

MetaXpress® Image Acquisition and Analysis Software

Analysis Guide

Version 2.0 for Microsoft Windows XP

0112-0170
September 2008

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Printed in USA

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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:

- **AutoRun** — 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 plates already stored in your MDCStore™ database and/or File server.
- **Batch Analysis** — Manually add analysis jobs on plates stored in your MDCStore database and/or File server to the AutoRun queue.



Note: You can also use the AcuityXpress cellular informatics software to further analyze your data.



Note: For more information about the MetaXpress acquisition workflow, refer to the “Acquisition guide” for your 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 1-1 Document Conventions

Bold type	Indicates a chapter or section heading, or is used for emphasis
Courier font	Indicates the name of a file or folder, the output of command, or text that you must type
Italic type	Indicates the name of a command or field, or text from a field, within a dialog box

Obtaining Support

Part of effective communication with MDS Analytical Technologies is determining the channels of support for the MetaXpress® application. 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. Press *F1* to access the online help for an active dialog box. In the Help window, click *See Also* to view and choose from a list of related topics.

2. Explore the MetaXpress Literature website for application notes — http://www.moleculardevices.com/product_literature/family_links.php?prodid=114.
3. Explore the Molecular Devices Support website — The support site, located at <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.
4. Internet Support — Fill out the Technical Support Request Form at http://www.moleculardevices.com/cgi-bin/support_request.cgi to send an e-mail to a pool of technical support representatives.
5. Call Customer Service — Contact Customer Service 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 — <http://www.microscopyu.com>
- ♦ The Molecular Probes handbook— <http://www.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:

- ♦ <http://www.chroma.com>
- ♦ <http://www.semrock.com>
- ♦ <http://www.omegafilters.com>



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.

Analyzing image data with the MetaXpress® software 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 how to use these tools to configure analysis and explains the common conventions used by the MetaXpress application modules.

MetaXpress application modules are software modules 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 to create an effective workflow. For more information, see [Chapter 6](#).

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.

Determining 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, see [Chapter 5](#).

1. In the **Review Plate Data** dialog box, locate and select the plate of interest. For information on the Review Plate Data dialog box, see [Using the Review Plate Data dialog box for Analysis on page 37](#).
2. Set viewing characteristics to improve the visibility of information.
3. Determine the analysis process to use – prepared application modules or custom journals.
4. Determine the settings needed. For specific instructions on performing manual analysis for the purpose of running a trial analysis, see [Chapter 7](#).
 - ◆ Make the initial settings.
 - ◆ Test the settings by running the analysis on selected wells.

- ◆ Examine and evaluate the initial results.
- ◆ Adjust settings to correct for inaccurate results.
- ◆ Repeat process until good results are obtained.
- ◆ Prepare to run automated analysis. For information, see [Chapter 8](#).

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 the automatic splitting of touching cells or objects to give a more accurate count.
- **Adaptive Background Correction™ (ABC) process** — 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 the 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 information on configuring application modules, see [Chapter 7](#).

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 apply 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.



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. For more information, see [Preparing For Analysis on page 37](#) 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.

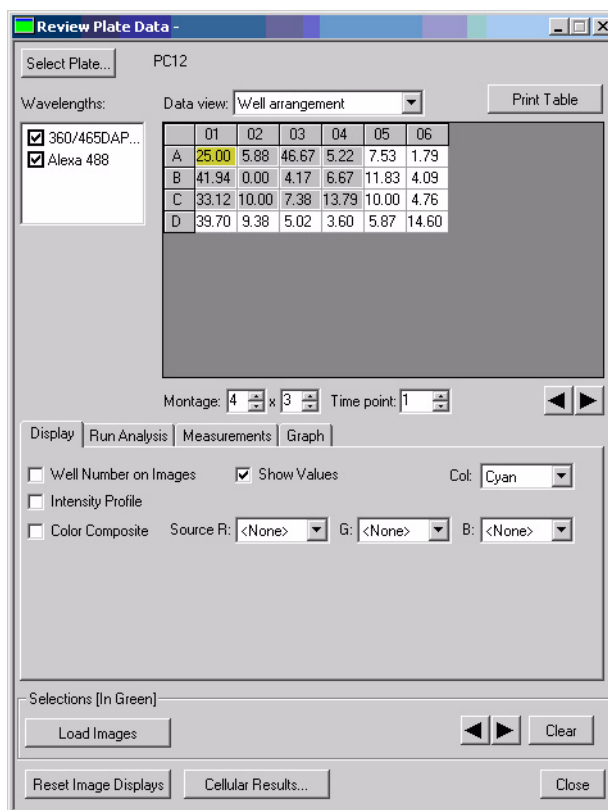


Figure 4-1 Review Plate Data dialog box – Analyzed plate open

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 Image Montage window in the layout in which the wells are presented in the microplate.

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 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, 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**.
2. In the **Review Plate Data** dialog box, 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 lower 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** field, clear the checkboxes for each wavelength to initially inhibit the display of images for each wavelength.
7. In the **Data view** drop-down list, select 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.
 - ◆ **Measurement vs Well** — 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 number 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]** section.
 - ◆ Click either the left or right arrow in the **Selections [In Green]** section 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.

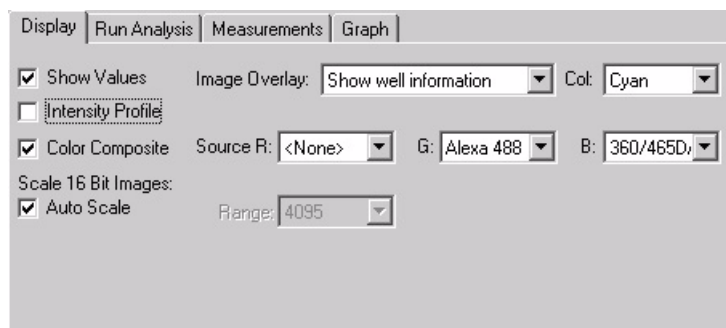


Figure 4-2 Review Plate Data dialog box – Display tab

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.



Note: 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.

To configure the display settings for the Review Plate Data dialog box

1. In the **Image Overlay** drop-down list, select 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. In the **Col** drop-down list, select a text color for the Well Number and/or the measurement values overlaid on the image.
3. Select the **Intensity Profile** check box to transform the image displayed into a three-dimensional intensity profile graph.
4. Select the **Color Composite** check box to combine images for two or three wavelengths into a single image using the color assignments in the **Source R/G/B** drop-down lists.
5. If the **Intensity Profile** and/or **Color Composite** check box is selected, select the **Auto Scale** check box to turn on auto scaling for 16-bit images, or clear to turn off auto scaling and manually specify the scaling range.
6. If the **Intensity Profile** and/or **Color Composite** check box is selected, and **Auto Scaling** is off, select the appropriate scaling range in the **Range** drop-down list.

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.

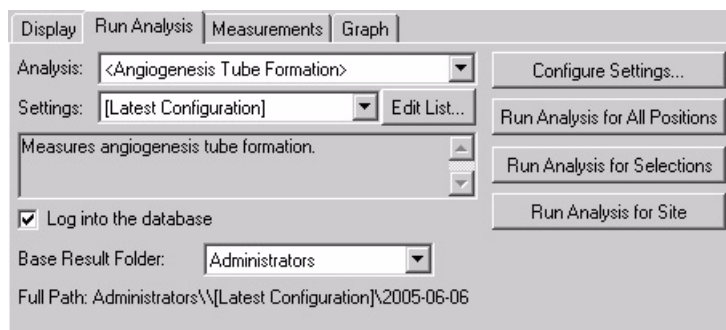


Figure 4-3 Review Plate Data dialog box – Run Analysis tab

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 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 the MetaMorph® software, 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.

To run an application module or an analysis created from a journal



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

1. In 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 list, select 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, go to [Step 10](#).
4. In the **Settings** drop-down list, select 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**.
7. In the **Edit List of Settings for <Neurite Outgrowth>**, click **New Settings**.
8. In the **New Settings for <Neurite Outgrowth>** dialog box, type a name in the **Name** field, 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. Use the help information for Neurite Outgrowth to 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. Select the **Log into the database** check box to log the measurement data from your analysis 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, select **Base folder for results**.
13. In the **Measurements Sets** dialog box, select the **Measurements Sets** folder and click **OK**.

All measurement data from your analysis will be stored as a subfolder under this folder.



Note: This step is necessary only the first time you run the analysis.



Note: Measurement sets and subfolders can be moved and renamed from within the AcuityXpress software as needed.

14. If you are running the analysis for all wells and all defined sites in the database, click **Run Analysis for All Positions**.
15. To run your analysis for only the selected wells, click **Run Analysis for Selections**.
16. To run your analysis for a specific site, select the site, and then click **Run Analysis for Site**.

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.

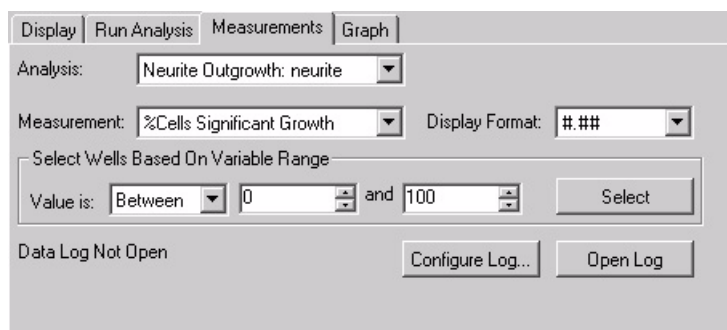


Figure 4-4 Review Plate Data dialog box – Measurements tab

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.

To specify well selection based on specified measurements query criteria

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

2. In the **Analysis** drop-down list, select the analysis that contains the measurements to query.
3. In the **Measurement** drop-down list, select the name of the measurement that you want to use to query your images in the database.
4. In the **Display Format** drop-down list, select the number of decimal places that you want to display in the grid.
5. In the **Value is** drop-down lists, select the variable range limit specifier and the numerical value(s) of the range.
6. Click **Open Log** to open either a Dynamic Data Exchange or a text log file, then click **OK**.
7. Click **Configure Log** to select either Column and Row labels, Plate info, or both, and 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 **F9** 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.



