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Chapter 1
Introduction

The MetaXpress software workflow is divided into two major parts — acquisition and analysis. The acquisition workflow involves configuring settings, acquiring images, and storing plate data in a database. The analysis workflow discussed in this manual consists of processing, enhancing, and analyzing acquired plate data. Analysis can be performed using the following methods:

**Auto Run** — automatically start application modules or custom journals to run on plates as soon as they are acquired. This can be done over a network using dedicated analysis machines or locally.

**Manual** — Manually run application modules or custom journals on plate data residing in your MDCStore database and/or Fileserver.

**Notes:**

- You can also use Molecular Devices Corporation’s (MDC) AcuityXpress cellular informatics software to further analysis your data.
- For more information about the MetaXpress acquisition workflow, refer to the Acquisition guide for your MDC hardware platform.

The following topics are discussed in this guide:

- Identifying and using Online Help, PDF Documentation, Web-based Documentation, and Support resources.
- Determining the image quality requirements for your experiments
- Viewing your images
- Viewing different wavelengths in one image or multiple images
- Changing the display options of an image
- Using MetaXpress Application Module procedures to:
  - Define application module settings
  - Run application modules
  - Review plate data
  - Store plate data into your database
  - Export data to Excel/text file
- Importing images into the database
**Documentation Conventions**

Before you begin using the MetaXpress system, familiarize yourself with the stylistic conventions used in this manual:

<table>
<thead>
<tr>
<th><strong>Bold Type</strong></th>
<th>Indicates a chapter or section heading, or is used for emphasis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Courier font</strong></td>
<td>Indicates the name of a file or folder, the output of command, or text that you must type.</td>
</tr>
<tr>
<td><strong>Italic Type</strong></td>
<td>Indicates the name of a command or field, or text from a field, within a dialog box.</td>
</tr>
</tbody>
</table>

**Obtaining Support**

Part of effective communication with MDC is determining the channels of support for MetaXpress. MDC provides a wide range of support materials that should be your first step when troubleshooting problems. Please complete the following steps in order when attempting to resolve any MetaXpress issues:

1. Consult the Documentation — Check the manuals that shipped with the system, as well as the online help available within the MetaXpress application. Online help for an active dialog box can be accessed by pressing the <F1> key. In the Help window, click **See Also** to view and choose from a list of related topics.


3. Explore the Molecular Devices Support website — The support site, located at [http://www.moleculardevices.com/pages/support.html](http://www.moleculardevices.com/pages/support.html) has links to technical notes, software upgrades and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Request Form. Refer to the section, *Gathering Support Information*, for what information to include in the email.

4. Internet Support — Fill out the Technical Support Request Form at [http://www.moleculardevices.com/cgi-bin/support_request.cgi](http://www.moleculardevices.com/cgi-bin/support_request.cgi) to send an email to a pool of technical support representatives. Refer to the section, *Gathering Support Information*, for what information to include in the email.

5. Call Customer Service — You can contact MDC’s Customer Service department at (800)-635-5577 (U.S. only) or +1 408-747-1700. Please have the system ID number, system serial number, software version number and the system owner’s name available when you call.

Additional support resources include the following:

- Nikon web-based microscopy course — [www.microscopyu.com](http://www.microscopyu.com)
- The Molecular Probes handbook— [probes.invitrogen.com](http://probes.invitrogen.com) offers advice on fluorescent probes and help determining if there are better stains available for your analysis.
The following sites offer filter information:

- [www.chroma.com](http://www.chroma.com)
- [www.semrock.com](http://www.semrock.com)
- [www.omegafilters.com](http://www.omegafilters.com)
Chapter 2
Defining Analysis Criteria

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

**Plate Specifications – How many wells 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. Factors like this 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. Since 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, the potential exists for creating 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 is linked to a settings file that you can access from the application module dialog box. After you store your settings, you can recall and reuse these settings at any time. Saving and reusing your settings enables you to streamline your experiment workflow and ensure a high level of consistency and accuracy.
View Characteristics – What viewing characteristics do you want?
The viewing characteristics that you choose on the Review Plate Data Display tab enable you to enhance the appearance of up to three wavelengths. You can view these wavelengths in separate image windows or you can click Color Composite to combine the images into a single overlay, composite image. You can also choose to display the well number on the image as well as the value that is shown for each well or site on the table.

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 MetaXpress application modules create two different types of logs: a Summary log and a Data log. 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 also referred to as cell-by-cell measurements. These measurements are logged for each individual cell in the image that has been identified by the software. 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.
Chapter 3
Determining Analysis Workflow

Analyzing image data in MetaXpress involves using several dialog boxes and toolbars in sequence to create a workflow. Most of these dialog boxes are located in the Screening and Apps menus. This chapter provides an overview of using these tools to configure analysis and explains the common conventions used by the MetaXpress application modules.

MetaXpress application modules contain assays designed to automate analysis procedures such as segmentation and cell scoring. Application modules, along with user-defined journals and manual analysis, make up the methods available create an effective workflow. Refer to Chapter 6, Selecting Application Modules, for more information.

Note that after you design a workflow, you can further automate the process by creating custom MetaXpress tool bars and task bars. For more information on custom MetaXpress tool bars and task bars, refer to the Acquisition Guide for your hardware platform.

Getting Started
The most logical place to begin the analysis process is to locate and select the plate datasets in your database that you want to analyze. You can start this process in one of two available dialog boxes: Plate Data Utilities or Review Plate Data. In both cases, your ultimate goal is to open the Plate Dialog, from which you can select the plate(s).

Recommended procedure to determine analysis settings
Complete the following procedure to determine the best settings to analyze your plate image data:

Note: This procedure is a broad overview and assumes you know how to open images from the Review Plate Data dialog box. For instructions on using the Review Plate Data dialog box, refer to Chapter 5, Preparing for Analysis.

1. Using the Review Plate Data dialog box, locate the plates in your database containing the image data to analyze.
2. Load the plate that you want to examine.
3. View acquired images using the Review Plate Data dialog box.
4. Set viewing characteristics to improve the visibility of information.
5. Determine the analysis process to use – prepared application modules or custom journals.
6. Determine the settings that are needed. Refer to Chapter 7, Performing Manual Analysis for specific instructions on performing manual analysis for the purpose of running a trial analysis.
7. Make the initial settings.
8. Test the settings by running the analysis on selected wells.
9. Examine and evaluate the initial results.
10. Adjust settings to correct for inaccurate results.
11. Repeat process until good results are obtained.
12. Test an entire plate and examine and evaluate the results.
13. Prepare to run automated analysis.
14. Refer to Chapter 8, *Performing Automated Analysis* for the procedure to run automated analysis.

**Application Module Conventions**

Most MetaXpress application modules share similar configuration steps, such as selecting images to process or determining an object’s width. An understanding of these similar steps helps when learning to use different application modules. The following capabilities and conventions are shared across most of the MetaXpress application modules:

- **Principles of Segmentation** – Image segmentation is the process of spatially partitioning an image into meaningful objects. Generally for fluorescent imaging, objects are bright and are segmented from a darker background. Additionally, segmentation may include automatic splitting of touching cells or objects to give a more accurate count.

- **Adaptive Background Correction™ (ABC)** – The system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and image analysis repeatability. The ABC process is used solely for defining object boundaries and is not used to modify reported intensity measurements. Intensity measurements such as Average Intensity and Integrated Intensity come directly from the original pixel data unless otherwise indicated.

- **Note:** Some modules, such as Translocation-Enhanced, indicate that an overall constant background intensity value can be uniformly subtracted from all pixel intensities during measurement. However, this uniform subtraction is completely independent of the ABC segmentation process.

- **Size Parameters** – The modules all require that the user supply an approximate size or range of sizes for the desired object detection. These values help to fine-tune automated algorithms that analyze objects according to the biology of the application. Appropriate values can be determined using the software’s interactive Region Tools applied directly to the image being analyzed.

- **Intensity Parameters** – The modules all require that the user supply an *Intensity above local background* detection parameter. This parameter specifies the minimum intensity level needed to detect the presence of a fluorescent stain and can be used to adjust the sensitivity of the detection. Since the ABC system adjusts for uneven background, these values are taken locally for each object.
detected, relative to local background levels, rather than on a global absolute basis.

**Note**: For detailed instructions on configuring application modules, refer to Chapter 7, *Performing Manual Analysis*.

## Setting Application Module Parameters

Before you can use a MetaXpress application module effectively, you must make settings in the application module dialog box based on the properties of the image or images that you are analyzing.

Depending on the dialog box, most of the modules have three to five major areas that contain controls. The following settings are used in most or all of the MetaXpress application module dialog boxes.

- **Stained area** – This drop-down list box enables you to choose whether you are applying your measurement to only the nucleus, only the cytoplasm, or both.

- **Approximate minimum width** – This measurement specifies the smallest object that you want to detect.

- **Approximate maximum width** – This measurement specifies the largest object that you want to detect.

- **Intensity above local background** – This value is used to adjust the sensitivity of detection to effectively delineate objects from the background. Since even background values within an image are common, the software automatically accounts for unevenness using the ABC system and compares intensities relative to local background rather than a single global value.
Chapter 4
Determining Settings for Analysis

Settings for analysis are made in the following dialog boxes:

- Review Plate Data dialog box
- Application module dialog boxes

Settings made in the Review Plate Data dialog box define the overall characteristics of your analysis. These settings are used to adjust the view of existing data and to select data for analysis. They do not affect image analysis or other measurements and cannot be saved and reused.

Settings made in application module dialog boxes define setting characteristics specific to the selected application module. These settings can be saved and reused.

Application module dialog boxes can be accessed either through the Review Plate Data dialog box, or directly through the Apps menu. When you begin to make your settings, determine whether you will be modifying an existing group of settings or creating completely new settings.

**Settings in the Review Plate Data Dialog Box**

You can configure settings in the Review Plate Data dialog box only after you have selected a plate for analysis. Refer to and complete the procedure in Chapter 5, *Preparing for Analysis* for selecting and loading a plate from your database.

Settings on the Review Plate Data dialog box are primarily made on the four tabbed areas of the dialog box. Since settings made in the Review Plate Data dialog box are simply for looking at the data, they cannot be stored in a setting file to be used again in the future.

From the *Run Analysis* tab of the Review Plate Data dialog box, you can select and access individual application module dialog boxes. Settings for application module dialog boxes can be saved to a settings file that is specifically used by that application module and can be saved and recalled for future use.

The following section lists and describes each of the available dialog box options on the main dialog area and the four tabs of the Review Plate Data dialog box. You should become familiar with these settings and their use before beginning an analysis.
Review Plate Data Dialog Box Options – Main

Figure 4-1
Review Plate Data dialog box – Analyzed plate open

Select Plate
Opens the Plate Dialog box. Use this dialog box to select Plate Data Sets of stored microwell plates from the database for viewing. To perform other operations, such as deleting plates or individual images, use the Plate Data Utilities dialog box.

Data view
Selects and indicates the well arrangement as shown in the Microplate Selection Grid and the Image Montage window.

Well arrangement – Organizes your viewable images in the montage in the layout in which the wells are presented in the microplate.
**Determining Settings For Analysis**

*Time vs well* – Compares timepoints against wells.

*Well vs measurement* – Compares wells against the available measurements.

**Print**
Prints the data in the table on the selected windows printer.

**Wavelengths**
Determines whether or not images from available wavelength will be displayed.

**Sites**
Selects display of one or all the sites in each selected well in your experiment. Available sites are indicated by a dash. Click on any available site to view only that site for all selected wells in the thumbnail view. Click *All Sites* to view all sites for all selected wells. If you run an Analysis on wells with sites, the values displayed in the table refer to the selected sites or if "All Sites" is selected, the average for the total number of sites in the well.

