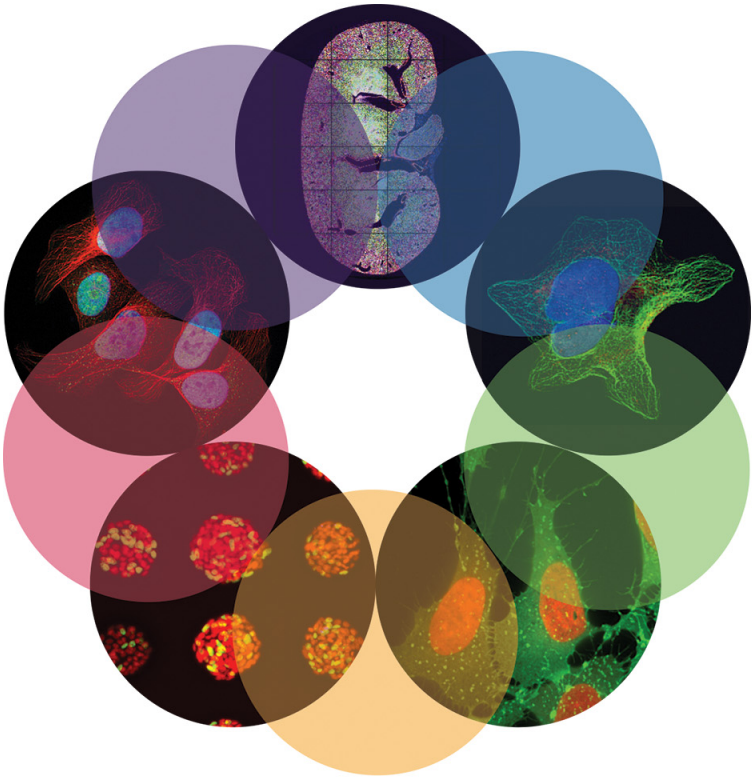

MetaXpress®

High Content Image Acquisition & Analysis Software

Version 6.2

Analysis Guide

5039429 C
April 2016

MOLECULAR DEVICES

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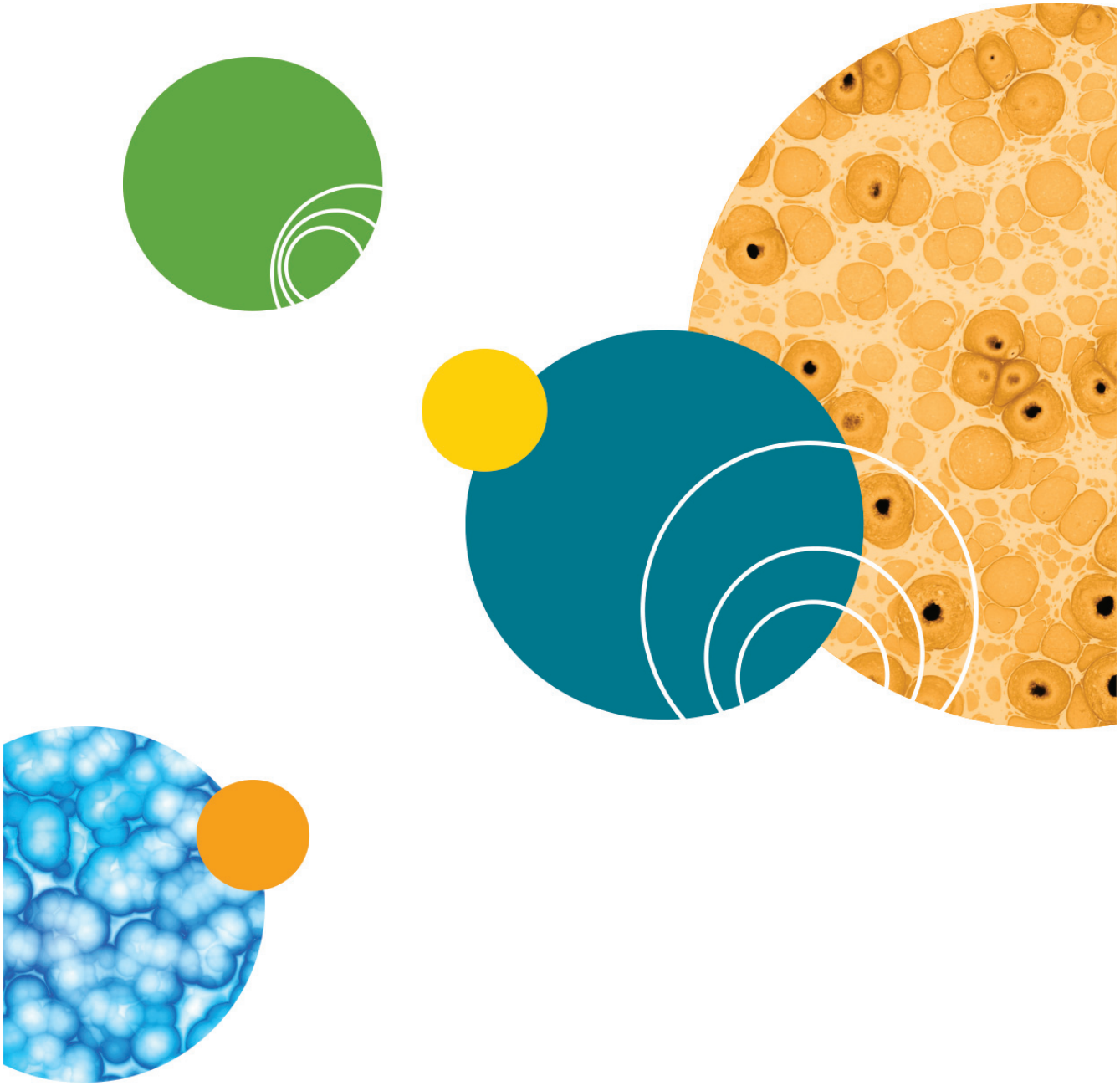
Contents



Introduction

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

- **Acquisition** 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® Micro 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.
- **Analysis** consists of selecting, measuring, assessing, and managing acquired images and plate data.

In addition to this introduction, this guide describes the topics that are pertinent to the general analysis workflow:

- Chapter 2: Before You Start Your Analysis on page 11
- Chapter 3: Selecting and Analyzing Plates on page 13
- Chapter 4: Viewing Analysis Results on page 49
- Chapter 5: Application Modules on page 67
- Chapter 6: Custom Modules on page 93
- Chapter 8: Automating and Monitoring an Analysis on page 107
- Chapter 9: Batch Analysis on page 115
- Chapter 10: Managing Plate Data on page 119

Simplified Menu Structure

An optional simplified menu structure can be installed to reduce the number of top-level menus. All the features of the MetaXpress Software are available in this reorganized menu structure.

The procedures in this guide describe both the default menu structure and the simplified menu structure.

You can use the **Menu Map** in the **Help** menu to help you find the locations of features in the simplified menu structure.

1. Click **Help > Menu Map**.
2. In the **Menu Map** dialog, select to view the **Default to customized** menu map.
3. Click the menu path where the software feature you want is found in the default menu structure.

   The simplified menu path appears to the right of the desired feature in the menu.
4. Click the menu path in the software window to access the desired feature.

For example, if you want to make a duplicate of an image, then use the following procedure:

1. Click **Help > Menu Map**.
2. In the **Menu Map** dialog, select to view the **Default to customized** menu map.
3. Click **Edit > Duplicate**.

   The simplified menu path **-> Edit: Image: Duplicate Image/Plane** appears to the right of the **Image** option in the submenu.
4. In the software window, click **Edit > Image > Duplicate Image/Plane**.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, http://www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Part of effective communication with Molecular Devices is determining the channels of support for the ImageXpress System, including the MetaXpress Software. Molecular Devices provides a wide range of support:

- Documentation: Check the guides that are included on the installation media and the help that is available within the MetaXpress Software. Help for an active dialog can be accessed by pressing F1 on your keyboard.
- Online knowledge base: The knowledge base has links to technical notes, software upgrades, newsletters, user guides, and other resources. Visit the Molecular Devices Support web page at http://www.moleculardevices.com/support and follow the links to the knowledge base.
- MetaMorph Software forum: This forum has information on journal scripts and custom modules, and has links to videos and webinars that can help you troubleshoot problems and be more productive using the software. Visit the forum at metamorph.moleculardevices.com/forum.

- Technical Support:

  Phone: Contact Technical Support at (800)-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

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

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

  - To find your system ID number, in the MetaXpress Software, click **Help > About MetaXpress**. The About dialog displays your system ID number.
  - The system serial number is located on your instrument.
- Additional support resources include:
  - Nikon web-based microscopy course: http://www.microscopyu.com
  - The Molecular Probes Handbook: http://www.lifetechnologies.com/us/en/home/references/molecular-probes-the-handbook.html
    This resource 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.semrock.com
    - http://www.chroma.com

Before You Start Your Analysis

2

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

- **Available wavelengths**

  Some application modules require certain stains and a specific number of wavelengths for analysis. Other modules analyze plates with a single wavelength, or with a flexible number of wavelengths. In addition, some modules can analyze transmitted light images, which have dark objects on a light background, while others expect fluorescent images, which have light objects on a dark background. If you are planning on using a particular analysis module, check the requirements for the module and verify that it is suitable for your assay.

- **Cell or Object Identification**

  To obtain a cell count, or measure features on a cell-by-cell basis, the analysis module must identify the individual cells. The most common and generally, the most reliable method is to use a nuclear stain. Other stains or transmitted light can be used, but sometimes this can require a custom module or journal.

- **Features of Interest**

  If subcellular features must be identified, then they should have a distinct appearance in the images that are being analyzed. Commercial stains are available for various subcellular organelles (for example, mitochondria) and these can be helpful to include in your assay when trying to analyze these features.

- **Measurements of Interest**

  One of the most important considerations in designing an analysis is selecting the measurements of interest. Ideally, the selected measurements should clearly distinguish the different possible phenotypes that are expected from the experiment. Sometimes, you might not know ahead of time which specific measurements will give the best results. For example, when measuring objects that change size with treatment, is the most useful measurement the area, the perimeter, or the length and breadth? And over a population of cells, should you compare the average area of all the cells, or the percentage of cells that fall within a certain size range? If you are unsure, then run some

tests using known controls and generate all the available relevant measurements to determine which ones give the most consistent results before scaling up to a large screen.

- **Statistics**

  Verify that your assay has a sufficient sample size to give you statistically significant results. Are you sampling enough of cells per well, and enough of replicates per treatment? If the event that you are observing is rare (affects a low percentage of cells), then you might need an increased sample size.

- **Data Storage Requirements**

  When reviewing analysis results, do you need to view results for each individual cell, or only summary data for the site? Do you need to store the segmentation overlay for all your plates? Cell-by-cell data and segmentation overlays can require a significant amount of database space, and should be saved only when required. In general, Molecular Devices recommends using these options during assay development, but not during screening unless it is essential for your particular assay.

- **Throughput Needs**

  If you are analyzing many images, then analysis throughput can be important to you. Keeping the analysis as simple as possible helps to improve the analysis speed. Consider using the optional MetaXpress PowerCore Software, which uses parallel processing to significantly accelerate image analysis using either application modules or custom modules.

- **Plate to Plate Variation**

  If you must compare results across plates or across time, then it is important to control for variation in staining intensity, cell density, and other assay parameters that can vary from plate to plate. Make sure that the appropriate positive and negative controls are included on each plate, and that your analysis settings will measure these assay parameters.

Selecting and Analyzing Plates

3

The first step in the image analysis process is to select the plate in the database that contains the images to analyze. You can then view and arrange the images in various ways to examine and compare them to decide which wells in the plate are appropriate for analysis, and how you might need to analyze the wells. 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. Based on all the information that you collect from arranging and viewing the images, you can select the appropriate wells for analysis. Finally, after you select the appropriate wells in the plate, you analyze the selected wells.

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.

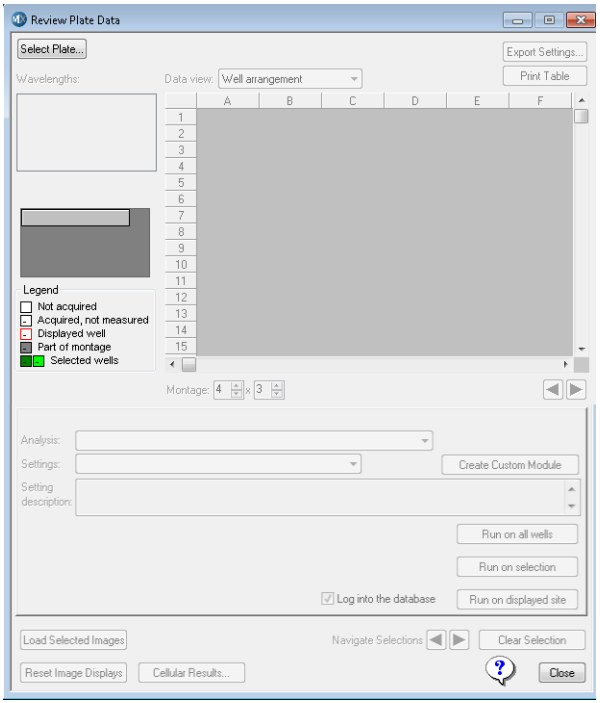
Settings that are made in an application module dialog (described in Chapter 6: Configuring Application Modules on page 55) define the characteristics that are specific to the selected application module. These application module settings can be saved and reused.

This chapter includes the following topics:

Selecting a Plate for Analysis

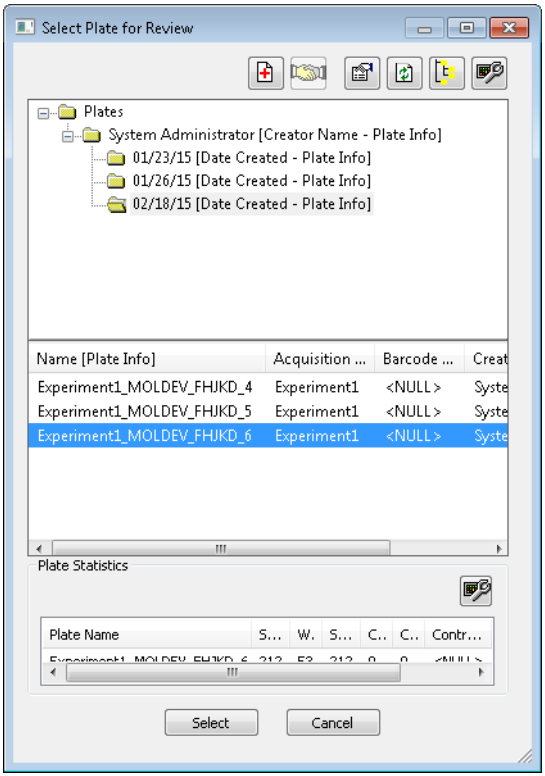
Note: For information about using the **Review Plate Data** dialog, see the video: https://www.youtube.com/watch?v=ks4UKc8vq-A.

1. Click **Screening > Review Plate Data**.
   In the simplified menu structure, click **Screening > Review Plate**.



2. In the **Review Plate Data** dialog, click **Select Plate**.
3. In the **Select Plate for Review** dialog, expand the plates folder in the top pane to view the subfolders that contain plates that have been saved in the database.

Tip: To select the information such as a barcode or plate description by which to organize the subfolders and plates, click the **Configure Branches** button in the upper-right corner of the dialog.



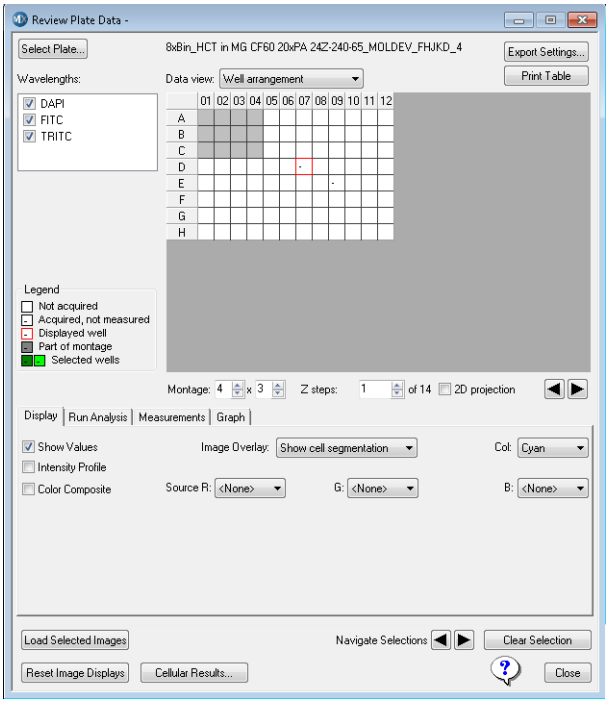
4. Double-click a subfolder in the top pane to display its contents in the middle pane.
5. In the middle pane, select a plate, and then click **Select**.

Tip: After you select a plate, default information about the selected plate is displayed in the bottom pane of the **Select Plate for Review** dialog. To add or modify the information that is to be displayed for a selected plate, click the **Configure Displayed Columns** button in the upper-right corner of the dialog.

The **Review Plate Data** dialog opens. The **Run Analysis** tab is the open tab. The image data for the selected plate is displayed in the dialog.

6. Click the **Display** tab, and then continue to Viewing and Arranging Images on page 17.

Tip: After you load a plate into the **Review Plate Data** dialog, as long as the plate images have *not* been imported, you can click **Export Settings** to export the protocol settings that were used to *acquire* the plate to an .hts file. You must name the file and select the location in which to save the file. You can then select this saved protocol file when acquiring data for other plates.

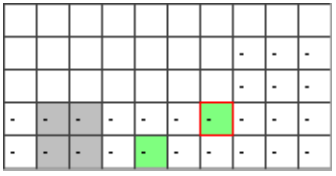


Viewing and Arranging Images

In the **Review Plate Data** dialog, you use the Plate map and the options on the **Display** tab to view, arrange, and then ultimately select images for analysis. Each square in the Plate map represents a single well in a plate. Markings, color highlighting, or shading are used to indicate the following:

- Wells that are marked with a hyphen (-) indicate that the wells contain image data.
- Wells that are highlighted in gray indicate wells that are displayed in a Montage window.
- Wells that are highlighted in bright green indicate wells that have been selected for analysis.
- Wells that are highlighted in dark green indicate wells that are included in the Montage window and that have also been selected for analysis.
- A well that is outlined in red indicates a well that is being displayed in its own individual image window.

  For example:



The **Data view** drop-down list at the top of the Plate map is useful for arranging images according to time points and measurements after you have run an analysis on the images. Before analysis, the Plate map is arranged in the same order that the wells are arranged in the microwell plate (**Well arrangement**).

1. In the **Review Plate Data** dialog, in the **Wavelengths** section, select one or more wavelengths to display in the images.
2. Do one or more of the following on the **Display** tab as needed to help in the viewing and arranging of images. See:
   - Selecting wells for the Montage window and viewing well images in a Montage window on page 18.
   - Viewing and arranging images for multi-site wells on page 20.
   - Generating a 3-D intensity profile graph on page 20.
   - Assigning colors to acquisition wavelengths on page 21.

- Creating a composite image on page 21.
- Scaling 24-bit color composite images on page 22.
- Auto scaling of 16-bit single wavelength images for display on page 22.
- Viewing time point data on page 24.
- Viewing Z series images on page 28.
- Collating the images of multiple wells on page 33.

Selecting wells for the Montage window and viewing well images in a Montage window

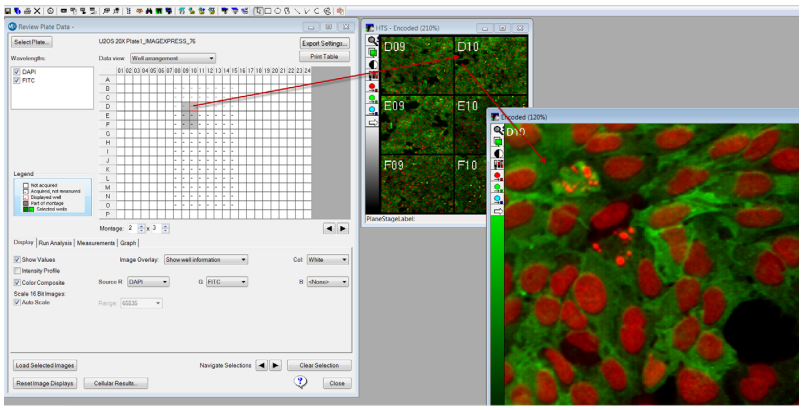
You can view a thumbnail for each well in a plate in a Montage window and compare the overall density and distribution of the sample material in each well. Samples showing an overall high density and even distribution are generally better candidates for analysis than wells with very low sample density and uneven distribution. To select a well or wells for displaying in the Montage window, do any of the following as needed:

- To select a single well in a plate for the Montage window, click once on the appropriate square in the Plate map.
- To select multiple wells in a plate for the Montage window, click and hold the left mouse button, and then drag the cursor over the appropriate wells in the Plate map.
- To select all the wells in a plate for the Montage window in a single step, click in the upper left corner of the Plate map (the blank cell between “A” and “01”).

  The Montage windows that open 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, then the window is labeled HTS-Encoded.

Note: The montage dimensions that are displayed in the **Montage** fields determine the total number of sites that are displayed across all the wells that have been selected for inclusion in the montage. Molecular Devices recommends that you do not manually adjust these values. Instead, allow the MetaXpress software to automatically adjust these dimensions as you select or de-select wells.

You can open an image in a Montage window in a separate window at full resolution. To do so, click the image in the Montage window or right-click the appropriate well in the Plate map. For example, the following figure shows the image for well D10 in the Montage window. If you click D10 in the Montage window, the full resolution image opens in a separate window.



