



ImageXpress® Micro Confocal

High-Content Imaging System

User Guide

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Safety Information

This section describes the safe use of the instrument. Safety includes an understanding of the information in this guide, the safety labels on the instrument, and the precautions that you must follow before and during operation of the instrument.

It is important to confirm 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.
- Read and understood all instrument documentation, including all warnings and cautions.

Always remember that the key to safety is operating 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 amount 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. The warning symbol can vary depending on the warning. 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 found 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.

Symbol	Indication
	Indicates that the product documentation must be consulted.
	Indicates a potential laser hazard. See Laser Safety on page 10 .
	Indicates a potential lifting hazard. For information about the weight of the instrument, see Instrument Specifications on page 249 .
	Indicates a potential pinch hazard.
	Indicates a potential heat hazard.
	Indicates power on. See Powering on the Instrument on page 25 .
○	Indicates power off. See Shutting Down the System on page 28 .

Protective Housing and Safety Interlocks

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

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



WARNING! Do not override any interlocks, open the protective housing, or attempt to gain access to the interior of the instrument. Doing this can damage the instrument components and result in hazardous exposure to laser light, hot surfaces, moving parts, or high voltage.

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 244](#).

Non-Interlocked Doors and Panels

The side access doors are not interlocked. These doors do not allow access to or provide harmful exposure to the laser autofocus system. Some moving parts exist inside these doors. See [Moving Parts Safety on page 12](#).

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 appears in this guide, 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-1: 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/cm^2).



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. Operate the instrument 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.

This light source is a solid-state light source that has a rated lifetime of more than 20,000 hours. There are no user-replaceable parts in the 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, inspect all electrical equipment before use and immediately report all electrical deficiencies. Contact Molecular Devices Technical Support to service equipment that requires the removal of covers or panels.



WARNING! The ImageXpress 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 power switch on the front of the ImageXpress 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 Systems Power and Options Controller

A single power cable connects the instrument to the external ImageXpress 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 Confocal System, the ImageXpress 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 instrument contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts.

To prevent injury:

- 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! LIFTING HAZARD. The ImageXpress Micro Confocal System weighs approximately 104 kg (230 lb). 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 of the instrument.

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 required precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. When working with potentially hazardous liquids, take applicable safety precautions, such as wearing safety glasses and protective clothing.
- 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 an instrument that is powered on.
- 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

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

Do the following before you clean 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 13](#).

Perform only the maintenance tasks described in this guide. Contact Molecular Devices to inspect and perform preventive maintenance service on the instrument each year. See [Obtaining Support on page 244](#).



WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before you return parts to Molecular Devices for repair. Molecular Devices does 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 193](#).

Water Immersion Option Safety

If your ImageXpress system includes the Water Immersion option, review the following information to ensure proper operation and to reduce the risk of damage to the instrument and components.



CAUTION!

- Do not remove or change the water immersion objectives that are installed within the instrument. A Molecular Devices Field Service Engineer must install and remove water immersion objectives.
- Do not touch the leak detection sensor, which is the brass color ring around the top of the objective below the cap.
- Do not place the water immersion controller on top of the instrument or near electronics. The water immersion controller is not watertight. Water will not be contained within the controller if water spills into the water immersion controller.
- Do not move the water immersion controller to a location that is above or below where it is installed by the Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. If you change the elevation of the water immersion controller, you can negatively affect the calibration.
- Do not attempt to remove the water immersion controller cover. With the exception of the water bottles, the controller does not contain any user-serviceable parts.
- Use only distilled water in the source water bottle. Deionized water can be used, but is not required. Do not use tap water, and do not reuse the water that is in the waste bottle.

Chapter 1: ImageXpress Micro Confocal System

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 cell imaging, moves fluids to and from cells for compound addition and cell washing, and uses transmitted light capability for label-free imaging.

