MetaXpress Image Acquisition and Analysis Software

for ImageXpress MICRO™

Version 1.7 for Microsoft XP®

Acquisition Guide

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Chapter 1
Introduction

The MetaXpress screening system running on the ImageXpress^{MICRO} hardware platform incorporates unique features that enable it to surpass the performance of a conventional automated microscope system. Primary among these unique features are the light path components, which are completely motorized and controlled by software. The filters and light path are designed for maximum light throughput and conservation of image registration between probes. Additionally, the excitation light for the system is more powerful and serviceable than a typical microscope. Standard features of the ImageXpress^{MICRO} system include the following:

- High-efficiency, custom imaging optics
- Fully integrated imaging software
- CoolSNAP $_{ES}$ digital CCD camera
- 4-position automated objective changer
- 5-position automated filter cube changer
- 300W xenon light source
- Fully automated X-Y sample stage and Z focus stage with better than 100 nm resolution
- Full support for Microsoft® SQL and ORACLE® database archiving and retrieval

Optional features include the following:

- Nikon objectives (4x-100x supported)
- Filter cubes configured for specific dye excitation and emission
- High-speed laser auto-focus
- CoolSNAP $_{HQ}$ digital CCD camera
- Offline analysis workstations
- Analysis application modules

Robotic Options available include the following:

- Thermo CRS CataLyst Express ^{TM}
- Polara software

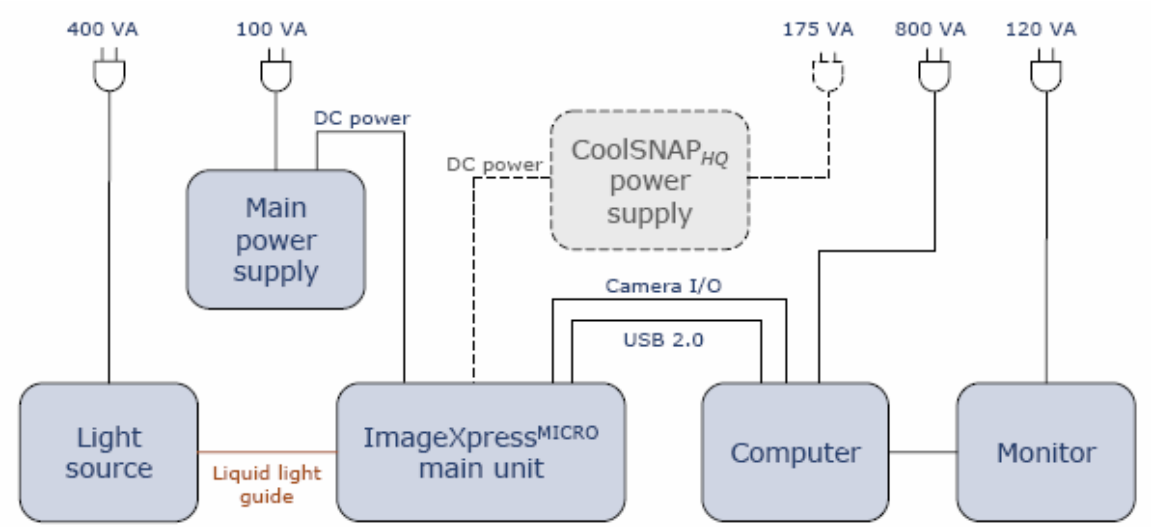
The following subjects are discussed in this chapter:

- ImageXpress^{MICRO} System Components
- Obtaining Support
- Administrator Tasks
- Training Opportunities

ImageXpress^{MICRO} System Components

Figure 1-2 shows the components that comprise the basic ImageXpress^{MICRO} system:

Figure 1-2
ImageXpress^{MICRO} System Components



Documentation Conventions

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

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

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

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. Refer to the section, *Gathering Support Information*, for what information to include in the email.
4. Internet Support — Fill out the Technical Support Request Form at http://www.moleculardevices.com/cgi-bin/support_request.cgi to send an email to a pool of technical support representatives. Refer to the section, *Gathering Support Information*, for what information to include in the email.
5. Call Customer Service — You can contact MDC's Customer Service department at (800)-635-5577 (U.S. only) or +1 408-747-1700. Please have the system ID number, system serial number, software version number and the system owner's name available when you call.

Additional support resources include the following:

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

Gathering Support Information

If you need to contact MDC for support, it is very important to have the following information available to help Customer Support personal troubleshoot the problem you are experiencing:

- The steps that led up to the occurrence of the problem
- The settings of any dialog boxes used when the problem occurred
- The text of any error messages

You should also collect the following information from your system whenever reporting software problems:

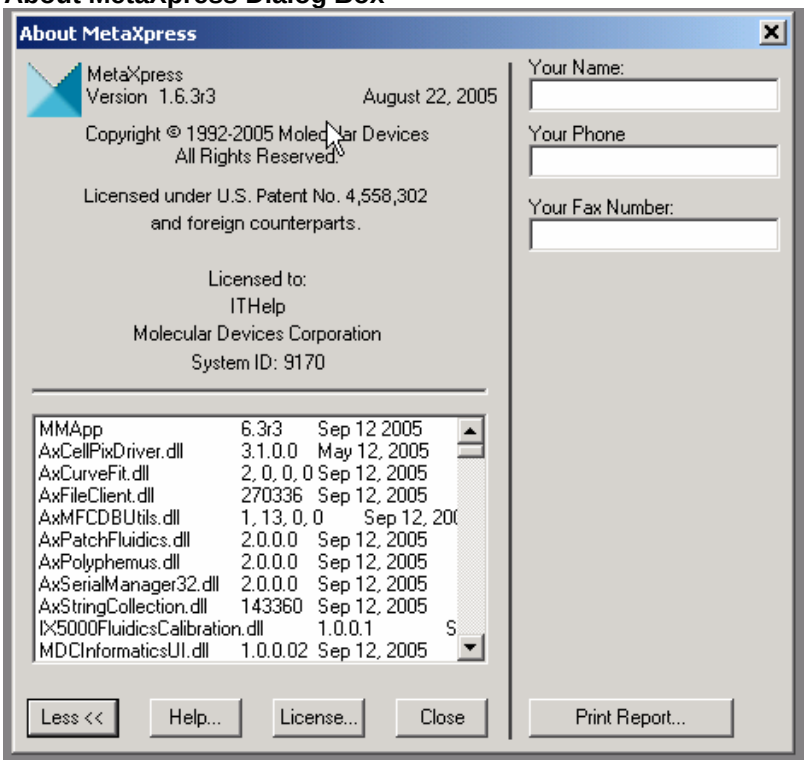
- **Copy of the Plate Acquisition Settings file** – By default, the plate acquisition settings file is saved to the database. To save the settings to a file, go to the *Experiment* tab of the Plate Acquisition Setup dialog box, click *Save settings*, then select *Save to file rather than database*. The settings file will then be saved to the **`C:\MX\HTSSTATE`** folder by default.

- **Journal files –** If you were running a journal when the problem occurred, include copies of the journal files that you were using. By default, journal files are saved in the **`C:\MX\app\mmproc\journals`** folder.
- **System Information Report –** This report contains information about many system settings and the release levels of all the .dll files in your currently installed MetaXpress software. The following section describes how to create a System Information report for emailing to MDC.

Creating a System Information Report

Much of the required system setting information can be obtained by creating a System Information Report. You create this report from the About MetaXpress dialog box on the Help menu. This report can be printed on a printer connected to your system or a network printer, or it can be "printed" to a PDF file or to an ASCII text file. Once the report is in the form of a PDF file or a text file, you can send this report to appropriate MDC support personnel as an E-mail attachment. Figure 6-1 shows the About MetaXpress dialog box:

Figure 6-1
About MetaXpress Dialog Box



Administrator Tasks

Most of the items in this document are for the general users. However, we recommend you identify one or more users as advanced users or system administrators. The responsibilities of the system administrator vary from site to site. Variables include the number of users on the system, the type of database used, and the type of work done. Some common MetaXpress system administrator tasks include:

- Installation overview with MDC representative
- Post-installation hardware and software testing
- Database planning and implementation
- Custom user and group settings creation
- Maintenance scheduling and software updating

Training Opportunities

Proper training for the System Administrator and the end users is essential to ensure that your MetaXpress screening system is used correctly and effectively. Training (at different levels) can be delivered to you and the MetaXpress users by one or more of the following methods:

- Onsite Installation and Training — provided with the purchase of the system
- Advanced Analysis Training at an MDC Facility — Please speak to your sales representative for a quote.

Onsite Installation and Training

When your system is delivered, an MDC representative will go to your site and install both the hardware and software components of MetaXpress. After the system is installed, two days of onsite training is provided. This training covers systems operation, hardware usage, and basic software, image acquisition and basic data analysis tutorials. It is essential that the System Administrator is present for this training. The total class size is limited to three people from your organization; this ensures that the training is extensive and targeted to your group's screening goals.

Advanced Analysis Training at MDC Facilities

Advanced training into MetaXpress or advanced AcuityXpress usage can be purchased. The training session is conducted in MDC's facilities in Downingtown, PA or Union City, CA. To contact MDC and learn more about training:

> Fill out the Technical Support Request Form at http://www.moleculardevices.com/cgi-bin/support_request.cgi to send an email with your training request.
>
> OR
>
> Call (800)-635-5577 and follow the voice-mail system instructions for reaching Customer Support for cellular imaging. Note that you must talk to your Sales Representative to receive quotes on advanced training.

In-house Training Using the CD ROM Tutorial

In addition to the above training opportunities, you can provide basic level MetaMorph training to MetaXpress users with the MetaMorph Tutorial CD-ROM.

In-house Follow-up Training

Members of your organization who attend MDC training classes receive training materials and have available to them copies of the PowerPoint presentations and samples used to conduct the class. These materials can be used to conduct small classes and seminars within your organization to help familiarize other MetaXpress users with MetaMorph and MetaXpress operation methods. Even if other members of your organization have previously attended MetaMorph or MetaXpress training, this information can be valuable for familiarizing these users with information about additions and modifications to the product found in the latest release of the software.

Chapter 2
Starting the ImageXpress^{MICRO} System

This chapter explains how to safely power up the ImageXpress^{MICRO} hardware and login to MetaXpress. The following topics are discussed in this chapter:

- Turning on the ImageXpress^{MICRO} System
- Starting MetaXpress
- Turning off the ImageXpress^{MICRO} System

Turning on the ImageXpress^{MICRO} System

Complete the following procedure to start the ImageXpress^{MICRO} and MetaXpress:

1. Ensure that the power cords from ImageXpress^{MICRO} and lamp power supplies are connected to a 100 VAC to 120 VAC or 220 VAC to 240 VAC power source.
2. Turn on the power switch on the front of the lamp power supply unit.

   Note: Turn on this power supply first to minimize electrical pulse interference with the electronic components in your system.
3. Turn on the power switch on the front of the ImageXpress^{MICRO} power supply unit.
4. If you have the optional CoolSNAP$_{HQ}$ camera, ensure that it is plugged in and turned on.
5. Turn on the power switch on the computer.
6. Turn on the power switch on the LCD monitor.
7. Once the computer has started and Windows is running, log in to Windows using the User Name/Password combination provided for you by your system administrator.

   Caution: Do not log into your system as “Guest” unless you are specifically instructed to do so by your system administrator.
8. Continue to the next procedure, *Starting MetaXpress*.

Starting MetaXpress

To start the MetaXpress application, complete the following procedure:

Notes:

- This procedure assumes that your ImageXpress^{MICRO} system and your MetaXpress Image Acquisition and Analysis software have been properly installed and configured by your MDC technical representative and your System Administrator.
- If you encounter or observe actions or results that are inconsistent with your expected results when using ImageXpress^{MICRO} system and your MetaXpress software, contact your system administrator before continuing your experiment.

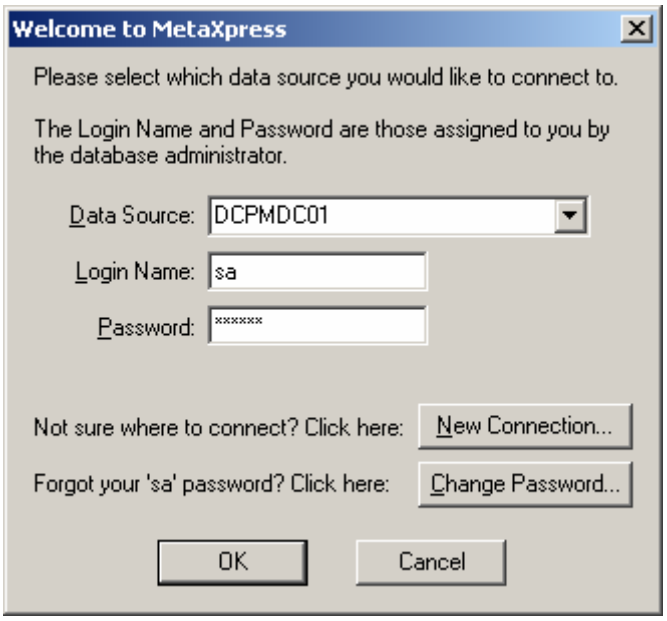
1. Double-click the MetaXpress icon on your desktop to start MetaXpress. The MetaXpress title window opens, as shown in Figure 2-1:

Figure 2-1
MetaXpress Title Window

Note: This window only opens when MetaXpress is configured to run in multi-user mode from within the Meta Imaging Series Administrator. If you do not see this dialog box, MetaXpress is in single-user mode; continue to Step 3.

2. In the *User Name* drop-down list select the user name to use and click *OK*. MetaXpress starts and a progress bar at the bottom of the dialog box that indicates the loading progress of the program. After the program loads, the Welcome to MetaXpress dialog box opens, as shown in Figure 2-2:

Figure 2-2
Welcome to MetaXpress Dialog Box



3. Select the data source to connect to (if there is more than one), enter your login name and password, and then click *OK*. A dialog box opens prompting you to select a Group.

   Note: The default System Administrator *Login Name* is **`sa`** and the *Default Password* is either **`mdc`** or **`imagexpress.`** If needed, you can change the password using the *Change Password* command.

4. Select one of the groups assigned in the Meta Imaging Series Administrator and click *OK*. The MetaXpress application will start and initialize the various components of the ImageXpress^{MICRO} system. If you receive error messages when the system is initializing, ensure that all hardware connections are plugged in and fully seated.

Turning off the ImageXpress^{MICRO} System

Complete the following procedure to power down the ImageXpress^{MICRO}:

1. Exit the MetaXpress application. You will be prompted to save any open images.
2. Shut down the computer from the Windows desktop: Start>Shutdown>Shutdown.
3. Turn off the power switch on the LCD monitor.
4. If you have the optional CoolSNAP$_{HQ}$ camera, turn off the power from the power supply.
5. Turn off the power switch on the front of the lamp power supply unit.
6. Turn off the power switch on the front of the ImageXpress^{MICRO} power supply unit.

Chapter 3
Post Installation and Testing

Your ImageXpress^{MICRO} system will be installed and configured by an MDC representative. After the installation is complete, the system will be ready for initial testing and use. The following topics are reviewed in this chapter:

- Verifying Device Settings in the Meta Imaging Series Administrator:
  - XY Stage
  - Objective(s)
  - Filter Cube
  - Shutter
- Verifying Camera Settings in the Meta Imaging Series Administrator
- Verifying Settings in MetaXpress:
  - Magnification Settings
  - Illumination Settings
  - Calibration Settings
  - Laser Auto Focus Sensor Settings (if applicable)
  - Plate Settings
  - Shading Correction Files

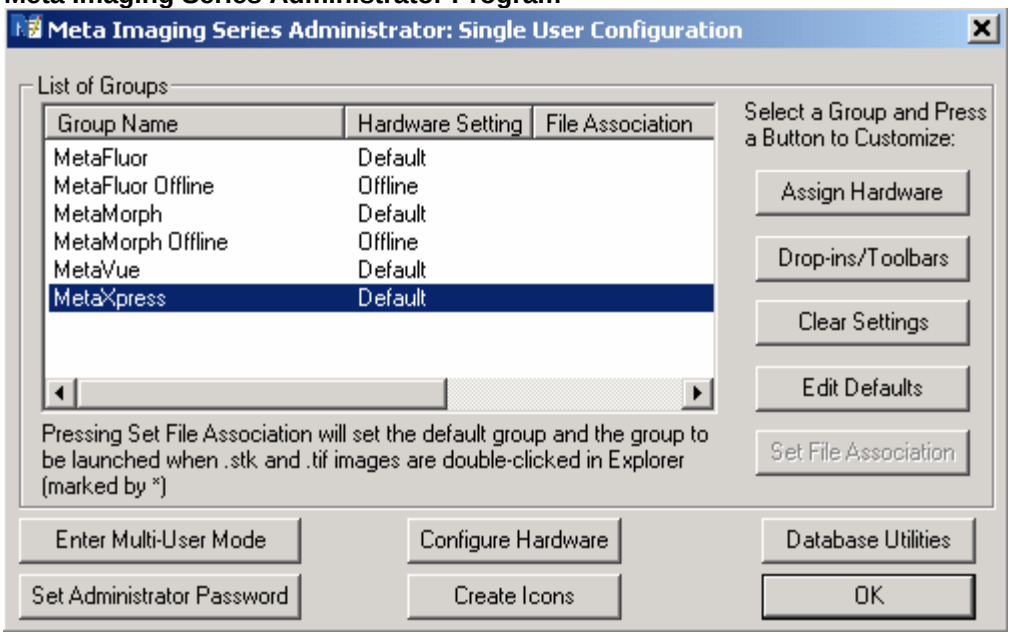
Verifying Device Settings in the Meta Imaging Series Administrator

This procedure ensures that the ImageXpress^{MICRO} hardware components are properly configured in the Meta Imaging Series Administrator application and the MetaXpress application. All hardware and software configuration settings are implemented in the Meta Imaging Series Administrator application. To check the hardware configuration in the Meta Imaging Series Administrator application, complete the following procedure:

> **Note:** For additional information about any of the dialog boxes in the Meta Imaging Series Administrator, press the [F1] key to access the online help for the active dialog box. The Meta Imaging Series Administrator and the MetaXpress application can not be run simultaneously.

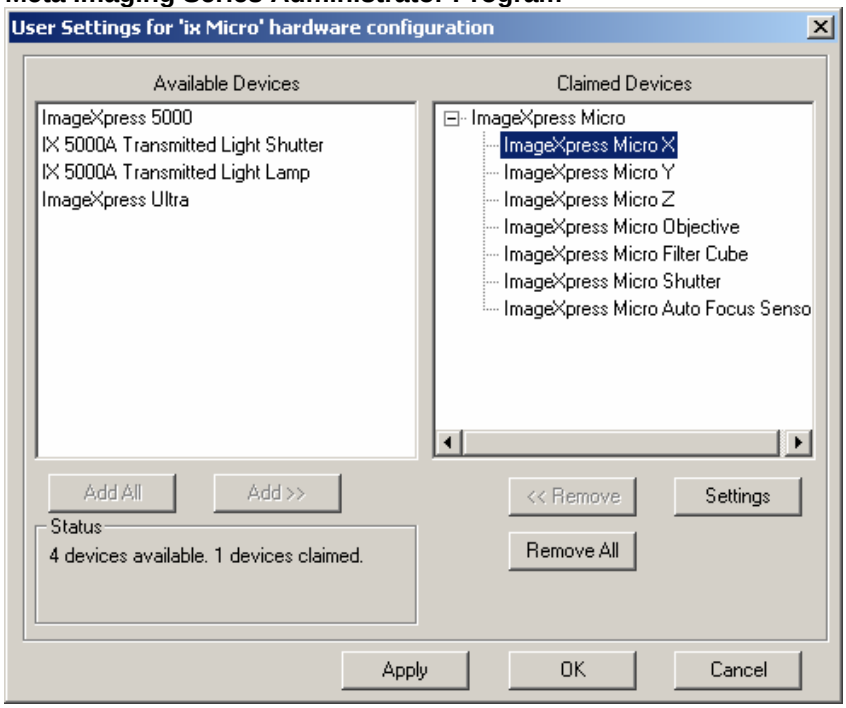
1. Use the procedure described in Chapter 2, *Starting the ImageXpress^{MICRO} System*, to power up the system, but do not start MetaXpress.
2. From the Windows Start menu, go to Programs>MetaXpress> Meta Imaging Series Administrator. The Meta Imaging Series Administrator program opens.
3. Select *MetaXpress* from the *List of Groups* field, as shown in Figure 3-1:

Figure 3-1
Meta Imaging Series Administrator Program



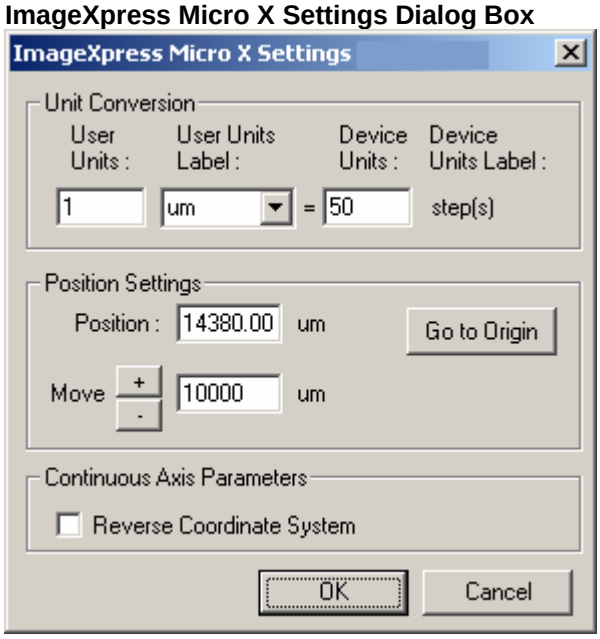
4. Click *Configure Hardware*. The *Configure Hardware* dialog box opens.
5. Click *Configure Devices*. The *User Settings hardware configuration* dialog box opens, as shown in Figure 3-2:

Figure 3-2
Meta Imaging Series Administrator Program



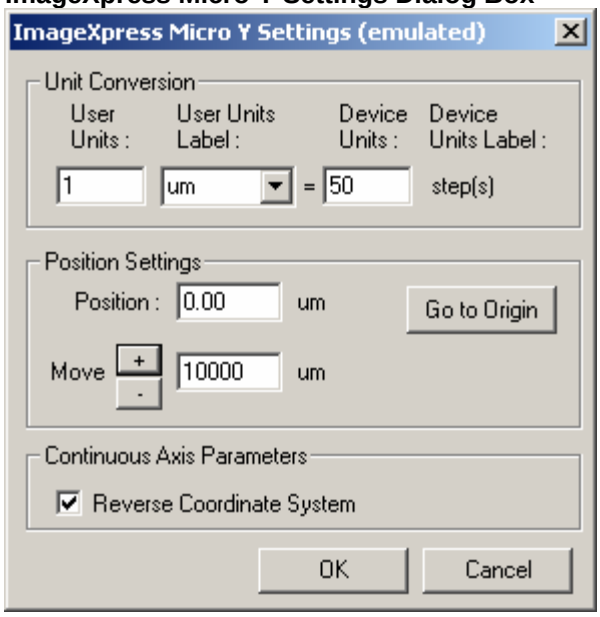
6. Select *ImageXpress Micro X* from the *Claimed Devices* list and click *Settings*. The ImageXpress Micro X Settings dialog box opens.
7. Increase the step size to 10,000 um, as shown in Figure 3-3:

Figure 3-3
ImageXpress Micro X Settings Dialog Box



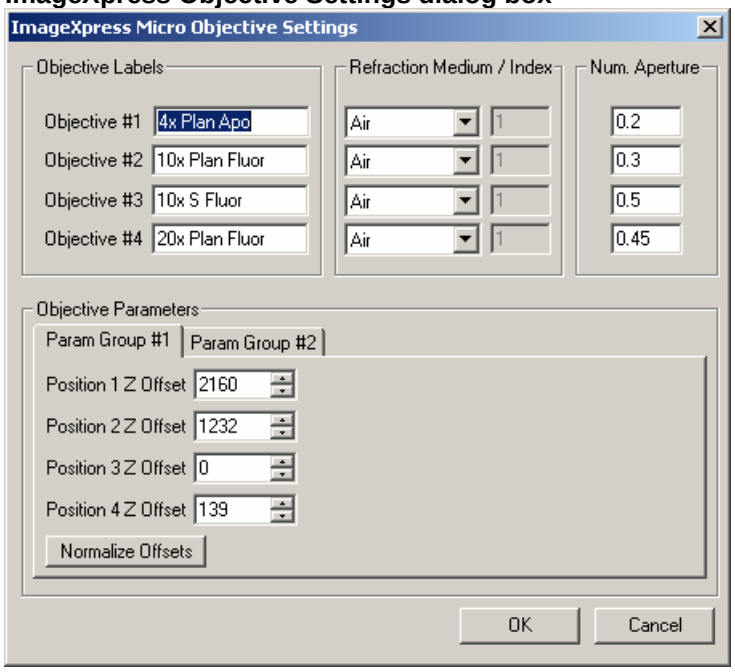
8. Ensure that *Reverse Coordinate System* is NOT checked.
9. Click the + icon and confirm that the stage responds to the control.
10. Change the step size back to 10 um and Click *OK*.
11. Select *ImageXpress Micro Y* from the *Claimed Devices* list and click *Settings*. The ImageXpress Micro Y Settings dialog box opens, as shown in Figure 3-4:

Figure 3-4
ImageXpress Micro Y Settings Dialog Box



12. Increase the step size to 10,000 um, as shown in Figure 3-4.
13. Ensure that *Reverse Coordinate System* IS checked.
14. Click the + icon and confirm that the stage responds to the control.
15. Change the step size back to 10 um and click *OK*.
16. Select *ImageXpress Z* from the *Claimed Devices* list.
17. Click *Settings*. The ImageXpress Z Settings dialog box opens.
18. Verify that the value in the *Device Units* field is *50*.
19. Increase the step size to 1000 um.
20. Click the + icon and confirm that the Z Motor responds to the control.
21. Change the step size back to 10 um and click *OK*.
22. Select *ImageXpress Objective* from the *Claimed Devices* list and click *Settings*. The ImageXpress Objective Settings dialog box opens, as shown in Figure 3-5:

Figure 3-5
ImageXpress Objective Settings dialog box

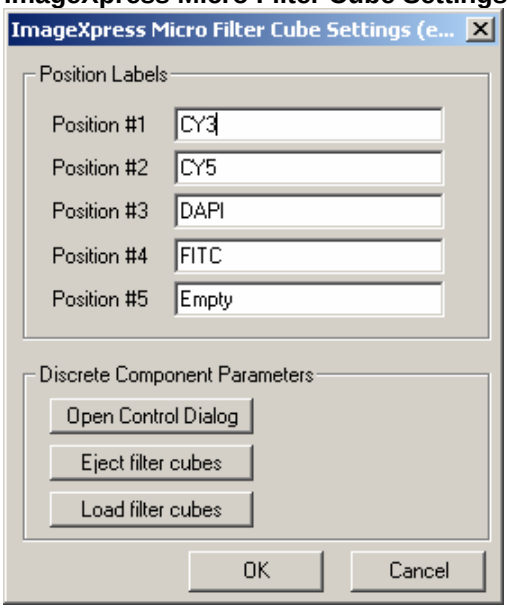


23. Confirm that the *Objective labels* and values in the *Num Aperture* fields match each objective on your system (the Numerical Aperture (NA) values are written on each objective). Position 1 is the position on the right if you are facing the filter cube access door at the front of the instrument.
24. Click the *Param Group #1* tab in the in the *Objective Parameters* section on the bottom half of the dialog box. This tab contains the Z offset positions in microns for the objectives. Confirm that these are valid numbers and all but one are greater than 0.

    Note: If you need to determine the offset values, refer to the *Configuring Parfocality* procedure found in the *Advanced Procedures* chapter of this document.

25. Click *Param Group #2*, then click *Open Control Dialog*. The Control – ImageXpress Micro Objective dialog box opens.
26. Click the arrow buttons and confirm that the objective changer is moving appropriately.
27. Click *Done*, then click *OK* to close the dialog box.
28. Select *ImageXpress Micro Filter Cube* from the *Claimed Devices* list and click *Settings*. The ImageXpress Micro Filter Cube Settings dialog box opens, as shown in Figure 3-6

Figure 3-6
ImageXpress Micro Filter Cube Settings dialog box



29. Confirm that the filter sets listed in the *Filter Labels* field are correct. Position 1 is the position closest to you if you are facing the front of the instrument (the side with the filter cube access door).
30. Click *Open Control Dialog*. The Control – ImageXpress Micro Filter Cube Settings dialog box opens.
31. Click the arrow buttons to confirm that the filter cube is responding to the program.
32. Click *Done*, then click *OK* to close the ImageXpress Micro Filter Cube Settings dialog box.
33. Select *ImageXpress Micro Shutter* from the *Claimed Devices* list and click *Settings*. The ImageXpress Micro Shutter Settings dialog box opens, as shown in Figure 3-7:

Figure 3-7
ImageXpress Micro Shutter Settings Dialog Box

34. Confirm that the *Open Delay* and *Close Delay* fields are both set to 20 milliseconds.

35. Click *Open Shutter Control Dialog*. The Control – ImageXpress Micro Shutter Settings dialog box opens.
36. Click *Toggle* to confirm that the shutter is responding.
37. Click *Done*, then click *OK* to close the ImageXpress Micro Shutter Settings dialog box.

    Note: If you have a CRS plate handling robot attached to the ImageXpress^{MICRO}, You should confirm those settings as well. Refer to the procedure, *Verifying External Control Settings*, located in the Appendix of this document.

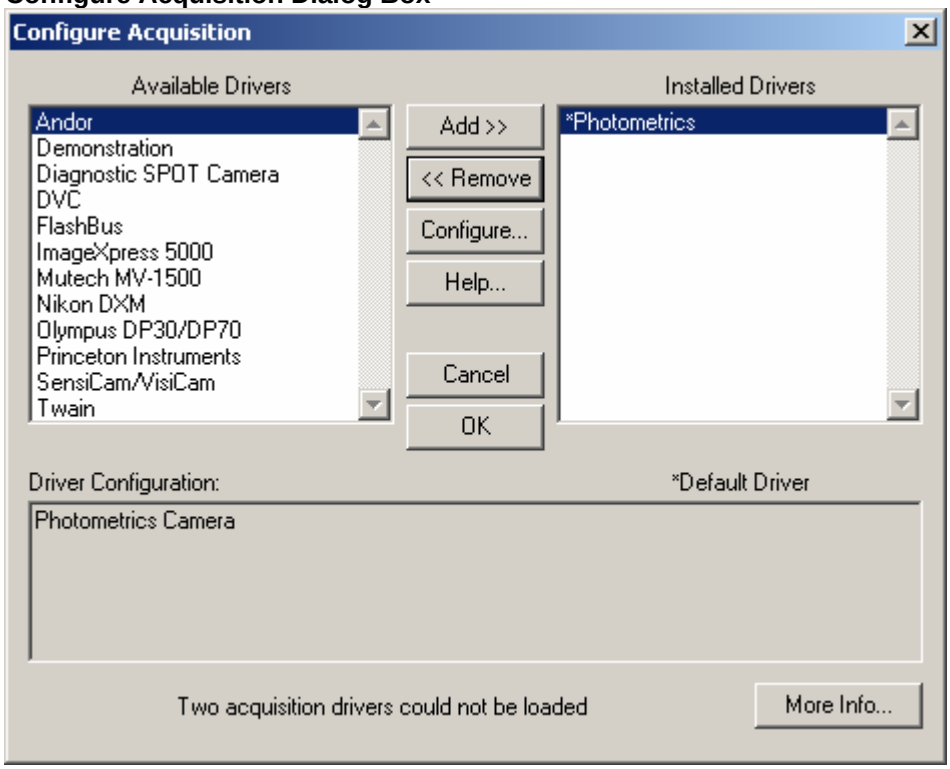
38. Click *OK* to close the User Settings hardware configuration dialog box and continue to the next procedure.

Verifying Camera Settings in the Meta Imaging Series Administrator

Complete the following procedure to ensure that the ImageXpress^{MICRO} camera driver is installed:

1. From the Configure Hardware dialog box, click *Configure Acquisition*. The Configure Acquisition dialog box opens.
2. Ensure that the *Photometrics* driver is listed in the *Installed Drivers* column, as shown in Figure 3-8:

Figure 3-8
Configure Acquisition Dialog Box



3. If the *Photometrics* driver is not listed in the *Installed Drivers* column, select it from the *Available Drivers* list and click *Add* to move it to the *Installed Drivers* column.
4. Click *Configure* to open the Photometrics Camera driver dialog box. Click the *Version* tab to bring it forward.
5. Click *Query for Version*. A dialog box opens confirming that the camera information was queried successfully. This confirms that the camera is responsive. If the camera was not queried successfully, ensure that the cabling is correct from the ImageXpress^{MICRO} main unit to the computer (if you are using the optional CoolSNAP$_{HQ}$, also ensure that the power supply is plugged in and turned on).
6. Click *OK* to exit the Photometrics Camera Driver dialog box, then click *OK* as needed to exit the Meta Imaging Series Administrator and proceed to the next section.

Verifying and Backing Up Settings in MetaXpress

After confirming hardware settings in the Meta Imaging Series Administrator, you should also check the following settings from within the MetaXpress:

- Magnification Settings
- Illumination Settings
- Calibration Settings
- Laser Auto Focus Sensor Settings

During the verification process it is recommended that you backup these settings as described in the procedures. This will allow you to restore the settings in case they are lost.

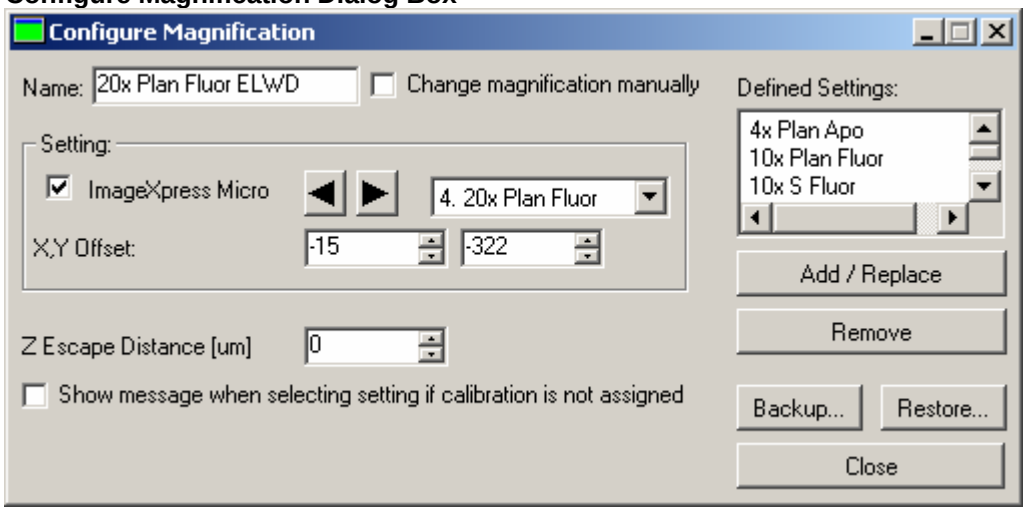
Verifying and Backing Up Magnification Settings

You will need to confirm magnification settings for the ImageXpress^{MICRO} objectives before using your system. Complete the following procedure to check the magnification settings in the MetaXpress application:

Note: For additional information about any of the dialog boxes in MetaXpress, press the F1 key to access the online help for the active dialog box.

1. Open MetaXpress and log into the database.
2. Select Configure Magnification from the Devices menu. The Configure Magnification dialog box opens as shown in Figure 3-9:

Figure 3-9
Configure Magnification Dialog Box



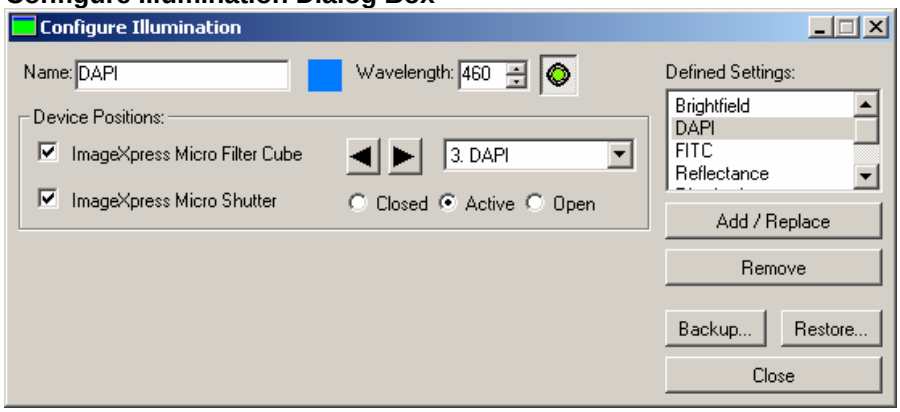
3. Ensure that the *ImageXpress Micro* setting exists and its checkbox is selected in the *Settings* field.
4. Confirm that the *Defined Settings* field contains a setting for each objective on your system.
5. Click *Backup*. The Backup All Magnification Settings dialog box opens.
6. Select a name and location for the backup and click *Save*.
7. Settings can be restored by clicking *Restore* and choosing the saved file.
8. Click *Close* to exit the Configure Magnification dialog box.

Verifying Illumination Settings

You will need to confirm illumination settings for the ImageXpress^{MICRO} before using your system. Complete the following procedure to check the Illumination settings in the MetaXpress application:

1. Open MetaXpress and log into the database.
2. From the Devices menu, select Configure Illumination. The Configure Illumination dialog box opens.
3. Ensure that ImageXpress Micro Filter Cube is selected in the *Device Positions* field.
4. Ensure that the ImageXpress Micro Shutter is selected as “Active” for each filter set.
5. Ensure that the correct illuminations are listed in the *Defined Settings* field, as shown in Figure 3-10:

Figure 3-10
Configure Illumination Dialog Box



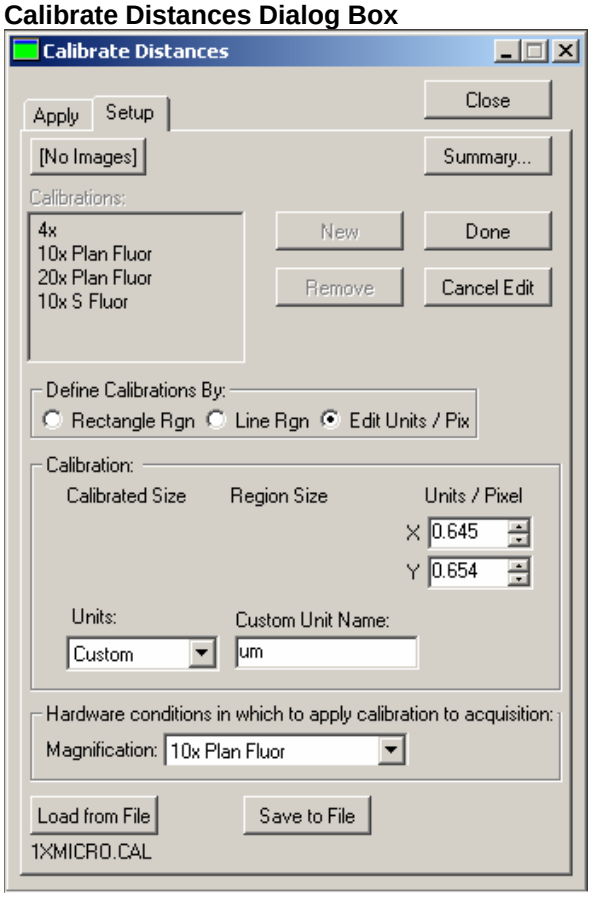
6. Set up other illumination settings if needed. Note that the value in the Wavelength field should match the center wavelength for the emission filter, as shown in Figure 3-10.
7. Click *Backup*. The Backup All Illumination Settings dialog box opens.
8. Select a name and location for the backup and click *Save*.
9. Settings can be restored by clicking *Restore* and choosing the saved file.
10. Click *Close* to exit the Configure Illumination dialog box.

Verifying and Backing Up Calibration Settings

Complete the following procedure to confirm and backup calibration settings in MetaXpress:

1. Select Calibrate Distances from the Measure menu. The Calibrate Distances dialog box opens.
2. Click the *Setup* tab. Confirm that there are calibration settings in the *Settings* field that match the objective settings from the Configure Magnification dialog box, as shown in Figure 3-11:

Figure 3-11
Calibrate Distances Dialog Box



3. Click *Save to File*. The Save Spatial Calibrations dialog box opens.
4. Select a name and location for the backup and click *Save*.
5. Settings can be restored by clicking *Load from File* and choosing the saved file.
6. Click *Close* to exit the Calibrate Distances dialog box.