Note: If you right-click a well, then the well is outlined in red and highlighted in green to indicate that is selected for analysis. To de-select the well for analysis, right-click it again.

To display images in the Montage window for a different group of wells, do one of the following:

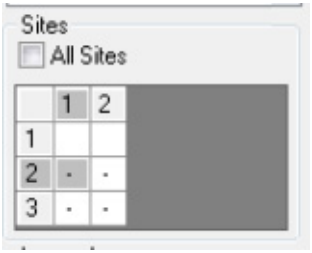
- Click another well in the Plate map to indicate the top left well in the new group.
- Use the left or right arrow beneath the lower right corner of the Plate map to move the group of selected wells.

The group of wells that is selected has the same number of wells (rows and columns) as the wells that were originally selected for the montage.

To toggle the display of the well number in the Montage window, select **Show well information/No overlay display** on the **Image Overlay** drop-down list. To change the color of the well number in the Montage window, select a different color on the **Col** drop-down list.

Viewing and arranging images for multi-site wells

If images were acquired at multiple sites in the wells in a plate, then a **Sites** section is displayed to the left of the Plate map. The Sites graphic indicates the sites for each well for which data was acquired as specified on the **Sites to Visit** tab on the **Plate Acquisition Setup** dialog. If data was not acquired for a specified site, then a blank white pane is displayed for site in the graphic. If data was acquired for a site, then a hyphen (-) is displayed. For example, in the following graphic, data was acquired for only four of the six sites that were visited in each well.



If images were acquired at multiple sites in a well, then:

- Click any available site in the **Sites** section to view only this site for all selected wells in the Montage window.
- Click **All Sites** to view all sites for all selected wells in the Montage window.

Note: If you select **All Sites**, then the MetaXpress software automatically adjusts the Montage dimensions to display all the sites in the selected wells in the Montage window.

Generating a 3-D intensity profile graph

To transform the image into a three-dimensional intensity profile graph, select **Intensity Profile**. The MetaXpress Software uses the colors that are assigned to the image and displays the highest intensities as the highest peaks in the graph.

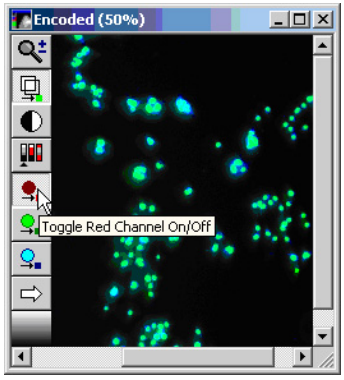
Assigning colors to acquisition wavelengths

You can assign a color to each acquisition wavelength in your data set. How you assign colors depends on whether your images were acquired with a single wavelength or with multiple wavelengths.

- For single wavelength images, select an appropriate LUT on the image window.
- For multi-wavelength images, select the appropriate wavelength on the **Source R** (red), **G** (green), or **B** (blue) drop-down list on the **Review Plate Data** dialog.

Creating a composite image

To combine images that were acquired with multiple wavelengths into a single composite image, select **Color Composite**. If images were acquired with multiple wavelengths, and **Color Composite** is not selected, then a separate Montage window displays the images for each wavelength that is selected in the **Wavelengths** section. Use the **Toggle Channel Color** buttons to turn on and off wavelengths as needed.



Note: If **Color Composite** is selected but all colors are set to [None] for **Source**, then no images are displayed for the plate and there are no error messages.

Tip: Although the **Color Composite** option is helpful for reviewing images, it does not produce the best quality images for presentation or publication. For better quality images, clear **Color Composite**, open single wavelength full resolution images for the site of interest, and then use the **Overlay Images** function (**Display > Overlay Images** in the default menu or **Edit > Display > Overlay Images** in the simplified menu).

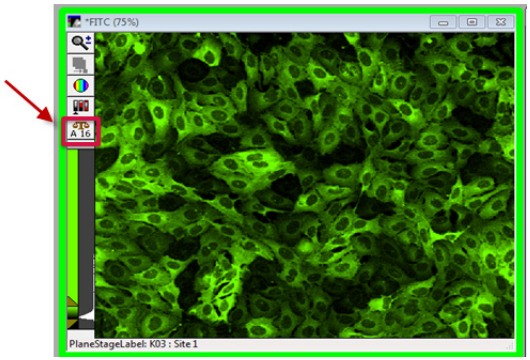
Scaling 24-bit color composite images

To manually define a range for scaling 24-bit color composite images, clear **Auto Scale**, and then in the **Range** field, select the upper range for the scaling.

Note: **Color Composite** must be selected for scaling. To automatically scale 24-bit color composite images, select Auto Scale.

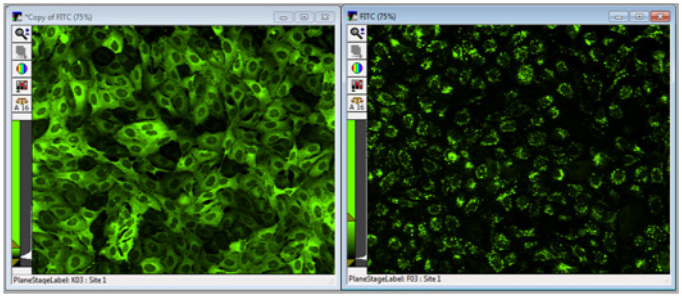
Auto scaling of 16-bit single wavelength images for display

Auto Scale automatically adjusts the scaling based on the minimum and maximum intensities in an image. By default, when you open an image in the MetaXpress Software, Auto Scale is turned on. This is indicated by the letter “A” displayed in the **Image Scale** button on the toolbar of a Montage window, or an individual image window. With Auto Scale turned on, pixels in the upper 1% of the intensity range are assigned the maximum possible intensity (generally 65,535) and pixels in the lower 1% of the intensity range are assigned the minimum possible value (0, or black), therefore making the image brighter on the display.



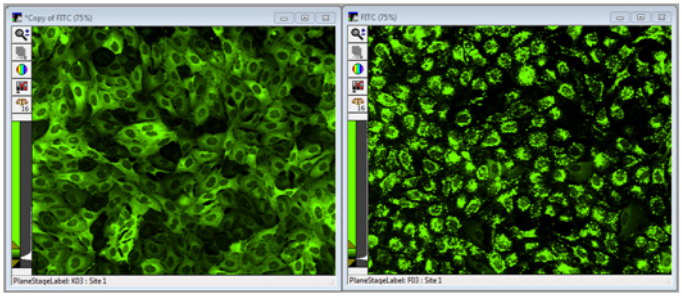
When comparing different objects between multiple images, it is not helpful to have Auto Scale turned on, as both bright and dim samples appear with the same brightness.

Auto Scale On



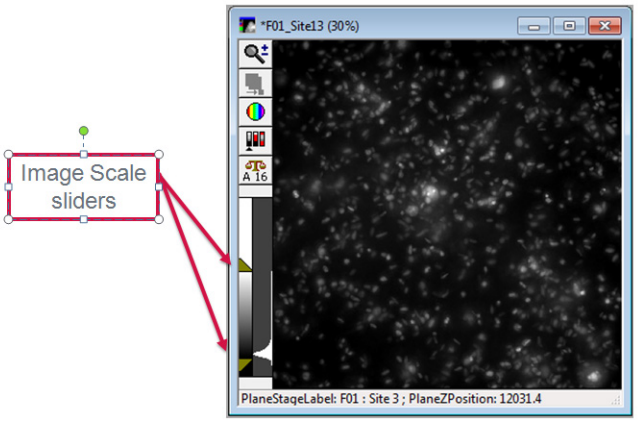
If Auto Scale is turned off, only then do the objects or images appear with their true intensity levels.

Auto Scale Off



When comparing the intensity levels of objects across multiple images, turn off Auto Scale and use the Image Scaling sliders to adjust the image display, as shown in the following figure:

- Drag the top Image Scale slider to set the intensity at which images are turned brighter (white) or dimmer (black). Moving the top slider up turns the image darker. Conversely, moving the top slider down turns the image brighter.
- Drag the bottom Image Scale slider to set the intensity at which the background is turned brighter (white) or dimmer (black). Moving the bottom slider up turns the background darker (black). Conversely, moving the bottom slider down turns the background brighter (white).



Viewing time point data

If you have acquired data for time point experiments, then multiple options are available for viewing this data. You can:

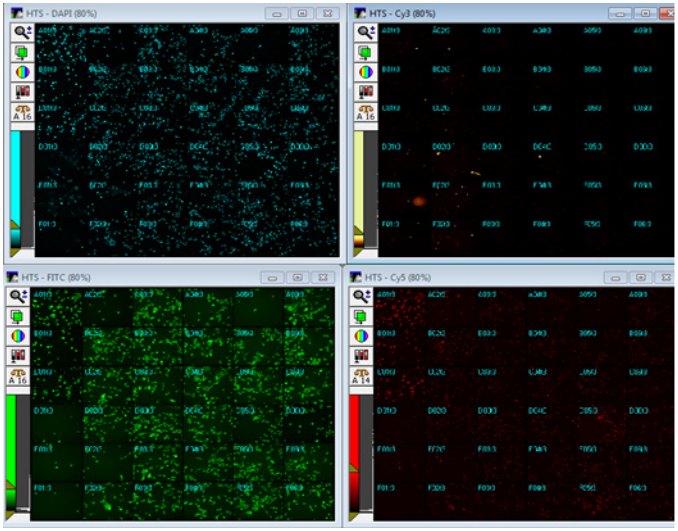
- View a thumbnail montage of multiple wells at a single time point. See To view a thumbnail montage of multiple wells at a single time point on page 25.
- View multiple wells at multiple time points. See To view multiple wells at multiple time points on page 25.
- View high-resolution images from selected wells/time points. See To view high-resolution images from selected wells or time points on page 26.
- View time points for a single site/well as a stack. See To view a stack (.stk) file of a series of time points from one site/well on page 27.

To view a thumbnail montage of multiple wells at a single time point

1. In the **Data view** field, select **Well arrangement**.
2. In the **Time points** field, select the appropriate time point; for example, 3 of 5.
3. Select the wells that are to be displayed in the Montage window. See Selecting wells for the Montage window and viewing well images in a Montage window on page 18.

Note: You might need to use the scroll bars in the Sites graphic to scroll to a site to select it.

The well ID/time point for each selected well is displayed in the upper left corner of each thumbnail image in the Montage window; for example, A01t3, B01t3, and so on.



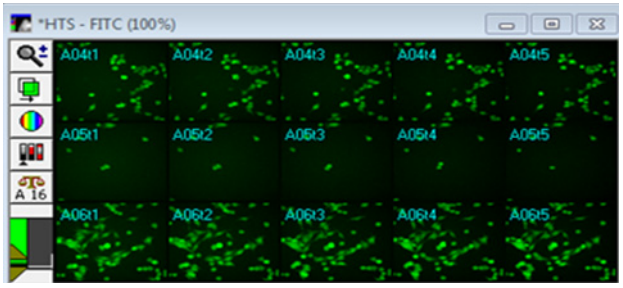
To view multiple wells at multiple time points

1. In the **Data view** field, select **Time Point vs Well** to change the layout of the Plate map. (The time points are displayed in the “X” direction and the well IDs are displayed in the “Y” direction.)

2. Select the wells that are to be displayed in the Montage window, which tiles the images at each time point in a filmstrip-like format across the montage. See Selecting wells for the Montage window and viewing well images in a Montage window on page 18.

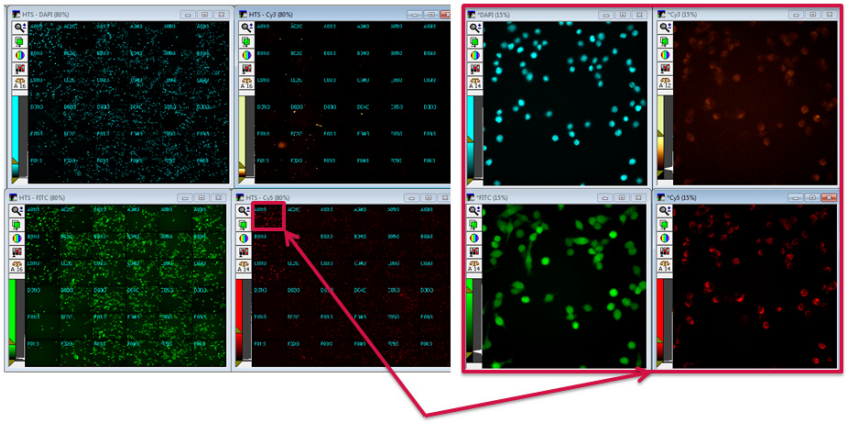
Note: You might need to use the scroll bars in the Sites graphic to scroll to a site to select it.

The well ID/time point for each selected well is displayed in the upper left corner of each thumbnail image in the Montage window; for example, A01t3, A01t2, and so on.



To view high-resolution images from selected wells or time points

Click any low-resolution thumbnail image in an existing montage to open the corresponding high-resolution image set.

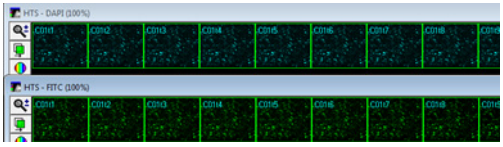


To view a stack (.stk) file of a series of time points from one site/well

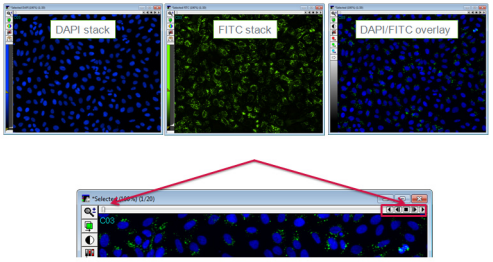
1. In the **Data view** field, select **Time Point vs Well** to change the layout of the Plate map. (The time points are displayed in the "X" direction and the well IDs are displayed in the "Y" direction.)
2. Do one of the following:
   - If only a single site per well was acquired, then right-click a single well of interest (for example, C18) in either the Plate map or a thumbnail in the Montage window.
   - If multiple sites per well were acquired, de-select **All Sites**, select the site of interest, and then right-click a single well of interest (for example, C18) in either the Plate map or a thumbnail in the Montage window.
3. Click **Load Selected Images**.

   The well ID/time point for each selected well is displayed in the upper left corner of each thumbnail image in the Montage window; for example, A01t1, A01t2, and so on.

Note: If needed, to clear all selected wells, click **Clear Selection**.



4. After you have created a stack file of a series of time points from one site or well, you can use the image slider or the playback controls at the top of a stack window to scroll through the images in the stack.



Viewing Z series images

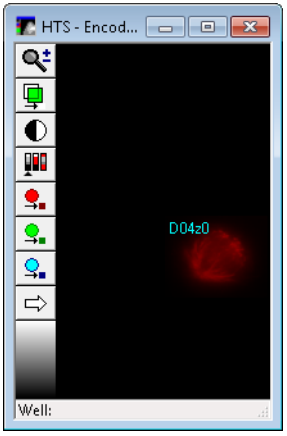
If you have acquired Z series images for experiments, then multiple options are available for viewing this data. You can:

- View a 2D projection image for the Z series. See To view a 2D projection image for the Z series.
- View a thumbnail montage of multiple wells at a single Z step. See To view a thumbnail montage of multiple wells at a single Z step on page 29.
- View multiple Z steps from a single well or multiple wells. See To view multiple Z steps from a single well or multiple wells on page 30.
- View a series of Z steps for a single site/well as a stack. See To view a stack (.stk) file of a series of Z steps from one site or well on page 31.
- View high resolution, single Z step images. See To view high resolution, single Z step images on page 32.

To view a 2D projection image for the Z series

1. In the **Data view** field, select **Well arrangement**.
2. Select **2D projection**.
3. Select the wells that are to be displayed in the Montage window. See Selecting wells for the Montage window and viewing well images in a Montage window on page 18.

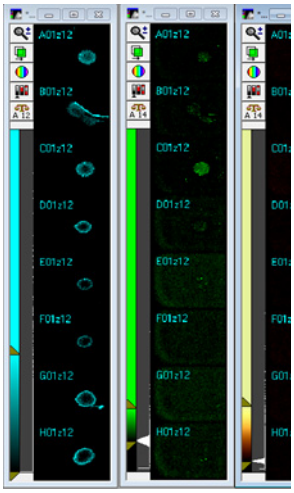
   The well ID/2D projection for each selected well—always labeled as well ID/z0—is displayed in the upper left corner of each thumbnail image in the Montage window, for example, D04z0.



To view a thumbnail montage of multiple wells at a single Z step

1. In the **Data view** field, select **Well arrangement**.
2. In the **Z steps** field, select the appropriate Z step, for example, 12 of 16.
3. Select the wells that are to be displayed in the Montage window. See Selecting wells for the Montage window and viewing well images in a Montage window on page 18.

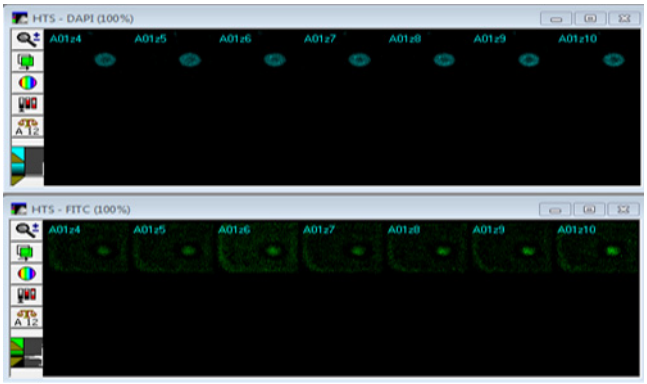
   The well ID/Z step for each selected well is displayed in the upper left corner of each thumbnail image in the Montage window, for example, A01z12, B01z12, and so on.



To view multiple Z steps from a single well or multiple wells

1. In the **Data view** field, select **Z Step vs Well** to change the layout of the Plate map. (The Z steps are displayed in the “X” direction and the well IDs are displayed in the “Y” direction.)
2. Do one of the following:
   - To select a single well, click and drag your cursor across the Z Steps Plate map for the well. You can select a subset of Z steps, or the full Z step course.
   - To select multiple adjacent wells, click and drag your cursor over the appropriate range of wells.

   As shown in the following figure, images at each Z step are tiled in a filmstrip-like format across the montage. The well ID/Z step for each selected well is displayed in the upper left corner of each thumbnail image in the Montage window, for example, A01z1, A01z2, and so on.



To view a stack (.stk) file of a series of Z steps from one site or well

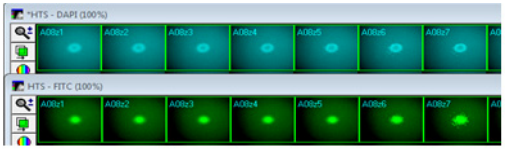
1. In the **Data view** field, select **Z Step vs Well** to change the layout of the Plate map.

   The Z steps are displayed in the “X” direction and the well IDs are displayed in the “Y” direction.

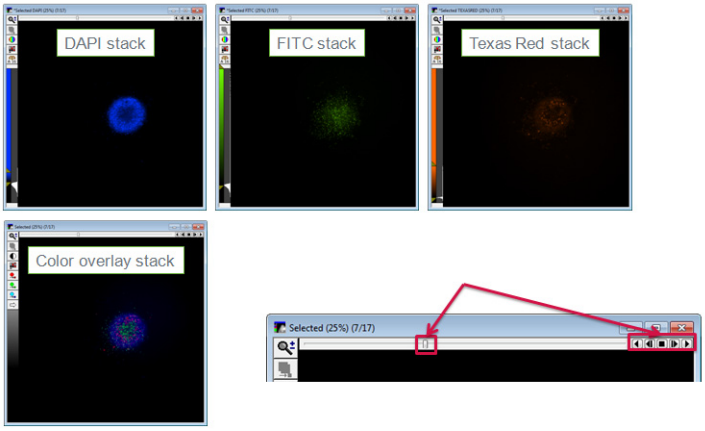
2. Do one of the following:
   - If only a single site per well was acquired, then right-click a single well of interest (for example, C18) in either the Plate map or a thumbnail in the Montage window.
   - If multiple sites per well were acquired, de-select **All Sites**, select the site of interest, and then right-click a single well of interest (for example, C18) in either the Plate map or a thumbnail in the Montage window.
3. Click **Load Selected Images**.

   The well ID/Z step for each selected well is displayed in the upper left corner of each thumbnail image in the Montage window, for example, A01z3, B01z3, and so on.

Note: If needed, to clear all selected wells, click **Clear Selection**.

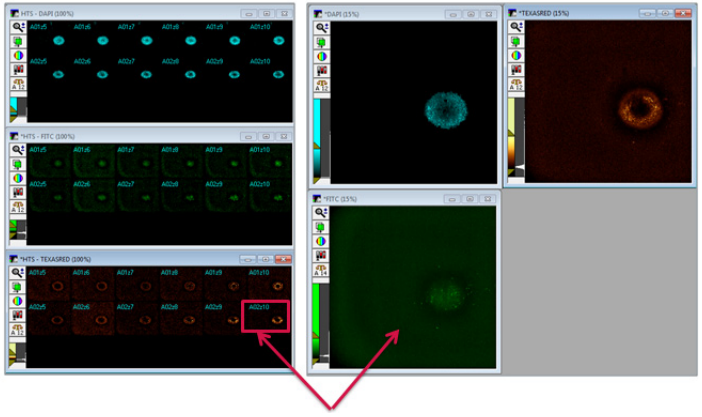


4. After you have created a stack file of a series of Z steps from one site/well, you can use the image slider or the playback controls at the top of a stack window to scroll through the images in the stack.