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 water immersion objectives
- Optional plate-handling robot with an automated barcode reader

ImageXpress Micro Confocal System Instrument Features

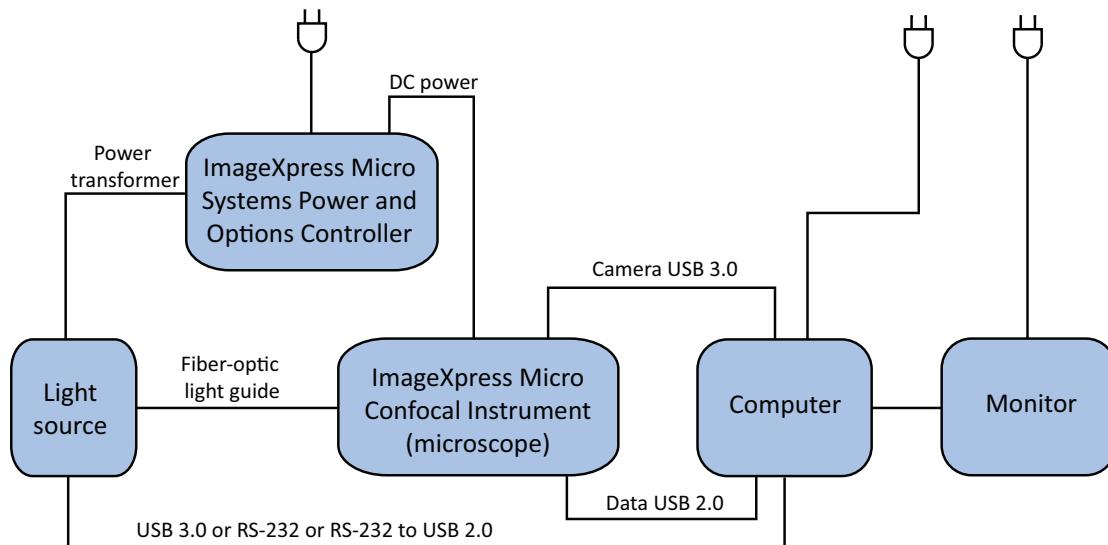


Figure 1-1: ImageXpress Micro Confocal System Components Without Options

Illumination System: Excitation

Light Source

This light source is a solid-state light source that has a rated lifetime of more than 20,000 hours. There are no user-replaceable parts in the 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	377/54 nm
FITC	475/34 nm
Cy3	531/40 nm or 543/22 nm (Contact Molecular Devices Technical Support to verify.)
TEXAS RED	560/32 nm
CY5	631/28 nm



Note: The warranty on the light source will be voided if you attempt to open the light source for any reason. For service contact Molecular Devices.

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 to provide 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. You must order the dichroic mirror filters through Molecular Devices.

Objective (Z) Stage

Motorized Z Stage

A linear encoder that features better than 100 nm resolution monitors the Z stage position.

Objectives

The standard objectives are Nikon CFI60 series. The objective lens focuses excitation light onto the sample and collects light that the sample emits. See [Compatible Objectives on page 251](#).



CAUTION! You must contact Molecular Devices to schedule a Field Service Engineer to install or remove a water immersion objective.

Motorized Objective Changer

The instrument includes a 4-position objective changer. Only the selected objective moves up and down when you change the position.

Sample (X-Y) Stage

Sample

The plate holder is designed to scan 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, you can use the glass slide adapter (included in the accessory kit) to image glass slides. 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

A linear encoder that features better than 100 nm resolution monitors the X-Y stage position.

Autofocus Laser

The ImageXpress Micro System uses a laser to autofocus the image. The red (690 nm) diode laser projects a laser spot onto the sample. Reflections of this spot from the bottom of the plate and the plate-sample interface are imaged by a dedicated, fast-focus sensor and the spot acts as a reference for focus by the autofocus feature in the MetaXpress Software.

Imaging System: Emission

Tube Lens

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

Camera

The ImageXpress Micro Confocal System uses a scientific sCMOS camera. This camera has a 2048×2048 image sensor format ($6.5 \times 6.5 \mu\text{m}$ pixel size) and has a peak quantum efficiency of 82%.

Confocal Disks

There are three available Confocal disk box configurations. For the confocal performance details with different objectives, see [Compatible Objectives on page 251](#).

Table 1-2: Confocal Disks Geometries

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

Electronics

The base ImageXpress Micro Confocal System includes the following components:

- External ImageXpress Systems Power and Options Controller and cables
- External computer, monitor, 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 standard acquisition and analysis protocols or to develop custom protocols to fit your acquisition and analysis needs. The MetaXpress Software workflow has 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 47](#) and [Configuring Plate Acquisition Options on page 57](#).
- 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 flash drive or the software application help.

Simplified Menu Structure

ImageXpress Micro Systems ship with the simplified menu installed. Legacy installations use the standard menu that displays more top-level menus. All software features are available from both menu implementations and the instructions in this guide list both paths to accomplish your goals. The simplified menu provides a map to where items were once located in the legacy standard menu.