The following estimated values can be used for ImageXpress^{MICRO} calibration settings:

Objective	Estimated Calibration
2x	3.225 um/pixel
4x	1.6125 um/pixel
10x	0.645 um/pixel
20x	0.3225 um/pixel
40x	0.16125 um/pixel
60x	0.1075 um/pixel
100x	0.0645 um/pixel

Make sure the appropriate magnification setting is selected for each calibration.
For additional information on creating calibrations settings, refer to the MetaXpress online help (press the F1 key when the Calibrate Distances dialog box is open to access

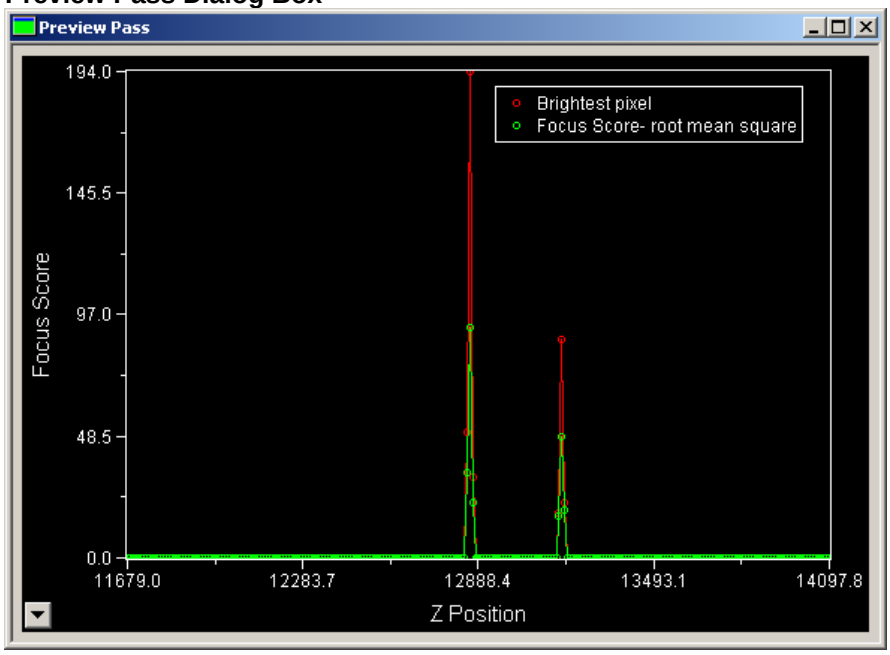
its online help). Also refer to the technical note Spatially calibrating images in MetaMorph, available online.

Verifying the Laser Auto Focus Sensor

This procedure uses a bead plate to test that the laser auto focus (LAF) sensor is enabled and functional. Use the following procedure to confirm that the Laser Auto Focus is responding in MetaXpress:

1. Power on the system and open MetaXpress if it is not already open.
2. From the Screening menu, select Plate Acquisition Setup.
3. Select the *Acquisition Loop* Tab and ensure that *Enable laser-based focusing* is selected.
4. Click the *Plate* tab to highlight it.
5. Select the included Costar *96-well Plastic* plate type from the *Plate name* drop-down list.
6. From the Screening menu, select Plate Acquisition and Control. The Plate Acquisition and Control dialog box opens.
7. Click *Eject Plate* to move the stage to the load position.
8. Load the bead plate and click *Load Plate*.
9. Click *Go To A1* to move the stage to the A1 position.
10. Go back to the Plate Acquisition Setup dialog box and click the *Autofocus* tab to highlight it.
11. Click *Configure Laser Sensor*. The Configure Laser Sensor dialog box opens.
12. Click *Preview Pass*. A window opens displaying a graph of focus intensities vs. Z-position, as shown in Figure 3-12:

Figure 3-12
Preview Pass Dialog Box



If the preview pass window contains at least one peak, the Laser Auto Focus Sensor is enabled and functional. If it does not contain any peaks, ensure that the plate is properly seated, increase the Exposure value in the Configure Laser Sensor dialog box, and try again. If the chart still does not contain a peak, contact MDC Customer Support and report the issue. For more information on the Preview Pass window, refer to the Confirming Laser Auto Focus Settings for Plate Files section of this document.

13. Click *Close* to exit the Configure Laser Sensor dialog box.
14. Continue to the next procedure.

Verifying the Plate Reference Point (A1 Center)

Complete the following procedure to ensure that the plate reference point (A1 center) is properly set:

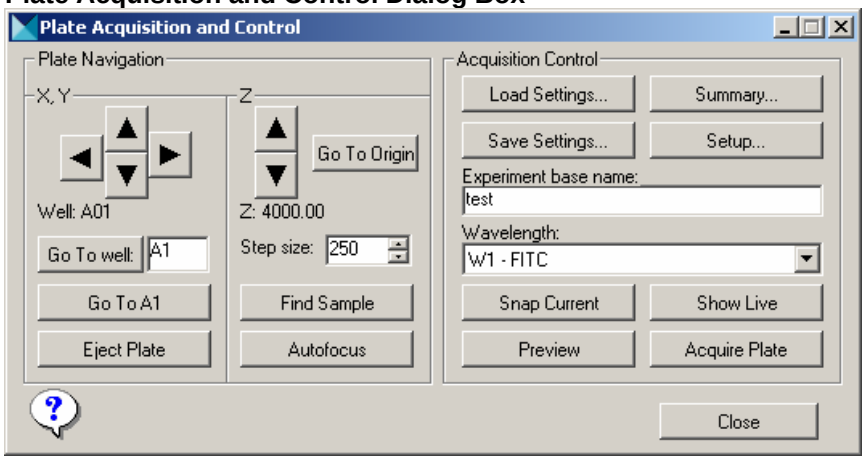
Note: You need the metal X/Y calibration plate/slide holder that ships with the ImageXpress ^{MICRO} to complete this procedure.

1. Power on the system and start MetaXpress if it is not already running.
2. From the Screening menu, select Plate Acquisition Setup.
3. Click the *Objective and Camera* tab to highlight it.
4. Select the lowest power objective from the *Magnification* drop-down list — Usually this is a 4x Enter a binning value of *1* in the *Camera Binning* field.
5. Select a gain value of *2* in the *Gain* field.
6. Click the *Plate* tab to highlight it.
7. Select the included *96 Wells (8x12)* plate type from the *Plate name* drop-down list.
8. Click the *Wells to Visit* tab to highlight it.
9. Ensure that *Visit multiple sites per well* is not selected.
10. Click the *Wl (Wavelength) 1* tab to highlight it.
11. Select *FITC* from the *Illumination setting* drop-down list and enter an exposure time of *100 msec* in the *Exposure* field.

    Note: Cubes other than the FITC are acceptable to use however the contrast may not be as high as with the FITC. Exposure times will vary significantly depending on your light source and filter cube choice.

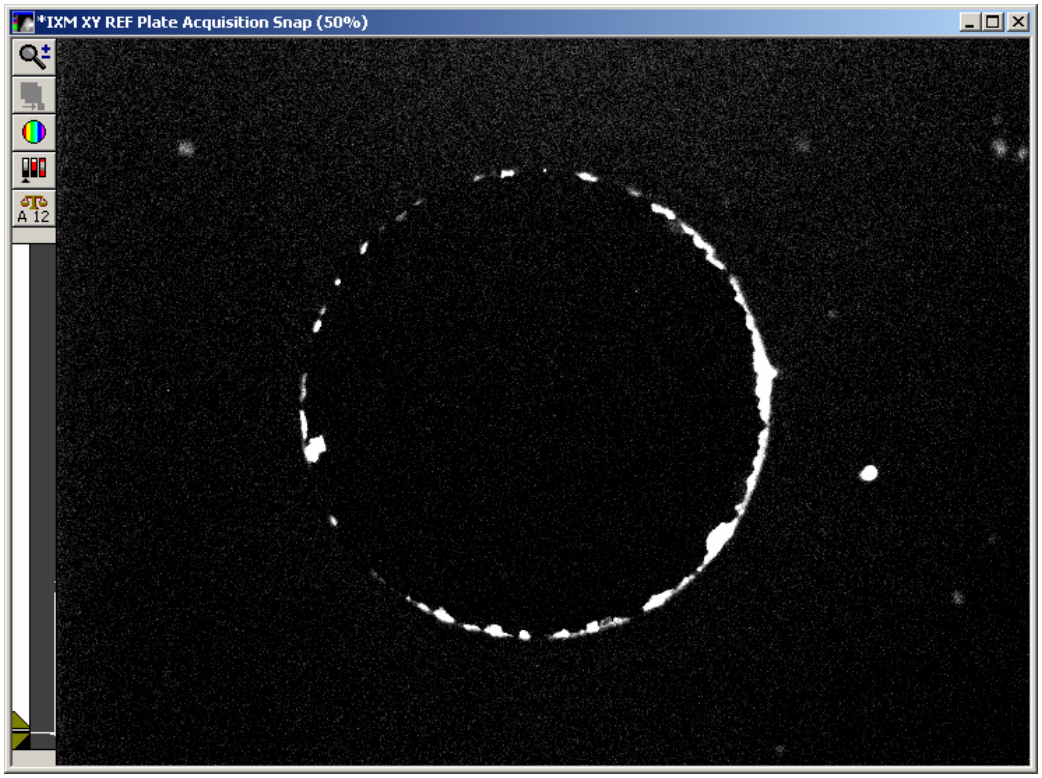
12. From the Screening menu, select Plate Acquisition and Control. The Plate Acquisition and Control dialog box opens.
13. Ensure that *W1 – FITC* is selected in the *Wavelength* field.
14. Enter a step size of *250* in the *Step size* field, as shown in Figure 3-13:

Figure 3-13
Plate Acquisition and Control Dialog Box



15. Click *Eject Plate* to move the stage to the load position.
16. Load the metal X/Y calibration plate. Ensure the notch in the plate is in the A1 position on the stage.
17. Click *Load Plate* to load the plate.
18. Click *Go To A1* to move the stage to the A1 position.
19. Click *Show Live* to open a live image window.
20. If you are not using the 4X objective skip to step 23
21. Use the Z control arrows to step the Z-motor (reducing the step size as you get closer to focus if needed) until the A1 pinhole comes into focus, as shown in Figure 3-14:

Figure 3-14
A1 Pinhole in Focus at 4X



22. Verify that the hole is visually centered in the field of view. If it is not, or if you cannot find the hole, contact MDC Customer Support.
23. If your lowest magnification objective is greater than 4x:
    a. Move the stage up until you are close to focus and left until you see the edge of the insert hole.
    b. Align the left side of the hole with the left side of the image window and record the stage X position.
    c. Move the stage to the right until you see the edge of the insert hole.
    d. Align the right side of the hole with the right side of the image window and record the stage X position.
    e. Calculate the horizontal center of the reference point.
    f. Repeat steps a – e for the vertical center of the reference point.
    g. Compare this stage position with the position of the stage when you click *Go To A1*.
24. Click *F2: Stop* to stop the live image.
25. Eject the X/Y calibration plate and continue to the next procedure.

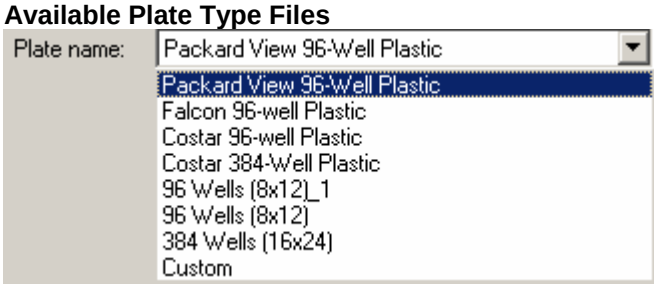
Verifying Plate Types

Complete the following procedure to ensure that the preconfigured plate type files included with MetaXpress are available from the Plate Acquisition Setup dialog box:

1. Open MetaXpress if it is not already open.
2. From the Screening menu, select Plate Acquisition Setup.

3. Click the *Plates* tab to highlight it.
4. Select the *Plate name* drop-down arrow to view the available plate type files, as shown in Figure 3-15:

Figure 3-15
Available Plate Type Files



5. If there are a number of custom plate types available, as shown above, then you are finished with this procedure.

   OR

   If there are no plate types listed other than the three defaults: *96 Wells (8x12)*, *384 Wells (16x24)*, and *Custom*, then the preconfigured plate type files are not loaded. Continue with this procedure to load the preconfigured plate type files.

6. Load the MetaXpress CD into the CD/DVD ROM drive of the computer.
7. When the MetaXpress Installation menu opens, select *Explore the CD*. Windows Explorer opens showing the contents of the cd.
8. Open the Plates folder on the CD. This folder contains the preconfigured plate type files (.plt).
9. Copy the plate files that you want available to the Plates directory of your MetaXpress installation directory (by default, `C:\MX\Plates`). These files will then appear in the *Plate name* drop-down list in the Plates tab of the Plate Acquisition Setup dialog box.

   Note: The plate files will be read-only after they are copied off the CD. You must turn off the Read-only attribute of these files before you can use them in MetaXpress.

10. Using Window Explorer, select all the plate files that you copied to the Plates directory ([**`Shift`**] + click to select multiple continuous items), right-click the selected files, and select Properties. The Properties dialog box opens. Under *Attributes*, uncheck *Read-only*, then click *OK*.

Confirming Laser Auto Focus Settings for Plate Files

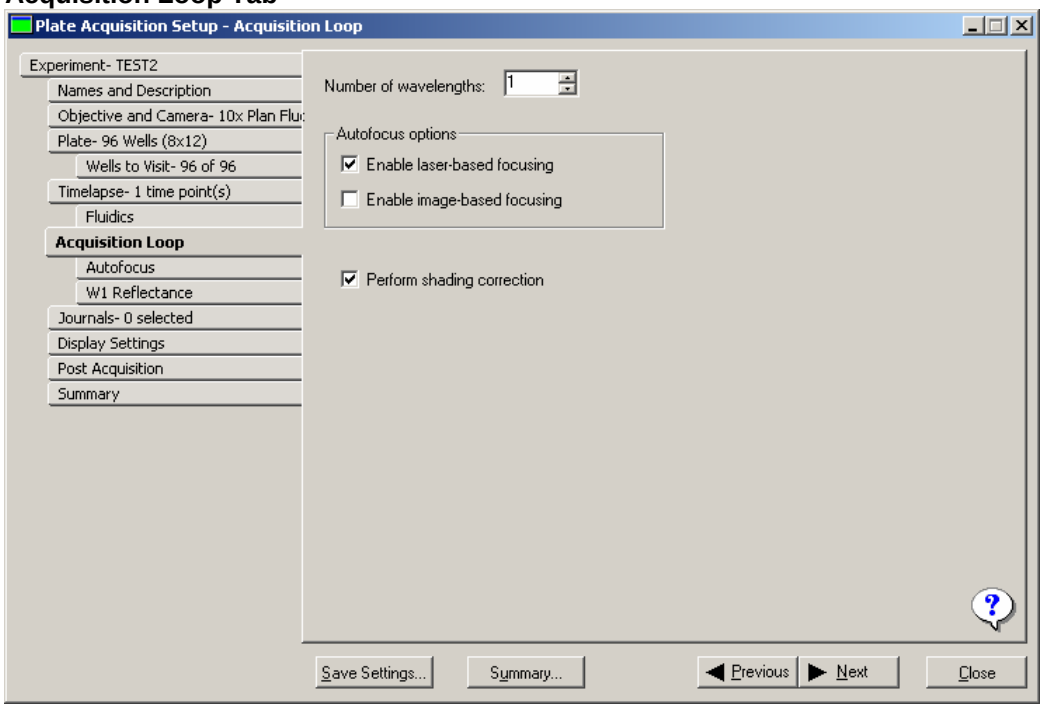
Before using a plate file, you need to confirm that the Laser Auto Focus (LAF) settings are optimal for the plate. Complete the following procedure to confirm the LAF settings:

Notes:

- Before beginning this procedure, prepare the plate that you are going to test by putting water or buffer in several of the wells.
- If you are using an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you are using. For information on configuring the correction collar, refer to Appendix C, *Adjusting a spherical aberration correction collar on ELWD objectives.*
- Do NOT use oil/water immersion objectives for this procedure. Use air objectives only.

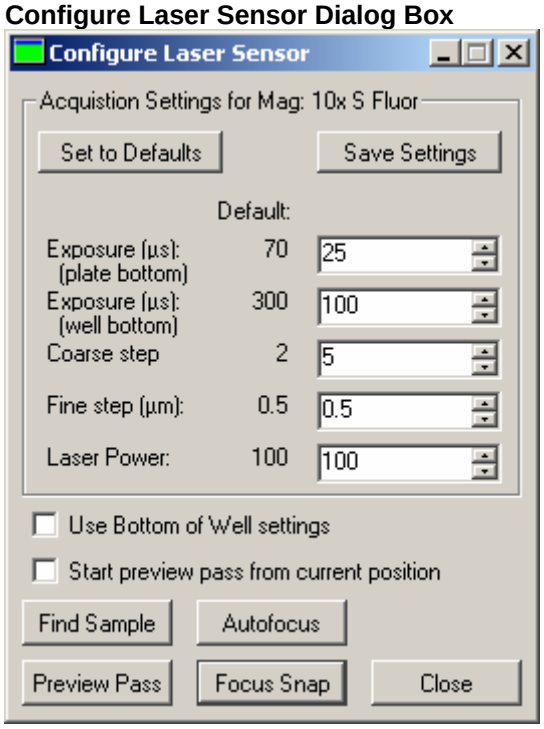
1. If they are not already open, open both the Plate Acquisition Setup dialog box and the Plate Acquisition and Control dialog box from the Screening menu. Also open the Plate Acquisition toolbar.
2. Click the Stage Load/Eject button on the Plate Acquisition toolbar and load the plate on the stage, then click the Load/Eject button again.
3. Use the Plate Acquisition toolbar or the Plate Navigation controls in the Plate Acquisition Control dialog box to move to a well containing water.
4. Click the *Plate* tab in the Plate Acquisition Setup dialog box and ensure that the correct plate file is selected in the Plate name drop-down box.
5. Click the *Objective and Camera* tab in the Plate Acquisition Setup dialog box and select an objective.
6. Click the *Acquisition Loop* tab and ensure that *Enable laser-based focusing* is selected and *Enable image-based focusing* is not selected, as shown in Figure 3-16:

Figure 3-16
Acquisition Loop Tab



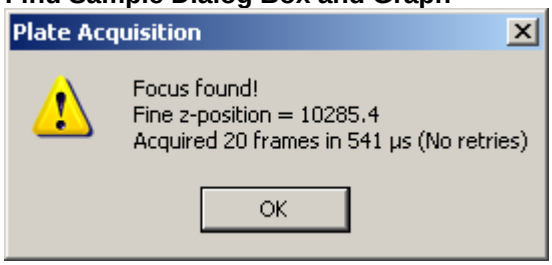
7. Click the *Autofocus* tab on the Plate Acquisition Setup dialog box and then click *Configure Laser Sensor* to open the Configure Laser Sensor dialog box, as shown in Figure 3-17:

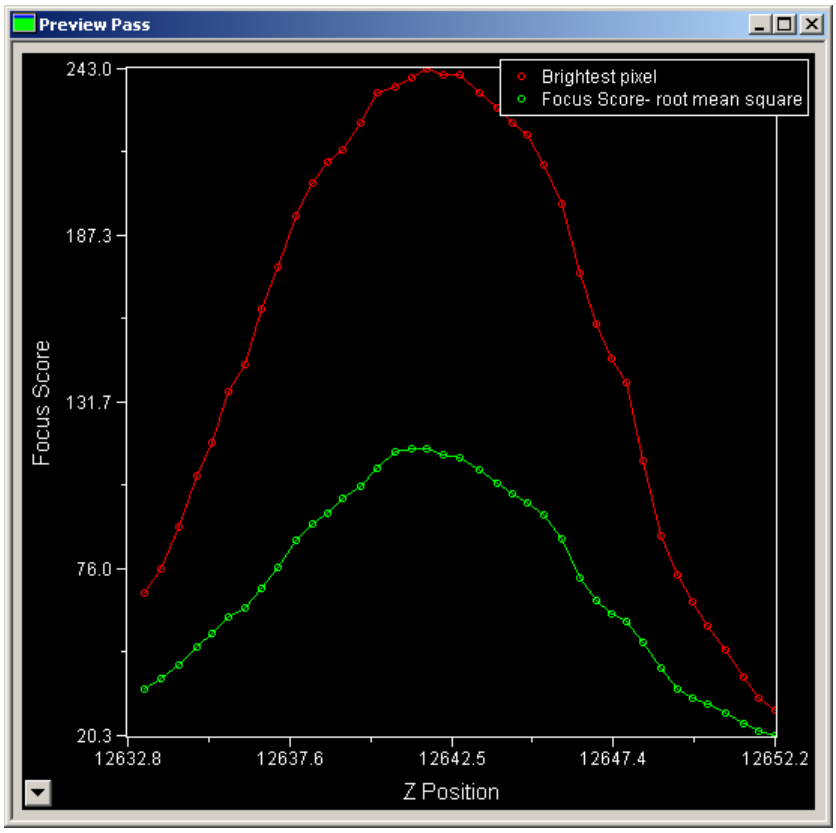
Figure 3-17
Configure Laser Sensor Dialog Box



8. From the Configure Laser Sensor dialog box, click *Find Sample*. The ImageXpress will use the current settings to focus. If the *Find Sample* command is successful, a confirmation dialog box opens, along with a Preview Pass graph, as shown in Figure 3-18:

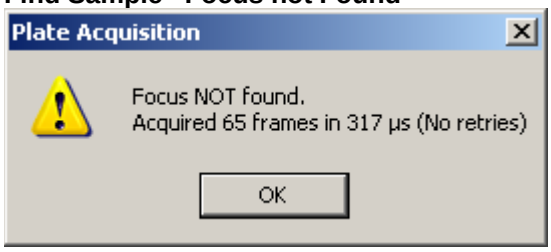
Figure 3-18
Find Sample Dialog Box and Graph





9. If the Find Sample command was not successful, a dialog box similar to the following opens:

Figure 3-19
Find Sample– Focus not Found

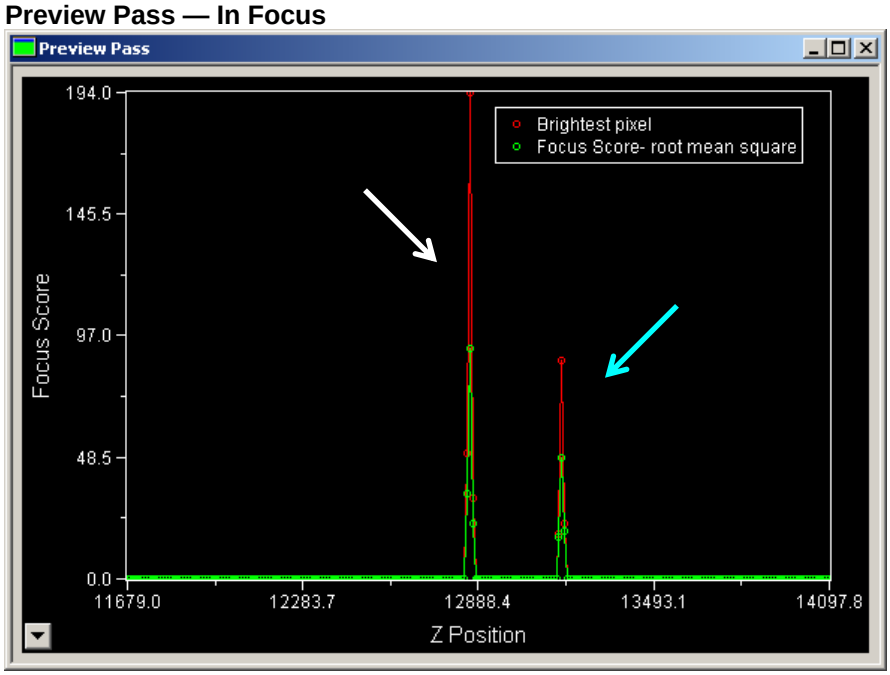


10. If the Find Sample command failed, perform the following checks in order:

- Ensure that the correct plate file is loaded in the Plates tab.
- Ensure that the correction collar is set appropriately for the plate you are using.
- Ensure that there is not a problem with the well you are using. Try moving to one or two other wells using the Plate Acquisition and Control dialog box and repeating Step 8. If focus is still not found, continue to the next step.

11. From the Configure Laser Sensor dialog box, click *Preview Pass*. A window opens displaying a graph of focus intensities vs. Z-position. Ideally, the graph should contain two sharp peaks made up of a red line and a green line, as shown in Figure 3-20:

Figure 3-20
Preview Pass — In Focus



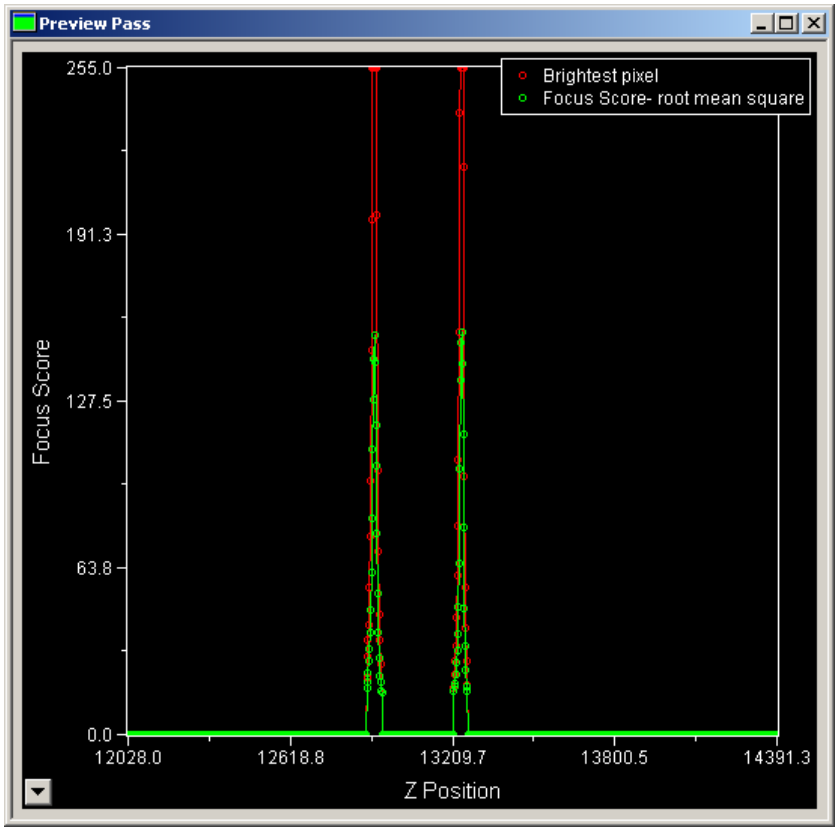
Note: The first peak (white arrow) represents the plate bottom — the second peak (blue arrow) is the well bottom. The top of each red peak represents the brightest pixel of the preview pass. The top of each green peak represents the highest focus score.

However, since the *Find Sample* command failed, you may see one of the following conditions:

- One or both of the peaks are saturated — the maximum intensity for the laser intensity is 255
- One of the peaks is very flat
- There is only one peak
- There are no peaks

Figure 3-21 shows two saturated peaks:

Figure 3-21
Preview Pass — Not Focused



12. Experiment with the *Exposure Time (plate bottom)* and *Laser Power* values until the *Preview Pass* command shows a sharp peak similar to the first peak in Figure 3-20.
13. Select *Use Bottom of Well Settings*, then experiment with the *Exposure Time (well bottom)* value until the *Preview Pass* command shows a sharp peak similar to the *second* peak in Figure 3-20. Note that when you have *Use Bottom of Well Settings* selected, your first peak will probably be saturated. This is expected behavior.
14. Click *Find Sample* again. If you still cannot find focus, refer to *Troubleshooting Laser Auto Focus Settings for Plate Types* in Chapter 7 of this document.

Note: Some plate/objective combinations may show a very weak signal for the bottom of the well when there is liquid in the well. If you are experiencing a very weak second peak (bottom of well focus value) even with a long exposure time, go to the *Autofocus* tab of the Plate Acquisition Setup dialog box, select *Focus on plate bottom only, then offset by bottom thickness* and try the *Find Sample* command again.

When you have finished making changes to the Configure Laser Focus dialog box, click *Save Settings*, then go to the Plate tab of the Plate Acquisition Setup dialog box and click *Save Settings* to save laser focus settings for the current plate

settings file. Note that when you click *Save Settings* in the Configure Laser Sensor dialog box, settings are saved only for the current objective. You must make and save settings for each objective as needed.

Note: If you need to create new plate files or create/troubleshoot LAF settings for a plate file, refer to Chapter 7 of this document.

Verifying Shading Correction files

Complete the following procedure to ensure that properly named shading correction files exist for each objective/filter set combination:

Notes:

- In order for shading correction images to be used during Plate Acquisition, the *Perform shading correction* checkbox must be selected in the *Acquisition Loop* tab of the Plate Acquisition Setup dialog box.
- The shading correction images must be named in the following format: **`shading_<magnification setting>_<wavelength>.tif`**. For example, **`C:\shading_4x Plan Apo_DAPI.tif`**.
- By default, MetaXpress looks in the root **`C:\`** directory for shading correction images. You can change this location by clicking the Directory button on the *Acquisition Loop* tab of the Plate Acquisition Setup dialog box and selecting a new location.

1. Use Windows Explorer to navigate to the root C:\ directory (or to another location if you have changed the default).
2. Locate the shading correction files. Ensure that they are named in the format listed above and that a file exists for each objective/filter set combination.

   Shading correction files are needed for each objective/filter combination and must be generated whenever an objective or filter is replaced or added to the system, or whenever the lamp is replaced. For information on creating shading correction files, refer to *Updating Shading Correction Settings* in Chapter 7 of this document.

Chapter 4
Preparing For Acquisition

This chapter provides plate acquisition guidelines to consider before acquiring experiment plate data. These guidelines help ensure that the images you acquire are the best possible quality. Review these guidelines before you define your plate acquisition experiment criteria.

Preparing to Acquire Fluorescence Images

When acquiring fluorescence images in a screening environment, follow a basic set of rules and guidelines to acquire quality images. As with any biological assays, the assay conditions need to be correctly evaluated to obtain a meaningful result. Include in your sample preparation both negative and positive controls so you can judge the validity of your assay. MDC recommends using a stain such as Hoechst or DAPI to stain your cells, since this stain can be used for focusing as well as segmentation for your analysis.

Despite the image enhancement tools and options available to you in the MetaXpress software, it is difficult to analyze a poor quality image. Starting with quality images helps ensure that your image data is more meaningful, and yields more information.

The following are the criteria to consider for attaining the highest possible fluorescence image quality:

Choice of Fluoroprobes – Ensure that you use probes that provide good staining and an excitation/emission pattern suitable for the filter cubes you have chosen for your ImageXpress^{MICRO}.

Illumination – Check that your light source is functioning correctly. The basic design of the ImageXpress^{MICRO} light source and light path within the imager help ensure that the light reaching your sample is the best possible quality. If you find that the light quality has become degraded, contact your system administrator or your MDC representative to correct the problem.

Objective choice – The magnification setting will depend on the type of information you are interested in obtaining. In general, for counting cells, a 10x objective is suitable. For translocation, a 10x or 20x objective is appropriate.

Wavelength – Select the correct wavelength for your fluoroprobes. More information on the supplied filter sets can be found in the *ImageXpress Hardware User's Manual*. The Chroma website at www.chroma.com is a good resource to help you determine the best filters to use with your stain of interest.

Exposure – The correct exposure time is crucial for your acquisition and analysis. Using the *Auto Expose* button on the one of the *Wavelength* tabs in the Plate Acquisition Setup dialog box will provide you with a good starting point. Adjust the exposure time after that so the grayscale intensity within a cell is about three times the intensity of the

background. In general, that will mean that you can reduce your exposure time, which will decrease your acquisition time.

Evaluating your Experiment Requirements

Nearly all settings for plate acquisition are made in the Plate Acquisition Setup dialog box. The settings that you choose are dependent on the content and distribution of your samples, as well as the requirements of your experiment. To help you determine what your settings should be, a brief checklist is included in this chapter.

Experiment requirements that you should consider include the following:

- What is the nature of your sample material? Is it very dense or very thin?
- Which stains will you apply to your samples?
- How many wells are in each plate?
- Which wells need to be imaged?
- Will you acquire multiple sites per well?
- Will you use a MetaXpress standard Application Module in conjunction with a prepared assay to analyze your data?

What is the nature of your sample material? Is it very dense or very thin?

Dense sample material requires more light and may mean that the Z-setting for focus will vary from sample to sample. If your sample is very dense, you might need to choose the *Low Signal* algorithm on the *Auto Focus* tab. If the focal plane varies greatly from well to well, you should set up a focus configuration to compensate for this.

Which stains and filter cubes will you use for your samples?

Your ImageXpress^{MICRO} system uses standard filter cubes to create the correct excitation wavelength and the corresponding emission wavelength filtration for your experiment. It also uses dichroic filtration to separate the excitation wavelength from the emission wavelength. Specific filter cubes are designed to be used with specific stains. It is important that your filter cubes are a correct match for your stains.

How many wells are in each plate?

For each experiment, you must specify the number of wells in the plates that you are using. You must use the same type of plate consistently throughout the experiment, and you must be sure that the plate dimensions are correctly specified. It is recommended that for any given experiment, you use only one brand of plate from a single manufacturer. Mixing various plate types from different manufacturers could introduce unknown variables and contribute to creating flawed data.

Which wells need to be imaged?

You can acquire images from any or all wells in a plate. The *Plate* tab on the Plate Acquisition Setup dialog box enables you to choose the specific wells from which you want to acquire images. However, you must apply this well selection to all plates in the experiment.

Will you acquire multiple sites per well?

Using multiple sites in a single well enables you to acquire images from a greater area of the well. If you have not checked the *Visit multiple sites per well* checkbox on the *Wells to visit* tab, an image will be acquired for only a single site located in the center of the well. The multiple sites option enables MetaXpress to acquire separate images of contiguous areas. Using the stitch command, you can assemble the smaller separate images into a single large image. This capability enables you retain or improve image resolution while increasing the image area of coverage. Unless you use a journal to change settings during the experiment, the sites you select are used during the entire experiment.

Sites can also be used to include specific areas of the wells in your experiment data, while at the same time excluding other areas of the well.

Will you use a standard MetaXpress Application Module to analyze your data?

You need to base your acquisition settings on the requirements of the application module used. The most important requirements are to prepare your samples correctly and to ensure that you use the appropriate filter set for each stain that you have applied.

Conditions that Interfere with Obtaining Quality Images

The following conditions or situations can interfere with obtaining the highest quality images:

- **Optics have been degraded by dust, dirt, fingerprints, or oil contamination** – If you detect any contamination on your objectives, you should inform your system administrator, who can take steps to clean the optics and correct the problem.
- **Uneven background** – The first step to correct an uneven background is to check for uneven illumination. If the illumination is uneven during fluorescence acquisition, contact your system administrator.
- **Uneven illumination** – Your ImageXpress system is designed to provide high-quality, evenly distributed illumination across the image field. If you observe that the light across your field is uneven, contact your system administrator or MDC representative.
- **Poor quality microplates** – Not all microplates are the same quality. The composition of the material of the bottom of the microplate needs to be of optical quality, or the images may be degraded. For fluorescence imaging, microplates with black well sides usually work the best. Plastic bottom plates are usually more uneven and distort light more than glass bottom plates.
- **Incorrect microplates** – Some experiments call for black, opaque microplates instead of clear ones. To additionally improve quality, you can use black opaque covers on your microplates. Also, if you are screening multiple plates, be sure that all plates are the same type from the same manufacturer. Well spacing can vary slightly from one manufacturer to another, and it is not possible to continuously change the settings for well spacing.

- **For laser autofocus, bottom of plate has dust, dirt, fingerprints, or oil contamination** – Since the laser measures the reflection from the bottom of the plate or from within the sample, interferences with reflection caused by dust particles, dirt, fingerprints, and scratches will affect the performance of the autofocus. To improve the autofocus, it is suggested that you clean the bottom of the plate using lens tissue and an optical cleaning solution.

Additional Guidelines

Magnification

Magnification selection depends on what measurements are needed. If you are interested in the total number of cells present a 10x objective may be adequate. But if you are interested in co-localization of two probes, a higher magnification might be needed. Counting or localizing small organelles may call for using objectives above 40x.

Exposure Time

The ratio between the Signal (intensity of the interested objects) and the Noise (the background and other forms of noise) (S/N) determines how hard it will be for the software to discriminate important features in an image. If this ratio is relatively small, it is more difficult to discriminate between objects and background. One method of increasing S/N is to increase the image exposure. Longer exposures provide higher signal in an image and, depending upon the sources of the noise, might not increase the noise to the same degree.

Conversely, longer exposures can cause photo bleaching damage and saturate the camera. Intensity measurements of an overexposed image are not accurate and these images should be avoided. One exception to this rule is when you are interested in extremely dim features of your sample that are otherwise not visible. A good example of this is overexposing a Neurite image where you are not interested in the bright cell body but you are interested in the weakly stained outgrowths.

Binning

Another method of increasing the S/N is to bin the pixels from the camera. Binning combines the electrons from adjacent pixels to create the effect of a single, larger pixel.. Binning increases the S/N at the expense of decreased resolution. Binning is often used to decrease the exposure time dramatically while maintaining the same S/N. Another positive feature of binning is that it produces smaller images that require less storage space.

Objective Choice

The choice of objective determines the magnification of the image, the depth of field of the image, and the brightness of the image. Another attribute of the objective is its numerical aperture (NA). With the magnification constant, brightness is proportional to NA^4. Higher NA objectives produce a sharper picture due to a narrower depth of field; this may or may not be an advantage if some of your objects are in different Z positions. Unfortunately, higher NA objectives, such as a SuperFluor 20x, cannot reach the outer

rows and columns of some multi well plates (such as a 384-well plate) because of the of the plate's skirt height.

Use of Different Fluorochromes

Individual fluorochromes have unique characteristics that help determine their best use. Some fluorochromes provide brighter intensities and require shorter exposure time, while others do not bleach as quickly and enable a longer exposure time. There also might be toxicity issues with some cell types or bleed through issues between pairs of fluorochromes. These factors should be considered when choosing a fluorochrome.

Shading

Shading is an artifact that can come from the objective, damaged optics, misalignment of the light source, and/or background light from the room. Shading should be addressed in the hardware first and if this fails, by using the available shading correction within the software.

Plate Choice

There are numerous types of multiwell plates available from a variety of vendors. MDC recommends determining what plates to use for your screening experiments based on the following guidelines:

- **Compatibility** —Verify that your cells are compatible with the plate material. Given the wrong surface, some cells might not bind and will act in an unusual manner, such as rounding up, or migrating to the edges of the well. If you are using immunohistochemistry you might require a much higher background staining in plastic as compared to glass.
- **Fluorescence background** — There is a large difference in auto fluorescence between glass and plastic. Also, there can be up to a 5X difference in auto fluorescence among plates from different manufacturers. If your signal is low, switching plate brands is a good troubleshooting tool.
- **Plate skirt height** — You should use a plate with a small skirt height (the height difference between the edge of the plate and the bottom surface of the wells) if you are using a high magnification or SuperFluor objective. If the skirt height is too high, you may not be able to image the outermost parts of a plate (the outermost rows and columns on a 384 well plate or some sites on the outermost rows and columns on a 96 well plate).
- **High magnification image clarity** — When using high magnifications, there are significant differences in clarity between standard plastic plates, optically clear plastic plates, and glass bottom plates.
- **Plate flatness and/or reproducibility of the Z pattern** — A truly flat plate is faster to scan than an uneven plate because the search range can be made smaller. The reproducibility of a plate allows you to set tighter focus ranges specifically for that plate type. This reduces the amount of focusing needed and speeds up acquisition. The major component in plate flatness is the variation from well to a neighboring well.

- **Outside edge of the plate** — If you use a plate handling robot, some types of plates do not work well with the fingers supplied with the robot and will require custom fingers to work correctly. If one or more plate types do not work with your robot grippers, contact MDC for assistance.

Correction Collars

If you are using an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you are using. For information on configuring the correction collar, refer to Appendix C, *Adjusting a spherical aberration correction collar on ELWD objectives*.

Chapter 5
Setting up Plate Acquisition

Before configuring an experiment, it is important to become familiar with the tools used in the MetaXpress screening software. The foundation of the MetaXpress software is MetaMorph. The MetaMorph software contains numerous dialog boxes for image acquisition, processing, and analysis. However, the majority of the dialog boxes available are not needed for a typical MetaXpress Plate Acquisition. This chapter explains the dialog boxes used to configure a screening experiment

Note: For more information on any MetaXpress dialog box, consult the online help available within the MetaXpress application. Online help for an active dialog box can be accessed by pressing the <F1> key.

Your MetaXpress main menu includes the Screening menu, which is used specifically for MetaXpress Image acquisition and analysis. Other menus, such as the Devices menu, provide additional dialog boxes needed to correctly configure MetaXpress.

To enable you to become more familiar with MetaXpress and the dialog boxes that you need to use, the following pages include sample images of the dialog boxes that are essential for acquiring plates and a brief explanation of each one.

