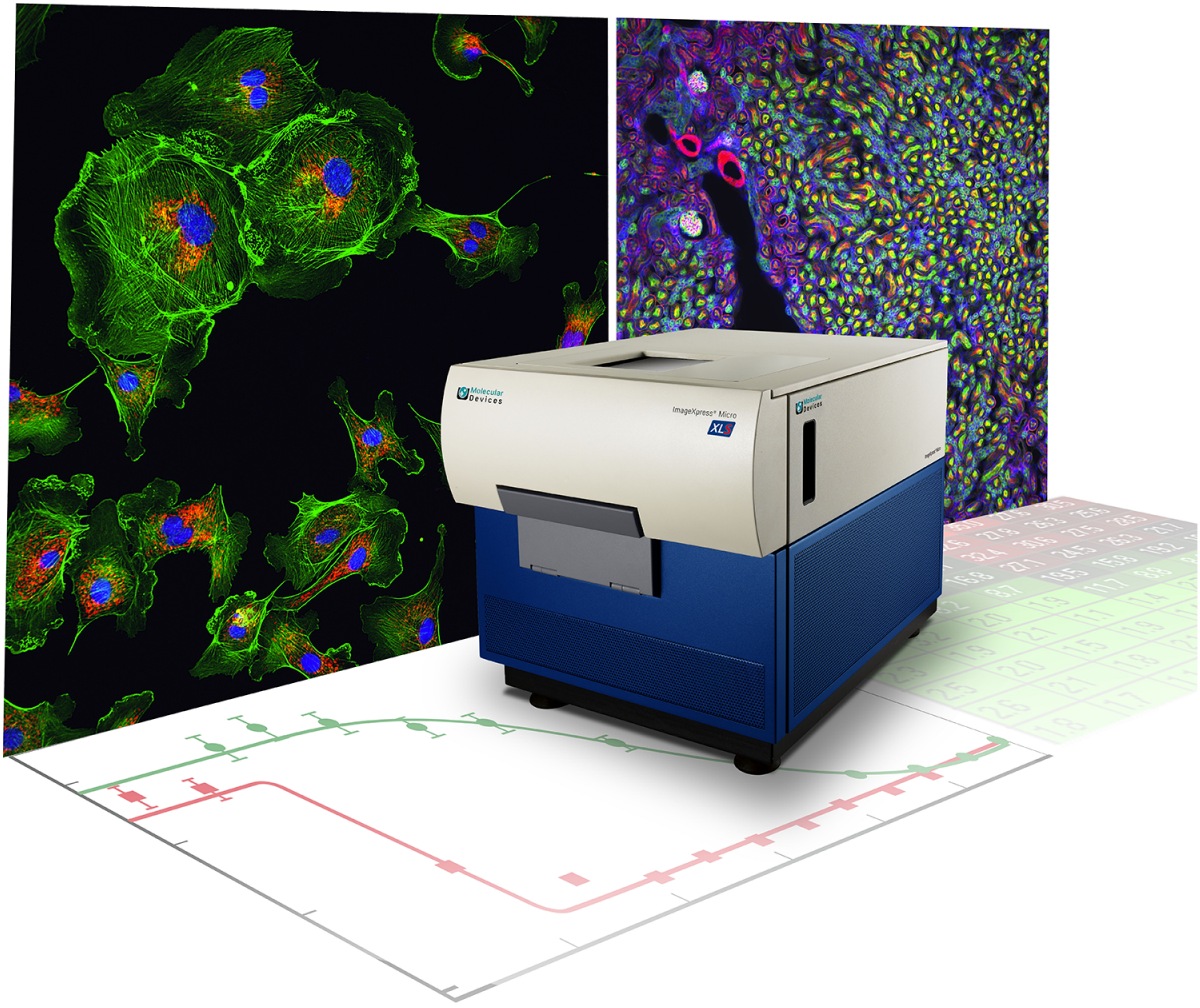


ImageXpress® Micro

Widefield High Content Screening System

User Guide

MOLECULAR DEVICES

5015321 C
April 2015

Product manufactured by Molecular Devices, LLC.

1311 Orleans Drive, Sunnyvale, California, United States of America 94089.

Molecular Devices, LLC is ISO 9001 registered.

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 3-1: 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 175).

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 10000+ 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 175 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.

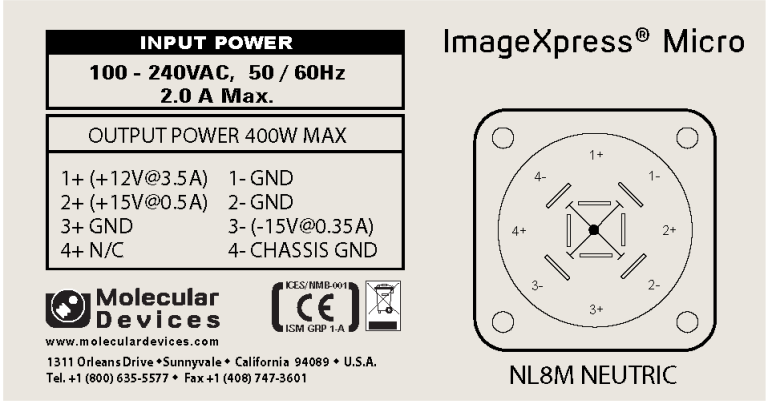


Figure 3-1: Power supply label

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.

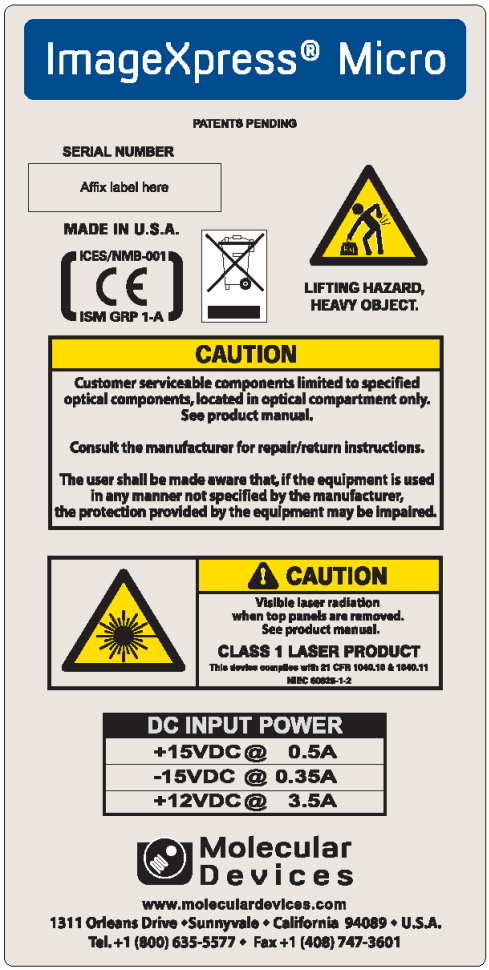


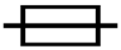
Figure 3-2: Safety label

Symbol Explanations

Table 3-2: 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

Table 3-2: Symbols (cont'd)

Symbols	Indication
	Indicates that you must not discard this electrical/electronic product in domestic household waste
	Indicates fuse

Introduction

1

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 XLS 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

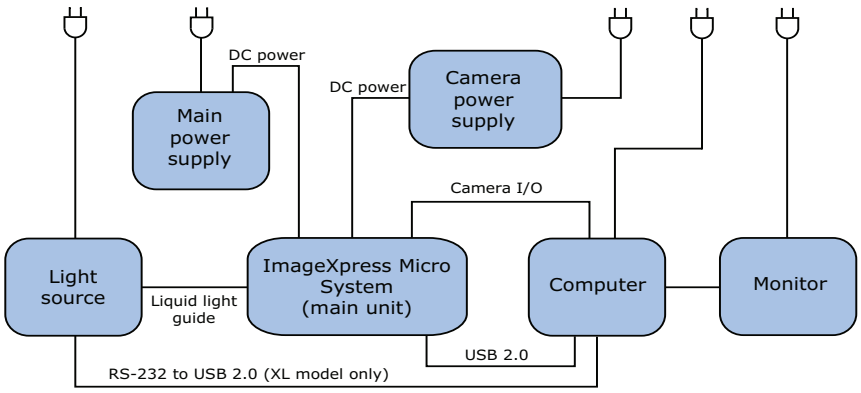


Figure 1-1: ImageXpress Micro System Components

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 10000+ 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

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 59, Setting Up Plate Acquisition on page 67, and Acquiring Plates on page 143.
- 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*.

Simplified Menu Structure

An optional simplified menu structure can be installed to reduce the number of top-level menus. All the features of the software are available in this reorganized menu structure.

The procedures in this guide describe both the default menu structure and the simplified menu structure.

You can use the **Menu Map** in the **Help** menu to help you find the locations of features in the simplified menu structure.

1. Click **Help > Menu Map**.
2. In the **Menu Map** dialog, select to view the **Default to customized** menu map.
3. Click the menu path where the software feature you want is found in the default menu structure.

The simplified menu path appears to the right of the desired feature in the menu.

4. Click the menu path in the software window to access the desired feature.

For example, if you want to make a duplicate of an image, then use the following procedure:

1. Click **Help > Menu Map**.
2. In the **Menu Map** dialog, select to view the **Default to customized** menu map.
3. Click **Edit > Duplicate**.

   The simplified menu path -> **Edit: Image: Duplicate Image/Plane** appears to the right of the **Image** option in the submenu.
4. In the software window, click **Edit > Image > Duplicate Image/Plane**.

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.

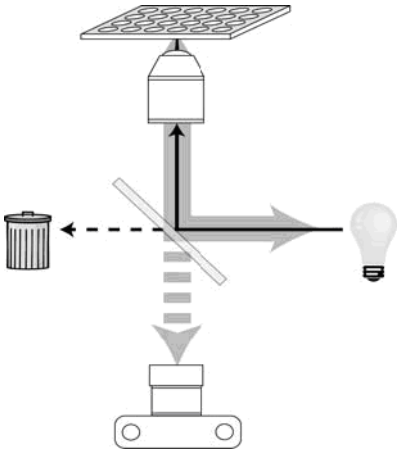


Figure 1-2: Dichroic mirror creates two light pathways

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.

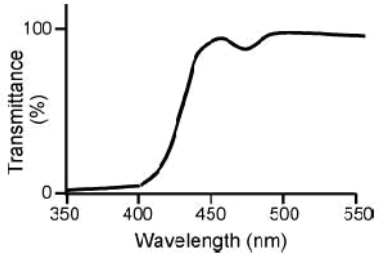


Figure 1-3: Example of a transmission spectrum of a dichroic

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 211.

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 175 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**.
2. In the MetaXpress Software title screen, in the **User Name** field, select the user name to use and click **OK**.

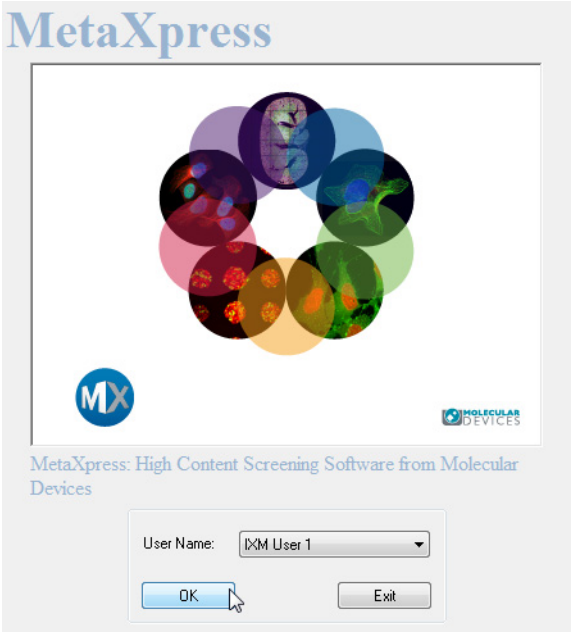


Figure 2-1: MetaXpress Software title screen

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.

The MetaXpress Software starts and a progress bar at the bottom of the dialog indicates the loading progress of the program.

3. In the **Welcome to MetaXpress** dialog, select the data source to connect to (if there is more than one), enter your login name and password, and then click **OK**.

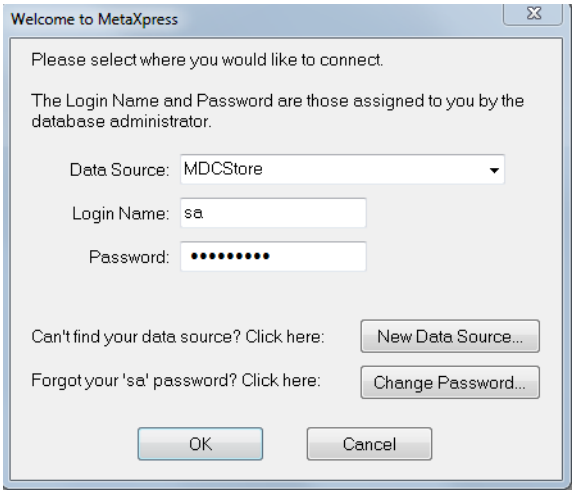


Figure 2-2: Welcome to MetaXpress dialog

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. In the dialog to select a **Group**, 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 59
- Setting Up Plate Acquisition on page 67
- Acquiring Plates on page 143

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 175.

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:\MX6\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:\MX6\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 38.

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.

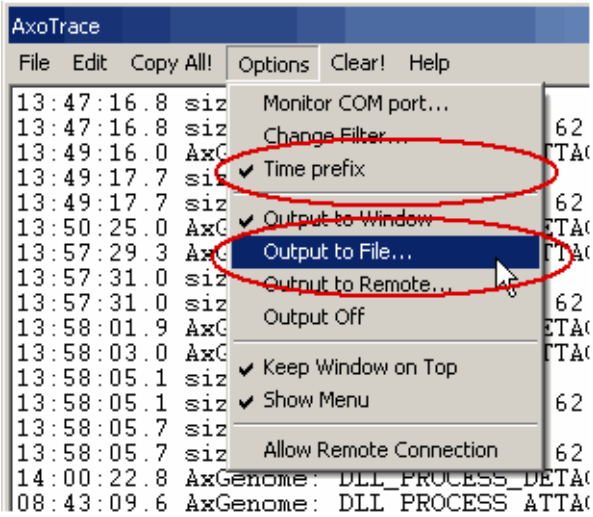


Figure 2-4: AxoTrace Options menu

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.

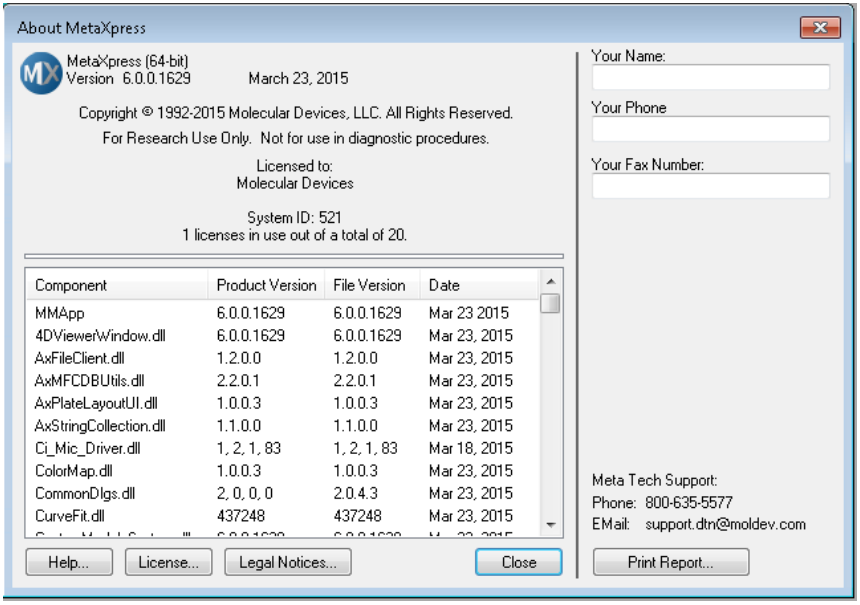


Figure 2-5: About MetaXpress Software

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. See Obtaining Support on page 34.

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.

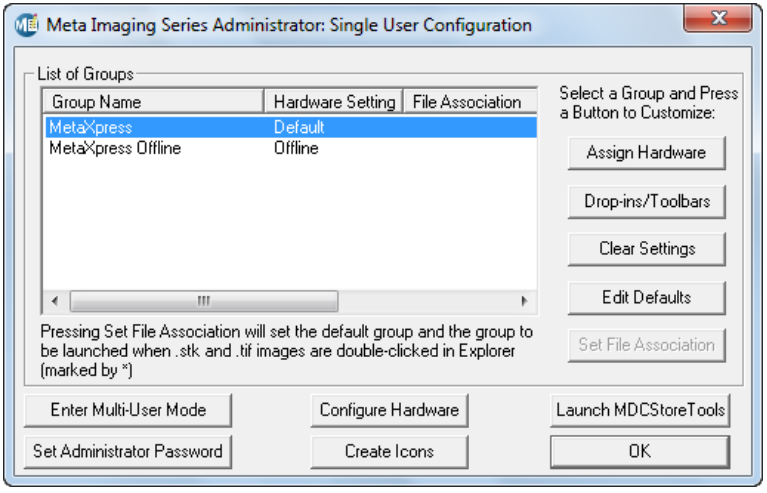


Figure 3-1: Meta Imaging Series Administrator Program

4. Click **Configure Hardware**.
5. In the **Configure Hardware** dialog, click **Configure Devices**.

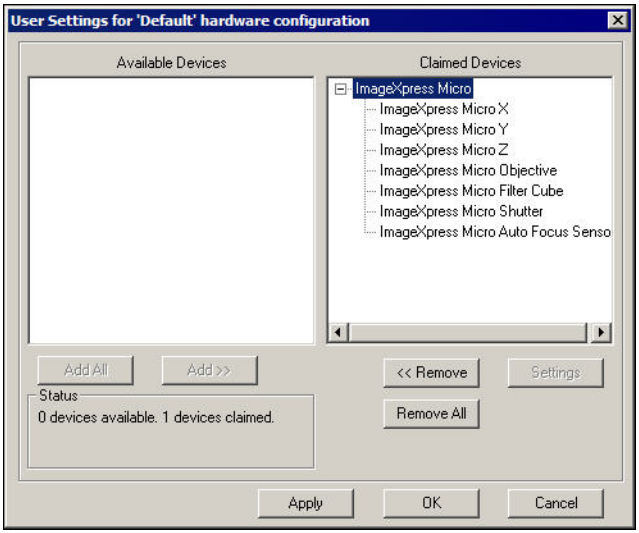


Figure 3-2: User Settings hardware configuration dialog

6. In the **User Settings hardware configuration** dialog, select **ImageXpress Micro X** from the **Claimed Devices** list and then click **Settings**.
7. In the **ImageXpress Micro X Settings** dialog, increase the step size to **10,000 µm**.

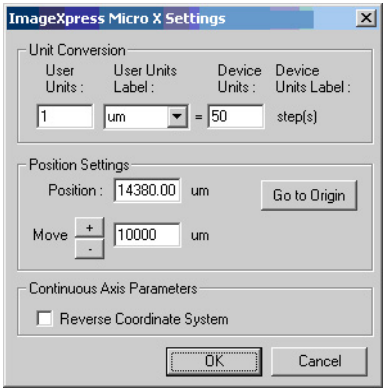


Figure 3-3: ImageXpress Micro X Settings dialog

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**.

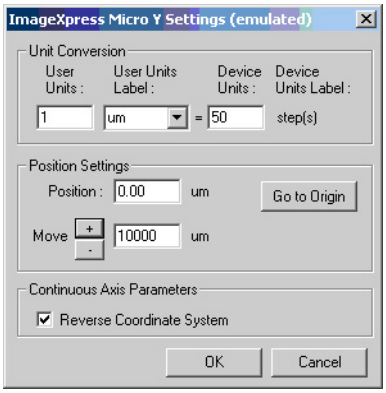


Figure 3-4: ImageXpress Micro Y Settings dialog

12. In the **ImageXpress Micro Y Settings** dialog, 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**.

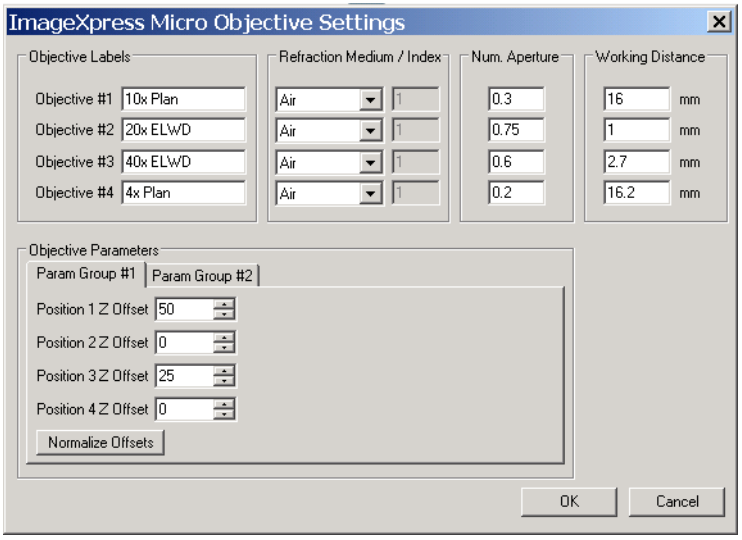


Figure 3-5: ImageXpress Objective Settings dialog

23. In the **ImageXpress Objective Settings** dialog, 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 µm 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 186.

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**.

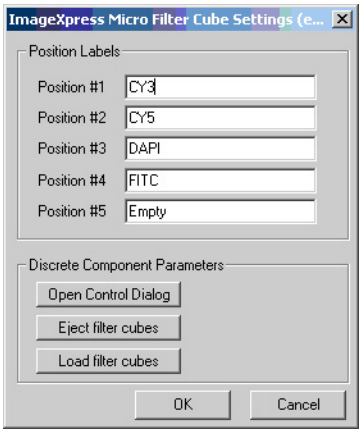


Figure 3-6: ImageXpress Micro Filter Cube Settings dialog

29. In the **ImageXpress Micro Filter Cube Settings** dialog, 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**.
31. In the **Control – ImageXpress Micro Filter Cube Settings** dialog, 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**.

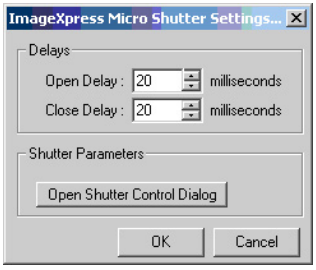


Figure 3-7: ImageXpress Micro Shutter Settings dialog

34. In the **ImageXpress Micro Shutter Settings** dialog, confirm that the **Open Delay** and **Close Delay** fields are both set to 20 milliseconds.
35. Click **Open Shutter Control Dialog**.
36. In the **Control – ImageXpress Micro Shutter Settings** dialog, 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 221.

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 and log into the database.

2. Click **Devices** > **Configure Magnification**.

   In the simplified menu structure, click **Control > Devices > Configure Magnification**.

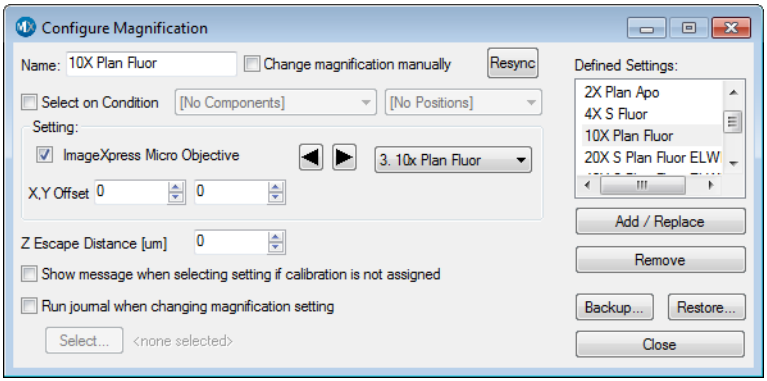


Figure 3-8: Configure Magnification dialog

3. In the **Configure Magnification** dialog, 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**.
3. In the C**onfigure Illumination** dialog, 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.

