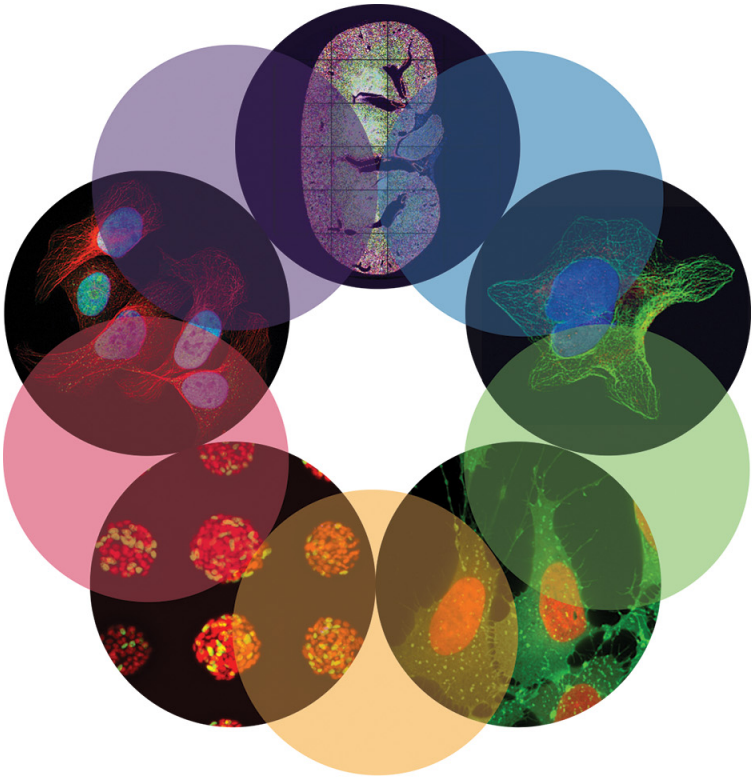

# MetaXpress®

## High Content Image Acquisition & Analysis Software

Version 6.1

## Analysis Guide

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September 2015

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® Ultra Confocal High Content Screening System. Both user guides are provided on the MetaXpress Software installation media and are available in the Molecular Devices knowledge base at http://www.moleculardevices.com/support.
- **Analysis** consists of selecting, measuring, assessing, and managing acquired images and plate data.

This guide describes the general analysis workflow:

## 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 4000 Q TRAP system 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.chroma.com
    - http://www.semrock.com
    - http://www.omegafilters.com

# Before You Begin Your Analysis

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

- **Plate Specifications**

  How many wells are on the plate?

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

- **Wells**

  Were images acquired from all wells?

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

- **Sites**

  How many sites were acquired for each well?

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

- **Wavelengths**

  How many wavelengths and which specific wavelengths were acquired?

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

- **Images**

  What is the total number of images acquired on the plate?

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

- **Settings**

  What unique analysis settings do you need to make?

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

- **Expected Results**

  What are the anticipated results of your experiment?

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

- **Data Log Measurement Selections**

  Which measurements are the most appropriate to select for logging?

  Most application modules create two different types of logs:

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

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

# Selecting and Analyzing Plates

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The first step in the image analysis process is to select the plate in the database that contains the images that are to be analyzed. You can then view and arrange the images in a variety of ways to examine and compare them to decide which wells in the plage 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.

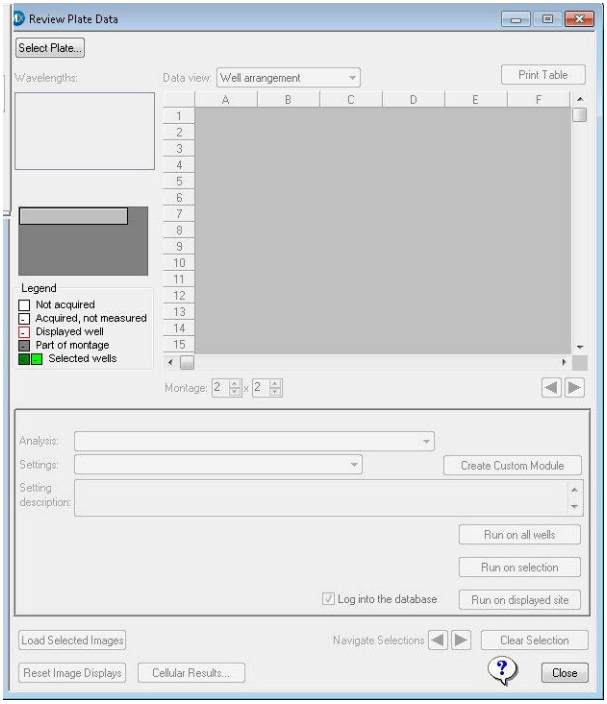
Settings made in application module dialogs (described in Chapter 6: Configuring Application Modules on page 55) define characteristics specific to the selected application module. These application module settings can be saved and reused.

This chapter includes the following topics:

## Selecting a Plate for Analysis

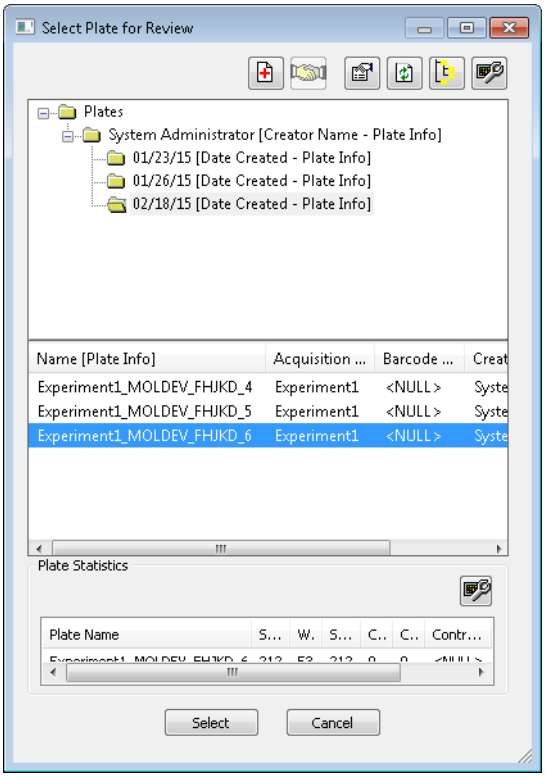
**Note:** For information about using the **Review Plate Data** dialog, see the video: https://www.youtube.com/watch?v=ks4UKc8vq-A.

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 sub-folders 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 sub-folders and plates, click the **Configure Branches** button in the upper right corner of the dialog.

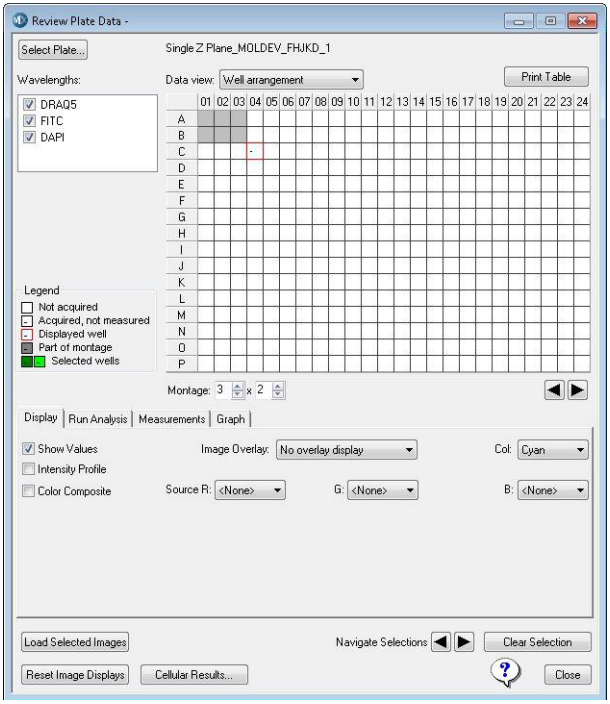



4. Double-click a sub-folder in the top pane to display its contents in the middle pane.
5. In the middle pane, select a plate, and then click **Select**.

   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.

**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.

6. Click the **Display** tab, and then continue to Viewing and Arranging Images on page 14.

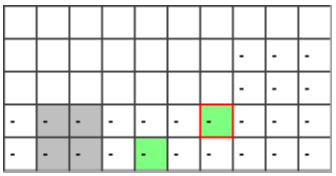



## Viewing and Arranging Images

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 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 any or all of the following on the **Display** tab as needed to assist in the viewing and arranging of images. See:
   - Selecting wells for the Montage window/viewing well images in a Montage window on page 15.
   - Viewing and arranging images for multi-site wells on page 17.
   - Generating a 3-D intensity profile graph on page 18.
   - Assigning colors to acquisition wavelengths on page 18.
   - Creating a composite image on page 18.
   - Scaling 16-bit color composite images on page 19.
   - Auto scaling of images for display on page 19.
   - Viewing time point data on page 21.
   - Viewing Z series images on page 25.
   - Collating the images of multiple wells on page 29.

## Selecting wells for the Montage window/viewing well images in a Montage window

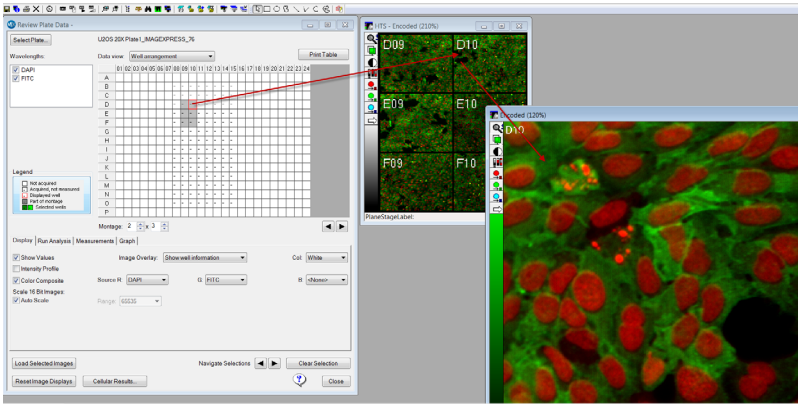
You can view a thumbnail for each well in a plate in a Montage window and compare the overall density and distribution of the sample material in each well. Samples showing an overall high density and even distribution are typically better candidates for analysis than wells with very low sample density and uneven distribution. To select a well or wells for displaying in the Montage window, do the 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 not only outlined in red but it is also 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:

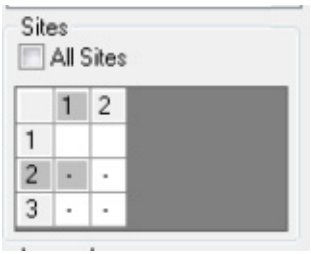
- Click on another well in the Plate map to indicate the top left well in the new group.
- Use the left or right arrow beneath the lower right corner of the Plate map to move the group of selected wells.

