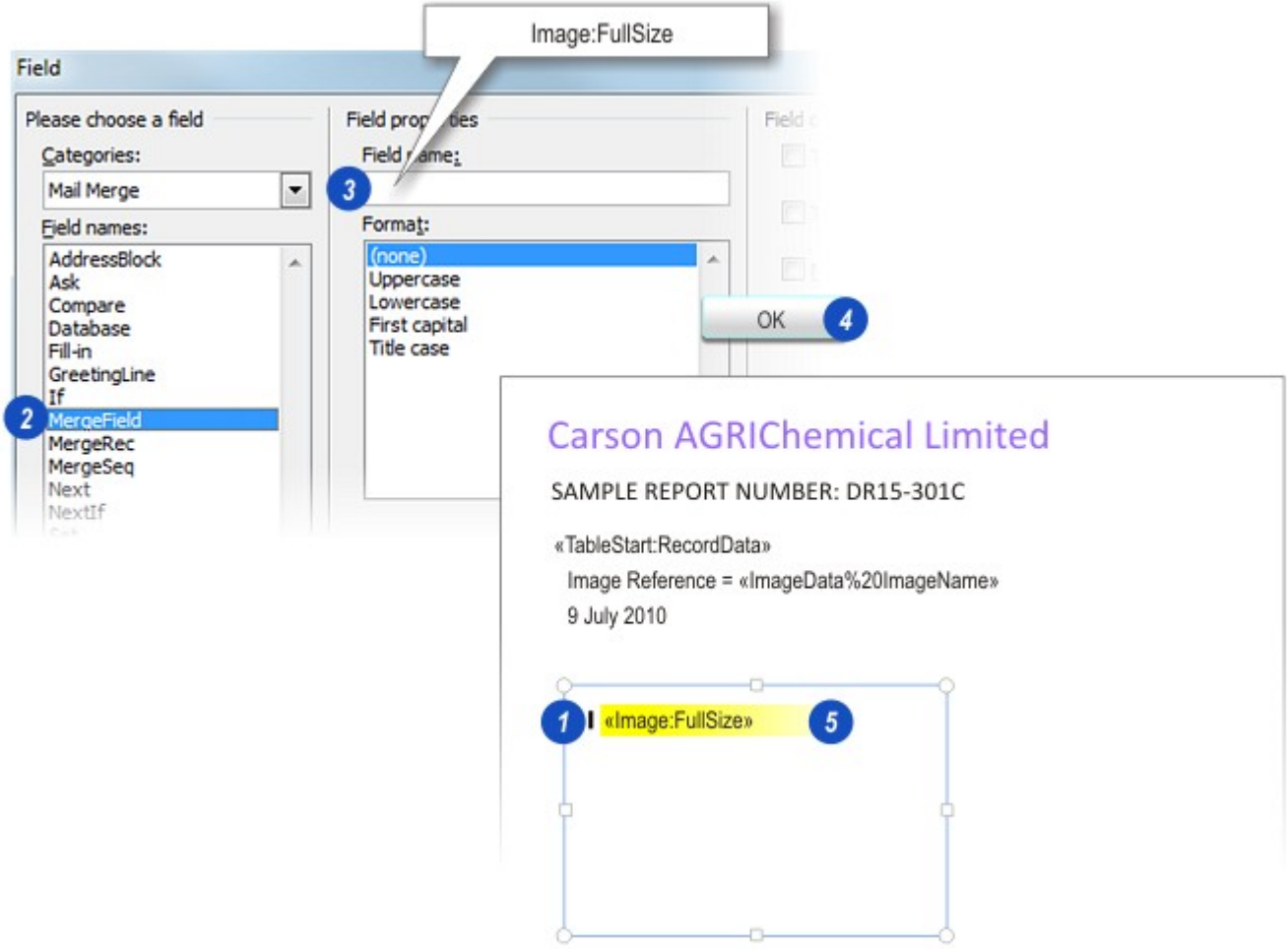
- A colon between *Image* and *FullSize*.
- No space between *Full* and *Size*.
- No need to add the « and » markers.

- **4:** Click *OK*.

5: The field appears inside the text box.

Only a single image text box is required on the new template even if it is required to show multiple images. LAS will export all of the image selected in the Gallery, drawing and placing them in sequence on the report. New report pages are created automatically as they are required.

Continued... 445



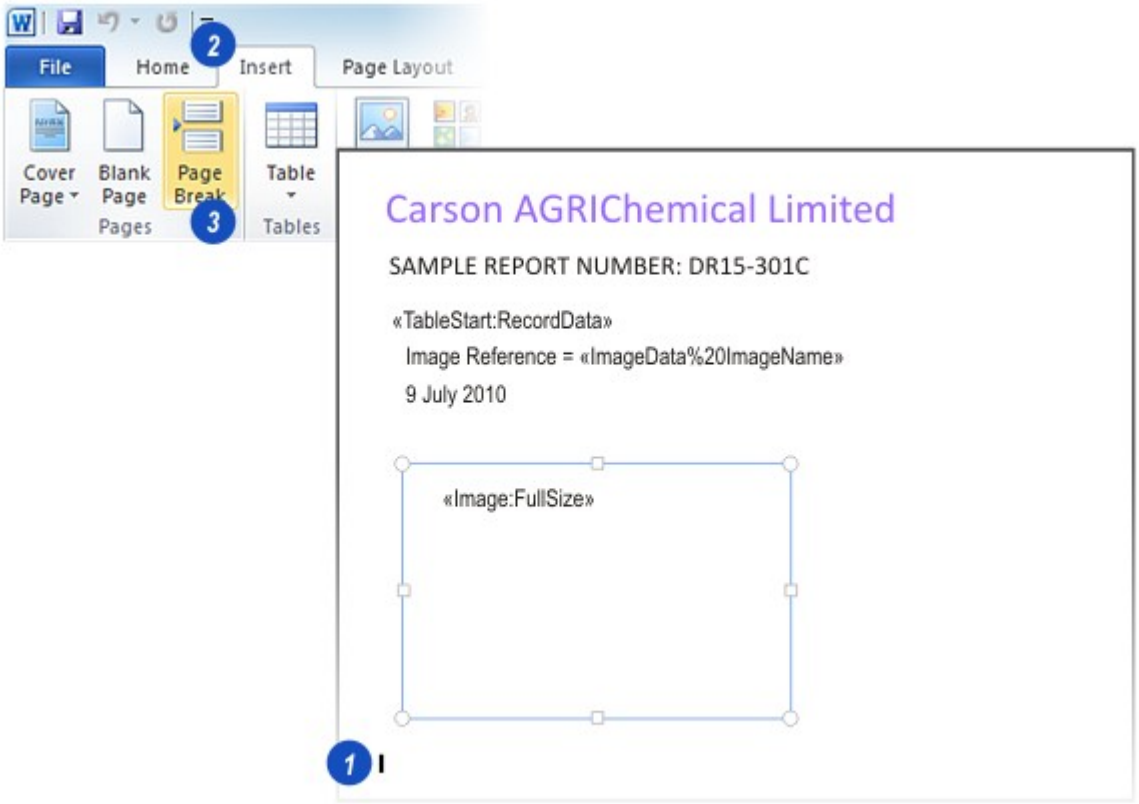
If only a single image is to be displayed on each report page, a *Page Break* can be inserted after the fields and image text box to force a new page for the next image.

1: After the fields and image text box, click to mark where the *Page Break* is to occur.

2: Click on the Word *Insert* tab.

3: Click the *Page Break* option. This will vary with different versions of Word.

Continued... 445



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The final step in creating a new template is to indicate the end of the data. *Field* Insert is used again:

On the Word *Field* dialog:

1: On the *Mail Merge* menu, click to select *MergeField*.

2: Click inside the *Field properties* text box and type:

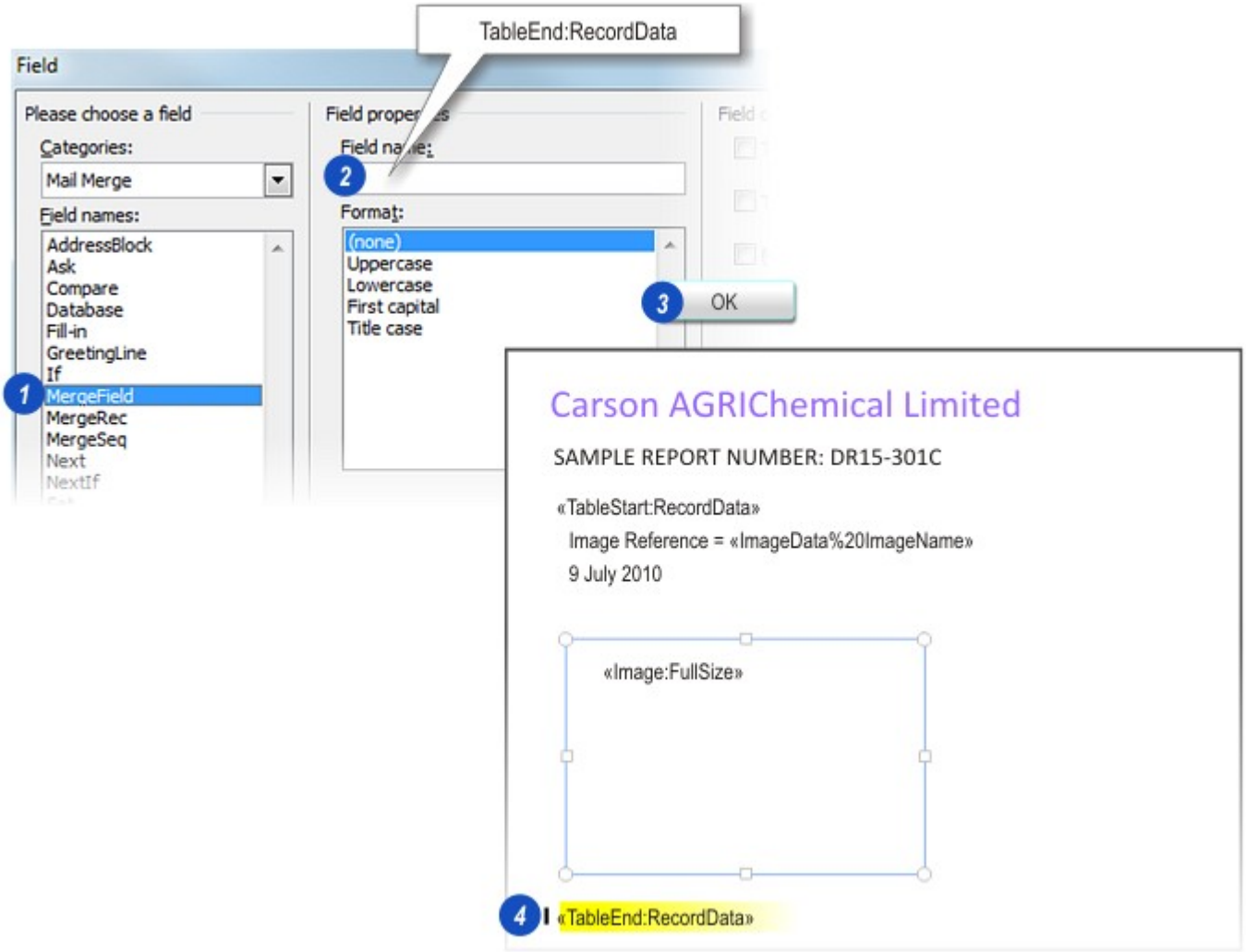
TableEnd:RecordData

- There are no spaces between the words,
- A colon between *TableEnd* and *RecordData* and
- No need to type the « and » markers.

3: Click *OK*.

4: The *TableEnd* field appears at the cursor on the template with the markers inserted.

Finally, save the new template, close Word and test it: *Go there...* 453



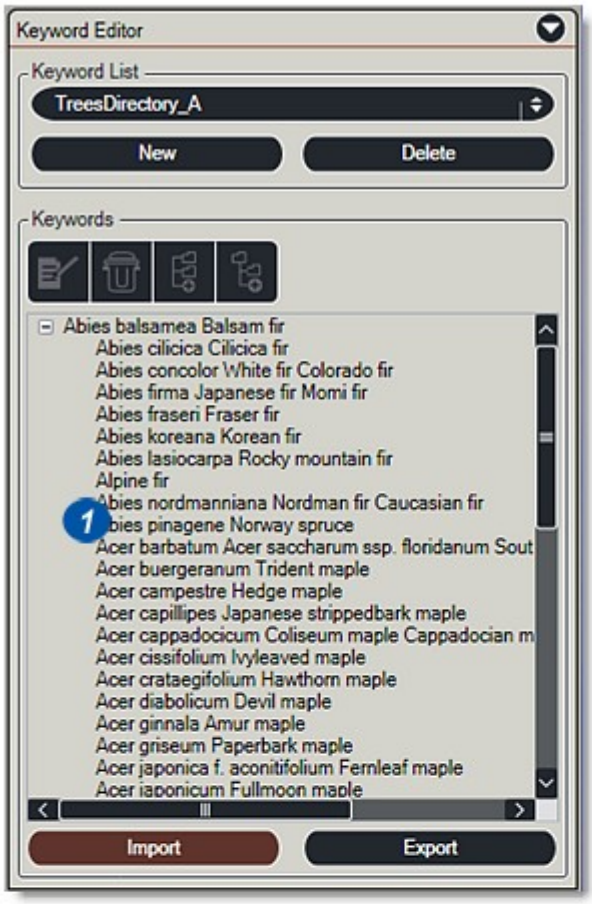
The *Keyword* feature can create lists of words and associate them with archives so that they can be used to populate selected fields.

This means that spellings – especially for complex subjects, possibly in another language – only have to be captured once and after that can be used indefinitely, confident that spelling and 'cases' are consistent **(1)**

So, *Keywords* are fast and accurate and can ensure that field searches will be precise.

Keyword lists can be shared across any number of archives with any changes to the list be reflected in all of the archives. This is because each archive maintains a link to the *Keyword* list rather than copying it.

New lists can be created by typing, by cutting and pasting words or phrases from an existing document or by importing a complete existing list.



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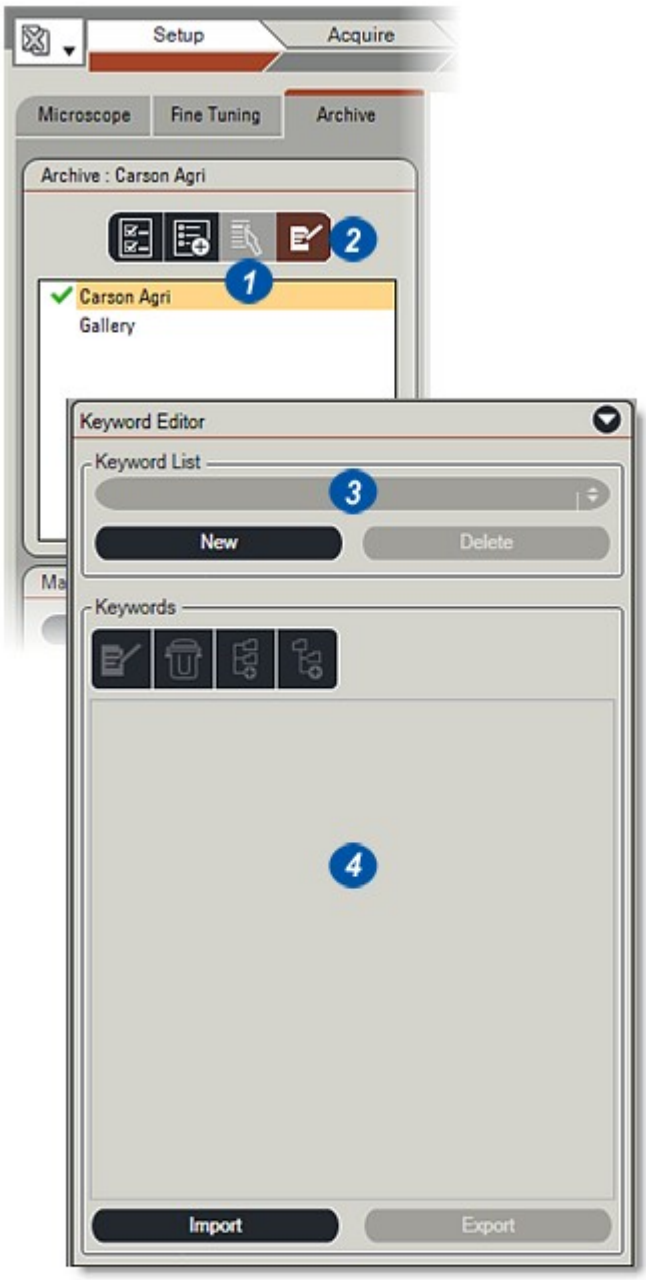
When a new archive is created the Keyword dialog automatically appears on screen ready for a new list to be created or imported. For existing archives:

1: Select the archive and if necessary make it active current by double-clicking it or clicking the *Set Archive as Current* button.

2: Click on the *Edit* button. The field structure and the Keyword dialog will appear. If there is a Keyword List already associated with the archive, its name will be displayed in the *Keyword List* window **(3)** and its contents in the main window **(4).**



1: On the *Keyword* dialog click on the *New* button.

2: When the *New Keyword List* dialog appears, type a name for the new list and click *OK*.

3: The name appears in the *Keyword List* window and the other controls become active.

4: Change the name and start again by clicking the *Delete* button and returning to step **(1).**

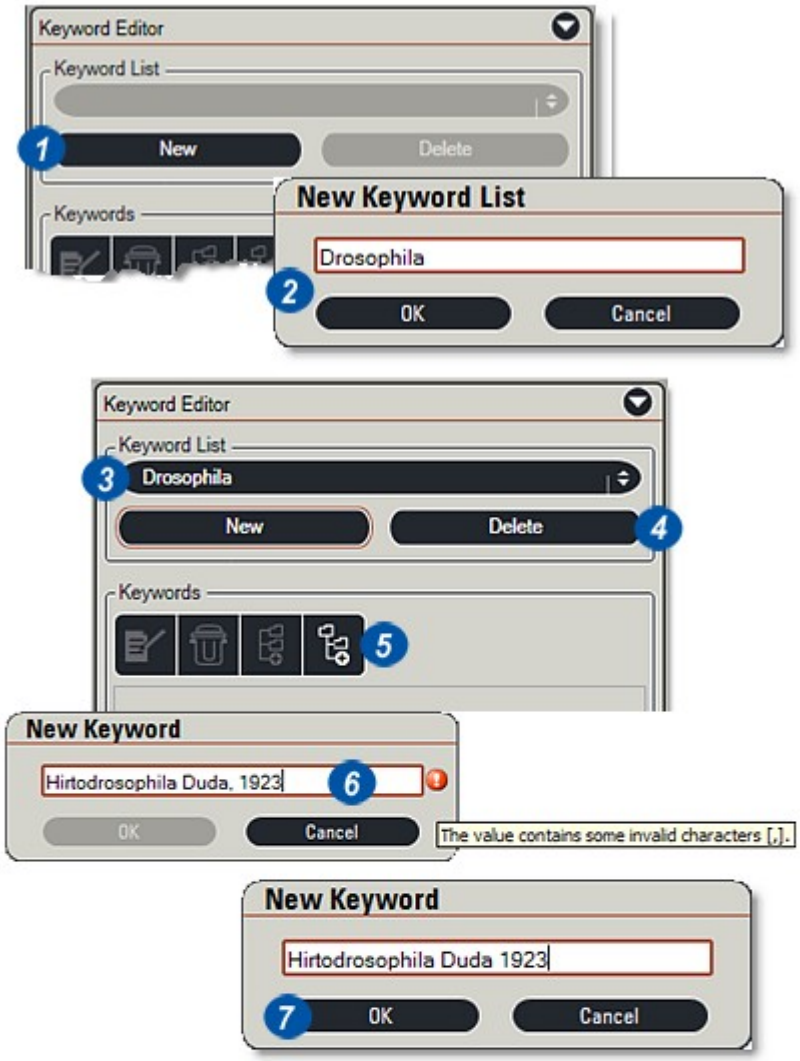
Typing the Keyword List:

5: The first word or phrase must be preceded by clicking the *Add a Child* button. The *New Keyword* dialog appears.

6: Type the first word or phrase. Words can be copied from other open documents – a text editor or internet browser for example – and pasted into the New Keyword text box using the *Ctrl+C (Copy)* and *Ctrl+V (Paste*) keyboard combinations.

Generally, only letters and numbers are allowed in keywords; If an invalid character is typed a red **(!)** appears to the left of the entry text box and the flyout prompt itemises the invalid character.
In the example it is the comma between *Duda* and *1923* shown as **[,].** Delete the invalid character and…

7: …click *OK*.



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1: The first word or phrase appears in the *Keyword Editor* main window.

2: To add more words click the *Add New Word at this Level* button and when the *New Word* dialog appears…

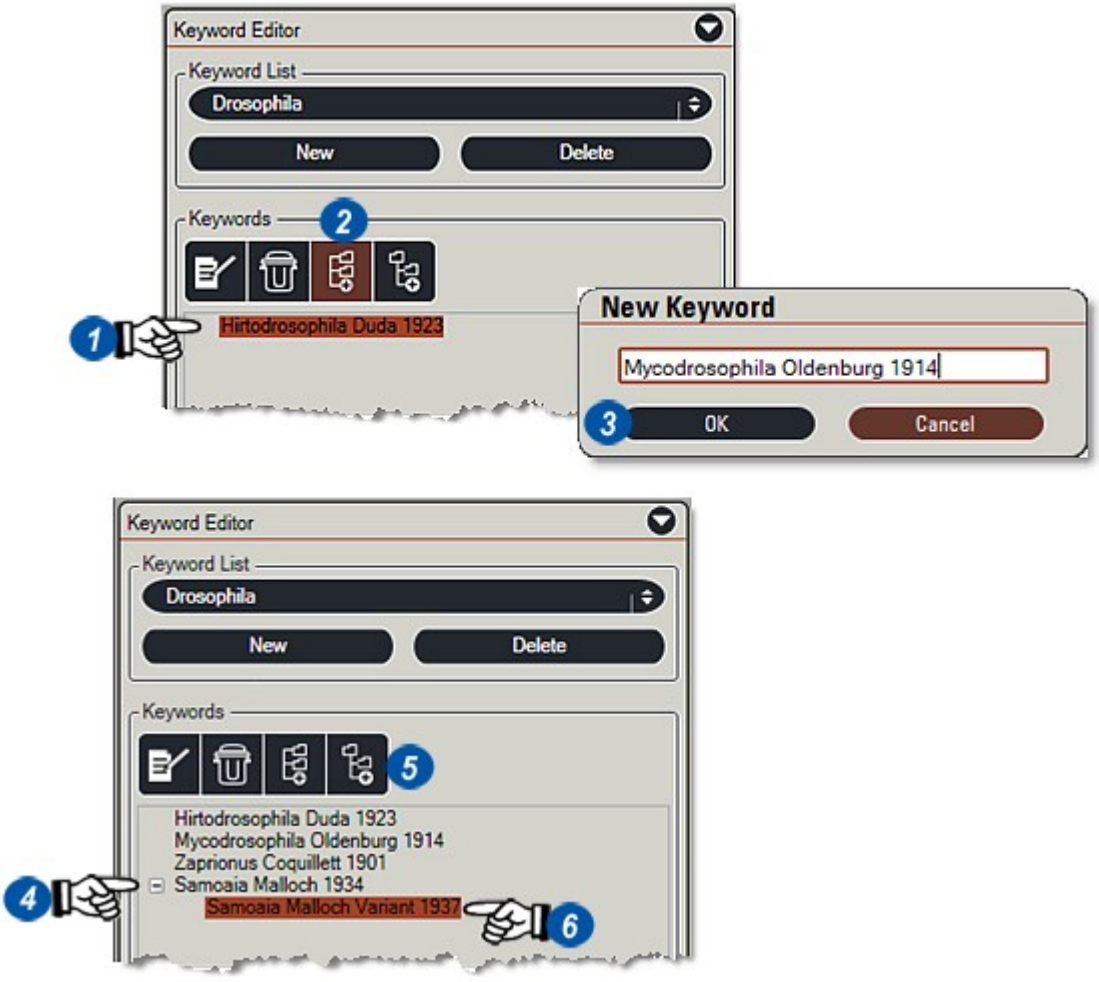
3: …type a word or phrase and click *OK*.

Keyword Grouping:
To make long lists of keyword easier to read or to group related entries, the list can be indented by:

4: Clicking on the entry that is to 'head' the group.

5: Click on the *Add a Child* button and type the word or phrase to be indented **(3).**

6: The word will appear indented in the main window. The last word or phrase added becomes the selected item automatically, so to continue to indent use the *Add New Word at this Level* button.
To revert to the original level, click to select the entry that 'headed' the group and use the *Add New Word* at this Level button.
Several indent levels are permissible.



To edit a word or phrase:

7: Click to select the word and…

8: …click the *Edit* button.

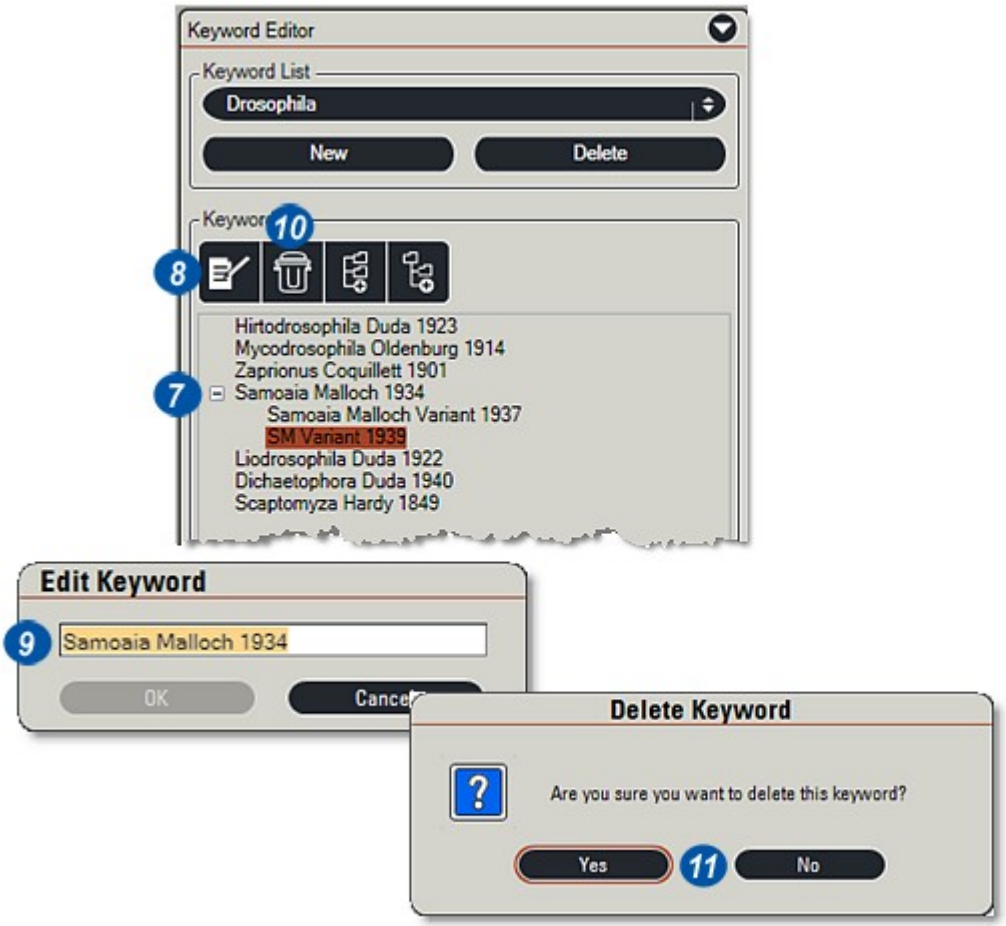
9: The word appears in the *Edit Keyword* dialog. Make the changes and click *OK*.

Delete a Keyword:

7: Click to select the word and…

10: …click the *Trashcan* (Delete) button.

11: Confirm (or cancel) the deletion.



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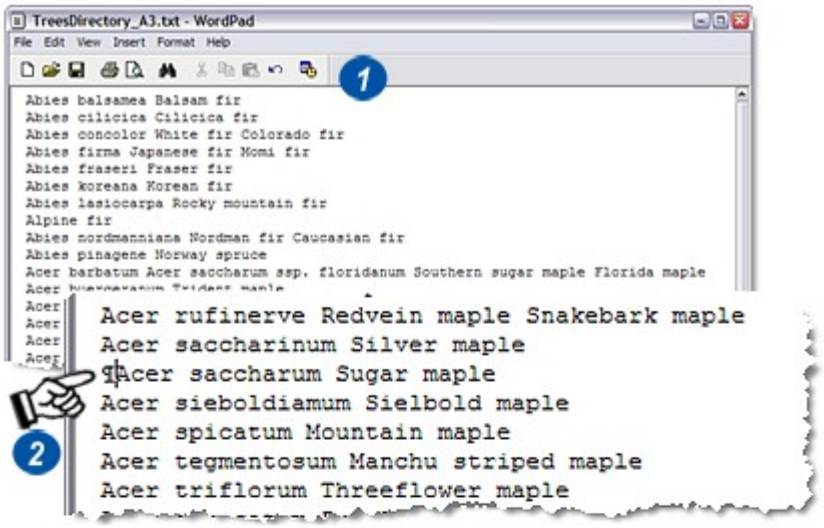
New lists can also be created from existing documents and imported as Keywords.
The list is created in a simple test editor such as *Wordpad* or *Notepad* – **DO NOT** use Microsoft Word to create the list, but individual words or phrases can be cut from Word and pasted into Wordpad for example.



1: Either type directly into the text editor or cut and paste from another source. Avoid punctuation characters especially commas *(Keyword Lists* are comma-delimited), using mainly letters and numbers.

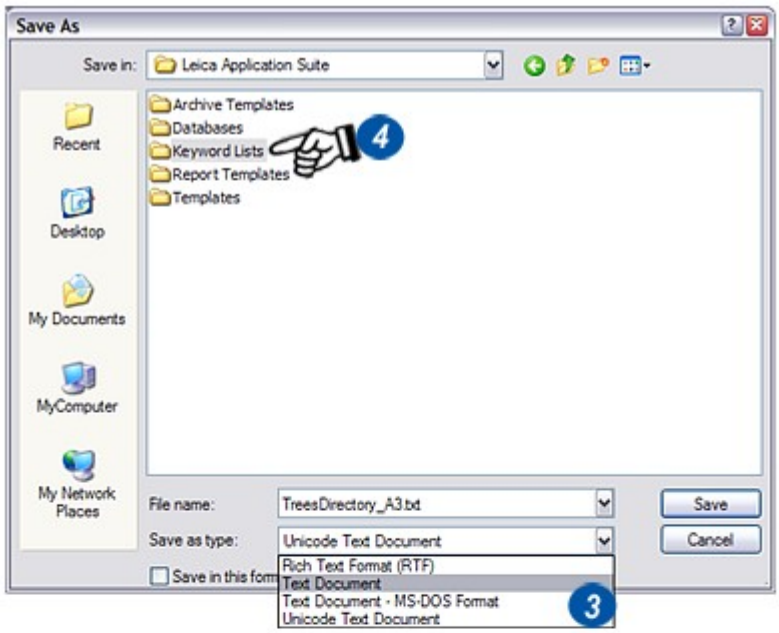
2: To create an indented entry or block, insert the *Paragraph* (sometimes called Pilcrow) character at the beginning of the line. These will create an indent when the list is imported into the *Keyword Editor.*
The *Paragraph* character can be inserted by holding down the keyboard Alt key and typing 0182 on the *Numeric Keypad* usually situated to the right of the main keyboard.
The character can be copied (highlight and use *Ctl+C*) and then pasted (*Ctrl+V*) if there are many entries to indent.
Do not use tabs or additional spaces to make an indent.

3: Save the file as a .txt document either as *Text (ASCII), MS-DOS Text (ASCII)* or *Unicode (UTF-8)* but not as Rich Text Format (RTF) which, like Microsoft Word includes extensive formatting.

4: Although *Keyword Lists* can be saved to any folder on the hard drive, Leica Application Suite has a default location:

C:\Documents and Settings\All Users\Shared Documents\Applications\Leica Application Suite\Keyword Lists

…which is recommended.



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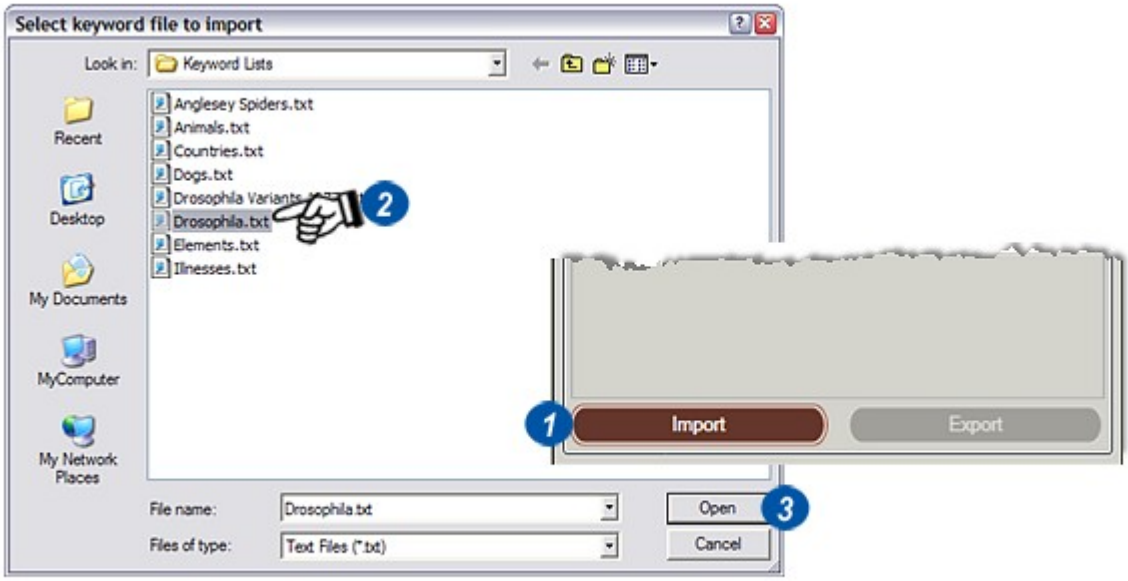
With a new *Keyword List* created in a text editor and saved as a text (.txt) file, it can now be 'imported' into an archive. The import process actually establishes a link between archive and *Keyword List* so that any changes made to the list will be reflected in the archive.

To make major changes in he list and **NOT** have the archive affected, save the text file under a different name and use it as a completely separate list.

1: Click on the *Keyword Editor Import* button.

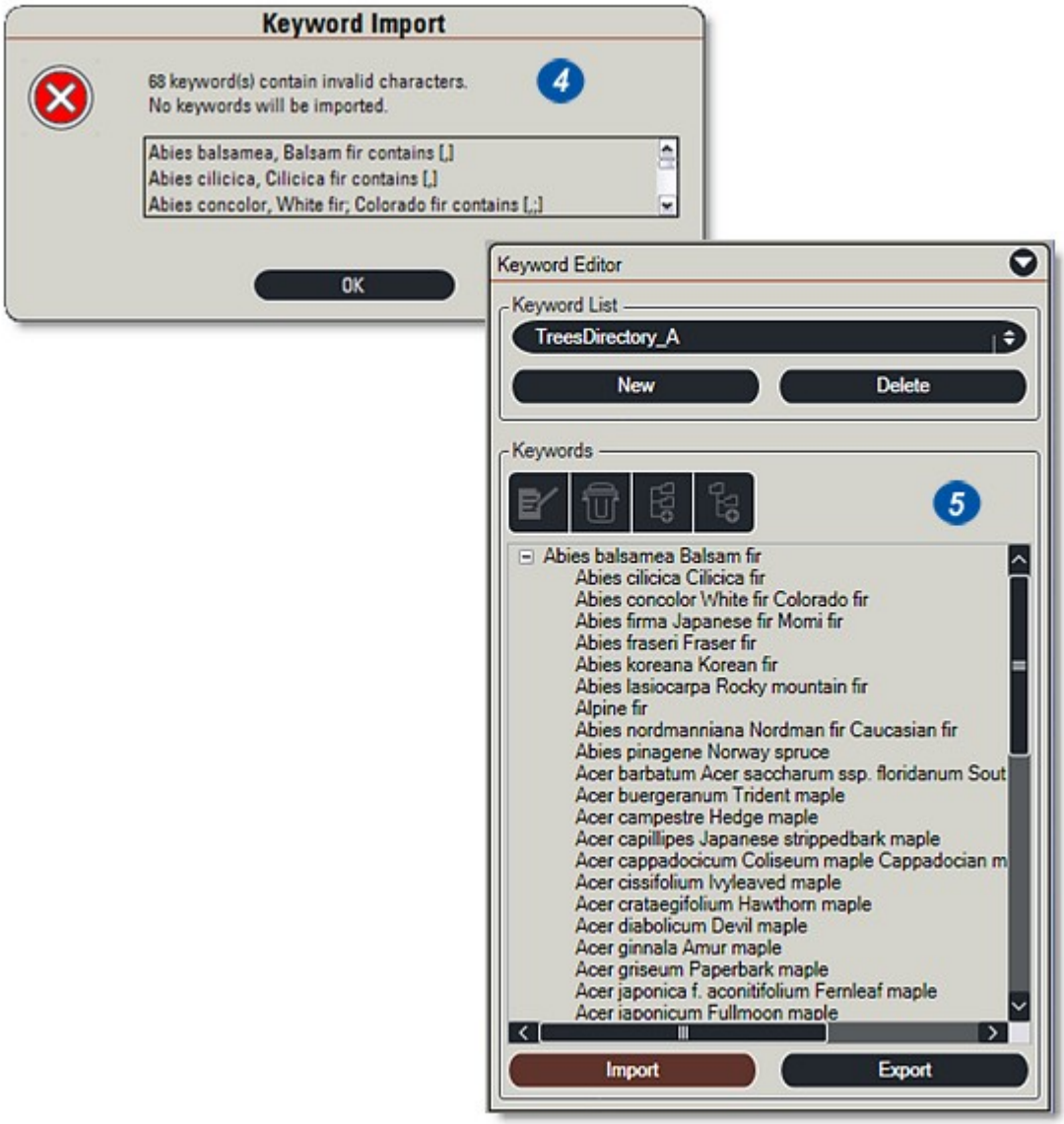
2: On the Windows dialog, navigate to the folder in which the new *Keyword List* is stored. The example shows the LAS default folder, *Folder Lists*. Click to select the list and…

3: …click *Open*.



4: If there are invalid characters in the List a warning appears indicating the problem characters and where they are located. The list will have to be corrected and saved with the text editor.

5: When import succeeds the list appears in the *Keyword Editor* main window. Long and wide lists automatically have scroll bars to the right and bottom.



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Keywords can only be copied to archive fields that has been designated as *Keyword Fields*.

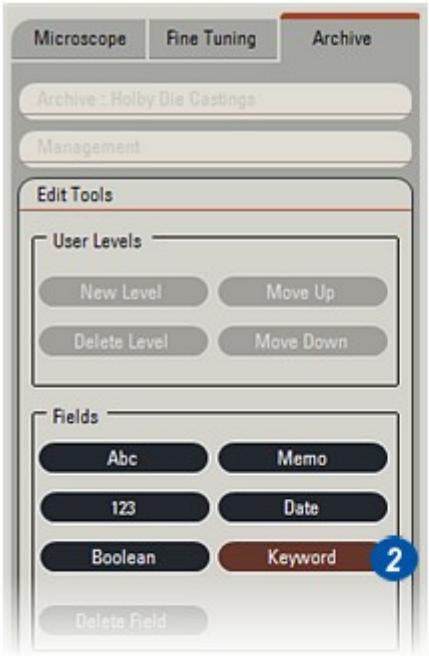
When a *Keyword List* (or lists) is associated with an archive, the *Keyword* button on the *Archive* panel becomes active:

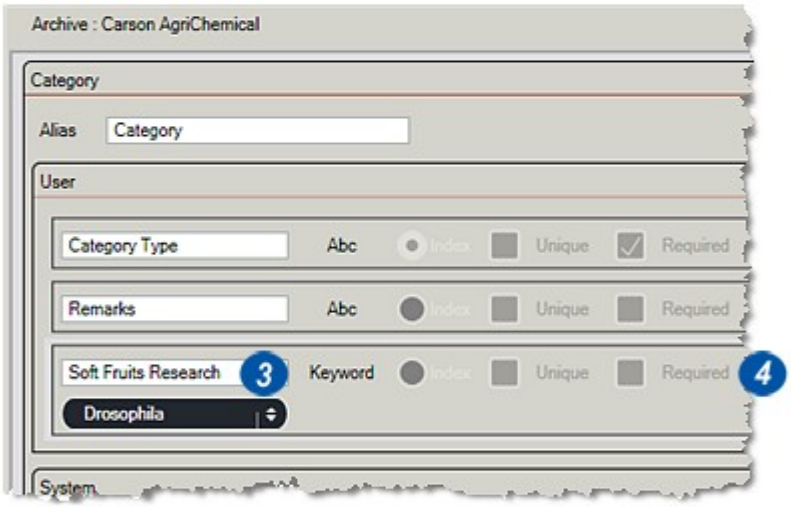
1: Click to select either the *Category (Record Group)* or *Image* panel. Keywords in the Category will appear on every image captured within that group; Keywords associated with the image will only appear if they are selected for that image.

2: Click on the *Keyword* button. A keyword text box opens on the selected panel.

3: Click in the *Keyword* text box and type a meaningful name for the Keyword Field.

4: If it important that a keyword always appears in the field, click to enable the *Required* check box. With Required enabled, a user will be forced to select a keyword from the List before an image can be saved.





5: In cases where several *Keyword Lists* are associated with an archive, select the appropriate list from the drop down menu by clicking on the arrows to the right of the *Keyword List* name and…

6: …clicking to select the list required.

Several *Keyword Fields* may be added to the *Record Group* or Image parts of the archive and this, together with the facility to associate a range of *Keyword Lists* with the archive, makes *Keywords* a powerful and fast method of gathering data. And if, for example, a list comprises the cost centres within an organisation and is set as *Required*, every image without fail could become the property of a particular department.

7: Click *Save*.

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Keywords are copied to keyword fields in the *Browse Workflow*. For *Required* keywords in the *Category* (Record Group), these are selected when the group is created as follows:

1: Click on the *Create a New Category* button.

2: Type a name for the new category.

3: If the keyword is *Required*, a red **(!)** will flash to the right of the *Archive Navigator*. This means that the new category cannot be saved until a keyword has been selected.

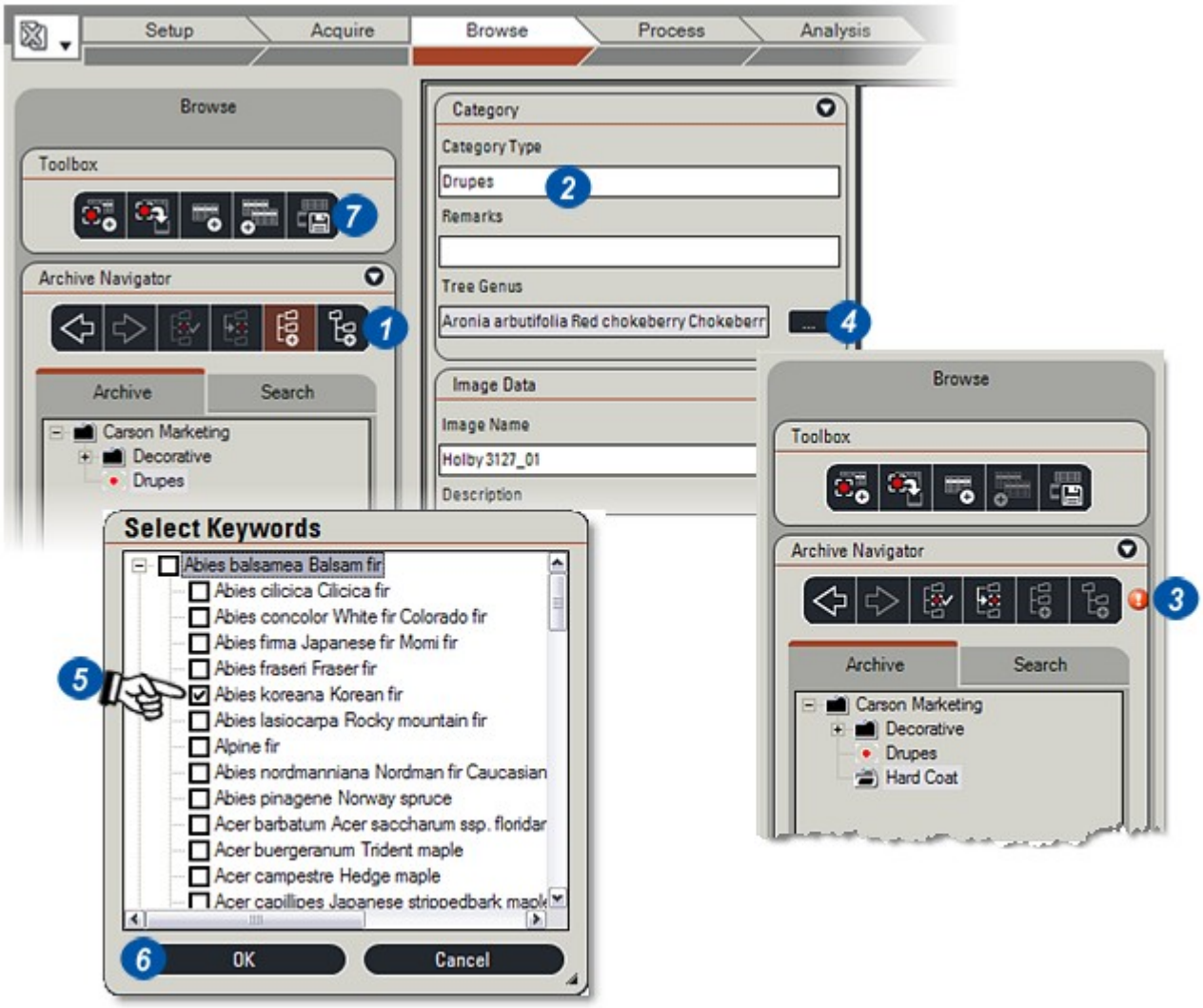
Use this process any time a keyword is required:

4: Click on the button to the right of the *Keyword Field* – this could be in *Category* or in Image. The *Select Keyword* dialog appears.

5: Scroll through the *Keyword List* to the required entry and then click in the check box to the left of it. A tick mark should appear. Clicking the entry itself is not sufficient.

6: Click *OK* and the keyword will be copied to the field.

7: Click on the *Save* button.



Keyword Lists may be exported to any folder on the computer or to an external storage device such as a memory stick. The *Export* feature makes a copy of the *Keyword List* at the new location; The original remains intact.

If there are several lists associated with an archive:

1: Click on the arrows to the left of the *Keyword List* on the Keyword Editor and from the menu…

2: …click to select the List to be exported.

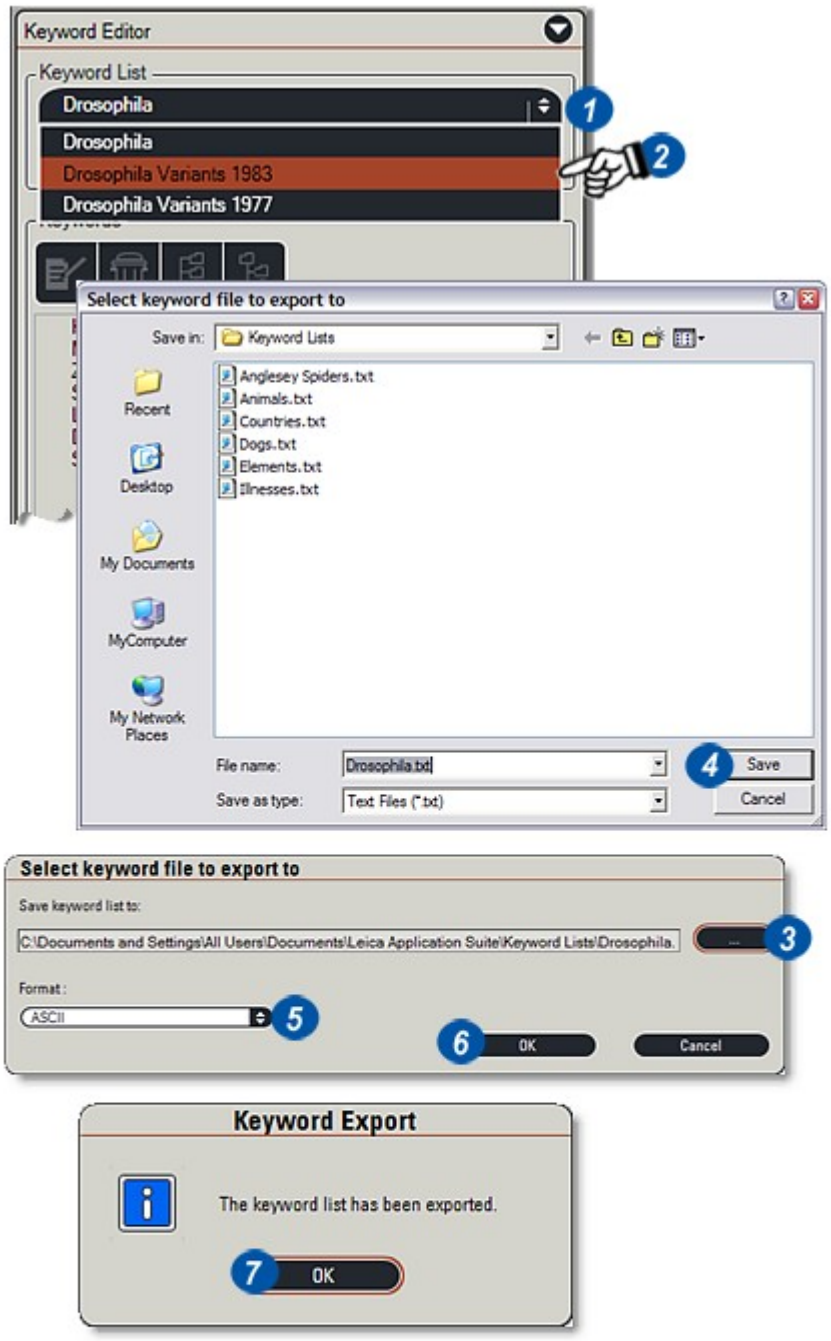
3: Navigate to the export target folder by clicking on the browse button to the right of the *Save Keyword List to* window, and…

4: …selecting it on the Windows dialog. Click *Save*.

5: Click on the arrows to the right of the *Format* text box and from the menu click to select the character coding – *ASCII* is the default and generally preferred.

6: Click *OK*.

7: A message indicates that the Export is complete. Click *OK*.



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A *Keyword List* may be deleted – disassociated with the archive – at any time BEFORE a *Keyword Field* has been created. After that, clicking the *Delete* button will result in the *Cannot Delete* message **(1).**

If you wish to simply transfer and image from an LAS Archive to a memory stick then is it simples to use the Export Image facility.

See Export from LAS Archive to File System 461

For those organisations using Leica *Image Manager (IM)* as a networked archive store and organiser, features are provided in LAS that allow images from workstations running under *Leica Application Suite* to be sent to Leica IM (from version 5 onward) and managed like any other IM image.

See Sending Images from LAS into Leica IM V5 462

Images stored in an IM archive can also be transferred into LAS.

See Receiving Images into LAS from Leica IM V5 473

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The *Side Tool Bar* is displayed on the right hand edge of the *Image Viewer*. The *Export* button is situated in the top group.

To *Export* the image being displayed using *LAS Archive* to a selected destination folder:

1: Click a thumbnail to select and display the image to export.

2: Click on the *Export* button.
For detailed help on the *Export* procedure look in *Functions Widely Available: Go there...*

To be able to use this feature, the Leica IM Image Data Read-in module must be licensed.

Leica Image Manager can store all of the data fields captured with the image in LAS providing they are nominated but it cannot itself display them – they are stored in a special file associated with the image. However, when the image is retrieved from Leica Image Manager by LAS, the data will be displayed in the *Browse* and *Process Workflows* in the usual way.

Setting up Image Manager to receive LAS images and data:
Images and data from LAS can be sent to either an existing or a new archive in Leica Image Manager. Run Leica Image Manager and to use an existing archive:

1: Click on *File* on the main menu and from the drop down menu…

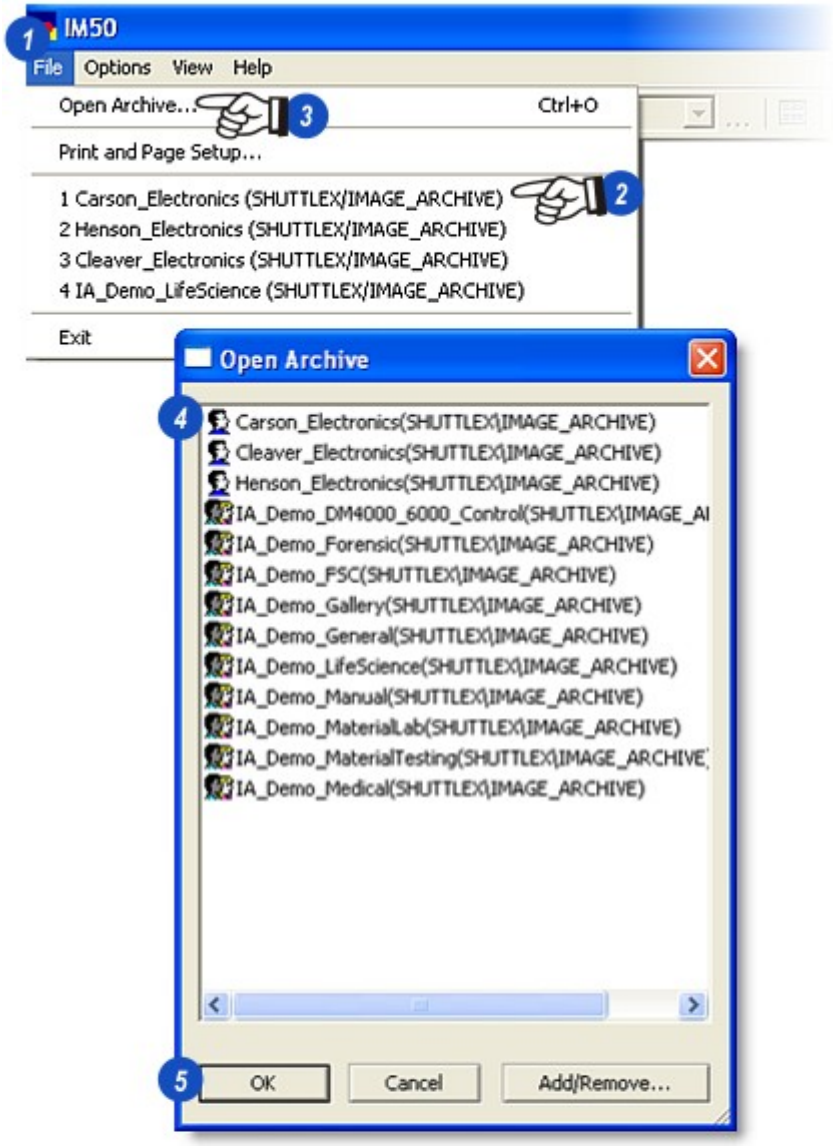
2: …click to select an archive from the *Recent* list or…

3: …click the *Open Archive* option. This will reveal the *Open Archive* dialog.

4: Click to select an archive from the list.

5: Click *OK*.

Continued… 464



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To create a New Archive:

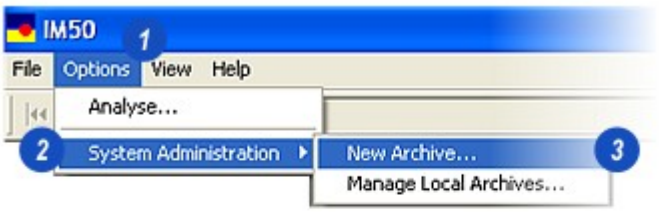
1: Click on *Options* on the main menu.

2: Click on *System Administration* and…

3: …click to select *New Archive*. The *New Archive Wizard* will appear. Follow the instructions.

A new archive need not have a range of fields – these will be selected later and the data for them will be supplied by LAS.

However, a new archive MUST have a *Text* field for every level and that must be chosen as the *Identifier (Key)* , as well as at least one *Image* field at the lowest level. If either are missing the archive will not be created.



Continued... 464

The *AutoSave* option in Leica Image Manager must be enabled so that the incoming images (from LAS) are directed to the selected archive and all of the data is correctly stored.

1: Select *Options* on the main menu…

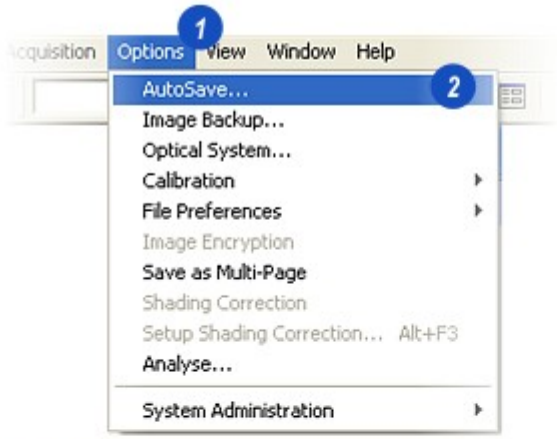
2: …and click *AutoSave*.

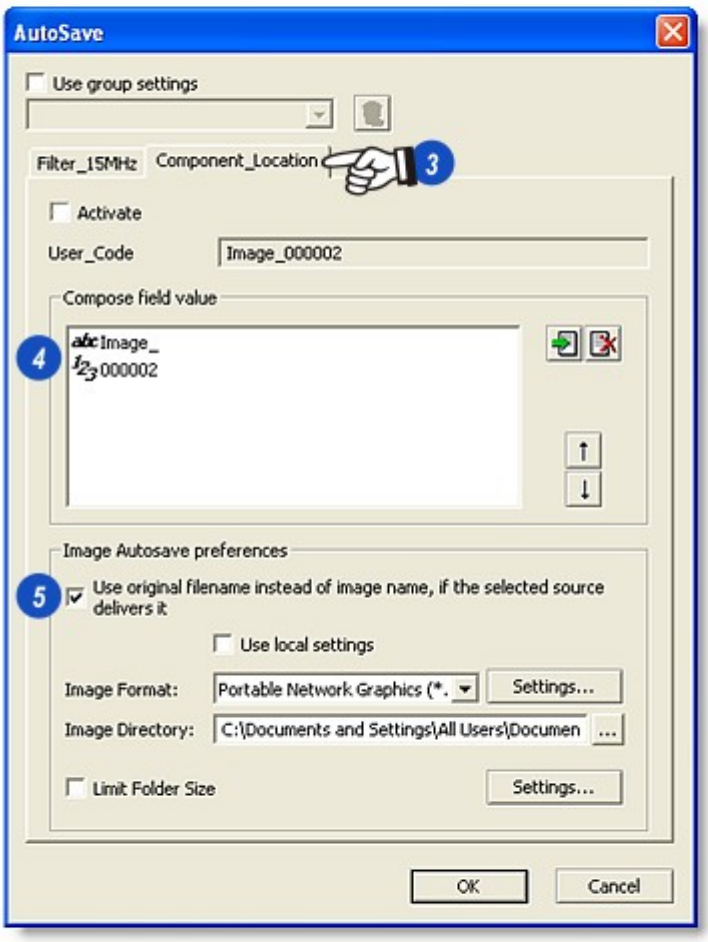
3: On the *AutoSave* dialog, click the right-hand tab that represents the new data sheet – the label on our example is *Component_Location.*

4: The fields existing in the chosen archive are displayed in the *Compose* window.

5: If it is intended that the *LAS Image Names* are used to refer to them within Leica Image Manager, click to enable *Use original filename…*

Continued… 465





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The image storage format can be changed as it is imported into Leica Image Manager. Jpeg compression is recommended because images are more compact and large archives will import quicker.

To change the image format:

1: Click on the small arrows to the right of the Image *Format* header and from the drop down list…

2: …click to select the required format.

3: The *Image Directory* (in Image Manager) will be set to that of the selected archive but can be changed by clicking on the browse button to the right of the *Image Directory* header and…

4: …using *Windows Browse for Folder* dialog to…

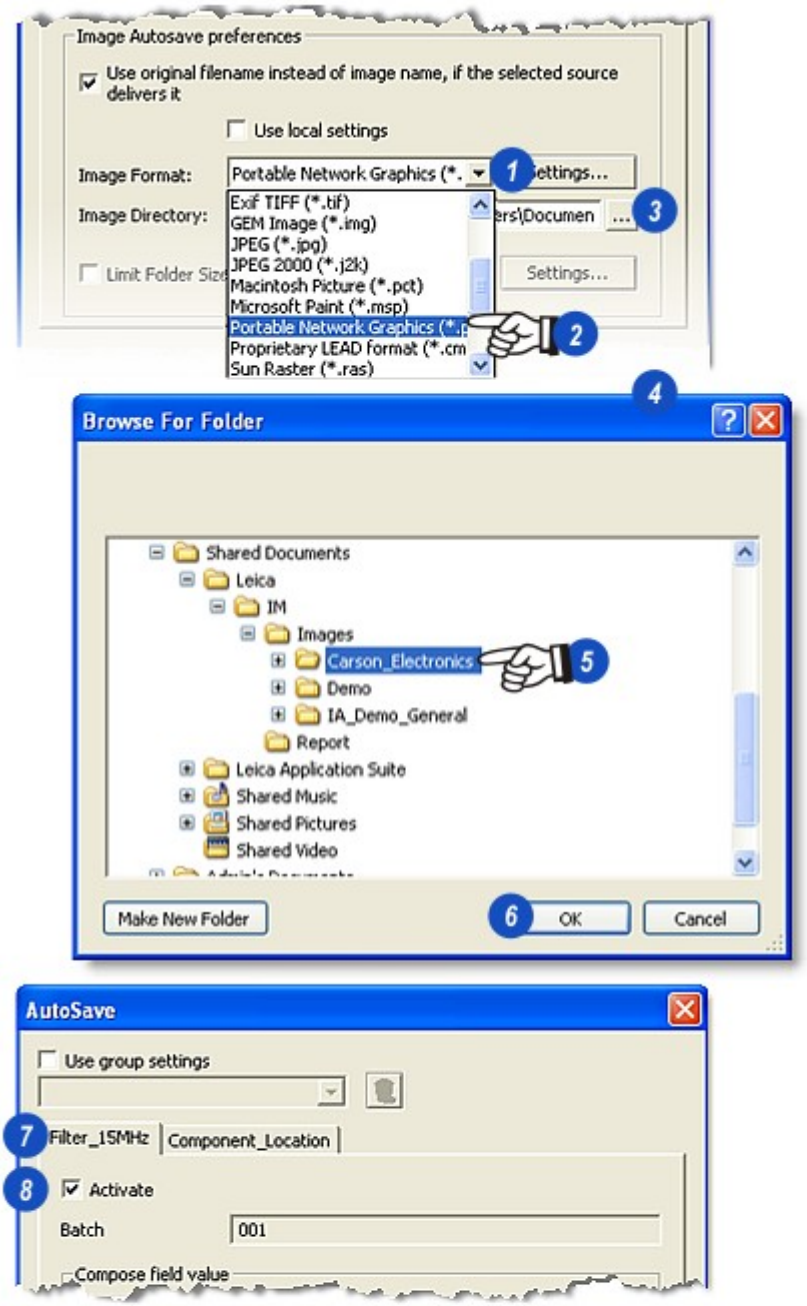
5: …navigate to the required directory.

6: Click *OK* to close the dialog.

7: Click on the left-hand tab and also…

8: …on the *Activate* checkbox to enable AutoSave.

Continued… 466



When Leica Application Suite is installed it makes available to Leica Image Manager an *Import Plug-in*, a small software application that will 'instructs' Leica IM how to store the data fields that will be sent from LAS.

The next step is to link Leica Image Manager to the *Plug-in:*

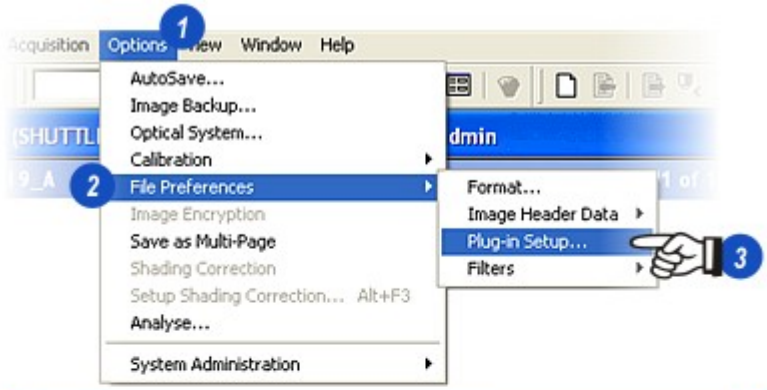
1: Click *Options* on the main menu.

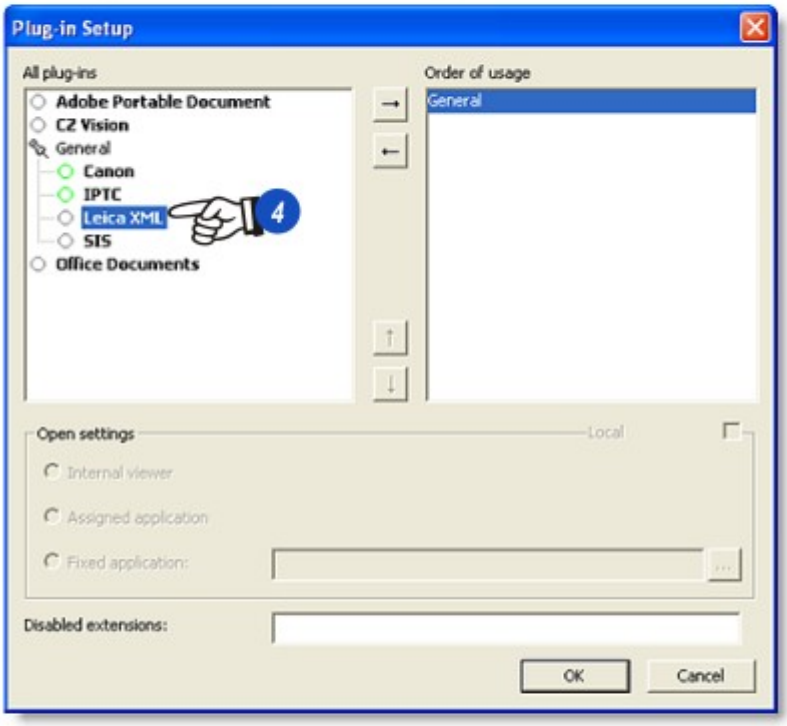
2: From the drop down list, click on *File Preferences* and then…

3: …on *Plug-in Setup.*

4: On the *Plug-in* dialog, click to select *Leica XML*.

Continued… 467





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With the *Leica XML Plug-in* selected:

1: Click on the *Include* button.

2: The *Leica XML Plug-in* is listed in the *Order of Usage* window and…

3: …the *Plug-in* is marked with a pin in the *All Plug-ins* window.

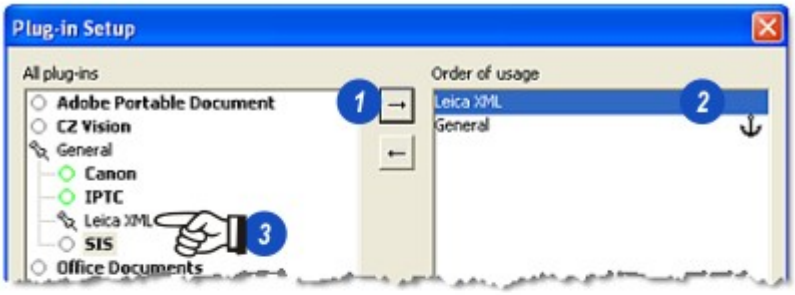
4: Check that the *Fixed Application* check box is enabled and…

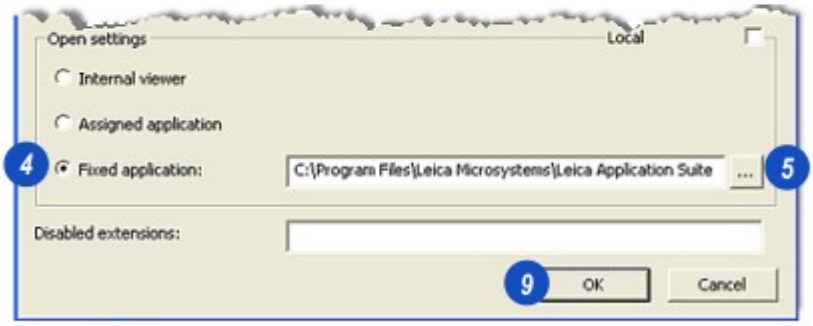
5: …the application being pointed to is *ImagePackageImporter.exe.* If it is not, click on the browse button to the right of the *Fixed Application* window and…

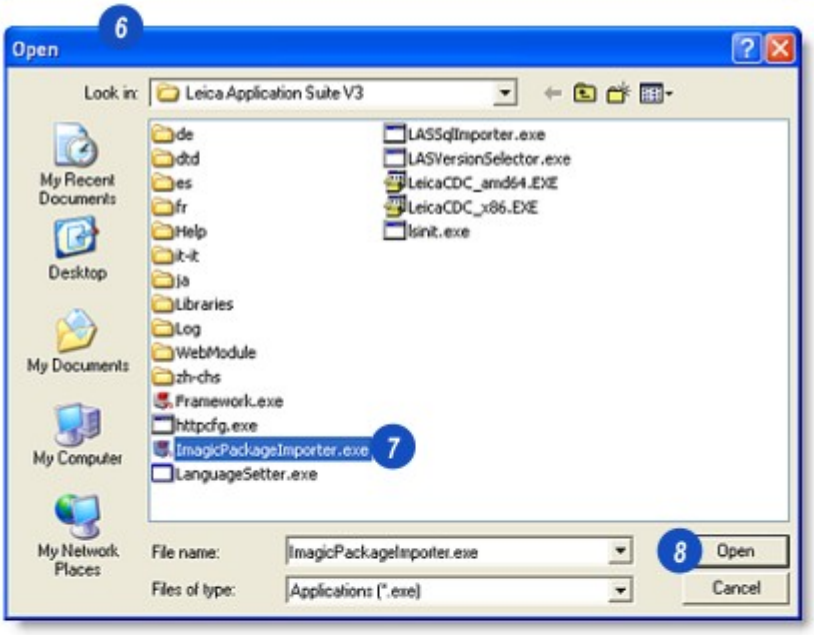
6: …on the *Browse (Open)* dialog, navigate to the *Leica Application Suite* directory and select the *ImagePackageImporter* application **(7).**

8: Click *Open*.

9: Click *OK* on the *Plug-in Setup* dialog.







Continued… 468

With the *Leica XML Plug-in* linked to Image Manager all of the LAS data fields are available to Leica IM. Now they can be displayed and selected for inclusion with the imported images.

1: Click *Options* on the main menu.

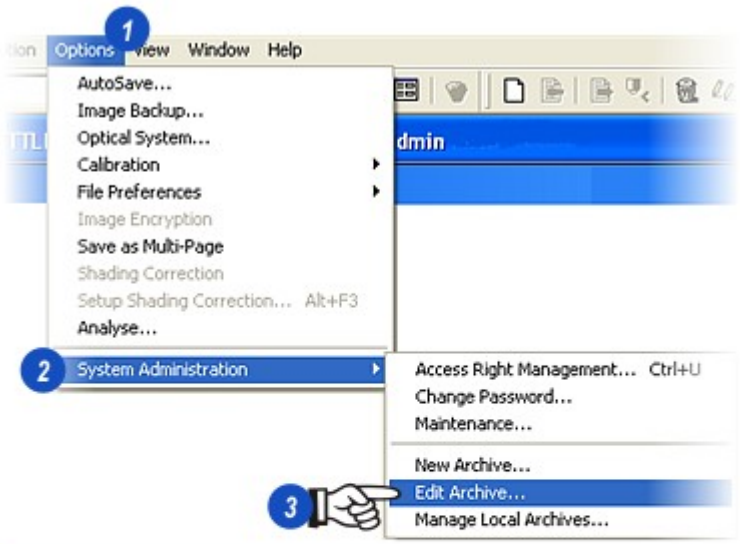
2: From the drop down list click to select *System Administration* and…

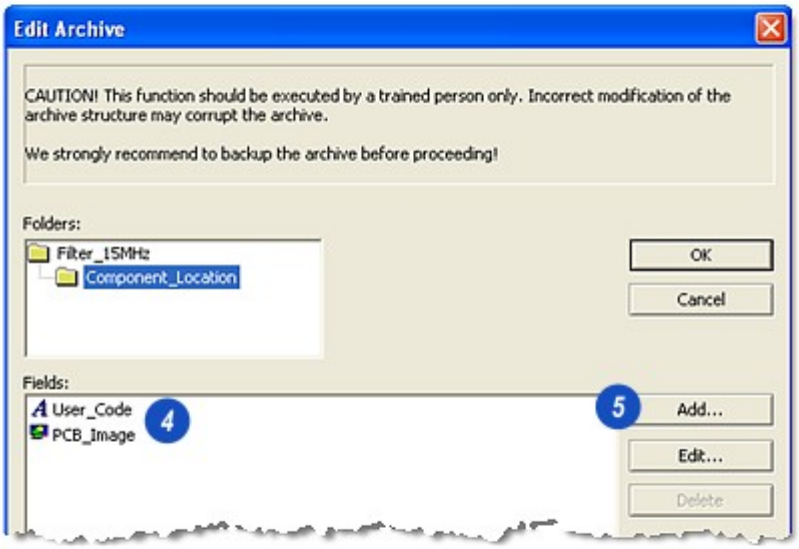
3: …click to sleect *Edit Archive.*

4: The *Edit Archive* dialog appears with the current *Image Manager* archive displayed including the Leica IM fields: Shown on the illustration are the minimum that would be allowed – one *Text* field as the key (Indicator) and one *Image* field.

5: Click on the *Add* button.

Continued… 469





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On the Field Properties dialog:

1: Click on the small arrow to the right of the *Type* header.

2: From the drop down list click to select *Image Header Data.*

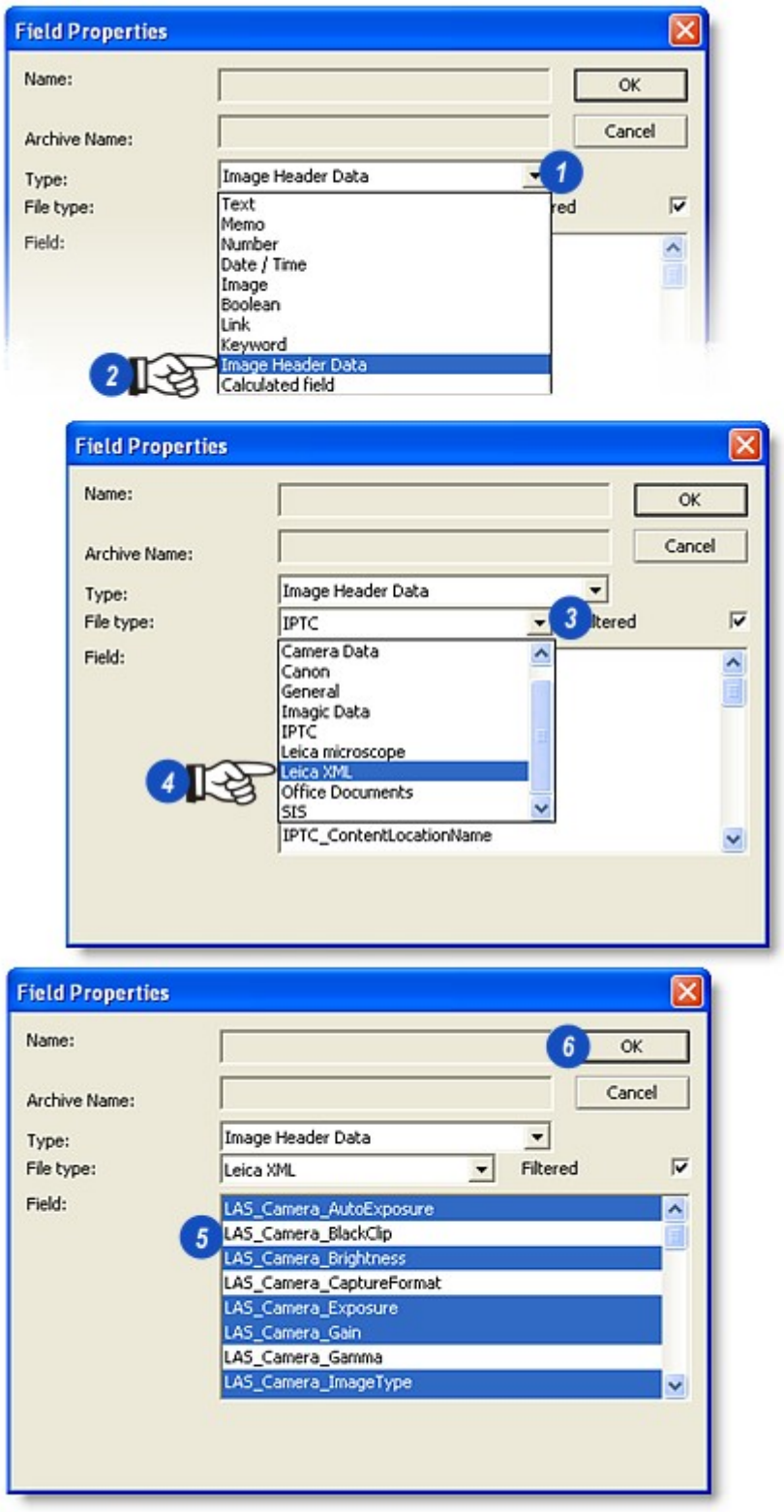
3: Click on the arrow to the right of the *File Type* and from the drop down…

4: …click to select *Leica XML.*

5: A list of all the available LAS data fields now appears in the *Field* window. Not all will be appropriate to the imported images so click to select only those required. The selected data will be imported into Image Manager but will be packaged in a separate file that is associated with the image. The fields are not available to Leica Image Manager but will be retrieved and displayed by LAS along with image.

6: Click *OK*.

Continued… 470



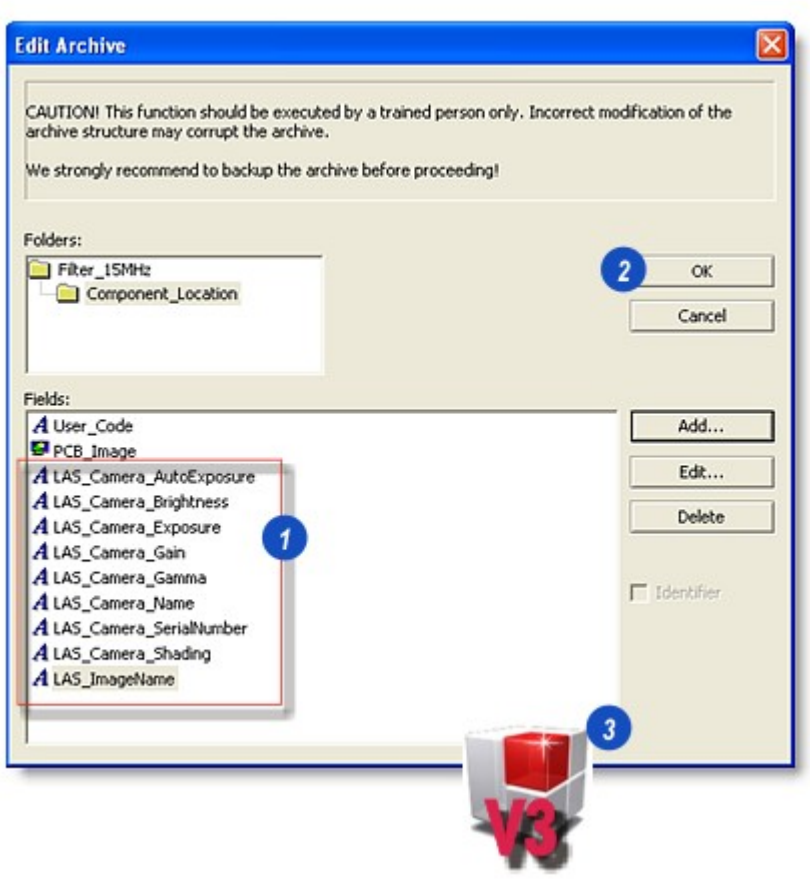
Returning to the *Edit Archive* dialog:

1: The selected LAS fields have been added to the list.

2: Click *OK* to complete the Leica Image Manager setup.

3: Double-click the desk-top icon to start *Leica Application Suite.*

Continued… 471



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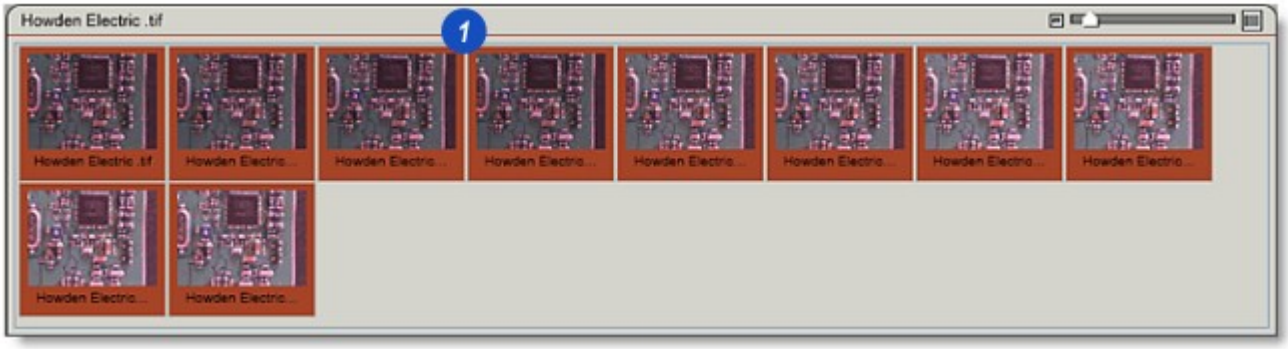


In Leica Application Suite, select the images to be exported to Leica Image Manager – it can be a single image or an entire range.

1: Click to select a thumbnail in the *Gallery* or if a range of images is required click on the first thumbnail to be included and holding down the keyboard *Shift* key, click on the last thumbnail in the range. All of the images encompassed by the two selections are highlighted.

2: Click on the *Export* button.

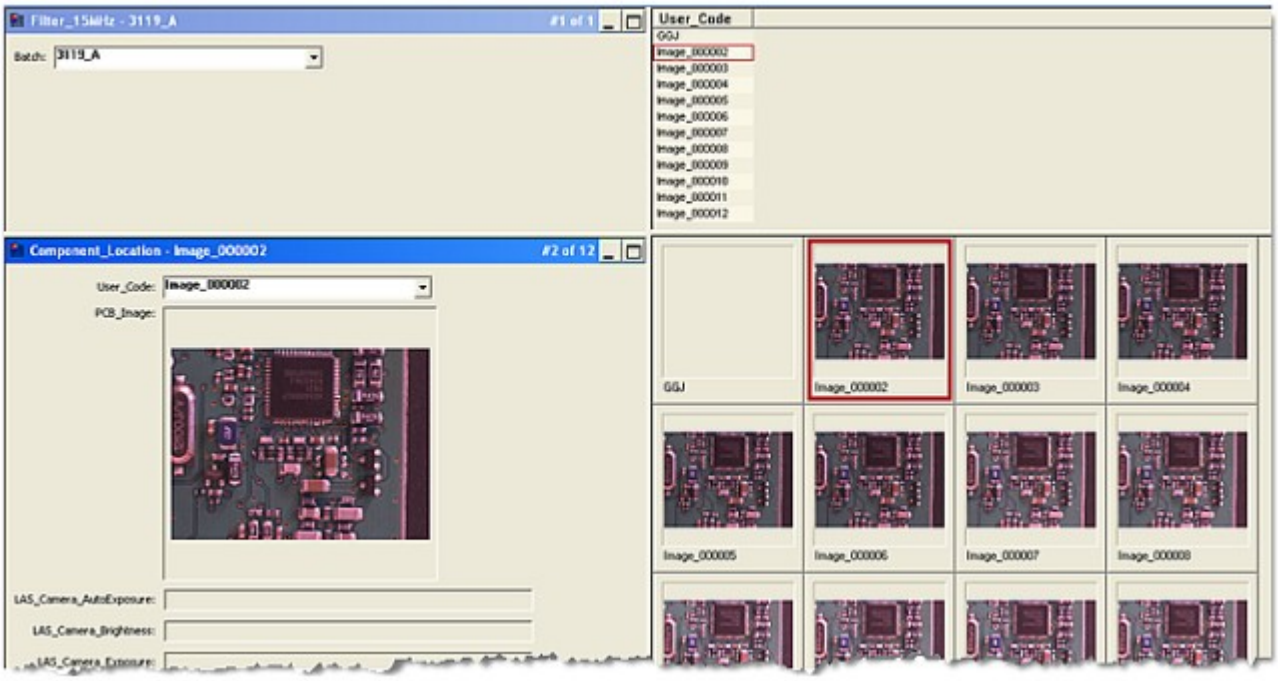
3: On the *Export Images* dialog, click to select *Image Manager* and if required…

4: …click on the *Don't ask again* check box to skip the *Export Images* dialog in the future.

5: Click *OK* and the export begins.

Continued… 472

Images exported from Leica Application Suite in Leica Image Manager.



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Existing images, sequences or movie clips stored in *Leica Image Manager 5 (IM V5)* prior to and including *Leica Application Suite 3.3*, can be easily imported into an LAS Archive or Folder created after Version 3.3. The process is slightly different for archives or folders.

Start LAS and then...

Start Image Manager.

Import into an LAS Archive:
Importing is carried out on a field-by-field basis so the target (receiving) archive must have the same structure and field names as the source otherwise data will be lost.

1: Click on the *Setup Workflow.*

2: Select the *Archive* tab.

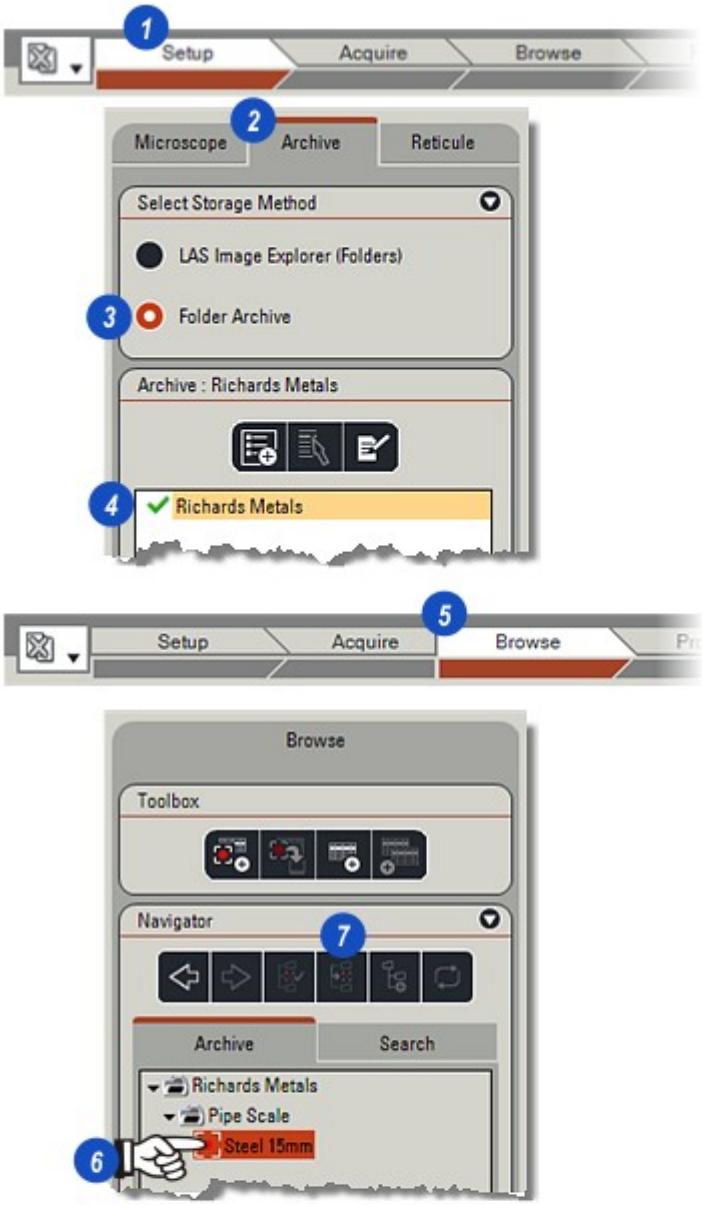
3: Click on the *Folder Archive* button.

4: On the list of available archives click to select the target archive into which the images will be imported.

5: Return to the *Browse Workflow.*

6: Click the target archive and...

7: ...select it as the *Capture Archive*.

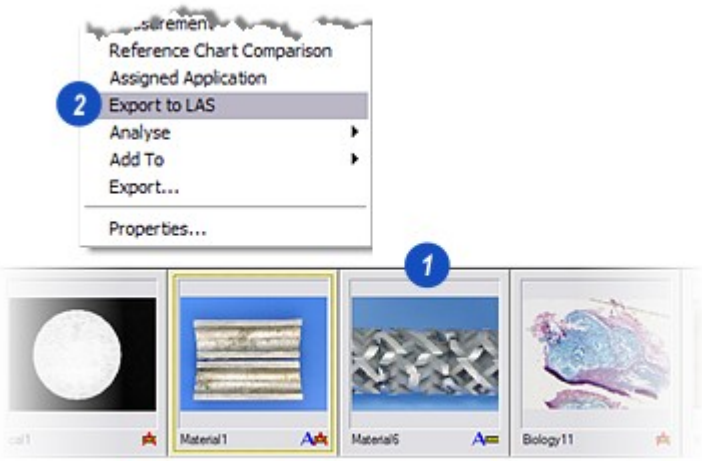


Continued... 474

Go to Image Manager and navigate to the location of the image(s) to be sent to LAS Archive:

1: Right click on the thumbnail of the image to be sent to LAS in the *Leica IM5* gallery.

2: From the *context menu*, click to select *Export to LAS*.
If this option is not available on the menu see the instructions in the Appendix: Go there... 477



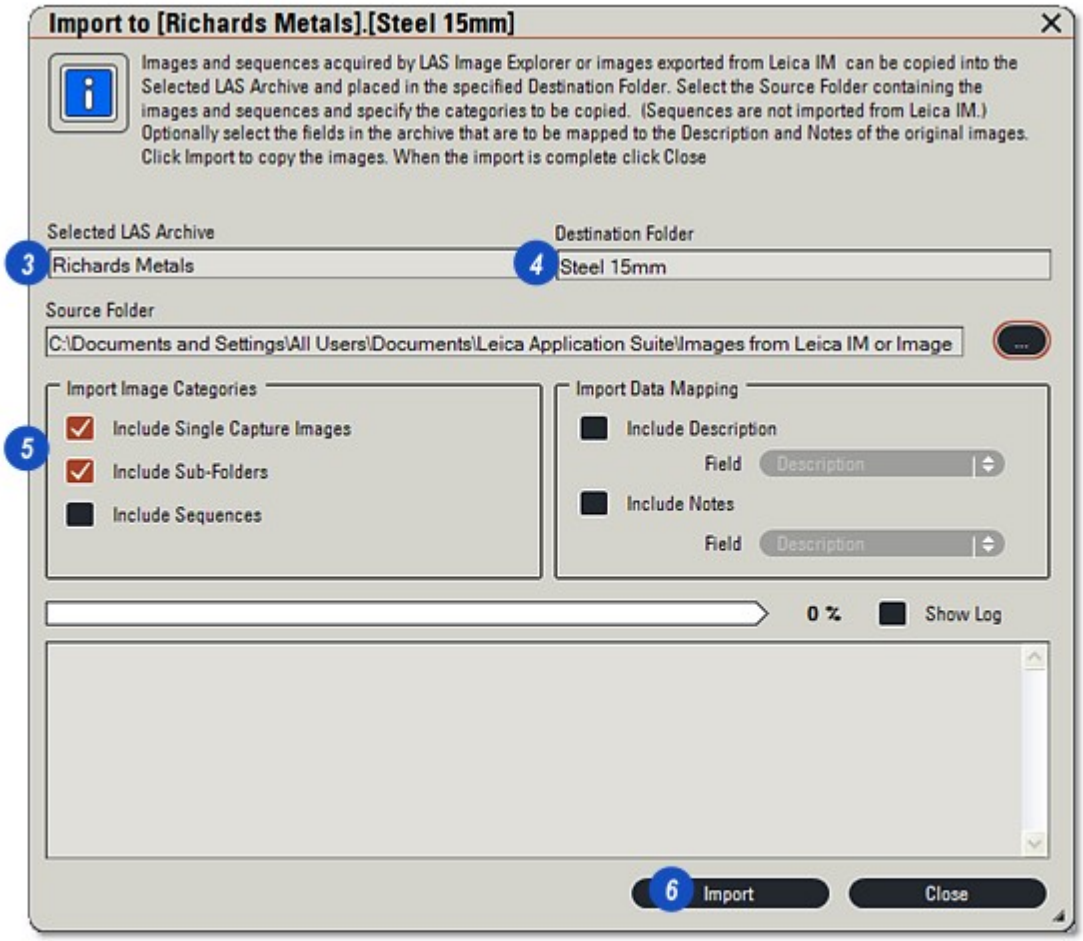
3: The *Import to* dialog appears with the target archive in LAS and...

4: ...the destination folder in the appropriate window.

5: Click to enable both *Include Single Capture Images* and *Include Sub-Folders.*

6: Click *OK* and the image will be sent to LAS.

Continued... 475



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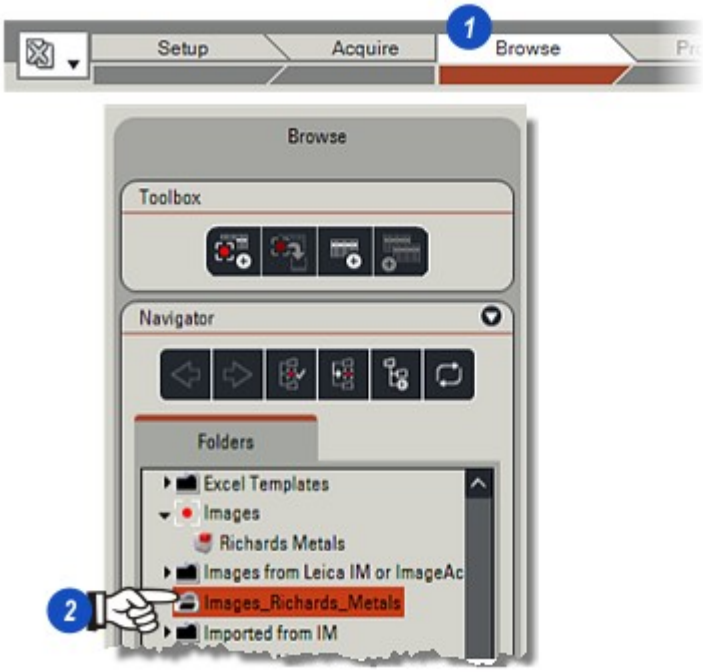
1: Click on the *Browse Workflow*.

2: On the *Folders* tab, navigate to the target folder in LAS into which the images from *Leica IM5* will be imported.

Continued... 476

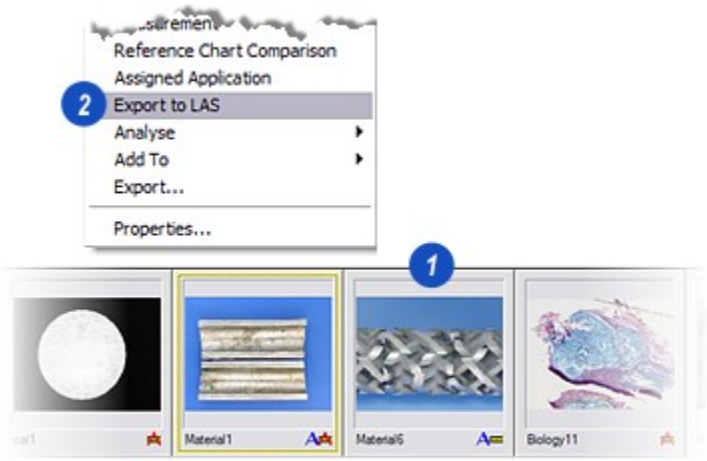


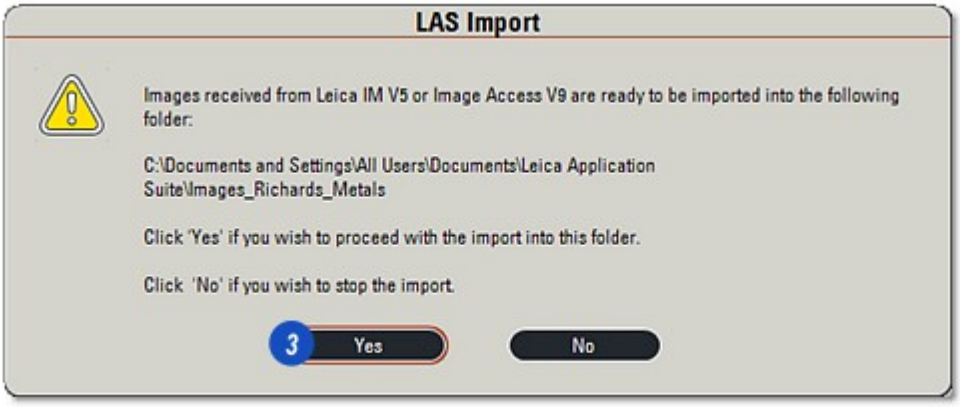
Go to Leica Image Manager and navigate to the location of the image(s) to be sent to LAS Archive:

1: Right click on the thumbnail of the image to be sent to LAS in the *Leica IM5* gallery.

2: From the *context menu*, click to select *Export to LAS*.
If this option is not available on the menu see the instructions in the Appendix: Go there... 477

3: The *LAS Import* dialog appears with the target archive in LAS listed. Click *Yes* to confirm the import and the image will be imported.





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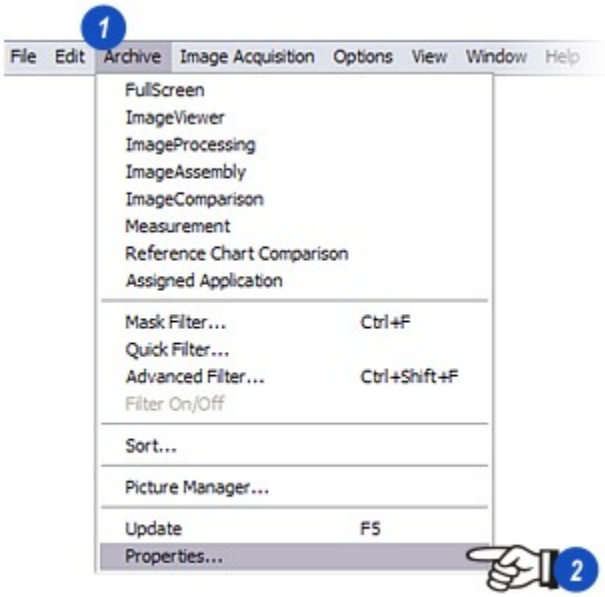
Right clicking on a thumbnail in the *Image Manager* gallery, reveals a context menu. To be able to export images from *Leica IM5* into *LAS* archives or folders, a small program must be enabled. The program resides in *Leica Application Suite* but is launched from *Leica Image Manager 5* by clicking on an entry in the context menu called *Export to LAS*.



To put the option on the context menu:

1: In *Leica IM5*, click on *Archive* on the main menu and...

2: ..from the drop down menu click the *Properties* option.

3: On the *Archives* dialog, click to enable *Assigned application*.

4: Click in the *Name* text box and type *Export to LAS*.

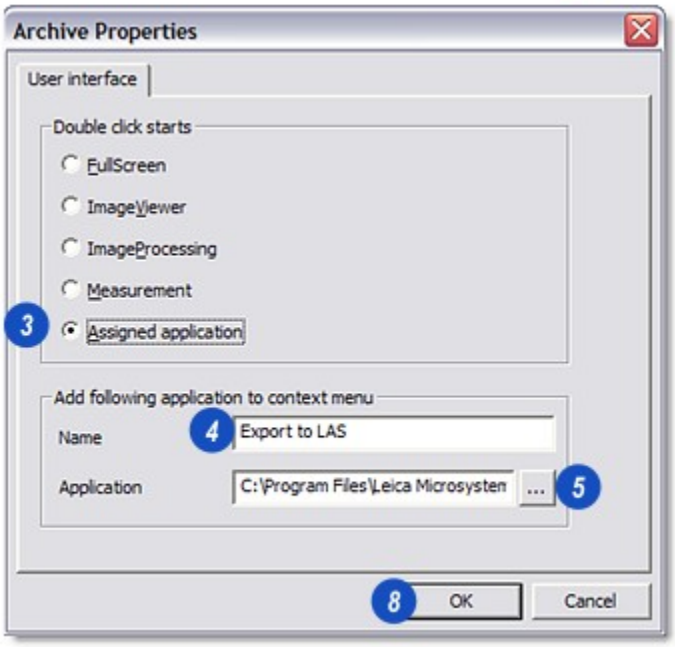
5: Click on the browse button to the right of the *Application* text box and...

6: ...on the *Open* dialog navigate to: *C:\Program Files\Leica Microsystems\Leica Application Suite V3...*

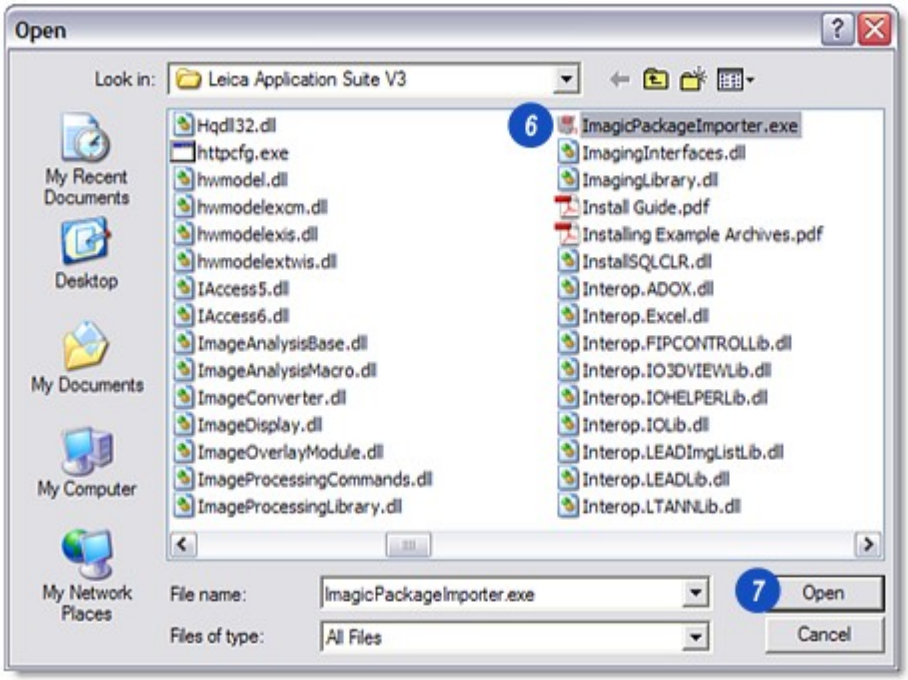
...and click to select *ImagicPackageImporter.exe*

7: Click *Open.*

8: Click *OK*



The *Export to LAS* option will now be available on the *Leica IM5* context menu.



Extended Annotation

Extended Annotations is an optional module that adds considerable power and flexibility to the image annotation tasks.

With three line styles, two shapes - *Rectangle*, including *Square*, *Ellipse* also including *Circle* - and an adaptable *Caption Label* text tool, Extended Annotations provides all of the essential features to produce high quality, professional annotated images.

A large number of graphic objects can be created and, if required permanently merged into an image.

The *Object Grid* that can be hidden or revealed, improves productivity by listing all of the graphic objects and their basic properties. Clicking on an entry immediately selects the object on the image, especially convenient when dealing with large, complex annotations.

Feature details include - click on a feature to go there...

- Drawing Lines 488: 488
- Drawing Rectangles and Ellipses: 489
- Drawing a Caption Label: 490
- Inserting an Image: 491
- Merging Graphics with an Image: 492

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The Extended Annotation tools are available on all of the Workflows except Setup. To launch the Extended Annotations interface:

1: Click on the *Extended Annotations* icon on the *Side Tool Bar* and click to select the *Extended Annotation* option.

The Toolbox tools are:
2: The *Pointer* used to select drawn items.

3: *Text* tool for creating labels captions.

4: Three Line tools – *Plain Line, Arrow* and *Distance Line.*

5: *Rectangle* tool.

6: *Circle* and *Ellipse* tool.

7: *Insert Image* tool.

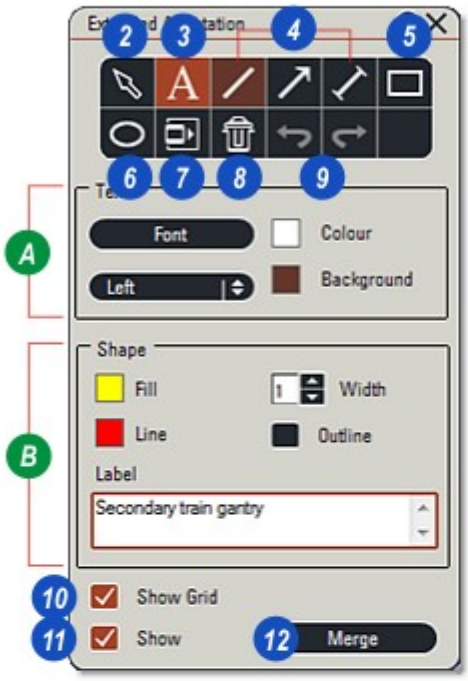
8: *Trash Can* (Delete) button.

9: *Undo* and *Redo* tools.

10: Display the *Object Grid*, a list of all of the drawn objects.

11: The *Show* check box must be enabled for the *Control Panel* to be displayed and the tools activated.

12: *Merge* incorporates the drawn objects with the image permanently.



A & B: These areas of the panel change according to the tool selected.

Continued… 480

The graphic objects drawn on an image are listed on the *Object Grid* to make access and editing quick and easy.

1: Graphic objects – rectangles, text and lines…

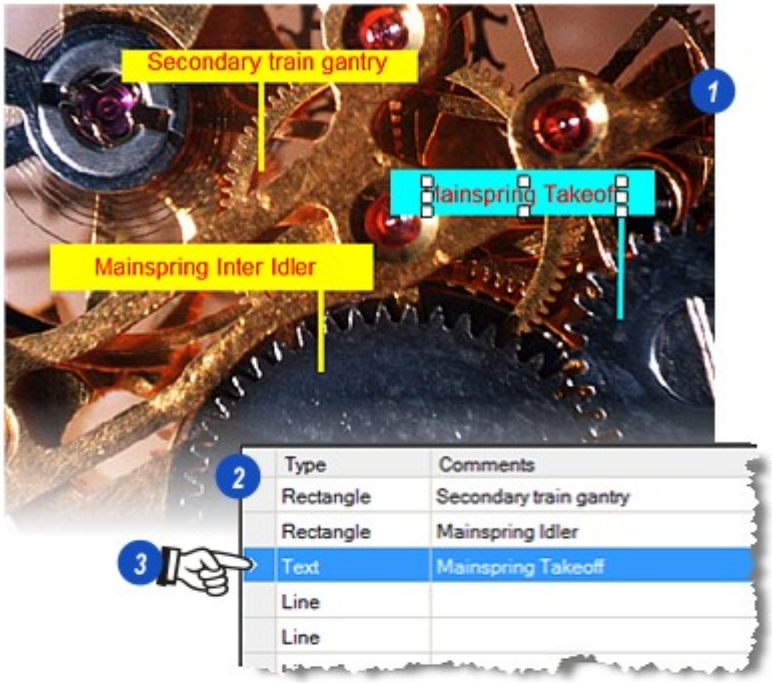
2: …are listed on the *Object Grid* if the *Show Grid* check box is enabled.

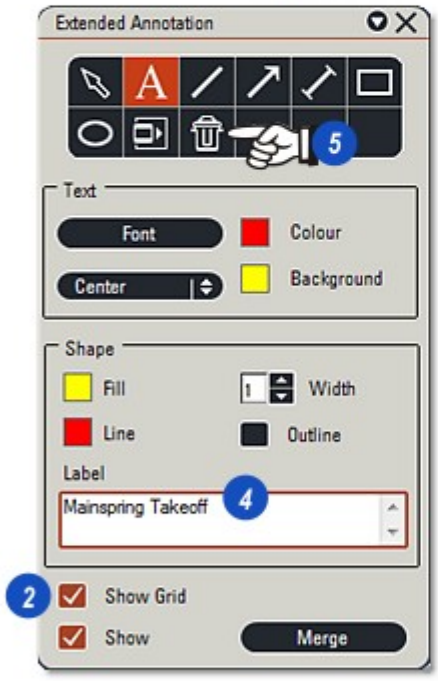
3: Clicking on a *Grid* entry immediately selects the object on the image - control 'handles' appear around it.

4: The caption text can be edited in the *Label* text box or…

5: …the object deleted by clicking on the *Trash Can* icon.

Continued… 481





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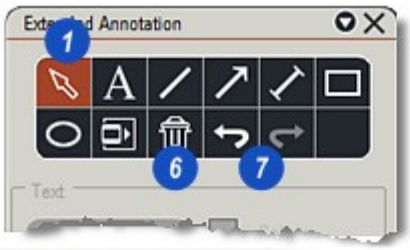
A graphics object drawn on an image can be selected and moved by dragging, edited or deleted as follows:



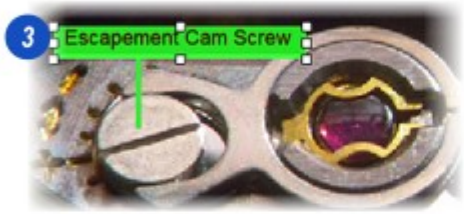
1: Click to select the *Pointer* in the toolbox.

2: Click on the object to be moved…

3: …eight small 'handles' appear around the periphery.

To Move the Object:

4: Click in the centre of the object – the cursor changes to a four-quadrant arrow - and holding down the mouse button, drag the object to a new location and release the button.



To Edit the Object…

5: …by stretching it, click on one of the small 'handles' – the cursor changes to a double-ended arrow - and holding down the mouse button, drag it to change the shape.

To Delete the Object:

6: Click on the *Trash Can* (Delete) icon in the toolbox.

To Navigate between Actions:

7: Click the left pointing arrow (*Undo*) to go back to the previous action - the right pointing arrow (*Redo*) becomes active. Click it to return to the last action.

Continued… 482

1: Select a *Line*, *Rectangle* or *Ellipse* tool from the toolbox.

Set the Line Thickness:

2: Increase or decrease the *Line* thickness by clicking the Up/Down arrows to the right of the *Width* text box.

Select the Line Colour and Transparency:

3: Set the line colour by clicking on the *Line* button and…

4: On the *Select Colour* dialog, choose a colour from the *Basic Colours* swatches, from the *Colour Wheel* by dragging the 'target' or…

5: …by clicking in the *Current Colour* text boxes and typing new *Red, Green* and *Blue* values.

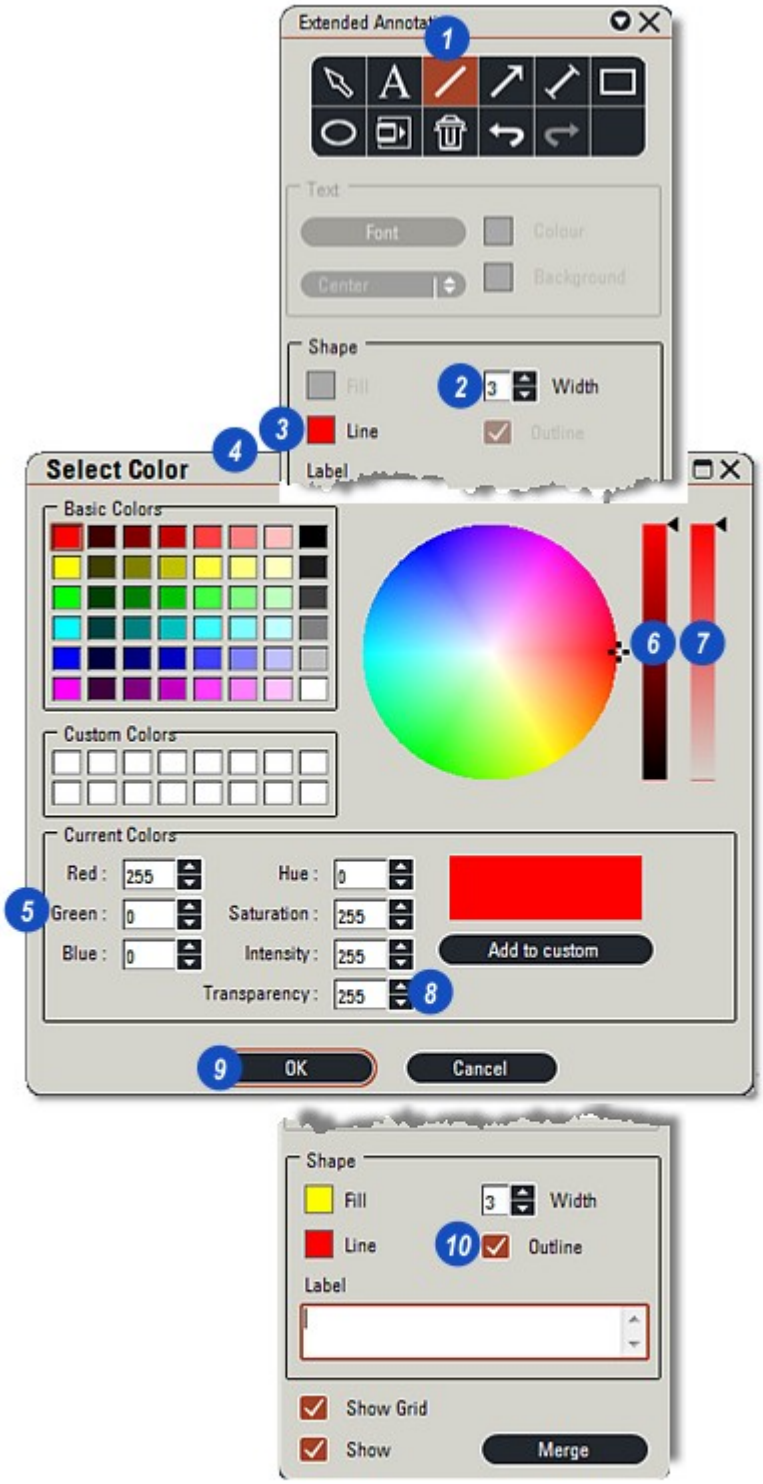
6: If necessary, adjust the *Shade* by clicking and dragging the slider.

7: Adjust *Transparency* by clicking and dragging the slider.

8: The transparency value is displayed in the *Transparency* text box. A value of 255 represents a solid colour and 0 is totally transparent.

9: Click *OK*.

10: Shapes can have an outline that is enabled by clicking the *Outline* check box. The *Outline* thickness and colour is set by following the steps above.



Continued… 483

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Rectangles and *Ellipses* can be filled with a colour and its transparency set to solid (Value 255) or completely transparent (Value 0) and all of the graduations between.

1 & 2: Click on either the *Rectangle* or *Ellipse* tool on the toolbox.

3: Set the shape fill colour and transparency by clicking on the *Fill* button.

4: On the *Select Colour* dialog, choose a colour either from the *Basic Colour* swatches, by dragging the 'target' on the *Colour Wheel* or…

5: …clicking in the *Current Colour* text boxes and typing new values for *Red, Green* and *Blue.*

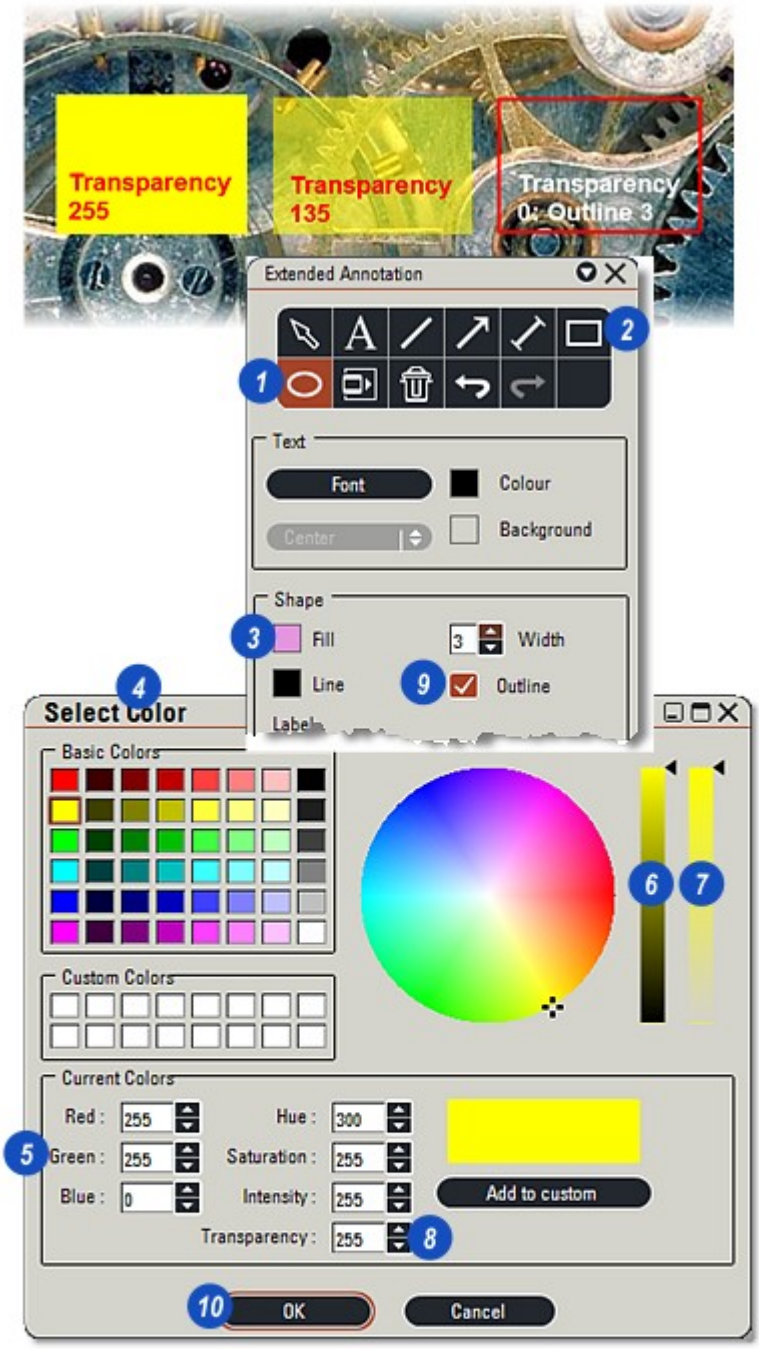
6: Adjust the *Shade* by clicking and dragging the slider on the *Shade Bar*.

7: Set the transparency by clicking and dragging the *Transparency* slider downward or…

8: …typing a value in the *Transparency* text box. Set the value to 0 (Completely transparent) and enable *Outline* **(9)** for an 'open' rectangle or ellipse.

10: Click *OK*.

Continued… 484



Caption Labels are created using the Text tool. The *Font Type Face, Style, Size* and *Colour* can be selected as well as the *Label Background Colour* and *Transparency*. The illustration **(1)** shows a typical Caption Label drawn directly onto the image.

To select the Font Type Face, Style and Size:

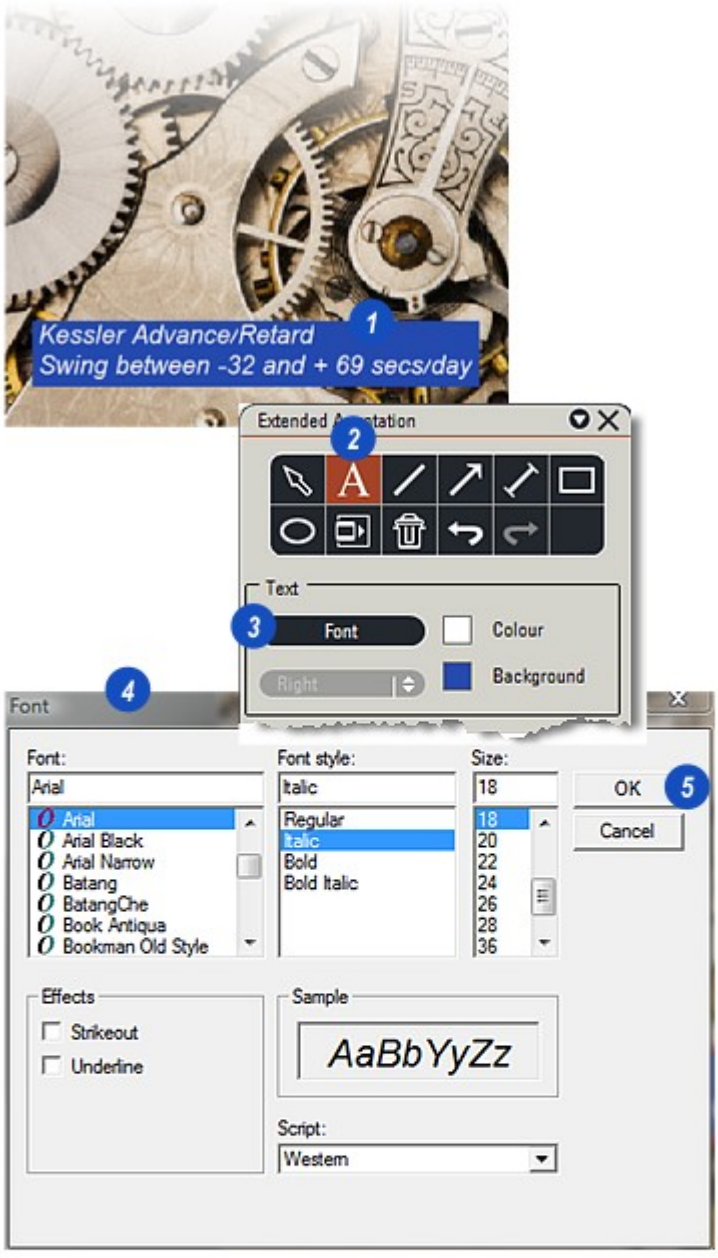
2: Click on the *Text* icon on the toolbox.

3: Click on the *Font* button and…

4: …on the *Font* dialog select the *Type Face, Font Style* and *Size.*

5: Click *OK*.

Continued… 485



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The colour of the text and the background are both changed in the same way. In Figure **(1)** the Background Colour transparency has been set to 0 (Totally transparent) so that the Caption text appears directly on the image.

2: Click on the *Text* icon in the toolbox.

3: Click either the (Font) *Colour* button or the *Background* button.

4: On the *Select Colour* dialog, choose a colour either from the *Basic Colours* swatches, from the *Colour Wheel* by dragging the 'target' or…

5: …by clicking in the *Current Colour* text boxes and typing new *Red, Green* and *Blue* values.

6: If necessary, adjust the *Shade* by clicking and dragging the slider.

7: Adjust *Transparency* – mainly applicable to the *Background* – by clicking and dragging the slider.

8: The transparency value is displayed in the *Transparency* text box. Set the value to *255* for a solid colour, *0* for a completely transparent *Background* or ant value between for different transparency levels.

9: Click *OK*.

Continued… 486

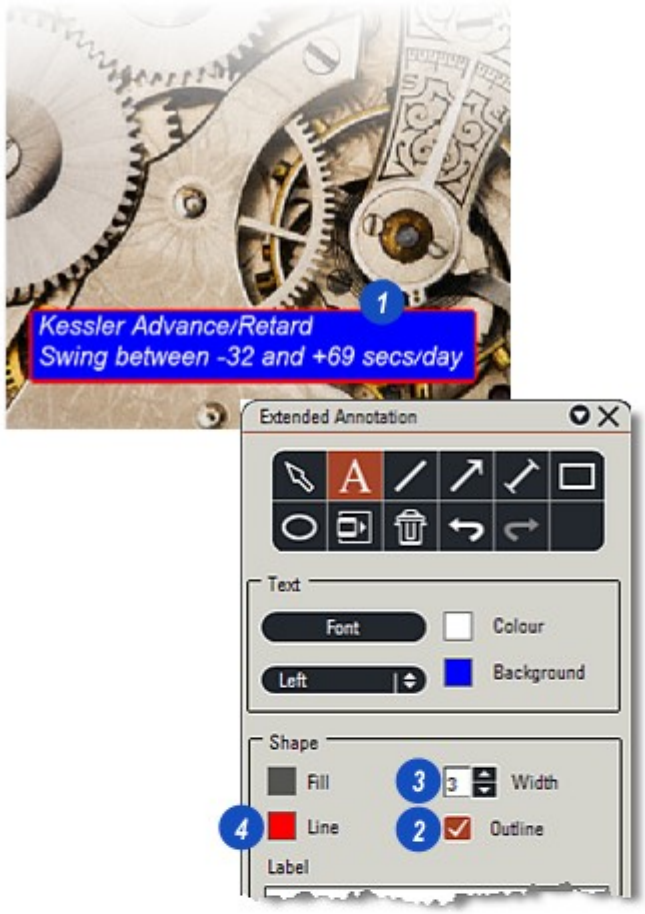
The Text Box in Figure **(1)** has a red outline created as follows:

2: Click on the *Outline* check box to enable outlining.

3: Set the width of the *Outline* by clicking on the small Up/Down arrows to the right of the *Width* text box to increase/ decrease the *Outline* thickness.

4: Change the colour of the *Outline* by clicking on the *Line* button and following the steps described previously: *Go there…* 482

Continued… 487



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There are three options for positioning text on a *Caption Label*:

1: Set *Left*,

2: Set *Centre* and

3: Set *Right*.

The text is automatically positioned as the Label is drawn.

4: Click the *Text* icon in the toolbox.

5: Click on the small arrows to the right of the *Position* header and…

6: …from the drop down list click to select the text position.

There are three *Line* styles available in Extended Annotations:

1: *Plain* Line.

2: *Arrow* and

3: *Distance Line* that displays a distance in pixels between the end strokes.

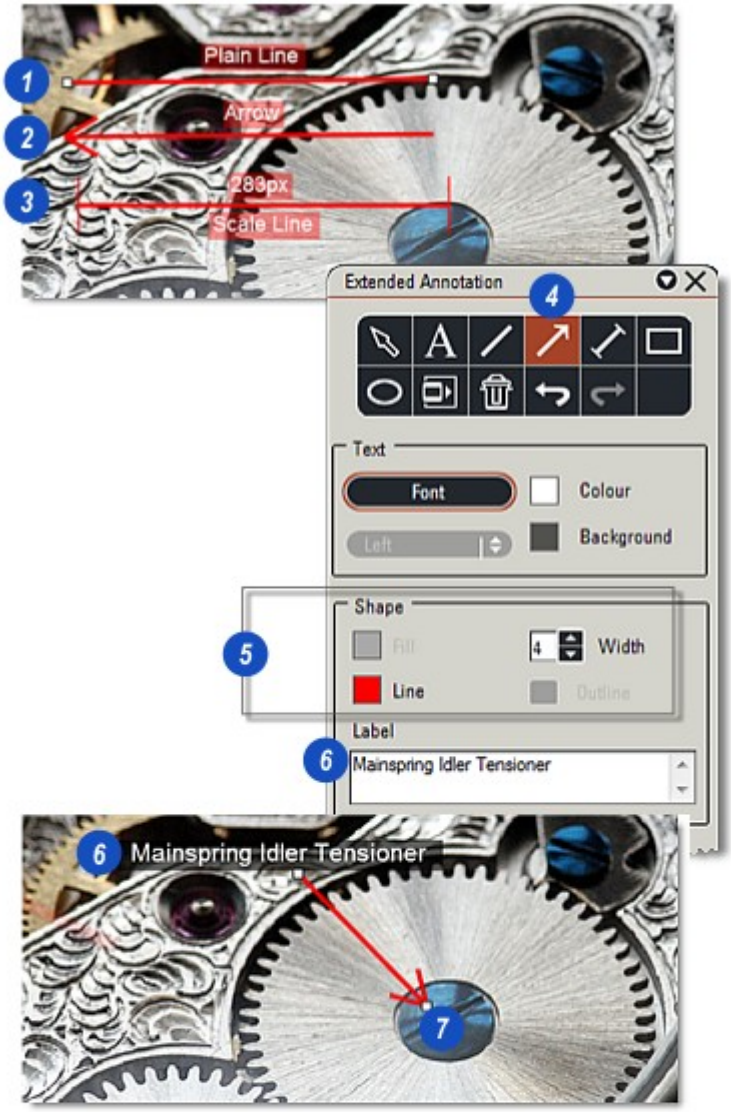
4: To draw a line, click on the *icon* of the style required in the toolbox.

5: Adjust the width and set the colour: *Go there…* 482

6: To include a *Caption Label* with the line, click in the *Label* text box and type the required text.
Set the *Text* and *Background* properties: *Go there…* 484

7: Click on the image at the starting point for the line and, holding the mouse button down drag in the required direction to the required length. Release the mouse button. The head for the *Arrow* line appears at the starting point.
Edit position, length and angle as described previously: *Go there…* 481

When lines are selected and dragged to a new location the *Caption Label* follows: To re-position the *Caption Label* only, select it using the *Point* tool, click in its centre and drag to a new position.



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There are two shape tools included with Extended Annotations, the *Rectangle* (including Square**1**) and the *Ellipse* (including Circle **2**).

1 & 2 Click on either the *Rectangle* or *Ellipse* tool on the toolbox.

3: Set the shape fill colour and transparency: *Go there…* 483

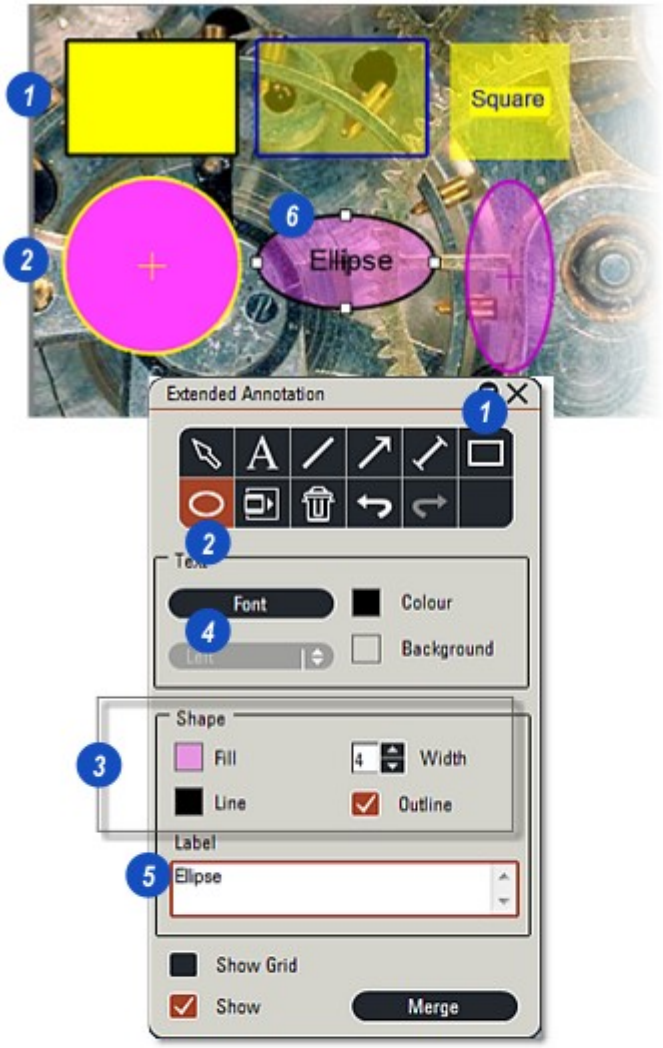
If a Caption Label is required:

4: Set the font attributes and colours: *Go there…* 484

5: Click in the *Label* text box and type the label text.

To draw the Rectangle or Ellipse:

6: Click on the image and holding down the mouse button, drag diagonally to create a shape of the required size. Edit the position and size: *Go there…* 481



To draw a Caption Label:

1: Click on the *Text* icon in the toolbox.

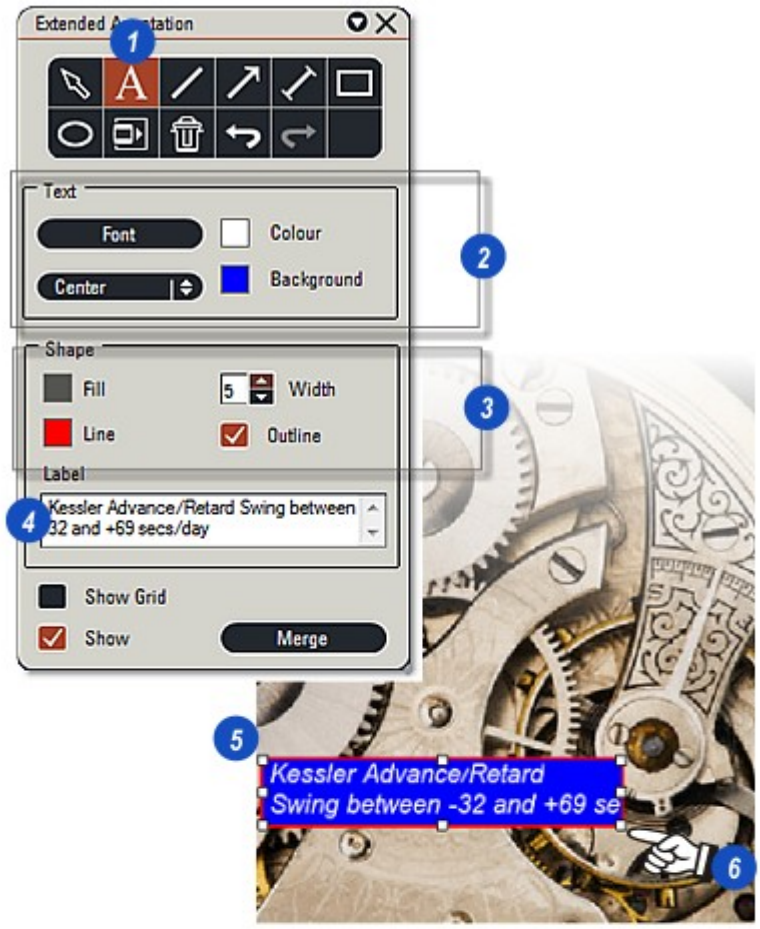
2: Set the *Font* attributes, *Font* and *Background* colours: *Go there…* 484

3: If an *Outline* is required set the thickness by clicking on the Up/Down arrows to the right of the *Width* text box and enable the *Outline* check box.
Set the *Outline* colour: *Go there…* 482

4: Click inside the *Label* text box and type the caption text.

5: Click on the image and whilst holding down the mouse button, drag diagonally to create the *Caption Label* .

6: When the label shape is correct, release the mouse button.
Edit the *Caption Label* size and position: *Go there…* 481



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It is quick and easy to insert smaller images over the main image. Inserted images should be typically low resolution - 640 x 480 pixels or less - so that they do not slow loading.

If an outline is required around the inserted image:

1: Click to enable the *Outline* check box.

2: Adjust the outline thickness by clicking on the arrows to the right of the *Width* text box.

3: Select the *Outline* colour: *Go there…* 482

To insert the image:

4: Click on the *Insert Image* icon in the toolbox.

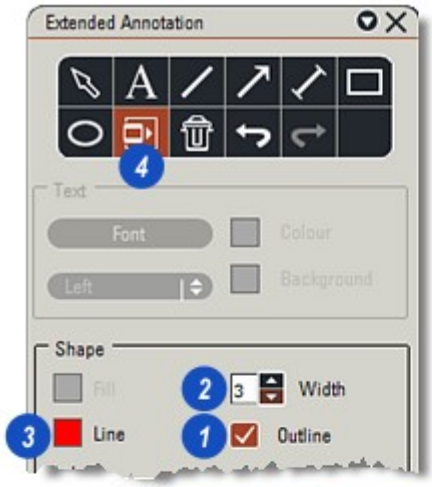
5: Click on the main image and holding down the mouse button…

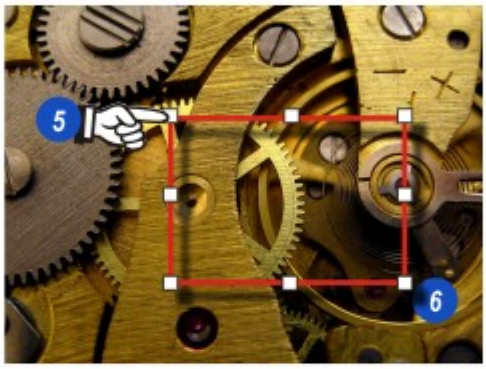
6: …drag down and to the right to draw a box. The inserted image will be scaled to fit inside the box.

7: Release the mouse button and the *Windows Open* dialog appears.

8: Navigate to the folder containing the image to be inserted and click on its name. Click *Open*.

9: The image is inserted into the box. Edit the insert size and position: *Go there…* 481



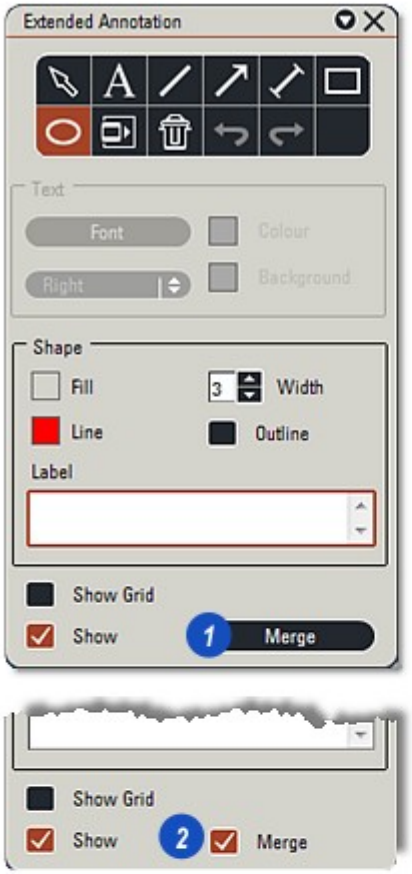


Continued… 492

When *Merging* is enabled all of the Caption Labels, Shapes and Lines drawn on the image will be permanently included as part of the image and cannot be altered.

1: Merge on a captured image is a ‘one shot’ button and only merges annotations with the current image. Click to merge.

2: Merge on live images is a check box setting and whilst it remains enabled will merge annotations with all captured images. Click to enable - click again to disable merging.



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The Leica Application Suite *MultiTime* optional module is a highly effective solution for the automatic acquisition of images over time. The time span and acquisition may range from many images per second or just a few delayed by minutes.

After acquisition they may be visualised, enhanced and documented. Other LAS modules can perform analysis and measurement of the images.

There are 2 distinct components to *MultiTime* that are operated independently:

- *MultiTime Time-lapse:* Images are acquired with a delay starting at 1 sec .
- *MultiTime Movie:* Images are acquired in a compressed image stream directly to the hard drive as fast as possible, equivalent to making a video recording.

If images are to be collected over a long time, the user must ensure that the specimen and microscope are unaffected by changes in temperature, focus position and the electrical supply. Achieving these conditions is not the remit of the *MultiTime* module.

Time Lapse is an imaging technique that acquires images with a predefined delay between each image acquired.

The images are stored to the hard drive at defined intervals and can be recalled individually, in a continuous loop or as an AVI movie file. This delay time is the distinguishing feature between normal imaging and *Time Lapse* imaging.

Time Lapse imaging is best suited for continuous image acquisition over long periods, or where there is no need for image data at full frame rates during the operation.

Time Lapse imaging typically has a significant time delay between each image processed. The camera can acquire images at its full frame rate, but only one image is processed per period. This is because it is not efficient to process every single image if only certain images are of interest.

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With the *MultiTime* module installed and enabled:

1: Click on the *Select Acquisition Mode* button.

2: From the menu, click to select *MultiTime*. The 'T' symbol will appear on tabs in both the *Acquire* and *Browse Workflows.*

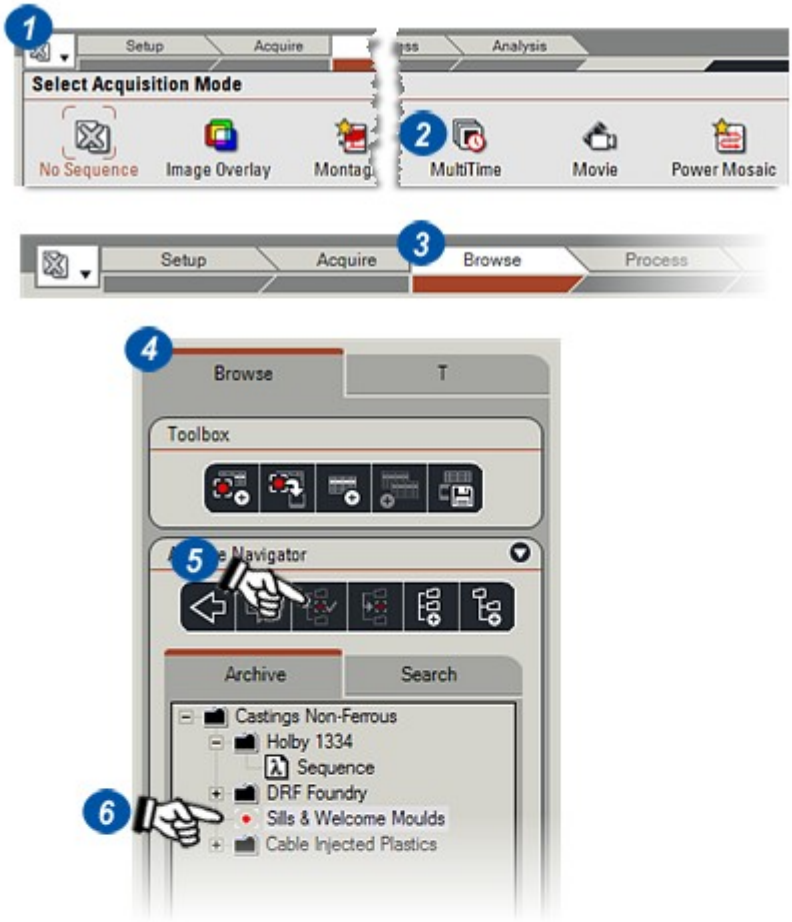
Select the Fixed Capture Location:
The *Time Lapse* images will be captured into the fixed location.

3: Click on the *Browse Workflow* and...

4: ...if necessary click on the *Browse* tab. Click to select the capture archive and then...

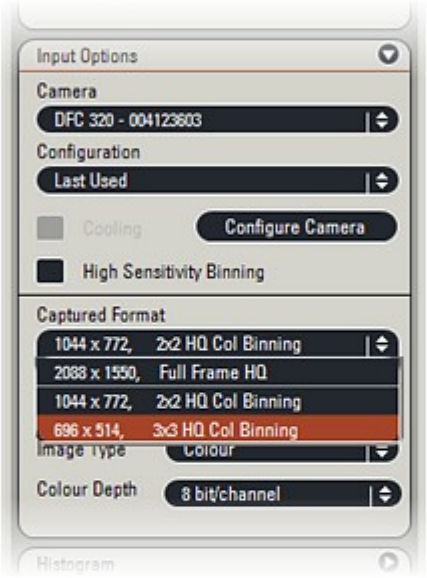
5: ...click on the *Set Fixed Location* button.

6: To indicate the fixed capture location archive, a red dot appears to the left of it.



1: Click on the *Acquire Workflow* tab. Both exposure and capture format depend to a great extent on the time lapse duration between individual images.

For example, a short time lapse of say 1 second, will demand a short exposure time to allow the image to be captured, processed and written to disk, for the control files to be created and written, and for the imaging elements in the camera to be reset ready for the next image. If these functions together take longer than the time lapse, images will be lost.



For a short time lapse:

Make sure the exposure time is also short – as a guide 150mS should be a maximum. Adjust the microscope light settings if necessary to achieve a short exposure.
Consider using VGA or 4x4 Binning for the *Captured Format*, avoid using *High Quality* (HQ) and set the *Captured Bitdepth* to 8 bits keeping disk files small and processing times low.
Turn off *Store and Recall* in *Preferences*.

See: Store and Recall. 495

Long time lapse:

Whilst the exposure time may not be as critical, still consider the lower resolution capture formats. Using high resolution for multiple images will rapidly consume disk space.

Always carry out an *Auto White Balance.*

Check for a *Shading Link* or create a new one.

Regularly backup *MultiTime* projects to CD or DVD and free up disk space.

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The *MultiTime* module must be installed and enabled. See: *Registration.*

1: Click on the *Acquisition Mode* selector.

2: From the menu, click on the *MultiTime* icon.

3: Click on the *Acquire Workflow* if it is not already visible.

4: An additional tab marked *'T'* will be displayed. Click on it to reveal the MultiTime controls.

The main panel – Define Time Sequence – is further divided into 3 smaller panels:

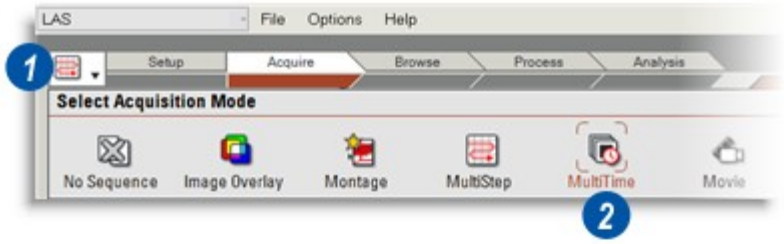
Start

Sequence and

Test Acquire Time

There is an additional panel – *Options* – which can be revealed by:

5: Clicking on the arrows to the right of the header bar.

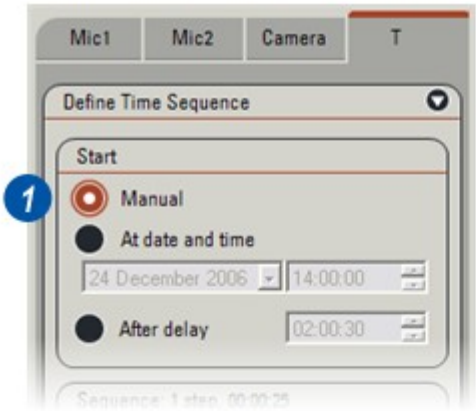


The Start panel provides three start up options for the MultiTime sequence:

Manual: The Sequence starts as soon as the *Acquire Time Lapse* button is clicked.

At date and time: Starts the capture on a specific date and at a specified time.

After delay: Waits for a specified time before starting the capture.



Manual start:

1: Click on the *Start: Manual* button and go directly to the Sequence panel to set up the time lapse.

At date and time start:

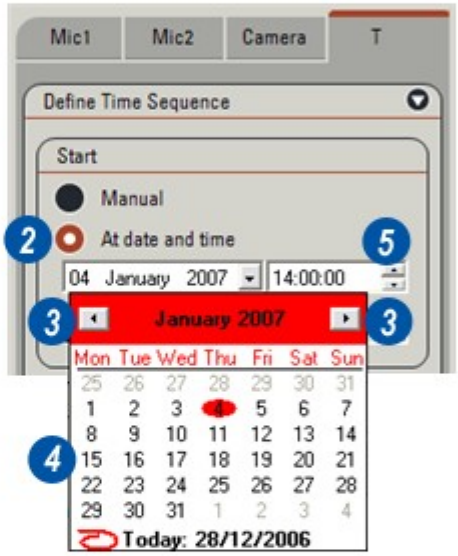
2: Click on the *At date and time* button.

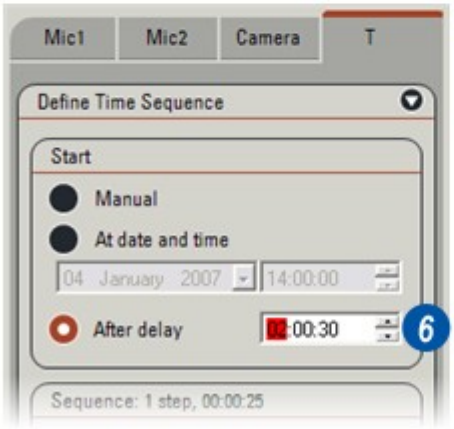
3: Click on the arrow to the right of the date window and the calendar appears.

4: Use the left/right arrows to scroll through the months and years.

5: Click on the day date on which the sequence will start.

6: The time of day is divided into three fields – *hours: minutes: seconds.* Double-click on a field to select and then use the up/down arrows to the right of the window to set the required value. Click and hold on an arrow for a fast scroll. The hours field (24 hour clock) rolls over at a count of 24, and the minutes and seconds at 60.





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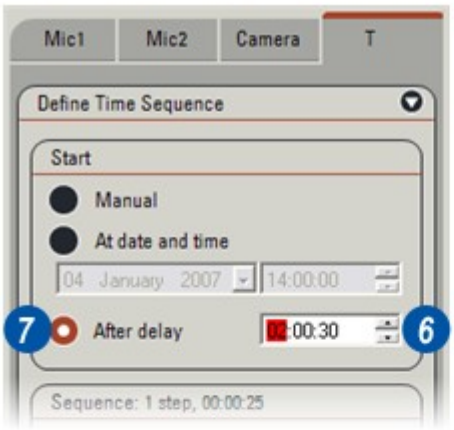
After delay start:
The delay can be from 1 second to 23 hours: 59 minutes: 59 seconds.

7: Click on the *After delay* button.

6: The time is divided into three fields – *hours: minutes: seconds*. Double-click on a field to select and then use the up/down arrows to the right of the window to set the required value. Click and hold on an arrow for a fast scroll. The hours field (24 hour clock) rolls over at a count of 24, and the minutes and seconds at counts of 60.



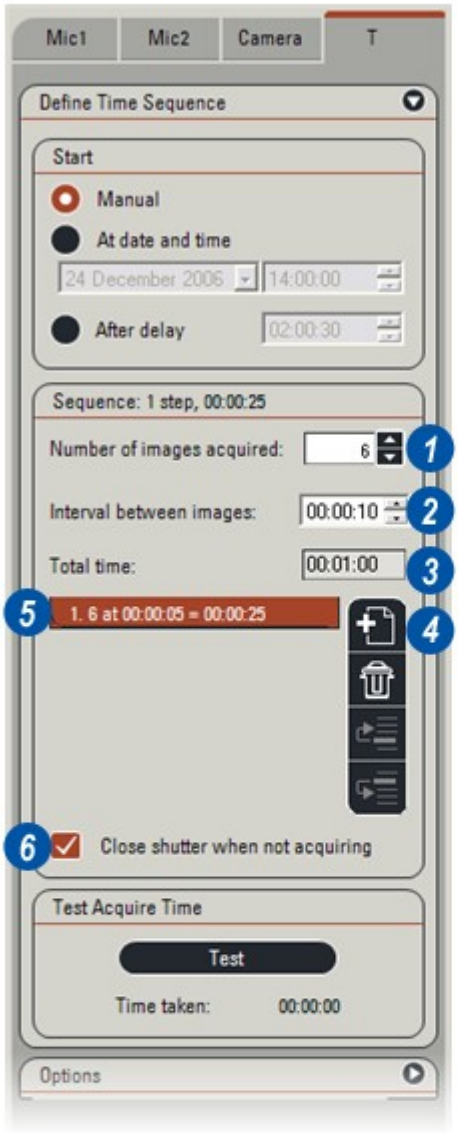
1: On the *Sequence* panel, click the up/down arrows to the right of *Number of images acquired* window to set the number of images in the sequence.

2: The *Interval Between Images* (time lapse) window is divided into three fields – *hours: minutes: seconds.* Double-click on a field to select and either type a value or use the up/down arrows to the right of the window to set the required value. Click and hold on an arrow for a fast scroll. The hours field (24 hour clock) rolls over at a count of 24, and the minutes and seconds at counts of 60.
Intervals may extend from 1 second to 23 hours: 59 minutes: 59 seconds.

3: The number of images is multiplied by the interval and the overall sequence duration is shown in the *Total time* window. In the example, 6 images are required with an interval of 10 seconds. Including an interval for capture and data writing for the 6th. image (in case further images are added to the sequence), this equates to 60 seconds or 1 minute (00:01:00). However, if no further images are added, the final interval will be ignored.

4: Click on the *Add Sequence* icon and the image count and interval appear in the Sequence window **(5).**

6: When using Fluorescence techniques, enable *Close shutter when not acquiring* to avoid damage to the specimen.



See: Testing MultiTime. 501

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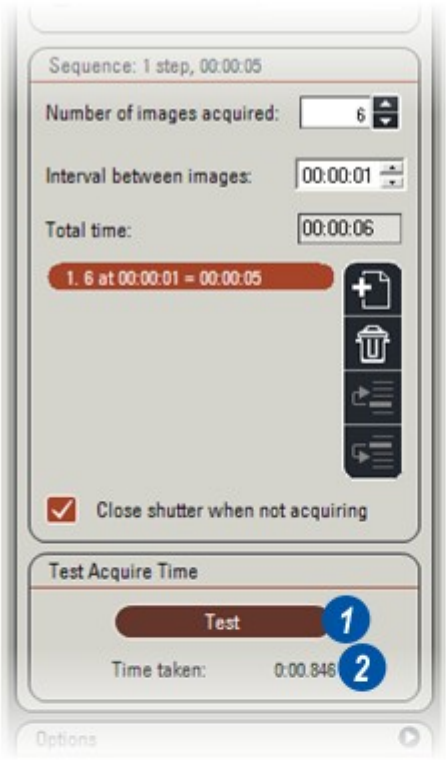
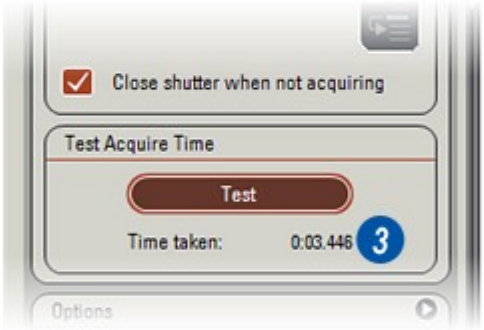
With the number of images required and the interval between each capture set, and the sequence loaded to the *Sequence* window, it is easy to check that the exposure and data capture will fit within the interval:

1: Click the *Test* button.

2: The result (in seconds) of exposing the image and emulating a write to disk together with the necessary data files is displayed beneath the *Test* button.
In the example, the interval between images is set to 1 second. The test capture and write amounted to 0.846 seconds so this sequence would have succeeded with a small margin to spare.

3: This test fails because either the exposure is too long or the captured image format results in a very large file – or both!
The test result here is 3.446 seconds, much longer than the 1 second interval, so most images are going to be missed.





See: Image Exposure and Capture Format. 496

To achieve complete flexibility, *MultiTime* can be configured with any number of capture sequences.

1: The *Start* option is executed first – in the example 'After delay' of 2 hours has been selected. When the delay has elapsed each of the sequences will be executed in turn.

The example sequences are:

2: 10 images with a 30 minute delay between each.

3: 100 images with a short 1 second delay between each image, and finally…

4: ...25 images with 1 minute between.

Each sequence was added to the overall program by clicking on the '*Add Sequence* ' icon.

Changing the Sequence order:
To move a sequence up or down the execution order:

4: Click on the sequence to be moved.

5: Click on the up/down order icons.

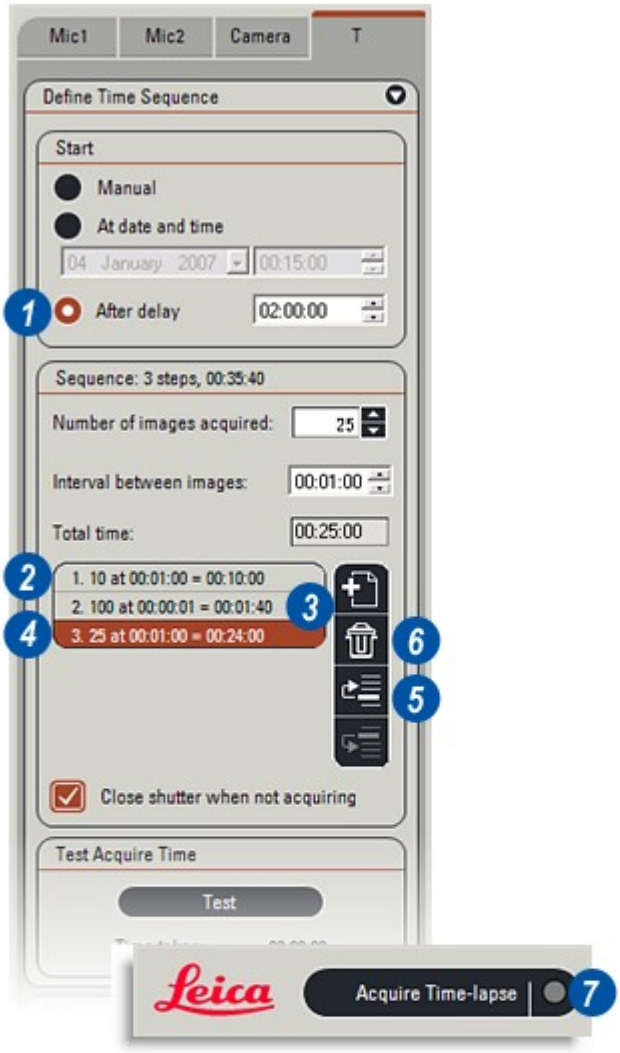
Deleting a sequence:

4: Click on the sequence to be deleted.

6: Click on the Trash Can icon.

Starting the *MultiTime* sequence:

7: To start a sequence or sequence program, click on the *Acquire* button.



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A sequence or sequence program, may be saved as a file for future use as follows:

1: Click on the arrows to the right of the *Options* header to reveal the panel.

2: Click in the *Configuration* text box and type a name for the sequence.

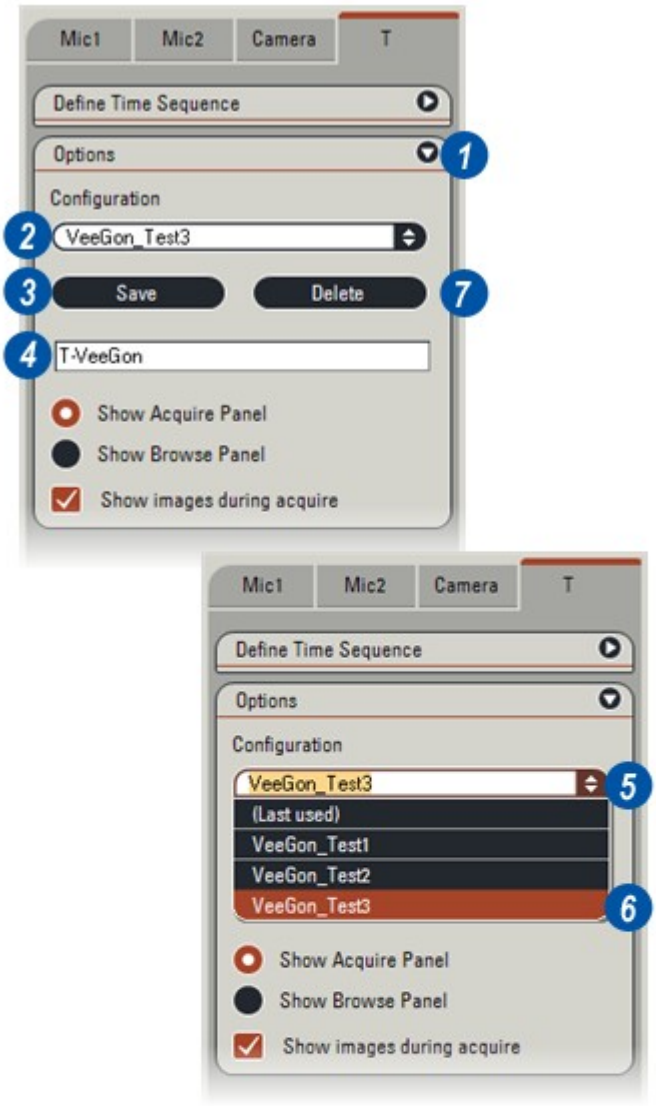
3: Click *Save*.

Project name:
When a MultiTime sequence starts, a new folder is created into which all of the images and their data files will be loaded.

By default, these folders are given the name *T-MultiTime* n where 'n' is a sequential number if other folders of the same name exist.

The prefix 'T' is always present because it denotes a *MultiTime* project, but change the folder name to one of your choice by:

4: Clicking in the *Folder Name* text box and delete by backspacing the word *MultiTime.*
Type in the new name. In the example the word *VeeGon* (the name of the test) has been typed so the folder will be named *T-VeeGon.*



To retrieve an existing configuration:

5: Click on the arrows to the right of the *Configuration* text box.

6: From the drop down list, click to select a configuration.

To delete a configuration:

6: Select a configuration from the list.

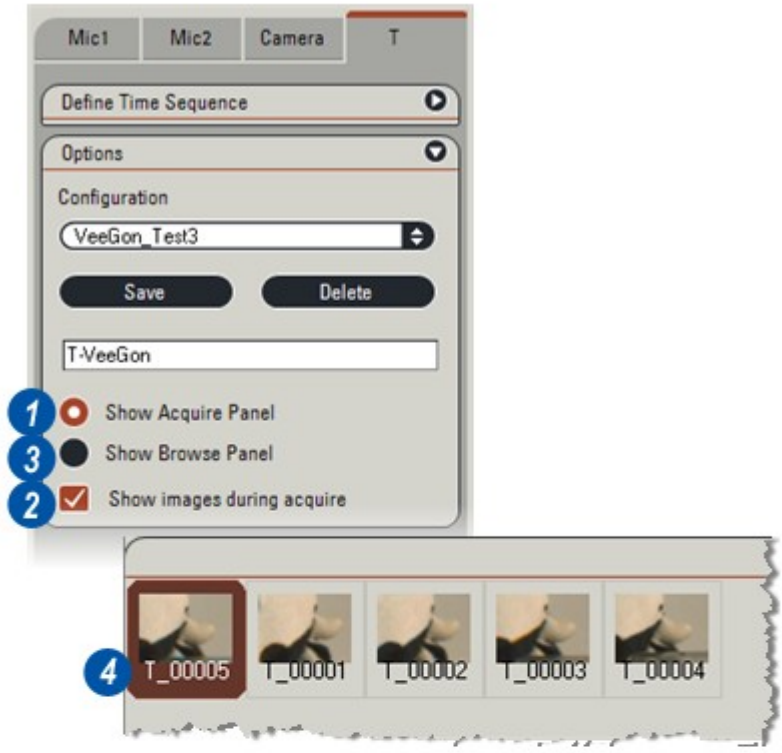
7: Click on the *Delete* button.

Three options are available to determine the state of the *Viewer* during the capture sequence:

1: Click the *Show Acquire Panel* to remain in the *Acquire Workflow* and watch the images in real time. To momentarily 'freeze' the image as it is captured. But there must be a sufficient interval between images to allow the *Viewer* to refresh.

2: Enable the *Show images during acquire* checkbox. This option works only if the Show Acquire Panel is selected.

3: Click *Show Browse Panel* to watch the sequence on the *Browse Workflow*. This has the advantage that the captured images are displayed on the *Viewer* as they are made and the *Gallery* **(4)** is progressively populated.



Continued... 505

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Progress, Pause and Stop panel:
During the capture sequence progress is displayed as:

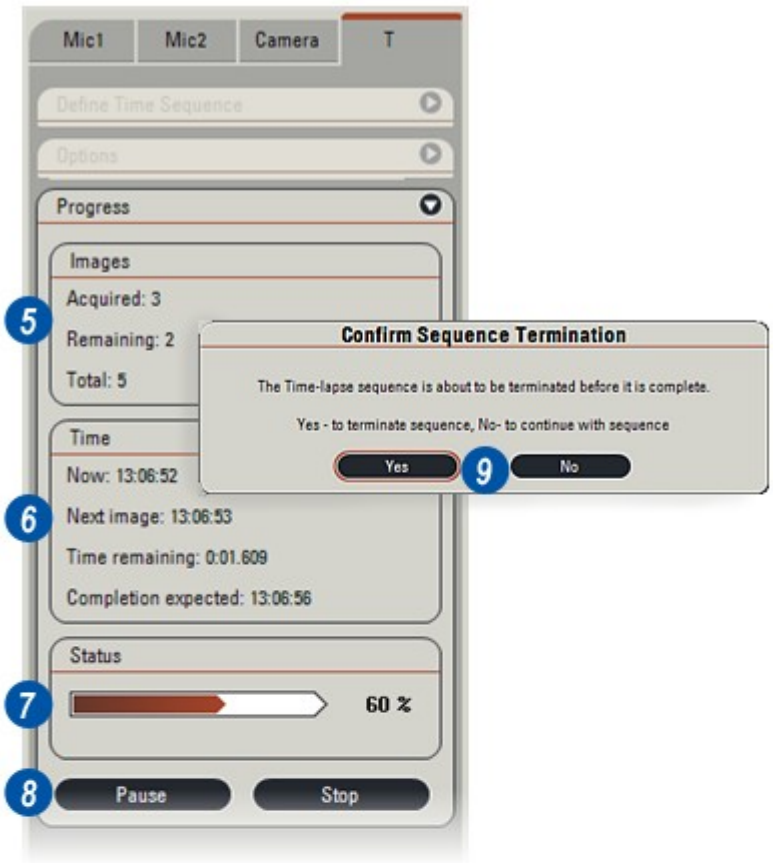
5: The number of images acquired so far and the number remaining to be captured.

6: The current time, *Next* image acquire time, sequence time remaining and estimated time of completion. If there are several sequences in a project, the completion time is for the entire project not just for the current sequence.

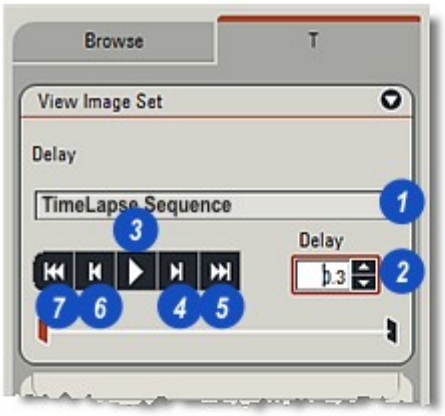
7: Graphical representation of progress, both as a bar and a percentage.

8: To suspend the sequence, click on the *Pause* button. The button label changes to *Re*sume. Click again to continue the capture. Note however, that images may be lost during *Pause* .

9: Stop the sequence by clicking the *Stop* button. The *Confirm Sequence Termination* message appears **(10)**. Click *Yes* to stop the sequence completely or *No* to continue the sequence.



When the capture sequence finishes, the *Browse Workflow* opens…



1: …with the name of the sequence in the *View Image Set* window.

2: Normally, the *MultiTime* images are played continuously, but an interval may be set and inserted between the images by clicking on the up/down arrows to the right of the *Delay* window. The delay interval is in seconds.

3: Start/Pause the sequence by clicking the *Play* button.

4: Skip one image forward button.

5: Move to the end of the sequence button.

6: Skip back one image button.

7: Move to the start of the sequence button.

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A sequence of images captured as a *MultiTime* project may be converted into a movie file which can then be played on most media software.

To convert a project into a movie:

1: Click on the arrow to the right of the *Save Time Lapse Image Set as Movie* header to reveal the panel.

2: Click on the arrows to the right of the *Movie File Type* text box and from the list select the movie file format. The .avi (Audio Video Interleave) format is the default and the most popular for video exchange.

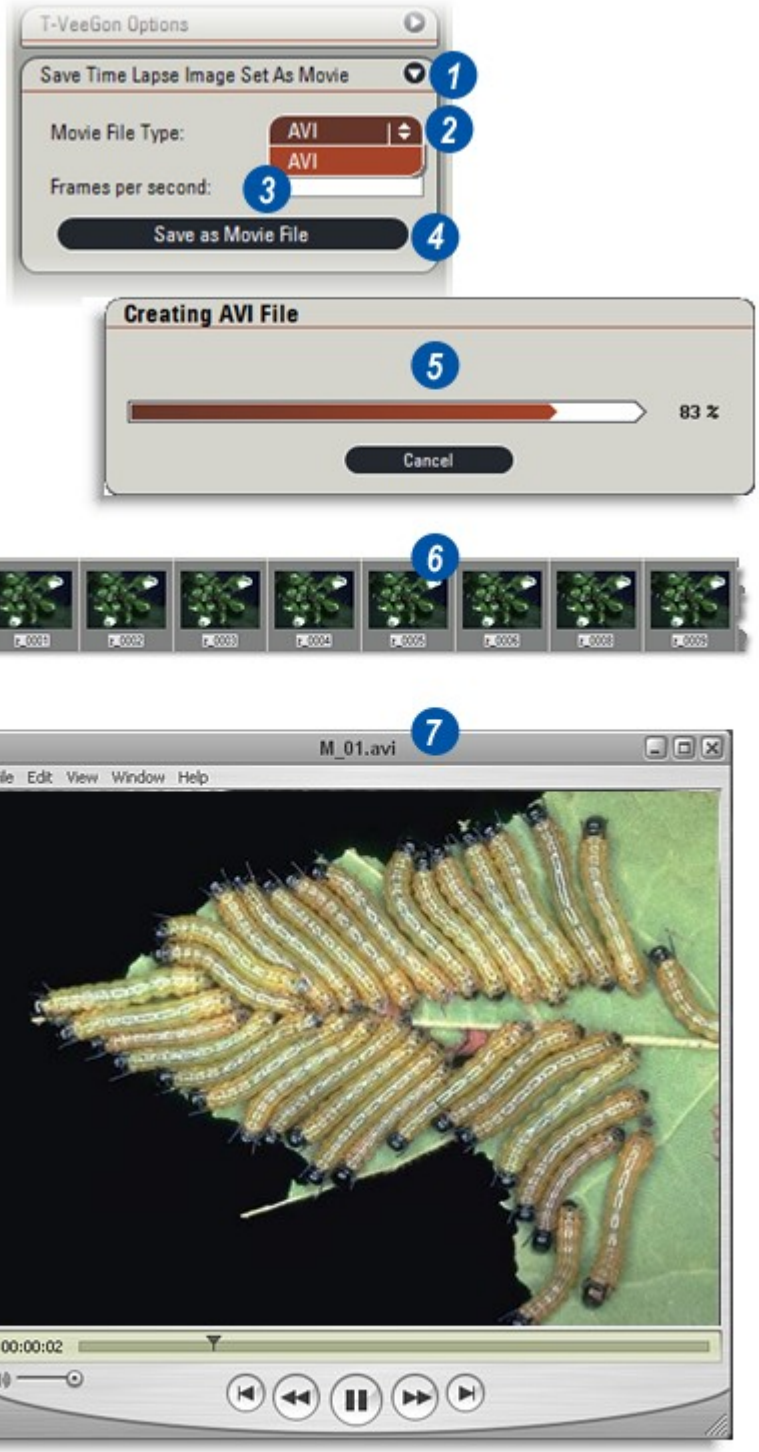
3: Set the number of frames per second. The 'smoothest' – least jerky – movies will have a larger number of frames per second. The range is 1 to 25. Double click on the *Frames* text box and type a value.

4: Click on the *Save as Movie File* button and the *Creating xxx File* progress panel appears **(5).** *xxx refers to the file format.*

6: Movie files are stored in the same folder as the *MultiTime* project and can be recognised by the file extension – in this case .avi – and they also appear in the *Gallery* as thumbnails.

7: The resulting movie file can be shown most popular video software packages – in this case Windows ® Media Player.

Preferences > Play Movie: *Go there...* 51



The *Movie* module must be installed and enabled before it is available. See: Registration for details of installation.

The *Movie* module captures images or 'frames' in real time as a single, continuous file directly to the computer hard drive. Frames are captured as quickly as the camera and the computer will allow broadly governed by the resolution and exposure time. Aim for low resolution without compromising viewing quality with fast exposure to achieve a frame rate of 20 to 25 frames per second. This will provide smooth playback.

Movies may be split into a number of chunks or 'clips' all stored as separate files. Between the clips, capture is suspended so time and disk space are not wasted on periods of specimen inactivity.

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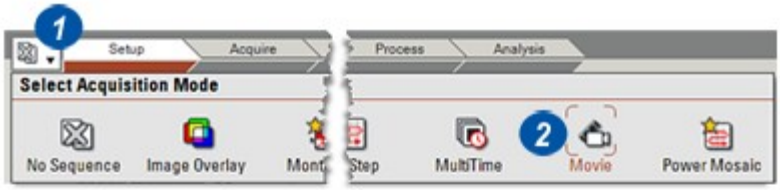
1: Click on the *Select Acquisition Mode* button and from the menu...

2: ...click to select *Movie*. The *Movie* caption will appear on tabs in Acquire and Browse Workflows.



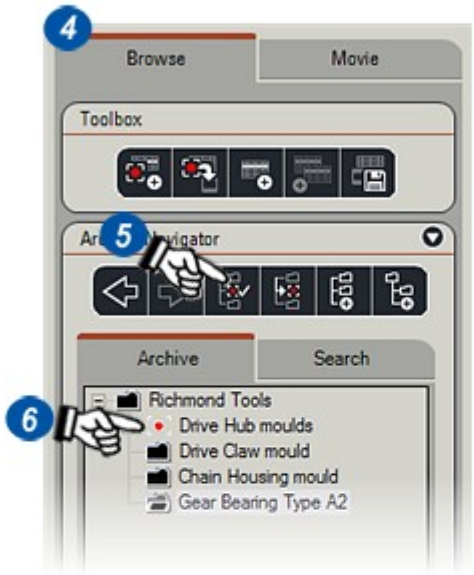
Select the Fixed Capture Location:
The Time Lapse images will be captured into the fixed location.

3: Click on the Browse Workflow and...

4: ...if necessary click on the *Browse* tab. Click to select the capture archive and then...

5: ...click on the *Set Fixed Location* button.

6: To indicate the fixed capture location archive, a red dot appears to the left of it.



Continued... 510

6: If it is not already selected, click on the *Acquire Workflow* tab. The *Movie* tab should now have been added to the Acquire panels.

7: Click on the *Camera* tab. The number of frames that can be captured each second and therefore, the overall 'real time detail' of the film, depends upon the Exposure time, the Live Format and the Camera.

8: Make sure the image quality is good and the Exposure time is as short as possible commensurate with a good image. Carry out an Auto White Balance if necessary.

9: Consider using 3 x3 Binning or Progressive VGA for the Live Format because although High Quality (HQ) formats may be used they can result in very large file sizes and very long exposure times. For smooth playback aim for 20 to 25 frames per second.

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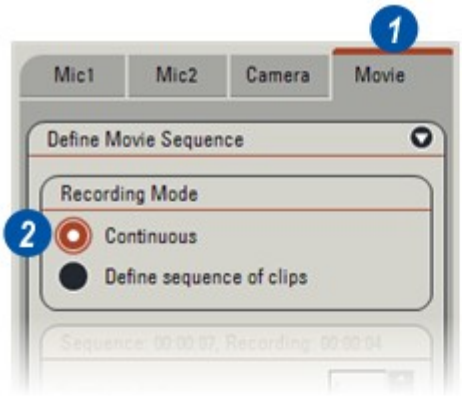
1: In the *Acquire Workflow* click on the *Movie* tab.



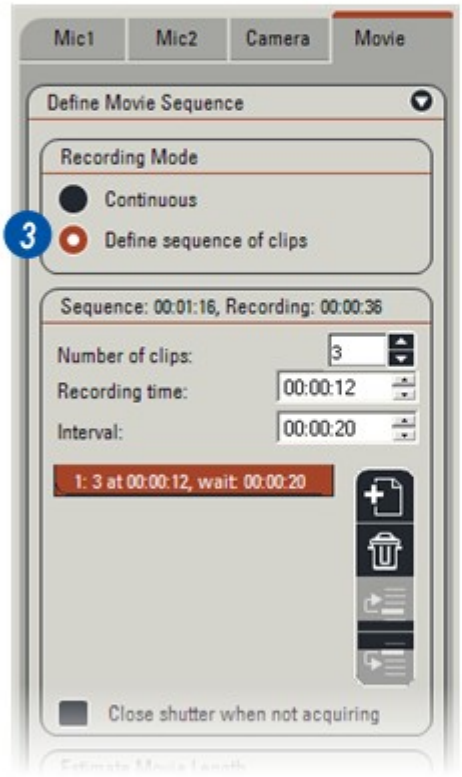
Two recording options are available:

Continuous results in a single clip lasting as long as frames can be captured within the constraints of the hard disk space available.

Preferences > Movie Settings: *Go there...*

Define a sequence of clips creates a single file for each clip. Clip duration and interval may be set individually. When the clips are replayed in Browse they are 'assembled' into a single, continuous movie.

The movie starts and records for the duration of the first clip. It then stops until the first interval has passed. The next clip then runs for its designated duration, recording stops and the second interval starts, and so on until all the clips have been recorded.



To select the Recording Mode:

2: Click on the *Continuous* button and then go to *Options.*

3: Click on the *Define sequence* button. With this option the selected sequence setup panel appears, then. go to *Defining Clip Sequence.*

Continued... 512

On the Sequence panel:

1: Set the number of clips for the sequence by clicking the up/down arrows to the right of the *Number of clips* text box.

2: Set the recording time for the clip(s) by clicking on the appropriate field in the *Recording time* window and typing in a number, or using the up/down arrows to the right of the window. The fields are hours: minutes: seconds (hh:mm:ss). If more than one clip is required, each will record for the same time.

3: If there is more than one clip, set the interval between them by clicking on the appropriate field in the Interval window and typing in a number, or using the up/down arrows to the right of the window. The fields are hours: minutes: seconds (hh:mm:ss). Each clip will be separated by the same interval.

4: Click on the *Add Sequence* icon to load the sequence to the program window **(5)**.

Almost any number of sequences may be added to a program. In the example there are 3 sequences with a total of 5 clips.

6: Remove a sequence by clicking on it and then on the *Delete* icon.

7: Change the order by clicking on a sequence and then on the up/down icon as appropriate.

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To give the movie a name:

1: Click in the *Movie name* text box and type an appropriate name.

2: The first time a movie is captured, a 'common' folder named *Movies* is created in the capture directory.

3: Within this common folder, a new folder is created for every new movie, with the name specified in the Movie name text box.

4: The movie '.avi' file(s) together with several control files, are stored in this folder.

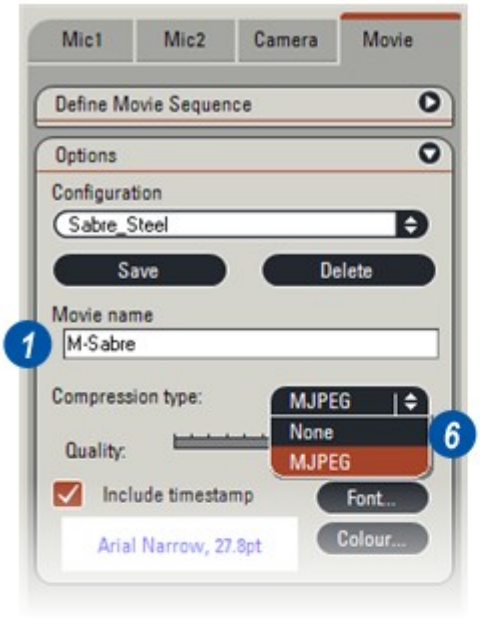
Select the *Compression* option:
Compression results in smaller movie files but can affect image quality. The compression is set on the *Quality* slider. Values up to about 50% on the slider, have little or no noticeable reduction in quality: Higher figures represent a greater level of compression and a smaller file size, but may result in a noticeable loss of quality.

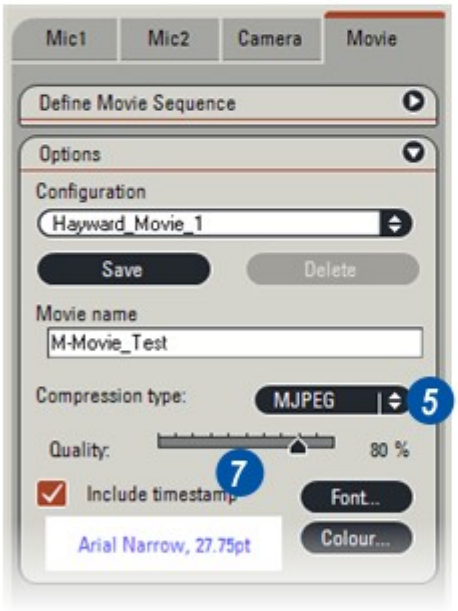
Start with a value of 80% and then experiment by gradually decreasing the to achieve acceptable quality.

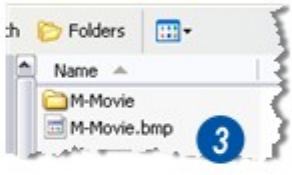
5: Click on the arrows to the right of the *Compression type* window and…

6: from the drop down menu select either *None* for no compression, or *MJPEG* to apply compression.

7: If *MJPEG* is selected, the *Quality* slider appears. This controls the degree of compression applied to the images and therefore the display quality. Click and hold the slider and drag to the left to decrease the quality or to the right to increase it.







To display a progress time stamp on the movie (1):

2: Click on the *Font* button and from the Font dialog…

3: …select a font type, style and size and click *OK*.

4: Click on the *Colour* button, select a font colour from the Select Colour dialog and click *OK*.

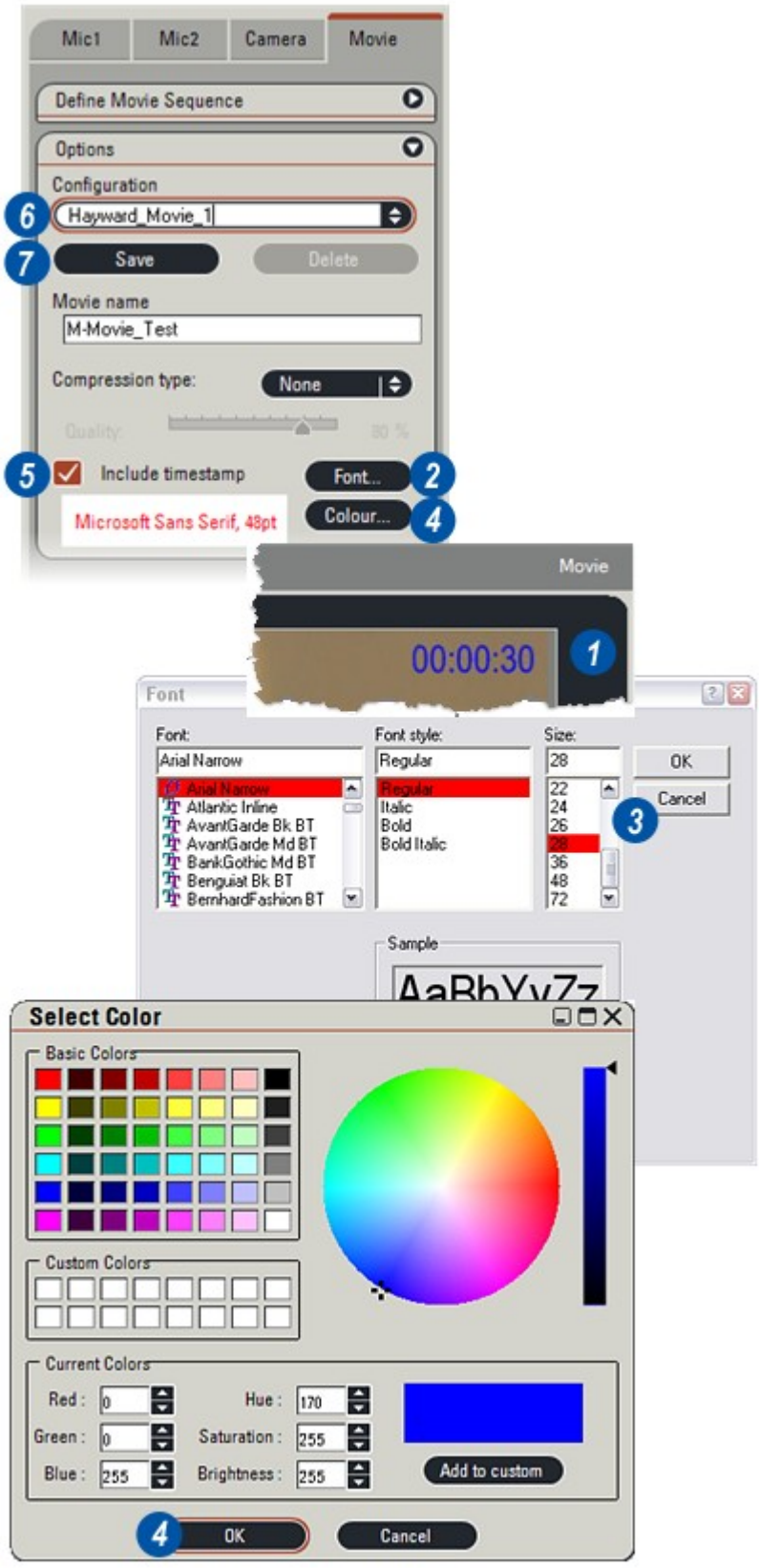
5: Click on the *Include timestamp* check box to enable the time stamp.

Saving the Configuration:
The current settings may be saved with a unique name and recalled for another movie at a later date.

To save the current setting:

6: Click in the *Configuration* text box to highlight the text, type a unique name for the current configuration and press *Enter* on the keyboard.

7: Click on the *Save* button which will save all of the current settings.



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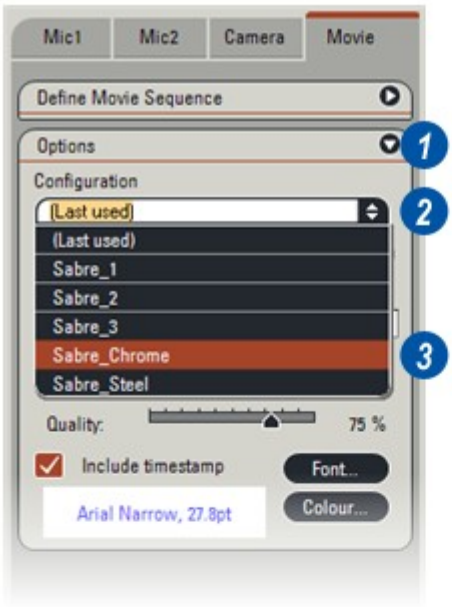
Previously saved configurations:
These may be retrieved and used again to load the settings for a new movie.

1: Click on the arrows to right of the *Options* bar to reveal the *Options* panel.

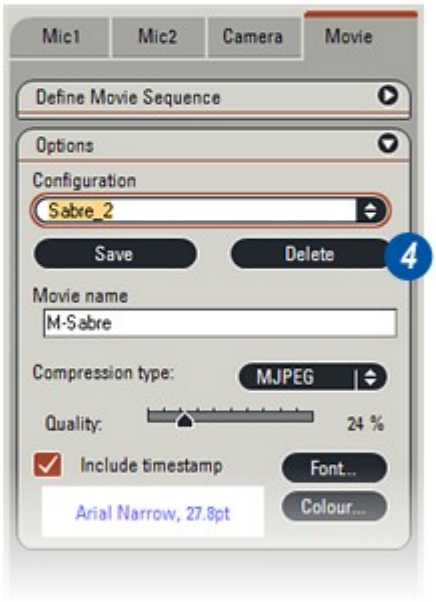
2: Click on the arrows to the right of the *Configuration* text box and from the drop down list…

3: Select the existing configuration to use.



Deleting a Configuration:

4: When a configuration is selected the *Delete* button becomes active. Click it to delete the selected configuration.



Testing the Movie length:
With all of the settings made or an existing configuration selected, check the movie length by…

5: Clicking the *Test* button.
After a short delay the *Approximate maximum length* **(6)** and *Frames per second count* **(7)** will appear.
Aim for a frame count of at least 15 frames/second (fps) and preferable closer to 26 fps.

If the frame count is too low, adjust the exposure time on the *Camera* tab, or increasing the light level at the microscope, or by selecting a *Live Capture* binning option that results in smaller image sizes.
After making changes, click the *Test* button to review the movie length and frame count.

To start recording the movie:

1: Click on the *Acquire Movie* button.

The *Recording Progress* panel shows the Current Clip progress **(2)** with…

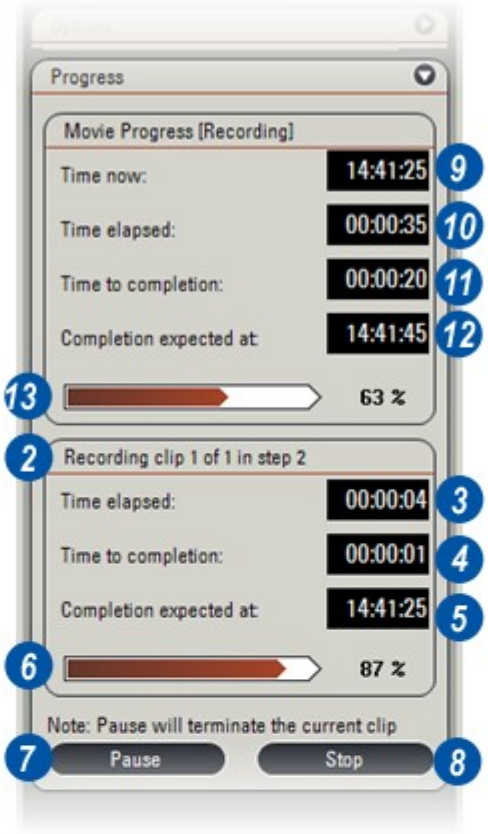
3: *Time elapsed* as hh:mm:ss,

4: Computed *Time to completion* as hh:mm:ss and…

5: Real time *Completion expected at.* There is also a progress bar **(6)** which provides a graphical representation of time elapsed for the current clip.

7: To stop the current clip and skip to the next (if there is one), click on the *Pause* button.

8: To halt the entire movie click on the *Stop* button.



Overall progress is detailed in the upper panel.

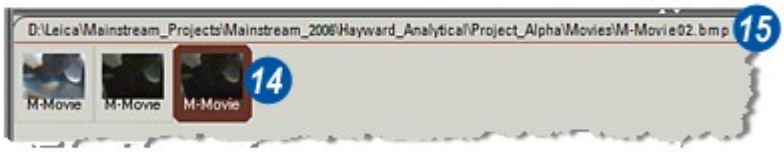
9: *Time now* is the real time clock.

10: *Time elapsed* for the entire movie, all clips so far.

11: Computed *Time to completion* as hh:mm:ss.

12: Real time *Completion expected at.* There is also a graphical display of time elapsed so far **(13).**

When the movie recording is complete, a sample reference image **(14)** is placed in the capture directory with a thumbnail. Placing the cursor over the thumbnail reveals the path and name **(15).**



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After capture the display will open in the *Browse Workflow* (**1)** in the specified movie capture folder. The movie will normally be played in the Viewer, but the playback application can be changed by clicking the *Play with* button **(2)** which will launch the program specified in *Preferences: Image Output Settings: Play Movie with…*

The playback controls **(3)** on the *Browse Viewer* are located on the *Play Movie* panel. Click the icons:

4: To *Start/Stop* the movie.

5: Skip a frame *forwards.*

6: Skip a frame *backwards.*

7: Skip to the *end of the movie.*

8: Skip to the *start of the movie.*

9: Skip to the next or previous clip by clicking on the left/right arrows to the right of the *Current movie clip* window.

10: The playback speed may be adjusted in the text box **(10)**. The default is 100% which is playback speed = recorded speed. Increase or decrease the speed by clicking in the text box and typing a speed (as a % of the recorded speed), or clicking on the up/down arrows to the right of the text box.

1: Click on the *Start/Stop* button. The button icon changes to Pause on a brown background.
Click the button again to halt the movie.

2: The *Progress Indicator* moves from left to right as a graphical indication of running time.
Click and hold the *slider* and then move it left or right to re-position the movie playing position.

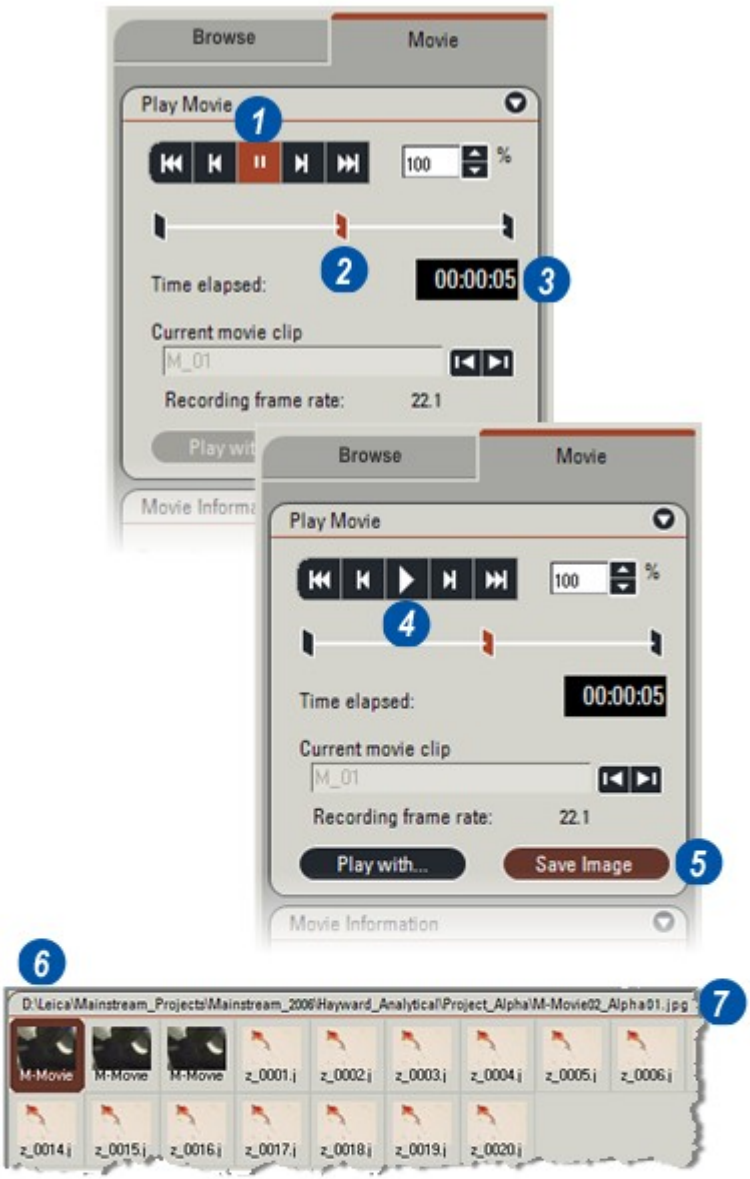
3: Running *Time elapsed* as hh:mm:ss is displayed in the window.

Frame Capture:
Individual movie frames may be captured and saved for future examination:

4: Click on the *Start/Stop* button to stop the movie at the frame required.
Alternatively, use the *Progress Indicator* to position the movie at the frame and then click on the *Start/Stop* button.

5: Click on the *Save Image* button.

6: The frame is stored in the current capture folder (not in the movie folders) with the name of the movie, the name of the capture folder and a sequential number which is automatically generated **(7).** The file name extension represents the capture format (.jpg etc).



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1: Click on the *Browse Workflow.*

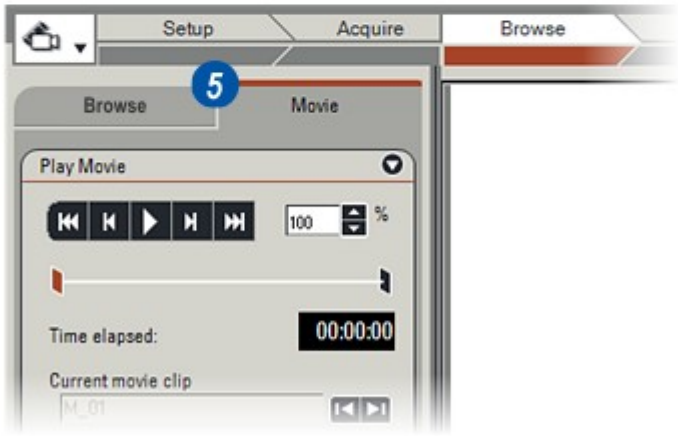
2: If necessary, click on the *Browse* tab to reveal the *Archive Navigator.*

3: Click to select the archive that contains the movie file to be played.

4: A thumbnail representing the movie (s) appear in the *Gallery*.

5: Click on the *Browse: Movie* tab to reveal the *Play Movie* controls.

See: Movie play controls: 517
See: Movie play and frame capture: 518



Images obtained from microscopes have a known and limited depth-of -focus. For specimens with varying surface height, when the Z-position of the specimen is adjusted, different regions of the specimen appear in focus. By collecting digital images at these Z-positions, they can be combined by an image processing algorithm into one single sharp composite image that the effectively extends the depth-of-focus of the image.

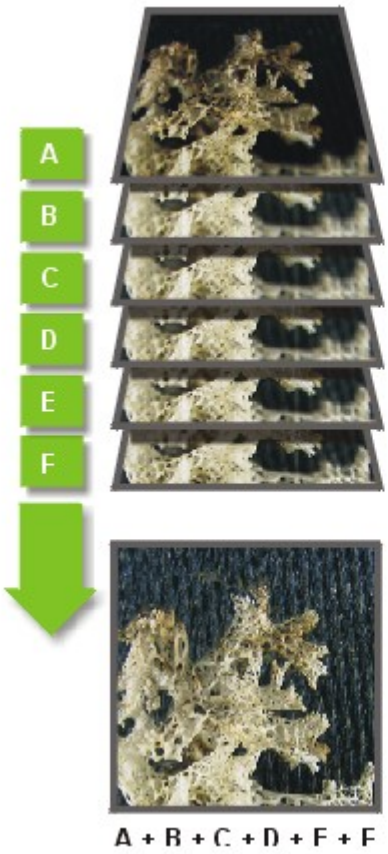
Specimens that can benefit from *LAS MultiFocus* are predominately those that are imaged by light reflecting from the surface such as geological and fossil specimens, plant and marine biology, histology and materials such as paper, electronic components, metallurgy, surface coatings and fractures

LAS MultiFocus provides fully integrated software controlling microscopes with motorised focus, cameras with high resolution and colour fidelity that are combined with a computer that comfortably handles digital images. The performance and relative economy of modern computing makes the use of sophisticated imaging algorithms practicable for acquiring and processing Z-Stacks of multiple images.

The principle is simple: An image that varies in focus, is taken at steps across the thickness of the specimen. These ‘slices’ are then mathematically combined to form a *MultiFocus* Image – all of the slices blended into a single, uniformly sharp image.

Almost any number of slices (together called a Z-Stack) can be captured – the higher the number, generally the better the *MultiFocus* Image – and each may be saved and examined.

The MultiFocus application is suitable for both manual and automatic microscopes.