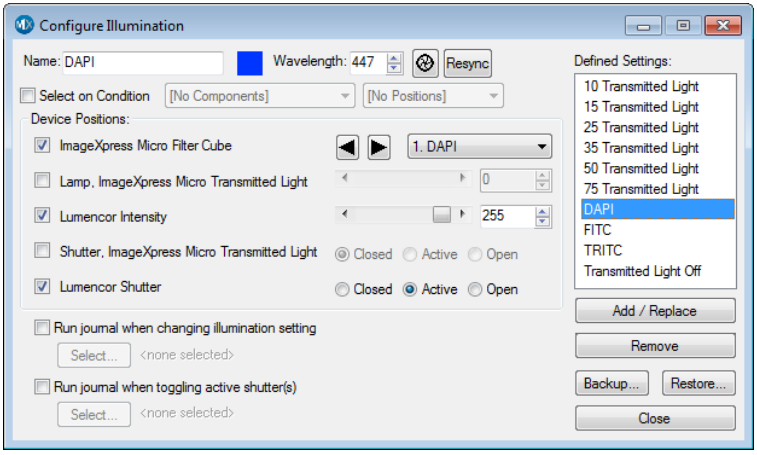


Figure 3-9: Configure Illumination dialog

6. Set up other illumination settings if needed. The value in the **Wavelength** field should match the center wavelength for the emission filter.
7. Click **Backup**.
8. In the B**ackup All Illumination Settings** dialog, 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. Click **Measure > Calibrate Distances**.

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

2. In the **Calibrate Distances** dialog, confirm that there are calibration settings in the **dialog** that match the objective settings from the **Configure Magnification** dialog.

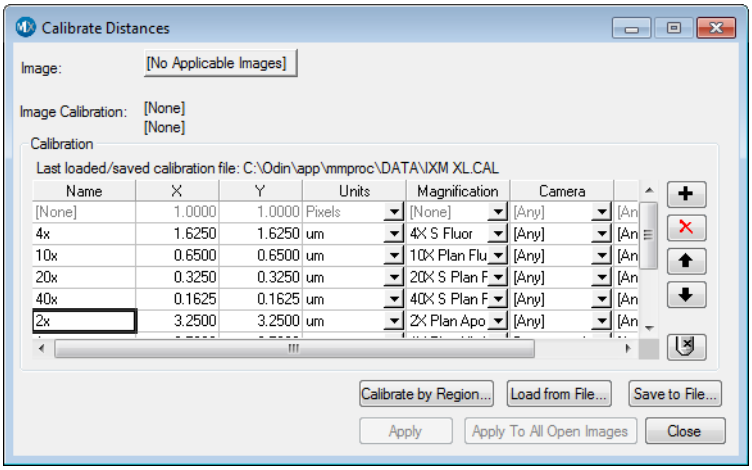


Figure 3-10: Calibrate Distances dialog

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

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

Objective	Estimated Calibration
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: Make sure the appropriate magnification setting is selected for each calibration.

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. Click **Screening > Plate Acquisition Setup**.

   In the simplified menu, click **Screening > Acquisition Setup**.
3. Click **Acquisition Loop**.

   In the simplified menu, click **Configure > Acquisition.**
4. In the **Autofocus options** section of the dialog, ensure that **Enable laser-based focusing** is selected.
5. Click the **Plate** tab to highlight it.
6. Select the included Costar **96-well Plastic** plate type from the **Plate name** field.
7. From the **Screening** menu, select **Plate Acquisition and Control**.
8. The Plate Acquisition and Control dialog appears.
9. Click **Eject Plate** to move the stage to the load position.
10. Load the bead plate and click **Load Plate**.
11. Click **Go To A1** to move the stage to the A1 position.
12. On the **Plate Acquisition Setup** dialog, click the **Autofocus** tab to highlight it.
13. Click **Configure Laser Settings**.

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

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

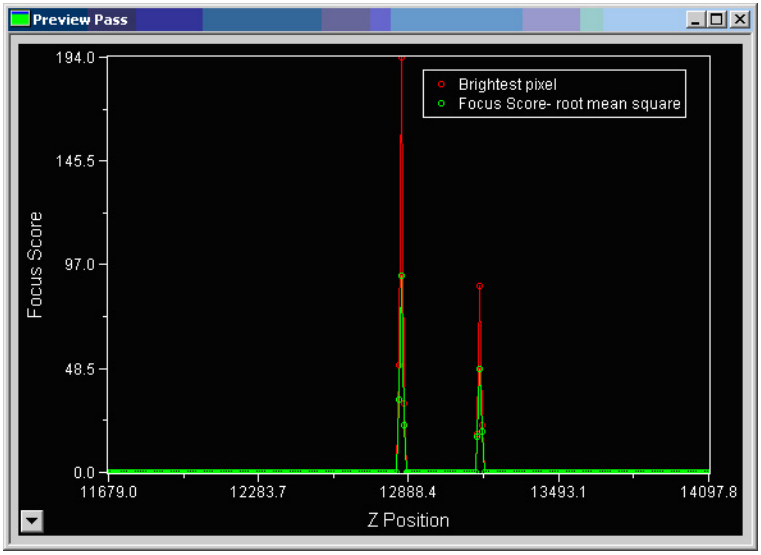


Figure 3-11: Preview Pass graph

15. If the **Preview Pass** window contains at least one peak, the **Laser Auto Focus Sensor** is enabled and functional.
16. 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.
17. If the **Preview Pass** window still does not contain a peak, contact Technical Support and report the issue. (See Obtaining Support on page 34.) For more information on the **Preview Pass** window, see Confirming Laser Auto Focus Settings for Plate Files on page 57.
18. 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 **Wl (Wavelength) 1** tab to highlight it.
11. Select **FITC** from the **Illumination setting** drop-down list and enter an exposure time of **100 msec** in the **Exposure** field.

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

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

    The Plate Acquisition and Control dialog appears.
13. Ensure that **W1 – FITC** is selected in the **Wavelength** field.
14. Enter a step size of **250** in the **Step size** field.

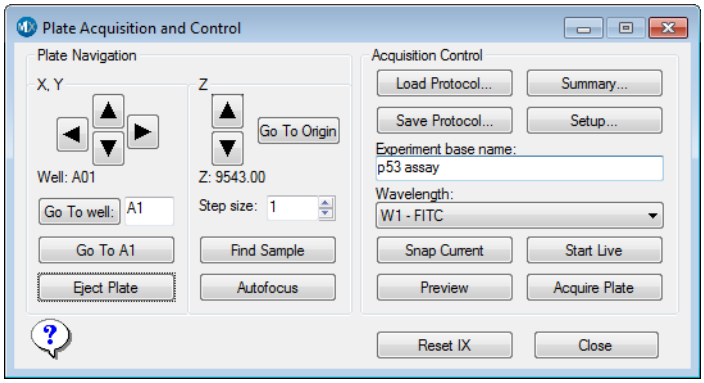


Figure 3-12: Plate Acquisition and Control dialog

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.

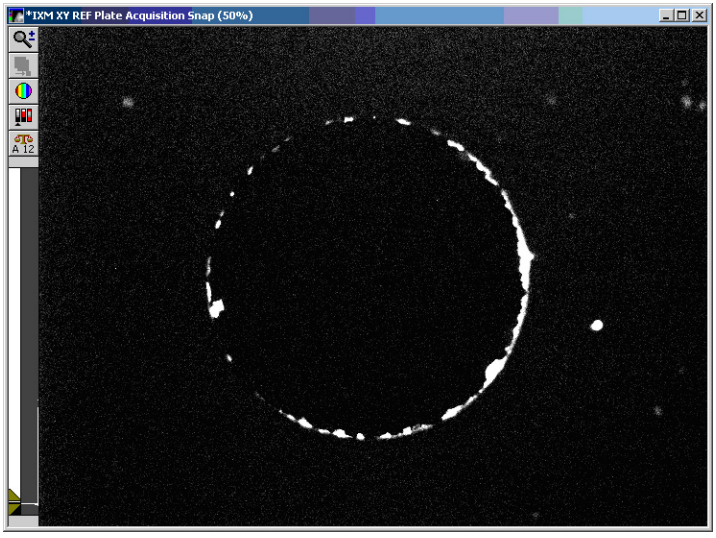


Figure 3-13: A1 Pinhole in focus at 4X

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. See Obtaining Support on page 34.
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. Click **Screening** > **Plate Acquisition Setup**.
3. Click **Configure > Plates**.
4. Select the **Plate name** drop-down arrow to view the available plate type files.

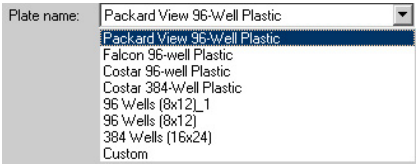


Figure 3-14: Available plate type files

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:\MX6\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 204.

Verifying Shading Correction Files

Shading correction files are needed for each objective and filter combination. They 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 200.

To use shading correction images during Plate Acquisition:

- Select **Plate Acquisition Setup > Acquisition Loop** > **Perform shading correction**.

Name shading correction images in the following format:

- **shading_<magnification setting>_<wavelength>.tif**

  For example: **shading_4x Plan Apo_DAPI.tif**

By default, the MetaXpress Software application looks in the **C:\Shading Images** directory for shading correction images. To change the default image location:

1. Click **Plate Acquisition Setup > Acquisition Loop > Directory**.
2. Select a new location.

To ensure that properly named shading correction files exist for each objective and filter set combination, do the following:

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.
3. Ensure that the file names are formatted correctly and that a file exists for each objective and filter set combination.

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.

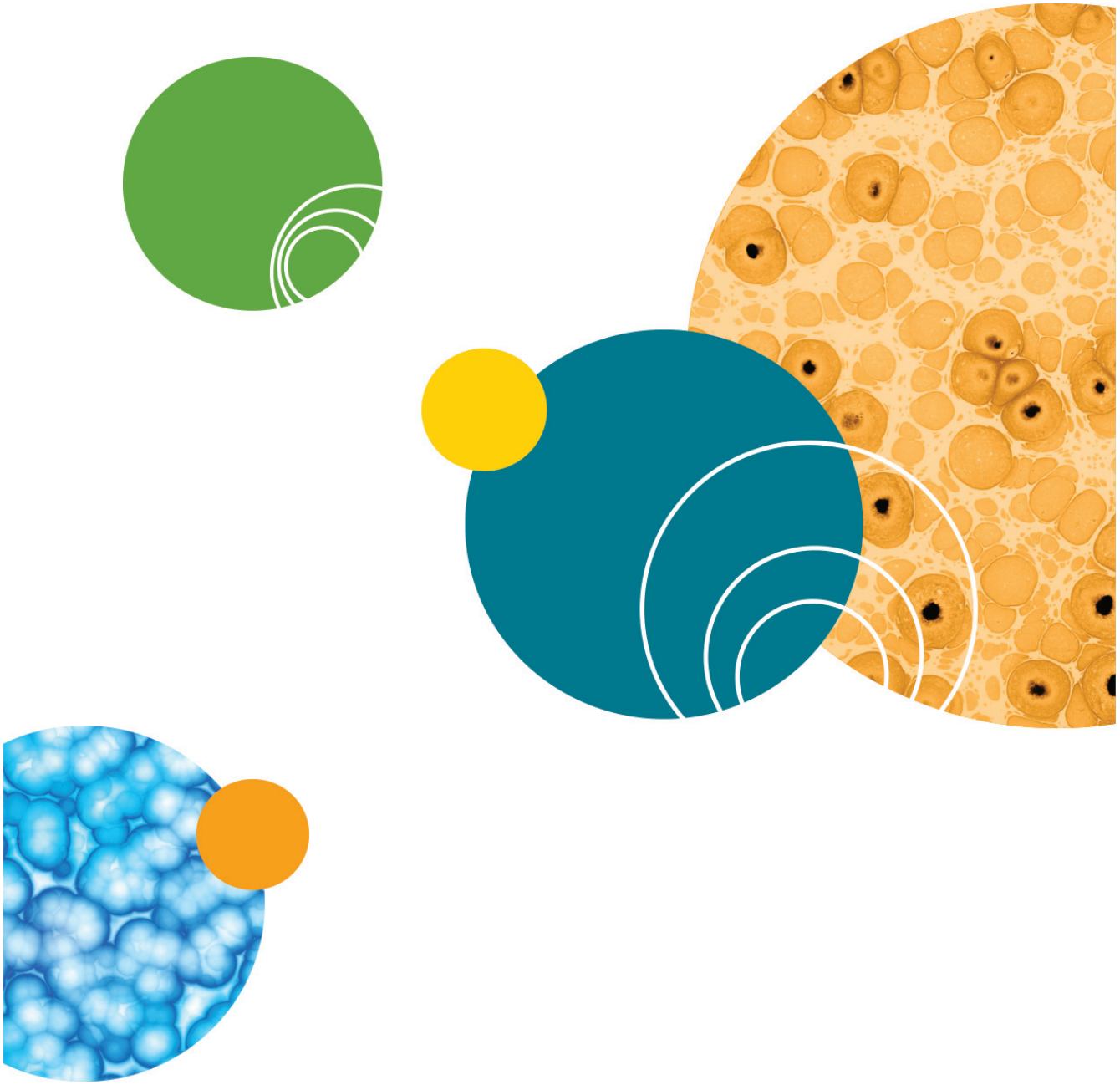
Plate Choice

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 204.



Setting Up Plate Acquisition

5

Before configuring an experiment, it is important to become familiar with the configuration tools that you use in the MetaXpress® Software. The foundation of the MetaXpress Software is the MetaMorph® Microscopy Automation and Image Acquisition Software. The MetaMorph Software contains numerous dialogs for image acquisition, processing, and acquisition. The MetaXpress Software adds database integration and tools for controlling the ImageXpress® Micro imaging systems and acquiring and analyzing microplates. This chapter explains the **Plate Acquisition Setup** dialog, which is the dialog that is used to *configure* a screening experiment.

Note: For information about the various dialogs (**Plate Acquisition**, **Plate Acquisition Setup**, and **Plate Acquisition and Control**) that are that are used to *acquire* plate data, see Chapter 6: Acquiring Plates on page 143.

Accessing the Plate Acquisition Setup Dialog

The MetaXpress Software main menu includes the **Screening** menu, which is used specifically in the MetaXpress Software for protocol configuration, image acquisition and acquisition. The **Screening** menu provides access to all plate configuration and acquisition-specific dialogs, including the **Plate Acquisition Setup** dialog. Figure 5-1 and Figure 5-2 show the options that are available from the **Screening** menu.

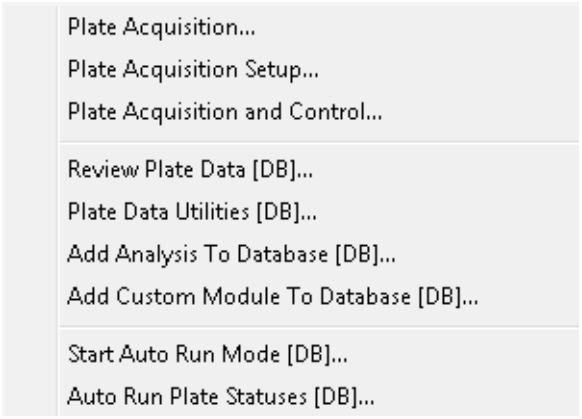


Figure 5-1: Default Screening menu

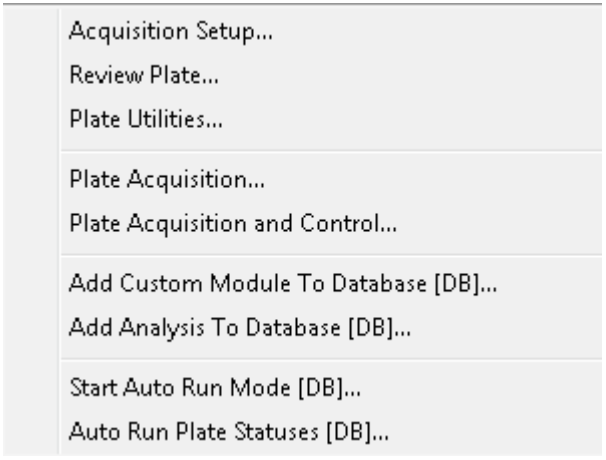


Figure 5-2: Simplified Screening Menu

Plate Acquisition Setup Dialog Layout

The **Plate Acquisition Setup** dialog provides three primary functions.

- Plate Navigation that provides manual control of some of the movable physical components of the ImageXpress® Micro imager
- Configuration Control on the **Configure** tab for configuring plate acquisition protocols
- Acquisition Control on the **Run** tab for carrying out a plate acquisition according to a selected protocol

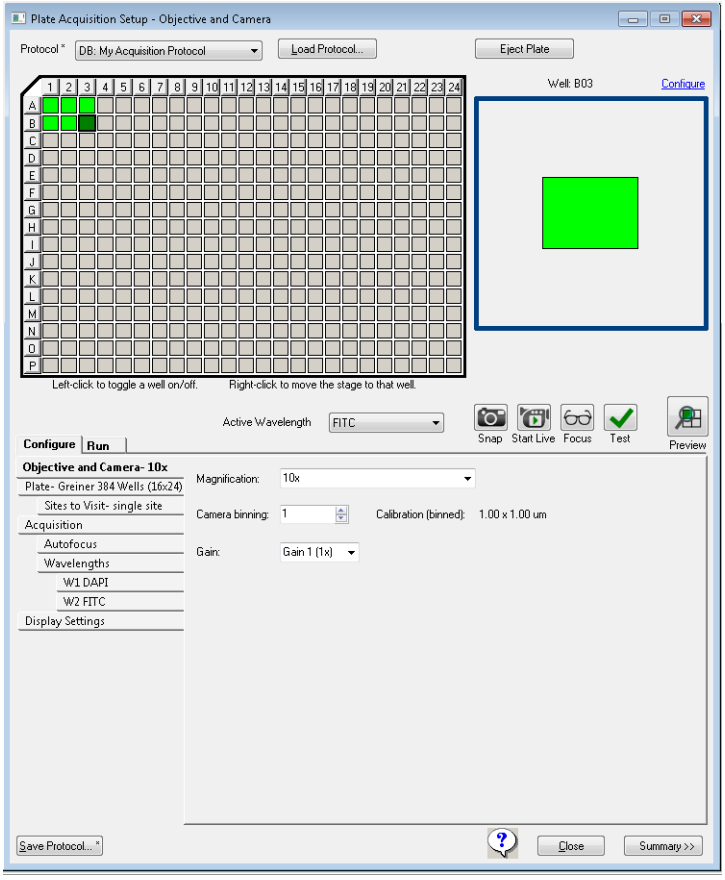


Figure 5-3: Plate Acquisition Setup dialog

The dialog has the following layout.

- **Protocol**: A drop-down list of the eight most recently used plate acquisition protocols.
- **Load Protocol** button: Opens the **Load Plate Acquisition Protocol** dialog in which you select a protocol for plate acquisition.
- **Eject Plate/Load Plate** button: Toggles between these two options. **Eject Plate** opens the sample door for loading or removing a plate. **Load Plate** closes the sample door so that the plate can be acquired.

Note: Beginning with Version 6.0 of the MetaXpress Software, the **Plate map** and **Site map** navigation functions that were previously available only from the **Plate Acquisition and Control** dialog are now also available from the **Plate Acquisition Setup** dialog. You can continue to use the **Plate Acquisition and Control** dialog, but for efficiency and convenience, you can just use the single **Plate Acquisition Setup** dialog.

- **Plate map**: The top left graphic. A graphical representation of the type of plate for which you are configuring the wells. The graphic is interactive to provide manual control for moving a stage to a specific well. When the **Plate Acquisition Setup** opens the first time before any protocols are configured and loaded, all the wells in the **Plate map** are a bright green, which indicates that they are all turned on and that data will be acquired for all of them. You use this interactive graphic to configure only those wells for which data is to be acquired.

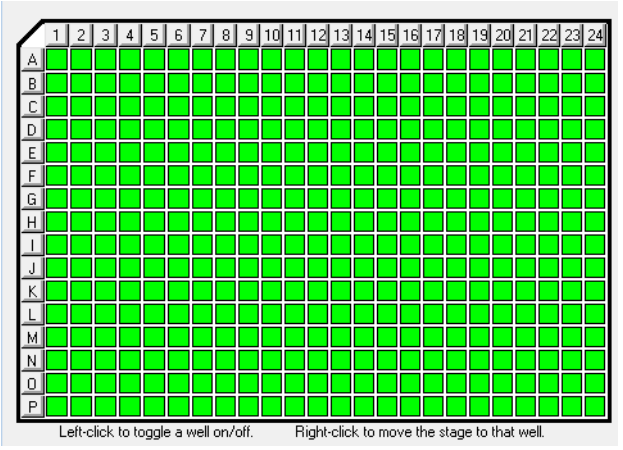


Figure 5-4: Plate Acquisition Setup dialog, Plate map

- **Site map**: The top right graphic. A graphical representation of the number of sites in each well and the position of the sites that are to be acquired for each well. The graphic is interactive to provide manual control for some of the movable physical components of the ImageXpress® Micro imager, including moving the focus. You use this interactive graphic to configure the sites that are to be acquired.

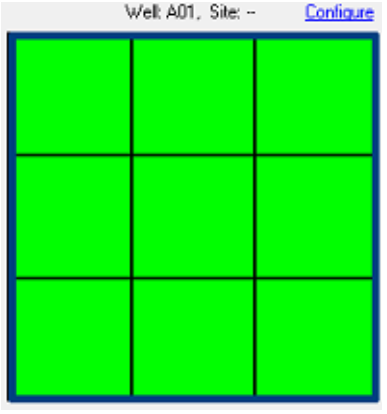


Figure 5-5: Plate Acquisition Setup dialog, Site map

- **Active Wavelength** field: Displays the currently selected acquisition wavelength.

- **Active Wavelength** tools: Provide shortcuts to various functions that can be carried out for the currently selected acquisition wavelength.

Figure 5-6: Active Wavelength tools

Note: There are a few applicable use cases for the **Active Wavelength** tools when configuring a plate acquisition protocol and these are discussed where appropriate in this chapter; however, you use these tools primarily when *acquiring* plate data. As a result, these tools are discussed in detail in Chapter 6: Acquiring Plates on page 143.

- **Configure** tab: Provides the necessary options for configuring a plate acquisition protocol. The **Configure** tab is organized in a "top-to-bottom" tab structure that runs down the left side of the dialog that is designed to guide you through the process of setting up your protocol configuration in a logical order. Each tab is dedicated to a specific type of function or setting. The tabs are dynamically updated according to the options that you have selected and the number of wavelengths that you are acquiring. See:
  - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
  - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
  - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
  - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
  - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136
- **Run** tab: Provides the necessary options for acquiring a plate according to a selected protocol.

Note: This chapter details the Configuration Control functions on the Configure tab. For detailed information about the Plate Navigation function and the Acquisition Control functions that are available on the **Run** tab, see Chapter 6: Acquiring Plates on page 143.

- **Save Protocol** button: Opens the **Save Acquisition Setting** dialog in which you can save the current plate acquisition settings as a protocol. See Saving a Plate Acquisition Protocol on page 141.

Figure 5-7: Save Acquisition Setting dialog

- **Summary** button: Opens the **Summary** panel, which displays a summary of all the current acquisition settings. See Summary Panel on page 140.
- **Close** button: Closes the Plate Acquisition Setup dialog. If you made changes to the configuration settings for the loaded protocol, then a message opens asking if you want to save the current configuration before closing.

Note: When you are configuring a protocol on the **Plate Acquisition Setup** dialog, red or yellow Warning icons might be displayed. A yellow icon can mean that an optional field is not filled in or could indicate another minor error. A red icon means that a required field is either not filled in or contains invalid data that must be changed. Figure 5-8 shows the dialog with several Warning icons.

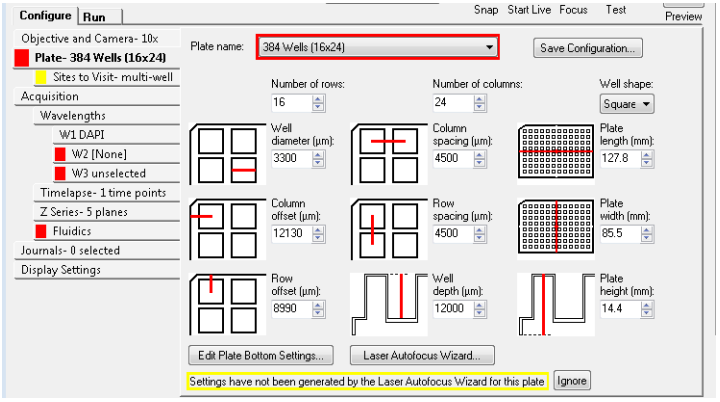


Figure 5-8: Plate Acquisition Setup dialog with Warning icons

Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab

You set the camera binning and gain as well as the magnification setting for a protocol the **Objective and Camera** tab. Based on these settings, you can improve either the image acquisition speed or the image quality.

