ImageXpress® Micro
Widefield High Content Screening System

User Guide

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Molecular Devices

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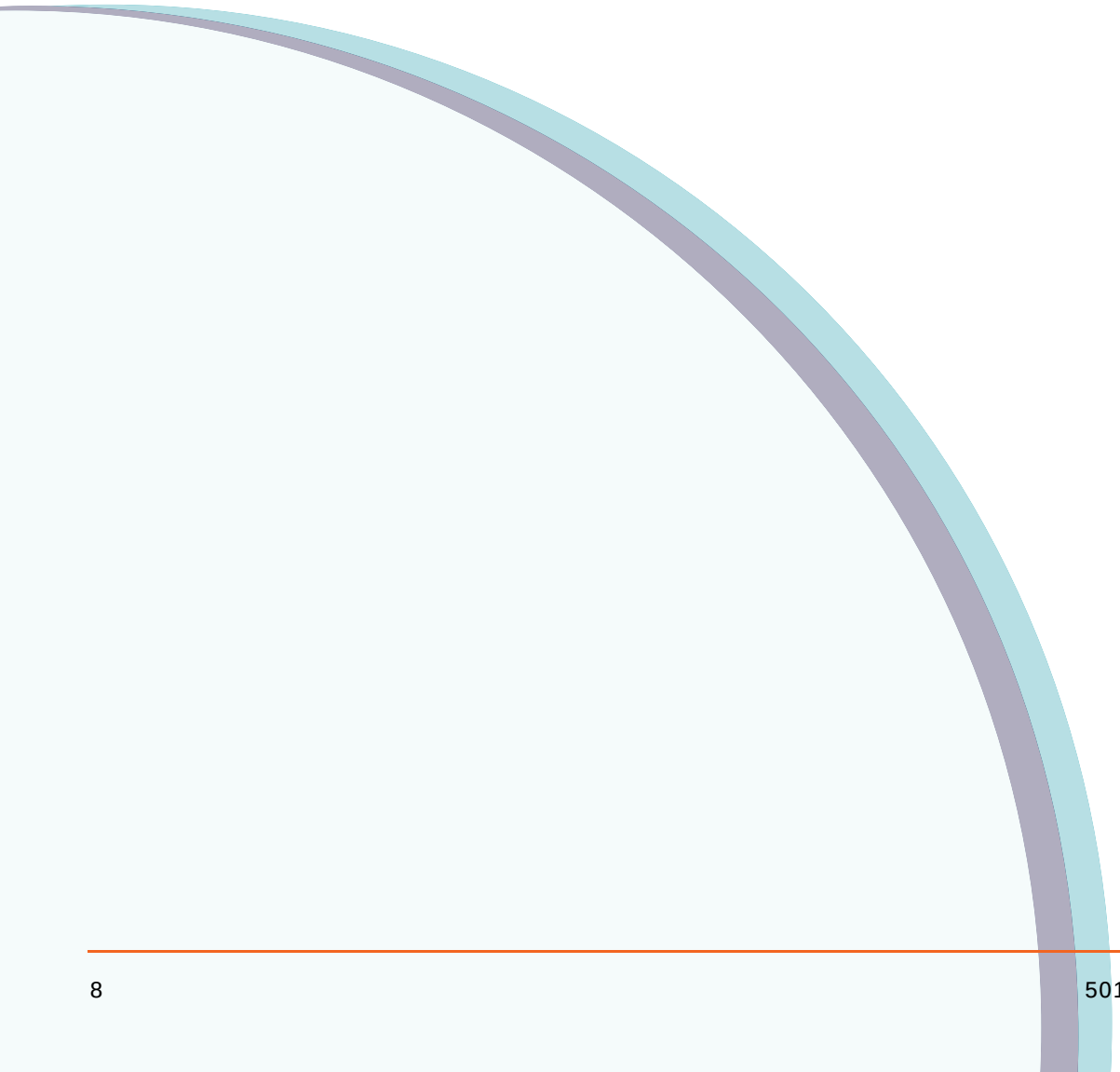
Contents



Safety

The operator of the ImageXpress® Micro System is assumed to be trained in the correct operation of the instrument and the safety issues. Throughout the *ImageXpress® Micro Widefield High Content Screening System User Guide*, the word “you” refers to this trained operator. Using controls, making adjustments, or performing procedures other than those specified in this guide can result in hazardous exposure to laser light, high voltage, hot surfaces, or moving parts. Exposure to these hazards can cause severe or fatal injury.

Safety Text Used in this Guide

Make sure you follow the precautionary statements presented in this guide.

WARNING! Indicates a possibility of severe or fatal injury to the user or other persons if the precautions are not observed.

CAUTION! Indicates that damage to the instrument, loss of data, or individual injury could occur if the user fails to comply with the advice given.

Note: Highlights information that is critical for optimal performance of the system or identifies items of general interest.

The Protective Housing

The protective outer housing and instrument panel interlocks are designed to protect you from exposure to laser light, hot surfaces, or moving parts.

WARNING! Do not defeat the interlocks, open the protective housing, or try to gain access to the interior of the instrument through any other openings, unless specifically instructed by one of the user procedures outlined in this user guide. Read each procedure carefully and follow all outlined safety precautions. Incorrectly opening the outer protective housing can damage the instrument components and result in hazardous exposure to laser light, hot surfaces, or moving parts.

Interlock Failure

There are three safety interlocks on the automated door. Do not operate this instrument with the door open.

WARNING! Do not disable any of the interlocks. When the automated door is opened, the interlocks trigger both the laser light source and the motion control electronics to turn off to prevent hazards associated with laser emission or moving parts.

If you experience any of the following symptoms, you might have interlock failure:

- The focusing laser stays on after the automated door is opened.
- The sample stage or filter mechanisms continue to move after the automated door is opened.

If this is the case, it is unsafe to continue using the ImageXpress Micro System. Please contact Technical Support immediately (see Obtaining Support on page 34).

Non-Interlocked Panels

There are several other panels on the instrument that are intended for use by field service personnel and are not interlocked. All non-interlocked service panels are secured to the protective housing using screws and require a special tool to remove.

WARNING! Make sure the instrument is powered OFF and power cable unplugged in the event you are instructed to remove non-interlocked panels. Absolutely do not operate or access the interior of this instrument with any covers or panels removed.

Laser Safety

The ImageXpress Micro System is a Class 1 LASER product. Contained within the instrument is an embedded Class 3b high power LASER (used for autofocus), which the user cannot and should not attempt to access. The classification and power of the embedded LASER are as follows:

Table S-1 Embedded laser classification and power

Wavelength	Power	Duration	Embedded Laser's Class
690 nm	20 mW	Continuous	Class 3b

Safety interlocks within the instrument allow samples to be loaded and unloaded without exposure to LASER radiation. If the top panel is removed for service, the LASER beam remains safely contained within the optical system until it passes through the microscope objective, which diverges the beam and renders incident power levels below Class 1 (1 mW/cm^2).

WARNING! Do not attempt to repair or adjust the LASER. Deliberate removal of the top panel, safety interlocks, and microscope objective while staring into the LASER beam can cause severe eye injury and blindness.

Lamp Safety

The ImageXpress Micro System is equipped with an external light source. The light source for the Standard model of the ImageXpress Micro System is equipped with a 300 W Xenon lamp. These lamps have a limited lifetime of approximately 500 hours and will need to be replaced upon failure (see Maintaining the ImageXpress Micro Instrument on page 153).

WARNING! In the event that a lamp requires replacement, ensure that you have allowed the lamp to cool for at least 30 minutes. The lamp generates an extreme amount of heat and attempting to remove the lamp immediately after use can result in injury.

Note: The XL model of the ImageXpress Micro System is equipped with an external solid-state light source that has a rated lifetime of more than 15000 hours. There are no user-replaceable parts in this light source.

Liquid Light Guide

The ImageXpress Micro System uses a liquid light guide between the external light source and the instrument. The light source generates a very bright light. In addition, the Standard model of the ImageXpress Micro System uses a high-powered Xenon lamp. The infrared and ultraviolet radiation generated by these lamps can cause significant skin burns and eye damage.

WARNING! Do not remove the light guide from the instrument or the light source when the lamp is powered on.

High-Voltage Hazard

There are no high-voltage electronics found inside the ImageXpress Micro System. High-ignition voltages do exist inside the external Xenon lamp light-source housing, which can be lethal.

WARNING! Do not operate the light source with the housing open or powered ON.

Moving Parts

The ImageXpress Micro System contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts. The interlocks and protective housing are designed so you cannot access the moving parts during a scan.

WARNING! Do not try to gain access to the interior of the instrument unless specifically instructed to do so. The moving parts inside the instrument can cause injury. Do not operate this instrument with covers or panels removed.

Fuses

In the Standard model of the ImageXpress Micro System, the Xenon light source contains a fuse. In the event of fuse failure, disconnect the power cord from the light source and consult Maintaining the ImageXpress Micro Instrument on page 153 for instruction on how to replace the fuse.

In the XL model of the ImageXpress Micro System, the supplied external power supply for the solid-state light source provides over-current protection for the light source limited to 7.9A maximum. The solid-state light source and power supply contain no user-serviceable parts.

The instrument power supply contains a circuit-breaker switch with a trip point of 18.75 amps.

Power Supply

The ImageXpress Micro System has one cable running from the instrument to the external power supply (input voltage range is from 100 to 240 VAC, 50/60 Hz, 2 A).

WARNING! Make sure this cable is unplugged before accessing any part of the instrument. Failure to do so can result in serious harm.

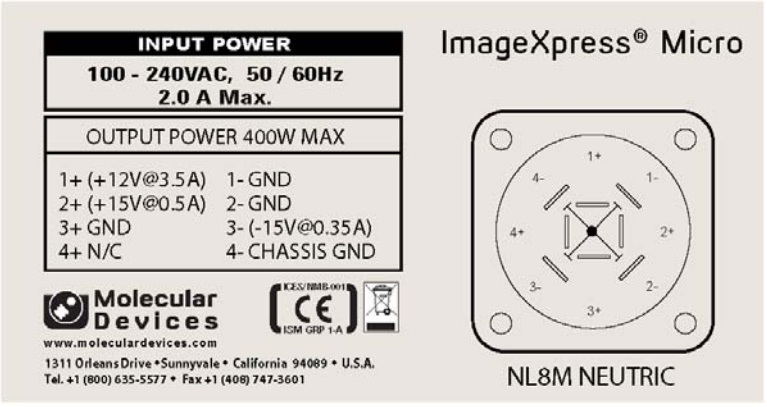


Figure S-1 Power supply label

Lifting Hazard

WARNING! The ImageXpress Micro System weighs approximately 180 lbs (82 kg). Do not attempt to lift or move the instrument without assistance. Moving your instrument can disrupt sensitive optical alignments. Molecular Devices recommends that you contact Technical Support to schedule a Field Service engineer to help with moving your instrument. Your warranty or service contract will not cover problems caused during or as a result of shipment or relocation.

Maintenance and Service

User service and maintenance is strictly limited to the procedures outlined in this user guide. Access for the majority of these procedures is through the interlocked panels described in The Protective Housing on page 10. No other user service that is not outlined within this user guide is permitted. If there is a problem or you have questions, please contact Technical Support.

List of Controls

This guide constitutes a list of controls.

WARNING! Use of controls, adjustments, or performance of procedures other than those specified within this user guide can result in hazardous conditions or injury.

Hazardous Material Precautions

Use standard laboratory procedures and cautions when working with chemicals.

WARNING! Always follow the manufacturer's precautions when working with chemicals. Molecular Devices is not responsible or liable for any damages caused by or as a consequence of the use of any hazardous material.

Safety Label

If the label becomes illegible or is missing for any reason, please contact Technical Support for a free replacement label. While waiting for a replacement label, copy the label below and attach a copy of the label to the instrument.

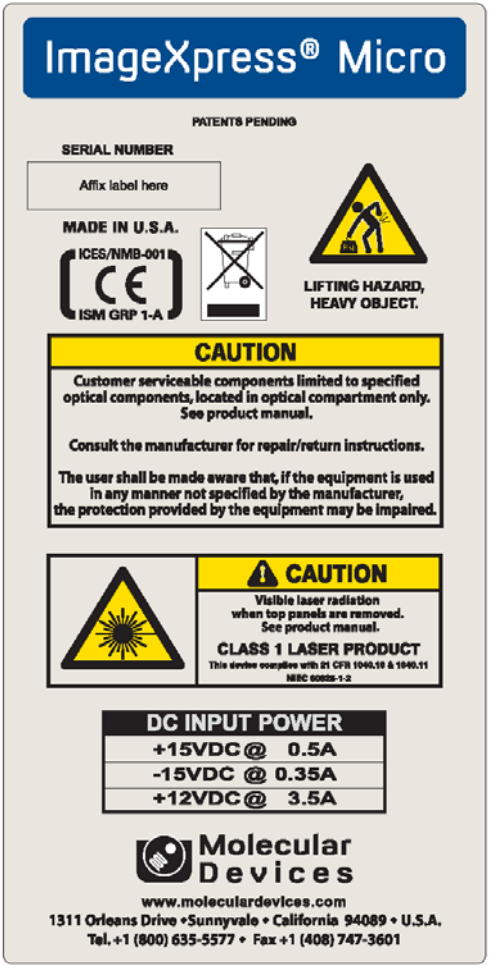


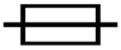
Figure S-2 Safety label

Symbol Explanations

Table S-2 Symbols

Symbols	Indication
	Indicates a potential pinch hazard location
	Indicates that the product contains a laser radiation source
	Indicates that product documentation needs to be consulted
	Indicates heavy object, lifting hazard
	Indicates Alternating Current
	Indicates Direct Current
	Indicates power on
	Indicates power off
	Indicates the location of the Protective Earth Ground Terminal
	Indicates that you must not discard this electrical/electronic product in domestic household waste

Table S-2 Symbols (cont'd)

Symbols	Indication
	Indicates fuse

1 Introduction

Overview

The ImageXpress® Micro Widefield High Content Screening System from Molecular Devices is an integrated cellular imaging and analysis system that is designed for rapid, automated screening of fluorescently labeled biological samples in microplates.

The core hardware component of the imaging system is a custom-designed, fully automated, epi-illumination fluorescence microscope, with rapid autofocus and precision sample movement features that allow large numbers of high-resolution images to be acquired in the shortest possible time. All key optical and mechanical elements are motorized, with asynchronous command execution, thereby allowing complete real-time control of the instrument configuration through the MetaXpress® High Content Image Acquisition and Analysis Software interface.

When used in combination with the powerful imaging capabilities of the MetaXpress Software, the instrument becomes an extremely flexible and programmable device, ideally suited for user-defined, high-speed automated assays.

Key components of the instrument include the following:

- External light source connected by a liquid light guide
- User-specified camera
  - A cooled CCD camera in the standard model
  - A scientific CMOS camera in the XL model
- Fast laser autofocus system with precision motorized Z (focus) stage
- Image-based autofocus
- Precision motorized X-Y (sample) stage
- High-quality user-changeable Nikon objectives in a four-position linear selector
- User-changeable filter cubes in a five-position slider
- Selectable binning modes to decrease exposure time and increase throughput
- Motorized selection of stage position, filter cubes, and objectives with asynchronous operation
- High-transmission fluorescence imaging optics with world-class chromatic aberration correction, resolution, and image flatness
- Operation and configuration by integrated MetaXpress Software
- Plate-handling robot (optional) with automated barcode reader

ImageXpress Micro Instrument

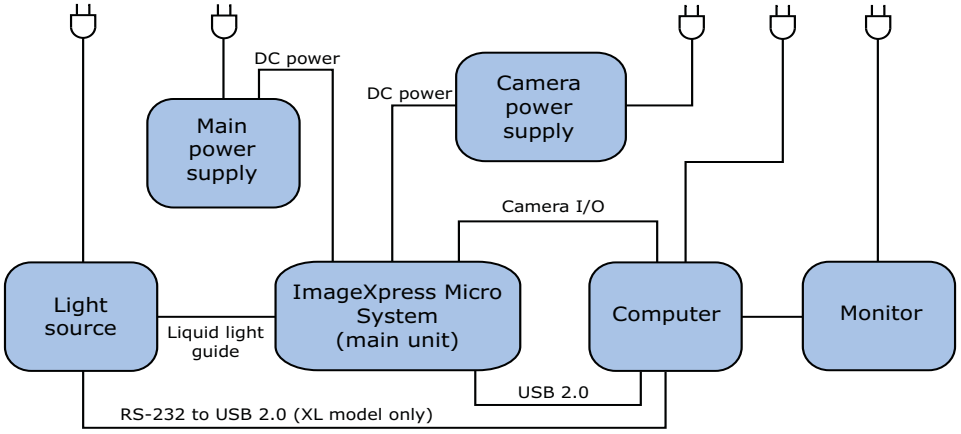


Figure 1-1 ImageXpress Micro System Components

Illumination System: Excitation

Light Source

The ImageXpress Micro System has an external light source, which provides continuous, high-intensity broadband illumination.

- In the Standard model of the ImageXpress Micro System, the 300 W, long-life Xenon lamp has an expected lifetime of more than 500 hours.
- In the XL model of the ImageXpress Micro System, the solid-state light source has an expected lifetime of more than 15000 hours.

Cold Mirror

In the Standard model of the ImageXpress Micro System, the Xenon lamp light source incorporates a cold mirror which prevents light with wavelengths longer than 675 nm from reaching the sample. Limiting the wavelengths of the light source minimizes sample heating and stress on optical components.

Note: In the XL model of the ImageXpress Micro System, the solid-state light source is limited to between 380 nm and 680 nm.

Light Guide

A liquid light guide couples the light from the light source to the illumination optics in the ImageXpress Micro System unit. The light source assembly is precisely aligned with the light guide during manufacture, and requires no further position adjustment.

Illumination Optics

The output end of the liquid light guide is imaged onto the sample by a set of internal optics and the objective, providing bright and uniform illumination of the specimen over a wide field of view. This constitutes an Abbé illumination system (also called critical illumination).

Shutter (standard model only)

A solenoid-activated mechanical shutter controls the exposure of the sample to excitation light to minimize sample degradation and photobleaching.

Filter Cube Changer

The 5-position filter cube changer takes standard Nikon TE2000 filter cubes. The ImageXpress Micro System uses Semrock filters.

Objective (Z) Stage

Motorized Z Stage

The Z stage is controlled by a linear encoder and has better than 100 nm resolution.

Objectives

Standard objectives are Nikon CFI60 series. The selected objective lens focuses excitation light onto the sample, and collects fluorescence light emitted by the sample. Molecular Devices offers a wide range of objectives to suit your experimental needs.

Motorized Objective Changer

There is a 4-position objective changer. Only the selected objective moves up and down in Z.

Sample (X-Y) Stage

Sample

The plate holder is designed for scanning multi-well plastic and glass bottom microplates in standard ANSI (SBS) formats, but can accommodate other plate formats that have standard microplate footprint dimensions. For example, glass slides can be imaged using a slide adapter included in the tool kit. Optimum image quality depends on plate flatness and optical clarity.

Plate Holder and Plate Clamp

A spring-loaded mechanical clamp holds the sample plate securely in the plate holder. The clamp automatically opens when the X-Y stage moves to the load/unload position, and automatically closes when the X-Y stage moves the plate into position for imaging.

Motorized X-Y Stage

The X-Y stage is controlled by a linear encoder and has better than 100 nm resolution.

Autofocus Laser (optional)

A red (690 nm) diode laser projects a laser spot onto the sample. Reflections of this spot from the bottom of the microplate and the plate-sample interface are imaged by a dedicated, fast-focus sensor, and are used as a reference for focusing using the autofocus feature of the MetaXpress Software.

Imaging System: Emission

Tube Lens

The tube lens collects collimated light from the objective and focuses it onto the detector plane of the camera. The emission wavelength range is 400 nm to 750 nm.

Camera

The Standard model of the ImageXpress Micro System is shipped with a cooled CCD camera. This camera has a 1392 × 1040 pixel resolution (6.45 × 6.45 µm pixel size), and has a peak quantum efficiency greater than 60% at 550 nm.

The XL model of the ImageXpress Micro System is shipped with a scientific CMOS camera. This camera has a 2560 × 2160 image sensor format (6.5 × 6.5 µm pixel size), and has a peak quantum efficiency of 60% at 550 nm. The center 2160 x 2160 pixels are extracted during plate acquisition to ensure adequate illumination uniformity.

Electronics

The ImageXpress Micro instrument also contains the following components:

- External power supply and cable.
- USB 2.0 port and cable to computer for device control.
- Camera cabling to camera board in computer.

MetaXpress Software

Use the MetaXpress® Software with the ImageXpress Micro System to select a standard image analysis routine or to develop a custom protocol to fit your specific acquisition and analysis needs. The MetaXpress Software workflow is divided into two major parts: acquisition and analysis.

- The acquisition workflow involves configuring settings, acquiring images, and storing plate data in a database. See Preparing For Acquisition on page 61, Setting Up Plate Acquisition on page 67, and Acquiring Plates on page 129.
- The analysis workflow consists of processing, enhancing, and analyzing acquired plate data. See the *MetaXpress High Content Image Acquisition and Analysis Software Analysis Guide* included on the MetaXpress Software installation USB flash drive.

Administrator Tasks

Most of the procedures in this document are for the general users. However, Molecular Devices recommends that 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 Software system administrator tasks include:

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

Basic Operational Theory

Fluorescence Imaging

Fluorescence is a phenomenon observed in certain species of molecules (fluorochromes, or dyes) in which photons of a specific wavelength are absorbed (excitation), and as a result a very short time later photons are emitted at a longer wavelength (emission). The utility of fluorescence imaging in biological applications stems from the ability to conjugate fluorescent molecules with biologically active probe molecules, so that application of the combined dye/probe molecule (fluorophore) to the specimen highlights the specific substances or regions to which the probe is targeted.

Further, by attaching different probes to a set of dye molecules with non-overlapping excitation and emission spectra, one can stain a specimen with multiple fluorophores, and either simultaneously or sequentially image different structures or substances within the same specimen. The absorption and emission peaks for each dye in a given environment are physical characteristics of that molecule, and their specific properties determine the initial selection of the optical components to be used: emission and excitation filters, and dichroic mirror.

Excitation and Emission Filters

In the ImageXpress Micro System, the excitation and emission filters are located in a filter cube.

To selectively excite one fluorophore more intensely than another, or to minimize excitation channel crosstalk, it is necessary to provide illumination containing only photons with a wavelength range matched to the target dye's absorbance (excitation) spectrum. If the primary illumination source provided is broadband, such as in the ImageXpress Micro System, a bandpass filter in the illumination optical path (called the excitation filter, since it filters the excitation light) is used to restrict the illumination spectrum to a narrow range of wavelengths.

Similarly, when imaging the illuminated sample, it is desirable to collect only the emission photons from the target fluorophore, rejecting as much as possible any reflected or scattered excitation light, any light from other dyes, and autofluorescence from the sample and substrate. This is accomplished by placing a filter in the collection light path, called the emission filter. Emission filters can either be of the bandpass variety, for maximum specificity, or longpass, to maximize the amount of emission light collected.

Dichroic Mirror

In the ImageXpress Micro System the dichroic mirror is in a filter cube.

A dichroic mirror is a specially designed beam splitter that transmits light above a certain cutoff wavelength, and reflects light at shorter wavelengths. This is the essential component that allows the construction of an epi-illumination fluorescence imaging system such as ImageXpress Micro System, in which the illumination and imaging optical paths overlap at the objective lens. That is, the same objective lens is used to focus the illumination light onto the sample as well as collect the emitted fluorescent light to form the image.

In the illumination path, the dichroic mirror reflects shorter wavelengths from the light source up through the objective onto the specimen, as shown in Figure 1-2.

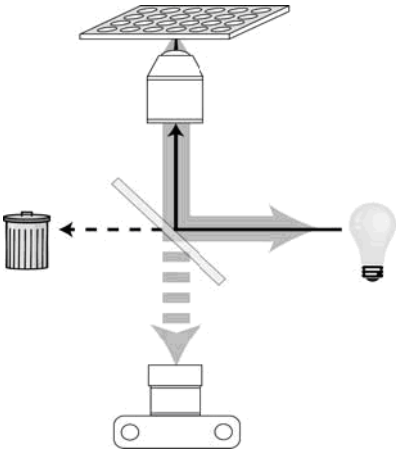


Figure 1-2 Dichroic mirror creates two light pathways

Incident light from the illumination source that is longer wavelength than the cutoff is transmitted to a beam dump that absorbs and diffuses the waste light to prevent it from entering the imaging optical path.

In the imaging optical path, longer wavelength fluorescence light emitted by the excited fluorophores in the specimen is collected by the objective lens, and transmitted by the dichroic through to the tube lens and camera. Incident light from the sample that is shorter wavelength than the cutoff (mostly reflected illumination light from the sample) is reflected by the dichroic (and further blocked by the emission filter), and is therefore prevented from entering the imaging system of tube lens and camera.

Dichroic mirrors are interference filters made by depositing a number of thin film coatings on a glass support. Dichroics need to be kept thin for high image quality, so the supporting glass is quite fragile, and generally the film coating is not protected with a cover glass. This means that unprotected dichroics are delicate and easily damaged components, and so care must be taken when handling them.

Dichroic Transmission Spectrum

An ideal dichroic mirror would have an infinitely sharp cut-off: that is, it would have unity transmittance coefficient at wavelengths longer than the cut-off, and zero transmittance (and therefore unity reflectance in a non-absorbing dichroic mirror) shorter wavelengths. In practice, the characteristic transmission spectrum for a dichroic looks something like Figure 1-3.

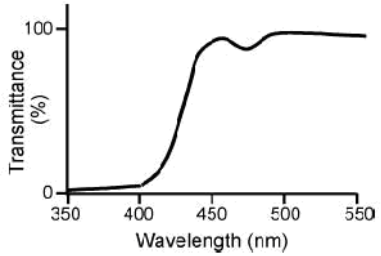


Figure 1-3 Example of a transmission spectrum of a dichroic

In principle, the cutoff wavelength (or midpoint of the cutoff region) of the dichroic should be chosen to lie halfway between the absorption and emission peaks of the chosen fluorochrome, as this will simultaneously maximize the amount of excitation light available at the sample, and also the amount of collected fluorescence emission that is transmitted to the camera. In practice, however, additional considerations such as fluorochrome efficiency can dictate that the cutoff region is biased towards one peak or the other-allowing, for example, greater transmission of longer wavelength image photons at the expense of less reflection of shorter wavelength excitation light.

Objective Lenses

The ImageXpress Micro System can be configured with any of the high quality Nikon objectives listed in Appendix C: Objectives Compatible with the ImageXpress Micro System on page 189.

If the objective you want to use is not listed, please contact us to verify compatibility with the ImageXpress Micro System.

Note that the extra-long working distance (ELWD) objectives have adjustable spherical-aberration correction collars for imaging through thick substrates such as most microplates. Please see Maintaining the ImageXpress Micro Instrument on page 153 for details on how to calculate and set their correct values.

Several of the other objectives (such as, CFI SUPER FLUOR 40X) also have correction collars for adjustment according to the thickness of the glass cover slip being used. Setting of these collars should be done according to the cover slip manufacturer’s specifications or through optimization of image quality.

Objectives are classified here according to optical correction, flatness of field, numerical aperture, and working distance. Before choosing additional objectives to use with your system, it is important to consider the types of plates you will be imaging. The plate material (plastic vs. glass) and thickness are major considerations when choosing an objective. Another important practical note is that generally the greater an objective's correction, the greater the number of lens elements it contains, with correspondingly reduced light transmission, especially in the UV spectrum. In particular, apochromatic (“Apo”) objectives tend to have poor UV transmission characteristics.

For detailed information on objectives, please see the Nikon website (www.nikon.com).

Getting Started

2

This chapter provides a quick overview of the start-to-finish workflow for using the ImageXpress® Micro System. The following topics are included in this chapter:

Starting the ImageXpress Micro System

This section explains how to safely power up the ImageXpress® Micro instrument and log in to the MetaXpress® Software.

Powering On the Instrument

1. Ensure that the power cords for the instrument and the lamp 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 light source power supply unit.

Note: Turn on this power supply first to minimize electrical pulse interference with the electronic components in your system.

3. Wait for the light source to stabilize before acquiring images.
   - In the Standard model of the ImageXpress Micro System, the Xenon lamp takes 15 to 20 minutes to stabilize.
   - In the XL model of the ImageXpress Micro System, the solid-state light source takes less than a minute to stabilize.
4. Turn on the power switch on the front of the ImageXpress Micro power supply unit.
5. Ensure that the camera power supply is plugged in and, if required, turned on.
6. Turn on the power to the host computer and the monitor.

7. After 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 the Software on page 30.

Starting the Software

To start the MetaXpress Software, complete the following procedure:

Note: This procedure assumes that your ImageXpress Micro System and your MetaXpress Software have been properly installed and configured by your Molecular Devices representative and your System Administrator.

Note: 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 or click **Start > Programs > MetaXpress > MetaXpress**.

The MetaXpress Software title screen appears.

Figure 2-1 MetaXpress Software title screen

Note: This screen appears only when the MetaXpress Software is configured to run in multi-user mode from within the Meta Imaging Series Administrator. If you do not see this screen, the MetaXpress Software is in single-user mode. Continue to Step 3.

2. From the **User Name** field, select the user name to use and then click **OK**.

The MetaXpress Software starts and a progress bar at the bottom of the dialog indicates the loading progress of the program. After the program loads, the Welcome to MetaXpress dialog appears.

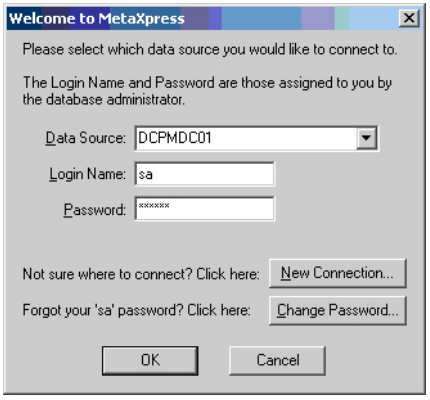


Figure 2-2 Welcome to MetaXpress dialog

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 appears prompting you to select a Group.

Note: For versions 4.0 and 5.0 of the MetaXpress Software, the default User Login Name is **MolDev**, the default System Administrator Login Name is **sa**, and the default password for both is **moldev**. For earlier versions of the software, the default User Login Name and password was **mdc**. You might need to log in using **mdc** if you had an earlier version of the software on your instrument workstation. If needed, you can change the password by clicking **Change Password**.

4. Select one of the groups assigned in the Meta Imaging Series Administrator and click **OK**.

   The MetaXpress Software application starts and initializes 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.

Acquiring Data

The acquisition workflow involves configuring settings, acquiring images, and storing plate data in a database.

For detailed information, see the following chapters:

- Preparing For Acquisition on page 61
- Setting Up Plate Acquisition on page 67
- Acquiring Plates on page 129

Analyzing Data

The analysis workflow consists of processing, enhancing, and analyzing acquired plate data. See the *MetaXpress High Content Image Acquisition and Analysis Software Analysis Guide* included on the MetaXpress Software installation USB flash drive.