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MultiFocus is an optional module which must be installed and operational before use

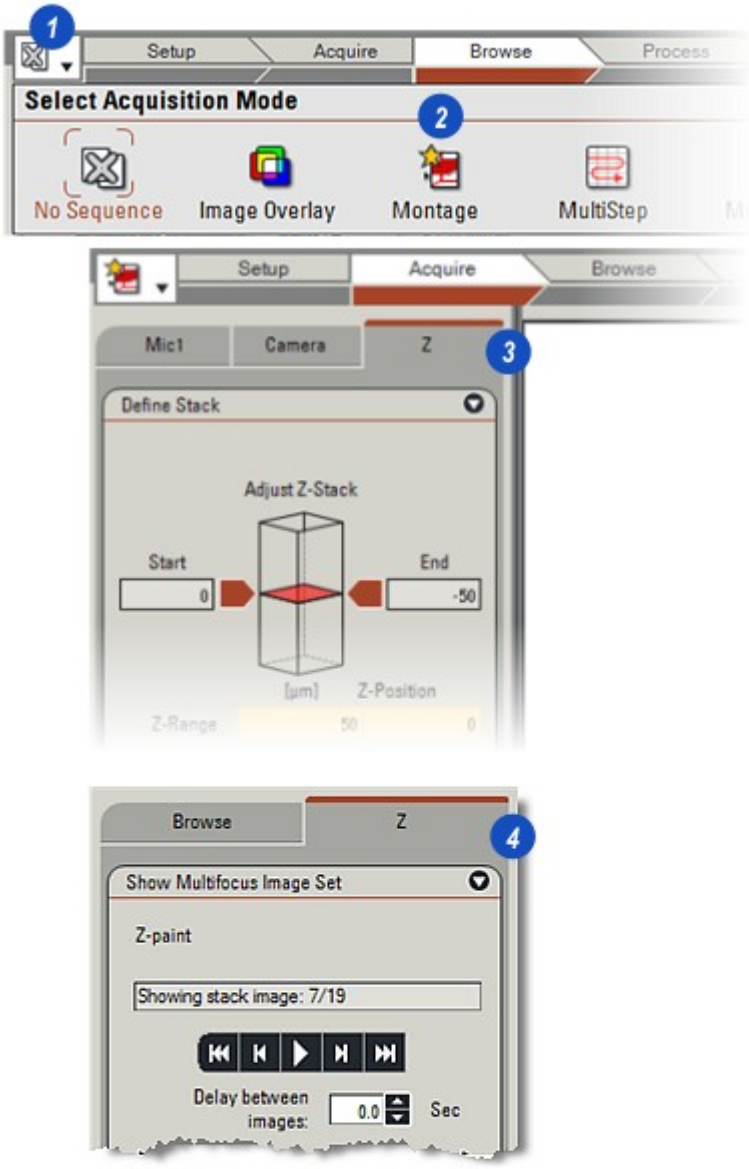
To enable MultiFocus:

1: Click on the *Module S*elect icon.

2: From the drop down menu click on the *MultiFocus (Montage)* icon to enable it. The icon will only be available if *MultiFocus* is installed.

3: The ‘Z’ logo appears on a new tab in the *Acquire Workflow* and…

4: Also in the *Browse Workflow.*

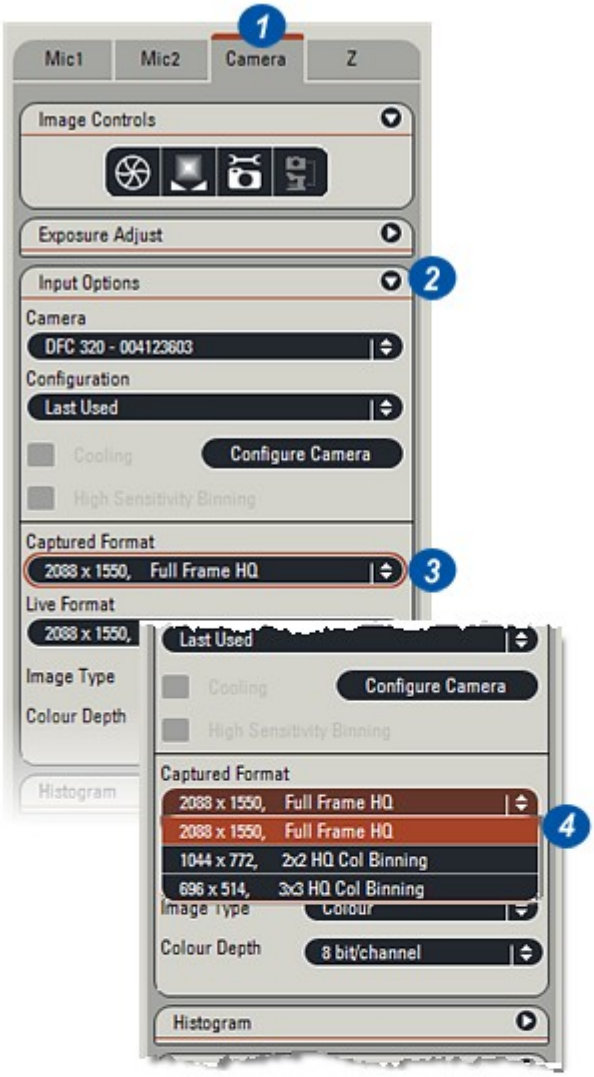


1: On the *Acquire Workflow* click on the *Camera* tab.

2: Click on the arrow to the right of the *Options* panel to reveal it.

3: Click on the arrow to the right of the *Captured Format* header bar and from the list of options…

4: Click to select the appropriate format. Thick specimens may require a large number of steps so consider a more 'compact' format that does not compromise quality but speeds the capture time, reduces disk space and makes processing quicker.



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Check the *Preferences* settings to ensure images have the correct settings.
Preferences: Go there...

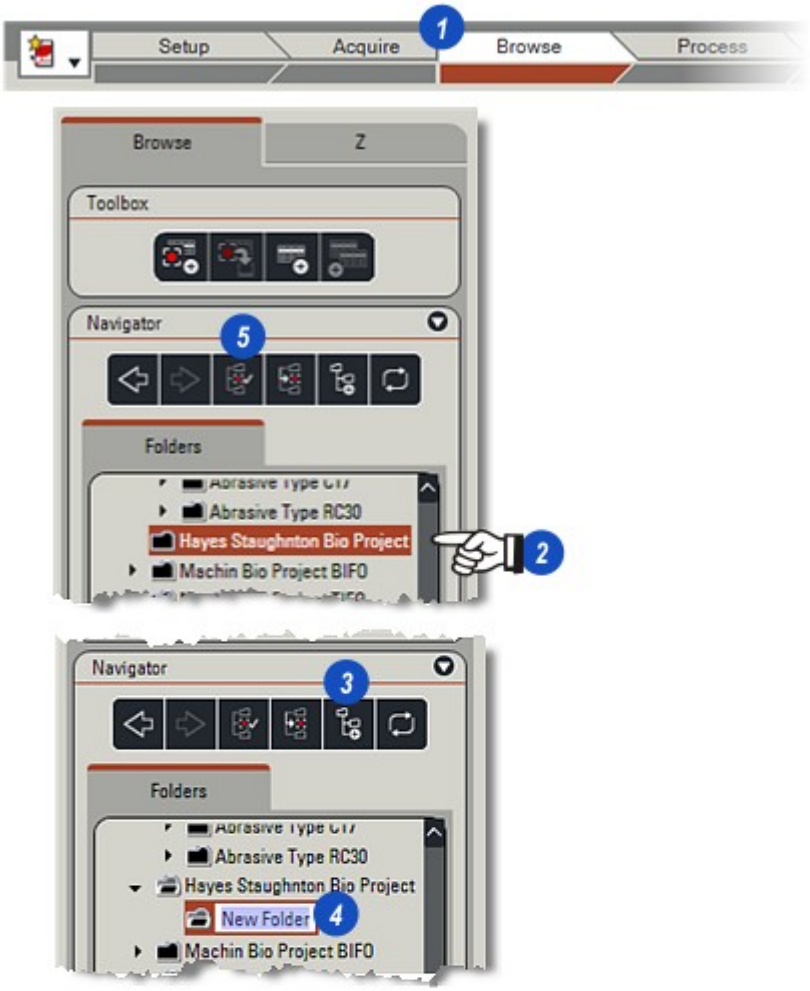
1: Click on the *Browse* Workflow and...

2: ...navigate to the folder in which to capture the images.

3: If a new folder is required click on the *New Folder* button and...

4: ...rename the folder appropriately.

5: Click on the *Set Capture Location* button to automatically save the images to the selected folder. Make sure *Capture to Fixed Location* checkbox in *Preferences* is enabled.



1: Click on the *Acquire Workflow* to select it.

2: Click on the *Z* tab to display the *MultiFocus*control panels.

3: If necessary, click on the arrow to the right of the *Options* header bar to reveal the Options panel.

Create MultiFocus after Stack Acquire:

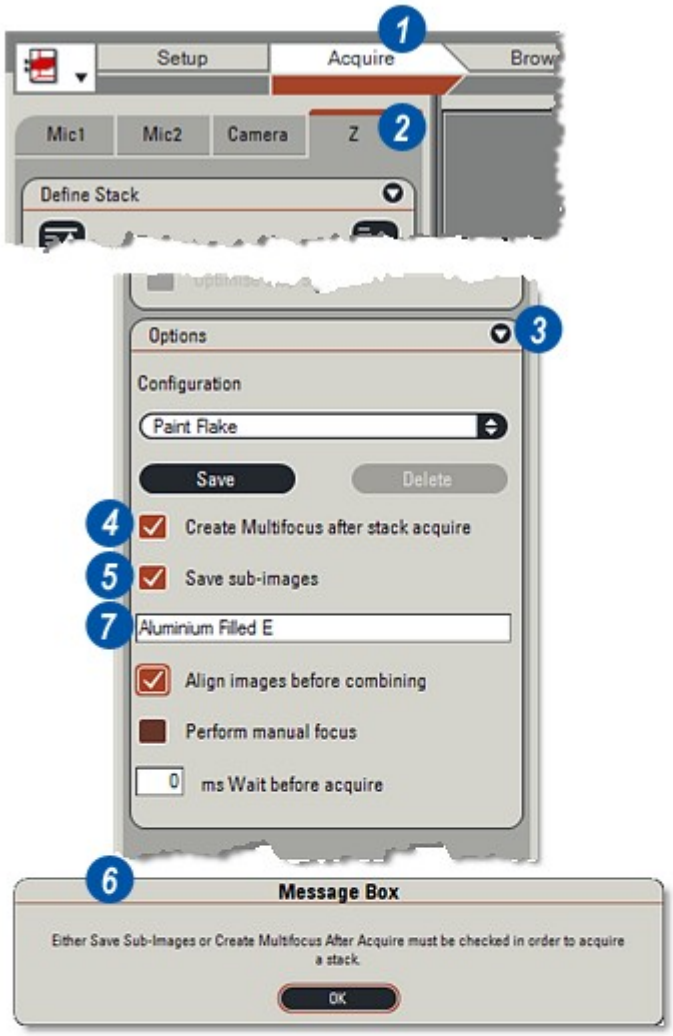
4: Selecting this option – clicking the checkbox – will automatically create a MultiFocus Image when the last Z-slice is acquired. Left un-checked, the *MultiFocus* Image can be created manually on the Viewer.

Save Sub-images:

5: If the *MultiFocus* Image is created automatically (See **4** above), it may not be necessary to save all of the Z-slices or sub-images. Check this option to discard the layers. *Save sub-images* must be selected if *Create MultiFocus* is unchecked otherwise the prompt **(6)** appears. If *Save sub-images* is checked, the *MultiFocus* Image cannot be re-created again; the entire process will have to be repeated.

Enter the Stack Name:

7: Click in the *Stack Name* text box and type an appropriate name for the Z-Stack. The letter Z is automatically prefixed to denote a *MultiFocus* group, and a sequential number is appended every time a new stack is acquired.



Continued 525

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Align Images before combining:

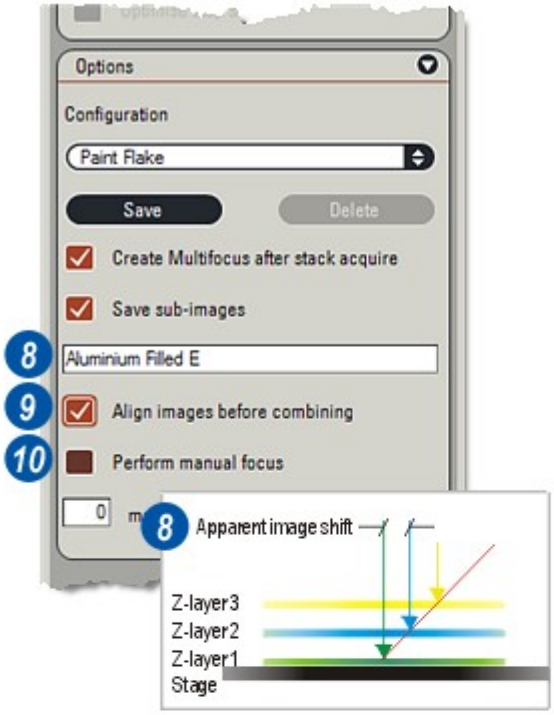
8: Optical variations - predominantly in stereo microscopes - can cause an apparent shift in the point of focus from layer to layer. The shift will be corrected in software if the *Align Images* option is checked so that all X-Y points of focus are in exact alignment.

Perform Manual Focus:

9: Check this option if the microscope does not have a motorised focus and each image is to be focussed manually.

Wait Before Acquire:

10: Measured in milli-seconds, this option inserts a delay between the *Acquire* button being clicked and the acquisition of the first layer. Click in the text box and type a value.



The *Configuration* facility allows *MultiFocus* Z-step settings and options to be saved, retrieved and used again.

Create a New Configuration:

1: Click in the *Configuration* text box and type an appropriate file name for the MultiFocus session.

2: Click on the *Save* button. The settings are saved to disk and the file name added to existing *Configurations*. After the *Z-Stack* has been set up, the Configuration will be saved again to include the new stack settings.

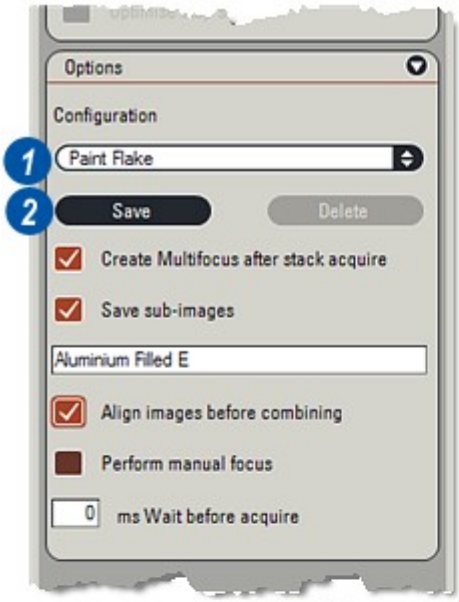
Retrieve an Existing Configuration:

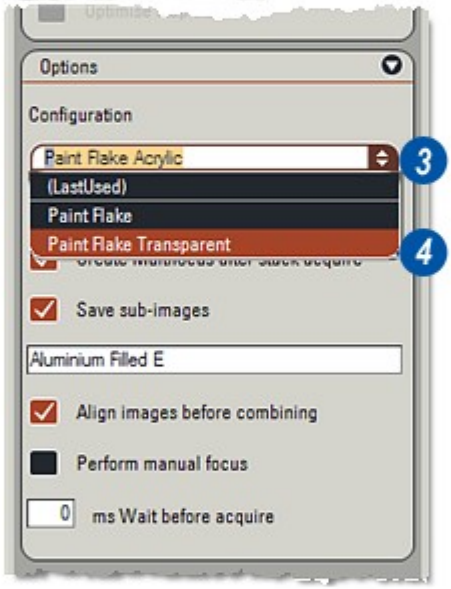
3: Click on the arrows to the right of the *Configuration* text box.

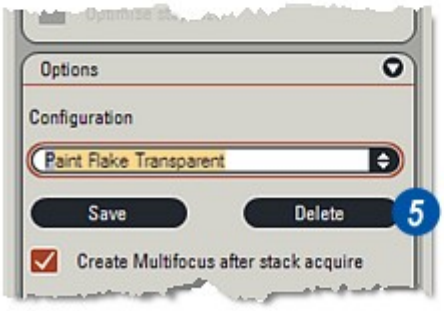
4: From the drop down list click to select the Configuration required. The *Last Used* option will automatically load the settings used during the last *MultiFocus* session.

Delete a Configuration:

5: Click the *Delete* button to remove the Configuration displayed in the text box. The settings cannot be retrieved. The *Last Used* option may not be deleted.







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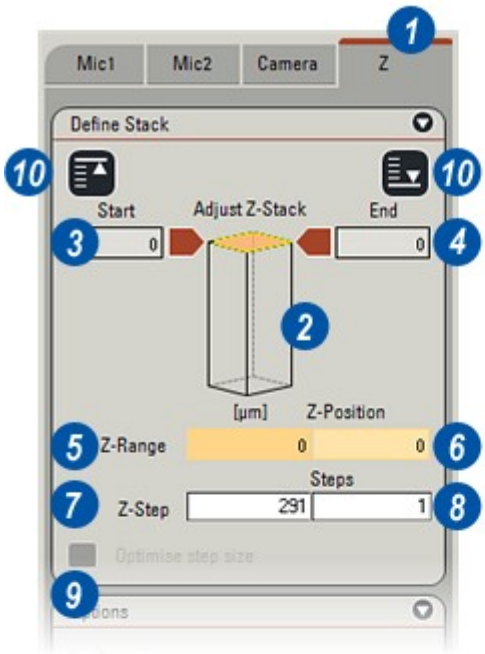
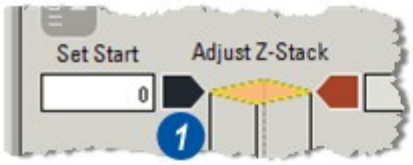
1: If necessary, click on the *Acquire: Z* tab to reveal the *MultiFocus* control panels.
Defining the stack consists of setting the first Z position on the specimen, called the *Start*, setting the last Z position called the *End*, and then deciding the number of focussed 'slices' or steps to capture between *Start* and *End*.

Start and *End* may encompass either the whole specimen from bottom to top or any part of the specimen. The positions chosen are displayed on a graphical 'stack' **(2)** with the *Start* position (**3)** shown as an arrow on the left hand side, and the *End* position as an arrow on the right hand side. Windows attached to the arrows display the microscope focus values.

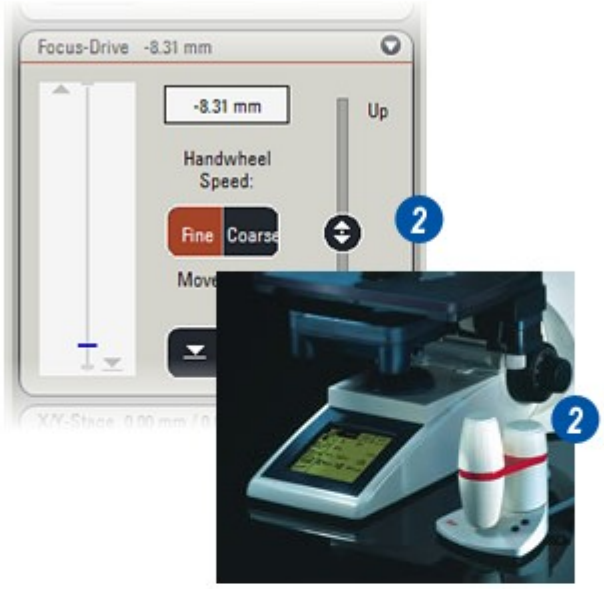
The *Z-Range* window **(5)** will show the numerical difference between the *Start* and the *End* positions.
Z-Position **(6)** is the current microscope focus position.
Z-Step **(7)** represents the distance between each of the Z-steps and *Steps* **(8)** is the number of images that will be captured.
The *Optimise step size* button **(9)** will determine the best step size based upon the microscope and the optics.
Limit buttons **(10)** will automatically move the objective to either the *Start* or *End* position.



Generally, the *Start* position is the part of the specimen closest to the stage. It provides the opportunity to place the specimen on a textured background which, when in sharp focus is a precise reference for the software. In subsequent 'slices' the background will be out of focus and so ignored.



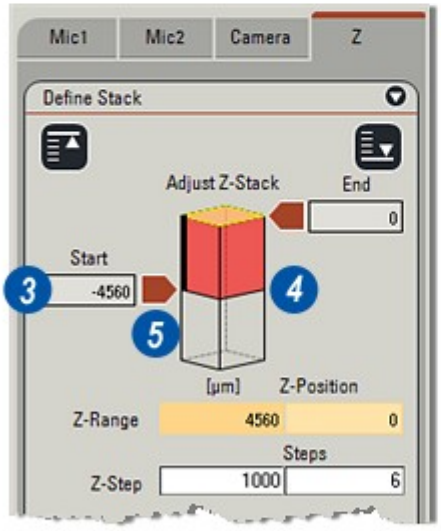
1: Click on the *Set Start* arrow. It will turn black indicating it is active and will automatically track the microscope movements.



2: Adjust the microscope either with its manual controls or by using the *Application Suite* interface, until the specimen at the Start position is in focus.

3: The microscope settings are reflected in the *Start* window and the Start arrow moves down the virtual ‘stack’ **(4).**

5: Click on the *Start* arrow which will become red and locked at the focussed position.



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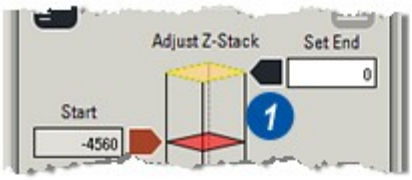
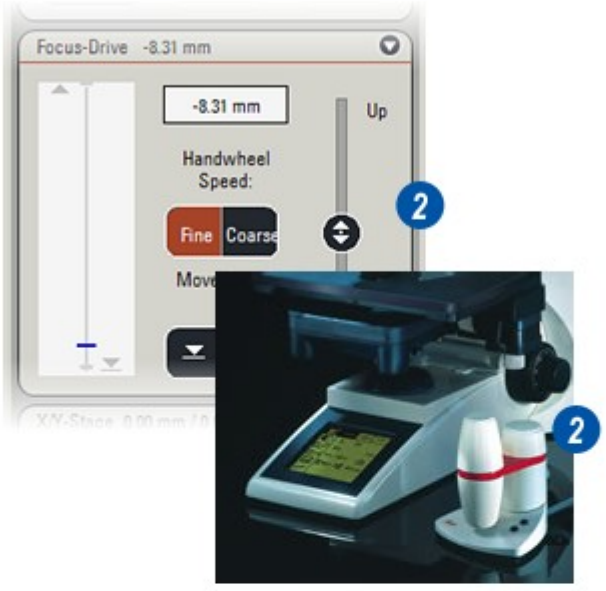
1: Click on the *Set End* arrow. It will turn black to indicate it is active and tracking the microscope movements.

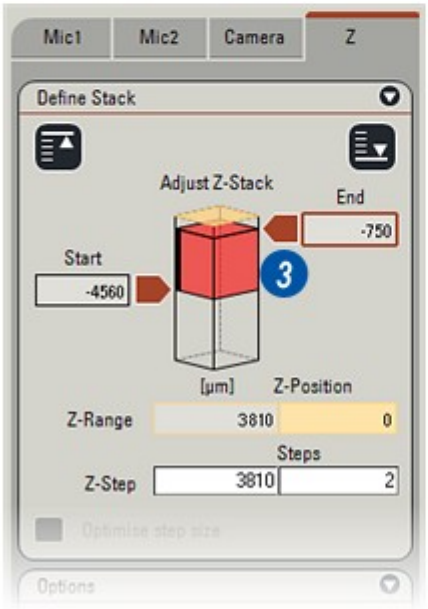
2: Focus on the part of the specimen that represents the last Z-step using either the microscopes manual controls or the *Application Suite* interface. The *End* arrow will move along the virtual 'stack' in response to the microscope.

3: Click on the *End* arrow which will become red again and locked in the *End* Z-step position.

Continued... 529







The illustrations show two images:

1: The *Start Z-layer* with part of the specimen in focus together with a sharp, textured background, and…

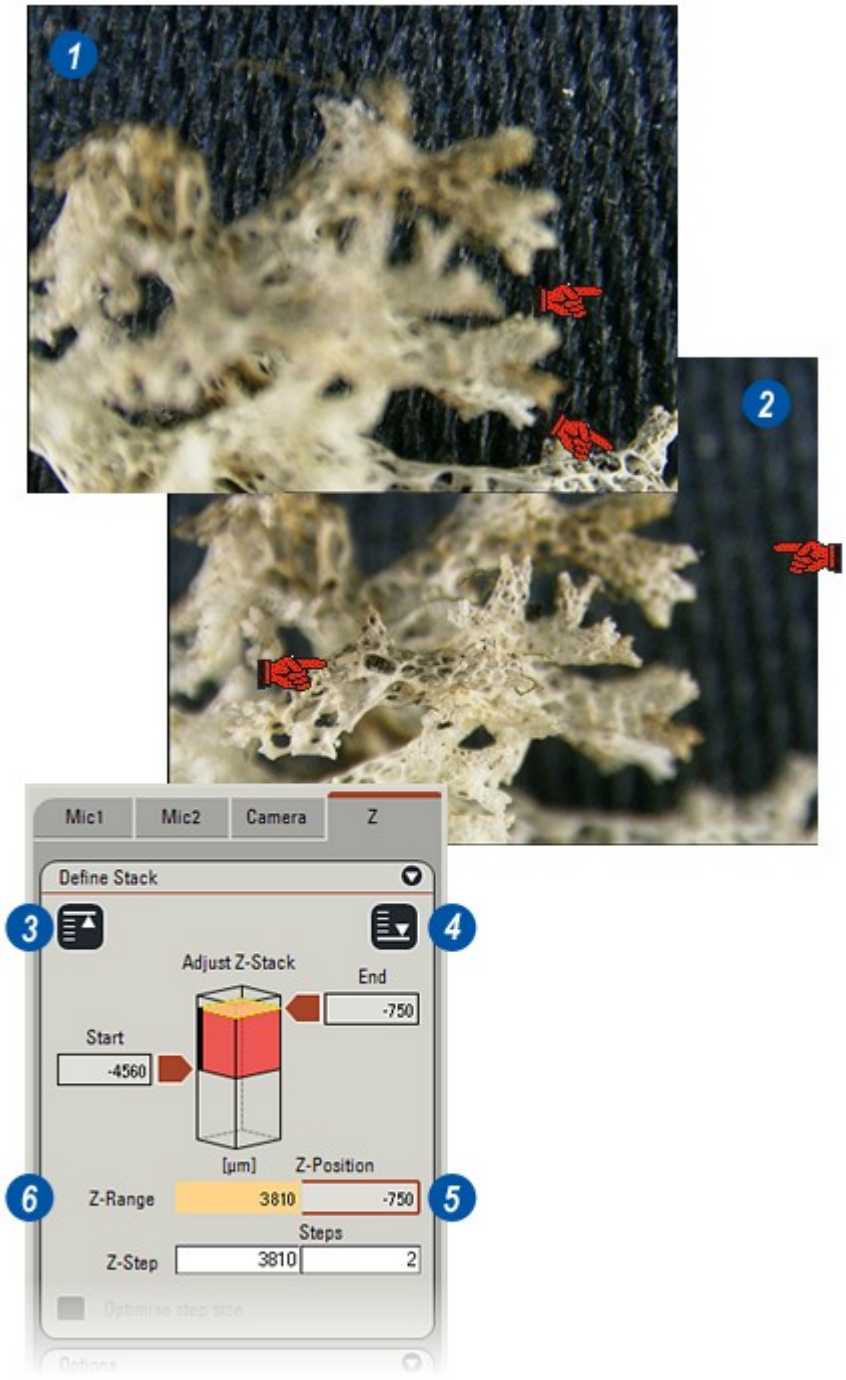
2: The *End Z-layer* with a different part of the specimen 'sharp' but the background out of focus.

Using the 'Go To' buttons:

3: The *Go To Start* button when clicked will drive the microscope to the Start position with the value displayed in the *Z-Position* window **(5)** for a focussing check.

4: The *Go To End* button drives the microscope to the End position for a focussing check. Again, the microscope value is displayed in the *Z-Position* window **(5).**

6: The *Z-Range* window shows the distance between the *Start* and *End* positions.



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With the *Start* and *End* points set, the distance between the two is calculated and displayed in the *Z-Range* and *Z-Step* windows.

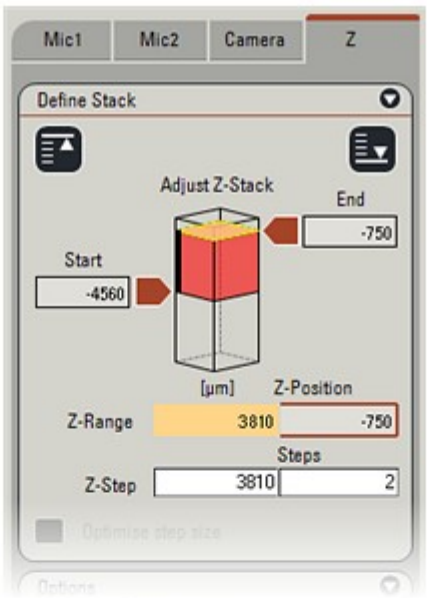
There are two methods of setting the number of layers or steps:

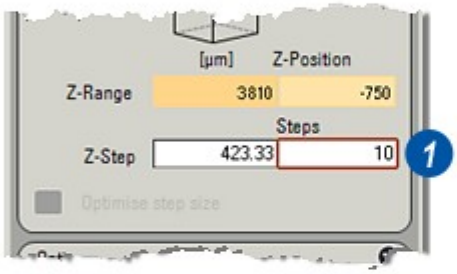
1: Click in the *Steps* text box and type the number of steps required. The distance between each is calculated and shown in the *Z-Step* text box.

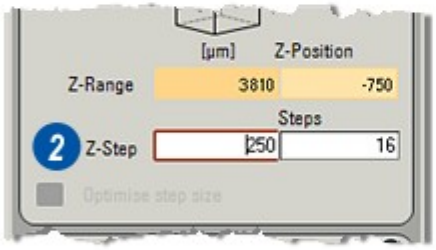
2: Click in the *Z-Step* text box and type the distance required between each step. The range value is divided by the entered figure and the resulting number of steps displayed in the *Steps* text box.

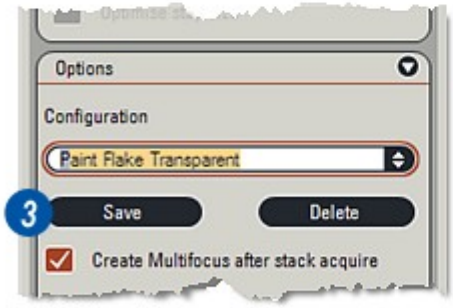
3: With the Stack Definition complete, click the *Options: Save* button to save the settings.

See: 532 *Acquire Z-Stack Images.* 532







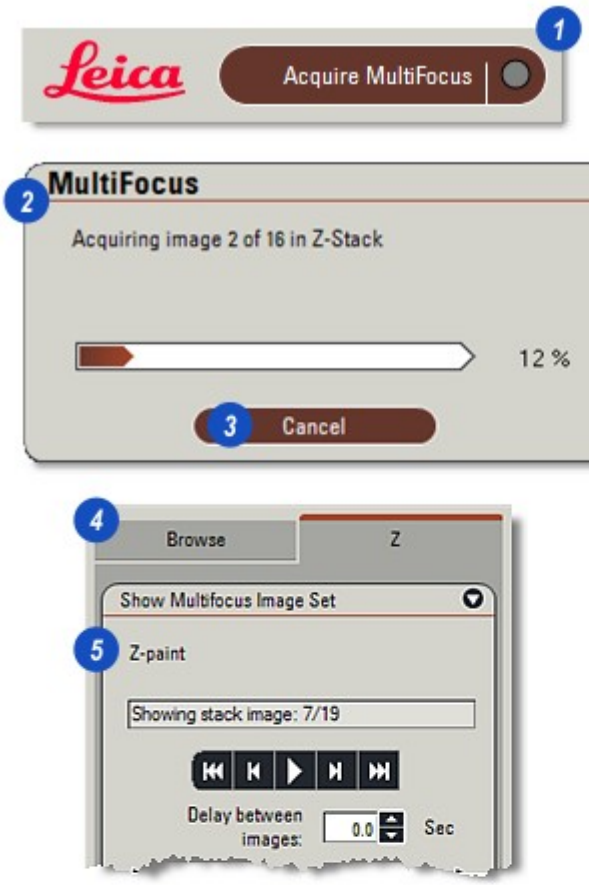


1: Click on the *Acquire MultiFocus* button to start the acquisition.

2: At each step position, the microscope will automatically move to the next step and focus. A progress message is displayed.

3: To cancel the sequence, click on the *Cancel* button.

4: When all of the images have been captured, the *Browse Workflow* opens with the *Show MultiFocus Image Set* control panel displayed **(5).**



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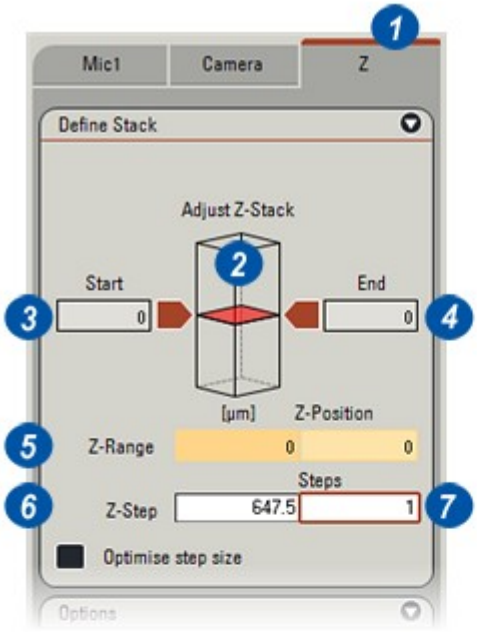
1: If necessary, click on the *Acquire: Z* tab to reveal the *MultiFocus* control panels.

Defining the stack consists of setting the first Z position on the specimen, called the *Start*, setting the last Z position called the *End*, and then deciding the number of 'slices' or steps to capture between *Start* and *End*.

Start and *End* may encompass either the whole specimen from bottom to top or any part of the specimen. The positions chosen are displayed on a graphical ‘stack’ **(2)** with the *Start* position **(3)** shown as an arrow on the left hand side, and the *End* position as an arrow on the right hand side. Windows attached to the arrows display the microscope focus positions.

The *Z-Range* window **(5)** will show the numerical difference between the *Start* and the *End* positions.

Z-Step **(6)** represents the distance between each of the Z-steps and *Steps* **(7)** is the number of images that will be captured.



Generally, the *Start* position is closest to the stage because the specimen can be set against a textured background which will also be in focus. This gives the software a benchmark for the background so that the out-of-focus background of subsequent steps will be ignored.

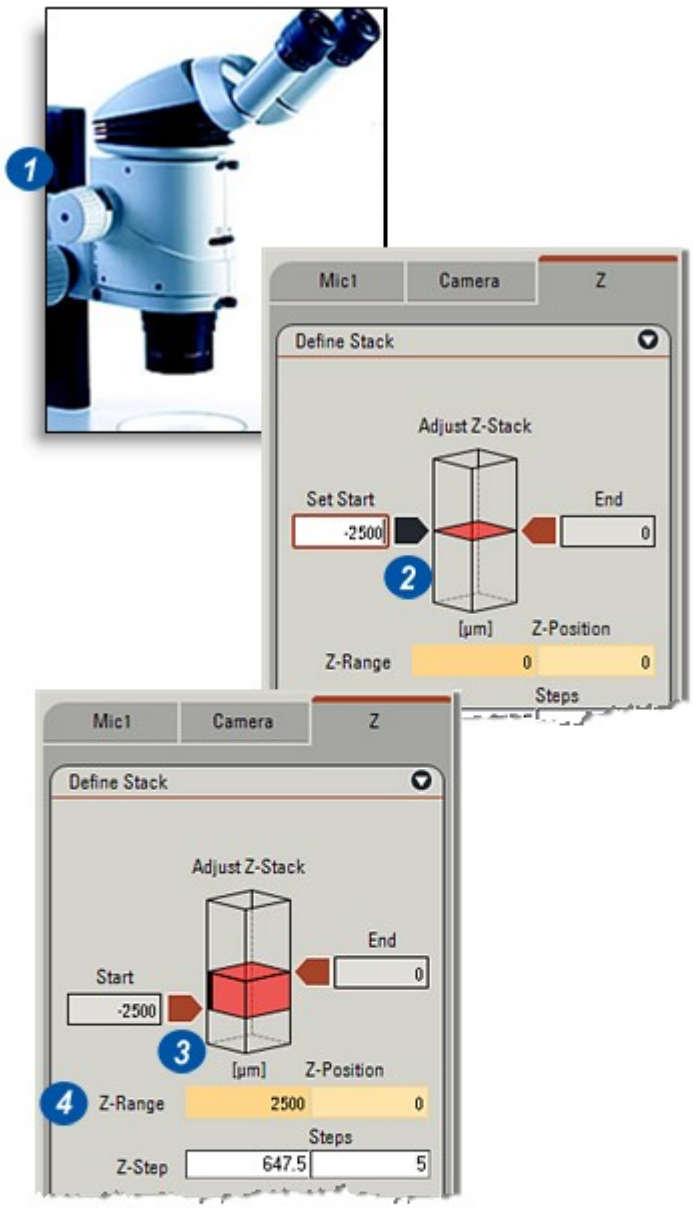
1: Focus on the start region of the specimen making a note of the microscope scale reading.

2: Click on the *Set Start* arrow which will turn black, and type in the scale reading.

3: Click on the *Set Start* arrow which will become red again. The virtual 'stack' displays a graphical representation of 'depth'.

4: The *Z-Range* value reflects the entered *Start* position.

See: Set End position: Manual Microscopes. 535



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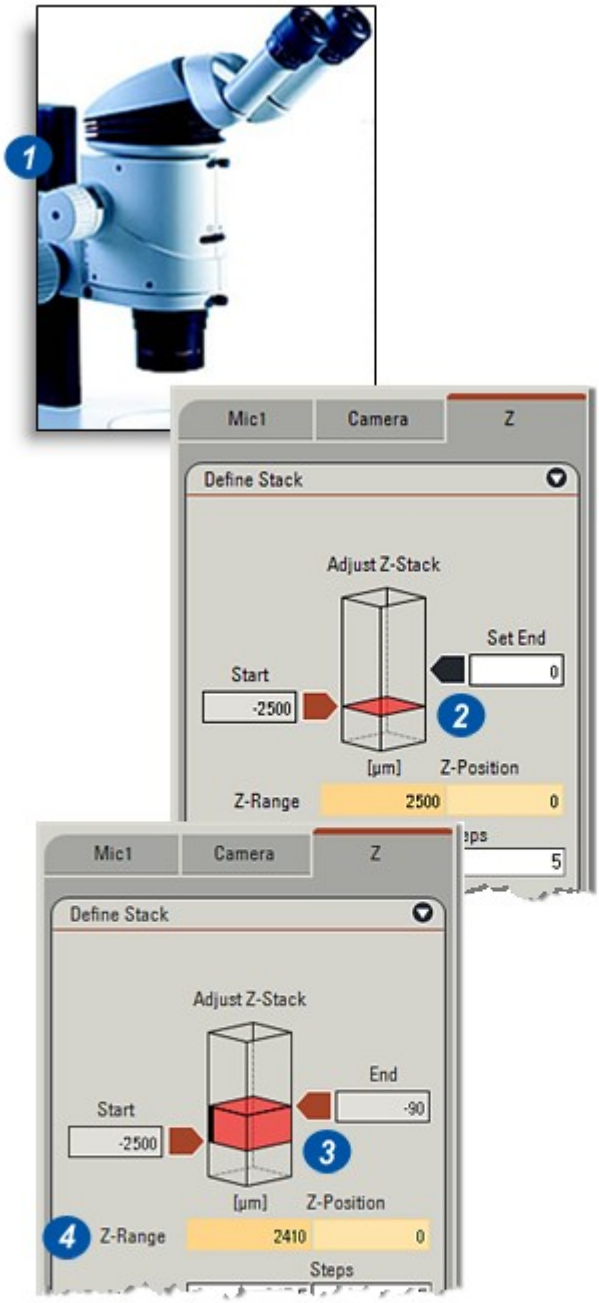
1: Focus on the region of the specimen that represents the *End* position. Make a note of the microscope scale reading.

2: Click on the *Set End* arrow which will turn black, and type the scale reading in the window.

3: Click on the *Set End* arrow which will become red. The virtual 'stack' will display a graphical representation of the distance between the *Start* and *End* positions.

4: The *Z-Range* window displays the difference between the *Start* and *End* microscope readings.

See: Set Start position: Manual Microscopes. 534



With the *Start* and *End* points set, the distance between the two is calculated and displayed in the *Z-Range* and *Z-Step* windows.

There are two manual methods of setting the number of layers or steps:

1: Click in the *Steps* text box and type the number of steps required. The distance between each is calculated and shown in the *Z-Step* text box, or...

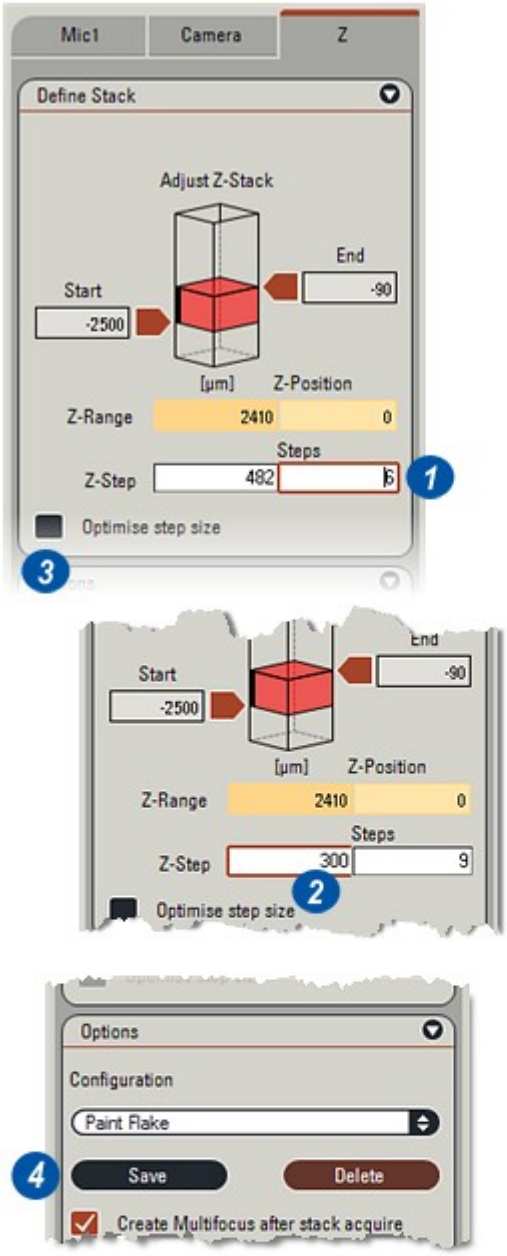
2: Click on the *Z-Step* text box and type the distance required between each step. The range value is divided by the entered figure and the resulting number of steps displayed in the *Steps* text box.

Optimise step size:

3: Enable the *Optimise step size* button to have the steps calculated automatically using the microscope aperture. On stereo microscopes check that the actual and displayed iris settings correspond.

4: With the Stack Definition complete, click the *Options: Save* button to save the settings.

See: Acquire Z-Stack images: Manual Microscopes. 538



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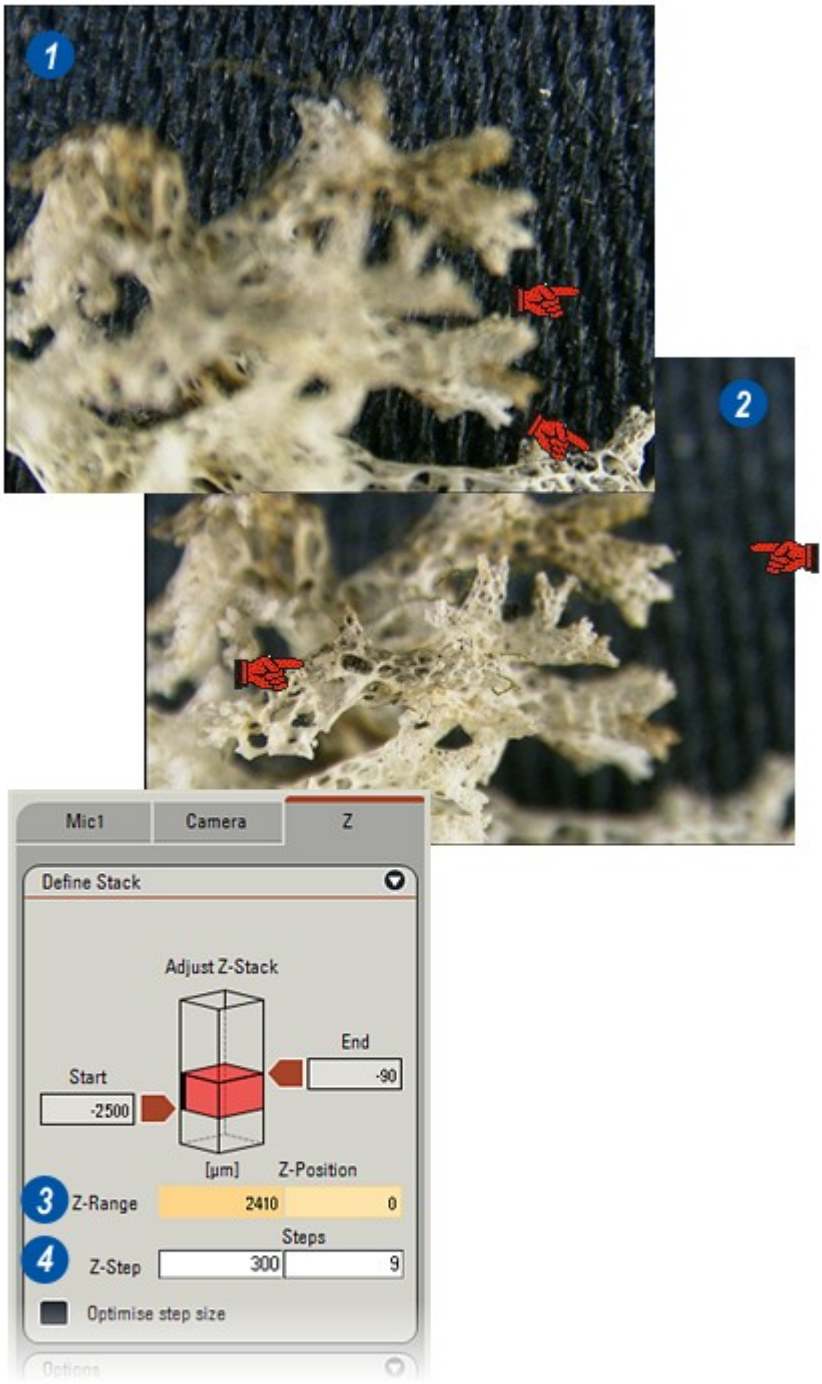
The illustrations show two images:

1: The *Start Z-layer* with part of the specimen in focus together with a sharp, textured background, and…

2: The *End Z-layer* with a different part of the specimen 'sharp' but the background out of focus.

3: The *Z-Range* window shows the distance between the *Start* and *End* Z-layers, and…

4: The number of steps and the 'size' of each step in the *Z-Step* window.

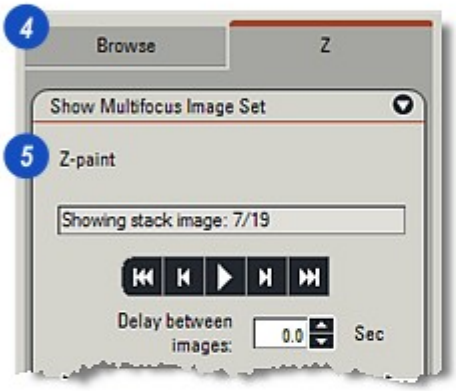


1: Click on the *Acquire MultiFocus* button to start the acquisition.

2: At each step position, the prompt appears. Re-focus the microscope using the *Z-Step* value, always focussing in the same direction.

3: Click *OK* to capture the image. To cancel the sequence if it is believed sufficient slices have been captured, click on the *Cancel* button. The number of captured images does not have to correspond with the calculated or entered number of steps.

4: When all of the images have been captured, the *Browse Workflow* opens with the *Show MultiFocus Image Set* control panel displayed **(5).**



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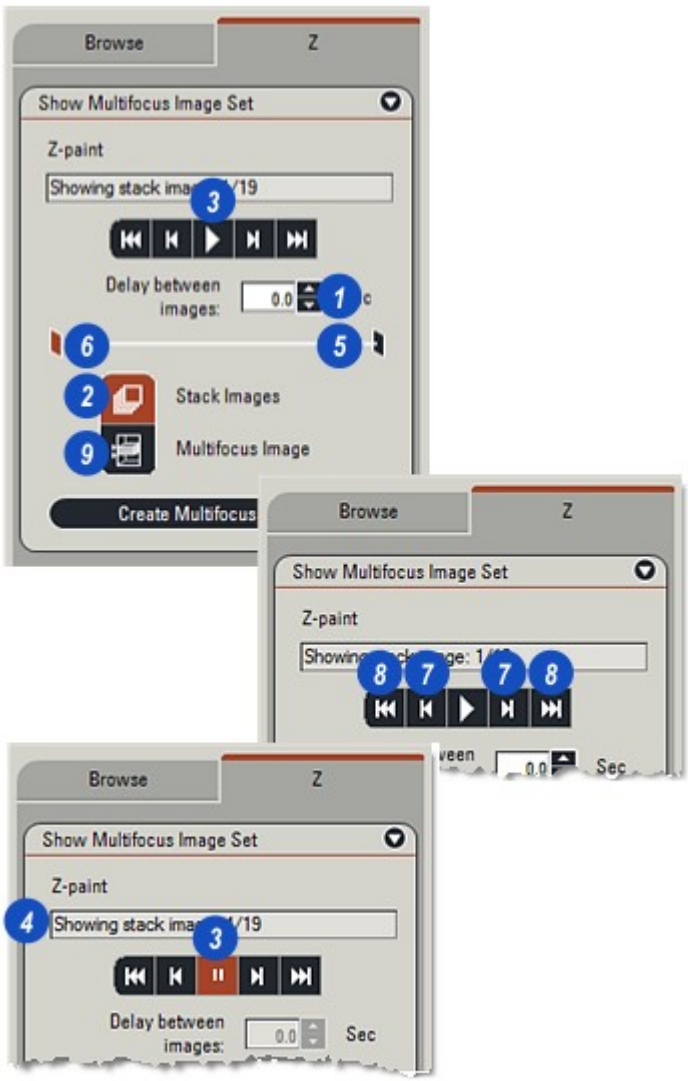
When all of the *MultiFocus* images are captured, the *Browse Workflow* opens with the *Show MultiFocus Image Set* panel displayed.

The panel provides the controls necessary to view the entire set as a 'slide show', a single layer or the *MultiFocus* Image.



1: Set the delay between images in seconds by:
Either clicking on the *Delay Between* text box and typing a number or…
Using the up/down arrows to the right of the text box.

2: Click on the *Stack Images* button to select the images.

3: Click the *Play* button. It will change to red with the pause symbol.

4: The current image and total images are displayed in the *Progress Window* and…

5: An indicator moves across the *Progress Bar*. Each image is displayed in turn in the *Viewer*.
Click the *Play* button again to 'freeze' the current image.

6: Move to individual images in the set by clicking and holding the *Progress Bar* indicator and dragging it left or right.

7: Skip an image backward or forward by clicking the *Skip* buttons.

8: Choose the first or last images by clicking the *Beginning* or *End* buttons.

9: Select and display the *MultiFocus* image by clicking the *MultiFocus Image* button.

Montage Multifocus:

Leica Application Suite *Montage Multifocus* provides advanced, versatile features for producing excellent extended depth-of-focus images using the renowned technology of *Auto-Montage* from *Syncroscopy*.

Digital images from a Z-Stack, spread over the focus range of the specimen, are acquired using the same features provided by *LAS Multifocus* but because *LAS Montage* provides tunable algorithms is able to create excellent extended depth-of-focus images covering a wider range of microscopy contrast methods.

LAS Multifocus provides only one montage method and is effective only on narrow range of specimen types. There are no facilities for adapting to different situations and no means of enhancing result image or alternative means of visualising the result image.

LAS Montage has many additional capabilities to extend the imaging conditions that can be used and improve the quality of the resulting image.

LAS *Montage* extends *Multifocus* by adding functions to provide:

- *Depth Map:* An image which contains depth information for all points on the image,
- *Confidence Map:* An image which contains an estimate of the accuracy of the depth map at all points on the image..

Additionally, the image can be viewed in several different ways to examine the surface in greater detail:

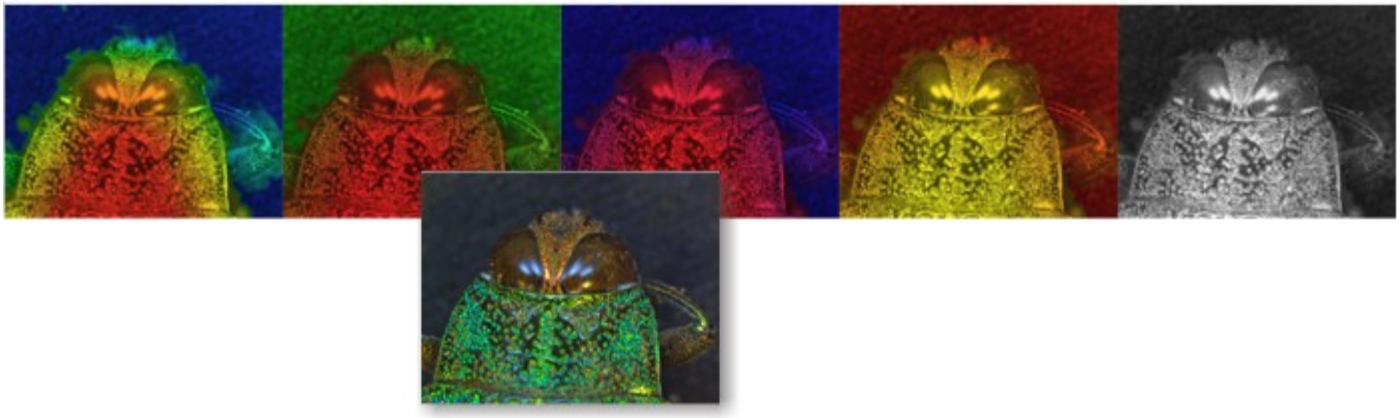
- *Anaglyph*.
- *Stereo Pair*
- *Colour Relief*
- *3D Model (Optional extra module)* which provides a perspective visualisation of the extended focus image for which the user can change the viewpoint so that the image give an impression of depth. *Note*: *LAS Montage* is a pre-requisite for the use of *LAS 3D* Viewer.

The extensive *Measure Tools* include a fast

- *Profile* display..

...that maps and measures the contours of a user-drawn path.

Z-Stack acquisition is the same for both *Multifocus* and *Montage*. To find out more: *Go there...* 520



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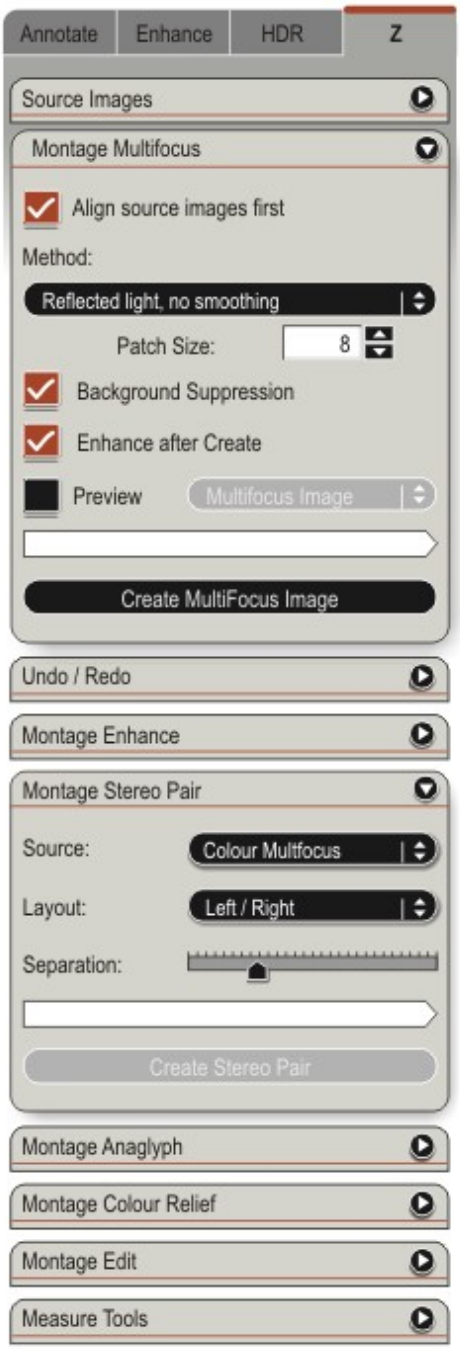
Launch *Montage* by:

1: Clicking on the *Acquisition Mode* button...

2: ...and then on the the *Montage* icon.

Montage Multifocus OperatingSteps:

- Acquire the Z-Stack: *Go there..* 520, 520
- Select the Z-Stack: *Go there...* 542
- Select the Z-Stack images to use: *Go there...* 544
- Create the Montage Multifocus image: *Go there...* 545
- Make Enhancements: *Go there...* 547
- View the Results: *Go there...* 552
- Edit the Results: *Go there...* 560
- Create additional views if needed: *Go there...* 554
- Use the Measure Tools: *Go there...* 563



1: Click to select the *Browse Workflow.*

2: Click the *Browse* tab and...

3: ... select the required Z-Stack on the *Navigator*.

4: Click on the ***Z*** tab to open the *Montage* control panel.

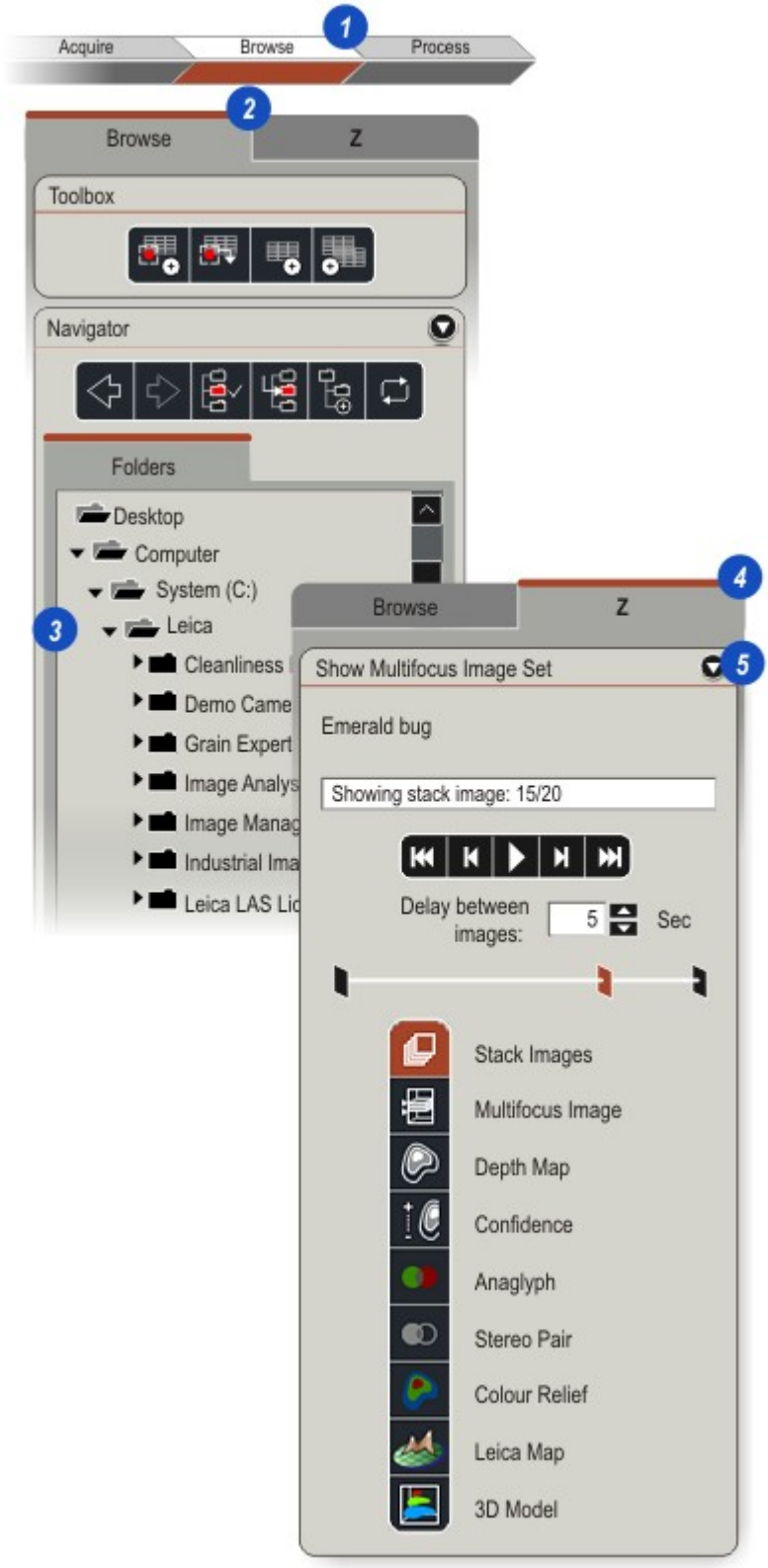
5: If necessary, click to expand the *Show Multifocus Image Set* panel.

The Z-Stack images will be loaded into the gallery together with any associated *Multifocus* image and maps.

Clicking on a thumbnail in the *Gallery* will display the image.

From this panel click to review the following images:

- *Stack Images* 543, 543
- *Multifocus Image* 545
- *Depth Map* 552
- *Confidence Map* 553
- *Anaglyph* 554
- *Stereo Pair* 555
- *Colour relief* 556
- *3D Model* 558
- *Leica Map, if it is installed, has its own help:* *Go there...* 571



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1: To view the Z-Stack sequence click on the *Stack Images* button. This will display the first image in the stack and enable the controls to view the stack.

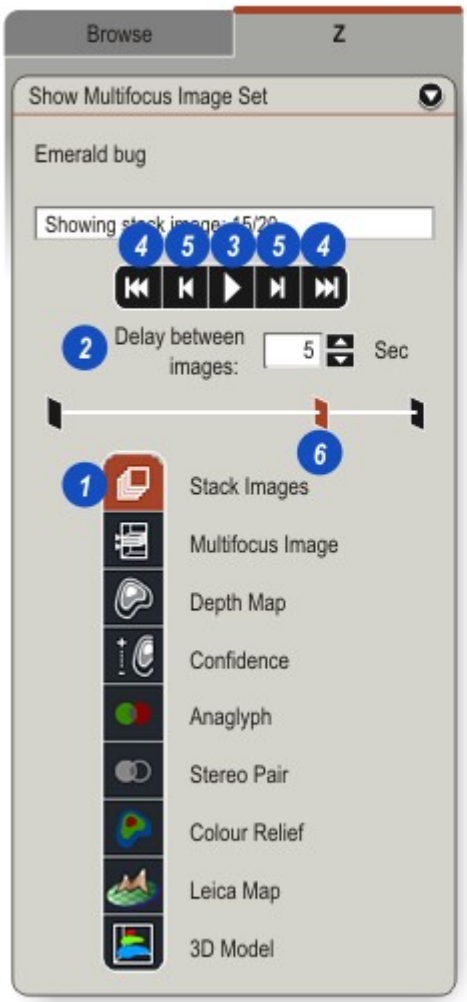
2: Enter a delay time between images and then use the control buttons to scan the images.

3: Click to play the image sequence continuously with the specified delay time between each image. Click again to stop.

4: Moves to first and last image in the sequence.

5: Steps forward or back in the sequence.

6: Click and drag on the red slider to move forward or back through the sequence. The display also shows the position in the sequence while the sequence is played.



The stack images are listed in the *Source Images* panel from where they can be selected for inclusion in the *Montage.*

Click to select the *Process Workflow* and then the ***Z*** tab.

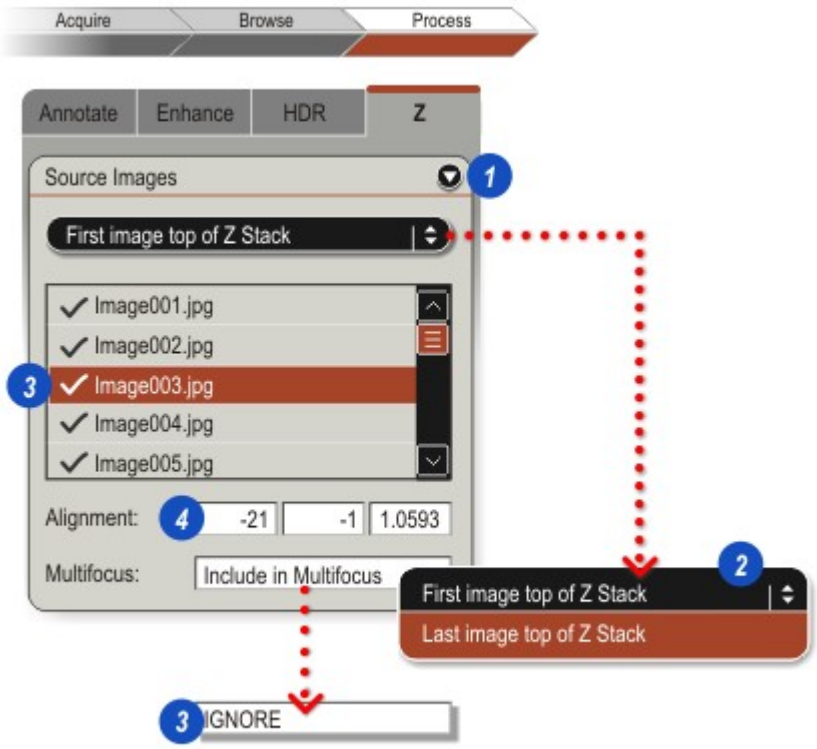
1: Click on the arrow to the right of the *Source Images* header to reveal the panel.

2: The image sequence can be swapped between starting with the top of stack or starting with the bottom of the stack by clicking the arrows and selecting the option from the drop-down list.

3: Clicking on an image in the list will select it and display it in the *Image Viewer.*

Clicking again toggles between *IGNORE* and *Include in Multifocus*. Any image that is ignored will not be used in the generation of the *Multifocus* image.

4: If the *Align Images* before combining box is checked on the *Montage Multifocus* panel (*Go there...* 545) then relative alignment data will be shown here.



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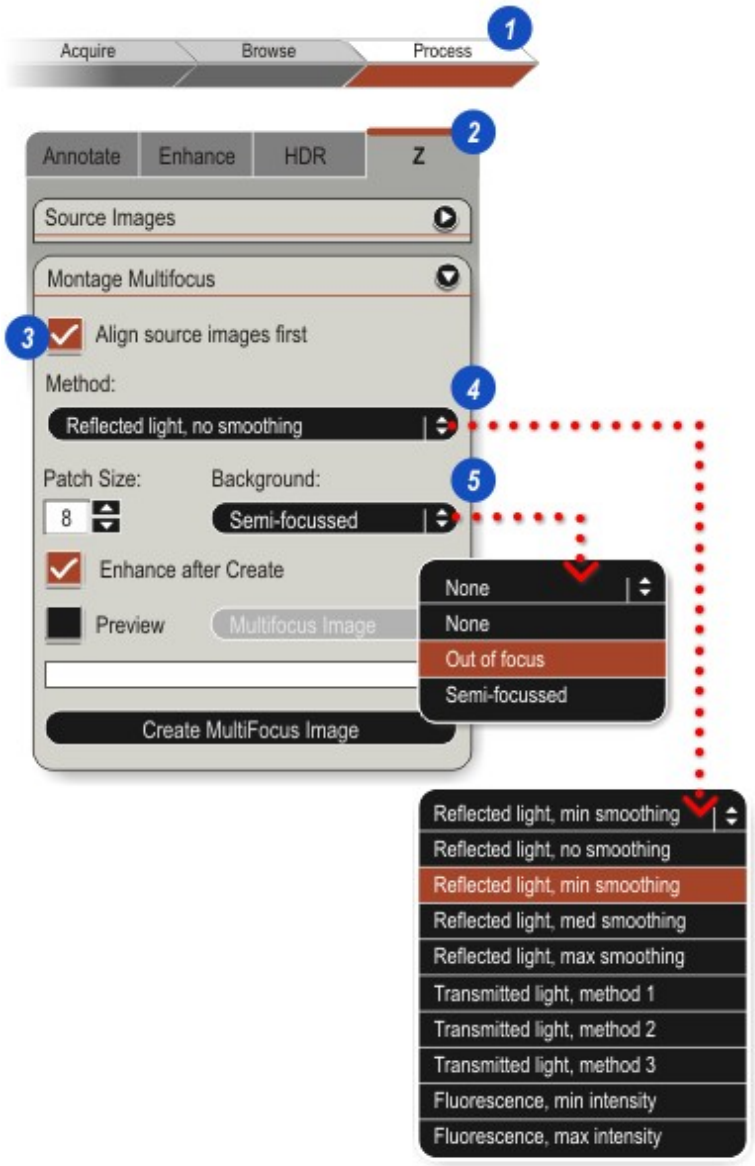
If the source images are not exactly registered with each other, having been captured with a stereo microscope or from scanned photographs, this should be corrected with the *Align Source Images* function before proceeding with the *Montage* operation.

1: Click on the *Process Workflow.*

2: Click on the **Z** tab.

3: On the *Montage Multifocus* panel, click to enable (tick mark visible) *Align source images first.*



4: Choose the *Montage Method* that is closest to the contrast method used when the Z-Stack was created by clicking on the arrows to the right of the *Method* header and selecting from the drop-down list:

- *Reflected light:* Four degrees of smoothing - none, minimum, medium or maximum ...
- *Transmitted light*: Methods 1, 2 and 3.
- *Fluorescence:* Minimum and maximum intensity.

5: Select the *Background* to reduce the effect the background has on the *Multifocus* image.

Continued... 546

1: The *Patch Size* refers to the spread of pixels used to calculate the montage. A recommended range is 8 to 20: Too small and and the detail will be good but noise could be a problem. Too large and whilst the depth maps will be smoother there could be less detail.

Adjust the *Patch Size* by clicking the up/ down arrows to increase/decrease it.

2: Click to enable the *Enhance after Create* check box to automatically apply any settings made on the *Enhance* dialogs (*Go there..* 547, 547). This is a convenient way of creating a final *Multifocus* image but enhancements can always be made after the image is created.

3: To preview a result, click to enable the *Preview* check box and...

4: ...click on the arrows to the right of the header and choose the image to be previewed from the drop-down list.

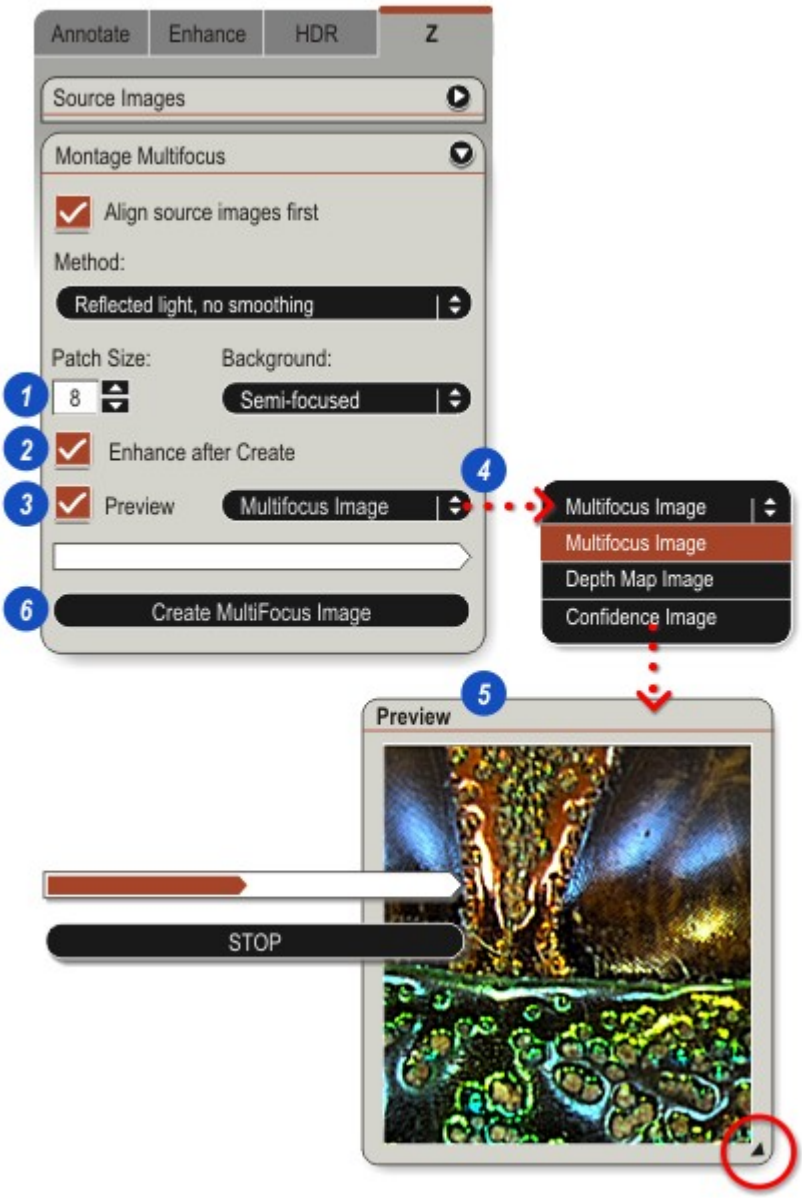
5: The *Preview* is automatically generated and the area can be enlarged or reduced by clicking and dragging the corner arrow.

Click the *STOP* button to halt the preview generation.

Click and drag inside the *Preview* window to move the area of interest.

To close the *Preview* window, click to disable the *Preview* check box (2).

6: Click the *Create Multifocus Image* button.



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The *Montage* and *Depth Map* images may be processed prior to publication. Smoothing the *Depth Map* can be used to remove artefacts that may appear in regions that lack in focus detail. Sharpening the *Montage* image may improve the images appearance.

The *Montage Enhance* panel is divided into three main control areas:

- *Background Confidence* ***(A)***..
- *Enhance Multifocus Image* ***(B)*** and
- *Enhance Depth Map Image* ***(C)***.

Additionally, there is a *Preview* facility and the *Enhance Images* button that applies the enhancements.

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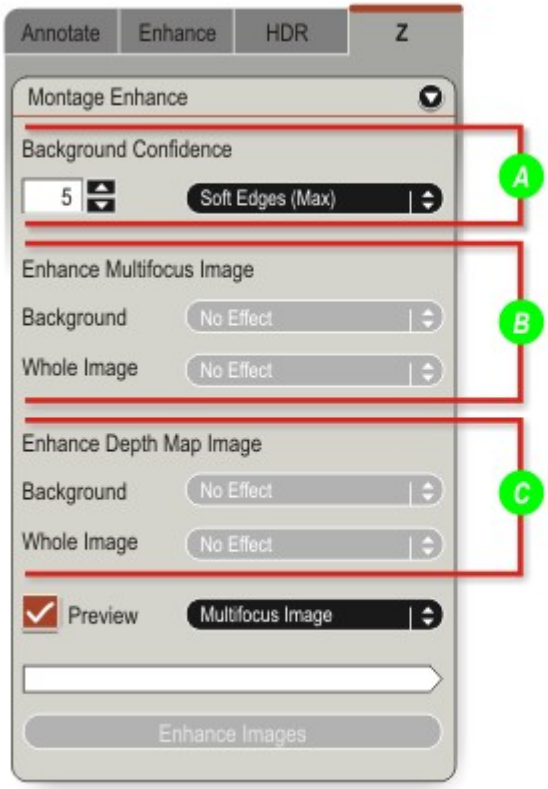
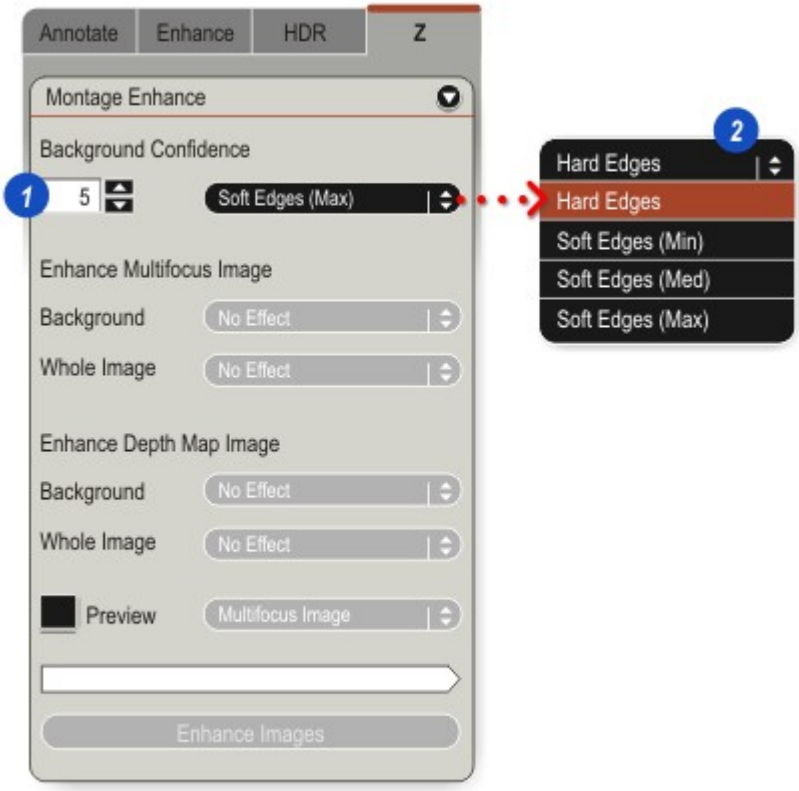


Note - background is now removed using the Background selection from the Montage MultiFocus panel. This information is retained only for reference.

Please set control **1** to 0.

Adjusting the *Background Confidence* value will define a new *Background Mask* layer which can be applied to both the *Multifocus* and *Depth Map* images.

The *Background Mask* can be derived from any or all of the layers and is simply the parts of the images that remains after all of the desirable image information has been extracted.



1: The threshold for the *Background Confidence* can be set to a value between 0 and 100% by clicking inside the text box and typing a value or using the up/down arrows.

Any pixel with a confidence value in the range *0* to *Background Confidence%* will be regarded as a background pixel.

2: The boundary between the *Background Mask* layer and the rest of the image may be smoothed depending on the value of the smoothing factor. Click on the arrows to the right of the header and from the drop-down list select from:

- *Hard edges.*
- *Soft Edges(Min)*
- *Soft Edges(Med)*
- *Soft Edges(Max)*

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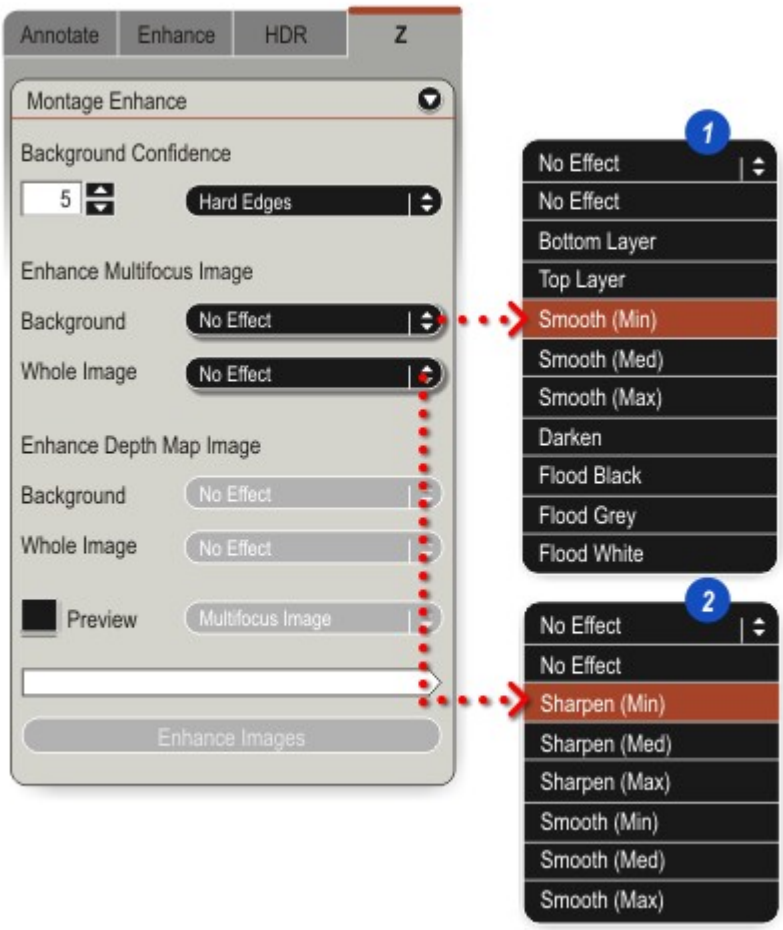
Background:
Apply various effects to the *Montage* image, but only within the region where a pixel has a confidence value in the range *0 - Background Confidence%* - the *Background Mask.*

1: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Montage* image..
- *Background*: Over the *Background Mask* region the background layer is copied to the *Montage* image
- *Smooth: (3 levels):* Over the *Background Mask* region a smoothing filter is applied to the *Montage* image
- *Flood Black, Grey or White:* Over the *Background Mask* region, the *Montage* image is set to a solid colour
- *Darken*: Over the *Background* mask region, the intensity of the *Montage* image is reduced



Whole Image:
Sharpen or smooth the parts of the *Multifocus* image where the confidence is higher than the *Background Confidence%*.

2: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Montage* image..
- *Sharpen (3 levels):* or...
- *Smooth (3 levels).*

Continued... 550

Background:
Apply various effects to the *Depth Map* image, but only within the filter region where a pixel has a confidence value in the range *0 - Background Confidence%* - the *Background mask.*

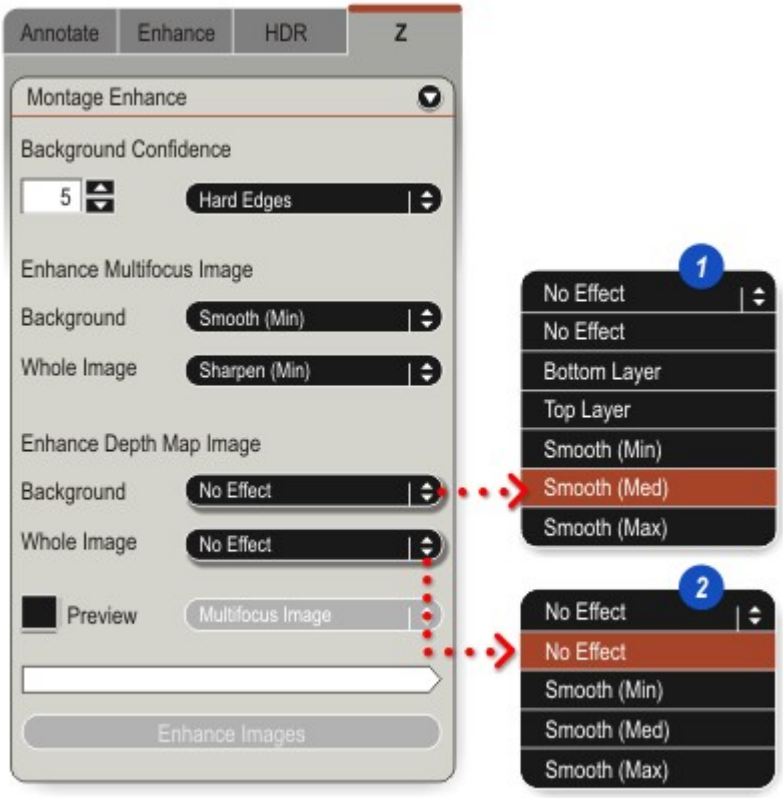
1: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Depth Map* image.
.
- *Smooth: (3 levels):* Over the *Background* mask region a smoothing filter is applied to the *Depth Map* image

Whole Image:
Sharpen or smooth the parts of the *Depth Map* image where the confidence is higher than the *Background Confidence%*.

2: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Depth Map* image or..
- *Smooth (3 levels).*



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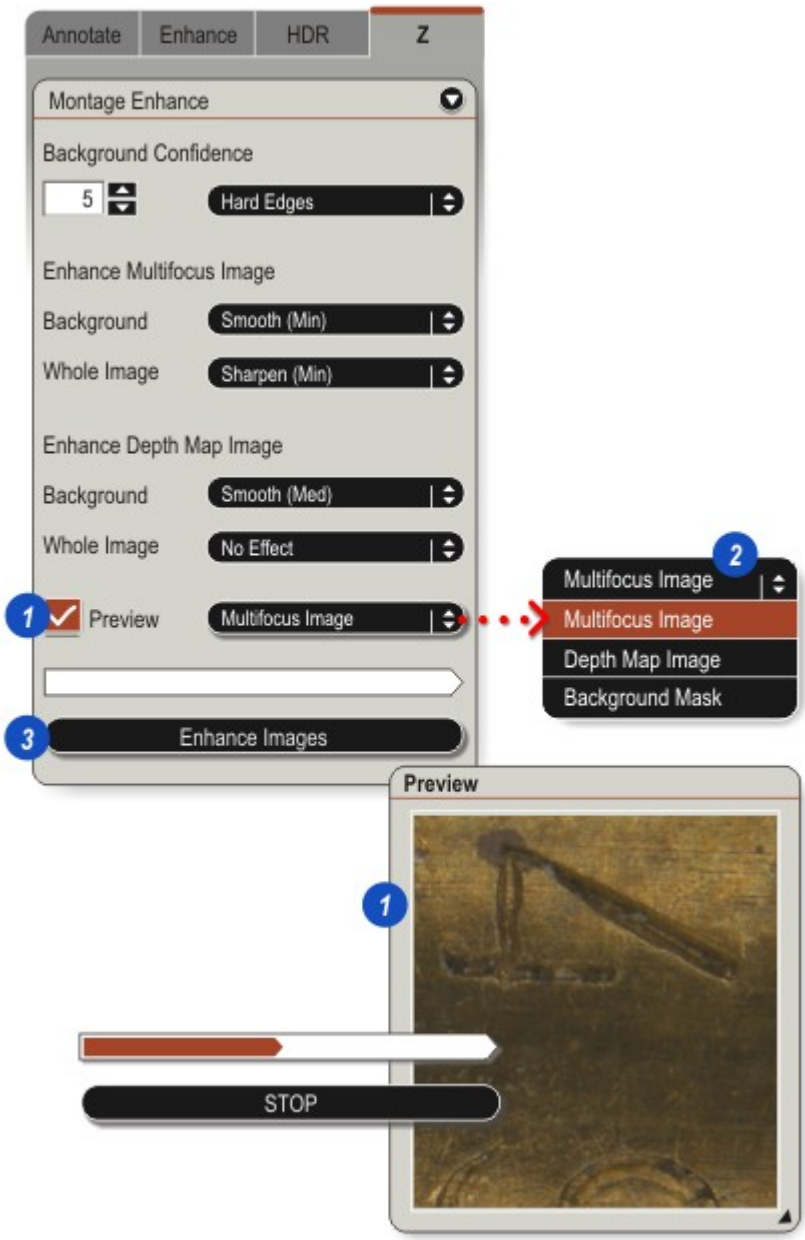
To preview the *Montage Enhance* settings:

1: Click to enable the *Preview* check box.

2: Select the image type to preview by clicking on the arrows to the right of the header and choosing from the drop-down list. The *Preview Viewer* opens.
To move the area of interest within the *Viewer*, click and drag the image.

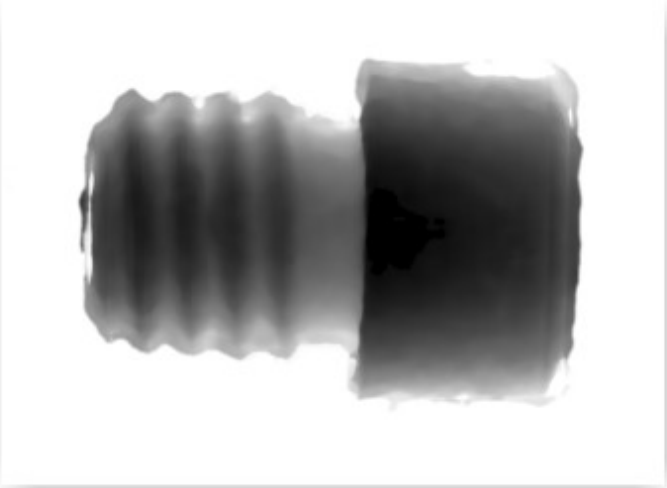
3: When the *Enhance* settings are acceptable, click on the *Enhance Images* button to apply them to the images.



A *Depth Map* image is generated as part of the Montage process.

The *Depth Map* is a record of which source image provided the in-focus region for the montage image at each pixel location, expressed as a grey level.

The *Depth Map* image is the same size as the source images used to generate it, but is always monochrome. The grey levels depend on the particular *Montage Method* used to generate the depth map.



The *Depth Map* image is displayed in the depth map image window and is saved in the dataset file itself, but can also be exported to an image file if required.

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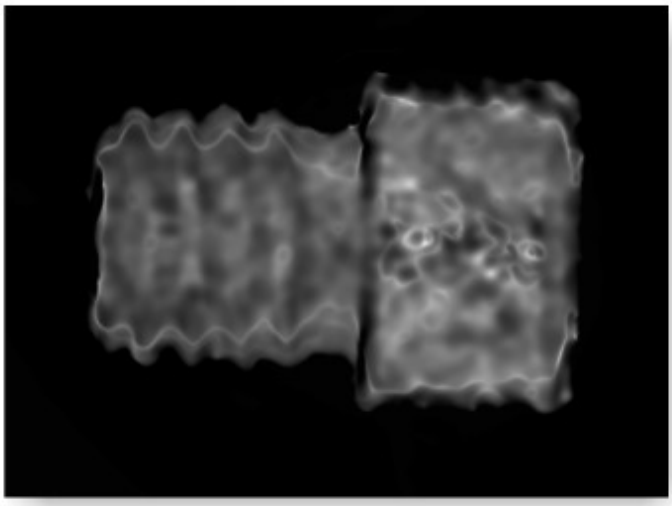
A *Confidence Map* is also generated for each montage operation. It is an estimate of accuracy of the depth map image at each pixel location, expressed as a grey level.

White represents high confidence and therefore an accurate depth value. Black represents low confidence and an uncertain depth value.

Low confidence will often represent areas of low contrast where several planes will appear to be in focus.

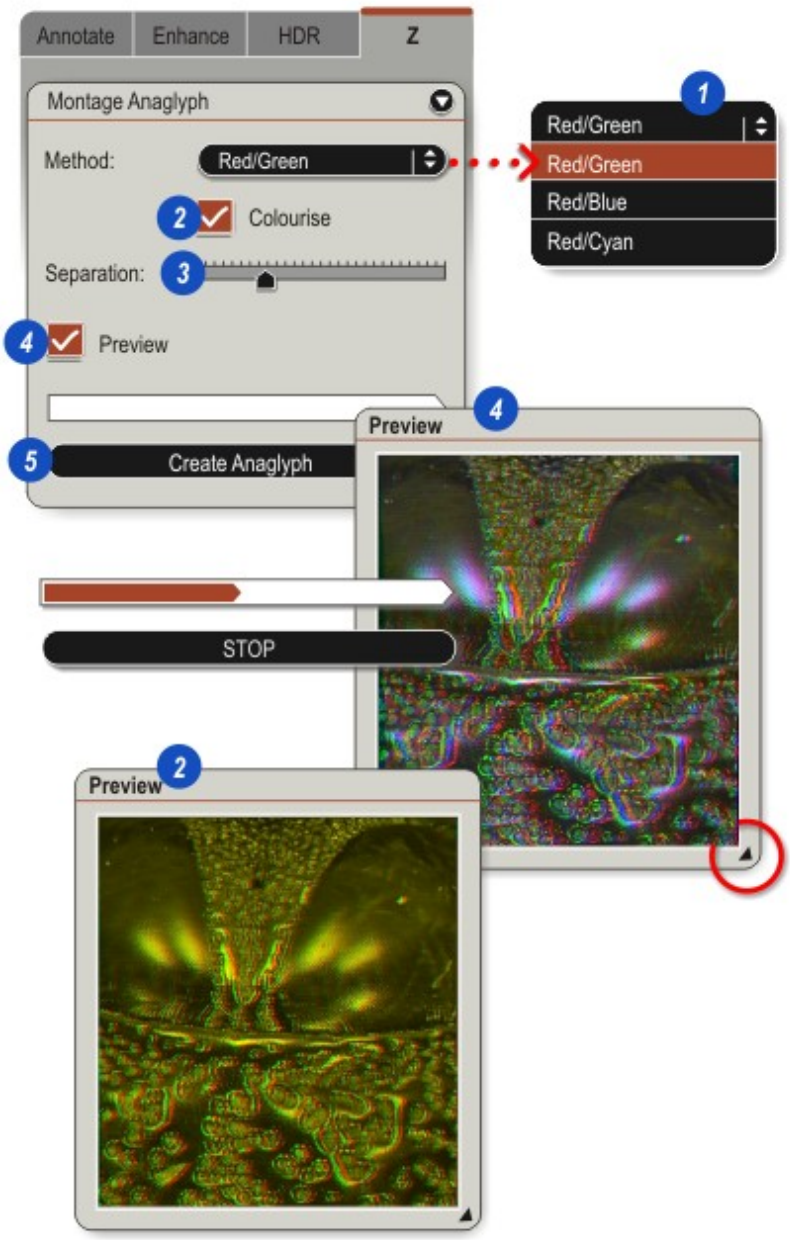


The *Confidence* image is the same size as the source images used to generate it, but is always monochrome. Values are expressed as percentages, ranging from 0% (no confidence that depth map is correct for that pixel) to 100% (complete confidence).

The *Montage Anaglyph* operation generates the anaglyph image, which when viewed through suitable red and green/blue lenses offers a 3-dimensional monochrome view of the montage image, with depth information synthesized from the depth map.

It is the same size as the *Montage* image used to generate it, but always comprises 3 RGB planes.

The anaglyph image will be saved as one of the result image files.



1: Select *Method* from drop-down list. Choose *Red/Green* or *Red/Blue* to match the glasses to be used. Choose *Red/Cyan* for better printed results - the effect depends on the *Montage* image.

2: Check C*olourise* to add a hint of the original *Montage* image colour back to the *Anaglyph* image - the final effect depends on the *Montage* image.

Colourise is not available with *Red/Cyan* option because all 3 colours are effectively already used.

3: Set the amount of *Separation* - the maximum amount, in pixels, by which left and right-eye images are displaced to provide the stereo effect. Lower values are recommended in most cases.
Click and drag the slider or if the mouse has a wheel for scrolling, click on the slider and use the wheel to adjust the parameter value.

4: Check *Preview* to see the effect of the settings in the Preview window

5: Click *Create Anaglyph* to create see the finished *Anaglyph* image in the Viewer.

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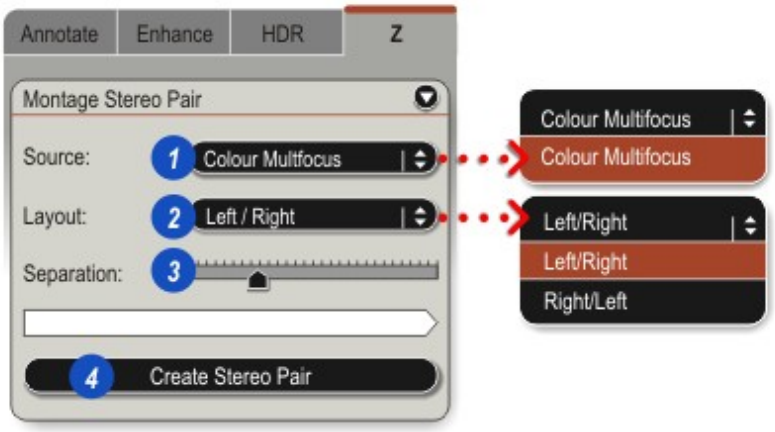
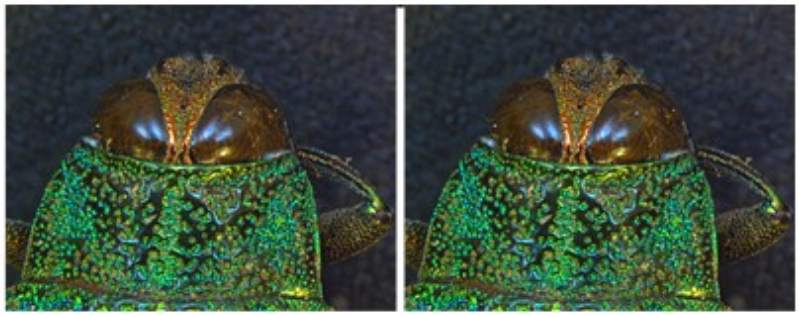
The *Montage Stereo Pair* operation generates the *Stereo Pair* image, which when viewed correctly offers a 3-dimensional colour view of the montage image - with depth information synthesized from the depth map.

The stereo pair image comprises two similar images, in colour or greyscale depending on the source image, each the same size and pixel depth as the *Montage* image used to generate it.

The *Stereo Pair* image will be saved as one of the result image files.





1: Select *Source* from drop down box. The stereo pair may be generated from:

- A monochrome representation of the *Montage* image,.
- From the original colour *Montage* image - if the source images is colour, or
- From the colour relief image if that has been generated.

2: Select *Layout*. For normal use, select *Left/ Right.* However, some people find it easier to view a stereo pair with the images flipped *Right/Left*

3: Set the amount of *Separation* - the maximum amount, in pixels, by which left and right-eye images are displaced to provide the stereo effect. Lower values are recommended in most cases.
Hint**:** *If your mouse has a wheel for scrolling, click on the slider and use the wheel to adjust the parameter value.*

4: Click *Create Stereo Pair.*

Montage Colour Relief generates the colour relief image that offers a view of the *Montage* image colour-coded with depth information from the *Depth Map.*

The *Colour Relief* image is the same size as the *Montage* image used to generate it, but always comprises 3 RGB planes.

Experiment with the controls to find a representation which works well with your particular images:

Images of surfaces consisting mainly of highlight detail tend to look good with mode set to *Light Features* and *Saturation* slightly left of centre.
Images of biological samples with dark fibres tend to look good with mode set to *Invert*.

A pair of *red/gr*een or *red/cy*an spectacles as used for anaglyphs, may help to interpret colour as depth.

The *Colour Relief* image will be saved as one of the result image files.

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1: Select *Method* from drop down box. Experiment with this control to find which mode works best with the images:

- *Light Features* applies the colour effect mostly to the lighter parts of the montage image.
- *Dark Features* applies the colour effect mostly to the darker parts of the montage image.
- *Invert* applies the colour effect to the darker parts of the montage image and darkens the background.

2: Click on *Apply Confidence* to use the confidence image to suppress unfocused parts of the montage image.

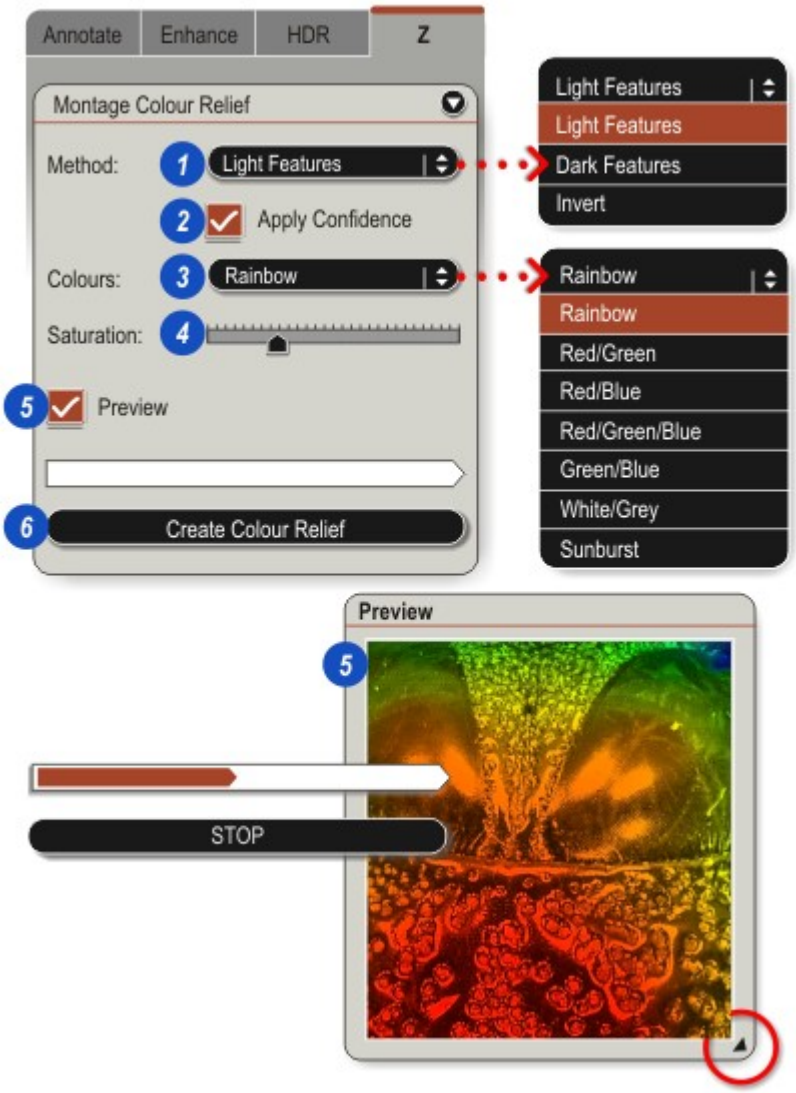
3: Select the *Colours* scheme in which to plot the colour relief image.

4: Adjust the *Saturation*. Saturation varies from purely monochrome intensity (left-most position) to full-coloured depth map with no intensity information (right-most position).

Click and drag the slider or if the mouse has a wheel for scrolling, click on the slider and use the wheel to adjust the parameter value.

5: Check *Preview* to see the effect of your settings in the *Preview* window

6: Click *Create Colour Relief* to see the finished image in the *Viewer*.



The *3D Model* is an Optional Module that must be licensed and installed. If it is not, the button will not appear in the tool list.

The module is launched from the *Browse Workflow:*

1: Click on the *Browse Workflow*.

2: Click to select the ***Z*** Tab.

3: On the tool list, click the *3D Model* button.

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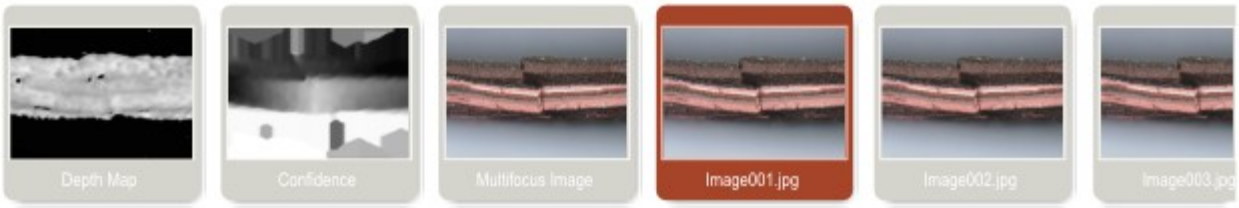

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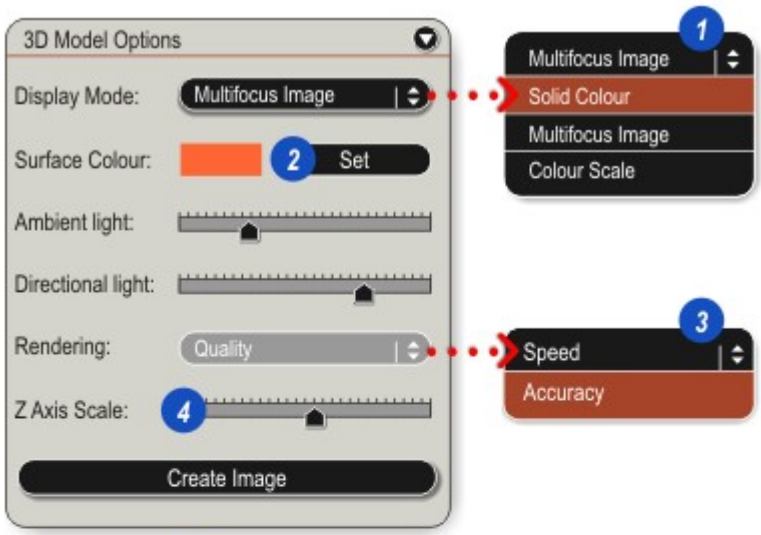
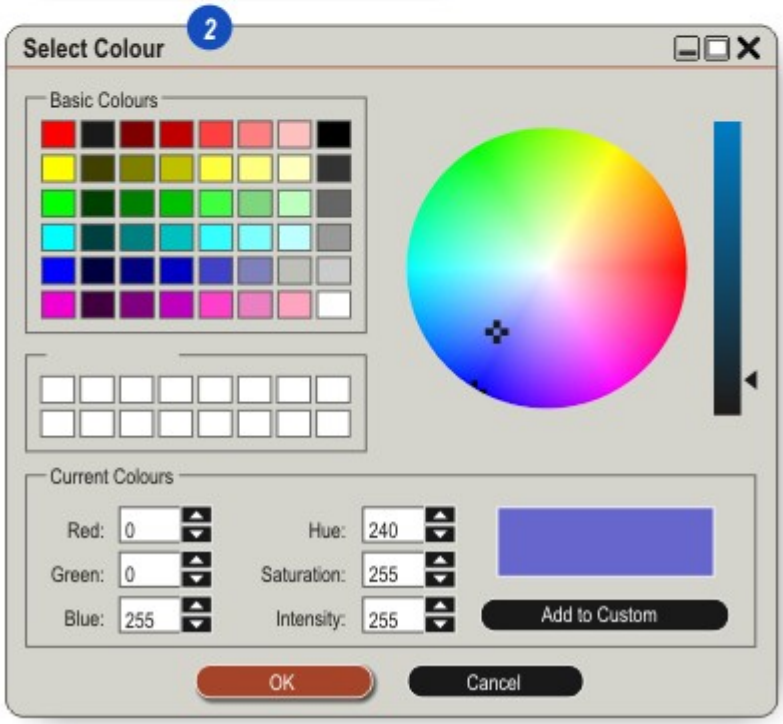
The *3D Model* is a 3D surface view of the image based on the *Depth Map* information and can be viewed from different angles by clicking on the model and dragging the mouse.

The model can be created in three different *Display Modes*:

- *A: Solid Colour:* The model is displayed as a surface relief with a single colour. The colour can be set by clicking on the Set button..
- *B: Multifocus Image:* The model is displayed as a surface relief overlayed with the *Multifocus* image.
- *C: Colour Scale:* The model is displayed as a surface relief overlayed with the colour relief image showing the depth map contours.





1: Select the display mode from the drop down box.

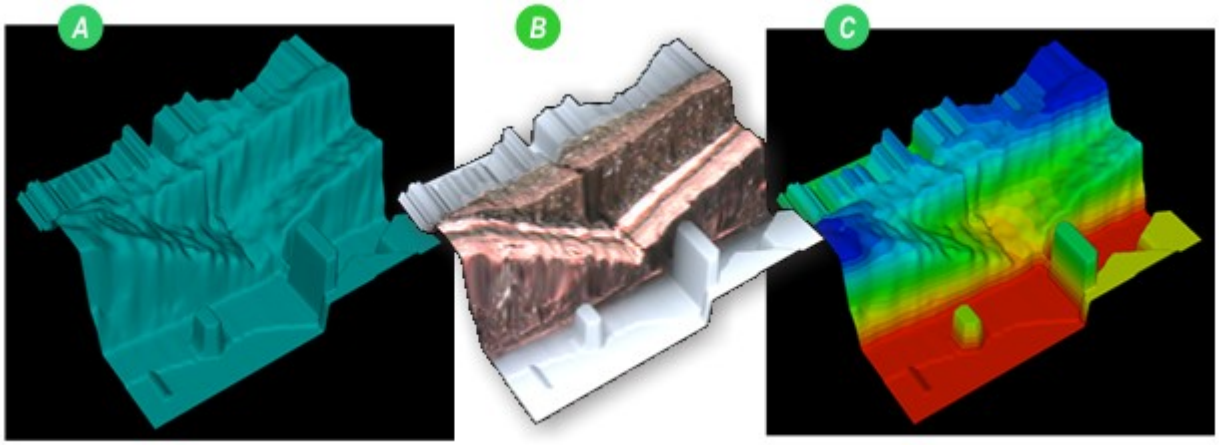
The brightness of the image can be adjusted using the *Ambient light* slider and the illumination direction with the *Directional light* slider.

2: With the *Solid Colour* mode selected, the colour can be changed by clicking the *Set* button and choosing a colour from the dialog.

3: Set *Rendering* to *Speed* or *Accuracy* to change the response time of the model display in *Colour Scale* mode.

4: The image can be 'stretched' along the Z axis by clicking and dragging the *Z Axis Scale* slider.

5: Click the *Create Image* button.



Montage Edit:

The *Montage Edit* tool is used to copy and blend pixels from one image - the *source*, - to another - the *target*. It is best described using a hypothetical example.

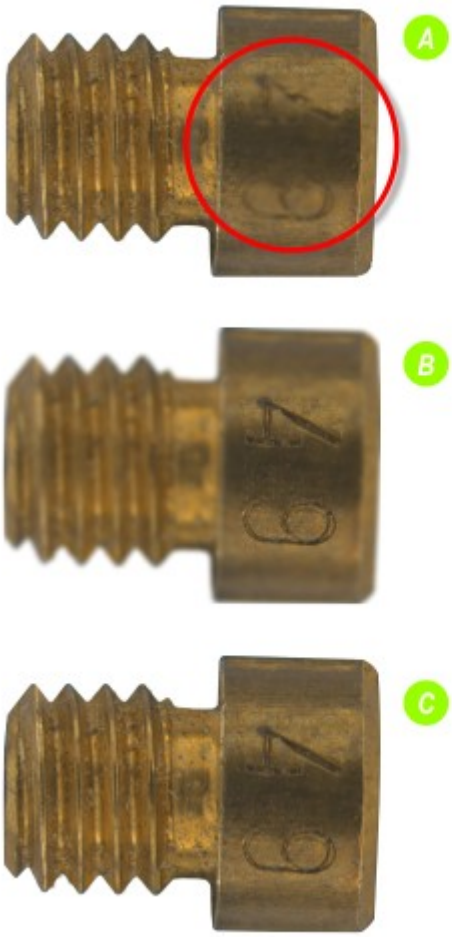
Image **(A)** is the resulting *Montage* from the Z Stack of a bolt. Most of the image is sharp with the exception of the serial number (circled) which is important to the job but out of focus and indecipherable. This will be the target of some edited pixels.

Image **(B)** is Z Stack image number (10) and whilst it is mainly out of focus, the serial number is sharp and readable. This will be the source of the edited pixels.

(C) *Montage Edit* allows the user to extract a section of a selected stack image and 'blend' it into the final montage.
Now the *Montage* is sharp and the serial number from Z Stack image (10) has been included and maintains its sharpness.

The *Dual Viewer* facility allows the user to view both the final montage and the edit source side-by-side so that the result can be seen immediately and editing is an easy and fast operation.

The *Depth Map* can also be the target of the edit.



Continued... 561

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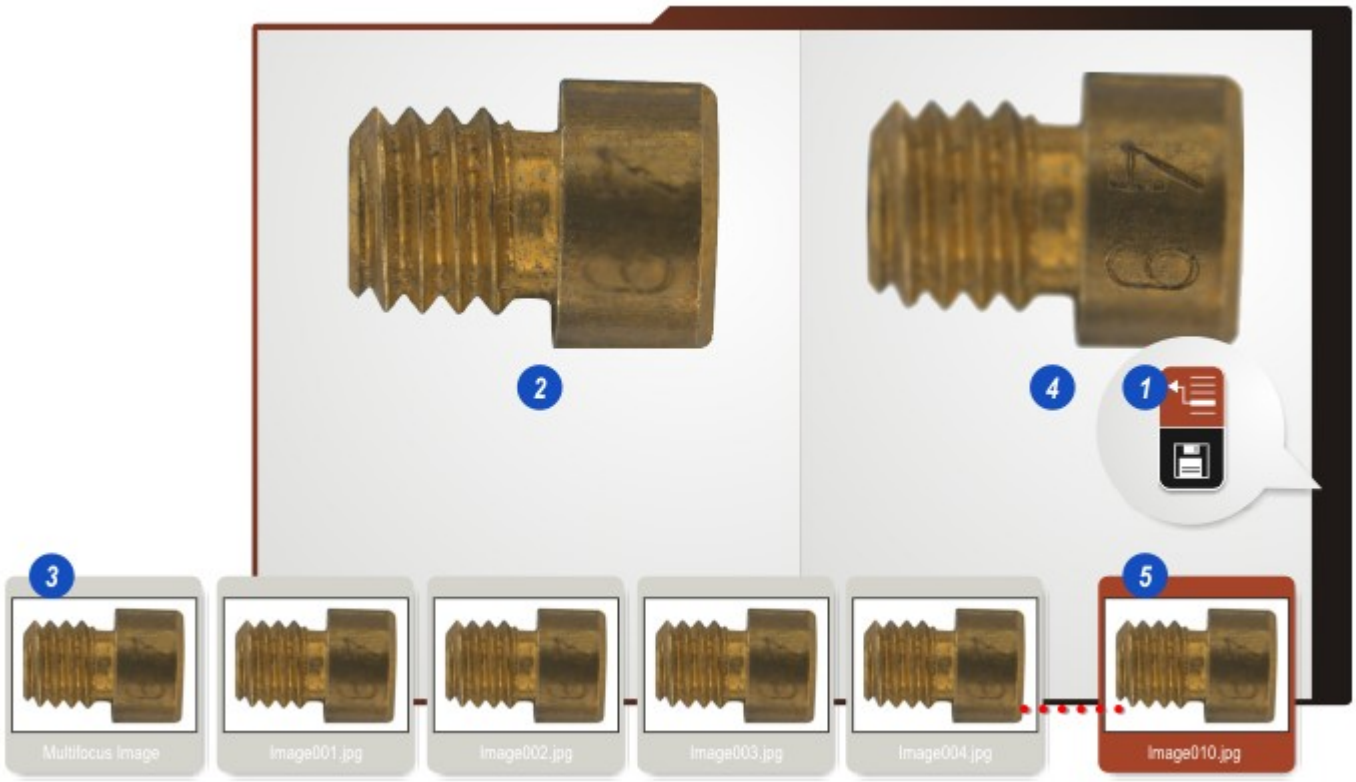

Both the source and target of the edited pixels can be viewed side-by-side by using the *Dual Viewer* facility:

1: Click on *Viewer Options* icon on the *Side Tool Bar*. From the drop-down list click to select *Dual Viewer*. The *Viewer* separates into two panes.

2: Click in the left-hand pane.

3: In the *Gallery*, click on the target thumbnail - in this case the *Montage Multifocus* image but it could be the *Depth Map*. The image appears in the left-hand pane.

4: Click in the right-hand pane.

5: Click on the source of the pixels - in this case *Image010.jpg.* The image appears in the right-hand pane.

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1: Click the *Edit Mode* check box to enable editing.

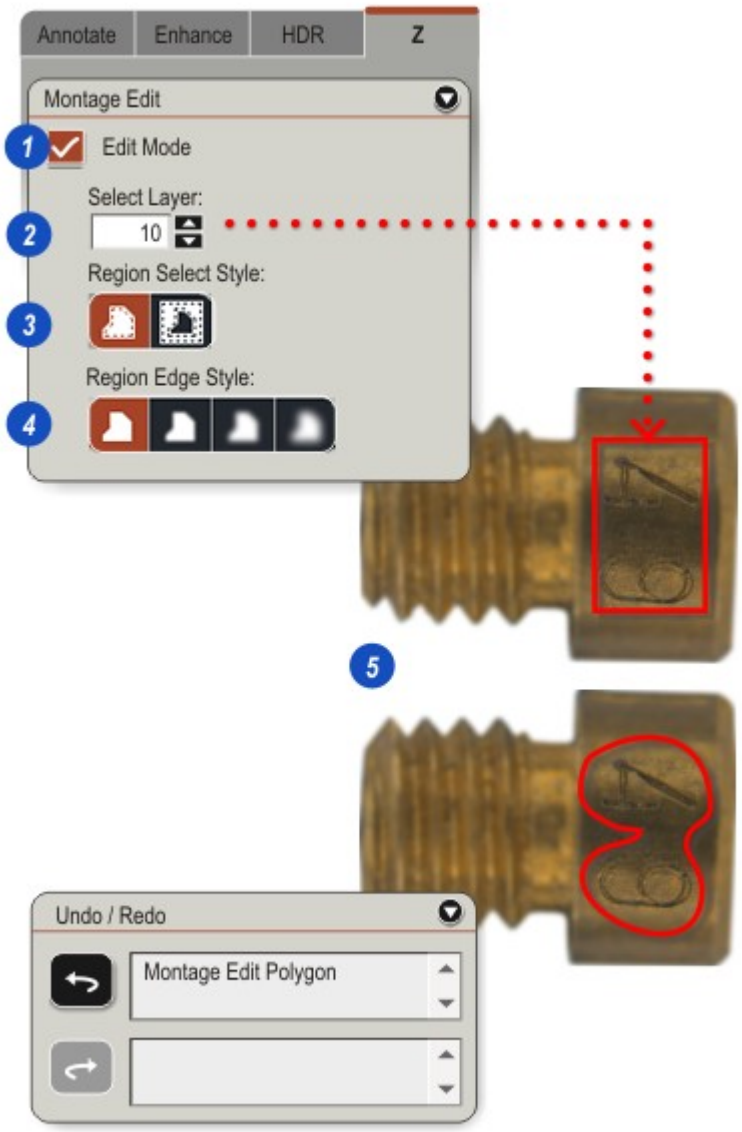
2: Click inside the *Select Layer* text box and type the source image number - in the example image 10.
Alternatively, click inside the text box and then click on the image thumbnail which will automatically transfer the image number.

3: *Region Select Style* allows the easy selection of an area using the mouse. The selected area is outlined with a red line.

4: There are 4 possibilities for the *Region Edge Style*:
The left-most selection uses a hard edge with no blending of the edited area and the background image.
Each of the other 3 edge styles add progressively heavier blurring of the compound image and the selected layer, while still remaining within the selected outline. Click to select.

5: Define the source edit area:

- Create a *rectangular* area by clicking at each corner..
- Create a *freehand* area by clicking and dragging to draw the edit area.
- Double-click to close the shape and define the edit area.



If a mistake is made while defining an outline, a right mouse click can be used to delete the last point. This can be repeated until the line is entirely removed.
To quickly remove an entire line, uncheck the *Edit Mode* checkbox.
The *Undo / Redo* panel can be used if the results of an edit operation are not as desired.

The edited target is automatically saved. To revert to the original *Montage Multifocus* image create a new image (*Go there...* 545) that is not influenced by the edit.

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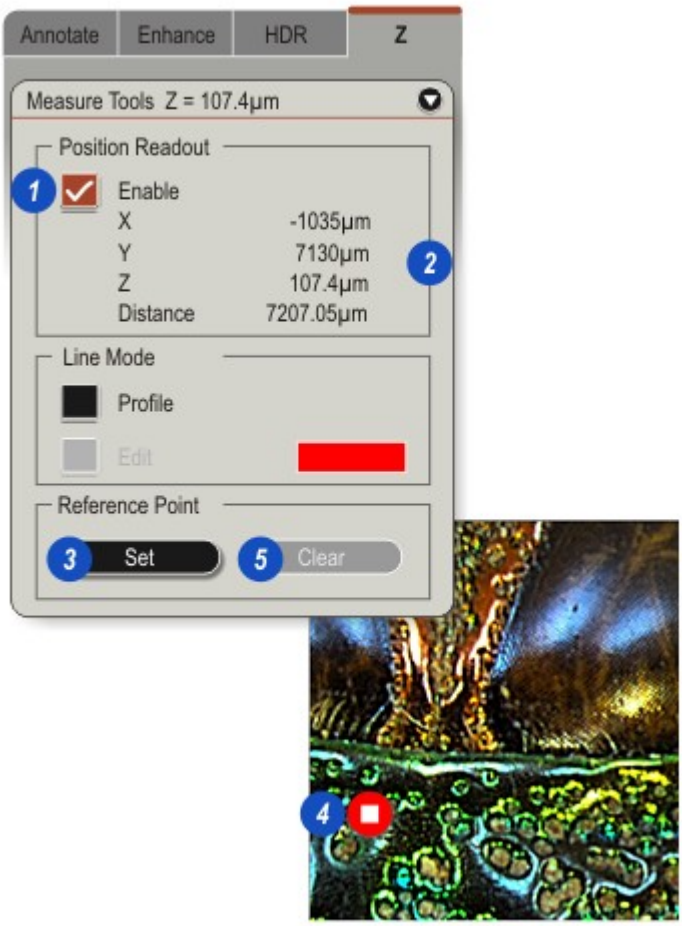
Measure Tools allows the user to read depth information from a point or line on a *Montage Multifocus* Image and display a profile of the surface as a graph on a chart. Measurements may be absolute or relative to a reference point set by the user.

This mode is controlled by several checkboxes on the panel. The panel is inactive if no depth map is available for the *Multifocus* image. (The depth map is created when *Montage Multifocus* makes a new *Multifocus* image.)



1: Check to enable the *Position Readout* check box to give a continuous readout of the current mouse position as the mouse is moved over the image.

2: The ***X*** and ***Y*** positions are given in calibrated units relative to the top left corner of the image.

The ***Z*** position is taken from interpreting the depth map and is displayed in microns.

Distance is measured from the top left corner of the image or from a *Reference Point* placed on the image. *Distance* follows a path constrained to the surface and prescribed by the *Depth Map.* The *Distance* along the surface will be longer than the straight-line distance between the start and end point.

3: Set a *Reference Point* on the image by clicking the *Set* button and...

4: ...clicking on the image where the *Reference* is to be placed.

5: Click the *Clear* button to remove a *Reference Point.*

- It is best practice to use calibrated images for *Distance* measurement. A nominal one micron per pixel is used as a default for un-calibrated images.

Continued... 564

Two check boxes control the *Profile* display:

1: Enable *Profile* (with *Edit* disabled) to draw...

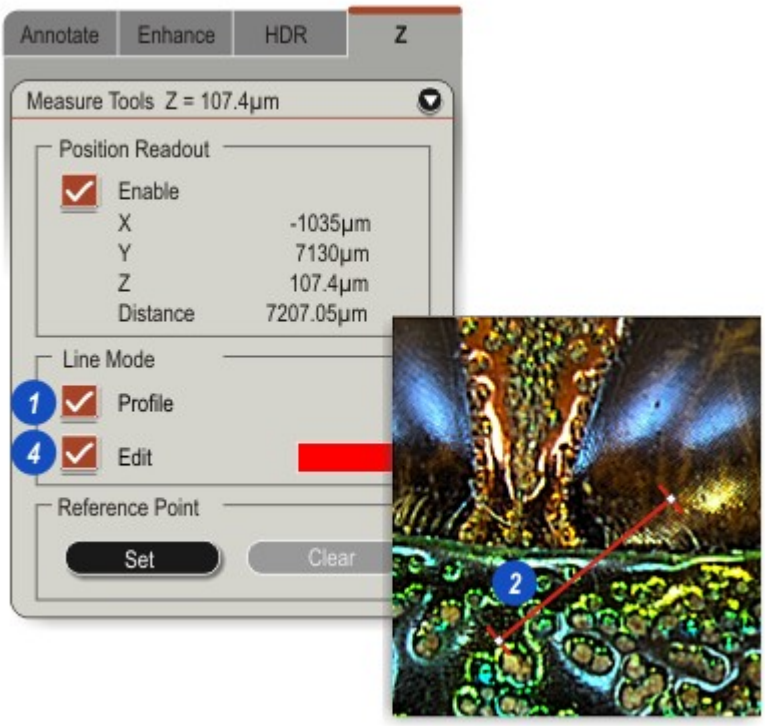
2: ... a straight line on the image with the mouse. Each new line will replace any previous one.

3: The *Profile* display will show the graph of the surface section. Use the controls top-right to display the *Profile* full screen or to close it.

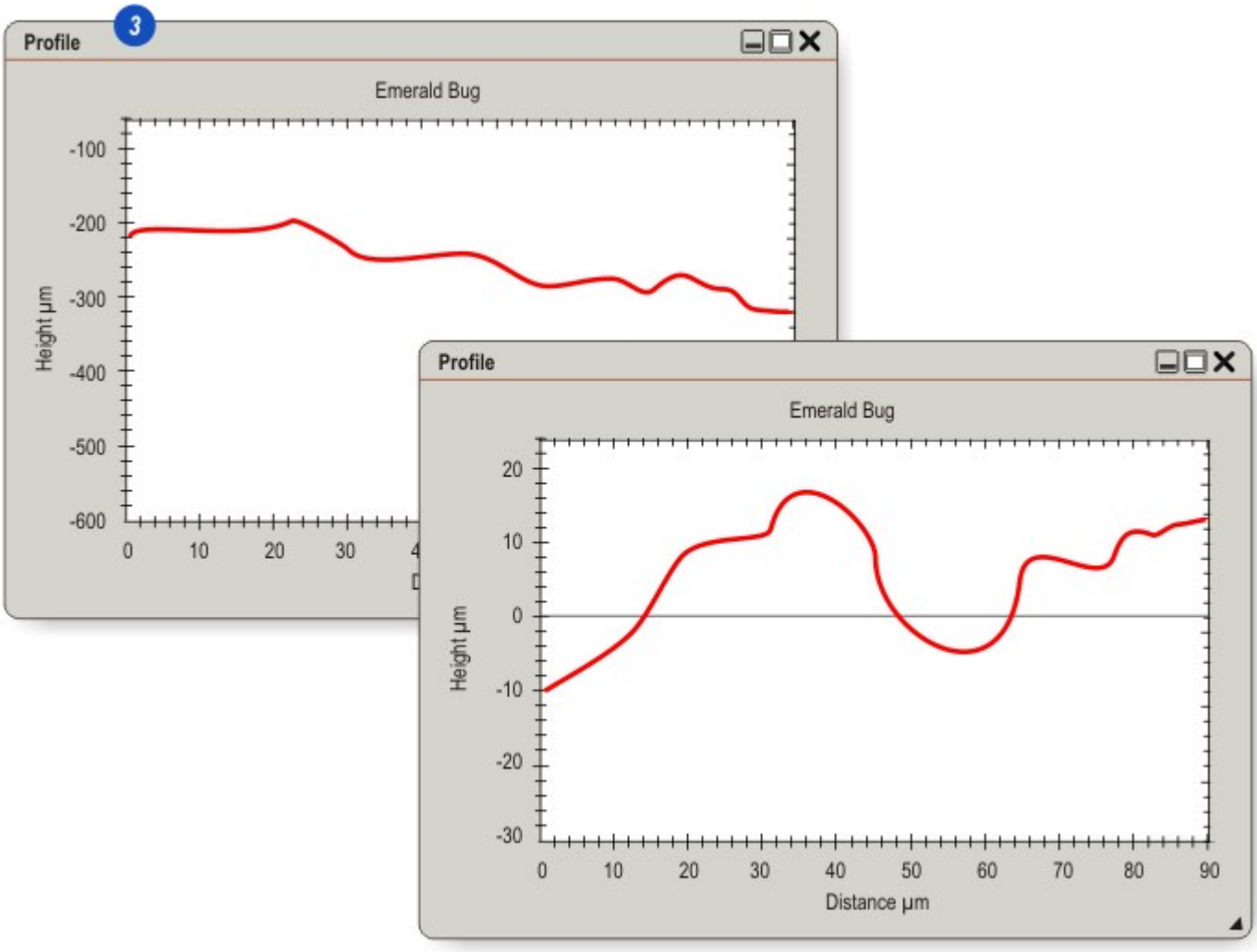
4: Click to enable *Edit* to interact with an existing line or move a reference point. The line can be moved by clicking and dragging it or rotated by clicking a dragging an end point.

A *Reference Point* can also be repositioned. The *Profile* graph changes accordingly.

Placing a *Reference Point* on the image sets a zero level for ***Z*** values and the display will change to include a Zero line with heights both above and below it.



Continued... 565



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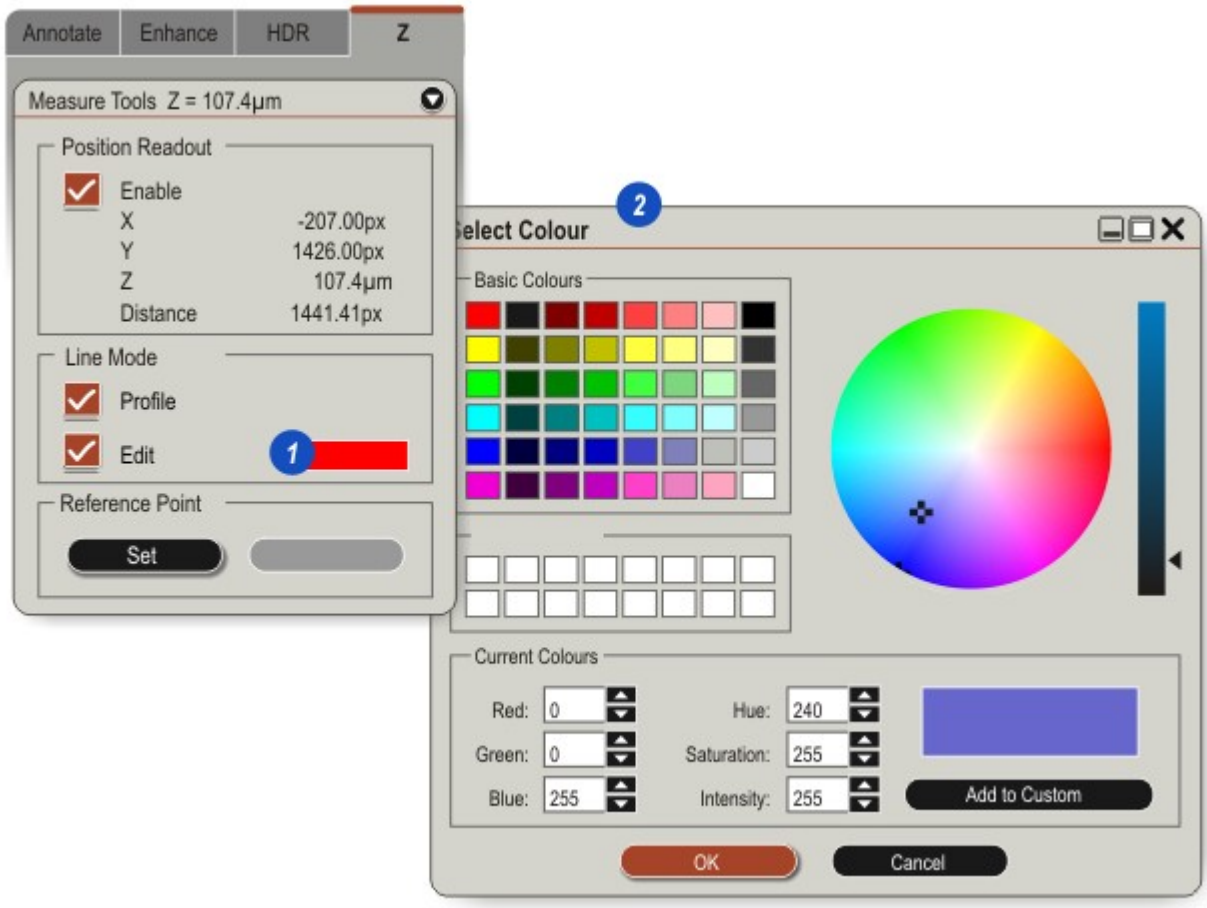
Change the *Line* and *Reference Point* colour by:

1: Click on the *Line Mode* colour button.

2: From the *Select Colour* dialog click to select a basic colour, drag the target to the required colour on the circle or enter values in the *Current Colours* text boxes.

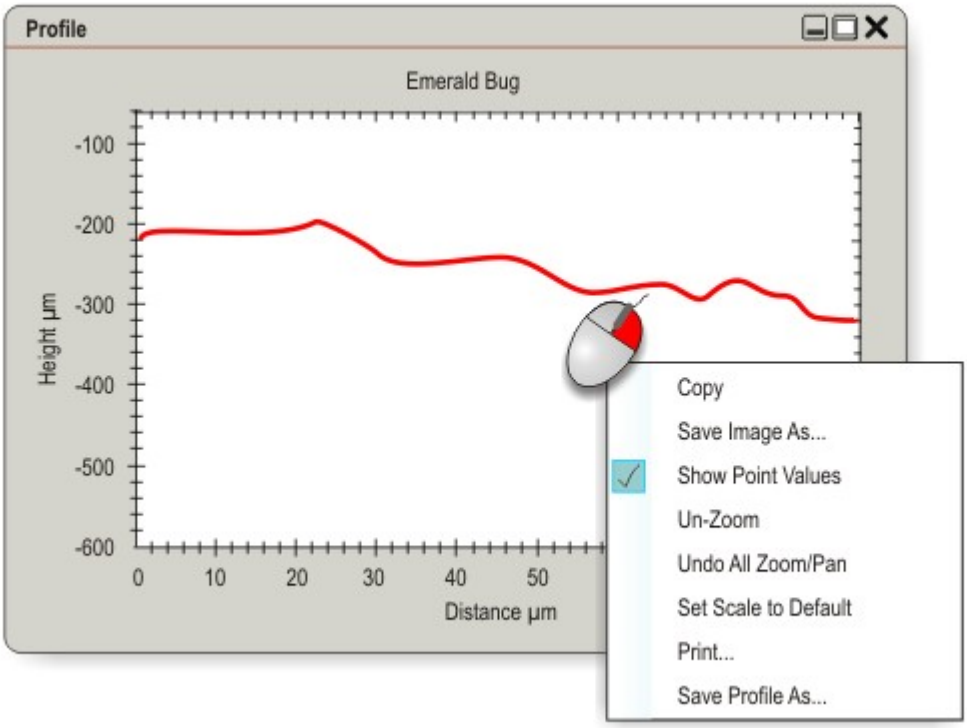
Continued... 566



Profile Panel Context Menu:

The *Profile Panel* has a context menu selected with a right-click.

- The *Profile* can be copied to the clipboard,.
- Saved to a named file and...
- Printed.
- Selecting *Show Point Values* provides a read-out of the graphs co-ordinates when the cursor is placed near the graph.
- To zoom a portion of the graph, left-click and drag a rectangle around the area of interest.
- Select *Un-Zoom* to remove the zoomed area.
- To *Pan* a zoomed chart hold down the left *Shift* key, click and drag the mouse.
- *Save Profile As* exports the profile to a text file that can be imported into Microsoft Excel.



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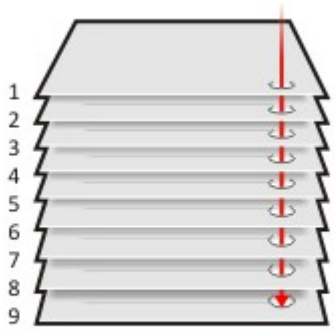
The purpose of the *Montage Methods* is to create a single in-focus image by combining parts of multiple images taken at different Z positions. Each individual image may contributes some focussed areas to the final image.

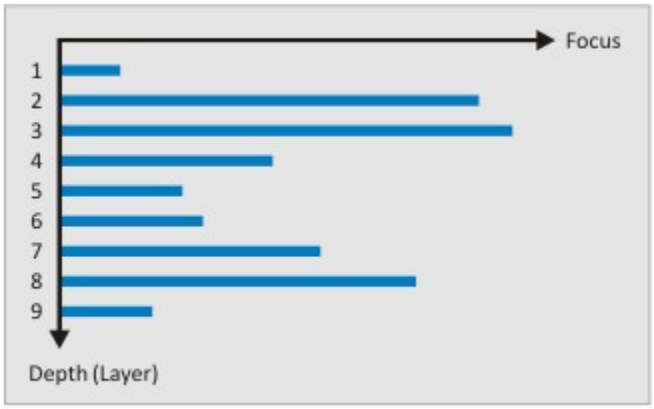
The multiple images are acquired in order of Z position ideally at steps in the Z position that are separated by less than the depth of focus of the objective being used. This set of images is called an image- or Z- 'stack' and the individual images are referred to as slices.

Consider a single pixel from each source image (slice) at the same *X/Y* location. Nine source images comprise the Z-Stack in the illustration with the single pixel location represented bottom right of each.

On comparison with neighbouring pixels in the same image, a numerical measure of 'sharpness of focus' is calculated for that pixel within each source image.
A graph of focus against depth (*Source Image Index*) is then plotted for that pixel location in all source images.

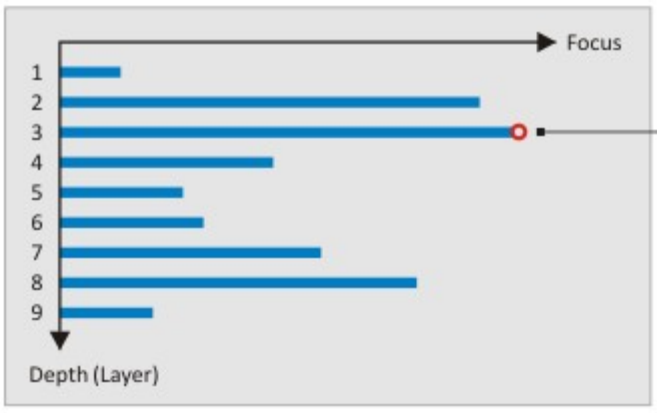
The focus comparison and the algorithms described in the following illustrations are repeated for every pixel *X/Y* location within the series of source images.





Reflected Light: No Smoothing: Fixed:
Selects the single source image plane which is in best focus at each pixel location.

The resulting *Depth Map* - a list of each in-focus pixel with its source slice and X/Y co-ordinates - is generated with whole integer values referring to a single source image index for each location and the depth map image will be seen to have a 'layered' appearance.



The resulting *Montage* image is generated by pasting in the single pixel value from the selected source image at that location.

Reflected Light No Smoothing is the quickest algorithm, so is useful for determining optimal parameter values before performing a *Reflected Light with Smoothing* or *Transmitted Light* montage operation. The *Depth Maps* produced are not suitable for 3D viewing or measurement.

Note - this method is used by the MultiFocus module.

Depth Map image value (3).
Montage Image value copied from single source image (3).

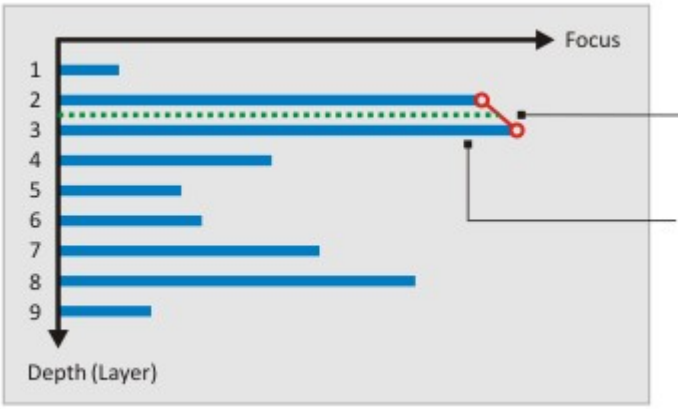
Reflected Light: Smoothing: Blended:
Takes into account the effects of adjacent in-focus planes at any one pixel location, and attempts to predict the point of maximum focus more accurately than the no smoothing method.

The *Depth Map* is generated with fractional floating-point values which shows slopes within the sample more accurately.

The *Depth Map* image will be seen to have a 'smooth' appearance.

The *Montage* image is generated by interpolating between adjacent source images according to the fractional part of the depth map value.

Reflected Light with Smoothing gives good results with many types of specimen.



Depth Map image value 2.7.
Montage Image value calculated from relative proportions of source images (2) and (3).

Blended Variations Min, Med & Max:
Blended (Min): The best focus for each pixel is compared with its focus in the two immediately adjacent source images and an interpolation is performed between those three values. This is expected to give the best results for most cases

Blended (Med): The best focus for each pixel is biased towards whichever of the immediately adjacent source images has stronger focus in a ratio calculated using all three values. This tends to squeeze the *Depth Map* a bit more enthusiastically and should cope better when the sample has a continuous gradient but focus has been moved too far between successive source images.

Blended (Max): This is like *Med* above but then applies a gentle smoothing to the *Depth Map* only. This should probably be viewed as a last resort to pull out acceptable-looking results when the other two have failed.

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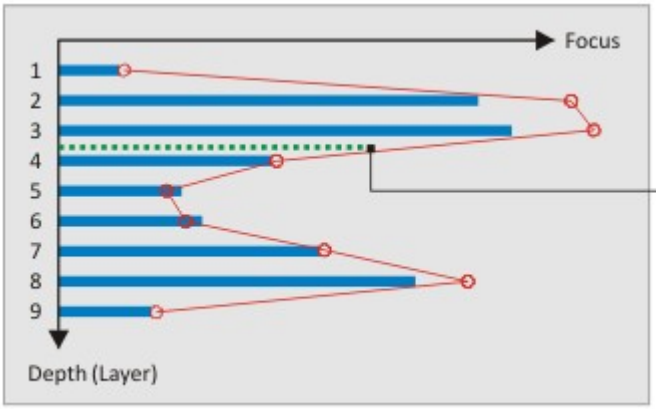
Transmitted Light: Method 1: Weighted:
Takes into account multiple in-focus planes at any one pixel location.

The *Depth Map* is generated with fractional floating-point values but may contain inaccurate depth values which do not correspond to an optimally-focussed source image.

The *Montage* image is generated by calculating relative proportions of all source images.

Transmitted Light: Method 1 depth is particularly effective on transparent biological samples, where more than one plane may contain focussed detail; The result is a combination of all focused planes, rather than a choice between them.

The result is expected to be a 'soft' focus not sharp.



Depth Map image value of 3.6 is not accurate.

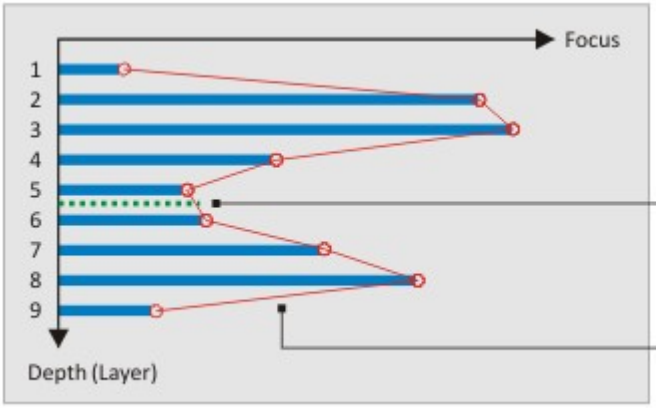
Montage Image calculated from relative proportions of all source images.

Transmitted Light: Method 2: Weighted Exponentially:
Takes into account multiple in-focus planes at any one pixel location, but biased more strongly towards the best focus.

The *Depth Map* is similar to that generated by the weighted depth algorithm, although it will usually be more accurate.

The resulting *Montage* image is generated by calculating relative proportions of all source images.

Particularly effective on biological samples with intrusive background.



Depth Map image value of 5.4 is not accurate.

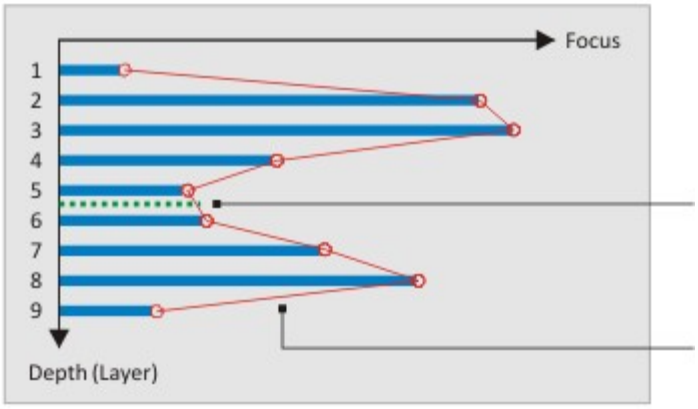
Montage Image calculated from relative proportions of all source images.

Transmitted Light: Method 3: Compound Weighted:
Takes into account multiple in-focus planes at any one pixel location, but biased more strongly towards the best focus.

The *Depth Map* is similar to that generated by the weighted depth algorithm, although it will usually be more accurate.

The resulting *Montage* image is generated by calculating relative proportions of all source images.

Particularly effective on biological samples with intrusive background.



Depth Map image value of 5.4 is not accurate.

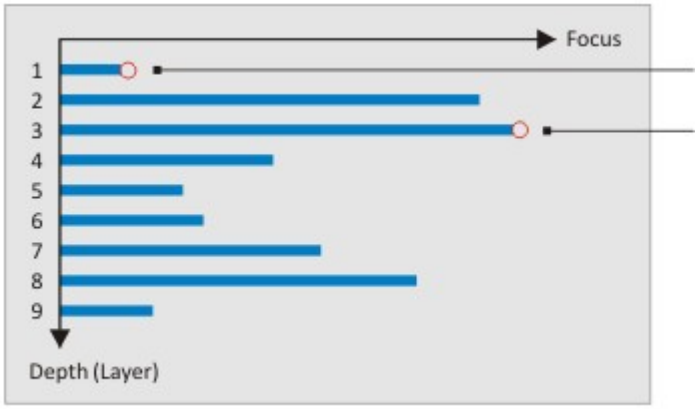
Montage Image calculated from relative proportions of all source images.

Fluorescence Min and Max Intensity: Projection:
A single pixel from each source image at the same X/Y locations is used as in all other cases.

Determine the maximum or minimum value that is found.

Place this value in the result image and repeat for all X/Y locations.

Often used on fluorescent images where light is being generated from various Z positions in the specimen.



Minimum

Maximum

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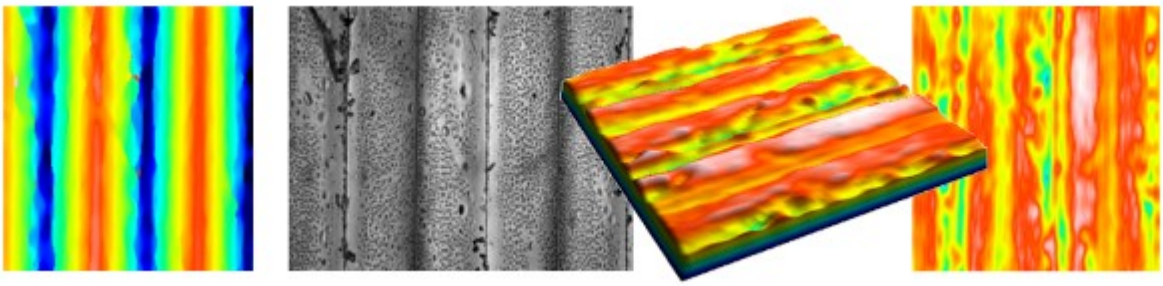
Leica Map is a powerful and sophisticated metrological application for analysing complex surface profiles and structures.

It works in conjunction with the *LAS Montage* module to produce comprehensive reports using a wide range of tools for both surface data manipulation and display. The reports can be customised to suite corporate or project styles and guidelines.

The working data is sourced from a *Montage* image sequence that has a processed montage image and depth map. This is the only data 'route' so *Montage* must be installed and licensed for *Leica Map* to work properly. The *Montage* stack must be captured using a well-maintained motorised microscope stand because the Z-positions must be accurate. *Refer to the Montage help: Go there...* 540

The following pages show how to export data to and launch *Leica Map,* and also provides a brief illustration of how it can be used. It has its own extensive help that can be launched from the *Main Menu* or used contextually by pressing key *F1.*

Leica Map is a very flexible and adaptable program but outcomes are always dependent upon the suitability of the original specimen and the users interpretation of the results.



Leica Map is a Windows application *not* an optional module that runs independently from LAS.and is installed from the LAS DVD. The dongle provided must be fitted to a USB slot on the computer whilst the program is running.

Leica Map can be used initially in demo mode with full functionality for a limited period. It is recommended that it is not installed until you are ready to start an evaluation.

However after the demo period expires, a license must be purchased for continued operation. A Leica Map dongle (separate from the LAS dongle) will be .supplied to enable the license. It is recommended that the Leica Map dongle is attached to a USB port on the computer before it is booted to make sure of validation before *Leica Map* is launched.

Leica Map can be launched directly from the desktop icon once installed for use with images directly from the computer hard drive.

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Leica Map must be configured to accept the Montage file formats and layer structure.

To select the file format:

1: Click on *Options* on the main menu and...

2: ...from the drop-down menu click to select *General Preferences*. Alternatively, press *F7* on the keyboard.

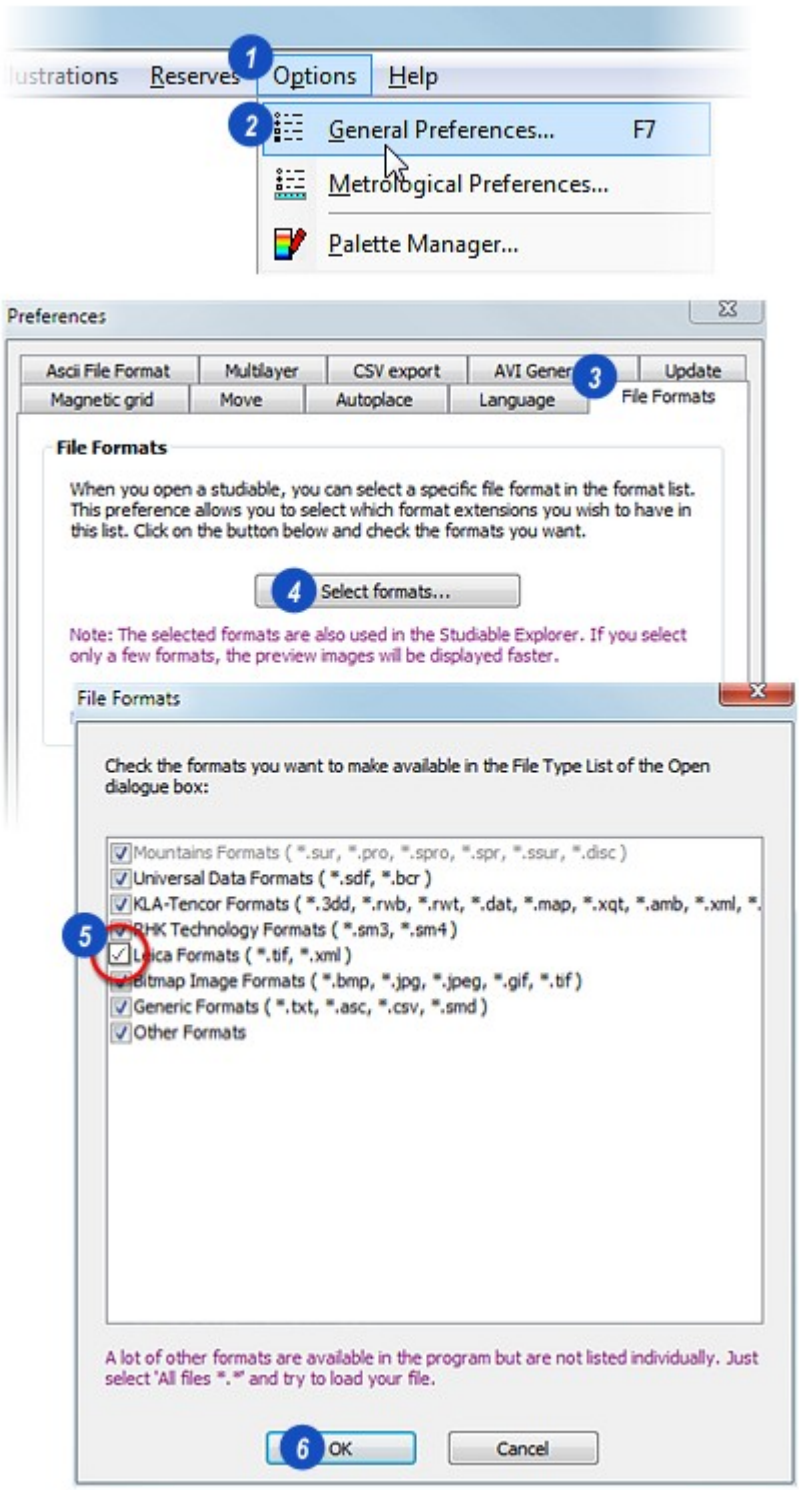
3: On the *Preferences* panel, click the *File Formats* tab and...

4: ...the *Select Formats* button.

5: The list of file formats appears. Scroll down the list until the Leica Formats entry appears and click to enable the check box.

6: Click *OK*.

Continued... 574



Select the Layer type as follows:

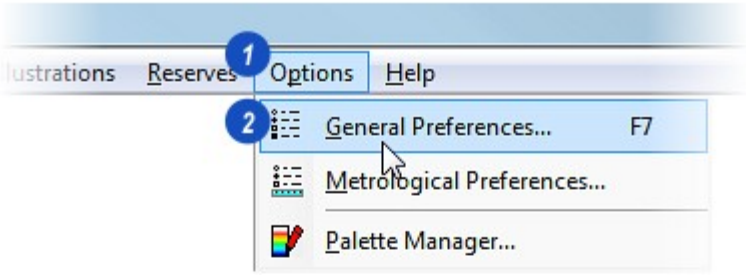
1: Click on *Options* on the main menu and...

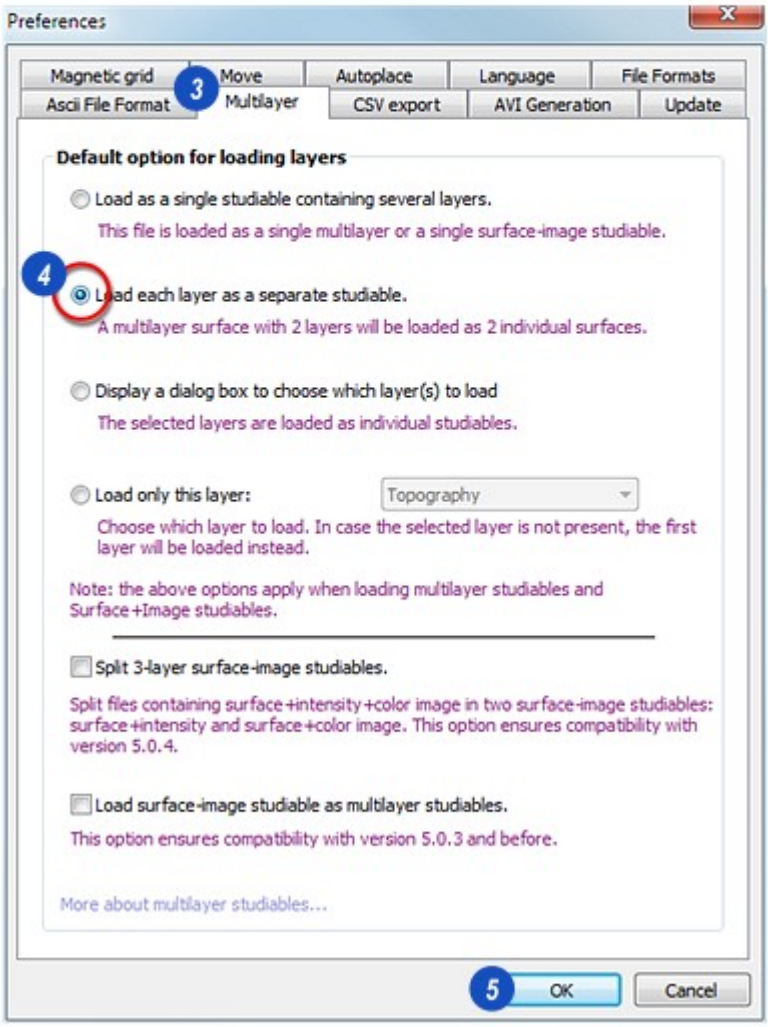
2: ...from the drop-down menu click to select *General Preferences*. Alternatively, press *F7* on the keyboard.

3: On the *Preferences* panel click the *Multilayer* tab.

4: Click to select the *Load each layer as a separate studiable* radio button.

5: Click *OK*.





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Users that have just captured a Z-Stack sequence in *Acquire* and have automatically come to the *Browse Workflow* because of the *'After Capture'* settings in *Preferences*, can skip this page.

For a previously captured Z-Stack sequence:

1: The *Montage* module must be active. If it is not click on the *Selector* button and...

2: ...from the *Acquisition Mode* list click to select *Montage*.

3: Click on the *Browse Workflow* and...

4: ...on the *Browse* tab.

5: In the *Navigator* locate the folder containing the *Montage Z-Stack* sequence and open it.

6: In the Z-Stack sequence there must be a *Depth Map* and...

7: ...a *Montage* extended focus image.

Continued...

To launch the *Leica Map* application from *Leica Application Suite* with *Montage* active:

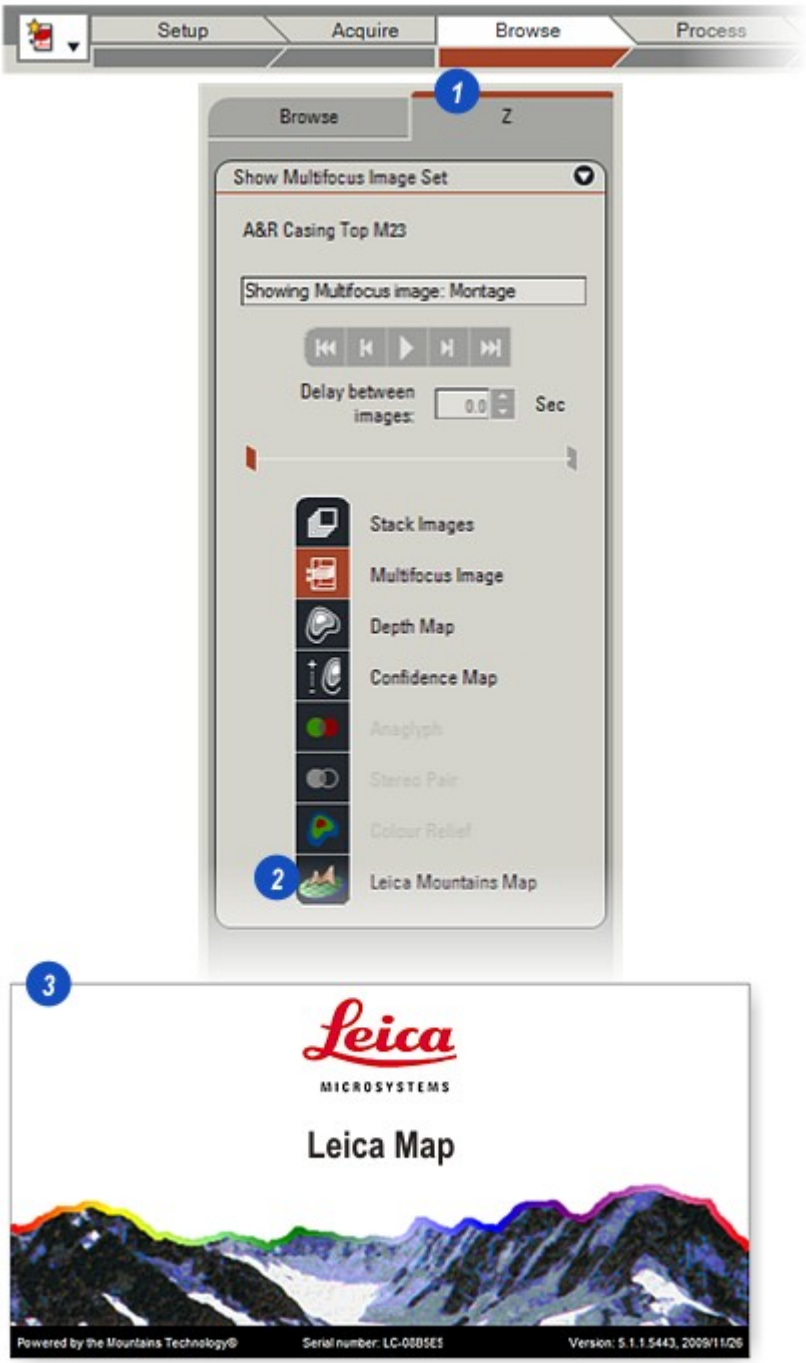
1: Still in the *Browse Workflow*, click on the *Z* tab.

2: Click on the *Leica Map* button. This will only be available if Leica Map is installed and the dongle is present and validated.

3: *Leica* Map is launched and the flash screen appears.

If *Leica Map* is already active it is detected and the *Montage* data transferred to it automatically.

Continued...



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Before image analysis begins, the user has the template option:

- Use an existing template - either the default provided with Leica or one created by the user, or...
- A basic report which can have specific analyses added to suit the project.

Using a Template:

1: To use a template for the report, click to enable the *Template* check box - a tick mark appears.

2: Select a template by clicking the *browse* button and...

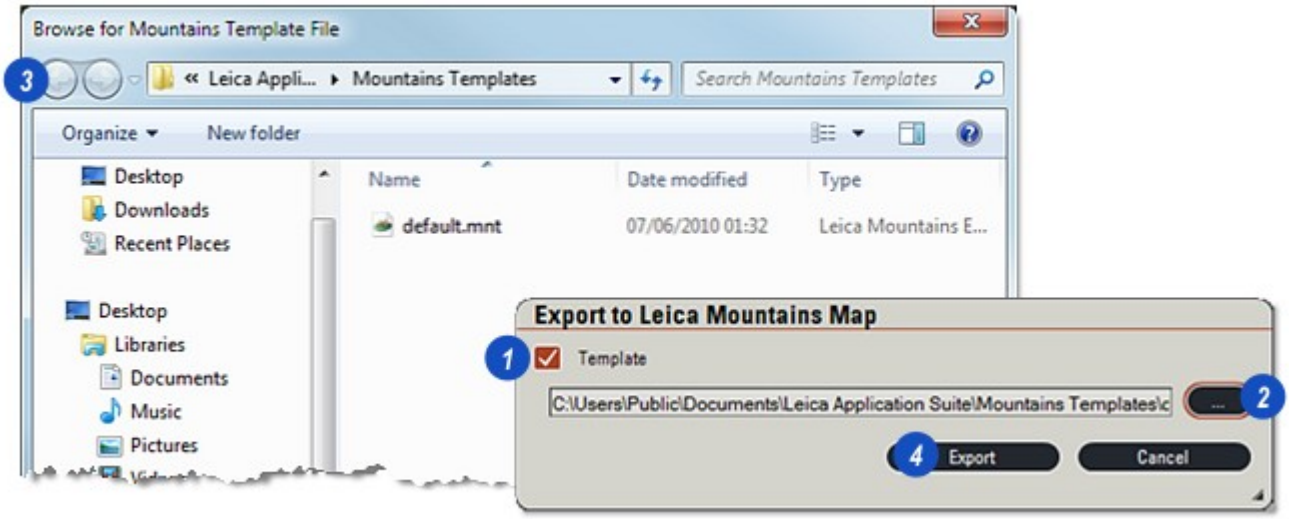
3: ...navigating to the folder in which the templates are stored. The location for the default template is:

C:\Users\Public\Documents\Leica Application Suite\Leica Map Templates\default.mnt.

4: Click the *Export* button.

Any previously saved *Leica Map* report with the *.mnt* extension stored in any location can be used as a template. However, but some *Montage* images may not be appropriate to the *Operators* and *Studies* used in the template and in this case *Leica Map* will automatically close.

Template examples are shown on the following pages.



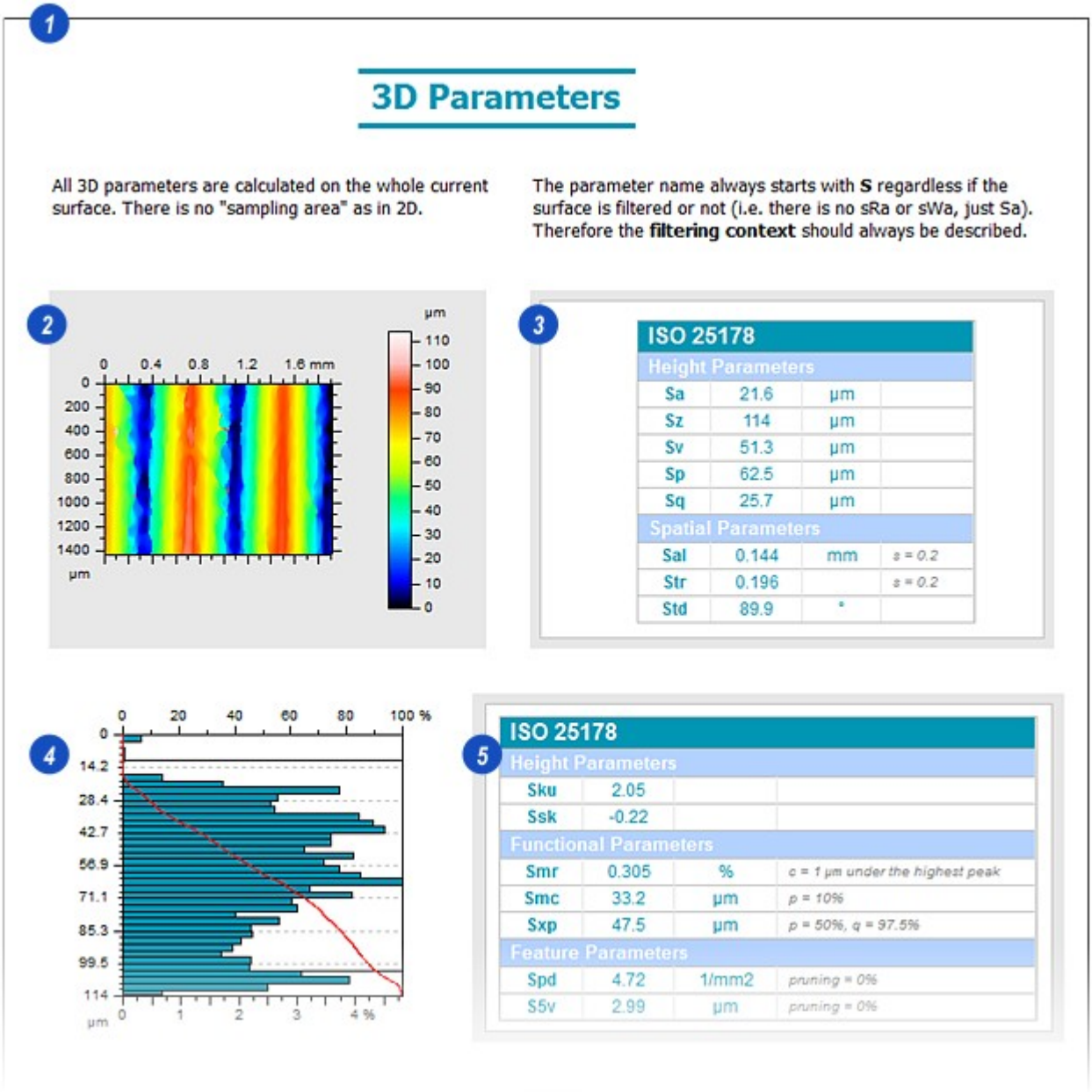
The default *3D Parameters* template **(1)** creates a report that contains much of the data prescribed in the *ISO 25178 Surface Texture Standards.*

A *Topography* pseudo colour image **(2)** is accompanied by a *Height Parameters* table **(3)** containing data such as *Mean Height of the Surface, Maximum Height of the Peaks, Valleys and the entire Surface,* and the *Root Mean Square Height. Spatial Parameters* include *Texture Aspect Ratio* and *Texture Direction.*

The *Abbot-Firestone Curve* **(4)** is represented as well as a table **(5)** showing *Height Parameters (*such as *Kurtosis Height distribution* and *Skewness), Functional Parameters* (*Surface Bearing* and *Extreme Height*) and *Features* (*Peak Density* etc) derived from segmentation.

Also included but not shown on the illustration, are *Waviness* and *Roughness* pseudo colour images.

Pages can be added to the report together with a wide range of specific data and images. *Operators and Studies: Go there...* 580



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1: If the *Template* option is cleared the browse button is no longer available and a basic report is created.

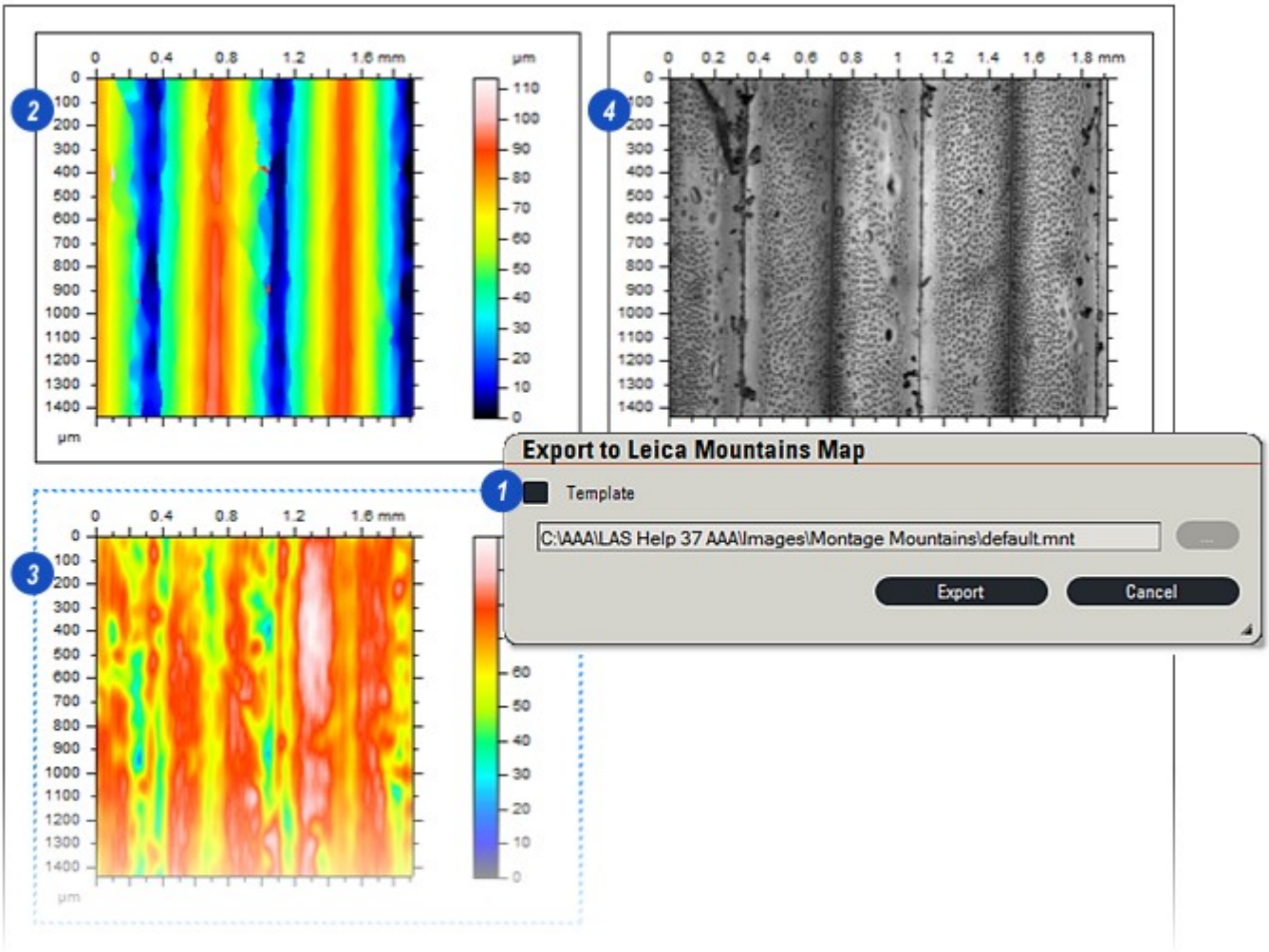
2: Click *Export* and only essential images such as the *Topography*...

3: ...and *Intensity* layers are included on an otherwise blank report sheet.

4: The report example shown here also includes the original *Montage* image.

The user can then add tables, analyses and images - called *Studiables* - appropriate to the project using *Operators and Studies:* 580 *Go there...* 580

More pages can be added and the various components moved around with borders, text, captions, company logos, names and report headers added as required. The resulting document can then be used as a template for other, similar projects.

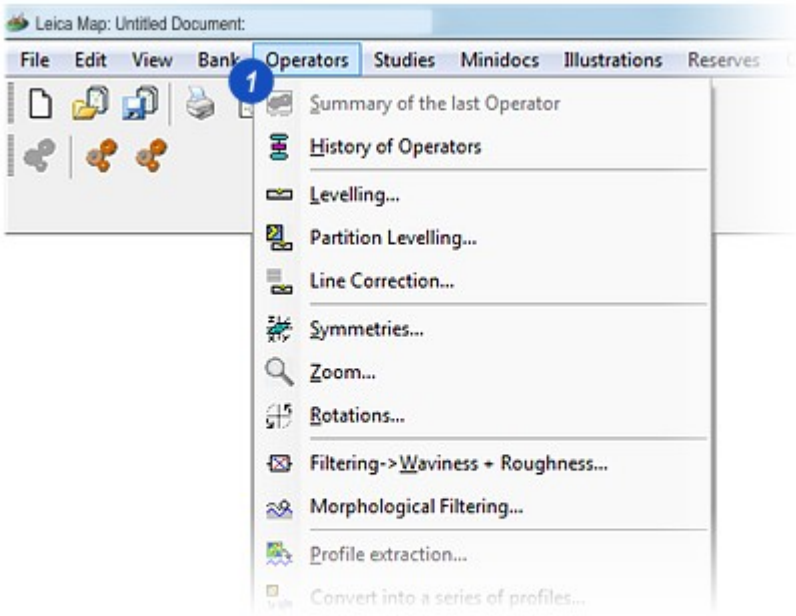


Leica Map performs surface analysis by applying *Studies* and *Operators* to the data being analysed (known as a Studiable), in this case *Depth Map* and *Montage Images*.

Studies are graphical representations of a studiable such as a 3D view or an analysis performed on a studiable such as a step height measurement.

Operators are mathematical transformations used to modify a studiable by adjusting settings in an associated dialog box. Examples would be to level a surface or to extract the waviness by filtering.

Many *Operators* are interactive providing controls to adjust for example, filter structures, thresholds and edge detection.

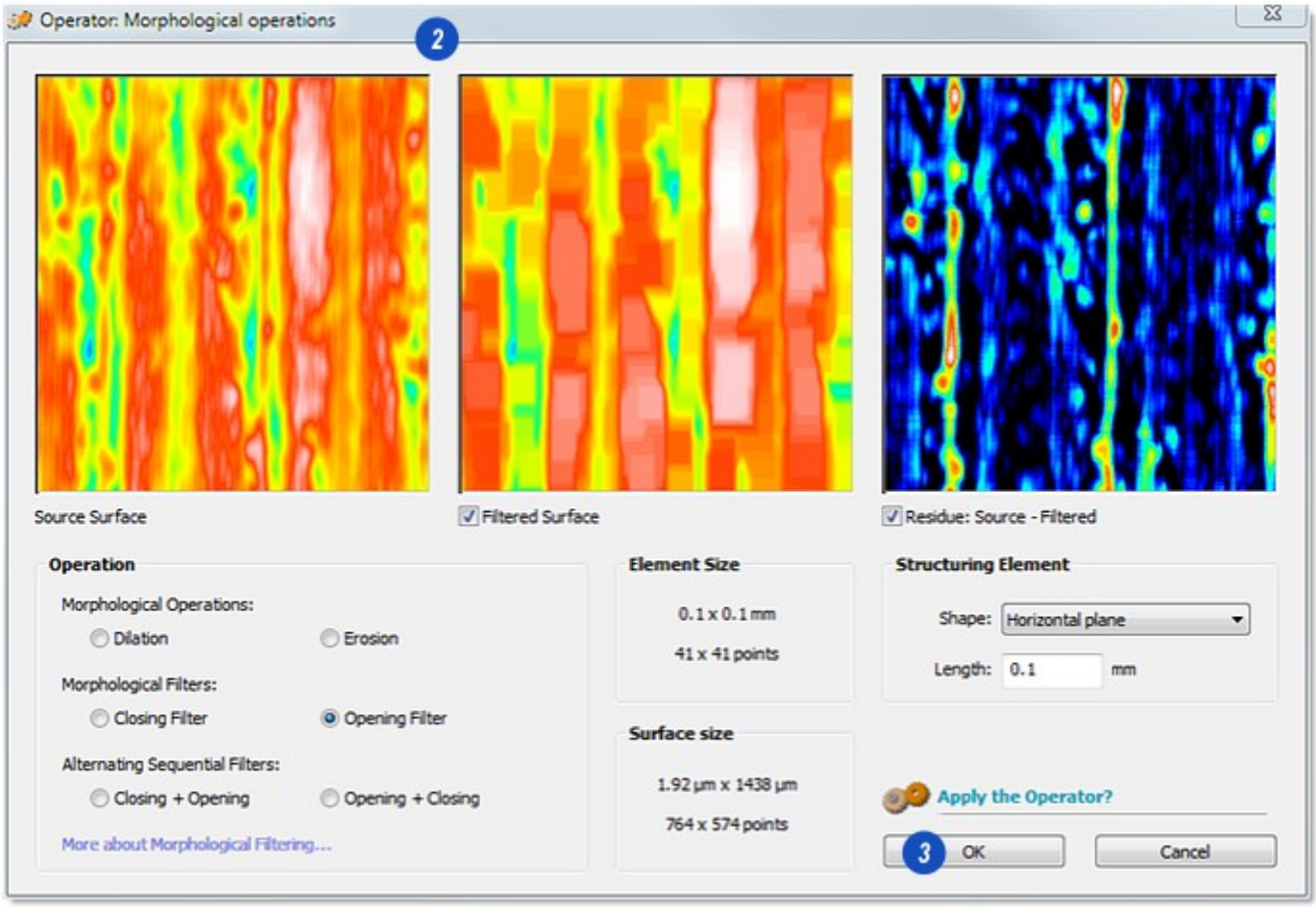


1: With an image on the report selected, on the *Main Menu* click the *Operators* option.

2: The *Operator* dialog appears. The controls and output displays differ with the *Operator* chosen. Change the parameters as needed and...

3: ...to include the results in the report click the *OK* button. Clicking the *Cancel* button will close the dialog and not include the results in the report.

Continued...



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Studies provide different methods for representing the surface data.

1: On th *Main Menu* click the *Studies* option.

2: From the drop-down, click to select the required study type.

3: If necessary, a new page is added automatically to the report and the study graphic displayed. *3D View* and *Distance Measurement* are illustrated.

Some studies have built-in interactivity. For example, on a corner of the *3D View* click and hold down the mouse button whilst moving the mouse will rotate and tilt the image. Similarly, the dotted lines on the *Distance Measurement* can be dragged to measure a specific distance or angle.

4: Right-click a study to reveal and change its parameters.

5: Display features such as axis colours and graduation, image tinting and slicing for both *Operators* and *Studies* can be changed using the mini-toolbars available when an image is selected.

An additional set of tools can change border colour and thickness and alter the image sequence if they are overlapping.

Illustrations and *Reserves*, both available from the *Main Menu* can be used to add captions, images and and a range of graphical features to enhance and customise a report.

Leica Map has a dedicated help file accessed through the help option on the *Main Menu* or contextually by pressing the *F1* key when the program is running.

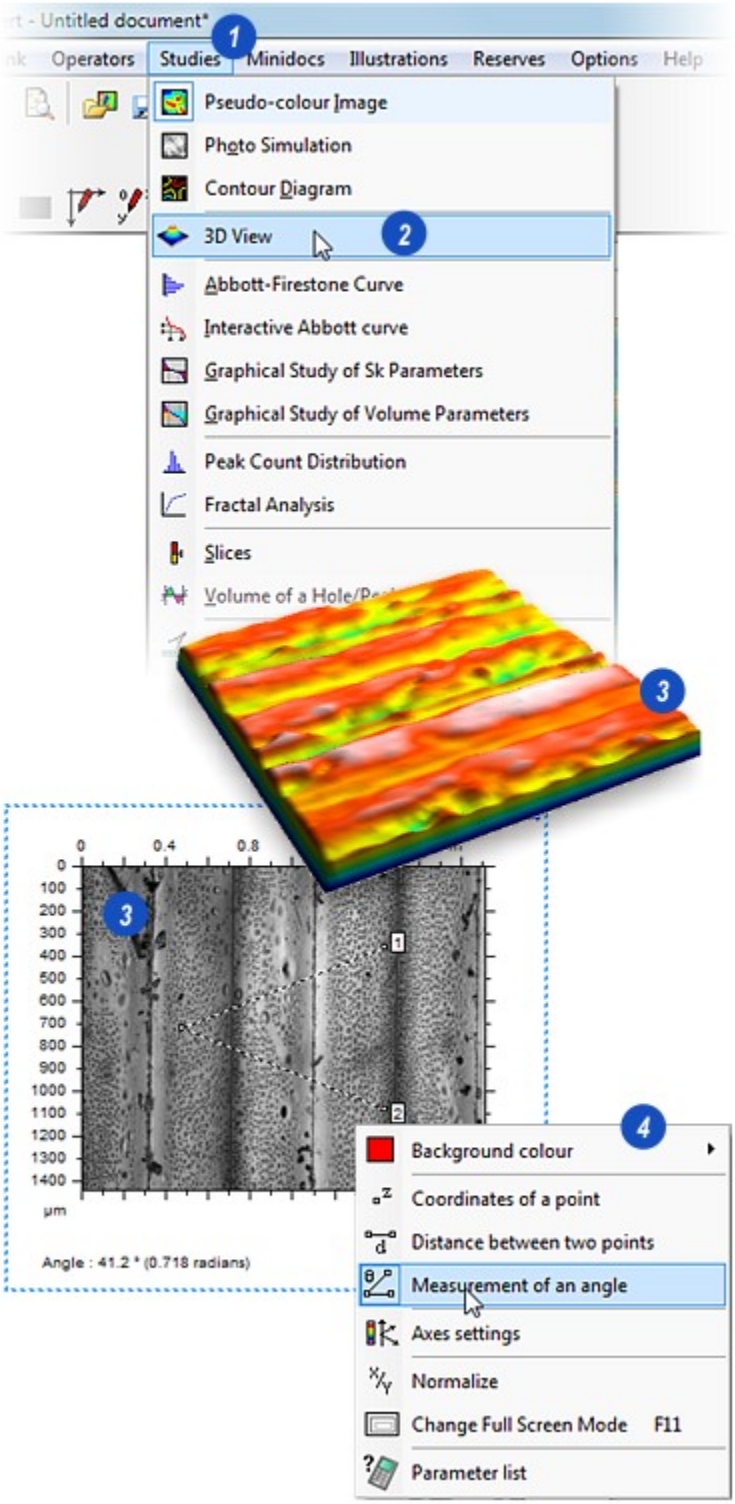
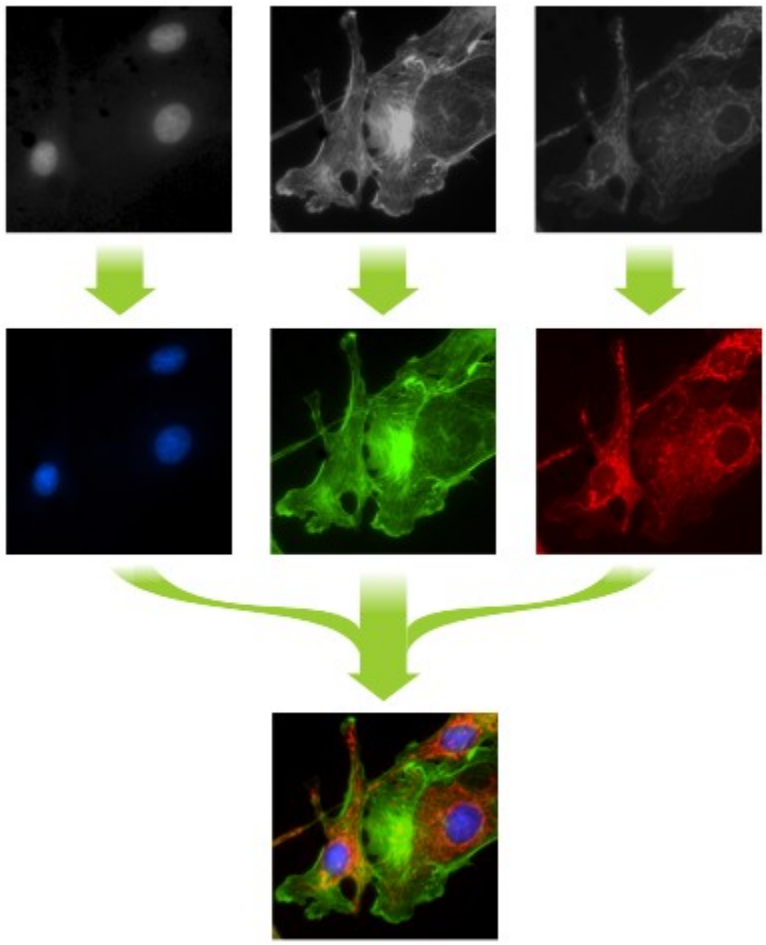


Image Overlay

Image Overlay is an LAS module that enables the acquisition of Channel images with a LAS compatible microscope and the creation of a composite image from a sequence of these images. The Channels are typically Fluorescent channels but can also be any other available contrast method.



Sequences of channels may be defined and for each channel the microscope and camera settings set and saved. Once a sequence is acquired a coloured overlay image may be made using a variety of methods.

If using a colour camera the *Image Type* should be set to greyscale.

See: Acquire: Camera: Image Formats: 239

Fully motorised microscopes will automatically select filters, but users of manual machines may still use Image Overlay simply by physically turning the filter turret when prompted.

Between 2 and 8 images are captured during an Overlay sequence. The subject and magnification are the same for all, but individual images use a different filter and exposure to optimise contrast for a specific part of the image.

Additional enhancement and ‘separation’ is achieved with pseudo colour – a digital staining technique – all controlled within Leica Application Suite.

The final step is to bring all of the images together in a single combined overlay in which individual parts can still be easily identified to illustrate their place in the ‘whole’.

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The *Image Overlay* module must be installed and enabled; If it is not the icon will not appear on the *Acquisition* menu **(2).**

To start Image Overlay:

1: Click on the *Select Acquisition* icon and from the menu…

2: Click to select *Image Overlay*. After it is selected an additional tab marked with the Lambda (**λ**) symbol **(3)** appears in both the *Browse* and *Acquire Workflows*.

1: Select the *Acquire W*orkflow by clicking on its tab.

2: Click on the *Image Overlay* tab marked with the Lambda (λ) symbol to reveal the Channel Setup panel comprising…

3: The *Setup Tools* and…

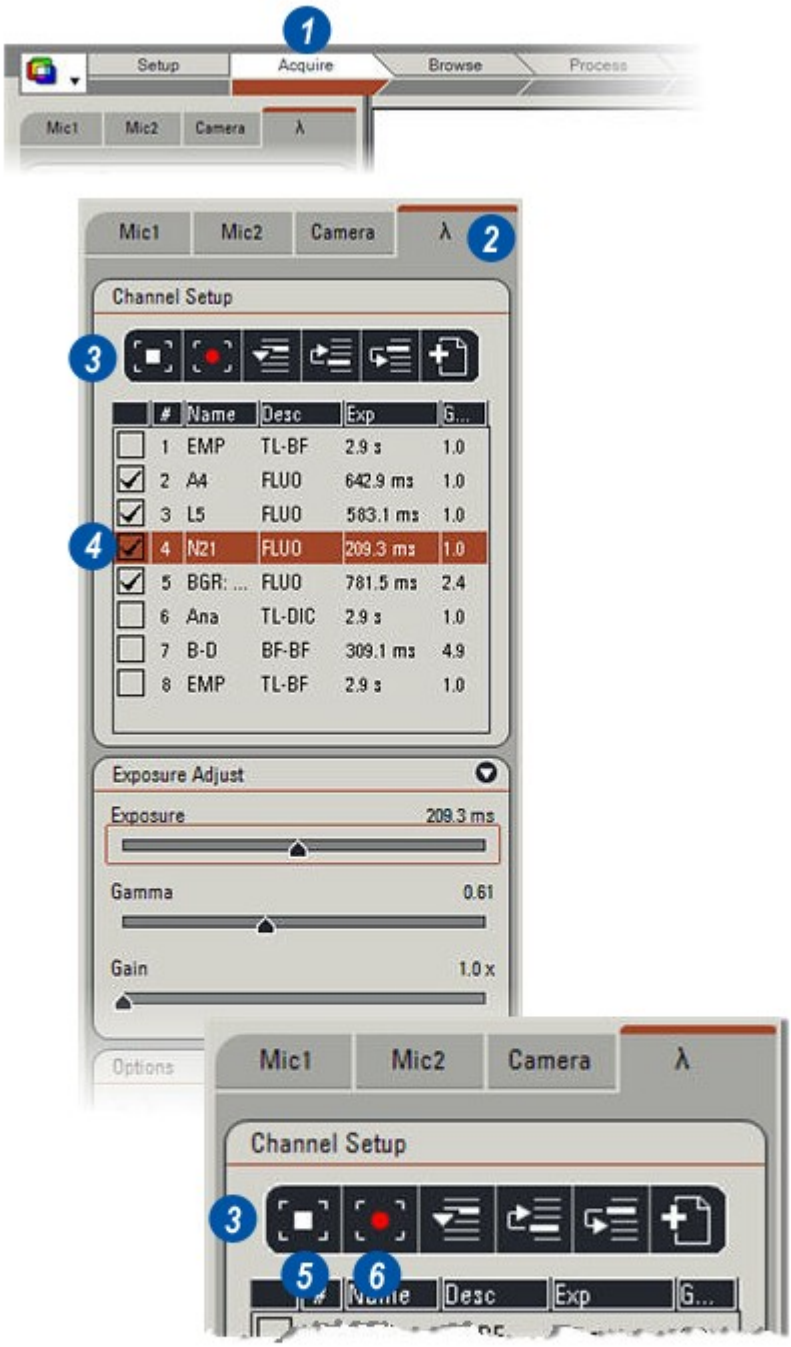
4: The *Channel Dialog.*

Using the Setup Tools:

5: *Camera Live* 'freeze' halts the camera activity leaving the latest image on the Viewer. Click again to resume taking live images. Use the *freeze* button in combination with…

6: *Capture Single Image* which will save the current image to the capture folder. The filename is created automatically and a thumbnail of the image is displayed in the Working Gallery.

Continued... 585
See: Channel Dialog 587



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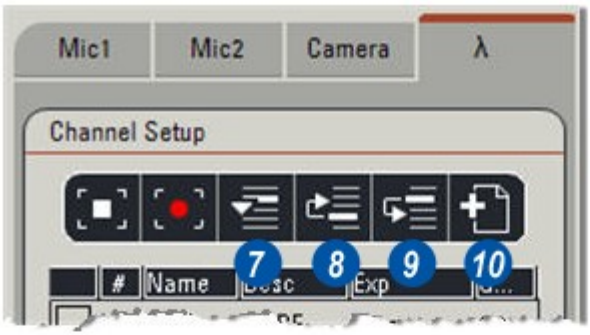
7: The *Next Filter* button moves the focus of interest to the next available filter on the Channel dialog. The focus 'rolls over' from bottom to top automatically.

8: Click the *Move Up* button to move the selected channel up in the filter sequence.

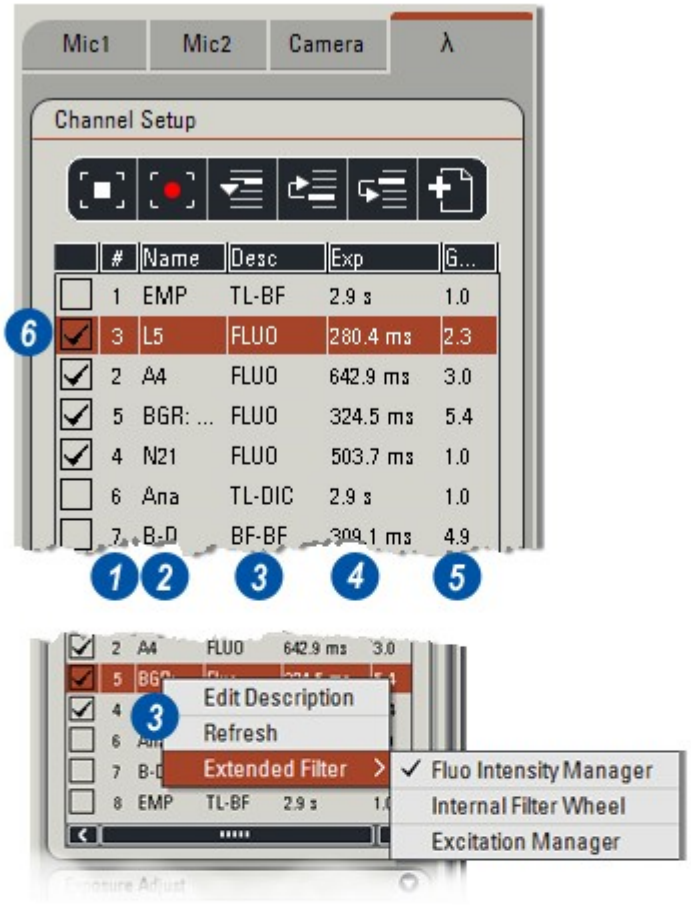
9: Click the *Move Down* button to move the selected channel down in the filter sequence.

10: To save the channel dialog sequence, click the *Create New Sequence* button.



See: Channel Dialog 587

The Dialog is a list of the filters available on the microscope with details of:



1: The filter position on the turret. Initially, this will also represent the image capture sequence in automatic mode but may be changed.

2: The filter name.

3: The contrast method. Right click on the entry to reveal a menu with options based upon the filter type:
Edit Description to change the description:
Refresh to update the filter information:
Reset Pseudo Colour reverts to the filter's default value. This is only available on monochrome cameras.
Extended Filter reveals an additional menu to determine how the filter works with the internal filter wheel.

4: Image exposure time. Initially, this value will be the same for all filters but may be changed individually to achieve the best results.

5: The Gain value. Again, these will be set to the same common value but may be altered for each filter.

6: To the left of each filter entry is a check box. When this is enabled (ticked) the filter will be actively used in the sequence and, with its associated image becomes a Channel.

Continued 586...

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Select a Channel by:

7: Clicking anywhere on a filter entry.

8: Clicking to enable the checkbox. At least two and up to the total filters available may be chosen for a complete Channel sequence.

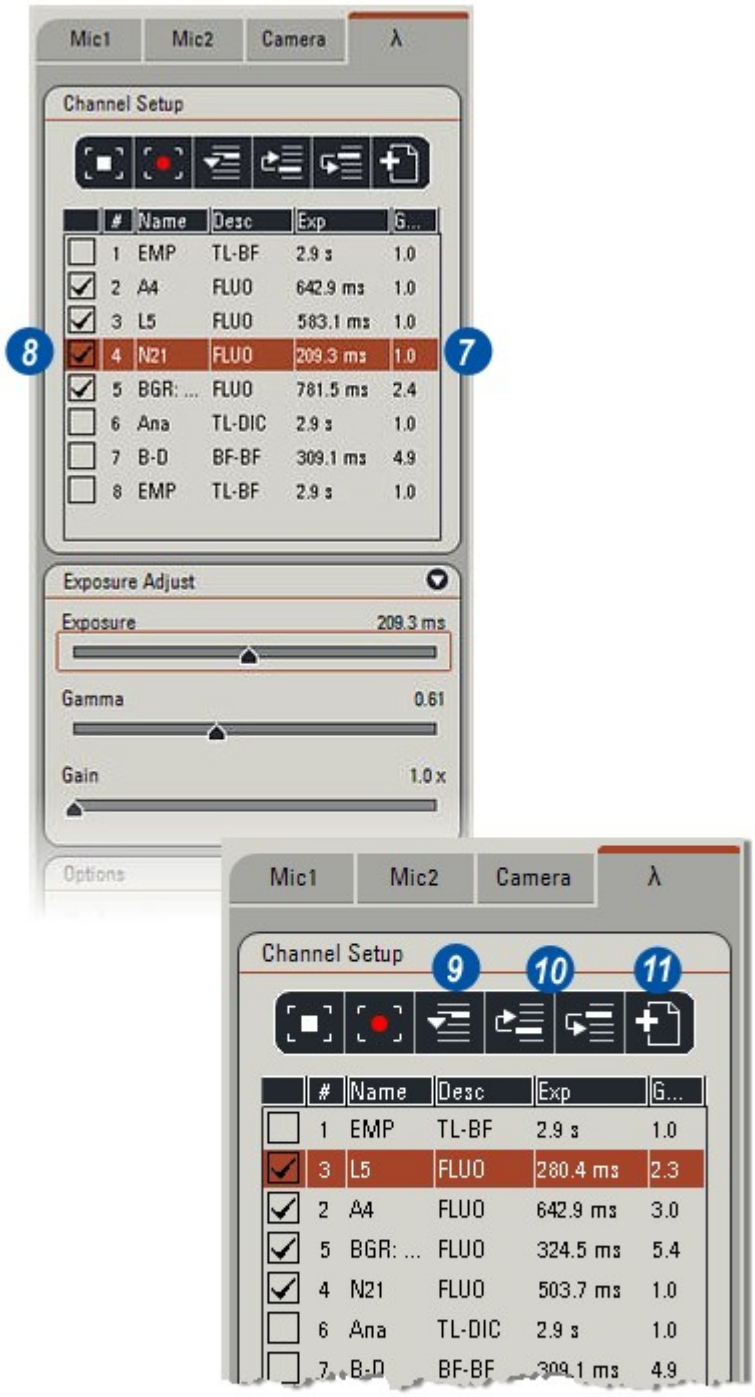
Changing sequence order:

9: Select an enabled Channel by clicking the *Next Filter* button.

10: Click either the *Move Up* or *Move Down* buttons to change the order in which images will be captured.

Saving a Channel sequence:
When all of the required filters have been selected:

11: Click on the *Create new sequence* button to save the Channel sequence.