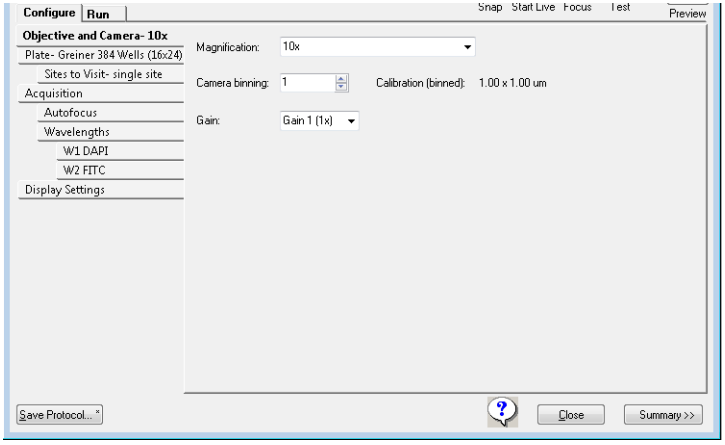


Figure 5-9: Plate Acquisition Setup dialog: Configure tab, Objective and Camera tab

Table 5-1: Plate tab configuration options

Option	Description
Magnification	Selects the magnification setting for the protocol. Magnification settings assign X and Y offset values (parcentricity) and a Z offset (parfocality) to a specific objective. When you select a specific objective, the system is physically moved to this objective position. **Note:** The available magnifications are created with the **Configure Magnification** dialog. See Verifying and Backing Up Magnification Settings on page 46. You must assign a calibration to each magnification setting. See Verifying and Backing Up Calibration Settings on page 49.
Camera binning	Specifies the binning value that is 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. **Note:** If sufficient light is available, lower camera binning increases the image resolution, but higher binning improves the signal-to-noise ratio. Higher binning also improves speed.
Camera gain	Specifies the amplification that is to be applied to the camera output. **Note:** Some cameras that are offered with the ImageXpress® Micro system do not have adjustable gain.

To configure Objective and Camera options

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Objective and Camera** tab.
2. Select the magnification setting for the protocol to move the selected objective into position.
3. Specify the binning value that is to be applied to the camera.
4. If applicable, select the amount of gain.

Note: The resulting pixel size (image calibration) is updated based on the current magnification and binning settings.

5. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

Plate Acquisition Setup dialog: Configure Tab, Plate Tab

You configure the plate dimensions that are necessary to correctly control the X, Y, and Z movements of the ImageXpress Micro System on the **Plate** tab. Correct entry of the plate dimensions ensures that the imager does not make any movements that might create a potentially hazardous situation. The dimensional information that you define for the plate also ensures that the laser-based autofocusing is as accurate as possible.

Note: The MetaXpress Software installation USB memory stick comes with a variety of common plate types already configured. Check the **Plates** folder of the MetaXpress Software CD for plate files. To use these plate files in the MetaXpress Software, you must copy them from the **Plates** folder on the memory stick into the **Plates** directory of your MetaXpress Software installation (the default plates directory is C:\MX#\Plates). After copying the plate files, make sure that the file properties are "writeable" and not read-only. These plate types are then be available from the **Plate** name drop-down list on the **Plate** tab. When you select a pre-configured plate type, the default values for the plate dimensions are displayed in the appropriate fields on the tab. After you have added a given plate type to C:\MX#\Plates, you must run the Laser Autofocus Wizard to define the laser settings for the plate type.

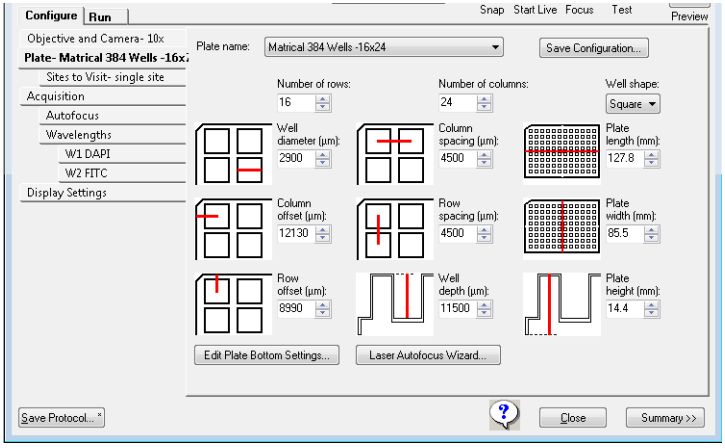


Figure 5-10: Plate Acquisition Setup dialog: Configure tab, Plate tab

Table 5-2: Plate tab configuration options

Option	Description
Plate name	Specifies the plate type that you are using for the protocol. You can select an existing configuration, or you can create a new custom plate configuration. The tab fields are automatically populated with values according to the plate type that you select. If your plate type is not available on the **Plate name** list, then you must manually type the manufacturer's plate specifications for these values. **Note:** The manufacturer generally cannot provide values for the **Plate Bottom** settings, including **Optical thickness** (which is not the same as plate thickness) and the **Bottom variation**. You must run the Laser Autofocus Wizard to measure these values on the instrument to ensure proper focusing. See Plate Dimensions Considerations on page 80.
Save Configuration	Opens the **Save Configuration** dialog, which you can use to name and save a a new custom plate configuration based on the currently displayed plate type values.
Number of rows	Indicates the number of rows for the selected plate type.
Number of columns	Indicates the number of columns for the selected plate type.
Well shape	Indicates the shape of the well on the plate: either **Circle** or **Square**.
Well diameter	Specifies the diameter of the well in µm. **Note:** The wells for many plate types have a slight conical shape. If you are creating a new custom plate configuration, then you must provide the diameter of the bottom of the well, not the top.
Column spacing	Specifies the spacing in µm between each well on the X axis. **Note:** Generally, this value should be the same for both the X and Y axis; however, if you are creating a new custom plate configuration, you can specify different values if needed.

Table 5-2: Plate tab configuration options (cont'd)

Option	Description
Plate length	Specifies the plate length in mm. The ANSI standard is 127.8 mm.
Column offset	Specifies the distance in µm between the center of well A01 and the left edge of the plate.
Row Spacing	Specifies the spacing in µm between each well on the Y axis. **Note:** Generally, this value should be the same for both the X and Y axis; however, if you are creating a new custom plate configuration, you can specify different values if needed.
Plate width	Specifies the plate width in mm. The ANSI standard is 85.5 mm.
Row offset	Specifies the distance in µm between the center of well A01 and the top edge of the plate.
Well depth	Specifies the well depth in µm. **Note:** The correct **Well depth** value is required for autofocusing and fluidics events.
Plate height	Specifies the plate height in mm. **Note:** The correct **Plate height** value is required for autofocusing and fluidics events.
Edit Plate Bottom Settings	Opens the **Configure Plate Bottom Settings** dialog, which you can use to adjust plate bottom settings. **CAUTION!** This dialog is intended primarily for informational and diagnostic purposes, and only advanced users should use this feature to adjust plate bottom settings. If you adjust plate bottom settings incorrectly, you might damage the objective or plate. Molecular Devices strongly recommends that you use the Laser Autofocus Wizard instead to calculate the plate bottom measurements.

Table 5-2: Plate tab configuration options (cont'd)

Option	Description
Laser Autofocus Wizard	Opens the Laser Autofocus Wizard, which guides you step by step through the process of automatically calculating plate bottom dimensions and the exposure times that are required for each objective.

Plate Dimensions Considerations

Even if you use an existing plate type configuration, Molecular Devices recommends that you use the Laser Autofocus Wizard to verify the accuracy of the **Bottom thickness** and four different variation measurements for your plates, where:

- **Bottom thickness** is a value in µm that is the average thickness for the well bottom.
- The four different measurements are the following.
  - Bottom thickness max variation
  - Adjacent well max variation
  - Intra-well max variation
  - Plate max variation

Note: All plate bottom thickness values (also known as reduced thickness measurements) are optical thickness as measured using the objective. The values are not the physical thickness measurements that the manufacturer provides. The optical thickness is the physical thickness of the plate bottom divided by the refractive index of the material.

Although plate manufacturers generally provide reliable plate and well dimensions, you must calculate the plate bottom measurements, such as average thickness and maximum variation in thickness of the entire plate. These parameters are critical and can vary from lot to lot. Also, plate manufacturers can change plate parameters without changing plate names. The Laser Autofocus Wizard walks you through steps to automatically calculate plate bottom dimensions as well as the exposure times that are required for each objective.

Note: The Laser Autofocus Wizard calculates measurements as accurately as possible. Some manual verification and adjustment of the settings might be necessary to optimize the results, particularly for thin-bottom plates. The MetaXpress Software online help topic "Configure Laser Autofocus Settings - Dialog Box Options" provides detailed information about the settings that the wizard calculates. Press **F1** with the dialog open to access the help. Contact Technical Support if you need additional assistance. See Obtaining Support on page 34.

CAUTION! You must not use the Laser Autofocus Wizard for slides as it calculates incorrect settings for slides. If you need assistance with slides, contact Technical Support. See **Obtaining Support on page 34****.**

To configure the dimensions for a new custom plate configuration

If your custom plate is 96-well or 384-well format, then you can select the appropriate template, and modify any values as needed. If your custom plate is any other size, then you must specify *all* the values for the plate.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Choose the appropriate size for your custom plate.
2. Click **Save Configuration**.
3. In the **Plate Acquisition - Save Configuration** dialog, type the name for your custom plate configuration, and then click **Save**.
4. In the **Well shape** field, select the well shape for the plate.
5. Complete the remaining configuration fields as required. See Table 5-2 on page 78.

CAUTION! You must enter the Well depth and Plate height values and all other plate dimensions correctly before you run the Laser Autofocus Wizard, or the wizard will more than likely fail

6. Click **Laser Autofocus Wizard** and follow the steps that the wizard provides to calculate plate bottom measurements.
7. If required, in the **Well depth** and **Plate height** fields, edit the values.
8. Click **Save Configuration**.
9. In the **Plate Acquisition - Save Configuration** dialog, click **Save,** and then in the **Plate Acquisition** message dialog, click **Yes** at the prompt to overwrite the existing custom plate type.

Note: If needed, adjust any objectives with correction collars to match the plate type.

10. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
    - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
    - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
    - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
    - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab

You configure the number of sites that are to be acquired for each well in a plate acquisition protocol on the **Sites to Visit** tab. You can acquire a single site per well, or multiple sites per well. 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.

The dialog tab is dynamically updated with the appropriate configuration options based on the site option that you select. The **Site map**, which is a graphical representation of the sites that you are configuring, is dynamically updated based on the configuration options that you select and the values that you specify for these options. See:

- To acquire a single site in each well on page 83.
- To acquire a fixed number of sites in each well on page 85.
- To configure adaptive acquisition for well sites on page 89.
- To configure a multi-well acquisition on page 96.

Note: You use the **Plate map** and **Site map** that are at the top of the **Plate Acquisition Setup** dialog to select and move to the wells and sites that are to be analyzed in a plate acquisition protocol. This topic and all other plate navigation dialogs are covered in detail in Chapter 6: Acquiring Plates on page 143.

To acquire a single site in each well

The **Single site** option acquires a single site that is centered in each well.

Note: To acquire a single off-center site, you should select **Fixed number of sites**, configure the site layout, and then deselect the unwanted sites. See To acquire a fixed number of sites in each well on page 85.

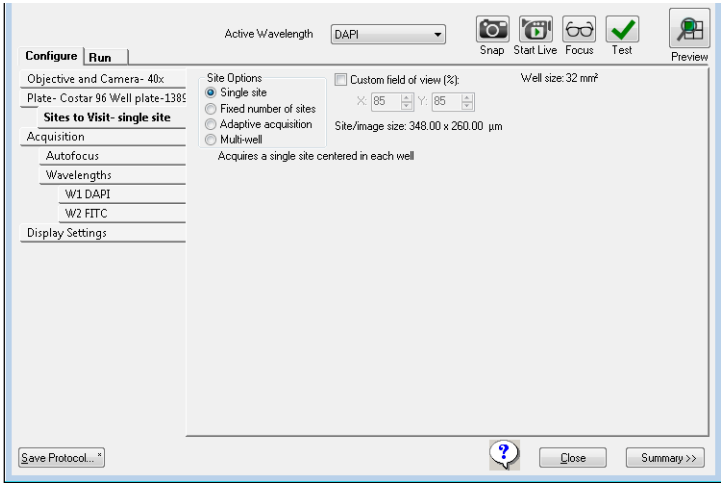


Figure 5-11: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Single site option selected

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Sites to Visit** tab.
2. Select **Single site**.
3. Optionally, to specify a percentage of the width (X) and height (Y) of the camera's field of view to acquire, select **Custom Field of View**, and then type the new X and Y values.

Note: The full field of view for the camera 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 type 50 in the X field and 50 in the Y field. This feature is particularly useful if the image that is acquired using the full field of view is not evenly illuminated or if the image that is 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.

Note: If a shading correction image was acquired with the full field of view, then you can use the **Custom Field of View** feature with shading correction. See To carry out shading correction on acquired images on page 134.

4. If data is not going to be acquired for all the wells in a plate, then on the **Plate map**, do any of the following as needed to configure the wells for which data is to be acquired.

- To turn off a well, click it. The well turns light gray, which indicates that data will not be acquired for the well. To turn it back on, click the well again. The well turns light green again.
- To turn off all the wells in the column in a single step, click the column header. To turn all the wells back on, click the column header again.
- To turn all the wells in a row off in a single step, click the row ID. To turn all the wells back on, click the row ID again.
- To turn off a contiguous group of wells in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate wells. To turn the wells back on, click and hold the left mouse button, and then drag the cursor across the wells again.
- To turn on or off all wells in a plate in a single step, click in the upper left corner of the plate just to the outside of the first well, A01, on the plate.

Note: You can configure the wells for which data is to be acquired as part of a protocol, or you can select the wells "on the fly" at the time that you run the protocol. See Chapter 6: Acquiring Plates on page 143.

5. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To acquire a fixed number of sites in each well

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Sites to Visit** tab.
2. Select **Fixed number of sites**.

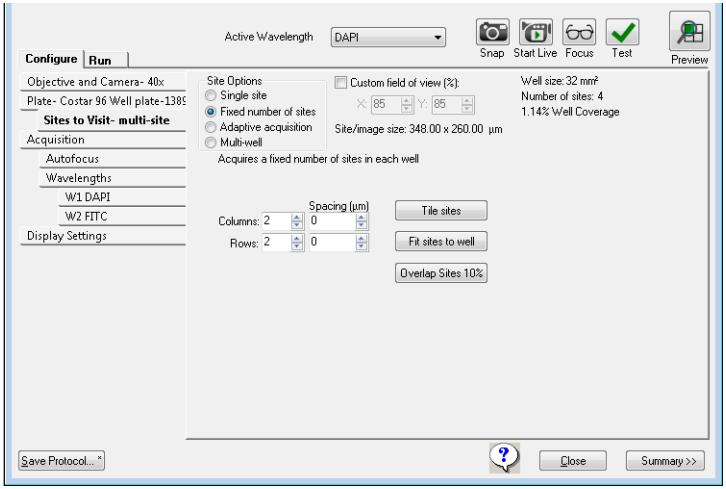


Figure 5-12: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Fixed number of sites option selected

3. Optionally, to specify a percentage of the width (X) and height (Y) of the camera's field of view to acquire, select **Custom Field of View**, and then type the new X and Y values.

Note: The full field of view for the camera 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 type 50 in the X field and 50 in the Y field. This feature is particularly useful if the image that is acquired using the full field of view is not evenly illuminated or if the image that is 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

Note: If a shading correction image was acquired with the full field of view, then you can use the **Custom Field of View** feature with shading correction. See To carry out shading correction on acquired images on page 134.

4. In the **Columns** and **Rows** fields, specify the maximum number of sites that are to be visited. For example, specify 2 columns and 2 rows, which are the default values, to acquire up to four sites, specify 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.

5. If data is not going to be acquired for all the wells in a plate, then on the **Plate map**, do any of the following as needed to configure the wells for which data is to be acquired.
   - To turn off a well, click it. The well turns light gray, which indicates that data will not be acquired for the well. To turn it back on, click the well again. The well turns light green again.
   - To turn off all the wells in the column in a single step, click the column header. To turn all the wells back on, click the column header again.
   - To turn all the wells in a row off in a single step, click the row ID. To turn all the wells back on, click the row ID again.
   - To turn off a contiguous group of wells in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate wells. To turn the wells back on, click and hold the left mouse button, and then drag the cursor across the wells again.

Note: You can configure the wells for which data is to be acquired as part of a protocol, or you can select the wells “on the fly” at the time that you run the protocol. See Chapter 6: Acquiring Plates on page 143.

   - To turn on or off all wells in a plate in a single step, click in the upper left corner of the plate just to the outside of the first well, A01, on the plate.

6. If data is not going to be acquired for all the sites in a well, then on the **Site map**, do any of the following as needed to configure the sites for which data is to be acquired.

- To turn a site off, click it. The site turns gray, which indicates that data will not be acquired for the site. To turn the site back on, click the site again. The site turns bright green again.
- To turn off a contiguous group of sites in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate sites. To turn the sites back on, click and hold the left mouse button, and then drag the cursor across the sites again.

Note: The site controls apply to all sites in all wells at the same time. When you turn on or off sites for a selected well, then all wells in the plate will have data acquired for the same sites.

Note: You can configure the sites in a well for which data is to be acquired as part of a protocol, or you can select the sites "on the fly" at the time that you run the protocol. See Chapter 6: Acquiring Plates on page 143.

7. Optionally, do one of the following as needed.

- To manually adjust the spacing between adjacent sites, where the default value is zero, or no spacing, type the X and Y values for the spacing in the **Spacing (µm)** fields.
- To manually adjust the spacing between adjacent sites so that the sites overlap, type *negative* X and Y values for the spacing in the **Spacing (µm)** fields.

Note: The camera size limits these values, and therefore, the amount of overlapping that you can specify.

- To automatically adjust the spacing between adjacent sites so that there is zero spacing between them, click **Tile Sites**. The X and Y spacing values are set to zero.
- To automatically adjust the spacing between the adjacent sites to the maximum allowed value based on the selected plate type, click **Fit sites to well**. The X and Y spacing values are updated accordingly.
- To automatically overlap the sites by 10%, click **Overlap Sites 10%**. The X and Y spacing values are updated to the

appropriate *negative* values based on the selected plate type.

8. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To configure adaptive acquisition for well sites

The **Adaptive Acquisition**™ option is a computational algorithm that is designed to analyze cell number on the fly during sample acquisition to increase the chances of collecting valid data in every well. If this option is selected, then the number of sites that are acquired per well is based on the number of cells per well.

Note: The **Adaptive Acquisition** option can significantly reduce acquisition time for multi-wavelength acquisition that requires a minimum number of cells per well or for samples with differing conditions across the plate.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Sites to Visit** tab.
2. Select **Adaptive Acquisition**.

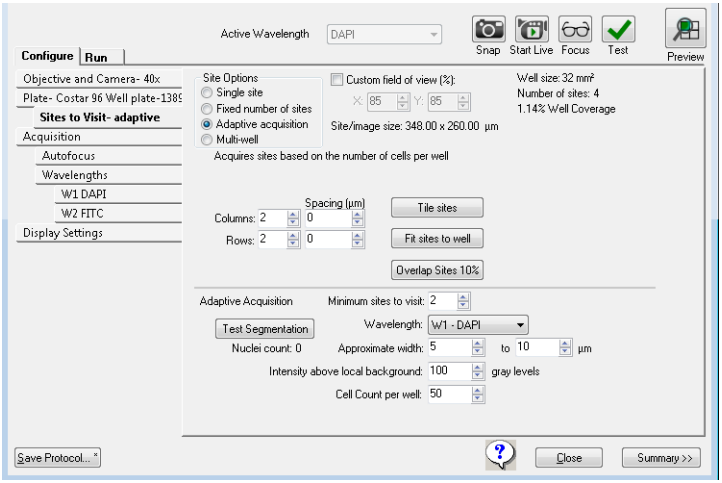


Figure 5-13: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Adaptive Acquisition option selected

3. Optionally, to specify a percentage of the width (X) and height (Y) of the camera's field of view to acquire, select **Custom Field of View**, and then type the new X and Y values.

Note: The full field of view for the camera 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 type 50 in the X field and 50 in the Y field. This feature is particularly useful if the image that is acquired using the full field of view is not evenly illuminated or if the image that is 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.

Note: If a shading correction image was acquired with the full field of view, then you can use the **Custom Field of View** feature with shading correction. See To carry out shading correction on acquired images on page 134.

4. In the **Columns** and **Rows** fields, specify the maximum number of sites that are to be visited. For example, specify 2 columns and

2 rows, which are the default values, to acquire up to four sites, specify 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.

5. If data is not going to be acquired for all the wells in a plate, then on the **Plate map**, do any of the following as needed to configure the wells for which data is to be acquired.
   - To turn off a well, click it. The well turns light gray, which indicates that data will not be acquired for the well. To turn it back on, click the well again. The well turns light green again.
   - To turn off all the wells in the column in a single step, click the column header. To turn all the wells back on, click the column header again.
   - To turn all the wells in a row off in a single step, click the row ID. To turn all the wells back on, click the row ID again.
   - To turn off a contiguous group of wells in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate wells. To turn the wells back on, click and hold the left mouse button, and then drag the cursor across the wells again.
   - To turn on or off all wells in a plate in a single step, click in the upper left corner of the plate just to the outside of the first well, A01, on the plate.

Note: You can configure the wells for which data is to be acquired as part of a protocol, or you can select the wells “on the fly” at the time that you run the protocol. See Chapter 6: Acquiring Plates on page 143.

6. If data is not going to be acquired for all the sites in a well, then on the **Site map**, do any of the following as needed to configure the sites for which data is to be acquired.

- To turn a site off, click it. The site turns gray, which indicates that data will not be acquired for the site. To turn the site back on, click the site again. The site turns bright green again.
- To turn off a contiguous group of sites in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate sites. To turn the sites back on, click and hold the left mouse button, and then drag the cursor across the sites again.

Note: You can configure the sites in a well for which data is to be acquired as part of a protocol, or you can select the sites "on the fly" at the time that you run the protocol. See Chapter 6: Acquiring Plates on page 143.

7. Optionally, do one of the following as needed.

- To manually adjust the spacing between adjacent sites, where the default value is zero, or no spacing, type the X and Y values for the spacing in the **Spacing (µm)** fields.
- To manually adjust the spacing between adjacent sites so that the sites overlap, type *negative* X and Y values for the spacing in the **Spacing (µm)** fields.
- To automatically adjust the spacing between adjacent sites so that there is zero spacing between them, click **Tile Sites**. The X and Y spacing values are set to zero.
- To automatically adjust the spacing between the adjacent sites to the maximum allowed value based on the selected plate type, click **Fit sites to well**. The X and Y spacing values are updated accordingly.

Note: Overlapping sites is not recommended for use with **Adaptive Acquisition** because it might result in some cells being counted more than once.

8. Specify the **Adaptive Acquisition** settings.

Table 5-3: Adaptive Acquisition options

Option	Description
Minimum sites to visit	The minimum number of sites to visit in a well. The MetaXpress Software acquires at least this minimum number of sites and then it continues to acquire sites until the total number of cells counted per well value as specified in the **Cell count per well** field is reached.
Wavelength	The wavelength that is used to differentiate nuclei in the source image. **Note:** This wavelength must be the same as the wavelength that is designated as being the first to be acquired. See To configure the acquisition wavelengths on page 108.
Approximate width	The approximate minimum width and the approximate maximum width of the nuclei that are expected to be detected. **Note:** Nuclei patterns in the source image that fall below this range are considered as noise. The algorithm might split larger objects that are above the maximum width into smaller objects, which can affect the cell count.
Intensity above local background gray levels	The intensity threshold of nuclei in the source image compared to the neighboring background values. This setting controls the sensitivity of detection. **Note:** For information about setting the intensity threshold, see To calculate the Intensity Above Local Background gray level value on page 96.

Note: The **Approximate width** and **Intensity above local background gray levels** work in conjunction – that is, how big must an object be and how much greater must its intensity be above the background to be considered a nucleus?

Table 5-3: Adaptive Acquisition options (cont'd)

Option	Description
Cell count per well	The total number of nuclei in each well that the MetaXpress Software must acquire before stopping acquisition of the well. **Note:** The MetaXpress Software always acquires the minimum number of sites, even if the **Cell count per well** can be obtained with a fewer number of sites. As the **Adaptive Acquisition** mode runs, a real-time count of the cells in the currently selected well is displayed as the images are acquired. **Adaptive Acquisition** stops acquiring when the maximum number of sites that have been defined is reached, even if the specified cell count is not attained.