Maintaining the Instrument

Specific user-level maintenance can be performed on the ImageXpress Micro System for changing the lamp and fuse, changing filter cubes, Changing and cleaning objectives, and cleaning the instrument as described in Maintaining the ImageXpress Micro Instrument on page 153.

For all other service and maintenance needs, please contact Molecular Devices. See Obtaining Support on page 34.

Shutting Down the System

1. Exit the MetaXpress Software application.

   The software prompts you to save any open images.
2. Turn off the power to the computer and the monitor.
3. Turn off or unplug the power to the camera power supply.
4. Turn off the power to the light source unit.

Note: Do not power cycle the light source too frequently. It is better to leave the light source on for a short while when it is not being used than to turn it on and off frequently. If the light source is unused for a longer period of time, turn it off.

5. Turn off the power switch on the front of the ImageXpress Micro System power supply unit.

Obtaining Support

Part of effective communication with Molecular Devices is determining the channels of support for the ImageXpress Micro System, including the MetaXpress® High Content Image Acquisition and Analysis Software. Molecular Devices provides a wide range of support:

1. Documentation — Check the manuals that are included on the installation media and the help that is available within the MetaXpress Software. Help for an active dialog box can be accessed by pressing the [F1] key.
2. Online knowledge base — The knowledge base has links to technical notes, software upgrades, newsletters, manuals, and other resources. Visit the Molecular Devices Support web page at www.moleculardevices.com/support and follow the links to the knowledge base.
3. Technical Support —

   Phone: Contact Technical Support at (800)-635-5577 (U.S. only) or +1 408-747-1700.

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

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

   - To find your system ID number, from the Help menu, select About MetaXpress. The dialog that appears displays your system ID number.
   - The system serial number is located on your instrument.
4. Additional support resources include:
   - Nikon web-based microscopy course — http://www.microscopyu.com
   - The Molecular Probes handbook — http://www.probes.invitrogen.com offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis.

   The following sites offer filter information:

   - http://www.chroma.com
   - http://www.semrock.com
   - http://www.omegafilters.com

Gathering Support Information

If you need to contact Molecular Devices for support, it is very important to have the following information available to help Technical Support personnel troubleshoot the problem you are experiencing:

- The steps that led up to the occurrence of the problem
- The settings of any dialogs 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, click **Save Settings**, and then select **Save to file rather than database**. The settings file will then be saved to the **C:\MX5\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:\MX5\app\mmproc\journals** folder. If you are running journals through the review plate data tab, then the journals are saved in **c:\analysis** and **c:\assay**.
- **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. For information on how to create a System Information report to email to Molecular Devices, see Creating a System Information Report on page 37.

Logging AxoTrace Messages to a .txt File

AxoTrace tracks the hardware status of the ImageXpress Micro System and logs all the hardware activities of the system. If the error occurs downstream of the initial cause, AxoTrace provides a log that Molecular Devices Technical Support can use to trace the error back to the original cause and pinpoint the problem. Use the procedure below if a reproducible hardware error occurs. Follow the AxoTrace setup procedure and record the steps that lead to the error. If the error results in the instrument not starting up, not responding, or the error cannot be reproduced, contact Molecular Devices Technical Support immediately.

To log AxoTrace messages to a .txt file

1. Open the MetaXpress Software and **AxoTrace** starts automatically.

   The AxoTrace icon appears in the toolbar.

Figure 2-3 AxoTrace icon in toolbar

2. Close the MetaXpress Software.
3. Double-click the **AxoTrace** icon in the toolbar.
4. In the **AxoTrace** window, click **Options > Output to File** and make sure that the **Time prefix** option is selected.

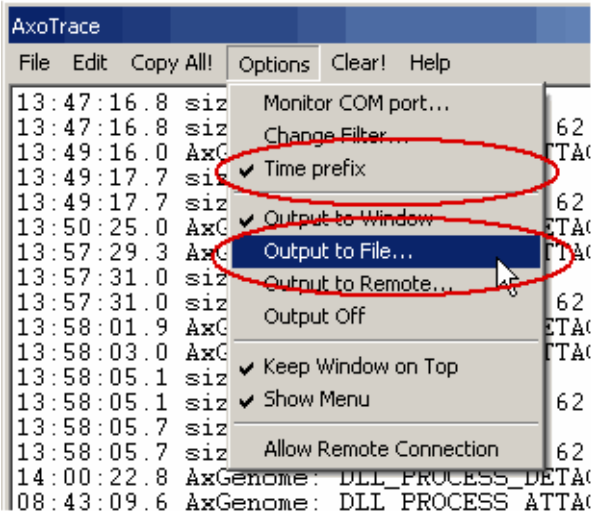


Figure 2-4 AxoTrace Options menu

5. Click **File > Save As**.
6. In the **Save As** dialog that appears, in the **Name** field, navigate to the location where you want to save the log file, type the file name (for example, axotrace log.txt) and then click **Save** to save the log file.
7. Close the **AxoTrace** dialog and then start the MetaXpress Software.

8. Turn off the ImageXpress Micro System and then turn it back on. The light source can remain on during this step.
9. Reproduce the error and after the error is reproduced, close the MetaXpress Software.
10. Send the AxoTrace log as an e-mail attachment to Molecular Devices Technical Support.

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 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. After the report is in the form of a PDF file or a text file, you can send this report to appropriate Molecular Devices support personnel as an email attachment. Figure 2-5 shows the About MetaXpress Software dialog.

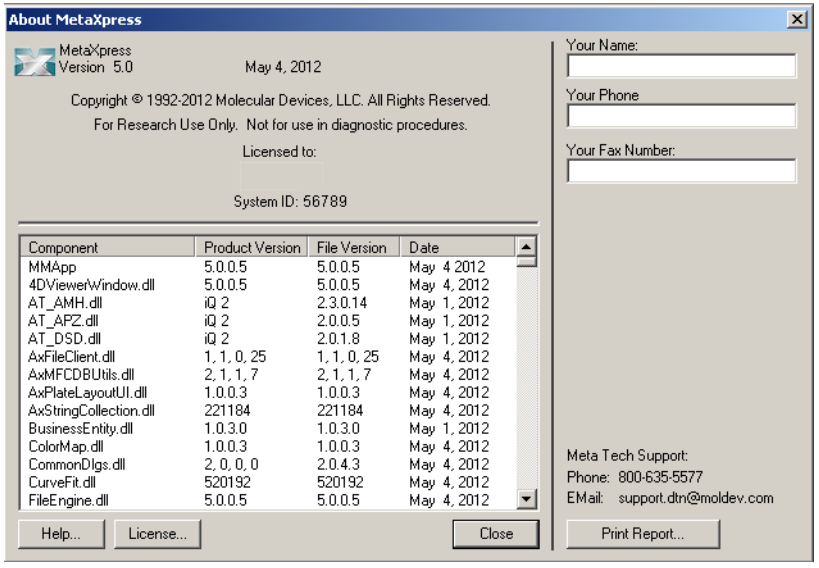
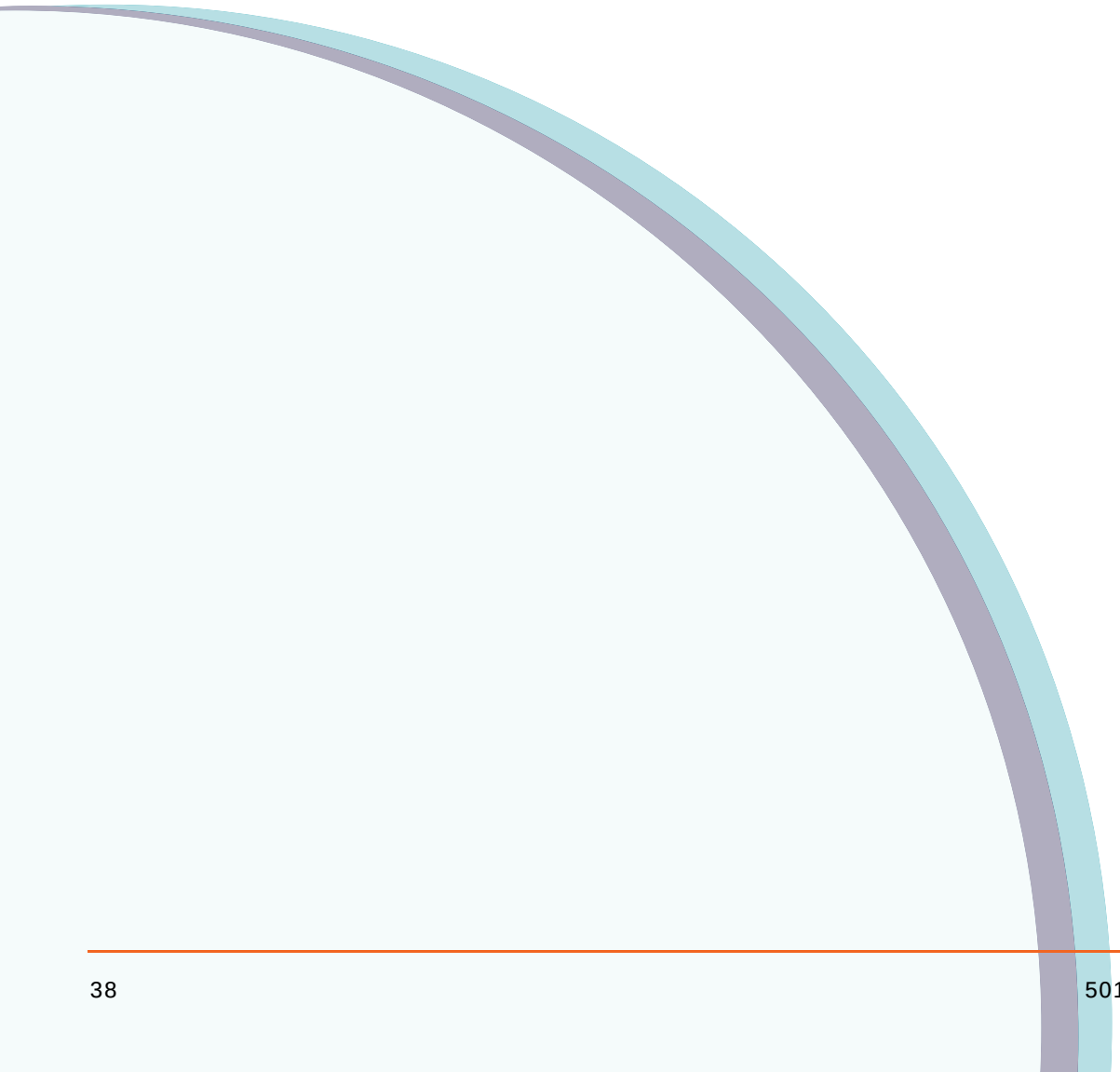


Figure 2-5 About MetaXpress Software



System Installation and Testing

3

System Installation

The ImageXpress® Micro System is shipped fully configured, and is installed at your site by a Molecular Devices field service engineer. The base system includes the imaging unit, host computer, and toolkit that includes a slide holder, three calibration slides, hex wrenches for changing filter cubes, and a TetraSpeck bead plate.

The ImageXpress Micro System host computer is shipped with the MetaXpress Software already installed, and the instrument is connected to the host computer during installation. There are four main connections, excluding power cords:

- Power supply to the ImageXpress Micro System
- Light guide from the external lamp to the ImageXpress Micro System
- USB 2.0 data cable from the ImageXpress Micro System to the host computer
- Cable from the camera to the computer
- Camera power supply cable to the ImageXpress Micro System.

If you need to install or re-install the MetaXpress Software on a new computer, please see the *MetaXpress High Content Image Acquisition and Analysis Software Installation Guide* included on the MetaXpress Software installation USB flash drive, or contact Technical Support.

Verifying Device Settings in the Meta Imaging Series Administrator

This procedure ensures that the ImageXpress Micro System hardware components are properly configured in the Meta Imaging Series Administrator application and the MetaXpress Software application. All hardware and software configuration settings are implemented in the Meta Imaging Series Administrator application.

Note: For additional information about any of the dialogs in the Meta Imaging Series Administrator, press the **F1** key to access the application help for the active dialog. The Meta Imaging Series Administrator and the MetaXpress Software application cannot be run simultaneously.

To check the hardware configuration in the Meta Imaging Series Administrator application, complete the following procedure:

1. Use the procedure described in Powering On the Instrument on page 29, to power up the system, but do not start the MetaXpress Software application.
2. From the Windows Start menu, click **All Programs > MetaXpress > Meta Imaging Series Administrator**.

   The Meta Imaging Series Administrator program opens.
3. Select **MetaXpress** from the **List of Groups** field.

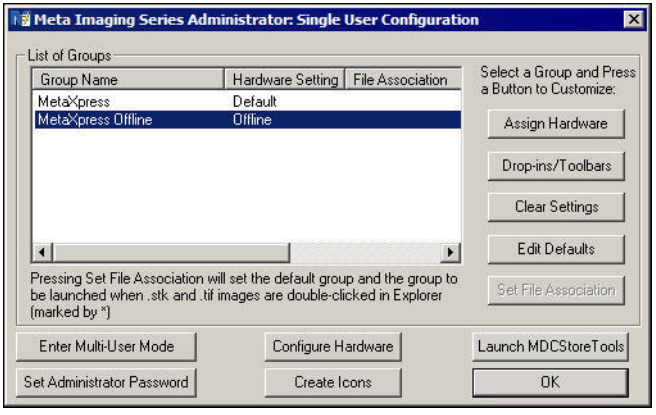


Figure 3-1 Meta Imaging Series Administrator Program

4. Click **Configure Hardware**.
   The Configure Hardware dialog appears.
5. Click **Configure Devices**.
   The User Settings hardware configuration dialog appears.

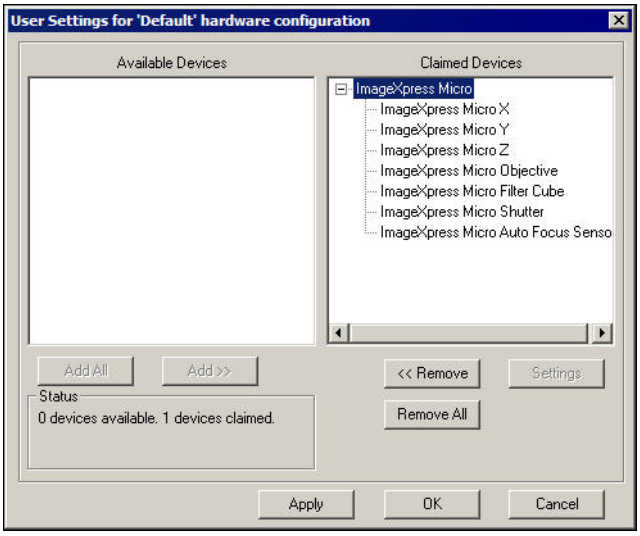


Figure 3-2 User Settings hardware configuration dialog

6. Select **ImageXpress Micro X** from the **Claimed Devices** list and then click **Settings**.
   The ImageXpress Micro X Settings dialog appears.
7. Increase the step size to **10,000 µm**.

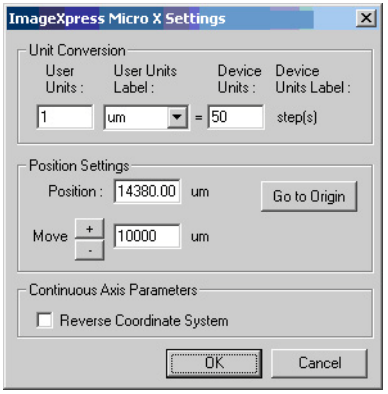


Figure 3-3 ImageXpress Micro X Settings dialog

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 µm** and click **OK**.
11. Select **ImageXpress Micro Y** from the **Claimed Devices** list and click **Settings**.

    The ImageXpress Micro Y Settings dialog appears.

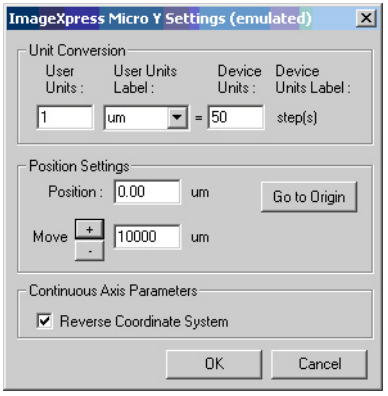


Figure 3-4 ImageXpress Micro Y Settings dialog

12. Increase the step size to **10,000 µm**.
13. Ensure that **Reverse Coordinate System** check box is selected.
14. Click the icon and confirm that the stage responds to the control.
15. Change the step size back to **10 µm** and click **OK**.
16. Select **ImageXpress Z** from the **Claimed Devices** list.
17. Click **Settings**.

    The ImageXpress Z Settings dialog appears.
18. Verify that the value in the **Device Units** field is **50**.
19. Increase the step size to **1000 µm**.
20. Click the icon and confirm that the Z Motor responds to the control.
21. Change the step size back to **10 µm** and click **OK**.
22. Select **ImageXpress Objective** from the **Claimed Devices** list and click **Settings**.

    The ImageXpress Objective Settings dialog appears.

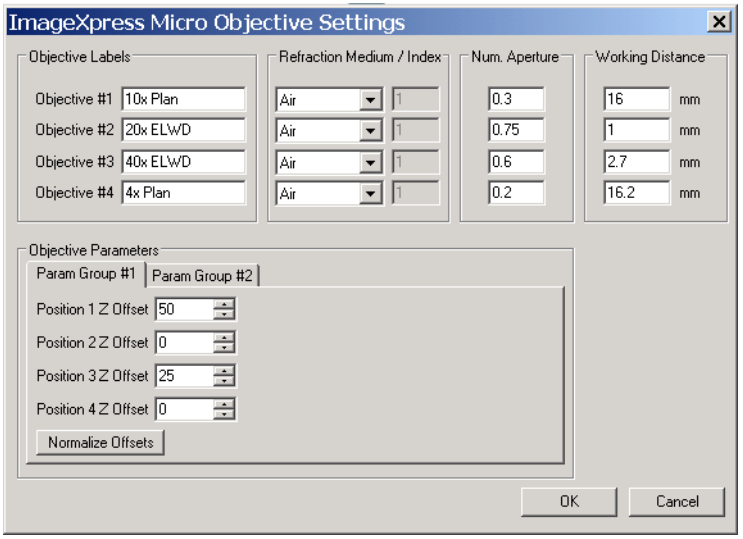


Figure 3-5 ImageXpress Objective Settings dialog

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 **Objective Parameters** section on the bottom half of the dialog. 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, see Configuring Parfocality after Changing Objectives on page 165.

25. Click **Param Group #2**, then click **Open Control Dialog**.

    The Control – ImageXpress Micro Objective dialog appears.
26. Click the arrow buttons and confirm that the objective changer is moving appropriately.
27. Click **Done**, then click **OK** to close the dialog.
28. Select **ImageXpress Micro Filter Cube** from the **Claimed Devices** list and click **Settings**.

    The ImageXpress Micro Filter Cube Settings dialog appears.

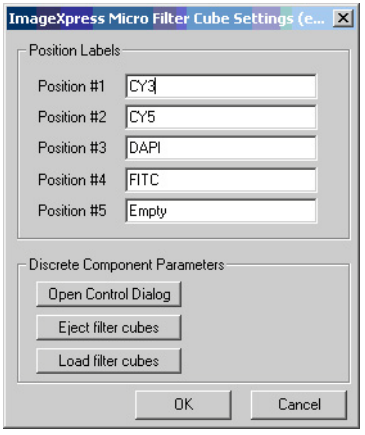


Figure 3-6 ImageXpress Micro Filter Cube Settings dialog

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 appears.
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.
33. Select **ImageXpress Micro Shutter** from the **Claimed Devices** list and click **Settings**.

    The ImageXpress Micro Shutter Settings dialog appears.

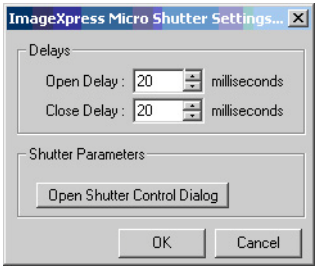


Figure 3-7 ImageXpress Micro Shutter Settings dialog

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 appears.
36. Click **Toggle** to confirm that the shutter is responding.
37. Click **Done**, then click **OK** to close the **ImageXpress Micro Shutter Settings** dialog.

Note: If you have a plate handling robot attached to the ImageXpress Micro System, you should confirm those settings as well. For information, see Verifying External Control Settings on page 197.

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

Verifying Camera Settings in the Meta Imaging Series Administrator

Complete the following procedure to ensure that the correct version of the ImageXpress Micro System camera driver is installed:

1. From the **Configure Hardware** dialog, click **Configure Acquisition**.
2. In the **Configure Acquisition** dialog, ensure that a driver is listed in the **Installed Drivers** column.
3. If a driver is not listed in the **Installed Drivers** column, or if more than one driver is listed, contact Molecular Devices support to determine which driver is required for your system.
4. In the **Installed Drivers** column, select the appropriate driver.
5. Click **Configure** to query the camera.
6. In the camera driver dialog, ensure that the camera is available in channel 1. This confirms that the camera is responsive.
7. If the camera was not queried successfully, ensure that the cabling is correct from the ImageXpress Micro System main unit to the computer and that the camera power supply is plugged in and, if required, turned on.
8. Click **OK** to close the camera driver dialog.
9. Click **OK** to close the **Configure Acquisition** dialog.
10. Click **OK** to close the **Configure Hardware** dialog.
11. Click **OK** to close the Meta Imaging Series Administrator and then continue to the next procedure.

Verifying and Backing Up Settings in the MetaXpress Software Application

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

- Magnification Settings
- Illumination Settings
- Calibration Settings
- Laser Autofocus 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 System objectives before using your system. Complete the following procedure to check the magnification settings in the MetaXpress Software application:

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

1. Open the MetaXpress Software application and log into the database.
2. Select **Configure Magnification** from the **Devices** menu.

   The Configure Magnification dialog appears.

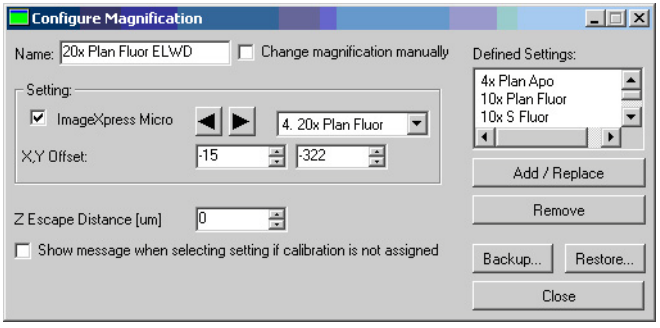


Figure 3-8 Configure Magnification dialog

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 appears.
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.

Verifying Illumination Settings

You will need to confirm illumination settings for the ImageXpress Micro instrument before using your system. Complete the following procedure to check the Illumination settings in the MetaXpress Software application:

1. Open the MetaXpress Software application and log into the database.
2. From the **Devices** menu, select **Configure Illumination**.

   The Configure Illumination dialog appears.
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.

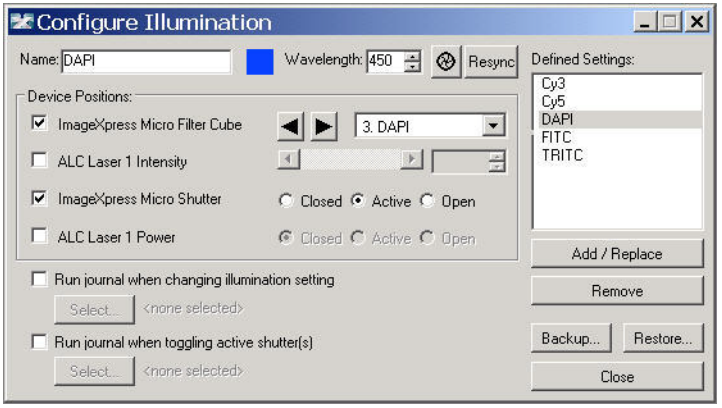


Figure 3-9 Configure Illumination dialog

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.
7. Click **Backup**.

   The Backup All Illumination Settings dialog appears.
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.

Verifying and Backing Up Calibration Settings

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

1. From the **Measure** menu, select **Calibrate Distances**.

   The Calibrate Distances dialog appears.

2. Confirm that there are calibration settings in the dialog that match the objective settings from the **Configure Magnification** dialog.

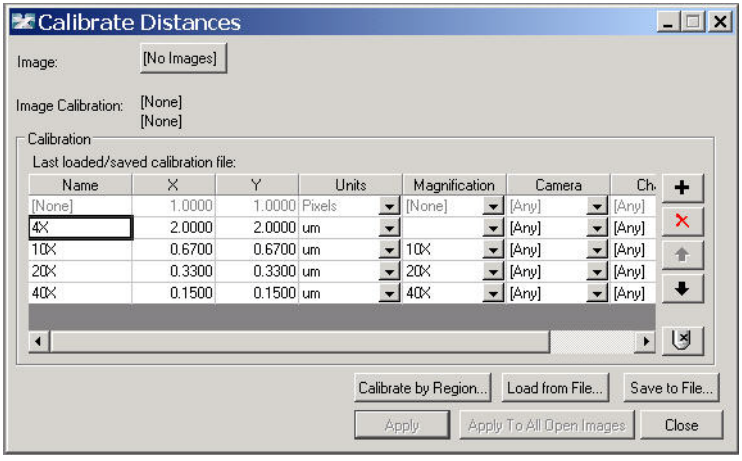


Figure 3-10 Calibrate Distances dialog

3. Click **Save to File**.

   The Save Spatial Calibrations dialog appears.

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.

   Table 3-1 shows estimated values that can be used for ImageXpress Micro System calibration settings.

Table 3-1 Estimated Calibration Settings

Objective	Estimated Calibration
1x	6.50 µm/pixel
2x	3.25 µm/pixel
4x	1.63 µm/pixel

Table 3-1 Estimated Calibration Settings (cont'd)

Objective	Estimated Calibration
10x	0.65 µm/pixel
20x	0.33 µm/pixel
40x	0.16 µm/pixel
60x	0.12 µm/pixel
100x	0.07 µm/pixel

Note: Make sure the appropriate magnification setting is selected for each calibration.

Note: For additional information on creating calibrations settings, refer to the MetaXpress Software online help. Press the F1 key when the Calibrate Distances dialog is open to access its online help. Also refer to the technical note "Spatially calibrating images in MetaMorph" available in the Molecular Devices support knowledge base at www.moleculardevices.com/Support.html.

Verifying the Laser Autofocus Sensor

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

1. Power on the system and open the MetaXpress Software application 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** field.
6. From the **Screening** menu, select **Plate Acquisition and Control**.
7. The Plate Acquisition and Control dialog appears.
8. Click **Eject Plate** to move the stage to the load position.
9. Load the bead plate and click **Load Plate**.
10. Click **Go To A1** to move the stage to the A1 position.
11. On the **Plate Acquisition Setup** dialog, click the **Autofocus** tab to highlight it.
12. Click **Configure Laser Settings**.

    The Configure Laser Autofocus Settings dialog appears.
13. Click **Preview Pass**.

A window opens displaying a graph of focus intensities vs. Z-position.

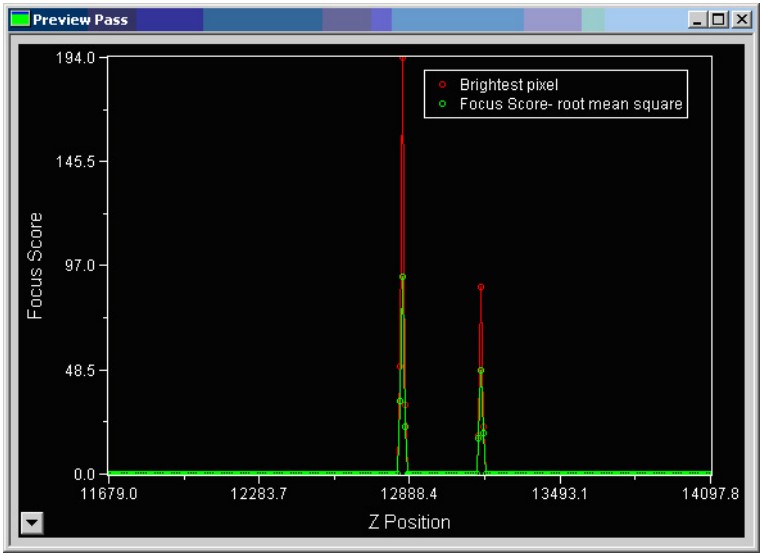


Figure 3-11 Preview Pass graph

14. If the **Preview Pass** window contains at least one peak, the **Laser Auto Focus Sensor** is enabled and functional.
15. If the **Preview Pass** window does not contain any peaks, ensure that the plate is properly seated, increase the **Exposure** value in the **Configure Laser Autofocus Settings** dialog, and try again.
16. If the **Preview Pass** window still does not contain a peak, contact Technical Support and report the issue. For more information on the **Preview Pass** window, see Confirming Laser Auto Focus Settings for Plate Files on page 58.
17. Click **Close** to exit the **Configure Laser Autofocus Settings** dialog.

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 instrument to complete this procedure.

1. Power on the system and start the MetaXpress Software application 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 magnification), and then 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 **96 Wells (8x12)** plate type from the **Plate name** field.
8. Click the **Sites to Visit** tab to highlight it.
9. Ensure that **Site Acquisition Option** is set to **Single site**.
10. Click the **WI (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 appears.
13. Ensure that **W1 – FITC** is selected in the **Wavelength** field.
14. Enter a step size of **250** in the **Step size** field.

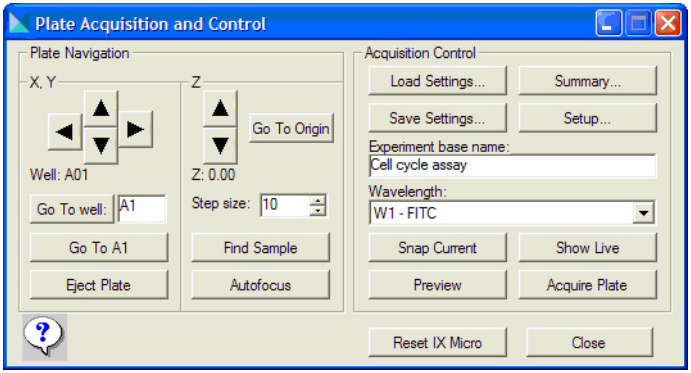


Figure 3-12 Plate Acquisition and Control dialog

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, go 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.

