MetaXpress® High Content Image Acquisition & Analysis Software

Version 5.0

Analysis Guide

5020933 A
July 2012

Molecular Devices

For research use only. Not for use in diagnostic procedures.

Product manufactured by Molecular Devices, LLC.
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

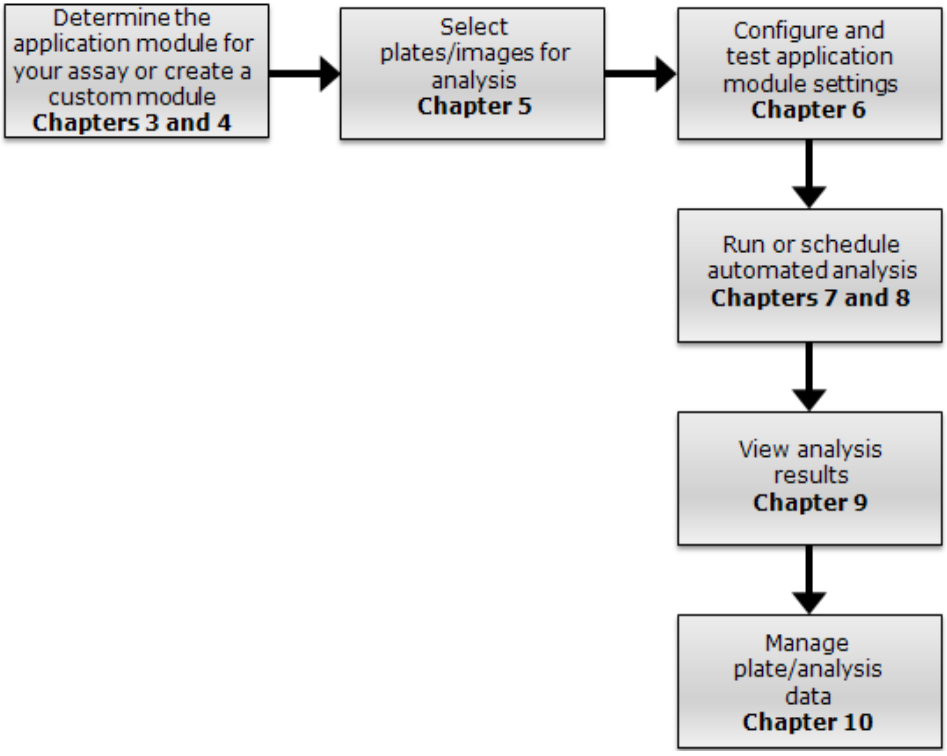
Contents

1 Introduction

The MetaXpress® High Content Image Acquisition & Analysis Software is divided into two major parts:

- Acquisition, which involves configuring settings, acquiring images, and storing plate data in a database. For information about image acquisition, see the user guide for the ImageXpress® Micro Widefield High Content Screening System or the ImageXpress® Ultra Confocal High Content Screening System. Both user guides are provided on the MetaXpress Software installation media and are available in the Molecular Devices knowledge base at http://www.moleculardevices.com/support.html.
- Analysis, which consists of selecting, measuring, assessing, and managing acquired images and plate data.

  This manual describes the general analysis workflow:



Obtaining Support

Molecular Devices provides a wide range of support for the MetaXpress Software:

1. Documentation — Check the manuals that are included on the installation media and the help that is available within the MetaXpress Software. Help for an active dialog box can be accessed by pressing the [F1] key.
2. Online knowledge base — The knowledge base has links to technical notes, software upgrades, newsletters, manuals, and other resources. Visit the Molecular Devices Support web page at www.moleculardevices.com/support and follow the links to the knowledge base.
3. Technical Support —

   Phone: Contact Technical Support at (800)-635-5577 (U.S. only) or +1 408-747-1700.

   Online: Visit www.moleculardevices.com/support and follow the links in the knowledge base to the Technical Support Request Form to send an e-mail to a group of experienced Technical Support representatives.

   Please have the system ID number, system serial number, software version number, and the system owner's name available when you call.

   - To find your system ID number, from the Help menu, select About MetaXpress. The dialog that appears displays your system ID number.
   - The system serial number is located on your instrument.
4. Additional support resources include:
   - Nikon web-based microscopy course — http://www.microscopyu.com
   - The Molecular Probes handbook — http://www.probes.invitrogen.com offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis.

   The following sites offer filter information:

   - http://www.chroma.com
   - http://www.semrock.com
   - http://www.omegafilters.com

Before You Begin Your Analysis

2

When preparing to analyze plates, consider the following plate characteristics:

- **Plate Specifications** — How many wells are on the plate?

  Plate size is a determining factor in the number of images included in the experiment. Experiments containing large numbers of images will need more time to be analyzed than ones from smaller experiments. These types of factors might influence the options and settings that you choose for your analysis.

- **Wells** — Were images acquired from all wells?

  Your plate might contain images for all wells, but because of the requirements of your experiment, you might not want to analyze all wells on all plates.

- **Sites** — How many sites were acquired for each well?

  The number of sites acquired for each well can influence how to analyze the data and organize your analyzed data for review. Unless you are using adaptive acquisition, the number of sites value applies to all wells from which you are acquiring data; the quantity of data collected is multiplied by the number of sites visited in each well.

- **Wavelengths** — How many wavelengths and which specific wavelengths were acquired?

  Similar to sites, the number of wavelengths acquired contributes to the amount of data acquired from each well. Some application modules require a minimum number of wavelengths to be acquired in order to produce meaningful data, while other modules will produce good results with only a single wavelength.

- **Images** — What is the total number of images acquired on the plate?

  The total number of images that you acquire is influenced not only by the number of wells that you acquire, but also the number of time points, the number of wavelengths for each time point, and the number of sites in each well. Therefore, it is possible to create very large data sets. Keep in mind that the amount of time available to process your data can become an important element in designing your experiment.

- **Settings** — What unique analysis settings do you need to make?

  Each available application module can be saved with unique settings. After you save your settings for a module, you can retrieve and reuse these settings at any time. Saving and reusing your settings helps streamline your analysis workflow and ensures a high level of consistency and accuracy.

- **Expected Results** — What are the anticipated results of your experiment?

  The results that you expect to obtain from your experiment can be one of the best guides in helping to properly design your experiment. By “working backward” from your anticipated results, you can ensure that you correctly identify all of the steps needed to design a successful experiment.

- **Data Log Measurement Selections** — Which measurements are the most appropriate to select for logging?

  Most application modules create two different types of logs:

  - Summary log measurements are measurements that apply to the entire image and include a number of default measurements that apply to every image.
  - Data log measurements are measurements of each individual cell in the image that has been identified by the software. Data log measurements are also called “cell by cell measurements.”

  Some application modules have High Throughput (HT) versions. Because of the increased throughput, these modules log only summary measurements and not cell-by-cell measurements.

About Application Modules

3

Application modules are the foundation of the MetaXpress® Software analysis workflow. These modules provide automated image analysis for biology-specific applications, interactive image segmentation and data results, and options for data recording and management. Some examples of application modules include Count Nuclei, Transfluor, Micronuclei, and Cell Cycle.

An application module can be run in a variety of ways:

- within the Review Plate Data dialog (see Chapter 6)
- as a stand-alone analysis using an option on the Screening menu (see the application Help)
- as part of a custom module (see Chapter 4 for an overview and the application Help for details)
- within a custom journal (see the application Help)
- in a queue of automated analyses (see Chapter 7)
- automatically in conjunction with the plate acquisition process (see Chapter 8)

All modules share common conventions and settings. Once you learn how to use one application module, you can easily use other application modules. For example, each module asks you to provide certain basic information about your images, such as the size, shape, and intensity of objects to be included in your analysis. Application modules require grayscale 16-bit fluorescent images (one for each wavelength that will be analyzed); some modules will work with transmitted light images as well.

Molecular Devices offers numerous application modules. The application modules that are available with your version of the software depends on the terms of your license. For a list of currently available application modules, visit www.moleculardevices.com.

Note: You can create your own custom module by combining a variety of image processing and segmentation steps in the Custom Module Editor. These steps can include the application modules described in this manual. For a brief introduction to custom modules, see Chapter 4. For detailed information about creating custom modules, see the help provided in the Custom Module Editor. You can open the Custom Module Editor from the Run Analysis tab in the Review Plate Data dialog.

When choosing an application module, consider what your assay needs to measure. Your assay might encompass activation of a receptor, apoptosis, and proliferation and so on, but to obtain meaningful numerical data from screening, you need to describe a phenotype that can be measured by the imaging system. Imaging systems do well at determining intensity, area, number of objects, or any combination of these measurements. To effectively translate your assay into useful measurements, evaluate the requirements of your assay in the simplest possible terms. For example, when counting cells, the brightest spots are usually of the greatest interest. Of these, only certain sizes and shapes might be of interest, since the other shapes might be dead cells or debris in the culture. Use the table below to determine which application module is best suited for your assay.

Assay Type	Description	Recommended Application Modules
Protein Localization/ Translocation	Typically a measurement of co-localization. The protein of interest is labeled or bound to by a labeled antibody and used with other probes specific to cell types, organelles, or cytoskeletal structures. The assay determines how much area or intensity of the protein-of-interest co-localizes with another probe.	• Translocation • Translocation-Enhanced • Multi Wavelength Translocation • Nuclear Translocation HT • Custom module
Cell Proliferation	Typically a count of the cells or nuclei in an image.	• Count Nuclei • Multi Wavelength Cell Scoring • Cell Proliferation HT
Cell Viability/Apoptosis	Typically a count of objects having specific characteristics. The cells are rounded up (shape and area), the cells label or do not label with a specific probe (intensity and count), or specific proteins localize to a sub cellular compartment (intensity and count); for example, the mitochondria.	• Live/Dead for a two-wavelength assay • Cell Health for a three-color assay, such as DAPI, Annexin, or Propidium Iodide (PI)

Assay Type	Description	Recommended Application Modules
Receptor Internalization and other Punctuate Staining	Usually measured by a probe moving to coated pits or vesicles, such as in the Transfluor assay for GPCR activation. Count and measure labeled pits or vesicles, or other punctate staining.	• Granularity • Transfluor • Transfluor HT • Custom module
Angiogenesis	Typically an area measurement. Either the length of tubules is measured or the creation of holes in a cell monolayer is measured.	Angiogenesis
Cell Physiology (Calcium/pH)	Almost always involves measurements of intensity. Typically a probe is used that changes its fluorescence intensity using one or two wavelengths under different physiological conditions. Two examples of this are Fluo-3 which increases its fluorescence with increasing free calcium concentration or Fura-2 in which the fluorescence with 340 nm excitation increases and 380 nm excitation decreases with increasing free calcium concentration.	• Cell Scoring • Multi Wavelength Cell Scoring • Custom module
Kinase Activity Assays	Generally involve measuring the phosphorylated epitope of the kinase by measuring fluorescence intensity. This value should be normalized to the number of cells expressing the kinase – a counting measurement.	• Cell Scoring • Multi Wavelength Cell Scoring
Neurite Outgrowth	Assesses changes in shape and lengths. The outgrowth lengths, number of outgrowths, branching, and other parameters are counted.	• Neurite Outgrowth • Custom module

Assay Type	Description	Recommended Application Modules
Cell Cycle	Typically involves the classification and counting of cells in specified stages of the cell cycle and analyzing the distribution of cells within these classes in response to compounds.	• Cell Cycle for detailed classification using one to three wavelengths — from a single nuclear stain to combinations including optional mitosis-specific and/or apoptosis-specific stains. • Mitotic Index for a simple two-wavelength application with a nuclear stain and a mitosis-specific stain to measure the percent of cells that are mitotic. • Monopole Detection for specific analysis of spindle formation and the disruption of centrosome separation.
Polynucleation and Genotoxicity	Typically with micronuclei, which are small nuclei produced during cell division by a lagging chromosome fragment or an entire chromosome and their induction is a highly quantitative measurement of chromosomal damage.	• Micronuclei for genotoxicity by detection of micronuclei in populations of mono-, bi- and multi-nucleated cells. Can also be used for detection of yeast budding. • Custom module

About Custom Modules

4

Not all samples can be meaningfully or accurately assessed with just one standard application module. Often, you need to combine an array of pre-processing and segmentation methods to effectively analyze sets of images. Applications such as protein subcellular patterns or fluorescence co-localization of objects are often best performed with a sequence of steps.

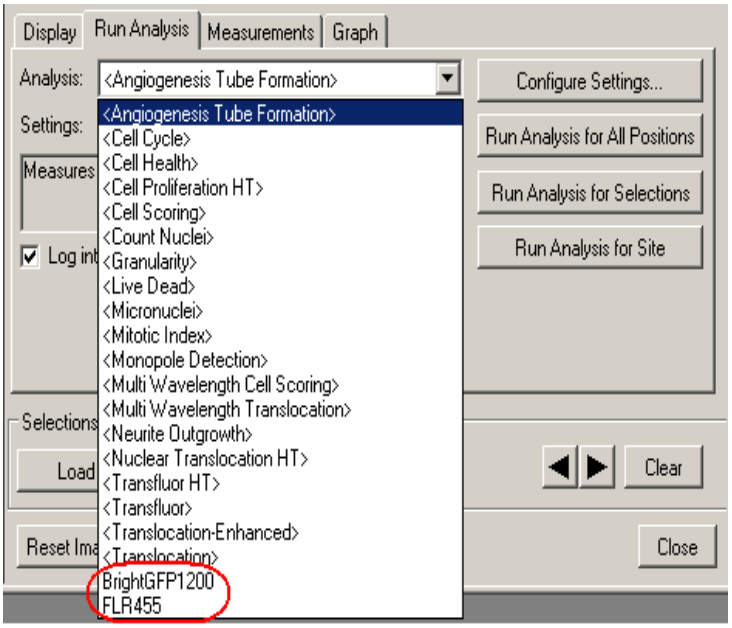
For example, you may want to remove noise, invert an image, increase intensity levels, extract objects of interest, shrink or remove objects, and then measure the objects in images acquired with different channels. A custom module makes this type of complex, repetitive task simple to perform. You can re-use a saved custom module with your copy of the software or you can share a custom module with other researchers for use with their data sets.

The Custom Module Editor is a flexible, interactive environment where you essentially create and test a template for an image analysis that can be re-used on multiple data sets. Simply choose from galleries of processing options, morphology filters, application modules, and segmentation methods to construct a sequence of analysis steps.

Note: For detailed information about how to create a custom module, see the help provided in the Custom Module Editor.

Once you save the final version of your custom module, the module automatically appears in the list of available analyses on the Run Analysis tab in the Review Plate Data dialog.

Application modules are distinguished from custom modules in the list by brackets. For example, the list below includes two custom modules:



To add a custom module to the database

In the Custom Module Editor, a custom module can be saved as an XML file so that it can be provided to other users for use with their copies of the MetaXpress Software. In this case, because the custom module is not automatically added to the list of analyses on the Run Analysis tab, to use the custom module you must add it to the list by following these steps:

1. From the **Screening** menu, select **Add Custom Module to Database**.
2. Locate and select the custom module XML file and click **Open**.