To use the Menu Map to find the legacy location of features:

1. Click **Help > Menu Map** to display the Menu Map dialog.
2. Select the **Default to Customized** Menu option to display the standard menu layout in the Menu Map dialog.
3. Click the menu path where the software feature was in the standard menu to display the path to the feature in the simplified menu.

For example, to duplicate an image:

1. From the simplified menu, click **Help > Menu Map** to display the Menu Map dialog.
2. Select the **Default to Customized** Menu option. The standard menu path to duplicate an image is to click the Edit menu and select Duplicate.
3. In the Menu Map dialog, click **Edit > Duplicate**. The corresponding simplified menu path displays: **-> Edit: Image: Duplicate Image/Plane**. This indicates that the simplified menu path to duplicate an image is to 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, and the type of work done. Some MetaXpress Software system administrator tasks include:

- Installation overview with a Molecular Devices representative
- Post-installation hardware and software tests
- Database planning and implementation
- Custom user and group settings creation
- Maintenance schedules and software updates

Theory of Operation

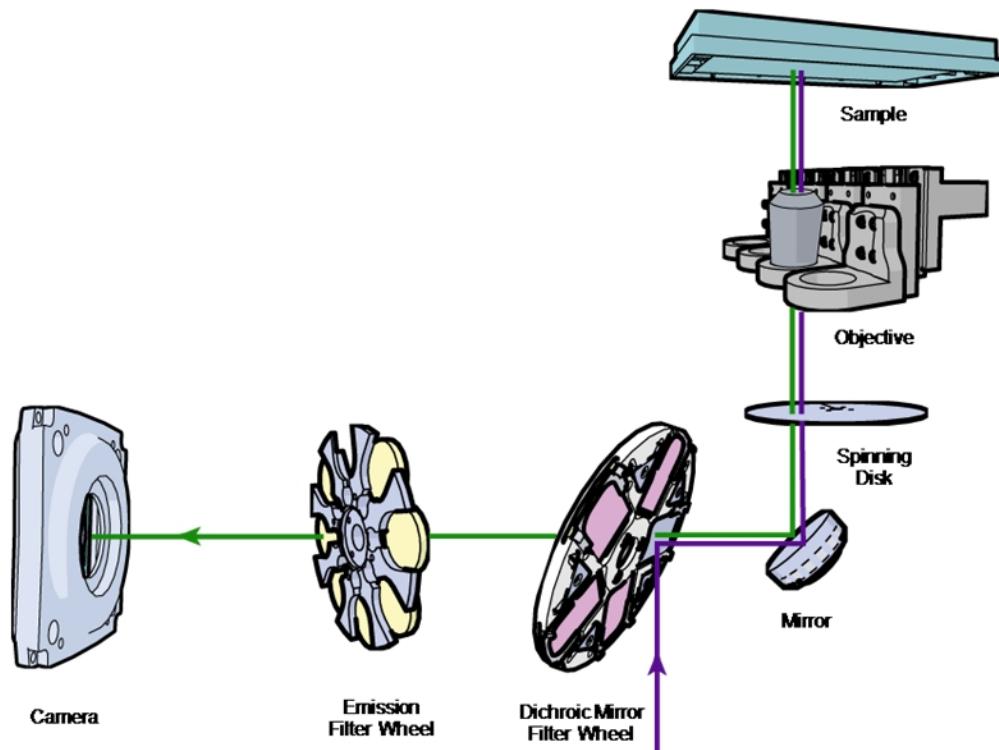


Figure 1-2: ImageXpress Micro Confocal System Optical Path

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

- [Fluorescence Imaging, see below](#)
- [Confocal Imaging, see below](#)
- [Excitation and Emission Filters, see below](#)
- [Dichroic Mirror on page 22](#)
- [Objective Lenses on page 23](#)

Fluorescence Imaging

Fluorescence is a property of certain classes of molecules (fluorophores) in which photons of a specific wavelength are absorbed (excitation), and, as a result, photons are emitted at a longer wavelength (emission) a very short time later. The utility of fluorescence imaging in biological applications stems from the ability to conjugate fluorescent molecules with biologically significant probe molecules, so that visualization of the combined fluorophore in the specimen highlights the specific distribution of the molecules in question.