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

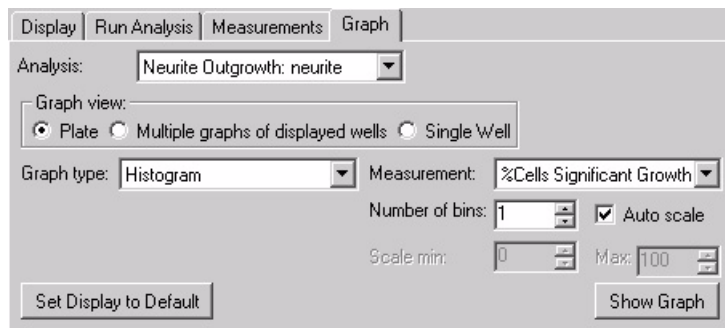


Figure 4-5 Review Plate Data dialog box – Graph tab

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:
- ◆ $\text{Well number} = \text{Current Column} + (\text{Current Row} * \text{Number of Columns})$
- ◆ 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 use 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: 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.

To display screen data on a graph

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

2. Select the data from the **Analysis** drop-down list.
3. In the **Graph view** section, select the source location for the data.
4. Select a graph type from the **Graph type** drop-down list.



Note: The options available for each graph type vary depending on the Graph View setting.

5. Select the measurement(s) from the **Measurement** and **Measurement2** (if applicable) drop-down list(s).
6. If **Histograms** is selected in the **Graph type** drop-down list, select the number of bins to display in the resulting histogram in the **Number of bins** list.
7. Select the **Auto Scale** check box to automatically scale the bin(s) based on the range of data from the selected Measurement.



Note: This option is only available when Histogram is selected from the Graph type drop-down list.

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 arrow on the bottom left corner of the graph and click **Graph Settings**. For examples of graphs, see [Graph Examples on page 33](#).
10. To set the display parameters to the default view, click **Set Display to Default** in the **Review Plate Data** dialog box.

There are separate graph defaults for each combination of Graph View and Graph Type. For examples of graphs, see [Graph Examples on page 33](#).

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.

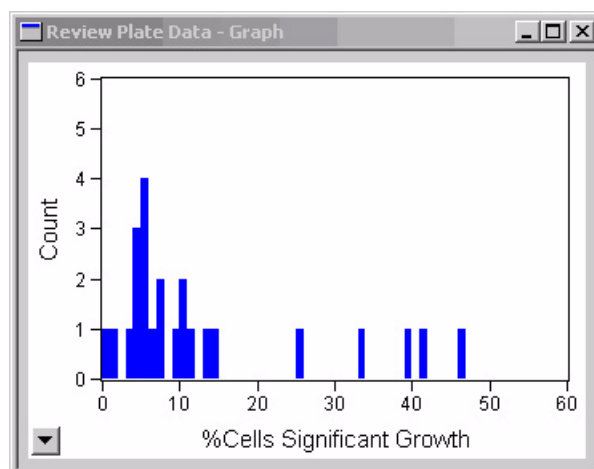


Figure 4-6 Histogram – Number of Cells (Count) versus the Percent of Cells of Significant Growth

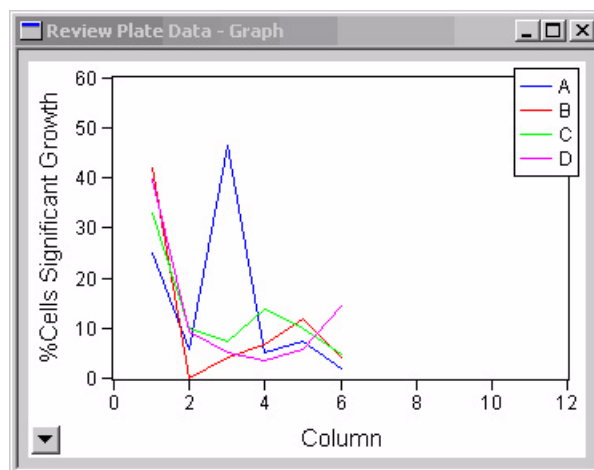


Figure 4-7 Measurement versus Well Column – Percent of Cells of Significant Growth versus Column

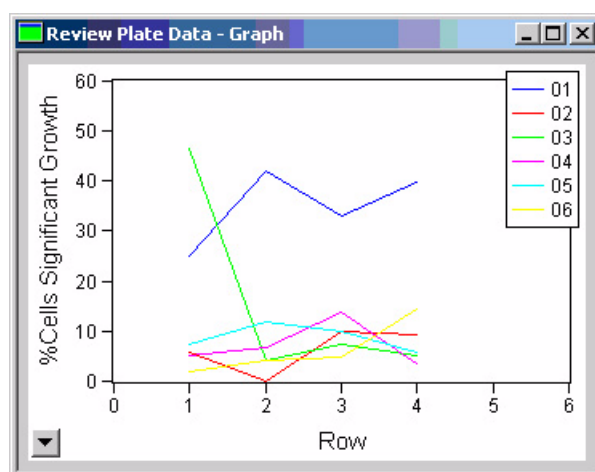


Figure 4-8 Measurement versus Well Row – Percent of Cells of Significant Growth versus Row number

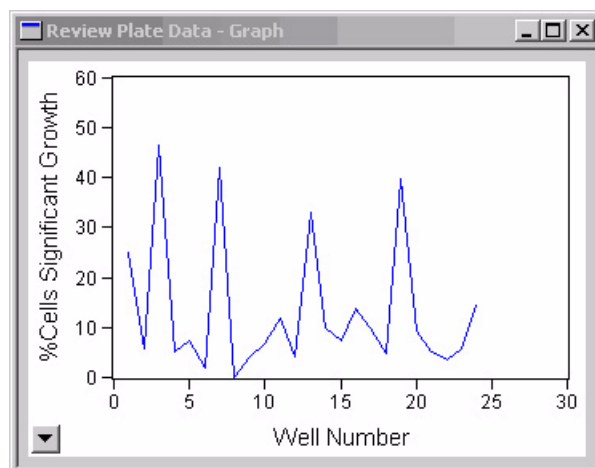


Figure 4-9 Measurement versus Well Number – Percent of Cells of Significant Growth versus the Well Number

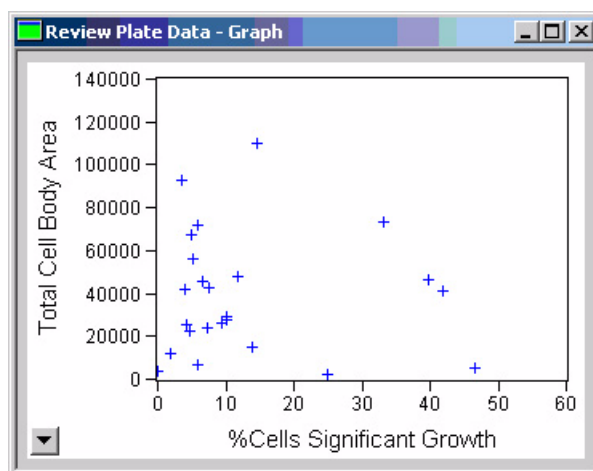


Figure 4-10 Scatter Plot – Total Cell Body Area versus the Percent of Cells of Significant Growth

There are two methods available to analyze acquired data — automated and manual. This chapter provides an overview of each, then explains how to use the Review Plate Data dialog box to manually open data for review. For information on the complete procedures, see [Chapter 7](#), and [Chapter 8](#).

Automated Analysis

The first opportunity to run a selected analysis on a plate is in conjunction with the acquisition of your plate image data. If analysis of the plate has been scheduled, automated analysis will begin after the acquisition of the last well. For information on how to initiate automated analysis, see [Chapter 8](#). With automated analysis, a single application module or journal set can be used to complete your analysis. Image data is retrieved from the MDCStore™ database and analyzed; the analysis results are subsequently stored back in the MDCStore 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

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

When you open the Review Plate Data dialog box without any plates open, it will appear as shown in [Figure 5-1](#).

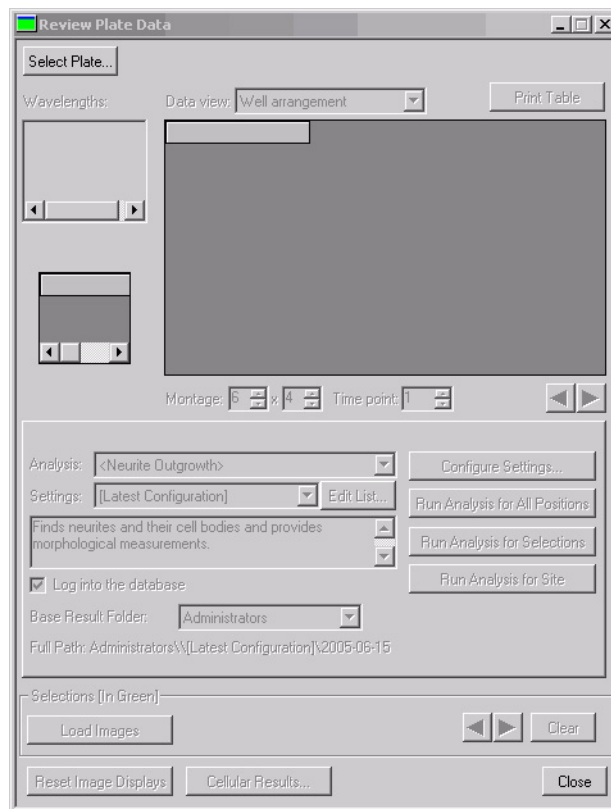


Figure 5-1 Review Plate Data dialog box with no plates selected

2. Click **Select Plate** to open the **Plate** dialog box and select one or more plates to analyze.

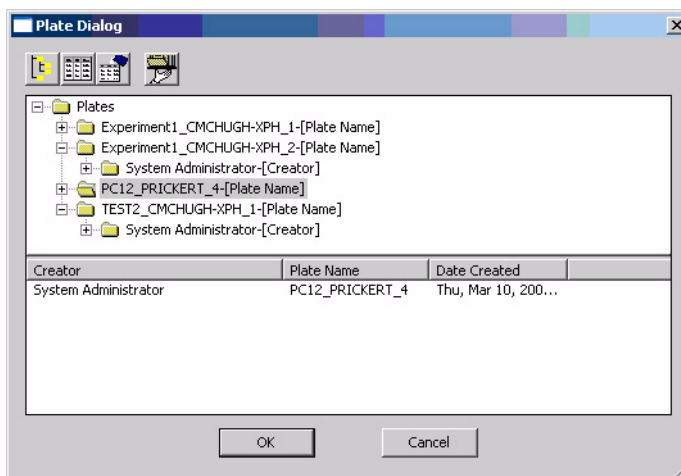


Figure 5-2 Plate Dialog with a plate selected

3. In the **Plates** folder, locate the plate that you want to analyze, and double-click the plate name.

The name of the plate and its associated attributes appear on the lower part of the Plate Dialog window.

