Molecular Devices

ClonePix™ 2

SOFTWARE APPLICATIONS MANUAL
SOFTWARE RELEASE 1.2.80.1071



07MAN1180.A1
Effective Date: 30-Jan-11
ECO #: 3093

Contents

Overview **5**
Workflows **6**
Pick Run **6**
Batch Pick Run **7**
Imaging Run **8**
Start up the ClonePix 2 ..... **9**
ClonePix 2 Software **9**
Main Navigation Screen **10**
New Process tab 10
Templates tab 10
Recent Processes tab 10
Menu Options **10**
File Menu 10
View Menu 11
Tools Menu 11
Help Menu 11
ClonePix 2 Processes **12**
Prepare for Pick Run **12**
Pin Fire Test 12
Alignment 13
Check Wash Bath 15
Wash Bath Utility 15
Sanitize Pins in Sterilizing Agent 16
Ultra Violet Sanitize 16
Check Bottles 17
Sanitize Pins 17
Finish 17
Pick Run **18**
Modify Settings 19
Picking 23
Imaging Run **44**
Modify Settings 45
Start Imaging 46
Review Results **47**

Maintenance Processes **49**
Commissioning Process **49**
Utility Processes **49**
Picking Head Management **49**
Remove Head 49
Replace Head 49
Utility Process **51**
Plate Handling 51
Sanitize Pins 51
UV Sanitize 51
Wash Bath Utility 51
Replacement Parts and Optional Extras **52**
Replacement Parts **52**
Optional Extras **52**
Reagents and Supplies **53**
CloneDetect Detection Reagents **53**
CloneMatrix **54**
CloneMedia / XPMedia **54**
PetriWell Cell Culture Plates **55**
Other **57**
Appendix A: Imaging Definitions **58**
Statistics for Prime Configurations **58**
Appendix B: Imaging Definitions **60**
Statistics for Fluorophore Configurations **60**
Appendix C: Guide to Fluorophore Configurations **61**
Measuring Fluorescence **61**
Example of Fluorescent Statistic Generation **62**
Interior Intensity Statistics **62**
Exterior Intensity Statistics Calculation **63**
Exterior Intensity Statistics **64**
Appendix D: Licensing **65**
Requesting a license **65**
Installing a license **66**
Appendix E: Reviewing and Exporting Data **67**
Review Results **67**
Remote Data Viewer **67**

Results Report 67
Data Tracking Logs 68
Automatic Logs 68
Raw Data 69
Appendix F: Plate Compatibility Matrix 70
Plate Holders 70
Lid Lifter Assembly 71
Appendix G: Glossary of Terms 72
Index 75
Contact Details 77

Overview

ClonePix 2 software controls the process of selective mammalian cell picking by ClonePix 2. It has been designed to simplify the day-to-day interaction with the instrument, permitting multiple users to select and collect colonies of suspension cells from semi-solid medium or adherent colonies from liquid medium. The colony detection feature contains powerful algorithms for selecting cell colonies based on physical characteristics and proprietary multi-channel fluorescence technology for detecting protein secretion or production.

There are two software packages. ClonePix 2 software is required to run the instrument and ClonePix 2 Remote Data Viewer is available for accessing results remotely. Copies of the Remote Data Viewer are available. (Please contact Customer Support for further information).

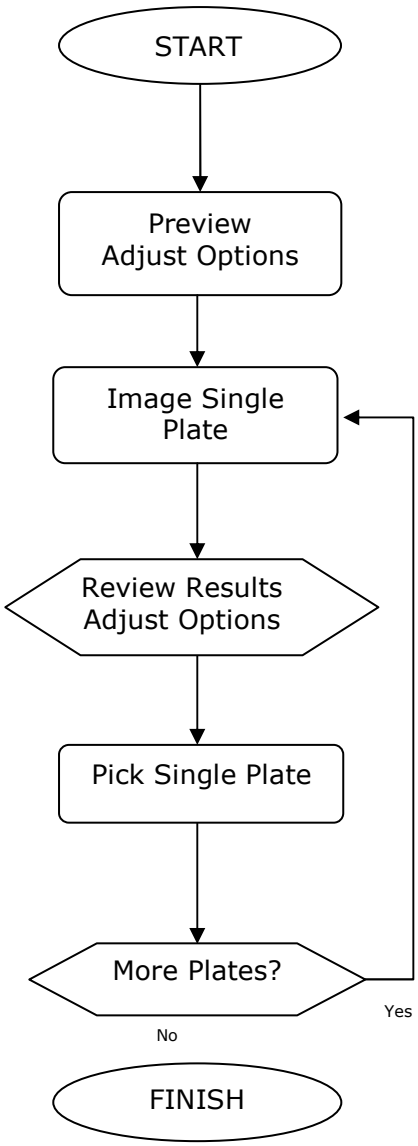
Workflows

There are three different workflow processes on ClonePix 2:

- Pick Run
- Batch Pick Run
- Imaging Run

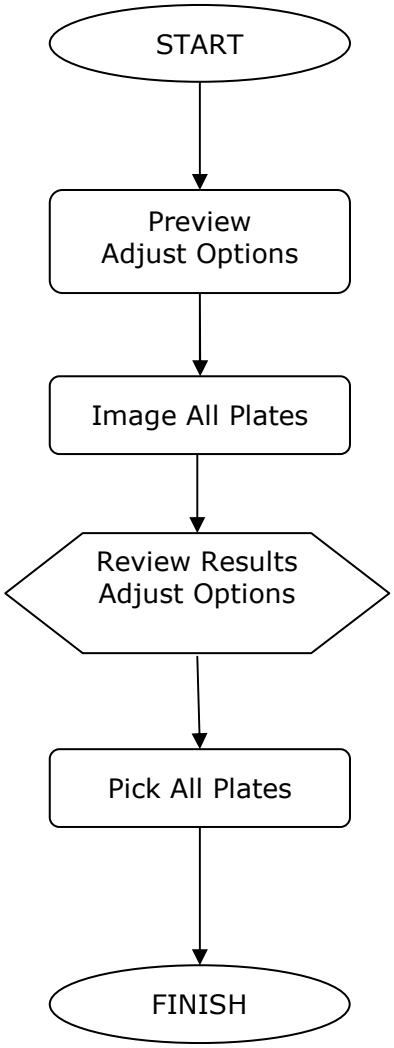
Pick Run

This process images and picks the first plate, then the second plate and so on, from however many plates the operator chooses.



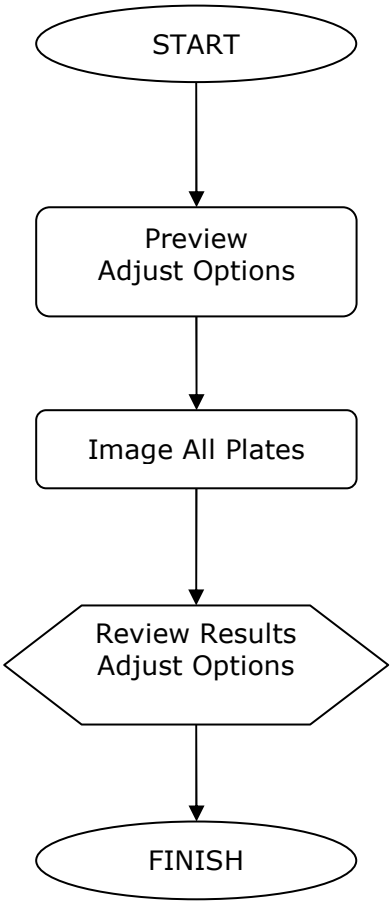
Batch Pick Run

This process images all plates in the cassette and aggregates statistics for all plates before the required clones are picked from the whole batch.



Imaging Run

This process images all plates without picking. Once the plates are imaged, the results can be opened on ClonePix 2 or loaded into the Remote Data Viewer (installed on another PC or laptop) and the images re-analyzed with different statistical criteria. This process is used to observe the status of plates during incubation, or to monitor growth and secretion over a period of time.



Start up the ClonePix 2

Before using the ClonePix 2, please refer to the ClonePix 2 Robot Manual for important setup, maintenance and safety information.

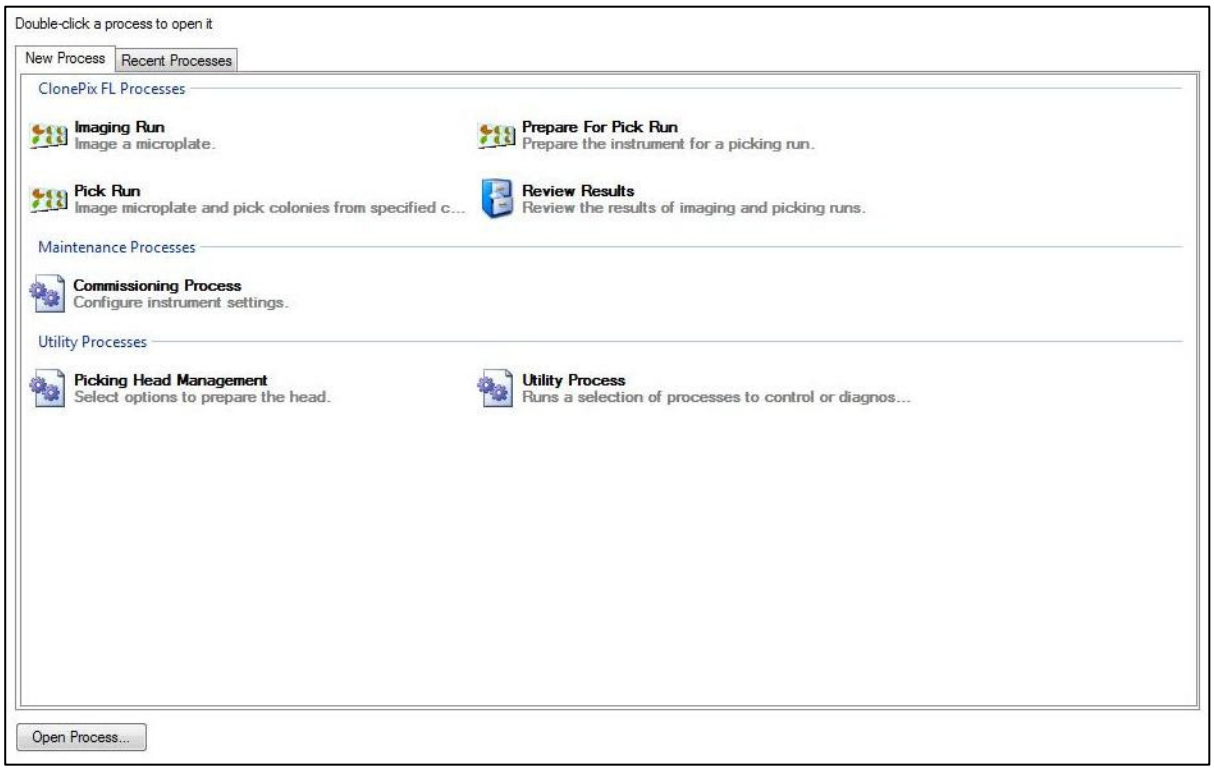
ClonePix 2 must be turned on for approximately 2 minutes before opening the software to ensure all drives are homed correctly.

ClonePix 2 Software

The software controls the ClonePix 2 system via Processes. A Process is a standard program for carrying out a task. The instrument has a set of processes that can be modified and saved. Processes are managed from the **Main Navigation Screen**, which is the view displayed when the software starts.

To start ClonePix 2 software, double-click the icon on the Windows desktop.

This will load the robot configuration, initialize the drives and display the **Main Navigation Screen**.





The interior light can be activated or deactivated at any time by double clicking the interior light icon in the bottom right corner of the screen.

The **Open Process** button can be used to browse for any saved Process or Template.

Main Navigation Screen

A Process is a standard program for ClonePix 2 to carry out a task such as a series of similar experiments, or a maintenance task.

New Process tab

The New Process tab provides default ClonePix 2 processes for use as templates for new imaging or picking processes. It also contains Maintenance and Utility Processes.

Templates tab

Saved templates can be retrieved by clicking on this tab.

Recent Processes tab

Recent processes can be retrieved by clicking on this tab.

Menu Options

The same menu is displayed in all windows. Items available may vary between windows.

File Menu

Open Process:
Allows a previously saved process to be opened.

Save Process:
Saves the current process.

Save Process as:
Allows the current process to be saved with a different name or location.

Close Process:
Closes the current process and returns to the Main Navigation Screen.

Save As Template:
Saves the current process as a template.

Recent Processes:
Allows a recent process to be selected and opened.

Switch User:
Allows the logged on user to switch to a different user. This option is only available with User Authorization.

Exit:
Exits ClonePix 2 software.

View Menu

Properties:

The view of the process window when a process's properties are being set and prior to starting the process.

Progress:

The view of the process window when a process is running.

Administrative properties:

Can be used to alter the way properties and default values are displayed.

Tools Menu

Configuration:

Provides access to the configuration settings for the instrument. It is strongly recommended that only trained personnel alter these settings.

Prepare Error Report:

Provides information to Customer Support to help diagnose recent problems.

Help Menu

About:

Displays the version numbers of the software modules. Most of these have the same version number so, if asked to provide the software version number, give this number unless otherwise directed.

Online Support:

This opens the web browser on the online support web page. This function only operates if the workstation has an internet connection, e.g. via a network.

ClonePix 2 Processes

Prepare for Pick Run

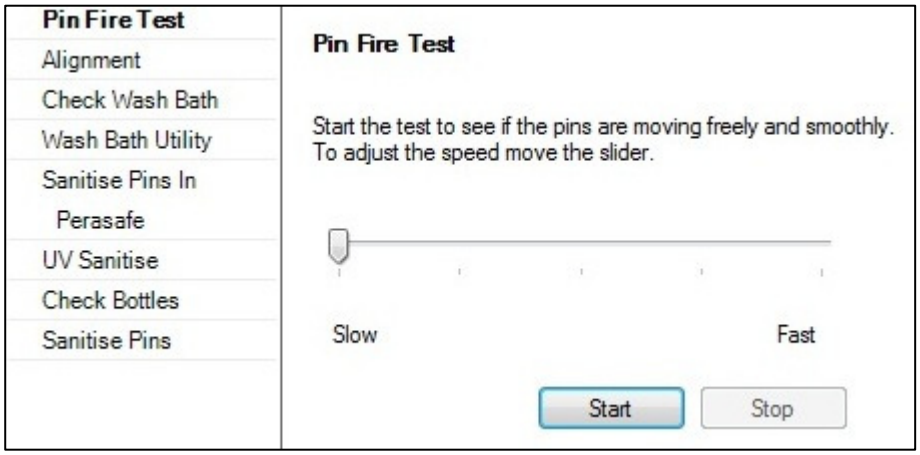
This process should be carried out prior to a **Pick Run** to ensure that ClonePix 2 is ready for picking. Any section of the following process can be bypassed by clicking **Next**.



Clicking **Start** will begin the Process.

Pin Fire Test

This test is for checking that the pins are firing correctly.

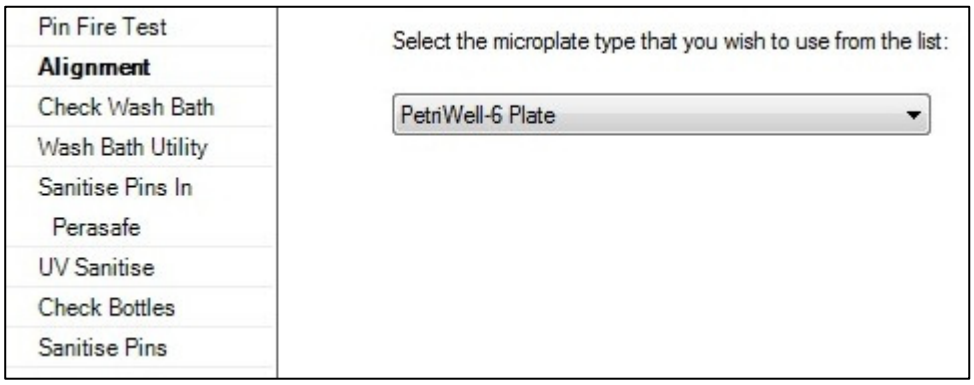


- Set the speed to medium/fast and click Start.
- Observe that the pins are moving freely.
- Click Stop to end the test.
- Click Next to move to Alignment.

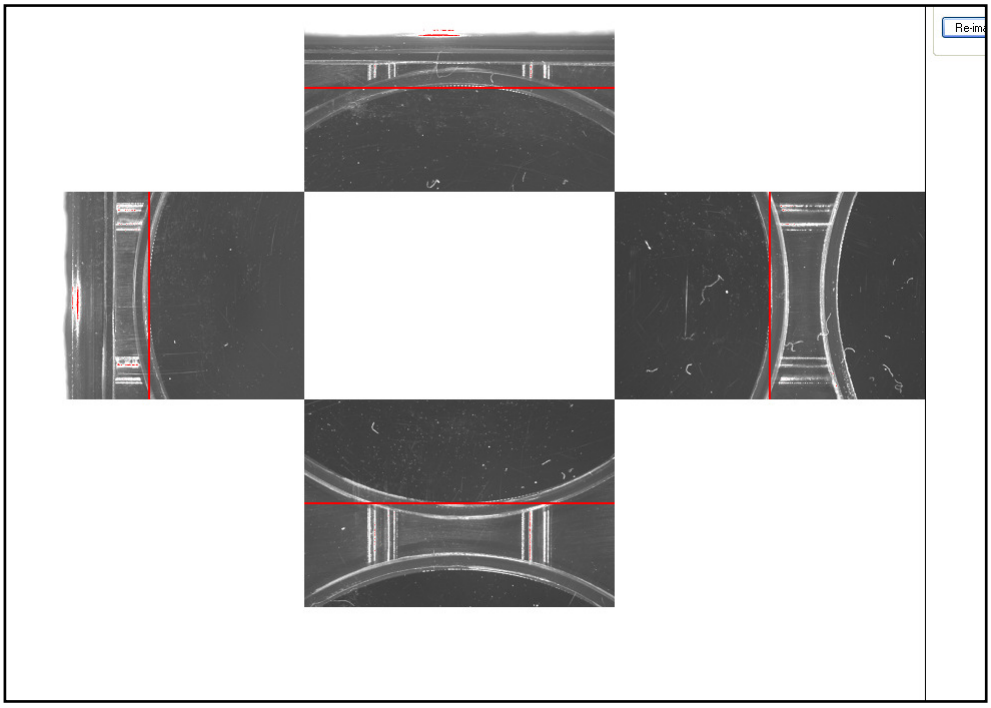
Important Note: If any pin is not firing and retracting freely do not continue. Run the **Pin Fire Test** continually for up to 1 hour. If this does not free up the pin action do not continue. Remove the Picking Head and return to Customer Support for reconditioning. The Picking Head cleaning procedures described in the **General Maintenance** section of the **Robot Manual** are for sterilization purposes only and are unlikely to resolve any pin movement issue.

Alignment

This section aligns the source plate and primary pin to the camera.



- Select the plate type from the drop-down list and click Next.
- The next screen will enable the stackers to be lowered or raised if required.
- Load a blank source plate into the source stacker cassette.
- Click Next to initiate imaging of the edges of well A1.

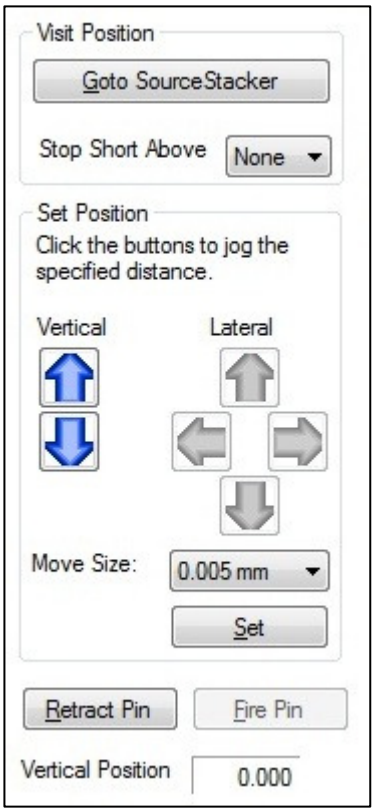


- If alignment is necessary, drag the red lines to line up the well and then click the Re-image button.
- Click Next to move to the alignment of the primary pin Z height.

Important Note: In normal usage the Z height of the primary pin should not require adjustment. Moving onto the next stage without modification is recommended.

If pin height adjustment is required follow the instructions below:

- Open the ClonePix 2 door and remove the picking tube from the pin 1.
- Attach the red tube from the air pressure gauge to the pin 1.
- The air pressure gauge will display a value in red.
- Set Stop Short Above to 5mm then click Goto SourceStacker.
- Note: The datum point can only be set with the pin extended.
- Using the Vertical arrow buttons lower the pin until it just touches the bottom of the well.
- When the pin is in the required position, the pressure gauge will display a value in yellow. Click Set to set this datum point.
- Click Next to move to alignment of the primary pin X & Y coordinates.

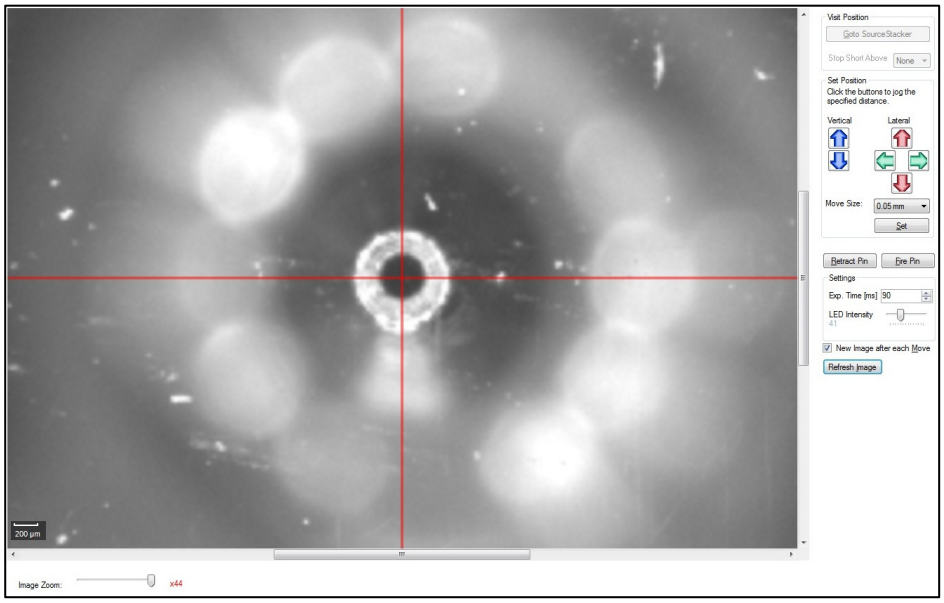


To align the pin correctly:

- Click Goto SourceStacker, there is no need to choose Stop Short Above as the pin already stops short by 0.5mm.
- Using the Lateral jog buttons move the pin until the pin hole is centered on the red cross.
- When the pin is in the required position click Set.