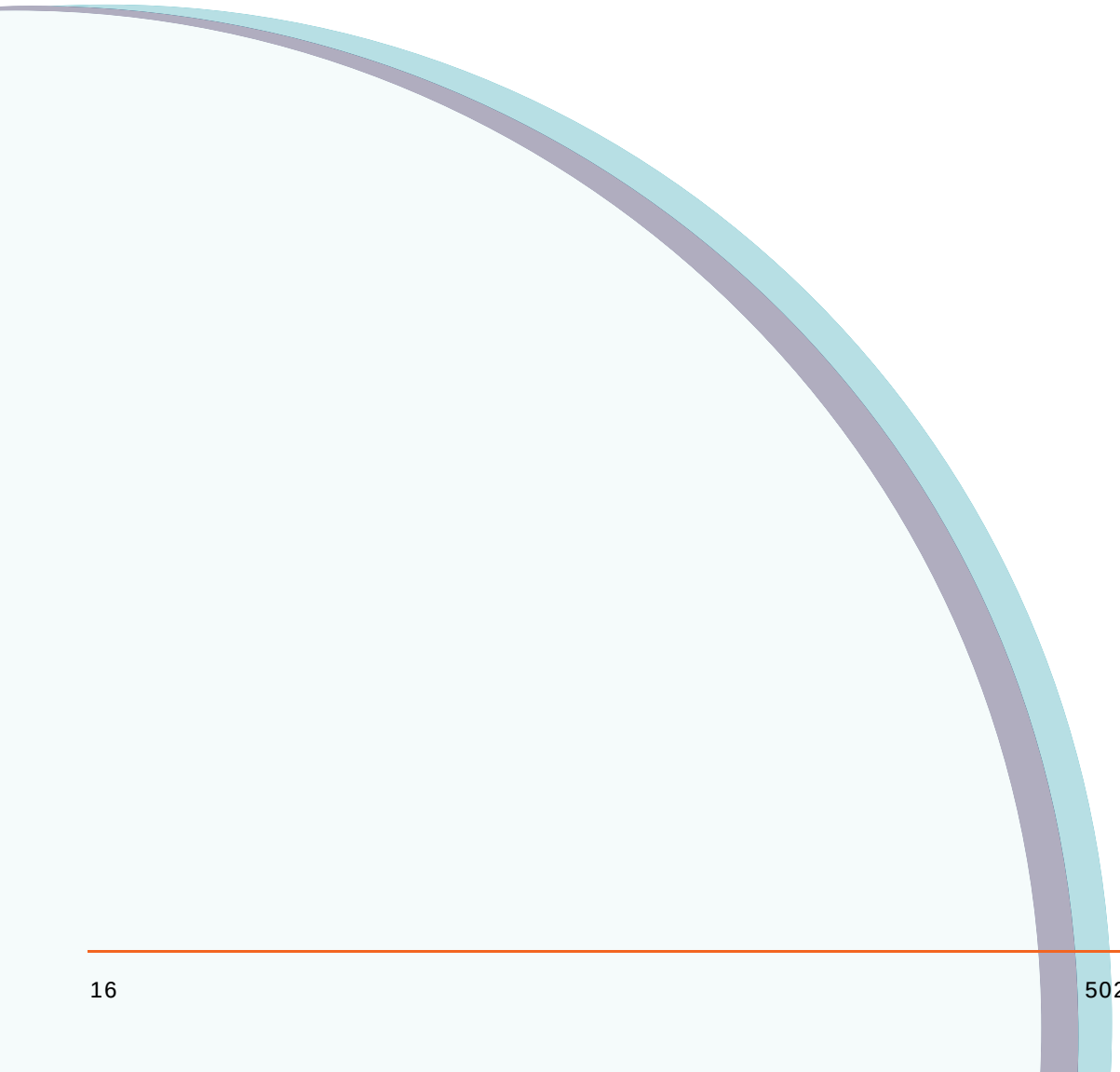
   The software uses the custom module file name as the setting name. If the module already exists in the database, you can add a new setting or overwrite an existing setting for the module.
3. Keep the setting name or enter a new setting name for the custom module and click **Add**.

The custom module is appended to the list of analyses on the Run Analysis tab.

To remove a custom module from the list of analyses

To delete a custom module, you must delete all of the settings associated with the module by following these steps:

1. From the **Screening** menu, select **Review Plate Data**.
2. On the **Run Analysis** tab, in the **Analysis** field, select the custom module.
3. Click **Edit List**.
4. Delete each setting associated with the custom module; when you delete the last setting, click **Yes** to confirm that you want to remove the custom module from the list of analyses.



Selecting a Plate and Viewing Images

5

The first step in the image analysis process is to select the plate in the database that contains the images that you want to analyze. Then, you can select, view, and arrange the images that you want to analyze in a variety of ways to examine and compare them.

For example, you can specify the wavelengths to display in the images, combine wavelengths into a composite image, and display information such as the well number on the images. You can also scale 16-bit images and generate an intensity profile to help you determine the areas of the image that have the highest intensity.

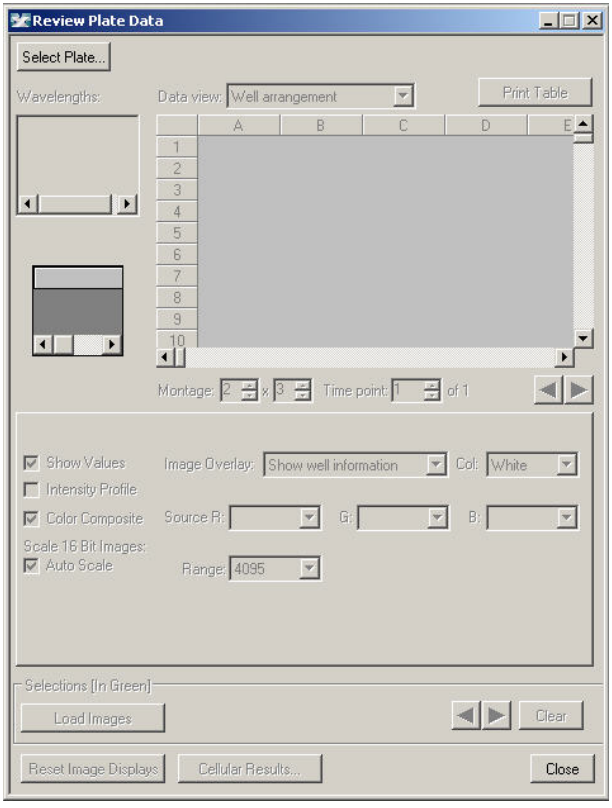
Note: The settings that you use to select and view images for analysis in the Review Plate Data dialog are for display purposes only and cannot be saved to a settings file. They do not affect image analysis or other measurements and cannot be saved and reused.

Settings made in application module dialogs (described in Chapter 6) define characteristics specific to the selected application module. These application module settings can be saved and reused.

This chapter includes the following topics:

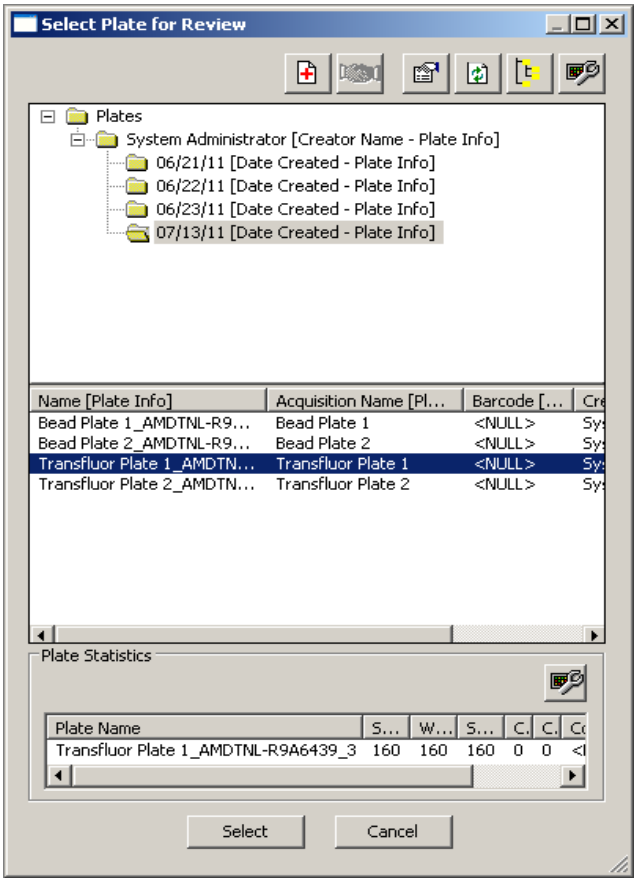
Selecting a Plate for Analysis

1. From the **Screening** menu, click **Review Plate Data**. The Review Plate Data dialog appears.



2. Click **Select Plate**.

3. In the **Select Plate for Review** dialog, expand the plates folder in the top pane to view folders containing plates that have been saved in the database.



4. Double-click a folder to display its contents in the middle pane.
5. In the middle pane, select the plate and then click **Select**.

   The image data for the plate that you selected appears in the Review Plate Data dialog.

Note: You can rearrange, add, and remove columns in the Select Plate for Review dialog by using the Configure Displayed Columns button at the top right corner of the dialog.

Viewing and Arranging Images

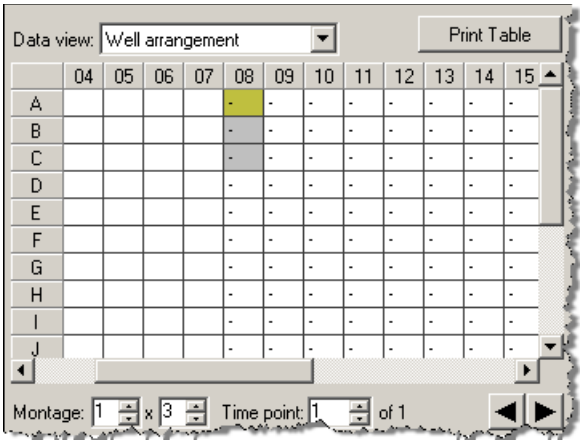
In the Review Plate Data dialog, use the well selection table and the options on the Display tab to view, arrange, and select images for analysis.

1. In the **Review Plate Data** dialog, in the **Wavelengths** section, select one or more wavelengths to display in the images.

   The well selection table serves several purposes. Each square represents a well. Markings, color highlighting, or shading are used to indicate the following:

   - Wells marked with a (-) hyphen — Indicates the wells containing image data.
   - Wells highlighted in gray — Indicates the wells included in the Montage window.
   - Wells highlighted in yellow — Indicates the images selected for individual display.
   - Wells highlighted in green — Indicates the wells that you selected (by right-clicking the wells).

   For example:



2. Click once on a square in the table to display the image of the well.

   The image that corresponds to the well that you selected opens in a separate “Montage” window. Additional images for other wells may appear in the Montage window depending on the dimensions that are indicated in the Montage fields.

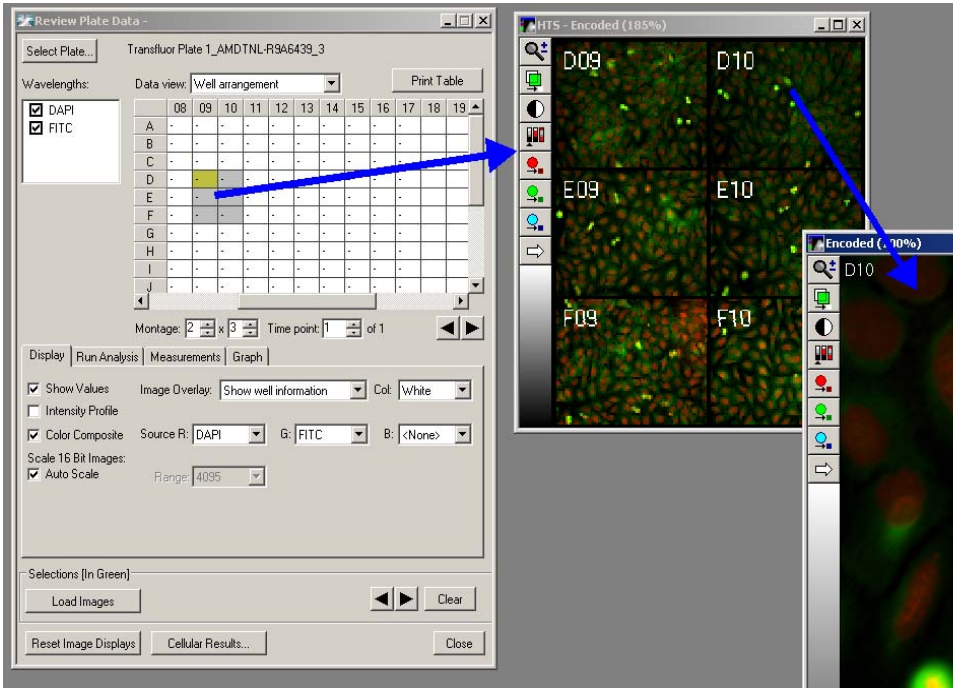
Note: Image Montage windows are labeled HTS- followed by the name of the stain or wavelength that you assigned to the wavelength. For example, HTS-DAPI or HTS-FITC. If you are viewing a color composite image, the window is labeled HTS-Encoded.

In the Montage window, you can view thumbnail images of each image from the plate and compare the overall density and distribution of the sample material in each well. Samples showing an overall high density and even distribution are typically better candidates for analysis than wells with very low sample density and uneven distribution.

The Data view field is useful for arranging images according to time points and measurements after you have run an analysis on the images. Before analysis, the table is arranged in the same order that the wells are arranged in the microwell plate.

3. To view the images of additional or different wells, click on the well or wells in the table and adjust the values in the Montage fields accordingly.
4. To open one of the images in the Montage window in a separate, individual window at full resolution, click on an image in the Montage window or right-click on a well in the table.

   For example, the figure below shows the image for well D10 in the Montage window; if you click on that image in the Montage window, the image opens in a separate window:



Note that if you right-click on a well, the well is highlighted in green to indicate that is selected for analysis. To de-select the well, right-click on it again.

5. If images were acquired at multiple sites in a well:
   - Click on any available site in the **Sites** section to view only that site for all selected wells in the Montage window.
   - Click **All Sites** to view all sites for all selected wells.

Note: If **All Sites** is selected, the Montage dimensions determine the sites that are displayed in the Montage window. To see all four sites in all selected wells, set the Montage dimensions to 4 X 4. Otherwise, to see just the upper two sites in all selected wells, set the dimensions to 2X1, and so on.