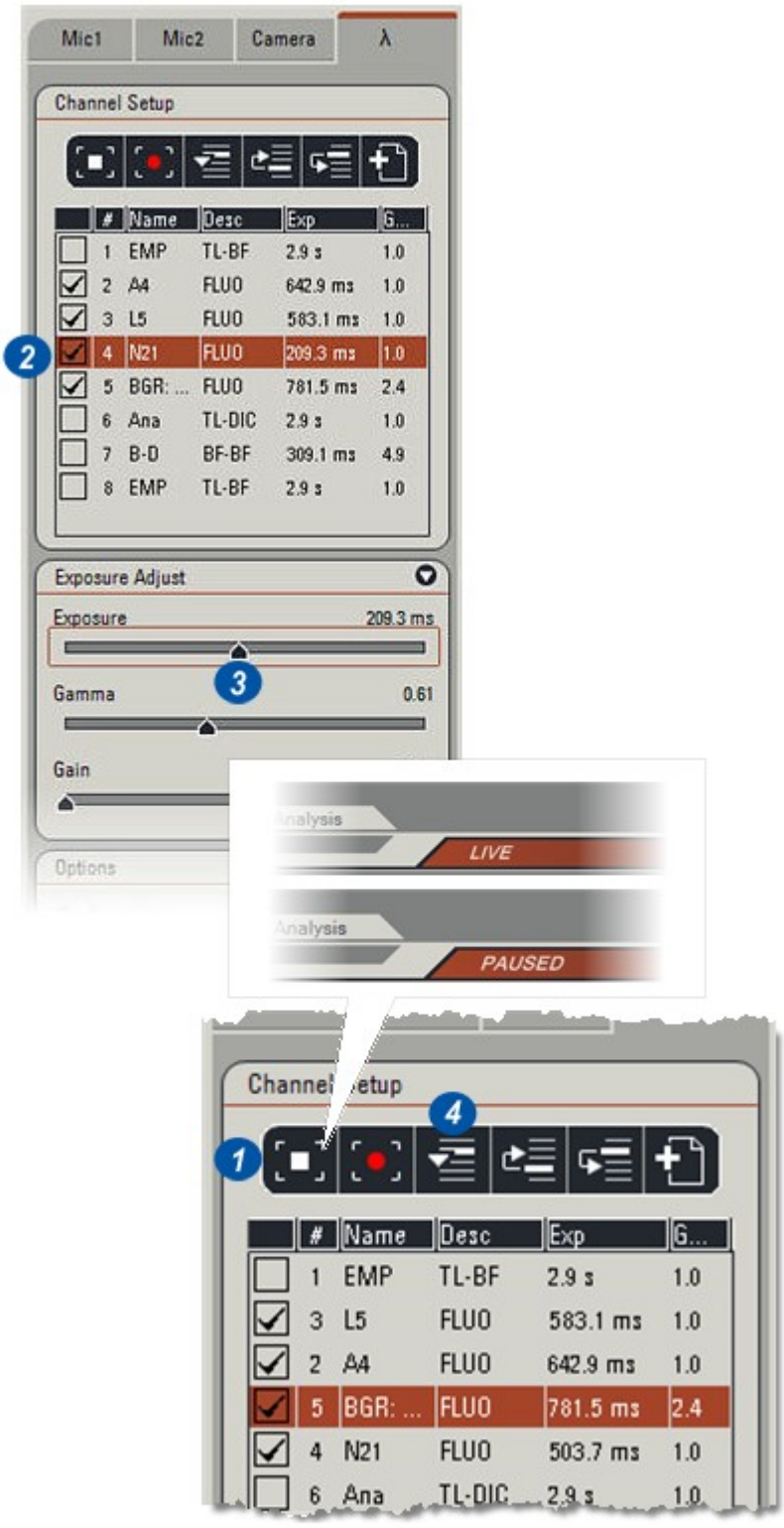
Each Channel may have *Exposure, Gamma* and *Gain* values set individually to achieve the best image.

1: Check that the *Camera* is in live mode. The message on the top bar of the *Viewer* indicates the camera state. If it reads *Paused* then click the *freeze* button to return to live. The button icon should have a small square in the centre, not an arrow.

2: Click on the *Next Filter* button to move to the Channel required. Motorised microscopes will automatically turn the filter turret to the correct position, but non-motorised machines will have to be set manually. The filtered image will appear in the *Viewer*.

3: If necessary, alter the *Exposure* time by clicking and holding the *Exposure* slider and drag it left, to decrease exposure, or right to increase it.

Continued 589...



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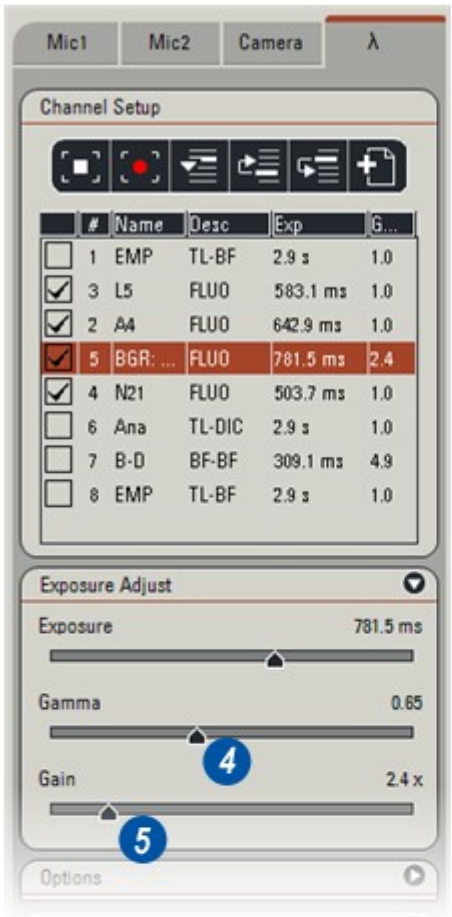
4: The *Gamma* value is normally set to best suit the viewing medium – in this case a colour monitor. Small changes can have a dramatic effect on colour density but in some cases can help to improve contrast. Click and hold the *Gamma* slider and drag left to reduce the *Gamma* value or right to increase it.

5: *Gain* will brighten or darken the image without affecting the *Exposure* time. Try to keep *Gain* to low values to avoid introducing 'noise' into the images. Click and hold the *Gain* slider and drag it left to darken the image or right to lighten it.



The *Capture Single Image* function provides a simple ‘ snapshot’ method of checking images from individual Channels after making changes to the Exposure settings.

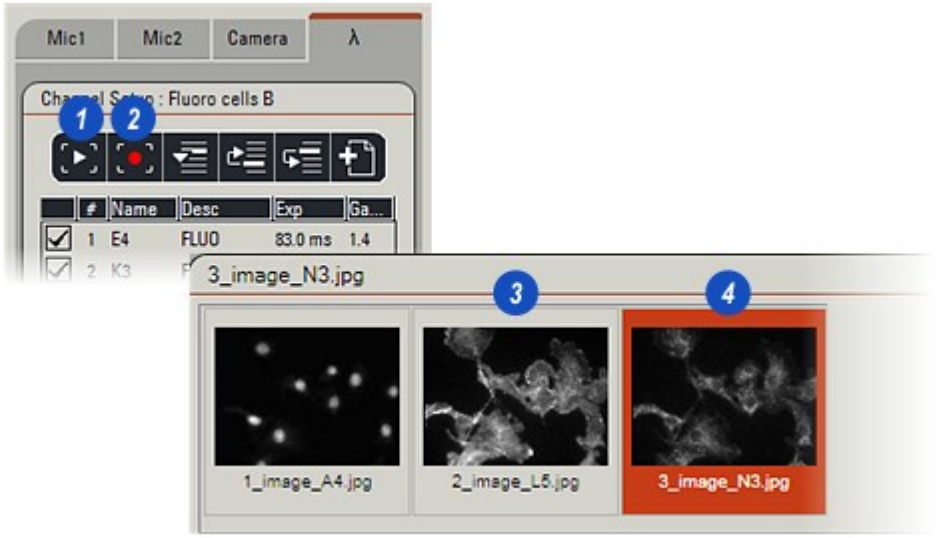
If necessary, click on the *Channel* to snap. Wait for the *Viewer* to settle and then:

1: Click on the *Camera Live* button to halt the *Camera*. Check the *Viewer* top bar: The message should read *Paused.*

2: Click on the *Capture Single Image* button.

3: The 'snapped' image appears in the *Gallery*.

4: Remove snapshots from the *Gallery* by clicking on the thumbnail to select it and then pressing the keyboard *Delete* key.



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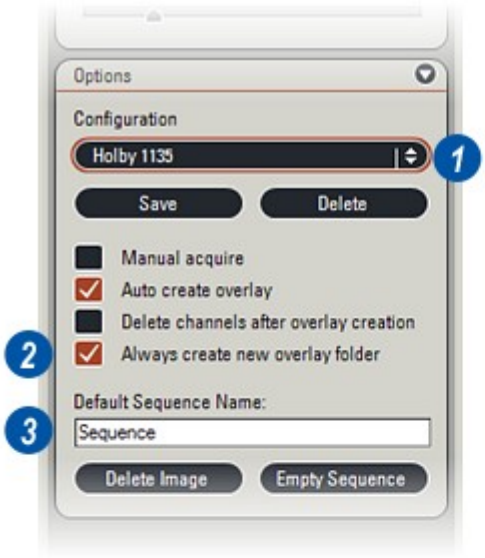
Changes made to any of the Channel settings are immediately reflected on the live image in the Viewer and also appear in the appropriate columns in the Channel dialog.

Always check that the correct filters are in the specified positions on the filter turret – some microscopes can do this automatically, but most require a manual check.

Final settings are made on the Options panel:



1: Click on the arrows to the right of the *Options* bar to reveal the panel.

2: If the *Always create new overlay folder* check box is enabled, every time an Overlay sequence is acquired a new folder within the current capture archive will be created. Situations, in which a substantial throughput is expected, will benefit from this time-saving option.

3: If *Auto create overlay* is selected, the text in the *Default Sequence Name* is used as part of the folder name.

Continued 592*...*

4: The folder name comprises the *Default Sequence Name* and a sequential number. Creation and naming are fully automatic.

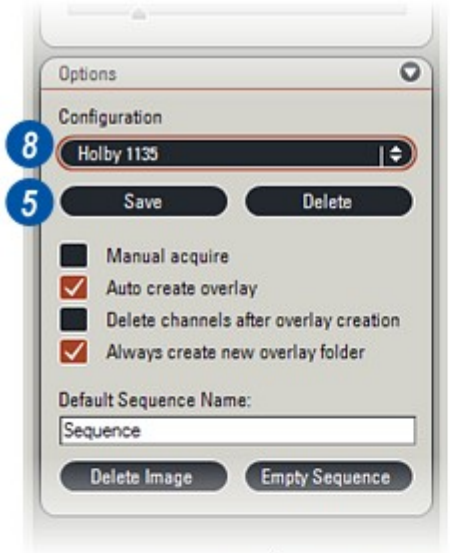
5: Save the configuration, including the Channel sequence by clicking on the *Save* button and…

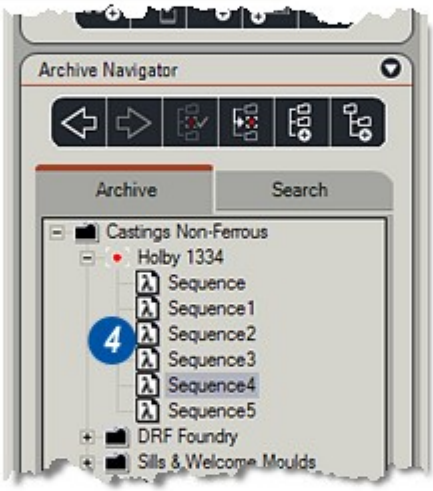
6: Entering an appropriate name for the configuration and…

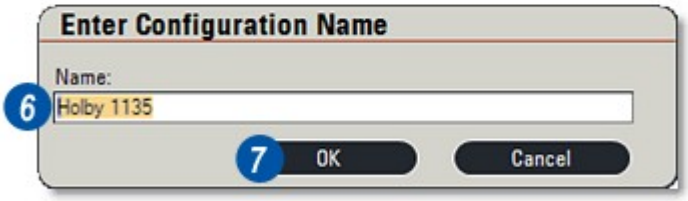
7: Clicking the *OK* button.

8: The new name appears in the *Configuration* window and the settings may be used again for future overlays.

See: Selecting an existing Configuration. 596







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For speed and efficiency, select:

1: *Auto create overlay* by clicking the check box and…

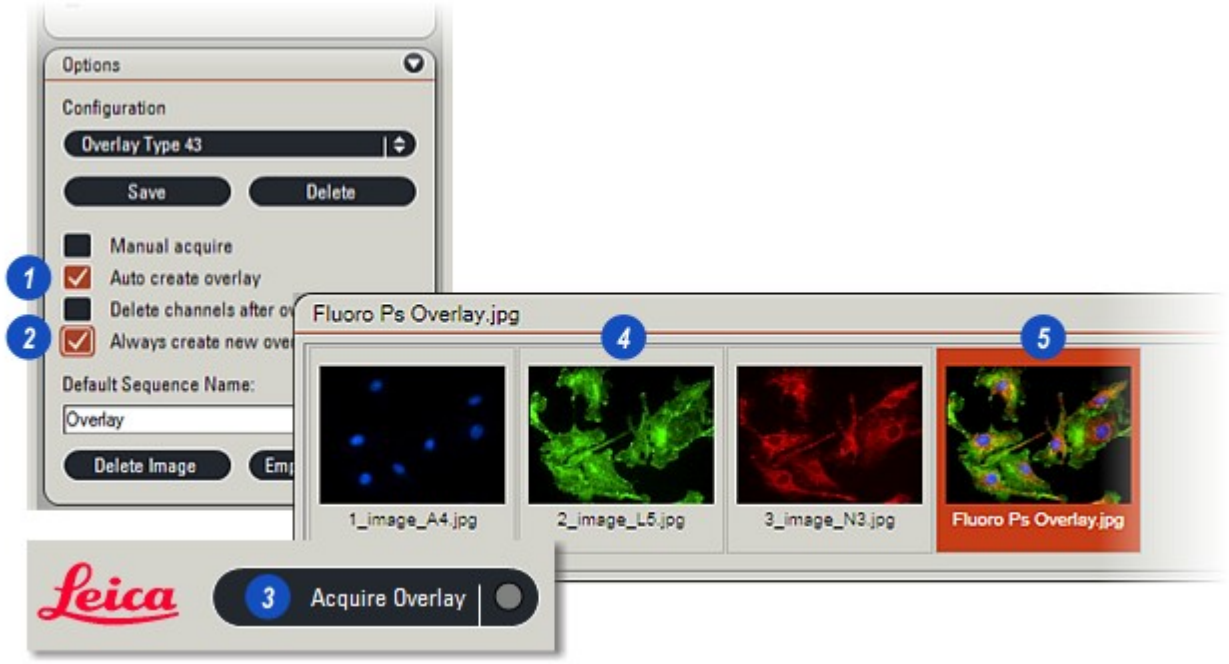
2: Click on the *Always create new overlay folder* check box.

3: Click on the *Acquire Overlay* button.

These options will create a new folder within the capture folder with the *Default Sequence Name* as part of its name. The images for each of the filter channels **(4)** will be saved inside the folder and they will be automatically combined to produce the Overlay **(5).**

Adjusting Channels before creating:

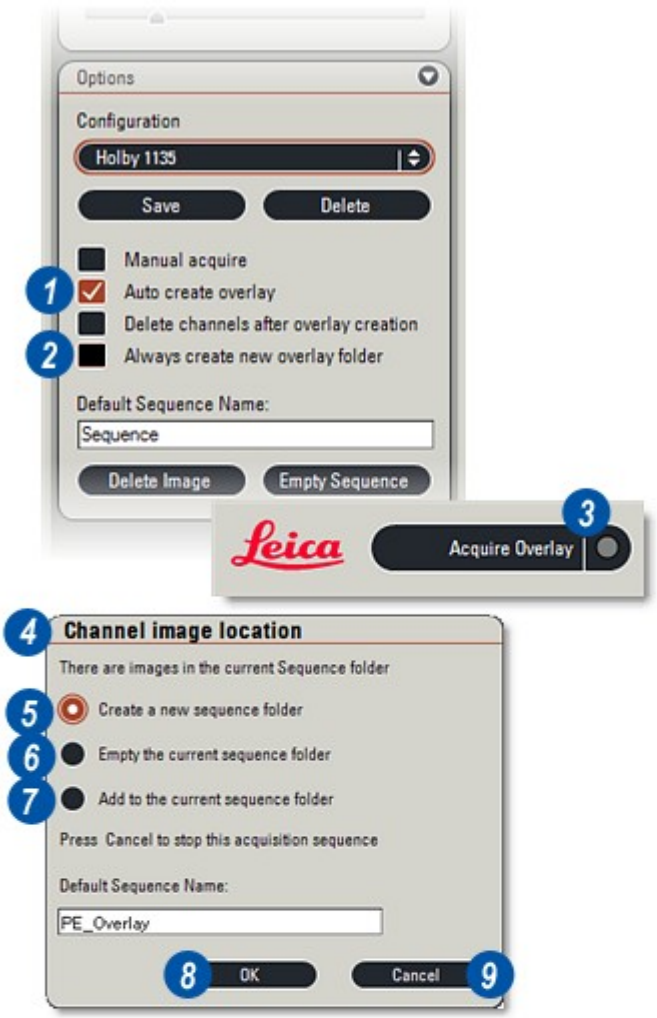
1: Uncheck the *Auto* create overlay check box for a preview before committing. Each of the images may then be examined and adjusted for colour balance and brightness before creating the overlay.



1: Click on the *Auto create overlay* check box to enable it.

2: Disable *Always create new overlay folder* by clicking the check box until the tick symbol disappears.

3: Click on the *Acquire Overlay* button. The Channel image location dialog appears (**4).**



There are 3 options on the dialog:

Create a new sequence folder **(5)** will create a new folder within the capture folder and save the images to it – the equivalent of checking the *Always create new overlay folder* check box.

Empty the current sequence folder **(6)** will delete all of the images inside the current folder and replace them with the new acquisition.

Add to the current sequence folder **(7)** is a powerful option that allows any number of channel images and subsequent overlays to be stored in the same folder. Each acquisition is given its own sequential number to differentiate between the sets**.**

Select the required option by clicking the button next to it and clicking on the *OK* button **(8).**

9: The entire acquisition sequence may be stopped by clicking the *Cancel*

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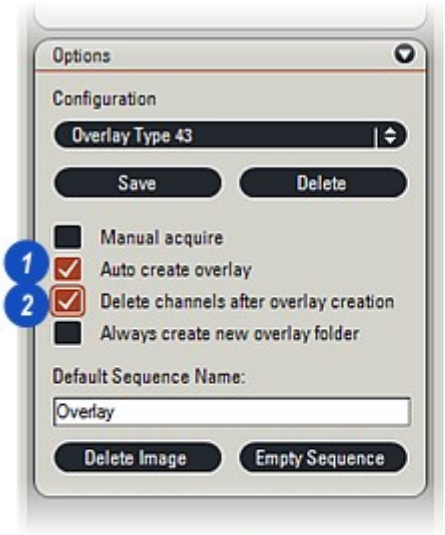
Enabling the *Delete channels after overlay creation* option, removes the individual Channel images after the overlay has been created automatically. This is an excellent ‘ housekeeping’ arrangement for very high throughput where there is no requirement to add pseudo colour to the images.

1: Check the *Auto create overlay* check box and…

2: ...the *Delete channels after Overlay creation* check box.

3: Click on the *Acquire Overlay* button. The Channel images are deleted after the overlay is created and saved in the current *Capture Folder* – not in a newly created folder or the last newly created folder.



Previously saved configurations stored in the current capture folder can be re-called by clicking on the arrows to the right of the *Configuration* window **(1)** and then choosing from the list **(2)**. Changes may then be made to the exposures and to the Options with the altered settings saved as a new configuration **(3).**

Manual Acquire:
Primarily designed for non-motorised microscopes, the *Manual Acquire* facility prompts for the filter turret to be moved manually to the correct position before the image is captured.

4: Click to enable the *Manual acquire* option.

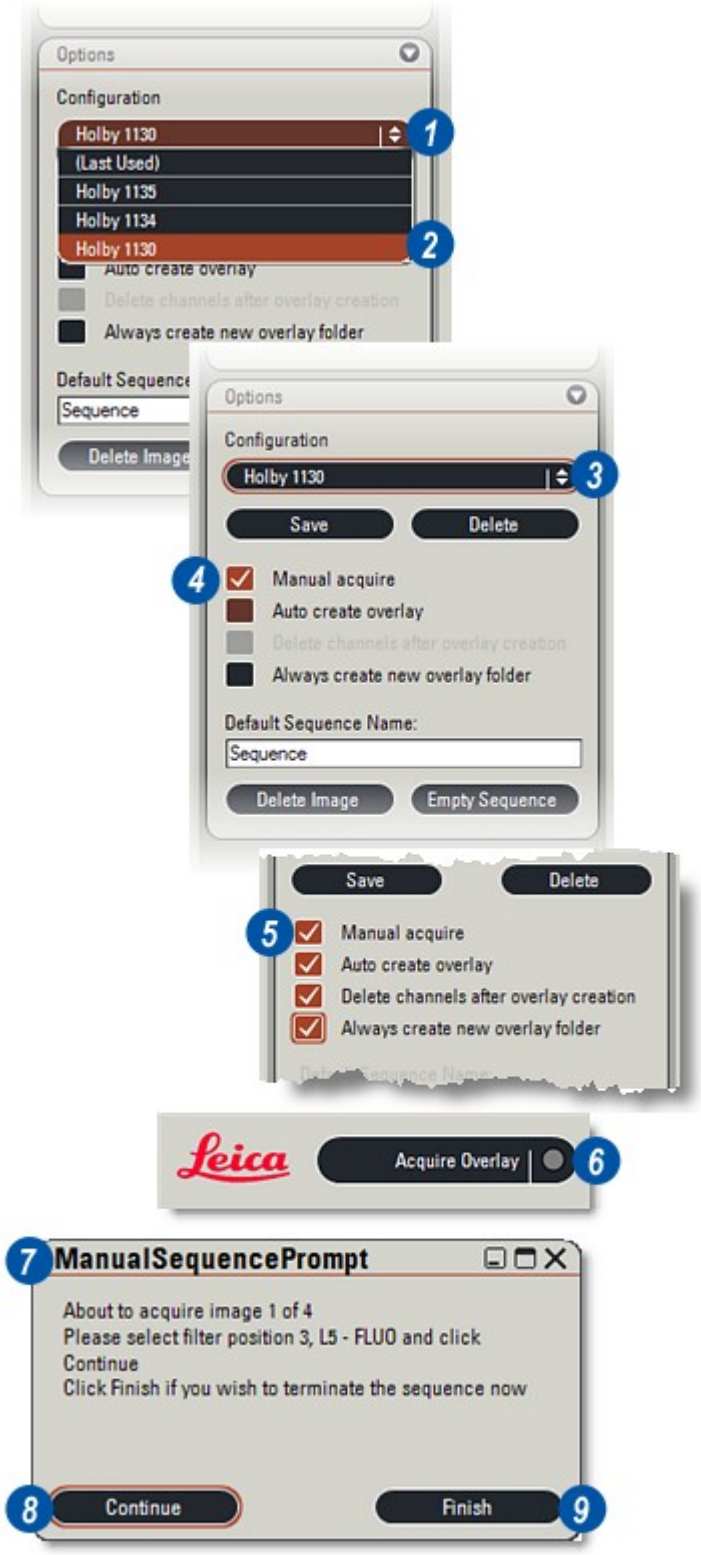
5: Any or all of the other options may be selected in *Manual Acquire* mode.

6: Click on the *Acquire Overlay* button.

7: When the *Manual Sequence Prompt* appears, turn the turret to the filter position specified on the prompt and…

8: Click *Continue*. The image will be acquired and appear as a thumbnail in the working *Gallery*. The prompting process repeats for all of the selected filters.

9: To end a capture sequence prematurely, click on the *Finish* button.



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When the Channel images have been captured…

1: The *Browse Workflow* opens with…

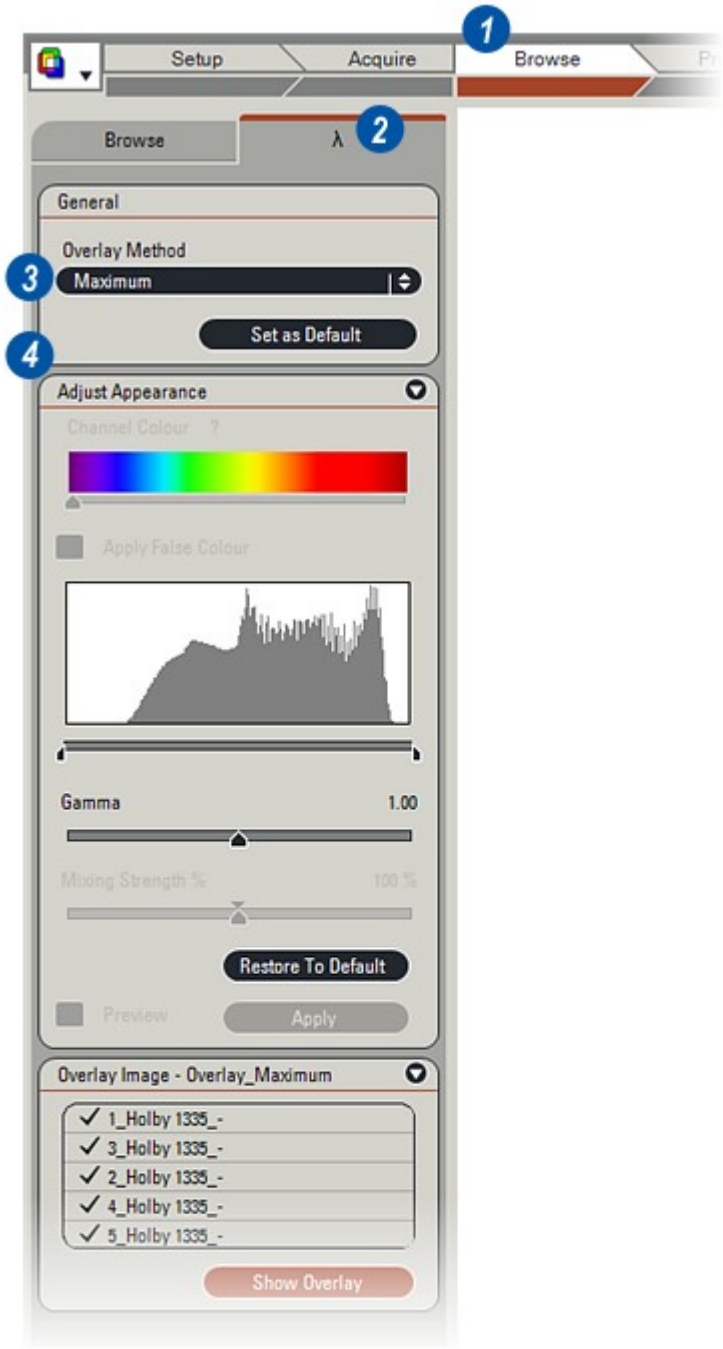
2: ...the *Image Overlay* or a preview selected. Thumbnails of the captured channel images together with the composite overlay if auto-create were selected, will be displayed in the *Gallery*. The *Viewer* shows the last image captured or the overlay.

There are three panels on the Image *Overlay* tab:

3: The *General* panel on which the *Overlay Method* determines the manner in which the channel images are combined to create the overlay.

4: The *Image Groups* and *Overlay Method* settings can be saved as the ongoing default.

Continued 598 ...



The *Adjust Appearance* panel provides the controls for:

6: Adding false (pseudo) colour to monochrome images.

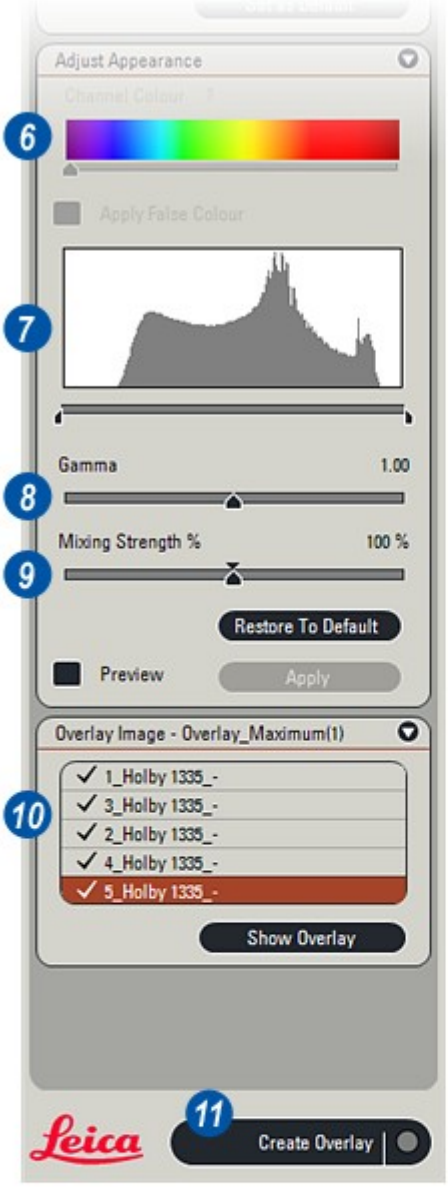
7: Adjusting colour balance with *Histogram* controls.

8: Changing the *Gamma* value of the channel images and the overlay, and

9: ...varying the 'mix' strength or dominance of a channel image. Images captured in colour will not have all of the colour controls available.

The *Overlay Image* panel **(10)** determines which of the channel images are to be included in the overlay. At least 2 channels must be selected before an overlay can be created.

11: The *Create Overlay* button creates a new Overlay based upon the selected settings. Once the channel images have been captured they may be altered at will and almost any number of different overlays created.



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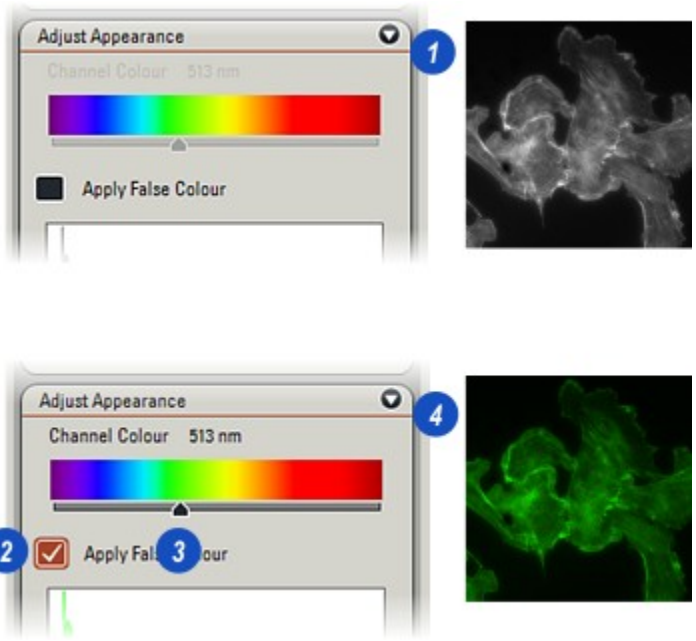
Monochrome Channel images can have false or pseudo colour applied to them before they are combined into a single overlay to improve contrast and clarity of the final image.



1: The *Monochrome Channel* image with the *Adjust Appearance* panel as it looks when capture is complete. Had this been a colour image the *Apply False Colour* check box would not be available.
Select another image by clicking on its thumbnail in the *Gallery*. It will be displayed in the *Viewer*.

2: To apply colour, click on the *Apply False Colour* check box to enable it. A basic colour may be applied to the image based upon the type of filter used in its capture.

3: Move the *Channel Colour* slider to the desired colour.

4: The applied colour is immediately displayed on the *Viewer*.

Continued 600...

5: Refine the image by adjusting the sliders below the *Histogram* and also...

6: ...the *Gamma* value.

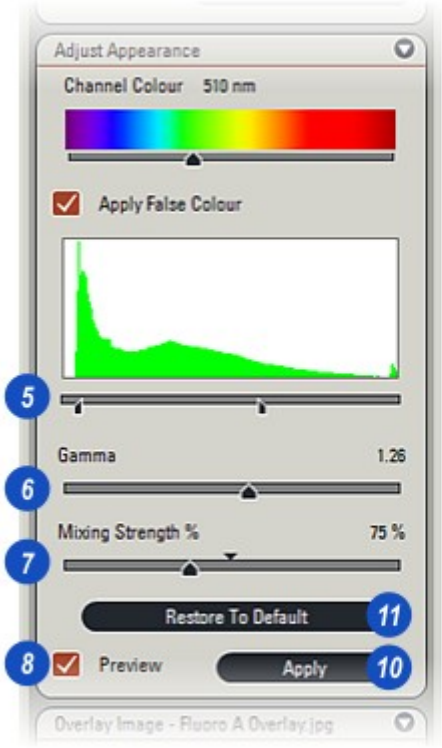
7: The *Mixing Strength* slider controls the predominance the Channel image will have in the overall result. The range is from 0 to 200%. The higher the value, then the greater the 'presence' of the image in the overlay.

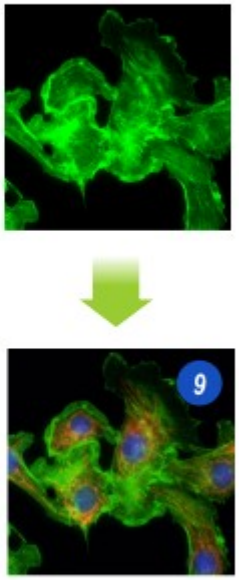
8: Test the result by clicking the *Preview* check box.

9: All of the channels are combined in a temporary overlay.

10: If the result is acceptable click on the *Apply* button to create an overlay. A new thumbnail will appear in the *Gallery*. In this way any number of overlays can be created to illustrate various aspects of the image.

11: To reset the channel images to their original filter colour values, click on a thumbnail and then on the *Restore To Default* button.





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1: To select the method of combining the Channel images to create the overlay, click on the arrows to the right of the *Overlay Method* text box and from the drop down list…

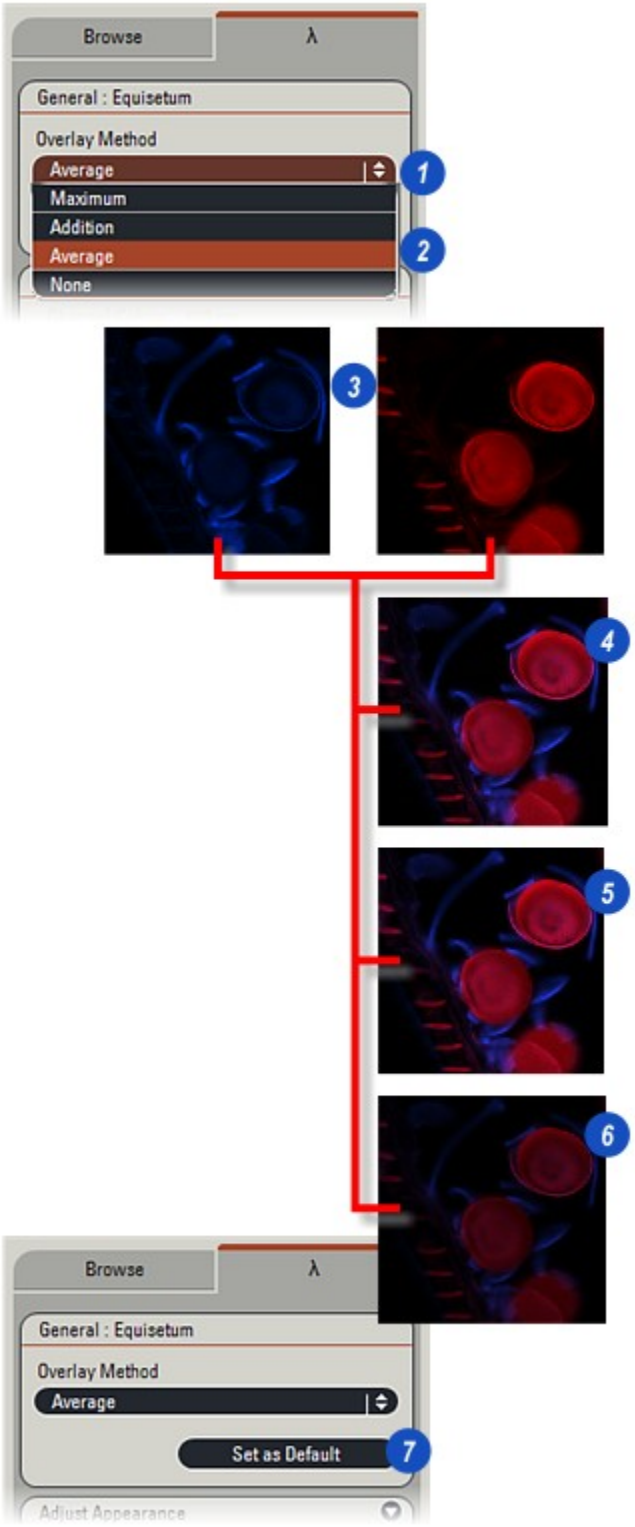
2: ...click to select the required method. In the illustrations, images **(3)** are the original, common channel images that have had false (pseudo) colour applied.

4: Represents the overlay combining the three common channel images using the *Maximum* option. The highest pixel value from the same location in the three common images is used to create the overlay.

5: The same process using the *Addition* option. The pixel value from the same location in all three channel images are added together.

6: Shows the result with the *Average* option. The pixel values in the same location in all three channel images are added together and then averaged.

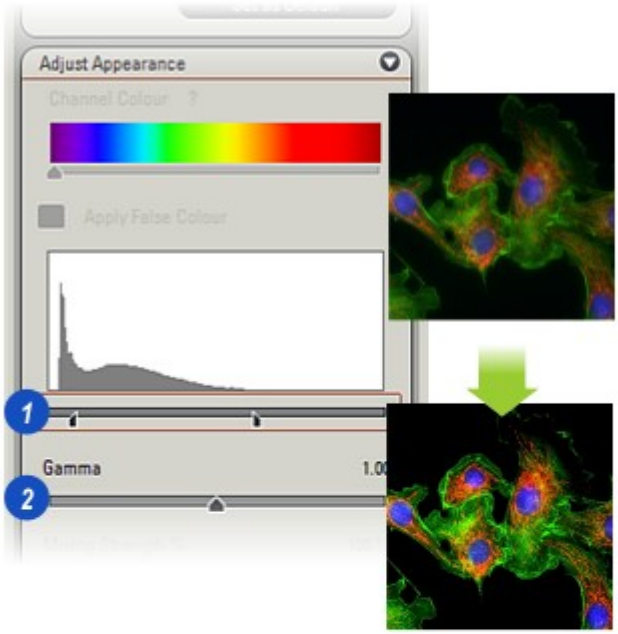
7: To save the settings as default, click the *Set as Default* button.



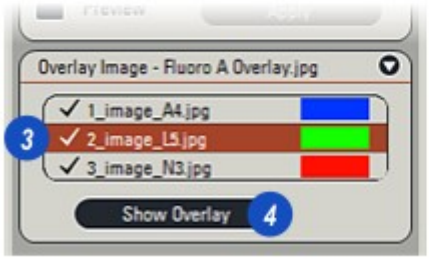
False colour is applied to a channel image electronically by association only – the original remains intact and unchanged. This means that colour may be 'removed' completely or 'fine tuned' at any time after capture and a new overlay created.

Individual colours in overlays cannot be altered, but the *Histogram* **(1)** and the *Gamma* **(2)** controls are available to lighten or intensify overall effect and in the process enhance a particular feature.

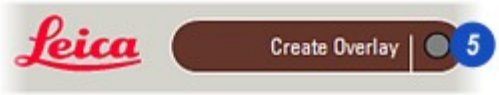
It is not necessary, nor always desirable to include all of the channel images in an overlay. The *Overlay Image* panel provides the means to include or exclude individual channels.



3: A channel is included when the check box to its left is checked. Click to un-check and exclude.
At least two channels must be selected in order to create an overlay. If only two are still selected the program will prevent any more exclusions.
The colour bar to the right of a channel name indicates the filter colour used in its capture.



4: Click the *Show Overlay* button to select and display the last overlay created or…



5: Click on the *Create Overlay* button to produce a new overlay using the revised channel images.

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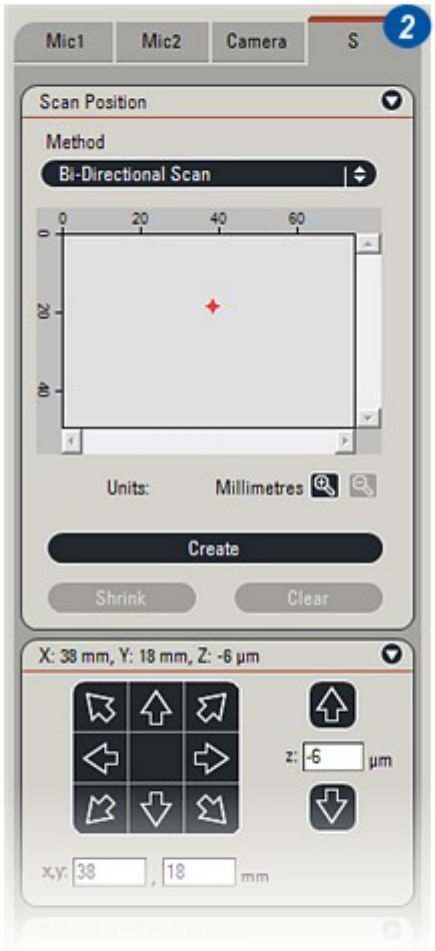
This section contains the following topics:

Introduction
This module allows for the creation of Scan Patterns using Motorised X/Y Stage control through the Leica Application Suite interface. The subsequent images may then be joined together to create one large image or mosaic.

1: On installation of the *MultiStep* module and the subsequent start up of LAS, the *MultiStep* module can be found by selecting the *Select Acquisition Mode* button to the left of the Workflow bar.

2: On selection of the *MultiStep* mode icon a new tab will appear in both the *Acquire* and *Browse Workflow* items labelled *S*.



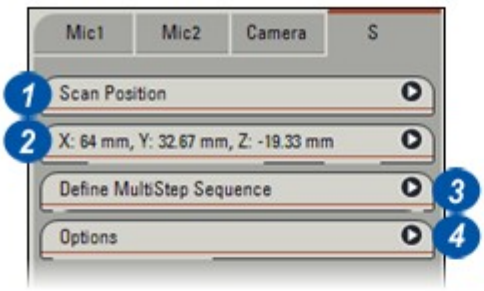
On the *Acquire Workflow* panel an *S* tab will be visible if the *MultiStep* option is active. Selection of this tab reveals four control panels:

1: Scan Position

2: Stage Position

3: Define MultiStep Sequence

4: Options



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This shows the position of the current field of view. The user can mark points on the specimen by driving the stage manually. The live window will be updated to reflect the true position on the stage.

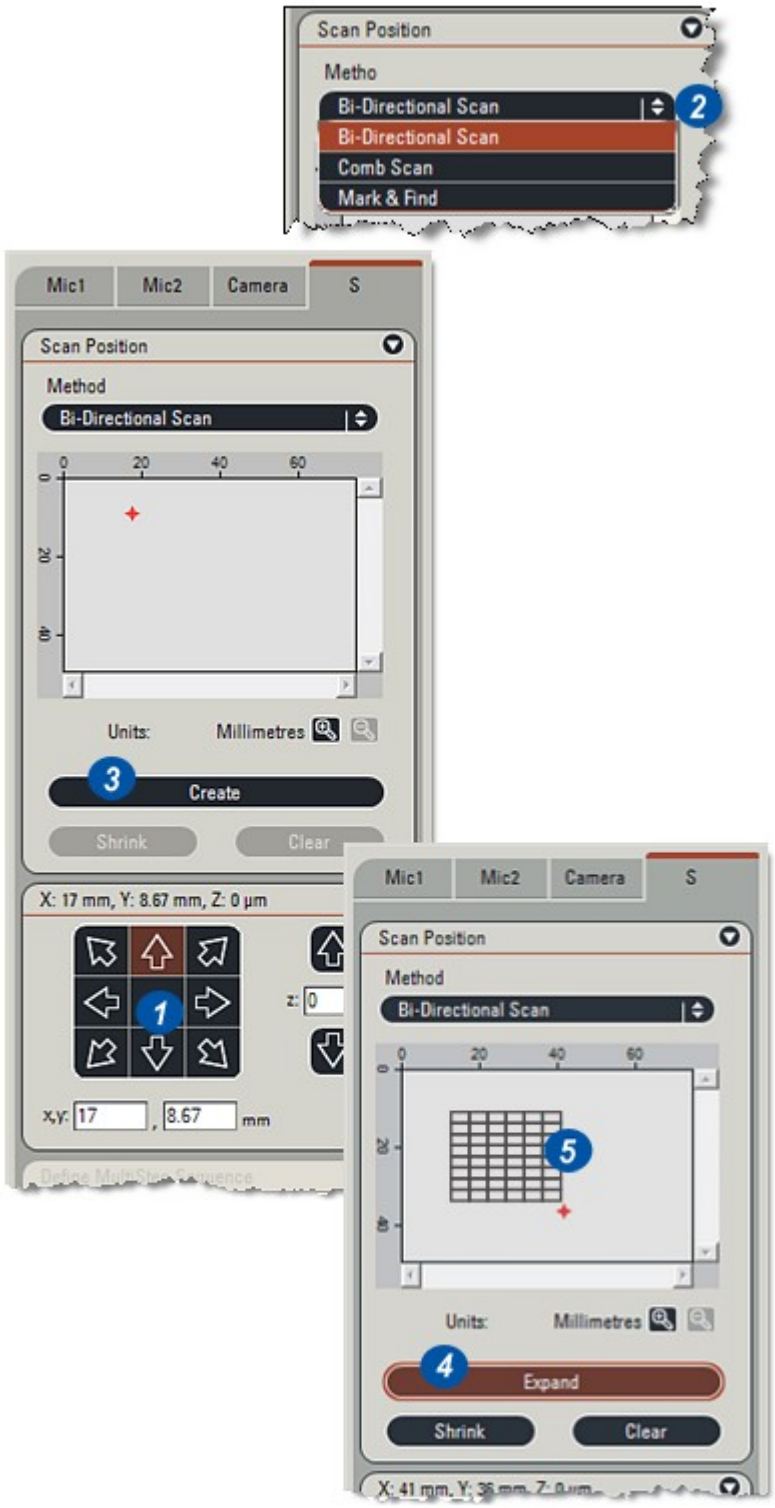
1: The *Navigation* or *Soft Joystick* can also be used to navigate to new positions.

2: Select *Scan Method* from the drop-down menu. A position is located using the microscope (e.g. the corner of an area of interest) to mark the initial field in the scan pattern.

3: The *Create* button is then pressed to mark this co-ordinate on the graphic. *(See right: Cross hair in red).*

4: The *Create* button then changes to *Expand*. When a second coordinate is added (again by driving the stage to the desired position and then selecting the *Expand* button), a graphic representation of the scan area is shown **(5).**

Continued... 606



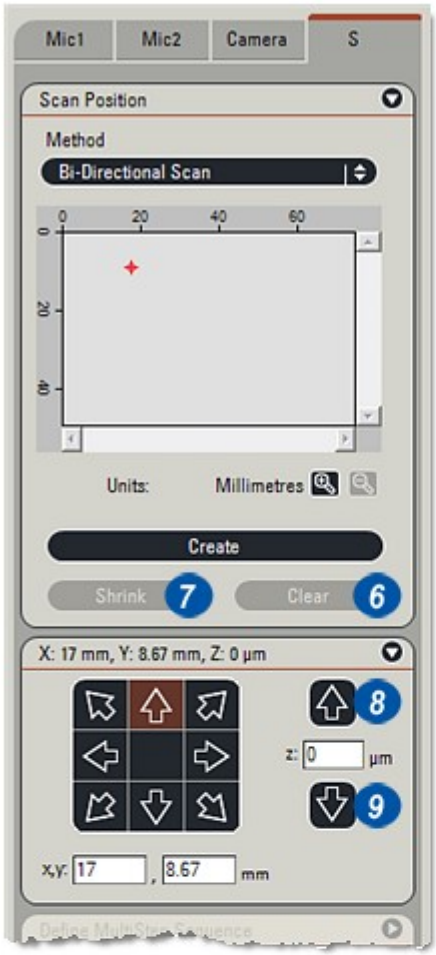
6: The *Clear* button allows the user to remove the scan pattern and start the process again.

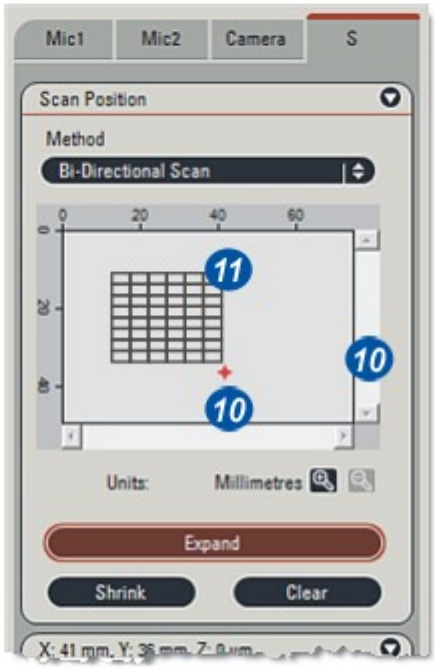
7: The *Shrink* button allows the user to reduce the size of the scan area. This is achieved by placing the red cross hair at the position the Scan Pattern is to shrink to.

8 and 9: The *Zoom In and Zoom Out* buttons allow the user to enlarge the Scan Position display.

10: This display can then be navigated using the scroll bars that will appear, or by holding the mouse button down over the scan position graphic and panning the position by moving the mouse. Releasing the mouse stops the panning.

11: Double clicking on the stage graphic causes the stage to move to that position.





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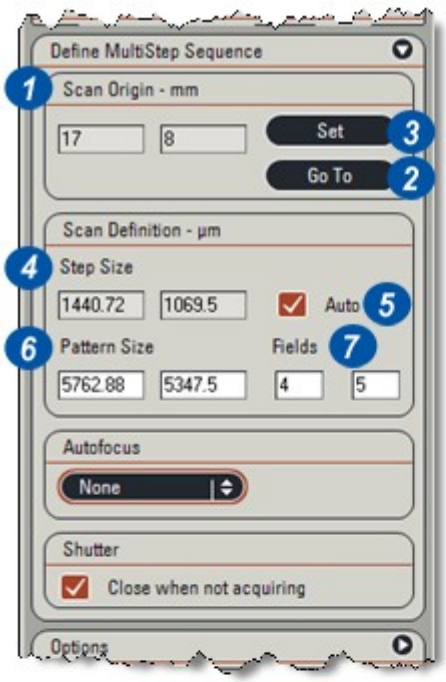
This is a data/textual representation of the scan pattern which allows fine tuning of the scan pattern.



1: *Scan Origin* is the location of the first field of view in the pattern and is the central point of that field.

2: *Go To* drives the stage to the Scan Origin.

3: *Set* takes the current stage position and sets it as the Scan Origin and the scan pattern is shifted appropriately. Scan Definition:

4: *Step Size* is the distance between one field and the next, both horizontally and vertically.

5: If *Auto* is selected the component images will be adjoining. De-selecting Auto allows the user to acquire images with overlaps or images with spaces between them.
If the Auto checkbox is selected this value will equal the dimensions of one field of view. If the Auto checkbox is unselected the step size is user defined.

6: The *Pattern Size* gives the x and y dimensions of the rectangular pattern.

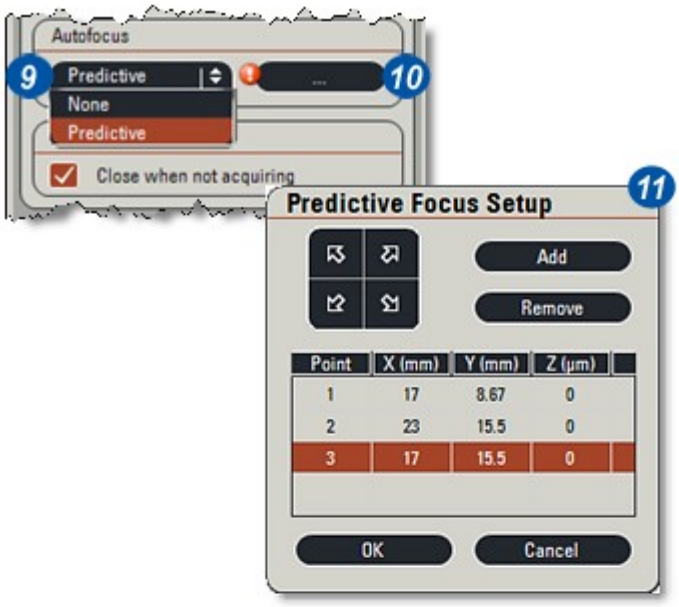
7: The *Fields* boxes show the number of whole fields of views in the pattern.
Changing the Step Size will change the Pattern Size as will changing the number of fields of view.

Continued... 608

9: This option allows the user to turn on *Predictive Focus.*

10: Pressing the associated button that appears when *Predictive Focus* is selected displays the *Predictive Focus Setup* dialog **(11).**

Continued... 609



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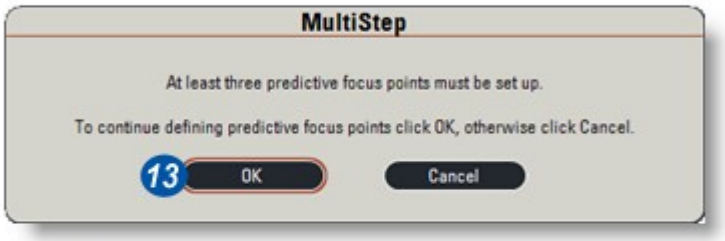
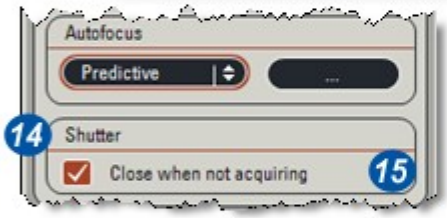
12: Selecting *Add* creates a new predictive focus point based on the current stage position.

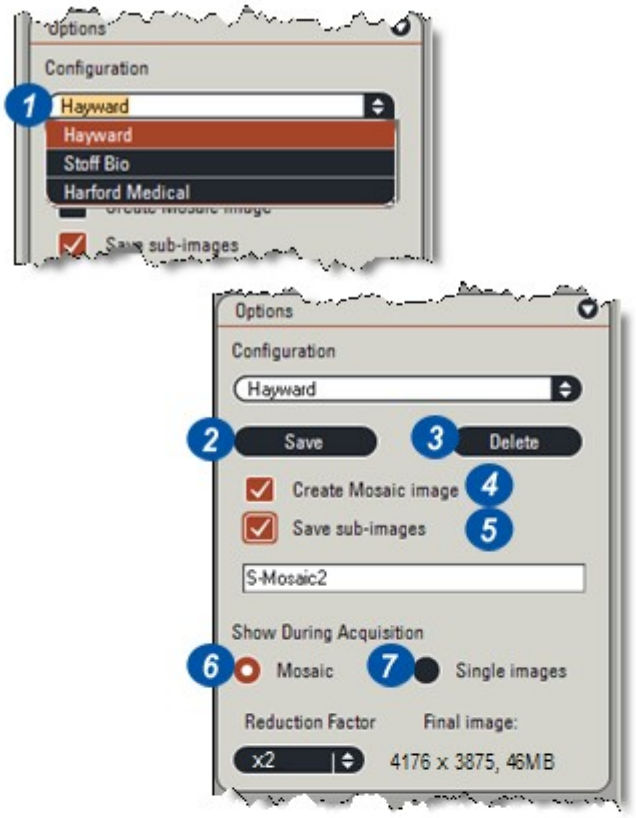
13: A prompt panel reminds the user that the *Predictive Focus Setup* focus dialog requires a minimum of three predictive focus points.

14: The *Shutter* allows the user to define whether the shutter is closed during stage moves and only opened prior to an acquisition by selecting *Close when not acquiring* **(15).** This is particularly useful if not essential when undertaking fluorescence specimens.





The *Configuration* section allows the user to store and recall previously saved configurations.



1: To save a new configuration based on the current settings, type a name into the configuration drop-down and then select *Save* **(2).** This entry defines the mosaic name and the sub-folder name if sub-images are saved .

3: To remove a previously saved configuration, select it from the drop-down list and then press the *Delete* button.

4: The *Create Mosaic image* if checked means that a mosaic image will be created after the last image in the Scan Pattern has been acquired. This will be displayed and stored in the Gallery.

5: If *Save sub-images* is selected, all acquired images will be saved as an image set. If de-selected these images will not be saved.

Show During Acquisition

6: *Mosaic* shows the dynamic build up of the mosaic on the screen as each image is acquired

7: *Single Images* shows each acquired image on the main screen as it is captured in turn.

Continued... 611

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Auto provides a final image at a size comparable with an individual field image size.

None provides an image at max size -- size of final image is sum of sizes of individual images.

8: Values (e.g. x2) indicate an image of size Max Size / (value* value). Highest value is capped to give an image of approx 640 x 480. It displays the image size and amount of MB storage that will be used.

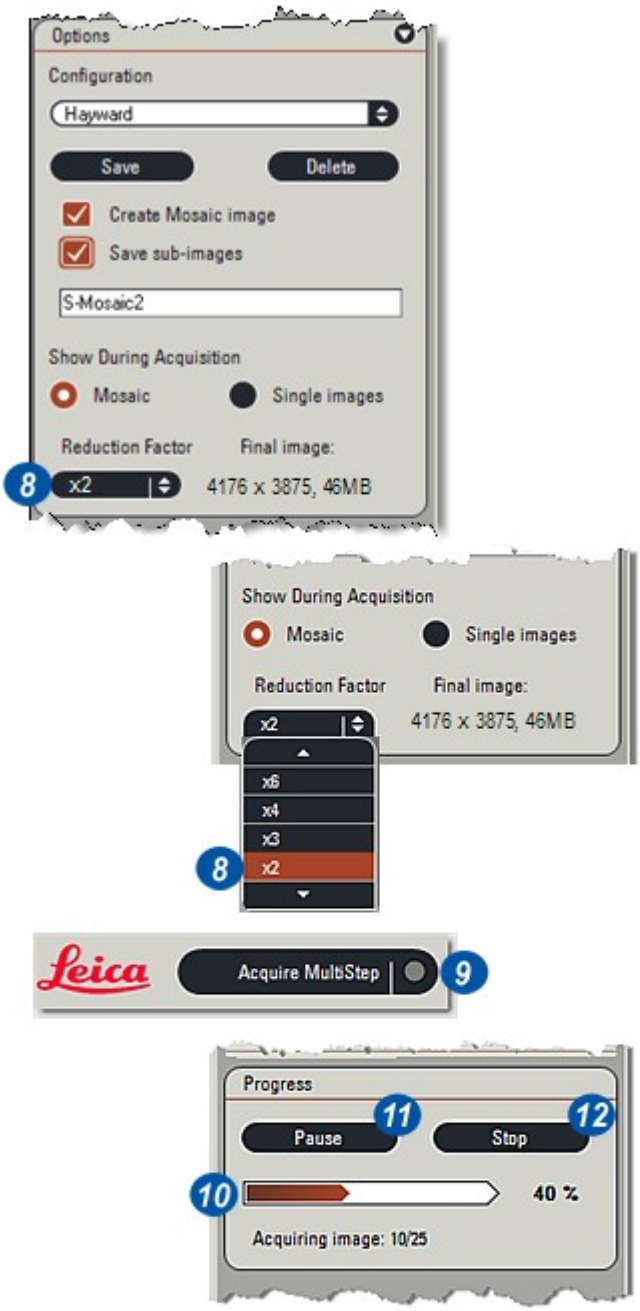
NB: Memory requirements can only be shown accurately if the image is to be saved as in BMP or TIF formats. JPGs will show the maximum size for the image. Disk space requirements are additional because field images need disk space too. Even if system is set up to acquire 16-bit images, mosaic images will be acquired in 8 bits

9: Selecting the *Acquire MultiStep* button will start the Scan Pattern acquisition process as defined above.

10: A *Progress* bar will show how the process is proceeding:

11: *Pause*: Will halt the acquisition which can then be restarted.

12: *Stop*: Will halt the acquisition where it is resulting in either a partial mosaic being created and/or a partial set of images depending upon your settings.



Under the MultiStep tab *S* **(1)** there are two control panels:

- View Image Set
- MultiStep

View Image Set
This allows the user to select a previously saved set of *MultiStep* acquisition images.

2: The images may then be stepped through (like a slide show), by using the video play controls which allow the user to go to the beginning or end of the sequence or step through images one at a time forward or back or play the sequence at the specified frame rate.

3: The rate at which images can be displayed is altered by entering a value into the *Delay* box.

4: The red slider in the sequence visualization can also be grabbed using the mouse (like using a scroll bar) and the sequence navigated by moving this back or forth through the image sequence.

5: The image being displayed is listed in the text box.

Continued.. 613

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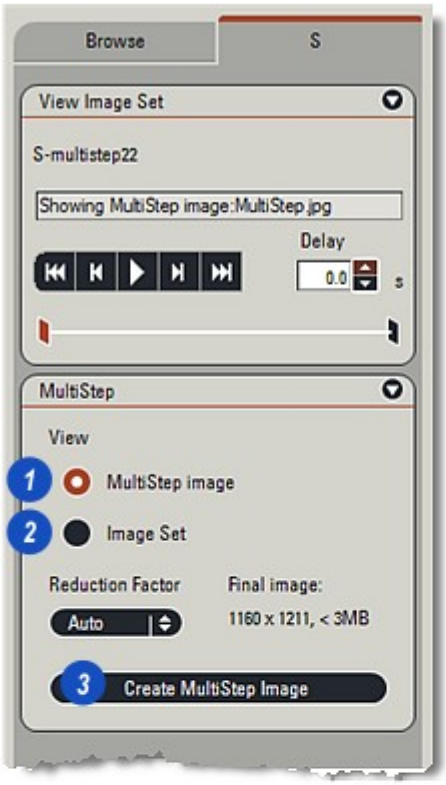
After selecting the sequence in *Browse*, the *MultiStep* panel allows the user to view the images and create a Mosaic image.

1: *MultiStep Image*: Selecting this automatically loads the *MultiStep* image from the currently selected image set in the gallery. The *View Image Set* controls will be disabled as the mosaic image is being displayed.

2: *Image Set*: Selecting this option loads the first image from the image set

3: The *Create MultiStep Image* button allows the user to re-create the *MultiStep* Image from the current image set.

Continued... 614

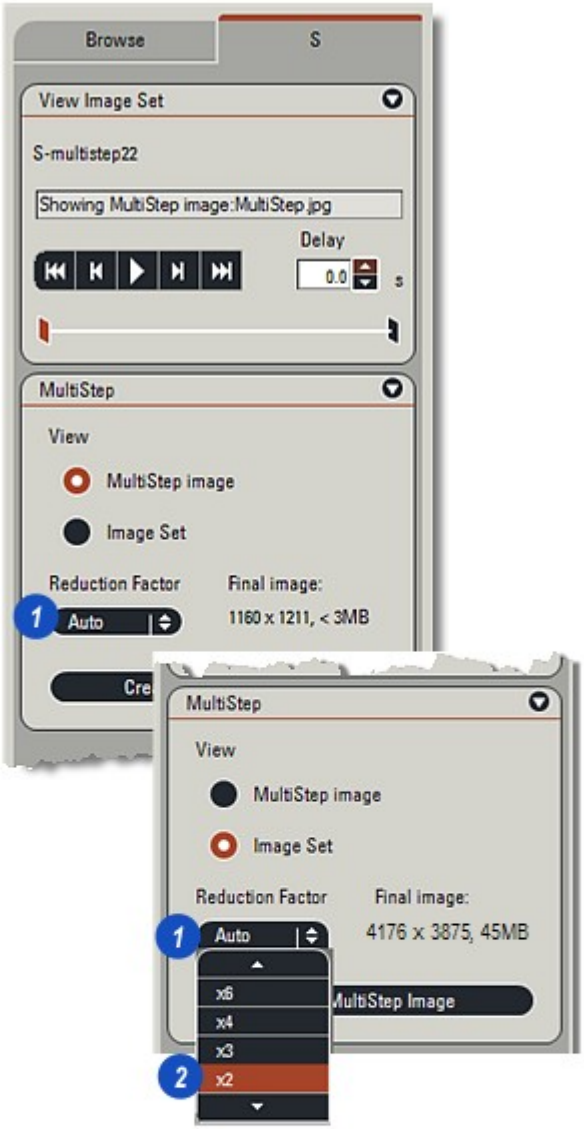


1: *Auto* provides a final image at a size comparable with an individual field image size.

None provides an image at max size. Size of final image is sum of sizes of individual images

2: *Values* (e.g. x2) indicate an image of size Max Size / (value* value). Highest value is capped to give an image of approx 640 x 480. It displays the image size and amount of MB storage that will be used.

Note: Memory requirements can only be shown accurately if the image is to be saved as in BMP or TIF formats. JPGs will show the maximum size for the image. Disk space requirements are additional because field images need disk space too. Even if system is set up to acquire 16-bit images, mosaic images will be acquired in 8 bits.



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LAS Power Mosaic software integrates high-performance specimen scanning into the Leica Application Suite to provide an easy-to-use application for creating, viewing, and saving ultra-high resolution mosaic images. LAS Power Mosaic is using in conjunction with a Leica microscope, Leica DFC digital camera and a stepping stage.

With LAS Power Mosaic you can scan a selected area or an entire slide quickly and accurately, and then effortlessly relocate to areas of interest with a simple click. Additionally, with the LAS Power Mosaic Plus software, it is possible to acquire images at multiple focus positions and view the complete mosaic while scrolling through focus.

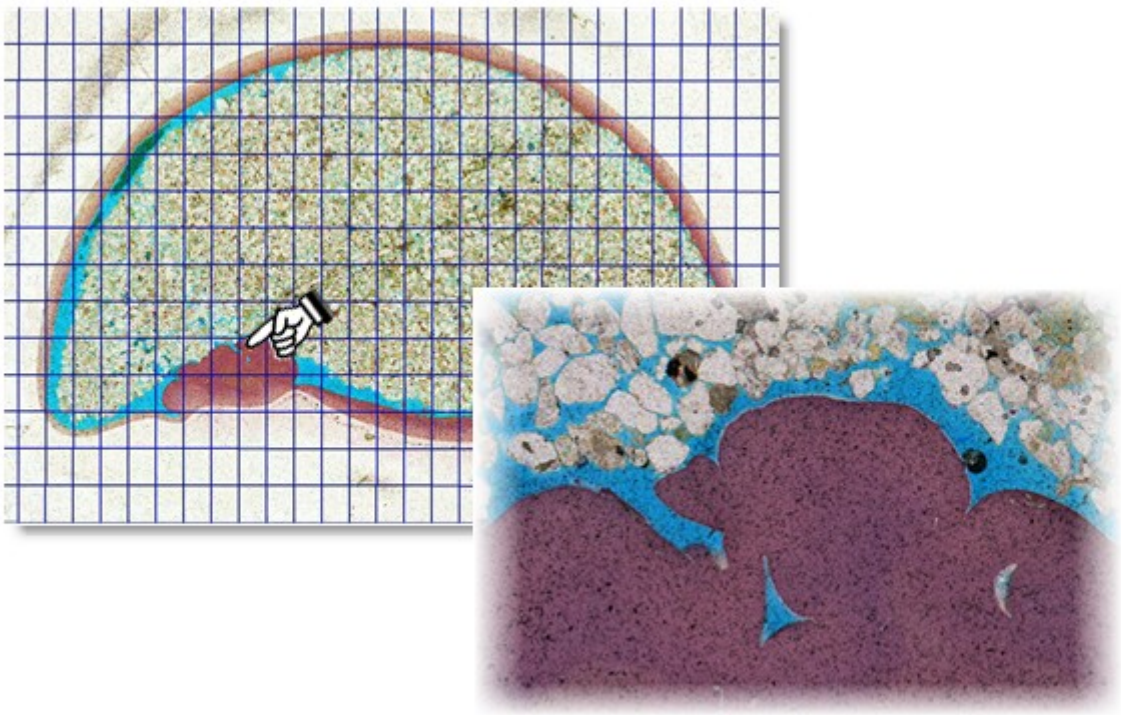
The ability to generate high-resolution mosaic images provides a powerful and novel method of visualising a specimen. The specimen overview is an aid to understanding the relationships between microscopic features and overall structure.

Once you have created a scanned mosaic, you can save your specimen's details as a workspace, or export the full-resolution mosaic for use with image publishing and analysis packages.

The software is designed to offer a comprehensive range of facilities with a strong emphasis on versatility and ease of use. With minimal experience it is possible to produce excellent mosaic images. In particular, the accurate calibration of the camera and motorised stage is essential and is made convenient by being performed automatically.

Good results depend, however, on the system being well configured and set-up and on the user becoming reasonably familiar with the available facilities and functionality.

The principal purpose of this manual is to provide practical guidance on configuration, set-up and use of LAS Power Mosaic.



Navigate specimens from LAS

- Power Mosaic integrates seamlessly within LAS.
- Power Mosaic images can be acquired and reviewed.
- Images from LAS Power Mosaic can be saved and measured in LAS.
- Specimen movement is performed by a stepper stage.
- A Leica DFC camera acquires images.

Power Mosaic Acquisition

- Uses triggered image capture for very fast continuous scan and acquire on suitable cameras
- Standard scan using step and acquire for low light applications.
- Image streaming for mosaic sizes limited only by free disk space.
- Additional scans can be added to the initial scan to include all parts of a specimen.

Scan Patterns

- Rectangle, Circular, Annular, Cross (+ and x), Random
- Overlap of tiles allows smooth merging of joins.
- Correction for camera rotation is performed
- Save / load scan patterns

Continued 617 ...

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Export/Import

- Save and load mosaic scan pattern and Power Mosaic environment as a ‘workspace’.
- Select workspace for recall from LAS.
- Load last used workspace on start up.
- Images exported to tif, bmp or jpg.
- Export mosaic as full resolution bitmap.
- Export mosaic as reduced resolution bitmap.
- Export user-selected region of interest.
- Export image to current LAS capture folder.

Specimen Map

- Switch between display of stage overview and pattern view.
- Drag to create pattern scan or enter exact details.
- Point and click relocation of stage to indicated position.
- Real-time graphic display of current stage position on specimen map.
- Zoom and pan over entire mosaic.

Continued... 618

Calibration

- Automatically measures calibration and updates scan patterns according to selected magnification on microscope using a correlation-based calibration procedure.
- Camera rotation and sage skew is measured to allow convenient adjustment.
- Small rotation of camera is compensated for in the mosaic creation.
- Automatic stage orthogonality correction.

Microscope Automation

- Oasis XY stage and Z focus control drive board is used.
- Software joystick for stage and focus movement.
- Compatible with Leica Microsystems LAS configured microscopes controlling focus, turret, condenser, and lamp control as available
- Stage and focus speed defined per objective linked to LAS.

Automatic Focus

- Multi-point predictive focus for continuous focus tracking.
- Predictive focus setup by combined auto scan with user review.
- Autofocus can be used for specimens that are not flat.
- Predictive and Autofocus can be combined.

LAS Power Mosaic Plus

This is an extended version of Power Mosaic and includes all the features of Power Mosaic and in addition:

- Z-Scan optional at each field.
- Definition of Z-Scan positions, step number and step width.
- Extended focus imager can be created.
- Mosaic image can be reviewed while sweeping through all focus positions.
- Navigator for multiple scan patterns

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The following procedure lists the steps required to obtain high-quality mosaic images. Once the system is correctly aligned and adjusted, some of the steps will not be necessary again.
It is assumed that the system is already installed according to the installation procedure detailed in the release notes.

1: Prepare the specimen:
Check the following:

- The specimen is as flat as possible to minimise the need for focus changes.
- The slide surface is clean.
- The specimen is firmly fixed to the stage - loose slides are a common problem.

2: Setup the microscope:

- To gain experience with Power Mosaic, start with an x5 objective as this magnification probably does not require focus compensation during the scan. This makes it easier to check that the scanning is working correctly.
- Ensure the condenser is in focus and apertures are set for Koehler illumination. With an automatic microscope this should be checked for all of the objectives expected to be used.

3: Initialise the Stage and Focussing:

- This checks the stage travel limits, finishing at the centre point. The small green 'target' in the stage area indicates current position. The 'hatched' border around the periphery indicates the 'soft' limit switch area.
- Lower the condenser and check that stage will not collide with objectives.
- Check/adjust the stage speed: *See: Stage Initialisation.* 634
- Initialise/adjust the focus limits: *See Focus Initialisation.* 635
- *See Autofocus Setup:* 636

Continued.. 620

4: Check the camera and adjust the exposure:
On a clear field of view, debris in the optical path will be seen as fixed dark regions on the live image when the specimen is moved. If cleaning is necessary, a qualified technician should perform it.

- Check that there is no visible debris in the optical path.
- For a colour camera Saturation = 1.75: Gamma = 0.6: Gain = 1: Make fine tuning adjustments around these values to achieve the required image.
- For a mono camera: Saturation = 1.75: Gamma = 1.0: Gain = 1.
- Ensure that the black and white levels on the histogram are reset.

See: Input Options: 631

Turboscan and Standard scan require different adjustments:

Adjusting the camera for Turboscan:
Turboscan, the fastest scanning method, can be used if:

- The camera has progressive scan mode.
- There is sufficient light to give an exposure of less than 200 µs and
- The specimen is flat enough to use no focus change or predictive focus.
- Select an exposure mode with Progressive scan.
- Set Exposure to approximately 100 µs.
- On the Histogram display, click to enable *'Show Under/Over Exposure'.*
- Adjust the lamp voltage until red flecks appear on the image white highlights. *Note:* On a DM microscope, fine lamp voltage control is achieved by pressing both stand lamp buttons together. Expect a high lamp voltage and very bright image.
- If there is insufficient light, increase the camera gain by small amounts to give the correct exposure. Check that this does not increase significantly image noise.

WARNING: Switch 100% light to the camera. DO NOT look at the specimen through the eyepieces.

Adjusting the camera for Standard scan
Standard scan is used when the conditions for Turboscan are not met.

- Select the camera image format to suit the specimen detail required. Choose the lowest resolution without compromising image quality to save disk space.
- Set the lamp voltage to give a comfortable image in the eyepiece.
- Adjust lamp voltage and camera exposure to until red flecks appear on a white region of the image.

Continued 621...

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5: Set shading
Shading correction has to be set in Power Mosaic. LAS settings are not used.

- Shading correction must be repeated every time the objective is changed.
- Move the specimen so a clear region without artefacts is visible over the whole image.
- Set the shading correction: *See: Shading:* 640

6: Calibration for each objective
LAS Power Mosaic derives its calibration from the stage movement to accurately align the tile edges. Calibration must be carried out for every objective on first use.
A warning message appears if an objective has not been calibrated.
Calibration tests that the values returned are reasonable: If they are not a warning is displayed. Camera rotation must be less than 0.1°. Conditions can alter over time and systems are susceptible to dirt, heat and vibration. Periodically check the calibration to ensure that it remains accurate.

- Check microscopes with a manual turret, that the selected objective matches that selected on the Acquire:Mic1 tab. The same applies to the Mag Changer if it is fitted.
- Set calibration: *See: Calibration:* 638
- If necessary adjust the camera rotation: *See: Camera rotation:* 639

7: Create and perform a test scan

- Select the Split Screen view. The 'crosshair' shows the current stage position.
- Move the stage so that the specimen can be seen in the live image window.
- Select the New Pattern tool, click close to the stage 'crosshair' and drag a small scan region.
- Click Acquire Power Mosaic.
- Use Zoom and Pan to check that the mosaic is formed correctly. *See: Create Pattern Grid:* 657

8: Extend the scan pattern to the required region
To include parts of the specimen not included in the test scan:

- Click on the Create/Expand tool.
- Click on the stage in an area outside the boundary of the test scan and drag to include the required parts of the specimen. *See: Create Pattern Grid:* 657

Continued 622...

9: Select the Focus Method:
During scanning, two focussing methods are available:

- *Predictive Focus* is best suited to uniform specimens and low magnification - up to x10. Focussing is performed either manually or automatically on a number of points across the specimen to create a table of values. Points which have not been pre-focussed use the table to predict a focus position without going through the time-consuming process of an Autofocus on every field.
- *Autofocus* should be used for irregular specimens at any magnification. Focussing is carried out at regular intervals across the specimen with options to set the repetitiveness.
- Both *Predictive* and *Autofocus* may be used in combination to benefit from the speed of Predictive and the precision of Autofocus.

Using Predictive focus:

- Select the focus points manually or use the automatic Grid.
- Select and focus each point manually or choose *'Autofocus on all points'*
- Run Predictive focus: *See: Predictive Focus:* 652

Using Autofocus:

- Set the repeat pattern for fields to be focussed.
- Enable/disable field skipping on focus failure. *See: Focus Methods:* 651

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Power Mosaic: Loading the module:

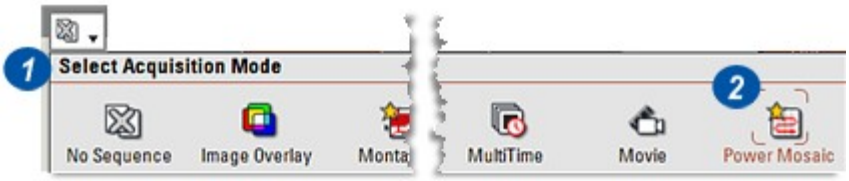
1: Click on the *Select Acquisition* button to reveal the available modules. If a module is already loaded and running the button icon will differ from the illustration.

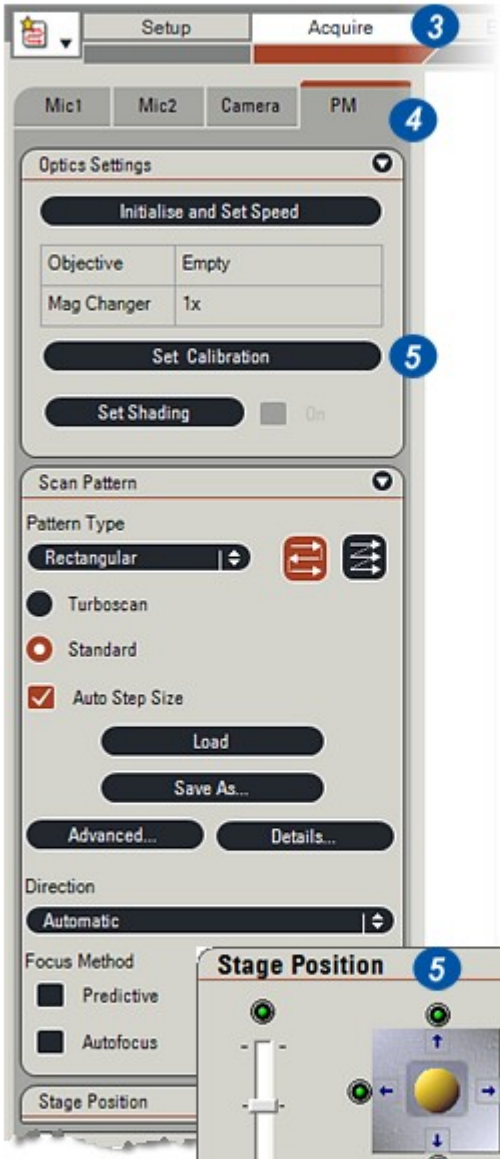
2: The *Power Mosaic* icon will be present only if it is installed. However, it will also need to be enabled: *See: Installing, Configuration and Licensing: Registration Information.* Click on the icon.

3: Click on the *Acquire Workflow* tab.

4: When Power Mosaic is loaded and running the PM tab will be present with the main panels displayed.

5: An additional panel – the On-screen Joystick – is available by clicking the *Show Joystick Tools* button. Click and hold on the *Joystick* header to drag and dock it on any part of the screen.





The User Interface is shown in the illustration with the Acquire Workflow selected and the Power Mosaic tab active**.**

1: *Workflow* tabs.

2: *Control Panels* and function tabs.

3: *Acquire* scan button.

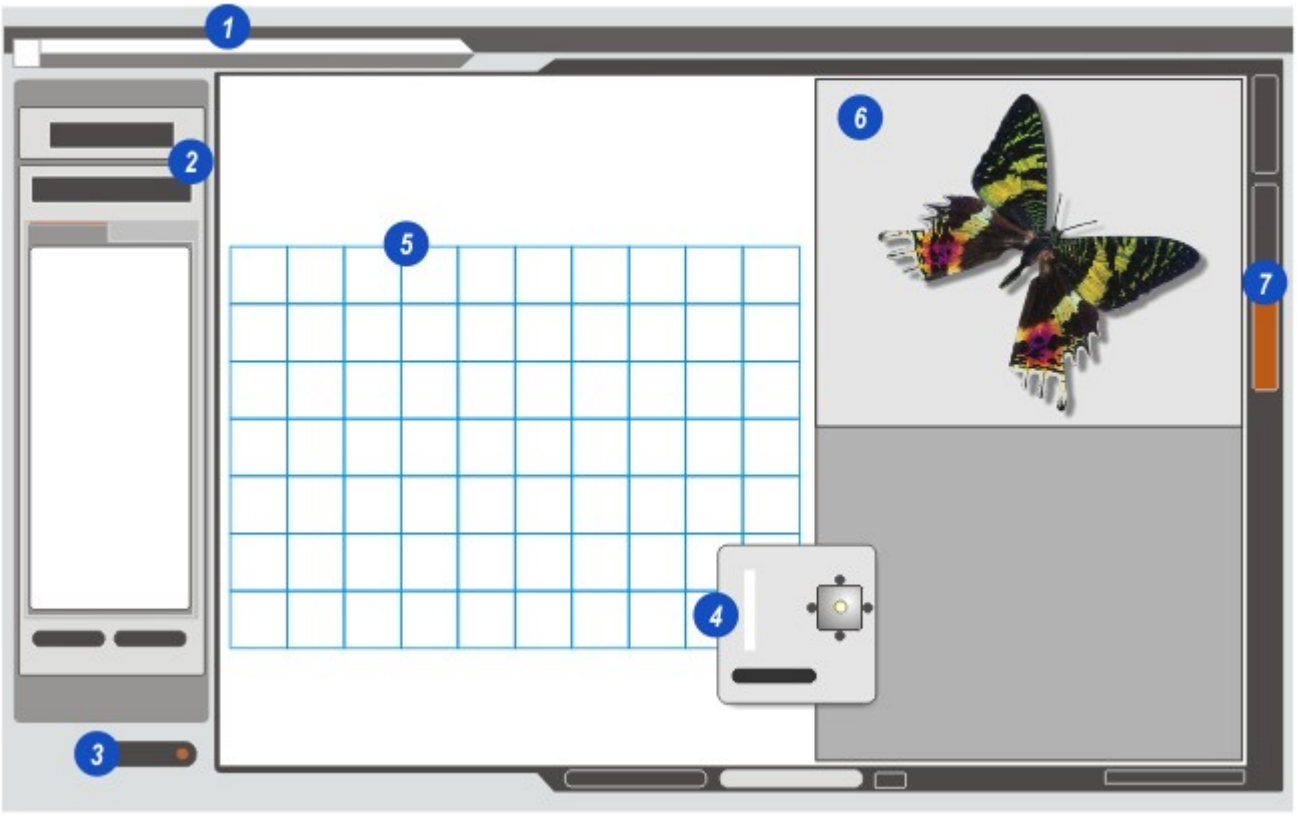
4: *Scan* viewing area with on-screen Joystick shown.

5: *Scan Grid Pattern.*

6: *Live Image Field:* Split screen view is selected.

7: *Tool Bar.* The tools are explained on the following page.

Continued... 625



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The Toolbar is located on the right-hand side of the screen. The tools descriptions are:

1: *Live Screen:* The entire screen is devoted to the live image.

2: *Split Screen*: Divided between viewer part of live screen.

3: *Grid Pattern View:* Entire screen is devoted to the scan grid.

4: *No Tool* selected.

5: *Stage Area:* displayed on Viewer.

6: *Display Grid Pattern.*

7: *Hide/Display Grid Pattern.*

8: *Zoom In/Zoom Out.*

9: *Pan.*

10: *Create a New Scan Grid.*

11: *Extend/Contract* existing scan grid.

12: *Go to Stage point.*

13: *Move Scan Pattern.*

14: *Clear scanned Tiles.*

Continued... 626

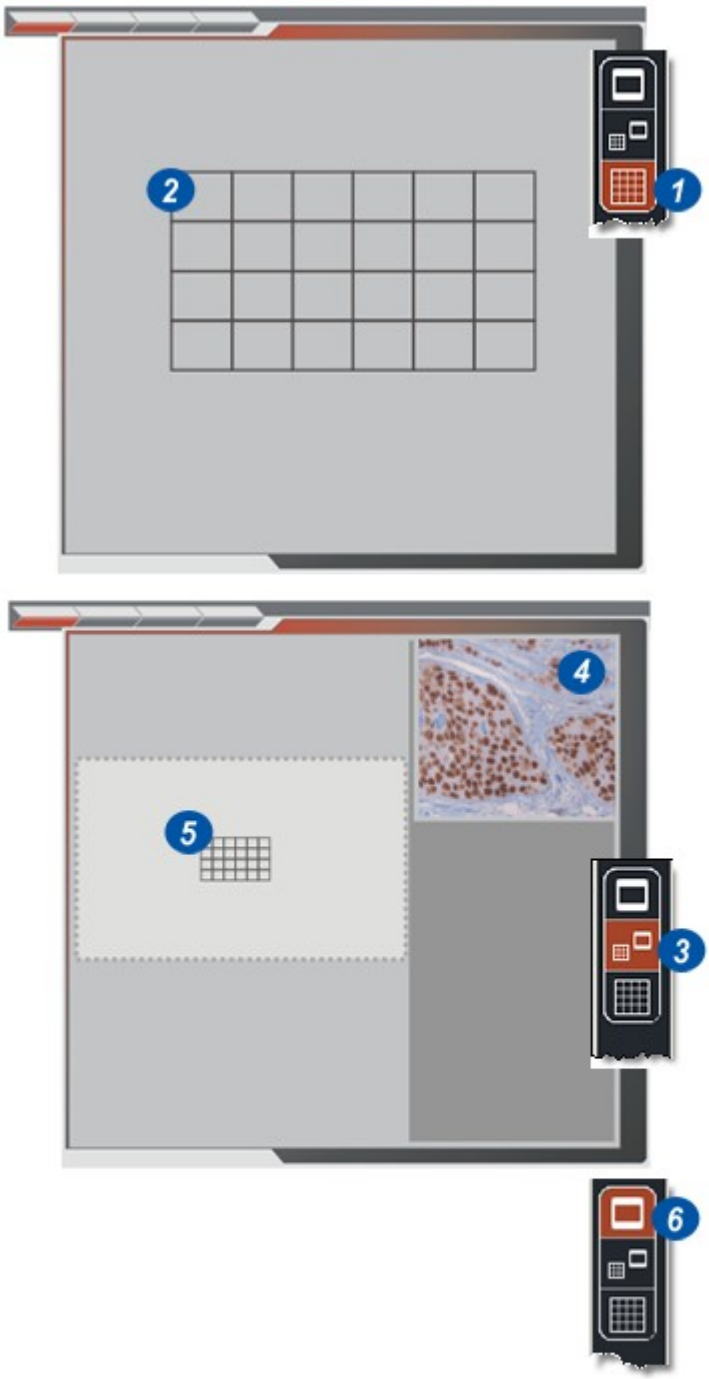
There are three viewing options available, each selected by clicking the appropriate button.

1: *View Stage Map:* fills the viewing area with…

2: …the scan pattern grid and scanned tiles scaled to fit.

3: *Split View:* displays the pattern grid, and scanned tiles on the left **(5)** and a live image on the right **(4)**.

6: *Live Image:* fills the viewer with a live image.



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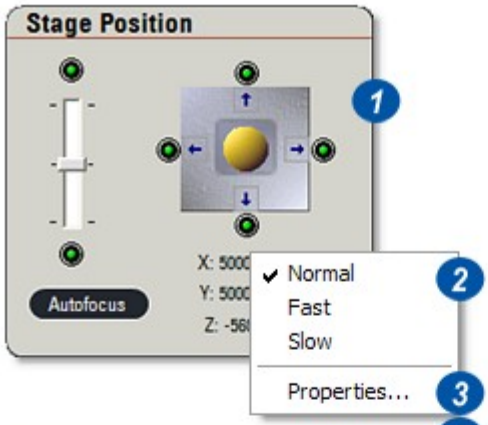
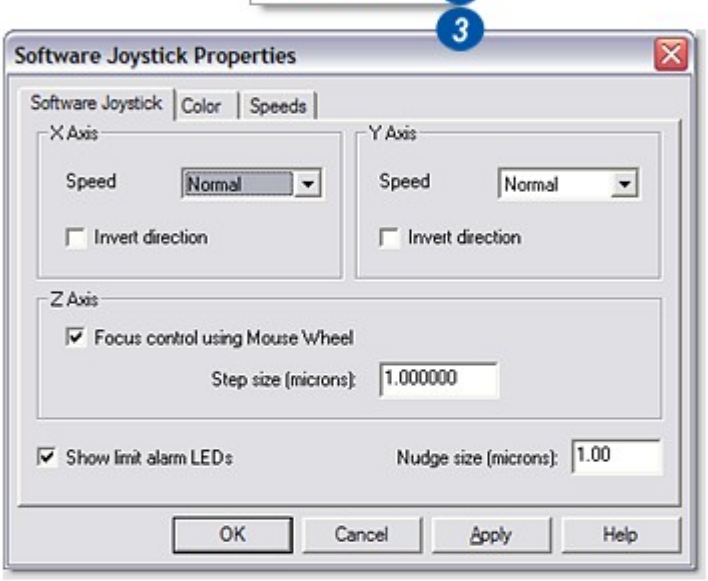
With the On-Screen Joystick visible, it can be customised to suit individual preferences.





1: Right click on the *Joystick* to reveal the *Speed* and *Properties* menu.

2: Click to select the speed at which the stage will be driven during when the joystick is moved.
Three options are available: *Normal, Fast or Slow.*
Actual speed will depend upon the stage type so 'select and test' to find the most appropriate.

3: Click on *Properties* to reveal the Properties Dialog. Three tabs provide:

Speed: Similar to **(2)** above but on this tab X and Y speeds may be selected individually and the travel direction can be inverted.

Z-Axis: Allows the Mouse Wheel (if fitted) to act as a focussing control and sets the focus step size. To change it, click on the Step size window, press the Delete key to clear the existing value and type a new value in µms.

Limit Alarm: There is a small indicator at each of the joystick quadrants and also top and bottom of the focus slider. Normally they are green when the stage and the focus are within limits. If the Show Limit Alarm LEDs box is checked, the indicators will turn red as the travel limits are approached.

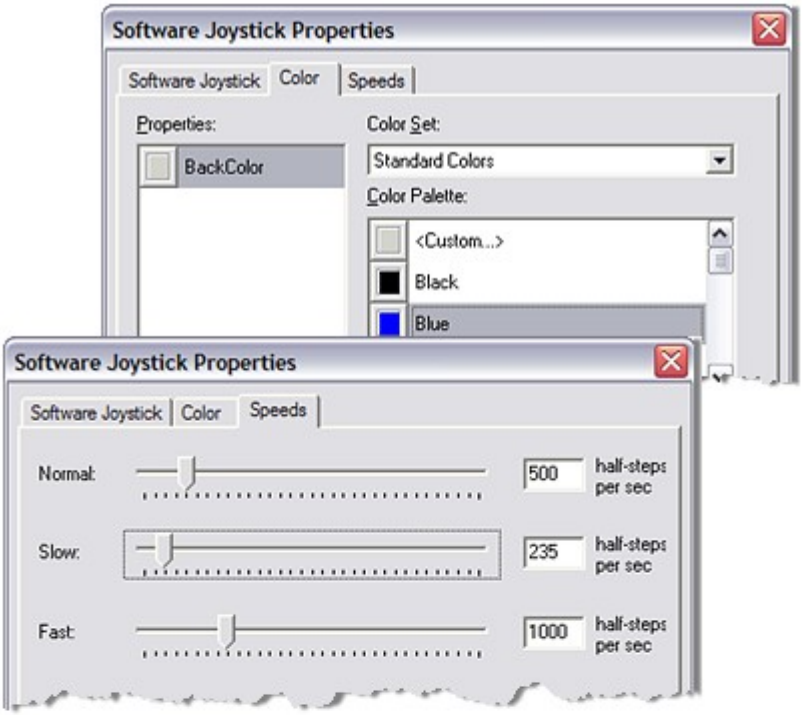
Nudge size: Each joystick quadrant also has a small arrow displayed. Clicking an arrow 'nudges' the stage in that direction. It is a very useful facility for precise positioning. Set the nudge value in µms by clicking the window, deleting the existing value and typing a new one.

Continued... 628

.

Color: The area around the joystick defaults to pale grey (silver) but may be changed by clicking to select a required color. Various color sets are available.

Speeds (Calibration): The three speed options may be calibrated individually by clicking and dragging the appropriate slider. Again, actual speeds will vary with the stage type: Change and test to achieve the best settings



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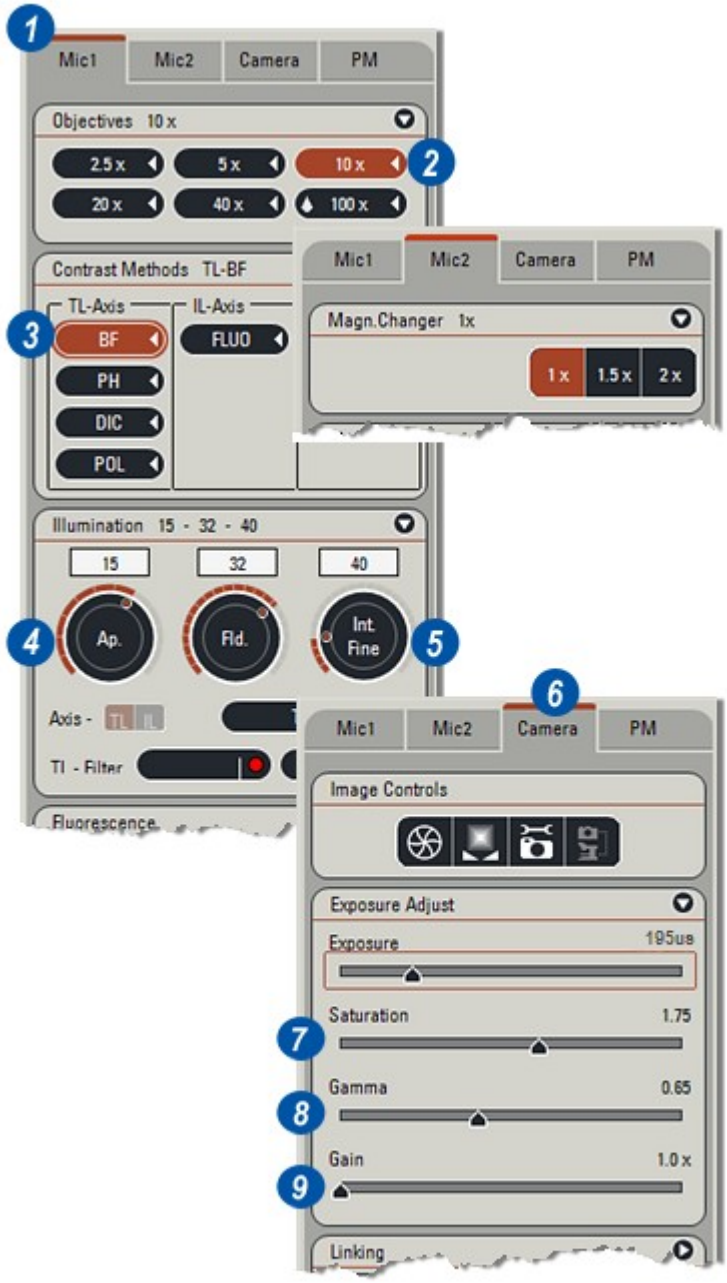
If Power Mosaic Turboscan is going to be used, the exposure times must be very short – typically less than 200µs (microseconds). This requires high light levels making some contrast methods unsuitable. If the required exposure times cannot be achieved, choose Standard scan instead for which light levels and exposure times are not critical.



1: Click on the appropriate Mic(roscope) tab and…

2: …select the required Objective, Magnification and…

3: …Contrast method.

4: Set the Aperture and...

5: …Intensity to suit specimen and scan type.

Often, a good image can be achieved quickly by setting basic exposure parameters, using Auto Exposure and then fine-tuning the result with white balance. This is especially suitable for Standard scan - Turboscan will probably require closer attention to exposure:

6: Click on the Camera tab to reveal the exposure controls.

7: Set the Saturation to 1.75 by clicking on the slider and dragging it – to the left to reduce the value and to the right to increase it.

8: Set the Gamma to 0.6 (1.0 for greyscale).

9: Gain should be as low as possible – start with a value of 1.

See: Acquire: Camera for more detailed information.

Continued... 630

The image must be properly focussed.

On the Histogram:

1: Set the *Black level* to 0 and…

2: …the *White level* to 255 by clicking and dragging the sliders.

3: Enable *Under/Over Exposure* by clicking the check box.

Run Auto Exposure:

4: Click on the *Auto Exposure* icon and then click again to turn Auto Exposure off.

Manual Exposure setting for Turboscan

5: Adjust the Exposure slider to give a reading of about 100µs. Gradually increase the light intensity (see previous page) until intermittent red flecking indicating slight over-exposure, appears.

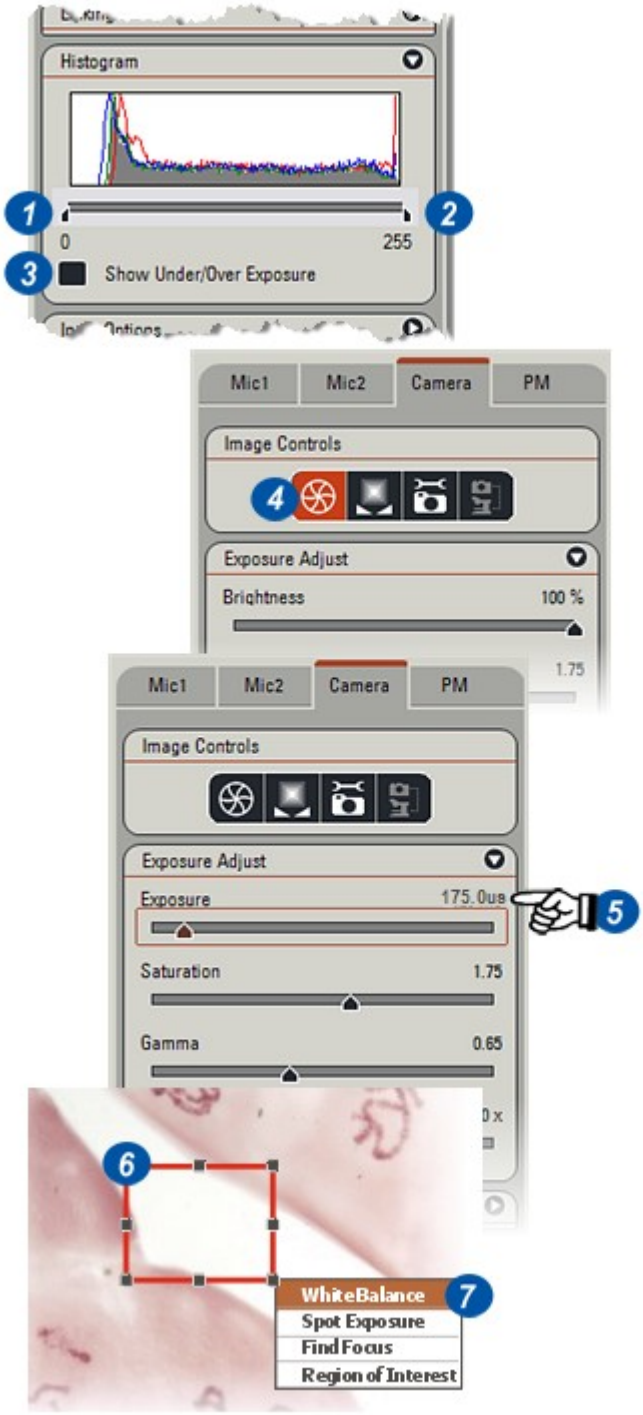
Set the white balance:

6: Click and drag a Region of Interest around a white area.

7: Select White Balance from the menu.

Fine tuning:

If necessary, fine tune the image with the Saturation and Gain controls.



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Selecting the Input Options:

1: Click on the *Camera* tab.

2: If the *Input Options* panel is concealed click on the arrow to the right of the header to reveal it.

The panel provides options for:

3: Using a pre-saved Input Options Configuration or creating a new one.

4: Selecting the *Image Type* – colour or greyscale.

5: Setting the *Colour Depth*.

6: Determining the *Captured Format* – how the image will be saved and stored on disc and…

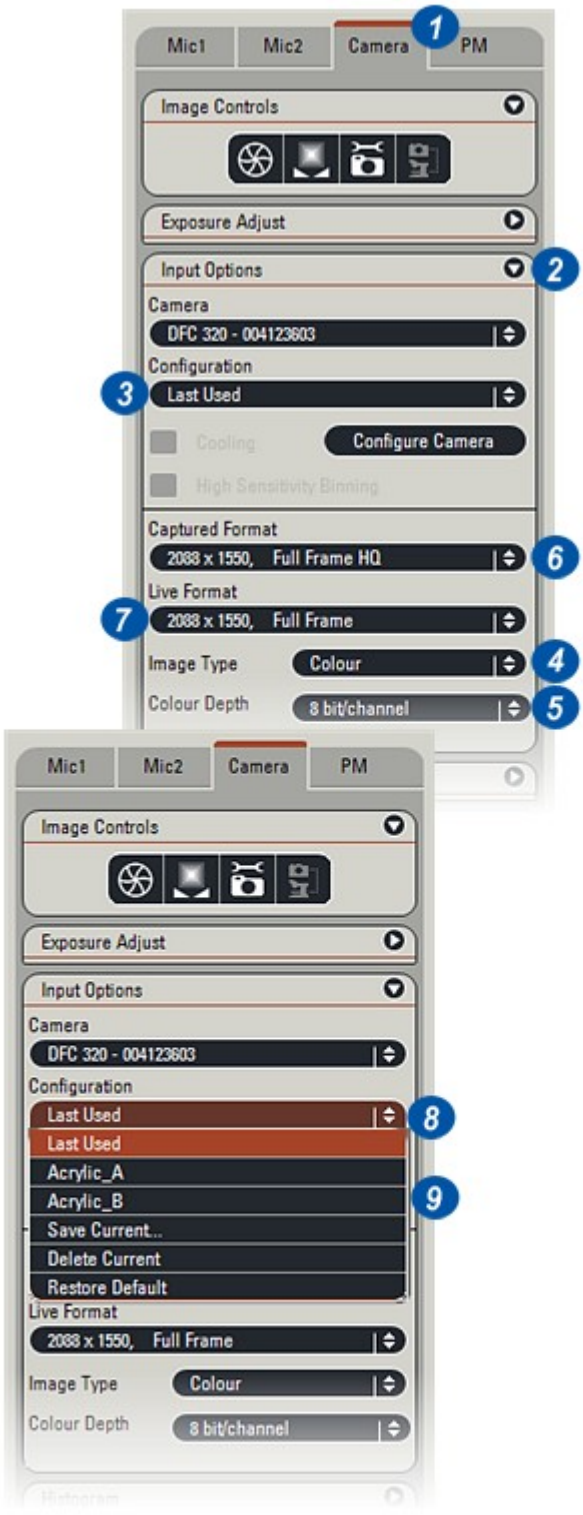
7: Selecting the *Live Format* – how the images will be displayed on the Viewer.

Selecting the Camera:
If Turboscan is going to be used, only designated high speed, progressive scan cameras with a trigger facility can be selected.

Choosing a Configuration:
If an Input Options configuration has been previously saved, it can be recalled from the Configuration menu.

8: Click on the arrows to the right of the *Configuration* header.

9: From the drop down menu, click to select the saved configuration. Each will have a unique name. All of the saved settings will be loaded and the remainder of the Input Options may be skipped.
A newly created configuration can also be saved using the *Configuration* menu and selecting the '*Save Current* ' option.



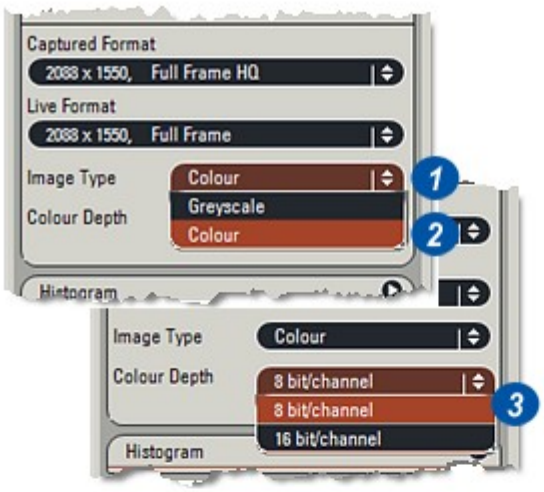
See: Acquire:Camera:Input Options for detailed procedures.

Continued... 632

Image Type:
Colour cameras may be used in either colour or monochrome (Greyscale) mode.

1: Click on the arrows to the right of the *Image Type* header and from the drop down menu…

2: …select either *Colour* or *Greyscale*. If Turboscan is being used with a Progressive Capture Format, the Image Type will automatically revert to Greyscale.



Captured Colour Depth:

3: Click on the arrows to the right of the *Captured Colour Depth* header and select 8 bit. Avoid the 16 bit option if it is available. It will greatly increase stored image size, slow the scan and may not be compatible with other image processing software.

Continued... 633

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Captured Format:
Power Mosaic has been designed to capture high quality images so avoid using the low resolution options. For Turboscan, select one of the Progressive options (but not VGA):

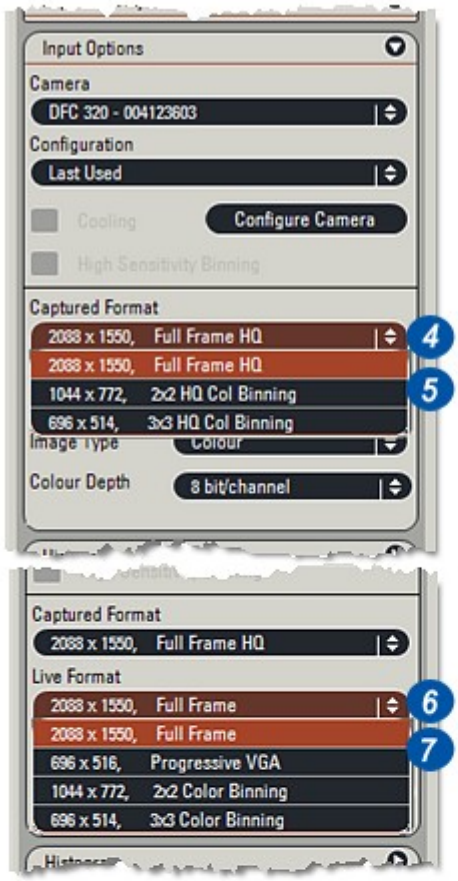
4: Click on the arrows to the right of the *Captured Format* header and from the drop down menu…

5: …choose the highest possible resolution. The 'binning' options will save disk space but are unlikely to improve scan speed. Use the navigation arrows to reveal other options.

Live Format:
Live format is not used during Power Mosaic, but for the sake of completeness keep the Live Format the same as the Captured Format.

6: Click on the arrows to the right of the *Live Format* header and from the drop down menu…

7: Select the same format as the *Captured Format*.



See: Acquire: Camera for more detailed information: 241

The Stage must be initialised the first time an objective is selected and again if the stage stalls for any reason.

Initialising determines the limits of travel in both the X and Y directions and 'matches' the stage speed to the objective.

Warning: Ensure that there are no obstructions before initialising – turn to an empty turret position and lower the sub-stage condenser.

To compensate for different stage types, ages, slackness and stiffness in the mechanisms as well as various imaging setups, the stage speed can be de-rated from its maximum. Lowering stage speed also helps prevent 'stalling' which results in a loss of initialisation values.

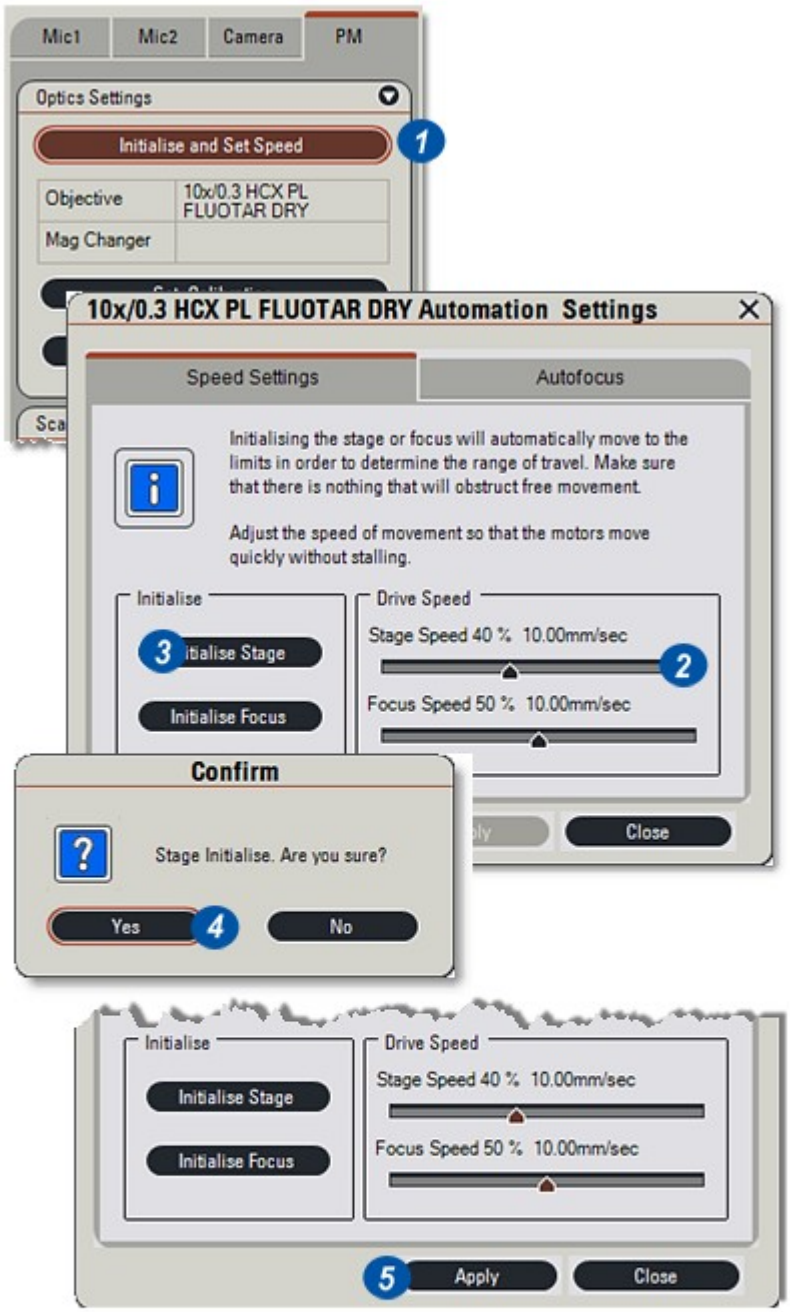
1: Click on the *Initialise and Set Speed* button.

2: On the *Automation Settings* dialog, set the Drive Speed slider to a high value - start at 90%.

3: Click the *Initialise Stage* button.

4: Click *Yes* on the *Confirm* message. If the stage stalls, reduce the speed and re-initialise.

5: After initialisation completes successfully, reduce the speed by 10% and click on the *Apply* button.



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The speed of the focus drive mechanism can be adjusted below its maximum to allow for possible overrun and minimise shaking.

Additionally, the limits between which the focus mechanism can operate are set to prevent the specimen colliding with the objective (Upper) and allow sufficient clearance for the specimen to be accessed (Lower).

Select Live Image and focus.

1: Click on the *Initialise and Set Speed* button.

2: On the *Automation Settings* dialog, adjust the *Focus Speed* by moving the slider. Determining the actual value will depend upon the microscope and the imaging setup and may require some trials.

3: Click on the *Initialise Focus* button.

4: Set the *Upper Focus Limit* (relative to the current position) by clicking on the text box and entering a new value. For example, to limit the focus position to 100um above the current position, type in '100'.

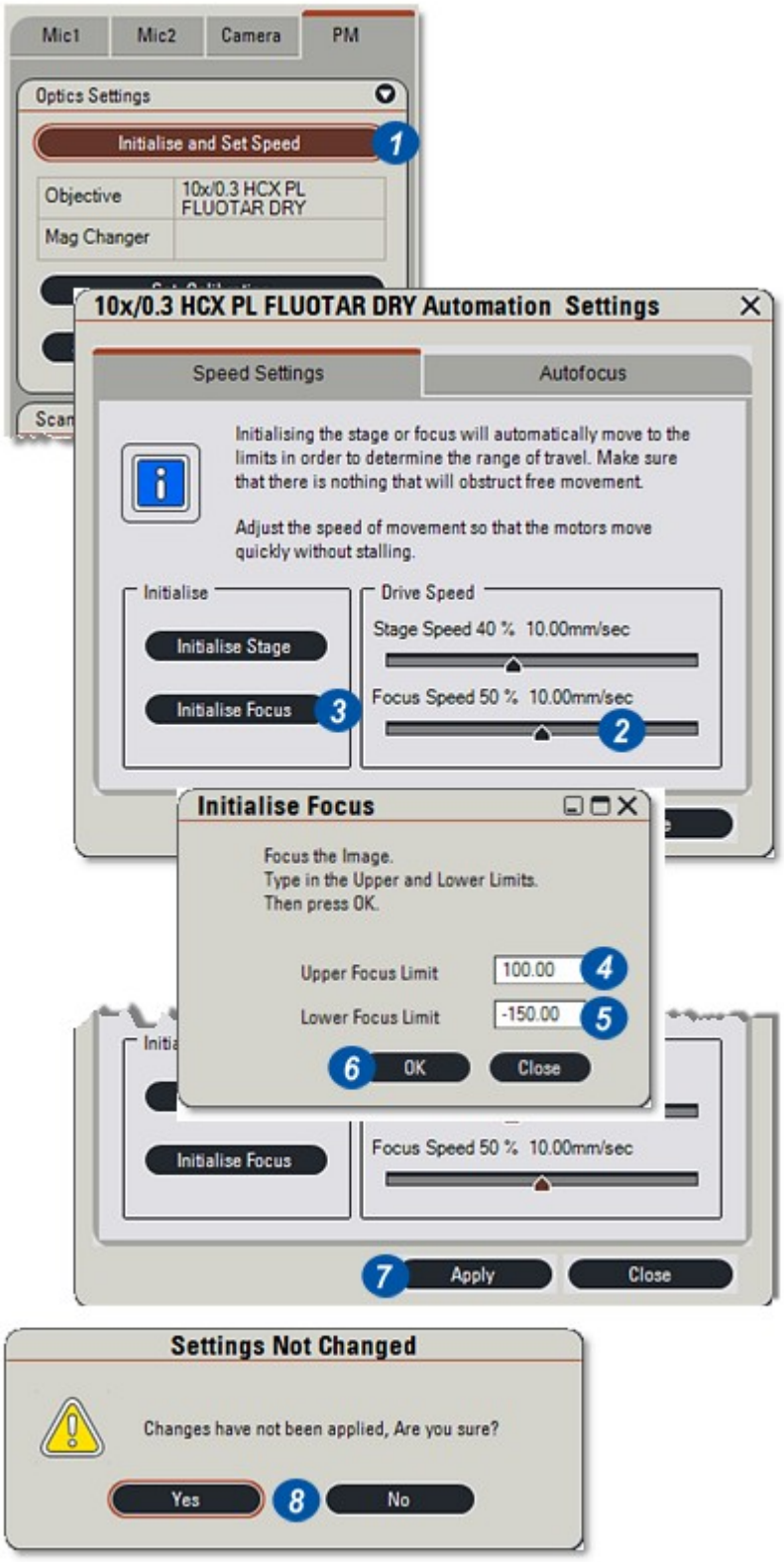
5: Set the *Lower Focus Limit* in the same way. Positive numbers will be automatically converted to a negative value so no need to type the leading sign.

6: Click *OK.*

7: Click *Apply* to apply the settings and Close to save them.

8: If Apply is not clicked, the *Settings Not Changed* message appears. Click *No* to apply the new settings

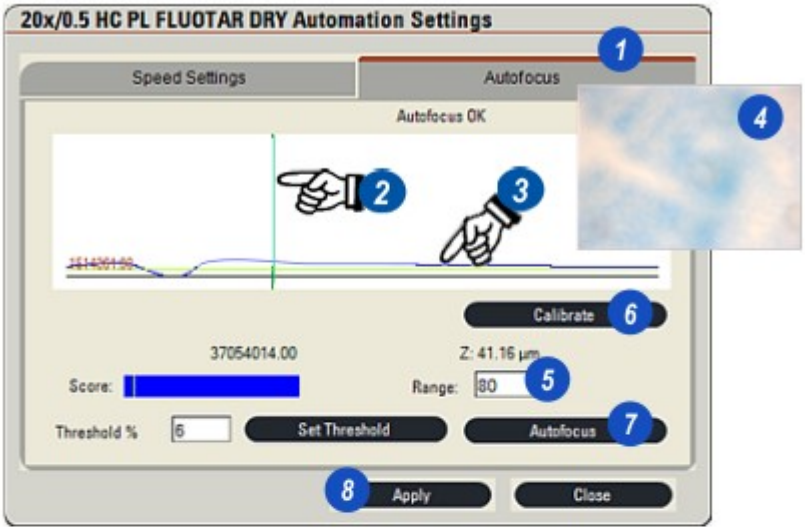
Go to Autofocus Setup: 636



Auto Focus initialisation sets the travel range of the focussing mechanism and also the threshold value for focussing ' success'.

Select the split screen display option.

1: Click on the *Auto Focus* tab.



2: The current stage position on the Z Axis is shown by a vertical green line.

3: A blue horizontal line represents the *Focus Curve*.

4: Using the on-screen Joystick, SmartMove or stage controls, navigate in the live image pane to a part of the specimen that contains some detail, avoiding repetitive or symmetrical patterns. It may be helpful in 'proving' the Auto Focus effectiveness to de-focus the specimen.

5: The *Range* value (in microns) determines the travel limits of the focussing mechanism. It reflects the thickness of the specimen. Click in the Range text box and enter a value – the mechanism will drive upward from the current stage Z position by 50% of the value, and downward by an identical distance. Between these limits, Auto Focus will achieve the best focus possible.

6: Find a field on the image that can be sharply focussed and click the *Calibrate* button. This will measure any drive backlash and store the value to be used with the current objective.

7: Click the *Apply* button. *ALWAYS click Apply when changing values.*

8: Click on the *Auto Focus* button.

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1: If there is sufficient detail and contrast in the specimen area chosen, the curve will look similar to this centred about the new stage Z Axis position, and…

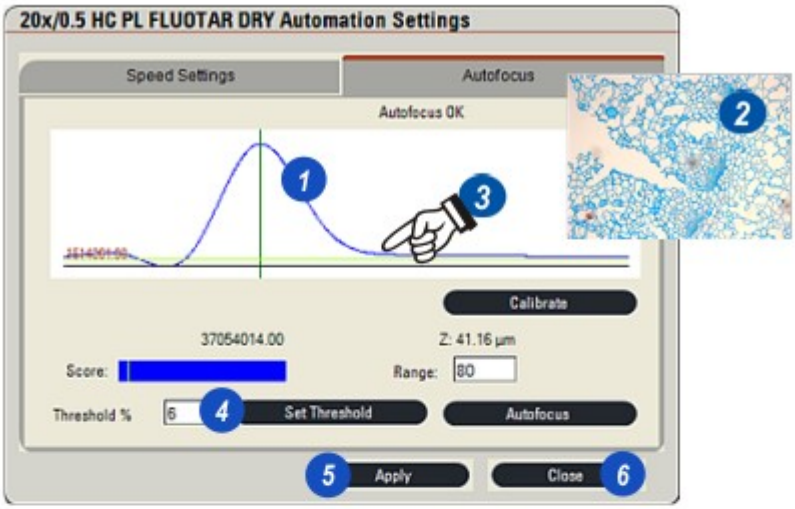
2: …the image should be sharp. The *Score* bar will extend across to the right.

3: The *Threshold* value represents a difference (%) of the lightest to darkest pixels in the image. For Auto Focus to 'succeed' the calculated value must be at or above the Threshold. Below the Threshold and there probably was not sufficient detail to guarantee accuracy.

4: Set the *Threshold* by clicking in the text box, typing a new value and clicking the *Set Threshold* button.

5: Click *Apply*. The Threshold line will be drawn to reflect the new value.
After changing Threshold repeat Auto Focus to ensure that the value is not so high it prevents focussing.

6: Click *Close.*



Note: In the unlikely event that the Z focus shows at the peak but the Score indicates something less than peak, gearing backlash could have caused the discrepancy. In this situation change the Z scan direction to 'Up' (bottom to top of specimen). Please refer to the Oasis manual.

Input options and lighting levels must be set and the specimen in place.

The Calibration process is automatic: It will determine the 'pixel/micron' value for the objective being used. As objective magnification increases so the actual field of view on the specimen decreases. Calibration correlates the field dimensions (microns) to the number of camera elements (pixels) required to capture it. It must be carried out at the start of a session, and whenever the optics are changed.

Calibration also checks for camera rotation – the angle of rotation from the Stage X and Y axes.

1: Select the *Live Screen* view.

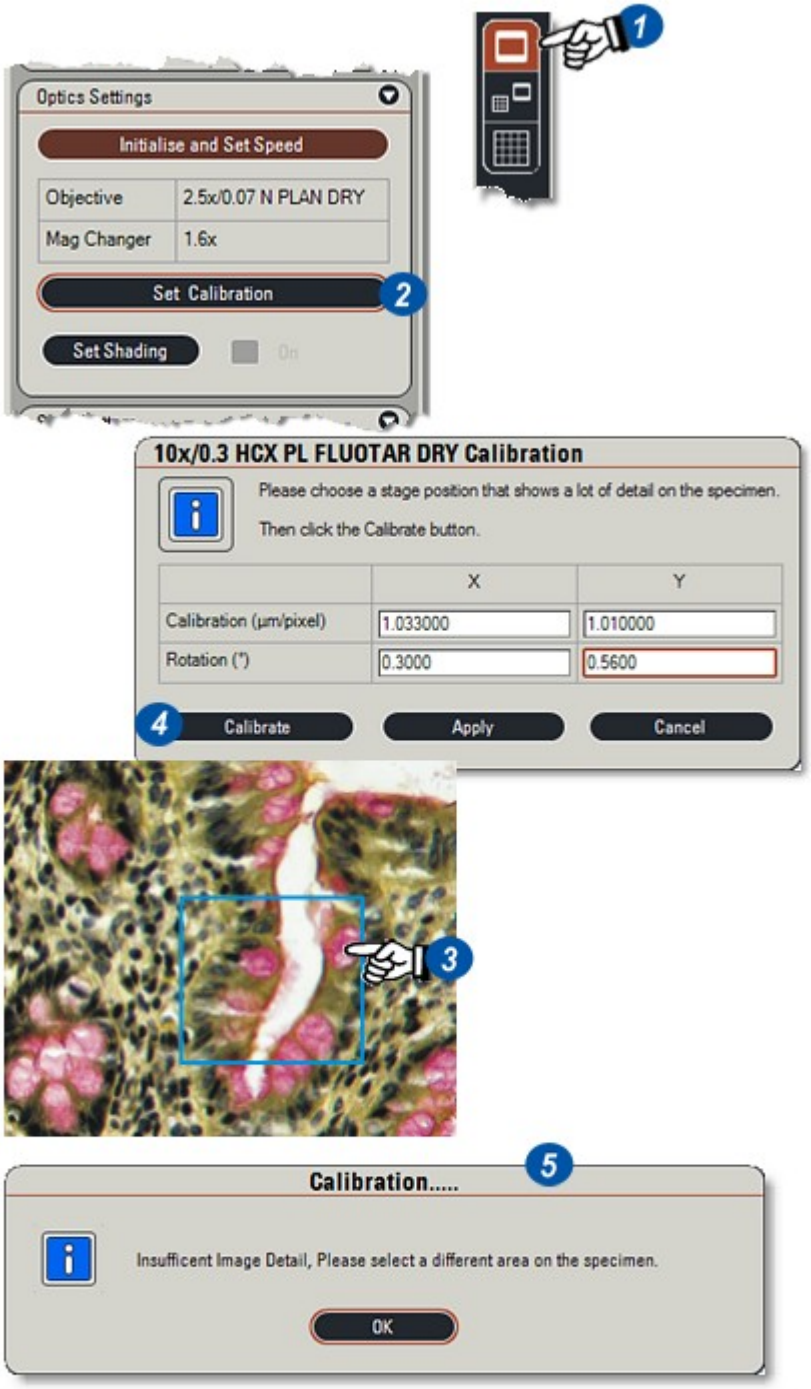
2: Click on the *Set Calibration* button. When the Calibration Dialog appears…

3: …a small rectangle is displayed on the live image. Use the on-screen joystick or stage controls to navigate to a well defined and detailed region of the image lying within the rectangle. Avoid uniform patterns.

4: Click on the *Calibrate* button.

5: If there is insufficient detail in the selected region a warning will appear. Repeat the process from Step **(3)**.

Continued... 639



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The values for camera rotation returned by the Calibration, relate to the stage X and Y axes. If the rotation is excessive a warning will be displayed **(2).**

Rotation for both axes should not exceed 0.10 degrees **(1).**

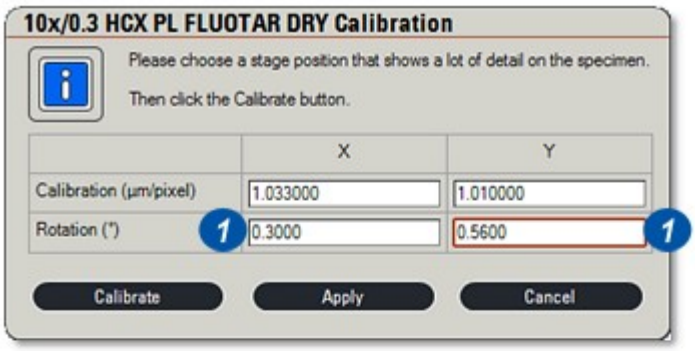
Check that the camera mount is secure.

Loosen slightly the camera clamp **(3)** so that the camera may be rotated.

Rotate it by very small amounts and repeat the calibration until the rotation angle is within limits.

Tighten the clamp.

Once set correctly and providing the camera and stage do not change, rotation should seldom require adjustment.



The Shading Reference electronically corrects any light level fall-off toward the edges of the image that is often inherent in optical systems.

The exposure, light intensity, required objective and mag level must all be properly set with the specimen in place. If there is a change in objective, the Shading Reference must be carried out again.

Select Live Screen view:

1: Navigate to a portion of the image that is free of detail with a clear background.

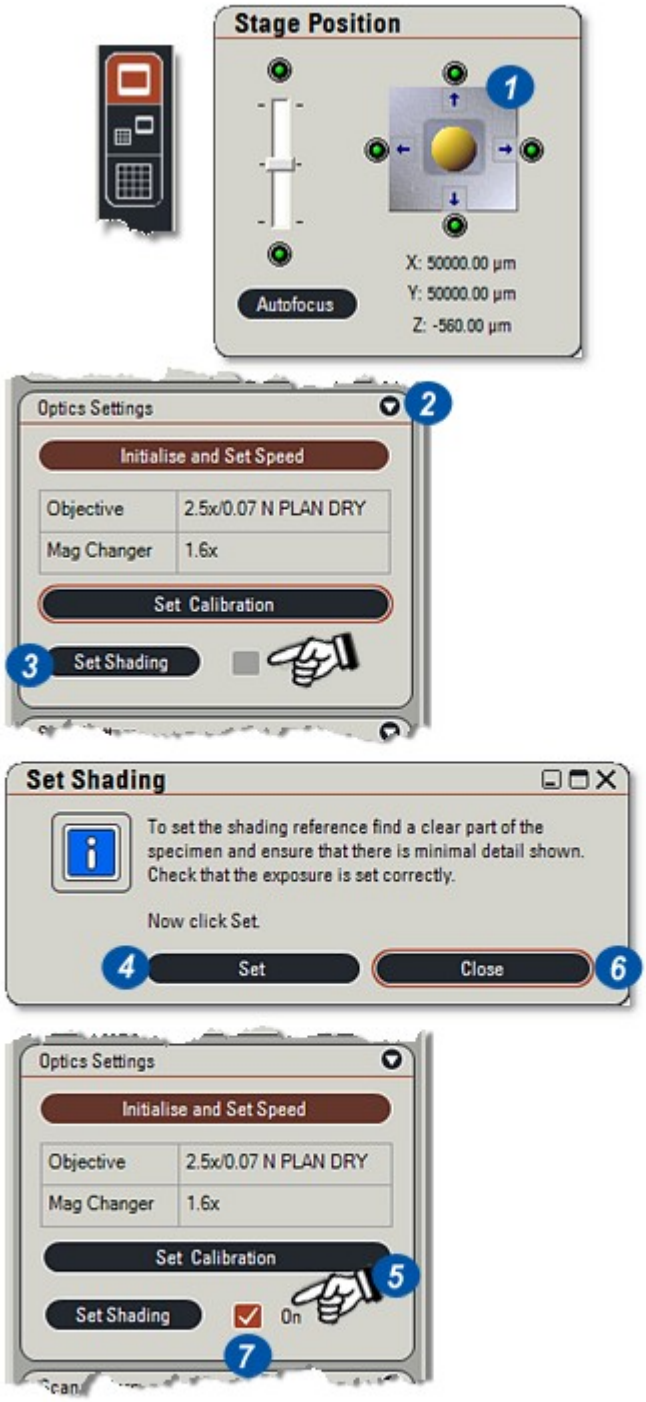
2: If necessary, click on the *Optics Setting* header to reveal the panel. If a Shading Reference has not been carried out, the check box will appear greyed out.

3: Click the *Set Shading* button and…

4: …on the dialog click the *Set* button.

5: When complete, the *Set Shading* box **(7)** will be checked and shading will be enabled.
If required, it can be disabled by clicking the check box.

6: Click the *Close* button on the dialog.



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A Scan Pattern configuration may be saved for future recall as a fast and accurate way of loading settings.

To retrieve and automatically load a saved configuration:

1: If necessary, click on the arrow to the right of the *Scan Pattern* panel to reveal it.

2: Click on the *Load* button.

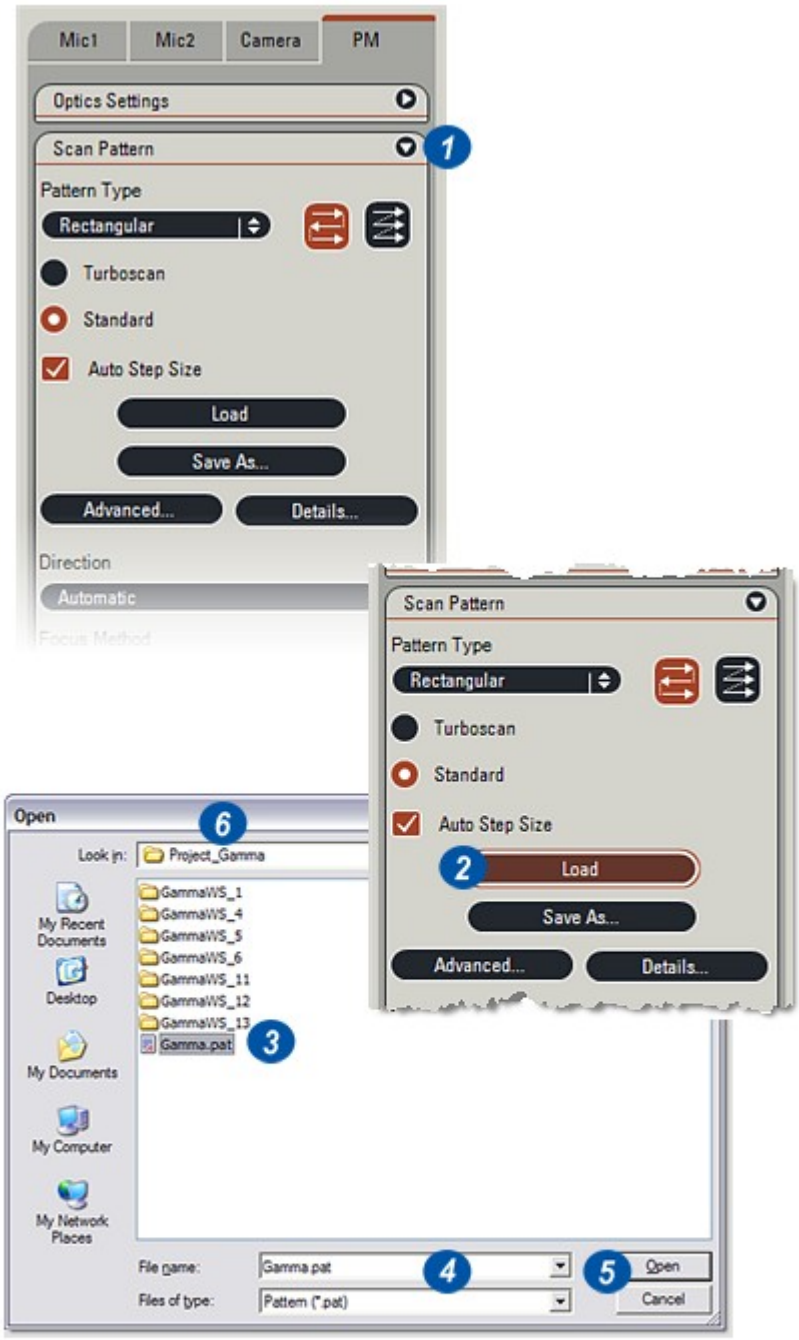
3: On the *Open* dialog, click to select the configuration required – the selected file name appears in the window **(4)**. Scan Pattern configuration files have the file extension *.pat*.

5: Click on the *Open* button.
Only the Scan Pattern settings are loaded; Optics and Z-Stack settings must be adjusted for each session.

6: The Folder defaults to the selection made either in *Preferences* or *Browse* . Use Windows navigation to open an alternative folder.

See: Preferences:Save in Directory:

See: Browse:Select Default Directory.



There are two scan pattern options which govern the stage tracking as the image tiles are collected – bi-directional and uni-directional.

The bi-directional pattern scans left to right, moves down to the next row, reverses and scans right to left.

The uni-directional pattern scans left to right, returns to the left, moves to the next row and scans from left to right again. (Depending upon the specimen shape the scan direction may be from top to bottom).

1: Choose bi-directional for speed.

2: Choose uni-directional for greater accuracy.

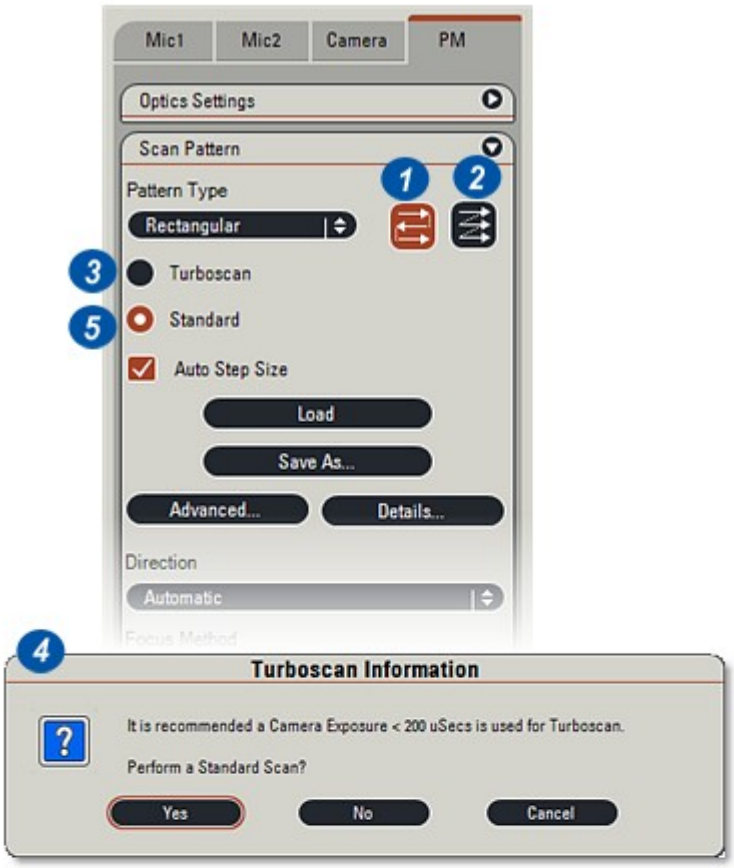
The selected check box is coloured red.

Select Scan Speed:
Turboscan is the fastest way to capture a image, but it does require a very fast exposure time - 200µs (micro seconds) or less and a progressive scan camera with trigger facility. During Turboscan, the stage moves continuously – it does not stop to make an exposure – which is why a short exposure is needed.

During Standard scan the stage halts at every tile position so the exposure time is immaterial. Standard scan can also operate with non-progressive cameras and does not require a trigger facility.

3: Choose *Turboscan* for speed but only if the exposure time is less than 200µs. Trying to scan with a higher exposure time will display the warning message **(4).**

5: Click to select *Standard* scan speed for exposure times greater than 200µs and non-progressive cameras.



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The Step Size represents the distance between adjacent tiles in µm (micro-meters). Normally, Auto Step Size is enabled to allow mosaic creation, and the program calculates the number of tiles required to cover the image on the basis that they will all abut adjacent tiles **(2).** Turning off Auto Step Size allows the step to be adjusted so that tiles may be overlapped or spaced apart **(8).**

1: Enable *Auto Step Size*. The check box will be coloured red with a tick. Auto Step Size must be enabled for a mosaic to be created.

2: The tiles are butting and arranged to cover the image. In these examples the Cross Scan Pattern has been used for clarity.

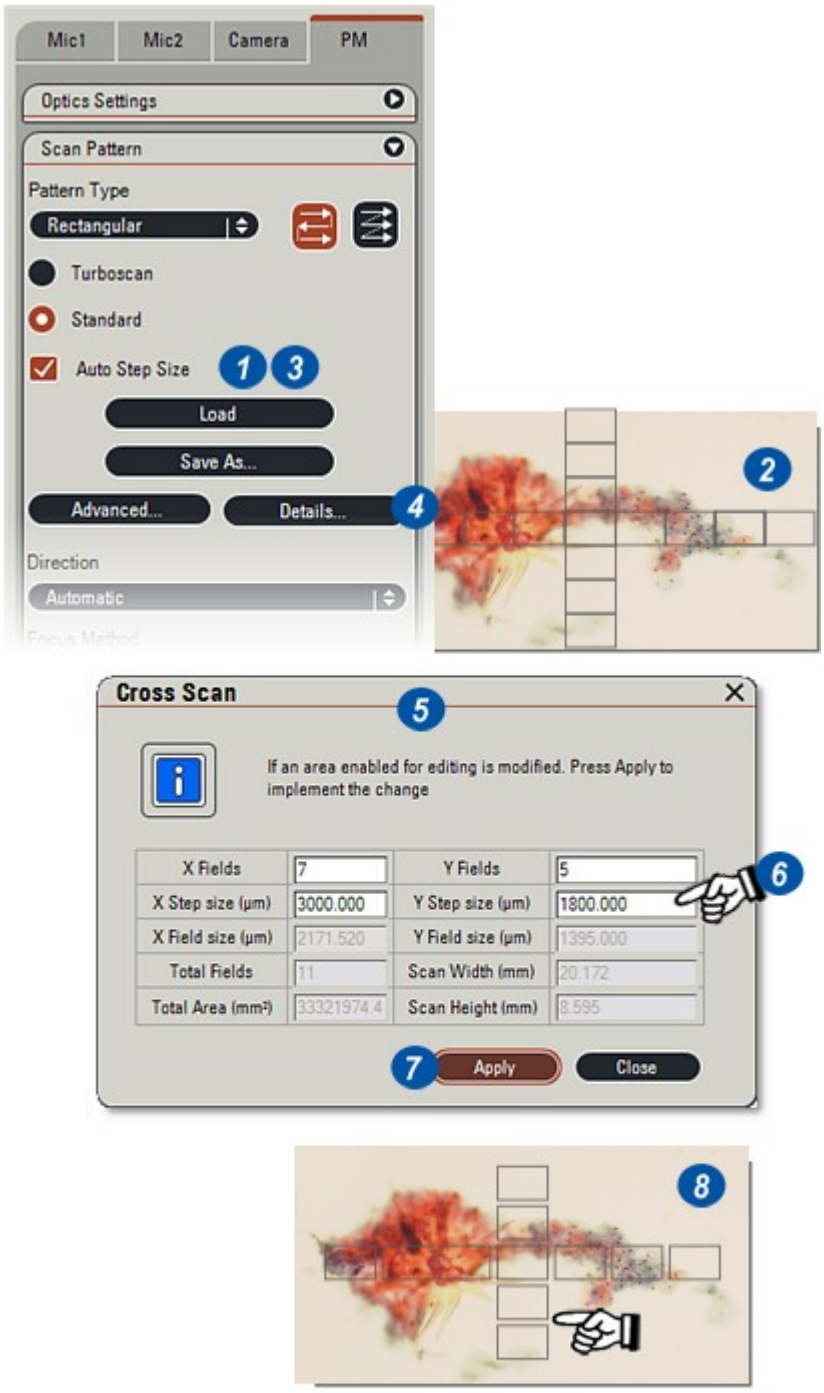
3: Click to disable *Auto Step Size*. The check box becomes grey.

4: Click the *Details* button.

5: The *Details Dialog* appears with…

6:…the tile X and Y co-ordinates enabled for editing. To change the step size, click on either the X or Y text box and type a new value.

7: Click *Apply*. If necessary, re-enter the step values to get the required spacing or overlap **(8).**

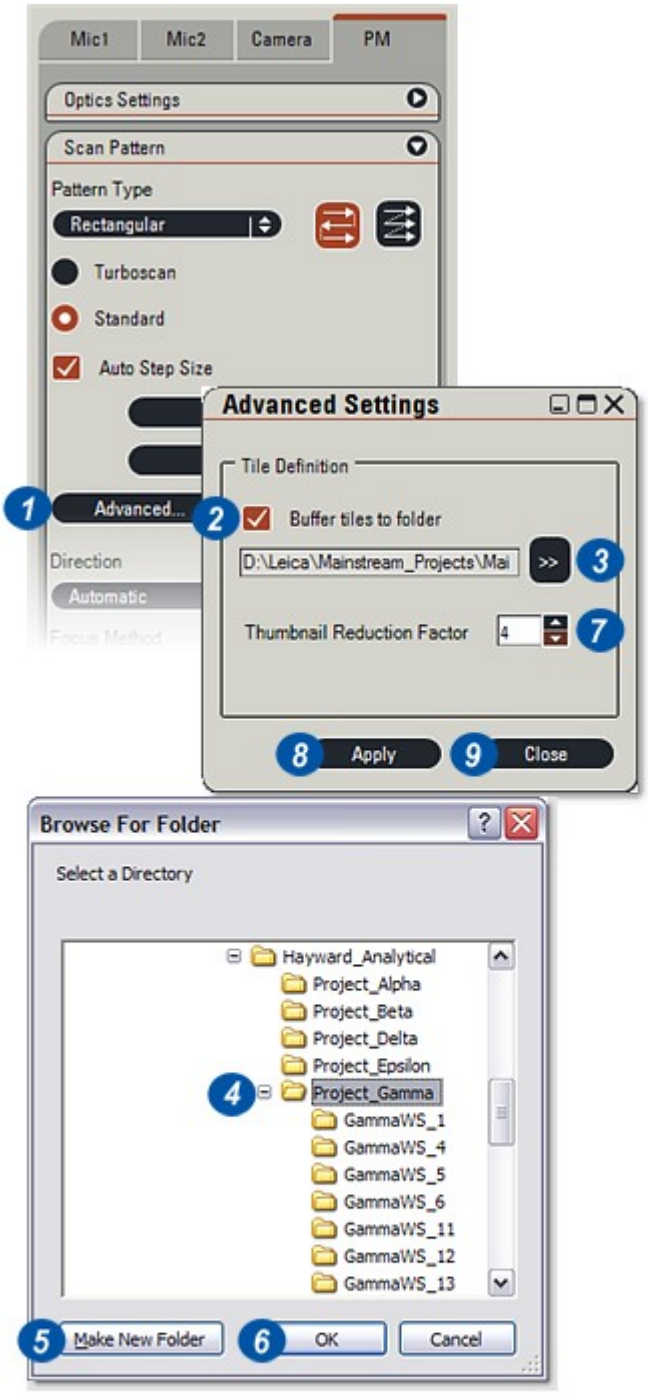


The Advanced options allow individual image tiles to be saved temporarily (Buffered) to a nominated folder and, if they are saved, set the size of the thumbnails in that folder.

Saving tiles has the advantage of identifying and keeping relevant parts of the image, and discarding less important parts so saving valuable disc space.

It is important that there is sufficient disk space to accommodate all of the images otherwise the scan will stop. The recommended approach is to have a partitioned section of the disk – D: PMTemp for example – in which to buffer the images. Make sure that computer privileges extend to the partition.

Thumbnail size is also important in that they are initially stored in RAM for fast access. If the thumbnails are too large, volatile RAM becomes clogged and the speed advantage is lost.



1: Click on the *Advanced* button.

2: On the dialog click to enable or disable tile buffering.

3: If tile buffering is required, click on the *Browse* button to reveal the *Browse for Folder* dialog.

4: Click to select a folder or…

5: …create a new folder. Use Windows navigation to reach other levels or directories.

6: Click *OK.*

7: If necessary, use the arrows to the right of the *Thumbnail Reduction Factor* text box. The larger the number the greater the thumbnail detail – and also the amount of disc space occupied.

8: Click *Apply* and…

9:…click *Close.*

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The stage Scan Direction may be either side-to-side or front-to-back. The options are:

Automatic: Allow the software to determine the best direction,

Horizontal: Select side-to-side, or

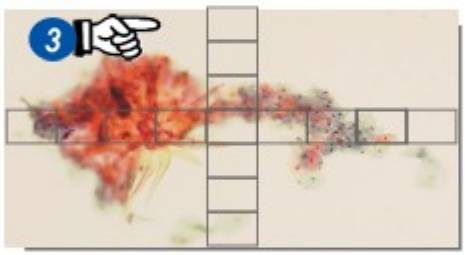
***Vertical*:** Select front-to-back.

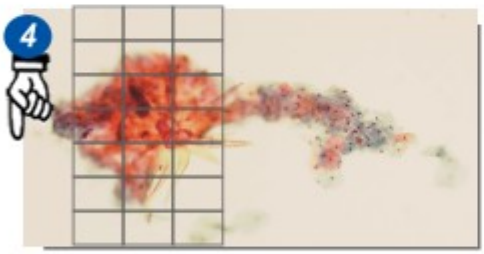
Generally, Automatic is the best option to ensure the most efficient scan, especially when Turboscan is being used.

1: Click on the arrows to the right of the *Direction* header to reveal the options drop down menu.

2: Click to select the required direction.

Although both specimens **3** and **4** illustrated are the same, a vertical scan on **Figure (4)** would be best because there are fewer direction changes.





Select Pattern Type:
There is a wide range of scan patterns available designed to provide total flexibility and efficiency in the capture and storage of images.

Each pattern type can be configured to best suit the task in hand. On the following illustrations, each small rectangle represents a separate tile.

1: Click on the arrows to the right of the *Pattern Type* header.

2: From the drop down menu select the pattern required.

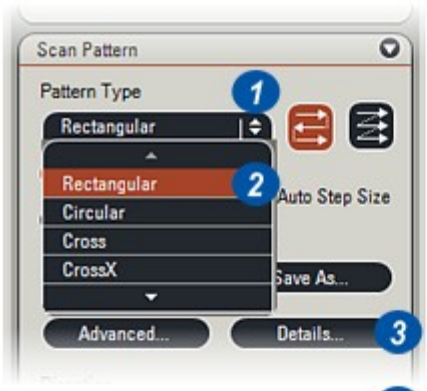
3: Click on the *Details* button. This opens the appropriate pattern dialog **(4).**

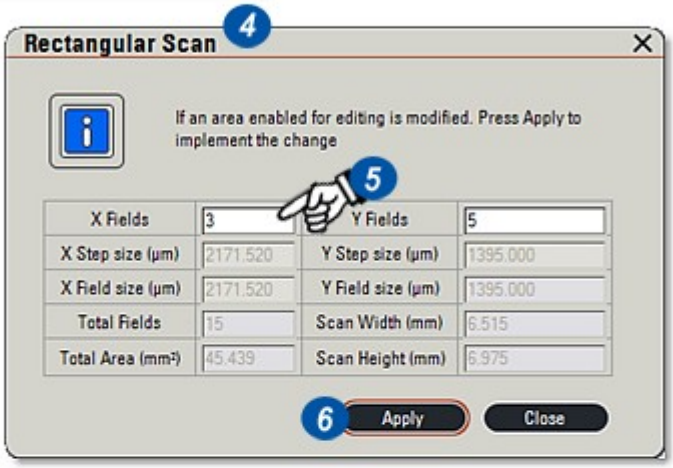
5: For the *Rectangle Pattern* **(7)**, the X and Y tile counts are available for editing. Click in the X or Y text box and enter a new value.

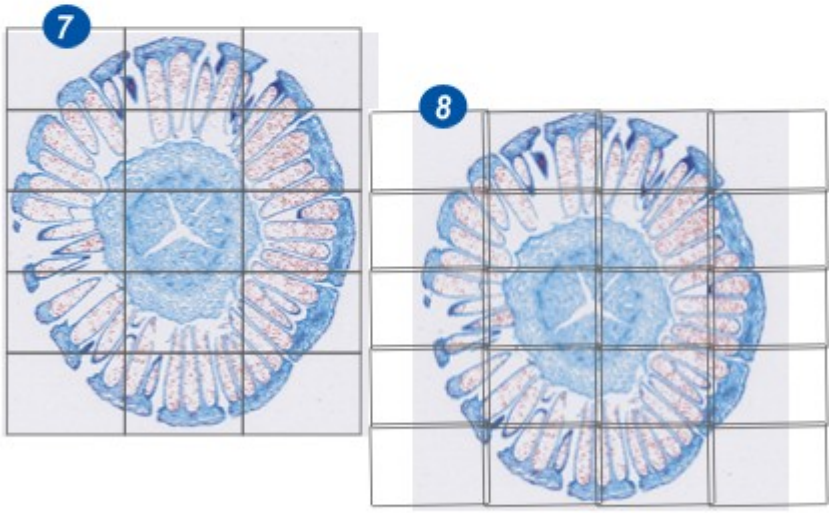
6: Click on the *Apply* button and the new configuration is applied to the image. Repeat steps **(5 and 6)** until the pattern is suitable.

For clarity, the tile layouts on the following pages are shown neatly abutting each other. However, if the camera rotation is not perfect the tiles may appear at an angle and possibly overlapping **(8)**. The software has been designed to accommodate these variations.

See: Create the Pattern Grid: 657







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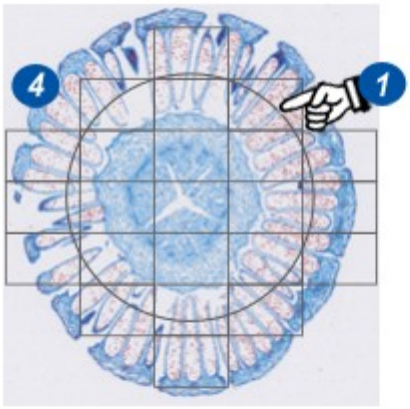
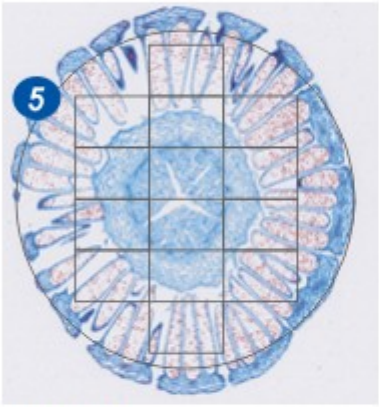
The following pages illustrate the Pattern Types available and the options for each.

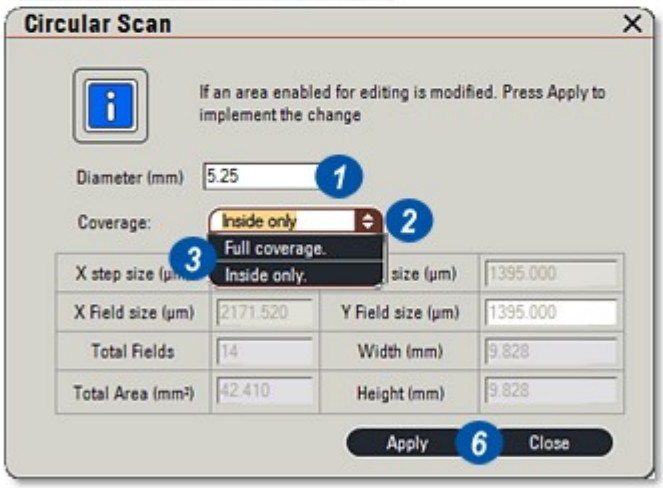
Circular Pattern Type:
The options are:

Full Coverage and

Inside only.







1: Click on the *Diameter* text box and enter a value for the circle diameter in millimetres. The circle does not have to cover the entire image.

2: Click on the arrows to the right of the *Coverage* text box and…

3:…select either *Full Coverage* **(4)** or *Inside only* **(5)** from the drop down menu.
Full Coverage covers the entire circle with overlap where necessary.
Inside only places tiles within the circle.

6: Click *Apply* to apply the values to the image. Repeat from Step **(1)** if necessary to adjust the pattern.
Click *Close* to save the pattern and exit.

Cross and CrossX Pattern Types:
The options are:

X and Y tiles for the Cross pattern:

Tile count only for the CrossX pattern*:*

1: For the *Cross* pattern:

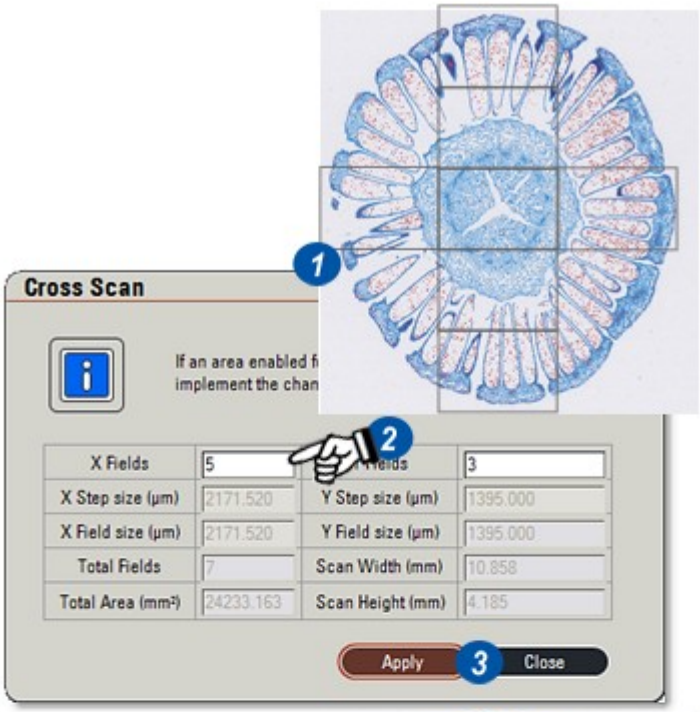
2 Click on the *X or Y text boxes* and enter a value. The X value represents the horizontal tile count and the Y value the vertical count.

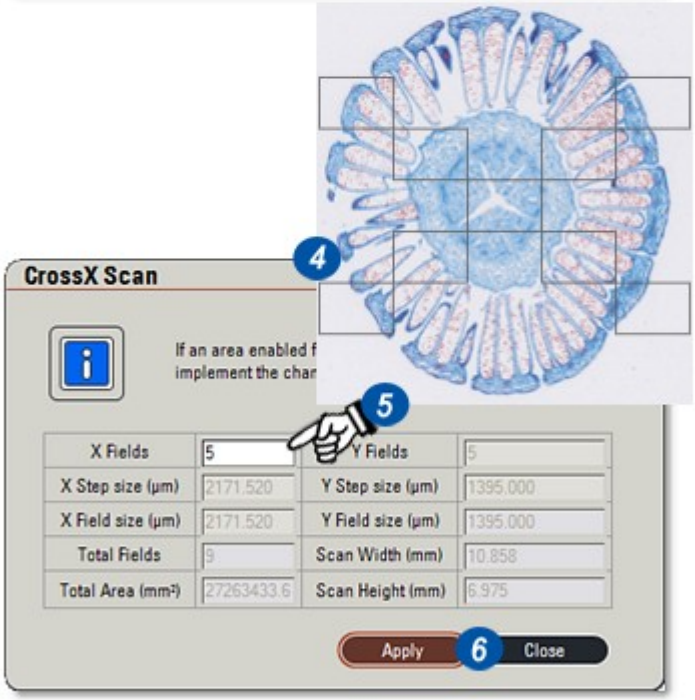
3: Click *Apply* to apply the settings.
Repeat from Step **(2)** to change the pattern.
Click *Close* to save and exit.

4: For the *CrossX* pattern which is a regular X:

5: Click on the *X text box* to enter a new total tiles values.

6: Click *Apply* to apply the setting.
Repeat from Step **(5)** to change the pattern.
Click *Close* to save and exit.





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Random and Random without overlap patterns:
The options are:

Random the number of tiles some of which may overlap:

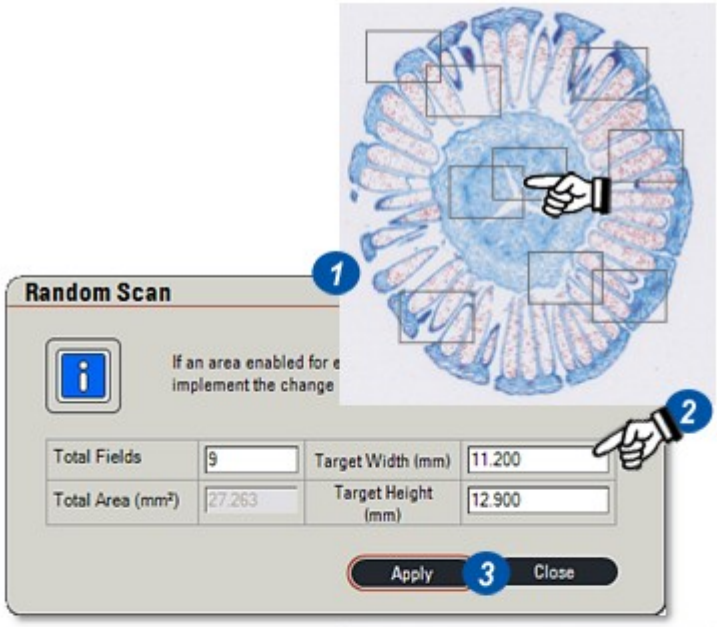
Random without Overlap the number of tiles none of which will overlap:

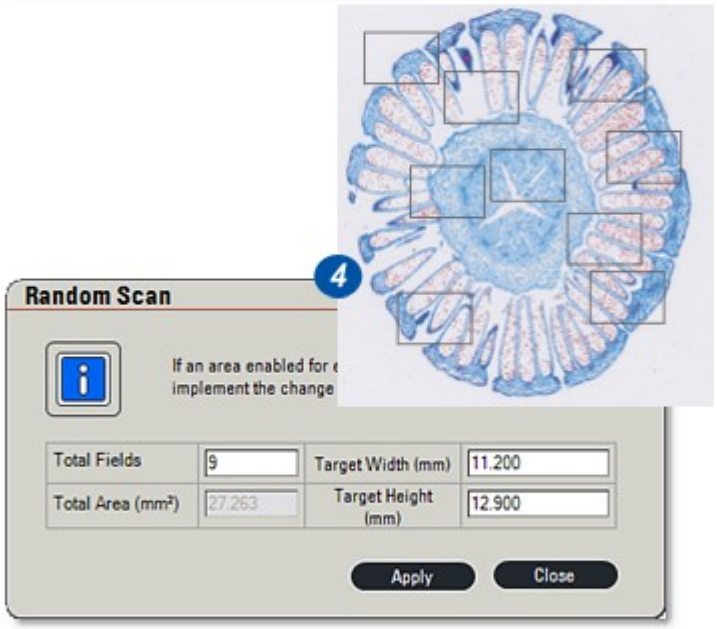
1: *Random* pattern creates a specified number of tiles randomly inside a specified boundary. Some of the tiles may overlap.

2: Click on the *Target Width and Target Height* text boxes and enter values for the scan area boundary. The scan area does not have to cover the entire image.
Click on the *Total Fields* (tiles) text box and enter a value for the number of tiles.

3: Click *Apply* to apply the settings.
Repeat from Step **(2)** the change the values.
Click on the *Close* button to save and exit.

4: *Random Pattern without Overlap*:
This option is the same as Random except that none of the tiles will overlap.





Annular:
Options are:

Full Coverage and

Inside only.

This option automatically populates an area defined between two concentric circles with a calculated number of tiles.

1: Click on the *Diameter* text box and enter a value for the outer (larger) circle in millimetres. The circle does not have to cover the entire image.

2: Click on the *Inner Diameter* circle text box and type a value for the smaller circle (shown coloured on the illustrations for clarity). It must be smaller than the outer diameter otherwise the settings will be ignored.

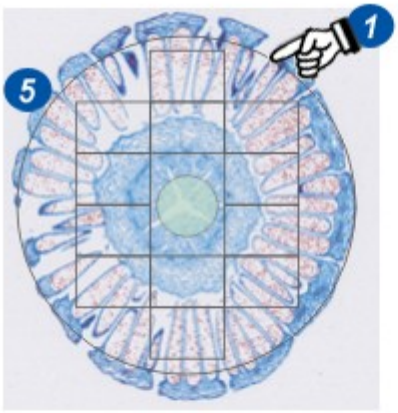
3: Click on the arrows to the right of *Coverage* text box and from the drop down menu…

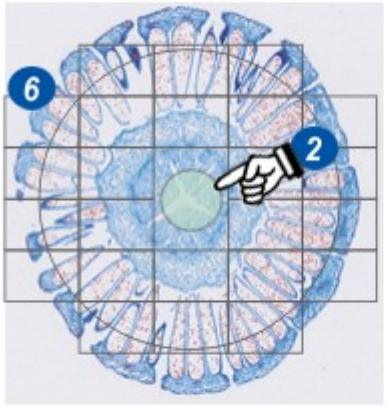
4: …click to select either *Full Coverage* or *Inner only*.

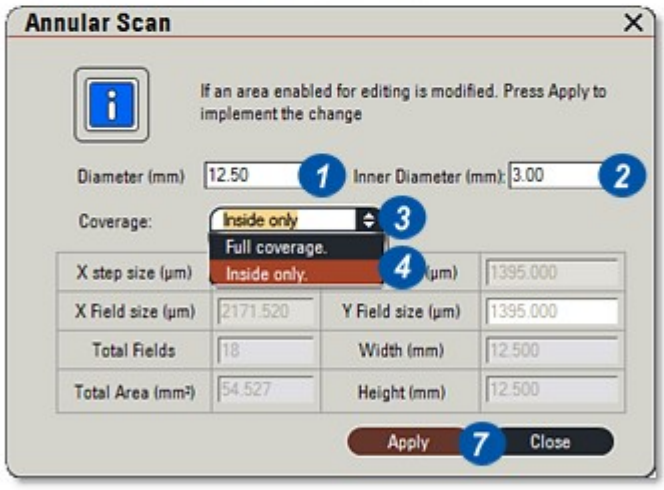
5: *Inner only* places the tile within the outer circle avoiding inner circle.

6: *Full Coverage* ensures the entire outer circle is covered without including the area of the smaller circle.

7: Click *Apply* to apply the settings.
Repeat from Step **(1)** to change the values.
Click on the *Close* button to save and exit.







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Three automatic focus methods are available:

Predictive Focus: which uses a set of known focus points to interpolate (predict) any unknown points. Predictive Focus is fast with good results but should be used only when the specimen is either flat or has a uniform, slope across focus points. Use objectives up to 10x or for very flat specimens up to 20x.

Autofocus: uses contrast differences in groups of adjacent pixels to establish sharpness. Because it is a continuous process of focussing and checking, Autofocus is slower than Predictive but yields extremely good results. especially over irregular specimens.

Predictive and *Autofocus:* in combination uses the speed of Predictive and the quality of Autofocus to achieve very good results, quickly. Use for specimens that are predominantly uniform but also have local irregularities. Predictive will come close to focus and the Auto will fine tune it.

Both *Predictive* and *Autofocus* methods and the combination are selected by enabling the check boxes **(1).**

When a method is enabled its setup button becomes active **(2)**. Setup is explained on the following pages.

For perfectly flat specimens both methods can be turned off.

Continued... 652

The Predictive Focus function, focuses on a number of predetermined points on the image and creates a 'table' of their focus values. The focus position for any other point can be predicted by interpolating the table values.

The greater the number of pre-determined points then the greater the accuracy of the prediction and better the overall focus. This is especially true for very specimens that have irregular focus.

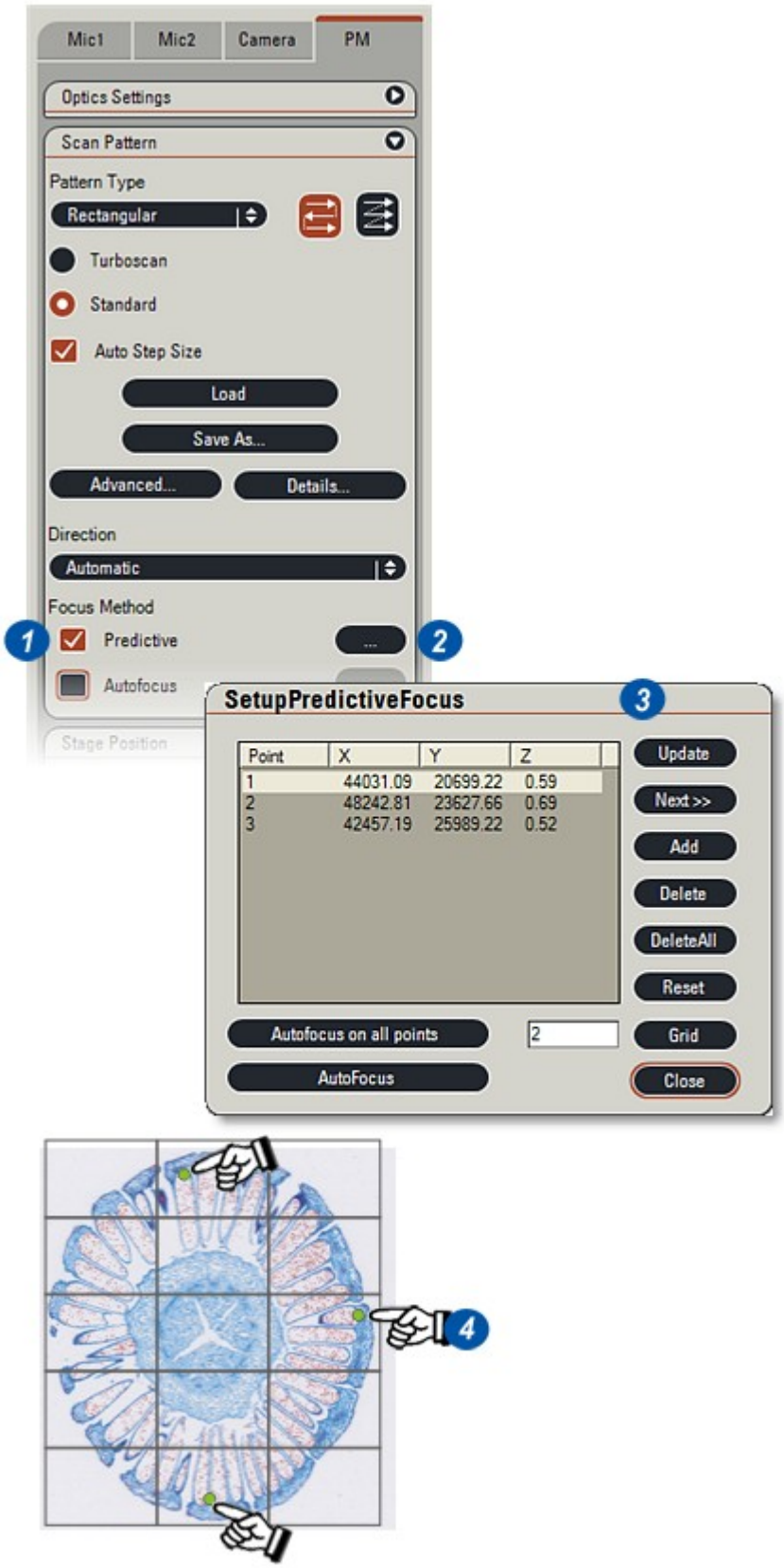
1: Click on the *Focus Method: Predictive* checkbox to enable it.

2: Click on the *Setup* button to display…

3: …the *Predictive Focus* dialog.

4: Three pre-determined points are automatically placed on the image. Their positions are displayed in the X/Y columns on the dialog. Normally, the set points will be coloured red to indicate that they require focussing. If they are green and have a value in the Z column denoting that they are already focussed, possibly as the result of a previous scan, the points should be focussed again.

Continued... 654



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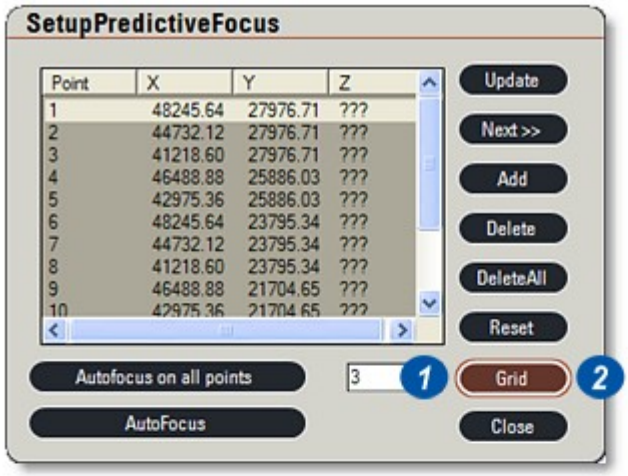
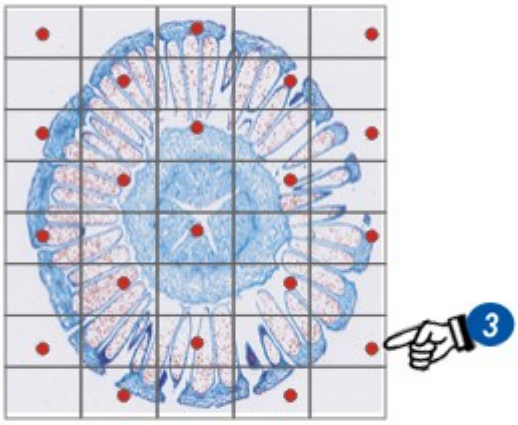
The Grid feature automatically creates a regular pattern of points across the image. The number of points can be established by typing a value.

1: Click on the *Grid* text box to high light the existing value. Press the keyboard delete key to remove the value. Type a new value.
The value represents the number of points that will be created along the top row of the grid. On the second row the number of points is reduced by one. The next row is increased by 1 and so on across the entire grid. In the illustration, a value of 3 has been typed.



2: Click on the *Grid* button and the new points will be created and listed on the dialog.
Points sitting on completely clear areas of the image **(3)** will result in a failed Autofocus because there is no detail to focus on. To avoid a failure, either delete the points *(See: Delete a focus set point)* 654 or adjust the Focus Threshold Setting upward to include values closer to white (clear). However, setting the threshold to a very high value can affect the precision of Autofocus.*See: Initialisation: Focus* 635



Continued... 660

Reset Focus Point values:

1: To clear all focus point values, click on the *Reset* button. The values will clear and '???' will be displayed in the Z column of the dialog.

Delete a Focus Point:

2: To delete a focus point click on the point on the dialog list.

3: Click the *Delete* button. The list entry and the point disappear.

To create a New Point:

4: Click on the *Move Stage To Point* button.

5: Select a new point on the image and click. The point appears on the image coloured red to indicate that it requires focussing.

6: Click on the *Add* button.

Delete all Points:

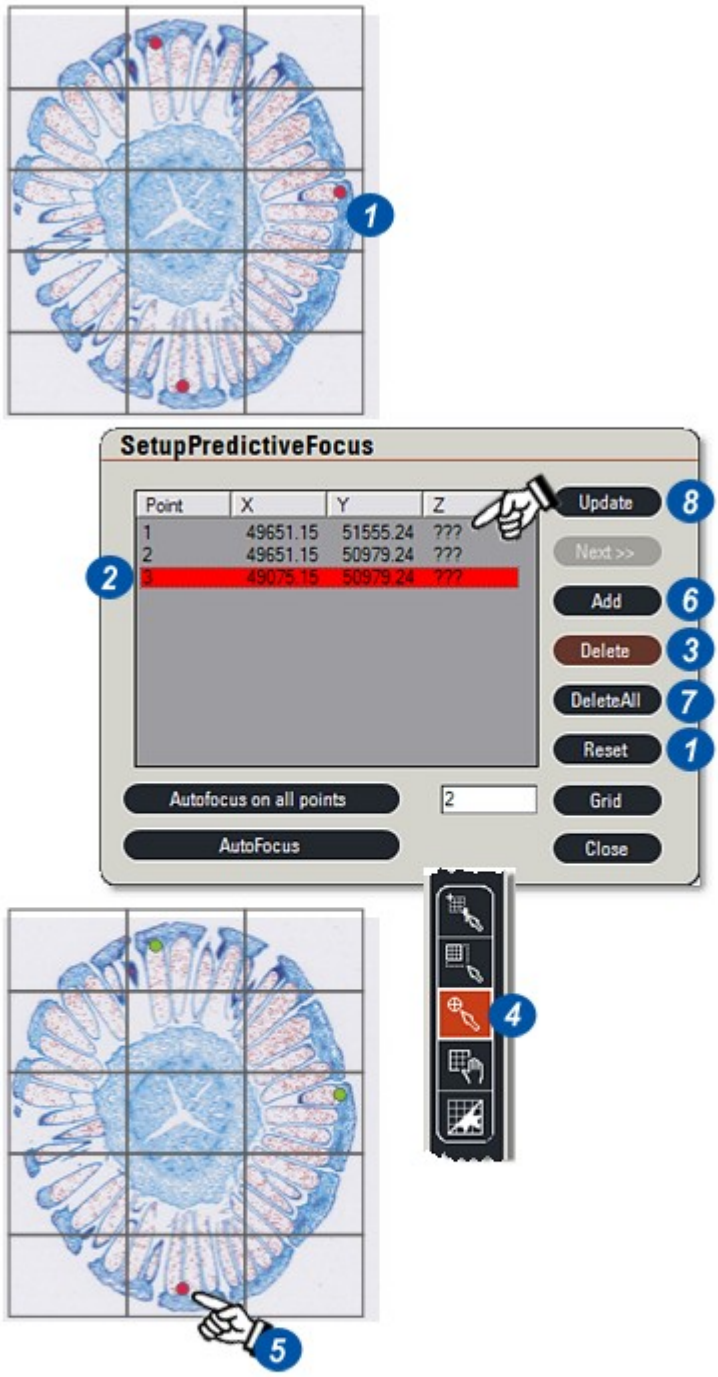
7: All of the points may be deleted by clicking on the *Delete All* button. The dialog list will clear and the points will disappear from the image.

Update:

Having added or deleted points, update the list and displayed points by…

8: …clicking on the *Update* button.

Continued... 660



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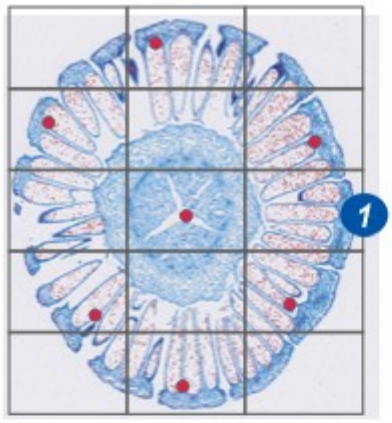
For multiple focus points:

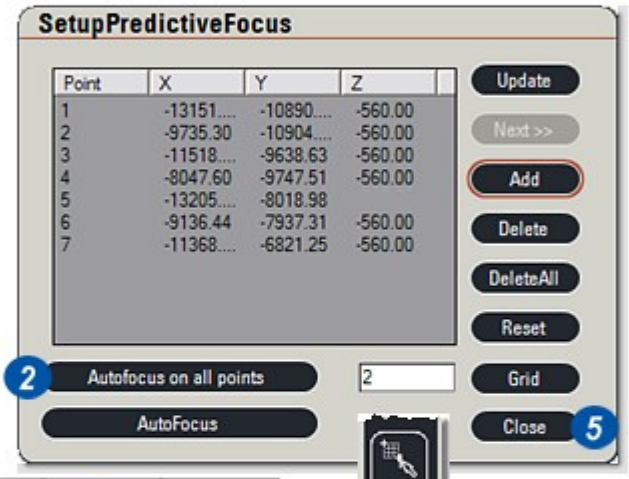
1: The illustration shows a range of pre-determined points that have been added manually to comprehensively cover the specimen.

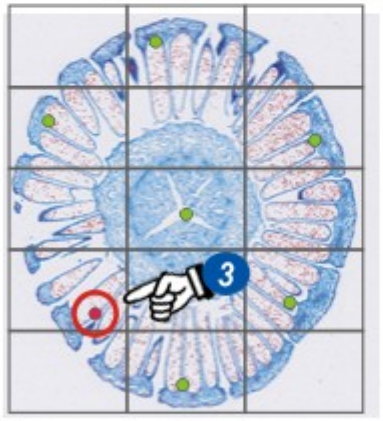


2: Click on the *Autofocus on all Points* button, or for a single point, click on the *Autofocus* button.
The program will cycle through each of the set points automatically focussing. As each is completed the point turns green and the value appears in the Z column of the dialog.

3: Check 'failed' points (circled for clarity) by…

4: …selecting the *Move Stage To* button and then on the focus point. Manually check the point for detail. Either delete the point or adjust the Focus Threshold Setting, reset the points and repeat the process.





Setup Autofocus Skipping:
To speed up the scan especially on specimens that are more-or-less uniform, automatic focussing can be skipped on some tiles. The number of tiles to skip is set up with Autofocus.

It is also possible to resume focussing on a tile immediately following a focus 'failure' rather than skipping tiles, to maintain focus integrity.

Autofocus failure generally occurs when there is insufficient detail in the specimen.

1: Click to enable the *Autofocus* check box.

2: Click on the *Setup* button and the Setup Autofocus dialog appears.

3: To enable tile skipping click on the *Autofocus every…* check box.

4: Set the number of tiles to skip by either clicking on the text box, deleting the existing value and typing another, or clicking on the increase/ decrease arrows to the right of the text box.

5: To *Retry on Failure*, click the check box to enable.

6: Click on the *Apply* button and…

7: Click on the *Close* button.

Go to Create the Pattern Grid... 657

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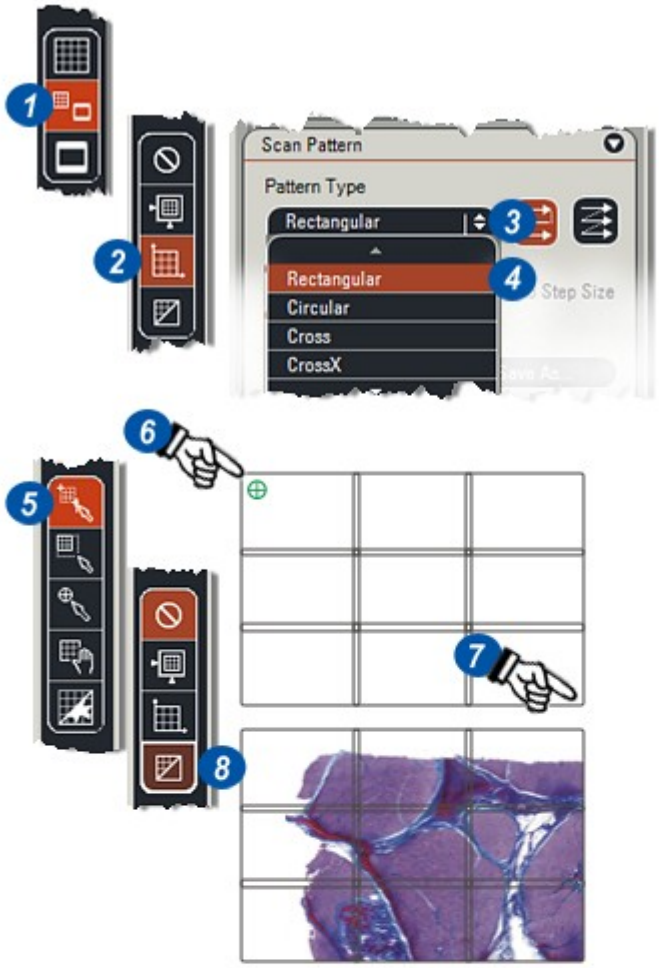
This step draws a pattern grid representing the scan tiles over the image. Power Mosaic will use this grid as a scanning 'map'. Because the specimen has yet to be scanned, its precise position on the stage is unknown so the first grid drawing locates it.



1: Click on the *Split View* button and navigate to an appropriate part of the specimen using the on-screen joystick.

2: Click on the *Pattern View* button.

3: Click on the arrows to the right of the *Pattern Type* header and from the menu...

4: …select by clicking the *Rectangular* option. A different pattern may be selected later.

5: Click on the *Draw Pattern* tool and…

6: …positioning the cursor close to the stage marker (small, green 'crosshair'), click and hold...

7: …and drag diagonally to the right. An arbitrary grid pattern should appear. If it does not, …

8: …click on the *Show Grid Lines* button to reveal it.

Continued... 658

9: Click on the *Clear Tiles* button to clear any previous images.

10: Confirm clearing the images on the warning message by clicking *Yes*.

11: Click on the *Acquire Power Mosaic* button. The specimen will be scanned with the tiles filled in sequence.
The first drawing usually encloses only part of the image as shown in the illustration. If this is the case go to the next page.

9: If none of the specimen is found click on the *Clear Tiles* button and repeat the process starting the drawing at a different location.

Continued... 659

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With the pattern grid enclosing only part of the specimen, it can be extended so that the specimen is fully covered.

1: Click on the *Create/Expand* button. This feature allows the existing grid to be expanded.
The Create/Expand button may also be used to reduce the pattern grid if it is too large.

2: Click on the top left corner of the existing pattern grid, hold and drag diagonally to the right. A dotted rectangle follows the cursor to indicate the extent of the grid. Release the mouse button when the specimen is considered to be completely enclosed **(3).**

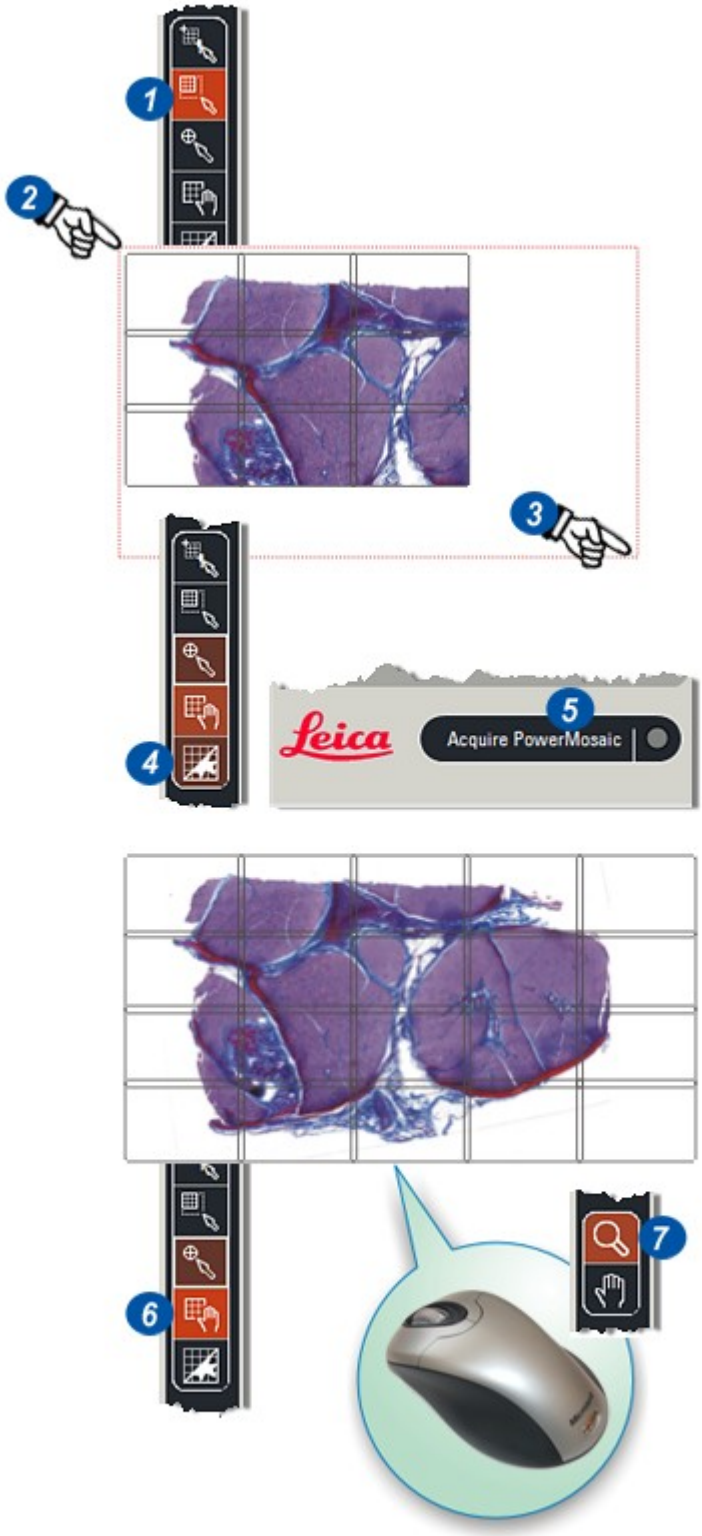
4: Click on the *Clear Tiles* button and confirm the clear on the warning message.

5: Re-scan by clicking the *Acquire Power Mosaic* button.

6: To re-position the scanned image within the grid, click on the *Move Scan Pattern* button, click and hold on the scanned image and drag it to reposition.

7: The *Magnifier* may help in the repositioning - click on the tool and use the mouse buttons to enlarge (left button) or reduce (right button) the view.

Continued... 660



Having re-positioned the image within the pattern grid, it is possible that there are too many tiles. Excess tiles may be removed as columns or rows only by:

1: Click on the *Details* button and on the dialog, change the fields (tiles) to remove any excess.

2: Click the *Apply* button and…

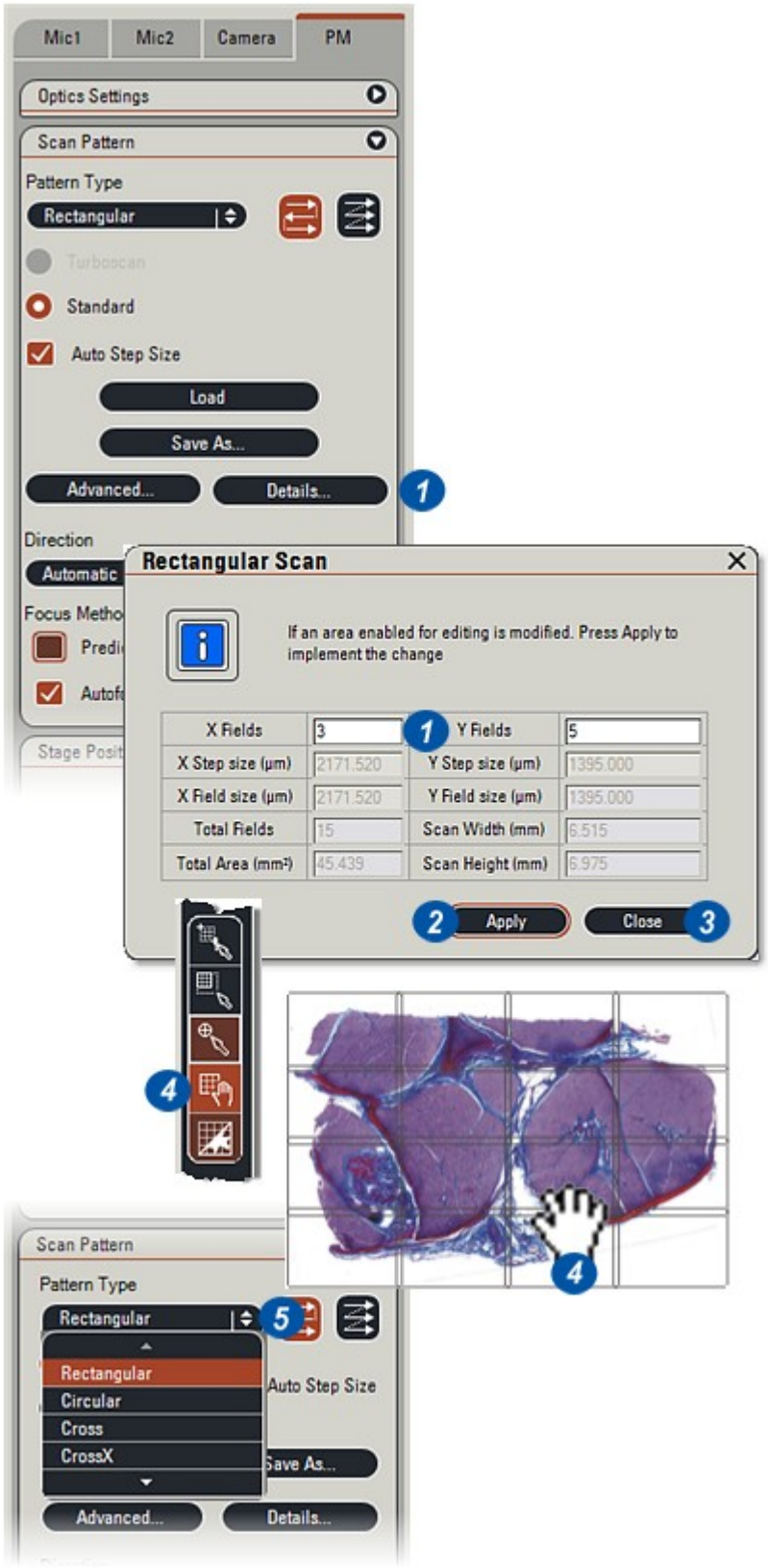
3: …click the *Close* button.

4: Select the *Move Scan Pattern* button and re-position the grid to make sure the fit over the specimen is acceptable.

5: If an alternative *Pattern Type* is required, click on the arrows to the right of the Pattern Type header and from the drop down menu click to select the required pattern.

It may be necessary to re-adjust the pattern grid-to-specimen fit using the *Move Scan Pattern* button.

Go to Save Configuration... 661



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With the Power Mosaic configuration complete, it is possible to save the settings for instant use in the future.

To save the current settings:

1: On the *Scan Pattern* panel click the *Save As* button.

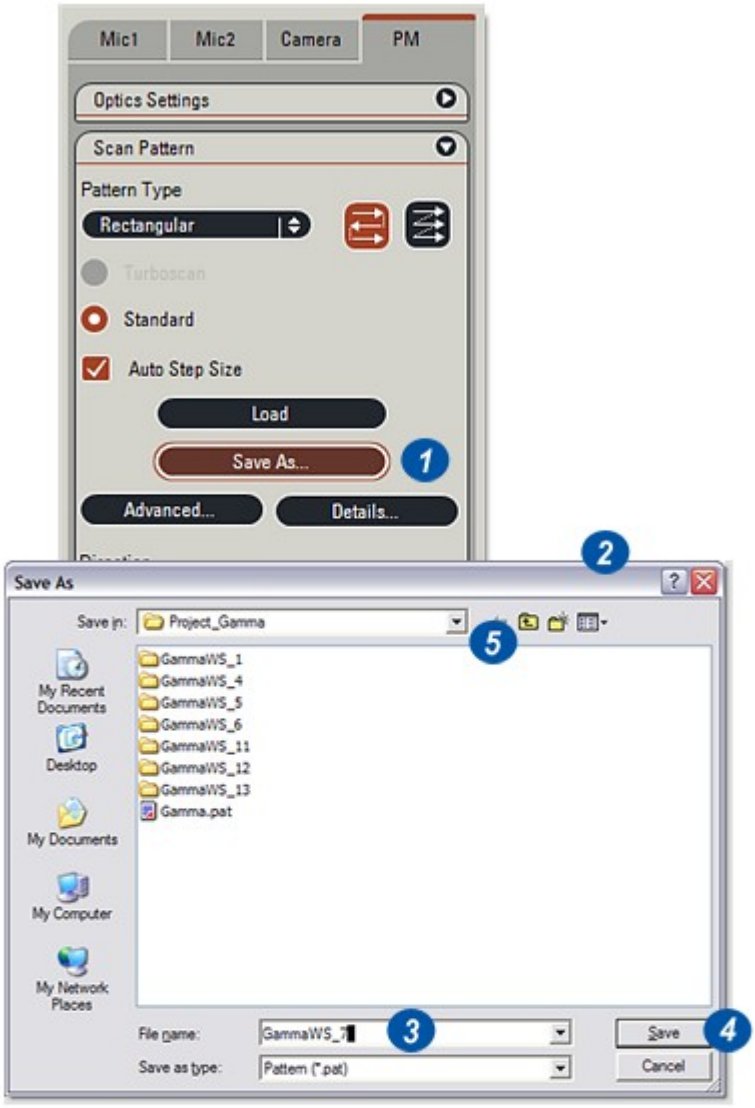
2: On the *Save As* dialog…

3: …type a file name and…

4: …click the *Save* button. Files are automatically saved with the *.pat* extension.
The *Save As* dialog defaults to the folder selected in *Preferences* or *Browse.*

See: Preferences:Save in Folder:
See: Browse:Select Default Folder:

5: The *Save As* folder may be changed on the Save As dialog using Windows navigation.



Before starting a Power Mosaic scan, the thumbnails and tiles from previous scans, if not required, should be deleted. This is not mandatory – keeping previous information can be a vital part of an ongoing session in which the specimen is in several parts for instance.

Thumbnails of each tile are stored in RAM to make access fast and immediate. They are used to 'paint' the mosaic on the Viewer. Keeping thumbnail size as small as possible *(See: Advanced Options: Thumbnail Reduction Factor 644)* will help prevent filling the RAM too quickly.

The tiles are stored initially in a temporary file on the hard drive. The individual images are much larger and used to paint the mosaic at greater resolution to preserve detail.

Clearing previous scans removes the thumbnails from RAM and the tiles from the temporary file. If the previous scan is left intact, new thumbnails and tiles will be added to the existing with sequential image numbers.

1: Click on the *Clear Tiles* button to clear the previous scan. This is only the previous scan and does not remove any prior to that.

2: The *Clear Mosaic* message appears. Click *Yes* to continue.

3: Click on the *Acquire Power Mosaic* button.

4: If Turboscan is selected and the exposure speed is too high, a dialog appears giving the option to use Standard scan instead or to cancel.

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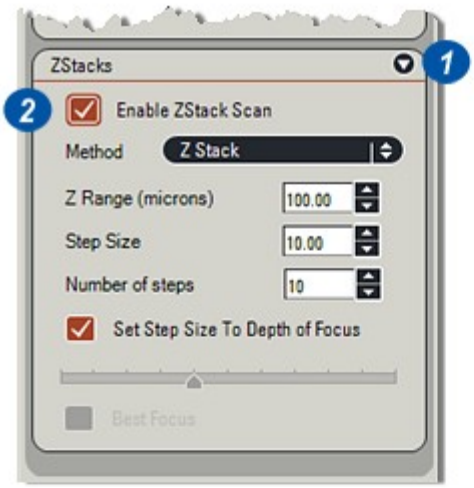
If Power Mosaic Plus is installed and enabled, the Z-Stack option is available. For every tile position on the XY plane, Power Mosaic Plus creates a stack of tiles in the third dimension or Z plane.

The number of tiles in the stack and the distance between them can be set to reflect the specimen.

Once captured the individual stack tiles can be processed in several ways and eventually into a single composite image that represents the best focus across the entire specimen regardless of thickness.

Z-Stacks can occupy very large amounts of disc space especially if all of the tiles are saved, so ensure that a liberal amount of space is available. The best arrangement for image storage is to have a partitioned area on the hard drive *(Drive D:* for example) separate from other programs and data, or a completely independent drive.



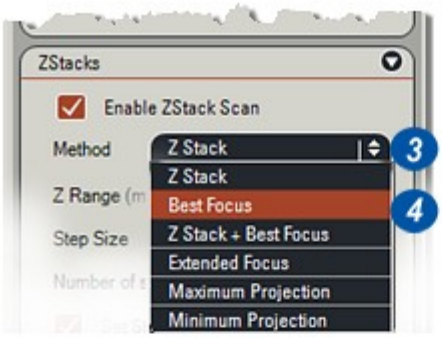
1: Click on the arrows to the right of the *Z-Stacks* panel to reveal it.

2: Click the *Enable Z-Stack* check box to enable scanning.

Continued... 664

3: To select the required processing method, click on the arrows to the right of the *Method* header and from the drop down menu…

4: Select the method:



Z-Stack will save all of the captured stack tiles for further, individual viewing.

Best Focus chooses the tile with the sharpest image from each stack and discards the rest.

Z-Stack + Best Focus combines both options, retaining all of the stack tiles but also selecting the best from each stack which it places at the lowest level.

Extended Focus examines all of the tiles from a stack, choosing the 'best' pixel from each at a given location. These are then combined into a single tile and the rest discarded.

Maximum and *Minimum Projections* emulate Extended Focus to create composite images based upon the darkest and lightest pixels in each pixel column in each stack tile.

Continued... 665

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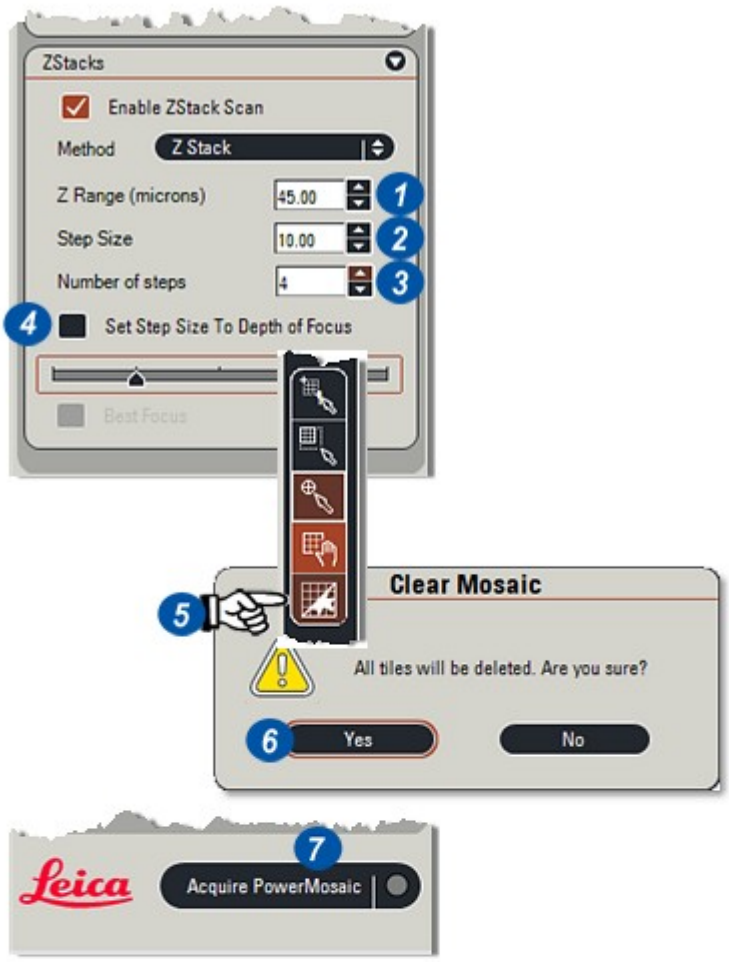
The Z-Range – the focussing distance based upon objective depth of focus - in microns that will be travelled – Step Size and Number of Steps, are inter-related; Change one value and the others will be calculated and changed automatically. Actual settings will depend upon the specimen.