9. Optionally, to determine if your current acquisition settings are appropriate for differentiating nuclei, select a well or wells and a site or sites for viewing, click **Focus** to bring the sample into focus, and then click **Test Segmentation**.

CAUTION! Focus carries out a large range autofocus on the currently selected well and site. To ensure that your cell counting settings are accurate, you must bring the sample into focus before you can click **Test Segmentation**.

After you click **Test Segmentation**, an image is snapped and the nuclei are counted. Both an acquisition overlay and the total number of cells counted in the image are displayed.

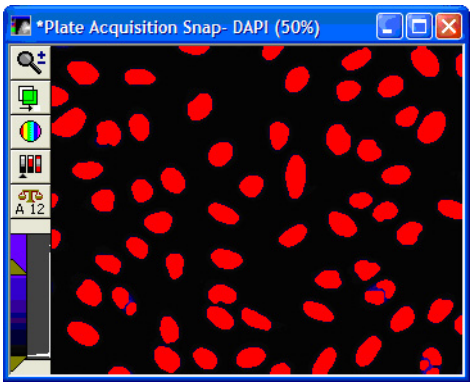


Figure 5-14: An example of Test Segmentation results in the Adaptive Acquisition mode. The red shows the segmentation overlaid on the original DAPI image.

10. If your test results are satisfactory, then continue to Step 11; otherwise, repeat Step 8 and Step 9 until your test results are satisfactory.
11. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
    - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
    - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
    - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
    - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To calculate the Intensity Above Local Background gray level value

When calculating the **Intensity Above Local Background gray level** value, you should set this value to one that is approximately half of the final calculated value.

1. On the source image, locate one of the dimmest objects (for example, a nucleus or a micronucleus, if applicable) that the MetaXpress Software must be able to detect.
2. Position your mouse pointer on the outside edge of the object and make note of the gray level value that is displayed in the status bar.

   For example, (48, 158) -> 18000 indicates that 18000 is the average gray level value of the nucleus.
3. Position your mouse pointer just outside of the object and make note of the gray level value that is displayed in the status bar.

   For example, (48, 162) -> 2000 indicates that 2000 is the average gray level value of the background just outside the object.

Note: Instead of carrying out Step 2 and Step 3, you might consider drawing a line region across a cell and its local background, and then use the **Linescan** tool that is available from the **Measure** menu to see more exact intensity values. See the MetaXpress Software online help for information about this tool.

4. Subtract the gray level value of the background from the gray level value of the object. The result is the intensity above local background value.

   Using the examples above, 18000 – 2000 = 16000 for the **Intensity Above Local Background gray level** value. You would then set this value to approximately 8000.

To configure a multi-well acquisition

To acquire high density plates faster, you can carry out a multi-well acquisition. A multi-well acquisition uses the size of the camera field of view to acquire several wells simultaneously while taking a single image, thereby reducing plate acquisition time. The MetaXpress Software automatically calculates the number of wells in which a site can be simultaneously acquired and this value depends on the plate size and the magnification that is configured for the protocol. Common configurations for multi-well acquisitions include a 1536 well plate with a 4x

magnification objective or a 384 well plate with a 2x magnification objective.

Note: During a multi-well acquisition, unselected wells might be exposed to excitation light from imaging neighboring selected wells; however, images are not be saved for the unselected wells.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Sites to Visit** tab.
2. Select **Multi-well**.

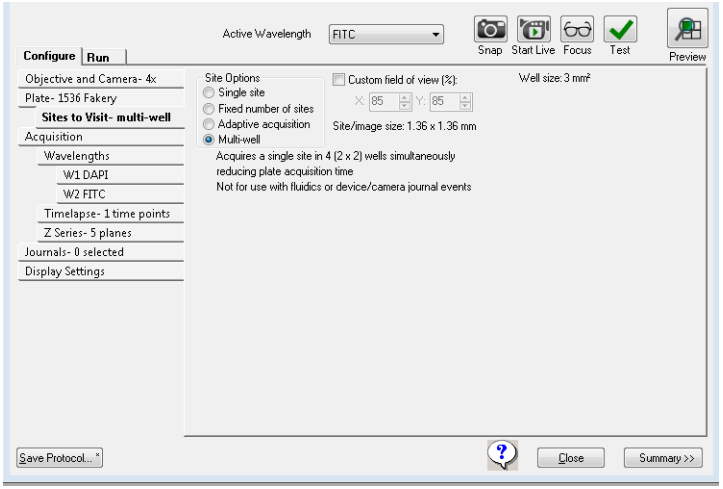


Figure 5-15: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Multi-well option selected

3. Optionally, to specify a percentage of the width (X) and height (Y) of the camera's field of view to acquire, select **Custom Field of View**, and then type the new X and Y values.

Note: The full field of view for the camera 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 type 50 in the X field. This feature is particularly useful if the image that is acquired using the full field of view includes the area outside the well boundary. By reducing the field of view, you can acquire an image of only the area that is within the well.

Note: If a shading correction image was acquired with the full field of view, then you can use the **Custom Field of View** feature with shading correction. See To carry out shading correction on acquired images on page 134.

4. If data is not going to be acquired for all the wells in a plate, then on the **Plate map**, do any of the following as needed to configure the wells for which data is to be acquired.
   - To turn off a well, click it. The well turns light gray, which indicates that data will not be acquired for the well. To turn it back on, click the well again. The well turns light green again.
   - To turn off all the wells in the column in a single step, click the column header. To turn all the wells back on, click the column header again.
   - To turn all the wells in a row off in a single step, click the row ID. To turn all the wells back on, click the row ID again.
   - To turn off a contiguous group of wells in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate wells. To turn the wells back on, click and hold the left mouse button, and then drag the cursor across the wells again.
   - To turn on or off all wells in a plate in a single step, click in the upper left corner of the plate just to the outside of the first well, A01, on the plate.

Note: You can configure the wells for which data is to be acquired as part of a protocol, or you can select the wells “on the fly” at the time that you run the protocol. See Chapter 6: Acquiring Plates on page 143.

5. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab

At a minimum, you must specify the autofocus options and acquisition wavelengths for your protocol on the **Acquisition** tab. Optionally, you can also specify various settings such as fluidics events and shading correction. Depending on the options that you select, other tabs with additional configuration options might also be displayed.

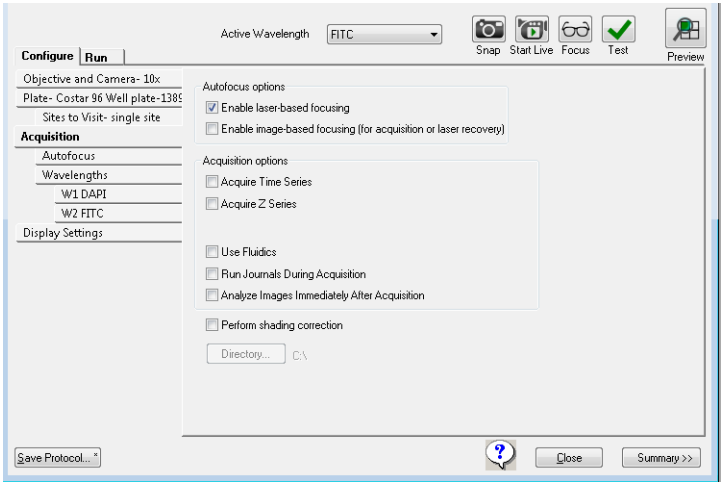


Figure 5-16: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

See:

- To configure autofocus options on page 100.
- To configure the acquisition wavelengths on page 108.
- To configure series acquisition options on page 119.
- To configure journals to run during acquisition on page 127.
- To configure post-acquisition analysis options on page 130.
- To carry out shading correction on acquired images on page 134.

Note: To configure fluidic stations, select **Use Fluidics** to open the **Fluidics** tab. For information about configuring fluidics stations and properties, see the *ImageXpress*® Micro *Widefield High Content Screening System Options User Guide*, or the MetaXpress Software help topic "Fluidics Events."

To configure autofocus options

Two autofocus configuration options are available on the **Acquisition** tab.

- Laser-based autofocusing is generally set to find the bottom of the well, and then moves the objective a specified distance up from the well bottom. This method is generally the fastest and does not cause photo damage to your specimen; however, this method might not be sufficient if the distance above the bottom of the well varies in your sample. Thumbprints or scratches at the bottom of the plate can also affect laser-based autofocusing.
- Image-based focusing uses an algorithm to identify the best focus image. This method works best for experiments that use low power objectives or when the sample distance above the bottom of the plate varies; however, this method can be slower than laser-based autofocusing and it can fail if out-of-focus debris is present in a sample.

You can configure one or both of these autofocus options for a plate acquisition protocol. Generally, with the exception of oil-immersion objectives, you should use laser-based autofocusing. Certain types of samples generally benefit from image-based focusing in addition to the laser-based focusing, including live organisms, suspension cells, tissue samples, or assays where the best focus position varies with the phenotype. If you select both laser-based and image-based autofocusing, then laser-based autofocusing is used to obtain a specified position above the bottom of the well and the image-based focusing is used to

fine tune the position. For oil-immersion objectives, you should use only image-based autofocusing.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Acquisition** tab.

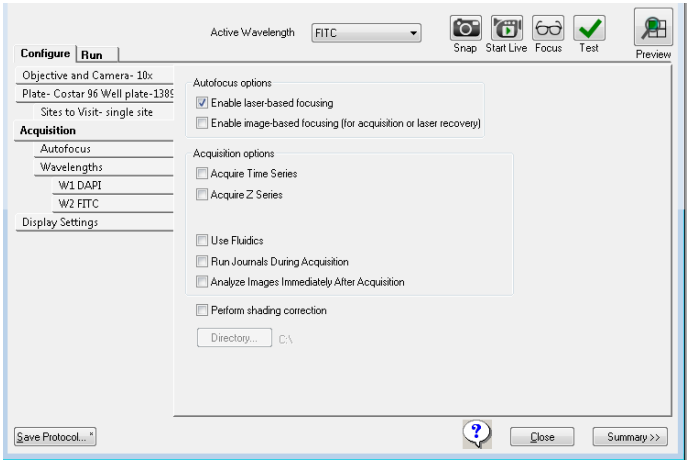


Figure 5-17: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

2. Under **Autofocus options**, select one or both options.
   - **Enable laser-based autofocusing**
   - **Enable image-based focusing (for acquisition or laser recovery)**

Note: Image-based focusing is best for complex samples with variations in distance between the surface of the plate and the sample.

3. Open the **Autofocus** tab.

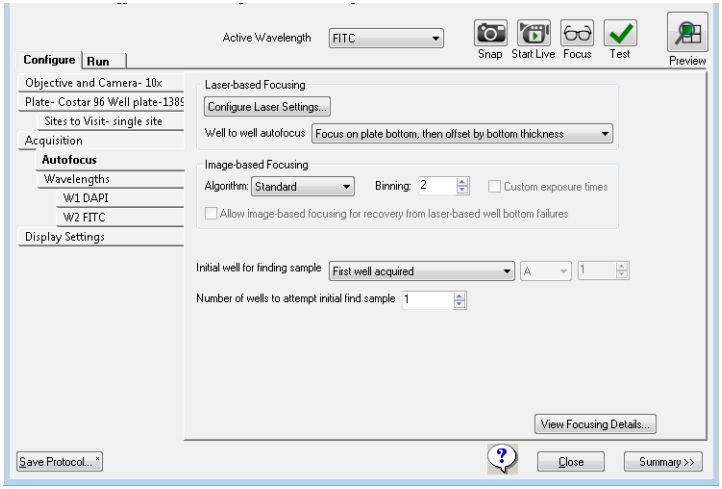


Figure 5-18: Plate Acquisition Setup dialog: Configure tab, Autofocus tab

4. Configure the autofocus options. See Table 5-4 on page 103.

Table 5-4: Autofocus configuration options

Option	Description
Laser-based focusing: Available only if **Enable laser-based autofocusing** is selected on the **Acquisition** tab.	
Configure Laser Settings	Opens the **Configure Laser Autofocus Settings** dialog. The settings for this dialog will have been calculated by running the Laser Autofocus Wizard. If the wizard has not been run for a selected plate and objective, then dialog might be blank. You can modify the settings that the wizard has calculated for this dialog. **Note:** The MetaXpress Software help topic "Configure Laser Autofocus Settings - Dialog Box Options" provides detailed information about the settings that the wizard calculates. Press F1 with the dialog open to access the help. For tips about using the Laser Autofocus Wizard, contact Technical Support for assistance. See Obtaining Support on page 34.
Well to well autofocus: Settings for adjusting the focus as the acquisition moves from well to well.	
• Focus on well bottom	The default value and the recommended option for most plate acquisition protocols. The initial focus on the plate, **Find Sample**, focuses on both the plate bottom and well bottom. As the system moves from well to well, the camera focuses on the well bottom only, using the **Bottom variation** settings from the plate configuration to determine the focus range.

Table 5-4: Autofocus configuration options (cont'd)

Option	Description
• Focus on plate bottom, then offset by bottom thickness	Offsets the laser by the **Bottom thickness** of the plate from the plate configuration. Select this option if you are using any of the following. • A thin-bottom plate with an objective with a large depth of field because of low magnification or low numerical aperture. This is typically for 4x and below. • A slide. • Multi-well acquisition.
• Focus on plate and well bottom	The laser focuses on both the plate bottom and the well bottom at every well. **Note:** This is recommended for plates with extreme bottom variation.
Note: Chamber slides with wells can use any of the above options.	
Image-based focusing	
Algorithm: The focusing algorithm.	
• Standard	The default algorithm, which is based on a standard group of settings including a normal camera signal level.
• Low Signal	Based on a set of values that are designed to compensate for the following. • The camera has a low signal level. • Some pixel intensities are somewhat brighter when slightly out of focus.
The following options are camera configuration options.	
Binning	Sets the binning used by the camera during the **Auto Focus** and **Show Live** commands. Horizontal and vertical binning are always set to the same value.

Table 5-4: Autofocus configuration options (cont'd)

Option	Description
Custom exposure times	Allows for setting custom exposure times for individual wavelengths during autofocus. If this is option is not selected, then the exposure time is calculated based on autofocus binning and acquisition exposure time. If this option is selected and you select either or both of the following options: • **Laser and Image** or **Laser with Image Recovery** for the first acquisition wavelength (W1) • **Image-based** for all subsequent acquisition wavelengths (W2, W3, and so on) then you must also specify values for **Exposure** and **Gain** on the wavelength tabs. See To configure the acquisition wavelengths on page 108.
Allow image-based focusing for recovery from laser-based well bottom failures	Enabled only if both **Enable laser-based focusing** and **Enable image-based focusing** are selected. Use image-based focusing only 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 using the plate bottom position that was found during the last successful laser autofocus, and then adding the plate bottom thickness and post-laser offset values to this plate bottom thickness.
Initial well for finding sample - The well to use when carrying out the initial Find Sample autofocus.	
• First well acquired	Finds the sample autofocus using the first well that is acquired. This is the recommended setting for most protocols.

Table 5-4: Autofocus configuration options (cont'd)

Option	Description
• Specific well	Finds the sample autofocus using a well that you specify. **Note:** After you select this option, you must specify the well row and well number. A1 is the default value.
• Skip Find Sample (select if sample is already in focus)	Disables the initial **Find Sample** autofocus when starting to acquire a plate. Select this option if your sample is already in focus. **Note:** You should select this option if you are using an oil-immersion objective, and you must manually find the focus before starting the plate acquisition.
Number of wells to attempt initial find sample	Enabled only if **First well acquired** is selected. The default value is one. The first well in which a sample is found is the well that is used for autofocusing. **Note:** You should set this value to three or greater, particularly if you are running a robot for automated plate loading.
Site Autofocus: Displayed only if **Fixed number of sites** or **Adaptive Acquisition** is selected. Determines how autofocusing is carried out for each site.	
First site only	Autofocuses in the top-left site in the well.
Center of well only	Autofocuses in the center of the well. **Note:** You should select this option when the magnification is low and the sites are relatively close together.

Table 5-4: Autofocus configuration options (cont'd)

Option	Description
All sites	Autofocuses for each site. **Note:** You should select this option with higher magnification, if the sites are spread far apart, and/or there is extreme variation in the plate bottom.
Timelapse Autofocus: Displayed only if the **Time Series** acquisition option is selected on the **Acquisition** tab. See To configure series acquisition options on page 119.	
First timepoint only	Recommended for fast kinetic acquisitions.
All timepoints	Recommended for long timelapse acquisitions.
Every Nth timepoint	You should set this to a value that is less than the number of specified timepoints. See To configure time series acquisition options on page 120.
View Focusing Details	Opens the **Auto Focus Details** dialog, which details the current autofocus parameters. This information can be useful for diagnostic purposes when troubleshooting focusing issues. You can click **Copy** to copy the parameters to the clipboard, and then paste them into a third party application such as Microsoft word. This can be used, for example, if you need to supply this information to Technical Support. See Obtaining Support on page 34.

5. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:

- Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
- Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
- Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
- Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To configure the acquisition wavelengths

You configure exposure time, autofocus, and timelapse settings for each acquisition wavelength on a wavelength tab. The total number of wavelength tabs depends on the number of wavelengths that are selected on the **Wavelengths** tab.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Wavelengths** tab.

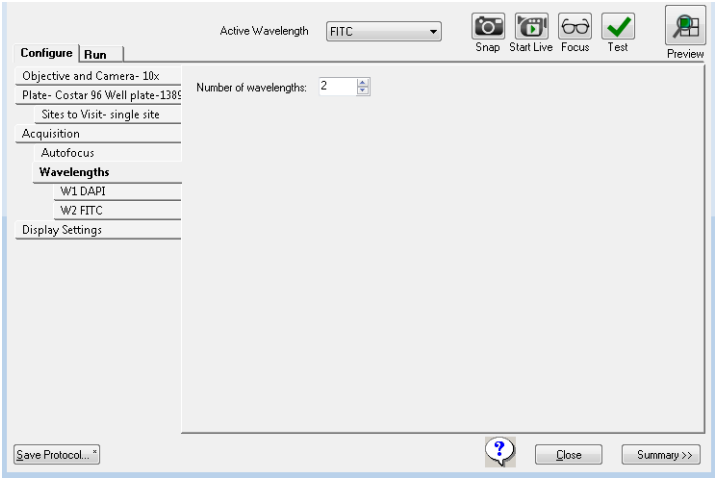


Figure 5-19: Plate Acquisition Setup dialog: Configure tab, Wavelength tab

2. In the **Number of wavelengths** field, select the total number of wavelengths that are to be used for acquisition.

Note: You must select at least one acquisition wavelength. You can select up to a maximum of eight wavelengths. Individual placeholder tabs are displayed that correspond to the number of acquisition wavelengths that you have selected (**W1**, **W2**, **W3**, and so on). The red Warning icon indicates that you have yet to configure the acquisition wavelengths. See Figure 5-20 on page 109.

Figure 5-20: Plate Acquisition Setup dialog, Configure tab: Three placeholder wavelength tabs

3. For *each* placeholder tab, do the following in the order listed.
   - Click the tab to open it.
   - In the **Illumination setting** field, select the illumination wavelength; for example, DAPI.

Note: After you select a wavelength, its name is displayed on a placeholder tab and in the **Active Wavelength** field at the top of the tab.

   - Configure the remaining acquisition wavelength options, where:

Offset (µm) is the Z motor offset distance for each wavelength that is defined. For the first wavelength, if you are using laser-based focusing, then the offset is the distance between the bottom of the well and the in-focus plane. If you are using just image-based focusing, then no offset is required for the first wavelength. For the second and subsequent wavelengths, with laser-based 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.

Table 5-5: Acquisition wavelength configuration options

Option	Description
Exposure	Specifies the exposure time in milliseconds that is to be associated with the active wavelength. You can type a value in this field or click **Auto Expose** to automatically determine an exposure time. **Note:** When you are configuring exposure times, you should check both positive and negative controls. If you use a dim sample to set the exposure time, then a bright sample might end up saturating. **Note: Auto Expose** is simply a manual tool to help configure the plate. It does *not* auto expose while the plate is being acquired.
Target max intensity	Specifies the intensity that auto exposure should attempt to attain for the brightest pixel in the image. **Note:** The recommended target intensity value is 75% of the maximum gray level that the camera driver reports as possible to obtain.
Autofocus options: Specifies the type of autofocus that is to be used when acquiring images. The options that are available depend on the **Autofocus** options that are specified on the **Acquisition** tab.	

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
• None	No autofocusing is carried out. If you select this option, then no other autofocusing configuration options are available.
• Laser with Z-offset	Displayed as an option only if **Enable laser-based focusing** is selected on the **Acquisition** tab. If selected, the laser autofocus is used based on the settings that the Laser Autofocus calculates, or as configured in the **Configure Laser Settings** dialog. Specify the post-laser offset in µm in the **Offset** field, or click **Calculate Offset** to automatically calculate the offset that is to be used for the first wavelength.
• Calculate Offset icon (>)	Click to display the options that are used to calculate the offset. You can also do the following. • Click **Z stack**: Shows distinct Z planes so that you can select the best in-focus plane. **Note:** If **Z stack** is not selected, then the MetaXpress Software carries out an image autofocus and determines the best in-focus plane. • Click **Custom Range**: Configure the total search range and step size that is used to calculate the offset. **Note:** If **Custom Range** is not selected, then default values are used for the **Calculate Offset** tool using either Z stack or image autofocus.

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
• Laser and image	Displayed as an option for only the first acquisition wavelength (W1) and only if **Enable laser-based focusing** *and* **Enable image-based focusing** are selected on the **Acquisition** tab. If this option is selected, the laser autofocuses first, and then image-based focusing is used to fine tune the image. If this option is selected, then you must also specify values for the following. • **Image-based range +/- µm**: Specifies the range that is to be used for the image-based portions of autofocusing. • **Max. step**: Specifies the maximum step size in µm of a single Z move that is to be used to attain the correct focus position. This setting is dependent on the objective that is used. You should set this option to a value of 25 µm or less. Use a smaller step size with higher NA objectives because the focus peak is narrower. If **Custom exposure time** is selected on the **Acquisition** tab, then with **Laser and image** selected, you must also specify values for the following. • **Exposure (ms)**: Specifies the exposure time in milliseconds that is to be used for the acquisition wavelength when autofocusing. • **Gain**: Sets the sensitivity of the camera when autofocusing. **Note:** The **Gain** option is not available for every camera type.

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
• Laser with image recovery	Displayed only for the first acquisition (W1) wavelength and only if **Enable laser-based focusing** *and* **Enable image-based focusing** are selected on the **Acquisition** tab and **Allow image-based focusing for recovery from laser-based well bottom failures** is selected on the **Autofocus** tab. If this option is selected, then you must also specify the following options. • **Image-based range +/- µm**: Specifies the range that is to be used for the image-based portions of autofocusing. **Note:** You should adjust the range based on the sample variability. Note that a larger range requires a longer time to focus. • **Max. step**: Specifies the maximum step size in µm of a single Z move that is to be used to attain the correct focus position. This setting is dependent on the objective that is used. Use a smaller step size with higher NA objectives because the focus peak is narrower. **Note:** Smaller step sizes generally require more steps to arrive at the final focus position. Increasing the number of image autofocus steps increases the chances of photobleaching or phototoxicity. If **Custom exposure time** is selected on the **Acquisition** tab, then with **Laser with image recovery** selected, you must also specify values for the following. • **Exposure (ms)**: Specifies the exposure time in milliseconds that is to be used for the acquisition wavelength when autofocusing. • **Gain**: Sets the sensitivity of the camera when autofocusing. **Note:** The **Gain** option is not available for every camera type.

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
• Z-offset from W1	Available only for the second or greater (W2, W3, and so on) acquisition wavelengths. If this option is selected, then the specified offset is moved from the W1 focus position. Specify the post-laser offset in µm in the **Offset** field, or click **Calculate Offset** to automatically calculate the offset that is to be used for all subsequent acquisition wavelengths. **Note:** The offset for W1 must be calculated before you can set this value.