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 graphic below, 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.

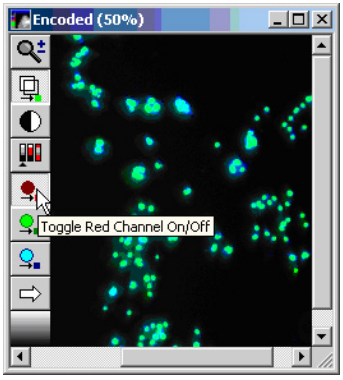
## Assigning colors to acquisition wavelengths

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 will be 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).

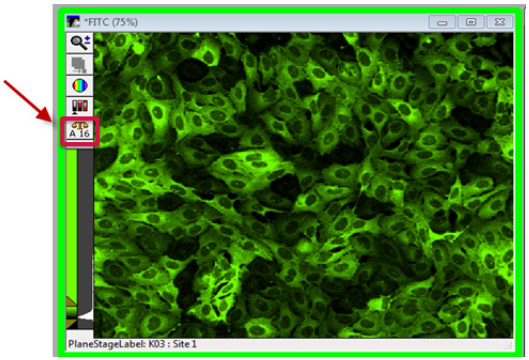
## Scaling 16-bit color composite images

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

**Note:** **Intensity Profile** must be selected for scaling. To automatically scale 16-bit color composite images, select Auto Scale.

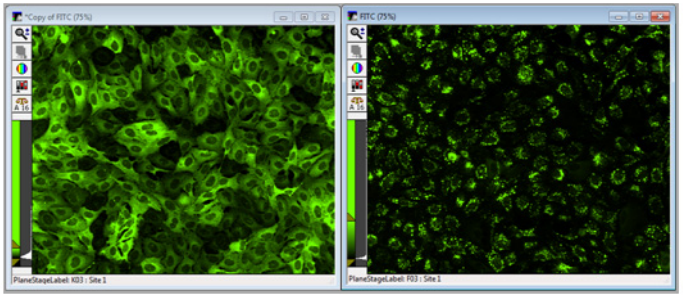
## Auto scaling of images for display

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 (usually 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.



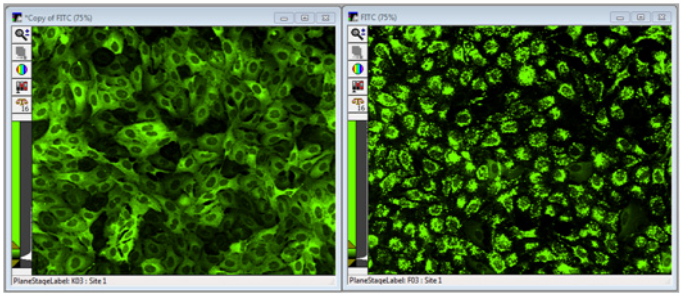

When comparing different objects between multiple images, it is not helpful to have Auto Scale turned on, as both bright and dim samples appear with the same brightness.

**Auto Scale On**



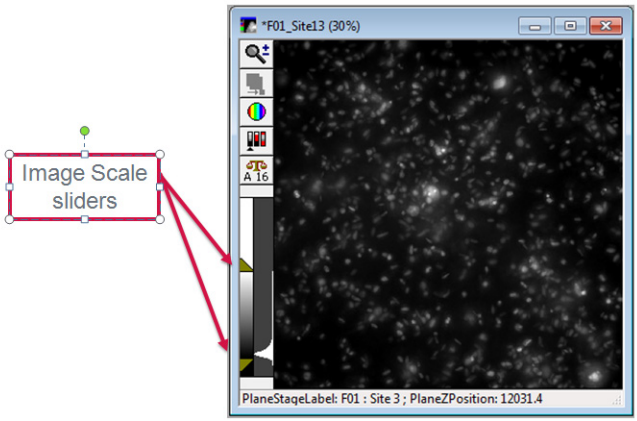
If Auto Scale is turned off, only then do the objects or images appear with their true intensity levels.

**Auto Scale Off**



When comparing the intensity levels of objects across multiple images, you should 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:

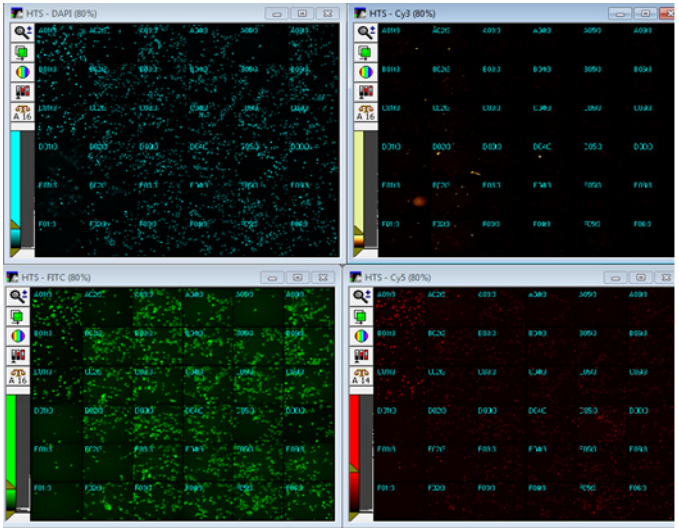
- View a thumbnail montage of multiple wells at a single time point. See To view a thumbnail montage of multiple wells at a single time point on page 22.
- View multiple wells at multiple time points. See To view multiple wells at multiple time points on page 22.
- View high resolution images from selected wells/time points. See To view high resolution images from selected wells/time points on page 23.
- 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 24.

### 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/viewing well images in a Montage window on page 15.

**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.



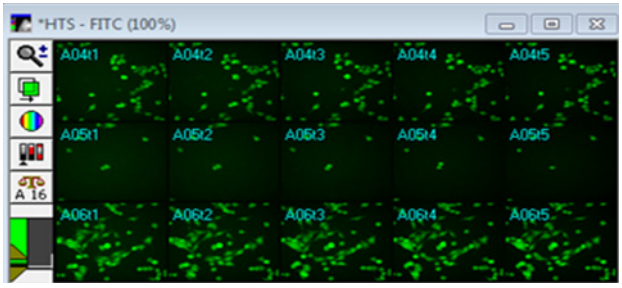

### To view multiple wells at multiple time points

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/viewing well images in a Montage window on page 15.

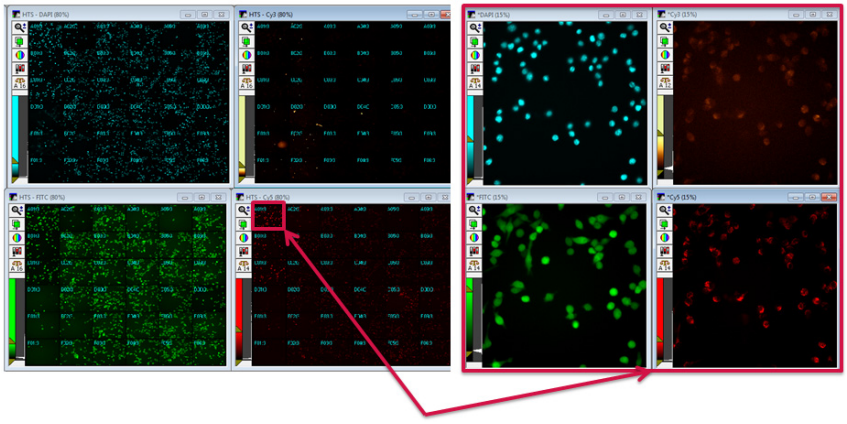
**Note:** You might need to use the scroll bars in the Sites graphic to scroll to a site to select it.

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/time points

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

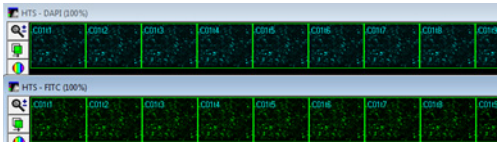


### To view a stack (.stk) file of a series of time points from one site/well

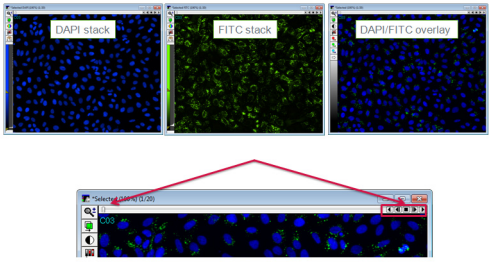
1. In the **Data view** field, select **Time Point vs Well** to change the layout of the Plate map. (The time points are displayed in the "X" direction and the well IDs are displayed in the "Y" direction.)
2. Do one of the following:
   - If only a single site per well was acquired, then right-click 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 remove all selected wells from the stack, click **Clear Selection**.



4. After you have created a stack file of a series of time points 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.



## Viewing Z series images

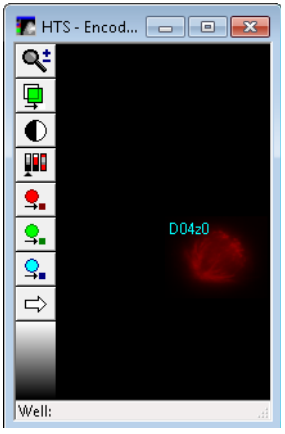
If you have acquired Z series images for experiments, then multiple options are available for viewing this data. You can:

- 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 26.
- 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 26.
- 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/well on page 27.
- View high resolution, single Z step images. See To view high resolution, single Z step images on page 28.