1, 2 and **3**: Use the up/down arrows to the right of each text box to enter a value. For large values, click on the appropriate text box and type a value.

4: The *Set Step Size* check box if enabled will automatically load a step distance based upon the depth of focus and adjust the Z-Range and Number of Steps accordingly. Changing the Step Size manually will turn off Set Step Size.

5: Click on the *Clear Tiles* button to discard any previous scans. The *Clear Mosaic* warning will appear…

6:…click *Yes* to clear.

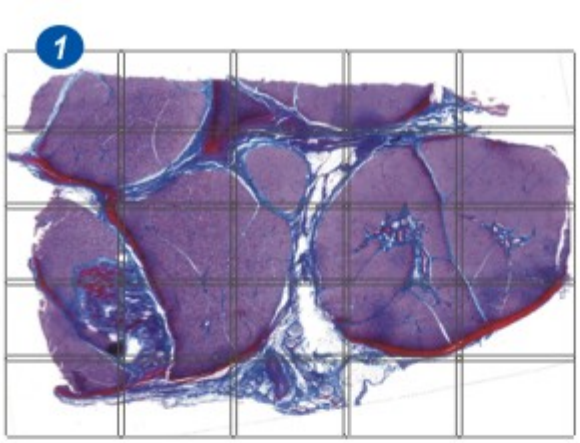
7: Click the *Acquire Power Mosaic* button.
Z-Stacks are only available in Standard Scan mode. If Turboscan has been selected it will be ignored

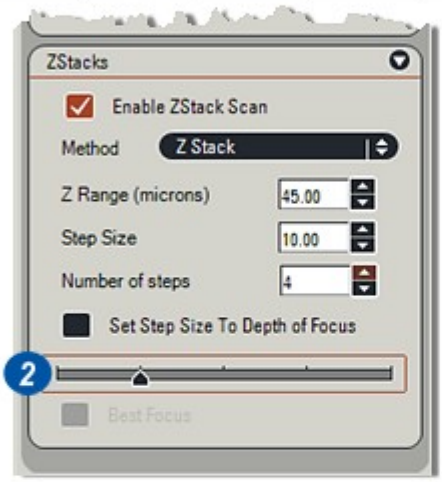
Continued... 666

When the scan is complete the entire image is displayed with the tile grid overlaid **(1).**

Two methods – *Z-Stack* and *Z-Stack + Best Focus* – have a slider **(2)** associated with them which allows each stack step to be viewed individually, a powerful image analysis tool.

The slider scale represents the number of steps or multiples of steps. Click, hold and drag the slider to display the step results in turn.





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Pattern Navigator is a module that is part of and licensed by *Power Mosaic Plus.* It allows users to create *Scan Patterns* randomly in any position across the stage and then scan them either all together or at selected sites. The great advantage of this facility is that only areas of interest are captured substantially reducing both processing time and required disk space.

Since patterns can be saved and restored, tasks that routinely use the same specimen layout on a slide (say), benefit from the speed that having a pre-defined scanning template can offer. For example, illustration **(A)** shows a standard 75 x 25 microscope slide (enlarged for clarity) divided into 12 specimen 'wells'. The wells are not spread evenly across the slide – there is a larger area at the top than there is at the bottom - nor are they of uniform size. To complicate matters even further, only 6 of the specimens are usually scanned – they are marked with a red border in the illustration. It is a simple task for *Pattern Navigator* to locate the required wells, create a scan pattern them and to store that as a template for future processing.

Continued... 668

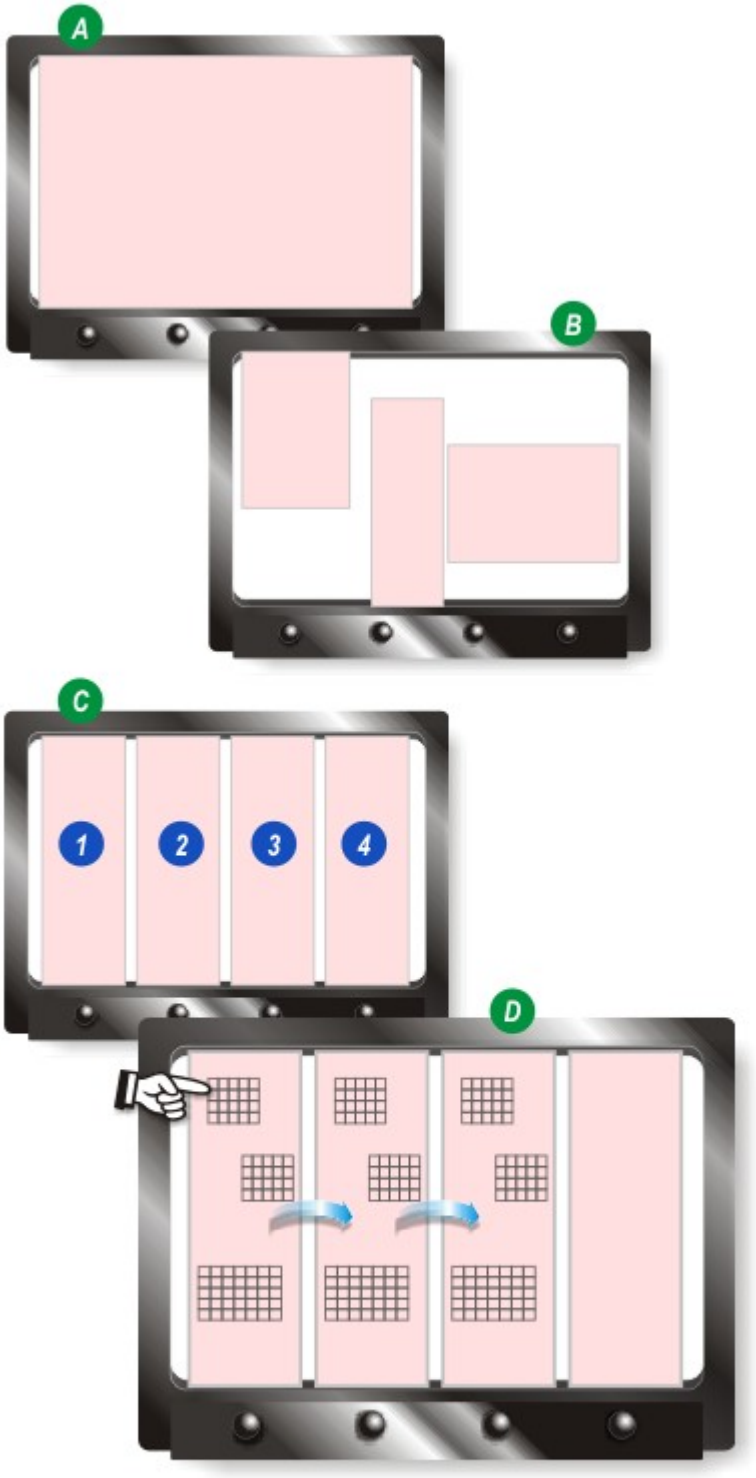
Pattern Navigator allows the user to divide the viewable stage into distinct areas called *Workspaces*. The number and position of the *Workspaces* is determined by the users for ease of working. The entire stage could be classed as a single *Workspace* **(A)**; It could be divided into several *Workspaces* of differing areas **(B)** or a group of uniform *Workspaces* like 4 slides in a row for example **(C)**. The only restriction is that *Workspaces* must not overlap.

Each *Workspace* encloses its own group of *Scan Patterns* **(D)**. An individual pattern can be re-sized and moved within its *Workspace* providing it does not overlap with another. *Scan Pattern* shapes – rectangular, square, circular, cross etc – can be mixed within a *Workspace* to achieve the most efficient and economical image capturing.

Because a *Workspace* acts as a container for its *Scan Patterns*, it can be moved to any location on the stage and its patterns will move precisely with it.

Workspaces can also be copied, so having set up a group of patterns the entire *Workspace* can be duplicated and 'pasted' to other parts of the stage. This is especially useful for repetitive specimens – four identical slides in a row for example.

By default the Workspace outline and fill is turned off. To turn it on and change its properties: *Go there...* 696



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Capturing specimen mosaics using *Pattern Navigator* comprises three simple steps:

1: Creating and mapping the *Workspace* .

2: Plotting the individual *Scan Patterns* within the *Workspace*.

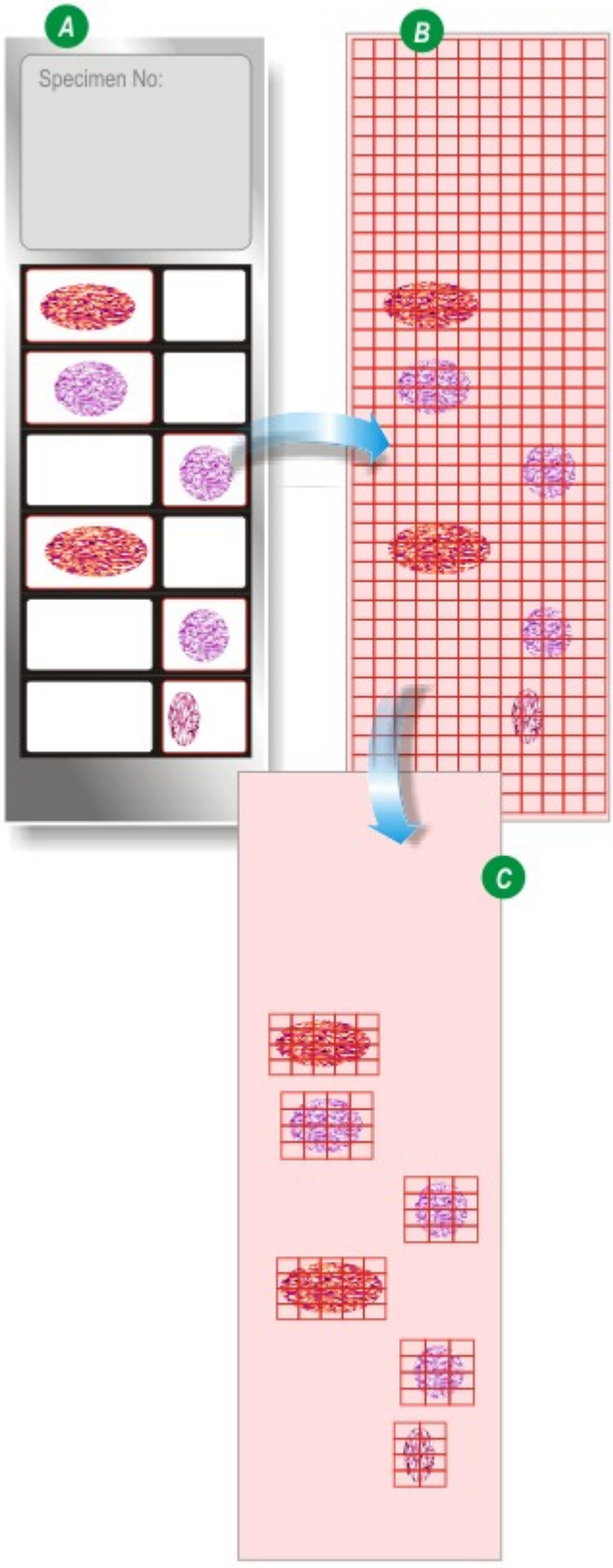
3: Scanning using the *Scan Patterns.*

Creating and Mapping the Workspace: Illustration **(A)** shows the sample microscope slide described earlier.

To map the locations of the required specimens, a rapid scan is made using a scan pattern that covers the entire *Workspace* – in this case the slide area **(B)**.

Using this as a guide, individual *Scan Patterns* are created and placed (just by dragging and dropping) over the specimens. The overall scan pattern is then removed **(C).**

Continued... 670



To map the *Workspace* only low resolution images are required because they are to be used only as a guide to the specimen location. Low resolution capture formats also reduce the scanning time.

1: In the *Acquire Workflow* click on the *Camera* tab.

2: Click on the arrow to the right of the *Image Formats* panel.

3: Click on the arrows to the right of the image type header and…

4: …select *Colour* or *Greyscale* to suit the image.

5: Click on the *Mic* tab and...

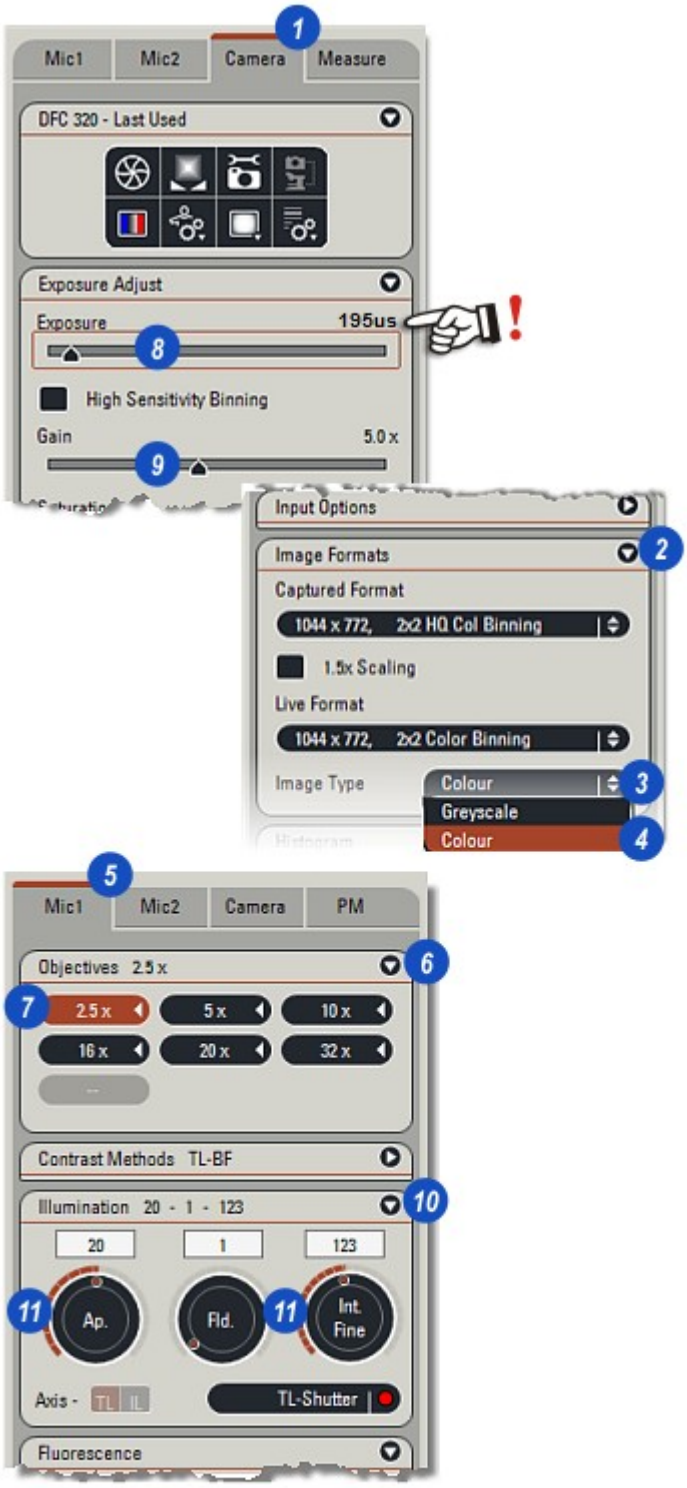
6: ..on the arrow to the right of the *Objectives* header.

7: Select the lowest magnification objective available.

8: Adjust the *Exposure* and…

9: …the *Gain* to achieve an acceptable image with an exposure time no greater than *200µs*, necessary to run *Power Mosaic* in *TurboScan* mode.

10: It may be necessary to adjust the *Aperture* and lamp *Intensity* **(11)** on the *Microscope* tab.



Continued... 671

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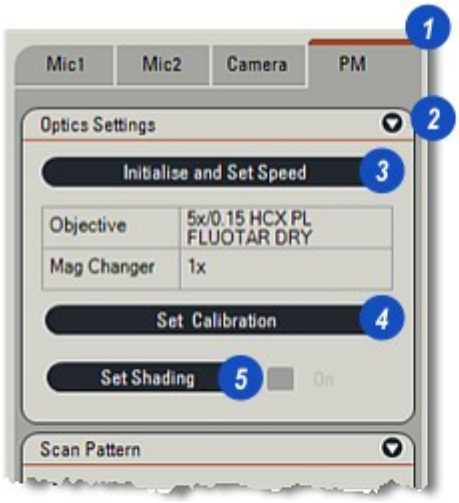
1: On the *Acquire Workflow*, click on the *PM* (Power Mosaic) tab.

2: Click on the small arrow to the right of the *Optics Settings* header to reveal the panel.



Stage Initialisation:

3: Click on the *Initialise and Set Speed* button: Follow the Initialisation procedure: *Go there…*

Objective Calibration:

4: Click on the *Set Calibration* button: Follow the Calibration procedure: *Go there…*

Shading:

5: Click on the *Set Shading* button and follow the *Shading* procedures: *Go there…*

Continued... 672

On the *PM* tab:

1: Click on the small arrow to the right of the *Scan Pattern* header to reveal the panel.

2: Click on the arrows to the right of the *Pattern Type* menu and from the drop down list click to select *Rectangular* pattern.

3: Click to enable the *TurboScan* button.

4: Click to enable the *Auto Step Size* check box.

5: Click to enable bi-directional scanning, the fastest option.

6: Disable both *Focus Method* options.

Continued... 673

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On the *Side Tool Bar* **(1)** click to select:

2: …the *Stage and Live Image* option,

3: …the *Stage View,*

4: …and *Show Scan Pattern.*

With this arrangement:

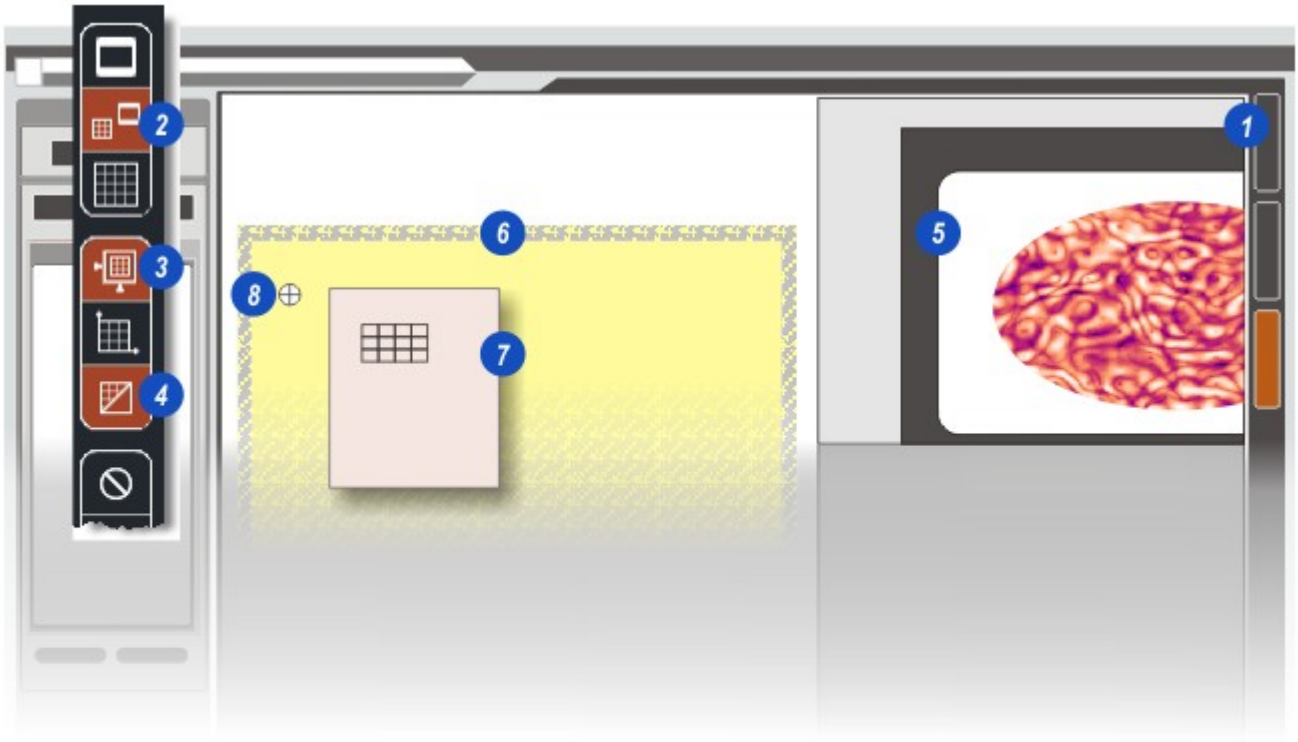
5: The Live Image appears top right of the *Viewer*.

6: The *Stage Viewable* area shown as a hatched outline.

7: A *Workspace* with *Scan Pattern* which at first use will be arbitrary sizes and positions. Subsequently, the last used *Workspace* will be displayed.

8: The *Stage Marker* which is the current stage position.

Continued... 674



On the *PM* (Power Mosaic) tab:

1: click the small arrow to the right of the *Power Mosaic Plus* header to reveal the panel.

2: On the *Pattern Navigator* panel, click to enable *Pattern Navigator* and…

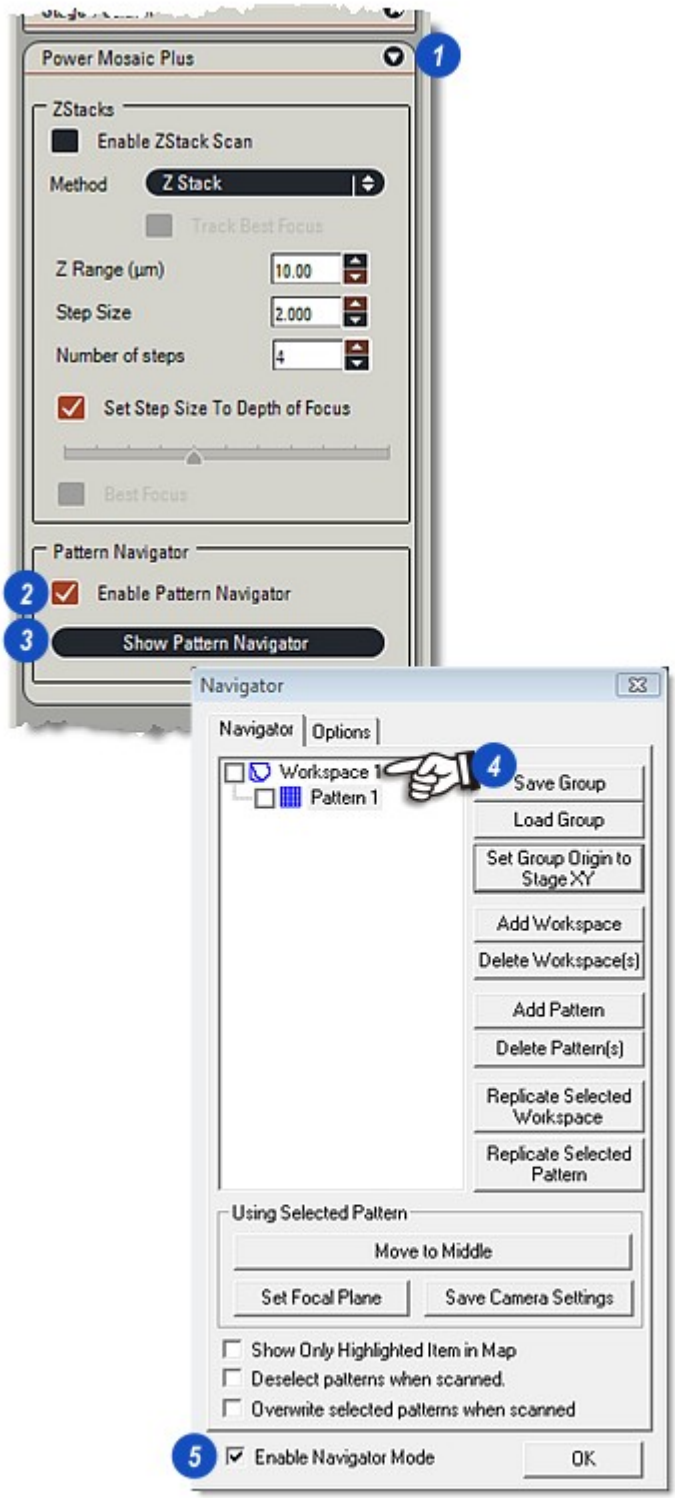
3: …click the *Show Pattern Navigator* button. The *Navigator* dialog appears.

4: On the dialog, the current displayed *Workspace* is listed together with the *Scan Pattern*(s) associated with it. Both *Workspace* and *Pattern* names can be changed to suit the user, so the words may differ but the layout will be the same.

5: The *Enable Navigator Mode* check box should be enabled.

Change Workspace and Scan Pattern Names: *Go there...* 689

Continued... 675



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In this example, the specimens are mounted on a standard 25 x 75mm microscope slide so the *Workspace* will be re-sized to the same dimensions.

1: *Right* click on the *Workspace* name.

2: The *Workspace* dialog appears.

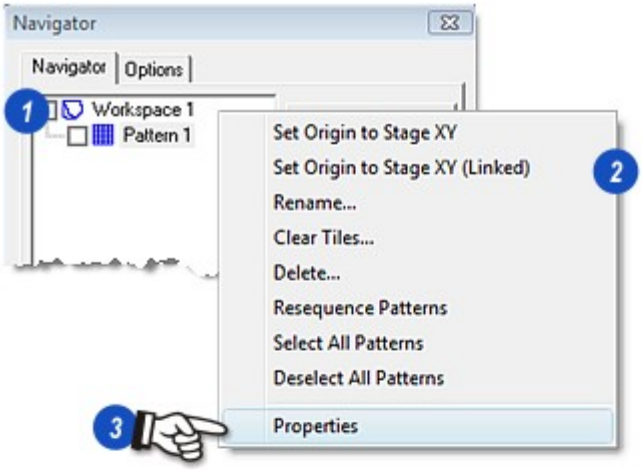
3: Click on the *Properties* option.

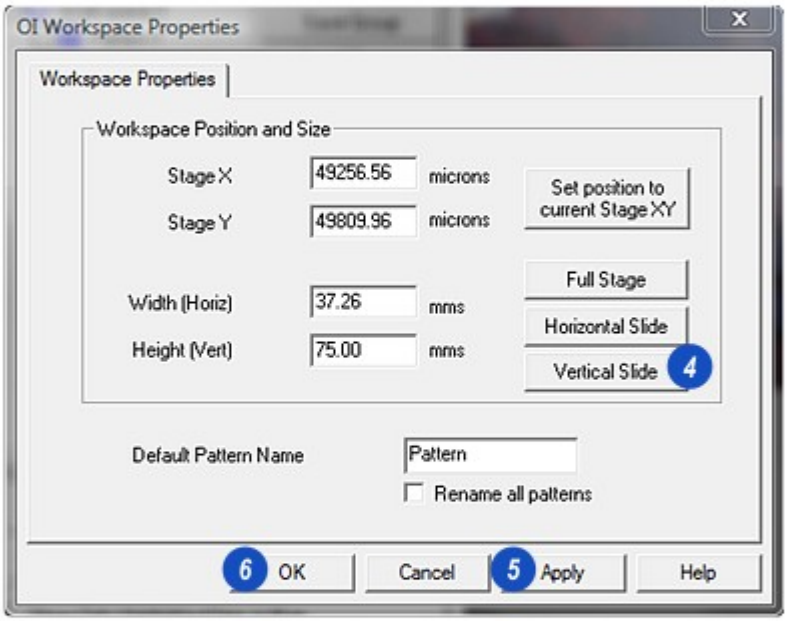
4: On the *Workspace Properties* dialog, click on the *Vertical Slide* button to automatically re-size the *Workspace* to 25 x 75mm orientated vertically.

5: Click *Apply* and…

6: …click *OK*.

Continued 676...





1: The *Workspace* has been re-sized to represent a standard 25 x 75 microscope slide.

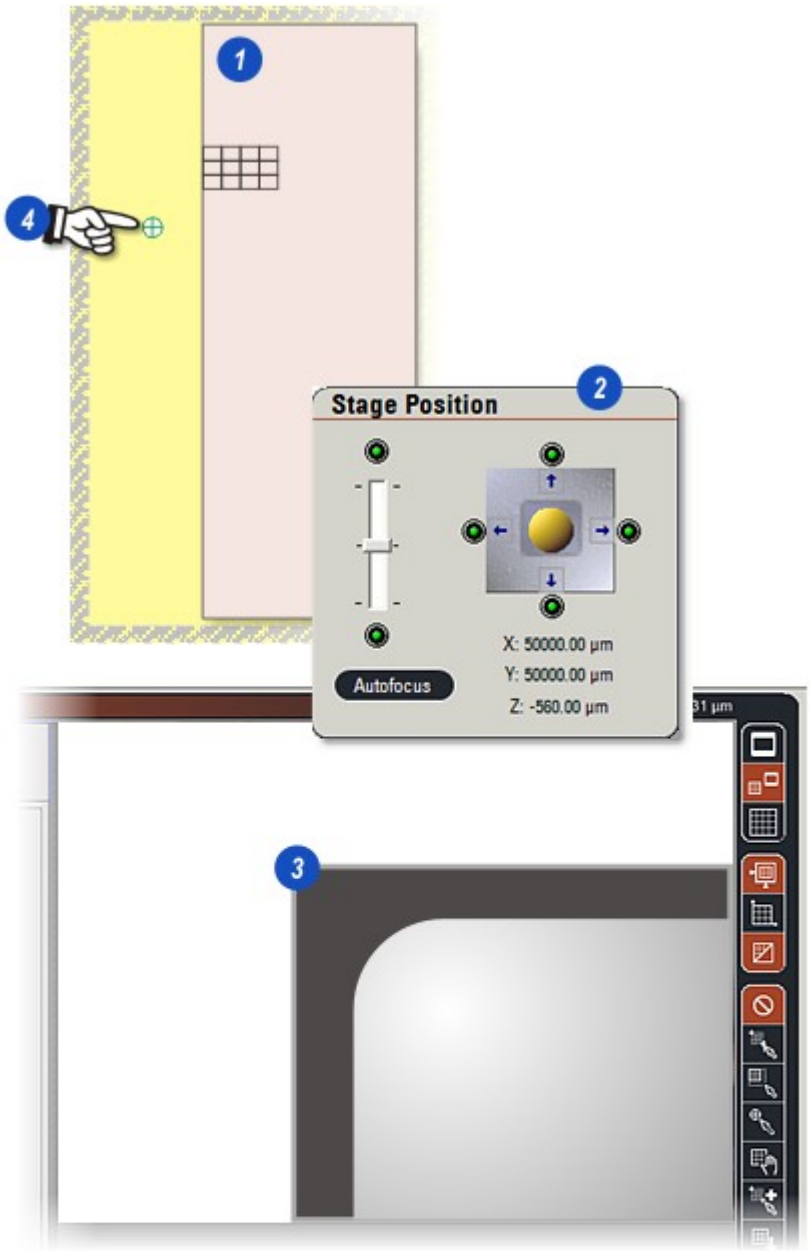
The next step positions the *Workspace* over the specimen slide with their top – left-hand corners aligned:

2: Using either the on-screen *Joystick* or *Leica SmartMove* (if fitted),

3: …drive the *Stage* to the top left-hand corner of the specimen slide.

4: The *Stage Marker* moves to reflect the current stage position.

Continued... 677



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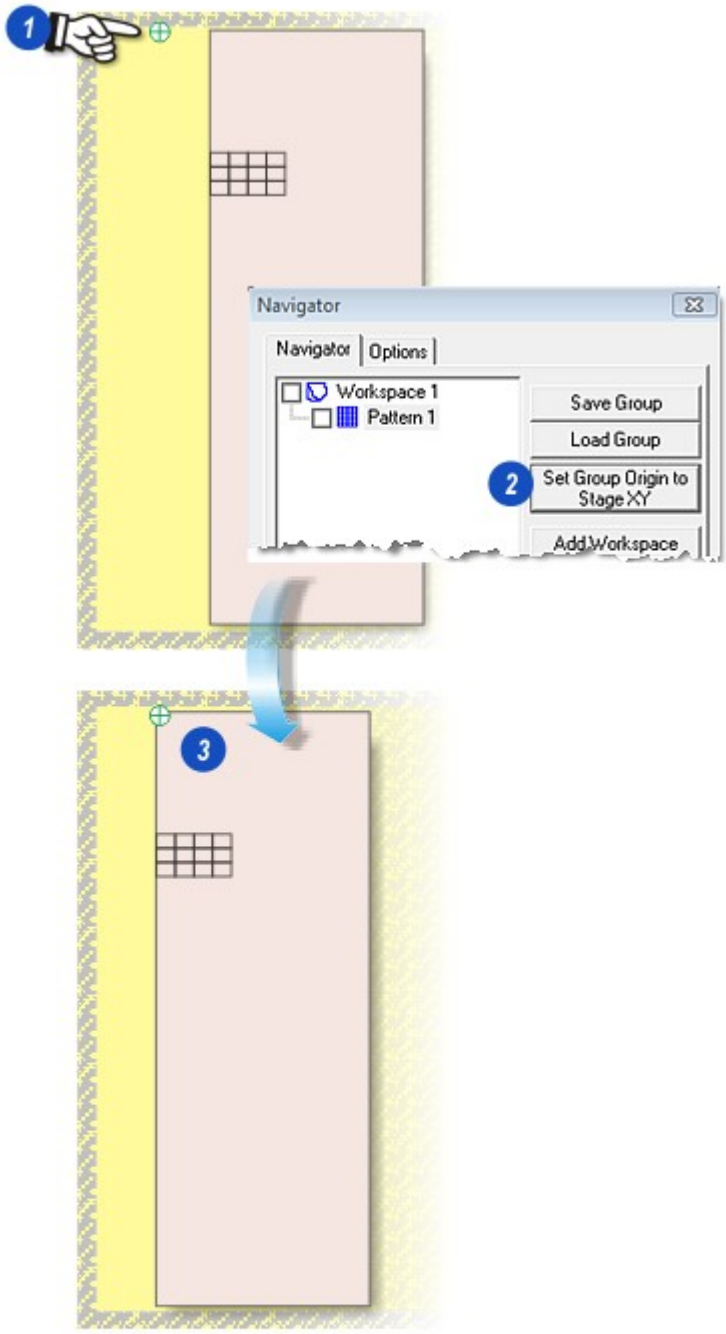
1: The *Stage Marker* has moved to the current *Stage* position which is now the top left-hand corner of the microscope specimen slide.

2: On the *Navigator* dialog, click the *Set Group to Stage XY* button.

3: The *Workspace* and *Scan Pattern* immediately move to align with the *Stage Marker* and therefore, the specimen slide.

Continued... 678



Create a *Scan Pattern* covering the entire *Workspace* – in this example the size and shape of a microscope slide.

1: Click on the *Create New Pattern* button.

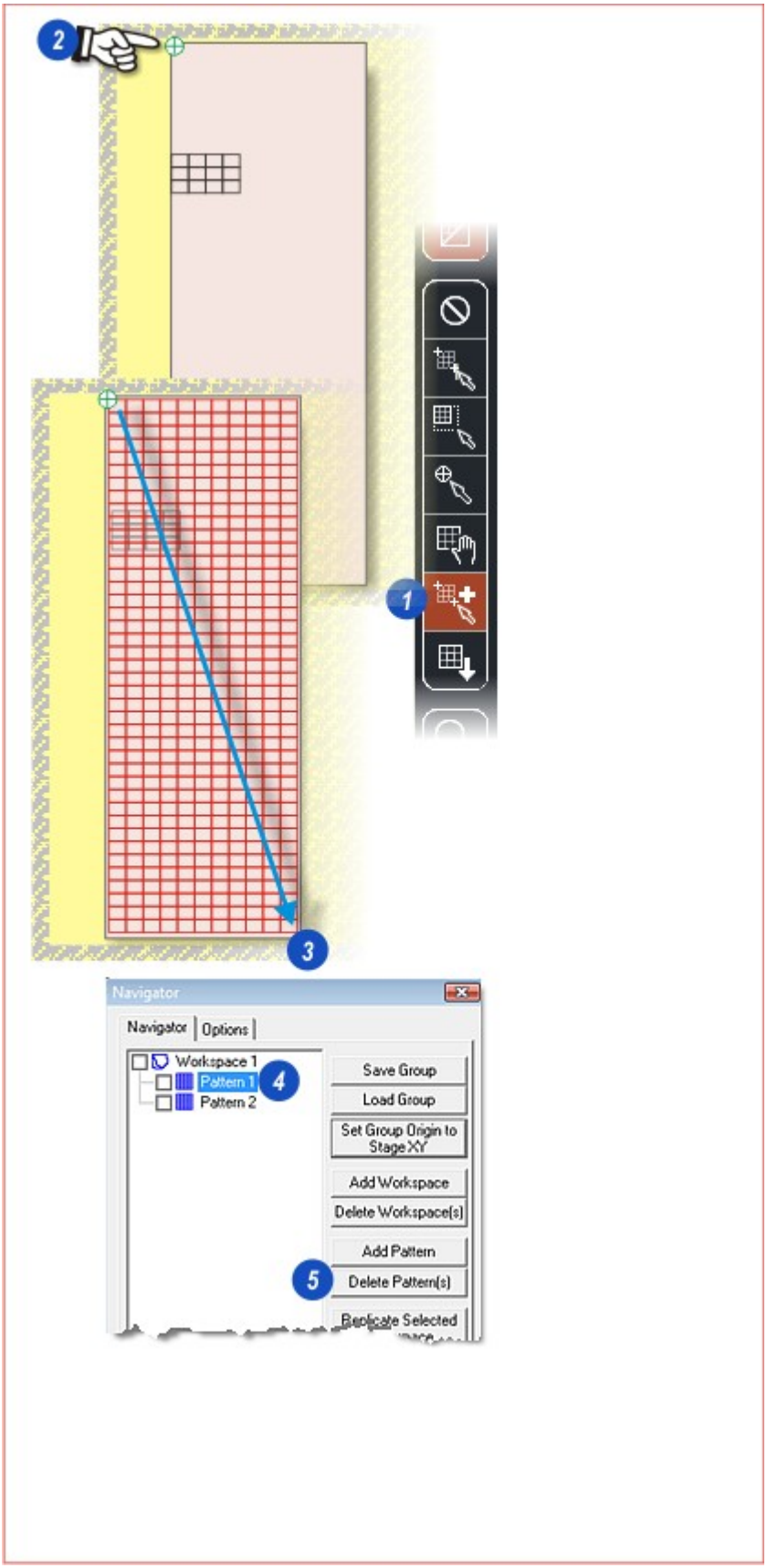
2: Click on the top left-hand corner of the *Workspace* and…

3: …holding the mouse button down, drag to the bottom right-hand corner and release the button.

4: Delete the original (small) *Scan Pattern* by, on the *Navigator* dialog, clicking the pattern name in the list.

5: Click on the *Delete Pattern(s)* button. Only the full-sized *Scan Pattern* now remains.

Continued… 679



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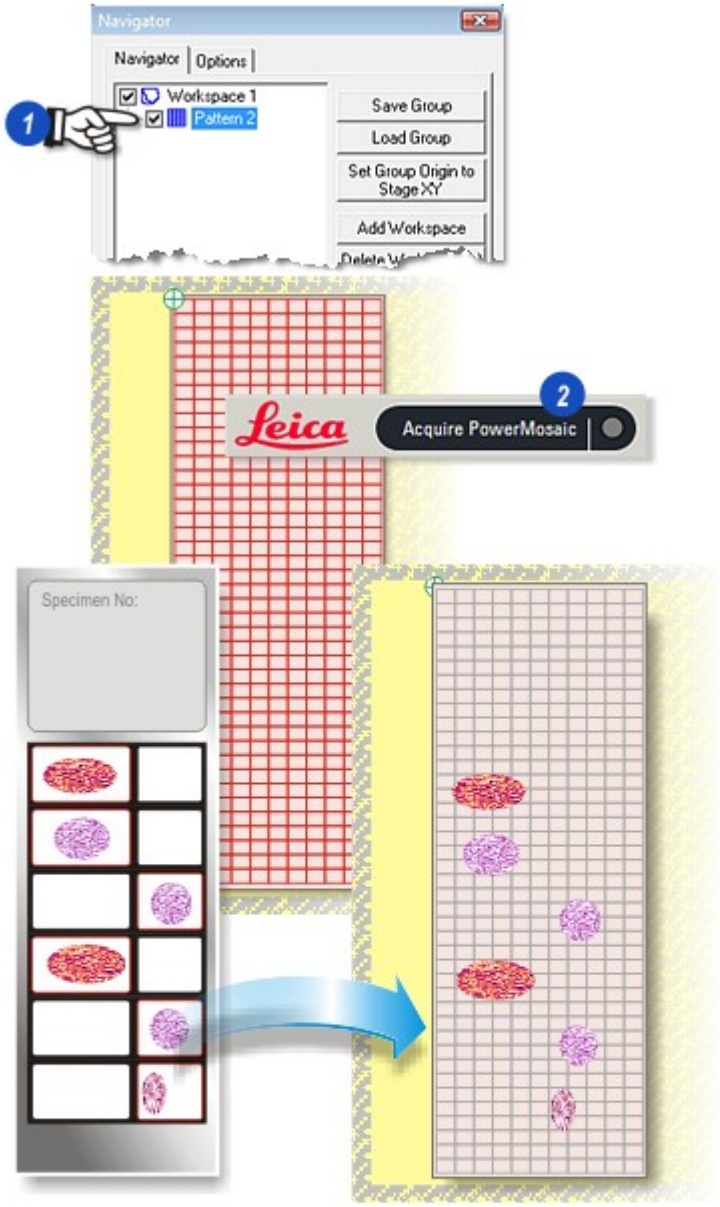
Only the *Workspaces* and *Scan Patterns* that are selected are scanned – the check box alongside has to be enabled:

1: Click to enable the check boxes to the left of the *Workspace* and *Scan Pattern.*

2: Start the scan of the entire specimen slide by clicking on the *Acquire Power Mosaic* button.

All of the specimens appear positioned against the *Scan Pattern* that will be used to precisely locate individual patterns ready for high quality image captures.

Continued… 680



Each specimen is now given its own individual *Scan Pattern* and it is these that will be used to capture the final, high quality images.

1: Click on the *Create New Pattern* button.

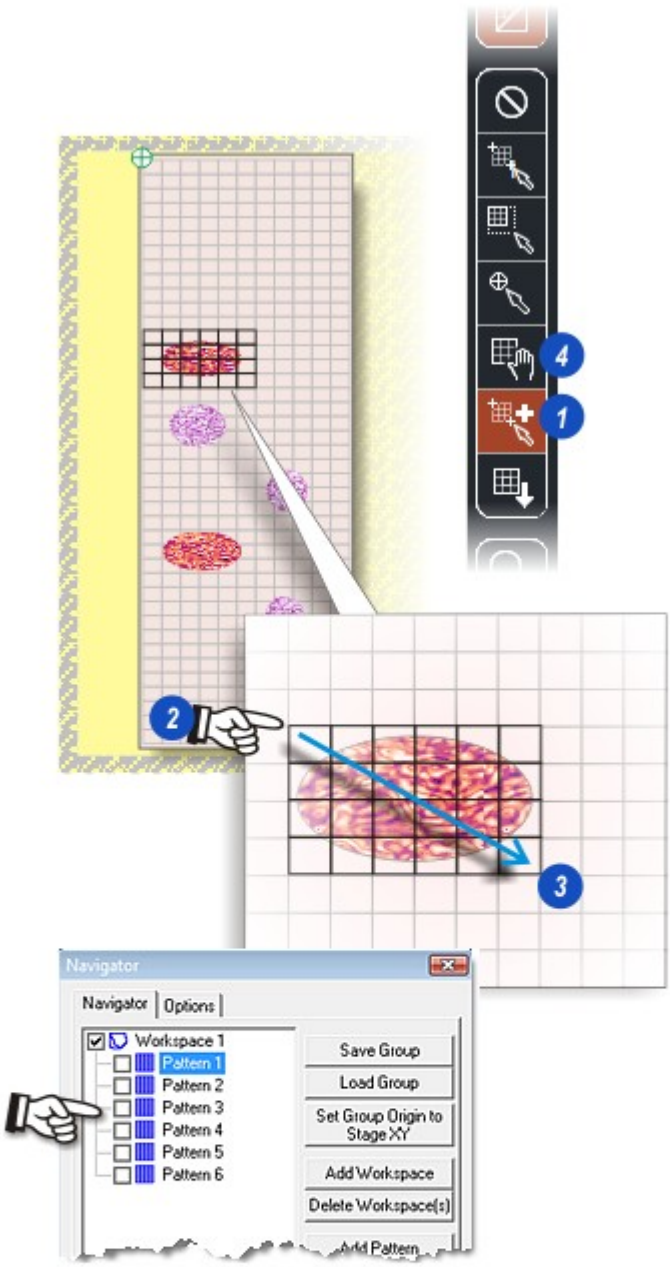
2: Click on the large *Scan Pattern* above and to the left of the specimen. Holding down the mouse button drag down and to the right to create a pattern that fully encloses the specimen.

3: Release the mouse button.

4: If necessary, click on the *Move Pattern* button and then on the new pattern. With the mouse button held down drag the *Scan Pattern* to the correct position.

Repeat the process for all of the other specimen areas. As the patterns are created they are added to the *Workspace* list.

Continued… 681



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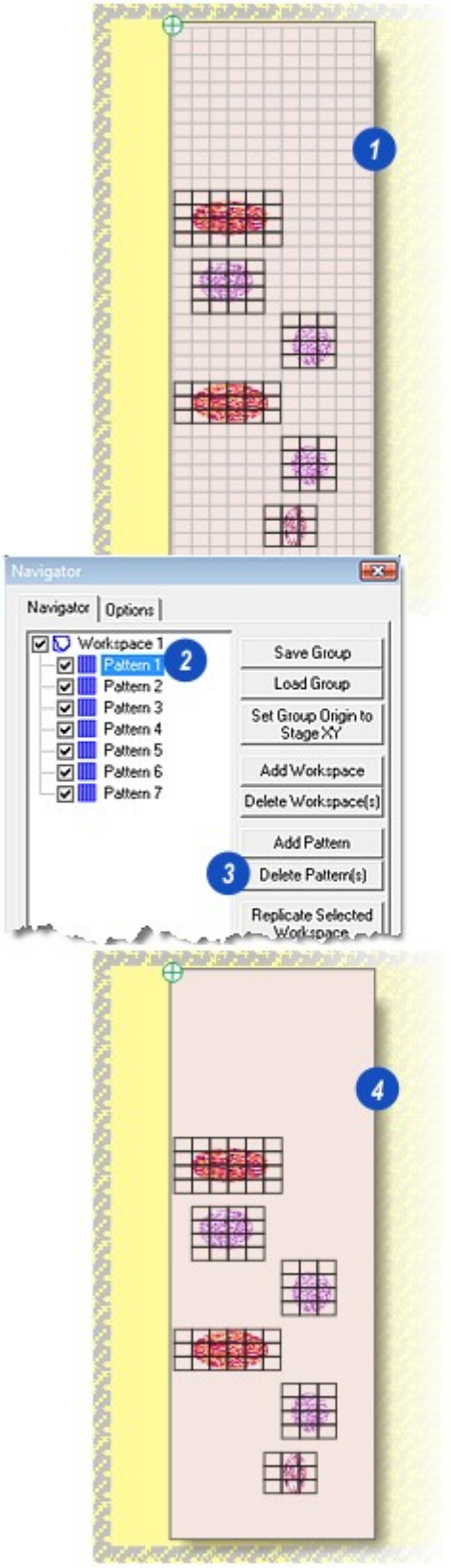
1: All of the required individual *Scan Patterns* are in place. The next task is to remove the large guide *Scan Pattern.*

2: Click on the large *Scan Pattern* on the *Navigator* dialog to select it. Selected *Scan Patterns* are highlighted on the *Workspace*.

3: Click on the *Delete Pattern(s)* button.

4: The large guide pattern is removed leaving just the individual patterns.

Continued… 682



At this point it is advisable to save the *Workspace* and its *Scan Patterns.*

1: On the *Navigator* dialog, click on the *Save Group* button.

2: The *Windows Save As* dialog appears.

3: The default folder for saved *Workspaces* is *OIGroups* but this can be changed to suit the user simply by navigating to an existing folder or creating a new one.

4: Type a unique name for the *Workspace* group.

5: Click *Save*.

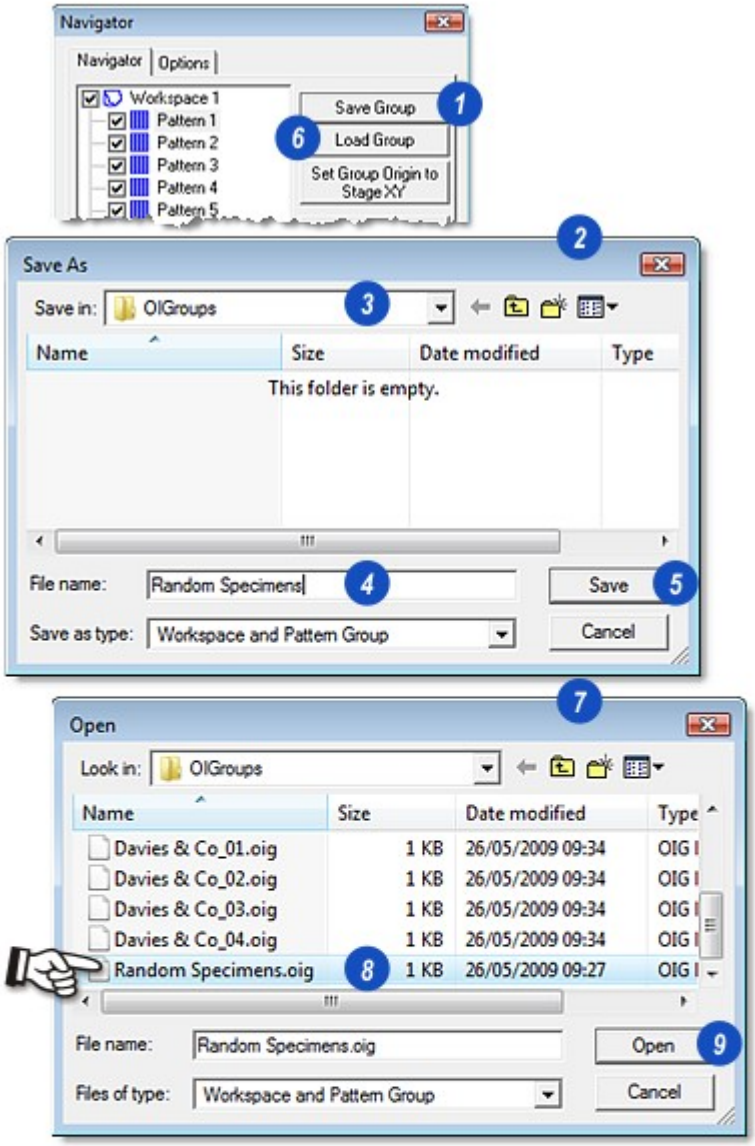
Loading a Saved Workspace Group:
To re-load a *Workspace* and its *Scan Patterns:*

6: Click on the *Navigator Load* button.

7: On the *Open* dialog…

8: …click on the required file with the ***.oig*** extension – ***not*** on the folder of the same name.

9: Click *Open* and the *Workspace* will be loaded and displayed.



Continued… 683

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Before scanning with the individual Scan Patterns:

1: On the *Camera* tab, select a higher quality *Captured Format.*

2: On the *PM* tab, select the *Scan Pattern* panel and decide upon *TurboScan* (high speed but lower precision) or *Standard Scan* (slower but higher precision). The higher quality image formats will often preclude using *TurboScan* due to the high capture time.

3: Decide upon bi-directional (faster but lower precision) or uni-directional scanning (slower but better precision).

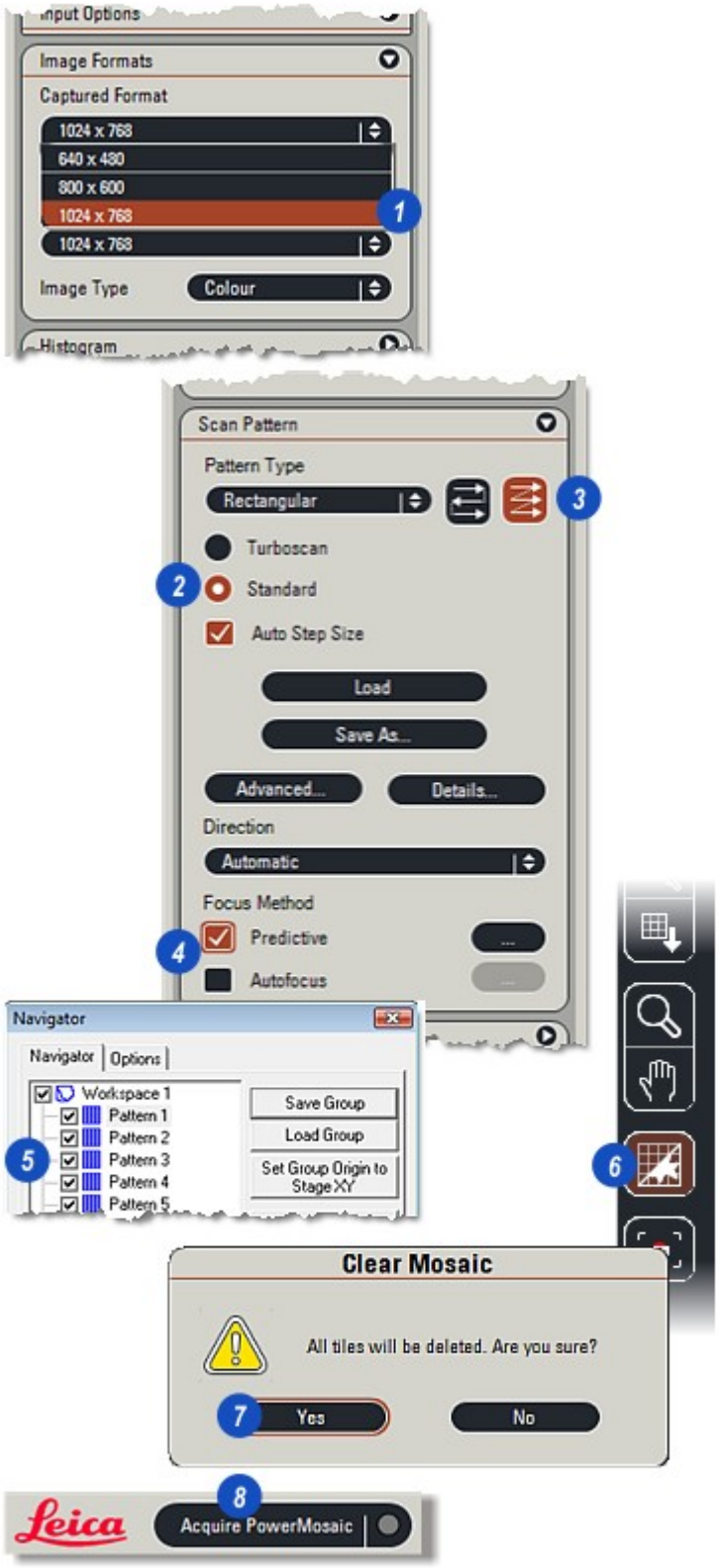
4: Select the *Focus Method*. This may require re-calibrating the focus.

5: Select the specimens to scan by enabling the check box to the left of each pattern.

6: Click on the *Clear Tiles* button to remove the previous scans and...

7: ...confirm the deletion.

8: Then start scanning…



This section describes the wide range of features available in *Pattern Navigator.*

On the *Navigator* dialog with a *Scan Pattern* selected:

1: Clicking *Move to Middle*, drives the stage to the centre of the selected *Scan Pattern.*

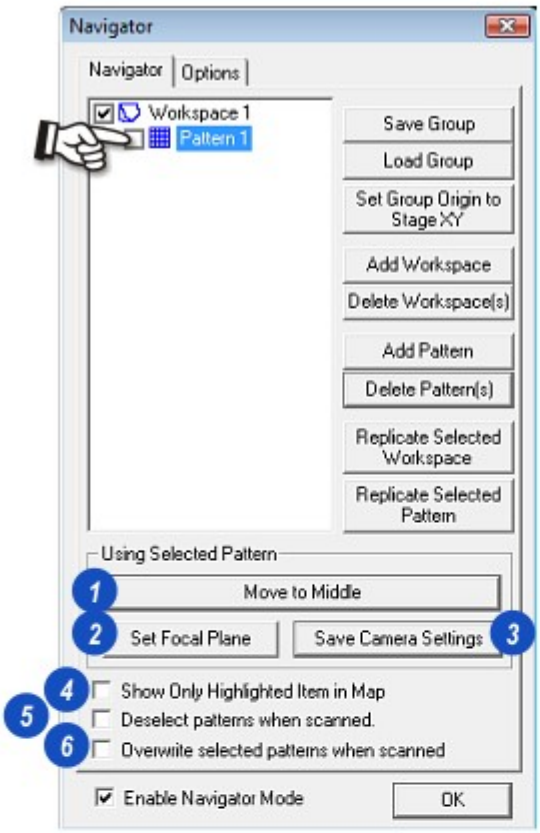
2: *Set Focal Plane* sets the Z axis to the centre of the current *Scan Pattern*.

3: *Save Camera Settings:* Not required.

4: Enable the check box to hide all of the *Scan Patterns* except the one selected.

5: Enable the check box to have *Scan Patterns* de-selected (check box to the left of the pattern name is cleared) after they have been scanned. The check box will have to be enabled before the pattern can be scanned again.

6: When *Overwrite* is enabled, any scanned images already present will be overwritten with a new image from the current scan.



Continued… 685

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To add a Workspace:

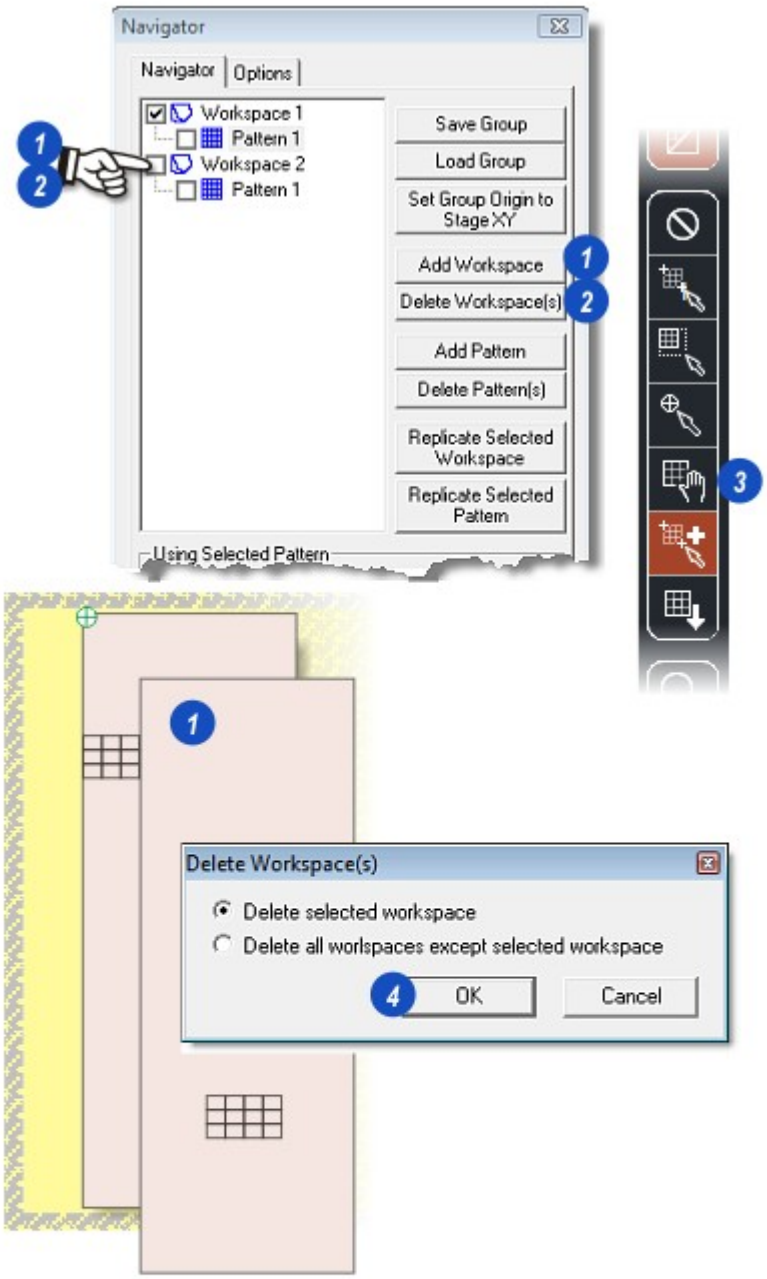
1: Click on a *Workspace* name on the *Navigator* list. This is the *Workspace* that will be copied and added. Click on the *Add Workspace* button. A duplicate of the selected *Workspace* appears with a new entry in the list. At least one *Scan Pattern* is created with a new *Workspace*.

Reposition the copy by selecting the *Drag and Drop* button **(3)** on the *Side Toolbar*, clicking on the *Workspace* copy and dragging it to a new location. A *Workspace* can be positioned precisely by using the *Workspace Properties* dialog*: Go there…*

Delete a Workspace:

2: Click on the *Workspace* name to be deleted on the *Navigator* list. Click on the *Delete Workspace(s)* button. On the *Delete Confirm* dialog **(4)** select either *Delete the Selected Workspace* or *Delete All but the Selected Workspace*. Click *OK* and the *Workspace(s)* will be removed permanently.

Continued… 686



To Add a Scan Pattern:

1: Click on the pattern name on the *Navigator* list to be duplicated and added. Click the *Add Pattern* button and a copy of the pattern will appear. Use the *Drag and Drop* tool **(3)** to re-position it.

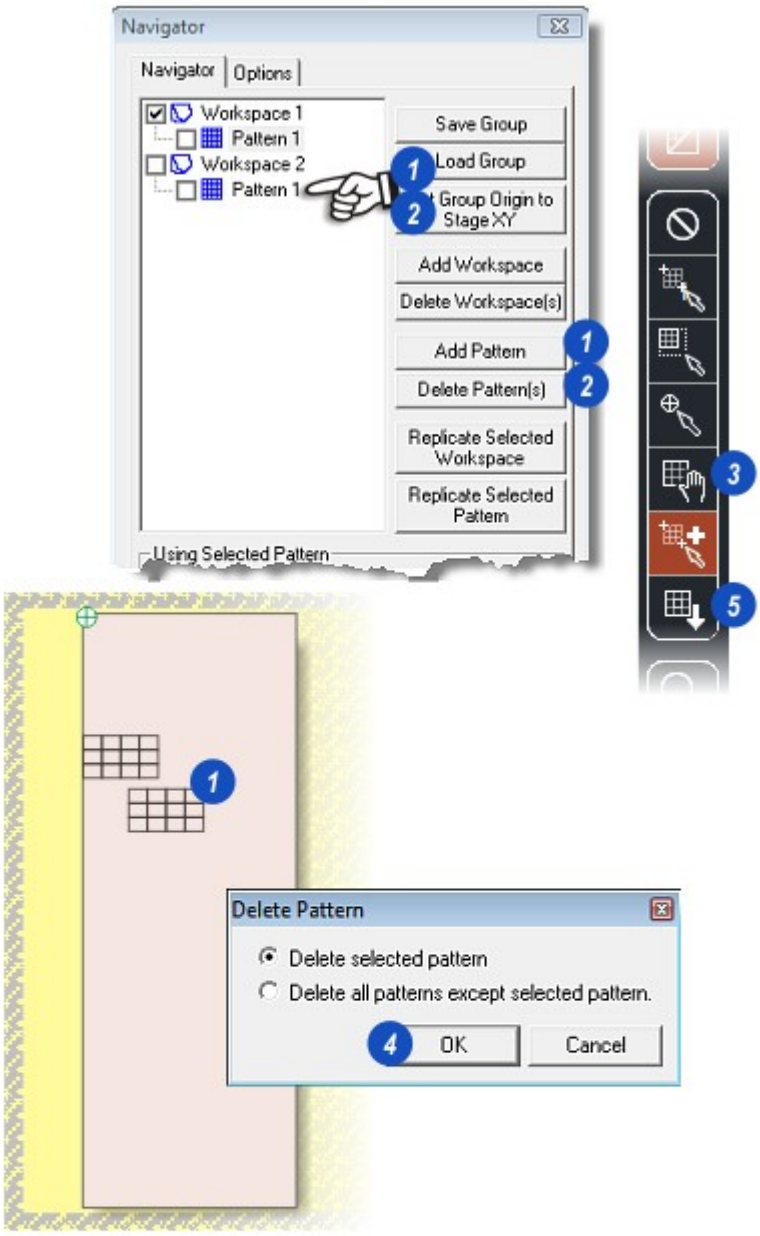
Delete a Scan Pattern:

2: Click on the pattern name to be deleted on the *Navigator* list. Click on the *Delete Pattern(s)* button. On the *Delete Confirm* dialog **(4)** select either *Delete the Selected Pattern* or *Delete All but the Selected Pattern.* Click *OK* and the patterns(s) will be removed permanently.

Copy a Scan Pattern:

5: Click on the *Copy Pattern* button to copy the currently selected *Scan Pattern* to the 'cursor' and click inside the Workspace to place a copy. Repeat for as many patterns required.

Continued... 687



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Workspace replication makes multiple copies of the selected *Workspace*, including any attached *Scan Patterns*, and places them in rows and columns determined by the user. The original *Workspace* is removed.

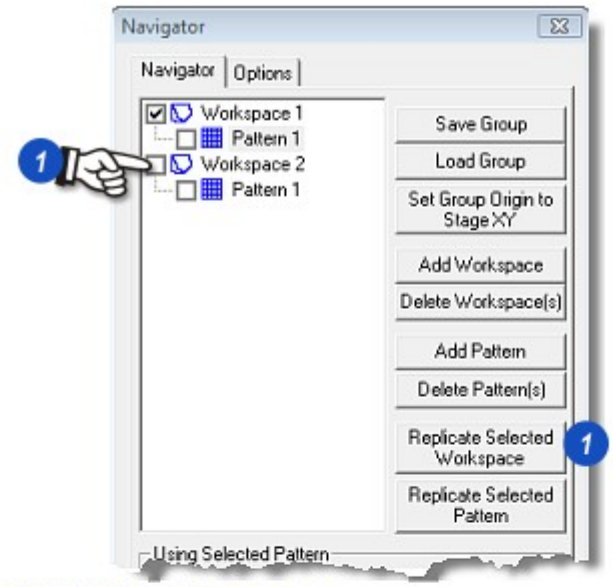
1: Click on the *Workspace* name on the *Navigator* list that is to be replicated. And click on the *Replicated Selected Workspace* button.

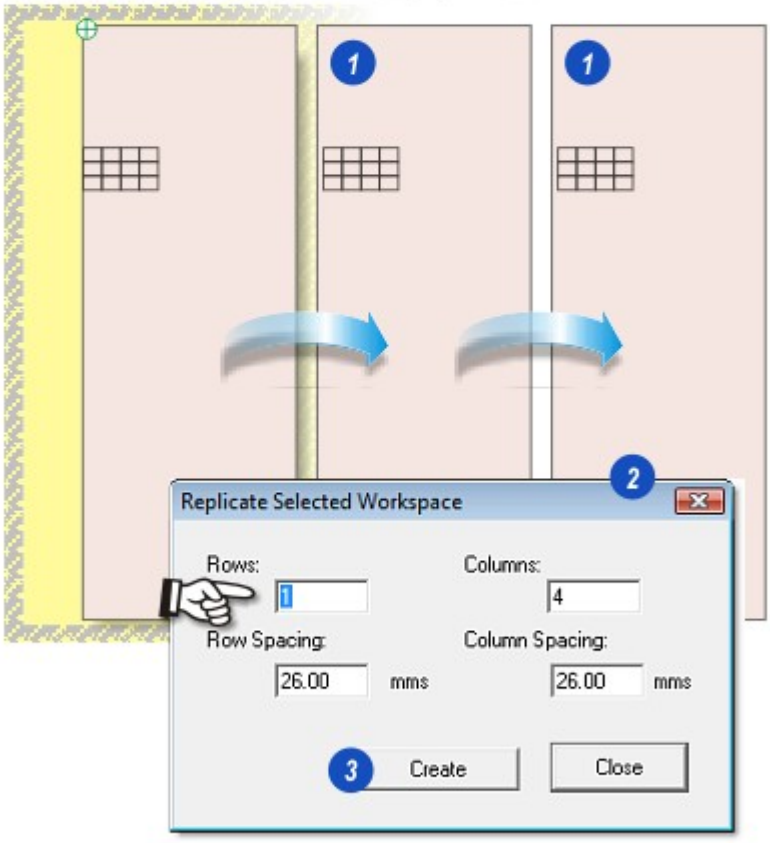
2: The *Replicate* dialog appears. Set the number of *Rows* and *Columns* required and their spacing by clicking in the appropriate window and typing a value.

3: Click **Create**. A warning appears advising that the original will be deleted and replaced by a new group each *Workspace* of which will be a faithful copy of the original.

The *Row* and *Column* settings can be adjusted and the *Create* button clicked again without closing the dialog.

Continued… 688





Scan Pattern replication, makes multiple copies of the selected pattern and arranges them precisely in a matrix either created by the user or based upon the range of templates supplied with the Navigator.

1: Click on the *Pattern* name on the *Navigator* list and click the *Replicate Selected Pattern* button.

2: On the *Replicate Pattern* dialog, enter the number of *Rows* and *Columns* as well as the spacing between, by clicking inside the appropriate text box and typing a new value.

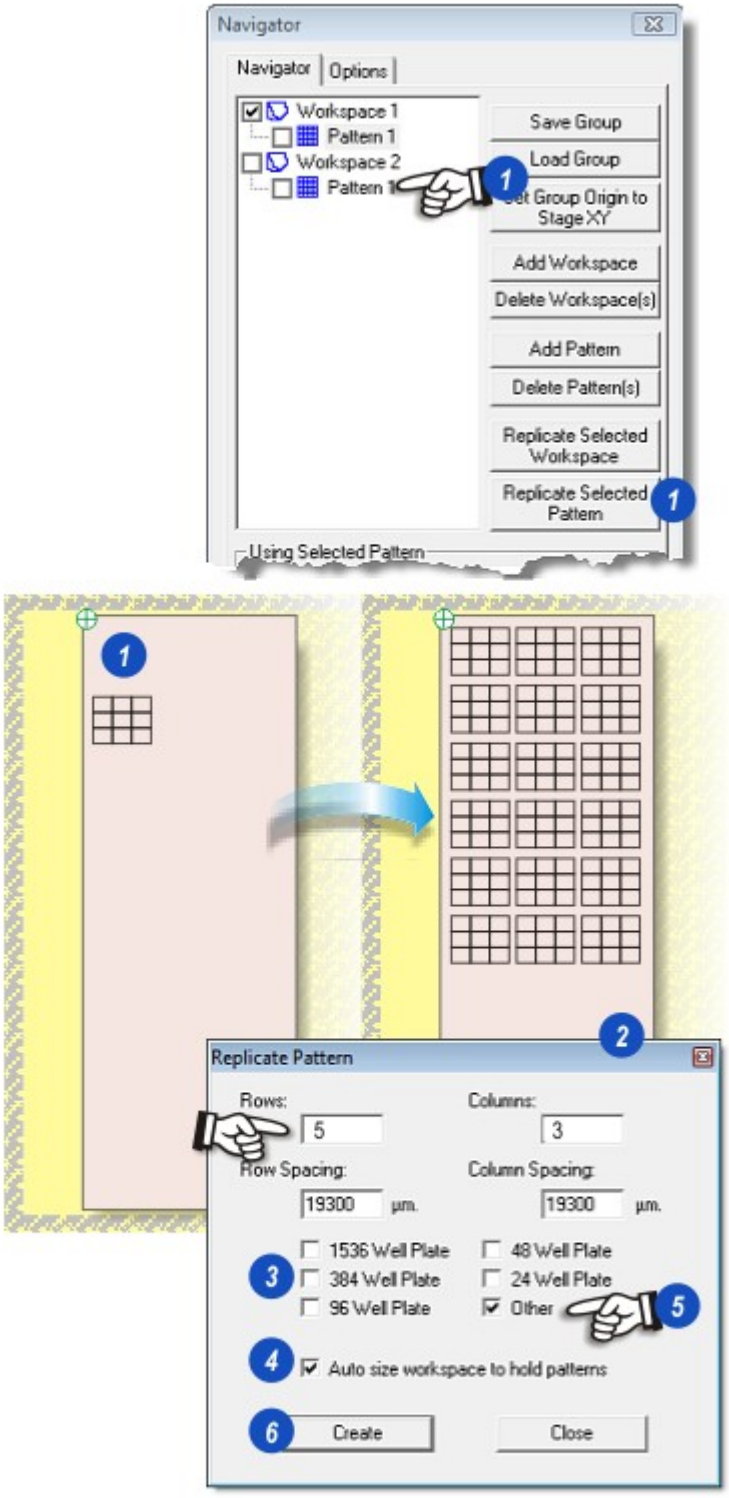
3: A range of templates for standard *Well Plate* configurations are provided and a template can be selected by clicking to enable the check box to its left.

4: If the current *Workspace* is too small to accommodate the selected template, it is automatically re-sized.

5: For user-defined column and row layouts click to enable the *Other* option and, if necessary, enable the automatic re-sizing facility **(4).**

6: Click the *Create* button. The selected pattern is replicated on its *Workspace* . The *Row*, *Column* and spacing values can be adjusted and the Create button clicked again without closing the dialog.

Continued… 689



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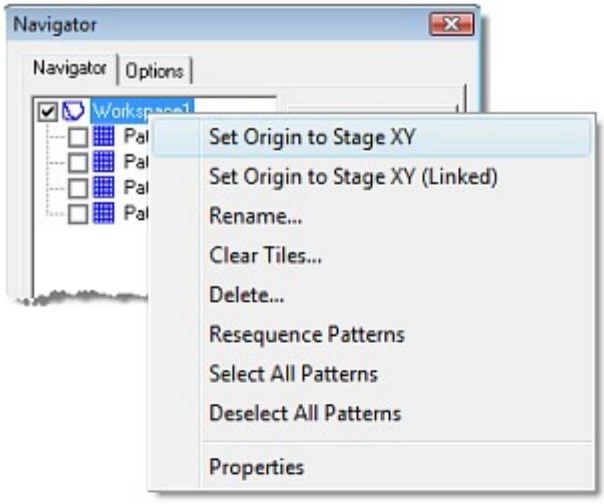
Right-clicking on a *Workspace* name on the *Navigator*, reveals an alternate menu. Click to select the high-lighted option:



Set Origin to Stage X/Y: Moves the entire selected *Workspace* and patterns to the current stage position. The *Stage Marker* is centred on the 'first' *Scan Pattern.*

Rename: Type a new name for the *Workspace*. It must be unique.

Clear Tiles: Clears all of the mosaic filed images form all of the patterns on the *Workspace*.

Delete: Deletes the selected *Workspace* and its patterns.

Re-sequence Patterns: Re-numbers the pattern sequence starting at '1'.

Select All Patterns: Selects and high lights all of the patterns on the *Workspace*.

Deselect All Patterns: Deselects all selected *Scan Patterns.*

Continued… 690

Right click on a *Workspace* name on the *Navigator* list and from the drop down menu:

1: Click on the *Properties* option.

2: The *Workspace* (top left corner) can be positioned precisely by setting the *Stage-X* and *Y* co-ordinates and clicking the *Apply* button **(7).**

3: *Set Position to Current Stage XY* when clicked will move the *Workspace* to the current stage position.

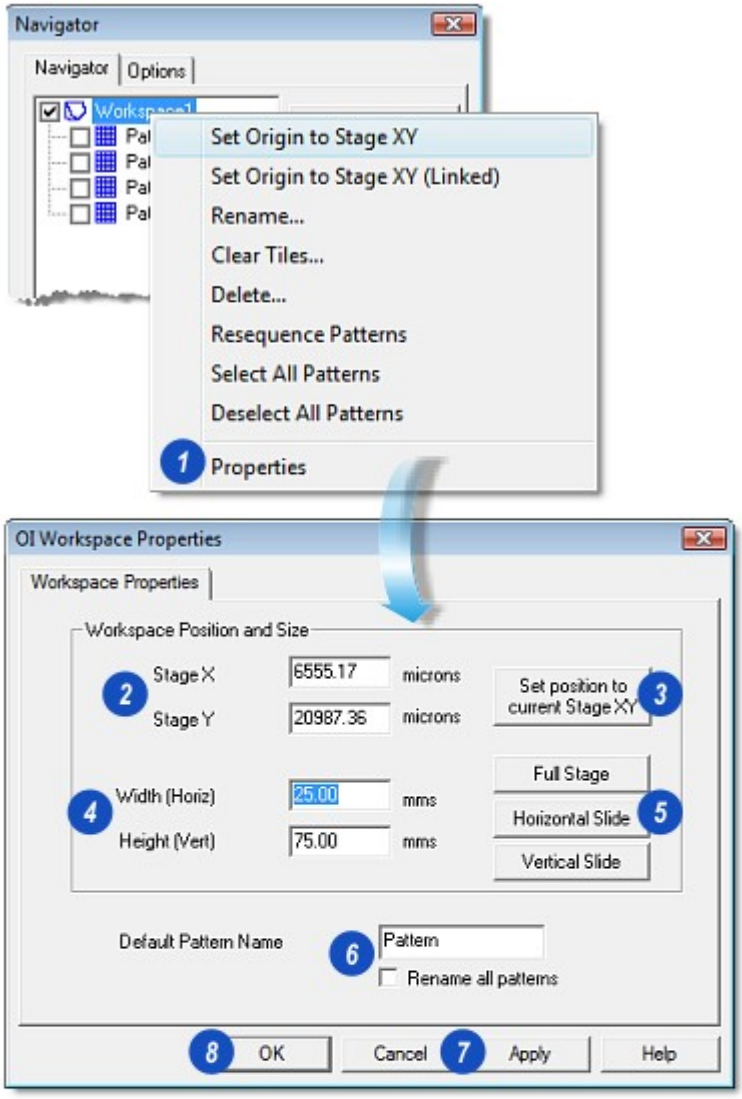
4: The *Workspace* can be re-sized by clicking in the *Height* and *Width* text boxes and typing a new value. Click the *Apply* button **(7).**

5: Three pre-set templates – *Full Stage, Horizontal* and *Vertical Slides* – are provided. Click on an option to automatically size the *Workspace*.

6: The default name for *Scan Patterns* is 'Pattern' followed by a sequential number as they are created. To change the default name, click in the *Default Pattern Name* text box and type a new name. To retrospectively rename all existing names, click to enable the *Rename All* check box.

7: Click *Apply* and…

8: …click *OK*.



Continued… 691

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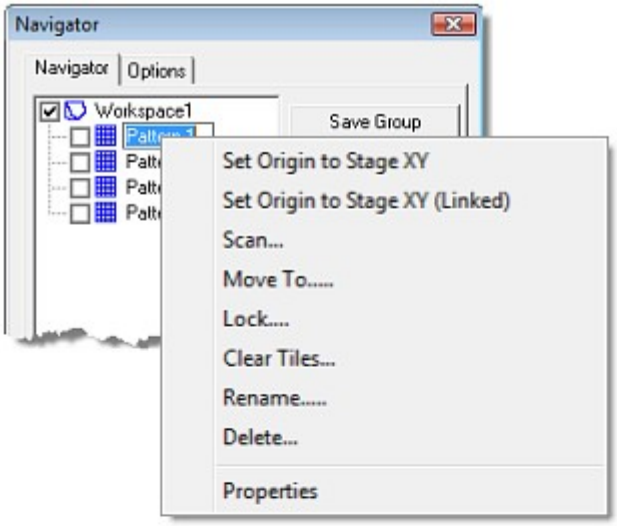
Right clicking on a *Scan Pattern* name on the *Navigator*, reveals an extended menu of options. Click to select a menu item:



Set Origin to Stage XY: Moves the *Scan Pattern* and its *Workspace* to the current stage position. The *Stage Marker* is located at the centre of the *Scan Pattern.*

Scan: Starts a scan for the selected pattern only.

Move To..: Selects the *Drag and Drop* tool. Click on the *Scan Pattern* and drag it to the required location.

Lock: Locks the *Scan Pattern* at its current position and a small padlock icon appears to the left of its name on the *Navigator*. Click *Lock* again to unlock it.

Clear Tiles: Removes any mosaic fields in the selected pattern only.

Rename: Type a unique name for the pattern.

Delete: Deletes the selected *Scan Pattern* only.

Continued… 692

To display the *Scan Pattern* properties:

1: Click on the arrow to the right of the *Scan Pattern* header to reveal the panel.

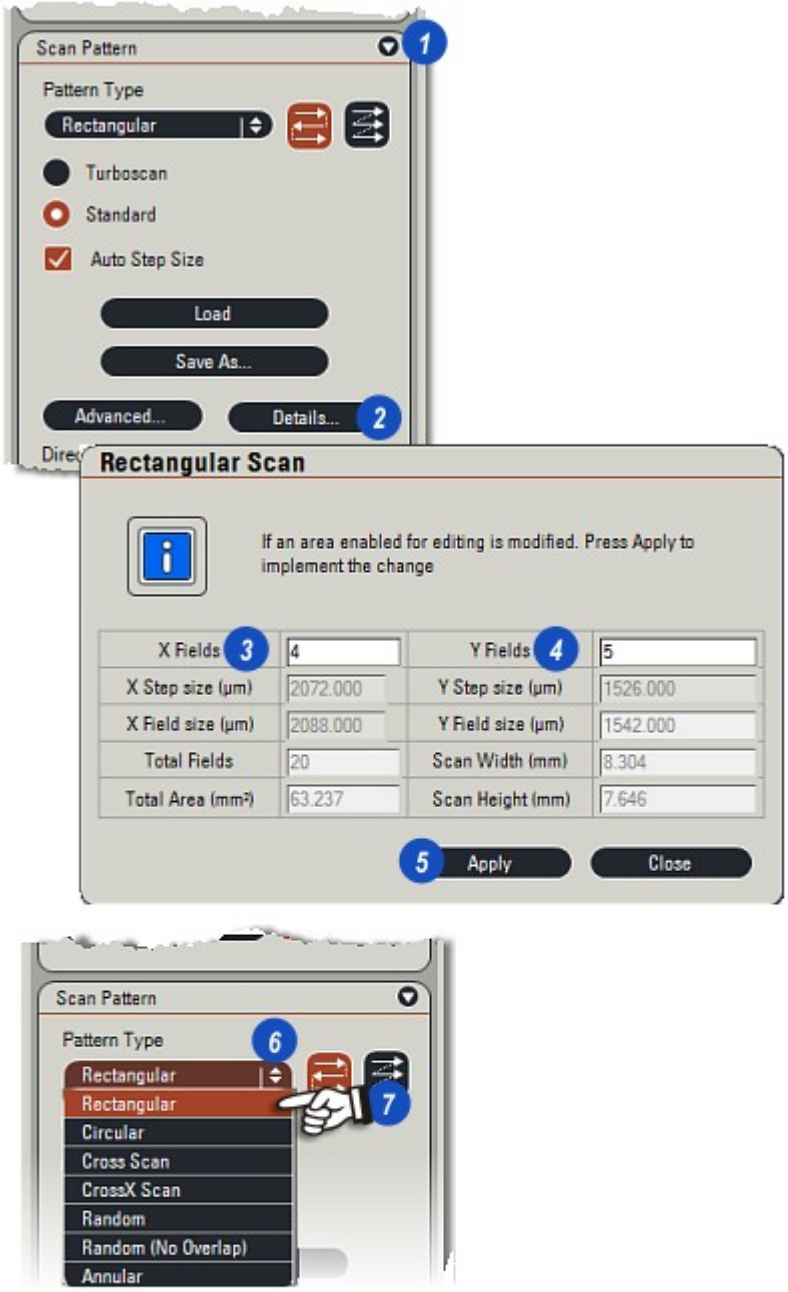
2: click on the *Details* button. The *Scan* dialog appears – in this case *Rectangular* layout.

3 & 4: Change the number of *Columns* (X) and *Rows* (Y) by clicking inside the appropriate text box and typing a value.

5: Click *Apply* and the selected pattern will change to reflect the new values.

6: A *Scan Pattern Type*– in this example the *Rectangular* layout has been used – can be changed to better suit the specimen shape and optimise the number of images required. Click on the arrows to the right of the *Pattern Type* header and from the drop down list…

7: …click to select the required type.



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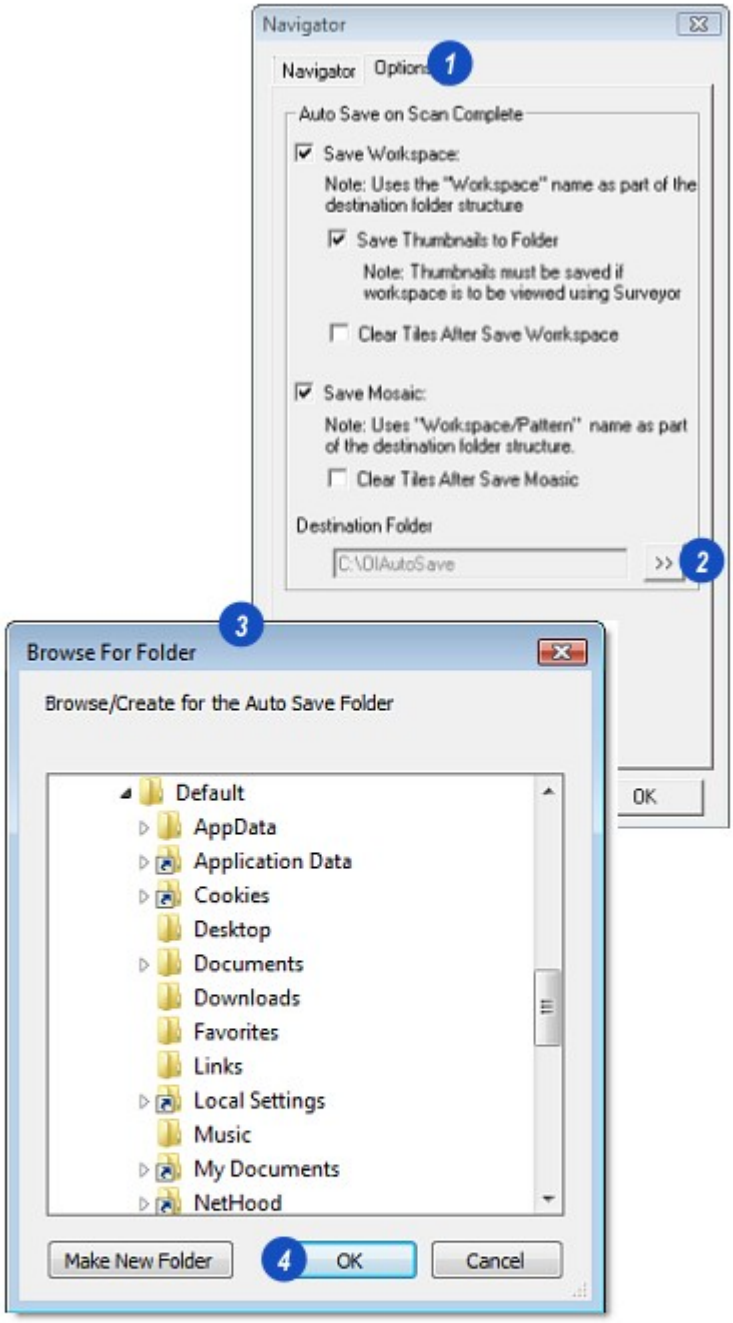
When the scan is complete, there are several options that can be invoked automatically – such as saving the mosaic.

1: Click on the *Navigator Options* tab to reveal the options. Check to enable the required options:

Save Workspace: Automatically saves the current *Workspace* with the word '*Workspace*' as the file name followed by a sequential number – *Workspace-05* for example.

Save Thumbnails to Folder: Saves a low resolution thumbnail of each scan tile within the *Workspace* folder to be used in the *Gallery*.

Clear Tiles After Save Workspace/ Mosaic: Clears the scan tiles from all *Scan Patterns*. Especially important where machine memory is at a premium. Use a greater Display Thumbnail Reduction Factor to reduce memory usage. *Go there...* 694



There are a large number of options that can be used to tailor *Pattern Navigator* exactly to a user's needs. The major options are explained here. The *Map Properties* dialog is reached by:

1: Click on the *Interactive Mouse* button on the *Side Tool Bar* and then right-click anywhere on the stage display area. The *Map Properties* dialog appears.

2: If necessary, click on the *Map* tab to reveal the main functions.

3: The *View* panel determines the current *Navigator* view:

Stage: The viewable *Stage* area with the *Workspaces* and *Scan Pattern(s).*

Pattern: The selected pattern is displayed enlarged.

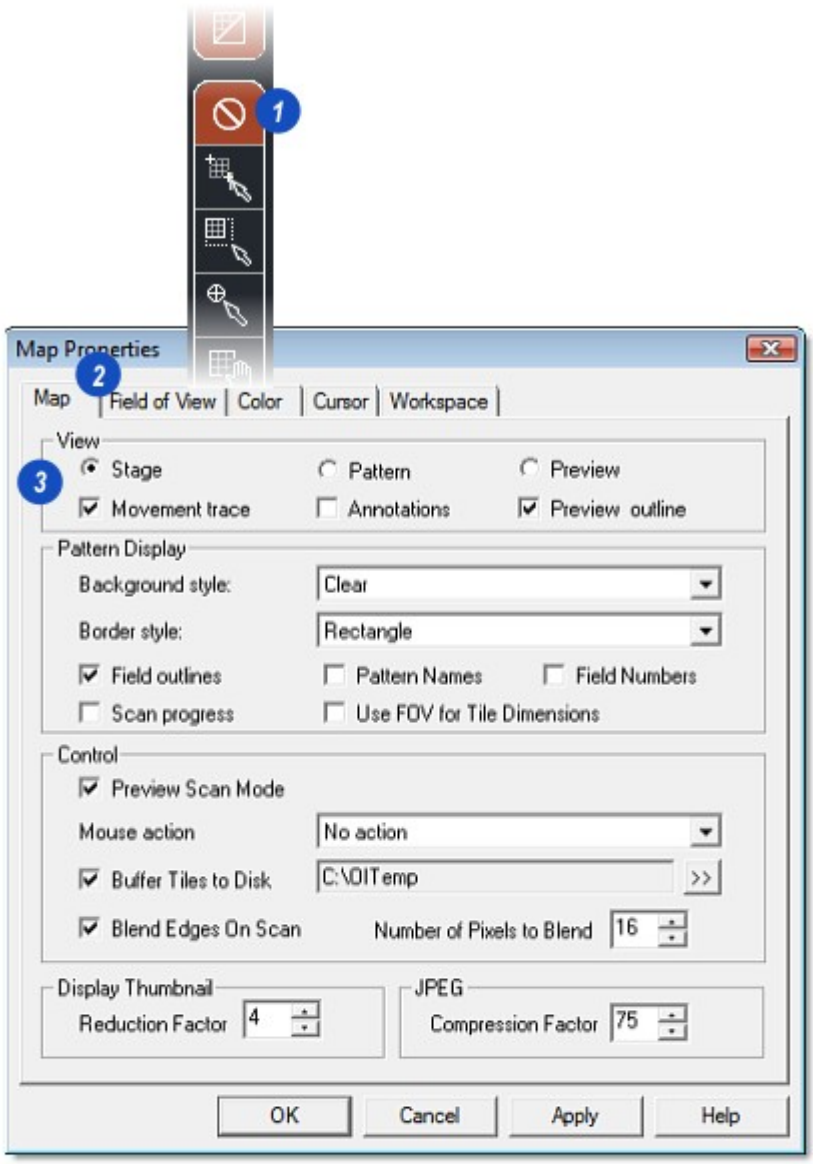
Preview: Not required.

Movement Trace: Displays the trace path over the *Scan Pattern* **(4).**

Annotations: Turns On and Off *Pattern Name* and *Field Numbers* if they are enabled.

Preview Outline: Not required.

Continued… 695



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In the *Pattern Display Panel*:

1: Click on the arrow to the right of *Background Style* menu to reveal how the *Scan Pattern Fields* should be highlighted and filled. Click a menu item to select it.

2: The *Scan Pattern* border style can be changed by clicking on the arrow to the right of the *Border Style* menu and clicking to select an option. *Rectangle* **(3)** is the 'normal' rectangle configuration and…

4: …*Circle* configures the pattern as a series of overlapping, circular fields.

Field Outlines: Displays an outline around each field.

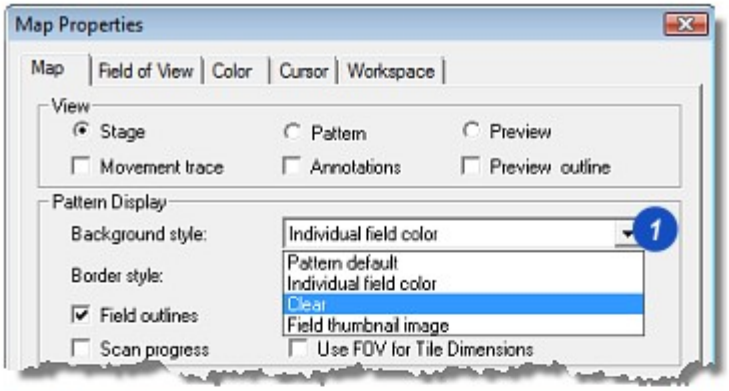
Pattern Names: Displays the *Pattern Name* beneath the pattern **(4).**

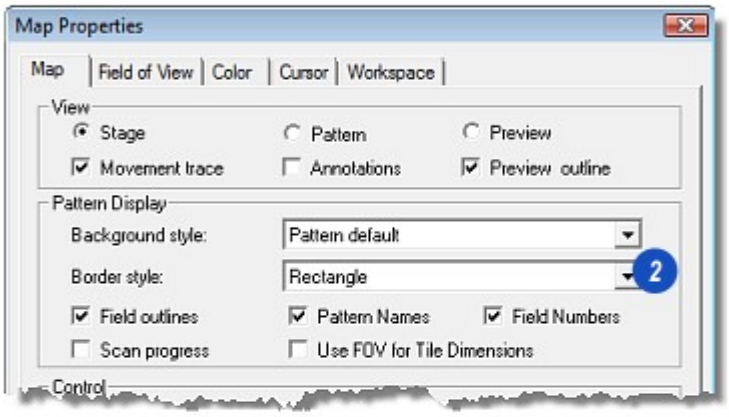
Field Numbers: Displays a sequential number against each field **(4)**.

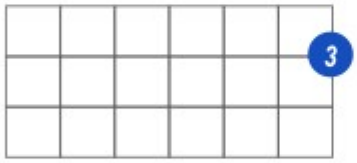
Scan Progress: Displays a progress bar during scanning.

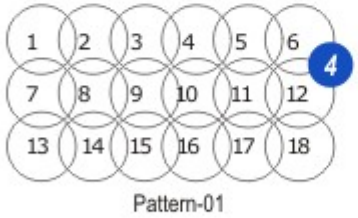
Use FOV for Tiles: The *Field of View* determines the field dimensions.

Continued… 696









1: Click on the *Colour* tab to reveal the colour options.

2: On the left-hand panel, click to select the item to have a colour change. Use the slider on the right to display all of the items.

3: Choose a colour from the right-hand display. The first colour entry has the option to select a user colour via the *Windows Colour* dialog.

4: Click on the *Cursor* tab to modify the *Cursor* – stage marker.

5: Adjust the values to construct the cursor of choice – even an elliptical outline **(6)**.

The *Workspace* can be displayed or hidden and its shape and colour changed by:

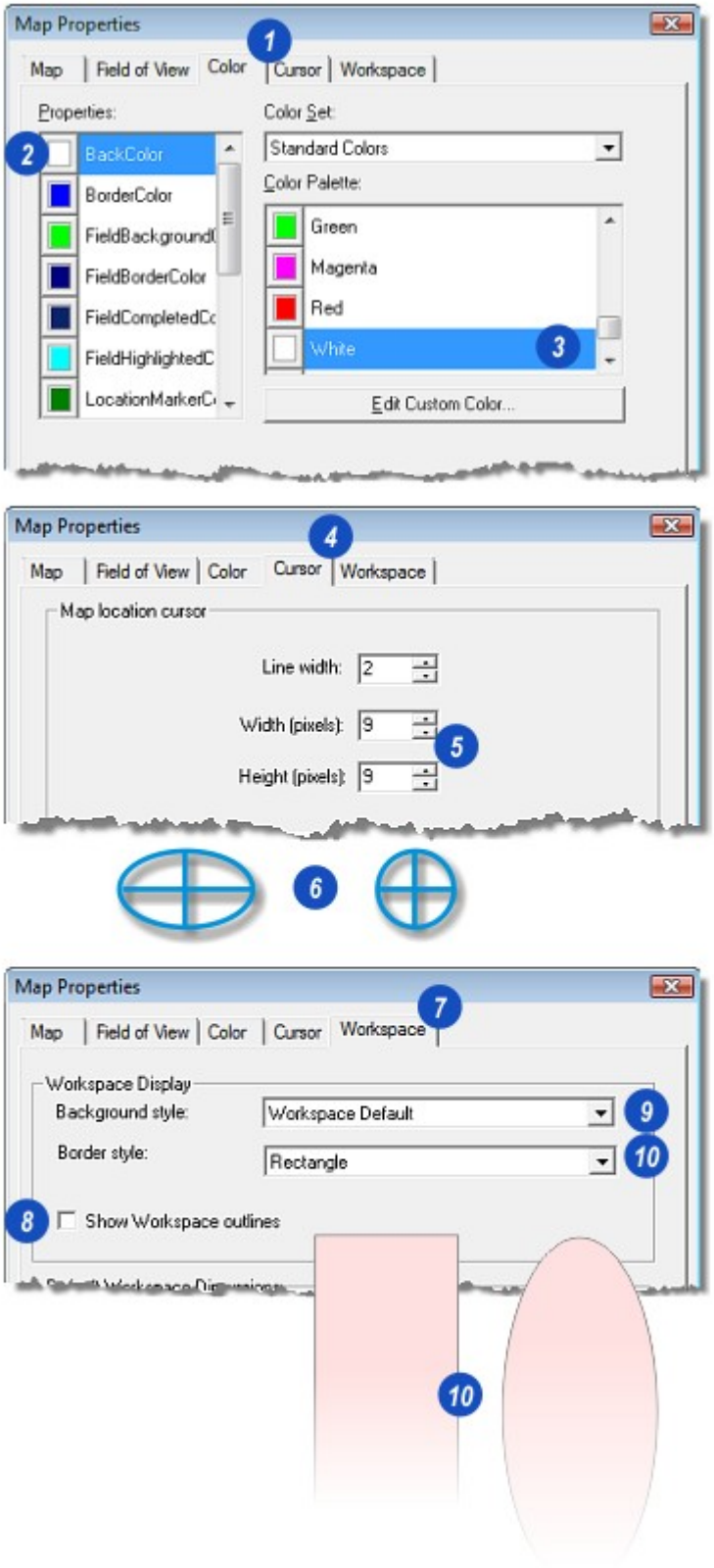
7: Click on the *Workspace* tab.

8: Display or hide the *Workspace* by checking or clearing the check box.

9: Click on the small arrow to the right of the *Background Style* menu to list the options - display in selected colour (See Step 2 above) or display the outline only.

10: The shape of the *Workspace* is set by clicking the small arrow to the right of the *Border Style* menu and selecting from *Rectangular* or *Circular* .

Continued... 697



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Some of the main *Pattern Navigator* properties can be accessed through the *LAS Power Mosaic* panels as follows:

1: Click on the small arrow to the right of the *Scan Pattern* header.

2: Click on the *Advanced* button.

On the *Advanced Settings* dialog:

3: Set the scan tiles capture folder by enabling the *Buffer Tiles to Folder* check box,…

4: …click on the browse button and, on the Windows dialog, navigate to and select the destination folder.

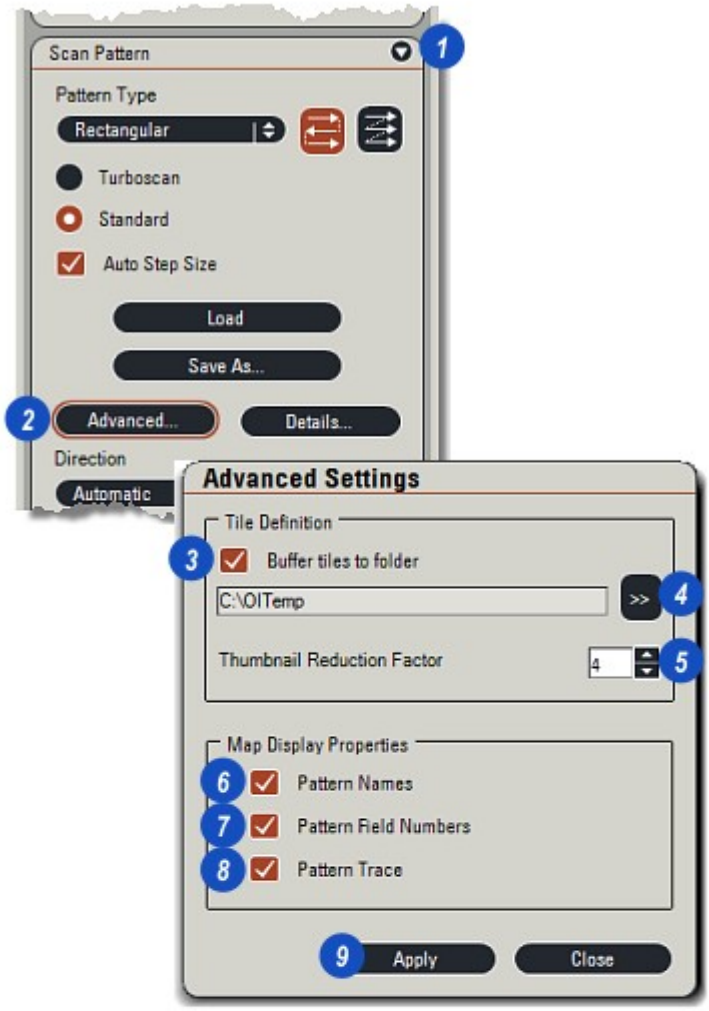
5: Set the *Thumbnail Reduction Factor* – a larger number results in more memory space-saving – by clicking on the Up/Down arrows to the right of the text box.

6: Display/Hide the *Scan Pattern Names* by checking or clearing the check box.

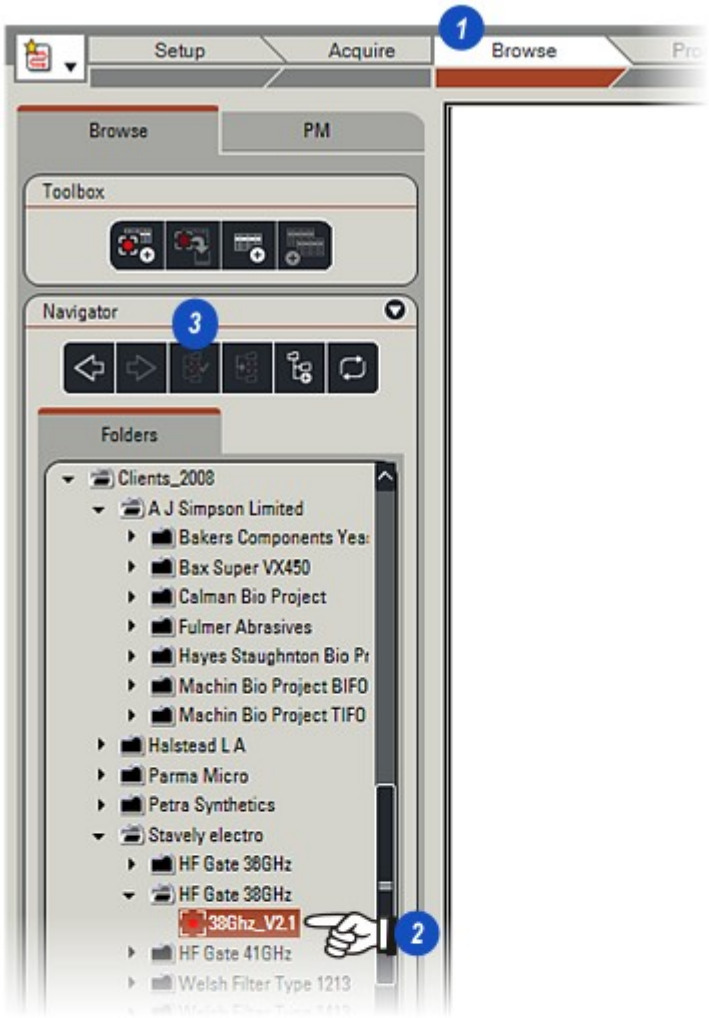
7: Display/Hide the *Scan Pattern Field Numbers* by checking or clearing the check box.

8: Enable/Disable the stage travel *Trace* by checking or clearing the check box.

9: Click *Apply* to load the settings and close the dialog.



After a scan (or scans), the tiles are saved in a temporary file on the hard drive and the thumbnails in RAM. Both may be saved to a nominated folder - the Capture Folder - on the hard drive.



Select the Capture Folder:

1: Click on the *Browse Workflow* tab.

2: Click on the archive folder in which to save the scans.

3: Click on the *Set Fixed Capture Location* button.
A red dot appears to the left of the selected folder.

Continued... 699

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There are two parts to saving the scanned images – saving the collection of individual tiles which at this point are still being stored in a temporary file, and thumbnails of the tiles that are in volatile RAM, - and then saving the tiles as a composite image or mosaic.

1: Click on the *PM* (Power Mosaic) tab.

2: Click on the *Save Workspace Location* button. A Workspace is a detailed description of the display and image settings saved as a file with the extension *.sws* within the Workspace Location.
Additionally, another folder to contain the individual tiles is created within the Workspace Location together with Thumbnail images of each tile.

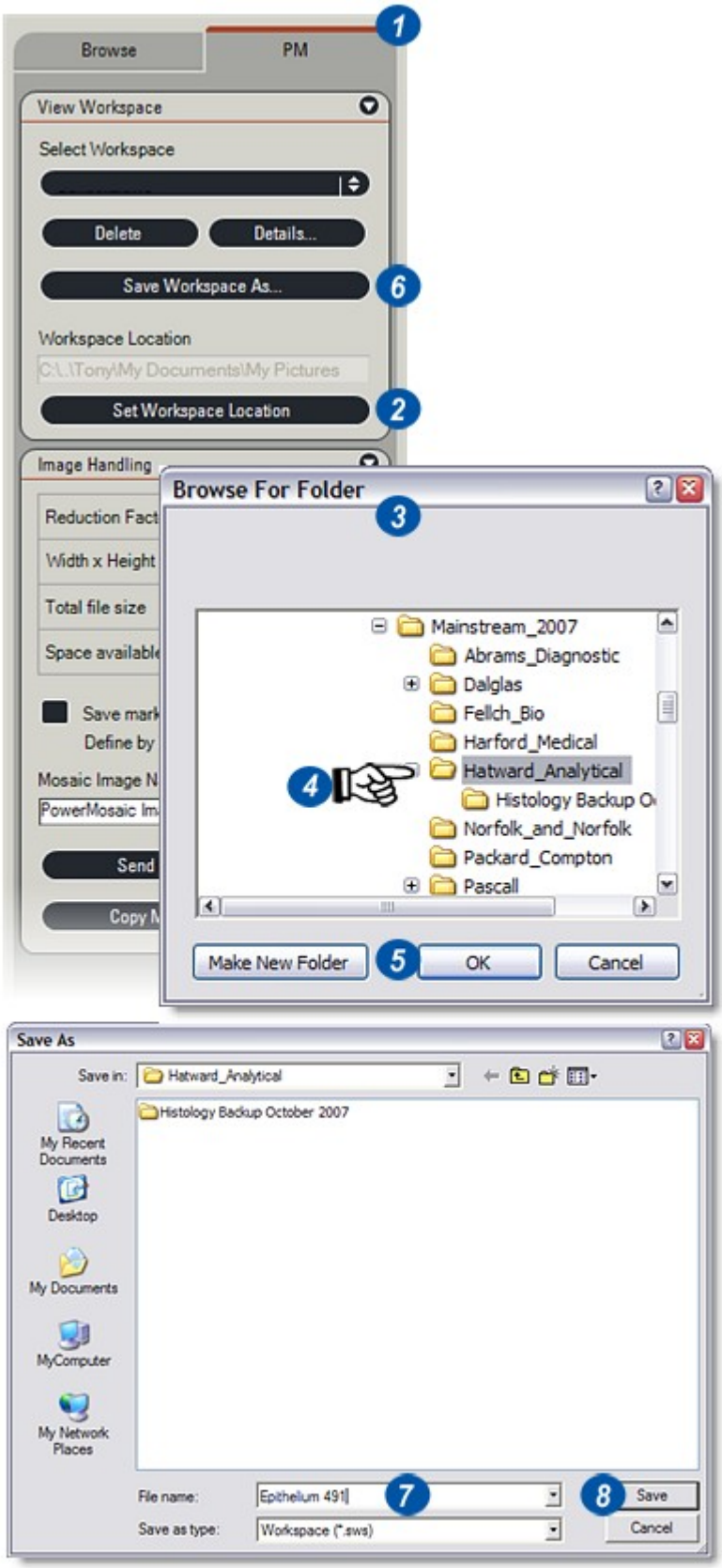
3: On the Browse For Folder dialog...

4: ...navigate to the folder in which to save the Workspace and...

5: ...click *OK*.

6: Click on the *Save Workspace As* button and on the *Save As* dialog...

7: ...type a unique name for the Workspace and click *Save* **(8)**.
A *Saving mosaic images progress* message appears and the tiles are saved at the format and resolution selected on Camera:Input Options.



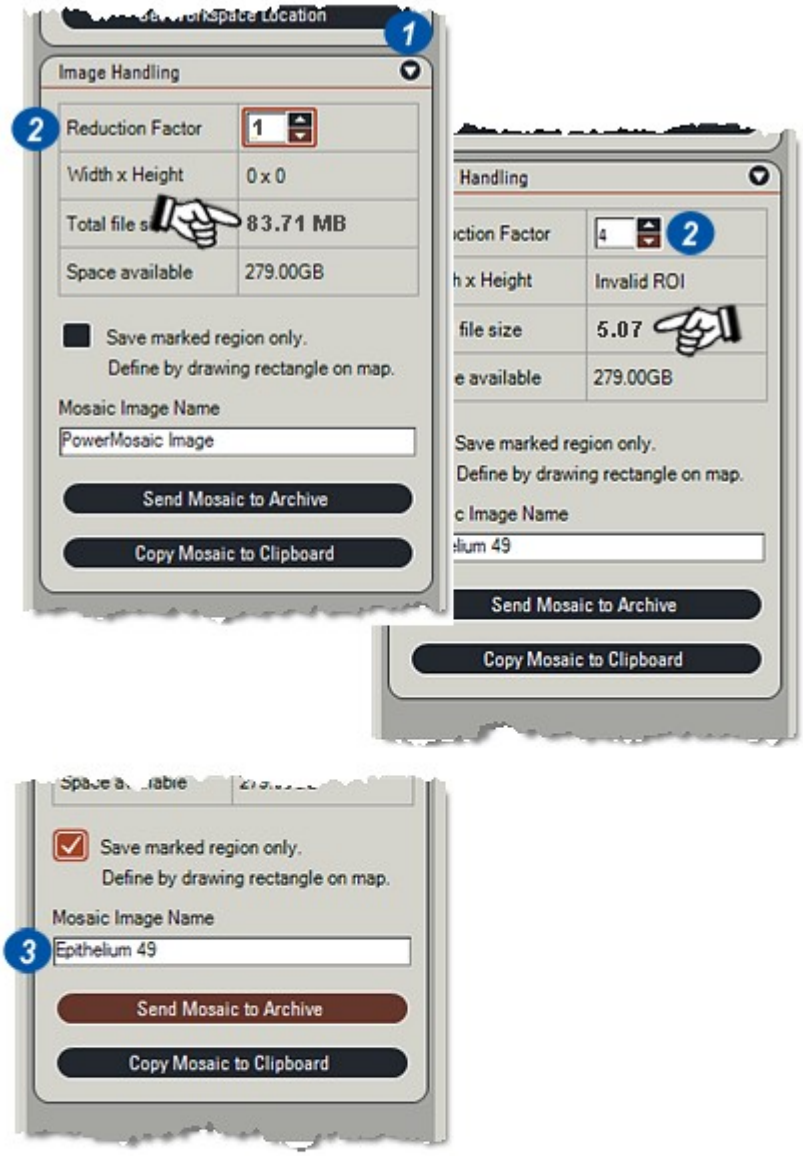
The composite image or mosaic file is saved directly in the Capture Folder with several associated control files. Because mosaic images can be very large in terms of disk space occupied – several gigabytes is not unusual – there is a facility to reduce the size of the saved mosaic. This is called the reduction factor.

1: Click on the arrow to the right of the *Image Handling* header to reveal the panel.

2: Using the arrows to the right of the *Reduction Factor* text box, increase or decrease the Reduction Factor. The illustrations show the considerable difference in image size with factors of 1 and 4.

3: Click in the *Mosaic Image Name* text box and type a name for the mosaic. The image will be stored with this name in the Capture Folder.

Continued... 701



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It is not necessary to save the entire mosaic – very often only one part is of real interest and the remainder can be discarded. It is possible to 'crop' the image to a selected region and save that alone. The individual tiles are not affected – they remain intact.

To save a selected part of the mosaic:

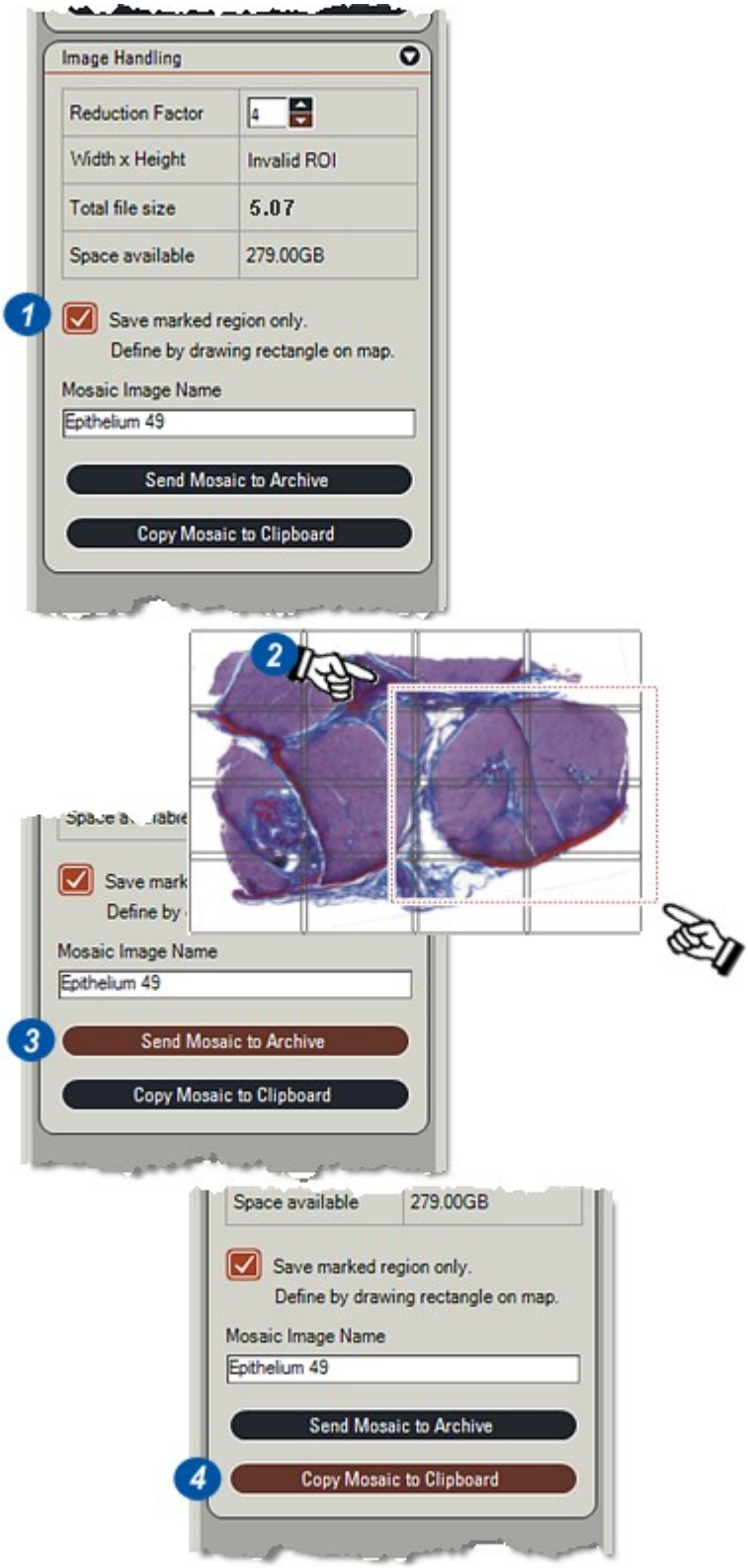
1: Click on the *Saved Marked Region* check box to enable it.

2: Click on the edge of the region to be saved, hold and drag diagonally to the right to encompass the region. Release the mouse button. For clarity, the illustration shows a red dotted line – on screen it is black.

3: Save the mosaic by clicking *Send Mosaic to Capture Folder.*

4: The mosaic can also be saved to the Windows Clipboard for loading into another application. Click on the Copy *Mosaic to Clipboard* button.

Go to Retrieving the Mosaic... 702



When a mosaic is retrieved and re-displayed, it is the Workspace and therefore the tiles that are targeted with a *new* full size mosaic created on the viewer. This could be saved again as a mosaic either as a selected region or at a different size.

To retrieve a mosaic:

1: Click on the arrows to the right of the *Select Workspace* header.

2: From the drop down list, click to select a *Workspace*.

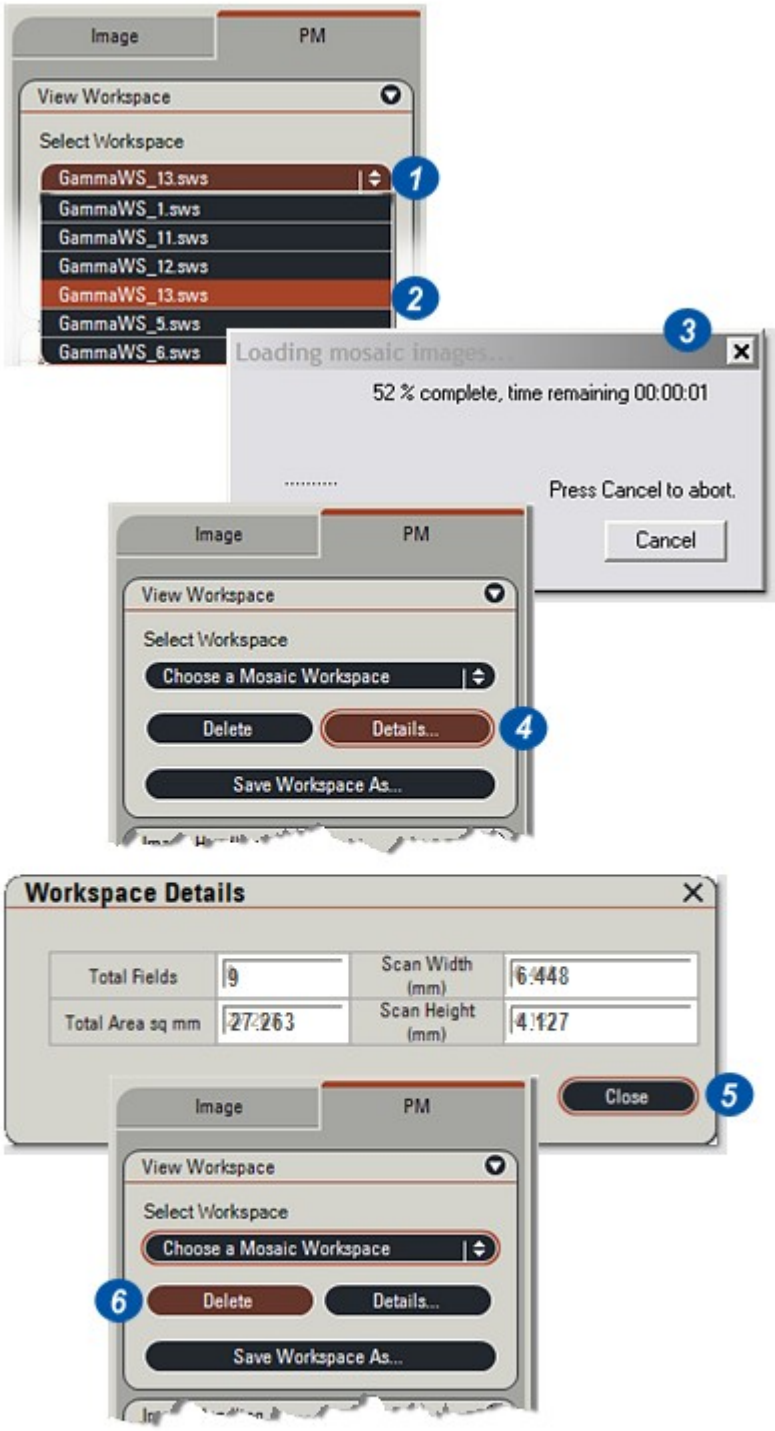
3: A progress panel (Loading Mosaic mages) appears and the tiles are displayed as a mosaic on the viewer.

4: Detail of the mosaic can be displayed by clicking the *Details* button and…

5: …the *Workspace Details* panel appears specifying the number of tiles and image area.

To delete a Workspace:

6: Click on the *Delete* button to delete the selected Workspace (Steps 1 and 2 above). Deleted Workspaces cannot be retrieved.



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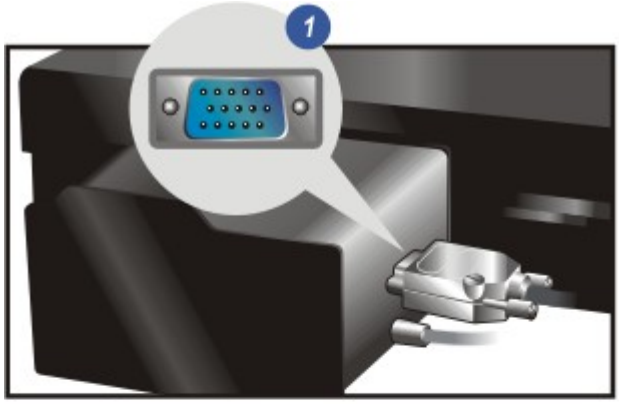
Although the Leica ISO Pro stage is driven directly by Leica Application Suite, it still requires an Oasis Controller Board to report the X and Y co-ordinates.

Connection between the stage and the computer is by a 15 pin (not the usual 9 pin) 'D' style DIN plug **(1).**

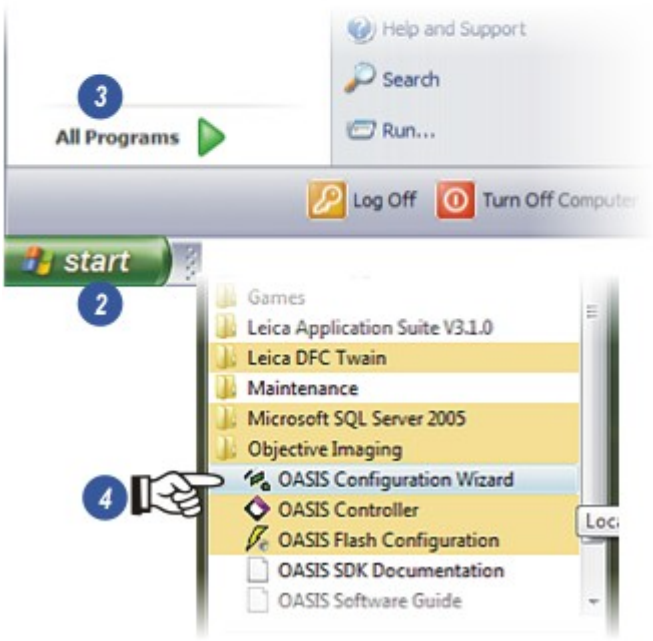


Selecting the Stage:
LAS needs to 'know' that an ISO Pro stage is to be used and this is setup using the Oasis Configuration Wizard. The illustrations are from a typical Windows XP operating system - Windows Vista is very similar.

2: Click on the *Start* button and...

3: ...on *All Programs.*

4: From the pop up menu, click to select the *Oasis Configuration Wizard.*



Continued... 704

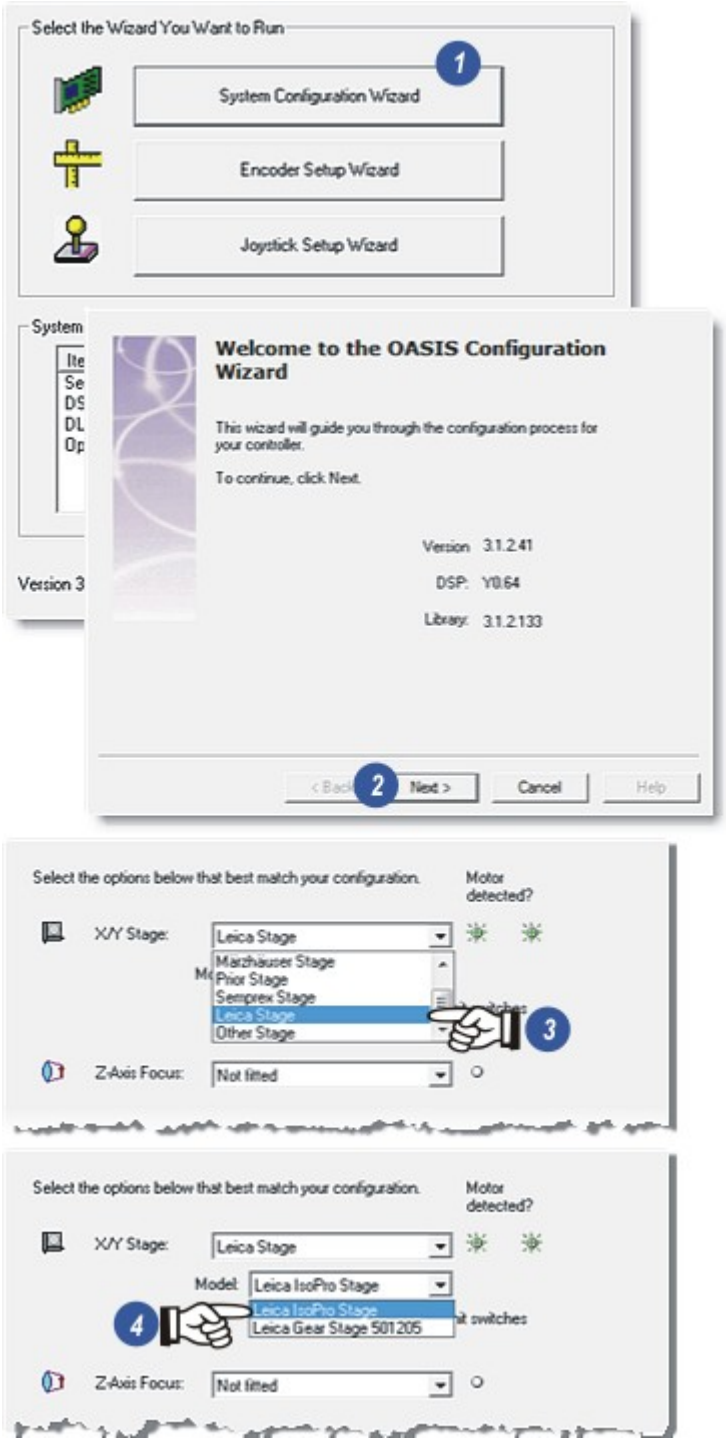
1: When the Oasis Configuration Wizard opens, click on the *System Configuration Wizards* button.

2: Click *Next* on the introduction dialog.

3: In the *X/Y* list box, use the scroll bar on the right to find the *Leica Stage* entry. Click to select it.

4: In the *Model* list box, click to select the *Leica ISO Pro* stage. Click *Next.*

Continued... 705



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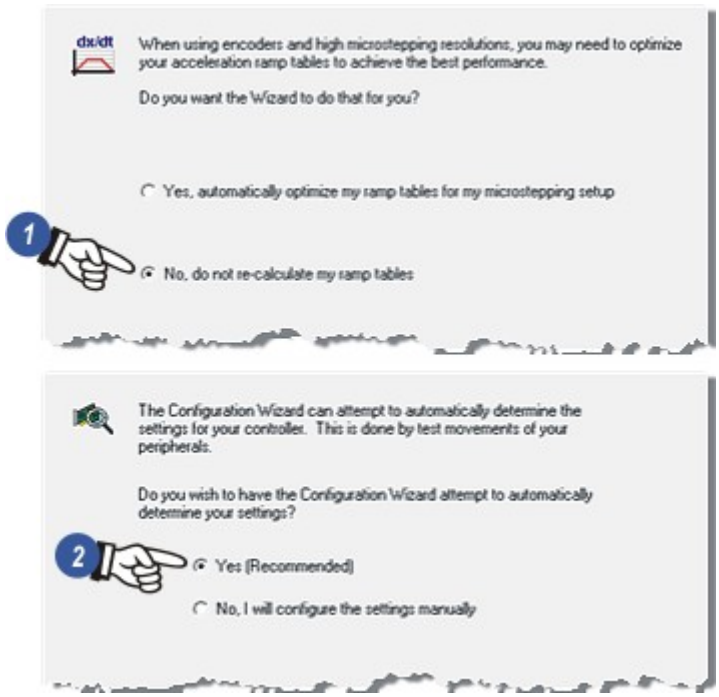
1: For the Acceleration Ramp tables click the *No, do not re-calculate* radio button. LAS automatically handles the motor power.

2: Most of the details of the data exchange between Oasis and LAS can be setup automatically so click to select *Yes (Recommended)*.

Make sure the stage is clear of any obstructions and click the **Finish** button to exit the wizard.

Continued...



On the Acquire Workflow:

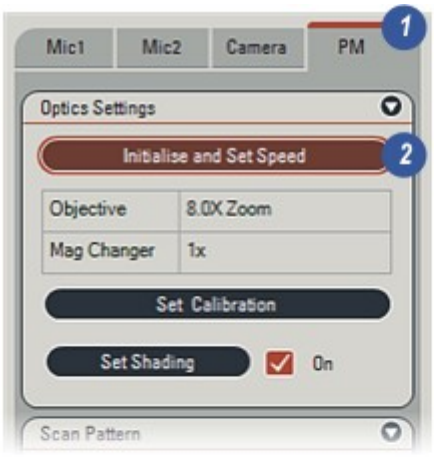
1: ...if necessary click the *PM* (Power Mosaic) tab to reveal the Optics Settings panel. If the PM tab is not visible the Power Mosaic module has not been loaded: *See Starting: Go there...* 623

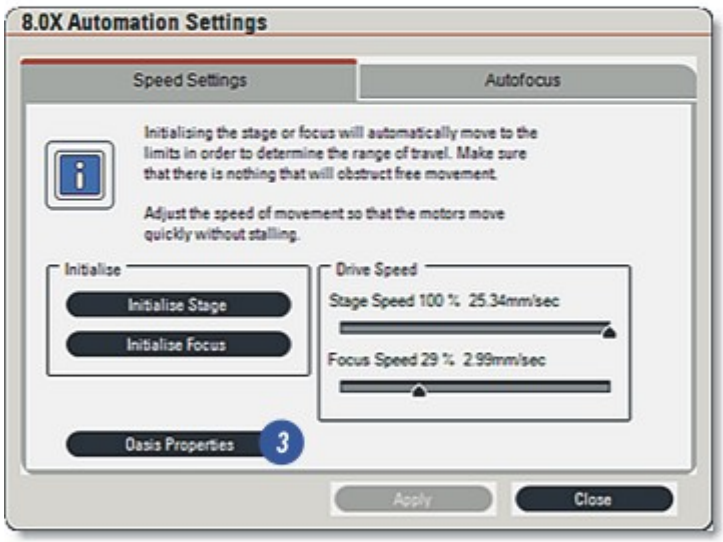
2: Click on the *Initialise and Set Speed* button.

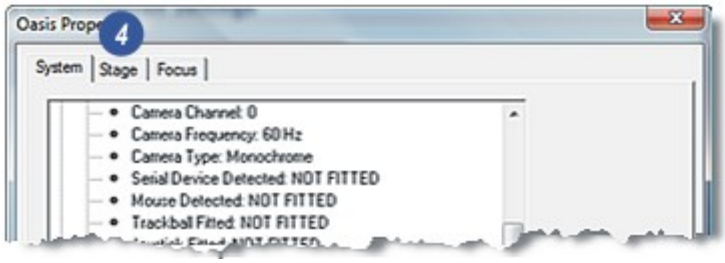
3: On the Automation Settings dialog, click on the *Oasis Properties* button.

4: When the Oasis Properties dialog opens, click on the *Stage* tab.

Continued... 707







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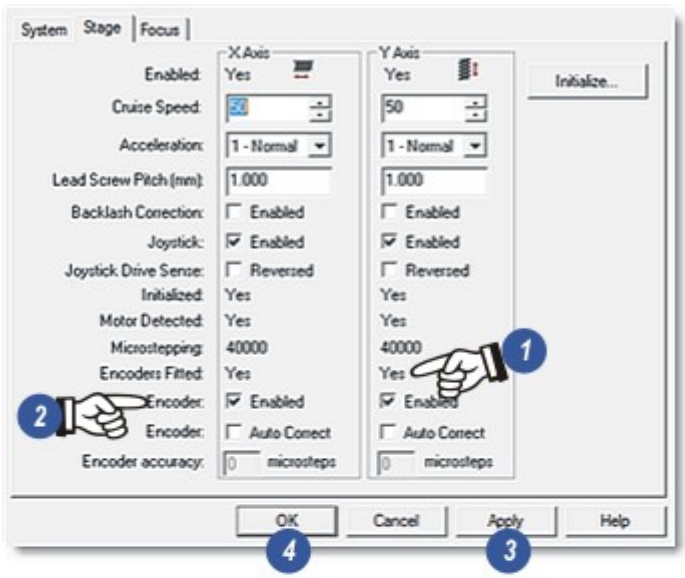
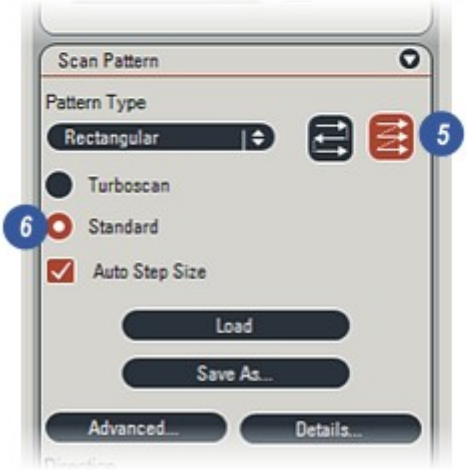
On the Oasis Properties > Stage dialog:





1: Check that *Encoders Fitted* is set to *Yes*. If it is not the cable connection may be loose or not fitted, or the wrong stage has been selected. Repeat the Oasis Configuration process: *Go there...* 703

2: Click to enable both of the *Encoder X* and *Y Axes* check boxes.

3: Click *Apply*.

4: Click *OK*.

5: The ISO Pro stage will perform well in both bi- and uni-direction modes, but for scanning precision the uni-direction mode is recommended.

6: Again, for accuracy and precision opt for the *Standard* scan speed in most situations.

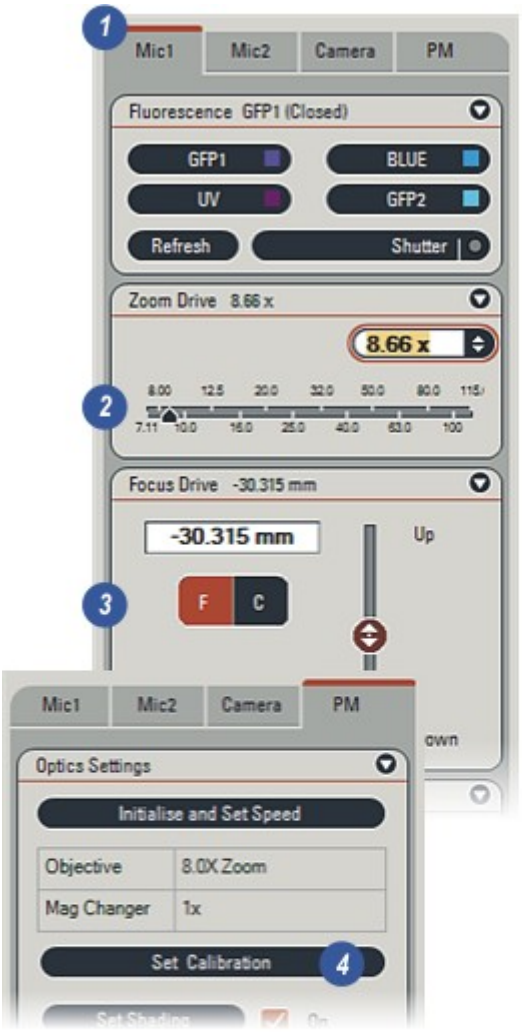
Continued... 708

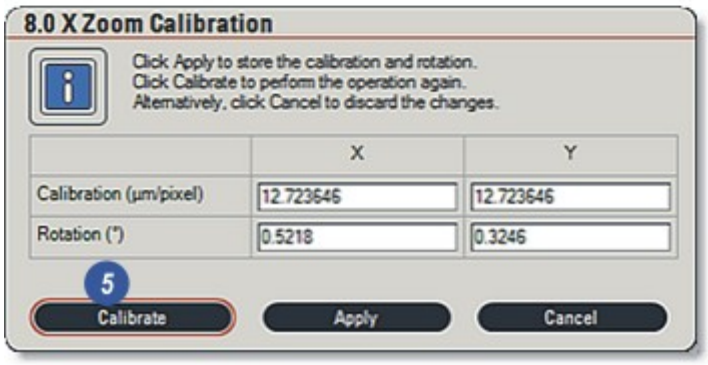
There are three essential steps to successful imaging using the ISO Pro stage with both motorised *(Select the Mic1 tab: **1**)* and manual stands:

Zoom: **(2)**

Focus: **(3)** and

Calibrate: **(4)** by clicking on the *Set Calibration* button and then on the *Calibrate* button **(5)** on the Zoom Calibration dialog. *For more details:* *Go there...* 638





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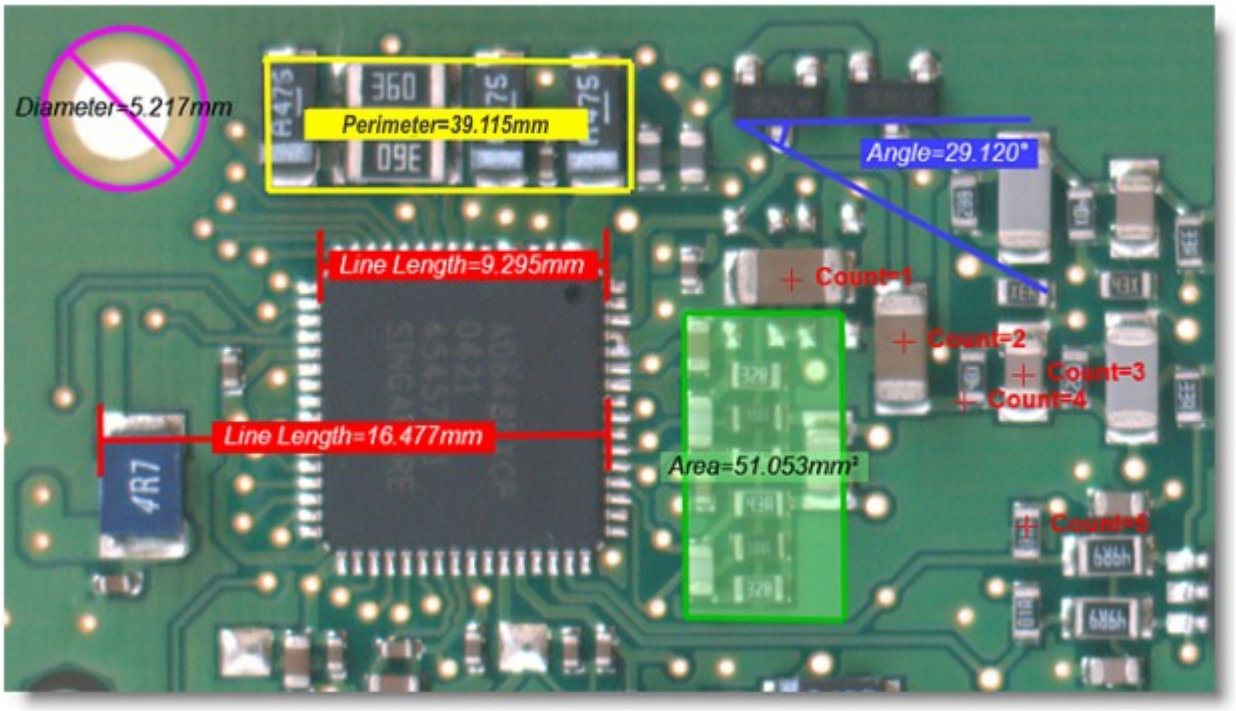
Optional modules Live Measurements and Interactive Measurements bring all of the flexibility and precision of Leica Application Suite to image measurement tasks. Live Measurements is optimised for working on the live image before it is captured, and Interactive Measurements is aimed at captured and stored images. Both modules are installed and licensed separately.

Because many of the procedures - measuring distance, area and so on - are common to both modules, Leica have created identical toolboxes and similar features that will make moving from live to captured images quick and seamless.

The toolbox contains a wide range of precision tools featuring:

- Configuration for line thickness, colour, label colour - including a transparency control - position and type font.
- Classes to reflect similarities with colour-coding make analysis much easier.
- Alternative Parameters so, for example a circle can be labeled with diameter, radius, area or circumference whichever is more appropriate.
- Display or Hide parameters to suit the image.
- Comments to display alongside and enhance the parameters.
- Configurations to save Classes and Settings together in a file that can be retrieved and applied to images at any time.
- Templates to be used as moveable 'overlays' to benchmark important positions and locations on live images.
- Display and analysis of selected measurements in the correct corporate style with Microsoft Excel.
- Snap to edge nearest to mouse cursor for improved consistency and speed in identifying measurement details on stored images.



The main areas of the user interfaces:

1: *Menu Bar*: For Options and Help.

2: *The Workflows*: Live Measurements is on the Acquire Workflow and Interactive is on Analysis.

3: *The Measure/Interactive Tab*: Click to reveal all of the tools and features.

4: *The Measurement Toolbox*: Click to select measurement type.

5: *The Control Tools*: Commands for actions such as New Measurements, Redo and Undo.

6: *Function Panels*: Reveal Properties, Classes, Configuration, Report and Template controls.

7: *The Acquire button*: Click to capture an image and data.

8: *Side Tool Bar*: Hide and reveal the Grid (for Results) and Thumbnail Gallery.

9: *Gallery Zoom:* Move the slider to resize the thumbnails.

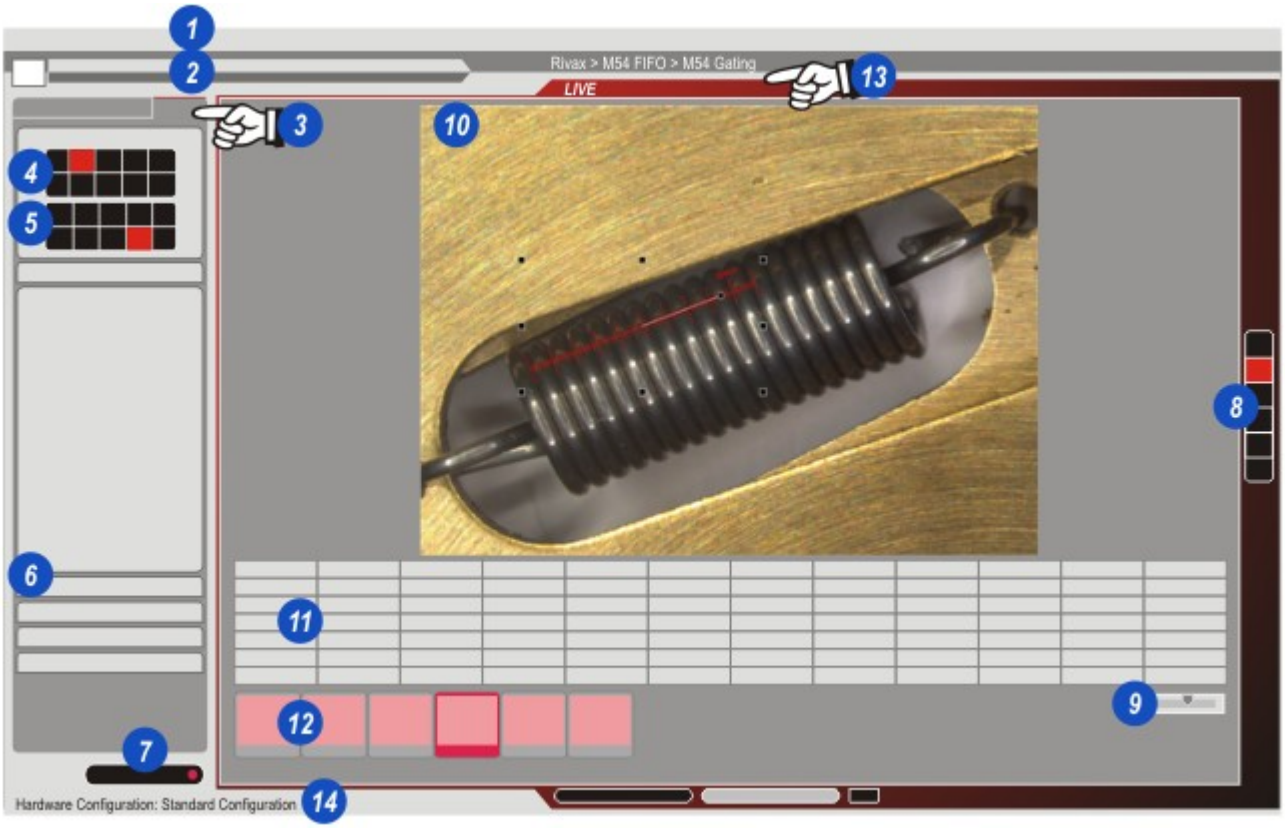
10: *The Viewer:* Displays the live or captured image.

11: *Results Grid:* Configure for Details or Summary of measurements.

12: *Thumbnail Gallery.*

13: *The Image Status Display.*

14: *Status Bar.*



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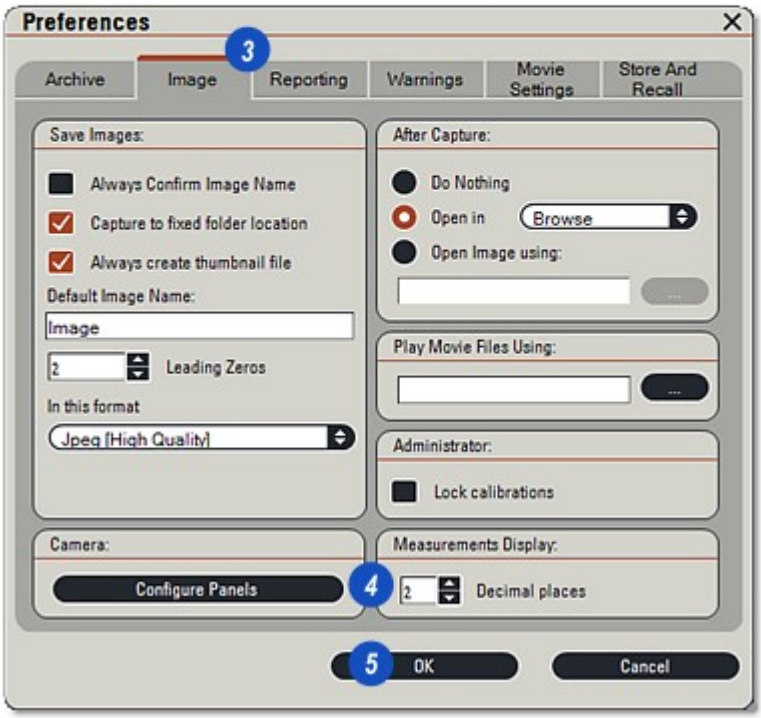
Direct links to all of the Live and Interactive Measurement features and tools:

To maintain ongoing measurement precision, calibration should be carried out at regular intervals. The Calibration functions can be found in the *Acquire Workflow* in the *Camera* section. *Go there...* 268



Measurement Precision:
The actual precision of LAS measurements is governed by the software and extends to 13 places after the decimal point. To display that precision would only clutter and confuse the images and so the number of integers displayed after the decimal point can be set by the user to aid clarity. Reducing the display places does not affect the calculation precision* which is still carried out internally using all 13 places.

To set the number of display places after the decimal point:

1: On the main header click on *Options* ...

2: ...and from the drop down click to select *Preferences*.

3: On the *Preferences* dialog, if necessary, click on the *Image* tab.

4: On the *Measurements Display* panel, in the *Decimal Places* text box, either click in the box and type a value or use the up/down arrows to the right of the text box to increase/decrease the value.

5: Click *OK* to save the value. Any measurements being displayed will be updated immediately.

*Precision in this context refers to the number of decimal places saved with the image and should not be confused with 'accuracy' that depends upon screen and image resolution, calibration and several other factors.

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Live Measurements Fast Track is a simple checklist of steps to take to get into precise measuring quickly.

- Measurements are made on a live camera image in the Acquire Workflow - no need to capture the image first.
- Carry out a calibration before making measurements: *Go there...* 268
- Make the image as large as possible - close the Gallery and Grid if they are open.
- Select an image Live Format that provides good resolution with a fast refresh rate: *Go there...* 241
- Determine if image measurements can be grouped together to make reporting and analysis quick and convenient. Set up Classes on the Measure tab to reflect the groups: *Go there...* 733
- If Classes are not being used, set up the Line, Background and Font properties for initial measurements. They can be altered later if necessary. *Go there...* 729
- Add a comment if needed: *Go there...* 732
- Select the items to display on the Display Labels: *Go there...* 732
- If required, save the settings as a Configuration: *Go there...* 737
- Start measuring...

Points to Note:

- Live Measurements cannot be used with sequence images created with modules such as MultiTime or Movie. If a sequence module is selected the Measure tab is not available.
- If a Stereo microscope zoom is being used, the measurement drawings will change size but their positions will be incorrect because of an image shift due to the focus change. Use the AX-Carrier option to correct.
- Microsoft Excel does not have to be installed on the computer to capture results, but the Excel Viewer at least will be needed to view them, and a complete Excel installation required if the template is to be changed.

The *Toolbox* for both *Interactive* and *Live Measurements* is the same and all of the tools work identically. The only obvious difference is the *Merge* control that works differently on live and captured images.

The *Interactive Measurements Toolbox* is reached by:

1: Click on the *Analysis Workflow.*

2: If necessary, click on the *Interactive* tab to reveal the main panel and *Toolbox*.

3: The *Merge* button is a 'one-shot' function that merges the measurements with the current captured image. Once merged, the measurements cannot be altered:
Merging: *Go there…*

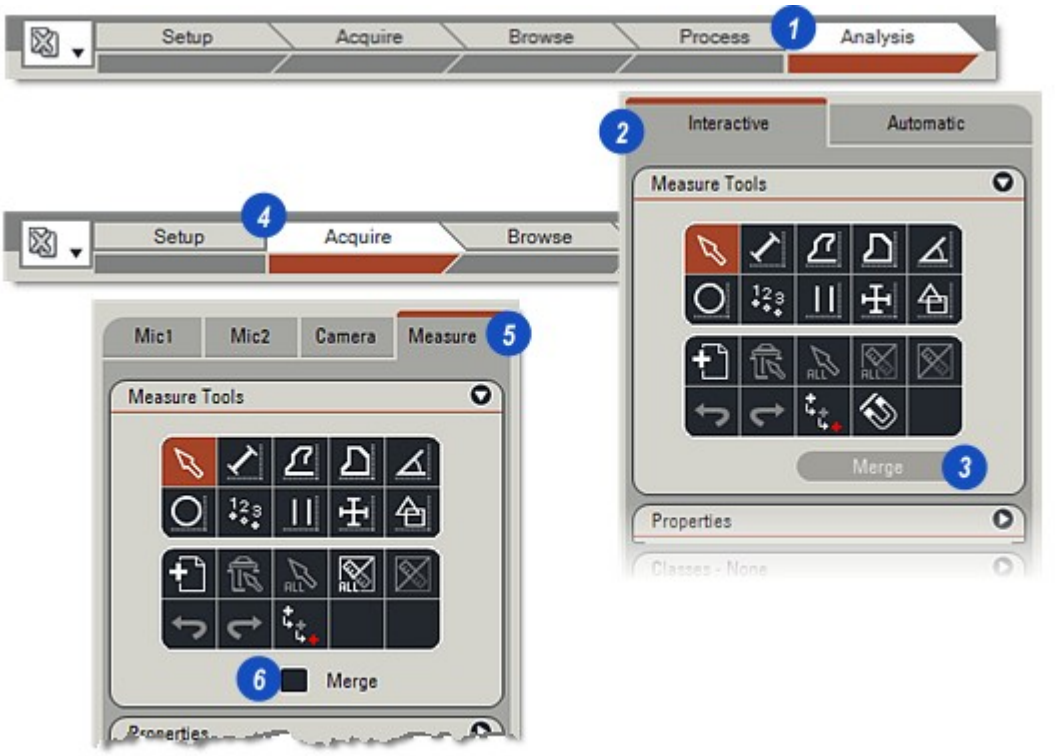
The *Live Measurement Toolbox* resides on the *Acquire Workflow:*

4: Click on the *Acquire Workflow*.

5: Click on the *Measure* tab to reveal the *Toolbox* and main panel.

6: The *Merge* feature is a check box which, when enabled merges measurements as the live image is captured. After merging the measurements cannot be changed.
Merging: *Go there…*

Continued…



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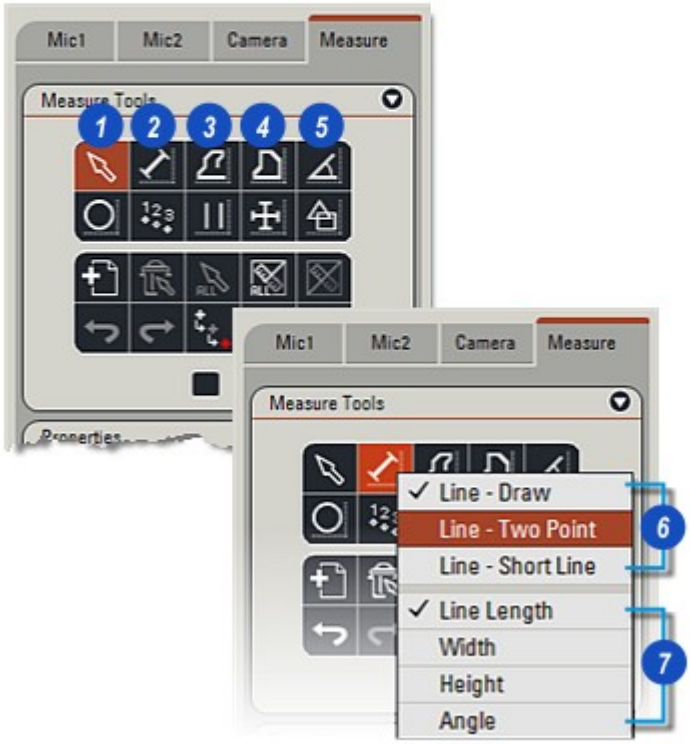
Each measurement tool - the top group in the Toolbox - is selected by clicking the appropriate button. Most tools have a range of parameters than can be selected by right-clicking the tool and choosing the parameter from the menu **(6)**. This will be the parameter that is displayed on the label. For example, *Circle* tools measure *Diameter*, *Radius*, *Area* and *Circumference* and any one may be chosen to be displayed with the appropriate result.

Some tools also have several modes. For example, the *Line* tool can be used to click and drag a line or click at the two ends with a line automatically drawn between, or short line in which the line is split across narrow features. Where various modes are available they appear at the top of the menu **(6)** with the parameters beneath **(7)**.



1: The Selection tool. Used to select and alter existing measurements or de-select the other tools. *Go there...* 717

2: Distance Line. Measures the distance in a straight line between 2 points. Also the line *Angle* and the *Width* and *Height* of an imaginary enclosing box - *Bounding Box* - from the horizontal. *Go there...* 718

3: Segment Line: Measures the distance around the *Periphery* of an irregular shape, a *Bounding Box Height* and *Width* and also the distance between the two *End Points*. *Go there...* 719

4: Area Tool: Measures the Area of an enclosed figure as well as the Periphery, individual Segments or Bounding Box Height and Width. *Go there...* 719

5: Angle Tool. Measures the Angle between two drawn lines and Bounding Box Height and Width. *Go there...* 720

Continued... 716

1: Circle and Ellipse: Draws a *Circle* using either a straight line representing the *Diameter* or *Radius* or alternatively, drawn around *Three Points* marking the *Circumference*. Also draws an *Ellipse*. *Go there...* 720

2: Count: Counts each item clicked and displays the count sequence number. *Go there...* 723

3: Multiple Distance Line: Establishes a base line or datum and then measures distances from the line to individual points as offsets or creates lines parallel to the datum with the distance between displayed. *Go there...* 723

4: Cross: Measures both the *Major* and *Minor* Axes of an object. *Go there...* 724

5: Triangle and Rectangle: Create triangular or rectangular areas and measure sides, enclosed area and angles. *Go there...*

6: Hide Labels: Sometimes, especially when making measurements that are close together or very small, the properties label can be obtrusive. Hide the label temporarily by pressing and holding down the *AltGr* key. The label re-appears when the *AltGr* is released.

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1: The Selection tool. Does not actually draw but is used to select measurements already made on the image ready to edit or move them. Click on the *Selection Tool* button and then on the measurement, the end-points of which will appear as small ‘ boxes’ or handles to indicate it is selected **(2)**.

Adjust a Measurement: Click and drag on a handle.

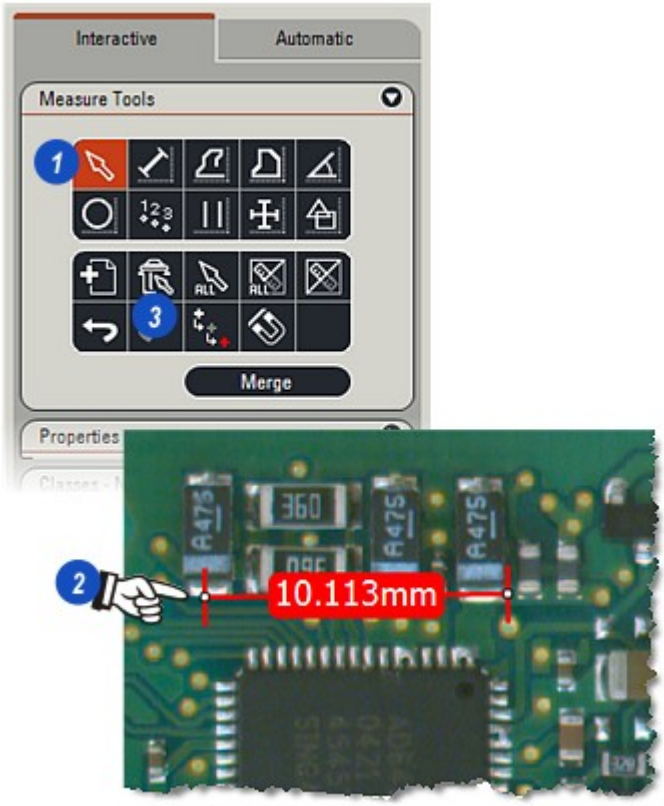
Delete: Press the Delete key or click on the Trash Can **(3)** to remove a selected measurement.

Undo/Redo Actions: Use the Undo button to restore the last deletion.

Re-position: Click and drag on a measurement line (not a handle) to re-position it. The Label follows.

Re-position Label: Click and drag on a Label to re-position it independently of the measurement.

Multiple Selection: Click and drag on the image (not a measurement) to draw an enclosing box or 'marquee' around any number of measurements to simultaneously select them.



Continued... 718

1: *Distance Line.* The distance in a straight line between 2 points.

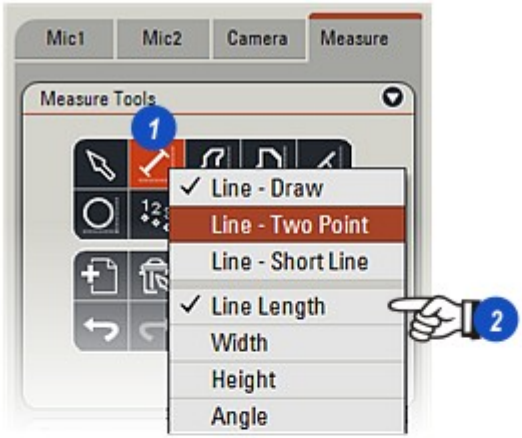
Option Draw: Click and hold down at the start point.
Drag to the end point and release the mouse button.

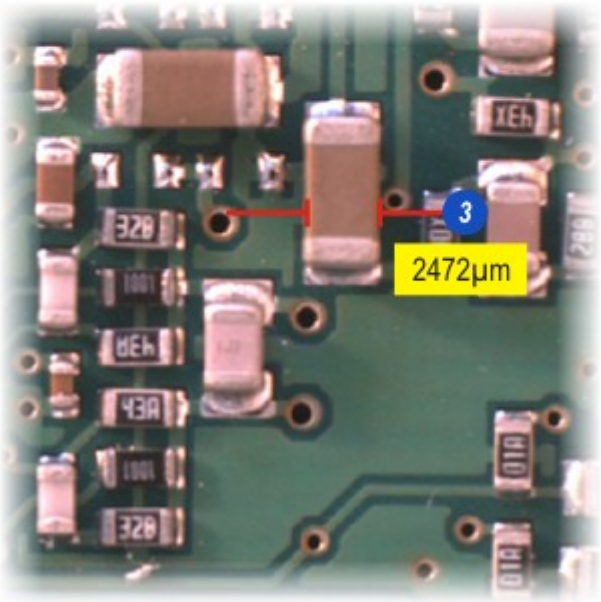
Option Two Point: Click at the starting point, move the cursor to the end point and click again.

Option Short Line: For situations in which the feature to be measured is so small a continuous line would be difficult to read and draw. Click on one side of the feature and holding down the mouse button drag to the opposite side of the feature. Can be dragged to any angle **(3)**.

Measurements Options: Length, Angle and Bounding Box Width and Height ***(2)***.

Continued... 719





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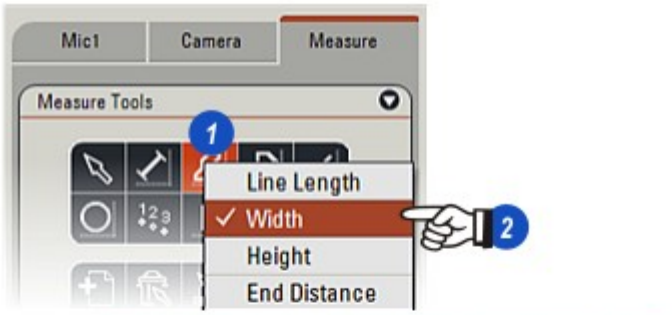
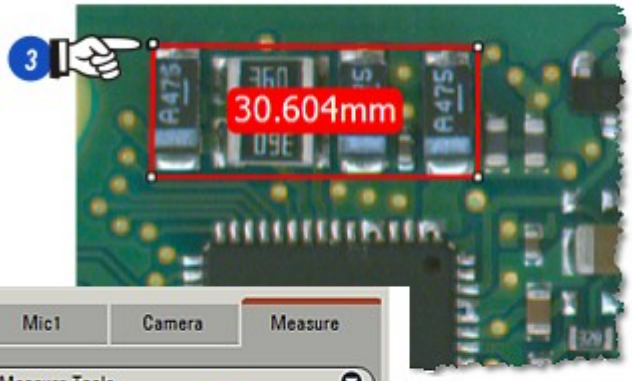
1: Segment Line: To measure the distance around the periphery of an irregular shape.
Click and release to start **(3).**
Click to end the segment and start another.
Repeat around the periphery.
Right click to end. The sum of all the segments is displayed.
Alternatively, click, hold and drag for a continuous line that tracks the mouse path.

Measurement Options: Length, Bounding Box Width and Height, the distance between the two End Points **(2).**

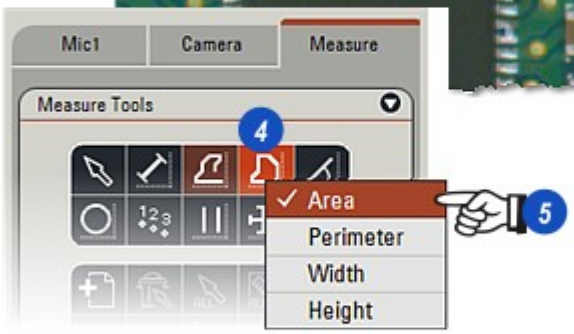


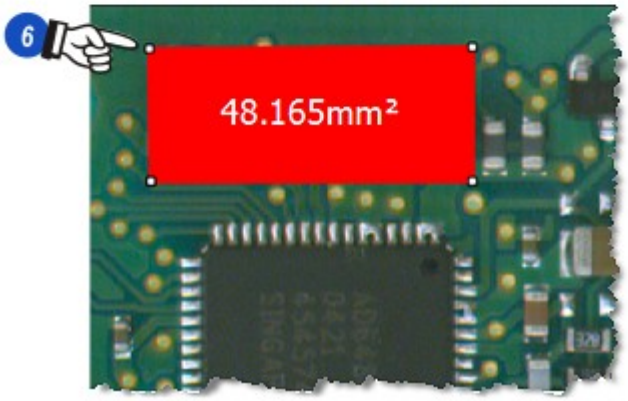


4: Area Tool: Measures the area of an enclosed figure.
Click and release to begin **(6).**
Move the cursor to the next vertex and click.
Repeat for all sides and back at the start point.
Right click to end.
Alternatively, click, hold and drag for a continuous line that tracks the mouse path.

Measurement Options: Area, Perimeter, Bounding Box Width and Height **(5).**

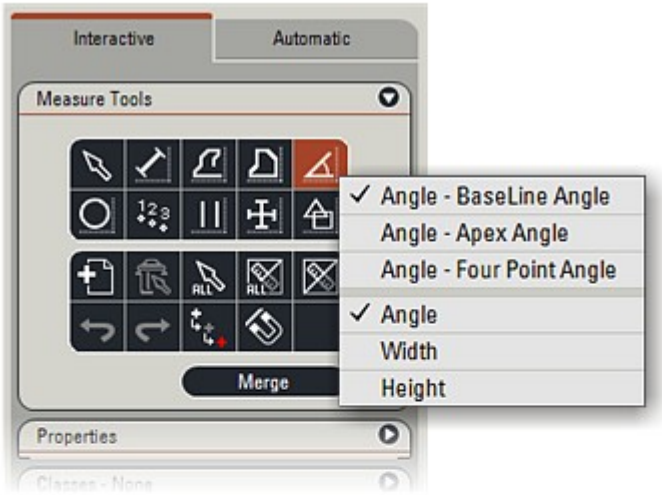
Note: If the drawn shape pulsates when complete, some points on the outline cross. These must be removed to give a correct area measurement.



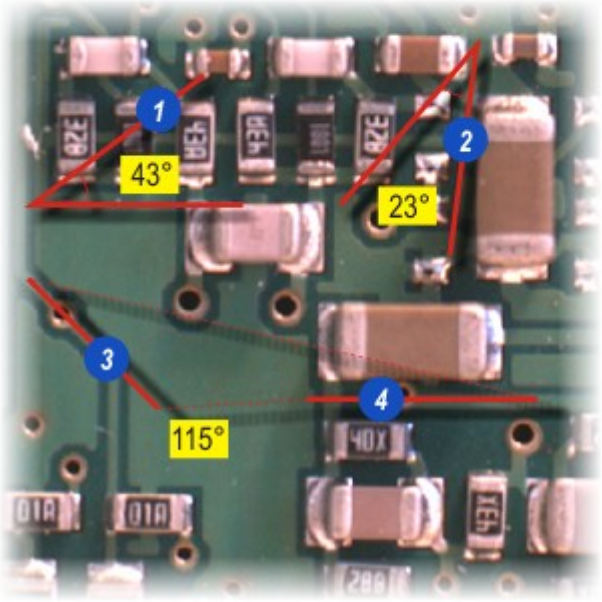


Continued... 720

Angle Tool. Measures the angle between two drawn lines.
For Baseline **(1)** and Apex **(2)** angles:
Click to set the start point of the first 'leg'.
Move the cursor to the end-point of the first line and click.
Move the cursor to the end-point of the second 'leg' and click.
Right click to end.
Baseline represents the angle between a horizontal or 'base' line and an extension **(1).**
Apex is the angle between the two legs irrespective of the inclination **(2).**



A Four Point Angle **(3)** is that between two lines that are not connected at a point. Furthermore, the lines projected intersection could be beyond the limits of the image.
Click on the starting point of the first line.
Move the cursor to the end of the first line and click again. The line is drawn **(3)**.
Click on the starting point of the second line.
Move the cursor to the end of the second line and click again. The line is drawn **(4)**.
Dotted lines are drawn between the ends of the lines.



If the lines are nearly parallel an imaginary point of intersection will be beyond the limits of the image or even the Viewer, but the software can accommodate this situation and return an accurate angle.
If the lines are parallel then the angle will be 0°.

Measurement Options: *Angle, Bounding Box Width and Height.*

Continued... 721

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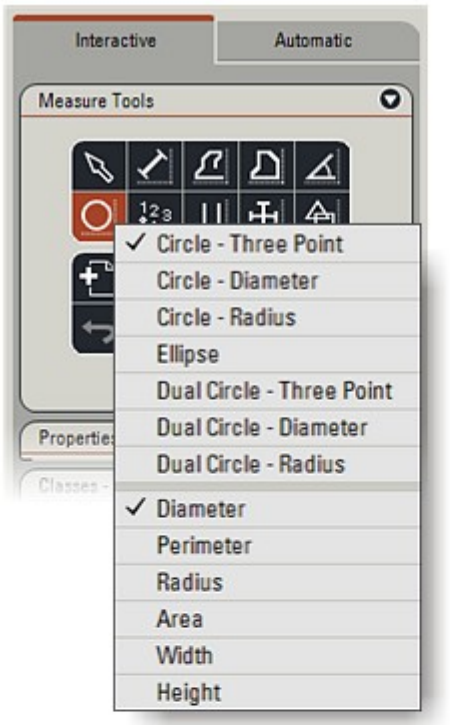
Circle: Draws a Circle or an Ellipse.
Using Three Points: Click on a point on the image **(1)**
Click on a second point on the image **(2)**.
Click on a third point **(3)** and drag the handle to describe the diameter required.

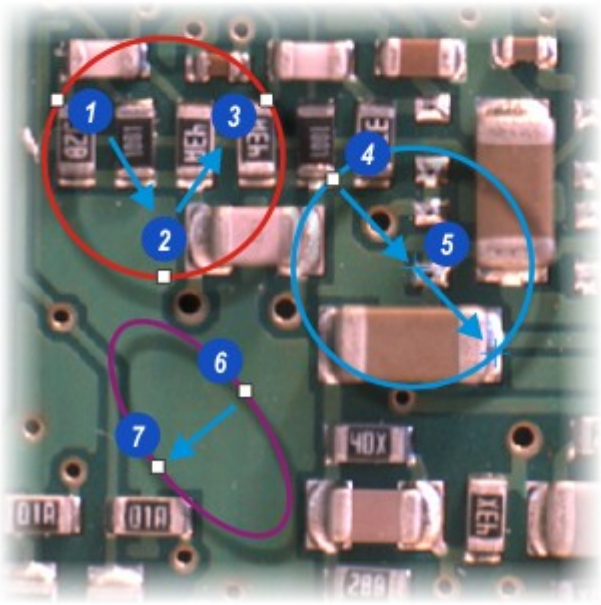
Using Diameter: Click and hold down on the first edge-point of the circle **(4)**.
Drag to the second edge point representing the diameter and release the mouse button.

Using Radius **(5)**: Click on a point on the image representing the centre of the circle.
Release the mouse button.

Drawing an Ellipse**)**: Click on a point on the image **(6)**.
Click on a second point **(7)** representing either the major or minor axis of the Ellipse and still holding down the mouse button drag to describe the other axis.
Click to complete.

Continued... 722





Dual Circle: Draws 2 circles using either the three-point, radius or diameter methods and provides a range of measurements.
Using the method preciously described, draw a circle around the first feature **(1)** and the around the second feature **(2)**.

Options: The Area, Width, Height etc parameters refer to the first circle. The parameters with the '2' suffix (for example Radius2) refer to the second circle. Distance Between Centres is the distance between the centres of the first and second circles. Closest Approch is the smallest distance between the two perimeters. The Centre X and Centre Y dimensions are the X (Horizontal) and Y (Verttical) distances from the top/left corner of the image.

Continued... 723

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1: Count: Counts each item clicked and displays the count sequence:

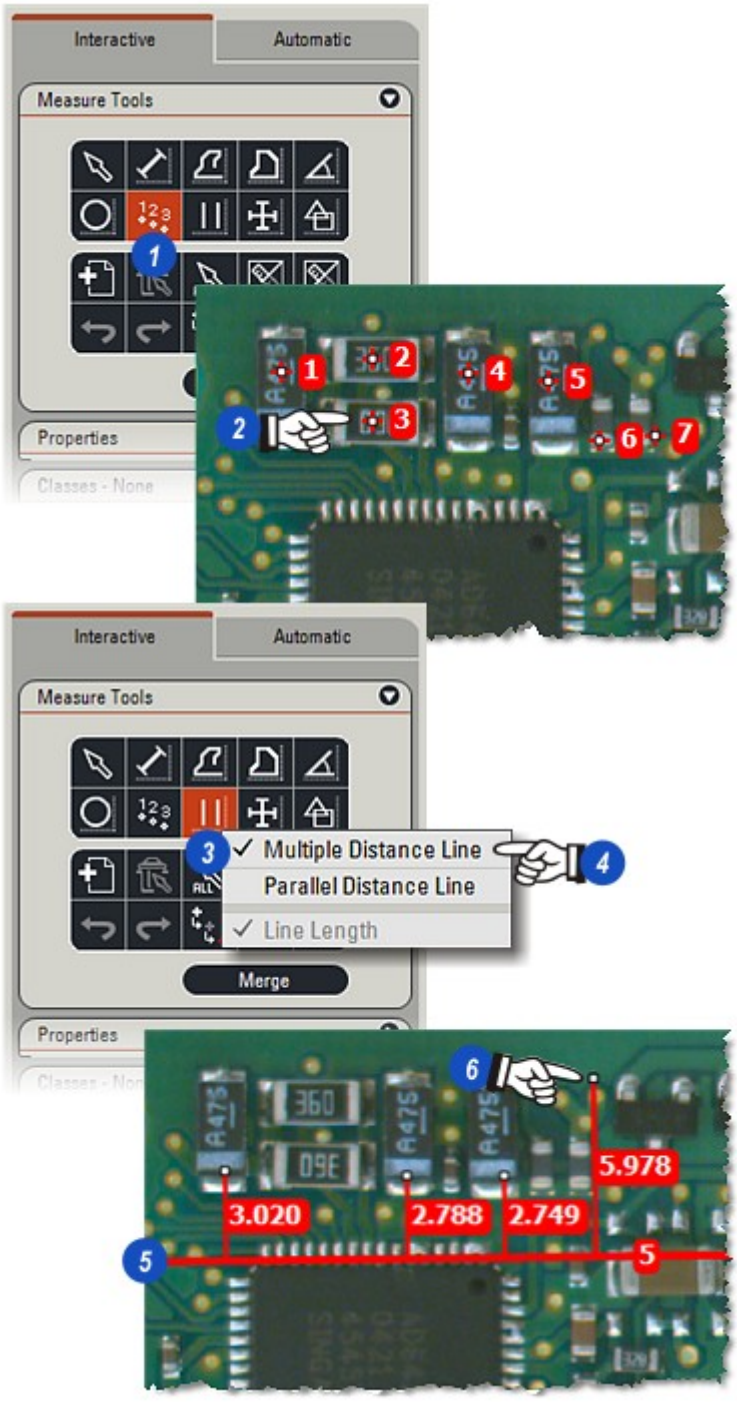
Click on each of the items to be counted **(2).** The count value automatically increments and a small 'target' is drawn over the clicked point. Individual 'target' and labels can be moved for clarity.

3: Multiple Distance Line: Using a single datum line and then measuring the distance of objects from that line: either by individual offset lines or additional lines parallel to the datum.

Multiple Distance Line **(4)**: Click on the image to establish the datum line starting position **(5).**
Click again to anchor it.
Move the cursor to the distance to be measured left or right, top or bottom of the datum.
Click on the end point of the distance measurement.
Repeat the process for subsequent lines ending with a right click **(6).**

Parallel Distance Lines: Click on the image to establish the datum line.
Click again to anchor it:
Move the cursor away from the datum line to a position to be measured and click to draw the parallel line.
Repeat as required ending with a right click.

Measurement Options: Length, either as the length of an offset line or the distance between the datum and a parallel line.



Continued... 724

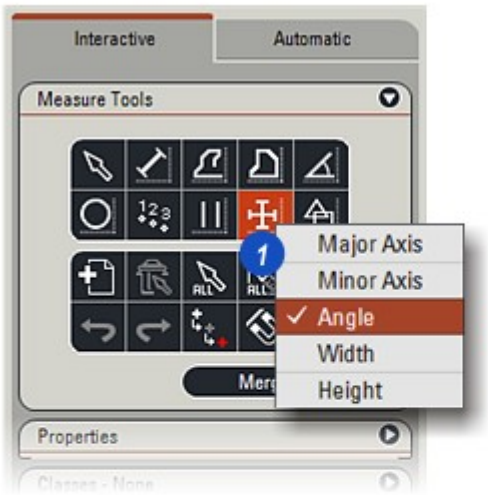
1: Cross: This tool measures both the major and minor axes of an object by drawing a cross over it. The *Cross* may be drawn at any angle.

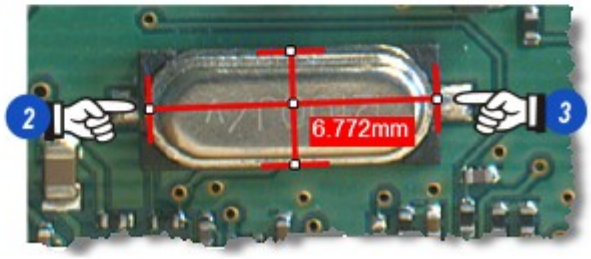
Major Axis Option: Measures the major (longest) axis.

Minor Axis Option: Measures the minor (shortest) axis: Click on an edge of the area to be measured **(2)**, and holding the mouse button down drag to the opposite side **(3).** The selected dimension is displayed. Drag the Cross at an angle if necessary.

Measurement Options: Angle measures the skew angle from the horizontal, Bounding Box Width and Height.

To adjust axis lengths, click on the *Selection* tool and then on the *Cross* to reveal handles for re-sizing and rotating. Click, hold and drag in the centre to re-position.





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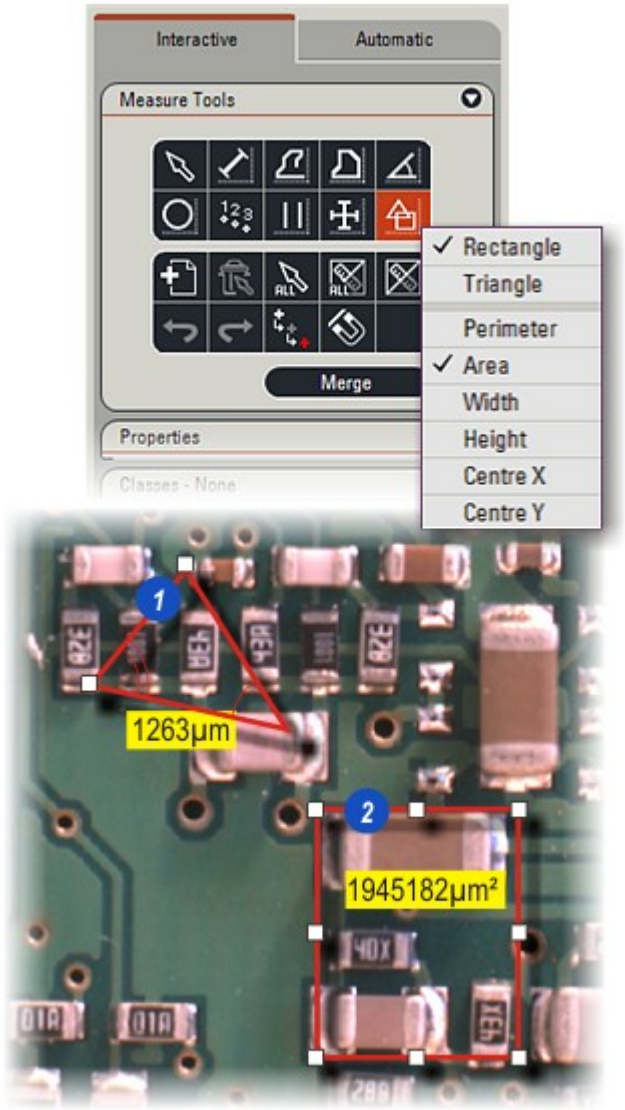
Triangle and Rectangle: Draws an enclosed 3 or 4-sided polygon.
Click on the starting point of the polygon.
Drag to the required side dimension and click again.
Drag to create the required dimensions.

To modify the polygon:
Click to choose the Select tool.
Click on the triangle to select it. Small handles appear at the vertices.
Click on handle and drag to change the dimensions.

Measurement Options: Perimeter, Area, Width at the greatest horizontal dimension, Height at the greatest vertical dimension. Centre X and Y the horizontal and vertical distances from the top/left corner of the image to the centre of the polygon.



The lower set of tools are utilities used to start new measurements, make multiple deletes and move back and forth between actions.

1: Start a new set of measurements: Deletes all existing measurements – confirm the deletion – ready to start a new set of measurements. Because this action also clears the *Undo/Redo* history, it cannot be reversed.

2: Delete the selected measurements: Click the icon to delete the existing and selected measurements. Measurements can be selected individually from the *Grid* or on the image, as a group by holding down the *CTRL* key and clicking them on the *Grid* or image, and by using the *Select All* tool **(3).**

3: Select all measurements: Selects all measurements on the image.

4: Hide/Reveal all measurements: This is a toggle action – click once to hide all of the measurements and click again to reveal them.

5: Hide/Reveal selected objects: Use this tool to hide or reveal a group of selected objects. Objects can be selected as a group by holding down the *CTRL* key on the keyboard whilst clicking the objects on the Grid or the image, or by using the *Selection* tool, clicking on the image and drawing a 'box' or marquee around the measurements to be selected.

6: Undo the last action and **Redo** the last action after an *Undo*. Individual measurements or points can be deleted or restored using the *Undo* and *Redo* button. Hover the cursor over the button to determine the action that will occur.

7: Toggle Cursor Colour: Each click on the button will switch the cursor between black and white. Right click on the button to set the cursor to a user-defined colour: *Go there…* 730

8: Snap to Edge: The *Snap* tool helps the user to make fast, precise measurements by automatically 'snapping' to a close, well contrasted edge: *Go there...* 728

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The *Zoom* feature allows parts of the image to be enlarged to achieve even higher levels of measurement accuracy.

1: Position the cursor close to the area of interest.

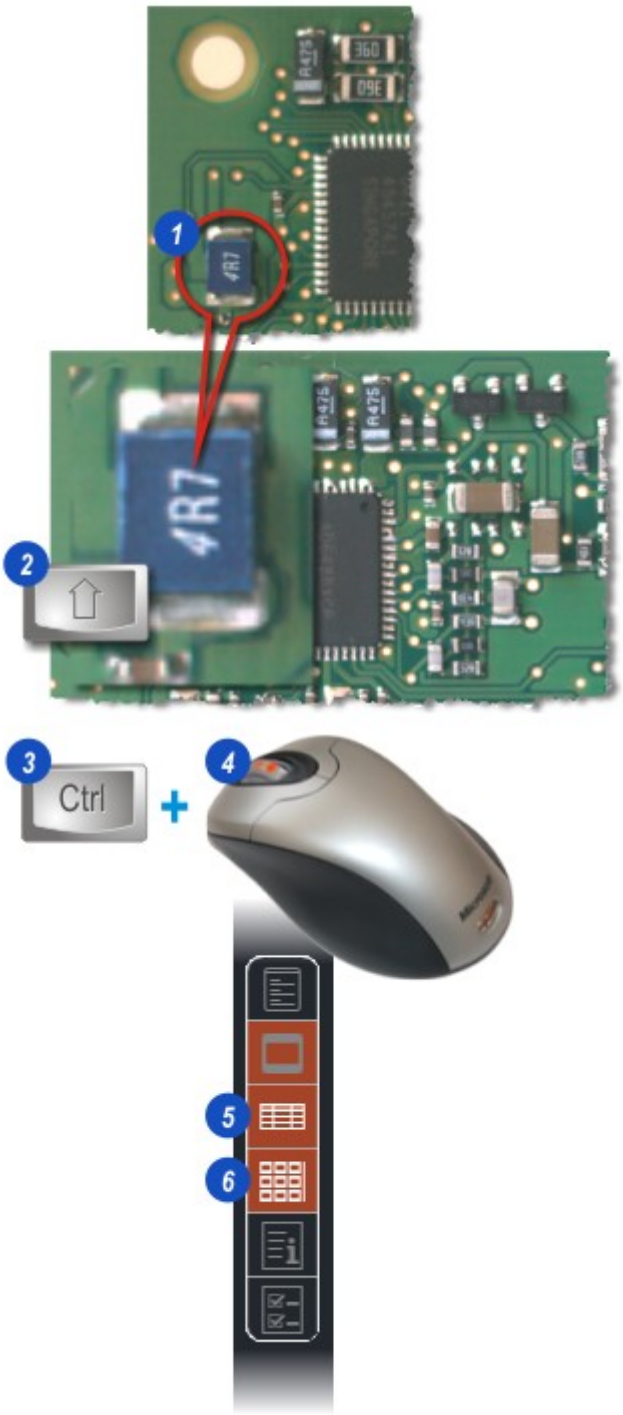
2: Press and hold down the *Shift* key. A Zoom window opens and will follow the cursor around the image.
Release the *Shift* to key to close the Zoom window.
The zoom level *within* the Zoom window can be increased or decreased by turning th mouse wheel.

3: The overall image zoom can be increased or decreased by holding down the keyboard *Control (Ctrl)* and...

4: ...turning the mouse wheel.

5: The *Results Grid* can be revealed or hidden by clicking the button on the side tool bar to the right of the *Viewer*. This is a toggle action - click once and the *Grid* is revealed, click again and it is hidden.

6: The *Thumbnail Gallery* can also be hidden and revealed by clicking the button on the side tool bar. Again, this is a toggle action button.



The *Snap* tool (not available with Live Measurements) allows the measuring tools - *Distance, Circle, Parallel, Angle and Cross* - to precisely 'snap' to adjacent sharp edges on the image.

1: Click on the *Snap* button to enable snapping.

2: Click to select the required measurement tool. The advancing end-point of the tool displays a small **'X'** in the *Snap* chosen colour.
Carry out the measurement using the techniques described earlier, but as the measurement line is extended, the advancing end-point will detect and snap to an edge as it get close.

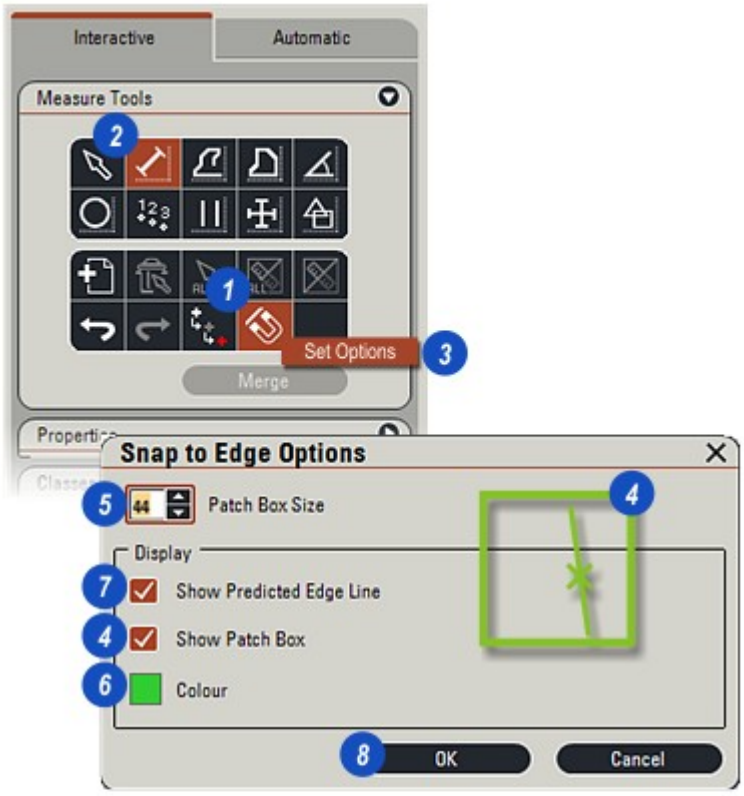
3: Change the *Snap* properties by right-clicking on the *Snap* tool and then left-clicking the pop-up *'Set Options'* button.

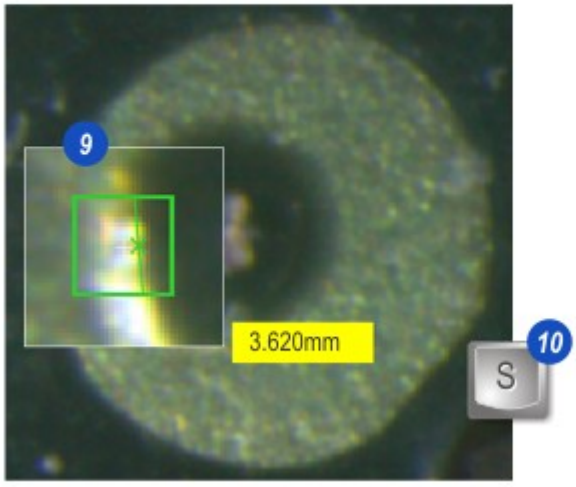
4: On the *Options* dialog, augment the advancing end-pont with an easy-to-detect *Patch Box* enclosing the Snap cursor.

5: Increase or decrease the size (maximum 50) of the *Patch Box* by clicking the up/down arrows. The box size determines the area that is 'searched' for a sharp edge.
If the box is too small edges may be difficult to detect; Too large and several edges may prove to be snap targets. Adjust the *Patch Box* size to suit the image complexity.

6: Change the *Patch Box, Predicted Line* and **'X'** cursor colour by clicking on the *Colour* button and from the *Colour Dialog* selecting a new colour.

7: Enable the *Predicted Edge Line* by clicking the check box. A line appears within the *Patch Box* and it rotates to indicate the *direction* of the nearest edge. Bring the *Patch Box* close to a circle and the *Edge Line* appears as a tangent, faithfully following the curve of the circle.





8: Click the *OK* button.

9: Use the *Zoom* feature (hold down the *Shift* key) to enlarge the image area within the *Patch Box* and aid precision.

10: Hold down the keyboard 'S' key to temporarily disable snapping and release the key to restore it. This overrides the *Snap* feature allowing the user to select and mark the target point.

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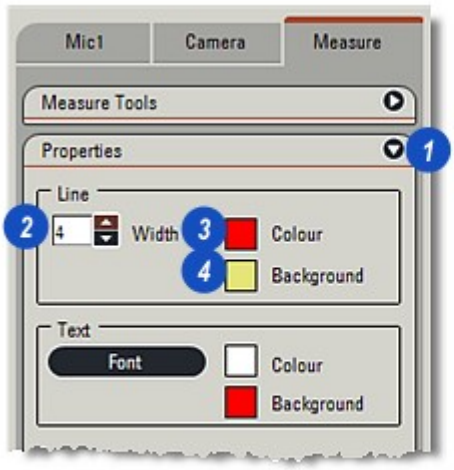
The *Properties Panel* provides all the facilities for tailoring the appearance of measurements to suit the user.

Change *Line Thickness* and *Colour*, the *Font,* its *Style, Size, Colour* and *Background*. Select the *Parameters* to be displayed on the labels and add *Comments* to them.



Set the Line Thickness and Colour:

1: Expand the *Properties* panel by clicking on the arrow to the right of the header.

2: Change the *Line Thickness* by clicking on the *Up/Down* (Increase/ Decrease) arrows to the right of the *Width* text box.

3: Change the *Line Colour* by clicking on the *Colour* button and on the *Select Colour* dialog choosing a new colour: *Go there…* 730

4: Change the *Fill* for closed shapes by clicking on the *Background* button and on the *Select Colour* dialog, choosing a new colour: *Go there…* 730

Continued… 731

The colour of lines, text and fills can be changed to user preferences. The procedure is the same for *Line, Font, Background* or *Class* colours.

1: On the *Select Colour* dialog select a new colour by…

2: …clicking on a swatch, …

3: …clicking and dragging the small 'target' on the *Colour Wheel*, or …

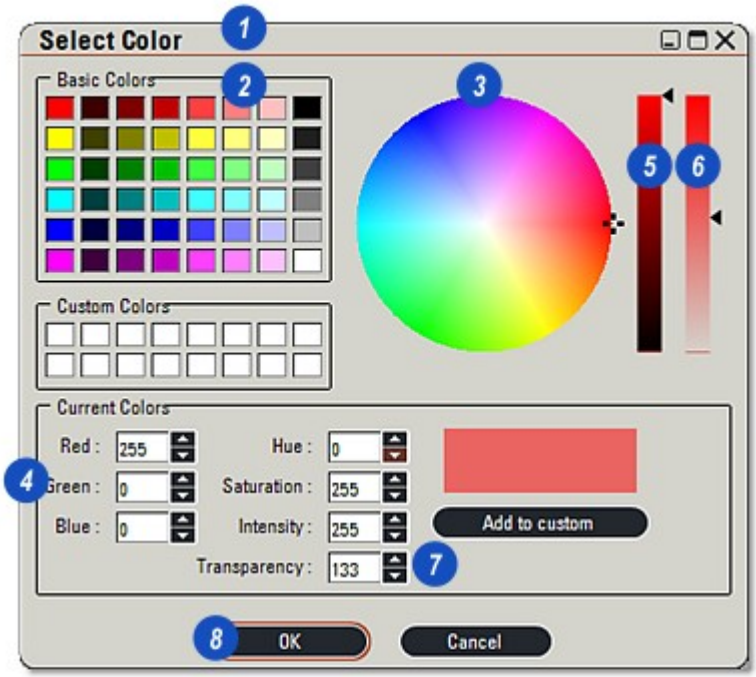
4: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range 0 to 255.

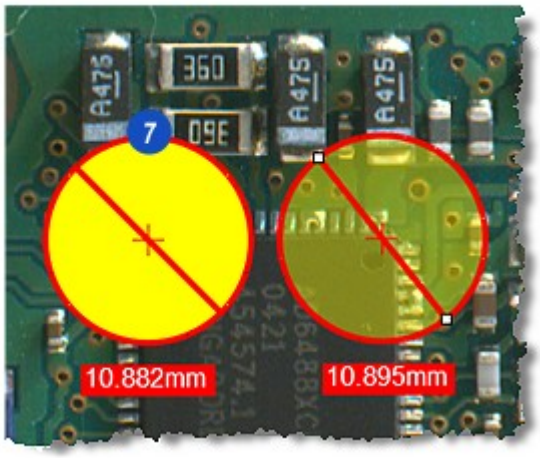
5: If necessary, adjust the shade by clicking and dragging the slider on the *Shade Bar*.

6: Colour transparency can be set by clicking and dragging the slider on the *Transparency Bar* or…

7: …clicking in the *Transparency* text box and typing a value in the range 255 for a solid colour to 0 for complete transparency which, when used with an outline will display an 'open' shape with no apparent fill. The circle on the right in the diagram has a transparency setting of 103.

8: Click **OK**.





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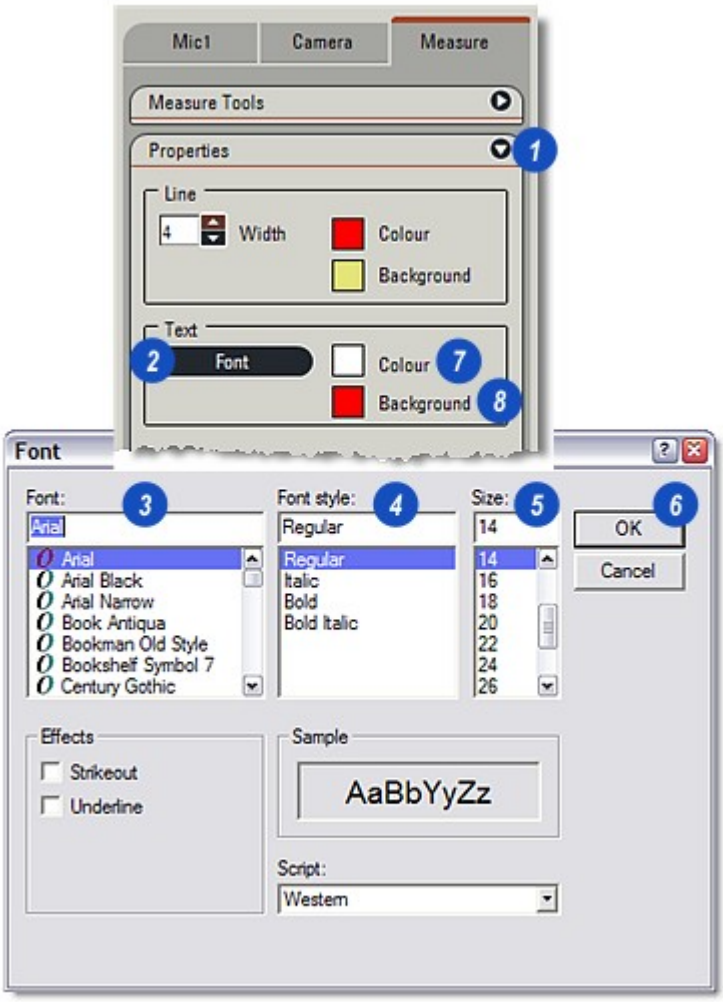
Change the *Font Type Face, Style, Size, Colour* and *Background.*



Select the Font, Style and Size:

1: Expand the *Properties* panel by clicking on the small arrow to the right of the panel header.

2: Click on the *Font* button.