6. To assign a color to a wavelength in your data set, on the **Display** tab, select the wavelength in the red (R), green (G), or blue (B) field in the **Source** field.
7. To combine the images that were acquired with multiple wavelengths into a composite image, select the **Color Composite** check box. If this check box is not selected, a separate Montage window displays images for each wavelength that is selected in the Wavelengths section.
8. To display images in the Montage window that are from a different group of wells, click on another well in the table to indicate the top left well in the new group or use the left or right arrow beneath the table to move the group of selected wells.
9. To collate the images of multiple wells into one window that you can scroll through and save as a stack (.stk) or multiplane TIF (.tif) file:
   - In the table, right-click on the wells to include in the stack (the wells will turn green as you click them), and then click **Load Image(s)** in the **Selections [In Green]** section. If **All Sites** is selected, the stack will contain images for all sites in all selected wells. A stack is created for each selected wavelength or, if Color Composite is selected, a single stack is created.
   - Use the slider at the top of the stack window to navigate through the images in the stack.
   - To remove all **Selections [In Green]** from the table, click **Clear**.
10. To display the well number in the Montage window, select **Show well information** in the **Image Overlay** field. To change the color of the well number in the Montage window, select a different color in the **Col** field.
11. To transform the image into a three-dimensional intensity profile graph, select the **Intensity Profile** check box. Using the

colors assigned to the image, the highest intensities are displayed as the highest peaks in the graph.

12. To define a range for scaling 16-bit color composite images, clear the **Auto Scale** check box and select the upper range for the scaling in the **Range** field.

Configuring Application Modules

6

Most application modules share similar configuration steps, such as selecting the source image to process, determining the width of an object, and providing the intensity above local background value. Once you are familiar with these basic steps, you will be able to use any of the various application modules.

This chapter includes the following topics:

Note: You can create your own custom module by combining a variety of image processing and segmentation steps in the Custom Module Editor. These steps can include the application modules described in this manual. For detailed information about creating custom modules, see the Help provided in the Custom Module Editor. You can open the Custom Module Editor from Run Analysis tab in the Review Plate Data dialog.

Basic Setup Procedure for Application Modules

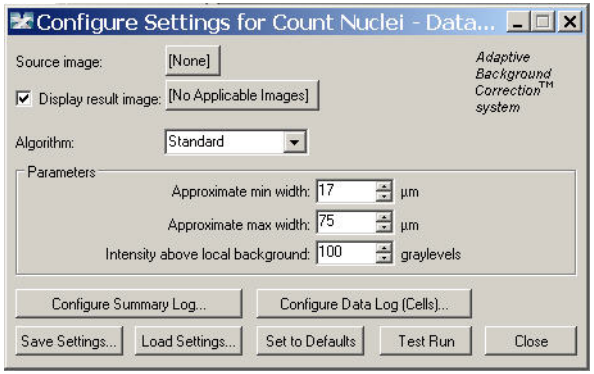
For best results, source images in all application modules should be calibrated in microns. If more than one image is used as a source image, they must have identical distance calibrations. Images acquired using an ImageXpress® Micro System or an ImageXpress® Ultra System are calibrated in microns. Images that have been imported from another system or that have been processed might not be calibrated.

Selecting the source image, result images, and algorithm

1. From the **Screening** menu, click **Review Plate Data** and then open the plate and images that you want to analyze as described in Chapter 5.
2. In the **Review Plate Data** dialog, click the **Run Analysis** tab.
3. Select an application module from the **Analysis** field.

Note: This procedure uses the Count Nuclei application module as an example.

4. Click **Configure Settings**.



Each application module needs at least one source image to process. Some modules need more than one source image. Examples of source image names in various application modules include:

- Count Nuclei: Source image
- Angiogenesis: Source image
- Translocation: Compartment image and Translocation Probe image
- Neurite Outgrowth: Neurite image and Nuclear Image

Note: The appropriate type of source image(s) varies depending on the application module used. Refer to the application help for the module you are using for more information about source images.

5. Click **Source image** and select one of the images that you opened in Step 1 (do not select images that start with HTS as these are thumbnail images).

Note: There are two ways to view the results of each application module: Displaying a result image or using an image overlay. Selecting Display result image will open or overwrite (see Step 7) a new image depicting what was measured. Using an image overlay creates an overlay on the main image. You can toggle the image overlay on or off with the Show/Hide overlay button on the side of the image window.

Note: To display a result image, the Display result image check box must be selected and an image must be selected.

6. To create a separate result image, select **Display result image**.

   The result images are not saved, but are useful when creating journals.

7. Open the image selector for **Display result image** and if needed change it to one of the following:
   - **Overwrite** — (Recommended) Overwrites a selected image or creates a new image if one does not exist.
   - **Add to** — Adds a plane to a stack.
   - **New** — Creates a new image every time the assay is run.
8. Click on the image name (typically indicated with [Source]) and select the appropriate image name from the field.

Note: Do not select images that start with HTS as these are thumbnail images.

9. For a journal, select **Specified** and give the image an appropriate name, such as **Result**.
10. If the application module provides an Algorithm option, select either **Standard** or **Fast**. The algorithm option determines how quickly the analysis will be performed. Both algorithms produce similar but not exactly identical results. If you select the Fast algorithm, then in the Intensity above local background field enter approximately half of the value that you calculate in Calculating the intensity above background value on page 32.

Note: The Standard algorithm is the algorithm that was used in version 3.1 and earlier of the MetaXpress Software.

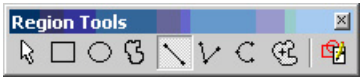
Determining object widths

Most application modules require that you specify object size measurements before processing the image(s). You can measure an object using one of the following:

- Region tools Line tool
- Caliper tool
- Show Region Statistics dialog

To measure the width of an object using the Region tools Line tool:

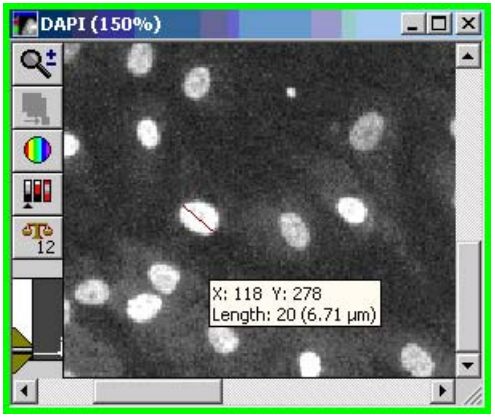
1. If the **Region** toolbar is not open, click **Region Tools** from the **Region** menu.



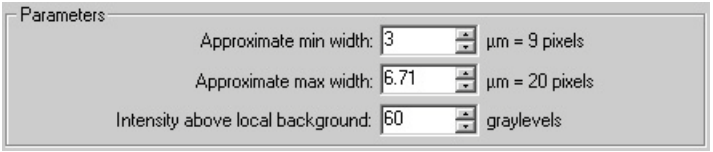
2. Select the **Line** tool.
3. Click on one of the edges of the largest object that you want to include in your analysis.

   A tooltip appears showing the current X and Y values of the pointer, as well as the length.
4. Move the cursor to the opposite edge of the object and note the Length value.

   In the following example the value is 20 pixels (or 6.71 µm). This number represents the cell width in pixels. If the image is calibrated, the length is in pixels and calibrated units.



5. Enter or select the value in microns in the **Approximate max width** field of the application module's **Configure Settings** dialog. The next figure shows the new Approximate max width value.



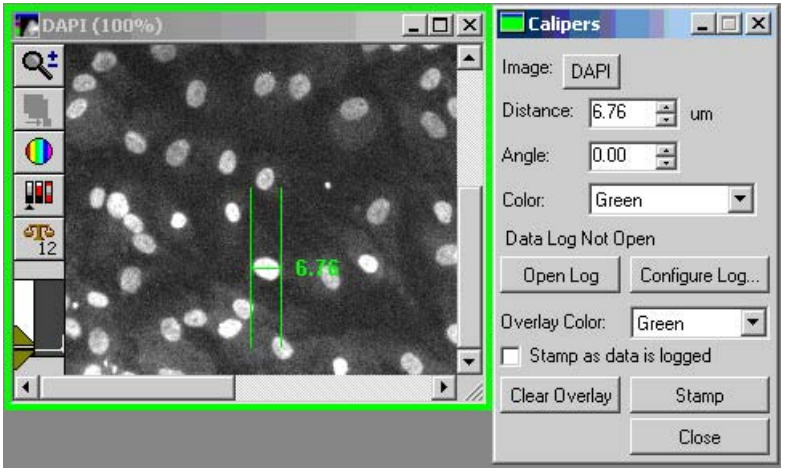
6. To remove the region lines, select **Clear Regions** on the **Regions** menu or right-click on the region and click **Delete Region**.
7. Repeat the steps above for the smallest object that you want to include in your analysis and enter the value in the **Approximate min width** field.

To measure the width of an object using the Caliper tool:

Note: The caliper feature is a drop-in option and may need to be loaded into the software. If the option is not present on the Measure menu, please contact your System Administrator or Technical Support to have this drop-in loaded.

1. From the **Measure** menu, click **Calipers**.
2. In the **Calipers** dialog, select the image to measure from the Image selector.

   The calipers appear on the selected image, as shown in the next figure.



3. To move the calipers, click the cross-bar so that it appears as a blinking line, and then drag the cross-bar to the desired location.
4. Click one of the caliper edge lines so that it appears as a blinking line, and then drag the line to the desired distance. The other caliper line will remain anchored.
5. Double-click the caliper cross-bar so that “nodes” appear at each end. With your mouse, drag one of the nodes away from the other to the desired distance.

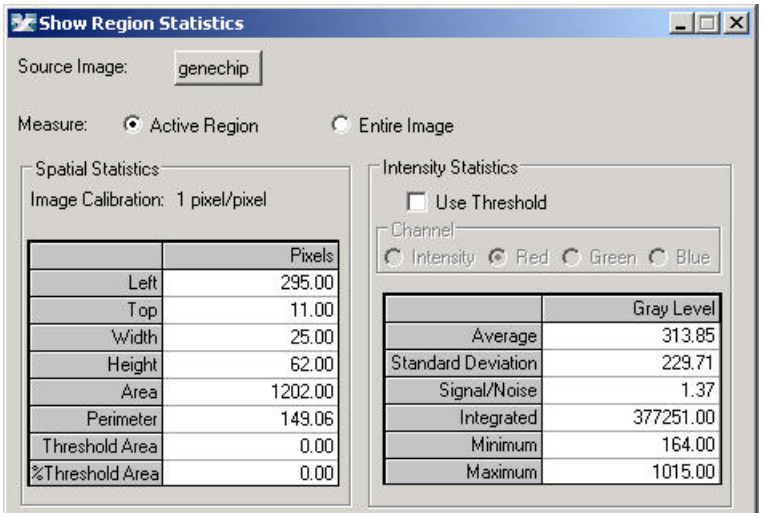
   The size of the line is displayed in the image and in the Caliper dialog.
6. In the application module’s **Configure Settings** dialog, in the **Approximate max width** field, enter the width of the largest object that you want to include your analysis.
7. Repeat steps 3-5 for the smallest object that you want to include in your analysis and enter its width in the **Approximate min width** field.

To measure the width and area of an object using the Show Region Statistics dialog:

1. If the **Region** toolbar is not open, click **Region Tools** from the **Region** menu.

2. Select the **Trace Region** tool as shown in the previous figure.
3. Click and hold the left mouse button to draw a region of interest around a typical compartment. Double-click the left mouse button to close the region.
4. Select the region (the region blinks when it is selected) and, on the **Measure** menu, select **Show Region Statistics**.

   The Show Region Statistics dialog appears.



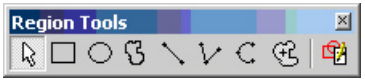
	Pixels
Left	295.00
Top	11.00
Width	25.00
Height	62.00
Area	1202.00
Perimeter	149.06
Threshold Area	0.00
%Threshold Area	0.00

	Gray Level
Average	313.85
Standard Deviation	229.71
Signal/Noise	1.37
Integrated	377251.00
Minimum	164.00
Maximum	1015.00

Calculating the intensity above background value

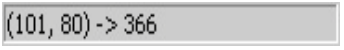
The Intensity above local background field is common for all application modules. It specifies a value for the intensity threshold of the object(s) of interest compared to the neighboring background gray-level values. This setting controls the sensitivity of the object detection and segmentation.

1. If the **Region** toolbar is not open, on the **Region** menu, select **Region Tools**.
2. Select the **Arrow** tool as shown in the next figure.



3. Move the arrow cursor over the dimmest part of the dimmest object in the image.

   As you move the cursor, the X,Y coordinates and the gray-level value of the pixel under the cursor are indicated at the bottom of the MetaXpress Software desktop. The X,Y coordinates are in parentheses and the gray-level value is to the right of the arrow. For example:



4. Note the gray-level value of the object.
5. Move the cursor just outside the object to the background of the image and note the gray-level value of the background.
6. Calculate the difference between the gray-level value of the object and the background. For example, using a cell's gray-level value of 366 and a background value of 304, the calculation would be: 366 - 304 = 62.
7. The value that you enter in the **Intensity above local background** field depends on whether the application module provides the Standard and Fast algorithms. If the application module does not provide either algorithm or if you select the Standard algorithm, enter a value that is slightly lower than the calculated value. In the previous example, a value of approximately 60 is appropriate. If you select the **Fast** algorithm, then in the Intensity above local background field enter approximately half of the value that you calculated.
8. Proceed to the next procedure without closing the application module dialog.

Testing and saving settings

After the module is configured, you should test the settings, make adjustments if needed, and then save the settings.

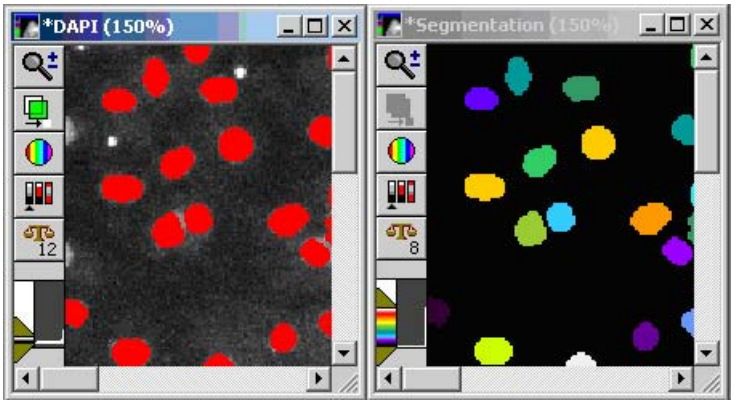
Note: Configure and save settings before you run an application module for the first time. After the settings are saved, they can be loaded as needed without further configuration.

1. In the application module dialog that you just configured, click **Test Run**.

Note: The Preview option, if it is available, shows the segmented image using just the selected wavelength. For the final test use the Test Run option as it uses all wavelengths.

The application module runs and an overlay image appears on the original image. A result image also opens if the Display result image check box is selected. The next figure shows a sample source image with an overlay (left) and a result image with the cells individually colored (right).

