



ImageXpress® Micro Confocal

High Content Imaging System

User Guide

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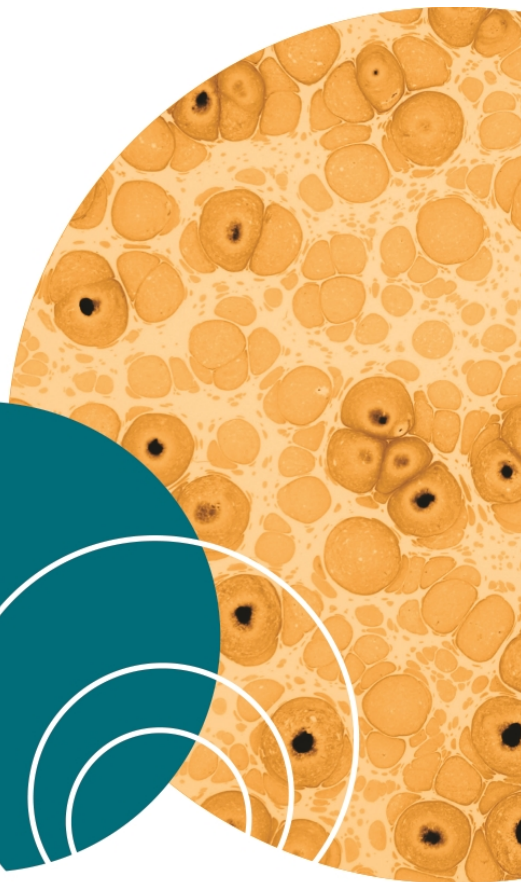
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Safety Information

The safety information section provides information on the safe use of the instrument, including the use of user-attention statements in this guide, a key to understanding the safety labels on the instrument, precautions to follow before operating the instrument, and precautions to follow while operating the instrument.

Please read and observe all warnings, cautions, and instructions. Remember, the most important key to safety is to operate the instrument with care.



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

Warnings, Cautions, Notes, and Tips

All warning symbols in the user guide are framed within a yellow triangle. An exclamation mark is used for most warnings. Other symbols can warn of other types of hazards such as biohazard, electrical, or laser safety warnings as are described in the text of the warning.

When warnings and cautions are displayed in this guide, be careful to follow the specific safety information related to them.

The following user-attention statements can be displayed in the text of Molecular Devices user documentation. Each statement implies a particular level of observation or recommended procedure as described:



WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed. Some warnings can have a different symbol on the left, such as electric shock, biohazard, and laser light warnings. The definition of the symbol is included in the text of the warning.



CAUTION! A caution indicates a situation or operation that could cause damage to the instrument or loss of data if correct procedures are not followed.



Note: A note calls attention to significant information.



Tip: A tip provides useful information or a shortcut, but is not essential to the completion of a procedure.

Symbols on Instrument Labels

Each safety label located on the instrument contains an alert symbol that indicates the type of potential safety hazard related to the label. The following table lists the alert symbols that can be found on Molecular Devices instruments.

Table S-1: Instrument Label Alert Symbols








Symbol	Indication
	This symbol indicates that the product documentation needs to be consulted.
	This symbol indicates a potential laser hazard. See Laser Safety on page 11 .
	This symbol indicates a potential lifting hazard. For information about the weight of the instrument, see Instrument Specifications on page 209 .
	This symbol indicates a potential pinch hazard.
	This symbol on the power switch indicates power on. See Powering On the Instrument on page 29 .
	This symbol on the power switch indicates power off. See Shutting Down the System on page 34 .
	This symbol on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. It indicates that you must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system.

Table S-1: Instrument Label Alert Symbols (continued)

Symbol	Indication
	For products under the requirement of the WEEE directive, please contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Before Operating the Instrument

Make sure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understood all Safety Data Sheets (SDS) for all materials being used.

Protective Housing and Safety Interlocks

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

The automated top door is interlocked. Do not operate this instrument with the top door open. Do not disable an interlock. When the automated top door is open, the laser light source is disabled to prevent hazards associated with laser emission.



WARNING! Do not defeat any 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 in this guide. Read each procedure carefully and follow all 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.

Safety Interlock Failure

If the focusing laser stays on when the automated top door is open, it is unsafe to continue using the instrument due to a safety interlock failure. Contact Molecular Devices Support immediately. See [Obtaining Support on page 203](#).

Non-Interlocked Doors and Panels

The side access doors are not interlocked. These doors do not provide access to exposure by the laser light source. Some moving parts exist inside these doors. See [Moving Parts Safety on page 13](#).

The instrument has several panels that are intended for use by field service personnel only, and are not interlocked. All service panels are secured to the protective housing with screws and require a special tool to remove.



WARNING! If you are instructed to remove non-interlocked panels, make sure that the instrument is powered OFF and the power cable is unplugged. Never operate this instrument with any covers or panels removed. Do not attempt to access the service-only areas inside the instrument when the power cable is connected.

Laser Safety



WARNING! LASER LIGHT. This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this guide, be careful to follow the specific safety information related to the symbol.

The ImageXpress Micro System is rated a Class 1 Laser Product because it houses a laser module, and the laser light cannot be accessed under normal use. The autofocus system uses a Class 3b high-power laser that the operator cannot and must not attempt to access.

Table S-2: Embedded Laser Module Specifications

Item	Description
Wavelength	690 nm
Maximum output power	20 mW, continuous wave
Laser class	Class 3b

The ImageXpress Micro System is equipped with a redundant laser safety system. When samples are being loaded or unloaded, hardware interlocks prevent the laser module from turning on until the automated door is closed.

The operator or the service engineer is not exposed to radiation from the laser module during operation, maintenance, or service. 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/cm²).



WARNING! LASER LIGHT. Do not attempt to repair or adjust the laser. Removal of the top panel, safety interlocks, and microscope objective, and then looking into the laser beam can cause severe eye injury and blindness.



WARNING! LASER LIGHT. The instrument must be operated only when all the doors and panels of the instrument are in place and closed.

Light Source Safety

The ImageXpress Micro System is equipped with an external light source connected to the instrument with a light guide.

The external light source for the ImageXpress Micro Confocal System is a solid-state light source that has a rated lifetime of more than 20,000 hours. There are no user-replaceable parts in this light source. The external light source uses an electronic shutter to control the exposure of the sample to excitation light. This helps to minimize sample degradation and photobleaching.

Electrical Safety

To prevent electrically-related injuries and property damage, properly inspect all electrical equipment before use and immediately report all electrical deficiencies. Contact Molecular Devices technical support for servicing of equipment that requires the removal of covers or panels.

Molecular Devices recommends turning the power off when the instrument is not in use.



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 systems power switch on the front of the ImageXpress Micro Systems Power and Options Controller.



WARNING! HIGH VOLTAGE. Do not operate the external light source with the external light source housing open. Do not open the external light source housing with the light source powered on.

ImageXpress Micro Systems Power and Options Controller

A single power cable connects the instrument to the external ImageXpress Micro Systems Power and Options Controller. The external power controller has an input voltage rating of 100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum. The power controller contains no user-serviceable parts.



WARNING! Before attempting to access any internal service areas of the instrument, unplug the power cable.

Fuses and Circuit Protection

In the ImageXpress Micro System, the external power supply for the solid-state light source provides over-current protection limited to 10 amp maximum. The solid-state light source contains no user-serviceable parts.

The ImageXpress Micro Systems Power and Options Controller provides over-current protection for the light source limited to 15 amps maximum. The power controller contains no user-serviceable parts.

Moving Parts Safety

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.

To prevent injury due to moving parts, observe the following:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.
- Avoid contact with the moving automated top door.
- Some user-controlled moving parts exist behind the access doors on the side of the instrument. When doing maintenance operations that require access through these doors, avoid contact with parts that are in motion.



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



Note: Observe all warnings and cautions listed for all external devices attached to or in use during the operation of the instrument. See the applicable user guide for the operating and safety procedures of that device.

Lifting Hazard



WARNING! The ImageXpress Micro Confocal System weighs approximately 104 kg (230 lbs). Do not attempt to lift or move the instrument without assistance.



CAUTION! Moving the 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 does not cover problems caused during or as a result of shipment or relocation.

Chemical and Biological Safety

Normal operation of the instrument can involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples based on good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original containers of solutions before their use.
- Dispose of all waste solutions based on the waste disposal procedures of your facility.
- Operate the instrument in accordance with the instructions outlined in this guide, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. Therefore, take applicable safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials.
- Observe the applicable cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the applicable cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.



WARNING! Never use the instrument in an environment where potentially damaging liquids or gases are present.

Cleaning and Maintenance Safety

Observe the cleaning procedures outlined in this guide for the instrument.

Do the following before cleaning equipment that has been exposed to hazardous material:

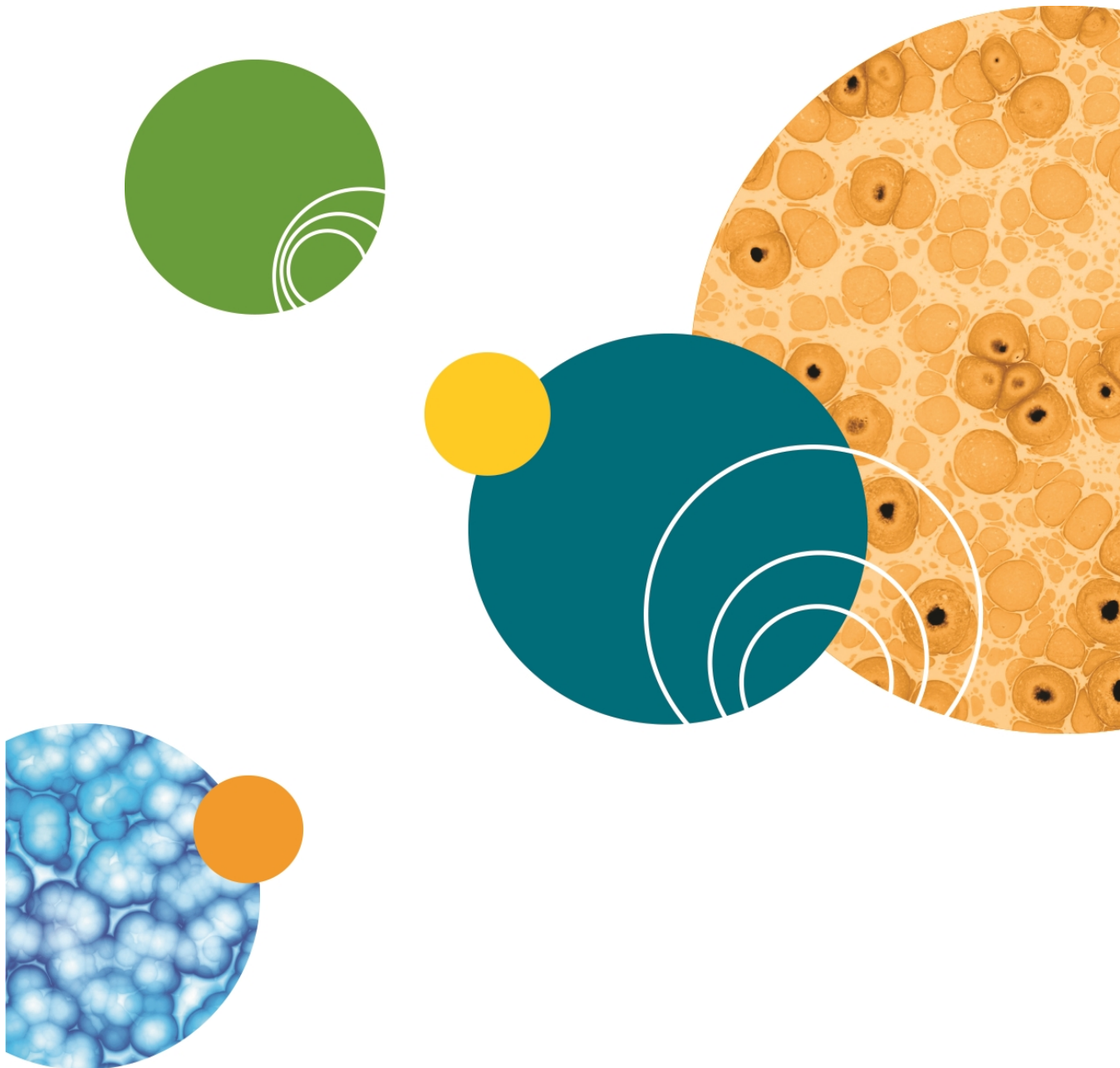
- Contact the applicable Chemical and Biological Safety personnel.
- Review the Chemical and Biological Safety information contained in this guide. See [Chemical and Biological Safety on page 14](#).

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See [Obtaining Support on page 203](#).



WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before returning parts to Molecular Devices for repair. Molecular Devices will not accept items that have not been decontaminated where it is applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

For approved cleaning and maintenance procedures, see [Maintenance on page 147](#).



Chapter 1: Introduction to the ImageXpress Micro Confocal System

1

The ImageXpress® Micro Confocal High Content Imaging 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. With the addition of modular options, the system provides environmental control for live cells, moves fluids to and from cells for compound addition and cell washing, and conducts transmitted light acquisition.

The core hardware component of the imaging system is a custom-designed, fully automated, epi-illumination fluorescence microscope. The rapid autofocus and precision sample movement features of the microscope 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, allowing complete real-time control of the instrument configuration through the MetaXpress® High-Content Image Acquisition and Analysis Software.

When used in combination with the powerful image analysis 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 solid state light source with selectable wavelength bands set by internal excitation filters connected by a fiber-optic light guide
- A scientific CMOS camera
- Laser autofocus system with precision motorized Z (focus) stage
- Image-based autofocus
- Precision motorized X-Y (sample) stage
- User-changeable, high-quality, Nikon objectives in a four-position linear selector
- User-changeable and selectable emission and dichroic filters in two separate controllable wheels inside the confocal module
- Configurable with an available spinning confocal disk box
- Selectable binning modes to decrease exposure time and increase throughput
- Motorized selection of stage position, filters, and objectives with asynchronous operation
- High-transmission fluorescence imaging optics with world-class chromatic aberration correction, resolution, and image flatness

- Operation and configuration control by the integrated MetaXpress Software
- Optional expansion solutions available for environmental control, phase contrast transmitted light, fluidics, and robotic plate handling

ImageXpress Micro Confocal System Instrument Features

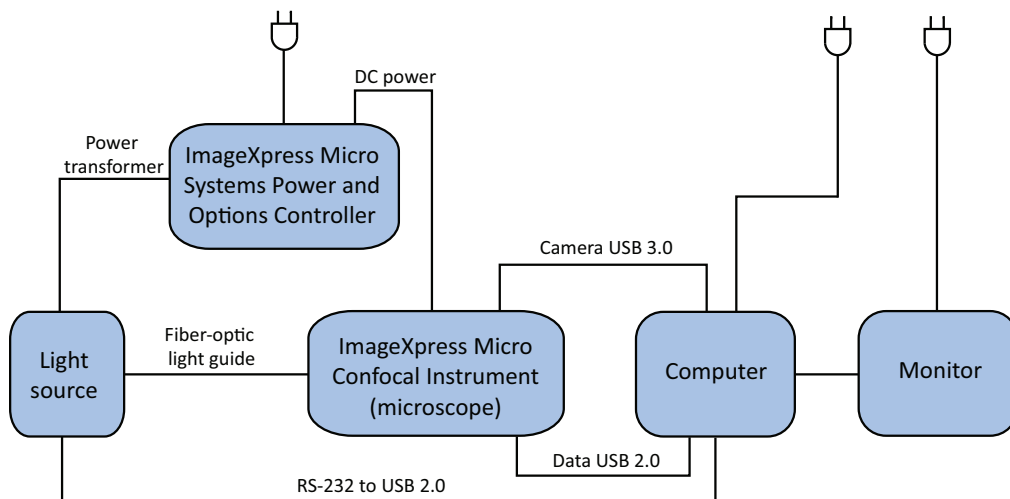


Figure 1-1: ImageXpress Micro Confocal System Components Without Options

Illumination System: Excitation

Light Source

The external light source for the ImageXpress Micro Confocal System is a solid-state light source that has a rated lifetime of more than 20,000 hours. There are no user-replaceable parts in this light source. Using an electronic shutter to control the exposure of the sample to excitation light helps to minimize sample degradation and photobleaching.

Light Source Filter Wavelength Ranges

Table 1-1: ImageXpress Micro Confocal System Light Source Excitation Spectra

Filter	Wavelength
DAPI	377/50 nm
FITC	475/34 nm
TRITC	531/40 nm
TEXAS RED	560/32 nm
CY5	631/28 nm

Light Guide

The ImageXpress Micro Confocal System uses a fiber optic light guide between the external light source and the instrument. 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 fiber optic 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.

Filter Changer

The 8-position emission filter wheel uses standard, commercially available filters. Molecular Devices offers and recommends Semrock filters, based on ImageXpress Micro Confocal System validation testing.

The 5-position dichroic filter wheel uses a unique frame, so the dichroic mirror filters can be ordered only through Molecular Devices.

Objective (Z) Stage

Motorized Z Stage

The Z stage position is monitored using a linear encoder that has better than 100 nm resolution.

Objectives

The 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. See [Compatible Objectives on page 217](#).

Motorized Objective Changer

The instrument has a 4-position objective changer. Only the selected objective moves up and down in the Z axis.

Sample (X-Y) Stage

Sample

The plate holder is designed for scanning multi-well microplates in standard ANSI (SBS) formats with plastic or glass bottoms. It 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 accessory kit. Optimal image quality depends on plate flatness, well bottom thickness, 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/eject position, and automatically closes when the X-Y stage moves the plate into position for imaging.

Motorized X-Y Stage

The X-Y stage position is monitored using a linear encoder that 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.

Confocal Disks

There are three available Confocal disk box configurations. For the confocal performance details with different objectives, see [Theoretical Calculation of Confocal Performance With Compatible Objectives on page 223](#).

Table 1-2: Confocal Disks Geometries

Disk			Part Number	Use
			5039730	Standard For basic imaging
Widefield	60 µm Pinhole			
			5039731	Optional For basic imaging, and for high-throughput imaging
Widefield	60 µm Pinhole	50 µm Slit		
			5040287	Optional For basic imaging, and for high-resolution imaging
Widefield	60 µm Pinhole	42 µm Pinhole		

Electronics

Without optional equipment, the ImageXpress Micro Confocal System also contains the following components:

- External computer, monitor, and cables
- External ImageXpress Micro Systems Power and Options Controller and cables
- External solid state light source, fiber, and cables
- USB 2.0 cable for computer to instrument control and instrument to computer data
- USB 3.0 cable for internal camera to computer

MetaXpress Software Features

Use the MetaXpress Software with the ImageXpress Micro Confocal System to select a standard acquisition and analysis protocol 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](#) and [Setting Up and Acquiring Data on page 69](#).
- 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 Suite installation USB flash drive, or the application help available when using the MetaXpress Software.

Simplified Menu Structure

An optional simplified menu structure can be installed to reduce the number of top-level menus in the MetaXpress Software. 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. The **Menu Map** is available only after the simplified menu installation.

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 guide are for 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

Theory of Operation

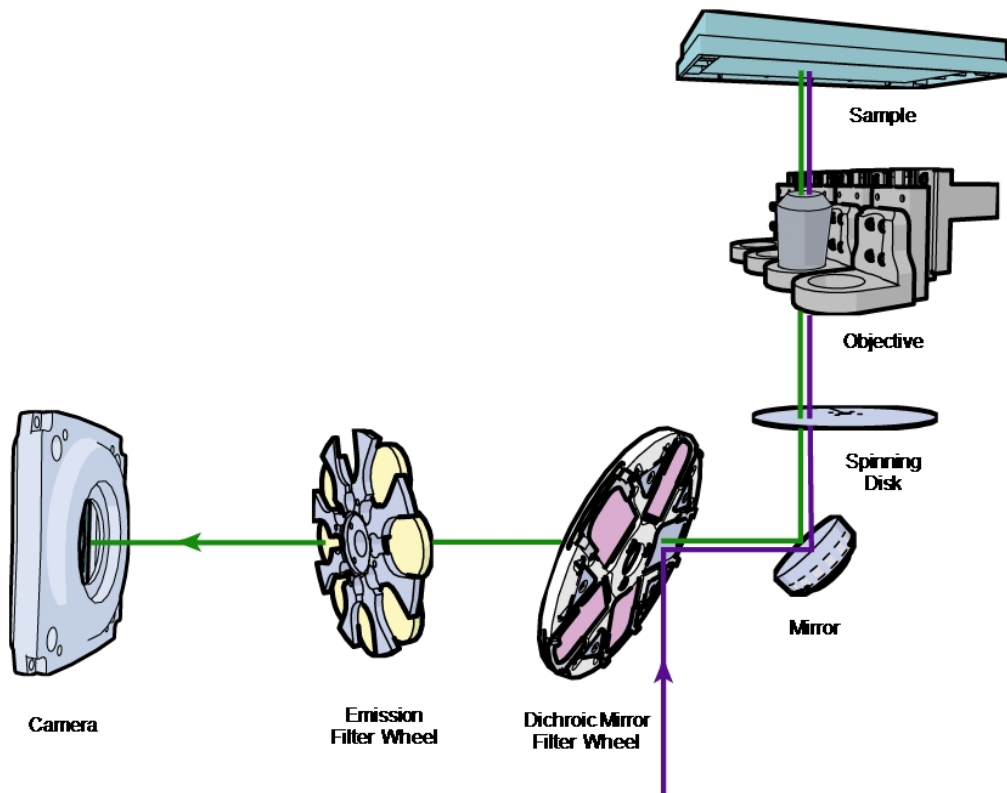


Figure 1-2: ImageXpress Micro Confocal System Optical Path

The ImageXpress Micro Confocal System uses the following components and functions:

- [Fluorescence Imaging on page 25](#)
- [Excitation and Emission Filters on page 25](#)
- [Dichroic Mirror on page 26](#)
- [Objective Lenses on page 27](#)

Fluorescence Imaging

Fluorescence is a phenomenon observed in certain species of molecules (fluorochromes, fluorescent proteins, 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.

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 or fluorescent protein 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, such as the emission and excitation filters, and the dichroic mirror.

Confocal Imaging

In standard widefield microscopy, fluorescent objects above or below the focal plane are seen as out-of-focus objects and increase the background fluorescence. Confocal microscopy rejects out-of-plane signals by using point-source illumination and a pinhole aperture ahead of the detector.

The main advantage of confocal imaging is the rejection of photons from out of focus portions of the sample and the resulting improved resolution and reduced background. By changing the size of the pinhole in front of the detector you can achieve a suitable balance between the degree of confocality and the sensitivity and speed of the instrument. A smaller pinhole gives better axial resolution, while a larger pinhole increases the collection efficiency resulting in higher sensitivity.

Excitation and Emission Filters

In the ImageXpress Micro Confocal System, the excitation filters are located inside the light source, and the emission filters and the dichroic mirrors are located within two separate, controllable wheels inside the confocal module.

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 absorbance (excitation) spectrum of the target dye. 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 Confocal System, framed dichroic mirrors are in a five-position filter wheel.

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 in which the illumination and imaging optical paths overlap at the objective lens. 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.

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 through the dichroic to the 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 very 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) at shorter wavelengths. In practice, the characteristic transmission spectrum for a dichroic looks something like [Figure 1-3](#).

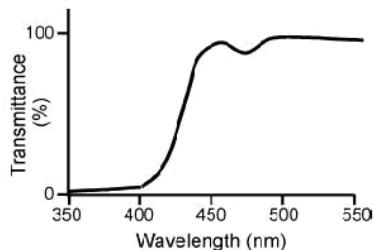


Figure 1-3: Example of a transmission spectrum of a dichroic mirror

In principle, the cutoff wavelength (or midpoint of the cutoff region) of the dichroic mirror should be chosen to lie halfway between the absorption and emission peaks of the chosen fluorochrome, as this simultaneously maximizes 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 toward one peak or the other. This allows, 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 Confocal System can be configured with any of the high quality Nikon objectives listed in [Compatible Objectives on page 217](#).

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



Note: Extra-long working distance (ELWD) objectives have adjustable spherical-aberration correction collars for imaging through thick substrates such as most microplates. For details on how to calculate and set their correct values, see [Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 178](#).

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 or thin plate bottom being used. Setting these collars should be done using the physical thickness of the plate bottom 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 and type of assay that you will be imaging. The plate material (plastic or glass) and thickness are major considerations when choosing an objective. Another important practical note is that generally the greater the correction of an objective, 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 web site (www.nikon.com).

Chapter 2: Using the ImageXpress Micro Confocal System

2

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

- [Starting the System on page 29](#)
- [Acquiring Data on page 33](#)
- [Analyzing Data on page 33](#)
- [Maintaining the Instrument on page 34](#)
- [Shutting Down the System on page 34](#)

Starting the System

The following procedures explain how to safely power up the instrument and computer and how to start and log in to the MetaXpress Software.

- [Powering On the Instrument on page 29](#)
- [Starting the Software on page 30](#)

Powering On the Instrument

Before starting the software, make sure the instrument is properly powered on.

1. Ensure that the power cords for the instrument and the light source are connected to the ImageXpress Micro Systems Power and Options Controller, and verify that the power button is switched on for the light source box.
2. Turn on the **Instrument** button on the front of the ImageXpress Micro Systems Power and Options Controller. This button also turns on the connected light source box.
3. Turn on the power to the host computer and the monitor.
4. After the computer has started and Windows is running, log in to Windows using the User Name and Password combination provided for you by your system administrator.



CAUTION! Do not log in to your system as **Guest** unless you are specifically instructed to do so by your system administrator.

5. Start the MetaXpress Software. See [Starting the Software on page 30](#).

Starting the Software

This procedure assumes that your ImageXpress Micro Confocal 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 the ImageXpress Micro Confocal 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 > All Programs > MetaXpress > MetaXpress**.

2. In the MetaXpress Software login dialog, from the **User Name** drop-down list, select the user name to use and then click **OK**.



Figure 2-1: MetaXpress Software login dialog



Note: The MetaXpress Software login dialog appears only when the software is configured to run in multi-user mode from within the Meta Imaging Series Administrator Software. If you do not see this screen, the software is in single-user mode and the dialog in [Figure 2-2](#) is displayed.

3. In the **Welcome to MetaXpress** dialog, select the **Data Source** to connect to (if there is more than one), type your **Login Name** and **Password**, and then click **OK**.

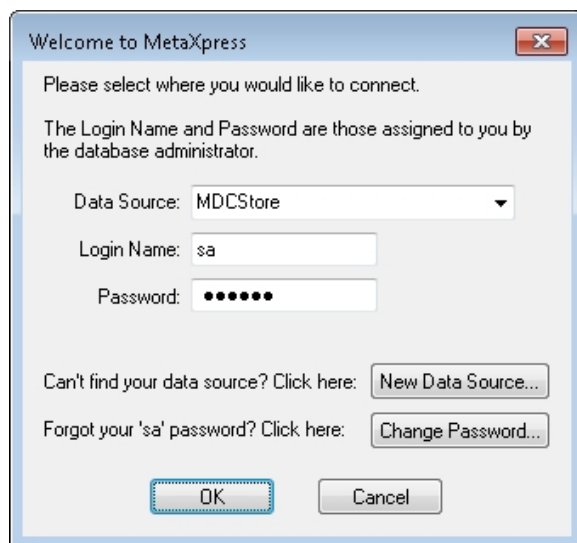


Figure 2-2: Welcome to MetaXpress dialog



Note: The default System Administrator **Login Name** is **sa**, and the default password is **moldev**. You can change the password by clicking **Change Password**.

4. In the dialog to select a security level, select one of the groups assigned in the MDCStore database and then click **OK**.

The MetaXpress Software starts and initializes the various components of the ImageXpress Micro Confocal System.

If you receive error messages when the system is initializing, try the following:

- Check that all hardware connections are plugged in and fully seated.
- Check that the plate stage is clear of blockages.
- Restart the system.

If the error message continues after the recommended troubleshooting, use AxoTrace and contact Molecular Devices Technical Support. See [Logging AxoTrace Software Messages to a .txt File on page 205](#).

Status Indicator Lights

The edges of the ImageXpress Micro Confocal System illuminate with colors that provide information about the instrument status.

Table 2-1: Status Indicator Colors

Color	Instrument Status
Orange	The instrument is powered on without software control.
Blue	The instrument is powered on with software control and is ready to use.
Green	The instrument is acquiring data.
Red	The instrument is in an error state or cannot communicate with the software.

If required, you can turn off the status indicator lights. See [Turning off the Status Indicator Lights on page 203](#).

Acquiring Data

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

For detailed information, see the following sections:

- [Preparing For Acquisition on page 59](#)
- [Setting Up and Acquiring Data on page 69](#)

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 Suite installation USB flash drive, or when using the MetaXpress Software, view the help by selecting **Help > Help Topics**, or pressing **F1** on your keyboard.

Maintaining the Instrument

Specific user-level maintenance can be done on the ImageXpress Micro Confocal System for cleaning the instrument, changing filters, and changing and cleaning objectives, as described in [Maintenance on page 147](#).

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See [Obtaining Support on page 203](#).

Shutting Down the System

1. Exit the MetaXpress Software.
The software prompts you to save any open images.
2. Turn off the **Instrument** button on the front of the ImageXpress Micro Systems Power and Options Controller. This also turns off the connected light source box.
3. Turn off the power to the computer and the monitor.

Chapter 3: System Installation and Testing

3

The ImageXpress Micro Confocal 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, the host computer, and accessory kit. See [Table 3-1](#) for kit contents.

The ImageXpress Micro Confocal System host computer is shipped with the MetaXpress® Software already installed. The instrument is connected to the host computer during installation. There are four main connections, excluding power cords. See [Figure 3-1](#).

- Power supply to the ImageXpress Micro Confocal System
- Fiber optic light guide from the external light source to the ImageXpress Micro Confocal System
- USB 2.0 data cable from the ImageXpress Micro Confocal System to the host computer
- USB 3.0 cable from the camera to the computer

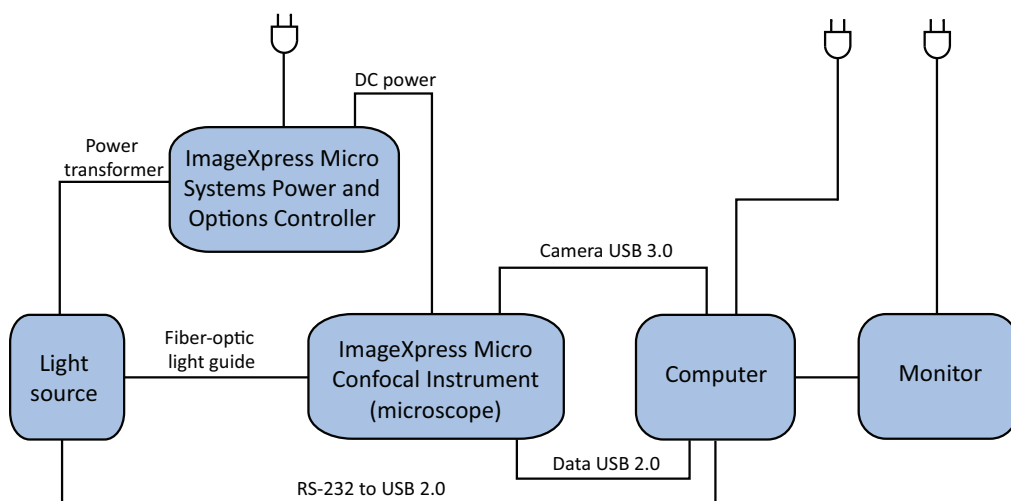


Figure 3-1: ImageXpress Micro Confocal System Components Without Options

Table 3-1: Accessory Kit Contents

Accessory	Description
Bead plate	TetraSpeck™ fluorescent microspheres test plate
Hex keys	1/16", 0.05", 2 mm, 4 mm, 2.5 mm
Slide holder	Single
Calibration slides	Spatial (GP-2) Red (GP-7) Green (GP-8) Blue (GP-9) Yellow (GP-11)
Shading correction plates	Fluorescent Green Fluorescent Red Fluorescent Pink
Filter tools	Emission and Dichroic
Bead slide	FocalCheck™ fluorescent microspheres test slide
Storage box	Empty accessory box for miscellaneous storage use

If you need to re-install the MetaXpress Software, or install it on a new computer, please see the *MetaXpress High-Content Image Acquisition and Analysis Software Suite Installation and Update Guide* included on the MetaXpress Software Suite installation USB flash drive, or contact Technical Support. See [Obtaining Support on page 203](#).

Verifying Device Settings in the Meta Imaging Series Administrator Software

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



Note: Molecular Devices recommends not running verification tests with a sample plate loaded on the stage.



Note: The Meta Imaging Series Administrator Software and the MetaXpress Software cannot be run simultaneously.

To verify the hardware configuration in the Meta Imaging Series Administrator Software, complete the following procedure:

1. Follow the procedure described in [Powering On the Instrument on page 29](#), except starting the MetaXpress Software.
2. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
3. In the **Meta Imaging Series Administrator**, from **List of Groups**, select **MetaXpress**.

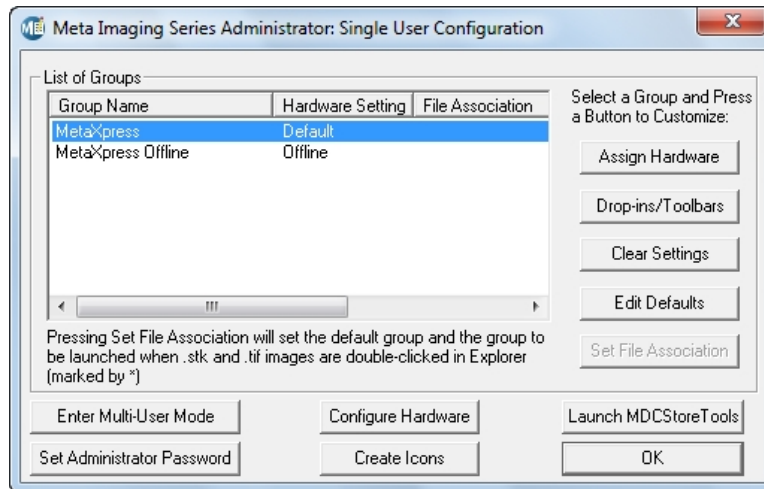


Figure 3-2: Meta Imaging Series Administrator Software

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

- In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro X** and then click **Settings**.

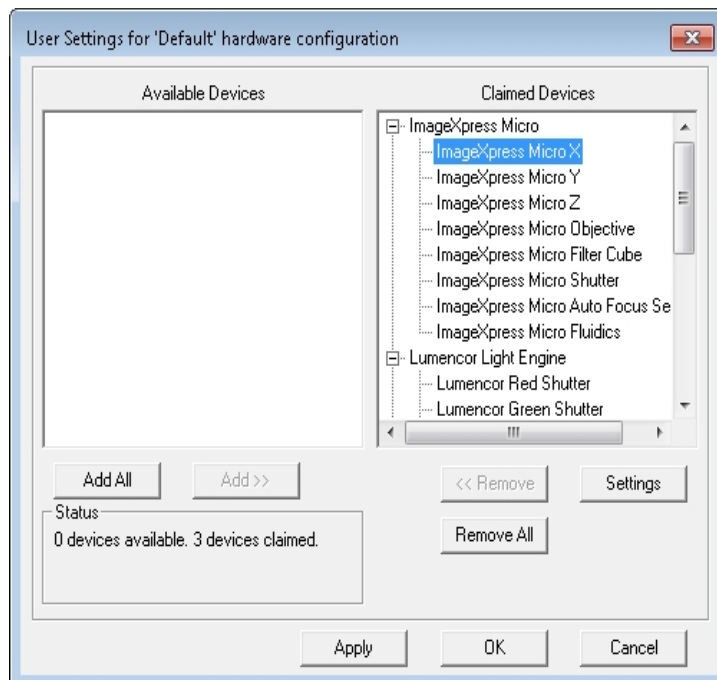


Figure 3-3: The User Settings dialog

- In the **ImageXpress Micro X Settings** dialog, in the **Move** field, increase the step size to 10,000 μm .

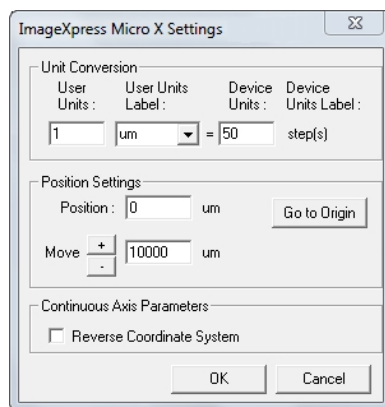


Figure 3-4: ImageXpress Micro X Settings dialog

- Ensure that the **Reverse Coordinate System** check box is deselected.

9. Click the + and – buttons and confirm that the stage responds to the control.



Note: To see the stage move, open the upper hinged side door on the instrument.

10. In the **Move** field, change the step size back to 10 μm and click **OK**.
11. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Y** and then click **Settings**.

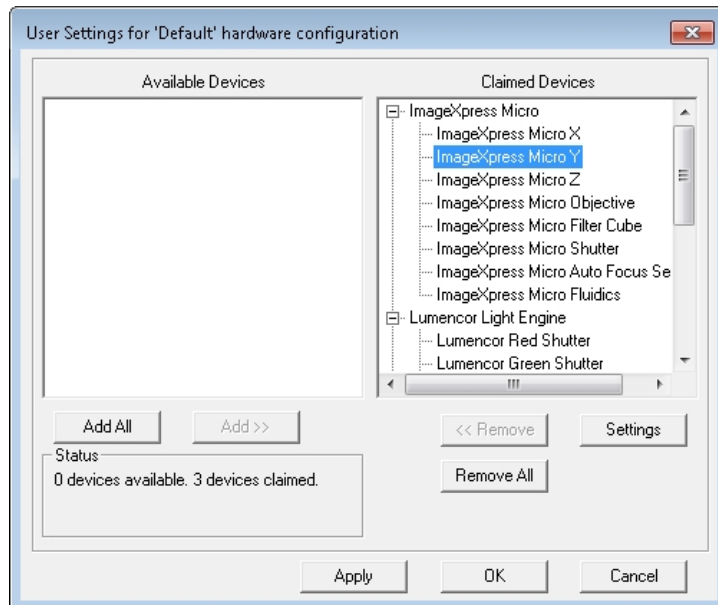


Figure 3-5: The User Settings dialog with ImageXpress Micro Y selection

12. In the **ImageXpress Micro Y Settings** dialog, in the **Move** field, increase the step size to 10,000 μm .

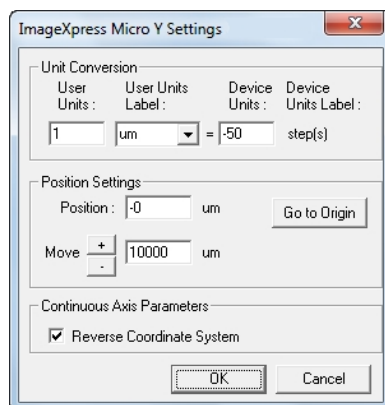


Figure 3-6: ImageXpress Micro Y Settings dialog

13. Ensure that the **Reverse Coordinate System** check box is selected.
14. Click the + and – buttons and confirm that the stage responds to the control.
15. In the **Move** field, change the step size back to 10 μm and click **OK**.
16. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Z** and then click **Settings**.

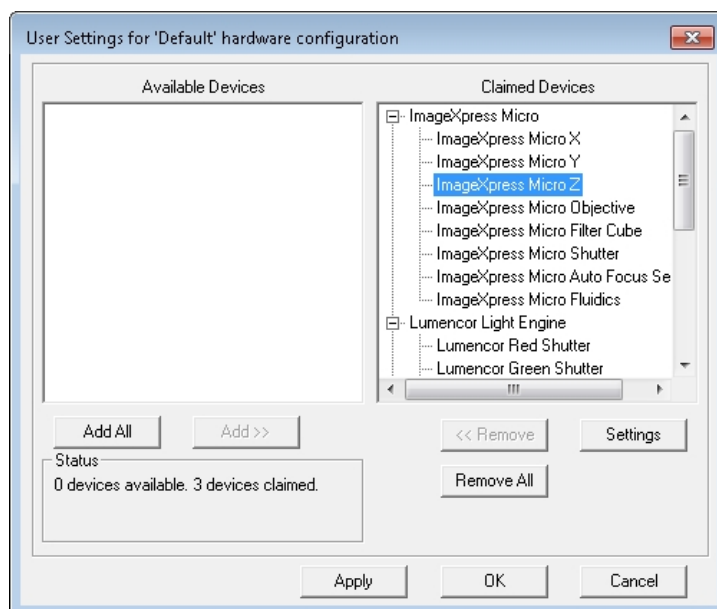


Figure 3-7: The User Settings dialog with ImageXpress Micro Z selection

17. In the **ImageXpress Micro Z Settings** dialog, verify that the value in the **Device Units** field is 50.

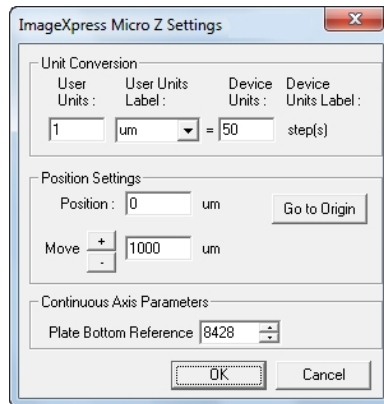


Figure 3-8: The ImageXpress Micro Z Settings dialog.