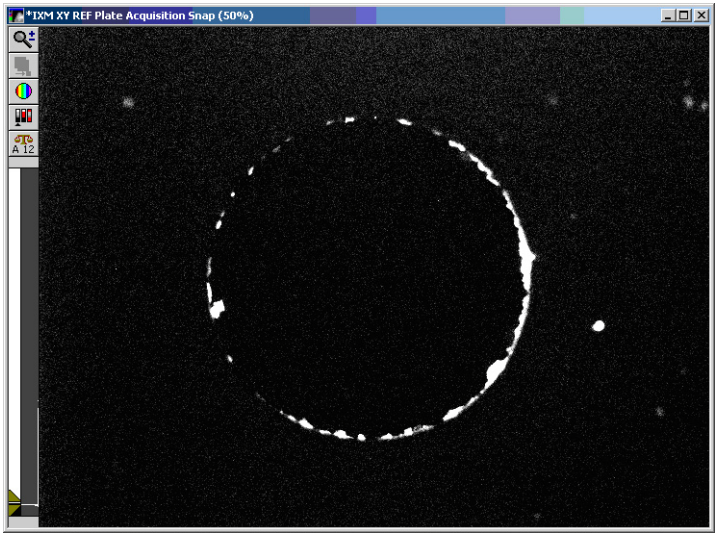


Figure 3-13 A1 Pinhole in focus at 4X

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 Technical Support.

23. If your lowest magnification objective is greater than 4x:

- Move the stage up until you are close to focus and left until you see the edge of the insert hole.
- Align the left side of the hole with the left side of the image window and record the stage X position.
- Move the stage to the right until you see the edge of the insert hole.
- Align the right side of the hole with the right side of the image window and record the stage X position.
- Calculate the horizontal center of the reference point.
- Repeat Step 23 for the vertical center of the reference point.
- 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 the MetaXpress Software application are available from the Plate Acquisition Setup dialog:

1. Open the MetaXpress Software application 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.

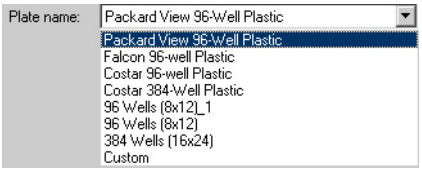


Figure 3-14 Available plate type files

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

   OR

   If there are no plate types listed except for the three defaults: **96 Wells (8x12)**, **384 Wells (16x24)**, and **Custom**, continue with this procedure to load the preconfigured plate type files.
6. Insert the MetaXpress Software Installation USB flash drive into the computer.
7. When the MetaXpress Software installation screen appears, click **Explore Installation Folders / Files**.

   Windows Explorer opens showing the contents of the flash drive.
8. Open the **Plates** folder.

   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 Software installation directory (by default, C:\MX5\Plates). These files will then appear in the **Plate name** filed in the **Plates** tab of the **Plate Acquisition Setup** dialog.

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

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 appears.

11. Under Attributes, uncheck **Read-only**, then click **OK**.

Confirming Laser Auto Focus Settings for Plate Files

Just before each site is acquired during plate acquisition, the laser autofocus system automatically moves the vertical (Z-axis) position of the objective to a point where the bottom of the well is in focus. Before using a plate file, confirm that the laser autofocus settings are optimal for the plate. You must prepare the plate that you are going to test by putting water or buffer similar to the buffer you will be using for real experiments in several of the wells.

Use the Laser Autofocus Wizard, available on the Plate tab, to confirm laser autofocus settings. The wizard walks you through the process. Additional information is available in the online help, in the topic "Configure Laser Autofocus Settings - Dialog Box Options."

Note: The Laser Autofocus Wizard calculates measurements as accurately as possible. Some manual verification and adjustment of the settings may be necessary to optimize the results.

Note: 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, see Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 182.

Verifying Shading Correction Files

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

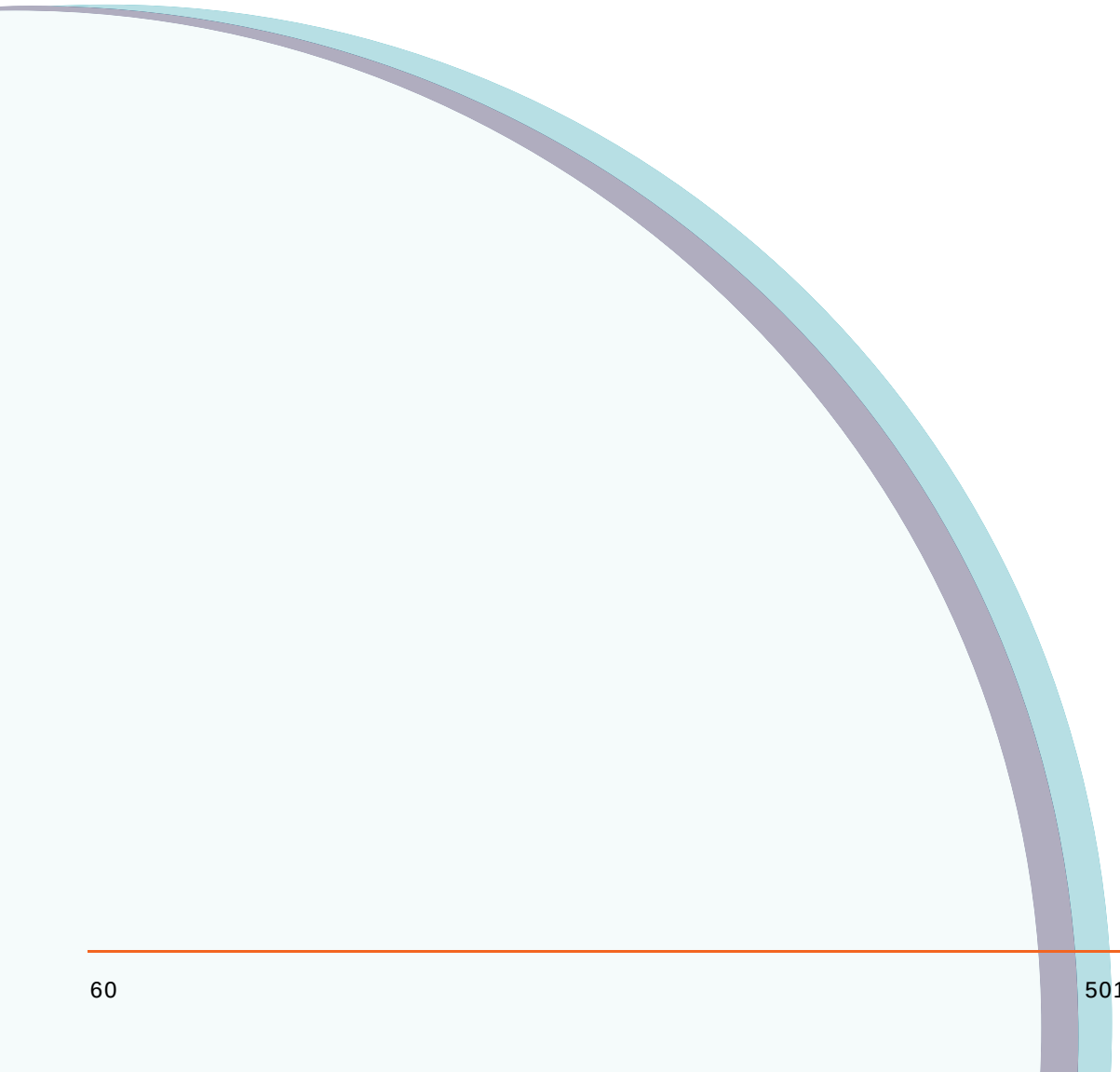
Note: In order for shading correction images to be used during Plate Acquisition, the Perform shading correction checkbox must be selected in the Acquisition Loop tab of the Plate Acquisition Setup dialog.

Note: The shading correction images must be named in the following format: **shading_<magnification setting>_<wavelength>.tif**. For example, **shading_4x Plan Apo_DAPI.tif**.

Note: By default, the MetaXpress Software application looks in the C:\Shading Images 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 and selecting a new location.

1. Use Windows Explorer to navigate to the C:\Shading Images 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, see Updating Shading Correction Settings on page 178.



Preparing For Acquisition

4

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. Molecular Devices 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 application, 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 System.
- **Illumination**: Check that your light source is functioning correctly. The basic design of the ImageXpress Micro System 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 Molecular Devices 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. 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. Clicking the Auto Expose button on the one of the Wavelength tabs in the Plate Acquisition Setup dialog provides 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 means you can reduce your exposure time, which decreases your acquisition time.

Evaluating your Experiment Requirements

Nearly all settings for plate acquisition are made in the Plate Acquisition Setup dialog. 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 thin? Dense sample material requires more light and might 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. Molecular Devices recommends 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 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 select the Single Sites option on the Sites 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 the MetaXpress Software application 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 Micro 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 Molecular Devices 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 can 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 the measurements needed. If you are interested in the total number of cells present, a 10x objective might be adequate. But if you are interested in co-localization of two probes, a higher magnification might be needed. Counting or localizing small organelles might require 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 faint features of your sample that are otherwise not visible. A good example of this is overexposure of 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 also produce a sharper picture due to a narrower depth of field; this might or might 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 multiwell plates (such as a 384-well plate) because of the of the plate 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, 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. Molecular Devices 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 might 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 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 a 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 Molecular Devices 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, see Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 182.

Setting Up Plate Acquisition

5

Before configuring an experiment, it is important to become familiar with the tools used in the MetaXpress® Software. The foundation of the MetaXpress Software is the MetaMorph® Microscopy Automation and Image Analysis Software. The MetaMorph Software contains numerous dialogs for image acquisition, processing, and analysis. However, the majority of the dialogs available are not needed for a typical MetaXpress Software plate acquisition. This chapter explains the dialogs used to configure a screening experiment.

Note: For more information on any dialog, consult the application help available within the MetaXpress Software. Help for an active dialog can be accessed by pressing the F1 key.

The MetaXpress Software main menu includes the Screening menu, which is used specifically for MetaXpress Software image acquisition and analysis. Other menus, such as the Devices menu, provide additional dialogs needed to correctly configure the MetaXpress Software application.

To help you to become more familiar with the MetaXpress Software application and the dialogs that you need to use, the following topics include sample images of the dialogs 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 dialogs. Figure 5-1 shows the options available in the Screening menu.

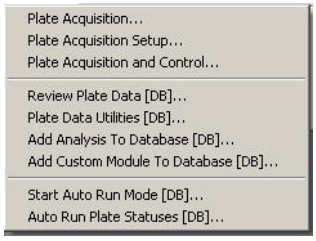


Figure 5-1 Screening menu

Plate Acquisition Dialog

The Plate Acquisition dialog is designed to be used after you have configured and saved settings using the Plate Acquisition Setup dialog. From this dialog, 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 shows the Plate Acquisition dialog.

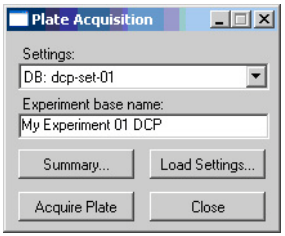


Figure 5-2 Plate Acquisition dialog

Load Plate Acquisition Setting Dialog

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

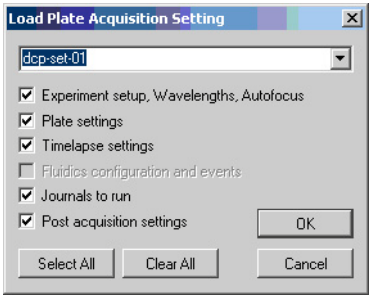


Figure 5-3 Load Plate Acquisition Settings dialog

Use the Load Plate Acquisition Settings dialog 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 selecting the associated checkbox. Settings for checkboxes that are not selected are not used.

Plate Acquisition and Control Dialog

The Plate Acquisition and Control dialog provides two primary functions: Plate Navigation that provides manual control of some of the movable physical components of the ImageXpress® Micro imager, and Acquisition Control that enables you to prepare and start a plate acquisition. In the Plate Navigation group, click 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.

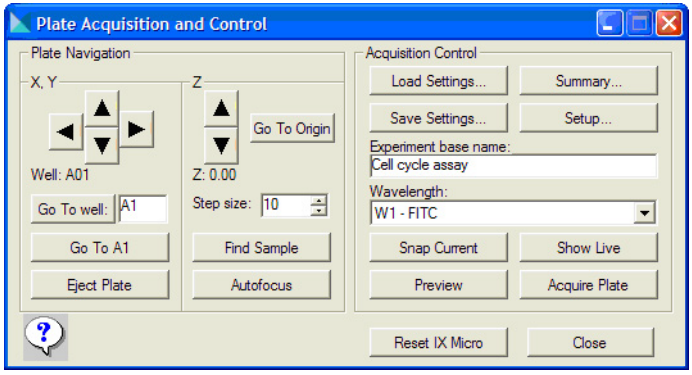


Figure 5-4 Plate Acquisition and Control dialog

The Acquisition Control group provides a consolidated organization of commands that can also be found in other dialogs. These controls enable you to save settings, load previously saved settings, open the Plate Acquisition Setup dialog, specify a new experiment base name, choose a wavelength, 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

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

The Plate Acquisition Setup dialog has a number of visible tabs that 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 is dedicated to a specific type of function or setting.

Note: When configuring the Plate Acquisition Setup dialog, 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 with several warnings.

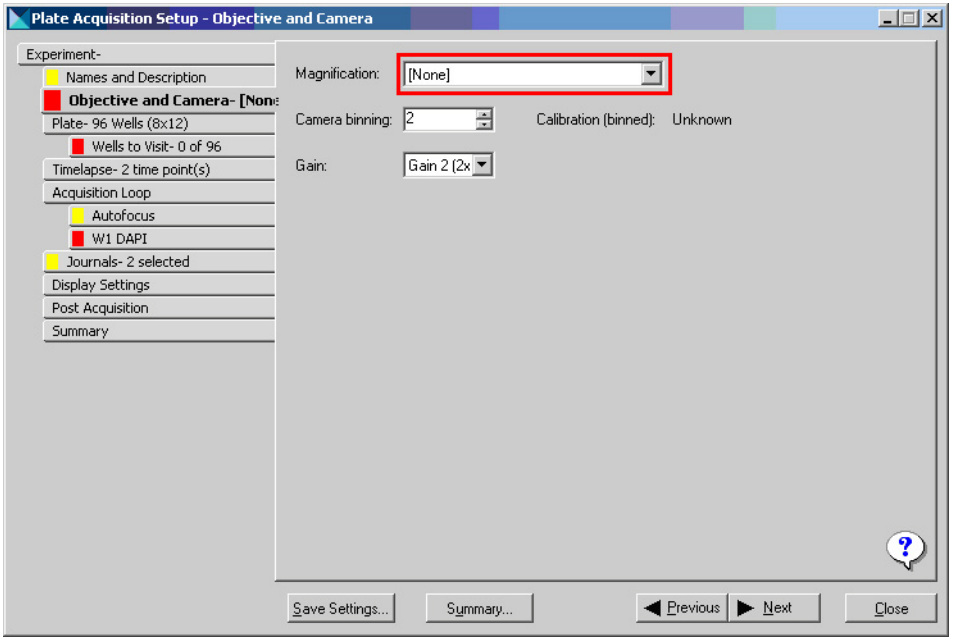


Figure 5-5 Warning Examples in Plate Acquisition Setup dialog

Using the Experiment Tab

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

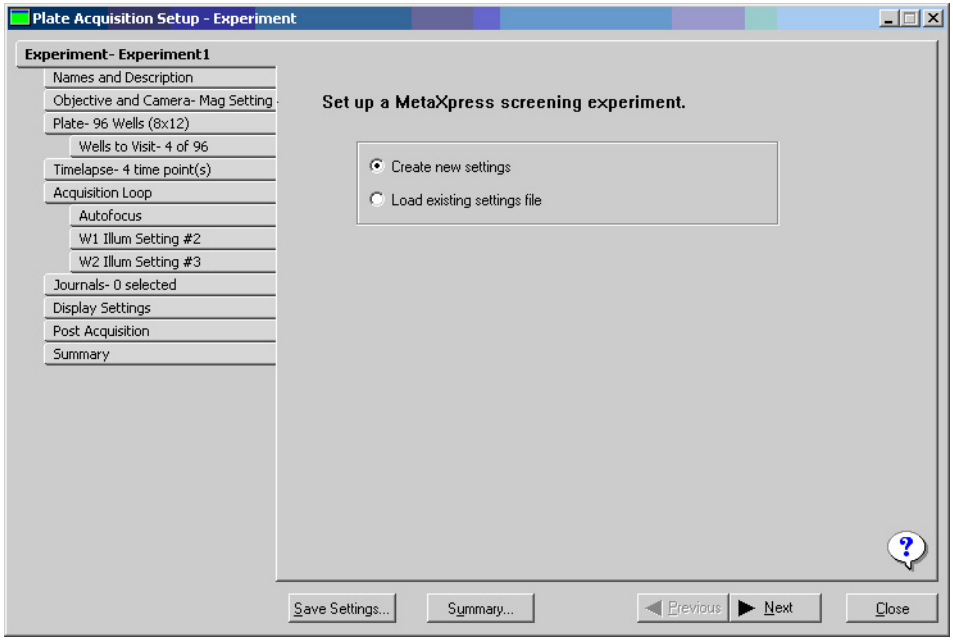


Figure 5-6 Plate Acquisition Setup dialog: Experiment tab

To load settings on the Experiments tab

The Experiments tab enables you to create a new setting or load a previous setting for revision.

1. From the **Screening** menu, select **Plate Acquisition Setup**.

   The Plate Acquisition Setup dialog appears with the Experiment tab displayed.

2. If you are creating new settings, select **Create new settings** and click **Next**. Proceed to Defining the Names and Description on page 73.

3. If you are using an existing settings file, select **Load existing setting file**.

   The Load Settings button appears.

4. Click **Load Settings**.

   The Load Plate Acquisition Settings dialog appears.

5. If you are loading a settings file that was saved to the database, select the settings file from the **Settings File** field. Select the individual settings to load from the file using the checkboxes next to each settings group.

   OR

   If you are loading a settings file that was saved outside the database, select **From File** in the field, and then select the individual settings to load from the file using the checkboxes next to each settings group.

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

6. If you are using a settings file from the database, click **Load**. The file loads from the database and the **Load Plate Acquisition State** dialog closes.

   OR

   If you are loading a settings file saved outside the database, click **Load**. The Load Screen state dialog appears. Navigate to the state file (.HTS) you want to open and click **Open**. The state file you selected loads and the **Load Screen Acquisition** dialog closes.

7. Click **Summary** to view the **Summary** tab and confirm your settings.

   OR

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

Defining the 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 using the Names and Description tab. This information is stored with the settings file in the database.

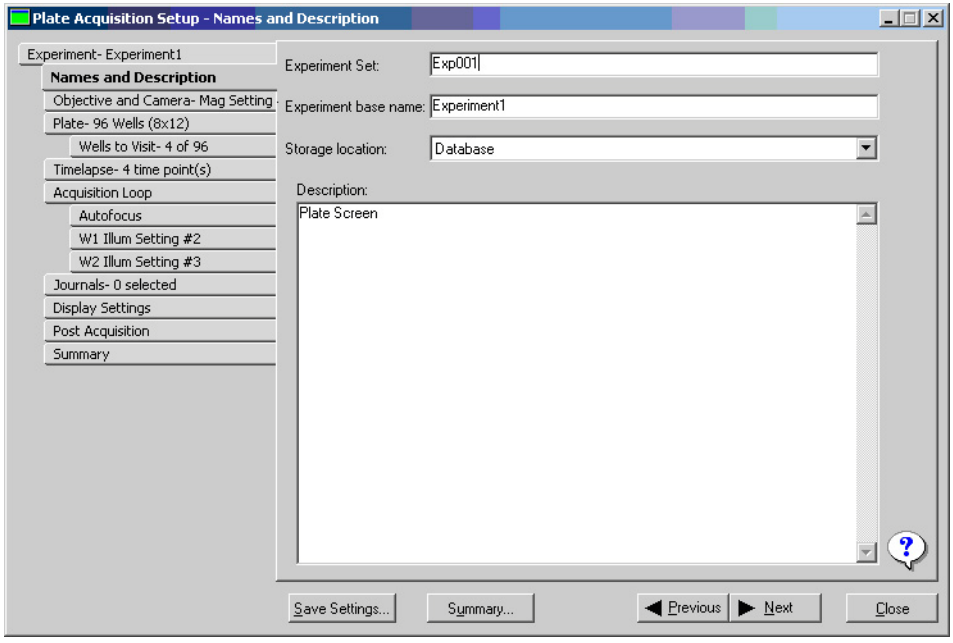


Figure 5-7 Plate Acquisition Setup dialog: Names and Description tab

To add an experiment set name, base name, and description

1. Type an experiment set name into the **Experiment Set** field.

Note: The Experiment Set name can be used to help sort and group experiments when you view them using the Review Plate Data command.

2. Type an experiment base name into the **Experiment base name** field.
3. Select a location where screening images are saved from the **Storage Location** field.

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

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

Defining 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.

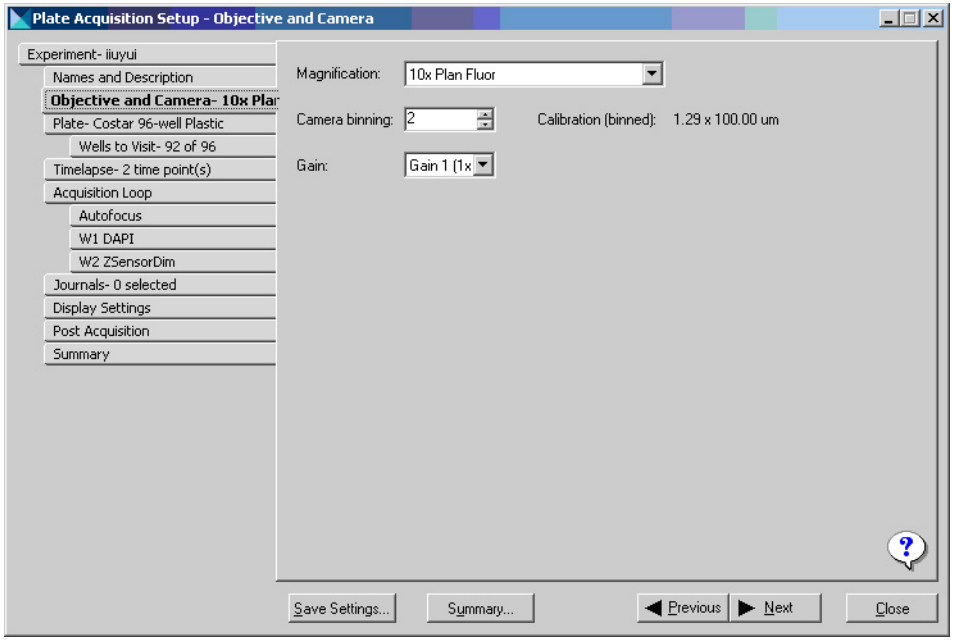


Figure 5-8 Plate Acquisition Setup dialog: Experiment tab

Option	Description
Magnification	Selects the magnification setting that you want to use in your experiment. 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 accessed from the Measure menu. For more information, see the application help in the MetaXpress Software by pressing F1 while the Calibrate Distances dialog is active.

Figure 5-8 Plate Acquisition Setup dialog: Experiment tab (cont'd)

Option	Description
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.

To change settings on the Objective and Camera tab

1. Select the magnification setting to use from the **Magnification** field at the top of the dialog.

Note: The settings available in the list are created in the Configure Magnification dialog in the Devices menu.

2. Select the amount of binning from the **Camera Binning** box.
3. Select the amount of gain from the **Camera gain** field, if applicable.

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

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

Defining the Plate Dimensions

Use the Plate tab to 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 the MetaXpress Software application 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 autofocusing is as accurate as possible.

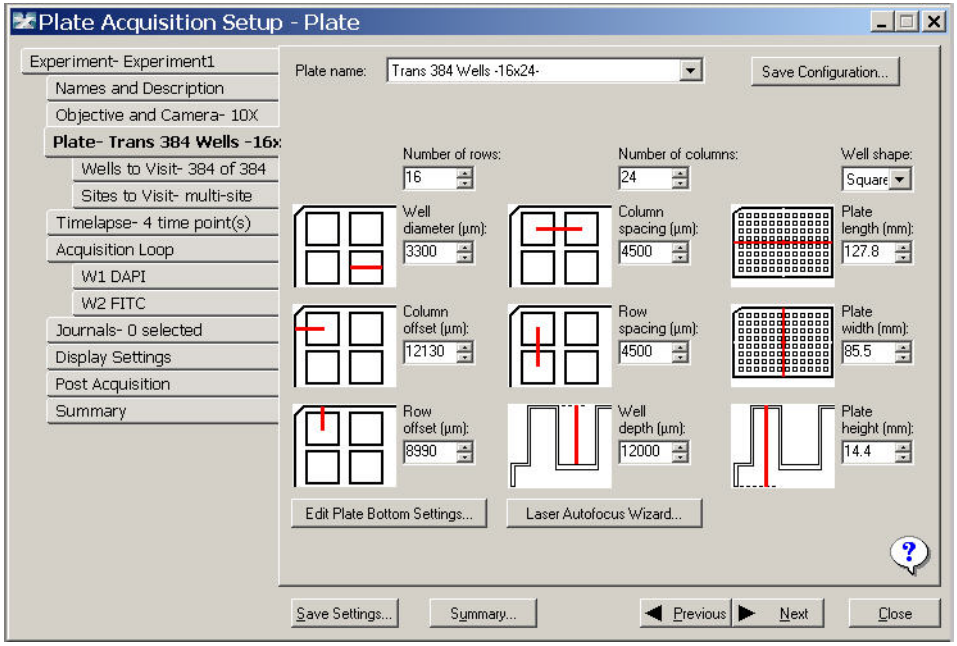


Figure 5-9 Plate Acquisition Setup dialog: Plate tab

Option	Definition
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 the plate selected. If your plate is not included in the list, you will need to type these values. Type the manufacture's plate specification in the setting boxes on this tab. There are two values that the manufacturer will probably 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. For more information on configuring plate types, see Laser Focus Troubleshooting Tips on page 127.

Figure 5-9 Plate Acquisition Setup dialog: Plate tab (cont'd)

Option	Definition
Save Configuration	Opens the Save Configuration dialog and enables you to name and save a custom configuration based on the current values.
Number of columns	Contains the number of columns for the selected plate type. This value can be changed to create a custom configuration if a custom plate is selected.
Number of rows	Contains the number of rows for the selected Plate type. This value can be changed to create a custom configuration. This option is available only if a custom plate 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	Specifies the diameter of the well in microns. This value can be changed to create a custom configuration 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 available only if a custom plate type is selected.
Plate Length	Specifies the plate length in microns. This option is available only if a custom plate type is selected.
Column Offset	Specifies the 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 available only 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 available only if a custom plate type is selected.

Figure 5-9 Plate Acquisition Setup dialog: Plate tab (cont'd)

Option	Definition
Plate Width	Specifies the plate width in microns. This option is available only 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 available only if a custom plate type is selected.
Well Depth	Specifies the well depth in microns.
Plate Height	Specifies the plate height in microns.

To configure plate settings on the Plate tab

Use the Plate tab to configure and save plate settings for your acquisition. The MetaXpress Software application comes with a variety of common plate types already configured.

Even if you use an existing plate type file, Molecular Devices recommends 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. For more information on configuring these values, see Laser Focus Troubleshooting Tips on page 127.

Molecular Devices strongly recommends that you use the Laser Autofocus Wizard, available on the Plate tab, to obtain plate bottom measurements. Although plate manufacturers generally provide reliable plate and well dimensions, you need to calculate the plate bottom measurements, such as average thickness and maximum variation in thickness of the entire plate. The Laser Autofocus Wizard walks you through steps to automatically calculate plate bottom settings as well as exposure times for each objective. The MetaXpress Software online help topic "Configure Laser Autofocus Settings - Dialog Box Options" provides detailed information about the settings that the wizard calculates.

Note: The Laser Autofocus Wizard calculates measurements as accurately as possible. Some manual verification and adjustment of the settings may be necessary to optimize the results, particularly for thin-bottom plates. Contact Technical Support if you need assistance.

All plate bottom values are optical thickness (also known as reduced thickness) measurements, as measured using the objective. The values are not physical thickness measurements.

Note: 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, see Laser Focus Troubleshooting Tips on page 127.

Note: The correct Well depth and Plate height values are needed for autofocusing.

To configure plates

1. In the **Plate name** field, 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 you select

   If you select 96 Wells (8×12) or 384 Wells (16×24), only the following options will be available for configuration: Well shape, Well depth, Plate height, Optical Thickness, and Bottom Variation.

   If you select Custom, all options will be available for configuration, including Number of columns and Number of rows.
2. In the **Well shape** field, select a square or circular well shape. The graphics of the plate are updated to reflect your selection.

3. Click **Laser Autofocus Wizard** and follow the steps that the wizard provides to calculate plate bottom measurements.

   The optical thickness is a value in microns that is the average measured thickness for the well bottom using the Laser Autofocus. It is proportional but not equal to the physical thickness of the bottom plate.

   The bottom variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms.

Note: The Laser Autofocus Wizard calculates measurements as accurately as possible. Some manual verification and adjustment of the settings may be necessary to optimize the results.

   The MetaXpress Software online help topic “Configure Laser Autofocus Settings - Dialog Box Options” provides detailed information about the settings that the wizard calculates.

4. If required, in the **Well depth** and **Plate height** fields, change the values.

   Correct Well depth and Plate height values are required for autofocusing.

5. If you selected **Custom**, complete the fields as required.

6. After you have configured the plate settings, click **Save Configuration** to type a name for your setting and save the plate configuration. Configurations that have been saved are then available in the Plate name list.

Note: Adjust any objectives with correction collars to match the plate type.

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

Specifying the Wells to Visit

The Wells to Visit tab enables you to configure which wells to acquire on your plate. This dialog 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.

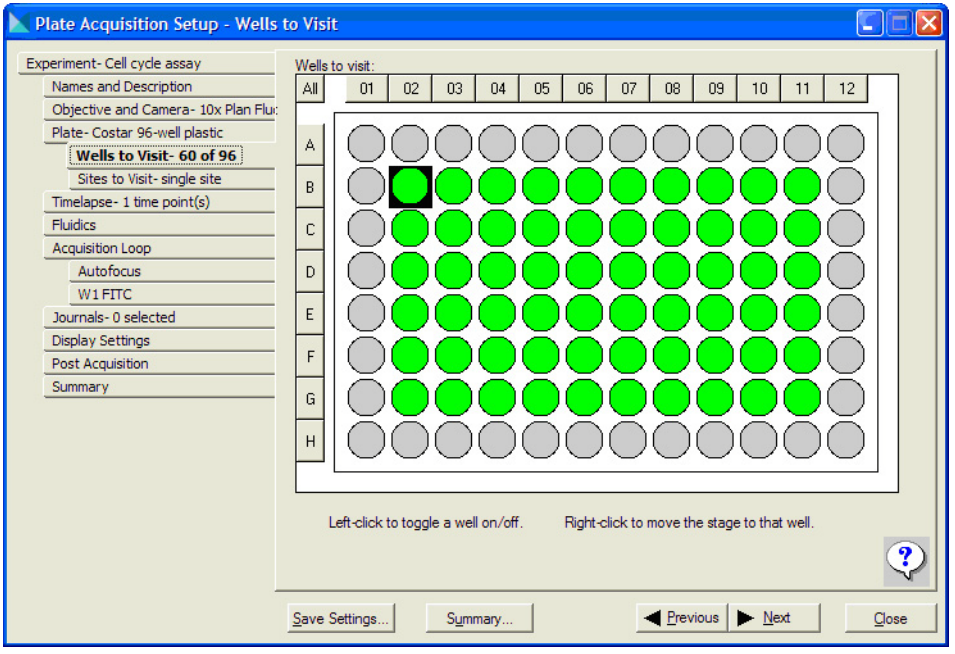


Figure 5-10 Plate Acquisition Setup dialog: Wells to Visit tab

Select the wells to sample during the experiment. You can select from 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.