By attaching different probes to a set of dye molecules with non-overlapping excitation and emission spectra, you can stain a specimen with multiple fluorophores and either simultaneously or sequentially image different structures or substances within the same specimen. The excitation 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 use, 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 uses point-source illumination and a pinhole aperture ahead of the detector to reject out-of-plane signals.

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. 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 that contains only photons with a wavelength range that matches the excitation spectrum of the target fluorophore. A bandpass filter in the illumination optical path (called the excitation filter, since it filters the excitation light) restricts 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 done by placing a filter in the collection light path, called the emission filter. Emission filters can either be a bandpass filter (for maximum specificity) or a longpass filter (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. See [Figure 1-2](#).

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 on 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.

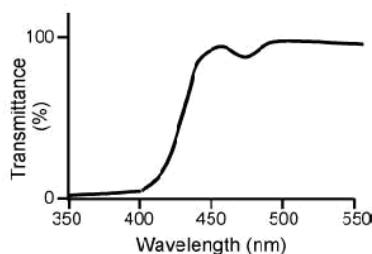
In the imaging optical path, longer wavelength fluorescence light emitted by the excited fluorophores in the specimen is collected by the objective lens and transmitted through the dichroic mirror to the camera. Incident light from the sample that is shorter wavelength than the cutoff (mostly reflected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

The optics in a filter cube are interference filters made by depositing thin film coatings on a glass support. These components are delicate and can be easily damaged. Always use care when you handle a filter cube.

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 mirror looks like the following graph.



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 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 high-quality Nikon objectives. See [Compatible Objectives on page 251](#).

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



Note: Some objectives have adjustable spherical aberration correction collars for imaging through thick substrates such as most microplates. Use the software to calculate and set the correct values. See [Adjusting Objective Correction Collars on page 221](#).

Several objectives (such as, 40x Super Plan Fluor ELWD) have correction collars for adjustment according to the thickness of the glass cover slip or thin plate bottom you use. Use the physical thickness of the plate bottom or through optimization of image quality to set these collars.

Objectives are classified according to optical correction, flatness of field, numerical aperture, and working distance. Before you choose the objectives to use with the system, it is important to consider the types of plates and type of assay you plan to image. The plate material (plastic or glass) and thickness are major considerations when you choose an objective. Another important note is that generally the more optically corrected an objective, the greater the number of lens elements it contains, with correspondingly reduced light transmission, especially in the UV spectrum. The levels of correction in descending order are: 1) Achromatic, 2) Fluor, 3) Plan Fluor, 4) Plan Apo.



CAUTION! You must contact Molecular Devices to schedule a Field Service Engineer to install or remove a water immersion objective.

For detailed information on objectives, see the Nikon web site (www.nikon.com).



Chapter 2: Using the ImageXpress Micro Confocal System

The topics in this section provide an overview of the start-to-finish workflow to use the ImageXpress Micro Confocal System.

- [Starting the System, see below](#)
- [Understanding Status Lights on page 28](#)
- [Maintaining the Instrument on page 28](#)
- [Shutting Down the System on page 28](#)

Starting the System

This section describes how to power on the instrument and how to start the MetaXpress Software.

Powering on the Instrument

Power on the instrument before you start the software.

To power on the instrument:

1. Ensure that the power cords for the instrument and the light source are connected to the ImageXpress 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 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 starts, log in to Windows using the User Name and Password combination that your system administrator provides.



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

-
5. Start the MetaXpress Software. See [Starting the Software on page 26](#).

Starting the Software

This procedure assumes that the ImageXpress Micro Confocal System and the MetaXpress Software are properly installed and configured by your Molecular Devices representative and your System Administrator.



Note: If you encounter actions or results that are inconsistent with the expected results when you use the instrument and the software, contact your system administrator before you continue the experiment.

To start the MetaXpress Software:

1. Double-click the MetaXpress icon on the computer desktop or click **Start > All Programs > MetaXpress > MetaXpress** to display the MetaXpress Software Login dialog.
2. Click the **User Name** drop-down and select the user name to use and then click **OK**.



Figure 2-1: MetaXpress Software Login Dialog



Note: The MetaXpress Software login dialog displays when you configure the software to run in multi-user mode from the Meta Imaging Series Administrator Software. When the software is in single-user mode the Welcome to MetaXpress dialog displays. See [Figure 2-2](#).

3. In the Welcome to MetaXpress dialog, click the **Data Source** drop-down and select a data source, enter your **Login Name** and **Password**, and then click **OK**.