**All Sites**
Specifies that all sites for all wells are to be included in each thumbnail image, and that each image in the thumbnail will be represented as an individual image in the image selection grid for the Montage. However, the number of sites shown in both the thumbnails in the Montage and in the image viewer also depends on the Montage dimensions that you specify. When this box is checked, the sites in the well are included in the Montage dimensions. For example, if each well has four sites, and the Montage dimension is 1X1, only the upper left site in well A01 is shown; if you change the dimension to 2X1, the two upper sites are shown. Only when the dimension is set to 4X4 are all four sites in A01 shown. If this box is not checked, only a single, selected site is shown in the Montage, and each selection box in the image selection grid represents all sites for each well. Therefore, when not checked, when you select a different site in the Sites box, both the thumbnail in the montage and the image in the image window will be updated.

**[Microwell Plate Selection Grid]**
Indicates the wells containing image data, the images included in the montage area, and the images selected for display.

**Montage**
Specifies the dimension in image thumbnails of the Image Montage window. **Note:** Image Montage windows are labeled as HTS- followed by the name of the stain or wavelength that you assigned to the wavelength. For example, HTS-DAPI or HTS-FITC.

**Time point**
Specifies the number of timepoints that you want to display and/or include in the image montage.

**Selections (In Green)**
Controls the selection and loading of images that you selected in the table. Select images by right-clicking on an image selection box, or by right-clicking on the associated image in the thumbnail view.

**Load Image(s)**
Loads the images you selected in the table into a stack for each wavelength, or a single stack if Color Composite View is selected. Each of the three available data views, *Well Arrangement*, *Time vs well*, and *Measurement vs Well*, can be selected and used to generate an image stack, providing three different types of image stack results.

**(Arrow Buttons)**
Changes the displayed selected image to the previous or next selection (Selections [In Green]).

**Clear**
Clears all Selections from the table.

**Reset Image Displays**
Resets the view settings in all image displays.

**Cellular Results**
Opens the Cellular Results dialog box. Use this dialog box to view and browse available analysis measurements. These measurements are configured in the Configure Settings>Configure Data Log (Cells)
dialog box.

**Note:** The results shown are those from the analysis selected on the Measurements tab.

**Close**
Closes the Review Plate Data (DB) dialog box.

### Reviewing Plate Data
To view and analyze images from a plate acquisition, complete the following procedure:

1. From the Screening menu, click *Review Plate Data*. The Review Plate Data dialog box opens.
2. Click *Select Plate*. The Plate dialog box opens, and the Review Screen Data dialog box temporarily closes.
3. Expand the plates folder in the top pane of the dialog box to view folders containing plates saved in the database.
4. Double-click a folder to view its contents in the bottom pane.
5. Select the plate containing the data to view and click *OK*. The Plate dialog box closes, and the Review Screen Data dialog box reopens.
6. In the *Wavelengths* box, if the check boxes for the selected wavelengths are already checked when you load the plate data, click the check box next to each wavelength to initially inhibit the display of images for each wavelength. Next, complete the remaining steps for this procedure. Then, re-check the boxes for each wavelength to display the associated images that you want to view.
7. In the *Data view* box, choose the type of view that you want to use:
   - *Well arrangement* – Arranges your viewable images in the montage in the order in which the wells are presented in the microwell plate.
   - *Time vs well* – Compares timepoints against wells or well selections.
   - *Well vs measurement* – Compares wells against the available measurements.
8. Using the table features, define the wells that you want to view. You can view "thumbnails" in a montage of images and select the size of the thumbnail images. You can select images from the Montage and view each image separately in an image window. You can select images from anywhere in the table, and load the images into a stack.
   - To define the images that you want to include in the thumbnail view, type or select an area of number of wells in the *Montage* boxes.
   - Click *Apply* to implement the # of Wells and Timepoint settings that you made.
   - Click either the left or right arrow to move thumbnail selector left or right in the table;

**OR**
Click anywhere on the table to place the first selection box at that location.
To load images into a stack, right click on the squares for the images that you want to include in the stack, then click *Load Image(s)* in the Selections [In Green] area.

Click either the left or right arrow in the Selection area to open a full image view and/or to change the view to the next or previous selected image. The image currently displayed in the image view is indicated by yellow highlight.

Click on an image in the montage to see full resolution of the displayed image. You can make measurements on the images as they are displayed.

Click *Clear* to remove all Selections [In Green] from the table.

9. Click the *Display* tab to set display options applicable to the analysis you will run.

10. Click the *Run Analysis* tab to specify settings for running your selected analysis.

11. Click the *Measurements* tab to specify measurement criteria for your selected analysis, as required.

   **Note:** The *Measurements* tab is used to view images for which an appropriate analysis has already been run. Each measurement included in the application module log file can be viewed interactively. Use the *Well Selection* option to choose the wells that you want to view.

12. Click the *Graph* tab to configure a graph for the data.

13. Click *Reset Image Displays* to reset any open image displays to the default values.

14. Click *Close* to close the Review Plate Data dialog box.

### Display Tab Settings

The settings on the *Display* tab do not affect your analysis results. These settings are used to improve the visual appearance of the images on your plate, and to add additional information to the display, such as the well number and certain analysis values. It also enables you to generate an intensity profile to help you determine the areas of the image that have the highest intensity.

### Review Plate Data Dialog Box Options - Display Tab

**Figure 4-2**

**Review Plate Data dialog box – Display tab**

<table>
<thead>
<tr>
<th>Display</th>
<th>Run Analysis</th>
<th>Measurements</th>
<th>Graph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Show Values</td>
<td>Image Overlay:</td>
<td>Col:</td>
<td></td>
</tr>
<tr>
<td>Intensity Profile</td>
<td>Show well information</td>
<td>Cyan</td>
<td></td>
</tr>
<tr>
<td>Color Composite</td>
<td>Source R:</td>
<td>G:</td>
<td>B:</td>
</tr>
<tr>
<td>Scale 16 Bit Images:</td>
<td></td>
<td>Alexa 488</td>
<td>360/465Dv</td>
</tr>
<tr>
<td>Auto Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Show Values**
Displays average Analysis values on the table and in the upper right corner of each image in the montage if *Show well information* is selected under *Image Overlay*. For each site, the values shown both on the table grid and the montage display are the average of all the values for all defined objects in the site. To see individual values for identified objects, click *Cellular Results*. The Cellular Results dialog box will open and display a table of values for all objects in the selected well or site.

**Image Overlay**

Overlays information onto thumbnails in the Image Montage and onto individual images for each selected wavelength. The following selections are available:

- **No overlay display** – No data is overlaid on the image.
- **Show well information** – The data for the measurement selected on the Measurements tab is overlaid on the image.
- **Show cell segmentation** – The segmentation image generated by the application (if available) is overlaid on the image.

**Col**

Selects text color to apply to well numbers and values displayed in the image.

**Intensity Profile**

Transforms the image into a three-dimensional intensity profile graph, using the colors assigned to the image and assigning the highest intensities to the highest peaks in the graph.

*Note:* This enables the Scale 16-bit Image fields that are described under *Scale 16-bit Images* in the following paragraphs.

**Color Composite**

Creates a single color composite of two or three wavelengths based on the colors (R/G/B) that you assign to the wavelengths in the Source boxes.

*Notes:*
- This enables the Scale 16-bit Image fields that are described under *Scale 16-bit Images* in the following paragraphs.
- It is recommended that you do not activate Color Composite before you have configured your settings as it prevents images from updating while you are making your configuration settings.

**Source (R, G, B)**

Assigns one or more of the primary colors to one or more of the wavelengths that you are using in your experiment.

**Scale 16-bit Images**

Enables you to apply scaling to 16-bit images either automatically or manually when color composites of intensity profile images are created. If source images are displayed, then their scaling can be set through the scale image dialog.

**Range**

Assigns the upper limit of the 16-bit scaling range when using manual scaling for 16-bit images. This box is inactive when Auto Scale is checked.

**Reviewing Plate Data - Display Tab**

To configure the display settings for the Review Plate Data dialog box, complete the following procedure:

1. In the *Image Overlay* drop-down list, choose one of the following selections:
   - **No overlay display** – No data is overlaid on the image.
   - **Show well information** – The data for the measurement selected on the Measurements tab is overlaid on the image.
**Show cell segmentation** – The segmentation image generated by the application (if available) is overlaid on the image.

2. Click the arrow in the **Col:** box to select the text color for the Well Number and/or the measurement values overlaid on the image.

3. Click **Intensity Profile** to transform the image displayed into a three-dimensional intensity profile graph.

4. Click **Color Composite** to combine images for two or three wavelengths into a single image using the color assignments in the **Source R/G/B** boxes.

5. If Intensity Profile and/or Color Composite is selected, click **Auto Scale** to turn on auto scaling for 16-bit images, or click to turn off auto scaling and manually specify the scaling range.

6. If **Intensity Profile** and/or **Color Composite** is selected, and **Auto Scaling** is off, click the arrow in the range box to select the appropriate scaling range for your image.

### Run Analysis Tab Settings

The settings on the **Run Analysis** tab are used to choose the application module or journal that you want to run, select the setting file to be used during the analysis, and to run the analysis on either the entire plate, the selections that you made previously in the well selection grid area, or the currently selected site.

This tab also enables you to open the Edit List of Settings dialog box for a specific application module or journal and choose the setting file that you want to use, modify an existing setting, create a new setting file, or delete a setting file.

#### Review Plate Data Dialog Box Options - Run Analysis Tab

**Figure 4-3**

**Review Plate Data dialog box – Run Analysis tab**

<table>
<thead>
<tr>
<th>Display</th>
<th>Run Analysis</th>
<th>Measurements</th>
<th>Graph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis:</td>
<td>Angiogenesis Tube Formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Settings:</td>
<td>[Latest Configuration]</td>
<td>Edit List...</td>
<td></td>
</tr>
<tr>
<td>Measures angiogenesis tube formation.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>✔ Log into the database</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base Result Folder: Administrators</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Path: Administrators[Latest Configuration]\2005-06-06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analysis**

Selects and indicates the name of the analysis that you are running. This can be either a prepared analysis, such as Neurite Outgrowth, or analyses created from journals.

**Settings**
Selects and indicates the settings name from the available settings associated with the analysis that you are using. Use the Configure Settings button on this tab to make different settings easily available for use with your selected Analysis.

**Edit List**
Opens the Edit List of Settings for <Neurite Outgrowth> dialog box. The Edit list of Settings for dialog box is used to edit settings applied to application modules included in MetaMorph, such as Neurite Outgrowth. Analyses that you create from journals must have all possible settings stored in the database at the time the journal is created and stored in the database C:\Assay folder.

**<Description>**
Displays one of the following types of descriptions. For application modules that are MetaMorph Drop-ins, such as Neurite Outgrowth, this area displays the same description as the Meta Imaging Series Administrator Configure Drop-in dialog box displays for the module. For analyses defined by journals, it displays the description information typed into the Analysis Description area when the analysis was added to the database.

**Log into the database**
Opens the database login dialog box if you are not logged in when you run the analysis.

**Base Result Folder**
Enables you to select the location within the database where the results data from the application module is stored.

**Configure Settings**
Opens the Configure settings for <Neurite Outgrowth> dialog box or the associated dialog box for the selected application module. This option is only available for application modules. Analyses created from journals must be configured in the originating journal.