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
• Image-based	Available for the second or greater (W2, W3, and so on) acquisition wavelengths and only if **Enable image-based focusing** is selected on the **Acquisition** tab. Specify the offset from W1 in µm in the **Offset** field, or click **Calculate Offset** to automatically calculate the offset that is to be used for all subsequent acquisition wavelengths. If this option is selected, then you must also specify the following options. • **Image-based range +/- µm**: Specifies the range that is to be used for the image-based portions of autofocusing. **Note:** You should adjust the range based on the sample variability. Note that a larger range requires a longer time to focus. • **Max. step**: Specifies the maximum step size in µm of a single Z move that is to be used to attain the correct focus position. This setting is dependent on the objective that is used. Use a smaller step size with higher NA objectives because the focus peak is narrower. **Note:** Smaller step sizes generally require more steps to arrive at the final focus position. Increasing the number of image autofocus steps increases the chances of photobleaching or phototoxicity. If **Custom exposure time** is selected on the **Acquisition** tab, then with **Image-based** selected, you must also specify values for the following. • **Exposure (ms)**: Specifies the exposure time in milliseconds that is to be used for the acquisition wavelength when autofocusing • **Gain**: Sets the sensitivity of the camera when autofocusing. **Note:** The **Gain** option is not available for every camera type.

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
Acquisition options - The options that are displayed are determined by the series option that is selected on the **Acquisition** tab.	
Acquire Time Series selected	
Timelapse - Specifies the image collection intervals to use for the wavelength.	• at all time points - The default value. Acquires an image at the indicated wavelength at each time point in the experiment. • at start of experiment - Acquires an image at the indicated wavelength at the first time point only. • at start/end of experiment - Acquires an image at the indicated wavelength at the first time point and the last time point for the experiment. • every nth timepoint - Acquires an image at the indicated wavelength at the indicated time point interval, for example, every 5th time point, beginning with the first time point for the experiment.
Acquire Z Series selected	
Z series	• Single plane - The acquisition wavelength acquires only a single plane in the middle of the Z Series range. It is not going to acquire the entire Z series. • 2D Projection Image Only - The acquisition wavelength is going to acquire the Z series, but every plane is not going to be retained. Instead, a projection is going to be acquired and this single image is saved. • Z Series and 2D Projection Image - Displayed if **Acquire Z Series** is the only series acquisition option that is selected on the **Acquisition** tab. **Acquire Time Series** *cannot* be selected. The acquisition wavelength is going to acquire the Z series, and every plane and the projection image are retained.

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
2D Projection Image	Displayed for all Z series acquisition options other than **Single Plane**. Indicates how the resulting 2D projection image will be produced. • Best Focus - The default value. The MetaXpress Software estimates the regions of best focus in the image stack to within one-tenth pixel accuracy along the Z axis. Two resolution grid sizes are used to enhance the criterion of focus through the stack. • Maximum - Recommended only for fluorescence. For each corresponding pixel position in the images, the pixel that has the highest intensity value out of all the planes is determined, and this is the value that is output to the resulting image. • Minimum - Recommended only for transmitted light. For each corresponding pixel position in the images, the pixel that has the lowest intensity value out of all the planes is determined, and this is the value that is output to the resulting image. • Sum - The intensities of the pixels in the stack planes are added for each corresponding pixel position, and this is the value that is output to the resulting image.
Digital confocal	Displayed only if your organization has purchased the optional Digital confocal feature. Select to carry out deconvolution-based image sharpening. **Note:** For information about the Digital confocal option, go to http://www.moleculardevices.com/support, click on the **Knowledge Base** link, and search for "Digital confocal."

Table 5-5: Acquisition wavelength configuration options (cont'd)

Option	Description
Shading correction	Always displayed, regardless of the series option that is selected on the **Acquisition** tab. Indicates whether shading correction has been turned on for the protocol. See To carry out shading correction on acquired images on page 134.

Note: The tabs are displayed in the order in which you configured the active wavelengths, which, in turn, corresponds to the acquisition order. For example, if you configured the DAPI wavelength first, and then the FITC wavelength second, then two wavelength tabs would be displayed. The DAPI tab would be displayed first, and the FITC tab would be displayed second. To save time, you should configure the acquisition wavelengths in the order that minimizes filter movement.

4. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To configure series acquisition options

Two series acquisition options are available for configuration on the **Acquisition** tab.

- **Acquire Time Series** acquires images at multiple time points. If you select this option, then you must also specify the set of images that are to be acquired at each time point.
- **Acquire Z Series** acquires individual optical sections (planes) in sequence through a sample to produce a 3-D image of the sample. If you select this option, then you must also specify the number of steps and the step size for moving through the sample.

You can select one or both of these options.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Acquisition** tab. See Figure 5-21 on page 119.

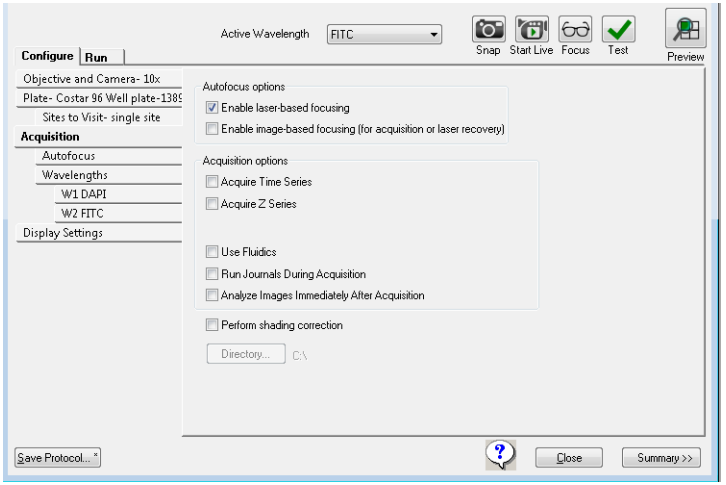


Figure 5-21: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

2. Under **Acquisition options**, select one or both of the following.
   - **Acquire Time Series**
   - **Acquire Z Series**
3. If you selected:

- **Acquire Time Series**, then continue to To configure time series acquisition options on page 120.
- **Acquire Z Series**, then continue to To configure Z series acquisition options on page 123.

To configure time series acquisition options

1. Open the **Timelapse** tab.

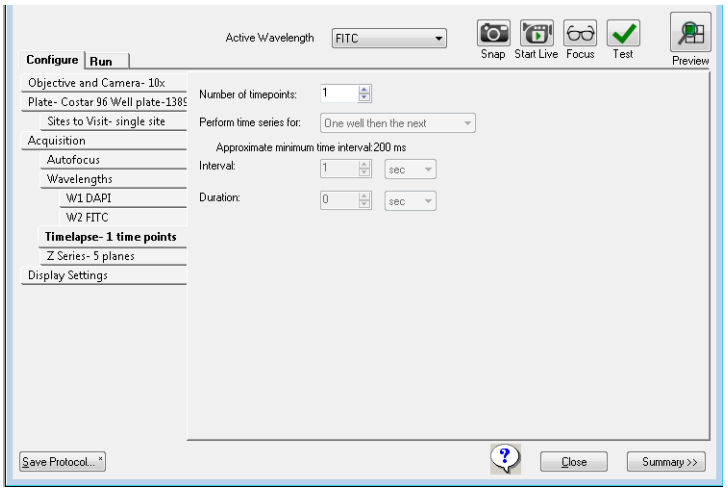


Figure 5-22: Plate Acquisition Setup dialog, Configure tab, Timelapse tab

2. Configure the time series acquisition options. See Table 5-6 on page 121.

Table 5-6: Time series acquisition configuration options

Option	Description
Number of timepoints	Specifies the total number of time points that are to be acquired. The default value is one. If you change this value, then the **Duration** field is automatically updated by calculating the duration from the number of time points and the time interval.
Perform time series for - Enabled only if the value for the **Number of time points** is > 1. Specifies the loop order that is to be used when acquiring images at multiple time points and also determines the set of images that are to be acquired at each time point.	
• One well then the next	A set of wavelength images is acquired at each site in the well at each time point. **Note:** This option is most common with a fluidics experiment, or with a photoactivation event.
• One row then the next	All the images in one row's worth of wells are collected at each time point. After the series is collected, the next row is acquired. **Note:** This option requires a longer time interval because all the wells in a row are acquired.
• One column then the 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.
• All selected wells	Every well that is selected for acquisition is acquired at each time point. The well selection is determined at the start of the first acquisition.
Note: Any refocusing that is carried after the images for the first timepoint are acquired is determined by the **Timelapse Autofocus** setting that is specified on the **Autofocus** tab. See To configure autofocus options on page 100.	

3. In the **Interval** field, type the amount of time between the start of an acquisition at one timepoint and the start of an acquisition at the next time point. The default unit of time is seconds (**sec**), but you can select milliseconds (**ms**), minutes (**min**), or hours (**hr**).

Note: The **Approximate minimum time** interval is calculated as the sum of the exposure times per time point based on the well selection, site selection, wavelength settings, and loop order selection. If the actual acquisition time for one loop exceeds the **Interval** setting, then the next timepoint commences as soon as the previous timepoint completes.

Note: The value in the **Duration** field is the result of multiplying the number of time points by the interval. If you change the number of timepoints or the interval, then the value in the **Duration** field is automatically updated.

4. Optionally, change the value in the **Duration** field.

Note: The time unit for the duration values does not have to be the same as the time unit for the interval value. If you change the value for the duration, then the value for the **Number of timepoints** is automatically updated.

5. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To configure Z series acquisition options

1. Open the **Z series** tab.

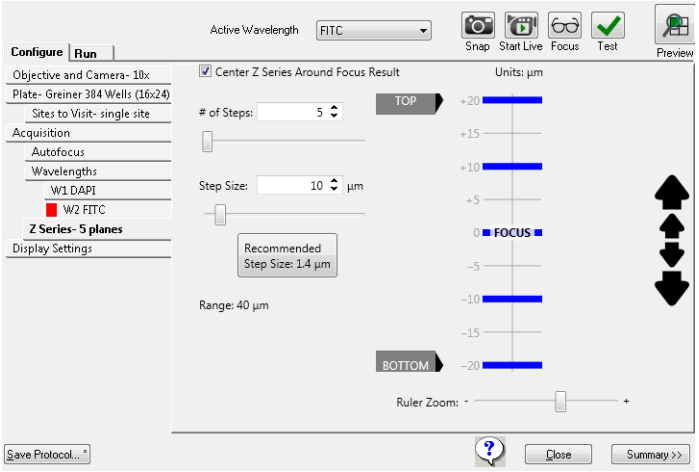


Figure 5-23: Plate Acquisition Setup dialog: Configure tab, Z Series tab showing the Z Series diagram

Note: The blue horizontal bars in the Z series diagram represent the planes that are to be acquired for a site.

2. Specify the Z series configuration options.

Table 5-7: Z series configuration options

Option	Description
# of Steps	The number of planes at a given site/well position for which images are to be taken; for example, if set to 12, which is the default value, then 12 images will be taken along the Z axis of the image. You can do the following to adjust this value. • Manually type a value in the field. • Click the Up/Down arrows in the field. • Use the slider bar that is displayed below the field.
Step Size	The distance in µm between the image planes in the Z series. You can do the following to adjust this value. • Manually type a value in the field. • Click the Up/Down arrows in the field. • Use the slider bar that is displayed below the field. **Note:** Based on the numerical aperture (NA) setting for the objective, the MetaXpress Software automatically calculates a recommended **Step Size** that would provide the best detail for your Z series. You can click this **Recommended Step Size** message to automatically set the **Step Size** to this recommended value.
Note: As you adjust one or both of these values, then the MetaXpress Software automatically calculates the **Range**, which is the overall image depth from top to bottom of the image sample, and displays this value below the **Step Size** on the tab.	

3. Optionally, to navigate the Z series for site/well position and modify the coordinates for the Z motor before you acquire any images, do any of the following as needed.

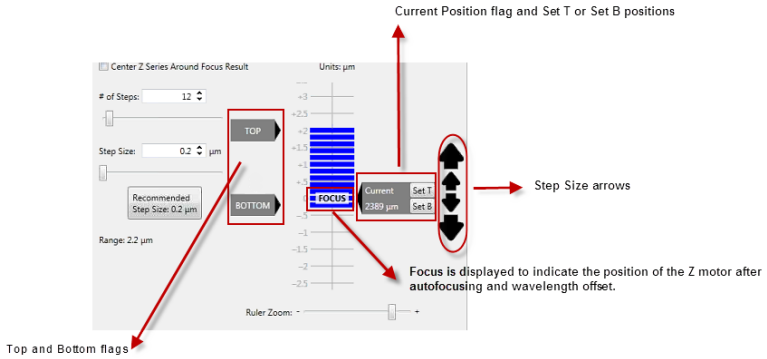


Figure 5-24: Z Series diagram

- Click and drag the **Ruler Zoom** slider bar that is displayed below the Z series diagram to zoom in/zoom out on the diagram.
- **Focus** is displayed on the Z series diagram to indicate the position of the Z motor after autofocusing and wavelength offsets are applied. You can do the following based on this location.
  - Click the **Focus** active wavelength tool to place the Z motor at the **Focus** indicator in the Z series diagram and display its current position in µm. The ruler markings indicate the distance in µm above and below the **Focus** position.
  - Click and drag the **Top** or **Bottom** flags to manually set the top and bottom positions for the Z series acquisition relative to the **Focus** position. As you manually adjust one or both of these values, the MetaXpress Software automatically calculates and displays the appropriate values for the **# of Steps** and the **Range**. The position in µm to which a flag is moved is temporarily displayed on the flag.
- Select **Center Z Series Around Focus Result** to evenly space the Z series planes above and below the **Focus** position. Now, if you click and drag a **Top** or **Bottom** flag on the Z series diagram to manually set the position for the Z series acquisition, the MetaXpress Software automatically moves the other position by exactly the same amount.

- Click and drag the **Current Position** flag on the Z series diagram to update the current position for the Z motor. After you move the flag, you can then click **Set T** or **Set B** to set the top and bottom positions, respectively.

Note: If the **Current Position** flag is not displayed, use the **Focus** active wavelength tool first.

Tip: Use this function with the **Start Live** active wavelength tool. Click **Start Live**, and then with the **Focus** set, click and drag the **Current Position** flag to view the different positions in an image in real time. From this, you could determine such things as the location for the top of the cells in the sample. You could then click **Set T** on the **Current Position** flag to accurately set the position for the top of an image.

- Click on a blue bar in the Z series diagram to move the **Current Position** flag to this plane. (Remember, this flag indicates the current position for the Z motor.) Optionally, you can then click the **Snap** active wavelength tool to snap and display a real time image of the selected plane in an image **Snap** window. As you click on different planes in the Z series diagram, the image in the **Snap** window is updated accordingly.

Note: You can also click and drag the Z slider bar at the top of the **Snap** image window to move through each plane in the acquired stack. To prevent asynchronous hardware moves, the position of the **Current Position** flag on the Z series diagram (i.e., the current position of the Z motor) is *not* updated.

- To move the Z motor up or down one step at a time by the *recommended* Step Size, click the small step size arrows, which are the small black vertical arrows to the right of the Z series diagram. To move the Z motor up or down one step at a time by the *defined* Step Size, click the large step size arrows, which are the large black vertical arrows to the right of the Z series diagram. If you click and hold a Step Size arrow, then the Z motor is moved continuously.

4. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To configure journals to run during acquisition

You use the **Journals** tab to configure specific journals to run during different stages of the acquisition. You can configure only a single journal to run at an acquisition step.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Acquisition** tab.

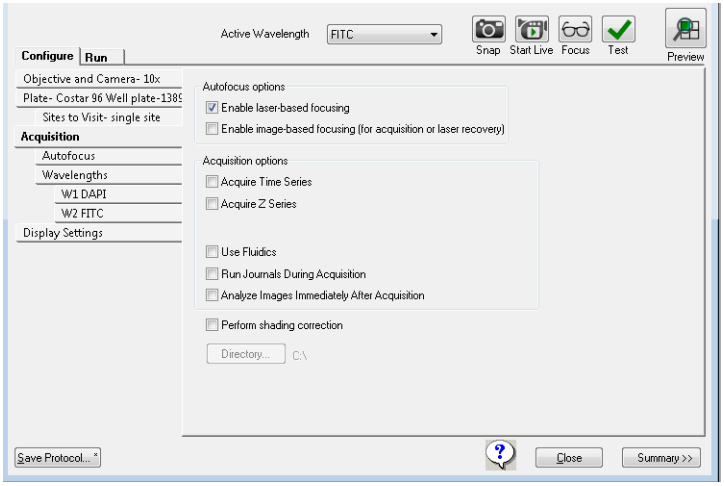


Figure 5-25: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

2. Select **Run journals during acquisition**.
3. Open the **Journals** tab. See Figure 5-26 on page 128.

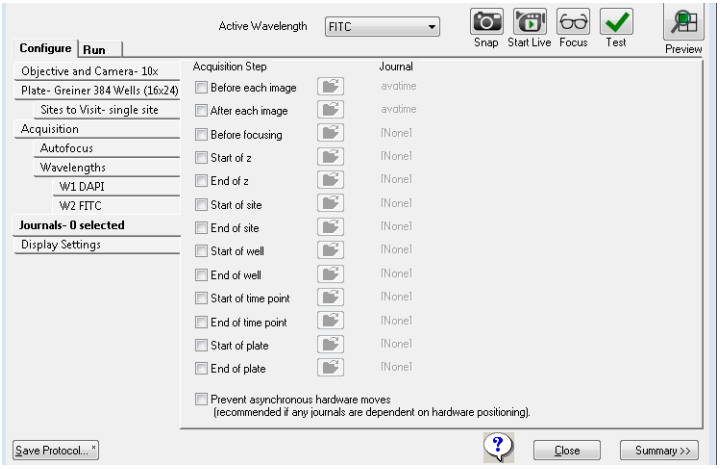


Figure 5-26: Plate Acquisition Setup dialog, Configure tab, Journals tab

4. Select the specific acquisition step for the journal.
5. Click the Select Plate Acquisition Journal icon , for the step, and then in the **Select Plate Acquisition Journal** dialog, scroll to and select the appropriate journal file.
6. After you select the journal, click **Open** to close the **Select Plate Acquisition Journal** dialog and return to the **Journals** tab.

Table 5-8: Journal options

Option	Description
Before each image	Runs only during the acquisition loop, after the illumination is set and focusing is done.
After each image	Runs only during the acquisition loop, after the shutter is closed and before images are saved.
Before focusing	Runs only during the acquisition loop, just before the focus algorithm begins.
Start of z	Runs before a Z series is started. The journal runs when the Z motor is at the lowest step in the series and before an image is acquired. The journal runs once for each wavelength that is configured to use the Z series.

Table 5-8: Journal options (cont'd)

Option	Description
End of z	Runs after a Z series is completed. The journal runs when the Z motor is at the highest step in the series and after any projection images are generated. The journal runs once for each wavelength that is configured to use the Z series.
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 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.
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.
Prevent asynchronous hardware moves	Optional. Select this option if any of the journals that are to be run move hardware such as changing shutters, moving focus, and so on. This option ensures that the journals run correctly. **Note:** Do not select this option if no journals are being used as acquisition is unnecessarily slowed down.

7. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To configure post-acquisition analysis options

You can select a specific analysis to run on a plate after the acquisition is complete on the **Analysis** tab. If you select this option, then the analysis job is added to the Auto Run queue in the MDCStore database for analysis either by a MetaXpress system that is set to Auto Run mode or by the MetaXpress PowerCore Software. You can select from a list of saved assays and settings files from any application module, custom module, or journal assay that has been saved to the MDCStore database.

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Acquisition** tab.

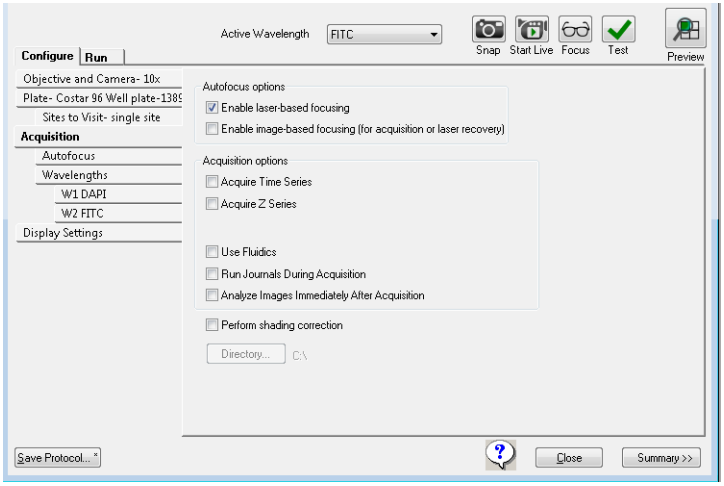


Figure 5-27: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

2. Select **Analyze Images Immediately After Acquisition**.
3. Open the **Analysis** tab. See Figure 5-28 and Figure 5-29 on page 132.

Note: The options that are displayed on the **Analysis** tab depend on the series acquisition mode (**Acquire Time Series** or **Acquire Z Series**) that is selected on the **Acquisition** tab as well as the image that is to be acquired and retained: **Single plane**, **2D Projection Image Only**, or **Z Series and Projection Image**.

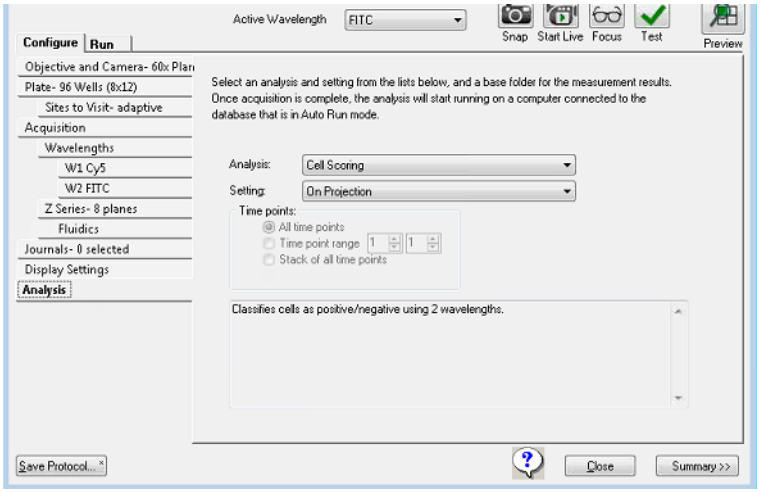


Figure 5-28: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Time Series)

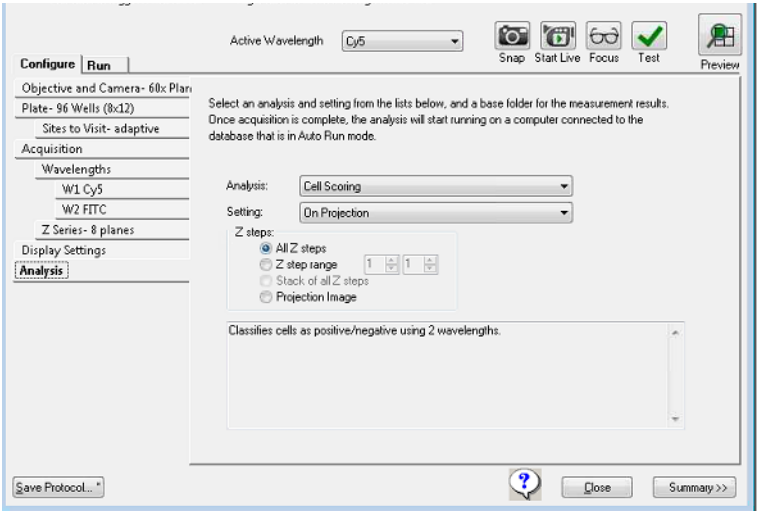


Figure 5-29: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Z Series and 2D Projection Image Only)

4. On the **Analysis** drop-down list, select the post-analysis assay to run.

Note: This list includes any application modules or journal assays that have been saved to the MetaXpress Software database. The list of available assays is the same list that is available on the **Run Analysis** tab on the **Review Plate Data** dialog.

5. On the **Setting** drop-down list, select the appropriate settings file for this module or journal assay.

Note: The list includes all setting files that have been previously saved to the MetaXpress Software database.

6. Specify the acquisition time points for which the analysis is to be run.

Table 5-9: Time point options - Time series acquisition

Option	Description
All time points	The default value. Analyze all the acquisition time points.
Time point range	Analyze only those acquisition time points that fall within the indicated range.
Stack of all time points	Available if the selected analysis is a journal.

Table 5-10: Time point options - Time series acquisition with Z series acquisition for at least one acquisition wavelength; 2D Projection Image Only

Option	Description
All time points	The default value. Analyze all the acquisition time points.
Time point range	Analyze only those acquisition time points that fall within the indicated range.
Stack of all time points	Available if the selected analysis is a journal.