Note: If Set is not clicked the new datum point will not be fixed.

- It is possible to change the Exposure time and LED Intensity here if required. For example; if the image is not bright enough, the Exposure time or LED Intensity can be increased.
- Click Next to set up for sterilization of the fluid system.

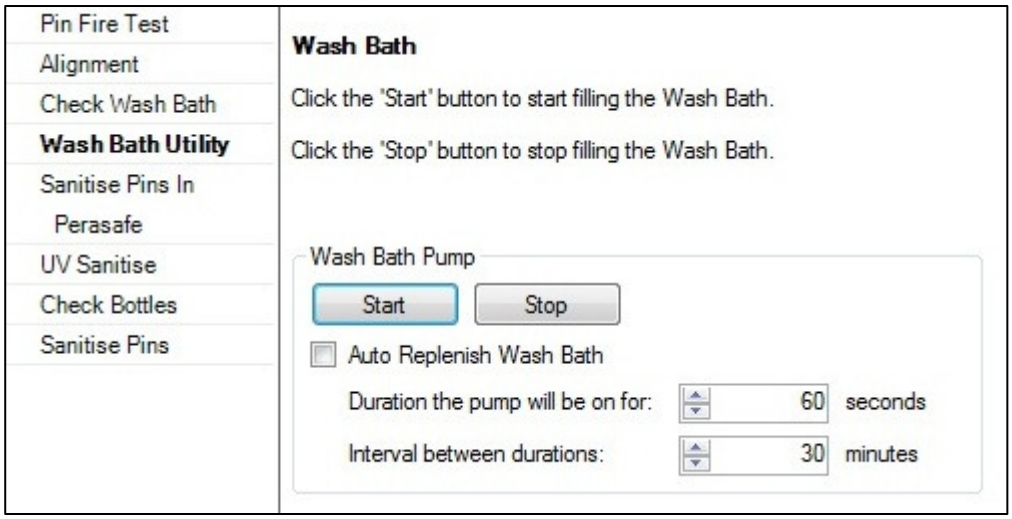


Check Wash Bath

- Check to see if there is any 70% ethanol in the wash bath. If the system has not been used, the bath will be empty and will need to be filled in the next stage of the process. If the system has been used already, the bath may be filled enough.
- Click Next.

Wash Bath Utility

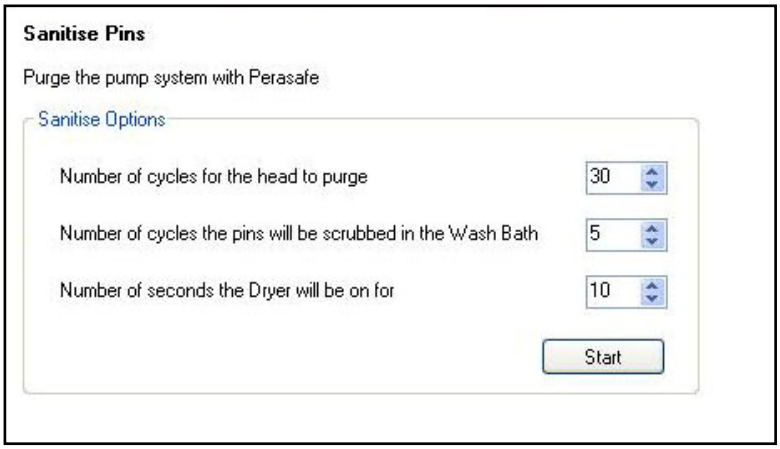
This step allows the Wash Bath to be filled and the Auto Replenish to be set as required.



- Click Start to fill the Wash Bath.
- Click Stop when the Wash Bath is filled.
- Click the Auto Replenish box to turn on the Auto Replenish function. This will automatically keep the wash bath filled whilst the software is running. The default timings can be changed if required.

Sanitize Pins in Sterilizing Agent

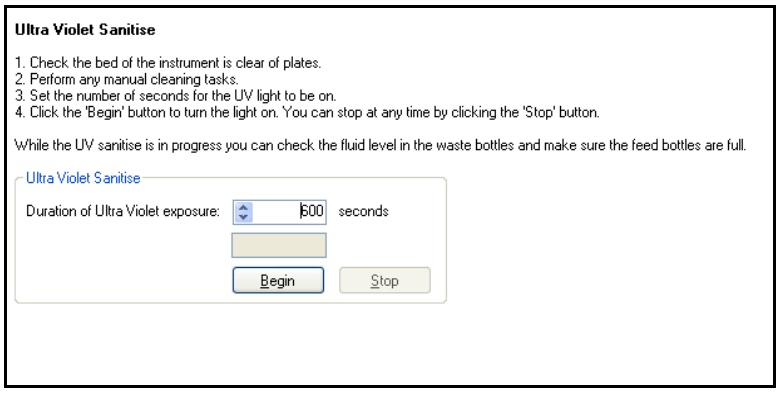
This step sanitizes the pump system between the sterile wash bottle and the picking pins.



- Connect a wash bottle containing freshly prepared Sterilizing Agent (Catalogue Number K8080) to the wash supply line.
- Click Start to pump the Sterilizing Agent through the entire system. This will take several minutes. The Sterilizing Agent needs to be left for 10 minutes - this can be done in parallel with UV sterilization (see next step).
- Click Next.

Ultra Violet Sanitize

This step activates the interior germicidal lamp.



- Set the required duration - 10 minutes (600 seconds) is recommended.
- Click Begin. The lamp will switch off after the set time. If the protective door is opened the lamp will switch off until the door is closed. Once the door has been closed the duration time will reset to 10 minutes and can be activated again.
- Click Next.

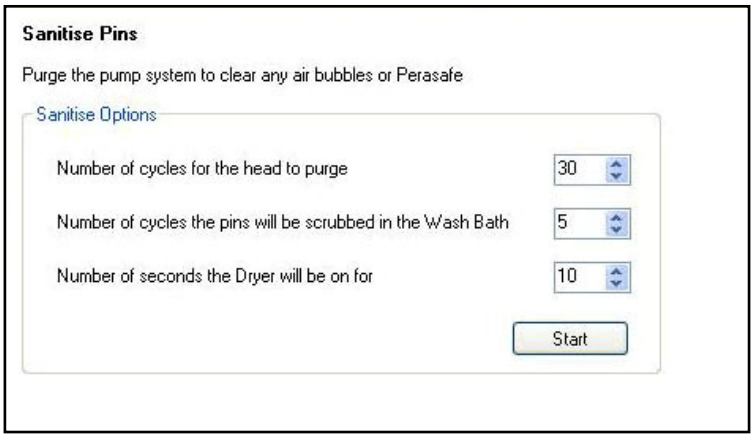
Check Bottles

At this point the Sterilizing Agent needs to be purged with sterile water.

- → Disconnect the fluid supply line from the bottle containing Sterilizing Agent and connect to an autoclaved bottle of high quality sterile water.
- → Empty the waste bottle.
- → Click Next to set up purge conditions.

Note: The connectors seal automatically when disconnected but it is advisable to liberally spray both ends with 70% ethanol before connecting the wash bottle.

Sanitize Pins



- → Click Start to pump sterile water through the entire system. This will take several minutes.

Note: This step should never be bypassed. Even if the system does not need to be sterilized, it is still essential to remove any air bubbles that have appeared if the system has been idle for any time (e.g. overnight). Air in the line can seriously affect picking success rates.

- → Click Next.
- → ClonePix 2 will automatically park the head safely, return the source plate and close the illumination cover.

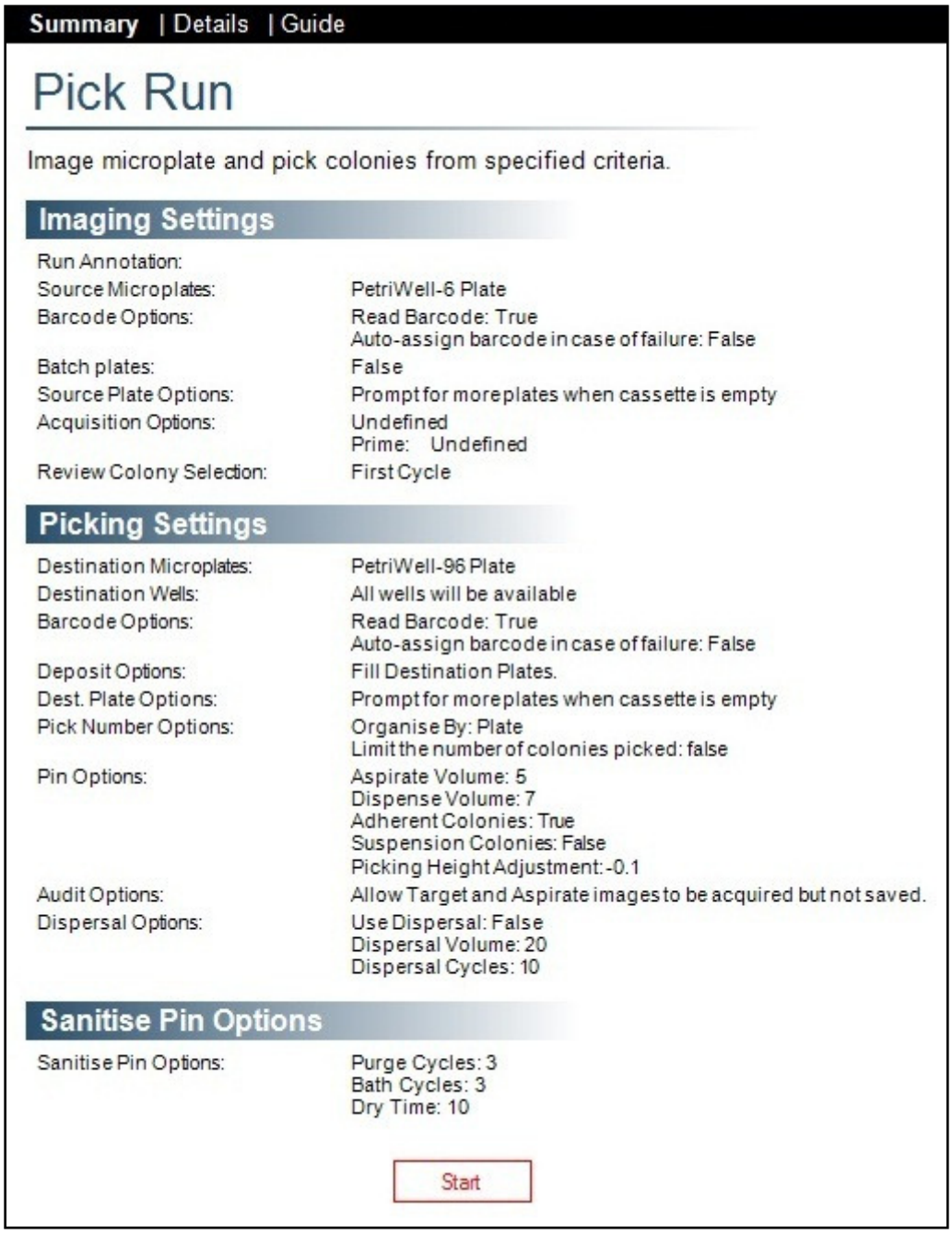
Finish

- → Click Finish to return to the Prepare for Pick Run process start page.
- → Click Close Process to return to the Main Navigation Screen.

Pick Run

The **Pick Run** process contains default parameters required for a full imaging and picking run, including image acquisition, colony detection, group selection and automated picking.

Using this process, it is possible to create multiple user-defined **Pick Run** or **Batch Pick Run** processes allowing different users to quickly reconfigure the robot to their own individual preferences. After completion of a **Pick Run**, the data is fully accessible in **Review Results**. **Pick Run** data is locked and cannot be modified.



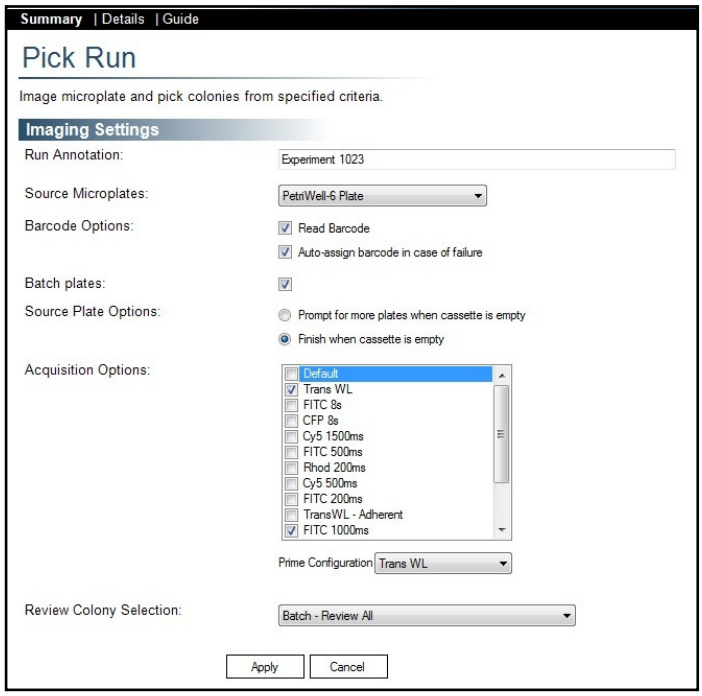
→ Use this window to modify the Imaging, Picking and Sanitize settings, and then start the Pick Run. If any incompatible options are selected, the appropriate text will be highlighted in red to indicate that a change is required. A run cannot be commenced while text is highlighted in red.

Modify Settings

The settings can be edited by clicking the section headings or selecting the Details or Guide tab.

Imaging Settings

Click on the **Imaging Settings** title or the Details/Guide tab to edit imaging settings.



Run Annotation

Enter a name to identify the run.

Source Microplates

Specify the type of source well plates.

Barcode Options

Selecting Read Barcode activates the source plate barcode reader. Using barcodes is recommended. Barcodes must be placed on the front short side of the microplate (A1 end). Read barcode must be activated for a Batch Pick Run.
Auto assign barcode in case of failure automatically generates a unique plate identifier if the barcode fails to read. If this box is not checked, the machine will request an identifier to be entered manually.

Note: Compatible barcodes for the system are 39, 93 and 128.

Batch Plates

Selects the Batch Pick Run option where all plates in the cassette are imaged and statistics are generated as an aggregate for all plates. The colony selection for the whole series of plates can be reviewed and adjusted prior to picking.

Source Plate Options

Two options are available relating to the Source plate supply:
Prompt for more plates when cassette is empty – The software will display a prompt asking for more plates to be added to the cassette once it has imaged all plates present in the cassette.
Finish when cassette is empty – The software will not look for more plates once it has found there are no more plates present in the cassette.

Acquisition Options

This section lists the acquisition options that have already been created e.g. White Light, FITC. Select the desired options by ticking the relevant boxes. The acquisition options can be further modified in Preview (see page 23).

Prime Configuration

This is the image acquisition option used for colony detection. Normally, this will be the white light acquisition option, although a fluorescent option specifically designed for colony detection could be used, e.g. viability assay such as LiveDetect.

Review Colony Selection

One of the following options for reviewing colony selection after imaging should be selected:

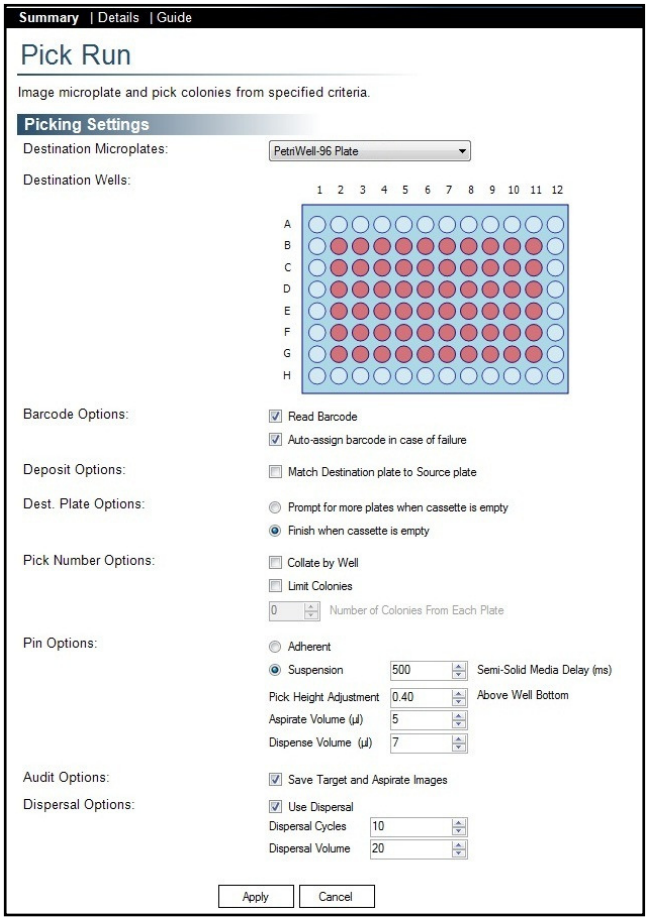
After First Microplate Only – This will bring up the Results Screen after the first microplate only is imaged to allow the Groups to be adjusted before committing to picking. These settings will be applied to any other plates in the cassette.

After Every Microplate – This will bring up the Results Screen after each microplate is imaged to allow the Groups to be adjusted before committing to picking.

Batch – Review All – This option must be selected when using Batch Pick Run. All source plates will be imaged to allow the groups to be adjusted for the whole series of plates before committing to picking.

Picking Settings

Click on the Picking Settings title or the Details/Guide tab to edit picking settings.



Destination Microplates

The type of destination well plates can be selected from the drop-down menu.

Destination Wells

The deposit wells to be used can be specified here. Right click on the mouse to select wells and left click to deselect wells. All destination plates will be filled using this template.

Barcode Options

Selecting Read Barcode activates the source plate barcode reader. Using Barcodes is recommended. Barcodes must be placed on the front short side of the microplate (A1 end). Read barcode must be activated for a Batch Pick Run.

Auto-assign barcode in case of failure automatically generates a unique plate identifier if the barcode fails to read. If this box is not checked, the machine will request an identifier to be entered manually.

Note: Compatible barcodes for the system are 39, 93 and 128.

Deposit Options

The default deposit option (box unchecked) sequentially fills all destination plate wells. Checking this box matches destination plate to source plate such that the completion of picking from each source plate will prompt the return of the current destination plate.

Destination Plate Options

Two options are available relating to the Source plate supply:

Prompt for more plates when cassette is empty – The software will display a prompt asking for more plates to be added to the cassette once it has imaged all plates present in the cassette.

Finish when cassette is empty – The software will not look for more plates once it has found there are no more plates present in the cassette.

Pick Number Options

ClonePix 2 can pick colonies by source plate or by source well. By default the software assumes that all wells of the current plate contain the same sample and are organized by source plate.

Collate by Well – When selected the system will pick all specified colonies from well A1 first then proceed to the next well. This should be selected when wells of a multi-well source plate contain different samples.

Limit Colonies – This feature permits the number of colonies picked from each plate or well to be limited.

Pin Options

The following pin options can be set:

Adherent or Suspension – The Adherent option is selected for collection of colony monolayers and the Suspension option is selected for collection of colonies from semi-solid medium. By default, there is a 500ms delay for Suspension picking to permit the colonies to be fully collected from the semi-solid medium.

Pick Height Adjustment – The pick height is automatically adjusted for optimal Adherent or Suspension picking (by default these are 0.1mm below and 0.4mm above well bottom, respectively).

Aspirate Volume – Recommended volume is 5µL.

Dispense Volume – Recommended volume is 7µL.

Audit Options

Selecting this option will save the target and aspirate images before and after picking a colony. If this option is selected all images will be saved. If this option is not selected, it is possible to toggle the images on and off whilst picking but the images will not be saved.
Note: This option slows picking dramatically.

Dispersal Options

Selecting dispersal separates the cells of a picked colony by aspirating and dispensing in the destination plate. If dispersal is required the following parameters can be set:

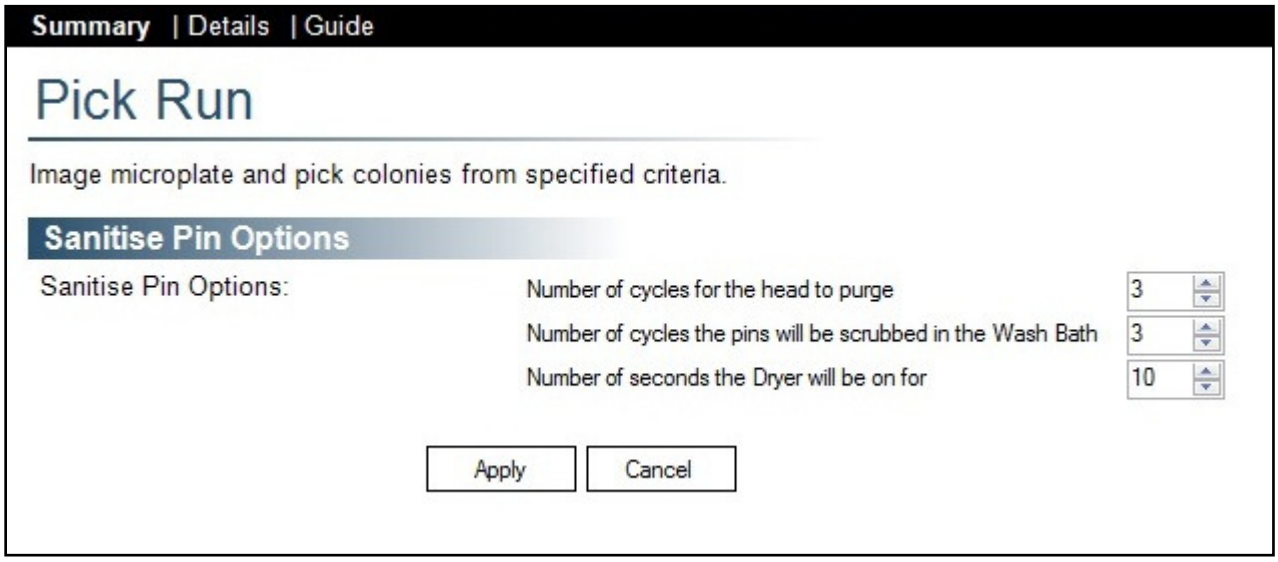
Dispersal Cycles – Recommended is 3-6 for CHO cells and 6-10 for hybridomas. Small colonies in chemically defined media may be best left undispersed.