18. In the **Move** field, increase the step size to 1000 μm .



Note: Do not change the **Plate Bottom Reference** value.

19. Click the + and – buttons and confirm that the Z motor responds to the control.
20. Click **Go to Origin**.
21. In the **Move** field, change the step size back to 10 μm and click **OK**.
22. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.

23. In the **ImageXpress Objective Settings** dialog, confirm that the **Objective Labels** and the values in the **Num Aperture** fields match each objective on your system.

The Numerical Aperture (NA) values are written on each objective.

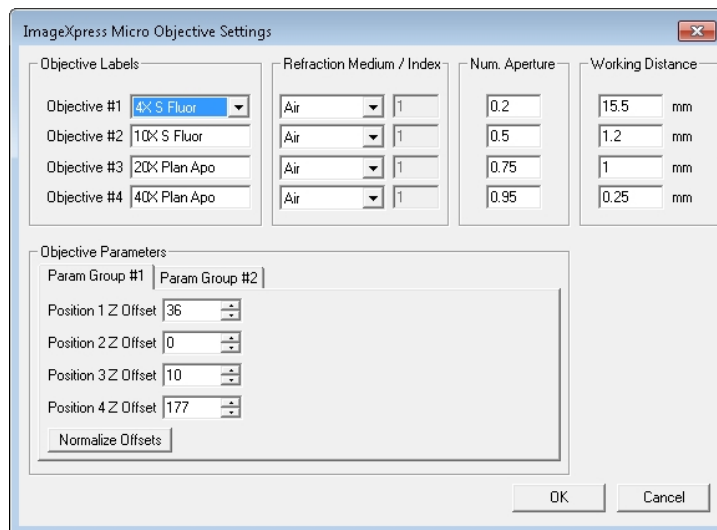


Figure 3-9: ImageXpress Micro Objective Settings dialog

24. In the **Objective Parameters** section on the bottom half of the dialog, click the **Param Group #1** tab.

This tab contains the Z offset positions in microns (μm) for the objectives.

25. Confirm that the values are valid numbers and at least one is set to zero.



Note: If you need to determine the offset values, see [Configuring Parfocality after Changing Objectives on page 183](#).

26. Click the **Param Group #2** tab.
27. Click **Open Control Dialog**.

28. In the **Control - ImageXpress Micro Objective** dialog, click the arrow buttons and confirm that the objective changer is moving appropriately.

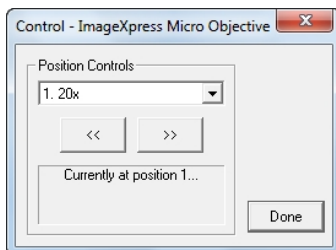


Figure 3-10: The Control - ImageXpress Micro Objective dialog.

29. Click **Done**.
30. In the **ImageXpress Micro Objective Settings** dialog, click **OK**.



Note: If you have a plate handling robot attached to the ImageXpress Micro Confocal System, confirm those settings as well. For information, see [Verifying External Control Settings on page 231](#).

31. Click **OK** to close the **User Settings** dialog.

Verifying Camera Settings in the Meta Imaging Series Administrator Software

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

1. With the ImageXpress Micro Confocal System powered on, make sure that the Meta Imaging Series Administrator Software is open to the **Configure Hardware** dialog.
2. In the **Configure Hardware** dialog, click **Configure Acquisition**.
3. In the **Configure Acquisition** dialog, ensure that a driver is listed in the **Installed Drivers** column.

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. Click **OK** to close the camera driver dialog.

8. Click **OK** to close the **Configure Acquisition** dialog.
9. Click **OK** to close the **Configure Hardware** dialog.
10. Click **OK** to close the **Meta Imaging Series Administrator**.
11. Verify and back up settings in the MetaXpress Software. See [Verifying and Backing Up Settings in the MetaXpress Software on page 44](#).

Verifying and Backing Up Settings in the MetaXpress Software

After confirming hardware settings in the Meta Imaging Series Administrator Software, check the settings in the MetaXpress Software as described in the following procedures:

- [Verifying Magnification Settings on page 45](#)
- [Verifying Illumination Settings on page 45](#)
- [Verifying Calibration Settings on page 47](#)
- [Verifying the Laser Autofocus Sensor on page 48](#)
- [Verifying the Plate Reference Point \(A1 Center\) on page 50](#)
- [Verifying Plate Types on page 53](#)
- [Confirming Laser Autofocus Settings for Plate Files on page 55](#)
- [Verifying Shading Correction Files-Legacy on page 56](#)

During the verification process, Molecular Devices recommends that you backup these settings as described in the procedures. This lets you restore the settings in case they are lost.



Tip: While using the MetaXpress Software, you can view the software help to get more information about an active dialog by pressing **F1** on your keyboard.

Verifying Magnification Settings

You need to confirm magnification settings for the ImageXpress Micro Confocal System objectives before using your system. Complete the following procedure to verify the magnification settings in the MetaXpress Software:

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

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

3. In the **Configure Magnification** dialog, in the **Setting** section, verify that there is an **ImageXpress Micro Objective** setting and that the check box is selected.

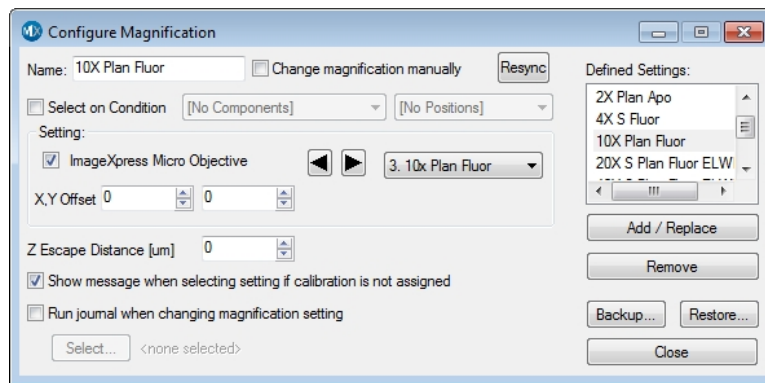


Figure 3-11: The Configure Magnification dialog

4. Confirm that the **Defined Settings** field contains a setting for each objective on your system.
5. Click **Backup**.
6. In the **Backup All Magnification Settings** dialog, select a name and location for the backup and then click **Save**.

Settings can be restored by clicking **Restore** and choosing the saved file.

7. Click **Close**.

Verifying Illumination Settings

You need to confirm illumination settings for the ImageXpress Micro Confocal System objectives before using your system. Complete the following procedure to verify the illumination settings in the MetaXpress Software:

1. Open the MetaXpress Software and log in to the database.
2. Click **Devices > Configure Illumination**.
In the simplified menu structure, click **Control > Devices > Configure Illumination**.
3. In the **Configure Illumination** dialog, in the **Defined Settings** list, ensure that the correct illuminations are listed.

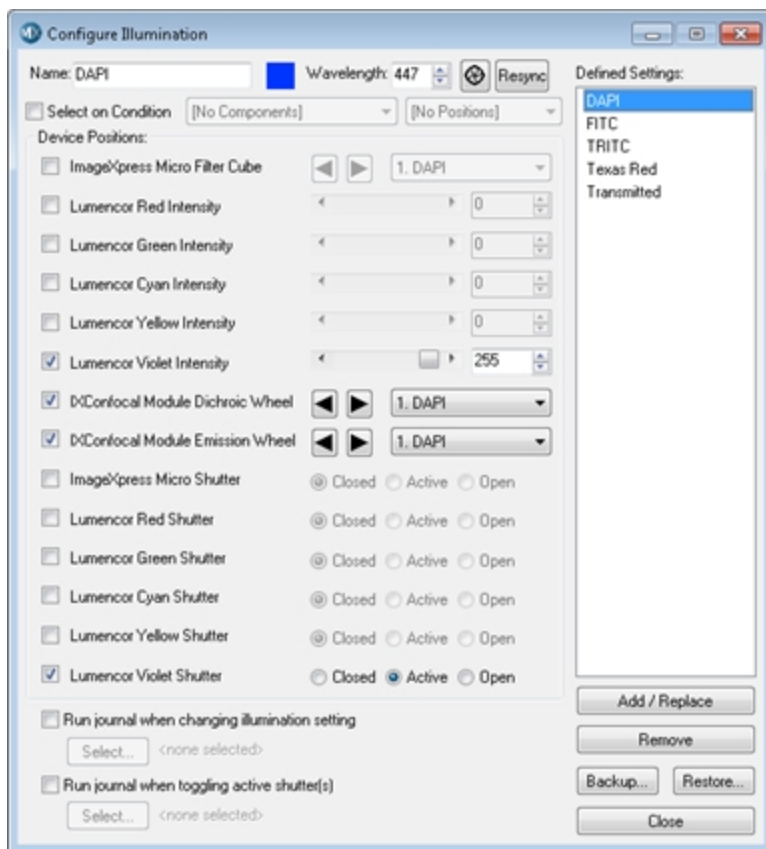


Figure 3-12: The Configure Illumination dialog

4. Ensure that the appropriate shutter is selected as **Active** for each filter set.
5. Set up other illumination settings if needed.

The value in the **Wavelength** field should match the center wavelength for the emission filter.



CAUTION! Never set more than one Lumencor Shutter to **Active**.

6. Click **Backup**.

- 7. In the **Backup All Illumination Settings** dialog, select a name and location for the backup and then click **Save**.
Settings can be restored by clicking **Restore** and choosing the saved file.
- 8. Click **Close**.

Verifying Calibration Settings

Complete the following procedure to verify and back up the calibration settings in the MetaXpress Software:

- 1. Open the MetaXpress Software and log in to the database.
- 2. Click **Measure > Calibrate Distances**.
In the simplified menu structure, click **Measure > Distances > Calibrate Distances**.
- 3. In the **Calibrate Distances** dialog, confirm that there are calibration settings in the dialog that match the objective settings from the **Configure Magnification** dialog.

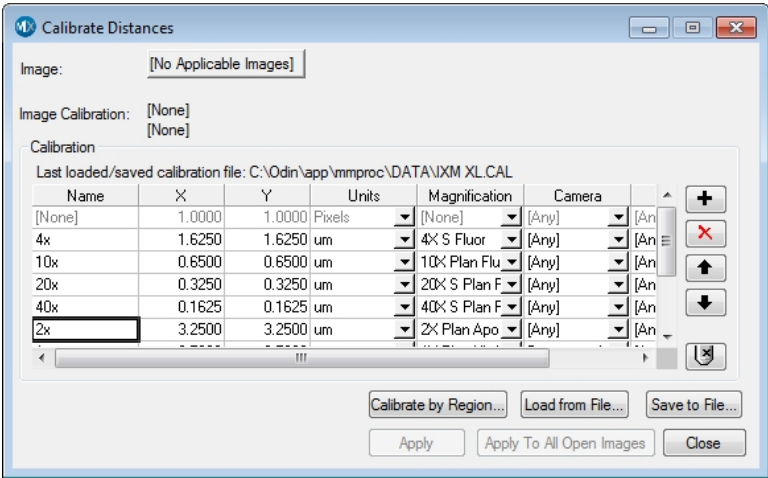


Figure 3-13: The Calibrate Distances dialog

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



Note: Use the same calibration setting for both X and Y.

The following estimated values can be used for ImageXpress Micro Confocal System calibration settings:

Table 3-2: Estimated Calibration Settings

Objective Magnification	Estimated Calibration
1x	6.83 $\mu\text{m}/\text{pixel}$
2x	3.42 $\mu\text{m}/\text{pixel}$
4x	1.70 $\mu\text{m}/\text{pixel}$
10x	0.68 $\mu\text{m}/\text{pixel}$
20x	0.34 $\mu\text{m}/\text{pixel}$
40x	0.17 $\mu\text{m}/\text{pixel}$
60x	0.11 $\mu\text{m}/\text{pixel}$
100x	0.07 $\mu\text{m}/\text{pixel}$



Note: For more information on creating calibration settings, with the **Calibration Distances** dialog open, press **F1** to view the MetaXpress Software application help.

To measure pixel sizes more accurately, in the **IXM Taskbar**, click **System Maintenance** and then click **Measure Pixel Sizes**.

If **IXM Taskbar** is not installed, contact Technical Support.

5. Click **Save to File**.
6. In the **Save Spatial Calibrations** dialog, select a name and location for the backup and then click **Save**.
7. In the **Calibrate Distances** dialog, click **Close**.

Verifying the Laser Autofocus Sensor

This procedure uses a bead plate to test that the laser autofocus (LAF) sensor is enabled and functional.

To verify that the Laser Autofocus sensor is responding in the MetaXpress Software:

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.
In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Acquisition** tab.
5. Select the **Enable laser-based focusing** check box.
6. Click the **Objective and Camera** tab.
7. From the **Magnification** list, select 10x.
8. Click the **Plate** tab.
9. From the **Plate name** list, select **Bead Plate invitrogen**.
10. Click **Eject Plate**.
11. Load the Tetra Speck bead plate and then click **Load Plate**.
12. On the image of the plate, right-click well A1 to move the stage to that well.
13. In the **Configure** tab, click the **Autofocus** tab.
14. In the **Autofocus** tab, click **Configure Laser Settings**.
15. In the **Configure Laser Autofocus Settings** dialog, click **Preview Pass**.

The **Preview Pass** window is displayed with a graph of focus intensities compared to the Z-position. You want to see graphs of sharp peaks in red and green.

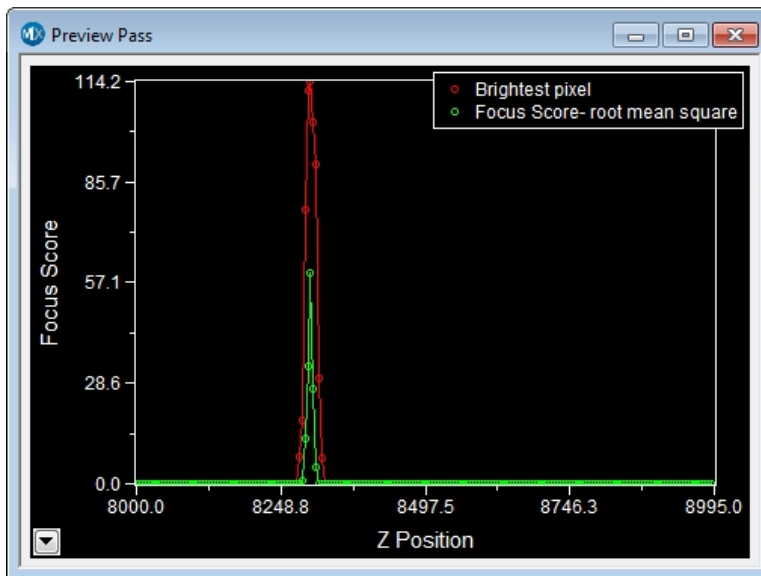


Figure 3-14: The Preview Pass window

16. In the **Preview Pass** window, ensure that there is at least one peak.
 - If the **Preview Pass** window shows at least one peak, the Laser Auto Focus Sensor is enabled and functional.
 - If the **Preview Pass** window does not show any peaks, ensure that the plate is properly seated. Then, in the **Configure Laser Autofocus Settings** dialog in the **Preview Pass** section, select **Exposure > Override exposure**, increase the value, and click **Preview Pass** again.
 - If the **Preview Pass** window still does not show a peak, click **Laser Autofocus Wizard**. Then, in the **Plate Acquire LAF Setup Wizard**, follow the on-screen instructions. For more information while using the **Laser Autofocus Wizard**, you can view the software help by pressing **F1** on your keyboard.
 - If the **Preview Pass** window still does not show a peak, contact Molecular Devices Technical Support and report the issue. See [Obtaining Support on page 203](#).

For more information on the **Preview Pass** window, see [Confirming Laser Autofocus Settings for Plate Files on page 55](#).
17. Close the **Preview Pass** window.
18. In the **Configure Laser Autofocus Settings** dialog, click **Close**.
19. In the **Plate Acquisition Setup** dialog, click **Eject Plate**.
20. Remove the Tetra Speck bead plate and then click **Load Plate**.
21. Click **Close**.

Verifying the Plate Reference Point (A1 Center)



Note: To complete this procedure, you need the metal slide holder plate that ships with the ImageXpress Micro Confocal System.

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.
In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Objective and Camera** tab.
5. From the **Magnification** list, select the lowest power objective. 10x is the highest that should be used.
6. In the **Camera Binning** field, type or select **1**.
7. Click the **Plate** tab.

8. From the **Plate name** list, select **96 Wells (8x12)**.
9. Click the **Sites to Visit** tab.
10. Under **Site Options**, select **Single site**.
11. Under the **Acquisition > Wavelengths** tab, click the **W1** tab.
12. From the **Illumination setting** list tab, select **FITC**.



Note: Filters other than FITC are acceptable to use, but the contrast might not be as high as with the FITC. Exposure times vary significantly depending on your light source and filter choice.

13. In the **Exposure** field, type or select **100** milliseconds (ms).
14. Click the **Screening > Plate Acquisition and Control**.
15. In the **Plate Acquisition and Control** dialog, from the **Wavelength** list, select **W1 - FITC**.

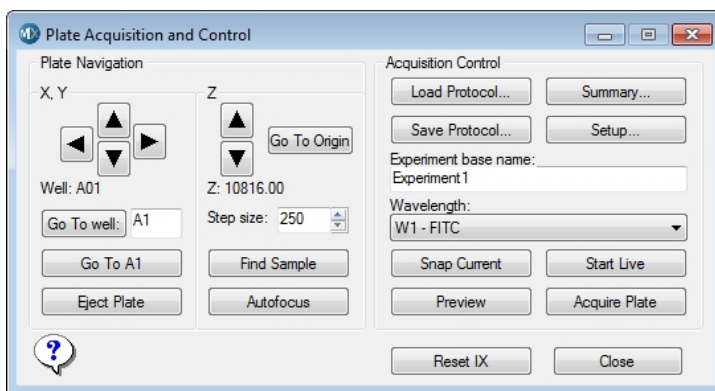


Figure 3-15: The Plate Acquisition and Control dialog

16. In the **Step Size** field, type **250**.
17. Click **Eject Plate**.
18. Load the metal slide holder plate and calibration slide, and ensure the notch in the plate is in the A1 position on the stage.
19. Click **Load Plate**.
20. Click **Go To A1** to move the stage to the A1 position.
21. Click **Start Live** to open a live image window.

22. If you are using a 4x objective, click 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 then verify that the hole is visually centered in the field of view.

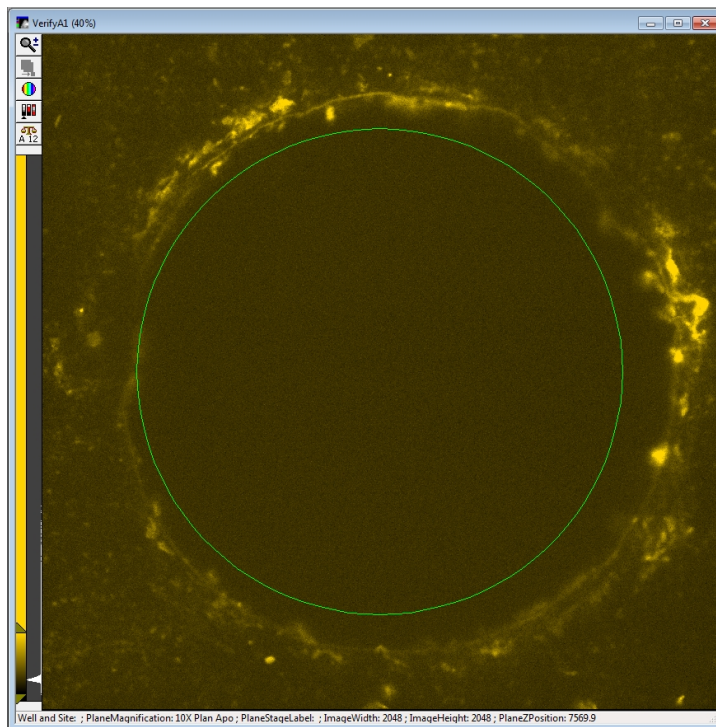


Figure 3-16: The A1 Pinhole in focus at 10X

If the A1 pinhole is not centered, or if you cannot find the hole, contact Molecular Devices Technical Support. See [Obtaining Support on page 203](#).

23. If your lowest magnification objective is greater than 10x, do the following:
 - a. Move the stage up until you are close to focus and then left until you see the edge of the pinhole.
 - b. Align the left side of the hole with the left side of the image window and record the stage X position.
 - c. Move the stage to the right until you see the edge of the pinhole.
 - d. Align the right side of the hole with the right side of the image window and record the stage X position.
 - e. Calculate the horizontal center of the reference point.
 - f. Move the stage left and then up until you see the top edge of the pinhole.
 - g. Align the top of the hole with the top of the image window and record the stage Y position.
 - h. Move the stage to down until you see the edge of the pinhole.
 - i. Align the bottom of the hole with the bottom of the image window and record the stage Y position.
 - j. Calculate the vertical center of the reference point.
 - k. Compare the calculated stage position with the position of the stage when you click **Go To A1**.
24. Click **F2: Stop** to stop the live image.
25. Click **Eject Plate**.
26. Remove the metal slide holder plate and then click **Load Plate**.
27. Click **Close**.
28. In the **Plate Acquisition Setup** dialog, click **Close**.

Verifying Plate Types

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.
In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Plates** tab.

- Click the **Plate name** drop-down list to view the available plate types.

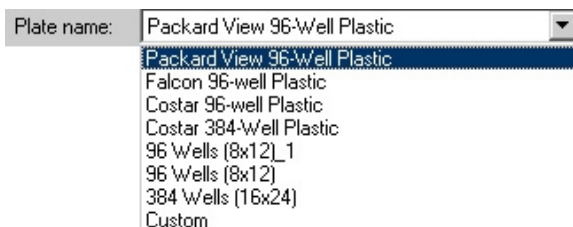


Figure 3-17: Available plate types

- If there are several custom plate types available in the list, then you are finished with this procedure.
 - If only **96 Wells (8x12)**, **384 Wells (16x24)**, and **Custom** are listed, then continue with this procedure to load the preconfigured plate type files.
- Insert the MetaXpress Software Suite installation USB flash drive into the computer.
 - When the MetaXpress Software installation window appears, click **Explore Installation Folders/Files**.
 - In Windows Explorer, open the **Plates** folder to view the preconfigured plate type files (.plt).
 - Select the plate type files that you need to be available in the MetaXpress Software.
 - To select adjacent files, click the first file and then hold down the **SHIFT** key and click the last file.
 - To select non-adjacent files, hold down the **CTRL** key and click the files.
 - Place a copy of the selected files in the **Plates** folder in your MetaXpress installation folder.

The default installation path is **C:\MX6\Plates**.

These files will then appear in the **Plate name** drop-down list.



Note: The plate files are 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.

- In the **Plates** folder in your MetaXpress installation folder, select all the copied files.
- Right click the selected files and click **Properties**.
- In the **Properties** dialog, in the **General** tab, clear the **Read-only** check box.
- Click **OK**.

Confirming Laser Autofocus 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 optimized for the plate.

To confirm the laser autofocus settings:

1. 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.
2. Open the MetaXpress Software and log in to the database.
3. Click **Screening > Plate Acquisition Setup**.
In the simplified menu structure, click **Screening > Acquisition Setup**.
4. In the **Plate Acquisition Setup** dialog, click **Eject Plate**.
5. Place the test plate in position and then click **Load Plate**.
6. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
7. In the **Configure** tab, click the **Plates** tab.
8. From the **Plate name** drop-down list, select the plate type you are testing.
9. In the **Objective and Camera** tab, select a magnification.
10. In the **Acquisition** tab, verify that **Enable laser-based focusing** is selected.
11. Using the navigation tools at the top of the **Plate Acquisition Setup** dialog, move to an appropriate well and site.
12. In the **Autofocus** tab, click **Configure Laser Settings**.



Note: If the **Configure Laser Settings** button is highlighted in red, the autofocus was not configured for this plate and objective configuration. To correct this, run the **Laser Autofocus Wizard**.

13. Click **Find Sample**, and verify that the focus status reports **Focus Found** and that the resulting focus position is appropriate for the plate.



Note: If the focus status reports **Focus Not Found**, or if the **Fine z-position** result is wrong, optimize the laser autofocus settings using the **Laser Autofocus Wizard**.

14. When finished, close the focus status report dialog and the **Configure Laser Autofocus Settings** dialog.



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.



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 178](#).

Verifying Shading Correction Files-Legacy



Note: This procedure is required if **Legacy Correction** is selected for one or more wavelengths in an acquisition protocol.

If **Legacy Correction** is selected as the **Shading Correction** method for an acquisition wavelength, then shading correction image files are needed for each **Magnification** and **Illumination setting**, and these images must be generated whenever an objective or filter set is replaced or added to the system, or whenever the light guide is replaced.



Note: For information about creating **Legacy Correction** shading correction image files, see [Updating Shading Correction Settings-Legacy on page 198](#).

To verify that the shading correction images are available for plate acquisitions using the **Legacy Correction** setting, do the following:

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.
In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Acquisition** tab.
5. During your initial on-site system installation, the shading correction image files are configured to be found in the **C:\Shading Images** folder.
To change the default image location, click **Directory for Stored Correction Images**, and then in the **Browse for Folder** dialog, select the new location.

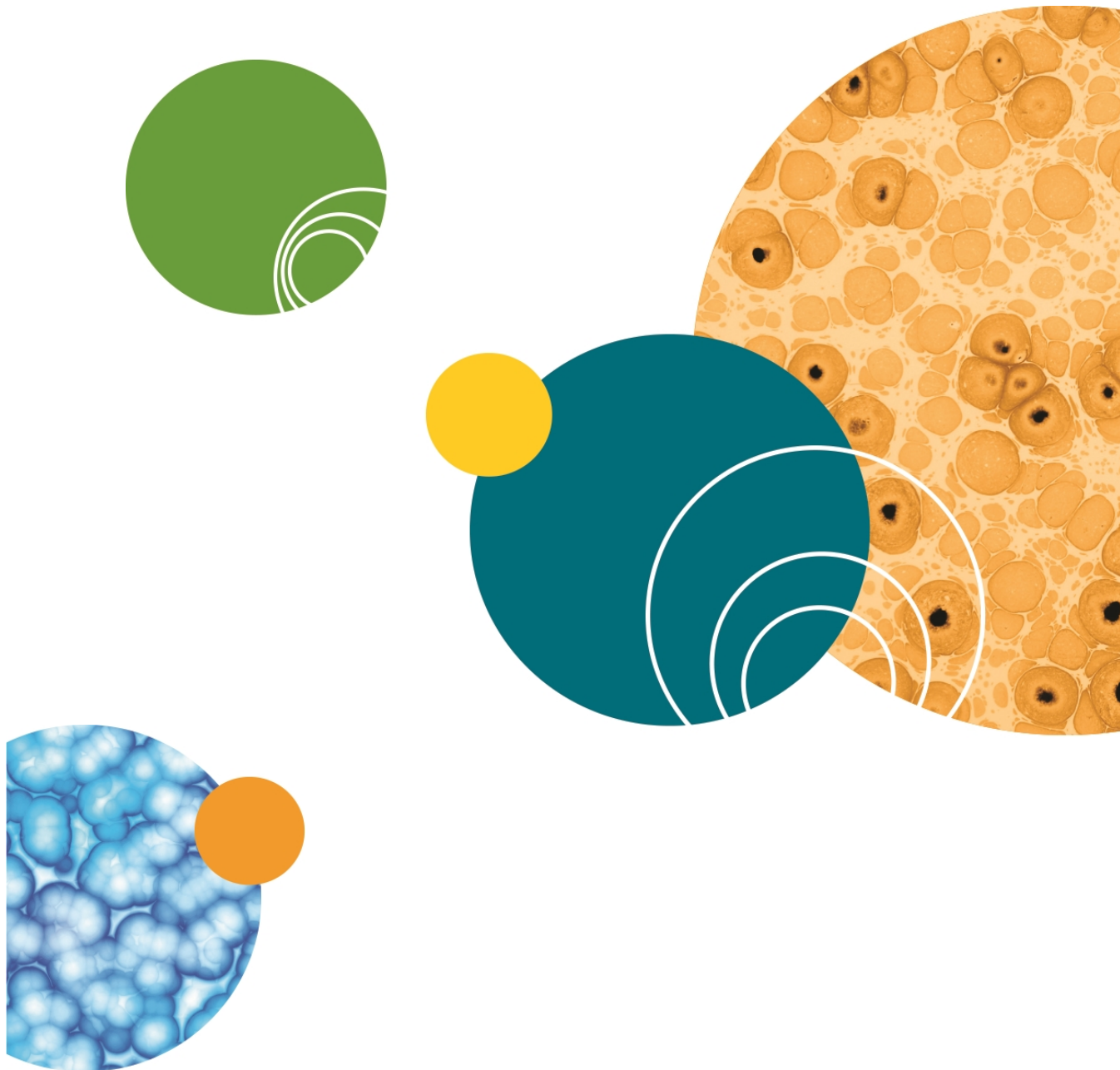
To ensure that the file names are formatted correctly:

1. Use Windows Explorer to navigate to the **C:\Shading Images** folder, or to another location if you have changed the default.

2. Locate the shading correction files.
3. Ensure that a file exists for each **Magnification** and **Illumination setting**.

Shading correction images use the following naming format:

- **For Widefield:** shading_<magnification name>_widefield_<illumination setting>.tif
For example: shading_4x Plan Apo_widefield_DAPI.tif
- **For Confocal:** shading_<magnification name>_confocal<disk size>um<slit or pinhole>_<illumination setting>.tif
For example: shading_20x_confocal 60um pinhole_FITC.tif



Chapter 4: Preparing For Acquisition

4

This section provides general 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 experiment settings.

The following are some criteria to consider to get the highest possible fluorescence image quality:

- [Assay Design on page 59](#)
- [Plate Selection on page 61](#)
- [Sample Preparation on page 63](#)
- [Plate Acquisition Settings on page 64](#)
- [Instrument Maintenance on page 68](#)

Assay Design

Evaluating your Experiment Requirements

When designing a high-content screening assay, it is important to consider the downstream image analysis steps. Despite the image enhancement tools and options available to you in the MetaXpress Software, it is difficult to analyze a poor quality image. Starting with quality images helps ensure that your image data is more meaningful, and yields more information.

As with any biological assays, the assay conditions need to be correctly evaluated to obtain a meaningful result. Include both negative and positive controls in your sample preparation so you can judge the validity of your assay. Run a small-scale version of the assay for optimization of the assay conditions before running a large-scale screen.

Selection of Different Fluorochromes

Typical high-content assays include one or more fluorochromes, including fluorescent proteins, antibody-based stains, and/or chemical-based stains. In general, Molecular Devices recommends including a nuclear stain such as Hoechst or DAPI to help with identification of the cells during image analysis. If the assay involves movement of a protein of interest to or from a particular cellular compartment or organelle, it may also be helpful to include a probe specific to that cellular compartment or organelle. If you are planning on using a standard MetaXpress Software module to analyze your data, review the requirements of that module.

Individual fluorochromes have unique characteristics that help determine their best use. Use probes that provide bright, specific staining and that have excitation and emission spectra suitable for the filter sets in your ImageXpress Micro Confocal System. For experiments using multiple stains, select fluorophores that have sufficient spectral separation. Some fluorochromes provide brighter intensities and require a 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.

If your ImageXpress Micro Confocal System has the Transmitted Light option, then it may be possible to identify cells using Transmitted Light images instead of fluorescence.

Cell-Based Assays

The most important consideration when selecting cells for a high-content assay is whether they are compatible with the biology being studied. The assay should give a robust response with clear distinction between positive and negative phenotypes. In addition, it is important to select a source of cells where it is possible to obtain consistent results from batch to batch, whether they are primary cells or cell lines, and whether they are transfected or not. Compared to widefield imaging, confocal imaging provides higher-quality images of thick samples, 3D cell masses, or tissues. However, uniformly staining thick samples for confocal imaging can be difficult.

Organism-Based Assays

The ImageXpress Micro Confocal System can be used for imaging whole organisms, such as nematodes or zebrafish. For best results, immobilize the organisms by treating with anesthetic and/or using a gel preparation.

Homogeneous Assays

Confocal imaging reduces background fluorescence from out of focus sample volume, thereby improving signal-to-background ratios, and allowing for homogeneous assays that would not work when widefield imaging.

Plates or Slides

Molecular Devices recommends the use of multi-well plates for high-content screening. The well layout is consistent from one plate to another, plates are easier to handle during sample preparation and imaging, and it is easier to scale up for a larger screen. However, some assays, such as imaging of tissue sections, require the use of slides. In this case, a slide holder is provided in the accessory kit, and there are software tools available to streamline a typical slide imaging workflow.

Plate Selection

The specific type of plate used can have a significant impact on image quality. Therefore, it is recommended to assess various plates for their compatibility with your assay. Molecular Devices recommends that for any experiment, you use plates of only one brand from a single manufacturer. Mixing various plate types from different manufacturers could introduce unknown variables and contribute to creating flawed data.

The following are factors to consider when selecting plates:

- Availability and cost
- [Plate Format on page 61](#)
- [Plate Material on page 62](#)
- [Fluorescence Background on page 62](#)
- [Bottom Thickness on page 62](#)
- [Plate Flatness or Reproducibility of the Z-Pattern on page 62](#)
- [Plate Skirt on page 63](#)
- [Batch-to-Batch Consistency on page 63](#)
- [Robot Compatibility on page 63](#)

Plate Format

- How many wells are in each plate?
- Is the well size compatible with the assay, and will the plates allow for the desired throughput?
- Do you have the equipment needed for pipetting into and washing the plates?

Plate Material

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 and a single-piece clear bottom usually work the best. Plastic bottom plates are usually more uneven and distort light more than glass bottom plates. When using high magnifications, there are significant differences in clarity between standard plastic plates, optically clear plastic plates, and glass bottom plates.

Verify that your cells are compatible with the plate material. There are some cells that adhere to and perform better on plastic. Given the wrong surface, some cells fail to bind and behave unusually, such as rounding up, or migrating to the edges of the well. In some cases, coating the plates or using pre-coated plates can be beneficial.

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.

Bottom Thickness

The thickness of the plate bottom should be compared with the working distance of the objective lens to be used to ensure that it is compatible. In general, objectives with higher numerical aperture (NA) tend to require thin-bottom plates. The extra-long working distance (ELWD) objectives are compatible with a larger range of plate thicknesses but tend to have lower NA. Plates with a bottom thickness comparable to a standard coverslip (0.17 mm) work well with most of the objectives.



Note: Plates with ultra-thin bottoms can be more uneven, possibly causing focusing issues.

Plate Flatness or Reproducibility of the Z-Pattern

A flat plate is faster to scan than an uneven plate because the autofocus 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.

Plate Skirt

Some manufacturers offer low-skirt or no-skirt options, allowing you to use all of the wells of the plate. With short working distance objectives, imaging the edge and corner wells can cause the objective to bump into the skirt of the plate, which requires the outer wells be omitted from the experiment.

Batch-to-Batch Consistency

Some plate manufacturers may be more consistent in producing plates than others. If parameters such as the plate bottom thickness vary from batch to batch, the plate settings must be optimized for each batch.

Robot Compatibility

If you will be using a plate-loading robot, ensure that the plates can easily be held by the robot grippers. Some types of plates do not work well with the grippers supplied with the robot and will require custom grippers to work correctly. If one or more plate types do not work with your robot grippers, contact Molecular Devices for assistance. Also make sure that there is a consistent location to affix a barcode on the side of the plate.

Sample Preparation

There are many variables involved in sample preparation. It is best to test these variables as appropriate during the assay optimization phase, before preparing a large number of plates for screening.

The following are some specific items to consider for imaging assays:

- [Cell Density on page 63](#)
- [Fixation and Staining Conditions on page 64](#)
- [Final Buffer or Media on page 64](#)
- [Plate Handling and Storage on page 64](#)

Cell Density

Cell density can affect the performance of the cells as well as downstream image analysis. If the cells are very sparse, you may need to acquire many sites in order to have a sufficient population for statistical analyses. If the cells are very dense, it may be difficult to identify individual cells accurately during cell segmentation.

Fixation and Staining Conditions

Fixation, permeabilization, and washing steps that are too harsh or aggressive can damage the cells and affect image quality. Typically, fixation in freshly-prepared, pre-heated, 4% methanol-free formaldehyde works well for many cell types. When optimizing the assay, it is also helpful to test a range of antibody and stain concentrations to determine the best conditions for your cells.

Final Buffer or Media

To reduce background in the fluorescent images, make sure that the buffer or media that the cells are left in is free of fluorescent components such as Phenol Red. This is most important for widefield assays. Solutions with a high percentage of glycerol, such as mounting media, are not recommended. Glycerol can interfere with the laser autofocus, and the high viscosity can cause pipetting difficulties, resulting in air bubbles. Finally, a low volume of liquid can also interfere with the laser autofocus and with transmitted light images. In general, make sure that wells are at least halfway full. Avoid letting cells dry out while the plate sits for extended time before imaging.

Plate Handling and Storage

Since the laser measures the reflection from the bottom of the plate or from within the sample, dust particles, dirt, fingerprints, and scratches will interfere with the reflection and affect the performance of the autofocus. To improve the autofocus, clean the bottom of the plate using lens tissue and an optical cleaning solution. Plates should be stored in the dark, and typically, fixed plates should be stored at 4°C. An opaque plate seal may be helpful. Avoid condensation of air humidity on the bottom of plates. Before imaging, allow chill-stored plates to be brought back to room temperature.

Plate Acquisition Settings

Nearly all settings for plate acquisition are made in the MetaXpress Software 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 your settings, use the following topics as a checklist.

- [Magnification on page 65](#)
- [Correction Collars on page 65](#)
- [Binning on page 65](#)
- [Widefield vs. Confocal Imaging Mode on page 66](#)

- [Site Selection on page 66](#)
- [Shading Correction on page 66](#)
- [Autofocus on page 67](#)
- [Illumination Settings on page 67](#)
- [Exposure Time on page 67](#)
- [Z Series Acquisition on page 68](#)

Magnification

The magnification setting depends on the type of information you want to get. Typically, higher magnification gives higher resolution of the objects of interest, but it produces a smaller field of view, which requires more sites to give the equivalent number of cells. Typically, for counting cells, a 4x objective is suitable. For translocation, a 20x or 40x objective can be appropriate. Counting or localizing small organelles might require objectives above 40x. Other considerations include the numerical aperture (NA) of the objective, the working distance, and the optical corrections built into the objective. With the magnification constant, brightness is proportional to the square of NA. Higher NA objectives also produce a sharper picture when used with plate bottom thickness matched to the objective correction thickness, typically 170 μm . Unfortunately, higher NA objectives, such as a 40X Plan Apo, fail to reach the outer rows and columns of some multi-well plates because of the plate skirt height.

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 178](#).

Binning

Another method of increasing the signal-to-noise (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 signal intensity. Another positive feature of binning is that it produces smaller images that require less storage space and are analyzed more quickly. Some assays benefit greatly from binning, while others will require unbinned images.

Widefield vs. Confocal Imaging Mode

Widefield imaging includes fluorescence from parts of the sample that are above or below the focal plane. Confocal imaging excludes fluorescence from parts of the sample that are out of focus. The depth of focus depends on the optics, particularly the objective lens, and the degree of confocality will depend on the size and shape of the hole that is used. Small pinholes achieve higher confocality and better sectioning capability than widefield, at the expense of reduced light intensities.

Therefore, if it is important to measure the total intensity of a particular fluorophore in cells, it may be preferable to use widefield imaging. Widefield imaging also tends to perform better with cells that are flat. If it is important to measure colocalization between two markers, particularly in a thick sample, then confocal imaging is recommended. In some cases, collecting a Z-series of multiple focal planes may also be needed to obtain suitable images of thick samples.

Site Selection

Using multiple sites in a single well enables you to acquire images from a greater area of the well. If you select the **Single Site** 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 **Multi-well** option enables the MetaXpress Software to acquire separate images within a given well, and those images can be of contiguous or distributed areas.

Using the stitch command, you can assemble the smaller separate images into a single large image. This capability enables you to 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. For example, the center of the well may exhibit a pipetting artifact from an automated pipettor, or the cells may clump more at the edges of the wells.

Shading Correction

All microscopes exhibit some degree of illumination variation, or shading, across the field of view. Shading is an artifact that can come from the objective, optics, light source, or background light from the room. Your ImageXpress Micro Confocal System is designed to minimize shading effects. If needed, use the available shading correction within the software. This is especially recommended for assays where comparison of individual cell intensities is critical, such as a cell cycle assay.