Screening Menu

The Screening menu provides access to all Plate Acquisition-specific dialog boxes. Figure 5-1 shows the options available in the Screening menu:

Figure 5-1
Screening Menu

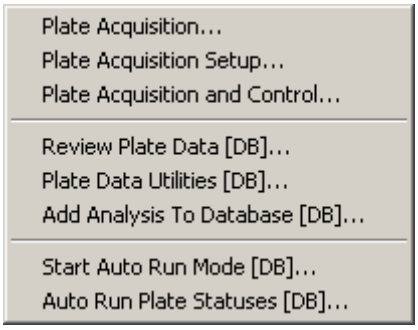
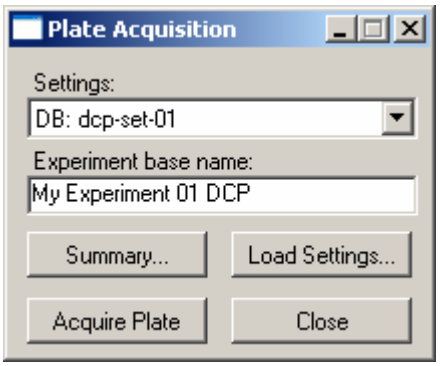


Plate Acquisition Dialog Box

The Plate Acquisition dialog box is designed to be used after you have configured and saved settings using the Plate Acquisition Setup dialog box. From this dialog box, you can choose a settings file, specify an Experiment Base Name, view a summary of your current settings, load specific settings from the selected file, and acquire a plate. Figure 5-2 illustrates the Plate Acquisition dialog box:

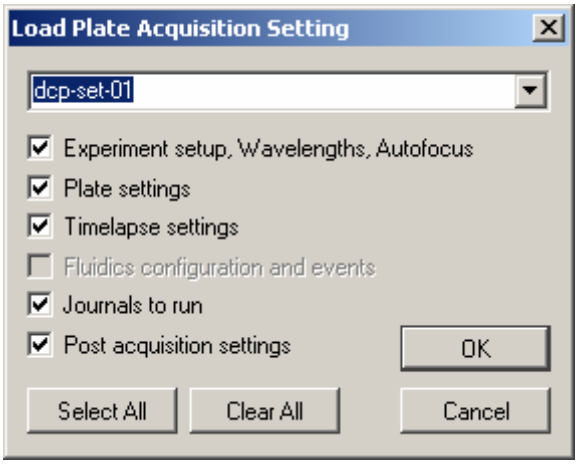
Figure 5-2
Plate Acquisition Dialog Box



Load Plate Acquisition Setting Dialog Box

After you have selected a settings file, click Load Settings to open the Load Plate Acquisition Setting dialog box, as shown in Figure 5-3:

Figure 5-3
Load Plate Acquisition Settings Dialog Box



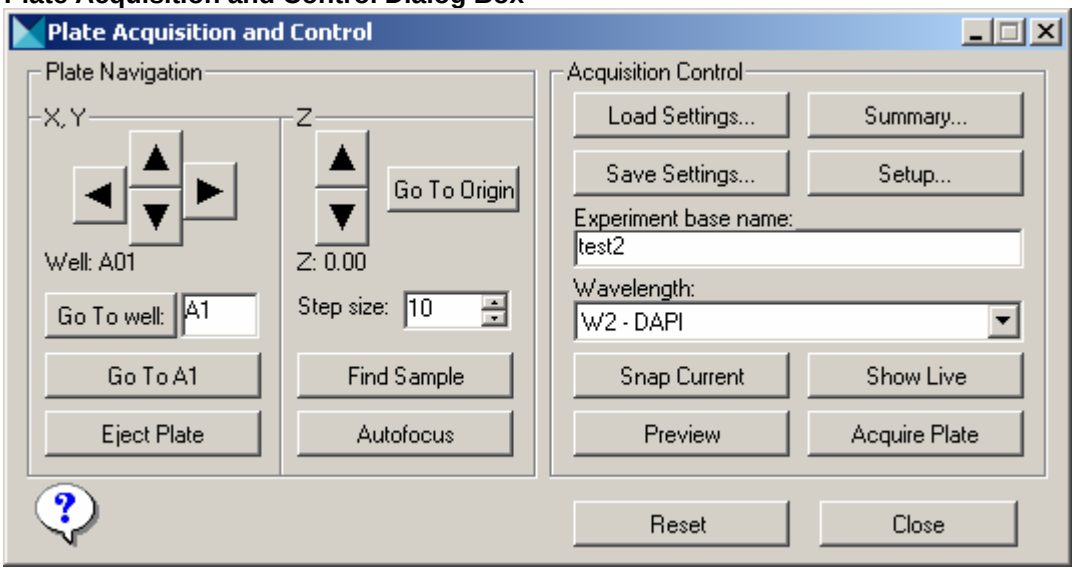
Use the Load Plate Acquisition Settings dialog box to load an existing settings file and choose the setting groups that you want to apply to your experiment. When you load a settings file, you can select one or more specific groups of settings to be applied to your experiment by checking the associated checkbox. Settings for boxes not checked are not used.

Plate Acquisition and Control Dialog Box

The Plate Acquisition and Control dialog box provides two primary functions. *Plate Navigation*, which provides manual control of some of the movable physical components of the ImageXpress^{MICRO} imager, and *Acquisition Control*, which enables you to prepare and start a plate acquisition. In the *Plate Navigation* Area, use the X, Y arrow buttons on the left that are organized in a cluster of four to manually move the plate from one well to another on the X and Y axes.

Note: If sites are enabled, the currently selected site is displayed below the arrow buttons along with the selected well. When you move from well to well, the selected site will remain the same. To change the site selection, use the site selection buttons on the Plate Acquisition tool bar, or the *Sites to Visit* field on the *Sites* tab of the Plate Acquisition Setup dialog box.

Figure 5-4
Plate Acquisition and Control Dialog Box



The Acquisition Control area provides a consolidated organization of commands that can also be found in other dialog boxes. These controls enable you to save settings, load settings that have previously been saved, open the Plate Acquisition Setup dialog box, specify a new experiment base name, choose the wavelength that you want to use, view a live image, snap an image using the current wavelength, preview the current selection of wells, and acquire an entire plate or all wells selected on the plate.

Plate Acquisition Setup Dialog Box

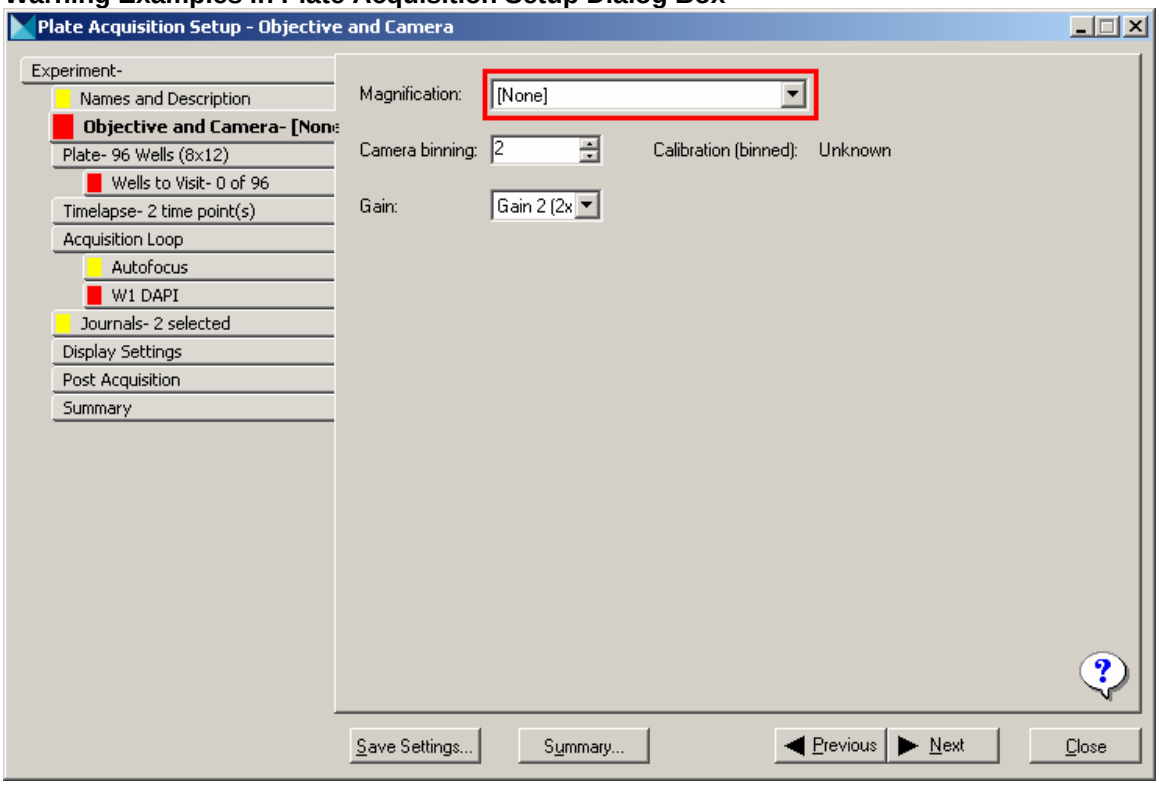
To prepare to use the MetaXpress software, you need to make the necessary configuration settings in the Plate Acquisition Setup dialog box. The Plate Acquisition Setup dialog box is organized in a “top-to-bottom” structure designed to guide you through the process of setting up your MetaXpress configuration in the correct order.

The Plate Acquisition Setup dialog box is a multi-tabbed dialog box in which the number of visible tabs can change according to the options that you have selected and the number of wavelengths that you are acquiring. Each tabbed area of the dialog box is dedicated to a specific type of function or setting.

Note: When configuring the Plate Acquisition Setup dialog box, you will encounter settings highlighted either in yellow or red. A yellow highlight can mean that an optional field is not filled in or could indicate another minor error. A red highlight means that a required field is either not filled in or contains invalid data that should be changed. These

visual reminders help when configuring an experiment. Figure 5-5 shows the dialog box with several warnings:

Figure 5-5
Warning Examples in Plate Acquisition Setup Dialog Box



Configuring the Experiment tab

Use the *Experiment* tab to specify whether you want to create new settings or load the setting stored in an existing settings file.

Figure 5-6
Plate Acquisition Setup dialog box – Experiment tab

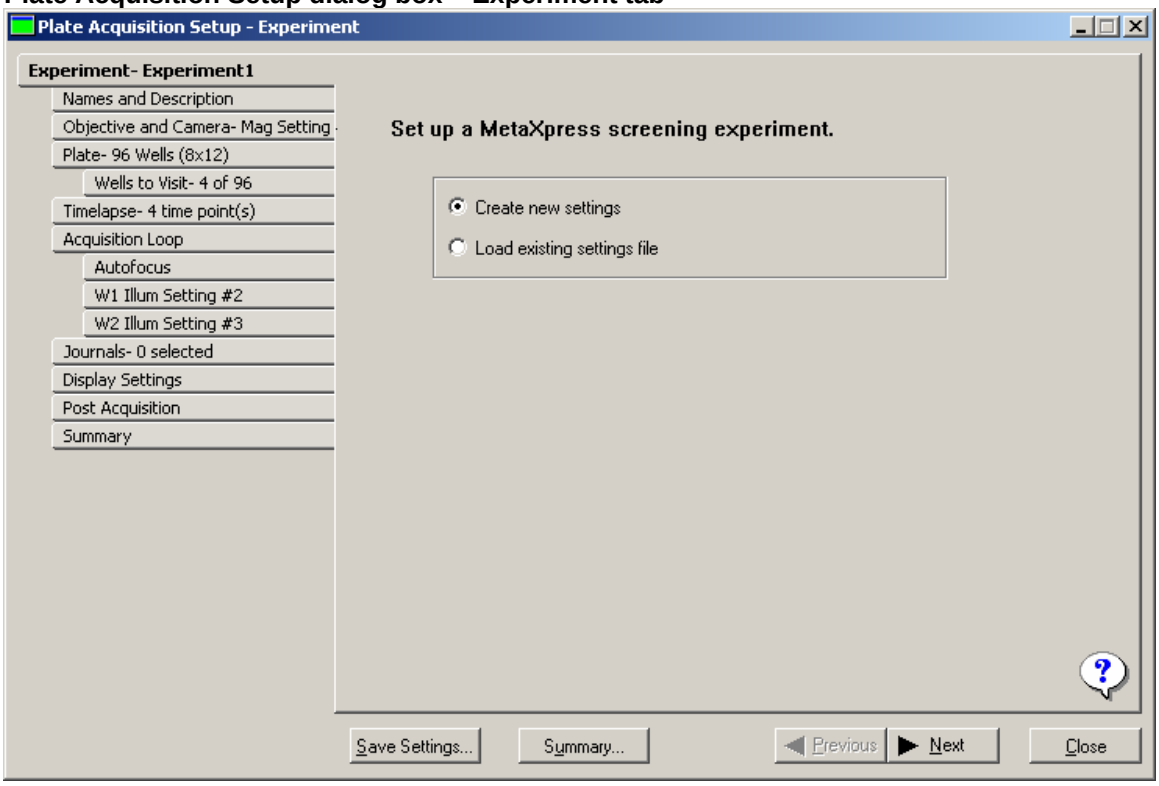


Plate Acquisition Setup Dialog Box Options - Experiments Tab

Create New Settings

Specifies that you are creating a new set of settings. Use this option to begin creating new acquisition settings. This is the default setting for starting a new experiment.

Load existing settings file

Enables the Load settings button. Click Load Settings to locate and load an existing settings file.

Load Settings

Opens the Load Settings dialog box and enables you to select a previously saved settings file.

Experiments Tab – Procedure

When you open the Plate Acquisition Setup dialog box, it opens to the Experiment tab. The Experiment tab enables you to create a new setting or load a previous setting for revision. If you are creating new settings, click *Next* to move to the next tab. To load an existing settings file, complete the following procedure:

1. From the Screening menu, select Plate Acquisition Setup. The Plate Acquisition Setup dialog box opens with the Experiment tab displayed.
2. Select *Load existing setting*. The Load Settings button appears.
3. Click *Load Settings*. The Load Plate Acquisition Settings dialog box opens.
4. If you are loading a settings file that was saved to the database, select the settings file to load from the Settings File drop-down list. Then select the individual settings to load from the file using the check boxes next to each settings group.

   OR

   If you are loading a settings file that was saved outside the database, select *From File* in the drop-down list and then select the individual settings to load from the file using the check boxes next to each settings group.

   Note: The settings listed here are configured on various tabs of the Plate Acquisition Setup dialog box.

5. If you are using a settings file from the database, click *Load*. The file will load from the database and the Load Plate Acquisition State dialog box will close.

   OR

   If you are loading a settings file saved outside the database, click *Load*. The Load Screen state dialog box opens. Navigate to the state file (.HTS) you want to open and click *Open*. The state file you selected will load and the Load Screen Acquisition dialog box will close.

6. Click *Load*. The selected settings will load and the Load Screen Acquisition dialog box will close.
7. Click *Summary* to view the *Summary* tab and confirm you settings.

   OR

   Click *Next* to move to the next tab.

Names and Description

After you have selected your settings file or chosen to create a new settings file, you need to designate a name and description for your experiment and choose the storage location for your experiment's data. Click the Names and Description tab, and complete the fields on the tab. The Names and Description tab enables you to assign an author name, base name, and description to your settings file. This information is stored with the settings file in the database.

Figure 5-7
Plate Acquisition Setup dialog box – Names and Description tab

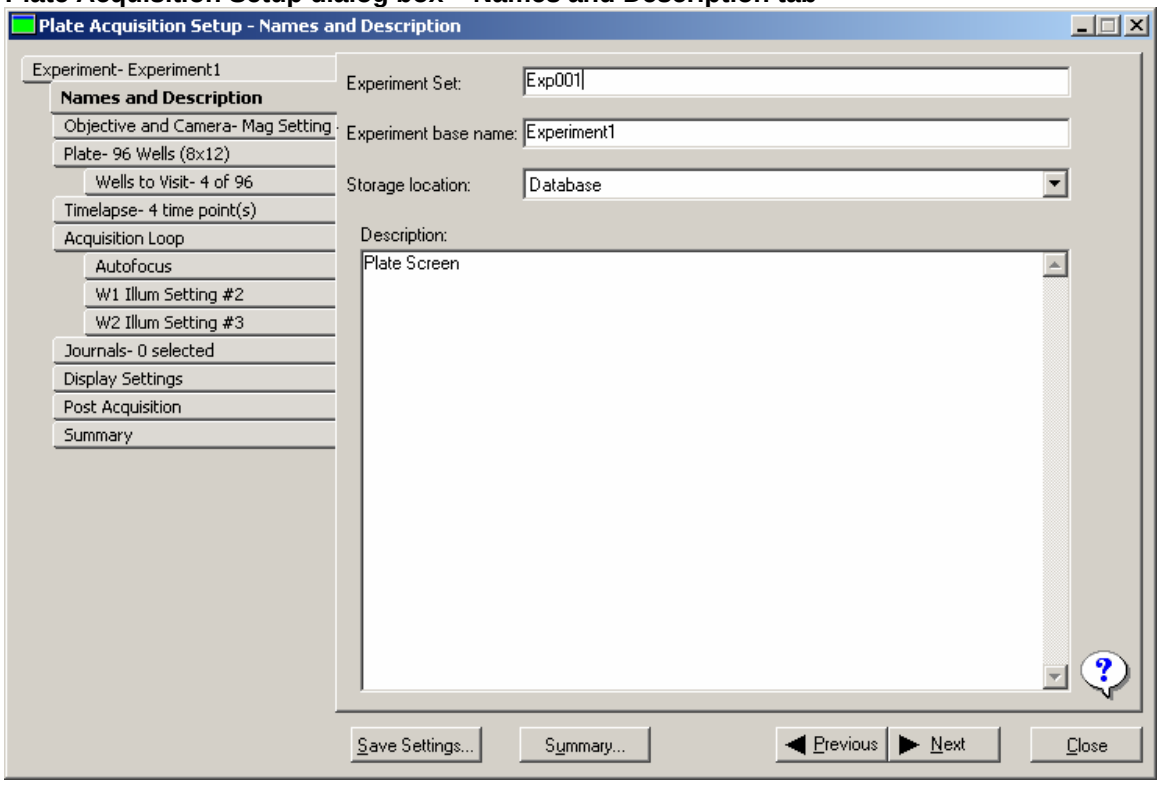


Plate Acquisition Setup Dialog Box Options - Names and Descriptions Tab

Experiment Set

Enables you to add an additional field in the database associated with the experiment. This label can be used to help sort and group experiments when view them using the Review Plate Data command.

Experiment base name

Defines the base file name.

Storage Location

Enables you to select a location where screening images are saved.

Note: You must configure the image locations using the Meta Imaging Series Administrator command Database Utilities command.

Description

Enables you to type an experiment description to be stored with the image information in the database.

Names and Description Tab – Procedure

To add an author name, base name, and description, complete the following procedure:

1. Type an experiment base name into the *Experiment base name* field.
2. Select a location where screening images are saved from the *Storage Location* Drop-down list.

   Note: Image locations are configured in the using the Meta Imaging Series Administrator command Database Utilities command.

3. Type a description into the *Description* field.
4. Click *Next* to move to the next tab.

Objective and Camera Magnification Settings

For each magnification setting that you have defined, this tab enables you to alter camera binning and gain. These settings enable you to either improve the image acquisition speed or improve the image quality. If sufficient light is available, lower camera binning and lower gain values will both increase the image resolution and improve the signal-to-noise ratio.

Note: Binning of an image can be beneficial for low intensity images, since it increases the signal-to-noise ratio and reduces the image file size. In this process, you lose resolution since you are grouping pixels together. For example, a 2 x 2 binning will combine the signals of four pixels into a single pixel, resulting in a reduction of your image from 100% maximum size to 25% of its maximum size.

Figure 5-8
Plate Acquisition Setup dialog box – Experiment tab

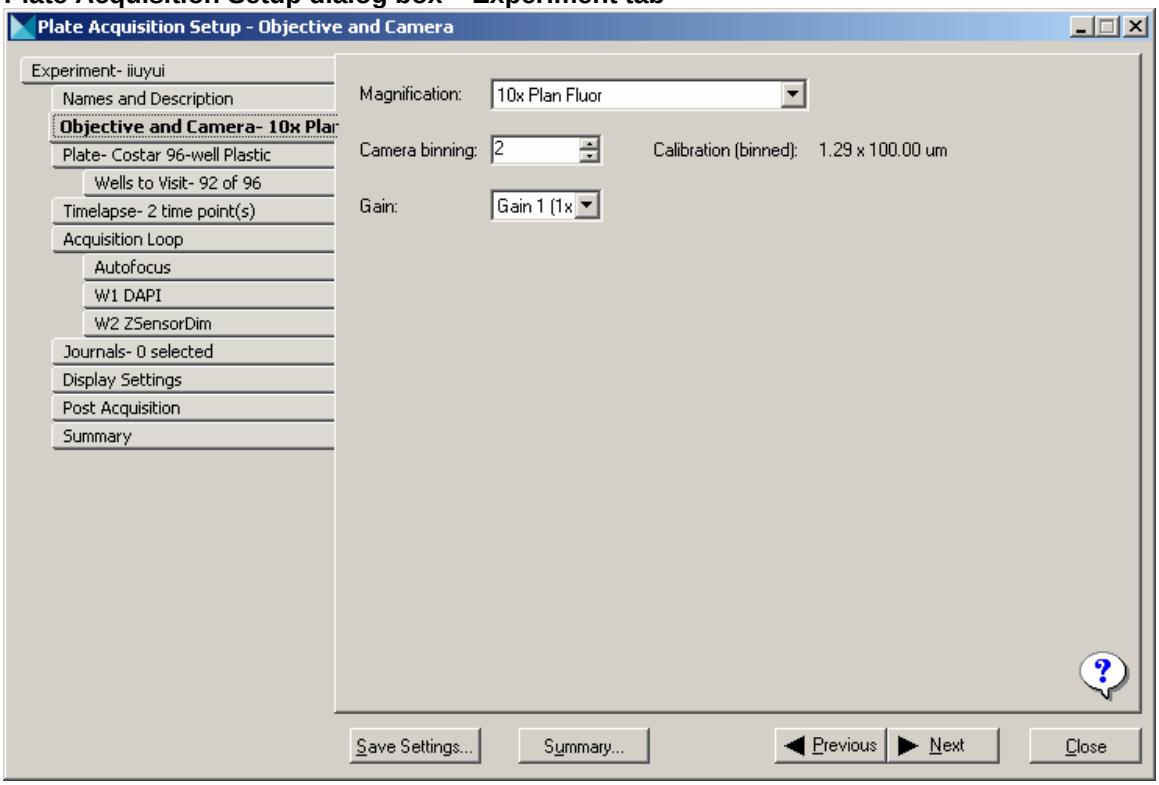


Plate Acquisition Setup Dialog Box Options - Objective/Camera Tab

Magnification

Selects the magnification setting that you want to use in your experiment. Magnification settings are made using the Configure Magnification dialog box. Magnification settings assign X and Y offset values and a Z escape distance to a specific objective.

Note: You must assign a calibration to the magnification setting using the Calibrate Distances dialog box. For more information, refer to the application note, *Spatially calibrating images in MetaMorph*, available from the Calibrate Distances online help page.

Camera binning

Specifies the binning value to be applied to the camera. Binning combines the output of adjacent pixels in square multiples. For example, a camera binning value of 1 is only one pixel, a binning value of 2 combines 2x2 or four pixels in a square, a binning value of 3 combines 3x3 or nine pixels in a square, and so on. This reduces the image file size and resolution, but increases signal-to-noise ratio.

Camera gain

Specifies the amplification to be applied to the camera output. The higher the gain, the greater the potential for background noise in the signal.

Objective and Camera Magnification settings - Procedure

Use the Objective/Camera tab to select magnification, binning, and gain settings for your acquisition. To change these settings, complete the following procedure:

Note: The settings available in the Magnification drop-down list were created in the Configure Magnification dialog box in the Devices menu. Magnification settings have a calibration and assign X and Y offset values and a Z escape distance to a specific objective.

1. Select the magnification setting to use from the *Magnification* drop-down list at the top of the dialog box.
2. Select the amount of binning from the *Camera Binning* box.

   Note: Binning is the process of combining data from multiple pixels (for example, 4 pixels — 2 x 2) into a single pixel during acquisition. Directing the camera to use binning causes the resulting acquired image to be brighter and smaller, but the resolution will be lower as a result. Because the image is smaller, both the time required to transfer the image and the storage requirement are significantly reduced. See the *Understanding Binning* online help page for more information.

3. Select the amount of gain from the *Camera gain* drop-down box (if applicable). The gain value specifies the amplification to be applied to the camera output.

   Note: For most experiments, use the highest gain possible for increased sensitivity.

4. Click *Next* to move to the next tab.

Plate Tab

Use the Plate Tab provide the necessary plate dimensional information needed to correctly control the X, Y, and Z movements of the ImageXpress^{MICRO} system. Correct entry of the plate dimensional specifications into MetaXpress ensures that the imager will not make any movements that might create a potentially hazardous situation. The dimensional information that you enter for the plate also ensures that the laser-based auto focusing is as accurate as possible.

Figure 5-9
Plate Acquisition Setup dialog box – Plate tab

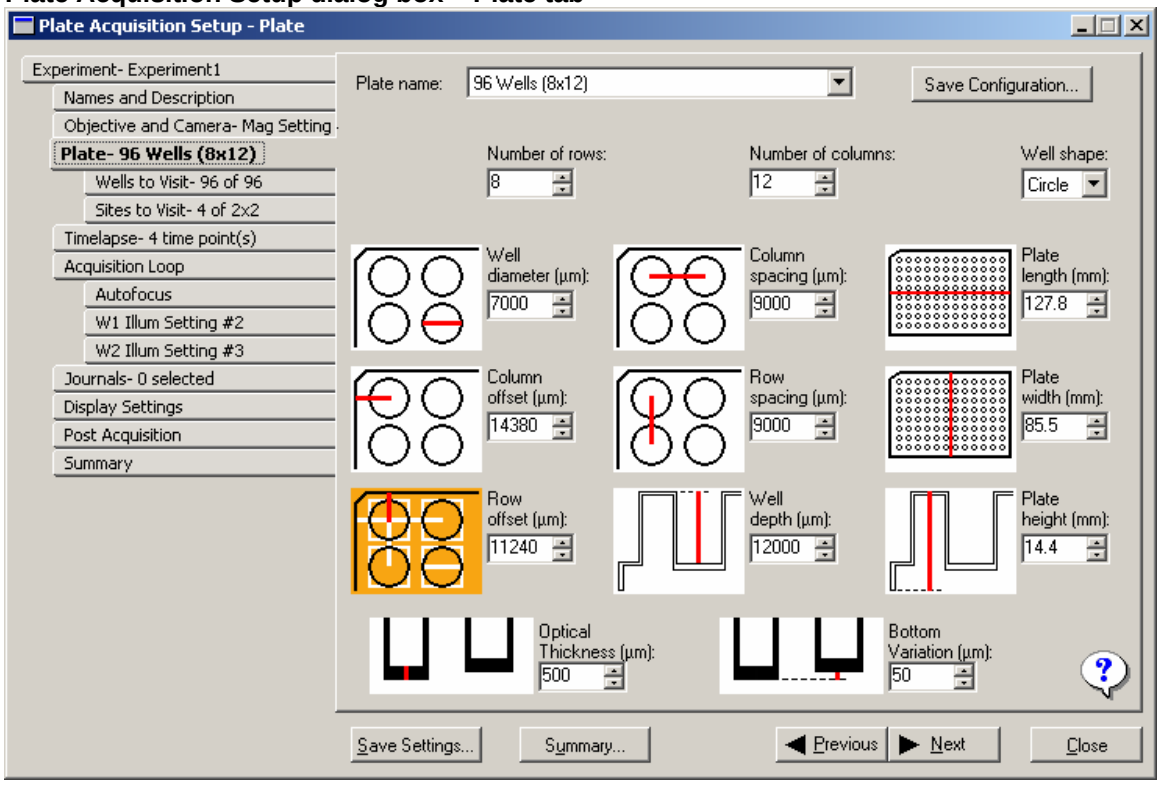


Plate Acquisition Setup Dialog Box Options - Plate Tab

Plate type

Specifies the plate type you are using. Choices include generic configurations such as Generic 96 well, custom configurations, or a previously defined configuration. Select the plate size that corresponds to your plate, or select *Custom* to specify a non-standard plate configuration. The values in the remaining boxes are pre-filled according to what is selected here.

To optimize acquisition, you need to specify the measurements of the plate that you will use. If your plate is not included in the drop down list, you will need to enter these values. Enter the manufacture's plate specification in the setting boxes on this tab. There are two values that the manufacturer will most likely not be able to supply: the optical plate thickness (this is not the same as plate thickness) and the bottom variation. These two values must be measured on the instrument to ensure proper focusing. Refer to Chapter 7, *Advanced Procedures*, for information on configuring plate types.

Save Configuration

Opens the Save Configuration dialog box and enables you to name and save a custom configuration based on the current values.

Number of columns

Contain the number of columns for the selected Plate type. This value can be changed to create a custom configuration. This option is only available if a custom plate type is selected.

Number of rows

Contain the number of rows for the selected Plate type. This value can be changed to create a custom configuration. This option is only available if a custom plate type is selected.

Well Shape

Selects the shape of the well – either Circle or Square.

Note: each of the following fields has a graphic that illustrates the measurement defined:

Well diameter

Contains a value in microns that is the diameter of the well. This value can be changed to create a custom configuration. This option is only available if a custom plate type is selected.

Column spacing

Specifies the spacing in microns between each well on the X axis. Normally this value should be the same for both the X and Y axis. However, you can specify different values for X and Y for plates that use different spacing between well on the X and Y axis. This option is only available if a custom plate type is selected.

Plate Length

Specifies the plate length in microns. This option is only available if a custom plate type is selected.

Column Offset

Specifies distance in microns between the center of a well in the first column of a plate and the left edge of the plate. This option is only available if a custom plate type is selected.

Row spacing

Specifies the spacing in microns between each well on the Y axis. Normally this value should be the same for both the X and Y axis. However, you can specify different values for X and Y for plates that use different spacing between well on the X and Y axis. This option is only available if a custom plate type is selected.

Plate Width

Specifies the plate width in microns. This option is only available if a custom plate type is selected.

Row Offset

Specifies distance in microns between the center of a well on the top row of a plate and the top edge of the plate. This option is only available if a custom plate type is selected.

Well Depth

Specifies the plate width in microns.

Plate Height

Specifies the plate height in microns.

Optical thickness

Contains a value in microns that is the average thickness for the well bottom measured using the laser autofocus. This value can be changed to create a custom configuration. Refer to Chapter 7, *Advanced Procedures*, for information on configuring this value.

Bottom variation

Contains a value in microns that is the maximum variation in Z direction between adjacent well bottoms. This value can be changed to create a custom configuration. Refer to Chapter 7, *Advanced Procedures*, for information on configuring this value.

Plate Tab Settings - Procedure

Use the Plate tab to configure and save plate settings for your acquisition. MetaXpress comes with a variety of common plate type already configured. Check the Plate name drop-down list in the Plate tab of the Plate Acquisition Setup dialog box to see what plate types are preconfigured.

Even if you use an existing plate type file, we recommend that you verify the accuracy of the optical bottom thickness and the bottom variation on your plates. These parameters are critical and can vary from lot to lot. Also, plate manufacturers can change plate parameters without changing plate names. Refer to Chapter 7, Advanced Procedures, for information on configuring these values.

If the plate type you are using is not listed in the Plate name drop-down list, you will need to complete the following procedures to assign and save correct plate values for the plate type.

The options available to you vary depending on what is selected in the Plate name drop-down list. To create and save plate settings, complete the following procedure:

Notes:

- You must know the optical thickness and bottom variation for your plate. The optical thickness is a value in microns that is the average thickness for the well bottom—see the graphic next to the *Optical Thickness* field. The bottom variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms. For instructions on determining the optical thickness and bottom variation for your plate, refer to the Chapter 7, *Advanced Procedures*.
- The correct *Well depth* and *Plate height* values are needed in order for the autofocus *Find Sample* command to work. These values can be obtained from the manufacturer.

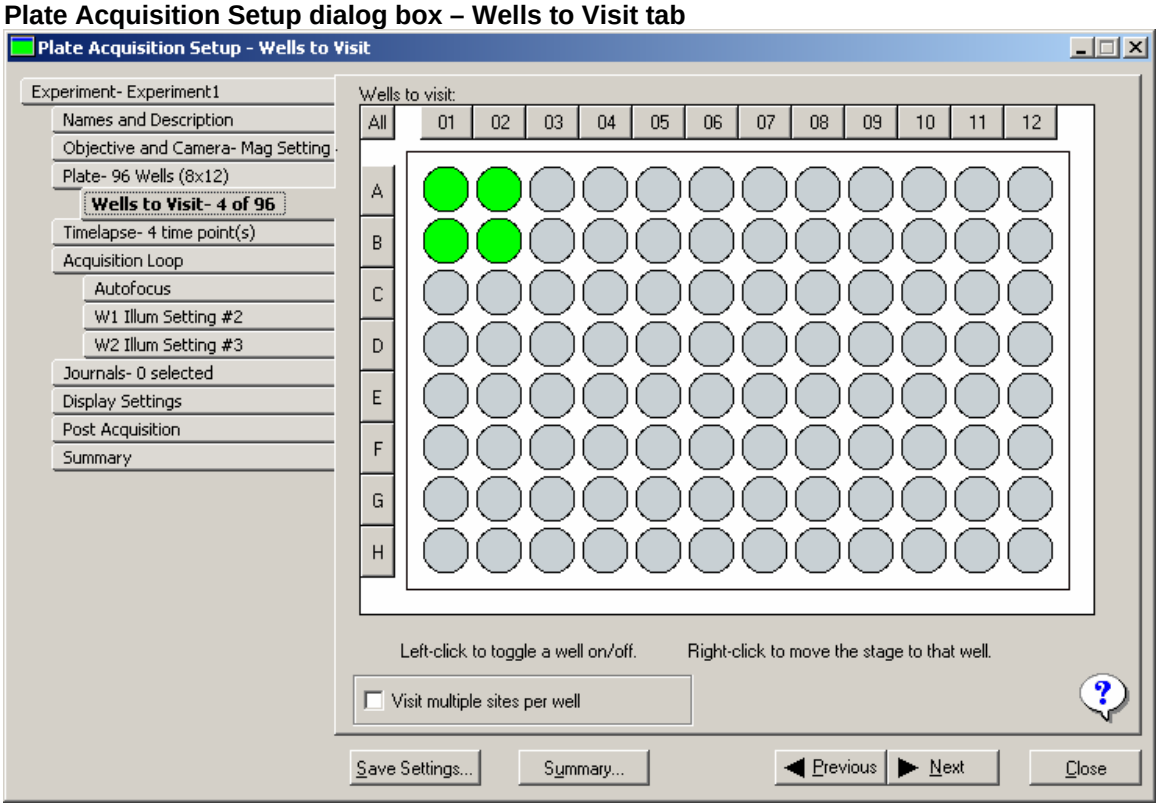
1. Select a plate that corresponds with the type you are using in the Plate name drop-down list.
2. If you select *96 Wells (8x12)* or *384 Wells (16x24)*, the following options will be available for configuration: Well Shape, Well depth, Plate height, Optical thickness, and Bottom variation.
3. If you select *Custom*, all options will be available for configuration.
4. Select your square or circular well shape from the *Well shape* drop-down list. The graphics of the plate are updated to reflect your selection.
5. Enter values in the *Optical Thickness* and *Bottom Variation* fields (see note above).
6. If needed, change the values in the *Well depth* and *Plate height* fields (see note above).
7. If you selected *Custom*, enter other values as needed.

8. After you have configured the plate settings, click *Save Configuration* to enter a name for your setting and save the plate configuration. Configurations that have been saved are then available to select in the *Plate Name* drop-down list.
9. Click *Next* to move to the next tab.

Setting Wells to Visit

The Wells to Visit tab enables you to configure which wells to acquire on your plate. You can also enable acquisition from multiple sites per well from this tab.. This dialog box tab displays a graphical representation of the type of plate that you are using, based on the settings that you made on the *Plate* tab.

Figure 5-10
Plate Acquisition Setup dialog box – Wells to Visit tab



Wells to visit

Select the wells to sample during the experiment. You can select a single well up to all the wells in the plate. Click individual well positions to toggle wells off and on separately. Right-click a well to move the stage to that well.

Click the column or row buttons to activate or deactivate an entire row. Click the *All* button in the upper left corner to toggle all wells on the plate simultaneously.

Visit multiple sites per well

Enables the Sites to Visit tab. When selected, there is a *Sites to Visit* tab in the tree-tab view and clicking *Next* opens the Sites to Visit dialog box where you can configure multiple sites per well.

Wells to Visit - Procedure

The Wells to Visit tab enables you to configure which wells to acquire on your plate. You can also enable acquisition from multiple sites per well from this tab. To configure which wells to visit, complete the following procedure:

Note: If you select Visit multiple sites per well, the Sites to Visit tab will become available and opens when you click *Next*.

1. In the Wells To Visit box, click to select the wells that you want to visit:

    - Click individual wells to select or deselect each well.
    - Click lettered buttons to select or deselect an entire row.
    - Click numbered buttons to select or deselect an entire column.
    - Click the *All* button in the upper-left corner to select or deselect all wells on the plate.
    - Right-click a well to move the stage to the well.

2. To enable visiting multiple sites per well, click *Visit multiple sites per well*. The *Sites to Visit* tab becomes available.
3. Click *Next* to move to the next tab.

Configuring Sites to Visit

Use this optional setting to specify acquisition of more than one location in a well. The number of sites in a well that you can acquire is dependent on the size of the well, the objective magnification, the distribution of sample material in the well, the type of plate, and the fluid content of the well. Other factors are the distribution of the material that you want to acquire, and the amount of spacing that you want to have between each acquisition location. All of these factors have the ability to influence the image quality.

The *Site arrangement in well* setting specifies the number of sites based on the setting value multiplied by itself. Thus a setting of 2 specifies four sites. You can further modify the Site arrangement by clicking any individual sites to deactivate them.

Note: The focus for individual sites is set on the *Autofocus* tab, described later in the document.

Figure 5-11
Plate Acquisition Setup dialog box – Sites to Visit tab

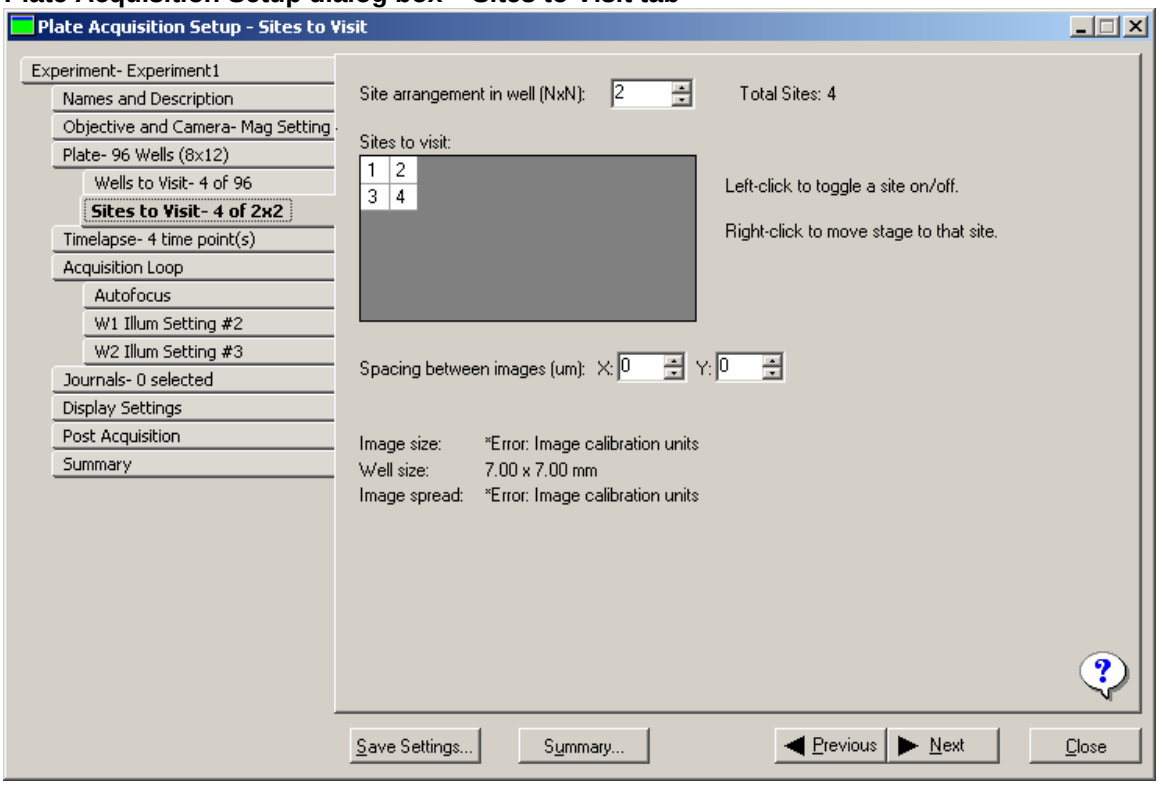


Plate Acquisition Setup Dialog Box Options - Sites to Visit Tab

Site arrangement in well (NxN)

Specifies the number of sites in the array, from 2 X 2 to a maximum of 127 X 127. Within the selected array of sites, you can click on any site to toggle the site on and off and right-click a site to move the stage to that site. You can have as few as one site or as many as 16,129 sites. Practical usage suggests that the

maximum number of sites that you select and use would be significantly less than 127 X 127.

Sites to visit

Select the sites to sample during the experiment. Click individual site positions to toggle sites on and off separately. Right-click a site to move the stage to that site.

Spacing between images

Specifies the distance between adjacent sites for both X and Y spacing. A negative value will overlap a portion of the data between adjacent sites images.

Sites to Visit Tab - Procedure

The Sites to Visit tab enables you to configure acquiring images from multiple sites in a well. You can also configure the vertical and horizontal spacing between sites in an image. Both positive and negative values can be entered. This enables you to either add a barrier between images (using positive values) or overlap images using negative values. To configure the Sites to visit tab, complete the following procedure:

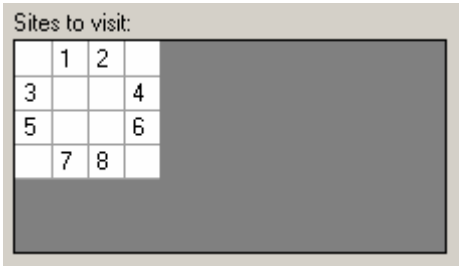
Note: The Sites to Visit tab is only available if you selected Visit multiple sites per well on the Wells to visit tab.

1. Enter a value in the *Site arrangement in well (NxN)* field:
    - Select 2 to acquire up to four sites
    - Select 3 to acquire up to nine sites
    - Select 4 to acquire up to 16 sites, etc.