Dispersal Volume – Maximum volume that can be dispersed is 20µL.

Sanitize Pin Options

Click on the **Sanitize Pin Options** title or the Details tab to edit pin sanitizing settings.

The picking pins are sanitized immediately prior to picking, between each round of picking, and at the end of a Pick Run. There are three steps to sanitizing pins which should be set up as follows:



- Select the number of purge cycles.
- Select the number of cycles the picking pins will be scrubbed in the ethanol bath.
- Select the number of seconds the halogen dryer will be on for.

The default settings are appropriate for most situations.

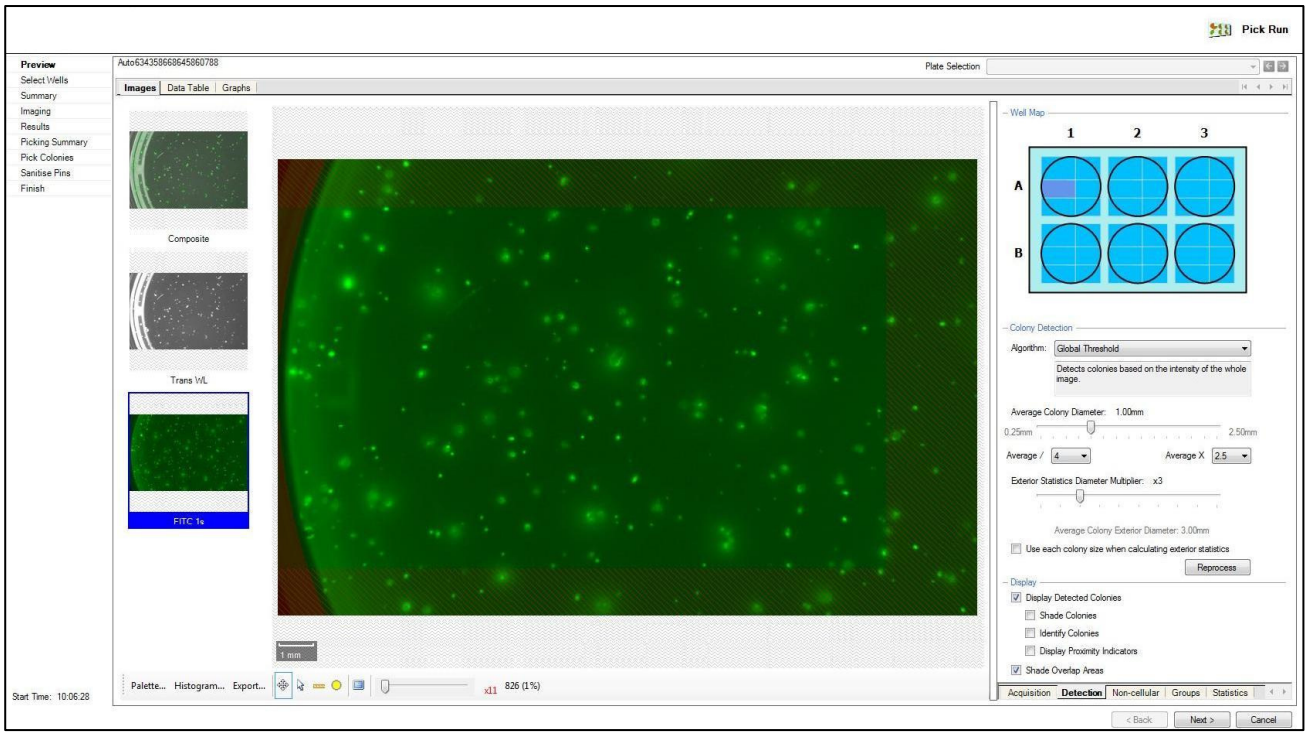
If new settings have been created it is advisable to **Save Process** at this stage.

Picking

Clicking **Start** will commence the picking process. The software will display a window prompting for the source plates to be loaded into the source stacker. The source stacker can be raised or lowered at this point if required.

Preview

The Preview screen permits the correct image acquisition and colony detection to be set up.



In **Preview** the first screen presented is the **Images** tab. This will initially appear as a grey screen until an image is selected on the well map to acquire the images. The tab is divided into two panels: the main panel shows the acquired images, with composite, white light and fluorescent channel thumbnails to the left of the main image. These thumbnails can be clicked on to display them in the central image frame.

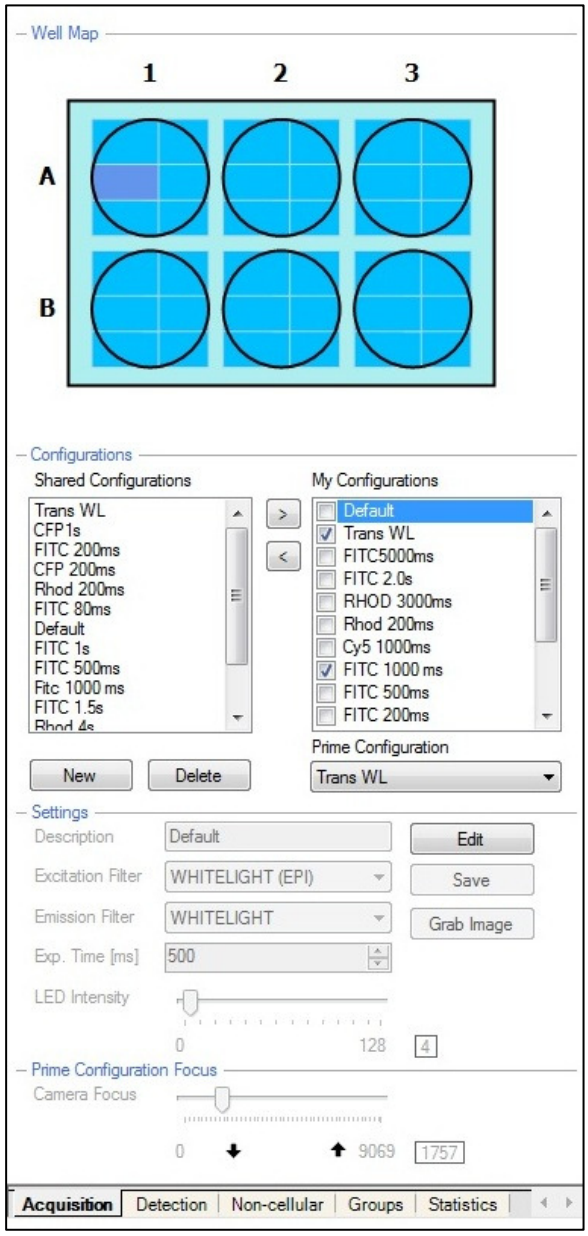
In **Preview** mode, only one image area of the well can be captured and viewed at a time, represented by a light blue rectangle in the **Well Map**.

To the right, a second panel shows the **Well Map** and several tabs (**Acquisition**, **Detection**, **Non-cellular**, **Groups** and **Statistics** tabs) that allow imaging settings to be set up. These are brought to the front by clicking on the tabs at the bottom of the panel.

Note: When in **Preview** mode the most important settings to set up correctly are the image acquisitions and the detection of the colonies because altering these settings later on in the process may take a long time.

IMAGE ACQUISITION

The **Acquisition** tab is used to define optical settings for image capture. It is important at this stage to make sure the images are bright but not over exposed to the white or fluorescent light.



Well Map

A map of the plate and the currently selected well is displayed above each of the settings tabs. Clicking on any of the wells imaged (displayed in light blue) will show the images for that well.

Configurations

Defined acquisition options are shown in the My Configurations box once they have been created. Personal configurations can be shared with other users by clicking once on the appropriate option in My Configurations to highlight the text in blue and then clicking the '<' button to transfer to the Shared Configurations box.

Prime Configuration: This is the acquisition option that will be used for colony detection. Normally this will be the white light acquisition option or a fluorescent option specifically designed for colony detection, e.g. viability assay such as LiveDetect.

Settings

These settings are used to set up and modify acquisition options. To modify a configuration, click once to highlight the text in blue and then click Edit. Select appropriate settings and then click Save. To create a new configuration click New, then click on the new configuration to highlight the text in blue and then click Edit. Select appropriate settings and then click Save. For white light imaging, use the Trans (transillumination) option. For fluorescent imaging, use the maximum LED intensity.

Prime Configuration Focus

This is the focus value that the camera will use when acquiring the images for each configuration.

ClonePix 2 software automatically detects image overexposure, shown as red pixels on the image. Where these are seen, it is recommended that the exposure time be reduced.

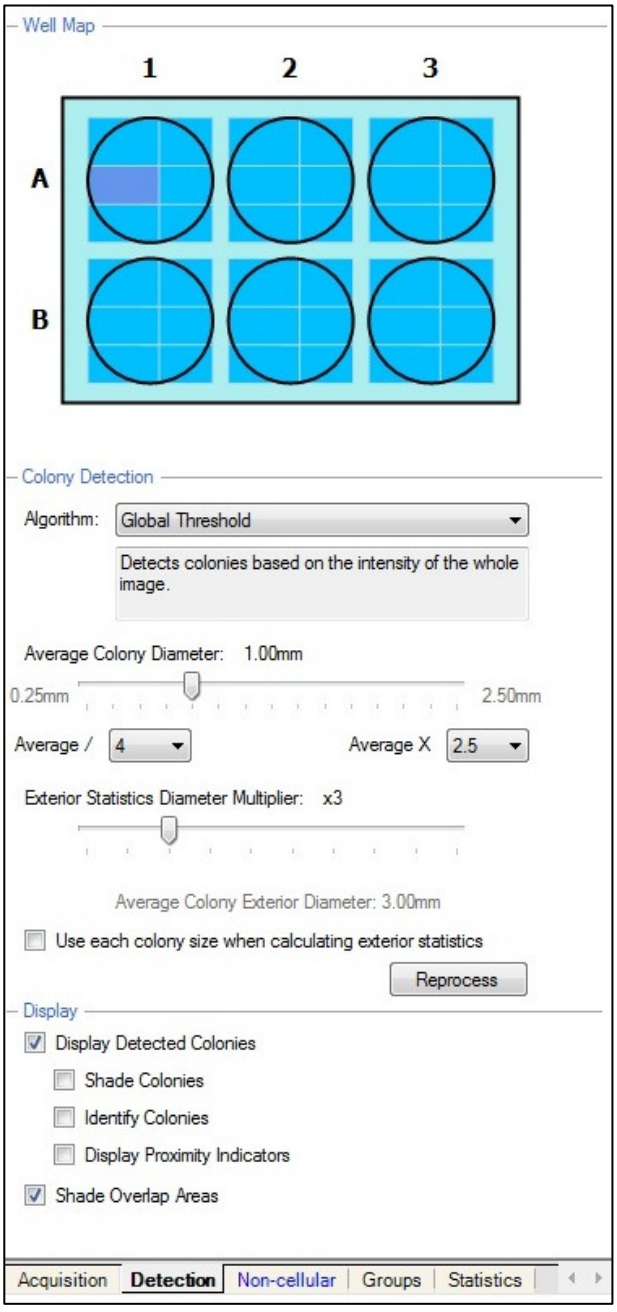
To select configurations for image acquisition, double-click on each configuration to put a tick in the adjacent box. Click **Grab Image** to preview the chosen images in the current image area.

COLONY DETECTION

This **Detection** tab is used to define settings for detecting colonies within the prime configuration (white light). It is important at this stage to make sure the all the desired colonies are detected. Detected colonies are displayed with a colored circle around the perimeter of the colonies (the color of the circle corresponds to the groups it is automatically assigned to by default).

Well Map

The well map remains visible.



Colony Detection

There are various algorithms available to best detect the colonies:

Global Threshold – Detection based on single background threshold intensity of the whole image.

Local Threshold – Detection based on background intensity around the vicinity of the colony.

Flatten Detection - Locates features by first removing background variations.

Edge Detect – Locates colonies by detecting the transition at the edge of each colony. This is better suited to detect adherent colonies.

Average Colony Diameter

The scale bar on the image is used to set the most appropriate average colony diameter. The software searches for colonies within a ten fold range of colony diameters thus excluding any large or small features that are not colonies. Minimum and maximum diameters are shown for the specified diameter. It is possible to extend this range by changing the upper and lower limits displayed as drop-down menus. The default values are the average colony diameter divided by 4 for the lower limit and the average colony diameter multiplied by 2.5 for the upper limit.

Exterior Statistics Diameter Multiplier

Moving the mouse pointer over any colony on the image shows a red circle around the colony. This defines the area that is used to calculate Exterior statistics. The Diameter Multiplier slide bar is used to set the required diameter to the red circle as multiples of the Average Colony Diameter.

Use each colony size when calculating exterior statistics

When selected the actual size of each colony, rather than the Average Colony Diameter, is used so that the larger colonies will have a larger exterior red circle.

Note: Click Reprocess to view the effect of any change.

Display

This section allows various aspects of the images to be displayed. The various options below enable this:

Display Detect Colonies - Deselecting the box hides colony detection from view to aid visualization of the underlying raw image.

Note: Be sure to reactive the display otherwise subsequent processing will not be visible.

Shade colonies - Selecting this option will fill the colony outline with the corresponding color for better visualization.

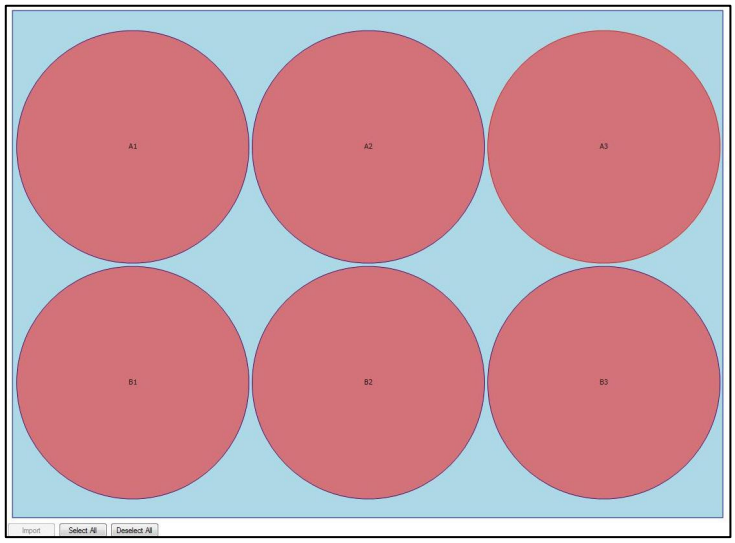
Identify Colonies - Superimposes a feature ID for each colony.

Display Proximity Indicators - When selected the nearest adjacent colony for every detected colony in the image is displayed. This is not the same as the Group Proximity (colony exclusion).

Shade Exclusion Zone - When selected the exclusion areas are visible on the image as hatched red lines. When de-selected the exclusion areas around the image are removed.

Select Wells

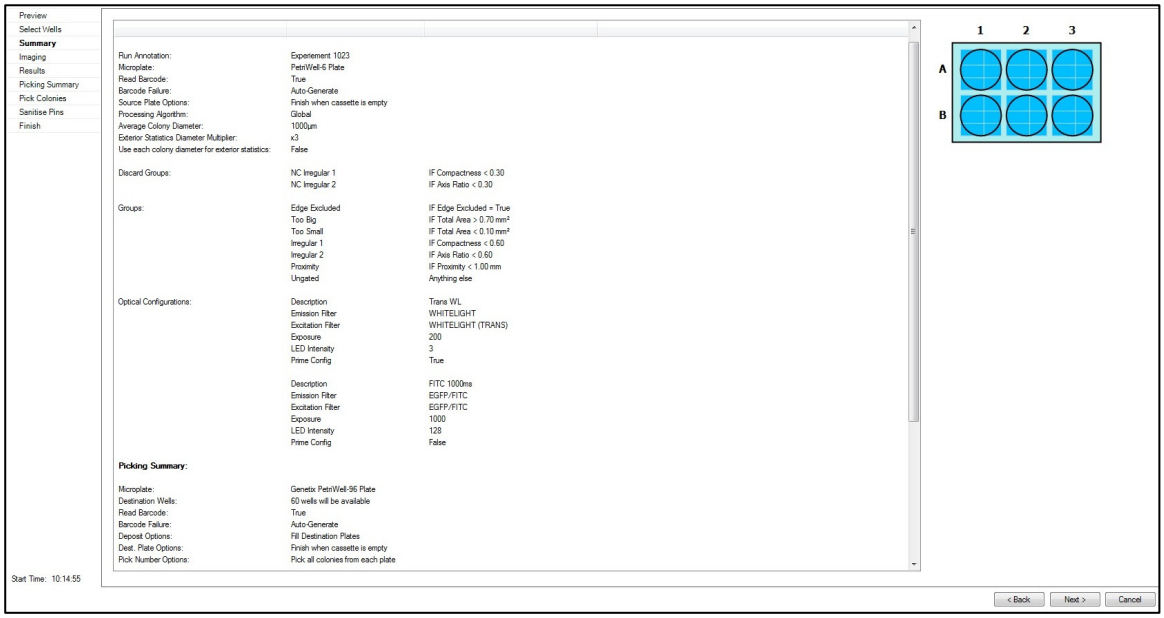
This screen provides the ability to choose which wells to image.



Clicking the wells with the right button of the mouse will deselect the wells (showing them as white). Clicking the well with the left button of the mouse will select the wells (showing them as pink). The **Select All** or **Deselect All** buttons can also be used to add or removed wells to be imaged.

Summary

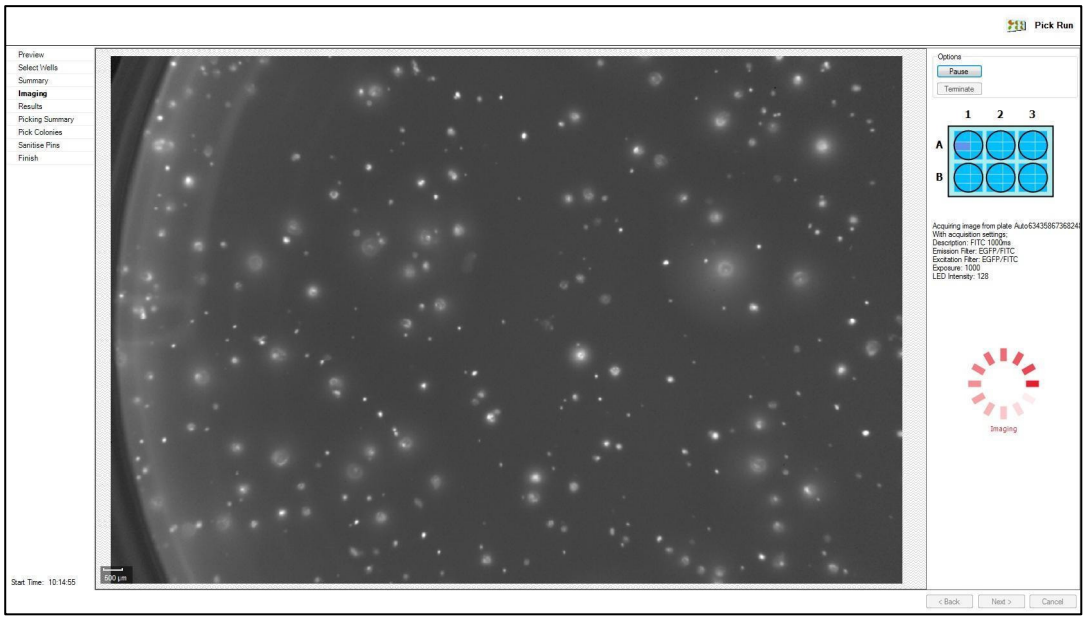
This screen provides a summary of the imaging and picking that will take place. This is a good point to check all setting are correct before proceeding to imaging.



Both imaging and picking summaries are displayed. A well map to the right of the summary identifies the wells that will be imaged.

Imaging

This screen displays the images that are being captured as imaging is taking place. The selected wells will be imaged in sequence for each of the acquisition options selected. The images will then be processed together for colony detection according to the defined settings



It is possible to cancel during imaging by pressing the **Pause** button and then clicking **Terminate**. A well map is also displayed to show the wells being imaged (displayed in light blue) and the current image being taken (displayed in dark blue). A small summary is displayed below the well map showing the acquisition details of the image being captured.

Results

After imaging has completed the results are displayed in several tabs within the **Results** screen. This screen has similar format to the **Preview** screen (see page 23) except that;

1. In the Results screen, all image areas can be instantly navigated using the Well Map.
2. In the Acquisition tab, only the configurations used during imaging are shown. The only settings that can be altered are selection of Prime Configuration.

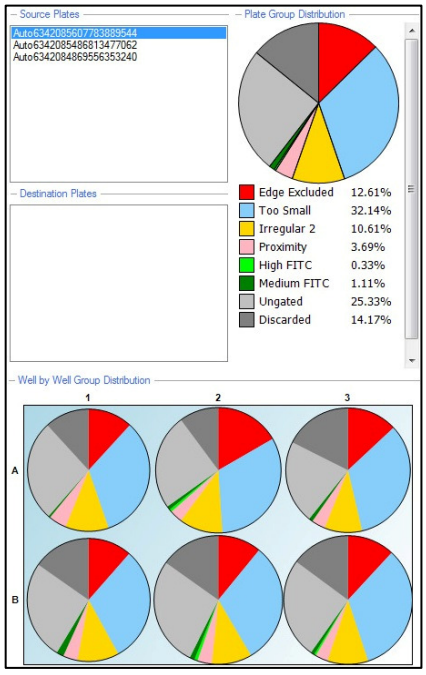
The various tabs within the results screen can be used in the next stage to discard debris or unwanted colonies and then create selection groups for picking in the next stage.