4. Select the plate in the lower part of the Plate Dialog window and click **OK**.

The results of your query open in the Review Plate Data dialog box.

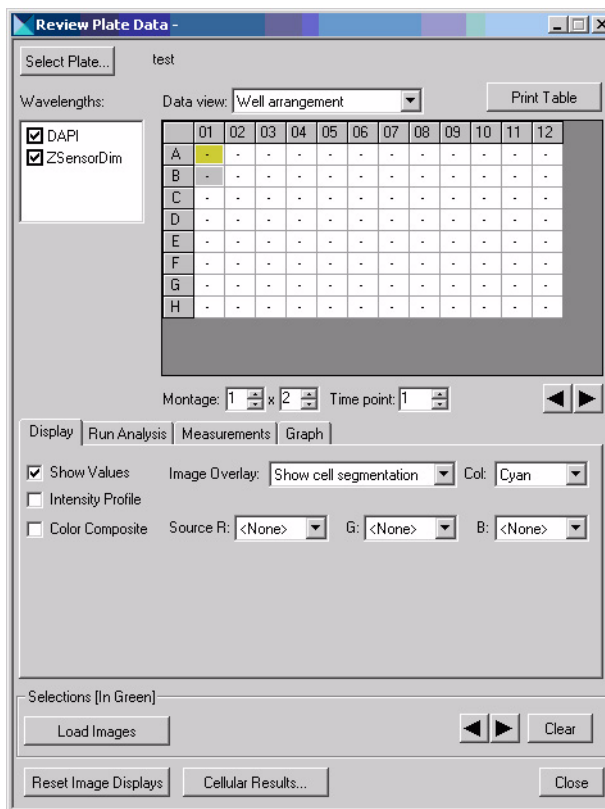


Figure 5-3 Review Plate Data dialog box with plate loaded for analysis

Plate Dialog Box Options

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



Figure 5-4 Plate Dialog options



Note: If you place the cursor 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. This button opens the Plate Query Attributes dialog box shown in [Figure 5-5](#).

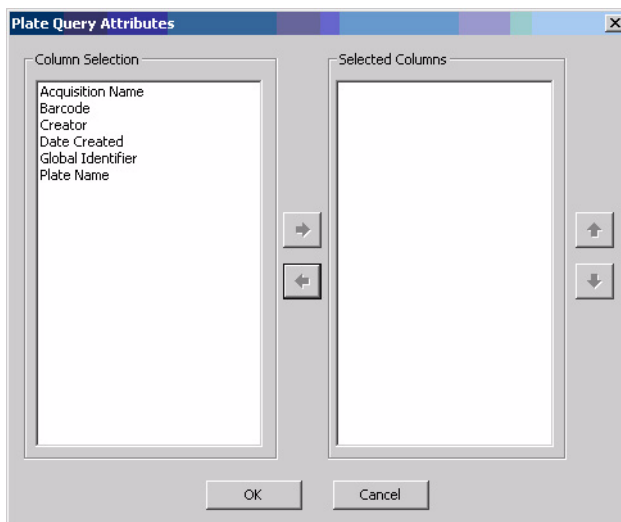


Figure 5-5 Plate Query Attributes window

This dialog box lists available query attributes in the Column Selection field.

To create a query

1. Highlight the attributes to include in your query, and click the right-facing arrow.

The attributes will be moved to the Selected Columns filed.

2. Use the up and down arrows to arrange the query sort order.

For example, if you want the primary sort to be based on all available users, place Creator at the top of your Selected Columns list. Then if you want to find all plates created on a specific date for each user, place Date Created next on the list. Finally, if you want to find all plates assigned to a specific acquisition name, 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 appears in the plate area:

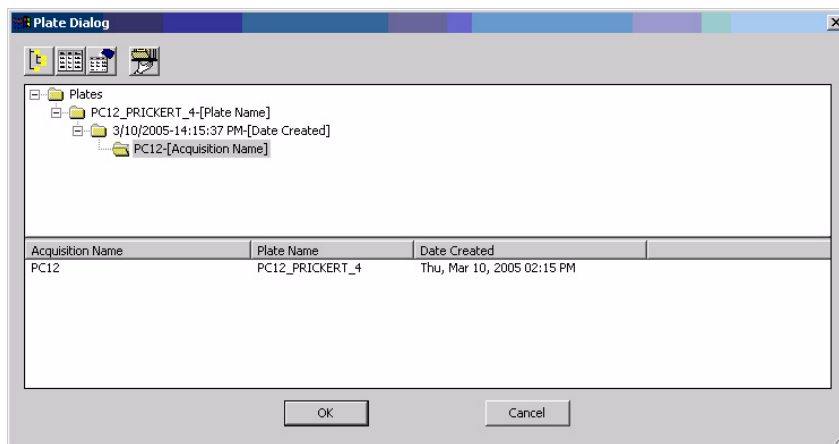


Figure 5-6 Plate Dialog showing query retrieval

Changing from Horizontal to Vertical Arrangement

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

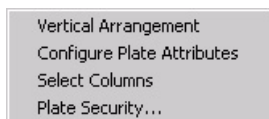


Figure 5-7 Plate Dialog menu

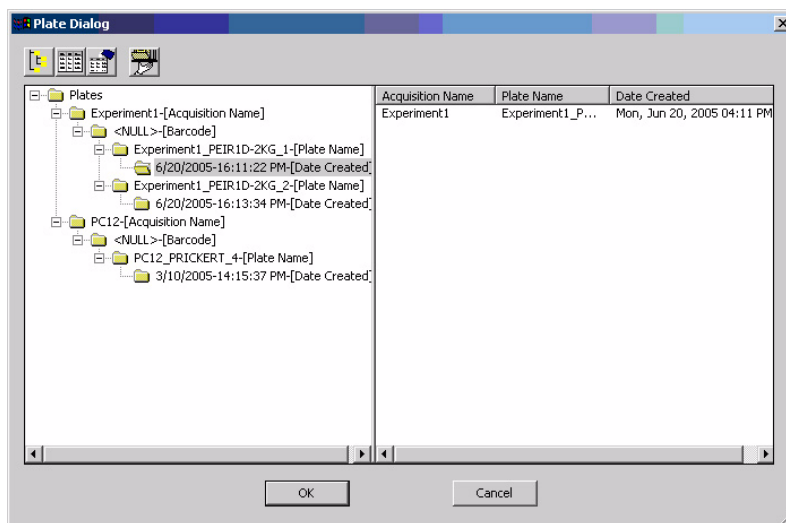


Figure 5-8 Plate Dialog showing query retrieval results in the vertical arrangement

Using the Plate Data Utilities Dialog Box for Manual Analysis

Use the Plate Data Utilities dialog box to run analysis on more than one plate at a time.

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

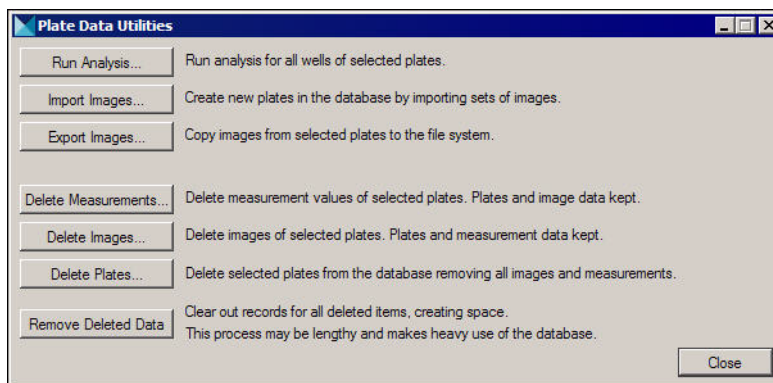


Figure 5-9 Plate Data Utilities dialog box

2. Click **Run Analysis**.
3. In the **Plates Data** 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. Press **Ctrl**, and click to select multiple plates.

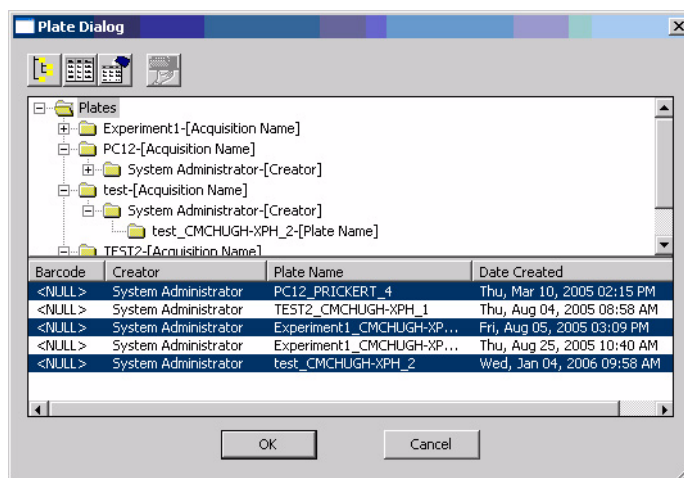


Figure 5-10 Plate Dialog with multiple plates selected

4. Click **OK**.

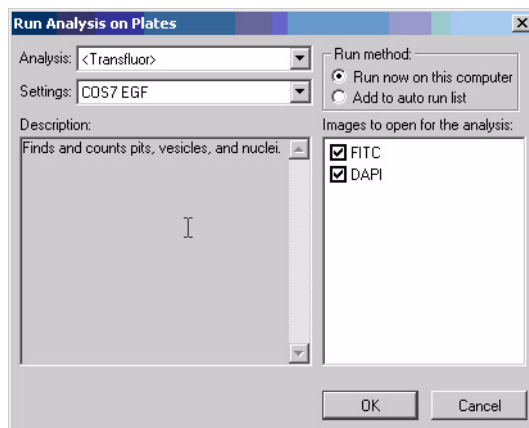


Figure 5-11 Run Analysis on Plates dialog box

5. In the **Run Analysis on Plates** dialog box, select the analysis, settings, and the results folder for the selected plates.
6. Select one of the following from the **Run method** section:
 - ♦ **Run now on this computer** — Runs all selected analyses on the computer being used.
 - ♦ **Add to auto run list** — Adds the selected analyses to the **Auto Run** queue.
7. Click **OK** to start the analysis for the selected plates.