A Cellular Results table containing individual object data also opens.



2. Click on a cell in the image to highlight the data in the Cellular Results table for that selected cell. Use Ctrl+Click or Shift+Click to select more than one row of data. If the table does not open when you run an application module, from the **Window** menu, select **Show Cellular Results**.
3. Compare the result image with the source image to determine if all the objects of interest have been detected. If not, lower the value in the **Intensity above background** field and run the module again before modifying the **Approximate max width** and **Approximate min width** fields.
4. Click **Configure Summary Log** to specify how application module data will be recorded.
5. In the **Configure Log** dialog, select or clear individual settings. For more information on the data that can be logged, refer to the online help Dialog Box Options topic for the application module you are using.

Note: The image overlay is saved to the database with the image after automated analysis only if you select the **Save Segmentation Overlay to Database** option when configuring the summary log for the application module. This option is not available for the Cell Proliferation HT, Nuclear Translocation HT, and Transfluor HT application modules.

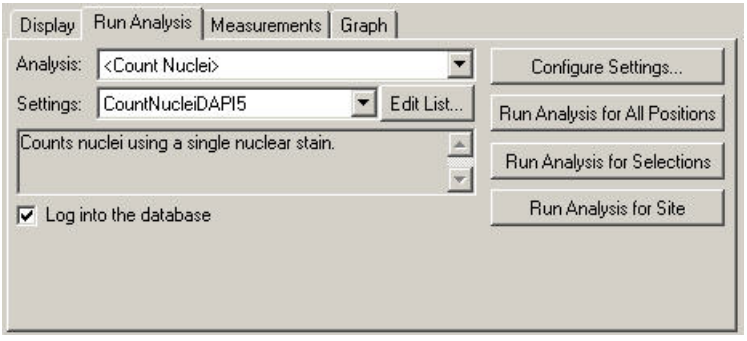
6. Click **Configure Data Log** to configure the data log if applicable.
7. Click **Save Settings**.
8. Enter a name and description for the settings in the **Save Settings** dialog and click **OK**.

   The settings are saved to the database.
9. Click **Close**.

Running the application module

In most circumstances, despite the automated capabilities, you should manually run preliminary tests on one or more wells, such as both positive and negative controls, to ensure valid results.

Use the Run Analysis tab to select the application module, custom module, or journal that you want to run, and then run the analysis on either the entire plate, on the selections that you made previously in the well selection table, or on the currently selected site.



On this tab, you can also open the Edit List of Settings dialog for a specific application module or journal and choose a settings file, modify an existing setting, create a new settings file, or delete a settings file. (Analyses that you create from journals must have all possible settings stored in the database at the time the journal is created and stored in the database `C:\Assay folder`.)

1. On the **Run Analysis** tab of the **Review Plate Data** dialog, select an application module in the **Analysis** field.
2. Select the appropriate saved setting in the **Settings** field.
3. Select the **Log into the database** check box to log the measurement data from your analysis into the database.
4. If you are running a custom analysis derived from a journal and have created a setup journal for it, click **Run Setup for Analysis** to run the setup journal. The setup journal must be in

the same folder as the main analysis and must be named in the format: `EXAMPLEJOURNAL_SETUP.JNL`.

5. Select one of the following options:
   - Click **Run Analysis for All Positions** to run the assay for all positions on the plate.
   - Click **Run Analysis for Selections** to run the assay on all sites in the selected wells. To select wells, right-click them in the well selection table in the **Review Plate Data** dialog (selected wells are highlighted in green).

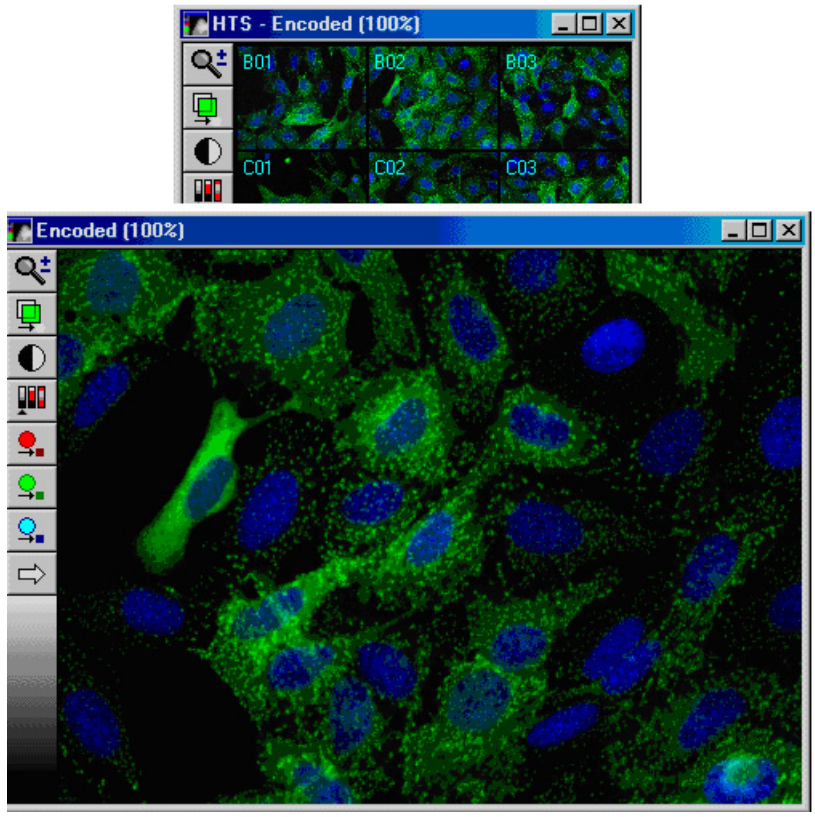
Note: To run an analysis on only certain sites in wells, you must use a journal.

   - Click **Run Analysis for Site** to run the assay for the currently selected site.
6. After the analysis is run, view the results in the Cellular Results table, on the **Measurements** tab, or on the **Graph** tab. See Chapter 9 for more information.

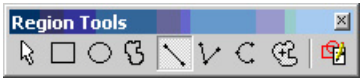
Example: The Transfluor Application Module

The procedures in the previous section work with any application module. The procedure in this section is specific to the Transfluor application module. This section assumes you have purchased the Transfluor module with your license for the MetaXpress Software.

1. Select a plate using the **Review Plate Data** dialog as described in the previous procedures.
2. Click on one of the wells to display an image.

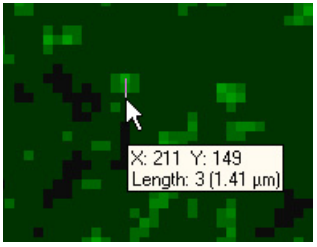


3. In the **Review Plate Data** dialog, click the **Run Analysis** tab.
4. Select **<Transfluor>** from the **Analysis** field.
5. Click **Configure Settings**.
6. In the **Configure Settings for Transfluor** dialog, click **Pits and Vesicle image**, and then select the wavelength image representing the pits and vesicles.
7. Click on the pits and vesicles image, and then increase the zoom by clicking the magnifying glass icon on the image window or by pressing **Page Up** on the keyboard.
8. Use the **Single Line** tool of the **Region** toolbar to measure a granule width by following these steps:
   - From the **Regions** menu, click **Region Tools**.
   - Select the **Single Line** tool.



   - In the image window, locate a small pit.
   - Move the cursor to one edge of the pit and click to anchor the line in position.
   - Move the cursor to the other edge of the pit and read the length of the line region.

   The figure below shows that, in this example, the length is 3 pixels or 1.41µm.



   - Enter this number in the **Approximate min width** field in the **Pits** section.
   - Repeat Step 8 for a larger pit. Enter that number in the **Approximate max width** field in the **Pits** section.

9. To determine the **Intensity over local background** value, select the **Locator Arrow** on the **Region** toolbar and follow Steps 10 - 14.
10. Find the dimmest pit.
11. Position the cursor over the dimmest pit and read the gray-level value.

    The gray-level value is the number that is displayed at the middle of the bottom of the screen after the right arrow. In the example below it is 162.

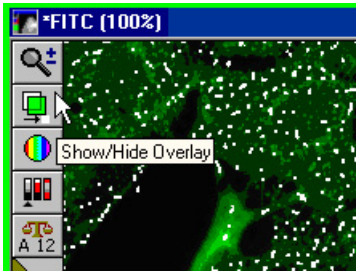
12. Move the cursor to just outside the pit to measure the background.
13. Calculate the difference between the gray-level value of the pit and the background. For example, 2030 - 1029 = 1001.
14. In the **Intensity above local background** field, enter or select a value that is slightly lower than the calculated value. For this example, a value of 800 is appropriate.
15. Repeat the steps above for vesicles, if applicable (if not, then clear the **Vesicles** check box).

Note: Vesicles are much larger and brighter than pits.

16. Make sure that the **Nuclear stain** check box is cleared.
17. Click **Test Run** to test your settings.

    The pits and vesicles segmentation appears as an overlay on top of the image.

18. Use the **Show / Hide Overlay** tool on the toolbar to toggle the results on or off to make sure all the pits are detected.



19. If the pits are not all detected, then lower the **Intensity above local background** value. If this does not produce the result that you want, then change the other parameters.
20. Select a negative control image and click **Test Run** to make sure that your settings are correct.
21. If you are using a nuclear stain, then select the **Nuclear stain** check box and repeat the steps above for the nuclei.
22. Click **Test Run**.

    The Cellular Results table appears, which you can use to interactively view individual cellular results. Clicking a cell in the image highlights the data for the selected cell in the table. Clicking on a row in the table highlights the corresponding cell in the image. To select and view more than a single line of data, press Ctrl+click; to select non-contiguous cells or lines of data can, press Shift+click.
23. Click **Configure Summary Log** to specify how data should be recorded.

    The Configure Log dialog opens.
24. Select or clear individual settings; to display the cellular results table after the application module runs, select **Save Segmentation**. For more information, refer to the online help Dialog Box Options page for the application module you are using.
25. Click **Configure Data Log** to configure the Data log if needed.
26. Click **Save Settings** and then close the dialog.
27. In the **Review Plate Data** dialog, on the **Run Analysis** tab, select the saved setting in the **Settings** field.
28. Select one of the following options:
    - Click **Run Analysis for All Positions** to run the assay for all positions on the plate.

- Click **Run Analysis for Selections** to run the assay on all sites in the selected wells. To select wells, right-click them in the well selection table in the **Review Plate Data** dialog (selected wells are highlighted in green).

Note: To run an analysis on only certain sites in wells, you must use a journal.

- Click **Run Analysis for Site** to run the assay for the currently selected site.

29. After the analysis is run, view the results in the Cellular Results table, on the **Measurements** tab, or on the **Graph** tab. See Chapter 9 for more information.

Cell-by-cell Multiplexing with Application Modules

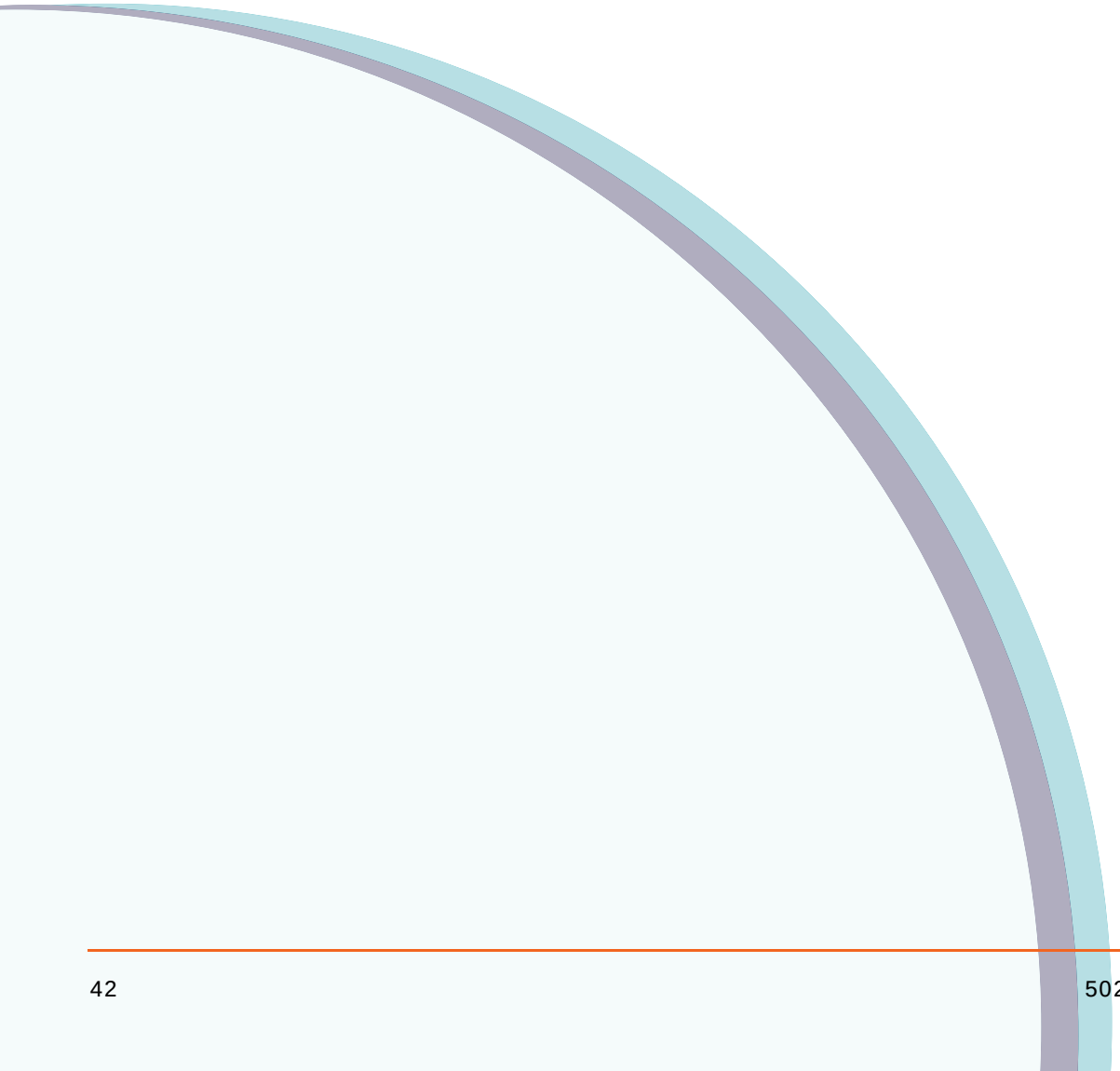
One of the advantages of high content screening is the ability to multiplex assays by running multiple analyses on the same samples. For example, you could run a Cell Scoring assay to measure transfection efficiency and a Transfluor assay to measure receptor internalization on the same sample. In the MetaXpress Software, these analyses may be multiplexed on cell-by-cell data.