To view high resolution, single Z step images

Click any low-resolution thumbnail image in an existing montage to display the corresponding high-resolution image set.



Collating the images of multiple wells

To collate the images of multiple wells into one window that you can scroll through and save as a stack (.stk) or as a multi-plane TIF (.tif) file, do the following:

1. In the Plate map, select the appropriate wells.
   - To select a single well, right-click the well in the Plate map. Repeat this step to de-select the well.
   - To select multiple wells, click and drag your cursor over the appropriate wells in the Plate map. Repeat this step to de-select the wells.
   - To select all the wells in a plate in a single step, click in the upper left corner of the Plate map (the blank cell between “A” and “01”). To de-select all wells at once, repeat this step.
2. Click **Load Selected Images**.

   A stack is created for each selected wavelength and displayed in an individual **Selected** (stack) window or, if **Color Composite** is selected, a single stack is created and displayed in a single **Selected** (stack) window. If **All Sites** is selected, then the stack contains the images for all sites in all selected wells.
3. Do any of the following as needed:
   - To navigate through the images in the stack, drag the slider at the top of the **Selected** (stack) window.
   - To clear all selected wells, click **Clear Selection**.

Selecting Wells for Analysis

Based on all the information that you collect from arranging and viewing the images, you can then select the appropriate wells for analysis. Wells that are selected for analysis are highlighted in a bright green. To select wells for analysis, do the following:

- To select a single well for analysis, right-click the well in the Plate map. Repeat this step to de-select the well for analysis.
- To select multiple wells for analysis, press and hold the Control key or the Shift key, click and hold the left mouse button, and then drag the cursor over the appropriate wells in the Plate map. Repeat this step to de-select the wells for analysis.
- To select all the wells in a plate for analysis in a single step, press and hold the Control key or the Shift key, and then click in the upper left corner of the Plate map (the blank cell between “A” and “01”). To de-select all wells for analysis, repeat this step.

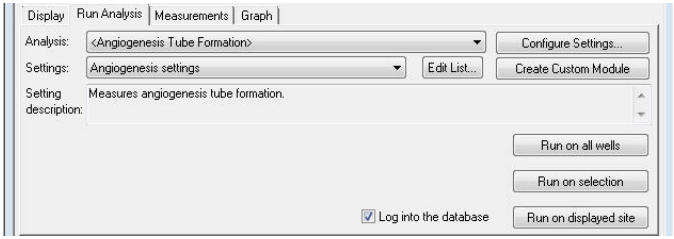
The following functions on the **Display** tab are applicable for these wells: **Load Selected Images** and **Navigate Selections**. The **Run on selection** function on the **Run Analysis** tab is also applicable for these wells.

Running an Analysis

After you select the appropriate wells in the plate, you analyze the selected wells. In most circumstances, despite the automated capabilities, manually run preliminary tests on one or more wells, such as both positive and negative controls, to ensure valid results.

1. On the **Analysis** drop-down list on the **Run Analysis** tab of the **Review Plate Data** dialog, select an application module, a custom module, or a journal.

Note: If the appropriate application module is not available, then you can create a custom module, or you can import a custom module. For information about creating a custom module, see Chapter 6: Custom Modules on page 93. For information about importing a custom module, see Importing a Custom Module on page 38. For assistance in developing or importing a journal, contact technical support. If the appropriate analysis journal is not available, then you can add it to the database. See Chapter 7: Journals on page 97.



2. Do one of the following:
   - To use an existing Settings file, on the **Settings** drop-down list, select the file.

Note: You must select a file that has the correct settings based on the wavelengths that were used to acquire the plate.

   - To create an entirely new Settings file for an application module, see Configuring the Settings file on page 72 in Chapter 5: Application Modules on page 67.
   - To modify an existing Settings file for an application module before using it, or import a needed Settings file for an application module, then click **Edit List** to open the **Edit List of Settings** dialog, and then continue to Working with a Settings file on page 40.
3. Optionally, to log the measurement data from your analysis into the database, select **Log into the database**.

4. If you are running a custom journal, or if the plate contains multiple time points, or Z step data, then do any of the following as applicable. Otherwise, continue to Step 5.
   - If you are running a custom analysis that is derived from a journal and you have created a setup journal for it, then click **Run Setup for Analysis** to run the setup journal.

Note: The setup journal must be in the same folder as the main analysis and must be named in the format: EXAMPLEJOURNAL_SETUP.JNL. See Adding a Setup Journal to the MetaXpress Software Database on page 104.

- If the plate contains multiple time points, then select from the **Time points** options.
  - **All time points**: Analyzes all the acquisition time points.
  - **Time point range**: Analyzes only those acquisition time points that fall within in the defined range. To use a single time point, type the same value in both fields.
  - **Selected time point**: Analyzes the currently selected time point.

Note: **Well arrangement** must be selected for **Data view** to show the currently selected time point in the **Time points** field.

  - **Stack of all time points**: Available when you select a time lapse journal that analyzes planes in a stack as separate time points.

- If the plate contains Z data, then select from the **Z steps** options.
  - **All Z points**: Analyzes all the acquisition planes.
  - **Z step range**: Analyzes only those planes that fall within the defined range. To use a single Z step, type the same value in both fields.
  - **Selected Z step**: Analyzes the currently selected Z step.

Note: Well arrangement must be selected for **Data view** to show the currently selected Z step in the **Z steps** field.

  - **Stack of all Z steps**: Available only if the selected analysis is a journal.
  - **2D projection**: Analyzes only the two-dimensional projection image.

5. To analyze the data, select one of the following options:
   - Click **Run on all wells** to run the assay for all sites in all wells on the plate.

Note: You can select this option with or without all wells in the Plate map selected for analysis.

   - Click **Run on selection** to run the assay on all sites in all selected wells.

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

   - Click **Run on displayed site** to run the assay for the currently selected site.

Note: This option requires an opened high-resolution single image file for each wavelength that is analyzed.

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 4: Viewing Analysis Results on page 49 for more information.

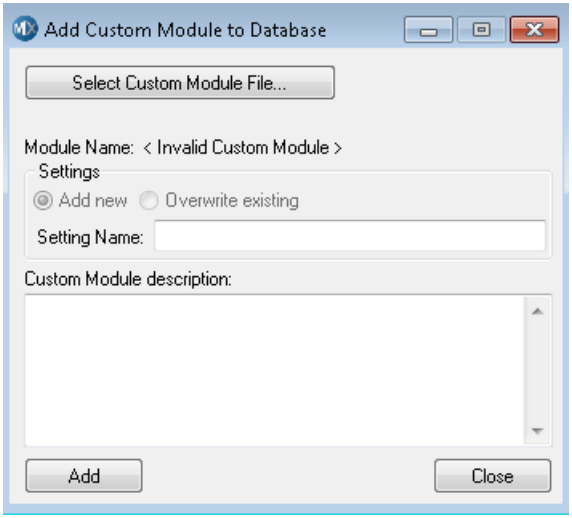
Importing a Custom Module

Any custom module that was created in the current MetaXpress Software database, even if from a different computer, is always available on the **Analysis** drop-down list on the **Run Analysis** tab on the **Review Plate Data** dialog. You can use the Import Custom Module and Export Custom Module options to move a custom module between databases.

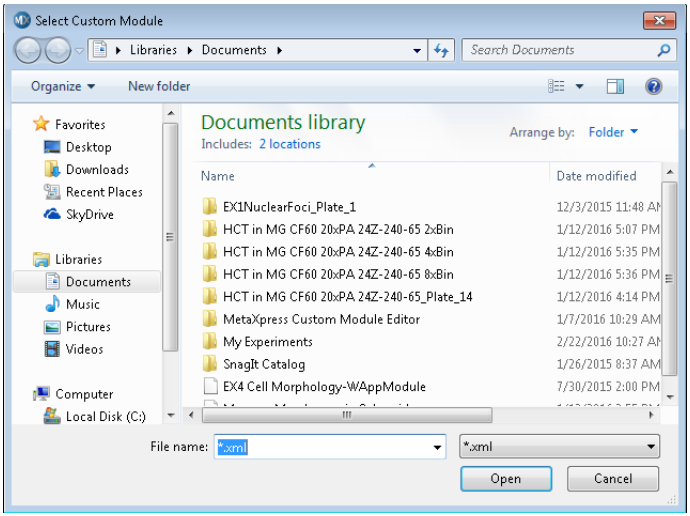
Note: For information about using the Custom Module Editor to create a custom module, and export a custom module, see Chapter 6: Custom Modules on page 93 or the help for the Custom Module Editor.

Note: To delete a custom module, you must delete all Settings files for the module. After you delete a custom module, it is no longer displayed on the **Analysis** drop-down list on the **Run Analysis** tab of the **Review Plate Data** dialog. See Deleting a Settings File on page 45.

1. On the **Screening** menu, select **Add Custom Module to Database**.



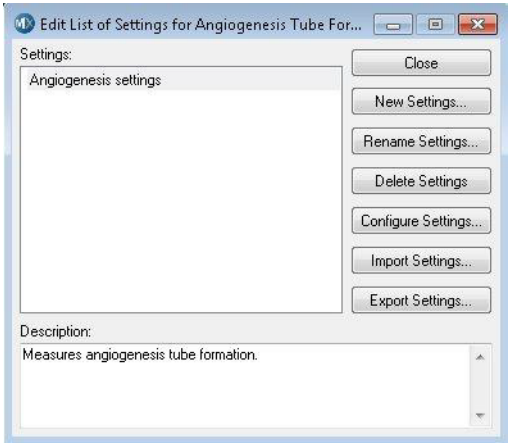
2. In the **Add Custom Module to Database** dialog, click **Select Custom Module File**.



3. In the **Select Custom Module** dialog, browse to and select the custom module file (the .xml file) that is to be imported, and then click **Open**.
4. In the **Custom Module Editor** dialog, under **Settings**, do one of the following:
   - To add the custom module file as a new file, leave **Add New** (the default value) selected, and then in the **Setting Name** field, type the name of the new custom module file.
   - To overwrite the existing custom module file in the database, select **Overwrite existing**. The Setting Name field is automatically populated with the name for the selected custom module file.
5. Optionally, in the **Custom Module description** field, type a description for the custom module file.
6. Do one of the following:
   - If you are adding the custom module as a new file, then click **Add**.
   - If you are overwriting an existing custom module, then click **Overwrite**.
7. At the prompt that indicates that the module was successfully added or overwritten, click **OK** to close the prompt and the **Add Custom Module to Database** dialog.

Working with a Settings file

When running an analysis, you can create a new Settings file for an analysis, or you can select an existing Settings file. Options are also available for creating a new Settings file for analysis by modifying an existing file, modifying an existing Settings file for an analysis, or importing an existing Settings file for an analysis. You can also rename a Settings file, delete a Settings file, and export a Settings file.



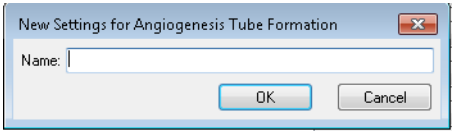
See:

- Creating a Standard Application Module Settings File by Modifying an Existing Settings File.
- Modify an Existing Standard Application Module Settings File on page 42.
- Importing an Existing Standard Application Module Settings File on page 43.
- Renaming a Settings File on page 44.
- Deleting a Settings File on page 45.
- Exporting a Settings File in its Current State on page 46.

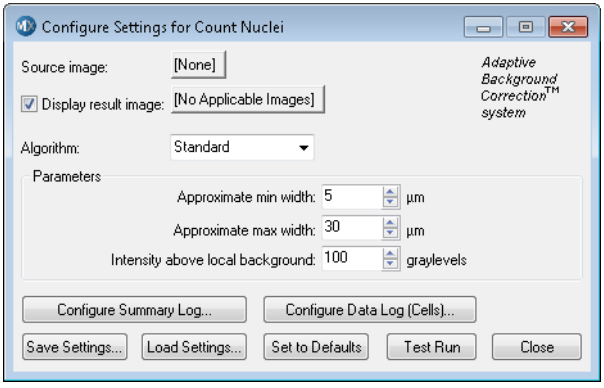
Creating a Standard Application Module Settings File by Modifying an Existing Settings File

When you create a new Settings file for a standard application module by modifying an existing file, the settings for the original Settings file remain untouched. Both the original Settings file and the newly created Settings file are displayed in the **Settings** pane on the **Edit List of Settings** dialog.

1. Make sure that the appropriate application module is selected on the **Analysis** drop-down list.
2. Click **Edit List**.
3. In the **Edit List of Settings** dialog, in the **Settings** pane, select the appropriate Settings file.
4. Click **New Settings**.



5. In the **New Settings** dialog, type the name for the new Settings file, and then click **OK**.



6. In the **Configure Settings** dialog, modify the analysis settings as required, and then click **Close**. See Configuring the Settings file on page 72.

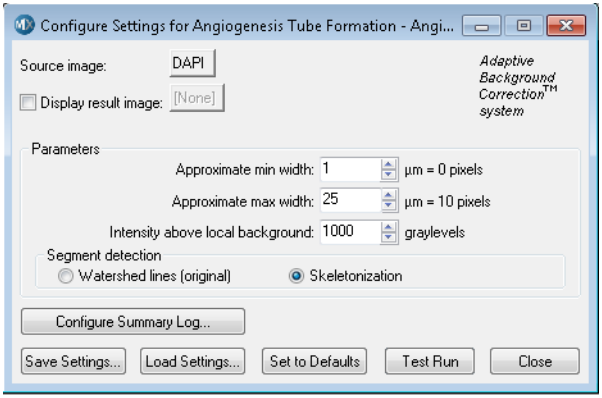
Modify an Existing Standard Application Module Settings File

When you modify the settings for an existing Settings file, any analyses that were done with the original settings are unaffected. Going forward, the settings are applicable only for new analyses. If required, you can also just modify the description for an existing Settings file.

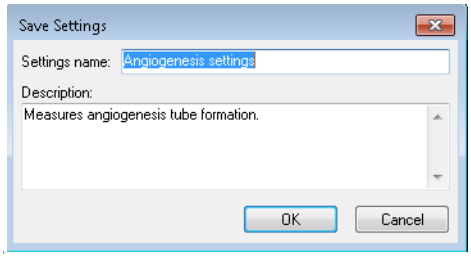
1. Make sure that the appropriate application module is selected on the **Analysis** drop-down list.
2. Click **Edit List**.
3. In the **Edit List of Settings** dialog, in the **Settings** pane, select the appropriate Settings file.
4. Optionally, in the **Edit List of Settings** dialog, edit the description for the Settings file.

Tip: To modify only the description for the Settings file, and no other values, then click **Close** on the **Edit List of Settings** dialog and return to the **Review Plate Data** dialog.

5. Click **Configure Settings**.



6. In the **Configure Settings** dialog, modify the analysis settings as needed. See Configuring the Settings file on page 72.
7. Click **Save Settings**.



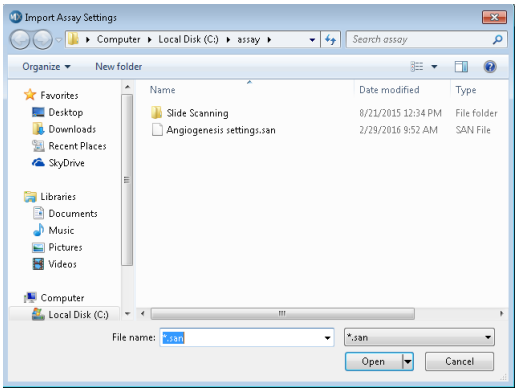
8. Click **OK** to close the **Save Settings** dialog, and save the Settings file with the modified settings.

Importing an Existing Standard Application Module Settings File

You can import an existing Settings file only for a standard application module. The Settings files that are available for importing depend on the selected application module. When you import a Settings file for a standard application module, it is imported into the database for the MetaXpress instance. After you import a Settings file, it is available for selection on the **Analysis** drop-down list on the **Run Analysis** tab of the **Review Plate Data** dialog.

Note: To import a custom module, see Importing a Custom Module on page 38. To add a journal, see Chapter 7: Journals on page 97.

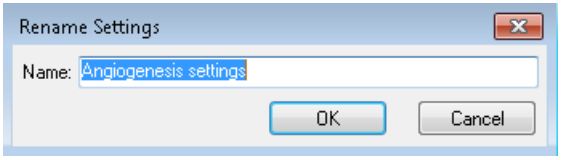
1. Make sure that the analysis for which you are importing the settings is selected on the **Analysis** drop-down list.
2. Click **Edit List**.
3. In the **Edit List of Settings** dialog, click **Import Settings**.



4. In the **Import Assay Settings** dialog, browse to and select the appropriate Settings file, and then click **Open**.

Renaming a Settings File

1. Make sure that the analysis for which you are renaming the Settings file is selected on the **Analysis** drop-down list.
2. Click **Edit List**.
3. In the **Edit List of Settings** dialog, in the **Settings** pane, select the appropriate Settings file.
4. Click **Rename Settings**.



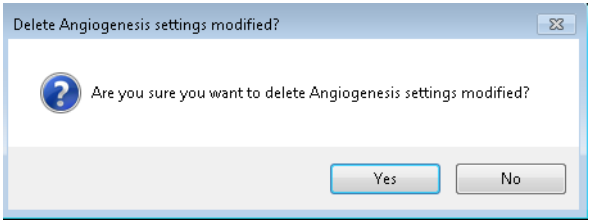
5. In the **Rename Settings** dialog, type the modified name for the Settings file, and then click **OK**.

Deleting a Settings File

When you delete a Settings file, any analyses that were done with the original settings are unaffected. Going forward, the Settings file is not available for selection for new analyses.

CAUTION! If you delete all Settings files for a custom module or a journal, then the module or the journal is deleted from the **Analysis** drop-down list.

1. Click **Edit List**.
2. In the **Edit List of Settings** dialog, in the **Settings** pane, select the appropriate Settings file.
3. Click **Delete Settings**.



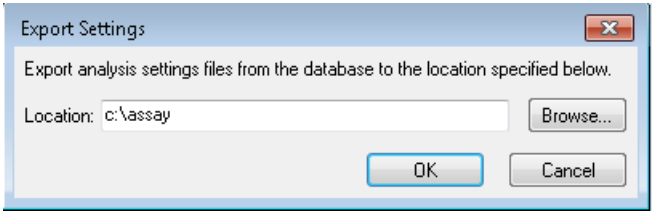
4. In the **Delete Settings** dialog, click **Yes** to delete the selected Settings file.

Exporting a Settings File in its Current State

The **Export Settings** option on the **Edit Settings List** dialog exports the Settings file in its current state - that is, the settings that are currently contained in the file. When you export a Settings file, the files are exported from the database to a default location of **c:\assay**, or you can select a different location. The Settings file is exported as named and with a set extension based on the module or journal that is associated with the file, and you cannot change either of these values.

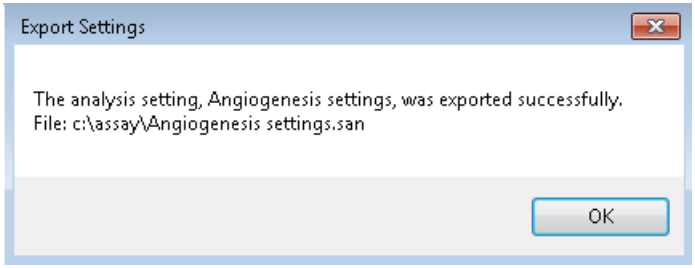
Note: To export the Settings file with the settings that were actually used for the measurement of a plate, see Exporting the Settings File Used to Measure the Plate on page 60.

1. Make sure that the analysis for which you are exporting the settings is selected on the **Analysis** drop-down list.
2. Click **Edit List**.
3. In the **Edit List of Settings** dialog, in the **Settings** pane, select the appropriate Settings file.
4. Click **Export Settings**.