To aid with viewing data between tabs, a population can be selected by clicking and dragging on the **Scatter** / **Ranking** graphs or clicking while holding the **Control** key in the image or statistics tabs. Colonies that have been selected are visualized as follows:

3. In the Scatter graph or Ranking plot within the Graphs tab, the data points are colored dark blue.
4. Within images, the colonies are highlighted with a blue/white circle.
5. In the Data Table tab, the data points are highlighted with a blue line above and below the row.

Note: The **Results** screen is bypassed for subsequent plates if **After First Microplate Only** is selected under **Review Colony Selection** is selected (see page 19).

OVERVIEW TAB



This tab displays a summary of the imaging and picking settings. A summary of the plates imaged is also displayed to the right of the main screen.

Source Plates

The source plates imaged will be listed here. When clicked on (it will be highlighted in blue) the well map will be displayed below in the Well by Well Group Distribution.

Destination Plates

The destination plates used in the picking process will be displayed here. At this stage in the process no plates will be visible as no colonies have been picked.

Well by Well Group Distribution

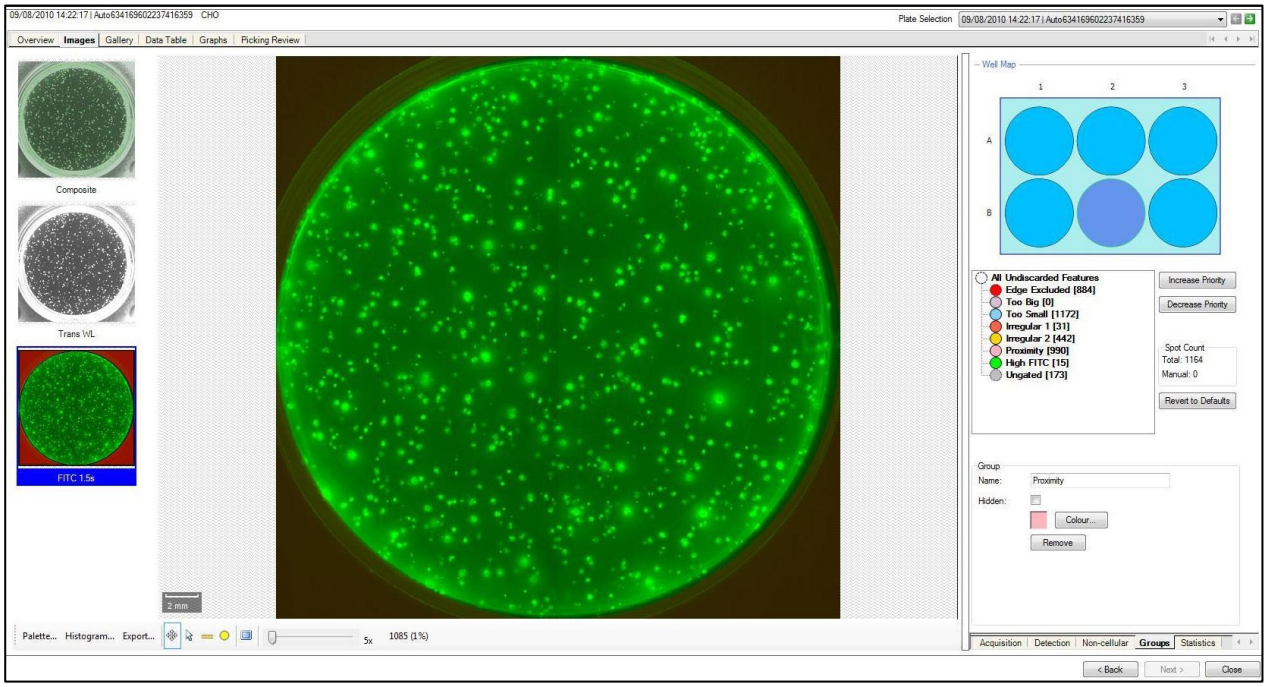
A selected plate will be displayed here. Each imaged well is displayed as a pie chart with each slice of the pie representing a group. The pie size is also proportional to the number of colonies detected in the well.

Plate Group Distribution

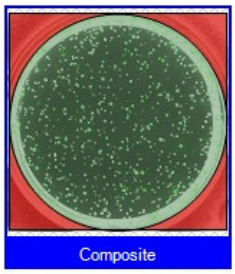
This pie chart displays the group distribution of all the wells in all the plates imaged. The percentage of each group is displayed below the pie chart. When moving the mouse over this key, the percentages will be displayed as the actual number of colonies in each group. Any discard groups created in the Non-Cellular tab (see page 35) will be displayed together as the Discarded proportion of the pie chart in dark grey.

IMAGES TAB

This tab displays whole-well images of the plates imaged. It is possible to view all the wells imaged by selecting a different well on the **Well Map**. Along the left hand side of the main image are thumbnails of the different filters used to image the plate. Acquired images can be viewed by clicking on the relevant thumbnails.

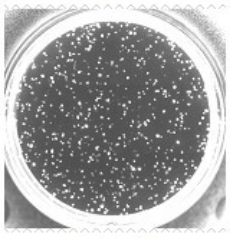


Underneath the **Well Map** are the **Acquisition**, **Detection**, **Non-cellular**, **Groups** and **Statistics** tabs. Clicking on the **Acquisition** and **Detection** tabs will display settings that were selected during the Preview stage (see page 23). These settings can be changed at this point if required but when Reprocess is clicked, all of the captured images will be reprocessed which may take some time especially if using batch mode and 10 plates were imaged. For descriptions of **Non-cellular**, **Groups** and **Statistics** tabs see pages 35, 36, 39.



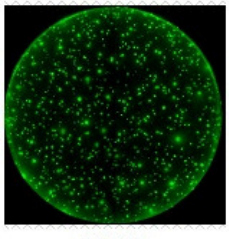
Composite Thumbnail

Fluorescent (non-prime) images can be superimposed onto the prime image in the Composite thumbnails. It is possible to change the brightness etc of the image by clicking on the thumbnail and then using Palette.



Trans WL Thumbnail

This displays the white light images acquired during imaging. It is possible to change the brightness etc by clicking on this thumbnail and then using the Palette.



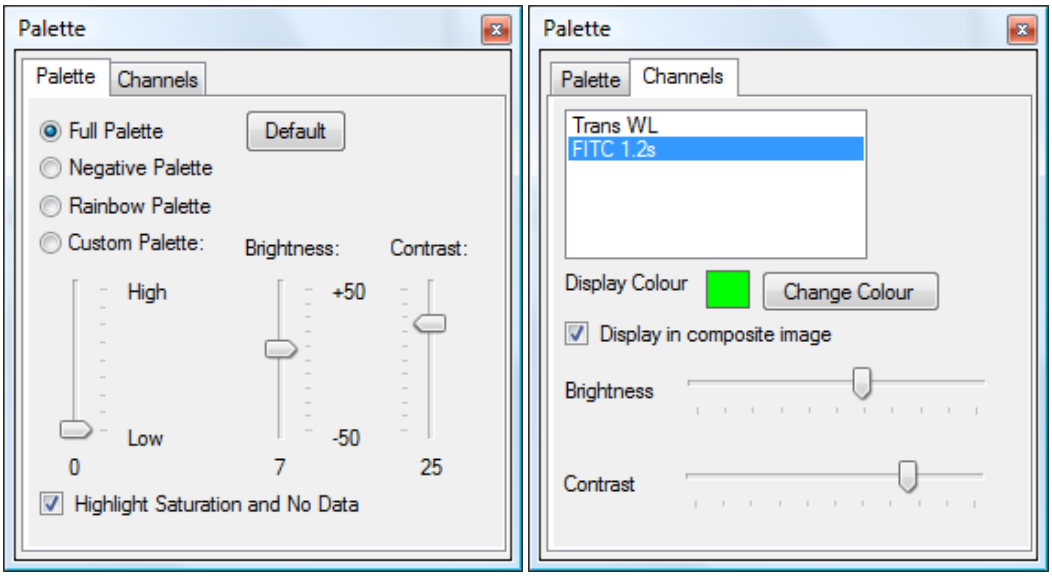
FITC 1.5s Thumbnail

Fluorescent thumbnails are displayed below the white light image. If there are several fluorescent channels used (e.g. FITC, RHO, CY5) they will all be displayed individually below the white light thumbnail. It is possible to change the brightness etc of the image by clicking on the desired thumbnail and then using the Palette.

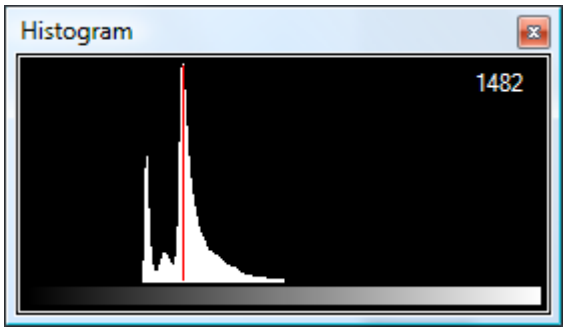


Palette

Palette button allows the images on-screen to be enhanced or modified. There is a palette available for each thumbnail (Composite, White Light and Fluorescent channels). To change any of the settings, select the desired thumbnail and click palette. There are various palettes to choose from as well as improving the brightness and contrast of the image. The Highlight Saturation and No Data option (selected as default) displays the red (saturated) and blue (No Data) pixels on the image. This can be removed by de-selecting this box.



Colors can also be applied to the images by clicking the Channels tab, selecting the channel of interest and giving it a color using the Change Color option. The fluorescent channels can be displayed in the composite image by selecting the Display in composite image box. The brightness and contrast of the composite image can only be altered using the sliders in this section of the palette.



Histogram

This displays the level of intensity within the selected image. The peaks in the histogram will show the distribution of the colonies' intensities.

Export

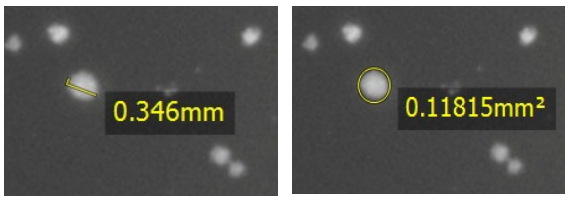
Export Image permits the current image to be exported. The exported image can be generated with or without detection (user defined) and is shown in the color specified in the acquisition option. The image can be exported as a .bmp, .jpg or a .png file.

Pan & Zoom

This allows the imaged to be zoomed and moved around the screen as required.

Select / Ruler / Area

Ruler and Area are tools that permit the direct measurement of a colony's diameter and area, respectively. To measure diameter, select the Ruler icon and then click and drag the mouse across a colony. To measure area, select the Area icon and click on the centre of the colony and drag out to the perimeter. Return to the Select icon after measurement.



Full Screen

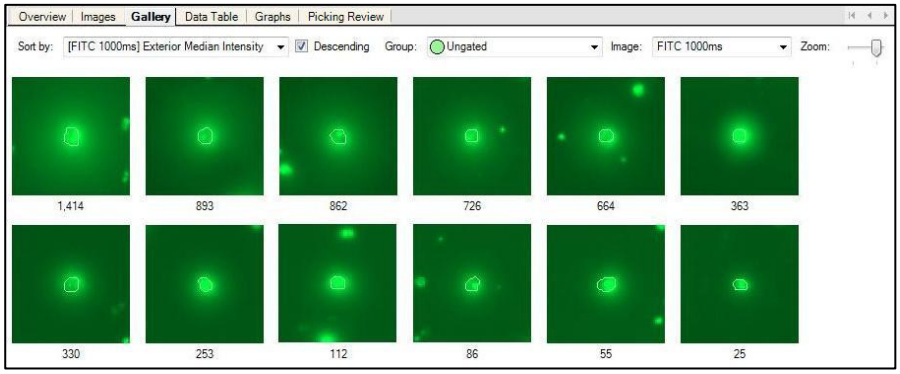
Clicking this button will enlarge the imaged being displayed to fill the full screen. Press Esc to return to regular view.

Zoom Control

Images can be digitally zoomed using Zoom Control. The magnification is shown to the right of the Image Zoom bar, which turns red when the actual image size is exceeded. Beyond this point the image will become pixelated. The scale bar in the bottom left hand corner of the image pane automatically adjusts with the Zoom Control.

GALLERY TAB

This tab enables colonies destined to be picked to be visualized in a defined order. It is possible to order them according to a fluorescent or morphological statistic, group and the acquisition option used (White light, FITC).



The colonies can be displayed using the following settings:

Sort by

This will sort the colonies according to the statistic chosen from the drop-down menu. This can be a fluorescent statistic or a morphological statistic.

Descending

By default this option is selected arranging the colonies in descending order from left to right. If the box is not selected the colonies will be arranged in ascending order from left to right.

Group

This drop-down menu allows all of the features or any of the groups to be displayed.

Image

This drop-down menu allows the white light or fluorescent image of the colonies to be displayed.

Zoom

The image can be zoomed into and out of by moving the slider from left to right.

Viewing Colonies

The number of colonies being displayed will be written here.

DATA TABLE TAB

Displays the statistics for colonies detected in the selected image. Each line represents a detected colony colored according to its Group. Fluorescent image parameters denoted by the prefix [acquisition option] are shown to the left and Prime (white light) image parameters are shown to the right. By default, parameters are listed in alphabetical order. Data can be ordered by any parameter by clicking on the chosen header. A second click will inverse the order.



The data table is interactive. A double-click on any row of colony data will hyperlink to the image of the colony (identified with a blue circle). Alternatively, right-clicking on a row will bring up a list of hyperlink options.

This right-click menu also allows colonies to be manually assigned to a particular **Group**. Any user-defined modifications to automatic grouping are logged in the statistics column **'Manual Group'**.



When any row is selected the statistics for that colony are also displayed in the **Statistics** tab to the right of the data table. This tab orders the statistics in an easy to view manner.

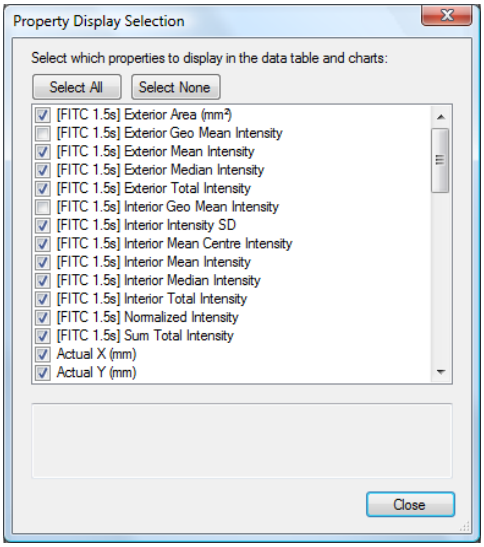
Colony Images

When a colony is selected in the data table the corresponding image will be displayed to the left of the table. It is possible to change the size of the colonies in the thumbnails by using the Zoom slider at the bottom.

Select Statistics

There are various statistics that are displayed in the Data Table as default, however it is possible to display only the desired statistics here. To do this, open the select statistics window and select the desired statistics. After clicking Close, only the chosen statistics will be displayed in the Data Table and the drop down menus within Graphs tab.

Note: Deselecting any of the statistics only hides the data from view and can be re-selected at any time.



Export Statistics

Selecting this will open a window for exporting data. Only statistics for picked colonies or all detected colonies can be exported. Data can be exported in 1) CSV format for opening as a single sheet in Excel and other statistics software, 2) in Excel-XML for opening in multiple sheets in Excel and other statistics software, or 3) XML format for compatibility with LIMS and other bespoke software. This is not functional in Preview.

Generate Report

When selected, a wizard for generating an XML-based summary of a Run is opened.

The following information can be exported into the Report for each plate processed:

Imaging Settings – All settings applied during set up.

Picking Settings – All settings applied during set up. Only available for a completed Pick Run.

Statistics – All of the selected statistics in the data table are entered into the report. It is recommended that these are excluded from the report as the data table is usually extensive and this will make the report large. It is recommended that statistics are exported via Export Statistics…

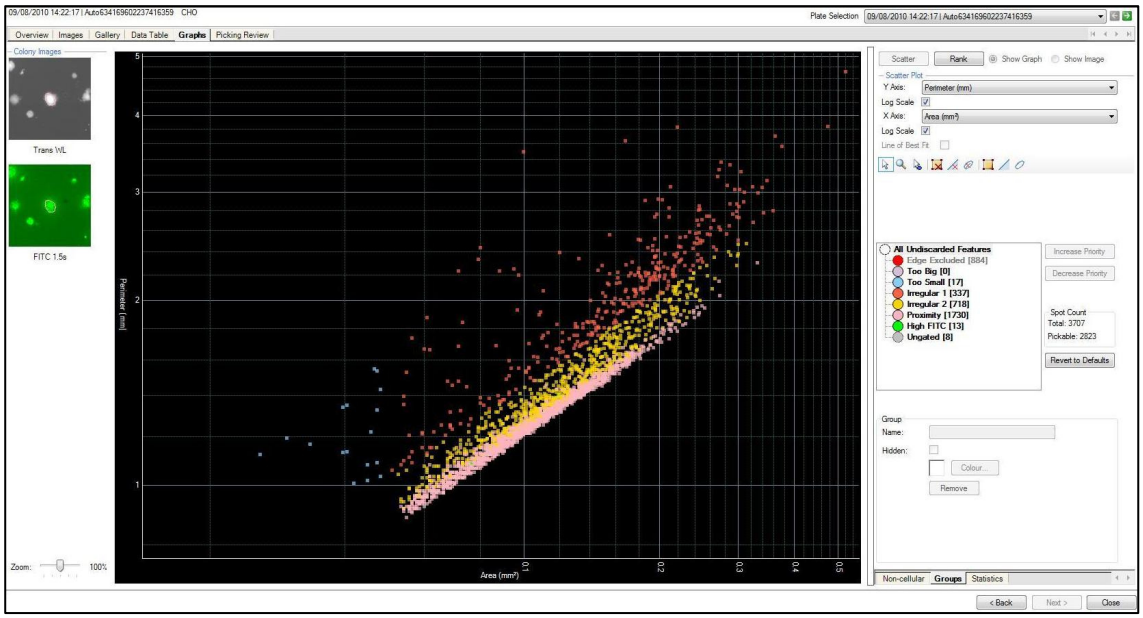
Scatter Plot - The last Scatter plot to be created in the Graphs tab will be entered into the report.

Rank Plot – The last Rank plot and Histogram created in the Graphs tab will be entered into the report.

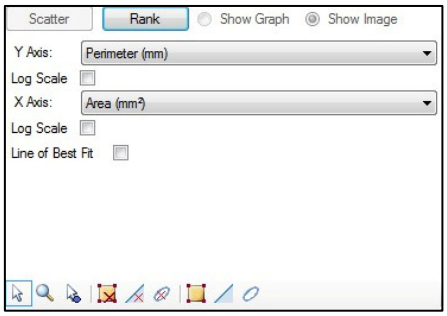
Pick Summary – All of the details from the Picking Review tab will be entered into the report.
As well as giving the Report a name, the following information can also be entered into the Wizard; Title, Cell Type, Project Details, Plating Efficiency, Incubation Time, Summary.

GRAPHS TAB

This is the main tab used for creating groups. Each point displayed within the graphs is a detected colony. The color of each point indicates the group that the colony has been assigned to. Hovering of each point on the graphs will display the corresponding colony in the image thumbnails to the left of the graphs.

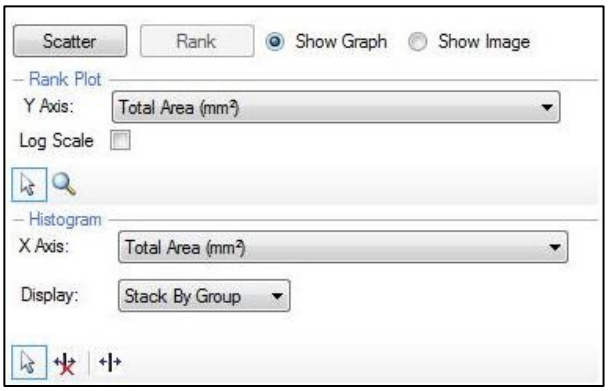


Scatter



There are various options available when looking at results and creating groups in the scatter graph. Any combination of two statistics can be plotted on the scatter plot by choosing them from the Y and X axis drop-down menus. It is possible to log the Y and X axis by selecting the 'Log Scale' boxes below the drop-down menus. A line of best fit can also be fitted to the data by selecting the 'Line of Best Fit' box. There are various tools that can only be used with the scatter plot; these are Spot Selection cursor, Zoom cursor, Gate Selection cursor, Discard Tools (denoted by a red cross on the icon) and Groups Tools. The Scatter Plot is interactive. A double-click on any data point will hyperlink to the image of the colony (identified with a blue circle). Alternatively, right-clicking on a data point will bring up a list of hyperlink options. Right-clicking on the background permits the current graphic image to be copied.

Rank



There are various options when looking at results and creating groups in the ranking plot. When Rank is selected the Rank Plot is displayed at the top of the screen and a Histogram is displayed beneath the rank plot.

Rank Plot - It is possible to display either the rank plot or a well image. When Show Graph is selected, the ranking plot will be displayed above the histogram. When Show Image is selected, the image of the currently selected well will be displayed above the histogram.