Compatible modules include Cell Cycle, Cell Health, Cell Scoring, Count Nuclei, Granularity, Mitotic Index, Monopole Detection, Multi Wavelength Cell Scoring, Multi Wavelength Translocation, Neurite Outgrowth, and Transfluor assays.

1. Configure each application module. Make sure that the same nuclear detection settings and algorithm are selected for each module.
2. Run each application module separately for selected wells or for all wells.
3. Review the data in either the AcuityXpress Software or in another application such as Microsoft Excel.

Note: You can also multiplex assays by creating a custom module that includes multiple application modules. For detailed information about creating custom modules, see the help provided in the Custom Module Editor. You can open the Custom Module Editor from the Analysis tab in the Review Plate Data dialog.

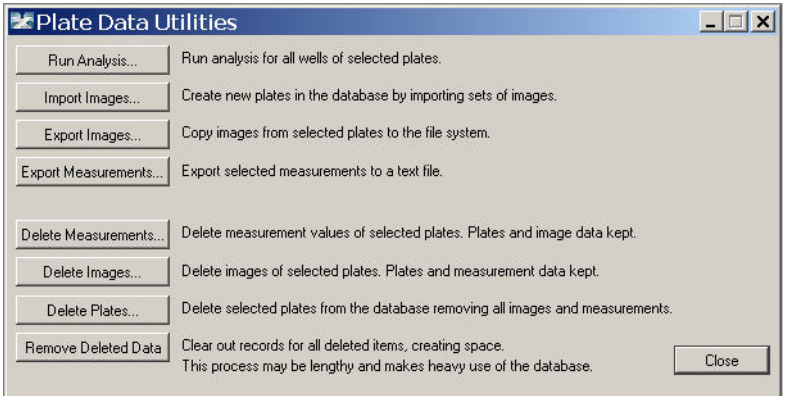
For more information about techniques for multiplexing assays, please contact Technical Support.



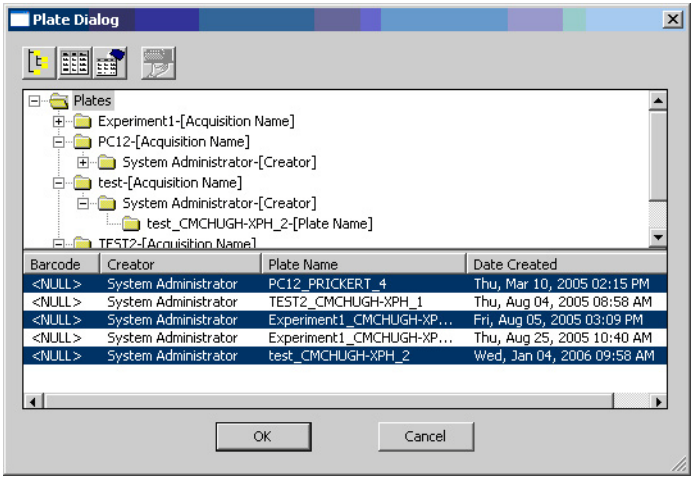
Manually Initiating an Analysis

Although the MetaXpress® Software provides features for automating analyses in conjunction with image acquisition, you can use the Plate Data Utilities dialog to manually initiate an analysis on more than one plate at a time.

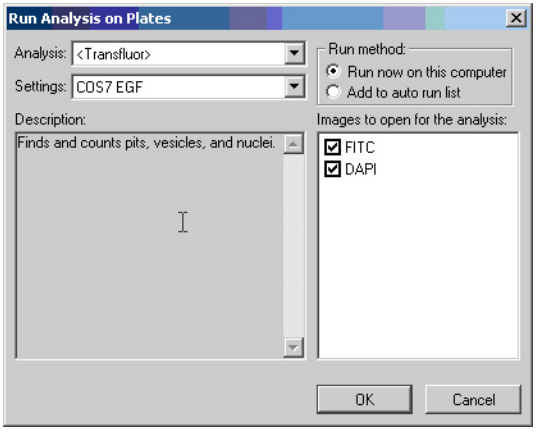
1. From the **Screening** menu, click **Plate Data Utilities**.



2. In the **Plate Data Utilities** dialog, click **Run Analysis**.
3. In the **Plate Dialog** dialog, use the query tools or expand a folder so several plates open in the bottom window, and then use Ctrl+click or Shift+click to select multiple plates.



4. Click **OK**.

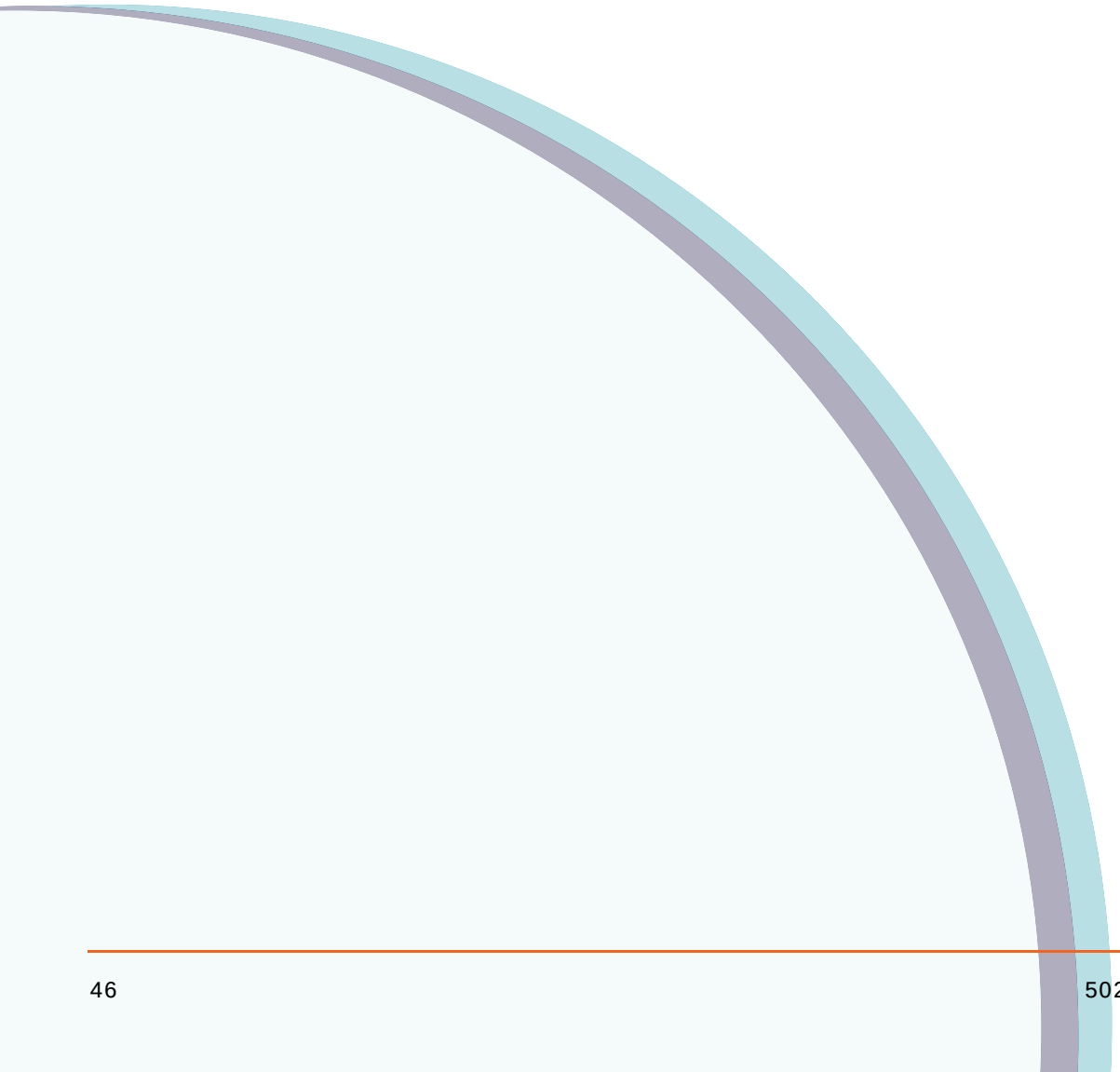


5. In the **Analysis** field, select the assay that you want to run.
6. In the **Settings** field, select the setting that you want to use.
7. In the **Images to open for the analysis** section, select the wavelengths to use with the selected assay.
8. Select **Run now on this computer** to start the analysis only on this computer or select **Add to auto run list** to add the job to the autorun queue to run on other computers which are in

autorun mode or to run with the MetaXpress® PowerCore™ Software.

Note: If you select **Add to auto run list**, you must also start the Auto Run mode available on the Screening menu or the MetaXpress PowerCore High Content Distributed Image Analysis Software must be running. The MetaXpress PowerCore Software processes application modules and custom modules but does not process journals.

9. Click **OK**.



Automating and Monitoring an Analysis

8

After you have tested the analysis settings for a specific application module or a custom set of journals and are confident in the results, you can use the automated analysis features in the MetaXpress® Software to increase your data analysis throughput.

Automated analyses can be started at one or more analysis workstations concurrently with acquisition. You can also automatically run analyses on an acquisition system after the acquisition is complete.

Note: Automated analysis using application modules and custom modules can also be run with the MetaXpress® PowerCore™ Software after the acquisition has been completed. Analysis of custom journals must be performed using the MetaXpress Software. For more information about the MetaXpress PowerCore Software, see the product documentation available in the Molecular Devices knowledge base at http://www.moleculardevices.com/support.html.

An analysis can run on systems running either the full or offline (analysis only) version of the MetaXpress Software. The systems must be connected to your database and logged in with privileges to view plates and write data. As image data is retrieved from the MDCStore™ database storage location that is specified during acquisition, the systems need privileges to access this storage location as well. Once the images are analyzed, the analysis results are stored back in the MDCStore database.

Using automated analysis provides several benefits, including the ability to dedicate designated workstations for running specific analysis application modules. Also, by dedicating a workstation for performing acquisitions only, the overhead for the acquisition system is significantly reduced.

Setting up and running an automated analysis involves two main steps:

1. Before starting an acquisition, configure the analysis settings on the acquisition workstation using the Post Acquisition tab in the Plate Acquisition Setup dialog.
2. Initiate automated analysis using the Auto Run Mode dialog.

Note: If the analysis that is configured on an acquisition workstation calls for an application module to run, the application module drop-in must be installed on the computer that is running in Auto Run Mode.

After the Auto Run Mode has started, you can use the Auto Run Plate Statuses dialog to monitor and control the progress of each analysis running for each plate being analyzed.

This chapter includes the following topics:

Setting Up an Automated Analysis

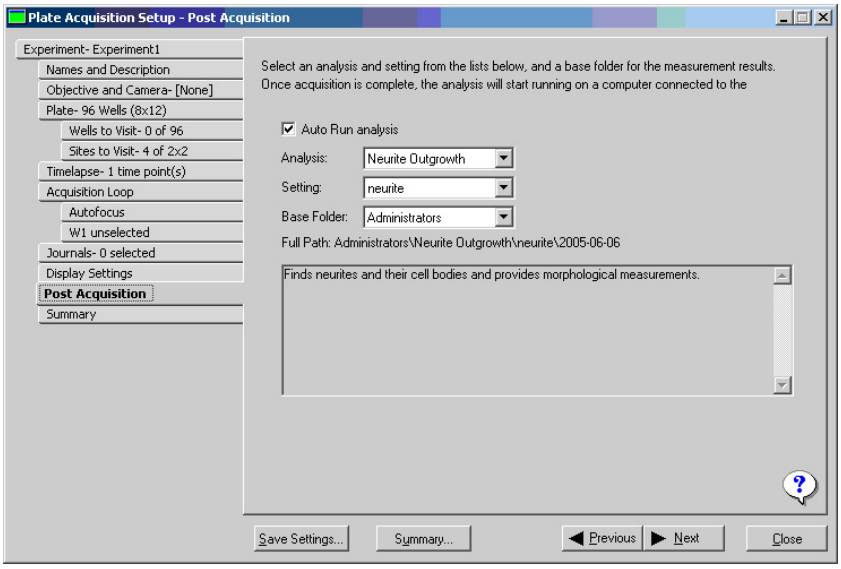
Use the Post Acquisition tab in the Plate Acquisition Setup dialog to choose a specific analysis to run on a data set after the acquisition is complete. The data set will be added to the Auto Run queue for analysis by a system set to Auto Run mode. You can select from a list of saved settings for any application module or journal assay saved to the database.

Note: If you do not want to automatically run post-acquisition analysis, ensure that Auto Run analysis after acquisition is not selected.

Note: The list of available assays and settings is a subset of the list that is on the Run Analysis tab of the Review Plate Data dialog. Analyses that do not have settings defined are not shown.

To select an analysis to automatically run after acquisition:

1. In the **Plate Acquisition Setup** dialog, on the **Post Acquisition** tab, select the **Auto Run analysis** check box.



2. In the **Analysis** field, select the assay (application module, custom module, or journal assay) to run after acquisition.

Note: Only analyses that have defined settings will be listed.

3. Select a settings file from the **Settings** field.

   A description of the settings file appears below the Settings field.

Note: You can configure and save settings in the Review Plate Data dialog.

Initiating an Automated Analysis

Using the Auto Run Mode, a networked system can run an analysis on plates automatically after they are acquired. After each plate is acquired on the main MetaXpress Software system, information regarding analysis of the plate is placed in a queue in the database. When other MetaXpress Software computers that are connected to the database are set in Auto Run Mode, they check the queue and run analyses on plates as the data becomes available.

Having separate computers to acquire and analyze your screening data greatly reduces the overall screening time by freeing up the main MetaXpress Software system to continue acquisition. You can also set up more than one MetaXpress Software computer to run in Auto Run Mode, further reducing the time it takes to process multiple plates.

Note: When the MetaXpress Software is in Auto Run Mode, the application cannot be used for any other purpose.

To set up and use Auto Run Mode:

1. On the computer that is acquiring images, start the MetaXpress Software and, from the **Screening** menu, click **Plate Acquisition Setup**.
2. In the **Plate Acquisition Setup** dialog, select the **Post Acquisition** tab.
3. Select the **Auto Run analysis** check box, and select an analysis in the **Analysis** field.
4. Continue to set up and run your acquisition. After the plate is acquired, it will be added to the Auto Run queue.
5. On the computer(s) running the analysis, start the MetaXpress Software and, from the **Screening** menu, click **Start Auto Run Mode**.

   The Auto Run Mode dialog appears.

6. To run the analysis on more than one plate, clear the **Leave auto run mode when plate is finished** check box. (If the check box is selected, only one plate will be analyzed.) Select this option if you want to use the system for other tasks and do not want the MetaXpress Software to start analyzing the next plate once it is finished with the one it is currently analyzing. Note that while in Auto Run Mode, all other MetaXpress Software options are disabled.
7. After the analysis is completed for all plates, click **Cancel** to close the **Auto Run Mode** dialog.