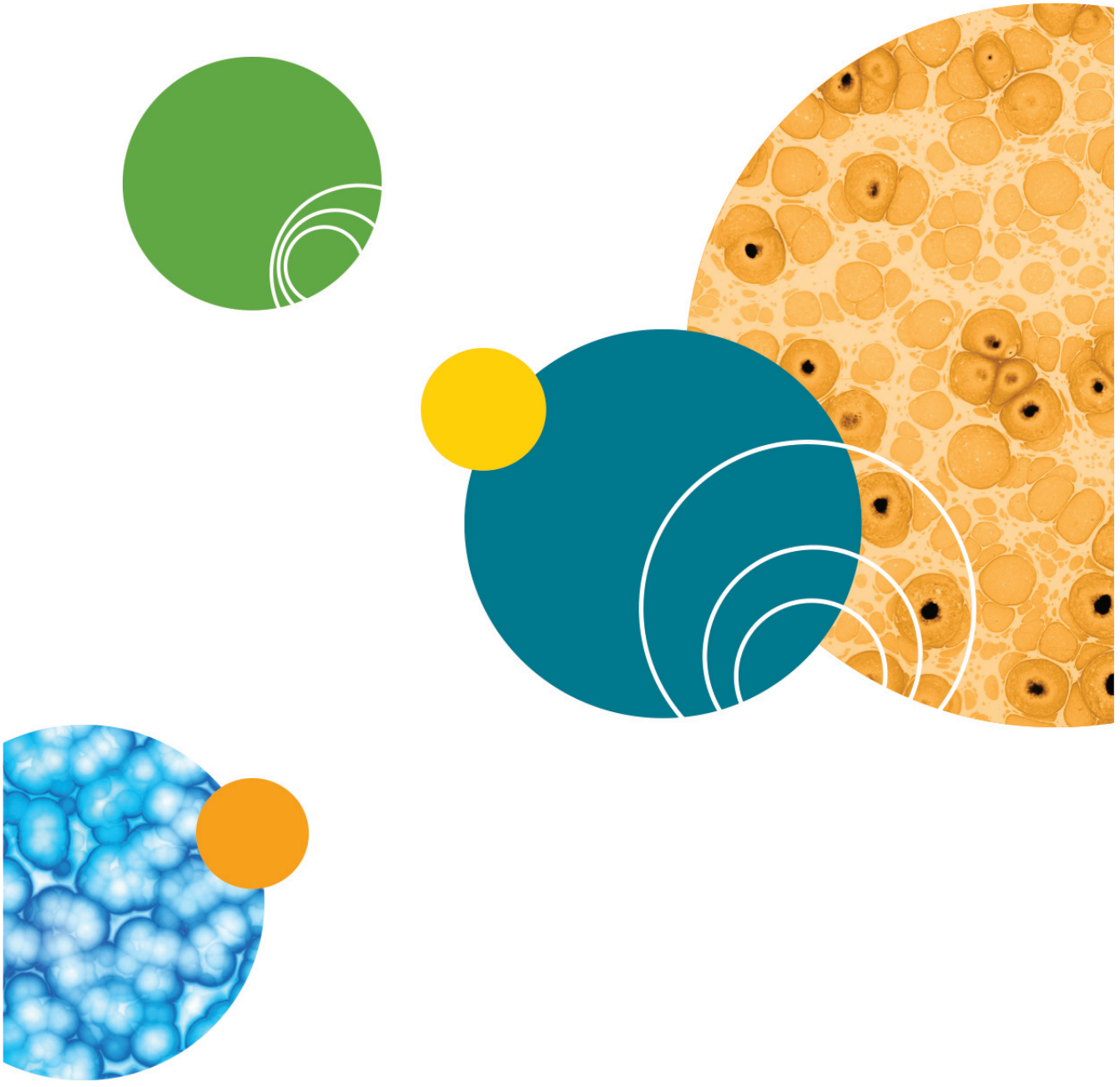


5. In the **Export Settings** dialog, do one of the following:
   - Leave the default location set to **c:\assay**.
   - Click **Browse** and in the **Browse for Folder** dialog, browse to and select a different location to which to export the file, and then click **OK**.

6. Click **OK**.



7. In the **Export Settings** message dialog, click **OK** to close the message indicating that the file was successfully exported and return to the **Edit List of Settings** dialog.



Viewing Analysis Results

4

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

This chapter includes the following topics:

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

Viewing Analysis Results

After running an analysis, you can click **Show Values** on the **Display** tab to display the analysis results for each well in the Montage window and on the Plate map. Values in the Montage window and the Plate map are automatically updated based on a selected measurement.

- If a single site is selected for display, then the value that is displayed for a well in the Montage window and the value that is displayed for the corresponding well in the Plate map are identical.
- If all sites are selected for display, then the individual values are displayed for the sites in each well in the Montage window and the average of all the site values is displayed for the corresponding well in the Plate map.
- If the selected measurement is a “Cell” measurement, then the values that are displayed in the Plate map are the average of all cells that were found in the well.

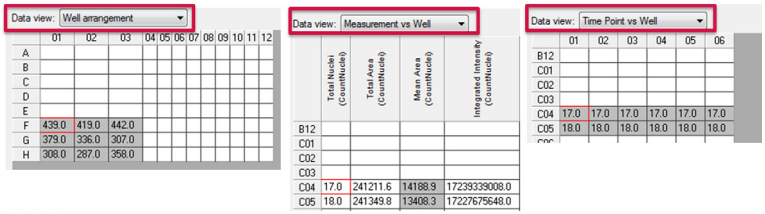
1. On the Display tab of the Review Plate Data dialog, click **Show Values**.
2. Open the **Measurements** tab, and then do the following:
   - On the **Analysis** drop-down list, select the appropriate analysis.
   - On the **Measurement** drop-down list, select the appropriate measurement.

Note: Measurements that are preceded by “Cell:” are cell-by-cell data. To view cell-by-cell data for the selected site or well, use the **Cellular Results** table. See Using the Cellular Results Table.

3. By default, analysis data is displayed in **Well Arrangement** (the plate format). Optionally, to change the **Data view**, select one of the following:

Table 4-1: Data view options for analysis results

Option	Description
Time point vs well	A matrix view of select measurements across all time points. Columns are time points and rows are well ID.
Z Step vs well	A matrix view of select measurements across all Z steps. Columns are Z steps and rows are well ID.
Measurement vs well	A matrix view of all measurements for a single plane. Columns are measurements and rows are well ID.



Note: If you are viewing time lapse data or Z series data, then there are special considerations. See Viewing analysis results for time lapse experiments or Z step experiments on page 52.

4. Continue to any of the following as needed for viewing and evaluating your data:
   - Using the Cellular Results Table on page 53.
   - Filtering Data Analysis Values on page 55.
   - Generating a Heat Map for Analysis Results on page 56.
   - Viewing Image Overlay Information on page 59.
   - Exporting Selected Data on page 61.
   - Graphing Analysis Results on page 65.

Viewing analysis results for time lapse experiments or Z step experiments

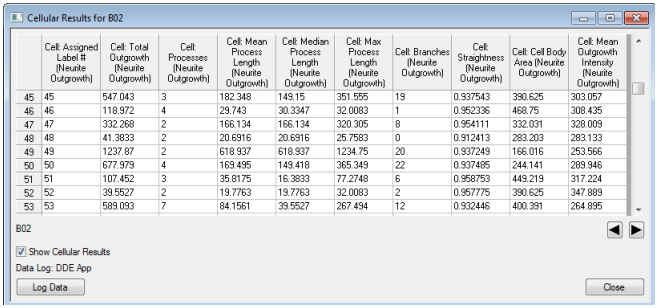
If you are viewing analysis results for time lapse experiments, or Z step experiments, then make note of the following:

- For time lapse experiments:
  - If **Well arrangement** or **Measurement vs well** is selected for the **Data view**, then the data that is displayed is for the time point that is selected in the **Time point** fields.
  - If a particular time point was not analyzed for the current measurement set, then no data is displayed when this time point is selected.
  - To view the data from all time points (for example, to identify trends over time), select **Time point vs well** for the **Data view**.
- For Z step experiments:
  - If **Well arrangement** or **Measurement vs well** is selected for the **Data view**, then the data that is displayed is for the Z step that is selected in the **Z step** fields.
  - If a particular Z step was not analyzed for the current measurement set, then no data is displayed when this Z step is selected.
  - If the 2D projection was selected for analysis, then select **2D projection** to view the resulting data. If the analysis was done on the 2D projection, then each of the individual Z planes displays the results of the analysis on the projection. If the 2D projection was not analyzed, then no data is displayed when **2D projection** is selected.
  - To view the data from all Z steps (for example, to identify trends through the Z-stack), select **Z Step vs well** for the **Data view**. Data from the 2D projection is not available from this view.

Using the Cellular Results Table

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

1. In the Plate map, make sure that you have a well selected for display in its own individual image window.
2. If the **Cellular Results** table has not opened automatically after running an application module analysis, then in the **Review Plate Data** dialog on the **Display** tab, click **Cellular Results**.



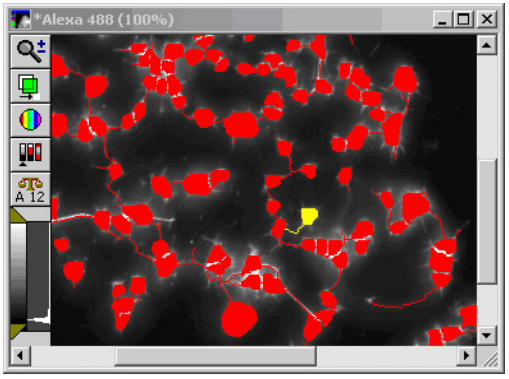
3. Do one or both of the following as required to review the cellular data:
   - Select one of the segmented cells in the image to highlight the corresponding data for the cell in the **Cellular Results** table.

Note: Use Ctrl+Click to select multiple cells from the image.

   - Select a row in the **Cellular Results** table to highlight the corresponding cell in the segmented image.

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

Note: This segmentation is absent if **Save segmentation overlay to database** was not selected. See Viewing Image Overlay Information on page 59.



4. Optionally, to prevent the **Cellular Results** table from opening each time an application module runs, clear **Show Cellular Results**.

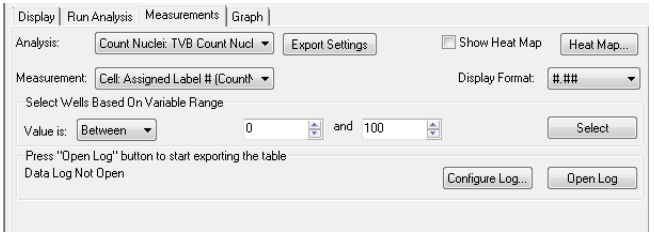
Note: If you clear **Show Cellular Results**, but later want to view the **Cellular Results** table when running an application module, click **Window > Show Cellular Results**. In the simplified menu, click **Measure > Show Cellular Results**.

Filtering Data Analysis Values

Use the options in the **Select Wells Based on Variable Range** section to highlight wells that fit criteria that you specify for a selected measurement.

Tip: The **Select Wells Based on Variable Range** provides a means of carrying out a subpopulation analysis by automatically selecting wells of interest which you can then use with the **Run Analysis for Selections** option on the **Run Analysis** tab.

1. In the **Review Plate Data** dialog, open the **Measurements** tab.



2. On the **Analysis** drop-down list, select the analysis that contains the measurements that are to be filtered.
3. On the **Measurement** drop-down list, select the measurement for which values are to be filtered.
4. In the **Display Format** field, select the number of decimal places that are to be displayed for your data.
5. Make sure that **Show Heat Map** is not selected.

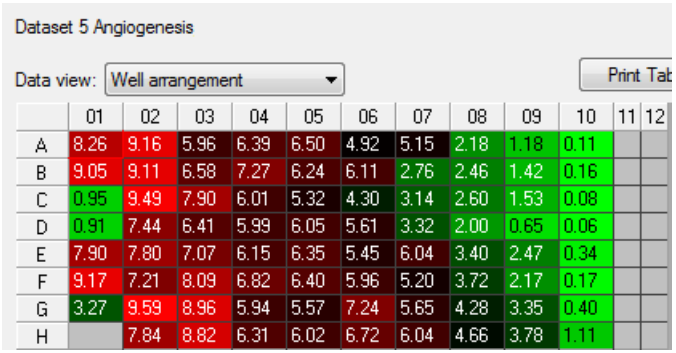
Note: If a heat map is enabled, selected wells on the grid cannot be distinguished; however, the selected wells are outlined in green on the thumbnail montage.

6. In the **Select Wells Based on Variable Range** section, specify the criteria for automatically selecting wells.
7. Click **Select** to highlight the wells that meet the criteria in green in the Plate map.

8. Do either one or both of the following as needed:
   - To clear the selection, click **Clear Selection**.
   - To log the data that is displayed in the Plate map, and if applicable, the **Cellular Results** table, see Exporting Selected Data on page 61.

Generating a Heat Map for Analysis Results

Visual representation of a data in a heat map provides a simple way to quickly identify general patterns; for example, clusters of similar values. By default, the heat map uses a green-red color scheme and distributes all 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. You can distribute the data on a linear or logarithmic scale, set low and high thresholds, and change the color scheme.



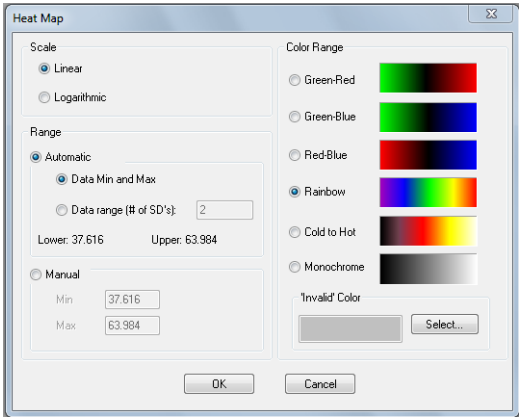
	01	02	03	04	05	06	07	08	09	10	11	12
A	8.26	9.16	5.96	6.39	6.50	4.92	5.15	2.18	1.18	0.11		
B	9.05	9.11	6.58	7.27	6.24	6.11	2.76	2.46	1.42	0.16		
C	0.95	9.49	7.90	6.01	5.32	4.30	3.14	2.60	1.53	0.08		
D	0.91	7.44	6.41	5.99	6.05	5.61	3.32	2.00	0.65	0.06		
E	7.90	7.80	7.07	6.15	6.35	5.45	6.04	3.40	2.47	0.34		
F	9.17	7.21	8.09	6.82	6.40	5.96	5.20	3.72	2.17	0.17		
G	3.27	9.59	8.96	5.94	5.57	7.24	5.65	4.28	3.35	0.40		
H		7.84	8.82	6.31	6.02	6.72	6.04	4.66	3.78	1.11		

1. In the **Data View** field, select **Well arrangement**, and then on the **Measurements** tab, select **Show Heat Map**.

Note: If a heat map is enabled, selected wells on the grid cannot be distinguished; however, the selected wells are outlined in green on the thumbnail montage.

2. Click **Heat Map**.

   The **Heat Map** dialog opens.



3. Optionally, do one or more of the following:

   - Select the type of scale that the heat map uses to distribute the data.

   Table 4-2: Heat Map scale options

Option	Description
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	**D**istributes 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). **Note:** The logarithmic scale presents a wide range of data on a more manageable scale. The scale is useful when you want to analyze values that are at the lower end of a wide range of data.

- Select the method that the heat map uses to calculate the range of data, and then define the range.

Table 4-3: Heat Map data calculation options

Option	Description
Manual	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) are displayed with the "lowest" color and values at or above the maximum (with rounding) are displayed with the "highest" color. Values between the minimum and maximum are distributed along the scale and are displayed with corresponding colors.
Automatic	If you select this option, then do one of the following for defining the range of data: • To use the full range of data, select **Data Min and Max**. • 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 SDs),** and then type the 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) are displayed with the "lowest" color and values at or above the maximum (with rounding) are displayed with the "highest" color. Values between the minimum and maximum are distributed along the scale and are displayed with corresponding colors.

- Select a different color scheme for the heat map.
- 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 opens, click a color and then click **OK**.

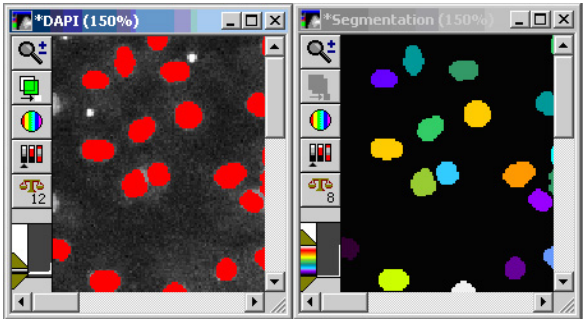
4. Click **Close** to close the **Heat Map** dialog and update the Heat Map display.

Viewing Image Overlay Information

Two options are available on the **Image Overlay** drop-down list on the **Display** tab on the **Review Plate Data** dialog for viewing image overlay information: **Show well information** and **Show cell segmentation**.

If **Show well information** is selected, then the well, site (if multiple site data), time point (if time lapse data), and Z plane (if Z series data) are displayed as a text overlay on the upper left corner of the image. If the plate has been analyzed, then the measurement value based on the current measurement set and selected measurement for the displayed site is displayed as a text overlay on the lower right corner of the image. You select the color of the overlay from the **Col** drop-down list on the **Display** tab on the **Review Plate Data** dialog. You can toggle the overlay on or off from the source image with the Show/Hide overlay button on the side of the image window. If you use **File > Save As** to save the source image, then the overlay is also saved.

If **Show cell segmentation** is selected when you are reviewing the data for a plate that has been analyzed and for which **Save segmentation overlay to database** was selected, then the overlay is displayed on top of the image.



Note: The overlay is saved to the database after automated analysis only if you select **Save segmentation overlay to database** when configuring the summary log for the application module, or **Create Object Overlay** when configuring the **Measure** tab for a custom module. The **Save segmentation overlay to database** option is not available for all application modules.

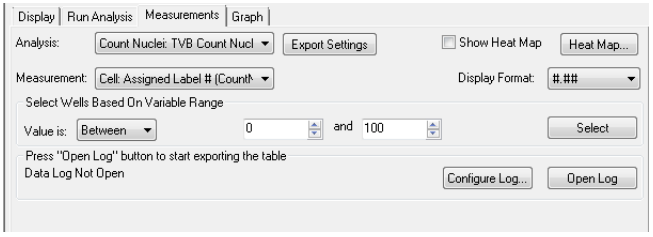
You can toggle the overlay on or off from the source image with the Show/Hide overlay button on the side of the image window. If you use **File > Save As** to save the source image, then the overlay is also saved.

Exporting the Settings File Used to Measure the Plate

After you load a Settings file for an analysis, you can modify the settings before you do the analysis. After you do the analysis, you have the option of exporting these settings. The values that are exported are the values that were used to measure the plate and acquire the plate data. When you export the Settings file, by default, the file name is used as-is and the file is exported to **c:\assay**, but you can change one or both of these values. If you export the settings for an application module, then the file extension depends upon the application module that was used to measure the plate. If you export the settings for a custom module, then the settings are exported to an .xml file. If you export the settings for a journal, then the settings are exported to a .zip file.

Note: To export a Settings file in its current state (the settings for the file at the time it was last saved), see Exporting a Settings File in its Current State on page 46.

1. In the **Review Plate Data** dialog, open the **Measurements** tab.



2. Next to the **Analysis** drop-down list, click **Export Settings**.
3. In the **Save Analysis Settings to file** dialog, leave the default values of file name and location as-is, or modify one or both values as needed.
4. Click **Save**.

Exporting Selected Data

After you display analysis results, you can use the **Log Data** option that is available on the **Measurement** tab to export the results to an Excel spreadsheet in the same format in which the data is displayed in the Plate map. You can also export the results to a text file. If the data are cell-by-cell results, then you export this data using the **Log Data** option that is available on the **Cellular Results** table.

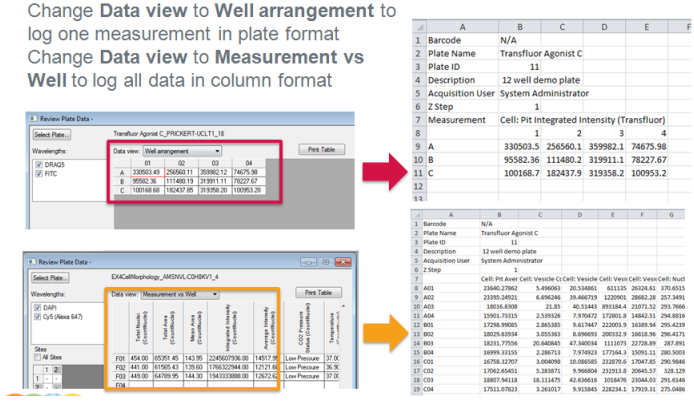
Note: Alternatively, you can use the **Export Measurements** option on the **Plate Data Utilities** dialog to export selected data of one or more measurement sets to a text file. For more information, see Exporting Measurements through a Query on page 123.

See:

- To export data to an Excel spreadsheet.
- To export data to a text (.log) file on page 63.
- To export cell-by-cell data to a text (.log) file on page 64.

To export data to an Excel spreadsheet

1. Do one of the following:
   - To log a single measurement in plate format, select **Well Arrangement** for **Data View**.
   - To log all data in column format, select **Measurement vs Well** for **Data View**.



2. Click **Configure Log**, and in the **Configure Log** dialog, configure header information (**Plate info**, **Column and row Labels**, or both).
3. Click **Open Log**, and then in the **Open Data Log** dialog, select **Microsoft Excel or other (DDE)**, and then click **OK**.
4. In the **Export Log Data** dialog, do the following:
   - On the **Application** drop-down list, select **Microsoft Excel**.
   - Type any of the following information: **Sheet name**, **Starting Row**, and **Starting Column**.
5. Click **OK** to close the **Export Log Data** dialog and link the measurement data to the Excel spreadsheet.
6. With the Excel spreadsheet open and connected to the MetaXpress Software, click **Log Data** to export the data that is currently displayed in the Plate map to the spreadsheet.
7. Optionally, if **Data View** is set to **Well Arrangement**, then to export another measurement set to the log file, select the appropriate measurement on the **Measurements** drop-down list, and then click **Log Data**.

Note: Repeat Step 7 as many times as required to export different measurement set data for the same analysis. Every time that you click the **Log Data** button, the newly exported data is displayed below the previously exported data. As long as the connection between the Excel spreadsheet and the MetaXpress Software is maintained, existing data is not overwritten.

8. Optionally, to stop exporting the data to the Excel spreadsheet at any time, click **Log > Close Data Log** (the default menu) or **Measure > Log > Close Data Log** (the simplified menu).

To export data to a text (.log) file

Because Excel has a row limit, export data to a text file if you are exporting many data points, or if you are exporting cell-by-cell data.

1. Click **Configure Log**, and in the **Configure Log** dialog, configure header information (**Plate info**, **Column and row Labels**, or both).
2. Click **Open Log**, and then in the **Open Data Log** dialog, select **A text file**.
3. In the **Open Data Log** file dialog, browse to the appropriate location for saving the log file, type a name for the log file, and then click **Save** to close the dialog and create and link the text file.

Note: The .log file must remain closed while logging data. If you open the file through Windows Explorer, your data is not logged.

4. Click **Log Data** to export the data that is currently displayed in the Plate map to the text file.
5. Optionally, if **Data View** is set to **Well Arrangement**, then to export another measurement set to the log file, select the appropriate measurement on the **Measurements** drop-down list, and then click **Log Data**.