**Run Setup for Analysis**
Runs the setup journal for the selected analysis if it exists. The setup journal must be in the same folder as the main analysis and must be named in the format: **EXAMPLEJOURNAL_SETUP.JNL**.

**Note:** This option is only available if the selected analysis is created from a journal and not a prepared analysis like Neurite Outgrowth.

**Run Analysis for All Positions**
Runs the analysis for all wells that are indicated to contain a sample. Wells containing valid samples are indicated by a dash in the grid location for the well.

**Run Analysis for Selections**
Runs the analysis for all selected wells. Right-click on a well to select it. Selected wells are indicated by a green rectangle.

**Run Analysis for Site**
Runs the analysis for just the selected and displayed image site.
Determining Settings For Analysis

Reviewing Plate Data - Run Analysis Tab

To run either an application module or an analysis created from a journal, complete the following procedure:

**Note:** The following procedure uses the Neurite Outgrowth application module as an example of how to use this tab.

1. From the Review Plate Data dialog box, click the Run Analysis tab. The Run Analysis tab moves to the front.
2. In the Analysis drop-down box, choose the analysis that you want to run, or use the prefilled selection.
3. If you are using the default settings of a MetaXpress application module, such as Neurite Outgrowth, or a custom analysis derived from a journal, skip to step 10.
4. In the Settings box, choose the setting that you want to apply to the analysis, if more than one selection is available.
5. If you need to modify the configuration of an application module such as Neurite Outgrowth, click Configure Settings. The dialog box for the application module opens.
6. If you need to add another setting to an existing application module, click Edit List. The Edit List of Settings for <Neurite Outgrowth> opens.
8. Type a name for the new Settings in the Name box, then click OK. The Configure Settings for Neurite Outgrowth dialog box opens. This is a special version of the Neurite Outgrowth dialog box specifically for defining new settings for your Neurite Outgrowth application module.
9. Using the help information for Neurite Outgrowth, make the appropriate settings changes, then click Close.
   **Note:** To access Help associated with a specific dialog box, press the F1 key when the dialog box is active. When the Help window opens, click See Also to view and select from a list of procedures and dialog box options.
10. To log the measurement data from your analysis into the database, click Log into the database.
11. If you are running a custom analysis derived from a journal and have created a setup journal for it, click Run Setup for Analysis to run the setup journal.
12. To select a database location for your analysis data, click the box next to Base folder for results. The Measurements Sets dialog box open. Select the Measurements Sets folder and click OK. All measurement data from your analysis will be stored as a subfolder under this folder.

**Notes:**
- This step is necessary only the first time you run the analysis.
- Measurement sets and subfolders can be moved and renamed from within AcuityXpress as needed.
13. If you are running the analysis for all wells and all defined sites in the database, click *Run Analysis for All Positions*.

14. To run your analysis for only the selected wells, click *Run Analysis for Selections*.

15. To run your analysis for a specific site, select the site, then click *Run Analysis for Site*. 
Determining Settings For Analysis

Measurements Tab Settings
The Measurements tab enables you to select and view a previously run analysis and choose a single measurement from the analysis to view within the montage window and the well selection grid area. It also enables you to select wells for viewing based on a variable value range that you specify in Select Wells Based On Variable Range. Values can be logged in a typical log file, such as an Excel Spreadsheet or a text file.

Review Plate Data Dialog Box Options - Measurements Tab

The Review Plate Data (DB) Measurements Tab enables you to query the database for specific measurements that fall within your specified limiting range.

**Analysis**
Selects the completed analysis that contains the measurements to extract.

**Measurement**
Selects a single measurement to use for querying measurement data stored in the screening database. Click the down arrow to open the list of available measurements. The list of measurements can vary for each image or for each experiment's group of images. The selected measurement will be displayed in the data table when Well Arrangement or Time vs Well is selected. The selected measurement will also be displayed in the show values on the montage when appropriate.

**Display Format**
Specifies the number of decimal places to be included in the result data. The display format selection can vary depending on the type of measurement selected.

**Select Wells Based on Variable Range**
Specifies the range within which you want to run the query for the measurement that you selected.

**Value is**
Selects and specifies the query qualifier. Qualifiers are equals (=), greater than (>), less than (<), between, outside, or like.

**Select**
Runs the specified query and selects all wells or sites that fall within the query requirements.

**Configure Log**
Configures the open log file. Opens the Configure Log dialog box. Select Column and Row labels, and/or Plate info, or neither.

**Open Log/Log Data**
Opens the Open Data Log dialog box. Choose to log measurements to either a Dynamic Data Exchange (DDE), a text file, or both. After you have opened the log file, this button becomes Log Data. The data in the table of this dialog box is logged to the destination(s) that you selected.

**Note:** The data in your table is logged locally to your selected destinations and not to the MDCStore database.

**Reviewing Plate Data - Measurements Tab**

To specify well selection based on your specified measurements query criteria, complete the following procedure:

1. From the Review Plate Data dialog box, click the **Measurements** tab. The measurements tab moves to the front.
2. In the **Analysis** drop-down box, choose the analysis that contains the measurements to query.
3. In the **Measurement** drop-down box, choose the name of the measurement that you want to use to query your images in the database.
4. In the **Display Format** box. Select the number of decimal places that you want to display for your data in the grid.
5. In the **Value is** box, select the variable range limit specifier, and the numerical value(s) of the range in the adjacent box(es).
6. Click **Open Log** to open either a Dynamic Data Exchange or a text log file, then click **OK**.
7. Click **Configure Log** to choose either Column and Row labels, Plate info, or both, then click **OK**.
8. Click **Select** to query the image database using your selected measurement and variable range limits. The selected wells or sites will be highlighted in green.
9. Click **Log Data** or press the **F9** key to log your query data.
Graph Tab Settings

The Graph tab enables you to create a graph for a single Application Module analysis or a single Journal analysis. You can specify that the graph view include the entire plate, that it consist of multiple graphs of displayed wells, or that it is for a single, selected well. Make your well choices in the Montage window, then choose the graph type and measurements that you want to use.

Note: Additional options for visualizing your image analysis data are available in the AcuityXpress software.

Review Plate Data Dialog Box Options - Graph Tab

Figure 4-5
Review Plate Data dialog box – Graph tab

<table>
<thead>
<tr>
<th>Analysis: Neurite Outgrowth: neurite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graph view: Plate, Multiple graphs of displayed wells, Single Well</td>
</tr>
<tr>
<td>Graph type: Histogram</td>
</tr>
<tr>
<td>Measurement: % Cells Significant Growth</td>
</tr>
<tr>
<td>Number of bins: 1, Auto scale</td>
</tr>
<tr>
<td>Scale min: 0, Max: 100</td>
</tr>
</tbody>
</table>

Analysis
Selects the completed analysis that contains the data to graph

Graph View
Determines the source of the data plotted in the graph. Valid options include the following:

Plate
Graphs the summary well measurement for all the wells in the plate. Select this option to view the data for an entire plate.

Multiple graphs of displayed wells
Displays separate graphs for each well displayed in the montage. If you change the appearance of the graph using the Graph Settings dialog box, each mini-graph is updated.

Single Well
Graphs all values of a measurement within a well.

Graph Type
Determines the type of data and how it is displayed for the graph. The choices available depend on what is selected in the Graph View field. The following options are available for each Graph View option:

Plate:
Histogram— Displays a bar chart, in the format of Measurement (Area, Correlation, Coefficient, etc.) vs. Count.
Measurements vs Well Column — Displays a graph with one trace for each column of the plate, in the format of Measurement (Area, Correlation Coefficient, etc.) vs. Column. You can view the data as one trace...
with error bars by clicking the Down Arrow button directly below the left side of the graph and selecting *Show Mean with Error Bars* from the drop-down menu.

**Measurements vs Well Row** — Displays a graph with one trace for each row of the plate, in the format of Measurement (Area, Correlation Coefficient, etc.) vs. Row. You can view the data as one trace with error bars by clicking the Down Arrow button directly below the left side of the graph and selecting *Show Mean with Error Bars* from the drop-down menu.

**Measurements vs Well Number** — Displays a graph with one trace for each well number in the format of Measurement (Area, Correlation Coefficient, etc.) vs. well number. The well number is calculated as follows:

Well number = Current Column + (Current Row * Number of Columns)

**Scatter Plot**— Displays a scatter plot graph containing two measurement variables for the plate.

**Multiple Graphs of displayed wells:**

- **Time**— Displays multiple graphs of the selected wells in the format of Measurement (Area, Correlation Coefficient, etc.) vs. time point number. If the selected measurement is not recorded at specific time points, but applies to all data, the trace will be flat. If the value is only recorded at some time points, only those time points will be used in the graph.

- **Single Measurement**—Displays a bar graph for each of the well in the montage view. If the *Data View* field is set to *Measurement vs Well or Well Arrangement*, then the measurement shown is taken from the time point indicated in the Time Point field. If the *Data View* field is set to *Time vs Well*, then the graph shows the measurement for each time point that is shown in the montage.

- **Measurement Pair**—Displays a bar graph for each of the selected wells that contains data for two Measurement selections.

**Single Well:**

- **Time**— Displays a graph with one trace for each cell, in the format of Measurement (Area, Correlation Coefficient, etc.) vs. Time Point.

- **Histogram**— Displays a bar graph of the counts of values sorted into bins.

- **Scatter Plot**— Displays a scatter plot graph containing two measurement variables (Area, Correlation Coefficient, etc.) for each cell in the well.

**Measurement**

Selects the measurement to be graphed. The options available are extracted from the active experiment and include all numerical data measurements configured in the application module or analysis. The y-axis is autoscaled to contain the measurement data selected in this field.

**Measurement2**

Selects the second measurement option when available. The range of this data is used to scale the x-axis in both scatter plots and measurement pair graphs.

**Number of Bins**

Selects the number of bins used when creating the histogram. This option is only available when *Histogram* is selected from the Graph Type field.

**Auto Scale**

Automatically scales the bins based on the range of data from the selected *Measurement*. This option is only available when *Histogram* is selected from the Graph Type field.

**Scale Min/Max**

Manually selects the minimum and maximum range for histogram based on the data in the Measurement field. This option is only available when *Histogram* is selected from the Graph Type field and *Auto Scale* is not checked.

**Set Display to Default**

Sets the display parameters for the current graph to its default. There are separate graph defaults for each combination of Graph View and Graph Type.

**Show Graph**
Determining Settings For Analysis

Opens the graph using the current settings. If a graph is already open and the settings are changed, Show Graph will update the open graph.

Reviewing Plate Data - Graph Tab

For a graph to display your screen data, complete the following procedure:

1. From the Review Plate Data dialog box, click the Graph tab. The Graph tab moves to the front.
2. Select the analysis containing the data you wish to graph from the Analysis field.
3. In the Graph View field, select the source location for the data you want graphed.
4. Select a graph type from the Graph Type drop-down box. The options available for each graph type vary depending on the Graph View setting.
5. Select the measurement(s) to be graphed from the Measurement and Measurement2 (if applicable) field(s).
6. If you selected Histograms from the Graph Type field, select the number of bins to display in the resulting histogram in the Number of Bins field.
7. Check the Auto Scale checkbox to automatically scale the bin(s) based on the range of data from the selected Measurement. This option is only available when Histogram is selected from the Graph Type field.
8. Click Show Graph to open the graph based on the current settings.
   Note: If the data on the graph is not displaying properly, click and drag one of the corners of the graph window to resize it.
9. To configure the graph settings, double-click inside the graph or click the Show Graph Menu arrow on the bottom left corner of the graph and select Graph Settings. See the next section, Graph Settings, for examples of graphs.
10. To set the display parameters for the current graph to the default view, click Set Display to Default. There are separate graph defaults for each combination of Graph View and Graph Type. See the next section, Graph Settings, for examples of graphs.