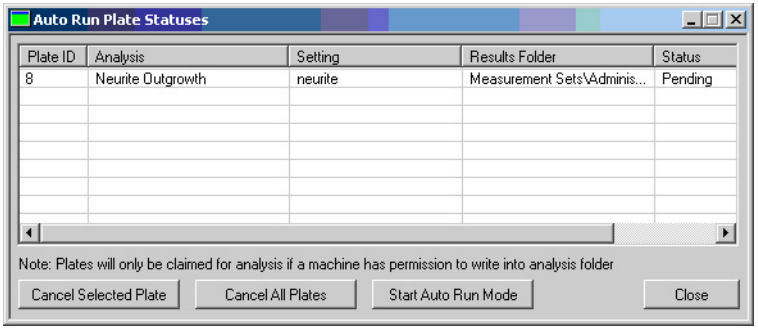
   Data from sites analyzed will remain in the database, but the plate will be removed from the Auto Run queue.

Monitoring the Status of Automated Analyses

1. On the computer running the analysis, from the **Screening** menu, click **Auto Run Plate Statuses [DB]**.

   OR

   From the **Screening** menu, click **Start Auto Run Mode [DB]**, and in the **Auto Run Mode** dialog, click **Status**.



Note: This dialog also shows any plates to be analyzed from the Plate Data Utilities dialog. However, these plates will not be claimed by computers in Auto Run Mode.

The following statuses are possible:

- **Running** — Indicates that the analysis is currently running on the plate. After the analysis is completed for the plate, the plate will be removed from the auto run plate status list.
- **Timeout** — Indicates that the analysis has not completed on a well or site in the expected time. The maximum time

allowed for analysis is set in the MDCStoreTools™ Data Management Utility with the Set Auto Run Timeout option. A timeout is normally caused by an error on the machine running the analysis. To diagnose the cause of the timeout, inspect the machine that has timed out for error messages or other problems. In some cases the problem can be resolved and the analysis can continue. If this happens, the status will return to Running. In other cases, the Auto Run must be canceled and the analyses run again. Some analyses, particularly custom ones created through the journaling system, take a long time to complete. In this case, increase the timeout value set for the Set Auto Run Timeout option to allow enough time to run the analysis.

- **Pending** — Indicates the analysis has not yet started on the plate.

2. To stop running an analysis on a plate, select the plate from the table in the **Auto Run Plate Statuses** dialog, and click **Cancel Selected Plate**.
3. To stop running the analysis on all plates, click **Cancel All Plates**.

Viewing Analysis Results

9

The MetaXpress® Software provides several ways to review your analysis results, including image overlays, graphs, and tables.

This chapter includes the following topics:

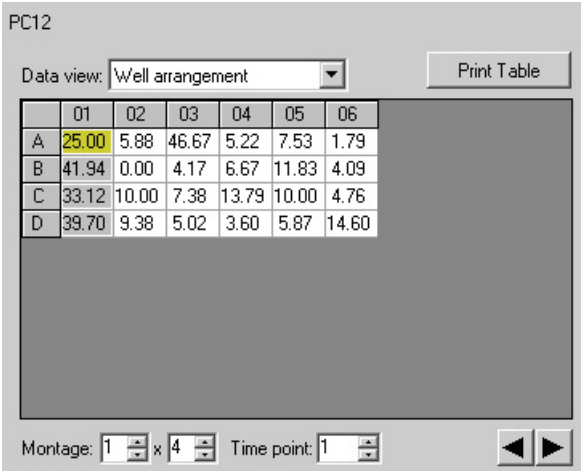
Note: In addition to the analysis review features of the MetaXpress Software discussed in this chapter, the AcuityXpress Software can be used for further visualization and investigation of your analysis results. Please contact your Molecular Devices representative for more information about the AcuityXpress Software.

Using the Review Plate Data Dialog Well Selection Table and Image Montage Window

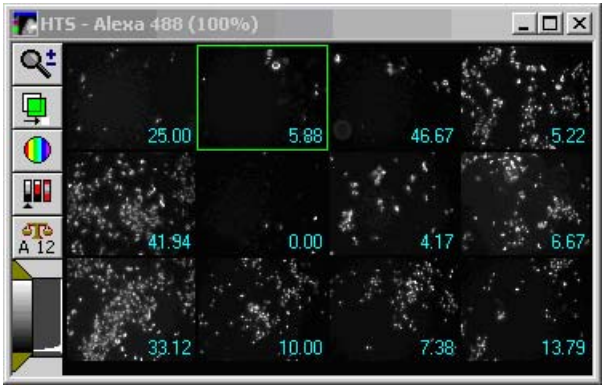
The well selection table serves several purposes. Markings, color highlighting, or shading are used to indicate the following:

- Wells marked with a (-) hyphen — Indicates the wells containing image data.
- Wells highlighted in gray — Indicates the wells included in the Montage window.
- Wells highlighted in yellow — Indicates the images selected for individual display.
- Wells highlighted in green — Indicates the wells that you selected (by right-clicking the wells).

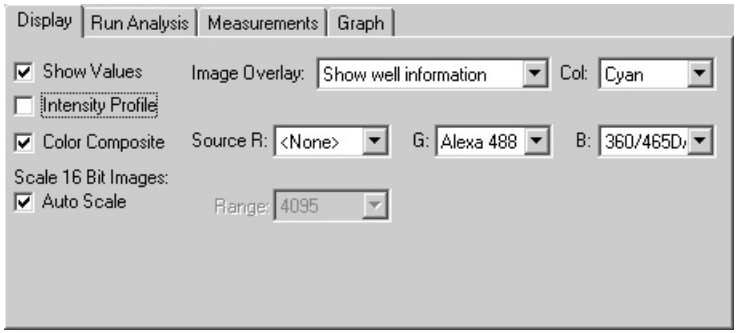
For example, the well selection table in the next figure shows the measurement results for an analysis.



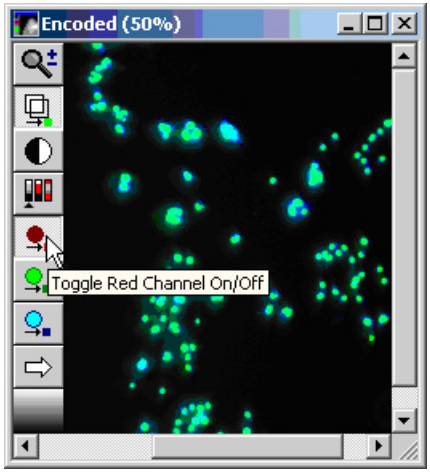
The image Montage window provides thumbnail views of the images associated with the data in the table. The contents of the Montage window depend on the settings made in various areas of the Review Plate Data dialog. For example, as described in Chapter 5, the Montage dimensions that you specify determines the number of images included in the Montage window.



Other settings that affect the Montage window are located on the Display tab. For example, to display the well number associated with the image, select the Well Number on images check box. To change the color of the values displayed on the thumbnail images, choose a different color in the Col field.



Use the Color Composite option to open a composite image in an image window and use the Toggle Color Channel buttons to turn on and off one or two of the wavelengths as shown in the next figure.



The Show Values option displays average analysis values in the well selection table and on each image in the montage. For each site, the values shown both in the table and on the images are the average of all the values for all defined objects in the site. Open the Cellular Results table to view values for all objects for the selected well or site as described in Using the Cellular Results Table on page 57.

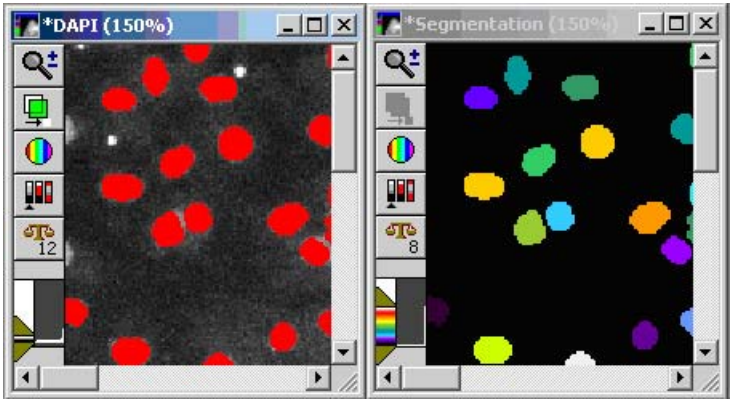
For more information on viewing and arranging images, see Chapter 5.

Viewing a Segmentation Image Overlay

When you run an application module interactively, such as when you test the module or run it on a selected site, an overlay image that shows the object segmentation opens. A result image also opens if the Display result image check box was selected when configuring the module.

Note: The overlay is saved to the database with the image after automated analysis only if you selected the Save Segmentation option when configuring the summary log for the application module. This option is not available for the Cell Proliferation HT, Nuclear Translocation HT, and Transfluor HT application modules.

You can toggle the overlay on or off from the source image with the Show/Hide overlay button on the side of your image window. If you save the source image, the overlay is also saved.



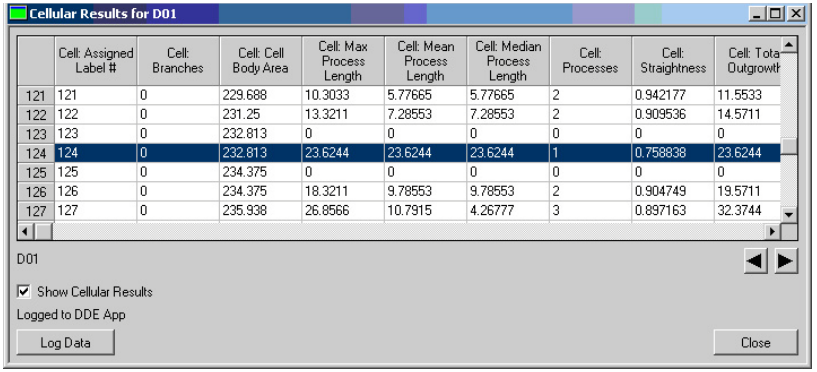
The result image does not contain the original image data — it is a graphical representation of the application module results.

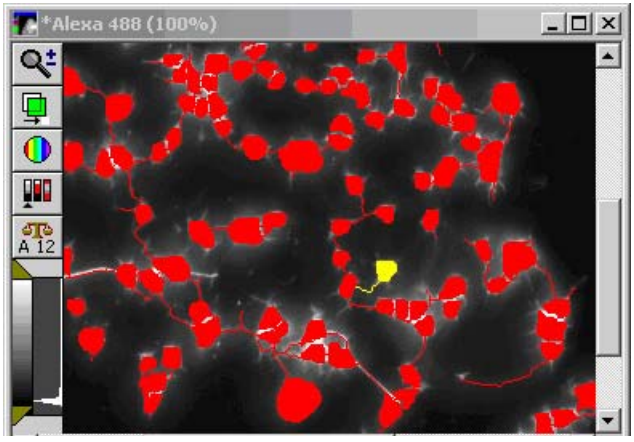
Using the Cellular Results Table

The Cellular Results table provides a quick way to correlate individual cells in a well plate image with data obtained from an application module. The data displayed in the Cellular Results table is the same data configured using the Configure Data Log (Cells) option for the application module you are using. However, you are not required have a log open to view the Cellular Results table.

1. If the table has not opened automatically, after running an application module analysis, in the **Review Plate Data** dialog, click **Cellular Results**.
2. In the table that appears, select a row in the Cellular Results table; the corresponding cell is highlighted in the segmented image as shown in the next figure. Or, select one of the segmented cells in the image; the corresponding data for the cell is highlighted in the Cellular Results table.

Note: Use Ctrl+Click to select multiple cells from the image or the Cellular Results table, use Shift+Click to select a range of cells from the Cellular Results table.





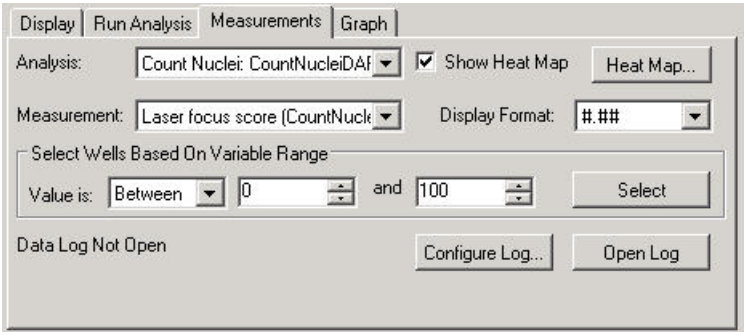
3. If you do not want the Cellular Results table to open each time an application module runs, clear the **Show Cellular Results** check box.

Note: If you have cleared the check box and later want to view the Cellular Results table when running an application module, from the **Window** menu, select **Show Cellular Results**.

Viewing Measurements

To view measurements of selected wells:

1. On the **Review Plate Data** dialog, select the **Measurements** tab.

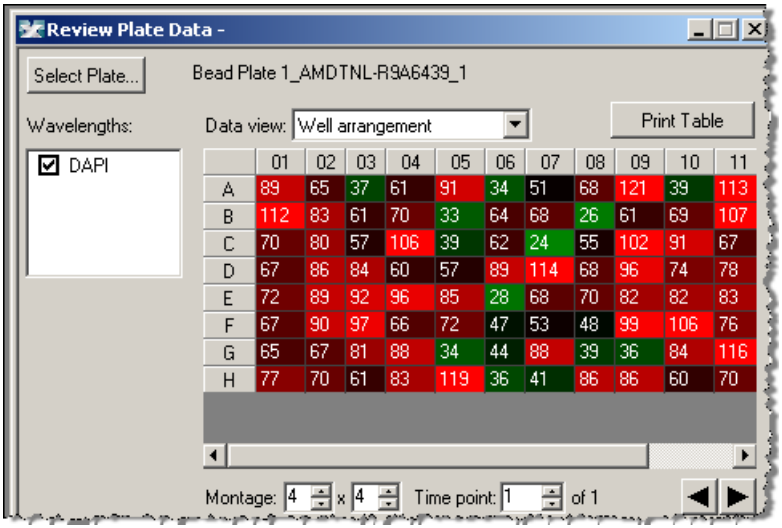


2. In the **Analysis** field, select the analysis that contains the measurements that you want to view.
3. In the **Measurement** field, select the name of the measurement that you want to use to query your images in the database.
4. In the **Display Format** field, select the number of decimal places that you want to display for your data in the grid.
5. In the **Select Wells Based on Variable Range** section, you can select wells automatically based on a range of measurements. The selected wells will be highlighted in green (make sure the Show Heat Map check box is cleared so that the Heat Map is not displayed).

   This feature highlights wells of interest based on a measurement so you can visually see them. It also provides a way to do a subpopulation analysis by automatically selecting wells of interest with which you can then use the Run Analysis for Selections option on the Run Analysis tab.