Note: For information on performing analysis, see [Chapter 5](#), and [Chapter 8](#).



Molecular Devices offers optional MetaXpress® application modules that automate common analysis tasks such as segmentation and cell counting. These tasks can be run from the Review Plate Data dialog box, opened as a stand alone dialog box, or used within a custom journal. These tasks 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 to 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, Multi Wavelength Translocation, 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 TransfluoTM assay for GPCR activation. To count and otherwise measure the labeled pits or vesicles, or other punctuate staining, use the Granularity, Transfluo, or Transfluo 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.



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
- Cell-by-cell multiplexing with application modules in the MetaXpress 2.0 software



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



Note: 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.

To open the image(s) to process with the application module

1. From the **Screening** menu, click **Review Plate Data**.
2. In the **Review Plate Data** dialog box, click **Select Plate**.
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.

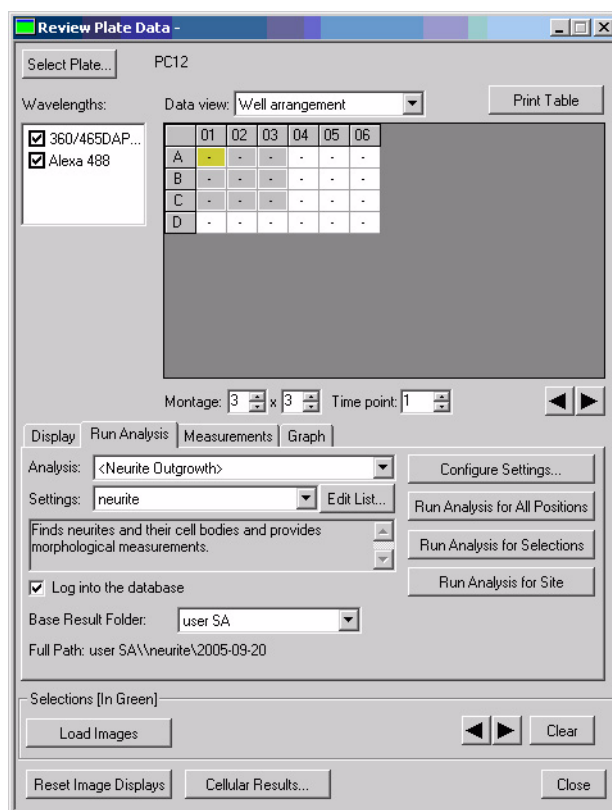


Figure 7-1 Review Plate Data dialog box



Note: For information on opening plates from the Plate dialog box, see [Chapter 5](#).



Note: The montage windows for each wavelength do not open automatically even if the wavelength boxes are checked.

4. To view the montage window for an individual wavelength, perform one of the following:
 - ◆ Click well A1

- ◆ Clear and then select 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**
5. Select a site or well to display a montage window for each selected wavelength.
 6. Click a thumbnail image in the montage window to open the full size image(s) for the site or well. For information on opening images from a montage window, see [Selecting an Image from the Montage View on page 63](#).
 7. 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.

To select source and result images

1. In the **Review Plate Data** dialog box, click the **Run Analysis** tab.
2. Select an application module from the **Analysis** drop-down list.



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

3. Click **Configure Settings**.

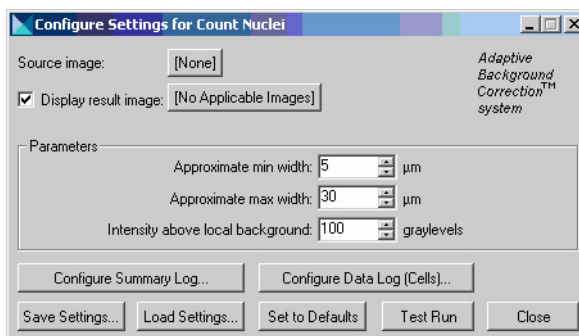


Figure 7-2 Configure Settings for Count Nuclei dialog box

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.



Note: There are two ways to view the results of each application module: Displaying a result image or using an image overlay. Selecting Display result image will open or overwrite (see [Step 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 **Display result image**.
6. Open the image selector for **Display result image** and if needed change it one of the following:
 - ◆ **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.

To determine measurements

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 determine the width of objects using the Region tools Line tool:

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



Figure 7-3 Region Toolbar with Line Tool selected

2. Select the **Line** tool as shown in [Figure 7-3](#).
3. Place the cursor on the image and locate the object that you want to measure.
4. Move the cursor 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 cursor to the opposite edge of the object and note the Length value.

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

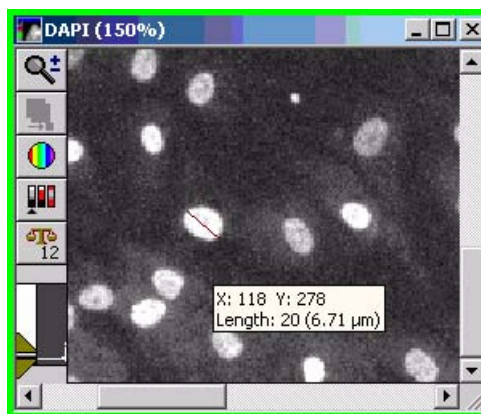


Figure 7-4 Sample Region Width Measurement

6. Type or select the value in microns in the **Approximate max width** field of the **Configure Settings** dialog box. [Figure 7-5](#) shows the new Approximate max width value.

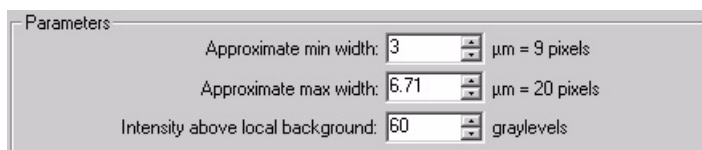


Figure 7-5 Changing the Approximate max width value

7. Click **Clear Regions** from the **Regions** menu to remove the region lines, or right-click on the region and click **Delete Region**.

To measure the width of an object using the caliper tool

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.

1. From the **Measure** menu, click **Calipers**.

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

The calipers appear on the selected image, as shown in [Figure 7-6](#).

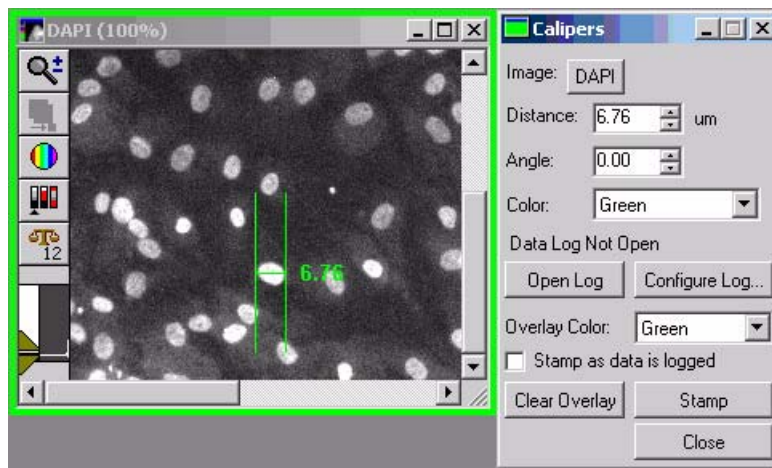


Figure 7-6 Calipers Sample Image and dialog box

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

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

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

To determine the width of an object using the Show Region Statistics 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.

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



Figure 7-7 Region Toolbar with Trace Region tool selected

2. Select the **Trace Region** tool as shown in [Figure 7-7](#).
3. Place the cursor 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, click **Show Region Statistics**.
The Show Region Statistics dialog box opens.

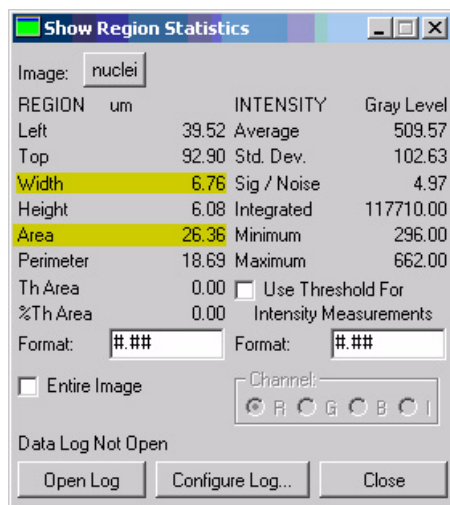


Figure 7-8 Show Region Statistics dialog box

5. Clear the **Entire Image** check box to view the area and/or width measurements for the active region created in [Step 3](#).

To determine accurate intensity above background values

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.

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



Figure 7-9 Region Toolbar with Arrow tool selected

2. Select the **Arrow** tool as shown in [Figure 7-9](#) (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](#).




Figure 7-10 Bottom of the MetaXpress® desktop with current gray-level highlighted

5. Move the cursor 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. Proceed to the next procedure without closing the application module dialog box.