Graph Examples

The following graphs are examples of the types of graphs that you can create using the capabilities of the settings on the Graph tab.
Figure 4-6
Histogram – Number of Cells (Count) versus the Percent of Cells of Significant Growth

Figure 4-7
Measurement versus Well Column – Percent of Cells of Significant Growth versus the column
Figure 4-8
Measurement versus Well Row – Percent of Cells of Significant Growth versus row number

Figure 4-9
Measurement versus Well Number – Percent of Cells of Significant Growth versus the Well Number
Figure 4-10
Scatter Plot – Total Cell Body Area versus the Percent of Cells of Significant Growth
Chapter 5
Preparing For Analysis

There are two methods available to analyze acquired data — automated and manual. This chapter provides an overview of each, then explains using the Review Plate Data dialog box to manually open data for review. Procedures for completing both methods are provided in Chapter 7, *Performing Manual Analysis*, and Chapter 8, *Performing Automated Analysis*.

**Automated Analysis**
The first opportunity to run a selected analysis on a plate is in conjunction with the acquisition of your plate image data. After image data for an entire plate has been acquired, if analysis of the plate has been scheduled, automated analysis will begin after the last well has been acquired. The procedure to initiate automated analysis is described in Chapter 8, *Performing Automated Analysis*. With automated analysis, a single application module or journal set can be used to complete your analysis. Image data is retrieved from the MDC Store Database and analyzed; the analysis results are subsequently stored back in the MDC Store database.

**Manual Analysis**
In most circumstances, in spite of the automated capabilities, you still should run manual preliminary tests on one or more wells to set the necessary dialog box options and settings needed to obtain valid results. After you determine effective settings for your experiment, you can store the settings for future use.

To begin manual analysis of images already stored in your database, open the Review Plate Data dialog box from the Screening menu. The Review Plate Data dialog box enables you to configure settings for preparing for and conducting manual image analysis. You can open this dialog box without having any plates open.

**Using the Review Plate Data dialog box for Analysis**
When you open the Review Plate Data dialog box without having any plates open, it will appear as shown in Figure 5-1:
Figure 5-1
Review Plate Data dialog box with no plates selected

Click *Select Plate* to open the Plate dialog box and choose one or more plates to analyze:
In the plates folder in the upper window, locate the plate that you want to analyze, and double-click the plate name. The name of the plate and its associated attributes is shown on the lower window of the Plate Dialog, as shown above.

To open the selected plate in the Review Plate Data dialog box, select it from the bottom window and click OK. The results of your query open in Review Plate Data dialog box, as shown in Figure 5-3:
Plate Dialog Box Options

The buttons on the top of the Plate dialog enable you to configure how the plate data is displayed:

Note: If you place your mouse pointer momentarily on a button, a tool-tip opens describing or naming the button.

Configure Plate Query

The first button is the Configure Plate Query button. Click this button to open the Plate Query Attributes dialog box, as shown in Figure 5-5:
Preparing For Analysis

Figure 5-5
Plate Query Attributes window

This dialog box lists available query attributes in the Column Selection field. To create a query, highlight the attributes that you want to include in your query, and click the right-facing arrow. The attributes will be moved to the Selected Columns field. Use the up and down arrows to arrange the query sort order.

For example, if you wanted the primary sort to be based on all available users, place Creator at the top of your Selected Columns list. Then if you wanted to find all plates created on a specific date for each user, place Date Created next on the list. Finally, if you wanted to find all plates assigned to a specific acquisition name, you would place Acquisition Name as the last entry in your query list.

Initiating a Query

To initiate the query, click the Retrieve Data button, or double-click the plate name in the query list. The name of the plate that you want to analyze will be displayed in the plate area:
Figure 5-6
Plate Dialog showing Query retrieval results in the horizontal arrangement

Changing from Horizontal to Vertical Arrangement
The Plate Dialog box default arrangement for the Query Select and Query Result windows is Horizontal. To change from a horizontal to vertical configuration, right-click inside either window area and click *Vertical Arrangement*:

Figure 5-7
Plate Dialog menu options

Figure 5-8 shows the window with a vertical arrangement:
Using the Plate Data Utilities Dialog Box for Manual Analysis

You can also use the Plate Data Utilities dialog box to run analysis on more than one plate at a time. Open the Plate Data Utilities dialog box from the Screening menu:

Click Run Analysis to open the Plate Dialog. When the Plate dialog is opened from the Plate Data Utilities dialog box, you can initiate analysis on multiple plates. Use the Query tools or manually select a node so several plates open in the bottom window. You can then use control-click to select multiple plates, as shown in Figure 5-10:
Click OK. The Run Analysis on Plates dialog box opens:

From this dialog, you can select the analysis, settings, and results folder for the selected plates. Click OK to start the analysis for the selected plates.

Molecular Devices offers optional MetaXpress application modules that automate common analysis tasks such as segmentation and cell counting. They can be run from the Review Plate Data dialog box, opened as a stand alone dialog box, or used within a custom journal. They can also be scheduled to run automatically in conjunction with the plate acquisition process.

This chapter describes several common analysis tasks and recommends application modules that help automate the tasks. Many of the modules share common conventions and settings. An understanding of the general principles of using application modules enables you use any of these modules by referring to the associated online help or this document.

Note: For a list of currently available application modules, see http://www.moleculardevices.com/pages/software/metaxpress_modules.html

Determining what needs to be measured
Consider what needs to be measured by your assay. 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. This is just one basic example; other typical assays are described in the following paragraphs.

Protein Localization/Translocation
Protein localization is 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 question then becomes how much area or intensity of my protein-of-interest co-localizes with another probe. For these instances, use the Translocation module, Translocation-Enhanced module, or the Nuclear Translocation HT module.

Cell Proliferation
Cell proliferation is typically a process of counting the cells or nuclei in an image. The Count Nuclei module or Cell Proliferation HT module is best suited for this task.
Cell Viability/Apoptosis
Cell viability is typically a counting of objects having specific characteristics – the cells are rounded up (shape/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. Use Live/Dead for a two wavelength assay or use Cell Health for a three color assay, such as DAPI, Annexin, or PI.

Receptor Internalization and other Punctuate Staining
Receptor internalization is usually measured by a probe moving to coated pits or vesicles, such as in the Transfluor® assay for GPCR activation. To count and otherwise measure the labeled pits or vesicles, or other punctuate staining, use the Granularity, Transfluor®, or Transfluor HT module.

Angiogenesis
Angiogenesis is typically an area measurement. Either the length of tubules is measured or the creation of holes in a cell monolayer is measured. Use the Angiogenesis module for this type of measurement.

Cell Physiology (Calcium/pH)
Cell physiological measurements are almost always measurements of intensity. Typically a probe is used that changes its fluorescence intensity using one or two wavelengths under different physiological conditions. Two examples of this are Fluo-3 which increases its fluorescence upon with increasing calcium or Fura-2 in which the fluorescence with 340 nm excitation decreases and 380 nm excitation increases with increasing calcium. Use the Cell Scoring or the Multi Wavelength Cell Scoring module for this.

Kinase activity assays
Generally kinase assays 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. It is recommended that you use either the Cell Scoring module or the Multi Wavelength Cell scoring module for this measurement.

Neurite outgrowth
Neurite outgrowth looks at changes in shape and lengths. The outgrowth lengths, number of outgrowths, branching, and other parameters are counted. It is recommended that you use the Neurite Outgrowth module for this measurement.

Cell Cycle
Cell Cycle assays typically involves the classification and counting and of cells in specified stages of the cell cycle and analyzing the distribution of cells within these classes in response to compounds. Use the Cell Cycle module for detailed classification using one to three wavelengths — from a single nuclear stain to combinations including optional mitosis-specific and/or apoptosis-specific stains. Use the Mitotic Index module.
for a simple two-wavelength application with a nuclear stain and a mitosis-specific stain to measure the percent of cells that are mitotic. For specific analysis of spindle formation and the disruption of centrosome separation, use the Monopole Detection module.
Chapter 7
Performing Manual Analysis

This chapter provides examples of setting up manual analysis using the optional MetaXpress application modules. The first section provides generic instructions that can be used for most application modules. The second section contains a procedure specific to the Transfluor application module. The following topics are discussed in this chapter:

- MetaXpress Application Module Configuration
  - Configuring an Application Module
  - Testing and Saving Settings
  - Running an Application Module
  - Selecting an Image from the Montage View

- Running the Transfluor Application Module
  - Configuring the Transfluor Module
  - Running the Transfluor Assay

- Viewing Analysis Results in the Cellular Results Table

- Transferring Data to an Excel Spreadsheet

Notes:

- The application modules discussed in this document can be purchased as Drop-ins to your existing MetaXpress installation. Contact your MDC representative for more information.

- The sample image in this chapter was calibrated using the Calibrate Distances command located in the Measure menu. For best results, source images in all application modules should be calibrated in microns. If more than one image is used as a source image, both images must have identical distance calibrations.

**MetaXpress Application Module Configuration**

This section describes some common procedures that can be applied to all MetaXpress application modules. Most application modules share similar configuration steps, such as selecting images to process or determining an object’s width. An understanding of these similar steps helps when learning to use different application modules.

**Manually Configuring an Application Module**

The following procedures explain how to configure an application module from the Review Plate Data dialog box:
Open the appropriate images from the Review Plate Data dialog box.
Select the application module and choose the source image(s) and result image.
Determine measurements.

Complete the following procedure to open the image(s) to process with the application module:

1. From the Screening menu, select Review Plate Data. The Review Plate Data dialog box opens.
2. Click Select Plate. The Plate Dialog opens.
3. Select the plate containing the images to analyze from the Plate list and click OK. The Review Plate Data dialog box reopens with the plate loaded, as shown in Figure 7-1:

**Figure 7-1 Review Plate Data Dialog Box**

![Review Plate Data Dialog Box](image)

**Notes:**

For more detailed instructions on opening plates from the Plate dialog box, refer to Chapter 5, *Preparing For Analysis*. 
Performing Manual Analysis

After you have made your plate selection, and the Review Plate Data dialog box reopens, the montage windows for each wavelength do not open automatically open even though the wavelength boxes are checked. You must do one of the following steps to view the montage window for an individual wavelength.

- Click well A1
- Uncheck, then check the wavelength(s) that you want to view
- Change a montage dimension to a different value
- Click a montage position arrow
- Click Reset Image Displays

4. Select a site or well to display a montage window for each selected wavelength.
5. Click a thumbnail image in the montage window to open the full size image(s) for the site or well.

For more information on opening images from a montage window, refer to Selecting an Image from the Montage View

6. Once you have the images that you want to analyze open, keep the Review Plate Data dialog box open and continue to the next procedure.

Complete the following procedure to select source and result images:

1. In the Review Plate Data dialog box, select the Run Analysis tab to highlight it.
2. Select an application module from the Analysis drop-down list. This procedure uses the Count Nuclei application module as an example.
3. Click Configure Settings. The Configure Settings for Count Nuclei dialog box opens:
Each application module needs at least one Source image to process. Some modules need more than one source image. Examples of Source image names in various application modules include:

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

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

4. Click *Source image* and select one of the images you opened in the previous procedure.

There are two ways to view the results of each application module: Displaying a result image or using an image overlay. Enabling *Display result image* will open or overwrite (see Step 6) a new image depicting what was measured. Using 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 your image window.