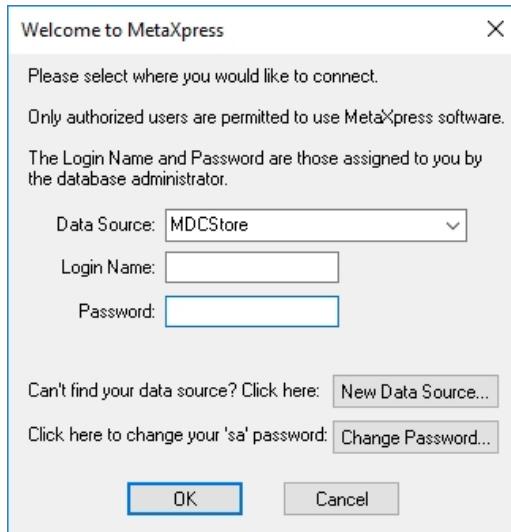


Figure 2-2: Welcome to MetaXpress Dialog



Note: The initial user Login Name is **moldev** and the customer-specific documentation provided with your instrument includes the unique password. You should click Change Password to change the password.

4. Select a security level, select a group that is 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 error messages display when the system initializes, try the following:

- Check that all hardware connections are plugged in and fully seated.
- Check that the plate stage is clear of blockages.
- Check that the Options Controller box and the light source are powered on, if present.
- Restart the system.

If the message continues, use AxoTrace and contact Molecular Devices Technical Support. See [Logging AxoTrace Software Messages to .txt Files on page 246](#).

Understanding Status Lights

The status light on the ImageXpress Micro Confocal System illuminates to provide information about the instrument status. You can turn off the status lights. See [Turning Off the Status Indicator Lights on page 244](#).

Table 2-1: Status Light 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.

Maintaining the Instrument

You can do specific user-level maintenance on the ImageXpress Micro Confocal System to clean the instrument, change filters, and change and clean objectives. Perform only the maintenance tasks this guide describes. Contact Molecular Devices to inspect and perform preventive maintenance service on the instrument each year. See [Maintenance on page 193](#) and [Obtaining Support on page 244](#).



CAUTION! You must contact Molecular Devices to schedule a Field Service Engineer to install or remove a water immersion objective.

Shutting Down the System

To shut down the ImageXpress Micro Confocal System:

1. Exit the MetaXpress Software.
2. Turn off the **Instrument** button on the front of the ImageXpress Systems Power and Options Controller. This also turns off the light source box.

Chapter 3: System Installation and Testing

The ImageXpress Micro Confocal System ships fully configured, and a Molecular Devices Field Service Engineer installs it at your site. The base system includes the imaging unit (instrument), host computer, and accessory kit. See [Table 3-1](#) for kit contents.

The ImageXpress Micro Confocal System host computer ships with the MetaXpress Software already installed. The instrument connects to the host computer during installation. There are four main connections, excluding power cords.