Testing and Saving Settings

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



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

1. Click **Test Run** in the application module dialog box 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.

A Cellular Results table containing individual object data also opens.

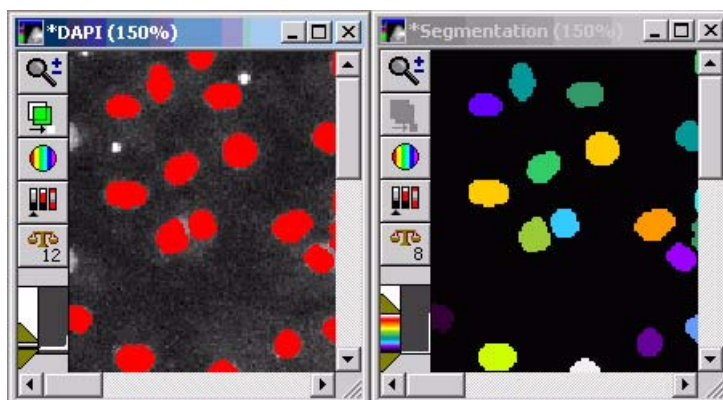


Figure 7-11 Sample Source and Result images

2. Click a cell in the image to highlight the data in the table for that selected cell. To select and view more than a single segment line of data, click and press **Ctrl**. To select non contiguous cells or lines of data, click and press **Shift**. If the table does not open when you run an application module, select **Show Cellular Results** in the **Window** menu.
3. 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.
4. Click **Configure Summary Log** to configure logging the application module data.

The Configure Log dialog box opens.

5. Select and/or clear 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.
6. Click **Configure Data Log** and configure the Data log if needed.
7. Click **Save Settings**.
8. Enter a name and description for the settings in the **Save Settings** dialog box and click **OK**.

The settings are saved to the database.

Selecting an Image from the Montage View

In 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.

1. Select the **Well Number on Images** check box on the **Display** tab to make it easier to identify and locate your sample well or site.

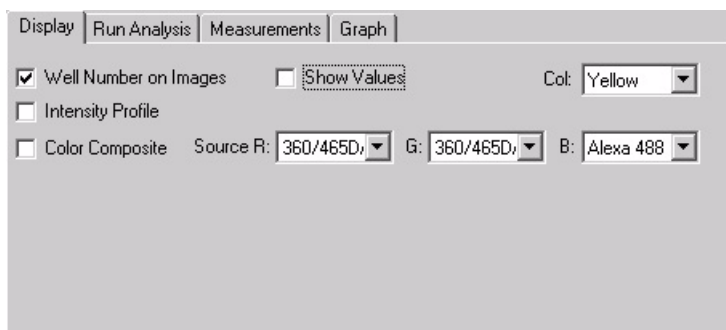


Figure 7-12 Review Plate Data dialog box – Display tab

Figure 7-13 shows a montage window without well numbers:

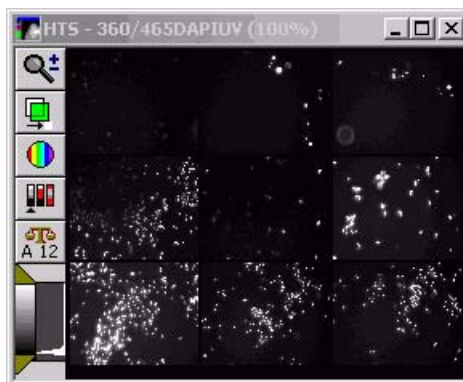


Figure 7-13 Montage window without well numbers

Figure 7-14 shows a montage window with well numbers. The well numbers on the Montage window are an image overlay.

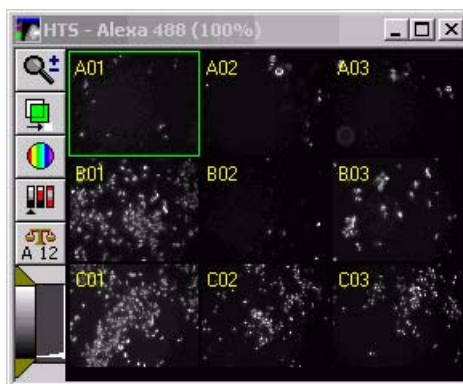


Figure 7-14 Montage window with well numbers and well A1 selected

2. Click the **Overlay** button on the upper left side to toggle the overlay (Montage) on and off.
3. Click the image to view an individual image in the Montage window.
4. Right-click an image to select the image in the well plate table.

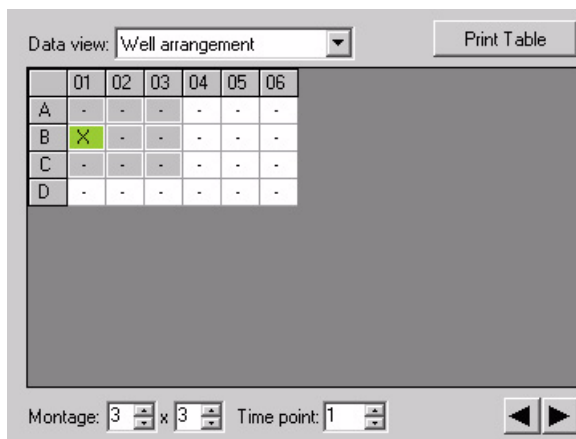


Figure 7-15 Review Plate Data - Well selection table

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.

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® software.

Configuring the Transfluor™ Module

1. In the MetaXpress® software, load the experiment through the **Review Plate Data** dialog box as described in the previous procedures.

2. Click on one of the wells to display an image.

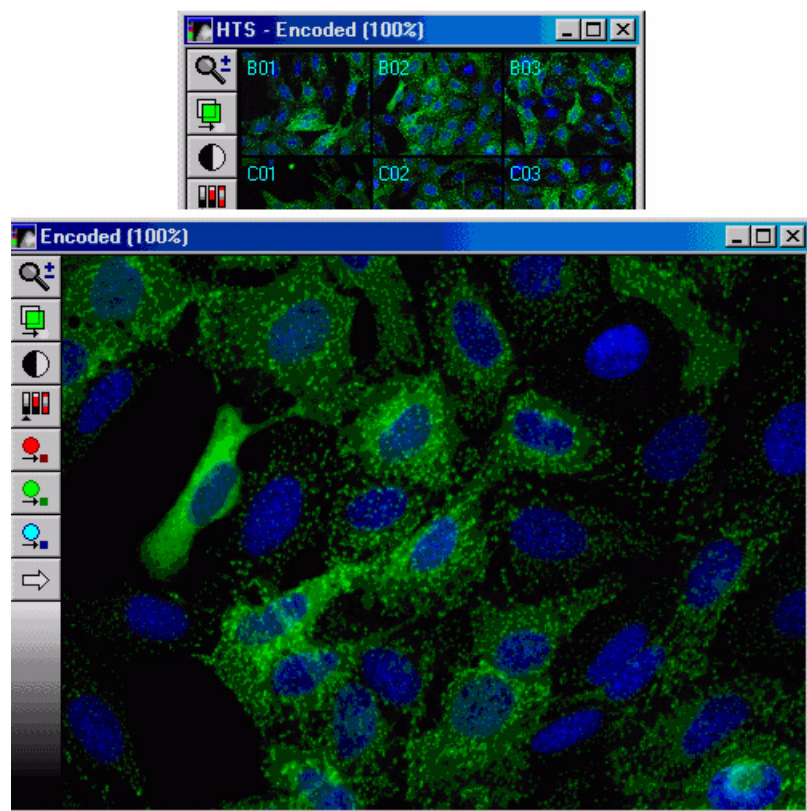


Figure 7-16 Montage window and open image

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**.
6. In the **Configure Settings for Transfluor** dialog box, click **Pits and Vesicle image**, then select the wavelength image representing the pit and vesicles opened in [Step 3](#).
7. Click on the pits and vesicles image, then increase the zoom by pressing **Page Up** on the keyboard.
8. Use the **Single Line** tool of the **Region** toolbar to measure a granule width by following these steps:
 - ◆ From the **Regions** menu, click **Region Tools**.
 - ◆ Select the **Single Line** tool



Figure 7-17 Region Tools with the Single Line tool selected

- ◆ Move to the image window from [Step 5](#) and locate a small pit.
- ◆ Move the cursor to one of edge of the pit and click to anchor the line in position.
- ◆ Move the cursor to the other edge of the pit and read the length of the line region.

In [Figure 7-18](#), the length is 3 pixels or 1.41 μm .

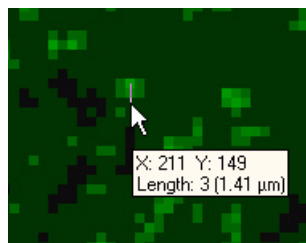


Figure 7-18 Montage window and open image

- ◆ Enter this number in the **Approximate min width** field in the **Pits** section.

- ◆ Repeat [Step 8](#) for a larger pit. Enter that number in the **Approximate max width** field in the **Pits** section.
- 9. To determine the **Intensity over local background** value, select the **Locator Arrow** on the **Region** toolbar.
- 10. Find the dimmest pit.
- 11. Position the cursor over the pit and read the gray value.
This is the number that is displayed at the middle of the bottom of the screen after the arrow. In the example below it is 162.



Figure 7-19 Gray value

- 12. Move the cursor to just outside the granule to measure the background.
- 13. 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** field. For this example, a value of 800 is appropriate.
- 15. Repeat [Step 6](#) to [Step 12](#) for vesicles, if applicable (if not, then clear the **Vesicles** checkbox).



Note: Vesicles are much larger and brighter than pits.

- 16. Clear the **Nuclear stain** checkbox 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.

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



Figure 7-20 Montage window and open image

19. If the pits are not all detected, then lower the **intensity above local background** parameter. If this does not produce the result that you want, then change the other parameters.
20. Select a negative control image and click **Test Run** to make sure that your settings are correct.
21. If you are using a nuclear stain, then reselect the **Nuclear stain** checkbox and repeat [Step 6](#) to [Step 12](#) for the nuclei.
22. Click **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 line of data, press Ctrl+click; to select non contiguous cells or lines of data can, press Shift+click.
23. Click **Save Settings**.
24. Click **Close**.