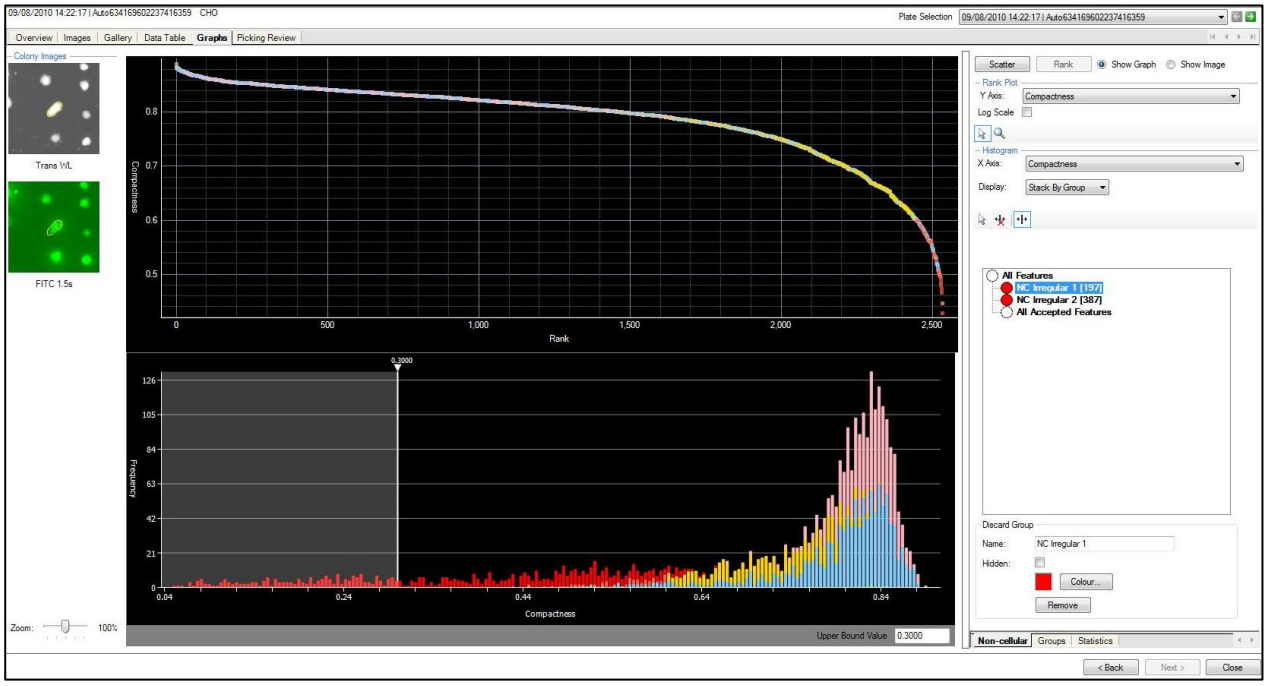
When Show Image is selected, as new groups are created or edited, the color change of the colonies will be automatically updated on the image.
The Rank Plot and Histogram statistics are linked. When a statistic is selected in one graph, it will also be displayed on the other.
It is possible to Log the scale of the Y Axis in the Rank Plot and also zoom into a desired area on the graph.
The Rank Plot is interactive. A double-click on any data point will hyperlink to the image of the colony (identified with a blue circle). Alternatively, right-clicking on a data point will bring up a list of hyperlink options. Right-clicking on the background permits the current graphic image to be copied.

Histogram – It is possible to create groups using this histogram by selecting a statistic from the drop-down menu and drawing a line gate where required. It is also possible to change group default values here (see page 36).

Display enables the grouping histogram to be visualized by Stack By Group or by Totals Only.

NON-CELLULAR TAB

The **Non-Cellular** tab, within **Graphs** tab, enables any kind of debris, e.g. hairs, small scratches, small bubbles etc, that are not colonies to be removed form the data set before deciding which colonies to pick. By removing debris from the data set before creating groups they are not considered as being part of the data. This means that real colonies will not be put into the **Proximity** exclusion group if they are too close to debris. There are two discard groups already created by default; **NC Irregular 1** and **NC Irregular 2**. These groups exclude debris based on compactness and axis ratio respectively. These groups can be deleted if not required. Non-Cellular discard groups can be created by selecting a statistic from the drop-down menu and drawing the discard line (icon denoted by a red cross) on the histogram.



Several of these **Non-Cellular** discard groups can be created if required and these will be listed in the **Non-Cellular** tab. By default all discard groups listed in the **Non-Cellular** tab

are colored red but this can be changed if required. Discard groups can also be hidden by selecting the group and clicking on the **Hidden** box.

GROUPS TAB

The **Groups** tab, within the **Graphs** tab, is used to visualize detected colonies and their corresponding groups as well as create new groups for picking. Groups can be optimized and/or set up at the **Preview** stage but are best created after imaging. The grouping system is designed to generate exclusion groups e.g. Too Small, Irregular, Proximity, and inclusion groups for picking specific populations, e.g. High FITC, High Rhodamine. Multiple groups can be picked in the same picking run but the choice of which groups to pick will be made later in the Picking Review tab (see page 40).

Image thumbnails are displayed to the left hand side of the graphs so that the colonies can be visualized when hovering over the points on the graphs.

There is the option to change between **Rank** and **Scatter** graph for plotting statistics and creating new groups.

The grouping system works by priority with the group at the top having highest priority. For example, a colony that would fall into two groups will be placed in the highest priority group.

Group priority can be changed by using the **Increase Priority** and **Decrease Priority** buttons. Any colonies that do not fall into an exclusion group are retained in the bottom category. By default, this is called **Ungated** as these are 'good' colonies that have not been rejected by morphological characteristics or close neighbor proximity.

The bracketed number to the right of the group name indicates the number of colonies in the group.

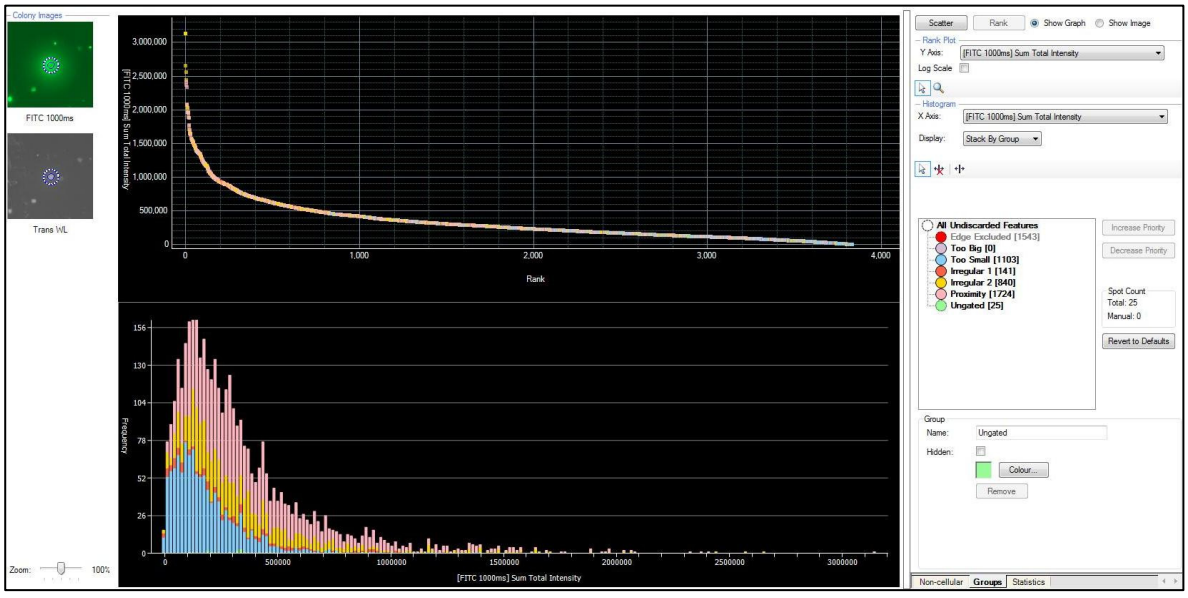
Spot Count

Total – When a group is selected, the total number of colonies assigned to that groups is displayed here.

Manual – If there are any manually assigned colonies to this group, the number will be displayed here.

Pickable – When All Undiscarded Features is selected the total number of pickable colonies will be displayed here.

Revert to Defaults – Clicking this button will change all of the group values back to the original default values.



There are six exclusion groups provided by default:

Edge Excluded
Edge excluded colonies are outside the pickable region of the well and cannot be selected. This group has highest priority and cannot be moved to a lower priority.

Too Big
Colonies greater in size than 0.7mm2 fall into this exclusion group.

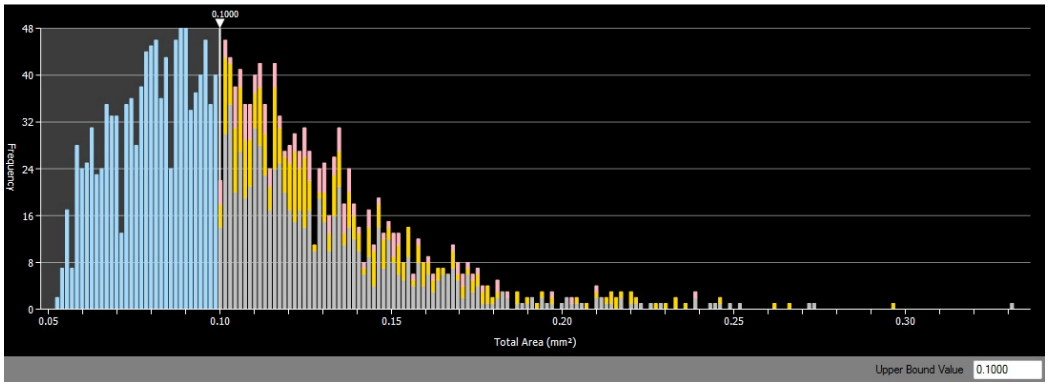
Too Small
Colonies smaller in size than 0.1mm2 fall into this exclusion group.

Irregular 1
Colonies that have a Compactness value less than 0.6 fall into this exclusion group.

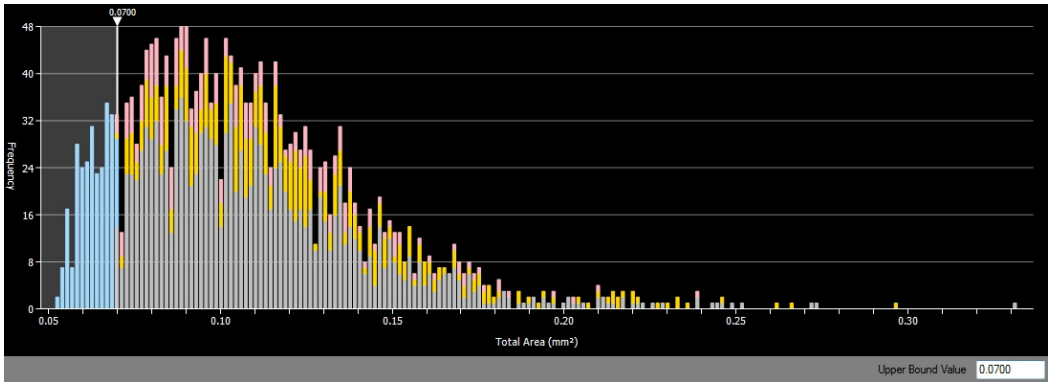
Irregular 2
Colonies that have an Axis Ratio value less than 0.6 fall into this exclusion group.

Proximity
Colonies that have a Proximity value less than 0.1mm fall into this exclusion group.

To edit a **Group**, double click once on the group name (e.g. Too Small) – the selected group and its gate will appear on the histogram.



The slider on the histogram can then be moved to the desired new value or the new value can be typed into the **Upper Bound Value** box in the bottom right corner of the histogram (press **Enter** to apply changes). The group gate will display the colonies that now fall into the altered group.



At the bottom of the **Groups** tab, a selected group can be renamed by typing in a new name and clicking **Enter**. The group can be hidden from view by ticking the **Hidden** box. This hides them from view on all images and in the **Data Table** and **Graphs** tabs. The group color can be changed using the **Color** button.

The bottom category (**Ungated** by default) is not a defined group and so does not have a gate on the histogram that can be changed. This group can not be deleted.

With the exception of the **Edge Excluded** group, any **Group** can be permanently deleted by selecting it and clicking the **Remove** button.

Creating New Groups

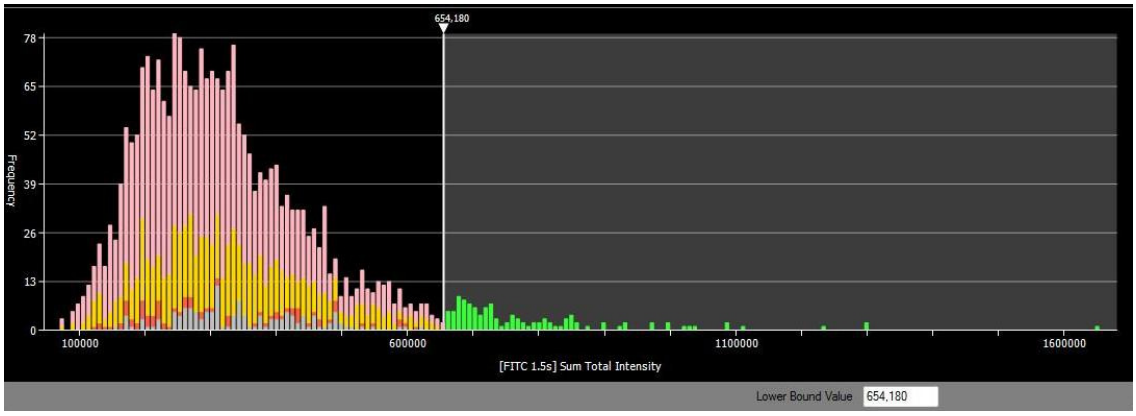
Example: Creating a group for picking high FITC-expressing colonies.

(A white light Prime image and a FITC fluorescent image need to be acquired to carry out this example).

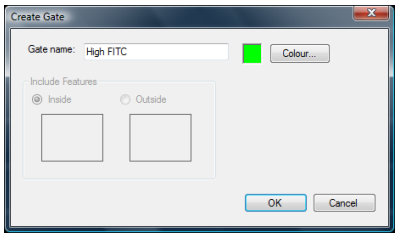
In the **Graphs** tab, click **Rank** to display the rank plot and histogram.

Select the relevant fluorescent statistic from the drop down menu (e.g. [FITC] Exterior Mean Intensity or [FITC] Sum Total Intensity). This will be displayed on both graphs.

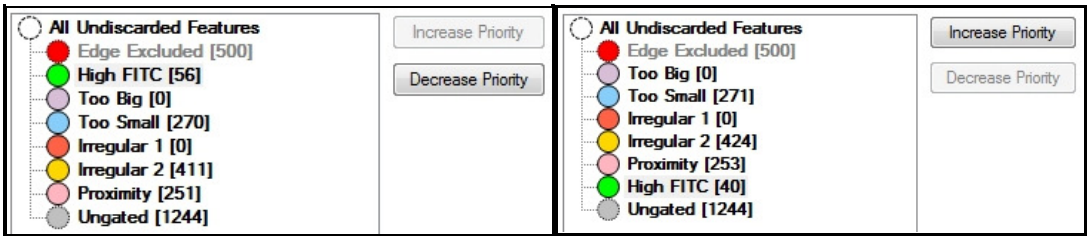
 Click on the **New line gate** icon, click on the histogram where the group will start and drag the line off the end of the histogram to create the group.



Name the group (e.g. High FITC), give it a color if required and click **OK**.



The group will be placed beneath the **Edge Excluded** group. Select the newly created group and decrease its priority so that it sits just above the **Ungated** category.

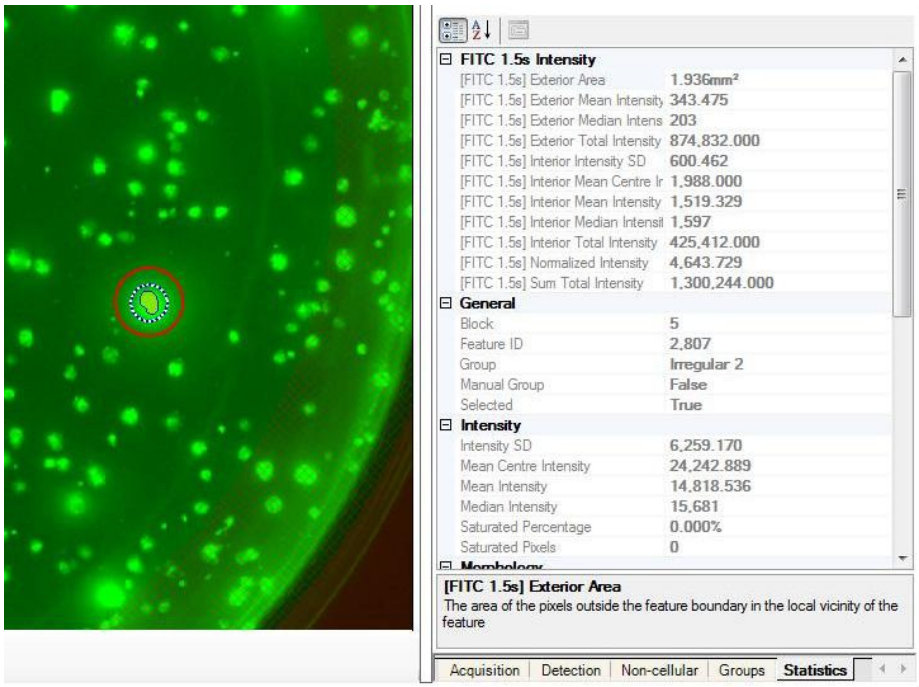


Decreasing the priority means that only colonies with good morphological and proximity characteristics can be put into the new group.

All colonies in this group are now shown in the **Group** color in the **Images**, **Statistics** and **Graphics** tabs.

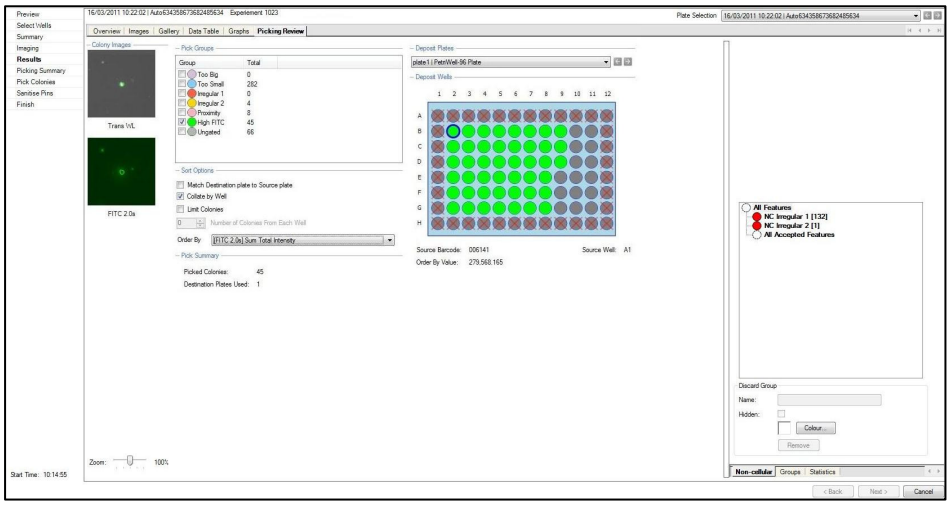
STATISTICS TAB

The statistics for any colony can be seen in the right hand panel in the **Images**, **Data Table**, **Graphs** and **Picking Review** tabs. Clicking on the statistics tab and then the colony of interest will display all the statistics available for that colony. The statistics in this list can be categorized (Fluorescent Intensity, General, Intensity, Morphology, Position) or ordered alphabetically by clicking on the relevant buttons.



PICKING REVIEW TAB

This screen summarizes the colonies to be picked. Any number of groups can be selected to be picked here as well as being ordered using various criteria.



Colony Images

When a well in Deposit Wells is selected, the corresponding colony image will be displayed to the left. It is possible to zoom in and out of these colony images using the Zoom slider below the images.

Picking Groups

All the groups available to be picked are listed here, including exclusion groups such as Too Small and new groups that have been created with specific characteristics.

Sort Options

This provides a number of ways colonies can be sorted.

Order by - Allows colonies within a checked group to be picked in order of any of the parameters in the drop-down menu, for example Fluorescent Exterior Mean Intensity. If several groups have been selected it is advisable to order by Group.
Match Destination late to Source plate, Collate by Well and Limit Colonies descriptions are on page 20.

Pick Summary

This summary displays the number of colonies to be picked and the number of destination plates that will be used to pick these colonies into.

Deposit Plates

This lists the number and type of destination plates (as previously defined in Modify Settings, Picking Settings) that will be required for picking.

Deposit Wells

This well map displays the wells that the colonies are going to be picked into. The wells are colored according to the corresponding colony that will be picked into them. Any wells that have been excluded from picking are displayed with red crosses over them. Underneath the well map is the following information which is displayed when hovering the mouse pointer over the colony assigned wells:

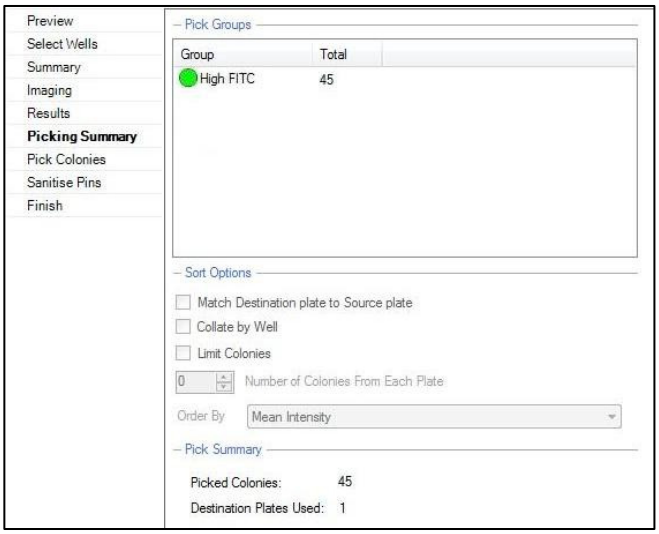
Source Barcode – Displays the barcode of the source plate where the picked colony will come from.

Source Well - Provides the source well number of the picked colony.

Order By Value - Provides the order value (assigned from the sort options drop-down menu) of the colony to be picked.

Picking Summary

This screen will display the pick information before the picking will commence. It is possible to click **Next** or **Back** at this stage. Clicking **Next** will commence picking or clicking **Back** will display the Results where changes can be made if required. **Note**: Selecting back at this point will reprocess all the images captured which may take a while if several plates have been imaged.



Pick Groups

This lists the groups and the number of colonies within a group that have been selected for picking.

Sort Options

This displays the various sort options that were available to be selected in the Picking Review tab of the Results. These cannot be changed at this point.