Autofocus

Your ImageXpress Micro Confocal System includes an advanced laser autofocus system. Laser autofocus is fast, reliable, does not photobleach the sample, and is not dependent on the quality of the staining. Most experiments only require laser autofocus with a defined Z-offset for each wavelength. Some assays, such as those using whole organisms or suspension cells, might also require some image-based autofocus in addition to the laser autofocus. Laser autofocus settings are defined for each plate type and objective. The **Laser Autofocus Wizard** provides assistance with optimizing these settings. If you see frequent focus failures, contact your system administrator or Molecular Devices representative.

Illumination Settings

Select the appropriate illumination setting for your assay. The Semrock website at www.semrock.com is a good resource to help you determine the best filters to use with your fluorophores. Your ImageXpress Micro Confocal System uses a standard, selectable light source filter to create the correct excitation wavelength, and a filter wheel to select the corresponding emission wavelength filtration for your experiment. It also uses dichroic mirrors to separate the excitation wavelength from the emission wavelength. Specific filter sets are designed to be used with specific stains. See [Table 7-1](#).

Exposure Time

An appropriate exposure time for each wavelength is crucial for your acquisition and analysis. Clicking the **Auto Expose** button on one of the **Wavelength** tabs in the **Plate Acquisition Setup** dialog provides you with a good starting point. If needed, adjust the exposure time after that so the grayscale intensity within a cell is at least three times the intensity of the background. If the background comes from out-of-plane fluorescence, consider using confocal imaging to improve the Signal-to-Background ratio. If the intensity is expected to vary with the phenotype, adjust the exposure time with both positive and negative controls so that the bright samples are not saturating, and the dim samples are still visible.

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.

Longer exposure times also increase the overall plate acquisition time.

Z Series Acquisition

What is the nature of your sample material? Is it very dense or thin? Dense sample material requires more light and might require collection of multiple focal planes in a Z Series. Depending on the analysis needs, individual planes can be saved and/or collapsed into a single 2D projection image.

Instrument Maintenance

For best performance, your ImageXpress Micro Confocal System should have regular preventive maintenance (PM) services. In between PM services, the system administrator can clean dust off the optics as needed (see [Maintenance on page 147](#)). Wear gloves when handling any optical components to avoid contaminating them with dirt or skin oils.

The basic design of the ImageXpress Micro Confocal 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.

Chapter 5: Setting Up and Acquiring Data

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 Analysis 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 Confocal System and for acquiring and analyzing microplates.

This section explains the **Plate Acquisition Setup** dialog that is used to configure and run a screening experiment.



Beginning with Version 6.0 of the MetaXpress Software, the well and site 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.

- [Accessing the Plate Acquisition Setup Dialog on page 70](#)
- [Plate Acquisition Setup Dialog Layout on page 71](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#)
- [Viewing the Summary Panel on page 137](#)
- [Saving a Plate Acquisition Protocol on page 138](#)

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 and image acquisition. The **Screening** menu provides access to all plate configuration dialogs 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.

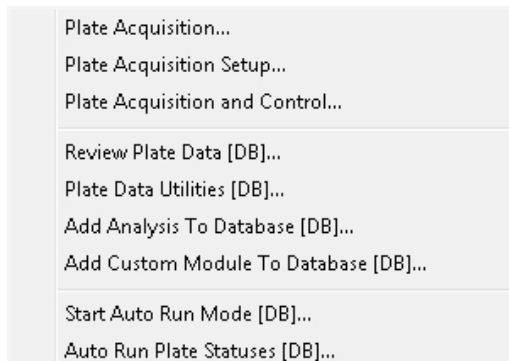


Figure 5-1: Default Screening Menu

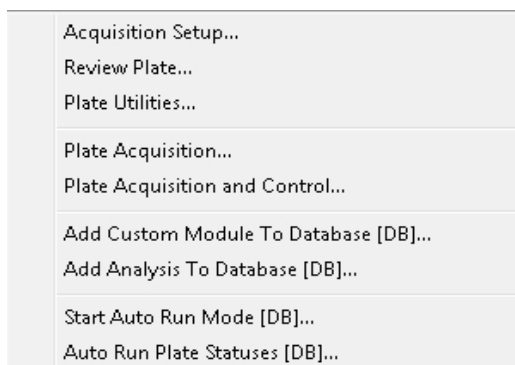


Figure 5-2: Simplified Screening Menu

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 Confocal System.
- Configuration control on the **Configure** tab for setting up plate acquisition protocols.
- **Acquisition Control** on the **Run** tab for doing a plate acquisition according to a selected protocol.

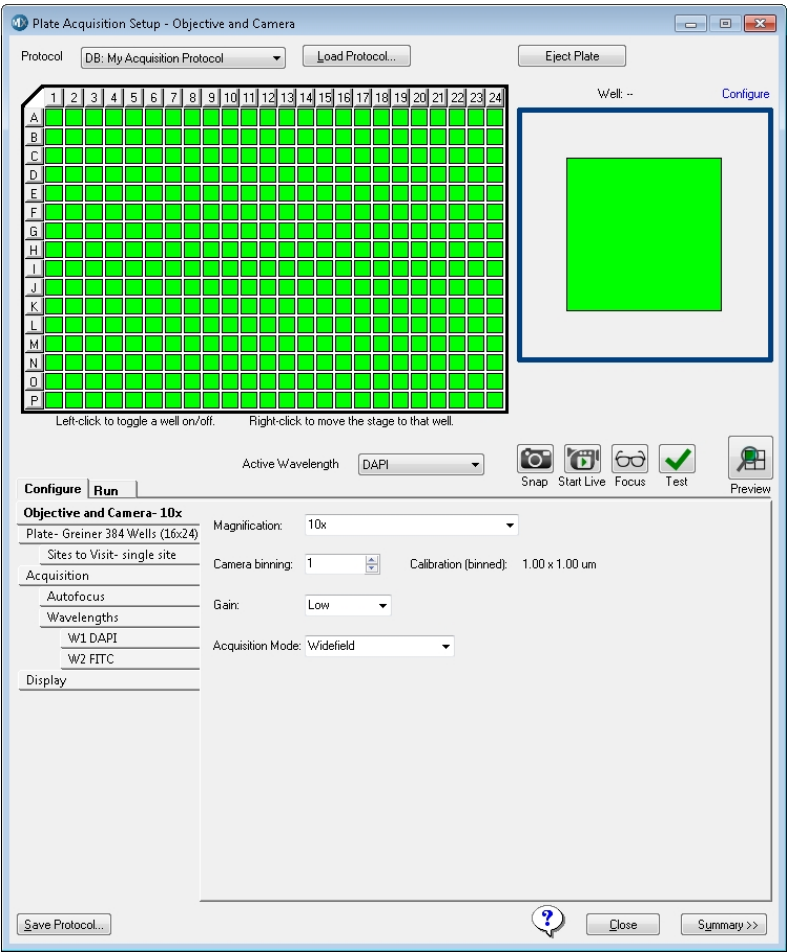


Figure 5-3: Plate Acquisition Setup Dialog

The following functionality is available in the **Plate Acquisition Setup** dialog:

- **Protocol:** provides a 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: switches between the following two options:
 - **Eject Plate:** opens the top door for loading or removing a plate.
 - **Load Plate:** closes the top door so that the plate can be acquired.
- **Plate map:** (upper left) is 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. By default, when the **Plate Acquisition Setup** dialog opens the first time, and before any protocols are configured and loaded, all the wells in the plate map are selected for data acquisition. Selected wells in the plate map are displayed in bright green. To turn off any data acquisition selection, click on the specific well in the graphic, click and drag over a section of wells, or click the row or column header. Disabled wells in the plate map are displayed in gray.

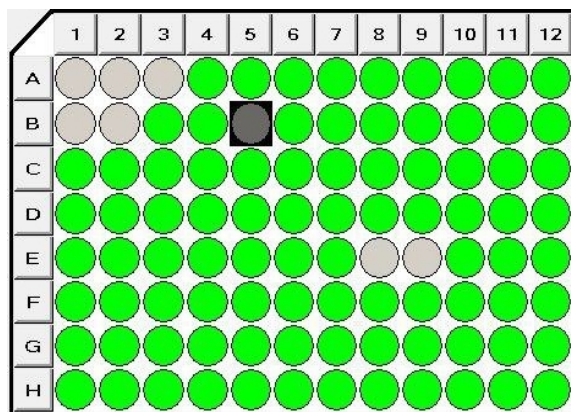


Figure 5-4: Plate Acquisition Setup Dialog, Plate map

- **Site map:** (upper right) is 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 Confocal System, including moving the focus. You use this interactive graphic to configure the sites that are to be acquired. Selected sites in the map are highlighted in bright green. Disabled sites in the map are highlighted in gray.

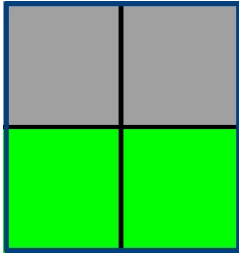


Figure 5-5: Plate Acquisition Setup dialog, Site map

- **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.

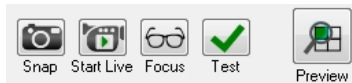


Figure 5-6: Active Wavelength tools



Note: There are a few use cases when configuring a plate acquisition protocol with the **Active Wavelength** is applicable, and these use cases are discussed where appropriate in this section. These tools, however, are primarily used when acquiring plate data.

- **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 and 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 76](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#)
- **Run tab:** provides the necessary options for acquiring a plate according to a selected protocol.



Note: This section details the **Configure > Configuration Control** functions.

- **Save Protocol:** 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 138](#).

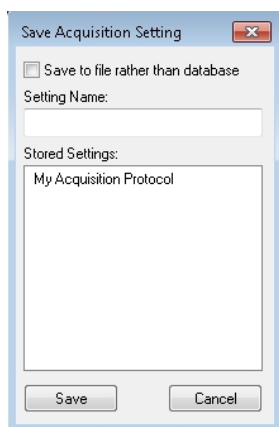


Figure 5-7: Save Acquisition Setting Dialog

- **Summary** button: opens the **Summary** panel, which displays a summary of all the current acquisition settings. See [Viewing the Summary Panel on page 137](#).



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.

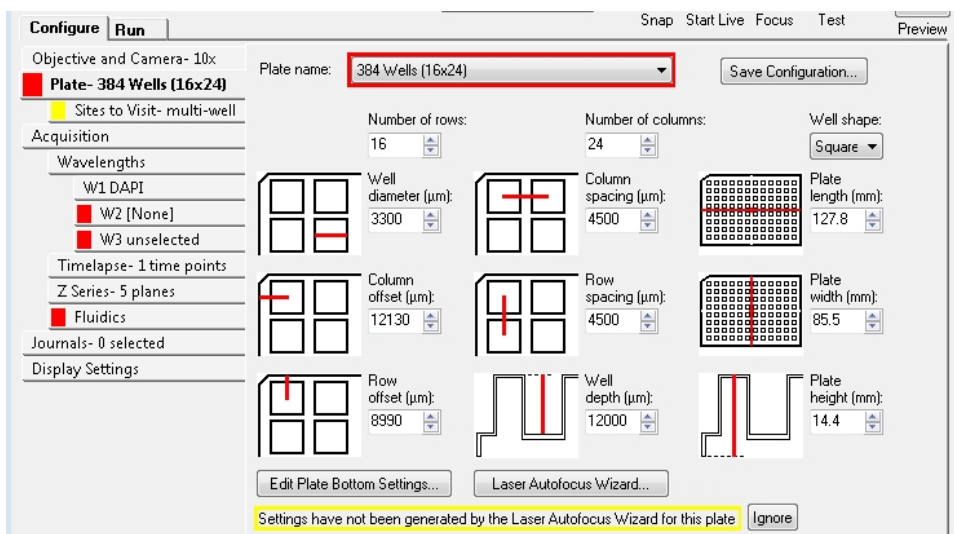


Figure 5-8: Plate Acquisition Setup Dialog with Warning Icons

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

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

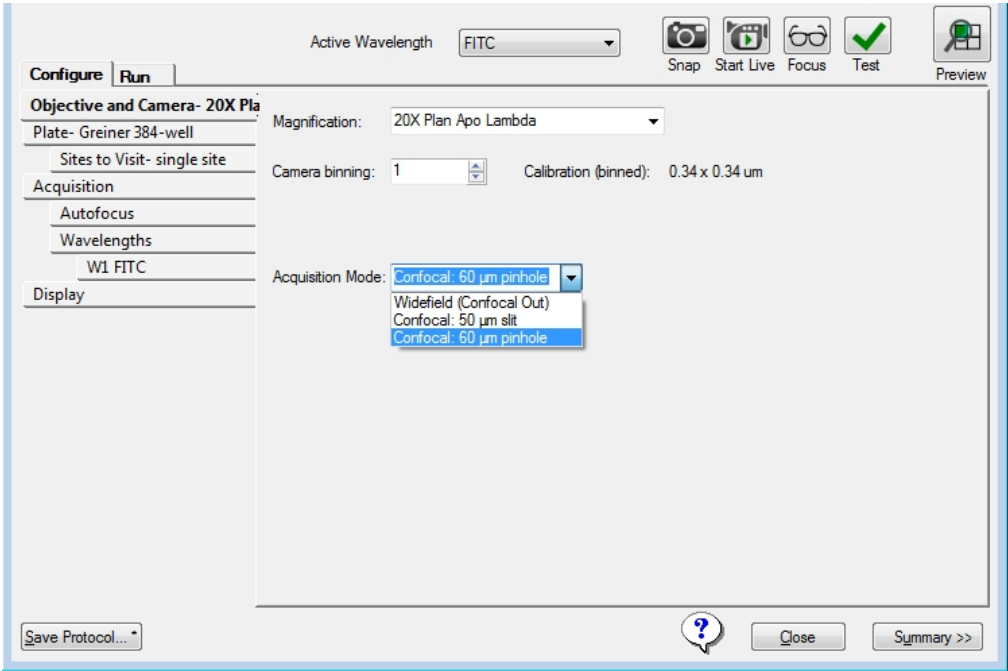


Figure 5-9: Plate Acquisition Setup dialog: Configure tab, Objective and Camera tab

Table 5-1: Plate Acquisition Setup dialog: Configure tab, Objective and Camera 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 objective is physically moved to the acquisition position. Note: The available magnifications are defined with the Configure Magnification dialog. See Verifying Magnification Settings on page 45 . You must assign a calibration to each magnification setting. See Verifying Calibration Settings on page 47 .
Camera binning	Specifies the binning value that is to be applied to the camera. Binning

Table 5-1: Plate Acquisition Setup dialog: Configure tab, Objective and Camera tab configuration options (continued)

Option	Description
	<p>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, and increases signal-to-noise ratio.</p> <p>Note: If sufficient light is available, lower camera binning increases the image resolution, but higher binning increases the signal-to-noise ratio for a given exposure time. Higher binning also improves the speed of the acquisition for a given target signal per bin.</p>
Acquisition Mode	<p>Specifies widefield or confocal imaging mode options, which are dependent on your instrument configuration.</p> <p>Note: An entire acquisition is either widefield or confocal. You cannot change the acquisition mode from timepoint to timepoint or from wavelength to wavelength.</p>

Configuring Objective and Camera Options



Tip: 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 [Viewing the Summary Panel on page 137](#).

1. Click the **Objective and Camera** tab.
2. Select the **Magnification** setting for the protocol to move the selected objective into position.
3. Specify the **Camera binning** value that is to be applied to the camera.



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

4. Specify the **Acquisition Mode** that is to be used, which is dependent on your instrument configuration.

5. If you are done configuring the acquisition protocol, then continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Plate Acquisition Setup Dialog: Configure Tab, Plate Tab

You configure the required plate dimensions on the **Plate** tab to accurately control the X, Y, and Z movements of the ImageXpress Micro Confocal System. Defining accurate plate dimensions prevents the imager from making potentially hazardous movements. Your defined plate dimensions also ensure that the laser-based autofocus is as accurate as possible.

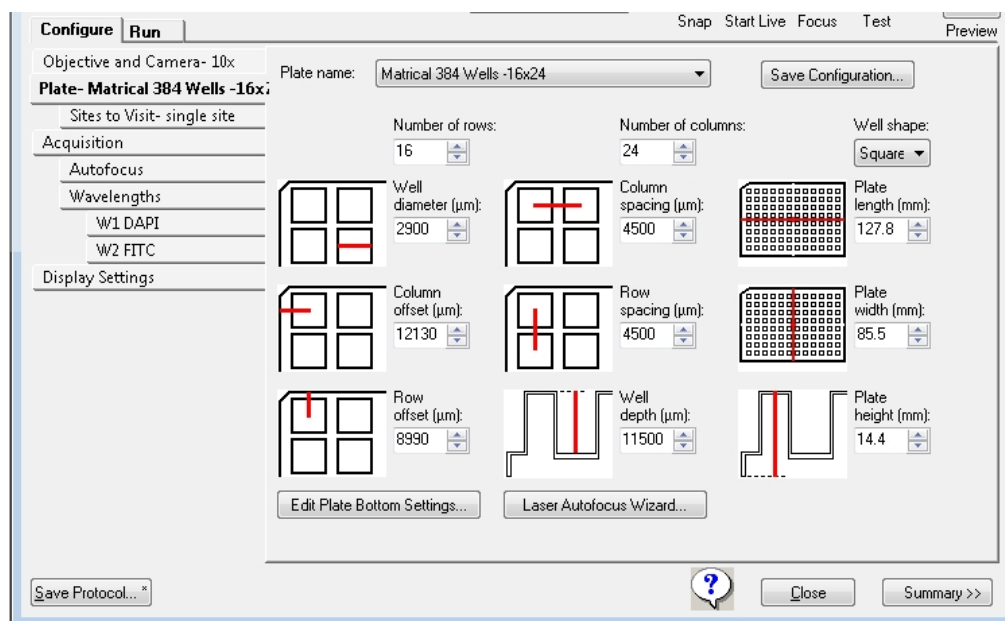


Figure 5-10: Plate Acquisition Setup dialog: Configure tab, Plate tab



Note: The MetaXpress Software installation flash drive comes with a variety of common plate types already configured. From the MetaXpress Software flash drive, find the **Plates** folder for plate files. To use these plate files in the MetaXpress Software, copy the **Plates** folder from the flash drive into the **Plates** directory of your MetaXpress Software installation. The default plates directory is C:\MX6\Plates. After copying the plate files, go to the file properties for each file and make sure that the **Read-only** check box is not selected. These plate types are then 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:\MX6\Plates, you must run the **Laser Autofocus Wizard** to define the laser settings for the plate type.

Table 5-2: Plate Settings

Option	Description
Plate name	<p>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.</p> <p>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 Considering Plate Dimensions on page 81.</p>
Save Configuration	<p>Opens the Save Configuration dialog that you use to name and save a new custom plate configuration based on the currently displayed plate type values.</p>
Number of rows	<p>Indicates the number of rows for the selected plate type.</p>
Number of columns	<p>Indicates the number of columns for the selected plate type.</p>
Well shape	<p>Indicates the shape of the well on the plate, either Circle or Square.</p>
Well diameter	<p>Specifies the diameter of the well in μm.</p> <p>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</p>

Table 5-2: Plate Settings (continued)

Option	Description
	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.
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 autofocus and fluidics events.
Plate height	Specifies the plate height in mm. Note: The correct Plate height value is required for autofocus and fluidics events.
Edit Plate Bottom Settings	Opens the Configure Plate Bottom Settings dialog that you 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 to calculate the plate bottom measurements instead of editing them here.
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.

Considering Plate Dimensions

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 the 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. For detailed information about the settings that the wizard calculates, see the “Configure Laser Autofocus Settings Dialog Options” topic in the MetaXpress Software help. With the Laser Autofocus wizard open, press **F1** to access the help. If you need more assistance, contact Technical Support. See [Obtaining Support on page 203](#).



CAUTION! Do not use the **Laser Autofocus Wizard** for slides because it calculates incorrect settings for slides. If you need assistance with slides, contact Technical Support. See [Obtaining Support on page 203](#).

Configuring 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.



Tip: 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 [Viewing the Summary Panel on page 137](#).

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: Plate Settings on page 79](#).



CAUTION! You must enter the **Well depth** and **Plate height** values and all other plate dimensions correctly before you run the **Laser Autofocus Wizard** to prevent the wizard from failing.

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



Note: If needed, adjust any objectives with correction collars to match the plate thickness. See [Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 178](#).

10. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

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:

- [Acquiring a Single Site in Each Well on page 83](#)
- [Acquiring a Fixed Number of Sites in Each Well on page 86](#)
- [Configuring Adaptive Acquisition for Well Sites on page 89](#)
- [Configuring 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.

Acquiring a Single Site in Each Well

To acquire a single site in each well, use the following procedure.



Tip: 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 [Acquiring a Fixed Number of Sites in Each Well on page 86](#).

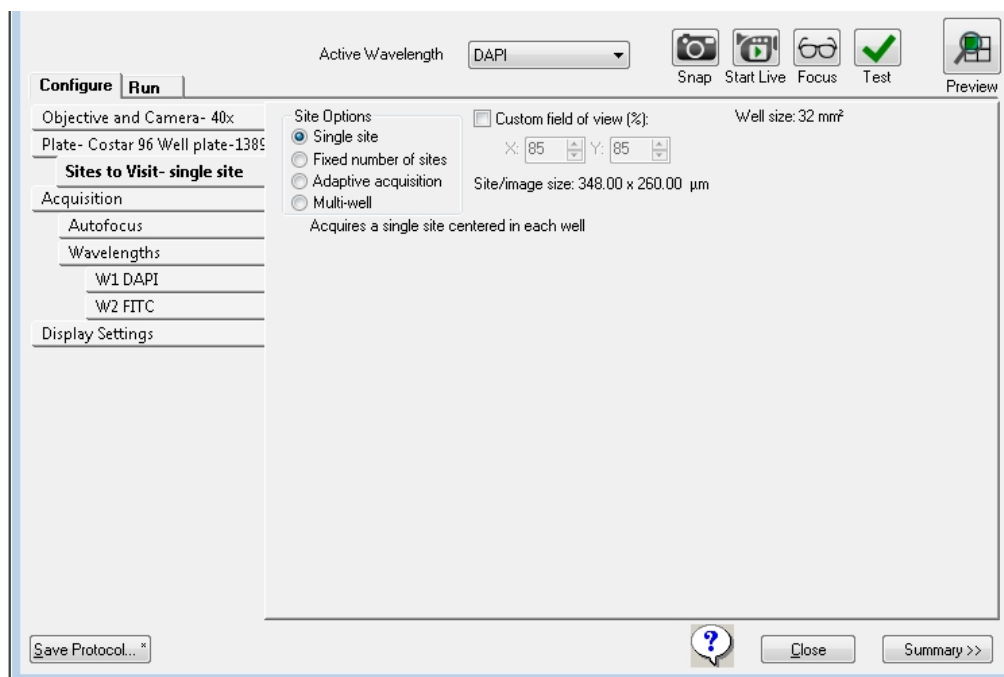


Figure 5-11: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Single site option selected

*** Tip:** 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 [Viewing the Summary Panel on page 137](#).

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



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 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.



Note: The **Custom Field of View** feature is compatible with full-size shading correction images, including the use of **Legacy Correction**. See [Correcting Image Shading on Acquired Images—Legacy on page 115](#).



Tip This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include 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 that is within the well.

4. Use the plate map to specify a subset of wells within a plate for data acquisition. All active wells are green in the plate map. Inactive wells are gray in the plate map.
 - To turn off a single well, click on a green well graphic. The well turns gray to indicate that this well will be skipped during data acquisition. To turn it back on, click the well again. The well turns green again.
 - To turn off a column of wells, click on the number header. The column of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the number header again. The wells turn green again.
 - To turn off a row of wells, click on the letter header. The row of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the letter header again. The wells turn green again.
 - To turn off a contiguous group of wells, click and drag the red-outline across the group of wells for selection. The selection group turns gray. To turn them back on, click and drag the red-outline across the group of wells for selection again. The wells turn green again.
 - To turn off or on all wells in a plate in a single step, click the white triangle in the upper-left corner of the plate map.



Note: You can configure automated data acquisitions for the wells as part of a protocol, or you can manually select the wells during the protocol run.

5. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Acquiring a Fixed Number of Sites in Each Well

To acquire a fixed number of sites in each well, use the following procedure.



Tip: 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 [Viewing the Summary Panel on page 137](#).

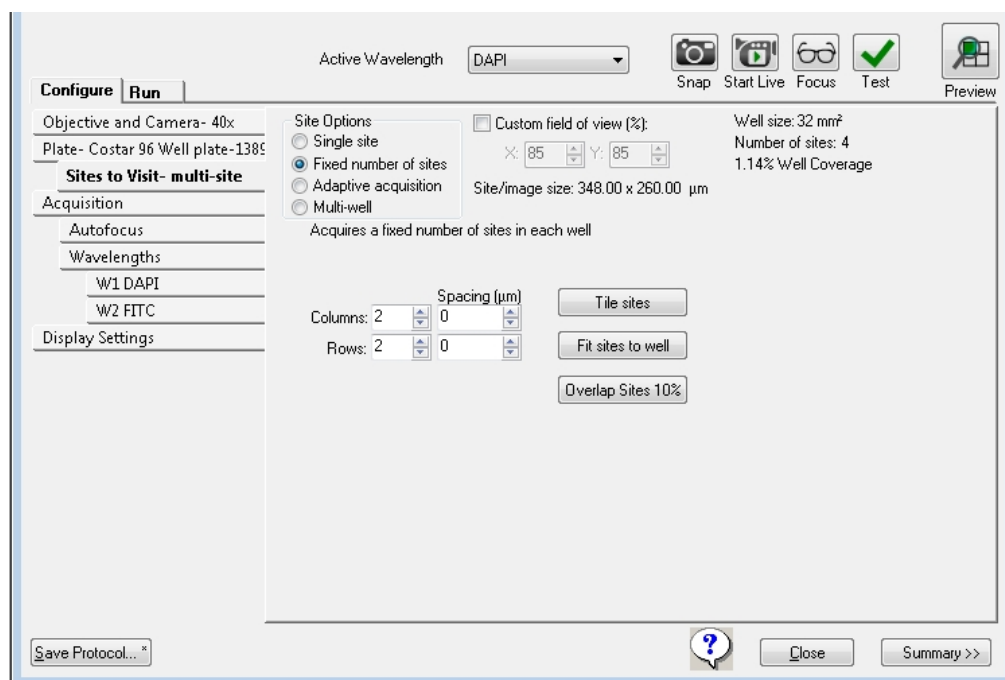


Figure 5-12: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Fixed number of sites option selected

1. Open the **Sites to Visit** tab.
2. Select **Fixed number of sites**.
3. Optionally, to specify a percentage of the field of view the camera will acquire, select **Custom Field of View (%)**, and then type the **X** (width) value and **Y** (height) value.



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 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.



Note: The **Custom Field of View** feature is compatible with full-size shading correction images, including the use of **Legacy Correction**. See [Correcting Image Shading on Acquired Images—Legacy on page 115](#).



Tip This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include 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 that is within the well.

4. In the **Columns** and **Rows** fields, specify the maximum number of sites to be visited. For example, the default values Columns 2 and Rows 2, acquire up to four sites, while specifying Columns 3 and Rows 3 acquires up to nine sites.



Note: **Columns: 45** and **Rows: 35** are the maximum allowable configuration.

5. Use the plate map to specify a subset of wells within a plate for data acquisition. All active wells are green in the plate map. Inactive wells are gray in the plate map.
 - To turn off a single well, click on a green well graphic. The well turns gray to indicate that this well will be skipped during data acquisition. To turn it back on, click the well again. The well turns green again.
 - To turn off a column of wells, click on the number header. The column of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the number header again. The wells turn green again.
 - To turn off a row of wells, click on the letter header. The row of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the letter header again. The wells turn green again.
 - To turn off a contiguous group of wells, click and drag the red-outline across the group of wells for selection. The selection group turns gray. To turn them back on, click and drag the red-outline across the group of wells for selection again. The wells turn green again.
 - To turn off or on all wells in a plate in a single step, click the white triangle in the upper-left corner of the plate map.



Note: You can configure automated data acquisitions for the wells as part of a protocol, or you can manually select the wells during the protocol run.

6. Use the site map to specify a sub-set of sites within a well for data acquisition.
 - 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 automated data acquisitions for the wells as part of a protocol, or you can manually select the wells during the protocol run. .

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, enter 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, enter 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 your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
- [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Configuring Adaptive Acquisition for Well Sites

The **Adaptive acquisition** option is a computational algorithm that is designed to analyze the number of cells 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.



Tip: 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 [Viewing the Summary Panel on page 137](#).

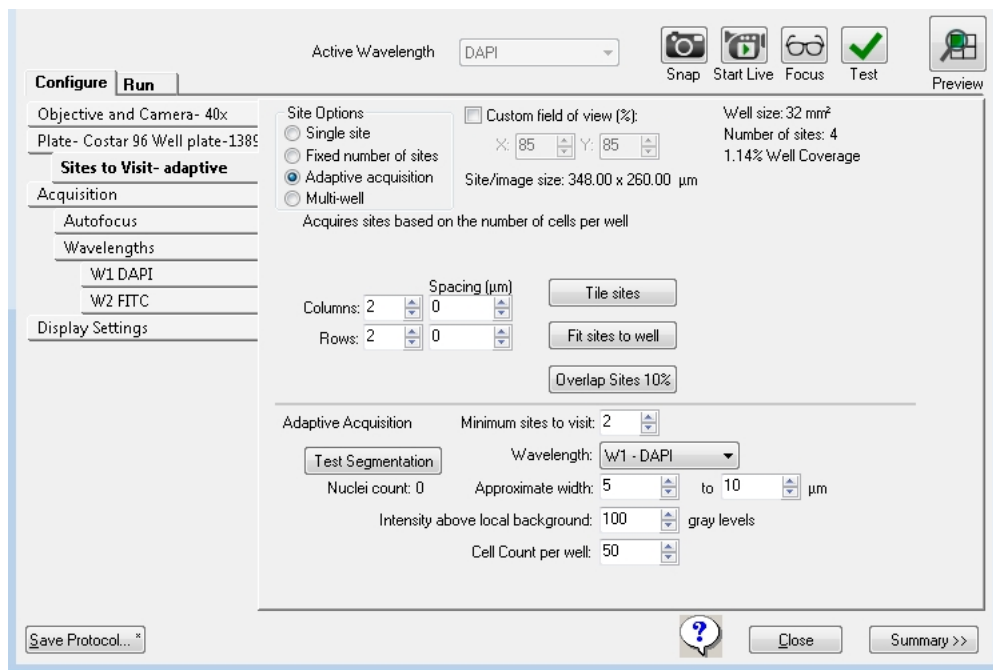


Figure 5-13: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Adaptive Acquisition option selected

1. Open the **Sites to Visit** tab.
2. Select **Adaptive Acquisition**.
3. Optionally, to specify a percentage of the field of view the camera will acquire, select **Custom Field of View (%)**, and then type the **X** (width) value and **Y** (height) value.



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 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.



Note: The **Custom Field of View** feature is compatible with full-size shading correction images, including the use of **Legacy Correction**. See [Correcting Image Shading on Acquired Images—Legacy on page 115](#).



Tip This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include 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 that is within the well.

4. In the **Columns** and **Rows** fields, specify the maximum number of sites to be visited. For example, the default values Columns 2 and Rows 2, acquire up to four sites, while specifying Columns 3 and Rows 3 acquires up to nine sites.



Note: **Columns: 45** and **Rows: 35** are the maximum allowable configuration.

5. Use the plate map to specify a subset of wells within a plate for data acquisition. All active wells are green in the plate map. Inactive wells are gray in the plate map.
 - To turn off a single well, click on a green well graphic. The well turns gray to indicate that this well will be skipped during data acquisition. To turn it back on, click the well again. The well turns green again.
 - To turn off a column of wells, click on the number header. The column of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the number header again. The wells turn green again.
 - To turn off a row of wells, click on the letter header. The row of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the letter header again. The wells turn green again.
 - To turn off a contiguous group of wells, click and drag the red-outline across the group of wells for selection. The selection group turns gray. To turn them back on, click and drag the red-outline across the group of wells for selection again. The wells turn green again.
 - To turn off or on all wells in a plate in a single step, click the white triangle in the upper-left corner of the plate map.



Note: You can configure automated data acquisitions for the wells as part of a protocol, or you can manually select the wells during the protocol run.

6. Use the site map to specify a sub-set of sites within a well for data acquisition.
 - 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 automated data acquisitions for the wells as part of a protocol, or you can manually select the wells during the protocol run. .

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, enter 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, enter 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. Specify the **Adaptive Acquisition** settings.

Table 5-3: Adaptive Acquisition Settings

Setting	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: For the most efficient acquisition, this wavelength should be the same as the wavelength that is designated as being the first to be acquired. See Specifying the Number of Acquisition Wavelengths on page 106 .
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 Calculating the Intensity Above Local Background Gray Levels Value on page 95 .
Note: The Approximate width and the Intensity above local background gray levels work in conjunction. A nucleus is determined by an object size and relative intensity above the background.	
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 acquired 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 acquired.

9. Optionally, to determine if your current acquisition settings are appropriate for differentiating nuclei, do the following:
 - Select a well or wells, and a site or sites for viewing.
 - Click **Focus** to bring the sample into focus.



Note: **Focus** does a large-range autofocus on the currently selected well and site. To ensure that your cell counting settings are accurate, bring the sample into focus before you click **Test Segmentation**.

- Click **Test Segmentation** to snap an image and count the nuclei. Both an acquisition image and the total number of cells that were counted in the image are displayed.

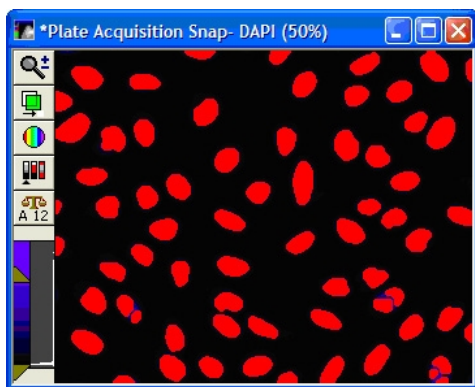


Figure 5-14: An example of Test Segmentation results in the Adaptive Acquisition mode. The red shows the segmentation overlaid on the original DAPI image.

If your test results are satisfactory, then continue to the next step. Otherwise, repeat Step 8 and Step 9 until your test results are satisfactory.

10. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Calculating the Intensity Above Local Background Gray Levels Value

To calculate the **Intensity above local background gray levels** value, use the following procedure:

1. If the **Region** toolbar is not open, then click **Regions > Regions Tools** (default menu), or **Measure > Regions > Regions Tools** (simplified menu).
2. Select the Arrow tool as shown in the following figure:

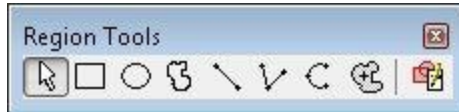


Figure 5-15: Region tools on the Region toolbar

3. 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.
4. Position your mouse pointer on the dimmest part of the object and make note of the gray level value that is displayed in the status bar.

As you move the cursor, the X, Y coordinates and the gray level value of the pixel that is under the cursor are indicated at the bottom of the MetaXpress Software desktop. The X and Y coordinates are enclosed in parentheses and the gray level value is displayed to the right. For example, (48, 158) -> 18000 indicates that 18000 is the average gray level value of the pixel.

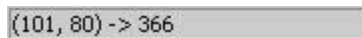


Figure 5-16: Gray level indicators

5. Position your mouse pointer just to the 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.



Tip: Instead of doing Step 4 and Step 5, you can draw 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. For more information about this tool, see the MetaXpress Software application help.

6. Subtract the gray level value of the background from the gray level value of the object.

The value that you enter in the **Intensity above local background** field depends on whether the application module provides the Standard and Fast algorithms.

- If the application module does not provide either algorithm, or if you select the Standard algorithm, then enter a value that is slightly lower than the calculated value. Using the examples above, $18000 - 2000 = 16000$, a value of 15900 is appropriate.
- If you select the Fast algorithm, then enter a value that is approximately half of the value that you calculated. Using the examples above, $18000 - 2000 = 16000$, a value of 8000 is appropriate.

Configuring a Multi-Well Acquisition

To acquire high density plates faster, you can run 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, wells that are not selected might be exposed to excitation light from imaging neighboring selected wells; however, images are not saved for the wells that are not selected.

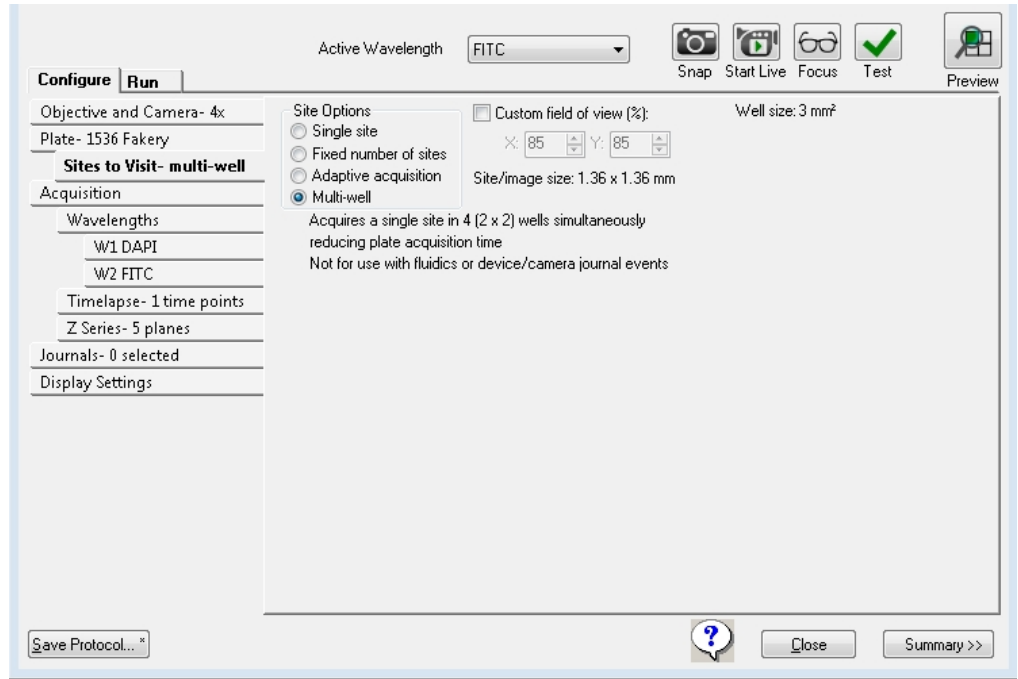


Figure 5-17: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Multi-well option selected

✳ **Tip:** 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 [Viewing the Summary Panel on page 137](#).

1. Open the **Sites to Visit** tab.
2. Select **Multi-well**.
3. Optionally, to specify a percentage of the field of view the camera will acquire, select **Custom Field of View (%)**, and then type the **X** (width) value and **Y** (height) value.



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 μm , then to acquire a width of 500 μm , enter **50** in the **X** field.



Note: The **Custom Field of View** feature is compatible with full-size shading correction images, including the use of **Legacy Correction**. See [Correcting Image Shading on Acquired Images—Legacy on page 115](#).



Tip This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include 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 that is within the well.

4. Use the plate map to specify a subset of wells within a plate for data acquisition. All active wells are green in the plate map. Inactive wells are gray in the plate map.
 - To turn off a single well, click on a green well graphic. The well turns gray to indicate that this well will be skipped during data acquisition. To turn it back on, click the well again. The well turns green again.
 - To turn off a column of wells, click on the number header. The column of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the number header again. The wells turn green again.
 - To turn off a row of wells, click on the letter header. The row of wells turn gray to indicate that they will all be skipped during data acquisition. To turn them back on, click the letter header again. The wells turn green again.
 - To turn off a contiguous group of wells, click and drag the red-outline across the group of wells for selection. The selection group turns gray. To turn them back on, click and drag the red-outline across the group of wells for selection again. The wells turn green again.
 - To turn off or on all wells in a plate in a single step, click the white triangle in the upper-left corner of the plate map.



Note: You can configure automated data acquisitions for the wells as part of a protocol, or you can manually select the wells during the protocol run.

5. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab

At a minimum, you must specify the autofocus options and the acquisition wavelengths for your protocol. Optionally, you can also specify various settings such as **Use Fluidics** and **Run Journals During Acquisition**. Depending on the options that you select, other tabs with additional configuration options can be displayed.