Running the Assay

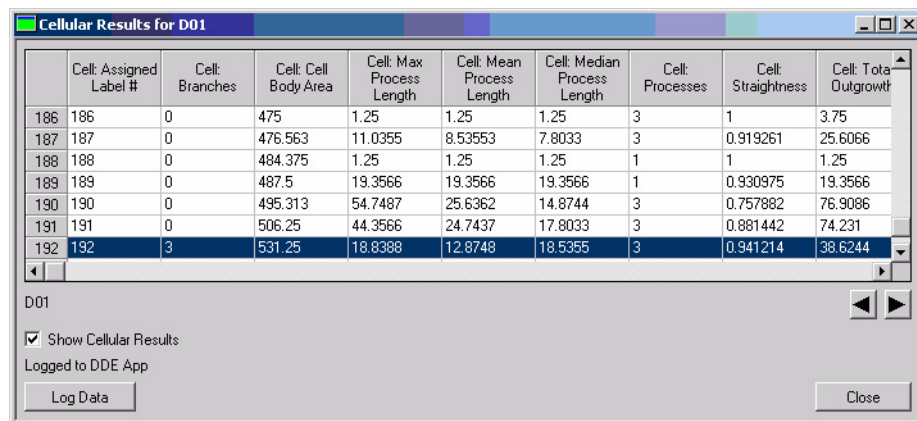
1. On the **Run Analysis** tab of the **Review Plate Data** dialog box, select **<Transfluor>** from the **Analysis** drop-down list.

2. Select the saved setting in the **Settings** drop-down list.
3. Click one of the following options:
 - ◆ Click **Run Analysis for All Positions** to run the assay for all positions on the plate.
 - ◆ Click **Run Analysis for Selections** to run the assay for the selected sites. Select sites by right-clicking on different wells in the **Review Plate Data** dialog box.
 - ◆ Click **Run Analysis for Site** to run the assay for the current active site.
4. After the analysis is run, view the results by measurements (in the **Measurements** tab of the **Review Plate Data** dialog box) or by graph (in the **Graph** tab of the **Review Plate Data** dialog box).

For more information on Transfluor™ screening, see <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 line of data, use Ctrl+click; to select non contiguous cells or lines of data, use Shift+click.



	Cell: Assigned Label #	Cell: Branches	Cell: Cell Body Area	Cell: Max Process Length	Cell: Mean Process Length	Cell: Median Process Length	Cell: Processes	Cell: Straightness	Cell: Total Outgrowth
186	186	0	475	1.25	1.25	1.25	3	1	3.75
187	187	0	476.563	11.0355	8.53553	7.8033	3	0.919261	25.6066
188	188	0	484.375	1.25	1.25	1.25	1	1	1.25
189	189	0	487.5	19.3566	19.3566	19.3566	1	0.930975	19.3566
190	190	0	495.313	54.7487	25.6362	14.8744	3	0.757882	76.9086
191	191	0	506.25	44.3566	24.7437	17.8033	3	0.881442	74.231
192	192	3	531.25	18.8388	12.8748	18.5355	3	0.941214	38.6244

D01

☒ Show Cellular Results

Logged to DDE App

Log Data

Close

Figure 7-21 On-screen cellular results table for Neurite Outgrowth



Note: For more information about using the Cellular Results table, see [Cellular Results Table on page 101](#) or the 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.

1. Click **Open Log** in the **Cellular Results** window or click **Open Data Log** on the **Log** menu to open a **Data Log**.

The Open Data Log 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.

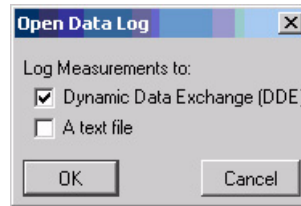


Figure 7-22 Open Data Log dialog box

2. Select the **Dynamic Data Exchange (DDE)** check box to log your data to an Excel Spreadsheet, and then click **OK**.

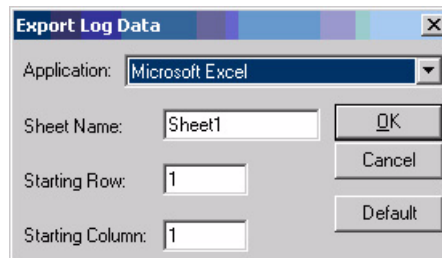


Figure 7-23 Export Log Data dialog box

3. In the **Export Data Log** dialog box, select **Microsoft Excel** from the **Application** drop-down list. Specify the **Sheet Name**, the **Starting Row**, and the **Starting Column**. Click **OK**.

The Excel application will launch and an empty spreadsheet will appear.

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

An Excel spreadsheet will appear similar to the following table:

	A	B	C	D	E	F	G	H	I	J
1	Cell: Assid	Cell: Total	Cell: Proce	Cell: Mean	Cell: Mediz	Cell: Max	Cell: Branc	Cell: Straic	Cell: Cell	Body Area
2	1	1.25	1	1.25	1.25	1.25	0	1	34.375	
3	2	1.25	1	1.25	1.25	1.25	0	1	40.625	
4	3	1.25	1	1.25	1.25	1.25	0	1	40.625	
5	4	25	2	12.5	12.5	22.5	0	1	43.75	
6	5	26.6421	2	13.3211	13.3211	23.6244	0	0.865523	53.125	
7	6	8.01777	1	8.01777	8.01777	8.01777	0	0.95086	57.8125	
8	7	0	0	0	0	0	0	0	57.8125	
9	8	0	0	0	0	0	0	0	57.8125	
10	9	14.7855	1	14.7855	14.7855	14.7855	1	0.929963	59.375	
11	10	0	0	0	0	0	0	0	60.9375	
12	11	20.8211	4	5.20527	3.38388	11.5533	0	0.874152	64.0625	

Figure 7-24 Excel spreadsheet containing the same data as the cellular results table

Cell-by-cell Multiplexing with Application modules in the MetaXpress® Software

One of the advantages of doing high-content screening is the ability to multiplex assays by running multiple analyses on the same samples (for example, Cell Scoring to measure transfection efficiency, and a TransfluoTM assay to measure receptor internalization). In the MetaXpress® software, version 2.0, these analyses may now be multiplexed on cell-by-cell data.

MetaXpress® image analysis

1. Configure your analysis settings for each application module appropriately. Make sure that the same nuclear detection settings are selected for each module.
2. Run each application module separately for selected wells, or for all positions as desired.
3. Compatible modules include: Cell Cycle, Cell Health, Cell Scoring, Count Nuclei, Granularity, Mitotic Index, Monopole Detection, Multi Wavelength Cell Scoring, Multi Wavelength Translocation, Neurite Outgrowth, and TransfluoTM assays.
4. Review the data in either the AcuityXpressTM application or in another application such as Microsoft Excel.

For more information, please contact your MDS Analytical Technologies representative.



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 run on systems running the MetaXpress application or the MetaXpress offline application 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. After the Auto Run has started, use the Auto Run Plate Statuses dialog box to monitor and control the progress of each analysis running for each plate being analyzed.

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. In the **Post Acquisition** tab, complete the steps described in [Plate Acquisition Setup - Post Acquisition Tab on page 76](#).
3. Complete the steps in the [Auto Run Mode on page 78](#).
4. To view the **Auto Run Status**, complete the steps in the [Auto Run Status on page 80](#).

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:



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



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

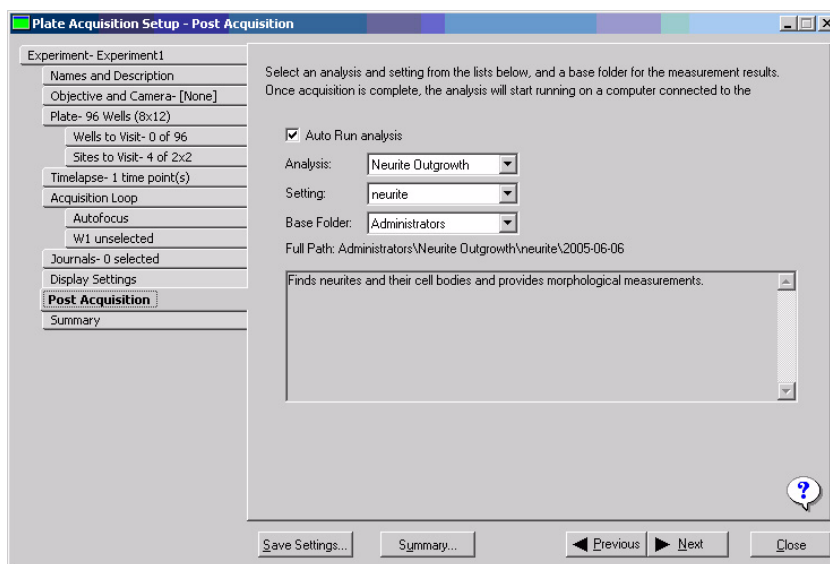


Figure 8-1 Plate Acquisition Setup dialog box - Post Acquisition tab

1. In the Post Acquisition tab, select the **Auto Run analysis** check box.
2. Select the assay (Application module or Journal Assay) to run after acquisition from the **Analysis** drop-down list.



Note: Only analyses 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 an 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 to acquire and analyze your screening data greatly reduces the overall screening time by freeing up the main MetaXpress computer to continue acquisition. 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 the Auto Run mode, the application cannot be used for any other purpose.

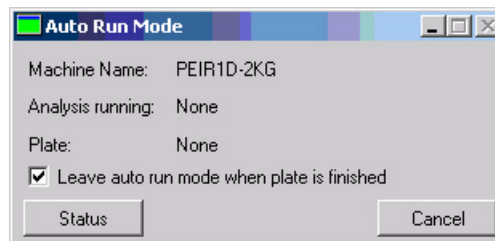


Figure 8-2 Auto Run Mode dialog box

Use the following procedure to set-up and use the Auto Run Mode command:

1. On the computer acquiring the images, open the MetaXpress® software and from the **Screening** menu, click **Plate Acquisition Setup**.
2. In the **Plate Acquisition Setup** dialog box, click the **Post Acquisition** tab.
3. Select the **Auto Run analysis** checkbox, and select an analysis in the **Analysis** drop-down list.
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 the MetaXpress software and, from the **Screening** menu, click **Start Auto Run Mode**.