To configure the Wells to Visit tab

The Wells to Visit tab enables you to configure which wells to acquire on your plate.

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

Specifying the Sites to Visit in a Well

Use this optional setting to specify acquisition of more than one location in a well. You can configure the vertical and horizontal spacing between sites in an image. Both positive and negative values can be entered. This enables you either to add a barrier between images (using positive values) or to overlap images using negative values.

The number of sites that you can acquire in a well depends 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 to consider when configuring multiple sites include 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.

Note: If single site is specified, one site per well is collected at the center of each well.

The focus for individual sites is set on the Autofocus tab. See Defining Autofocus Settings on page 96.

The Adaptive Acquisition™ option is a computational algorithm to analyze cell number on the fly during sample acquisition, which increases the chances of collecting valid data in every well. The Adaptive Acquisition option can also significantly reduce acquisition time for multi-wavelength acquisition requiring a minimum number of cells per well or for samples with differing conditions across the plate. If this option is selected, a new tab, Cell Counting, will appear as described below. In addition, you will specify a minimum number of sites to be collected.

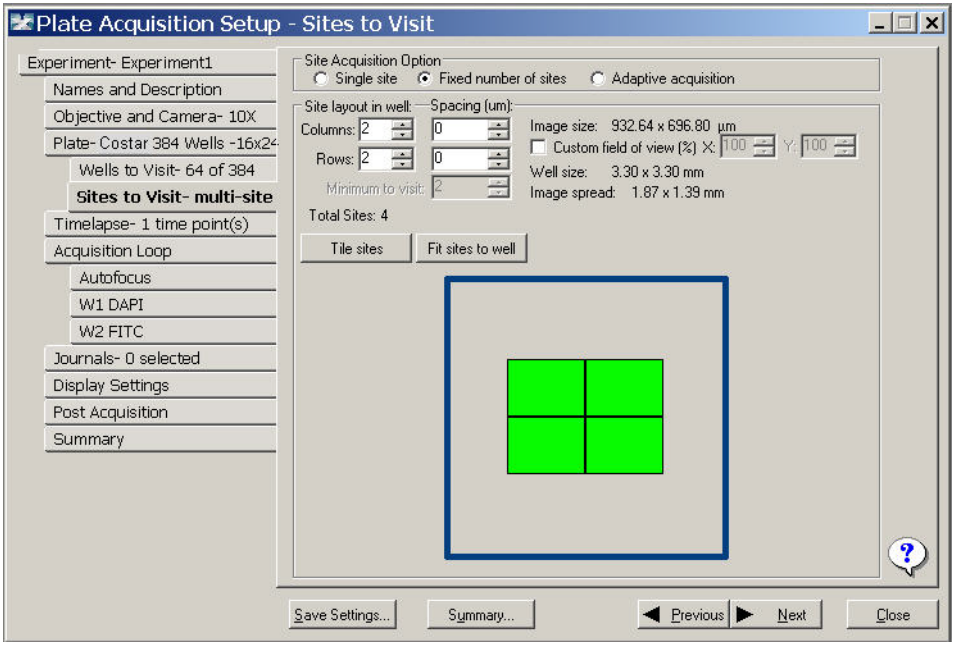


Figure 5-11 Plate Acquisition Setup dialog: Sites to Visit Tab.

To configure the Sites to Visit tab

1. Specify the number of columns and rows (for example, 2 columns and 2 rows to acquire up to four sites; 3 columns and 3 rows to acquire up to nine sites, and so on).

Note: The maximum number of columns and rows that can be configured is 45 × 35.

2. To specify a percentage of the width (X) and height (Y) of the camera's field of view to acquire, select the **Custom Field of View** check box.

The camera's full field of view is 100 percent (100% in the X field and 100% in the Y field). So, for example, if the full field of view width is 1000, to acquire a width of 500, you would enter 50 in the X field.

This feature is particularly useful if the image acquired using the full field of view is not evenly illuminated or if the image acquired includes the area outside the well boundary. By reducing the field of view, you can acquire an image of only the properly illuminated area or of only the area within the well.

A custom field of view can be used for a single site or for multiple sites.

Note: The custom field of view feature can be used with shading correction, but shading correction images must have been acquired with the full field of view.

3. Click **Tile Sites** to configure the sites with zero spacing between them (adjacent).
4. Click **Fit Sites to Well** to maximize the spacing between the sites within the well.
5. In the graphical display, left-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. You can also click and drag to select or deselect a rectangular block of sites. Right-click a site to move the stage to that site.

   Figure 5-12 shows the configuration of the Sites to Visit tab to acquire images at the edges of a round well configured with a 4x4 arrangement of sites.

   Figure 5-13 shows the configuration of the Sites to Visit tab to acquire images at the edges of a square well with a 4x4 arrangement of sites.

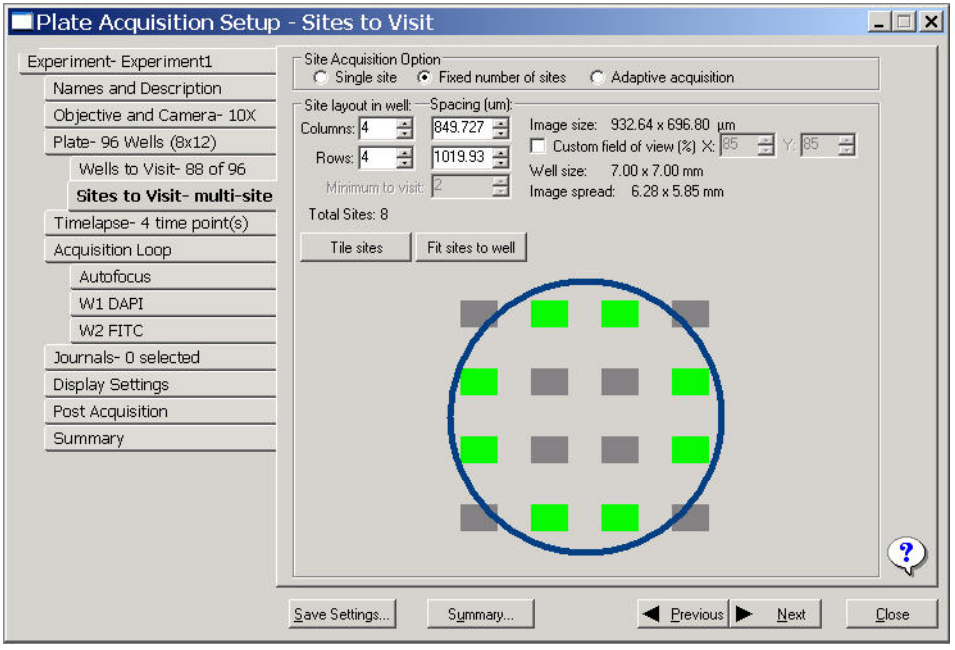


Figure 5-12 Sites to Visit tab showing the acquisition of images at the edges of a round well with a 4x4 arrangement of sites

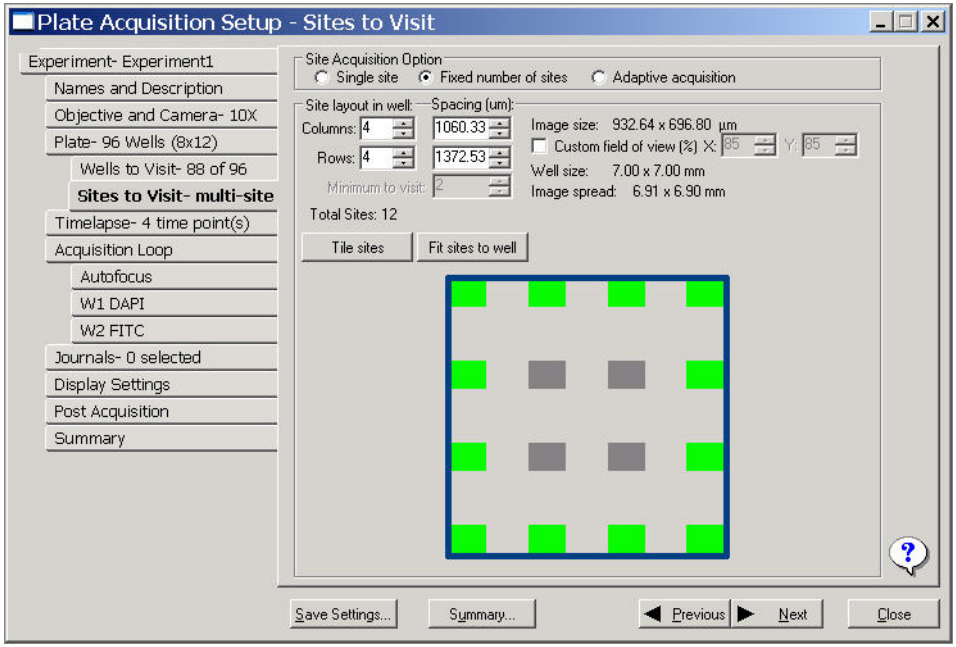


Figure 5-13 Sites to Visit tab showing the acquisition of images at the edges of a square well with a 4x4 arrangement of sites

6. To include a distance between adjacent sites, type the X and Y values of the spacing in the **Spacing between images (µm)** field.

   Typing a negative value results in overlapping data between adjacent images. You can view the current image size, well size, and image spread values on the tab. These values update to reflect changes in configuration.

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

Using the Cell Counting Tab

When in the Adaptive Acquisition mode (set in the Sites to Visit tab), the Cell Counting tab appears. In this tab, first select the channel that will be used for cell counting. Next, enter approximate values for the width and intensity of your nuclei, and then use the Test Settings option to optimize your settings on a representative site (see Figure 5-15). Note that you should be in an appropriate location on your sample, and in focus on the sample. Test Settings will snap an image (see Figure 5-16), and count the cells using the current settings, displaying both an analysis overlay and the total number of cells counted in the image.

Note: In this mode, the instrument collects a minimum number of sites based on the **Minimum to visit** setting, and a maximum number of sites based on the configuration in the Sites to visit tab.

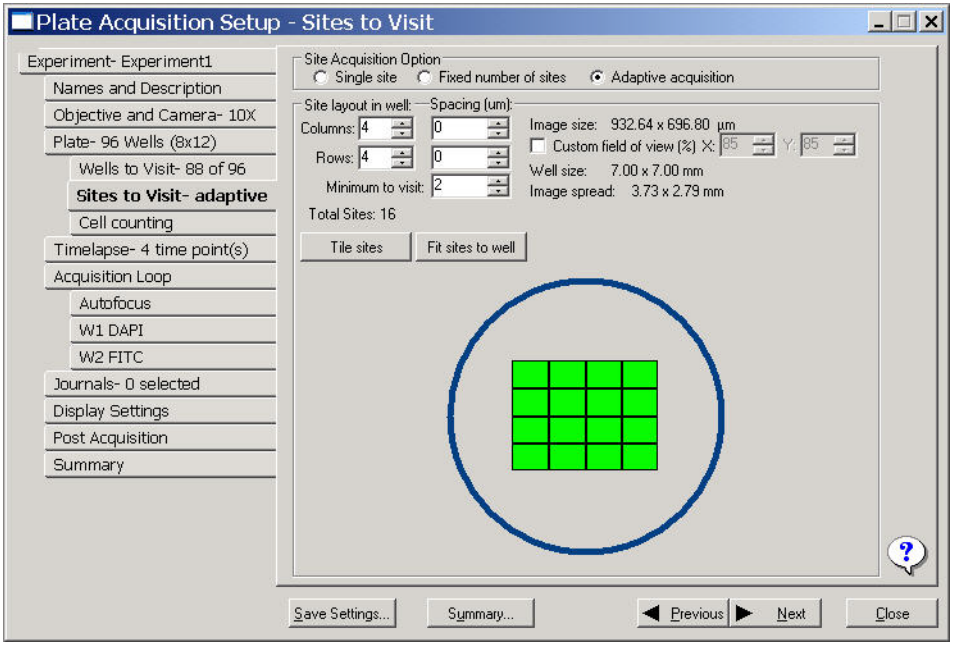


Figure 5-14 Sites to Visit tab with the Adaptive Acquisition mode set

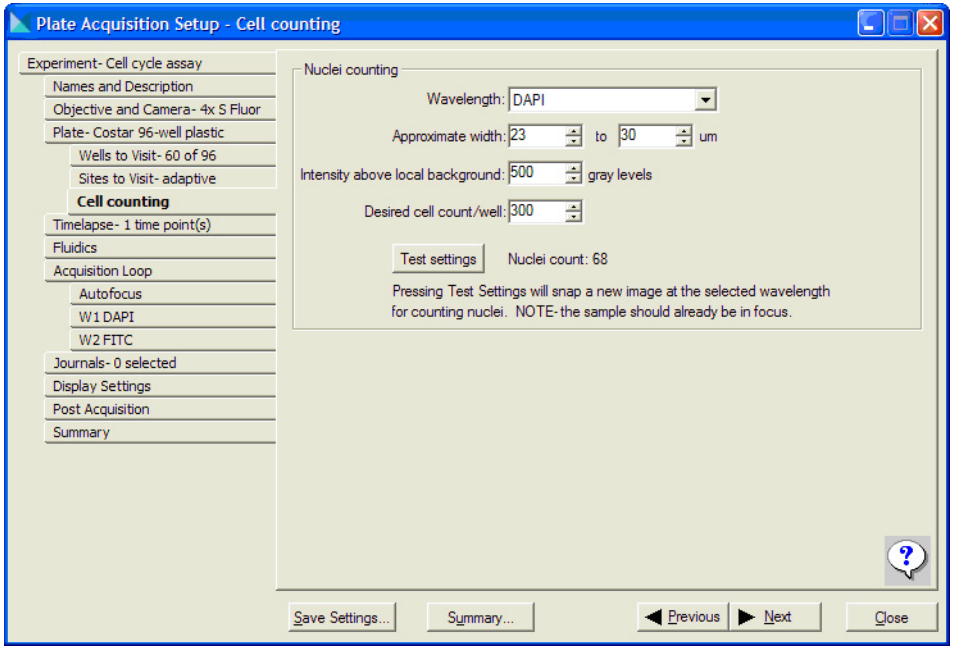


Figure 5-15 Cell counting tab in the Plate Acquisition Setup dialog

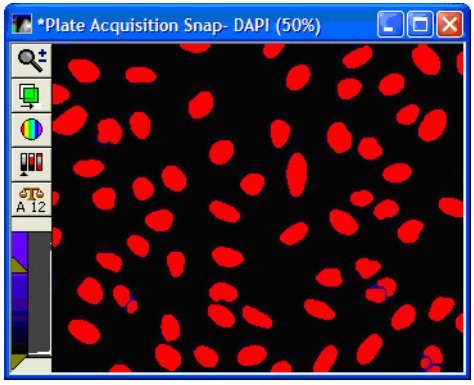


Figure 5-16 Results from Cell counting test settings in the Adaptive Acquisition mode

If you are running in the Adaptive Acquisition mode, a real-time count of the cells in the current well will be displayed as the images are being acquired.

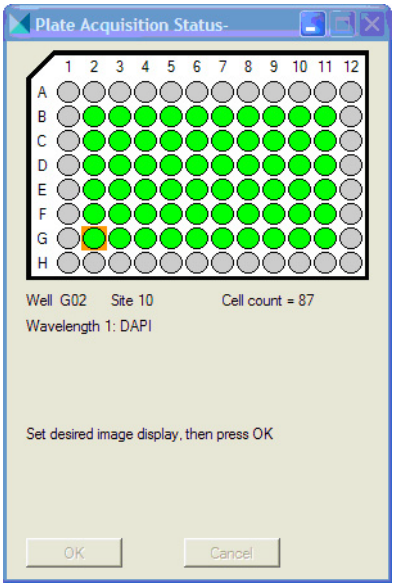


Figure 5-17 Plate Acquisition Status dialog in the Adaptive Acquisition mode

Setting Timelapse Options

The Timelapse 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.

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 x Interval = Duration

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

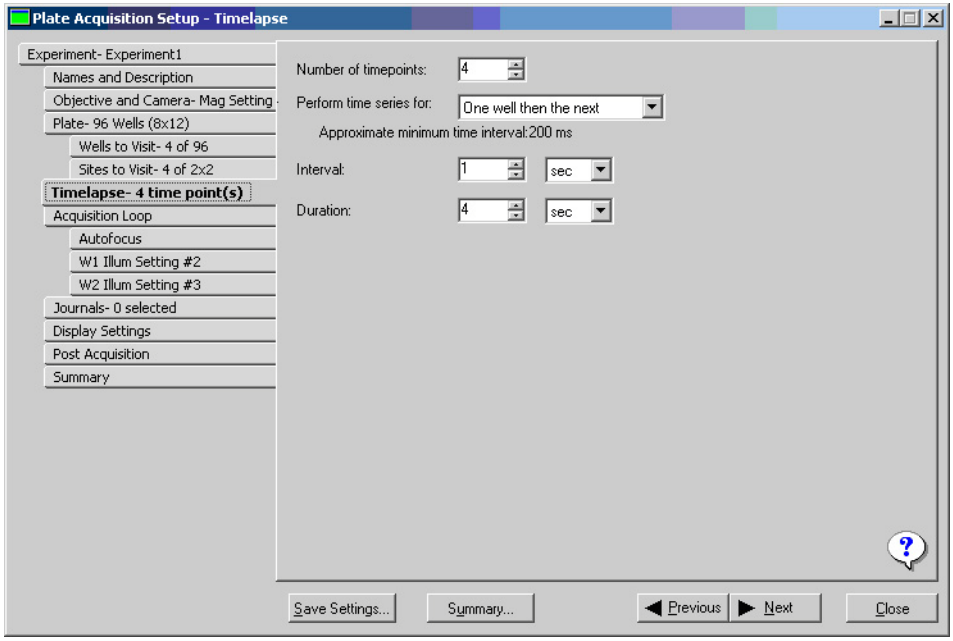


Figure 5-18 Plate Acquisition Setup dialog: Timelapse tab

Option	Description
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.

Figure 5-18 Plate Acquisition Setup dialog: Timelapse tab (cont'd)

Option	Description
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 after the first acquisition is complete. No warning notice will be given if the acquisition time is longer than the specified interval. Select a time unit of 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 time 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. Select a time unit of ms, sec, min, or hr.

To configure the Timelapse tab

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.
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. After 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. After 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. After 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. Select a time unit of ms, sec, min, or hr.
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.
5. Click **Next** to move to the next tab.

Defining Acquisition Loop Options

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

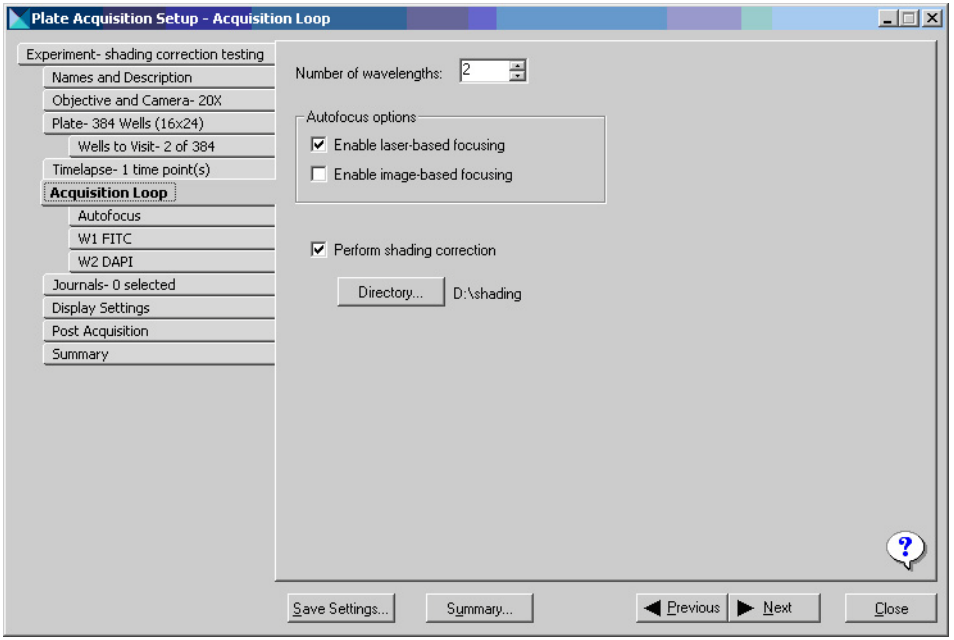


Figure 5-19 Plate Acquisition Setup dialog: Acquisition Loop tab

Option	Description
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. Configure laser autofocusing in the Autofocus tab.
• Enable image-based focusing	Selects image-based focusing for the acquisition. 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: **shading_10x_DAPI.tif**.

Figure 5-19 Plate Acquisition Setup dialog: Acquisition Loop tab (cont'd)

Option	Description
Directory	Opens the Browse dialog. Use this command to change the location where the MetaXpress Software looks for shading correction images. The default location is the C:\Shading Images directory.

To configure the Acquisition Loop tab

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 one or both of the autofocus options.

Note: Laser-based focusing finds the bottom of the well and moves a specified distance up from the bottom. This method is generally the 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.

Note: Image-based focusing uses a specified algorithm to identify the best focus image. This method works 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.

Note: Selecting both Laser-based and Image-based focusing 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: **shading_10x_DAPI.tif**.

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

Defining Autofocus Settings

Autofocus settings are made on a combination of three different tabs in the Plate Acquisition Setup dialog and in additional dialogs within the MetaXpress Software application. 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.

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 samples in the remaining wells. Offset values can also be used to achieve focus for different wavelengths without using the laser to recalculate the focus distance.

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

- Plate Acquisition Setup dialog
  - Acquisition Loop tab
  - Autofocus tab
  - Wavelength tabs
- Configure Laser Autofocus Settings dialog
- Focus dialog (optional)
  - Focus tab (optional)
  - Autofocus tab (optional)
- Configure Laser Autofocus dialog (optional)

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

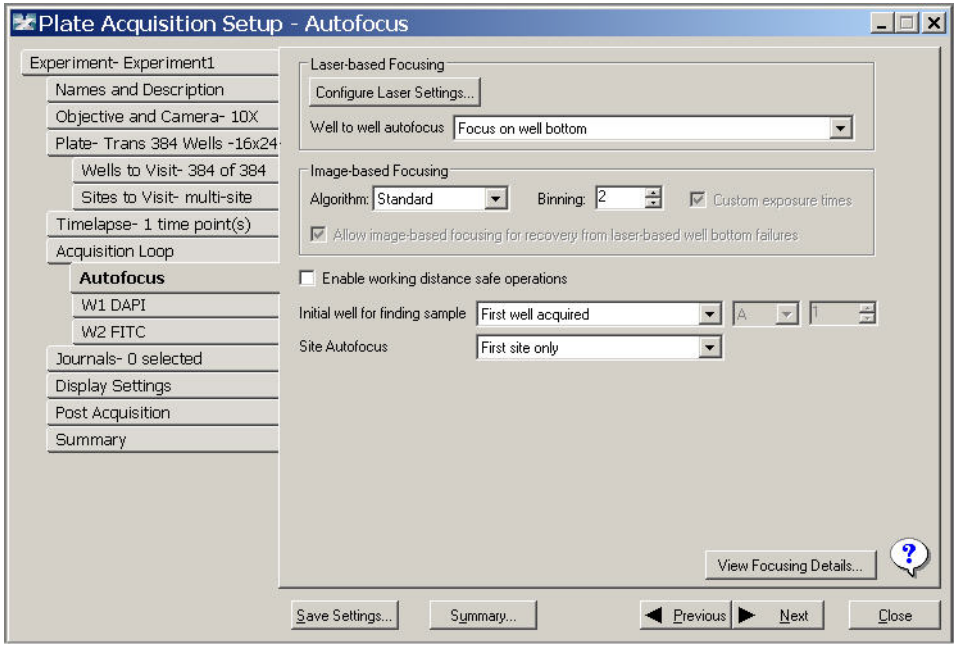


Figure 5-20 Plate Acquisition Setup dialog: Autofocus tab

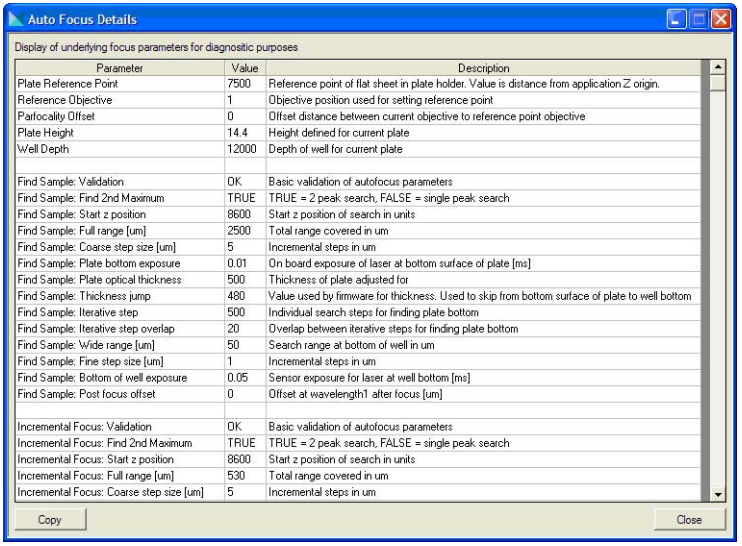
Option	Description
Laser-based Focusing options:	
• Configure Laser Settings	Opens the Configure Laser Autofocus Settings dialog. These settings are calculated using the Laser Autofocus Wizard. The MetaXpress application help topic “Configure Laser Autofocus Settings - Dialog Box Options” provides detailed information about the settings that the wizard calculates.
• Well to well autofocus:	
◆ Focus on well bottom	This is the recommended option for most situations. The initial focus on the plate (Find Sample) will focus on both the plate bottom and well bottom. As the system moves from well to well, it will focus on the well bottom only, using the bottom variation from the Plate configuration as the focus range.

Figure 5-20 Plate Acquisition Setup dialog: Autofocus tab (cont'd)

Option	Description
◆ 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 thin-bottom plate with an objective with a large depth of field (low magnification or low numerical aperture), if you are using a glass slide, or if you are using an oil-immersion objective.
◆ Focus on plate and well bottom	The laser will focus on both the plate bottom and well bottom at every well. This is recommended for plates with significant variation in their optical thickness.
Image-based Focusing options:	
• Algorithm:	Enables you to select the algorithm to use when focusing. Valid choices include Standard and Low Signal
◆ Standard (default)	Algorithm is based on a standard group of settings including a normal camera signal level.
◆ Low Signal	Algorithm is 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 autofocus.
• First well acquired	Finds sample autofocus using the first well.
• Specific well	Finds sample autofocus using the well that you specify

Figure 5-20 Plate Acquisition Setup dialog: Autofocus tab (cont'd)

Option	Description
• Skip Find Sample (select if sample is already in focus)	Disables initial find sample autofocus when starting a plate. Select this option if your sample is already in focus.
Site Autofocus options:	Determines how autofocusing is done for each wavelength when sites are configured. The options include First site only, Center of well, and All sites.
• First site only	Autofocuses in the top-left site in the well.
• Center of well	Autofocuses in the center of the well. This is the recommended method.
• All sites	Autofocuses 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.
Timelapse Autofocus options:	Determines how autofocusing is performed when multiple timepoints are selected. The options include First timepoint only
• First timepoint only	Recommended for fast kinetic acquisitions.
• All timepoints	Recommended for long timelapse acquisitions.
• Every Nth timepoint	This option is available only when more than one timepoint is configured in the Timelapse tab.
View Focusing Details button	Opens a window with detailed focus parameters, useful for diagnostic purposes when troubleshooting focusing issues. You can click the Copy button to copy the parameters as necessary, for example when sending them to Technical Support.



Parameter	Value	Description
Plate Reference Point	7500	Reference point of flat sheet in plate holder. Value is distance from application Z origin.
Reference Objective	1	Objective position used for setting reference point
Parfocality Offset	0	Offset distance between current objective to reference point objective
Plate Height	14.4	Height defined for current plate
Well Depth	12000	Depth of well for current plate
Find Sample: Validation	OK	Basic validation of autofocus parameters
Find Sample: Find 2nd Maximum	TRUE	TRUE = 2 peak search, FALSE = single peak search
Find Sample: Start z position	8600	Start z position of search in units
Find Sample: Full range [um]	2500	Total range covered in um
Find Sample: Coarse step size [um]	5	Incremental steps in um
Find Sample: Plate bottom exposure	0.01	On board exposure of laser at bottom surface of plate [ms]
Find Sample: Plate optical thickness	500	Thickness of plate adjusted for
Find Sample: Thickness jump	480	Value used by firmware for thickness. Used to skip from bottom surface of plate to well bottom
Find Sample: Iterative step	500	Individual search steps for finding plate bottom
Find Sample: Iterative step overlap	20	Overlap between iterative steps for finding plate bottom
Find Sample: Wide range [um]	50	Search range at bottom of well in um
Find Sample: Fine step size [um]	1	Incremental steps in um
Find Sample: Bottom of well exposure	0.05	Sensor exposure for laser at well bottom [ms]
Find Sample: Post focus offset	0	Offset at wavelength1 after focus [um]
Incremental Focus: Validation	OK	Basic validation of autofocus parameters
Incremental Focus: Find 2nd Maximum	TRUE	TRUE = 2 peak search, FALSE = single peak search
Incremental Focus: Start z position	8600	Start z position of search in units
Incremental Focus: Full range [um]	530	Total range covered in um
Incremental Focus: Coarse step size [um]	5	Incremental steps in um

Figure 5-21 Autofocus details

Configuring Laser Autofocus Settings

Use the Configure Laser Autofocus Settings dialog in conjunction with the Laser Autofocus Wizard and the Plate Acquisition Setup Autofocus tab to configure the settings for the MetaXpress Software laser focus system.

To configure the Autofocus tab (Laser Autofocus only)

1. From the **Screening** menu, click **Plate Acquisition Setup**.
   The Plate Acquisition Setup dialog appears.
2. Click the **Acquisition Loop** tab.
3. On the Acquisition Loop tab, verify that **Enable laser-based focusing** is selected.
4. Click the **Autofocus** tab.