### 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/viewing well images in a Montage window on page 15.

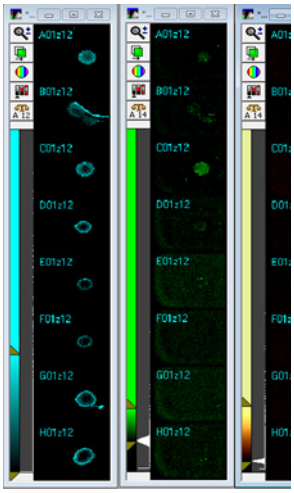
   The well ID/2D projection for each selected well—always labeled as well ID/z0—is displayed in the upper left corner of each thumbnail image in the Montage window, for example, D04z0.



### 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/viewing well images in a Montage window on page 15.

   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.

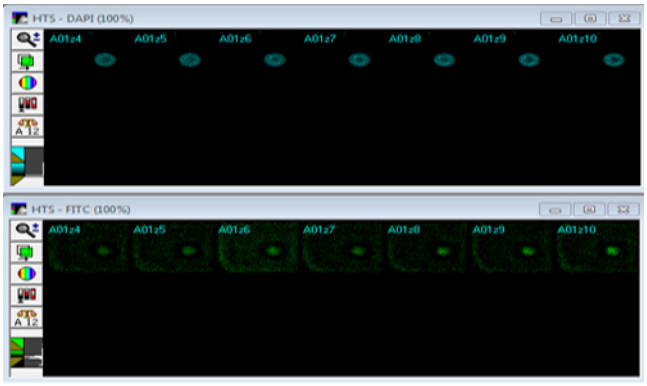



### To view multiple Z steps from a single well or multiple wells

1. In the **Data view** field, select **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:
   - To select a single well, click and drag your cursor across the well's Z Steps Plate map. 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 figure on the next page, 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.

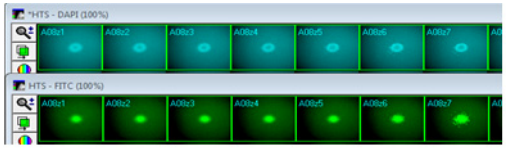



### To view a stack (.stk) file of a series of Z steps from one site/well

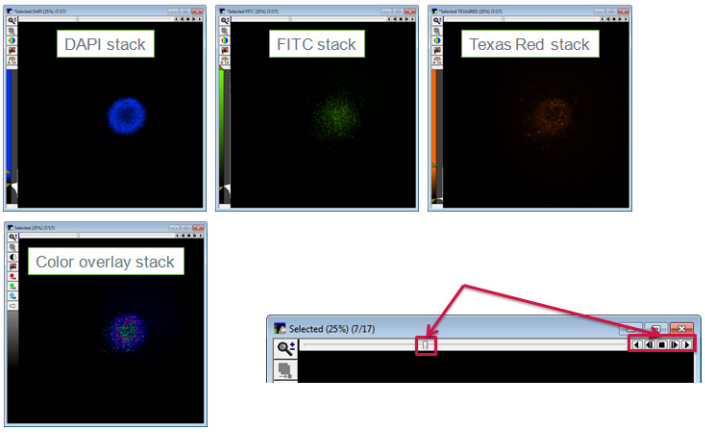
1. In the **Data view** field, select **Time Point vs Well** to change the layout of the Plate map. (The time points are displayed in the "X" direction and the well IDs are displayed in the "Y" direction.)
2. Do one of the following:
   - If only a single site per well was acquired, then right-click 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/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 remove all selected wells from the stack, click **Clear Selection**.

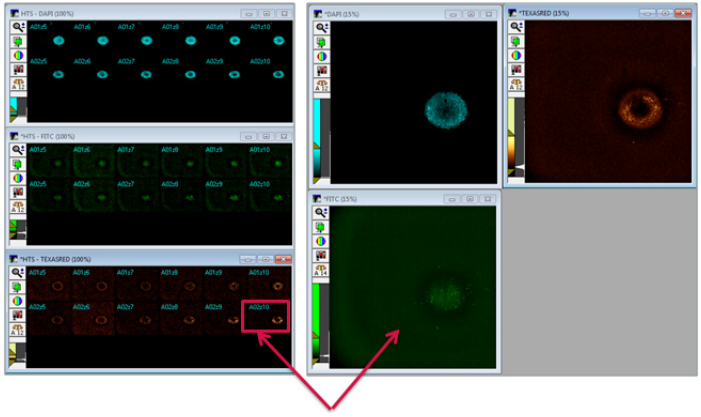


4. After you have created a stack file of a series of Z steps from one site/well, you can use the image slider or the playback controls at the top of a stack window to scroll through the images in the stack.




### To view high resolution, single Z step images

Click on 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 remove all selections from the stack, click **Clear**.

## 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.

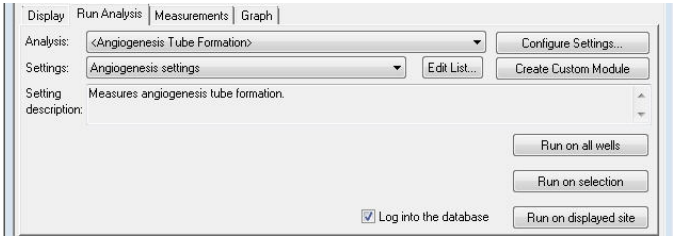
The following functions on the **Display** tab are applicable for these wells: **Load Selected Images** and **Navigate Selections**. The **Run on selection** function on the **Run Analysis** tab is also applicable for these wells.

## Running an Analysis

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

You use the **Run Analysis** tab to select the application module, custom module, or journal to run, and then run the analysis. The first time that you run a selected analysis, you must create the appropriate settings file. Otherwise, you can select from a list of available settings files.

1. In the **Analysis** field on the **Run Analysis** tab of the **Review Plate Data** dialog, select an application module, a custom module, or a journal.




2. On the **Settings** drop-down list, select the appropriate settings file.

**Note:** You must make sure to select a file that has the appropriate settings based on the wavelengths that were used to acquire the plate. If this is the first time that you are running a selected analysis, then you must first create the appropriate settings file. See Chapter 6: Configuring Application Modules on page 55 and Chapter 7: About Custom Modules on page 71.

3. Optionally, to log the measurement data from your analysis into the database, select **Log into the database**.

4. If you a 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`.

- 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 timelapse journal that analyzes planes in a stack as separate time points.
- If the plate contains Z data, then select the 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 time plane, 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 33 for more information.

# 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. Please 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 as well as 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.

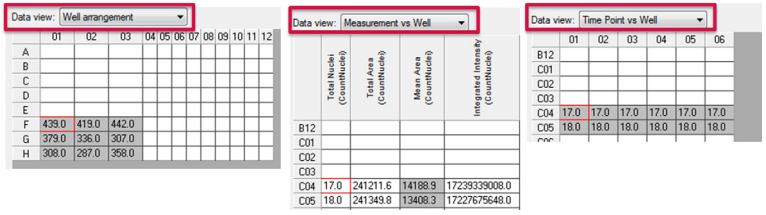
1. On the Display tab of the Review Plate Data dialog, click **Show Values**.
2. Open the **Measurements** tab, and then do the following:
   - On the **Analysis** drop-down list, select the appropriate analysis.
   - On the **Measurement** drop-down list, select the appropriate measurement.

**Note:** Measurements that are preceded by "Cell:" are cell-by cell data. To view cell-by cell data for the selected site/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 timelapse data or Z series data, then there are special considerations. See Viewing analysis results for timelapse experiments or Z step experiments on page 35.

4. Continue to any of the following as needed for viewing and evaluating your data:
   - Using the Cellular Results Table on page 36.
   - Filtering Data Analysis Values on page 38.
   - Generating a Heat Map for Analysis Results on page 39.
   - Viewing Image Overlay Information on page 42.
   - Exporting Selected Data on page 43.
   - Graphing Analysis Results on page 47.

## Viewing analysis results for timelapse experiments or Z step experiments

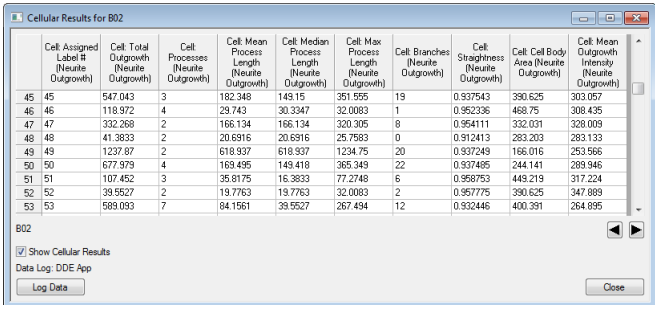
If you are viewing analysis results for timelapse experiments, or Z step experiments, then make note of the following:

- For timelapse 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 performed 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**.



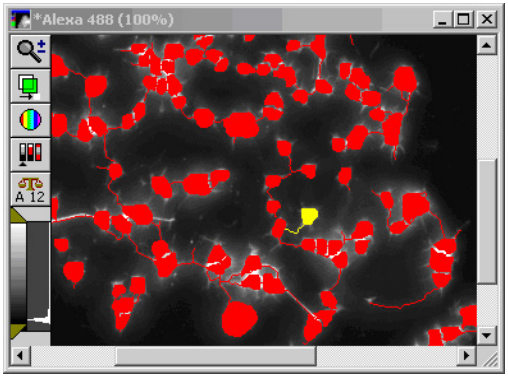

3. Do one or both of the following as needed to review the cellular data:
   - Select one of the segmented cells in the image to highlight the corresponding data for the cell in the **Cellular Results** table.

**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 42.




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

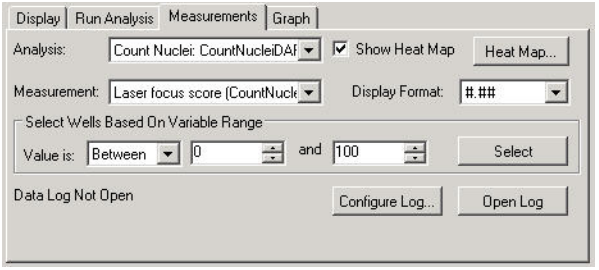
**Note:** If you clear **Show Cellular Results**, but later want to view the **Cellular Results** table when running an application module, click **Window > Show Cellular Results**. In the simplified menu, click **Measure > Show Cellular Results**.