The Auto Run Mode dialog box opens.

6. To run the analysis on more than one plate, clear the **Leave auto run mode when plate is finished** check box.
7. To check the status of the analysis, click **Status** to open the **Auto Run Status** dialog box on the computer running the assay.

OR

On another computer running the MetaXpress software and logged into the database, click **Start Auto Run Plate Status** in the **Screening** Menu.

8. After the analysis is 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

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

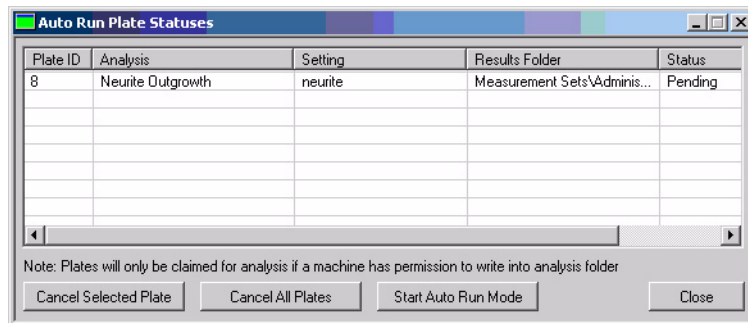


Figure 8-3 Auto Run Plate Statuses dialog box



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.

1. On the computer running the analysis, from the **Screening** menu, click **Auto Run Plate Statuses [DB]**.
OR
From the **Screening** menu, click **Start Auto Run Mode [DB]**, and in the **Auto Run Mode** dialog box, click **Status**.

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

Auto Run Plate Statuses 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** — 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. After the analysis is completed for the plate, the plate will be removed from the auto run plate status list.
 - ◆ **Timeout** — Indicates that the analysis has not completed 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 diagnose the cause of the timeout, inspect the machine that has timed out for error messages or other problems. In some cases the problem can be resolved and the analysis can continue. If this happens the status will return to Running. In other cases, the Auto Run must be canceled and the analyses run again. Some analyses, particularly custom ones created through the journaling system, take a long time to complete. In this case 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 that 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 the Auto Run mode, the application cannot be used for any other purpose.

- **Close** — Closes the dialog box.

The Plate Data Utilities and Plate dialog boxes enable 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 the selection of 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.

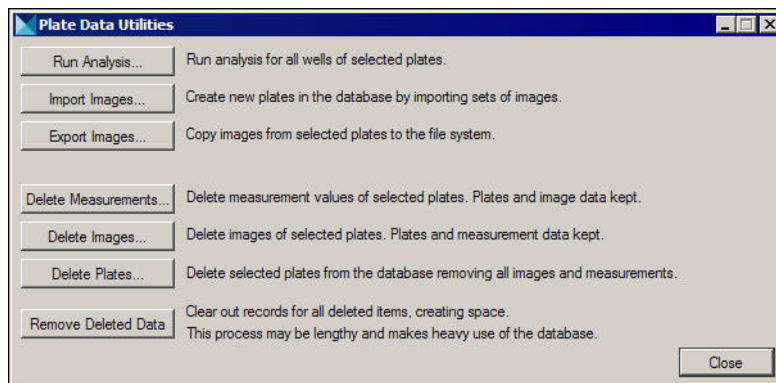


Figure 9-1 Plate Data Utilities dialog box

Most of the commands in the Plate Data Utilities dialog box open the Select Plates for Analysis dialog box. The Select Plates for Analysis 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.

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

The Run Analysis on Plates dialog box opens.



Note: To run an analysis on more than one plate, use Ctrl+click to select multiple plates, as shown in [Figure 9-2](#).

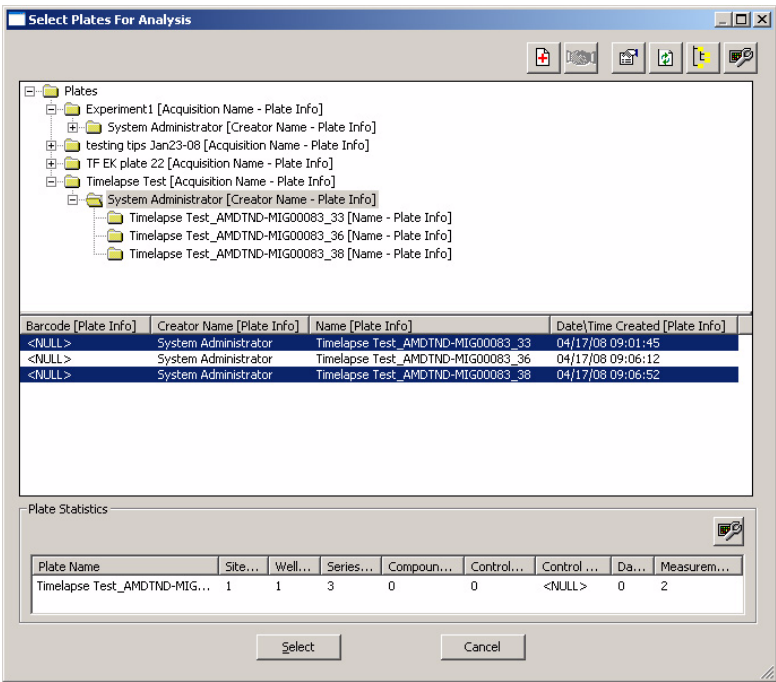


Figure 9-2 Select Plates for Analysis dialog box

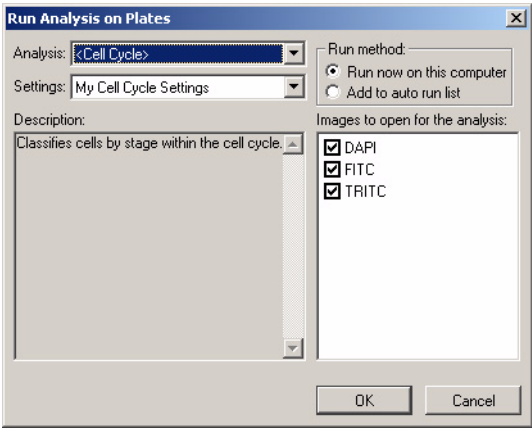


Figure 9-3 Run Analysis on Plates dialog box

6. In the **Analysis** drop-down list, select the assay that you want to run.
7. In the **Settings** drop-down list, select the setting that you want to use.
8. In the **Images to open for the analysis** field, select the wavelengths to use with the selected assay.
9. 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 MDS Analytical Technologies 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: The MetaXpress software and the MDCStore database do not import third party images by default.

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

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

The path is displayed next to the Select Directory button and the HTD files are displayed in the HTD Files field.

4. Select the data set(s) to be imported by selecting 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** drop-down list.



Note: The locations in this list were configured using the 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, proceed to [Importing Cellomics Data on page 88](#).
8. Click **Cancel** to close the dialog box.

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 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 check boxes 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 Special: Opens the Import Special dialog box, which enables you to import Cellomics data.

Cancel: Cancels the command.

Importing Cellomics Data

Use the Import Special 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) dialog box or the Review Plate Data (DB) dialog box. The Import Special 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. A default calibration of 1 μm = 1 pixel will be applied to the imported images unless you enter a specific calibration when you import the data.

Use the following procedure to import Cellomics data:

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

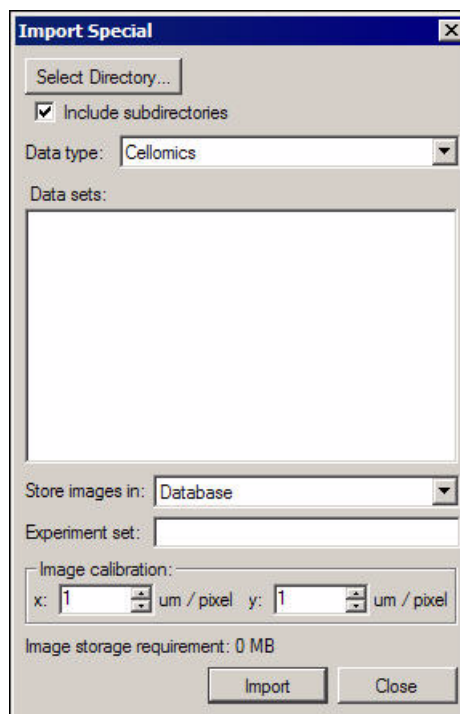


Figure 9-4 Import Special dialog box

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

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

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

The files are imported and will be available for review in the Review Plate Data (DB) dialog box.
8. Click **Close** to close the dialog box.

Import Special 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.

Data type: Enables you to select the Cellomics data type.

Data sets: 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 are configured using the 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 $1\ \mu\text{m} = 1\ \text{pixel}$.

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, click **Plate Data Utilities**.

2. In the **Plate Data Utilities** dialog box, click **Export Images** to export images to a local or networked server.
3. In the upper pane of the **Select Plate for Export** dialog box, expand the **Plate** folder to view folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Select the plate to export from the lower pane and click **Select**.
6. In the **Browse for Folder** dialog box, navigate 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 Select Plates for Deletion dialog box. After that, use the Plate Data Utilities dialog to permanently remove the plates from the database or files server.

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



Note: This step removes the data from the Select Plate for Deletion dialog box but not from the database/files server. To remove the data from the database/files server, you must click Remove Deleted Data after using the Select Plate for Deletion 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 Select Plates for Image Deletion 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, click **Plate Data Utilities**.
2. In the **Plate Data Utilities** dialog box, click **Delete Images**.
3. In the upper pane of the **Select Plates for Image Deletion** dialog box, expand the **Plate** folder to view folders containing plates saved to the database.
4. Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the lower pane.
5. Select the plate containing the images to delete from the lower pane and click **Select**.



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 **Selected Plates for Image Deletion** 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 in the Select Plates for Measurement Deletion dialog box. After that, use the

Plate Data Utilities dialog to permanently remove the measurements from the database or fileserver.