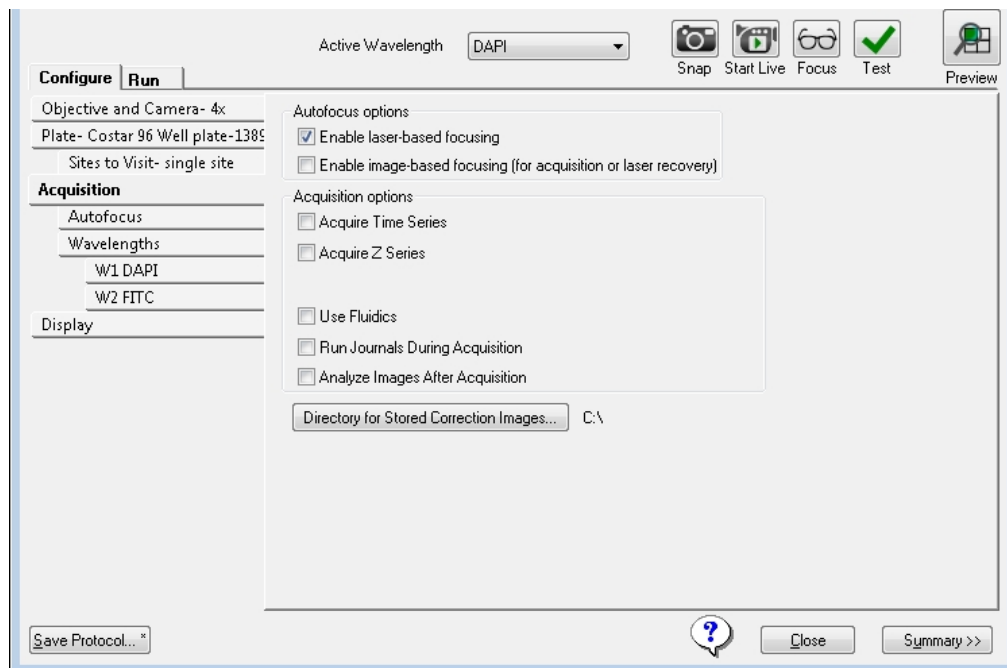


Figure 5-18: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

See:

- [Configuring Autofocus Options on page 100](#)
- [Specifying the Number of Acquisition Wavelengths on page 106](#)
- [Configuring Series Acquisition Options on page 117](#)
- [Configuring Journals to Run During Acquisition on page 125](#)
- [Configuring Post-Acquisition Analysis Options on page 129](#)



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

Configuring Autofocus Options

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

- **Laser-based Focusing** 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 affect focus performance.
- **Image-based Focusing** uses an algorithm to identify the best focus image. This option works best for experiments that use low power objectives or when the sample distance above the bottom of the plate varies. Performance can be slower than **Laser-based Focusing** and focusing can fail if out-of-focus debris is in a sample.

You can configure one or both of the **Autofocus Options** for a plate acquisition protocol. With the exception of oil-immersion objectives, Molecular Devices recommends primarily using **Laser-based Focusing**. For oil-immersion objectives, only use **Image-based Focusing**.

Certain types of samples can benefit from using both **Image-based Focusing** and **Laser-based Focusing**, including live organisms, suspension cells, tissue samples, and assays where the best focus position varies with the phenotype. When you select both, the **Laser-based Focusing** 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.



Tip: 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 [Viewing the Summary Panel on page 137](#).

To configure autofocus options, use the following procedure:

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



Note: Molecular Devices recommends using **Image-based Focusing** with complex samples that have variations in distance between the surface of the plate and the sample.

3. Click **Autofocus**.

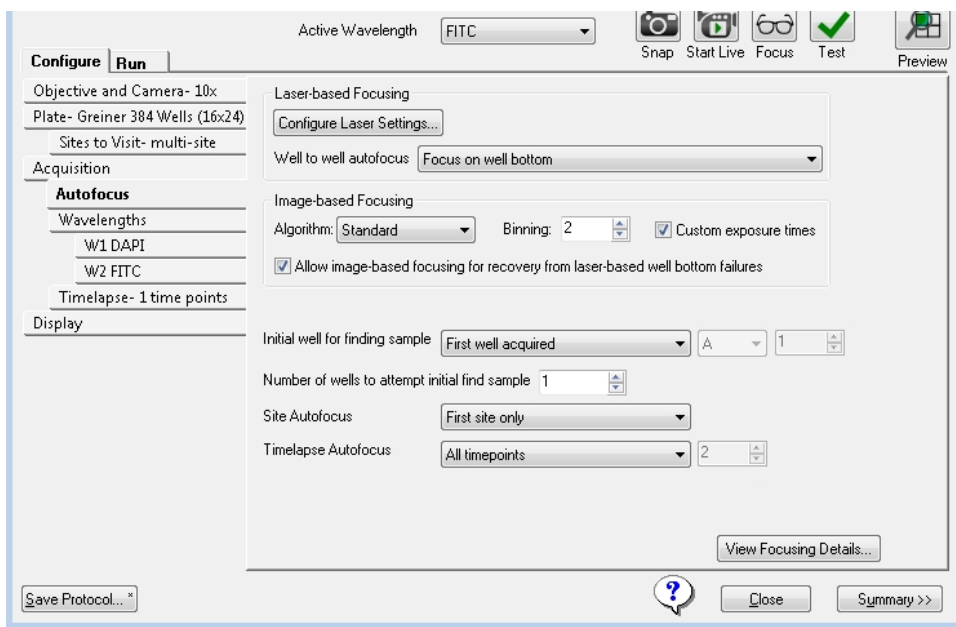


Figure 5-19: Plate Acquisition Setup dialog: Configure tab, Autofocus tab

4. Configure the **Autofocus options**.**Table 5-4: Autofocus options**

Options	Description
Laser-based Focusing is available only if Enable laser-based focusing is selected on the Acquisition tab.	
Configure Laser Autofocus Settings	<p>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 the dialog can be blank. You can modify the settings that the wizard has calculated for this dialog.</p> <p>Note: The MetaXpress Software help topic “Configure Laser Autofocus Settings - Dialog 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 203.</p>
<p>Well to well autofocus adjusts the focus as the acquisition moves from well to well.</p> <p>Note: Chamber slides with wells can use any of these options.</p>	
Focus on well bottom	<p>The default value and the recommended option for most plate acquisition protocols.</p> <p>The initial focus on the plate for finding the samples, 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 Edit Plate Bottom Settings option on the Plate tab to determine the focus range.</p>
Focus on plate bottom, then offset by bottom thickness	<p>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:</p> <ul style="list-style-type: none"> – A thin-bottom plate with a large depth of field objective, needed because of low magnification or low numerical aperture. This is generally for magnifications of 4x and below. – A slide – Multi-well acquisition
Focus on plate and well bottom	<p>The laser focuses on both the plate bottom and the well bottom at every well.</p> <p>Recommended setting for plates with extreme bottom variation.</p>
Note: Chamber slides with wells can use any of the previous options.	

Table 5-4: Autofocus options (continued)

Options	Description
Image-based Focusing	
Algorithm: provides the focusing algorithm options.	
Standard	Default algorithm, based on a standard group of settings including a normal camera signal level.
Low Signal	Based on a set of values designed to compensate for a low signal level on the camera, and pixel intensities that are brighter when slightly out of focus.
Camera configuration options:	
Binning	Sets the binning used by the camera during Auto Focus and Show Live . Horizontal and vertical binning are always set to the same value.
Custom exposure times	For individual wavelengths during autofocus. If this 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 Laser and Image or Laser with Image Recovery for the first acquisition wavelength (W1), and 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 Specifying the Number of Acquisition Wavelengths on page 106 .
Allow image-based focusing for recovery from laser-based well bottom failures	<p>Enabled only if both Enable laser-based focusing and Enable image-based focusing are selected.</p> <p>Use Image-based focusing only if Laser-based focusing cannot find the plate or well bottom.</p> <p>The image-based recovery search is centered on an estimated well bottom offset position.</p> <p>The estimated position is calculated by 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.</p>
Initial well for finding sample: Sets the well to use for the first Find Sample autofocus.	
First well acquired	Finds the sample autofocus using the first well that is acquired. Recommended setting for most protocols
Specific well	Finds the sample autofocus using a well that you specify.

Table 5-4: Autofocus options (continued)

Options	Description
	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 when your sample is already in focus. Note: Select this option when 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 the First well acquired option is selected. The first well in which a sample is found is the well that is used for autofocusing. The default value is one. Note: If you are using a robot for automated plate loading, set this value to three or greater.
Site Autofocus is enabled only if Fixed number of sites or Adaptive Acquisition is selected on the Sites to Visit tab. It determines how to do autofocusing 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: Select this option when the magnification is low and the sites are relatively close together.
All sites	Autofocuses for each site. Note: Select this option with higher magnification, when the sites are spread far apart, or there is extreme variation in the plate bottom.
Timelapse Autofocus is enabled only if Acquire Time Series is selected on the Acquisition tab. See Configuring Series Acquisition Options on page 117 .	
First timepoint only	Use for fast kinetic acquisitions.
All timepoints	Use for long timelapse acquisitions.
Every Nth timepoint	Set a value that is less than the number of specified timepoints. See Configuring Time Series Acquisition Options on page 118 .

Table 5-4: Autofocus options (continued)

Options	Description
View Focusing Details	Opens the Auto Focus Details dialog, which lists the current autofocus parameters. This information can be useful for diagnostic purposes when troubleshooting focusing issues. Click Copy to copy the parameters to the clipboard, and then paste them into a third party application, such as Microsoft Word.

5. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
- [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Specifying the Number of Acquisition Wavelengths

You specify the total number of acquisition wavelengths for an experiment on the **Wavelengths** tab. You must specify at least one acquisition wavelength, but you can specify up to a maximum of eight. You configure exposure time, autofocus, and timelapse settings for each acquisition wavelength on an individual wavelength tab. Individual placeholder tabs (**W1**, **W2**, **W3**, and so on) are enabled that correspond to the number of acquisition wavelengths that you have set. For example, if you set the **Number of wavelengths** to three, then a **W1** tab, a **W2** tab, and a **W3** tab are enabled. The red warning icon indicates that you have yet to configure the acquisition wavelengths. See [Figure 5-20](#).

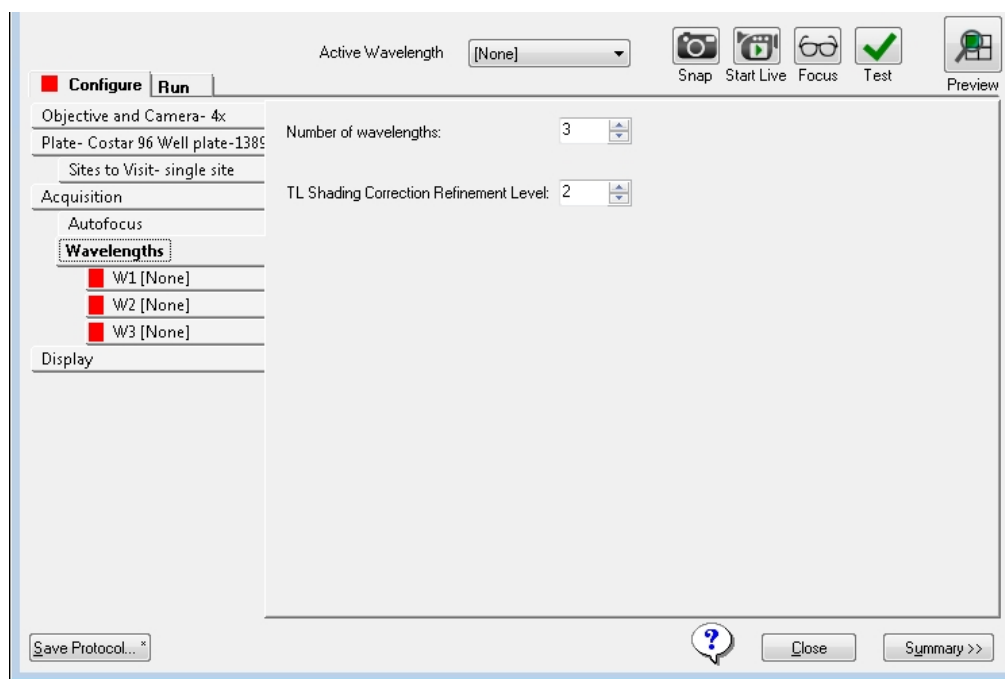


Figure 5-20: Plate Acquisition Setup dialog: Configure tab, Wavelength tab: Three placeholder Wavelength tabs



Tip: 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 [Viewing the Summary Panel on page 137](#).

To specify the number of acquisition wavelengths, do the following:

1. Click **Wavelengths**.



Note: **TL Shading Correction Refinement Level** is always displayed on the **Wavelengths** tab. The default value is two. This option is applied only if **Autocorrect for TL** is selected for **Shading Correction** for one or more acquisition wavelengths. The larger the value for the refinement level, the flatter the transmitted light will be, but the longer the correction will take. The single value that is specified here applies to all acquisition wavelengths for which **Auto Correction for TL** is selected as the shading correction method. See [Configuring an Acquisition Wavelength on page 108](#).

2. In the **Number of wavelengths** field, specify the total number of wavelengths that are to be used for acquisition, where the minimum is one and the maximum value is eight.
3. Configure each acquisition wavelength. See [Configuring an Acquisition Wavelength on page 108](#).

Configuring an Acquisition Wavelength



Tip: For efficiency, configure the acquisition wavelengths in the order that minimizes filter movement. The order in which you configure the acquisition wavelengths corresponds to the acquisition order. For example, if you configure the W1 tab as a DAPI wavelength and then configure the W2 tab as a FITC wavelength, then the DAPI acquisition wavelength runs first and the FITC acquisition wavelength runs second.

For each individual wavelength placeholder tab that is marked with a red warning icon, do the following in the order listed:

1. Click the individual wavelength tab, for example, **W1**.
2. In the **Illumination setting** field, select the illumination wavelength, for example, DAPI.

The selection name is displayed in the **Active Wavelength** field under the plate map, and on the individual tab.

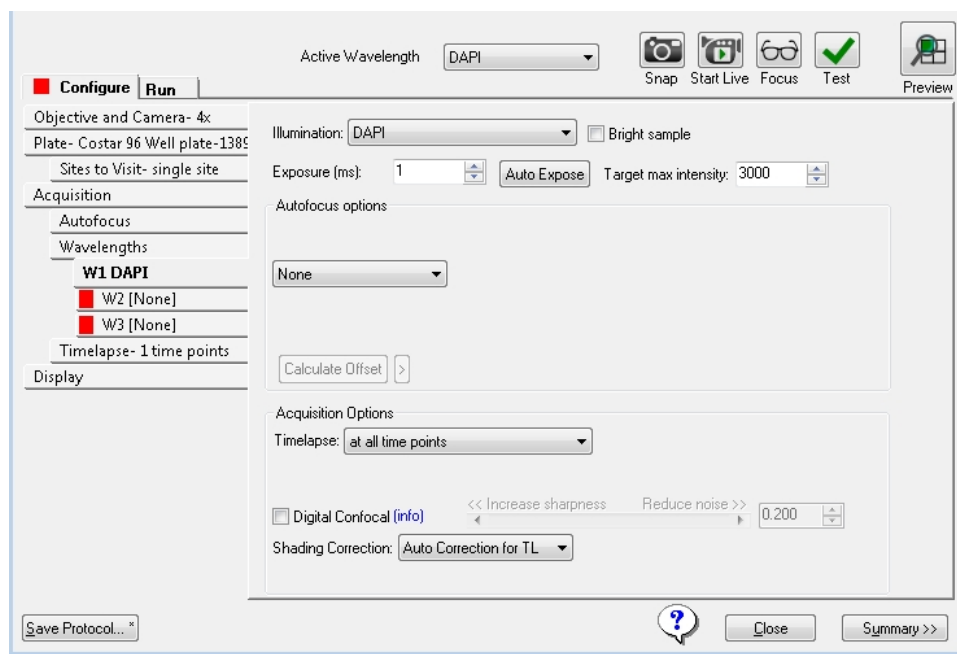


Figure 5-21: Plate Acquisition Setup dialog: Configure tab, Wavelength tab: Setting placeholder Wavelength tab

3. 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 Settings

Options	Description
Bright Sample	If your sample has a high intensity, but a low (1 ms to 7 ms) exposure time, then select this option to reduce the light power for the acquisition wavelength and prevent saturation of the sample. After you select this option, then adjust your exposure time accordingly to control the precision of the sample brightness.
Exposure (ms)	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. Exposure times should be determined using an in-focus image. Note: When you are configuring exposure times, check both the positive and the negative controls. If you use a dim sample to set the exposure time, then a bright sample might become saturated.
Auto Expose	Click to automatically set the exposure time that is required to obtain the Target max intensity . If Auto Expose results in a 1 ms to 3 ms value to achieve this intensity, then you are automatically prompted to activate Bright Sample mode to prevent saturation of your sample. Click Yes to turn on Bright Sample mode and automatically rerun Auto Expose , or click No to leave all settings as-is. Note: Auto Expose is a manual tool to help configure the plate. It does not auto expose while the plate is being acquired. Exposure times should be determined using an in-focus image.
Target max intensity	Specifies the intensity that Auto Expose 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 is possible with your camera driver.
Autofocus options specify the type of autofocus that is to be used when acquiring	

Table 5-5: Acquisition Wavelength Settings (continued)

Options	Description
	images. The available options depend on the acquisition options that are selected on the Acquisition tab.
None	No autofocusing is done. 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, then the Laser-based Focusing is used based on the settings that the Laser Autofocus Wizard calculates, or as configured using Configure Laser Settings on the Acquisition tab. Specify the Post-laser offset value in μm , or click Calculate Offset to automatically calculate the first (W1) wavelength offset.
Calculate Offset icon (>)	Click > to display the options that are used for calculating the offset. Use Z stack shows distinct Z planes so that you can select the best in-focus plane. Note: Without Use Z stack , MetaXpress Software carries out an image autofocus and determines the best in-focus plane. Custom Range determines the total search range and step size for calculating the offset. Note: Without Custom Range , the default values are used for the Calculate Offset tool using either Z stack or image autofocus.
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 selected, the laser autofocuses first, and then image-based focusing fine tunes the image. You must also specify values for the following: Image-based range +/- μm specifies the range for the image-based portions of autofocusing. Max. step specifies the maximum step size of a single Z move in μm that is required to obtain the correct focus position. This setting depends on the objective that is used. Because the focus peak is narrower, use a smaller step size with higher NA objectives. 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 for the acquisition wavelength when autofocusing.

Table 5-5: Acquisition Wavelength Settings (continued)

Options	Description
Laser with Image Recovery	<p>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.</p> <p>When selected, you must also specify the following options:</p> <p>Image-based range +/- μm: Specifies the range that is to be used for the image-based portions of autofocusing.</p> <p>Note: You should adjust the range based on the sample variability. A larger range requires a longer time to focus.</p> <p>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. Because the focus peak is narrower, use a smaller step size with higher NA objectives.</p> <p>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.</p> <p>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:</p> <p>Exposure (ms) specifies the exposure time in ms that is to be used for the acquisition wavelength when autofocusing.</p>
Z-offset from W1	<p>Available only for the second or later (W2, W3, and so on) acquisition wavelengths. The offset for W1 must be calculated before you can set this value. Selecting this option moves the W1 focus position to the specified offset. Specify the post-laser offset in μm in the Offset field, or click Calculate Offset to automatically calculate the offset for all subsequent acquisition wavelengths.</p>
Image-based	<p>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 Z-offset from W1 in μm in the Offset field, or click Calculate Offset to automatically calculate the offset that is to be</p>

Table 5-5: Acquisition Wavelength Settings (continued)

Options	Description
	<p>used for all subsequent acquisition wavelengths. If this option is selected, then you must also specify the following options:</p> <p>Image-based range +/- μm specifies the range that is to be used for the image-based portions of autofocus.</p> <p>Note: You should adjust the range based on the sample variability. A larger range requires a longer time to focus.</p> <p>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.</p> <p>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.</p> <p>If Custom exposure times is selected on the Acquisition tab, then with Image-based selected, you must also specify values for the following:</p> <p>Exposure (ms) specifies the exposure time in milliseconds that is to be used for the acquisition wavelength when autofocus.</p>
<p>Acquisition options are displayed depending on the Series option that is selected on the Acquisition tab.</p>	
<p>Acquire Time Series enables Timelapse</p>	<p>Specifies the image collection intervals to use for the wavelength.</p> <p>at all time points is the default value. It acquires an image at the indicated wavelength at each time point in the experiment.</p> <p>at start of experiment acquires an image at the indicated wavelength at the first time point only.</p> <p>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.</p> <p>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.</p>
<p>Acquire Z Series enables Z Series</p>	<p>Single plane acquires only a single plane based on the autofocus options for the wavelength, just as if the Acquire Z Series option were disabled. It is used in a multi-wavelength acquisition when the Z Series acquisition is not required for all wavelengths.</p>

Table 5-5: Acquisition Wavelength Settings (continued)

Options	Description
	<p>2D Projection Image Only acquires the Z Series, and a single projection image, which is saved.</p> <p>Z Series and 2D Projection Image acquires the Z Series, and every plane and the projection image are saved.</p>
2D Projection Image	<p>Available for all Z Series acquisition options other than Single Plane indicates how the resulting 2D projection image is generated.</p> <p>Best Focus is 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.</p> <p>Maximum is 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.</p> <p>Minimum is 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.</p> <p>Sum is 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.</p> <p>Note: If the sum overflows the 16 bit chip capacity, then a warning message opens that indicates that the maximum possible intensity of 65535 was exceeded. The MetaXpress Software cuts the pixels off at this value as a result.</p>
Digital Confocal	<p>Enabled only if your organization has purchased the optional Digital Confocal feature. Select to do deconvolution-based image sharpening.</p> <p>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.”</p>
Shading Correction	<p>Indicates whether shading correction has been enabled for the acquisition wavelength. Options are the following, where FL represents fluorescence and TL represents transmitted light:</p>

Table 5-5: Acquisition Wavelength Settings (continued)

Options	Description
	<p>Off indicates that no shading correction is carried out.</p> <p>Auto Correction for FL is the most complete approach. It provides both a shading correction and a background subtraction. Background subtraction removes stray light that is unrelated to the light that is emitted by the sample. The shading correction is tuned for fluorescence and it adjusts for uneven illumination of the sample. The shading reference is generated once and applied over all samples.</p> <p>FL Subtraction Only does the background subtraction but not the shading correction once per run. This method should be used at high magnification, or when there is a concern about the algorithm being able to correctly determine background, which can occur if the fluorescent target takes up the majority of the field of view. For example, in a scenario in which images were being taken of a tissue sample and the sample of interest covered the entire field of view, then the shading algorithm might interpret some of the signal as shading and slightly over-correct the image.</p> <p>FL Shading Only provides only shading correction once per run. It does not provide background subtraction. The shading correction is tuned for fluorescence. The shading reference is generated once and applied over all samples. Used primarily for oil immersion objectives where subtraction should not be done because it would require the breaking of the oil droplet.</p> <p>Auto Correction for TL is a shading algorithm that is specifically tuned for transmitted light instead of fluorescence. It does shading correction but not background subtraction. If you select this option, then you must set the TL Shading Correction Refinement Level on the Wavelengths tab. The shading correction uses this value. The shading reference is collected at every site. See Specifying the Number of Acquisition Wavelengths on page 106.</p> <p>Legacy Correction, from earlier versions of the MetaXpress Software, requires the use of preset magnification, illumination, and acquisition mode reference images that are located in a specified directory. See Correcting Image Shading on Acquired Images—Legacy on page 115.</p> <p>Note: The new methods are recommended over the Legacy Correction method. With the new methods, correction is always based on the current state of the system and is not subject to variation over time.</p>

4. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Correcting Image Shading on Acquired Images—Legacy



Note: The following information is applicable if **Legacy Correction** is selected as the shading correction method for an acquisition wavelength. See [Configuring an Acquisition Wavelength on page 108](#).

Before you can do legacy shading correction, the appropriate shading correction images must have been acquired for each acquisition wavelength and saved to a directory that has been specified as the Stored Correction Images directory. During your initial on-site system installation, the shading correction image files are configured to be found in the **C:\Shading Images** folder (see [Verifying Shading Correction Files-Legacy on page 56](#)), but you always have the option of changing this default directory.

The file name for a shading correction image must always include the exact matches for the **Magnification**, **Acquisition Mode**, and **Illumination** settings, for example, *Shading_10XPlan Fluor_widefield_DAPI.tif*. See [Updating Shading Correction Settings-Legacy on page 198](#).



Tip: 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 78](#).



Note: If a shading correction image cannot be found for a particular combination of **Magnification**, **Acquisition Mode**, and **Illumination** settings in the directory that has been specified as the Stored Correction Images directory, then no shading correction is done for the acquisition wavelength.

To change the default directory location for shading correction images, use the following procedure:

1. Click **Acquisition**.

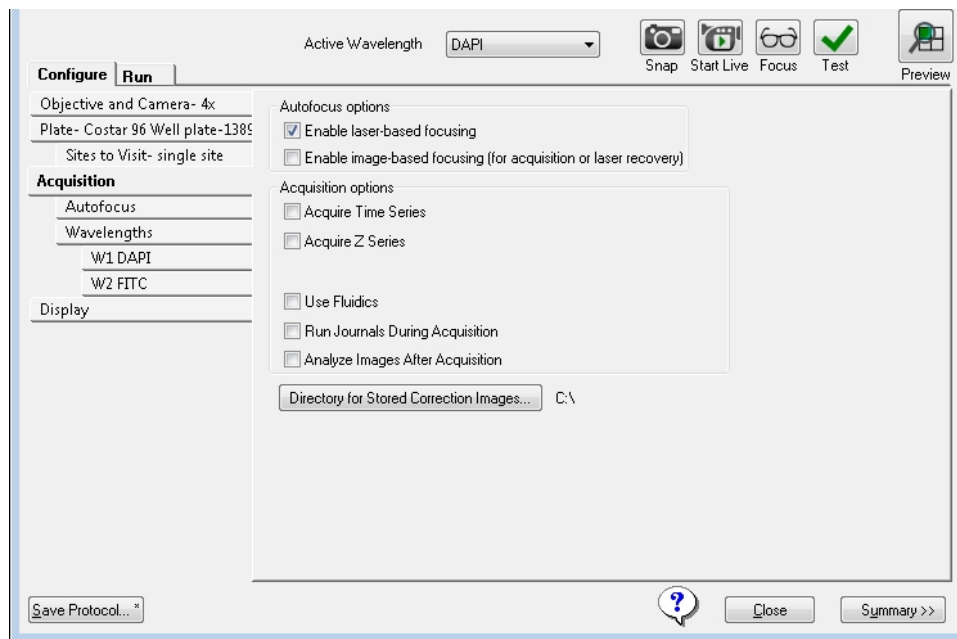


Figure 5-22: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

2. Click **Directory for Stored Correction Images**, and then in the **Browse for Folder** dialog, select the new location.
3. Click **OK** to close the dialog and return to the **Acquisition** tab.
4. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#)
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Configuring Series Acquisition Options

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

- **Acquire Time Series** acquires images at multiple time points. When selecting this option, 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. When selecting this option, 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.

*** Tip:** 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 [Viewing the Summary Panel on page 137](#).

1. Click **Acquisition**.

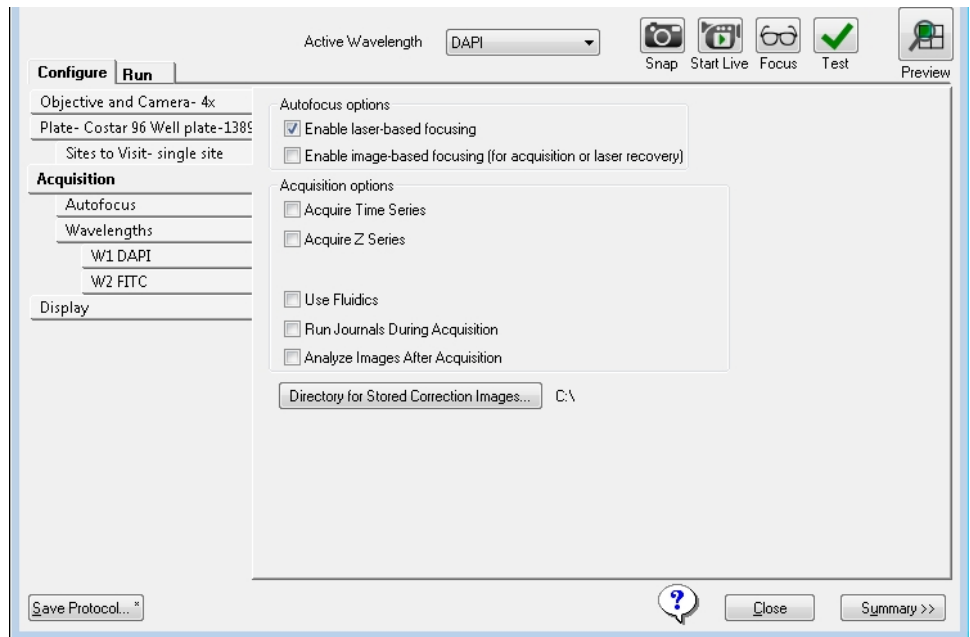


Figure 5-23: Acquisition Setup dialog, Configure tab, Acquisition tab, Acquisition options, Series options

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

3. Continue according to your selection:

- For **Acquire Time Series**, continue to [Configuring Time Series Acquisition Options on page 118](#).
- For **Acquire Z Series**, continue to [Configuring Z Series Acquisition Options on page 121](#).

Configuring Time Series Acquisition Options

1. Click **Timelapse**.

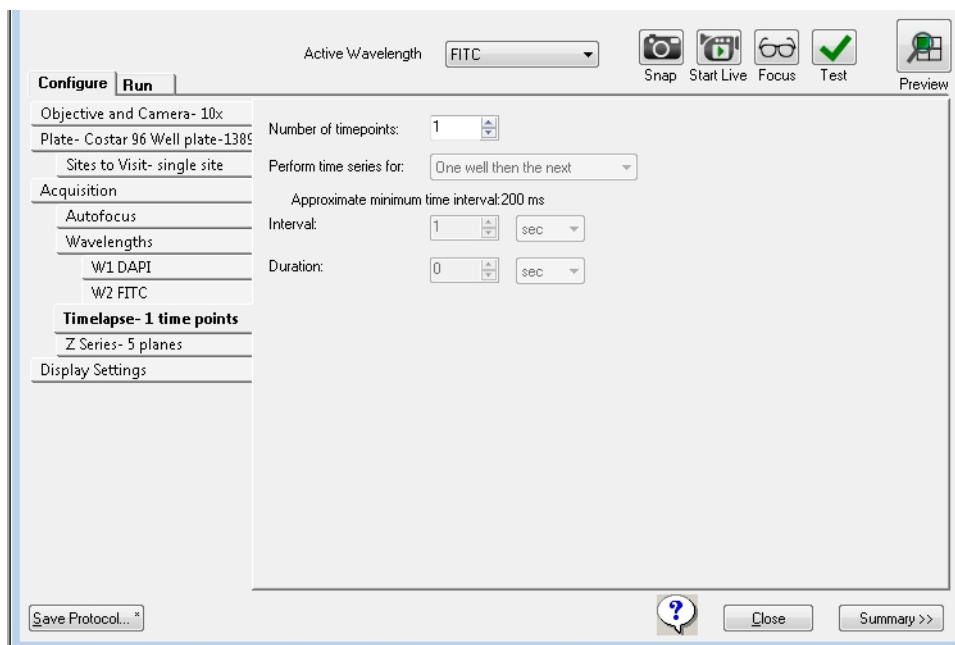


Figure 5-24: Plate Acquisition Setup dialog, Configure tab, Timelapse tab

2. Configure the time series acquisition options.

Table 5-6: Times series acquisition options

Options	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 Interval .
Perform time series for is enabled only when the value for the Number of time points is > 1. It specifies the loop order that is to be used for 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	Acquires a set of wavelength images 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	Collects all the images that are in one row of wells 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	Collects all the images in one column of wells at each time point. After the series is collected, the next column is acquired.
All selected wells	Determines the well selection at the start of the first acquisition, and then acquires every selected well at each time point.
Note: Any refocusing that is done after the images for the first timepoint are acquired is determined by the Timelapse Autofocus setting that is specified on the Autofocus tab. See Configuring 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** value 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** value, then the next timepoint starts as soon as the previous timepoint finishes.



Note: The **Duration** value results from multiplying the number of time points by the interval. If you change the **Number Of Timepoints** value, or the **Interval** value, then the value in the **Duration** value is automatically updated.

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



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

5. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Configuring Z Series Acquisition Options

1. Click **Z Series**.

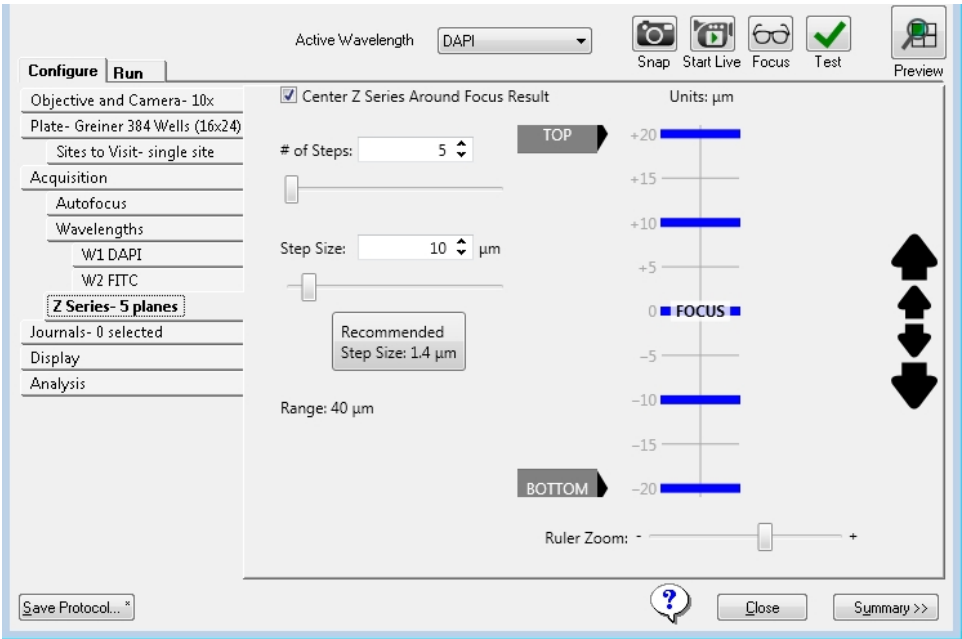


Figure 5-25: Plate Acquisition Setup dialog: Configure tab, Z Series tab showing the Z Series diagram



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

2. Configure the Z series acquisition options.

Table 5-7: Z series acquisition options

Option	Description
# of Steps	<p>The number of planes at a given site or 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:</p> <ul style="list-style-type: none">–Manually type a value in the field.–Click the Up or Down arrows in the field.–Drag the slider bar that is displayed below the field.
Step Size	<p>The distance in μm between the image planes in the Z series. You can do the following to adjust this value:</p> <ul style="list-style-type: none">–Manually type a value in the field.–Click the Up or Down arrows in the field.–Drag the slider bar that is displayed below the field. <p>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 to this recommended value.</p>
<p>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 then displays this value below the Step Size on the tab.</p>	

- Optionally, to navigate the Z Series for a site or well position and modify the coordinates for the Z motor before you acquire any images, do any of the following as needed:

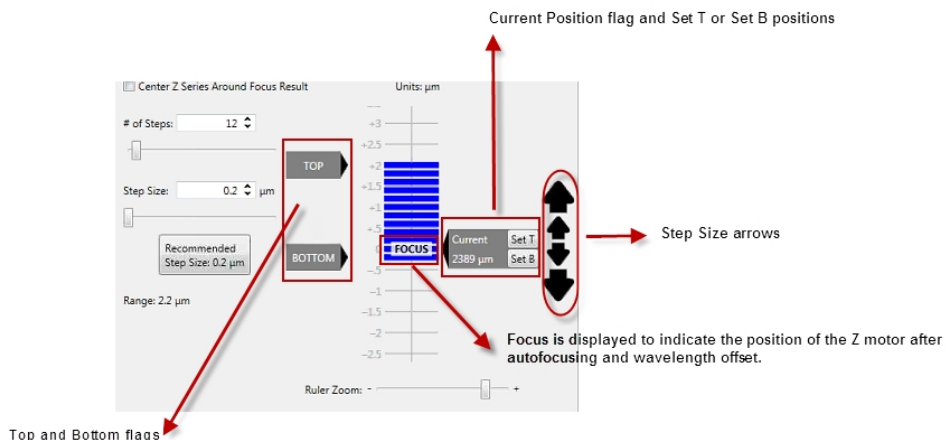


Figure 5-26: Z Series diagram

- Drag the **Ruler Zoom** slider bar that is displayed below the Z series diagram to zoom in or 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.
 - 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, when you 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.

- 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. (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 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 (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 your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Configuring Journals to Run During Acquisition

You use the **Journals** tab to configure specific journals to run during different stages of the acquisition. Only one journal configuration runs per acquisition step.

*** Tip:** 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 [Viewing the Summary Panel on page 137](#).

1. Click **Acquisition**.
2. Select **Run Journals During Acquisition**.

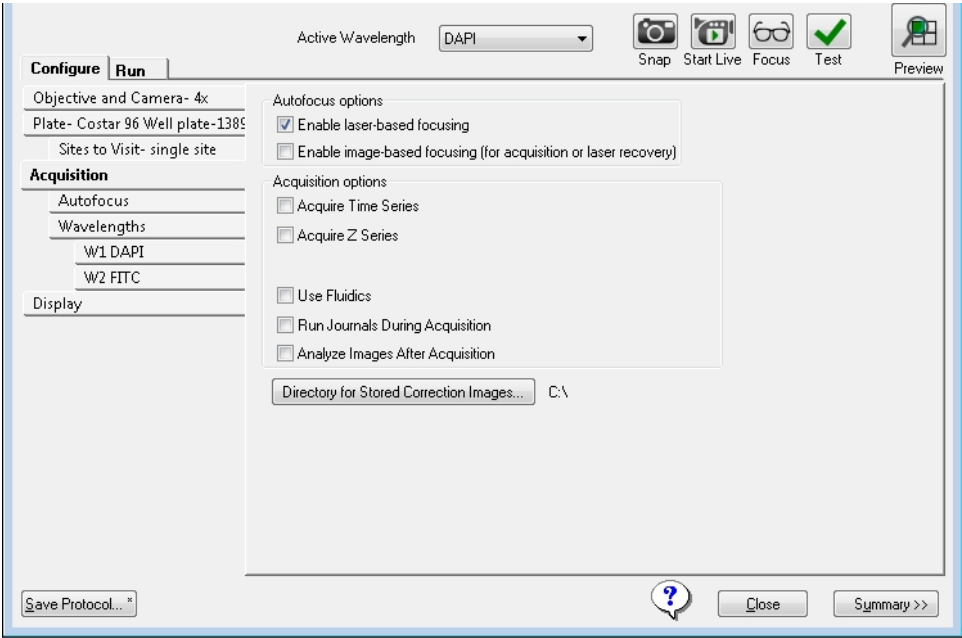


Figure 5-27: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

3. Click **Journals**.

4. Select an **Acquisition Step** for the journal to run.

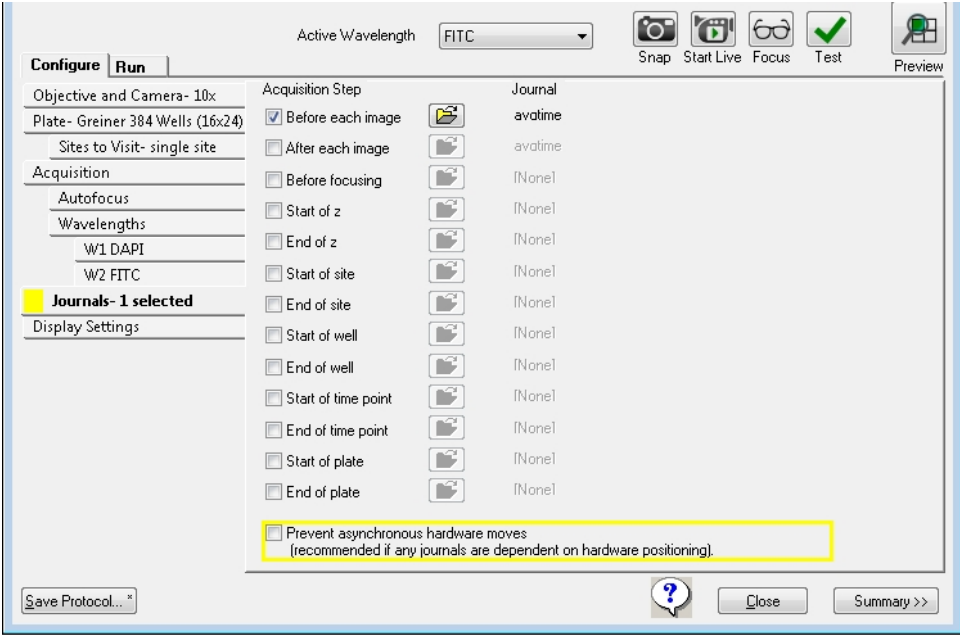


Figure 5-28: Plate Acquisition Setup dialog: Journals tab, Acquisition Step Selection and Loaded Journal

Table 5-8: Journal acquisition steps

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.
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

Table 5-8: Journal acquisition steps (continued)

Option	Description
	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	<p>(Optional) Select this option only if a selected journal moves hardware, such as changing filters, or moving focus. This option ensures that the journals run correctly.</p> <p>Note: Do not select this option without the use of a journal because it unnecessarily slows the acquisition.</p>

- Click the folder icon for the selected acquisition step.