Note: Repeat Step 5 as many times as required to export different measurement set data for the same analysis. Every time that you click the **Log Data** button, the newly exported data is displayed below the previously exported data. As long as the connection between the log file and the MetaXpress Software is maintained, existing data is not overwritten.

6. Optionally, to stop exporting the data to the text file, at any time, click **Log > Close Data Log** (the default menu) or **Measure > Log > Close Data Log** (the simplified menu).

To export cell-by-cell data to a text (.log) file

Cell-by-cell data is indicated by the word “Cell” preceding the measurement.

1. Click **Cellular Results** to open the **Cellular Results** table.
2. In the Montage window, click the appropriate well.

Note: Do not click in the Plate map as this changes the wells that are displayed in the Montage window.

3. Click **Open Log**.
4. Click **Open Log**, and then in the **Open Data Log** dialog, select **A text file**.
5. In the **Open Data Log** file dialog, browse to the appropriate location for saving the log file, type a name for the log file, and then click **Save** to close the dialog and create and link the text file.

Note: The .log file must remain closed while logging data. If you open the file through Windows Explorer, your data is not logged.

6. Click **Log Data** to export the data that is currently displayed in the Plate map to the text file.
7. Optionally, if **Data View** is set to **Well Arrangement**, then to export another measurement set to the log file, select the appropriate measurement on the **Measurements** drop-down list, and then click **Log Data**.

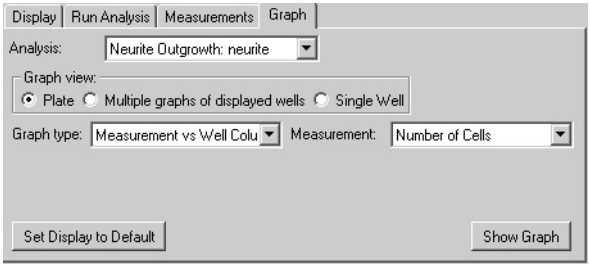
Note: You can repeat Step 5 as many times as required to export different measurement set data for the same analysis. Every time that you click the **Log Data** button, the newly exported data is displayed below the previously exported data. As long as the connection between the log file and the MetaXpress Software is maintained, any newly exported data does not overwrite any existing data.

8. Optionally, to stop exporting the data to the text file, at any time, click **Log > Close Data Log** (the default menu) or **Measure > Log > Close Data Log** (the simplified menu).

Graphing Analysis Results

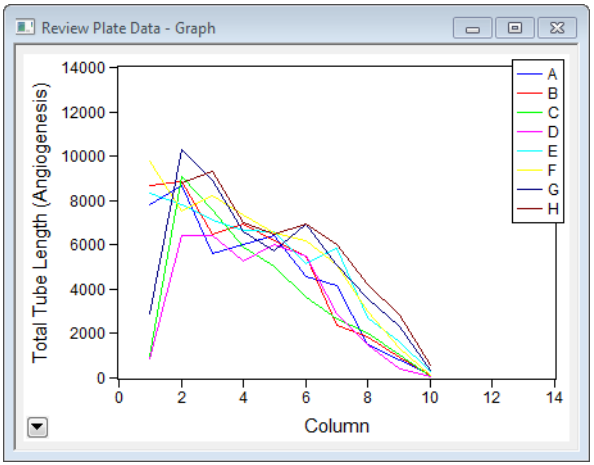
To configure a graph to display your data, use the following procedure:

1. In the **Review Plate Data** dialog, open the **Graph** tab.



2. On the **Analysis** drop-down list, select the analysis that contains the data that is to be graphed.
3. In the **Graph view** section, select the source location for the data.
4. On the **Graph type** drop-down list, select a graph type. The options available for each graph type vary depending on the selected **Graph view**.
5. On the **Measurement** drop-down list and, if applicable, the **Measurement2** drop-down list, select the measurements that are to be graphed.
6. If you selected **Histograms** for the **Graph type**, then do the following. Otherwise, go to the next step.
   - Select the number of bins to display in the resulting histogram in the **Number of Bins** field.
   - Select **Auto Scale** to automatically scale the bins based on the range of data from the selected measurement.

7. 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 resize it.

8. Optionally, do one or both of the following as needed:
   - 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**.
   - To reset the display parameters for the current graph to the default view, click **Set Display to Default**.

Note: Each combination of **Graph view** and **Graph type** has its own unique graph default.

Application Modules

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**.

This chapter includes the following topics:

About Application Modules

An application module can be run in various ways:

- Automatically with the plate acquisition process. See the MetaXpress Help.
- Within the **Review Plate Data** dialog. See Configuring an Application Module on page 71.
- As a stand-alone analysis using an option on the Screening menu See the MetaXpress Help.
- As part of a custom module. See Chapter 6: Custom Modules on page 93 for an overview and the Custom Module Editor Help for details.
- Within a custom journal. See the MetaXpress Help.
- In a queue of automated analyses. See Chapter 9: Batch Analysis on page 115.

All modules share common conventions and settings. After 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 sizes, shapes, and intensities of the objects that are to be included in your analysis. Application modules require grayscale 16-bit fluorescent images, with one image for each wavelength that is to be analyzed. Some modules 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.

When choosing an application module, consider what your assay must measure. Your assay might encompass activation of a receptor, apoptosis, proliferation, and so on, but to obtain meaningful numerical data from screening, you must 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 generally of the greatest interest. Of these, only certain sizes and shapes might be of interest, as the other shapes might be dead cells or debris in the culture. Use the guidelines provided in Table 5-1 on page 68 to determine which application module is best suited for your assay.

Table 5-1: Application Module Guidelines

Assay Type	Description	Recommended Application Modules
Protein Localization/ Translocation	Generally 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	Generally a count of the cells or nuclei in an image.	• Count Nuclei • Multi Wavelength Cell Scoring • Cell Proliferation HT
Cell Viability/Apoptosis	Generally 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)

Table 5-1: Application Module Guidelines (cont'd)

Assay Type	Description	Recommended Application Modules
Receptor Internalization and other Punctuate Staining	Generally 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	Generally 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)	Nearly always involves measurements of intensity. Generally 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. Normalize this value 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

Table 5-1: Application Module Guidelines (cont'd)

Assay Type	Description	Recommended Application Modules
Cell Cycle	Generally 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	Generally involves micronuclei, which are small nuclei produced during cell division by a lagging chromosome fragment or an entire chromosome. 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

Configuring an Application Module

Most application modules share similar configuration steps, such as selecting the source image to process, calibrating the source images, determining the width of an object, and providing the **Intensity Above Local Background** value. After you are familiar with these basic steps, you can use any of the various application modules. See:

- Calibrating the source images on page 71.
- Configuring the Settings file on page 72.
- Determining object widths on page 75.
- Measuring the area of an object on page 79.
- Calculating the intensity above background value on page 80.
- Testing and saving settings on page 81.

Note: You can create your own custom module by combining various image processing and segmentation steps in the **Custom Module Editor**. These steps can include the application modules described in this guide. For detailed information about creating custom modules, see Custom Modules on page 93 for an overview and the Custom Module Editor Help for details.

Calibrating the source images

For best results, calibrate the source images in all application modules in microns. Make sure that the X and Y calibration for an image match, and if more than one image is used as a source image, then the images must have identical distance calibrations. Images that were acquired using an ImageXpress® Micro System are calibrated in microns. Images that have been imported from another system or that have been processed might not be calibrated.

Configuring the Settings file

Every application module needs an associated Settings file that details the values that are to be used for the module-specific settings. You can select a pre-existing Settings file, or if the appropriate Settings file is not available, then either configure a Settings file for the module or import a Settings file.

Every application module also 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 images varies depending on the application module used. For more information about source images, view the application help for the module you are using. The following procedure uses the **Count Nuclei** application module as an example, with a new Settings file being created for the module.

Two options are available for viewing a result image in an application module:

- You can display a separate result image.
- You can display an image overlay.

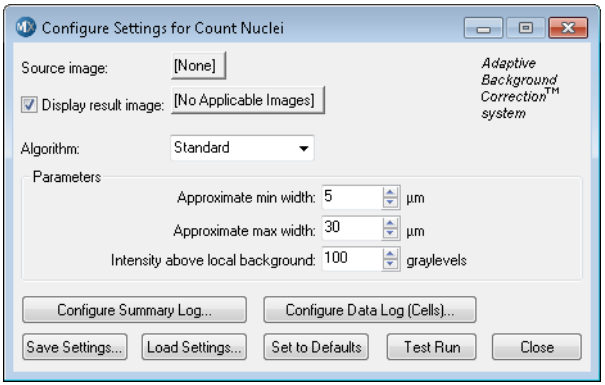
If you display a separate result image, the image is not saved, but separate result images are useful when creating journals.

Finally, an **Algorithm** option—**Standard** or **Fast**—is provided for some application modules. The **Algorithm** option determines how quickly the analysis is done. Both algorithms produce similar but not identical results.

To configure the Settings file

1. Click **Screening > Review Plate Data**.

   In the simplified menu structure, click **Screening > Review Plate**.
2. Open the plate and images that are to be analyzed as described in Chapter 3: Selecting and Analyzing Plates on page 13.
3. In the **Review Plate Data** dialog, click the **Run Analysis** tab.
4. From the **Analysis** drop-down list, select an application module.
5. Click **Configure Settings**.



Note: If the appropriate Settings file has previously been created, then you can click the **Settings** drop-down list and select it, and then continue as needed.

6. Click **Source image** and select one of the images that you opened in Step 2.

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

7. Do one of the following to view the results of the module:
   - To display a separate result image, continue to Step 8.
   - To display or create an image overlay, use the **Show/Hide Overlay** option on the side of the image window after the application module completes its analysis. See Viewing Image Overlay Information on page 59. Continue to Step 11.

8. Select **Display result image**.
9. Open the image selector for **Display result image**, and then select 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.

Note: If **Display result image** is selected, but an image is not selected (**None** is displayed), then you receive an error if you try to run the application module.

10. Click the result image name (the default value is the [Source] image name), and then do one of the following in the **Specify Image Name** dialog:
    - Select the appropriate image name from list of available images.

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

    - Select **Specified**, and then modify the image name as appropriate.

Note: If you are running the module within a journal, then Molecular Devices recommends that you select **Specified** and modify the image name accordingly.

11. If the application module provides an **Algorithm** option, select either **Standard** or **Fast**, and if you select **Fast**, then in the **Intensity above local background field**, type approximately half of the value that you calculate in Calculating the intensity above background value on page 80.

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

12. Continue as needed to specify the values that the application module requires, for example, determining object widths, before processing the images.

Determining object widths

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

- Region tools Line tool. See To measure the width of an object with the Single Line Regions tool on page 75.
- Caliper tool. See To measure the width of an object using the Calipers tool on page 77.

To measure the width of an object with the Single Line Regions tool

1. If the **Region** toolbar is not open, click **Regions > Region Tools**.

   In the simplified menu structure, click **Measure > Regions > Region Tools**.

2. Click the **Single Line** tool.

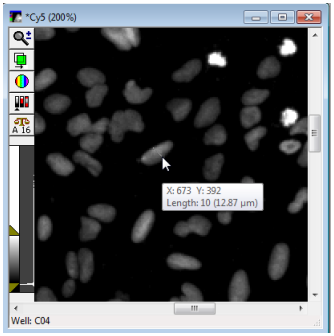
   

3. Click one of the edges of the smallest object that is to be included in your analysis.

   A tooltip opens that shows the current X and Y values of the pointer. A placeholder is displayed for the Length.

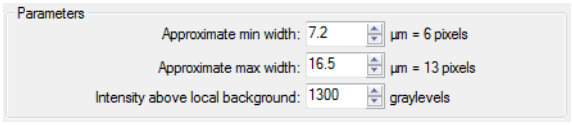
4. Move the cursor to the opposite edge of the object and note the Length value.

   In the following example, the value is 10 pixels (or 12.87 µm). This value represents the object width in pixels. If the image is calibrated, then the length is in pixels and calibrated units.



5. In the **Configure Settings** dialog, in the **Approximate min width** field, type or select the value in microns.
6. To remove the region lines, select **Clear Regions** on the **Regions** menu or right-click on the region and click **Delete Region**.
7. Click one of the edges of the largest object that is to be included in your analysis.

   A tooltip opens that shows the current X and Y values of the pointer. A placeholder is displayed for the Length.
8. Move the cursor to the opposite edge of the object and note the Length value.
9. In the **Configure Settings** dialog, in the **Approximate max width** field, type or select the value in microns.



10. To remove the region lines, select **Clear Regions** on the **Regions** menu or right-click on the region and click **Delete Region**.
11. Continue as needed to specify the values that the application module requires, for example, determining the Intensity above local background, or if you are done configuring the module, then continue to Testing and saving settings on page 81.

To measure the width of an object using the Calipers tool

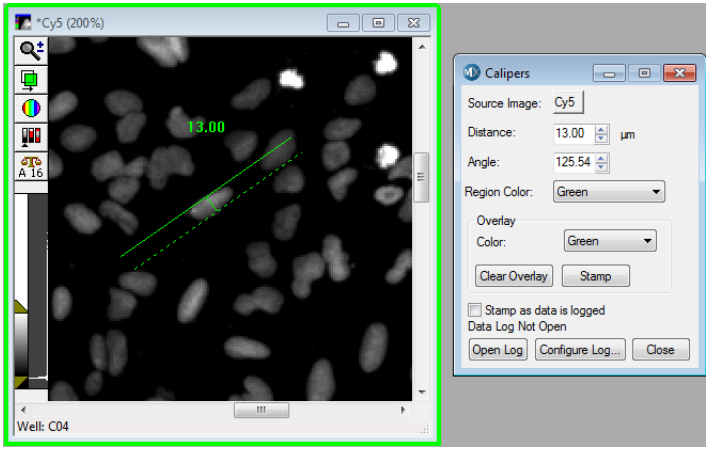
Note: The **Calipers** feature is a drop-in option and might need to be loaded into the MetaXpress Software. If the option is not present on the **Measure** menu (default menu), or on the **Measure > Distance** menu (simplified menu), contact your System Administrator or Technical Support to have this drop-in loaded.

1. Click **Measure > Calipers**.

   In the simplified menu structure, click **Measure > Distances > Calipers**.

2. In the **Calipers** dialog, from the Image selector, select the image to measure.

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



3. To move the calipers to the smallest object that is to be included in your analysis, click the cross-bar so that it appears as a blinking line, and then use the cross-bar to drag the calipers to the object.

4. To measure the width of the object, do the following:
   - Click one of the caliper edge lines so that it appears as a blinking line, and then drag the line to the edge of the object. The other caliper line remains anchored.
   - Click the other caliper edge lines so that it appears as a blinking line, and then drag the line to the opposite edge of the object.

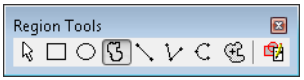
   The size of the line is displayed in the image and in the **Calipers** dialog.
5. In the **Configure Settings** dialog, in the **Approximate min width** field, type or select the value in microns.
6. Repeat Step 3 through Step 5 for the largest object that is to be included in your analysis and type or select its width in the **Approximate max width** field.
7. Click **Close** to close the **Calipers** dialog.
8. Continue as needed to specify the values that the application module requires, for example, determining the Intensity above local background, or if you are done configuring the module, then continue to Testing and saving settings on page 81.

Measuring the area of an object

1. If the **Region** toolbar is not open, click **Regions > Region Tools**.

   In the simplified menu structure, click **Measure > Regions > Region Tools**.

2. Select the **Trace Region** tool.

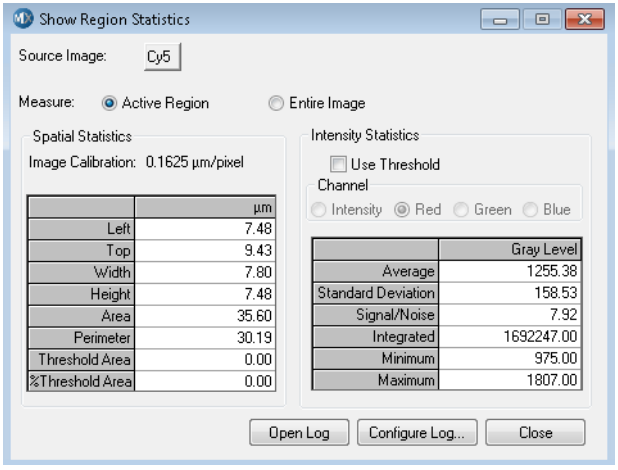


3. Click and hold the left mouse button to draw a region of interest around a typical compartment. Double-click the left mouse button to close the region. The region line blinks to indicate that the region is selected.
4. Click **Measure > Show Region Statistics**.

   In the simplified menu structure, click **Measure > Distances > Show Region Statistics**.

5. For **Measure**, select **Active Region**.

   The area of the object is displayed in the **Area** field of the Measurements table (the left table) on the dialog. This value represents the object area in pixels. If the image is calibrated, then the area is in pixels and calibrated units.



6. Click **Close** to close the **Show Regions Statistics** dialog.
7. Continue as needed to specify the values that the application module requires, for example, determining the Intensity above local background, or if you are done configuring the module, then continue to Testing and saving settings on page 81.

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 objects of interest compared to the neighboring background gray-level values. This setting controls the sensitivity of the object detection and segmentation.

The value that you type 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, then type a value that is slightly lower than the calculated value. If you select the **Fast** algorithm, then in the **Intensity above local background** field, type a value that is approximately half of the calculated value.

1. If the **Region** toolbar is not open, click **Regions > Region Tools**.

   In the simplified menu structure, click **Measure > Regions > Region Tools**.

2. Select the **Arrow** tool.

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 gray-level value for a cell of 366 and a background value of 304, the calculation is: 366 – 304 = 62. Based on the guidelines, then, in this example, you would specify a value of approximately 60 for the Intensity above local background.

Tip: You can also consider **Linescan** as an alternate method for intensity measurements. Draw a line across a dim object and its local background, and then use the **Linescan** tool that is available from the **Measure > Intensities** menu (simplified menu) or the **Measure** menu (default menu) to see more exact intensity values. See the MetaXpress Software application help for more information about this tool.

7. Continue as needed to specify the values that the application module requires, or if you are done configuring the module, then continue to Testing and saving settings on page 81.

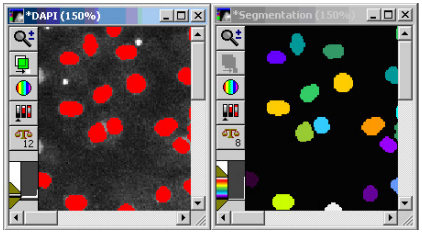
Testing and saving settings

After the module is configured, test the settings, make adjustments if needed, and then save the settings. When testing the module settings, the **Preview** option shows the segmented image using just the selected wavelength, and you can use this option or the **Test Run** option. The Test Run option uses all wavelengths, and this must be the option that you use for the final test.

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**.

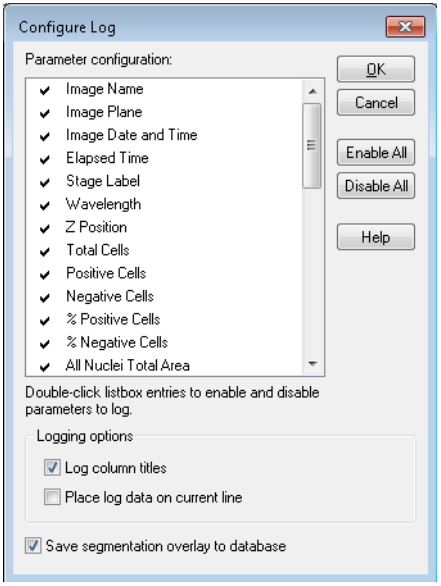
   The application module runs and an overlay image appears on the original image. If **Display result image** is selected, then a result image opens as well. A **Cellular Results** table that contains individual object data also opens. The following figure shows a sample source image with an overlay (left) and a result image with the cells individually colored (right).



2. Click a cell in the image to highlight the data in the **Cellular Results** table for the cell. Use Ctrl+Click or Shift+Click to select more than one row of data.

Note: If the table does not open when you run an application module, then from the **Window** menu, you can select **Show Cellular Results**

3. Compare the result image with the source image to determine if all the objects of interest are being detected.
   - If all objects of interest are being detected, then continue to Step 4.
   - If all objects of interest are not being detected, then 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, or other required values. After you are satisfied that all objects of interest are being detected, continue to Step 4.
4. To specify the site by site data that is to be recorded for the application module, click **Configure Summary Log**, and in the **Configure Log** dialog, select or clear the appropriate settings.



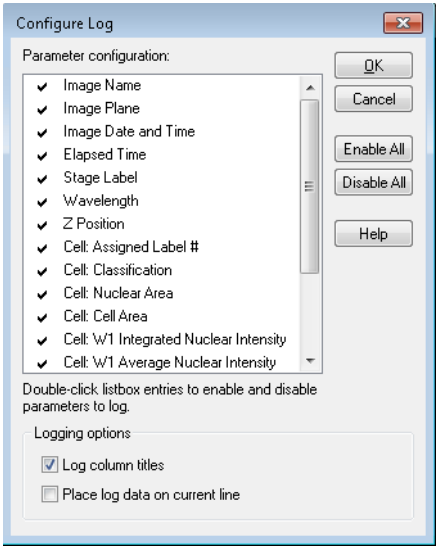
5. Click **OK** close the dialog and apply the settings.

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.