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



Note: This step does not remove the data from the database/fileserver. To remove the data from the database/fileserver, you must 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.



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 the AcuityXpress™ software.

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

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. Select the 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.

PC12

Data view: Well arrangement Print Table

	01	02	03	04	05	06
A	25.00	5.88	46.67	5.22	7.53	1.79
B	41.94	0.00	4.17	6.67	11.83	4.09
C	33.12	10.00	7.38	13.79	10.00	4.76
D	39.70	9.38	5.02	3.60	5.87	14.60

Montage: 1 x 4 Time point: 1

Figure 10-1 Neurite Outgrowth – Well table showing analysis results for a single measurement

Review Plate Data dialog box options

Data view: Allows you to select how to view the data in 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 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.

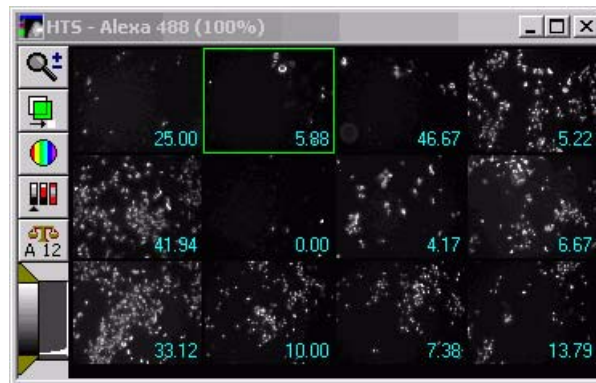


Figure 10-2 Neurite Outgrowth – Montage window showing analysis results for a single measurement overlaid on the image thumbnails

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, select the Well Number on images check box. If you want the value shown in the table displayed on the image, select the Show Values check box. To change the color of these values displayed on the thumbnail images, open the Col drop-down list and choose a different color.

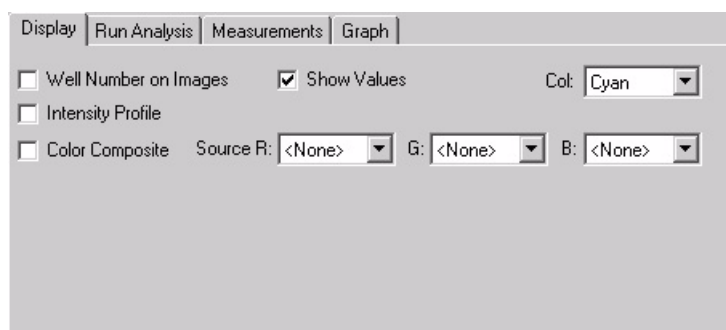


Figure 10-3 Review Plate Data dialog box – Display tab

Display tab options

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 select 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 selected.

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 select 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 on and off one or two of the wavelengths to enable you to view the image data for each wavelength separately.

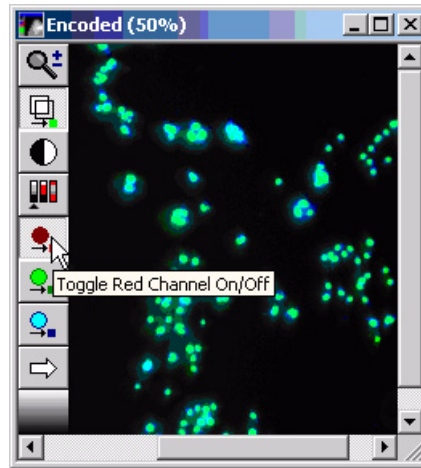


Figure 10-4 Neurite Outgrowth Color Composite image window with the Red Channel turned off

Application Module Segmentation Image Overlay

After an application module has 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:

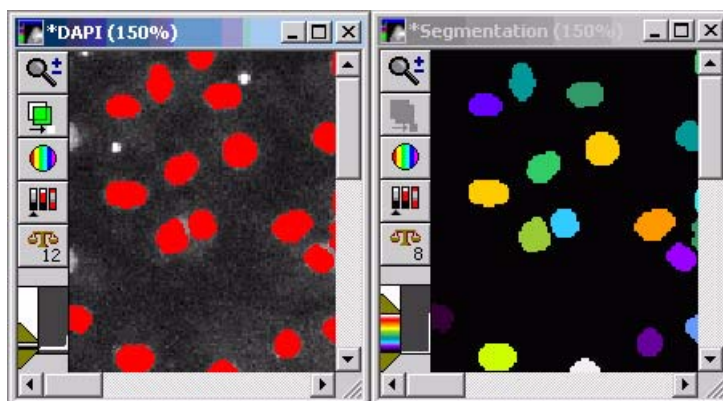


Figure 10-5 Sample Source and Result images

The source image contains the 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 graphical 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 the 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 interactively 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 using Ctrl+Click. You can also select a range of cells from the Cellular Results table using Shift+Click.



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.

Cellular Results for D01									
	Cell: Assigned Label #	Cell: Branches	Cell: Cell Body Area	Cell: Max Process Length	Cell: Mean Process Length	Cell: Median Process Length	Cell: Processes	Cell: Straightness	Cell: Total Outgrowth
121	121	0	229.688	10.3033	5.77665	5.77665	2	0.942177	11.5533
122	122	0	231.25	13.3211	7.28553	7.28553	2	0.909536	14.5711
123	123	0	232.813	0	0	0	0	0	0
124	124	0	232.813	23.6244	23.6244	23.6244	1	0.758838	23.6244
125	125	0	234.375	0	0	0	0	0	0
126	126	0	234.375	18.3211	9.78553	9.78553	2	0.904749	19.5711
127	127	0	235.938	26.8566	10.7915	4.26777	3	0.897163	32.3744

D01

☒ Show Cellular Results
Logged to DDE App

Log Data Close

Figure 10-6 Neurite Outgrowth – Cellular Results dialog box

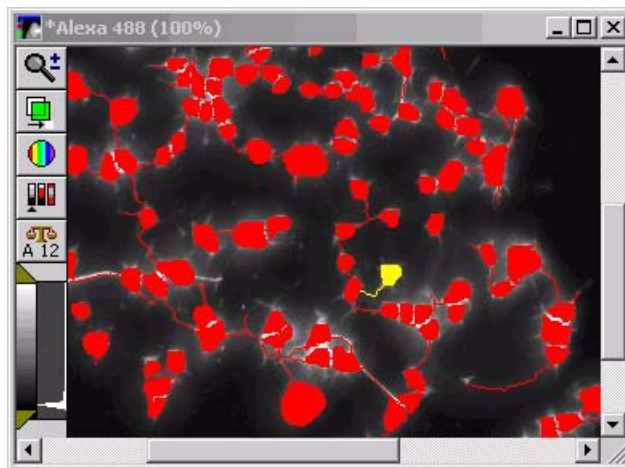


Figure 10-7 Neurite Outgrowth – Cellular Results – Image overlay of neurite outgrowth data with a single cell/neurite selected

To use the Cellular Results table

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.



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

5. If you do not want the Cellular Results table to open each time an application module runs, clear the **Show Data On App. Module Run** check box.



Note: If you have cleared the check box 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.

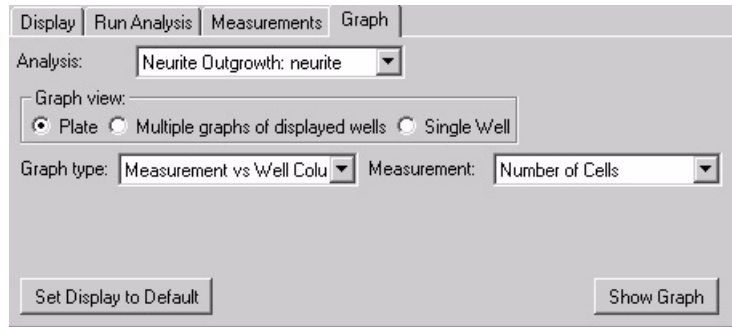


Figure 10-8 Graph Tab

2. Select the analysis containing the data you wish to graph from the **Analysis** field.
3. In the **Graph View** section, select the source location for the data you want graphed.
4. Select a graph type from the **Graph Type** drop-down list. 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) drop-down list(s).
6. If you selected **Histograms** from the **Graph Type** drop-down list, select the number of bins to display in the resulting histogram from the **Number of Bins** field.
7. Select the **Auto Scale** checkbox to automatically scale the bin(s) based on the range of data from the selected Measurement.



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

- Click **Show Graph** to open the graph based on the current settings.

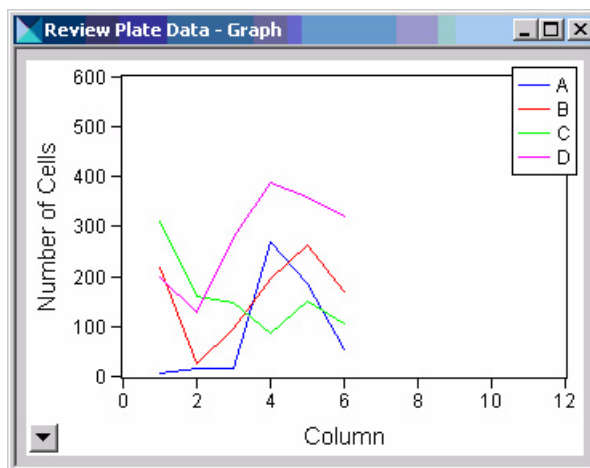


Figure 10-9 Sample Graph



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

- 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**.
- 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 selections based on your specified measurements query criteria, complete the following procedure.

- From the **Review Plate Data** dialog box, click the **Measurements** tab.

Figure 10-10 Measurements Tab

2. In the **Analysis** drop-down list, select the analysis that contains the measurements to query.
3. In the **Measurement** drop-down list, select the name of the measurement that you want to use to query your images in the database.
4. In the **Display Format** drop-down list, select the number of decimal places that you want to display for your data in the grid.
5. In the **Value is** drop-down list, 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, and then click **OK**.
7. Click **Configure Log** to select either Column and Row labels, Plate info, or both, and 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 **F9** 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 select 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.