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

5. To create a separate result image, select the *Display result image* check box.

6. Open the image selector for *Display result image* and if needed change it from *None* to *New*, *Overwrite*, or *Add to*. *Overwrite* is the recommended selection.
→ **Overwrite**: Overwrites the selected image or creates a new image if one does not exist. This is the recommended selection.

→ **Add to**: Adds a plane to a stack.

→ **New**: Creates a new image every time the assay is run.

7. Click on the image name (typically indicated with [Source]).
8. Select **Specified** and give the image an appropriate name, such as *Result*.
9. Continue to the next procedure to configure the settings.

Most application modules require you to specify a number of size and contrast measurements before processing the image(s). These measurements often include the following:

- Minimum, average, and/or maximum width of an object
- Area of an object
- Intensity above background of an image

Complete the following procedure to determining the width of objects using the Region tools Line tool:

1. If the Region toolbar is not open, select Region Tools from the Region menu. The region toolbar opens, as shown in Figure 7-3:

   **Figure 7-3**
   *Region Toolbar with Line Tool Selected*

2. Select the Line tool as show above.
3. Place your mouse pointer on the image and locate the object that you want to measure.
4. Move the mouse pointer to one of the edges and click. A tool-tip appears showing the current X and Y values of the pointer, as well as the length.
5. Move the mouse pointer to the opposite edge of the object and note the Length value. In the following example the value is 20 pixels (or 6.71 um). This number represents the cell width in pixels. If the image is calibrated, the length in pixels and calibrated units:
6. Type or select the value in microns in the *Approximate max width* field. Figure 7-5 shows the new *Approximate max width* value.

![Figure 7-4 Sample Region Width Measurement](image)

Figure 7-5
Changing the *Approximate max width* value

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximate min width</td>
<td>3 ( \mu \text{m} = 9 \text{ pixels} )</td>
</tr>
<tr>
<td>Approximate max width</td>
<td>8.71 ( \mu \text{m} = 20 \text{ pixels} )</td>
</tr>
<tr>
<td>Intensity above local background</td>
<td>60 graylevels</td>
</tr>
</tbody>
</table>

7. You can remove the region lines by selecting Clear Regions from the Regions menu, or right-click on the region and select Delete Region.

You can also use the Caliper tool to measure the width of an object. The caliper function is a drop-in and may need to be loaded into the software. If the command is not present in the Measure menu, please see your System Administrator to have this drop-in loaded. Complete the following procedure to use the Caliper tool to measure the width of an object:

1. From the Measure menu, select Calipers. The Calipers dialog box opens.
2. Select the image to measure from the Image selector. The calipers appear on the selected image, as shown in Figure 7-6:

![Figure 7-6 Calipers Sample Image and Dialog Box](image)
3. The calipers can be moved by single-clicking the cross-bar so that it is displayed as a blinking line, indicating that it is active, and then dragging the cross-bar to the desired location with your pointer.

4. Single-click one of the caliper edge lines so that it is displayed as a blinking line. Then drag the line to the desired distance. The other caliper line will remain anchored.

5. Double-click the caliper cross-bar so that "nodes" appear at each end. With your pointer, drag one of nodes away from the other to the desired distance. The size of the line is displayed in the image as well as in the Caliper dialog box.

6. Enter the value in the *Approximate max width* field of the application module dialog box.

A third way to determine the width of an object is by using the Show Region Statistics dialog box. This also displays the area of the selected object. Complete the following procedure to use the Show Region Statistics dialog box to view the width and area of an object:

1. If the Region toolbar is not open, select Region Tools from the Region menu. The region toolbar opens, as shown in Figure 7-7:

![Figure 7-7 Region Toolbar with trace region Tool Selected](image)

2. Select the Trace Region tool as show above.

3. Place your mouse pointer on the image, 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. From the Measure menu, select Show Region Statistics. The Show Region Statistics dialog box opens, as shown in Figure 7-8:
5. Ensure that *Entire Image* is not selected in order to view the area and/or width measurements for the active region created in Step 3.

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 values. This setting is for controlling the sensitivity of the detection and/or segmentation. Complete the following procedure to determine accurate intensity above background values:

1. If the Region toolbar is not open, select Region Tools from the Region menu. The region toolbar opens, as shown in Figure 7-9:

![Figure 7-9 Region Toolbar with arrow Tool Selected](image)

2. Select the Arrow tool as show above (this should be selected by default).
3. Place the arrow cursor in the image window and move it over the visibly dimmest part of the dimmest object in the image.
4. Note the gray-level value of the object. The current gray-level value, along with the current X and Y coordinate values, are displayed on the bottom of MetaXpress desktop, as shown in Figure 7-10:
Performing Manual Analysis

5. Move the mouse 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 cell signal and the background. For the
example, using the cell signal value of 366 noted in Figure 10 and a background
value of 304, the calculation would be as follows: 366 - 304 = 62.

7. Enter a slightly lower value in the **Intensity above local background** field of the
application module dialog box. In this example, a value of approximately 60 is
appropriate.

8. Move on to the next section, Testing and saving settings. Do not close the
application module dialog box that you have configured.

**Testing and Saving Settings**

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

*Note:* Configuring and saving settings only needs to be done before you run an
application module for the first time. After the settings are saved, they can be loaded as
needed without further configuration.

Complete the following procedure to test and save your settings:

1. Click **Test Run** in the application module to measure using the settings that you
just configured. The application module will run and an overlay image will appear
on the original image. A result image will also open if the **Display result image**
checkbox was selected. Figure 7-11 illustrates a sample source image with an
overlay and a result image with the cells individually colored:

**Figure 7-10**
Bottom of MetaXpress Desktop with current gray-level highlighted

(101, 80) -> 366

**Figure 7-11**
Sample Source and Result images
A Cellular Results table containing individual object data also opens. Clicking a cell in the image highlights the data for the selected cell in the table. To select and view more than a single segment of line of data, use Ctrl+Click; to select non contiguous cells or lines of data can, use Shift+Click. If the table is not opening when you run an application module, ensure that Show Cellular Results is select in the Window menu.

2. Compare the result image with the source 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 other parameters.

3. To configure logging the application module data, click Configure Summary Log. The Configure Log dialog box opens.

4. Check and/or uncheck individual parameter configuration settings. For more information on the settings that can be logged, refer to the online help Dialog Box Options page for the application module you are using.

5. Click Configure Data Log and configure the Data log if needed.

6. To save you settings, click Save Settings. The Save Settings dialog box opens.

7. Enter a name and description for the settings and click OK. The settings are saved to the database.

Selecting an Image from the Montage View

Using the Montage image feature of the Review Plate Data dialog box, you can view thumbnail images of each image from the plate and compare the overall density and distribution of the sample material in each well. Samples showing an overall high density and even distribution are typically better candidates than are wells with very low sample density and uneven distribution.

To make it easier to identify and locate your sample well or site, check the Well Number on Images option on the Display tab:

![Figure 7-12 Review Plate Data dialog box – Display tab](image)

The following figure shows a montage window without well numbers:
The next figure shows a montage window with well numbers. The well numbers on the Montage window are an image overlay. Therefore, if you click the Overlay button on the upper left side, you can toggle the overlay (Montage) on and off.

To view an individual image in the Montage window, click the image. You can also select an image in the well plate table by right-clicking the image.
Figure 7-15 shows one well selected. Each well with a hyphen represents wells containing valid sample data. The gray wells indicate the wells selected to be included in the montage. Increase or decrease the montage dimensions to add or remove wells from the montage.
Performing Manual Analysis

Running the Transfluor Application Module
The earlier procedures in this chapter were generic in that they work with any application module. This procedure is specific to the Transfluor® Application Module. This section assumes you have purchased the Transfluor module, which is optional and does not come standard with MetaXpress.

Configuring the Transfluor Module
1. In MetaXpress, load your experiment through the Review Plate Data dialog box as described earlier in this chapter.
2. Click on one of the wells to display an image.

Figure 7-16
Montage Window and Open Image

3. Click the Run Analysis tab in Review Plate Data dialog box.
4. Select <Transfluor> from the Analysis drop-down list.
5. Click Configure Settings. The Configure Settings for Transfluor dialog box opens.
6. Click Pits and Vesicle image, then select the wavelength image representing the pit and vesicles that was opened in step 3.
7. Click on the pits and vesicles image, then increase the zoom by pressing the Page Up button on the keyboard.
8. Use the Single Line Region of Interest tool to measure a granule width by following these steps:
   a. Select Regions from the main menu, then Region Tools.
   b. Select the Single Line Region tool.
   c. Move to the image window from step 5 and locate a small pit.
   d. Move the mouse pointer to one of edge of the pit and click to anchor the line in position.
   e. Move the mouse pointer to the other edge of the pit and read the length of the line region. In the following example, the length is 3 pixels, or 1.41 μm.

![Figure 7-17 Montage Window and Open Image](image)

Enter this number in the Approximate min width box under “Pits”.

f. Repeat steps a through e for a larger pit. Enter that number in the Approximate max width box under “Pits”.

9. To determine the Intensity over local background value, select the Locator Arrow on the Region toolbar.

10. Find the dimmest pit.

11. Position the mouse pointer over the pit and read the gray value. This is the number that is displayed at the middle of bottom of the screen after the arrow. In the example below it is 162.

![Gray value example](image)

12. Move the mouse pointer just outside the granule to measure the background.

13. Now calculate the difference between the signal and the background. For the example image, the result is 2030 - 1029 = 1001.

14. Type or select a slightly lower value in the Intensity above local background box. For this example, a value of 800 is appropriate.

15. Repeat steps 6 through 12 for vesicles, if applicable (if not, then uncheck the Vesicles checkbox). Vesicles are much larger and brighter than pits.

16. Deselect the nuclear stain if selected.

17. Click Test Run to test your settings. The pits and vesicles segmentation should appear as an overlay on top of the image. Use the Show/Hide Overlay tool on the left toolbar to toggle the results on or off to make sure all the pits are detected.
If they are not, then lower the intensity above local background parameter first. If this does not produce the result that you want, then change the other parameters.

18. Select a negative control image and redo Test Run to make sure that your settings are correct for it as well.

19. If you are using a nuclear stain, then recheck the Nuclear stain checkbox and repeat steps 6 through 12 for the nuclei. Once you’ve performed a Test Run, the Cellular Results table will come up, allowing you to interactively view individual cellular results. Clicking a cell in the image highlights the data for the selected cell in the table. To select and view more than a single segment of line of data, use Ctrl+Click; to select non contiguous cells or lines of data can, use Shift+Click.

20. Save your settings by clicking Save Setting.

21. Hit the Close button.

**Running the Assay**

1. From the Review Plate Data dialog box, select the Run Analysis tab and select <Transfluor>.
2. Select your saved setting from the Settings drop-down list.
3. Click Run Analysis for All Positions to run the assay for all position on the plate.
   OR
   Click Run Analysis for Selections to run the assay for the selected sites. Select sites by right-clicking on different wells in the Review Plate Data dialog box).
   OR
   Click Run Analysis for Site to run the assay for the current active site.
4. Once the analysis is run, you can view the results by measurements (under the Measurements tab of the Review Plate Data dialog box) or by graph (under the Graph tab of the Review Plate Data dialog box).