6. To log data to an Excel spreadsheet or a text log file, click **Open Log** and then click **OK**.
7. To select either Column and Row labels, Plate information, or both, click **Configure Log** and then click **OK**.
8. To log your query data, click **Log Data** or press **F9**.
9. To display the measurement values in a range of colors that you specify, in the **Data View** field, select **Well arrangement**, and then, on the **Measurements** tab, select the **Show Heat Map** check box.

   A color is displayed for each measurement value in the well selection table. For example:



This type of visual representation of data provides a simple way to quickly identify general patterns (for example, clusters of similar values). You can distribute the data on a linear or logarithmic scale, set low and high thresholds, and change the color scheme.

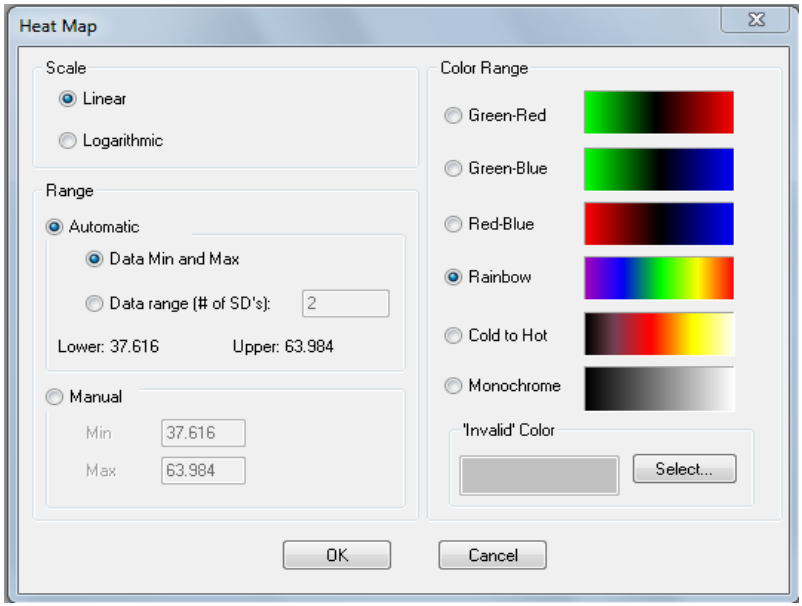
Note: The heat map is not available for the Measurement vs Well data view.

By default, the heat map uses a green-red color scheme and distributes all of the measurement values on a linear scale. The lowest (“cold”) value is green, while the highest (“warm”) value

is red. Measurements between the low and high ends are displayed with colors that correspond to the relative position of the values on the scale.

10. To change the range of data, select a different color scheme, or use a logarithmic scale to distribute the data, click **Heat Map**.

The Heat Map dialog appears.



11. Select the type of scale (Linear or Logarithmic) that the heat map will use to distribute the data:

- **Linear** distributes the data using the actual measurement values. This type of scale is ideal for data that does not cover a wide range of values.
- **Logarithmic** distributes the data on a logarithmic scale, starting with .0001 and exponentially increasing the values using a base of 10 (.0001, .001, .01, .1, 1, 10, 100, 1,000, 10,000). This type of scale presents a very wide range of data on a more manageable scale. The logarithmic scale is particularly useful when you want to analyze values that are at the lower end of a wide range of data.

12. Select the method that the heat map will use to calculate the range of data (Automatic or Manual) and then define the range:

- If you select **Automatic**, then to use the full range of data, select **Data Min and Max**.

OR

To have the MetaXpress Software distribute the data evenly below and above the mean value by a specific number of standard deviations, select **Data range (# of SD's)** and then type a number of standard deviations. This option calculates the range of data using the following formulas:

Minimum Value = Mean - (# Standard Deviations X Standard Deviation)

Maximum Value = Mean + (# Standard Deviations X Standard Deviation)

For example, if the mean value of the range of data is 50, the standard deviation between values in the data is 2, and you type 5 as the # standard deviations, then the minimum value is 40 (50 - (5 X 2)) and the maximum value is 60 (50 + (5 X 2)). Values at or below the minimum (with rounding) will be displayed with the "lowest" color and values at or above the maximum (with rounding) will be displayed with the "highest" color. Values between the minimum and maximum will be distributed along the scale and are displayed with corresponding colors.

- If you select **Manual**, then in the **Min** and **Max** fields, type the lowest and highest values to include in the range of data.

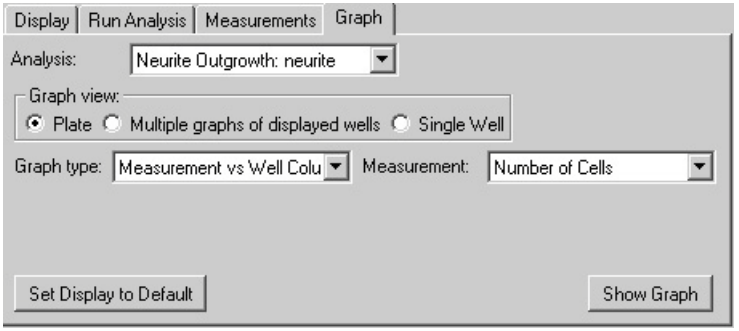
  Values at or below the minimum (with rounding) will be displayed with the "lowest" color and values at or above the maximum (with rounding) will be displayed with the "highest" color. Values between the minimum and maximum will be distributed along the scale and are displayed with corresponding colors.

13. Optionally, select a different color scheme for the heat map.
14. Optionally, to select a different color for invalid data in the heat map (for example, for wells that were not measured and for which data was not collected), click **Select** and in the dialog that appears, click a color and then click **OK**.
15. Click **Close** to close the Heat Map dialog.

Graphing Results

To configure a graph to display your data:

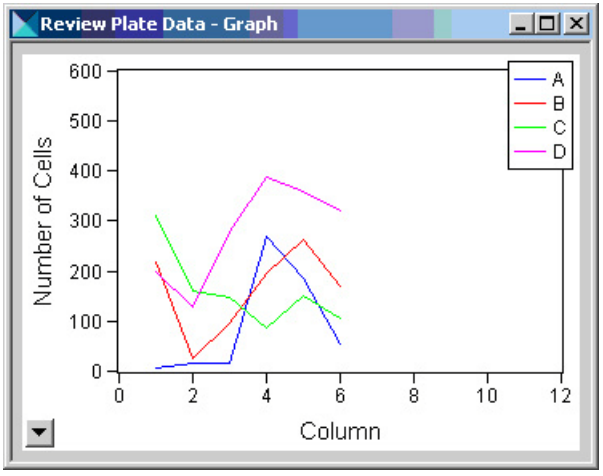
1. On the **Review Plate Data** dialog box, select the **Graph** tab.



2. In the **Analysis** field, select the analysis containing the data that you want to graph.
3. In the **Graph View** section, select the source location for the data.
4. In the **Graph Type** field, select a graph type. The options available for each graph type vary depending on the **Graph View** setting.
5. In the **Measurement** and **Measurement2** (if applicable) fields, select the measurement(s) to be graphed.
6. If you selected **Histograms** from the **Graph Type** field, select the number of bins to display in the resulting histogram in the **Number of Bins** field.
7. Select the **Auto Scale** check box to automatically scale the bin(s) based on the range of data from the selected measurement.

Note: This option is only available when Histogram is selected from the Graph Type field.

8. Click **Show Graph** to open the graph based on the current settings. For example:



Note: If the data on the graph is not displayed properly, click and drag one of the corners of the graph window to re-size it.

9. To configure the graph settings, double-click anywhere on the graph or click the **Show graph menu** arrow on the bottom left corner of the graph and select **Graph Settings**.
10. To reset the display parameters for the current graph to the default view, click **Set Display to Default**.

    There are separate graph defaults for each combination of Graph View and Graph Type.

Exporting Logged Data

Logged results data can be exported to an ASCII data file and made available to other programs that are able to process data in this format.

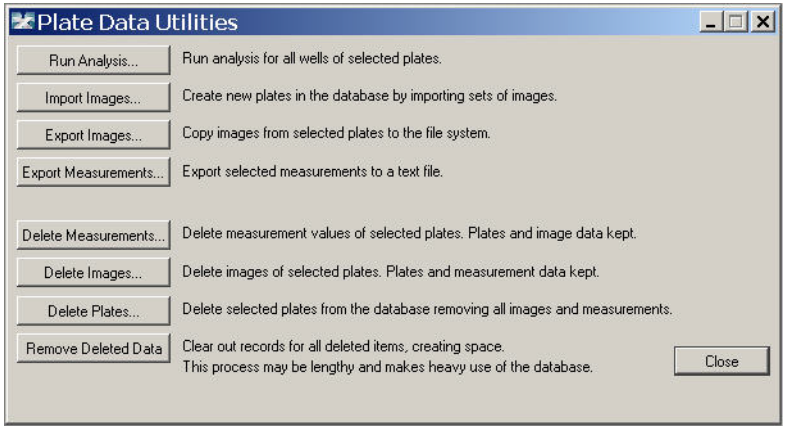
You can also log your data to a Microsoft Excel spreadsheet. In this case, the Excel program starts automatically and displays your data in a spreadsheet. You can use the capabilities of the Excel program to enhance your data for improved visual presentation.

You can also use the Export Measurements option on the Plate Data Utilities dialog to export selected data of one or more measurement sets to a text file. For more information, see Exporting Measurements on page 69.

Managing Plate Data

10

Use the Plate Data Utilities option on the Screening menu to manage images and data acquired during plate acquisition:



Note: The Delete Measurements, Delete Images, and Delete Plates options mark data for deletion but they do not delete the data from the database/fileserver. To permanently remove the data from the database/fileserver, you must use the MDCStoreTools™ Data Management Utility. For more information, see the *MDCStoreTools™ Data Management Utility User Guide*.

This chapter includes the following topics:

For information about the Run Analysis option on the Plate Data Utilities dialog, see Chapter 7.

Importing Images

Use the Import Images option to import one or more data sets created by other Meta Imaging Series® applications into the configured image storage location in the MDCStore database. Only images that have been saved with the appropriate file naming conventions and with an associated HTD file may be imported. The data will then be accessible using either the Plate Data Utilities dialog or the Review Plate Data dialog. You can also use this dialog to import a plate of Cellomics DIB files into the MDCStore fileserver or database as described in the next section.

Note: To import third party images or images with varying file name conventions into the MDCStore database, use the MDCStore™ Xchange Data Conversion Service that is available as an optional component of the MetaXpress Software installation.

1. In the **Plate Data Utilities** dialog, click **Import Images**.
2. In the **Import Images** dialog, click **Select Directory**.
3. In the **Browse for Folder** dialog, navigate to the local or network folder containing the data set(s) to import, and then click **OK**.

   The path is displayed next to the Select Directory button and the HTD files are displayed in the HTD Files field.
4. Select the data set(s) to import by selecting the check boxes next to the HTD files in the **HTD Files** field.
5. Select a location (either the fileserver or database) to import the data sets to from the **Move images** field.

Note: The locations in this list are configured using the MDCStoreTools™ Data Management Utility.

6. Click **Import**.

   The files are imported and are available for review in the Review Plate Data (DB) dialog.

Note: Depending on the number of files being imported, this step may take several minutes to complete.

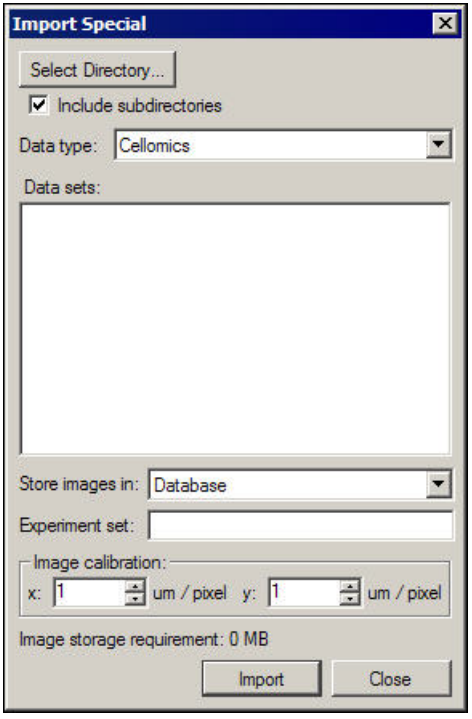
Importing Cellomics Data

Use the Import Special option to convert sets of Cellomics DIB files into data sets in the database that can be accessed using either the Plate Data Utilities dialog or the Review Plate Data dialog. The Import Special option creates MetaXpress HTD files based on the data in the DIB files and converts the images to the TIFF format. It then moves the data into your MetaXpress Software screening database or a selected local or network folder.

Note: You can also import third party images into the MDCStore database using the MDCStore™ Xchange Data Conversion Service that is available as an optional component of the MetaXpress Software installation.

Note: Cellomics DIB files do not include image calibration data. A default calibration of 1 µm = 1 pixel will be applied to the imported images unless you enter a specific calibration when you import the data.

1. In the **Plate Data Utilities** dialog, click **Import Images**, and then click **Import Special**.



2. In the **Import Special** dialog, click **Select Directory**.
3. In the **Browse for Folder** dialog, navigate to the local or network folder containing the Cellomics data to import, and then click **OK**.

   The path is displayed next to the Select Directory button and the Cellomics files are displayed in the Data sets field.
4. Select the check box next to the Cellomics file set that you want to import.
5. Select a location (either the database or an available network folder) to import the Cellomics data to from the **Store images in** drop-down field.
6. Enter a calibration ratio (µm per pixel) for the **x** and **y** values of the imported images in the **Image calibration** section. The default calibration is 1 µm = 1 pixel.
7. Click **Import**.

   The files are imported and will be available for review in the Review Plate Data dialog.

Exporting Measurements

The Export Measurements option exports selected data of one or more measurement sets to a text file. The Export Measurements wizard provides a query builder that helps you select the data to export. For example, of all the cell data in a measurement set, you might want to export data only from cells with a nuclear intensity greater than some threshold. Or, of all the image data, you might want to export data only from images where the number of cells is above some threshold.

When using the wizard, keep in mind the following:

- On both the Step 1 page and the Step 2 page of the wizard, the pane on the left contains the measurement sets or data types that can be queried, and the pane on the right displays the query as you build it.
- In the Query pane, multiple measurement set queries are combined with an OR by default, causing all selected measurement sets to be included.
- In the Query pane, multiple data type queries are combined with an AND by default, requiring that all conditions defined be met at the same time.
- To change the way queries are combined (known as Boolean expressions), in the Query pane, use Ctrl-click or Shift-click to select the individual queries for measurement sets or data types, and then click the OR or AND button. To undo or separate a combination, select the Boolean expression and click Break Up.
- To remove an expression from the Query pane, select the expression and click Remove.
- To save the query for later use, click Save. To load a query that was saved earlier, click Load.