3: On the *Font* dialog, select the *Font Type Face*,…

4: …the *Font Style* – bold, italic etc, and…

5: …the *Size* in points.

6: Click *OK*.

Change the Font colour:

7: Click on the *Colour* button and on the *Select Colour* dialog choose a new colour: *Go there…* 730

Change the Font Background colour:

8: Click on the *Background* button and on the *Select Colour* dialog choose a new colour: *Go there…* 730

1: Click on the small arrow to the right of the *Properties* header to reveal the *Properties* panel.

Adding Comments:
Comments are often a useful addition to a measurement and can be added by:

2: Click in the *Comments* text box and type a comment or note.

Displaying Label Parameters:
In the *Display Labels* window click to select an entry so that it will displayed as part of the object label. A tick mark appears to the left of the selection. Click again to hide the property – the tick mark disappears.

2: *Comment*: A comment or note entered in the *Comment* text box.

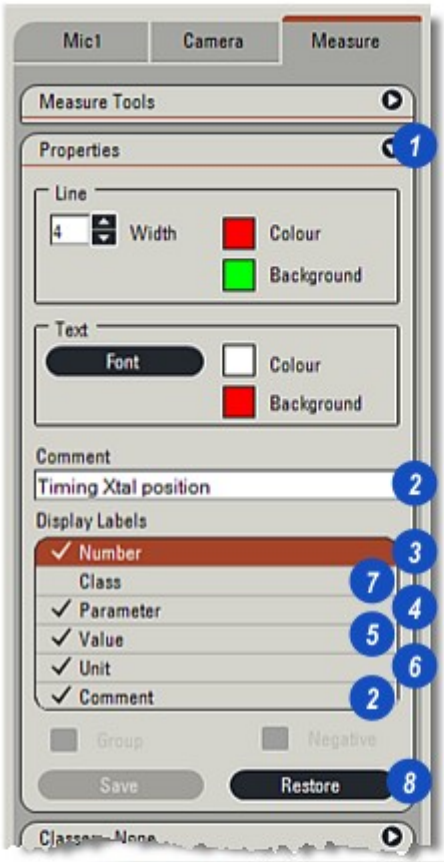
3: *Number*: Is a sequential measurement number.

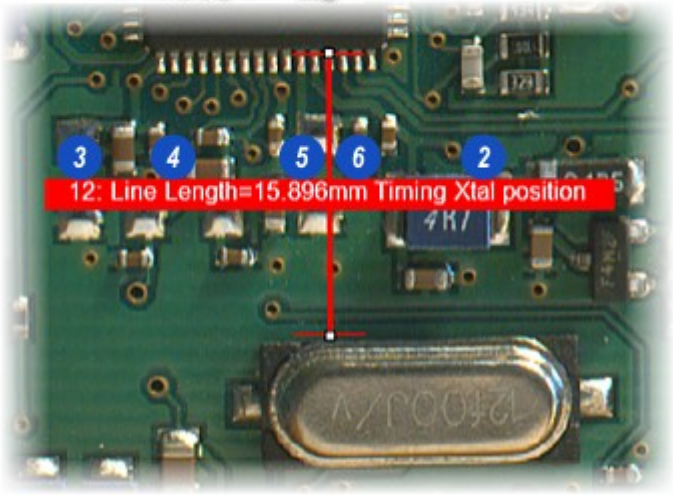
4: *Parameter*: Shows the tool value – *Length, Area, Angle* etc.

5: *Value*: The actual measurement.

6: *Unit*: The measurement units chosen on *Camera* > *Calibration* – mm for example. *Go there…* 268

7: *Class*: The *Class Name* if one has been selected. About Classes: *Go there...* 731





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Classes represent a quick and simple method of grouping items to distinguish them from other parts of the image. Classes are saved globally but are grouped and saved in configurations. A configuration can be attached to an image and that same configuration used for all of the images in an experiment.

Apart from visual colour coding on the image, classes also group measurements together on result reports making reading and analysis far easier. Any or all of the measurement tools can be used within a class.

This example shows part of an electronic printed circuit board:

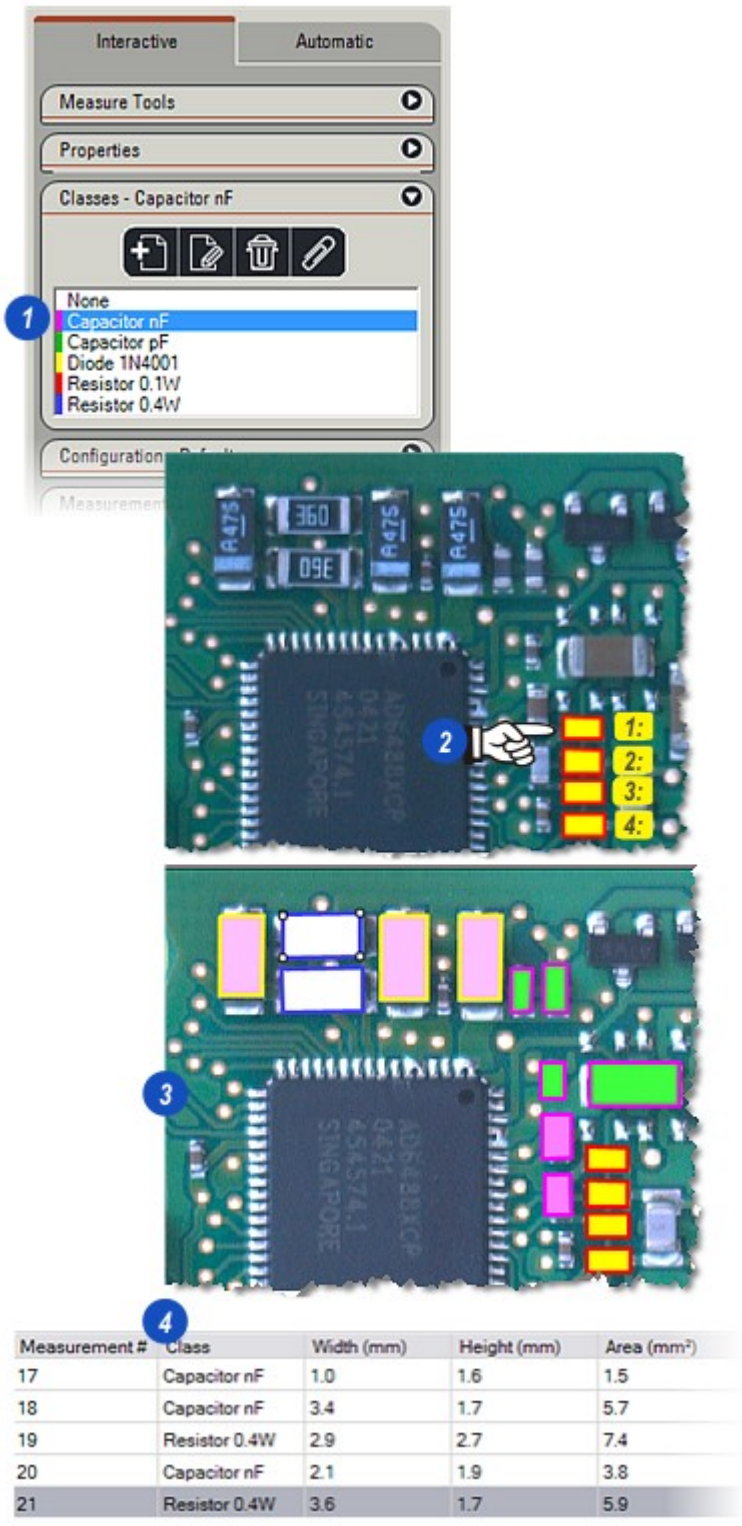
1: The required class is selected by clicking it. The class name also appears in the *Classes* panel header.

2: The *Area* tool has been used to highlight the small diodes. The outline colour, thickness and the area fill can be defined for a class as well as the font style, size and colour. On the illustration sequential numbering has been enabled and appears as labels alongside each component, one of many description options that can be turned on or off and saved with the image.

3: In this illustration all of the 5 classes have been used to highlight and colour code different components.

4: A combination of *Class Name* and *Measurement Number* can quickly identify a component on a *Results Report,* another integral part of the Live and Interactive Measurement modules.

Continued… 734



Measurement #	Class	Width (mm)	Height (mm)	Area (mm²)
17	Capacitor nF	1.0	1.6	1.5
18	Capacitor nF	3.4	1.7	5.7
19	Resistor 0.4W	2.9	2.7	7.4
20	Capacitor nF	2.1	1.9	3.8
21	Resistor 0.4W	3.6	1.7	5.9

If necessary, click on the arrow to the right of the *Classes* header to reveal the *Classes* panel.

1: Click on the *New Class* button and…

2: …on the *Create New Class* dialog type a name for the class.

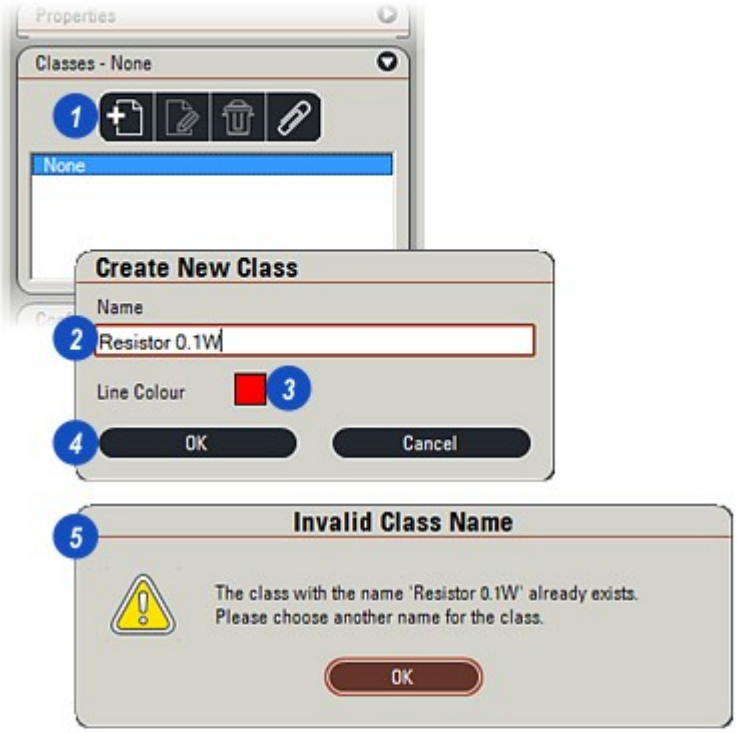
3: The line colour for the class can be set by clicking the *Line Colour* window and on the *Select Colour* dialog choosing a new colour: *Go there…* 730

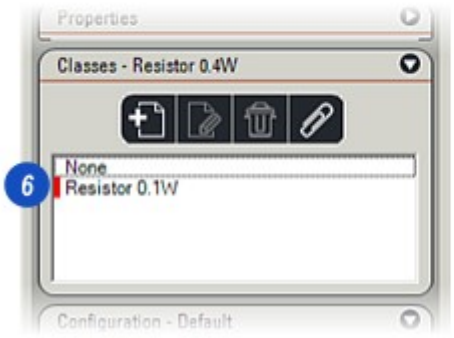
4: Click *OK*.

5: The new class name must be unique. If it is not a warning appears and the name is not accepted unless it is removed from the *Master Class List*: *Go there...*

6: The new *Class Name* appears in the *Classes* window with its selected colour shown to the left.

Continued… 735





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Edit Class:
To edit the *Class Name* and/or *Line Colour*:

1: Click to select the *Class* to be edited.

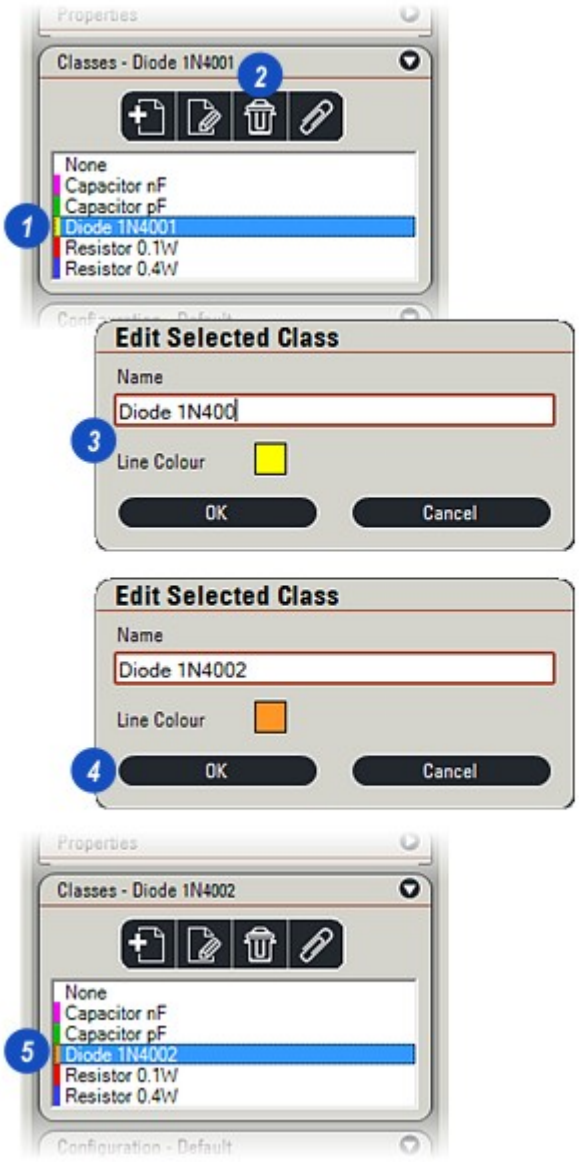
2: Click on the *Edit* button.

3: On the *Edit Selected Class* dialog, click to select the existing name and type a new name or click in the *Line Colour* window and choose a new colour from the *Select Color* dialog: *Go there...* 730

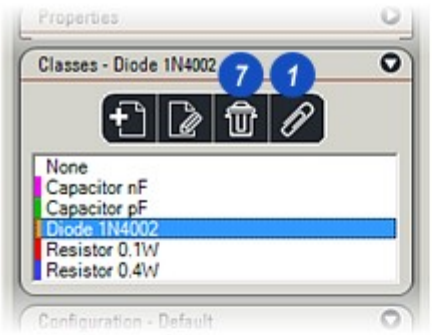
4: Click the *OK* button.

5: The edited Class appears in the *Classes* window.

Continued....



All class descriptions are stored in a single file called the *Master Class File*. Classes may be retrieved from the file (Attach) to serve in new projects and they can be moved back into the file if they are no longer required (Detach). A class can be shared among any number of projects.



Attach/Detach a Class:

1: Click on the 'paper clip' *Attach/Detach* button.

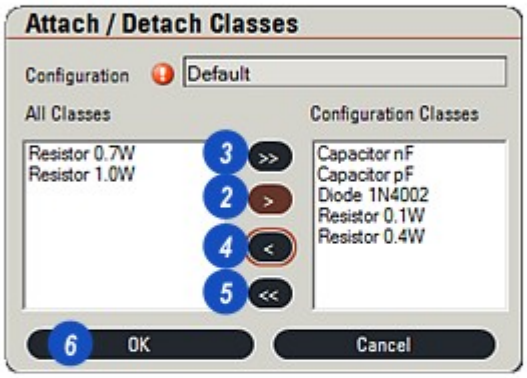
2: On the *Attach/Detach* dialog all of the classes in the *Master File* are listed in the left-hand pane and those in the current project in the right-hand pane. Attach a class by clicking on it in the left-hand pane and then clicking the right arrow.

3: Attach all classes by clicking on the right double-arrow.

4: Detach a class by clicking to select it in the right-hand pane and then clicking the left arrow.

5: Detach all classes by clicking the left double-arrow.

6: Click the *OK* button.

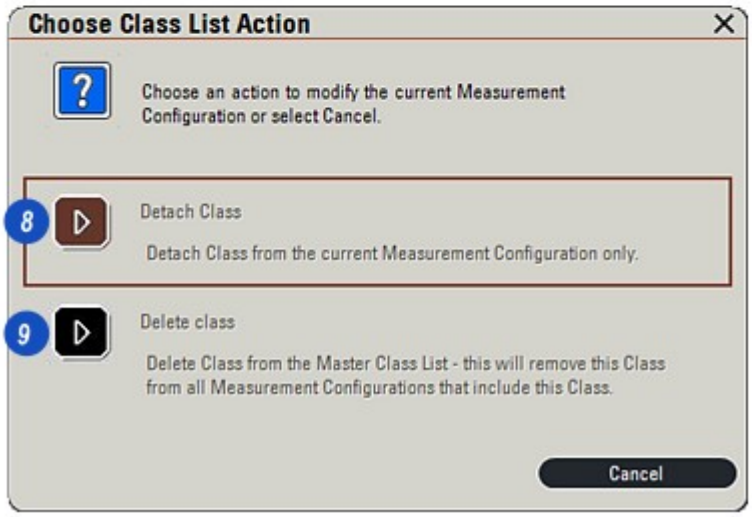


Delete a Class:

7: Delete a class from the *Master File* by clicking the 'Trash Can' *Delete* button.

8: The *Choose Class Action* dialog allows the user either to simply detach the class and move it back into the *Master File* or...

9: ...removing it from the *Master File* completely.



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The current *Classes* and properties can be saved as a *Configuration* file associated with the image, and retrieved to be used at a later date.

1: Each *Configuration* has a unique name and can be accessed by clicking on the arrow to the right of the *Current Configuration* window and from the drop down list…

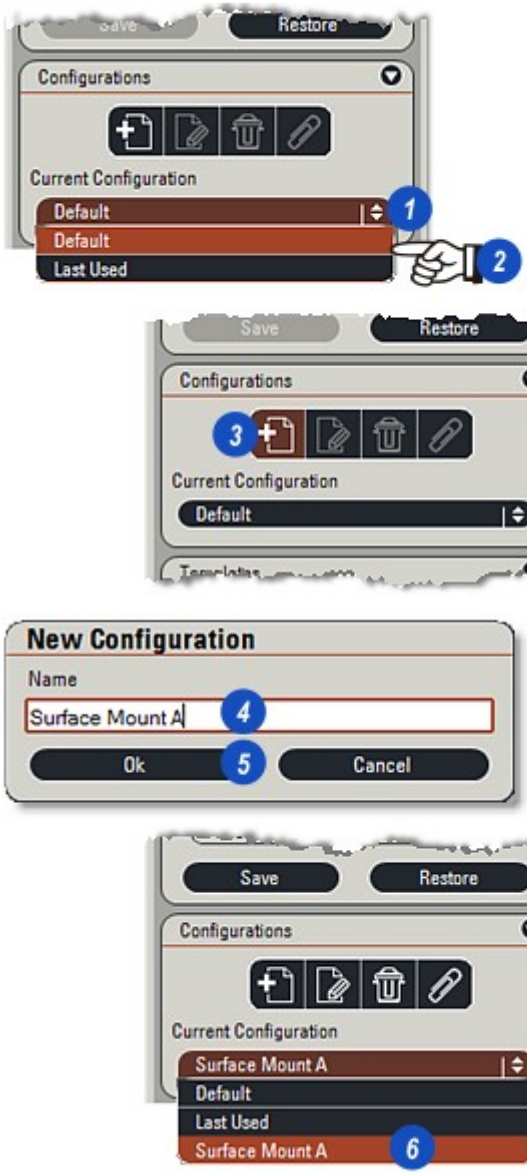
2: …clicking to select the required configuration.
Two configurations are provided with Leica Application Suite – *Default*, which are 'factory' settings that can be subsequently changed in *Preferences*, and *Last Used* which, as its name implies, re-loads the configuration that was last being used and had been saved - that could be the *Default*.

3: To save the current settings as a new configuration, click on the *New* button and…

4: …type a unique name for the new configuration.

5: Click *OK* to save the setting.

6: The new configuration appears in the drop down list.



Continued… 738

To Edit a Configuration Name:

1: Select the configuration to be changed from the drop down list.

2: Click on the *Edit Configuration* button.

3: On the *Edit Configuration* dialog, change the name by clicking in the text box and typing a new name and…

4: …clicking *OK*.

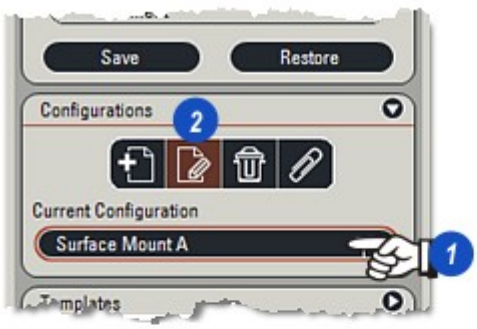
To Delete a Configuration:

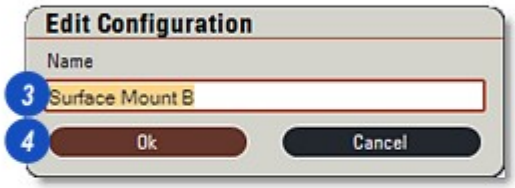
5: Select the configuration to be deleted from the drop down list.

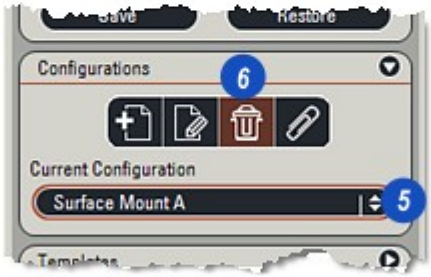
6: Click the *Delete* (Trash Can) button.

7: Confirm the deletion and the *Configuration* will be removed permanently. The operation cannot be reversed.

Continued… 739







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All classes are saved in a *Master Class File* that makes re-use in different configurations simple and fast.

1: Select the configuration to which classes will be attached or detached from the drop down list.

2: Click on the *Attach/Detach* 'paper clip' button.

3: The dialog shows all available classes from the *Master Class* file in the left hand pane **(4)** and all of the classes (if any) currently attached to the configuration in the right hand pane **(5)**.

To attach a Class to the Configuration:

6: Click the *Class* entry and...

7: ...click on the *Attach Selected* button. The Class appears in the right hand window **(8)**.

To Detach a Class from the Configuration:

8: Click the Class to be detached in the right-hand pane.

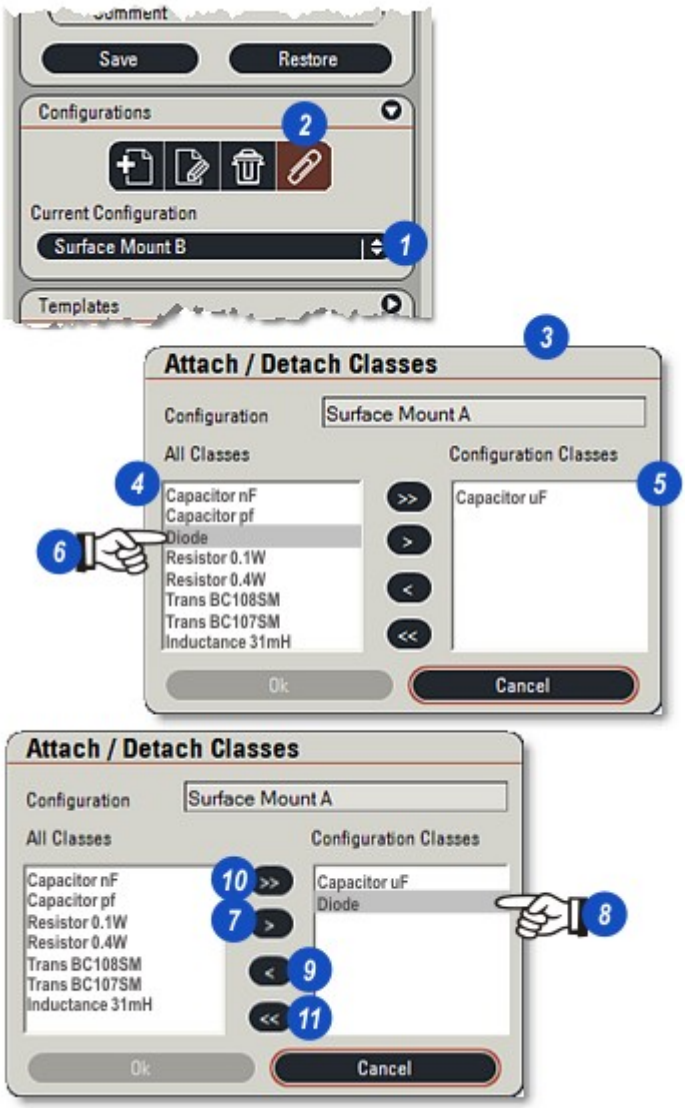
9: Click on the *Detach Selected* button. The Class is removed from the right-hand pane and appears again in the left-hand pane.

Attach/Detach All Classes:

10: Click on the *Attach All* button to attach all of the classes to the configuration.

11: Click on the *Detach All* button to detach all of the classes in the configuration.

Click *OK.*



A 'live' *Template* is a measurement or a collection of measurements used as a ‘ standard’ that can be overlaid on a live image to check for conformity.

The illustration **(1)** represents a ‘perfect’ product and boundary lines have been drawn around three components to map their precise relationship to each other. These measurements are saved as a *Template*, retrieved at any time and placed over a live image to check that it conforms to tolerances for example. Templates are saved as discreet files and are not associated with a specific image.

Having created the measurements:

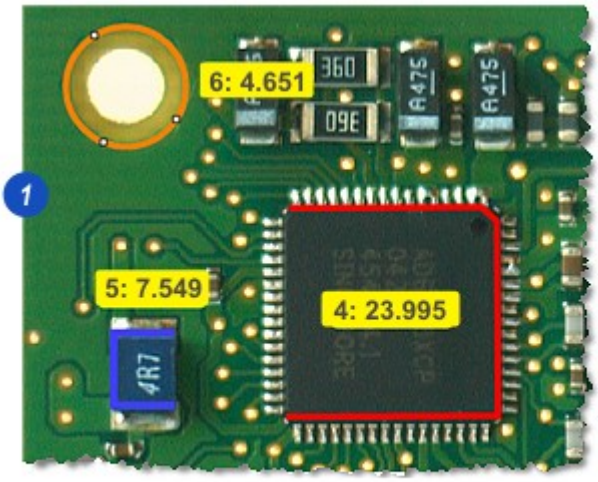
2: If necessary, click the arrows to the right of the *Template* header to reveal the panel.

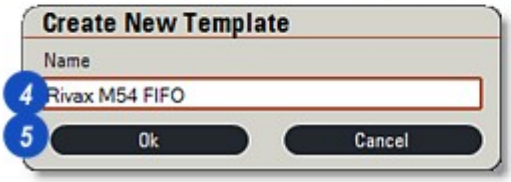
3: Click on the *Create New Template* button and…

4: …on the dialog give the *Template* a unique name.

5: Click *OK*.

Continued…





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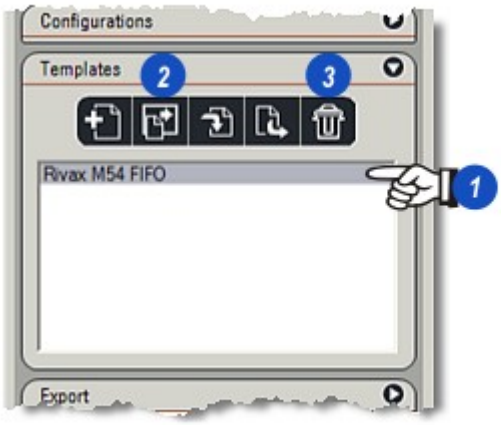
To Retrieve and Apply a Template:

1: Click on the arrows to the right of the *Template* drop down and click to select a template.

2: Click on the *Template Apply* button and the template will appear as an overlay to the live image. Alternatively, double click on the entry name.



Remove a Live Image Overlay:

3: Remove an overlay by clicking the *Delete* (Trash Can) button and on the *Confirm Delete* dialog clicking *Yes*.

Continued... 742

The *Template Export* facility allows a template to be saved in a location other than the default *Measurement Templates* folder.

To export a template to a selected folder, ensure that the template to be exported is highlighted in the *Templates Window*:

1: Click on the *Export* button.

2: On the *Browse for Folder* dialog, navigate to the folder to which the template will be exported.

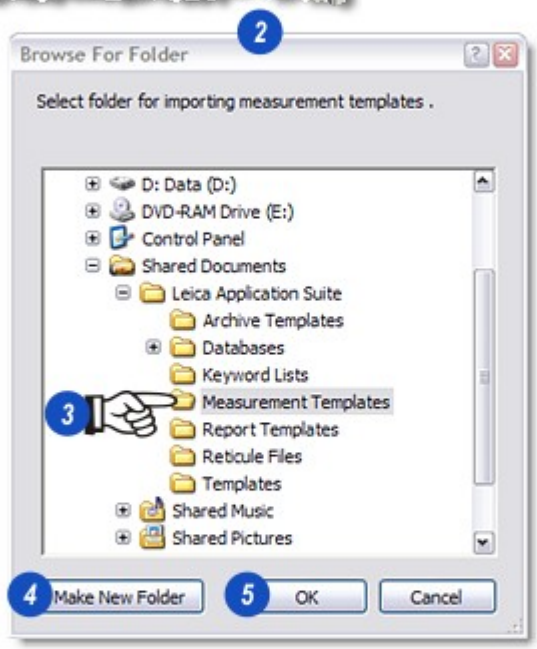
3: There is a default folder created when LAS is installed called *Measurement Templates,* and is the recommended folder for all templates.

4: If required, create a new folder by clicking the *Make New Folder* button.

5: Click *OK*.

6: The *Export Template Report* confirms that the template has exported successfully (or not).

Continued... 743



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Templates conforming to the LAS configuration (.tem file extension) can be imported from any folder.

1: Click on the *Import Template* button.

2: On the *Open* dialog...

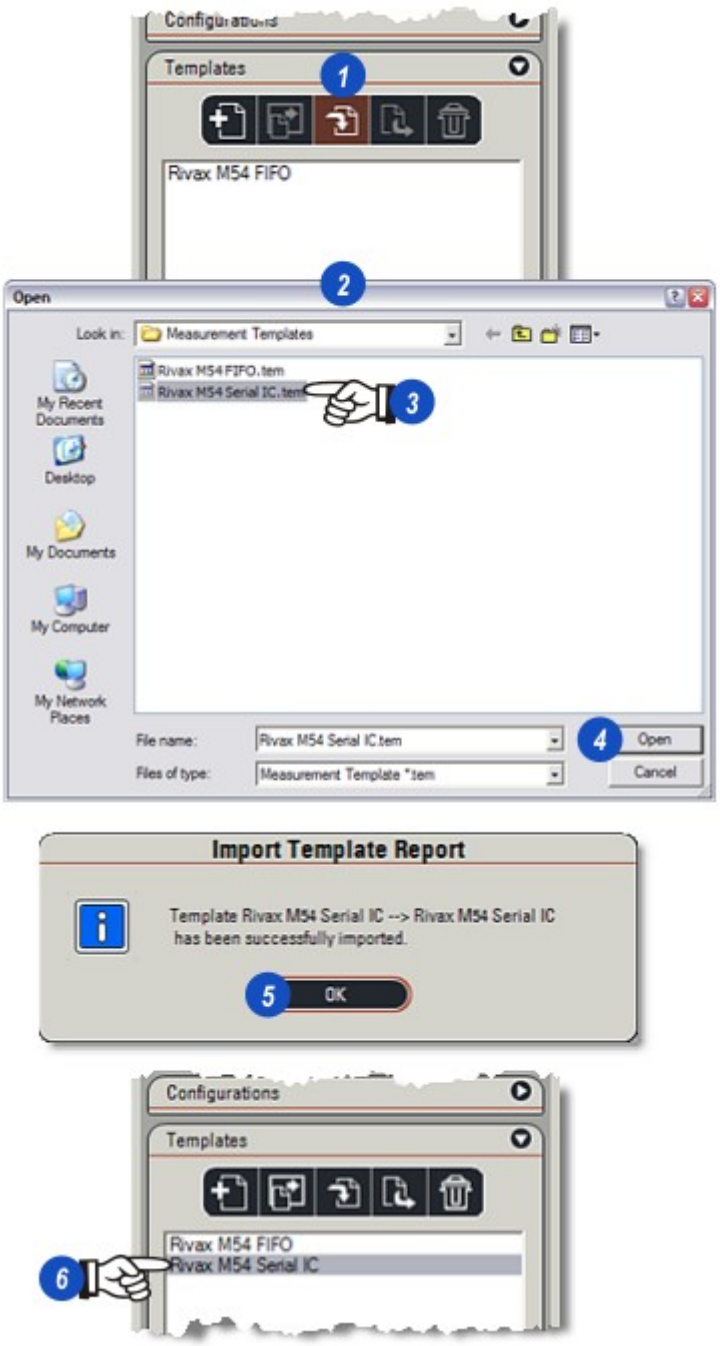
3: ...navigate to the source folder and click to select the template.

4: Click on the *Open* button.

5: The template is imported and success noted on the *Import Template Report*..

6: ...with the template appearing in the *Templates* window.

Although a template is a separate file, a copy of it as an overlay can be saved with a captured image by merging it into the image: *Go there…*



Measurements provides a simple method of creating a range of measurement overlays for a single, captured image.

1: Click on the arrow to the right of the *Measurements* header to reveal the panel.

2: The *Default* entry represents the original image.

3: Make a measurement on the image.

4: Click on the *New Measurement* button and…

5: …on the *Create New Measurement* dialog type a unique name for the measurement.

6: Click *OK*.

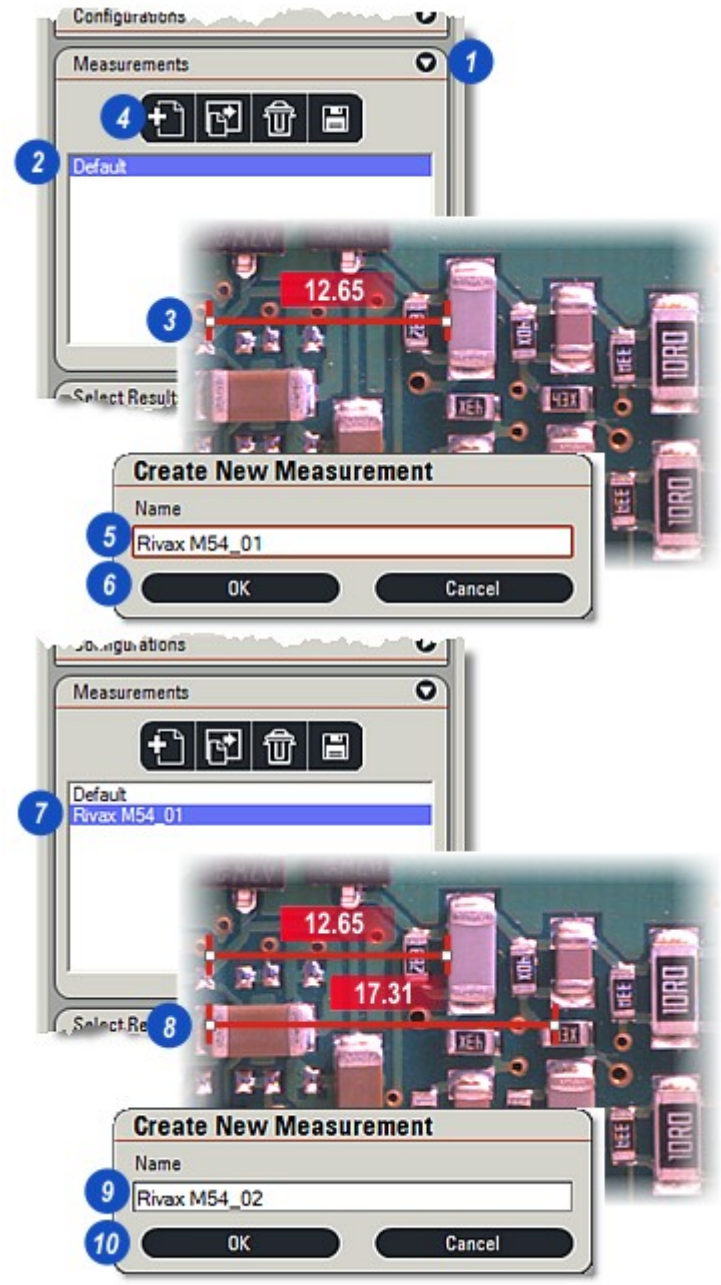
7: The new measurement name appears in the window.

8: Make another measurement – or delete the first and then make another. Click on the *New Measurement* button **(4).**

9: Type a name for the new measurement and…

10: …click OK.

Continued… 745



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1: Continue to add or change measurements giving each version a new name.

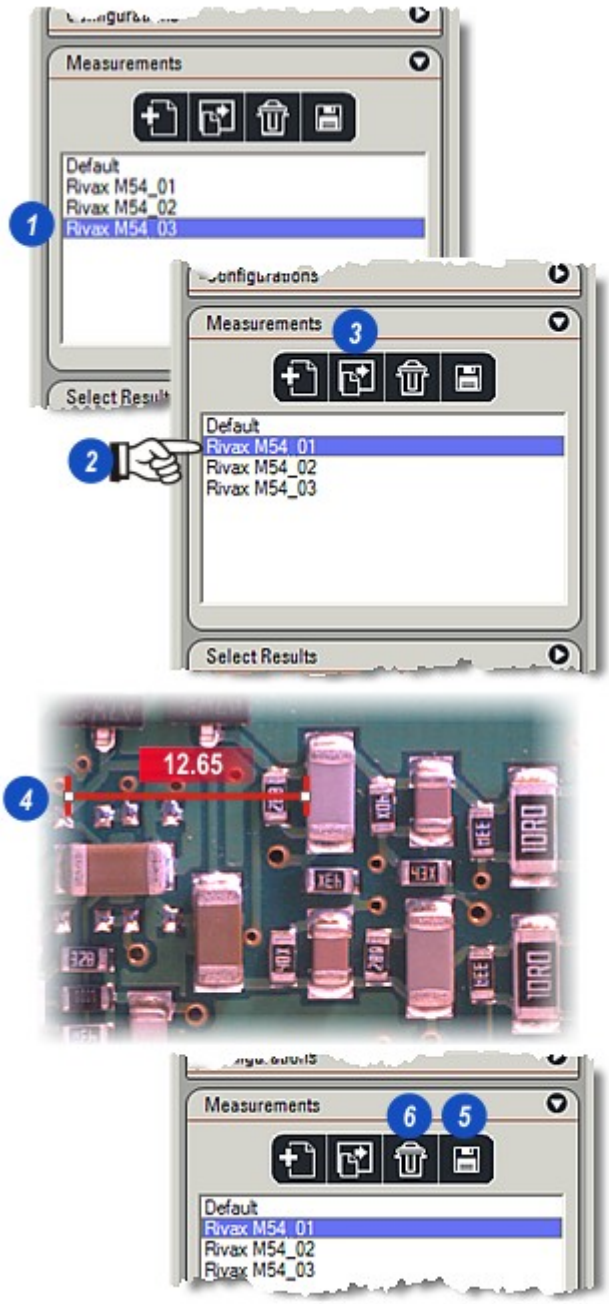
2: retrieve a measurement by clicking on its name in the window and then…

3: …clicking on the *Load* button and…

4: …the measurement is retrieved and placed in position over the image.

5: Edit a measurement by adding, re-positioning or deleting measurements and then clicking the *Save* button.

6: Delete a measurement by clicking on its name to select it and then on the *Trash Can* (Delete) button.



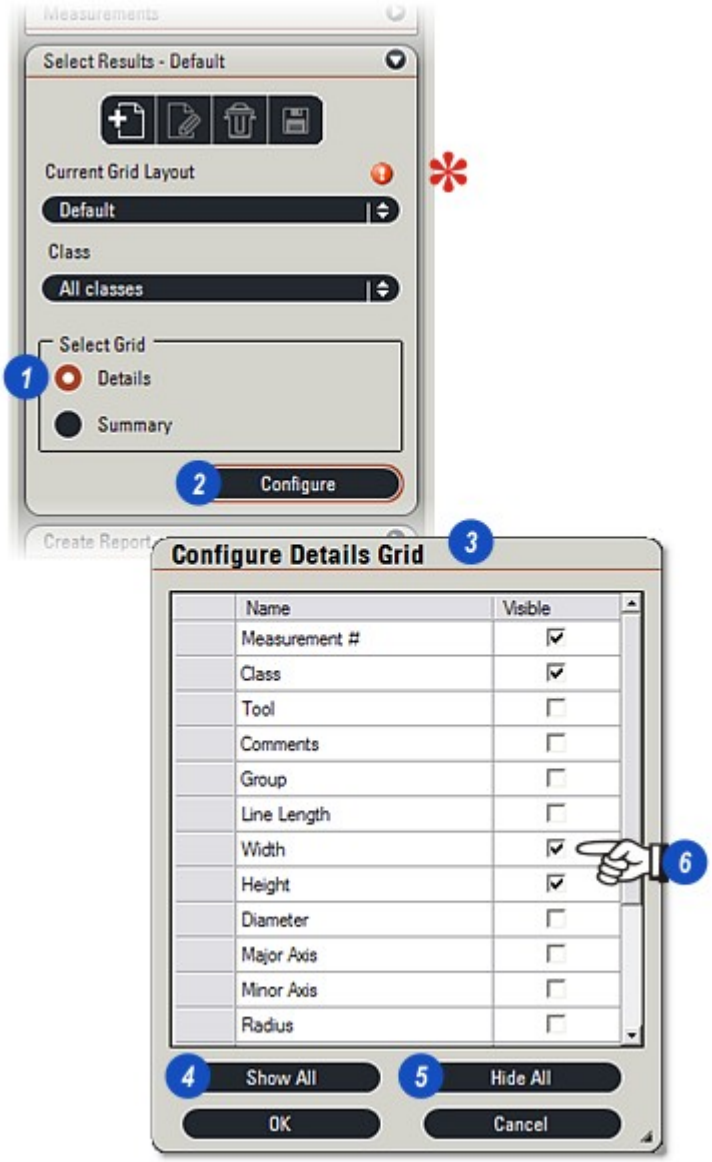
The *Select Results* function displays an analysis of all the measurements taken on an image and presents them as:

Details: Each measurement is shown with all its parameters (or those that are selected in the *Configuration*), or

Summary: The measurements are treated as a complete set of data tabulated statistically.

Both options present the information as a grid arranged below the image. The information can be later edited, deleted or attached to the image.

If necessary, click on the small arrow to the right of the Select Results panel.



Grid Setup for Details:

1: Click to enable the *Details* button.

2: Click the *Configure* button.

3: On the *Configure Details Grid* dialog...

4: ...click the *Show All* button to enable all of the parameters or...

5: ...click the *Hide All* button to disable all of the parameters.

6: Individual parameters can then be enabled or disabled.

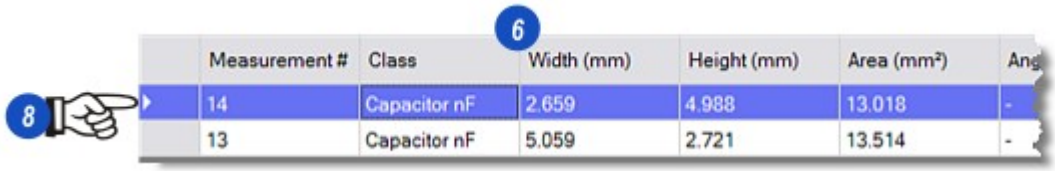
7: Click *OK*.

8: Those parameters that have been enabled will appear as a heading on the *Grid* with the appropriate results beneath. If a chosen parameter is not applicable to a measurement - for instance, *Line Length* is not a parameter of a *3-Point Circle* - then the value is reported as a dash (-).

*

When changes are made to the Grid either in Details or Summary, the warning **!** flashes. Save the new Grid Layout as a configuration: Go there... 748

Continued... 747



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To display the results using the Summary information:

1: Click to enable the *Summary* button.

2: Click the *Configure* button.

3: On the *Configure Summary Grid* dialog...

4: ...click the *Show All* button to enable all of the parameters or...

5: ...click the *Hide All* button to disable all of the parameters.

6: Individual parameters can then be enabled or disabled.

7: Click *OK*.

8: Those parameters that have been enabled will appear as a heading on the grid with the appropriate results beneath. If a chosen parameter is not applicable to a measurement - for instance, *Line Length* is not a parameter of a *3-Point Circle* - then the value is reported as a dash (-).

9: Hide/Reveal the *Results Grid* by clicking the *Grid* button on the *Side Tool Bar.*

The results can be configured further by selecting if only a specified class is to be displayed or alternatively no classes or all classes.

To select the Class configuration:

1: Click on the arrows to the right of the *Class* header and…

2: …from the drop down list click to select *None*, *All Classes* or an individual *Class*.

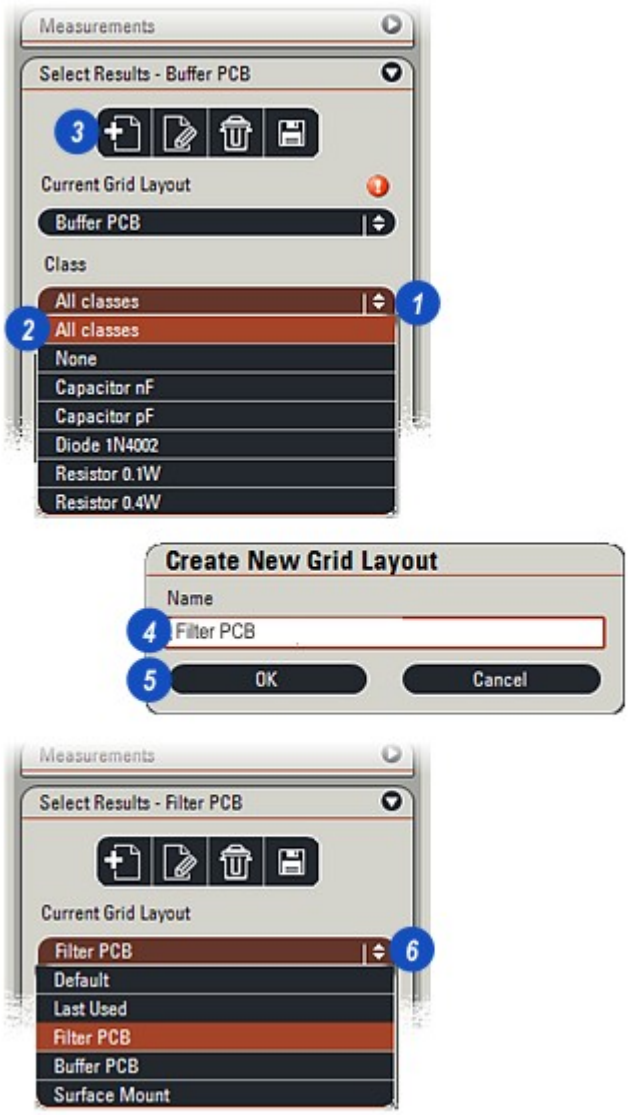
Save the Grid Configuration:
To save the Grid settings as a Configuration that can be retrieved and used later:

3: Click on the *New Grid Configuration* button.

4: Click inside the *Name* text box and type a unique name for the configuration.

5: Click *OK*.

6: To see the new configuration in the list, click the small arrow to the right of the *Current Grid Layout* header to reveal the list.



Continued… 746

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Edit a Results Grid Configuration:

1: Click on the *Edit* button.

2: On the *Edit Configuration* panel, click inside the **Name** text box and type a new, unique name for the configuration.

3: Click *OK*.

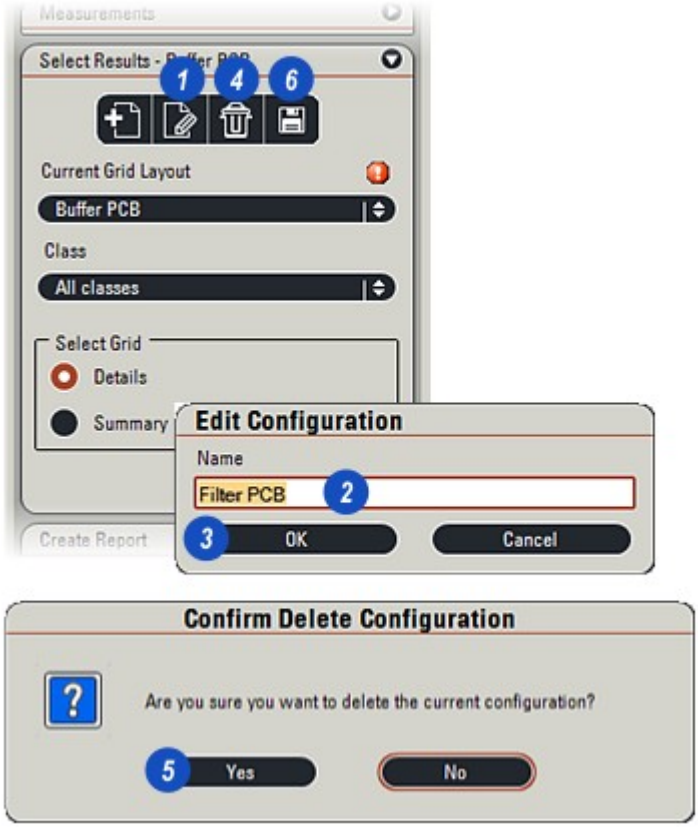
Delete a Results Grid Configuration:

4: Click on the 'Trash Can' *Delete* button.

5: Confirm or abort the the deletion. If a configuration is deleted it cannot be retrieved.

Save the Results Grid Configuration with the Image:

6: Click on the *Save* button. This will attach a copy of the *Results Grid Configuration* to the image so that when it is opened again in Measurements the configuration will be loaded automatically.



A report collects the measurement information and stores it in a readable format – either to be displayed in *Microsoft Excel* or as a *Comma Separated Value* (CSV) file. CSV files are stored as plain text and are very compact, making them ideal as e-mail attachments. They can also be used in a wide range of text processing applications.

The parameters that are exported reflect those chosen to display in Select Results. *Go there...* 748

There are a few differences between *Live* and *Interactive Measurements* detailed on the following pages.

1: A typical *Live Measurement* image with a range of measurement tools being used.

2: Click on the arrows to the right of the *Create Report* header to reveal the panel.

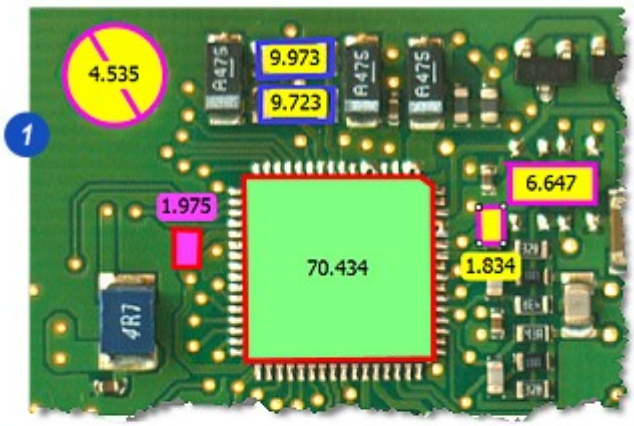
Live Measurements Panel:

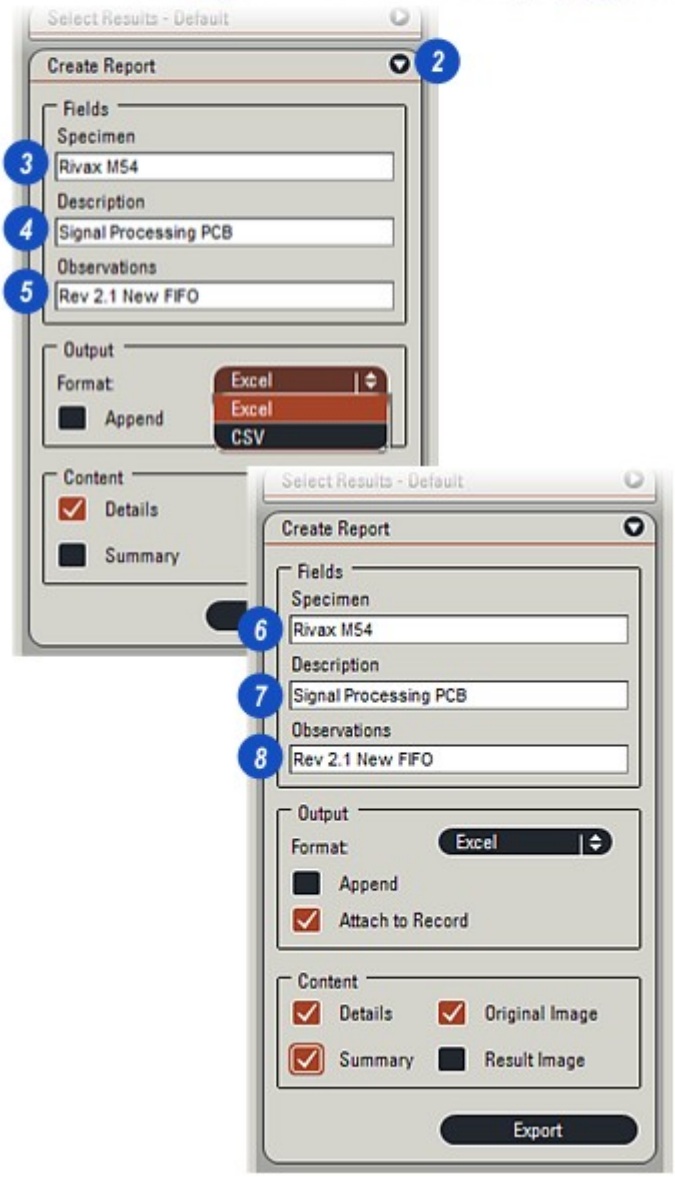
3, 4 and 5: Three *Field* text boxes – *Specimen*, *Description* and *Observations* – are provided as optional headers for the measurement file. They do not have to contain information but to add text to them simply click in a text box and type.

Interactive Measurements Panel:

6, 7 and 8: The *Field* text boxes are the same as Live Measurements and if they were completed when the image was captured they will be displayed automatically.

Continued… 751





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1: Click on the arrows to the right of the *Format* header to reveal the output options.

2: Select *Excel* from the drop down menu.

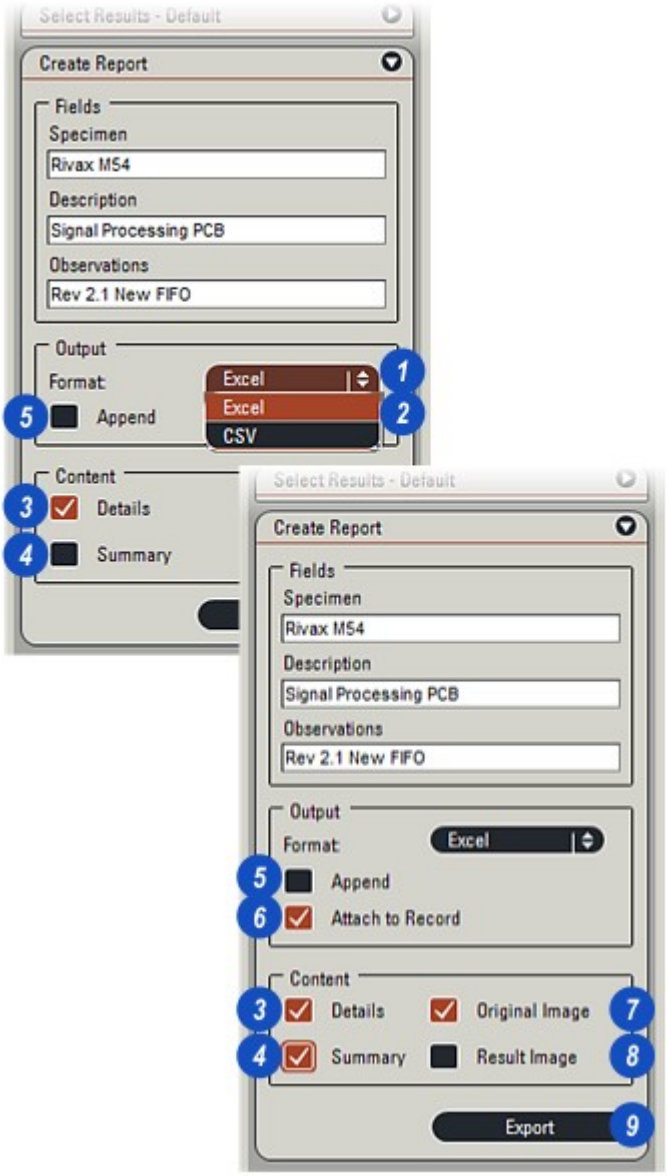
3: The report content can be *Details* – the actual measurements, classes and results, or…

4: …*Summary* that is a detailed analysis of the measurements.

...or both *Details* and *Summary* combined in a single Excel book.

5: To add the report data to an existing Excel spreadsheet as a separate page, click the Append button. This arrangement allows results to be analysed individually or as a group.

Click the *Export* button **(8).**



Interactive Measurements Options:

6: The report can be attached as a file to the image record by enabling the *Attach to Record* check box.

7: Enable the *Original Image* check box to include the original image in the report and…

8: …select *Result Image* to include the image together with the overlaid measurements.

9: Click the *Export* button.

Continued... 752

1: On the *Export* dialog, click the browse button and…

2: …navigate to a folder on the *Select a Report File* dialog, …

3: …give the file a name – the proper file extension (.xls) is automatically added and...

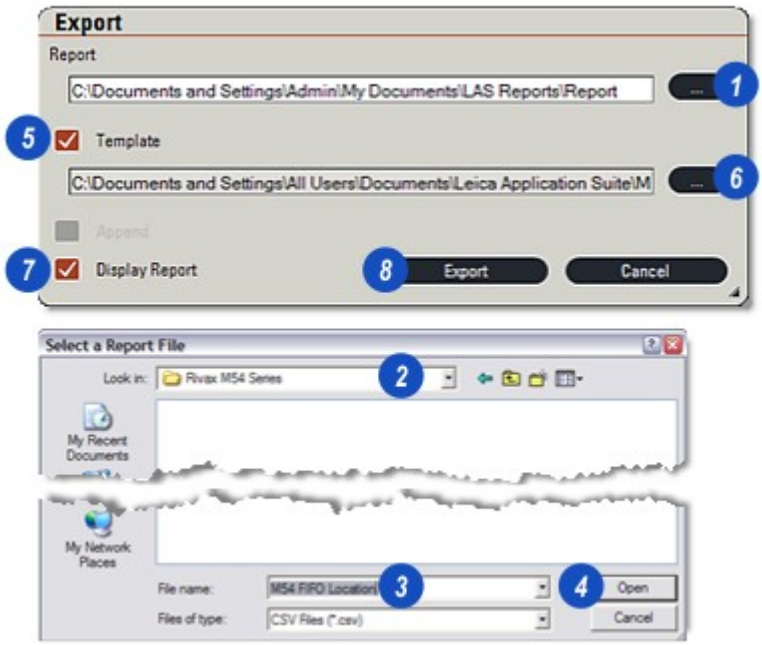
4: …click the *Open* button.

5: To use a template to lay out the report, click to enable the *Template* check box and…

6: …navigate to the *Template* folder. On first use this is automatic for *Leica Excel Templates* that have been specially created for fast, efficient reporting and can be easily modified to suit users' precise requirements. If a template is not used images are not exported.

7: To display the file on screen after the report is complete, click to enable the *Display Report* button.

8: Click *Export* and the file will be written. If the *Display Report* check box is enabled the appropriate application (if installed) will be launched with the information displayed in it.



Continued… 753

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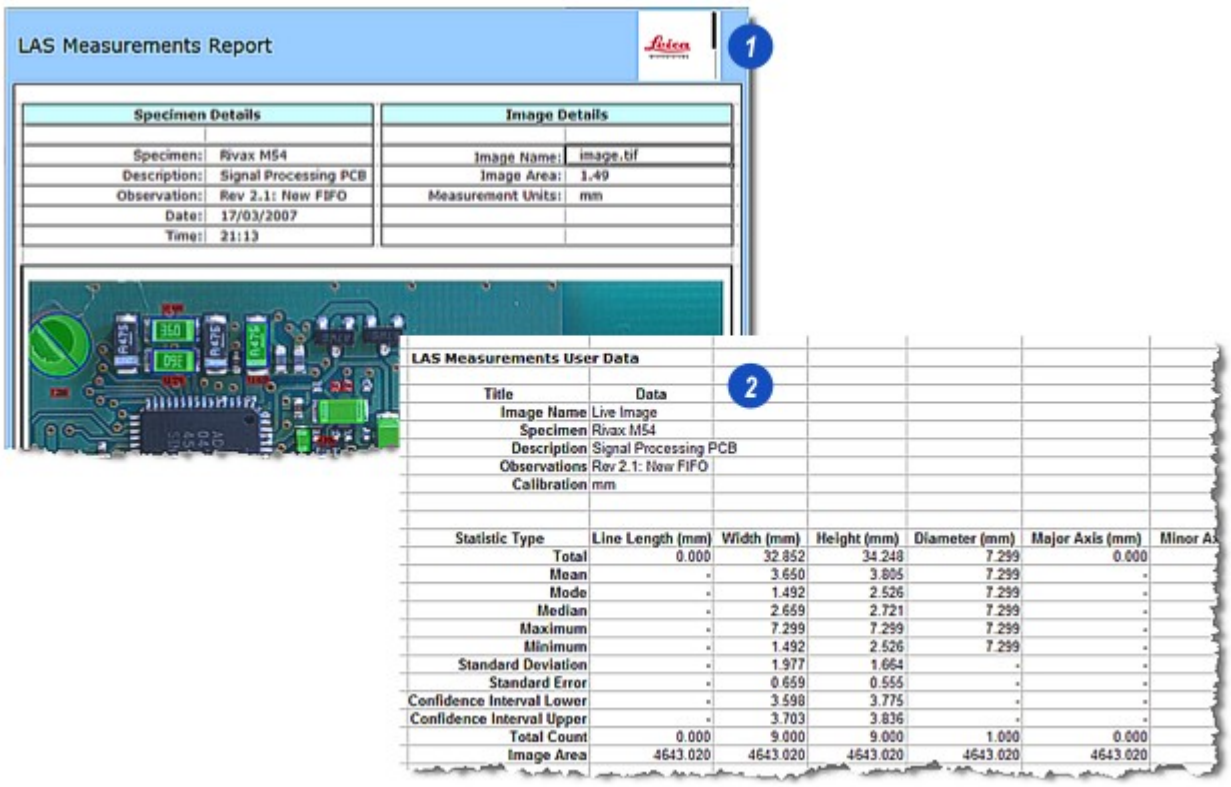
1: The *Excel Front Page* with *Summary*: The image appears only with Interactive Measurements..

2: The *Statistics* sheet. If *Summary* is not included then the lower part of the sheet - the statistics - will be blank. See *Appendix* for explanations of the parameters: *Go there...* 759



LAS Measurements Report

1

Specimen Details		Image Details	
Specimen:	Rivax M54	Image Name:	image.tif
Description:	Signal Processing PCB	Image Area:	1.49
Observation:	Rev 2.1: New FIFO	Measurement Units:	mm
Date:	17/03/2007		
Time:	21:13		

LAS Measurements User Data

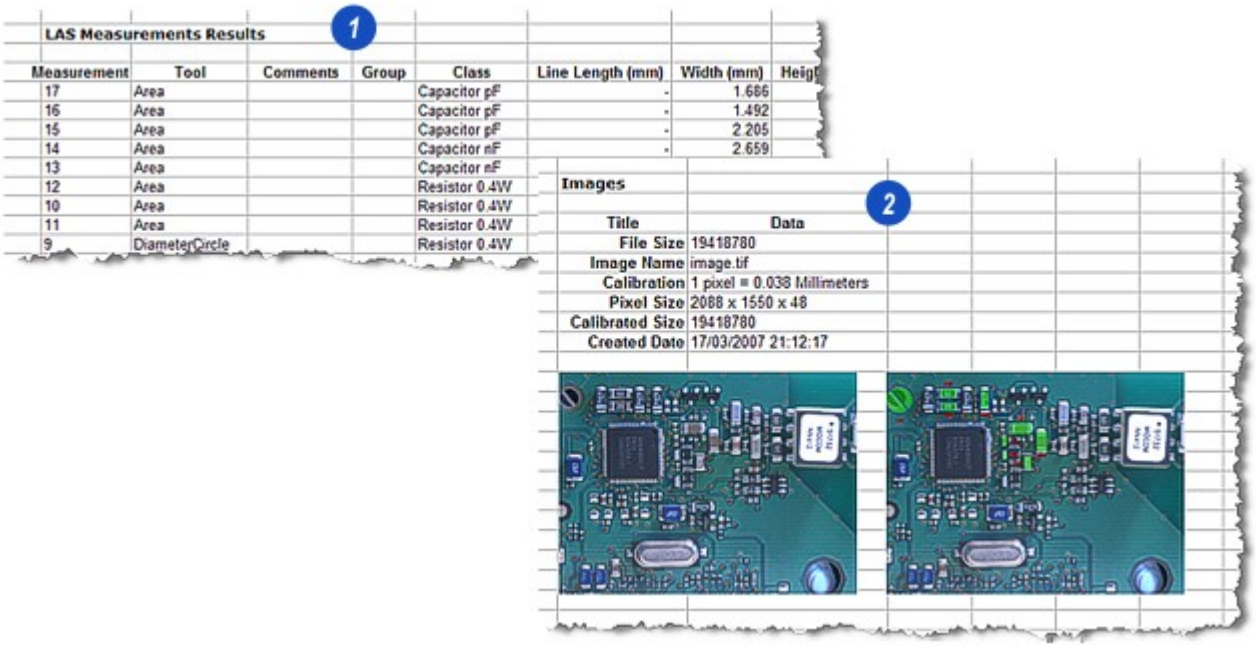
2

Title	Data
Image Name	Live Image
Specimen	Rivax M54
Description	Signal Processing PCB
Observations	Rev 2.1: New FIFO
Calibration	mm

Statistic Type	Line Length (mm)	Width (mm)	Height (mm)	Diameter (mm)	Major Axis (mm)	Minor A
Total	0.000	32.852	34.248	7.299	0.000	
Mean	-	3.650	3.805	7.299	-	
Mode	-	1.492	2.526	7.299	-	
Median	-	2.659	2.721	7.299	-	
Maximum	-	7.299	7.299	7.299	-	
Minimum	-	1.492	2.526	7.299	-	
Standard Deviation	-	1.977	1.664	-	-	
Standard Error	-	0.659	0.555	-	-	
Confidence Interval Lower	-	3.598	3.775	-	-	
Confidence Interval Upper	-	3.703	3.836	-	-	
Total Count	0.000	9.000	9.000	1.000	0.000	
Image Area	4643.020	4643.020	4643.020	4643.020	4643.020	

1: An *Excel Details* sheet lists all of the measurements and chosen parameters.

2: The *Excel Images* sheet. The images only appear with Interactive Measurements- on the left the image without measurements and on the right with the measurements overlaid.



LAS Measurements Results

Measurement	Tool	Comments	Group	Class	Line Length (mm)	Width (mm)	Heigh
17	Area			Capacitor pF	-	1.686	
16	Area			Capacitor pF	-	1.492	
15	Area			Capacitor pF	-	2.205	
14	Area			Capacitor nF	-	2.659	
13	Area			Capacitor nF			
12	Area			Resistor 0.4W			
10	Area			Resistor 0.4W			
11	Area			Resistor 0.4W			
9	DiameterCircle			Resistor 0.4W			

Images

Title	Data
File Size	19418780
Image Name	image.tif
Calibration	1 pixel = 0.038 Millimeters
Pixel Size	2088 x 1550 x 48
Calibrated Size	19418780
Created Date	17/03/2007 21:12:17

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1: Click on the arrows to the right of the *Format* header and select *CSV* from the options.

2: The *Content* selection differs from the *Excel* options in that *Details* or *Summary* can be selected but *not* both. Click on the content required.

Interactive Measurements Option:

3: Interactive Measurements has the option to attach the CSV report to the image record. Click to enable the *Attach to Record* check box.

4: Click the *Export* button.

5: On the *Export* dialog, click on the browse button and…

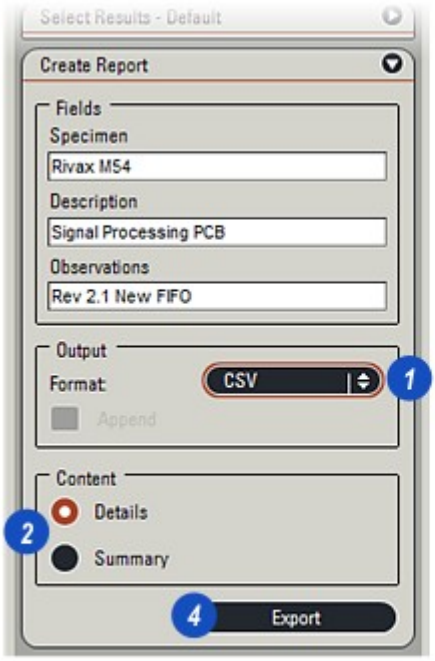
6: …on the *Select a Report File* dialog navigate to a folder in which to store the report.

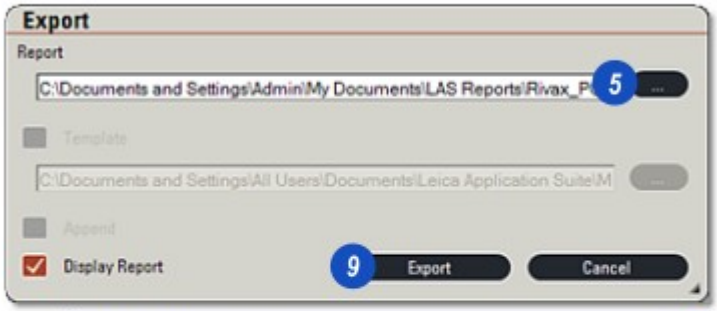
7: Give the report a name and…

8: …click *Open*.

9: Click *Export*.

Continued… 756





1: Example of a report based on a *Comma Separated Values (CSV)* output. CSV output is a single page, very compact report ideal for data transmission. Here the data is displayed in a Microsoft Excel format.

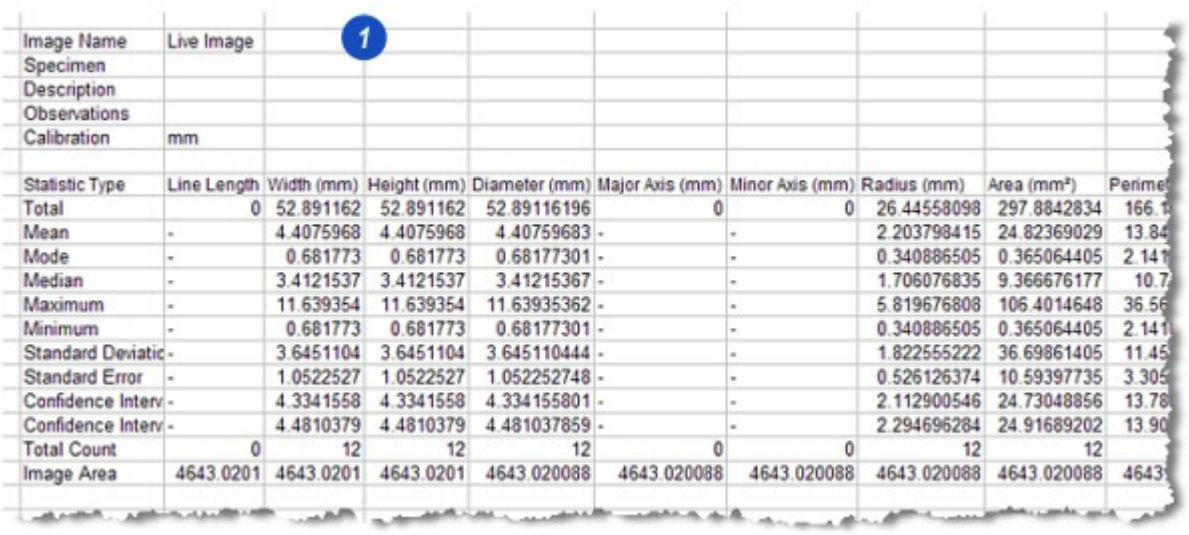


Image Name	Live Image								
Specimen									
Description									
Observations									
Calibration	mm								
Statistic Type	Line Length	Width (mm)	Height (mm)	Diameter (mm)	Major Axis (mm)	Minor Axis (mm)	Radius (mm)	Area (mm²)	Perime
Total	0	52.891162	52.891162	52.89116196	0	0	26.44558098	297.8842834	166.1
Mean	-	4.4075968	4.4075968	4.40759683	-	-	2.203798415	24.82369029	13.84
Mode	-	0.681773	0.681773	0.68177301	-	-	0.340886505	0.365064405	2.141
Median	-	3.4121537	3.4121537	3.41215367	-	-	1.706076835	9.366676177	10.7
Maximum	-	11.639354	11.639354	11.63935362	-	-	5.819676808	106.4014648	36.56
Minimum	-	0.681773	0.681773	0.68177301	-	-	0.340886505	0.365064405	2.141
Standard Deviatic	-	3.6451104	3.6451104	3.645110444	-	-	1.822555222	36.69861405	11.45
Standard Error	-	1.0522527	1.0522527	1.052252748	-	-	0.526126374	10.59397735	3.305
Confidence Interv	-	4.3341558	4.3341558	4.334155801	-	-	2.112900546	24.73048856	13.78
Confidence Interv	-	4.4810379	4.4810379	4.481037859	-	-	2.294696284	24.91689202	13.90
Total Count	0	12	12	12	0	0	12	12	
Image Area	4643.0201	4643.0201	4643.0201	4643.020088	4643.020088	4643.020088	4643.020088	4643.020088	4643

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To 756 retrieve a report, when using the optional modules LAS Archive, previously attached to an image:

1: Click on the *Browse Workflow* and...

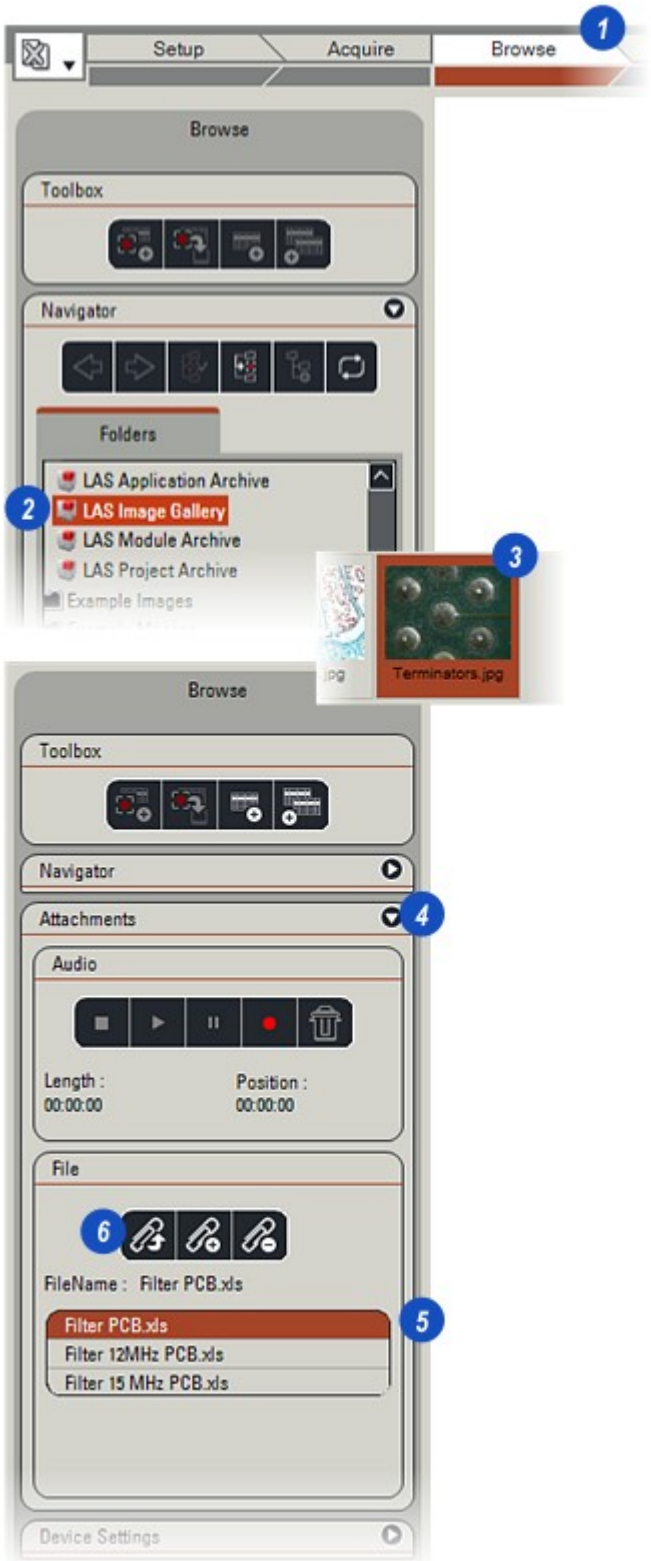
2: ...open the image archive.

3: Click on the *Gallery* thumbnail to select and display the image.

4: Expand the *Attachments* panel by clicking on the small arrow to the right.

5: Attached reports are listed in the *File* drop-down. Click to select the required report and...

6: ...click the *Open* button to start Microsoft Excel and display the report spreadsheet.



Merging is the process of combining measurements with an image so that they become a single entity.

Measurements merged with an image cannot be edited and altered.

The *Merge* control differs in appearance and function between Live and Interactive Measurements.

Live Measurements Merge:

1: Clicking to enable the *Merge* check box will merge measurements with images as they are captured whilst it is enabled.

2: Click on the *Acquire Image* button.

Interactive Measurements Merge:

3: The Interactive *Merge* is a 'one shot' button – click it to merge measurements with the captured image.

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Total: Is the total for all tools beneath that heading. For example, Total Line Length is the total measurement for all the tools - Distance and Line - that have length as a parameter.

Mean: Represents the Total divided by the number of measurements made (Total/Total Count).

Mode: The most frequently occurring parameter value from measurements using this parameter. If multiple modes exist, that with the lowest value is displayed.

Median: The actual mid-value in a list of values. For example 676 is the Median of 214, 676 and 1031. For an even numbered list of values, the values either side of the mid-point are averaged.

Maximum and Minimum: The largest and smallest measurements made regardless of the tool used.

Standard Deviation: A measure of the spread of a parameter values from the measurements using that parameter. It is based upon a random sample taken from the values.

Standard Error: Uses the convention Standard Deviation/Square root(n) where 'n' is the number of measurements made or Total Count.

Confidence Interval Lower: The lower range of parameter values within which 95% of parameter values are likely to fall. It is based upon the assumption that the values are normally distributed with a mean of the same value as Mean.

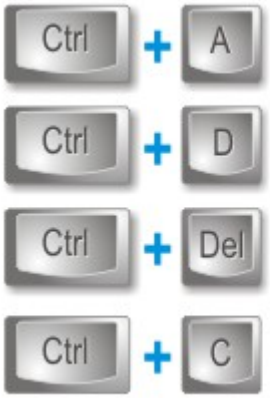
Confidence Interval Upper: The upper range of parameter values within which 95% of parameter values are likely to fall. It is based upon the assumption that the values are normally distributed with a mean of the same value as Mean.

Total Count: The number of measurements made with this parameter.

Total Image Area: Is the area (selected units^2) of the entire image.

Statistic Type	Width (mm)	Height (mm)	Area (mm²)	Angle (°)	Count
Total	35.11	35.11	330.35	0.00	0.00
Mean	11.70	11.70	110.12	-	-
Mode	9.29	9.29	67.74	-	-
Median	12.25	12.25	117.84	-	-
Maximum	13.58	13.58	144.77	-	-
Minimum	9.29	9.29	67.74	-	-
Standard Deviation	2.20	2.20	39.09	-	-
Standard Error	1.27	1.27	22.57	-	-
Confidence Interval Lower	11.70	11.70	110.11	-	-
Confidence Interval Upper	11.70	11.70	110.12	-	-
Total Count	3.00	3.00	3.00	0.00	0.00
Image Area	4643.02	4643.02	4643.02	4643.02	4643.02

When the *Results Grid* is selected, a range of Shortcut Key combinations are available.



For all Shortcuts, press and hold down the *Control (Ctrl)* key and at the same time press the auxiliary key:

Ctrl + A: *Selects all of the measurements.*

Ctrl + D: *Deselects the measurements.*

Ctrl + Del(Delete): *Deletes all of the selected measurements.*

Ctrl + C: *Copies any selected text to the Windows clipboard. It can then be pasted to another application by using Ctrl + V.*

For a complete list of all the LAS Keyboard Shortcuts: *Go there...* 22

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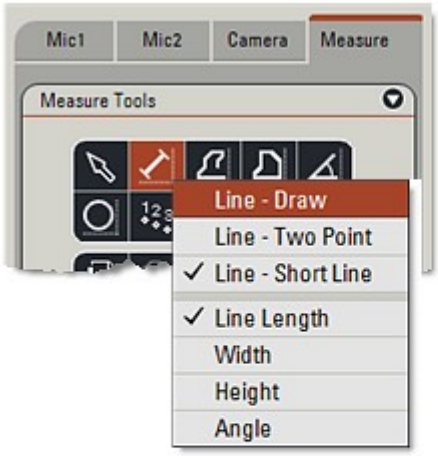
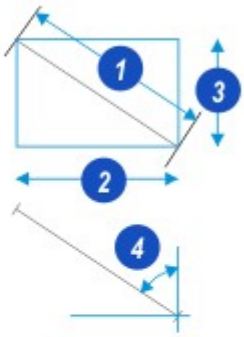
All distances are calculated from the centre of the enclosing line:





Line:

1: *Length:* Overall length of the drawn line measured to the centre of the line ending strokes.

2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the line.

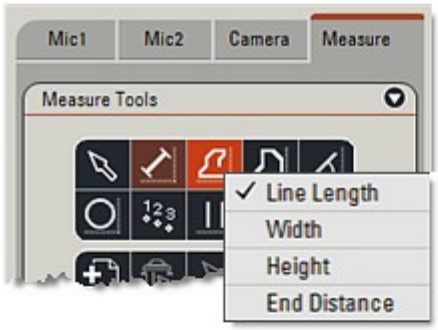
4: *Angle:* The angle swept between the line and the horizontal.

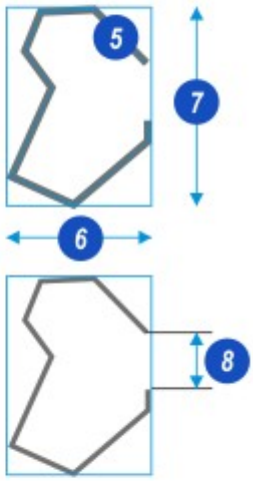
Segment Line:

5: *Length:* The total length of all the segments added together.

6 & 7: *Width and Height:* The dimensions of a bounding box enclosing the figure.

8: *End Distance:* The distance between the two end points of the figure.



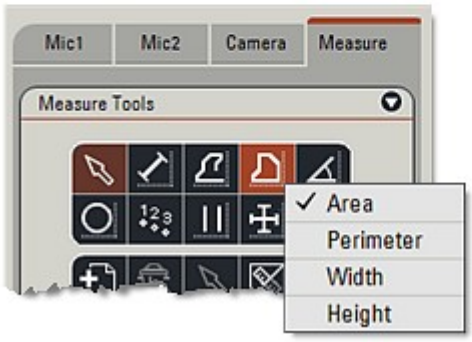


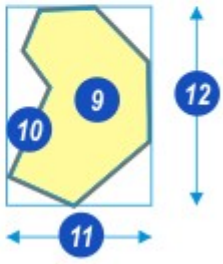
Area:

9: *Area:* The enclosed space returned in the selected measurement squared (for example mm²).

10: *Perimeter:* The distance around the figure.

11 & 12: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the figure.





Continued... 762

Circle:
All distances are calculated from the centre of the enclosing line:

1: *Diameter*: Distance across the circle in any direction.

2: *Perimeter*: The distance around the circle.

3: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.

4: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

5: *Width*: Distance across the circle horizontally.

6: *Height*: Distance across the circle vertically.

Width and *Height* are the same as *Diameter* but are retained because the circle is part of the ellipse class that will have definite *Width* and *Height* dimensions

Ellipse:

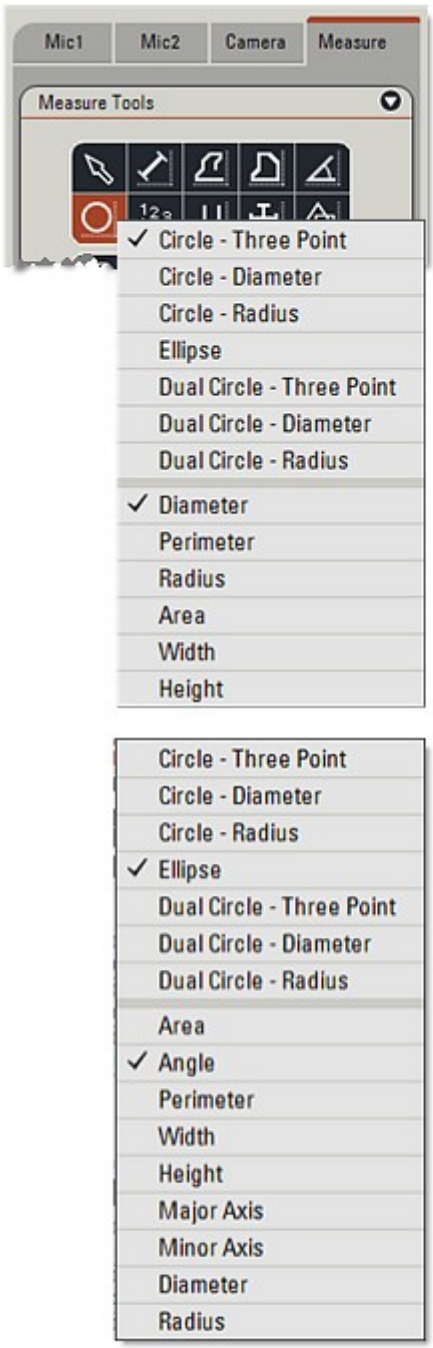
7: *Angle*: Angle between the horizontal and the Major Axis.

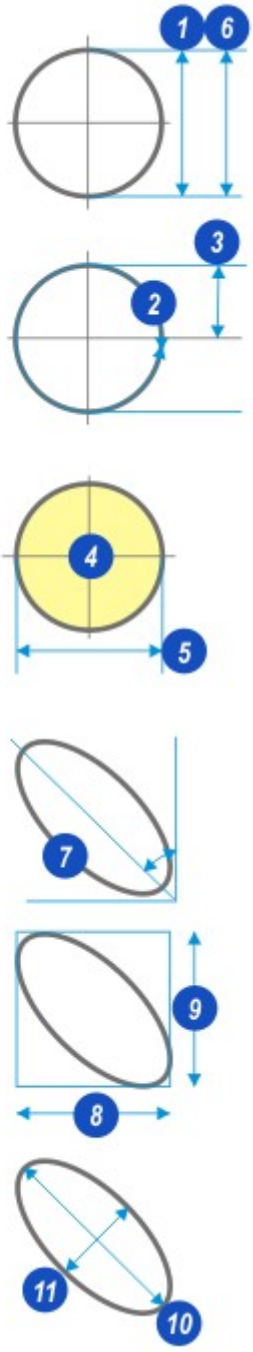
8 & 9: *Width and Height:* Horizontal and vertical sides of a bounding box enclosing the Ellipse.

10: *Major Axis:* The greatest dimension.

11: *Minor Axis:* The smallest dimension.

Continued... 763





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All distances are calculated from the centre of the enclosing line. Parameters without the '2' suffix refer to the first circle drawn and those with the '2' suffix to the second circle.

1: *Diameter*: Distance across the circle in any direction.
Width and Height are the same as Diameter but are retained because the circle is part of the ellipse class that will have definite Width and Height dimensions

2: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.

3: *Perimeter*: The distance around the circle.

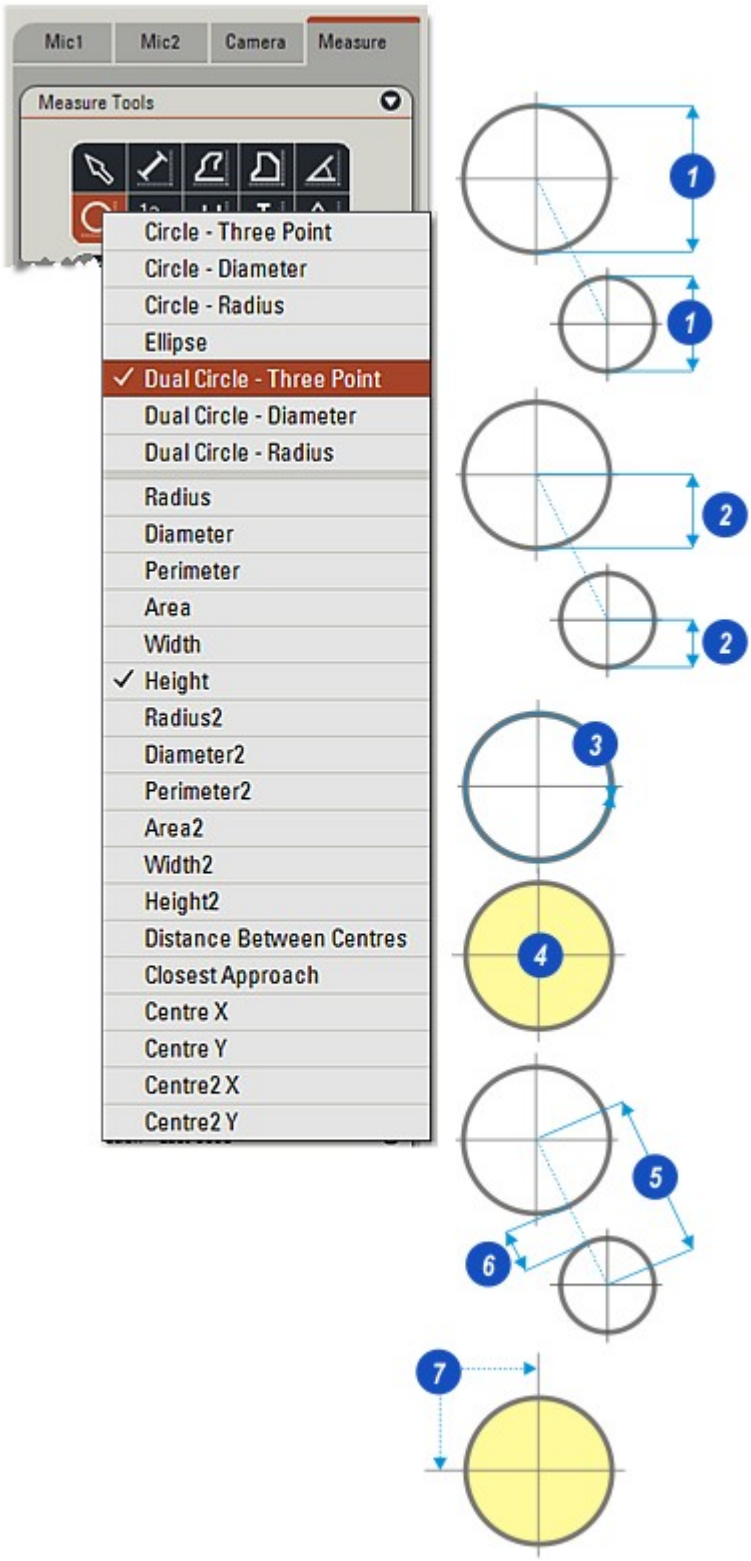
4: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

5: *Distance Between Centres:* Distance between the centres of the first circle and the second circle.

6: *Closest Approach:* Smallest distance between the perimeters of the two circles.

7: *CentreX and CentreY:* The distances between the centre of the circle and the left edge of the image (CentreX) and that from the centre of the circle to the top of the image (CentreY).

Continued... 764



Baseline Angle:

1: *Angle*: The angle described with reference to the horizontal.

2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the drawn angle.

Apex Angle:

4: *Angle*: The uppermost angle described between the two drawn 'legs'.

Four Point Angle:

5: *Angle*: The angle described at the intersection of two extended lines.

Multiple Distance Line:

6: *Length*: The distance between the Datum line and the selected feature.

Parallel Distance Line:

7: *Length*: The distance between the Datum line and the selected parallel line.

Cross:

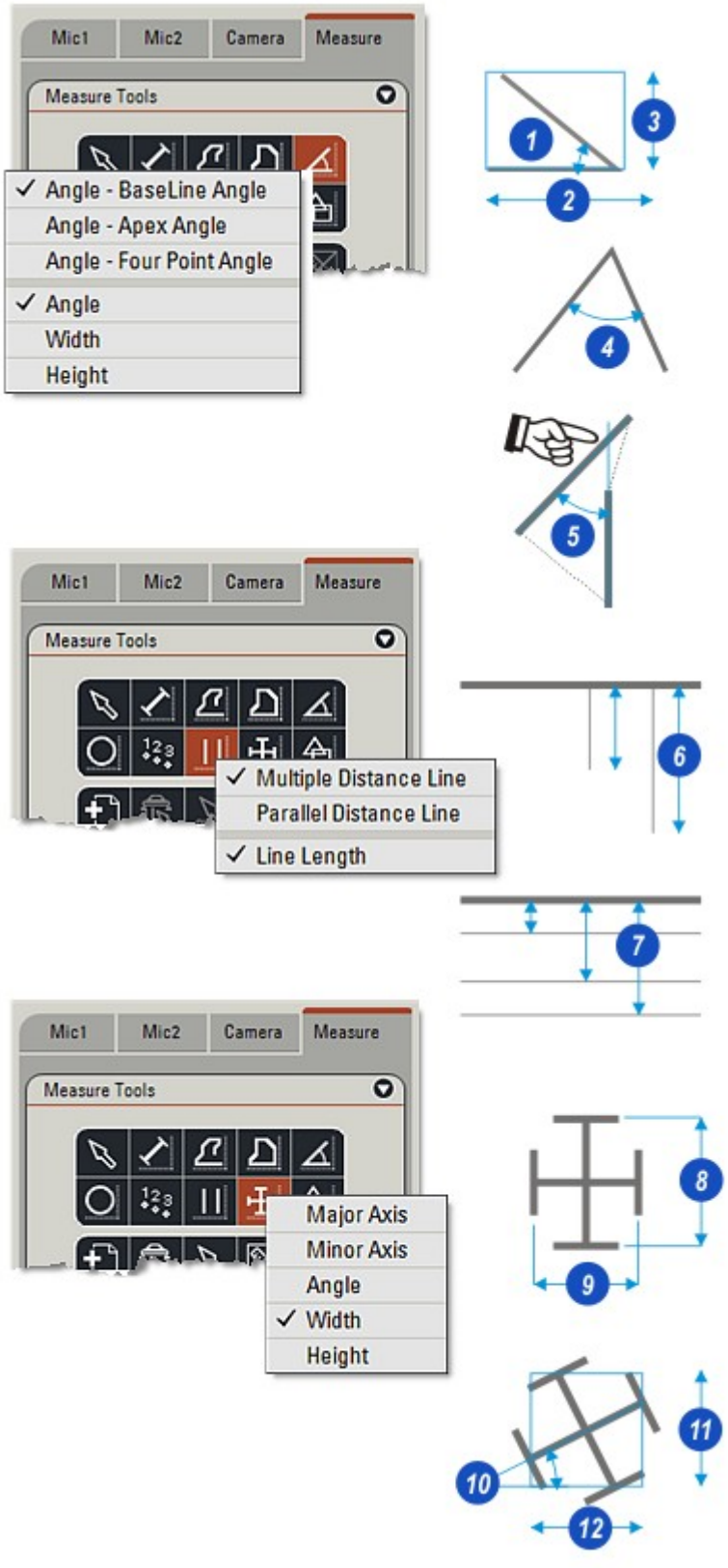
8: *Major Axis:* The length of the longest arm.

9: *Minor Axis:* The length of the shortest arm.

10: *Angle*: The angle at the intersection of the horizontal and the axis of the Cross.

11 & 12: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the Cross at the intersection of the axis and the line ending.

Continued... 765



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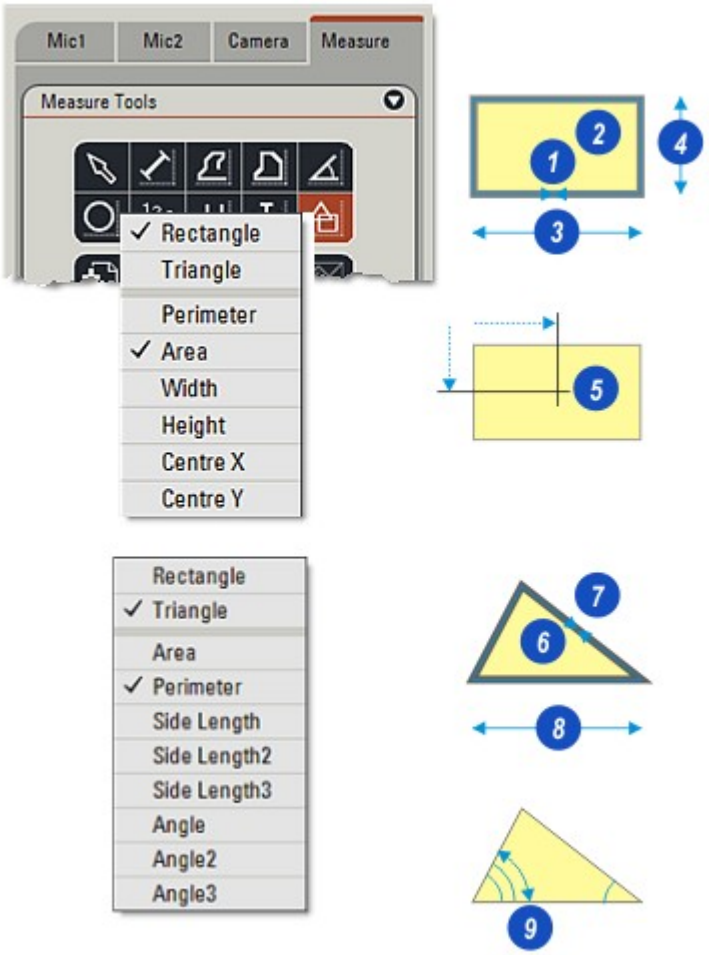
All distances are calculated from the centre of the enclosing line:



Rectangle:

1: *Perimeter*: The distance around the Rectangle.

2: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

3 & 4: *Width and Height:* Horizontal and vertical dimensions of the Rectangle.

5: *CentreX and CentreY:* The distances horizontally (X) and vertically (Y) from the edge of the image to the centre of the Rectangle.

Triangle:

6: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

7: *Perimeter*: The distance resulting in the sum of the sides.

8: *Side Length:* The length of the side commencing with the longest (Side Length) and finishing with the shortest (Side Length 3).

9: *Angle*: The angle described between two sides commencing with the smallest (Angle) and finishing with the largest (Angle 3).

Image Analysis

Leica Application Suite (LAS) *Image Analysis* is an optional software module for image processing and analysis in quantitative microscopy. LAS *Image Analysis* allows the user to:

- Measure size, shape, position, orientation, intensity parameters for individual features (eg cells, fibres, nodules, particulates, pores etc).
- Measure the area percent, total area, perimeter and other parameters that are summed for the entire Field of View (eg bone sections, reflective minerals, tissue sections etc.)
- Analyse multiple images and accumulates data for them.
- Show a list of selected parameters for all features measured.
- Calculate a range of statistics.
- Create histograms to display the distribution of sizes and shapes.
- Store and display images in a gallery.
- Export data to Excel to create user defined reports.

The combination of digital camera and microscope control makes LAS Image Analysis the optimum application for automatic measurement in a diverse range of imaging tasks including:

- Analysis of the size distribution of porosity.
- Characterisation of the shape of a population of features.
- Measurement of particulate dimensions
- Counting powders from pharmaceutical preparations
- Fibre cross-section dimension and shape

- Optional LAS Macro capability is described the LAS Macro Editor help file. *Go there..* 912

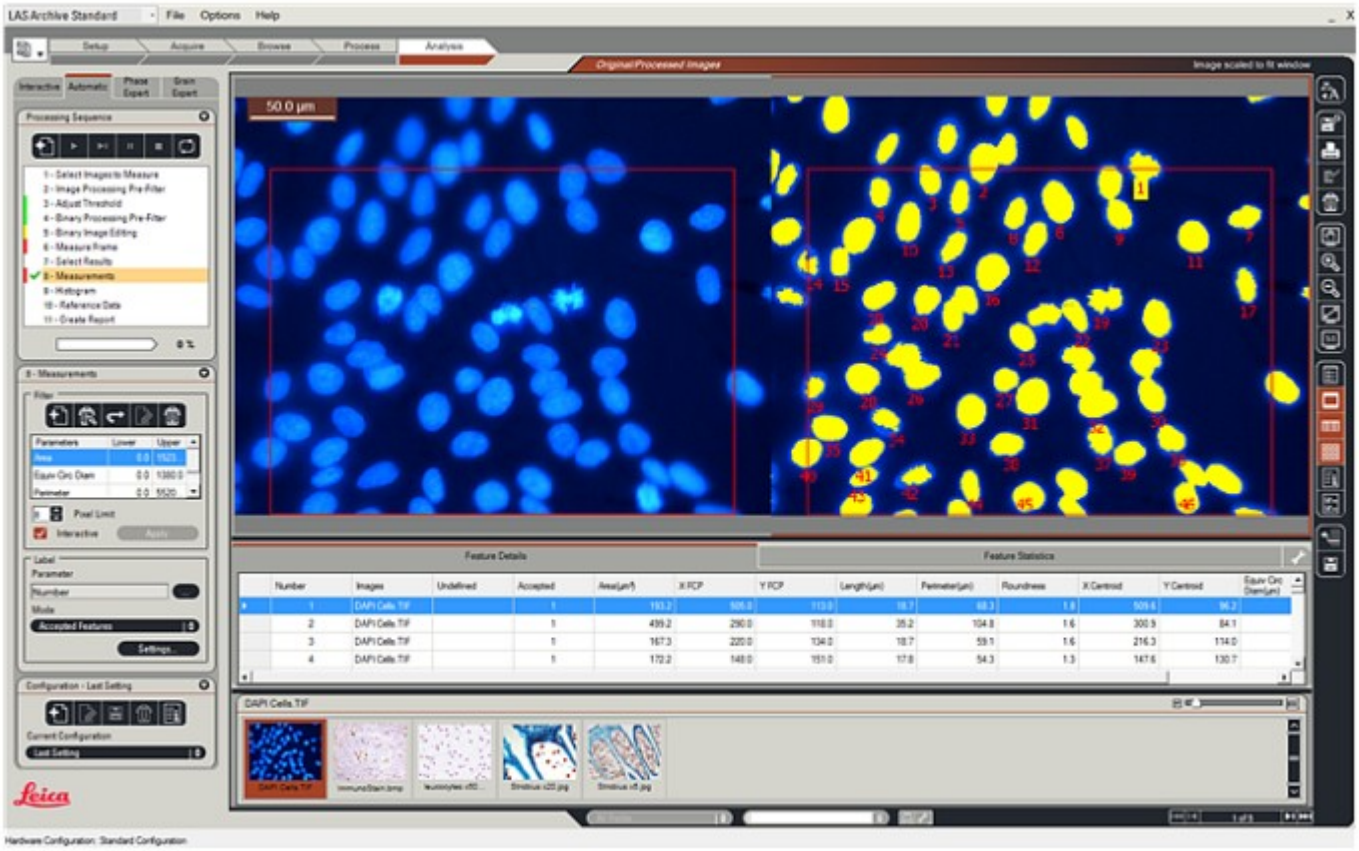


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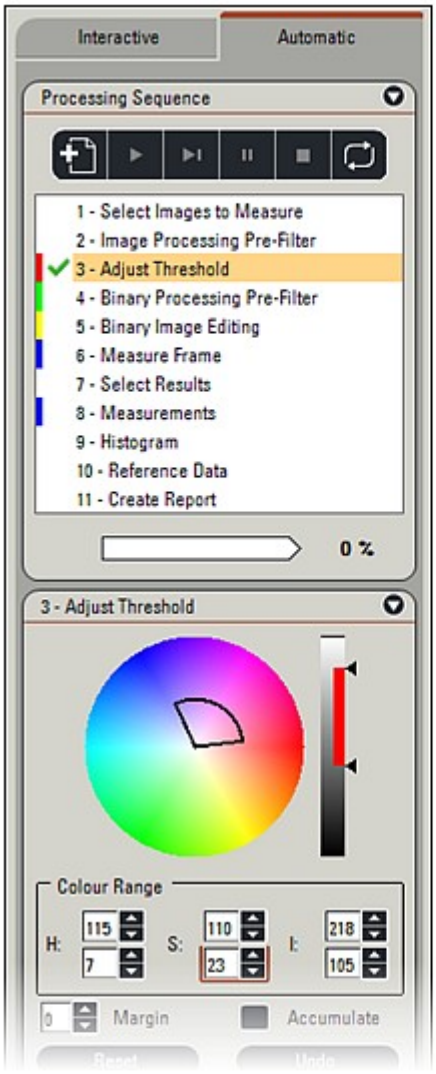
Image Analysis requires a number of steps to be performed in a defined order. This process starts with the acquisition of the initial image of the specimen, through to the detection of the features, and finally to the presentation and interpretation of results. These steps are:

- Image Acquisition.
- Feature Detection
- Measurement
- Results and Interpretation

LAS *Image Analysis* is designed to make each of these stages as simple as possible. The Image Analysis Sequence guides you through the acquisition, detection and measurement set-up steps and once established, these settings can be used repetitively.



The User Interface conveniently groups tools, controls, images, results and image thumbnails so that using LAS Image analysis is both fast and intuitive.

1: The *Analysis Workflow* that is the launch area for LAS Image Analysis optional module.

2: The *Automatic Tab* reveals all of the Image analysis controls.

3: Automatic *Processing Sequence* tool bar.

4: The Main *Processing Menu* - click to select a specific component and reveal...

5: Specific Component *Control Panels.*

6: The *Viewer* can be split to simultaneously show both original and processed images with *Scroll Bars* to aid navigation around enlarged views.

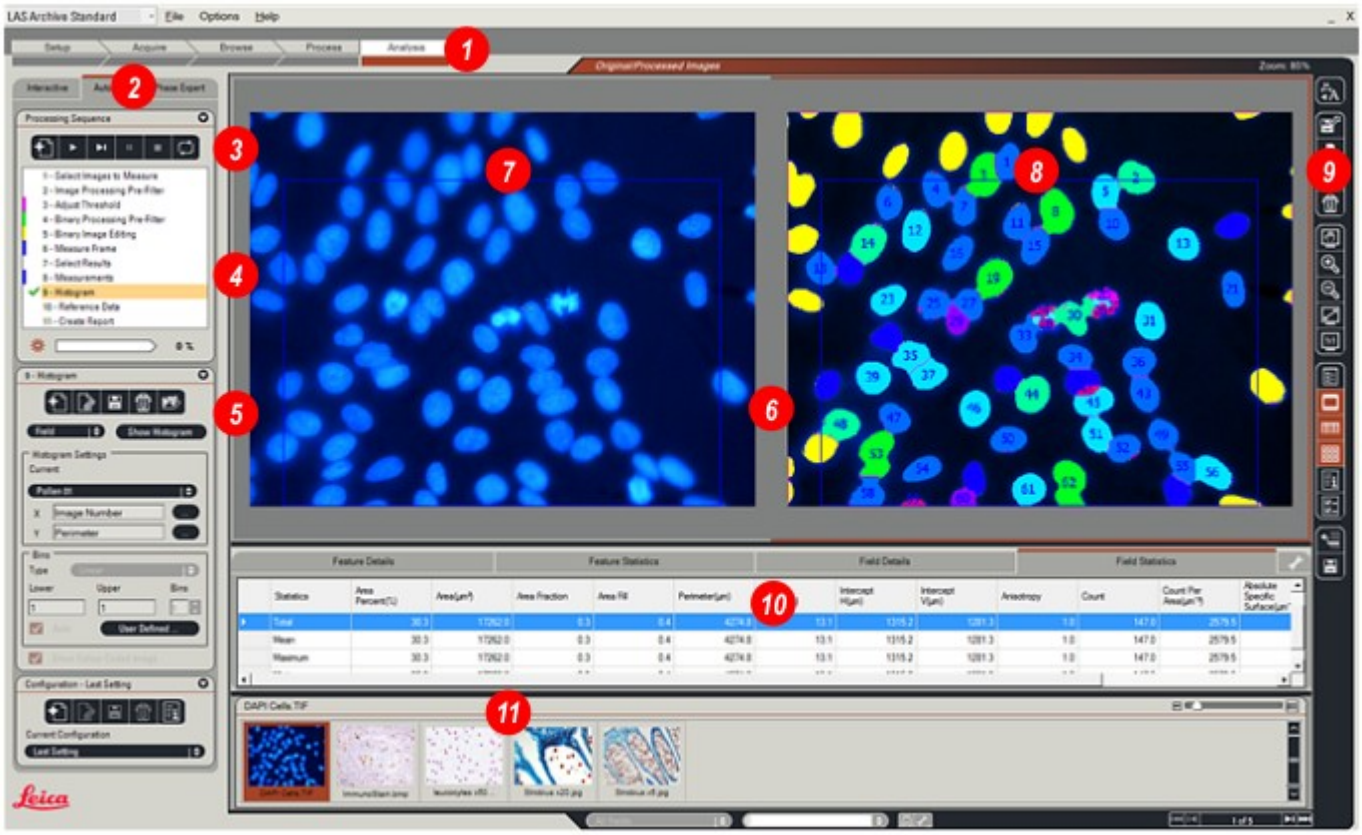
7: The split *Viewer* can be locked so that both displays respond together to navigation and setup - in this case a *Measure Frame* created on the original...

8: ... is duplicated automatically on the processed image.

9: The *Side Tool Bar.*

10: The *Grid* displays measurement results.

11: The *Gallery* displays thumbnails of the images.



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LAS Image Analysis is part of the Analysis Workflow:

1: Click on the *Analysis Workflow* and...

2: ...click on the *Automatic* tab.

3: Select one of the main components by clicking on it in the main *Processing Sequences* menu. A green tick mark appears to the left and the selection is highlighted. The colour bars against each option indicate the colour chosen (on the appropriate panel) to represent the option processing.

4: Processing progress is indicated by the rotating 'star' and the *Progress Bar.*

5: Some of the control panels are collapsed and can be expanded by...

6: ...clicking on the arrows to the right of the header.

7: Most Control Panels can be detached from the 'parking' area on the Automatic tab and moved by clicking, holding and dragging the header, to any other part of the screen.

8: Restore a detached Control Panel by clicking on the *'X'* to the right of the panel header.

9: Many panel feature drop-down menus that are revealed by clicking on the small arrows to the right of the menu header and then clicking on the list to select an item.

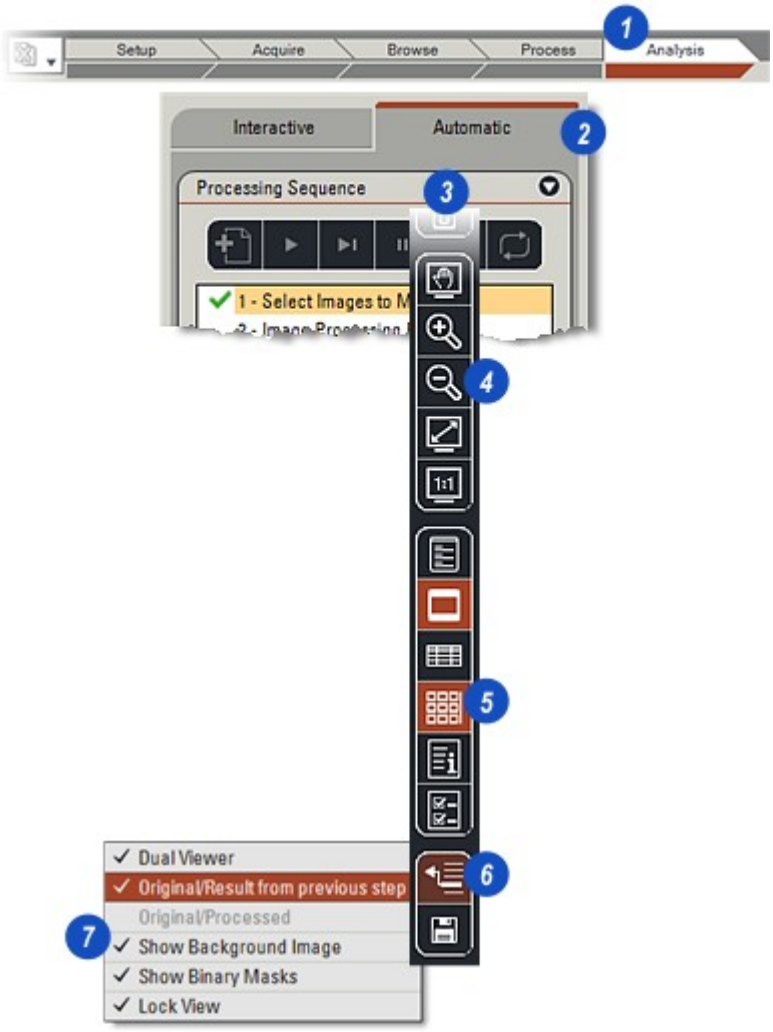
10: Some panels have *Tool Bars*; Hover the cursor above a tool button to display a description of the tool.

The Image Analysis module is located on the *Analysis Workflow.*

1: Click on the *Analysis* Workflow and…

2: …click on the *Automatic* tab.

3: The Side Toolbar is situated on the right-hand side of the *Viewer* with the buttons grouped according to function:



Viewer primary controls **(4)** pan image, zoom in and out, fit the image to the full *Viewer* area and display the image at the actual size.

Image source and data controls **(5)** hide or reveal the *Data Form*, the image, the *Data Grid* and the *Gallery.* Additionally, they allow the *Data Form* to be configured so that only information needed by the user is displayed.

Image Analysis controls **(6)** comprises 2 buttons - the upper expands to reveal a list of options **(7)** that can be turned on and off by clicking the entry.

Single/Dual Viewer splits the *Viewer* to show the original and processed image side by side; *Original/Result* displays the previous processed image; *Original/ Processed* toggles the original and processed images at full *Viewer* size; *Show Background Image* turns the background image off leaving only the processed image; *Show Binary Mask* displays processed image areas only and shows the *Binary Mask*. Additionally, the split *Viewer* display can be locked to ensure that both images zoom together and show the *Measure Frame* on both.

Continued 771...

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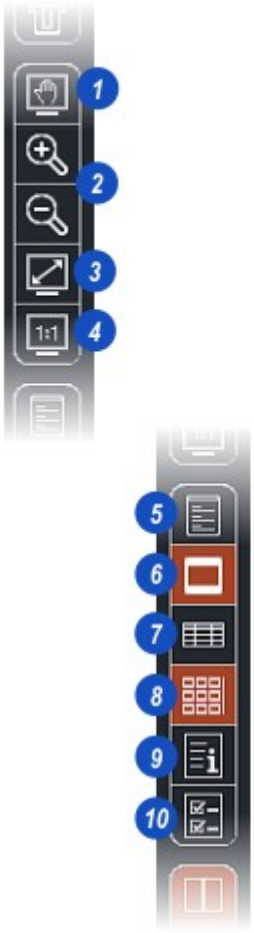
The *Side Toolbar*: Click on a link for details:



Viewer primary controls:

1: Pan the Image: Go there...

2: Zoom in and zoom out: Go there...

3: Fit the image to Viewer: Go there...

4: Display the Image at actual size: Go there...

Image source and data controls:

5: Display Data Form: Go there...

6: Hide and reveal Image: Go there...

7: Hide and Reveal the Data Grid: Only available if an LAS Archive is installed: Go there...

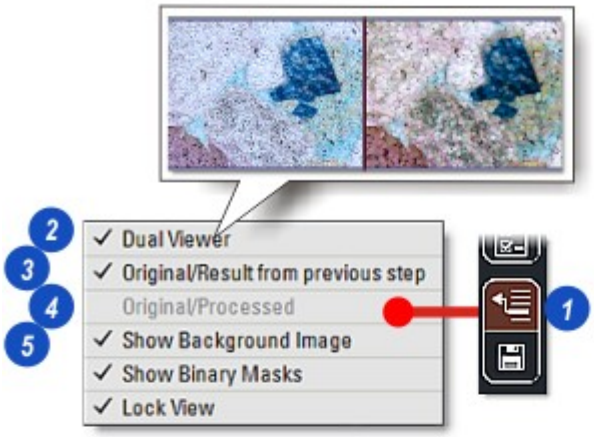
8: Hide and reveal the thumbnail Gallery: Go there...

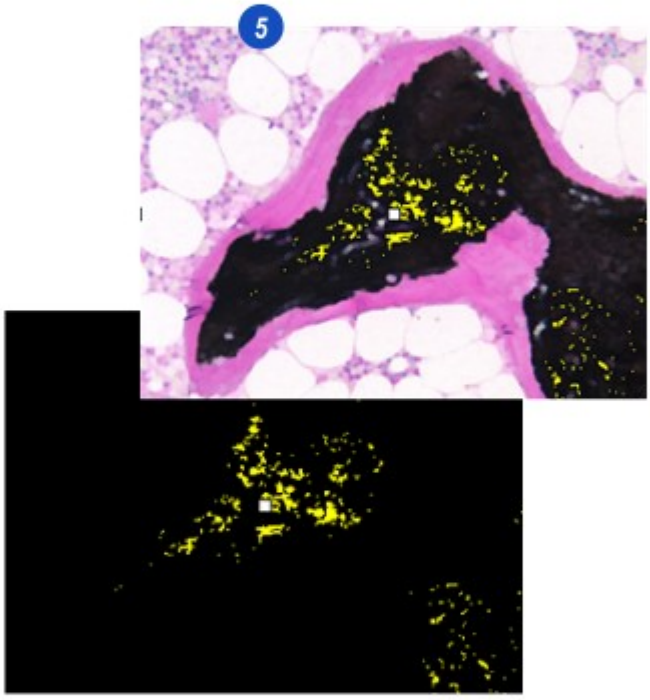
9: Display the Archive Record details: Go there...

10: Select the Record Fields to be displayed: Go there...

Continued… 772

The lower group of buttons relates specifically to Automatic Measurement operations.





1: Click on the Viewer options button. When an option is enabled a tick mark appears to the left of the selection. Click again to disable the option.

2: *Single or Split Screen* toggle. When enabled the Viewer is split into 2 panes with the original image to the left and the processed image on the right. This is a toggle action button – click again to revert to a single image in the Viewer and use the *Original/ Processed* button (Item 4 below) to select either the original image or the processed image.

3: Clicking the *Original/Result from Previous Step* button displays the processed image from the previous operation in the left-hand screen area. This is a toggle button so click again to return to the work. Use *Previous Step* as a fast means of comparing processing steps.

4: The *Original/Processed Image* button toggles between the original image and the processed image when the Viewer is set to display full screen single image (not split).

5: *Show Background Image* is a toggle that hides or reveals the processed underlying image to show only those areas that have been modified. The illustrations show an image that has been passed through a Binary filter with processed pixels coloured yellow. Turning off the background image displays only the mask pixels. Click the button again to reveal the background image.

Continued... 773

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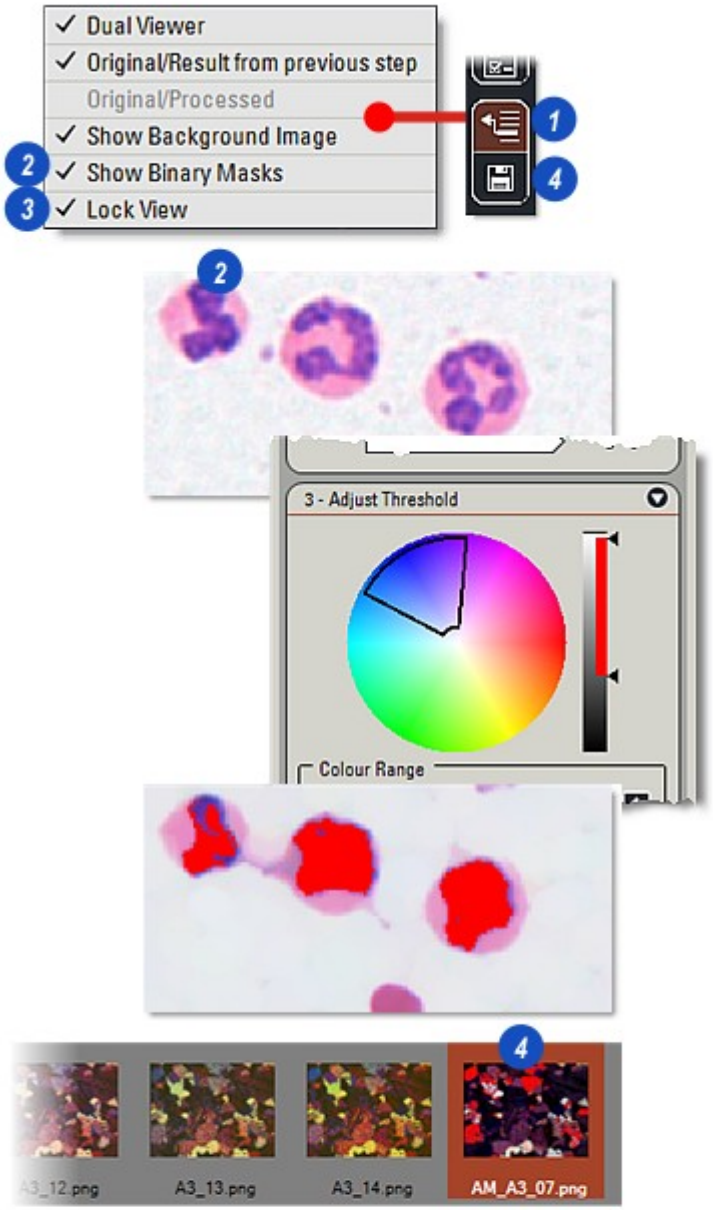
1: Click on the *Viewer Options* button to reveal the options list.

2: The illustrations show an original image – pink, more-or-less circular areas containing dark blue features – with the *Threshold Adjust* set to process mainly dark blue. Enabling the *Show Binary Mask* button shows the processed areas – those falling within the threshold values - as bright red pixels (the colour is selectable). Use the *Show Binary Mask* button to quickly show the effects of binary editing.

3: If the *Zoom* control is used to enlarge the images beyond the *Viewer* area, scroll bars are automatically displayed around them. The original and processed images can be scrolled independently or, if the *Lock Image* button is enabled, moved together from any of the scroll bars. When the *Lock* is enabled both images are automatically synchronised.

4: Saves the results image including all masks currently displayed. A thumbnail appears in the *Gallery* and the image can be used at a later date for documentation.

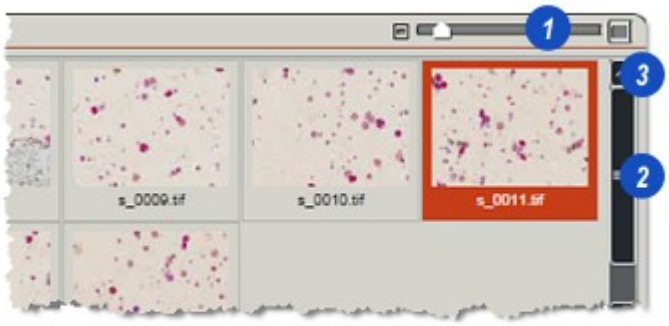
Continued 774...



The size of the thumbnails in the *Gallery* can be altered with the slider **(1)** – slide left for smaller, right for larger.

The *Gallery* depth can be changed by clicking on the horizontal bar that separates it from the *Viewer* and dragging it - up to increase the *Gallery* area and down to reduce it.

Scroll through the rows of thumbnails by clicking and dragging the scroll button **(2)** or clicking on the arrows **(3)** to the top and bottom of the scroll bar.



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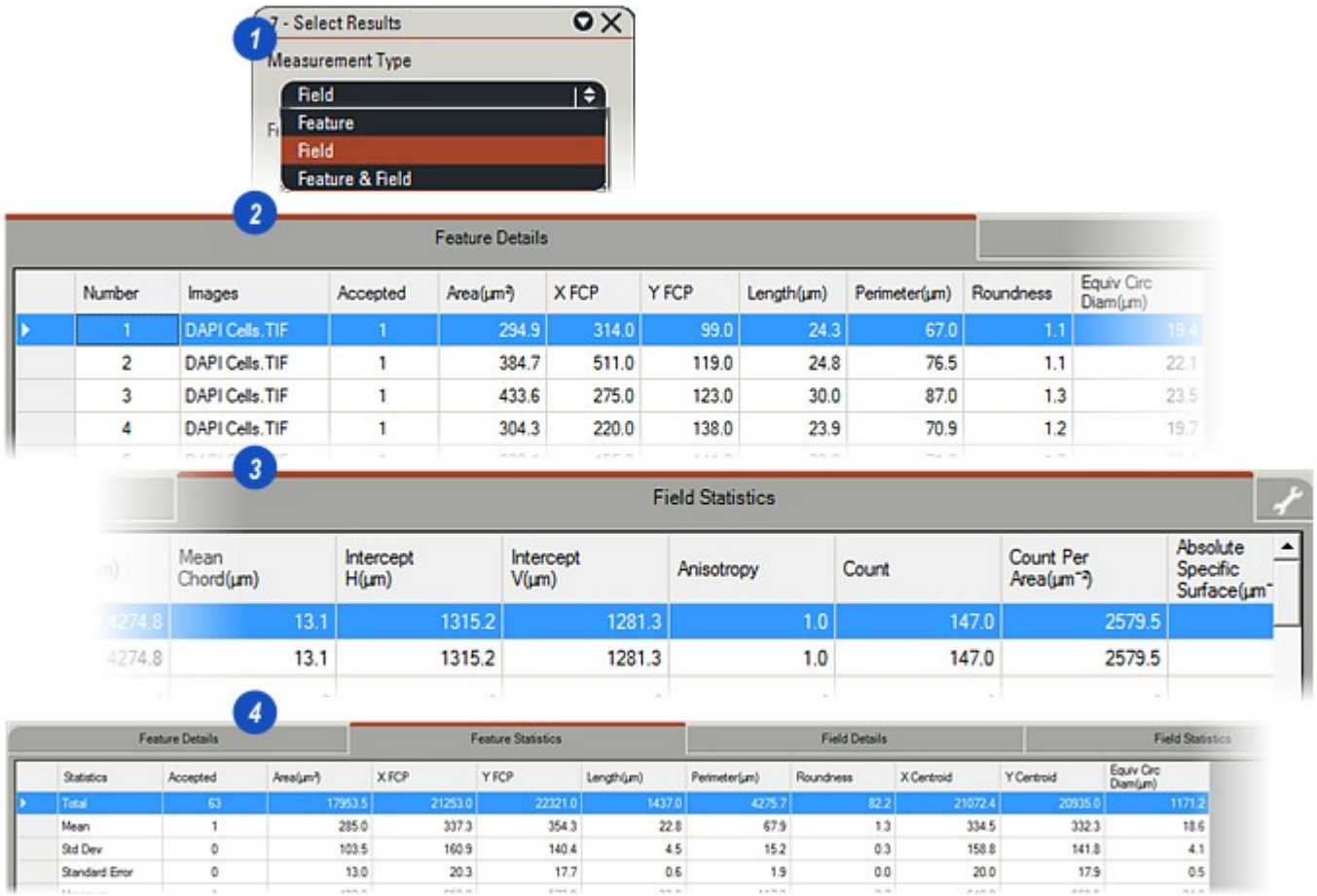
1: The *Measurement Type* determines whether only the selected *Features* are measured, or the entire *Field* or both *Features and Field*. Generally, measuring only the features is faster.

2: Depending upon the *Measurement Type* chosen, the results displayed in the *Grid* will have two tabs - *Feature Details* and *Feature Statistics* or...

3: *Field Details* and *Statistics* on two tabs or...

4: ...both *Field* and *Feature Details* and *Statistics* displayed on four tabs.

Continued... 776



1: Measurement results can be displayed on screen by clicking to enable the *Grid View.*

2: Click on an entry in the *Grid* to reveal the feature on the image the label of which will be shown in a contrasting colour. In the same way, clicking on a feature on the *Binary Output* Image will highlight the results for that feature in the *Grid*. If a sequence is being measured, the appropriate image will be automatically displayed. Hold down the keyboard *Ctrl* key whilst clicking on rows to make multiple selections. Hold down the *Shift* key to make a sequential selection.

3: The *Grid* headings are determined by the range of results chosen in *Select Results* – either a *Predefined range* or *All Measurements.*

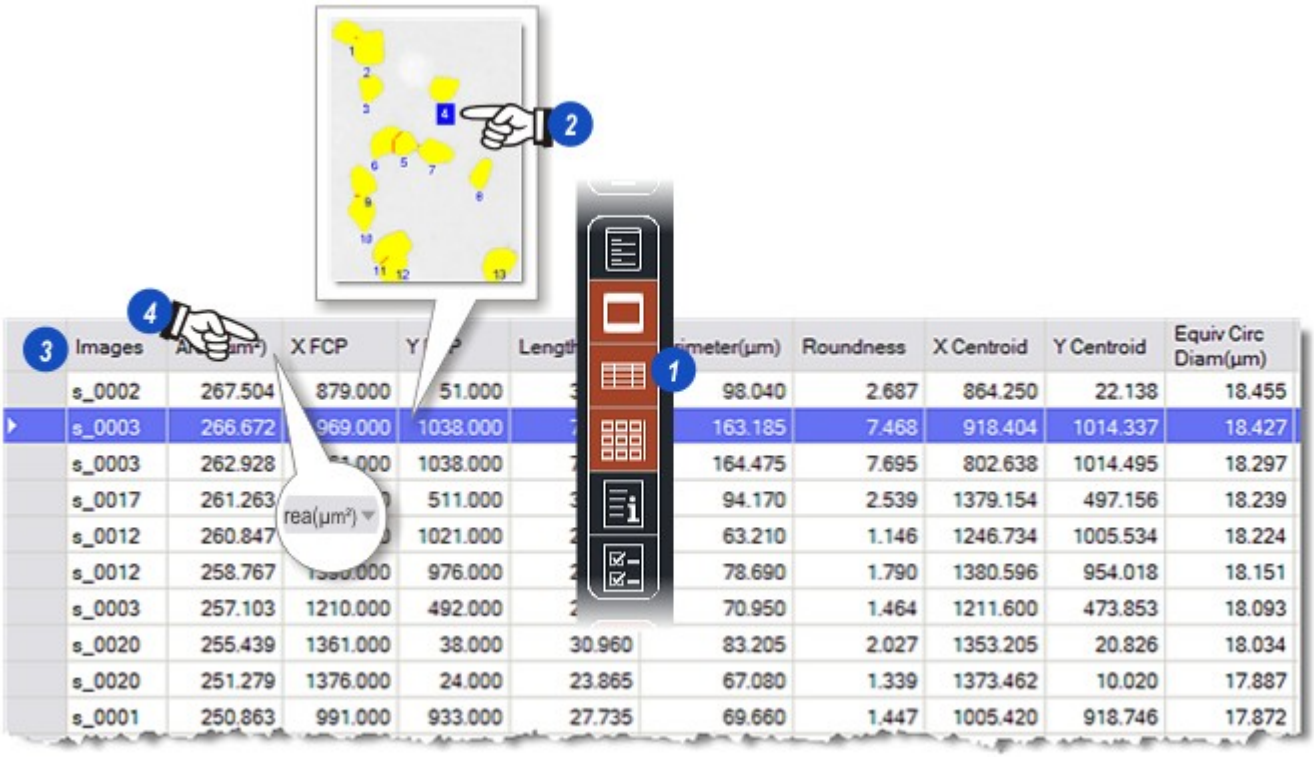
4: These can be further filtered by selecting those which are to be displayed on the *Grid* by clicking on the *Configure* tool that...

5: ...displays the *Select Feature Details* dialog. Click to enable a check box to the right of the parameter to be displayed. Click *OK.*

6: Most headings when clicked will display a small arrow to the right. Clicking on the arrow will sort the results low-to-high or high-to-low.

Windows control keys can also be used to copy data from the *Grid*.
Ctrl + C: Copy selected items to the clipboard.
Ctrl + A: Select all the grid data. Use *Ctrl C to* copy it.

Select Results to display on the Grid: *Go there…* 856



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This section describes three approaches to using *LAS Image Analysis* each depending upon the type of image being processed and the features that need to be measured.

Image Analysis works on images that have already been captured in *Browse* or *Acquire* or that have been imported - Image Analysis itself does not have the ability to capture original images. They can be individual images or part of a sequence captured with another module such as *MultiStep*.

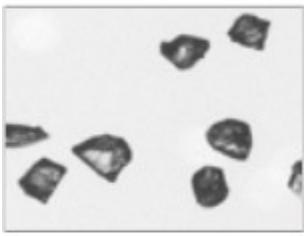
The detail to be processed must be different in either contrast or colour from the background and should be evenly illuminated. Shading correction should have been used.

- *Fast Track Automatic:* For clear, evenly illuminated images with defined features. No image processing needed - just detect features, measure and display results.
- *Fast Track with User Interaction:* Images need some work to define and isolate the features of interest. Minimal image processing but some artefacts need to be removed.
- *Advanced Measurements:* Images are highly detailed and less well defined, perhaps due to a lack of contrast or a focus problem. A range of the processing tools need to be used to pin point the features to produce accurate results.

Continued... 778

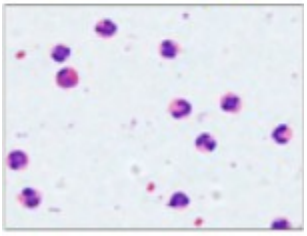
Use **Fast Track Image Analysis** for images with:
- Well defined features,
- Evenly illuminated with good contrast.
- 'Clean' backgrounds free from artefacts.

Turn off all Image Processing: *Go there...* 798
Select *No Filter* options to turn off *Image Processing, Binary Image Processing and Binary Image Editing*



Select Images to Measure: *Go there...* 782
- Images should have good contrast.
- Be properly focussed
- Have features that are separate and well defined.

Adjust Threshold: *Go there...* 814
- Select the features to be measured.
- Separates features from background
- Output is a Binary Image ready for measurement.



Measure Frame: *Go there...* 851
- Use *Typical* to define the area enclosing the features to measure.

Select Results: *Go there...* 856
- Choose either *Pre-determined* or *All Parameters*.
- Select the *Measurement Type*.

Measurements: *Go there...* 860
- Set *Parameter Filters*.
- Choose *Name/Unit* display options.
- Select *Font* and *Style* and make measurements.

Histogram and Results: *Go there...* 870
- Show results as *Histogram*.
- View details in the *Grid*.

Display: Add Reference Data: *Go there...* 776
- Add user-defined project and test information.

Create Report: *Go there...* 885
- Create, save and display Report.
- Analyse results.

Continue with Fast Track User Interaction: 779

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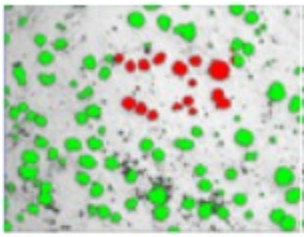
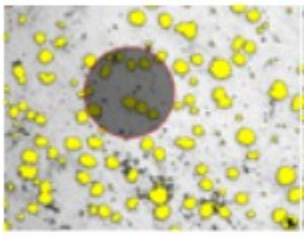
Fast Track with User Interaction is ideal for images that have:
- Well defined features that may be touching or overlapping.

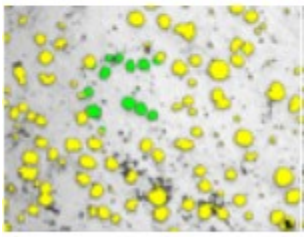


Turn off Image Processing and Binary Image Processing:
Select the *No Filter* options: *Go there...* 798

Select Images to Measure: *Go there...* 782
- Images should have reasonable contrast.
- Properly focussed and evenly illuminated.
- Features can be touching and overlapping but defined.



Adjust Threshold: *Go there...* 814
- Selects the features to be measured.
- Separates features from background.
- Output is a Binary Image ready for measurement.



Binary Image Editing: *Go there...* 838
- *Remove and Create features.*
- *Draw shapes*
- *Select features to be included or ignored*

Measure Frame: *Go there...* 851
- *Use Typical to define the area enclosing the features to measure.*

Select Results: *Go there...* 856
- Choose either *Pre-determined* or *All Parameters*.
- Select the *Measurement Type*.

Measurements: *Go there...* 860
- Set *Parameter Filters*.
- Choose *Name/Unit* display options.
- Select *Font* and *Style* and make measurements.

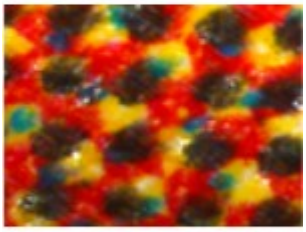
Histogram and Results: *Go there...* 870
- Show results as *Histogram*.
- View details in the *Grid.*

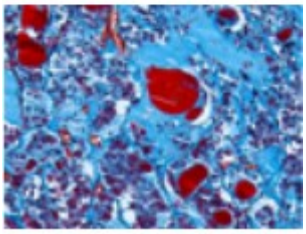
Add Reference Data: *Go there...* 776
- Add user-defined data and notes that will be included in the report.

Create Report: *Go there...* 885
- Create, save and display *Report*.
- Analyse results.

Continue with Advanced Measurements: 780

Detailed images with many features, noisy backgrounds and unwanted artefacts, require the addition of the powerful *Greyscale Image* and *Binary Image Processing* tools to separate and identify even the most obscure features.





Select Images to Measure: *Go there...* 782
- Many details overlapping and touching.
- Focussing and illumination could be better.
- Many and varied artefacts and noise.

Image Processing Pre-Filter: *Go there...* 787
- Improve definition of wanted areas and features.
- Start to close holes and fissures.

Adjust Threshold: *Go there...* 814
- Select the features to be measured.
- Separates features from background.
- Output is a Binary Image ready for measurement.

Binary Image Processing Pre-Filter: *Go there...* 825
- Adjusts the Binary Output Image to remove unwanted features.
- Close holes and separate features.

Binary Image Editing: *Go there...* 838
- Remove and create features.
- Draw shapes.
- Select features to be included or ignored.

Measure Frame: *Go there...* 851
- User Define the area enclosing the features to measure.

Select Results: *Go there...* 856
- Choose either *Pre-determined* or *All Parameters*.
- Select the *Measurement Type*.

Measurements: *Go there...* 860
- Set *Parameter Filters*.
- Choose *Name/Unit* display options.
- Select *Font* and *Style* and make measurements.

Histogram and Results: *Go there...* 870
- Show results as *Histogram*.
- View details in the *Grid*.

Add Reference Data: *Go there...* 776
- Add user-defined data that will be included in the report.

Create Report: *Go there...* 885
- Create, save and display *Report*.
- Analyse results.

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Main Steps:

This section describes the major components and tools of the LAS *Image Analysis* module. Generally, they are used in the menu sequence, but it is possible to move back and forth between components without loosing settings.

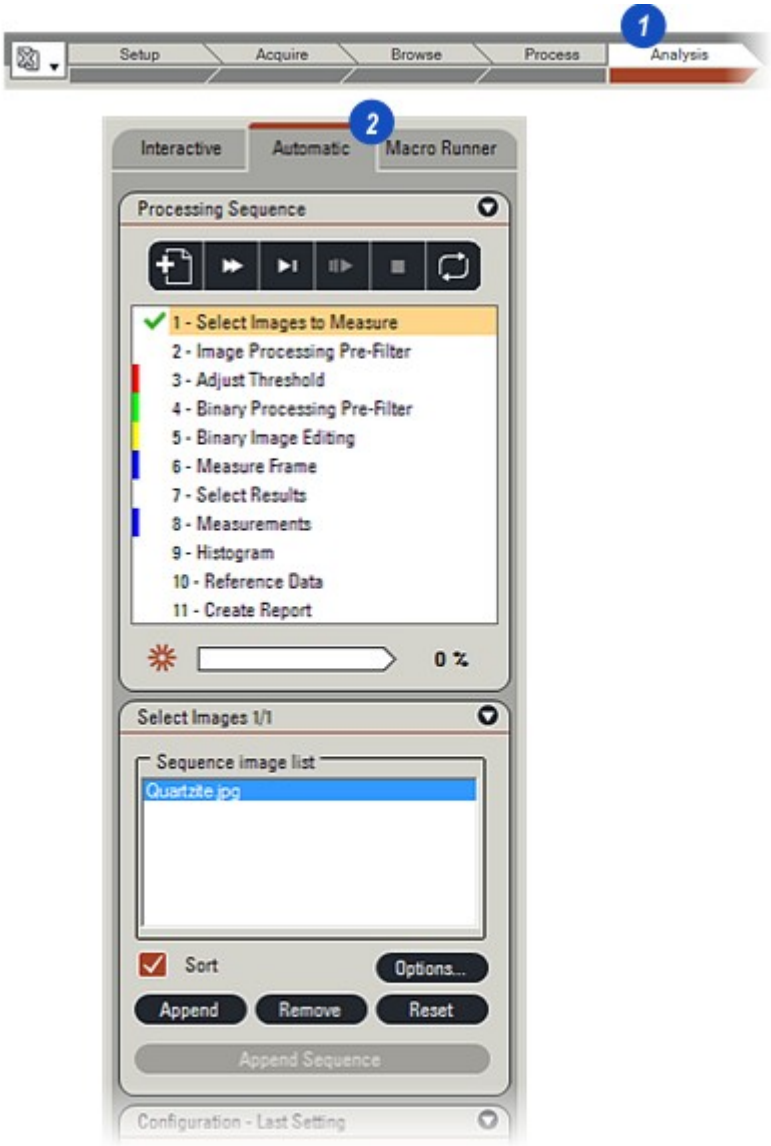
- *Select Images:* Choose the image folders, groups and sequences: Image Analysis can be used to process single images and sequences - the *Progress Star* next to the *Progress Bar* only appears when a sequence is being processed. *Go there...* 782
- *Greyscale Processing:* Improve and clarify features to measure: *Go there...* 787
- *Adjust Threshold:* Isolate features and create binary output image prior to measurement: *Go there...* 814
- *Binary Processing:* Fill holes and fissures in features: *Go there...* 825
- *Measure Frame:* Create a frame to contain the features to be measured: *Go there...* 851
- *Select Results*: Select and configure the results to display in the *Grid* and report: *Go there...* 856
- *Measurements:* Count and measure the selected features: *Go there...* 860
- *Histogram:* Display the measurement results graphically: *Go there...* 860
- *Reference Data:* Information to be displayed on the *Report*: *Go there...* 883
- *Create Report:* Configure, display and print the *Report* data and images: *Go there...* 885

Select Images: Choose the Images to be Processed:

LAS *Image Analysis* is automated which means that if measurements are to be made on a series of similar images only ***one*** need be processed and measured. The processing steps are 'remembered' by the program and then applied *automatically* to the remaining images - often without any further work by the user.

1: Select LAS Image Analysis by clicking on the *Analysis* Workflow...

2: ...and if necessary clicking on the *Automatic* tab.



The folder and images to be used in Image Analysis must be selected in *Browse* - either with *Image Explorer* or *LAS Archives* (if installed).

The image selected in *Browse* is displayed in the *Image Analysis Viewer* and thumbnails in the *Gallery*.

To change the selected folder: 783 *Go there...* 783

Continued… 784

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If the required images reside in an LAS Archive it must be selected and made active. This is done on the *Setup Workflow* as follows:

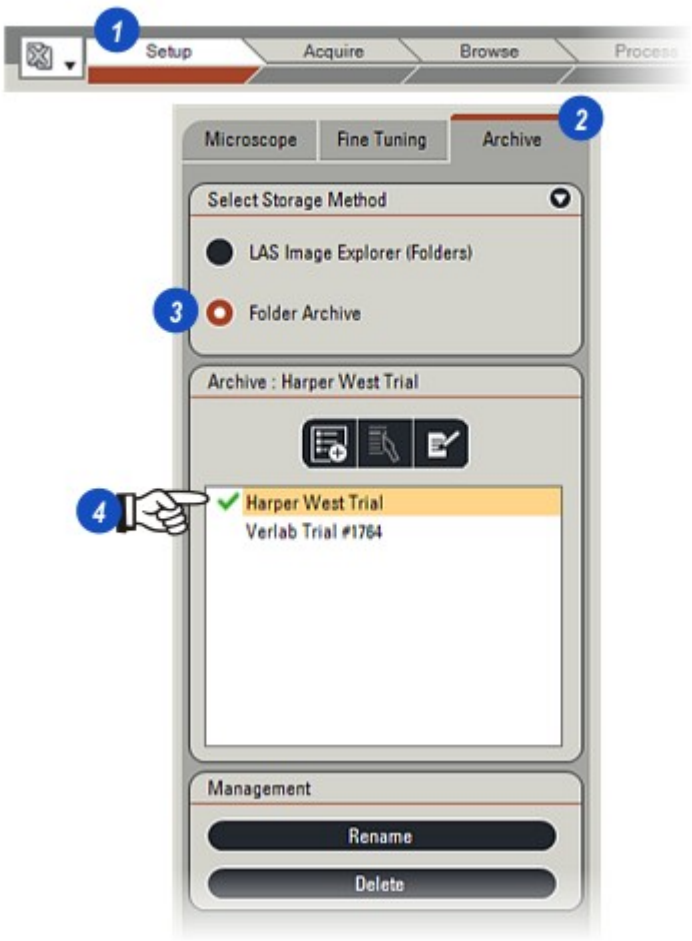
1: Click on the *Setup* Workflow tab.

2: Click on the *Archive* tab to reveal the control panels.

3: To select *LAS Archives,* click on the *Folder Archive* button.

4: Double click the required archive entry to make it active.

Continued… 784



If an image sequence or collection of individually selected images is going to be processed automatically, they have to be added to the *Sequence List.*

1: Click on the arrow to the right of the *Sequence* header to reveal the *Sequence List* panel.

Loading Sequences:

2: If the images to be processed form part of a sequence, click on a thumbnail and…

3: …click the *Append* button. The program will recognise that the image is part of a sequence and…

4: …the *Append Sequence* button will become active. Click it and...

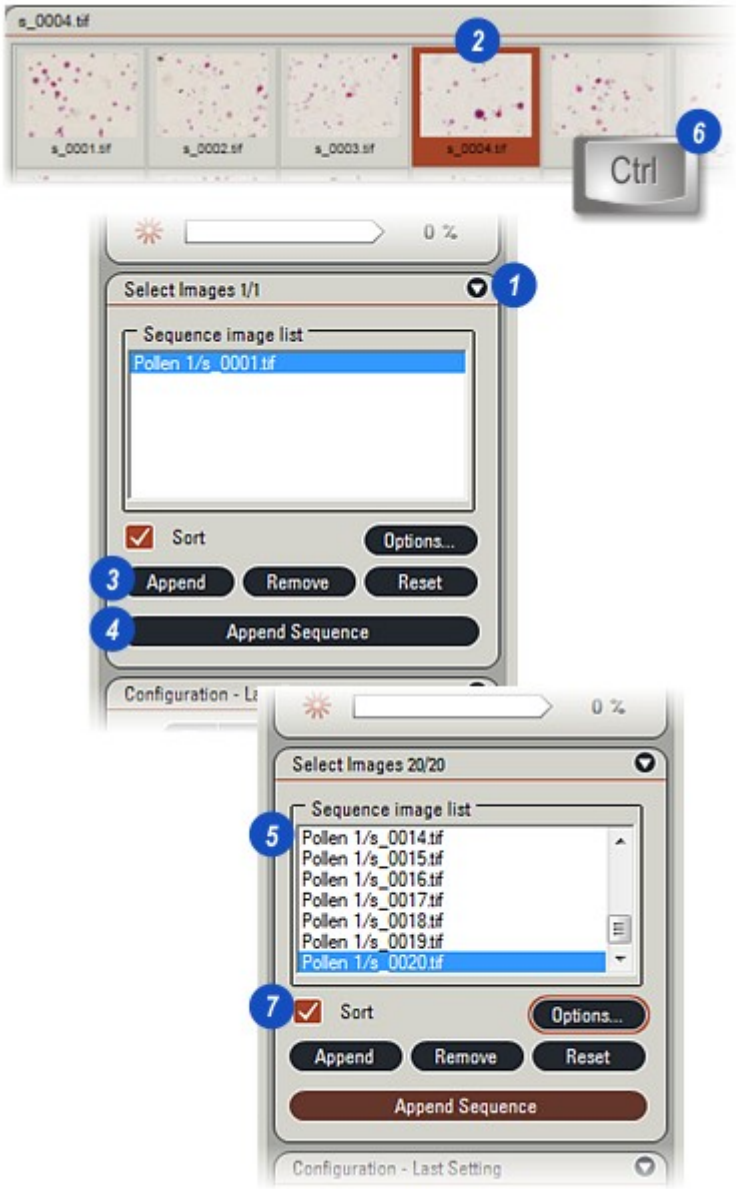
5: ...all of the sequence images will be loaded to the *Sequence List* automatically.

Loading Random Selections:

2: To selected images at random for processing, click on the first thumbnail and with the *CTRL* key **(6)** on the keyboard held down, click on the thumbnails of the other images to be included.

3: Click the *Append* button and all of the image names will appear in the list.

7: To sort the images in numerical order, click to enable the *Sort* check box.



Creating a Process Sequence: Go there… 896

Continued… 785

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Remove an Image from the Sequence List:

1: Individual images can be removed from the measurement list (but not the Archive) by clicking to select the image and then…

2: …clicking the *Remove* button.

Remove all Images from the List:

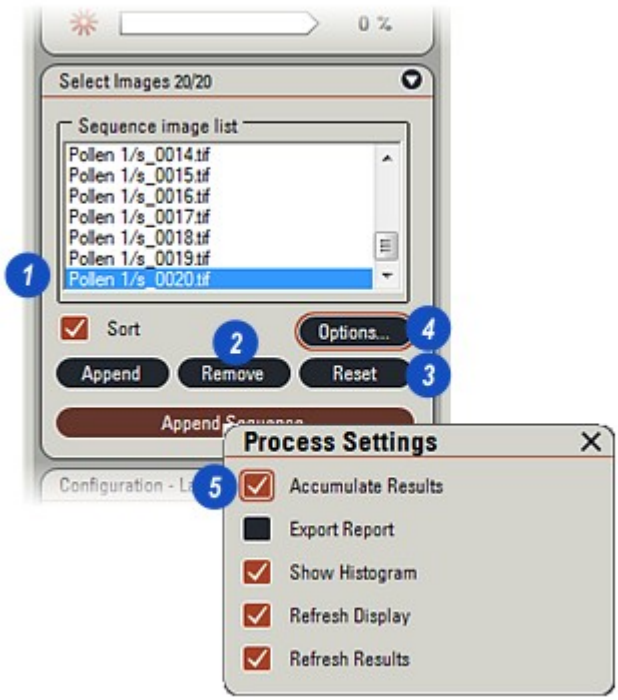
3: Remove all of the images from the measurement list by clicking the *Reset* button.

Accumulate Results:

Enabling *Accumulate Results* will produce a continuous list, the results for each image following the previous. This option allows comparisons across the entire image collection.

In *Select Results* *(Go there...)* 856 enable display *Image Name* to easily locate the results for individual images.

Disable *Accumulate Results* to have the results associated only with individual images - use this option if there are a large number of images in the sequence to speed up processing. Clicking on a *Gallery* thumbnail will display the image and its results in the *Grid*.

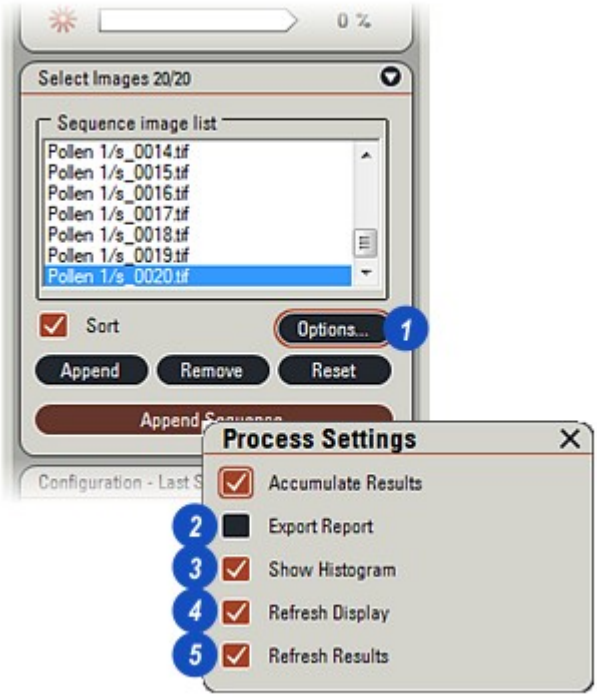


4: To enable *Accumulate Results*, click on the *Options* button and...

5: ...from the *Process Settings* menu, click to enable the *Accumulate Results* check box.

Continued... 786

1: Click on the *Options* button. From the *Process Settings* menu...



Export Report:

2: ...click to enable the *Export Report* check box and the results of the *Processing Sequence* will be loaded automatically to the chosen *Report* option (Excel or CSV) when processing is complete.

Show Histogram:

3: During the *Processing Sequence* the *Histogram* for each image can be displayed if the *Show Histogram* check box is enabled.

Refresh Display:

4: Enable the *Refresh Display* check box to refresh the display between each image analysis.

Refresh Results:

5: The *Grid* results display will be updated for every image if the *Refresh Results* check box is enabled.

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The Greyscale Processing component of LAS Image Analysis provides selected tools to enhance feature recognition in both colour and monochrome images.

Skip Greyscale principles: 797

Greyscale Processing Principles:
At the heart of most image processing is a concept called a *Structuring Element*, shown in its simplest form in Illustration **(A).** It comprises a matrix of cells, each of which will be a binary value – either 1 or 0. The centre (red) cell is called the *Origin*.

In Illustration **(B):**

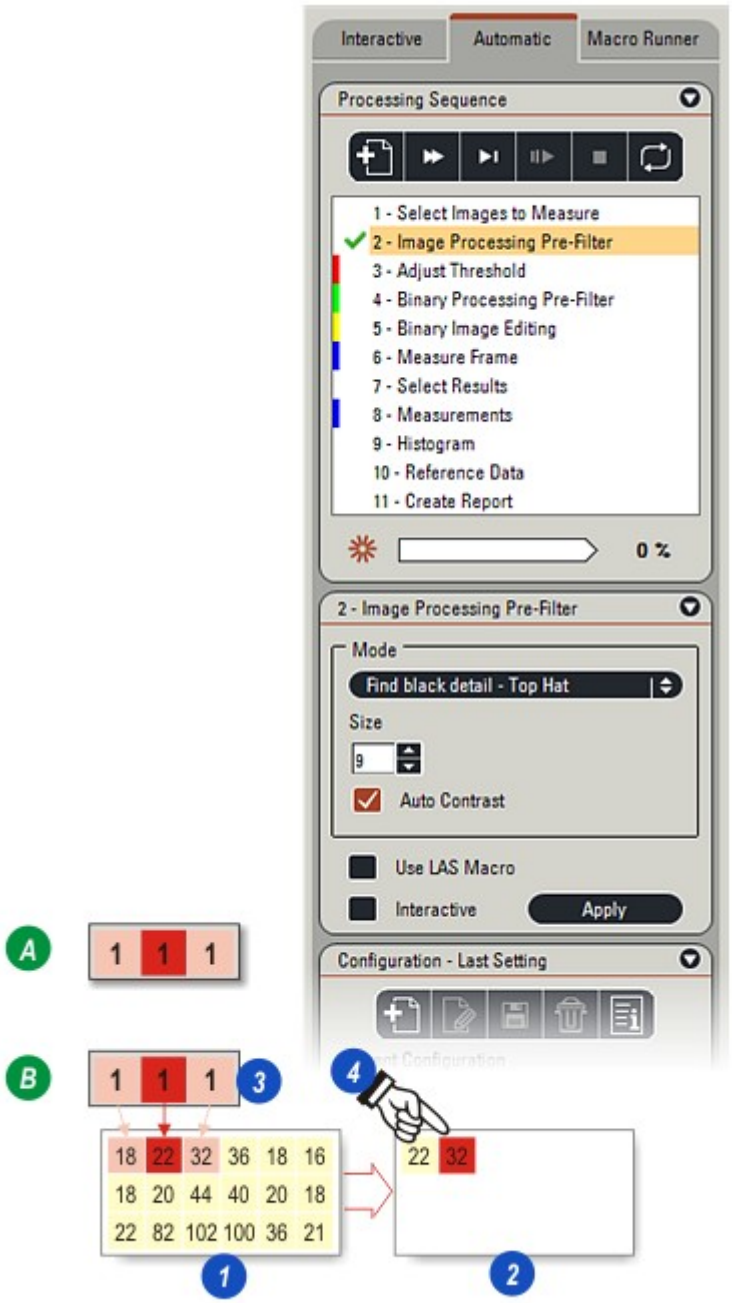
1: The original *Greyscale Input Image* with each pixel represented as a value in the range 0 to 255 - black to white.

2: **T**he *Output Image* which will be created using values determined by the Input Image and the *Structuring Element.*

3: The Structuring Element is used as an 'overlay' with the *Origin (red) Cell* positioned over a pixel on the Input Image. This is also coloured red and called the *Input Pixel.*
Those pixels either side of the *Input Pixel* are called *Neighbours*, shaded pink. The value of the *Neighbour* pixels are used to create...

4: ...a new *Output Pixel* in the Output Image.

Continued... 788



A *Neighbour* pixel is only tested if the corresponding cell in the *Structuring Element* is set (=1). In the example both cells in the *Structuring Element* are set so the *Neighbour* pixels either side of the *Input Pixel* will be evaluated.

Brightening detail: Dilation:
In this process, the *Neighbour* pixel with the *greatest* value determines the value of the *Output* pixel. Since, in a greyscale image the lighter pixels have the greatest values (closer to white at 255), the effect is to increase the area of lighter detail and reduce that of darker detail.

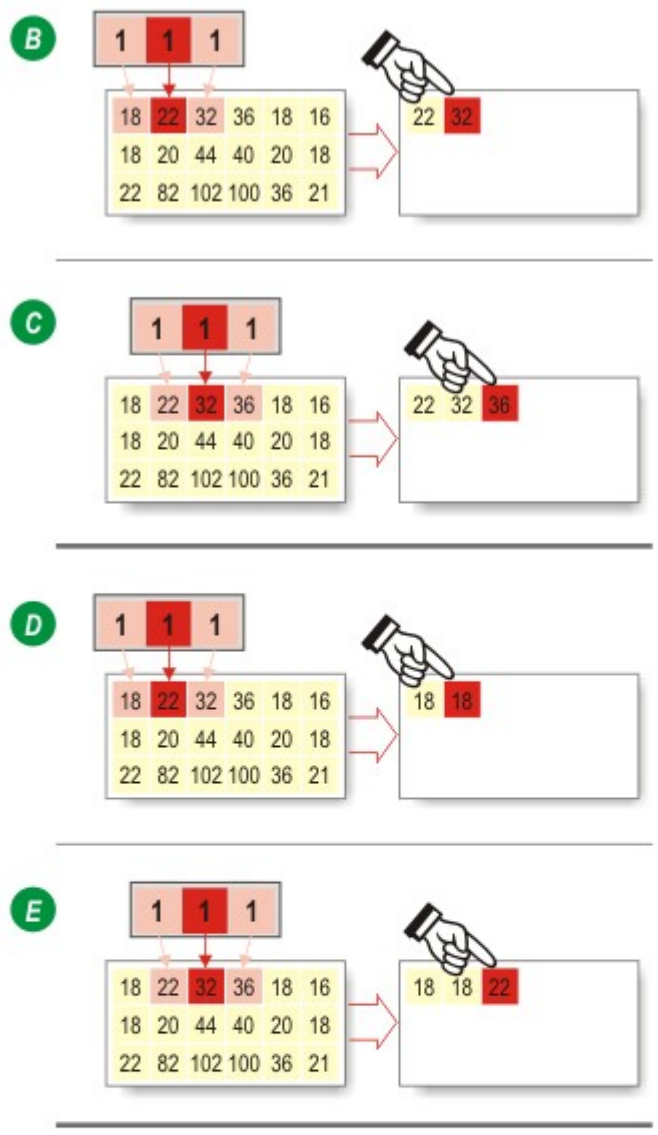
In the Illustration **(B)** the *Structuring Element Origin* (red) is positioned above an *Input Pixel* with the value 22. The *Neighbour* pixels values are 18 and 32. The intention is to brighten the image and so the *Neighbour* pixel with the *greatest* value is used for the new *Output Pixel* – in this case 32.

In Illustration **(C)** the *Structuring Element* has moved right to the next *Input Pixel*. Now the values in the *Neighbour* pixels are 22 and 36 and again the *greatest* is used for the *Output Pixel*.

Darkening detail: Erosion:
To darken the image the same evaluation process is used but in this process the *Neighbour* pixel with the *lowest* value (closer to black at 0) is used as the *Output Pixel*. Illustrations **(D)** and **(E)** show the process.

All of the greyscale *Input Image* pixels are tested until the *Output Image* is complete.

Continued... 789



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A simple example:
Illustration **(A)** is a greyscale image – a small block of pale grey pixels each with a value of 120, surrounded by dark grey pixels with a value of 30.

A 2 x 3 *Structuring Element* **(1)** is to be applied to the image, first as a *Dilation* and then as an *Erosion*.

Dilation uses the *Neighbour* pixels with the *greatest* values so in Illustration **(B)** the pale grey block has 'grown' adding pixels to the top and sides.

The process is reversed by *Erosion* in Illustration **(C).** *Erosion* uses the *smallest* values found in the *Neighbour* pixels so the pale grey pixels (with a value of 120 closer to white) have been replaced by dark grey pixels (with a value of 30 closer to black).

The image shift – the pale grey block has moved upward by a row of pixels – is due to the design of the *Structuring Element*, and can be prevented by using a more appropriate matrix.

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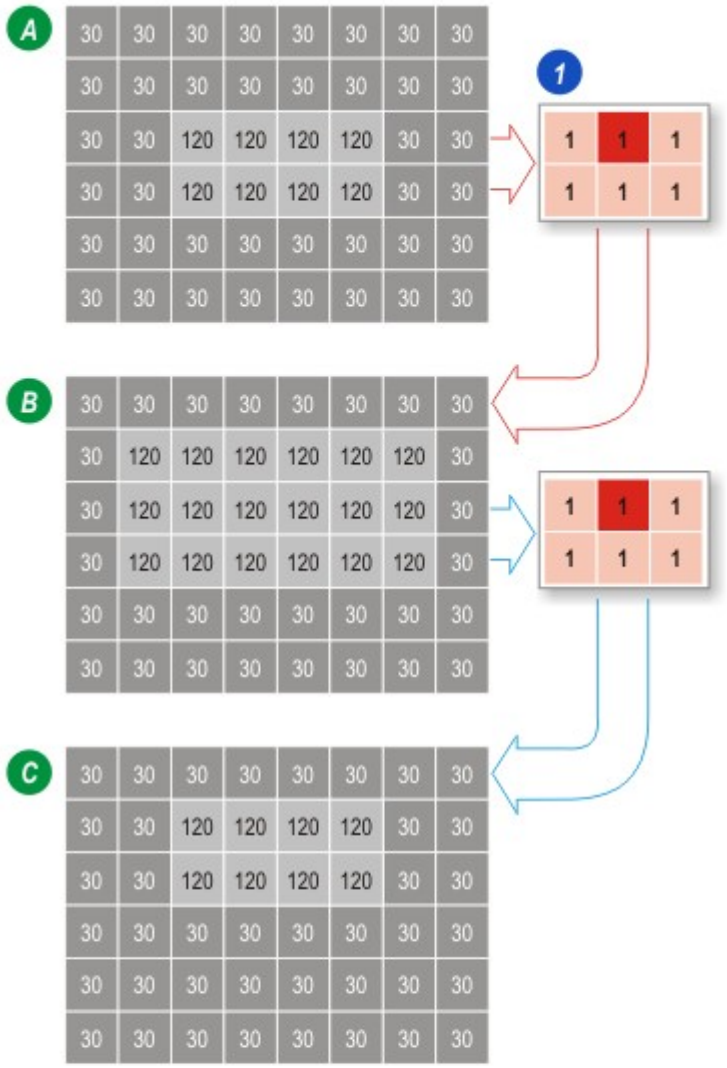
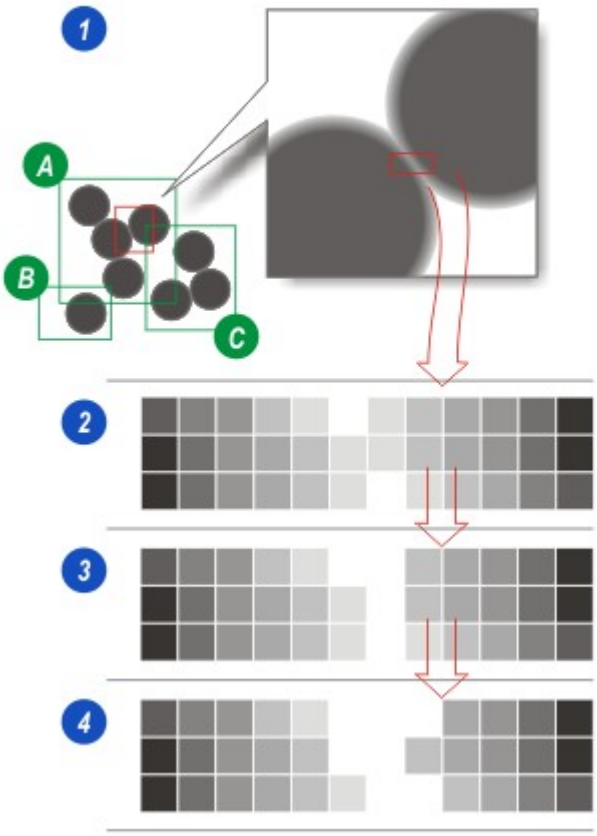


A practical example:
Greyscale image **(1)** shows a group of 8 black discs on a white background. The transition between black (0) and white (255) on an image is seldom that distinct – very often there will be several grey pixels around the boundary of a dark feature giving it a 'blurred' appearance. The blurring may be due to incorrect focussing, poor lighting or perhaps the feature itself is fuzzy.



The blurred boundary can make adjacent features appear to be touching each other and, if the intention was to count the features in an image, the discs in Figure **(1)** would be treated as 3 features – groups **(A), (B)** and **(C) -** instead of 8.

The boundaries between 2 adjacent discs may look like the pixels in Illustration **(2),** the black pixels of the discs blurring through shades of grey with white background pixels appearing at the extreme edges.

A *Dilation* (Illustration **3**) uses the greatest *Neighbour* pixel values to create an *Output Image* so at the edges of the blurring, white (value of 255) would replace pale grey pixels (value say of 210). The 'new' white background pixels can be seen in the centre.

The amount of *Dilation* depends upon the *Structuring Element* matrix. Illustration **(4)** shows the effect of applying a *Structuring Element* with a greater number of cells testing a wider group of *Neighbours*. Here several grey pixels have been replaced with white pixels to create a larger gap between adjacent discs.

Continued... 792

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1: The same greyscale image but this time 8 white discs on a black background and apparently 'grouped' because of edge blurring. The task is the same – separate the discs without losing important dimensional data. Light features against a dark background are often better separated by using *Erosion* followed by *Dilation*.

2: The original 'blurred' image.

3: *After Erosion – Neighbour* pixels with the smallest value (nearer to black at 0) replace those with *greater* value (closer to white at 255) – the grey pixels around the boundaries of the discs have been replaced with black.

4: *After Dilation – Neighbour* pixels with the greatest value (closer to white at 255) replace those with closer to black (at 0). The effect is to 'grow' the white discs and, with the appropriate *Structuring Element*, to closely re-establish the discs' diameter without causing them to touch again.

An *Erosion* followed by a *Dilation* is called an *Opening Filter.*

Continued... 793

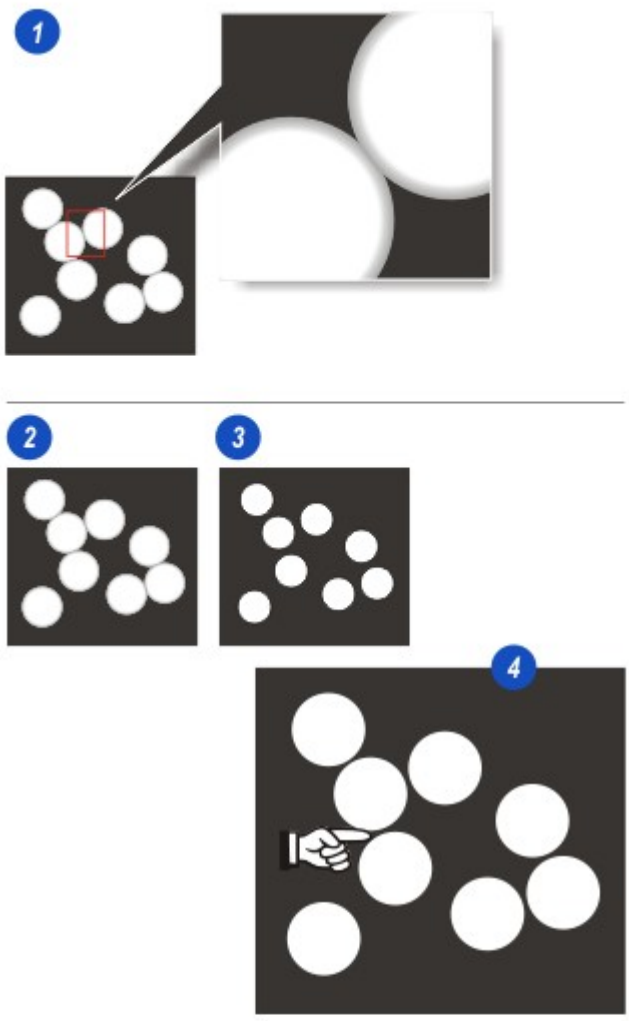
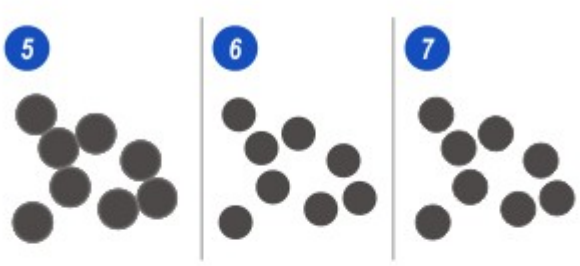


Illustration **(5)** is the original blurred image and **(6)** is the same image with successive *Dilations* until all of the grey pixels have been replaced with white – only distinct, separate black discs and white background remain – 8 features now instead of 3.



However, removing the grey pixels may have also reduced the actual size of the discs which could be detrimental if areas or dimensions are to be measured. An *Erosion* will make the black discs grow – *smallest* value *Neighbour* pixels (those nearest black at 0) replace higher value pixels (those closest to white 255). *Erosions* continue until the black discs are close but not touching to complete the transition **(7)**.

Dilation followed by *Erosion* is called a *Closing Filter.*

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Open and *Closed Filters* have a wide range of applications in image processing. Noise removal is one most frequently used.

1: A greyscale image with clumps of small features in the background which could be 'noise' or just insignificant parts of the image, unwanted artefacts that need to be removed before accurate measurements can be made.

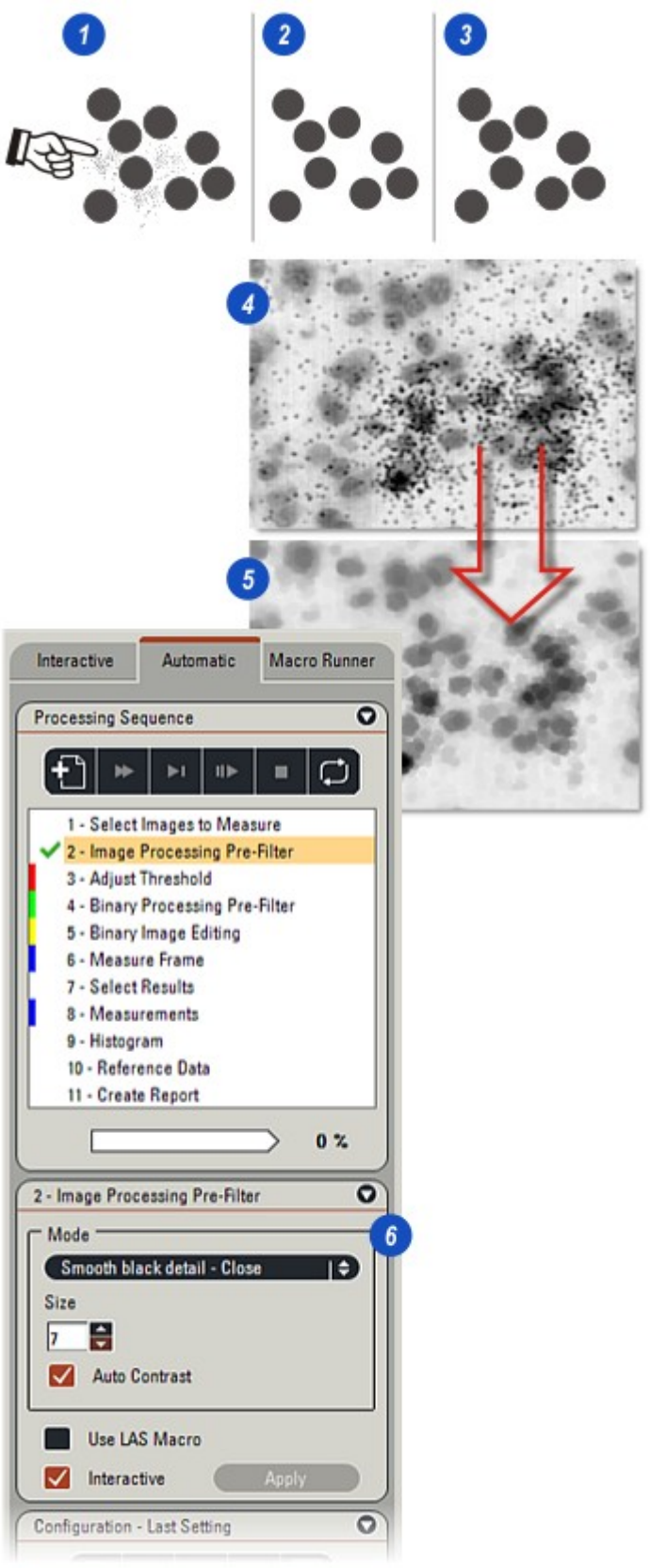
2: Successive Dilations have not only separated the black discs but also removed the 'noise' artefacts completely – simply because they are small.

3: Erosion enlarges the discs to their original size but because the 'noise' no longer exists there is nothing to enlarge.

4: Removing small artefacts in Image Analysis; The original Input Image and...

5: ...the Output Image with the artefacts removed and the grey features restored to their original size.

6: The Image Analysis panel with *Image Processing Pre-Filter* tools in *Smooth Black Detail* Mode.



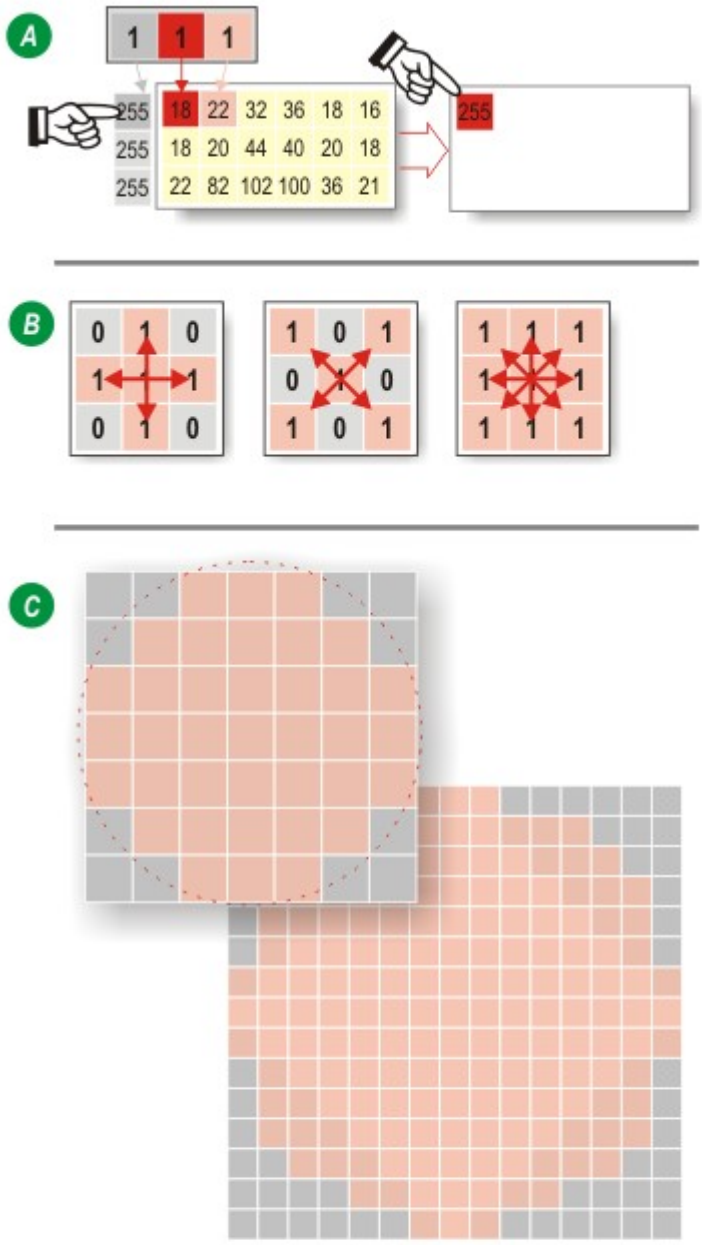
Smooth Black Detail Mode: Go there… 800

Continued... 794

On the edge of the Input Image, the first *Neighbour Cell* of the Structuring Element will point to a non-existent pixel beyond the edge of the image. For greyscale images **(**Illustration **A)** and depending upon the process – *Dilation* or *Erosion* – these pixels are assumed to have values of 0 for *Dilation* and 255 for *Erosion*. This arrangement prevents border effects – fine, dark lines appearing around the output image.

Almost any layout can be applied to a *Structuring Element*; **(**Illustration **B)** shows a common 3 x 3 cell configuration in which a set cell (=1) is an active *Neighbour* used to evaluate the Input Image pixel values, whereas a cell cleared (=0) will be ignored. The *Structuring Element* matrix – how many cells it contains – generally reflects the size and shape of the features being detected within the Input image. Image Analysis uses a circular configuration which expands or contracts depending upon the size selected by the user **(** Illustration **C).**

Continued... 795



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The *Structuring Elements* using more complex mathematical principles are used in a wide range of greyscale filters to detect and improve the quality of features of interest in an image. They are used extensively in Image Analysis and the following describes just two of them.

Median Noise Removal:
Illustration **(A)** depicts a *Structuring Element* with a 3 x 3 matrix – the Origin has 8 *Neighbour Cells*. Only those cells set (=1) are used in the filter; Those cleared (=0) are ignored. The filter extracts the *Neighbour* pixel information from the Input Image – in this case 22, 18, 44 and 82 – and calculates a Median value, that is a value at the mid-point of the range, not an average.

For an odd-numbered range the middle value is chosen, but for even-numbered ranges the two values closest to the middle are averaged. Our example is even-numbered (4 values) and so the two mid values (18 and 44) are averaged (18 +44 = 62/2 = 31) which becomes the value of the new *Output Pixel.*

More refined versions can then manipulate the values in the Input and Output Images. For example, having established the Median *Output Pixel* value, subtracting it from the *Input Pixel* value (20 – 31 = -11) and then adding back the Input value (-11 + 20 = 9), darkens the image and improves contrast.

Illustration **(B)** shows Image Analysis in action with the *Median Noise Removal* tool selected. **(1)** is the Input Image and **(2)** the Output Image.

Noise removal - Median: *Go there...* 802

Continued… 796

Some of the most useful morphological filters, *Tophats* belong to a sub-class of operations called residues, since they produce something which is 'left over' from the original image.

Tophats can be defined as:

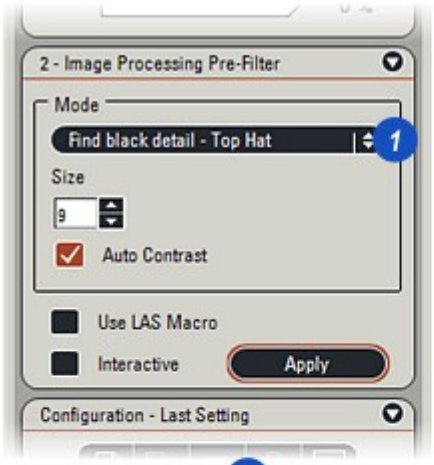
Smoothing > Difference > Tophat

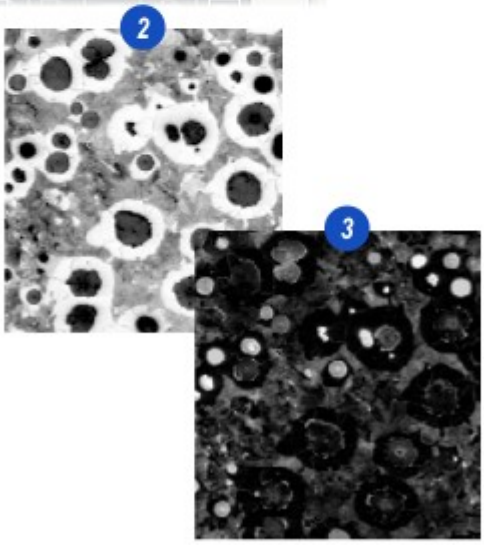
The net effect is to enhance whatever was removed during the smoothing process, which will usually be the detail of the image.

The most common *Tophats* are constructed by using grey openings and closings as the smoothing operations. The Find black detail-*Tophat* is defined as:

Tophat = Closed Image - Original Image

The effect of a black *Tophat* **(1)** is to 'pick out' the small dark features in the image. The closing will remove the dark features up to a size defined by the cycles used, replacing them with the grey level of the lighter regions which surround them, and the subtraction of the original image will thus show the grey level difference between these 'filled in' pixels and their grey levels in the original image.





2: The original image and...

3: ...after *Find black detail Tophat*.

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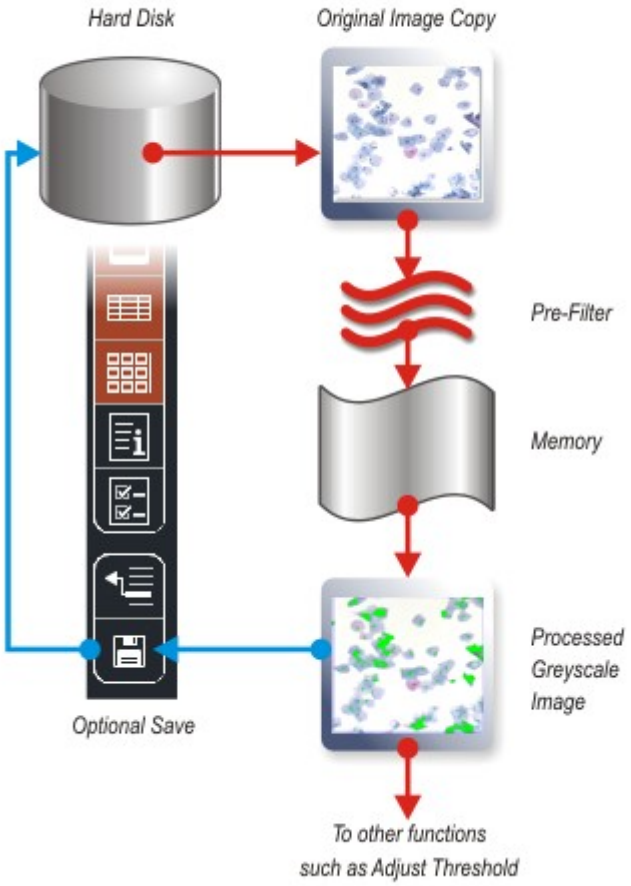
Experimentation is an inevitable part of improving image definition and this manual cannot possibly list methods for every image type or quality. Instead, the following pages illustrate examples to demonstrate some of the powerful potential in the *Image Processing* toolkit – it is up to the user to select and manipulate the best tool for the job in hand.

When *Greyscale Pre-Filters* are applied to an image the original is not affected at all – it remains intact on the hard drive. Applying a filter creates a *Processed Greyscale Output Image* in memory which is then passed on to other functions such as *Adjust Threshold*, for additional processing.
Although the processed Greyscale Output Image is created in memory it can be saved to disk and 'exported' to another application just like any other image:

Click on the *Save* button on the right side toolbar to save the processed image. A thumbnail of it also appears in the *Gallery*.

Continued… 798



To display the *Greyscale Image Processing* tool panel:

1: Click on the *Image Processing Pre-Filter* option in the main list.

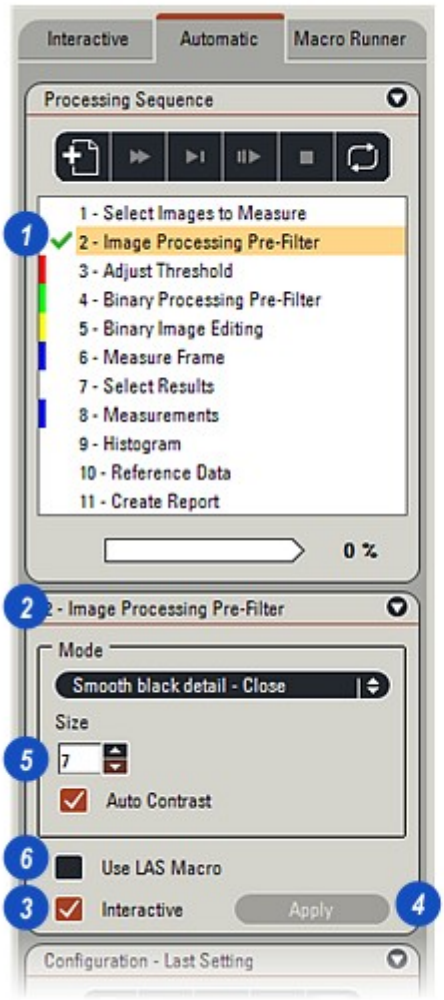
2: The *Pre-Filter* panel appears.

3: Each ‘pass’ of a structuring element can be displayed automatically by clicking the *Interactive* check box to enable it.

4: Disable Interactive and use the *Apply* button to update the Output Image when required. This option is preferable for larger, complex images for which selecting the filter and setting the *Size* and only then applying the changes is faster.

5: The structuring element matrix is determined by the *Size* text box. To change the value, click in the window and type a new value or use the *Up/ Down* arrows to the right of the box. Small increments are preferable because there will be a point at which the image cannot be improved by making another ‘pass’.

6: To use *LAS Macro Runner*, click to enable the check box. Detailed help for LAS Macro: *Go there...*



Continued… 799

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The contrast between features and background can be improved for most images by enabling Auto Contrast:

1: Click the checkbox (Tick mark visible) to enable *Auto Contrast.*

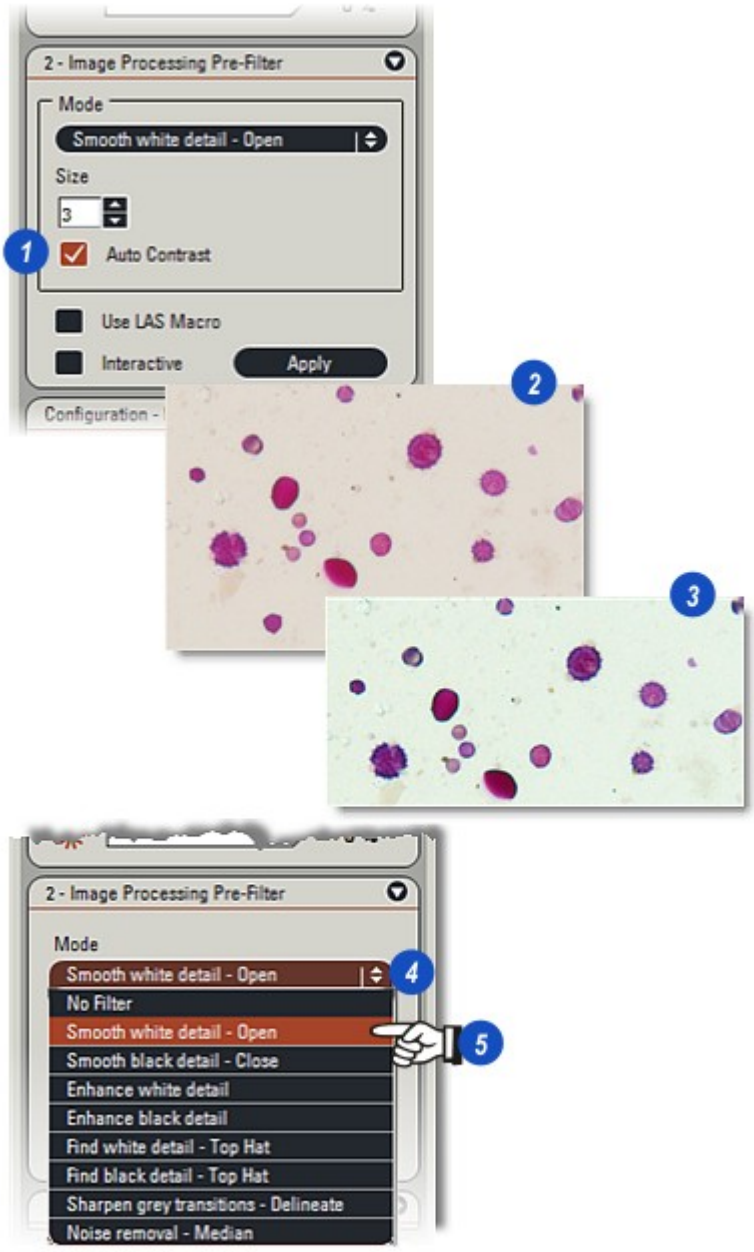
2: The original greyscale image and…

3: …the same image with *Auto Contrast* turned on. The improvement is very obvious.

4: To select a Pre-Filter, click on the arrows to the right of the *Mode* drop down window and…

5: …click to make a selection from the list.

Continued... 800



To access the filters:

1: Click on the arrows to the right of the *Mode* menu window and…

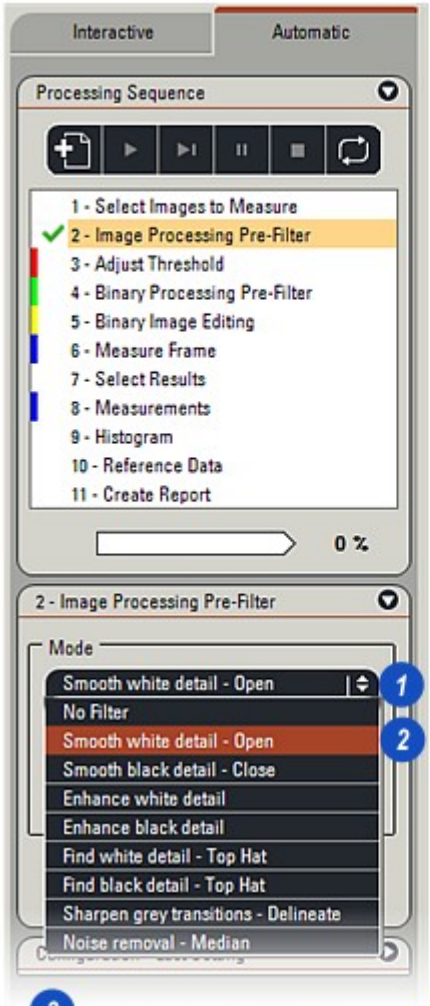
2: …click to select the filter required. The option *No Filter* disables all of the filters and can be used as a simple 'reset'.

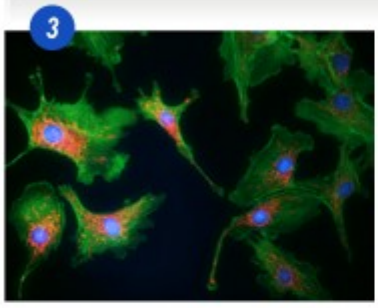
3: The original *Greyscale Input* Image.

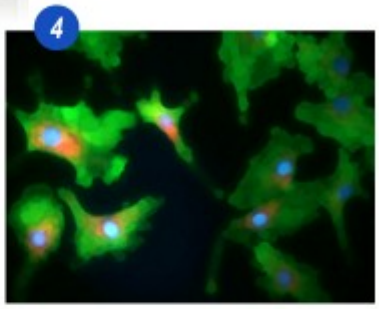
4: *Smooth White Detail* brightens the Output Image by accentuating the lighter tones. This is an open filter – *Erosion* followed by *Dilation*.

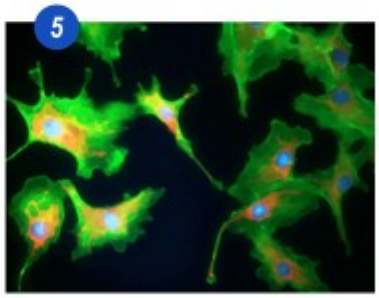
5: *Smooth Black Detail* darkens the Output Image by strengthening the darker tones. This is a close filter – *Dilation* followed by *Erosion*.

Continued… 801









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1: The original *Greyscale* Input Image.

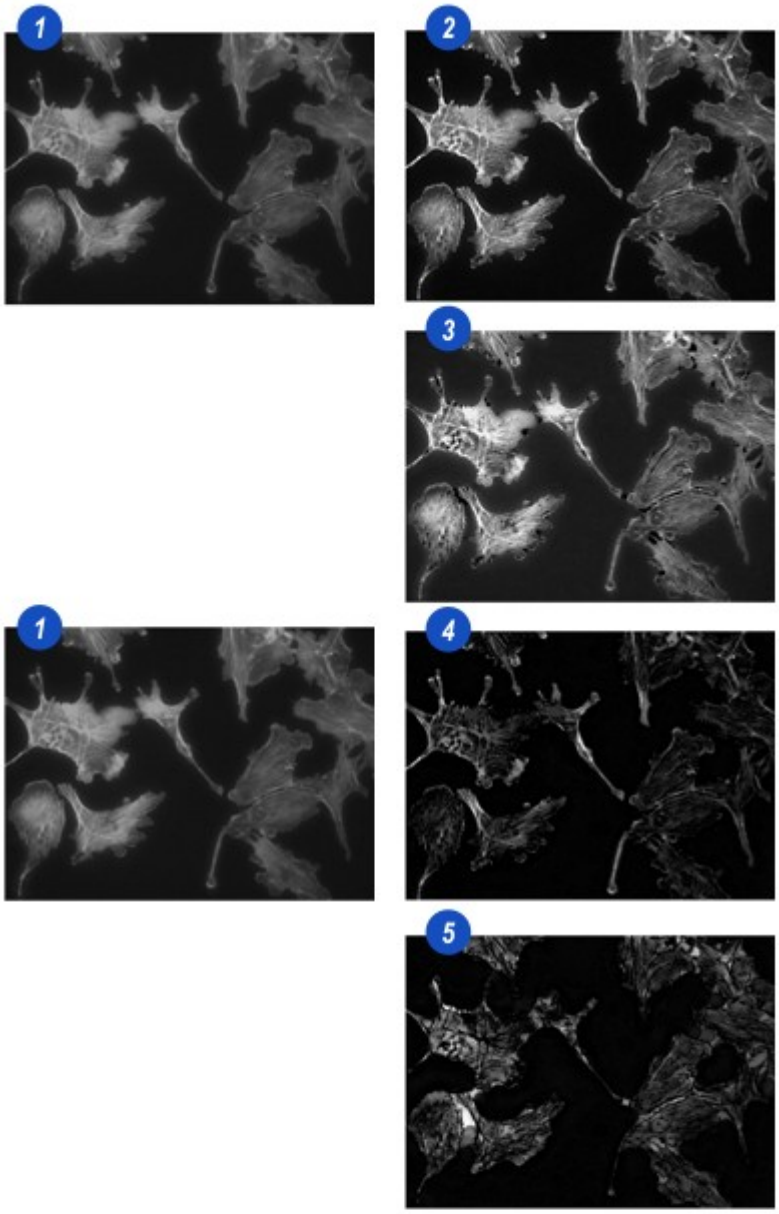
2: *Enhance White Detail* accentuates the white – values approaching 255 – areas of the image.

3: *Enhance Black Detail* improves the contrast between the black (values toward 0) and the lighter areas of the image.

4: *Find White Detail Top Hat* detects and removes lighter detail from the image depending upon the size of the Structuring Element.

5: *Find Black Detail Top Hat* detects and removes darker detail from the image depending upon the size of the Structuring Element.

Continued… 802

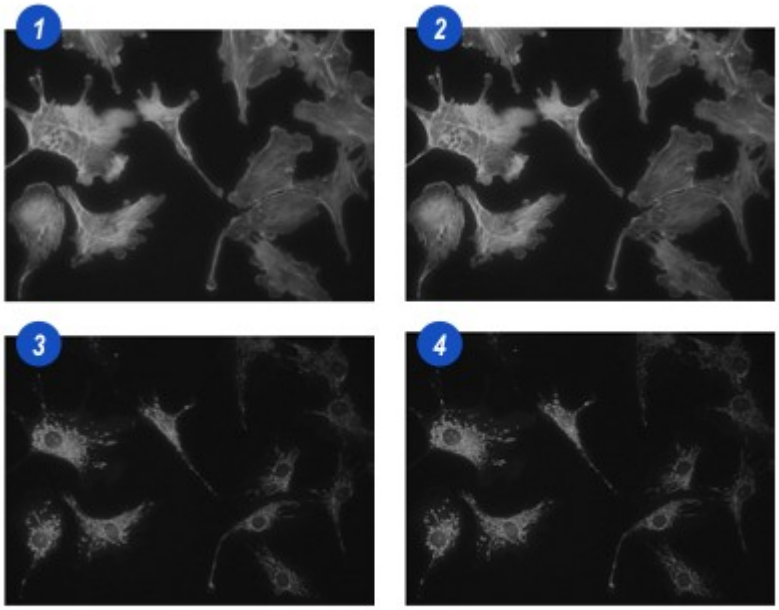


1: The original *Greyscale* Input Image and…

2: …after applying the *Sharpen Grey Transitions (Delineate)* filter. Intermediate grey tones are removed to provide greater contrast between areas of the feature.

3: The original *Greyscale* Input Image.

4: The *Noise Removal (Median)* filter removes artefacts down to pixel size from the image.