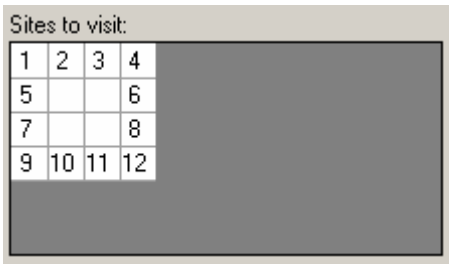
    Note: The box in the *Sites to Visit* field will grow or shrink according to the value entered in the *Site arrangement in well (NxN) field*.

2. In the *Sites to Visit* field click individual sites to turn off any sites that you do not want to acquire or to turn on any sites that are turned off. Right-click a site to move the stage to that site.

    Example:
    If you want to acquire images at the edges of a round well configured with 16 sites, configure the *Sites to visit* field as shown below:



    To acquire images at the edges of a square well configured with 16 sites, configure the *Sites to visit* field as shown below:



3. If you want to include a distance between adjacent sites, enter the X and Y values of the spacing in the *Spacing between images (um)* field.

   Note: Entering a negative value in these fields will result in overlapping data between adjacent images.

   You can view the current image size, well size, and image spread values on the lower half of the tab. These value update to reflect changes in configuration.

4. Click *Next* to move to the next tab.

Configuring Timelapse Settings

The *Time Lapse* tab enables you to set the loop order to use when acquiring images at multiple time points. It also enables you to set the number of time points to acquire and the total time needed for all time points.

Notes:

- If you are not using multiple time points in your acquisition, set the *Number of Timepoints* value to *1* and click *Next* to move to the next tab.
- The values in the *Number of Timepoints*, *Interval*, and *Duration*, fields have the following relationship:

  Number of Timepoints * Interval = Duration

  Changing any of the values will automatically update the others as needed.

Figure 5-12
Plate Acquisition Setup dialog box – Timelapse tab

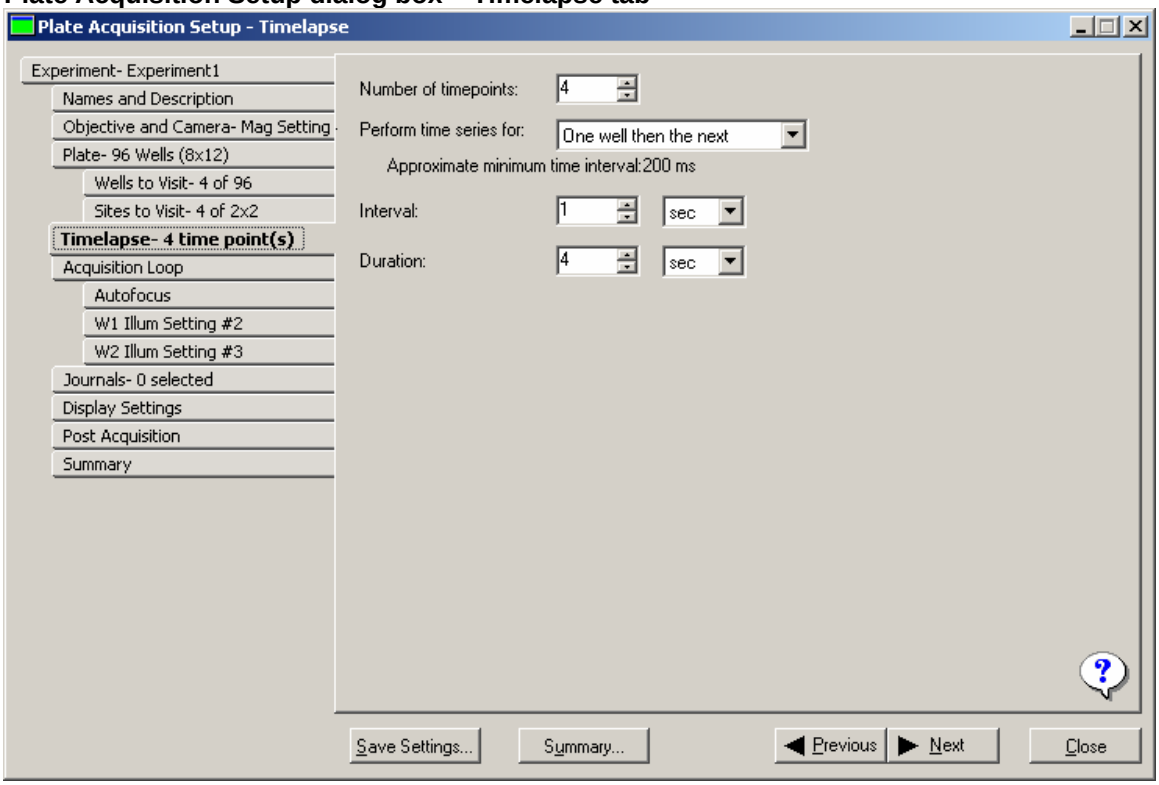


Plate Acquisition Setup Dialog Box Options - Timelapse Tab

Number of timepoints

Specifies the total number of time points to be acquired. When you change this field, the duration field is automatically updated by calculating the duration from the number of time points and the time interval.

Perform time series for

Selects the loop order to be used when acquiring images at multiple time points and determines the set of images to be acquired at each time point.

Valid acquisition sequences include the following:

- **One well, then next** – A set of wavelength images is acquired at each site in the well at each time point. No refocusing is done.
- **One row, then next** – All the images in one row's worth of wells are collect at each time point. Once the series is collected the next row is acquired. No refocusing done.
- **One column, then next** – All the images in one column's worth of wells are collected at each time point. Once the series is collected, the next column is acquired. No refocusing done.
- **All selected wells** – Every well selected for acquisition is acquired at each time points. The well selection is determined at the start of the first acquisition. No state file will be acquired. No refocusing is done.

Interval

Specifies the amount of time between the start of acquisition at one time point to the start of acquisition at the next time point. If the time interval is shorter than the length of time required to actually acquire the images, the next acquisition will occur as soon as possible once the first acquisition is complete. No warning notice will be given if the acquisition time is longer than the specified interval. Use the drop-down box to select a time unit. Units can be ms, sec, min, or hr. Changing the interval field updates the duration field by calculating the duration from the number of time points and the time interval. The interval field can be set to 0 to acquire images as fast as possible. If the interval is set to 0, the duration will be set according to the approximate minimum time, and the duration field will be inactive.

Duration

Specifies the time it will take to acquire the number of times points based on the interval. Changing this field updates the number of time points field by calculating the number of time points from the time interval and the duration. Use the drop-down box to select a time unit. Units can be ms, sec, min, or hr.

Time Lapse Tab - Procedure

To configure Timelapse for your experiment, complete the following procedure:

1. Select the number of time points to acquire in the *Number of Timepoints* field. Note that changing this value updates the *Duration field* as needed (see note above).
2. Select a loop order to use when acquiring images at multiple time points from the *Perform time series for* field. The following options are available:

    - ***One site, then next*** – Acquires all the images at the site and then collects the next set of wavelength images after the interval has elapsed. Once the series is collected, the next site is acquired. No refocusing is done after the first timepoint. This option is useful to collect large amounts of data from a well when image alignment is vital — for example, when performing cell mobility analysis.
    - ***One well, then next*** – A set of wavelength images is acquired at each site in the well at each time point. No refocusing is done. This option is most common for rapid acquisition from a well if multiple sites are selected. All sites are acquired per timepoint.
    - ***One row, then next*** – All the images in one row's worth of wells are collect at each time point. Once the series is collected the next row is acquired. No refocusing done. This option requires a longer time interval because all the wells in a row are acquired.
    - ***One column, then next*** – All the images in one column's worth of wells are collected at each time point. Once the series is collected, the next column is acquired. No refocusing done after the first timepoint.

- ***All selected wells*** – Every well selected for acquisition is acquired at each time point. The well selection is determined at the start of the first acquisition. No refocusing is done after the first timepoint.

3. Set the amount of time between the start of acquisition at one time point to the start of acquisition at the next time point in the *Interval* fields. Use the drop-down box to select a time unit. Units can be ms, sec, min, or hr.

   Notes:
   - If the time interval is shorter than the length of time required to actually acquire the images, the next acquisition will occur as soon as possible once the first acquisition is complete. No warning notice will be given if the acquisition time is longer than the specified interval. Under these circumstances, the actual duration will be longer than the duration time shown in the *Duration* field
   - The *Interval* field can be set to 0 to acquire images as fast as possible. If the interval is set to 0, the duration will be set according to the approximate minimum time, and the *Duration* field will be inactive.

4. If needed, change the amount of time for the entire time series in the *Duration* field. Note that the *Duration* field value is the result of multiplying the number of time points by the interval.

   Note: The *Number of timepoints* field will automatically update as the *Duration* field is changed.

5. To enable the *Fluidics* tab, select *Perform fluidics experiment*.

   Note: This option is currently N/A.

6. Click *Next* to move to the next tab.

Configuring the Acquisition Loop Tab

The Acquisition Loop tab configures options used to control the events of a single acquisition loop. Use this tab to configure the following settings:

- Number of wavelengths to acquire
- Autofocus options
- Activation of shading correction during acquisition

Figure 5-13
Plate Acquisition Setup dialog box – Acquisition Loop tab

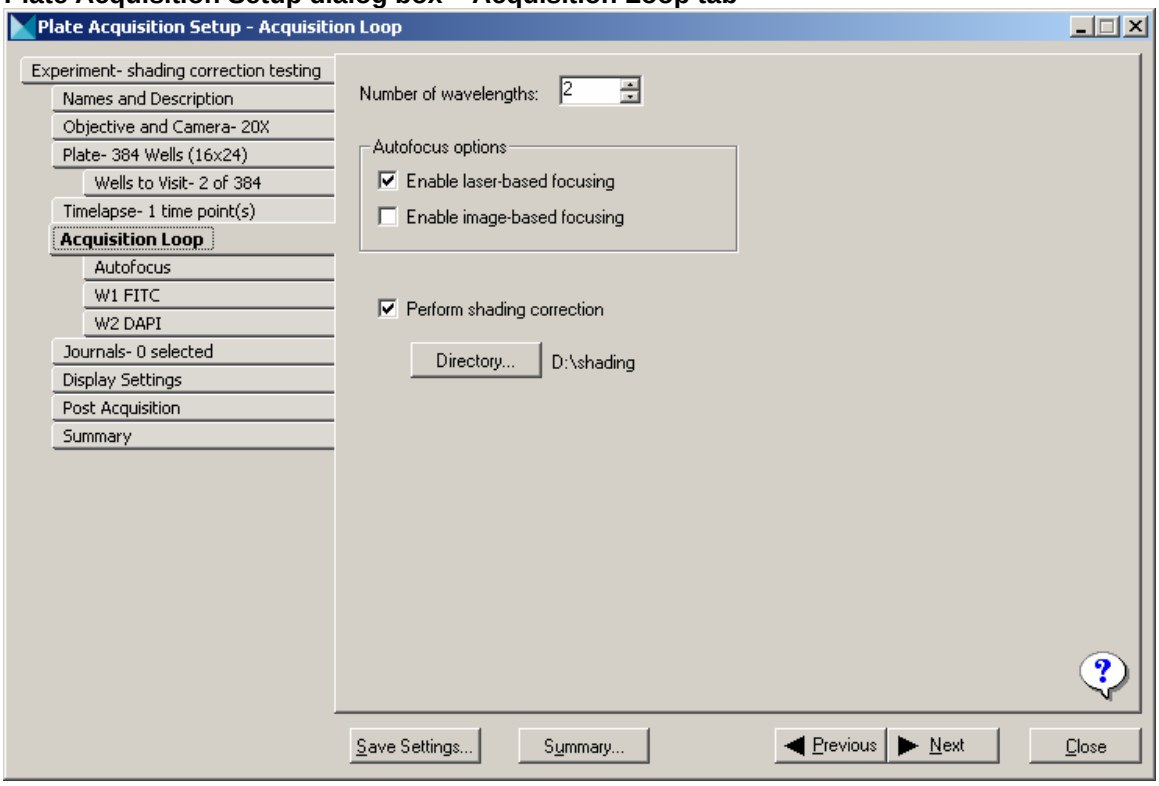


Plate Acquisition Setup Dialog Box Options - Acquisition Loop Tab

Number of wavelengths

The total number of wavelengths that you have configured for use during your experiment. Set from one to eight wavelengths in this box.

Autofocus options

Enable laser-based focusing

Selects laser-based focusing for the acquisition. You will be able to configure laser auto focusing in the *Autofocus* tab.

Enable image-based focusing

Selects image-based focusing for the acquisition. You will be able to configure image-based focusing in the *Autofocus* tab.

Perform shading correction

Enables shading correction for the acquisition. If a correction image exists for a wavelength, correction is performed. Correction images must be named in the following format: **`shading_<magnification setting>_<wavelength>_.tif`**. For example — **`C:\shading_10x_DAPI.tif`**.

Directory

Opens the Browse dialog box. Use this command to change the location where MetaXpress looks for shading correction images. The default location is the root C:\ directory.

Acquisition Loop Tab - Procedure

Use the settings on this tab to specify the type of Autofocus process to use, the number of wavelengths in your experiment, and whether Shading Correction will be applied to your images.

To configure the *Acquisition Loop* tab, complete the following procedure:

1. Select the total number of wavelengths to use during acquisition in the *Number of wavelengths* field.

   Note: You must enable at least one wavelength in this field.

2. In the *Autofocus options* field, select the autofocus options to enable in the *Autofocus tab* — laser-based, image-based, or both.

   Notes:
   - ***Laser-based***: Finds the bottom of the well and moves a specified distance up from the bottom. This method is normally fastest and will not cause photo damage to your specimen. This method does not work well if the distance above the bottom of the well changes in your sample.
   - ***Image-based***: Uses a specified algorithm to identify the best focus image. Best for experiments using low power objectives and when the sample distance above the bottom of the plate varies. This method can be slower than laser-based and can fail if there is out-of-focus debris present.
   - ***Both Laser- and Image-based***: Uses the laser to get to a specified position above the bottom of the well and image-based focusing to fine tune. This method works best when there is some variation in the distance above the plate bottom. It is especially useful at very high magnifications.

3. Select *Perform shading correction* to enable shading correction.

   Note: In order for shading correction to be applied during acquisition, correction images must exist for each wavelength used. The correction images must be named in the following format: **`shading_<magnification setting>_<wavelength>_.tif.`** For example — **`C:\shading_10x_DAPI.tif`**.

4. To change the location where MetaXpress looks for shading correction images, click *Directory* and selecting a new location using the Browse dialog box. The default location is the root C:\ directory.
5. Click *Next* to move to the next tab.

Configuring Autofocus Settings

Autofocus settings are made on a combination of three different tabs in the Plate Acquisition Setup dialog box and in additional dialog boxes within MetaXpress. Certain autofocus settings need to be made in advance by your system administrator to simplify the daily autofocus procedures needed when setting up an experiment. Refer to the *Advanced Procedures* chapter of this document for detailed procedures on initially setting up autofocus.

Within Laser Autofocus, you can choose to focus on one surface – the bottom of the plate, or two surfaces – the bottom of the plate and the bottom of the well. You can also choose to use offset values based on the laser autofocus position for a particular well to achieve proper focus on your sample(s) in the remaining wells. Offset values can also be used to achieve focus for different wavelengths without using the laser to re-calculate the focus distance.

The following tabs and dialog boxes are used to complete the process of configuring autofocus:

- Plate Acquisition Setup dialog box
  - Acquisition Loop tab
  - Autofocus tab
  - Wavelength tab
- Configure Laser Sensor dialog box
- Focus dialog box (optional)
  - Focus tab (optional)
  - Autofocus tab (optional)
- Configure Laser Autofocus dialog box (optional)

The *Autofocus* tab enables you to configure autofocus options for your acquisition. The choices available on this tab will vary depending on what you select in the *Autofocus Options* area of the *Acquisition Loop* tab.

Figure 5-14
Plate Acquisition Setup dialog box – Autofocus tab

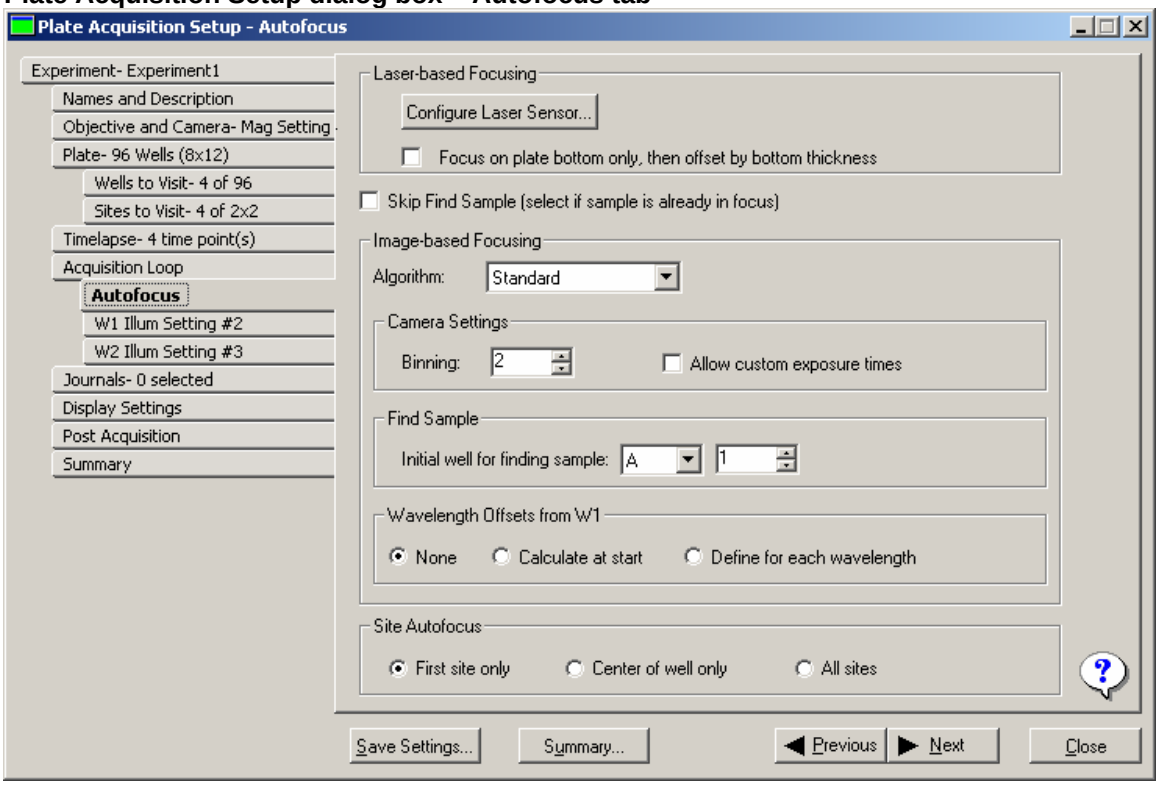


Plate Acquisition Setup Dialog Box Options - Autofocus Tab

Laser-based Focusing

Configure Laser Sensor

Opens the Configure Laser Sensor dialog box.

Focus on plate bottom only, then offset by bottom thickness

Offsets the laser by the bottom thickness of the plate, as defined in the Plate tab. Select this option if you are using a glass bottom plate and a liquid medium to increase the laser's accuracy.

Skip Find Sample (select if sample is already in focus)

Disables initial find sample auto focus when starting a plate. Select this option if your sample is already in focus.

Image-based Focusing

Algorithm

Enables you to select the algorithm to use when focusing. Valid choices include the following:

- **Standard** – Algorithm based on a standard group of settings including a normal camera signal level. (Default)
- **Low Signal** – Algorithm based on a set of values selected to compensate of a low signal level from the camera. This setting can compensate for situations in which some pixel intensities are somewhat brighter when slightly out of focus.

Camera settings

Binning

Sets the binning used by the camera during the Auto Focus and Show Live commands. Horizontal and vertical binning are always set the same and should be set to less than four.

Allow custom exposure times

Enables setting custom exposure times for individual wavelengths during autofocus. If this is not selected, the exposure time is calculated based on autofocus binning and acquisition exposure time.

Initial well for finding sample

Select the well to use when performing the initial find sample auto focus.

Wavelength offsets from W1

Defines how the wavelength offset from the first wavelength is determined when auto focusing. Valid choices include the following:

- **None** – No offsets are calculated.
- **Calculate at start** — Offsets are calculated at the start of acquisition.
- **Define for each wavelength** – Enables you to configure the offset for each wavelength in the Wavelength tabs.

Site Autofocus

Determines how autofocusing is done for each wavelength when sites are configured. The options are:

First site only — auto focuses in the top left site in the well.

Center of well — auto focuses in the center of the well. This is the recommended method.

All sites — auto focuses for each site.

Note: This option is available only if you have already configured the use of multiple sites in the Sites tab and enabled image-based focusing in the *Acquisition Loop* tab.

Setting up the Configure Laser Sensor Dialog Box

Use this dialog box in conjunction with the Plate Acquisition Setup *Autofocus* tab to configure the settings for the MetaXpress laser focus system. From this dialog box you can make settings that define the operating parameters of the focus sensor for each objective and plate type.

Notes:

- For complete instructions on how to configure this dialog box, refer to the *Advanced Procedures* chapter of this document.
- Once configured correctly, the settings in this dialog box usually do not need to be modified.

Configure Laser Sensor - ImageXpress Micro Dialog Box Options

Acquisition Settings for Mag: xx

Lists the laser sensor settings for the active magnification setting. The active magnification setting is the one currently selected in the Objective/Camera tab. Changes made to each setting are saved in a state file and used going forward for laser auto focusing unless the defaults are restored.

Notes:

- The default values are guidelines only and vary depending on the active objective. To obtain the best results, set these values so that the peaks on the Preview Pass graph are as sharp as possible.
- These settings are used only when auto-focusing using Laser Auto Focus and are not used during image acquisition. They are independent of the acquisition settings set in the in the Objective/Camera tab.

Set to Defaults

Restores the default settings to the active magnification setting.

Save Settings

Saves the settings to the current plate settings configured in the Plate tab.

Note: You must have a custom plate configuration selected on the Plate tab for the settings to be saved. The default 96- or 348-well settings files will not save your laser focus settings.

Exposure (plate bottom)

Specifies the exposure time in micro-seconds to be used when the laser is looking for the bottom of the plate.

Exposure (well bottom)

Specifies the exposure time in micro-seconds to be used when the laser is looking for the bottom of the well.

Coarse step

Z-motor step size used during the initial stage of autofocusing. The default value varies for each objective.

Fine step

Z-motor step size used during the second stage of autofocusing. The default value varies for each objective.

Laser Power

Sets the laser intensity. Valid values are 1-100. 100 is the default.

Use Bottom of Well Settings

Uses the bottom of well exposure setting when using *Preview Pass* or *Focus Snap*.

Start preview pass from current position

Use this option to run the *Preview Pass* function from the current Z position. This is useful in checking the current exposure values.

Find Sample

Performs a wide range auto focus on the current well position. Find Sample attempts to find the bottom of the plate, and then the bottom of the well (if configured on *Autofocus* tab). Displays a message box containing the results of the autofocus.

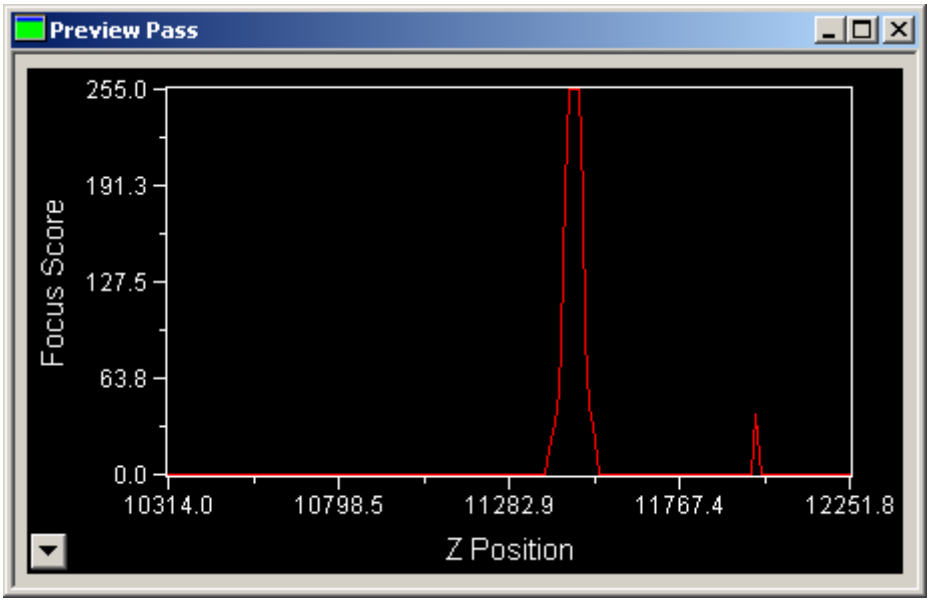
Preview Pass

Run the Z-motor through a range calculated from the current plate file (unless *Start preview pass from current position* is selected). Opens a graph that charts the focus values of each Z position. If *Use Bottom of Well settings* is checked, the well bottom exposure value, along with the *Fine Step* value, are used in the preview pass. If *Use Bottom of Well settings* is not checked, the plate bottom exposure and *Coarse Stop* values are used.

Use the Preview pass function to determine the optimal exposure settings to use during autofocus. Ideally, the graph will show two sharp peaks that represents the focus values at the bottom of the plate and the bottom of the well, as shown below:

Figure 5-15

Preview Pass dialog box



Autofocus

Performs auto focus on the current well position using the current settings. Autofocus attempts to find bottom of well only. If unable to search for bottom of well only, a full Find Sample search is done. Displays a message box containing the results of the autofocus.

Focus Snap

Acquires an image using the current auto focus settings.

Close

Closes the dialog box and saves the most recent settings.

Autofocus tab - Procedure

Note: For additional instructions on how to configure laser auto focus, refer to the *Advanced Procedures* chapter of this document. The following procedure only provides an overview of the dialog box.

Configuring Autofocus—Laser Auto Focus only

The laser autofocus settings in the Configure Laser Sensor dialog box are accessed by clicking the *Configure Laser Sensor* button on the *Autofocus* tab. Once these setting have been completed correctly, they typically do not need to be modified. If your current laser autofocus settings are not finding correct focus, use the *Preview Pass* and *Find Sample* commands to test your autofocus results, then adjust settings as needed to achieve correct laser focus.

To configure Laser Autofocus on your ImageXpress^{MICRO} System, complete the following procedure:

1. From the Screening Menu, click Plate Acquisition Setup. The Plate Acquisition Setup dialog box opens.
2. Click the *Wells to Visit* tab.
3. Right-click on the well that you want to use to set up and verify your focus accuracy.
4. Click *Visit multiple sites per well*, then click the *Sites to Visit* tab.
5. In the *Site arrangement in well* box type or select a multiplier that will provide enough sites to choose from, then click the site that you want to use for Laser Autofocus.
6. Click the *Acquisition Loop* tab.
7. On the *Acquisition Loop* tab, verify that *Enable laser-based focusing* is selected and that *Enable image-based focusing* is not selected.
8. Click the *Autofocus* tab.
9. In the *Site Autofocus* area, if sites are enabled on the Sites tab, choose the site pattern that you want to use. Choose *First Site only*, *Center of well only*, or *All Sites*:

   First site only — auto focuses in the top left site in the well.

   Center of well — auto focuses in the center of the well. This is the recommended method.

   All sites — auto focuses for each site.
10. If you plan to focus only on the bottom of the plate and then use the bottom thickness value to focus on your sample, click *Focus on plate bottom only, then offset by bottom thickness*.

11. In the *Laser-based Focusing* area, click *Configure Laser Sensor*. The Configure Laser Sensor dialog box opens.
12. In the Configure Laser Sensor dialog box, click *Preview Pass*, the Preview Pass runs and the Preview Pass dialog box opens. This dialog box displays a graph showing the focus scores for the bottom of the plate and the bottom of the well. If the preview pass fails to find focus, adjust the settings in this dialog box for Exposure, Course Step, Fine Step, and Laser Power. Refer to the detailed procedure in the Refer to the *Advanced Procedures* chapter of this document for more information about making these settings.

    Note: If settings in the Configure Laser Sensor dialog box are incorrect, a Red warning indicating Invalid laser parameters is displayed.
13. If the focus for the Preview Pass was successful, click *Find Sample.* Based on the results observed for Preview Pass, autofocus will attempt to find both the bottom of the plate and the bottom of the well, if selected. If *Find Sample* is not successful, adjust the settings in this dialog box for *Exposure, Course Step, Fine Step, and Laser Power* and try again.

    Note: If settings in the Configure Laser Sensor dialog box are incorrect, a *Red* warning indicating *Invalid laser parameters* is displayed.
14. Click *Autofocus* to run autofocus on the currently selected well with the current setting.
15. Click *Focus Snap* to acquire a single image based on your current settings. (refer to the *Advanced Procedures* chapter of this document for more information).
16. Click *Close* to close the Configure Laser sensor dialog box.
17. On the *Autofocus* tab, click *View Focusing Details*, if you need to view all the settings values that will be applied to your autofocus procedures.

Configuring Autofocus—Image-based Focus only

Complete the following procedure if you selected Enable image-based focusing on the *Acquisition Loop* tab:

1. From the *Algorithm* drop-down list, select from one of the following two image-based autofocus algorithms:

    Standard – Algorithm based on a standard group of settings including a normal camera signal level. (Default)

    Low Signal – Algorithm based on a set of values selected to compensate for a low signal level from the camera. This setting can compensate for situations in which some pixel intensities are somewhat brighter when slightly out of focus.

    Note: MDC recommends using this setting whenever you are using a 20x Apo lens.
2. In the *Binning* box, select the binning value that the camera will use during image-based auto focus.

    Note: It is recommended that your binning value is set to three or less.

3. If you do not want to use a calculated exposure time for each wavelength during autofocus**,** select *Allow custom exposure time*. This will enable you to set an exposure time for each wavelength on the Wavelength tab(s).
4. To disable the initial find sample auto focus routine when starting a plate, click *Skip Find Sample*.

   Note: Choose this option if your sample is already in focus.
5. If *Find Sample* is enabled, select the first well to be used when performing the initial find sample auto focus by setting the *Initial Well for Finding Samples* fields. A1 is the default and should not need to be changed if you are acquiring the entire plate.
6. In the *Wavelength Offsets form W1* field, Select how the wavelength offset from the first wavelength is determined when auto focusing. Valid choices include the following:

   None – No offsets are calculated.

   Calculate at start — Offsets between each of the wavelengths are calculated at the start of acquisition.

   Define for each wavelength – Enables you to configure the offset for each wavelength in the *Wavelength* tabs.
7. If you enabled *Visit multiple sites per well* in the *Wells to Visit* tab, select the site autofocus setting in the *Site Autofocus* field. The following choices are available:

   First site only — Auto focuses in the top left site in the well.

   Center of well — Auto focuses in the center of the well. This is the recommend method. This occurs even if the center of the well location is not configured to be acquired.

   All sites — Auto focuses at each site.

   Note: This option is only available if you have already configured the use of multiple sites in the *Sites* tab.
8. Click *Next* to move to the next tab.

Configuring Wavelengths

Use the Wavelength tab(s) to configure exposure, autofocus, and time-lapse settings for each wavelength. The total number of wavelength tabs depends on the number of wavelengths selected in the Acquisition Loop tab.

Figure 5-16
Plate Acquisition Setup dialog box – Wavelength tab

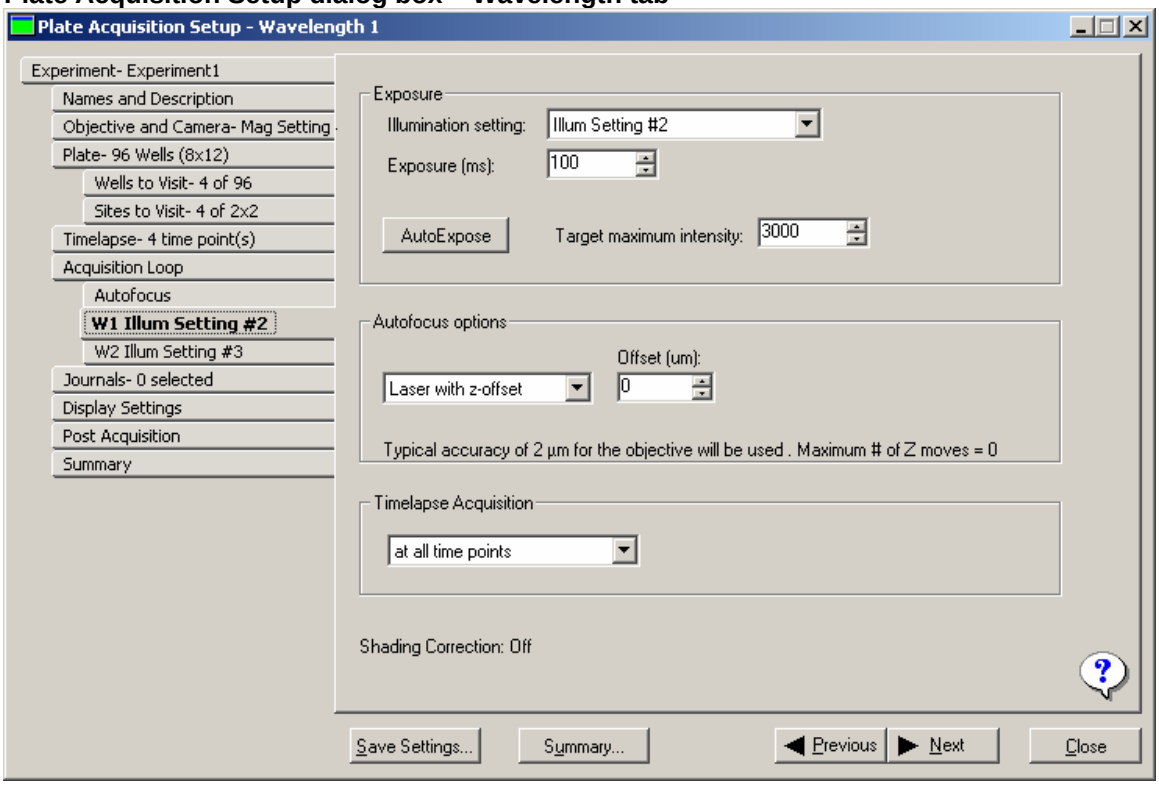


Plate Acquisition Setup Dialog Box Options - Wavelength Tab(s)

Note: There is one tab for each wavelength. The number of wavelengths is configured in the Acquisition Loop tab.

Exposure

Illumination setting

Selects an illumination setting to be used with the active wavelength. Illumination settings are defined in the Configure Illumination dialog box.

Exposure

Specifies the exposure time in milliseconds to be associated with the active wavelength. Type a value in this box or click Auto Expose to automatically determine an exposure time.

Auto Expose

Automatically determines the exposure time for the currently loaded sample, and applies it as the exposure value. Auto Expose works best when used with wells that have the most intense signal (for example, a positive control). You should set the *Target maximum intensity* to 75% of the maximum intensity of the camera.

Target max. intensity

Sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. The target

intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain.

Autofocus

Selects the type of autofocus to be used when acquiring images. The options available here depend on how the autofocus tab was configured. Valid autofocus choices include the following:

- **None** – No autofocusing is done. This selection enables no other options.
- **Laser with Z-offset** – Uses the laser autofocus as configured in the Configure Laser Sensor dialog box. This selection enables the Offset (um) setting and is only available for the first wavelength. This option is only available if *Enable laser-based focusing* is selected in the Acquisition Loop tab.
- **Z-offset from W1** – This option moves the specified offset from the W1 focus position and is only available for the second and subsequent wavelengths. Enter the Z-offset value (in um) in the Offset field. This option is only available if *Enable laser-based focusing* is selected in the Acquisition Loop tab.
- **Image Only** – This selection enables the *Range, Max. Step, Exposure,* and *Gain* fields. This option is only available if *Enable image-based focusing* is selected in the Acquisition Loop tab.
- **Laser and image** – This selection enables using both laser and image based autofocusing as configured in the Autofocus tab. It enables the Image-based range, Max. Step, Exposure, and Gain fields. This option is only available if both *Enable laser-based focusing* and *Enable image-based focusing* are selected in the Acquisition Loop tab.

Offset (um)

Laser offset to use for either the Laser with *Z-offset* option or the *Z-offset from W1* option.

Range

Specifies the total focus range that the focus operation is permitted to move. This is a plus or minus value from the current or previous focus position. Thus, if the range is +/- 500, the Z motor can move a maximum of 500 microns in either direction from the current or previous focus position.

Image-based range

Specifies the range to use for the image-based portions of auto-focusing. Laser auto-focusing is performed to an accuracy equal to this range before image-based auto-focusing begins. This option is only available if both *Enable laser-based focusing* and *Enable image-based focusing* is selected in the Acquisition Loop tab.

Max step (um)

Specifies the maximum step size in microns of a single Z move to be used in attaining the correct focus position. This setting is dependent on the objective used. Use a smaller step size with higher NA objectives because the focus peak is narrower.

Note: Smaller step sizes typically require more steps to arrive at the final focus position.

Exposure (ms)

Specifies the exposure time in milliseconds to be used when auto-focusing.

Gain

Sets the sensitivity of the camera when used with the Auto Focus command.

Timelapse acquisition

Specifies the image collection intervals to use for each wavelength. The following choices are available:

All time points – Acquires an image for each timepoint for this wavelength.

At start – Acquires an image at this wavelength for the first timepoint only.

At start and end – Acquires images at this wavelength for the first and last timepoints only.

Every nth timepoint – Acquires an image at this wavelength at the selected timepoint interval beginning at the first timepoint.

Image Shading Correction

Displays the current status of shading correction. This text is only visible if shading correction is enabled on the Acquisition Loop tab.

Wavelength(s) Tab - Procedure

To configure the *Wavelength* tab(s), complete the following procedure:

Note: The options available in the *Wavelength* tab(s) vary depending on the selection made in the *Acquisition Loop* and *Autofocus* tabs.

1. In the *Illumination* box, select the illumination setting for this wavelength. The illumination settings are defined in the Configure Illumination dialog box.
2. In the *Exposure* box, type or select an exposure time in milliseconds or, if you have an appropriate sample in view, click *Auto Expose* to set this value automatically.
3. Enter a value for the Target Intensity in the *Target Intensity* field or use the default value. This value sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. When *Auto Expose* is selected, the target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain.
4. Select the type of Autofocusing to perform for the wavelength in the *Autofocus* drop-down list:

   Note: Laser-based autofocusing is recommended under most circumstances. It is faster than image-based, less sensitive to the sample, and does not cause photo bleaching damage to the sample.

   Note: The number of options available in the drop-down list vary depending on selections made in the *Acquisition Loop* and *Autofocus* tabs. The following options are available:

   - ***None*** – no autofocusing will be performed on this wavelength.
   - ***Laser with z-offset*** – This option is available if *Enable laser-based focusing* is selected in the Acquisition Loop tab *and* this is the first wavelength. Select an offset to use for the first wavelength in the *Offset* field. No image-based focusing will be performed on this wavelength.
   - ***Image Only*** – This option is available if *Enable image-based focusing* is selected in the Acquisition Loop tab. It enables the *Range, Max. Step, Exposure, and Gain* (*Exposure, and Gain* are only available if Allow custom exposure time is selected in the Autofocus tab*).* This option is best used for complex samples with variations in distance between the surface of the plate and the sample. It is also the best choice for experiments using low magnification such as 2X or 4x.
   - **Laser and image** – This option uses the laser to focus, then uses image-based focusing to fine-tune the image. It is only available if both *Enable image-based focusing* and *Enable laser-based focusing* are selected in the *Acquisition Loop* tab. It enables the *Image-based range, Exposure*, and *Gain* fields(*Exposure, and Gain* are only available if Allow custom exposure time is selected in the Autofocus tab*).*

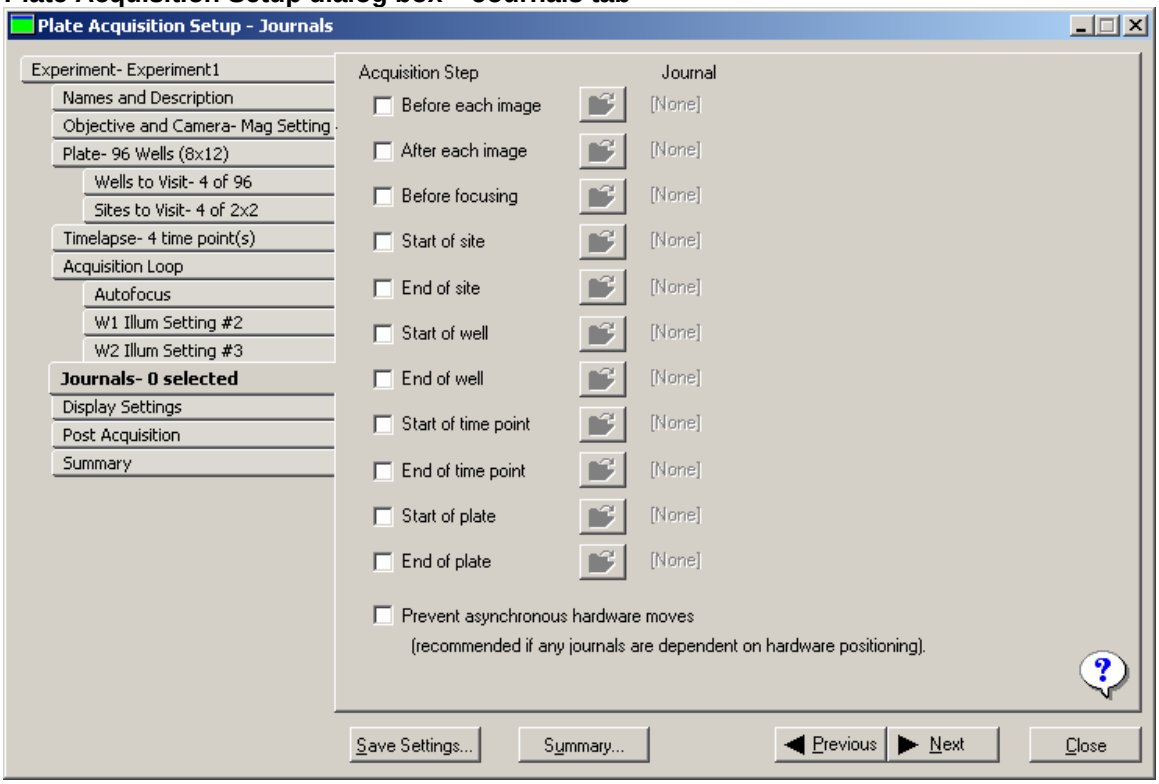
- **Z-offset from W1** — This option moves the specified offset from the wavelength 1 focus position and is only available for the second and subsequent wavelengths. Enter the Z-offset value (in um) in the *Offset* field. This option is only available if *Enable laser-based focusing* is selected in the *Acquisition Loop* tab.