5. In the **Well to well autofocus** field, select:
   - **Focus on plate bottom only, then offset by bottom thickness** if you plan to focus only on the bottom of the plate and then use the bottom thickness value to focus on your sample.
   - **Focus on plate and well bottom** if you are using thin-bottom plates. With this option, the autofocus always searches for both peaks in every well. This option is recommended if the plate bottom variation is at least half the thickness of the plate bottom.
   - **Focus on well bottom** if you plan to focus only on the bottom of the well.
6. In the **Site Autofocus** field, if sites are enabled on the **Sites** tab, select the site pattern that you want to use. Select from **First Site only**, **Center of well only**, or **All Sites**:
   - **First site only**: autofocuses in the top left site in the well.
   - **Center of well**: autofocuses in the center of the well. This is the recommended method.
   - **All sites**: autofocuses for each site.
7. On the **Autofocus** tab, click **Configure Laser Settings** to confirm the settings that will be used for autofocusing, including exposure times. If you need to change the exposure values, on the **Plate** tab, click **Laser Autofocus Wizard** and follow the steps that the wizard provides for measuring exposure times.

Note: The Laser Autofocus Wizard calculates measurements as accurately as possible. Some manual verification and adjustment of the settings may be necessary to optimize the results.

The MetaXpress online help topic “Configure Laser Autofocus Settings - Dialog Box Options” provides detailed information about the settings that the wizard calculates.

8. 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.
9. Click **Next** to move to the next tab.

To configure the Autofocus tab (Image-based Focus only)

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

1. From the **Algorithm** field, select from one of the following two image-based autofocus algorithms:
   - **Standard** (default): Algorithm based on a standard group of settings including a normal camera signal level.
   - **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: Molecular Devices recommends using Low Signal 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 autofocus.

Note: Molecular Devices recommends that your binning value is set to 3 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 tabs.
4. To disable the initial find sample autofocus 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 autofocus 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. 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**: Autofocuses in the top-left site in the well.
   - **Center of well**: Autofocuses in the center of the well. This is the recommended method. This occurs even if the center of the well location is not configured to be acquired.
   - **All sites**: Autofocuses at each site.

Note: This option is available only if you have already configured the use of multiple sites in the Sites tab.

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

Defining Wavelength Settings

Use the Wavelength tabs to configure exposure, autofocus, and timelapse settings for each wavelength. The total number of wavelength tabs depends on the number of wavelengths selected in the Acquisition Loop tab.

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

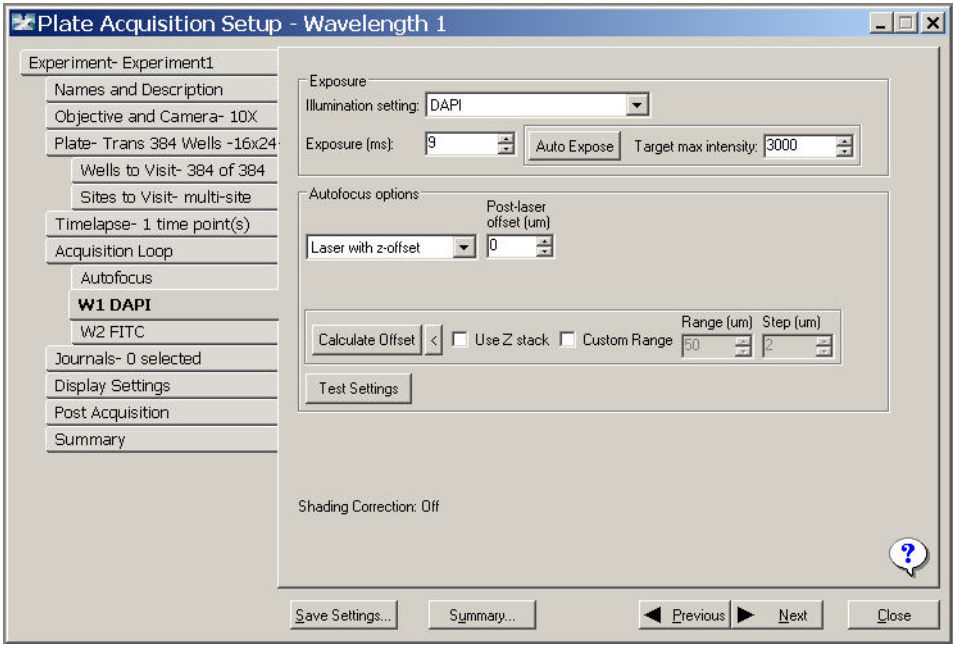


Figure 5-22 Plate Acquisition Setup dialog: Wavelength tab

Option	Description
Exposure options:	
• Illumination setting	Selects an illumination setting to be used with the active wavelength. Illumination settings are defined in the Configure Illumination dialog.
• 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.
• AutoExpose	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.

Figure 5-22 Plate Acquisition Setup dialog: Wavelength tab (cont'd)

Option	Description
• Target maximum 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% of the maximum gray level that the camera driver reports as possible to obtain.
Autofocus options:	Selects the type of autofocus to be used when acquiring images. The options available here depend on how the autofocus tab was configured.
• None	No autofocusing is done. This selection enables no other options.
• Laser with Z-offset	Uses the laser autofocus based on the settings calculated by the Laser Autofocus Wizard (or as configured in the Configure Laser Settings dialog). Specify an offset to use for the first wavelength in the Offset field. The next section provides instructions on using the Calculate Offset feature. No image-based focusing will be performed on this wavelength. This option is only available if you selected Enable laser-based focusing in the Acquisition Loop tab.
• Z-offset from W1	This option moves the specified offset from the W1 focus position and is available only for the second and subsequent wavelengths. Enter the Z-offset value (in µm) in the Offset field. The next section provides instructions on using the Calculate Offset feature. This option is available only if Enable laser-based focusing is selected in the Acquisition Loop tab.
• Image-based	This option is available for the second and subsequent wavelengths if you have selected Enable image-based focusing or both Enable laser-based focusing and Enable image-based focusing in the Acquisition Loop tab). Image-based focusing is best for complex samples with variations in distance between the surface of the plate and the sample. It is also useful for experiments using magnification settings of less than 10X.

Figure 5-22 Plate Acquisition Setup dialog: Wavelength tab (cont'd)

Option	Description
• Laser and image	Uses both laser- and image-based autofocusing as configured in the Autofocus tab. This option is only available for the first wavelength if you selected both Enable laser-based focusing and Enable image-based focusing in the Acquisition Loop tab. With this option, the laser autofocuses, then image-based focusing is used to fine-tune the image.
• Laser with image recovery	Attempts to perform a laser with z-offset focus (described above). Only uses image-based focusing if laser-based autofocusing cannot find the plate or well bottom. The image-based recovery search is centered on an estimated well bottom offset position. The estimated position is calculated by using the plate bottom position found during laser autofocus and then adding to that the plate bottom thickness and post laser offset values. This option is only available if both Enable laser-based focusing and Enable image-based focusing are selected in the Acquisition Loop tab and you have selected **Allow image-based focusing for recovery from laser-based well bottom failures** on the Autofocus tab.
• Offset (µm)	Z motor offset distance for each wavelength defined on the Plate Acquisition Setup tab. For the first wavelength, if you are using laser-based focusing, the offset is the distance between the bottom of the well and the in-focus plane (if you are using just image-based focusing, no offset is needed for the first wavelength). For the second and subsequent wavelengths, with laser- or image-based focusing, the offset is the difference between the focus position of the Z motor using the first wavelength and the focus position of the Z motor using the second or subsequent wavelength. The next section provides instructions on using the Calculate Offset feature.
• 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. For example, 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.

Figure 5-22 Plate Acquisition Setup dialog: Wavelength tab (cont'd)

Option	Description
• Image-based range	Specifies the range to use for the image-based portions of autofocusing. Laser autofocusing is performed to an accuracy equal to this range before image-based autofocusing begins. This option is available only if both Enable laser-based focusing and Enable image-based focusing is selected in the Acquisition Loop tab.
• Max step (µm)	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 autofocusing.
• Gain	Sets the sensitivity of the camera when used with the Autofocus command.
Timelapse acquisition options:	Specifies the image collection intervals to use for each wavelength.
• 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.

To configure the Wavelength tabs

Note: The options available in the Wavelength tabs 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.
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 in the **Target Intensity** field or use the default value.

   This value sets the intensity that the 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% 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 options** section (see Figure 5-22 for descriptions of the types of autofocusing available).

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 field vary depending on selections made in the Acquisition Loop and Autofocus tabs.

5. Optionally, in the **Autofocus options** section, in the **Offset** field, adjust the Z offset used during autofocusing. To use the Calculate Offset feature, which automatically determines the appropriate Z motor offset distance for each wavelength as defined on the Plate Acquisition Setup tab, follow Steps 6-10.

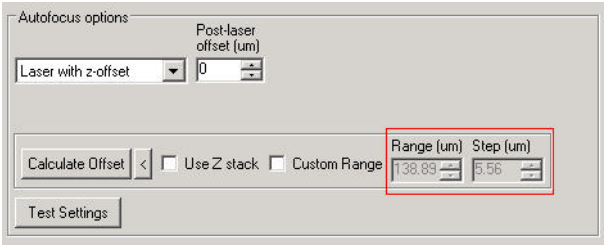
   For the first wavelength tab, the offset is the distance between the bottom of the well and the in-focus plane. For the second and subsequent wavelength tabs, the offset is the difference

between the focus position of the Z motor using the first wavelength and the focus position of the Z motor using the second or subsequent wavelength. As a simple example, suppose the position of the Z motor when the sample is in focus using Wavelength 1 is 500 and the position of the Z motor when the sample is in focus using Wavelength 2 is 700. The difference between the two positions is 200, so the offset for Wavelength 2 is 200.

Before using the Calculate Offset feature:

- Configure the plate parameters using all of the tabs above the Acquisition Loop tab.
- If you are using laser-based focusing, configure all laser autofocus parameters using either the Laser Autofocus Wizard (available from the Plate tab) or the Configure Laser Autofocus Settings dialog (available from the Autofocus tab). The MetaXpress online help topic “Configure Laser Autofocus Settings - Dialog Box Options” provides detailed information about the settings that the wizard calculates.
- On the Autofocus tab, in the Image-based Focusing section, select the algorithm and set the binning. These fields are available even if you selected only Laser-Based Focusing on the Acquisition Loop tab because part of the Calculate Offset feature uses image-based focusing to determine the offset. Custom exposure times are not used.
- Ensure that the signal/noise is set so that the system can focus appropriately (adjust the exposure time if necessary to correct a dim or saturated signal).

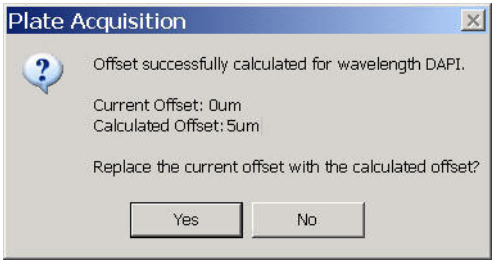
The default range and step size that will be used to calculate the offset is based on the numerical aperture of the objective in the selected setting. For example:



6. If you are using laser-based focusing, either with or without image-based focusing, to automatically calculate the Z offset using the default range and step size for the first wavelength, click **Calculate Offset**.

Note: If you are using only image-based focusing, you cannot set an offset for the first wavelength.

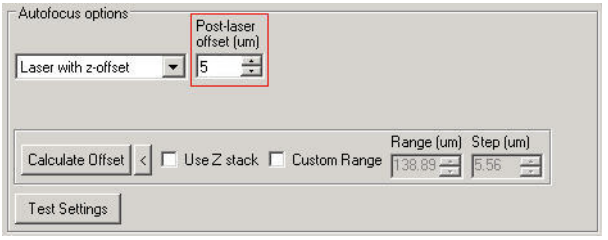
The software focuses on the sample, snaps an image, and displays a message indicating the calculated offset value. For example:



The offset may be a negative value.

7. Click **Yes** to replace the current offset value with the newly calculated value.

   The Post-laser offset field displays the newly calculated value. For example:



8. To use a custom range and step size rather than the default values, select the **Custom Range** check box, type a new range and step size, and click **Calculate Offset** again.

Note: If, in the Autofocus options section, you selected either "Laser and Image" or "Laser with Image Recovery," the offset is calculated using the range (total distance) and step size displayed in the Calculate Offset section. The offset is not calculated using the Image-based range and Max step displayed in the Autofocus section (next to the Post-laser offset value).

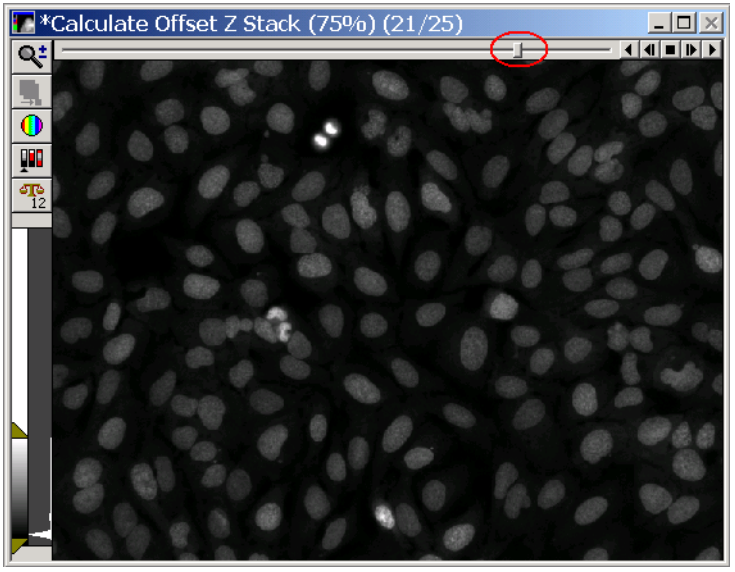
9. To have the software acquire a Z stack from which you can select the most in-focus image that will be used to calculate the offset:

- Select the **Use Z Stack** check box and click **Calculate Offset**.

The MetaXpress software acquires a Z stack of images and displays the following message:

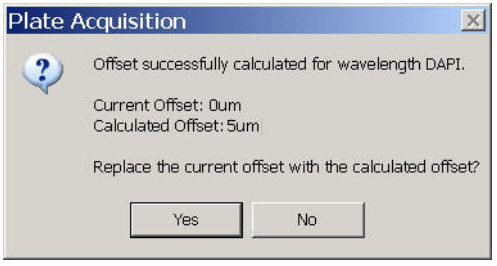
Note: To acquire fewer or more than the default number of planes (25), select the **Custom Range** check box and type new values for the range and step size of the Z stack (the number of planes that will be acquired is equal to approximately the range divided by the step size). You can acquire as many planes as needed.

- Use the slider at the top of the Z stack image window to navigate to the most in-focus image. For example:



- With the most in-focus image displayed, click **OK**.

The software calculates the offset and a message appears, indicating the newly calculated offset value. For example:



- To replace the current offset value with the newly calculated value, click **Yes**.
- Optionally, to save the Z stack, click **File > Save As**.

Tip! The Test Settings button focuses on the sample and snaps an image using the current settings for the selected wavelength.

10. On the second and subsequent wavelength tabs, repeat Steps 6-9.
11. If you enabled multiple timepoints in the **Timelapse** tab, select from the **Timelapse Acquisition** field 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.
12. 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 help page for the Journals tab in the Plate Acquisition Setup dialog.

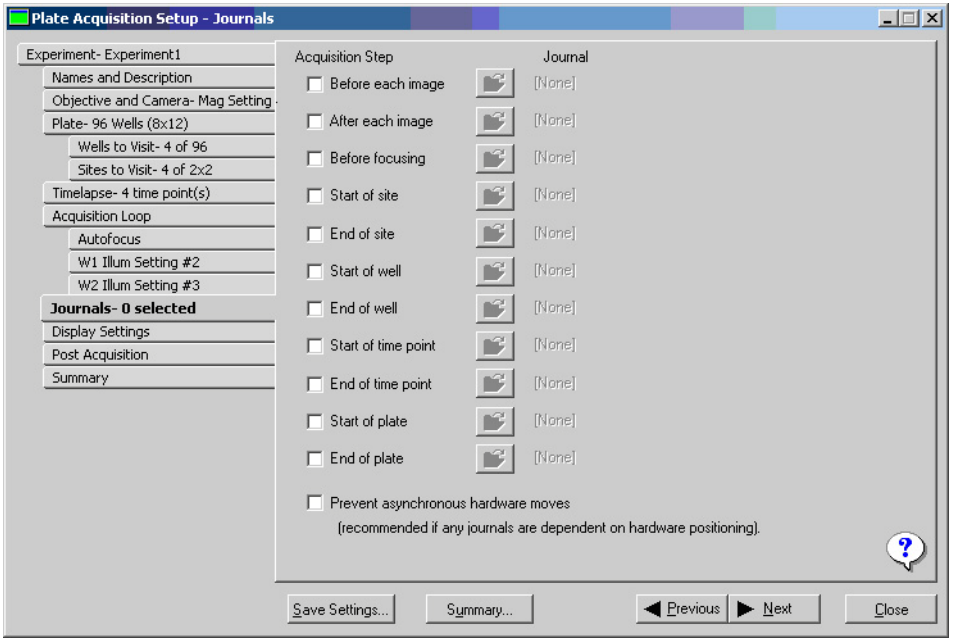


Figure 5-23 Plate Acquisition Setup dialog: Journals tab

Option	Description
Acquisition step:	Specifies that a journal should be run for the selected step. Only one journal can be assigned to each step. Select the checkbox next to the step that you want to use, and 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 clearing the checkbox for the step. To reactive a pre-assigned journal, select the checkbox.
• Before each Image	Runs only during the acquisition loop, after the illumination is set and focusing is done.

Figure 5-23 Plate Acquisition Setup dialog: Journals tab

Option	Description
• 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. Use this dialog to select and assign a journal to a step. Also use this dialog to clear a journal from a step. To assign a journal, select 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.

To configure the Journals tab

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

1. Select one of the checkboxes under the Acquisition Step to select when to run a journal.
2. Click the folder icon next to the selected acquisition step.

   The Select Screen Acquisition Journal dialog appears with the contents of the Journals folder displayed by default.
3. Select 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 Step 1 to Step 3 as needed to assign journals to run at additional acquisition steps.
5. If any of the journals you run move hardware (change shutters, move focus, etc.), select **Prevent asynchronous hardware moves** to ensure that the journals will run correctly.
6. Click **Next** to move to the next tab.

Defining Display Settings

Use the Display Settings tab to configure the MetaXpress Software 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 is unobstructed.

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

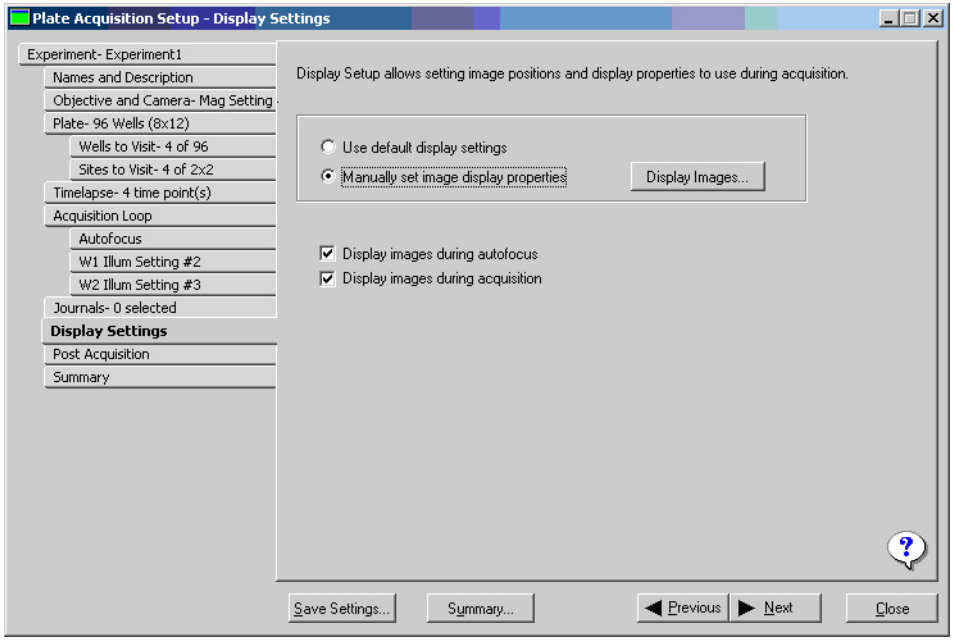


Figure 5-24 Plate Acquisition Setup dialog: Display Settings tab

Option	Description
Use default display settings	Uses the MetaXpress Software default settings for displaying images and dialogs during acquisition. Images are tiled and autoscaled and the status dialog is unobstructed.
Manually set image displays properties	Makes the Display Images button visible, which enables you to change the configuration of the MetaXpress Software desktop during acquisition.
Display Images	Previews the current display settings by opening the Screening Status dialog and acquiring an image for each wavelength. After all images have been acquired, you can change the configuration of the display by arranging the image windows and dialog and changing the size, scaling, and LUT of images. These new settings are saved and used during acquisition.
Display images during autofocus	Displays the images acquired during autofocus. Disabled by default.

Figure 5-24 Plate Acquisition Setup dialog: Display Settings tab (cont'd)

Option	Description
Display images during acquisition	Causes each image to be displayed as it is acquired. Disabled by default.

To configure the Display Settings tab

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 properties**.
2. Click **Display Images**.

   The Screening Status dialog appears and an image is acquired for each configured wavelength.
3. Change the configuration of the display by arranging the image windows and dialogs. You can change the location, size, zoom, scaling, gamma, and LUT of images. These new settings are saved and used during acquisition.
4. To display images acquired during autofocus, select **Display Images during autofocus**.

Note: The Display Images during autofocus is disabled by default.

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

Setting Post Acquisition Options

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.

Note: 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.

Note: The list of available assays and settings is the same list that is in the Assay tab of the Review Plate Data (DB) dialog.

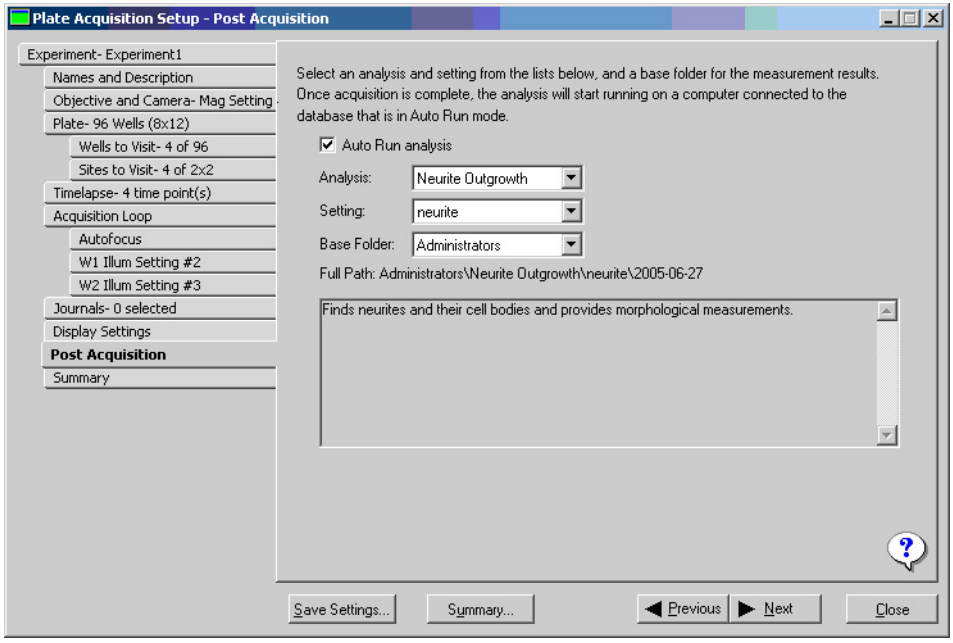


Figure 5-25 Plate Acquisition Setup dialog: Post Acquisition tab

Option	Description
Auto-Run analysis after acquisition	Activates the Analysis field that enables you to select an assay to auto-run on a separate MetaXpress Software computer after each plate that is acquired. For more information, refer to the Auto Run Mode help file.

Figure 5-25 Plate Acquisition Setup dialog: Post Acquisition tab (cont'd)

Option	Description
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 includes 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.
Base Folder	Shows available folders in the database to store measurement results.

To configure the Post Acquisition tab

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

1. Select **Auto Run analysis**.
2. Select the assay (Application module or Journal Assay) to run after acquisition from the **Analysis** field.
3. Select a setting file from the **Settings** field.

   The field below the Settings field displays a description of the settings if one exists.

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

4. Select a base folder in which to store measurement results from the **Base Folder** field. To select a new location, click **Select** and click the button to open the **Measurements Sets** dialog. 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.

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

Note: 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 or the Plate Acquisition Toolbar to perform these and other tasks.

Note: If an error message appears after you click Acquire, the most likely cause is a configuration error. Read the text in the message to determine the error.

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

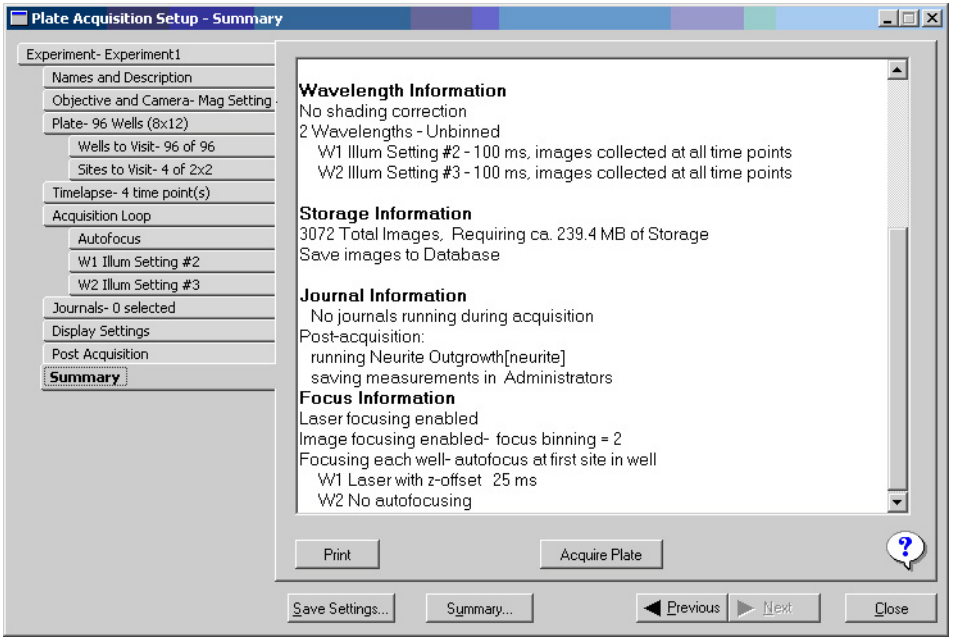


Figure 5-26 Plate Acquisition Setup dialog: Summary tab

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

To save settings and start acquisition

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

1. Click **Save Settings** to open the **Save Acquisition Settings** dialog and save the current settings to a file on the local hard drive or to the database. See Save Acquisition Setting Dialog on page 123.
2. If you want to print the settings summary, click **Print** to open the **Print Setup** dialog and then print the settings.
3. Click **Acquire Plate** to acquire images from a plate based on the settings made in the **Plate Acquisition Setup** dialog.

   The Plate Acquisition Setup dialog closes and the Screen Status dialog appears. Each image appears briefly on the MetaXpress Software desktop as it is acquired and saved to the database. After the last image is acquired and saved, the Screen Status dialog closes and the Plate Acquisition Setup dialog reopens.
4. Click **Close** to exit the **Plate Acquisition Setup** dialog.

Save Acquisition Setting Dialog

After configuring the Plate Acquisition Setup dialog, save the settings. The Save Acquisition Setting dialog enables you to save your settings to the database or a file on a local or network drive.

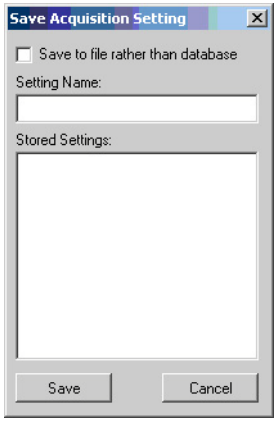


Figure 5-27 Save Acquisition Setting dialog

- **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.
- **Setting Name**: Type a State file name in this field.

- **Stored Settings**: 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 Settings Name field. If Save to file rather than database is selected, the Save button opens the Screen Acquisition State dialog.
- **Cancel**: Closes the dialog without taking any action.

To save the Plate Acquisition Settings

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 appears.
3. To save the State file on the local computer, select **Save to file rather than database** and proceed to Step 4. To save the State file to the database, go to Step 6.
4. Click **Save**.

   The Screen Acquisition State dialog appears.
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 **Setting Name** field, or select a listed state file from the **Stored Settings** field to overwrite an existing state file in the database and click **Save**.

Load Plate Acquisition Setting Dialog

The Load Plate Acquisition Setting dialog allows you access previously saved settings files that were created using the Plate Acquisition Setup dialog. You can load all or part of a saved settings file using the checkboxes to select specific conditions or groups of settings.

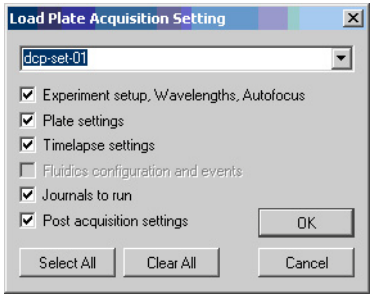


Figure 5-28 Load Plate Acquisition Setting dialog

Saved settings files can be loaded using any of the following dialogs from the Screening menu:

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

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

- **Settings File List**: Selects a saved settings file from the database or from a local file on your hard drive.
- **Checkboxes for Settings**: These checkboxes enable or disable the loading of specific settings from a settings file. The settings listed are configured on the various tabs of the Plate Acquisition Setup dialog. 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 selected settings file from the database or opens the Load Plate Acquisition Setting dialog, which enables you to select a saved settings file stored outside the database.

Note: To select a settings file stored outside the database, select From File in the Settings field.

- **Cancel**: Cancels the command and closes the dialog.

To load the Plate Acquisition Settings File

1. From the **Screening** menu, open one of the following:
   - Plate Acquisition Setup
   - Plate Acquisition and Control
   - Plate Acquisition
2. Click **Load Settings**.

   The Load Plate Acquisition Settings dialog appears.
3. If you are loading a settings file saved to the database, select the settings file from the field, select the individual settings to load selecting the checkboxes for each settings group, and then click **OK**.

   The file loads from the database and the Load Plate Acquisition Settings dialog closes.
4. If you are loading a settings file saved to your hard drive, select **From File** in the field, select the individual settings to load using the checkboxes for each settings group, and then click **OK**.
5. In the HTSSTATE folder, navigate to the state file (.HTS) you want to open and click **Open**.

   The selected state file loads and the Load Plate Acquisition Settings dialog closes.

Using a Saved Setting For a New Plate

This procedure explains how to use a previously saved settings file to rerun an experiment on a new plate. This procedure uses the Plate Acquisition and Control dialog to load the settings file and start the acquisition. You can also use Plate Acquisition, Plate Acquisition Setup, or the Plate Acquisition toolbar to perform these tasks.

Note: This procedure assumes that the settings file is configured correctly for the new plate. If you need to make changes to the settings file before acquiring, use the Plate Acquisition Setup dialog.