6. In the **Select Plate Acquisition Journal** dialog, select a listed journal file.

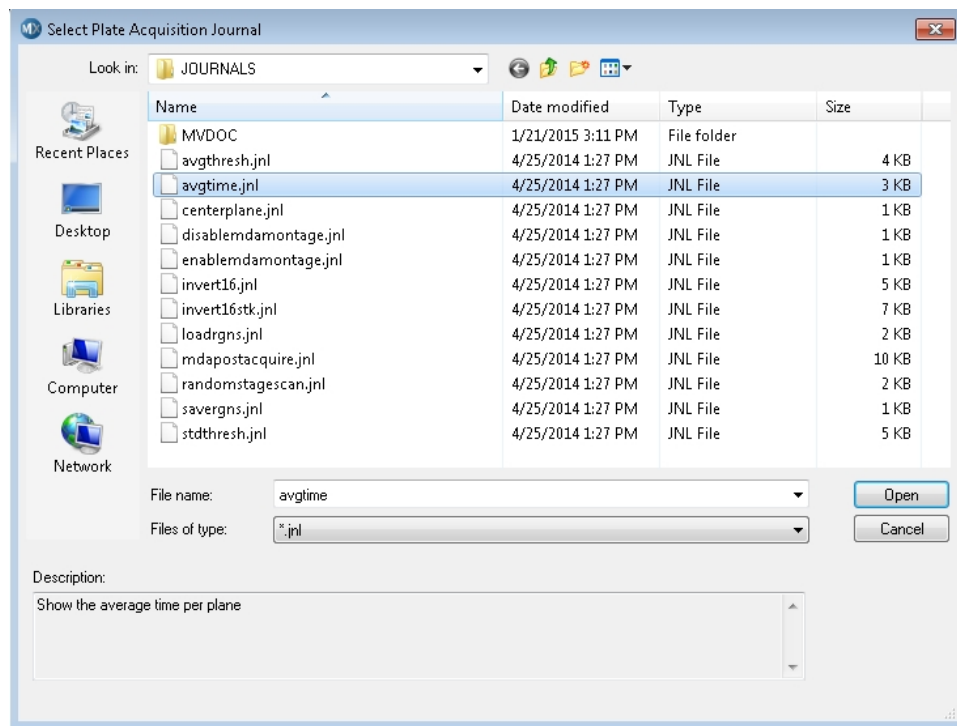


Figure 5-29: Select Plate Acquisition Journal dialog

7. Click **Open** to close the **Select Plate Acquisition Journal** dialog and return to the **Journal** dialog.
8. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

Configuring 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 MetaXpress Software 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.

*** Tip:** 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 [Viewing the Summary Panel on page 137](#).

1. Click **Acquisition**.

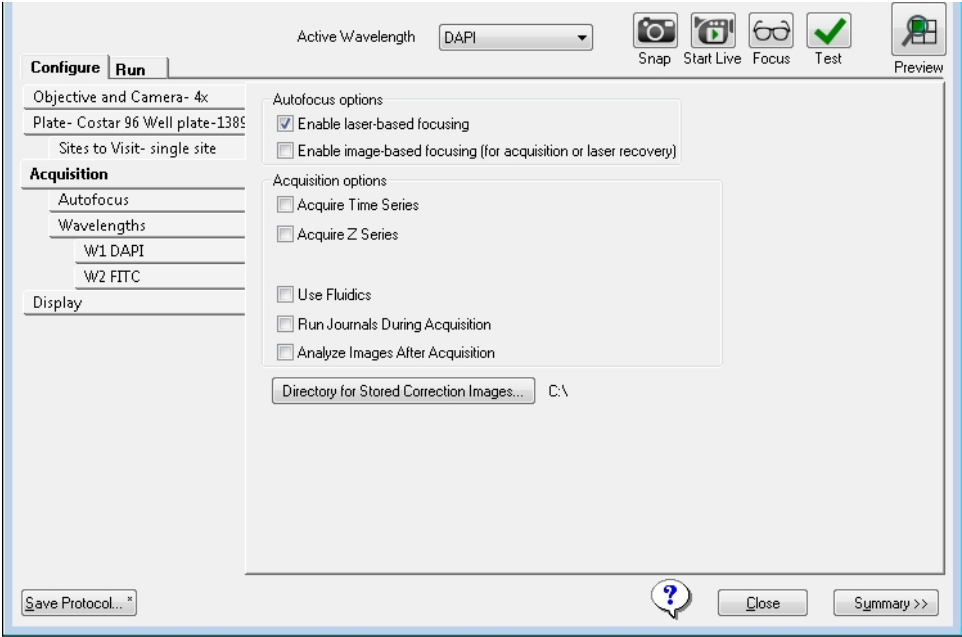


Figure 5-30: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

2. Select **Analyze Images Immediately After Acquisition**.

3. Click **Analysis**.

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**.

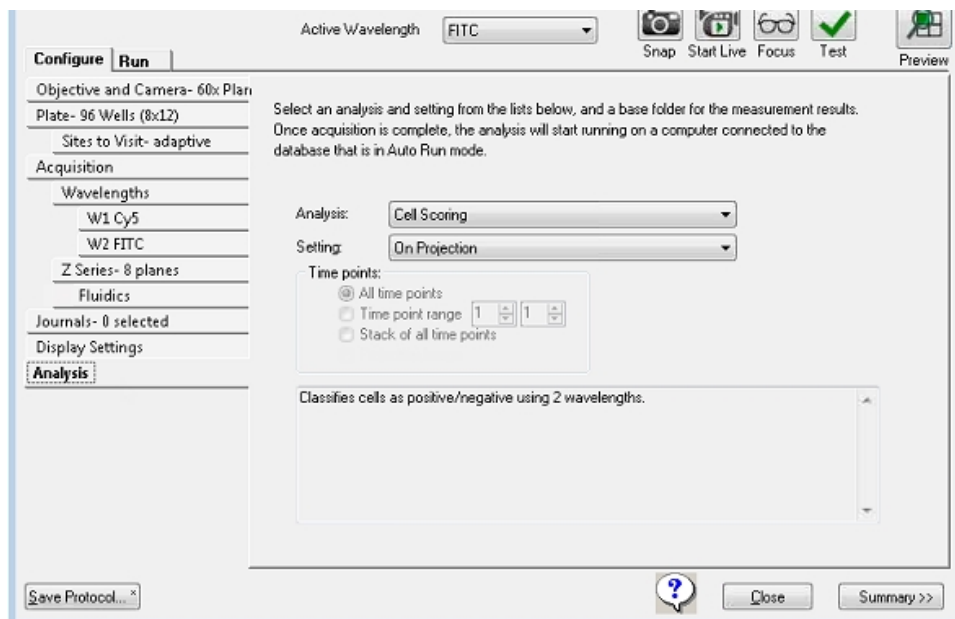


Figure 5-31: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Time Series)

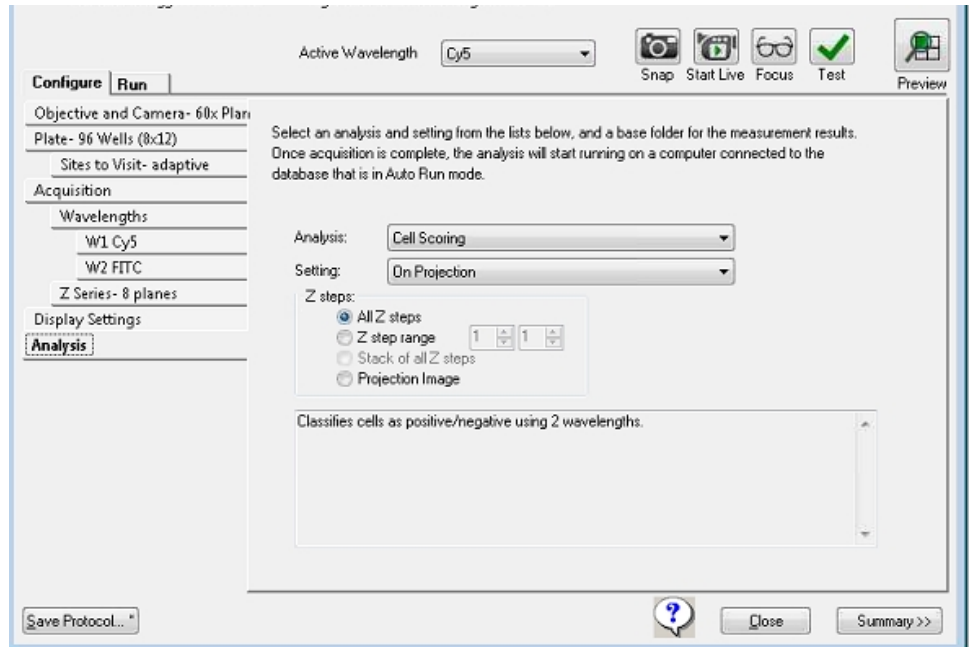


Figure 5-32: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Z Series and 2D Projection Image Only)

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



Note: This list includes any application modules, custom 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: Z step 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 your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
- [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 133](#).

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.

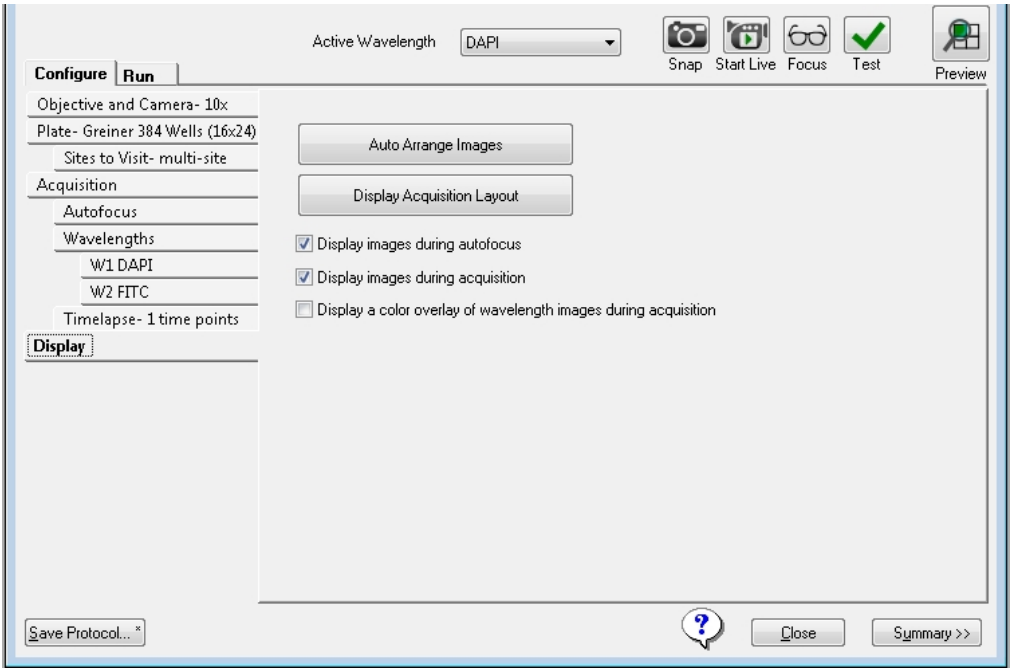


Figure 5-33: Plate Acquisition Setup dialog: Configure tab, Display tab

For a description of the options on this tab, see [Table 5-12: Display Settings on page 134](#).

Table 5-12: Display Settings

Options	Description
Auto Arrange Images	Use the MetaXpress Software default settings for displaying images and dialogs during acquisition. Images autoscale and are arranged in a default layout, and the status dialog is unobstructed. Note: Use the Active Wavelength Preview tool to generate a preview of an acquired image for each configured acquisition wavelength.
Display Acquisition Layout	Displays the current settings for displaying images and dialogs during acquisition. The Plate Acquisition Status dialog also opens. You can manually change the display configuration using a variety of options for this preview. Click OK to register the changes, or Cancel to revert to the previous settings. Note: Display settings are saved with the acquisition protocol.
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.

Configuring the Display Settings for an Acquisition Protocol



Tip: 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 [Viewing the Summary Panel on page 137](#).

1. Click **Display**.
2. To use the MetaXpress Software default settings for displaying images during acquisition, click **Auto Arrange Images**.
3. To display the current settings for displaying images and dialogs during acquisition, click **Display Acquisition Layout**.

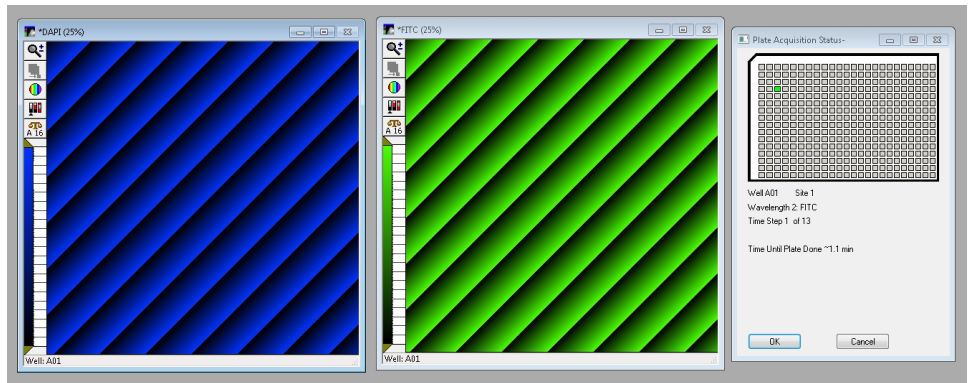


Figure 5-34: Display Settings tab, Display Acquisition Layout Settings

4. Leave the images in this default layout, or optionally, manually adjust them by doing any of the following:



Note: If the image windows are not visible, then click the **Active Wavelength Preview** 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 **Preview** window tools to change the size, scaling, or LUT for an image.

5. Optionally, select or clear any or all the image preview options:
 - **Display images during autofocus**
 - **Display images during acquisition**
 - **Display a color overlay of wavelength images during acquisition**
6. To save the changes, click **OK** or to revert to the previous settings, click **Cancel** . Display settings are saved with the acquisition protocol.
7. If you are done configuring your protocol, continue to [Saving a Plate Acquisition Protocol on page 138](#). Otherwise, continue to any other configuration as needed. See:
 - [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83](#).
 - [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99](#).

Viewing the 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. By default, when the **Plate Acquisition Setup** dialog first opens, the **Summary** panel is closed.

1. At the bottom of the **Plate Acquisition Setup** dialog, click **Summary >>** to open the 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 the content.
 - Click **Copy** to copy the contents to your clipboard, and paste the contents into another program, such as Microsoft Word.
 - Click **Print** to send the contents to a selected printer.

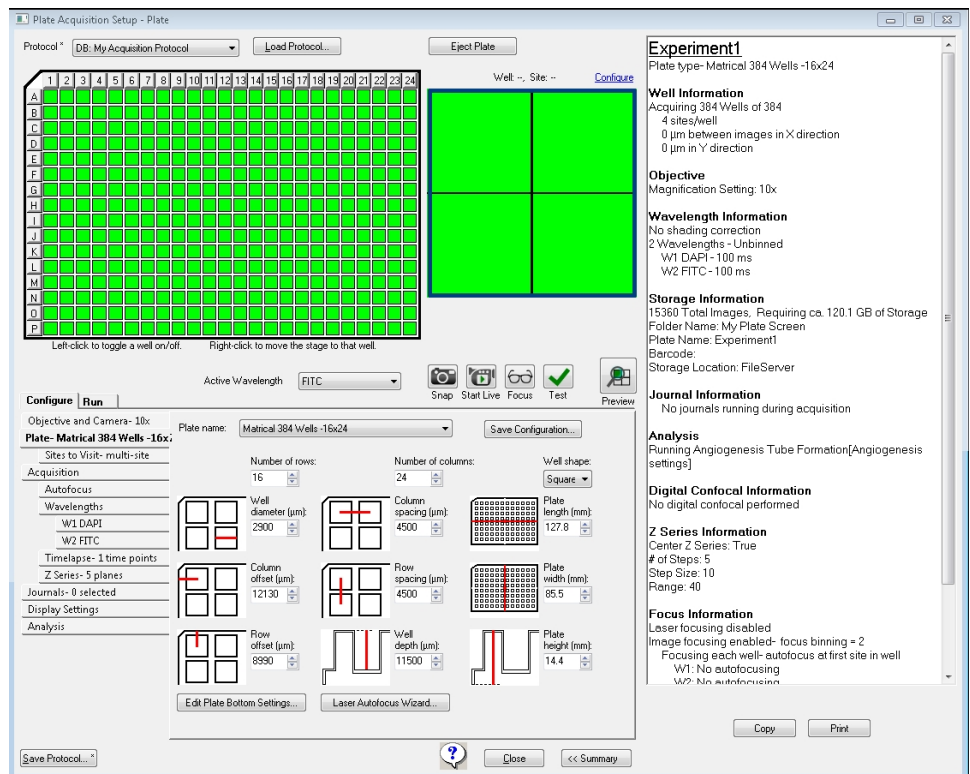


Figure 5-35: Plate Acquisition Setup dialog with open Summary panel

3. When you are done using the **Summary** panel, you can leave it open during the entire process of configuring 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 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 with a robot for automated plate loading, then you must save the protocol to a file.

Saving a Plate Acquisition Protocol to a Database

1. Click **Save Protocol**.
2. In the **Protocol Name** field, type a name for the protocol.
3. Click **Save**.

You can now acquire your plate data with the saved protocol.

Saving 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 can 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.

Chapter 6: Customizing the MetaXpress Software

6

A powerful feature of the MetaXpress Software is the ability to customize the operation of the software for individual users or groups of users. Different objectives and workflows call for customized settings that can be switched as needed. Programs within the MetaXpress Software Suite, such as the Meta Imaging Series Administrator Software and the **Create Taskbar** command, let you create settings that match the needs of your users. The following topics are included this section:

- [Users and Groups in the Meta Imaging Series Administrator Software on page 139](#)
- [Custom Toolbars and Taskbars on page 142](#)
- [Default Paths for Data on page 145](#)

An optional simplified menu structure can be installed to reduce the number of top-level menus in the MetaXpress Software. See [Simplified Menu Structure on page 22](#).



Note: The concepts of users and groups as discussed in this section are specific to custom hardware, and to drop-ins and toolbars settings for the MetaXpress Software. They are NOT related in any way to configuring users and groups within the database. For information on setting up users and groups within the database, see the *MDCStore High Content Data Management Solution Database Schema Installation and Update Guide* included on the MetaXpress Software Suite installation USB flash drive.

Users and Groups in the Meta Imaging Series Administrator Software

The Meta Imaging Series Administrator Software commands enable you to define and configure settings for individual users and groups in the MetaXpress Software. There are two modes for the Administrator:

- Single-User mode, where the Administrator enables you to select hardware settings and configure drop-ins and toolbars for groups that have already been created.
- Multi-User mode, where you create new groups, add users to different groups, and define hardware settings for groups.

As a System Administrator, you work in Multi-User mode creating groups and users for your MetaXpress Software.

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 6-1](#) shows the **Multiple User Configuration** window in the Meta Imaging Series Administrator Software.

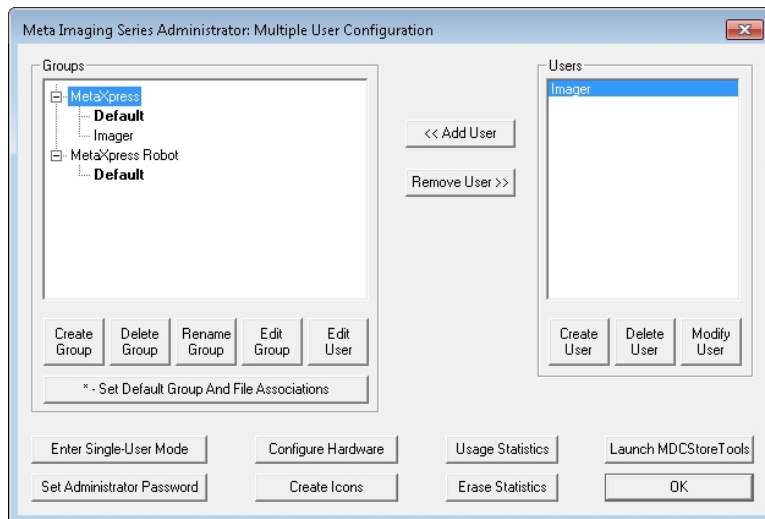


Figure 6-1: Example of the Multiple User Configuration window

This example shows a system with the following groups defined:

- **MetaXpress:** This is the ImageXpress Micro System and MetaXpress Software group.
- **MetaXpress Robot:** The hardware settings in this group enable an optional robot to be used with the MetaXpress Software.

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.

Creating an Offline Version of the MetaXpress Software

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 Software 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 Software group in the Meta Imaging Series Administrator Software:



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

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. In the Meta Imaging Series Administrator Software, if the program opens in **Single User Configuration** mode, click **Enter Multi-User Mode**.
3. Click **Create Group**.
4. In the **Create Group** dialog, in the **Group Name** field, type a group name, such as **MetaXpress Offline**.

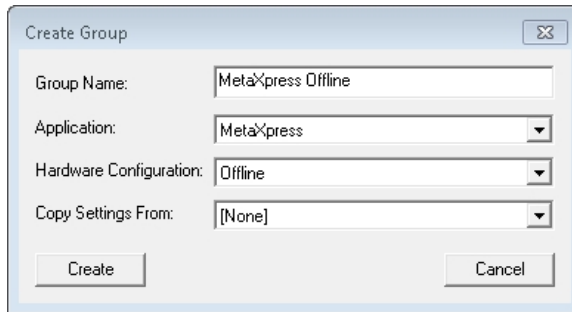


Figure 6-2: Create Group dialog

5. From the **Application** drop-down list, select **MetaXpress**.
6. From the **Hardware Configuration** drop-down list, select **Offline**.
7. From the **Copy Settings From** drop-down list, select **[None]**.
8. Click **Create** to close the **Create Group** dialog and add the new group to the **Groups** list.
9. In the **Groups** list, click the new group.
10. In the **Users** list, click a user that you want to add to the new offline group.
11. Click **<<Add Users** to add the user to the new group.
12. Continue to 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.

13. Click **OK** to exit the Meta Imaging Series Administrator Software.

Creating MetaXpress Software Group Icons and Adding Them to the Windows Desktop

After creating the **MetaXpress Offline** group and adding users with the previous procedure, 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 Windows desktop. These shortcuts can then be copied directly to the Windows desktop. This lets users choose which version to start from the desktop.

Use the following procedure to create and add group icons to the Windows desktop:

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. In the Meta Imaging Series Administrator Software, click **Create Icons** to create the icons.
3. Click **OK** to exit the Meta Imaging Series Administrator Software.
4. Double-click the **MetaXpress 6.0** shortcut on your desktop.
5. In the **MetaXpress 6.0** folder, confirm that a shortcut for the group that was created in the [Creating an Offline Version of the MetaXpress Software on page 140](#) is listed.
6. Right-click the shortcut (for example, **MetaXpress Offline**), and then select **Send To > Desktop** to create the shortcut on the desktop.
7. Continue to add shortcuts to the desktop, as needed.
8. Double-click the new desktop shortcut to open that instance of the software.

Custom Toolbars and Taskbars

After you have groups configured, 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 journals to or remove journals from toolbars.

Use the following procedure to customize the toolbars:

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. In the Meta Imaging Series Administrator Software, from the **List of Groups** list, select the group that has the toolbar you want to edit.
3. Click **Enter Multi-User Mode**.
4. From the **Groups** list, double-click the group that has the toolbar you want to edit.

5. In the **Edit Group** dialog, click **Drop-ins/Toolbars**.
6. In the **Configure Drop-ins/Toolbars** dialog, click the **Toolbars** tab.
7. If it is selected, deselect the **Use default toolbars** check box.
8. In the **Commands** section, select the item you want to add to the toolbar:
 - Select **Menus** to add menu commands to toolbars.
 - Select **Toolbars** to add toolbar commands to other toolbars.
 - Select **Journals** to add journals to any toolbar or to create new Journal toolbars.
9. To add any command to a toolbar, drag the command from the left pane to the appropriate toolbar folder in the right pane.



Note: You can use the **CTRL** or **SHIFT** keys in combination with a mouse-click to select multiple commands, and then drag the commands to the appropriate toolbar folder.

10. Click **OK** when finished.
 11. Click **Yes** in the message to confirm that you want the users in the group to use the modified configuration.
 12. Click **OK** to close the **Edit Groups** dialog.
 13. Click **OK** to exit the Meta Imaging Series Administrator Software.
- The modified toolbars will be available the next time you start the corresponding version of the MetaXpress Software.

Creating Taskbars

Create taskbars directly in the MetaXpress Software. Taskbars are a convenient way to access frequently used commands and journals. Each taskbar can have 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, because they let you add journals as well as commands. Molecular Devices recommends creating taskbars that combine commands and journals specific to your experiments.

To create and load a taskbar, use the following procedure:

1. Start the MetaXpress Software.
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. Define the number of rows and columns for the taskbar by dragging the edges of the **New Taskbar** window until the desired number of rows and columns appear in the window.
5. Define the width of all the buttons in the taskbar by dragging the right or left edge of the active button until the buttons are the desired width.
6. Under **Category**, select the type of item you want to add to the taskbar:
 - Select **Function** to add a software function to the taskbar.
 - Select **Journal** to add a journal to the taskbar.
 - Select **Taskbar** to add a link to another taskbar to the taskbar.



Note: If you select **Journal** or **Taskbar** as the **Category**, the directory names are displayed in square brackets in the list. 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 the active button in the taskbar as shown in [Figure 6-3](#).

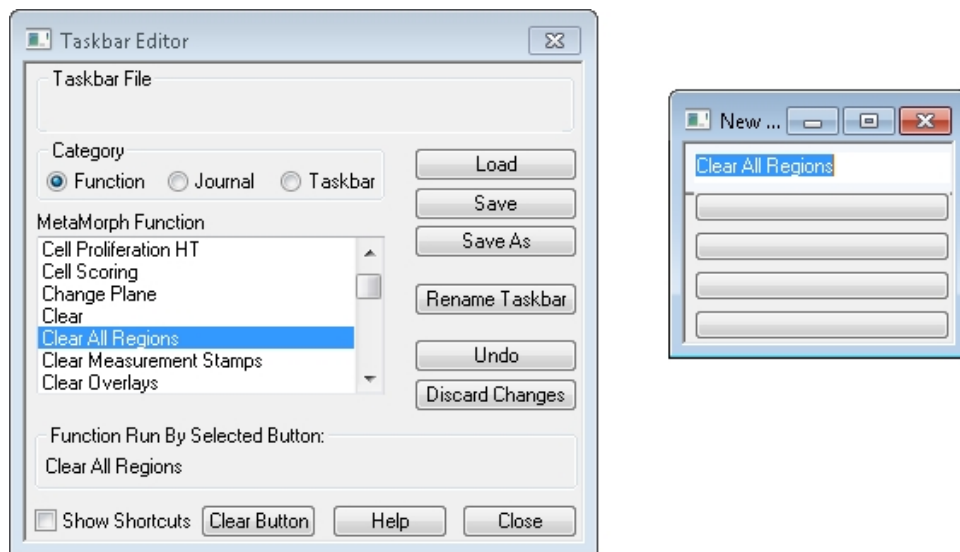


Figure 6-3: Adding a function to a taskbar

8. Continue to add items to the taskbar by clicking a blank button, selecting the **Category**, and double-clicking the entry in the list.
9. If necessary, click **Undo** to undo the last action or click **Clear Button** to clear an item from the active button.

10. To give the task bar a descriptive name, click **Rename Taskbar**.
11. In the **Rename Taskbar** dialog, in the **Taskbar Title** field, type the new name.
12. Click **OK**.
In the **New Taskbar** window, the new name becomes the title of the window.
13. In the **Taskbar Editor** dialog, click **Save**.
14. In the **Save As** dialog, type a name for the taskbar file and navigate to the appropriate drive and folder, if necessary.
15. Click **Save**.
16. In the **Taskbar Editor** dialog, click **Close**.
17. To use the new taskbar (or a different taskbar) immediately, click **Journal > Taskbars > Load Taskbar**.
18. In the **Select a Taskbar** dialog, navigate to the appropriate drive and folder and select the desired taskbar file.
19. Click **Open**.

Default Paths for Data

The **Configure Default Paths** command in the MetaXpress Software 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 that can be unique to each user. Molecular Devices recommends changing the following default paths:

- **Default Data Paths:** Your system 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\Username.
The following data file types should have their file paths changed to point to the data drive:
 - Log files
 - Memory lists
 - Calibrations
 - Illumination Settings
 - Magnification Settings
- **Default HTS State Path:** The MetaXpress Software settings file path should also point to the data drive. For example: D:\MX\HTSSTATE\.



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



Note: Molecular Devices recommends making monthly backups of the Data, and HTS State files.

To edit the default data paths for a group, use the following procedure:

1. Start the MetaXpress Software.
2. Click **Edit > Configure Default Paths**.
3. In the **Configure Default Paths** dialog, select the item with the default file path you want to modify.

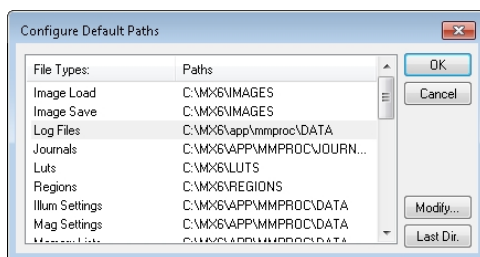


Figure 6-4: Configure Default Paths dialog

4. Click **Modify**.
5. In 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.

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See [Obtaining Support on page 203](#).

Before operating the instrument or performing maintenance operations, make sure that you are familiar with the safety information in this guide. See [Safety Information on page 7](#).

Safety Precautions

To avoid personal injury or damage to the equipment during service or maintenance procedures, observe the following precautions.

- Some procedures require that the power supply for the instrument is turned OFF and that the power cable is unplugged before doing the service or maintenance procedure. If the MetaXpress Software is running, exit the program before turning off the instrument.
- Some procedures require that you 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 as described in the procedure. Avoid contact with other components as they can be damaged or knocked out of alignment.



CAUTION! Do not touch the autofocus laser.

- To prevent dust from collecting inside the instrument, keep all access doors closed unless you are doing maintenance tasks.
- Ensure that all components and access doors are closed before starting the instrument.

The following topics describe maintenance procedures that can be done by users to ensure optimal operation of the instrument.

- [Doing Preventive Maintenance on page 148](#)
- [Cleaning the Instrument on page 148](#)
- [Light Source Maintenance on page 150](#)
- [Replacing a Confocal Disk Box on page 150](#)
- [Filter Maintenance on page 156](#)
- [Objective Maintenance on page 175](#)

- [Updating Shading Correction Settings-Legacy on page 198](#)
- [Turning off the Status Indicator Lights on page 203](#)



WARNING! Service or maintenance procedures other than those specified in this guide can be done only by Molecular Devices qualified personnel. When service is required, contact Molecular Devices technical support.

Doing Preventive Maintenance

To ensure optimal operation of the instrument, do the following preventive maintenance procedures as necessary:

- Wipe off visible dust with a lint-free cloth to avoid dust build up on the instrument.
- Wipe up all spills immediately.
- Follow applicable decontamination procedures as instructed by your laboratory safety officer.
- Respond as required to all error messages displayed by the software.

Molecular Devices recommends turning the power off when the instrument is not in use.

Cleaning the Instrument



WARNING! BIOHAZARD Always wear gloves when operating the instrument and during cleaning procedures that could involve contact with either hazardous or biohazardous materials or fluids.

Always turn the power switch off and disconnect the power cord from the main power source before using liquids to clean the instrument.

- Wipe up all spills immediately.

Cleaning the ImageXpress Micro Confocal System Instrument

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

Before cleaning the instrument, read and follow the [Safety Precautions on page 147](#).

The following cleaning procedure is compatible with disinfectant wipes, such as Kimwipes wipers with 70% ethanol.



CAUTION! Never use an autoclave to clean any instrument components.



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

1. Start the MetaXpress Software and log in to the database.
2. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.
If the **Plate Acquisition** tool bar is not visible, then click **Window > Toolbars > Plate Acquisition**.
In the simplified menu, click **Control > Window > Toolbars > Plate Acquisition**.
3. Exit the MetaXpress Software.
4. On the ImageXpress Micro Systems Power and Options Controller for the ImageXpress Micro Confocal System, power OFF the instrument. See [Shutting Down the System on page 34](#).
5. Ensure that no sample is loaded.
6. With gloved hands, use a damp wipe to wipe down the entire outer surface including side panels and top panels of the instrument.
7. Use an alcohol wipe or a disinfectant wipe and go over the entire surface again.
8. Use forceps wrapped with damp wipes to gently wipe the perimeter of the plate holder and stage region where a plate would normally be loaded.
9. Use forceps wrapped with alcohol wipes or disinfectant wipes to go over the plate holder and stage region again.
10. Use a fresh damp wipe to wipe down the stage area underneath and around the plate loading region.
The stage is freely moving without power, so to clean the plate holder and stage region underneath where the plate is loaded, you can slide the stage around.
11. Use a fresh alcohol wipe or a disinfectant wipe and go over the stage area underneath and around the plate loading region again.
12. Wait a few minutes for the alcohol to evaporate.
13. On the ImageXpress Micro Systems Power and Options Controller for the ImageXpress Micro Confocal System, power ON the instrument. See [Powering On the Instrument on page 29](#).
14. Start the MetaXpress Software and log in to the database.
15. If the top door stays open after restarting the instrument, in the **Plate Acquisition** toolbar, click **Stage Load/Eject** to close the door.

Light Source Maintenance

The ImageXpress Micro Confocal System is equipped with an external light source connected to the instrument with a light guide. The external light source for the ImageXpress Micro Confocal System is a solid-state light source that has a rated lifetime of more than 20,000 hours. There are no user-replaceable parts in this light source.



CAUTION! Do not fold or crimp the light guide. The light quality will be diminished and the fiber optics can break.

Replacing a Confocal Disk Box

In the ImageXpress Micro Confocal System, the Confocal disk box is installed in the Confocal module housing within the lower enclosure of the instrument [Figure 7-1](#). There are three available Confocal disk box configurations. The standard unit contains a single 60 μm pinhole Confocal disk for basic Confocal protocols. There are two optional Confocal disk box configurations. Each configuration includes two Confocal disks. For basic and high-throughput Confocal protocols, there is a dual disk box containing a 60 μm pinhole disk, and a 50 μm slit disk. For basic and high-resolution Confocal protocols, there is a dual disk box containing a 60 μm pinhole disk and a 42 μm pinhole disk. The accessory kit includes the necessary hex keys required for the replacement process.



Figure 7-1: Disk box location in the Confocal module housing

Before replacing a disk box, read and follow the [Safety Precautions on page 147](#).

Basic tools required for replacing all confocal disk boxes:

- Powder-free gloves
- Bulb duster
- 2.5 mm hex key (provided)
- 4 mm hex key (provided)

See the following for the on-instrument Confocal disk box removal and installation procedures:

- [Removing a Disk Box on page 151](#)
- [Installing a Disk Box on page 152](#)
- [Testing a Disk Box on page 154](#)

Removing a Disk Box

Use this procedure if you need to change from one type of Confocal disk box to another type of Confocal disk box, or to replace a Confocal disk box.



Note: To complete this maintenance procedure, the instrument and computer must be powered off.

To remove a disk box:

1. Shut down the system. See [Shutting Down the System on page 34](#).
2. After shutting down the system, wait one minute for the spinning disk in the disk box to stop.
3. Put on powder-free gloves, and open the lower hinged side door to access the Confocal module housing.
4. The disk box is located on the right of the module housing and protected by a secured cover. See [Figure 7-1](#).
5. Using the provided 2.5 mm hex key, loosen only the six 2.5 mm hex screws on the disk box cover, just enough to allow the cover to come off the housing.



Note: The screws should not come out of the cover screw holes.

6. Take the cover off and set it aside.
7. Using the provided 4 mm hex key, loosen only the two 4 mm hex screws securing the disk box to the rails.



Note: The screws should not come out of the screw holes.

8. On the bottom of the disk box, carefully unplug the ribbon cable. See [Figure 7-2](#).

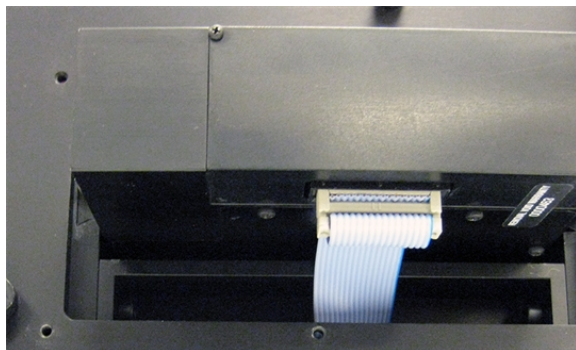


Figure 7-2: Ribbon cable on the bottom of a disk box

9. Slide the disk box out of the instrument, and place the disk box on one of the sides that keeps the field lens on the top and the window on the bottom uncovered and untouched.



Note: Keep dust off the field lens on the top and the window on the bottom.

Installing a Disk Box



Note: To do this procedure the instrument and computer must be powered off, you must wear powder-free gloves, and the installed disk box must have been removed from the instrument. See [Removing a Disk Box on page 151](#).

To install a disk box:

1. Before installing disk box, use bulb duster to blow visible particulates off of the disk box lens and window.
The use of canned air is not recommended, as this can blow fluids onto the optics.
2. Install the new Confocal disk box into the empty disk box slot with the heads of the side hex screws visible.



CAUTION! Do not install the disk box backwards. If you do insert the disk box into the slot backwards, the lens window will be in the wrong location and you will not be able to tighten the side hex screws.

Do not try to force the disk box into the slot. It is not possible to insert the disk box upside-down.

- Using the provided 4 mm hex key, tighten the two 4 mm side hex screws on the disk box so that the box is secured from moving in any direction.



CAUTION! Do not over tighten the hex screws. When properly tightened, the disk box does not move up and down at all, nor will it slide in and out at all.

- On the bottom of the disk box, plug in the ribbon cable. See [Figure 7-3](#).



Figure 7-3: Ribbon cable and cable socket on the bottom of the disk box

- Place the disk box cover over the six screw holes, and using the provided 2.5 mm hex key, tighten the six 2.5 mm hex screws on the disk box cover enough to secure it on the Confocal module housing.
- Close the lower hinged side door.
- Power on the system. See [Powering On the Instrument on page 29](#).
- When the system is powered on, continue by performing a functional test of the new disk box using the MetaXpress Software. See [Testing a Disk Box on page 154](#).

Testing a Disk Box

Use this procedure to verify Confocal disk box functionality.



Note: To complete this maintenance procedure, the instrument and computer must be powered on.

To test a disk box:

1. Open the MetaXpress Software and log in to the database.
2. Click **Devices > Device Control**.

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

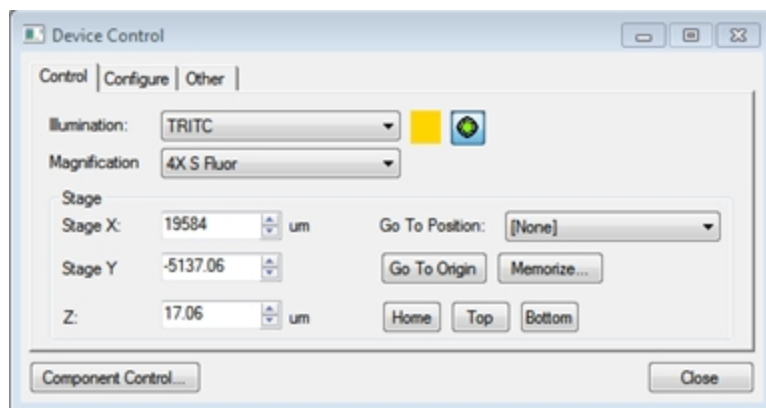


Figure 7-4: Device Control dialog

3. Select a low **Magnification**.
4. Click **Component Control**.

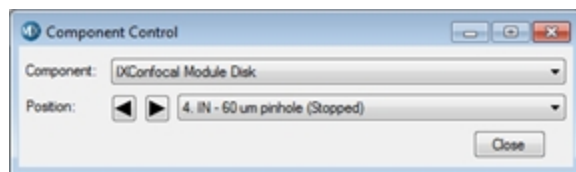


Figure 7-5: Component Control dialog

5. From the **Component** drop-down list, select **IXConfocal Module Disk**.

6. From the **Position** drop-down list, select **<position #>. IN - <Confocal disk type> (Stopped)**.



Note: **Position** options for **Stopped** are even numbers and for **Running** are odd numbers.

7. Click **Stage Load/Eject** to move the stage to the load position.
8. Click **Acquire > Acquire > Show Live**.

In the simplified menu structure, click **Control > Acquire > Acquire > Show Live**.