## 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.

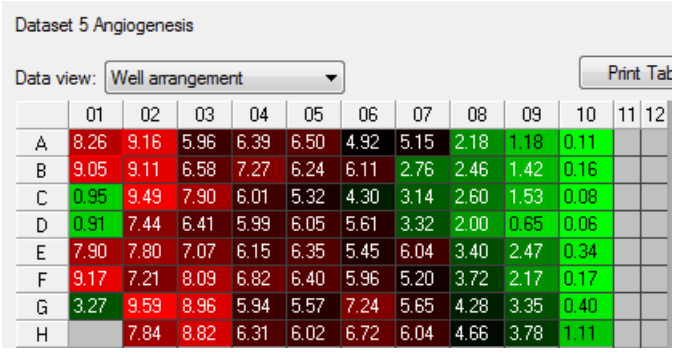
**Note:** If a heat map is enabled, selected wells on the grid cannot be distinguished; however, the selected wells are outlined in green on the thumbnail montage.

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 43.

## 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 of the measurement values on a linear scale. The lowest (“cold”) value is green, while the highest (“warm”) value is red. Measurements between the low and high ends are displayed with colors that correspond to the relative position of the values on the scale. You can distribute the data on a linear or logarithmic scale, set low and high thresholds, and change the color scheme.



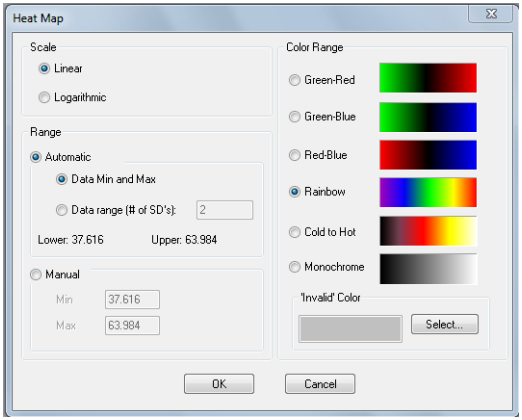

| | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 8.26 | 9.16 | 5.96 | 6.39 | 6.50 | 4.92 | 5.15 | 2.18 | 1.18 | 0.11 | | |
| B | 9.05 | 9.11 | 6.58 | 7.27 | 6.24 | 6.11 | 2.76 | 2.46 | 1.42 | 0.16 | | |
| C | 0.95 | 9.49 | 7.90 | 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 any or all 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).<br><br>**Note:** The logarithmic scale presents a very wide range of data on a more manageable scale. The scale is particularly 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:<br>• To use the full range of data, select **Data Min and Max**.<br>• To have the MetaXpress Software distribute the data evenly below and above the mean value by a specific number of standard deviations, select **Data range (# of SD's),** and then type a number of standard deviations. This option calculates the range of data using the following formulas:<br>◆ Minimum Value = Mean - (# Standard Deviations X Standard Deviation)<br>◆ Maximum Value = Mean + (# Standard Deviations X Standard Deviation)<br>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 then in the dialog that opens, click a color and then click **OK**.

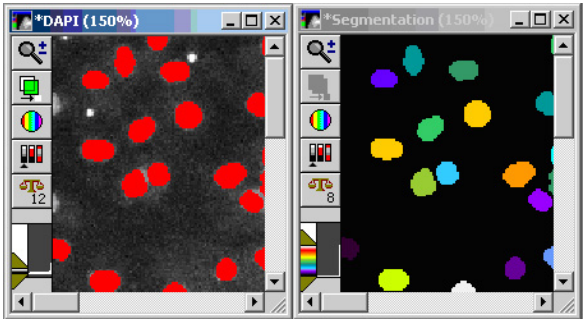
4. Click **Close** to close the **Heat Map** dialog and update the Heat Map display.

## 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 timelapse 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 selected **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 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 exactly 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.

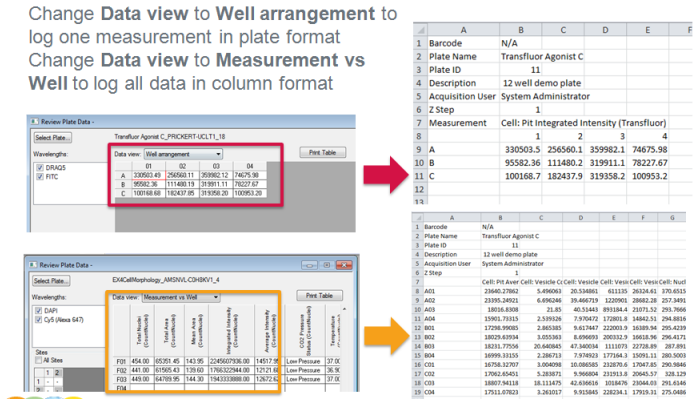
**Note:** Alternatively, you can use the **Export Measurements** option on the **Plate Data Utilities** dialog to export selected data of one or more measurement sets to a text file. For more information, see Exporting Measurements on page 89.

See:

- To export data to an Excel spreadsheet.
- To export data to a text (.log) file on page 44.
- To export cell-by-cell data to text (.log) file on page 45.

### 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 **Dynamic Data Exchange**.
4. In the Export Log Data dialog box, do the following:
   - On the **Application** drop-down list, select **Microsoft Excel**.
   - Enter any of the following information: **Sheet name**, **Starting Row**, and **Starting Column**.
5. Click **OK** to close the **Export Log Data** dialog box 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 needed 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, you should 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, enter 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 needed 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 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 simply 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, enter 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**.

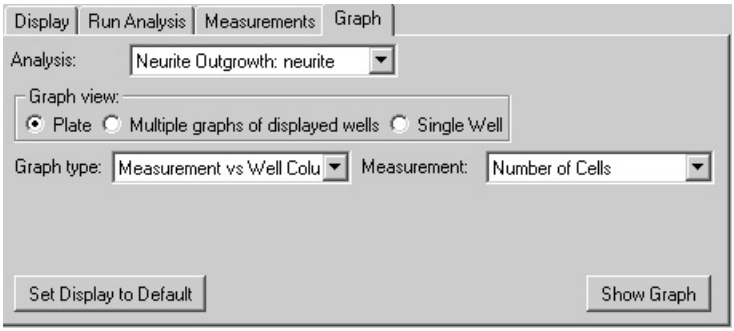
**Note:** You can repeat Step 5 as many times as needed to export different measurement set data for the same analysis. Every time that you click the **Log Data** button, the newly exported data is displayed below the previously exported data. As long as the connection between the log file and the MetaXpress Software is maintained, any newly exported data will 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

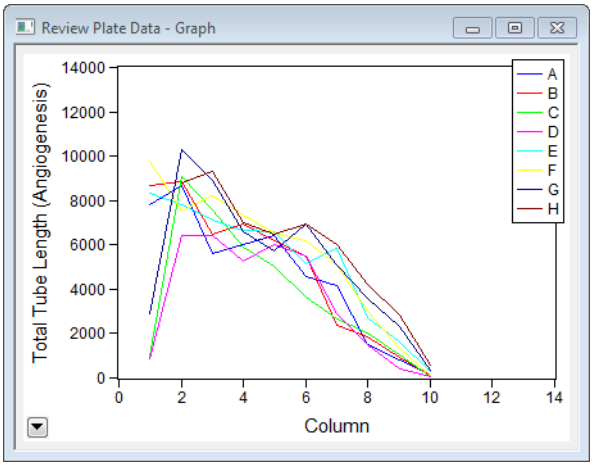
To configure a graph to display your data, use the following procedure:

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 measurement(s) 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 bin(s) 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.

# About 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.

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

- within the Review Plate Data dialog (see Chapter 6: Configuring Application Modules on page 55)
- as a stand-alone analysis using an option on the Screening menu (see the application Help)
- as part of a custom module (see Chapter 7: About Custom Modules on page 71 for an overview and the application Help for details)
- within a custom journal (see the application Help)
- in a queue of automated analyses (see Chapter 9: Batch Analysis on page 81)
- automatically in conjunction with the plate acquisition process (see Chapter 5: About Application Modules on page 49)

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

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

**Note:** You can create your own custom module by combining a variety of image processing and segmentation steps in the Custom Module Editor. These steps can include the application modules described in this manual. For a brief introduction to custom modules, see Chapter 7: About Custom Modules on page 71. For detailed information about creating custom modules, see the help provided in the Custom Module Editor. You can open the Custom Module Editor from the Run Analysis tab in the Review Plate Data dialog.

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

**Table 5-1:** Application Modules

| Assay Type | Description | Recommended Application Modules |
|---|---|---|
| Protein Localization/ Translocation | Typically a measurement of co-localization. The protein of interest is labeled or bound to by a labeled antibody and used with other probes specific to cell types, organelles, or cytoskeletal structures. The assay determines how much area or intensity of the protein-of-interest co-localizes with another probe. | • Translocation<br>• Translocation-Enhanced<br>• Multi Wavelength Translocation<br>• Nuclear Translocation HT<br>• Custom module |
| Cell Proliferation | Typically a count of the cells or nuclei in an image. | • Count Nuclei<br>• Multi Wavelength Cell Scoring<br>• Cell Proliferation HT |