5. If you enabled multiple timepoints in the *Timelapse* tab, use the *Timelapse Acquisition* drop-down box to set the image collection intervals to use for each wavelength. The following choices are available:
   - **All time points** – Acquires an image for each timepoint for this wavelength.
   - **At start** – Acquires an image at this wavelength for the first timepoint only.
   - **At start and end** – Acquires images at this wavelength for the first and last timepoints only.
   - **Every nth timepoint** – Acquires an image at this wavelength at the selected timepoint interval beginning at the first timepoint.
6. Click *Next* to move to the next tab.

Scheduling Journals

Use the Journals tab to configure specific journals to run during different stages of acquisition. For more detailed descriptions of each Acquisition step, refer to the Plate Acquisition Setup Dialog Box - Journals help page.

Figure 5-17
Plate Acquisition Setup dialog box – Journals tab



Acquisition step

Specifies that a journal should be run for the selected step. Only one journal can be assigned to each step. Click the check box next to the step that you want to use, then click *File Open* to assign the journal to the step. After you have assigned a journal to a step, you can temporarily deactivate the running of the journal by deselecting (un-checking) the check box for the step. To reactive a pre-assigned journal, simply click the check box.

Before each Image – Runs only during the acquisition loop, after the illumination is set and focusing is done.

After each image – Runs only during the acquisition loop, after the shutter is closed and before images are saved.

Before focusing – Runs only during the acquisition loop, just before focus evaluation begins.

Start of site – Runs only during the acquisition loop, before any images are acquired from each site.

End of site – Runs only during the acquisition loop, after all images have been acquired from each site.

Start of well – Runs only during the acquisition loop, at the beginning of each well, before any images are acquired from a well.

End of well – Runs only during the acquisition loop, at the end of each well, after all images have been acquired from a well.

Start of plate – Runs after the stage is moved to the find sample position, but before the find sample action is performed.

End of plate – Runs after the last acquisition for a plate is complete.

Start of time point – Runs only during the acquisition loop, at the beginning of each time point, before any images are acquired for a time point.

End of time point – Runs only during the acquisition loop, at the end of each time point, after all images have been acquired for a time point.

File Open 

Opens the Select Screen Acquisition Journal dialog box. Use this dialog box to select and assign a journal to a step. Also use this dialog box to deselect or unassign a journal to a step. To assign a journal, click the checkbox for the acquisition step and click the *File Open* button.

Journal

Lists the names of the journals that you have assigned to each step.

Prevent asynchronous hardware moves

Select this option if any of the journals you run move hardware (change shutters, move focus, etc.). This ensures that the journals will run correctly.

Journals Tab - Procedure

To configure the Journals tab, complete the following procedure:

Note: If you do not need to run any journals during the acquisition, click *Next* to move to the next tab.

1. Click the checkbox next to the Acquisition Step where you want to run a journal to select it.
2. Click the folder icon next to the selected acquisition step. The Select Screen Acquisition Journal dialog box opens with the contents of the Journals folder displayed by default.
3. Choose the journal that you want to run at the selected acquisition step, and click *Open*. If the journal is not located in the Journals folder, browse to the folder it is in, select it and click *Open*.
4. Repeat steps 1-3 for as needed to assign journals to run at additional acquisition points.
5. If any of the journals you run move hardware (change shutters, move focus, etc.), select *Prevent asynchronous hardware moves*. This ensures that the journals will run correctly.
6. Click *Next* to move to the next tab.

Configuring Display Settings

Use the Display Settings tab to configure the MetaXpress desktop appearance during acquisition. You can choose to use the default display settings or create custom settings. The default display settings tile and autoscale all acquired images, and ensure that the status dialog box is unobstructed.

Figure 5-18
Plate Acquisition Setup dialog box – Display Settings tab

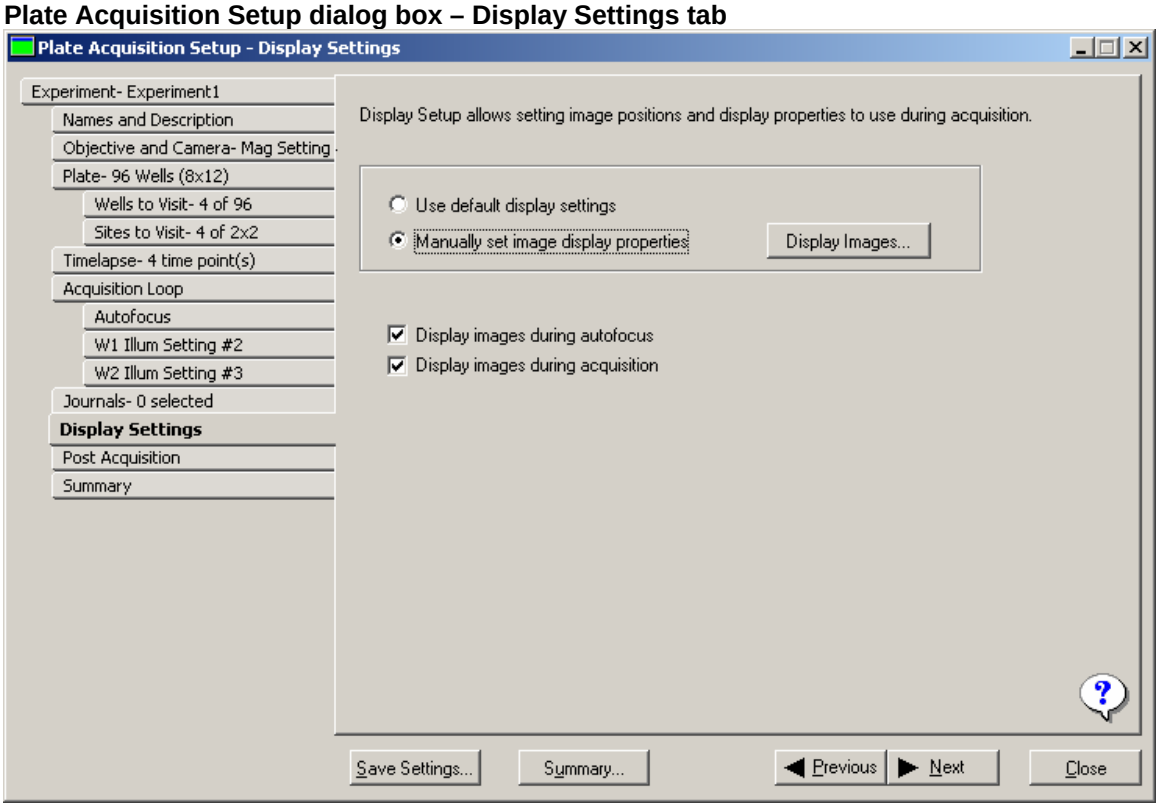


Plate Acquisition Setup Dialog Box Options - Display Settings Tab

Use this tab to configure the MetaXpress desktop appearance during acquisition.

Use default display settings

Uses the MetaXpress default settings for displaying images and dialog boxed during acquisition. Images are tiled and autoscaled and the status dialog box is unobstructed.

Manually set image displays settings

Makes the *Display Images* button visible, which enables you to change the configuration of the MetaXpress desktop during acquisition.

Display Images

Previews the current display settings by opening the Screening Status dialog box and acquiring an image for each wavelength. Once all images have been acquired, you can change the configuration of the display by arranging the image windows and dialog box and changing the size, scaling, and LUT of images. These new settings will be saved and used during acquisition.

Display images during Auto Focus

Displays the images acquired during autofocus. Disabled by default.

Display images during acquisition

Causes each image to be displayed as it is acquired.. Disabled by default

Display Settings - Procedure

To change the default display settings, complete the following procedure:

Note: If you do not need to change the display settings, ensure that *Use default display settings* is selected and click *Next* to move to the next tab.

1. Select *Manually set image display settings*.
2. Click *Display Images*. The Screening Status dialog box opens and an image is acquired for each configured wavelength.
3. Change the configuration of the display by arranging the image windows and dialog boxes; you can change the location, size, zoom, scaling, gamma, and LUT of images. These new settings will be saved and used during acquisition.
4. To display images acquired during auto focus, select *Display Images During Auto Focus*. This is disabled by default.
5. Click *Next* to move to the next tab.

Configuring Post Acquisition Settings

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 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 modules or journal assays saved to the database.

Notes:

- If you do not want to automatically run post-acquisition analysis, ensure that *Auto Run analysis after acquisition* is not selected and click *Next* to move to the next tab.
- The list of available assays and settings is the same list that is in the *Assay* tab of the Review Plate Data (DB) dialog box.

Figure 5-19
Plate Acquisition Setup dialog box – Post Acquisition tab

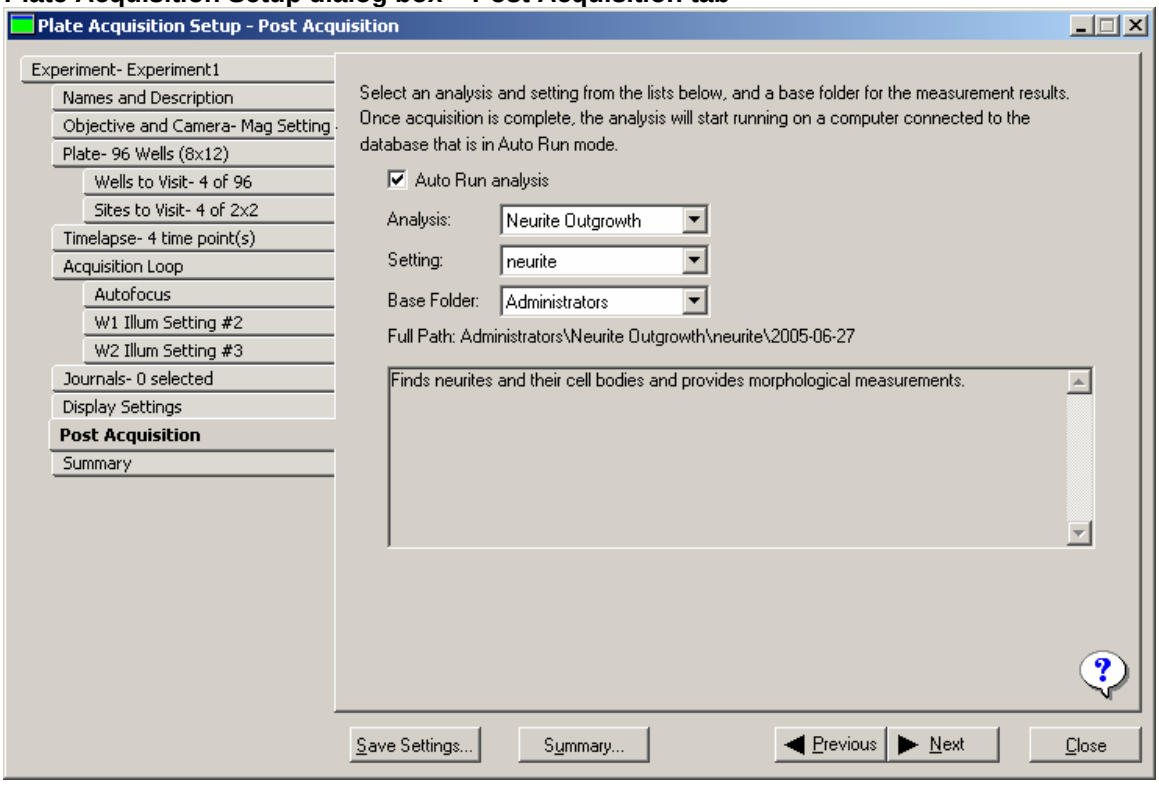


Plate Acquisition Setup Dialog Box Options - Post Acquisition Tab

Auto-Run analysis after acquisition

Activates the Analysis drop-down list that enables you to select an assay to auto-run on a separate MetaXpress computer after each plate is acquired. Refer to the Auto Run Mode help file for more information.

Analysis

Selects the assay to run. This list includes any application modules or journal assays saved to the database.

Settings

Selects the setting to use for the selected assay. The list included all settings previously saved to the database. The list of available assays and settings is the same list that is in the Assay tab of the Review Plate Data (DB) dialog box.

Base Folder

Shows available folders in the database to store measurement results. To select a new location, pick <*Select*> and click the  button to open the Measurements Sets dialog box. This enables you to select another base folder within the database to store measurements results.

Note: You can configure and save settings in the Review Plate Data (DB) dialog box.

Configuring Post Acquisition - Procedure

To select an analysis to automatically run after acquisition, complete the following procedure:

1. Select the *Auto Run analysis* checkbox.
2. Select the assay (Application module or Journal Assay) to run after acquisition from the *Analysis* drop-down list.
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 (DB) dialog box.
4. Select a base folder in which to store measurement results from the *Base Folder* drop-down list. To select a new location, pick <Select> and click the  button to open the Measurements Sets dialog box. This enables you to select another base folder within the database to store measurements results.
5. Click *Next* to move to the next tab.

Viewing the Summary Tab

Use the Summary tab to view a list of all current settings for the acquisition, save the settings to a file, and start acquiring images.

Notes:

- The information in the summary tab is identical to the information displayed when you click the Summary button on the bottom of the dialog box.
- If you want to make any changes to the stage or Z Position, or snap an image to test the current settings before starting the acquisition, use the Plate Acquisition and Control dialog box or the Plate Acquisition Toolbar to perform these and other tasks.
- If an error dialog box opens after you click *Acquire*, the most likely cause is a configuration error. Read the text in the dialog box to determine the error.
- After your acquisition is complete and the images have been saved to the database, you can use the Review Plate Data (DB) dialog box to view the images and setup analysis.

Figure 5-20
Plate Acquisition Setup dialog box – Summary tab

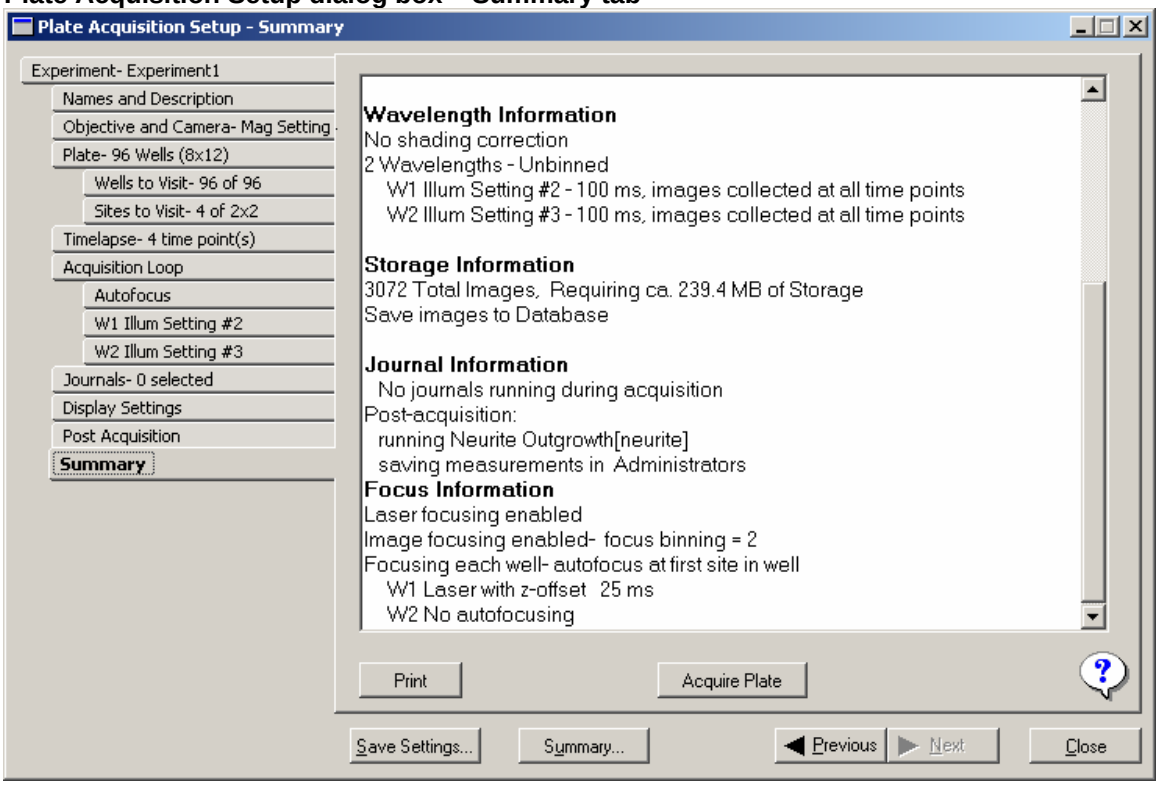


Plate Acquisition Setup Dialog Box Options - Summary Tab

Summary

Lists the current settings selected for your acquisition, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

Acquire

Starts the sequential acquisition of images from a plate based on the current settings.

Summary Tab - Procedure

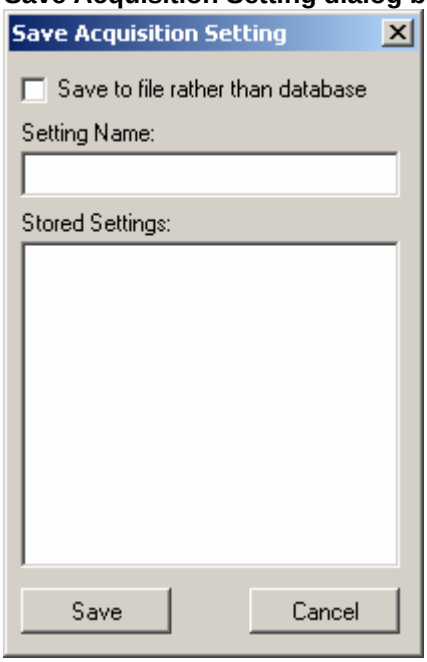
Complete the following procedure to save your settings file and start acquisition:

1. Click *Save Settings* to open the Save Plate Acquisition Settings dialog box and save the current settings to a file on the local hard drive or to the database.
2. If you want to print the settings summary, click *Print* to open the Print Setup dialog box and then print the settings.
3. Click *Acquire* to acquire images from a plate based on the settings made in the Plate Acquisition Setup dialog box. The expected behavior after clicking *Acquire* is outlined below:
    - The Plate Acquisition Setup dialog box closes and the Screen Status dialog box opens
    - Each image appears briefly on the MetaXpress desktop as it is acquired and saved to the database.
    - After the last image is acquired and saved, the Screen Status dialog box closes and the Plate Acquisition Setup dialog box reopens.
4. Click *Close* to exit the Plate Acquisition Setup dialog box.

Save Acquisition Setting

After you configuring the Plate Acquisition Setup dialog box, you should save the settings so they can be reused. The Save Acquisition Setting dialog box enables you to save your settings to the database or a file on a local or networked drive.

Figure 5-21
Save Acquisition Setting dialog box



Saving the Plate Acquisition Settings

To save the current Plate Acquisition settings, complete the following steps:

1. From the *Screening* menu, open one of the following:
   - Plate Acquisition and Control
   - Plate Acquisition
   - Plate Acquisition Setup
2. Click *Save Settings*. The Save Acquisition State dialog box opens.
3. To save the State file on the local computer, select *Save to file rather than database* and proceed to Step 3. To save the State file to the database, skip to Step 5.
4. Click *Save*. The Screen Acquisition State dialog box opens.
5. Type the name of a new state file that you want to create in the *File name* field, or select a listed state file name to overwrite an existing state file and click *Save*.
6. To save the State file to the database, ensure that *Save to file rather than database* is not selected and type the name of a new state file that you want to create in the State Name field, or select a listed state file from the Stored States field to

overwrite an existing state file in the database and click *Save*.

Save Acquisition State - Dialog Box Options

Save to file rather than database

Enables the state file to be saved to a file on the local computer instead of in the database.

State Name

Enter a State file name in this field.

Stored States

Contains a list State files saved in the screening database.

Save

Saves the current acquisition state to the database using the name selected in the State Name field. If *Save to file rather than database* is selected, the *Save* button opens the Screen Acquisition State

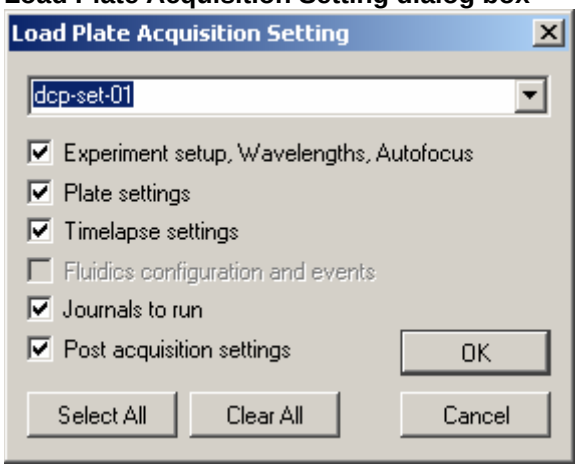
Cancel

Closes the dialog box without taking any action.

Load Plate Acquisition Setting

After you have created and saved settings using the Plate Acquisition Setup dialog box, you can use the Load Plate Acquisition Setting dialog box to load all or part of these saved settings for future experiments.

Figure 5-22
Load Plate Acquisition Setting dialog box



This dialog box loads the selected settings from an existing MetaXpress acquisition settings file. You can load all the settings from a saved file, or use the check boxes to select specific conditions or groups of settings to load.
After settings files are created and saved using the Plate Acquisition Setup dialog box, they can be loaded using the following dialog boxes, all found under the Screening menu of MetaXpress:

- Plate Acquisition Setup
- Plate Acquisition and Control
- Plate Acquisition

You can also use the Load Settings button on the Plate Acquisition toolbar to access the Load Plate Acquisition Settings dialog box.

Loading the Plate Acquisition Settings File

To load a saved screen acquisition settings file from one of the Plate Acquisition dialog boxes, complete the following steps:

1. From the Screening menu, open one of the following:
   - Plate Acquisition and Control
   - Plate Acquisition
2. Click *Load Settings*. The Load Plate Acquisition Settings dialog box opens.
3. If you are loading a settings file that was saved to the database, select the settings file to load from the *Settings File* drop-down list. Then select the individual settings to load from the file using the check boxes next to each settings group.

   OR

   If you are loading a settings file that was saved to your hard drive, select *From File* in the drop-down list and then select the individual settings to load from the file using the check boxes next to each settings group.
4. If you are using a settings file from the database, click *Load*. The file will load from the database and the Load Plate Acquisition Settings dialog box will close.

   OR

   If you are loading a settings file saved to your hard drive, click *Load*. The Load Screen state dialog box opens. Navigate to the state file (.HTS) you want to open and click *Open*. The state file you selected will load and the Load Screen Acquisition Settings dialog box will close.

Load Plate Acquisition State - Dialog Box Options

Settings File List

Selects a saved settings file from the database or from a local file on your hard drive.

These checkboxes enable or disable the loading of specific settings from a settings file. The Settings listed in the Load Plate Acquisition Settings dialog box are configured on the various tabs of the Plate Acquisition Setup dialog box.

Settings

Select the checkbox next to each setting to load it with your settings file.

Select All

Selects all the settings options

Clear All

Clears all the settings options.

OK

Loads the settings file selected in the Settings File drop-down list or opens the Load Screen State dialog box, enabling you to select a saved settings file stored outside the database.

> **Note**: you can only select a settings file stored outside your database if *From File* is selected in the Settings drop-down list

Cancel

Cancels the command and closes the dialog box.

More about Optical Thickness and Laser Focus

A plate bottom typically consists of plastic or glass material. The transitional point between the plastic or glass and air or liquid is referred to as an interface. The laser for the laser focus system reflects off both interfaces: First at the air to plastic or liquid to plastic interface (at the bottom of the well), and then at the plastic to air interface (when looking from the bottom of the plate through the plastic or glass material) at the bottom of the plate. The Z-position difference between these two interfaces is called the *Optical Thickness*.

When the system performs a focus search with the laser, it moves from bottom to top, looking for the first laser spot that it can locate. This represents the bottom of the plate. Then the machine moves the focus by the specified optical thickness and performs another search for the second laser spot. This is the interface of plastic to air or liquid. Correctly specifying the optical thickness is important since it ensures proper focusing.

Once the machine has found both interfaces, it will perform a search for only the second interface. To ensure that this is the correct range, the variation between wells must be accurately determined.

Using a Saved Setting on a New Plate

This procedure explains how to use a previously saved settings file to rerun an experiment on a new plate. After settings files are created and saved using the Plate Acquisition Setup dialog box, they can be loaded in several ways. This procedure uses the Plate Acquisition and Control dialog box to load the settings file and start the acquisition. See the note below for more options when loading a settings file.

Notes:

- This procedure uses the Plate Acquisition and Control dialog box to load the settings file and start the acquisition. You can also use either the Plate Acquisition dialog box or the Plate Acquisition toolbar to perform these tasks.
- This procedure assumes that the settings file you load is configured correctly for the plate you will use. If you need to make changes to the settings file before acquiring, use the Plate Acquisition Setup dialog box.

To load a saved plate setting and begin an acquisition, complete the following procedure:

1. From the Screening menu, select Plate Acquisition and Control. The Plate Acquisition and Control dialog box opens.
2. Select Load existing settings file, then click *Load Settings*. The Load Plate Acquisition Settings dialog box opens.
3. If you are loading a settings file that was saved to the database, select the settings file to load from the Settings File drop-down list. Then select the individual settings to load from the file using the check boxes next to each settings group.

OR

If you are loading a settings file that was saved outside the database, select *From File* in the drop-down list and then select the individual settings to load from the file using the check boxes next to each settings group.

4. If you are using a settings file from the database, click *OK*. The file will load from the database and the Load Plate Acquisition State dialog box will close.

   OR

   If you are loading a settings file saved outside the database, click *OK*. The Load Screen state dialog box opens. Navigate to the state file (.HTS) you want to open and click *Open*. The state file you selected will load and the Load Screen Acquisition dialog box will close.

5. Click *Summary* to open the Screen Summary dialog box and review your settings. Close the dialog box when finished.
6. If you need to make any changes to your settings, click *Setup* to open the Plate Acquisition Setup dialog box and make the changes.
7. Click *Acquire* to begin the acquisition with the loaded settings.

Chapter 6
Acquiring Plates

Plate Acquisition can be initiated from any of the following locations in MetaXpress:

- Plate Acquisition and Control dialog box
- Plate Acquisition Setup dialog box - Summary tab
- Plate Acquisition dialog box
- Plate Acquisition Tool Bar

Each of these locations is intended to serve a specific function in the plate acquisition flow. Depending on where you are in this process, you need to determine which dialog box is most appropriate to use for the type of plate acquisition that you need to run. For example, when you are first designing your experiment, you would most likely be initiating acquisition from either the Plate Acquisition and Control dialog box or the Summary tab of the Plate Acquisition Setup dialog box. Once all settings are complete, you would most likely initiate acquisition from the Plate Acquisition dialog box or the Plate Acquisition Tool Bar.

Note: If you are using a robotic plate handler to load your plates, acquisition is initiated from within the environment of the software that controls the plate handler device.

Plate Acquisition and Control Dialog Box

At the beginning of the process flow, while designing your experiment, you will most likely need to make manual changes to the X, Y and Z positions to accomplish correct plate and focus alignment. The Plate Acquisition and Control dialog box provides controls that enable you to simplify and expedite this process. You can also use this dialog box as a starting point for configuring the Plate Acquisition Setup dialog box. Click *Setup* to open the Plate Acquisition Setup dialog box. Other controls on this dialog box enable you to initiate autofocus, specify the Experiment base name, Load Settings, Save Settings, display the Summary, open a Live window, open a preview window, Snap an image, and Acquire a Plate. For additional information about this dialog box refer to the following paragraphs or this topic in the MetaXpress Online Help.

Using the Plate Acquisition and Control Dialog Box

Use this dialog box to acquire images from multi-well plates using the settings defined in the Plate Acquisition Setup command. You can also control the stage and Z-motor from this dialog box, as well as change the current wavelength and save and load settings.

Note: Most of the tools available in the Plate Acquisition and Control dialog box are also available in the Plate Acquisition toolbar. To display the Plate Acquisition toolbar, select Window>Toolbars> Plate Acquisition from the MetaXpress menu bar.

There are several possible workflows available for your acquisition. One typical workflow for multi-well plate acquisition is as follows:

- Configure and save your settings file using the Plate Acquisition Setup dialog box.
- Use the Plate Acquisition and Control dialog box or toolbar to do the following:
  - Load your settings file and review the settings using the Summary button.
  - Confirm your settings if needed using the available tools.
  - Enter an experiment base name.
  - Start the acquisition. During acquisition, the acquired images are saved into the database.
- If you want to make any changes to the stage or Z Position, or snap an image to test the current settings before starting the acquisition, use the Plate Acquisition and Control dialog box or the Plate Acquisition Toolbar to perform these and other tasks.
- Perform any post-acquisition analysis using the Review Plate Data or Plate Data Utilities dialog boxes. This can be configured to start automatically from the Plate Acquisition Setup dialog box if desired.

Figure 6-1
Plate Acquisition and Control dialog box

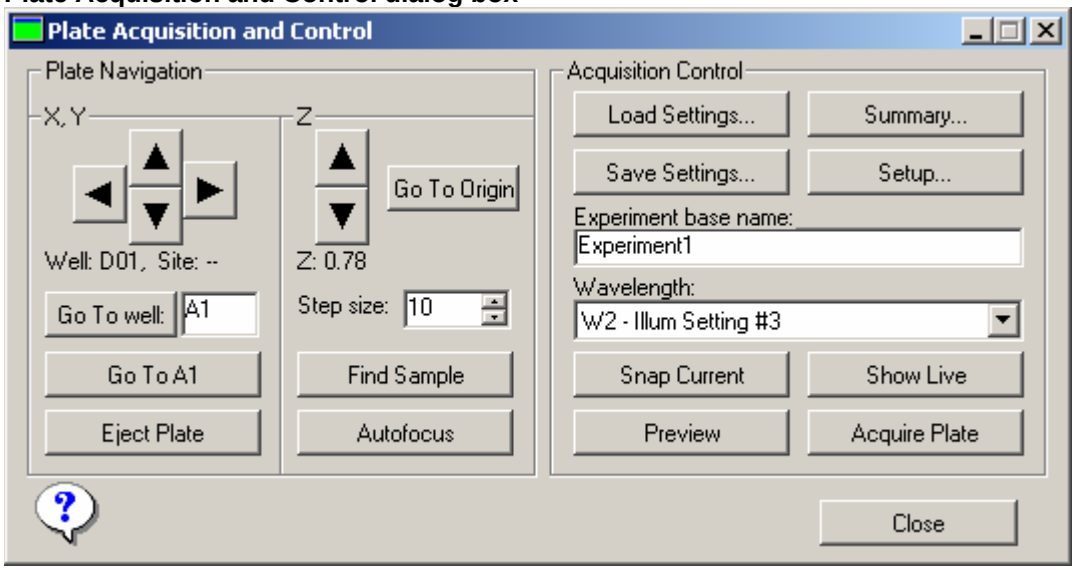


Plate Acquisition and Control - Dialog Box Options

Plate Navigation

X, Y Controls

Moves the stage in increments of one well in the direction of the selected arrow button.

Well

Indicates the well currently in position for image acquisition.

Site

Indicates a site within a specific well that is currently in position for image acquisition.

Go to well

Moves the stage to the well number that you type into the Go to well box.

Go To A1

Moves the stage to the A1 position.

Z Controls

Moves the Z-motor in one-step increments in the direction of the selected arrow button. The step size is set in the *Step Size* field.

Go to Origin

Moves the Z-motor to the focus position as defined in the Focus dialog box.

Step size

Sets the size of the individual focus increments used by the *Z* control arrows.

Find Sample

Performs a very coarse auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire.

Auto Focus

Performs auto focus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup tool.

Acquisition Control

Load Settings

Loads the selected settings from an existing screening settings file. Settings files are stored either in the database or on the file system. When you click *Load Settings*, the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear all* to clear all selections. Click *Load* to load your selected conditions. The Load Settings function is identical to the Load Settings option in the *Experiment* tab of the Plate Acquisition Setup dialog box.

Summary

Lists the current settings selected for your acquisition, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well. The Summary function is identical to the *Summary*

tab in the Plate Acquisition Setup dialog box.

Save Settings

Saves the current settings to a file on the local hard drive or to the database. When you click *Save Settings*, the Save Acquisition dialog box opens. Type the name of a new settings file that you want to create, or select a listed settings file name to overwrite an existing settings file.

Setup

Opens the Plate Acquisition Setup dialog box and enables you to change acquisition settings.

Experiment base name

Defines the base file name.

Wavelength

Selects the wavelength to use for your snap or live image.

Snap Current

Acquires a single image of the currently in place well at the current settings for stage (XY-position), focus (Z-position), wavelength, well, site, and exposure.

Show Live

Continuously acquires images based on the current settings, and updates the image as settings are changed.

Preview

Previews the current display and exposure settings by opening the Plate Acquisition Status dialog box and autofocusing and acquiring an image for each wavelength. Once all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog box and changing the size, scaling, and LUT of images. These window new settings will be saved and used during acquisition.

Acquire

Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Settings dialog box.

Close

Closes the dialog box.

Using the Plate Acquisition and Control Dialog Box

The controls on the Plate Acquisition and Control dialog box allow you to manually control certain microscope functions to enable you to test settings and conditions and acquire preliminary or test images of samples. The Acquire button is used to begin the automated acquisition process configured in the Plate Acquisition Setup dialog box. Use the following procedure to familiarize yourself with the controls on the Plate Acquisition and Control dialog box:

1. From the Screening menu, select Plate Acquisition and Control, the Plate Acquisition and Control dialog box opens.
2. Ensure that a plate is in place on the microscope stage.
3. To move the plate to A1, click *Go To A1*.

   OR

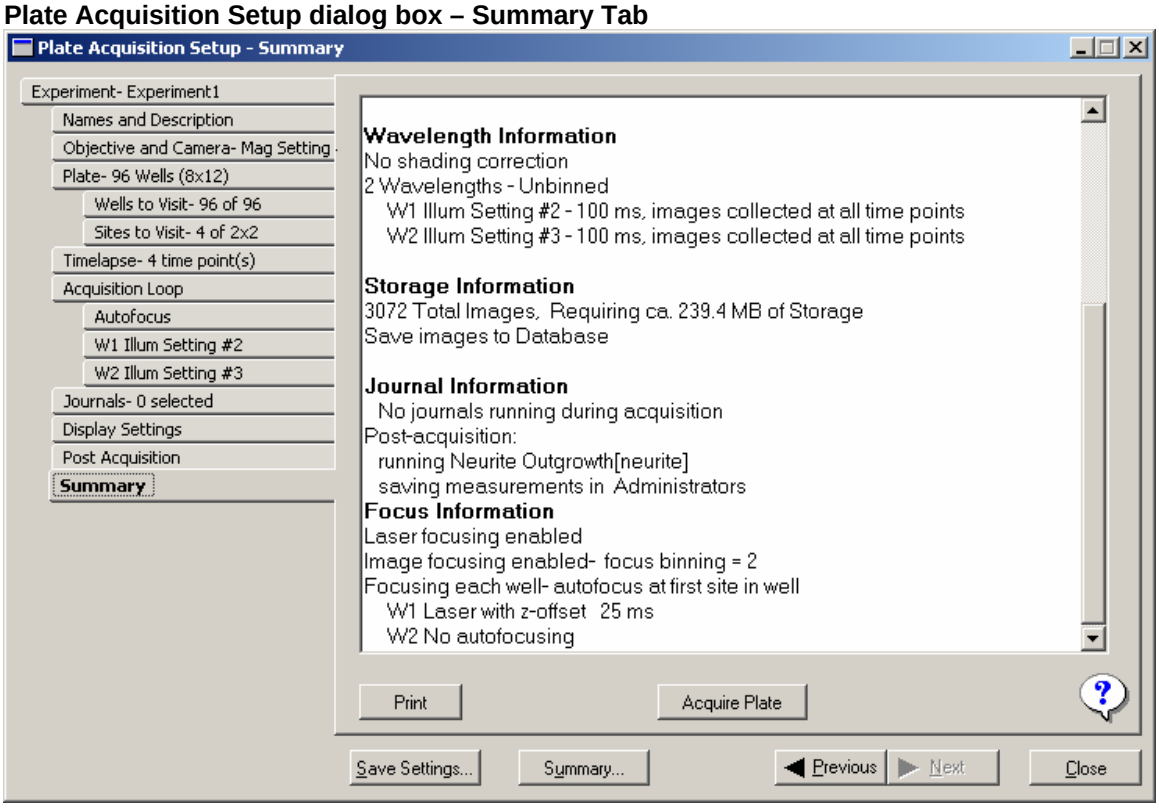
4. Type the well number for a specific well that you want to view in the *Go to well* box, and click **Go to well**. The plate moves to the desired location.
5. To change the Z-focus motor position, use the Z-control arrows.

6. To change the increment the arrows use in the *Z Current position* field, enter a value in the *Step size* field. Set a large value for course movement or a small value for fine movement.
7. Click *Go to origin* to move the Z focus motor to its origin position as defined in Focus dialog box.
8. Click *Find Sample* to initiate the Find Sample focusing routine on the current well.
9. Click *Autofocus* to perform auto focus on the current well using the wavelength selected in the *Wavelength* field.
10. Click *Load Settings* to open the Load Plate Acquisition Settings dialog box and load a previously saved Settings file.
11. Click *Save Settings* to open the Save Plate Acquisition Settings dialog box and save the current settings to a file on the local hard drive or to the database.
12. Click *Summary* to open the Plate Acquisition Summary dialog box and view your current settings.
13. Click *Setup* to open the Plate Acquisition Setup dialog box and change your acquisition settings.
14. Click the *Wavelength* drop-down box to select a wavelength that has been defined for the current setting in the Plate Acquisition Setup dialog box.
15. Click *Snap Current* to acquire a single image with the current settings.
16. Click *Show Live* to acquire images so you can manually focus the microscope.
17. Click *Preview* to open the Plate Acquisition Status dialog box and an image view dialog box for each wavelength. During this time, you can adjust the display of images and windows so that they will be appropriately sized and positioned for acquisition.
18. Click *Acquire* to acquire images from a plate based on the settings made in Plate Acquisition Setup dialog box.
19. Click *Close* to exit the dialog box.

Plate Acquisition Setup dialog box - Summary Tab

The Plate Acquisition Setup dialog box Summary Tab includes an Acquire Plate button from which you can initiate plate acquisition. This button is located on the last tab of the dialog box. Normally, you should arrive on the Summary tab after making all required settings for acquisition, thus it is a logical place from which to initiate plate acquisition.

Figure 6-2
Plate Acquisition Setup dialog box – Summary Tab



Use the Summary tab to view a list of all current settings for the acquisition, save the settings to a file, and start acquiring images. Use the following procedure to save your settings file and start acquisition:

Notes:

- The information in the summary tab is identical to the information displayed when you click the Summary button on the bottom of the dialog box.

- If you want to make any changes to the stage or Z Position, or snap an image to test the current settings before starting the acquisition, use the Plate Acquisition and Control dialog box or the Plate Acquisition Toolbar to perform these and other tasks.

- If an error dialog box opens after you click Acquire, the most likely cause is a configuration error. Read the text in the dialog box to determine the error.

- After your acquisition is complete and the images have been saved to the database, you can use the Review Plate Data (DB) dialog box to view the images and setup analysis.

Using the Plate Acquisition Setup Dialog Box – Summary Tab

To use the Summary tab, complete the following procedure:

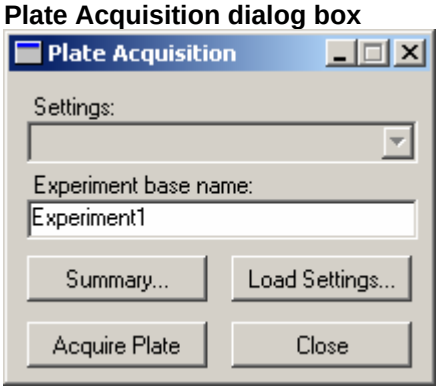
1. Click *Save Settings* to open the Save Plate Acquisition Settings dialog box and save the current settings to a file on the local hard drive or to the database.
2. If you want to print the settings summary, click *Print* to open the Print Setup dialog box and then print the settings.
3. Click *Acquire* to acquire images from a plate based on the settings made in the Plate Acquisition Setup dialog box. The expected behavior after clicking *Acquire* is outlined below:
   - The Plate Acquisition Setup dialog box closes and the Screen Status dialog box opens
   - Each image appears briefly on the MetaXpress desktop as it is acquired and saved to the database.
   - After the last image is acquired and saved, the Screen Status dialog box closes and the Plate Acquisition Setup dialog box reopens.
4. Click *Close* to exit the Plate Acquisition Setup dialog box.