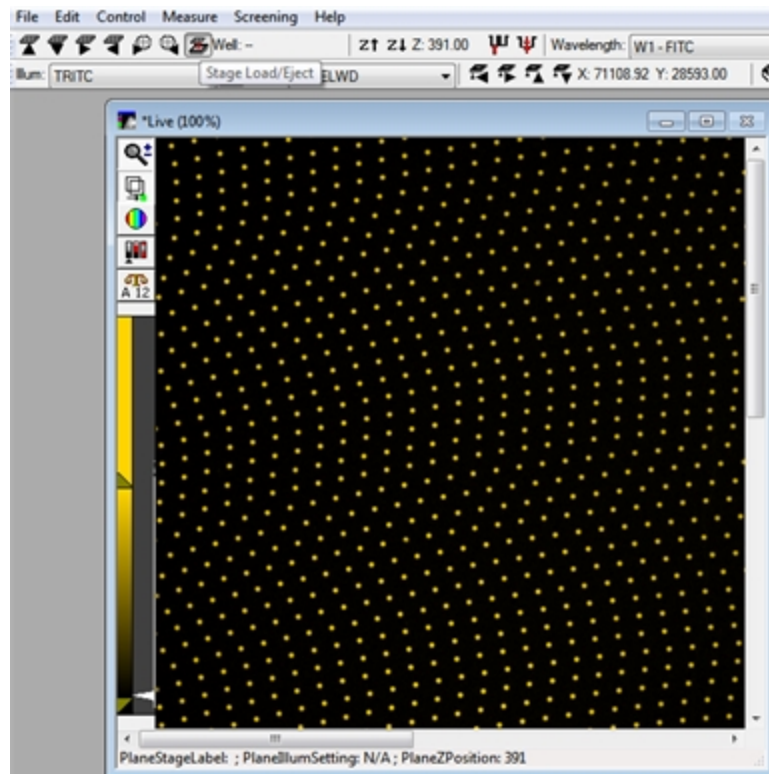


Figure 7-6: Live window showing a stopped confocal pinhole disk pattern when the top door is open

9. In the **Component Control** dialog, from the **Component** drop-down list, select **Component > IXConfocal Module Disk**.

10. From the **Position** drop-down list, select **<position #>. IN - <Confocal disk type> (Running)**.

When the disk spins, the **Live** window image should show a noise pattern.

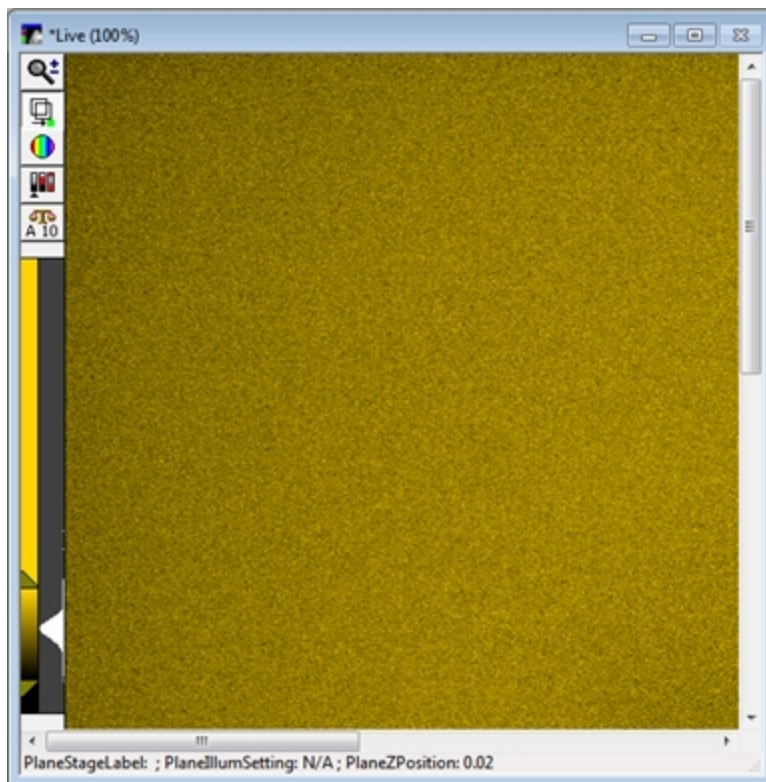


Figure 7-7: Live window showing a running confocal pinhole disk pattern



CAUTION! If the **Live** window pattern does not change, the confocal disk is not spinning. Before contacting support, verify that the disk box installation is correct. See [Installing a Disk Box on page 152](#).

11. If you have a dual confocal disk box, test the other disk next by going back through this procedure starting at step 2. Otherwise, you are finished with the disk box functionality testing.

Filter Maintenance

If you replace or add to the optical components in the factory-standard ImageXpress Micro Confocal System, you must physically change or add the component and then update the software to reflect the new hardware configuration.

Changing or adding a filter requires the following procedures:

- [Replacing Filters on page 157](#)
- [Editing the Filter Settings on page 170](#)
- [Editing the Illumination Settings in the Software on page 173](#)
- [Updating Shading Correction Settings-Legacy on page 198](#)

Replacing Filters

In the ImageXpress Micro Confocal System, the emission filters and the dichroic mirror filters install into separate wheels within the Confocal module housing [Figure 7-8](#). The emission wheel has eight positions for the filters, and the dichroic wheel has five positions for the mirrors. The accessory kit includes a specialized maintenance tool for replacing each of the filter types. Filters are delicate components, and special care is required when handling them.



Figure 7-8: Confocal module housing with filters access door open

Before replacing any filters, read and follow the [Safety Precautions on page 147](#).

Basic tools required, but not provided, for replacing all confocal filters:

- Flashlight
- Powder-free gloves
- Bulb duster



CAUTION! To prevent skin oils from damaging the optical coatings, and to reduce the chances of introducing dust particles to the filter optics, Molecular Devices recommends wearing powder-free gloves during the filter replacement procedures.

See the following for the on-instrument filter and mirror removal and installation procedures:

- [Replacing an Emission Filter on page 158](#)
- [Replacing a Dichroic Mirror on page 165](#)

Replacing an Emission Filter

In the ImageXpress Micro Confocal System, the emission filters install into a wheel within the confocal module within the instrument enclosure. Filters are delicate components, and special care is required when handling them.

Before replacing any filters, read and follow the [Safety Precautions on page 147](#).

Required tools:

- Basic tools required for replacing all filters. See [Replacing Filters on page 157](#).
- Emission filter
- Emission Filter tool

Replacing an emission filter involves the following procedures:

- [Removing an Emission Filter on page 159](#)
- [Installing an Emission Filter on page 163](#)

Removing an Emission Filter

Use this procedure to change from one kind of emission filter to another kind of emission filter, to replace an existing emission filter, or add additional emission filters.



Note: To complete this maintenance procedure, the instrument and computer must be powered on.

To remove an emission filter:

1. Exit the MetaXpress Software if it is running.
2. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, select a hardware configuration from the **Hardware Settings** list, and click **Configure Devices**.
5. In the **User Settings** dialog, in the **Claimed Devices** list, under **ImageXpress Confocal Module**, select **IXConfocal Module Emission Wheel** and click **Settings**.

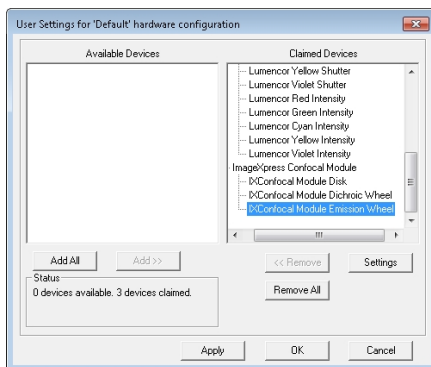


Figure 7-9: User Setting Selection of IXConfocal Module Emission Wheel Settings

6. In the **IXConfocal Module Emission Wheel Settings** dialog, set the **Filter Exchange Mode** to **On**.

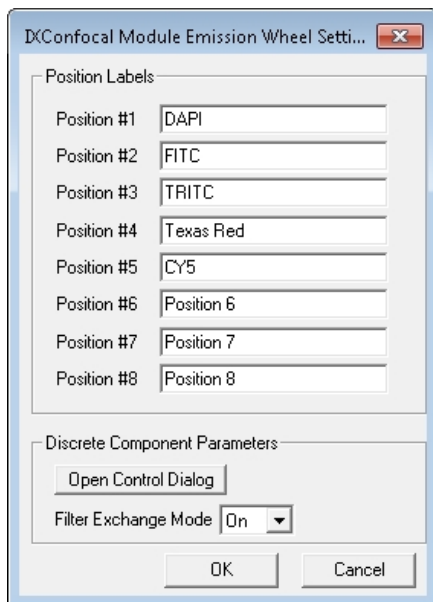


Figure 7-10: The IXConfocal Module Emission Wheel Settings dialog

7. Click **Open Control Dialog**.
8. From the **Position Controls**, select the numbered position for the filter to be removed.



CAUTION! For selecting **Position 1**, although **Position 1** shows as the default selection when the dialog opens, the filter wheel may not be in the corresponding position. Select a different position, wait for the wheel to move, then select **Position 1** again.

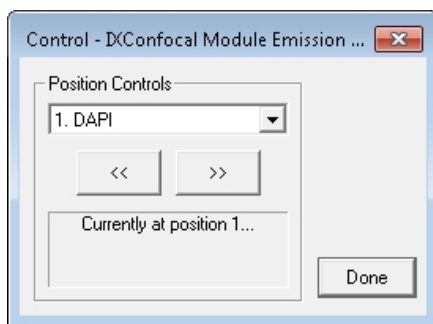


Figure 7-11: IXConfocal Module Emission Wheel Control dialog

9. When the wheel moves to your selected position, click **Done**.
10. Put on your gloves, and open the lower hinged side door to access the confocal module housing.
11. Open the filter access door on the left of the confocal module housing. The slot for the emission filter is on the left.

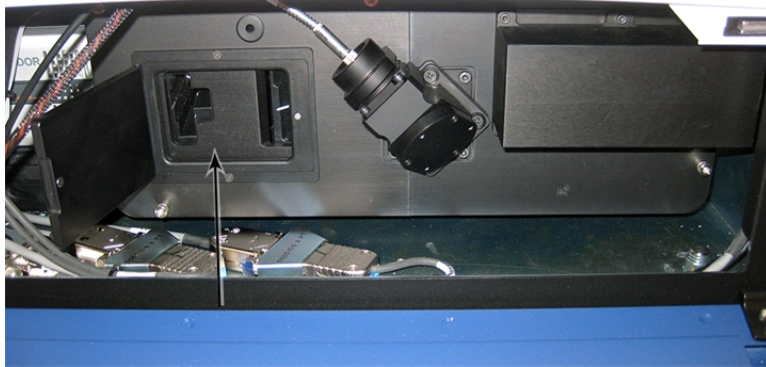


Figure 7-12: Confocal module housing with filters access door open

12. Use a flashlight to look into the emission access slot to get familiar with the tool alignment groove, and verify that the you can see the rim of the selected filter.



Note: If the rim of the filter is not visible, verify that you did steps 6 through 8 correctly.

13. Squeeze the spring-loaded handle of the emission tool with your thumb and first two fingers, and insert the tool, pushed to the left side, all of the way into the emission access slot.

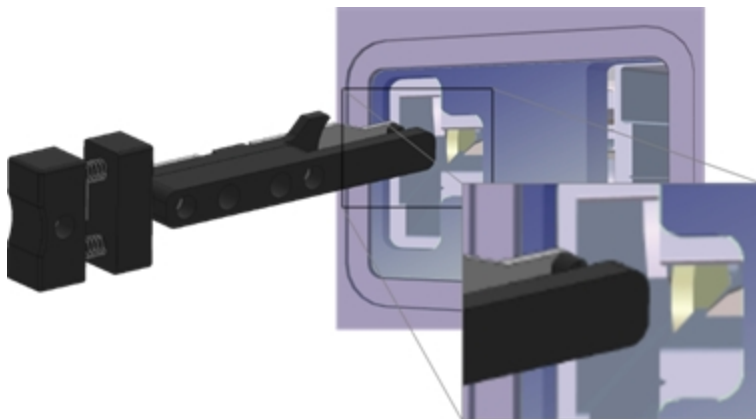


Figure 7-13: Orientation for Inserting the Emission Filter Tool

14. Move the tool to the right, and when you can feel the tool surround the filter rim, release your squeeze on the handle.
15. Set aside your flashlight, place this free and gloved hand under the emission filter access slot, and pull the tool straight out of the slot. Use your hand under the access slot to catch the possibly loose filter. The filter should be captured on the other end of the tool.
16. Carefully set aside the removed filter.
17. Continue to install an emission filter into the now empty wheel slot. See [Installing an Emission Filter on page 163](#).

Installing an Emission Filter



Note: It is assumed that you are continuing from having just removed an emission filter from a specified wheel slot position, or you have specified an empty wheel slot, and you are still using the Meta Imaging Series Administrator Software.

To install an emission filter:



CAUTION! To protect the emission filter, load the emission tool with the emission filter over a table or other work surface.

1. Squeeze the spring-loaded handle of the emission tool with your thumb and first two fingers, and place the new emission filter lens on the tool with the arrow on the rim pointing down.



Note: When installed, the arrow points toward the camera.

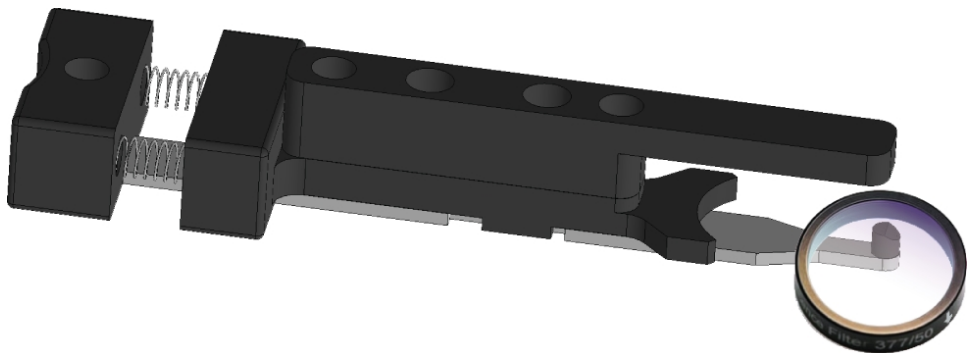


Figure 7-14: Emission Filter Tool and Emission Filter Orientation

2. Release your squeeze on the handle to secure the filter lens on the end of the emission tool.
3. Insert the filter secured on the emission tool into the emission access slot until you feel the filter click into place.



Note: Use a flashlight to help guide the installation, if needed.

4. Squeeze the handle on the tool to release the filter.
5. Move the tool to the left, pull the emission tool out of the access slot, and release your squeeze on the tool handle, and verify that the filter installed properly.
6. Close the filters access door of the confocal module housing.

7. Close the blue lower hinged side door.
8. If the filter is a different kind than the one removed, in the Meta Imaging Series Administrator Software, in the **IXConfocal Module Emission Wheel Settings** dialog, in the corresponding **Position Labels** field, enter the new filter name.

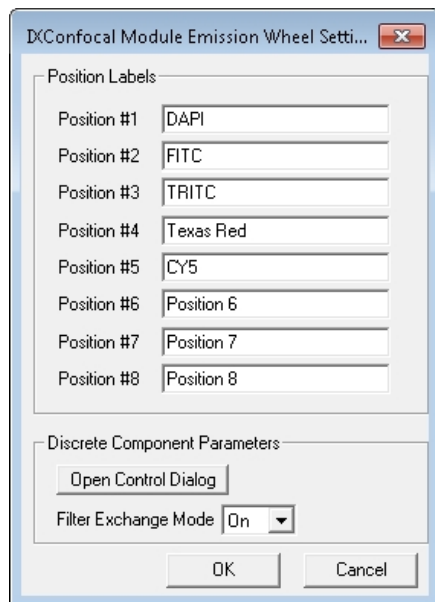


Figure 7-15: The IXConfocal Module Emission Wheel Settings dialog

9. In the **IXConfocal Module Emission Wheel Settings** dialog, set the **Filter Exchange Mode** to **Off**.
10. Click **OK** to close the **IXConfocal Module Emission Wheel Settings** dialog.
11. Click **OK** to close the **User Settings** dialog.
12. Click **OK** to close the **Configure Hardware** dialog.
13. If you are finished with administrator tasks, click **OK** to close the Meta Imaging Series Administrator Software.

Replacing a Dichroic Mirror

In the ImageXpress Micro Confocal System, the dichroic mirrors install into a wheel within the instrument enclosure. Mirrors are delicate components, and special care is required when handling them.

Before replacing any dichroic mirrors, read and follow the [Safety Precautions on page 147](#).

Required tools:

- Basic tools required for replacing all filters. See [Replacing Filters on page 157](#).
- Dichroic mirror in frames
- Dichroic Mirror tool

Replacing a dichroic mirror involves the following procedures:

- [Removing a Dichroic Mirror on page 165](#)
- [Installing a Dichroic Mirror on page 168](#)

Removing a Dichroic Mirror

Use this procedure to change from one kind of dichroic mirror to another kind of dichroic mirror, or to replace an existing dichroic mirror.



Note: To complete this maintenance procedure, the instrument and computer must be powered on.

To remove a dichroic mirror:

1. Exit the MetaXpress Software if it is running.
2. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, select a hardware configuration from the **Hardware Settings** list, and click **Configure Devices**.

5. In the **User Settings** dialog, in the **Claimed Devices** list, under **ImageXpress Confocal Module**, select **IXConfocal Module Dichroic Wheel** and click **Settings**.

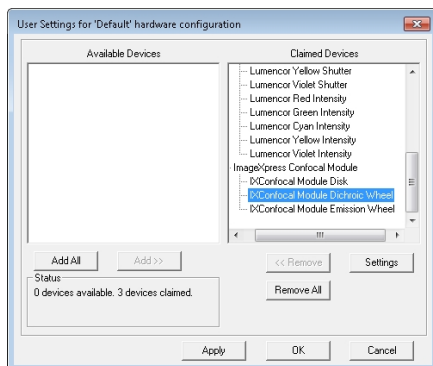


Figure 7-16: User Setting Selection of IXConfocal Module Dichroic Wheel Settings

6. In the **IXConfocal Module Dichroic Wheel Settings** dialog, set the **Filter Exchange Mode** to **On**.

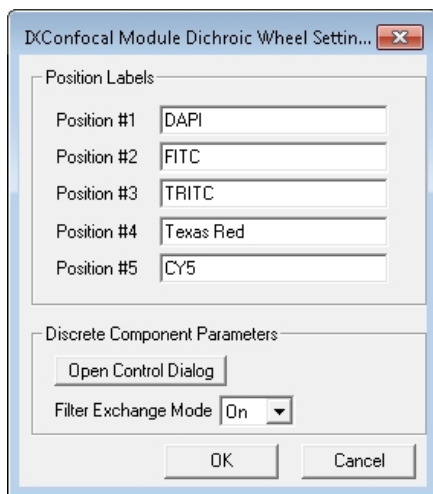


Figure 7-17: The IXConfocal Module Dichroic Wheel Settings dialog

7. Click **Open Control Dialog**.

8. From the **Position Controls**, select the numbered position for the filter to be removed.



CAUTION! For selecting **Position 1**, although **Position 1** shows as the default selection when the dialog opens, the filter wheel may not be in the corresponding position. Select a different position, wait for the wheel to move, then select **Position 1** again.

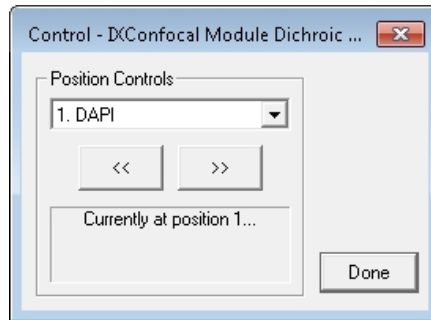


Figure 7-18: IXConfocal Module Dichroic Wheel Control dialog

9. When the wheel moves to your selected position, click **Done**.
10. Put on your gloves, and open the lower hinged side door to access the confocal module housing.
11. Open the filter access door on the left of the confocal module housing. The slot for the dichroic mirror is on the right.



Figure 7-19: Confocal module housing with filters access door open

12. Use a flashlight to look into the dichroic access slot to identify the tool alignment groove, and verify that you can see the rim of the selected dichroic mirror.



Note: If the rim of the dichroic mirror is not visible, verify that you did steps 6 through 8 correctly.

13. Squeeze the spring-loaded handle of the dichroic mirror tool with your thumb and first two fingers, and insert the tool all of the way into the dichroic mirror access slot.

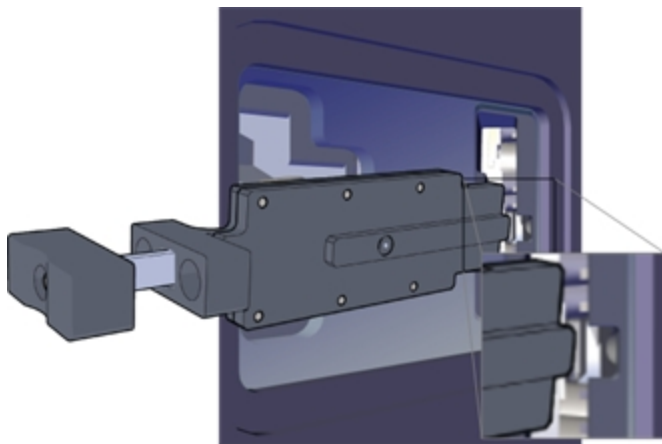


Figure 7-20: Orientation for Inserting the Dichroic Mirror Tool

14. When the tool is inserted so the depth indicator is flush with the housing and you can feel the tool press up against the outer edge of the mirror rim, release your squeeze on the handle.
15. Set aside your flashlight, place this free and gloved hand under the dichroic mirror access slot, and pull the tool out of the slot. Use your hand under the mirror access slot to catch the possibly loose mirror. The mirror should be captured on the other end of the tool.
16. Carefully set aside the removed mirror.
17. Continue to install a dichroic mirror into the now empty wheel slot. See [Installing a Dichroic Mirror on page 168](#).

Installing a Dichroic Mirror



Note: It is assumed that you are continuing from having just removed a dichroic mirror from a specified wheel slot position, and you are still using the Meta Imaging Series Administrator Software. See [Removing a Dichroic Mirror on page 165](#).

To install a dichroic mirror:



CAUTION! To protect the dichroic mirror, load the dichroic mirror tool with the dichroic mirror over a table or other work surface.

1. Squeeze the spring-loaded handle of the dichroic mirror tool with your thumb and first two fingers, and place the new dichroic framed mirror on the tool.



Note: When installed, the mirror will face the camera.

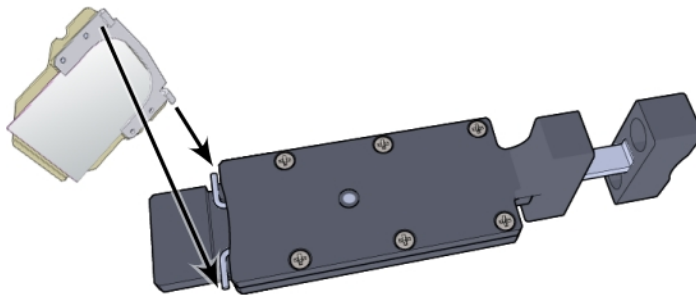


Figure 7-21: Dichroic Mirror Tool and Dichroic Mirror Installation Orientation

2. Release your squeeze on the handle to secure the mirror on the end of the dichroic mirror tool.
3. Verify that the mirror frame is captured by the springs on the tool.
4. Insert the mirror secured on the dichroic mirror tool into the dichroic access slot until you feel the mirror click into place.



Note: Use a flashlight to help guide the installation, if needed.

5. Squeeze the handle on the tool to release the mirror.
6. Pull the dichroic tool straight out of the access slot, and release your squeeze on the tool handle, and verify that the mirror installed properly.
7. Close the filter access door of the confocal module housing.
8. Close the blue lower hinged side door.

9. (Optional) In the Meta Imaging Series Administrator Software, in the **IXConfocal Module Dichroic Wheel Settings** dialog, in the corresponding **Position Labels** field, enter a new filter name.

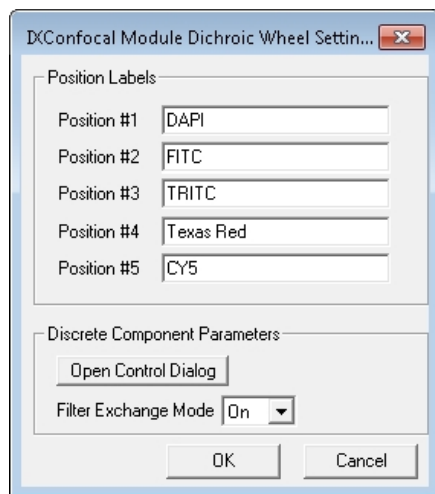


Figure 7-22: The IXConfocal Module Dichroic Wheel Settings dialog

10. In the **IXConfocal Module Dichroic Wheel Settings** dialog, set the **Filter Exchange Mode** to **Off**.
11. Click **OK** to close the **IXConfocal Module Dichroic Wheel Settings** dialog.
12. Click **OK** to close the **User Settings** dialog.
13. Click **OK** to close the **Configure Hardware** dialog.
14. If you are finished with administrator tasks, click **OK** to close the Meta Imaging Series Administrator Software.

Editing the Filter Settings

The ImageXpress Micro Confocal System uses emission filters and dichroic mirrors. Configure the settings in the Meta Imaging Series Administrator Software for both types of filters. There are separate settings dialogs.

See:

- [Editing the Emission Filter Settings on page 171](#)
- [Editing the Dichroic Mirror Settings on page 172](#)

Editing the Emission Filter Settings

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. In the **Meta Imaging Series Administrator**, from **List of Groups**, select **MetaXpress**.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, select a hardware configuration from the **Hardware Settings** list, and click **Configure Devices**.
5. In the **User Settings** dialog, in the **Claimed Devices** list, under **ImageXpress Confocal Module**, double-click **IXConfocal Module Emission Wheel**.
6. In the **IXConfocal Module Emission Wheel Settings** dialog, edit the name of the filter you are adding or replacing in the appropriate **Position Labels** field.

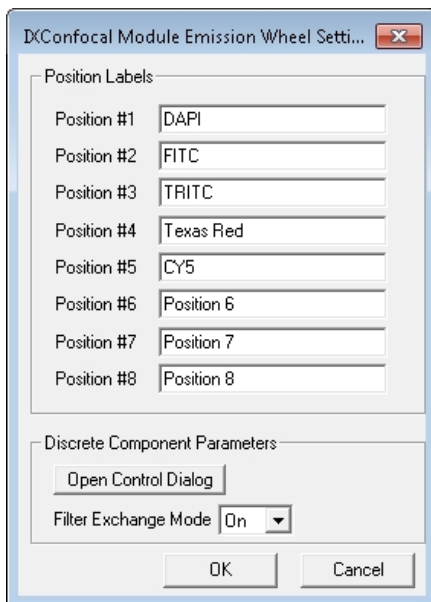


Figure 7-23: The IXConfocal Module Emission Wheel Settings dialog

7. Click **OK** to close the **IXConfocal Module Emission Wheel Settings** dialog.
8. Click **OK** to close the **User Settings** dialog.
9. If multiple hardware configurations are present and are affected by the change, repeat the preceding steps for each appropriate hardware configuration.
10. Click **OK** to close the **Configure Hardware** dialog.
11. If you are finished with administrator configuration tasks, click **OK** to close the Meta Imaging Series Administrator Software.

Editing the Dichroic Mirror Settings

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. In the Meta Imaging Series Administrator Software, from **List of Groups**, select **MetaXpress**.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, select a hardware configuration from the **Hardware Settings** list, and click **Configure Devices**.
5. In the **User Settings** dialog, in the **Claimed Devices** list, under **ImageXpress Confocal Module**, double-click **IXConfocal Module Dichroic Wheel**.
6. In the **IXConfocal Module Dichroic Wheel Settings** dialog, edit the name of the mirror you are adding or replacing in the appropriate **Position Labels** field.

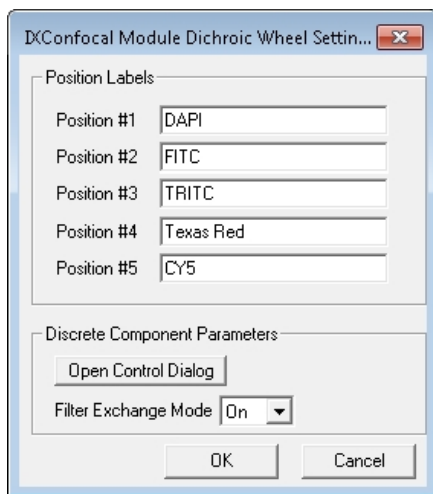


Figure 7-24: The IXConfocal Module Dichroic Wheel Settings dialog

7. Click **OK** to close the **IXConfocal Module Dichroic Wheel Settings** dialog.
8. Click **OK** to close the **User Settings** dialog.
9. If multiple hardware configurations are present and are affected by the change, repeat the preceding steps for each appropriate hardware configuration.
10. Click **OK** to close the **Configure Hardware** dialog.
11. If you are finished with administrator configuration tasks, click **OK** to close the Meta Imaging Series Administrator Software.

Editing the Illumination Settings in the Software

1. Start the MetaXpress Software and log in to 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 Settings** field and then select the filter that you installed from the corresponding option field.

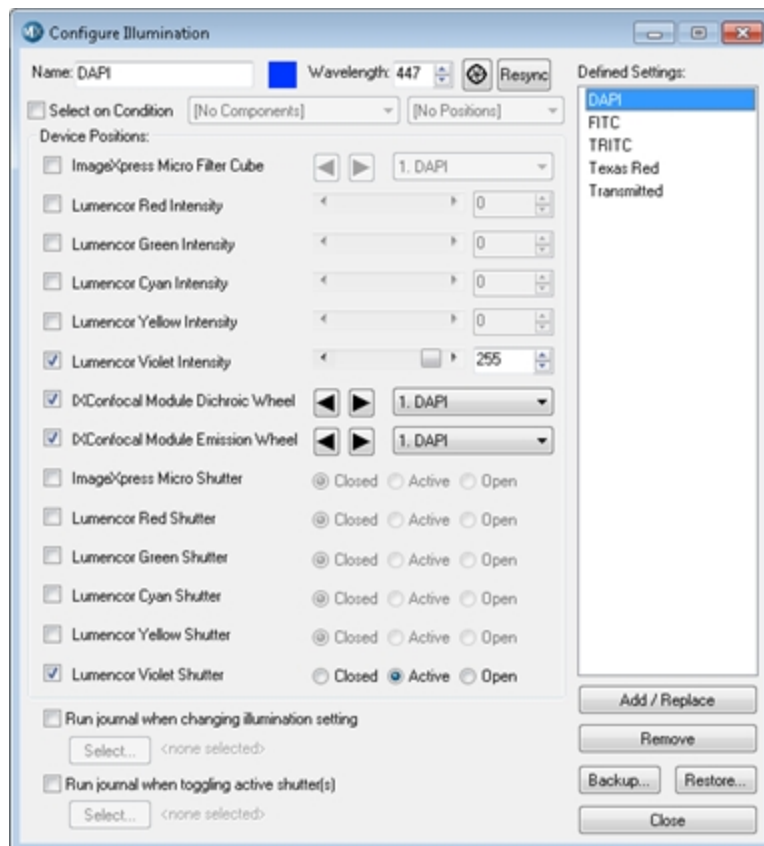


Figure 7-25: The Configure Illumination dialog

4. Ensure that the appropriate shutter is selected as **Active** for each filter set.

- 5. Set up other illumination settings if needed.
The value in the **Wavelength** field should match the center wavelength for the emission filter.



CAUTION! Never set more than one Lumencor Shutter to Active.

- 6. In the **Name** field, type the name of the new filter.
- 7. In the **Wavelength** field, type the emission wavelength of the new filter.
- 8. Click **Add/Replace** to add this setting to the **Defined Settings** list.
- 9. If you replaced a filter with an existing setting, select the old setting from the **Defined Settings** field and then click **Remove**.
- 10. After the settings are updated, click **Backup** to back up the modified settings to a file.
- 11. Click **Close**.

Settings for Common Fluorophores

Table 7-1: Settings for Common Fluorophores

Fluorophore	Shutter Color Channel	Excitation Filter
DAPI, Hoechst	Violet (380 to 420 nm)	377/50 nm
FITC, GFP, EGFP	Cyan (440 to 500 nm)	475/34 nm
TRITC, Cy3	Green (490 to 550 nm)	531/40 nm
Texas Red, mCherry	Yellow (530 to 610 nm)	560/32 nm
Cy5	Red (610 to 660 nm)	631/28 nm

Objective Maintenance

If you replace or add to the optical components in the factory-standard ImageXpress Micro System, you must physically change or add the component and then update the software to reflect the new hardware configuration.

You can clean an objective lens without removing it from the instrument. See [Cleaning an Objective on page 176](#).

If you are using oil-immersion objectives, see [Using Oil-Immersion Objectives on page 177](#).

Correct Objective Placement



Note: Molecular Devices recommends placing ELWD objectives (20X, 40X, 60X) or any objective with a correction collar in one of the outer positions (1 or 4). The outer positions give easy access through one of the side panels for adjusting the correction collar. See [Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 178](#).

After installing a new objective, you must update the objective settings in both the Meta Imaging Series Administrator Software and the main MetaXpress Software.

If the objective you are replacing was the one used to determine the plate bottom reference point, you also must do the procedures described in [Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 186](#), or contact Molecular Devices technical support. See [Obtaining Support on page 203](#).



Note: Molecular Devices recommends that you leave the reference objective in place and replace only the other objectives. The reference objective is typically a 10x objective.

Changing or adding an objective can require the following procedures:

- [Replacing an Objective on page 179](#)
- [Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 180](#)
- [Configuring Parfocality after Changing Objectives on page 183](#)
- [Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 186](#)
- [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 193](#)

- [Updating Magnification and Calibration Settings on page 196](#)
- [Updating Shading Correction Settings-Legacy on page 198](#)

Cleaning an Objective

If debris or contaminants have collected on an objective, follow these instructions for cleaning the objective lens.

Before cleaning an objective, read and follow the [Safety Precautions on page 147](#).



CAUTION! To prevent skin oils from damaging the optical coatings, Molecular Devices recommends wearing powder-free gloves during the following procedure.

1. Start the MetaXpress Software and log in to the database.
 2. In the MetaXpress Software select the objective that needs to be cleaned.
 3. Open the upper, hinged side panel door of the instrument.
 4. Exit the MetaXpress Software.
 5. On the ImageXpress Micro Systems Power and Options Controller for the ImageXpress Micro Confocal System, power OFF the instrument.
 6. Use a bulb duster to carefully to blow dust contaminants off the objective.
-



CAUTION! Do not use a product that disperses aerosol propellants or fluid onto the lens surface, such as a compressed-air can.

7. Use lens paper, and if necessary, lens cleaner like 100% methanol, to gently wipe the objective free of contaminants.

For the preferred cleansing solvent and procedure, see the information from the objective manufacturer.



CAUTION! Do not use Kimwipes wipes or similar lint-leaving products to wipe a lens.

Using Oil-Immersion Objectives

Oil-immersion objectives can be used with the ImageXpress Micro Confocal System for research-mode imaging. For ordering information, see your sales representative.



Note: Oil-immersion objectives are not recommended for scanning entire microplates.

To apply oil to the objective:

1. Eject the plate to open the top door.
2. Remove any plates in the instrument.
3. Use a dropper bottle to sparingly add oil to the top of the appropriate objective.
4. Insert the sample with either a thin glass coverslip or in a microplate with a thin glass bottom.



Note: Oil-immersion objectives are not compatible with plastic microplates.

5. In the MetaXpress Software, select **Plate Acquisition Setup > Configure > Acquisition > Autofocus options > Enable image-based focusing**.



Note: Only use **Image-based Focusing** with oil-immersion objectives.

6. Select **Autofocus > Initial well for finding sample > Skip Find Sample**.



Note: Oil-immersion objectives are not compatible with the **Laser Autofocus Wizard**.

7. Slowly step up the objective manually until you are near to focus.
8. When you are done with the oil-immersion objective, eject the plate, remove the sample, and use lens paper to clean the top of the objective.

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 Confocal System have adjustable correction collars, that are used to minimize spherical aberration in the image of the specimen. The collars have a range of 0 mm to 2 mm correction, and changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very much 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, set the correction collar for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined in one of the following ways:

- Get the plate specifications from the plate manufacturer.
- Break a spare plate and use calipers to measure the thickness.
- Measure the optical thickness with the laser autofocus and multiply it by the refractive index of 1.59 for polystyrene or 1.52 for glass.

After you have determined the thickness of your plate or slide, you can adjust the correction collar.



CAUTION! If the thickness of the intended plate, slide, or coverslip is out of the range of the correction collar, it cannot be used with the selected objective.

Before adjusting the correction collar on an objective, read and follow the [Safety Precautions on page 147](#).

1. Start the MetaXpress Software and log in to the database.
2. In the MetaXpress Software move the objective changer to an appropriate position for accessing 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.

Molecular Devices recommends placing ELWD objectives (20X, 40X, 60X) or any objective with a correction collar in one of the outer positions (1 or 4). The outer positions give easy access through one of the side panels for adjusting the correction collar.

3. Exit the MetaXpress Software.
4. On the ImageXpress Micro Systems Power and Options Controller for the ImageXpress Micro Confocal System, power OFF the instrument.

5. Open the upper hinged side door of the instrument.
6. Locate the correction collar on the objective that you want to adjust.



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

7. Rotate the correction collar to its new setting.
8. Close the upper, hinged side panel door.
9. Start the MetaXpress Software and log in to the database.
10. Test the correction collar setting by examining the image quality of acquired images.
If the quality has degraded, re-adjust the correction collar by repeating this procedure.

Replacing an Objective

The objectives in the ImageXpress Micro Confocal System are mounted in a four-position linear selector. Objectives are very delicate components, and special care is required when handling them.

Before replacing an objective, read and follow the [Safety Precautions on page 147](#).

To determine the correct position for the objective, see [Correct Objective Placement on page 175](#).



CAUTION! To prevent skin oils from damaging the optical coatings, Molecular Devices recommends wearing powder-free gloves during the following procedure.

1. Start the MetaXpress Software and log in to the database.
2. In the MetaXpress Software 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.
3. Exit the MetaXpress Software.
4. On the ImageXpress Micro Systems Power and Options Controller for the ImageXpress Micro Confocal System, power OFF the instrument.
5. Place the new objective in its protective casing on a clean work-area surface near the front of the ImageXpress Micro Confocal System.

6. Open the upper hinged side panel door of the instrument.



CAUTION! When moving objectives in and out, beware of the free-moving stage. It slides around loosely when the instrument is powered off. Use caution to prevent damage to the objective in your hand.

7. Reach in and unscrew the 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 correction collar on the objective, if applicable.
9. With the objective in your hand, reach into the instrument and screw the objective into its position.
10. Close the upper hinged side panel door of the instrument.
11. On the ImageXpress Micro Systems Power and Options Controller for the ImageXpress Micro Confocal System, power ON the instrument.
12. Update the Meta Imaging Series Administrator Software to reflect the new hardware configuration. See [Editing the Objective Settings in the Meta Imaging Series Administrator Software](#) on page 180.

Editing the Objective Settings in the Meta Imaging Series Administrator Software

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. In the **Meta Imaging Series Administrator**, from **List of Groups**, select **MetaXpress**.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, click **Install System Devices**.
5. In the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.

6. In the **ImageXpress Micro Objective Settings** dialog, edit the text in the appropriate **Objective #** field for the new objective, if necessary.

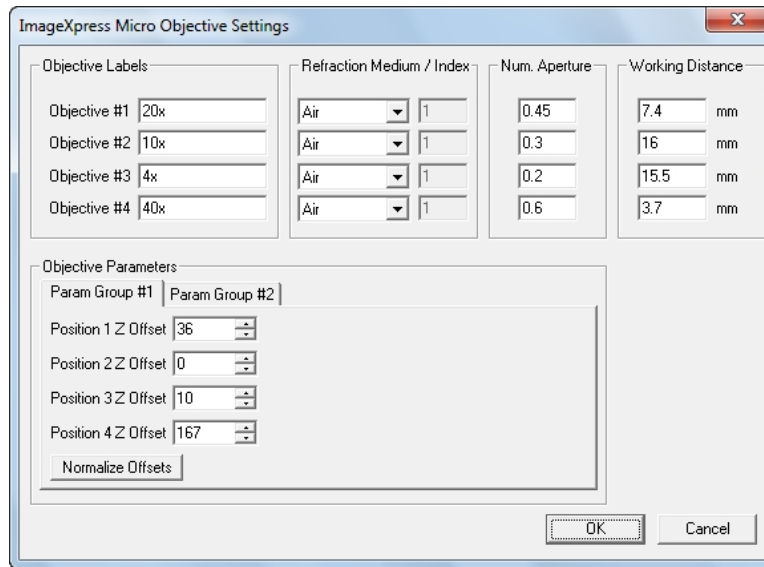


Figure 7-26: ImageXpress Micro Objective Settings Dialog

7. Edit the **Refractive Medium/Index** value, if necessary.
8. In the corresponding **Num. Aperture** field, type the numerical aperture for the new objective as is printed on the objective.