**Table 5-1:** Application Modules (cont'd)

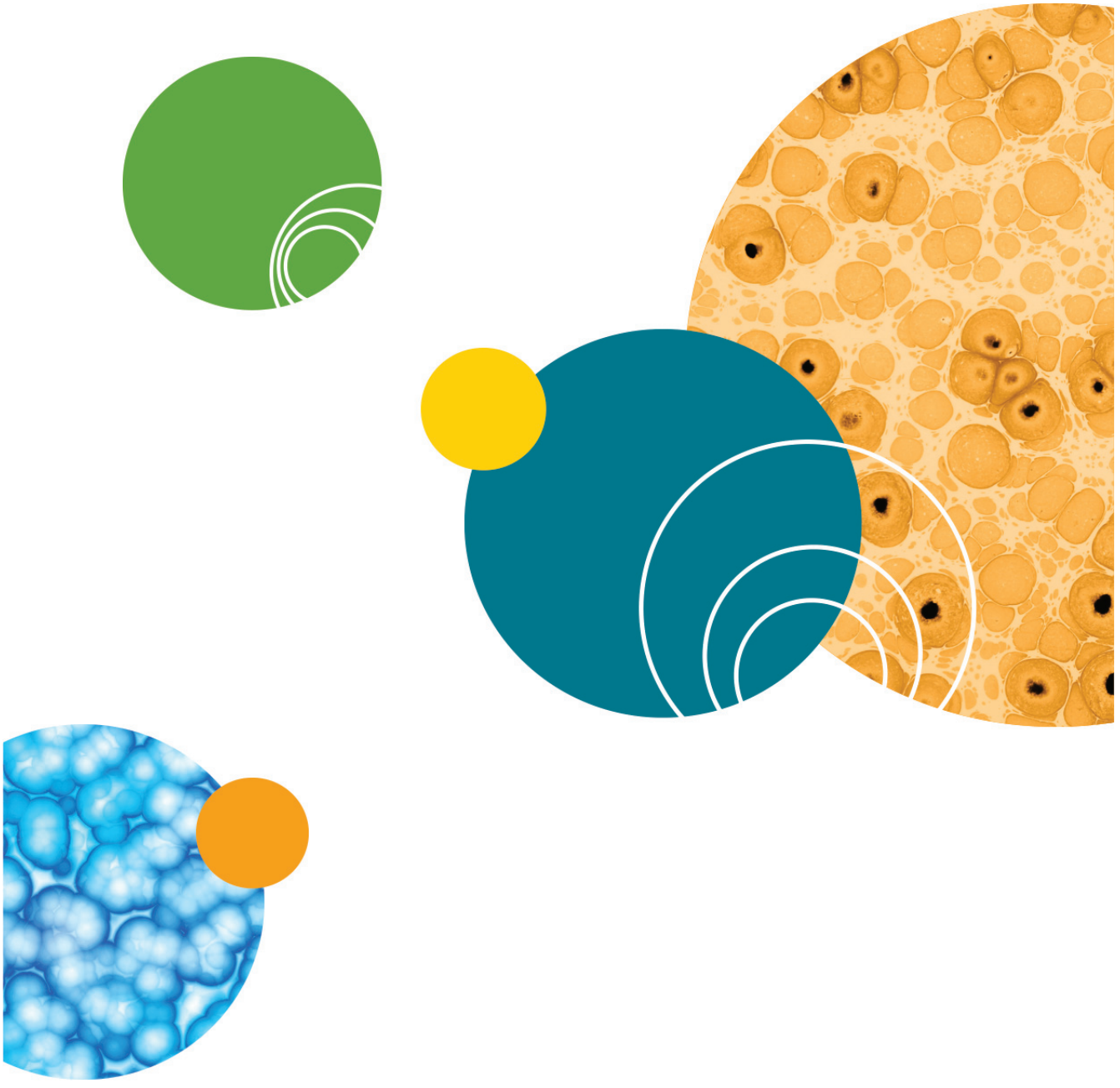
| Assay Type | Description | Recommended Application Modules |
|---|---|---|
| Cell Viability/Apoptosis | Typically a count of objects having specific characteristics. The cells are rounded up (shape and area), the cells label or do not label with a specific probe (intensity and count), or specific proteins localize to a sub cellular compartment (intensity and count); for example, the mitochondria. | • Live/Dead for a two-wavelength assay<br>• Cell Health for a three-color assay, such as DAPI, Annexin, or Propidium Iodide (PI) |
| Receptor Internalization and other Punctuate Staining | Usually measured by a probe moving to coated pits or vesicles, such as in the Transfluor assay for GPCR activation. Count and measure labeled pits or vesicles, or other punctate staining. | • Granularity<br>• Transfluor<br>• Transfluor HT<br>• Custom module |
| Angiogenesis | Typically an area measurement. Either the length of tubules is measured or the creation of holes in a cell monolayer is measured. | • Angiogenesis |
| Cell Physiology (Calcium/pH) | Almost always involves measurements of intensity. Typically a probe is used that changes its fluorescence intensity using one or two wavelengths under different physiological conditions.<br>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<br>• Multi Wavelength Cell Scoring<br>• Custom module |

**Table 5-1:** Application Modules (cont'd)

| Assay Type | Description | Recommended Application Modules |
|---|---|---|
| Kinase Activity Assays | Generally involve measuring the phosphorylated epitope of the kinase by measuring fluorescence intensity. This value should be normalized to the number of cells expressing the kinase – a counting measurement. | • Cell Scoring<br>• 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<br>• Custom module |
| Cell Cycle | Typically involves the classification and counting of cells in specified stages of the cell cycle and analyzing the distribution of cells within these classes in response to compounds. | • Cell Cycle for detailed classification using one to three wavelengths — from a single nuclear stain to combinations including optional mitosis-specific and/or apoptosis-specific stains.<br>• 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.<br>• Monopole Detection for specific analysis of spindle formation and the disruption of centrosome separation. |

**Table 5-1:** Application Modules (cont'd)

| Assay Type | Description | Recommended Application Modules |
|---|---|---|
| Polynucleation and Genotoxicity | Typically with micronuclei, which are small nuclei produced during cell division by a lagging chromosome fragment or an entire chromosome and their induction is a highly quantitative measurement of chromosomal damage. | • Micronuclei for genotoxicity by detection of micronuclei in populations of mono-, bi- and multi-nucleated cells. Can also be used for detection of yeast budding.<br>• Custom module |



# Configuring Application Modules

6

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

This chapter includes the following topics:



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

## Basic Setup Procedure for Application Modules

For best results, calibrate the source images in all application modules in microns. You must also 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 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.

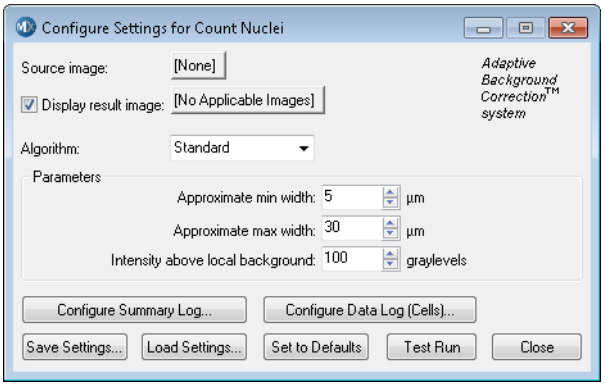
## Selecting the source image, result images, and algorithm

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

   In the simplified menu structure, click **Screening > Review Plate**.
2. Open the plate and images that you want to analyze as described in Chapter 3: Selecting and Analyzing Plates on page 11.
3. In the **Review Plate Data** dialog, click the **Run Analysis** tab.
4. From the **Analysis** drop-down list, select an application module.

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

5. Click **Configure Settings**.




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

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

**Note:** The appropriate type of source images varies depending on the application module used. For more information about source images, view the application help for the module you are using.

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

**Note:** There are two ways to view the results of each application module: Displaying a result image or using an image overlay. Selecting Display result image will open or overwrite (see Step 8) a new image depicting what was measured. Using an image overlay creates an overlay on the main image. You can toggle the image overlay on or off with the Show/Hide overlay button on the side of the image window.

**Note:** To display a result image, **Display result image** and an image must be selected. If **Display result image** is selected, but an image is not selected, then you receive an error if you try to run the application module.

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

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

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

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

10. For a journal, select **Specified** and give the image an appropriate name, such as **Result**.
11. If the application module provides an Algorithm option, select either **Standard** or **Fast**. The algorithm option determines how quickly the analysis will be performed. Both algorithms produce similar but not exactly identical results. If you select the Fast algorithm, then in the Intensity above local background field enter approximately half of the value that you calculate in Calculating the intensity above background value on page 62.

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

## Determining object widths

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

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

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

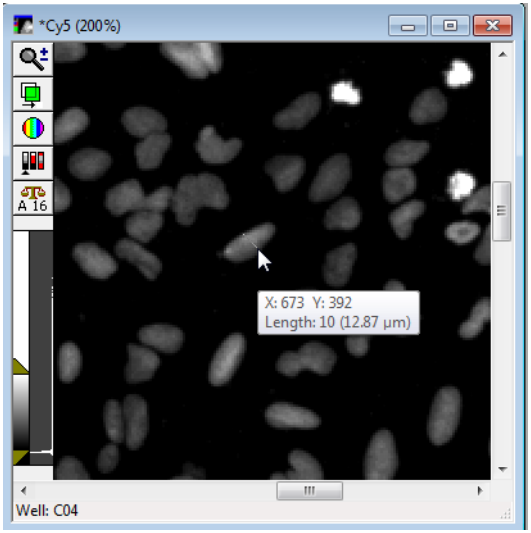
1. If the **Region** toolbar is not open, click **Regions > Region Tools**.

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

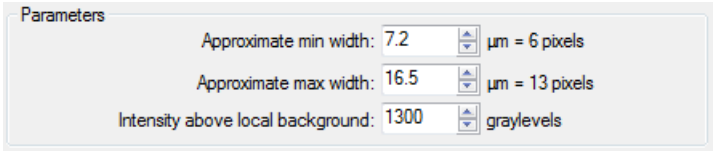

2. Click the **Line** tool.
3. Click on one of the edges of the largest object that you want to include in your analysis.

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

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




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




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

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

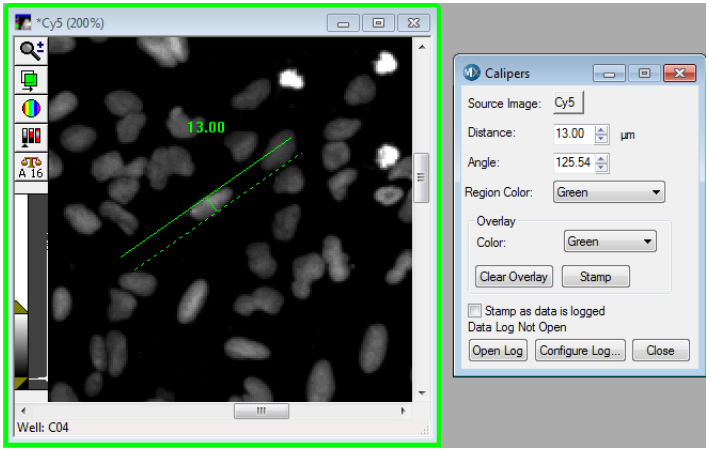
**Note:** The caliper feature is a drop-in option and might need to be loaded into the MetaXpress Software. If the option is not present on the **Measure > Distance** menu (simplified menu) or the **Measure** menu (default menu), please contact your System Administrator or Technical Support to have this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

1. Click **Measure > Calipers**.

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

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

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




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

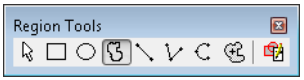
   The size of the line is displayed in the image and in the Caliper dialog.