To load a saved plate setting and begin an acquisition

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

   The Plate Acquisition and Control dialog appears.
2. Click **Load Settings**.

   The Load Plate Acquisition Settings dialog appears.

3. If you are loading a settings file saved to the database, select the settings file to load from the field, and then select the individual settings to load from the file using the checkboxes for each settings group.

   OR

   If you are loading a settings file saved outside the database, select **From File** in the field and then select the individual settings to load from the file selecting the checkboxes for 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 will close.

   OR

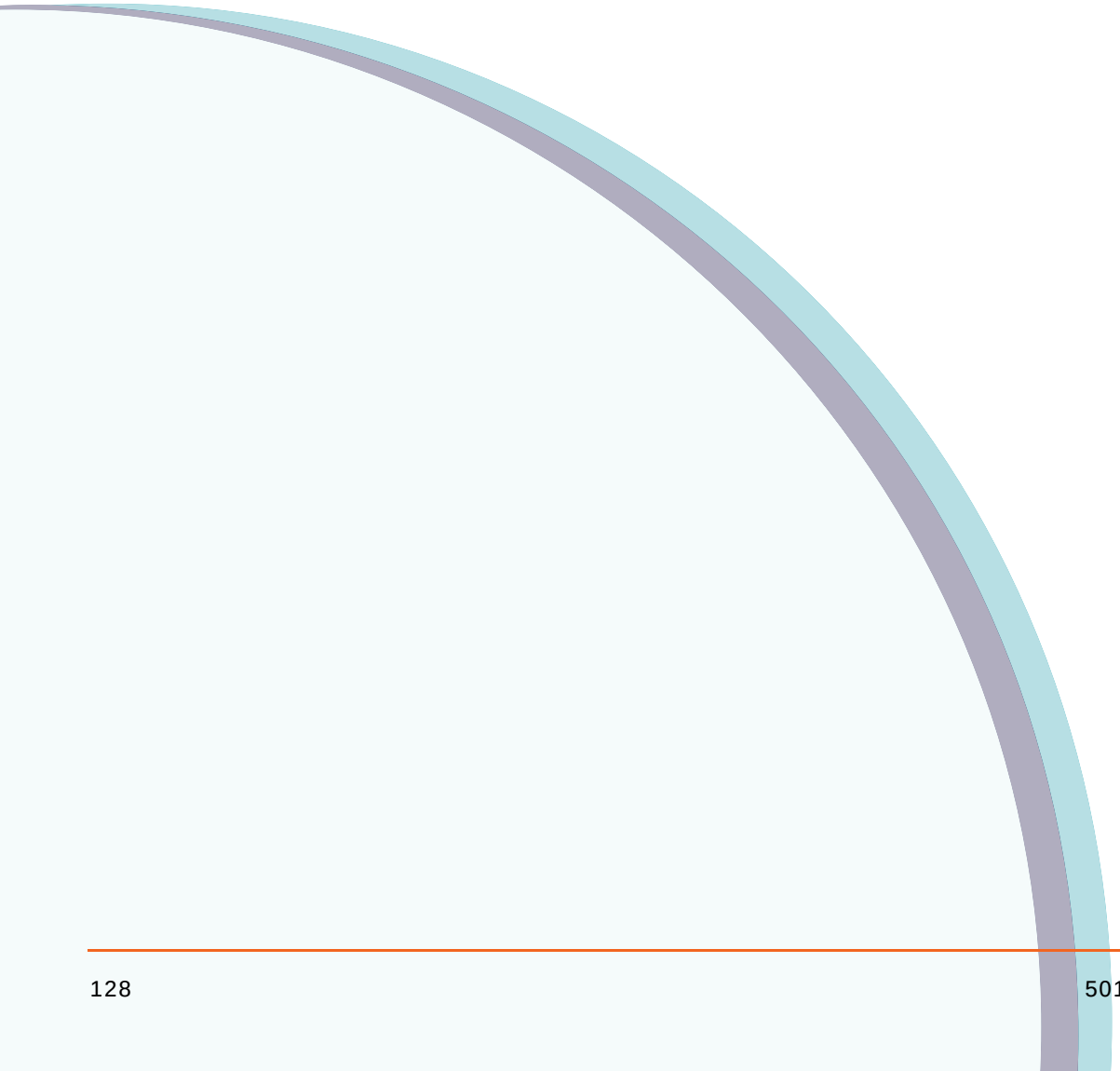
   If you are loading a settings file saved outside the database, click **OK**. In the **Load Plate Acquisition Setting** dialog, navigate to the state file (.HTS) you want to open and click **Open**. The state file you selected loads and the dialog closes.

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

Laser Focus 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 is not large enough. Increase the Bottom Variation value by 10 µm, or measure the wells in question to get a more accurate read. Repeat steps 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, select Find plate bottom only, and 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 Updating the System After Adding or Replacing an Objective on page 163.

  If you are still unable to successfully use the laser autofocus with your plate type, contact Technical Support.



Acquiring Plates

6

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

- Plate Acquisition and Control dialog
- Plate Acquisition Setup dialog: Summary tab
- Plate Acquisition dialog
- Plate Acquisition toolbar

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 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 or the Summary tab of the Plate Acquisition Setup dialog. After all settings are complete, you would most likely initiate acquisition from the Plate Acquisition dialog or the Plate Acquisition toolbar.

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

At the beginning of the process, while designing your experiment, you will most likely need to make changes to the X, Y, and Z positions to accomplish correct plate and focus alignment. The Plate Acquisition and Control dialog provides controls that enable you to simplify and expedite this process. You can also use this dialog as a starting point for configuring the Plate Acquisition Setup dialog. Click Setup to open the Plate Acquisition Setup dialog. Other controls on this dialog 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, see the following topics or refer to the application help available in the MetaXpress Software.

Using the Plate Acquisition and Control Dialog

Use the Plate Acquisition and Control dialog 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, 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 are also available in the Plate Acquisition toolbar. To display the Plate Acquisition toolbar, click **Window>Toolbars>Plate Acquisition**.

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.
- Use the Plate Acquisition and Control dialog 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 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 dialogs. This can be configured to start automatically from the Plate Acquisition Setup dialog, if desired.

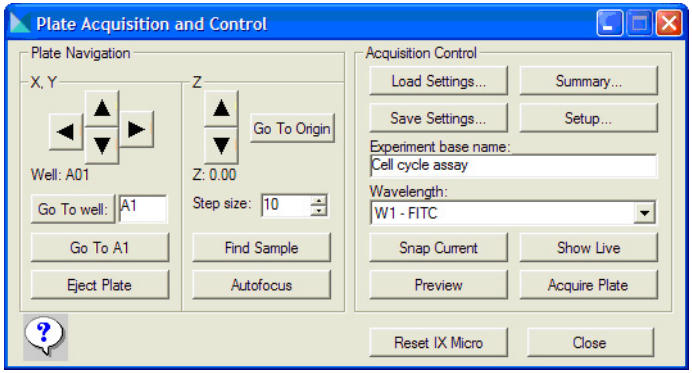


Figure 6-1 Plate Acquisition and Control dialog

Option	Description
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.
• Step size	Sets the size of the individual focus increments using 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.

Figure 6-1 Plate Acquisition and Control dialog (cont'd)

Option	Description
• Autofocus	Performs autofocus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup dialog.
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 appears. Check the boxes for the conditions and groups of settings that you want to load from the settings file, clear 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.
• 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.
• 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 appears. 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 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.

Figure 6-1 Plate Acquisition and Control dialog (cont'd)

Option	Description
• 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 and autofocusing and acquiring an image for each wavelength. After all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog 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.
Reset IX Micro	Turns off and then turns on the ImageXpress Micro instrument
Close	Closes the dialog.

To use the Plate Acquisition and Control dialog

The controls on the Plate Acquisition and Control dialog 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. Use the following procedure to familiarize yourself with the controls on the Plate Acquisition and Control dialog:

1. Click **Screening > Plate Acquisition and Control**.

   The Plate Acquisition and Control dialog appears.
2. Ensure that a plate is in place on the microscope stage.
3. Click **Go To A1** to move the plate to A1.

   OR

   Type the well number that you want to view in the **Go to well** box, and click **Go to well**. The plate moves to the desired location.

4. To change the Z-focus motor position, use the Z-control arrows.
5. Select a step size in the **Step size** field. Use a large value for course movement or a small value for fine movement.
6. Click **Go to Origin** to move the Z focus motor to its origin position as defined in the **Focus** dialog.
7. Click **Find Sample** to initiate the Find Sample focusing routine on the current well.
8. Click **Autofocus** to autofocus on the current well using the wavelength selected in the **Wavelength** field.
9. Click **Load Settings** to open the **Load Plate Acquisition Settings** dialog and load a previously saved settings file.
10. Click **Save Settings** to open the **Save Plate Acquisition Settings** dialog and save the current settings to a file on the local hard drive or to the database.
11. Click **Summary** to open the **Plate Acquisition Summary** dialog and view your current settings.
12. Click **Setup** to open the **Plate Acquisition Setup** dialog and change your acquisition settings.
13. Click the **Wavelength** field to select a wavelength that has been defined for the current setting in the **Plate Acquisition Setup** dialog.
14. Click **Snap Current** to acquire a single image with the current settings.
15. Click **Show Live** to acquire images so you can manually focus the microscope.
16. Click **Preview** to open the **Plate Acquisition Status** dialog and an **image view** dialog 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.
17. Click **Acquire** to acquire images.
18. Click **Close** to exit the dialog.

Plate Acquisition Setup dialog: Summary Tab

The Plate Acquisition Setup dialog 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. 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.

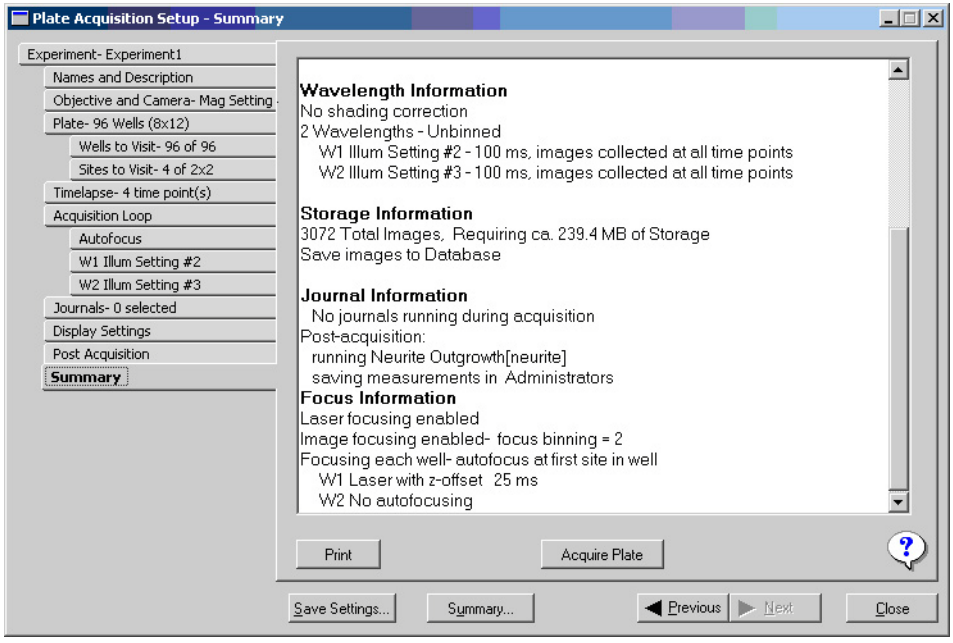


Figure 6-2 Plate Acquisition Setup dialog: Summary tab

Using the Plate Acquisition Setup dialog: 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.

Note: The information in the Summary tab is identical to the information displayed when you click the Summary button on the bottom of the dialog.

Note: 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 or the Plate Acquisition Toolbar to perform these and other tasks.

Note: If an error dialog appears after you click Acquire, the most likely cause is a configuration error. Read the text in the dialog to determine the error.

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

To use the Summary tab

1. Click **Save Settings** to open the **Save Plate Acquisition Settings** dialog 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 and then print the settings.
3. Click **Acquire** to acquire images.

   The Plate Acquisition Setup dialog closes and the Screen Status dialog appears. Each image appears briefly on the MetaXpress Software desktop as it is acquired and saved to the database. After the last image is acquired and saved, the Screen Status dialog closes and the Plate Acquisition Setup dialog reopens.
4. Click **Close** to exit the **Plate Acquisition Setup** dialog.

Plate Acquisition Dialog

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

- Select 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.

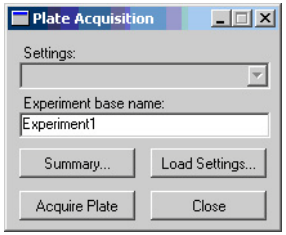


Figure 6-3 Plate Acquisition dialog

Using the Plate Acquisition Dialog

Use the Plate Acquisition dialog 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.

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

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.
- Use the Plate Acquisition and Control dialog 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 dialogs. This can be configured to start automatically from the Plate Acquisition Setup dialog if desired.

To use the Plate Acquisition dialog

1. Click **Screening > Plate Acquisition**.
   The Plate Acquisition dialog appears.
2. Ensure that a plate is in place on the microscope stage.
3. Select a Settings file to use from the **Settings** field.
4. To change the experiment name, type a name in the **Experiment Base Name** field.
5. Click **Summary** to view details about the current settings.
6. Click **Load Settings** to open the Load Plate Acquisition Setting dialog.
7. Select the checkboxes for the conditions and groups of settings that you want to load from the settings file, or click **Select All** to load all conditions.
8. Click **OK** to load your selected conditions.
9. Click **Acquire** to acquire images from a plate based on the current settings.
10. Click **Close** to exit the dialog.

Observing Acquisition Progress

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

- Screen Summary dialog
- Plate Acquisition Status dialog

Screen Summary Dialog

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

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

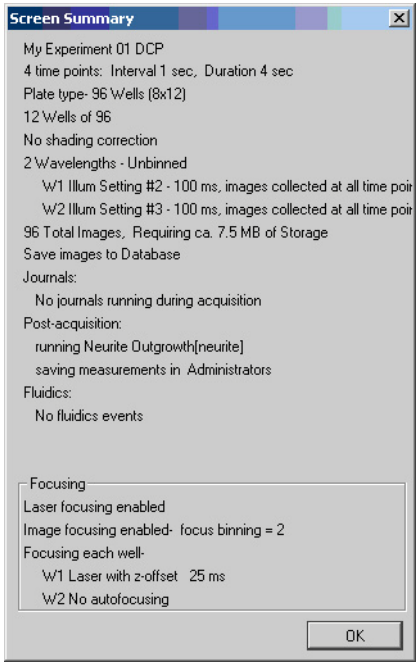


Figure 6-4 Screen Summary dialog

Plate Acquisition Status

This dialog 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, click Preview in the Plate Acquisition and Control dialog.

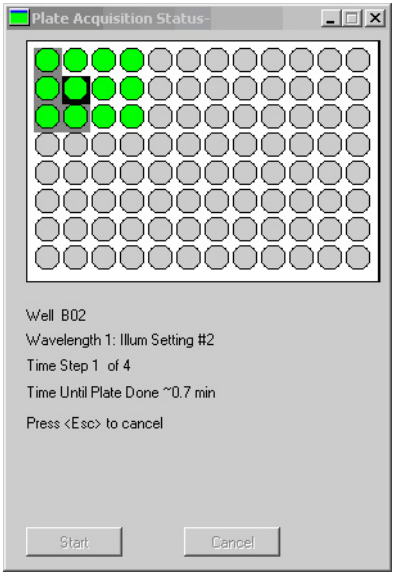


Figure 6-5 Plate Acquisition Status dialog

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

Using the Plate Acquisition Toolbar

The Plate Acquisition toolbar contains tools used to control the hardware on the ImageXpress Micro System.

If the Plate Acquisition toolbar is not currently loaded, click Window > Toolbars > Plate Acquisition.

Figure 6-6 Plate Acquisition toolbar

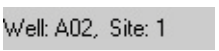
Icon	Description
	Moves the stage up in one-well increments
	Moves the stage down in one-well increments
	Moves the stage forward in one-well increments
	Moves the stage backward in one-well increments
	Moves the stage forward in one-site increments
	Moves the stage backward in one-site increments
	Moves the stage to the load/eject position.
	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

Figure 6-6 Plate Acquisition toolbar (cont'd)

Icon	Description
Wavelength:	Selects the wavelength to use for the 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 appears. Check the boxes for the conditions and groups of settings that you want to load from the settings file, clear 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 and autofocusing and acquiring an image for each wavelength. After all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog 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

Customizing the MetaXpress Software Application

7

A powerful feature of the MetaXpress® Software 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 the MetaXpress Software application, 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 Taskbars
- 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 the MetaXpress Software application. They are NOT related in any way to configuring users and groups within the database. For information on setting up users and groups with the database, refer to the *MDCStore High Content Data Management Solution Database Schema Installation and Update Guide* included on the MetaXpress Software installation USB flash drive.

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 the MetaXpress Software application. 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 7-1 shows the Multiple User Configuration screen in the Meta Imaging Series Administrator.

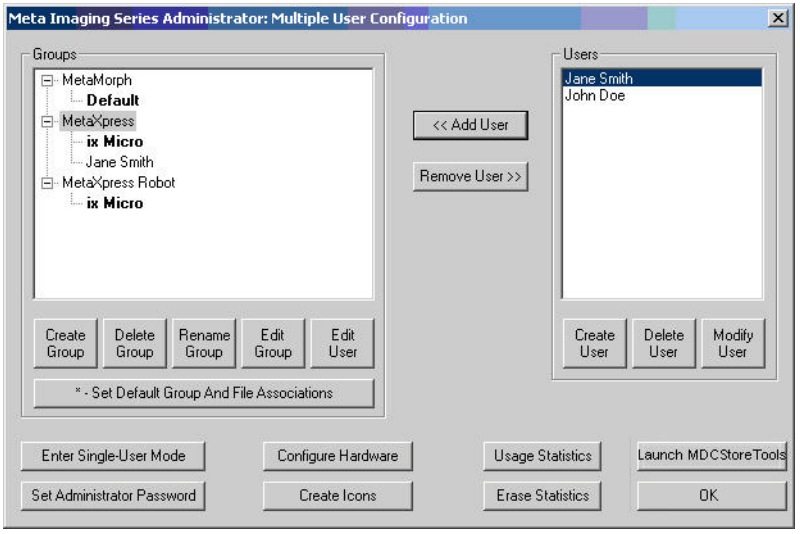


Figure 7-1 Sample of the Multiple User Configuration screen

This sample shows a system with the following groups defined:

- MetaMorph: This is the default MetaMorph® Software application group.
- MetaXpress: This is the ImageXpress Micro System and MetaXpress Software application 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 the application help (press F1 to access the help).

Creating an Offline Version of the MetaXpress Software Application

Molecular Devices recommends creating an offline group for MetaXpress Software users. This offline group has no hardware settings and is useful for analysis of acquired images. Since the offline group has no hardware settings, it does not attempt to establish communication with the other MetaXpress components. This allows the application to 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 Software 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 dialogs in the Meta Imaging Series Administrator, press the F1 key to access the application help for the active dialog.

1. From the Windows **Start** menu, click **All 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 appears.

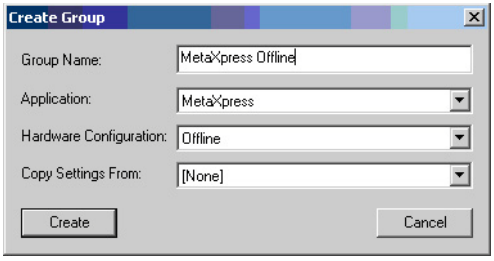


Figure 7-2 Create Group dialog

4. Type a group name into the **Group Name** field, for example, MetaXpress Offline.

5. Select **MetaXpress** from the **Application** field.
6. Select **Offline** from the **Hardware Configuration** field.
7. Select **None** from the **Copy Settings From** field.
8. Click **Create**.

   The Create Group dialog closes and the new group is listed in the Groups field.
9. Select the new group in the **Groups** list, select a user from the **Users** list, and then click **<<Add Users** to add the user to the new group. Add users to the groups as needed.

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 Software 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 Software desktop. These shortcuts can then be copied directly to the MetaXpress Software 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 Software desktop:

1. From the Windows **Start** menu, click **All Programs > MetaXpress >Meta Imaging Series Administrator**.
2. In the Meta Imaging Series Administrator, click **Create Icons** to create the icons.
3. Click **OK** to exit the Meta Imaging Series Administrator.
4. Double-click the **MetaXpress 5.0** shortcut on your desktop.
5. In the MetaXpress 5.0 folder, confirm that a shortcut for the group that was created in the Creating an Offline Version of the MetaXpress Software Application on page 145 is listed.
6. Select and right-click the shortcut (for example, MetaXpress Offline), and then select **Send To > Desktop**.

   The shortcut is created on the 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 desktop.

Custom Toolbars and Taskbars

Now that you have the groups configured and the 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 the toolbars:

1. From the Windows **Start** menu, click **All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. In the Meta Imaging Series Administrator, select the group that you want to edit the toolbar in from the **Groups** list and click **Edit Group**.
3. In the **Edit Group** dialog, click **Drop-ins/Toolbars**.
4. In the **Configure Dropins/Toolbars** dialog, click the **Toolbars** tab, and clear 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 a mouse click to select multiple commands, and then drag the commands to the appropriate toolbar folder.

7. Click **OK** when finished.
8. Click **Yes** in the dialog to confirm that you want the users in the group to use the modified configuration.
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 the MetaXpress Software application.

Creating Taskbars

Taskbars are created directly in the MetaXpress Software 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. Molecular Devices 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 Software application.
2. Click **Journal > Taskbars > Create Taskbar**.

   The Taskbar Editor dialog and Taskbar window open. Position them so that you can see both the dialog 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.

Note: If you selected Journal or Taskbar as the Category, the directory names are 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 to add it to active button in the taskbar as shown in Figure 7-3.

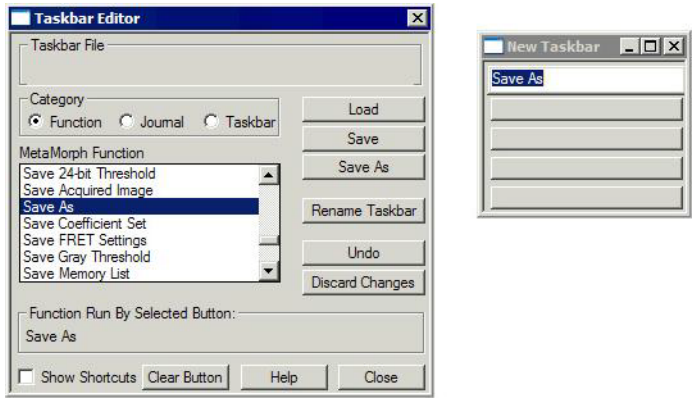


Figure 7-3 Adding a function to a taskbar

7. Repeat Step 5 and Step 6 for each item you want to add to the taskbar.
8. If necessary, click **Undo** to undo the last command or click **Clear Button** to clear an item from the active button.
9. If you want to rename the taskbar, click **Rename Taskbar**, and type the new name in the **Taskbar Title** field of the **Rename Taskbar** dialog. Click **OK**.
10. Click **Save**, and type a name in the **File Name** field of the **Save As** dialog. Navigate to the appropriate drive and folder, if necessary. Click **Save**.
11. To use the new taskbar (or a different taskbar) immediately, click **Journal > Taskbars > Load Taskbar**. Select the desired taskbar file and click **Open**.
12. Click **Close** to close the **Taskbar Editor** dialog.

Default Paths for Data

The Configure Default Paths command in the MetaXpress Software application is used to change the default file paths for each group or user. You can modify these paths so that the users on the system have their own dedicated data folders. These folders contain log files, calibration settings, and other data unique to each user. Molecular Devices 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: Molecular Devices 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 the MetaXpress Software application.
2. Click **Edit > Configure Default Paths**.

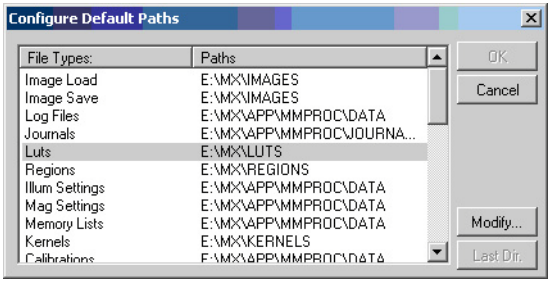
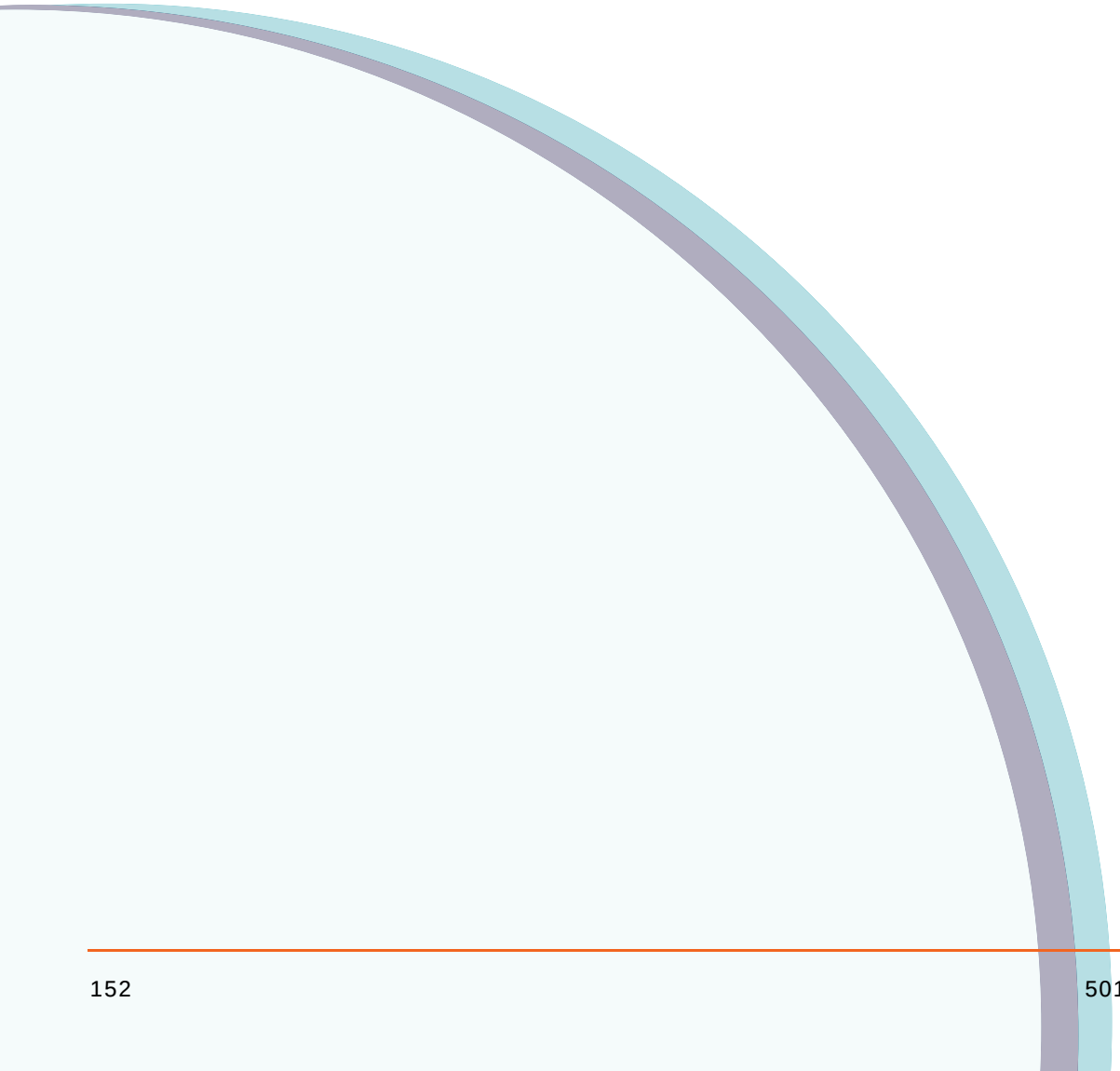


Figure 7-4 Configure Default Paths dialog

3. In the **Configure Default Paths** dialog, select the item whose default file path you want to modify.
4. Click **Modify**.

   The Browse for Folder dialog appears.
5. Select the folder that you want to use for the new default path, or click **New** to create a new folder.
6. Click **OK**.
7. In the **Configure Default Paths** dialog, click **OK** to apply the new default path and close the dialog.



Maintaining the ImageXpress Micro Instrument

8

User Safety Instructions

To avoid personal injury or damage to your equipment during user service and maintenance, it is important to strictly observe the safety information outlined below:

- Ensure that the power supply for the ImageXpress® Micro instrument is turned OFF and that the power cable is unplugged. If the MetaXpress® Software is running, first exit the program before turning off the instrument.
- Disconnect the USB connection to the hardware server (host) PC, and turn off any attached peripherals, such as the robot plate-loading arm.
- Access ONLY the user-serviceable components inside the enclosure. Avoid contact with other components as they can be damaged or knocked out of alignment.

CAUTION! Be sure not to touch the autofocus laser.

- Keep liquids, vapor, and dust well away from the interior of the instrument. Do not attempt to clean inside the enclosure.
- Do not leave the interlocked access panels open for extended periods of time.
- Ensure all components and access panels are replaced before restarting the instrument.

Light Source

The ImageXpress Micro System is equipped with an external light source. The light source for the Standard model of the ImageXpress Micro System is equipped with a fuse-protected 300 W Xenon lamp.

Note: The XL model of the ImageXpress Micro System is equipped with an external solid-state light source that has a rated lifetime of more than 15000 hours. There are no user-replaceable parts in this light source.

Changing the Lamp

When it is time to replace the lamp in the ImageXpress Micro System light source, be aware that the entire lamp assembly must be replaced, and not the bulb alone. For the Xenon lamp, this includes the bulb and heat sink.

Instructions for changing the lamp are available in the Molecular Devices knowledge base. Visit www.moleculardevices.com and follow the links to the knowledge base.

WARNING! In the event that a lamp requires replacement, ensure that you have allowed the lamp to cool for at least 30 minutes. The lamp generates an extreme amount of heat and attempting to remove the lamp immediately after use can result in injury.

Changing the Light Guide

A liquid light guide couples the light from the light source to the illumination optics in the ImageXpress Micro System unit. The light source assembly is precisely aligned with the light guide during manufacture, and requires no further position adjustment. However, the light guide needs to be replaced if it is worn or damaged.

Instructions for replacing the light guide are available in the Molecular Devices knowledge base. Visit www.moleculardevices.com and follow the links to the knowledge base.

Changing the Light Source Fuse

The ImageXpress Micro System light source ships with a spare fuse, if applicable.

Instructions for replacing the fuse are available in the Molecular Devices knowledge base. Visit www.moleculardevices.com and follow the links to the knowledge base.