Note: Make a note of the values you entered in the previous steps. 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. In the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro** and then click **Settings**.
11. In the **ImageXpress Micro Settings** dialog, ensure that the **Parameter Group #1** tab is active and then select the **Maintenance Mode** check box.
12. Click **OK** to close the **ImageXpress Micro Settings** dialog.
13. Click **OK** to close the **Install System Devices** dialog.



Note: In the next steps, type the previously entered values in the **Configure Devices** dialog to ensure that the settings carry over for all hardware profiles.

14. 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**.
15. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.
16. In the **ImageXpress Micro Objective Settings** dialog, edit the text in the appropriate **Objective #** field for the new objective with the same information you previously entered.
17. Edit the **Refractive Medium/Index** value with the same information you previously entered.
18. In the corresponding **Num. Aperture** field, type the numerical aperture for the new objective with the same information you previously entered.
19. Click **OK** to close the **ImageXpress Micro Settings** dialog.
20. Click **OK** to close the **User Settings** dialog.
21. Click **OK** to close the **Configure Hardware** dialog.
22. Close the **Meta Imaging Series Administrator**.
23. Configure parfocality. See [Configuring Parfocality after Changing Objectives on page 183](#).

Configuring Parfocality after Changing Objectives

To configure parfocality, you must use the MetaXpress Software to find valid focus values for each objective, and then enter them into the **MetaXpress Objective** dialog in the Meta Imaging Series Administrator Software. See [Filter Specifications and Calibration Compatability on page 225](#).

1. Start the MetaXpress Software and log in to the database.
2. Click **Devices > Configure Magnification**.

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

3. In the **Configure Magnification** dialog, select the **ImageXpress Micro Objective** check box.

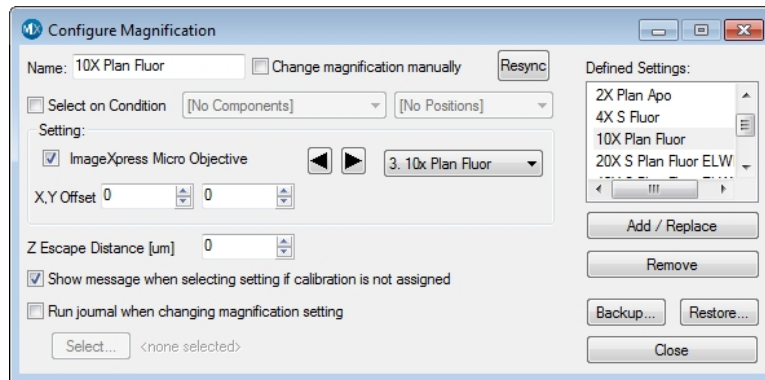


Figure 7-27: The Configure Magnification dialog

4. From the **Setting** drop-down list, select the objective with the highest numerical aperture (NA) that is in position #1.
5. In the **Device Control** toolbar, from the **Illum** drop-down list, select the **FITC** (or other visible light) illumination setting.



Figure 7-28: Illum setting in the Device Control toolbar

If the **Device Control** tool bar is not visible, then click **Window > Toolbars > Device Control**.

In the simplified menu, click **Control > Window > Toolbars > Device Control**.

6. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.
If the **Plate Acquisition** tool bar is not visible, then click **Window > Toolbars > Plate Acquisition**.
In the simplified menu, click **Control > Window > Toolbars > Plate Acquisition**.
7. Load the bead plate that shipped with the instrument on to the stage, and then click **Stage Load/Eject** again to return the stage to its previous position.
8. Click **Acquire > Acquire**.
In the simplified menu, click **Control > Acquire > Acquire**.
9. In the **Acquire** dialog, click **Show Live** to open a live image window.
10. Click **Devices > Move Stage to Absolute Position**.
In the simplified menu, click **Control > Devices > Move Stage to Absolute Position**.
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.



CAUTION! Never click the **Set Origin** button.

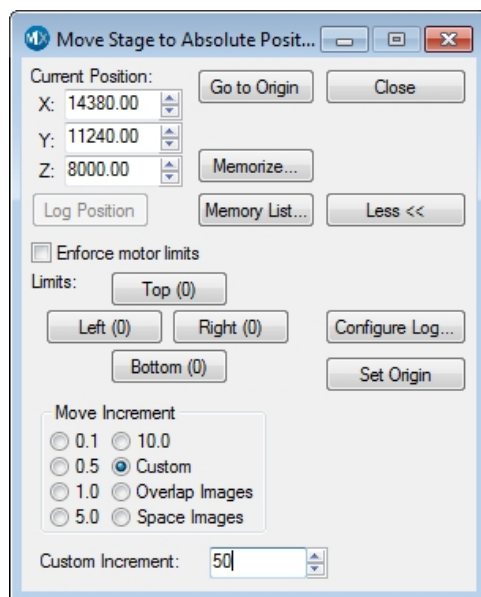


Figure 7-29: Move Stage to Absolute Position dialog

12. When the sample is in focus, write down the **Current Position: Z** value. You will need to refer to these values later in subsequent procedures.

For your convenience, you can write these values in the following table:

Table 7-2: Objective Focus Values

Objective Number	Focus Value (Current Position: Z)
1	
2	
3	
4	

13. From the **Setting** drop-down list, select the objective with the next highest numerical aperture (NA) that is in position #2.

Repeat the following steps for each objective find and write down the focus values for each. You will use these values to determine the parfocality for each objective. This is different than configuring Z offsets for laser autofocus.



Note: You must use the same filter set for all objectives.

14. In the **Move Stage to Absolute Position** dialog, use the **Current Position: Z** control to find and focus a sample in the live image window.
Since you found the X and Y coordinates of the sample for the first objective, you need to change only the Z position for the other objectives.
15. When the sample is in focus, write down the **Current Position: Z** value.
16. After you have written down the focus values for each objective, in the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.
17. Remove the bead plate, and then click **Stage Load/Eject** again to return the stage to its previous position.
18. Continue updating the software settings:
19.
 - If the objective you are changing is the one used to determine the plate bottom reference point, see [Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 186](#).
Molecular Devices recommends that you leave the reference objective in place and replace only the other objectives.
 - If the objective was NOT the one used to determine the plate bottom reference point, exit the MetaXpress Software and see [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 193](#).

Determining the Plate Bottom Reference Point after Changing the Reference Objective



Note: You do NOT need to do this procedure unless you replaced the reference objective. If you did not replace the reference objective, see [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 193](#).

The plate bottom reference point is a setting that the MetaXpress Software 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 or move this objective, you must determine the new plate bottom reference point and enter this value in the Meta Imaging Series Administrator Software.



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

Updating the plate bottom reference point requires two separate procedures:

- [Finding the Plate Bottom Reference Objective and Reference Point on page 187](#)
- [Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 192](#)

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:

```
[system calibration]  
PlateBottomReferenceObjective=X
```

where **X** is the position of the objective used as the plate bottom reference objective.

If you need to update the plate bottom reference point, the value in the calibration file must also be updated.



Note: Molecular Devices recommends that you contact technical support before attempting these procedures. See [Obtaining Support on page 203](#).

Finding the Plate Bottom Reference Objective and Reference Point

Before starting this procedure, make sure that the MetaXpress Software is in maintenance mode. The software was put in maintenance mode in [Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 180](#).

1. Start the MetaXpress Software and log in to the database, if the software is not already running.
2. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.

If the **Plate Acquisition** tool bar is not visible, then click **Window > Toolbars > Plate Acquisition**.

In the simplified menu, click **Control > Window > Toolbars > Plate Acquisition**.

3. Remove any plates from the stage and load one of the flat-field correction (FFC) plates that shipped with your system.
4. Click **Stage Load/Eject** again to return the stage to its previous position.
5. Click **Devices > Configure Magnification**.

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

6. In the **Configure Magnification** dialog, from the **Setting** drop-down list, select the objective to be used as the reference.

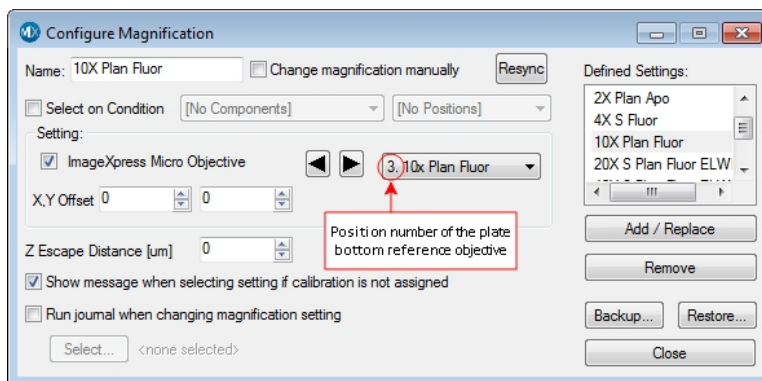


Figure 7-30: The Configure Magnification dialog

- 7. Write down the position number of the selected objective. You will need to refer to this value in [Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 192](#).

For your convenience, you can write the position number in the following table:

Table 7-3: Plate Bottom Reference Objective

Plate Bottom Reference Objective Position

- 8. Close the **Configure Magnification** dialog.
If a message appears prompting you to replace the stored setting, click **No**.
In the simplified menu, click **Control > Window > Toolbars > Plate Acquisition**.
- 9. Click **Devices > Stage > Move Stage to Absolute Position**.
In the simplified menu, click **Control > Devices > Move Stage to Absolute Position**.
- 10. In the **Move Stage to Absolute Position** dialog, in the **Current Position: Z** field, type **7700** and then press **ENTER** to move the Z-motor.
- 11. In the **Current Position: X** field, type **64000** and then press **ENTER** to move to the approximate center of the plate.
- 12. Click **Devices > Focus**.
In the simplified menu, click **Control > Devices > Focus**.
- 13. In the **Focus** dialog, click **Configure Laser**.

14. In the **Configure Laser Autofocus** dialog, select **Start from current z-position**.

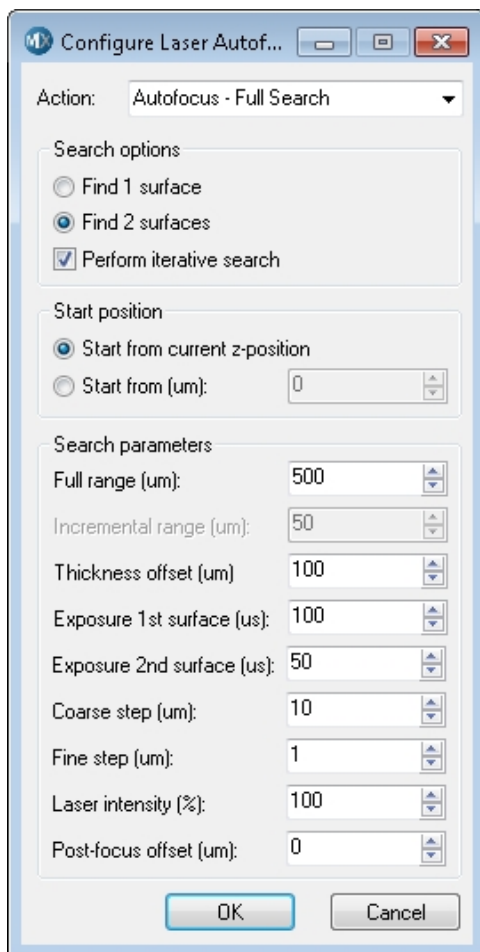


Figure 7-31: Configure Laser Autofocus dialog

15. Set the **Full range** value to **1000** microns (μm).
16. Set the **Exposure 1st surface** value to **10** microseconds (μs).
17. Set the **Coarse step** value to **5** microns (μm).



Note: The value to set depends on the objective. For the 10x Plan Apo objective, use 3 μm .

18. In the **Focus** dialog, in the **Autofocus** tab, click **Preview Pass**.

The **Preview Pass** window displays a graph of focus intensities compared to Z-position. Ideally, the graph contains sharp peaks in red and green.

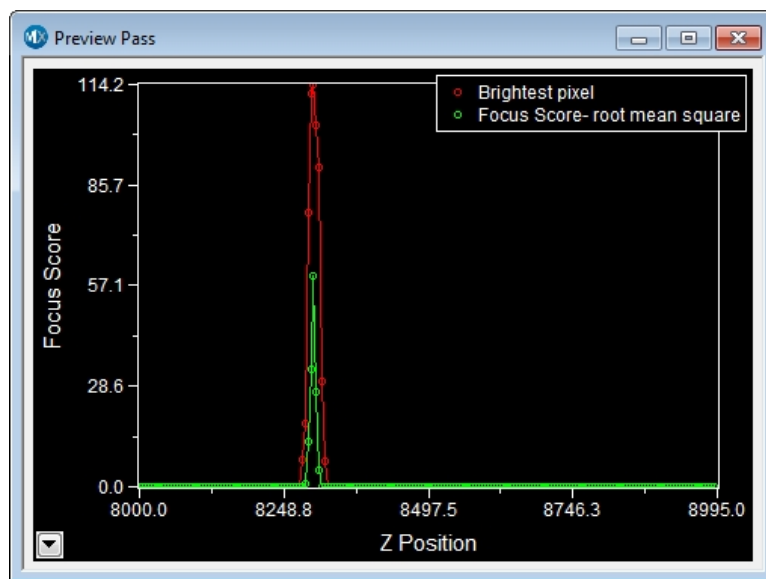


Figure 7-32: Preview Pass window

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.

If there is no peak in the graph, 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 6000 and 10000.
- If there still is no peak in the graph, then try moving to a new area of the FCC plate and click **Preview Pass**. If you still cannot find a peak after moving to a new area of the FCC plate twice and covering a z-range from 6000 to 10000, contact Technical Support. See [Obtaining Support on page 203](#).

19. In the **Preview Pass** window, click the trace line at the top of the green line of the first peak.

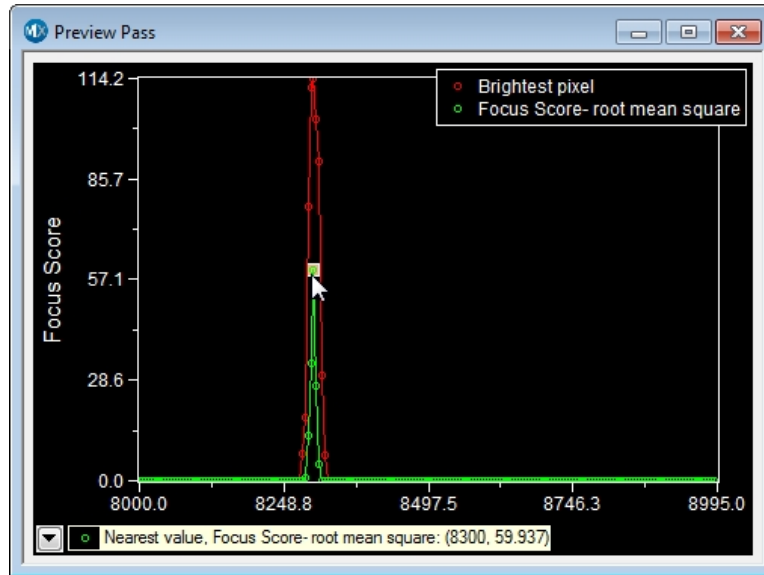


Figure 7-33: Z-position of peak

The X, Y coordinates on the graph are displayed as a tool tip. The X coordinate on the graph is the value for the Z-position.

In [Figure 7-33](#), the Z-position value is **8300**.

20. Write down the Z-position for the center focus position.

For your convenience, you can write the Z-position for the center focus position in the following table:

Table 7-4: Plate Bottom Reference Points

Position	X Value	Y Value	Z Value
Center	64000	33000	
Upper-Left	150000	11000	
Lower-Right	150000	74000	
Upper-Right	114000	11000	
Lower-Right	11400	74000	

21. In the **Move Stage to Absolute Position** dialog, in the **Current Position: X** field, type **15000**.

22. In the **Current Position: Y** field, type **11000** and then press **ENTER** to move to the upper-left corner of the plate.
23. In the **Focus** dialog, in the **Autofocus** tab, click **Preview Pass**.
24. In the **Preview Pass** window, click the trace line at the top of the green line of the first peak.
25. Write down the Z-position for the upper-left position.
26. Repeat **Step 21** through **Step 25** for the other three corners of the plate, using the X and Y values in [Table 7-4](#).
27. Circle the lowest Z-position value.
The circled 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 Software on page 193](#).
28. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.
29. Remove the flat-field correction (FFC) plate, and then click **Stage Load/Eject** again to return the stage to its previous position.
30. Exit the MetaXpress Software.
31. Edit the **MetaXpress.ref** file. See [Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 192](#).

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

Complete the following procedure to enter the value for the position of the plate bottom reference objective in the **MetaXpress.ref** configuration file.



Note: Only do this procedure if you needed to complete [Finding the Plate Bottom Reference Objective and Reference Point on page 187](#).

1. Click **Start > All Programs > Accessories > Notepad** and then open the **MetaXpress.ref** file in the **Hardware** folder.

The default installation path for this folder is **C:\MX6\Hardware**.



Note: Make sure that the copy of the **MetaXpress.ref** that you open is located in the **Hardware** folder of the MetaXpress Software installation folder (For example, **C:\MX6\Hardware**) and NOT in the root directory of your hard drive.

2. Find the following line in the **MetaXpress.ref** file:

```
[system calibration]
PlateBottomReferenceObjective=X
```

 where **X** is the position of the objective used as the plate bottom reference objective.
3. Replace the number at the end of the line with the objective position you wrote down.
 See [Table 7-3: Plate Bottom Reference Objective on page 188](#).
4. Save the modified file.



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

5. Exit Notepad.
6. Continue updating the software settings. See [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 193](#).

Entering the Focus Objective Values in the Meta Imaging Series Administrator Software

Do the following procedure to turn off maintenance mode, and to enter the focus objective values in the Meta Imaging Series Administrator Software.

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. In the **Meta Imaging Series Administrator**, from **List of Groups**, select **MetaXpress**.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, click **Install System Devices**.
5. In the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro** and then click **Settings**.
6. In the **ImageXpress Micro Settings** dialog, ensure that the **Parameter Group #1** tab is active and then clear the **Maintenance Mode** check box.
7. In the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.

8. In the **ImageXpress Micro Objective Settings** dialog, in the **Position 1 Z Offset** field, type the focus value that you wrote down for the objective 1. See [Table 7-2: Objective Focus Values on page 185](#).



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

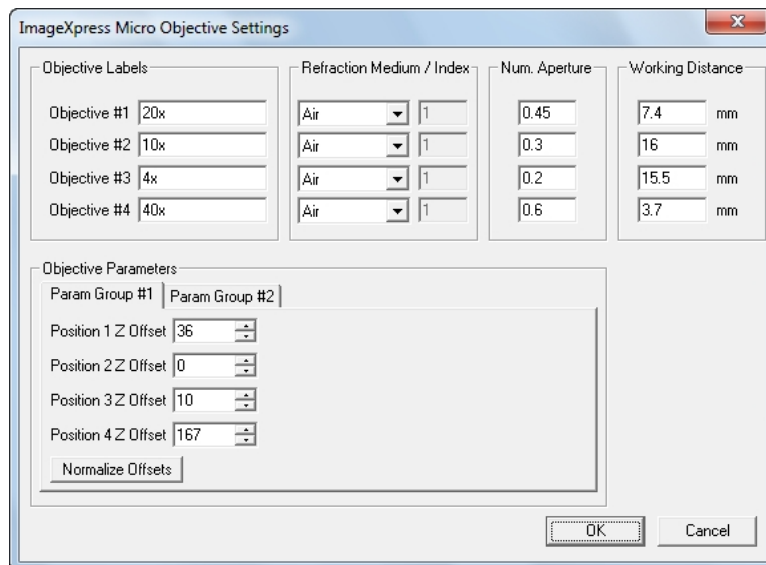


Figure 7-34: ImageXpress Micro Objective Settings Dialog

9. Type the focus values for the other three objectives in their appropriate Z Offset fields.



Note: In some cases, there might not be an objective in each position of the turret. If so, type the focus value for the highest magnification objective that you do have in each of the empty Position 1 through 4 Z Offset fields. Do not leave any of these values as 0.

10. Click **Normalize Offsets** to calculate the offsets for each Z position.
11. Click **OK** to close the **ImageXpress Micro Objective Settings** dialog.
12. If you completed [Finding the Plate Bottom Reference Objective and Reference Point on page 187](#), then in the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro Z** and then click **Settings**.

If you did not change or replace the reference objective, then go to **Step 15**.

13. In the **ImageXpress Micro Z Settings** dialog, in the **Plate Bottom Reference** field, type the lowest plate bottom reference point Z-position value that you wrote down and circled. See [Table 7-4: Plate Bottom Reference Points on page 191](#).
14. Click **OK** to close the **ImageXpress Micro Z Settings** dialog.
15. Click **OK** to close the **Install System Devices** dialog.



Note: In the next steps, type the previously entered values in the **Configure Devices** dialog to ensure that the settings carry over for all hardware profiles.

16. 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**.
17. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.
18. In the **ImageXpress Micro Objective Settings** dialog, in the **Objective Labels**, **Refraction Medium/ Index**, **Num. Aperature**, and **Working Distance** fields, type the values that you entered after you changed the objectives. See [Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 180](#).
19. In the **Position 1 Z Offset**, **Position 2 Z Offset**, **Position 3 Z Offset**, and **Position 4 Z Offset** fields, type the corresponding focus values that you wrote down for objectives 1, 2, 3, and 4. See [Table 7-2: Objective Focus Values on page 185](#).
20. Click **Normalize Offsets** to calculate the offsets for each Z position.
21. Click **OK** to close the **ImageXpress Micro Settings** dialog.
22. If you completed [Finding the Plate Bottom Reference Objective and Reference Point on page 187](#), then in the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro Z** and then click **Settings**.
If you did not change or replace the reference objective, then go to **Step 25**.
23. In the **ImageXpress Micro Z Settings** dialog, in the **Plate Bottom Reference** field, type the lowest plate bottom reference point Z-position value that you wrote down and circled. See [Table 7-4: Plate Bottom Reference Points on page 191](#).
24. Click **OK** to close the **ImageXpress Micro Z Settings** dialog.
25. Click **OK** to close the **User Settings** dialog.



Note: If you use more than one hardware profile, repeat **Step 16** through **Step 25** as needed for each hardware profile.

26. Click **OK** to close the **Configure Hardware** dialog.
27. Close the **Meta Imaging Series Administrator**.

28. Update the settings in the MetaXpress Software. See [Updating Magnification and Calibration Settings on page 196](#).

Updating Magnification and Calibration Settings

Before starting this procedure, make sure that the MetaXpress Software is NOT in maintenance mode. Maintenance mode was turned off in [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 193](#).

1. Start the MetaXpress Software and log in to the database.
2. Click **Devices > Configure Magnification**.
In the simplified menu, click **Control > Devices > Configure Magnification**.
3. In the **Configure Magnification** dialog, select the **ImageXpress Micro Objective** check box.

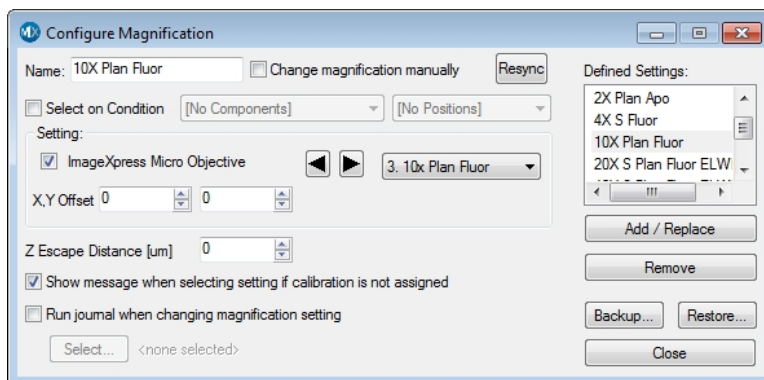


Figure 7-35: The Configure Magnification dialog

4. From the **Setting** drop-down list, select the installed objective.
5. In the **Name** field, type a name for the new objective.
6. Click **Add/Replace** to add this objective to the **Defined Settings** list.
7. If you replaced an objective with an existing setting, select the old setting from the **Defined Settings** field and then click **Remove**.
8. After the settings are updated, click **Backup**.
9. Click **Close** to close **Configure Magnification** dialog.
10. Click **Measure > Calibrate Distances**.
In the simplified menu, click **Measure > Distances > Calibrate Distances**.
11. In the **Calibrate Distances** dialog, click **Setup**.
12. Click **New**.
13. In the **Calibrations** field, type the name of your new objective and then press **ENTER**.

14. After the fields in the lower half of the dialog become active, from select **Define Calibrations By**, select **Edit Units/Pix**.
15. In the **X** and **Y** fields, type the calibration values for the new objective.
The following estimated values can be used for ImageXpress Micro Confocal System calibration settings:

Table 7-5: Estimated Calibration Settings

Objective Magnification	Estimated Calibration
1x	6.83 $\mu\text{m}/\text{pixel}$
2x	3.42 $\mu\text{m}/\text{pixel}$
4x	1.70 $\mu\text{m}/\text{pixel}$
10x	0.68 $\mu\text{m}/\text{pixel}$
20x	0.34 $\mu\text{m}/\text{pixel}$
40x	0.17 $\mu\text{m}/\text{pixel}$
60x	0.11 $\mu\text{m}/\text{pixel}$
100x	0.07 $\mu\text{m}/\text{pixel}$



Note: For more information on creating calibration settings, with the **Calibration Distances** dialog open, press **F1** to view the MetaXpress Software application help.

To measure pixel sizes more accurately, in the **IXM Taskbar**, click **System Maintenance** and then click **Measure Pixel Sizes**.

If **IXM Taskbar** is not installed, contact Technical Support.

16. From the **Magnification** field, select the new objective.
17. Click **Done**.
18. After the settings are updated, click **Save to File**.
19. Click **Close**.

Updating Shading Correction Settings-Legacy



Note: This procedure is required if **Legacy Correction** setting is selected as the **Shading Correction** method for one or more acquisition wavelengths.

Shading correction files are needed for each objective and filter set, and must be generated whenever an objective or filter is replaced or added to the system, or whenever the light guide is replaced.



Note: For shading correction images to be used during Plate Acquisition, the **Legacy Correction** setting must be selected in the **Shading Correction** field of an individual wavelength tab in the **Plate Acquisition Setup** dialog.

If you replaced or installed a filter, do the following procedures before updating the shading correction file:

- [Editing the Filter Settings on page 170](#)
- [Editing the Illumination Settings in the Software, see page 173](#)

If you replaced or installed an objective, do the following procedures before updating the shading correction file:

- [Editing the Objective Settings in the Meta Imaging Series Administrator Software, see page 180](#)
- [Configuring Parfocality after Changing Objectives, see page 183](#)
- [Determining the Plate Bottom Reference Point after Changing the Reference Objective, see page 186](#)

You do NOT need to do this procedure unless you replaced the reference objective.

- [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 193](#)
- [Updating Magnification and Calibration Settings on page 196](#)

Requirements

Shading Plates

To generate the shading correction files, you need a shading correction plate that is appropriate for the filter set you are using. Shading correction plates are part of the included accessory kit.

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.

To determine which shading plate to use for each filter set, see [Filter Specifications and Calibration Compatability on page 225](#).

System Maintenance Taskbar

To run the shading correction journal, you need the **System Maintenance Taskbar.jtb** that is part of the **Main Taskbar.jtb**. To determine if this taskbar is installed on your computer, do the following procedure:

1. Start the MetaXpress Software and log in to the database.
2. Click **Journal > Taskbars > Load Taskbar**.
In the simplified menu, click **Control > Journal > Taskbars > Load Taskbar**.
3. In the **Select a Taskbar** dialog, navigate to the **Taskbars** folder in the MetaXpress Software installation folder.
The default installation is in **C:\MX6\Taskbars**.
4. Select the **System Maintenance Taskbar.jtb** file.
If the **System Maintenance Taskbar.jtb** file does not exist on your computer, see [Importing the Main Taskbar Journal Suite on page 199](#).
5. Click **Open** to load the taskbar.

After you have met all the requirements, you can update the shading correction settings. See [Running the Shading Correction Journal from the Main Taskbar on page 203](#).

Importing the Main Taskbar Journal Suite

The IXM Taskbar is a collection of tools intended to enhance and streamline common imaging workflows.

Do the following procedure only if the **Main Taskbar** is not installed on your computer. If the **Main Taskbar** is installed, see [Running the Shading Correction Journal from the Main Taskbar on page 203](#).



Tip: If you plan to create additional groups (or configurations) in the Meta Imaging Series Administrator Software, do this procedure before creating the additional groups. You can then create the groups using the option to copy settings from existing groups.

-
1. Contact Technical Support to get the journal suite file **IXMTaskbar_v#.jzp**, where # is the current version of the software. See [Obtaining Support on page 203](#).
 2. Download the **IXM_Taskbar_v#.jzp** file to the workstation computer.
 3. Start the instrument.
 4. Start the MetaXpress Software and log in to the database.

5. Click **Journal > Import Journal Suite**.

In the simplified menu, click **Control > Journal > Import Journal Suite**.

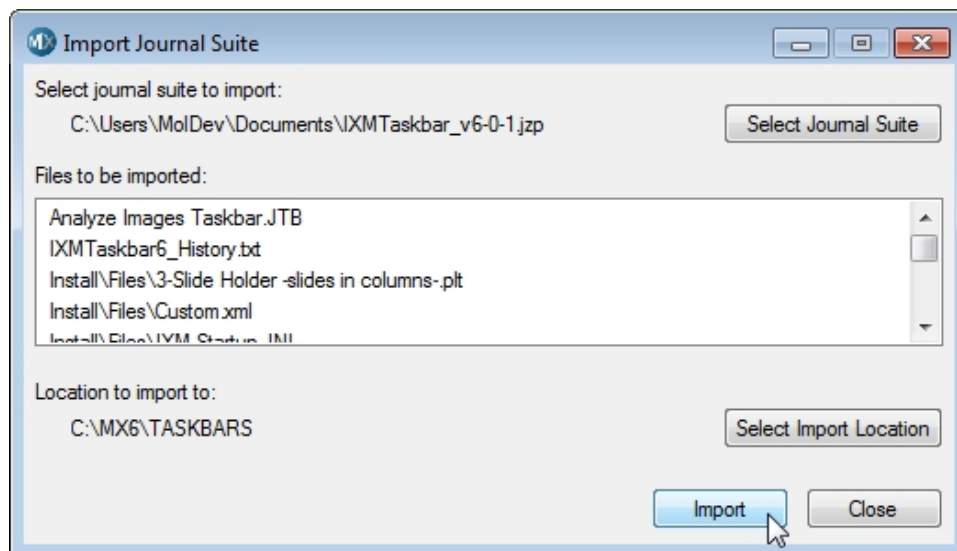


Figure 7-36: Import Journal Suite dialog

6. In the **Import Journal Suite** dialog, click **Select Journal Suite** and locate the **IXM_Taskbar_v#.jzp** file.

The path to the journal suite file is displayed at the top of the **Import Journal Suite** dialog.

7. Click **Select Import Location** and browse to the **Taskbars** folder within the MetaXpress installation directory.

The default installation is in **C:\MX6\Taskbars**. If it is missing, create the **Taskbars** folder.



Tip: To determine the correct installation folder, right-click the MetaXpress Software icon on you desktop and then click **Open file location**.

8. Select the **Taskbars** folder and click **OK**.
9. In the **Import journal Suite** dialog, click **Import**.
10. Click **Close** when done.



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

11. Click **Journal > Taskbars > Load Taskbar**.

In the simplified menu, click **Control > Journal > Taskbars > Load Taskbar**.

12. In the **Select a Taskbar** dialog, navigate to the **Taskbars** folder in the MetaXpress Software installation folder.

The default installation is in **C:\MX6\Taskbars**.

13. Select the **Main Taskbar.JTB** file and then click **Open**.

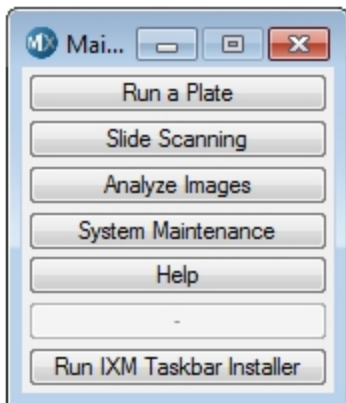


Figure 7-37: The Main Taskbar dialog

14. In the **Main Taskbar**, click **Run IXM Taskbar Installer**.

15. 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.

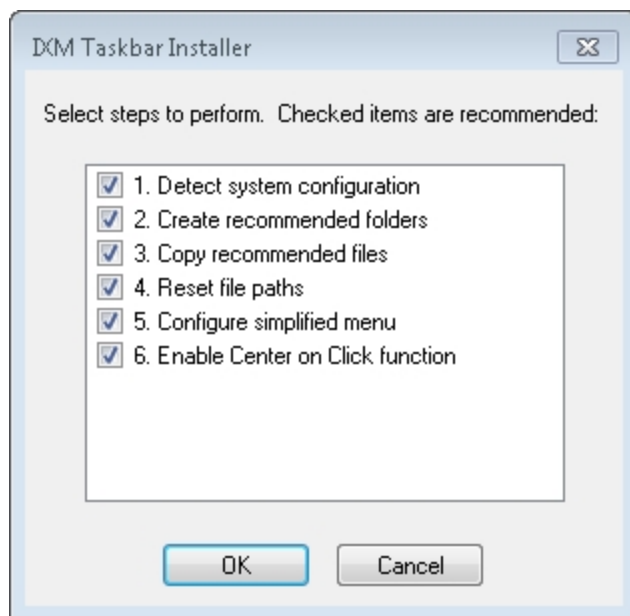


Figure 7-38: IXM Taskbar Installer



Note: The check boxes that are pre-selected in the **IXM Taskbar Installer** dialog are the recommended tasks for your configuration.

16. In the **IXM Taskbar Installer** dialog, click **OK**.
17. If the **Select Configurations** dialog appears, select the current group or **All groups**, and then click **OK**.
Molecular Devices recommends that you select **All groups**.
18. If the **Select Users** dialog appears, select the current user or **All users**, and then click **OK**.
Molecular Devices recommends that you select **All users**.
19. In the **Select directory for default paths** dialog, select the main MetaXpress installation directory.
The default installation is in **C:\MX6**.
20. Click **OK**.
21. In the **Configure Menu** dialog, select to **Install** or **Uninstall** the simplified menu.
Molecular Devices recommends using the simplified menu. See [Simplified Menu Structure on page 22](#).

22. Click **OK**.
23. Exit and restart the MetaXpress Software.

To run the shading correction journal, see [Running the Shading Correction Journal from the Main Taskbar on page 203](#).

Running the Shading Correction Journal from the Main Taskbar

1. From 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.



Tip: To show or hide the taskbar that was most recently used, press **F4** on your keyboard.

Turning off the Status Indicator Lights

The edges of the ImageXpress Micro System illuminate with colors that provide information about the instrument status. See [Status Indicator Lights on page 33](#).

If required, you can turn off the status indicator lights from the **Devices > Device Control > Other** tab, or in the simplified menu **Control > Devices > Device Control > Other** tab, and deselect the **Enable Microscope LED** check box.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Part of effective communication with Molecular Devices is determining the channels of support for the ImageXpress Micro System, including the MetaXpress Software. Molecular Devices provides a wide range of support:

- Documentation: Check the guides that are included on the installation media and the help that is available within the MetaXpress Software. Help for an active dialog can be accessed by pressing **F1** on your keyboard.
- Online knowledge base: The knowledge base has links to technical notes, software upgrades, newsletters, user guides, and other resources. Visit the Molecular Devices Support web page at www.moleculardevices.com/support and follow the links to the knowledge base.
- MetaMorph Software forum: This forum has information on journal scripts and custom modules, and has links to videos and webinars that can help you troubleshoot problems and be more productive using the software. Visit the forum at metamorph.moleculardevices.com/forum.
- Technical Support:

Phone: Contact Technical Support at (800)-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

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

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

 - To find your system ID number, in the MetaXpress Software, click **Help > About MetaXpress**. The About dialog displays your system ID number.
 - The system serial number is located on your instrument.
- Additional support resources include:
 - Nikon web-based microscopy course:
<http://www.microscopyu.com>
 - The Molecular Probes Handbook:
<http://www.lifetechnologies.com/us/en/home/references/molecular-probes-the-handbook.html>

This resource 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 the 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 Protocol**, and then select **Save to file** rather than to the database. The settings file is then 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 207](#).

Logging AxoTrace Software Messages to a .txt File

The AxoTrace Software tracks the hardware status of the ImageXpress Micro System and logs all the hardware activities of the system. If an error occurs downstream of the initial cause, the AxoTrace Software 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 following procedure if a reproducible hardware error occurs. Follow the AxoTrace Software 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 the AxoTrace Software starts automatically.
The AxoTrace Software icon appears in the Windows taskbar.



Figure 7-39: AxoTrace Software icon in the Windows taskbar

2. Exit the MetaXpress Software.
3. Double-click the AxoTrace Software icon in the Windows taskbar.
4. In the AxoTrace window, click **Options** and make sure that the **Time prefix** option is selected.

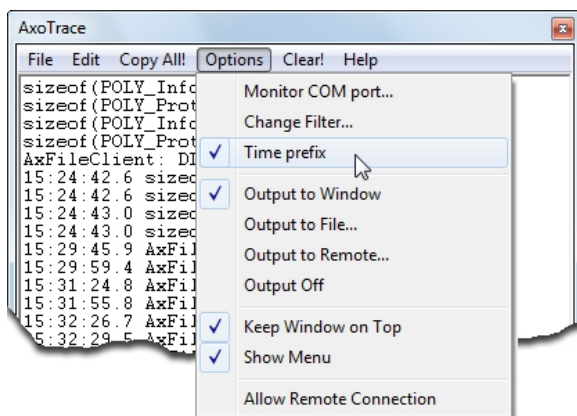


Figure 7-40: AxoTrace Software Options menu

5. Click **File > Exit** to close the AxoTrace window.
6. Start the MetaXpress Software.
7. Turn off the ImageXpress Micro System and then turn it back on.
The light source can remain on during this step.
8. Reproduce the error, and after the error is reproduced, exit the MetaXpress Software.
9. Double-click the AxoTrace Software icon in the taskbar.
10. In the AxoTrace window, click **File > Save As**.
11. In the **Save As** dialog, 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.
12. Click **File > Exit** to close the AxoTrace window.
13. Send the AxoTrace Software log as an email 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 can create this report from the **About MetaXpress** dialog using the following procedure.

1. In the MetaXpress Software, click **Help > About MetaXpress**.
2. In the **About MetaXpress** dialog, in the fields on the right, type your contact information.