6. In the **Configure Settings** dialog of the application module, in the **Approximate max width** field, type the width of the largest object that you want to include your analysis.
7. Repeat Step 3 through Step 5 for the smallest object that you want to include in your analysis and type its width in the **Approximate min width** field.

## Measuring the area of an object

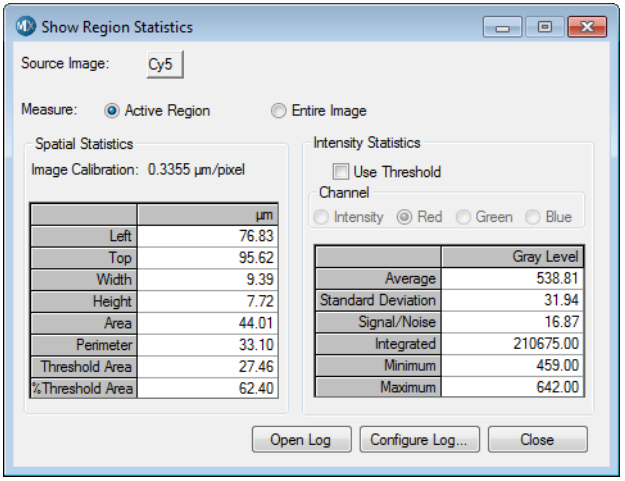
1. If the **Region** toolbar is not open, click **Regions > Region Tools**.

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




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

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



## Calculating the intensity above background value

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

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

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

2. Select the **Arrow** tool as shown in the following figure.


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

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


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

**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 online help for more information about this tool.

## Testing and saving settings

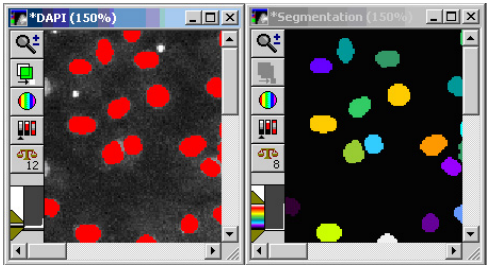
After the module is configured, you should test the settings, make adjustments if needed, and then save the settings.

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

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

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

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

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

**Note:** Segmentation overlay takes a significant amount of space in the database and Molecular Devices does not recommend to save it for large screens.

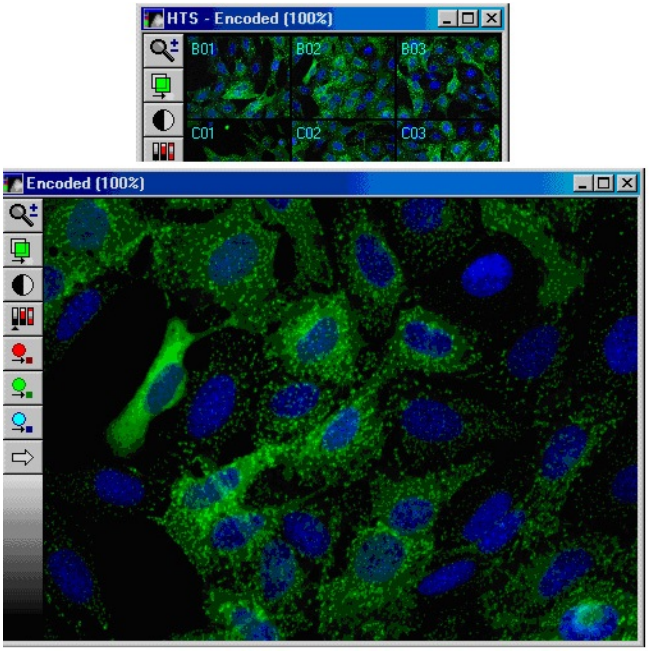
6. Click **Configure Data Log** to configure the data log if applicable.
7. Click **Save Settings**.
8. Enter a name and description for the settings in the **Save Settings** dialog and click **OK**.

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

## Example: The Transfluor Application Module

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

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

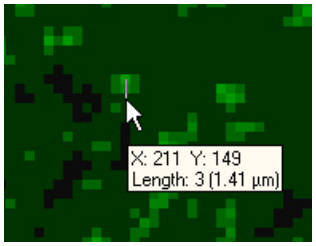



3. In the **Review Plate Data** dialog, click the **Run Analysis** tab.
4. Select **<Transfluor>** from the **Analysis** field.
5. Click **Configure Settings**.
6. In the **Configure Settings for Transfluor** dialog, click **Pits and Vesicle image**, and then select the wavelength image representing the pits and vesicles.
7. Click on the pits and vesicles image, and then increase the zoom by clicking the magnifying glass icon on the image window or by pressing **Page Up** on the keyboard.

8. Use the **Single Line** tool of the **Region** toolbar to measure a granule width by following these steps:
   - If the **Region** toolbar is not open, click **Regions > Region Tools**.
     In the simplified menu structure, click **Measure > Regions > Region Tools**.
   - From the **Region Tools** toolbar, select the **Single Line** tool.


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

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




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

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

11. Position the cursor over the dimmest pit and read the gray-level value.

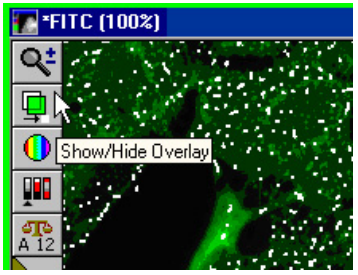
    The gray-level value is the number that is displayed at the middle of the bottom of the screen after the right arrow. In the example below it is 162.


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

**Note:** Vesicles are much larger and brighter than pits.

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

    The pits and vesicles segmentation appears as an overlay on top of the image.
18. Use the **Show/Hide Overlay** tool on the toolbar to toggle the results on or off to make sure all the pits are detected.



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

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

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

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

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

    - Click **Run on displayed site** to run the assay for the currently selected site.
29. After the analysis is run, view the results in the Cellular Results table, on the **Measurements** tab, or on the **Graph** tab. See Chapter 4: Viewing Analysis Results on page 33.

## Cell-by-cell Multiplexing with Application Modules

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

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

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

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

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

# About Custom Modules

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

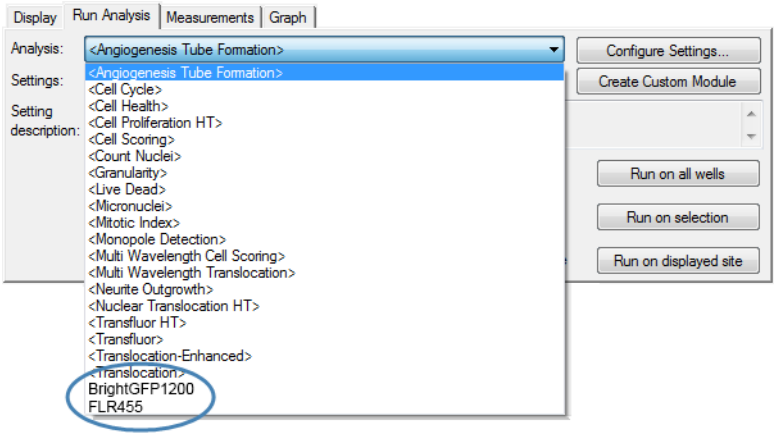
For example, you might want to remove noise, invert an image, increase intensity levels, extract objects of interest, shrink or remove objects, and then measure the objects in images acquired with different channels. A custom module makes this type of complex, repetitive task simple to perform. You can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

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

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

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

Application modules are enclosed in brackets in the list. Items that are not enclosed in brackets can be either custom modules or journals. For example, the following list includes two custom modules.




## To add a custom module to the database

In the Custom Module Editor, a custom module can be saved as an XML file so that it can be provided to other users for use with their copies of the MetaXpress Software. To use a custom module that has been transferred from another computer, you must add it to the list by following these steps:

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

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

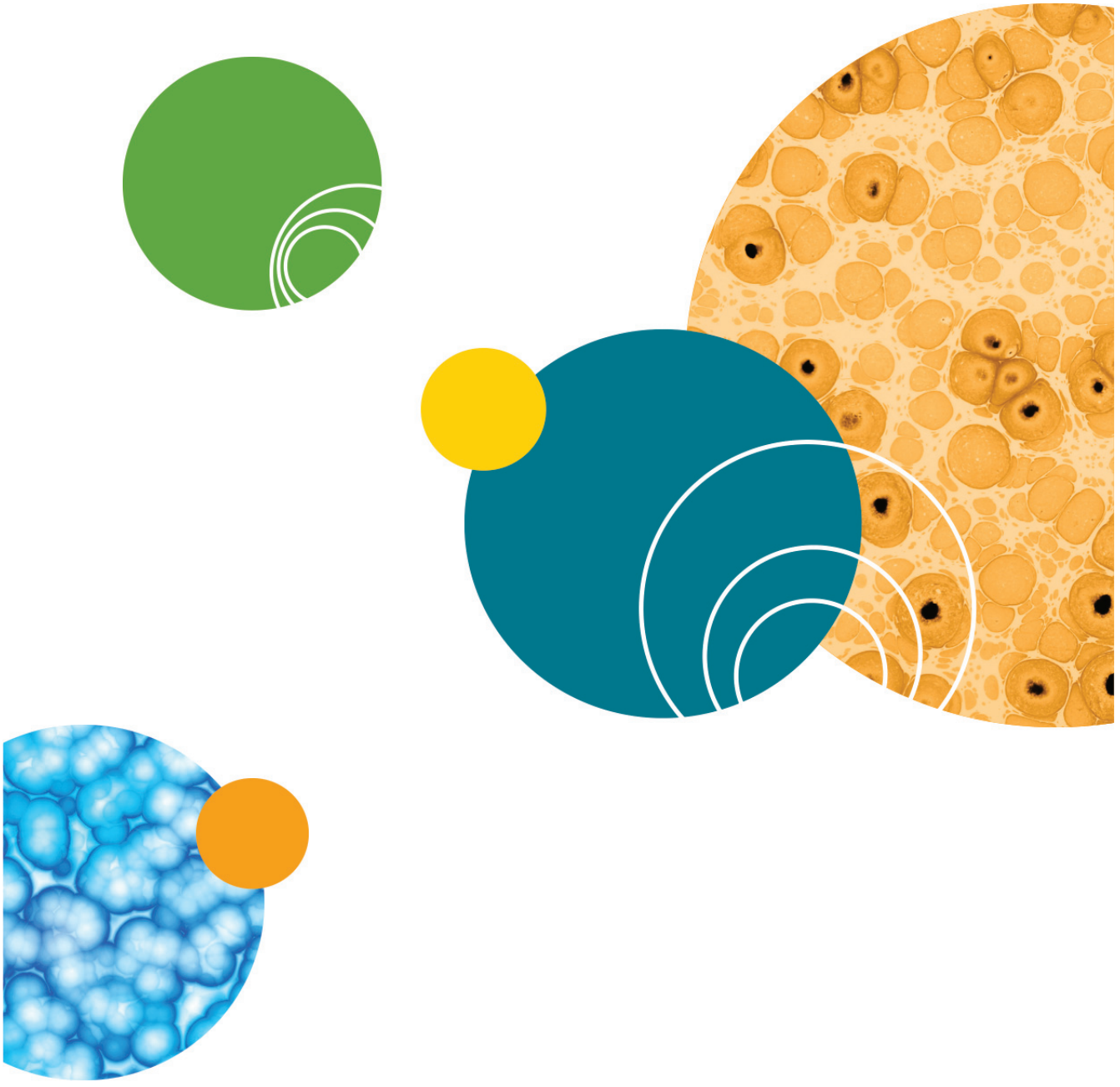
The custom module is appended to the list of analyses on the Run Analysis tab.