Replacing the Shutter (standard model only)

A solenoid-activated mechanical shutter controls the exposure of the sample to excitation light to minimize sample degradation and photobleaching. The shutter has an expected lifetime of 1 million cycles.

Instructions for replacing the shutter are available in the Molecular Devices knowledge base. Visit www.moleculardevices.com and follow the links to the knowledge base.

Filters Cubes

If you decide to replace or add to any of the optical components in the factory-standard ImageXpress Micro instrument, there are two procedures that need to be completed:

- Changing the component within the instrument.
- Updating the software to reflect the new hardware configuration.

Changing Filter Cubes

The ImageXpress Micro instrument's filter cubes are mounted in a five-position slider within the instrument enclosure. Filter cubes are delicate components, and special care is required when handling them. Please follow these outlined instructions for adding or replacing a filter cube.

You can leave the lamp powered on during this procedure.

CAUTION! We advise wearing powder-free gloves during the following procedure to prevent skin oils from damaging optical coatings. Read and follow the User Safety Instructions on page 153 for safe user-service procedures.

1. From the Windows **Start** menu, click **All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. Click **Configure Hardware**.
3. Click **Configure Devices**.
4. Select **ImageXpress Micro Filter Cube Changer**.
5. Click **Settings**.
6. Click the **Eject Filter Cubes** button.
7. Close the Meta Imaging Series Administrator program.
8. Power OFF the ImageXpress Micro instrument at the main power switch, which is located on the external power supply.
9. Open the door on the front of the instrument that allows access to the filter cube changer.

10. Pull up on the front latch to release the filter cube cassette from the filter cube changer.

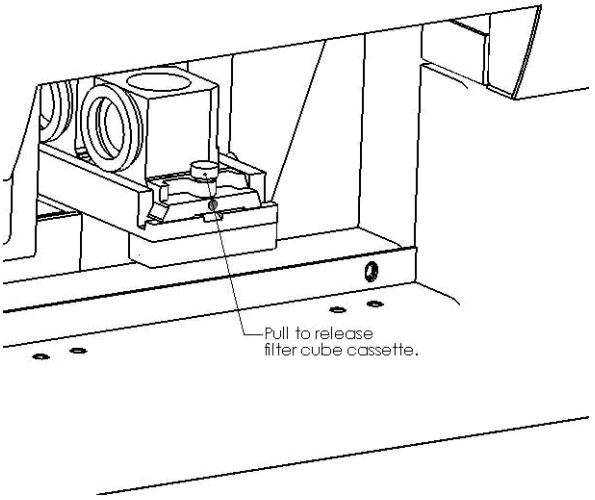


Figure 8-1 Removing filter cubes

11. Remove the filter cube cassette.
12. Loosen the hex screw that holds the filter cube in place, and remove the cube.
13. Slide the new cube into place and lightly tighten the hex screw.
14. Place a finger underneath the ejected filter cube changer in the instrument to hold it in place. Carefully line up the filter cube cassette with the changer, and push the filter cube cassette back into place until the latch engages.

CAUTION! If you feel resistance while replacing the filter cube cassette, do not proceed. Remove it and recheck to make sure that it is lined up correctly with the changer.

15. Power ON the ImageXpress Micro instrument at the main power switch, which is located on the external power supply.
16. Update the software to reflect the new hardware configuration. See Updating the System After Adding or Replacing a Filter Cube on page 157.

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 and then update settings within the main MetaXpress Software program.

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, click **All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. Select **MetaXpress** from the **List of Groups** field.
3. Click **Configure Hardware**.
4. Click **Install System Devices**.
5. From the **Installed Devices** list, select **ImageXpress Micro Filter Cube** and click **Settings**.

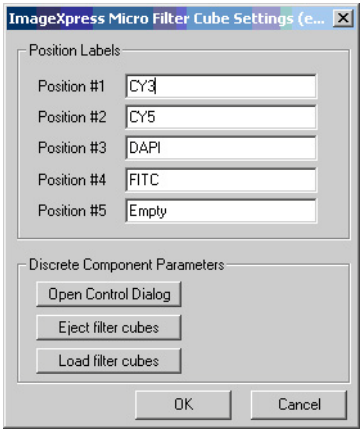


Figure 8-2 ImageXpress Cube Settings dialog

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

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

8. In the **Configure Hardware** dialog, ensure that the hardware settings you are using are selected in the **Hardware Settings** list and then click **Configure Devices**.

   The User Settings hardware configuration dialog appears.
9. From the **Claimed Devices** list, select **ImageXpress Micro Filter Cube** and click **Settings**.

   The ImageXpress Micro Filter Cube Settings dialog appears.
10. Again, edit the name of the filter cube you are adding or replacing in the appropriate **Position Labels** field.
11. Click **OK** to close the **User Settings** dialog and return to the **Configure Hardware** dialog.
12. Click **OK** to exit each dialog and close the Meta Imaging Series System Administrator.

Updating Illumination Settings

Complete the following procedure to update illumination settings within the MetaXpress Software application:

1. Open the MetaXpress Software application and log into the database.
2. Click **Devices > Configure Illumination**.

   The Configure Illumination dialog appears.

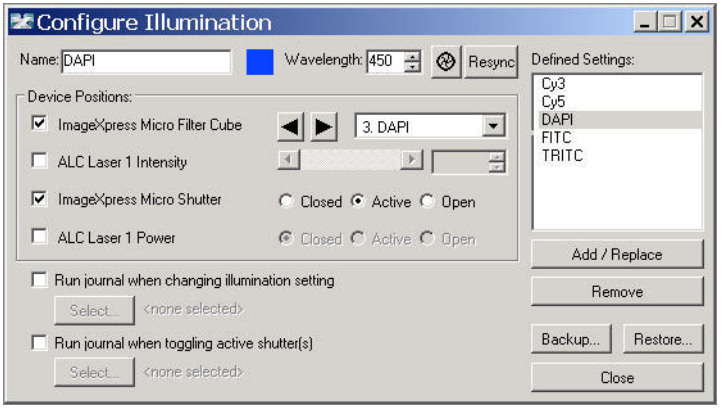


Figure 8-3 Configure Illumination dialog

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. Select the **ImageXpress Micro Shutter** checkbox and select **Open** for the shutter. If you are doing a florescence experiment with transmitted light, select **Closed**.
5. Enter the name of the new filter in the **Name** field.
6. Enter the emission wavelength of the new filter in the **Wavelength** field.
7. Click **Add/Replace** to add this setting to the **Defined Settings** list.
8. If you replaced a filter with an existing setting, select the old setting from the **Defined Settings** field and click **Remove**.
9. After the settings are updated, click **Backup**.
10. Click **Close**.

Objectives

If you decide to add or replace any of the optical components in the factory-standard ImageXpress Micro System, there are two parts to the procedure:

- Changing the component within the instrument.
- Updating the software to reflect the new hardware configuration.

CAUTION! Read the section Correct Objective Placement before installing or replacing an objective.

Correct Objective Placement

Molecular Devices recommends that users place the ELWD objectives (20X, 40X, 60X) or any other objective with a correction collar in one of the two outer positions (1 or 4) so that the correction collar can be accessed from one of the side panels.

Changing Objectives

The ImageXpress Micro instrument's objectives are mounted in a four-position linear selector. Objectives are very delicate components, and special care is required when handling them. Objectives can be added or replaced by following these steps.

You can leave the lamp powered on during this procedure.

CAUTION! We advise wearing powder-free gloves during the following procedures to prevent skin oils from damaging optical coatings.

1. Read and follow the User Safety Instructions on page 153 for safe user-service procedures.
2. Read and follow Correct Objective Placement on page 160.
3. In the MetaXpress Software or the Meta Imaging Series Administrator move the objective changer to an appropriate position for installing the objective.
   - Selecting position 1 (the position on the right) moves the objectives to the left.
   - Selecting position 4 (the position on the left) moves the objectives to the right.
4. Exit the MetaXpress Software and turn off the ImageXpress Micro instrument at the main power switch, which is located on the instrument's external power supply.

5. Place the new objective in its protective casing on a clean work area surface near the front of the ImageXpress Micro instrument.

   While moving objectives in and out, beware of the free-moving stage. It slides around loosely when the instrument is powered off. This can be a hazard to the objective in your hand.
6. Remove the left or right (as appropriate) side panel of the instrument by grasping the handle, pulling it away from the instrument, and supporting the back of the door with your other hand.
7. Reach in and unscrew any objective you want to remove.
   - To access position 2, you need to remove the objective in position 1.
   - To access position 3, you need to remove the objective in position 4.
8. Set the objective's correction collar, if applicable.
9. Reach in and screw in the objective.
10. Replace the side door by aligning the tabs at the back of the door, and then snapping the front of the door into place.
11. Turn ON the ImageXpress Micro instrument at the main power switch, which is located on the instrument's external power supply.
12. Update the software to reflect the new hardware configuration. See Updating the System After Adding or Replacing an Objective on page 163.

Cleaning Objectives

In the event that debris or contaminants have collected on an objective, follow these instructions for cleaning the objective lens:

1. Read and follow the User Safety Instructions on page 153 for safe user-service procedures.
2. In MetaXpress Software or the Meta Imaging Series Administrator Software select the desired objective.
3. Open the top door.
4. Exit MetaXpress Software and turn off the ImageXpress Micro instrument at the main power switch, which is located on the instrument's external power supply.
5. To remove dust, use compressed air to blow dust contaminants off objectives.

   CAUTION! Do not use a product that disperses aerosol propellants or fluid onto the lens surface.

CAUTION! Do not invert the compressed air can, as that disperses aerosol propellants.

6. To wipe the objective free of contaminants, use lens paper and solvent of choice. If unsure which solvent to use, consult the objective manufacturer for preferred cleansing solvent and procedure.

CAUTION! Do not use Kimwipes to wipe a lens.

Using Oil-Immersion Objectives

Oil-immersion objectives can be used with the ImageXpress Micro System for research-mode imaging. Please consult with your sales representative for ordering information.

To apply oil to the objective:

1. Eject the plate to open the top door.
2. Remove any plates in the system.
3. Sparingly add oil to the top of the appropriate objective using a dropper bottle.
4. Insert the sample, either with a thin glass coverslip or in a microplate with a thin glass bottom. Oil-immersion objectives are not compatible with plastic microplates.

In the MetaXpress Software, slowly step up the objective until you are near to focus. Please note that oil-immersion objectives are not recommended for scanning entire microplates.

When you are done with the oil-immersion objective, eject the plate, remove the sample, and clean the top of the objective with a piece of lens paper.

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 Software program. If the objective you are replacing was the one used to determine the plate bottom reference point, you will also need to repeat the procedure Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 167, or contact Molecular Devices for technical 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, click **All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. Select **MetaXpress** from the **List of Groups** field.
3. Click **Configure Hardware**.
4. Click **Install System Devices**.
5. Select **ImageXpress Micro Objective** from the **Installed Devices** list and click **Settings**.

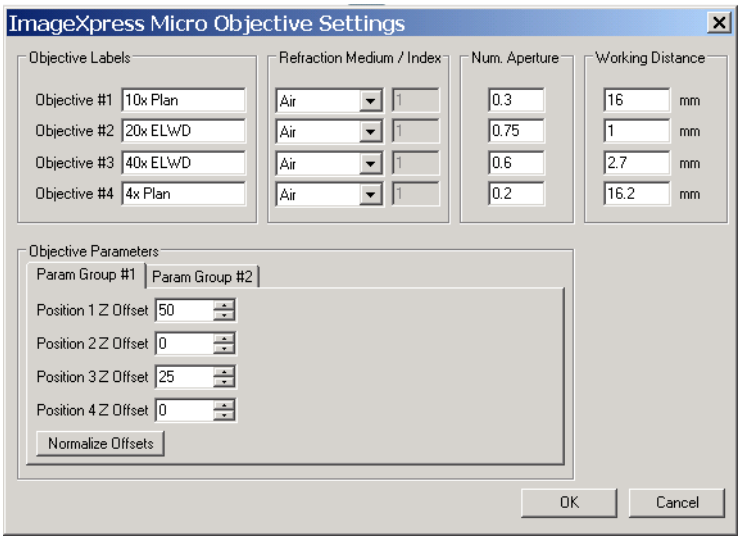


Figure 8-4 ImageXpress Objective Settings dialog

6. If necessary, edit the text 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 Step 6 and Step 8. You will need to enter this information again for your specific hardware settings.

9. Click **OK** to close the **ImageXpress Micro Objective Settings** dialog.
10. Select **ImageXpress Micro** from the **Installed Devices** list and click **Settings**.

    The ImageXpress Micro Settings dialog appears.
11. Ensure that the **Parameter Group #1** tab is active and select the **Maintenance Mode** checkbox.
12. Click **OK**, and then click **OK** again as needed to return to the **Configure Hardware** dialog.

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

13. In the **Configure Hardware** dialog, ensure that the hardware settings you are using are selected in the **Hardware Settings** list and then click **Configure Devices**.

    The User Settings hardware configuration dialog appears.
14. Double-click **ImageXpress Micro Objective** in the **Claimed Devices** list.

    The ImageXpress Micro Objective dialog appears.
15. Enter the same information about the new objective that you entered in Step 6 and Step 8.
16. Click **OK**, and then click **OK** as needed to exit the **Meta Imaging Series Administrator** and continue to Configuring Parfocality after Changing Objectives on page 165.

Configuring Parfocality after Changing Objectives

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

1. Open the MetaXpress Software application.
2. Open the following dialogs:
   - **Devices > Configure Magnification**
   - **Acquire > Acquire**
   - **Devices > Stage > Move Stage to Absolute Position**
3. If the following toolbars are not open, click **Window > Toolbars** and select the **Device Control** and the **Plate Acquisition** toolbars.
4. Click **Devices > Configure Magnification**.
5. If not selected, select the checkbox next to the **ImageXpress Micro Objective**.

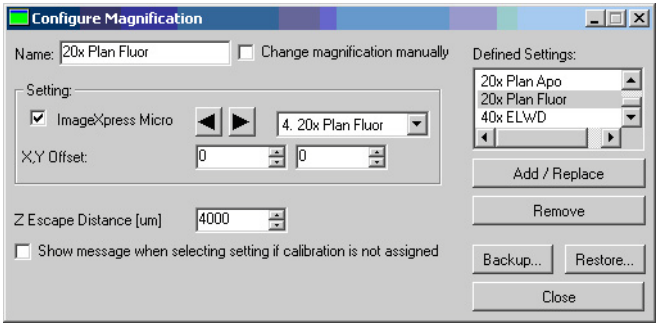


Figure 8-5 Configure Magnification dialog

6. Select the objective with the highest numerical aperture (NA) that is in **position #1** from the field.
7. Select the **FITC** (or other visible light) illumination setting from the **Illum** field on the **Device Control** toolbar, as shown in Figure 8-6.

Figure 8-6 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 instrument on to the stage, and then click the **Stage Load / Eject** button again to return the stage to its previous position.
10. In the **Acquire** dialog, click **Show Live** to open a live image window.
11. In the **Move Stage to Absolute Position** dialog, use the **Current Position X**, **Y**, and **Z** controls to find and focus a sample in the live image window.

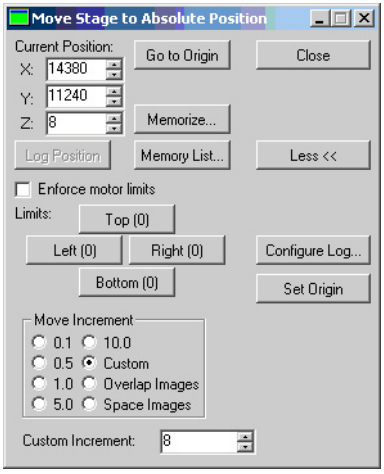


Figure 8-7 Move Stage to Absolute Position dialog

12. When the sample is in focus, write down the **Current Position Z** value in the **Focus Value** column in Table 8-1.

Table 8-1 Objective Focus Value

Objective Number	Focus Value
1	
2	
3	
4	

Note: You will need to refer to these values later in subsequent procedures.

13. Use the field in the **Configure Magnification** dialog to switch to the objective in **position #2** with the next highest NA.

Note: It is important that you use the same filter set for all objectives.

14. Repeat Step 9 to Step 12 for each objective, writing down the focus values for each in Table 8-1. You will use these values to determine the Z-offsets for each objective (which is different than configuring Z offsets for laser autofocus).

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

15. After you have written down the focus values, close the MetaXpress Software application.
16. If the objective you are changing was the one used to determine the plate bottom reference point, see Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 167.

    OR

    If the objective was NOT the one used to determine the plate bottom reference point, see Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 173.

Determining the Plate Bottom Reference Point after Changing the Reference Objective

The plate bottom reference point is a setting that the MetaXpress Software application 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.

Note: Molecular Devices recommends that you leave the reference objective in place and only replace other objectives.

Note: Molecular Devices recommends that you contact Technical Support before attempting this procedure.

Note: You do NOT need to perform this procedure unless you replaced the reference objective. If you did not replace the reference objective, see Editing the Objective Settings in the Meta Imaging Series System Administrator on page 163.

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:\MX5\Hardware** by default). The line is:

PlateBottomReferenceObjective=X

where X is the position of the objective used. If you need 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. Enter Maintenance mode. For information on how to enter Maintenance mode, see Editing the Objective Settings in the Meta Imaging Series System Administrator on page 163.
2. Remove any plates from the stage, and load one of the flat-field correction (FFC) plates that shipped with your system.
3. Click **Device > Configure Magnification**.

The Configure Magnification dialog appears.

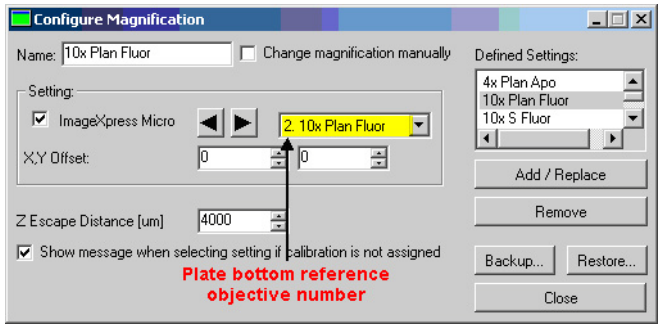


Figure 8-8 Configure Magnification dialog with objective # highlighted

4. Select the objective you want to use as the reference from the field, and write down the plate bottom reference objective number (highlighted in Figure 8-8) in Table 8-2.

Table 8-2 Plate Bottom Reference Objective

Plate Bottom Reference Objective

Note: You will refer to this value in Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 173.

5. Close the **Configure Magnification** dialog. If a dialog appears prompting you to replace the stored setting, click **No**.
6. Open the following dialogs if they are not already open:
   - **Devices > Stage > Move Stage to Absolute Position**
   - **Devices > Focus**
7. In the **Move Stage to Absolute Position** dialog, 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, select the **Autofocus** tab, and then click **Configure Laser**.

The Configure Laser Autofocus dialog appears.

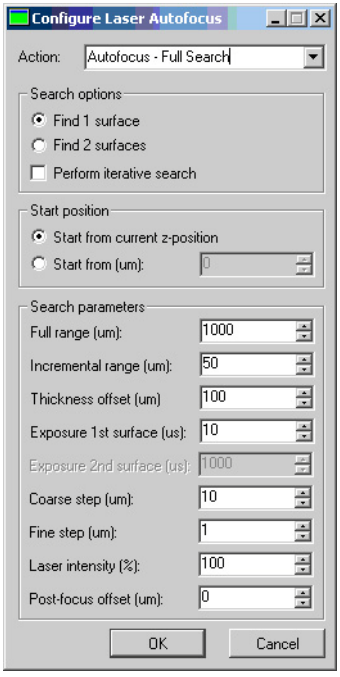


Figure 8-9 Configure Laser Autofocus dialog

10. Select **Start from current z-position**.
11. Set the **Full range** value to **1000** microns (µm).
12. Set the **Exposure 1st surface** value to **10** microseconds (µs).
13. Set the **Coarse step** value to **10** microns (µm).
14. On the **Autofocus** tab of the **Focus** dialog, 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 8-10.

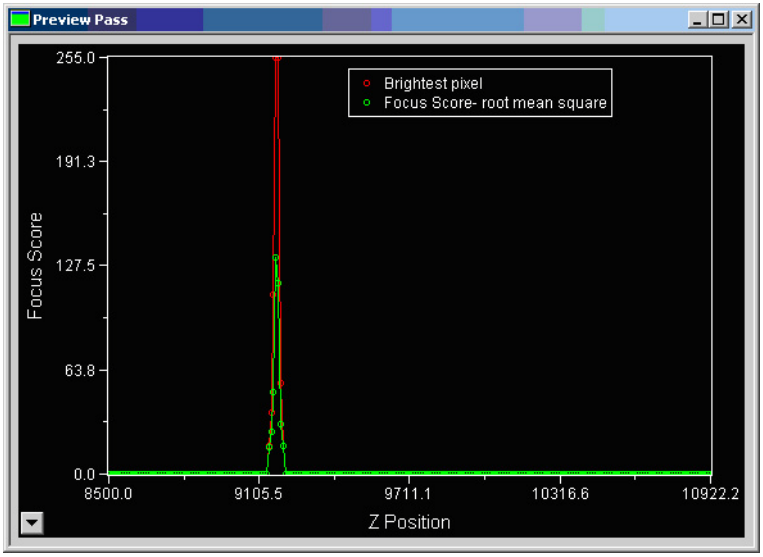


Figure 8-10 Preview Pass dialog

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, go to Step 16. If there is no peak, try the following:

- In the **Focus** dialog, increase the **Current Position** value by 1000 and click **Preview Pass**. Repeat as needed until you cover a range between 8000 and 12000. If there is a peak in the graph, go to Step 16. If there is no peak, continue to the next step.
- Try moving to a new area of the FCC plate and repeat Step 14. 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 Technical 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 as a tool tip as shown in Figure 8-11. This gives the Z-position of the plate bottom. In this example, the current Z-position value is 9172.8.

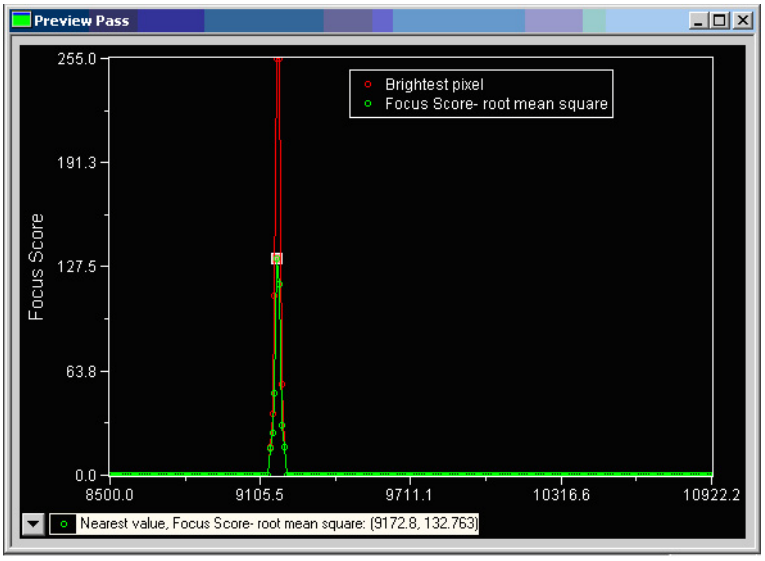


Figure 8-11 Z-position of peak

17. Write down the Z-position value for the center position in Figure 8-3.

Table 8-3 Plate Bottom Reference Point

	Position	X Value	Y Value	Z Value
1	Center	64000	33000	
2	Upper Left	150000	11000	
3	Lower Left	150000	74000	
4	Upper Right	114000	11000	
5	Lower Right	114000	74000	

18. Repeat Step 7 through Step 17 for each corner of the FCC plate. Enter the X and Y values in Figure 8-3 into the **Move Stage to Absolute Position** dialog. Write down the Z-position values (rounded off) for each corner in the Z Value column of Table 8-3.
19. Circle the lowest Z value in Figure 8-3.

    This value is the Plate Bottom Reference Point. You will refer to this value in Entering the Focus Objective Values in the Meta Imaging Series Administrator on page 174.
20. Exit the MetaXpress Software application and continue to Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 173.

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 Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 167.

1. Click **Start > All Programs > Accessories > Notepad** and then open the **MetaXpress.ref** file in the MetaXpress **Hardware** folder (**C:\MX5\Hardware** by default).

   Note: Make sure that the copy of the **MetaXpress.ref** that you open is located in the **Hardware** folder of the MetaXpress folder (For example, **C:\MX5\Hardware**) and NOT in the root directory.

2. Edit the following line in the file, replacing the number with the objective position you entered in Table 8-2.

   ```
   [system calibration]
   PlateBottomReferenceObjective=4
   ```
3. Save the modified file and close Notepad.

   Note: Do NOT use the Save As option in Notepad, as this 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 procedures. You also need to turn off Maintenance mode before you can continue to configure the device. Complete the following procedure to turn off Maintenance mode and enter the values in the Meta Imaging Series Administrator.

1. Start the Meta Imaging Series System Administrator.
2. Click **Configure Hardware**, and then click **Install System Devices**.
3. Double-click **ImageXpress Micro** in the **Installed Devices** list.
4. Clear **Maintenance Mode**.
5. Click **OK** to exit the **ImageXpress Micro** dialog
6. Double-click **ImageXpress Micro Objective** in the **Installed Devices** list.

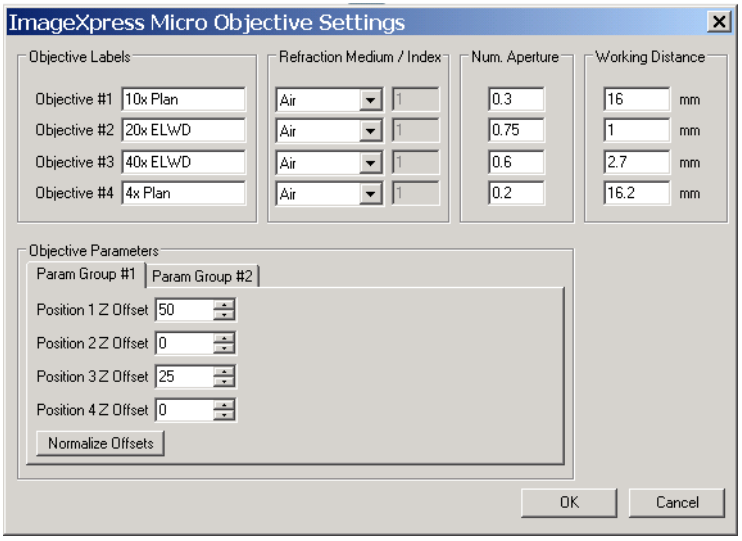


Figure 8-12 ImageXpress Objective Settings dialog

7. Type the focus value for Objective #1 from Table 8-1 into the **Position #1 Z Offset** field.
8. Repeat Step 7 for **Position #2** to **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 might 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.
11. If you needed to complete the Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 167, continue to Step 12.

    OR

    If you did not change or replace the reference objective, go to Step 15.
12. Double-click **ImageXpress Micro Z** in the **Installed Devices** list.

    The ImageXpress Micro Z dialog appears.
13. Type the value you circled in Step 19 of the Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 167 into the **Plate Bottom Reference** field.
14. Click **OK** to exit the **ImageXpress Micro Z Settings** dialog, and then click **OK** to return to the **Configure Hardware** dialog.

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

15. From the **Configure Hardware** dialog, ensure that the hardware settings you are using are selected in the **Hardware Settings** list and then click **Configure Devices**.

    The User Settings hardware configuration dialog appears.

16. Double-click **ImageXpress Micro Objective** in the **Claimed Devices** list.

    The ImageXpress Micro Objective dialog appears.

17. Edit the **Objective Labels**, **Refraction Medium / Index**, and **Num. Aperture** fields with the same values you entered in Step 6 to Step 8 of Editing the Objective Settings in the Meta Imaging Series System Administrator on page 163.

18. Repeat Step 7 through Step 9 to enter and normalize the Z offsets.

19. If you needed to complete the Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 167, double-click **ImageXpress Micro Z** in the **Claimed Devices** list and enter the Plate Bottom Reference number as in Step 13.

20. Click **OK** to exit each dialog and close the Meta Imaging Series System Administrator.

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

Updating Magnification and Calibration Settings

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

1. Open the MetaXpress Software application and log into the database.
2. Click **Devices > Configure Magnification**.

   The Configure Magnification dialog appears.

3. Ensure that **ImageXpress Micro Objective** is selected in the **Settings** field and select the installed objective from the field.
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. After the settings are updated, click **Backup**.

8. Click **Close** to exit the **Configure Magnification** dialog.
9. Click **Measure > Calibrate Distances**.

   The Calibrate Distances dialog appears.
10. Click the **Setup** tab.
11. Click **New**, and type the name of your new objective in the **Calibrations** field. Press **Enter**.

    The fields in the lower half of the dialog become active.
12. Select **Edit Units / Pix** in the **Define Calibrations By** field.
13. Enter the calibration value for the new objective in the **X** and **Y** fields. The following estimated values can be used for ImageXpress Micro System calibration settings:

    Table 8-4 Estimated Calibration Settings

Objective	Estimated Calibration
1x	6.50 µm/pixel
2x	3.25 µm/pixel
4x	1.63 µm/pixel
10x	0.65 µm/pixel
20x	0.33 µm/pixel
40x	0.16 µm/pixel
60x	0.12 µm/pixel
100x	0.07 µm/pixel

Note: For additional information on creating calibrations settings, refer to the application help available within the MetaXpress Software (press F1 when the Calibrate Distances dialog is open to access help).

14. Select the new objective from the **Magnification** field.
15. Click **Done**.
16. After the settings are updated, click **Save to file**.
17. Click **Close**.

Updating Shading Correction Settings

This section explains how to run a journal to create shading correction files to use during plate acquisition in the MetaXpress Software application. 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. You will need a flat-field correction (FFC) plate to focus the objective.

Note: The taskbar used for this procedure is the System Maintenance Taskbar.JTB which is part of the Main Taskbar.JTB. To determine if this is installed on your system, select **Journal > Taskbar > Load taskbar**, navigate to **C:\MX5\Taskbars** (or the directory where the current version of the software is installed) and then select the **System Maintenance Taskbar.JTB** file. If this taskbar is not installed on your system, contact Technical Support to obtain the journal suite file IXMTaskbar_v#.jzp. Once you have obtained the journal file, import it using the procedure in To import the journal suite into the MetaXpress Software on page 179.

Note: 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.

This procedure assumes that if you have added or replaced an objective, you have already performed the following procedures.

- Editing the Objective Settings in the Meta Imaging Series System Administrator on page 163.
- Configuring Parfocality after Changing Objectives on page 165.
- Updating Magnification and Calibration Settings on page 176.

This section includes the topics:

You will need FFC plates appropriate for the filter sets you are using to generate the shading correction files. These plates are part of the accessory kit that shipped with the instrument. Remove the paper backing from each plate before use. Handle the plates by the edges to avoid getting fingerprints on the imaging surface. Never use alcohol or other solvents to clean the plates. You can use compressed air to remove dust from the plates. Table 8-5 lists the correction plate used for each filter set.