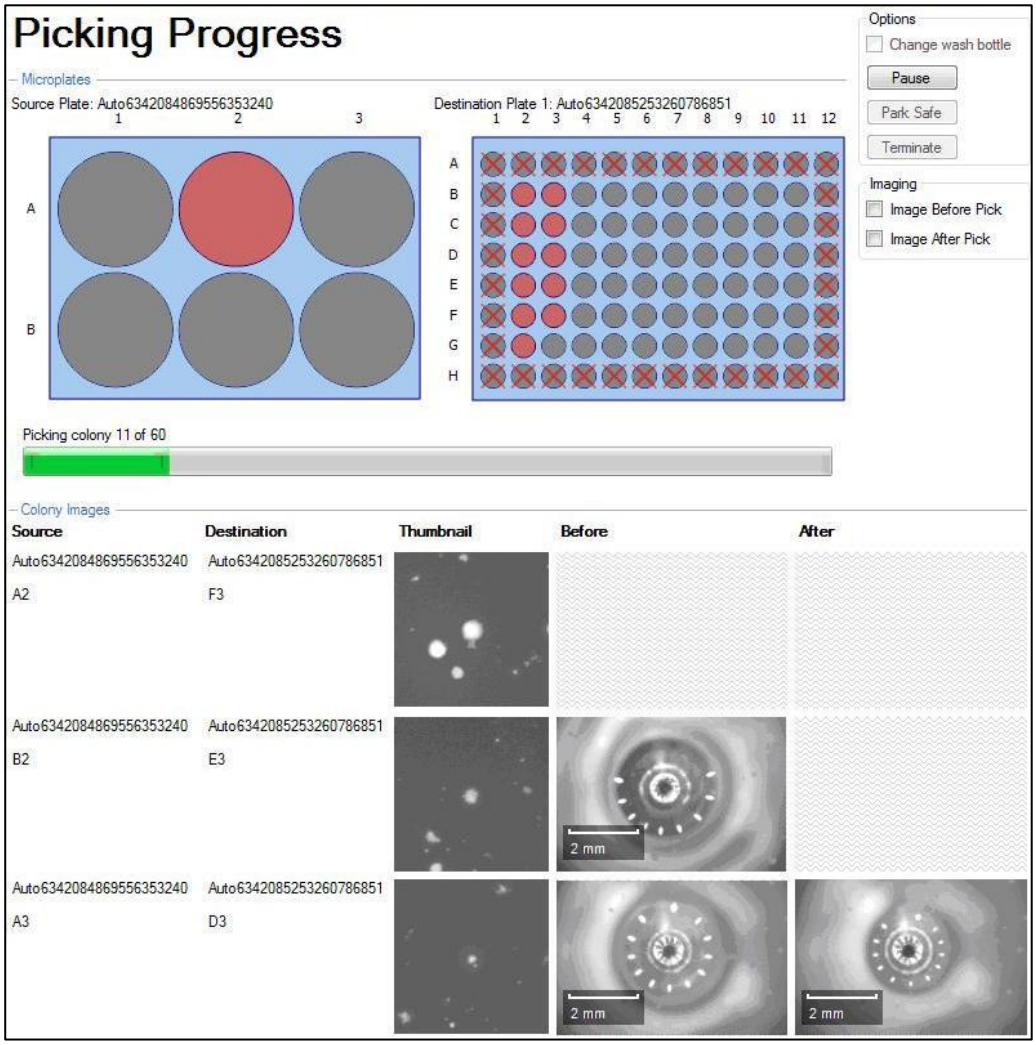
Pick Summary

This section displays the number of colonies in total to be picked and the number of destination plates required for the entire pick process.

Clicking **Start** will commence picking of the colonies. The software will display a window prompting for the destination plates to be loaded into the destination stacker cassette. The destination stacker can be raised or lowered at this point if required.

Picking Colonies

The screen displays the **Picking Progress** as the colonies are being picked. The automated picking step will initiate with a pin wash. ClonePix 2 will then commence to pick the first colonies.



Microplates

This section displays a schematic of the source plate and the destination plate. As colonies are being picked from a well of a source plate the well is colored pink. As the colonies are being picked into the destination plate the wells are colored pink. The barcodes of the plates being used are displayed and any wells not being used are highlighted by a red cross over the well.

Colony Images

As colonies are being picked several pieces of information are displayed; source plate barcode and well number, destination plate barcode and well number, a thumbnail image of the colony to be picked, a thumbnail image of the pin in place before the colony is picked and a thumbnail image of the pin in place after the colony has been picked. It is optional to have the before and after images shown on screen. To select or deselect these before and after images, click on the appropriate boxes.

Options

Various options are available here to stop the colony picking:

Change Wash Bottle – The function allows sterile water to be replaced during the picking. To activate, click Pause, select Change Wash Bottle, and then click Continue. Immediately prior to the next wash cycle, ClonePix 2 will display a prompt to swap over the bottle (if this is necessary), and will then commence the long purge. The next cycle of pin washing will revert to the original purge settings.

Note: Do not forget to empty the waste bottle each time that the wash bottle is replaced.

Pause – The picking can be paused and continued at any point by clicking this button.

Park Safe – Once in pause mode, the picking head can be moved to the Park Safe position. Clicking Continue will restart the picking from the Park Safe position.

Terminate – Once in pause mode, the picking can be terminated immediately. Warning: Please be sure termination is the right decision before clicking this button.

Imaging

If selected the camera will reposition and show on-screen images of the pin tip before and after picking.

Image Before Pick – This box should be selected to see each colony immediately prior to being picked.

Image After Pick - This box should be selected to see if each colony has been successfully collected by the pin.

These options can be switched on and off at any time during the run by using the Image Before Pick and Image After Pick check boxes.

Note: These images will not be saved. If saving the images is required refer to page 20 and select the **Save Target and Aspirate Images**.

Note: Activating these images significantly slows the picking speed and small colonies may not be visible.

Sanitize Pins

In between each pick and at the end of each run, the pins are automatically cleaned as specified in the set up.

Finish

When the picking has completed, the finish screen will appear. Click **Finish** to return to the **Pick Run** process start page. To return to the **Main Navigation Screen** click **Close Process**. If the process has not been saved, a window will appear prompting for the settings to be saved.

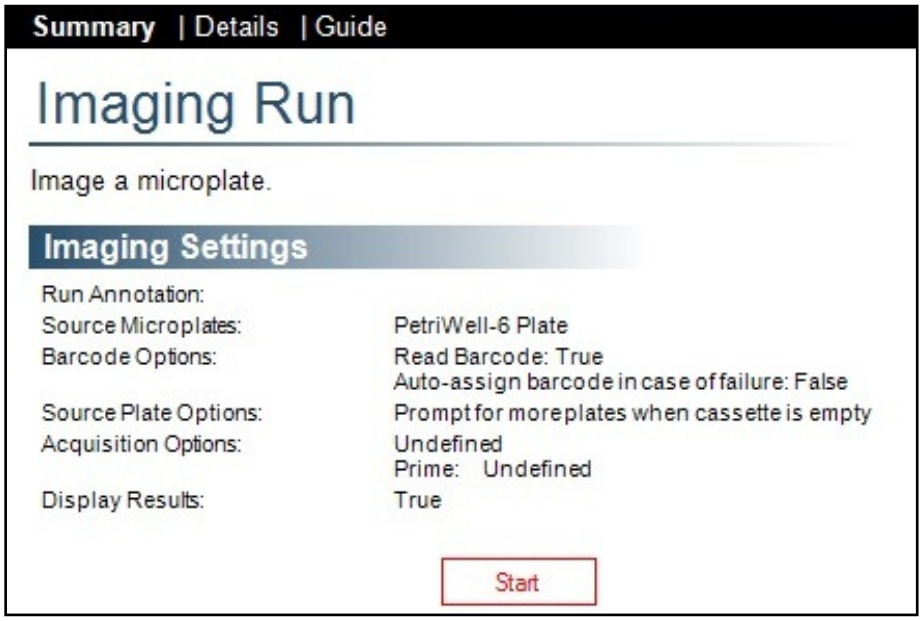
To view the results of the picking run, go to **Review Results** (Page 47).

Imaging Run

The **Imaging Run** process permits plate imaging without committing to a picking run. This default process contains all the parameters required for a standard imaging run, including image acquisition, colony detection and group settings.



After imaging, source plates are returned to the stacker cassette, and then the process ends. Imaged microplate results are accessible in **Review Results** with data for all detected colonies. Imaging run data can be reprocessed in **Review Results** (Page 47).

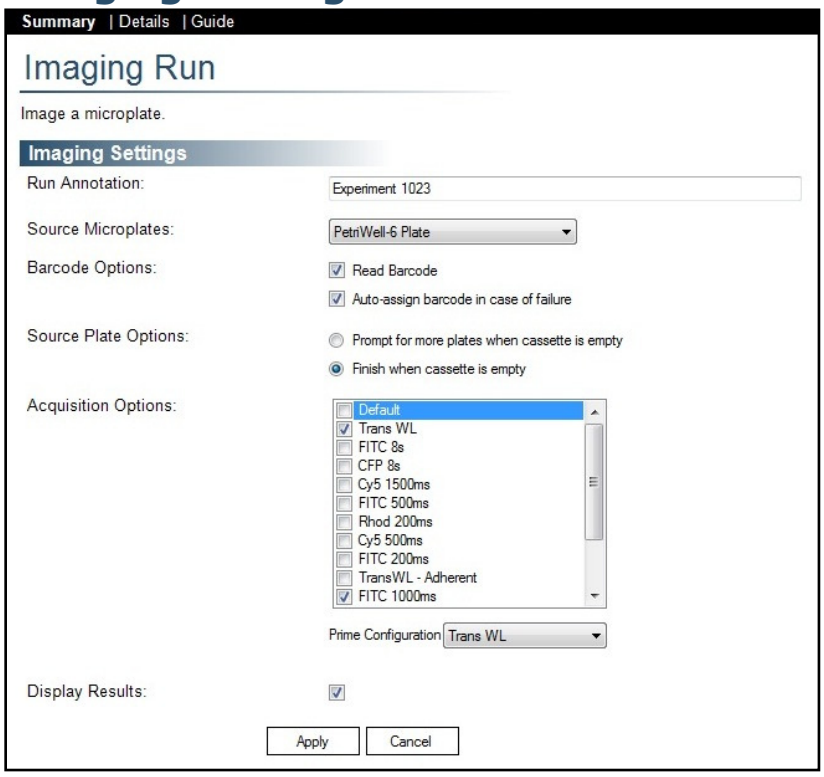


This screen is used to modify the **Imaging Settings** and then start the imaging process.

Modify Settings

The settings can be edited by clicking the section heading, **Details** or **Guide**.

Imaging Settings



Run Annotation

A name can be entered here to identify the run.

Source Microplates

The type of source microplates should be specified here by choosing a plate type from the drop-down menu.

Read Barcode

This box should be ticked if there is a barcode on the plate that needs to be read. If a barcode is not found while loading a plate, the machine will request one to be entered manually. If Auto-assign barcode in case of failure is selected and a barcode is not found the system will automatically give the plate a number with the prefix Auto. Using barcodes is recommended.

Source Plate Options

Two options are available relating to the source plate supply:

Prompt for more plates when cassette is empty – The software will display a prompt asking for more plates to be added to the cassette once it has imaged all plates present in the cassette.

Finish when cassette is empty – The software will not look for more plates once it has found there are no more plates present in the cassette.

Acquisition Options

This section lists the acquisition options that have already been created e.g. White Light, FITC. Select the desired options by ticking the relevant boxes. The acquisition options can be further modified in Preview (see page 23).

Prime Configuration

This is the image acquisition option to be used for colony detection. Normally, this will be the white light acquisition option, although a fluorescent option specifically designed for colony detection could be used, e.g. viability assay such as LiveDetect.

Display Results

Selecting this box will display the results at the end of the run. If this is not selected the run will end and results can be viewed via Review Results.

If these are new settings it is advisable to **Save Process** at this stage.

Start Imaging

When required settings have been created, clicking **Start** will begin the imaging process. The software will display a window prompting for the source plates to be loaded into the source stacker cassette. The source stacker can be raised or lowered at this point if required.

Preview

This screen permits the correct image acquisition and colony detection settings to be established. Please see **Preview** section under **Pick Run** for details (Page 23).

Select Wells

This screen permits the number of imaged wells of each plate to be specified. Please see **Select Wells** section under **Pick Run** for details (Page 26).

Summary

This screen provides a summary of the plates to be imaged.

Imaging

The selected wells will be imaged in sequence for each of the acquisition options selected and the plate(s) returned. The images will then be processed together for colony detection and groupings according to the defined settings.

Results

If the **Display Results** option was selected (see Image Settings above), the results will appear after imaging. The **Results Screen** has a similar format to the **Preview Screen**. All image areas can be instantly navigated to using the **Well Map** and collated statistics for the entire plate are now available. **Groups** options can be adjusted at this stage (see Groups tabs, Page 36). If the **Display Results** option was not selected, the images and results can be accessed via **Review Results** on the **Main Navigation Screen**.

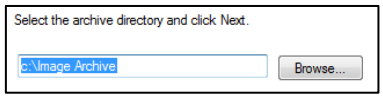
Finish

Once imaging is complete, the Finish screen will be displayed. Clicking on the **Finish** button will return the screen to the **Imaging Run Process** page. Clicking **Close Process** will return the screen to the **Main Navigation Screen**. If the process has not been saved a prompt advising this will appear. To view the results of the imaging run, go to **Review Results** (next section).

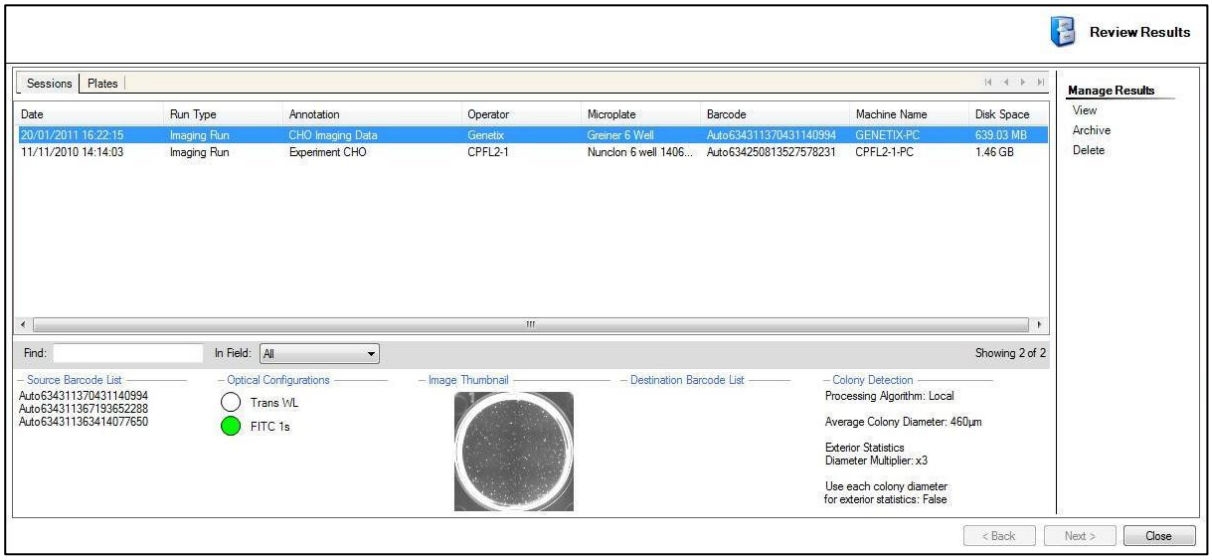
Review Results

Results of imaging and picking runs are reviewed here. The results can be viewed on ClonePix 2 or via the Remote Data Viewer (copies available for purchase from Customer Support).

By default, data is stored on the C: drive in a folder called **Image Archive**. Clicking **Next** will open the Image Archive or clicking **Browse** will allow data to be accessed remotely.



The following window will appear showing a list of completed runs:

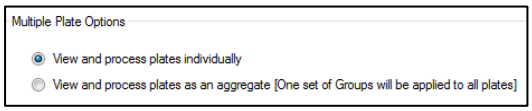


The runs will be displayed in two tabs: **Sessions** tab and in the **Plates** tab. In the **Session** tabs, results are displayed as one row even if the run consisted of several plates. In the **Plates** tabs the results are displayed as one row per plate processed so if there were three plates in the run, three individual rows would be displayed.

The results list can be re-ordered by a single click on the table headers, or a double click to invert the selection.

View

To view the data there are various options available:



In **Sessions**, either double click on the required row or click once on the required row and click View to open the data set.

In **Plates**, the above can be carried out or to view multiple plates use **Shift-Click** or **Control-Click** to highlight the required rows and then click on **View**.
There is also the option to combine the data from multiple plates and view and process the plates as an aggregate.

For each data set the following information is displayed (Under both Sessions and Plates tabs**): Date, Run Type, Annotation, Operator, Microplate, Barcode** and **Machine Name.**
It is possible to search the data sets using the Find option. The fields available to search in (drop-down menu) are the various information points mentioned above; Date, Run Type etc.

When a data set is selected the following information is displayed (Under both Sessions and Plates tabs):

Source Barcode List
This lists all the barcodes on the source plates used.

Optical Configurations
This lists all the optical configurations used in the selected run and the colors associated with them.

Image Thumbnail
The prime configuration well image is displayed here.

Destination Barcode List
This lists all the barcodes on the destination plates used.

Colony Detection
A small summary of the parameters used to detect the colonies is displayed here.

Once data has been opened for viewing, depending on whether imaging or picking data is being opened, all of the tabs available are the same as seen throughout the **Pick Run** and **Imaging Run stages**. In a picking run data set the **Picking Review** tab will be visible (see page 40).

Important note: For data integrity Pick Run data is fully accessible in Review Results but the data are locked and thus cannot be modified. Imaging Run data can be altered on-screen but cannot be saved.

Archive
Data stored to Image Archive can be easily copied or transferred to CD or DVD using the Archive result option. To do this, use Shift-Click or Control-Click to highlight the required rows and then click on Archive.

Delete
Clicking on a data set and then clicking Delete will permanently remove the data from the hard drive (Image Archive).

Maintenance Processes

Commissioning Process

The commissioning of the machine is to be carried out by our Approved Engineers. Some processes may be carried out under supervision by an Approved Engineer.



Utility Processes

Picking Head Management

Head management options should be used each time the head is removed and/or replaced.



Remove Head

Aids removal of the picking head only. If you wish to remove and replace head it may be more convenient to use Replace Head.

- Click on Remove Head icon to bring the head to a convenient location for removal.
- Remove Head by detaching the picking tubing and unscrewing the locking bolt.
- Close the door and click Next to return head to safe location.

Replace Head

Process to aid replacement of the picking head followed by preparation of the head for use.

- Click on Replace Head icon to bring the head to a convenient location for replacing.
- Replace Head by screwing in the locking bolt and pushing on the picking tubing. It is best to re-connect the tubes starting from the back.
- Click Next to advance to Pin Fire Test (See page 12).
- Click Next to advance to Alignment to align plate and primary pin (See page 13).
- Click Next to proceed to Pin Offsets:

Picking Head Pin Offsets

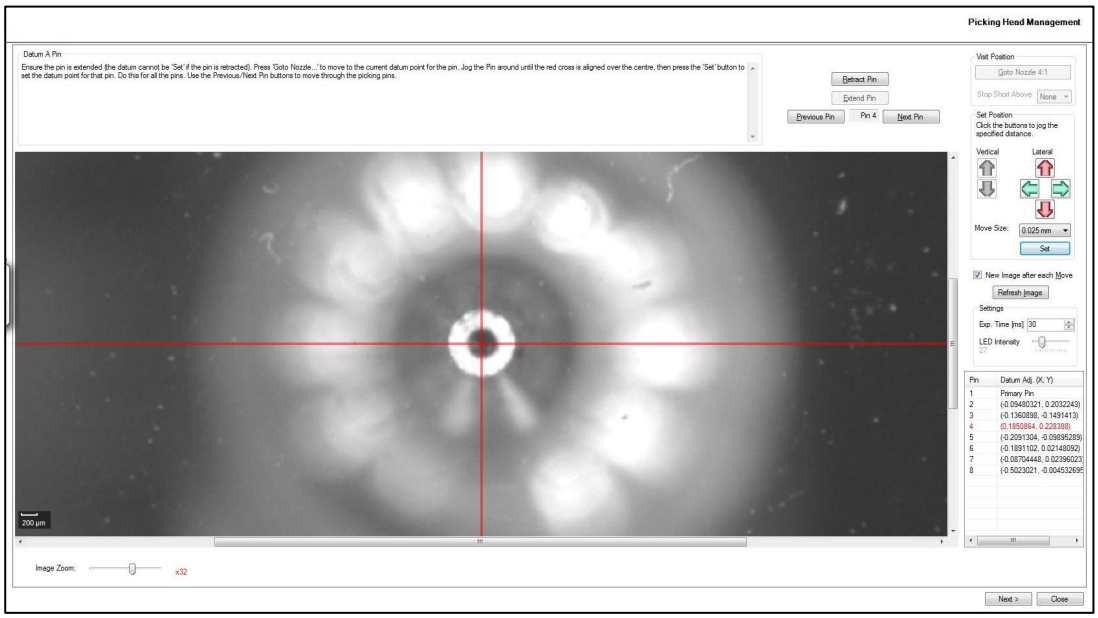
This process aligns the remaining seven pins relative to the primary pin. It must be carried out whenever the pins are removed or replaced to compensate for µm differences between pins.

- Following on from Alignment, a prompt will ask if the same plate is to be used. Click Next to continue or Close to cancel.
- Click Next to continue. This will extend the Primary Pin into well A1.

Note: The datum point for the primary pin cannot be set here; it should already have been set in the Alignment procedure above. If the primary pin is misaligned, exit the Replace Head process and start again.

- Click Next Pin. This will extend the next pin into well A1.
- Align the pin using the X and Y axis Jog buttons until the pin hole is centered on the red cross.
- When the pin is in the required position click Set. The new pin coordinates will be shown in red.
- Use the Next Pin button to move through and align/set the remaining pins.

Note: If the Previous Pin button is used the pin may appear not to be aligned because the actual position is shown rather than the offset on the assumption that you want to create the offset.



Pin correctly aligned to camera and pins 2 & 3 already aligned shown in red.