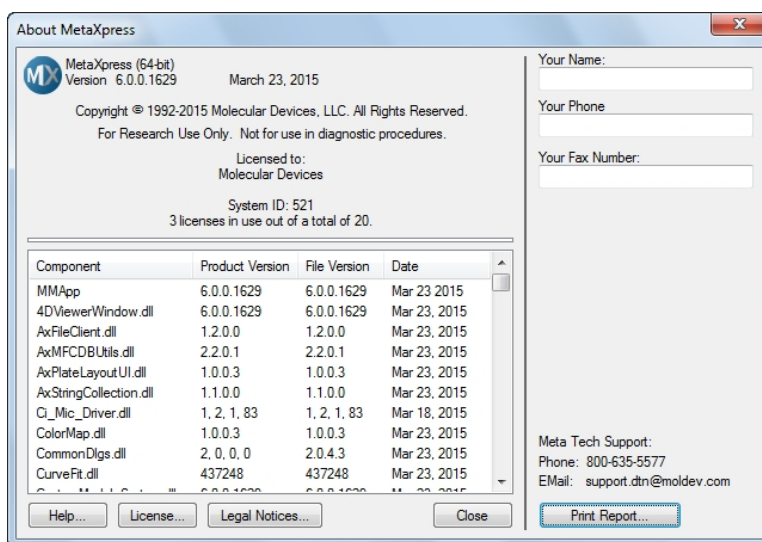
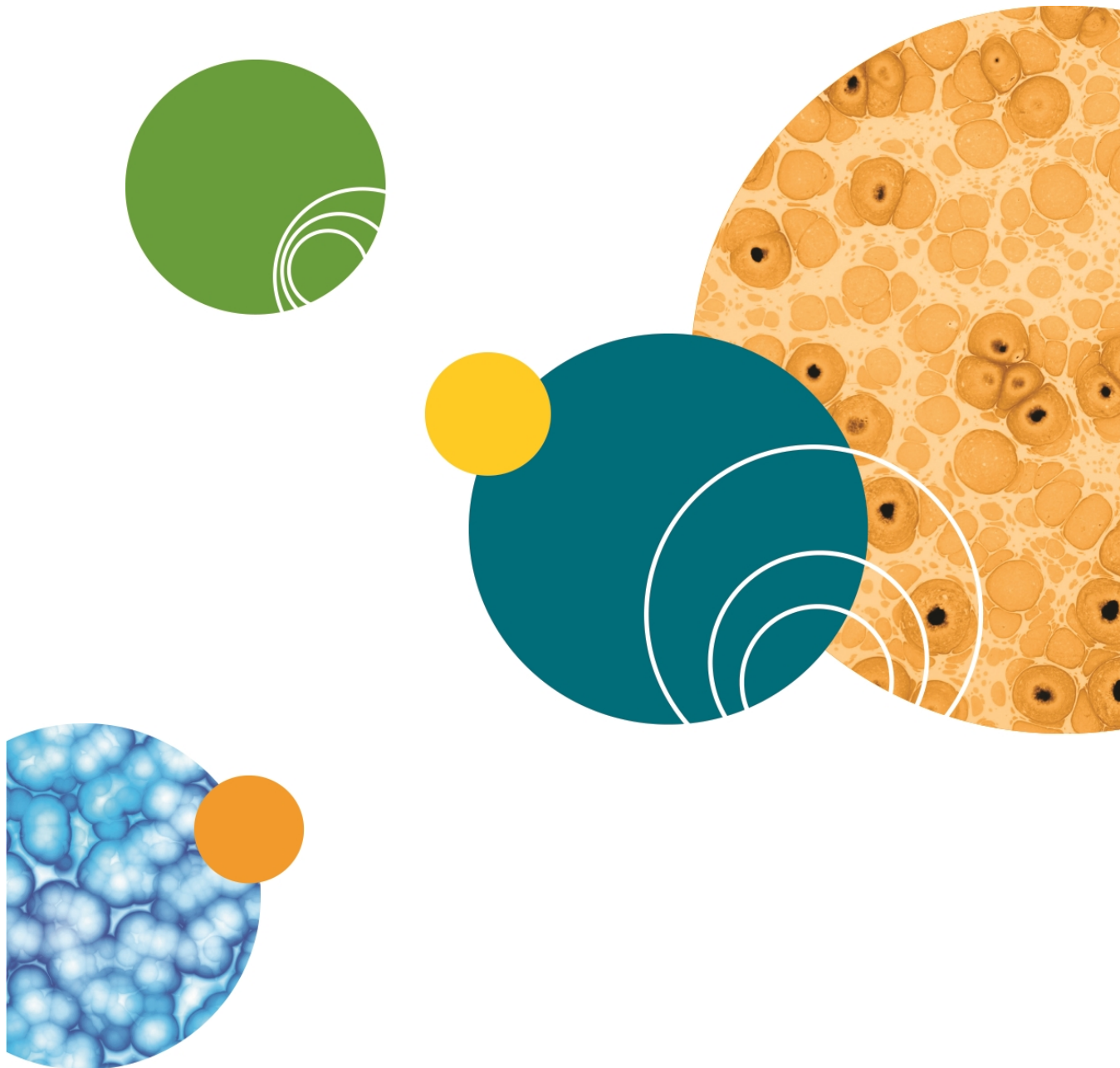


Figure 7-41: About MetaXpress Software

3. Click **Print Report**.
4. In the **Print Setup** dialog, select to print to a PDF file or to a text file and then click **OK**.
5. Send the report file to appropriate Molecular Devices support personnel as an email attachment.



Appendix A: Instrument Specifications

A



WARNING! If the instrument 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 systems power switch on the front of the ImageXpress Micro Systems Power and Options Controller.



Note: For Environmental Control Options requirements, see the *ImageXpress Micro High Content Imaging System Options User Guide*.

Table A-1: Operational and Environmental Specifications for the Instrument Without Options

Item	Description
Operating environment	Indoor use only
Systems power and options controller input	100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum
Light source power	220 W, 24 V, AC to DC converter included
Computer power input	100 VAC to 240 VAC, 50/60 Hz, 690 VA, 120 W
Computer monitor power input	100 VAC to 240 VAC, 50/60 Hz, 40 W
Weight, base unit, no options	104 kg (230 lbs.)
Ambient operating temperature	15°C to 30°C
Humidity restrictions	35% to 50% non-condensing
Altitude restrictions	Not to exceed 2000 m (6562 ft)
Equipment class	1
Installation category	II
Pollution degree	2
Ingress Protection	IP20

Instrument Dimensions

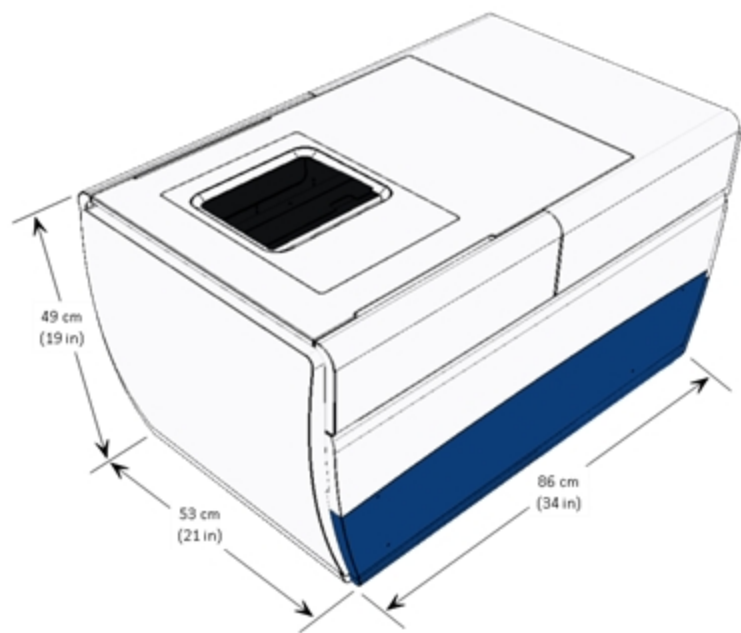


Figure A-1: Instrument Height, Width, and Length

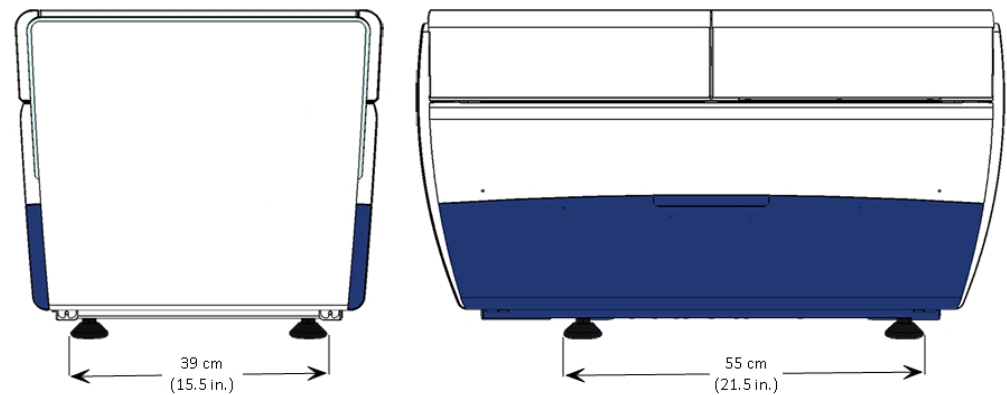
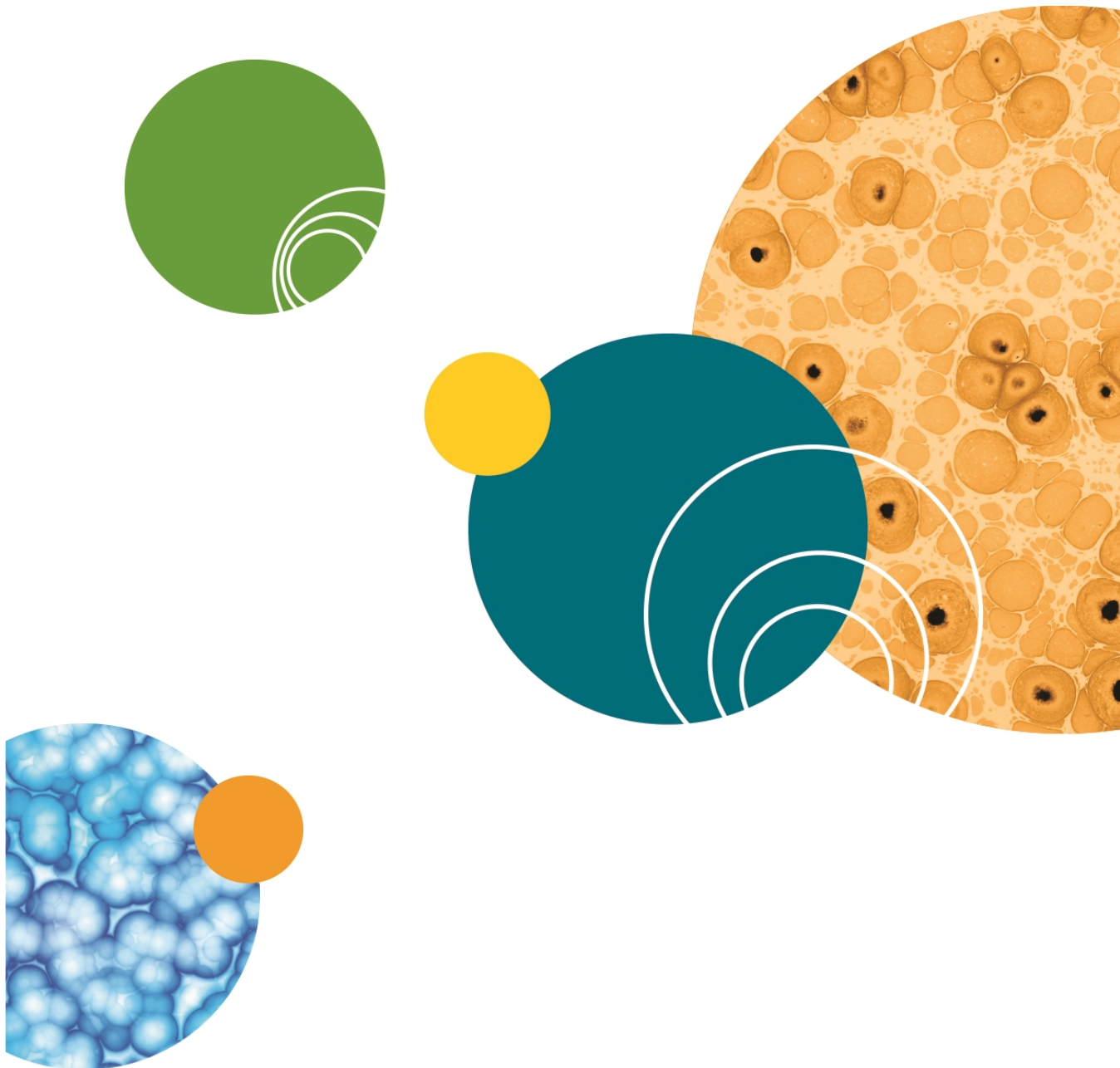


Figure A-2: Distance Between the Outside Edges of the Instrument Feet



Appendix B: Site Requirements

B

The ImageXpress Micro Confocal System is designed to operate indoors under laboratory conditions. For optimal performance, site requirements must be met. As with any precision optical instrument, take care to maintain a low-dust, low-vibration environment. Temperature and humidity extremes can compromise performance. Also see [Instrument Specifications on page 209](#).

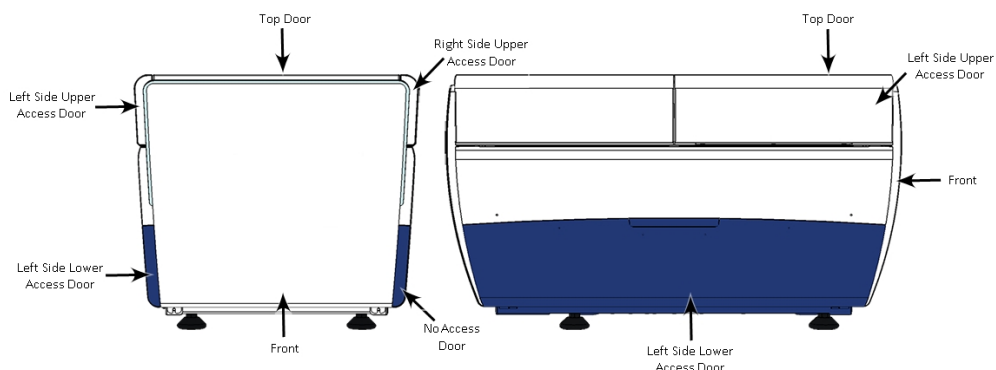


Figure B-1: Instrument Front and Sideways Installation Orientation Options

Instrument Installation Restrictions

Avoid the following lab conditions or situations:

- Avoid installation in or next to a room where there is high-motion equipment, like elevators, air conditioners, and heaters.
- Avoid placement directly in the path of air vents, because sudden temperature changes and air-flow vibrations can degrade performance.
- Avoid placement near external vibration caused by trains or excessive vehicle traffic.
- Avoid placement in any room with noticeable vibration on floors or walls.
- Avoid placement less than 5 m (15 ft) away from refrigerators.
- Avoid placement less than 2 m (6 ft) away from doors.
- Avoid using a table that is mechanically attached to a wall.
- Avoid sharing a table with shakers, stirrers, mixers, or centrifuges.



WARNING: Molecular Devices does not service BSL-3 and above laboratories.

Space requirements

- Table or workbench dimensions depend on your installation orientation. See [Figure B-1](#).

Front installation:

- 94 cm (37 in.) deep
- 61 cm (24 in.) wide
- 15 cm (6 in.) of open space between the wall and the back of the instrument for cables
- A minimum of 51 cm (20 in.) on the left, access door side to allow access for filter and objective changes
- 18 cm (7 in.) on the right, no access door side, for service access

Sideways installation:



Note: Molecular Devices recommends that the left side of the instrument face forward to allow optimal access to the lower door, as illustrated in [Figure B-1](#). There is no lower access door on the right side of the instrument.

-
- 94 cm (37 in.) wide
 - 61 cm (24 in.) deep
 - 15 cm (6 in.) of open space between the wall and the back of the instrument for cables
 - 30 to 45 cm (12 to 15 in.) of clearance between the wall and the side of the instrument for service access
 - The cables and light guide must hang loosely.
 - A minimum of 51 cm (20 in.) of top clearance for plate loading. Optional fluidics and transmitted light modules require additional top clearance.
 - Space below the table must accommodate the light source and ImageXpress Micro Systems Power and Options Controller such that the light guide and power cable can easily reach the back of the instrument.
 - Keep components within 1.2 m (4 ft) of the instrument to accommodate the length of the cables.

Weight support requirements

- Sufficient to support 104 kg (230 lbs.) with minimal vibration.

Power Requirements

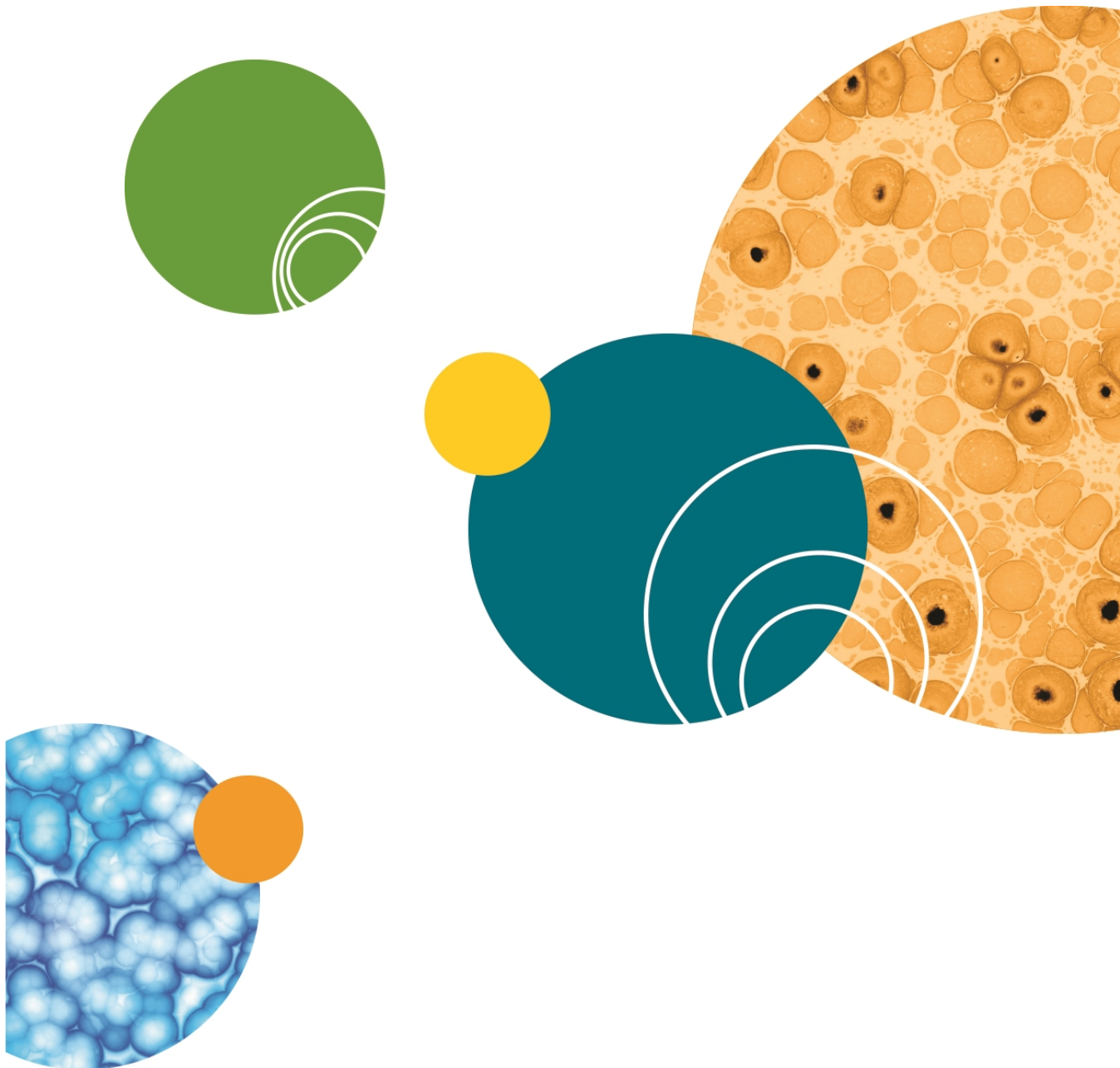
- Direct connections to all international supply voltages available.
- Use the included IEC power cord to connect the external ImageXpress Micro Systems Power and Options Controller to a GROUNDED power receptacle that is rated for 15 A.
- Input voltage range is from 100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum.
- Fluctuations must be within $\pm 10\%$ of the nominal voltage.
- When using a power strip, the computer should be connected to a different power strip than the ImageXpress Micro Systems Power and Options Controller that connects to the instrument and light source.



Tip: Molecular Devices recommends connecting the ImageXpress Micro Systems Power and Options Controller, the computer and the monitor, to uninterruptible power supplies (UPS) to protect the ImageXpress Micro Confocal System and your data from laboratory power disruptions.

Power Consumption

- 620 watts maximum, including system initialization and motor start current



Appendix C: Compatible Objectives



The following table details the Nikon objectives that are compatible with the ImageXpress Micro Confocal System. It also provides microplate compatibility information and theoretical calculation of confocal performance for the objectives used in the system.

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

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	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 Lambda	1-6300-0451	No	0.10	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	0.20	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 Lambda	1-6300-0121	No	0.20	20 mm	Thin bottom (0.17 mm), Thin bottom

Table C-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
					(0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)
4x Plan Fluor DL	1-6300-0292	Yes, PhL	0.13	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	1-6300-0190	No	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 S Fluor	1-6300-0122	No	0.50	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 Lambda	6500-0120	No	0.45	4.0 mm	Thin bottom (0.17 mm), Thin bottom (0.17 mm) No

Table C-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System (continued)

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

Table C-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
					(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, 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, Thick bottom (0.25 mm to 1 mm)
40x Plan Apo Lambda cc 0.11 mm to 0.23 mm	1-6300-0412	No	0.95	0.25 mm to 0.16 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 ,

Table C-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
					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	0.66 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 Apo Lambda	TBD	No	0.95	0.11 mm to 0.21 mm	Thin bottom (0.17 mm) ¹ , Thin bottom (0.17 mm) No Skirt
60x Plan Fluor	1-6300-0414	No	0.85	0.3 mm	Thin bottom (0.17 mm) ¹ , Thin bottom

Table C-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
					(0.17 mm) No Skirt
60x Plan Apo Oil	1.6300-0417	No	1.40	0.13 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 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)
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
100x CFI L Plan EPI cc 0 mm to 0.7 mm	1-6300-0419	No	0.85	1.2 mm to 0.85 mm	Thin bottom (0.17 mm), Thin bottom (0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)

¹Potential interference with microplate skirt when imaging edge wells.

²Image degradation above microplate thickness 0.3 mm.

³20x and 40x ELWD image through cover slips, but other objectives give better resolution and shorter exposures.



Note: When used with thin-bottom microplates, 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 a microplate skirt when imaging edge wells. Molecular Devices recommends to either omit the edge wells or to use a microplate with a low skirt.

Table C-2: Theoretical Calculation of Confocal Performance With Compatible Objectives

Objective	60 μm Pinhole Diameter Airy Units (AU)	42 μm Pinhole Diameter Airy Units (AU)	50 μm Slit Width Airy Units (AU)	Optical Thickness using 60 μm Pinhole ¹	Optical Thickness using 42 μm Pinhole ¹
10x Plan Fluor	2.9 AU	2.0 AU	2.4 AU	33.3 μm	25.6 μm
10x S Fluor	4.8 AU	3.4 AU	4.0 AU	18.6 μm	13.5 μm
10x Plan Apo Lambda	4.3 AU	3.0 AU	3.6 AU	20.9 μm	15.3 μm
10x Plan Fluor DLL	2.9 AU	2.0 AU	2.4 AU	33.3 μm	25.6 μm
10x Plan Fluor DL	2.9 AU	2.0 AU	2.4 AU	33.3 μm	25.6 μm
20x Super Plan Fluor ELWD	2.2 AU	1.5 AU	1.8 AU	11.8 μm	9.5 μm
20x S Fluor	3.6 AU	2.5 AU	3.0 AU	6.3 μm	4.6 μm
20x Plan Apo Lambda	3.6 AU	2.5 AU	3.0 AU	6.3 μm	4.6 μm
20x Plan Fluor DLL	2.4 AU	1.7 AU	2.0 AU	10.3 μm	8.1 μm
20x Super Plan Fluor ELWD DM	2.2 AU	1.5 AU	1.8 AU	11.8 μm	9.5 μm
40x Super Plan Fluor ELWD	1.4 AU	1.0 AU	1.2 AU	5.1 μm	4.3 μm
40x Plan Apo Lambda	2.3 AU	1.6 AU	1.9 AU	2.6 μm	1.9 μm
40x S Fluor	2.2 AU	1.5 AU	1.8 AU	2.8 μm	2.1 μm
40x Plan Fluor Oil	3.1 AU	2.2 AU	2.6 AU	2.8 μm	2.1 μm
40x Plan Fluor DLL	1.8 AU	1.3 AU	1.5 AU	3.6 μm	2.9 μm
40x Super Plan Fluor	1.4 AU	1.0 AU	1.2 AU	5.1 μm	4.3 μm

Table C-2: Theoretical Calculation of Confocal Performance With Compatible Objectives (continued)

Objective	60 μm Pinhole Diameter Airy Units (AU)	42 μm Pinhole Diameter Airy Units (AU)	50 μm Slit Width Airy Units (AU)	Optical Thickness using 60 μm Pinhole ¹	Optical Thickness using 42 μm Pinhole ¹
ELWD ADM					
60x Super Plan Fluor ELWD	1.1 AU	0.8 AU	0.9 AU	3.2 μm	2.8 μm
60x Plan Apo Lambda	1.5 AU	1.1 AU	1.3 AU	1.9 μm	1.5 μm
60x Plan Fluor	1.4 AU	1.0 AU	1.1 AU	2.3 μm	1.9 μm
60x Plan Apo Oil	2.3 AU	1.6 AU	1.9 AU	1.8 μm	1.3 μm
60x Plan Fluor ELWD ADL	1.1 AU	0.8 AU	0.9 AU	3.2 μm	2.8 μm
100x Plan Fluor Oil	1.3 AU	0.9 AU	1.0 AU	1.4 μm	1.2 μm
100x CFI L Plan EPI	0.8 AU	0.6 AU	0.7 AU	1.8 μm	1.6 μm

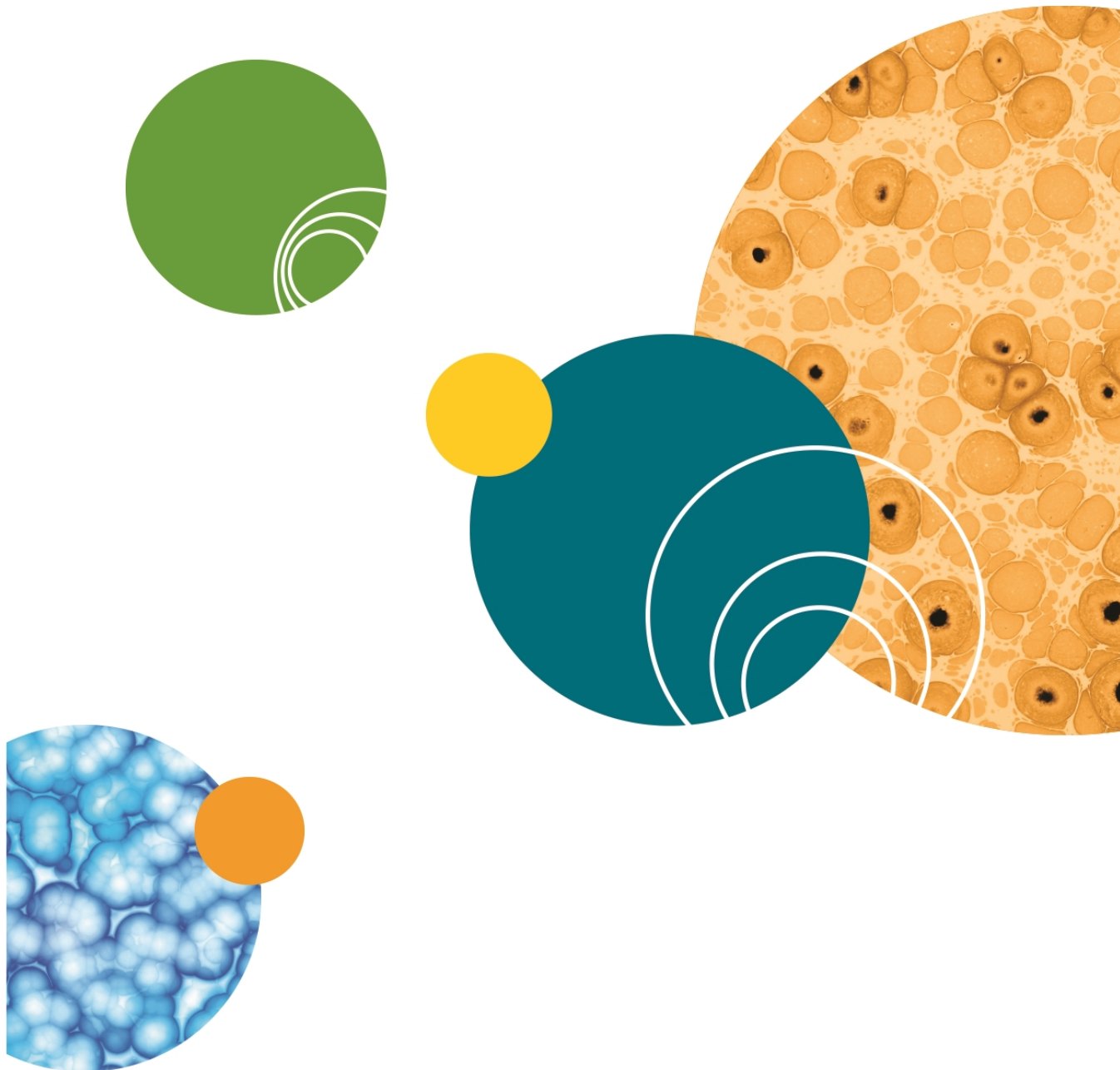
¹ $\text{FWHM}_{\text{axial}}$ for pinholes larger than 1 Airy Unit as set forth by Toomre, et alia.

Appendix D: Filter Specifications and Calibration Compatability



Table D-1: Filter Specifications and Calibration Compatibility

Filter	Wavelengths	Shading Correction Plate	Calibration Slide
DAPI	Excitation: 377/50 nm Emission: 447/60 nm Dichroic: 409 nm	Green Pink	Green (GP-8)
FITC	Excitation: 475/34 nm Emission: 536/40 nm Dichroic: 506 nm	Pink Red	Yellow (GP-11)
TRITC	Excitation: 531/40 nm Emission: 593/40 nm Dichroic: 562 nm	Red	Yellow (GP-11) Red (GP-7)
Texas Red	Excitation: 560/32 nm Emission: 624/40 nm Dichroic: 593 nm	Red	Yellow (GP-11) Red (GP-7)
Cy5	Excitation: 631/28 nm Emission: 692/40 nm Dichroic: 660 nm	Red	Blue (GP-9) Red (GP-7)



Appendix E: Replacement Parts and Optional Extras

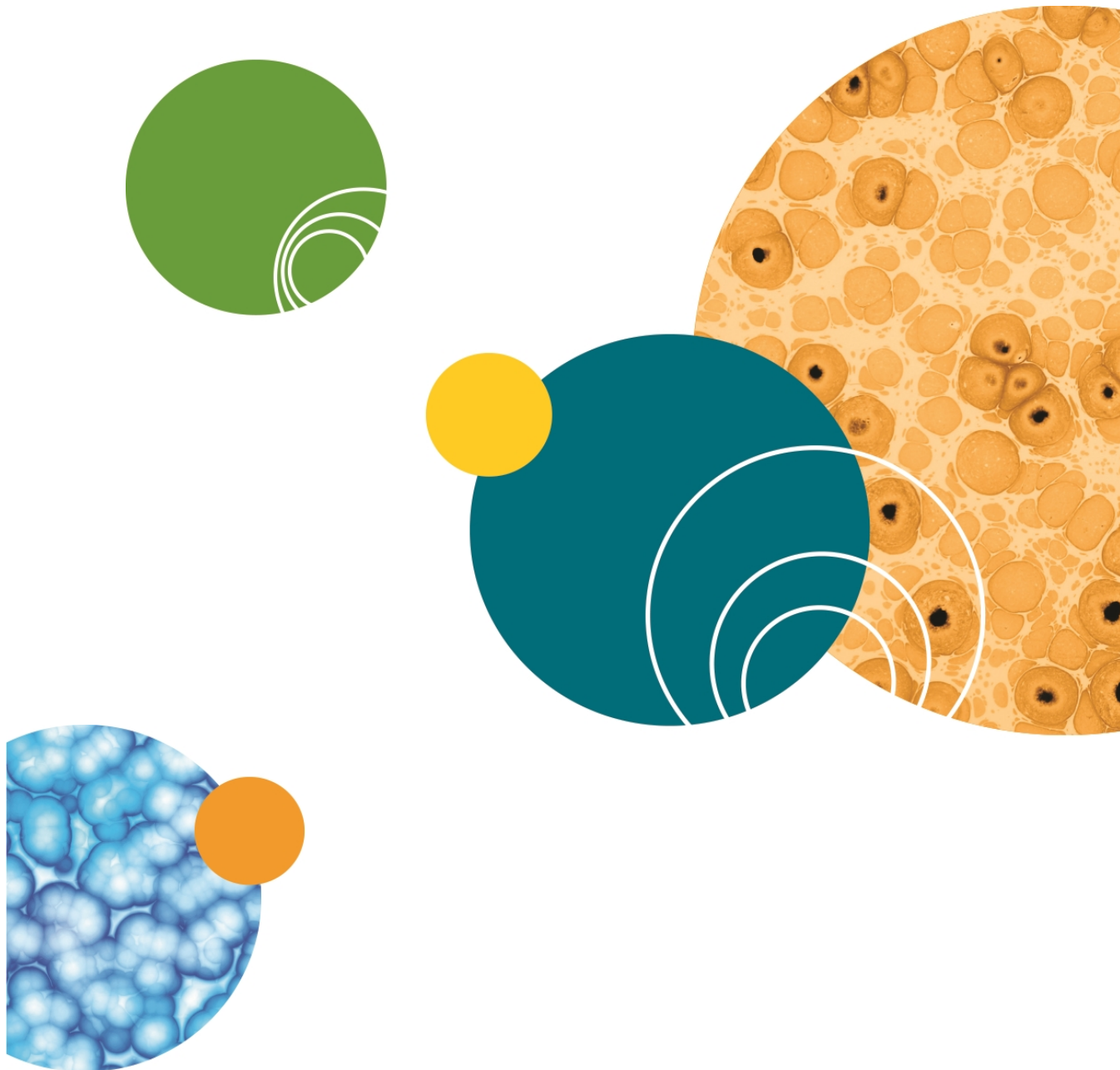
E

For an up-to-date list of replacement parts and optional extras, see the website at:

www.moleculardevices.com

Table E-1: Replacement Parts and Optional Extras for the ImageXpress Micro Confocal System

Part Number	Description
—	For a List of Compatible Nikon Objectives see Compatible Objectives on page 217
5039730	Confocal Single Disk Unit Includes: A) 60 micron pinhole disk for basic confocal imaging B) Widefield for non-confocal imaging
5039731	Confocal Screening Option Dual Disk Unit Includes: A) 60 micron pinhole disk for basic confocal imaging B) 50 micron slit disk for for high-throughput confocal imaging C) Widefield for non-confocal imaging
5040287	Confocal Research Option Dual Disk Unit Includes: A) 60 micron pinhole disk for basic confocal imaging B) 42 micron pinhole disk for high-resolution confocal imaging C) Widefield for non-confocal imaging
5039856	DAPI Filter Set
5039857	FITC Filter Set
5039858	TRITC/Cy3 Filter Set
5039859	Texas Red Filter Set
5037556	Cy5 Filter Set
5042555	Accessory Tool Kit
1-GP-2	GP-2 Spatial Calibration Slide
5039434	Lumencor Fiber-Optic Solid-State Light Source
5000284	Robot-friendly Three Slide Holder for Imaging



Appendix F: File Privileges for the MetaXpress Software



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



Note: The following information assumes that the MetaXpress Software has been installed to the default path of **C:\MX6**.



Note: There are no restrictions as to where the software is installed. Nothing prevents installing the software to the path of **C:\Program Files\MX6**.



Note: When your system has multiple configurations and multiple groups not named **MetaXpress**, substitute the appropriate group name for the default group name **MetaXpress** as needed in the following list of folder paths.

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 in the **C:\Shading Images** folder. 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 - 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

Appendix G: Robotic Plate Handling

G

If your ImageXpress Micro Confocal 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 Confocal System so 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 a robotic plate handler upgrade for your system, contact Molecular Devices sales or support.

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

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

Verifying External Control Settings

If you have a robot attached to the ImageXpress Micro Confocal System, you need to confirm that the **External Control** settings in the Meta Imaging Series Administrator Software are enabled and that the correct COM port is selected.



Note: The **External Control** module must be purchased and activated on the MetaXpress Software key. If it is not activated, try updating the MetaXpress Software. If it is still not activated, contact your Molecular Devices representative.

To confirm that the **External Control** settings in the Meta Imaging Series Administrator Software are enabled:

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. Click **Configure Hardware**.
3. In the **Configure Hardware** dialog, click **Install System Devices**.
4. In the **Install Systems Devices** dialog, ensure that **External Control** is listed in the **Installed Devices** list.

If it is not listed, select it from the **Available Hardware** list and click **Install>>**.

5. From the **Installed Devices** list, select **External Control** and click **Settings**.
6. In the **External Control Settings** dialog, click the **Connections Settings** tab.
7. Verify that the correct COM port is selected.
8. Verify that the option **Operate device in emulation mode** is deselected.
9. Verify that **Control** is listed in the **Included components** list.
If it is not listed, select it from the **Available Hardware** list and click **Install>>**.
10. Click **OK** to return to the **Install System Devices** dialog, then click **Apply** and **OK** to return to the **Configure Hardware** dialog.
11. Select an appropriate hardware configuration from the **Hardware Settings** list and click **Configure Devices**.
12. In the **User Settings** dialog, ensure that **External Control** is listed in the **Claimed Devices** list.
If it is not listed, select it from the **Available Hardware** list and click **Install>>**.
13. Click **Apply** and **OK** to return to the **Configure Hardware** dialog
14. Repeat steps 11-13 for each hardware configuration that needs the **External Control**.
15. Click **OK** and **OK** to exit the Meta Imaging Series Administrator Software.
16. Start the MetaXpress Software, and select the **Screening** menu to verify that the **External Control** option is available.

Appendix H: Electromagnetic Compatibility



H

Regulatory for Canada (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

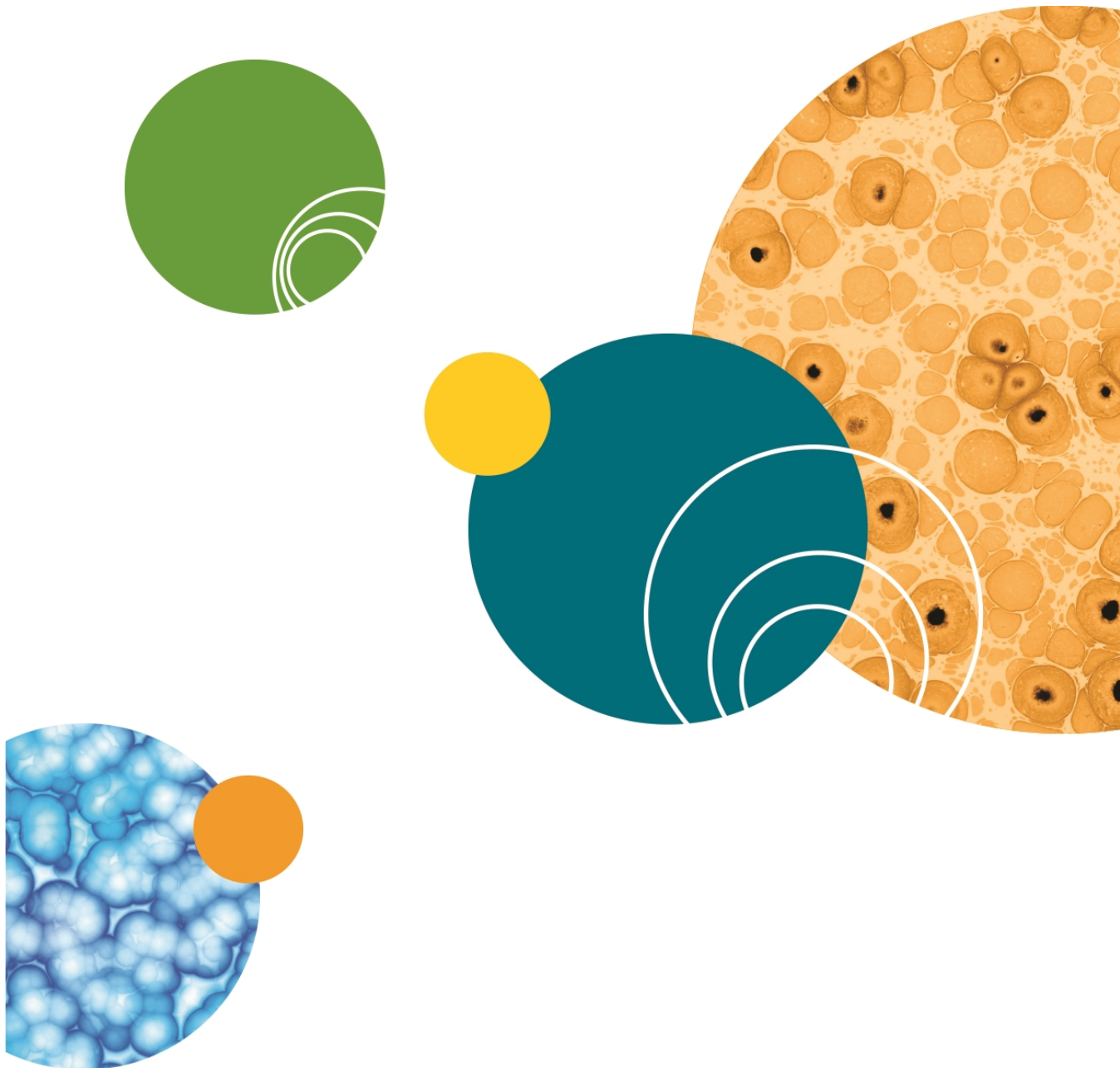
This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power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

Information to 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, might 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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