Table 8-5 Flat-field Correction Plates 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

To import the journal suite into the MetaXpress Software

Follow this procedure if the Main taskbar is not installed on your system. If the Main taskbar is installed, skip this section and proceed to To run the shading correction journal from the Main taskbar on page 181.

This procedure is easier to perform prior to creating additional groups (configurations) in the Meta Imaging Series Administrator. Once you have followed this procedure, you can then create additional groups by using the option to copy settings from an existing group.

1. Contact Technical Support to obtain the Journal Suite file, **IXMTaskbar_v#.jzp** (# is the current version of the software).
2. Download the **IXMTaskbar_v#.jzp** file to the ImageXpress Micro System workstation.

3. Start and log in to the MetaXpress Software.
4. Click **Journal > Import Journal Suite**.

   The Import Journal Suite dialog appears.
5. Click **Select Journal Suite**.

   The Select Import Suite File Name dialog appears.
6. Navigate to the **IXMTaskbar_v#.jzp** file and click **Open**.

   The path to the journal suite is displayed at the top of the Import Journal Suite dialog.
7. Click **Select Import Location**, and navigate to **C:\MX5\TASKBARS** and click **OK**.

   The path of the import location is displayed at the bottom of the Import Journal Suite dialog.

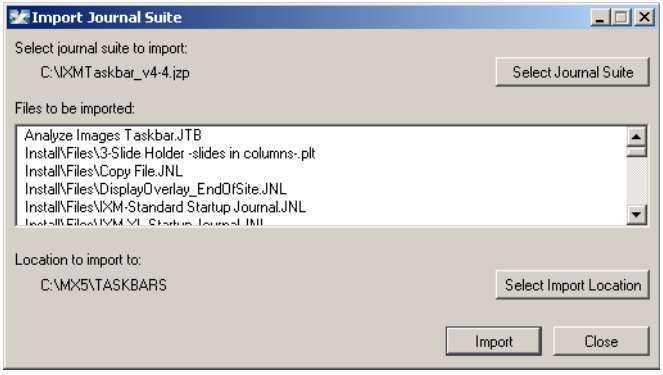


Figure 8-13 Import Journal Suite dialog

8. Click **Import**.

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

9. Click **Close**.
10. Click **Journal > Run Journal.**
11. Navigate to **C:\MX5\TASKBARS\Install**, select the **IXM Taskbar Installer.JNL** file, and click **Open**.
12. In the **Select System Type** dialog, select either **IXM (Standard)** or **IXM-XL**.

    The MetaXpress Directory dialog appears.

13. Follow the prompts in the subsequent dialogs to confirm that the current version of the MetaXpress Software is installed in the correct directory (typically C:\MX5).

Note: If the default file paths for journals, images, data, and so on do not go to the C:\MX5 folder, you will be prompted to reset them to go to that folder.

14. In the **Taskbar Installation Final Steps** dialog, click **Continue**.
15. Click **Journal > Journal Control > StartUp Journal.**
16. Navigate to **C:\MX5\StartUp,** select the startup journal (the name of the journal includes the appropriate ImageXpress Micro System model: Standard or XL), and click **OK**.
17. Exit and restart the MetaXpress Software.
18. Click **Journal > Recording Tools** and confirm that the **Pick Point** option is listed (it may be grayed out but it must be listed; if it is not listed, contact Technical Support for assistance).
19. Proceed to the next section to run the shading correction journal from the **Main** taskbar.

To run the shading correction journal from the Main taskbar

1. On the **Main** taskbar, select **System Maintenance** and then click **Set up Shading Correction**.
2. Follow the prompts to focus on the plates and create shading correction files.

Note: Press <F4> on your keyboard to show or hide the taskbar that was most recently used.

Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives

The ELWD (extra long working distance) Nikon objectives that can be supplied with the ImageXpress Micro System have adjustable correction collars, used to minimize spherical aberration in the image of the specimen. 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.

The settings to be used depend 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).

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

1. Read and follow the User Safety Instructions on page 153 for safe user-service procedures.
2. Follow the steps in Changing Objectives on page 160 for accessing the objective selector and lenses. If you put these objectives in the outer two positions, they can be accessed from the two side doors, which can be removed by hand.
3. Locate the correction collar on the objective that you want to adjust.

Note: The graduated scale on the barrel and its current setting. You might have to use a flashlight to view the markings.

4. Rotate the correction collar to its new setting.
5. Securely close the access doors.
6. Test the correction collar setting by examining the image quality of acquired images. If the quality has degraded, re-adjust the correction collar.

Cleaning the ImageXpress Micro System

Note: This procedure does not guarantee that your instrument is decontaminated or sterile.

The following procedure is designed to clean the plate-loading region of the instrument without damaging the internal components of the imaging system.

To prevent damaging the instrument, please read and follow these **precautionary guidelines** carefully when cleaning your instrument:

- To protect the ImageXpress Micro System optics and electronics, do not remove the front panels of instrument during the cleaning procedure.
- Do not use any cleaning agents other than those recommended in this procedure without first contacting Molecular Devices Technical Support.
- Do not use ultraviolet light for sterilization, as this can damage plastic components.
- Do not use any organic solvents.
- Do not pour or squirt water or alcohol directly onto the instrument, to prevent damaging internal components.

The following cleaning procedure is compatible with disinfectant wipes (or Kimwipes wipers with 70% ethanol).

You can leave the lamp powered on during this procedure.

To clean the ImageXpress Micro instrument

1. Read and follow the User Safety Instructions on page 153 for safe user-service procedures.
2. In MetaXpress Software, open the instrument's door.
3. Exit the MetaXpress Software and turn off the ImageXpress Micro instrument at the main power switch, which is located on the instrument's external power supply.
4. Ensure that the side panels have not been removed, the filter cube access door is closed, and no sample is loaded.
5. With gloved hands, use a damp wipe to wipe down the entire outer surface including side panels and top panels of the instrument. Then, use an alcohol wipe or a disinfectant wipe and go over the entire surface again.
6. Use forceps wrapped with Kimwipes to gently wipe the perimeter of the plate/stage region where a plate would normally be loaded. Wipe with damp Kimwipes first, and then with an alcohol or disinfectant wipe.

7. The stage is freely moving without power, so to clean the plate/stage region underneath where the plate is loaded, you can open the door and slide the stage around.
8. Use a fresh damp wipe to wipe down the stage area underneath and around the plate loading region, followed by wiping with an alcohol wipe or disinfectant wipe.

Operational and Environmental Specifications

A

Operational and environmental specifications are shown in Table A-1.

Table A-1 Operational and Environmental Specifications

Specification	Measurement
Base Unit Weight	82 kg
Base Unit Dimensions (H x W x D)	490 mm x 460 mm x 800.5 mm
Mains Power Input	100 to 240 VAC, 50/60 Hz, 400 VA, 2.0 A maximum
Options Controller	100 to 240 VAC, 50/60 Hz, 960 VA, 8 A
Camera Power Supply	**Standard:** 100 to 240 VAC, 50/60 Hz, 240 VA, 2.0 A **XL:** 100 to 240 VAC, 50/60 Hz, 120 VA, 1.0 A
Light Source Power Supply	**Standard:** 100 to 240 VAC, 50/60 Hz, 360 VA, 300 W **XL:** 100 to 240 VAC, 50/60 Hz, 288 VA, 2.5 A
Host Computer	100 to 240 VAC, 50/60 Hz, 690 VA, 5.8 A
Computer Monitor	100 to 240 VAC, 50/60 Hz, 35 W
Mains Voltage Fluctuations	Not to exceed 10% of nominal supply voltage
Equipment Class	1
Pollution Degree	2
Installation Category	2
Operating Environment	Indoor Use Only
Altitude	Not to exceed 2000 m
Operating temperature	15°C to 30°C
Humidity	35% to 50% non-condensing
Ingress Protection	IP20

WARNING! If the ImageXpress Micro System is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

WARNING! The ImageXpress Micro System is an Equipment Class 1 product that relies on protective earth grounding for safe operation. Any interruption of the protective earth ground conductor, inside or outside the instrument, or disconnection of the protective earth ground terminal can result in personal injury.

WARNING! Do not position the equipment so that it is difficult to operate the circuit breaker switches on the front of the equipment.

WARNING! For Environmental Control Options a tank or house CO_2/air mixture (for example, 5% CO_2/95% air) is required with an adequate CO_2 regulator that can supply a maximum of 20 PSI (138 kilopascal) to the CO_2 inlet on the rear of the Options Controller.

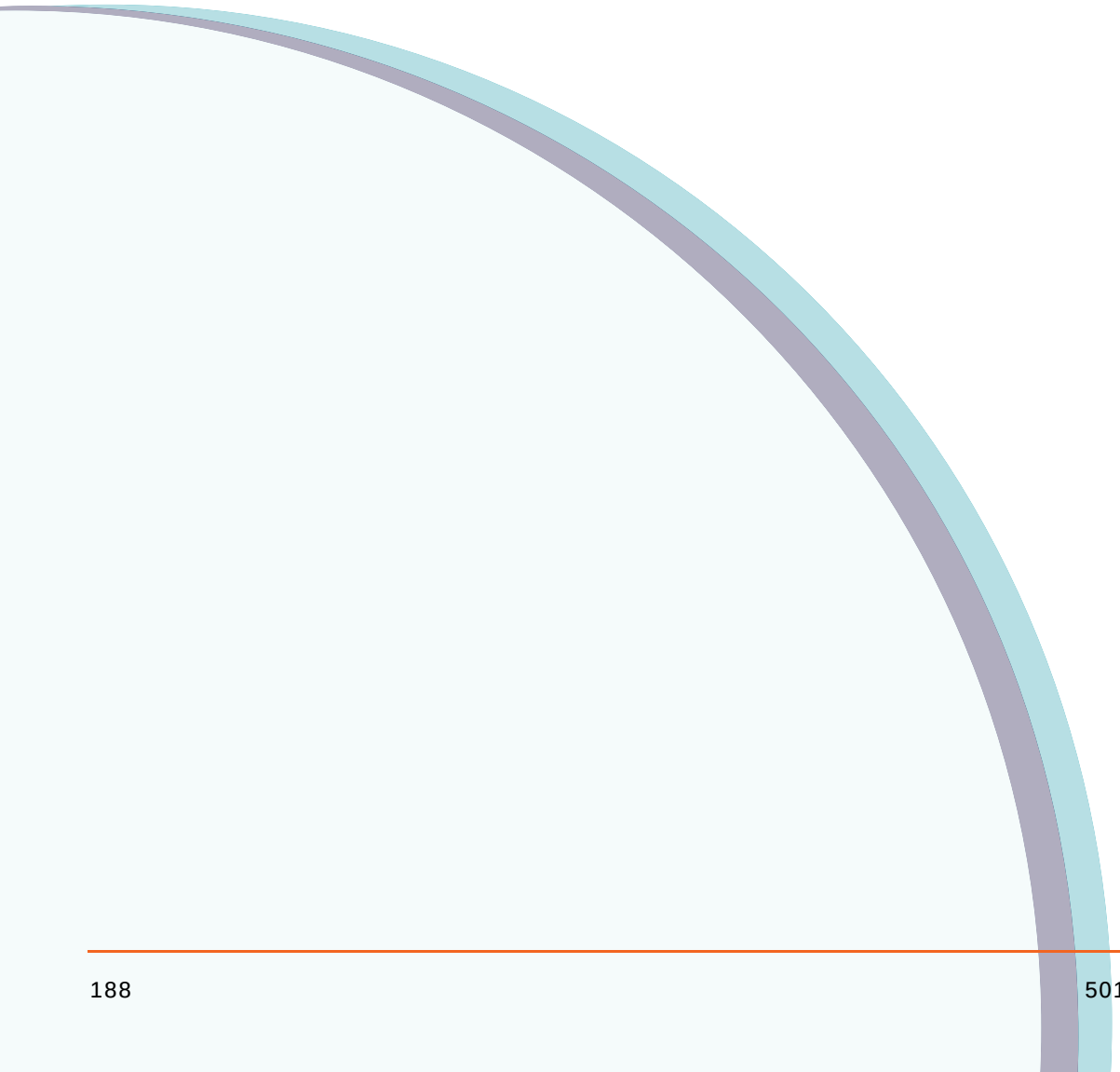
Site Requirements

B

The ImageXpress® Micro System is designed to operate indoors under laboratory conditions. For optimal performance, site requirements must be met. As with any precision optical instrument, care should be taken to maintain a low-dust, low-vibration environment. Temperature and humidity extremes can compromise performance.

Table B-1 Site Requirements

Item	Description
Environmental Temperature	50° to 86° F (10° to 30° C).
Environmental Humidity	5% to 95% non-condensing.
Altitude	Up to 1.25 miles (2000 m).
Power Requirements	The ImageXpress Micro System can be directly connected to all international supply voltages. The input voltage range is from 100 to 240 V~ and input frequency range 50 to 60 Hz. No range switching is required. Fluctuations must not exceed ±10% of the nominal voltage. Use the included IEC power cord to connect the external power supply to a GROUNDED power receptacle that is rated for 15 A. If using a power strip, do not connect the acquisition computer to the same power strip as the instrument and light source.
Power Consumption	ImageXpress Micro System power consumption is 1100 watts for 2 to 3 seconds at initialization, 800 watts average operating RMS.
Space Requirements	Table or bench top 30 inches (76 cm) deep. There needs to be space below the table for the light source and power supply such that the light guide and power cable can easily reach the back of the instrument.
Rear Clearance	The rear of the instrument should be no closer than 6 inches (15 cm) to a wall.
Weight Requirements	Sufficient to support 180 lbs (82 kg) with minimal vibration.



Objectives Compatible with the ImageXpress Micro System

C

Table C-1 details the Nikon objectives that are compatible with the ImageXpress® Micro System. It also provides plate compatibility information.

Table C-1 Nikon Objectives Compatible with the ImageXpress Micro System

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture	Working Distance	Plate Compatibility
1x Plan Achromat	6500-0119	No	0.04	3.2 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)[1]
2x Plan Apo	1-6300-0451	No	.010	8.5 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
4x S Fluor	1-6300-0189	No	.020	15.5 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
4x Plan Apo	1-6300-0121	No	.020	15.7 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
4x Plan Fluor DL	1-6300-0292	Yes, PhL	.013	16.2 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
10x Plan Fluor	1-6300-0790	No	.030	16.0 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)

Table C-1 Nikon Objectives Compatible with the ImageXpress Micro System (cont'd)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture	Working Distance	Plate Compatibility
10x S Fluor	1-6300-0122	No	.050	1.2 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)[2]
10x Plan Apo	6500-0120	No	0.45	4.0 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
10x Plan Fluor DLL	1-6300-0294	Yes, Ph1	0.30	16.0 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
10x Plan Fluor DL	1-6300-0293	Yes, Ph1	0.30	15.2 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
20x Super Plan Fluor ELWD cc 0 mm to 2 mm	6500-0108	No	0.45	8.1 mm to 7.0 mm	Thin bottom (0.17 mm)[3] Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)
20x S Fluor	1-6300-0411	No	0.75	1.0 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
20x Plan Apo	1-6300-0196	No	0.75	1.0 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
20x Plan Fluor DLL	1-6300-0295	Yes, Ph1	0.50	2.1 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)[2]

Table C-1 Nikon Objectives Compatible with the ImageXpress Micro System (cont'd)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture	Working Distance	Plate Compatibility
20x Super Plan Fluor ELWD DM cc 0 mm to 2 mm	6500-0111	Yes, Ph1	0.45	8.1 mm to 7.0 mm	Thin bottom (0.17 mm)[3] Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)
40x Super Plan Fluor ELWD cc 0 mm to 2 mm	6500-0109	No	0.60	3.7 mm to 2.7 mm	Thin bottom (0.17 mm)[3] Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)
40x Plan Apo	1-6300-0412	No	0.95	0.14 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
40x S Fluor cc 0.11 mm to 0.23 mm	1-6300-0197	No	0.90	0.3 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
40x Plan Fluor Oil	1-6300-0416	No	1.30	0.2 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
40x Plan Fluor DLL	1-6300-0297	Yes, Ph2	0.75	0.72 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
40x Super Plan Fluor ELWD ADM cc 0 mm to 2 mm	6500-0112	Yes, Ph2	0.60	3.7 mm to 2.7 mm	Thin bottom (0.17 mm)[3] Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)
60x Super Plan Fluor ELWD cc 0.1 mm to 1.3 mm	6500-0110	No	0.70	1.8 mm to 2.62 mm	Thin bottom (0.17 mm)[3] Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)
60x Plan Fluor	1-6300-0414	No	0.85	0.3 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
60x Plan Apo Oil	1-6300-0417	No	1.40	0.21 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt

Table C-1 Nikon Objectives Compatible with the ImageXpress Micro System (cont'd)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture	Working Distance	Plate Compatibility
60x Plan Fluor ELWD ADL cc 0.1 mm to 1.3 mm	6500-0113	Yes, Ph2	0.70	1.8 mm to 5.62 mm	Thin bottom (0.17 mm)[3] Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)
100x Plan Fluor	1-6300-0415	No	0.95	0.2 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
100x Plan Fluor Oil	1-6300-0418	No	1.30	0.2 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt

1 Potential interference with microplate skirt when imaging edge wells.
2 Image degradation above microplate thickness 0.3 mm.
3 20x and 40x ELWD will image through cover slips, but other objectives will give better resolution and shorter exposures.

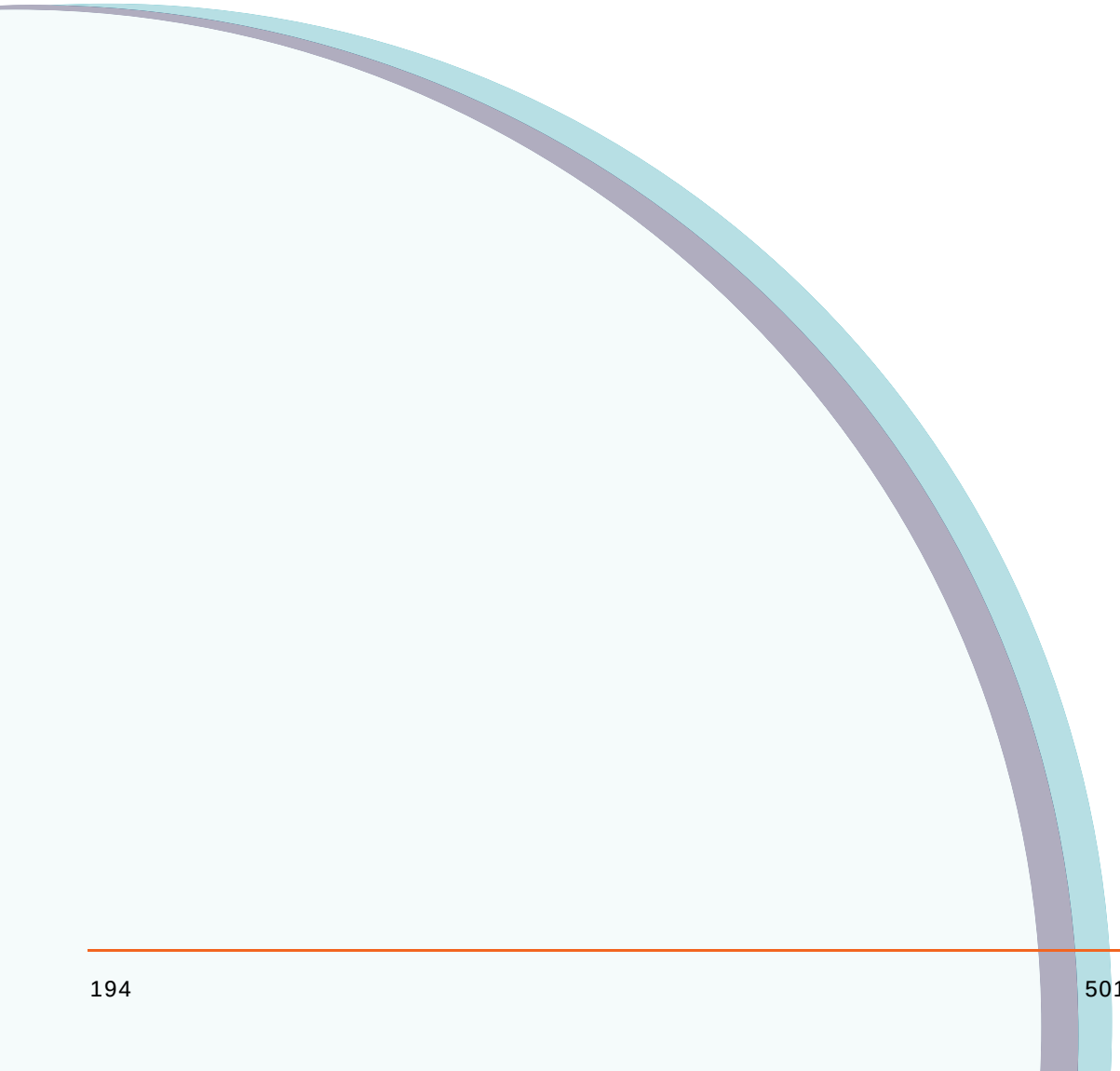
Note: When used with thin-bottom plates, the short working distance of 20X S Fluor, 20X Plan Apo, 40X S Fluor, 40X Plan Apo, 40X Plan Fluor Oil, 60X Plan Fluor, 60X Plan Apo, 100X Plan Fluor, and 100X Plan Apo objectives can cause interference with microplate skirt when imaging edge wells. Molecular Devices recommends to either omit the edge wells or use a plate with a low skirt.

Filter Set / FFC Plate Combinations

D

Table D-1 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



File Privileges for the MetaXpress Software Application

E

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

Note: The following assumes that the MetaXpress Software application has been installed in C:\MX5 (the default location).

Note: 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\MX5.

Software Administrator

Read/write access is required for everything under the C:\MX5 tree. The Software Administrator also needs to create and periodically modify shading correction images on the C:\Shading Images directory. Because this is a possible security violation, this operation can 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:\MX5
C:\MX5\app\mmproc
C:\MX5\app\mmproc\Dropins
C:\MX5\Help\C:\MX\Help\ - all subdirectories -
C:\MX5\Groups\

If the system is set up for multiple users:

C:\MX5\Groups\MetaXpress
C:\MX5\Groups\MetaXpress\Users

If the system is an acquisition computer:

C:\MX5\Hardware\
C:\MX5\Hardware\ - all subdirectories -
C:\MX5\Plates\
C:\MX5\Vinput\ - all subdirectories -

Read / Write and Modify Access needed:

C:\Analysis
C:\Assay
C:\Backup
C:\Shading

If the system is set up for a single user:

C:\MX5\Groups\MetaXpress

If the system is set up for multiple users:

C:\MX5\Groups\MetaXpress\Users\Individual user

Robotic Plate Handling

F

If your ImageXpress® Micro System has a robotic plate handler integrated with the base imaging system, there are additional hardware and user procedures to become familiar with. The robotic plate handler is integrated with the ImageXpress Micro System in such a way that plates can be scanned for barcodes, loaded onto the imaging system where images are acquired, and then returned to a home location.

If you want to upgrade your system with a robotic plate handler, contact Molecular Devices sales support.

A robotic plate handler is easily integrated with the ImageXpress Micro Imaging System. The Robot step-by-step protocol is as follows:

1. Fetch a microplate from a hotel shelf.
2. Scan the microplate barcode.
3. Load the microplate onto the ImageXpress Micro System.
4. Acquire images.
5. Unload the microplate from the ImageXpress Micro System.
6. Return the microplate to the original hotel shelf.

Verifying External Control Settings

If you have a robot attached to the ImageXpress Micro System, you need to confirm that the External Control settings in the Meta Imaging Series Administrator are enabled and that 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, click **All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. In the **Meta Imaging Series Administrator**, select **MetaXpress** from the **List of Groups** field.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, click **Install System Devices**.
5. In the **Install Systems Devices** dialog, 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**.
7. In the **External Control Settings** dialog, click the **Connections Settings** tab.

CRS Catalyst Express Robot User Procedures

Loading Plates onto the CRS Catalyst Express Robot

In order to use the CRS Catalyst Express robot to scan barcodes and to load and unload plates onto the ImageXpress Micro instrument, you must properly load your plates onto the hotels of the robotic plate handler. If the plates are not loaded in the correct orientation, two errors will occur:

- The barcode will not be scanned.
- Well position A1 will not be correctly located in the front-left corner of the plate-loading region on the ImageXpress Micro instrument.

The following steps will prevent these errors:

1. If you plan to scan barcodes, properly affix the barcode labels before loading plates onto the robotic plate handler. We recommend that labels are located on the front left side of the plate and they must be consistently located in the same position on all plates being scanned. For example:

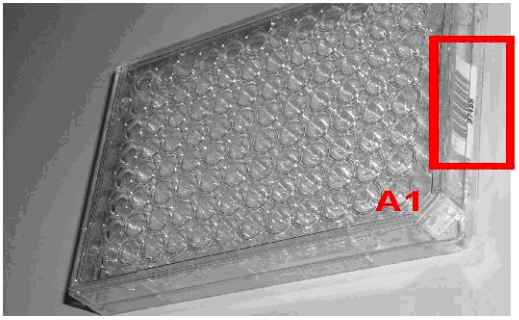


Figure F-1 Boxed region shows barcode affixed on front of microplate

Note: We suggest using labels with a minimum line width of no less than 7.5 mm, preferably 10 mm. For recommendations on compatible barcode vendors, please contact Molecular Devices Technical Support.

2. The CRS Catalyst Express has three vertical racks (“hotels”) onto which you can load microplates. The directions that follow assume you are positioned in front of the robot as pictured immediately below. The hotel numbers (1 to 3) are at the front of the robot.

Figure F-2 Numbers mark the three hotel racks where microplates are loaded

3. Plates need to be loaded into the hotels with the barcodes facing the front of the robot.

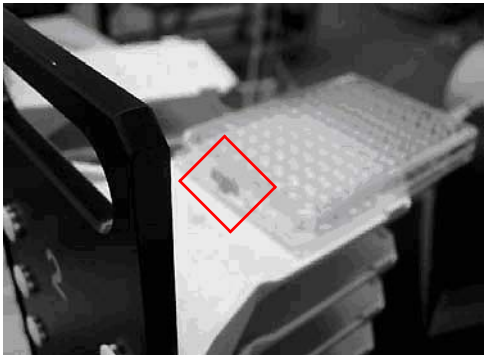


Figure F-3 Box shows barcode centrally located on the microplate, which should be facing toward the front of the hotel

4. When correctly placed in the hotels, well position A1 is located in the front left corner as viewed from the front of the robot.

5. If your plates are loaded with the barcode facing front and A1 in the front left location, then your plates will be scanned and correctly loaded onto the ImageXpress Micro System plate-loading region.

Electromagnetic Compatibility (EMC)

G

REGULATORY INFORMATION FOR CANADA (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est confomre à la norme NMB-001 du Canada.

ISM EQUIPMENT CLASSIFICATION (Group 1, Class A)

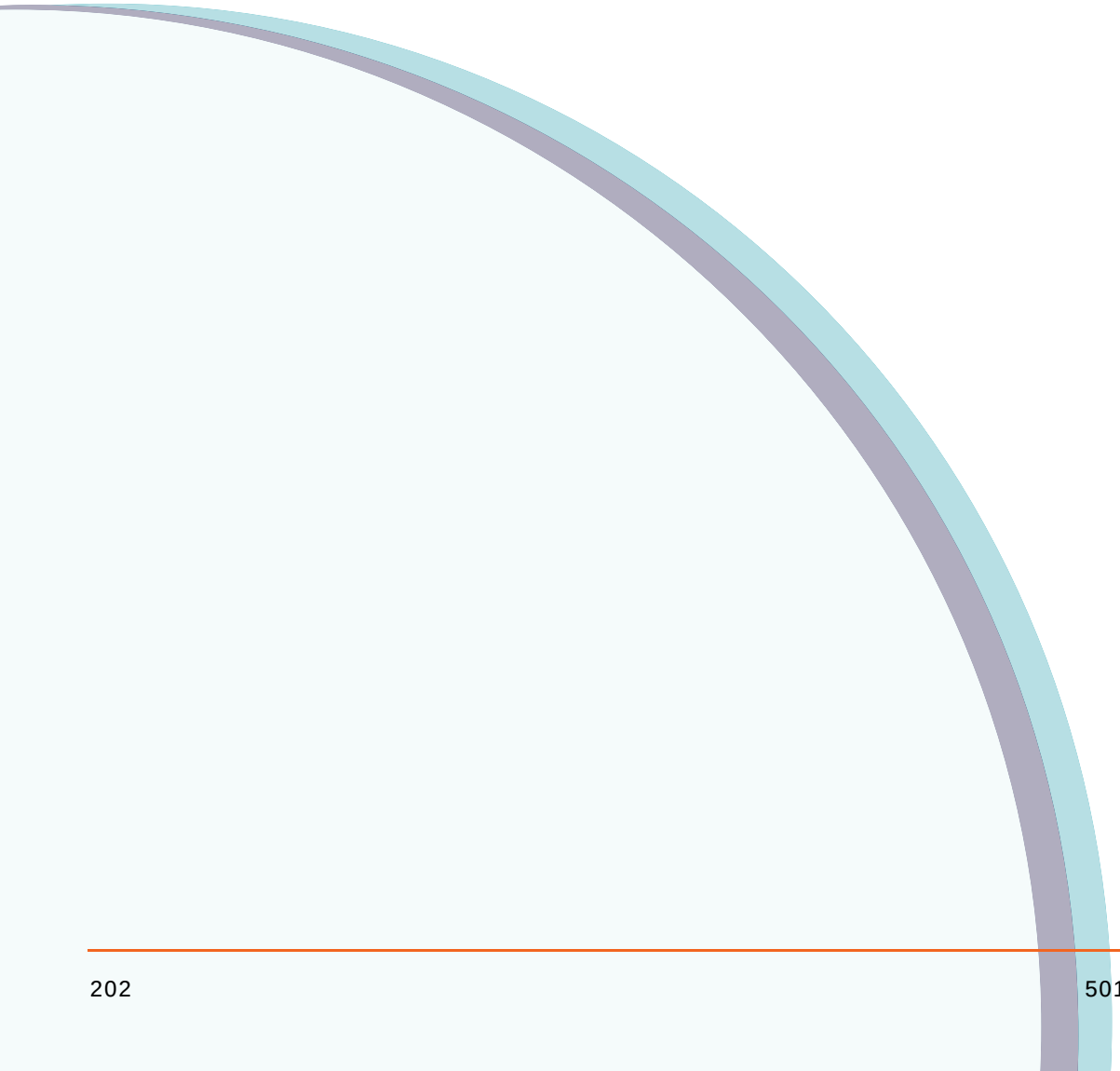
This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power supply network which supply buildings used for domestic purposes.

INFORMATION FOR THE USER (FCC NOTICE)

This equipment has been tested and found to comply with the limits for non-consumer ISM equipment, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a non-residential installation. This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and TV reception. The user is cautioned that changes and modifications made to the equipment without the approval of the manufacturer could void the user's authority to operate this equipment.



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