To find out more about Transfluor, please visit [http://www.moleculardevices.com/pages/reagents/transfluor.html](http://www.moleculardevices.com/pages/reagents/transfluor.html)

**Viewing Analysis Results**

The Cellular Results table opens after an application module runs. It provides a way to associate cell-by-cell data with individual cells in the cell body segmentation image. The
overlay segmentation is used in conjunction with this table. Clicking a cell in the image highlights the data for the selected cell in the table. To select and view more than a single segment of line of data, use **Ctrl+Click**; to select non contiguous cells or lines of data can, use **Shift+Click**.

**Figure 7-19**

On-screen cellular results table for Neurite Outgrowth

![Cellular Results for DO1 table](image)

**Note:** For more information about using the Cellular Results table, refer to Chapter 10 or your MetaXpress Help file (F1).

**Transferring Data to an Excel Spreadsheet**

When you open a log file, you have the option of either transferring your plate data to a text file or to an Excel Spreadsheet. The following figures illustrate the process of transferring your data to an Excel spreadsheet.

Before you can log your data to an Excel Spreadsheet, you must open a Data Log using the Open Data Log dialog box. You can access this dialog box either by clicking the Open Log button in the Cellular Results window, or by clicking Open Data Log on the MetaXpress Log menu.

**Figure 7-20**

Open Data Log dialog box

![Open Data Log dialog box](image)
Performing Manual Analysis

This dialog box enables you to choose whether you want to log your data to an Excel Spreadsheet using a Dynamic Data Exchange (DDE) link, log your data to a text file, or both.

Check *Dynamic Data Exchange (DDE)* to log your data to an Excel Spreadsheet, then click OK. The Export Data Log dialog box opens:

**Figure 7-21**
Export Log Data dialog box

In the Export Data Log dialog box, choose Microsoft Excel from the Application drop-down list. Specify the Sheet Name and the Starting Row and Starting Column. Click OK. The Excel application will launch and an empty spreadsheet will appear.

To log your data, click Log Data in the Cellular Results dialog box, click Log Data on the Log menu, or press the F9 key.

After you click Log Data or press F9, the Excel spreadsheet will appear similar to the following table:

**Figure 7-22**
Excel spreadsheet containing the same data as the cellular results table

<p>| | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
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<td>0</td>
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<tr>
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<td>11</td>
<td>20.8211</td>
<td>4</td>
<td>5.20527</td>
<td>3.38385</td>
<td>11.5633</td>
<td>0</td>
<td>0.874152</td>
<td>64.9526</td>
</tr>
</tbody>
</table>
Chapter 8
Performing Automated Analysis

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 MetaXpress' automated analysis features to improve your data analysis throughput.

Use the following dialog boxes to configure, run, and monitor the status of your automated analysis:

- Plate Acquisition Setup – *Post Acquisition* tab
- Auto Run Mode
- Auto Run Status

Automated analysis can be started at one or more analysis workstations concurrently with acquisition. You can also automatically run analysis on an acquisition system after the acquisition is complete. Note that for both options, you must configure the analysis settings on the acquisition workstation before starting the acquisition. This is done using the *Post Acquisition* tab of the Plate Acquisition Setup dialog box.

**Note:** If the analysis configured on an acquisition workstation calls for an application module to run, the analysis machine running in Auto Run mode must have the application module drop-in installed locally in order to complete the analysis.

The analysis can be run on systems running MetaXpress or MetaXpress Offline that are attached to your database and logged in with privileges to view plates and write data. Using automated analysis provides several distinct advantages including:

- Unique workstations dedicated to running specific analysis application modules.
- Acquisition workstation dedicated to performing acquisitions only, reducing the overhead for the acquisition system.

Before running in Auto Run Mode, you must configure Auto Run Analysis on the *Post Acquisition* tab of the Plate Acquisition Setup dialog box. Automated analysis can then be initiated using the Auto Run Mode dialog box. Once auto run is started, use the Auto Run Plate Statuses dialog box to monitor and control the progress of each analysis running for each plate being analyzed.
Monitoring Statuses
When running the Auto Run Plate Statuses dialog box, you can monitor the following conditions:

- All analysis workstations connected to the database into which the current acquisitions are being stored.
- Plate ID number
- Name of the Application Module or Journal set being used to analyze the plate
- Name of the settings being used
- Location of the result folder
- Status of the analysis on each plate

General Procedure for Automated Analysis
Complete the following general procedures to activate Auto Run analysis:

1. In the Plate Acquisition Setup dialog box, click the Post Acquisition tab.
2. On the Post Acquisition tab, complete the steps described under Plate Acquisition Setup - Post Acquisition Tab, which follows.
3. Complete the steps in the Running in Auto Mode procedure.
4. To view the Auto Run Status, complete the steps under Viewing the Auto Run Status.

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

Notes:

- If you do not want to automatically run post-acquisition analysis, ensure that Auto Run analysis after acquisition is not selected.
- The list of available assays and settings is a subset of the list that is in the Run Analysis tab of the Review Plate Data dialog box. Analyses that do not have settings defined are not shown.
Figure 8-1
Plate Acquisition Setup dialog box - Post Acquisition tab

1. Select Auto Run analysis after acquisition.
2. Select the assay (Application module or Journal Assay) to run after acquisition from the Analysis drop-down list.
   Note: Only Analysis that have defined settings will be shown.
3. Select a setting file from the Settings drop-down list. The Field below the Settings drop-down box displays a description of the settings, if one exists.
   Note: You can configure and save settings in the Review Plate Data dialog box.
**Auto Run Mode**

Enables a networked system to run analysis on plates automatically after they are acquired.

Use this command, along with the *Post Acquisition* tab of the Plate Acquisition Setup dialog box, to automatically start running analysis on plates after they have been acquired. After each plate is acquired on the main MetaXpress system, information regarding analysis of the plate is placed in a queue in the database. When other MetaXpress computers connected to the database are set in Auto Run Mode, they check the queue and run analysis on plates as the data becomes available.

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

Note: You can monitor the status of the Auto Run by either clicking the Status button within the command or running the Auto Run Plate Status command from the Apps Menu.

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

**Figure 8-2**

Auto Run Mode dialog box

Running in Auto Mode

1. Use the following procedure to set-up and use the Auto Run Mode command:
2. On the computer acquiring the images, open MetaXpress and, from the Screening Menu, click Plate Acquisition Setup; the Plate Acquisition Setup dialog box opens. Click the Post Acquisition tab to enable it.
3. Check the *Auto run analysis* checkbox and use the drop-down menu to select an analysis to run.
4. Continue to setup and run your acquisition as normal. After the plate is acquired, it will be added to the Auto Run queue.
5. On the computer(s) running the analysis, open MetaXpress and, from the Screening Menu, click Start Auto Run Mode; the Auto Run Mode dialog box opens.
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6. If you want to run analysis on more than one plate, ensure that Leave auto run mode when plate is finished is not checked

7. If you want to check the status of the analysis, On the computer running the assay, click Status to open the Auto Run Status dialog box.
   OR
   On another computer running MetaXpress and logged into the database, select Start Auto Run Plate Status in the Screening Menu.

8. After the analysis has completed for all plates, click Cancel to close the Auto Run Mode dialog box.

Auto Run Mode - Dialog Box Options

Machine Name
Lists the network name of the current MetaXpress computer.

Analysis Running
Lists the name of the currently running analysis. The analysis is selected in the Post Acquisition tab of the Plate Acquisition Setup dialog box on the MetaXpress computer doing the acquisition.

Plate
Lists the plate ID of the plate currently being analyzed.

Leave auto run mode when plate is finished
Exits Auto Run Mode after the current plate is analyzed. Select this option if you want to use the system for other tasks and do not want MetaXpress 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 functions are disabled.

Status
Opens the Auto Run Status dialog box.

Cancel
Cancels the analysis being run after the current site is completed and closes the dialog box. Data from sites already analyzed will remain in the database, but the plate will be removed from the Auto Run queue.
Auto Run Status

Enables you to view the status of an auto run analysis.

Use this command to view the status of analysis started with the Auto Run Mode command.

Figure 8-3
Auto Run Plate Statuses dialog box

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

Viewing the Auto Run Status

1. Use the following procedure to view the Auto Run status:
2. On the computer running the analysis, from the Screening Menu, click Auto Run Plate Statuses [DB]. The Auto Run Status dialog box opens.
   OR
   From the Screening Menu, click Start Auto Run Mode [DB]. The Auto Run Mode dialog box opens. Click Status; the Auto Run Status dialog box opens.
3. To stop running an analysis on a plate, select the plate from the table and click Cancel selected plate.
4. To stop running the analysis on all plates, click Cancel all plates.
5. To close the dialog box, click Close.

Auto Run Plate Status - Dialog Box Options

Plate ID
Lists the ID number of the plate.

Analysis
Lists the name of the analysis in the database being run on the plate. The analysis is selected on the MetaXpress computer doing the acquisition in the Screen Acquisition dialog box.

Setting
Performing Automated Analysis

Lists the setting name in the database associated with the analysis. The assay setting is selected on the MetaXpress computer doing the acquisition in the Screen Acquisition dialog box.

**Status**
Lists the current status of the plate. The following statuses are possible:

- **Running** - Indicates that the analysis is currently running on the plate. Once 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 progress on a well or site in the expected time. The timeout value is selected in the Meta Imaging Series Administrator with the `Set Auto Run Timeout` command. A timeout is normally caused by an error on the machine running the analysis. To diagnosis the cause of the timeout, inspect the machine that has timed out for error messages or other problems. In some cases the problem can be resolved and the analysis can continue. If this happens the status will return to `Running`. In other cases, the auto run must be canceled and the analyses run again. Some analyses, particularly custom ones created through the journaling system, take a long time to complete. In this case the timeout value set in the `Set Auto Run Timeout` command should be increased to allow enough time to run the analysis.
- **Pending** - Indicates the analysis has not started to run for the plate.

**Machine ID**
Lists the ID of the MetaXpress computer processing the analysis. The machine ID is the network name for the machine processing the analysis.

**Progress**
Lists the current well and site the analysis is analyzing.

**Cancel selected plate**
Stops the analysis from analyzing the selected plate. The computer running the analysis will not respond to this command until it completes analysis of the site it is currently processing. The selected plate will be removed from the Auto Run queue.

**Cancel all plates**
Stops the analyzing of all plates. The computer running the analysis will not respond to this command until it completes analysis of the site it is currently processing. All plates will be removed from the Auto Run queue.

**Start Auto Run Mode**
Starts running analysis on plates currently pending in the Auto Run queue and on new plates as they are added to the queue. This is the same command as Start Auto Run Mode [DB] on the Screening menu.

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

**Close**
Closes the dialog box.
Chapter 9
Managing Plate Data

The Plate Data Utilities and Plate dialog boxes enables you to import images into the database, run assays on selected images, and delete plates, images, and measurements. You can use the Plate Data Utilities command to manage images and data acquired during plate acquisition. The following features are supported:

- **Run Analysis** — Enables you to select one or more plates to open in the Run Analysis on Plates dialog box.
- **Export Images** — Copies the images of one or more selected plates from the database/fileserver to another local or networked location.
- **Delete Measurements** — Deletes the measurements associated with a selected plate(s) from the Plate dialog box.
- **Delete Images** — Deletes images from plates selected from the Plate dialog box.
- **Delete Plates** — Deletes plates selected from the Plate dialog box.
- **Remove Deleted Data** — Permanently removes plates, images, and/or measurements marked for deletion from the database/fileserver.