Plate Acquisition dialog box

The plate acquisition dialog box has the least number of controls and options compared to the other three locations from which you can initiate plate acquisition. Before acquiring a plate from this dialog box you can do only the following procedures:

- Choose or type an Experiment base name
- Select and load the Settings file that you want to use for the acquisition.
- View the summary of acquisition settings.
- Acquire a plate.

Figure 6-3
Plate Acquisition dialog box



Use this dialog box to quickly start acquiring plates using any of the settings defined in the Plate Acquisition Setup command. You can also view a summary of the current settings file and change the base name of the experiment from this dialog box.

Note: To perform additional configuration of the experiment before starting the acquisition, use the Plate Acquisition and Control dialog box.

There are several possible workflows available for your acquisition. One typical workflow for multi-well plate acquisition is as follows:

- Configure and save your settings file using the Plate Acquisition Setup dialog box.
- Use the Plate Acquisition and Control dialog box or toolbar to do the following:
  - Load your settings file and review the settings using the Summary button.
  - Confirm your settings if needed using the available tools.
  - Enter an experiment base name.
  - Start the acquisition. During acquisition, the acquired images are saved into the database.
- Perform any post-acquisition analysis using the Review Plate Data or Plate Data Utilities dialog boxes. This can be configured to start automatically from the Plate Acquisition Setup dialog box if desired.

Using Plate Acquisition

Use the following procedure to familiarize yourself with the controls on the Plate dialog box:

1. From the Screening menu, select Plate Acquisition, the Plate Acquisition dialog box opens.
2. Ensure that a plate is in place on the microscope stage.
3. Select a Settings file to use from the *Settings* drop-down list.
4. To change the experiment name, enter a name in the *Experiment Base Name* field.
5. Click *Summary* to view details about the current settings.
6. Click *Load* to open the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear all* to clear all selections. Click *Load* to load your selected conditions.
7. Click *Acquire* to acquire images from a plate based on the current settings.
8. Click *Close* to exit the dialog box.

Plate Acquisition - Dialog Box Options

Settings

Contains a list of all settings currently available. These settings are configured in the Plate Acquisition Setup dialog box.

Experiment Base Name

Defines the base file name.

Summary

Lists the current settings selected for your acquisition; the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

Load

Loads the selected settings from an existing screening settings file. When you click Load Settings, the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear all* to clear all selections. Click *Load* to load your selected conditions.

Acquire

Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Settings dialog box.

Close

Closes the dialog box.

Observing Acquisition Progress

The following dialog boxes enable you to observe and determine the status of the progress of your plate acquisition:

- Screen Summary dialog box
- Plate Acquisition Setup dialog box – Summary tab
- Plate Acquisition Status dialog box

Screen Summary dialog box

The Screen Summary dialog box shows a complete summary list of all of the settings in the Plate Acquisition Setup dialog box. This same information is also repeated on the Summary Tab of the Plate Acquisition Setup dialog box.

To view the Screen Summary dialog box, click the Summary button on the Plate Acquisition and Control dialog box.

Figure 6-4
Screen Summary dialog box

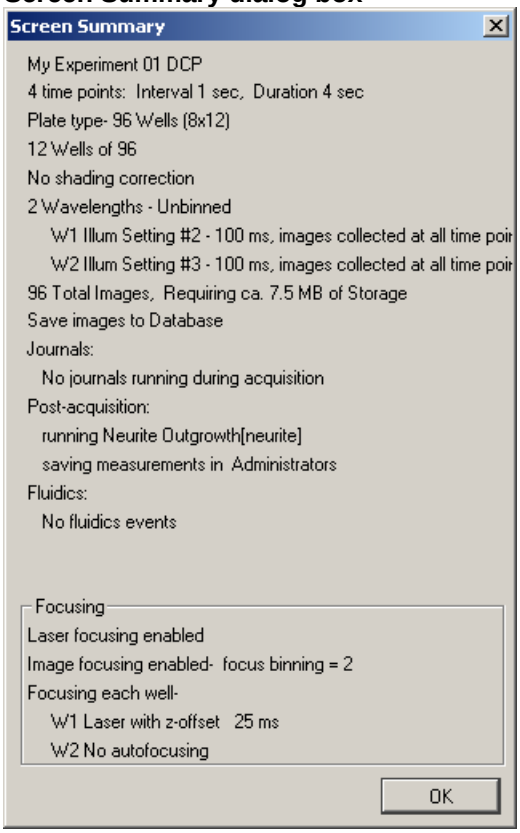


Plate Acquisition Setup dialog box – Summary Tab

The Plate Acquisition Setup dialog box – Summary Tab shows similar information to the Screen Summary dialog box about the settings for your experiment.

Note: You should always review the summary window before proceeding with an experiment. The amount of hard disk storage space required is indicated in the summary information. You should verify that the location where your images are being saved has sufficient free space before proceeding.

Figure 6-5
Plate Acquisition Setup dialog box – Summary Tab

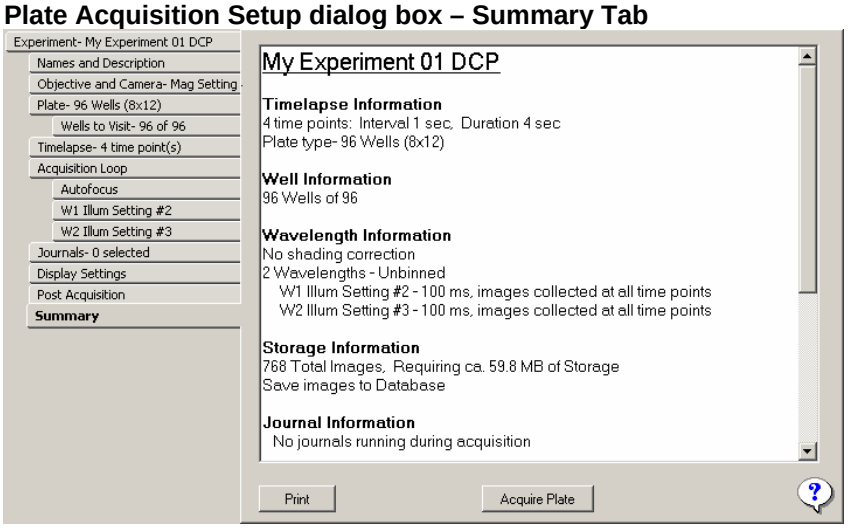


Plate Acquisition Status

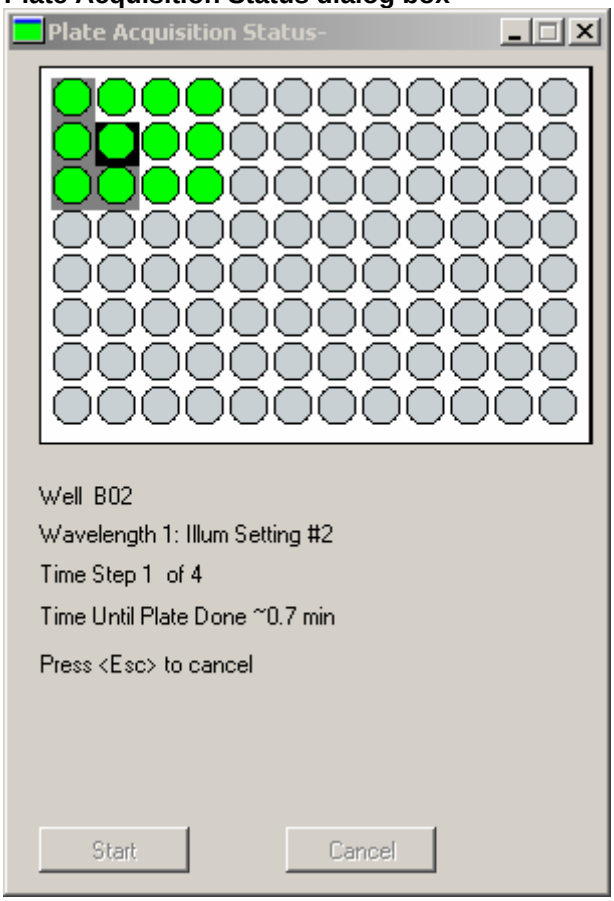
This dialog box shows the progress of your plate acquisition by indicating the wells scheduled for acquisition, the wells that have been completed, and the well currently being acquired.

To view the Plate Acquisition Status dialog box, click the Preview button in the Plate Acquisition and Control dialog box.

Legend:

White – Wells not scheduled for acquisition
Green – Wells scheduled for acquisition
Green with Gray outline box – Wells that have completed acquisition
Green with Black outline box – Well currently being acquired

Figure 6-6
Plate Acquisition Status dialog box



Using the Plate Acquisition Toolbar

The Plate Acquisition toolbar contains tools used to control the hardware on the ImageXpress^{MICRO} screening system.

Figure 6-7
The Plate Acquisition toolbar

If the Plate Acquisition toolbar is not currently loaded, you can enable it by going to the Window menu and selecting Toolbars>Plate Acquisition. The following tools are available on the Plate Acquisition toolbar:

Tool	Description
	Moves the stage up in one-well increments.
	Moves the stage down in one-well increments.
	Moves the stage forward in one-well increments.
	Moves the stage backward in one-well increments.
	Moves the stage forward in one-site increments.
	Moves the stage backward in one-site increments.
	Moves the stage to the load/eject position. This option is only available with the ImageXpress.
	Current well and/or site position.
	Moves the Z position (focus) upward in single step increments.
	Moves the Z position (focus) downward in single step increments.
	Performs a very coarse auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire.
	Performs auto focus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup tool.
	Selects the wavelength to use for your snap or live image.
	Acquires a single image of the currently in place well at the current settings for stage (XY-position), focus (z-position), wavelength, well, site, and exposure.

Show Live continuously acquires images based on the current settings, and updates the image as settings are changed.

Loads the selected settings from an existing screening settings file. Settings files are stored either in the database or on the file system. When you click Load Settings, the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load.

Screen Summary lists the current settings selected for your acquisition, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

Previews the current display and exposure settings by opening the Plate Acquisition Status dialog box and autofocusing and acquiring an image for each wavelength. Once all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog box and changing the size, scaling, and LUT of images. These new settings will be saved and used during acquisition.

Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Settings dialog box.

Chapter 7
Advanced Procedures

The procedures explained in this chapter are needed if you have made a hardware change to your ImageXpress^{MICRO} system. For example, if you have added or replaced an objective, you must configure the software before using the objective. The following subjects are discussed in this chapter:

- Updating the system after adding or replacing an objective:
  - Editing the objective settings in the Meta Imaging Series System Administrator
  - Configuring parfocality after changing objectives
  - Determining the Plate Bottom Reference Point After Changing the Reference Objective (this is only needed if you changed the objective used to determine the plate bottom reference point)
  - Updating calibration, magnification, and shading correction settings
- Updating the system after adding or replacing a filter:
  - Editing the hardware setting in the Meta Imaging Series System Administrator
  - Updating illumination and shading correction settings
- Defining a plate type
  - Defining and confirming Laser Auto Focus settings for plate types
  - Troubleshooting Laser Auto Focus Settings for Plate Types

Updating the System After Adding or Replacing an Objective

After installing a new objective, you must update the objective settings in both the Meta Imaging Series Administrator and the main MetaXpress program. If the objective you are replacing was the one used to determine the plate bottom reference point, you will also need to redo the procedure *Determining the Plate Bottom Reference Point after Changing the Reference Objective*, or contact MDC for Customer support.

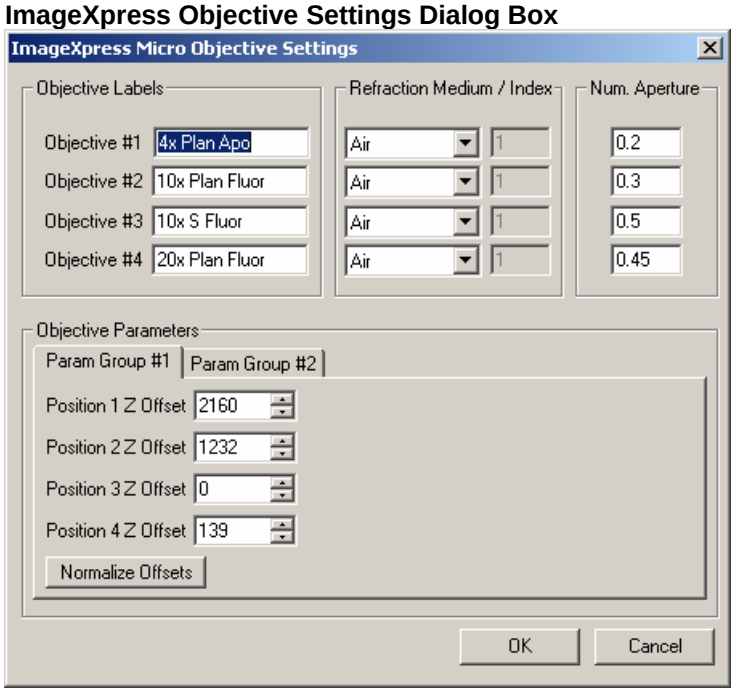
Editing the Objective Settings in the Meta Imaging Series System Administrator

Complete the following procedure to update your objective settings and enter Maintenance Mode:

1. From the Windows Start menu, go to Programs>Meta Imaging Series 6.xMetaXpress> Meta Imaging Series Administrator. The Meta Imaging Series Administrator program opens.
2. Select *MetaXpress* from the *List of Groups* field.
3. Click *Configure Hardware*. The *Configure Hardware* dialog box opens.
4. Click *Install System Devices*. The *Install Systems Devices* dialog box opens.

5. Select *ImageXpress Micro Objective* from the *Installed Devices* list and click *Settings*. The ImageXpress Micro Objective Settings dialog box opens, as shown in Figure 7-1:

Figure 7-1
ImageXpress Objective Settings Dialog Box



6. Edit the Objective Labels text as needed in the *Objective #* field for the new objective.
7. Change the *Refractive Medium/Index* value if needed.
8. Enter the numerical aperture for the new objective in the corresponding *Num. Aperture* field (the value is written on the objective).

   Note: Make a note of the values you entered in Steps 6-8. You will need to enter this information again for your specific hardware setting.

9. Click *OK* to close the ImageXpress Micro Objective Settings dialog box.
10. Select ImageXpress Micro from the Installed Devices list and click *Settings*. The ImageXpress Micro Settings dialog box opens.
11. Ensure that the *Parameter Group #1* tab is active and check the *Maintenance Mode* checkbox.
12. Click OK, then click OK again as needed to return to the Configure Hardware dialog box.

Note: The next steps involve entering some of the values again, this time starting from the Configure Devices dialog box. This is to insure that the setting will carry over for all hardware profiles.

13. From the Configure Hardware dialog box, ensure that the hardware setting you are using is selected in the *Hardware Settings* list and then click *Configure Devices*. The User Settings hardware configuration dialog box opens.
14. Double-click *ImageXpress Micro Objective* in the *Claimed Devices* list. The ImageXpress Micro Objective dialog box opens.
15. Enter the same information about the new objective that you entered in Steps 6-8.
16. Click *OK*, then click *OK* as needed to exit the Meta Imaging Series Administrator and continue to the next procedure.

Configuring Parfocality after Changing Objectives

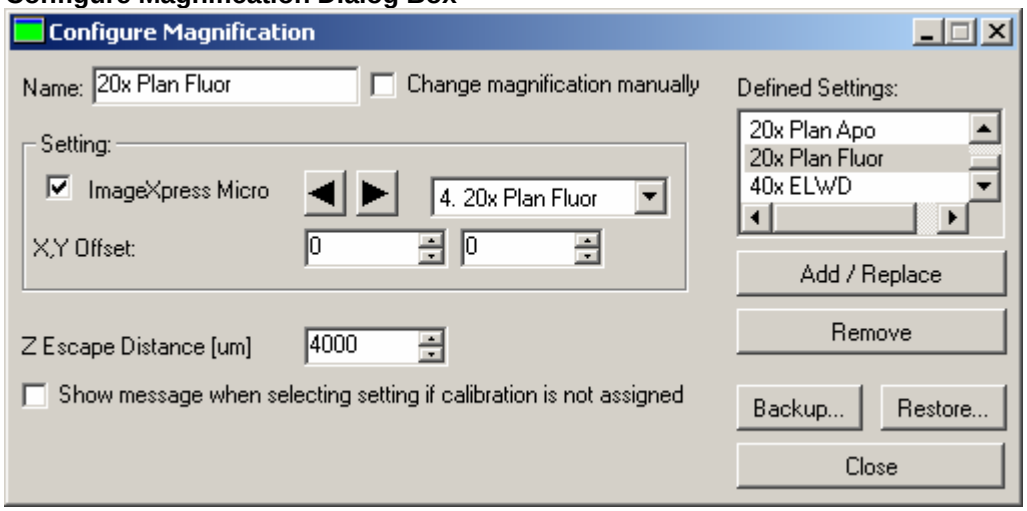
In order to configure parfocality, you must use MetaXpress to find valid focus values for each objective and then enter them into the MetaXpress Objective dialog box in the Meta Imaging Series System Administrator. Complete the following procedure to configure parfocality:

Note: You must be in Maintenance Mode before performing this procedure.

1. Open MetaXpress.
2. Open the following dialog boxes:

    - Configure Magnification (Devices menu)
    - Acquire (Acquire menu)
    - Move Stage to Absolute Position (Devices menu>Stage> Move Stage to Absolute Position)
3. From the Window Menu, go to Toolbars and ensure that both the Device Control and Plate Acquisition toolbars are selected. Open the Device control toolbar if it is not displayed: Window>Toolbars>Device Control.
4. From the Devices Menu, select Configure Magnification.
5. Ensure that the checkbox next to *ImageXpress Micro Objective* is selected, as shown in Figure 7-2:

Figure 7-2
Configure Magnification Dialog Box



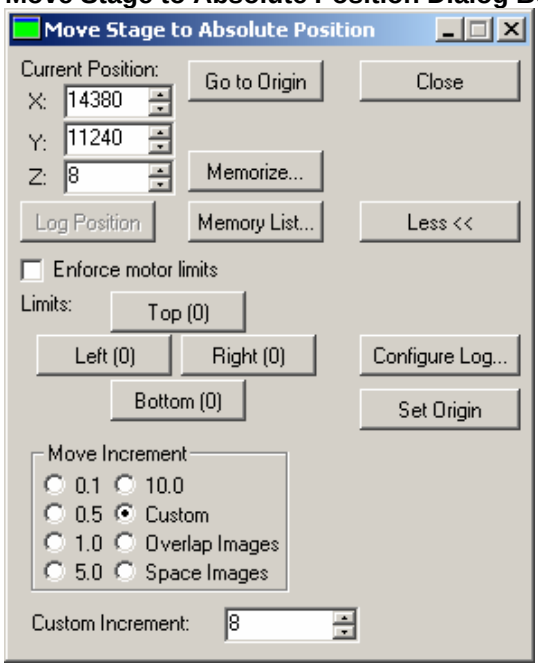
6. Select the objective with the highest numerical aperture (NA) that is in position #1 from the drop-down list.

7. Select the FITC (or other visible light) illumination setting from the *Illum* drop-down field on the Device Control toolbar, as shown in Figure 7-3:

Figure 7-3
Partial Device Control Toolbar

8. Click the Stage Load/Eject button on the Plate Acquisition toolbar to move the stage to the load position.
9. Load the bead plate that shipped with the ImageXpress^{MICRO} on the stage, then click the Stage Load/Eject button again to return the stage to its previous position.
10. In the Acquire dialog box, click *Show Live* to open a live image window.
11. In the Move Stage to Absolute Position dialog box, use the *Current Position* X, Y, and Z controls to find and focus a sample in the live image window. Figure 7-4 shows the Move Stage to Absolute Position dialog box:

Figure 7-4
Move Stage to Absolute Position Dialog Box



12. When the sample is in focus, write down the *Current Position Z:* value in the Focus Value column in Table 7-1 below:

Table 7-1
Objective Focus Values

Objective Number	Focus Value
1	
2	
3	
4	

Note: You will need to refer to these values later in this document.

13. Use the drop-down list in the Configure Magnification dialog box to switch to the objective in position #2 with the next highest NA. It is important that you use the same filter set for all objectives.
14. Repeat steps 9-12 for each objective, writing down the focus values for each in Table 7-1. You will use these values to determine the Z-offsets for each objective.

    Note: Since you found the X and Y coordinates of the sample in Step 11, you will only need to change the Z position for each additional objective.

15. If the objective you are changing was the one used to determine the plate bottom reference point, refer to the next procedure, *Determining the Plate Bottom Reference Point after Changing the Reference Objective.*

    OR

If the objective was NOT the one used to determine the plate bottom reference point, skip to the procedure, *Entering Objective Values in the Meta Imaging Series Administrator*.

After you have written down the focus values, Close MetaXpress and continue to the next procedure.

Determining the Plate Bottom Reference Point after Changing the Reference Objective

The plate bottom reference point is a setting that MetaXpress uses for autofocusing. It is set when your system is configured before shipment. The reference point is determined using a particular objective (usually 10x) in a specific objective position. If you change this objective, you must determine the new plate bottom reference point and enter this value in the Meta Imaging Series Administrator.

Notes:

- MDC recommends that you leave the reference objective in place and only replace other objectives.
- It is recommended that you contact MDC Customer Support before attempting this procedure.
- You do **NOT** need to perform this procedure unless you replaced the reference objective. If you did not replace the reference objective, skip to the procedure *Entering Objective Values in the Meta Imaging Series Administrator*.
- You must be in Maintenance Mode to perform this procedure. For instructions on entering Maintenance Mode, refer to the *Editing the Objective Settings in the Meta Imaging Series System Administrator* section of this chapter.

The objective position used for this setup is specified in a line of the system calibration file **`MetaXpress.ref`**, located in the Hardware folder of your root install directory (**`C:\MX\Hardware`** by default). The line is:

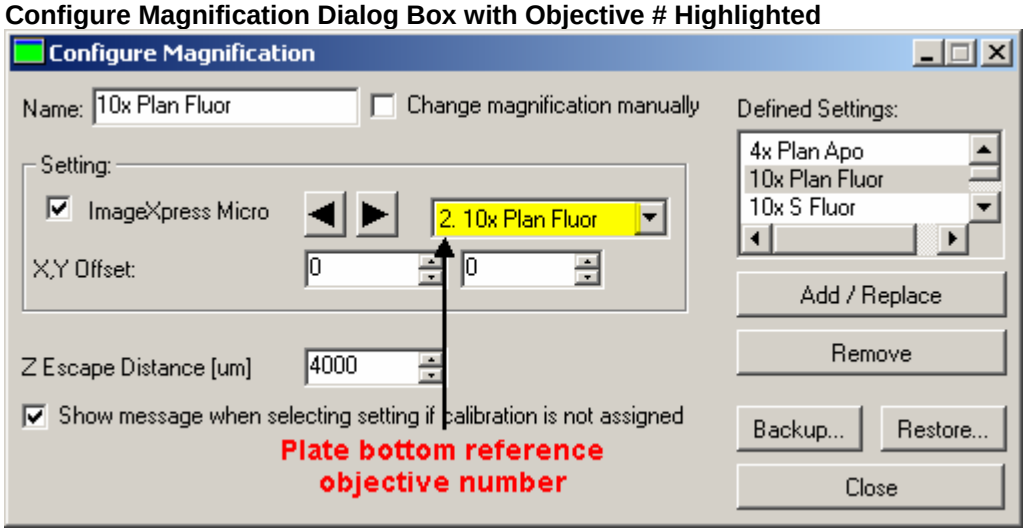
```
PlateBottomReferenceObjective=X
```

where X is the position of the objective used. If you have to update the plate bottom reference point, the value in the calibration file must also be updated. Complete the following procedure to determine both the plate bottom reference objective and the plate bottom reference point.

1. Ensure that you are in Maintenance mode. For instructions on entering Maintenance Mode, refer to the *Editing the Objective Settings in the Meta Imaging Series System Administrator* section of this chapter.

2. Remove any plates from the stage, and load one of the flat-field correction (FFC) plates that came with your system.
3. From the Device menu, select Configure Magnification. The Configure Magnification dialog box open, as shown below:

Figure 7-5
Configure Magnification Dialog Box with Objective # Highlighted



4. Select the objective you want to use as the reference from the drop-down list, and write down the plate bottom reference objective number (see example in Figure 7-5) in Table 7-2 below:

Table 7-2

Plate Bottom Reference Objective

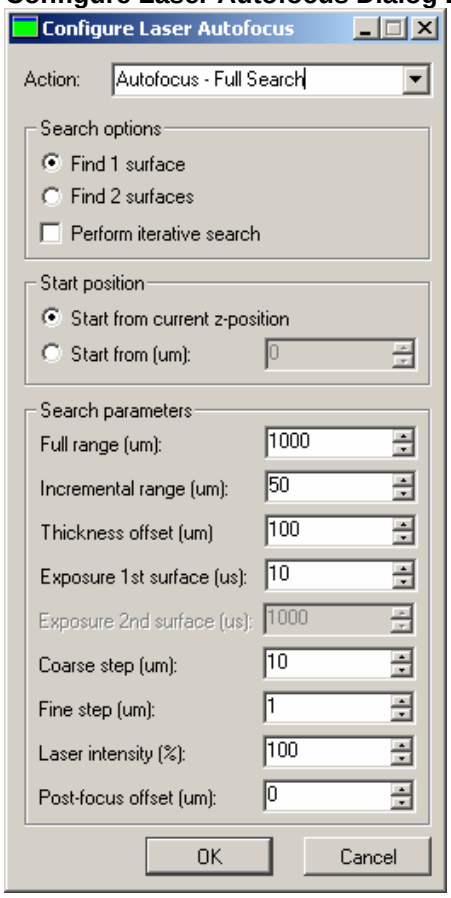
You will refer to this value in the next procedure, *Entering the Plate Bottom Reference Objective Value in the `MetaXpress.ref` Configuration File*.

5. Close the Configure Magnification dialog box. If a dialog box opens prompting you to replace the stored setting, click *No*.
6. If they are not open, open the following dialog boxes:

   - Move Stage to Absolute Position (Devices menu>Stage)
   - Focus (Devices menu)

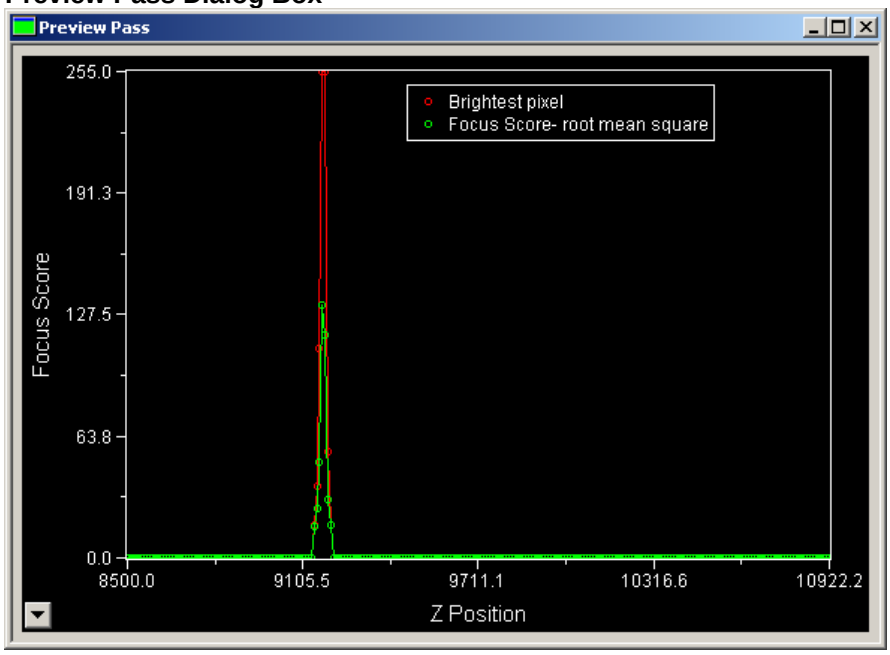
7. In the Move Stage to Absolute Position dialog box, type *8000* in the *Current Position: Z* field and press [**`Enter`**] to move the Z-motor.
8. Type *64000* in the *Current Position: X* field and *33000* in the *Current Position: Y* field and press [**`Enter`**] to move to the approximate center of the plate.
9. In the Focus dialog box, select the *Auto focus* tab, then click *Configure Laser*. The Configure Laser Autofocus dialog box opens, as shown in Figure 7-6:

Figure 7-6
Configure Laser Autofocus Dialog Box



10. In the Start position field, select *Start from current z-position.*
11. Set the *Full range* value to 1000 microns (um).
12. Set the *Exposure 1st surface* value to 10 microseconds (us).
13. Set the *Coarse step* value to 10 microns (um).
14. Go back to the *Auto Focus* tab of the Focus dialog box and click *Preview Pass*. A window opens displaying a graph of focus intensities vs. Z-position. Ideally, the graph should contain a sharp peak, made up of a red line and a green line, as shown in Figure 7-7:

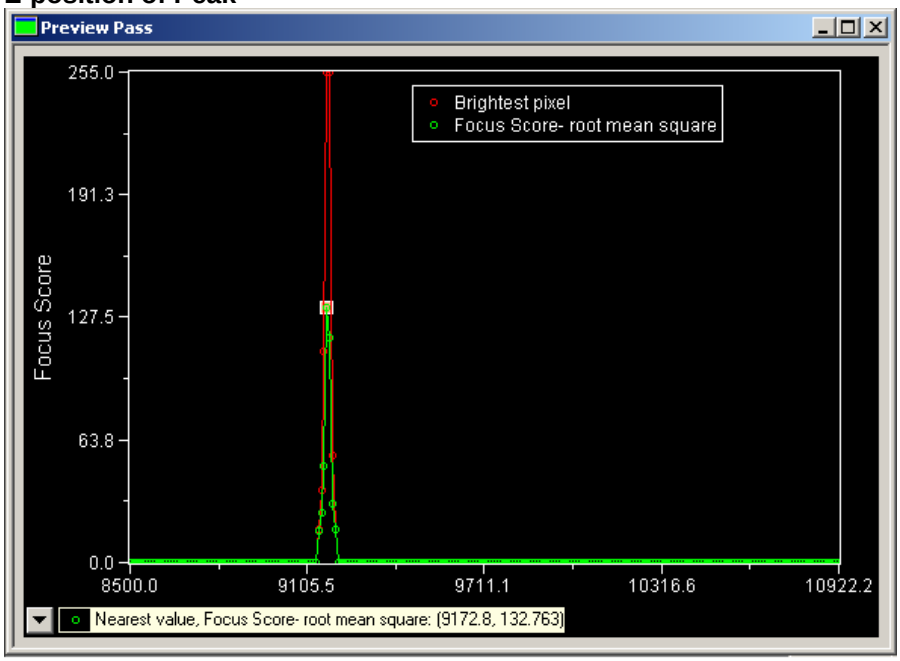
Figure 7-7
Preview Pass Dialog Box



Note: The top of the red peak represents the brightest pixel of the preview pass. The top of the green peak represents the highest focus score.

15. If there is a peak in the graph, skip to the next step. If there is no peak, try the following:
    a. In the Focus dialog box, increase the Current Position value by 1000 and click *Preview Pass* again. Repeat as needed until you cover a range between 8000 and 12000. If there is a peak in the graph, skip to the next step. If there is no peak, continue to Step b.
    b. Try moving to a new area of the FCC plate and repeat step 14 – 15a. If you still cannot find a peak after moving to a new area of the FCC plate twice and covering a z-range from 8000 to 12000, contact MDC Customer Support.
16. When you see a peak (or 2 peaks) on the preview pass, click the trace line at the top of the green line of the first peak. The X, Y position is displayed in a tool tip, as shown in Figure 7-8:

Figure 7-8
Z-position of Peak



Note the X value in the tool-tip. It is the Z-position of the plate bottom. In this example, the current Z-position value is 9172.8.

17. Write down the Z-position value for the center position in Table 7-3 below:

Table 7-3
Plate Bottom Reference Points — Circle the lowest Z value

	Position	X Value	Y Value	Z Value
1	Center	64000	33000	
2	Upper Left	15000	11000	
3	Lower Left	15000	74000	
4	Upper Right	114000	11000	
5	Lower Right	114000	74000	

18. Repeat Steps 7-17 for each corner of the FCC plate, writing down the Z-position values (rounded off) for each corner in the Z Value column of Table 7-3.

    To move to each corner of the plate using the Move Stage to Absolute Position dialog box, use the X and Y values in Table 7-3.

19. Circle the lowest Z value in Table 7-3. This value is the Plate Bottom Reference Point. You will refer to this value in the *Entering Objective Values in the Meta Imaging Series Administrator* section of this document.
20. Exit MetaXpress and continue to the next procedure, *Entering the Plate Bottom Reference Objective Value in the* `MetaXpress.ref` *Configuration File*.

Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File

Complete the following procedure to enter the value in the **MetaXpress.ref** configuration file:

Note: Only perform this procedure if you needed to complete the *Determining the Plate Bottom Reference Point after Changing the Reference Objective* procedure.

1. Open Notepad (Start>Programs>Accessories>Notepad) and use it to open the **MetaXpress.ref** file in the MetaXpress hardware folder — **C :\MX\Hardware** by default.

   NOTE: Ensure that the copy of the **MetaXpress.ref** that you open is located in the hardware folder of the MetaXpress folder (For example, **C:\MX\Hardware**) and NOT the root directory.

2. Edit the following line in the file, replacing the number with the objective position you entered in Table 7-2 of the *Determining the Plate Bottom Reference Point After Changing the Reference Objective* procedure:

   ```
   [system calibration]
   PlateBottomReferenceObjective=4
   ```

3. Save the modified file and close Notepad.

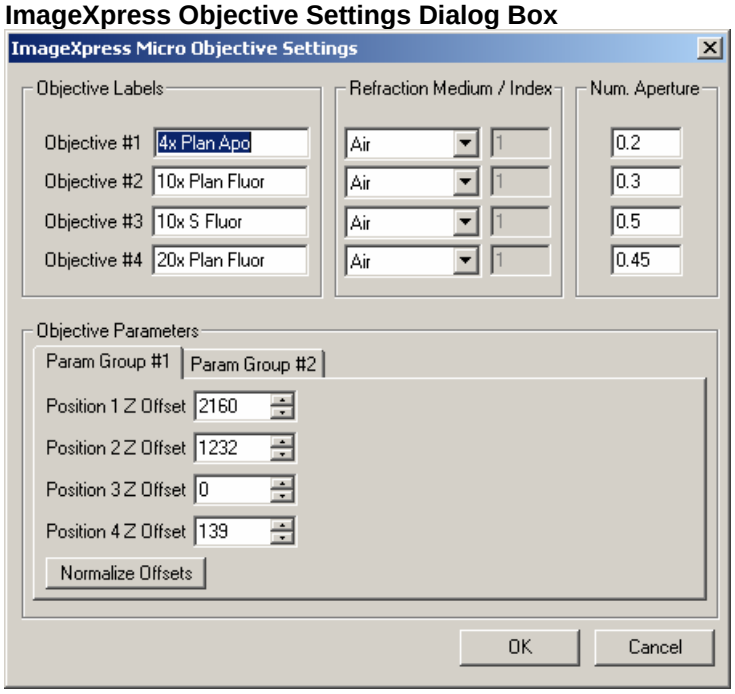
   Note: Do NOT use the *Save as* option in Notepad. Doing so causes a .txt extension to be added to the saved .ref file.

Entering the Focus Objective Values in the Meta Imaging Series Administrator

The next step is to open the Meta Imaging Series Administrator and enter the values you recorded in the previous procedure(s). You also need to turn off Maintenance mode before you can continue configuring the device. Complete the following procedure to turn off Maintenance mode and enter the values in the Meta Imaging Series Administrator and turn off Maintenance mode:

1. Start the Meta Imaging Series System Administrator.
2. Click *Configure Hardware*, then click *Install System Devices*. The Install System Devices dialog box opens.
3. Double-click *ImageXpress Micro* in the Installed Devices list. The ImageXpress Micro Settings dialog box opens.
4. Uncheck *Maintenance Mode*.
5. Click *OK* to exit the ImageXpress Micro dialog box
6. Double-click *ImageXpress Micro Objective* in the *Installed Devices* list. The ImageXpress Micro Objective dialog box opens as shown in Figure 7-9:

Figure 7-9
ImageXpress Objective Settings Dialog Box



7. Refer to Table 7-1 and enter the focus value that you wrote down for Objective #1 in the *Position #1 Z Offset* field.
8. Repeat Step 7 for *Position #2-4 Z Offset* fields.

   Note: The *Position 1-4 Z Offsets* refer to Objective numbers 1-4 and are not in order of magnification. Refer to the *Objective Labels* fields to match the objective number with its magnification.

   Note: In some cases, there may not be an objective in each position of the turret. If this is the case, enter the focus value for the highest magnification objective that you do have in each of the empty *Position 1-4 Z Offset* fields. Do not leave any of these values as 0.

9. Click *Normalize Offsets* to calculate the offsets for each Z position.
10. Click *OK* to exit the ImageXpress Micro Objective dialog box.
11. If you needed to complete the *Determining the Plate Bottom Reference Point after Changing the Reference Objective* procedure, continue to Step 12.

    OR

    If you did not change or replace the reference objective, skip to Step 15.

12. Double-click *ImageXpress Micro Z* in the *Installed Devices* list. The ImageXpress Micro Z dialog box opens.

13. Enter the value you circled in Step 19 of the *Determining the Plate Bottom Reference Point after Changing the Reference Objective* procedure in the *Plate Bottom Reference* field.
14. Click *OK* to exit the ImageXpress Micro Z Settings dialog box, then click *OK* to return to the Configure Hardware dialog box.

    Note: The next steps involve entering some of the values again, this time starting from the Configure Devices dialog box. This is to insure that the setting will carry over for all your hardware profiles.

15. From the Configure Hardware dialog box, ensure that the hardware setting you are using is selected in the *Hardware Settings* list and then click *Configure Devices*. The User Settings hardware configuration dialog box opens.
16. Double-click *ImageXpress Micro Objective* in the *Claimed Devices* list. The ImageXpress Micro Objective dialog box opens.
17. Edit the *Objective Labels*, *Refraction Medium/Index*, and *Num. Aperture* fields with the same values you entered in steps 6-8 of the *Editing the Objective Settings in the Meta Imaging Series System Administrator* section earlier in this chapter.
18. Repeat Steps 7-9 to enter and normalize the Z offsets again.
19. If you needed to complete the *Determining the Plate Bottom Reference Point After Changing the Reference Objective* procedure, Double-click *ImageXpress Micro Z* in the Claimed Devices list and again enter the Plate Bottom Reference number as in Step 13 above.
20. Click *OK* to exit each dialog box and close the Meta Imaging Series System Administrator.

    Note: If you use more than one hardware profile, repeat Steps 15-18 as needed for each hardware profile.

Updating Magnification and Calibration Settings

Complete the following procedure to update magnification and calibration settings within MetaXpress:

1. Open MetaXpress and log into the database.
2. From the Devices menu, select Configure Magnification. The Configure Magnification dialog box opens.
3. Ensure that *ImageXpress Micro* is selected in the *Settings* field and select the objective that you installed from the corresponding drop-down box.
4. Enter the name of the new objective in the *Name* field.
5. Click *Add/Replace* to add this setting to the *Defined Settings* list.
6. If you replaced an objective with an existing setting, select the old setting from the Defined Settings field and click *Remove*.
7. Once the settings are updated, click Backup and back up the new settings.
8. Click *Close* to exit the Configure Magnification dialog box.
9. From the Measure menu, select Calibrate Distances. The Calibrate Distances dialog box opens.

10. Click the *Setup* tab to highlight it.
11. Click *New*, type the name of your new objective in the *Calibrations* field, and press [Enter]. The bottom half of the dialog box becomes active.
12. Ensure that *Edit Units/Pix* is selected in the *Define Calibrations By* field and enter the calibration value to use for the new objective in the *X* and *Y* fields.
13. The following estimated values can be used for ImageXpress^{MICRO} calibration settings:

Objective	Estimated Calibration
2x	3.225 um/pixel
4x	1.6125 um/pixel
10x	0.645 um/pixel
20x	0.3225 um/pixel
40x	0.16125 um/pixel
60x	0.1075 um/pixel
100x	0.0645 um/pixel

For additional information on creating calibrations settings, refer to the MetaXpress online help (press the F1 key when the Calibrate Distances dialog box is open to access its online help). Also refer to the technical note *Spatially calibrating images in MetaMorph*, available online.

14. Select the new objective from the *Magnification* drop-down list.
15. Click *Done*. The calibration is saved and the bottom half of the dialog box becomes inactive.
16. Once the settings are updated, click *Save to file* and back up the settings.
17. Click *Close* to exit the Calibrate Distances dialog box.

Updating Shading Correction Settings

This section explains how to create shading correction files to use during plate acquisition in MetaXpress. Shading correction files are needed for each objective/filter combination and must be generated whenever an objective or filter is replaced or added to the system, or whenever the lamp or liquid light guide is replaced. This procedure uses a flat-field correction (FFC) plate to focus the objective and a MetaXpress journal to create the shading correction file.

The following procedures are explained in this section:

- Importing the Journal Suite
- Focusing on the FCC plate
- Running the Shading Correction Journal

Notes:

- The journal used in this procedure is named **`IXM_Shading_Correction.JNL`** and is located in the default location for journal files in MetaXpress – **`C:\MX\app\mmproc\journals`**. If the journal is not installed on your system, MDC Customer Support will provide you with a link to this file. You will then need to complete the procedure *Importing the Journal Suite* later in this chapter.

- This procedure assumes that if you have added or replaced an objective, you have performed the following procedures before generating the updated shading correction files:

    - Editing the objective settings in the Meta Imaging Series System Administrator
    - Configuring parfocality after changing objectives
    - Updating calibration and magnification settings in MetaXpress

- In order for shading correction images to be used during Plate Acquisition, the *Perform shading correction* checkbox must be selected in the *Acquisition Loop* tab of the Plate Acquisition Setup dialog box.