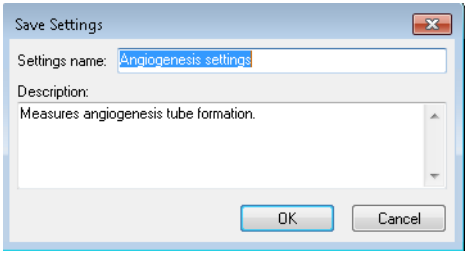
Note: Segmentation overlay takes a significant amount of space in the database and Molecular Devices does not recommend saving it for large images.

Note: For more information on the data that can be logged, see the application help Dialog options topic for the application module you are using.

6. If applicable, to configure the cell by cell data that is to be recorded for the application module, click **Configure Data Log**, and in the **Configure Log** dialog, select or clear the appropriate settings.



7. Click **OK** close the dialog and apply the settings.
8. Click **Save Settings**.
9. In the **Save Settings** dialog, type a name and description for the application module settings.



10. Click **OK** to close the dialog and save the application module settings to the database.
11. Click **Close**.

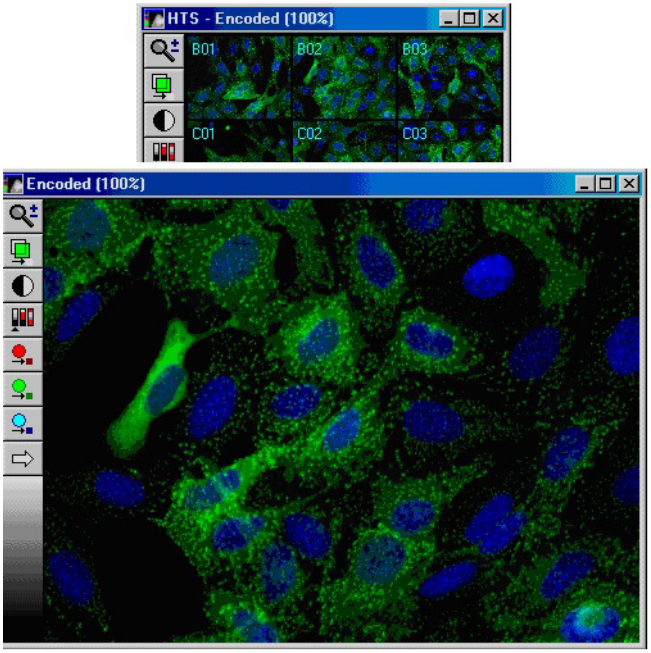
Example: The Transfluor Application Module

The procedures that were described in Configuring an Application Module on page 71 work with any application module. This section provides an example of procedures that are specific to a module, the **Transfluor** application module. See:

- To select the wavelength image on page 85.
- To determine the object widths on page 86.
- To determine Intensity above local background on page 87.
- To test the application module settings on page 88.
- To specify how data should be recorded on page 89.
- To run the application module on page 90.

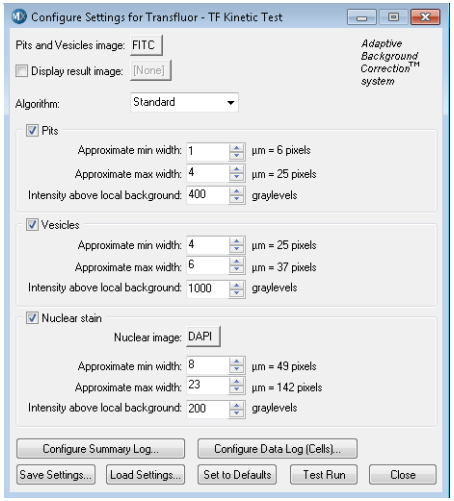
To select the wavelength image

1. Use the **Review Plate Data** dialog to select a plate as described in Chapter 3: Selecting and Analyzing Plates on page 13.
2. Click one of the wells to display an image.



3. In the **Review Plate Data** dialog, click the **Run Analysis** tab.
4. From the **Analysis** drop-down list, select <**Transfluor**>.

5. Click **Configure Settings**.
6. In the **Configure Settings for Transfluor** dialog, click the **Pits and Vesicle image** selector, and then select the wavelength image that represents the pits and vesicles.



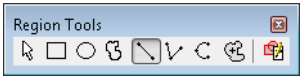
7. Click the pits and vesicles image, and then increase the zoom by clicking the magnifying glass icon on the image window or by pressing **Page Up** on the keyboard.
8. Continue to To determine the object widths.

To determine the object widths

To determine the objects, you can use the **Single Line** tool on the **Region** toolbar to measure a granule width according to the following steps:

1. If the **Region** toolbar is not open, click **Regions > Region Tools**.

   In the simplified menu structure, click **Measure > Regions > Region Tools**.
2. From the **Region Tools** toolbar, select the **Single Line** tool.

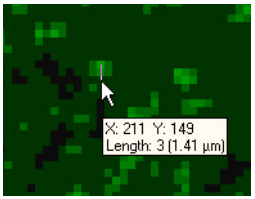


3. In the image window, click one of the edges of the smallest pit that is to be included in your analysis.

   A tooltip opens that shows the current X and Y values of the pointer. A placeholder is displayed for the Length.

4. Move the cursor to the opposite edge of the pit and note the Length value.

   The following figure shows that, in this example, the length is **3** pixels or **1.41**µm. This value represents the object width in pixels. If the image is calibrated, then the length is in pixels and calibrated units.



5. In the **Configure Settings** dialog, in the **PIts** section, in the **Approximate min width** field, type or select the value in microns.
6. To remove the region lines, select **Clear Regions** on the **Regions** menu or right-click on the region and click **Delete Region**.
7. Click one of the edges of the largest pit that is to be included in your analysis.

   A tooltip opens that shows the current X and Y values of the pointer. A placeholder is displayed for the Length.
8. Move the cursor to the opposite edge of the pit and note the Length value.
9. In the **Configure Settings** dialog, in the **Pits** section, in the **Approximate max width** field, type or select the value in microns.
10. To remove the region lines, select **Clear Regions** on the **Regions** menu or right-click on the region and click **Delete Region**.
11. Continue to To determine Intensity above local background.

To determine Intensity above local background

1. On the **Regions** toolbar, click the **Locator** tool.

2. 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. For example, in the following figure, the value is **162**.

3. Move the cursor to just outside the pit to measure the background.
4. Calculate the difference between the gray-level value of the pit and the background.
5. In the **Pits** section, **Intensity above local background** field, type or select a value that is slightly lower than the calculated value. For example, if you calculated an Intensity above local background value of **1000**, then a value of **800** is appropriate.
6. If applicable, repeat for vesicles. Otherwise, clear the **Vesicles** check box.

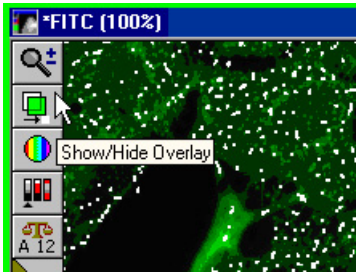
Note: Vesicles are much larger and brighter than pits.

7. Continue to To test the application module settings.

To test the application module settings

When you test the module, you first confirm that pits are being detected correctly. If you are using a nuclear stain, you then confirm that nuclei are being detected correctly.

1. Clear the **Nuclear stain** check box.
2. Click **Test Run** to test your settings to display the pits and vesicles segmentation as an overlay on the image.
3. On the Image window toolbar, use the **Show/Hide Overlay** tool to toggle the results on or off to make sure all the pits are detected.



4. Do one or both of the following as needed:
   - If all the pits are not detected, then lower the **Intensity above local background** value and check the results again with the **Show/Hide Overlay** tool.
   - If lowering the intensity value does not produce the needed results, then modify the other parameters or continue to modify the intensity value with the other parameters and check with the **Show/HIde Overlay** tool until the needed results are obtained.
5. Select a negative control image, and then click **Test Run** to make sure that your settings are correct. (Objects that are not pits are not being detected as such.)
6. If you are using a nuclear stain, then select **Nuclear stain** and repeat Step 2 through Step 5 for the nuclei in the image.
7. Click **Test Run** to open the **Cellular Result**s table.
8. Use the **Cellular Results** table to interactively view individual cellular results and confirm that the results are as expected. Otherwise, make adjustments as needed, and continue testing until the appropriate results are obtained.
   - Clicking a cell in the image highlights the data for the selected cell in the table.
   - Clicking 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.
9. Continue to To specify how data should be recorded.

To specify how data should be recorded

1. Click **Configure Summary Log**.
2. To specify the site by site data that is to be recorded for the application module, click **Configure Summary Log**, and in the **Configure Log** dialog, select or clear the appropriate settings.
3. Click **OK** close the dialog and apply the settings.

4. To save the image overlay to the database with the image after automated analysis, select the **Save Segmentation Overlay to Database.**
5. If applicable, to configure the cell by cell data that is to be recorded for the application module, click **Configure Data Log**, and in the **Configure Log** dialog, select or clear the appropriate settings.
6. Click **OK** close the dialog and apply the settings.
7. Click **Save Settings**.
8. In the **Save Settings** dialog, type a name and description for the application module settings.
9. Click **OK** to close the dialog and save the application module settings to the database.
10. Click **Close**.
11. Continue to To run the application module.

To run the application module

1. In the **Review Plate Data** dialog, on the **Run Analysis** tab, on the **Settings** drop-down list, select the saved Transfluor settings file that you created.
2. Run the analysis. See Running an Analysis on page 34.
3. After the analysis is run, view the results in the **Cellular Results** table, on the **Measurements** tab, or on the **Graph** tab. See Chapter 4: Viewing Analysis Results on page 49 for more information.

Cell-by-Cell Multiplexing with Application Modules

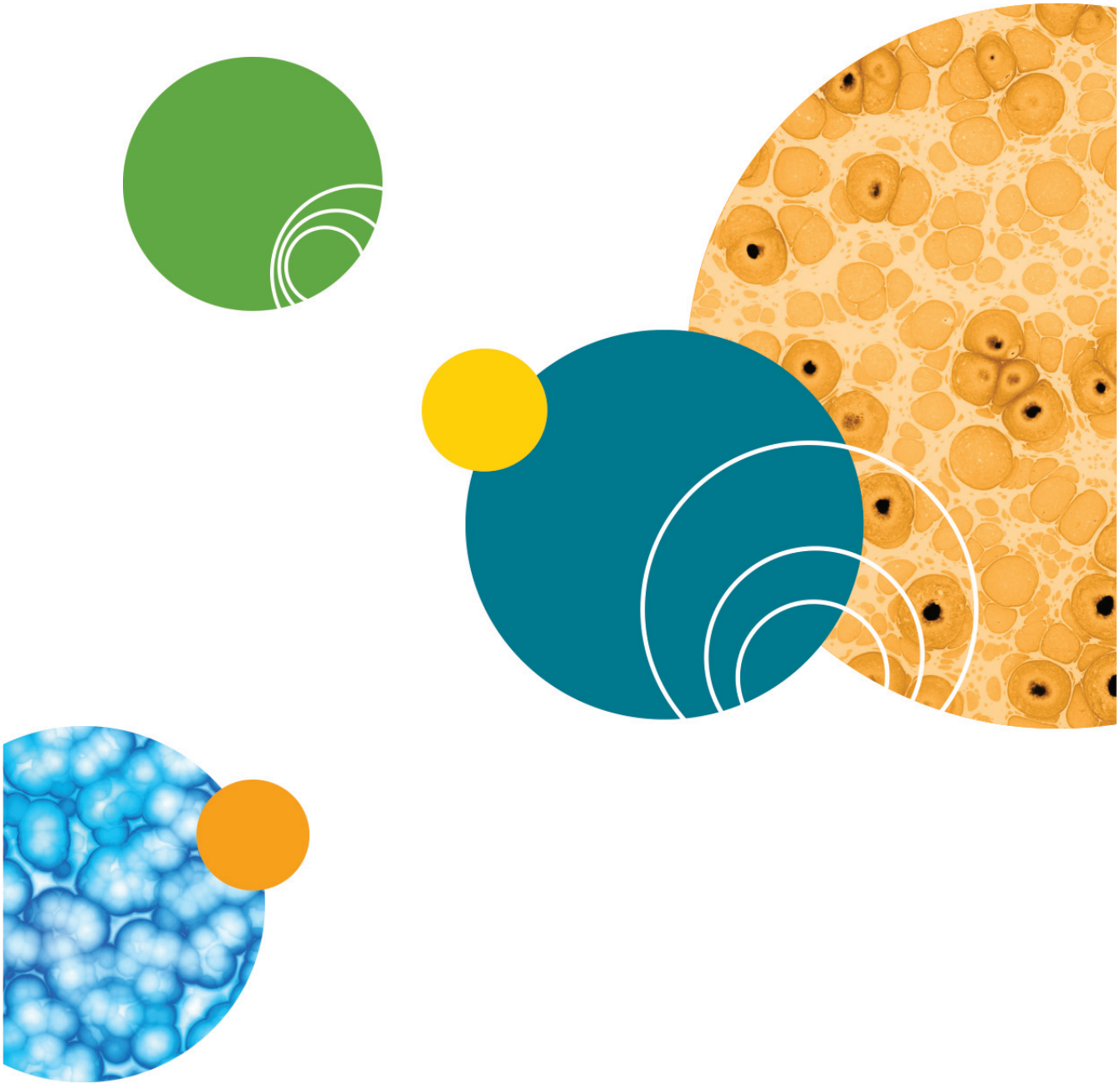
One of the advantages of high content screening is the ability to multiplex assays by running multiple analyses on the same samples. For example, you could run a **Cell Scoring** assay to measure transfection efficiency and a **Transfluor** assay to measure receptor internalization on the same sample.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

Compatible modules for cell-by-cell multiplexing include **Cell Cycle**, **Cell Health**, **Cell Scoring**, **Count Nuclei**, **Granularity**, **Mitotic Index**, **Monopole Detection**, **Multi Wavelength Cell Scoring**, **Multi Wavelength Translocation**, 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.

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

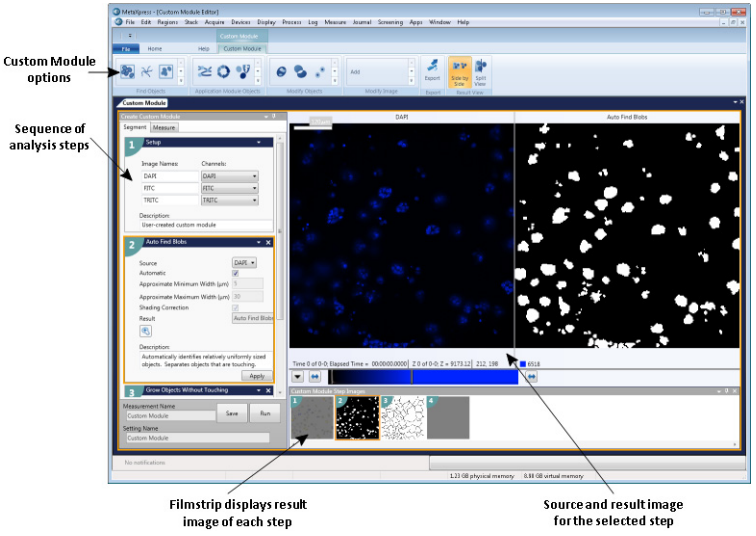


Custom Modules

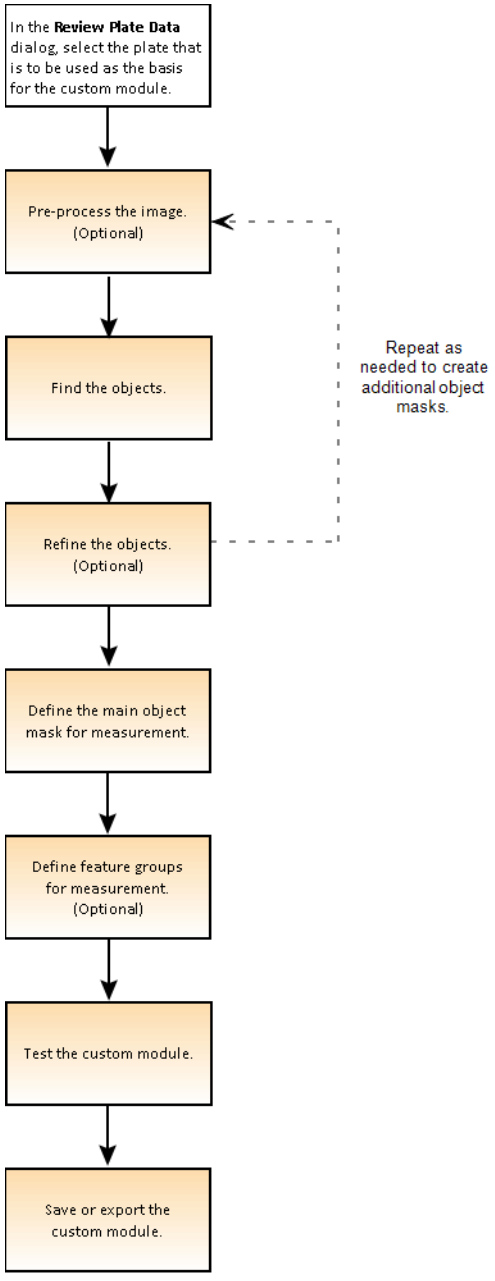
6

Not all samples can be meaningfully or accurately assessed with just one standard application module. Often, you must combine an array of preprocessing and segmentation methods to effectively analyze sets of images. Applications such as protein subcellular patterns or fluorescence co-localization of objects are often best done with a sequence of steps. For example, you might 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 do. Moreover, depending on your research goals, you might want to use the same sequence of steps to analyze numerous datasets, or share a custom module with other researchers for use in their datasets.

The MetaXpress Custom Module Editor is an optional, flexible, interactive environment in which you can create a template for an image analysis that can be distributed and re-used. You select from galleries of processing options, morphology filters, and segmentation methods to construct a sequence of analysis steps that are displayed in Step Cards in the Create Custom Module (left) pane of the workspace. When you test (apply) a step, a result image is automatically displayed in the Image Grid and the Filmstrip pane in the workspace.



The general sequence of tasks for creating a custom module are:



- **Select the plate**: In the **Review Plate Data** dialog, select the plate that to use as the basis for the custom module, and then click **Create Custom Module** to open the Custom Module Editor. Alternatively, to modify an existing custom module, on the **Analysis** drop-down list, select the appropriate custom module, and then click **Configure Custom Module**.

Note: If you have Z Series data loaded in the **Review Plate Data** dialog, and your organization has purchased and installed the optional 3D Custom Module (also referred to as a Volume Module), then when you click **Create Custom Module** or **Configure Custom Module**, the 3D Custom Module opens automatically with your data loaded.

In the Custom Module Editor:

- **Pre-process the image**: Generally, the first steps in configuring a custom module are pre-processing steps to improve the image quality so that objects of interest can then be clearly defined with segmentation options. The **Modify Image** group offers many options that enhance features, increase the level of detail, and remove unwanted image artifacts. These options can, among many other things, shrink and grow dark and light objects, smooth light and dark objects simultaneously, detect bright or dark features of a specific spatial size or shape, increase local contrast, detect edges, fill holes, and invert an image.
- **Find the objects**: When the image quality is optimal, then in the second series of steps, you use the segmentation options in the **Find Objects** and **Application Module Objects** groups to define the objects of interest. The result image from these steps is a black and white image called a mask image.
- **Refine the objects**: If needed, you can add a third series of steps from the **Modify Objects** group to further define the location and boundary of each object in the mask image. For example, you can remove border objects, invert objects, and keep or remove marked objects. Logical operations are available for combining or separating images. Optionally, you can filter the objects based on their properties, such as size or intensity.

- **Measure the objects**: After you are satisfied that the mask steps that you have specified define the locations and boundaries of objects in the image accurately and reproducibly, then you specify the **Measure Mask** step. The **Measure Mask** step, which is the final analysis step, defines the properties of the objects to be measured, for example, total area, area, hole area, average intensity, and so on. Optionally, you can then add one or more feature groups that specify the binary mask images to use to measure the features, which are the objects that are contained within the objects that are identified in the main mask.
- **Test the custom module**: Each time you run a custom module in the Custom Module Editor, the measurement results are displayed in a Measurement data table in the **Data Table** pane. You can select data in the Measurement data table to update the image view and you can also display the measurement results in a graph.
- Save and export the custom module: After you have defined all the required steps for the custom module, you then save the module. When you save a custom module, it is automatically added to the list of available modules on the **Analysis** drop-down list on the **Review Plate Data** dialog. To share the module with other users who are using different MetaXpress Software databases, export the module. When you export a custom module, it is saved as an XML file. After you save or export a custom module, you can import it into the Custom Module Editor to modify it or you can modify it directly from the **Review Plate Data** dialog.

Tip: You can also use the Export Custom Module function as a method of backing up a custom module.

Note: For a detailed explanation about using the Custom Module Editor to create and modify your needed custom modules, see the help for the Custom Module Editor.

Journals

7

Journals are powerful macros for the MetaXpress Software. Journals provide customizations beyond that of custom modules, and they can extend the capabilities of the MetaXpress Software for both image acquisition and image analysis. Some examples of analysis journal routines include the following:

- Load saved regions, and then measure the intensity within the regions.
- Score cells based on calculations using measurements from multiple analysis modules.
- Map objects in images to a grid.
- Stitch multiple sites together, and then do neurite outgrowth analysis.
- Analyze up to a specific number of cells per well, and then skip the remaining sites in the well.

All journals can be recorded, edited, saved, and run. After journals and any associated files are uploaded to the MDCStore database from one MetaXpress computer, they can be run on any MetaXpress computer that is connected to the same database. Selecting a journal analysis downloads a local copy of these files to the computer that is running the analysis.

This chapter includes the following topics:

Note: For more information on creating journals, visit the Molecular Device knowledge base at http://www.moleculardevices.com/support. Example journals can be downloaded from the MetaMorph/MetaXpress User Forum at http://metamorph.moleculardevices.com/forum/.