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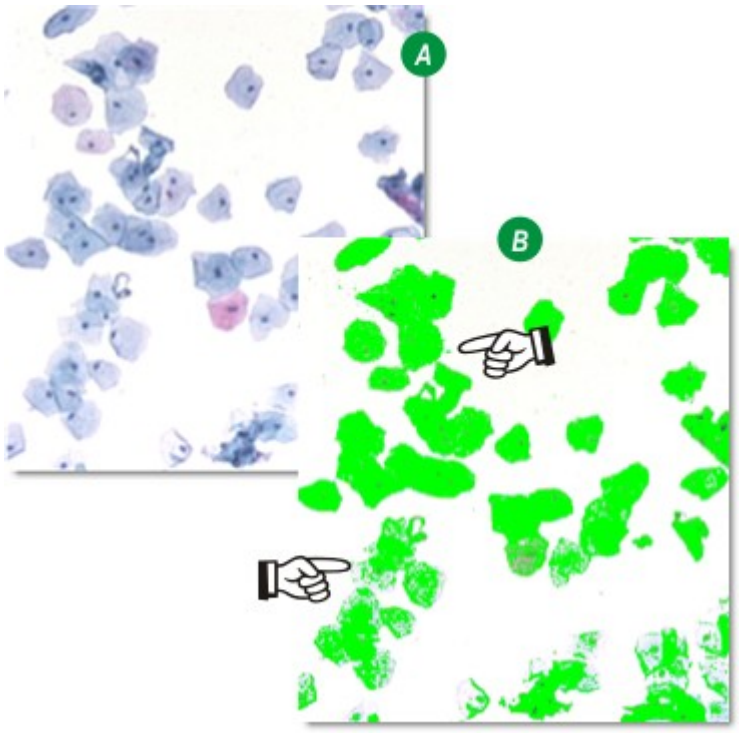
The aim of this example is to measure the number of biological cells on an image. The problem with the original image **(A)** is that the cells overlap, have a range of differing colours, have irregular outlines with varying contrast and some have coalesced into indeterminate 'blobs'.



Skipping a Greyscale Pre-Filter and going directly to *Adjust Threshold* (Illustration **B**) results in a fuzzy Binary Output Image with scattered additional artefacts all of which could be included in the measured count. Adjustments to intensity might have improved the image slightly, but it would be a time-consuming hit-and-miss process not necessarily resulting in a better count. Better to use a *Pre-Filter* to isolate individual cells allowing each to be counted with acceptable accuracy.

Because the cell outlines and contrast are so variable, an alternative solution would be to count the 'nuclei' – they have a more-or-less consistent colour and size – and where cells overlap the nucleus tends to show through and can be counted.

Continued… 804

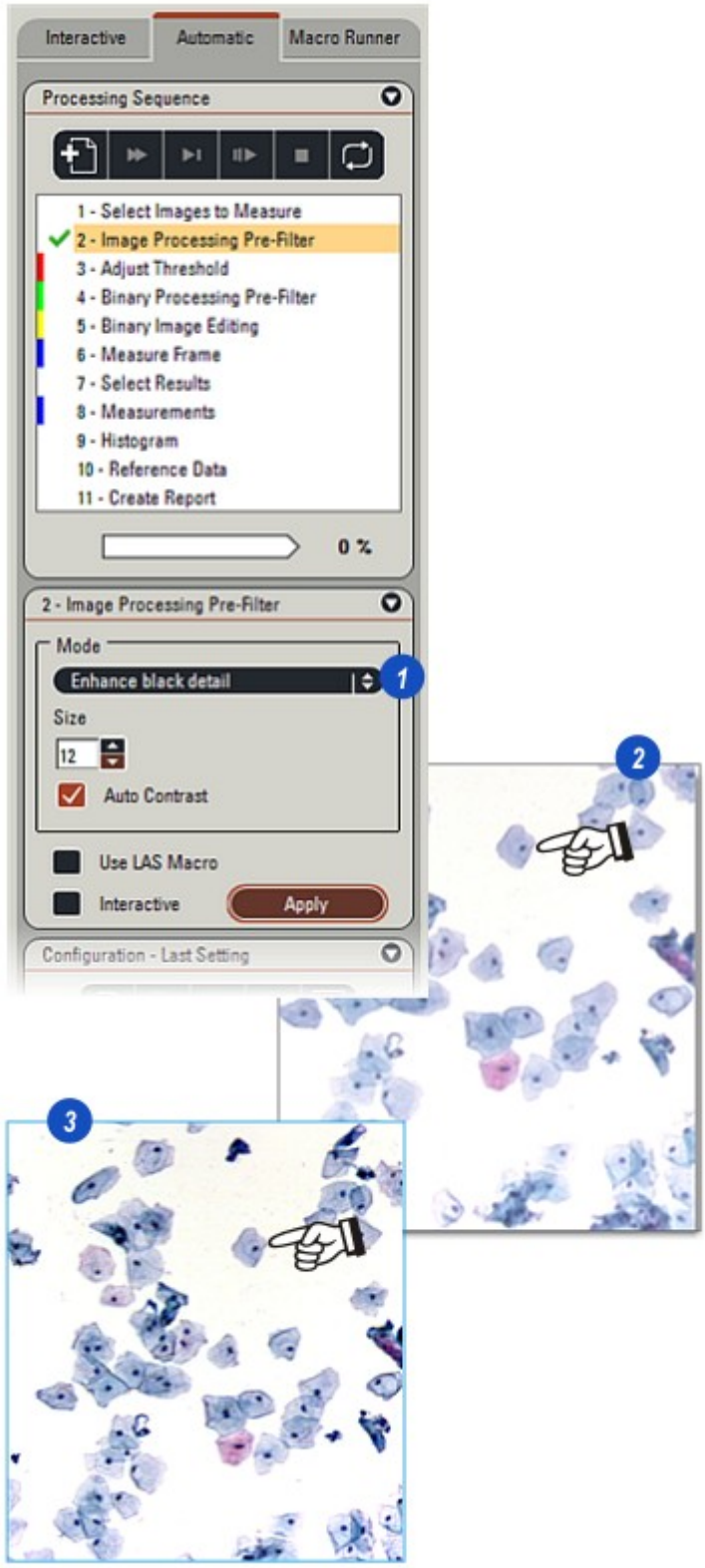
1: The *Enhance Black Detail* filter was chosen to improve the contrast between the nuclei and the surrounding cell tissue. Applying a structuring element of size 12, many of the intermediate greys have disappeared, the cell edges have become more clearly defined and the nuclei contrast is especially enhanced.
There are still areas of the image in which the cells have clumped together but these may be removed or more clearly defined during *Adjust Threshold* or with *Binary Image Edit*.

2: The original image and…

3: …the Greyscale Output Image.

Selecting a Pre-Filter 798: *Go there…* 798

Continued… 805



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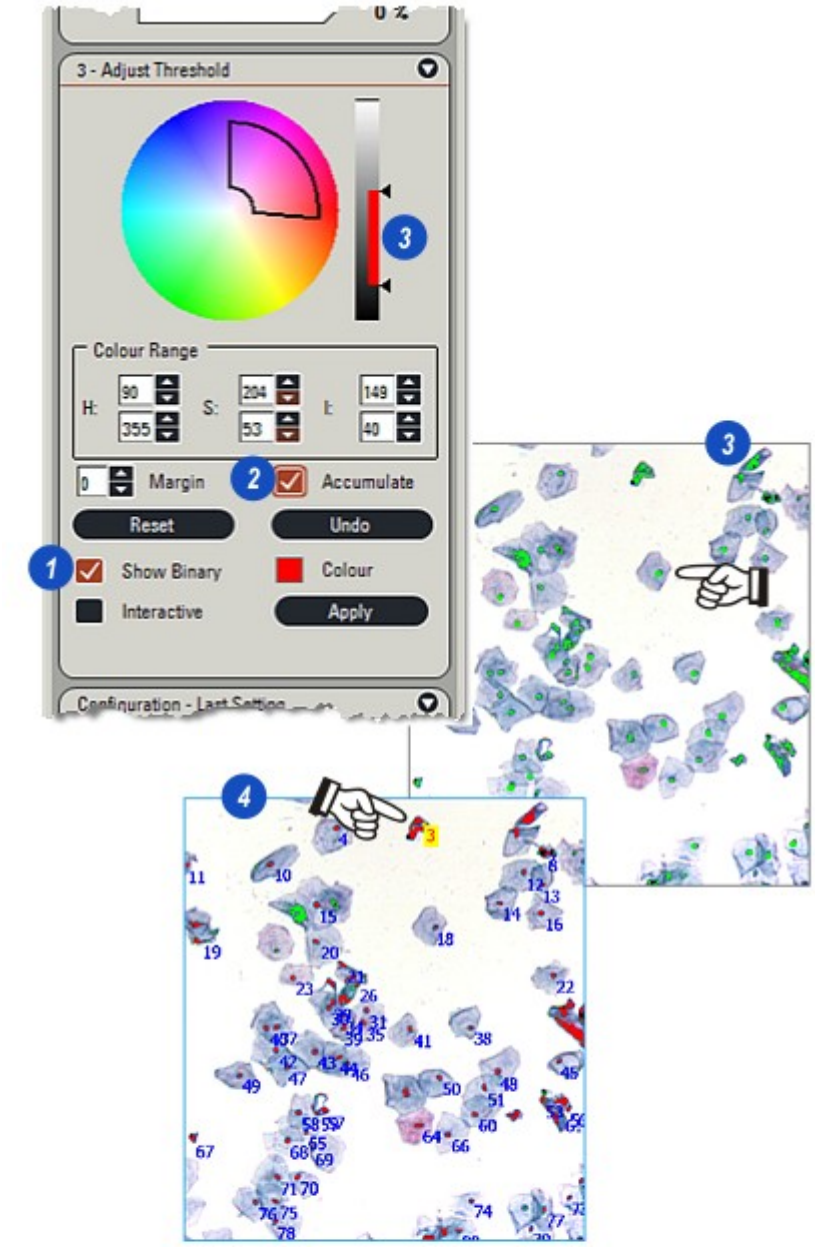
The next step is to apply the Thresholds. Check that *Show Binary* **(1)** and *Accumulate* **(2)** are both enabled.

3: With adjustments to the upper and lower Intensity Thresholds, the resulting Binary Output Image highlighted the nuclei and some clumps of artefacts all shown in green the default colour.

4: A first measurement for cell count yielded 86 but that included the clumps of artefacts as well. The feature highlighted in yellow is currently selected on the Grid view.

Adjust Threshold: Go there... 814
Measurements: Go there... 860
Grid View: Go there... 856

Continued… 806



There are several methods for removing unwanted features – *Reject* and *Delete* Modes in Binary Edit for example, but in this example they were excluded by refining the upper and lower limits of the *Area Measurement* tool.

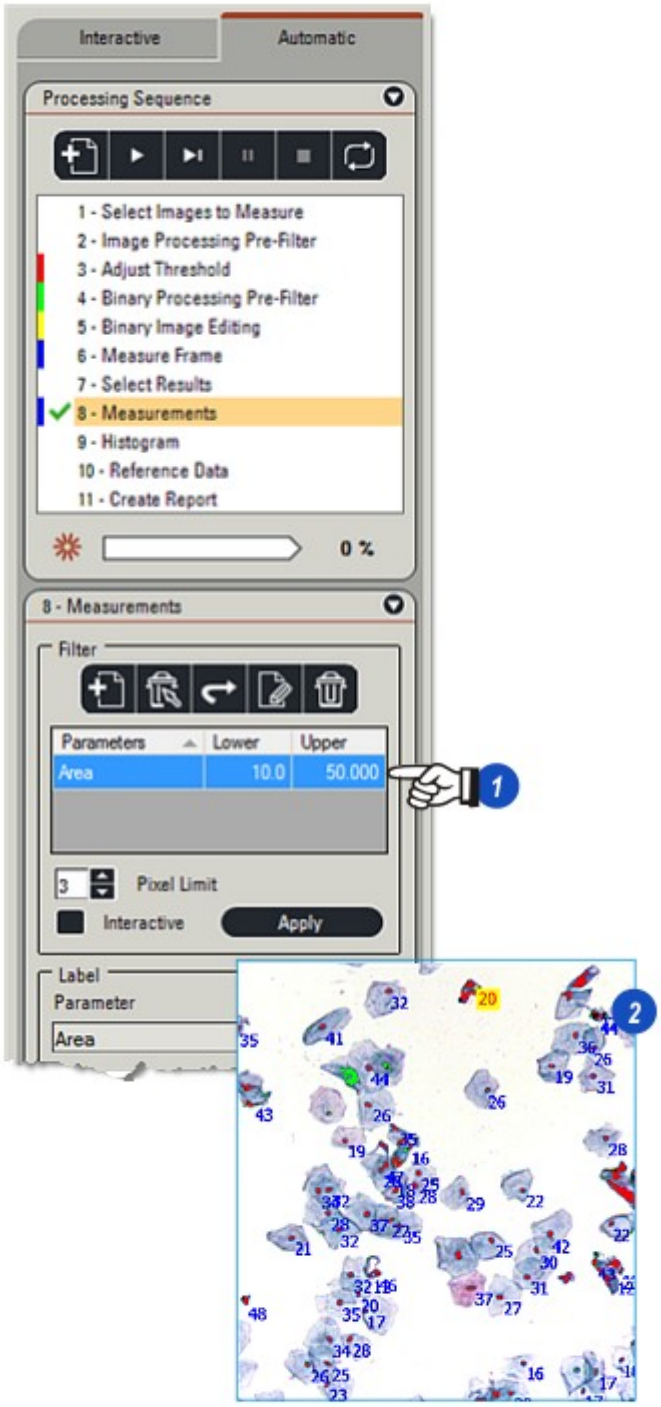
A first pass with a wide difference between the upper and lower limits produced an area value for all of the features, nuclei as well as unwanted artefacts. From this it could be seen that the nuclei areas fell between 10 and 50µm^2 whereas the unwanted artefacts were considerably larger.

The *Area Parameter* limits where then set to include the nuclei **(1)** with the result that all of the unwanted artefacts were ignored and the count reduced to 80 **(2)**.

In this example a *Measure Frame* was used to select just a small part of the image since the features were evenly distributed across the entire image and the smaller *Measure Frame* would yield a good mean result. It also speeds some of the processing time.

Once the correct filtering and thresholds have been established, the *Measure Frame* can be set to *Entire Image* and a further measurement made – in this case it resulted in a total count of 294 cells.

Measurement Tools: Go there... 860
Area Parameter: Go there... 904



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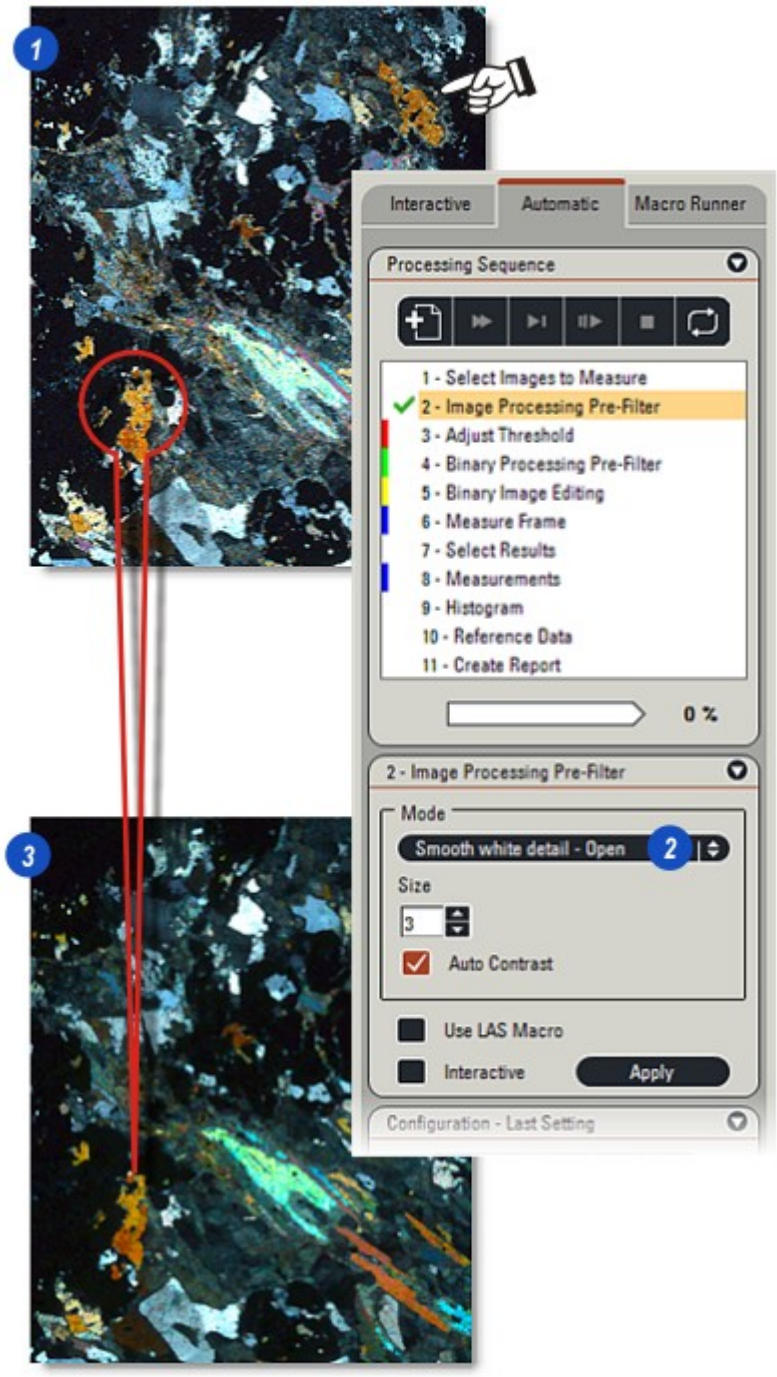
In this example a piece of schist, a crystalline rock, is the original image **(1).** The specimen contains some iron ore which shows up as red-orange areas scattered randomly across the image. The test is to determine what percentage of the sample comprises iron ore.

In such a complex image there are likely to be many single, unconnected pixels that are close to the iron ore colour range for which we will be searching. Leaving these pixels in place will slow the measurement process considerably without beneficially affecting the end result, so an overall 'tidy-up' to remove 'stray' pixels and consolidate larger groupings is a good starting point.

The Pre-Filter chosen was *Smooth White Detail* **(2)** with a structuring element of *Size* 3. Because *Smooth White Detail* is an open filter – *erosion* first and then *dilation* – some features disappear completely which is good for removing the stray pixels, but it can also start to reduce the area of the wanted features. Since this example will measure area, the filter has to be used sparingly.

(3). The result of the *Smooth White Detail* Pre-Filter.

Continued… 808



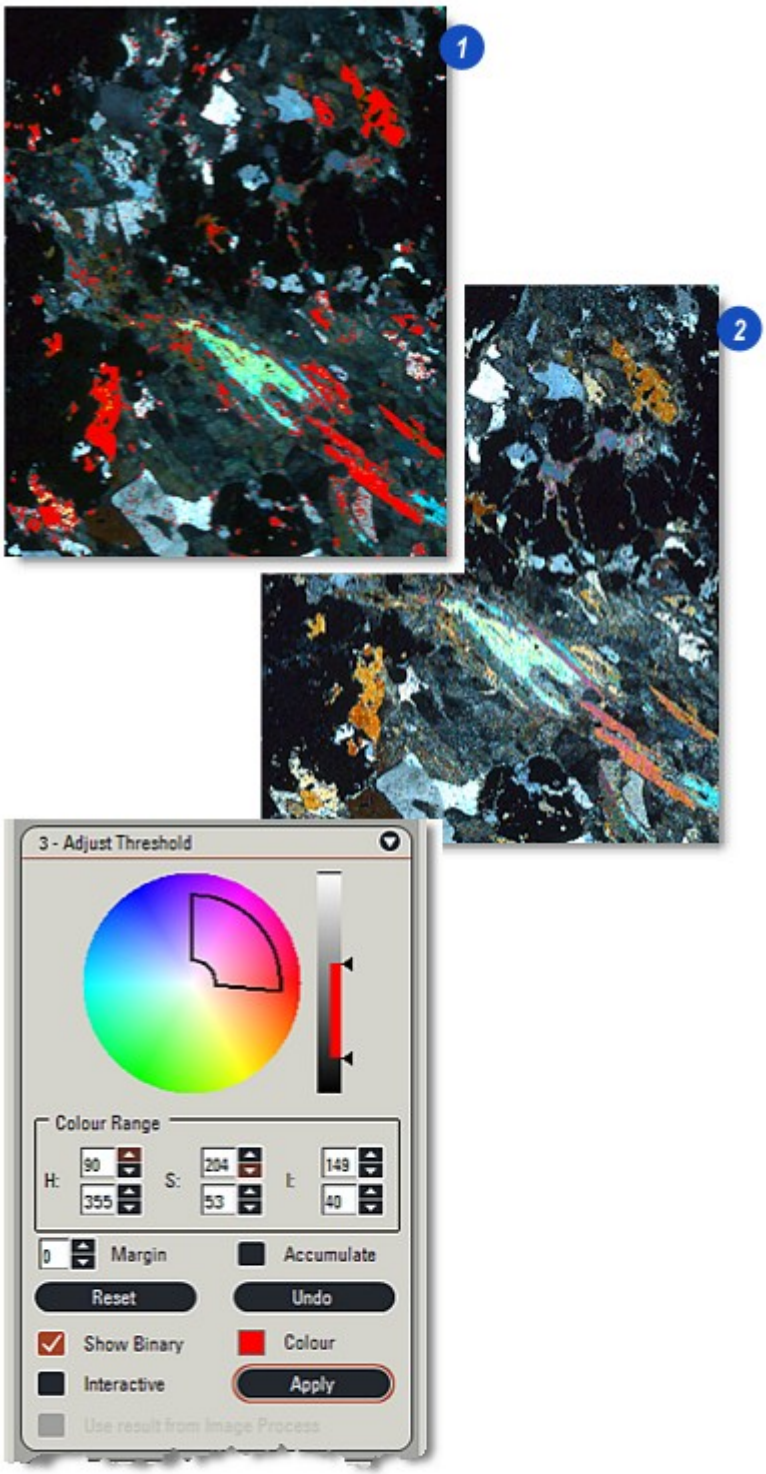
It took only minor alterations to the *Adjust Threshold* 814 intensity and saturation to produce a clean, well-delineated Binary Output Image **(1)** which, when compared with the original **(2)** proved to have faithfully pin-pointed the traces of iron ore and ignored everything else.

However, there were some holes and fissures in the main accretions which looked as though they should be included, but trying to fill them with the Threshold controls also tended to draw unwanted artefacts into the Binary Image.

There is an easier way to fill holes…

Adjust Threshold: Go there... 814

Continued 809...



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The *Combine Detail* filter, which is part of the *Binary Processing Pre-Filter collection* 825, can quickly, simply and precisely fill holes and fissures in a Binary Output Image **(1).**

2: The *Reconstruct* and *Fill* options were enabled by clicking the check boxes.

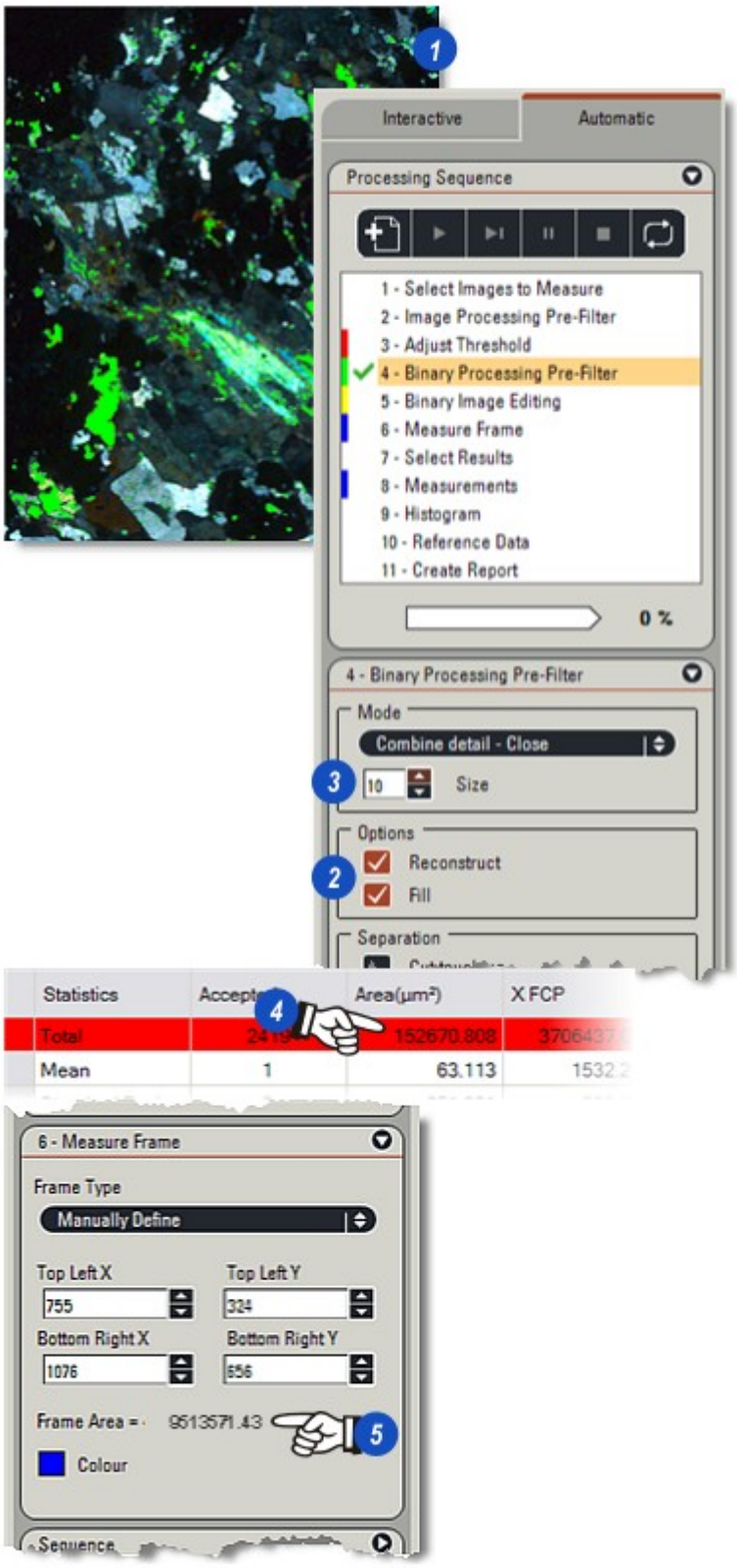
3: The structuring element *Size* control was incremented in single steps until the holes in the major features were filled without gathering together some of the more separate features.

4: A first measurement yielded a total feature (iron ore) area of 152670µm^2 which was displayed on the *Grid View* and this was...

5: ...within a manually defined *Measure Frame* of 2587631µm^2 giving a value of 5.9% iron ore.

To check that the measure frame represented an average part of the image, the frame was reset to *Entire* image and the area measurement was taken again. This yielded a feature area of 577912µm^2 within an image area of 9513571µm^2 - 6.0%.

Binary Processing Pre-Filters: 825 Go there... 825
Grid View: Go there... 776



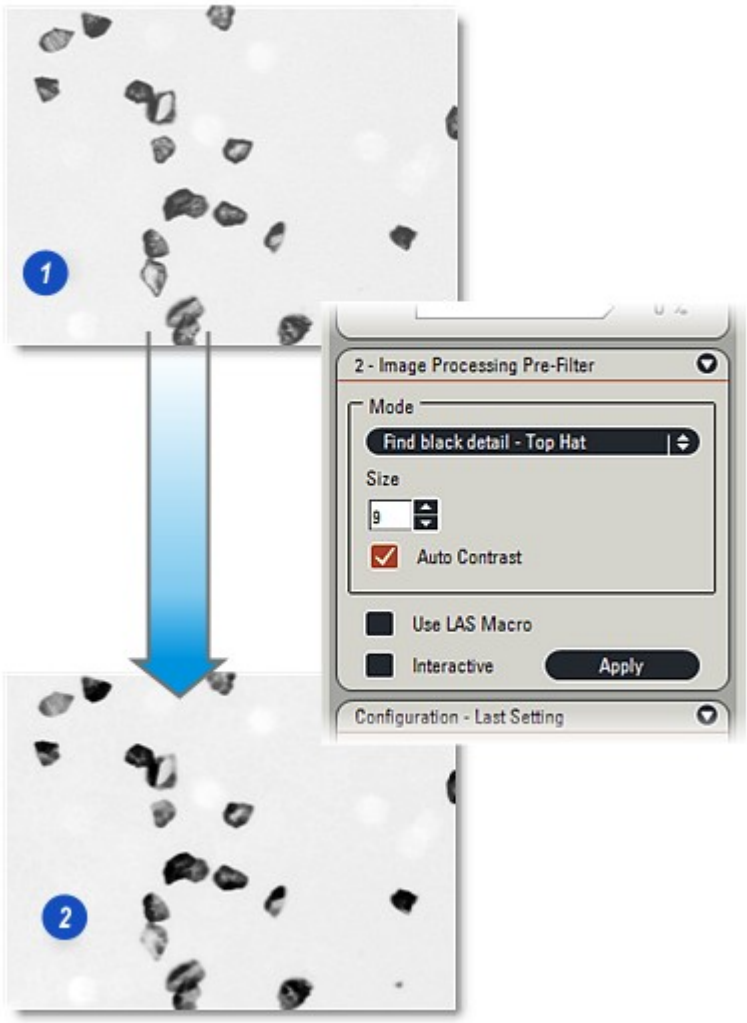
The diamond chips in the original image **(1)** produce an acceptable Binary Output image if passed directly through the Adjust Threshold, but some fast processing with a Greyscale Pre-Filter reduces the amount of fine adjustments made to the Threshold and can improve measurement precision considerably.

LAS Image Analysis are so fast that it is always beneficial to run an image through several greyscale filters to check for improvements.

In this example the image was processed with the Smooth Black Detail Pre-Filter that removed some of the intermediate grey values and increased the chip edge contrast **(2).**

However, it had to be used carefully since this was to be a feature count – how many chips in the image – and because several overlap the Smooth Black filter could have blended them together resulting in a single feature rather than two.

Continued… 811



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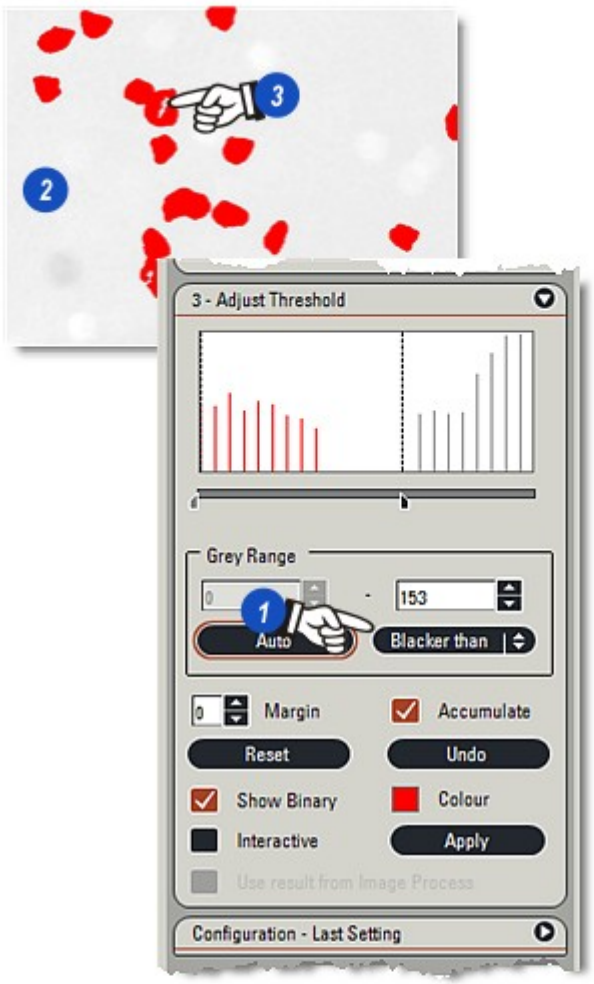
1: Diamond chips is a monochrome image with predominant greys closer to the black end of the scale, so the *Grey Range Selector* was set to *Blacker* than and minor adjustments made to the *Histogram* sliders.

2: The fast result is a very good *Binary Output Image* with small holes **(3)** in just two of the chips.

Continued… 812



1: In this example the holes in several of the diamond chips would not have made a difference to the final feature count, but since it was necessary to pass the image though a *Binary Pre-Filter* to make sure that there was good separation where the chips overlapped, the *Combine Detail* with…

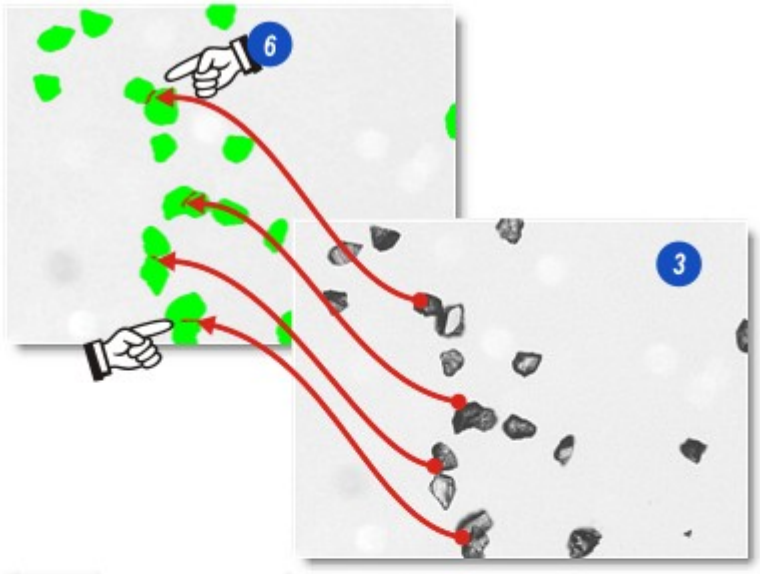
2: …the *Fill* option enabled, was used to fill the holes with the *Size* setting at 6. Again, this was used sparingly because some of the chips are close together and could have been combined.

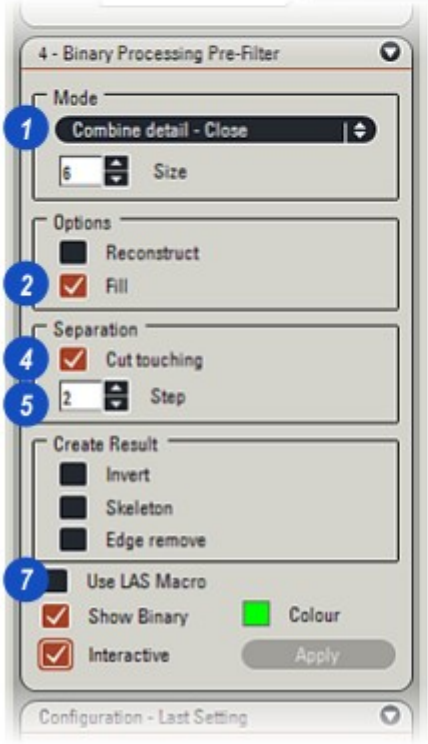
3: There were 4 chip pairs that were either overlapping or touching; They can be clearly seen on the original.

4: With *Separation* > *Cut Touching* enabled…

5: …two steps were sufficient to separate the overlaps, indicated by the red lines on the *Output Image* **(6).**

7: To use *LAS Macro Runner*, click to enable the check box. Detailed help for LAS Macro: *Go there...*





Continued… 813

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1: The final step before counting the diamond chips was to remove the fragment (bottom right) to prevent it being counted achieved with…

2: ...Binary Edit Delete Mode. The fragment was not actually removed from the Binary Output Image but only highlighted and ignored in the count.

3: The count shows 18 chips in the image and shows that the *Cut Touching* tool is working well.

Binary Edit Delete Mode: *Go there...* 849

Adjust Threshold is a quick and simple method of selecting the features to measure or edit and ignoring the rest.

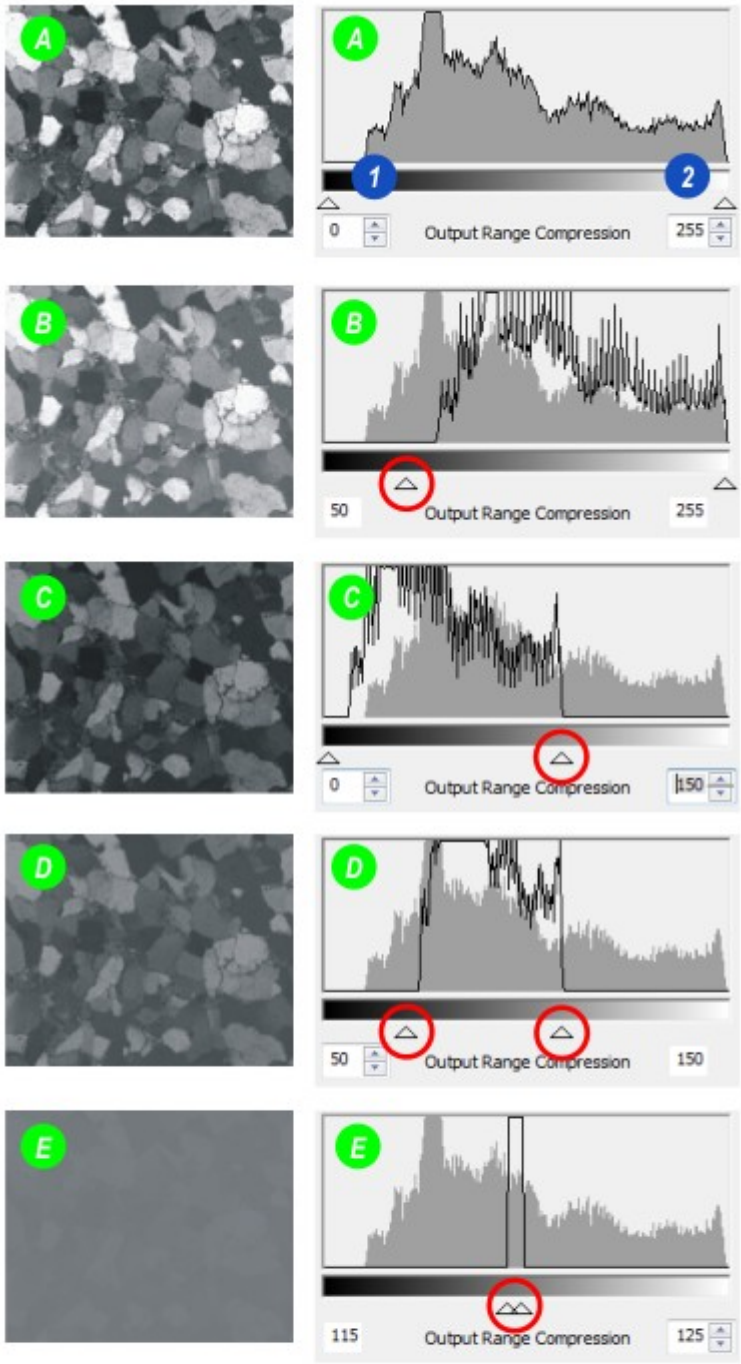
The Threshold principle:
Illustration **(A)** shows the original greyscale image on the left and its graphical representation called a *Histogram*, on the right. The *Histogram* comprises 256 vertical bars each representing a pixel value. A value of 0 represents black **(1)** on the left and a value of 255 represents white **(2)** on the right. This image of quartzite does not have any absolute black areas - the darkest being close to a value of 25 - very dark grey. The two *Histogram* sliders can be moved along the base line so that any values to the left of the black slider are ignored as well as any of those to the right of the white slider.

In Illustration **(B)** the black slider has been moved to position 50 so all pixels with a value from 0 to 49 have been ignored with the result that many of the darker features have been lost.

Illustration **(C)** shows the same effect but this time moving the white slider to the 150 position. All pixels with a value between 151 and 255 are ignored and the overall effect is a darker image.

Both sliders have been moved in Illustration **(D)** - black is at position 50 and white at position 150 - so only those pixels in the range 50 to 150 are reproduced in the image.

The sliders can be moved to isolate a very narrow range of pixels (Illustration **E**) to the extent that just a single pixel value could be selected.



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1: The Threshold dialog is opened by clicking on the *Adjust Threshold* option on the main menu.

2: For colour images the dialog with a *Colour Wheel* and controls for *Hue (H), Saturation* (S) and *Intensity (I)* opens, whereas…

3: …for monochrome images the scale is displayed as a *Histogram* with only the Intensity control available.

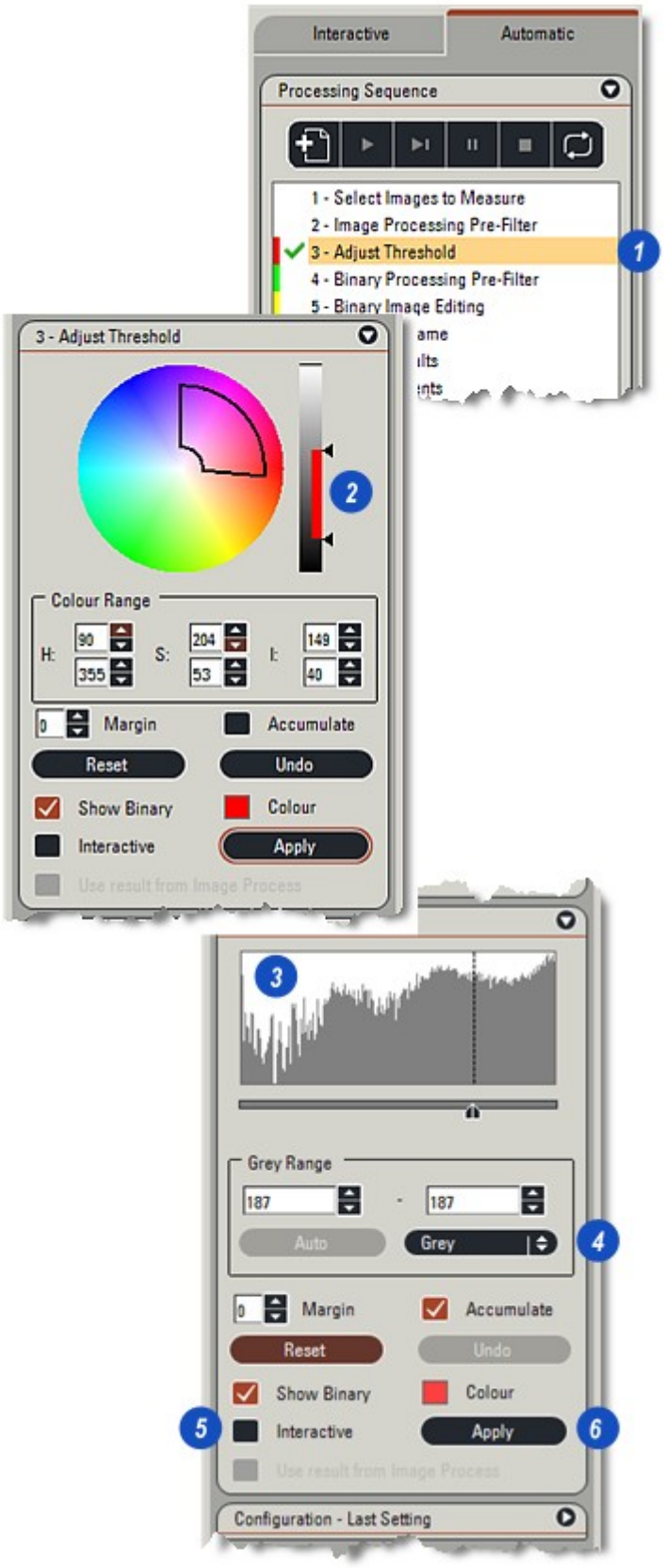
4: Additionally, the monochrome image dialog has a *Grey Range Selector*. The other buttons and controls are the same for both image types.

The Interactive Checkbox:

5: With the *Interactive* checkbox enabled (showing a tick mark) each change in the Threshold settings is automatically reflected in the Output Image. For simple images this is the best setting, but more complex images may take a little longer to refresh and so disable *Interactive* and click on…

6: … the *Apply* button to update the Output Image after settings have been altered.

Continued... 816



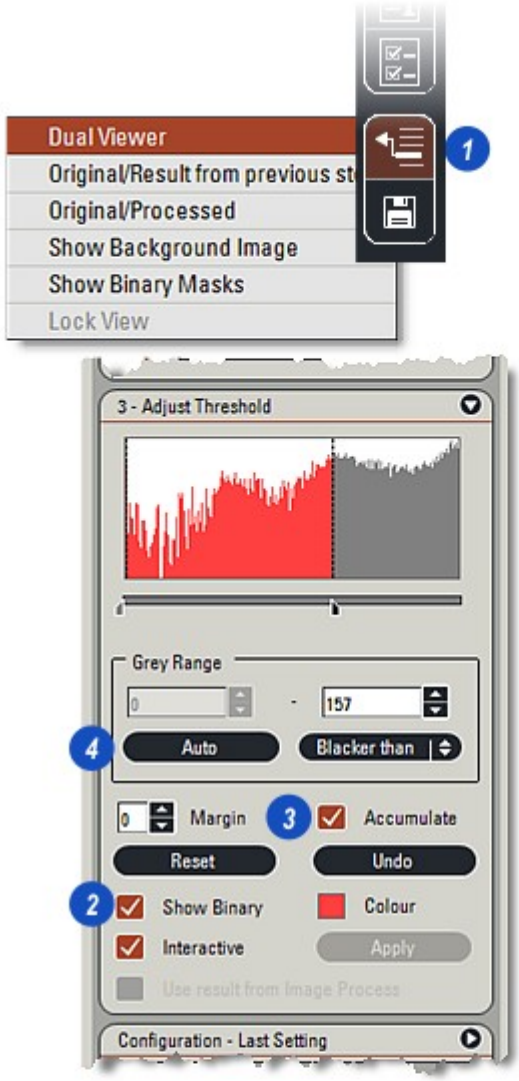
Dual Viewer and Binary enabled:

1: Click on the Viewer Options button on the Side Tool Bar and from the context menu, click to enable *Dual Viewer* – original *Input Image* to the left and adjusted *Output Image* to the right of the *Viewer*.

2: Click to enable the *Show Binary* checkbox which will then display the features selected for measurement in the right hand side of the *Viewer*. The features are shown in the default colour red or one of the user's choosing.

3: Checking the *Accumulate* check box allows a sequence of selections made on the original image to be added together - accumulated - on the *Binary Output Image*. *The Accumulate feature:* *Go there...* 819

4: The threshold for images with good contrast and well-defined features, can be established automatically by clicking the *Auto* button. Fine adjustment can then be made with the other controls.



The *Adjust Threshold* tool produces a *Binary Output* Image in computer memory that is passed on to subsequent filters and tools for enhancement or measurement. The original image is unaffected.

Position the cursor over the *Binary Output Image* and right click and hold down the mouse button. This will temporarily hide the selected features making it easier to compare with the original and determine if all of them have been included. Release the mouse button to restore the *Binary Output Image*.

Change the Binary Image Colour: Go there... 820

Continued… 820

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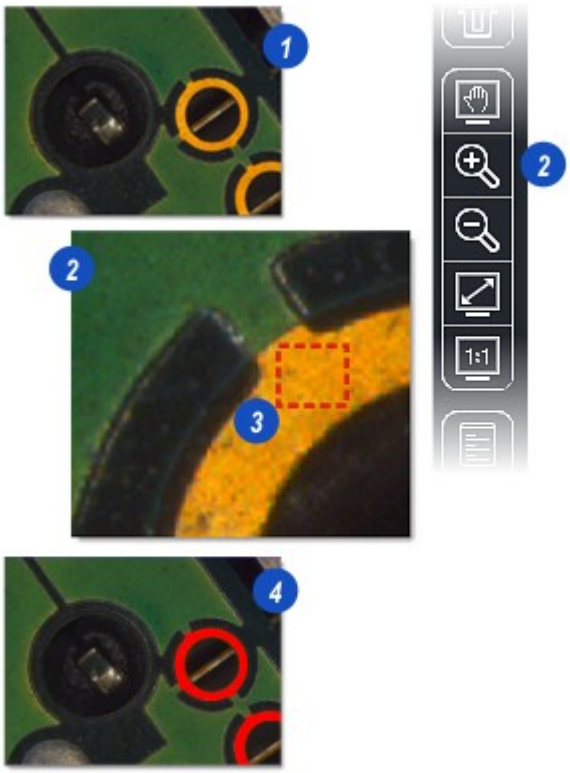
There are two methods of selecting features to be included in measurements:

- The Region of Interest can be used for both colour and monochrome images and,
- The Grey Range Selector used on monochrome images only.

Both are fast, precise and easy to use.

Region of Interest selection:
Drawing a *Region of Interest* around a feature to include a range of greyscale values is a fast and intuitive. Pixels across the image with values matching those inside the drawn region are selected and coloured on the *Binary Output Image*. The process is suitable for both greyscale monochrome and colour images.



1: The original image, part of an electronic circuit board. The gold-plated rings bottom right which have distinctive colour values are to be selected for measurement.

2: Use the *Zoom In* tool from the side toolbar to pin-point the feature.

3: Click on the feature and drag down and to the right to create a small rectangle – the *Region of Interest*. Keep the boundaries of the rectangle within the feature.

4: Release the mouse button and pixels with values that match those within the Region will be selected on the *Binary Output Image.*

Continued... 818

LAS Image Analysis recognises colour or monochrome images and as soon as a *Region of Interest* is drawn displays the appropriate control panel.

Colour Images: Colour and Intensity:
The *Colour Wheel* **(1)** can be used to adjust and experiment with the *Hue* and *Saturation* values. *Hue* is measured along the circumference of the *Wheel* and *Saturation* is represented by a position along the *Wheel*'s radius.

2: As the cursor moves over the *Wheel* both *Hue* and *Saturation* are displayed in a *Tooltip* box.

3: Click and drag on the *Wheel* segment to alter the *Hue* and *Saturation*. The actual values are displayed in the *Colour Range* windows **(5)**.

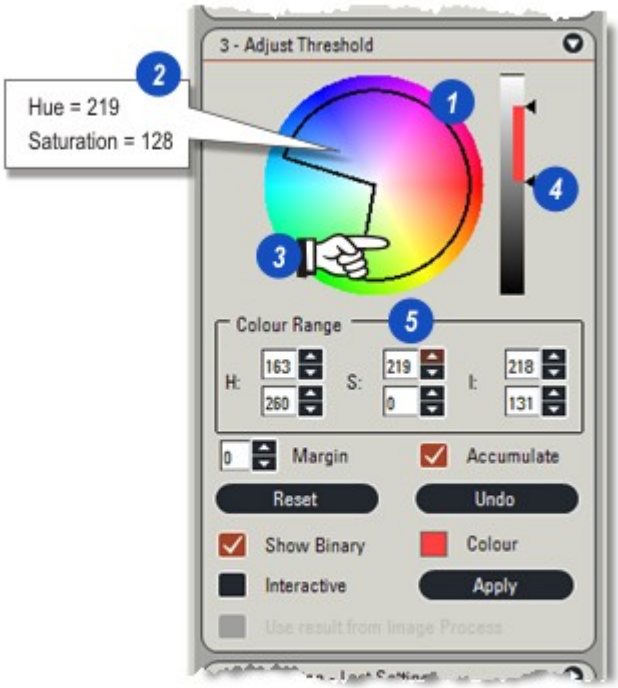
4: Intensity can be changed by clicking and dragging the *Intensity Bar Threshold* pointers up or down.

5: Fine-tune all three parameters with the Up/Down arrows to the right of the *Colour Range* windows. Each pair of windows represents the span of values for that parameter *H=Hue, S=Saturation* and *I=Intensity.*

Monochrome Images:
A *Histogram* **(6)** displays the greyscale values for monochrome images. Adjustments are made with the *Histogram Sliders* or with the *Grey Range Selector*.

The Grey Range Selector: Go there… 821

Continued… 819



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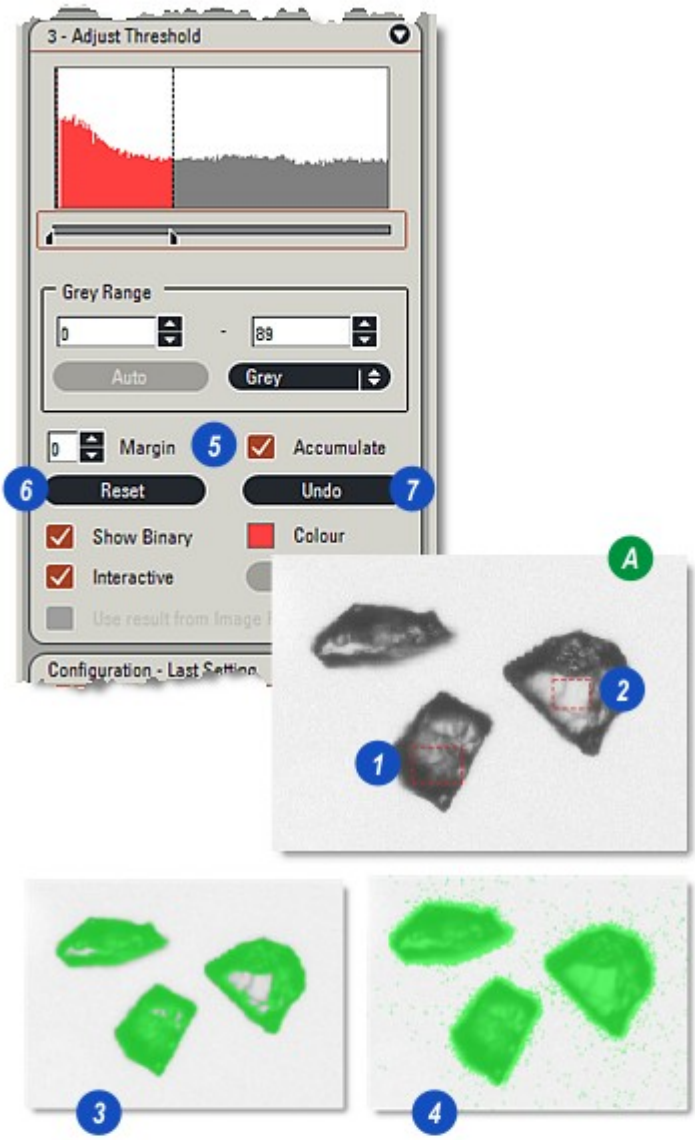
The *Accumulate* tool adds the values of successive drawn regions to extend the Threshold range and therefore, include more features.
For example, Illustration **(A)** shows three diamond chips that together include greyscale values from 45 (near to black) and 187 (near to white).
The first drawn region **(1)** encompasses value from 45 to 103 and all features falling within that range will be selected.
The second drawn region **(2)** includes values from 93 to 187 and with *Accumulate* enabled this range will be added to the previous to extend the scope from 45 to 187.
Disable *Accumulate* **(5)** if each Threshold selection is to be treated as a completely new operation.



In the examples,

3: ...represents the features selected after the first region was drawn and...

4: ...after both regions have been drawn and the range extended.
However, the higher values now capture some background artefacts but they can be removed with small adjustments to the *Grey Range Selector* value.

Reset and Undo:

6: The *Reset* button will clear all Threshold values whereas…

7: …clicking *Undo* will remove only the last pass.

Continued… 821

The colour of the selected pixels in the *Binary Output Image* can be changed to suit the user.

1: Click on the *Colour* button. *The Select Colour* dialog appears.

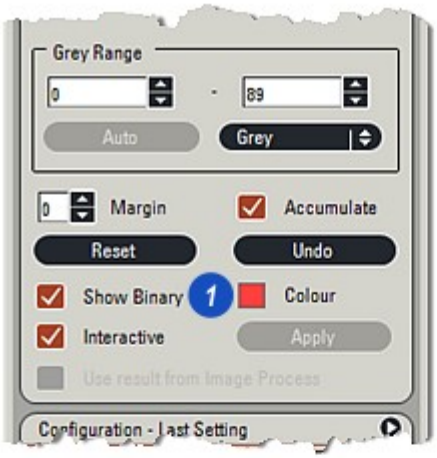
2: Choose a colour from the swatches by clicking on it or…

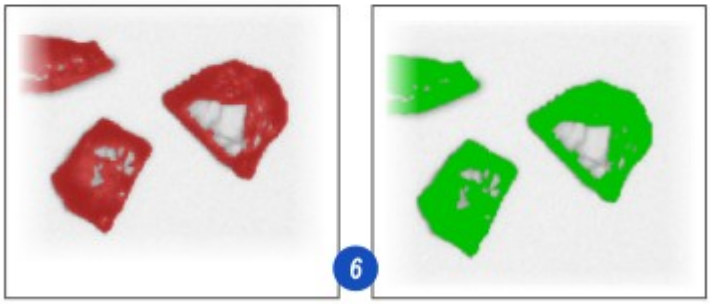
3: …click and drag the hue sliders or…

4: …click and drag the marker on the *Colour Wheel.*

5: Click *OK*. The new colour appears in the *Colour* button.

6: The illustrations show the effect of changing the binary *Colour* from red to green.





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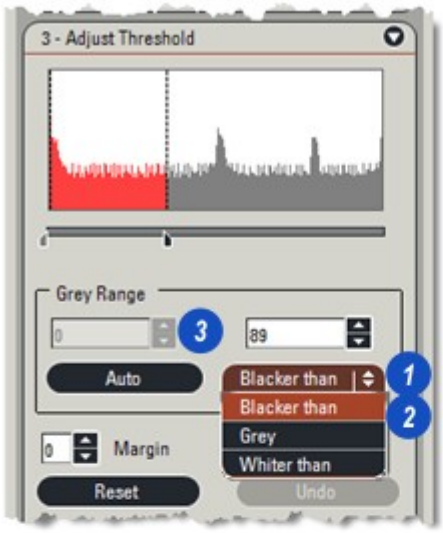
The *Grey Range Selector* is a fast and easy way to select features on a greyscale monochrome image. LAS Image Analysis automatically detects monochrome images and displays the histogram and *Grey Range Selector.*
Three user-set grey 'levels' are available as a drop down menu:



Blacker than selects all features at or below the entered grey value.

Whiter than selects all features at or above the entered value, and…

Grey automatically sets two values (Blacker than + 1 and Whiter than – 1) as a centre band.

To set the Threshold range:

1: Click on the small arrows to the right of the *Grey Range* menu header and…

2: …click to select a range. The Grey Range text boxes reflect the the set range selected.

3: To enter a value either click inside the window and type a new value or use the Up/Down arrows to the right of the box that are ideal for 'fine tuning'.

Changes made to the grey values are automatically reflected in the *Blacker than* and *Whiter than* settings.

Continued… 822

1: The entered values are displayed on the *Histogram…*

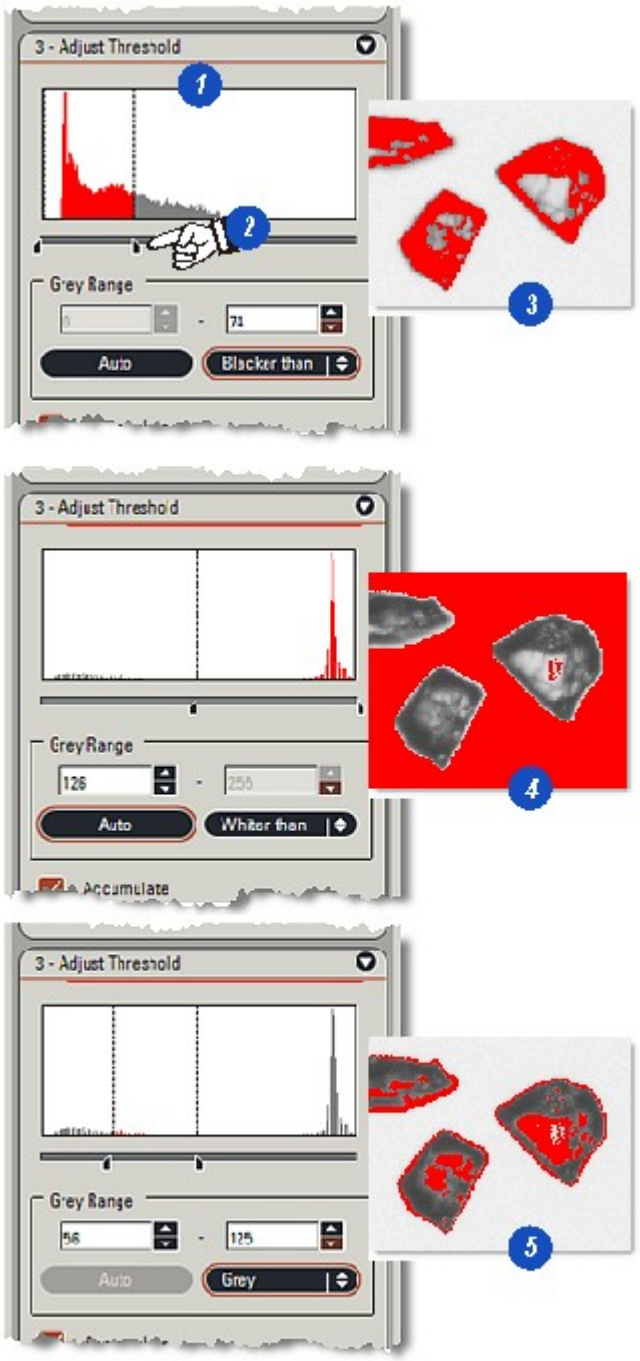
2: …the sliders of which can be clicked and dragged to adjust the settings. The selected range is shown as a colour on the *Histogram* display.

The *Binary Output Images* for the three *Grey Range Selector* settings are shown in the illustrations:

3: *Blacker than* setting.

4: *Whiter than* setting, and…

5: *Grey* setting.



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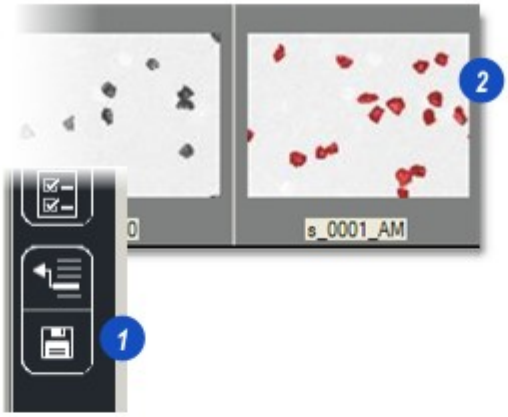
1: Save the *Binary Output Image* at any time by clicking on the *Binary Save* button on the side tool bar. The *Binary Image* is saved in the same folder as the original and…

2: …a thumbnail using the original name/number plus the suffix ‘AM’ as a caption. Saving subsequent *Binary Output Images* for any of the tools will replace and overwrite the preceding binary save.

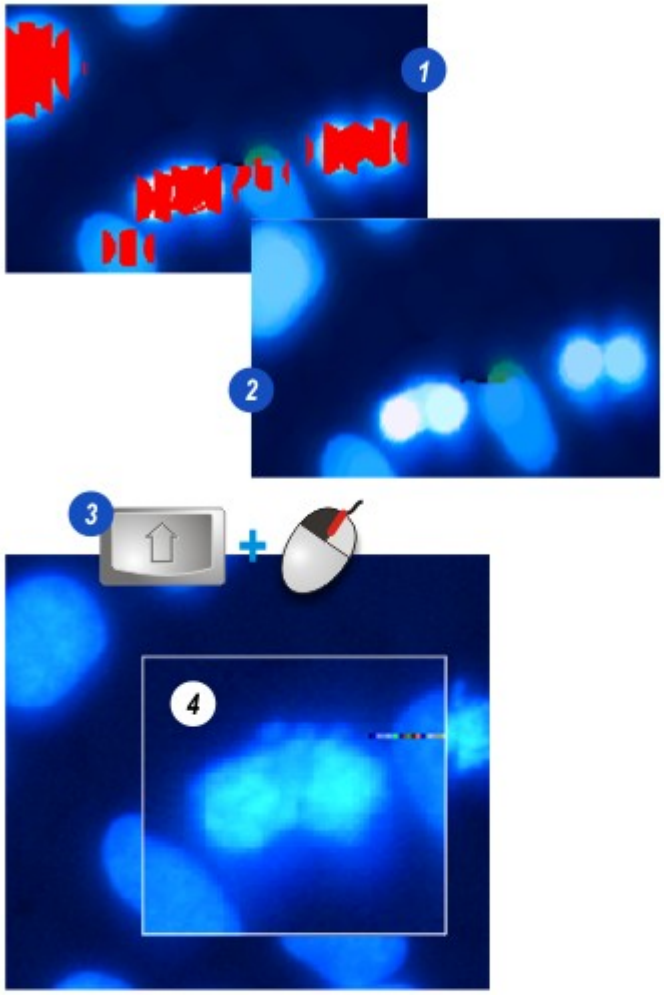


To switch between the *Binary Output Image* and the *Input Image:*

1: On the *Binary Output Image* click and hold down the right mouse button. This will hide the binary image and reveal the original input **(2).**

To enlarge parts of either the *Input Image* or the *Binary Output Image:*

3: Hold down the keyboard *Shift* key and click on a portion of the image to enlarge. The magnified area tracks the mouse movements **(4)**. Release the Shift key to close the magnifier.



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Binary Processing uses the same techniques as Greyscale Processing for improving and modifying images except that instead of working with values ranging from 0 to 255, only pixel values of 1 and 0 are used.

Usually, the image presented to the Binary Processing function has passed through Greyscale Processing and Adjust Threshold. Illustration **(A)** shows the sequence:

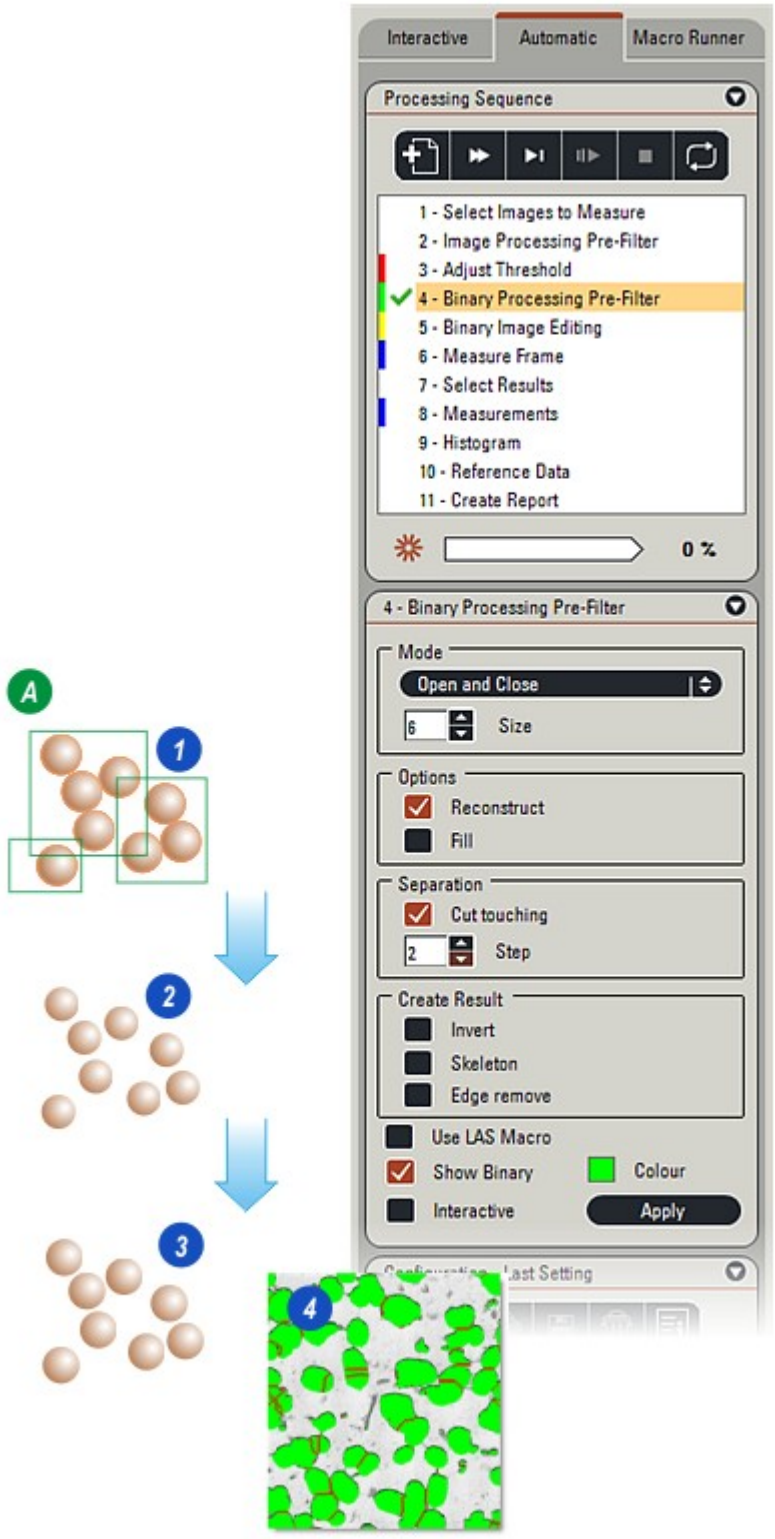
1: The Greyscale Input Image comprising 8 touching disks; the task is to count the disks and measure their areas. Performing a *Number* (Count) and *Area* calculation on this image would yield just 3 features with diverse areas because some of the disks are touching.

2: Shows the Output Image after a Greyscale Processing *Erosion* – the disks are no longer touching so a *Number* (Count) would be correct at 8. But some diameter has been lost so the *Area* calculation would be understated.

3: The disks after a Greyscale *Dilation*. They are still separate and much of their original diameter has been restored. A count measurement would be much more accurate.

Skip Binary Processing Principles: 830

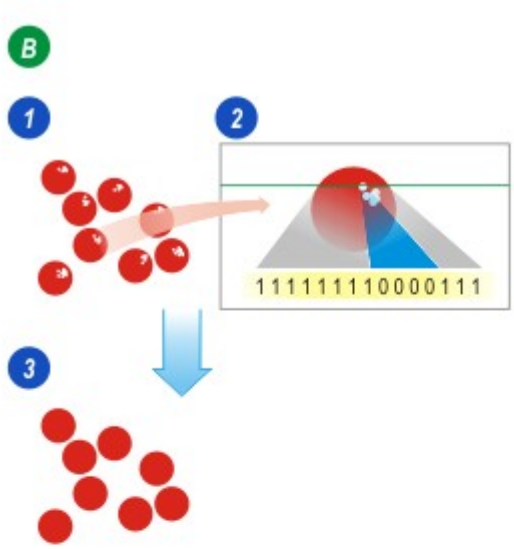
Continued… 826



In Illustration **(B)**:

1: The image has passed though *Adjust Threshold* and those grey values falling with the Threshold limits are coloured red. The highlights on the disks have not fallen within the Threshold limits and so remain white.

The *Output Image* from the *Adjust Threshold* has converted a Greyscale Input with grey value ranging from 0 (black) to 255 (white), to a *Binary Output Image* in which those pixels selected are set to a value of '1' and those not selected cleared to a value of '0' – hence Binary because only two values are used.



2: An enlarged disk. The string of binary digits below the illustration represents a single row of pixels where the green line cuts through the disk and highlight. The selected (red) areas of the disk are set to '1' and the unselected highlight (white) areas cleared to '0'.
There are 'holes' in the disks!

3: For a proper *Area* measurement those 'holes' need to be selected and filled. *Binary Processing* can do that quickly and efficiently.

Continued... 827

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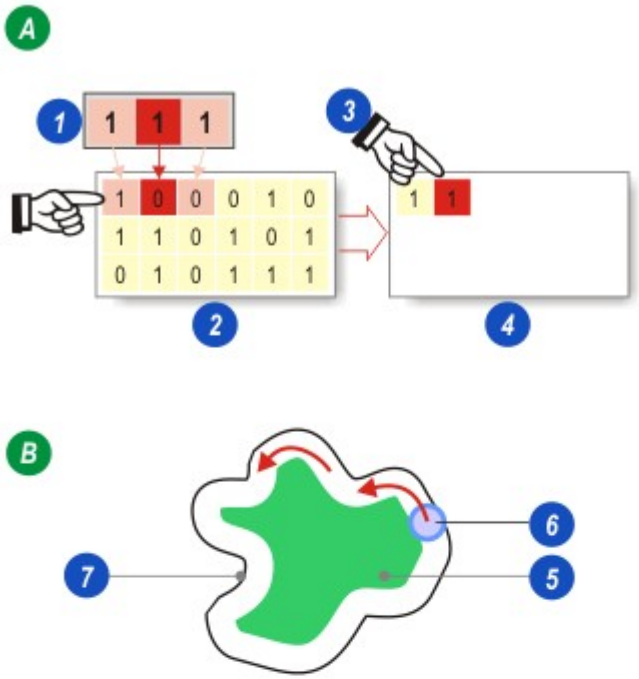
The *Binary Pre-Filters* provide the tools for modifying the *Binary Image* in the same manner as Greyscale Pre-Filters with the difference that values of '1' and '0' only are being tested.



Binary Dilation:
In Illustration **(A):**

1: Is the *Structuring Element*, an 'electronic overlay' with cells that are also set or cleared to binary values. In the illustration there are three cells all of which are set = 1.
The centre cell is called the *Origin* (coloured red) and it 'looks' at a single pixel (also coloured red) called the *Input Pixel …*

2: …in the Binary Input Image.
The process examines the pixels neighbouring the *Input Pixel* to determine whether the corresponding pixel **(3**) in the Binary Output Image **(4)** should be set or cleared. The *Structuring Element* settings determine if a neighbour is tested (Cell=1) or ignored (Cell=0).

The process described in this illustration is called a *Dilation* – if any of the neighbours are set (=1) then the *Output Pixel* is also set – which is the case in Illustration **(A)**.
A *Dilation* has the effect of increasing the number of selected (=1) pixels.

Illustration **(B)** shows a simplified form of dilation:

5: The original object.

6: An imaginary 'roller' tracing around the periphery of the object.

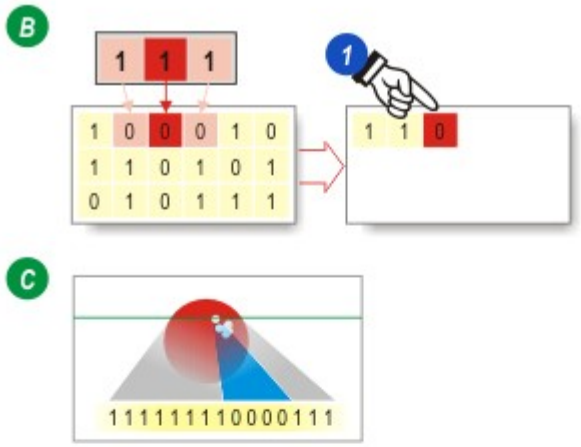
7: The new outline of the object.

Continued… 828

In Illustration **(B)** the *Structuring Element* has moved one pixel to the right. Now the *Input Pixel* (red) and its neighbours are all cleared (=0) and so the pixel in the *Binary Output Image* is also cleared **(1)**. The process continues for every pixel until a complete and new *Binary Output Image* has been created.

Binary *Dilation* would be a most suitable pre-filter for filling the highlight 'holes' in the example image **(C).**

Continued... 829



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Binary Erosion:

1: The *Structuring Element*.

2: The *Binary Input Im*age with pixels either set (=1) or cleared (=0).

3: The *Binary Output Image*. Set pixels are included in a measurement but cleared pixels are not.

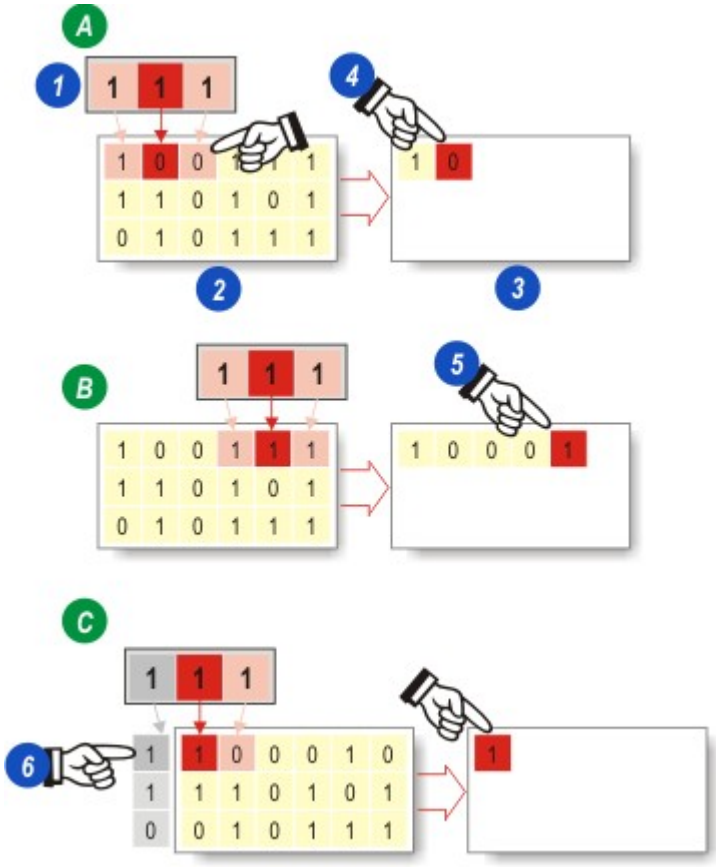
The *Erosion* process examines the *Input Pixel's* neighbours and if any one is cleared (=0) then the Output Pixel is also cleared. This has the effect of removing selected pixels from the image.
In Illustration **(A)** a neighbouring pixel is cleared (=0) and so the *Output Pixel* **(4)** is also cleared.

The *Structuring Element* has moved four pixels to the right in Illustration **(B)** and now both of the neighbours are set (=1) so the *Output Pixel* **(5)** is also set.

To determine the value of pixels on the extreme edges of the *Output Image*, Illustration **(C)**, the Input Pixel (coloured red) is 'ghosted' **(6)** to become a neighbour. The illustration represents a *Dilation* so the *Output Pixel* is set, but if this were an *Erosion* the *Output Pixel* would be cleared because the right-hand neighbour is cleared.

Erosion and *Dilation* can be used in combination to achieve specific results. *Dilation* followed by an *Erosion* is called a closing filter used for filling 'holes' in an image.

Erosion followed by *Dilation* is called an *opening filter* and is often used for removing small details such as noise and dust.



1: Open the *Binary Pre-Filter* controls by clicking on the entry in the main menu.

2: In the *Mode* section, three active filters can be selected as well as *No Filter* by clicking on the arrows to the right of the window and…

3: …clicking to select the required filter.

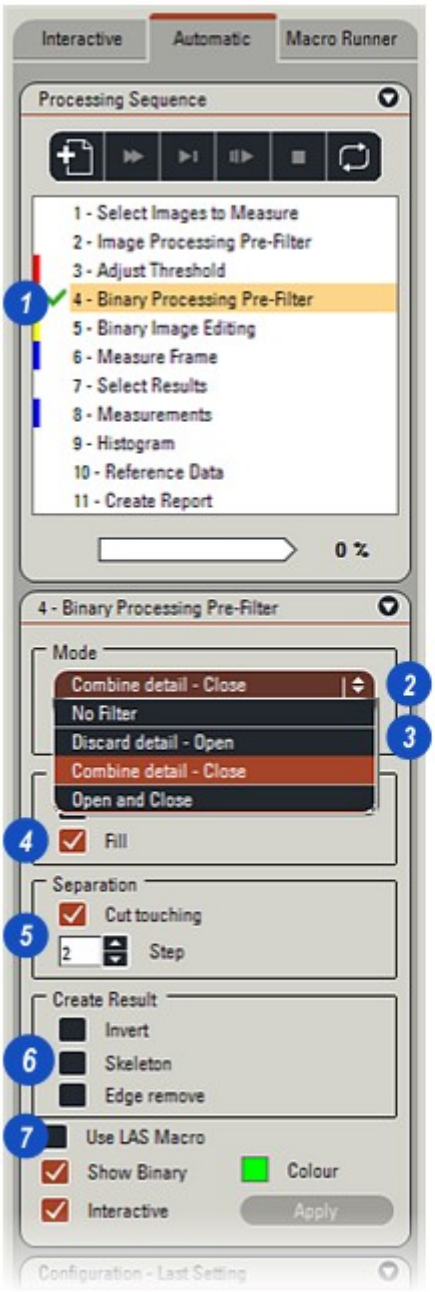
4: The Options panel contains the *Reconstruct* and *Fill* tools.

5: Separation, which helps to separate overlapping or touching features has a single tool – *Cut Touching* – enabled with a check box. There is a Step control associated with this tool.

6: Three tools – *Invert, Skeleton* and *Edge Remove* – are contained in the *Create Result* panel to modify the Input Binary Image in very specific ways.

7: To use *LAS Macro Runner*, click to enable the check box. Detailed help for LAS Macro: *Go there...*

Continued... 831



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1: The *Show Binary* control displays the *Binary Output Image* as it is modified. Generally, leave S*how Binary* enabled.

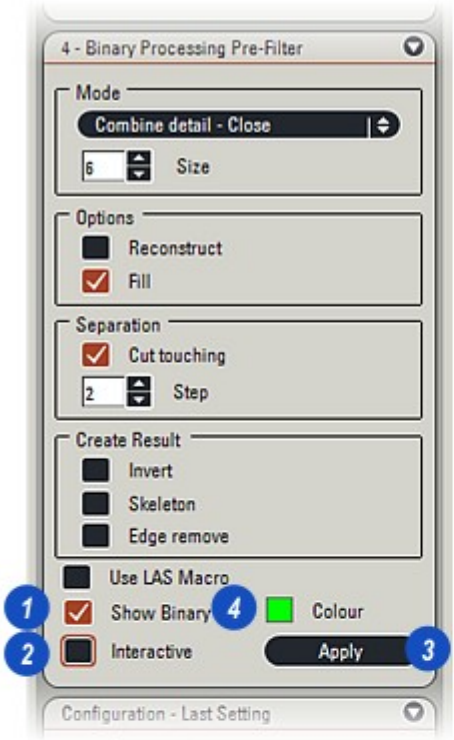
2: Click the *Interactive* check box to automatically start processing every time a change is made to the filters or controls. For complex image requiring a large number of 'passes' at different settings this can be time consuming. Better to disable *Interactive* and…

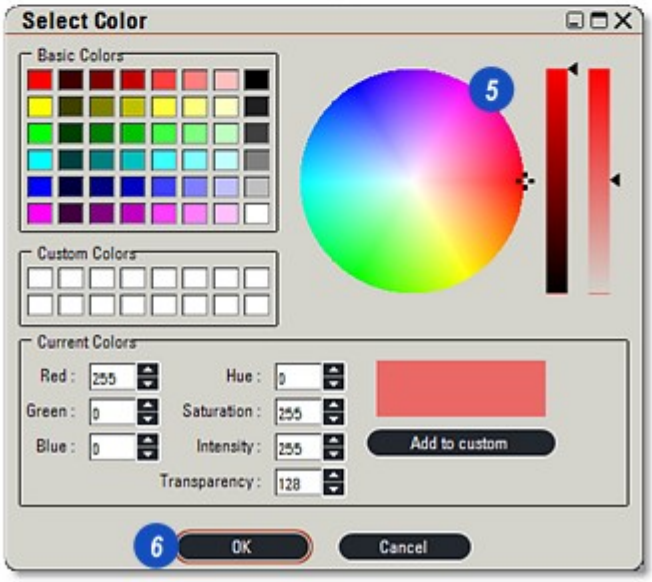
3: …use the *Apply* button instead. In this situation changes can be made to settings and only processed when the *Apply* button is clicked.

4: To change the default processing colour, click on the *Colour* button and…

5: …on the *Select Colour* dialog use the palette, colour wheel or sliders to select a new colour.

6: Click *OK* and the new selected colour will appear on the *Colour* button.





1: The original image comprising a group of pollen grains, ..

2: …is passed through a *Greyscale Pre-Filter > Smooth White Detail* that flattens and merges the background leaving the selected features in sharper contrast.

3: Using *Adjust Threshold* selects most of the pollen grains but leaves some with holes and fissures. Trying to fill them with the *Threshold Hue* and *Intensity* began to create noise on the background, and so…

4: …*Binary Processing* was used initially without filters and...

5: …the *Fill* tool only. Click the *Fill* check box to enable it. This filled the holes well but left some fissures open.

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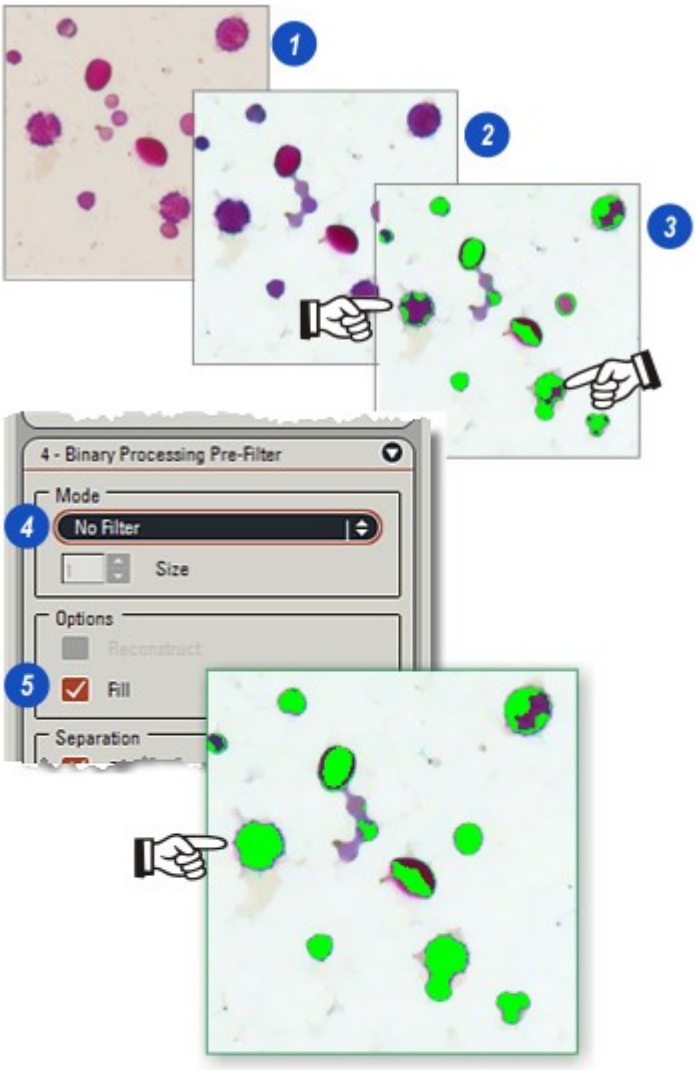

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The Combine Detail filter:

1: Using the *Combine Detail* filter and…

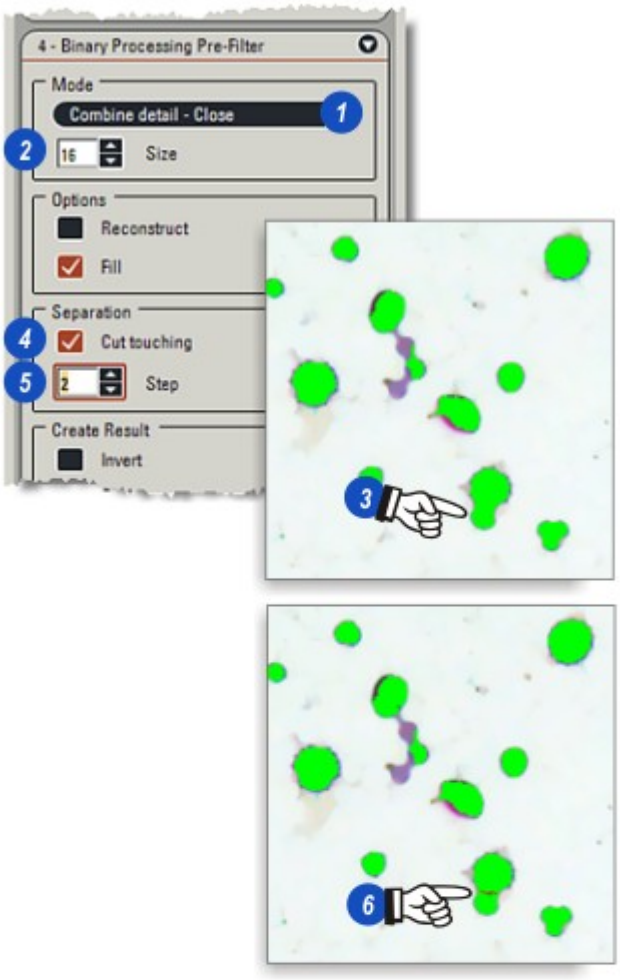
2: …gradually increasing the *Structuring Element Size* removes the fissures leaving the pollen grain features well defined ready for measurements.

The Cut Touching filter:

3: However, two of the pollen grains that were almost touching have now coalesced into a single feature and have to be separated.

4: Enable the *Cut Touching* tool by clicking the check box.

5: Gradually increase the *Step* value until a red line **(6)** appears to indicate that the features have been detected and separated.



Continued… 834

The Discard Detail filter:

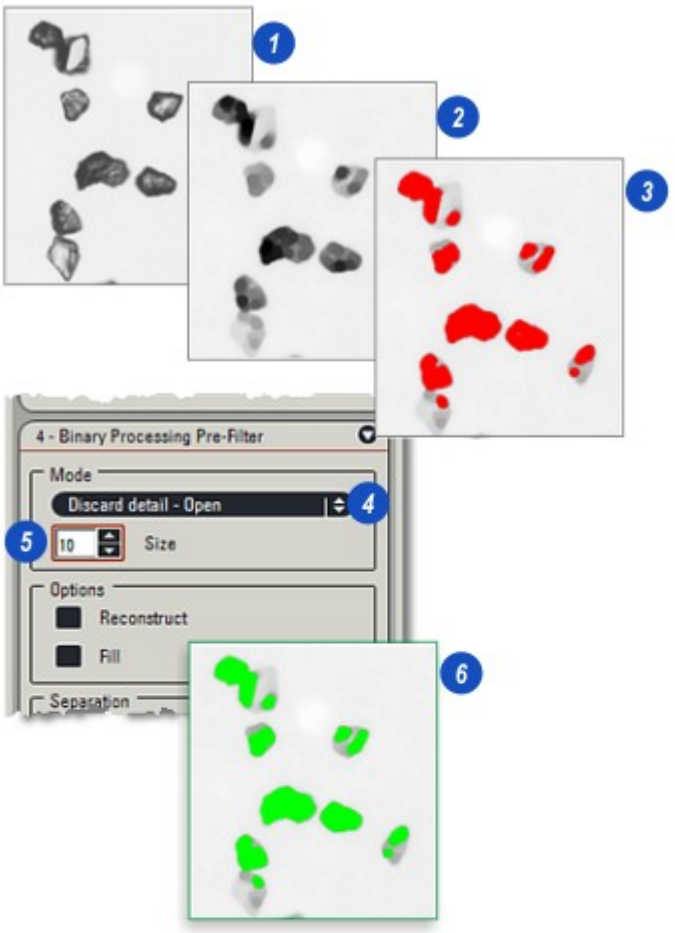
1: The original image of diamond chips, …

2: …has had the darker areas enhanced with the *Greyscale Pre-Filter > Smooth Black Detail.*

3: Applying *Adjust Threshold* has selected most of the darker areas but also included some mid-grey tones that are not required.

4: Using the *Binary Pre-Filter > Discard Detail* and gradually increasing the size of the *Structuring Element* **(5)** will shrink the selected areas, starting with the lighter tones, until only those required are highlighted for use in measurements.

6: The selected feature areas are highlighted in green, but a first step with a *Size* setting of 10 has had little effect on the Binary Image and so...



Continued… 835

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The Reconstruct tool:

1: ...the Size setting has been increased to 20 with the result that some of the lighter tones have been discarded and now show as red areas. The green areas are those required.

2: A final setting of 30 has retained only the very dark areas which was the aim of using the *Discard Detail* filter, and…

3: …a quick *Measurements > Number (Count)* check reveals that only the required features are included.

4: However, a part of the image has been rejected – possibly because two diamondchips were overlapping – and needs to be included without drawing in any other lighter features.

5: The *Options > Reconstruct* tool targets only small, closely related areas to include as selected.

6: The previously excluded area is now included and whilst this would not affect a *Number (Count)* measurement, it would be significant if *Area* was being measured.
To separate overlapping features enable the *Cut Touching* filter. In this case using Cut Touching with a setting of 3 resulted in a Number count of 4 rather than the original 2.

Separate touching features: Go there... 833

Continued... 836

The Invert tool:

1: The original image – part of a section through soft tissue…

2: …having been passed through the *Smooth White* greyscale filter and the *Threshold* filter to select the predominantly white area of the image. The selected areas are shown coloured green.

3: Enabling the *Create Result* > *Inver*t checkbox de-selects the previously selected areas – now displayed in colour mauve - and selects everything else. It is essentially a 'swap' tool.

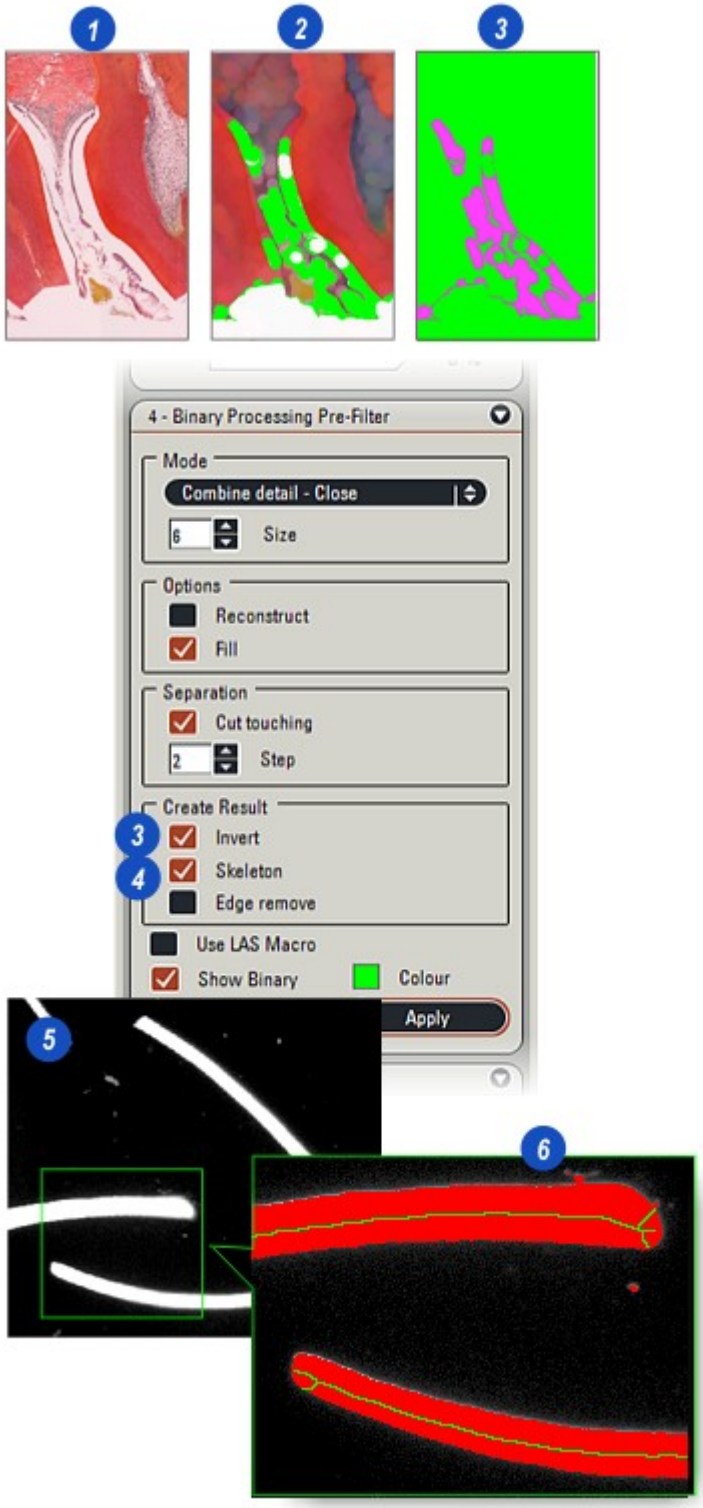
The Skeleton tool:

4: Enabling the *Skeleton* tool erodes selected features to the point where only a 'backbone', just 1 pixel wide remains.

5: The original image shows wool fibres.

6: The *Skeleton* tool applied showing the 'backbone' which traces the main Binary selection.

Continued... 837



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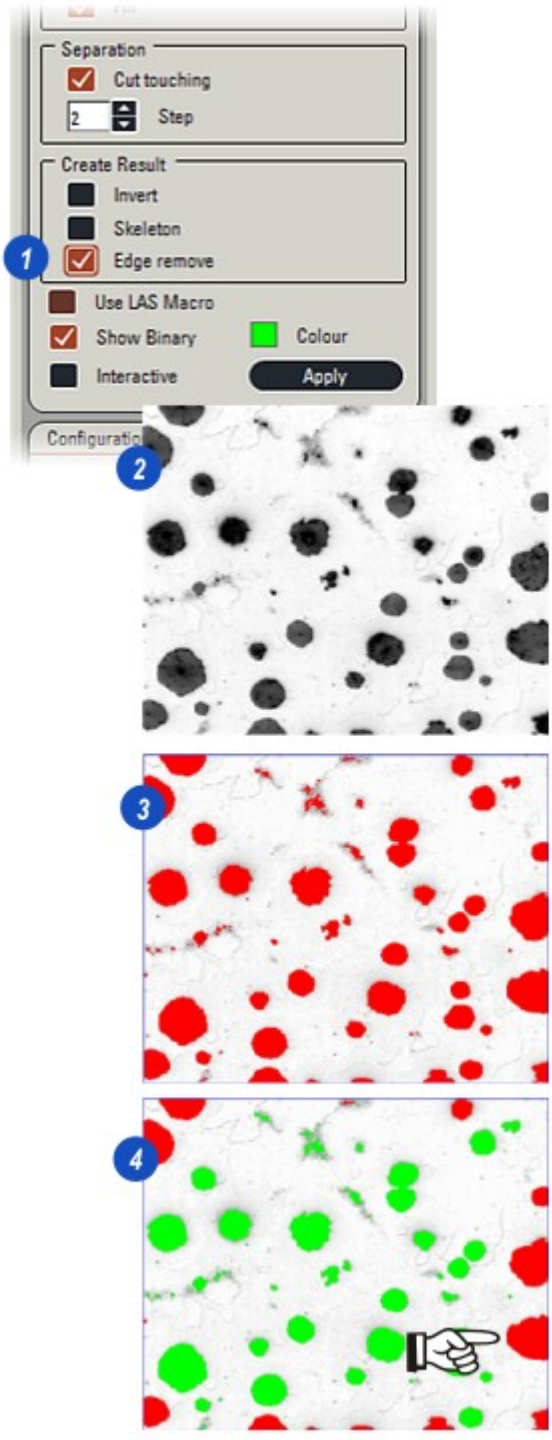
1: Click to enable the *Edge Remove* tool which will remove any features not wholly within the boundaries of the image.

2: The original image. The black features are to be counted but only if they are within the image.

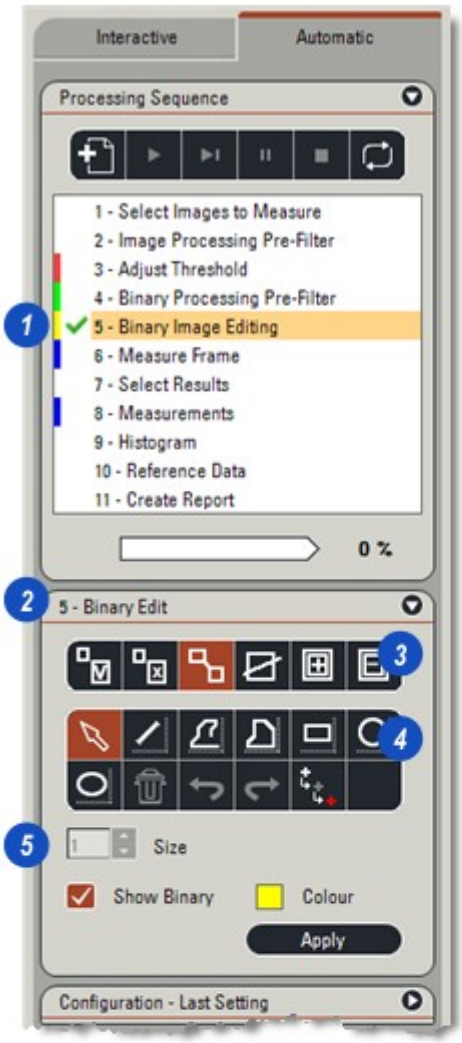
3: The illustration shows the *Binary Output* image after a *Threshold Adjust* - the red objects are selected for measurement.

4: With *Edge Remove* enabled any selected objects that are not completely within the image boundary - coloured red on the image - are ***not*** included in a measurement.



Binary Image Editing:

Binary Image Editing is a tool collection that provides the methods for working directly on Binary Images to add, remove, select and de-select features. Facilities also include drawing and filling shapes as well as grouping features.



1: On the Image Analysis main menu, click on the *Binary Image Editing* entry.

2: The Binary Image Editing panel appears. There are two groups of buttons:

3: *Mode* and…

4: *Tools*. Some of the Mode buttons affect the way that the Tools behave.

5: The *Size* window controls the line thickness for some of the Tools used for drawing.

The Binary Editing program uses three separate images:

- The original *Greyscale* image which remains unchanged.
- The *Binary Input Image* which is the Greyscale image converted to binary format showing the features of interest. The factory default colour for this is light green.
- The *Binary Output Image* which represents the edited image still in binary format. The colour for this is user selectable.

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1: Display the edited Binary Output Image by enabling the *Show Binary* check box. Disabled, and the unedited Binary Input Image as it was supplied to the editing program is displayed in the factory default colour green.

2: Click on the *Apply* button to apply to apply Mode changes to the Binary Output Image.

3: The colour of selected features on the Binary Output Image can be changed by clicking the *Colour* button.

4: On the *Select Colour* dialog, choose a colour from the swatches by clicking on it or…

5: …click and drag the hue sliders or…

6: …click and drag the marker on the Colour Wheel.

7: Click *OK*. The new colour appears in the *Colour* button.

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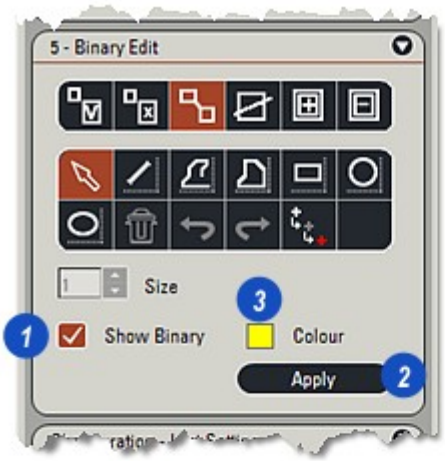
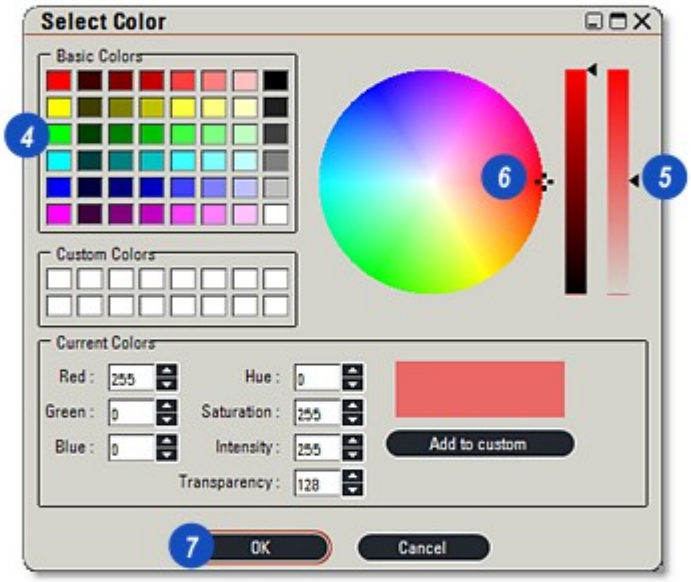


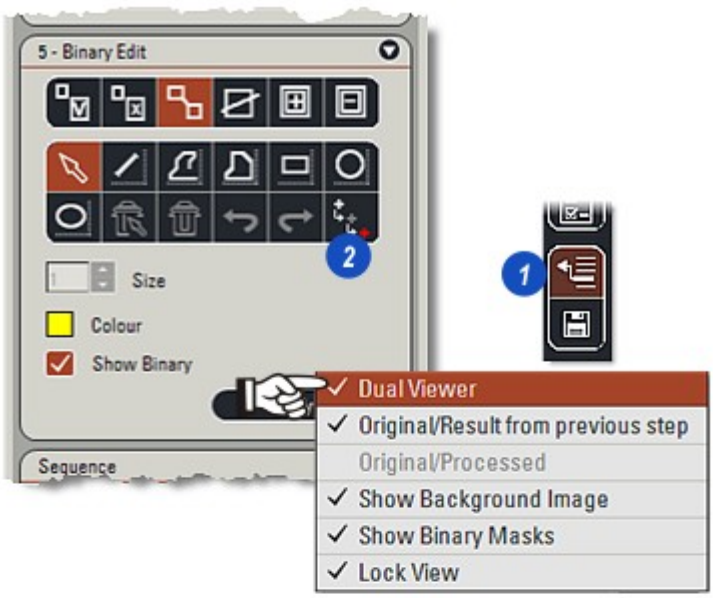


Binary Image editing is easier to use if the Viewer is set to display only the Binary Image – turn off *Split Screen* **(1).**

The Cursor colour can be toggled to either black or white to suit the image by clicking on the *Cursor Colour* button **(2)**.

Binary editing uses 3 images:

- The *Input Greyscale* (original) which remains unchanged,
- An *Input Binary* created with the Threshold tool with the selected features coloured green (factory setting), and
- The *Output Binary* which is the result of the editing process. This will be used with the measurement tools. The colour of the selected features is user defines.



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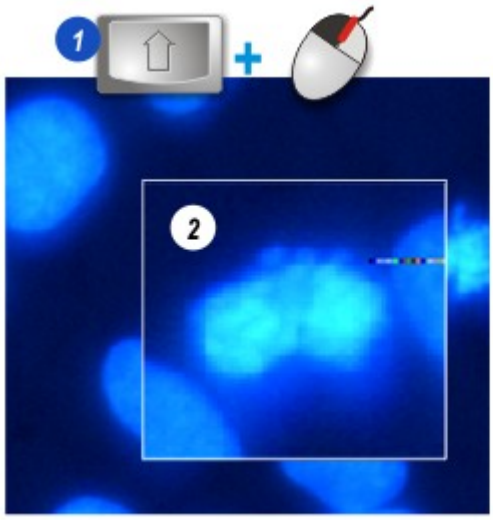
To enlarge parts of either the *Input Image* or the *Binary Output Image:*

1: Hold down the keyboard *Shift* key and click on a portion of the image to enlarge. The magnified area tracks the mouse movements **(2)**. Release the *Shift* key to close the magnifier.



To isolate a specific area – a Region of Interest (RoI) - on the image for measurements:

1: Click on the *Accept Mode* button. Four drawing tools are available to create the RoI...

2: The *Freehand* tool, the *Rectangle*, the *Circle* and the *Ellipse*. Click on the most appropriate tool – a *Rectangle* is shown on the illustrations.

3: Click on the top left corner of the Region to be selected and holding down the mouse button, drag to the bottom right corner. The drawn region is filled with a semi-transparent colour to make identifying the selected features easier.

4: To delete the Region and start again, click on the *Undo* button.

5: When the drawn region is correct click on the *Apply* button

6: Enabling *Show Binary* will display the selected features in the chosen binary mask colour.

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Isolate part of the image to prevent enclosed features from being included in the measurements as follows:

1: Click on the *Reject Mode* button.

2: Four drawing shapes are available:
- Irregular Fill Area,
- Rectangle,
- Circle and
- Ellipse.

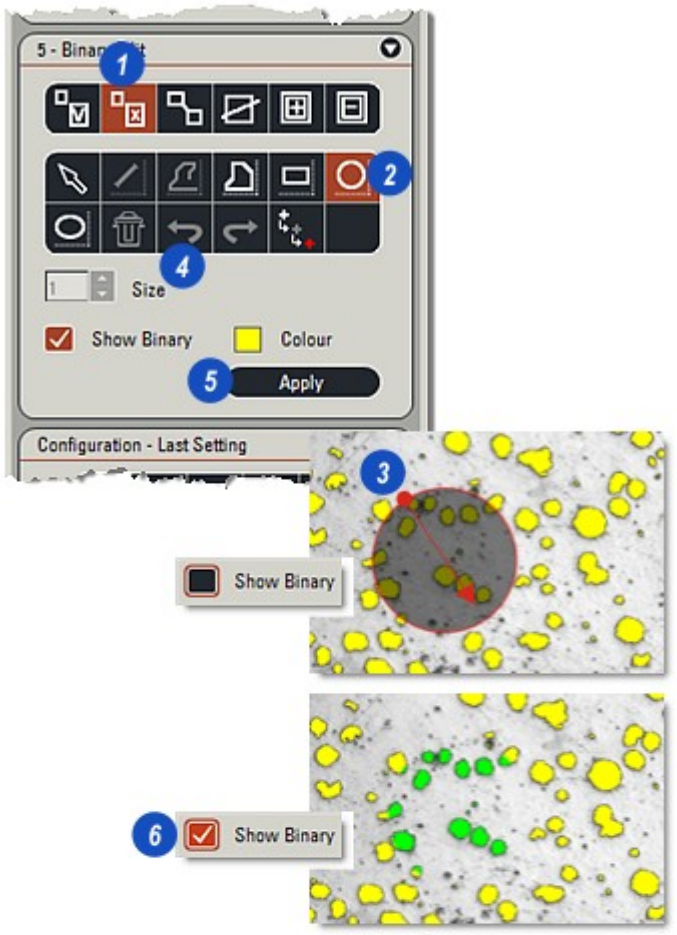
Click on the appropriate shape to select it and…

3: Click on the image and holding down the mouse button drag down and to the right to draw a *Region of Interest* the features within which will be excluded from the measurements. The Region has a translucent fill.

4: To delete the Region and start again click on the *Undo* button.

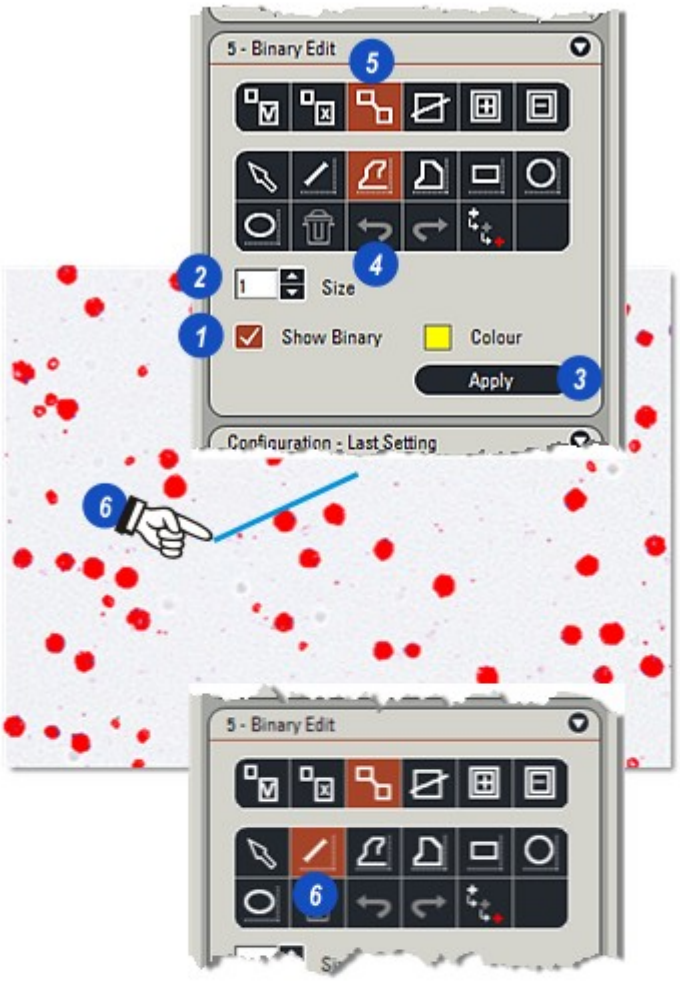
5: Click the *Apply* button to set the rejected Region.

6: Enable the *Show Binary* check box to view the rejected features in the chosen Binary Output image colour.



Continued... 844

In *Drawing Mode*, lines, open shapes and filled shapes can be drawn directly on to the *Binary Output Image*.



1: Enable the *Show Binary* checkbox otherwise the drawing will not appear. On the illustration the shapes are shown in blue for clarity; Normally, they appear in the chosen *Binary Output Image* colour which in this case would be red.

2: For the *Line* and *Irregular Freehand* tool, set the line thickness by clicking on the *Up/Down* arrows to the right of the Size text box.

3: When a drawing is satisfactory, click the *Apply* button.

4: Use the *Undo* button to delete individual drawings or the *Delete All* (Trash Can) button to remove all drawings from the image.

5: Click on the *Drawing Mode* button.

Draw a Straight Line:

6: To draw a straight line click on the *Line* button. Click and hold down the mouse button on the image where the line is to begin and drag to the line end point. Release the mouse button.

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Draw a Filled Circle:

1: Click on the *Circle* tool.

2: Click and hold down the mouse button on the image at a point representing the edge of the *Circle*.

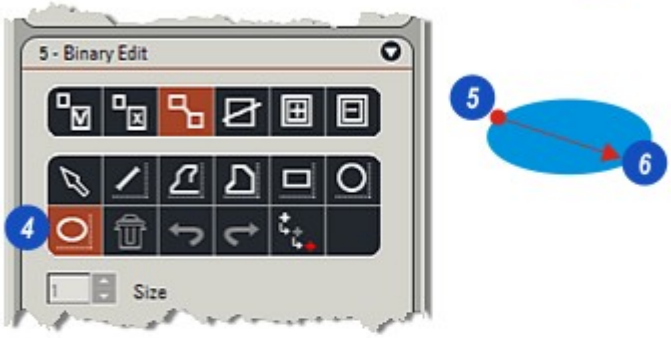
3: Drag in any direction. An outline of the *Circle* is drawn as the mouse is dragged. Release the mouse button when the *Circle* is complete.

Draw a Filled Ellipse:

4: Click on the *Ellipse* tool.

5: Click and hold down the mouse button on the image at a point representing the edge of the *Ellipse*.

6: Drag in any direction. An outline of the *Ellipse* is drawn as the mouse moves. Release the mouse button when the *Ellipse* is complete.



Continued... 847

Irregular Freehand Shape:

1: Click on the *Freehand* tool button.

2: Click and release the mouse button on the image at the shape's starting point.

3: Move the mouse to the next point on the shape and click again. A line will be drawn connecting the two points.

4: Repeat Step **(3)** until all of the points around the shape are plotted and at the final point double-click to complete it.

Irregular Freehand Shape with Fill:

5: Click on the *Freehand Fill* tool button.

6: Click and release the mouse button on the image at the shape's starting point.

7: Move the mouse to the next point on the shape and click again. Line will be drawn connecting the two points to create a closed shape which will be filled with solid colour.

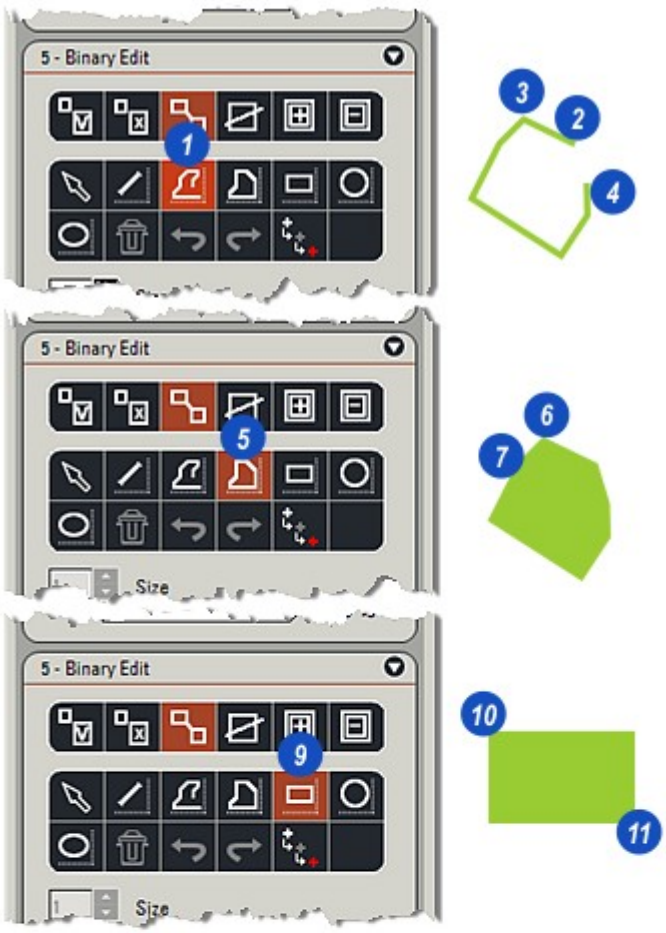
8: Repeat Step **(7)** until all of the points around the shape are plotted and at the final point ***double-click*** to complete it.

Draw a Filled Rectangle:

9: Click to select the *Rectangle* tool.

10: Click and hold down the mouse button on the image at the top left-hand corner of the *Rectangle*.

11: Drag down and toward the right-hand bottom corner of the *Rectangle*. Release the mouse.



Continued... 845

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A group of features selected on the Binary Output Image are ignored during measurements by:

1: Enable the *Show Binary* checkbox.

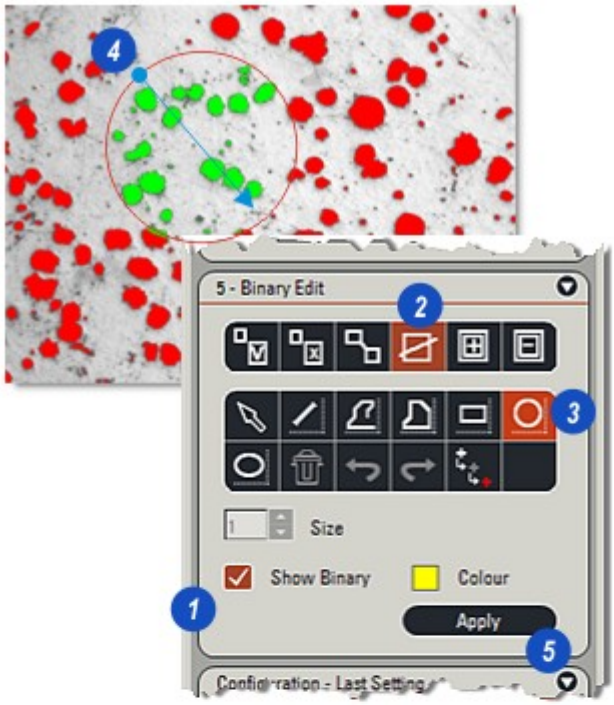
2: Click to select the *Erase* button.

3: Select either the *Outline, Rectangle, Circle* or *Ellipse* tool and…

4: …click on the *Input* or *Output* image and draw a shape to enclose the features to be ignored. The features are displayed in the default colour.

5: Click on the *Apply* button when the grouping is correct.

During the measurement process the selected features will be ignored and not included in result data.



Continued... 848

Includes only those features individually selected in the measurement data.

1: Click to enable *Show Binary*.

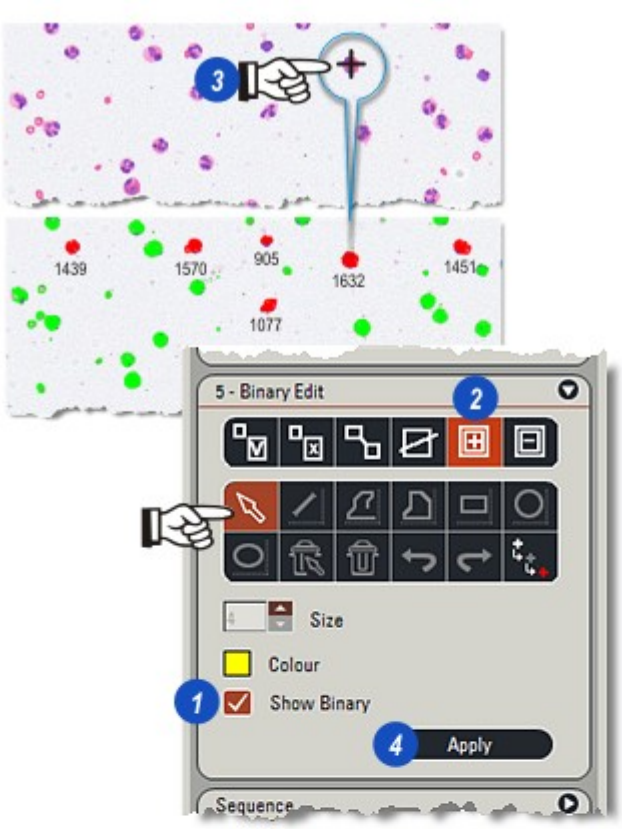
2: Click on the *Keep* button. The *Select* tool is automatically selected.

3: Click on individual features (the illustration cross hairs are shown emphasised for clarity) that will be measured.

4: Click the *Apply* button.

Features not selected will be ignored. Each selected feature is displayed in the default colour.

Continued... 849



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Individual features that will not be included in the measurements are selected in *Delete Mode* as follows:

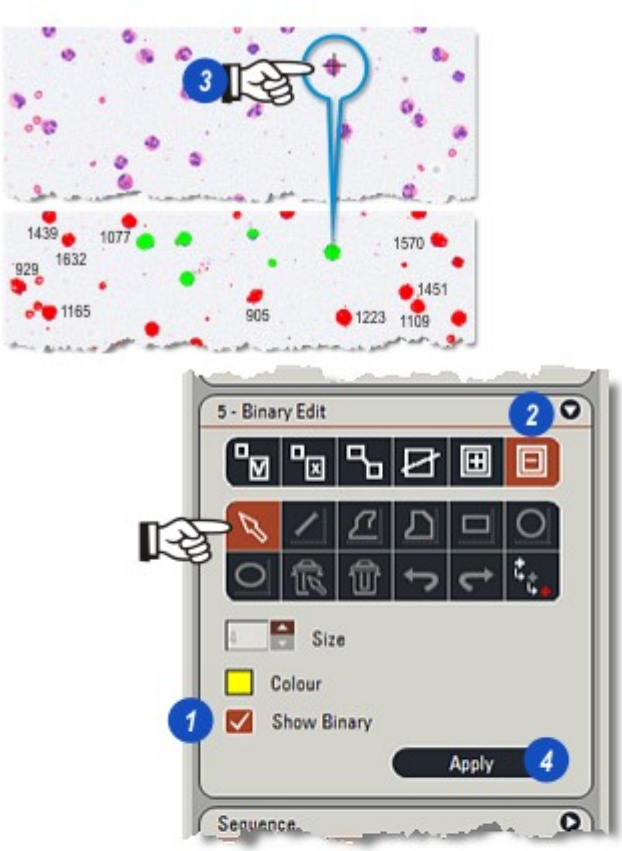
1: Enable *Show Binary* by clicking the check box.

2: Click to select the *Delete* Mode button. The *Select* tool is automatically enabled.

3: Click on the individual features to be excluded from the measurements. Each is displayed in the default *Binary Edit* colour green.

4: Click the *Apply* button.

When a measurement is made the features selected will be ignored.



1: *Undo* reverses the last edit action and *Redo* repeats the last action.

2: *Delete* all actions and drawings.

3: Delete the last drawing by selecting it with the *Selection* tool and then clicking the *Delete Selected* button.

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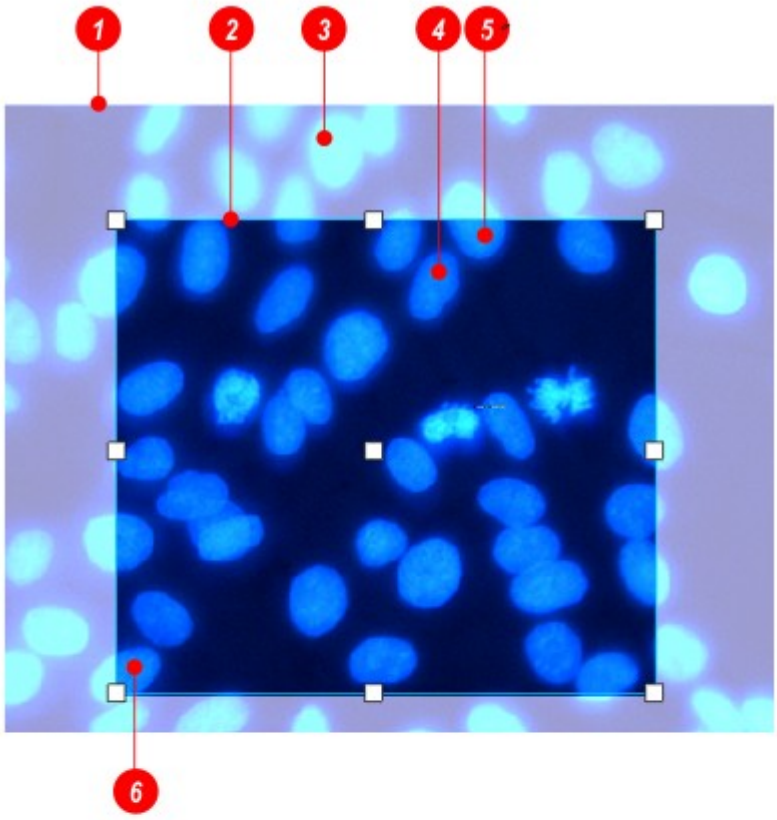
A *Measure Frame* determines the area of an image that will be used for analysis and measurement. It can be the entire image or more usually a selected, representative area. In the illustration:



1: Is the edge of the image.

2: A typical *Measure Frame* outlined in blue.

3: The Guard Region.

For a selected area, software rules concerning objects that straddle or touch the Measure Frame boundary dictate:

Any object that has its bottom right hand pixel lying within (not on) the Measure Frame boundary is included in the analysis even though any of its other pixels lie in the Guard Region – outside the Measure Frame boundary.
Field measurements are performed within the Measure Frame and any pixels outside the Frame are not included.

4: This object is completely within the Measure Frame and is included.

5: Although starting outside the Measure Frame, the bottom right-hand pixel of this object is inside the Measure Frame and *will* be included.

6: The bulk of this object is within the Measure Frame but because its bottom right-hand pixel is either touching or outside the frame boundary it is not included.

Continued... 852

To Create a Measure Frame:

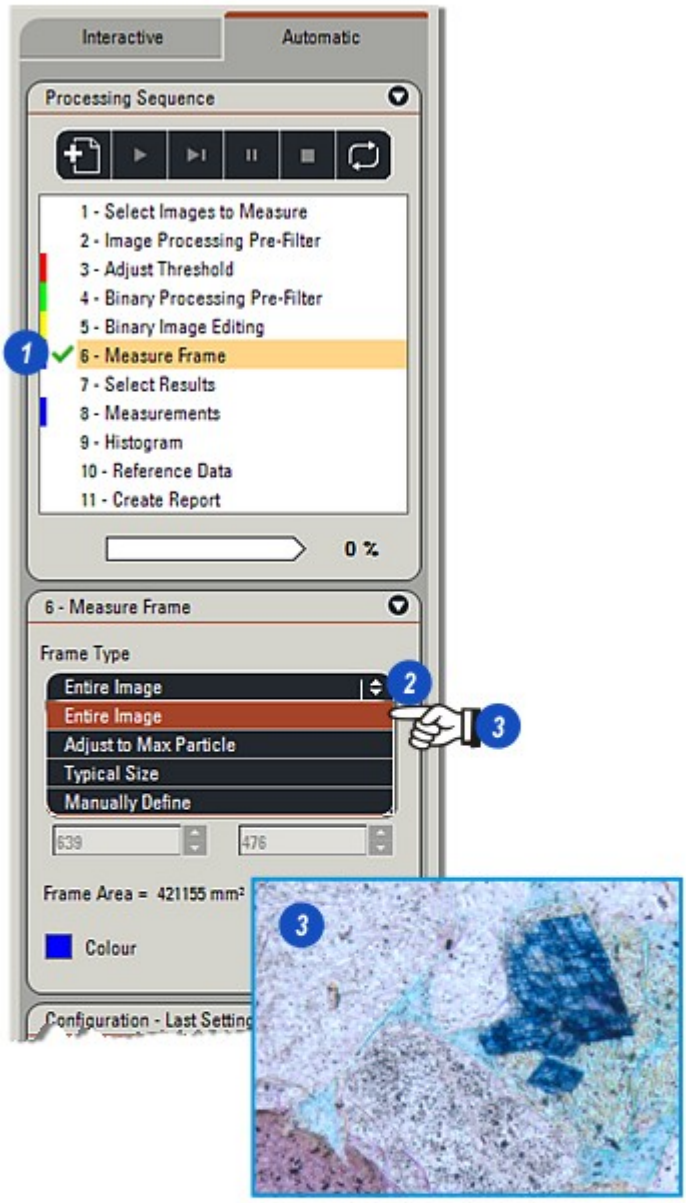
1: Click on the *Measure Frame* option in the *Processing Sequence* window.

2: There are 4 Measure Frame options available all accessed by clicking the arrows to the right of the *Frame Type* list box and clicking the required option from the drop down list.

Entire Image Measure Frame:

3: To include the entire image in the Measure Frame click to select the Entire Image option. The frame boundary coincides with the edges of the image.

Continued... 852



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Frame to Suit the Maximum Feature Size:
The *Adjust to Max Particle* option creates a frame based upon an feature size the user enters:

1: Swipe the *Particle Dia*meter text box and type a value in micro-metres.

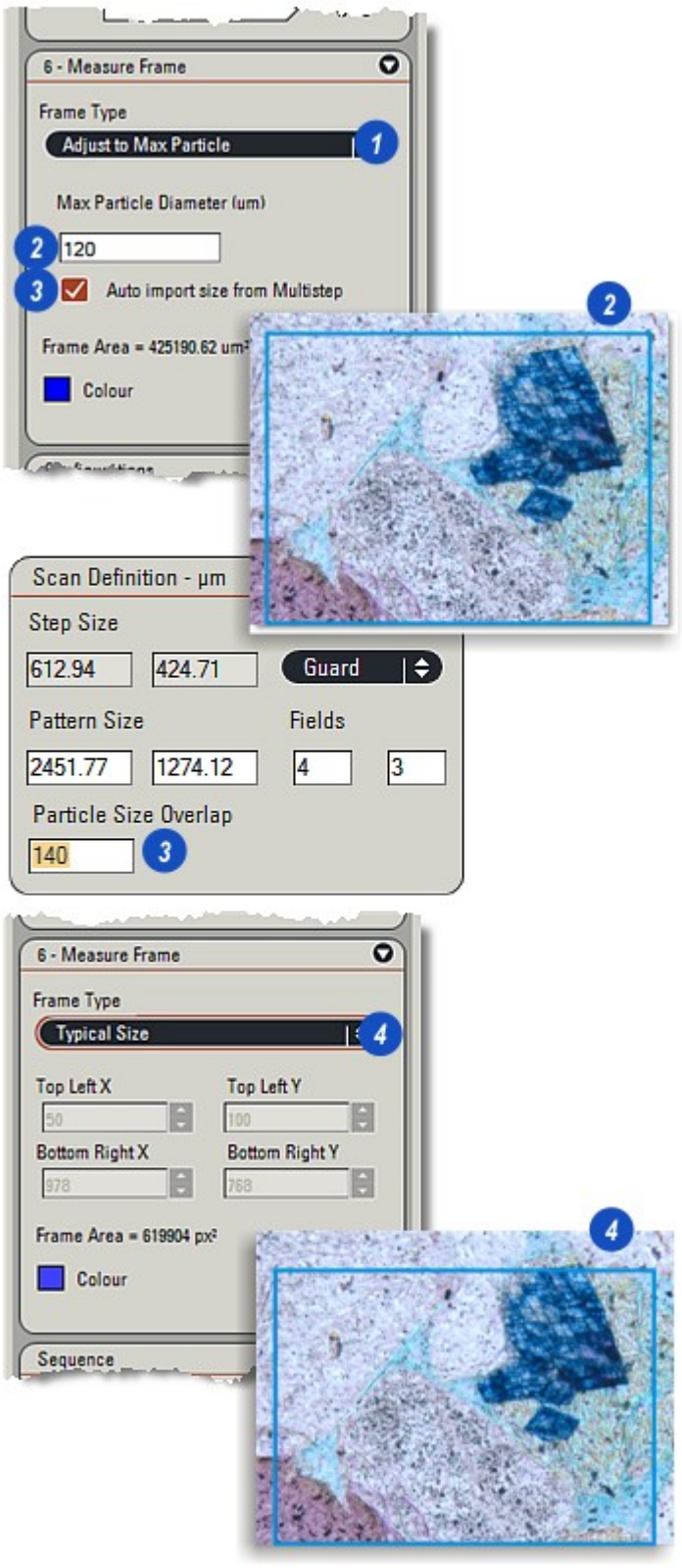
2: The *Guard Region* is based on:
100% of the particle diameter at the top:
50% of the particle diameter on the sides:
0% at the bottom.

3: To create a *Measure Frame* based upon the particle size in a sequence such as a series of *Multistep* images, click to enable the *Auto Import Size from MultiStep* check box. The check box is only available if the sequence is selected for measurement and appended.

Typical Measure Frame:

4: The *Typical Measure Frame* option will suit a wide variety of microscope images. Based upon an 'average' particle size, it creates a *Guard Region* with:
100% of average particle diameter at the top:
50% of average particle diameter on the sides:
0% at the bottom.

It is a quick and easy 'one-click' operation.



Continued... 853

Manually Defined Measure Frame:
This option allows the user to create a frame of specific dimensions and position it anywhere on the image.

1: Click on the arrows to the right of the *Frame Type* header and from the drop down menu click to select *Manually Define.*

2 & 3: Swipe the co-ordinate text boxes and type, or using the up/down arrows to the right of each box enter a value - two co-ordinates are required *Top Left X/Y* and *Bottom Right X/Y* – or…

4: …click and drag the frame handles to the required size.

5: The *Measure Frame* can then be positioned by clicking and dragging the handle in its centre.

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The *Measure Frame* colour can be changed by:

1: Click on the *Colour* button.

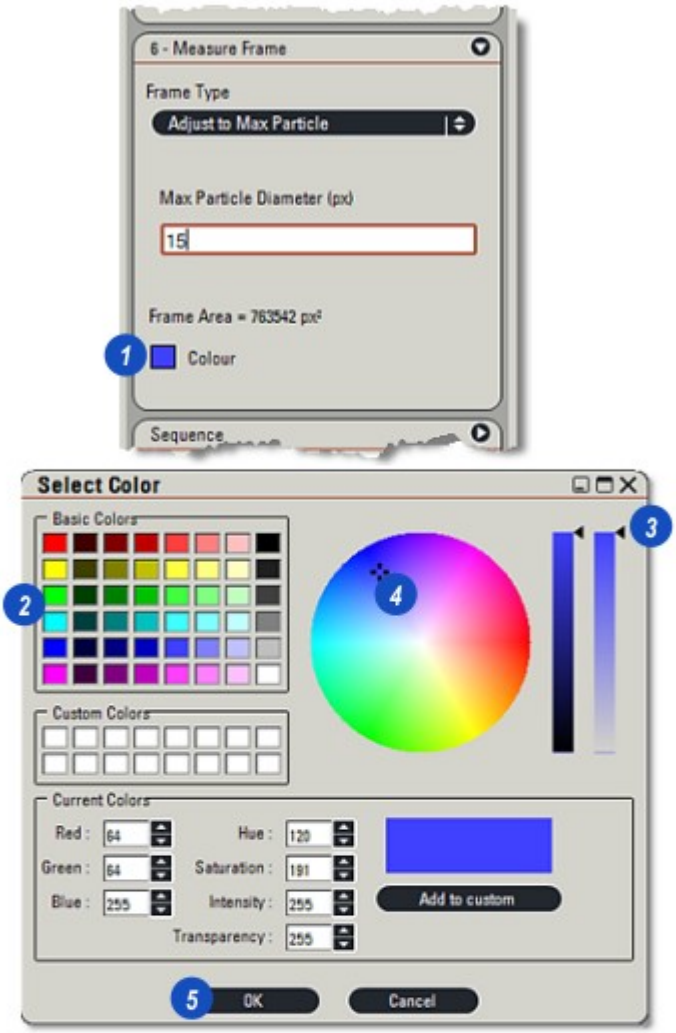
2: On the *Select Colour* dialog choose a new colour by clicking on a pallete well or...

3: ...clicking and dragging the Hue sliders or...

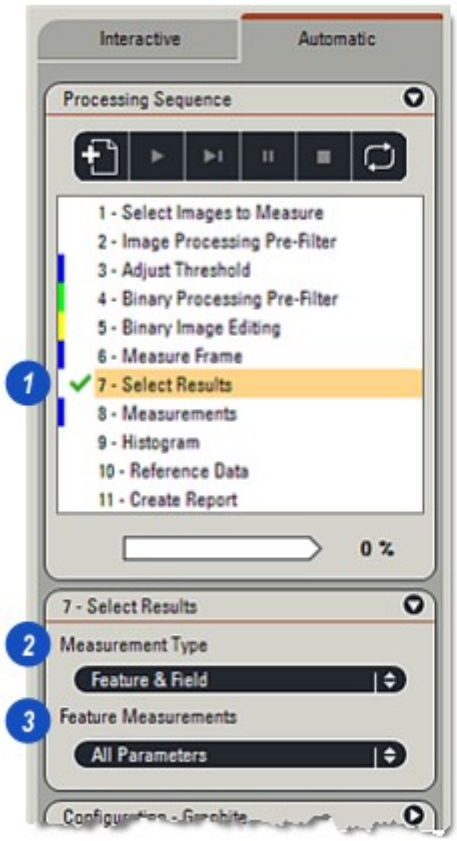
4: ...clicking and dragging the target mark on the *Colour Wheel.*

5: Click *OK*. The *Colour* button changes to reflect the new colour.

Continued... 852



Use *Select Results* to determine if all of the parameters are to be measured or just a predetermined, commonly used group. This option is particularly useful for images having many features with which there would be an appreciable processing delay to include parameters not really needed.
Select Results is also used to determine the results actually shown on the *Grid* and *Summary*.



1: Click on the *Select Results* entry on the main menu. The control panel is divided into two:

2: *Measurement Type:* The range of measurements to be displayed and reported, and…

3: *…Feature Measurement:* The measurements that will be made and available to display and report.

Continued… 857

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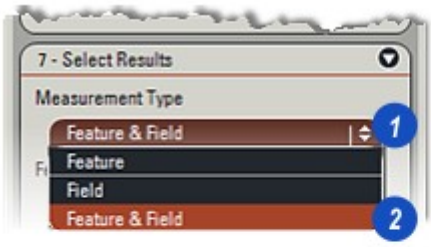
Measurement Type:
Select the range of measurements to be displayed by:

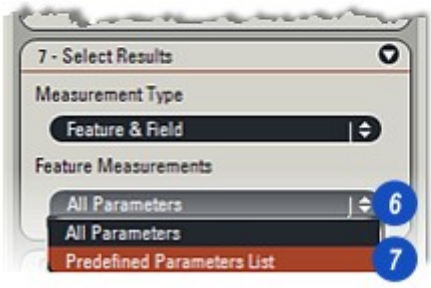
1: Click on the arrow to the right of the *Measurement Type* header to reveal the options.

2: Click to select:
Feature: **(3)** Displays only the selected feature results.

Field: **(4)** Displays results for *Field Data.* Measurements that describe a property of a complete *Field of View* are called *Field Measurements* and the data produced is called *Field Data.* Parameters such as *Field Area* and *Field Perimeter* are *Field Data*. It is also so-called because it represents the summed value for all objects within the *Measure Frame* regardless of whether they are touching or separate This is in contrast to *Feature Data* that produces a separate value for each isolated feature or 'blob' that ends inside the *Measure Frame*.

Feature & Field: **(5)** The most comprehensive results but with multiple images can result in a very large display and report.



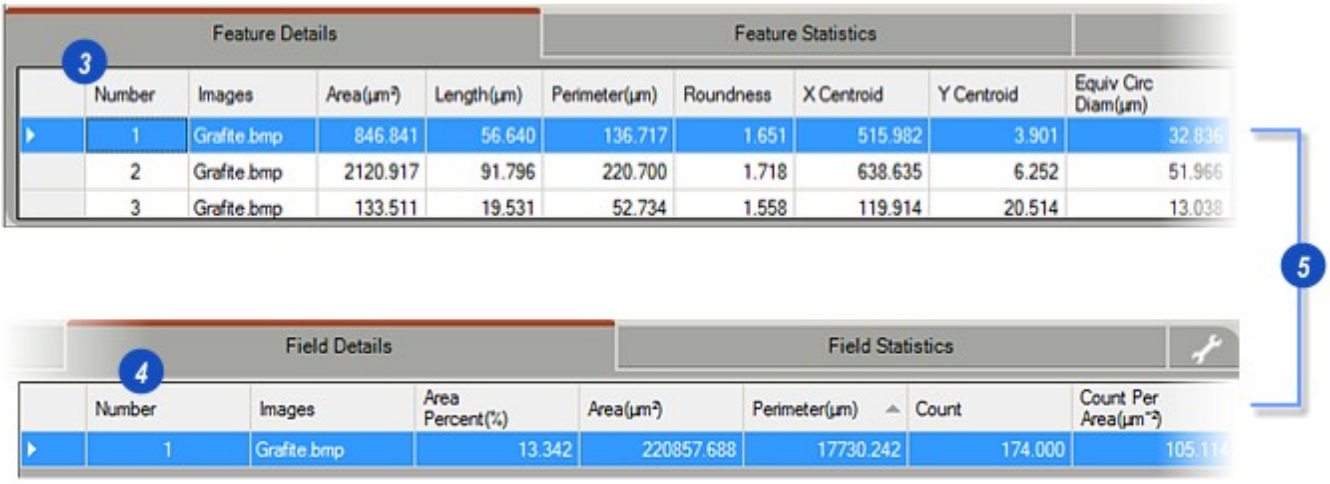


7: Click to select:
All Parameters: All possible measurements are made but the range can be modified: Go there...

Predefined Parameters: A selection of the more commonly used measurements.

Continued... 858

Feature Measurements:
6: Click on the arrow to the right of the *Feature Measurements* header to reveal the options.



The parameters calculated and then displayed on the *Grid* are determined by the settings selected on the *Select Field Details* and *Select Feature Statistics.* The dialog depends upon the results tab currently displayed, so first click on either *Feature* or *Field* tab.

1: Click on the Setup tool to the right of the results tabs.

2 & 3: Either the *Field Details* or *Feature Statistics* dialog will appear.

4: Click to enable the check box to the right of the item required to display.

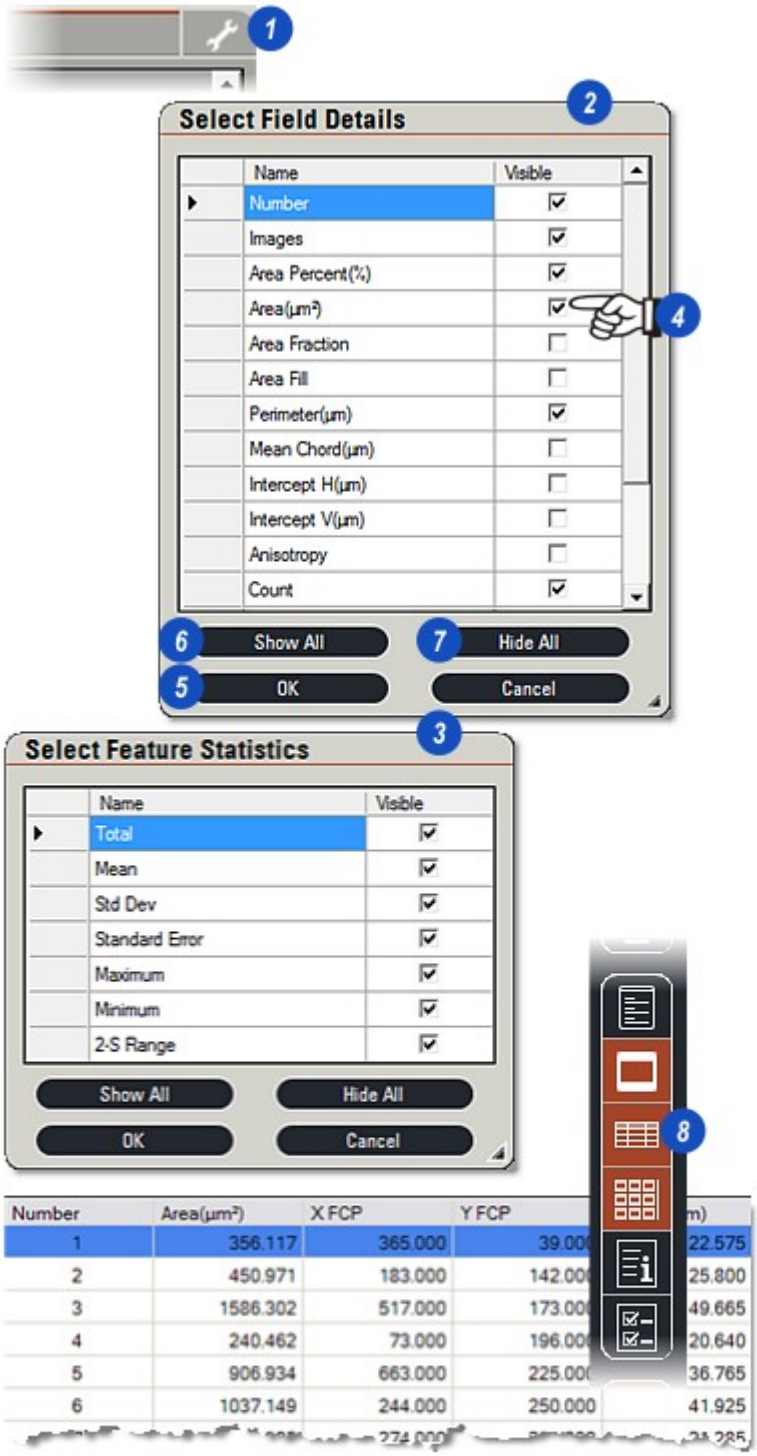
5: Click *OK* to save the settings.

6: …click on the *Show All* button to select all of the items.

7: Clicking on the *Hide All* button to de-select all of the items.

8: To display the Results Grid click on the *Side Toolbar Show/Hide Grid* button. This is a toggle – click to display, click again to hide.

Continued… 859



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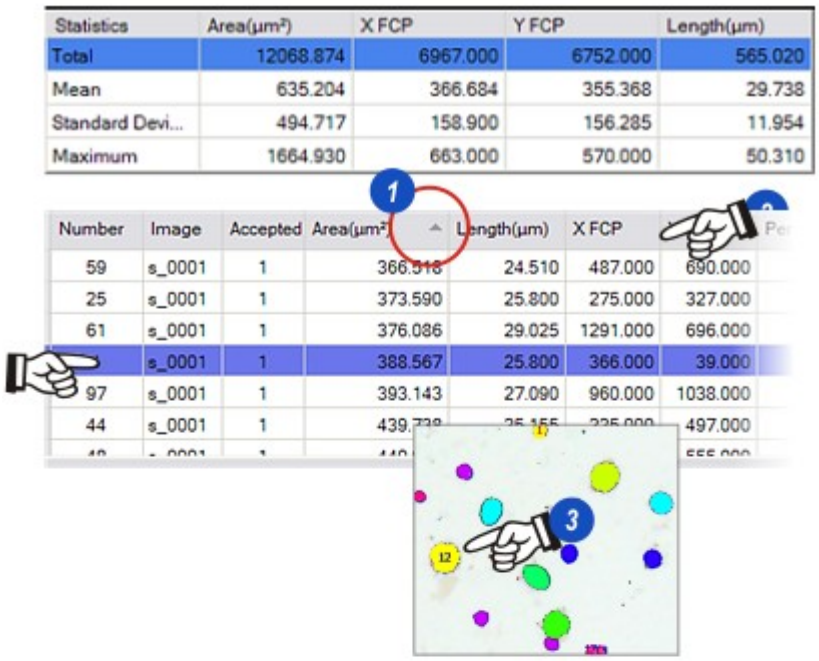
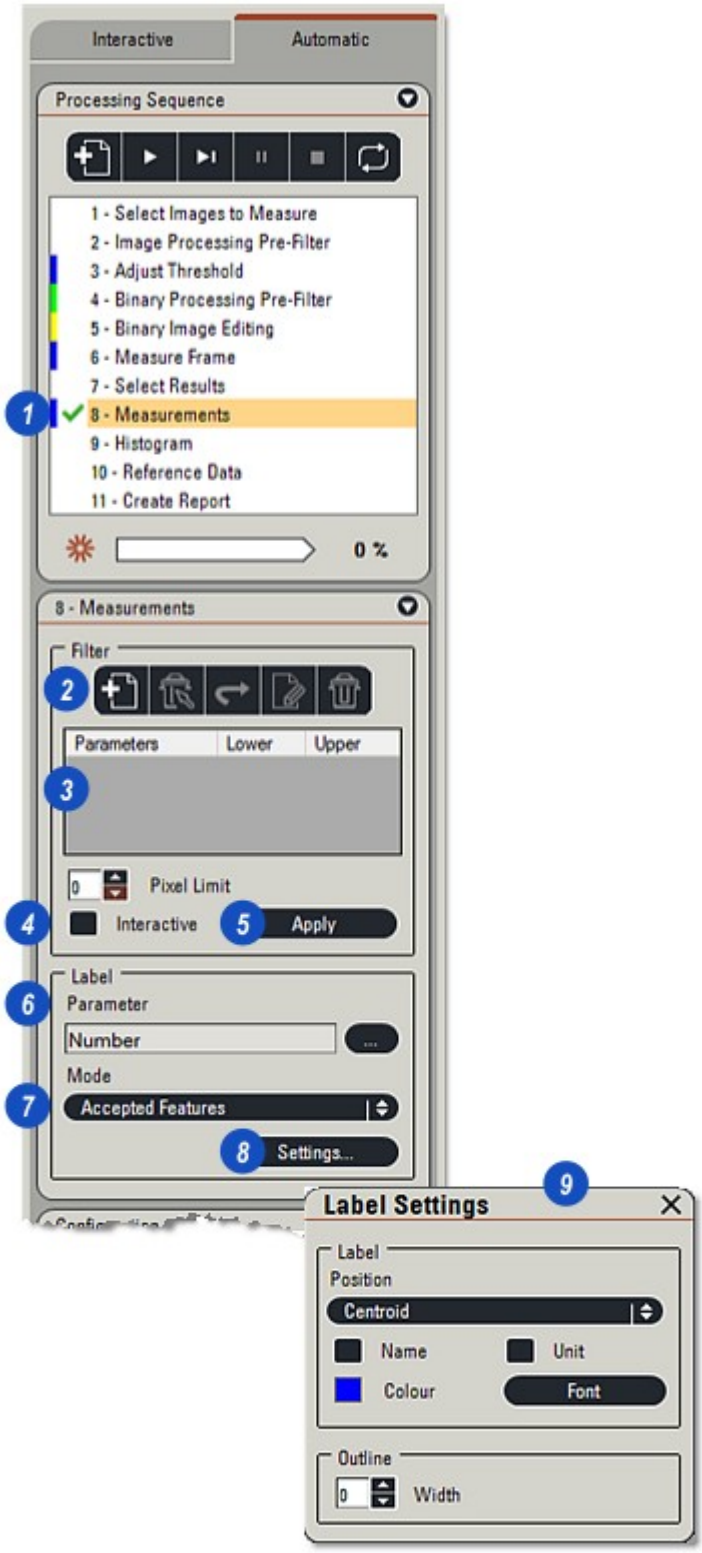
1: Columns that contain results can be order High-to-Low or Low-to-High by clicking on the area to the right of the column title. The small arrow that appears shows the direction of sort. This is a very fast option for isolating features of interest.

2: Column width can be changed by clicking on the dividing rule and dragging it to the desired size.

3: Click on an entry in the *Grid* to highlight the feature on the *Output Image.*



LAS Image Analysis Measurements uses the *Binary Output Image* of all the selected features and applies a wide range of fast, precise measurement algorithms. The scope of the measurements depends upon the *All* or *Predefined* options in the *Select Results* component.
Detailed descriptions of the *Measurement Parameters* can be found in the Appendix: *Go there...* 905



1: To reveal the control panel click on the *Measurements* entry on the main menu.

2: The *Filter* toolbar used to create and modify *Parameter* limits.

3: The *Parameter Filter* list window.

4: To automatically apply setting changes to the output image, click to enable the *Interactive* check box.

5: Disable *Interactive* to allow manual updates to images via the *Apply* button.

6: On the *Label* panel, *Parameter* determines the name that will appear against a feature - for example, *Area*.

7: Use the *Mode* menu to select the features to label.

8: Click on the *Settings* button to display the *Label* settings dialog **(9).**

Continued… 863

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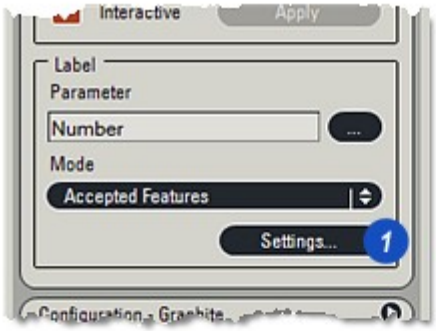
1: On the *Label* panel, click on the *Settings* button to reveal the *Label Settings* dialog:

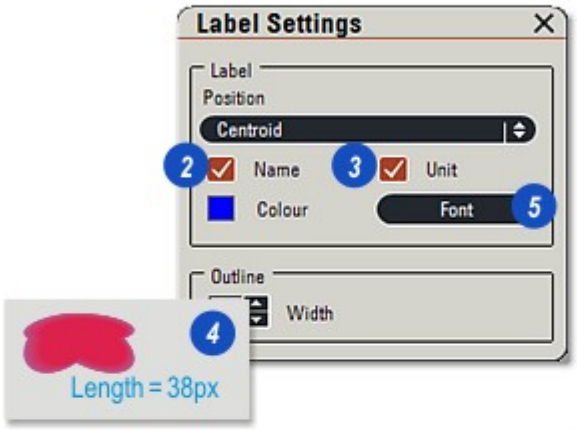


Display the Feature Name:

2: The *Parameter* – Length, Number, Diameter etc – can be displayed alongside the feature and result by enabling the *Name* checkbox.

Display the Feature Measurement Unit:

3: Enabling the *Unit* checkbox will display the measurement units – pixels (px), millimetres (mm) – next to the feature.
Figure **(4)** shows how the Name and Units displays appear.
To set the number of digits following the decimal place on the display, change the *Measurement Display* value on the *Preferences > Image* dialog. *Go there...*



Changing the Label Font:

5: Click on the *Font* button. The *Font* dialog appears.

6: Click to **c**hoose a *Font…*

7: …a *Font Style* and…

8: …a *Font Size* (in points).

9: Click *OK.*

Continued... 862

Label Position:

1: Click on the *Settings* button on the *Label* panel. The *Label Settings* dialog appears.

2: Click on the arrows to the right of the *Position* header to reveal the label position options.

3: Click to select a label display position:

Centroid displays the label in the centre of the object.

Below-Centre displays the label below and on the centre line of the object.

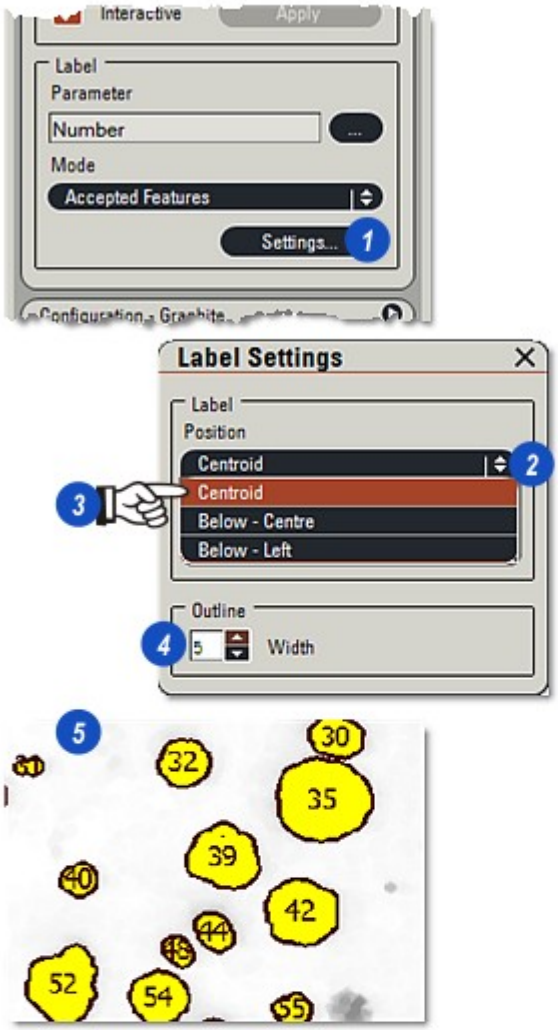
Below-Left displays the label below and to the left of the feature.

Feature Outline:

4: To draw an outline around the selected features, change the *Outline Width* by clicking the Up/Down (thicker/thinner) arrows to the right of the *Outline* text box. A value of '0' turns off outlining.

5: Outlined features. The *Outline* colour is the same as the *Label Font* colour.

Continued... 864



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The colour of the *Labels* and results displayed on the *Measurements Binary Output Image* can be changed to suit the user.

1: Click on the *Settings* button on the *Labels* panel. The *Label Settings* dialog appears.

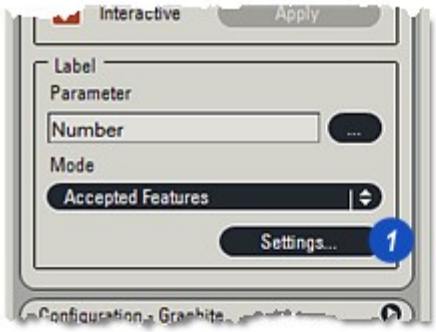
2: Click on the *Colour* button. The *Select Colour* dialog appears.

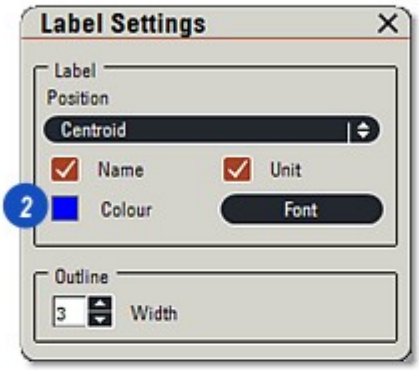
3: Choose a colour from the swatches by clicking on it or…

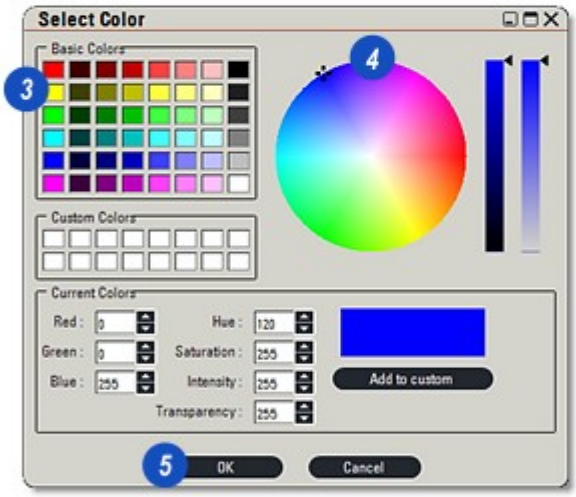
4: …click and drag the marker on the *Colour Wheel.*

5: Click *OK.* The new colour appears in the Colour button.

Continued.. 861, 864







Two options are available that determine the range of parameters to be measured:

All Parameters which calculates a value for every parameter against every selected object, and...

Predefined Parameters that calculates only a commonly used range of parameters.

The selection is made in *Select Results*: *Go there...* 856

The *Parameter* options that will be available on the *Select Measurement Parameters* dialog will depend upon the range selected.

1: Click on the browse button to the right of the *Parameter* text box.

2: If the *Predefined Parameters* option has been previously selected, the *Select Measurement Parameters* dialog will display only the most commonly used parameters.

3: If *All Parameters* had been chosen, all of the possible measurement parameters will appear - a much longer list.

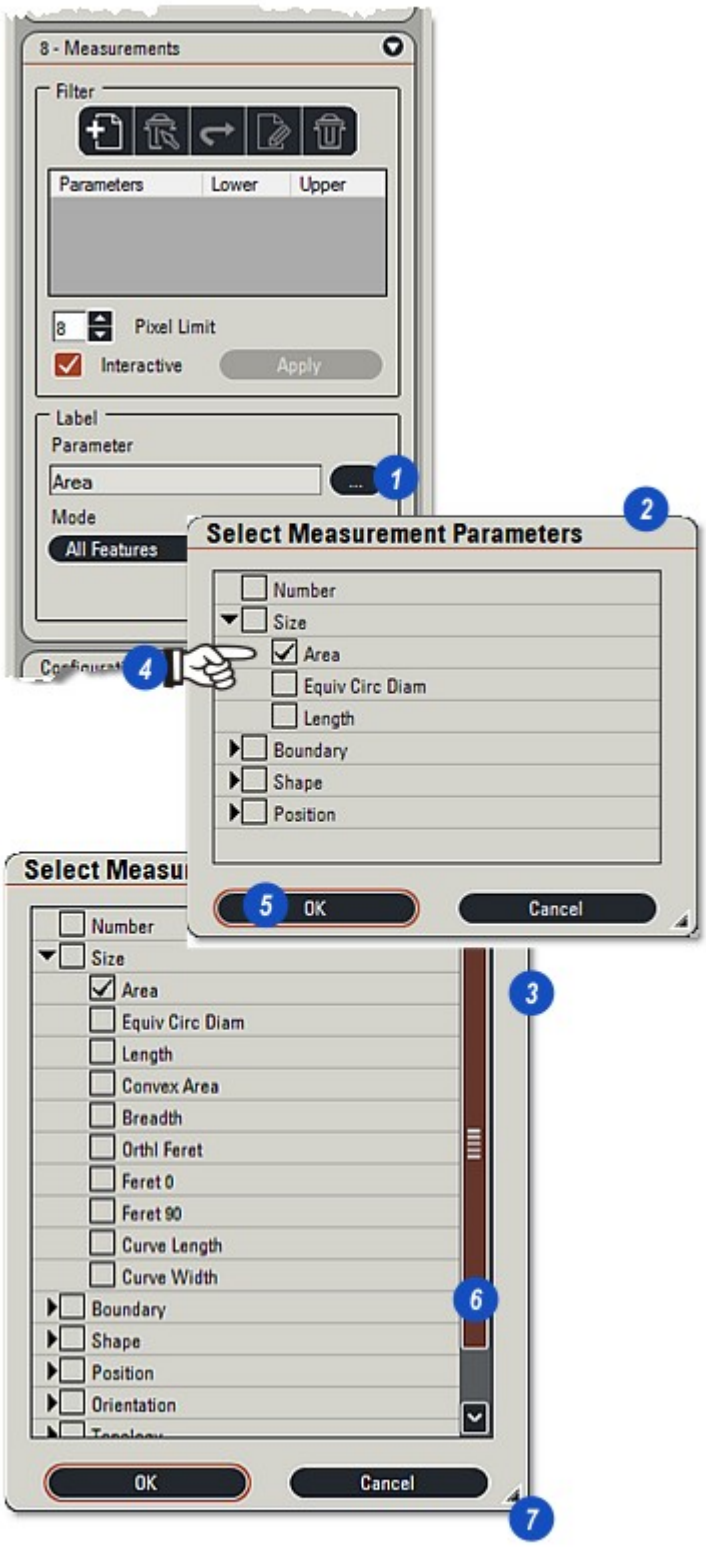
4: Select the parameter to display by clicking to enable the appropriate check box.

5: Click *OK*.

6: If *All Parameters* are displayed, click and drag the scroll bar to reveal more of the list.

7: The dialog can be enlarged by clicking and dragging the small arrow on the botton right corner of the dialog.

Continued… 865



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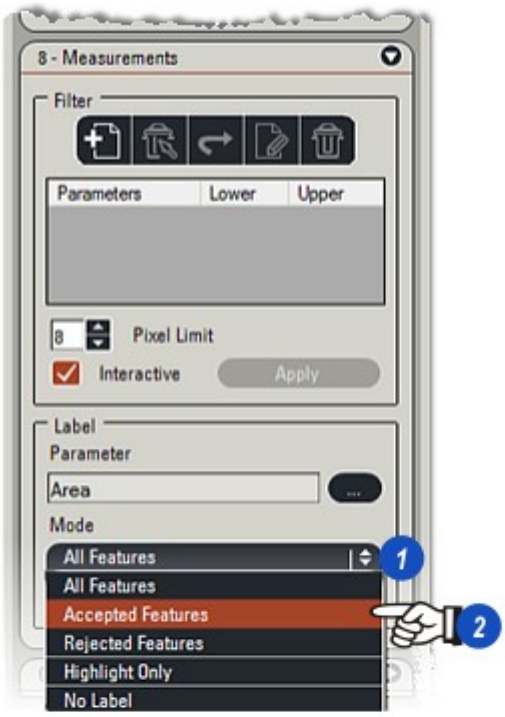
The *Labelling Mode* controls the features on the image that are labelled.



1: Click on the arrows to the right of the *Mode Listbox* and...

2: ... from the options click to select:

All Features: All the features highlighted on the *Binary Output Image* are labelled regardless of whether they fall inside limits or not.

Accepted Features: Features are labelled that fall within specified limits. For example, if the *Area* parameters are set to find image features no smaller than 50px2 and no greater than 150px2, then only those features that fell within the parameters would be labelled. All others would be ignored.

Rejected Features: Use this option to label features that fall outside the set parameters. Using the example above, features smaller than 50px2 and greater than 150px2 would be labelled.

None: Turns off labelling.

Continued... 864

Most Parameters can accept lower and upper limits so that only features falling within the scope of the limits are found and labelled.
These range pair values can be saved together with all of the other settings as a *Configuration* to be restored and used at any time.

To Create a New Filter:
The list of available parameters will depend upon the *All* or *Predefined* option in *Select Results.*

1: Click on the *New Filter* button and…

2: …on the *Set Measurement Parameters* dialog expand the Parameter Set and…

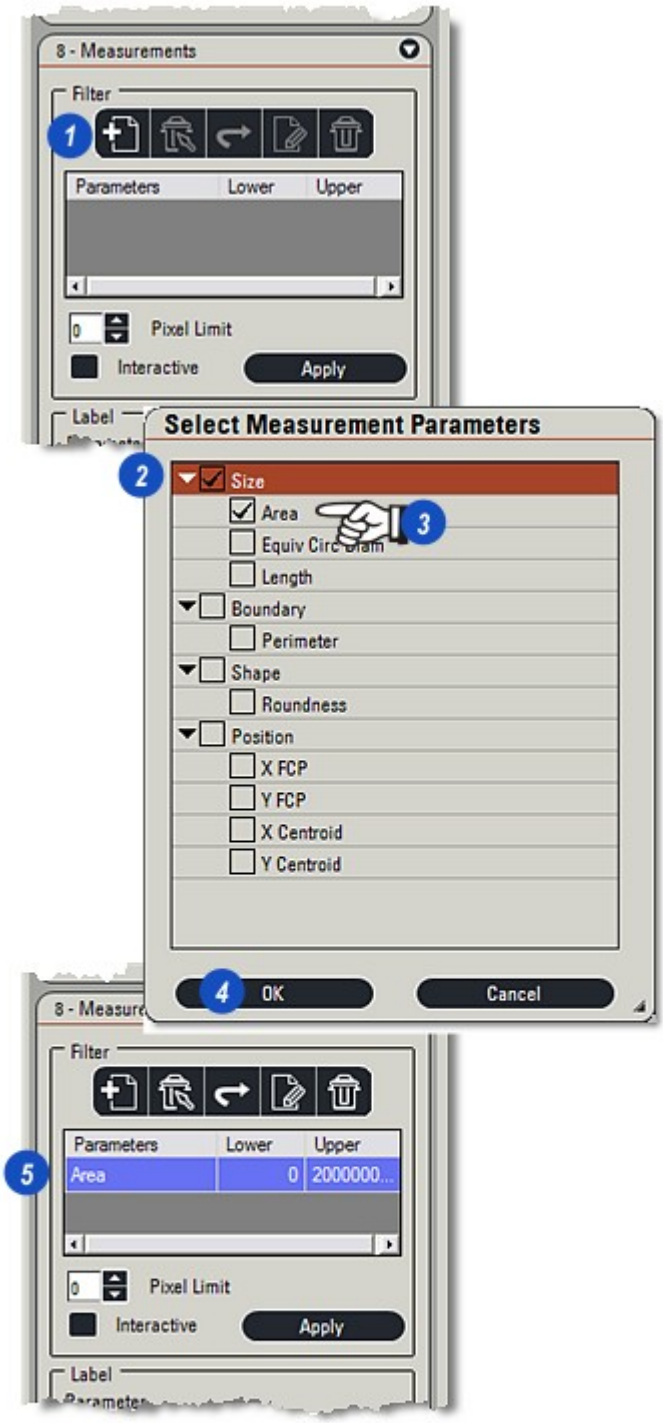
3: …click in the checkbox to the left of the desired *Parameter.*

4: Click *OK*.

5: The chosen *Parameter* name appears in the *Filter List* with the default lower and upper values. These values are based upon the image in the *Viewer*.

Select All or Predefined Parameter in Select Results: 866 Go there... 857

Continued… 867



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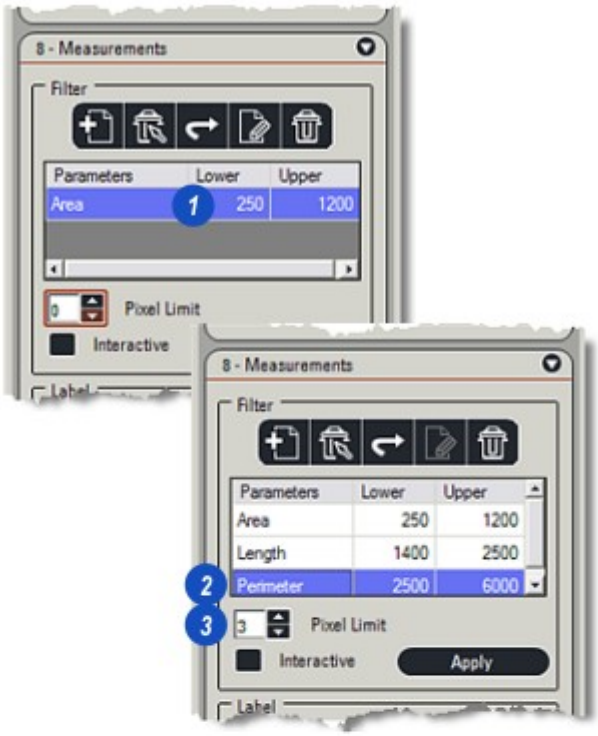
1: Set the required lower and upper limits for the *Filter* by clicking in the limit text box and typing a new value.

2: A different *Filter* for all appropriate Parameters can be added to the *Filter List* by repeating the process.
All parameter filters in the list will be used during measurement processing.

Filter Tolerance:
The lower and upper limits of a *Filter* can have an applied tolerance - in terms of *pixels* for un-calibrated images and *µm* for calibrated images - above and below the limit value - by:

3: …clicking the Up/Down arrows to the right of the *Pixel Limit* to reach the required value.

Continued... 868

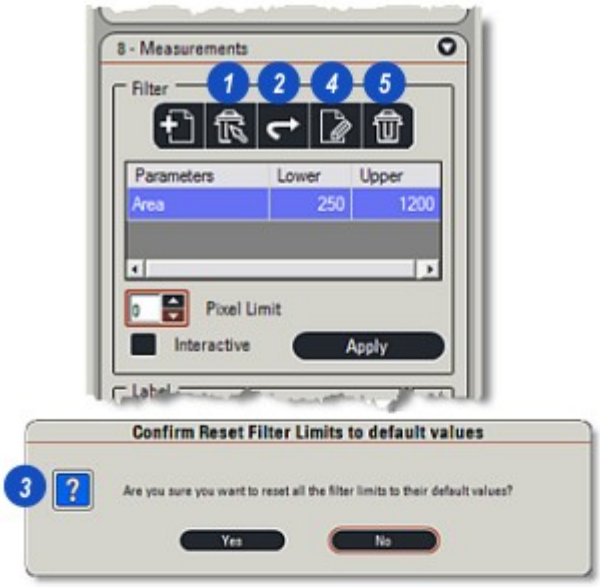


1: *Delete a Filter* by clicking on it in the list and then clicking the *Delete Filter* button.

2: *Restore Filter* limits to their default image-based settings. When this button is clicked a warning **(3)** appears; Click *OK* to continue.

4: *Restore Filter* limits to values based upon the current image. The lower and upper limits actually measured on the image are used in the filter. For example, if the Area Filter was originally set to 500 lower and 2500 higher, and a measurement yielded an feature low of 549 and an feature high of 2317, these two values would be used in the *Filter*. Again, a warning appears for confirmation.

5: *Clear all Filters*. Requires confirmation.



Save the Filters as a Configuration: Go there… 902

Continued... 869

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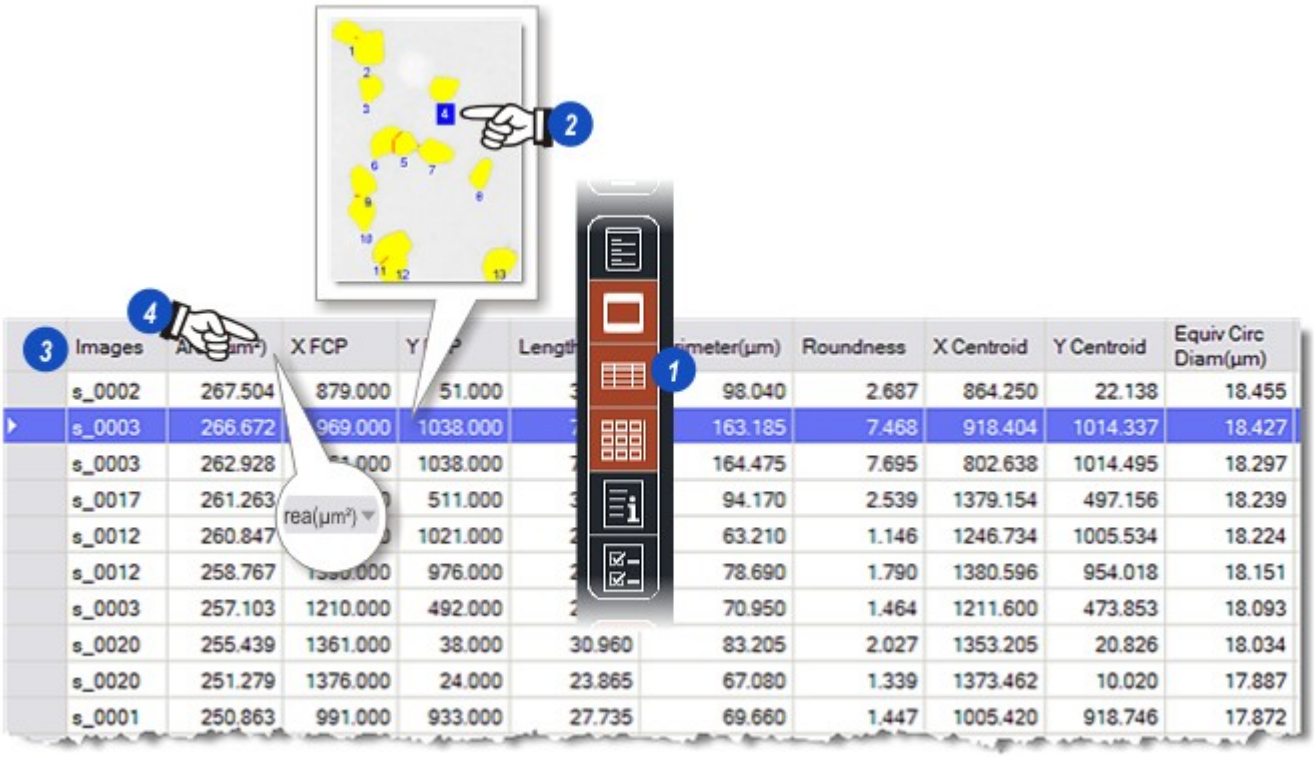
1: Measurement results can be displayed on screen by clicking to enable the *Grid View.*

2: Click on an entry in the *Grid* to reveal the feature on the image the label of which will be shown in a contrasting colour. In the same way, clicking on a feature on the *Binary Output Image* will highlight the results for that feature in the *Grid*. If a sequence is being measured, the appropriate image will be automatically displayed.
Pressing the keyboard *Delete* key will remove the selected feature from the *Grid* and measurement results but it will be coloured as not included. Reinstate the feature in *Binary Editing* using the *Keep* tool.

3: The Grid headings are determined by the range of results chosen in *Select Results* – either a Predefined range or All Measurements. These can be further filtered by selecting those which are to be displayed on the Grid.

4: Most headings when clicked will display a small arrow to the right. Clicking on the arrow will sort the results low-to-high or high-to-low.

Select Results to display on the Grid: Go there… 856



Histogram:

The *Histogram* function groups results together and displays them graphically as a vertical or horizontal bar chart or as a pie chart.

1: Select *Histogram* by clicking on the entry in the main menu.

The *Histogram* control panel is divided into three parts:

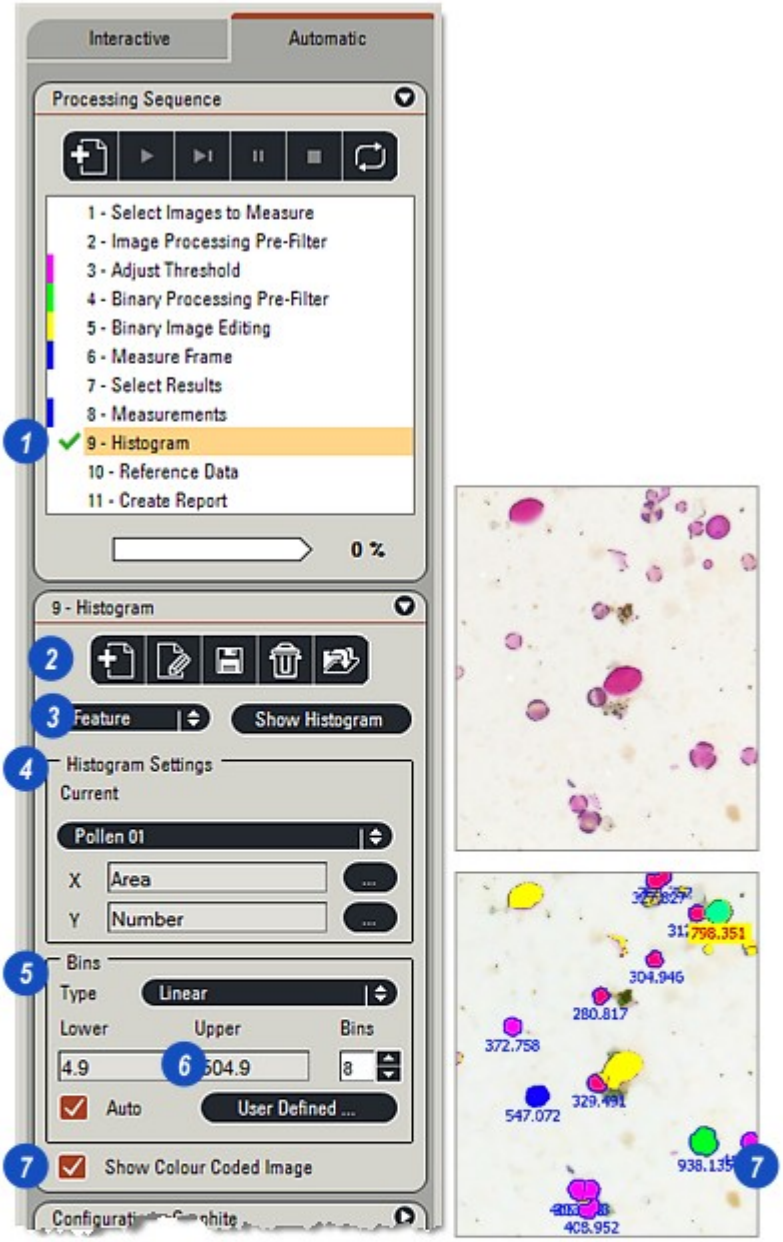
2: The *Tool Bar* with the display *Features or Fields* button **(3)** and the *Display Histogram* button.

4: The *Settings* group and…

5: The *Bins* configuration. On entry, the *Bins* – the groups into which the results will be collected – are configured and the upper and lower *Bin* values **(6)** shown on the display.

7: Each *Bin* can be colour coded and to show how the features are to be allocated to Bins, they too can be coloured accordingly by enabling the *Show Colour Coded Image* check box.

Continued... 871



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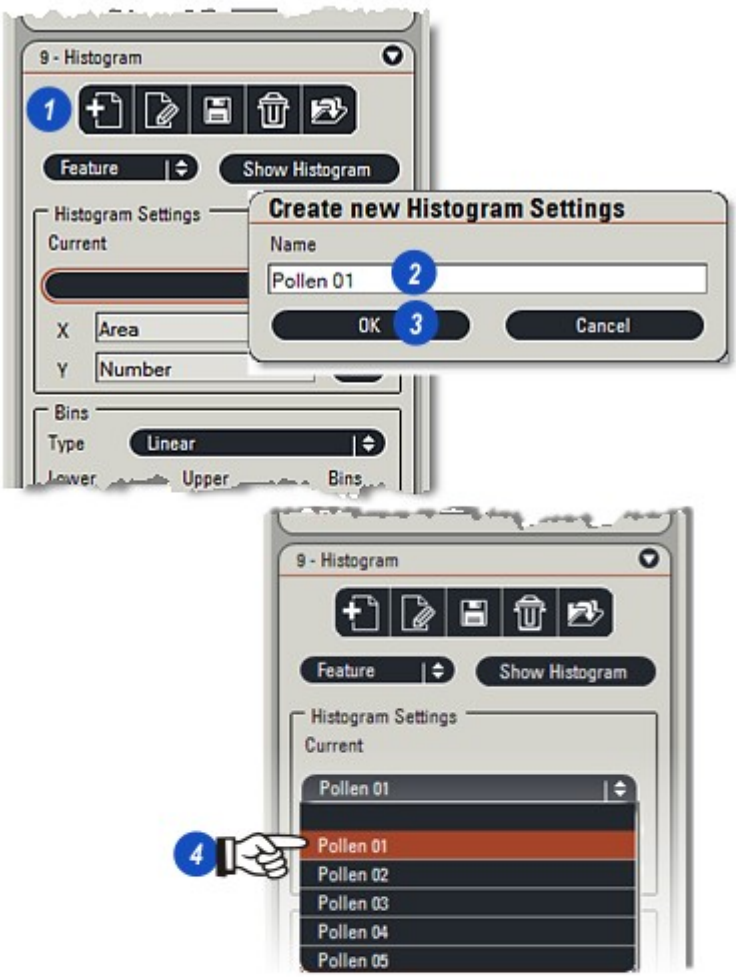
1: Click on the *New Histogram* button on the tool bar. The resulting file will contain the current settings and both the *Field* and *Feature* definitions. These are used in the report.

2: On the *Create New Histogram* dialog, click inside the *Name* text box and type a new name for the *Histogram*.

3: Click *OK*.

4: The name of the new *Histogram* now appears in the *Histogram* List which is revealed by clicking on the arrows to the right of the *Current* list box window.



Continued… 872

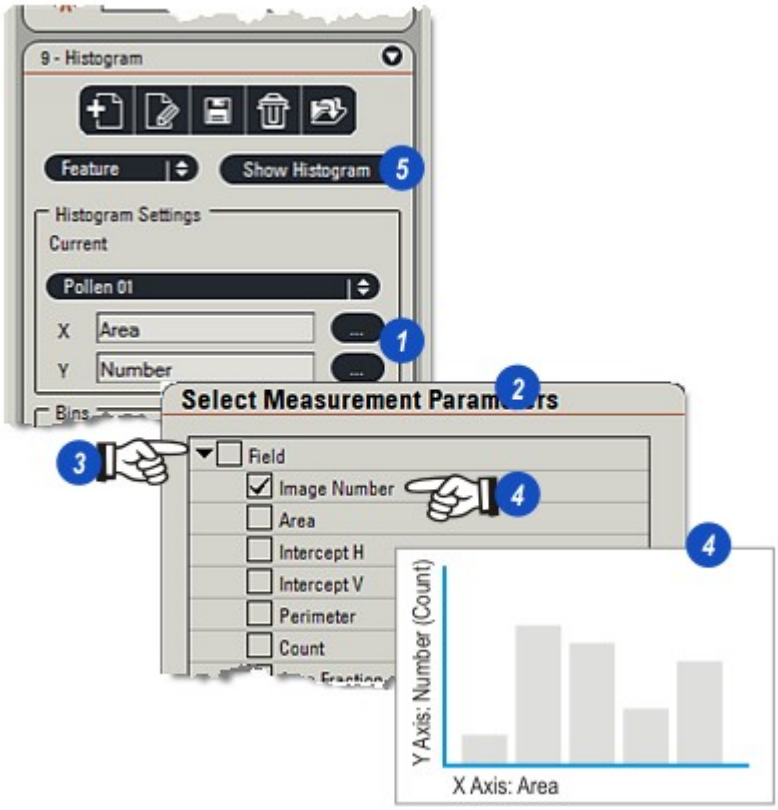
1: To select the parameter for the X-Axis, click on the *Browse* button to the right of the X window and…

2: …on the *Select Measurement Parameters* dialog, …

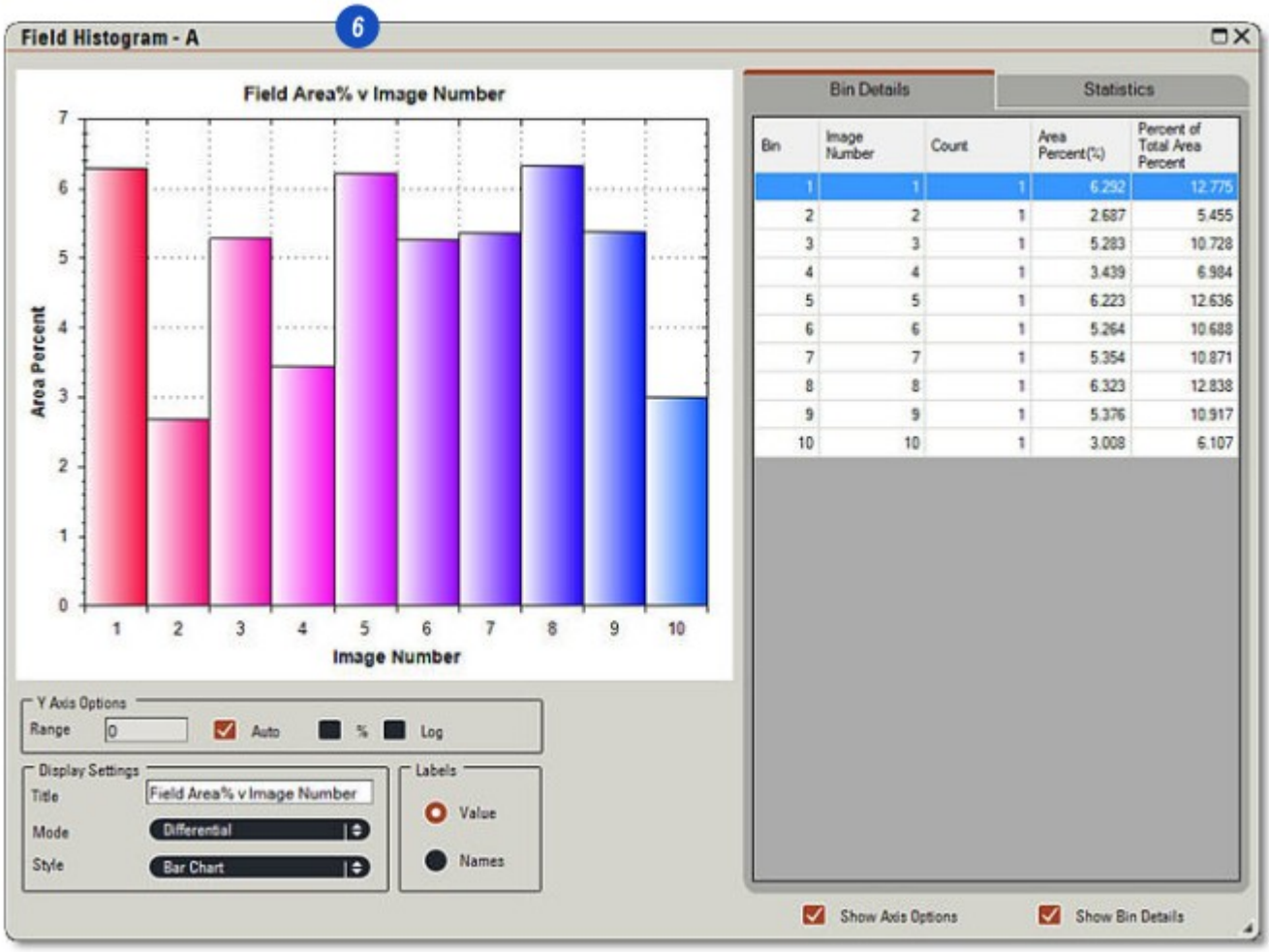
3: …expand the required parameter group by clicking on the arrow to the left of the heading and…

4: …clicking to choose the parameter that will be used as the X (horizontal) scale.
Repeat the process for the Y-Axis (vertical) scale.
The list of parameters is determined by the selections made in *Select Results - All* 873 or 873 *Predetermined Parameter* 873 list: 873 *Go there...* 856

5: Click on the *Show Histogram* button to display the *Histogram* **(6)**.



Continued… 873



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1: There are three options for the *Bin* scales, selected by clicking on the arrows to the right of the *Type* list window…

2: …and clicking on the required option:

Linear uses the value range between the lower and upper results and divides by the number of bins selected.

Log uses the same value range and bin count but applies logarithmic increments.

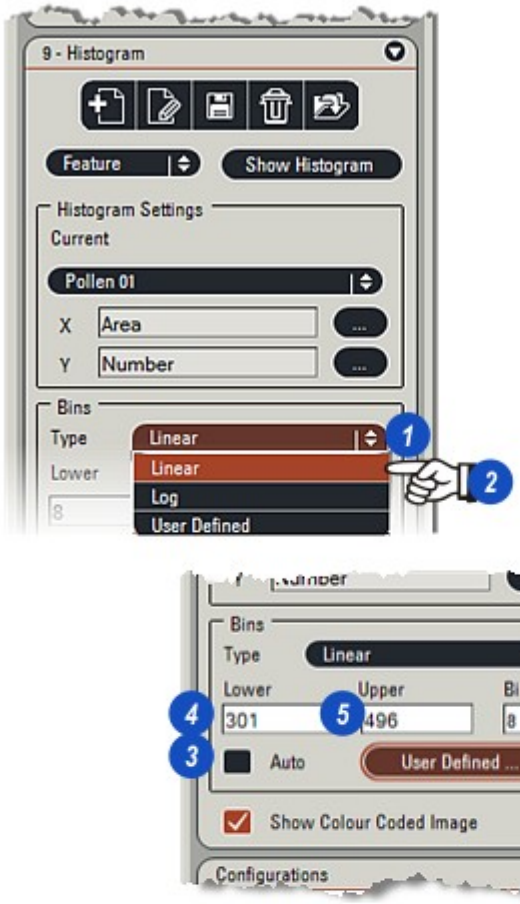
User Defined allows the value range to be set manually by:

3: Clicking to disable the *Auto* function...

4: …clicking in the *Lower Limit* text box and typing the lowest result to be displayed, and…

5: …repeating the procedure for the *Upper Limit*. These settings can be used to exclude some values from the *Histogram*.

6: Set the number of bins required by clicking inside the *Bin* text box and typing a value or using the Up/Down arrows to the right of the box.



Continued… 874

1: Enabling the *Auto* function by clicking the check box, will allow the software to determine the scale values and Bin colours.

2: Disable the Auto function and click *User Defined* to set the Bin colour to a personal preference.

3: The *Define Bins* dialog presents two options…

4: …the *Auto Range* will configure the Bin colours to preset shades, whereas…

5: …*Set All Same Colour* displays…

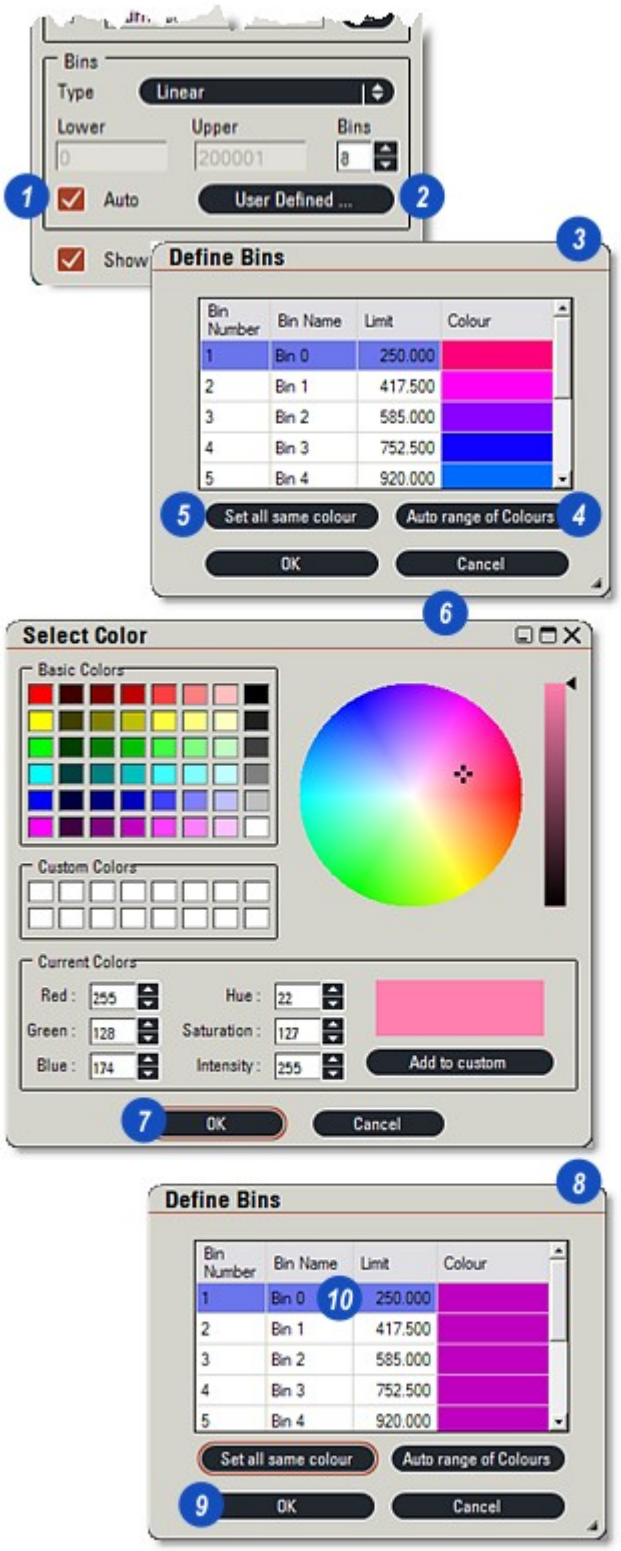
6: …the *Select Colour* dialog so that all of the Bins use the same display colour. Choose a colour from the wheel, palette or slider and…

7: …click *OK*.

8: The *Define Bins* dialog changes to show the selected colour.

9: Click *OK* to finish.

10: The default Bin labels are called *'Bin'* plus a sequential number, but that name can be changed by clicking in the *Bin Name* text box and typing a new name.



Continued… 875

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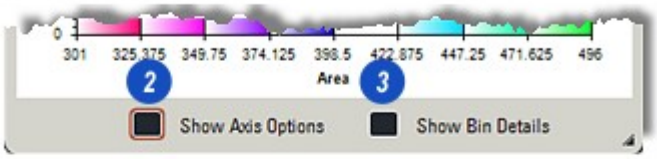
1: Click the *Save Histogram* button on the tool bar to save the settings so far. Any subsequent alterations to the *Histogram* settings can be saved and retrieved at a later date to replicate the *Histogram* display.

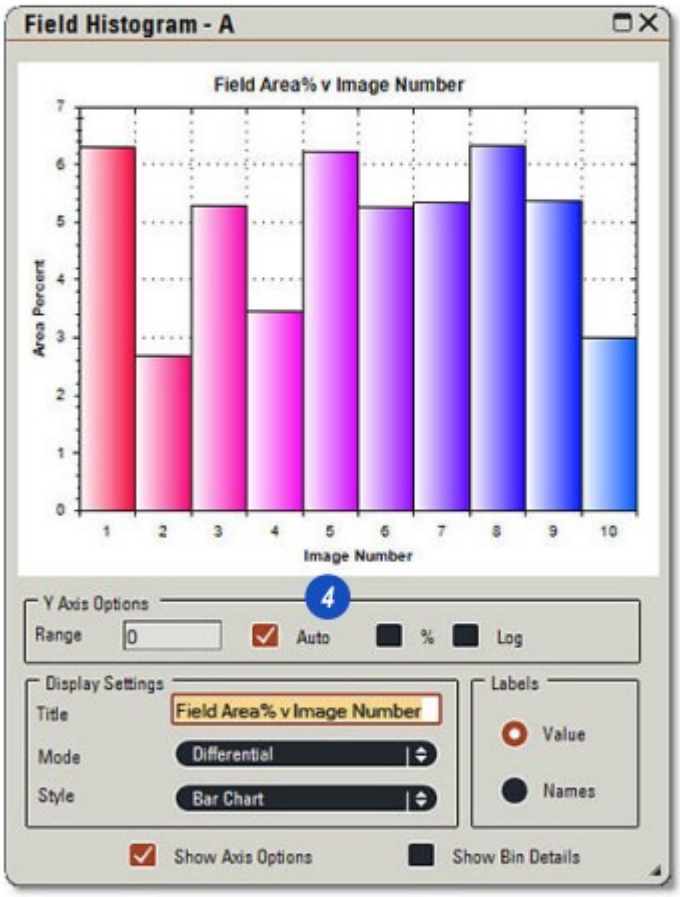
There are two check boxes at the bottom right of the *Histogram* display that will expand the scope of the display:

2: *Show Axis Option* when checked reveals the *Y Axis* configuration panels **(4)** to determine the display mode, style and scales.

3: Enabling *Show Bin Details* displays the results alongside the *Histogram* and also the configuration panels for the results and the Histogram labels.

Continued… 876





The user has extensive control on how the results are displayed on the *Histogram* so that data can be presented in the most effective way.

The *Y-Axis* scale has three options - *%, Log* and *Auto* - as well as a *Range* value to display the results appropriately.

1: Click to enable the *Show Axis Options*.

On Illustration **(A)** to use a Logarithmic scale:

2: Click to check the *Log* check box.

3: Click inside the *Range* text box and type a value to set an appropriate scale.

On Illustration **(B)** to display the results as a percentage of all the measurements:

4: Click to check the *%* check box and enter an appropriate *Range* value.