- Click Next to Wash Bath Utility (See page 15)
- Click Next to Sanitize Pins in Sterilizing Agent (See page 16).
- Click Next to Ultra Violet Sanitize (See page 16).
- Click Next to Sanitize Pins (purge the system with sterile water) (See page 17).
- Click Next twice to automatically park the head safely, return the source plate and close the illumination cover.

Utility Process

The **Utility Processes** provides a number of processes to control or diagnose the hardware.

Utility Processes available are:

Plate Handling

Source Plate

- Select Source Plate Station from the Plate Station drop down list.
- Select the type of plate from the microplate drop down list.
- Click Get Plate and ensure that the selected plate is collected from the Source Plate Station.
- Click Return Plate and ensure the selected plate is returned to the Source Plate Station.

Destination Plate

- Select Destination Plate Station from the Plate Station drop down list.
- Select the type of plate from the microplate drop down list.
- Click Get Plate and ensure that the selected plate is collected from the Destination Plate Station
- Click Return Plate and ensure the selected plate is returned to the Destination Plate Station

Sanitize Pins

- Select the number of purge cycles required.
- Select the number of cycles the picking pins will be scrubbed in the ethanol bath.
- Select the number of seconds the halogen dryer will be on for (recommend 10 seconds).

UV Sanitize

- This feature controls the germicidal lamp inside ClonePix 2 as described on page 16.

Wash Bath Utility

- This feature controls the ethanol wash bath inside ClonePix 2 as described on page 15.

Replacement Parts and Optional Extras

Please refer to the website for the latest replacement parts and optional extras.

Replacement Parts

Code	Description
X4941	Additional picking head populated with eight X4961 (F1 Pins)
X4942	Additional picking head populated with eight X4962 (F2 Pins)
X4949	Additional picking head without pins
X4961	F1 – ClonePix colony picking pin, 0.40mm diameter (Single pin)
X4962	F2 – ClonePix colony picking pin, 0.70mm diameter (Single pin)
X1036	O Rings for picking pins (Pack of 8)
X4970	Replacement picking tubing (Pack of 8)
X4948	Picking Pin Removal Key
X4975F	5L Feed (wash) bottle & fixings
X4975W	5L Waste bottle & fixings
X4976	Nylon bristle ethanol bath insert

Optional Extras

Code	Description
SL4950-A01	ClonePix Remote Data Viewer
X4990	ClonePix filter set ex 440 em 505 (CFP)
X4992	ClonePix filter set ex 500 em 550 (YFP)
X4993	ClonePix filter set ex 530 em 590 (CloneDetect 549, Rhodamine)
X4992	ClonePix filter set ex 622 em 700 (CloneDetect 622, Cy5)

Reagents and Supplies

Please refer to the website for the latest reagents & supplies, replacement parts and optional extras.

CloneDetect Detection Reagents

Cat No.	Description	Pack Size
K8295	Recombination CloneDetect anti-human IgG detection agent. FITC labeled.	10000U / 1ml
K8200	CloneDetect anti-human detection agent. FITC label. Sterile, azide free.	10000U / 1ml
K8201	CloneDetect anti-human detection agent. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8202	CloneDetect anti-human detection agent, FITC label. Sterile, azide free, BSA free.	10000U / 1ml
K8210	CloneDetect anti-human detection agent. 549 label. Sterile, azide free.	10000U / 1ml
K8211	CloneDetect anti-human detection agent. 549 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8212	CloneDetect anti-human detection agent. 649 label. Sterile, azide free.	10000U / 1ml
K8213	CloneDetect anti-human detection agent. 649 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8215	CloneDetect anti-human CIF (Complex Initiation Factor)	10000U / 1ml
K8205	CloneDetect human IgG (Fc-Chain) specific. FITC label. Sterile, azide free.	10000U / 1ml
K8206	CloneDetect human IgG (Fc-Chain) specific. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8252	CloneDetect anti-human detection agent. Kappa chain specific. FITC label. Sterile, azide-free.	10000U / 1ml
K8250	CloneDetect anti-human detection agent. Kappa chain specific. 549 label. Sterile, azide-free.	10000U / 1ml
K8251	CloneDetect anti-human detection agent. Kappa chain specific. 649 label. Sterile, azide-free.	10000U / 1ml
K8255	CloneDetect anti-human detection agent. IgM specific. FITC label. Sterile, azide-free.	10000U / 1ml
K8253	CloneDetect anti-human detection agent. IgM specific. 549 label. Sterile, azide-free.	10000U / 1ml
K8254	CloneDetect anti-human detection agent. IgM specific. 649 label. Sterile, azide-free.	10000U / 1ml
K8220	CloneDetect anti-mouse detection agent. FITC label. Sterile, azide free.	10000U / 1ml
K8221	CloneDetect anti-mouse detection agent. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8230	CloneDetect anti-mouse detection agent. 549 label. Sterile, azide free.	10000U / 1ml
K8231	CloneDetect anti-mouse detection agent. 549 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml

K8232	CloneDetect anti-mouse detection agent. 649 label. Sterile, azide free.	10000U / 1ml
K8233	CloneDetect anti-mouse detection agent. 649 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8225	CloneDetect mouse IgG (Fc-Chain) specific. FITC label. Sterile, azide free.	10000U / 1ml
K8226	CloneDetect mouse IgG (Fc-Chain) specific. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8235	CloneDetect anti-mouse CIF (Complex Initiation Factor)	10000U / 1ml
K8240	CloneDetect anti-rat detection agent. FITC label	10000U / 1ml
K8300	LiveDetect Green (494nm Ex/ 517nm Em). Sterile. In Atomizer applicator	50ul (for 5ml)

CloneMatrix

Cat No.	Description	Pack Size
K8510	CloneMatrix	1 x 40ml (100ml final media volume)
K8500	CloneMatrix	6 x 40ml (6 x 100ml final media volume)
K8530	CloneMatrix: optimized for CHO cells	1 x 40ml (100ml final media volume)
K8520	CloneMatrix: optimized for CHO cells	6 x 40ml (6 x 100ml final media volume)

CloneMedia / XPMedia

Cat No.	Description	Pack Size
K8610	CloneMedia (semi-solid media for hybridomas/myelomas)	1 x 90ml
K8600	CloneMedia (semi-solid media for hybridomas/myelomas)	6 x 90ml
K8685	CloneMedia-HEK (semi-solid media for serum-free HEK 293 cells)	1 x 90ml
K8680	CloneMedia-HEK (semi-solid media for serum-free HEK 293 cells)	6 x 90ml
K8710	CloneMedia-CHO (semi-solid media for CHO-s, CHOK1 and DG44 cells)	1 x 90ml
K8700	CloneMedia-CHO (semi-solid media for CHO-s, CHOK1 and DG44 cells)	6 x 90ml
K8740	CloneMedia-CHO-G (glutamine free semi-solid media for CHO-s, CHOK1 and DG44 cells)	1 x 90ml
K8730	CloneMedia-CHO-G (glutamine free semi-solid media for CHO-s, CHOK1 and DG44 cells)	6 x 90ml
K8725	CloneMedia-CHOK1SV (semi-solid media for CHOK1SV cells; glutamine-free)	1 x 90ml
K8720	CloneMedia-CHOK1SV (semi-solid media for CHOK1SV cells; glutamine-free)	6 x 90ml
K8712	CloneMedia-CHO DHFR (semi-solid media for DG44, DUXB11 and CHO-S cells)	1 x 90ml
K8742	CloneMedia-CHO DHFR (glutamine-free semi-solid media for DG44, DUXB11 and CHO-S cells)	1 x 90ml
K8650	XP Media (liquid media for cell line expansion of hybridoma/myeloma	500ml

	cells)	
K8690	XP Media-HEK (liquid media for cell line expansion of suspension HEK cells)	500ml
K8750	XP Media-CHO (liquid media for cell line expansion of CHO-S cells)	500ml
K8760	XP Media-CHO-G (Glutamine-free liquid media for cell line expansion of CHO-S cells)	500ml

PetriWell Cell Culture Plates

Cat No.	Description	Pack Size
W1000	PetriWell-1 Plate. Black with clear base. Polystyrene. TC treated	1/40
W1005	PetriWell-1 Plate. Black with clear base. Polystyrene. Non TC treated	1/40
W1007	PetriWell-1 Plate. Black with clear base. Polystyrene. Collagen coated	1/40
W1009	PetriWell-1 Plate. Black with clear base. Polystyrene. Poly-D-Lysine coated	1/40
W1050	PetriWell-1 Plate. Black with clear base. EquiGlass. TC treated	1/40
W1055	PetriWell-1 Plate. Black with clear base. EquiGlass. Non TC treated	1/40
W1055BC	PetriWell-1 Plate. Black with clear base. EquiGlass. Non TC treated. Bar-coded.	1/40
W1057	PetriWell-1 Plate. Black with clear base. EquiGlass. Collagen coated	1/40
W1059	PetriWell-1 Plate. Black with clear base. EquiGlass. Poly-D-Lysine coated	1/40
W1100	PetriWell-6 Plate. Black with clear base. Polystyrene. TC treated	1/40
W1105	PetriWell-6 Plate. Black with clear base. Polystyrene. Non TC treated	1/40
W1107	PetriWell-6 Plate. Black with clear base. Polystyrene. Collagen coated	1/40
W1109	PetriWell-6 Plate. Black with clear base. Polystyrene. Poly-D-Lysine coated	1/40
W1150	PetriWell-6 Plate. Black with clear base. EquiGlass. TC treated	1/40
W1155	PetriWell-6 Plate. Black with clear base. EquiGlass. Non TC treated	1/40
W1155BC	PetriWell-6 Plate. Black with clear base. EquiGlass. Non TC treated. Bar-coded.	1/40
W1157	PetriWell-6 Plate. Black with clear base. EquiGlass. Collagen coated	1/40
W1159	PetriWell-6 Plate. Black with clear base. EquiGlass. Poly-D-Lysine coated	1/40
W1500	PetriWell-96 Plate. Black with clear base. Polystyrene. TC treated	1/40
W1505	PetriWell-96 Plate. Black with clear base. Polystyrene. Non TC treated	1/40
W1507	PetriWell-96 Plate. Black with clear base. Polystyrene. Collagen coated	1/40
W1509	PetriWell-96 Plate. Black with clear base. Polystyrene. Poly-D-Lysine coated	1/40
W1510	PetriWell-96 Plate. Clear. Polystyrene. TC treated	1/40
W1515	PetriWell-96 Plate. Clear. Polystyrene. Non TC treated	1/40
W1515BC	PetriWell-96 Plate. Clear. Polystyrene. Non TC treated. Bar-coded.	1/40
W1517	PetriWell-96 Plate. Clear. Polystyrene. Collagen coated	1/40
W1519	PetriWell-96 Plate. Clear. Polystyrene. Poly-D-Lysine coated	1/40
W1550	PetriWell-96 Plate. Black with clear base. EquiGlass. TC treated	1/40
W1555	PetriWell-96 Plate. Black with clear base. EquiGlass. Non TC treated	1/40

W1557	PetriWell-96 Plate. Black with clear base. EquiGlass. Collagen coated	1/40
W1559	PetriWell-96 Plate. Black with clear base. EquiGlass. Poly-D-Lysine coated	1/40

Other

Cat No.	Description	Pack Size
K2505	aQu Clean pin cleaning solution	1L
K8080	Sterilizing Agent (1L per Sachet)	10
K8085	Sterilizing Agent (1L per Sachet)	50
K8150	CaliBeads: Fluorescent beads for ClonePix 2. 200µm diameter. Pan-wavelength.	100ml
K8010	Adherent Cell Picking Reagent – Type A	1 x 500ml
K8020	Adherent Cell Picking Reagent – Type B	1 x 500ml
K8030	Adherent Cell Picking Reagent – Type C	1 x 500ml
K8040	Adherent Cell Picking Reagent – Type D	1 x 500ml
K8005	Adherent Cell Picking Reagent Test Kit Types A – D	4 x 250ml
K8100	ProbeClean: Fluorescently labeled Antibody Clean-up Columns	50

Appendix A: Imaging Definitions

Statistics for Prime Configurations

Statistic	Description	Category	Unit
Actual X	The X co-ordinate of the centre of the feature in mm	Position	mm
Actual Y	The Y co-ordinate of the centre of the feature in mm	Position	mm
Area	The area covered by the feature (excluding 'child' features) in square mm	Morphology	mm^2
Axis Ratio	The ratio of the minimum and maximum radiuses of the feature measured from 0 (very elongated) to 1 (a perfect circle)	Morphology	
Block	The identifier of the block of features this feature is associated with	General	
Compactness	A measure of how compact the feature is, measured from 0 (not compact) to 1 (a perfect circle)	Morphology	
Deposit Barcode	Barcode of the plate the colony has been deposited in	Information	
Deposit Well	The well the colony has been deposited in	Information	
Edge Excluded	Whether the colony center lies within the exclusion zone	Position	
Feature ID	A unique identifier	General	
Group	Group the feature is assigned to	General	
Image Column	The column the image containing the feature is in	Position	
Image Row	The row the image containing the feature is in	Position	
Intensity SD	The standard deviation of the intensity of the pixels in the feature	Intensity	
Manual Group	Whether the feature was manually added to its current group	General	
Mean Centre Intensity	The mean intensity of the nine pixels at the centre of the feature	Intensity	
Mean Intensity	The mean intensity of all the pixels in the feature	Intensity	
Median Intensity	The median intensity of all the pixels in the feature	Intensity	
Perimeter	The length of the perimeter of the feature in mm	Morphology	mm
Picked	Flag to signify if the colony has been picked.	Information	
Pixel Area	The area covered by the feature (excluding 'child' features) in pixels	Morphology	pixels
Pixel Perimeter	The length of the perimeter of the feature in pixels	Morphology	pixels
Pixel Radius Max	The maximum radius of the feature in pixels	Morphology	pixels
Pixel Radius Min	The minimum radius of the feature in pixels	Morphology	pixels
Pixel Radius SD	The radius standard deviation of the feature in pixels	Morphology	pixels
Pixel Total Area	The total area of the feature (including 'child' features) in pixels	Morphology	pixels
Pixel X	The X co-ordinate of the centre of the feature in pixels relative to the top left of the image	Position	pixels
Pixel Y	The Y co-ordinate of the centre of the feature in pixels relative to the top left of the image	Position	pixels
Proximity	The distance to the closest neighboring colony in the same image	Morphology	mm
Radius Max	The maximum radius of the feature in mm	Morphology	mm

Radius Min	The minimum radius of the feature in mm	Morphology	mm
Radius SD	The radius standard deviation of the feature in mm	Morphology	mm
Saturated Percentage	The percentage of saturated pixels in the feature	Intensity	%
Saturated Pixels	The number of saturated pixels in the feature	Intensity	
Selected	Whether the feature is currently selected	General	
Source Barcode	The barcode of the plate that the feature is in	Position	
Source Well	The well the feature is in	Position	
Total Area	The total area of the feature (including 'child' features) in square mm	Morphology	mm^2
Volume Equivalent	The volume of a sphere having the same cross-sectional area as the feature in cubic mm	Morphology	mm^3
Well Index	A numerical annotation of the Source Well for graphical presentation	Position	

Appendix B: Imaging Definitions

Statistics for Fluorophore Configurations

Statistic	Description	Category	Unit
Exterior Area	The area of the pixels outside the feature boundary in the local vicinity of the feature	Intensity	mm²
Exterior Mean Intensity	The arithmetic mean intensity of all the pixels outside the feature boundary in the local vicinity of the feature	Intensity	
Exterior Geo Mean Intensity	The geometric mean intensity of all the pixels in the feature	Intensity	
Exterior Median Intensity	The median intensity of all the pixels outside the feature boundary in the local vicinity of the feature	Intensity	
Exterior Total Intensity	The total intensity of all the pixels outside the feature boundary in the local vicinity of the feature	Intensity	
Interior Intensity SD	The standard deviation of the intensity of the pixels in the feature	Intensity	
Interior Mean Centre Intensity	The mean intensity of the nine pixels at the centre of the feature	Intensity	
Interior Mean Intensity	The arithmetic mean intensity of all the pixels in the feature	Intensity	
Interior Geo Mean Intensity	The geometric mean intensity of all the pixels in the feature	Intensity	
Interior Median Intensity	The median intensity of all the pixels in the feature	Intensity	
Interior Total Intensity	The total intensity of all the pixels in the feature	Intensity	
Normalized Intensity	The total intensity of the feature divided by the primary area	Intensity	
Sum Total Intensity	The sum total intensity of the feature (interior and exterior)	Intensity	

Appendix C: Guide to Fluorophore Configurations

The ClonePix 2 acquires images of fluorescing colonies and generates a range of statistics from the images in order that the colonies can be screened to select the most 'fit'. The user can choose which parameters to use to meet their definition of 'fit' – the software does not enforce any particular definition of what the 'best' colonies are.

Most users wish to select either the highest expressing colonies or those that are positive for a specific parameter. The software generates a variety of statistics to facilitate this. This appendix explains how these statistics are generated and provides advice on how they might be used.

Measuring Fluorescence

The ClonePix 2 software works on the principal that all colonies can be detected under white light. All of the detection algorithms in the software process the white light (Prime Probe) images to detect colony boundaries.

There are two principal advantages to this approach; 1) colonies that are not fluorescing can still be detected and taken into account when deciding which colonies to select and 2) knowing the boundaries of all colonies allows for a more rigorous approach to processing the fluorescent images.

Fluorescent colonies can be divided into two types; 1) those whose fluorescence is purely 'internal', i.e. within the colony boundary as determined from the white light image and 2) those whose fluorescence is also present outside the colony boundary.

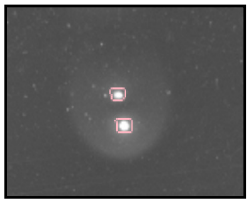
Generating statistics for the first type of fluorescent colony is straightforward because for any given pixel in the image the pixel is either within the boundary of one colony or it is a background pixel and can be ignored. The only complication is determining what proportion of the pixel's intensity is 'real' fluorescence and what proportion is 'background' fluorescence. Background fluorescence would exist even in the absence of any colonies since fluorescence images rarely have a background of zero – pure black.

Generating statistics for the second type of fluorescent colony (those whose fluorescence is also present outside the colony boundary) is more problematic. This is because when two or more of these colonies are close together, their fluorescence will mingle together and there is no deterministic mechanism to accurately decide what proportion of a given pixel's intensity can be attributed to each colony. The problem is compounded as the density of colonies increases (i.e. when there are more colonies in each plate). Additionally, fluorescence from the second type of colony can affect the first type of colony, which can therefore no longer be assumed to be independent of other colonies.

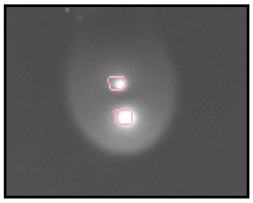
Because of this difficulty, the ClonePix 2 software does not attempt to determine what proportion of intensity of pixels outside of colony boundaries comes from any particular colony. Instead, all of the pixels surrounding each colony are included, as explained below. This results in some pixels being included in the total for several colonies, but this isn't a shortcoming as will become clear.

Finally, it is important to appreciate that the ClonePix 2 measures relative fluorescence, not absolute fluorescence. This means that fluorescent statistics can only meaningfully be compared when generated from the same plate or batch of identical plates at the same time. It is not necessarily meaningful to compare fluorescent statistics between different plates imaged on different days. This is because a number of things can vary that affect fluorescence, for example exposure time. Varying the exposure time used when acquiring fluorescent images will increase or decrease the absolute values of the fluorescent statistics. However, all colonies will in general get brighter or darker thus their brightness relative to each other is unaffected.

Example of Fluorescent Statistic Generation



The white light image (right) shows two colonies close together. Their approximate boundaries, as determined by the colony detection algorithm are shown in pink.



The corresponding fluorescent image is shown (right) with the colony detection algorithm in pink overlaid. A number of fluorescent statistics can be generated from this directly.

Note: a background subtraction algorithm is applied to all fluorescent images before intensity statistics are generated. This algorithm seeks to determine what level of fluorescence there would be in the absence of any colonies, and removes this from the image so that the intensity statistics only include 'real' fluorescence.

Interior Intensity Statistics

The simplest statistic is **Interior Total Intensity**. This is the sum of the intensities of all of the pixels of the fluorescent image within the colony boundary from the matching white light image. Larger colonies will have larger totals because they are larger. This statistic is not therefore especially useful on its own but several other statistics are derived from it.

Interior Mean Intensity is Interior Total Intensity divided by colony area (in pixels). It is the 'average' brightness of all pixels within the colony, or the arithmetic mean. Since it does take account of area, this statistic can be used to compare colonies with different areas. In fact, this is probably the most powerful statistic (along with Interior Median Intensity, described below) since colonies with a high Interior Mean Intensity are overwhelmingly likely to be bright colonies – rather than simply faint colonies that happen to be near a bright colony that is dispersing fluorescence into the surrounding medium.

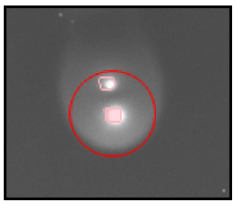
Interior Geo Mean Intensity is similar to the Interior Mean Intensity but is calculated as the geometric mean. This statistic will never be greater than the Interior Mean Intensity, since the way it is calculated tends to reduce the influence of the largest values. Whether the geometric mean is a 'better' statistic to use than the arithmetic mean depends entirely on the colonies in question.

Interior Intensity SD (Standard Deviation) is a measure of how the intensity of pixels within a colony boundary varies. Colonies with a low value for this statistic must have pixels that are all of a similar brightness. Conversely, those with high values will have both bright pixels and faint pixels with their colony boundaries. This statistic can be used to differentiate between colonies that have similar Interior Mean Intensity Values.

Interior Median Intensity is the intensity of the pixel midway between the faintest pixel and the brightest pixel within the colony boundary. This is similar to Interior Mean Intensity described above but tends to be more robust since its value is less affected by a few bright or a few faint pixels. As with Interior Mean Intensity, colonies with a high Interior Median Intensity are very likely to be bright colonies.

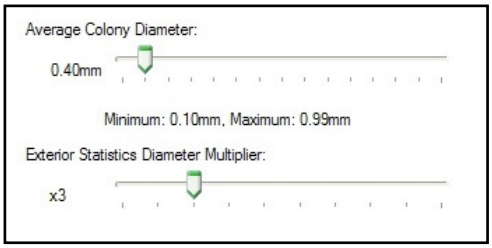
Interior Mean Centre Intensity is the mean intensity of the nine pixels closest to the centre of the colony (i.e. the center pixel and its immediate eight neighboring pixels). Often, colonies are brightest at their centre so this statistic provides an unbiased measure of the brightness of a colony.