Table 5-11: Time point options - Z series acquisition for at least one acquisition wavelength; Z Series and 2D Projection Image

Option	Description
All Z points	The default value. Analyze all the acquisition planes.
Z step range	Analyze only those planes that fall within the indicated range.
Stack of all Z steps	Available if the selected analysis is a journal.
Projection Image	Analyze only the projection image.

7. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

To carry out shading correction on acquired images

You specify whether to carry out shading correction in acquired images on the **Acquisition** tab. Before you can carry out shading correction, the appropriate shading correction images must have been acquired *for each acquisition wavelength* and saved to a directory that you have specified as the Plate Acquisition Shading Correction directory. The default value for this directory is C:\Shading Images. The name for a shading image must include the exact matches for the magnification and illumination settings; for example,

Shading_10XPlan Fluor_DAPI.tif

Note: If a shading correction image was acquired with the full field of view, then you can use the **Custom Field of View** feature on the **Plate** tab with shading correction. See Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.

Note: If a shading image is not present for a particular combination of magnification and illumination settings, then no shading correction is carried for the wavelength.

1. Open the **Acquisition** tab.

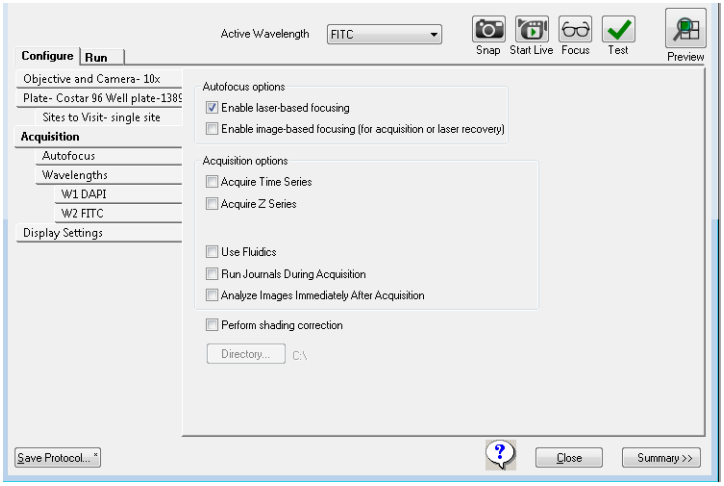


Figure 5-30: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

2. Select **Perform shading correction**.
3. If needed, click **Directory** and in the **Browse for Folder** dialog, browse to and select the directory that contains the required shading correction images. After you have selected the folder, click **OK** to close the dialog and return to the **Acquisition** tab.
4. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup dialog: Configure tab, Display tab on page 136.

Plate Acquisition Setup dialog: Configure tab, Display tab

You configure the settings for the MetaXpress Software desktop appearance such as image size and positions, the **Plate Acquisition Setup** dialog size and position and other image properties, during acquisition on the **Display** tab. You can choose to display images only during autofocus, only during acquisition, or both. You can also choose to display a color overlay of wavelength images during acquisition.

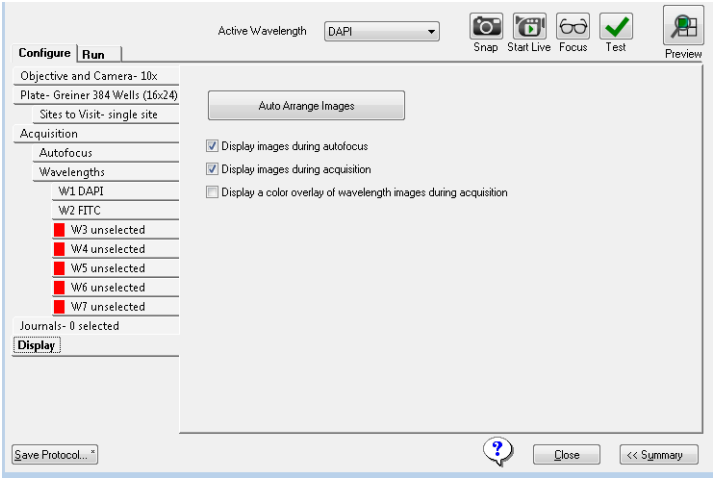


Figure 5-31: Plate Acquisition Setup dialog: Configure tab, Display Settings tab

Table 5-12: Configure tab, Display tab options

Option	Description
Auto Arrange Images	Use the MetaXpress Software default settings for displaying images and dialogs during acquisition. Images are tiled and autoscaled and the status dialog is unobstructed. The **Plate Acquisition Setup** dialog also opens. You can manually change the display configuration using a variety of options for this preview. **Note:** Use this option in conjunction with the Active Wavelength **Preview** tool to generate a preview of an acquired image for each configured acquisition wavelength.
Display images during autofocus	Selected by default. Displays the images that are acquired during image autofocus and during use of the **Calculate Offset** tool.
Display images during acquisition	Selected by default. Displays each image as it is acquired.
Display a color overlay of wavelength images during acquisition	Displays a color composite of the wavelengths that are acquired at each site.

To configure the display settings for an acquisition protocol

Note: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all the protocol settings. See Summary Panel on page 140.

1. Open the **Display** tab.
2. Click **Auto Arrange Images** to use the MetaXpress Software default settings for displaying images during acquisition. You can leave the images in this default layout, or you can manually adjust them by doing any of the following as needed.

Note: If the image windows are not visible, then click the Active Wavelength **Preview** tool to display an image window for each configured acquisition wavelength.

- Rearrange the position of the image windows.
- Rearrange the position of the **Plate Acquisition Setup** dialog.
- Change the size of the image windows.
- Change the size of the **Plate Acquisition Setup** dialog.
- Use the image preview tools to change the size, scaling, or LUT for an image.

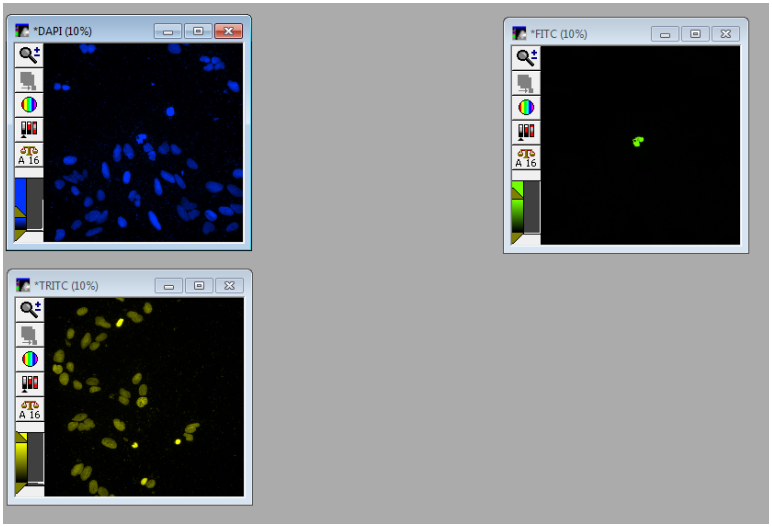


Figure 5-32: Display Settings tab, preview for manually adjusting the image display properties

3. Optionally, select or clear any or all the image preview options as needed: **Display images during autofocus**, **Display images during acquisition**, or **Display a color overlay of wavelength images during acquisition**.

4. If you are done configuring the plate acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 141; otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 74.
   - Plate Acquisition Setup dialog: Configure Tab, Plate Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 82.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.

Summary Panel

The **Summary** panel displays a summary of all the current acquisition settings. The **Summary** panel is displayed to the right of the **Plate Acquisition Setup** dialog. When the **Plate Acquisition Setup** dialog first opens, the **Summary** panel is closed.

After you open the **Summary** panel, you can leave it open at all times during the configuration of an acquisition protocol, or you can toggle its display, and open it only when it is convenient for you.

1. At the bottom of the **Plate Acquisition Setup** dialog, click **Summary >>**. See Figure 5-33 on page 140.

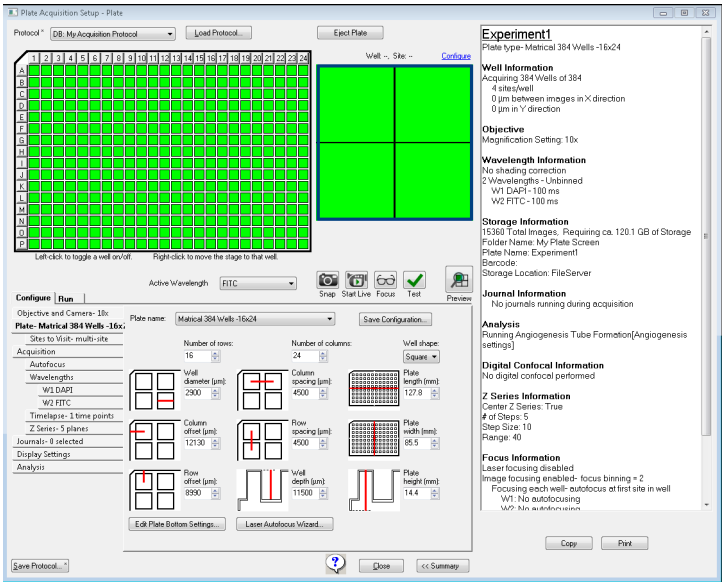


Figure 5-33: Plate Acquisition Setup dialog with open Summary panel

2. Optionally, do any or all of the following as needed.
   - Use the Scroll bar to the right of the **Summary** panel to scroll up and down through its display.
   - Click **Copy** to copy the contents to the client clipboard. You can then paste these copied contents into a third party application such as Microsoft Word as needed.

Note: This option is useful, for example, if you need to copy and paste the contents to send them to Technical Support when you are troubleshooting a protocol configuration. See Obtaining Support on page 34.

- Click **Print** to print the contents to a selected printer.

3. When you are done viewing or working with the contents of the **Summary** panel, you can leave it open at all times during the configuration of an acquisition protocol, or you can click **Summary <<** to close it.

Saving a Plate Acquisition Protocol

When you save a plate acquisition protocol, you have the option of saving the protocol to a database or to a file. The default location is to save to a database. Your organization's working needs should determine whether to save the protocol to a database or a file. For example, if your organization plans on integrating your ImageXpress® Micro system to a robot for automated plate loading, then you must save the protocol to a file.

To save a plate acquisition protocol to a database

1. Click **Save Protocol**.
2. In the **Save Acquisition Setting** dialog, in the **Setting Name** field, type the name for the plate acquisition protocol.
3. Click **Save**.

   You can now acquire your plate data with the saved protocol. See Chapter 6: Acquiring Plates on page 143.

To save a plate acquisition protocol to a file

When you save a protocol to a file, the file type is .HTS and you cannot change this. The default location for saving a protocol is C:\MX6\HTSSTATE, but you c an select a different location.

1. Click **Save Protocol**.
2. Select **Save to file rather than database**.
3. Click **Save**.
4. In the **File name** field on the **Plate Acquisition Setting** dialog, type a name for the protocol.
5. Optionally, select a different location in which to save the file.
6. Click **Save**.

   You can now acquire your plate data with the saved protocol. See Chapter 6: Acquiring Plates on page 143.

Acquiring Plates

6

You can initiate plate acquisition from any of the following four locations in the MetaXpress® Software application:

- Plate Acquisition Setup dialog
- Plate Acquisition and Control dialog
- Plate Acquisition dialog
- Plate Acquisition toolbar

Before Version 6.0 of the MetaXpress® Software, each of these locations served a specific function in the plate acquisition flow such as:

- Making changes to the X, Y, and Z positions to accomplish correct plate and focus alignment.
- Selecting or typing an Experiment base name.
- Selecting and loading the protocol (Settings file) to use for the acquisition.
- Viewing the summary of acquisition settings.
- Acquiring a plate.

Beginning with Version 6.0 of the MetaXpress® Software application, however, all the functions that were previously available only from the **Plate Acquisition and Plate Acquisition and Control** dialogs are now also available from the **Plate Acquisition Setup** dialog. You can continue to use the **Plate Acquisition and Plate Acquisition and Control** dialogs to carry out specific functions for acquiring plates in the MetaXpress® Software application and for historical purposes, the use of these dialogs is still documented in this chapter. For efficiency and convenience, however, Molecular Devices recommends that you use the **Plate Acquisition Setup** dialog.

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

Plate Acquisition Setup dialog

After you have configured a plate acquisition protocol, you will most likely need to move the stage or the focus to ensure correct plate and focus alignment before you can run the protocol to acquire plate data. The Plate Acquisition Setup dialog provides two maps—the **Plate map** and the **Site map**—for accomplishing these tasks. Other controls on this dialog are provided for initiating autofocus, specifying the protocol variables such as the storage name and location and the plate name, loading a protocol, specifying the wells and/or sites for which to acquire data, opening a Live window or a Preview window, snapping an image, and acquiring a plate.

Note: The procedures in this section are organized to guide you through the process of running a plate acquisition protocol in the most logical order, but you can carry out the procedures in the order that best suits your working needs.

To load a plate acquisition protocol

1. On the MetaXpress Software main menu, click **Screening > Plate Setup dialog**.

Note: If the correct protocol is already selected on the **Protocol** drop-down list when the **Plate Acquisition Setup** dialog opens, then you can skip to Step 4.

2. On the **Plate Acquisition Setup** dialog, click **Load Protocol**.
3. On the **Load Plate Acquisition Protocol** dialog, do one of the following:

Table 6-1: Loading a plate acquisition protocol

Option	Steps
To load a protocol from the database	• On the **Protocols in Database drop-down** list, select the protocol that is to be loaded. • Click **Load from DB**.
To load a protocol from a file	• Click **Load From File**. • In the **Load Plate Acquisition Protocol** dialog, browse to and select the correct protocol (a .HTS file), and then click **Open**.

4. Click **Eject Plate**, to open the door, and then load the plate for which data is to be acquired.
5. Click **Load Plate** to close the door.
6. After you have loaded a plate acquisition protocol, if the protocol specifies the correct wells and sites for which data is to be acquired, then continue to To verify the acquisition settings on page 148. Otherwise, continue to To configure the wells and sites for which to acquire data (Optional) on page 145.

To configure the wells and sites for which to acquire data (Optional)

After you have loaded a plate acquisition protocol, if the protocol does not specify the wells and sites for which data is to be acquired, or if you need to select different wells and sites, then you can configure the wells and sites at the time that you run the protocol.

The top left graphic on the **Plate Acquisition Setup** dialog is the **Plate map**. You use this interactive graphic to move the stage and to configure only those wells for which data is to be acquired.

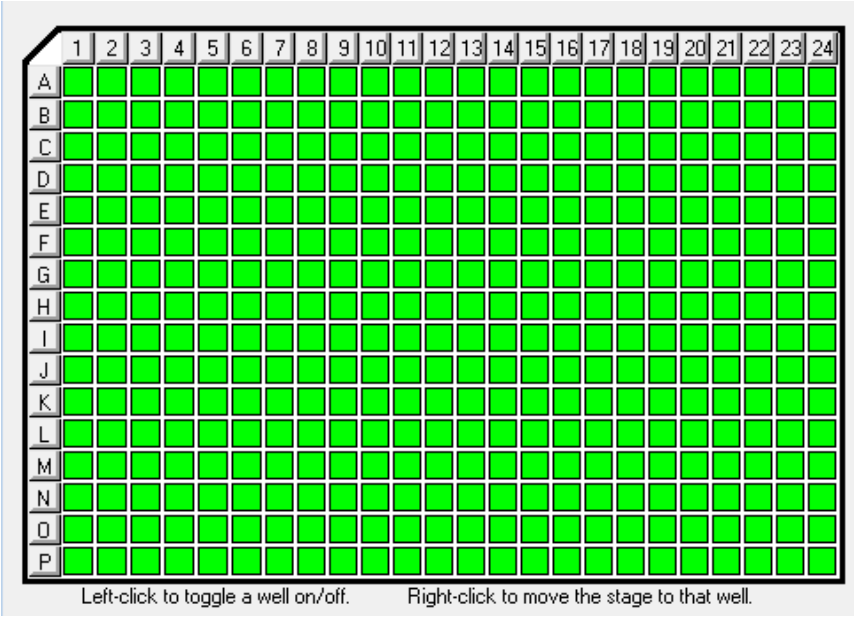


Figure 6-1: Plate Acquisition Setup dialog, Plate map

The top right graphic on the **Plate Acquisition Setup** dialog is the **Site map**. You use this interactive graphic to move the focus and to configure the position of the sites in a well that are to be acquired.

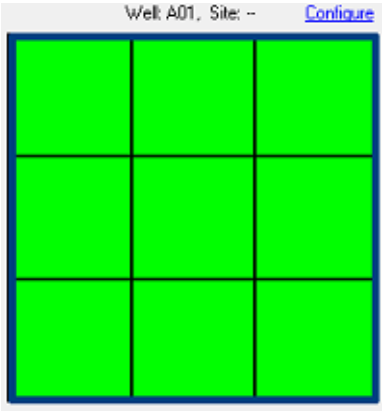


Figure 6-2: Plate Acquisition Setup dialog, Site map

To configure the wells and sites for which to acquire data:

1. Configure the wells for which data is to be acquired. The following actions are available for the **Plate map**.
   - To turn off a well, click it. The well turns light gray, which indicates that data will not be acquired for the well. To turn it back on, click the well again. The well turns light green again.
   - To turn off all the wells in the column in a single step, click the column header. To turn all the wells back on, click the column header again.
   - To turn all the wells in a row off in a single step, click the row ID. To turn all the wells back on, click the row ID again.
   - To turn off a contiguous group of wells in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate wells. To turn the wells back on, click and hold the left mouse button, and then drag the cursor across the wells again.
   - To turn on or off all wells in a plate in a single step, click in the upper left corner of the plate just to the outside of the first well, A01, on the plate.
2. Configure the sites in a well for which data is to be acquired. The following actions are available for the **Site map**.
   - To turn a site off, click it. The site turns gray, which indicates that data will not be acquired for the site. To turn the site back on, click the site again. The site turns bright green again.
   - To turn off a contiguous group of sites in a single step, click and hold the left mouse button, and then drag the cursor across the appropriate sites. To turn the sites back on, click and hold the left mouse button, and then drag the cursor across the sites again.

Note: The site controls apply to all sites in all wells at the same time. When you turn on or off sites for a selected well, then all wells in the plate will have data acquired for the same sites.

3. Continue to To verify the acquisition settings.

To verify the acquisition settings

After you have configured the wells and sites for which to acquire data, you should verify that your current acquisition settings are appropriate for the objects that are to be detected at each acquisition wavelength. You will most likely need to move the stage or the focus to verify correct plate and focus settings before you can run the protocol to acquire plate data. You use the functions that are available from the **Plate map** to move the stage to test settings on appropriate controls wells before you run the protocol. You use the functions that are available from the **Site map** to test settings on a different site that is within the well. After you have moved the stage or focus, you can use the **Snap**, **Test**, and **Preview** functions that are available for the **Active Wavelength** tools to verify your acquisition and focus settings.

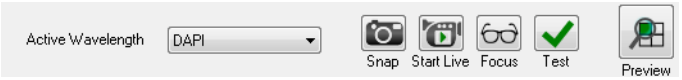


Figure 6-3: Wavelength selection and interactive Wavelength tools

Note: The **Snap** and **Test** functions for an acquisition wavelength are also available on the **Run** tab. See Figure 6-4: Plate Acquisition Setup dialog, Run tab on page 149.

1. To move the stage to a well, right-click the well.

   The well turns dark green and it is surrounded by a black box, which indicates that the well is currently in position for image acquisition.

   Note: You can move the stage to an unselected well. If you move the stage to a well that is turned off, then the well is displayed in dark gray.

2. To move the stage to a site within the current well, right-click the site.

   The site turns dark green, which indicates that the site is currently in position for image acquisition. The current well position and site location, for example, Well: A01, Site: 2, is displayed above the **Site map**.

   Note: You cannot move the stage to an unselected site.

3. Open the **Run** tab.

Note: The **Exposure Time** value and the **Focus Offset** value that are displayed for each acquisition wavelength at the bottom of the **Run** tab are the values that you specified for the wavelength during configuration of the loaded acquisition protocol. You can modify these values for an acquisition wavelength here, or from the individual wavelength tabs. See To configure the acquisition wavelengths on page 108.

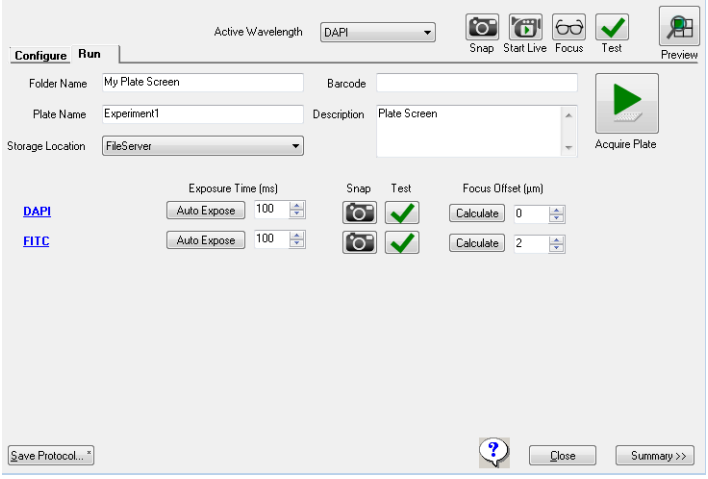


Figure 6-4: Plate Acquisition Setup dialog, Run tab

4. Verify your acquisition settings.

   You can verify settings on an individual wavelength basis, or you can verify your settings for all acquisition wavelengths in a single step. Test results are displayed for each acquisition wavelength in standalone image windows.

Table 6-2: Verifying acquisition settings

Option	Description
Individual wavelength	In the **Active Wavelength** field, select an acquisition wavelength, and then from the Active Wavelength tools, run the appropriate tests as needed. • **Snap**: Acquires a single image at the current well and site position (XY-position), focus (Z-position), wavelength, and exposure time. • **Test**: Autofocuses and acquires all planes for the currently selected active wavelength to test the acquisition of its autofocus, exposure time, Z-series and display settings. **Note:** You can also run these tests using the **Snap** and **Test** options that are displayed next to each acquisition wavelength at the bottom of the **Run** tab.
All wavelengths	**Preview**: Autofocuses and acquires all planes for all acquisition wavelengths at the same time to test the acquisition of their exposures and display settings. The results of this testing are displayed in a separate image window for each acquisition wavelength.

5. If you are satisfied with the results, then continue to To run a plate acquisition protocol on page 151; otherwise, modify your acquisition settings and repeat this procedure as needed until you are satisfied with the results, and then continue to To run a plate acquisition protocol on page 151.

Tip: If you need to modify your acquisition settings, then you can click **Start Live** to assist you. When you click **Start Live**, the MetaXpress Software continuously acquires images based on the current acquisition settings, and then updates these images as you modify the settings. **Be aware, however, that this might cause photobleaching of the sample**.

Note: If you modify the acquisition settings for a protocol, then you can run the protocol as-is, or optionally, you can click **Save Protocol** to save the protocol. You can save the modified protocol as a new protocol, or you can overwrite the existing protocol.

To run a plate acquisition protocol

After you have loaded a protocol, configured the wells and sites for which to acquire a data, and verified that your current acquisition settings are appropriate, then you can run the loaded protocol and acquire the plate data.

1. Enter the information for your experiment.

   Table 6-3: Experiment information

Option	Description
Folder Name	Recommended. The name of the folder in which the plate acquisition data is to be stored.
Plate Name	Required. The name/id of the plate for which data is being acquired.
Storage Location	Required. The location in which the folder that contains the acquisition data is to be saved. Your Administrator configures the available options.
Barcode	Optional. You can enter the value manually, or automatically using a hand-held barcode scanner. **Note:** If the instrument is being used with a robotic plate handler, then the automation software populates the barcode value as appropriate.
Description	Optional. A description of the experiment that you are running to acquire the plate data.

2. Click **Acquire Plate** to acquire images from a plate based on the settings for the loaded protocol.

   The **Plate Acquisition Setup** dialog closes and the **Plate Acquisition Status** dialog opens. The dialog displays an image of the plate wells that have been acquired (outlined in black) and the well that is currently being acquired (outlined in red). The

well/site ID for the well that is currently being acquired is displayed below this graphic. Wells are acquired in a serpentine fashion– going down one column, and then up the next column. After acquisition begins, the estimated time remaining is displayed. An image is displayed briefly for each acquisition wavelength in a standalone image window on the MetaXpress Software desktop as it is acquired and saved. After the last image is acquired and saved, the **Plate Acquisition Status** dialog closes and the **Plate Acquisition Setup** dialog reopens.