Continued... 877

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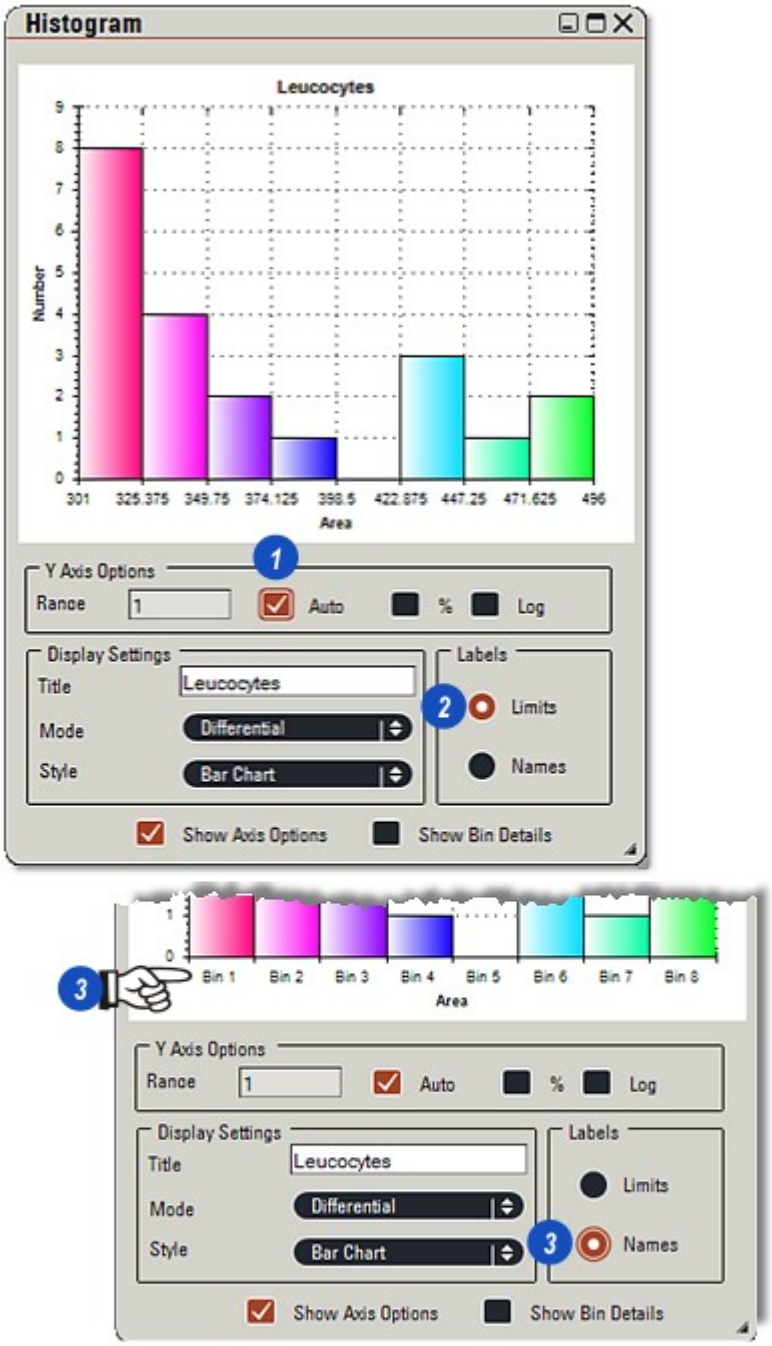
The program can setup the scale and the bin ranges by using the *Auto* function:

1: Click on the *Auto* check box to enable it.

2: With the *Limits* button selected the range of results allocated to each bin is shown along the *X-Axis*.

3: Click the *Names* button to display the *Bin Name* along the *X-Axis*.

Continued... 878



1: Enter a title for the *Histogram* by clicking in the *Title* text box and typing.

2: The *Mode* establishes how the results are spread across the bins. Click on the arrows to the right of the *Mode* header and...

3: ...click to select the required option:

Differential displays the results in each bin as a direct value against the Y-Axis scale.

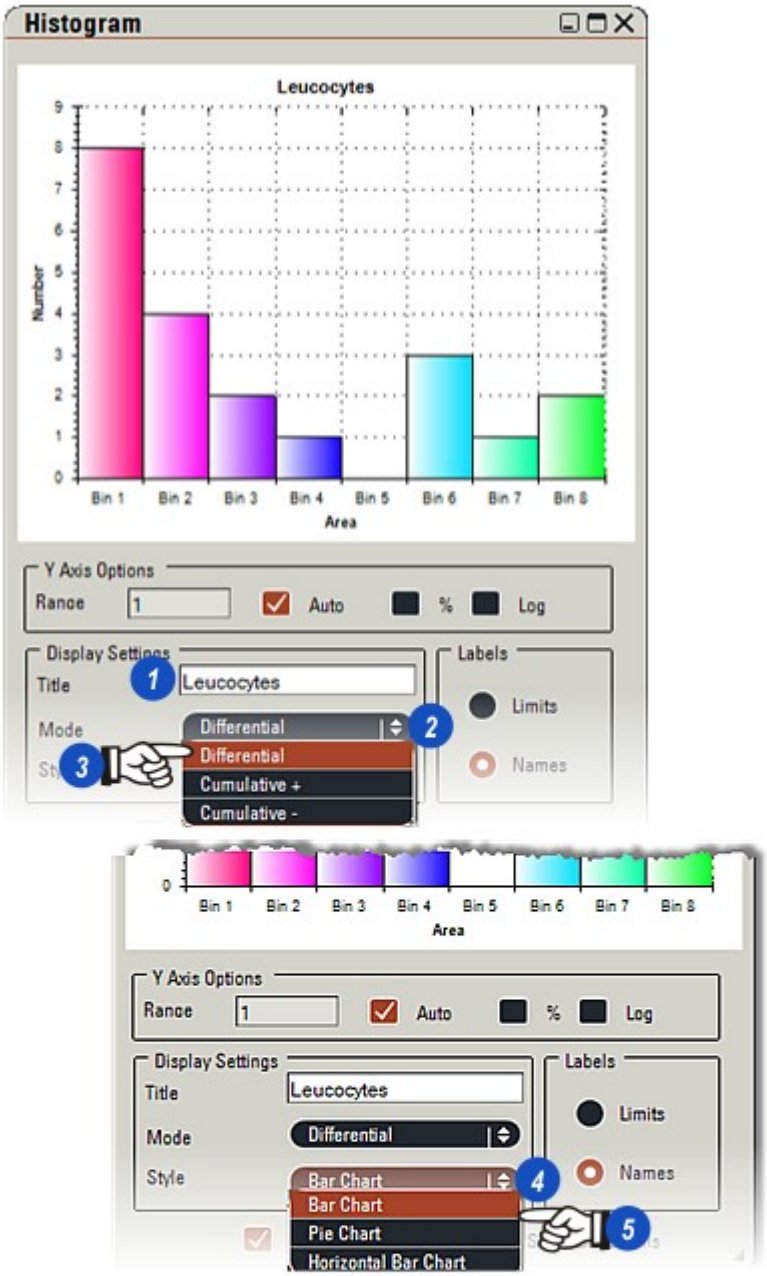
Cumulative+ adds the results progressively across the Histogram to display an ascending ramp.

Cumulative- subtracts the results across the bins resulting in a declining ramp.

4: Choose the display *Style* (illustrated on the following page) by clicking on the arrows to the right of the *Style* header and from the menu...

5 ...clicking to select a style.

Continued... 879



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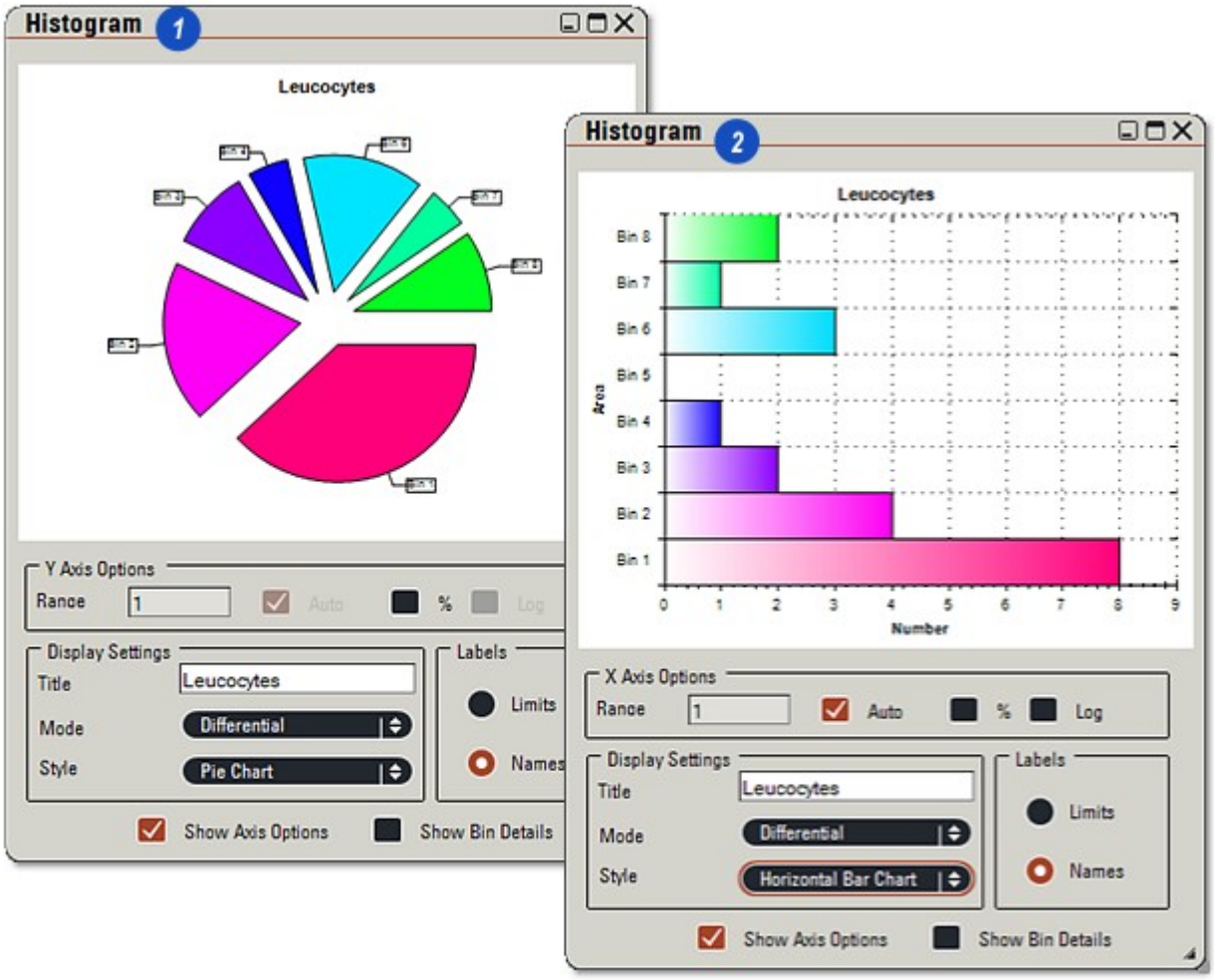
Examples of **(1)** *Pie* Chart display style and **(2)** *Horizontal Bar* chart.

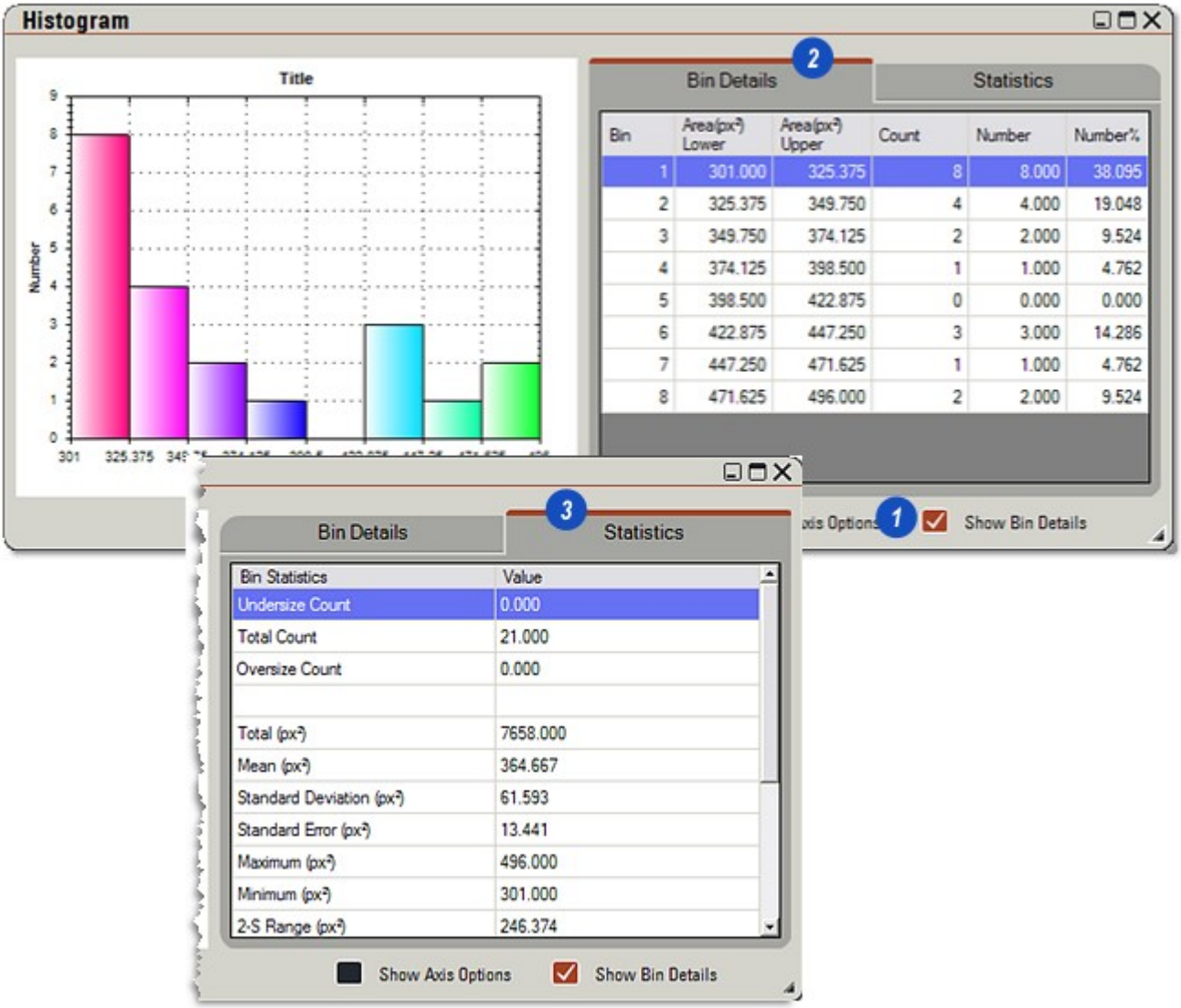


Both the details of the results in each bin and a list of statistics are available by clicking to enable the *Show Bin Details* check box **(1).**

Click on the *Bin Details* tab **(2)** to display a comprehensive breakdown of the results in each bin.

The statistics are revealed by clicking on the *Statistics* tab **(3).**

Continued... 881



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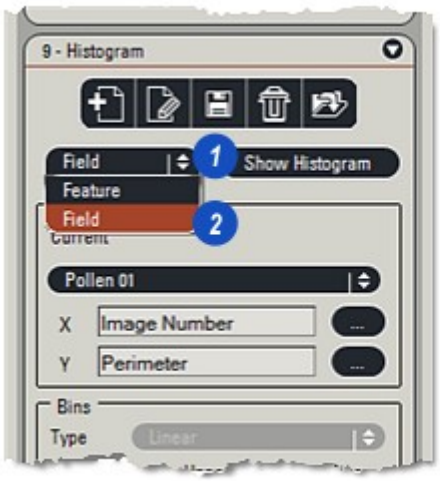
The illustrations so far have shown the results for all of the measured features, but the Field option will display results based upon the Field(s) in terms of the Y-Axis parameter.



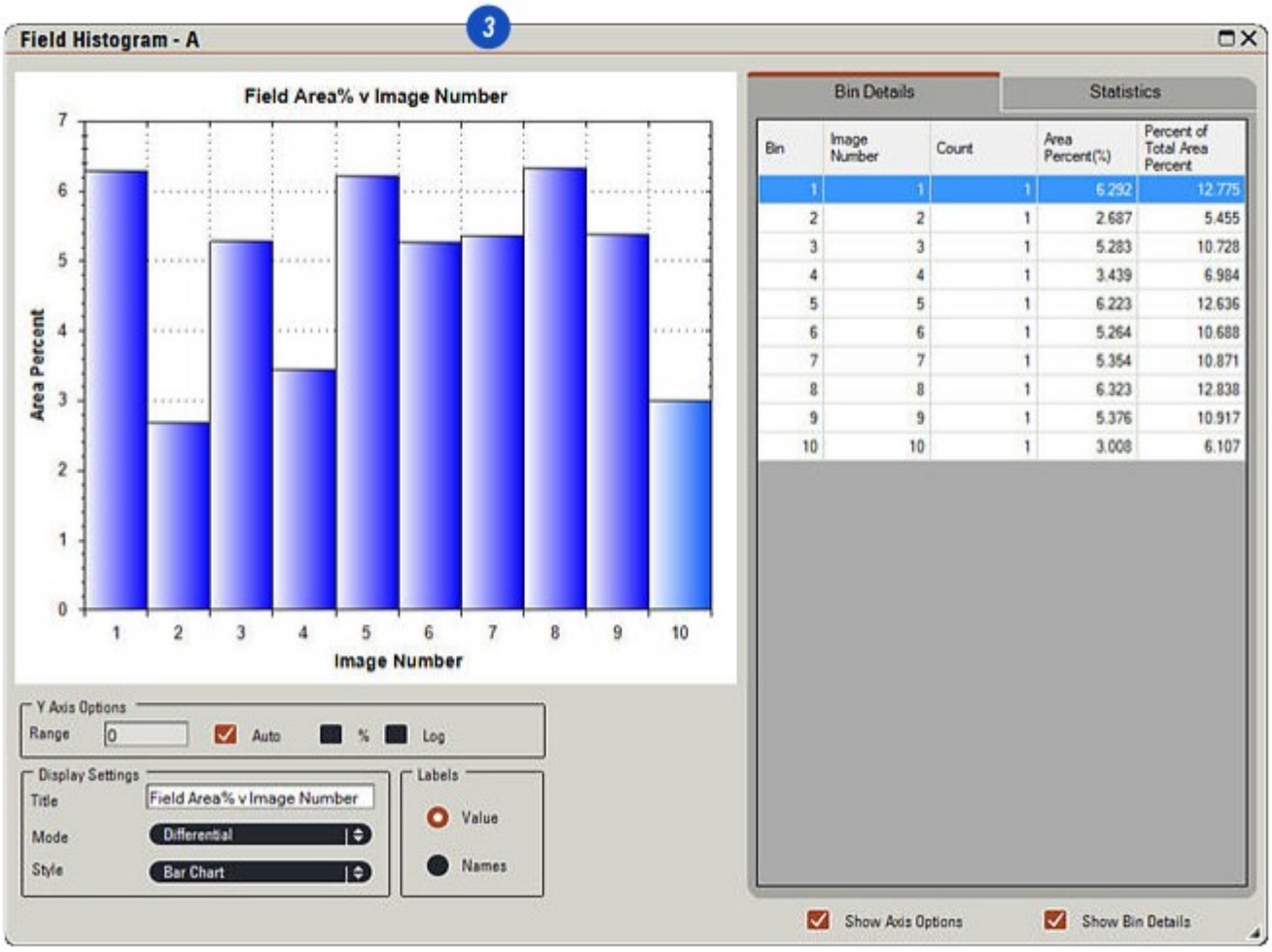
Switch between displaying Field or Features by:

1: Click on the small arrows to the right of the *Field/Feature* header and...

2: Click to select the display option required. There is no need to close the *Histogram* before changing display options.

3: The Field *Histogram*.

Continued... 882



1: Create a *New Histogram.*

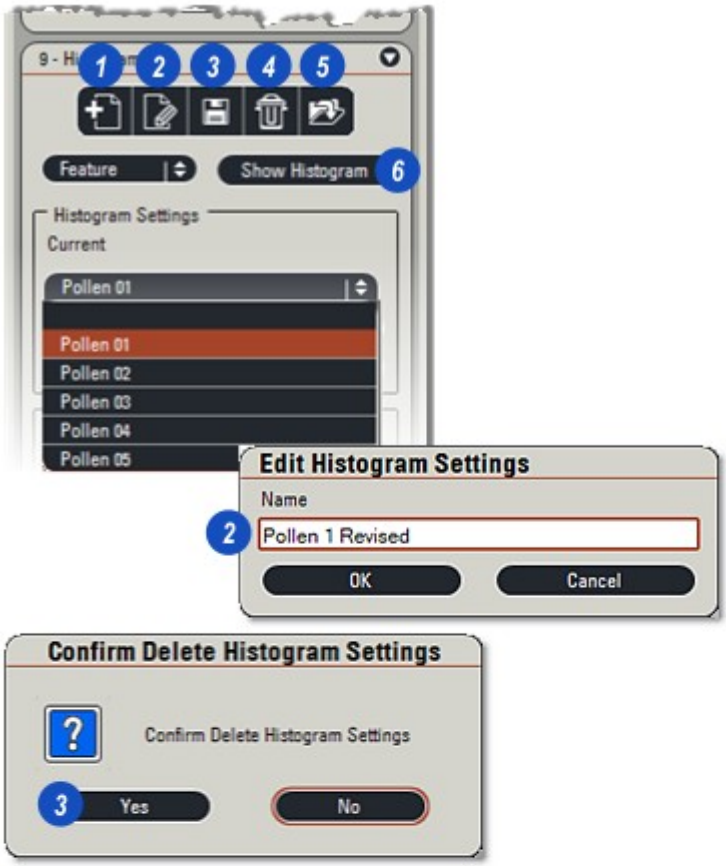
2: *Edit Histogram Settings* allows the name of the Histogram currently selected in the list to be changed. Click in the *Name* text box on the dialog and type a new unique name. Click *OK*.

3: To delete all of the Histogram settings click on the *Trash Can*. Confirm the deletion on the dialog. This action cannot be reversed.

4: *Save the Current Histogram* settings.

5: *Open Histogram* settings. Click on the arrows to the right of the *Current* drop down menu and click to select the required Histogram from the list. Click the *Open* button to load the settings.

6: *Show the Histogram.* Displays the current Histogram - hide it by using the usual controls on the upper right hand of the Histogram display.



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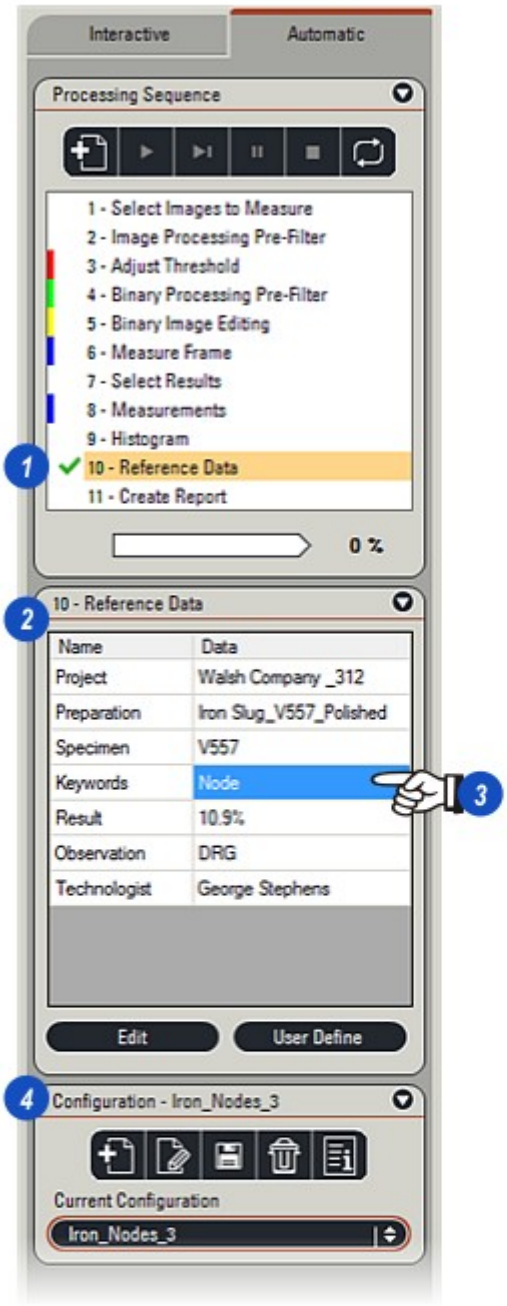
User *Reference Data* is attached and displayed on the Sequence Process Report.
The entries are simply text strings – and alpha/numeric characters are acceptable – entered into the appropriate text boxes on the *Reference Data* panel.

1: Click on the *Reference Data* entry in the main menu to reveal the entry panel **(2)**. Seven default topic text boxes are available – *Experiment, Preparation, Specimen, Keywords, Result, Observation* and *Technologist* - although others to suit the user may be added.

3: Click in a topic text box and type an entry.

4: The *Reference Data* is saved by creating a *Configuration*.



Create a Configuration: Go there... 902

Create a Report: Go there... 885

To edit any of the *Reference Data* topics

1: Click on the *Edit* button.

2: On the dialog (the heading shows the topic selected) type the revised text. Line returns are acceptable.

3: Click *OK*.

4: Edit another topic by navigating with the *Previous* and *Next* buttons.

To Hide, Reveal or Add a New Topic:

5: Click the *User Define* button. The dialog shows the existing topics and their display status. Click on a *Topic* check box to hide or display it.

6: Add a new *Topic* by clicking the New button. On the dialog type the new *Topic Name*. It will immediately appear in the list.

7: Hide or reveal the selected *Topic* with the *Visibility* button.

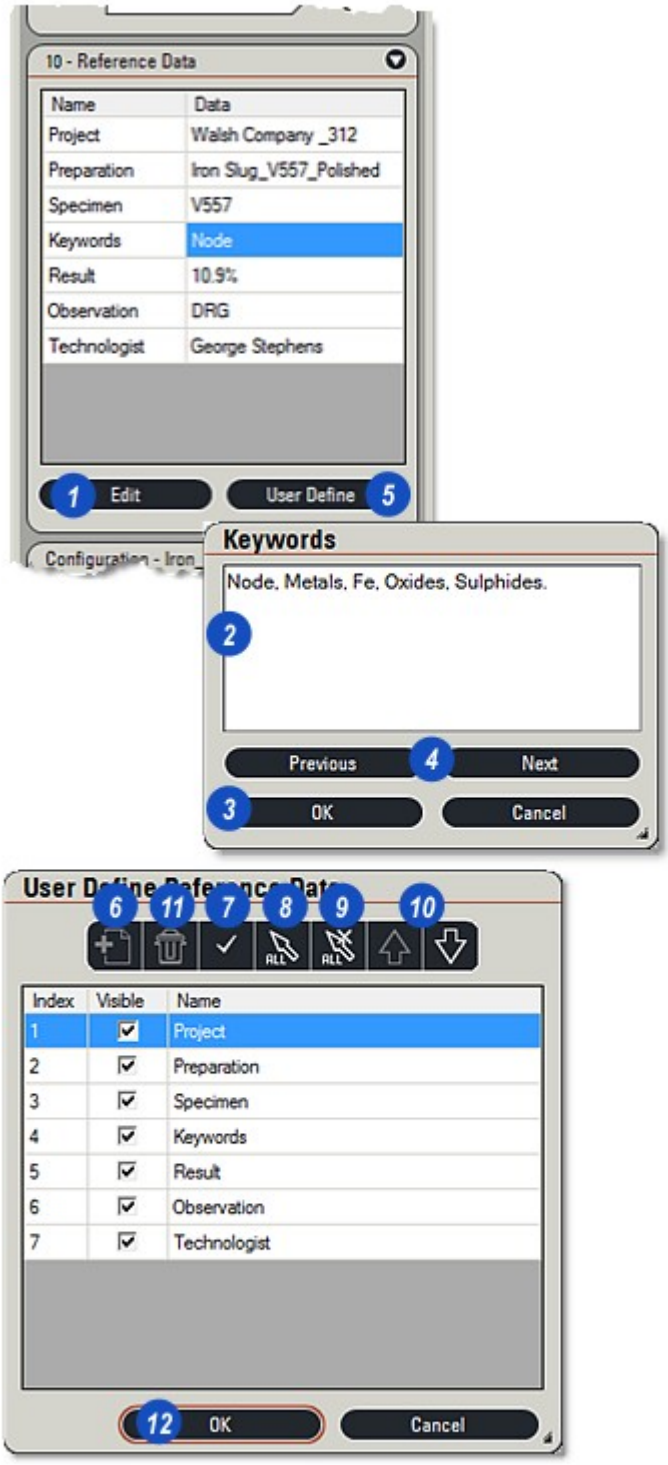
8: Click to reveal all topics.

9: Click to hide all topics.

10: Click the *Up/Down* buttons to move the selected *Topic* up or down the list.

11: Click *Delete* (Trash Can) to remove the selected Topic.

12: Click *OK*.



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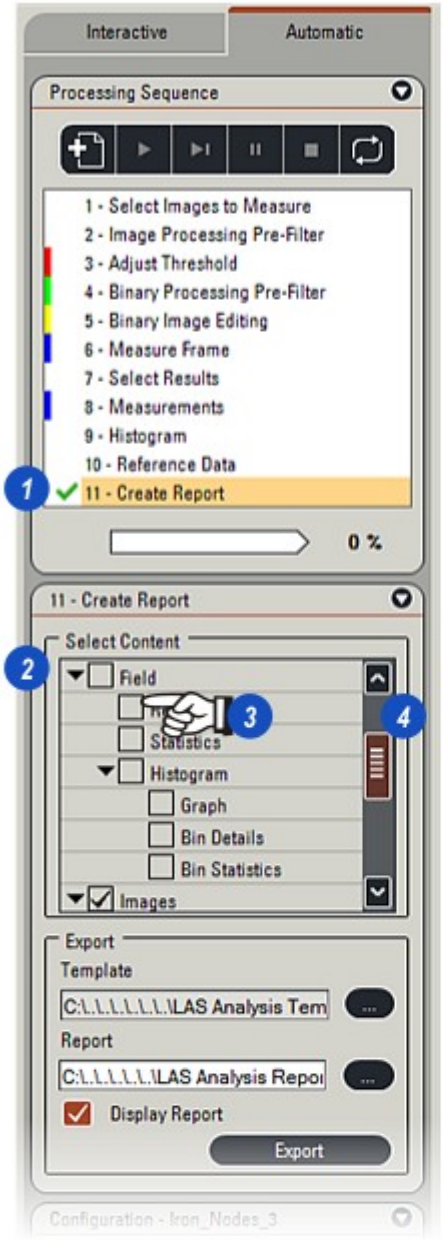
Reporting in LAS Image Analysis is both powerful and comprehensive. The reports save all of the data generated by a *Processing Sequence* in a Microsoft Excel spreadsheet. A standard Excel-compatible template is supplied with *Image Analysis* and data can be stored using it providing Excel is installed on the user's computer.



1: Click on the *Create Report* entry in the main menu to open the control panel.

2: The *Report Contents* are selected from the list by enabling the check boxes. Click on the arrow to the left of the headings to reveal the contents and…

3: …click to select the content item in the check box.

4: Click and drag the scroll slider to display further headings.

Template styling and layout can be changed by users using the basic Excel syntax, but before attempting changes make a copy of the original and work on that. See following page.

Continued… 886

1: Images can be displayed in the report by clicking to enable the *Image Sequence* or *Groups* header and then…

2: …clicking to select the individual images. No images are selected as a default with a maximum of 5 selectable.
It is recommended that the number of images selected be kept to a minimum otherwise problems with Excel memory usage could result.

Selecting the Template:

3: Click on the *Browse* button to the right of the *Template* window and…

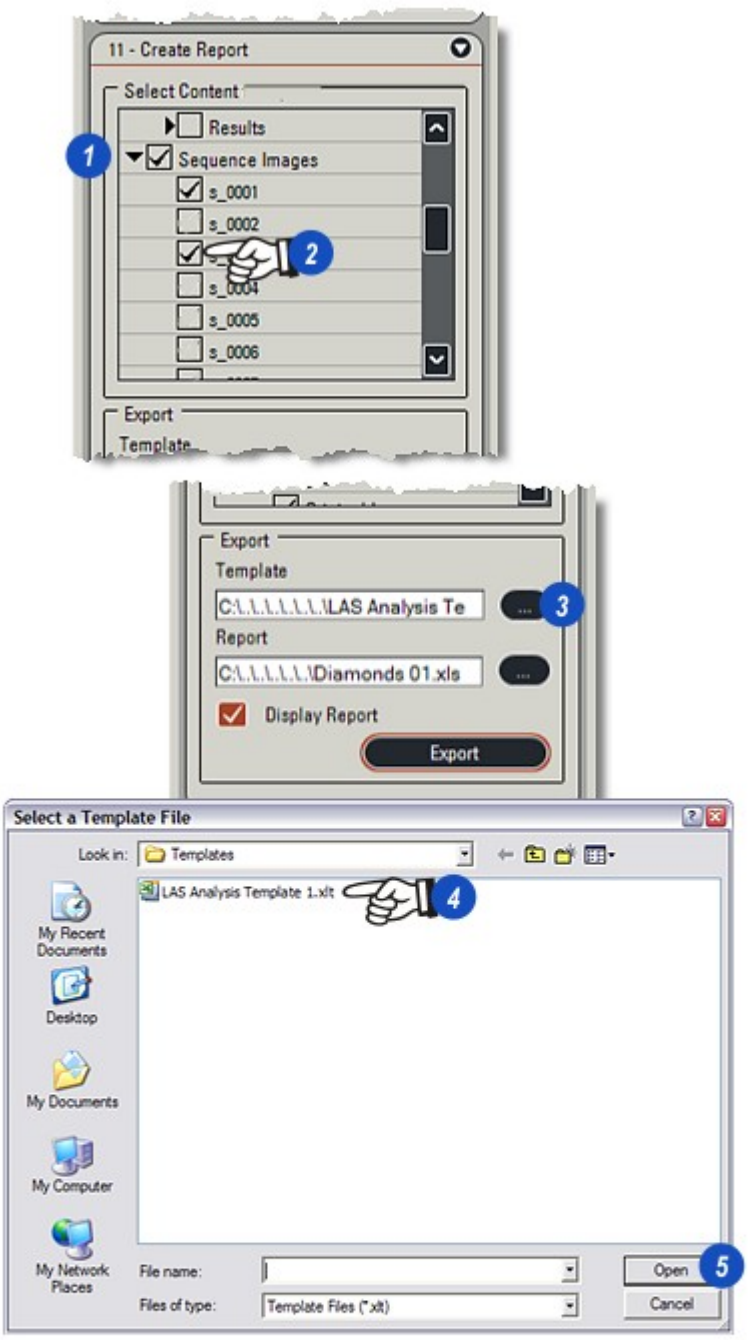
4: …on the *Select Template Dialog* click to select the required template. A comprehensive template *(LAS Analysis Template 1.xlt)* is supplied with LAS Image Analysis. It can be found at:

My Computer>Shared Documents> Leica Application Suite> Analysis>Templates

...and can be copied to another file name and used as the basis for end user templates.

5: Click *Open* and the template name appears in the Template window.

Continued… 887



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1: To select a file in which to save the Report, click on the *Browse* button to the right of the *Report* window and…

2: …on the *Select a Report File* dialog navigate to the required folder,…

3: Type a file name and…

4: ...click *Open*. The default file path is:

My Computer>Shared Documents> Leica Application Suite> Analysis>LAS Analysis Report.xls

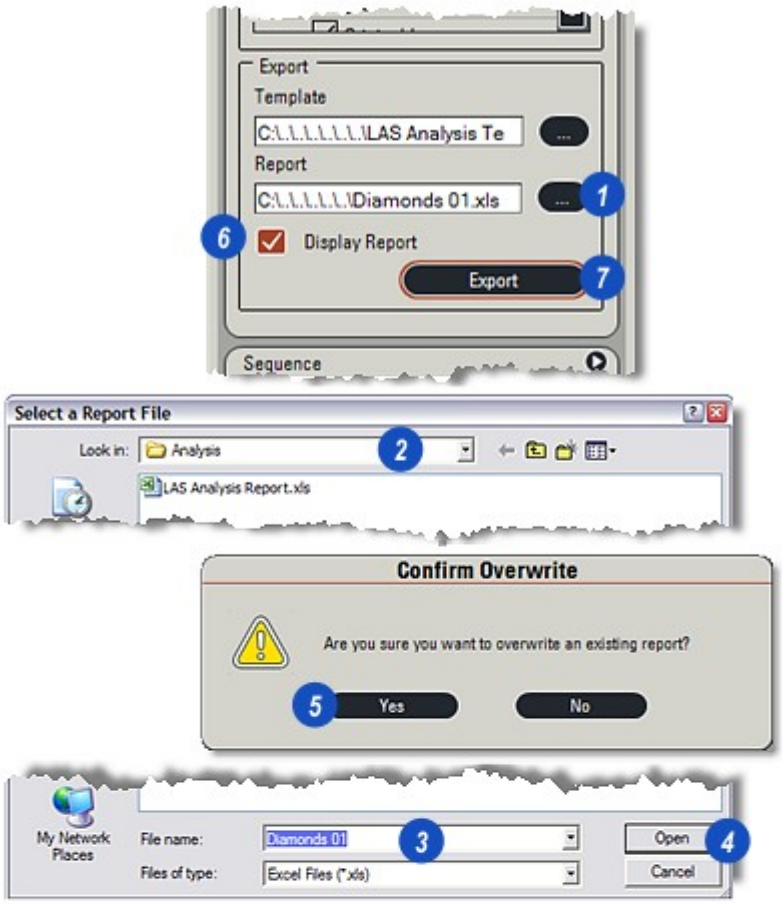
...but can be changed to any location using Windows navigation.

5: If the file name already exists, confirm (or abort) the overwrite.

6: Click the *Display Report* check box to display the report immediately.

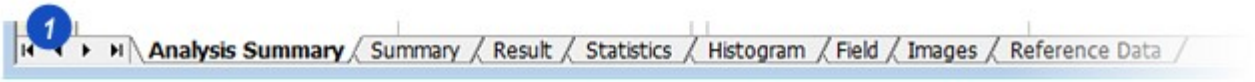
7: Click *Export* to generate the report.

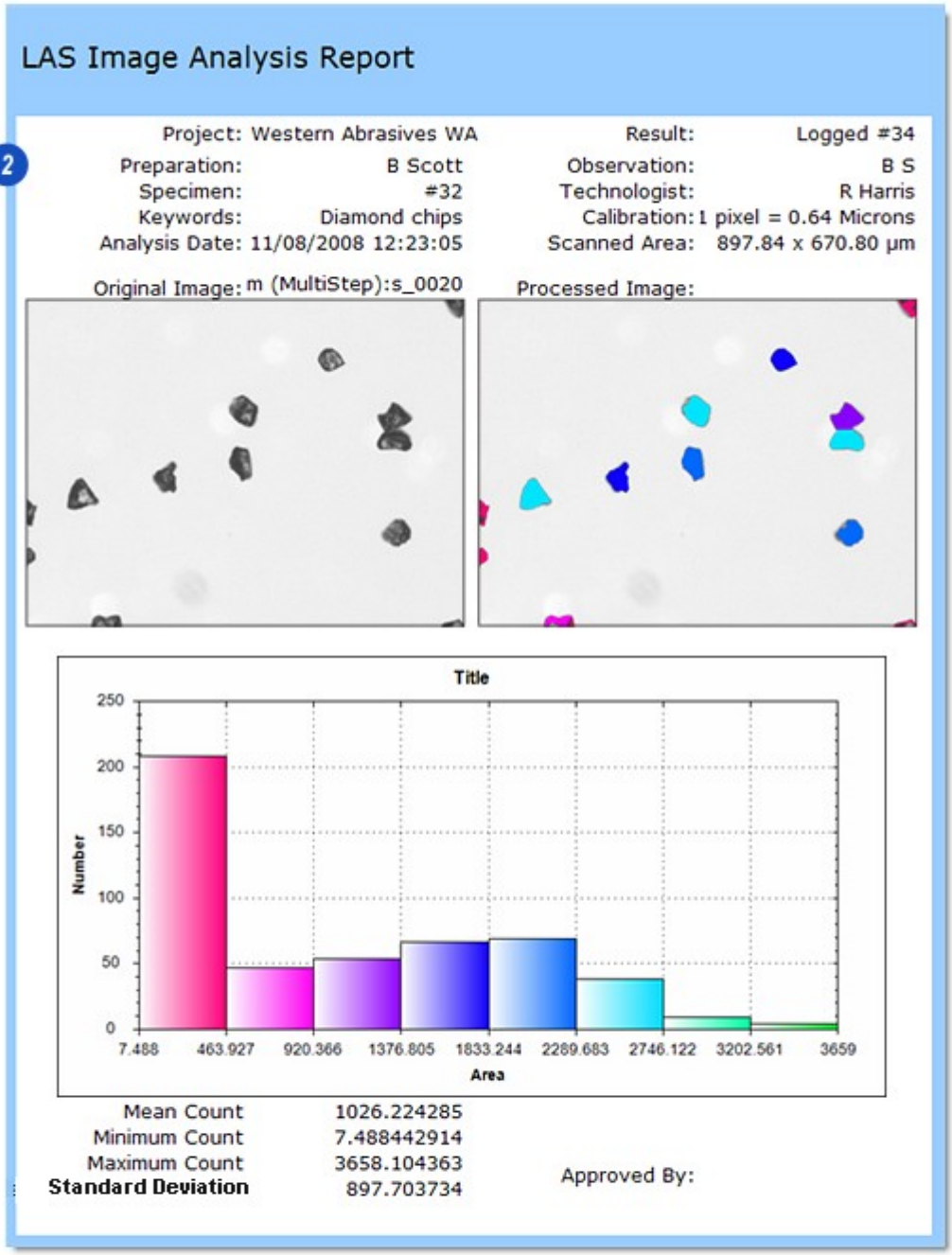
Continued… 888



1: The *Image Analysis Template* comprises 6 individual *Excel* sheets indicated by tabs along the bottom edge. Specific data in the sheets is linked to the front page to provide an analysis.

2: The *Analysis Summary Front Page*. Layout detail can be altered by the user.





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	A	B	C	D	E	F	G	H	I	J	K	L
1												
2												
3												
4		Title	Data									
5		Project										
6		Specimen										
7		Technologist										
8		Keywords										
9		Preparation										
10		Observation										
11		Result										
12												
13		Statistics	Accepted	Area	X FCP	Y FCP	Length	Perimeter	Roundness	X Centroid	Y Centroid	Equiv Circle
14				(µm²)			(µm)	(µm)				Diameter (µm)
15		Total	495	507981.021	370733.000	280853.000	21388.835	60453.886	809.205	369059.290	267539.183	15604.304
16		Mean	1	1026.224	748.956	567.380	43.210	122.129	1.635	745.574	540.483	31.524
17		Standard Deviation	0	897.704	432.393	318.741	21.346	62.528	0.693	431.302	319.206	17.688
18		Standard Error	0	40.349	19.435	14.326	0.959	2.810	0.031	19.386	14.347	0.795
19		Maximum	1	3658.104	1390.000	1038.000	96.750	256.710	7.695	1386.972	1033.389	68.247
20		Minimum	1	7.488	2.000	2.000	5.160	14.190	1.090	4.746	1.000	3.088
			0						2.771	1725.209	1276.823	

	A	B	C	D	E	F	G	H	I	J	K	L	M
1													
2													
3													
4		No:	Image	Accept	Area	X FCP	Y FCP	Length	Perimeter	Roundness	X Centroid	Y Centroid	Equiv Circle
5					(µm²)			(µm)	(µm)				Diameter (µm)
6		1	s_0020	1	251.279	1376.000	24.000	23.865	67.080	1.339	1373.462	10.020	17.887
7		2	s_0020	1	255.439	1361.000	38.000	30.960	83.205	2.027	1353.205	20.826	18.034
8		3	s_0020	1	247.119	1379.000	55.000	21.285	65.790	1.310	1377.448	41.012	17.738
9		4	s_0020	1	1643.297	958.000	225.000	54.180	159.315	1.155	963.163	190.953	45.742
10		5	s_0020	1	1253.066	1189.000	401.000	52.245	143.190	1.224	1181.645	365.276	39.943
11		6	s_0020	1	2363.020	703.000	403.000	65.790	193.500	1.185	690.101	354.463	54.852
12		7	s_0020	1	986.394	1185.000	411.000	52.890	131.580	1.313	1148.206	389.516	35.439
13		8	s_0020	1	2329.738	1205.000	479.000	70.950	199.305	1.275	1166.748	446.738	54.464
14		9	s_0020	1	2177.889	685.000	574.000	70.305	192.210	1.269	678.748	517.977	52.659
15		10	s_0020	1	1797.642	464.000	611.000	61.920	186.405	1.446	442.262	567.334	47.842
16		11	s_0020	1	60.324	10.000	652.000	12.255	33.540	1.395	5.441	645.821	8.764
17		12	s_0020	1	2457.457	154.000	665.000	66.435	205.110	1.280	168.960	622.012	55.937
18		13	s_0020	1	113.159	23.000	670.000	16.125	45.150	1.347	16.563	659.669	12.003

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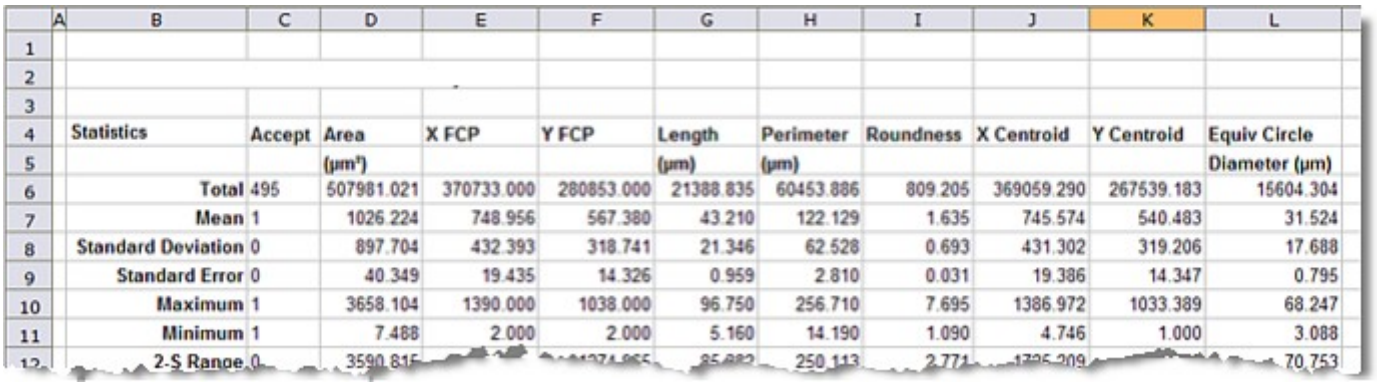
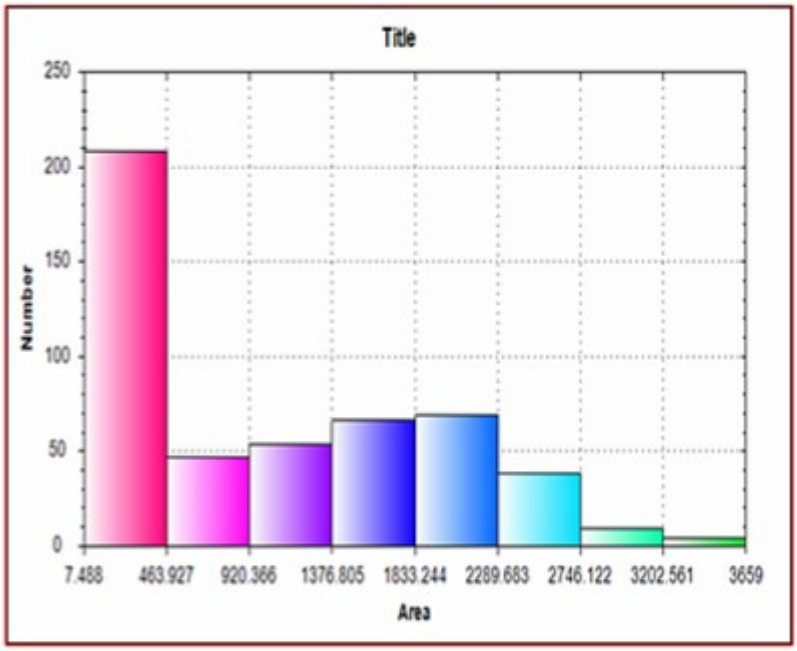


	A	B	C	D	E	F	G	H	I	J	K	L
1												
2												
3												
4		Statistics	Accept	Area	X FCP	Y FCP	Length	Perimeter	Roundness	X Centroid	Y Centroid	Equiv Circle
5				(µm²)			(µm)	(µm)				Diameter (µm)
6		Total	495	507981.021	370733.000	280853.000	21388.835	60453.886	809.205	369059.290	267539.183	15604.304
7		Mean	1	1026.224	748.956	567.380	43.210	122.129	1.635	745.574	540.483	31.524
8		Standard Deviation	0	897.704	432.393	318.741	21.346	62.528	0.693	431.302	319.206	17.688
9		Standard Error	0	40.349	19.435	14.326	0.959	2.810	0.031	19.386	14.347	0.795
10		Maximum	1	3658.104	1390.000	1038.000	96.750	256.710	7.695	1386.972	1033.389	68.247
11		Minimum	1	7.488	2.000	2.000	5.160	14.190	1.090	4.746	1.000	3.088
12		2-S Range	0	3590.815				250.113	2.771			70.753

Histogram Statistics

Bin Statistics	Value
Undersize Count	0
Total Count	495
Oversize Count	0
Total Count	507981.021
Mean Count	1026.224
Standard Deviation Count	897.704
Standard Error Count	40.349
Maximum Count	3658.104
Minimum Count	7.488
2-S Range Count	3590.815
Median Count	692.147
Mode Count	235.708
Skewness	0.483
Kurtosis	-1.051
Features	495
Specimen Area (µm²)	12045410.042
Normalised Count	41.094



Bin Data

Bin	Area(µm²) Lower	Area(µm²) Upper	Count	Number	Number%
1	7.488	463.927	208	208.000	42.020
2	463.927	920.366	47	47.000	9.495
3	920.366	1376.805	54	54.000	10.909
4	1376.805	1833.244	66	66.000	13.333
5	1833.244	2289.683	69	69.000	13.939
6	2289.683	2746.122	38	38.000	7.677
7	2746.122	3202.561	9	9.000	1.818

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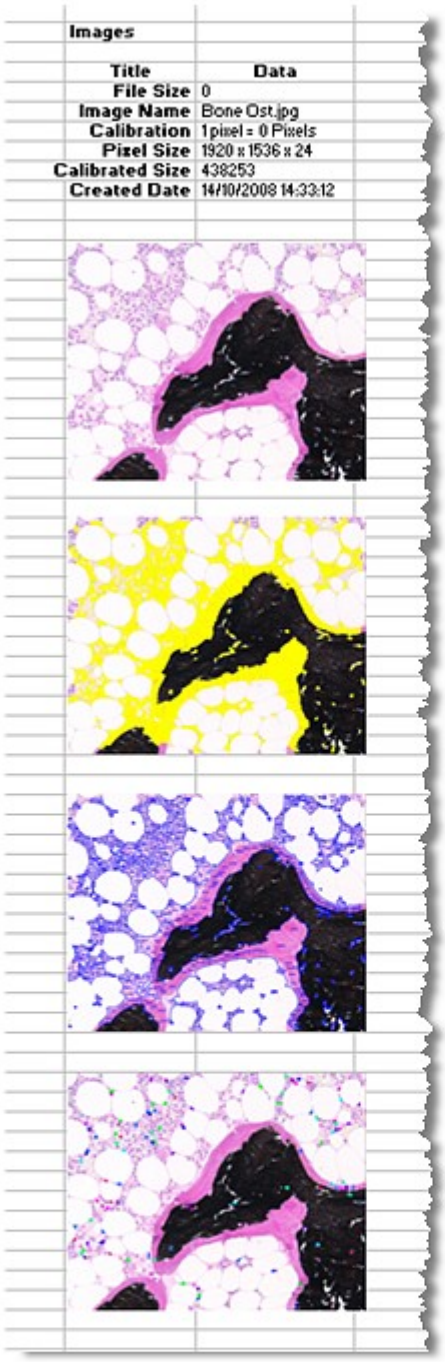
Excel display of a selected image with the *Binary Masks* produced during a *Process Sequence.*



Images

Title	Data
File Size	0
Image Name	Bone Ost.jpg
Calibration	1 pixel = 0 Pixels
Pixel Size	1920 x 1536 x 24
Calibrated Size	438253
Created Date	14/10/2008 14:33:12

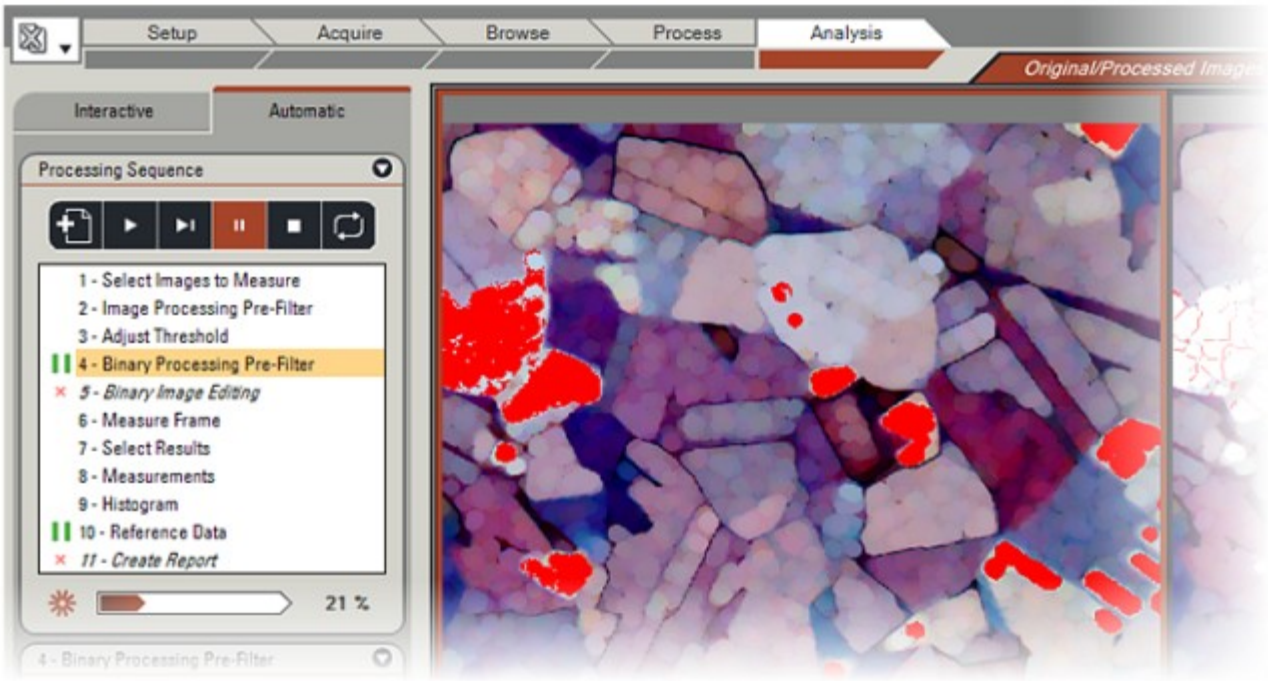
The *Processing Sequence* feature of LAS Image Analysis takes groups or sequences of images and applies any or all of the other tools and their settings *automatically* to produce rapid results, very often without any further user intervention.

For sequences – colour or monochrome - initial analysis is carried out on a single image with the program 'remembering' each step. Then, with a single button click all of the images will be processed using the step settings.

Optionally, a comprehensive report can be generated and displayed when processing is complete. Individual images can also be grouped and processed even if they have differing tool settings.

The sequence can be programmed to halt at any tool to allow refinement – deleting or separating features for example – before resuming the sequence.

The *Processing Sequence* can be saved as a *Configuration* to be recalled and re-run at any time.



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1: *Create a New Processing Sequence*. Clicking this button clears all existing sequence information so needs to be confirmed on the dialog.

2: *Run Processing Sequence*. Starts an existing sequence.

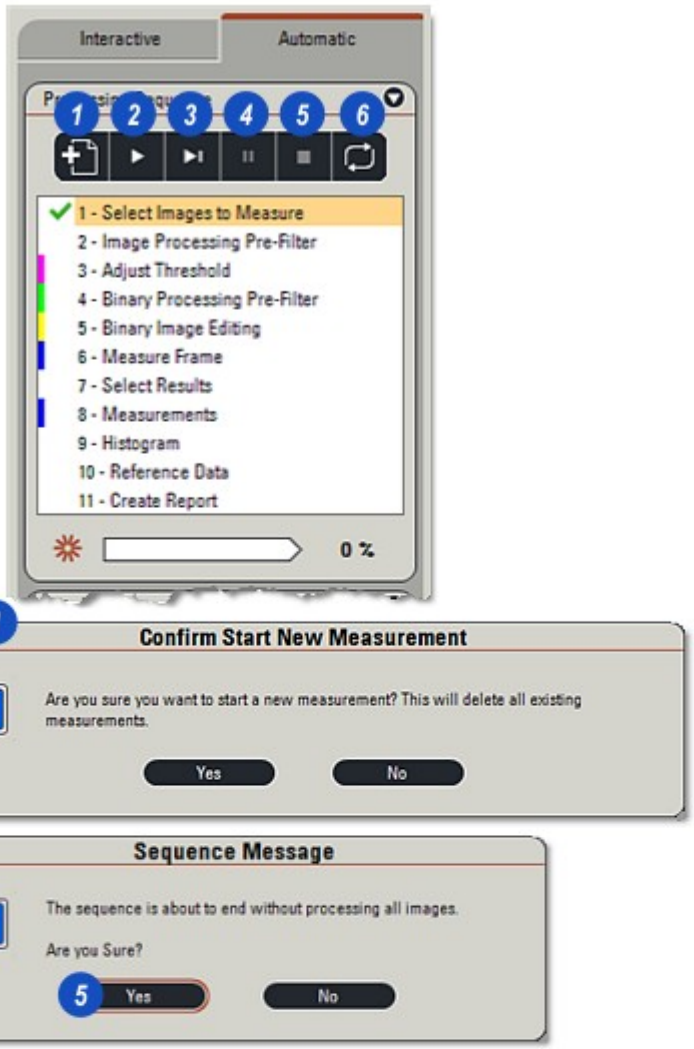
3: Clicking *Next Step* stops the automatic run and allows the user to move through the sequence step-by-step. Click the *Run* button to resume automatic processing.

4: The *Pause* button will stop the sequence at any time. Click it again to resume.

5: *Stop* the sequence. Requires confirmation on the dialog.

6: The *Update* button refreshes the processing sequence if any changes are made to individual images.

Continued… 896



1: The individual images shown in the *Gallery* illustration are part of a sequence with…

2: …the composite labelled *MultiStep*. There are a total of 25 images in the sequence.

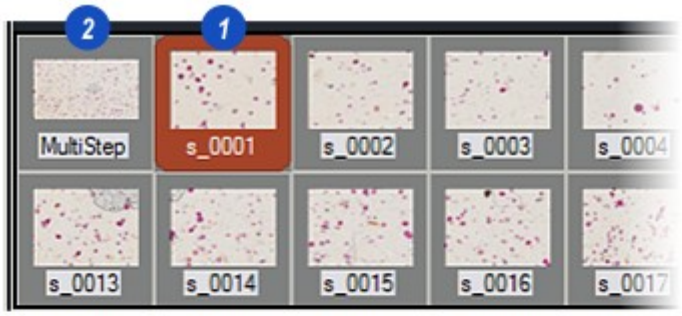
3: If necessary, click the arrow to the right of the *Sequence* panel header to expand it.

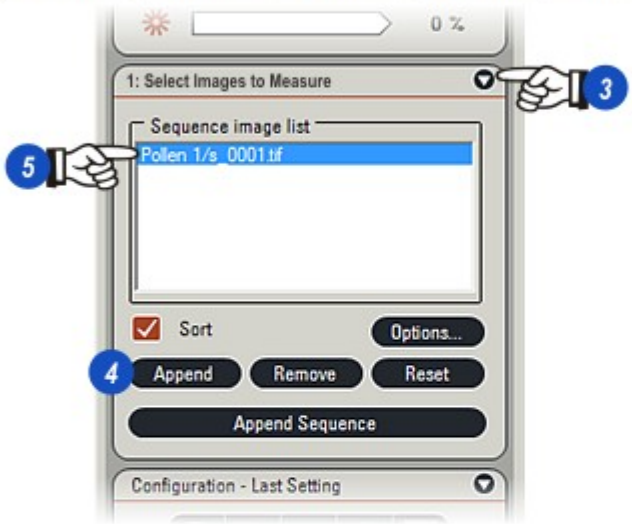
4: Load the first image to the *Sequence Image List* by clicking the *Append* button.

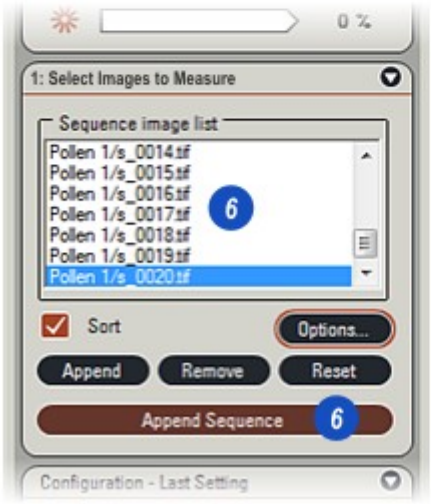
5: The image name appears in the list.

6: The program 'recognises' the image as part of a sequence and the *Append Sequence* button becomes active. Click to load all of the images to the list.

Continued… 897







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1: Remove individual images from the list by clicking to select the image and then clicking the *Remove* button.

2: To clear the list completely, click on the *Reset* button.

3: Sort the images in numerical order by enabling the *Sort* check box.

4: To output a sequential list containing all of the image results, click the *Options* button and...

5: ...from the *Process Settings* menu, enable the *Accumulate Results* check box.
If it is left unchecked results from individual images are displayed on the *Grid* during the *Processing Sequence* and at the end by clicking on an entry in the *Sequence List*.

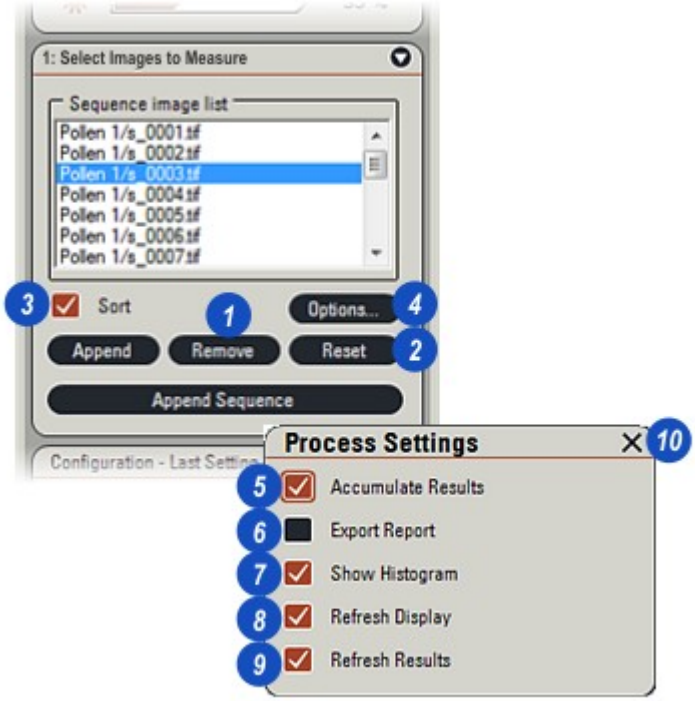
6: The *Export Report* check box when enabled will automatically create a report when processing is finished.

7: To display a *Histogram* for each image as it is processed click to enable the *Show Histogram* check box.

8: Enable the *Refresh Display* check box to refresh the display between each image analysis.

9: The *Grid* results display will be updated for every image if the *Refresh Results* check box is enabled.

10: Click the **'X'** to close the *Process Settings* menu.



Setup Report settings 898: Go there... 885

Continued... 898

To load individual images into the *Image List:*

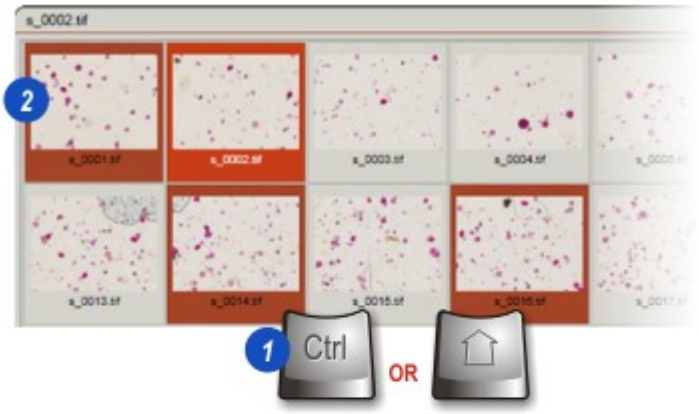
1: Press and hold either the *Ctrl* or *Shift* key on the keyboard and…

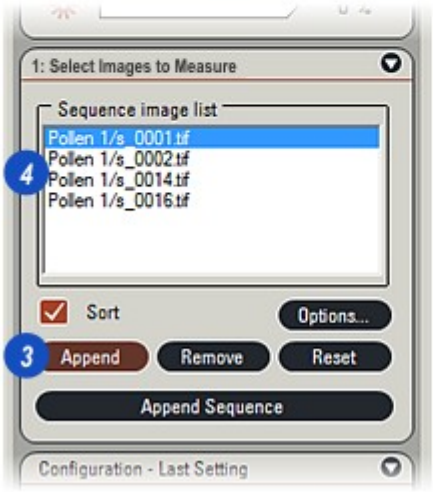
2: …click on each of the thumbnails in the *Gallery* of the images to be included. Those selected will have a brown frame.

3: Click on the *Append* button and...

4: ... the image names will appear in the list.

Continued… 899





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1: Click on the *New Sequence* button. Because this will delete all existing *Processing Sequence* results…

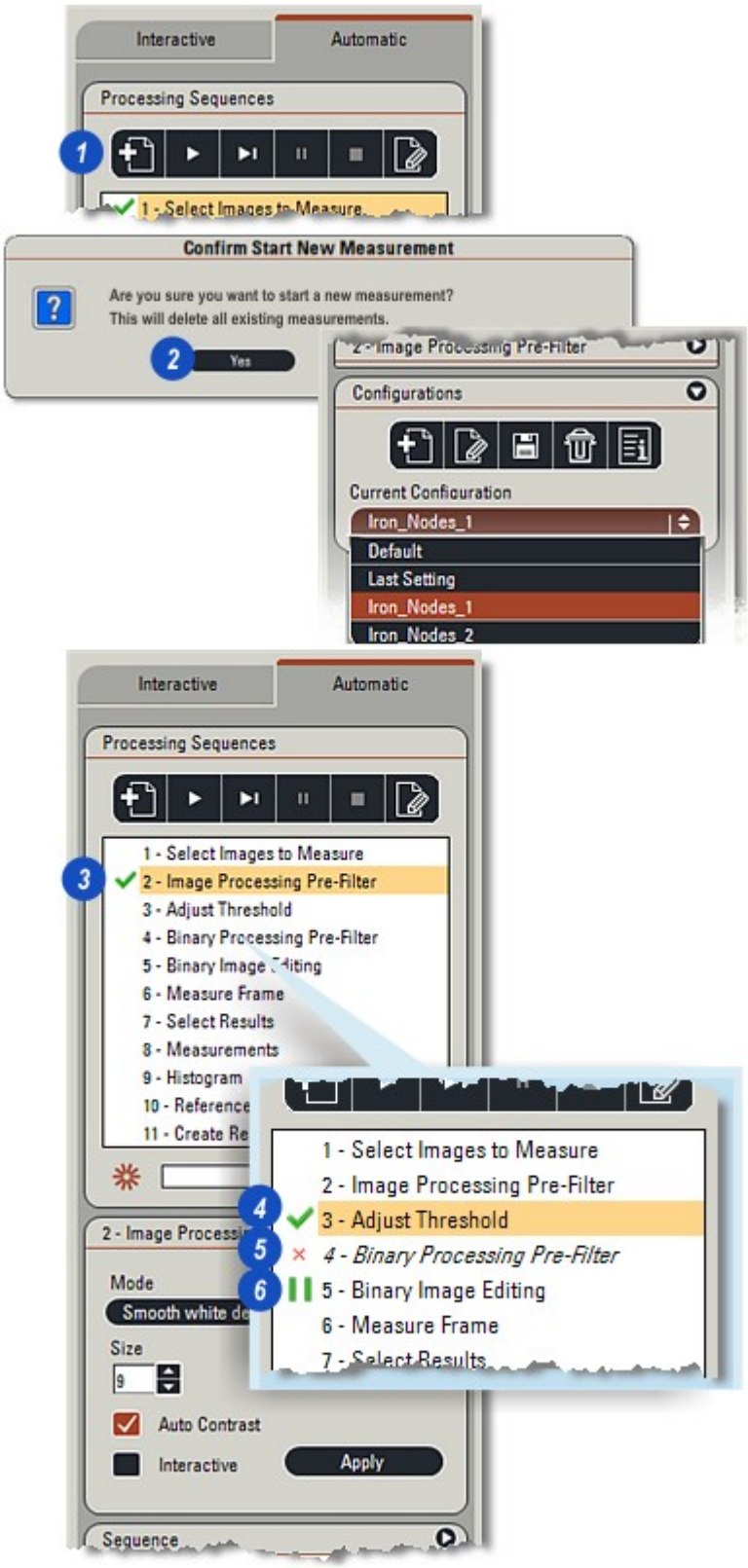
2: …confirm a new set of measurements by clicking *Yes* at the dialog. Click *No* if the current settings are going to be required later and save them to a configuration.

3: Select a typical image from the list and apply the processing tools in the usual way. As settings are made, the program keeps track of them displaying a green tick mark to the left of the name to indicate normal processing with the current settings.

4: Some images may require specific additional processing – for instance, artefacts may need to be removed on an image-by-image basis using the *Binary Edit* tool. The *Processing Sequence* can be configured to halt at any tool or to skip a tool completely by clicking to the left of the tool name until…

5: …a red cross appears to indicate this tool should be skipped or…

6: … two green vertical bars are shown to denote that the *Processing Sequence* will pause at this tool on every image for further manual changes to be made.



Saving settings as a Configuration: Go there... 902

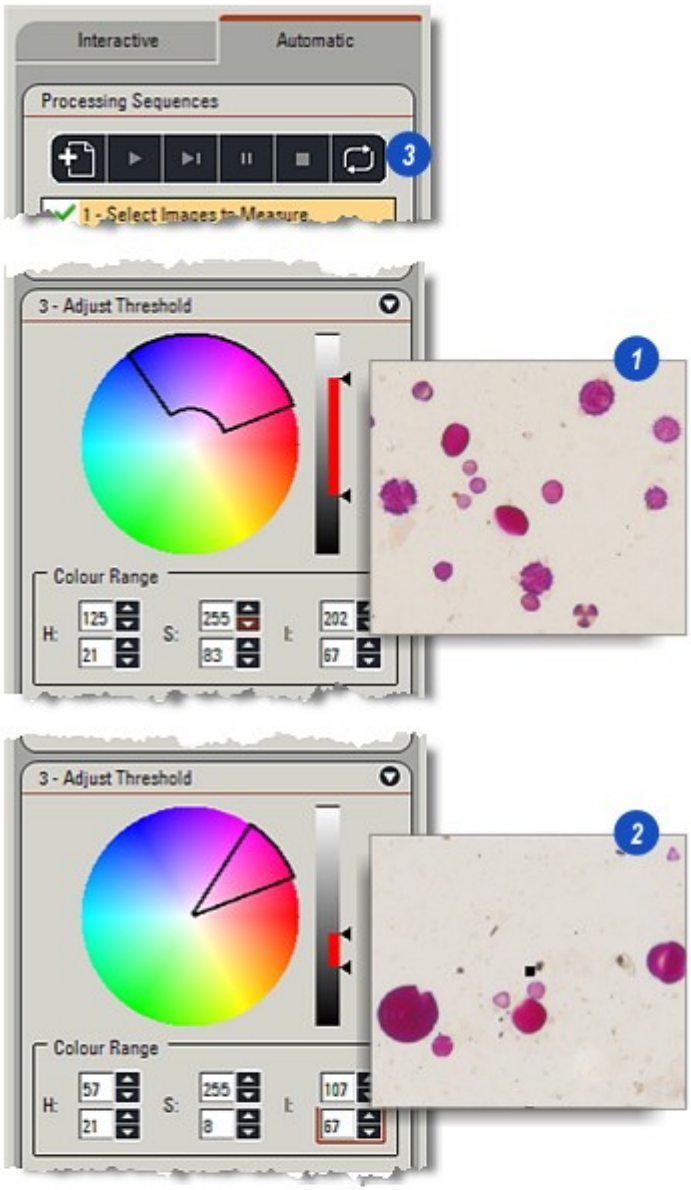
Continued… 900

Images in a sequence or group can have different filters or setting applied to them; The settings are automatically applied by the *Processing Sequence.*

1 & 2: In the illustration two images from the same sequence have different *Threshold* settings. Those for the first image would apply to all images in the sequence or group because they were made first.
Subsequently, the *Threshold* for image **(2)** was changed but this would not affect the remaining images, only image **(2).**

3: When changes are made to an individual image the *Update* button must be clicked to apply the new settings to the *Process Sequence*.

Continued… 901



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Sometimes, during the setup of a *Processing Sequence* it may be necessary to work on an image not included in the *Sequence* List. LAS Image Analysis is flexible enough to allow processing on a separate image whilst retaining the *Processing Sequence* information.

1: The image, although part of the sequence, has **not** been included in the *Sequence Image List*. However, work can be carried out on the image without affecting the *Process Sequence.*

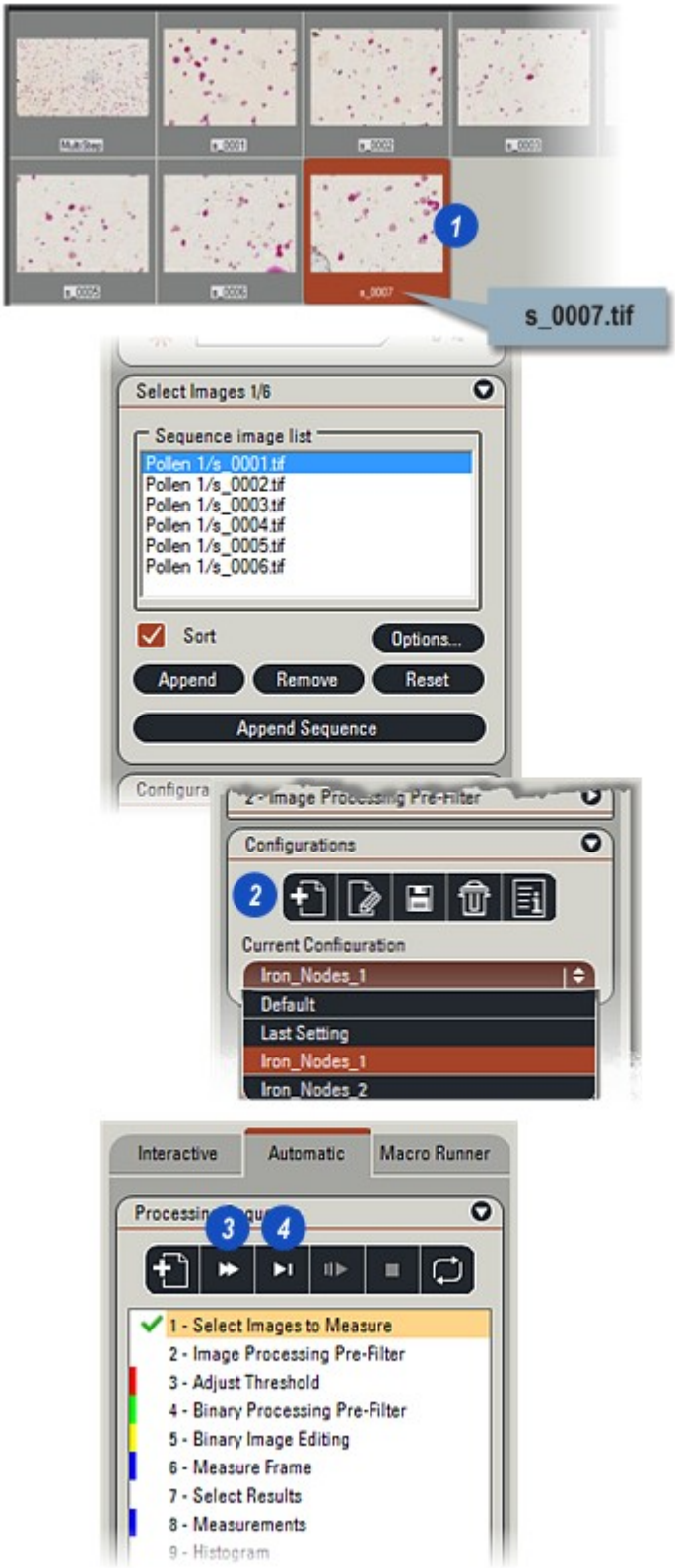
2: Having completed processing the separate image, create a new *Configuration*. This will include all of the current settings including those of the separate image, so its data can be recalled later.

Starting the Processing Sequence:

3: With the settings complete, click on the *Run Sequence* button. Each image will be processed in turn and the results displayed in the *Grid* if it is enabled. The image currently being processed is highlighted in the *Gallery* .

4: At any time the process can be halted and stepped through single process at a time by clicking on the *Next* button.

All of the Process Sequence data is saved in a Report as a Microsoft Excel file: Go there… 885

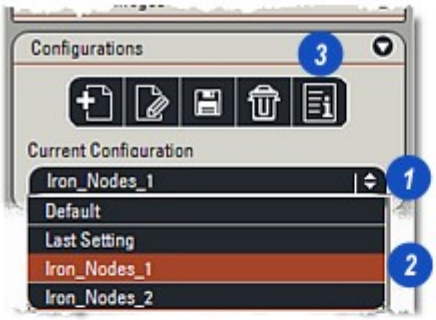


The current settings for most editing panels can be saved with the archive as a *Configuration* to be retrieved at a later date. Retrieved *Configuration* settings are applied to automatically to the tools

1: Each configuration has a unique name and can be accessed by clicking on the arrow to the right of the *Current Configuration* window and from the drop down list…

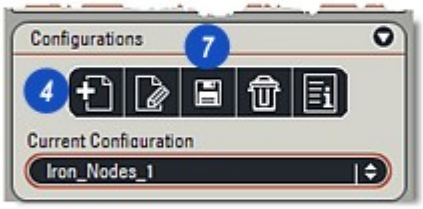
2: …clicking to select the required configuration.

3: Click the *Display Configuration Settings* button to list the configuration details.



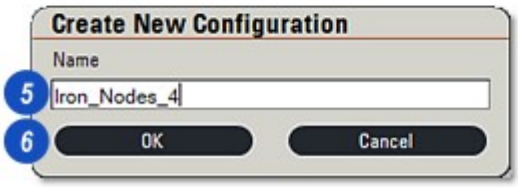
Save Configuration:
Saves all of the current settings and process sequences in a unique file.

4: To save the current settings as a new configuration, click on the *New* button and…



5: …type a unique name for the new configuration.

6: Click *OK* to save the setting.



7: Click on the *Save Configuration* button The new configuration appears in the drop down list.

Continued... 903

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Edit Configuration:
A *Configuration* name can be changed by:

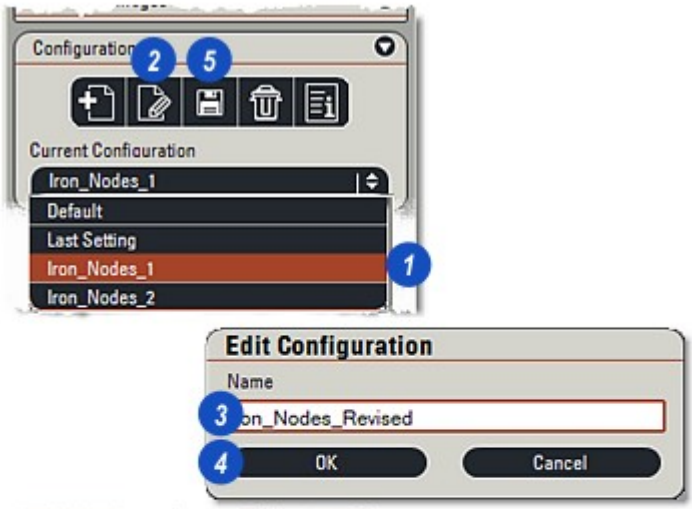
1: Select the configuration to be changed from the drop down list.

2: Click on the *Edit Configuration* button.

3: On the *Edit Configuration* dialog, change the name by clicking in the text box and typing a new name and…

4: …clicking *OK*.

5: Click the *Save Configuration* button.

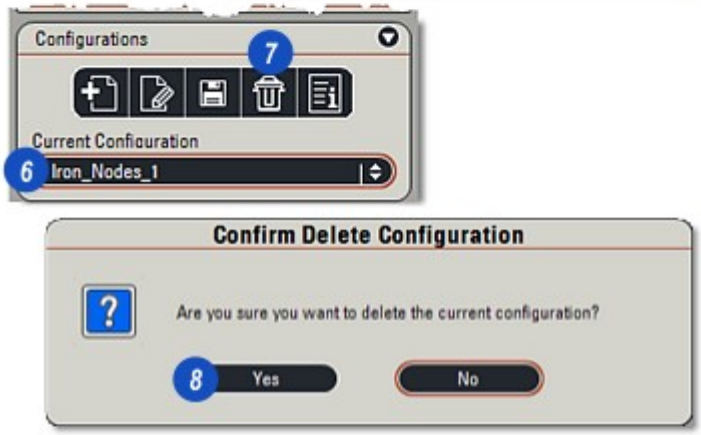


Remove a Configuration:
A *Configuration* can be removed from the list by:

6: Selecting the configuration to be deleted from the drop down list.

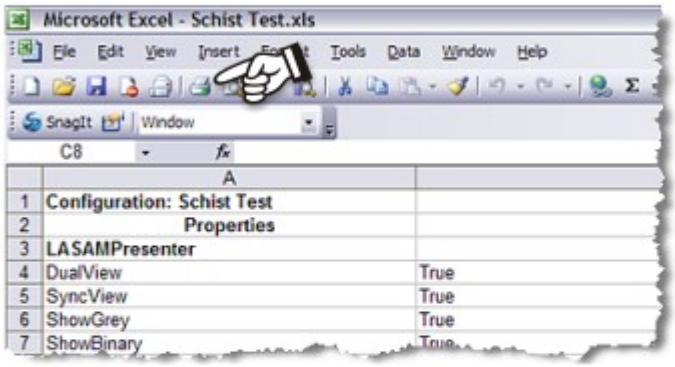
7: Clicking the *Delete* (Trash Can) button.

8: Confirm the deletion and the *Configuration* will be removed permanently. The operation cannot be reversed.



Print a Configuration:
Check that the printer is on and connected to the computer. The configuration is captured to an Excel spreadsheet and printed from there.

1: Select the *Configuration* to be printed from the drop down list. When the Configuration settings appears on an *Excel* spreadsheet, use the print function to print the settings list.



Appendix:

This Appendix contains useful reference information. The word *'Feature'* is used to indicate parts of the image that are of interest and need to be measured.

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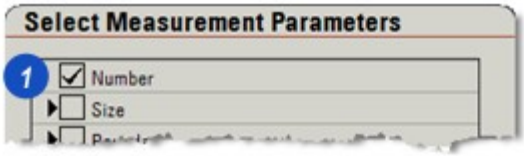
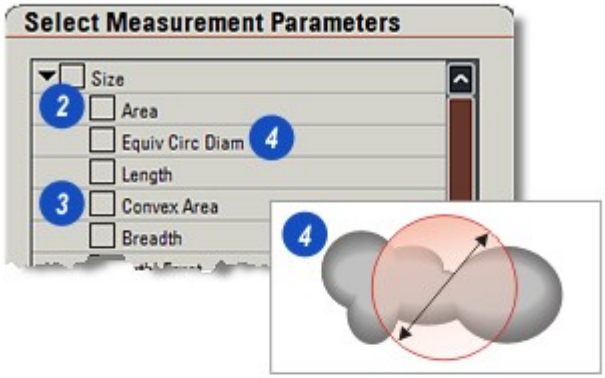
All measurements are quoted in calibrated units.





Number:

1: *Number:* Carries out a count of all the selected objects on the Binary Output Image.

Size:

2: *Area*: Measures the Area of every selected object.

3: *Convex Area:* Derived from the mean Feret diameter approximating to the enclosing polygon.

4: *Equivalent Circle Diameter:* The diameter of a circle that has the same area as the feature.

Continued... 906

1: *Length*: The greatest distance between parallel lines drawn through 2 points on a feature's boundary regardless of orientation. Also called *Max Feret Diameter.*

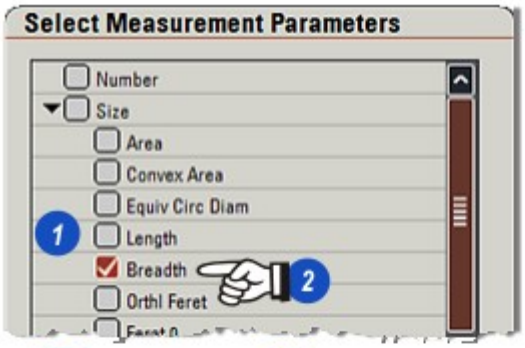
2: *Breadth*: The shortest distance between parallel lines drawn through 2 points on a feature's boundary regardless of orientation.

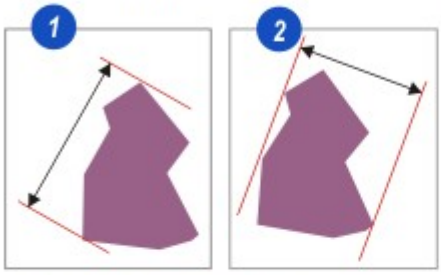
3: *Orthogonal Feret*: The Feret diameter perpendicular to the Max Feret diameter or length.

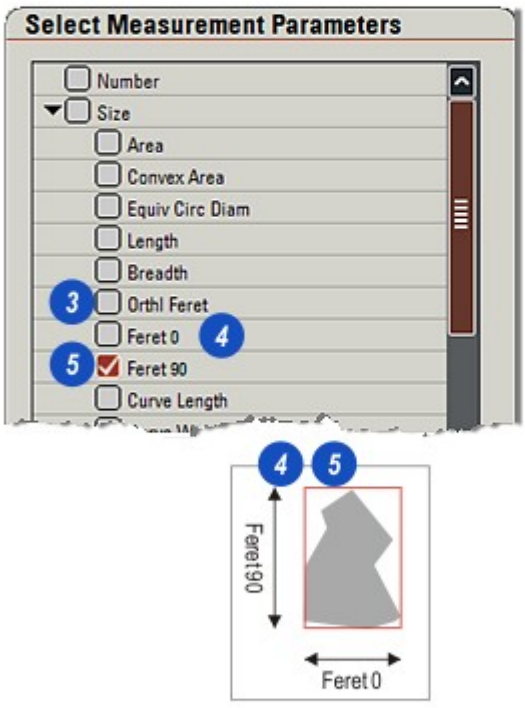
4: *Feret 0:* The greatest horizontal distance (width) measured in the horizontal direction.

5: *Feret 90:* The greatest vertical distance (height) measured between parallel lines in the vertical direction. Feret 0 and Feret 90 are the equivalent of the width and height of a Bounding Box around the feature.

Continued…. 908







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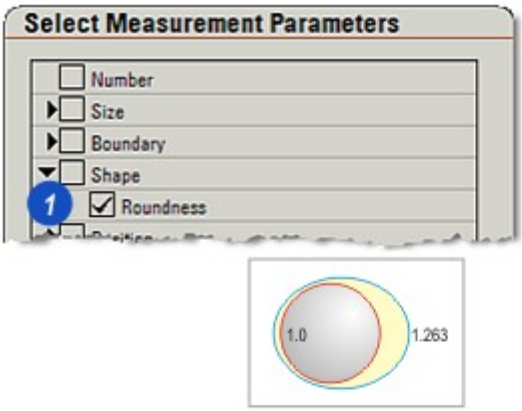
1: *Roundness*: The perfect circle has a notional value of 1.0. Any variations from the circular are reflected by an increase in the notional value.

2: *Aspect Ratio:* Object length divided by object breadth or Feret Length/Feret Breadth.

3: *Fullness Ratio:* The square root of the object area divided by the objects Convex Area. v(A/CA).

Continued… 909



1: *Curve Length:* The *Curve Length* can measure the actual length of an irregular feature – a piece of string for example - the curves of which may overlap.

2: *Curve Width:* Measures the width of the same piece of string.

Boundary:

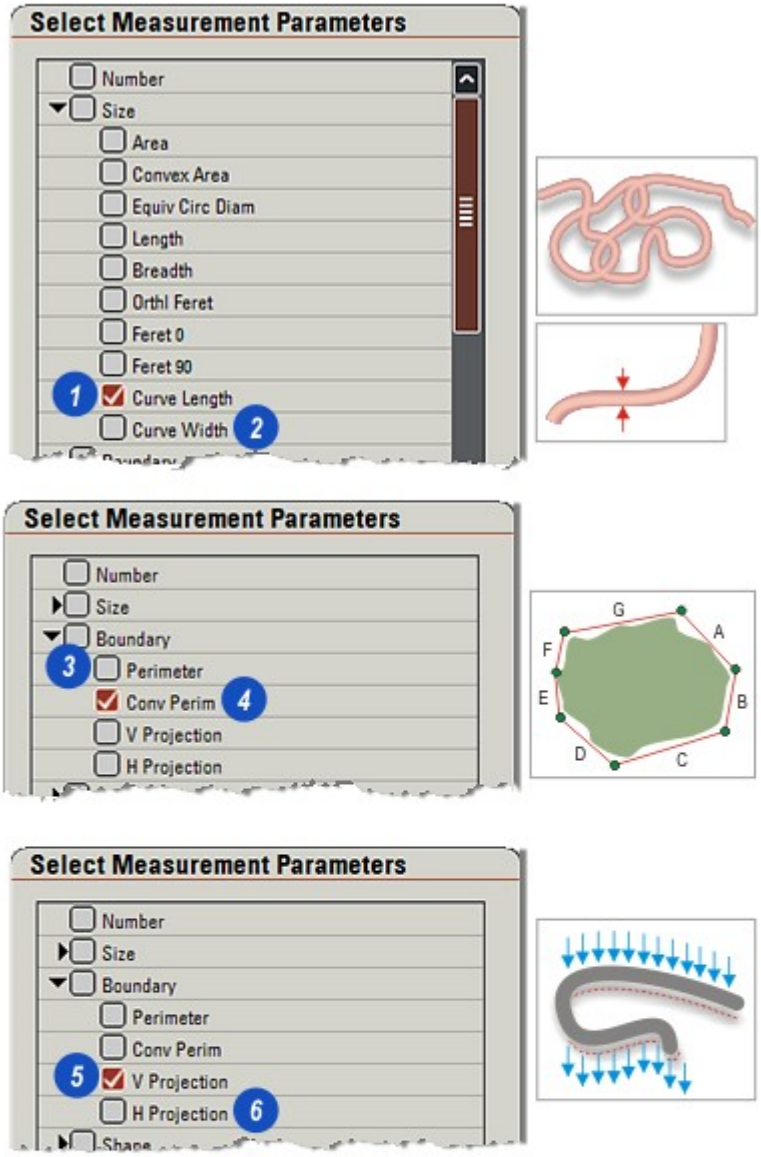
3: *Perimeter:* Count in µm of the distance around the Perimeter of a feature. It includes all inlets and projections and compensates for the edge orientation.

4: *Convex Perimeter*: Derived from the Feret diameter and approximates to the perimeter of the enclosing polygon.

5: *Vertical Projection*: The pixel count representing the shadow cast by a feature if light were impinging upon it from a vertical direction.

6: *Horizontal Projection:* The pixel count representing the shadow cast by a feature if light were impinging upon it from a horizontal direction.

Continued… 907



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FCP = Feature Count Point and represents the lowest and rightmost pixel in the feature.

1: *X-FCP* is measured from the left-hand edge of the image to the Feature Count Point, and…

2: *Y-FCP* is measured from the top edge of the image to the Feature Count Point.

Centroid is the *Centre of Mass* of an feature. Its position is measured from the edges of the image by the co-ordinates…

3: *X Centroid:* The distance from the left hand edge, and…

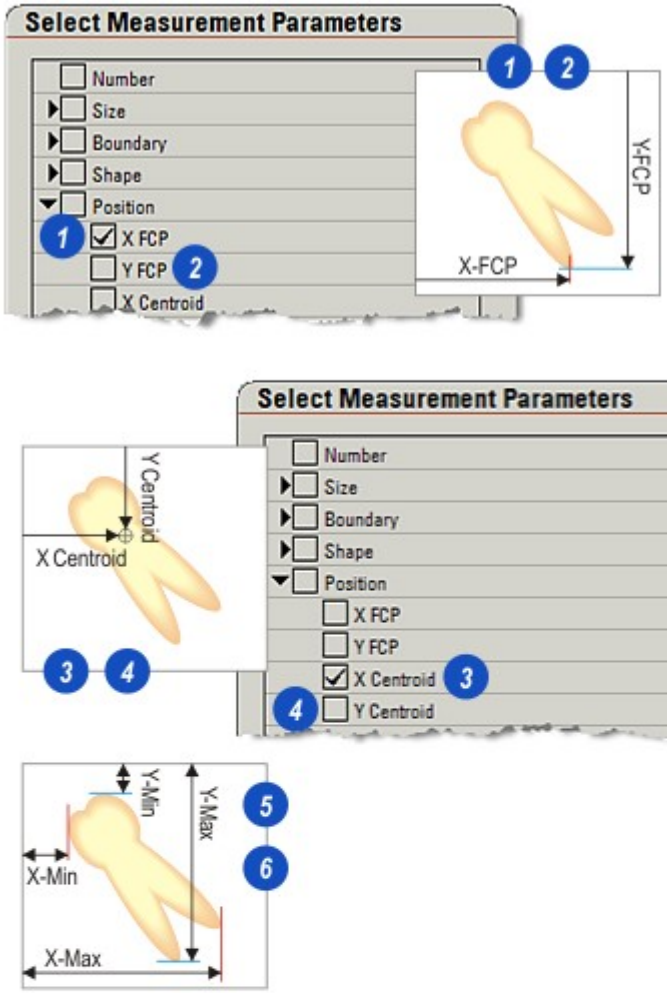
4: *Y Centroid:* The distance from the feature's top edge.

The feature's position co-ordinates on the image are measured with:

5: *X-Min and X-Max:* The boundary pixel closest to and furthest from, the left hand edge of the image.

6: *Y-Min and Y-Max*: The boundary pixel closest to and furthest from, the top edge of the image.

Continued… 910



1: *Orientation:* The angle of the Feret Length to the horizontal.

2: *Orthogonal Orientation:* The angle perpendicular to the Orientation (Feret Length) - essentially the Orientation minus 90º).

3: *Derived Orientation:* The angle representing a line plotted through a range of pixel co-ordinates.

Continued… 911

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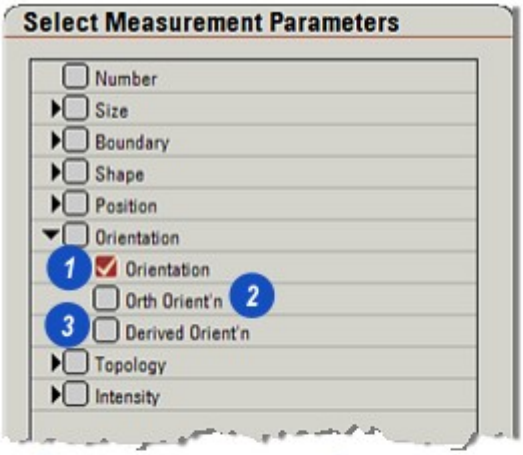

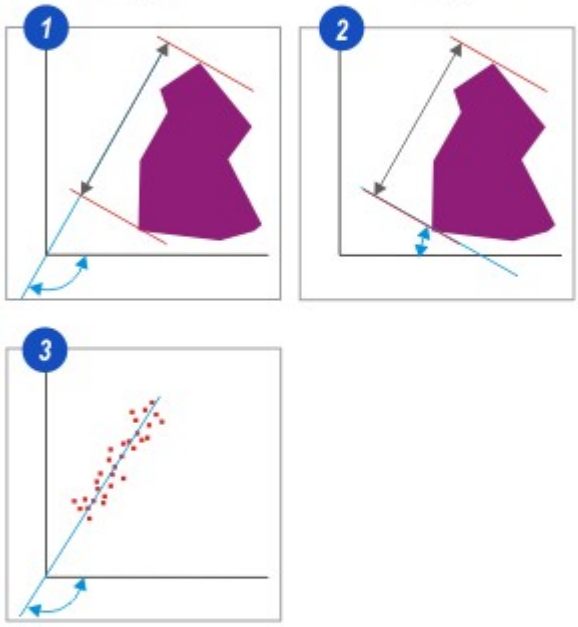

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Topology:

1: *Forks*: The number of downward projecting fork-shaped limbs.

2: *Joins*: The number of points at which a projection (limb) joins the feature.

3: *Tops*: The count of upward-extending projections (limbs).

4: *Ends*: The number of downward-extending projections (limbs).

Intensity:

5: *Integrated Grey*: Sum of the grey value of all of the pixels within the feature.

6: *Grey Mean:* Sum of the value of all of the pixels in the feature (Integrated Grey) divided by the number of pixels in the feature (Equivalent to Area).

7: *Grey Variance:* Result of the value of the darkest pixel in the feature subtracted from the lightest pixel value.

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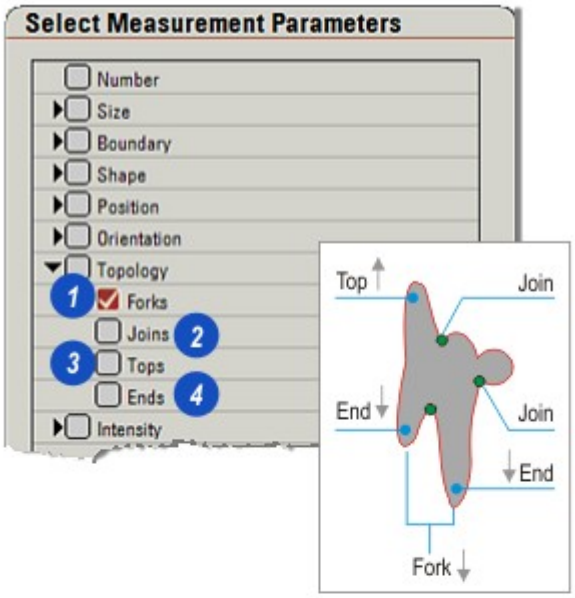
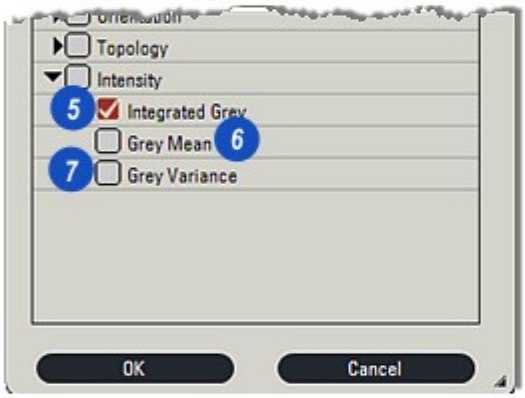




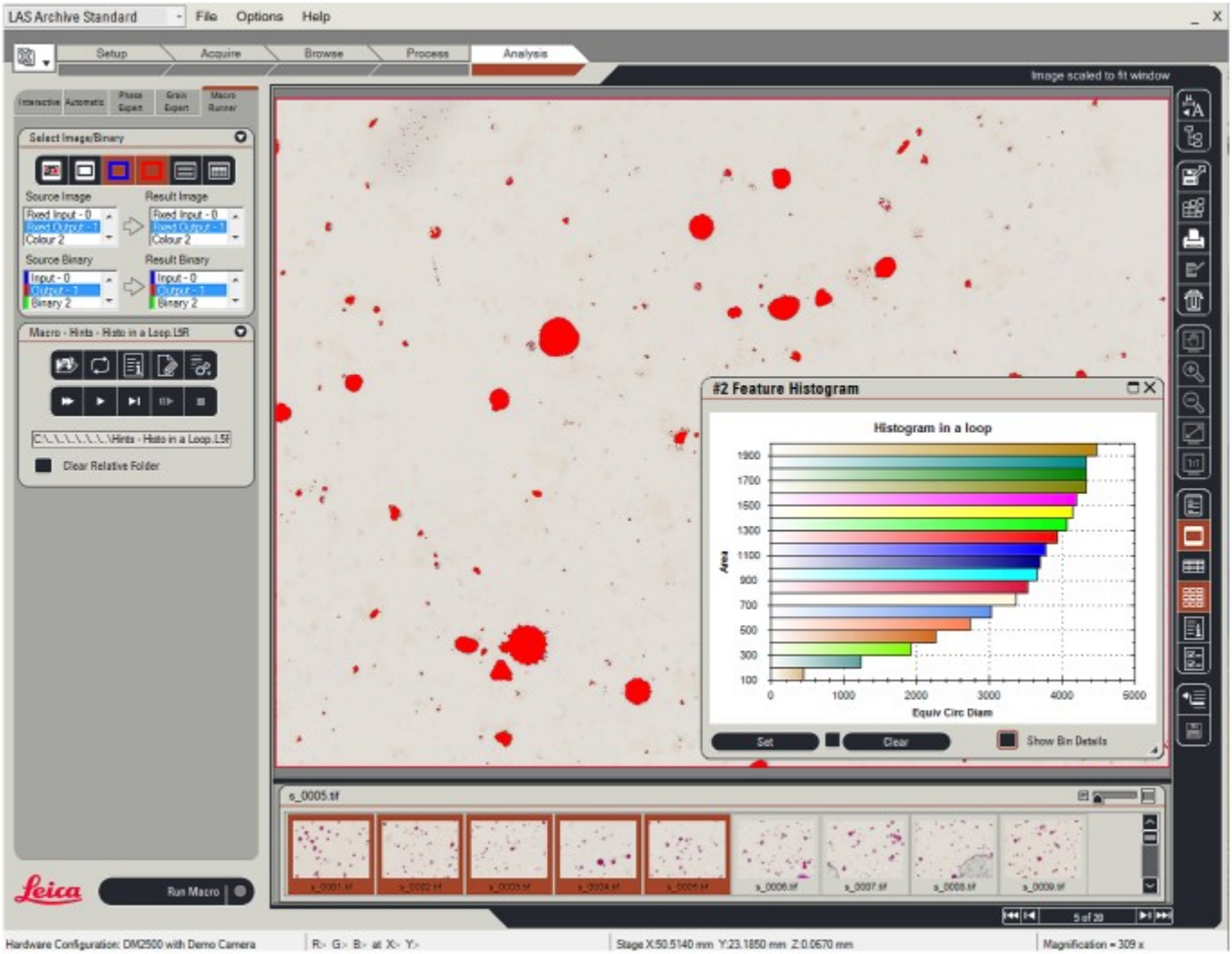
LAS Macros automate image processing, analysis and measurements for quantitative microscopy. The richness of image processing functions in LAS can be adapted to a diverse range of imaging tasks. LAS Macro allows repetitive tasks to be customised to the needs of particular applications, optimising imaging solutions in a wide range of fields. This versatile software processes images obtained by Leica digital cameras and digital microscopes by the software of the Leica Application Suite.

The *LAS Macro Editor* defines the instructions for image processing, binary processing and measurement. An LAS Macro routine with its image processing instructions is very much like a conventional computer program. The difference is that you do not create it by typing in its statements character by character; rather, you create it interactively, using facilities from the panels of the LAS Macro Editor. An instruction is created automatically and inserted into the LAS Macro by pressing the Insert button next to the instruction in the Pending Instruction window. There is no need to write any software code!

Macro programs are run within the LAS environment either using the *LAS Macro Runner* or in combination with *LAS* ***Image Analysis***. LAS Macros are included in the analysis sequence at the step for Image *Processing* or *Binary Processing.* This combination makes a complete application solution that can be repeatedly used by operators with no specialist knowledge of LAS Macros.

By adding the versatility of LAS Macros to the automation of the *LAS Image Analysis* sequence or to the simplicity of the *LAS Macro Runner*, provides an efficient solution to demanding and unconventional tasks in analytical microscopy.

For detail information about the optional LAS Macro Runner, please refer to the LAS Macro Editor help file. *Go there...* 912



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Phase Expert

Optional module *Phase Expert* has been designed to measure precisely up to ten different phases – regions of the image that can be identified by their homogenous colour or grey level.

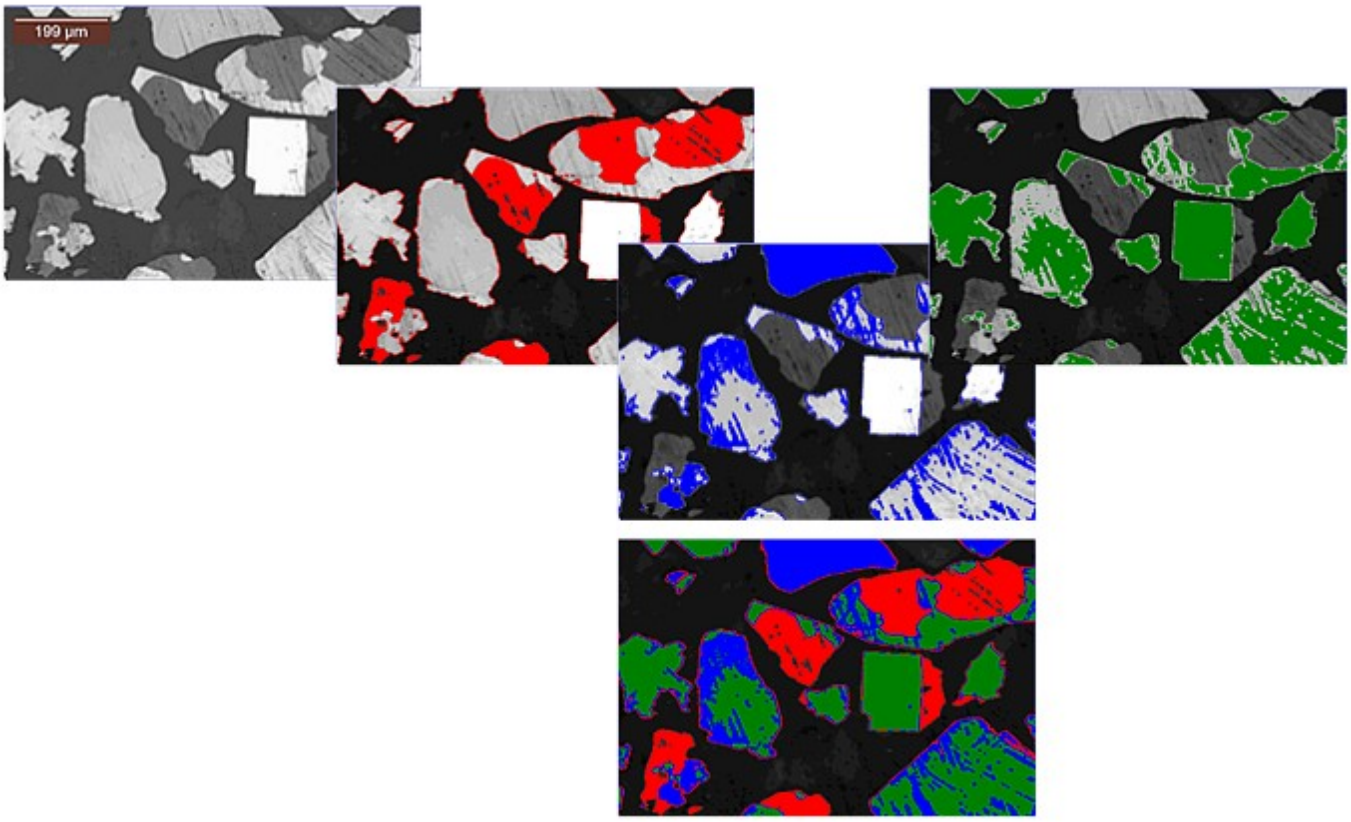
For example, regions of differing reflectivity in oil-shale; Colours due to the polarised light of different constituents in a rock section or stain variations in tissue or bone sections. Phase Expert can determine the occurrence of these phases both in terms of the overall image or with reference to just one of the selected phases.

Samples are typically embedded in a resin billet with the image face ground and polished to good reflective flatness.

Single or multiple images can be processed automatically once any required grey processing filters specified and thresholds set.

Results can be displayed on screen in a variety of formats both as graphics and as tabular in the *Grid*. And they can be saved as a report to *Microsoft Excel Spreadsheet* for easy distribution. Displayed results and reports provide wide scope for tailoring to the user's needs.

Phase Expert works in conjunction with *Image Analysis* which must be installed, licensed and enabled.



These steps represent the sequence users should follow to successfully carry out *Phase* measurements.

Because Phase Expert works in conjunction with Image Analysis, in most steps links are provided to both modules:
Red, will take the reader to the appropriate place in the Image *Analysis (Automatic)* help file for more detailed information, and
Blue, connects to help files that are specific to *Phase Expert*.
Sometimes, it will be beneficial to follow both links if they are provided.

Set up the Phases:
Each of the *Phases* to be measured is colour-coded and that colour is used on the processed image to distinguish it from other *Phases*.
- Names and colours of the *Phases* can be selected by the user.
- Up to 10 different *Phases* can be chosen for measurement.

Phase Expert help >>> 916

Select the Images to Process:
One or more images can be processed in sequence simply by selecting the *Thumbnail(s)* in the *Gallery* and then clicking the *Append* button on the *Select Images to Measure* panel.
Image Analysis help >>> 782 *Phase Expert help >>>* 920

Choose the Reference:
Phase Expert provides two paradigms for determining the measurements:
- Measure each *Phase* against the *Field* – the entire image, or:
- Measure each *Phase* against a selected *Phase* called the *Reference*.

If the entire Field is to be used check that none of the phases have the *(Ref)* marker set against and go to the next step.
Phase Expert help >>> 919

Set the Measure Frame:
The *Measure Frame* determines the part of the *Field* that is measured and analysed.
- *Phases* lying outside the *Measure Frame* will be ignored.
- For *Phase Expert* the usual option is *Entire Image* (Field).

Image Analysis help >>> 851 *Phase Expert help >>>* 922

Image Processing Pre-Filter:
A wide and powerful range of filters to improve *Phase* boundaries and recognition making measurement and analysis faster are more precise.
Image Analysis help >>> 797

Continued... 915

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Adjust Thresholds:

Adjust Thresholds allows the upper and lower pixel values for each *Phase* to be adjusted so that they accurately represent the desired boundaries. The *Phase* detection process uses the pixel range values set during *Threshold* adjustment.

- Clear, easy to use *Histogram* shows each *Phase* in its own colour with a simple *Bar* display beneath.
- Range adjustment can be made by dragging on the *Histogram* or by entering values.
- Two modes – *Continuous* and *Overlapping* – are available.

Image Analysis help >>> 814 *Phase Expert help >>>* 923

Binary Processing Pre-Filters:

The *Binary Pre-Filters* provide the tools for modifying the Binary Processed Image in the same manner as *Greyscale Pre-Filters* work on the original image but with finer precision – small, individual details can be targeted and modified.

- Discard and Combine details.
- Fill Holes and Separate Touching details.

Image Analysis help >>> 830 *Phase Expert help >>>* 929

Binary Image Editing:

Binary Image Editing is a tool collection that provides the methods for working directly on processed *Binary Images* to add, remove, select and de-select details.

- Facilities also include drawing and filling shapes as well as grouping features.

Image Analysis help >>> 838 *Phase Expert help >>>* 930

Results and Histogram:

There is a wide range of options for displaying *Phase Expert* results both as graphics – *Bar* and *Pie* charts – and tabular in *Detail* and *Summary*.

- Create personalised layouts and save as re-usable Configurations.
- Display tabular results for multiple fields as aggregates or individual fields and phases.

Image Analysis help >>> 870 *Phase Expert help >>>* 931

Reference Data:

A comprehensive range of *Data Items* can be appended to the *Phase Expert* results that will identify important details such as the *Project Name,* the *Specimen* and how it was prepared. Enter the information in the *Reference Data* dialog.

- Administrators can add to the supplied list of data headings to comply with corporate demands.

Image Analysis help >>> 883 *Phase Expert help >>>* 939

Create Report:

Phase Expert Reports are created and saved in *Microsoft Excel* spreadsheet format. *Excel* must be installed on the computer to create the report but only the *Excel Viewer* is needed to display it. Images can be included and each report is structured to show the results comprehensively on individual sheets.

- A flexible Template is supplied with Phase Expert.
- The default Template can be modified to suit the user's needs.
- The report can be displayed as it is created.

Image Analysis help >>> 885 *Phase Expert help >>>* 940

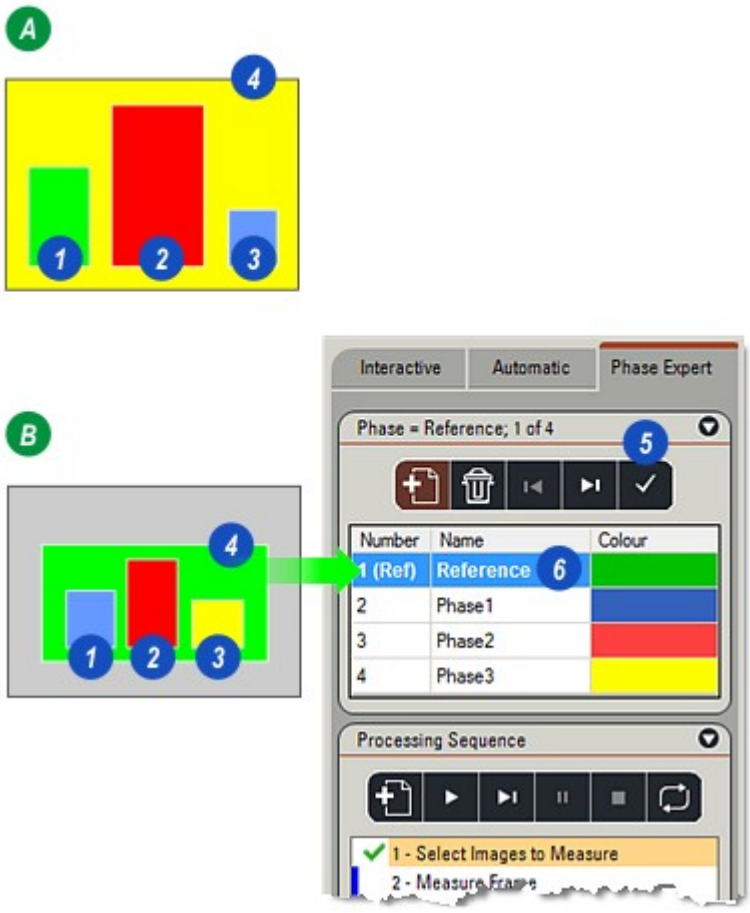
Phase Expert measurements can function in either of two modes:

A: *Field Mode*: In which each of the phases *Green* **(1),** *Red* **(2)** and *Blue* **(3)** in the diagram are measured with respect to the entire *Visible Field* **(4)**.

B: *Reference Mode:* The phases *Blue*, *Red* and *Yellow* are measured with respect to another phase coloured *Green* **(4)** in the illustration, that generally encompasses the others. The *Field* parameters are ignored in the measurement comparisons but are reported.

5: *Reference Mode* is selected by clicking the *Tick* button on the tool bar. Clicking again turns off *Reference Mode.*

6: When *Reference Mode* is active the tag ***'Ref'*** appears next to the chosen phase. In the illustration the word *Reference* has been typed in by the user.



Continued… 917

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Each phase to be measured is colour-coded on the binary output image. Set up the phases by:

1: Click on the arrow to the right of the *Phase* header to reveal the panel. On opening a single phase with the default name *Phase 1* is automatically displayed.

For some jobs a single Phase will be sufficient, but many images will contain several Phases all of which need to be identified. In these cases add further Phases as described on the following pages.

2: Change the *Phase Name* by clicking in the name text box and typing a new name.

3: To change the *Phase Colour*, double-click on the colour and…

4: …the *Colour* dialog appears. Choose a colour from the swatches, wheel or hue slider or type the required values.

5: Click *OK.*

6: An example of a *Phase Name* and *Colour* change.

Continued… 918

1: Add further *Phases* by clicking on the *Create New Phase* button. Change the *Phase Name* and *Colour* using the procedure described on the previous page.

As additional *Phases* are added they are given a sequential number shown in the left-hand column. The lowest number represents pixel values close to 0, the black end of the greyscale. Each additional *Phase* represents a higher span of pixel values so the three *Phases* in the illustration might represent the greyscale pixel values:

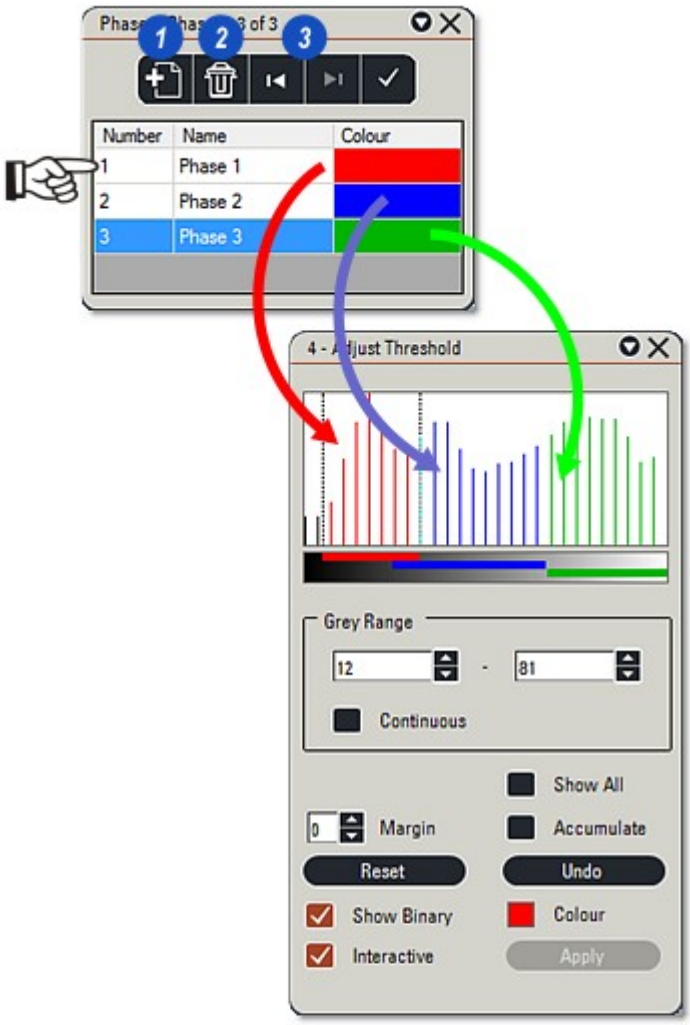
Phase 1: Red: 0 (Black) to 72.
Phase 2: Blue: 73 to 154.
Phase 3: Green: 155 to 255 (White).

...for example.

The initial range values are established by software based on a broad analysis of the image. The user will adjust them during a later step.

2: Delete a *Phase* by clicking to highlight it and then clicking on the *Trash Can* (Delete) button. If a *Phase* is deleted by mistake, immediately click the *Create New Phase* button and it will be restored.

3: Navigate through the *Phases* using the *Previous* (Left) and *Next* (Right) buttons.



Continued... 919

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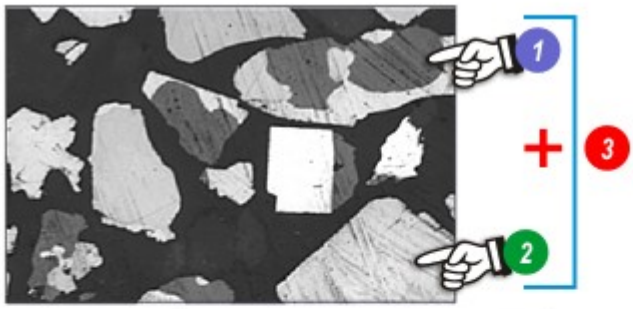
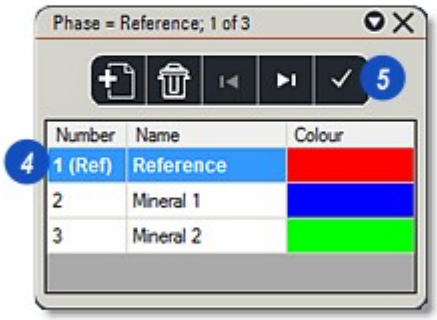
In this example, flakes of two minerals - dark grey **(1)** and light grey **(2)** on the image, exist blended together. The task is to determine what percentage of each is represented by the blend. The area of both minerals together **(3)** is represented by a combined phase called the *Reference*.



- The first step is to measure the area of *both* minerals to determine the combined *Reference*..
- The next step is to measure *Mineral 1* separately and finally...
- ...measure *Mineral 2* separately.



4: The combined measurement - the *Reference* - is colour-coded red. *Mineral 1* is coloured Blue and *Mineral 2,* green. The colours have been set up as phases with appropriate names.

5: Set the *Reference* phase - the combined measurement - by clicking on the phase and then on the *Set Reference* button. *(Ref)* appears against the phase to indicate it is the *Reference*.

To see the outcome of this example: *Go there…* 926

Continued… 920

1: On the *Processing Sequence* click on *Select Images to Measure.*

2: Click on a *Thumbnail* in the *Gallery*. *If a single image is to be processed it does not have to be added to the Sequence Image List*. Continue only if multiple images are going to be processed.

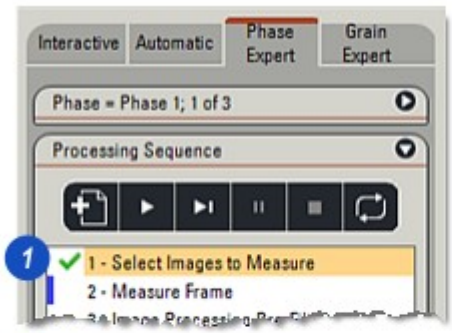
3: Click to *Append* button on the *Select images to measure* dialog.

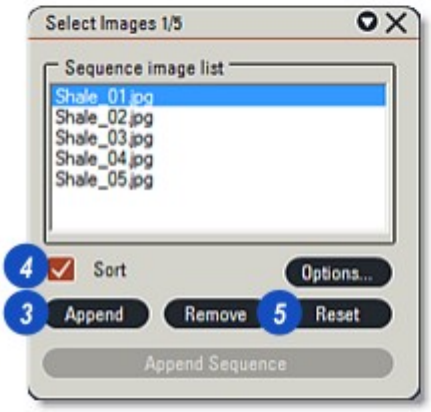
Repeat the steps to add more images to be measured as a batch.

All selected images must have the same type - for example .bmp, .png, .jpg - although it is recommended that images of type tif or jpg are used because these are not compressed. Sometimes artefacts of the compression process are seen in formats using compression.

4: Images can be selected in any order from the *Gallery*. To sort them into numerical sequence click to enable the *Sort* check box.

5: Use the *Remove* button to remove a single selected image from the list, or click the *Reset* button to remove all images.





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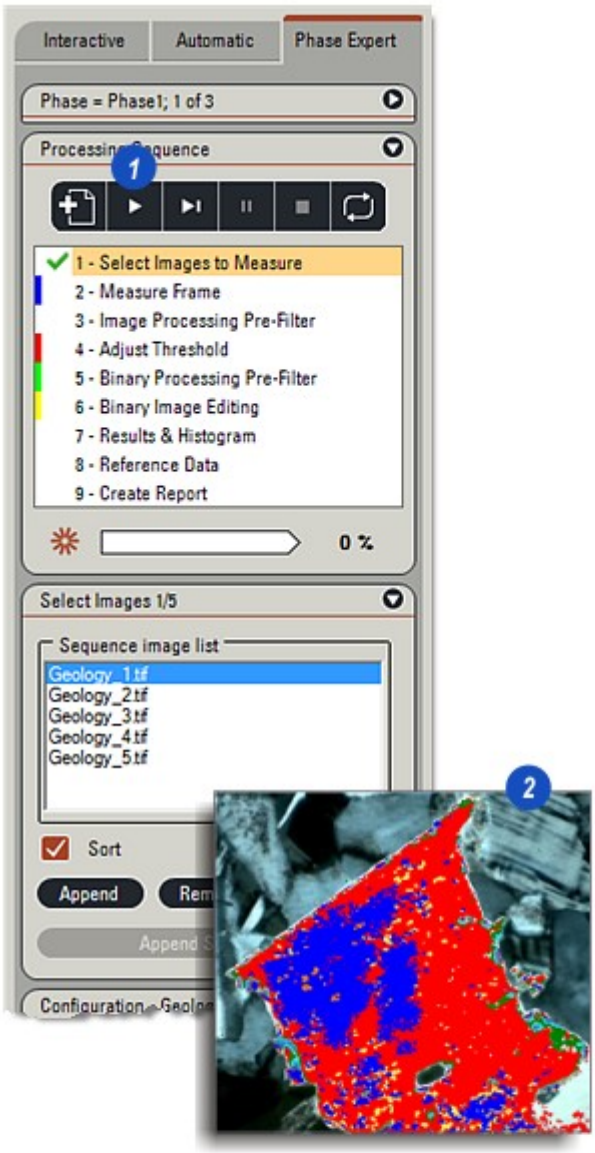
When multiple images have been added to the *Image List*, the process so far has been carried out on just one of them. But the program 'remembers' the settings so by clicking on the *Run Sequence* button **(1)** all of the images are processed one after the other and measured using the same settings. This is sometimes called Batch Mode.

The image **(2)** shows all of the phases displayed together. Use the *Show All* check box on the *Adjust Threshold* panel to see this result.

For further information about the Processing Sequence see *Image Analysis* help: *Go there...* 894

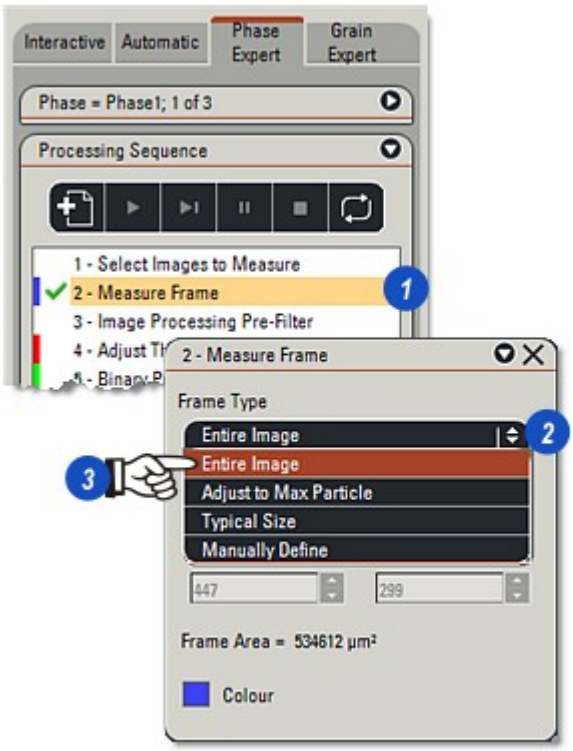
Continued... 926



1: In the *Processing Sequence* click to select *Measure Frame*.

2: On the *Measure Frame* panel click on the arrows to the right of the header.

3: Select the *Entire Image* option from the drop down menu. Although other options are available and can be used, *Entire Image* is the usual selection for *Phase Expert.*



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Adjust Threshold allows the pixel range values of each phase to be adjusted so that they accurately represent the phases boundaries. The phase detection process uses the upper and lower pixel values set during the *Threshold* adjustment.

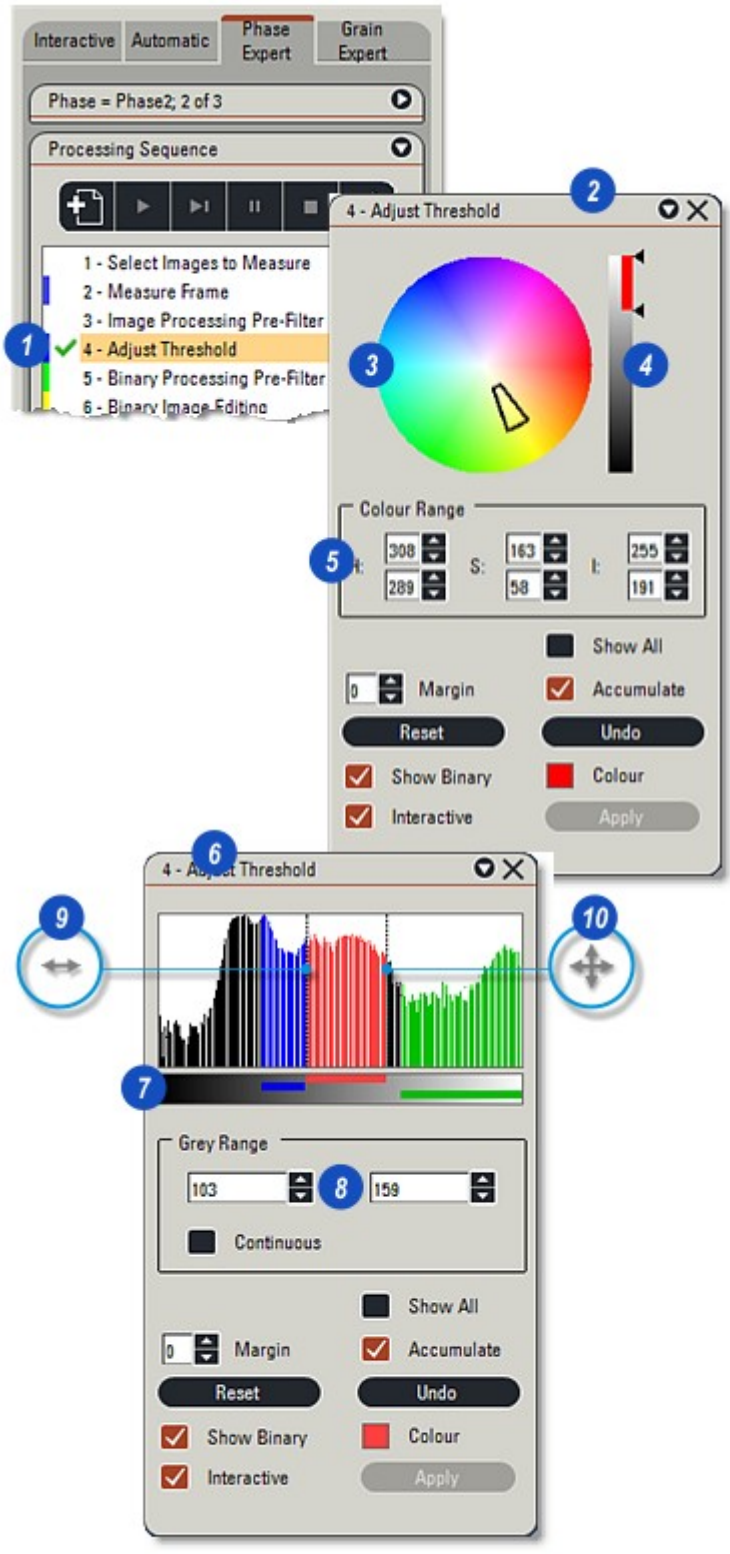
1: Click to select *Adjust Threshold* on the *Processing Sequence* menu.

Colour images use the colour wheel dialog **(2)** that allows *Hue* and *Saturation* to be set on the *Colour Wheel* **(3)** and Intensity controlled with the *Slider* **(4)**. Actual values can be typed into the *Colour Range* text boxes **(5)**.

Monochrome or greyscale images use the *Histogram* panel **(6)** that also has *Colour Bars* that, in some instances show how pixel values overlap each other. Values can be typed into the text boxes **(8)**. The value range on the *Histogram* can be adjusted by clicking and dragging on the vertical dashed bars when the cursor is represented by a double-ended arrow *(9)*, or the entire range can be shifted up or down when the four-ended arrow **(10)** is displayed.

The following pages illustrate *Threshold* adjustment for both colour and mono images.

Continued… 924



In this example three phases are going to be detected and measured with respect to the entire visible field, hence *Field Mode.*

On original image **(1),** the three areas of interest are the yellow flecks, the orange speckles and the pale blue blobs that surround the yellow area. Each area will be detected in turn starting with the yellow flecks – *Phase 1* detected in *Red*.

2: On the *Phase* panel, click to select the *Phase 1 (Red).*

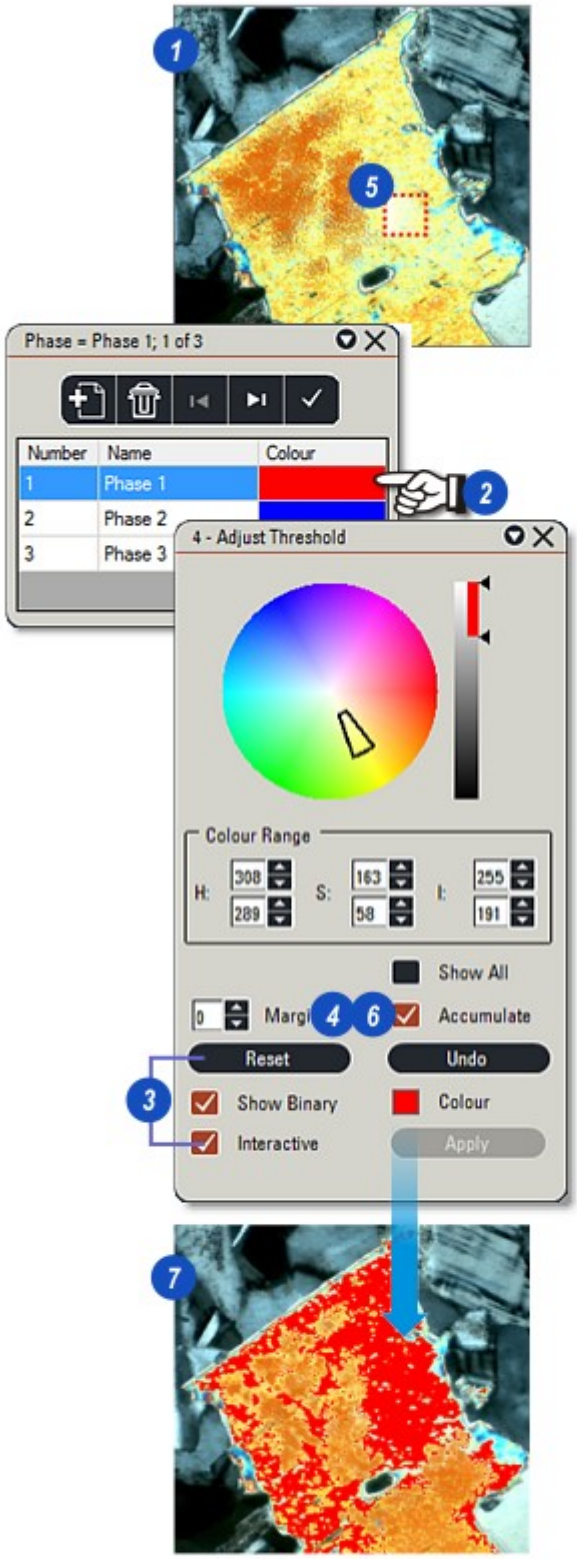
3: Enable *Interactive* and *Show Binary* and click the *Reset* button to remove any existing detection.

4: Disable *Accumulate*.

5: Click on part of the area of interest – in this case the yellow flecks – and drag a small rectangle. The pixel values contained within the rectangle are immediately displayed on the *Colour Wheel* and in the text boxes and the binary image **(7)** will be shaded with the phase colour – in this case *Red..*

6: Click to enable *Accumulate*. This will add any further selections to the existing. Keep repeating step **(5)** to include any (yellow flecked) area not yet detected. Make fine adjustments on the *Colour Wheel, Intensity Slider* and in the *Colour Range* text boxes.

7: The final image with *Phase 1 (Red)* detecting all of the yellow flecks.



Continued… 925

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The process for detecting the two remaining phases – orange speckles and pale blue blobs, *Phase 2* and *3* – is the same as threshold detection for *Phase 1* but selecting relevant areas on the image.

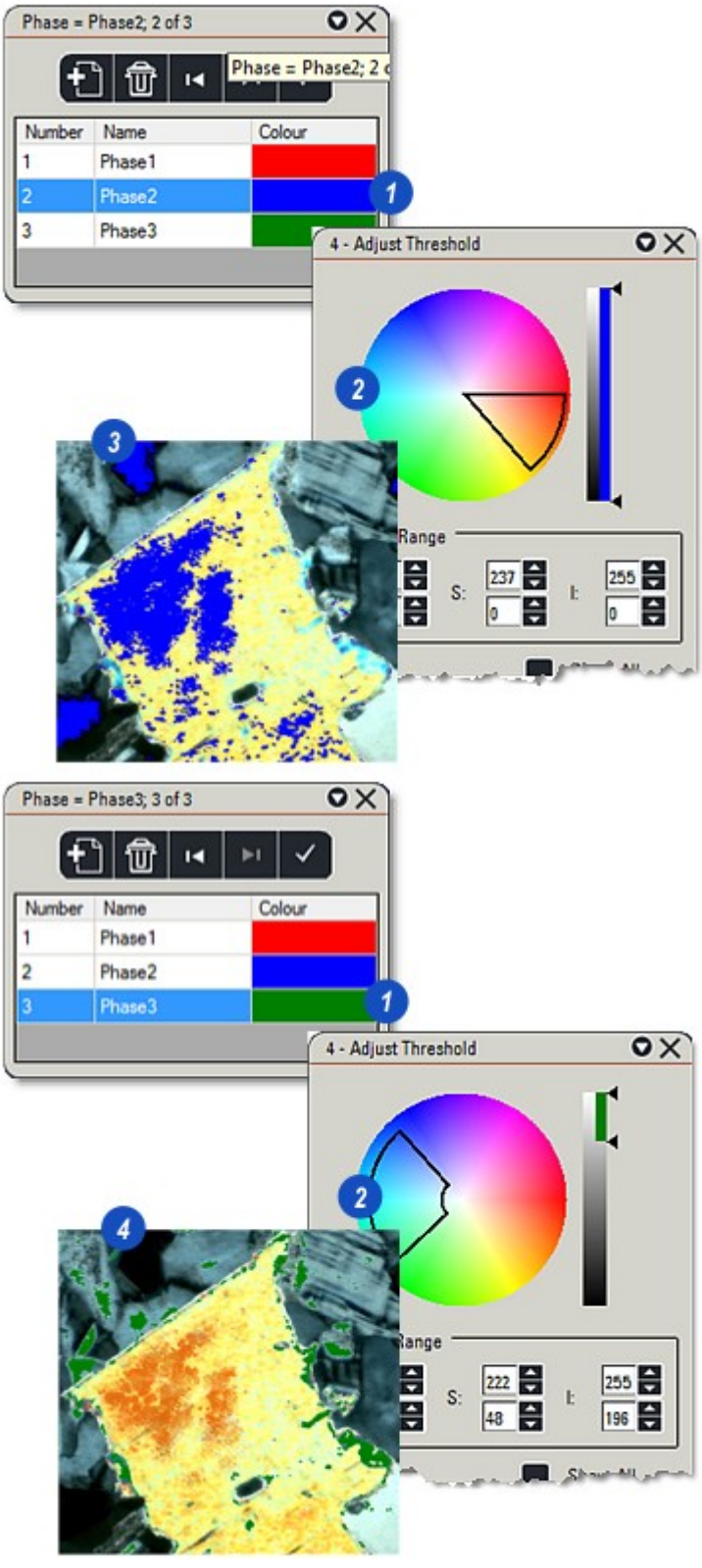
1: Click to select the phase to be detected.

2: Select and detect an appropriate area of the image and adjust the threshold on the *Colour Wheel* controls.

3: The result of *Adjust Threshold* for the orange speckles – *Phase 2 Blue*, and…

4: …for *Phase 3 Green*, the pale blue blobs.

Continued... 926



1: Click on the *Reference* phase on the phase list. This indicates the phase that is going to be selected. In this example the *Reference* is going to include both *Mineral 1* and *Mineral 2.*

2: Click to enable *Show Binary*, *Interactive* and also on *Reset* to clear any automatic detection.

3: Click to disable (OFF) *Accumulate*.

4: Disable the *Continuous* function on the *Histogram*.

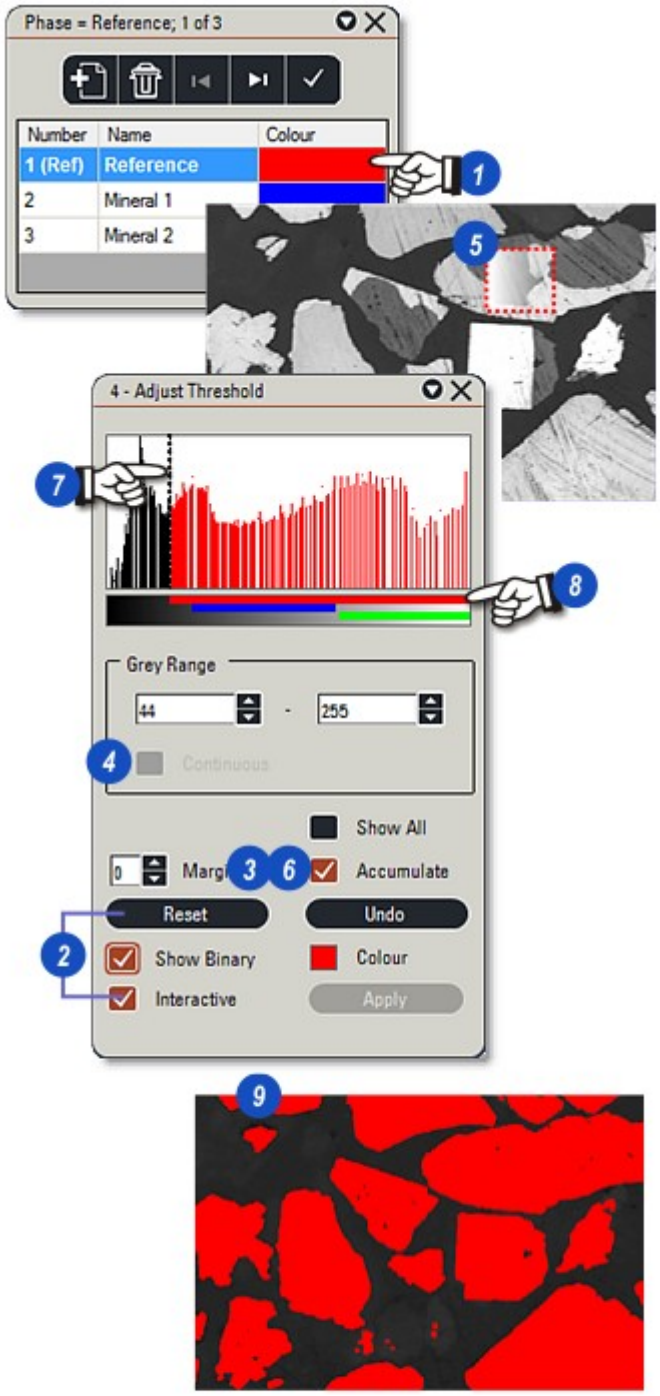
5: Select an area that represents either of the minerals, click and drag to create a region. All of the pixels values within the region will be displayed on the image and on the *Histogram*.

6: Click to enable (ON) *Accumulate* and repeat procedure **(5)** on another part of the minerals that is not yet detected. The *Accumulate* feature will add the selection to the previous values. Continue like this until all areas of the two minerals are detected and combined.

7: Adjust the detected phases by dragging the vertical dotted lines on the *Histogram* until only the areas covered by *Mineral 1* and *Mineral 2* are represented. Everything else is ignored.

8: On the *Histogram* the different phases are allowed to overlap because *Continuous* is disabled.

9: The *Reference* phase binary image.



Continued… 927

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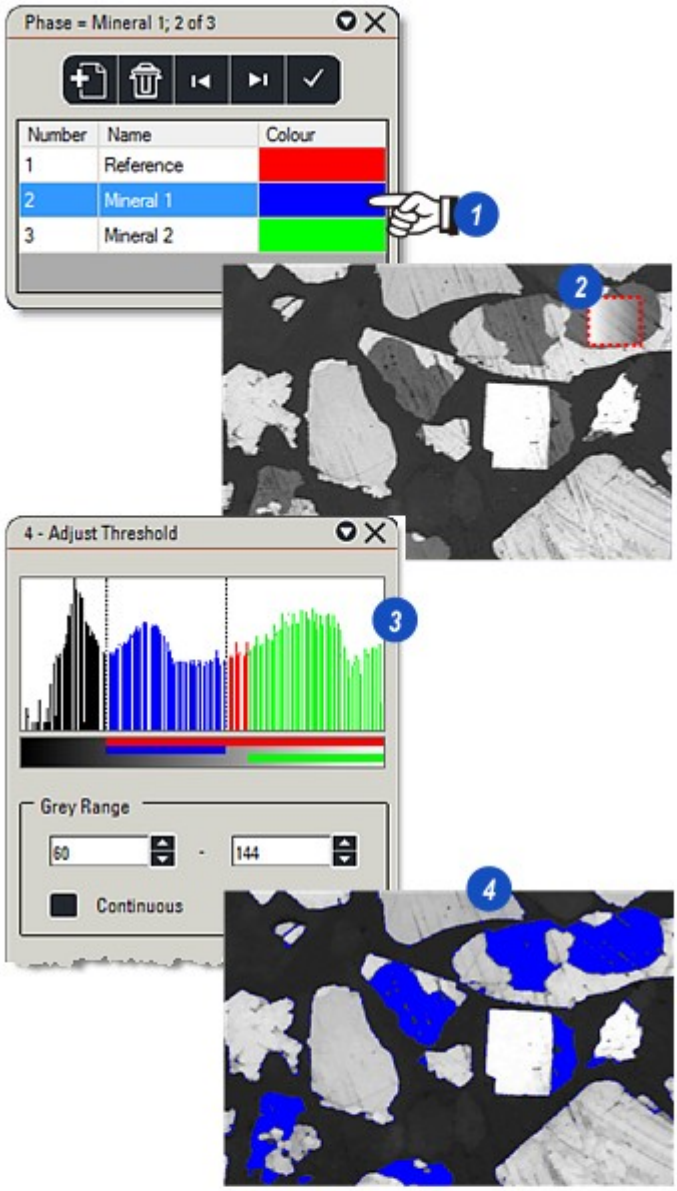
Detect *Mineral 1* that is colour-coded Blue on the phase list.

1: Click to select the *Blue (Mineral 1)* phase.

2: Follow the steps on the previous page to start the detection but this time select a region that represents only *Mineral 1*, repeating the selection until all of the phase has been detected.

3: Fine tune the detection with the *Histogram* controls – notice how the *Mineral 1 phase (Blue)* overlaps the *Reference (Red)* phase.

4: The *Mineral 1* phase binary image after detection.



Continued… 928

Detect *Mineral 2*, colour-coded Green on the phase list.

1: Click to select the *Green (Mineral 2)* phase.

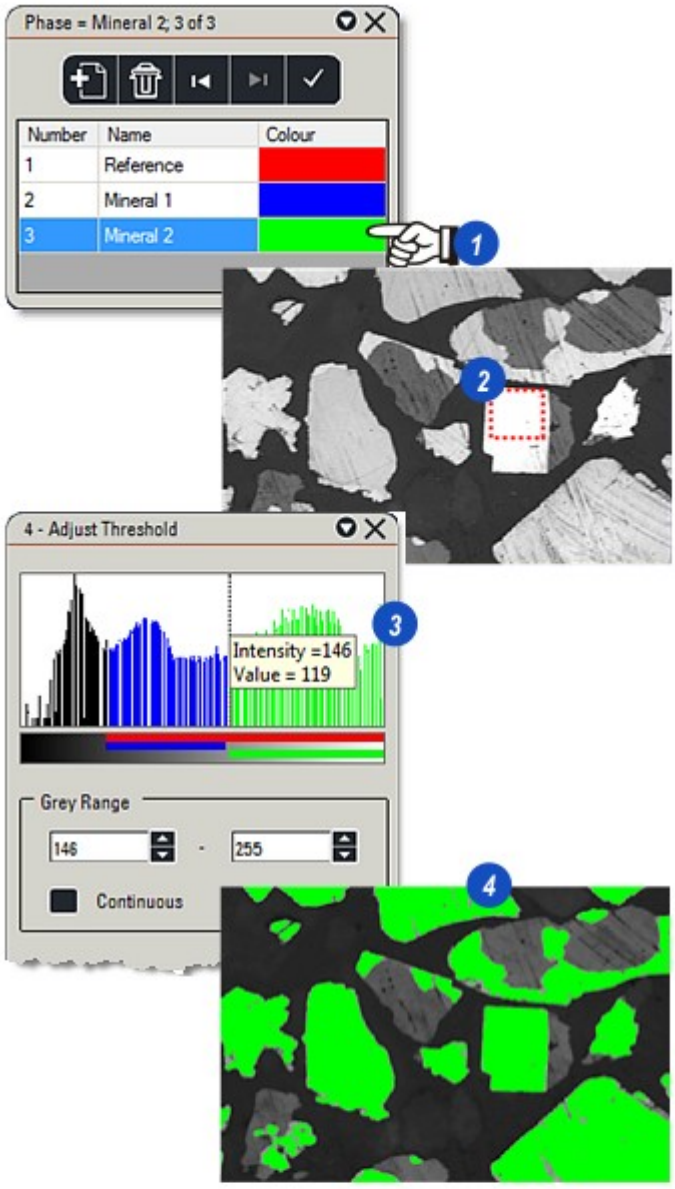
2: Follow the steps on the previous pages to start the detection but this time select a region that represents only *Mineral 2***,** repeating the selection until all of the phase has been detected.

3: Fine tune the detection with the *Histogram* controls – notice how the *Mineral 2* phase (*Green*) overlaps the *Reference (Red)* phase but butts against the highest value for the *Mineral 1* phase (*Blue*).

4: The *Mineral 2* phase binary image after detection.

That completes the *Threshold* phase detection for this example.

Continued… 929



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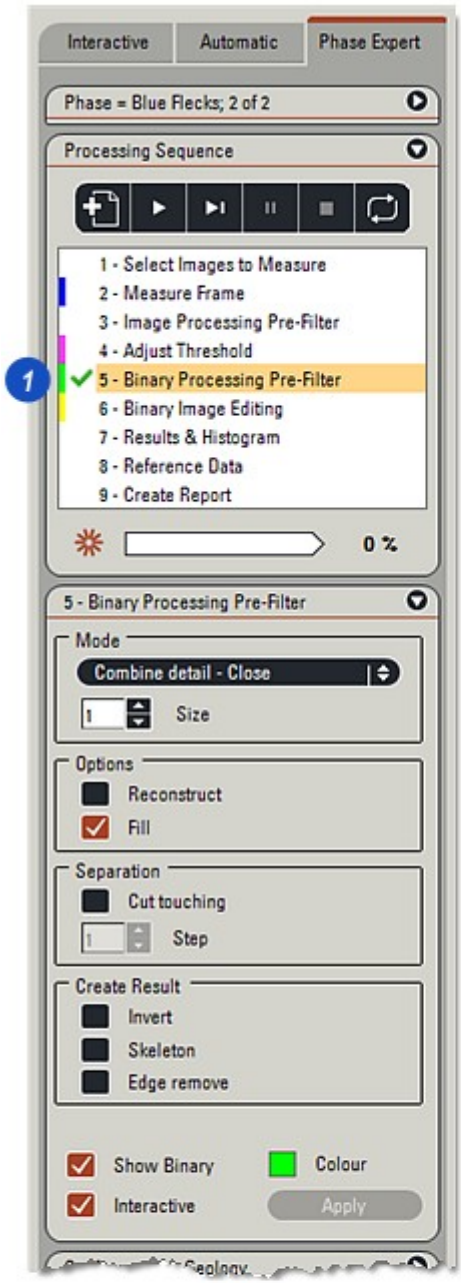
The *Binary Processing Pre-Filters* provides several filters and a range of tools that can quickly and easily improve a binary image - combining or separating detail, filling holes, separating phases that are touching and reconstructing 'fractured' phases.

1: Click on the *Binary Processing Pre-Filter* option on the *Processing Sequence* menu to open the *Pre-Filter* panel.

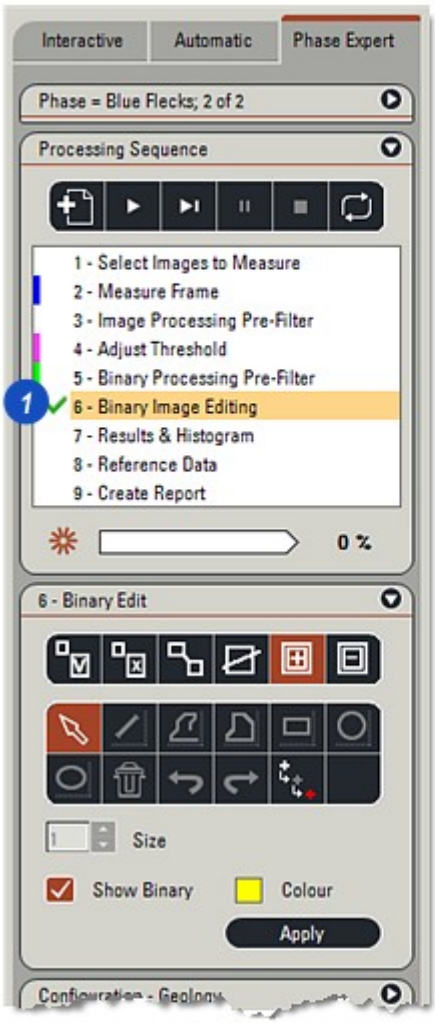
For detailed information and techniques about *Binary Processing Pre-Filters*, see *Image Analysis* help: *Go there...* 797



Binary Image Editing is a tool collection that provides the methods for working directly on *Binary Images* to add, remove, select and de-select features. Facilities also include drawing and filling shapes as well as grouping features.

1: To use the *Binary Editing* tools, click on the *Binary Image Editing* option on the *Processing Sequence* menu.

For detailed information about *Binary Editing*, see the *Image Analysis* help: *Go there...* 838



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1: Click to select *Results & Histogram* on the *Processing Sequence* menu.

2: Click on the arrows to the right of the *Phase/Field* header and…

3: …from the drop-down list click on the *Phase* option.

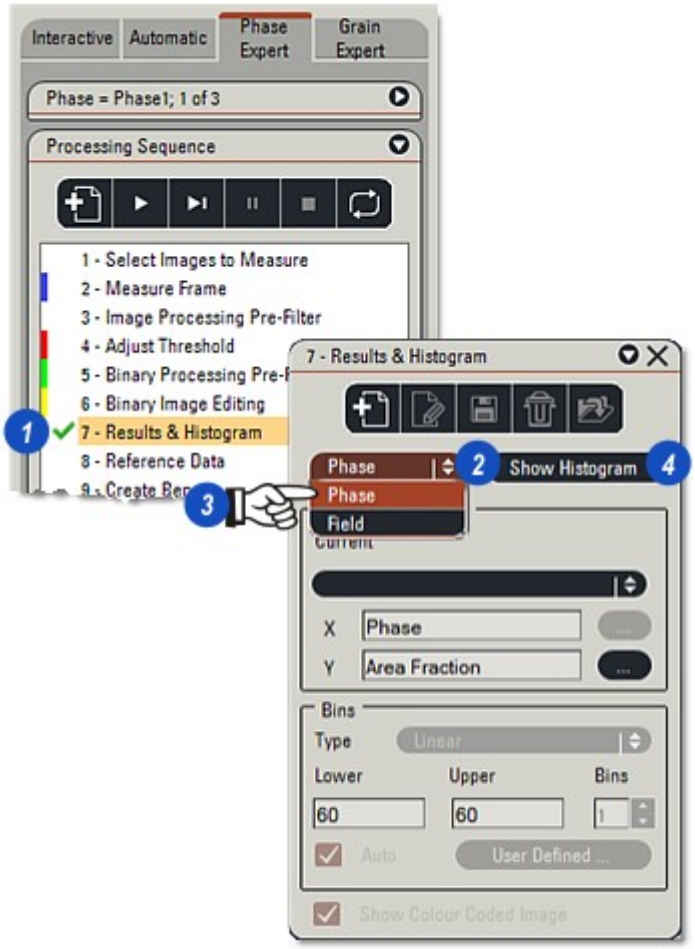
4: Click on the *Show Histogram* button.

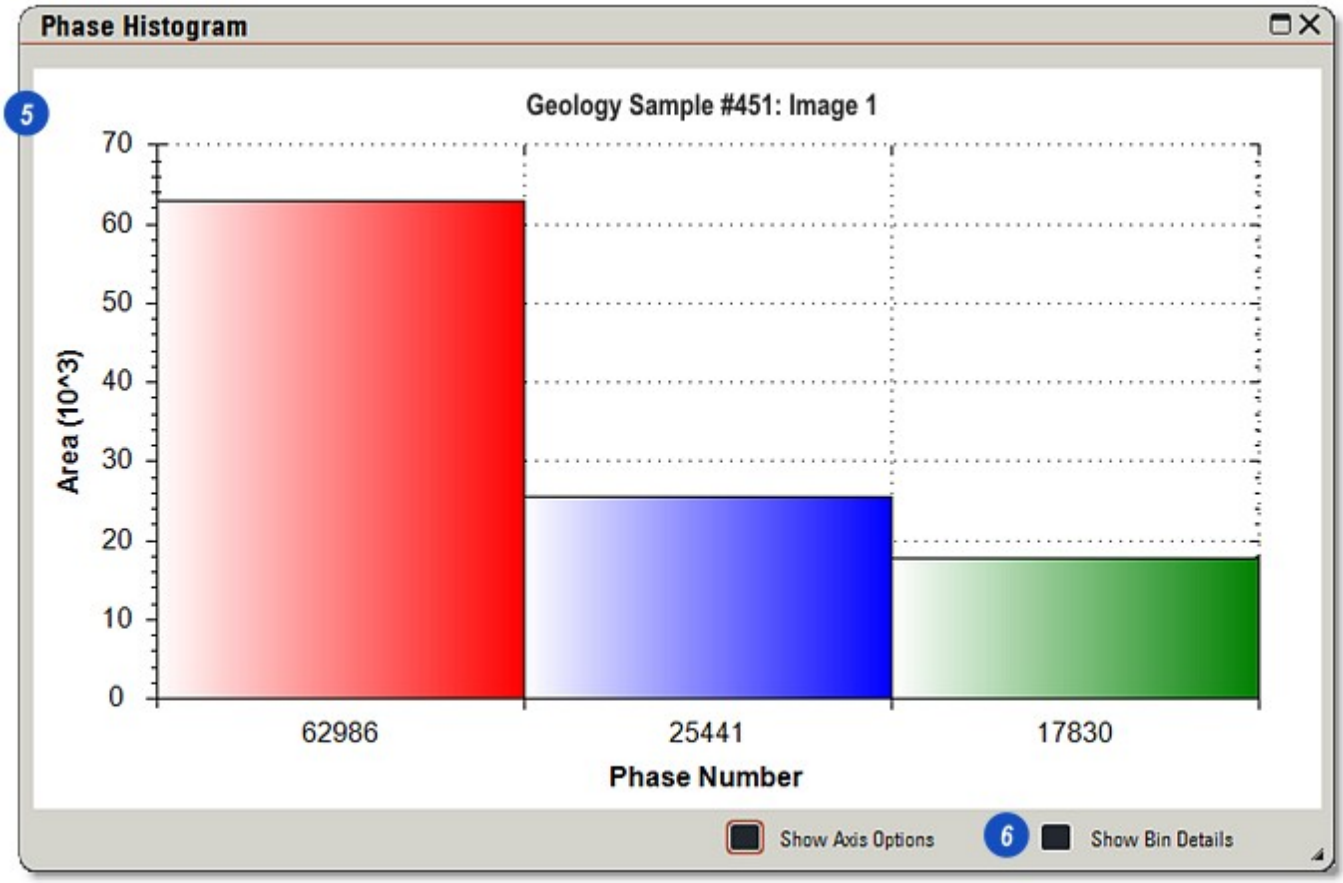
5: The *Phase Histogram* shows the phases and their results plotted against the parameter chosen for the *Y* axis.

With *Phase Histogram* selected, the *X* axis always shows the *Phases* and cannot be changed. The *Y* axis which represents the measured parameter, can be user selected. *Go there…* 933

6: For details of other *Axis* and *Bin* options, see *Image Analysis* 873 help.

Continued… 932





1: Click on the arrows to the right of the *Phase/Field* header.

2: From the drop-down menu click to select the *Field* option.

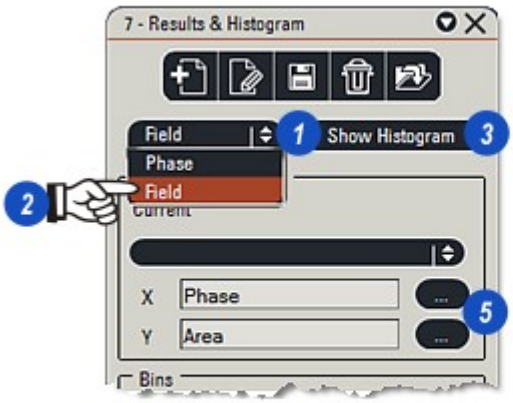
3: Click the *Show Histogram* button.

4: The *Field Histogram* is displayed. This is the result of the entire image – *Field* – plotted against the parameters chosen by the user for both the *X* and *Y* axes.

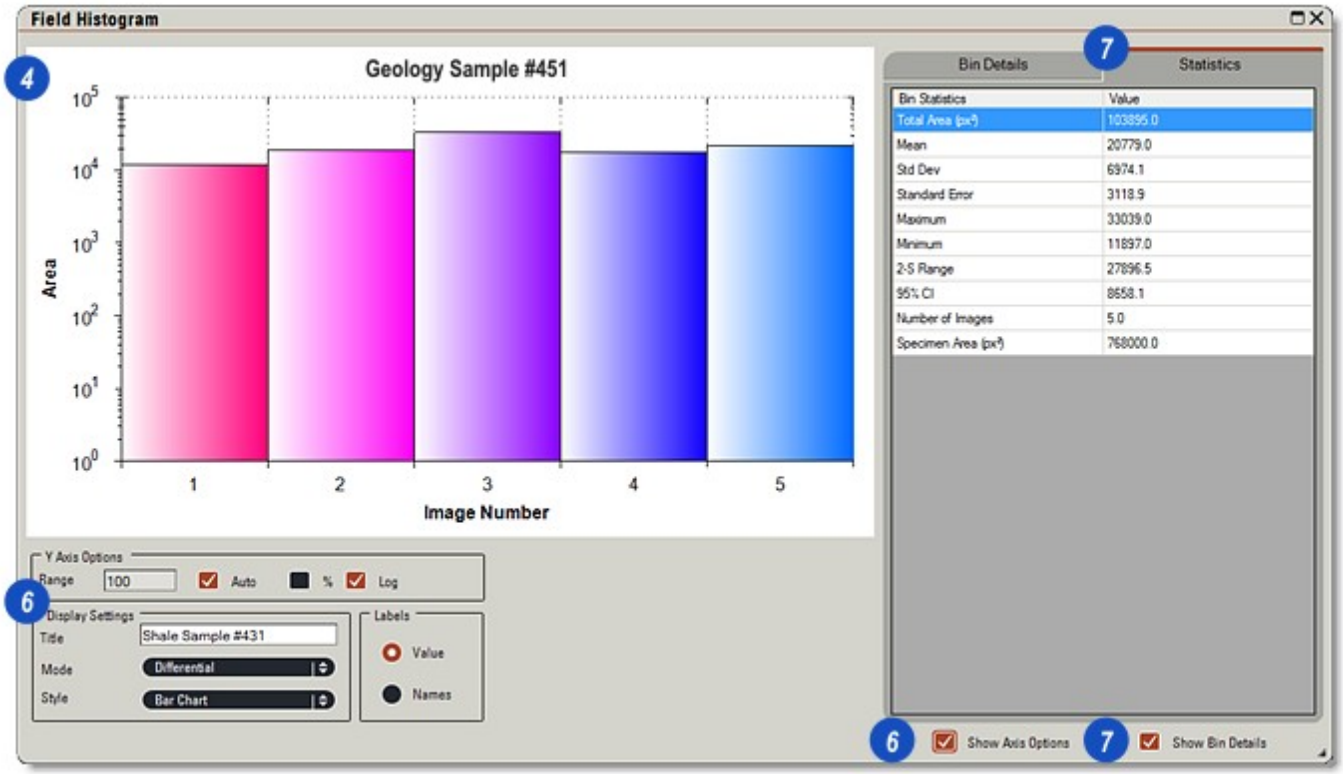
5: Selecting the parameters for the measurement axes: *Go there…* 933

6: The *Y* axis and *Display Settings* dialog revealed by enabling the *Axis Options* check box, and...

7: ...the *Bin Details* panel when the *Show Bin Details* check box is enabled.



Continued… 933



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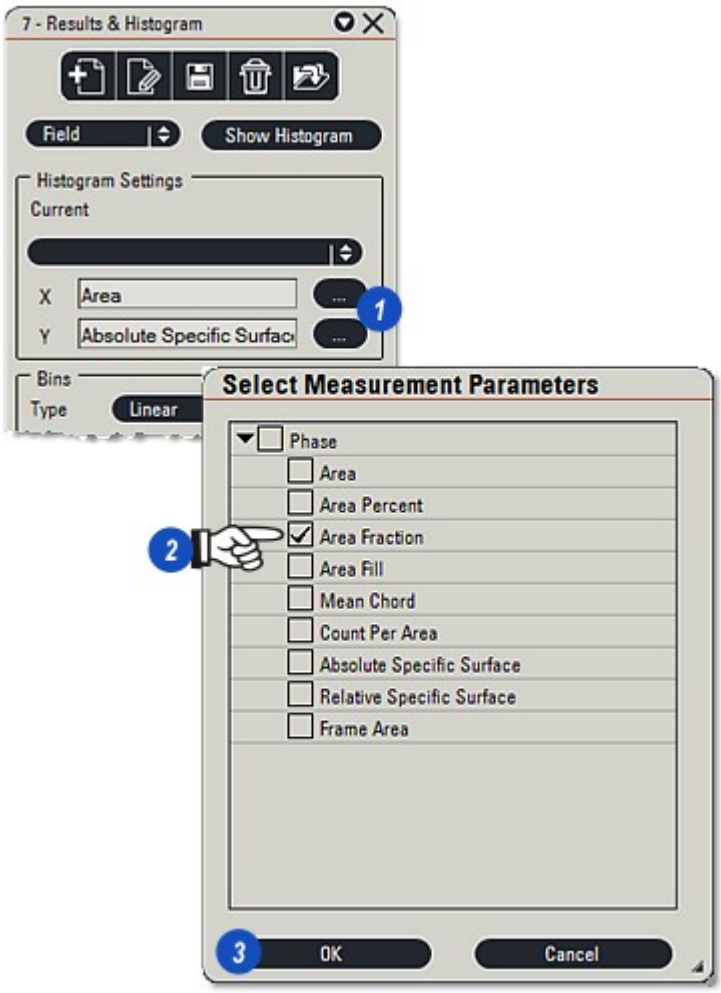
With the *Phase* option selected for the *Histogram* display, only the *Y* axis parameter can be changed. For the *Field* option both *X* and *Y* parameters can be selected.

1: Click on the *Browse* button against the axis to be changed. If the button is greyed out the option is not available.

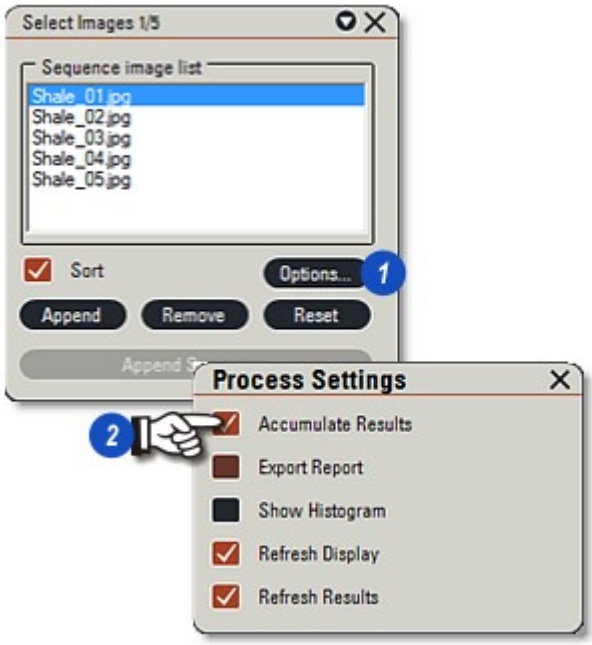
2: The *Select Measurement Parameters* list will depend upon the display option chosen and the axis to be altered. Click to select the parameter required.

3: Click *OK*.

Continued... 934



If multiple fields are measured, results can be aggregated or shown for individual fields by:



1: Clicking on the *Options* button on the *Select Images* dialog and ...

2: ...clearing/enabling the *Accumulate Results* check box on the *Select Images to Measure* panel.

The check box affects the display only so there is no need to re-process the images.

3: Click on the *Phase Details* tab to reveal the results as each *Phase* compared to the *Field* or compared to the *Reference Phase* is one has been set up.

4: Click on the *Phase Summary* tab to show each *Phase* as a set of user-defined statistics.

To change the oder of the results - low-to-high or high-to-low, double-click a column header.

Continued… 936

3

Phase Details

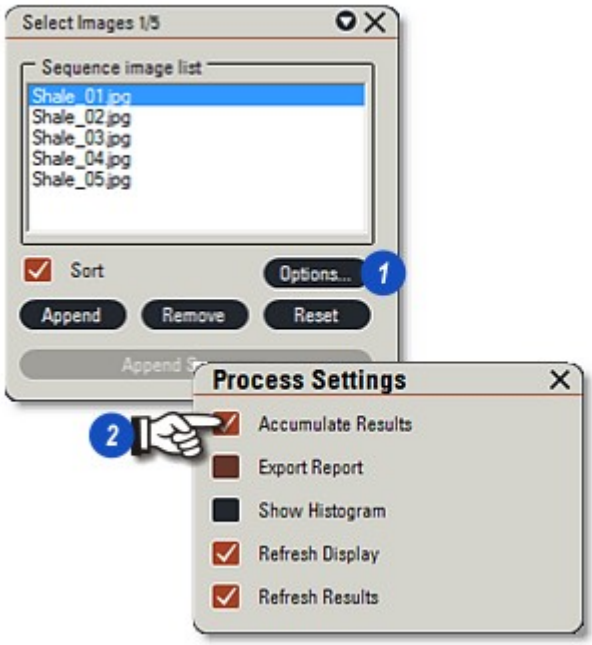
	Number	Images	Phase Name	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Mean Chord(px)	Count Per Area(px⁻²)	Frame Area(px²)
	1	Shale_01.jpg	Phase1	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
▶			Phase2	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
			Phase3	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000

4

Phase Summary

Phase	Statistics	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Mean Chord(px)	Count Per Area(px⁻²)	Frame Area(px²)
Phase1	Total	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
	Mean	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
	Std Dev	-	-	-	-	-	-	-
	Maximum	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
	Minimum	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
Phase2	Total	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
	Mean	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
	Std Dev	-	-	-	-	-	-	-
	Maximum	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
	Minimum	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
Phase3	Total	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000
	Mean	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000
	Std Dev	-	-	-	-	-	-	-
	Maximum	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000
	Minimum	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000

If multiple fields are measured, results can be aggregated or shown for individual fields by:



1: Clicking on the *Options* button on the *Select Images* dialog and ...

2: ...clearing/enabling the *Accumulate Results* check box on the *Select Images to Measure* panel.

The check box affects the display only so there is no need to re-process the images.

3: Click on the *Phase Details* tab to reveal the results as each *Phase* compared to the *Field* or compared to the *Reference Phase* is one has been set up.
Click on the *Phase Summary* tab to show each phase as a set of user-defined statistics.

To change the order of the results - low-to-high or high-to-low, double-click a column header.

Continued... 937

3

Phase Details

Number	Images	Phase Name	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Mean Chord(px)	Count Per Area(px⁻²)	Frame Area(px²)
4	Shale_04.jpg	Phase1	36.247	55675.000	0.362	0.569	10.996	7760.417	153600.000
2	Shale_02.jpg	Phase1	30.521	46881.000	0.305	0.439	9.710	7584.635	153600.000
1	Shale_01.jpg	Phase1	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
3	Shale_03.jpg	Phase1	26.269	40349.000	0.263	0.356	8.122	9479.167	153600.000
5	Shale_05.jpg	Phase1	25.982	39908.000	0.260	0.351	7.390	11217.448	153600.000
3	Shale_03.jpg	Phase3	24.098	37014.000	0.241	0.317	15.257	598.958	153600.000
1	Shale_01.jpg	Phase2	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
5	Shale_05.jpg	Phase2	20.327	31223.000	0.203	0.255	3.296	17207.031	153600.000
2	Shale_02.jpg	Phase2	20.243	31094.000	0.202	0.254	3.526	15071.615	153600.000
4	Shale_04.jpg	Phase2	17.050	26189.000	0.171	0.206	3.374	13170.573	153600.000
5	Shale_05.jpg	Phase3	16.867	25908.000	0.169	0.203	11.196	774.740	153600.000
3	Shale_03.jpg	Phase2	16.762	25747.000	0.168	0.201	3.305	13190.104	153600.000
2	Shale_02.jpg	Phase3	14.092	21645.000	0.141	0.164	12.938	501.302	153600.000
4	Shale_04.jpg	Phase3	13.482	20708.000	0.135	0.156	11.139	579.427	153600.000
1	Shale_01.jpg	Phase3	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000

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Shown below are the tabular results for the *Reference* example.

1: Click on the *Details* tab to show the overall relationship between the *Reference* phase (Highlighted) - always shown as representing 100% of the area - and...

2 & 3: ...*Minerals 1* (44.4%) and *2* (55.6%) that together add up to 100%.

4: Click on the *Summary* tab to show the basic statistics.

Continued… 938

1

Phase Details

Number	Images	Phase Name	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Frame Area(px²)
1	Pyrite_cr1.bmp	Reference (Ref)	100.0	73217.0	0.5	1.2	135000.0
1	Pyrite_cr1.bmp	Mineral 1	44.4	32542.0	0.4	0.8	73217.0
1	Pyrite_cr1.bmp	Mineral 2	55.6	40675.0	0.6	1.2	73217.0

2

3

4

Phase Summary

Phase	Statistics	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Frame Area(px²)
	Minimum	100.0	73217.0	0.5	1.2	135000.0
Mineral 1	Total	44.4	32542.0	0.4	0.8	73217.0
	Mean	44.4	32542.0	0.4	0.8	73217.0
	Standard Error	-	-	-	-	-
	Maximum	44.4	32542.0	0.4	0.8	73217.0
	Minimum	44.4	32542.0	0.4	0.8	73217.0
Mineral 2	Total	55.6	40675.0	0.6	1.2	73217.0
	Mean	55.6	40675.0	0.6	1.2	73217.0
	Standard Error	-	-	-	-	-
	Maximum	55.6	40675.0	0.6	1.2	73217.0
	Minimum	55.6	40675.0	0.6	1.2	73217.0

1: To change the *Phase Details* column header parameters or the *Phase Summary* statistic parameters, click on the appropriate results tab.

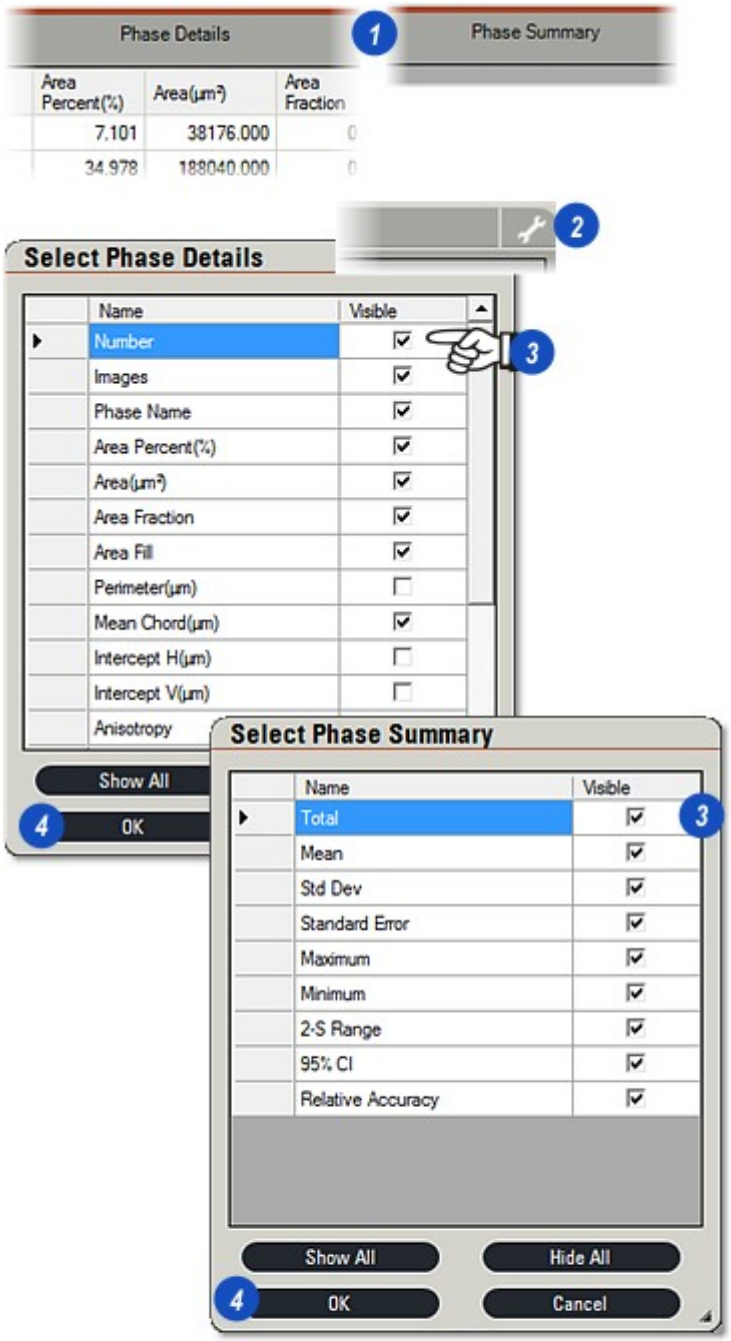
2: Click on the *Tool Tab* and, depending upon the results tab selected the *Select Phase Details* or *Select Phase Summary* dialog will appear.

3: Enable the required check boxes to include the parameter required.

4: Click OK.

The *Show All* option when enabled will select all of the options.

Click on the *Hide All* button to clear all of the options before starting a new range of selections.



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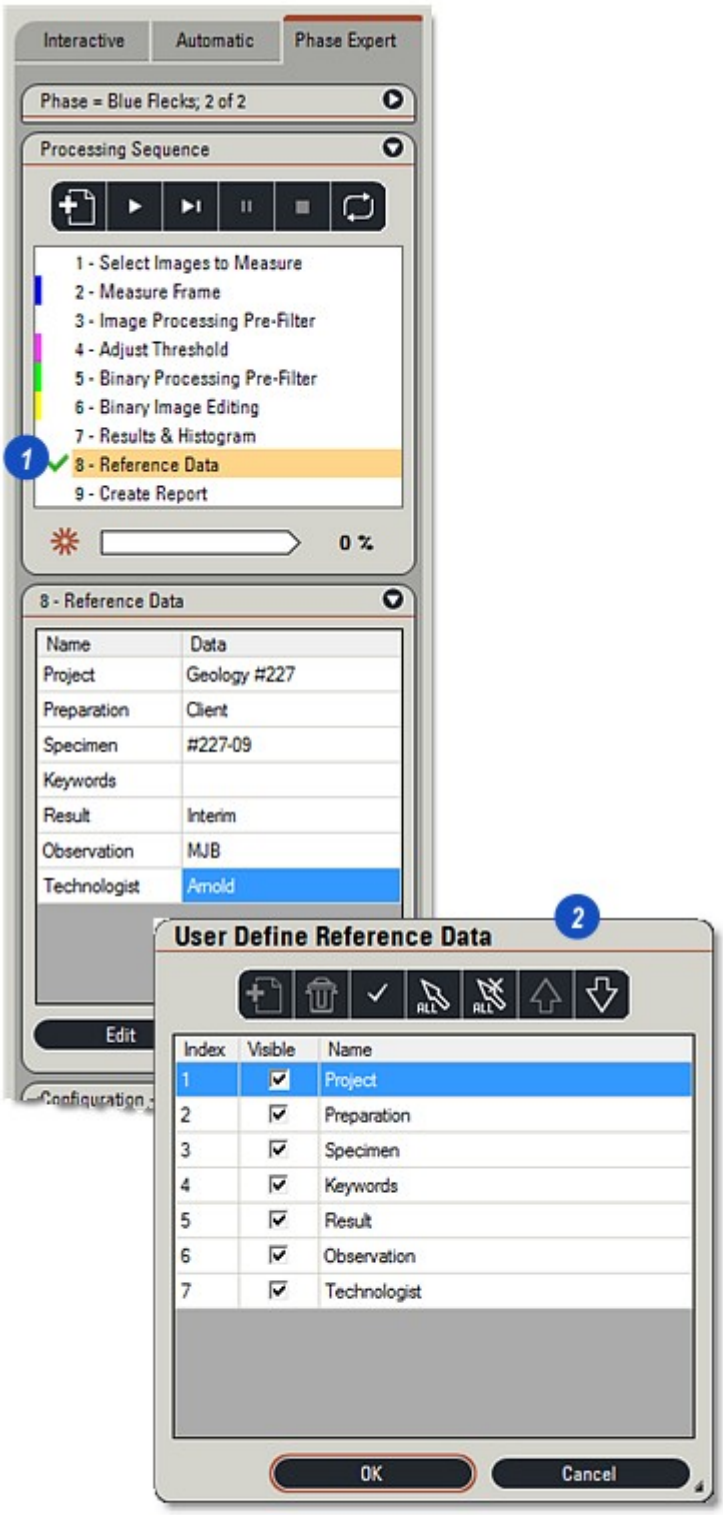
A comprehensive range of *Data* Items can be appended to the *Phase Expert* results that will identify important details such as the *Project Name*, the *Specimen* and how it was prepared. Enter the information in the *Reference Data* dialog.

1: Click on the *Reference Data* option on the *Processing Sequence* menu to reveal the *Reference Data* dialog.

Administrators can add to the supplied list of data headings to comply with corporate needs **(2)**.

For details refer to Image Analysis 883 help:



1: Click to select *Create Report* on the *Processing Sequence* menu.

2: On the *Create Report* panel, click to select/de-select the images to be included in the report.

3: Nominate a *Report Layout Template* by clicking on the browse button and…

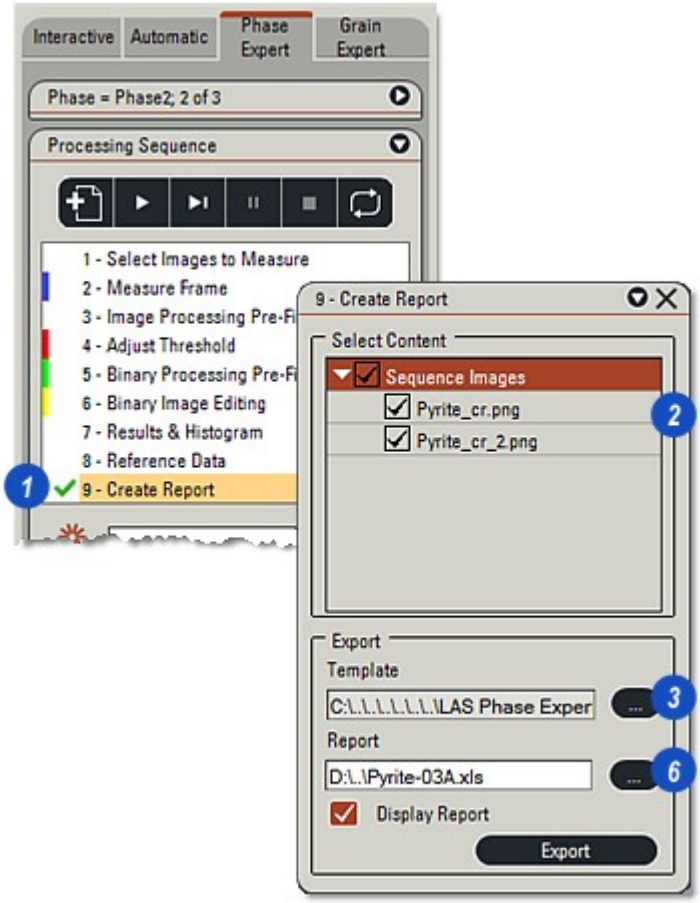
4: …navigating to and clicking to select a template. A default template is supplied with *Phase Expert* and is located in the *Excel Templates\Phase\LAS Phase Expert Templates* folder.
The supplied template can be modified to suit the user's requirements and saved under a separate file name.

5: Click *Open*.

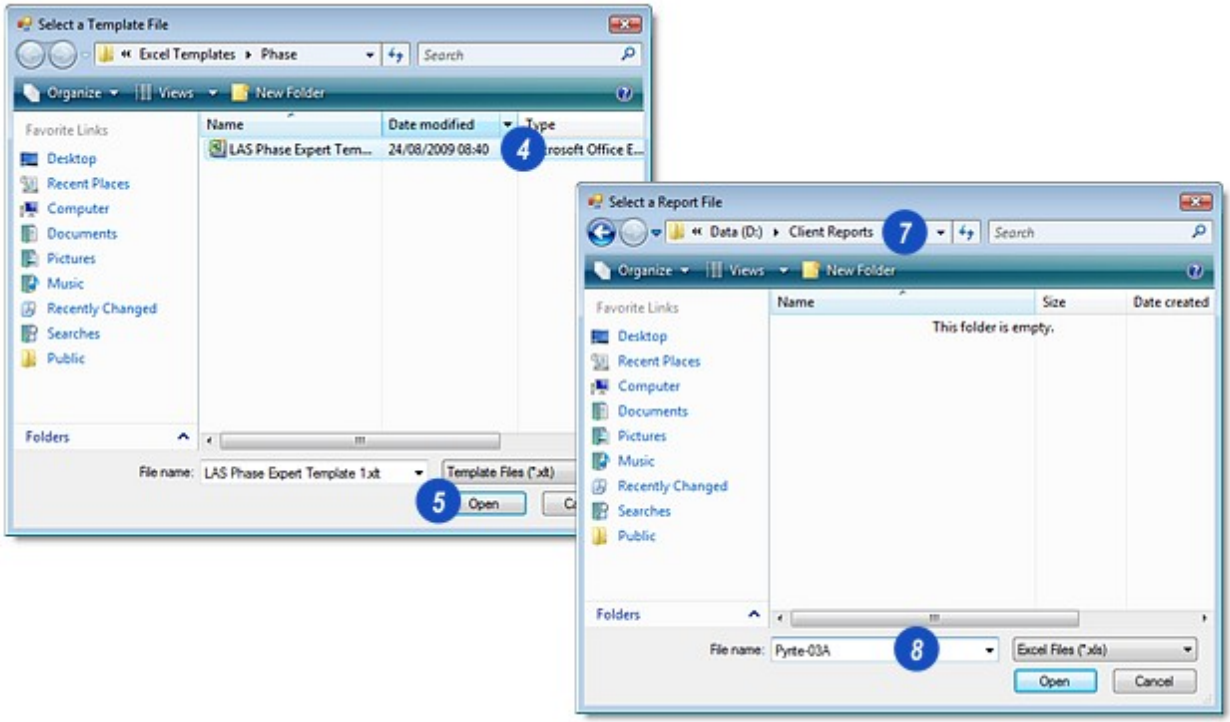
6: Specify where the report is to be saved by clicking the browse button to the right of the *Report* text box and…

7: …navigating to the destination folder, ...

8: …typing a file name for the report and clicking *Open*.



Continued… 941



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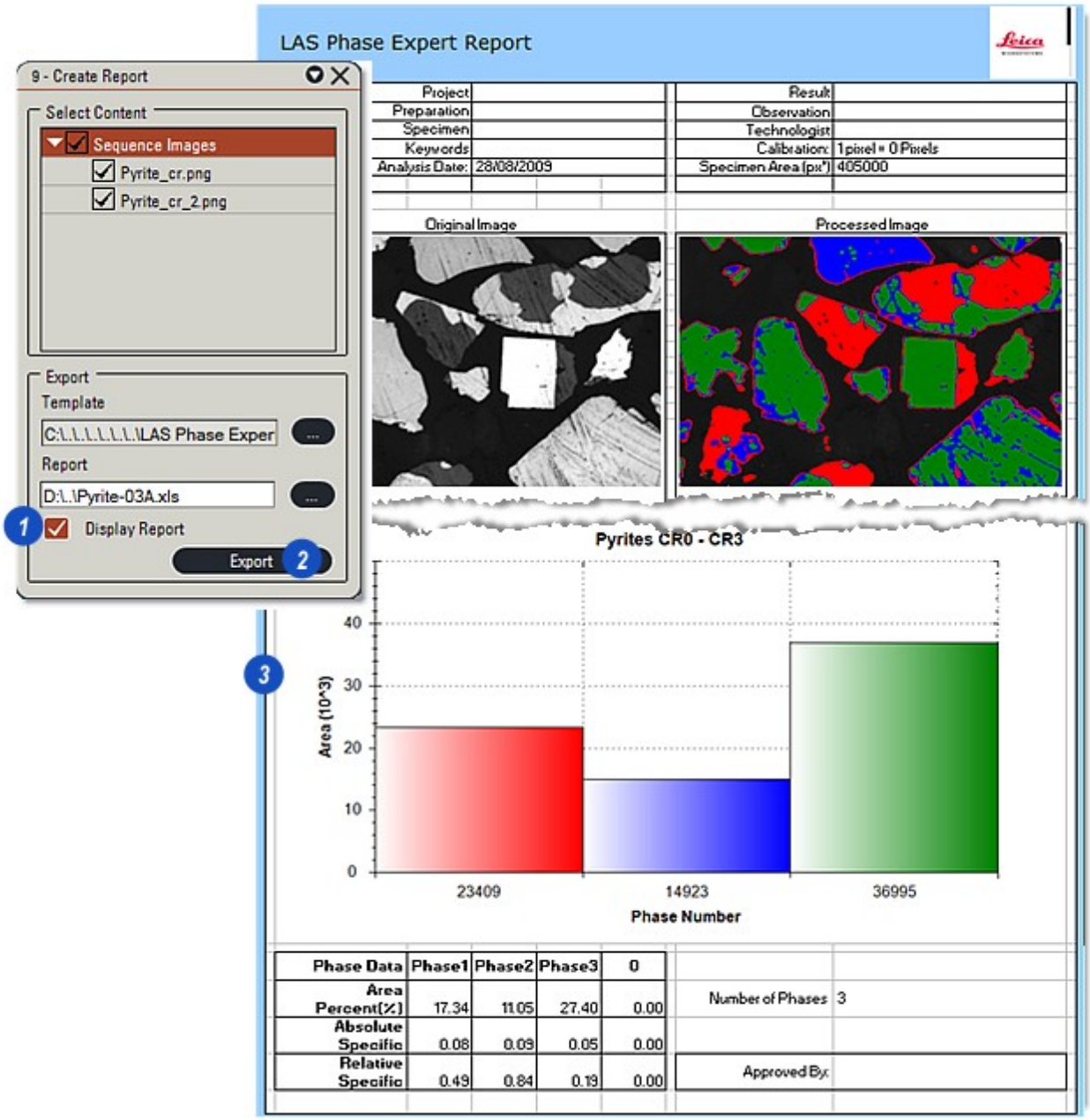
1: To display the report automatically as it is created, click to enable the *Display Report* button.

2: To create the report click the *Export* button.

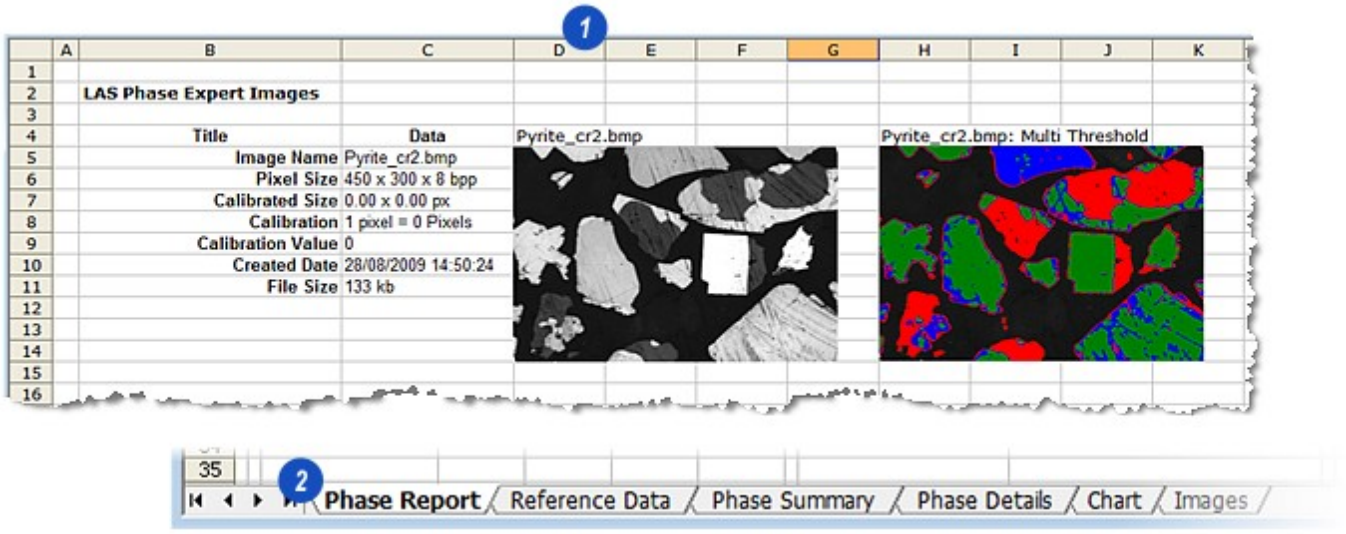
3: The front page of the report displayed in *Microsoft Excel*.

Continued… 942



1: Sample page from the *Microsoft Excel* report.

2: The *Excel Spreadsheet* tab structure.



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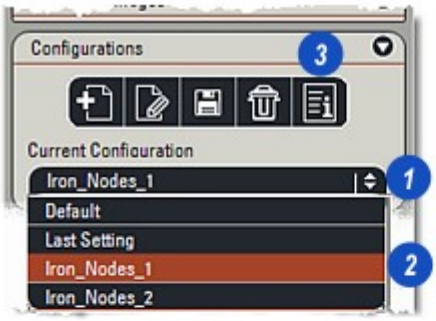
The current settings for most editing panels can be saved with the archive as a *Configuration* to be retrieved at a later date. Retrieved *Configuration* settings are applied to automatically to the tools

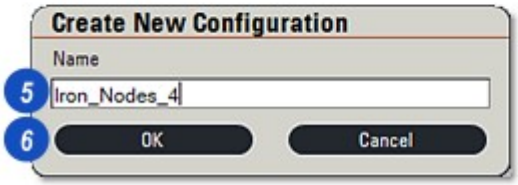


1: Each configuration has a unique name and can be accessed by clicking on the arrow to the right of the *Current Configuration* window and from the drop down list…

2: …clicking to select the required configuration.

3: Click the *Display Configuration Settings* button to list the configuration details.

Save Configuration:
Saves all of the current settings and process sequences in a unique file.



4: To save the current settings as a new configuration, click on the *New* button and…

5: …type a unique name for the new configuration.

6: Click *OK* to save the setting.

7: Click on the *Save Configuration* button The new configuration appears in the drop down list.

Continued... 944

Edit Configuration:
A *Configuration* name can be changed by:

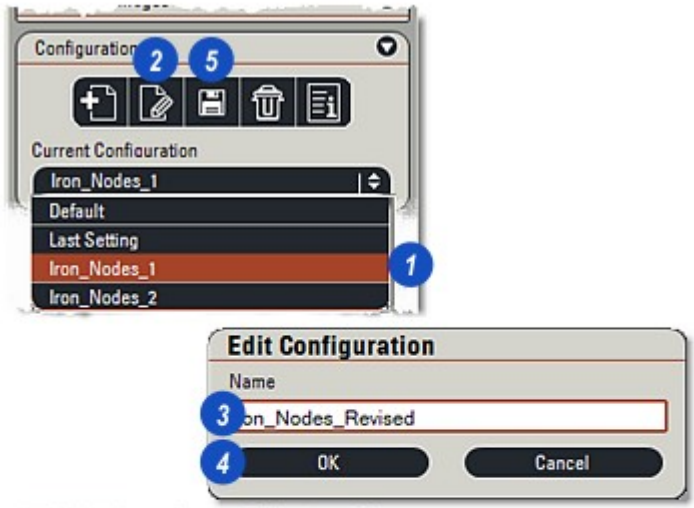
1: Select the configuration to be changed from the drop down list.

2: Click on the *Edit Configuration* button.

3: On the *Edit Configuration* dialog, change the name by clicking in the text box and typing a new name and…

4: …clicking *OK*.

5: Click the *Save Configuration* button.

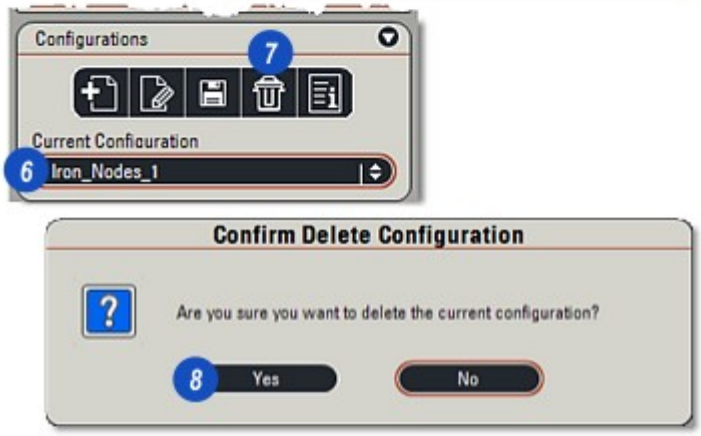


Remove a Configuration:
A *Configuration* can be removed from the list by:

6: Selecting the configuration to be deleted from the drop down list.

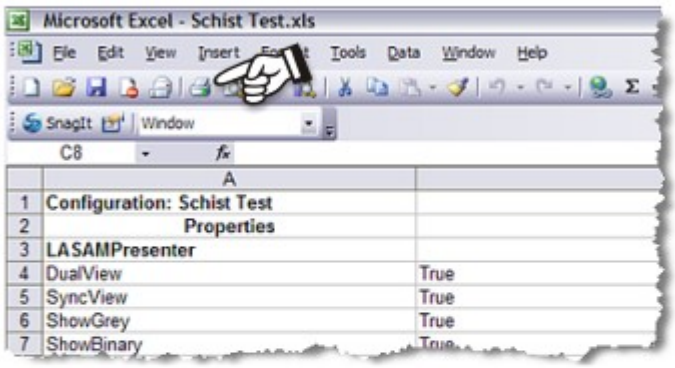
7: Clicking the *Delete* (Trash Can) button.