### To remove a custom module from the list of analyses

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

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

   In the simplified menu structure, click **Screening > Review Plate**.
2. On the **Run Analysis** tab, in the **Analysis** field, select the custom module.
3. Click **Edit List**.
4. Delete each setting associated with the custom module.
5. After you delete the last setting, click **Yes** to confirm that you want to remove the custom module from the list of analyses.



# Automating and Monitoring an Analysis

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

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

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

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

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

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

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

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

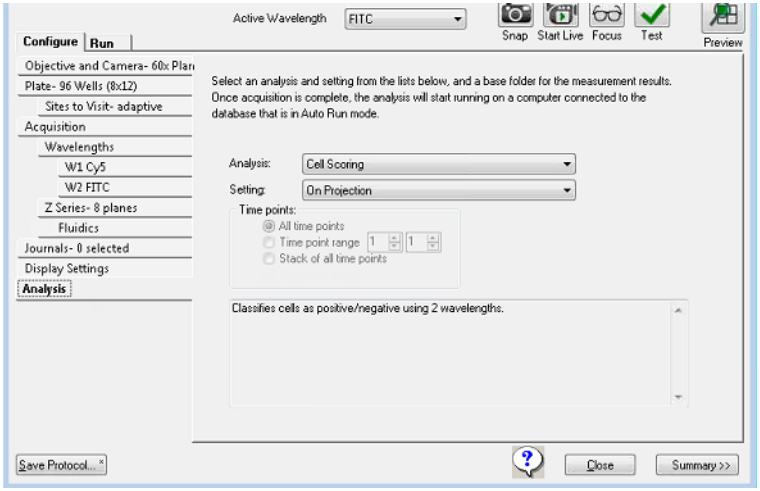
To choose a specific analysis to run on a data set after the acquisition is complete, select **Analyze Images After Acquisition** on the **Configure** tab on the P**late Acquisition Setup** dialog, and then specify the analysis options on the **Analysis** tab. The data set will be added to the Auto Run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

**Note:** Only analyses that have defined settings are listed.

3. On the **Setting** field, 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.

## Initiating an Automated Analysis

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

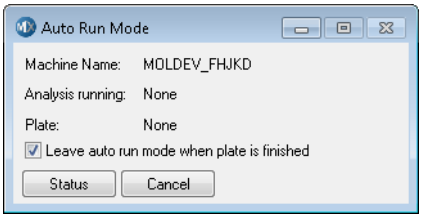
Having separate computers to acquire and analyze your screening data greatly reduces the overall screening time by freeing up the main MetaXpress Software system to continue acquisition. You can also set up more than one MetaXpress Software computer to run in Auto Run Mode, further reducing the time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

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

To set up and use Auto Run Mode:

1. On the computer that is acquiring images, start the MetaXpress Software.
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. In the **Analysis** field, 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 computers 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 the **Leave auto run mode when plate is finished** check box. If the check box is selected, only one plate is analyzed.

   Select this option if you want to use the system for other tasks and do not want the MetaXpress Software to start analyzing the next plate once it is finished with the one it is currently analyzing. Note that while in Auto Run Mode, all other MetaXpress Software options are disabled.

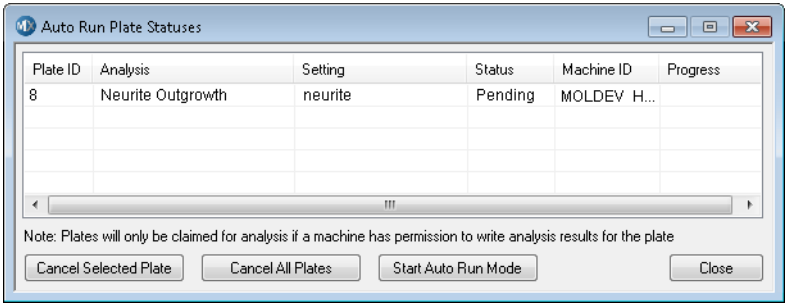
9. After the analysis is completed for all plates, click **Cancel** to close the **Auto Run Mode** dialog.

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

## Monitoring the Status of Automated Analyses

1. On the computer running the analysis, 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, these plates will not be claimed by computers in Auto Run Mode.

The following statuses are possible:

- **Running** indicates that the analysis is currently running on the plate. After the analysis is completed for the plate, the plate will be removed from the auto run plate status list.
- **Timeout** indicates that the analysis has not completed on a well or site in the expected time. The maximum time allowed for analysis is set in the MDCStoreTools™ Data Management Utility with the Set Auto Run Timeout option. A timeout is normally caused by an error on the machine running the analysis. To diagnose the cause of the timeout, inspect the machine that has timed out for error messages or other problems. In some cases the problem can be resolved and the analysis can continue. If this happens, the status will return to Running. In other cases, the Auto Run must be canceled and the analyses run again. Some analyses, particularly custom modules or journal analyses, take a long time to complete. In this case, increase the timeout value set for the Set Auto Run Timeout option to allow enough time to run the analysis.
- **Pending** indicates the analysis has not yet started on the plate.
- **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. To stop running an analysis on a plate, select the plate from the table in the **Auto Run Plate Statuses** dialog, and click **Cancel Selected Plate**.
3. To stop running the analysis on all plates, click **Cancel All Plates**.

# Batch Analysis

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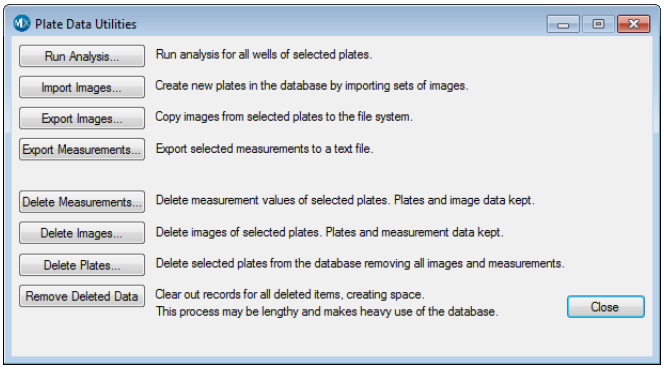
Although the MetaXpress® Software provides features for automating analyses in conjunction with image acquisition, you can use the **Plate Data Utilities** dialog to manually initiate an analysis on more than one plate at a time.

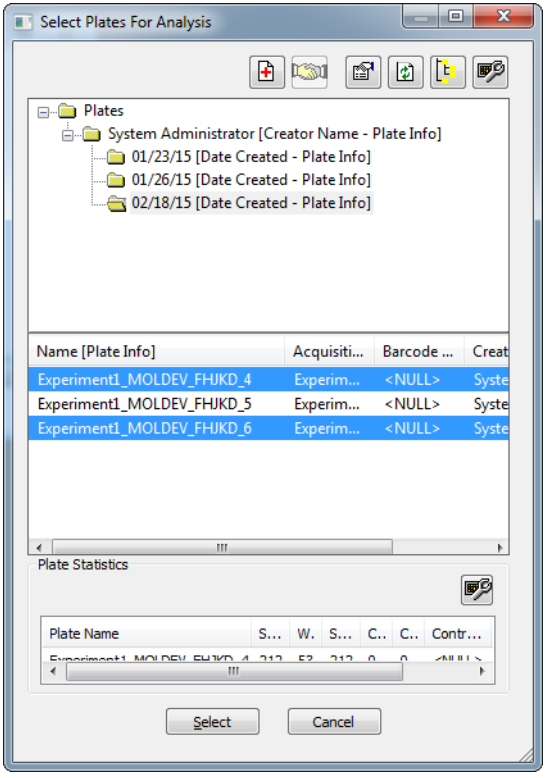
1. Click **Screening > Plate Data Utilities**.

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



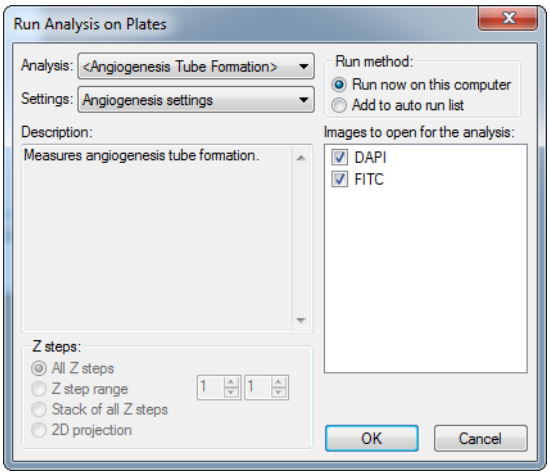

2. In the **Plate Data Utilities** dialog, click **Run Analysis**.

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, from the **Analysis** list, select the assay to run.