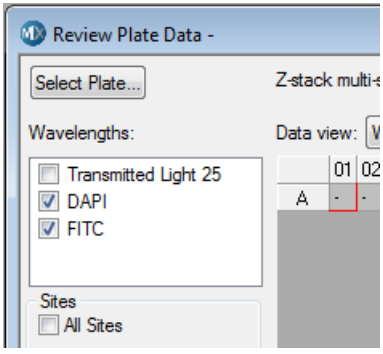
Considerations for Designing an Analysis Journal

Before you design an analysis journal, it is important to understand how the journal and the available images are looped as the journal runs across the plate. You must consider the following:

- Wavelengths
- Wells
- Sites
- Time lapse assays
- Database logging

Wavelengths

The wavelengths that are selected on the **Review Plate Data** dialog or the **Run Analysis on Plates** dialog (**Plate Data Utilities** > **Run Analysis** > **Select Plate for Analysis**) determine the images that are available for the analysis journal. All the wavelengths that are selected for the plate are opened for each site as the journal analysis runs. The images are named the same as the selected wavelengths. For example, as shown in the following figure, the images are named **DAPI** and **FITC**. The **Transmitted Light 25** image is not selected, and therefore, it is not available as the analysis journal runs.



Wells

The analysis starts with the upper-left well (A01), moving across to the right in the first row (A12 for a 96-well plate), and then down to the start of the next row (B01). Likewise, it moves across to the right and down, finishing with the last row of the plate from left to right. All sites within each well are run before the analysis moves to the next well.

Sites

Within each well, the analysis starts with site 1, then runs on site 2, and so on, until it has completed the final site. It then moves to the next well. All sites within each well are run before the analysis moves to the next well. For time-lapse assays and Z-series assays, there are more analysis-order considerations that depend on the time-lapse or Z-series option that is selected.

Time lapse assay

Table 7-1: Analysis order for a time lapse assay

Time Lapse Option	How the journal loops
• All timepoints • Time point range	Within each site, the analysis runs on the first selected time point, and then on the subsequent time points one at a time, until the final time point is completed. The analysis then moves to the next site.
• Stack of all time points	For each site, all available time points are loaded into a stack for each selected wavelength. The journal can then do stack arithmetic or loop through the planes as appropriate for the specific analysis.
• Selected time point	For each site, a single time point is run.

Table 7-2: Example of analysis order with a multi-site plate run using the **All time points** option

Well	Site	Analysis Order
A01	Site 1	Time point 1
		Time point 2
	Site 2	Time point 1
		Time point 2
A02	Site 1	Time point 1 Time point 2
	Site 2	Time point 1
		Time point 2

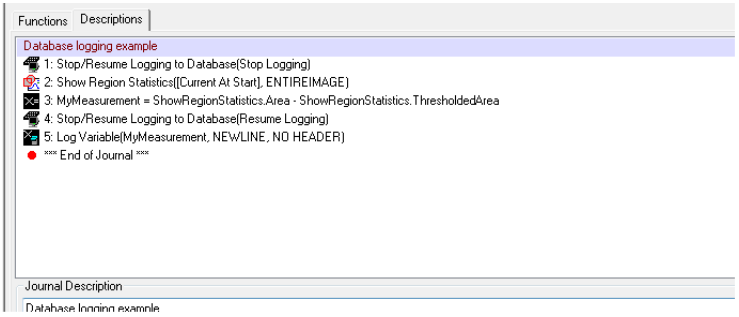
Z series assay

Table 7-3: Analysis order for a Z series assay

Time Lapse Option	How the journal loops
• All Z steps • Z step range	Within each site, the analysis runs on the first selected Z step, and then on the subsequent Z steps one at a time, until the final Z step is completed. The analysis then moves to the next site.
• Stack of all Z steps	For each site, all available Z steps are loaded into a stack for each selected wavelength. The journal can then do stack arithmetic or loop through the planes as appropriate for the specific analysis.
• Selected Z step • 2 D projection image	For each site, only the indicated single image for each wavelength is run.

Database logging

Any steps in a journal that log data (for example, **Show Region Statistics** or an application module), generally log data to the database. To avoid unwanted data appearing for intermediate steps and stop the database logging, use the **Stop/Resume Logging to Database** journal function. The following figure shows an example journal that uses the **Stop/Resume Logging to Database journal** function.



You must also consider how to manage database logging based on the type of data that the journal is measuring: cell data, time lapse data, or Z series data.

Cell data

If the analysis journal is measuring cell data by looping through objects or regions, then to log each data point as a separate cell, use the **Set Cell Number for Database Logging** journal function.

Time lapse data

If the analysis journal is running on a time lapse assay using the **Stack of all time points** option, then to log data specifically for each time point, use the **Set Time and Z Step for Database Logging** journal function.

Z series data

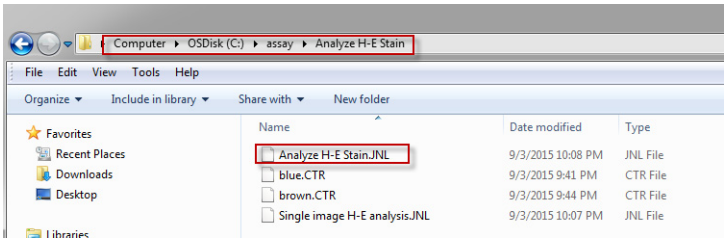
If the analysis journal is running on a Z series assay using the **Stack of all Z steps** option, then to log data specifically for each Z step, use the **Set Time and Z Step for Database Logging** journal function.

Adding an Analysis Journal to the MetaXpress Software Database

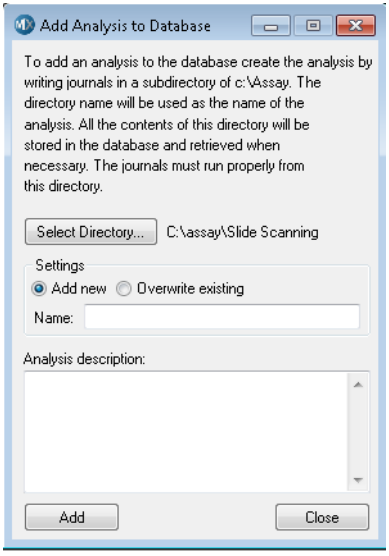
Before you can add an analysis journal to the MetaXpress Software database, you must verify that any computer that is to run the journal has the MetaXpress Software installed on it, that the folders **c:\analysis** and **c:\assay** are present, and that the Windows user has full control (Read, Write, and Modify) for these folders. When you add an analysis journal to the MetaXpress Software database, you can create new analysis settings, or you can overwrite existing analysis settings. You can add multiple settings for any analysis journal. These might include different analysis parameters such as threshold settings, region files, or IMA state files. After you add an analysis journal to the MetaXpress Software database, the journal is available for selection on the **Analysis** drop-down list on the **Run Analysis** tab of the **Review Plate Data** dialog.

CAUTION! If you modify an existing analysis journal by editing the journal file or other associated files inside the **c:\assay** folder on your MetaXpress computer, then you *must* add the analysis to the MetaXpress Software database to save the changes. Otherwise, running the analysis journal with the existing settings overwrites your local changes.

1. On the MetaXpress Software computer, in the **c:\assay** folder, create a subfolder that is named exactly the same as the analysis journal that is being added to the database: **c:\assay\Journal Name**.
2. Save the journal (*.jnl) and if applicable, any associated files to this subfolder.



3. In the MetaXpress Software, on the main menu, click **Screening > Add Analysis to Database**.



4. In the **Add Analysis to Database** dialog, click **Select Directory**, and then browse to and select the directory that contains the analysis journal.

5. Continue to one of the following:
   - To create new analysis settings on page 103.

Note: If you are adding a new analysis journal, then you must add new analysis settings.

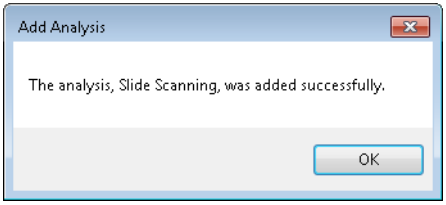
   - To overwrite existing analysis settings on page 103.

To create new analysis settings

1. Under **Settings**, select **Add new**.
2. In the **Name** field, type the name for the new analysis settings.

Note: Remember, the name for the new analysis settings and the name of the subfolder that contains the settings must be identical.

3. Optionally, in the **Analysis Description** field, type a description for the new analysis settings.
4. Click **Add**.

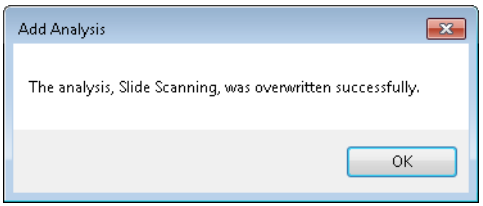


5. In the **Add Analysis** message dialog, click **OK**.

To overwrite existing analysis settings

1. Under **Settings**, select **Overwrite existing**.
2. On the **Name** drop-down list, select the name for the existing analysis settings.
3. Optionally, in the **Analysis Description** field, modify the existing description for the analysis settings.

4. Click **Overwrite**.

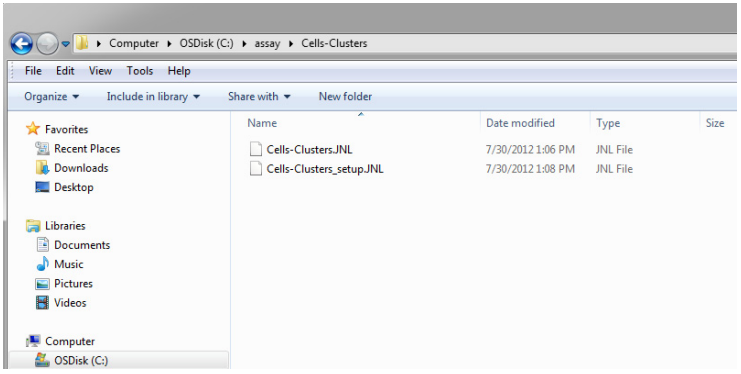


5. In the **Add Analysis** message dialog, click **OK**.

Adding a Setup Journal to the MetaXpress Software Database

A *setup journal* is always optional and it is run before the main analysis journal. A setup journal is used to do actions that are generally run before the main analysis such as assigning variables, opening a log, or loading an IMA state file. To create and run a setup journal, do the following:

1. Create the setup journal that contains the appropriate steps. When you name the setup journal, name it identically to the main analysis journal and add the suffix "_setup." See Adding an Analysis Journal to the MetaXpress Software Database on page 101.
2. Save the setup journal in the same **c:\assay** subdirectory as the main analysis journal.



3. To run the setup journal:
   - If you are using the **Review Plate Data** dialog to run the analysis, then after you select the main analysis journal on the **Analysis** drop-down list on the **Run Analysis** tab, a **Run Setup for Analysis** option is displayed. Click this option to run the setup journal. If no setup journal exists, then the

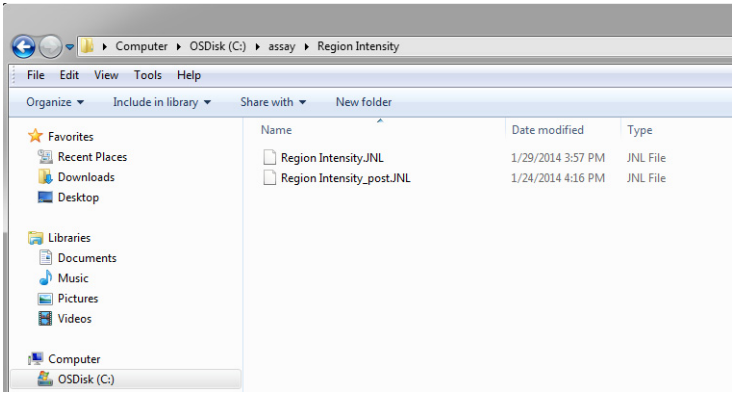
message “No setup journal exists for this analysis” is displayed.

- If you are running the analysis through the **Plate Data Utilities > Run Analysis > Run now on this computer** option, then the setup journal is automatically run at the start of every plate that is being analyzed. This is important to note if the setup journal includes any interactive steps, as the analysis pauses while it waits for user input.
- If you are running the analysis in **Auto Run** mode, then the setup journal does *not* run.

Adding a Post Journal to the MetaXpress Software Database

A *post journal* is always optional and it is run after the main analysis journal. A post journal is used to do actions that are generally run after the main analysis such as resetting variables or log files, or calculating plate-based statistics. To create and run a post journal, do the following:

1. Create the post journal that contains the appropriate steps. When you name the post journal, name it identically to the main analysis journal and add the suffix “_post.” See Adding an Analysis Journal to the MetaXpress Software Database on page 101.
2. Save the post journal in the same **c:\assay** subdirectory as the main analysis journal.



3. To run a post journal:
   - If you are using the **Review Plate Data** dialog to run the analysis, then the post journal does not run automatically after the main analysis is complete. Instead, run the post journal manually by clicking **Run > Run Journal** (or **Control > Journal > Run Journal** in the Simplified menu), and then selecting the appropriate post journal.
   - If you are running the analysis through the **Plate Data Utilities > Run Analysis > Run now on this computer** option, then the post journal is automatically run at the end of every plate that is being analyzed.
   - If you are running the analysis in **Auto Run** mode, then the post journal does *not* run.

Table 7-4 summarizes the behavior of the optional setup and post journals for the different methods of running a journal analysis.

Table 7-4: Journal behavior when using different methods to run journals

	Setup Journal	Main Analysis Journal	Post Journal
Review Plate Data dialog	Click **Run Setup for Analysis** to run.	Runs on all wells, or selected wells as defined by the user.	Does not run automatically. Run the journal manually instead.
Run Analysis > Run now on this computer	Runs automatically at the start of each plate.	Runs on all wells.	Runs automatically at the end of each plate.
Auto Run mode	Does not run.	Runs on all wells.	Does not run.

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 done 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.

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. After 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 doing 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 options on the **Post Acquisition** tab on 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. The application module drop-in must be enabled on the acquisition workstation before you can select it here.

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

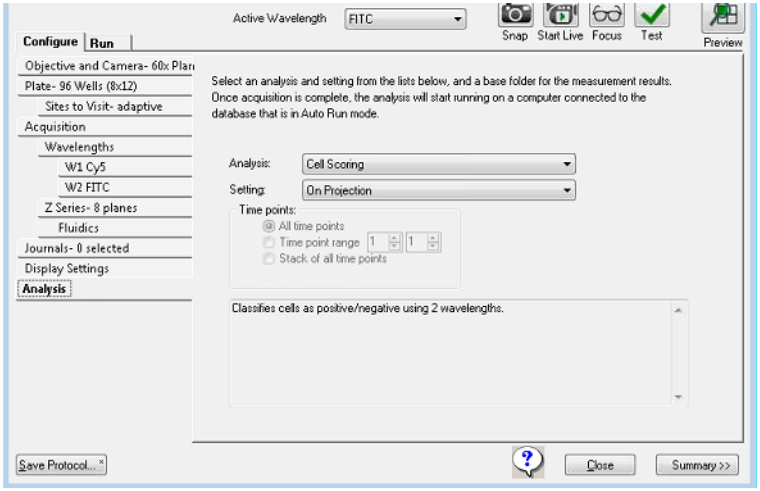
To choose a specific analysis to run on a data set after the acquisition is complete, select **Analyze Images After Acquisition** on the **Acquisition** tab on the P**late Acquisition Setup** dialog, and then specify the analysis options on the **Analysis** tab. If the system is set to Auto Run mode, then the data set is added to the Auto Run queue for analysis. You can select from a list of saved settings for any application module, custom module, or journal assay that is saved to the database.

Note: If you do not want to automatically run post-acquisition analysis, ensure that **Analyze Images After Acquisition** is not selected on the **Acquisition** tab.

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 run automatically after acquisition:

1. In the **Plate Acquisition Setup** dialog, on the **Acquisition** tab, select **Analyze Images After Acquisition**, and then open the **Analysis** tab.



2. On the **Analysis** drop-down list, select the assay (application module, custom module, or journal assay) to run after acquisition.

Note: Only analyses that have defined settings are listed.

3. On the **Setting** drop-down list, select a settings file.

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

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

4. Continue to Initiating an Automated Analysis on page 110.

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 significantly 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.
2. Click **Screening > Plate Acquisition Setup**.

   In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, on the **Acquisition** tab, select **Analyze Images After Acquisition**, and then open the **Analysis** tab.
4. On the **Analysis** drop-down list, select the assay (application module, custom module, or journal assay) to run after acquisition.
5. Continue to set up and run your acquisition. After the plate is acquired, it is added to the Auto Run queue.
6. On the computer that is running the analysis, start the MetaXpress Software.

7. Click **Screening > Start Auto Run Mode** [DB].

8. In the **Auto Run Mode** dialog, to run the analysis on more than one plate, clear **Leave auto run mode when plate is finished**. If this option is selected, then only one plate is analyzed.

Note: To use the system for other tasks and prevent the MetaXpress Software from starting the analysis on the next plate after it is finished with the plate that it is currently analyzing, then **Leave auto run mode when plate is finished** must be selected. While in Auto Run Mode, all other MetaXpress Software options are disabled.

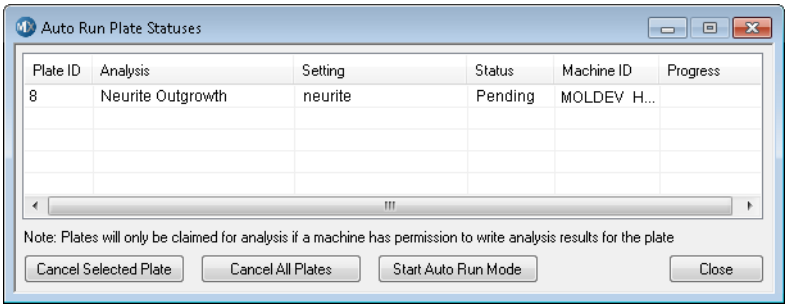
9. After the analysis is completed for all plates, click **Cancel** to close the **Auto Run Mode** dialog.

Note: Data from sites analyzed remains in the database, but the plate is removed from the Auto Run queue.

Monitoring the Status of Automated Analyses

1. On the computer that is running the analysis, click the **Screening > Auto Run Plate Statuses [DB]**.

   Alternatively, click **Screening > Start Auto Run Mode [DB]**, and then in the **Auto Run Mode** dialog, click **Status**.



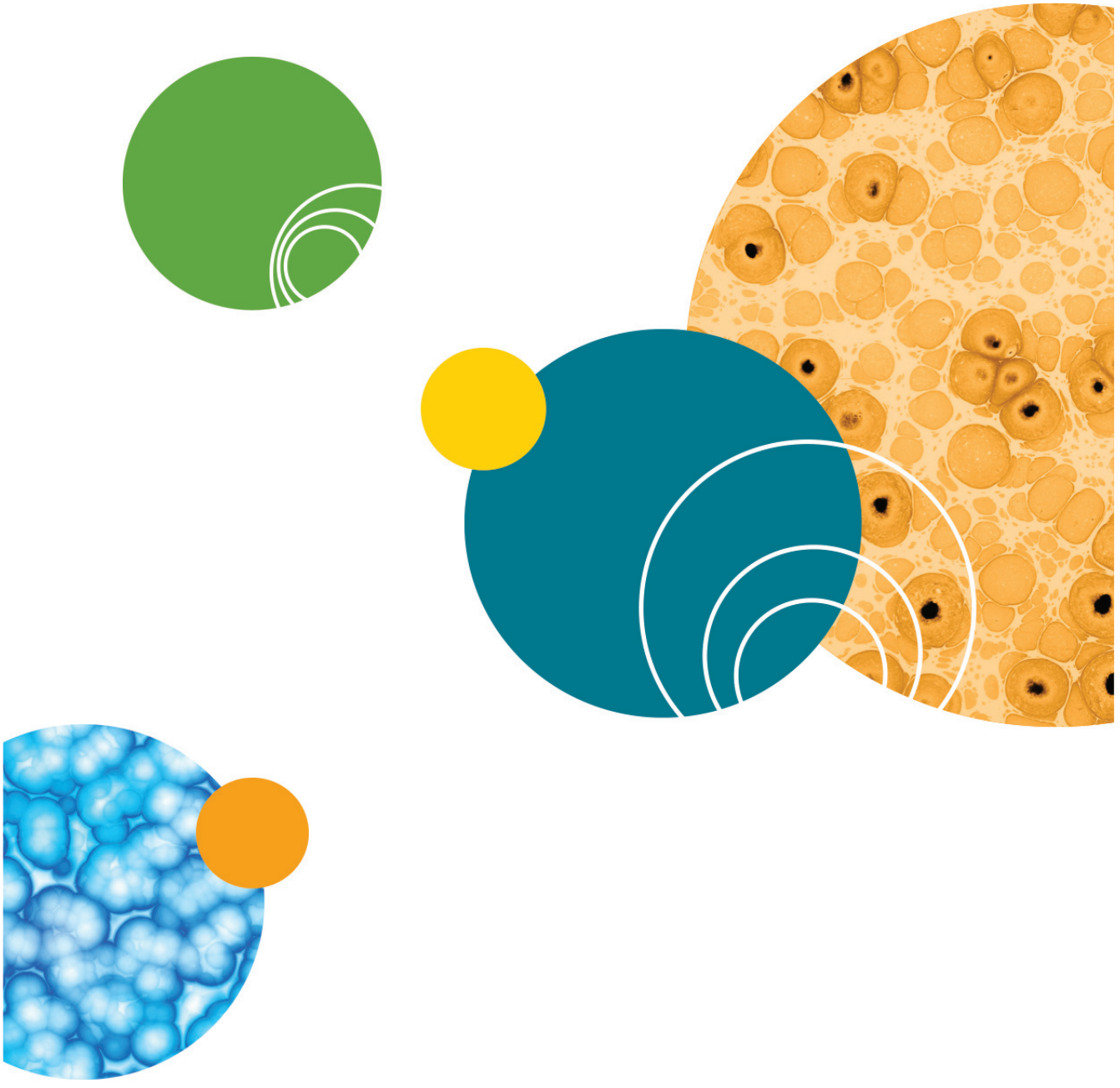
Note: The **Auto Run Plate Statuses** dialog also shows any plates to be analyzed from the **Review Plate Data** dialog. However, computers in Auto Run Mode do not claim these plates.