Note: The Delete Measurement, Delete Images, and Delete Plates commands remove data from the Plate dialog box but do not delete them from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

![Plate Data Utilities dialog box](image)

Most of the commands in the Plate Data Utilities dialog box open the Plate dialog box. The Plate dialog box interfaces with the MDCStore database and fileserver and enables you to perform a number of functions on acquired plates. The dialog box performs different actions depending on how it was called.
Running Analysis on Plates

You can use the Plate Data Utilities dialog box to run analysis on more than one plate at a time. Complete the following procedure to run analysis on one or more plates:

1. From the Screening menu, choose Plate Data Utilities. The Plate Data Utilities dialog box opens.
2. To run analysis on a selected plate, click Run Analysis. The Plates dialog box opens.
3. Expand the plate’s folder in the top pane of the dialog 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 bottom pane.
5. Select the plate to run analysis on and click OK. The Run Analysis on Plates dialog box opens:
   
   **Note:** You can select more than one plate to run analysis on using Ctrl+click to select multiple plates, as shown in Figure 9-2:

   **Figure 9-2**
   Plate dialog box
6. In the *Analysis* drop-down list, choose the assay that you want to run from the list of available analysis.

7. In the *Settings* drop-down list, choose the setting that you want to use from the list of available settings.

8. In the *Images to open for the analysis* box, select the wavelengths to use with the selected assay.

9. After all settings are complete, click **OK** to run your selected assay.

**Importing Images**

You can use the Import Images command to import one or more data sets created by other MDC applications, such as the non-database version of Screen Acquisition, into the MDCStore fileserver or database. The data will then be accessible using either the Plate Data Utilities dialog box or the Review Plate Data dialog box. You can also use this dialog box to open the Import Cellomics Data dialog box, which enables you to import a plate of Cellomics DIB files into the MDCStore fileserver or database.

**Note:** MetaXpress and the MDCStore Database do not import 3rd party images by default.

Use the following procedure to import data sets into the screening database or a local or network folder:

1. From the Plate Data Utilities (DB) dialog box, click *Import Images*. The Import Images dialog box opens.
2. Click *Select Directory*. The Browse for Folder dialog box opens.
3. Navigate to the local or network folder containing the data set(s) to import using the Browse for Folder dialog box, 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. Chose the data set(s) to be imported by checking the check boxes next to the HTD files to import in the HTD Files field.

5. Select a location (either the fileserver or database) to import the data sets to from the Move images to drop-down box.
   **Note:** The locations in this list were configured using Database Utilities command in the Meta Imaging Series Administrator.

6. Click Import. The files are imported and will be available for review in the Review Screen Data (DB) dialog box.
   **Note:** Depending on the number of files to import, this step may take several minutes to complete.

7. If you need to import Cellomics data, click Import Cellomics Data. The Import Cellomics Data dialog box opens.

8. To close the dialog box, click Cancel.

**Import Images - Dialog Box Options**

**Select Directory**
Opens the Browse for Folder dialog box and enables you to select a local or networked location for the folder containing the data sets (HTD files) to import.

**Move Images to**
Displays a drop-down list of available local or network location(s) where the data set should be imported. You can also import directly into the database. The locations in this list were configured using the Screen Database: Database Utilities command in the Meta Imaging Series Administrator.

**HTD Files**
Displays a list of valid HTD files (plates) contained in the folder selected in the Select Directory field. Use the checkboxes to select which files to import.

**Description**
Displays a description of the experiment. This is only available if the Description field in the Main tab of the Screen Acquisition dialog box was filled in when the plate was acquired.

**Import**
Imports the selected HTD files into the database or directory selected in the Move Images to field.

**Import Cellomics Data**
Opens the Import Cellomics Data dialog box.

**Cancel**
Cancels the command.

**Importing Cellomics Data**
Use the Import Cellomics Data command to convert sets of Cellomics DIB files into data sets in the database that can be accessed using either the Plate Data Utilities (DB) command or the Review Plate Data (DB) command. The command 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 screening database or a selected local or network folder.

**Note:** Cellomics DIB files do not include image calibration data. Unless you enter a specific calibration when you import the data, a default calibration of 1 um = 1 pixel will applied to the imported images.
Use the following procedure to import Cellomics data into MetaXpress:

1. From the Plate Data Utilities (DB) Import Images dialog box, click *Import Cellomics Data*. The Import Cellomics Data dialog box opens:

   **Figure 9-34**
   **Import Cellomics Data dialog box**
   ![Import Cellomics Data dialog box](image)

   1. Click **Select Directory**. The Browse for Folder dialog box opens.
   2. Navigate to the local or network folder containing the Cellomics data to import using the Browse for Folder dialog box, then click **OK**. The path is displayed next to the **Select Directory** button and the Cellomics files are displayed in the **Cellomics Data Files** field.
   3. Select the check boxes next to the Cellomics file sets to import in the **Cellomics Data Files** field.
   4. Select a location (either the database or an available network folder) to import the Cellomics data to from the **Store images in** drop-down box.
   5. Enter a calibration ratio (ums per pixel) for the x and y values of the imported images in the **Image calibration** fields.
   6. Click **Import**. The files are imported and will be available for review in the Review Plate Data (DB) dialog box.
   7. Click **Close** to close the dialog box.

### Import Cellomics Data - Dialog Box Options

**Select Directory**

Opens the Browse for Folder dialog box and enables you to select a local or networked location for the folder containing the Cellomics (.dib) files to import.
**Cellomics data files**
Displays a list of valid Cellomics file sets contained in the folder selected in the *Select Directory* field. Use the checkboxes to select which files to import.

**Move images to**
Displays a drop-down list of available local or network location(s) where the data set should be imported. You can also import directly into the database. The locations in this list were configured using the Screen Database: Database Utilities command in the Meta Imaging Series Administrator.

**Image Calibration**
Sets a calibration ratio for the x and y values of the imported images. The default calibration is 1 um = 1 pixel.

**Import**
Imports the selected Cellomics files into the database or directory selected in the *Move Images to* field.

**Close**
Closes the dialog box.

**Exporting Images**
Complete the following procedure to export images to a local or networked folder:

1. From the Screening menu, choose Plate Data Utilities. The Plate Data Utilities dialog box opens.
2. To export images to a local or networked server, click Export Images. The Plate dialog box opens.
3. Expand the plate’s folder in the top pane of the dialog 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 bottom pane.
5. Select the plate to export from the bottom pane and click *OK*. The Plate dialog box closes and the Browse for Folder dialog box opens.
6. Browse to the local or networked folder to export the images to and click OK. The images are exported and the Browse for Folder dialog box closes.

**Deleting Plates**
To delete plates from the database you must first delete them from the Plates dialog box. After that, use the Plate Data Utilities dialog to permanently remove the plates from the database or fileserver. Complete the following procedure to delete a plate:

1. From the Screening menu, choose Plate Data Utilities. The Plate Data Utilities dialog box opens.
2. To delete a plate, click *Delete Plates*. The Plates dialog box opens.
3. Expand the plate’s folder in the top pane of the dialog 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 bottom pane.
5. Select the plate to delete from the bottom pane and click *OK*. The Plate dialog box
closes.

**Note:** This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

6. In the Plate Data Utilities dialog box, click *Remove Deleted Data* to permanently remove the plate data from the database/fileserver.

### Deleting Images

To delete images from a plate you must first delete them from the Plates dialog box. After that, use the Plate Data Utilities dialog to permanently remove the images from the database or fileserver.

**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 box.

Complete the following procedure to delete images from a plate:

1. From the Screening menu, choose Plate Data Utilities. The Plate Data Utilities dialog box opens.
2. To delete images from a plate, click *Delete Images*. The Plates dialog box opens.
3. Expand the plate’s folder in the top pane of the dialog 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 bottom pane.
5. Select the plate containing the images to delete from the bottom pane and click *OK*. The Plate dialog box closes.
   
   **Note:** This step does not remove the images from the database/fileserver. To remove the images from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the images have been deleted they cannot be recovered.
6. In the Plate Data Utilities dialog box, click *Remove Deleted Data* to permanently remove the images from the database/fileserver.

### Deleting Measurements

To delete measurements from a plate you must first delete them from the Plates dialog box. After that, use the Plate Data Utilities dialog to permanently remove the measurements from the database or fileserver.

**Note:** If you delete 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 box.

Complete the following procedure to delete measurements from a plate:
1. From the Screening menu, choose Plate Data Utilities. The Plate Data Utilities dialog box opens.
2. Click Delete Images. The Plates dialog box opens.
3. Expand the plate’s folder in the top pane of the dialog 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 bottom pane.
5. Select the plate containing the measurements to delete from the bottom pane and click OK. The Plate dialog box closes.
   **Note:** This step does not remove the data from the database/fileserver. To remove the data from the database/fileserver, you must click Remove Deleted Data after using the Plate dialog box. Once the images have been deleted they cannot be recovered.
6. In the Plate Data Utilities dialog box, click Remove Deleted Data to permanently remove the measurements from the database/fileserver.

**Plate Data Utilities - Dialog Box Options**

**Delete Plates**
Opens the Plates dialog, which enables you to select the plate(s) to delete from the database.

**Delete Measurements**
Opens the Plates dialog, which enables you to select the plate(s). The measurements associated with the selected plates will be deleted from the database.

**Warning:** Once the data has been deleted it cannot be recovered.

**Delete Images**
Deletes images from plates stored in the database.

**Import Images**
Opens the Import Images dialog box. Use this dialog box to import a new data set into the database or to another defined local or network location. You can also access the Import Cellomics Data dialog box from here.

**Run Analysis**
Opens the Plates dialog box. Use this dialog box to select a plate it run and click OK. This will open the Run Analysis on Plates dialog for the selected plate. Choose settings and images to be run with the selected analysis. Click OK to run the analysis. The Run Analysis on Plates dialog box closes, and the Review Plate Data dialog box opens.

**Close**
Closes the Plate Data Utilities dialog box.

**Plate Dialog Box Options**

**Plate Query Attributes**
Opens the Plate Query Attributes dialog box. Use this dialog box to configure how the plates tree structure in the Plates Field (the top half of the dialog box) is organized.
Managing Plate Data

**Attribute Columns**
Opens the Attribute Columns dialog box. Use this dialog box to specify which columns are available in the Details field (the bottom half of the dialog box). You can also sort the left to right order of the columns.

**Retrieve Data**
Re-queries the database and updates the dialog box.

**Plate Security**
Opens the Sharing and Security dialog box. Use this dialog box to set up sharing of plates between groups.

**Plates Field**
Contains a tree-view organization of all available plates. Left-click a folder to expand it and display the contents in the Details Field.

**Details Field**
Displays the details of the available plates. Left-click a plate to make it active. Shift-click multiple plates or Control-click individual plates to select multiple plates (where applicable).

**OK**
Performs the appropriate action on the selected plate(s). See the main Plate dialog box help file for a list of commands that use the Plate dialog box.

**Cancel**
Cancels the command and closes the dialog box.
Chapter 10
Viewing and Understanding Analysis Results

This chapter describes the types of results available to you after analyzing your experiment image data. Types of image analysis results available include:

- Result images that with overlay information that provides visual highlighting to identify various cell areas.
- Image and cellular numerical result data. All data is stored in log files associated with the specific Application Modules(s) used to analyze your data.
- Graphs that provide a visual interpretation of your logged image data.
- Result data that can be further analyzed in AcuityXpress.

In addition to the analysis options discussed in this chapter, AcuityXpress software from MDC can be used for further visualization and mining of your analysis results. Please contact your MDC representative for more information on AcuityXpress.

Extracting Results after Running your Analysis

The results that you obtain from your experiment depend on the settings made before running the selected application module. Use the following steps as a guide for the process flow, from making your initial settings in the selected application module to reviewing the various types of results.