7. From the **Settings** 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 **From the Images to open for the analysis** 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 analyses the time points 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 timelapse journal that analyzes planes in a stack as separate time points from the **Analysis** list.
11. If the plate contains Z data, then select the 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.
    - **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

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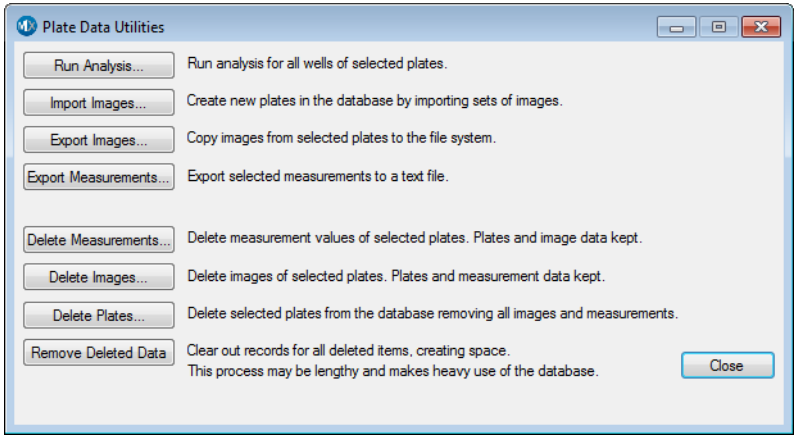
Use the Plate Data Utilities dialog to manage images and data acquired during plate acquisition:




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

This chapter includes the following topics:



For information about the Run Analysis option on the Plate Data Utilities dialog, see Chapter 9: Batch Analysis on page 81.

## Importing Images

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

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

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

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

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

6. Click **Import**.

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

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

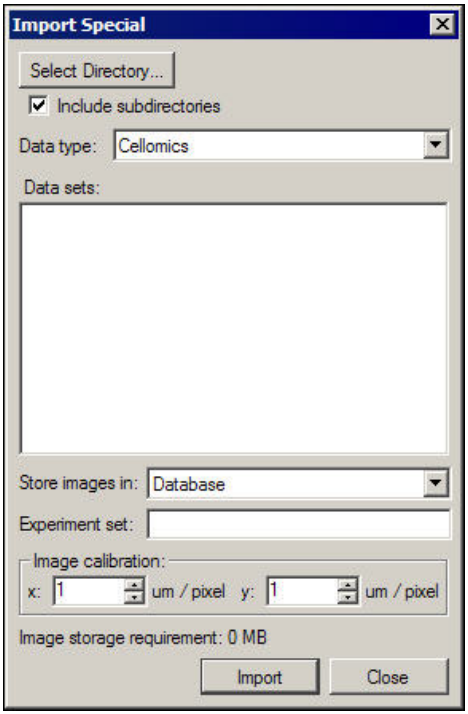
## Importing Cellomics Data

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

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

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

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




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

   The path is displayed next to the Select Directory button and the Cellomics files are displayed in the Data sets field.
4. Select the check box next to the Cellomics file set that you want to import.
5. Select a location (either the database or an available network folder) to import the Cellomics data to from the **Store images in** drop-down field.

6. Enter a calibration ratio (µm per pixel) for the **x** and **y** values of the imported images in the **Image calibration** section. The default calibration is 1 µm = 1 pixel.
7. Click **Import**.

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

## Exporting Measurements

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

When using the wizard, keep in mind the following:

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

### To construct a query

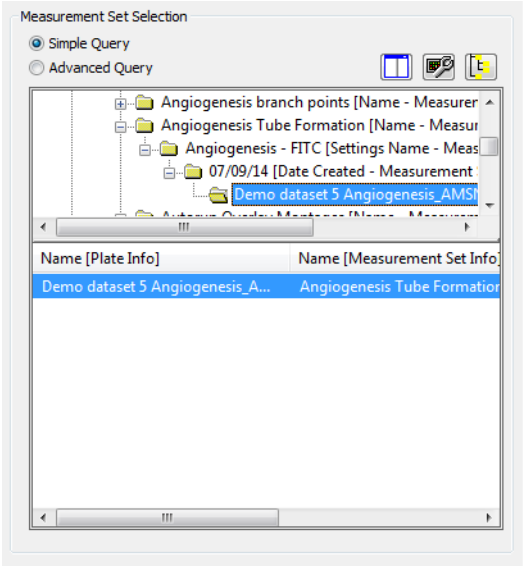
Two methods for constructing a query are available: simple and advanced. With either method, on the Step 1 page of the wizard, you select or specify the measurement sets to export and then, on the Step 2 page, you select the type of data to export and filter that data. The main difference between the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

- Simple Query - On the Step 1 page, select the measurement sets from a list.
- Advanced Query - On the Step 1 page, specify the attribute criteria that identify the measurement sets (for example, measurement sets that contain data for plates acquired on a certain date). To use this method, you must know some of the unique attributes that identify the measurement sets containing data that you want to export (for example, the date created, creator name, or name, or the plate information contained in the measurement set such as date annotated or global ID).

1. 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 that you want to export: cell measurements, image measurements, or both and then click **OK**.
4. On the **Export Measurements Wizard - Step 1** page, select **Simple Query** and proceed to Step 5 or select **Advanced Query** and proceed to Step 8.
5. To construct a simple query, in the top left pane, double-click the measurement set folder that contains the data that you want to export.

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



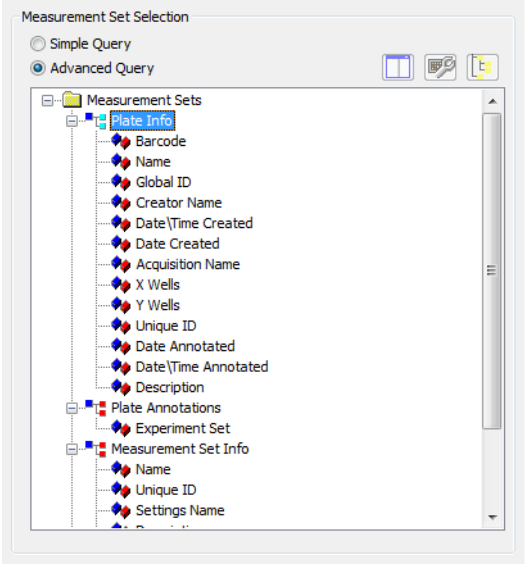

The attributes are displayed for informational purposes only to help you identify the measurement sets. As described in Step 11, you will select the data types to export on the next page of the wizard.

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

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

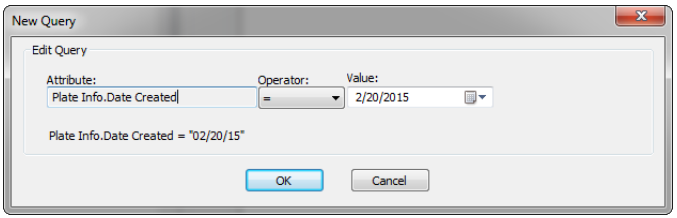
**Tip:** To quickly add all measurement sets to the lower left pane, double-click the top-level Measurement Set folder in the top left pane. Then, in the lower left pane, use Ctrl-click or Shift-click to select measurement sets to add to the query.

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



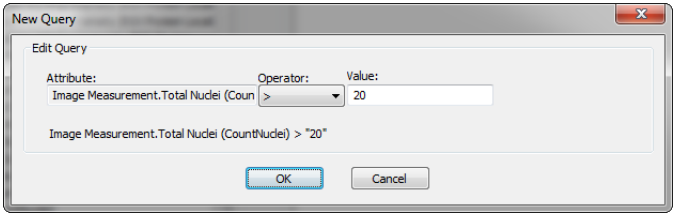

9. Double-click the attribute that you want to use to identify the measurement sets for the query and, in the New Query dialog, select an operator and type a value to filter the data that will be exported.

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




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

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




13. Click **OK**.

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

### To configure the data for export

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

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

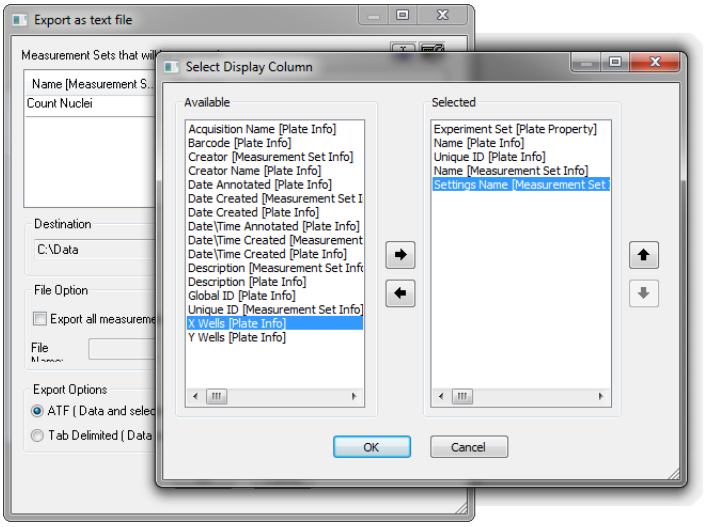
**Note:** To resize the width of the columns, drag the column headings or right-click and select Fit Columns. To sort the data in a column in ascending or descending order, click once in the column heading. To reorder the items in the right panes, click the up and down arrows.

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

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

   The Export as text file dialog that appears lists the measurement sets that contain the data to be exported. By default, the data for each measurement set is saved as a separate text file with a system-generated file name. By default, the data for each measurement set is saved as a separate text file with a system-generated file name.
5. To change the name of the system-generated file name, select the file name in the list, and then click the **Edit File Name** button  .
6. To combine the measurement data for all measurement sets into one text file, select **Export all measurements to one file** and type a file name.

7. To include information in the header of the text file such as the plate name, date the measurement set was created, or the plate description, click the **Configure Columns** button  . In the dialog that appears, use Ctrl-click or Shift-click to select columns, and then click an arrow between the panes to move the columns.




8. Click **OK**.

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

9. To create a separate text file that contains the computer name, destination folder, number of files, and file names related to the export, click **Save Summary**.

10. Click **Close**.

## Exporting Images

To export images to a local or networked folder:

1. 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. Select the plate to export from the lower pane and click **Select**.
6. In the **Browse for Folder** dialog, navigate to the local or networked folder to export the images to and click **OK**.

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

## Deleting Plates

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

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

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**.

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

## Deleting Images

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

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

To delete images from a plate:

1. 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 containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Select the plate containing the images to delete from the lower pane and click **Select** and then click **OK**.

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

## Deleting Measurements

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

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

1. 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 folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Use Ctrl+click or Shift+click to select the plates containing the measurements to delete from the lower pane and click **Select**.

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

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