To construct a query

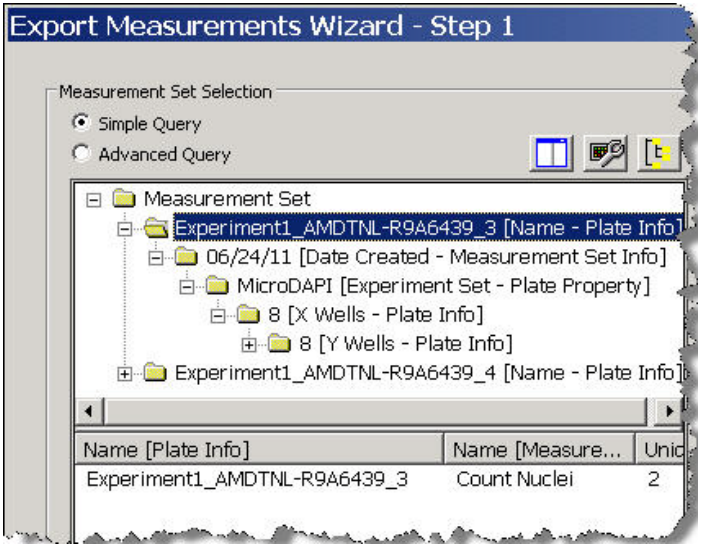
Two methods for constructing a query are available: simple and advanced. With either method, on the Step 1 page of the wizard, you select or specify the measurement sets to export and then, on the Step 2 page, you select the type of data to export and filter that data. The main difference between the two methods is the way in which you select or specify the measurement sets:

- Simple Query - On the Step 1 page, select the measurement sets from a list.
- Advanced Query - On the Step 1 page, specify the attribute criteria that identify the measurement sets (for example, measurement sets that contain data for plates acquired on a certain date). To use this method, you must know some of the unique attributes that identify the measurement sets containing data that you want to export (for example, the date created,

creator name, or name, or the plate information contained in the measurement set such as date annotated or global ID).

1. From the **Screening** menu, select **Plate Data Utilities**.
2. In the **Plate Data Utilities** dialog, click **Export Measurements**.
3. Select the type of measurement data that you want to export: cell measurements, image measurements, or both and then click **OK**.
4. On the **Export Measurements Wizard - Step 1** page, select **Simple Query** and proceed to Step 5 or select **Advanced Query** and proceed to Step 8.
5. To construct a simple query, in the top left pane, double-click the measurement set folder that contains the data that you want to export.

   The measurement set attributes are displayed in a "tree view" in the top left pane and the measurement set is added to the list in the lower left pane. For example:



   The attributes are displayed for informational purposes only to help you identify the measurement sets. As described in Step

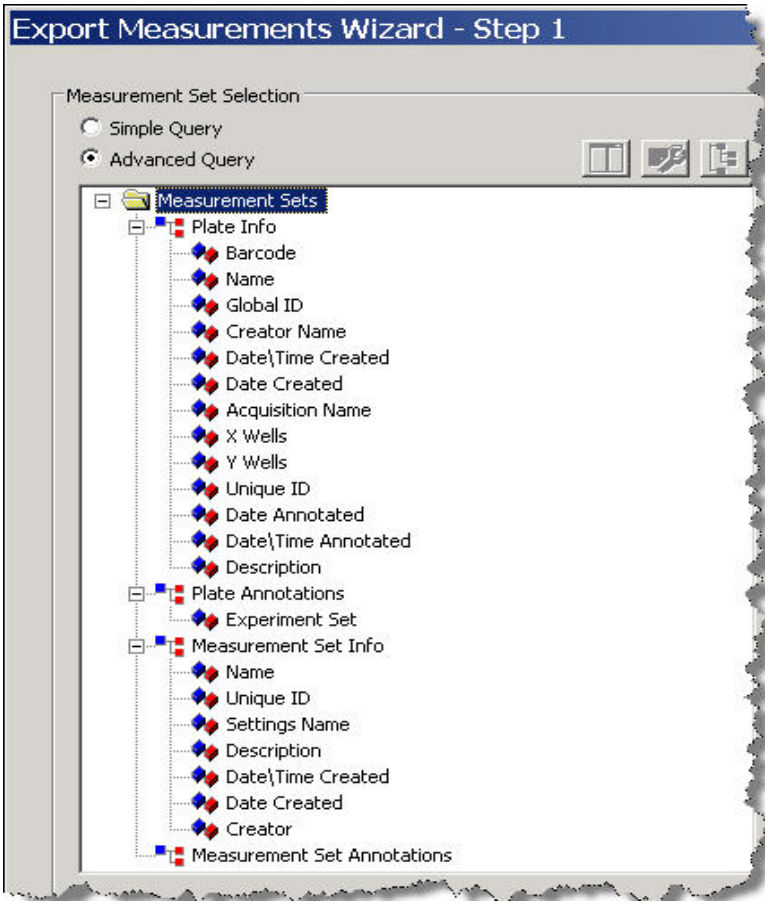
11, you will select the data types to export on the next page of the wizard.

Note: To add, remove, or rearrange columns in the lower left pane, click the **Configure Columns** button. In the dialog that appears, use Ctrl-click or Shift-click to select columns, and then click an arrow between the panes to move the columns.

6. In the lower left pane, select the measurement sets containing data that you want to export and then click the arrow between the panes to add the measurement sets to the Query pane.

Tip! To quickly add all measurement sets to the lower left pane, double-click the top-level Measurement Set folder in the top left pane. Then, in the lower left pane, use Ctrl-click or Shift-click to select measurement sets to add to the query.

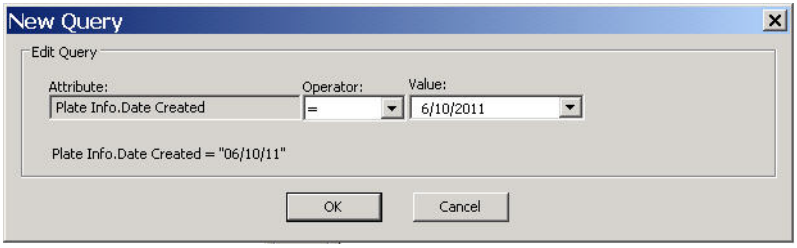
7. When all of the measurement sets containing data that you want to export are listed in the Query pane, click **Next**, and proceed to Step 11.
8. To construct an advanced query, in the left pane, expand the Measurement Sets folder, and then expand a folder containing measurement set attributes. For example:



9. Double-click the attribute that you want to use to identify the measurement sets for the query and, in the New Query dialog, select an operator and type a value to filter the data that will be exported.

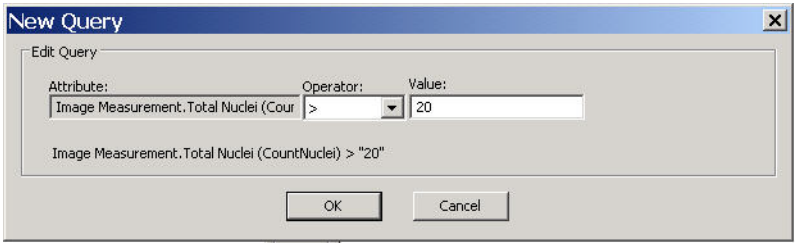
For example, to export just those measurement sets that contain data for plates acquired on June 10, 2011, expand the

Plate Info branch, double-click Date Created, select "=" for the Operator, and select the date in the Value field:



10. When you have included all of the criteria that identifies the measurement sets containing data that you want to export in the Query pane, click **Next**.
11. On the **Export Measurements Wizard - Step 2** page, expand the Data Types folder, expand a sub-folder, and then double-click the type of data that you want to export.
12. Define the criteria for the data that should be exported for the data type by selecting an operator and a value.

    For example, to export measurements that contain a total nuclei count of more than 20, select ">" for the Operator, and type "20" in the Value field:



13. Click **OK**.

    The query statement that defines the data that should be exported for that data type is added to the Query pane.
14. Continue to add as many data types as needed to the query.
15. Click **Finish**.
16. Proceed to the next section for instructions on specifying how the data will appear in the text file.

To configure the data for export

After you have used the wizard to configure the query, the Configure Data Export dialog appears. Use this dialog to specify how the data will appear in the text file and then export the data.

1. In the top left pane of the **Configure Data Export** dialog, select the data type that you want to display in rows in the text file (for example, sites, compounds, plates, cells, or wells) and then click the arrow to move the data type to the right pane.
2. In the lower left pane, select the measurement type that you want to display in columns for each data type (for example, total nuclei, total cell area, average intensity) and then click the arrow to move the measurement type to the right pane..

Note: To re-size the width of the columns, drag the column headings or right-click and select Fit Columns. To sort the data in a column in ascending or descending order, click once in the column heading. To reorder the items in the right panes, click the up and down arrows.

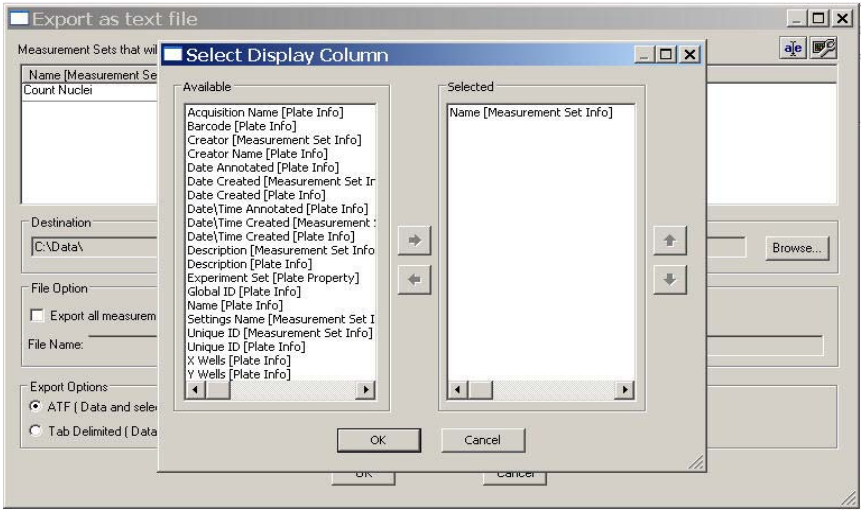
3. To calculate a statistic for the exported data (for example, if you are averaging the data for multiple cells or wells), select the statistic from the list in the **Apply Calculation** field. If you do not want to include a statistic in the text file, select **None**.

   The Data Layout View section shows how the data will appear in the text file.
4. Click **OK**.

   The Export as text file dialog that appears lists the measurement sets that contain the data to be exported. By default, the data for each measurement set is saved as a separate text file with a system-generated file name. By default,

the data for each measurement set is saved as a separate text file with a system-generated file name.

5. To change the name of the system-generated file name, select the file name in the list, and then click the **Edit File Name** button  .
6. To combine the measurement data for all measurement sets into one text file, select **Export all measurements to one file** and type a file name.
7. To include information in the header of the text file such as the plate name, date the measurement set was created, or the plate description, click the **Configure Columns** button  . In the dialog that appears, use Ctrl-click or Shift-click to select columns, and then click an arrow between the panes to move the columns.



8. Click **OK**.

   The data is saved in the text file or text files, and the Export Measurement Set Summary dialog appears.
9. To create a separate text file that contains the computer name, destination folder, number of files, and file names related to the export, click **Save Summary**.
10. Click **Close**.

Exporting Images

To export images to a local or networked folder:

1. From the **Screening** menu, click **Plate Data Utilities**.
2. In the **Plate Data Utilities** dialog, click **Export Images** to export images.
3. In the upper pane of the **Select Plate for Export** dialog, expand the **Plate** folder to view folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Select the plate to export from the lower pane and click **Select**.
6. In the **Browse for Folder** dialog, navigate to the local or networked folder to export the images to and click **OK**.

The acquired images (TIF), thumbnnail images (TIF) and the associated experiment plate information file (HTD) are exported.

Deleting Plates

To delete plates from the database you must first delete them using the Delete Plates option. After that, use the MDCStoreTools Data Management Utility to permanently remove the plates from the database or fileserver. If you create an archive or a backup of the images with the MDCSToreTools Data Management Utility, then you will be able to retrieve the plates with the images, but not the measurements. For more information, see the *MDCStoreTools™ Data Management Utility User Guide*.

Note: You must have the appropriate security privileges to select plates for deletion. Deleting plates will delete related images and measurements.

1. From the **Screening** menu, click **Plate Data Utilities**.
2. In the **Plate Data Utilities** dialog, click **Delete Plates**.
3. In the upper pane of the **Select Plates for Deletion** dialog, expand the **Plate** folder to view folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.

5. Use Ctrl+click or Shift+click to select the plates to delete from the lower pane, click **Select** and then click **OK**.

Note: This step removes the data from the Select Plate for Deletion dialog but not from the database/fileserver. To permanently remove the data from the database/fileserver, you must use the MDCStoreTools Data Management Utility. For more information, see the *MDCStoreTools™ Data Management Utility User Guide*.

Deleting Images

To delete images from a plate you must first delete them using the Delete Images option. After that, use the MDCStoreTools Data Management Utility to permanently remove the images from the database or fileserver. See the *MDCStoreTools™ Data Management Utility User Guide* for more information. (The image measurements and data will remain in the database.)

Note: If you delete only the images from a plate (and not the measurements or the plate itself) that has been analyzed and contains data, you can still view the data from the plate using the Review Plate Data dialog.

To delete images from a plate:

1. From the **Screening** menu, click **Plate Data Utilities**.
2. In the **Plate Data Utilities** dialog, click **Delete Images**.
3. In the upper pane of the **Select Plates for Image Deletion** dialog, expand the **Plate** folder to view folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Select the plate containing the images to delete from the lower pane and click **Select** and then click **OK**.

Note: This step does not remove the images from the database/fileserver. You must use the MDCStoreTools Data Management Utility to permanently remove the images from the database or fileserver. See the *MDCStoreTools™ Data Management Utility User Guide* for more information.

Deleting Measurements

To delete measurements from a plate you must first delete them using the Delete Measurements option. After that, use the MDCStoreTools Data Management Utility to permanently remove the measurements or a selected measurement from the database or fileserver. See the *MDCStoreTools™ Data Management Utility User Guide* for more information.

Note: This option deletes all the measurement sets associated with a plate. To delete just one measurement set from a plate, use the Manage Measurement Set option in the MDCStoreTools Data Management Utility. For more information, see the *MDCStoreTools™ Data Management Utility User Guide*.

1. From the **Screening** menu, click **Plate Data Utilities**.
2. In the **Plate Data Utilities** dialog, click **Delete Measurements**.
3. In the upper pane of the **Select Plates for Measurement Deletion** dialog, expand the **Plate** folder to view folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Use Ctrl+click or Shift+click to select the plates containing the measurements to delete from the lower pane and click **Select**.

Note: This step does not remove the data from the database/fileserver. To remove the data from the database/fileserver, you must use the MDCStoreTools Data Management Utility. For more information, see the *MDCStoreTools™ Data Management Utility User Guide*.