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 is 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 generally caused by an error on the computer running the analysis. To diagnose the cause of the timeout, inspect the computer that has timed out for error messages or other problems. In some situations, the problem can be resolved and the analysis can continue. If this happens, the status is reset to **Running**. In other situations, the Auto Run must be canceled and the analyses run again. Some analyses, particularly custom modules or journal analyses, take a long time to complete. For these situations, increase the timeout value for the **Set Auto Run Timeout** option to allow enough time to run and complete the analysis.
- **Pending** indicates the analysis has not yet started on the plate.

- **Acquiring** indicates that the plate is currently being acquired. Analysis does not happen until after the acquisition is complete.
- **Error** indicates an error in the analysis and that the analysis was unable to be completed as a result, for example, analysis settings that were defined for different wavelengths than those that are available on the plate.

2. Optionally, do one of the following as required:
   - 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**.
   - To stop running the analysis on all plates, click **Cancel All Plates**.



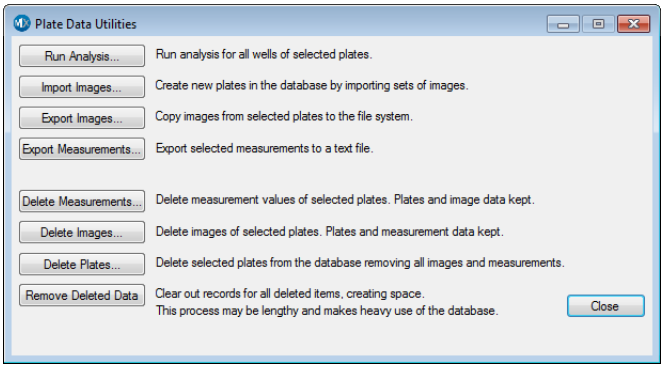
Batch Analysis

9

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

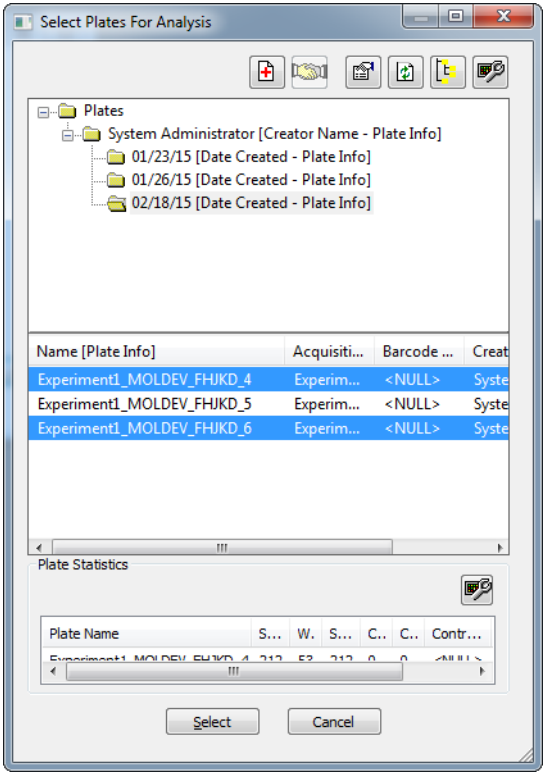
1. Click **Screening > Plate Data Utilities**.

   In the simplified menu structure, click **Screening > Plate Utilities**.



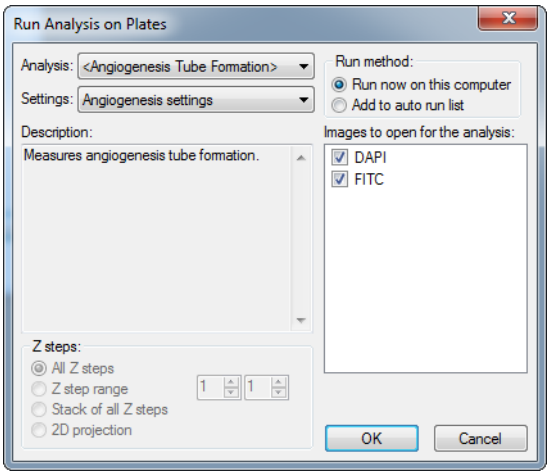
2. In the **Plate Data Utilities** dialog, click **Run Analysis**.

3. In the **Select Plates for Analysis** dialog, use the query tools or expand a folder so that several plates open in the bottom window.
4. Select a plate or use Ctrl+Click or Shift+Click to select multiple plates.



5. Click **Select**.

6. In the **Run Analysis on Plates** dialog, on the **Analysis** drop-down list, select the assay to run.



7. On the **Settings** drop-down list, select the appropriate setting.
8. Select a **Run method**:
   - Select **Run now on this computer** to start the analysis only on this computer.
   - Select **Add to auto run list** to add the job to the autorun queue to run on other computers that 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 that is available on the **Screening** menu, or the MetaXpress PowerCore Software must be running. The MetaXpress PowerCore Software processes application modules and custom modules, but it does not process journals.

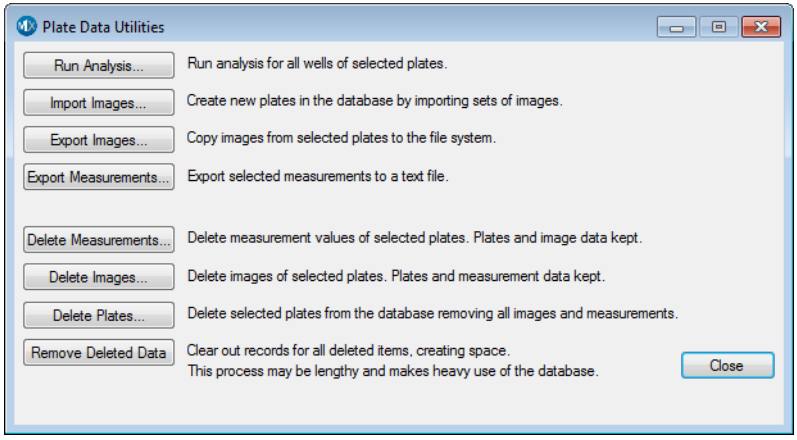
9. If you selected **Run now on this computer**, then on **the Images to open for the analysis** drop-down list, select the wavelengths to use with the selected assay.

10. If the plate contains multiple time points, then select from the **Timelapse** options:
    - **All time points** analyses all acquisition time points.
    - **Time point range** analyzes the time points that are in the defined range. To use a single time point, type the same time point in both fields.
    - **Stack of time points** is available when you select a time lapse journal that analyzes planes in a stack as separate time points from the **Analysis** drop-down list.
11. If the plate contains Z data, then select from the **Z Steps** options:
    - **All Z steps** analyzes all the acquisition planes.
    - **Z step range** analyzes only those planes that fall within the defined range. To use a single Z step, type the same step value in both fields.
    - **Stack of all Z steps** is available when the selected analysis is a journal.
    - **2D projection** analyzes only the two-dimensional projection image.
12. Click **OK**.

Managing Plate Data

10

Use the **Plate Data Utilities** dialog to manage the images and data that were 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 or fileserver. To permanently remove the data from the database or fileserver, use the MDCStoreTools™ Data Management Utility. For more information, see the *MDCStoreTools™ Data Management Utility User Guide*.

This chapter includes the following topics:

Note: For information about the **Run Analysis** option on the **Plate Data Utilities** dialog, see Chapter 9: Batch Analysis on page 115.

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 can be imported. The data is then accessible using either the **Plate Data Utilities** dialog or the **Review Plate Data** dialog. You can also use these dialogs to import a plate of Cellomics DIB files into the MDCStore fileserver or database. See Importing Cellomics Data on page 121.

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 sets 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. To select the data sets to import, in the **HTD Files** pane, select the check boxes next to the HTD files.
5. On the **Move images** drop-down list, select a location (either the fileserver, a UNC path location, or the database) to which to import the data sets.

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** dialog.

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

Importing Cellomics Data

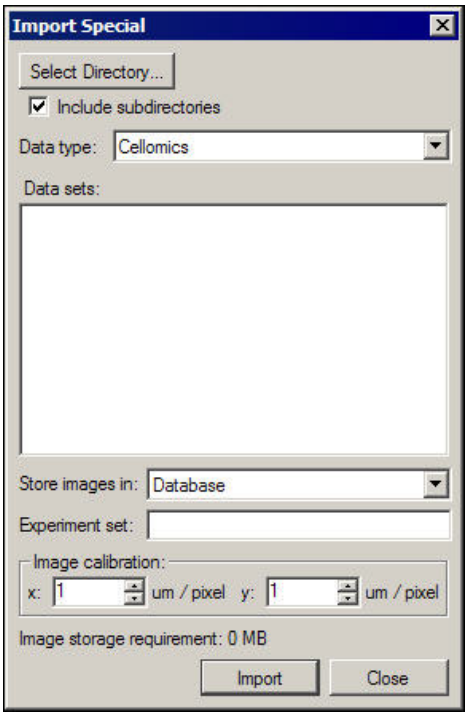
Use the **Import Special** option on the **Import Images** dialog to convert sets of Cellomics DIB files into data sets in the database that can be accessed using either the **Plate Data Utilities** dialog or the **Review Plate Data** dialog. The **Import Special** option creates MetaXpress HTD files 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. Unless you define a specific calibration when you import the data, a default calibration of 1 µm = 1 pixel is applied to the imported images.

To import cellomics 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**.

Note: To convert the sets of Cellomics DIB files that are contained in the selected directory and below, make sure that **Include subdirectories** is selected.

3. In the **Browse for Folder** dialog, navigate to the local or network folder containing the Cellomics data to be imported, and then click **OK**.

   The path is displayed next to the **Select Directory** button and the Cellomics files are displayed in the **Data sets** pane.

4. Select the check box next to the Cellomics file set to be imported.

5. On the **Store images in** drop-down list, select a location (either the database or an available network folder) to which to import the Cellomics data.
6. In the **Image calibration** section, type a calibration ratio (µm per pixel) for the **x** and **y** values of the imported images.

Note: The default calibration is 1 µm = 1 pixel.

7. Click **Import**.

   The files are imported and are available for review in the **Plate Data Utilities** or **Review Plate Data** dialog.

Exporting Measurements through a Query

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 out of all the image data, you might want to export data only from images where the number of cells is above some threshold.

Two methods for constructing a query are available: **Simple** and **Advanced**. The main difference between the two methods is how you select or specify the measurement sets:

- **Simple Query**: To create a Simple query, select the measurement sets from a list.
- **Advanced Query**: To create an Advanced query, specify the attribute criteria that identify the measurement sets, for example, measurement sets that contain data for plates acquired on a certain date. To create an Advanced query, you must know some of the unique attributes that identify the measurement sets containing data that you want to export such as the date created, the creator name, or name, or the plate information that is contained in the measurement set such as date annotated or global ID.

When using the **Export Measurements** 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 defined conditions are 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 **OR** or **AND**. 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 then 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

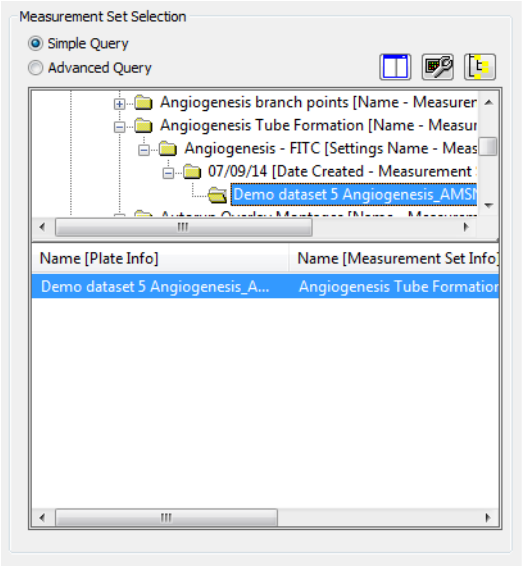
1. Click **Screening > Plate Data Utilities**.

   In the simplified menu structure, click **Screening > Plate Utilities**.
2. In the **Plate Data Utilities** dialog, click **Export Measurements**.
3. Select the type of measurement data to export: **Cell Measurements**, **Image Measurements**, or **Cell and Image Measurements**, and then click **OK**.
4. On the **Export Measurements Wizard - Step 1** page, do one of the following:
   - Select **Simple Query,** and then continue to To construct a Simple query
   - Select **Advanced Query**, and then continue to To construct an Advanced query on page 126.

To construct a Simple query

1. In the top left pane, double-click the **Measurement Set** folder that contains the data to export.

   The measurement set attributes are displayed in a “tree view” in the upper-left pane and the measurement set is added to the list in the lower-left pane. For example:



Note: The attributes are displayed for informational purposes only to help you identify the measurement sets. You 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.

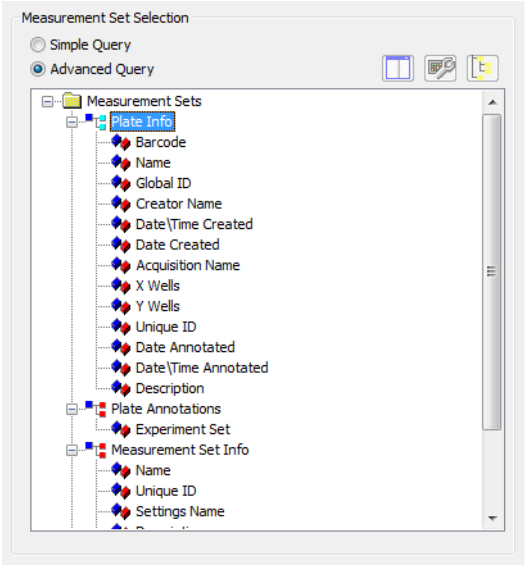
2. In the lower-left pane, select the measurement sets that contain the data to export, and then click the arrow between the panes to add the measurement sets to the **Query** pane.

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.

3. When all the measurement sets containing the data to export are listed in the **Query** pane, click **Next**.
4. Click **Finish**.
5. Continue to To configure the data for export on page 128 for instructions on specifying how the data appears in the text file.

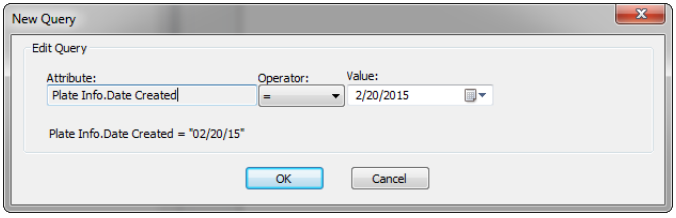
To construct an Advanced query

1. In the top left pane, expand the **Measurement Sets** folder, and then expand a folder that contains the measurement set attributes. For example:



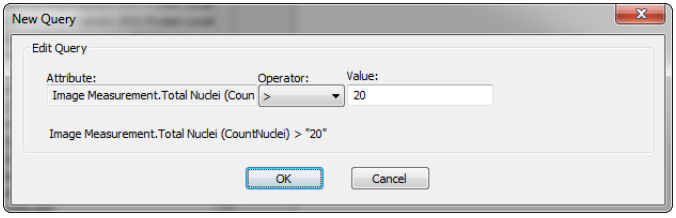
2. Double-click the attribute that identifies the measurement sets for the query, and then in the **New Query** dialog, select an operator and type a value to filter the data that to export, and then click **OK**.

   For example, to export just those measurement sets that contain data for plates acquired on February 20, 2015, expand the **Plate Info** branch, double-click **Date Created**, select "=" for the **Operator**, and then in the **Value** field, select the date.



3. Repeat Step 2 as required to include all the criteria that identifies the measurement sets containing the data to export in the **Query** pane.
4. Click **Next**.
5. On the **Export Measurements Wizard - Step 2** page, expand the **Data Types** folder, expand a subfolder, and then double-click the type of data to export.
6. To define the criteria for the data to export for the selected data type, in the **New Query** dialog, select 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 then in the **Value** field, type **20**.



7. Click **OK**.

   The query statement that defines the data that to export for the selected data type is added to the **Query** pane.
8. Repeat Step 5 through Step 7 to add as many data types as needed to the query.
9. Click **Finish**.

10. Continue to To configure the data for export for instructions on specifying how the data appears 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 opens. Use this dialog to specify the data display in the text file, and then export the data.

1. In the **Rows** pane, select the data type to display in rows in the text file such as sites, compounds, plates, cells, or wells, and then click the arrow to move the data type to the right pane.
2. In the **Columns** pane, select the measurement type to display in columns for each data type such as total nuclei, total cell area, average intensity, and then click the arrow to move the measurement type to the right pane.

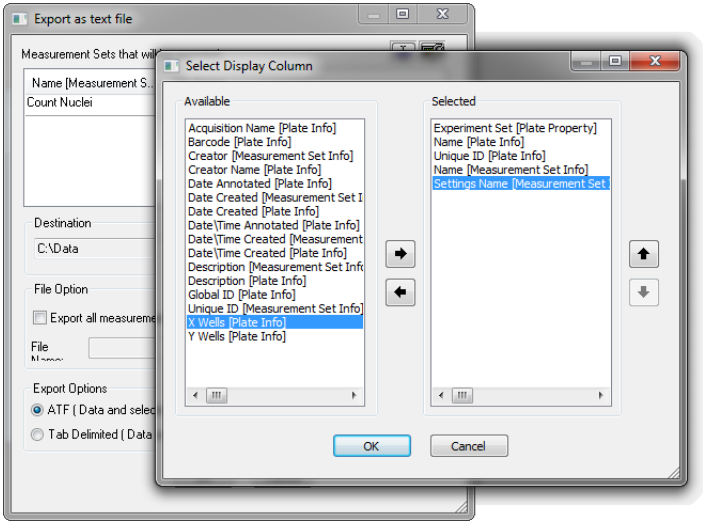
Note: To resize the width of the columns, drag the column headings or right-click and select **Fit Columns**. To sort the data in a column in ascending or descending order, click once in the column heading. To reorder the items in the right panes, click the up and down arrows.

3. To calculate a statistic for the exported data (for example, if you are averaging the data for multiple cells or wells), then on the **Apply Calculations** drop-down list, select the statistic. Otherwise, leave the value set to **None**.

   The **Data Layout View** pane shows how the data is to be displayed in the text file.
4. Click **OK**.

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

5. Do one or more of the following as required:
   - Optionally, 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  .
   - To combine the measurement data for all measurement sets into one text file, select **Export all measurements to one file,** and then in the **File Name** field, type a file name.
   - 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  , and in the **Select Display Columns** dialog, use Ctrl-click or Shift-click to select columns, and then click an arrow between the panes to move the columns.



6. Click **OK**.

   The data is saved in the text file or text files, and the **Export Measurement Set Summary** dialog opens.

7. Optionally, 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**.

8. Click **Close**.

Exporting Images

To export images to a local or networked folder, do the following:

1. Click **Screening > Plate Data Utilities**.

   In the simplified menu structure, click **Screening > Plate 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. In the lower pane, select the plate that to export, and then click **Select**.
6. In the **Browse for Folder** dialog, navigate to the local or networked folder to which to export the images, and then click **OK**.

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

Deleting Measurements

To delete measurements from a plate, you must first delete them using the **Delete Measurements** option. After you use the **Delete Measurements** option, you must then 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: The **Delete Measurements** option deletes all the measurement sets that are 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*.

To delete measurements

1. Click **Screening > Plate Data Utilities**.

   In the simplified menu structure, click **Screening > Plate 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 the folders that contain plates that have been saved to the database.
4. Double-click the appropriate folder to display all the plates and their associated information (name, date created, creator, and barcode (if applicable)) that are contained in the folder in lower pane.
5. In the lower pane, select the plate (use Shift+click or CTRL+click to select multiple plates) that contains the measurements to delete, click **Select**, and then click **OK**.
6. In the **Plate Data Utilities** message, click **OK** to delete all the measurements from the selected data sets.

Note: This step does not remove the data from the database or fileserver. To remove the data from the database or 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 you use the **Delete Images** option, you must then 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 remain in the database.

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

To delete images from a plate

1. Click **Screening > Plate Data Utilities**.

   In the simplified menu structure, click **Screening > Plate 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 that contains plates that have been saved to the database.
4. Double-click the appropriate folder to display all the plates and their associated information (name, date created, creator, and barcode (if applicable)) that are contained in the folder in lower pane.
5. In the lower pane, select the plate (use Shift+click or CTRL+click to select multiple plates) that contains the images to delete, click **Select**, and then click **OK**.
6. In the **Plate Data Utilities** message, click **OK** to delete all the images from the selected data sets.

Note: This step does not remove the images from the database or 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 Plates

To delete plates from the database, you must first delete them using the **Delete Plates** option. After you use the **Delete Plates** option, you must then 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 can 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 deletes related images and measurements.

To delete a plate

1. Click **Screening > Plate Data Utilities**.

   In the simplified menu structure, click **Screening > Plate 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**.
6. In the **Plate Data Utilities** message, click **OK** to delete all the selected plates.

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

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