8: Confirm the deletion and the *Configuration* will be removed permanently. The operation cannot be reversed.



Print a Configuration:
Check that the printer is on and connected to the computer. The configuration is captured to an Excel spreadsheet and printed from there.

1: Select the *Configuration* to be printed from the drop down list. When the Configuration settings appears on an *Excel* spreadsheet, use the print function to print the settings list.



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Grain Expert

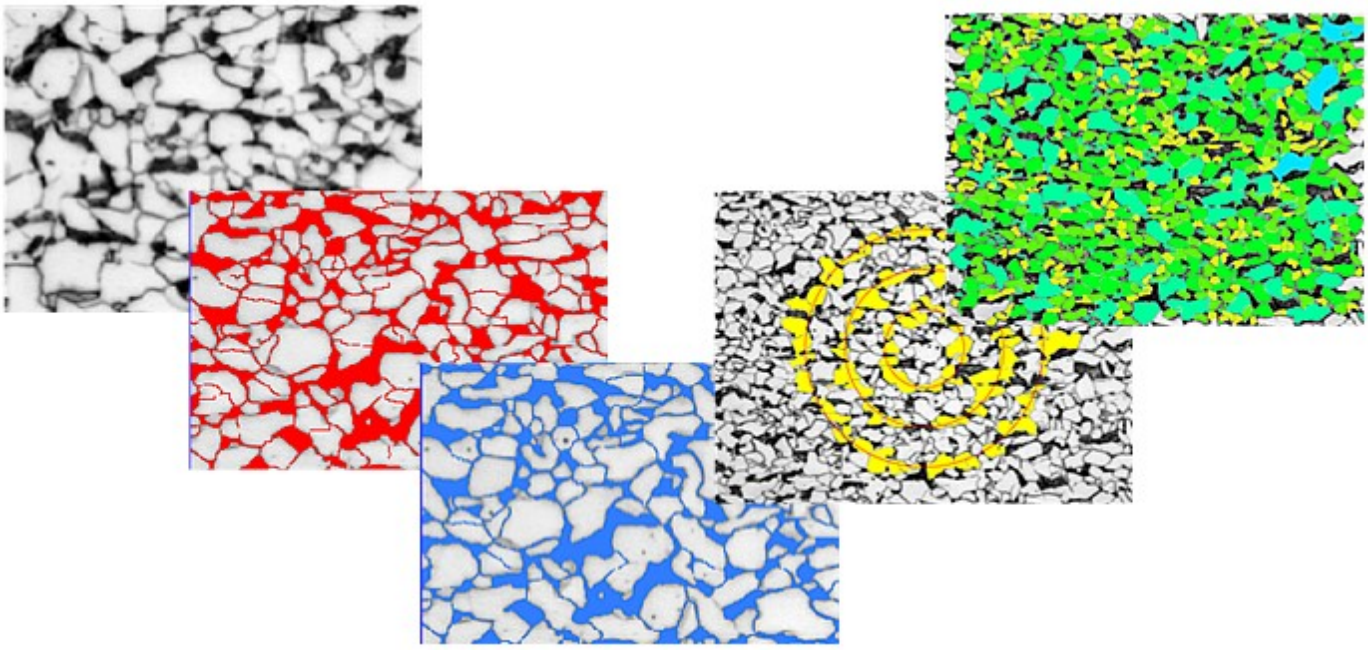
The grain sizes in a metal, plastic, composite or material is an important factor in determining its strength or machinability. *LAS Grain Expert* allows you to measure the average grain size of visible grains on the surface of a specimen. Grain boundaries are identified on the basis of contrast in the image. Grains can be measured using a number of different standards and techniques using automatic methods to identify boundaries. For example, on appropriate images:

- Automatically identify grains and measure their size.
- Count the intersections of grain boundaries with a test line
- Mark the diameter of selected grains on an image
- Edit the image to trace the shape of selected grains on an image
- Compare a sample image with a selection of typical example images to select the most appropriate boundary identification method.

LAS Grain Expert calculates:

- Average grain size, expressed in terms of a grain size number.
- Mean grain area.
- Maximum and minimum grain size.
- Confidence level.
- Relative accuracy.

The results obtained depend on the standard and technique employed.

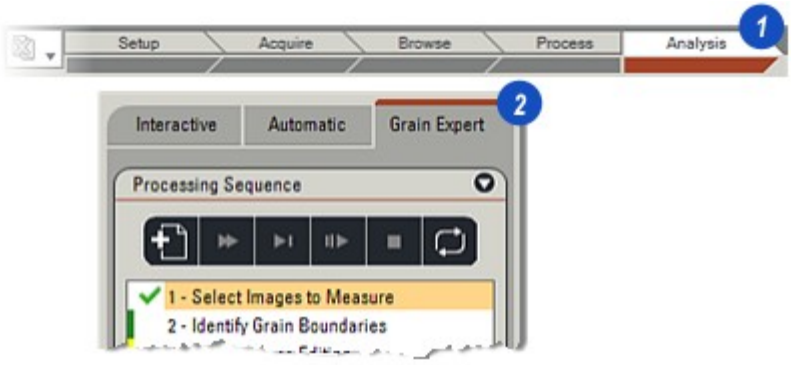


Grain Expert: Step by Step:

Grain Expert must be installed, licensed and enabled. Click on the *Analysis Workflow* **(1)** and then on the *Grain Expert* tab **(2)**.

If the tab is not present, *Grain Expert* has either not been installed or is not enabled: Refer to the *LAS Installation Guide*.

These steps represent the sequence users should follow to successfully carry out *Grain* measurements.



Because *Grain Expert* works in conjunction with *Image Analysis*, in most steps links are provided to both modules: *Red*, will take the reader to the appropriate place in the *Image Analysis (Automatic)* help file for more detailed information, and *Blue,* connects to help files that are specific to *Grain Expert*.
Sometimes, it will be beneficial to follow both links if they are provided.

Select the Images to Process:
One or more images can be processed in sequence simply by selecting the *Thumbnail(s)* in the *Gallery* and then clicking the *Append* button on the *Select Images to Measure* panel.
Image Analysis help >>> *Grain Expert help >>>* 947

Identify the Grain Boundaries:
A range of *Reconstruction Images* are supplied with *Grain Expert* to help to simplify and speed the task of finding the grain boundaries.
Additional close refinement can then be achieved with the *Threshold* and *Sensitivity* controls.
Grain Expert help >>> 949

Binary Image Editing:
Repair boundaries, remove features and generally fine-tune the *Binary Image* prior to analysis.
Image Analysis help >>> *Grain Expert help >>>* 957

Select Standard:
Grain Expert provides configurations for all of the internationally accepted standards for grain analysis together with a wide range of statistical methods.
Grain Expert help >>> 958

Results and Histogram:
Display the results using graphical *Histograms* - bar and pie charts available - as well as *Tabular* data in the *Grid* conveniently grouped under detail and statistic tabs for both *Fields* and *Grains*.
Image Analysis help >>> *Grain Expert help >>>* 961

Reference Data:
Add user and analysis references to make the job totally product and company specific. The references are automatically included in the report.
Image Analysis help >>> *Grain Expert help >>>* 967

Create Report:
Reports are created using *Microsoft Excel* and a suitable template. A standard template is supplied with *Grain Expert* and can be tailored to user and corporate needs.
Image Analysis help >>> *Grain Expert help >>>* 968

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1: On the *Processing Sequence* click on *Select Images to Measure*.

2: Click on a *Thumbnail* in the *Gallery*.

3: Click to *Append* button on the *Select Images* dialog to add the selected image to the *Sequence Image List*.

Repeat the steps to add more images to be measured as a batch.

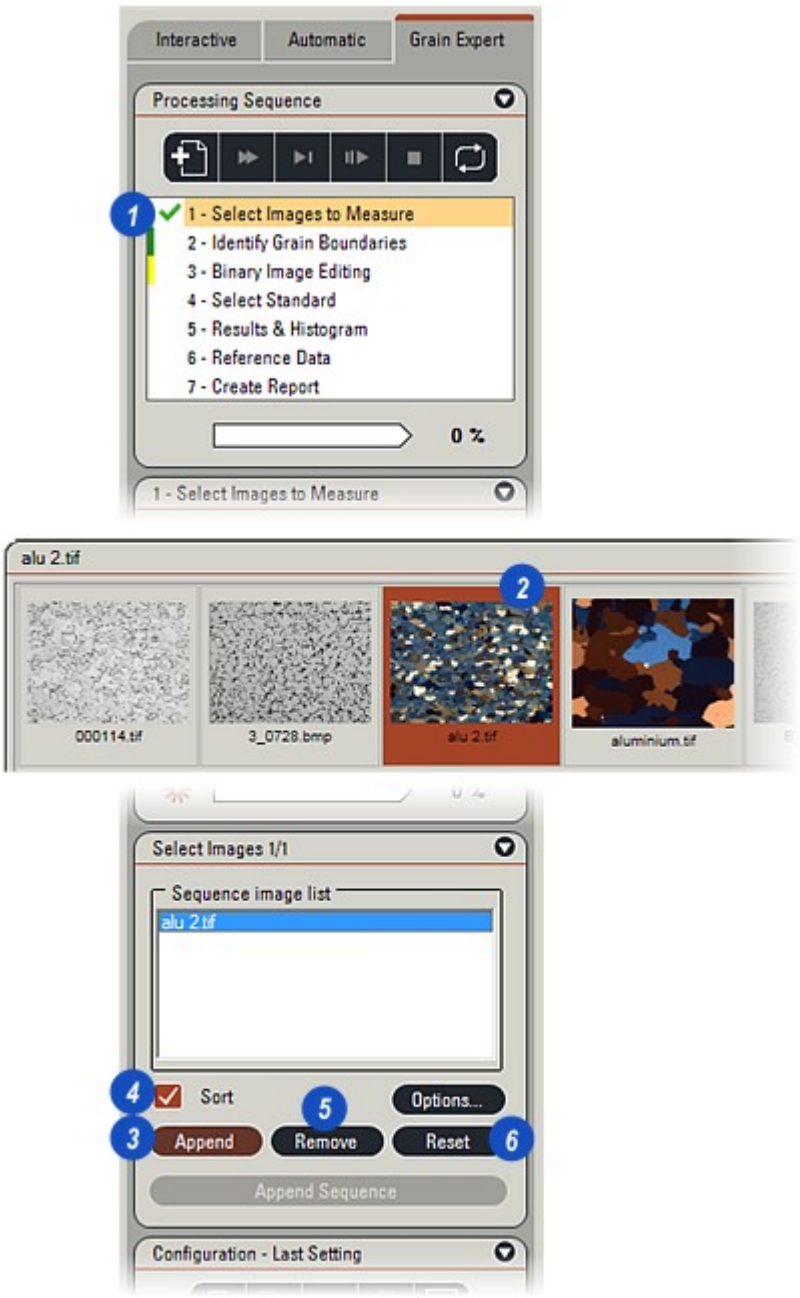
All selected images must have the same type - for example *.bmp, .png, .jpg* - without compression, and the same resolution.

4: Images can be selected in any order from the *Gallery*. To sort them into numerical sequence click to enable the *Sort* check box.

5: Use the *Remove* button to remove a single selected image from the list, or...

6: ...click the *Reset* button to remove all images.

Continued...



Additional controls on the *Select Images* panel are check boxes – click to enable, click again to disable - reached by:

1: Click on the *Options* button to reveal...

2: ...the *Process Settings* pop-up menu.

3: *Accumulate Results:* Adds the results of the current pass to those of the previous. Two or more images have to be selected.

4: *Export Report*: At the end of the analysis run an *Excel Report* is generated and displayed. Excel must be installed on the computer.

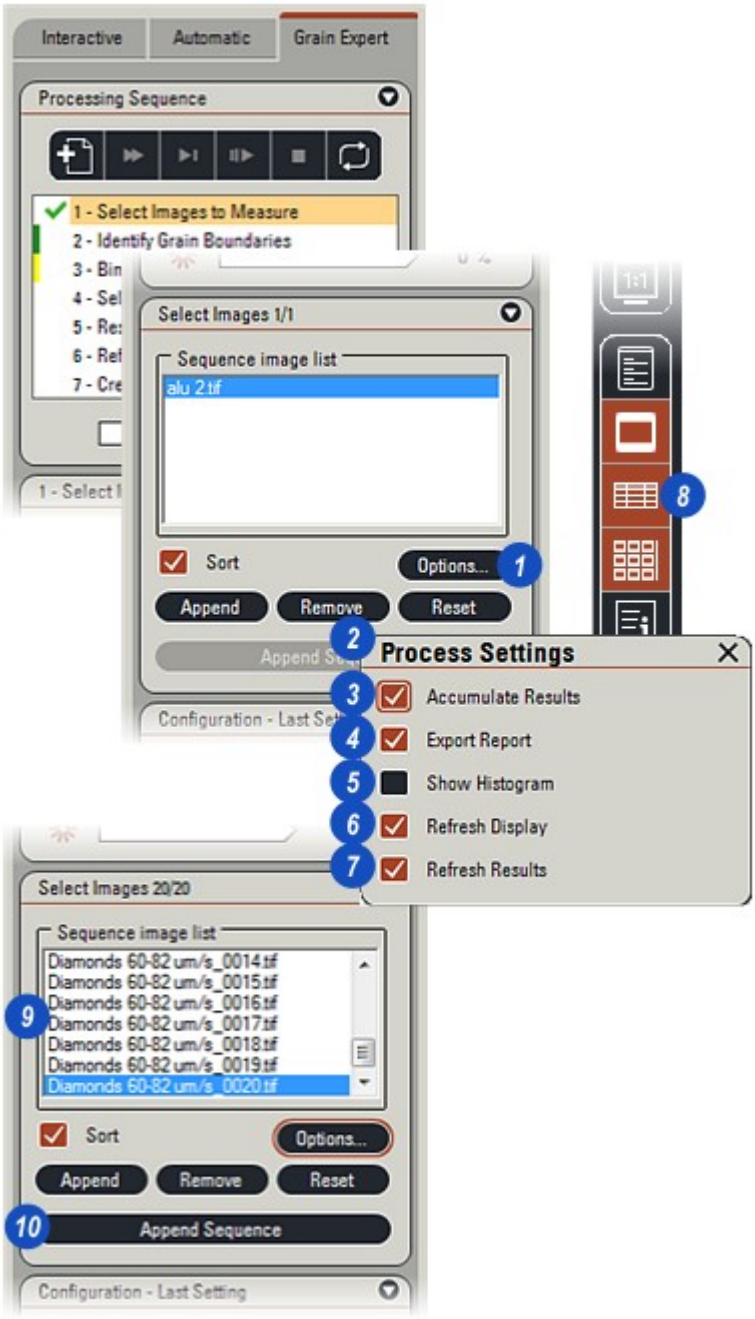
5: *Show Histogram*: Displays the *Histogram* at the end of the analysis run.

6: *Refresh Display*: Updates the display as processing proceeds independently of the *Interactive/Apply* setting on each sequence panel.

7: *Refresh Results*: Updates the *Grid* as processing proceeds. Turn the *Grid* on by clicking to enable the *Grid* display button **(8)** on the *Side Tool Bar*.

9: If multiple images are part of a sequence, the software recognises this and…

10: …the *Append Sequence* button becomes active. Click the button and the selected range is imported into the *Image List*. This avoids having to load images separately.
To select a range of images in the *Gallery*, click on the first image and then holding down the *Shift* key click on the last. All of the images between will be selected.



To select a range of images separately, click on the first image and then holding down the *Ctrl* button click on each of the others.
If *Sort* is enabled, the sequence will be sorted numerically.

Continued…

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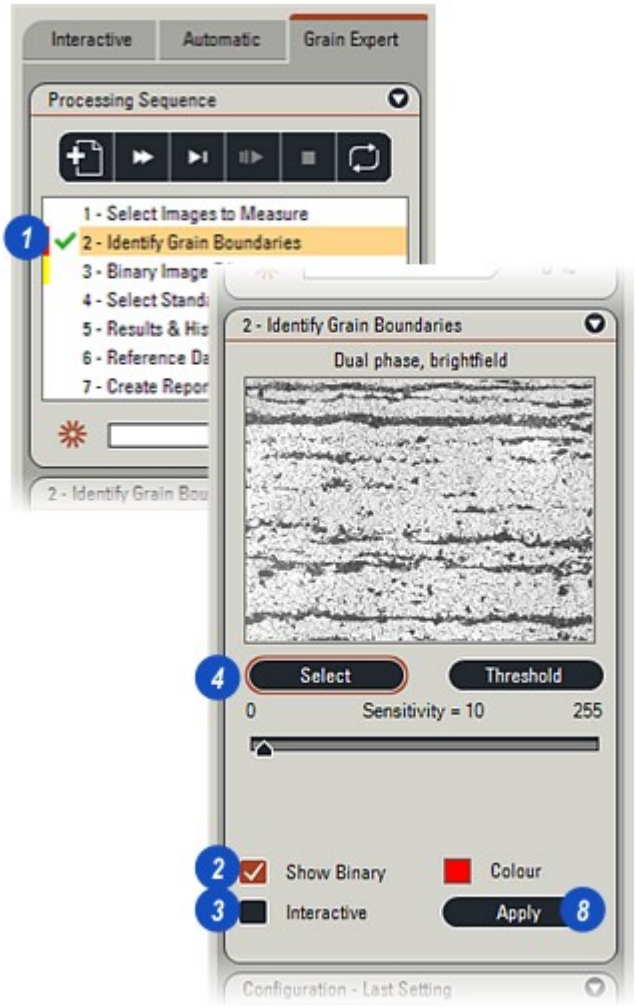
To speed grain boundary detection, several *Reconstruction Images* are provided with *Grain Expert*. By matching one of these to the selected specimen, a more appropriate and faster software algorithm can be used.



1: Click to select *Identify Grain Boundaries* in the *Processing Sequence* menu.

2: On the *Identify Boundaries* panel, click to enable *Show Binary* to view the processed image.

3: Click to disable *Interactive to* prevent processing whilst matching a *Reconstruction Image* to the specimen.

4: Click the *Select* button.

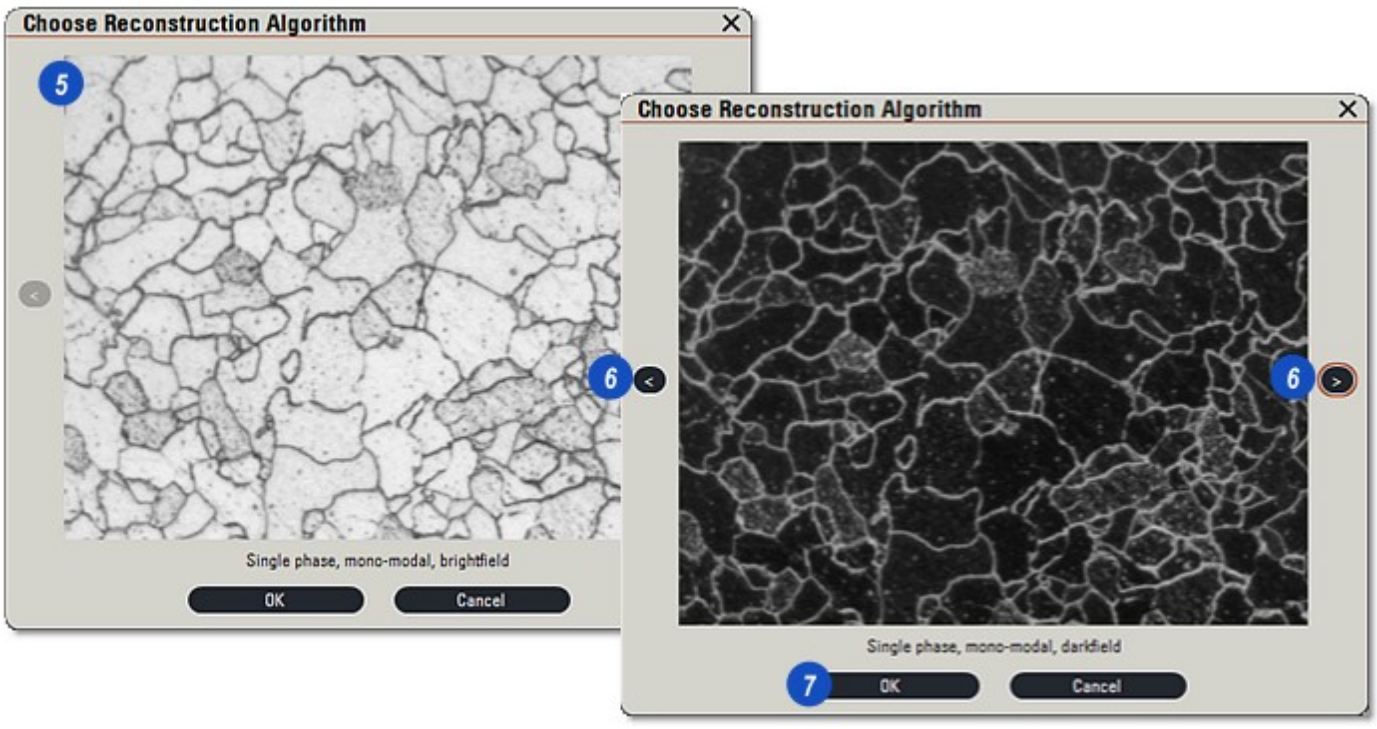
5: The first *Reconstruction Image* appears.

6: Move back and forth through the images until one appears that more closely matches the specimen than any of the others. It does not have to be identical.

7: Click *OK*.

8: Click on the *Apply* button and the initial boundary detection will appear in the binary window.

Continued…



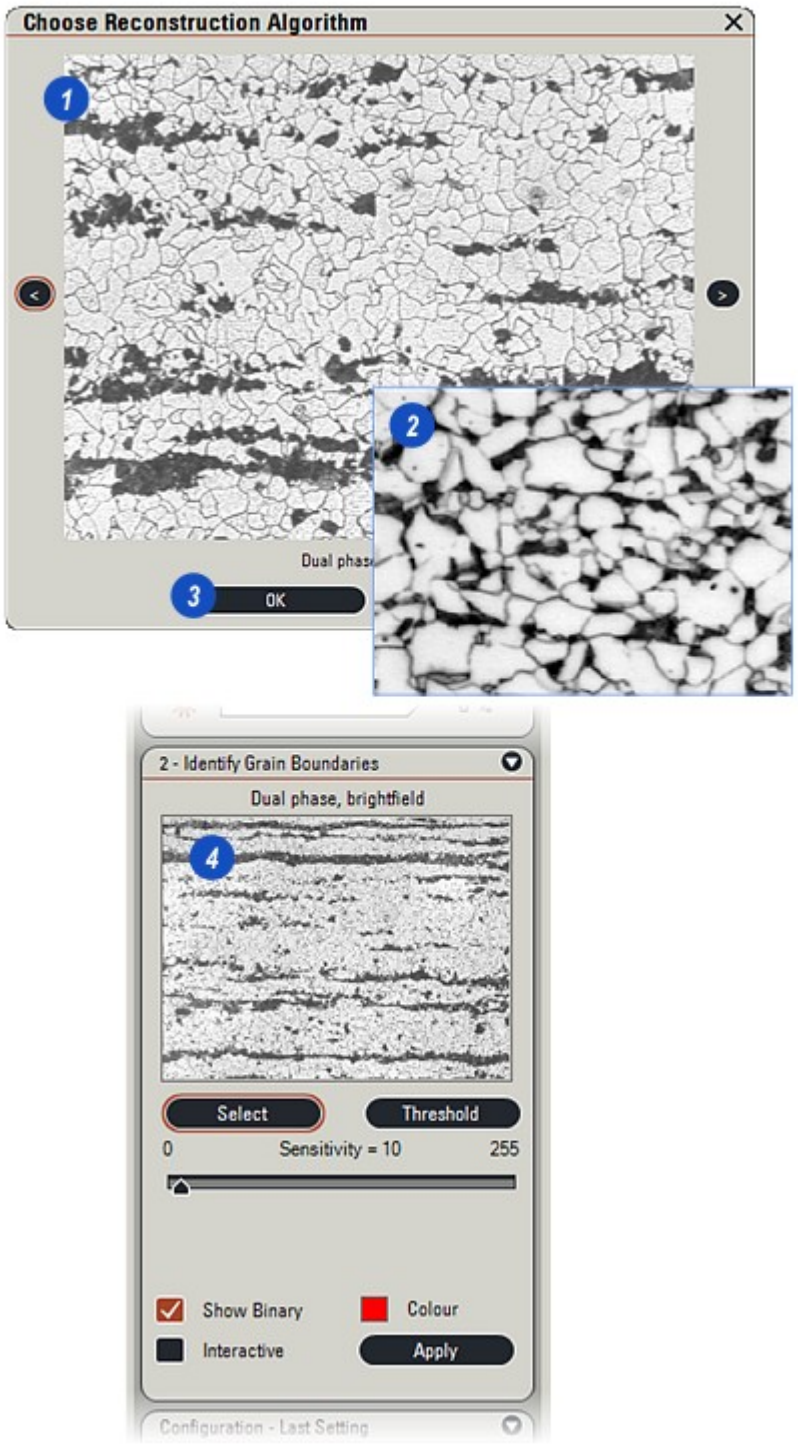
1: In the example the *Dual phase brightfield Reconstruction Image* has been chosen as quite closely matching that of…

2: …the specimen.

3: Click *OK* and…

4: …a scaled version of the *Reconstruction Image* appears in the *Identify Boundaries* window.

Continued…



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Grain Expert supports both greyscale and colour images, but using conventional bright- and darkfield illumination the grains will appear only as various shades of grey. Images captured using polarised light techniques will display in colour and the *Etched Polarised Reconstruction Image* should be selected.

1: Click to select *Identify Grain Boundaries* in the *Processing Sequence* menu.

2: On the *Identify Boundaries* panel, click to enable *Show Binary* to view the processed image.

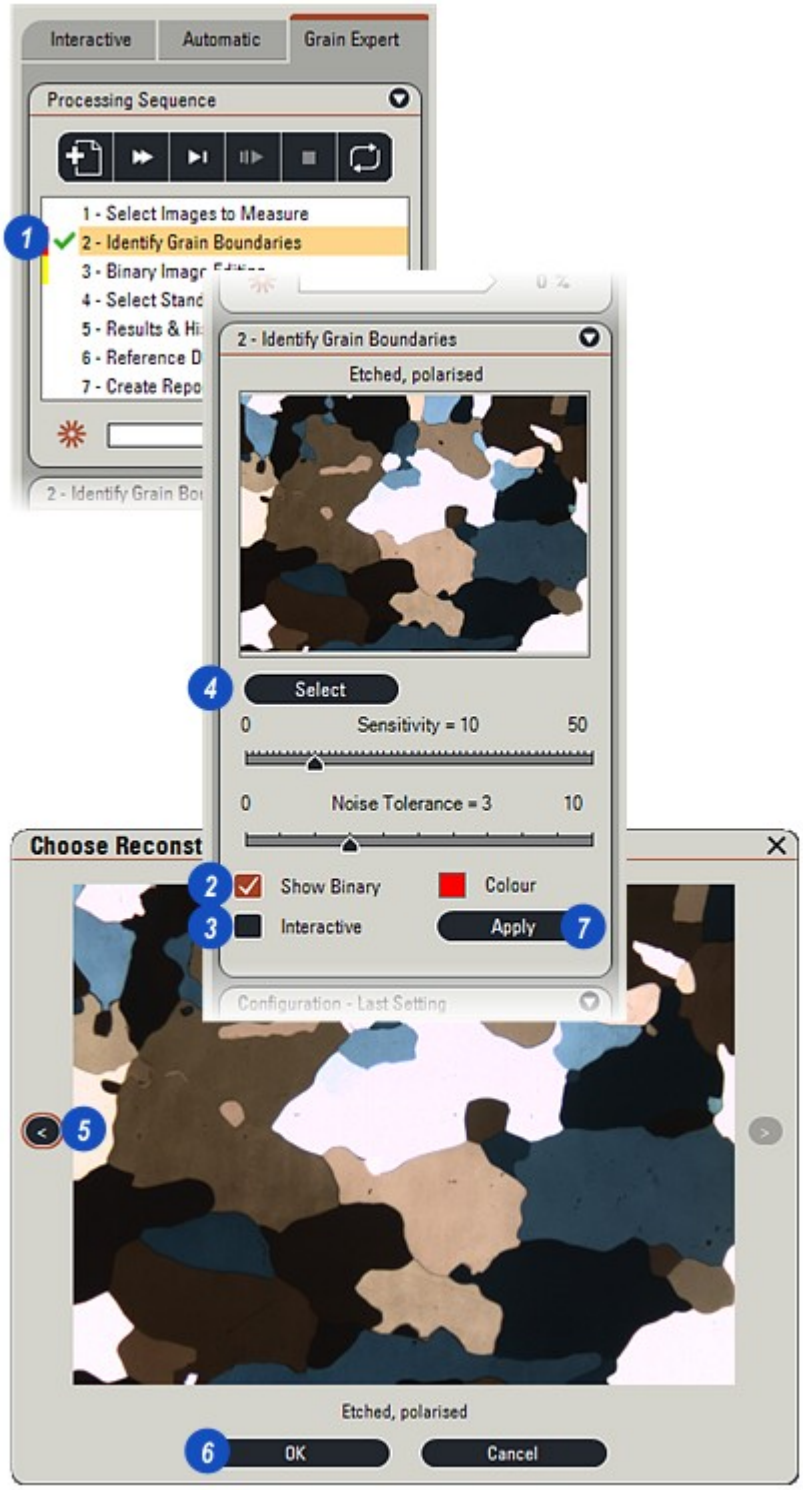
3: Click to disable *Interactive to* prevent processing whilst finding the *Reconstruction Image.*

4: Click the *Select* button.

5: The first *Reconstruction Image* appears. Move through the reconstruction samples to find *Etched, Polarised.*

6: Click *OK*.

7: Click on the *Apply* button and the initial boundary detection results will be displayed in the binary window.



The colour used to show the grain boundaries can be chosen to suit both the user and the image.

1: Click on the *Colour* box on the *Identify Boundaries* panel. The *Select Color* dialog appears.

2: Choose a new colour by dragging the target mark on the *Colour Wheel*,

3: …clicking to select a *Swatch* or…

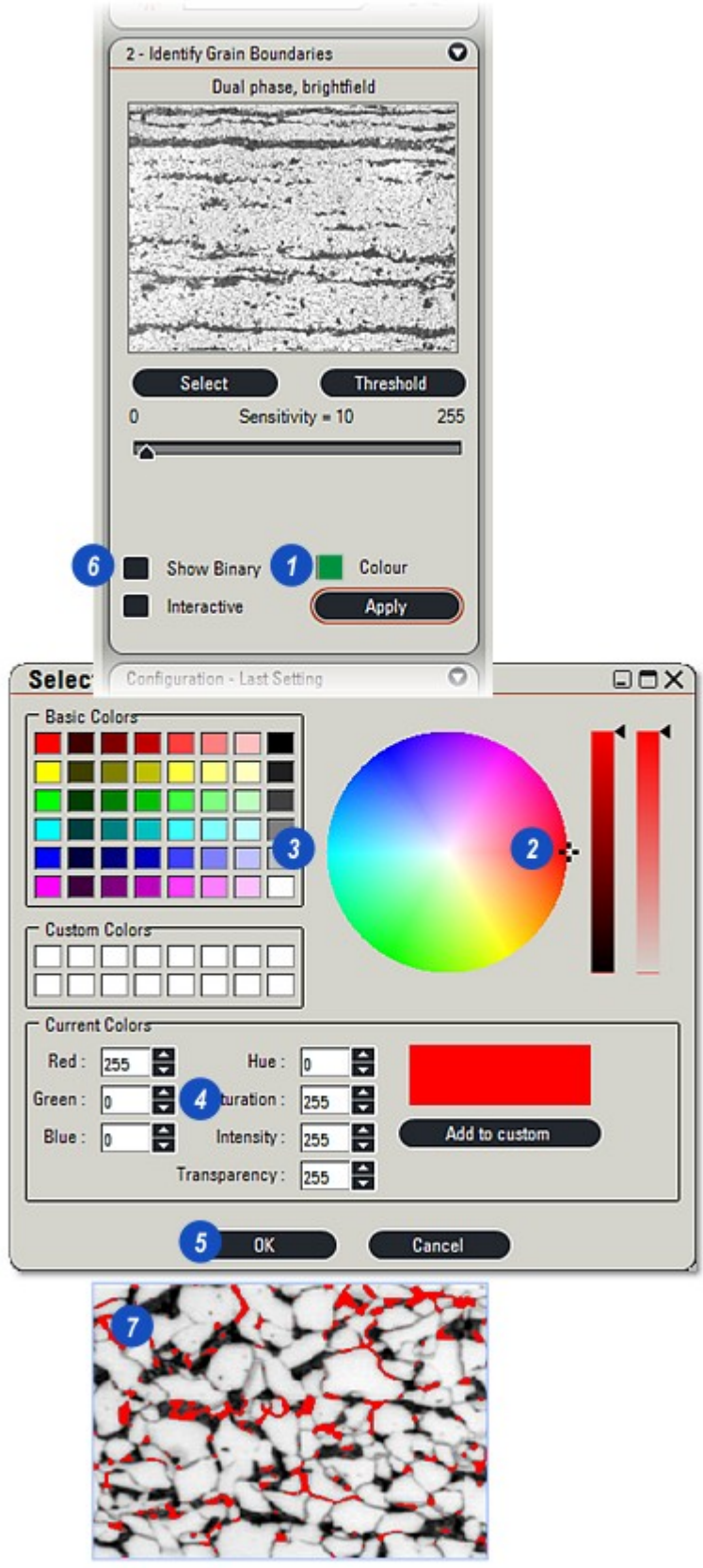
4: …typing *Values.*

5: Click *OK*. The new colour appears in the *Colour* box.

6: Click to enable the *Show Binary* check box and…

7: …the new colour appears on the binary output image.

Continued…



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The *Threshold* and *Sensitivity* controls together, serve to detect the boundaries between individual grains. Time spent at this and during the *Binary Editing* stage to identify the boundaries as closely as possible, will result in a more accurate analysis.

Set the Sensitivity to Mid-range:
This is the starting setting to provide initial boundary detection and will be adjusted later:

1: If necessary, click to enable *Show Binary*. This will display the binary output image with the detected boundaries shown in the selected colour.

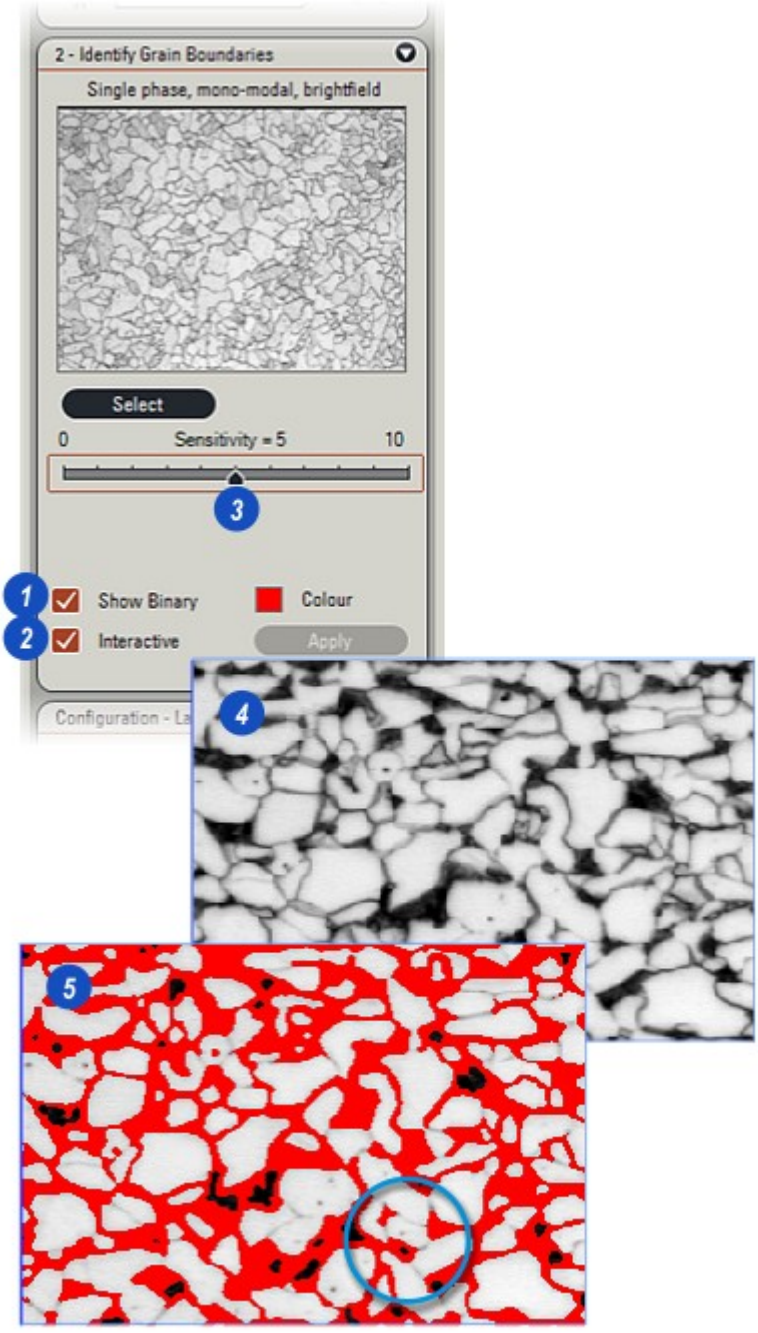
2: Click to enable Interactive update.

3: Click, hold and drag the *Sensitivity* slider to about the mid-point on the track.

4: Part of the original image and…

5: …after the *Sensitivity* has been changed. At this stage many of the boundaries remain undetected – the original grey instead of red.

Continued…



Depending upon the *Reconstruction Image* chosen, for greyscale images an additional control will be available to the user - the *Threshold* button that can fine-tune the boundary detection using a Histogram.

1: The binary image with *Sensitivity* set from the previous step.

2: On the *Identify Grain Boundaries* panel, click the *Threshold* button.

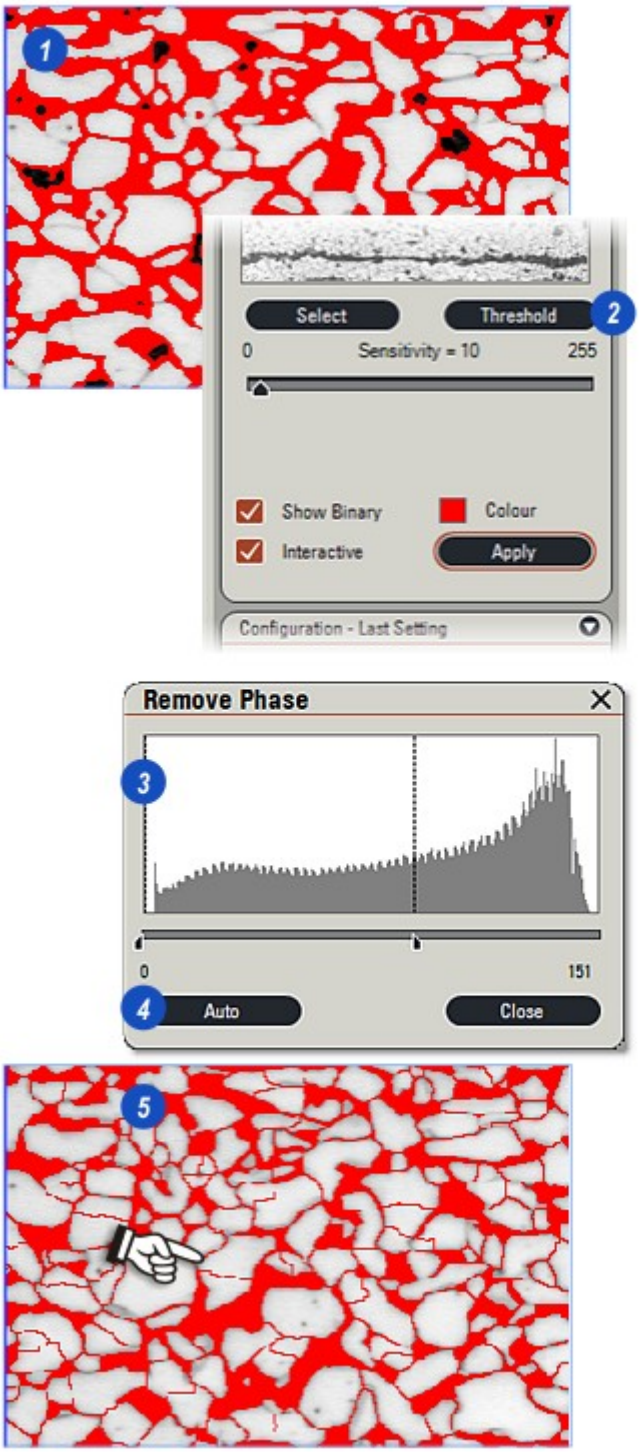
3: The *Threshold Remove Phase Histogram* appears representing the image with the total black pixels (Value 0) to the left and the white pixels (Value 255) to the right.

The vertical dotted bars and the sliders beneath the *Histogram* represent the span of pixel values included in the binary image. Pixels with a value greater than *151*, the setting on the illustration, are ignored.

4: Click on the *Auto* button. This will automatically detect detail in the image that could represent grain boundaries.

5: The binary image after *Auto* detect - far more boundaries have been detected and the entire image is more refined.

Continued…



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1: The binary image following the *Auto* detect stage.

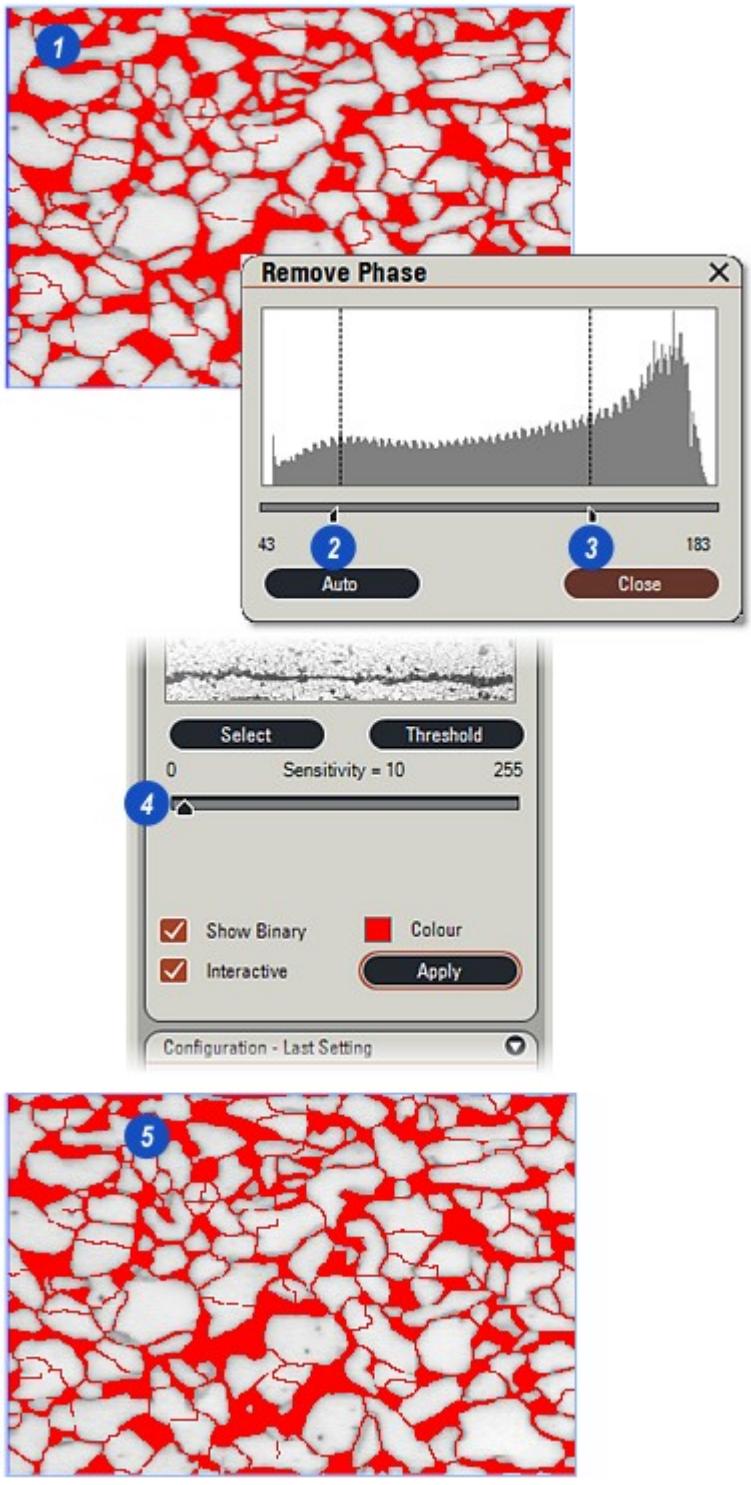
The *Histogram Remove Phase* dialog can be used to refine the detection process by:

2 & 3: Dragging the *Histogram* sliders to include or reject pixel values. On the illustration, the black and dark grey range has been excluded by dragging the left slider to value 43. The mid- and light grey range has been increased by dragging the right slider to value 183. In this way it is possible to detect boundaries that the *Auto* detect did not find.

4: Complete the detection by gradually dragging the *Sensitivity* slider to the left – approaching 0 – until there is no perceptible improvement in the boundary detection.

5: The binary output image so far.

Continued…



When colour images are selected, the Grain Boundaries panel has an additional control - the Noise Tolerance slider.

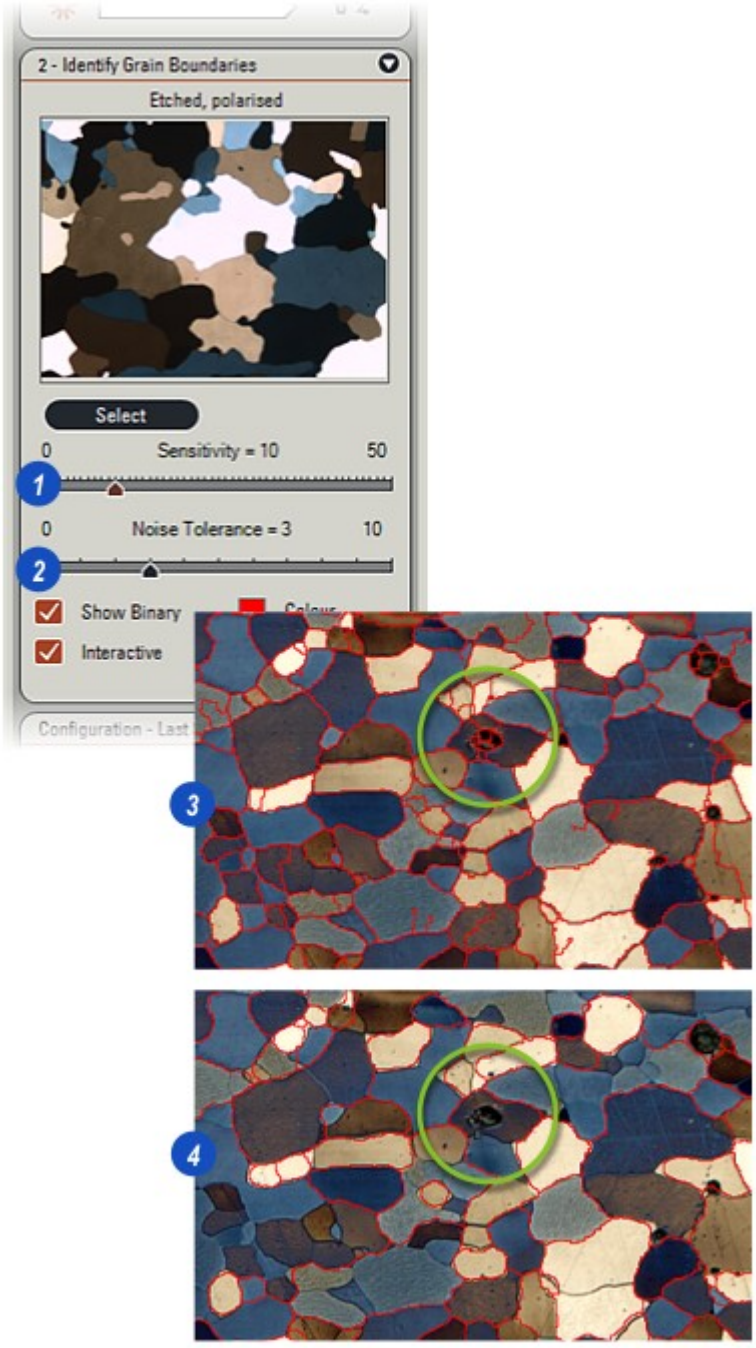
1: Click, hold and drag the *Sensitivity* slider to achieve the best boundary detection possible, and then refine the detection by...

2: ...dragging the Noise Tolerance slider to the left or right.

3: Part of the original image with Noise Tolerance set very low and possibly more boundary or 'edge' detection than is really necessary.

4: Noise Tolerance set too high at 7 and some boundaries have been missed. For this image a setting somewhere between the two would be appropriate and adequate.

Continued…



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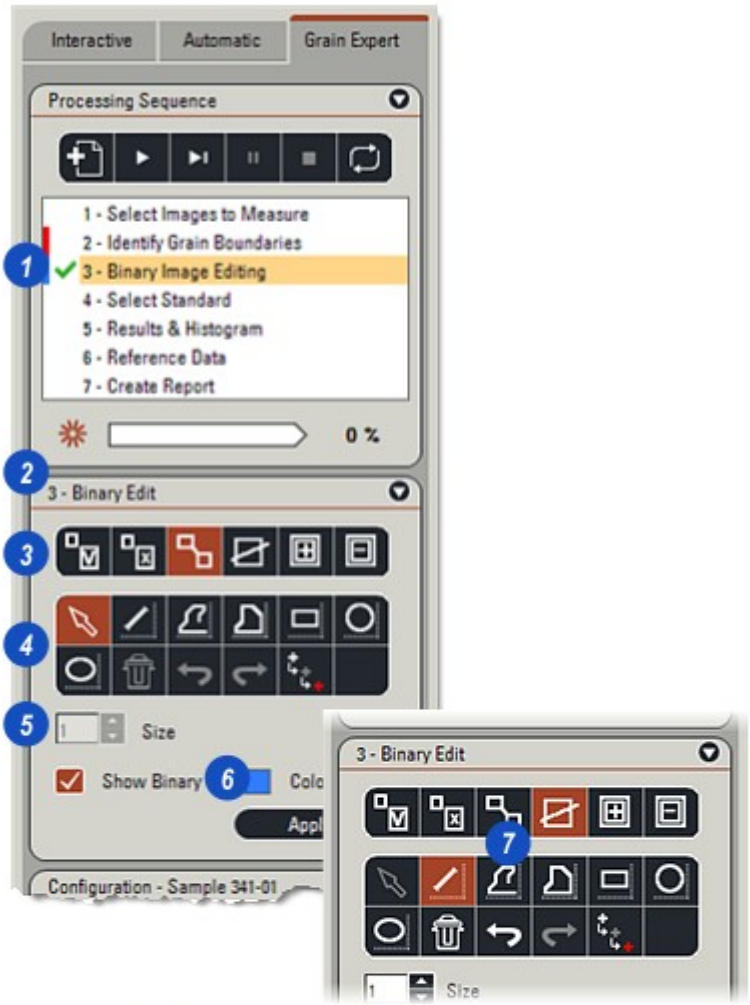
Binary Image Editing provides the tools for working directly on binary images to add, remove, select and de-select features. Facilities also include drawing and filling shapes as well as grouping features. Use *Binary Image Editing* to 'repair' incomplete boundaries and mark phases that should not be included in the analysis.



1: On the *Processing Sequence* menu, click on the *Binary Image Editing* entry.

2: The *Binary Image Editing* panel appears. There are two groups of buttons:

3: *Mode* and…

4: *Tools*. Some of the *Mode* buttons affect the way that the Tools behave.

5: The *Size* window controls the line thickness for some of the Tools used for drawing.

6: The colour of the binary output image is user selectable.

7: The fast-edit tool combination of *Erase* and *Draw Line*, can be used to remove detected boundaries - the image is not modified, only the binary overlay.

8: Click on the image and draw a short line intersecting the boundary to be removed.

9: The boundary is automatically detected and removed.

For detailed information about the *Binary Editing* tools and how to use them, read *Image Analysis* help: *Go there...*

Continued...

Three *Standards* are available within *Grain Expert:*

- *ASTM E112*,
- *JIS G 0551/2* and
- *ISO 643 2003.*

Select the Standard:

1: Click on the *Select Standard* entry on the main menu.

2: The *Select Standard* panel appears.

3: Click on the arrows to the right of the *Standard* header and…

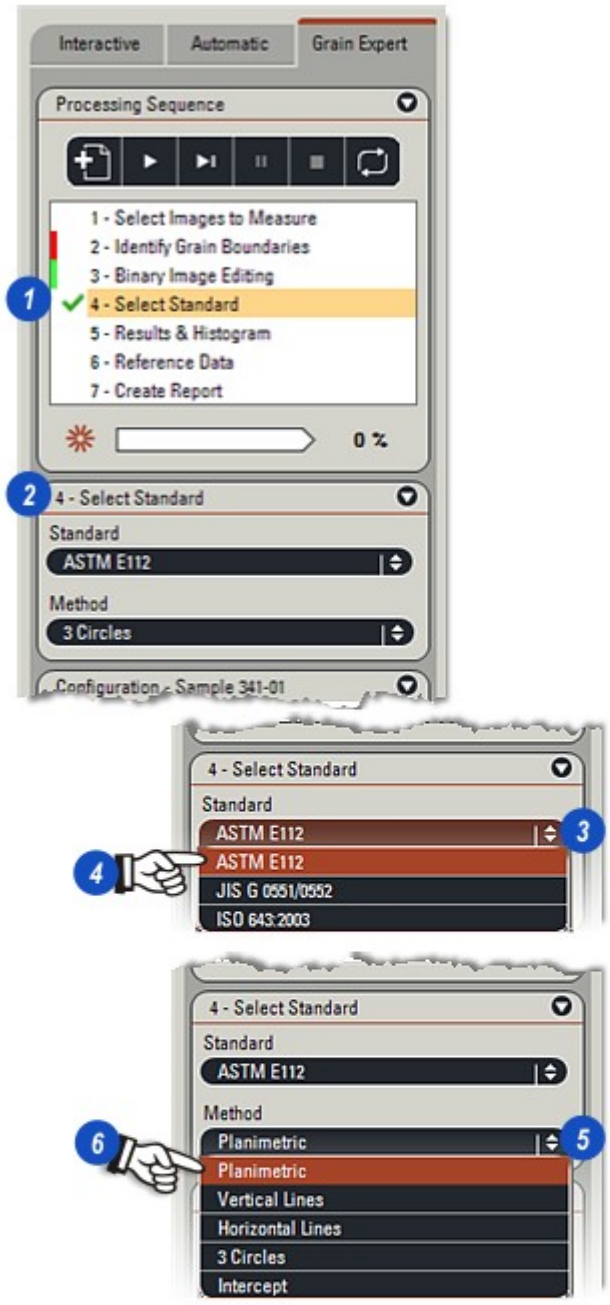
4: …from the drop down list, click to select the required standard.

Choose the Method:

5: Click on the arrows to the right of the *Method* header.

6: From the drop down list, click to select the *Method* required. Examples of the *Method* structures and the display results are shown on the following pages.

Continued…



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The following *Method* diagrams and result displays are applicable to the analysis of the entire *Field*:

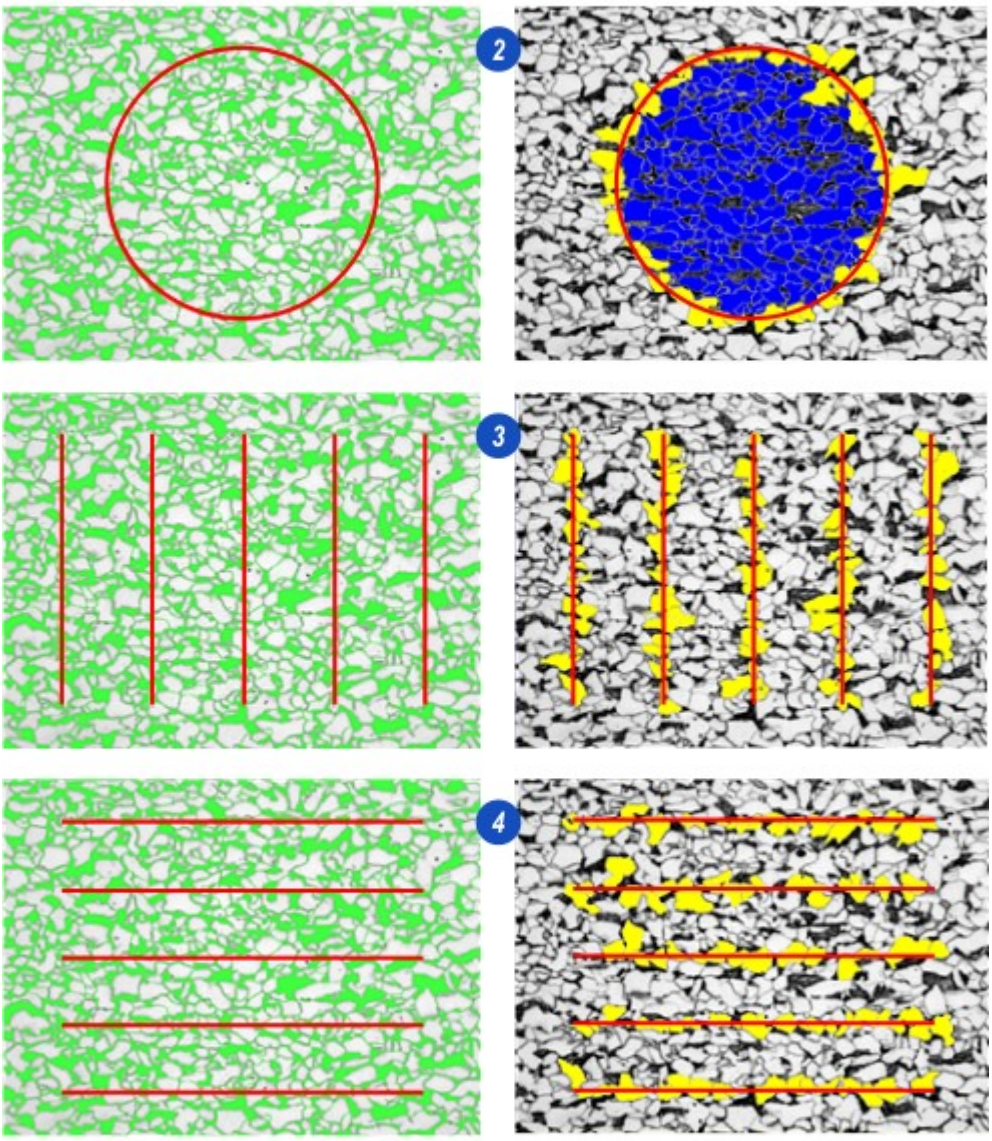
1: On the *Results and Histograms* panel, click on the arrows to the right of the *Field/Grain* header and select the *Filed* option.

2: *Planimetric* Method.

3: *Vertical Lines* Method.

4: *Horizontal Lines* Method.

Continued...



Continued from the previous page...

1: Select the *Field* option on the *Results and Histogram* panel.

2: *3 Circles* Method and…

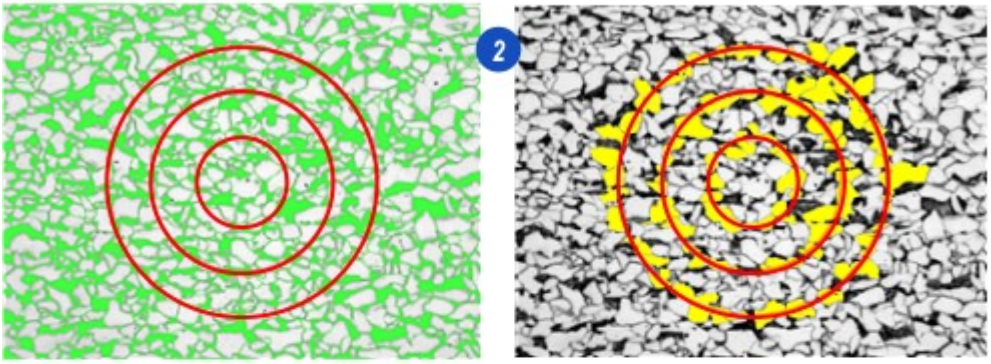
3: *Intercept* Method.

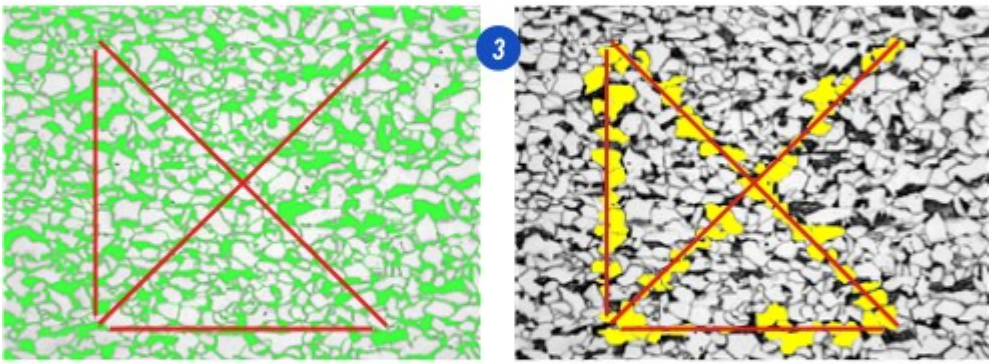
Grain Results:

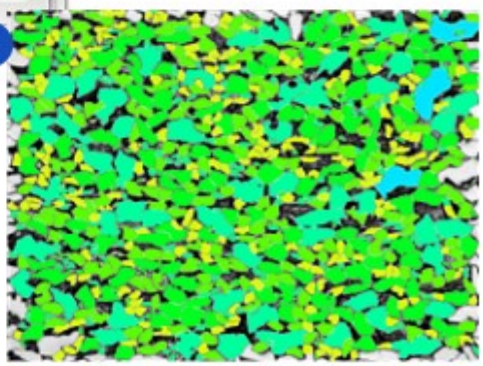
4: To display the *Grain* results binary output image in which each grain size is colour coded, on the *Results and Histogram* panel click on the arrows to the right of the *Field/Grain* header and select *Grain* from the options.

5: The *Grain* results binary output image.

Continued…







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1: Click to select *Results & Histogram* on the *Processing Sequence* menu.

2: Click on the arrows to the right of the *Grain/Field* header and…

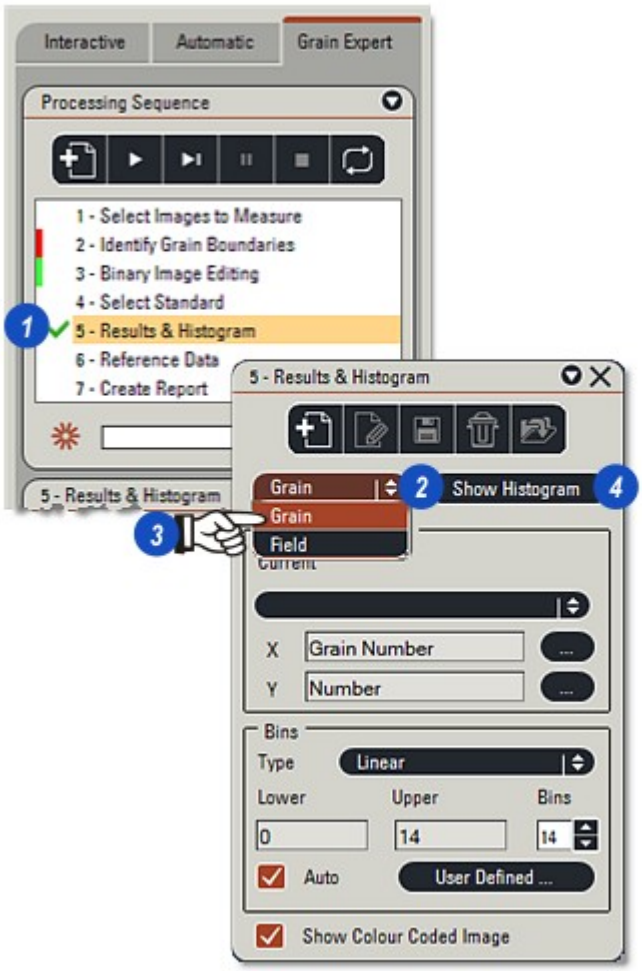
3: …from the drop-down list click on the *Grain* option.

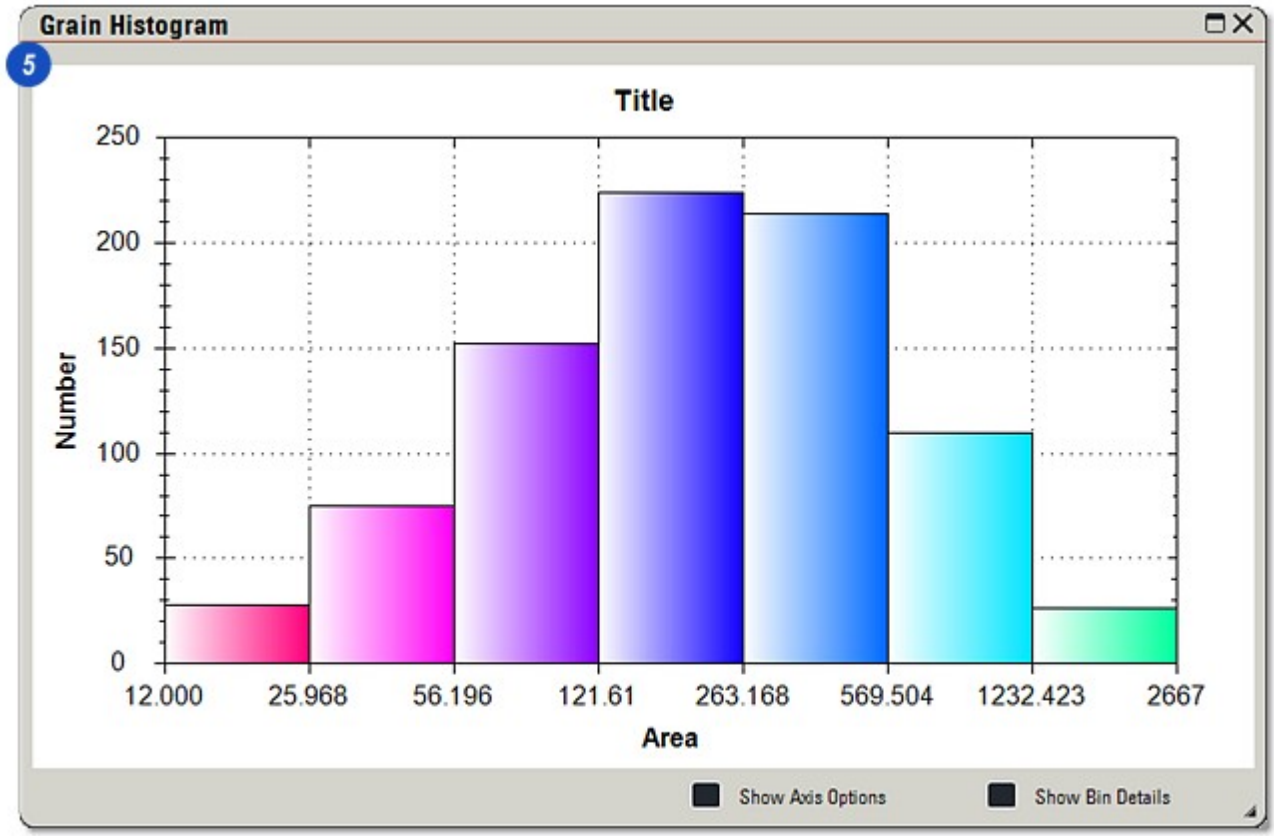
4: Click on the *Show Histogram* button.

5: The *Grain Histogram* shows the grains and their results plotted against the parameters chosen for the *X* and *Y* axes. Selecting the *X* and *Y* axes parameters: *Go there…*

For details of other *Axis* and *Bin* options, see *Image Analysis* help.

Continued…





1: For this analysis 5 fields were chosen.

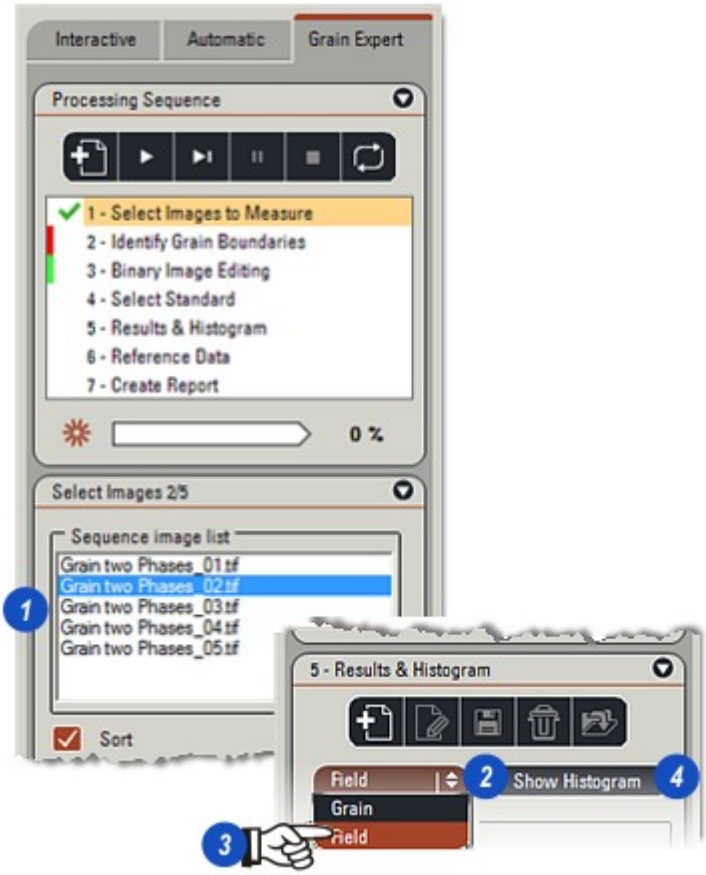
2: On the *Results and Histogram* panel, click on the arrows to the right of the *Grain/Field* header and from the options…

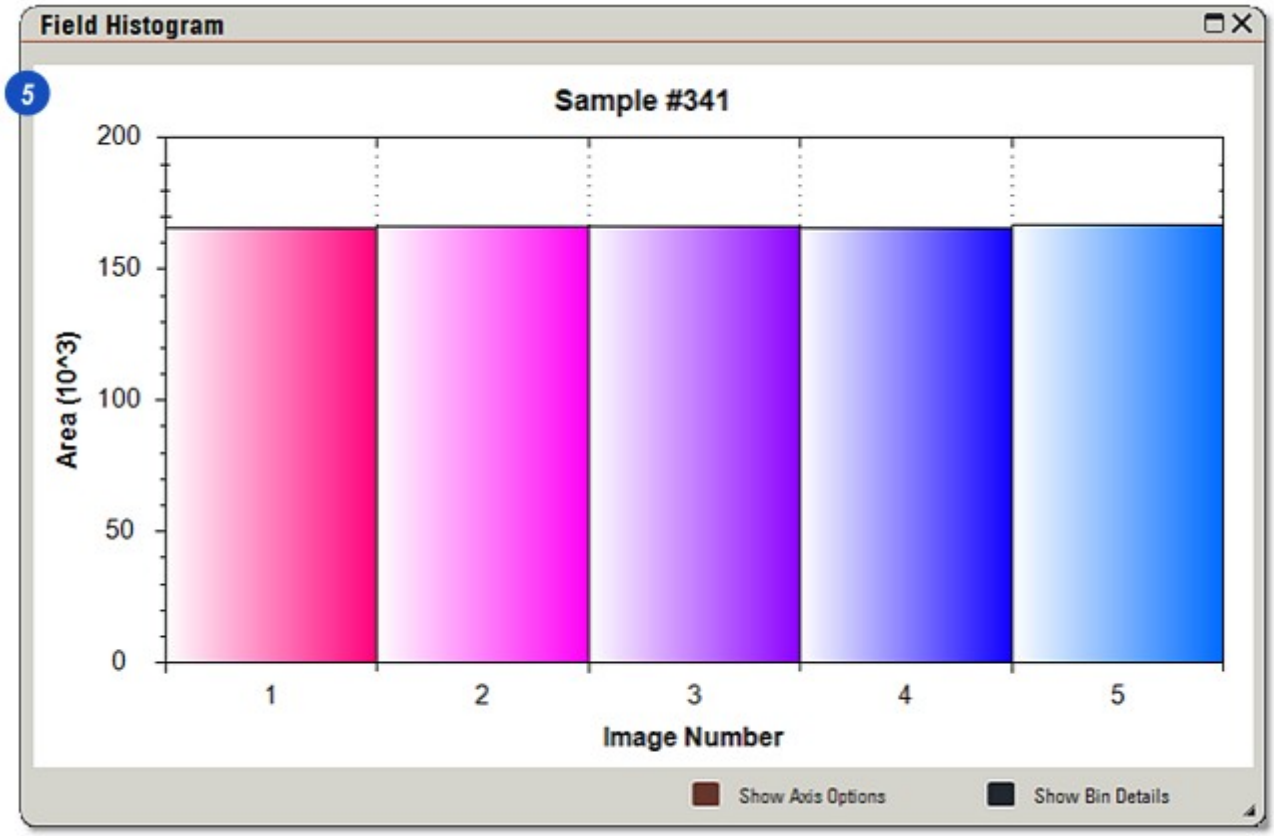
3: …click to select *Field*.

4: click on the *Show Histogram* button.

5: The *Field Histogram* shows the 5 fields along the *X* axis with their measured area on the *Y* axis.

Continued…





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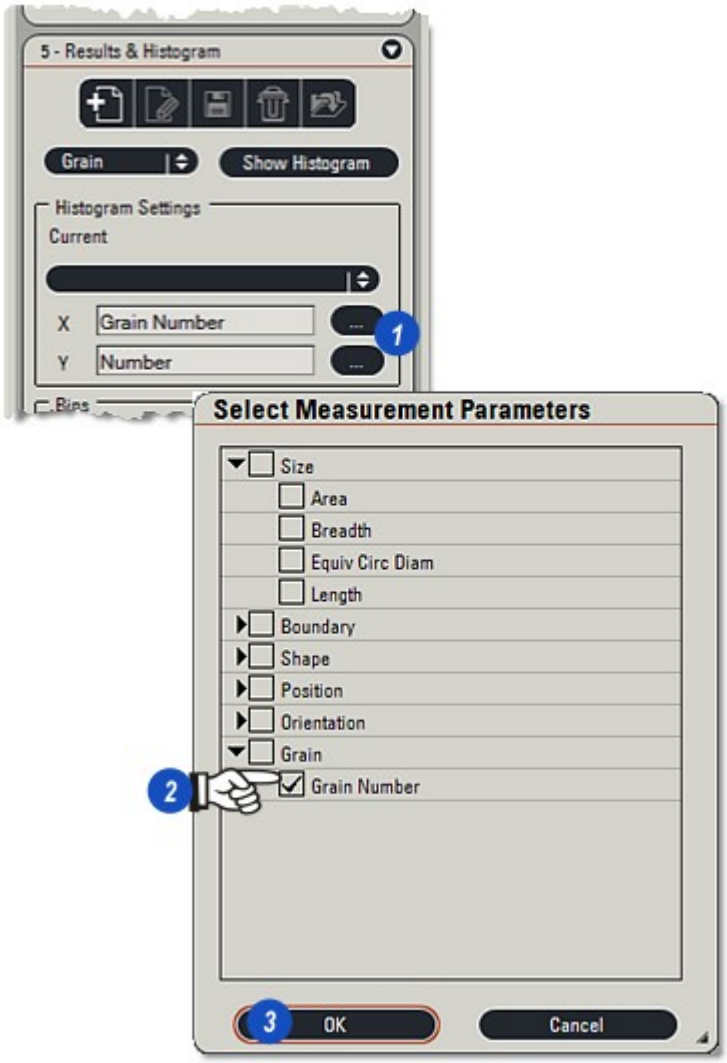
With the *Field* option selected for the *Histogram* display, only the *Y* axis parameter can be changed - the *X* axis will always read *Image Number*. For the *Grain* option both *X* and *Y* parameters can be selected.

1: Click on the browse button against the axis to be changed. If the button is greyed out the option is not available.

2: The *Select Measurement Parameters* dialog appears. The items in the list will depend upon the display option chosen and the axis to be altered. Click to select the parameter required.

3: Click *OK*.

Continued...

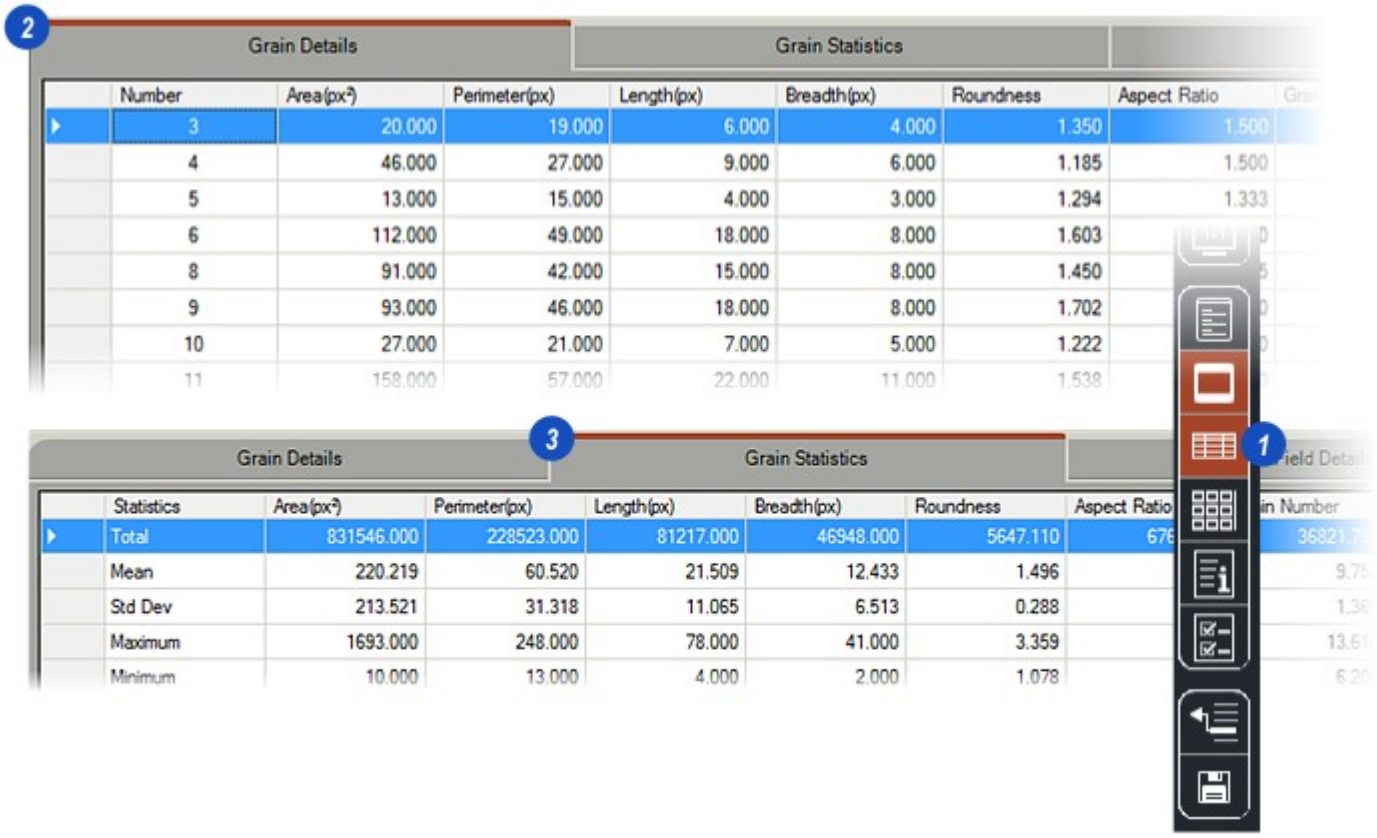


1: If necessary, click on the *Show Grid* button on the *Side Tool Bar* to display the results in tabular form.

Four tabs are displayed across the top of the *Grid*. To the left the *Grain* results – *Details* and *Statistics* – and to the right the *Field* results, *Details* and *Statistics* also (Shown on the following page).

2 & 3: Click on the appropriate tab to reveal the results. The result parameters can be selected to suit the job and user: *Go there…*

Continued…



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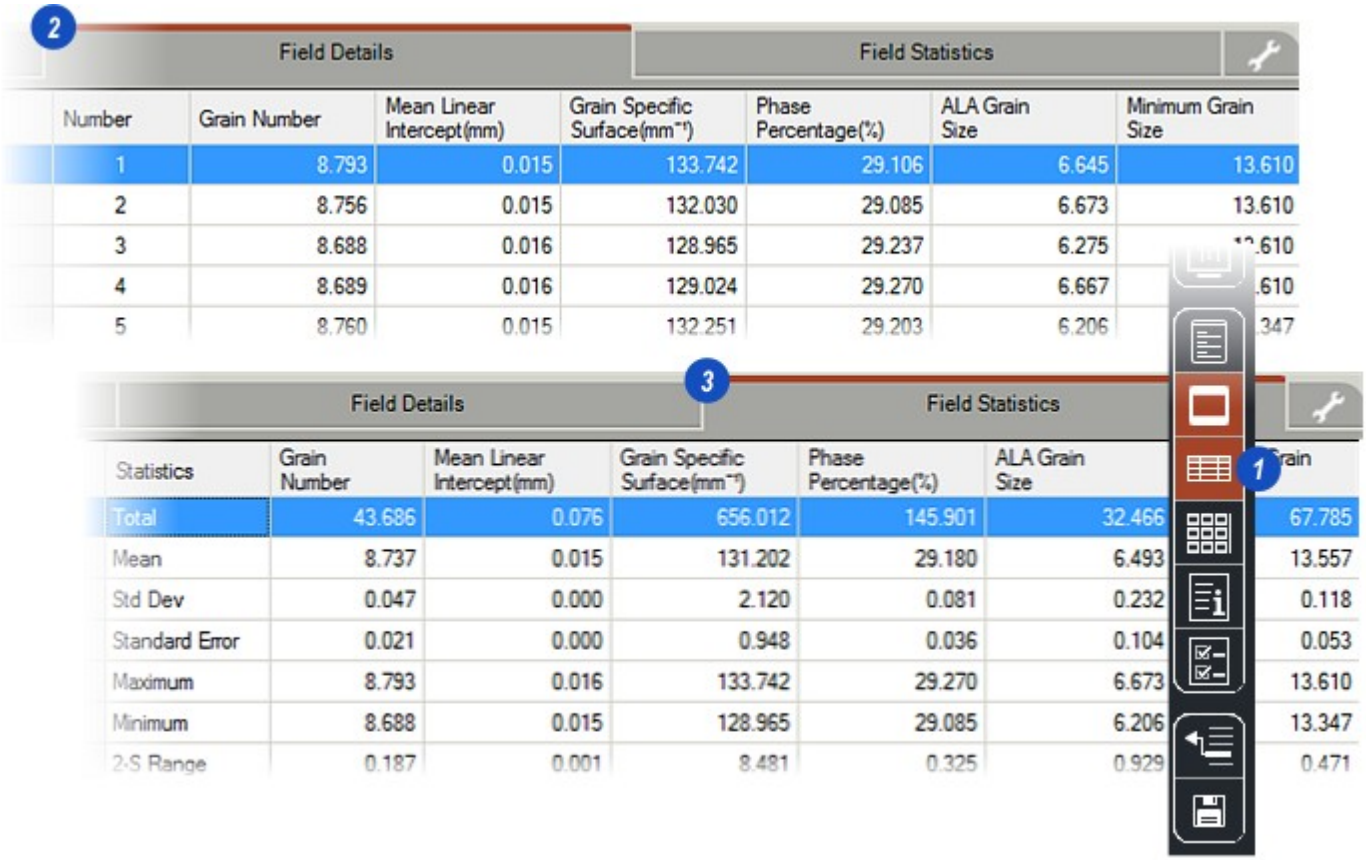
1: If necessary, click on the *Show Grid* button on the *Side Tool Bar* to display the results in tabular form.

Four tabs are displayed across the top of the *Grid*. To the left the *Grain results – Details* and *Statistics* (Shown on the previous page) – and to the right the *Field* results, *Details* and *Statistics* also.

2 & 3: Click on the appropriate tab to reveal the results. The result parameters can be selected to suit rhe job and user: *Go there…*

Continued…



1: To change the *Tabular (Grid)* display column header parameters for *Grain* or *Field*, click on the appropriate results tab.

2: Click on the *Tool Tab* and, depending upon the results tab selected the *Select Details* or *Select Statistics* dialog will appear.

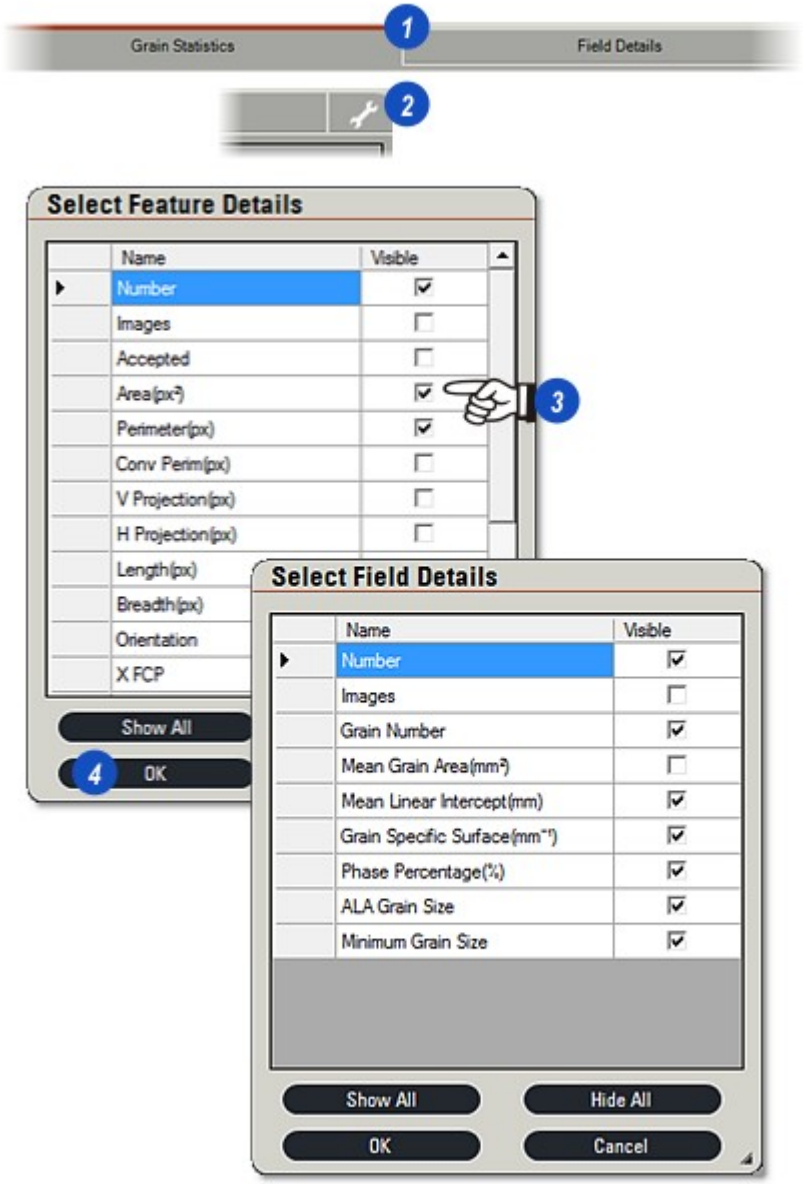
3: Enable the required check boxes to include the parameter required in the displayed results.

4: Click *OK.*

The *Show All* option when enabled will select all of the options.

Click on the *Hide All* button to clear all of the options before starting a new range of selections.

Continued...



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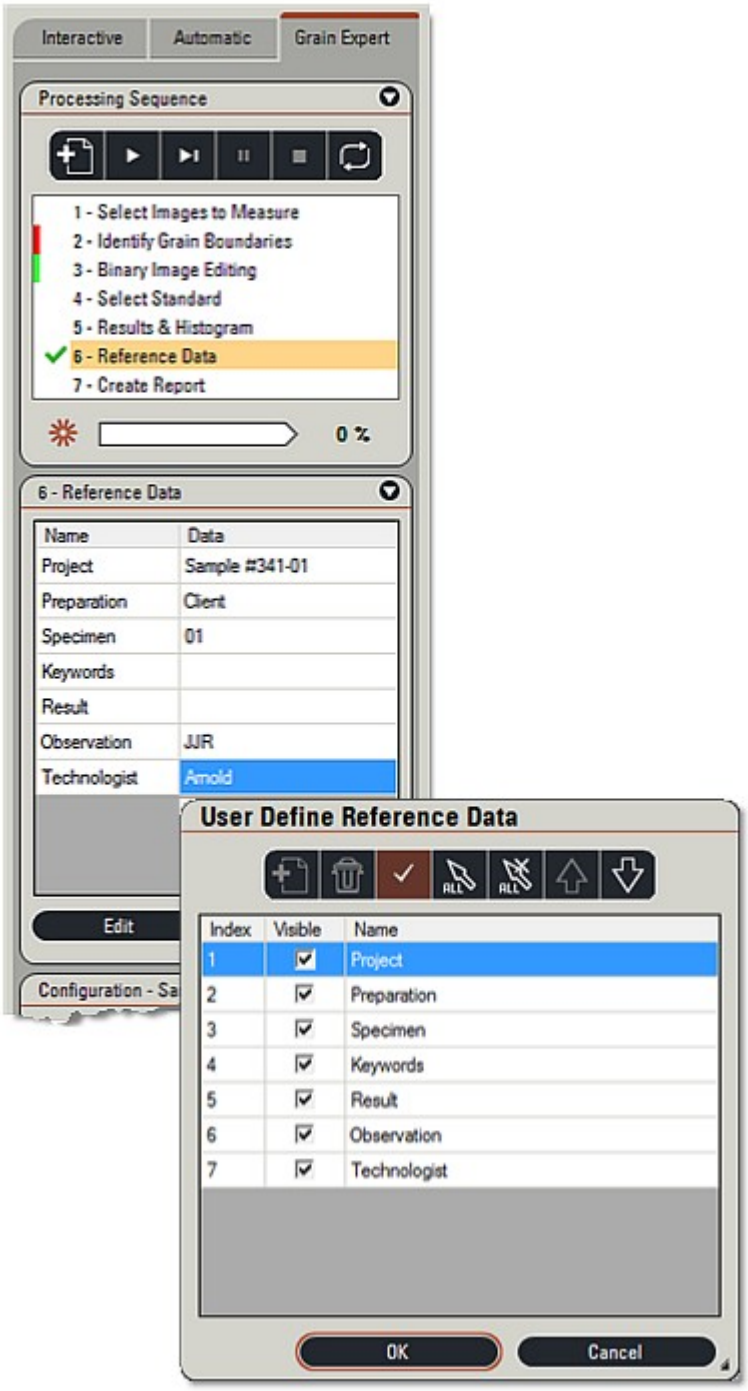
A comprehensive range of *Data* Items can be appended to the *Grain Expert* results that will identify important details such as the *Project Name*, the *Specimen* and how it was prepared. Enter the information in the *Reference Data* dialog.

Administrators can add to the supplied list of data headings to comply with corporate demands.

For details refer to Image Analysis help:

Continued…



The report is created using *Microsoft Excel* which must be installed on the computer.

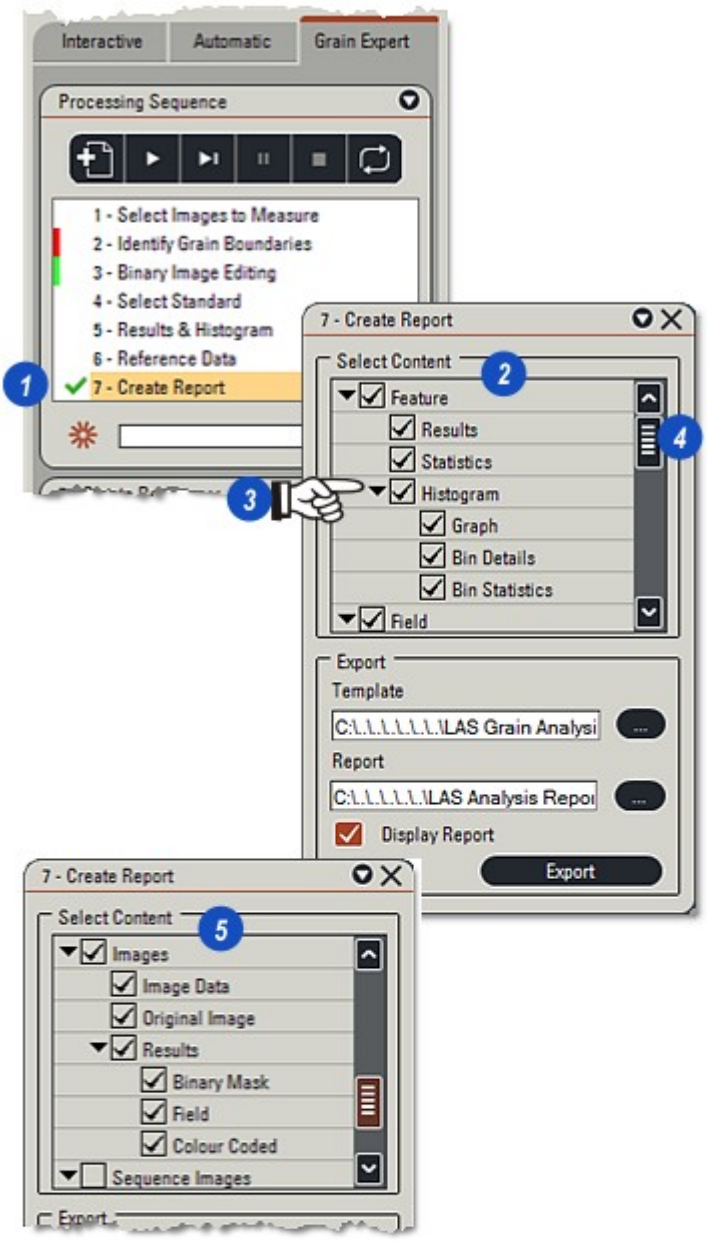
1: On the *Processing Sequence* menu, click to select *Create Report*.

2: The *Select Content* dialog lists all of the information and images that can be included in the report.

3: The list is divided into sub-sections – click on the small arrow to the left of a sub-section header to reveal all of the section options.
To include an item in the report, click to enable the check box to the right of the item.

4: The list is extensive so use the scroll bar to reveal more items **(5)**. Images tend to be large files that can make a report unwieldy, especially if it is to be transmitted electronically – by e-mail for example – so where possible keep the included images to a necessary minimum.

Continued…



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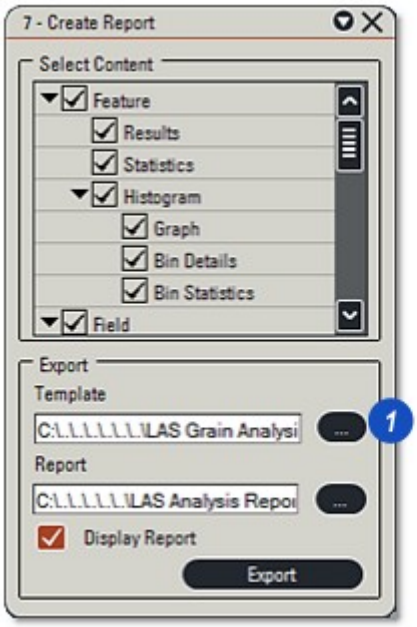
The reports are created using an *Excel* template; A standard template is provided with *Grain Expert* that will be suitable for many applications. It can be modified by the user to reflect the job or corporate style. Alternatively, use any appropriate existing *Excel* template.



Locate the Template:

1: Click on the browse button to the right of the *Template* text box.

2: Navigate to the *Template* folder…

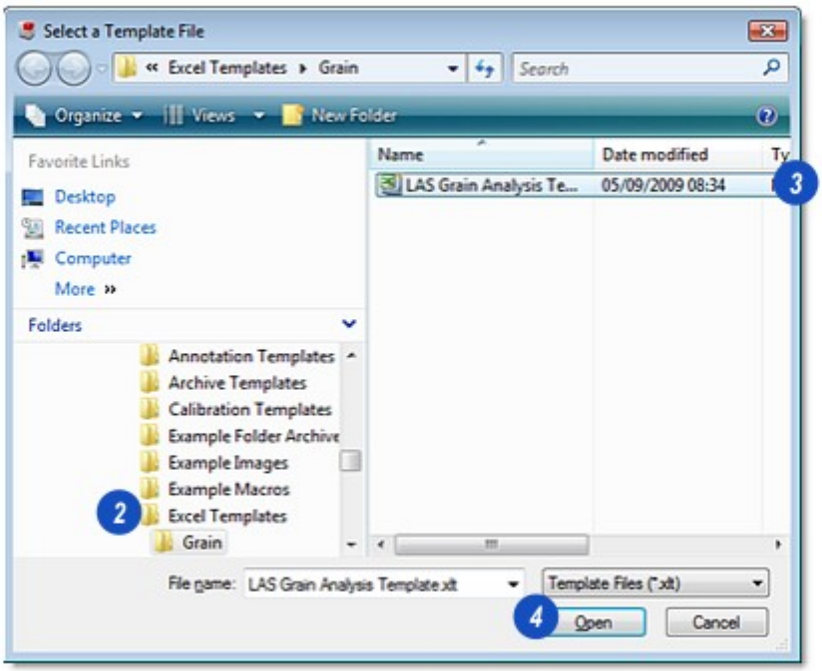
3: …and file and click to select it.

4: Click on the *Open* button.

A default template is supplied with *Grain Expert* and is located in the *Users/Excel Templates\Grain* folder with the name:
LAS Grain Analysis Expert Template.xlt.

The precise path may vary with the installed operating system.

Continued...



1: Click on the browse button to the right of the *Report* text box.

2: Navigate to the folder into which the report file will be saved.

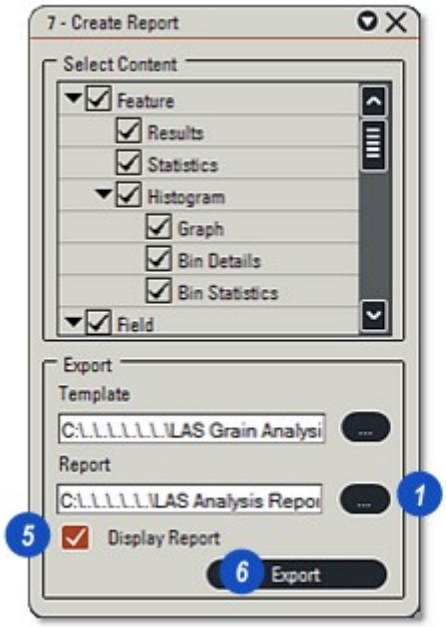
3: Type a name for the file and…

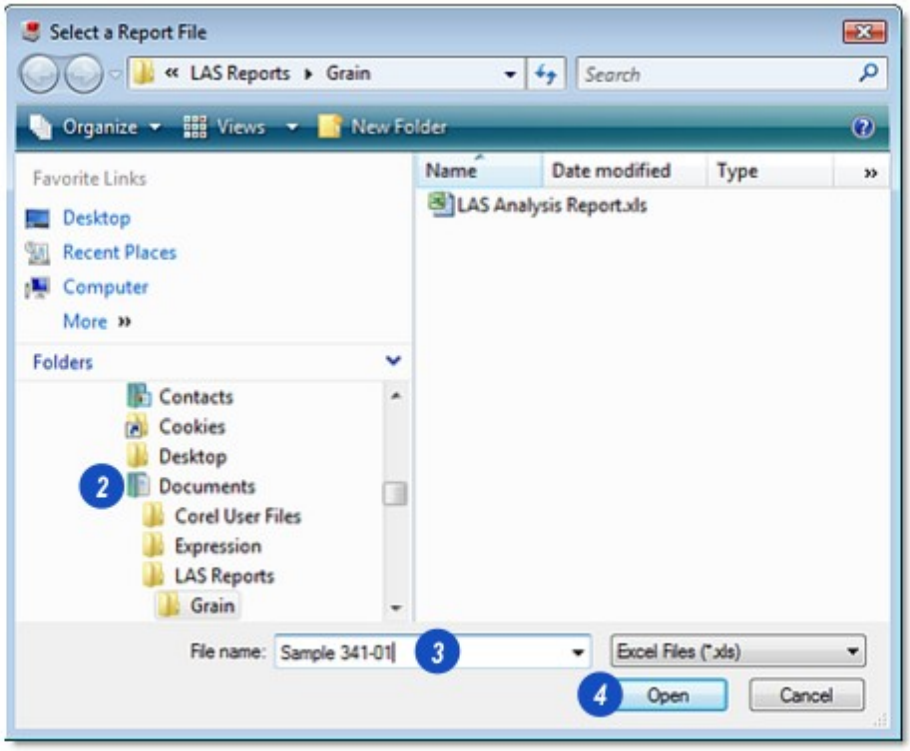
4: …click on the *Open* button.

5: If the report is to be displayed as soon as it is created, click to enable the *Display Report* check box.

6: Click on the *Export* button.

Continued...





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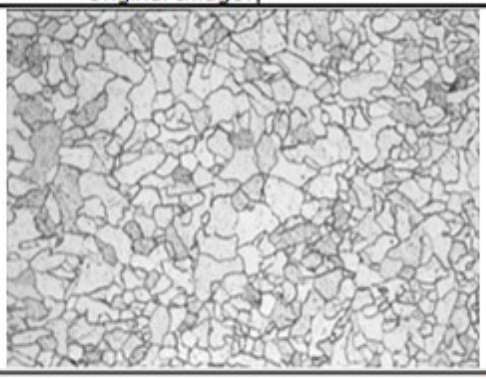
This report was created with Microsoft Excel using the the template supplied with *Grain Expert. The report can be displayed and read (but not created) using Excel Viewer.*

Continued...

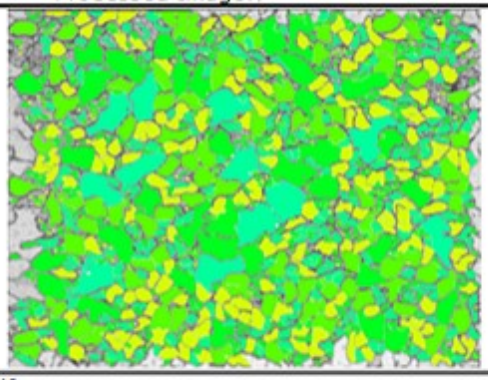
LAS Grain Analysis Report ISO 643:2003 Intercept

Project		Result	
Preparation		Observation	
Specimen		Technologist	
Keywords		Calibration:	1 pixel = 0.67 Pixels
Analysis Date:	05/09/2009	Scanned Area:	506.67 x 382.67 px

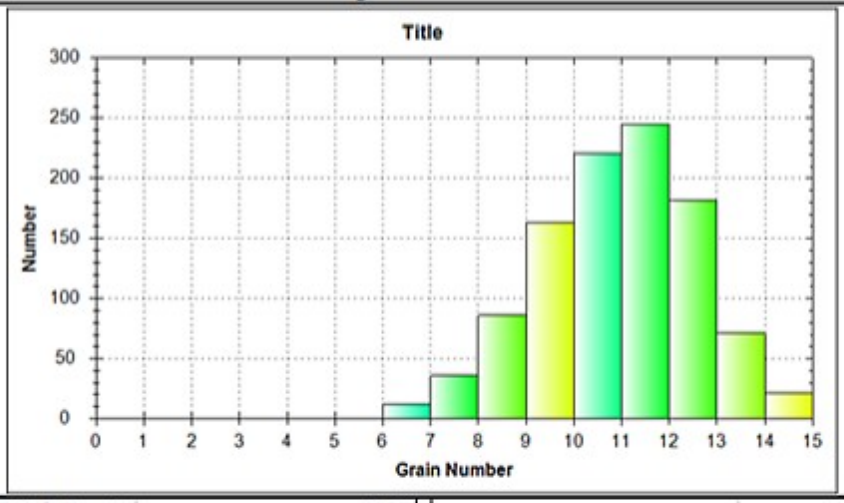
Original Image:



Processed Image:



grain20x2.tif

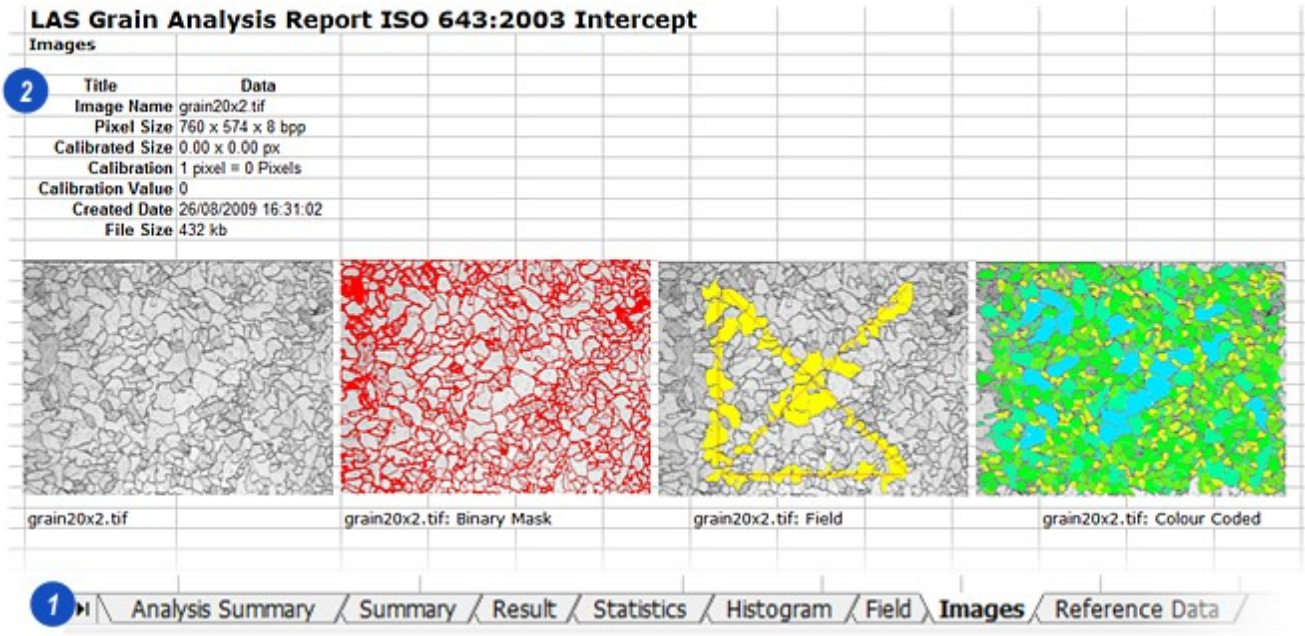


Total Count	1037		
Mean	10.9		
Total	11304.38	Approved By:	
Oversize Count	0		

This report was created with Microsoft Excel using the the template supplied with *Grain Expert. The report can be displayed and read (but not created) using Excel Viewer.*

1: Depending upon the information included in the report, it is divided into sheets access by clicking the tabs along the lower edge.

2: The illustration shows the *Images* sheet.



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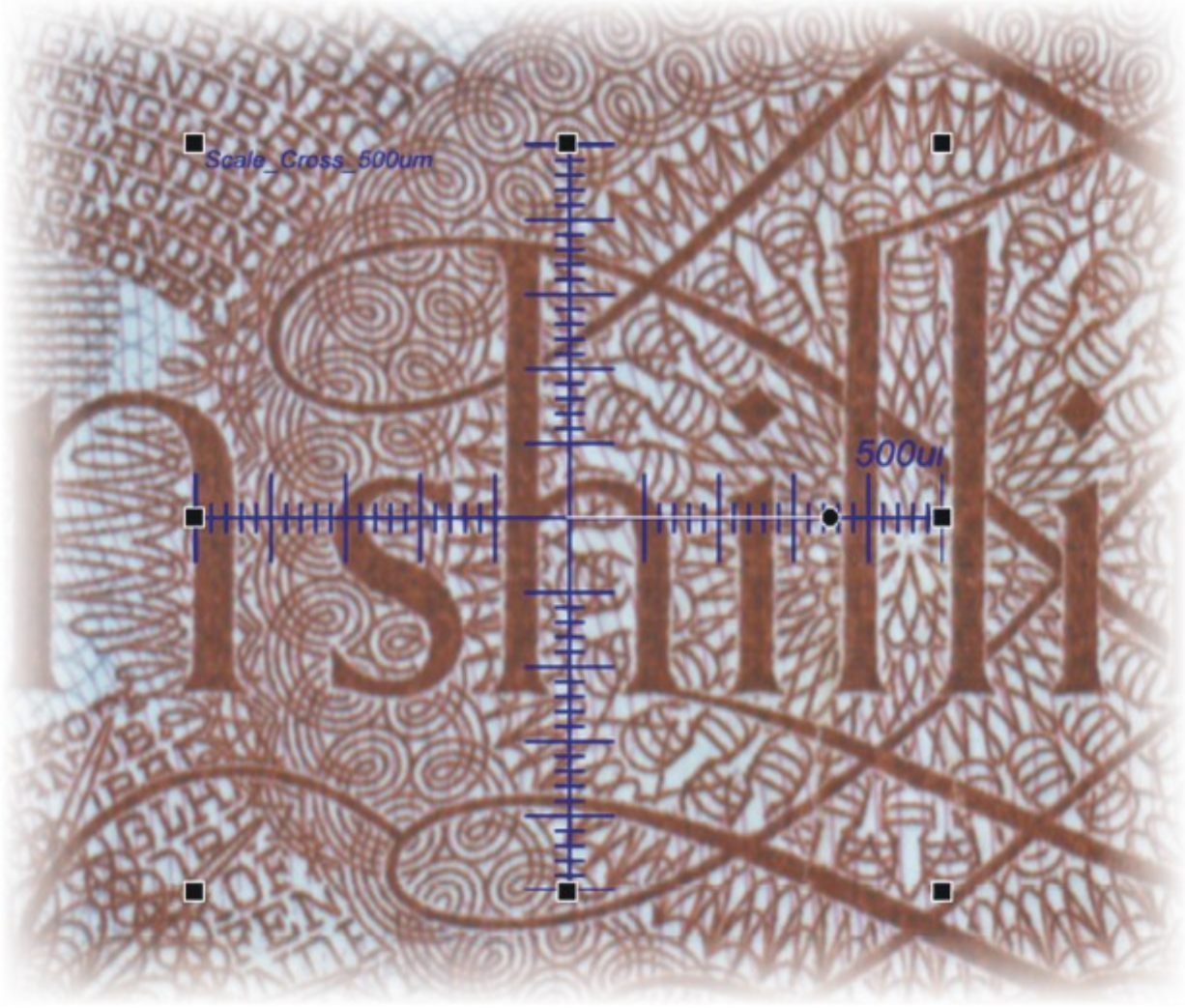
Reticule

Reticule is an optional module that allows a precise pre-defined measuring grid – a Reticule - to be overlaid on a live image.

- Software generated: Completely independent of eyepiece reticules.
- A fast and reliable method of selecting the best grid to suit the application.
- A reticule can be captured and merged with the image.
- Fixed and Scalable versions available from a comprehensive Library.
- Reticule patterns are stored as standard svg format allowing users to design and create to their own special requirements.

LAS Reticule has a wide and varied range of precision uses:

- Item counting and distribution.
- Comparison and location.
- Sizing.
- Volume estimation.
- Assessing image scale.



Reticule Fast Track is a checklist of the steps to take to get *LAS Reticule* operational quickly:

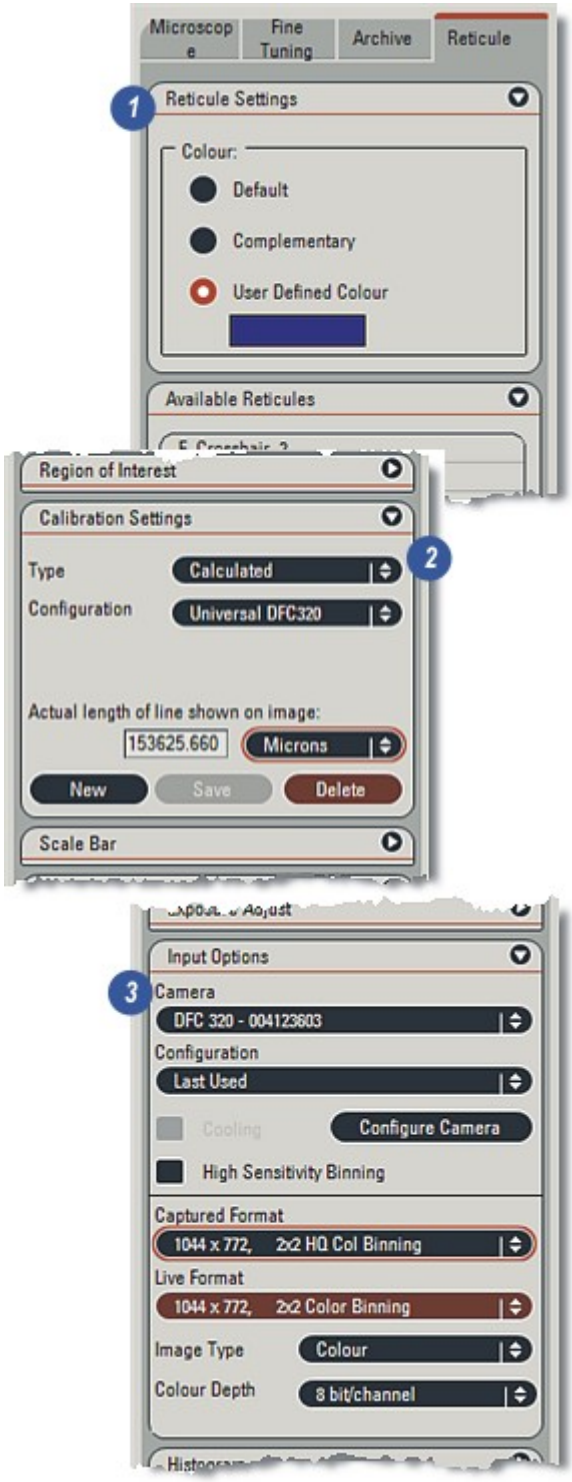
1: Select and load the required *Reticules* from the *Library:* *Go there...* 975

2: *Calibrate* the microscope: *Go there...* 268

3: Select the best live image format to suit the application and hardware. Good resolution but with an acceptable refresh rate is the essential aim: *Go there...* 240

If using a Stereo microscope the zoom is adjusted, the *Reticule* drawings adjust in size but due to image shift are no longer in the correct position. Use the AX-Carrier option to correct this,

Most suitable for use with a microscope having automatic magnification readout.



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The first task is to select the styles required from the *Reticule Library* and attach them to the *Reticule* software.

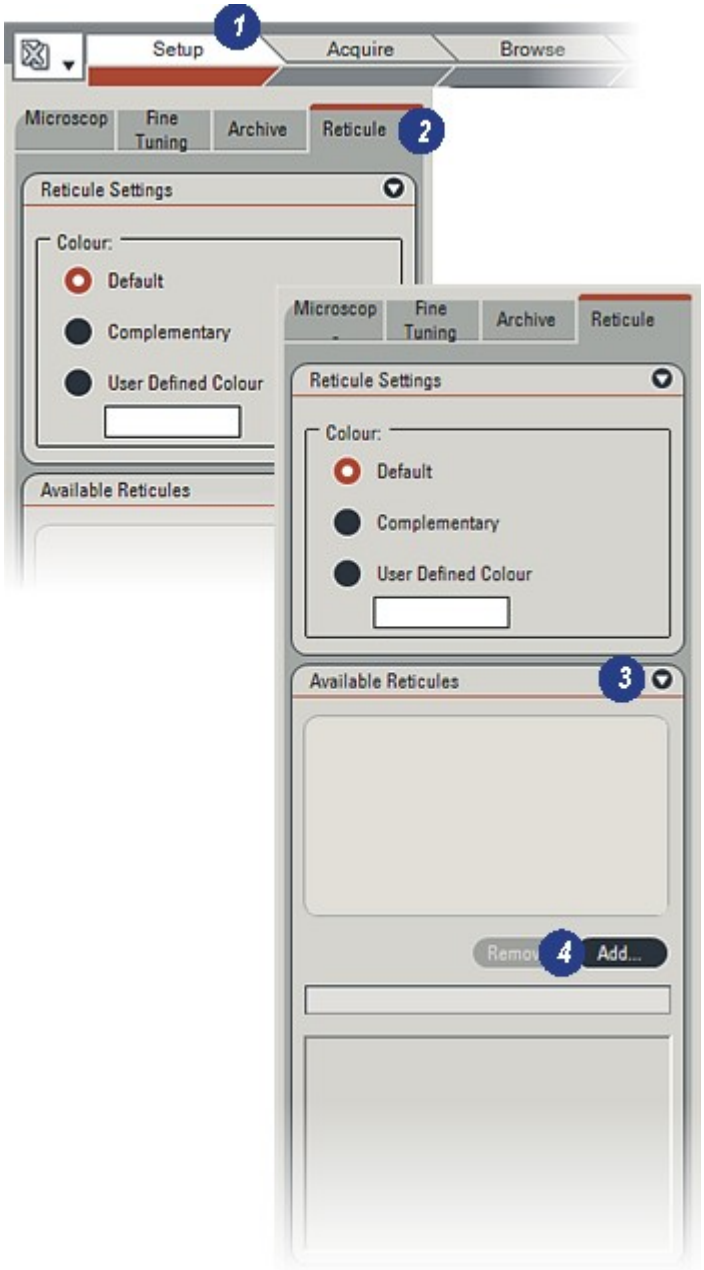
1: Click to select the *Setup Workflow* and…

2: Select the *Reticule* tab. If it is not present the module is not installed or is not licensed.

3: On first use the *Available Reticules* window will be empty, so to select the styles…

4: …click on the *Add* button.

Continued... 976



1: The Widows navigator dialog opens with the *Reticule Files* folder automatically selected.

2: A single style can be selected simply by clicking on its file name, but for multiple selections, hold down the *Ctrl* key on the keyboard whilst clicking individual files. The illustration shows 11 files selected and highlighted.

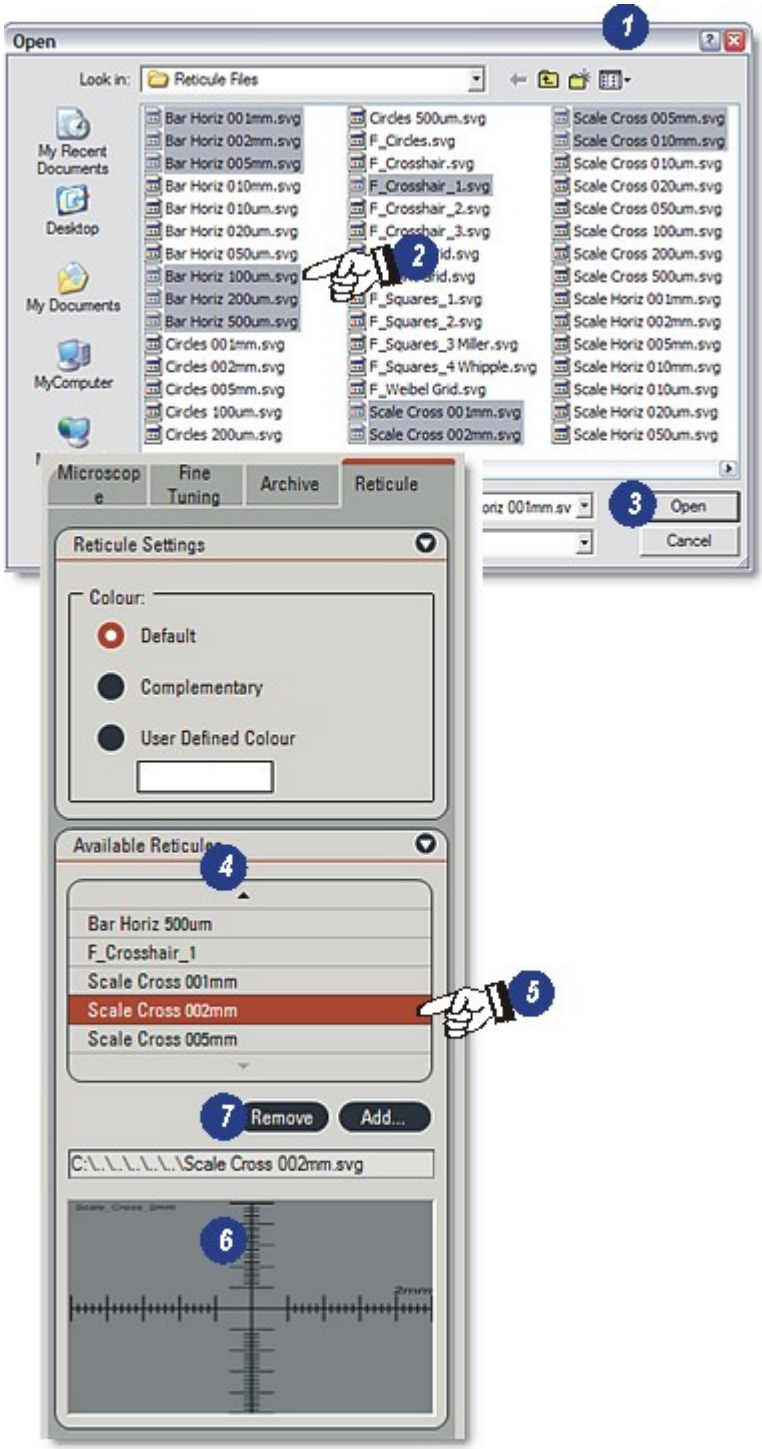
3: Click on the *Open* button.

4: The selected reticule files appear in the *Available Reticules* window.

5: Select an individual style by clicking its entry. It is highlighted and…

6: …displayed in the viewer.

7: To delete a reticule style, click to select it in the list (5) and then click the *Remove* button. If a style is inadvertently deleted it can be recovered from the library. *See previous page…* 975



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Fixed reticules are non-movable and not scalable. They are always centered in and displayed to fit within the image window - 'fit to window' where the window is the live image size.

For example, a crosshair displayed across the centre of the image window remains fixed and unchanged when the image is moved (by moving the camera or panning), or if the magnification is changed.

Examples here show:

1: *Weibel Reticule.*

2: *Squares Reticule* one of several variations and styles.

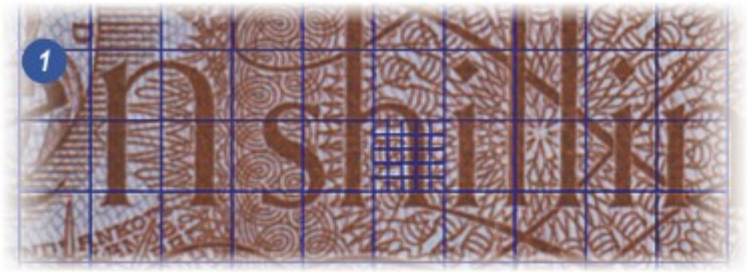
3: A *Point Grid Reticule.*

4: *Mertz Reticule.*

Fixed Reticules are defined in pixels.

Mertz, *Point* and Weibel are examples of stereological grids.

Continued... 978



Scalable Reticules have an absolute size associated with them and are defined in physical units for example millimetres. They can be positioned (moved) and rotated by the user; but cannot be re-sized manually.

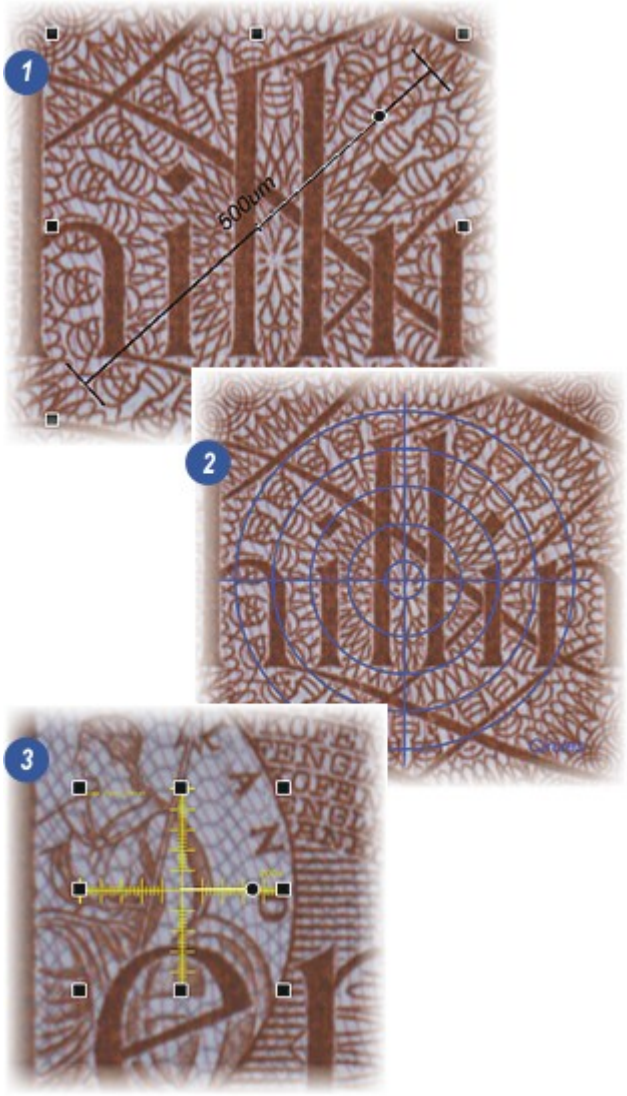
If the image is moved or zoomed, the centre of a *Scalable Reticule* will remain over the feature in the image where the user positions it and it will re-size to be correctly scaled to the image. The scaling information is obtained by reading it automatically from the connected microscope. So, for example, a rectangular reticule placed over a feature will *remain* centred over the feature and scaled to the image when the image zoomed in or out.

Scalable Reticule examples are:

1: Horizontal Bar 500µm

2: Circles 500µm

3: Cross 200µm.



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There are three colour options for the reticule which are selected on the *Reticule Settings* panel:

Default: Displays the reticule in the colour defined by the Reticule definition file.

Complementary: Automatically determines the display colour based upon the average hue of the image to maintain a good contrast.

User Defined: Allows the user to select the display colour as follows:

1: Click to enable the *User Defined Colour* radio button.

2: Click in the *Selected Colour* window and…

3: …the Windows *Select Color* dialog appears. Choose a colour from the wheel by clicking and holding the 'target' and dragging it to the desired colour, or clicking to select a *Basic Color* in the matrix. Precise colour selection can be made by typing the red, green and blue (RGB) values in the text boxes, and a colour can be saved by clicking the *Add to custom* button.

4: Click *OK* and the selected colour appears on the *Reticule Settings* panel **(5).**

The reticule style to be used is selected on the *Acquire Workflow*:

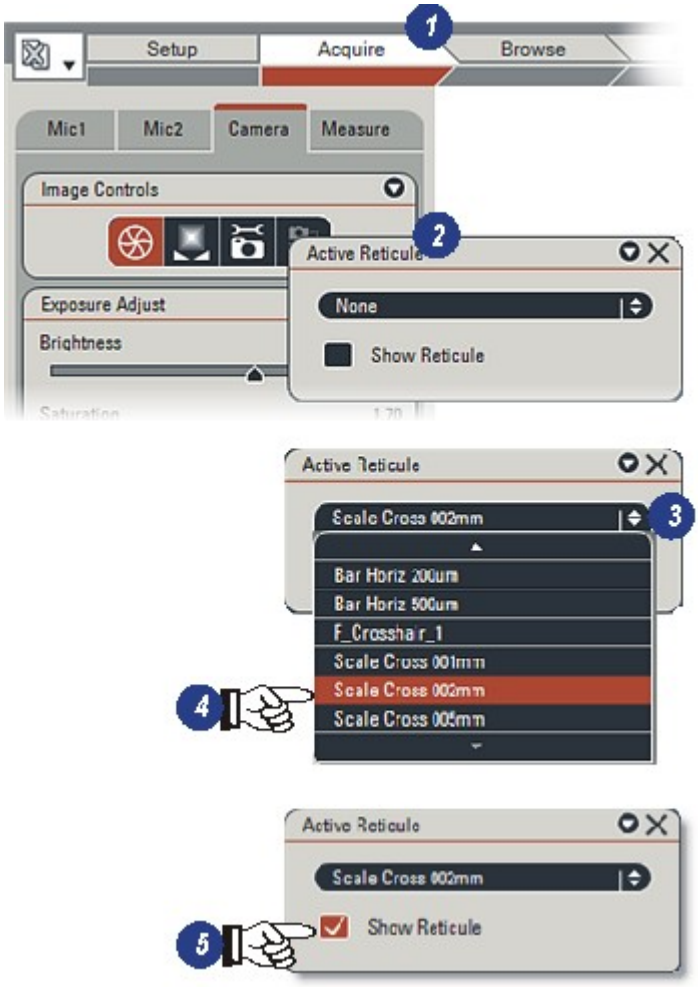
1: Click to select the *Acquire* Workflow and the *Camera* tab.

2: The *Active Reticule* control panel selects the desired reticule and determines if it is to be displayed or not. Like most other control panels, it can be moved to any part of the screen by clicking and holding on the header bar and dragging it to the required position. Return it to the normal location by clicking the 'X' on the header.

3: Click on the small arrow to the right of the *Active Reticule* list box and all of the reticule styles attached to the module appear.

4: Click to select the style required.

5: Click to enable the *Show Reticule* checkbox and the reticule will appear on the live image.



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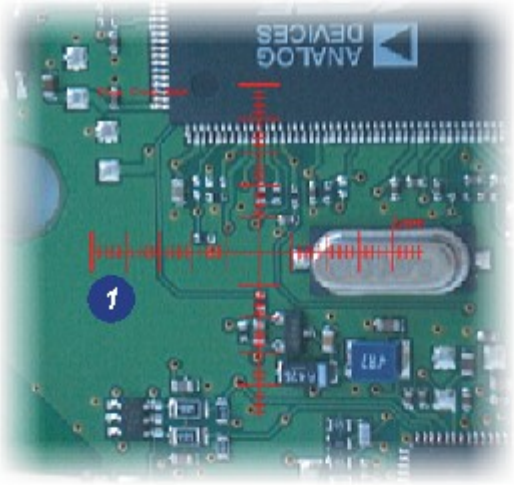
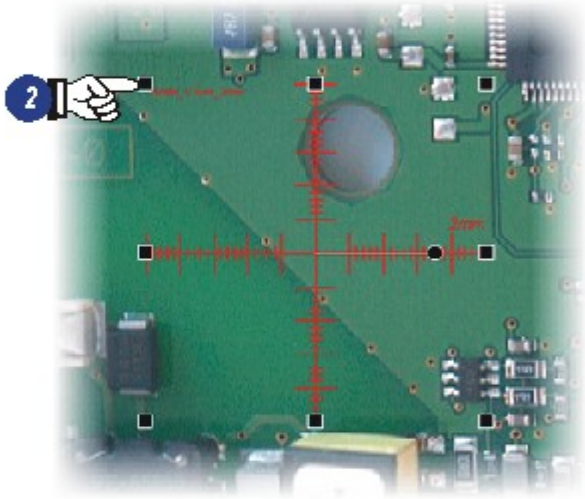
1: The selected reticule in the chosen colour on the live image.

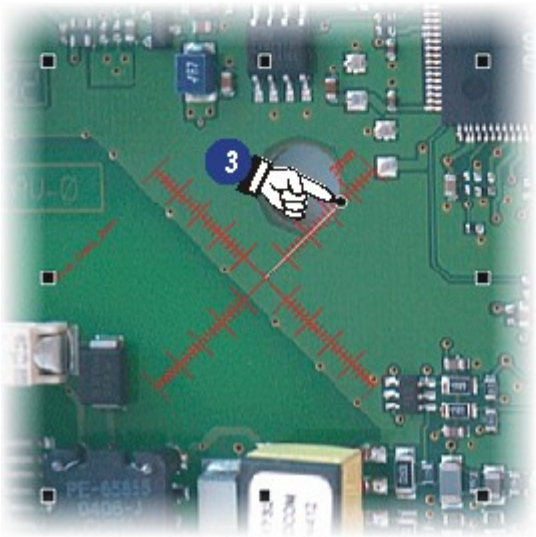
2: Move the reticule by clicking on it (8 'handles' appear) and dragging it to the desired location.

3: A *Scalable Reticule* can be rotated to any angle by clicking, holding and dragging on the *Rotate* 'handle'.

Images captured from the *Acquire Workflow* by either clicking on the *Acquire Image* button or pressing function key F3 on the keyboard, will merge the reticule with the image. To capture without the reticule, either switch it off with the *Show Reticule* checkbox or make the capture from the *Browse Workflow*.





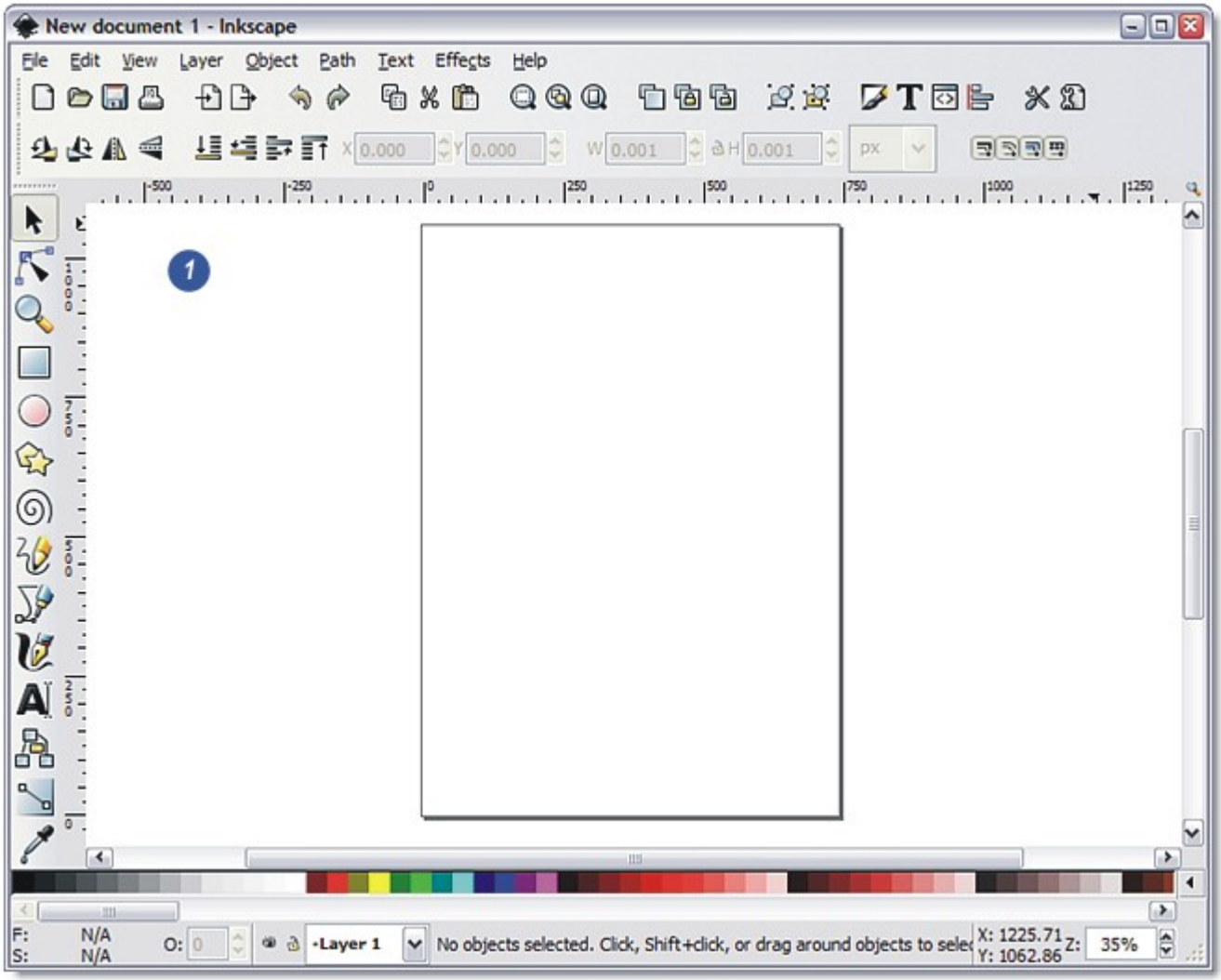


There are several vector graphic drawing packages available that are capable of creating svg *Reticule* files. An open source application - download and use the program free of charge but do not use it for commercial gain - called Inkscape (Google for the latest releases), will produce *Reticules* to import into LAS.

This and the following page is a general guide; Open source software is constantly changing and improving so some experimentation will be necessary.

- Download and install *Inkscape*.
- Run the program.
- From the *File* option, select *Document Properties* and set the default units to pixels (px), the *Canvas Size* to *Custom 640 x 640* (adjust as necessary to suit the LAS *Viewer* size and camera)
- Click *Fit Page to Selection.*

Continued... 983



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- Select a shape from the toolbar and draw it on the canvas area. The shape must not extend beyond the canvas.
- Use the various tools and dialogs to change line weights and colours.
- Save the drawing using the navigation dialogs and the Plain svg format.
- Open the file in a simple text editor - Notepad or Wordpad. Find the lines that specify:

width="680px" and height="680px"

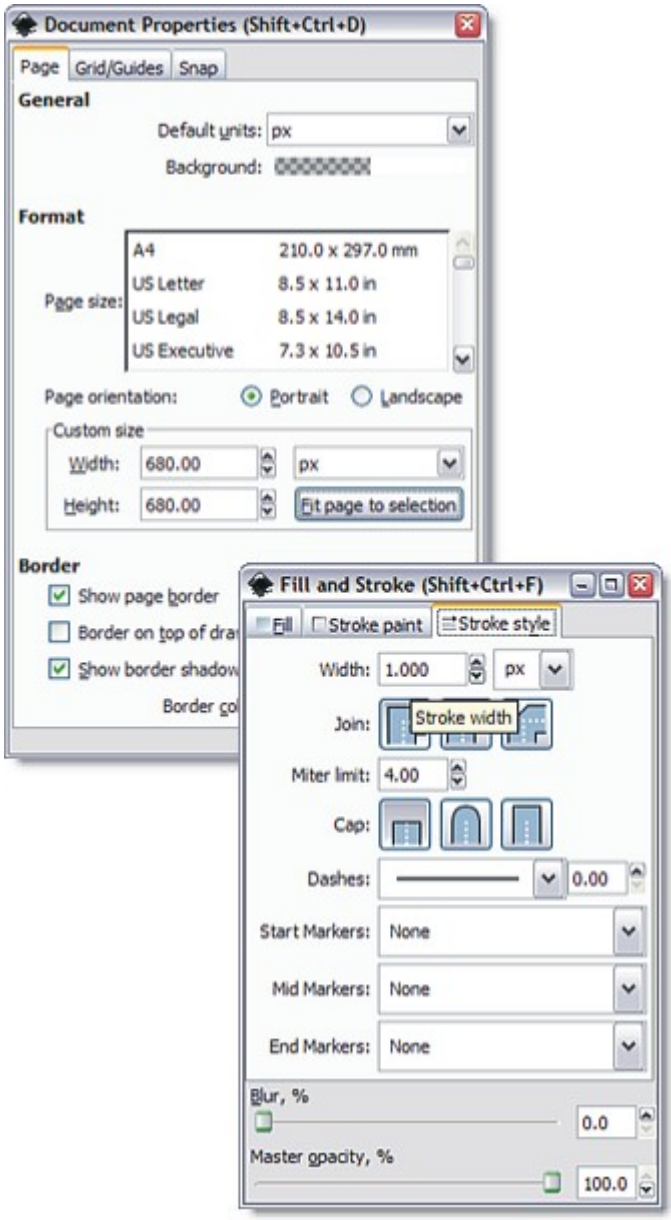
...and make sure the 'px' suffix is present. LAS needs these units. Save the file which will be a fixed reticule.

- To create a *Scalable Reticule* with the file open in the text editor and replace the width and height lines with:

width="0.2mm" height="0.2mm" viewBox="0 0 680 680"

...where 0.2mm is the scale required. Do not change the pixel size. Save the file.

- Launch LAS and import the 'personalised' *Reticule* in the normal way: *Go there...* 975



To determine just how *Reticule* files are constructed, open a *Reticule Library* file in Notepad and check that newly created files have the same structure.

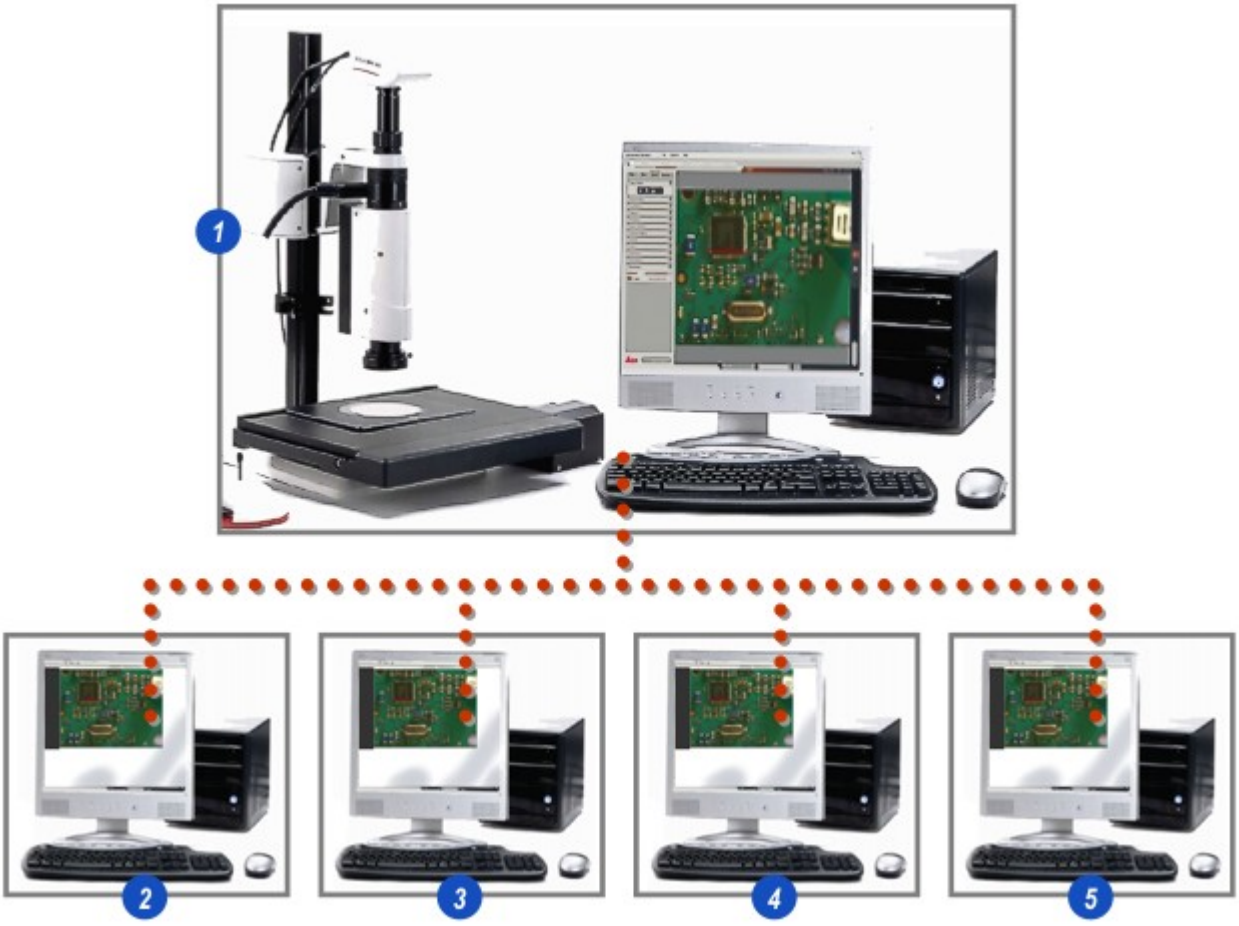
Web Sharing

Leica Application Suite Web Sharing module streams live images across a local network so that they can be seen in real time by other users.

In the illustration, the *Master PC* **(1)** running Leica Application Suite with Web Sharing installed and enabled, streams the microscope image to the local network. Other users – *Clients* – on the network view the image in real time using only free software, *Internet Explorer* with the *Silverlight* plugin. On the illustration the Clients are numbered **2** through to **5** but almost any number of clients can view the image simultaneously.

Web Sharing is an optional module, free to evaluate for 60 days; After that a chargeable license is required.

- Suitable for Local Area or Virtual Private networks, wired or wireless.
- Only the Master PC needs Leica Application Suite and the Web Sharing software.
- No special hardware required - only a standard DFC camera on the Master microscope.
- Other users – Clients – need only Internet Explorer with Microsoft Silverlight plugin to view the images.
- With the appropriate projection hardware, a Client can be used for large screen viewing in lecture halls and seminars.
- Three image size options – 640 x 480, 800 x 600 and 1024 x 768 pixels.
- Clients can see Scale Bar and Master Cursor movements, especially useful for pointing to areas of interest.
- Image Capture feature for Clients so that they can have a permanent, printable record on their own computer.



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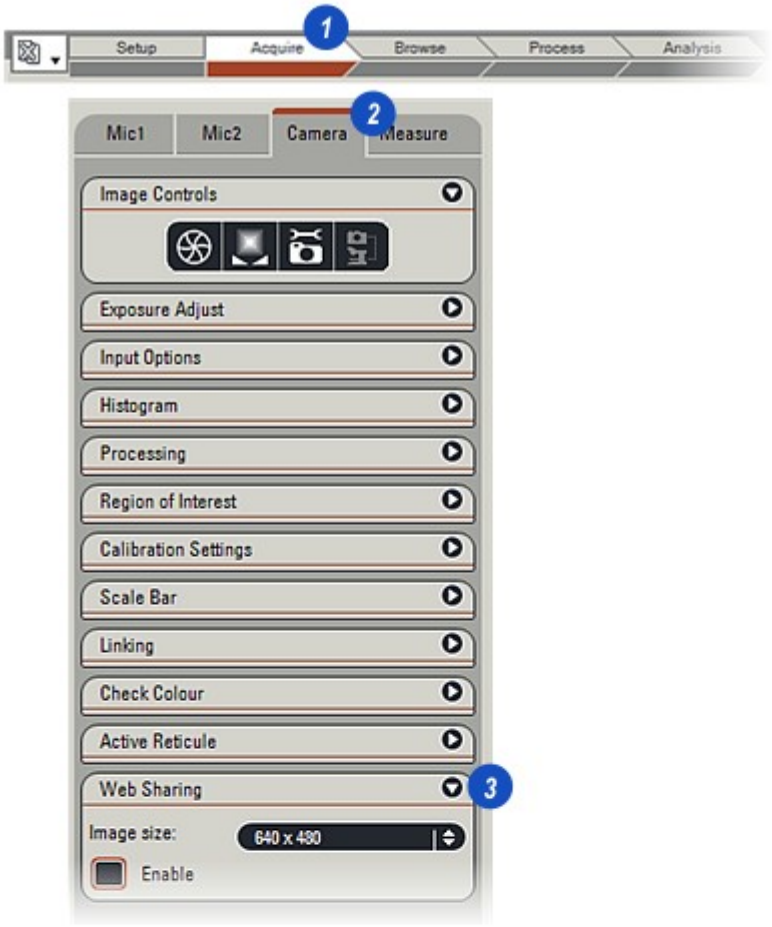
The Web Sharing launch panel is on the *Camera* tab of the Acquire Workflow.

1: Click on the *Acquire* Workflow.

2: If necessary, click on the *Camera* tab.

3: Click on the small arrow to the right of the *Web Sharing* header to expand the panel.

Continued… 986



Both the *Cursor* (and *Scale Bar* if it is enabled) on the Master monitor are available to the Clients.

If the Master Cursor is moved - to point out a particular feature for example - the movement will be reflected on the Client monitors. If the Scale Bar is moved to a new position, this too will change position on the Client display.

If it is intended that a Scale Bar is visible:

1: Click the small arrow to the right of the *Calibration Settings* header and…

2: …also on the *Scale Bar* header to access the settings and in particular to enable the Scale Bar by clicking the *Show* button **(3)** to reveal the Scale Bar **(4)**.

When Web Sharing is active, most of the other LAS functions are disabled to ensure rapid refresh at the Client monitors, so the Scale Bar setup can only be carried out with Web Sharing disabled.

Scale Bar setup details: *Go there…*

Calibration: Process > Calibration: *Go there...* 268

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The Camera Settings for both Live and Captured Formats, affect the speed at which Web Sharing images are downloaded to the Client monitors.
The Live Format affects the refresh rate and the Captured Format the download speed if the Client decides to save the image. Images saved at a Client machine will have the same resolution as the Master Camera Captured Format.

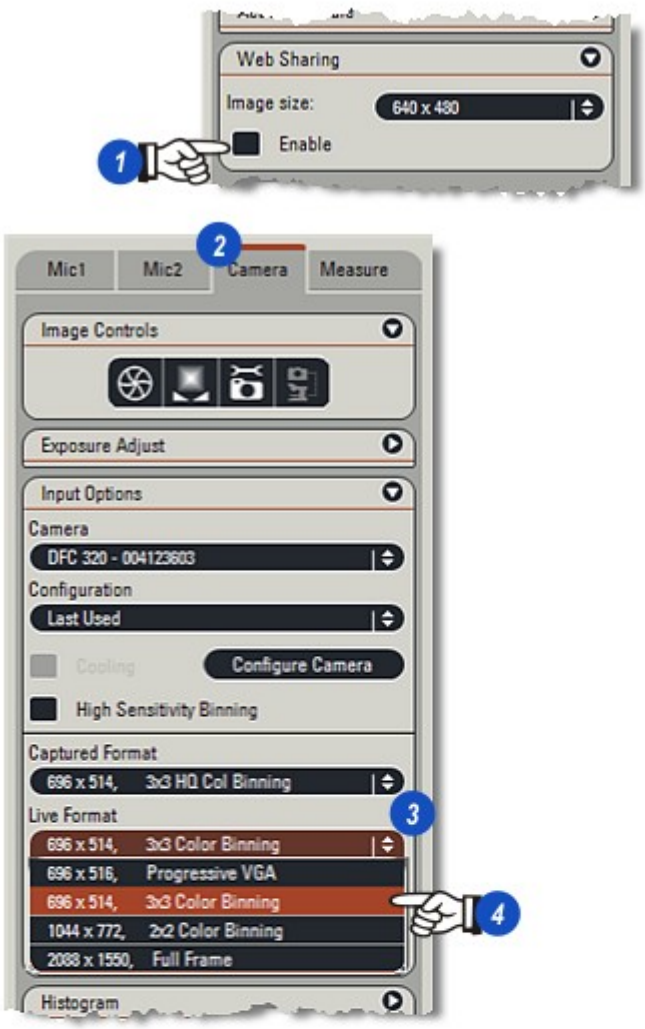
Before enabling Web Sharing check the Camera Formats; Start with a fairly low resolution – *696 x 514: 3 x 3 Color Binning* – for example, and increase the resolution if the refresh rate allows or more detail is required at the Client monitors.

1: Make sure that Web Sharing is disabled.

2: Click on the *Camera* tab on the *Acquire Workflow.*

3: Click on the small arrows to the right of the *Captured* and *Live Format* headers and…

4: …from the drop down menus choose one of the lower settings.



More detail about Camera Formats: *Go there...* 239

Continued… 991

Sharing Stored Images:

Usually, Web Sharing streams the current live image, but it is possible to temporarily disable the camera and instead stream images that have previously been captured.

The compression type of the stored image does not matter, but the resolution should be at least 1024 x 768.

The first step is to disable the camera and replace it with the Demo Camera loaded automatically with LAS Version 3.0 onwards. If Leica Application Suite is running, close it down.

1: On the *Desktop Task Bar* click on the *Start* button. The illustrations show Windows XP layout but Vista is very similar.

2: Click on the *All Programs* arrow.

3: Move the cursor to highlight *Leica Application Suite* in the list of programs.

4: On the sub-menu move the cursor to *License LAS* and click the option.

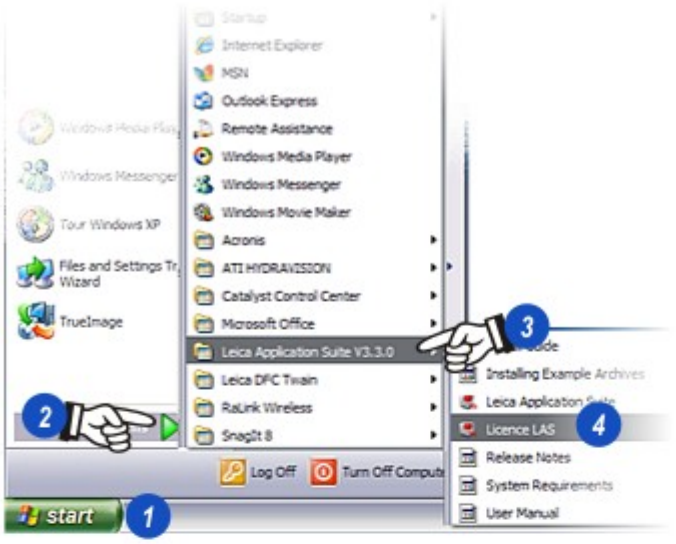
5: The *LAS Framework* will load but instead of launching the *User Interface* will stop when the *Main Menu* bar appears. Click on *Options*.

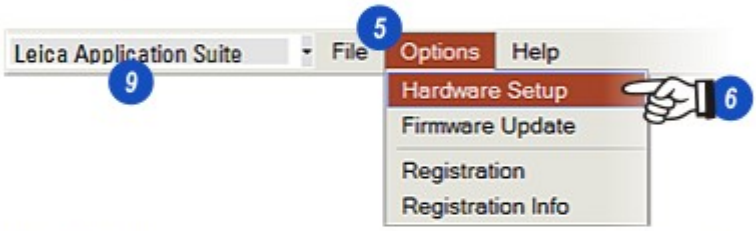
6: From the drop down menu click to select *Hardware Setup.*

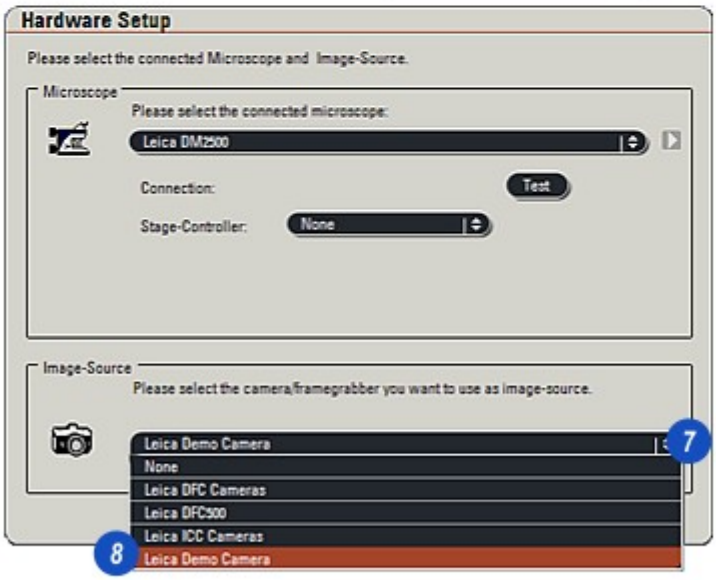
7: When the *Hardware Setup* dialog appears, click on the small arrows to the right of the Image Source header.

8: From the list of option click to select *Demo Camera* and then click *Save*.

9: Launch the program by clicking on the *Leica Application Suite* header.







Continued… 989

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With LAS running:

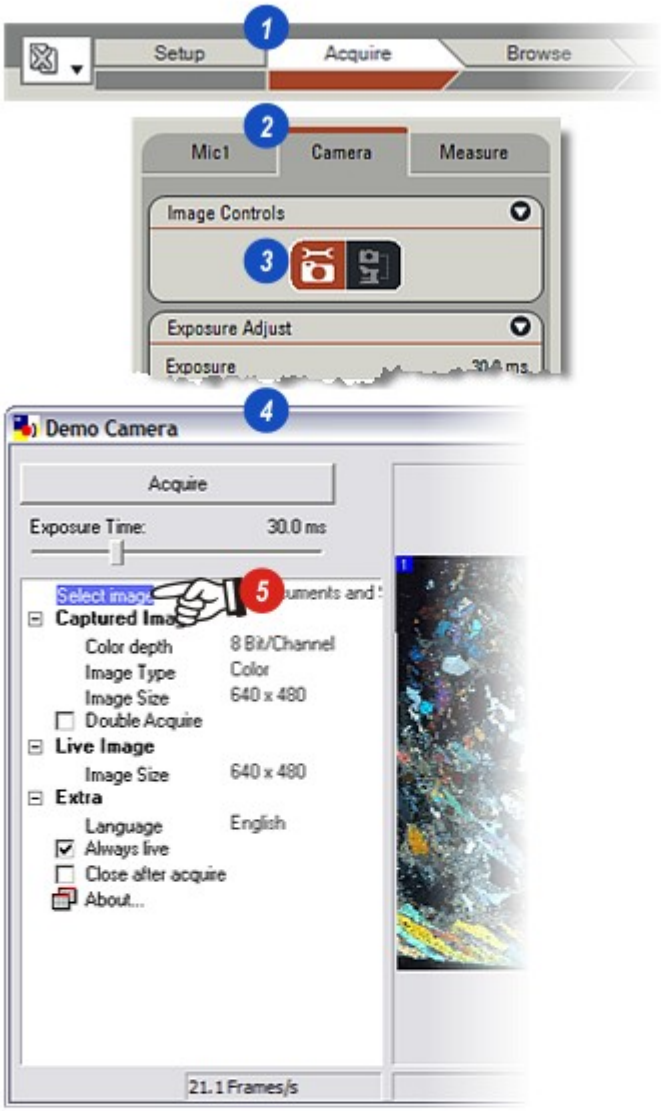
1: …click on the *Acquire Workflow*.

2: Click on the *Camera* tab to reveal the camera controls.

3: Click on the arrow to right of the *Image Controls* header to display the *Camera Twain* icon. Click it to open…

4: …the *Demo Camera* dialog.

5: Select the image to be shared by *right-clicking* the *Select image* caption.



Continued… 990

1: On the *Select Image File* dialog navigate to the folder that contains the image to be shared and…

2: …click the arrow to the right of the *Files of Type* window and chose the file compression type from the list by clicking it.

3: Click to select the image.

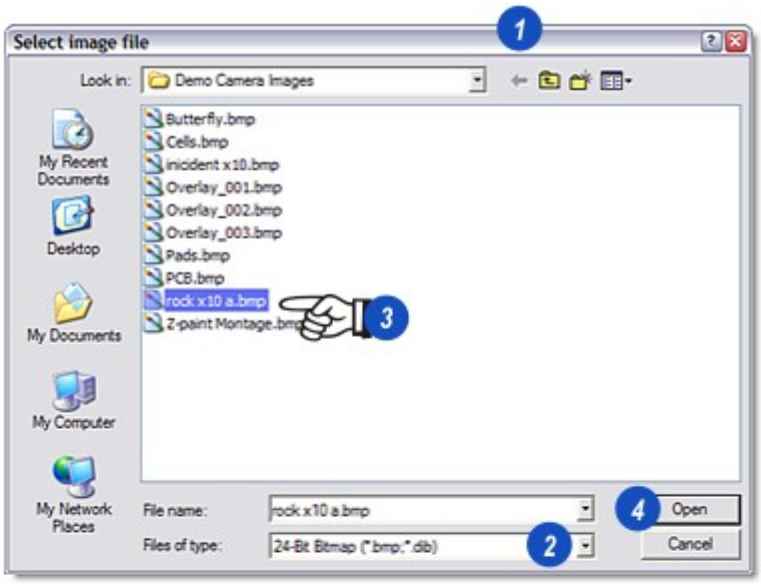
4: Click *Open*.

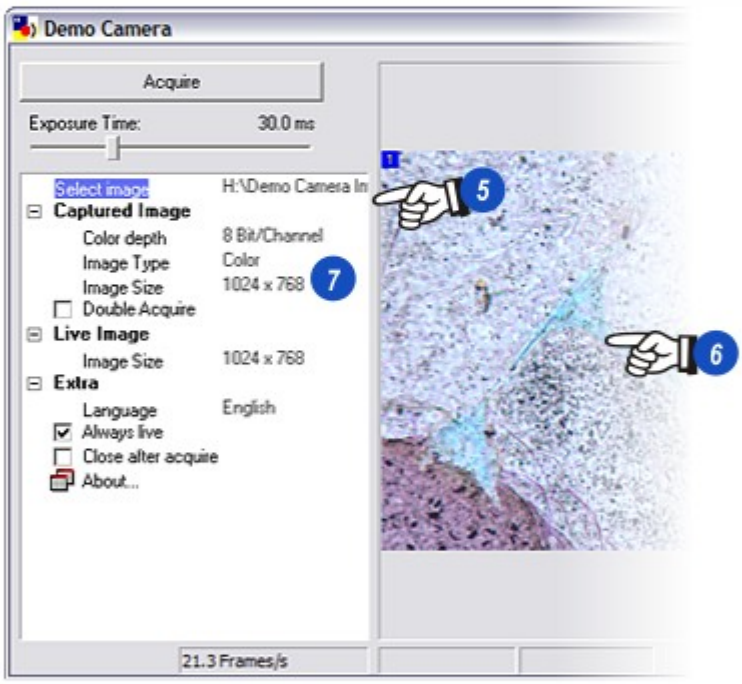
5: The *Demo Camera* dialog re-appears with the path of the chosen image and…

6: …the image itself displayed.

7: The image resolution is display under the *Captured Image* details - it should be at least 1024 x 768.

Close the Demo Camera dialog (small X top right of the window) and follow the normal Web Sharing procedure from here. *Go there...* 991





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Web Sharing: Select Image Size and Enable:

1: If necessary, expand the *Web Sharing* panel by clicking on the small arrow to the right of the header.

2: Web Sharing has three size options – that is the size that the clients will see on their monitors. Larger sizes are slower to download so the refresh rate at the client will also be slower. The actual refresh rate depends upon the network hardware and the number of active users.

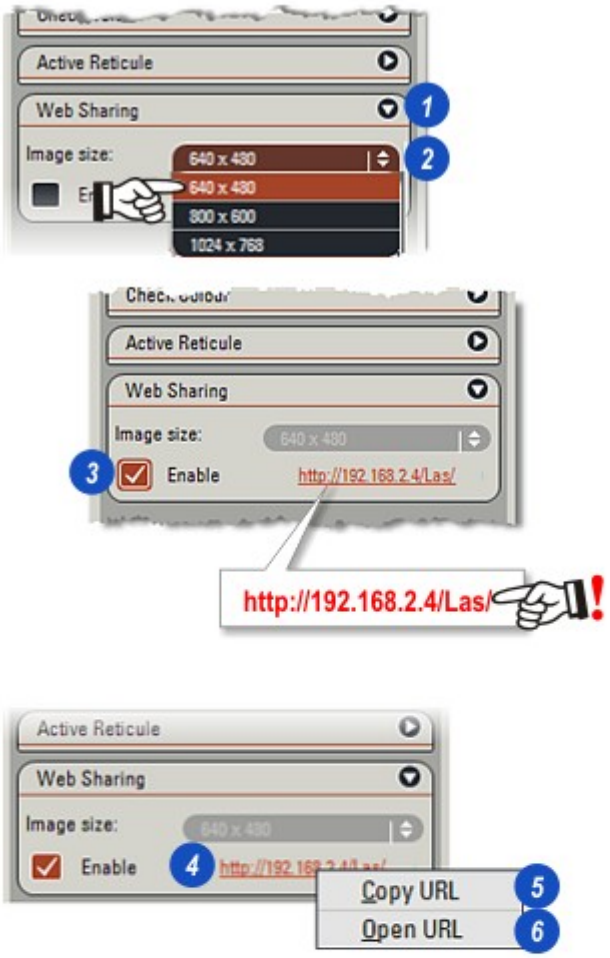
Check that Web Sharing is disabled – the *Enable* checkbox is blank – and click the arrows to the right of the header and from the drop down list click to select the required size.

3: Click to enable *Web Sharing*.

4: When the software successfully loads the *Master Address* appears on the panel. The Address is unique to the Master but can change every time LAS is started. Right click on the Address to reveal options to:

5: *Copy* the Address *(URL)* to the clipboard from where it can be saved or attached to an e-mail for example, or…

6: …*Open* Address *(URL)* to test the network connection by sending the image back to the Master for display.



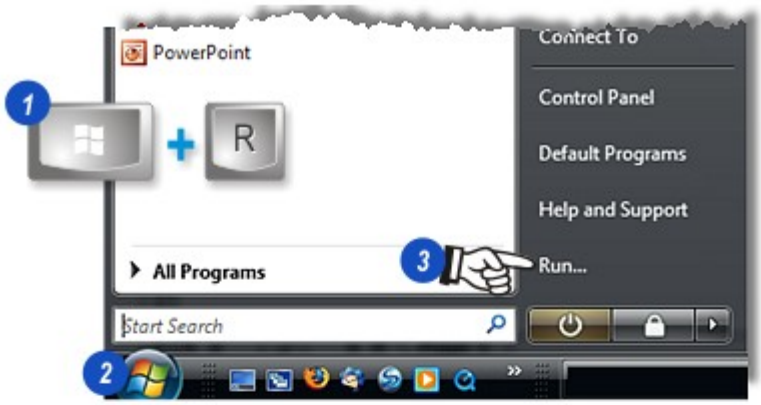
Continued… 992

A client can check if he is connected to the Master by sending the Master Address and waiting to see if there is a response. The process called 'pinging' is carried out at the Command level.

1: Hold down the keyboard *Windows* key and at the same time press the *R* key or…

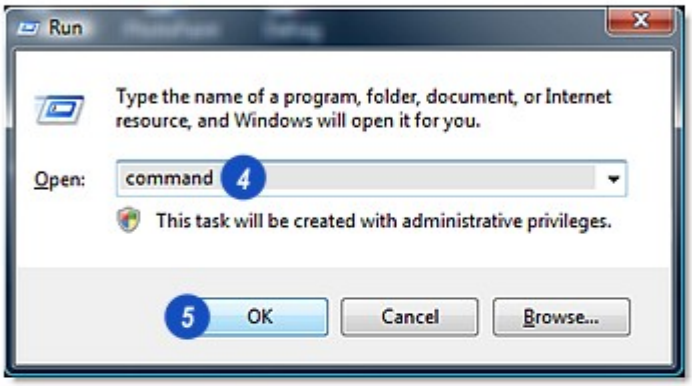
2: …click on the *Start* button and from the Start Menu…

3:… click on the *Run* option.



4: On the Run dialog type the word *'command'* in the text box and…

5: …click *OK.*



6: In the Command Window against the > prompt type *'ping'* and then the numeric part of the master Address – in the example 192.168.2.4. Do not include the *http* or the */Las/* parts of the string.

7: If connection is made successfully the details will be displayed in the window. An unsuccessful connection will report *'Address not found'*.

Continued… 993

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Clients do not need LAS or Web Sharing software to receive images; All that is required is *Microsoft Internet Explorer* Version 6, 7 or 8 with the *Microsoft Silverlight* plugin. Currently (January 2009) no other browser supports Silverlight.

1: Launch *Internet Explorer* either from a desktop shortcut or from Program Files.

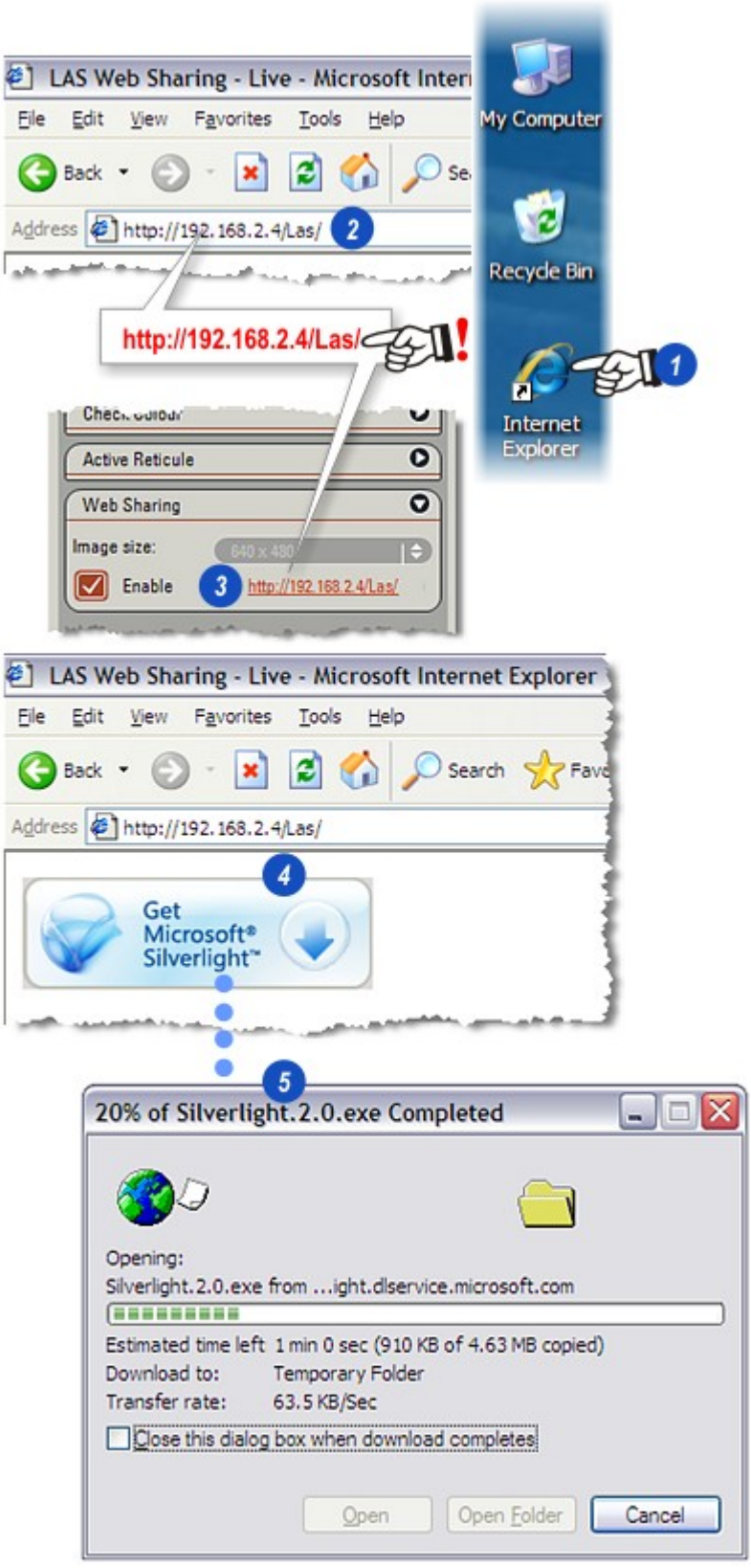
2: The illustration shows Internet Explorer 6 with the entire address string supplied by the Master *Web Sharing* module **(3)** typed (or copied) into the address text box. Ensure that the last forward slash (*/*) is included.

4: If Silverlight is not installed the link to Microsoft Silverlight appears. Click on the arrow to…

5: …download the plugin (4.6Mbytes) and follow the onscreen instructions to install it.

Silverlight is automatically installed with Internet Explorer versions 7 and 8.

Continued… 994



1: The illustration shows Internet Explorer Version 7 with the live image from the Master in the *Web Sharing* window.

2: The *Connection Status* – either buffering or connected - is shown on the side panel with…

3: …and indicator in the top right hand corner – yellow whilst buffering and green when connected.

4: The Master Cursor will appear if it is within the LAS Viewer area and also the Scale Bar if it is enabled. If the Master user moves the Cursor or the Scale Bar the new positions are displayed at the Client display.

Continued… 995

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If connection is temporarily lost or the Master goes 'off line' to change images:

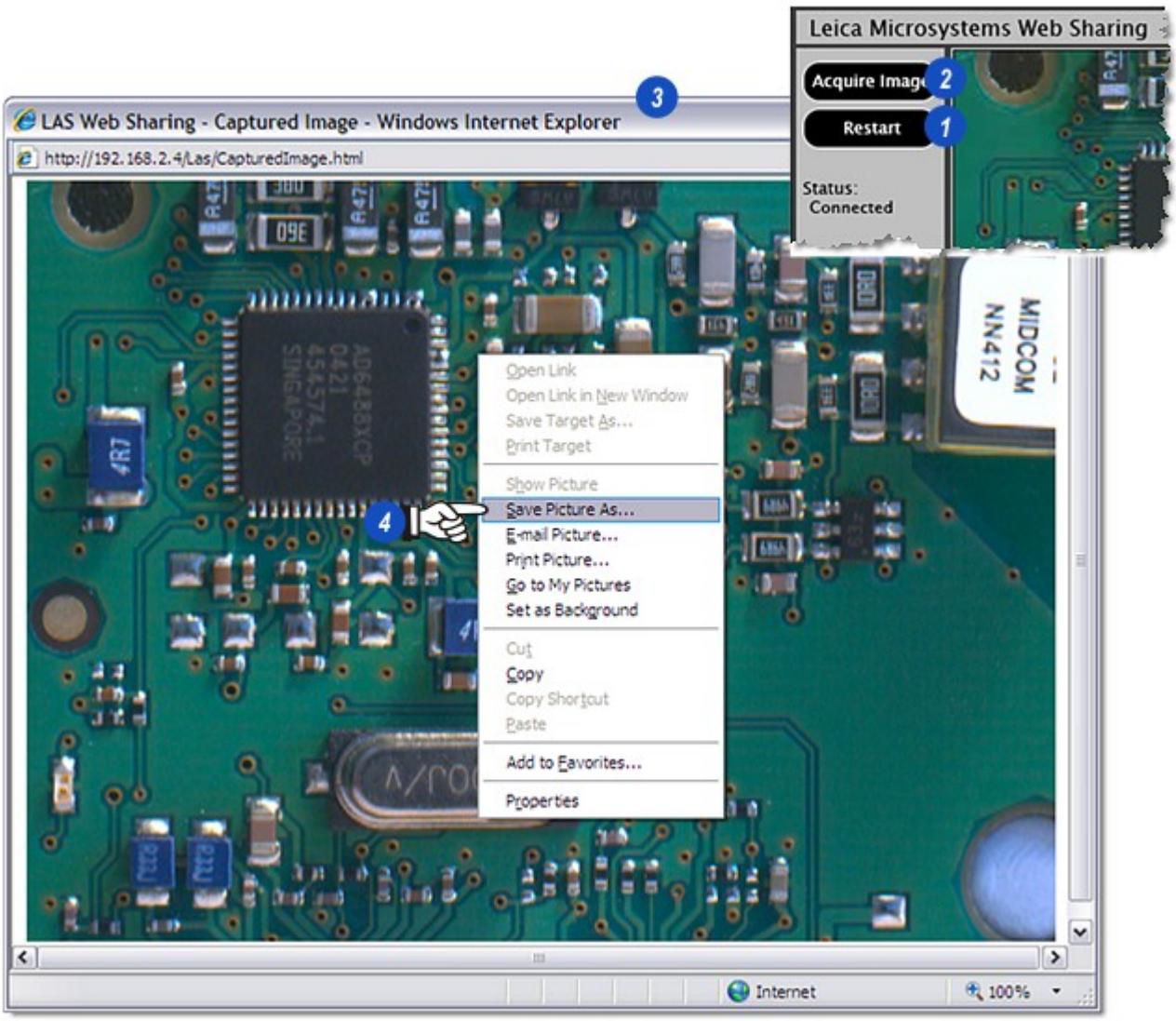
1: …click *Restart* to refresh the connection and current image.

2: To copy the image click the *Acquire Image* button and…

3: …when a new *Captured Image* window appears…

4: …right click on the image and from the menu click to select *Save Picture As…*

Continued… 996

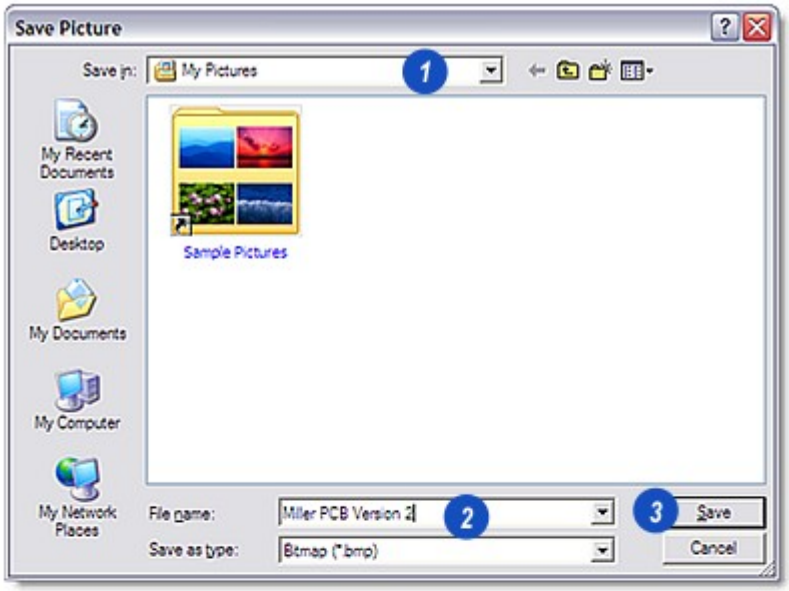


1: On the *Save Picture* dialog, navigate to the folder in which to save the image.

2: Type a name for the image and…

3: Click on the *Save* button.

Images are saved only in the Windows bmp format.



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This is an optional module for DM Motorised microscopes. It has to be installed and licensed before it can be used.

Using *Multi-User Package*, each user of the microscope can create unique profiles and store them for retrieval later. A profile comprises the microscope hardware set up together with individual settings such as the light source and intensity for a specific combination of objective and contrast method.

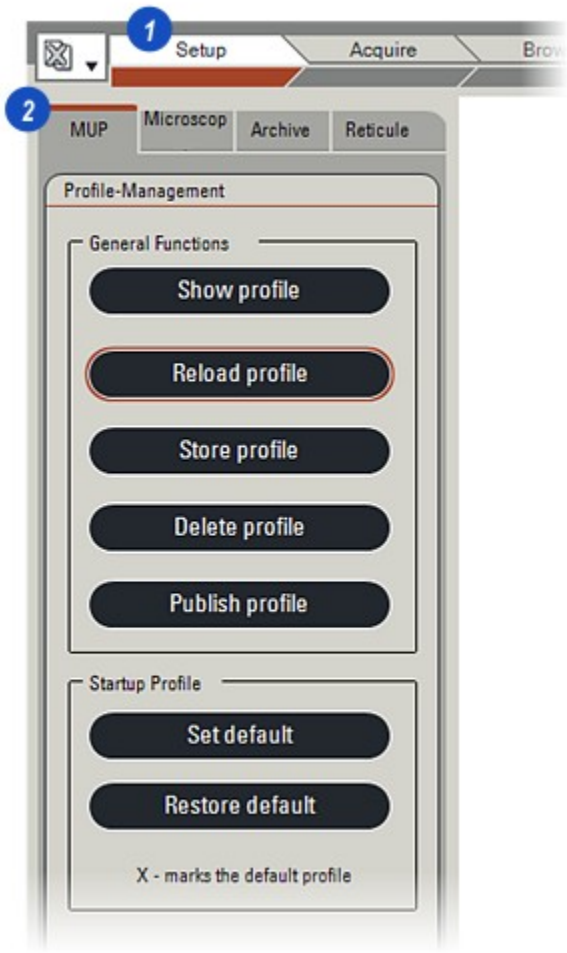
Both Administrators and Standard Users can create profiles but Administrators have an added facility in that they can make their profiles 'public' and available to all other microscope users. Standard Users can access only their own profiles.

Both Administrators and Standard Users can nominate a *Default Profile* from their Profile List that is automatically loaded when the microscope is switched on. If a *Default* has not been selected then the last set up used is loaded.

The *Multi-User Package* is available by:

1: Clicking on the *Setup Workflow* and...

2: ...on the *MUP* tab.



Continued... 998

The Multi-User software recognises users by their Windows log-in so an Administrator or Standard User can be identified, the appropriate control panel displayed and access allowed to the proper *Profile List.*

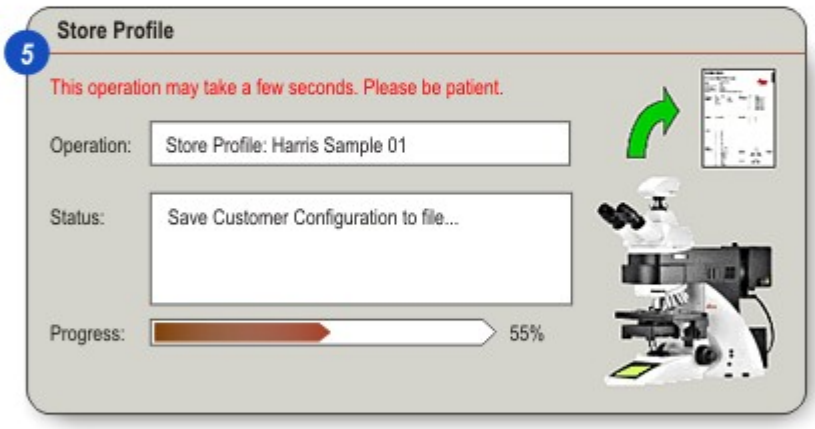
Standard Users:
The first time a Standard User opens the Multi-User Package an empty *Profile List* is created. After a task has been carried out on the microscope, the settings can be saved as a profile by clicking the *Store profile* button.

1: The *Store new profile* dialog appears.

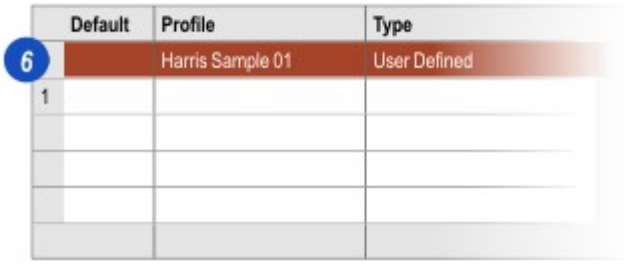
2: Click in the *Profile name* text box and type a unique name for the profile.

3: Click in the *Short description* text box and enter a relevant note about the settings.

4: Click *OK.*



5: The *Store Profile* message appears showing progress.



6: The profile is stored and appears in the *Profile List.* The *Type* entry, *User Defined* indicates that this profile is still private and owned by the user.

The user can then make changes to the microscope and store those as a separate profile which will also be added to the *List*. So, a 'library' of microscope settings are built up that can be re-loaded at will, saving the users time and guaranteeing consistency.

Continued... 999

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Reload a profile:
The microscope settings stored in a profile can be quickly re-loaded by:

1: Click on the required profile in the *Profile List.*

2: Click the *Reload profile* button.

3: The *Reload* progress indicator appears - there are usually sounds from the microscope as well if objectives, filters and focus are being changed.

Delete a profile:
To remove a profile from the List:

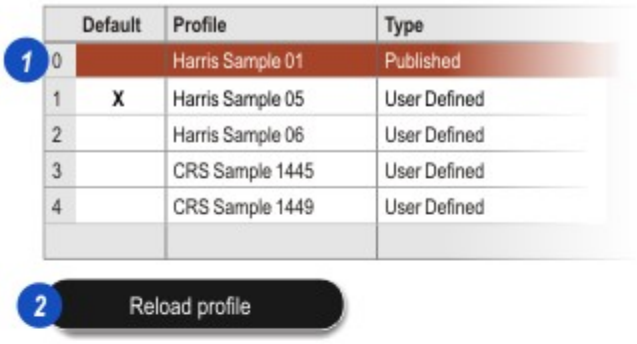
1: Click on the profile to be deleted in the *List*.

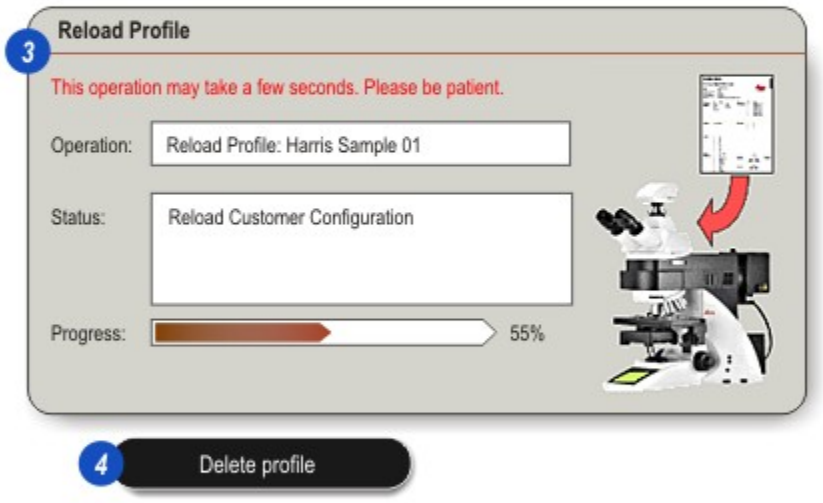
4: Click the *Delete profile* button.

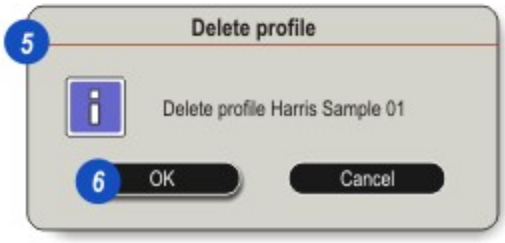
5: Confirm or cancel the deletion on the dialog.

6: Click *OK*. The deletion cannot be reversed.

Continued...







To select a profile that will be used as the *Default* switch on settings:

1: Click in the *Profile List* to select the profile to be used as *Default*.

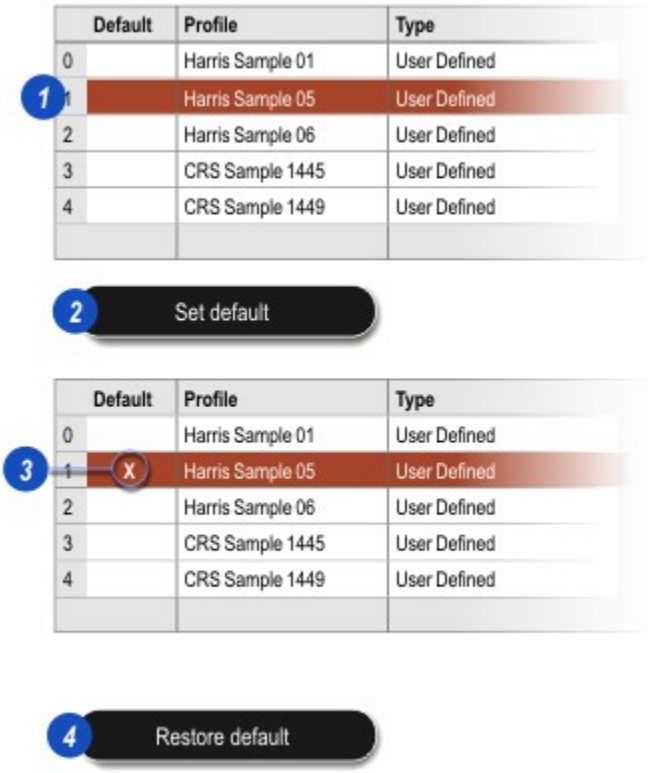
2: Click the *Set default* button.

3: An **'X'** appears in the *Default* column on the *Profile List*.

4: To remove the *Default* selector, click the *Restore default* button.

If a profile is not selected as the *Default*, the settings last used by the user will be loaded to the microscope at switch on.

Continued... 1001



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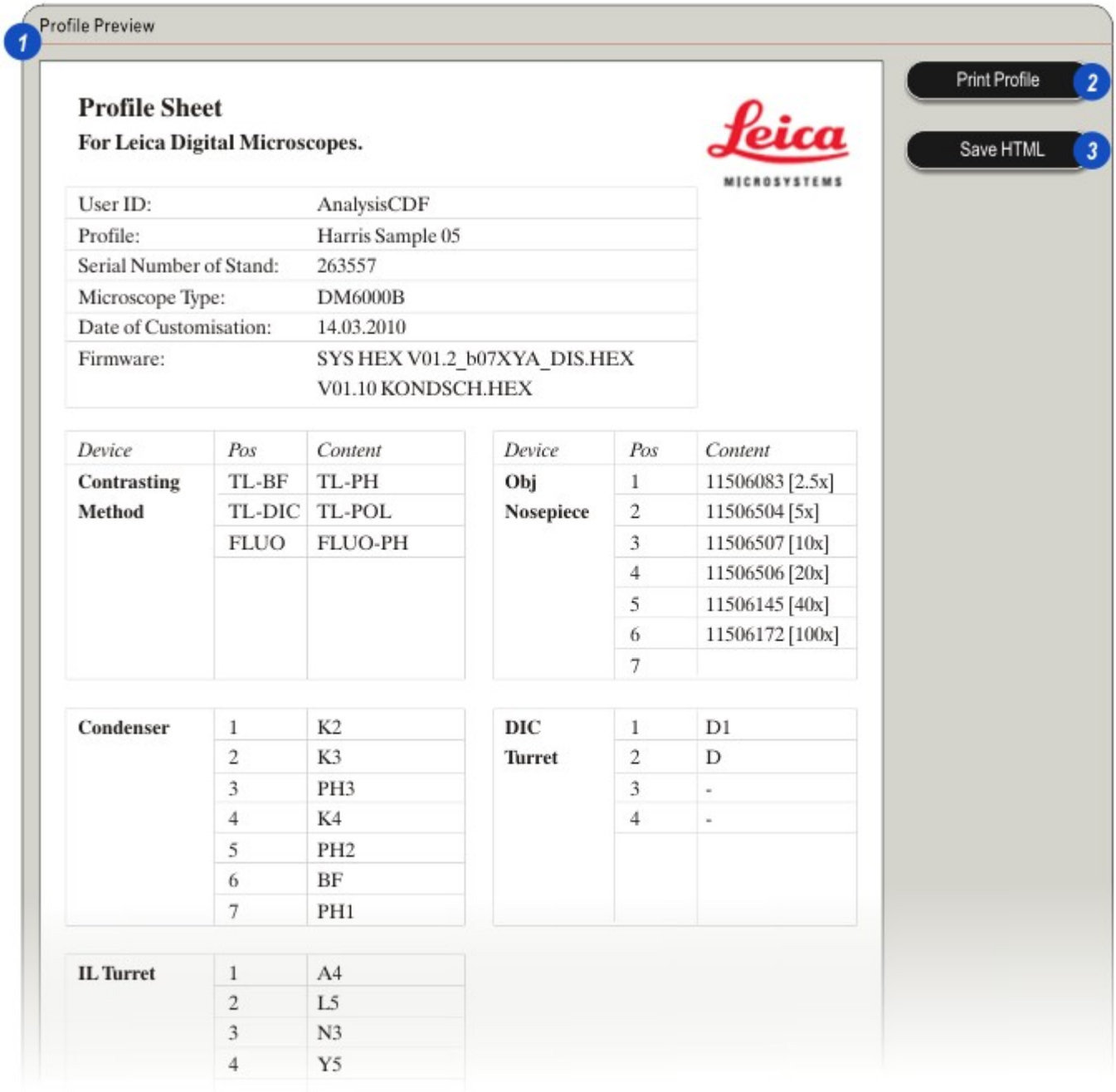
Profile settings can be displayed by clicking the *Show profile* button - the *Profile Preview* **(1)** appears.

2: Print the settings by clicking the *Print Profile* button.

Profile data is stored in HTML format in a folder created by Leica Application Suite. Copy the HTML to a destination of choice by clicking the *Save HTML* button **(3)** and then navigating to the required folder.

Continued... 1002



Profile Sheet

For Leica Digital Microscopes.

Leica MICROSYSTEMS

User ID:	AnalysisCDF
Profile:	Harris Sample 05
Serial Number of Stand:	263557
Microscope Type:	DM6000B
Date of Customisation:	14.03.2010
Firmware:	SYS HEX V01.2_b07XYA_DIS.HEX V01.10 KONDSCH.HEX

Device	*Pos*	*Content*
Contrasting Method	TL-BF	TL-PH
	TL-DIC	TL-POL
	FLUO	FLUO-PH

Device	*Pos*	*Content*
Obj Nosepiece	1	11506083 [2.5x]
	2	11506504 [5x]
	3	11506507 [10x]
	4	11506506 [20x]
	5	11506145 [40x]
	6	11506172 [100x]
	7	

Condenser	1	K2
	2	K3
	3	PH3
	4	K4
	5	PH2
	6	BF
	7	PH1

DIC Turret	1	D1
	2	D
	3	-
	4	-

IL Turret	1	A4
	2	L5
	3	N3
	4	Y5

Profile data is stored in HTML format in a folder created by Leica Application Suite.

The file can be copied to a folder of the users choice and from there can be distributed to other users, for example by e-mail. Copy the HTML to a destination of choice by:

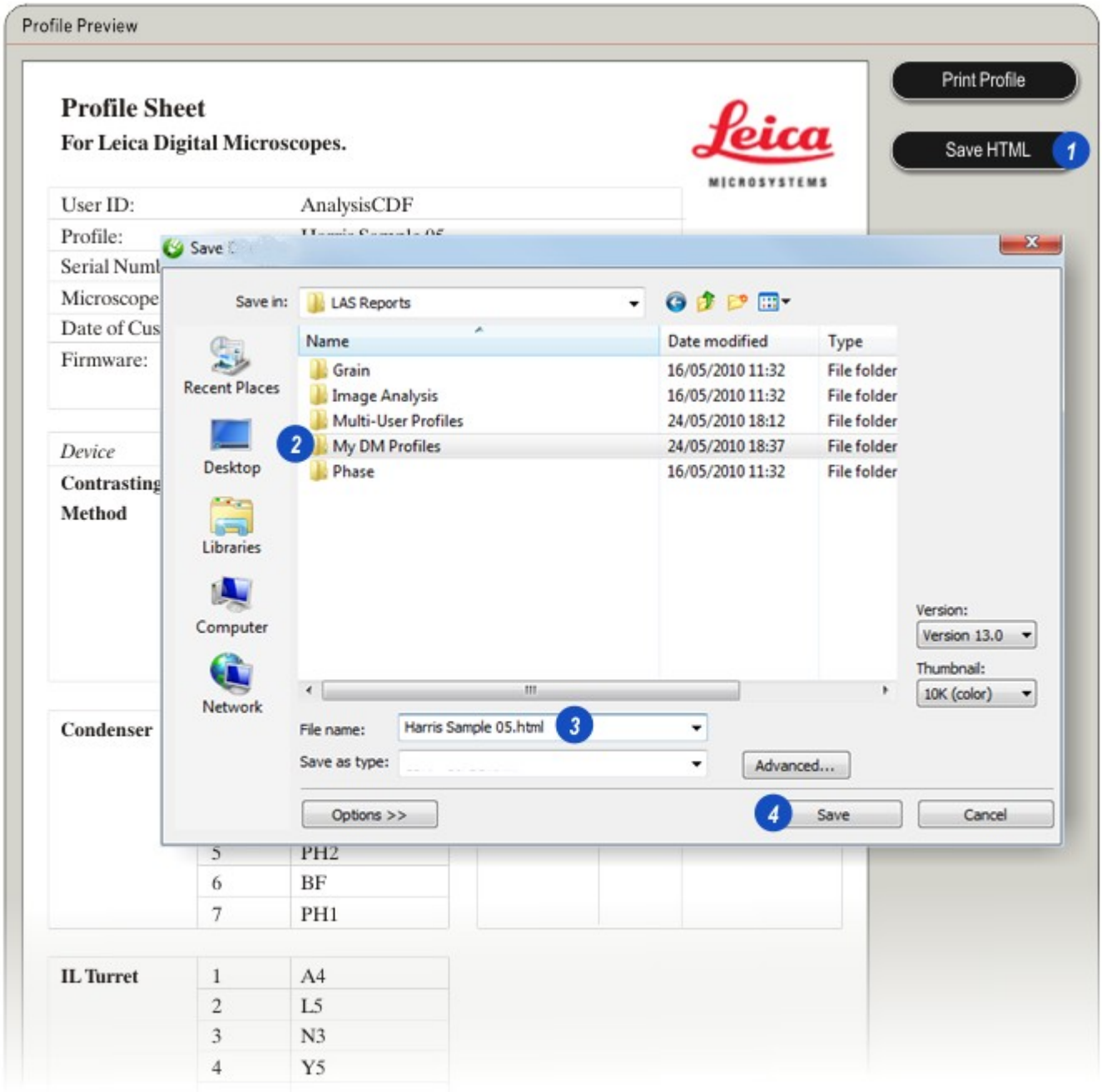
1: Clicking the *Save HTML* button...

2: ... and then navigating to the required folder.

3: Give the copy file a name and...

4: ...click *Save*.

Continued... 1003



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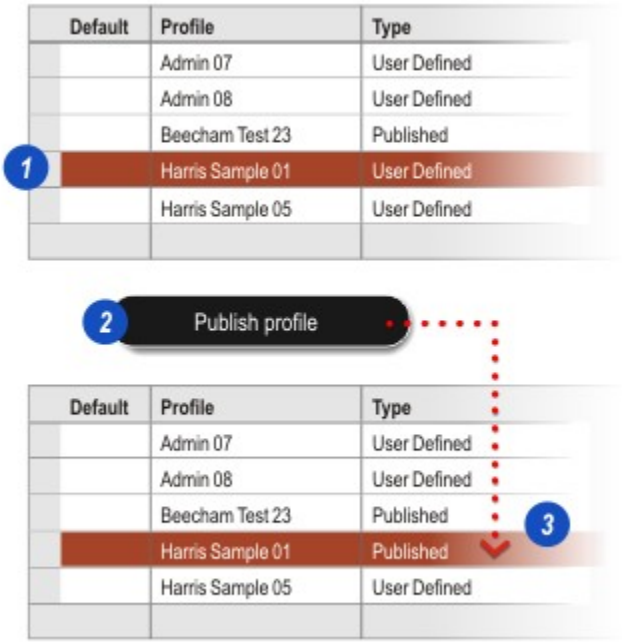
Administrators have the same tools as Standard Users plus the facility to publish any profile from their own list.

Publishing makes a profile available for sharing among all the other computer users.

1: From the *Administrator's Profile List* the profile to be published is clicked to select it.

2: Click the *Publish profile* button.

3: The profile becomes available to all users and is marked as such with the *'Published'* label.



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