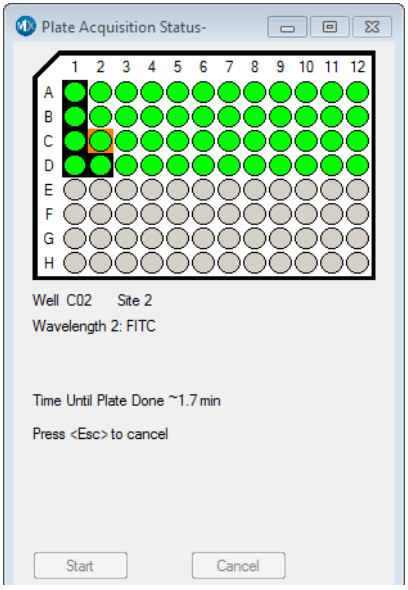


Figure 6-5: Plate Acquisition Status dialog

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 verify 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 Protocol**, **Save Protocol**, 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.

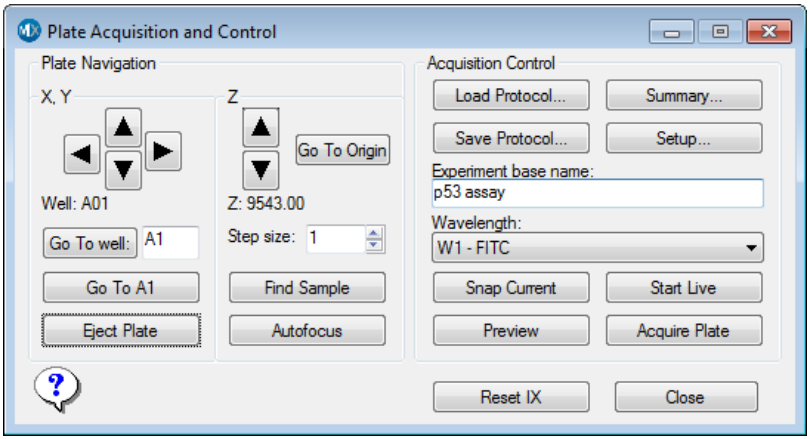


Figure 6-6: Plate Acquisition and Control dialog

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.

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

Option	Description
• 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.
• 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.

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

Option	Description
• 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.
• 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 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.

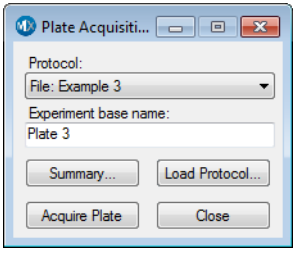


Figure 6-7: Plate Acquisition dialog

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.

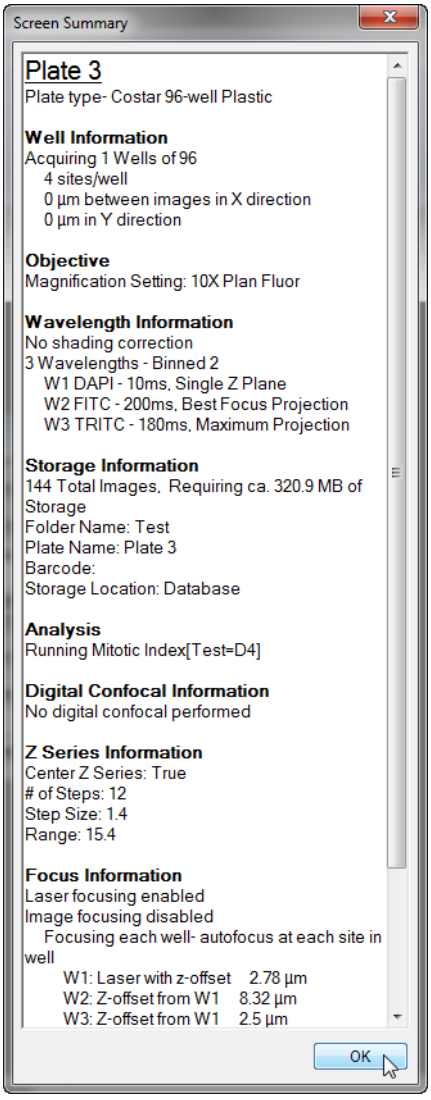


Figure 6-8: Screen Summary dialog

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.

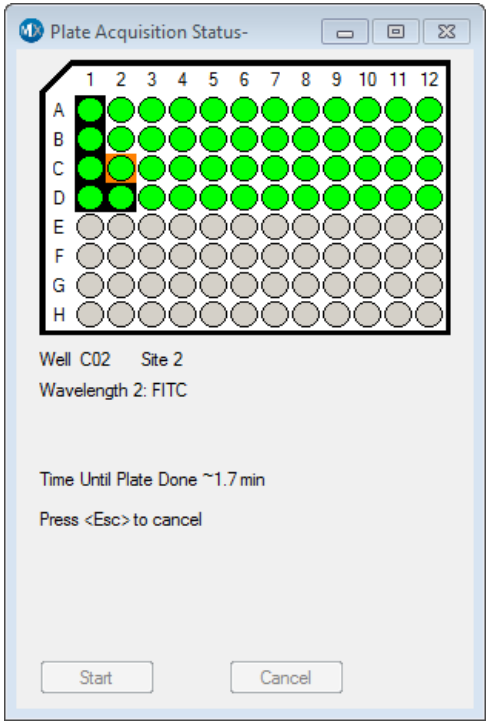


Figure 6-9: Plate Acquisition Status dialog

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**.

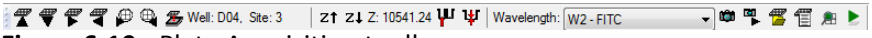


Figure 6-10: Plate Acquisition toolbar

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
	Selects the wavelength to use for the snap or live image

Figure 6-10: Plate Acquisition toolbar (cont'd)

Icon	Description
	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.

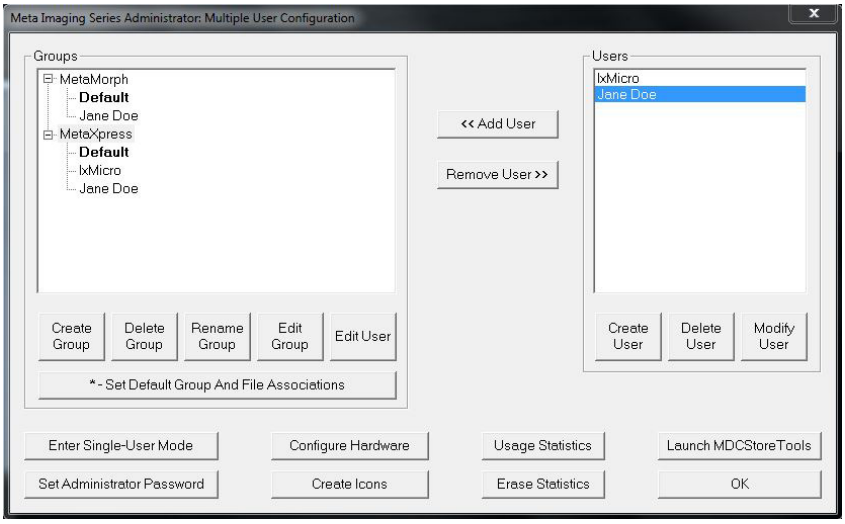


Figure 7-1: Sample of the Multiple User Configuration screen

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**.

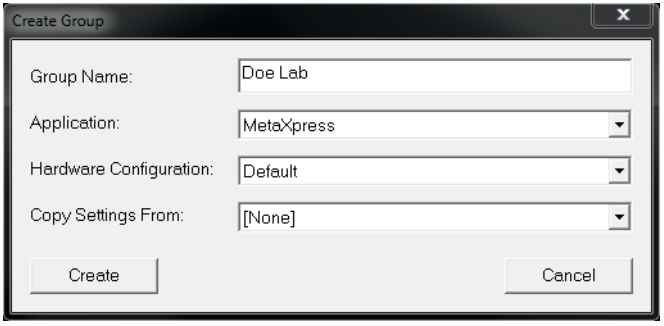


Figure 7-2: Create Group dialog

4. In the **Create Group** dialog, 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 167 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**.

   In the simplified menu, click **Control > Journal > Taskbars > Create Taskbar**.
3. When the **Taskbar Editor** dialog and **New Taskbar** window open, position them so that you can see both the dialog and the window at the same time.
4. Select the number of rows and columns for the taskbar by dragging the thick border of the **New Taskbar** window until the desired number of rows and columns appear in the window.
5. 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.
6. 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.

7. 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.

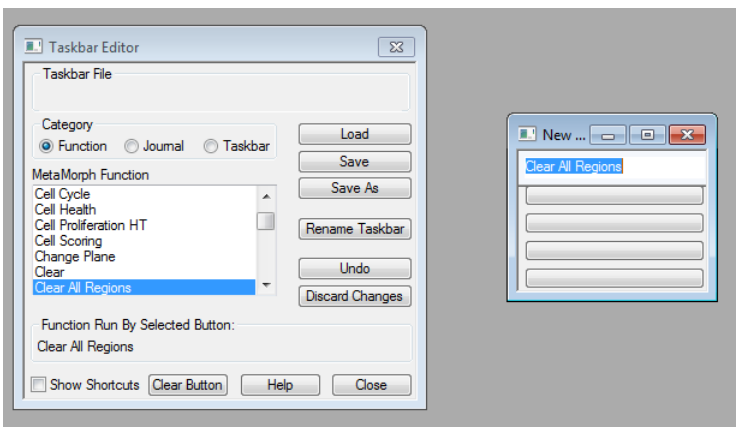


Figure 7-3: Adding a function to a taskbar

8. Repeat Step 6 and Step 7 for each item you want to add to the taskbar.
9. If necessary, click **Undo** to undo the last command or click **Clear Button** to clear an item from the active button.
10. 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**.
11. 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**.
12. To use the new taskbar (or a different taskbar) immediately, click **Journal > Taskbars > Load Taskbar**. Select the desired taskbar file and click **Open**.
13. 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**.

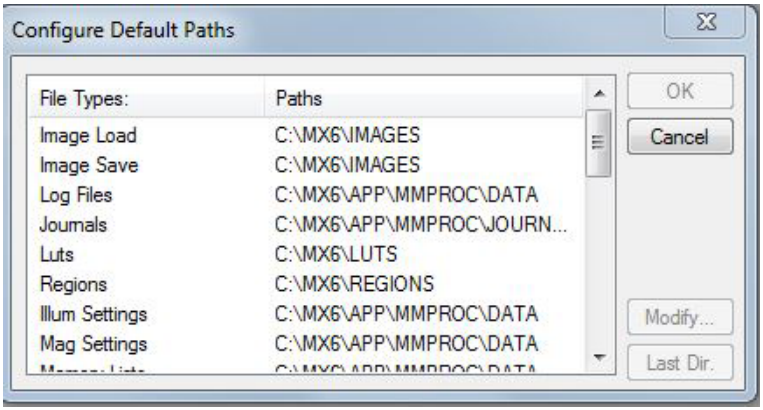
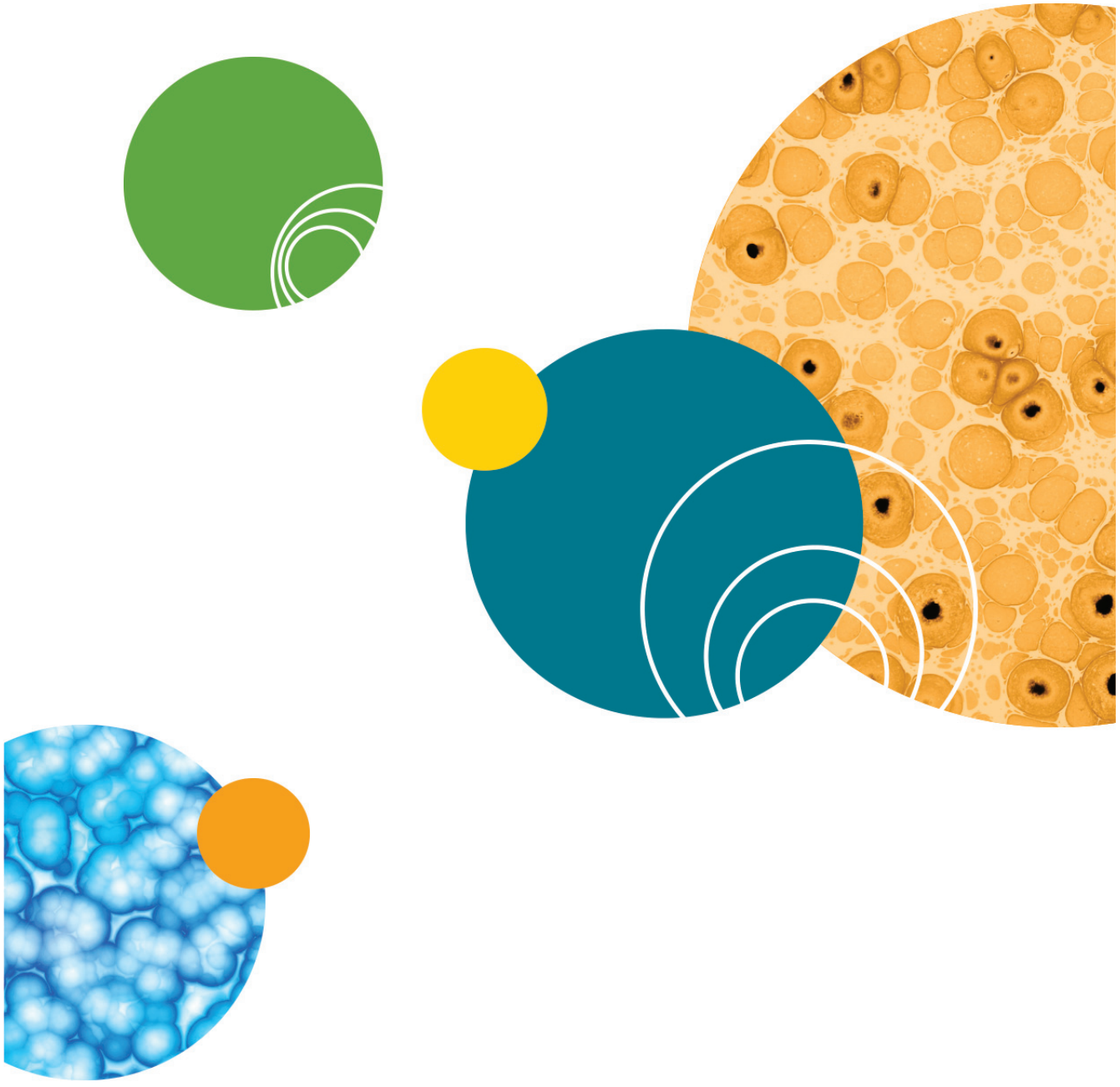


Figure 7-4: Configure Default Paths dialog

3. In the **Configure Default Paths** dialog, select the item whose default file path you want to modify.
4. Click **Modify**.
5. When the **Browse for Folder** dialog, 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 175 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.

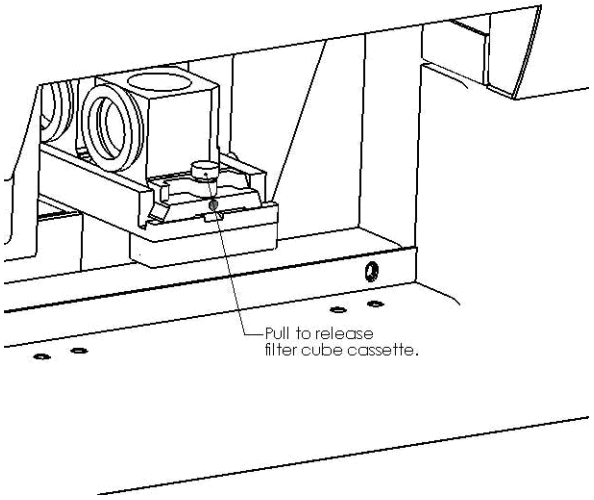


Figure 8-1: Removing filter cubes

11. Remove the filter cube cassette.
12. Slide the new cube into place and lightly tighten the hex screw.
13. 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.

14. Power ON the ImageXpress Micro instrument at the main power switch, which is located on the external power supply.
15. Update the software to reflect the new hardware configuration. See Updating the System After Adding or Replacing a Filter Cube on page 179.

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**.

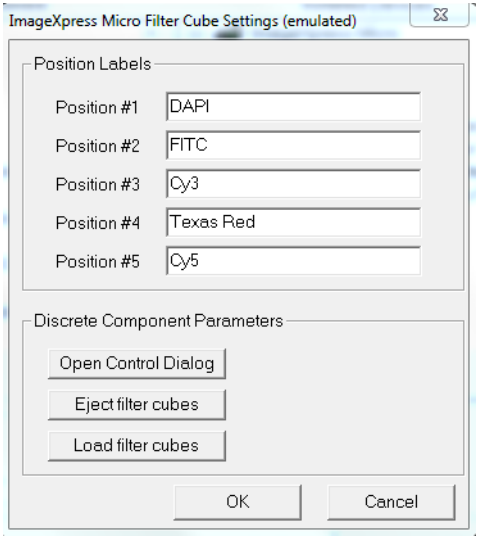


Figure 8-2: ImageXpress Cube Settings dialog

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**.

   In the simplified menu, click **Control > Devices > Configure Illumination**.

3. In the **Configure Illumination** dialog, ensure that the illumination device you changed is selected in the **Device Positions** field and select the filter that you installed from the corresponding option field. See Figure 8-3 on page 181.

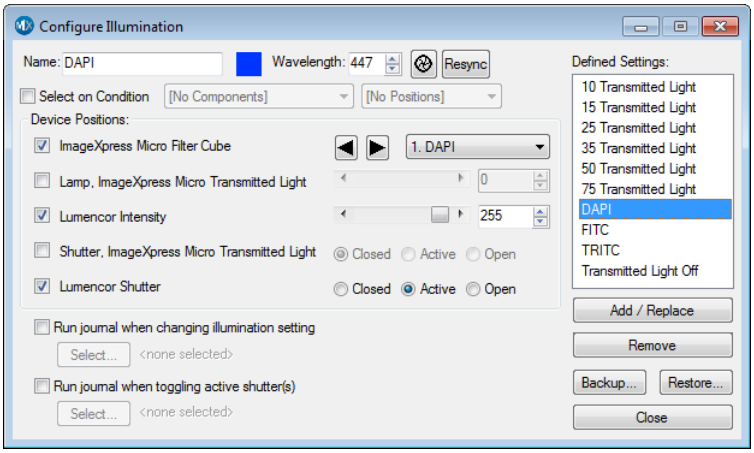


Figure 8-3: Configure Illumination dialog

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 175 for safe user-service procedures.
2. Read and follow Correct Objective Placement on page 182.
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 184.

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 175 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 190, or contact Technical Support. See Obtaining Support on page 34.

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**.

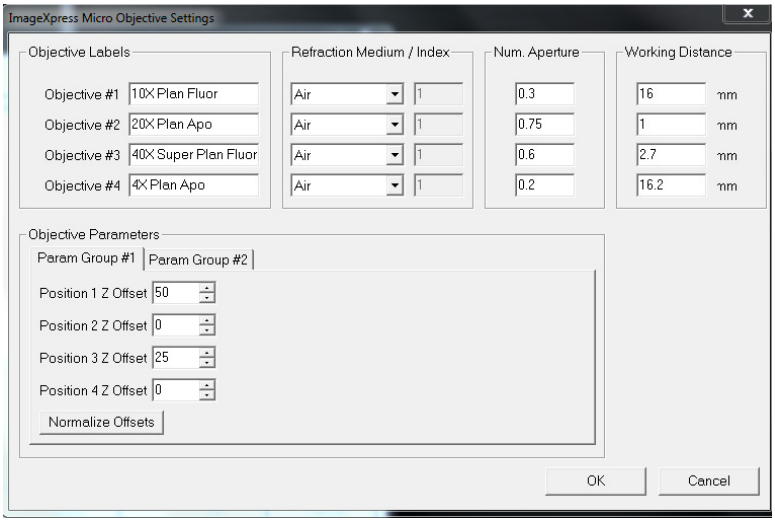


Figure 8-4: ImageXpress Objective Settings dialog

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**.
11. In the **ImageXpress Micro Settings** dialog, ensure that the **Parameter Group #1** tab is active and select the **Maintenance Mode** check box.
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**.
14. In the **User Settings hardware configuration** dialog, double-click **ImageXpress Micro Objective** in the **Claimed Devices** list.
15. In the ImageXpress Micro Objective dialog, 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 186.

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. See Table D-1: Recommended Filter set/FFC plate combinations on page 217. Complete the following procedure to configure parfocality:

1. Open the MetaXpress Software application.
2. Open the following dialogs:
   - **Devices > Configure Magnification**

     In the simplified menu, click **Control > Devices > Configure Magnification**.
   - **Acquire > Acquire**

     In the simplified menu, click **Control > Acquire > Acquire**.
   - **Devices > Stage > Move Stage to Absolute Position**

     In the simplified menu, click **Control > Devices > Move Stage to Absolute Position**.
3. If the following toolbars are not open, click **Window > Toolbars**, or In the simplified menu, click **Control > Window > Toolbars**, and select **Device Control** and **Plate Acquisition**.
4. Click **Devices > Configure Magnification**.
5. If not selected, select the check box next to the **ImageXpress Micro Objective**.

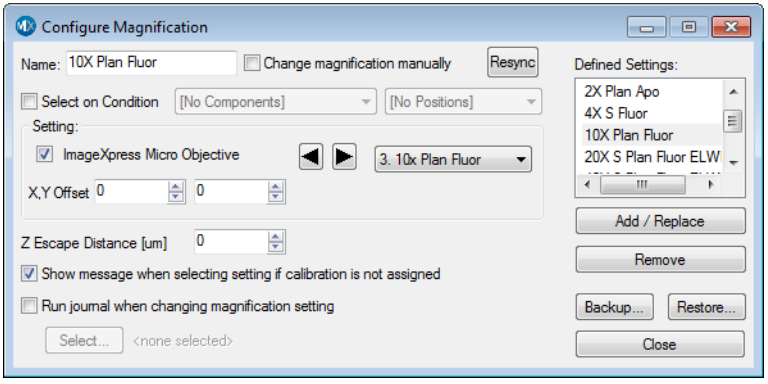


Figure 8-5: Configure Magnification dialog

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 on page 187.

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.

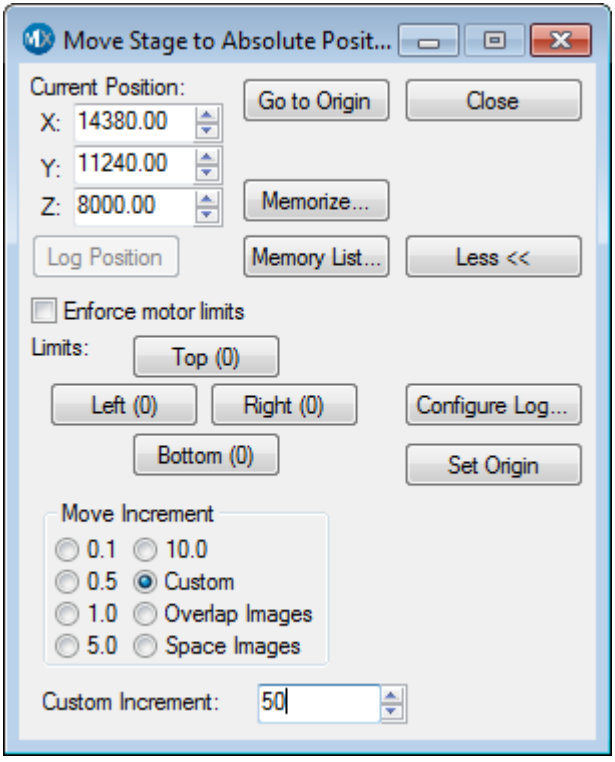


Figure 8-7: Move Stage to Absolute Position dialog

12. When the sample is in focus, write down the **Current Position Z** value in the **Focus Value** column in Table 8-1 on page 188.

Table 8-1: Objective Focus Value

Objective Number	Focus Value
1	

Table 8-1: Objective Focus Value

Objective Number	Focus Value
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 190.

    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 195.

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. See Obtaining Support on page 34.

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 185.

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:\MX6\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 185.
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**.

   in the simplified menu, click **Control > Devices > Configure Magnification**.