- You will need FFC plates appropriate for the filter sets you are using to generate the shading correction files. These plates are provided in the accessory kit that shipped with the instrument. Make sure that the paper backing has been removed from each plate before using it for this procedure. The plates should be handled by the edges to avoid getting fingerprints on the imaging surface. Never use alcohol or other solvents to clean the plates; you may use compressed air to remove dust from the plates. The table below lists the correction plate used for each filter set:

Flat-field correction plates to use for each filter set

FFC Plate	Filter Set
DAPI	Pink
Fura-2	Pink
CFP	Pink
YFP	Red
FITC	Red
Cy3	Green
Rhodamine	Red
Texas Red	Red
Cy5	Red

Importing the Journal Suite into MetaXpress

Note: You only need to complete this procedure if the journal is not installed on your system. The journal used in this procedure is named **`IXM_Shading_Correction.JNL`** and is located in the default location for journal files in MetaXpress – **`C:\MX\app\mmproc\journals`**. If the journal is not installed on your system, complete the following procedure:

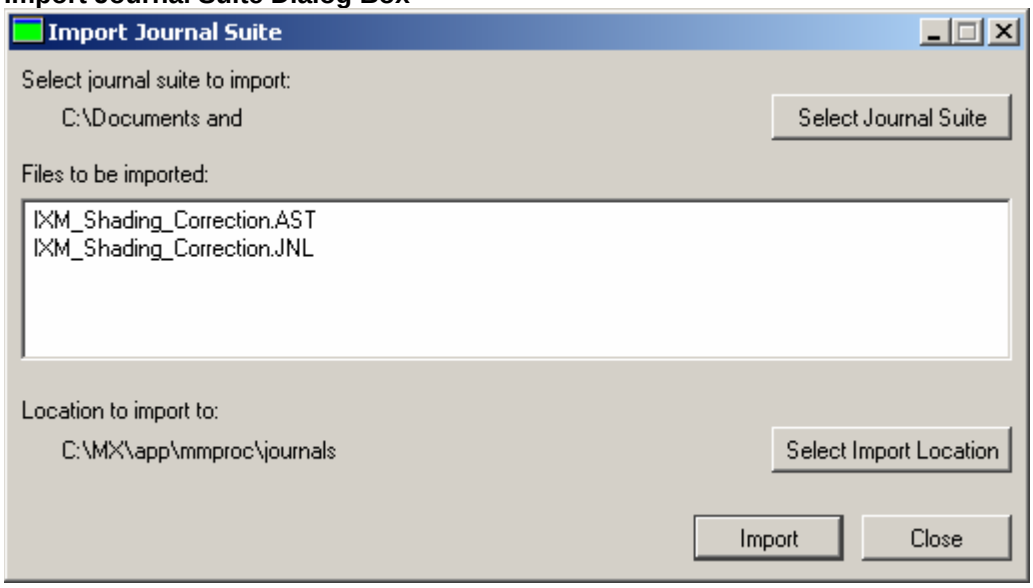
1. Download the journal suite file **`IXM_Shading_Correction.jzp`** from the MDC web site. Please contact MDC Customer Support for a link to the file.
2. Open and login to MetaXpress.

3. From the Journal menu, select Import Journal Suite. The Import Journal Suite dialog box opens.
4. Click *Select Journal Suite*. The Select Import Suite File Name dialog box opens.
5. Navigate to the **`IXM_Shading_Correction.jzp`** file and click *Open*. The path to the journal suite is displayed at the top of the Import Journal Suite dialog box.
6. Click *Select Import Location*, navigate to the location where the journals will be saved and click *OK*. The path of the import location is displayed at the bottom of the Import Journal Suite dialog box.

   Note: The default location for journal files in MetaXpress is **`C:\MX\app\mmproc\journals`**.

   Figure 7-10 illustrates the Import Journal Suite dialog box:

Figure 7-10
Import Journal Suite Dialog Box



7. Click *Import*. The journals are imported and can be opened using the Run Journal command.

   NOTE: There is no visual confirmation that the import is complete.

8. Click *Close* to close the Import Journal Suite dialog box and continue to the next procedure.

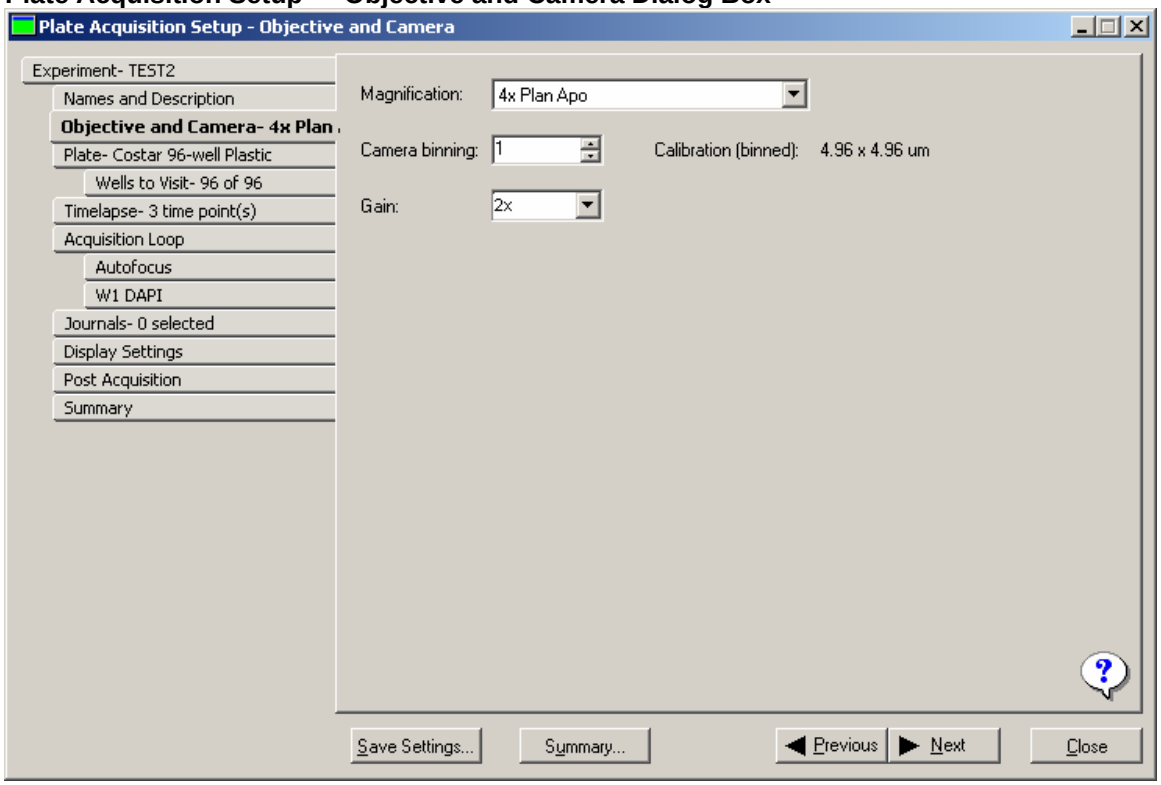
Focusing with the flat-field correction plates

In order to use the journal to create shading correction images, you must focus on the bottom of an appropriate FFC plate using the laser auto focus (or image auto focus if laser auto focus is not available). Complete the following procedure to focus on the bottom of a plate using laser auto focus:

1. From the Screening menu, select Plate Acquisition and Control.

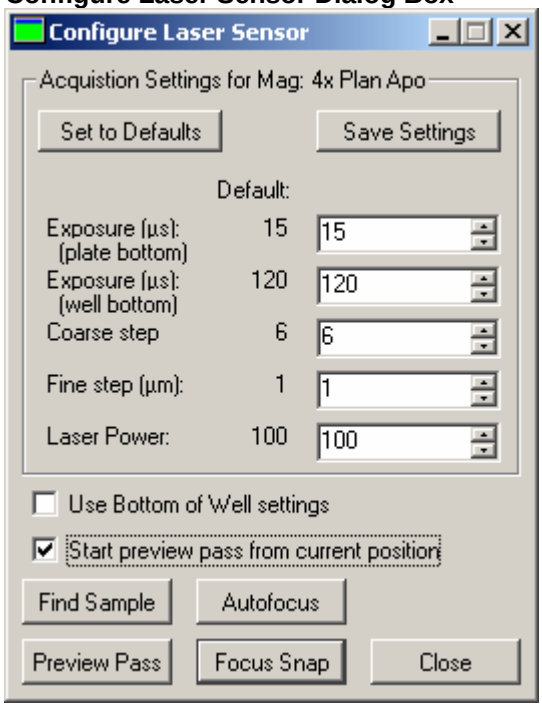
2. Click *Eject Plate* to move the stage to the load position.
3. Load the appropriate FFC plate for the filter set(s) of interest.
4. Click *Load Plate* to move the stage to its previous position.
5. From the Screening menu, select Plate Acquisition Setup.
6. Click the *Objective and Camera* tab and select the appropriate objective from the *Magnification* drop-down list.
7. Ensure that the *Camera binning* field is set to 1 and the Gain is set to 2, as shown in Figure 7-11:

Figure 7-11
Plate Acquisition Setup — Objective and Camera Dialog Box



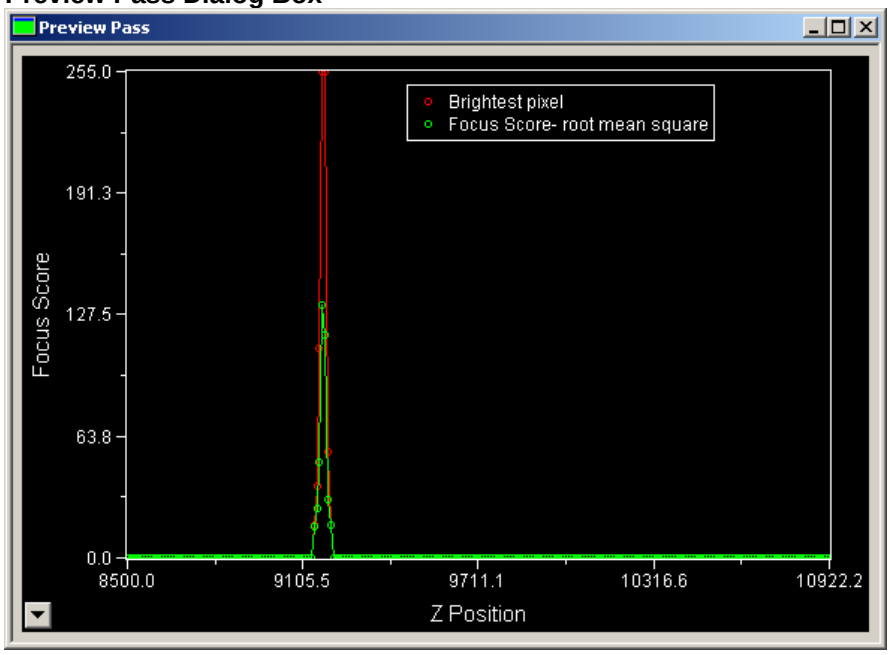
8. Click the *Wells to Visit* tab and right-click on a well near the middle of the plate. The stage moves to that well.
9. Click the *W1 tab* and ensure that the *Illumination* setting is set to the filter set you are creating the shading correction image for.
10. Click the *Acquisition Loop* tab and make sure that *Enable laser-based focusing* is checked.
11. Click the *Autofocus* tab, then click *Configure Laser Sensor*. The Configure Laser Sensor dialog box opens, as shown in Figure 7-12:

Figure 7-12
Configure Laser Sensor Dialog Box



12. Check *Start Preview Pass from current position*.
13. In the Plate Acquisition and Control dialog box, set the Z Step size to 1000.
14. Use the Z controls to step the Z motor down to the bottom of its range, then step up by 8000 um (8 steps).
15. From the Configure Laser Sensor dialog box, click *Preview Pass*. A window opens displaying a graph of focus intensities vs. Z-position. Ideally, the graph should contain a sharp peak, made up of a red line and a green line, as shown in Figure 7-13:

Figure 7-13
Preview Pass Dialog Box



Note: The top of the red peak represents the brightest pixel of the preview pass. The top of the green peak represents the highest focus score.

16. If the peak is either saturated or too low/not visible, adjust the *Exposure (plate bottom)* and/or *Laser power* values and retry.
17. Click on the arrow in the lower left hand corner of the Preview Pass window and select *Show Graph Data*. The Graph Data dialog box opens.
18. Click the point in the graph that represents the peak. You can click on either the green peak or the red peak.

    Clicking the peak highlights the appropriate row in the Graph Data dialog box that contains the focused Z position.

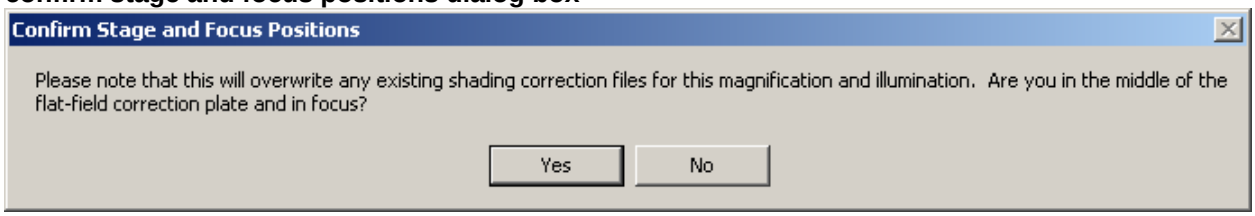
19. Use the Z controls in the Plate Acquisition and Control dialog box to navigate to the Z position found in the previous step.
20. Ensure that the appropriate wavelength is selected in the *Wavelength* field, and then click *Snap Current* to acquire an image and verify that your FFC plate is in focus. If the image is not in focus, adjust the exposure time in the *W1* tab of the Plate Acquisition Setup dialog box. If there are scratches, dust, or other imperfections on the FCC plate, they should be in focus. If there are no imperfections on the plate, the intensity should be at its maximum.
21. Close both the Plate Acquisition and Control and Plate Acquisition Setup dialog boxes and continue to the next procedure.

RUNNING THE SHADING CORRECTION JOURNAL

Now that the field is focused, you need to run the journal that creates the shading correction file. Complete the following procedure to create the file:

1. From the Journal menu, select Run Journal. The Run Journal dialog box opens.
2. Select the IXM_Shading_Correction.jnl journal and click *Open*. A dialog box open confirming the operation, as shown in Figure 7-14:

Figure 7-14
confirm stage and focus positions dialog box

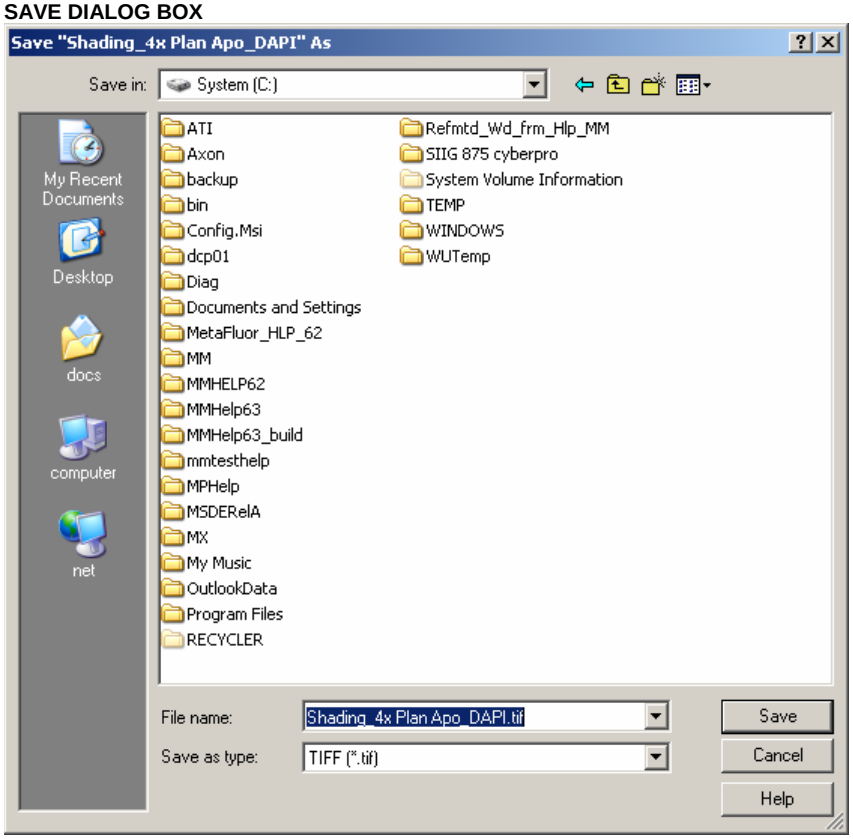


3. Click *Yes*. The Select Illumination dialog box opens.
4. Select the illumination that you used in the *Focusing with the flat-field correction plates* procedure and click *OK*. The journal creates a 27-plane shading stack and averages the stack into a final shading correction image.

   NOTE: The journal takes several minutes to create the final shading correction image.

   When the image is complete, the Save dialog box opens, as shown in Figure 7-15:

FIGURE 7-15
SAVE DIALOG BOX



5. Navigate to the root of the C directory (**`C:\`**) and save the image.

   Notes:

   - The shading correction images must be named in the following format: **`shading_<magnification setting>_<wavelength>.tif`**. For example, **`C:\shading_4x Plan Apo_DAPI.tif`**. The journal uses this naming convention by default.
   - By default, MetaXpress looks in the root **`C:\`** directory for shading correction images. You can change this location by clicking the Directory button on the *Acquisition Loop* tab of the Plate Acquisition Setup dialog box and selecting a new location.

6. After the image is saved, The Select Illumination dialog box opens with the *Illumination* field set to *[None]*. If you need to create additional shading correction files for other filters using this objective and FCC plate combination, select a filter from the *Illumination* drop-down box and click *OK*

   OR

   If you are finished, ensure that [None] is selected in the *Illumination* field and click *OK* to exit the journal.

Updating the System After Adding or Replacing a Filter Cube

After installing a new filter cube, you must update the filter settings in the Meta Imaging Series Administrator then update settings within the main MetaXpress program.

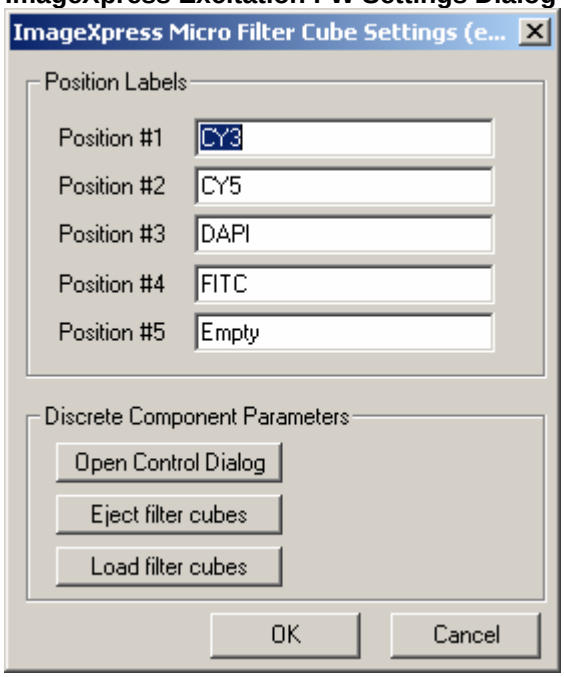
Note: For information on installing a new filter, refer to the *ImageXpress^{MICRO} Hardware User's Guide*.

Editing the Filter Settings in the Meta Imaging Series System Administrator

Complete the following procedure to update your filter settings:

1. From the Windows Start menu, go to Programs> MetaXpress Meta Imaging Series 6.x> Meta Imaging Series Administrator. The Meta Imaging Series Administrator program opens.
2. Select *MetaXpress* from the *List of Groups* field.
3. Click *Configure Hardware*. The *Configure Hardware* dialog box opens.
4. Click *Install System Devices*. The *Install Systems Devices* dialog box opens.
5. From the *Installed Devices* list, select ImageXpress Micro Filter Cube and click *Settings*. The ImageXpress Micro Filter Cube Settings dialog box opens, as shown in Figure 7-16:

Figure 7-16
ImageXpress Excitation FW Settings Dialog Box



6. Edit the name of the filter cube you are adding/replacing in the appropriate *Position Labels* field.
7. Click *OK* to close the Install System Devices dialog box and return to the Configure Hardware dialog box.

Note: The next steps involve entering the values again, this time starting from the Configure Devices dialog box. This is to ensure that the setting will carry over for all hardware profiles.

8. From the Configure Hardware dialog box, ensure that the hardware setting you are using is selected in the *Hardware Settings* list and then click *Configure Devices*. The User Settings hardware configuration dialog box opens.
9. From the *Claimed Devices* list, select ImageXpress Micro Filter Cube and click *Settings*. The ImageXpress Micro Filter Cube Settings dialog box opens.
10. Again, edit the name of the filter cube you are adding/replacing in the appropriate *Position Labels* field.
11. Click *OK* to close the User Settings dialog box and return to the Configure Hardware dialog box.
12. Click *OK* to exit each dialog box and close the Meta Imaging Series System Administrator.

Updating Illumination Settings

Complete the following procedure to update illumination settings within MetaXpress:

1. Open MetaXpress and log into the database.
2. From the Devices menu, select Configure Illumination. The Configure Illumination Dialog box opens.
3. Ensure that the illumination device you changed is selected in the *Device Positions* field and select the filter that you installed from the corresponding drop-down box.
4. Enter the name of the new filter in the *Name* field.
5. Enter the emission wavelength of the new filer in the *Wavelength* field.
6. Click *Add/Replace* to add this setting to the *Defined Settings* list.
7. If you replaced a filter with an existing setting, select the old setting from the *Defined Settings* field and click *Remove*.
8. Once the settings are updated, click *Backup* and backup the new settings.
9. Click *Close* to exit the Configure Illumination dialog box.

Defining a Plate Type

The MetaXpress CD comes with a variety of common plate types already defined. Check the Plates directory of the MetaXpress CD for these plate files. In order to use the plate files in MetaXpress, you must copy them from the Plates folder on the CD into the plates directory of your MetaXpress installation (C:\MX\plates by default). These plate types will then be available from the plate name drop-down list.

If the plate type you are using is not included on the CD, you need to complete the following procedures to assign and save correct plate values for the plate type.

Notes:

- You must know the optical thickness and bottom variation for your plate. The optical thickness is the distance (in um) between the Z position when in focus at the bottom of the plate and the Z position when in focus at the bottom of the well. The bottom variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms.

  The optical thickness is equivalent to the physical thickness of the plate divided by the refractive index. The bottom variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms.

- The correct Well depth and Plate height values are needed in order for the autofocus Find Sample command to work correctly.

  See the following websites for specifications on some common plate types:

  http://www.corning.com/lifesciences/products__services/microplate_equipment_compatibility_guide/

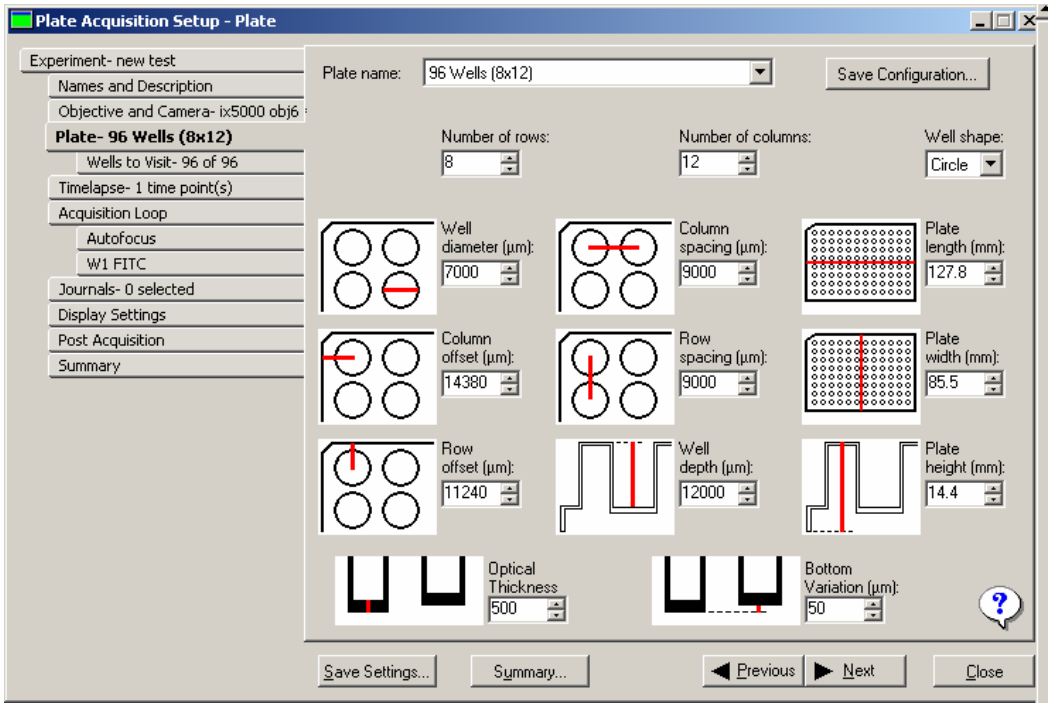
  http://www.bdbiosciences.com/discovery_labware/technical_resources/pdf/TB435_A.pdf

  http://www.sbsonline.org/msdc/approved.php

Complete the following procedure to define the plate types for MetaXpress:

1. Open MetaXpress.
2. From the Screening menu, select Plate Acquisition Setup. The Plate Acquisition Setup dialog box opens.
3. Click the *Plate* tab. The Plate tab opens, as shown in Figure 7-17:

Figure 7-17
Plate Tab



4. Select a plate that corresponds with the type you are using in the Plate name drop-down list.

   Note: If you select *96 Wells* (8x12) or *384 Wells* (16x24), the default values for Well Diameter, Column Offset, Row Offset, Column Spacing, Row Spacing, Plate Length, and Plate Width are the ANSI/SBS standards for multi-well plates. If the plate type you are configuring conforms to these standards you should not have to change these values. If you select *96 Wells* (8x12) or *384 Wells* (16x24), the following options will be available for configuration: Well Shape, Well depth, Plate height, Bottom thickness, and Bottom tolerance.

   If you select *Custom*, all options will be available for configuration.

5. Enter values from the manufacturers spec as needed for *Well diameter*, *Column offset*, *Row offset*, *Column spacing*, *Row spacing*, *Well depth*, *Plate length*, *Plate width*, and *Plate height*.
6. Select your square or circular well shape from the *Well shape* drop-down list. The graphics of the plate are updated to reflect your selection.
7. Enter values in the *Optical Thickness* and *Bottom Variation* fields (see the procedures that follow). If needed, change the values in the *Well depth* and *Plate height* fields.
8. If you selected *Custom*, enter other values as needed.

   Note: The default values for Well Diameter, Column Offset, Row Offset, Column Spacing, Row Spacing, Plate Length, and Plate Width are the ANSI/SBS

standards for multi-well plates. If the plate type you are configuring conforms to these standards you should not have to change these values.

9. After you have configured the plate settings, click *Save Configuration* to enter a name for your setting and save the plate configuration. Configurations that have been saved are then available to select in the *Plate Name* drop-down list.

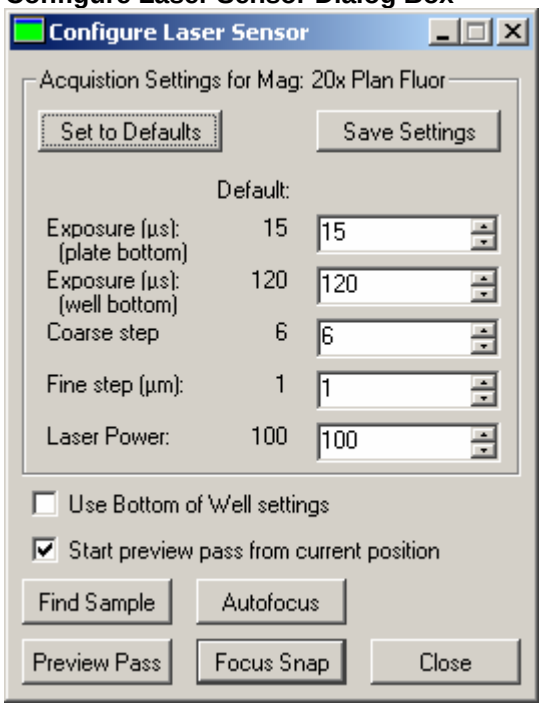
Determining Optical Thickness

Complete the following procedure to use the laser autofocus to determine the optical thickness for a plate:

Note: If you do not have the laser autofocus option, the physical thickness values is used instead of the optical thickness value. Use the plate manufacturer's specifications and/or calipers to determine the physical thickness.

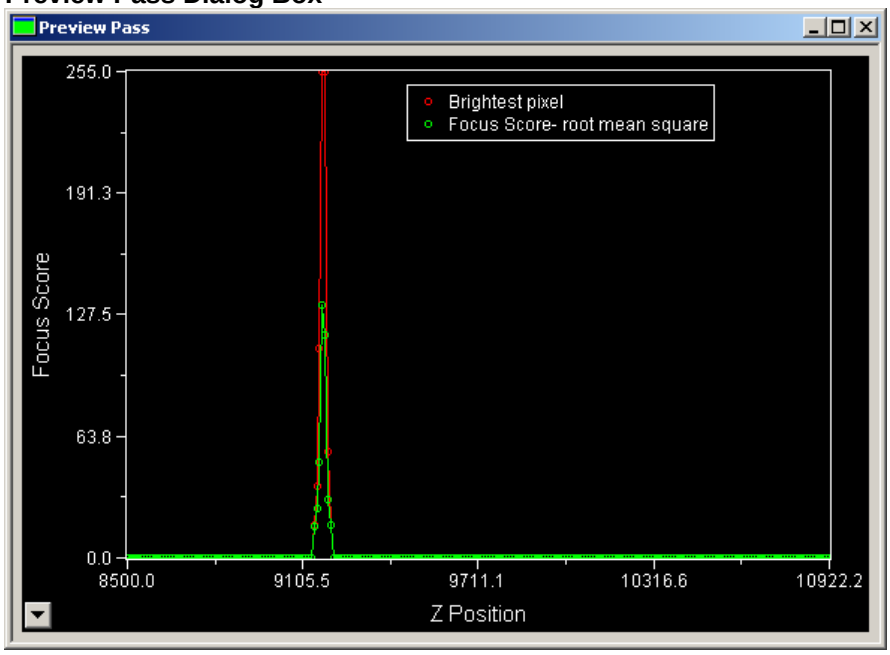
1. If they are not open, open both the Plate Acquisition Setup dialog box and the Plate Acquisition Control dialog box from the Screening menu. Also open the Plate Acquisition toolbar.
2. Click the *Objective and Camera* tab on the Plate Acquisition Setup dialog box and select the 20x objective. Note that this is the same objective used to determine the plate bottom reference value.
3. Click the Stage Load/Eject button  on the Plate Acquisition toolbar and load a plate on the stage.
4. Use the Plate Navigation controls in the Plate Acquisition Control dialog box or the Plate Acquisition toolbar to move to an empty well.
5. In the Plate Acquisition and Control dialog box, set the Z Step size to *1000.*
6. Use the Z controls to step the Z motor down to the bottom of its range, then step up by 8000 um (8 steps).
7. Click the *Autofocus* tab on the Plate Acquisition Setup dialog box and then click *Configure Laser Sensor* to open the Configure Laser Sensor dialog box, as shown in Figure 7-18:

Figure 7-18
Configure Laser Sensor Dialog Box



8. Click *Set to Defaults*.
9. Ensure that *Use Bottom of Well Settings* is not selected.
10. Ensure that *Start Preview Pass from current position* is selected.
11. Click *Preview Pass*. A window opens displaying a graph of focus intensities vs. Z-position. Ideally, the graph should contain a sharp peak, made up of a red line and a green line, as shown in Figure 7-19:

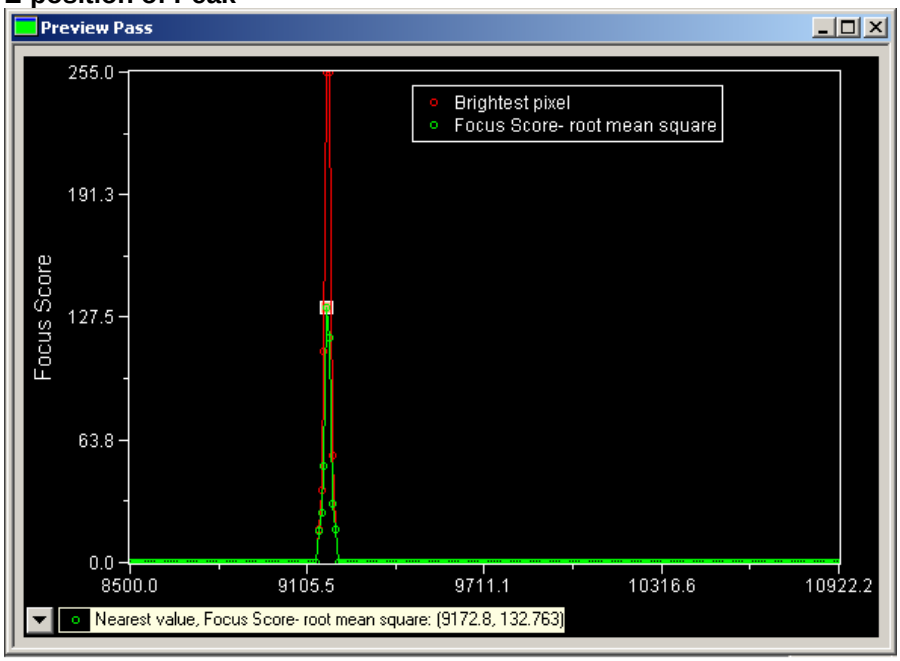
Figure 7-19
Preview Pass Dialog Box



Note: The top of the red peak represents the brightest pixel of the preview pass. The top of the green peak represents the highest focus score.

12. If there is a peak in the graph, skip to the next step. If there is no peak, try the following:
    a. If the current Z-position is < 12000, use the Focus dialog box to increase it by 1000 and click *Preview Pass* again.
    b. If the current Z-position is >12000, use the Focus dialog box to decrease it to 8000. In the Configure Laser Sensor dialog box, double the value in the *Exposure (plate bottom)* field, decrease the *Fine step* value by half, and click *Preview Pass* again. If you still cannot find a peak after adjusting the exposure/step size twice and covering a z-range from 8000 to 12000, contact MDC Customer Support.
13. When you see a peak (or 2 peaks) on the preview pass, click the trace line at the top of the green line of the first peak. The X, Y position is displayed in a tool tip, as shown in Figure 7-20:

Figure 7-20
Z-position of Peak



Note the X value in the tool-tip. It is the Z-position of the plate bottom. In this example, the current Z-position value is 9172.8.

Write down the Z-position value (rounded off) in the Well 1 Plate Bottom Value field of Table 7-4 below.

14. If there is a second, separate peak, click the point at the top of the green line in the second peak. The X, Y position is displayed in a tool tip. Note the X value in the tool-tip. It is the Z-position value of the well bottom. Record this value in the Well 1 Well Bottom Value field of Table 7-4:

Table 7-4
Optical thickness value

	Well Bottom Value —	Plate Bottom Value	= Optical Thickness Values
Well 1			
Well 2			
Well 3			
Well 4			
Well 5			

15. If there were two separate peaks when you clicked *Preview Pass* and you have both Well bottom and Plate Bottom Values for Well 1, skip to the next step. If there was only one peak, try the following:
    a. From the Devices menu, open the Focus dialog box.

b. Add 20 to the value you just recorded for plate bottom in the Current Position field and press [**Enter**] to raise the Z-motor.
c. In the Configure Laser Sensor dialog box, check *Use Bottom of Well settings*.
d. Enter a value of 25 in the *Exposure (well bottom)* field.
e. Click *Preview Pass*. A new window opens displaying a graph of focus intensities vs. Z-position for the bottom of the well. The graph should contain a sharp peak. If there is no peak, double the exposure value in the *Exposure (well bottom)* field and try again (up to a maximum exposure of 500).
f. When you see a peak on the preview pass, click the trace line at the top of the peak. The X, Y positions is displayed in a tool tip (the X value is actually the current Z focus value).
g. Write down the X position value displayed in the tool-tip in the Well 1 Well Bottom Value field of Table 7-4.

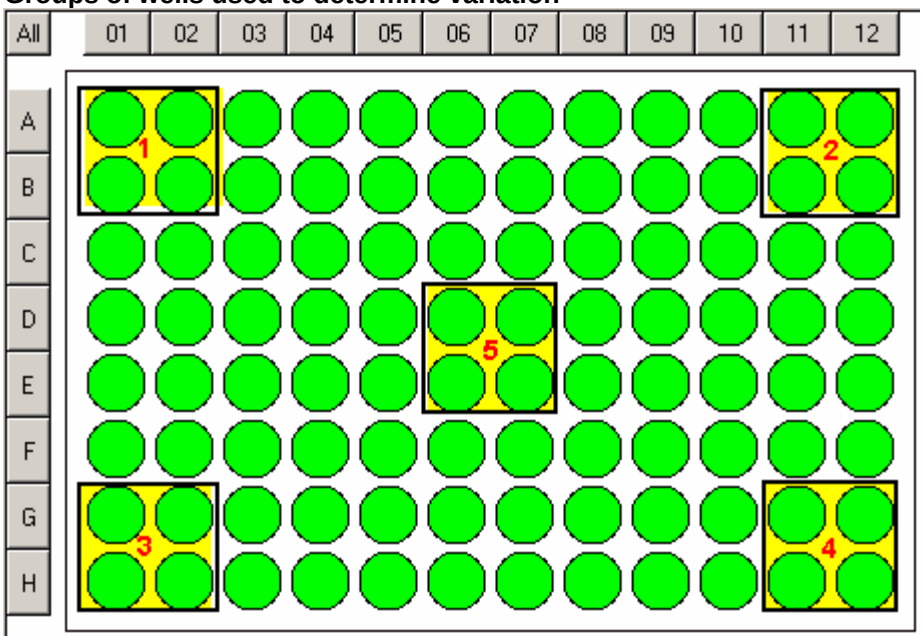
16. Subtract the Plate Bottom Value from the Well Bottom Value. The result is the Optical Thickness value for the first well. Enter this in the Optical Thickness field for well 1 in Table 7-4.
17. Repeat Steps 4-16 with four random wells from different areas of the plate and enter the values in Table 7-.4
18. Average all five of the Optical Thickness values in Table 7-4.
19. Enter this value in the *Optical Thickness* field in the *Plate* tab of the Plate Acquisition Setup dialog box.

Determining Bottom Variation

The bottom tolerance variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms. Determining the bottom variation of a plate involves measuring the plate bottom values of five groups of four wells across an entire plate and finding the absolute maximum value. Complete the following procedure to determine the Bottom Variation value for a 96-well plate:

1. Use the *Determining Bottom Thickness* procedure to find the **plate bottom** values for the groups of wells shown in Figure 7-21:

Figure 7-21
Groups of wells used to determine variation



2. Enter the plate bottom values for each group of wells in Table 7-5:

Table 7-5
Group 1 Plate bottom values

Well	Plate Bottom Value
A1	
A2	
B1	
B2	

3. Determine the variations for Group 1, then enter the absolute value for each result in Table 7-6:

Table 7-6
Group 1 Variations

	Variation	Absolute Value
A1 – A2	=	
A1 – B1	=	
B1 – B2	=	
A2 – B2	=	

4. Circle the largest absolute value in Table 7-6.
5. Repeat Steps 2-4 for each of the remaining four groups:

Table 7-7
Group 2 Plate Bottom Values

Well	Plate Bottom Value
A11	
A12	
B11	
B12	

Table 7-8
Group 2 Variations

	Variation	Absolute Value
A11 – A12	=	
A11 – B11	=	
B11 – B12	=	
A12 – B12	=	

Table 7-9
Group 3 Plate Bottom Values

Well	Plate Bottom Value
G1	
G2	
H1	
H2	

Table 7-10
Group 3 Variations

	Variation	Absolute Value
G1 – G2	=	
G1 – H1	=	
H1 – H2	=	
G2 – H2	=	

Table 7-11
Group 4 Plate Bottom Values

Well	Plate Bottom Value
G11	
G12	
H11	
H12	

Table 7-12
Group 4 Variations

	Variation	Absolute Value
G11 – G12	=	
G11 – H11	=	
H11 – H12	=	
G12 – H12	=	

Table 7-13
Group 5 Plate Bottom Values

Well	Plate Bottom Value
D6	
D7	
E6	
E7	

Table 7-14
Group 5 Variations

	Variation	Absolute Value
D6 – D7	=	
D6 – E6	=	
E6 – E7	=	
D7 – E7	=	

6. Take the largest absolute value of all five groups, multiply it by 1.1 and enter it in Table 7-15:

Table 7-15
Bottom Variation Value

Highest Absolute Value	Bottom Variable Value
x 1.1	=

7. Enter this value in the *Bottom Variation* field in the *Plate* tab of the Plate Acquisition Setup dialog box.

Creating Laser Auto Focus Settings for Plate Types

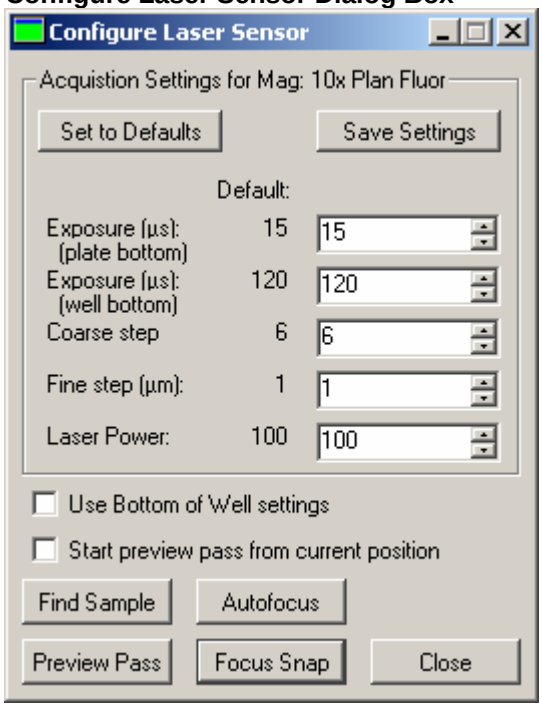
After creating a plate type, you need to configure laser auto focus (LAF) settings for it using the Configure Laser Sensor dialog box . Typically you need to adjust only 2 settings — *Exposure (us) (plate bottom)*, and *Exposure (us) (well bottom)*. The default values for the other settings should work in most cases, and should only be adjusted as a last resort. Complete the following steps to create laser settings for your plate type:

Notes:

- LAF settings are objective-specific; you need to create settings for each magnification setting that uses the plate type. The default values in the Configure Laser Focus dialog box are a good starting point.
- If at any point you have problems completing the following procedure, skip to the next section, *Troubleshooting Laser Auto Focus Settings for Plate Types*.
- This procedure assumes that you have a plate containing either water, buffer, or known samples loaded.