Exterior Intensity Statistics Calculation

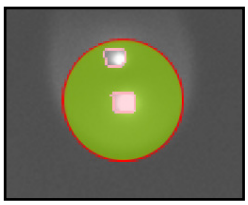


The fluorescent image is shown again on the right with the lower colony highlighted (its interior is shaded pink). Around the colony is a red circle, which is visualized by moving the mouse pointer over the colony. The diameter of the red circle is derived from a combination of the Average Colony Diameter and the Exterior Statistics Diameter Multiplier provided by the user in the Detection tab when the image was originally processed.

In this example, the user had selected an average colony diameter of 0.4mm and an Exterior Statistics Diameter Multiplier of x3 (left). Therefore the red circle will have a diameter of 1.2mm. It is important to appreciate that the size of this circle is the same for every colony in the image even though the colonies themselves are different sizes.



The significance of the red circle is that it defines the boundary within which pixels for a given colony are processed to generate the Exterior Intensity Statistics.

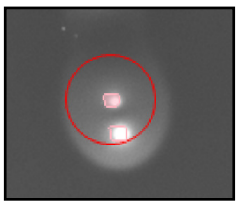


Exterior Intensity Statistics are generated by firstly considering all of the pixels within the red circle. Following this, any pixels within the colony of interest's boundary are excluded (the pixels shaded in pink). Next, all of the pixels that lie within any other colonies are excluded. So in the image on the right, all of the pixels within the boundary of the second unshaded colony would be excluded. This is because any fluorescence within a neighboring colony's boundary is quite likely to have come from the neighboring colony.

What remains are the pixels that are local to the colony of interest but not within any other colony boundary (highlighted in green in the image). These are the pixels that are used to generate the 'exterior intensity' statistics.

Note that if the colony is larger than the Average Colony Diameter specified by the user or if the colony is an irregular shape, it is possible that some of the pixels within the colony boundary would lie outside the red circle. In those circumstances, the red circle will not be a

good approximation of the area 'local' to the colony. Also it is not clear how to meaningfully compare irregular shaped colonies with the more circular shaped colonies because it is unlikely that the 'local' area values produced can be used for comparative purposes.



Neighboring colonies are treated in exactly the same way. In the image to the right, the top colony (shaded in pink) has been selected. Again, the red circle overlaps the lower colony. This means that many pixels will be used to generate exterior statistics for both colonies.

In this example both colonies are fluorescing and dispersing their fluorescence into the local medium so, for the pixels close to both colonies, it is not clear what proportion of the intensity of each pixel is derived from which colony. The area of fluorescence around the colonies looks quite uniform implying that the fluorescence from each colony isn't cumulative. This suggests that counting the intensities of the pixels twice (once from each colony) would be a reasonable approach.

Exterior Intensity Statistics

Exterior Area (mm2) is the area of the 'exterior' pixels (those shaded green in the image above). It could be used to exclude colonies that are in crowded regions of the plate. A low value for a given colony compared to other colonies in the image will indicate that the exterior intensity statistics for that colony may not be accurate. It is not in any way a measure of the actual size of the halo of fluorescence for any colony.

Exterior Total Intensity is the sum of the intensities of all of the 'exterior' pixels. If there are neighbouring colonies within the red circle, this value will be lower. On its own, this statistic is not very useful since it cannot be used comparatively with other colonies.

Exterior Mean Intensity is the Exterior Total Intensity divided by the number of pixels in the exterior area (arithmetic mean). This statistic provides a good approximation of how bright the area immediately surrounding a given colony is, though there is no implication that the brightness is coming from the colony as opposed to one or more of its neighbors.

Exterior Geo Mean Intensity is similar to the Exterior Mean Intensity but is the geometric mean. See the explanation of Interior Geo Mean Intensity for further details.

Exterior Median Intensity is the intensity of the pixel midway between the brightest and the faintest pixel within the exterior pixels. Just as the Interior Median Intensity is more robust than the Interior Mean Intensity, so the Exterior Median Intensity is more robust than Exterior Mean Intensity.

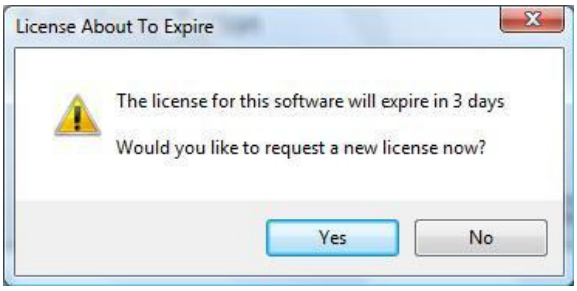
Sum Total Intensity seeks to approximate the sum of the Exterior Total Intensity and Interior Total Intensity if there were no neighboring colonies within the red circle. Instead of using the actual Exterior Total Intensity it uses the Exterior Mean Intensity multiplied by the number of pixels within the red circle. The calculation also excludes the pixels within the colony of interest's boundary but includes any pixels in neighboring colonies in the red circle. If this calculation were not taken into account it would not be meaningful to compare Sum Total Intensity between colonies because the value would vary depending on the presence of neighboring colonies.

Normalized Intensity is Sum Total Intensity divided by colony area (in pixels). The highest secreting colonies will have the highest values here but unfortunately in practice normalizing by area biases the statistic in favor of small colonies rather than bright colonies. This is because the interior intensity is always much brighter than the exterior intensity which decreases exponentially from the colony boundary.

Appendix D: Licensing

Requesting a license

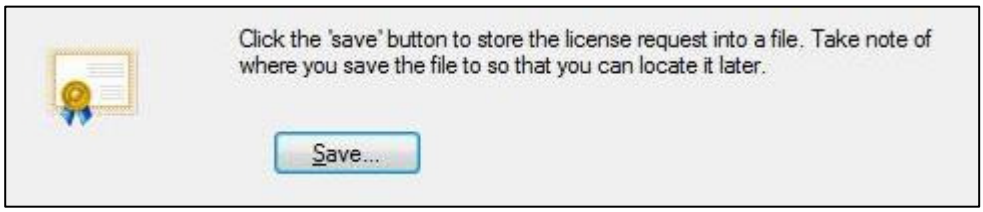
If the following message appears when booting ClonePix 2 software, a new license will need to be requested. This is easily done by creating a license request and then e-mailing it to Customer Support.



→ Click **Yes**.



→ Select "Request a new license" and click **Next**.



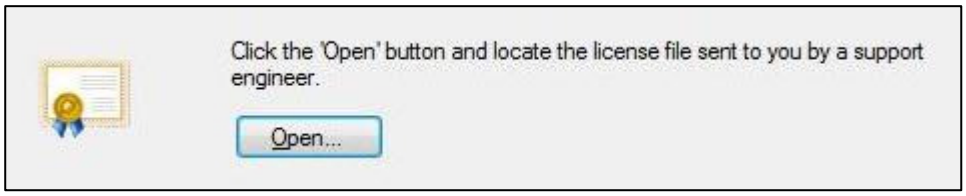
→ Save the file to a location where it is accessible to e-mail.
→ E-mail this *.req file to a Customer Support representative.
→ Click **Finish**.

Installing a license

- When a license file is sent back by a Customer Support representative, save the file to a place accessible on the computer of ClonePix 2.
- Re-open the ClonePix 2 software.
- Select "Install a license file".



- Click **Next**.



- Click **Open**, and browse to the location where the license (*.License) file was saved.
- Open the file.
- A window should appear to indicate that the license file was successfully installed.



- Click **Finish**. The software will now boot.

Appendix E: Reviewing and Exporting Data

The ClonePix 2 generates a large quantity of data, in the form of images, statistics and logs of samples transferred between well plates. This appendix explains the various options available for accessing this data.

Review Results

The simplest form of access is to use Review Results via the icon on the main screen. This provides access to all images and statistics generated on the ClonePix 2, including colony detection overlays and settings. Review Results is the best way to access historical data, since it is able to present the data in the way it was originally generated, including links between graphs, data tables and images. For Pick Runs, an extra tab called Output Results is displayed to present destination plate, well and barcode information. Pick Run data are non-modifiable to provide an accurate record of the run. Imaging Run data are modifiable to permit free play within the Detection and Group tabs, although such changes are not saved when the file is closed.

Graphs and images can be exported from within Review Results and either copied to the Windows clipboard or saved as a file. They can easily be pasted into presentations, etc. as required.

Statistics can also be exported, with a choice of data formats including comma separated values (CSV), XML and Excel-XML. Statistics are exported by clicking the 'Export Statistics' button on the 'Statistics' tab.

Remote Data Viewer

This is an optional software program for accessing ClonePix 2 results remotely on another PC or laptop (order code: SL4950-A01). The format is identical to the Review Results feature in ClonePix 2 software (see above), and permits results to be accessed on ClonePix 2 via a network or copies of runs transferred elsewhere.

Results Report

A Results Report can be generated to provide a concise summary of any run including user-defined settings. It can be created from within Review Results or the Remote Data Viewer and is generated in XML format so that it can be opened in a web browser and easily printed or stored electronically. Results Reports are generated by clicking the 'Generate Report' button on the 'Statistics' tab.

Data Tracking Logs

Important note: The editing of Data Tracking Logs requires entering the Configuration section of ClonePix 2. It is strongly advised that this should only be done under supervision by an Approved Engineer.

A Data Tracking log file is created automatically for each run. This is an XML file with the same schema used by other instruments. It includes some statistical information for each colony along with a 'replicate' element for each transfer from a source well to a destination well.

The Data Tracking log may be of interest for those wishing to automatically process the transfer of samples between plates. However the Automatic Logs option may be more useful (see below).

Data Tracking logs are generated in the 'Data Tracking' sub folder of the configuration folder. The configuration folder is stored under the local applications data folder. On Windows XP, the location is usually:

"C:\Documents and Settings\All Users\Application Data\Molecular Devices\Fusion\ClonePix 2\Data Tracking"

On Windows Vista and Windows 7:

C:\ProgramData\Molecular Devices\Fusion\ClonePix 2\DataTracking

One file is generated for each imaging or picking run. The file name is derived from the data and time of the run, and the file extension is XML.

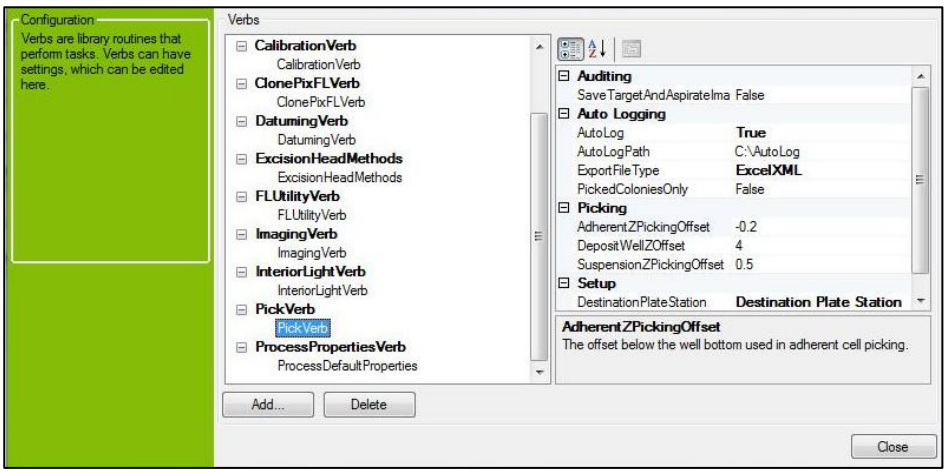
Automatic Logs

Important note: The editing of Automatic Logs requires entering the Configuration section of ClonePix 2. It is strongly advised that this should only be done under supervision by an Approved Engineer.

'Auto Logs' can be generated by the system for each run, and some level of control of their content is provided. Auto Logs are not generated by default, but when enabled one log is generated for each run in a location specified by the user.

The log is broadly the same format as that provided by the 'Export Statistics' button in Review Results or the Remote Data Viewer.

The settings to control Auto Logs are accessible from the 'PickVerb' in the Verbs section of the Configuration screen (available from the 'Tools' menu of the ClonePix 2 software).



There are four settings which control Auto Logs:

AutoLog

Controls whether Auto Logs are generated. Set to ‘True’ to enable.

AutoLogPath

The location to output the logs to. It must be a fully qualified path of a folder that the operator of the software has write access to. This can be a mapped drive, but be aware that if the drive is not available during a run, the run could abort abnormally.

ExportFileType

The format of file to generate. Can be comma separated values (CSV), XML or Excel-XML.

PickedColoniesOnly

Whether to only include in the output file statistics for colonies that were actually picked (‘True’) or all colonies (‘False’).

One folder is generated for each run, whose name is derived from the data and time of the run. A file is created in the folder with the name ‘Auto.csv’ or ‘Auto.xml’ depending upon the file type selected.

Raw Data

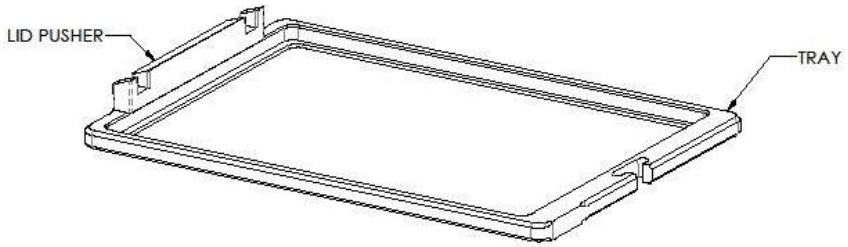
The default location for raw data (TIF images and XML files) is: “C:\image archive”. Viewing data from this location is not recommended.

Appendix F: Plate Compatibility Matrix

The ClonePix 2 stacker system uses plate holders and a lid lift system to transport the plate into the correct position ready for picking and depositing.

Plate Holders

There are two types of plate holder that are specifically designed for various plate types. The difference between the two types is the Lid Pusher situated at the back of the plate holder. This is an important part of the plate holder as it pushes the lid onto the lid lift. If the wrong plate holder is used the lid will not be pushed onto the lid lift correct and will result in stacker failure.



Source Plate	Lid Pusher Color	Lid Lifter Assembly
Petriwell-1	Blue	Silver
Petriwell-6	Blue	Silver
Greiner 6 well - #657160	Green	Red
Nunc Omni - #165218	Blue	Silver
Nunc – 6 Well	Green	White

Destination Plate	Lid Pusher Color	Lid Lifter Assembly
Costar 96 FB - #3300	Blue	Silver
Costar 96 FB - #3595	Blue	Silver
Costar 96 FB - #3596	Blue	Silver
Costar 96 FB - #3598	Blue	Silver
Falcon 96 FB - #353072	Blue	Silver
Petriwell-96	Blue	Silver
Greiner 96 FB - #655185	Blue	Silver
Matrix 96 FB - #4942	Blue	Silver
Nunc 96 FB - #167008	Blue	Silver
Nunc 96 FB - #143761 (Lids from 163320)	Blue	Silver

Lid Lifter Assembly

There are three different types of Lid Lifter Assembly used on the ClonePix 2. These assemblies have been designed to remove the lids of specific plate types. It is very important the correct lid lifter assembly is used with the correct plate otherwise plate jams are likely to occur. The table above lists the three types and the corresponding compatible plate types.

Appendix G: Glossary of Terms

Barcode

A unique label for source and destination microplates. Recommended barcoded plate types for colonies suspended in semi-solid medium are PetriWell-1 plates (Catalogue number W1055BC) and PetriWell-6 plates (Catalogue number W1155BC)

Colony

A clonal group of cells, typically of mammalian origin, that have grown either adherently or suspended in semi-solid medium.

Datum Point

A series of X, Y, Z co-ordinates that define a set position on the Robot bed.

Destination plate

96-well microplate(s) prefilled with liquid medium to collect picked colonies.

Dispersal

Dispersal Option separates out the cells of a picked colony by aspirating and dispensing in the destination plate.

Epi-illumination

The white light option normally used for configuring the robot and for visualising colonies during picking, using LEDs located under the source plate being imaged.

EquiGlass

A high quality polymer used as the base material for PetriWell plates. It has excellent properties for optimal fluorescent imaging.

Halogen dryer

Proprietary ultra-high temperature dryer used as part of the Pin Sanitise system.

Image Acquisition

The capturing of images using pre-defined Acquisition Options.

Image Archive

Default location for Pick Run and Imaging Run images and data. Accessible via Review Results.

LED intensity

ClonePix 2 uses only LEDs for consistent and reliable imaging. The intensity of the LEDs can be adjusted by the user to control image exposure under the Acquisition tab.

LiveDetect

A reagent for visualising only live cells. Can be used as a fluorescent alternative to imaging colonies by white light. Catalogue number K8300.

.gfp file

Format used to save a Process.

.gft file

Format used to save a Process Template.

Interior Light

Illuminates the interior of the instrument. Can be activated or deactivated at any time using the Interior Light icon in the bottom right corner of the screen. Interior Light is not used for imaging and will automatically switch off during imaging

PetriWell-1 plate

Single well plate optimized for visualizing plated colonies. The non-treated type (Catalogue number W1055) are recommended for colonies suspended in semi-solid medium. The TC-treated type (Catalogue number W1050) are recommended for colonies grown as adherent monolayers.

PetriWell-6 plate

Six well plate optimized for visualizing plated colonies. The non-treated type (Catalogue number W1155) are recommended for colonies suspended in semi-solid medium. The TC-treated type (Catalogue number W1150) are recommended for colonies grown as adherent monolayers.

Picking Pins

Reusable stainless steel tools used to collect colonies. F1 pins have an internal tip diameter of 400µm and are for picking suspended colonies. F2 pins have an internal tip diameter of 700µm and are for picking adherent colonies (monolayers).

Primary Pin

The picking pin nearest the front of the robot. All other pins are aligned relative to this one.

Prime Configuration

The Acquisition Option that will be used for colony detection. Normally this is based on white light imaging.

Process

A standard program for ClonePix 2 to carry out a task such as a series of similar experiments, or a maintenance task. A Process is saved as a .gfp file.

Proximity Indicators

Red lines between colonies on the image that show the nearest neighbour for each colony. Note: This feature is not the same as the colony exclusion feature in Groups.

Remote Data Viewer

An optional software program (order code: SL4950-A01) for accessing ClonePix 2 results remotely. Please contact Customer Support for further information.

Semi-solid medium

A viscous cell culture medium such as CloneMedia used to immobilize cells in suspension to permit growth of clonal colonies.

Source plate

One- or 6-well microplate(s) containing colonies for picking.

Sterilizing Agent

Recommended for sterilizing ClonePix 2 and its components and accessories. Catalogue number K8080.

Template

A program from which Processes can be generated. A Template is saved as a .gft file.

Transillumination

The white light option normally used to detect colonies using LEDs positioned on the robot head.

Wash bottle

4 liter bottle of sterile water used to purge the fluid system (1 liter in some older systems).

Waste bottle

4 liter bottle used to collect purged fluid (1 liter in some older systems).

White Light

Full spectrum LED illumination, of which there are two options: Transillumination (Trans) is normally used to detect colonies. Epi-illumination is normally used for configuring the robot and for visualising colonies during picking.

X Drive

Robot axis running from right to left.

XML

Extensible Markup Language. A simple text format derived from SGML Originally designed to meet the challenges of large-scale electronic publishing, XML is also used in the exchange of a wide variety of data on the Web.

Y Drive

Robot axis running from back to front.

Z Drive

Robot axis running vertically from high to low on the Robot bed.

Index

A

Acquisition Options 19
Alignment 13
Archive 48
Audit Options 21
Average Colony Diameter 25

B

Barcode Options 19
Batch Pick Run 7
Batch Plates 19

C

Check Bottles 17
Collate by Well 21
Configuration 9
Creating New Groups 38

D

Data Table tab 32
Drives 9

E

Edge Excluded 37

F

File Menu 10
Full Screen 31

G

Gallery tab 31
Generate Report 33
Graphs tab 34
Groups tab 36

H

Help Menu 11
Histogram 30

I

Image After Pick 43
Image Archive. 47
Image Before Pick 43
Images tab 29
Imaging Run 8
interior light 9
Irregular 1 37
Irregular 2 37

M

Main Navigation Screen 9, 10

N

Non-Cellular tab 35

O

Overview tab 28

P

Palette 30
Park Safe 43
Picking Review tab 40
Picking Summary 41
Pin Fire Test 12
Plate Holders 69
Plates tab 47
Prepare for Pick Run 12
Preview 23
Prime Configuration 19
Proximity 37

R

Rank 34
Review Colony Selection 20
Run Annotation 19

S

Sanitize Pins 17
Sanitize Pins in Sterilizing Agent 16
Scatter 34
Sessions tab 47
Sort Options 40
Source Microplates 19
Source Plate Options 19
Spot Count 36
Start up 9
Statistics tab 39

T

Thumbnail 29
Too Big 37
Too Small 37
Tools Menu 11

U

Ultra Violet Sanitize 16
Ungated 38

V

View Menu 11

W

Wash Bath Utility 15
Well Map 24
workflow 6

Contact Details

Molecular Devices

Queensway, New Milton
Hampshire BH25 5NN, UK

Tel: +44 (0) 1425 624 600
Fax: +44 (0) 1425 624 700
Web: www.moleculardevices.com/genetix

For all technical queries please contact your nearest Customer Support group. Visit www.moleculardevices.com/genetix for latest contact details.

Trademarks

ClonePix™, CloneSelect™, CellReporter™, *Half*BD®, '*N*Rich®, SlidePath™, Data Arena™ and Image Arena™ are trademarks of Molecular Devices (New Milton) Ltd.

Copyright © 2011 by Molecular Devices (New Milton) Ltd

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form by any means, electronic, mechanical, by photocopying, recording, or otherwise, without the prior written permission of Molecular Devices (New Milton) Ltd.

Information furnished by Molecular Devices (New Milton) Ltd is believed to be accurate and reliable; however, no responsibility is assumed by Molecular Devices (New Milton) Ltd, for its use; nor for any infringements of patents or other rights of third parties which may result from its use. No license is granted by implication or otherwise under any patent rights of Molecular Devices (New Milton) Ltd.

Revised June 2011