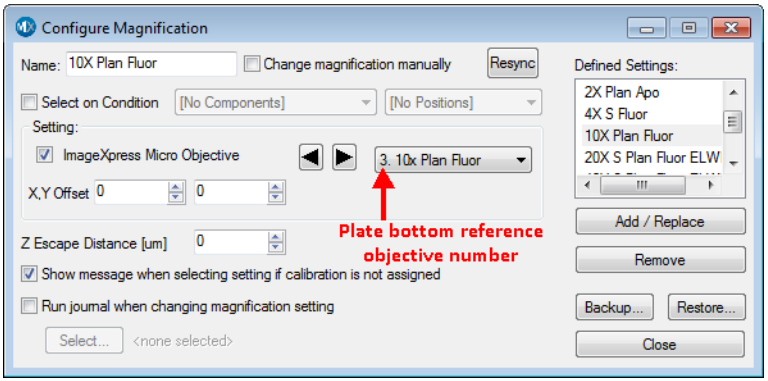


Figure 8-8: Configure Magnification dialog with objective # highlighted

4. In the **Configure Magnification** dialog, 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 195.

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**

     In the simplified menu, click **Control > Devices > Move Stage to Absolute Position**.
   - **Devices > Focus**

In the simplified menu, click **Control > 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, click **Autofocus > Configure Laser**.

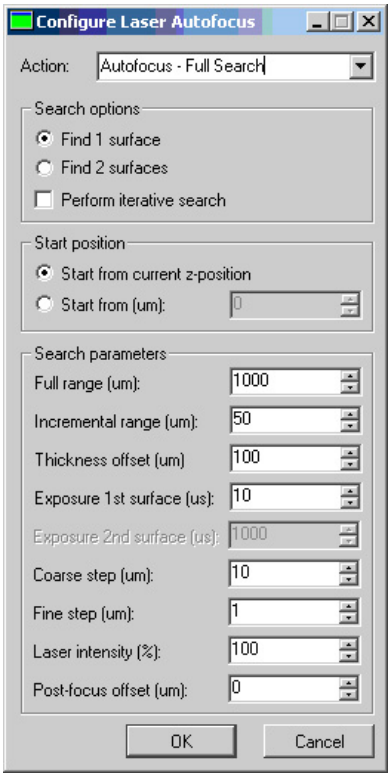


Figure 8-9: Configure Laser Autofocus dialog

10. In the **Configure Laser Autofocus** dialog, 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.

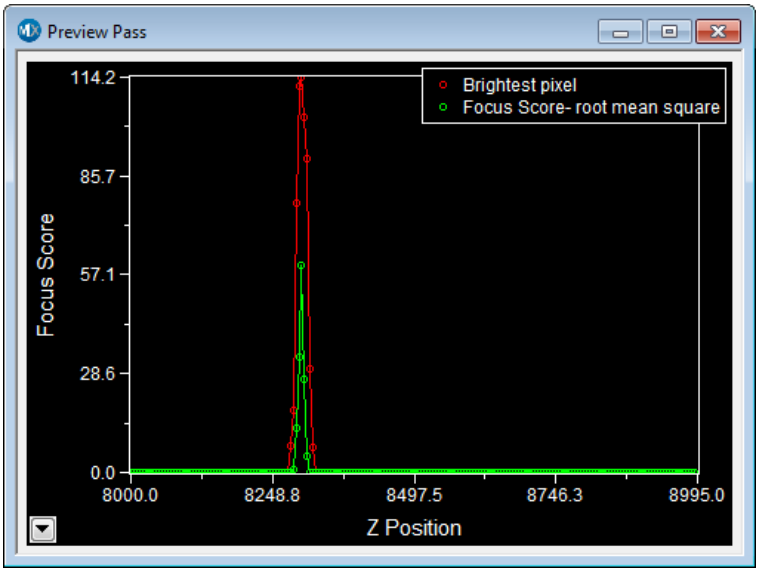


Figure 8-10: Preview Pass dialog

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. See Obtaining Support on page 34.

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.

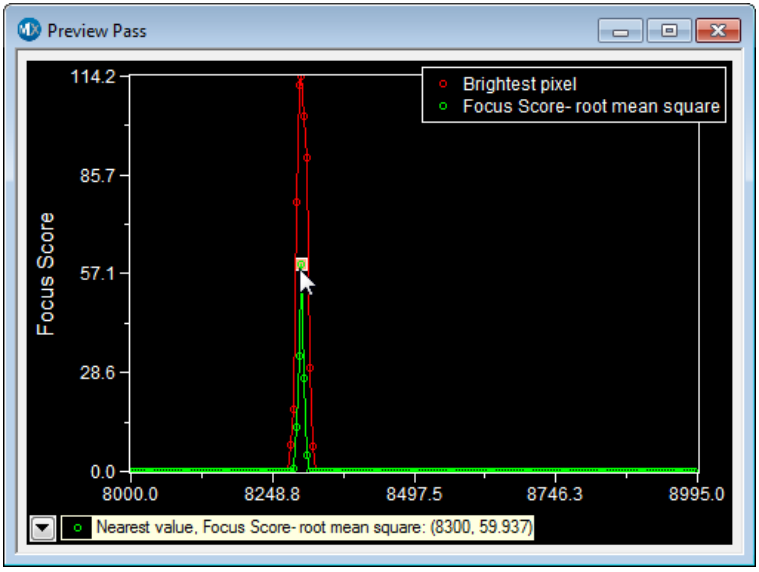


Figure 8-11: Z-position of peak

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 on page 194.

    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 196.

20. Exit the MetaXpress Software application and continue to Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 195.

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 190.

1. Click **Start > All Programs > Accessories > Notepad** and then open the **MetaXpress.ref** file in the MetaXpress **Hardware** folder (**C:\MX6\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:\MX6\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 on page 191.

   [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.

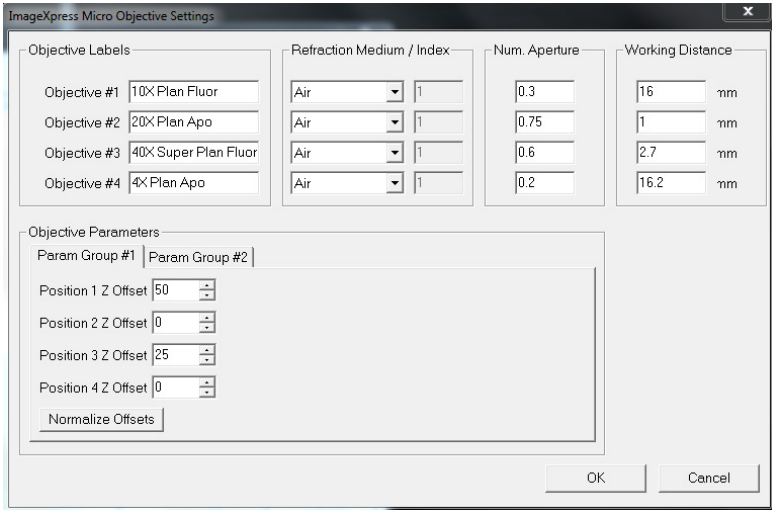


Figure 8-12: ImageXpress Micro Objective Settings dialog

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 190, continue to Step 14.
12. OR
13. If you did not change or replace the reference objective, go to Step 18.
14. Double-click **ImageXpress Micro Z** in the **Installed Devices** list.
15. The ImageXpress Micro Z dialog appears.
16. Type the value you circled in Step 19 of the Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 190 into the **Plate Bottom Reference** field.
17. 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.

18. 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**.
19. The User Settings hardware configuration dialog appears.

20. Double-click **ImageXpress Micro Objective** in the **Claimed Devices** list.
21. The **ImageXpress Micro Objective** dialog appears.
22. 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 185.
23. Repeat Step 7 through Step 9 to enter and normalize the Z offsets.
24. If you needed to complete the Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 190, double-click **ImageXpress Micro Z** in the **Claimed Devices** list and enter the Plate Bottom Reference number as in Step 16.
25. 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 18 to Step 23 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**.

   In the simplified menu, click **Control > Devices > Configure Magnification**.
3. In the **Configure Magnification** dialog, 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**.

   In the simplified menu, click **Measure > Distances > Calibrate Distances**.

10. In the **Calibrate Distances** dialog, click **Setup**.
11. Click **New**, type the name of your new objective in the **Calibrations** field, then press **Enter**.
12. When the fields in the lower half of the dialog become active, 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:\MX6\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.nal file, import it (See Obtaining Support on page 34.) After you have obtained the jour using the procedure in To import the journal suite into the MetaXpress Software on page 201.

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 185.
- Configuring Parfocality after Changing Objectives on page 186.
- Updating Magnification and Calibration Settings on page 198.

This section includes the topics:

- To import the journal suite into the MetaXpress Software on page 201
- To run the shading correction journal from the Main taskbar on page 203

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 203.

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)**. See Obtaining Support on page 34.
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**.

   In the simplified menu, click.
5. In the **Import Journal Suite** dialog, click **Select Journal Suite**.
6. In the **Select Import Suite File Name** dialog, 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:\MX6\TASKBARS** and click **OK**.

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

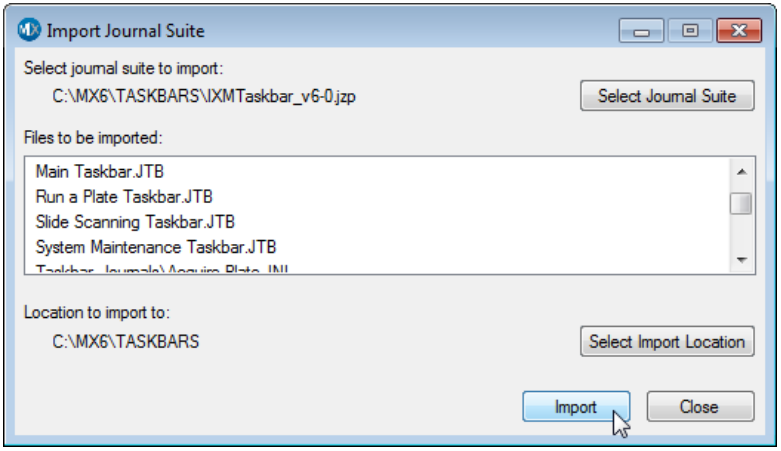


Figure 8-13: Import Journal Suite dialog

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:\MX6\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-XLS**.
13. In the MetaXpress Directory, 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:\MX6).

Note: If the default file paths for journals, images, data, and so on do not go to the C:\MX6 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.**

    In the simplified menu, click **Control > Journal > Journal Control > StartUp Journal.**
16. Navigate to **C:\MX6\StartUp,** select the startup journal (the name of the journal includes the appropriate ImageXpress Micro System model: Standard or XLS), 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 might be grayed out but it must be listed. If it is not listed, then contact Technical Support for assistance See Obtaining Support on page 34.)
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 175 for safe user-service procedures.
2. Follow the steps in Changing Objectives on page 182 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. See Obtaining Support on page 34.
- 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 175 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 **XLS:** 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 XLS: 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.

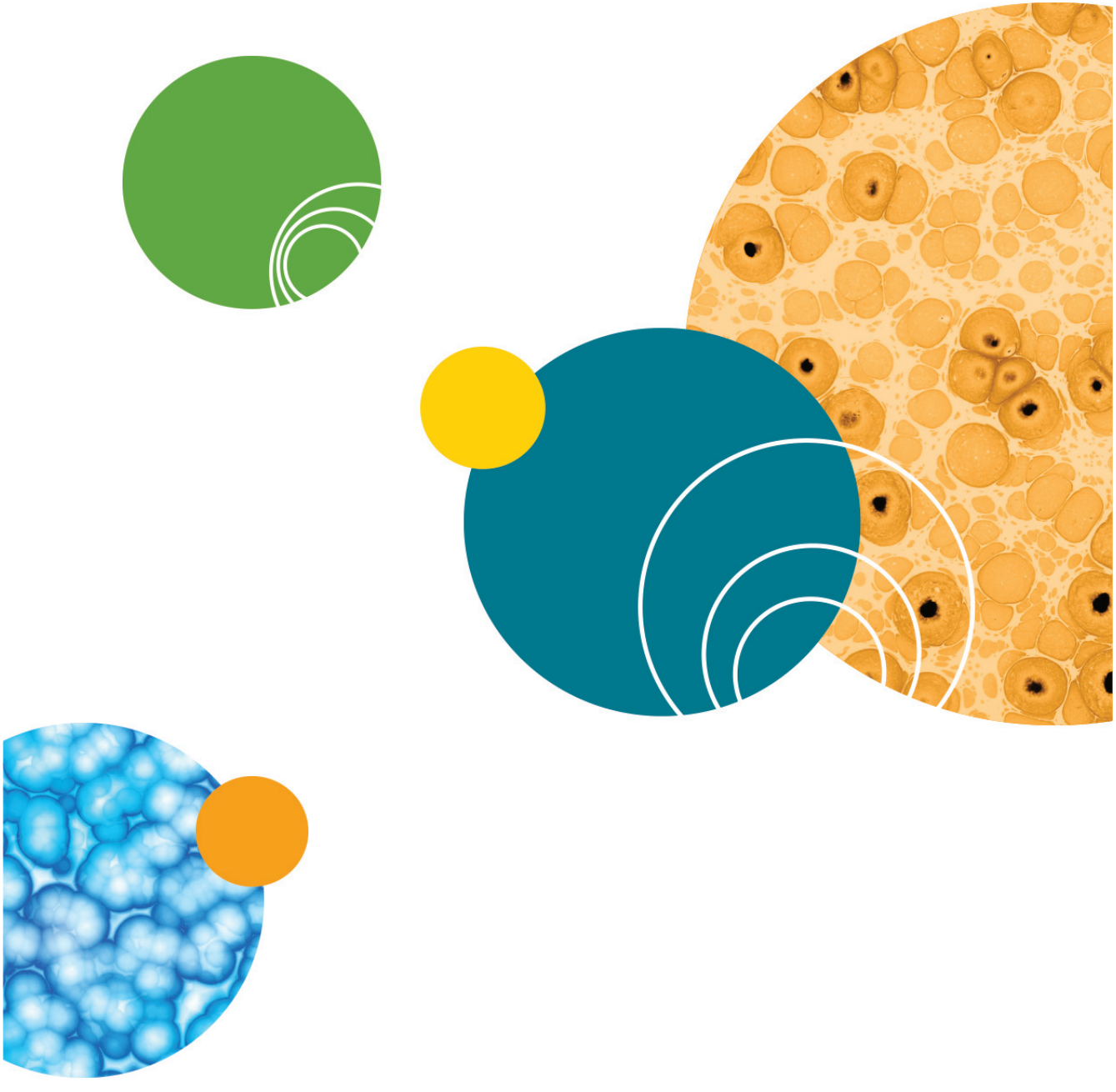
Site Requirements

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)

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
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)
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)

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 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]
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)

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

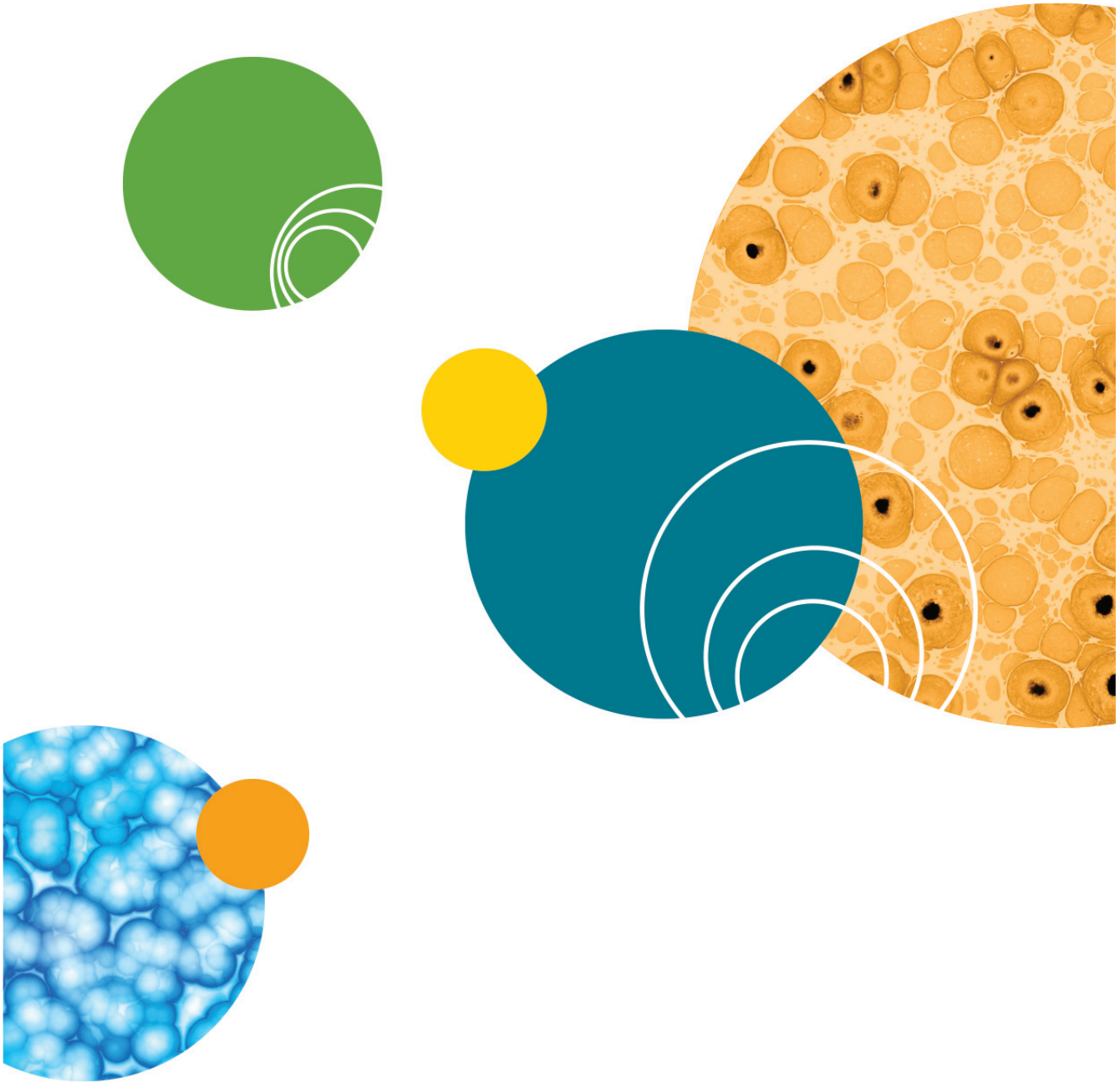
Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture	Working Distance	Plate Compatibility
40x Plan Apo	1-6300-0412	No	0.95	0.14 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
40x S Fluor cc 0.11 mm to 0.23 mm	1-6300-0197	No	0.90	0.3 mm	Thin bottom (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

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 Apo Oil	1-6300-0417	No	1.40	0.21 mm	Thin bottom (0.17 mm)[1] Thin bottom (0.17 mm) No Skirt
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.

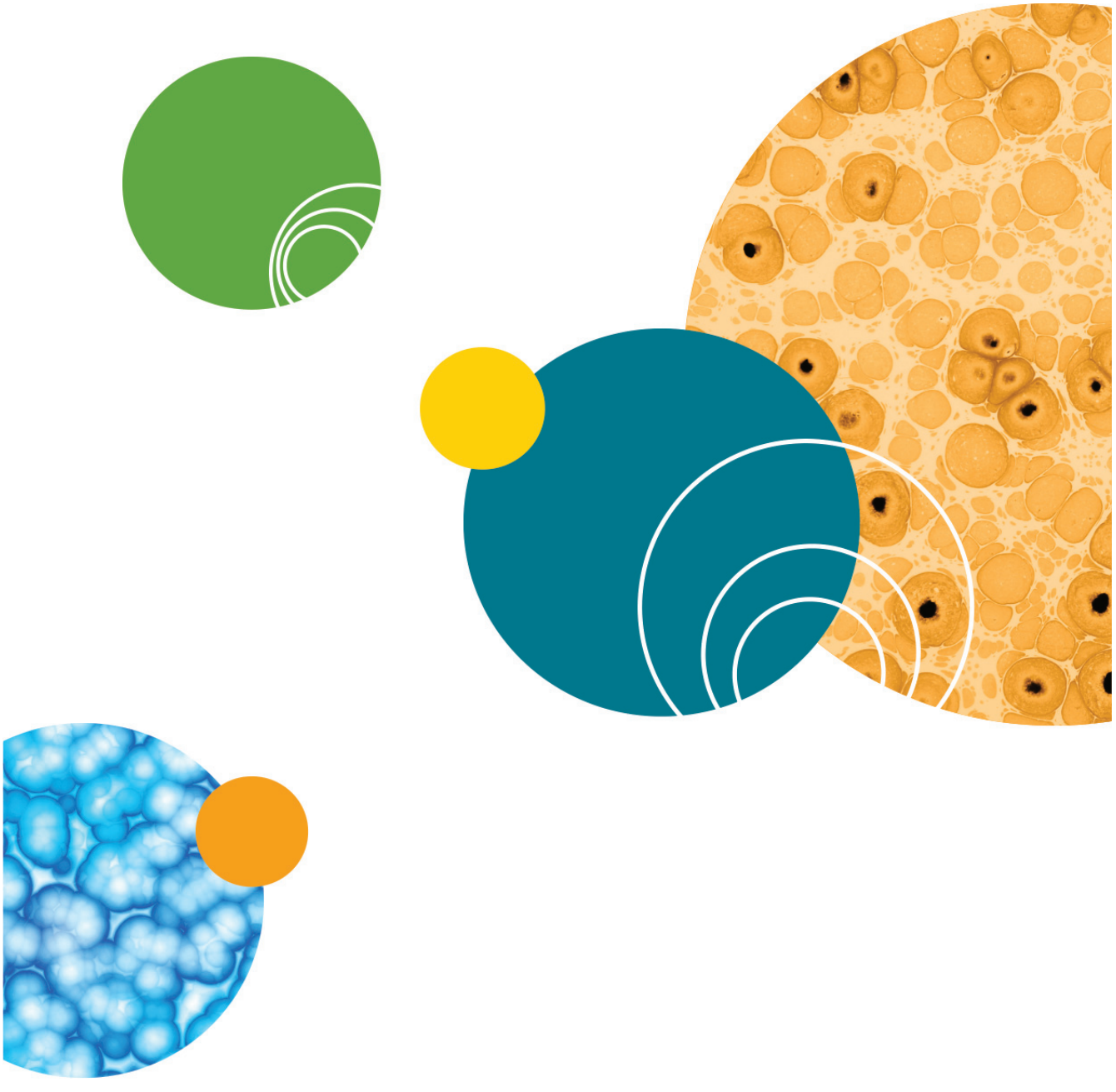


Filter Set/FFC Plate Combinations

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

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:\MX6** (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\MX6**.

Software Administrator

Read/write access is required for everything under the C:\MX6 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:\MX6
C:\MX6\app\mmproc
C:\MX6\app\mmproc\Dropins
C:\MX6\Help\C:\MX\Help\ - all subdirectories -
C:\MX6\Groups\

If the system is set up for multiple users:

C:\MX6\Groups\MetaXpress
C:\MX6\Groups\MetaXpress\Users

If the system is an acquisition computer:

C:\MX6\Hardware\
C:\MX6\Hardware\ - all subdirectories -
C:\MX6\Plates\
C:\MX6\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:\MX6\Groups\MetaXpress

If the system is set up for multiple users:

C:\MX6\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:

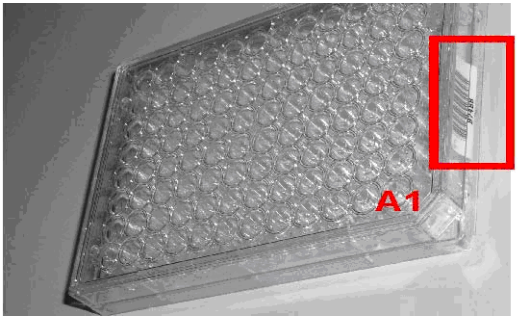


Figure F-1: Boxed region shows barcode affixed on front of microplate

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. See Obtaining Support on page 34.

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.

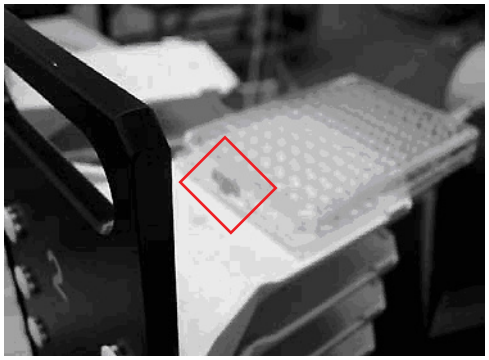


Figure F-3: Box shows barcode centrally located on the microplate, which should be facing toward the front of the hotel

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)

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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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