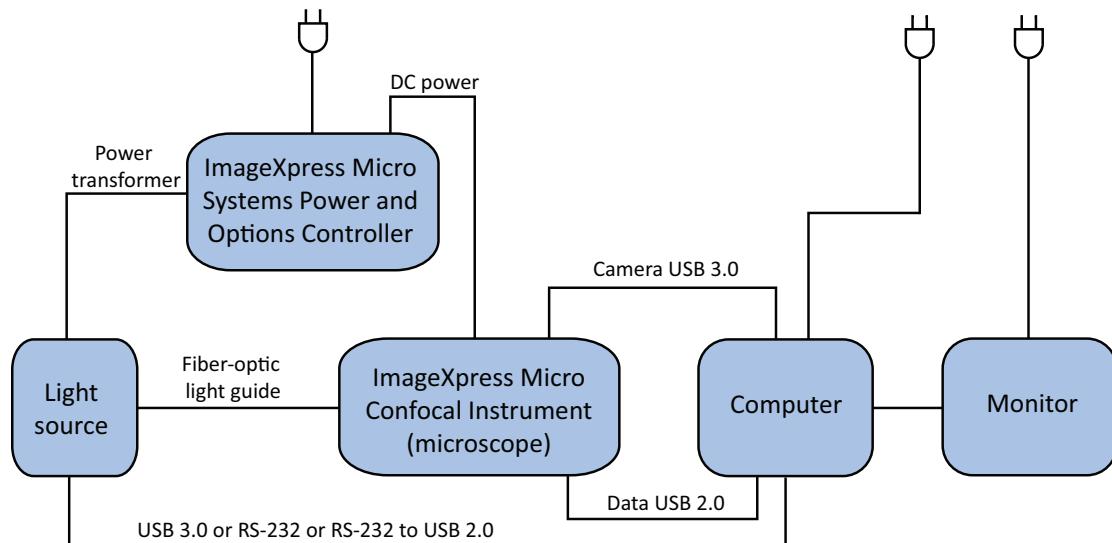


Figure 3-1: ImageXpress Micro Confocal System Components Without Options

- Power supply to the instrument
- Fiber optic light guide from the external light source to the instrument
- USB 2.0 data cable from the instrument to the computer
- USB 3.0 cable from the camera to the computer
- RS-232, USB 3.0, or RS-232 to USB 2.0 cable from the external light source to the computer

Table 3-1: Accessory Kit Contents

Accessory	Description
Bead Plate	TetraSpeck™ fluorescent microspheres test plate
Hex Keys	1/16", 0.05", 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	Accessory box for miscellaneous storage

If you need to re-install the MetaXpress Software or install the software on a new computer, see the *MetaXpress High-Content Image Acquisition and Analysis Software Suite Installation and Update Guide* included on the MetaXpress Software Suite flash drive or contact Technical Support. See [Obtaining Support on page 244](#).

Verifying Device Settings in the Meta Imaging Series Administrator Software

Use the Meta Imaging Series Administrator Software to configure all hardware and software components. This section describes how to verify that the ImageXpress Micro Confocal System hardware components are properly configured in the Meta Imaging Series Administrator Software and in the MetaXpress Software.



Note: Molecular Devices recommends that you run verification tests without a sample plate loaded on the stage except when verifying the Laser Autofocus Sensor.



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

To verify the hardware configuration:

1. Power on the instrument but do not start the MetaXpress Software.
2. Click **Start > All Programs > MetaXpress 6 > Meta Imaging Series Administrator** to display the Meta Imaging Series Administrator.

3. In the **List of Groups** list, select **MetaXpress**.

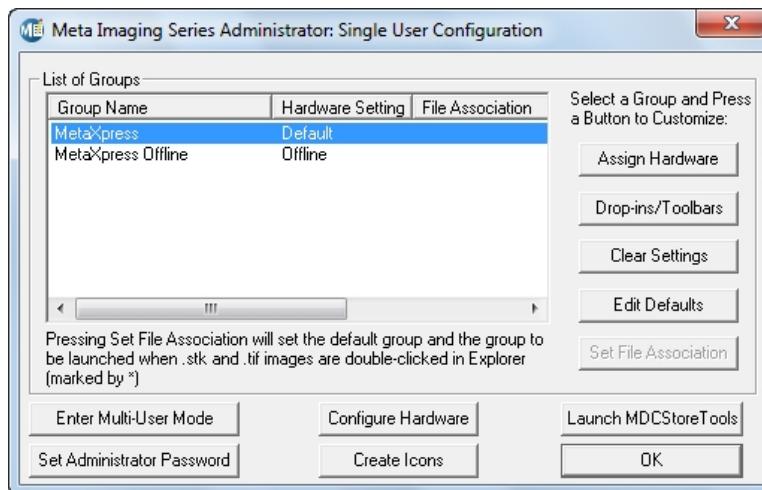


Figure 3-2: Meta Imaging Series Administrator Software Home Dialog

4. Click **Configure Hardware** to display the Configure Hardware dialog.
5. Click **Configure Devices** to display the User Settings dialog.