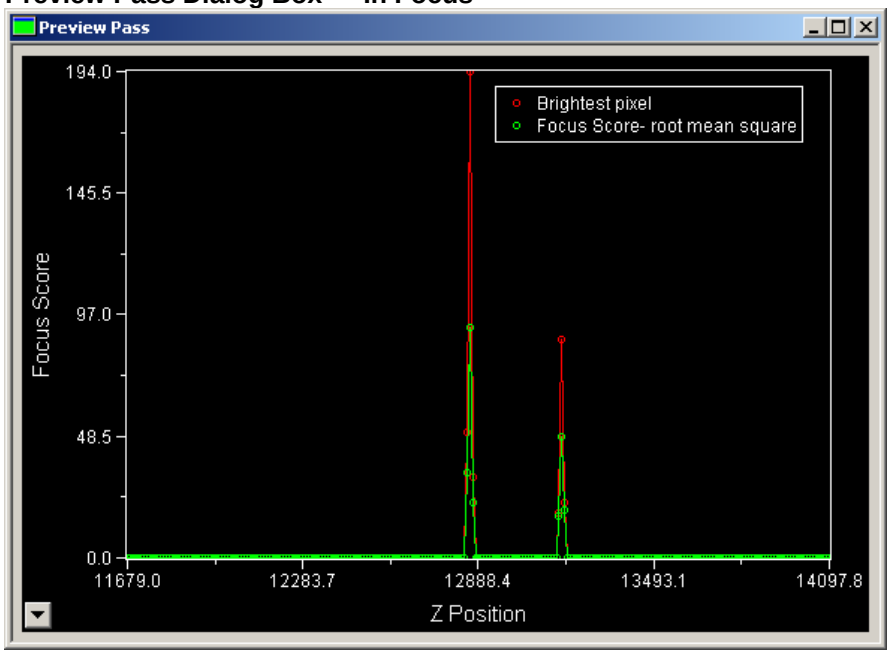
1. From the screening menu, select Plate Acquisition Setup. The Plate Acquisition Setup dialog box opens.
2. Click the *Objective and Camera* tab and select the objective to configure from the *Magnification* drop-down list.
3. Click the *Plate* tab and ensure that the correct plate is selected in the *Plate name* drop-down list.
4. Click the *Wells to Visit* tab and right-click a well that contains water, buffer, or a known sample to move to it.
5. Click the *Acquisition Loop* tab and make sure that *Enable laser based focusing is* selected and *Enable image-based focusing* is *not* selected
6. Click the *Autofocus* tab, then click *Laser Configuration*. The Configure Laser Sensor dialog box opens:

Figure 7-22
Configure Laser Sensor Dialog Box



7. Ensure that *Use Bottom of Well Settings* is not selected.
8. Starting with the default values, press *Preview Pass*.
9. A window opens displaying a graph of focus intensities vs. Z-position. Ideally, the graph should contain one or two sharp peaks, made up of a red line and a green line, as shown in Figure 7-23:

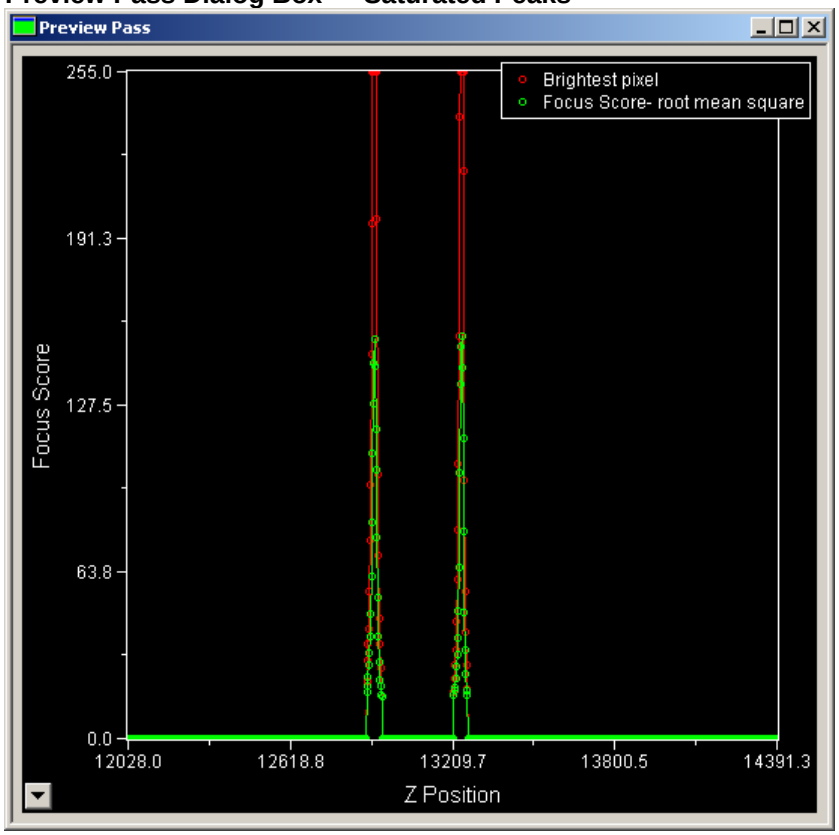
Figure 7-23
Preview Pass Dialog Box — In Focus



Note: The top of the red peak represents the brightest pixel of the preview pass. The top of the green peak represents the highest focus score.

10. If there is a peak in the graph, like the one shown in Figure 7-23, skip to the next step. If there is no peak, try the following:
    a. Double the value for *Exposure (us) (plate bottom)*.
    b. Click *Preview Pass*. Repeat up to three times until there is a peak displayed in the graph.
    c. If you still have no peak, refer to the next section, Troubleshooting Laser Auto Focus Settings for Plate Types.
11. Figure 7-24 shows two saturated peaks. Note that the maximum intensity is 255, represented by the red curve:

Figure 7-24
Preview Pass Dialog Box — Saturated Peaks



If the peaks are saturated, try the following:

a. Reduce the value of *Exposure (us) (plate bottom)* by half.
b. Click *Preview Pass*. Repeat this until the peak is not saturated (note that the minimum exposure value is 10). If the peak is still saturating with the exposure at 10, reduce the Laser Power value and try again. Note that in general laser power should be at 100% unless it is necessary to avoid saturation.

12. Now that the first peak — which represents the bottom of plate focus — is sharp, click *Use Bottom of Well Settings* to check the bottom of well focus (the second peak).
13. Click *Preview Pass*. Ideally, the graph should now display two sharp peaks.
14. If there are two peaks in the graph, skip to the next step. If there is only one peak, try the following:
    a. Double the value for *Exposure (us) (well bottom)*.
    b. Click *Preview Pass*. Repeat up to three times until there are two peaks displayed in the graph.
15. If two peaks are present, and the second one is saturated, try the following:
    a. Reduce the value for *Exposure (us) (plate bottom)* by half.
    b. Press Preview Pass. Repeat until the peak is not saturated (note that the minimum exposure value is 10).

16. Press *Save Settings* to save these values for your plate type using the current objective.
17. Repeat this procedure as needed for each plate type/objective combination.

Troubleshooting Laser Auto Focus Settings for Plate Types

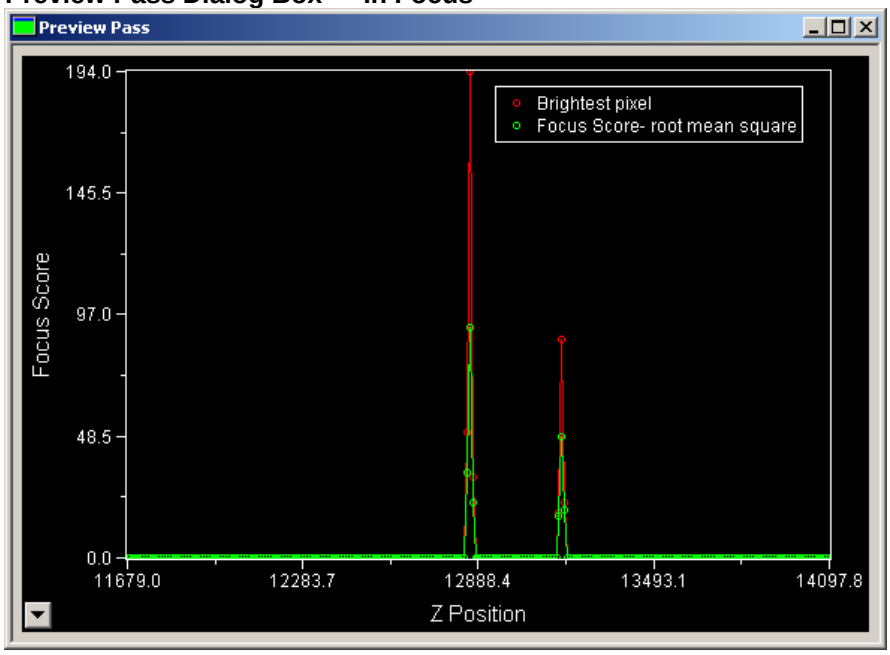
There are several things to consider when troubleshooting LAF settings for your plate type. You should perform the following procedures, in the order listed, until you resolve the problems you are experiencing:

Note: Before trying any of the following troubleshooting procedures, ensure that you have successfully completed the procedures for determining optical thickness and bottom variation.

Verifying Laser Auto Focus Search Range

1. Make sure that a plate of the type you are trying to troubleshoot is loaded.
2. From the screening menu, select Plate Acquisition Setup. The Plate Acquisition Setup dialog box opens
3. Click the *Plate* tab and ensure that the correct plate is selected in the *Plate name* drop-down list.
4. Click the *Wells to Visit* tab and right-click a well that contains water, buffer, or a known sample to move to it.
5. Click the *Acquisition Loop* tab and make sure that *Enable laser based focusing is* selected and *Enable image-based focusing* is *not* selected
6. Click the *Autofocus* tab, then click *Laser Configuration*. The Configure Laser Sensor dialog box opens.
7. Ensure that *Use Bottom of Well Settings* is not selected.
8. Ensure that *Start preview pass from current position* is not selected.
9. Click *Preview Pass*. A window opens displaying a graph of focus intensities vs. Z-position. Ideally, the graph should contain two sharp peaks, made up of a red line and a green line, as shown in Figure 7-25:

Figure 7-25
Preview Pass Dialog Box — In Focus



10. If two peaks are present, then the range covered is appropriate. Continue to the next procedure.
11. If no peaks are displayed, try doubling the *Exposure (us) (plate bottom)* value and click *Preview Pass* again. Repeat twice if needed.

    OR

    If only one peak is displayed, select *Use Bottom of Well settings*, increase the *Exposure (us) (well bottom)* value to double the *Exposure (us) (plate bottom)* value and click *Preview Pass* again. Repeat if needed.

12. If there are still no peaks present, note the starting Z-position (x-axis) in the Preview Pass graph and write it down for reference:

Starting Z-position		**Enter this value in the Current Position Field**
	— 2000	**=**

13. From the Devices menu, select Focus to open the Focus dialog box.
14. Subtract 2000 from the Starting Z-position value noted above, enter the result in the *Current Position* field, and press **`[Enter]`**. The Z-motor moves to the new position.
15. In the Configure Laser Sensor dialog, make sure that *Start preview pass from current position* is selected and click *Preview Pass* again.

16. If there are peaks present in this graph, continue to the next procedure, *Verifying Plate Height and Well Depth*. If no peaks are found, continue with this procedure.
17. Using the Focus dialog, move the Z-position to 1000 um above the starting position you wrote down in step 12.
18. Click *Preview Pass* again.
19. If there are peaks present in this graph, continue to the next procedure, *Verifying Plate Height and Well Depth*. If no peaks are found, continue with this procedure.
20. Repeat steps 14-15, increasing the value in the Current Position field by 1000 um until peaks are found. Do not enter a value above 12000 um.
21. If there are peaks present in this graph, continue to the next procedure, *Verifying Plate Height and Well Depth*. If no peaks are found, continue with this procedure.
22. If there are no peaks found, verify that the objective is currently centered in a well.

    Note: To verify that the objective is centered in a well, open one of the side panels on the ImageXpress^{MICRO} and open the shutter with a visible wavelength (e.g. FITC) selected. Visually verify that the light is centered under your well of interest. For information on removing the side panels of the ImageXpress^{MICRO}, refer to the *ImageXpress^{MICRO} Instrument User's Guide*.

23. If not currently centered in a well, then move the stage until centered and repeat steps 8- 17 as needed.
24. If you still cannot find peaks in a Preview Pass graph, then contact MDC customer support for assistance.

Verifying Plate Height and Well Depth

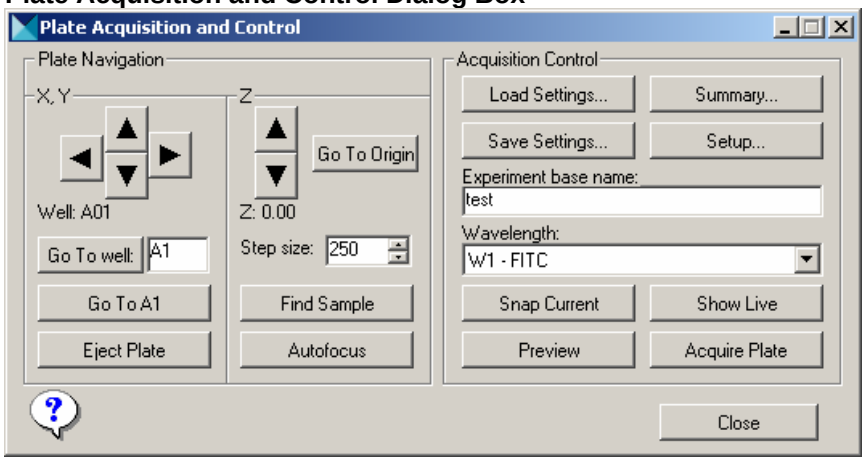
1. Obtain the manufacturers specifications for the plate type you are using and verify that the settings for your plate type match the specifications. If they do not match, enter the published values in the Plate tab of the Plate Acquisition Setup dialog box, resave the plate configuration, and retry the previous procedure, *Verifying Laser Auto Focus Search Range*.
2. If you are unable to obtain manufacturers specs, then you can measure the plate height (in mm) and well depth (in um) using calipers. Enter the measured values, and retry the previous procedure, *Verifying Laser Auto Focus Search Range*.
3. If still unable to perform laser autofocus correctly, continue to the next procedure to verify that objective parfocality settings are correct.

Verifying Objective Parfocality

1. Open the Device control toolbar if it is not displayed: Window>Toolbars>Device Control.
2. Select your highest magnification setting from the *Mag* drop-down list on the toolbar (for best results, check the 4x objective last).

3. From the Screening Menu, select Plate Acquisition and Control. The Plate Acquisition and Control dialog box opens, as shown in Figure 7-26:

Figure 7-26
Plate Acquisition and Control Dialog Box



4. Select FITC (or any visible spectrum filter cube) from the *Wavelength* drop-down list.
5. Press *Eject Plate*, then load the bead plate that shipped with the ImageXpress^{MICRO} on the stage.
6. Press *Load Plate* to return the stage to its previous position.
7. Use the X, Y, and Z controls to find and focus a sample. Record the z-position.
8. Select the next highest magnification objective from the *Mag* drop-down list on the Device Control toolbar.
9. Note the z-position, then use the Z controls to find and focus a sample.
10. Note the focus position. It should not be more than 25 um from the position noted in step 9.
11. Repeat steps 8 - 10 for each objective. For best results, check the 4x objective last.
12. If you have to move the z-motor more than 25 um after changing from one objective to another, then parfocality settings should be updated. Refer to the procedure *Configuring Parfocality after Changing Objectives*. After you have updated parfocality settings, restart troubleshooting with the procedure *Verifying Laser Auto Focus Search Range*.
13. If parfocality settings are appropriate, then try the following tips.

Other troubleshooting tips

- If the focus was found for some wells and lost for others, that usually means that the *Bottom Variation* value entered in the *Plate* tab of the Plate Acquisition Setup dialog box is not large enough. Try increasing the *Bottom Variation* value by 10 um (or measure the wells in question to get a more accurate read) and try again (repeat as needed).
- Check for scratches, dirt, or condensation on the bottom of the plate and correct as needed.

- Ensure that there is liquid in each well.
- If the well bottom has a very weak laser signal, which is possible with samples containing a high percentage of glycerol, try selecting the option *Find plate bottom only, then offset by bottom thickness* on the *Autofocus* tab in Plate Acquisition setup. This option is also necessary for low NA objectives (such as 2x or 4x).
- If you have recently changed objectives, ensure that you complete the procedures in the *Updating the System after Adding or Replacing an Objective* section of this chapter.

If still unable to successfully use laser autofocus with your plate type, contact MDC Customer Support.

Chapter 8
Customizing MetaXpress

A powerful feature of MetaXpress is the ability to customize the operation of the software for your users. Different objectives and workflows call for customized settings that can be switched as needed. Applications within MetaXpress, such as the Meta Imaging Series Administrator and the Create Taskbar command, allow you to create settings that match the needs of your users. The following topics will be covered in this section:

- Users and Groups in the Meta Imaging Series Administrator
- Custom Toolbars and Task Bars
- Default Paths for Data

Note: The concepts of users and groups discussed in this chapter are specific to custom hardware and drop-ins/toolbars settings for MetaXpress. They **DO NOT** have anything to do with configuring users and groups within the database. For information on setting up users and groups with the database, refer to the *MetaXpress Database Guide*.

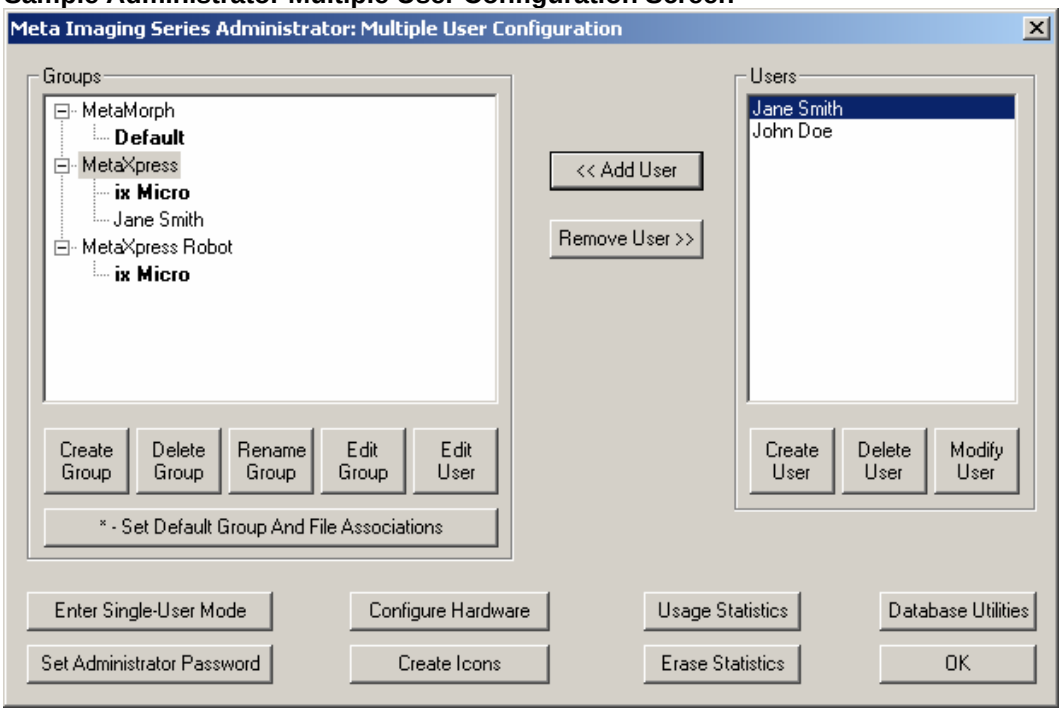
Users and Groups in the Meta Imaging Series Administrator

The Meta Imaging Series Administrator commands enable you to define and configure settings for individual users and groups in MetaXpress. There are two modes for the Administrator — Single-User Mode and Multi-User Mode.

In Single-User mode, the Administrator enables you to select hardware settings and configure drop-ins and toolbars for groups that have already been created. In Multi-User mode, you create new groups, add users to different groups, and define hardware settings for groups. As a System Administrator, you will be working in Multi-User mode, creating groups and users for your MetaXpress system. For detailed instructions on setting up users and groups, refer to the online help for the Meta Imaging Series Administrator.

Your ImageXpress^{MICRO} system ships with a number of groups and hardware settings predefined. The number of groups depends on the configuration of your system. Figure 8-1 illustrates a sample Meta Imaging Series Administrator Multiple User Configuration screen:

Figure 8-1
Sample Administrator Multiple User Configuration Screen



This sample shows a system with the following groups defined:

- **MetaMorph** — This is the default MetaMorph group.
- **MetaXpress** — This is the ImageXpress^{MICRO} MetaXpress group.
- **MetaXpress Robot** — The hardware settings in this group enable the optional CRS robot to be used with the MetaXpress system.

To enable a group — that is, to use the hardware and software settings created for a group — you must create users and assign them to the group.

Note: For more information on creating users and groups in the Meta Imaging Series Administrator, refer to its online help file (press `[F1]` while in the application).

Creating an Offline Version of MetaXpress

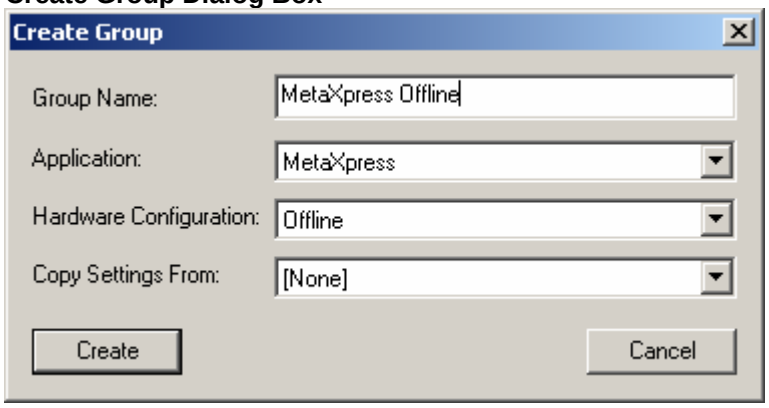
MDC recommends creating an offline group for MetaXpress users. This offline group has no hardware settings and is useful for analysis of already acquired images. Since the offline group has no hardware settings, it does not attempt to establish communication with the other MetaXpress components; this make the application start faster and allows you to run the software without turning on any hardware. Use the following procedure to create an offline MetaXpress group in the Meta Imaging Series Administrator:

> **Note:** You must exit the MetaXpress application before using the Meta Imaging Series Administrator. The two programs cannot run at the same time.

Note: For additional information about any of the dialog boxes in the Meta Imaging Series Administrator, press the [F1] key to access the online help for the active dialog box.

1. From the Windows Start menu, go to Programs>MetaXpress> Meta Imaging Series Administrator. The Meta Imaging Series Administrator program opens.
2. If the program opens in Single User Configuration mode, click *Enter Multi-User Mode*.
3. Click *Create group*. The Create Group dialog box opens, as shown in Figure 8-2:

Figure 8-2
Create Group Dialog Box



4. Type a group name in the *Group Name* text box, for example, MetaXpress Offline.
5. Select *MetaXpress* from the *Application* drop-down list.
6. Select *Offline* from the *Hardware Configuration* drop-down list.
7. Select *[None]* from *the Copy Settings From* drop-down list.
8. Click *Create*. The Create Group dialog box closes and the new group is listed in the *Groups* field.
9. Add users to the group as needed by selecting the new group in the *Groups* list, selecting a user from the *Users* list, then clicking <<Add Users.

   Note: You must add at least one user to the new offline group for it to be available.

10. Click *OK* to exit the Meta Imaging Series Administrator program.

Creating Group Icons and adding them to the MetaXpress desktop

After creating the MetaXpress Offline group and adding users using the above procedure, you should use the *Create Icons* command to create icons for the new group. This command installs shortcuts for any new groups to the Meta Imaging Series folder on the MetaXpress desktop. These shortcuts can then be copied directly to the MetaXpress desktop. This enables users to choose which version to start from the desktop. Use the following procedure to create and add group icons to the MetaXpress desktop:

1. From the Windows Start menu, go to Programs>MetaXpress>Meta Imaging Series Administrator. The Meta Imaging Series Administrator opens.
2. Click *Create Icons* to create the icons. No confirmation box will appear.
3. Click OK to exit the Meta Imaging Series Administrator.
4. Double-click the Meta Imaging Series 6.x shortcut on you MetaXpress desktop. The Meta Imaging Series folder opens.
5. Confirm that the group you created in the *Creating an Offline Version of MetaXpress* procedure is listed in this folder.
6. Select the shortcut you created (for example, MetaXpress Offline), right-click and select Send To>Desktop (create shortcut). The shortcut is created on the MetaXpress desktop.
7. Double-click the desktop shortcut to open that instance of the application.
8. Repeat Step 6 as needed to add other shortcuts to the MetaXpress desktop.

Custom Toolbars and Taskbars

Now that you have groups configured and icons on the desktop, you can create or modify custom toolbars and taskbars to include specific combinations of tools and commands.

Customizing Toolbars

With the Configure Dropins/Toolbars command, you can add menu commands to toolbars, move commands from one tool bar to another, and add or remove journals to toolbars. Use the following procedure to customize
MetaXpress toolbars:

1. From the Windows Start menu, go to Programs>MetaXpress> Meta Imaging Series Administrator. The Meta Imaging Series Administrator opens.
2. Select the group that you want to edit the tool bar in from the *Groups* list and click *Edit Group*. The Edit Group dialog box opens.
3. Click *Drop-ins/Toolbars*. The Configure Dropins/Toolbars dialog box opens.
4. Click the Toolbars tab, then uncheck the Use default toolbars checkbox.
5. Select *Menus* to add menu commands to toolbars.

   OR

   Select *Toolbars* to add toolbar commands to other toolbars.

   OR

   Select *Journals* to add journals to any toolbar or to create new Journal toolbars.

6. To add any command to a toolbar, click and drag a command from the left window to the appropriate toolbar folder in the right window.

Note: You can use the Control or Shift keys in combination with the mouse to select multiple commands, then drag the commands to the appropriate toolbar folder.

7. Click *OK* when finished. A dialog box opens to confirm that you want the users in the group to use the modified configuration.
8. Click *Yes.*
9. Click *OK* to exit the Edit groups dialog.
10. Click *OK* to exit the Meta Imaging Series Administrator. The modified toolbars will be available the next time you start the corresponding version of MetaXpress.

Creating Taskbars

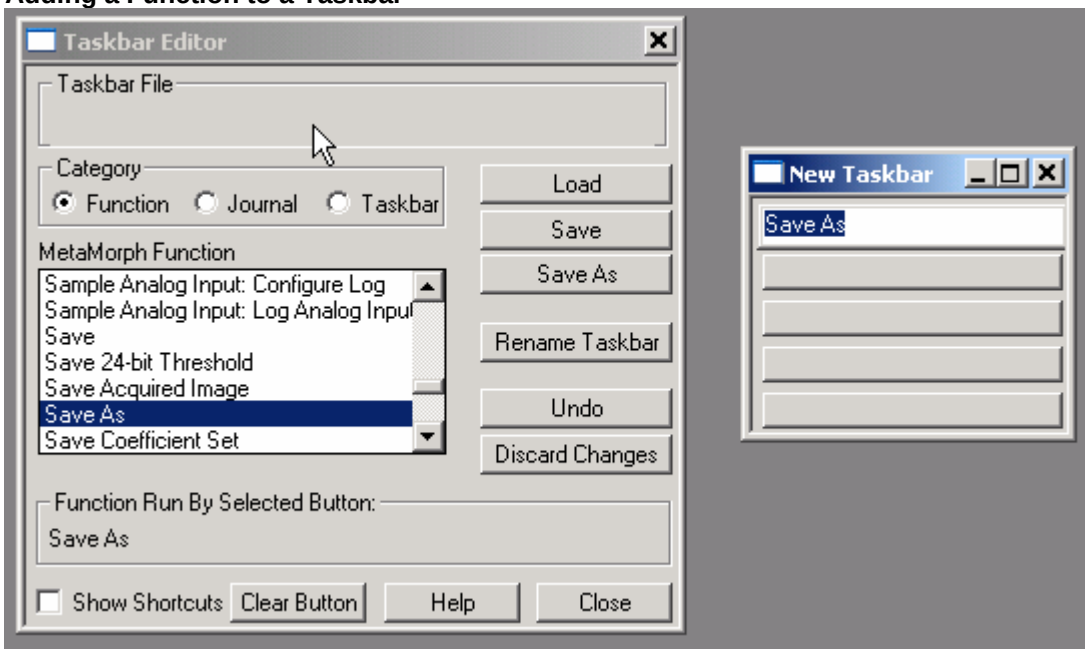
Taskbars are created directly in the MetaXpress application and are a convenient way to access frequently used commands and journals. Each taskbar can consist of up to 48 buttons in a configuration of rows and columns of your choosing. You can mix and match journals, commands, or other taskbars within the same taskbar. Taskbars differ from toolbars in that they enable you to add journals as well as commands. MDC recommends creating taskbars that combine commands and journals specific to your experiments. Use the following procedure to create and load a taskbar:

1. Start the MetaXpress application.
2. From the Journal menu, choose Taskbars>Create Taskbar. The Taskbar Editor dialog box and Taskbar window open. Position them so that you can see both the dialog box and the window at the same time.
3. Select the number of rows and columns for the taskbar by dragging the thick border of the Taskbar window until the desired number of rows and columns appear in the window.
4. Select the width of the buttons in the taskbar by dragging the thick border of the active button until the buttons are the desired width.
5. Select the desired category for the first item you want to add to the taskbar from the *Category* group.

   If you selected *Journal* or *Taskbar* as the *Category*, the directory names will be displayed in square brackets in the list box below *Category*. Double-click a directory name to display the appropriate files in that directory or double-click the double period ("..") to go up one level in the directory structure.

6. When you have located the item you want to add to the taskbar, double-click its entry in the list box to add it to active button in the taskbar as shown in Figure 8-3:

Figure 8-3
Adding a Function to a Taskbar



7. Repeat Steps 5 and 6 for each item you want to add to the taskbar. You can use the *Undo* command to undo the last item you added or you can use *Clear* Button to clear an item from the active button if needed.
8. If you want to rename the taskbar, click *Rename Taskbar*. The Rename Taskbar dialog box opens. Type the desired name in the text box, then click *OK*.
9. When finished, click *Save*. The Save As dialog box opens. Type the desired file name in the File Name text box. You can use the Save In list or Up One Level button to select the appropriate drive and folder, if necessary. Then click *Save*.
10. To use the new taskbar (or a different taskbar) immediately, select Load Taskbar from the Journal menu. Select the desired taskbar file, then click *Open*.
11. Click *Close* to close the Taskbar Editor.

Default Paths for Data

The Configure Default Paths command in the MetaXpress application is used to change the default file paths for each group or user. You can modify these paths so that each user on the system has their own dedicated data folders. These folders contain log files, calibration settings, and other data unique to each user. MDC recommends changing the following default paths on a MetaXpress system:

- **Default Data Paths** — Your MetaXpress computer has a dedicated hard drive partition for data. The default data file paths for each user should all point to this data drive. For example: **`D:\Data\Bob`**. The following data file types should have their file paths changed to point to the data drive:
  - **Log files**
  - **Memory lists**
  - **Calibrations**

- **Default HTS State Path** — The MetaXpress settings file path should also point to the data drive. For example: **`D:\MX\HTSSTATE\`**.

  Note: The MetaXpress settings file is saved to the database by default.

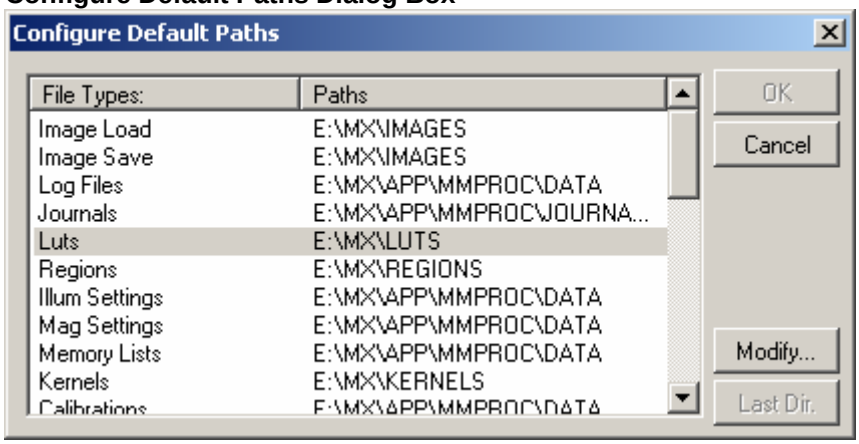
- **Default Assay Path** — This path should point to an assay folder on the root directory of your C drive. For example: **`C:\Assay\`**.

Note: MDC recommends making monthly backups of the Data, HTS State, and Assay files.

Use the following procedure to edit the default data paths for a group:

1. Start MetaXpress.
2. From the Edit menu, select Configure Default Paths. The Configure Default Paths dialog box opens, as shown in Figure 8-4:

Figure 8-4
Configure Default Paths Dialog Box



3. Select the item whose default file path you want to modify.
4. Click *Modify*. The Browse for Folder dialog box opens.
5. Select the folder that you want to use for the new default path, or click *New* to create a new folder.
6. After you have created or selected the appropriate folder, click *OK* to return to the Configure Default Paths dialog box.
7. Click *OK* to apply the new default path and close the dialog box.

Appendixes

Appendix A: Recommended Filter Set/FFC Plate Combinations

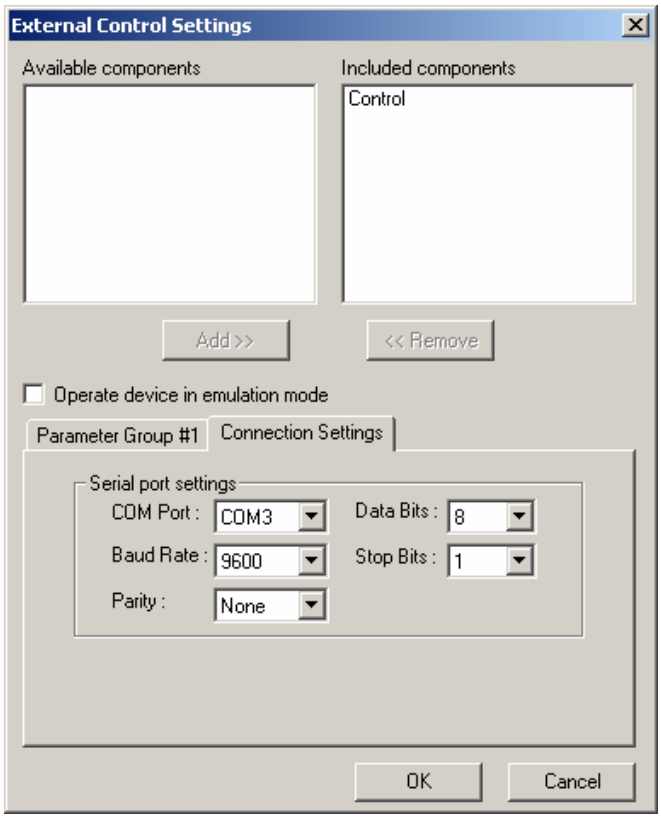
Filter Set	FFC Plate
DAPI	Pink
FITC	Red
TRITC	Red
Cy3	Green
Cy5	Red
CFP	Pink
YFP	Red
Texas Red	Red
GFP	Red
Fura-2 340x (must be installed adjacent to Fura-2 387x)	Pink
Fura-2 387x (must be installed adjacent to Fura-2 340x)	Pink

Appendix B: Verifying External Control Settings

If you have a robot attached to the MetaXpress system, you need to confirm that the External Control settings in the Meta Imaging Series Administrator are enabled and the correct COM port is selected.

Use the following procedure to confirm that the External Control settings in the Meta Imaging Series Administrator are enabled:

1. From the Windows Start menu, go to Programs>MetaXpress> Meta Imaging Series Administrator. The Meta Imaging Series Administrator program opens.
2. Select MetaXpress from the *List of Groups* field.
3. Click *Configure Hardware*. The *Configure Hardware* dialog box opens.
4. Click *Install System Devices*. The *Install Systems Devices* dialog box opens.
5. Ensure that External Control is listed in the *Installed Devices* list. If it is not, Select it from the *Available Hardware* list and click *Install>>*.
6. Select *External Control* from the *Installed Devices* list and click *Settings*. The External Control Settings dialog box opens.
7. Click the *Connections Settings* tab to display the Connection Settings, as show below:



8. Ensure that the correct *COM Port* setting is selected. If the COM port setting is not correct, select the correct port and click *OK* to close the External Control Settings dialog box. If you do not know what the COM port setting should be, contact your MDC representative.
9. Click *Apply*, then *OK* to close the Install System Devices dialog box.
10. Click *Configure Devices* in the Configure Hardware dialog box. The *User Settings for 'Default' hardware configuration* dialog box opens.
11. Ensure that *External Control* is listed in the Claimed Devices list. If it is not, select it from the *Available Devices* list and click *Add>>*.
12. Click *Apply*, then *OK* to close the *User Settings for 'Default' hardware configuration* dialog box.
13. Click *OK* to close the Configure Hardware dialog box, then click *OK* to exit the Meta Imaging Series Administrator program.

Appendix C: Adjusting spherical aberration correction collar on ELWD objectives

The ELWD (extra long working distance) Nikon objectives that can be supplied with the ImageXpress^{MICRO} have adjustable correction collars that are used to minimize spherical aberration in images. The collars have a range of 0–2 mm correction, and changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution is largely dependent on properly setting these collars. Note that

certain other objectives (for example, the 40X S Fluor or the 60X Plan Fluor) may have correction collars as well, and you can follow the same procedures described below.

The setting to be used depends on the thickness of the microplate well or slide on which the specimen is mounted. In general, the correction collar should be set for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined by:

- Obtaining the plate specifications from the plate manufacturer.
- Smashing a spare plate and using calipers to measure the thickness.
- Measuring the optical thickness with the laser autofocus and multiplying it by the refractive index (1.59 for polystyrene; 1.52 for glass).

Once you have determined the thickness of your plate or slide, follow these steps to adjust a given correction collar:

1. Use the software to move the objective to an accessible position.
2. Close the MetaXpress software and power off the instrument.
3. Follow the appropriate instructions for safely opening the access panels on the instrument.
4. Locate the correction collar on the objective that you want to adjust. Note the graduated scale on the barrel and its current setting. You may have to use a flashlight to view the markings.
5. Rotate the correction collar to its new setting.
6. Securely close the access doors.
7. Power on the instrument and start up the MetaXpress software.
8. Adjust your focus position and/or focus settings as necessary.

Test the correction collar setting by examining the image quality of acquired images. If the quality has degraded, re-adjust the correction collar.

Appendix D: Windows File Privileges Needed for MetaXpress

This appendix describes which directories must be accessible to MetaXpress administrators and users.

Notes:

- The following assumes that MetaXpress has been installed under C:\MX (the default location).
- There are no restrictions as to where the software is installed. There is nothing that prevents you from installing the software to the path: C:\Program Files\MX.

Software Administrator

Read/Write Access is required for everything under the C:\MX tree. The Software Administrator also needs to create and periodically modify shading correction images on the C:\root directory. Because this is a possible security violation, this operation could be accomplished by the System Administrator so that the Software Administrator is not given write access to the root directory.

Standard Users

Read Only access needed:

C:\MX
C:\MX\app\mmproc
C:\MX\app\mmproc\Dropins
C:\MX\Help\
C:\MX\Help\ - all subdirectories -
C:\MX\Groups\

If the system is set up for multiple users
C:\MX\Groups\MetaXpress
C:\MX\Groups\MetaXpress\Users

If the system is an acquisition computer
C:\MX\Hardware\
C:\MX\Hardware\ - all subdirectories -
C:\MX\Plates\
C:\MX\Vinput\ - all subdirectories-

Read Write Access needed:

C:\Assay
C:\Backup
If the system is set up for a single user:
C:\MX\Groups\MetaXpress
If the system is set up for multiple users
C:\MX\Groups\MetaXpress\Users*Individual user*