1. Open selected Application Module dialog box.
2. Choose settings file or create new settings.
3. Open a log file.
4. Configure both the Summary Log and the Data log to choose the measurements that you want to log into each log file.
5. Save the new settings to a settings file.
6. Run the analysis for the selected application module.
7. Log the measurement results into your log file.
8. View the results in the locations described in the following sections.
**Dialog Boxes and Tools for Viewing Results**

Your analysis results are available in the following formats:

- Review Plate Data dialog box well selection table
- Image montage or individual images in a MetaXpress image window
- Application module segmentation image overlay
- Cellular results table in the Cellular Results dialog box
- Graphs of various types from the Review Plate Data dialog box
- Measurements tab of the Review Plate Data dialog box
- Logged ASCII data exported to a text file
- Logged data exported to an Excel spreadsheet

**Review Plate Data Dialog Box Microwell Well Selection Table**

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

Wells marked with a (-) hyphen – Indicates the wells containing image data.
Wells highlighted in gray – Indicates the wells included in the montage area.
Wells highlighted in (orange) – Indicates the images selected for individual display.

![Figure 10-1](image.png)

**Neurite Outgrowth – Well table showing analysis results for a single measurement**

<table>
<thead>
<tr>
<th></th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25.00</td>
<td>5.88</td>
<td>46.67</td>
<td>5.22</td>
<td>7.53</td>
<td>1.79</td>
</tr>
<tr>
<td>B</td>
<td>41.94</td>
<td>0.00</td>
<td>4.17</td>
<td>6.67</td>
<td>11.83</td>
<td>4.99</td>
</tr>
<tr>
<td>C</td>
<td>33.12</td>
<td>10.00</td>
<td>7.38</td>
<td>13.79</td>
<td>10.00</td>
<td>4.76</td>
</tr>
<tr>
<td>D</td>
<td>39.70</td>
<td>9.38</td>
<td>5.02</td>
<td>3.60</td>
<td>5.87</td>
<td>14.60</td>
</tr>
</tbody>
</table>

**Data view**

You can select the data view for the table. This drop down list selects and indicates the well arrangement as shown in the Microwell Plate Selection Grid and the Image Montage window. You can choose one of the following data views:

*Well arrangement* – Arranges your viewable images in the montage in the order in which the wells are presented in the microwell plate.
**Time vs well** – Compares timepoints against wells or well selections.

**Well vs measurement** – Compares wells against the available measurements.

**Print Table**
The displayed data can be sent directly to an available printer and printed in ASCII format. The table borders are not preserved for printing. This is intended to quickly provide a printed record of the displayed data.

**Image Montage or Individual Images in a MetaXpress Image Window**
The image montage window provides thumbnail views of the images associated with the data in the table. The contents of the montage window depend on the settings made in various areas of the Review Plate Data dialog box. For example, the number of images included in the montage depends on the montage dimensions that you specify in the *Montage* field below the Plate Selection Table.

**Figure 10-2**
Neurite Outgrowth – Montage window showing analysis results for a single measurement overlaid on the image thumbnails

**Display Tab Settings**
Other settings that affect the Montage window are located on the *Display* tab. For example, if you want the Well Number associated with the image shown on the image, check *Well Number on images*. Also, if you want the value shown in the table displayed on the image, check *show values*. To change the color of these values displayed on the thumbnail images, open the *Col* drop-down list and choose a different color.
The Display tab also enables you to create an Intensity Profile or a Color Composite in place of the images in the Montage view.

**Intensity Profile**
Transforms the image into a three-dimensional intensity profile graph, using the colors assigned to the image and assigning the highest intensities to the highest peaks in the graph. This enables the Scale 16-bit Image fields. When you check *Intensity Profile*, the following additional settings used by Intensity Profile appear in the dialog box.

- **Scale 16-bit Images**
  Enables you to apply scaling to 16-bit images either automatically or manually when a color composite of intensity profile images are created. If source images are displayed, then their scaling can be set through the scale image dialog.

- **Range**
  Assigns the upper limit of the 16-bit scaling range when using manual scaling for 16-bit images. This box is inactive when Auto Scale is checked.

**Color Composite**
Creates a single color composite of two or three wavelengths based on the colors (R/G/B) that you assign to the wavelengths in the Source boxes. When you check *Color Composite*, you can associate a maximum of three wavelengths each with one of the primary colors.

- **Source (R, G, B)**
  Assigns one or more of the primary colors to one or more of the wavelengths that you are using in your experiment.

After you have activated *Color Composite*, you can open the composite image in an image window and use the *Toggle Color Channel* buttons to turn off and on one or two of the wavelengths to enable you to view the image data for each wavelength separately.
Application Module Segmentation Image Overlay

After an application module is run, the source image opens with an overlay image attached. A result image also opens if the Display result image checkbox was selected when configuring the module. Figure 10-5 illustrates a sample source image with an overlay and a result image with the cells individually colored:

The source image contains your original data with an overlay of the module results turned on by default. You can toggle the overlay on or off from the source image with the Show/Hide overlay button on the side of your image window. If you save the source image, the overlay is also saved.
The result image does not contain the original image data—it is a graphically representation of the application module results.

**Cellular Results Table**

The Cellular Results table is used to select individual cells in a well plate image for quick viewing of its data in table format when analyzing plate data using MetaXpress application modules. This table opens automatically after an application module finishes analyzing an image. You can also configure it not to open each time an application module is run.

Use this feature to interactivly view data belonging to an individual cell by clicking the cell in the image. This highlights the data for the selected cell in the Cellular Results table. Conversely, you can click a value in the Cellular Results table and the corresponding cell will become highlighted in the image. When selecting cells from the image or the Cellular Results table, you can select multiple cells by using the **Ctrl+Click** combination. You can also select a range of cells from the Cellular Results table using the **Shift+Click** combination.

**Note:** The data displayed in the Cellular Results table is the same data configured using the **Configure Data Log (Cells)** command for the application module you are using. However, you are not required have a log open to view the Cellular Results table.

**Figure 10-6**

**Neurite Outgrowth – Cellular Results dialog box**

```plaintext
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>0</td>
<td>229.68</td>
<td>10.3033</td>
<td>5.77685</td>
<td>5.77685</td>
<td>2</td>
<td>0.542177</td>
<td>11.553</td>
</tr>
<tr>
<td>122</td>
<td>0</td>
<td>231.25</td>
<td>13.3211</td>
<td>7.26553</td>
<td>7.26553</td>
<td>2</td>
<td>0.909536</td>
<td>14.5711</td>
</tr>
<tr>
<td>123</td>
<td>0</td>
<td>232.813</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>124</td>
<td>0</td>
<td>232.813</td>
<td>23.6244</td>
<td>23.6244</td>
<td>23.6244</td>
<td>1</td>
<td>0.788838</td>
<td>23.6244</td>
</tr>
<tr>
<td>125</td>
<td>0</td>
<td>234.75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>234.75</td>
<td>16.3211</td>
<td>9.26553</td>
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<td>2</td>
<td>0.934749</td>
<td>19.5711</td>
</tr>
<tr>
<td>127</td>
<td>0</td>
<td>235.938</td>
<td>26.6556</td>
<td>10.7815</td>
<td>4.26777</td>
<td>3</td>
<td>0.887163</td>
<td>32.3744</td>
</tr>
</tbody>
</table>
```
Using the Cellular Results Table

Complete the following procedure to use the Cellular Results table with application modules:

1. From the Apps menu, select an application module to run.
2. Configure the module (see the help file for the module for more information), and configure the data log using the Configure Data Log (Cells) command. You do not have to open a data log in order to view the Cellular Results table.
3. Click Apply to run the application module. The source image is processed and the Cellular Results table opens.
4. Select one of the segmented cells in the source image; the corresponding data for the cell will highlight in the Cellular Results table.
   OR
   Select a row in the Cellular Results table; the corresponding cell will highlight in the source image.
   Note: When selecting cells from the image or the Cellular Results table, you can select multiple cells by using the Ctrl+Click combination. You can also select a range of cells from the Cellular Results table using the Shift+Click combination.
5. If you do not want the Cellular Results table to open each time an application module is run, uncheck Show Data On App. Module Run.
   Note: If you uncheck this and later want to view the Cellular Results table when running an application module, select Show Data On App. Module Run from the Windows menu on the MetaXpress or MetaMorph menu bar.
6. Click Close to exit the Cellular Results table.

**Graph Settings**

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

1. From the Review Plate Data dialog box, click the *Graph* tab. The Graph tab moves to the front:

   ![Figure 10-8 Graph Tab](image)

   - **Figure 10-8 Graph Tab**
     
     - **Display** tab
     - **Run Analysis** tab
     - **Measurements** tab
     - **Graph** tab
     
     - **Analysis** field: Neurite Outgrowth: neurite
     
     - **Graph view** field:
       - Plate
       - Multiple graphs of displayed wells
       - Single Well

     - **Graph type** field:
       - Measurement vs Well Col
       - Measurement: Number of Cells

     - **Set Display to Default** button
     - **Show Graph** button

2. Select the analysis containing the data you wish to graph from the *Analysis* field.
3. In the *Graph View* field, select what the source location for the data you want graphed.
4. Select a graph type from the *Graph Type* drop-down box. The options available for each graph type vary depending on the *Graph View* setting.
5. Select the measurement(s) to be graphed from the *Measurement* and *Measurement2* (if applicable) field(s).
6. If you selected *Histograms* from the *Graph Type* field, select the number of bins to display in the resulting histogram in the *Number of Bins* field.
7. Check the *Auto Scale* checkbox to automatically scale the bin(s) based on the range of data from the selected *Measurement*. This option is only available when *Histogram* is selected from the *Graph Type* field.
8. Click *Show Graph* to open the graph based on the current settings:
Figure 10-9
Sample Graph

Note: If the data on the graph is not displaying properly, click and drag one of the corners of the graph window to resize it.

9. To configure the graph settings, double-click inside the graph or click the Show Graph Menu arrow on the bottom left corner of the graph and select Graph Settings.

10. To set the display parameters for the current graph to the default view, click Set Display to Default. There are separate graph defaults for each combination of Graph View and Graph Type.
Measurement Settings
To specify well selection based on your specified measurements query criteria, complete the following procedure.

1. From the Review Plate Data dialog box, click the Measurements tab. The measurements tab moves to the front:

   **Figure 10-10**
   **Measurements Tab**

   ![Measurements Tab Image](image)

   - Analysis: Neurite Outgrowth: neurite
   - Measurement: Number of Cells
   - Display Format: \# \#\#\#

   - Select Wells Based On Variable Range
     - Value is: Between 0 and 100
     - Select

   - Data Log: DDE App

2. In the Analysis drop-down box, choose the analysis that contains the measurements to query.

3. In the Measurement drop-down box, choose the name of the measurement that you want to use to query your images in the database.

4. In the Display Format box, select the number of decimal places that you want to display for your data in the grid.

5. In the Value is box, select the variable range limit specifier, and the numerical value(s) of the range in the adjacent box(es).

6. Click Open Log to open either a Dynamic Data Exchange or a text log file, then click OK.

7. Click Configure Log to choose either Column and Row labels, Plate info, or both, then click OK.

8. Click Select to query the image database using your selected measurement and variable range limits. The selected wells or sites will be highlighted in green.

9. Click Log Data or press the F9 key to log your query data.

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

Logged Data Exported to an Excel Spreadsheet
You can also choose to log your data to an Excel spreadsheet. When you make this selection, the Excel program is automatically started and your data is automatically displayed in an Excel spreadsheet. You can use the capabilities of the Excel program to enhance your data for improved visual presentation.