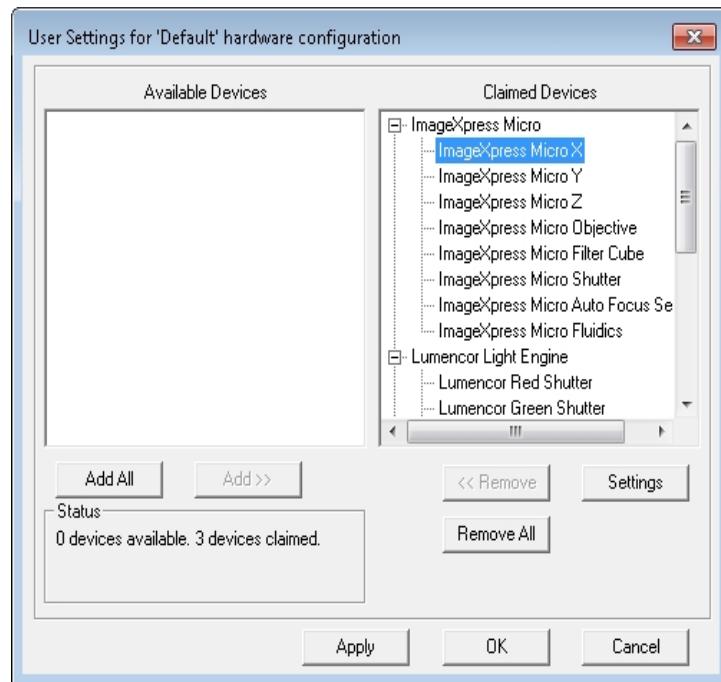


Figure 3-3: User Settings Dialog

- In the Claimed Devices list, select **ImageXpress Micro X** and then click **Settings** to display the ImageXpress Micro X Settings dialog.

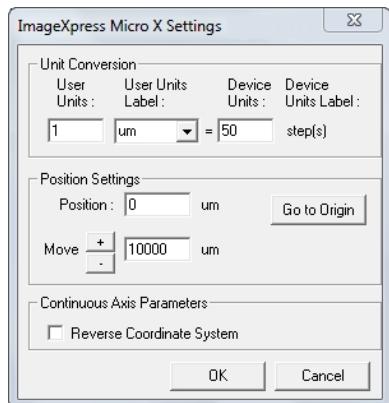


Figure 3-4: ImageXpress Micro X Settings Dialog

- In the **Move** field, increase the step size to 10,000 μm .
- Clear the **Reverse Coordinate System** check box.
- Click **[+]** and **[-]** and confirm that the stage responds to the control.



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

- In the **Move** field, change the step size back to 10 μm and click **OK** to display the User Settings dialog.

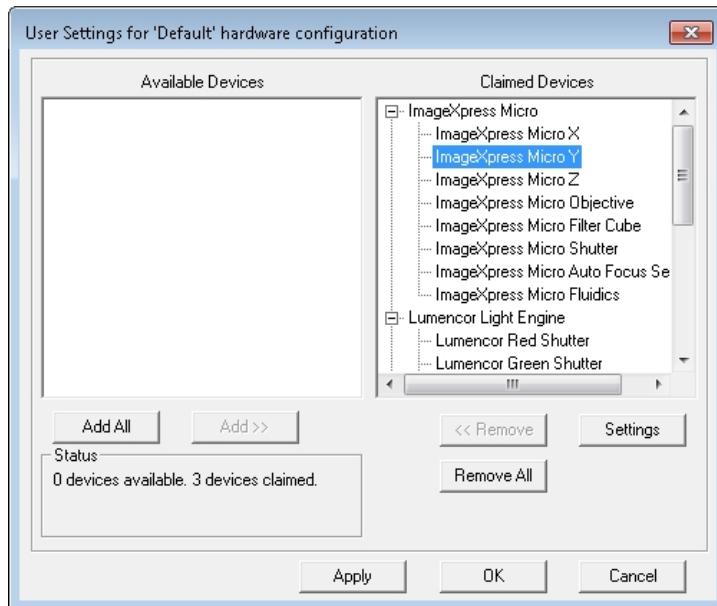


Figure 3-5: User Settings Dialog with ImageXpress Micro Y Selection

11. In the Claimed Devices list, select **ImageXpress Micro Y** and then click **Settings** to display the ImageXpress Micro Y settings dialog.

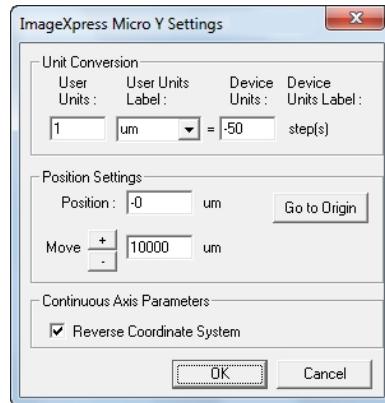


Figure 3-6: ImageXpress Micro Y Settings Dialog

12. In the **Move** field, increase the step size to 10,000 μm .
13. Select the **Reverse Coordinate System** check box.
14. Click **+** and **-** 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** to display the User Settings dialog.

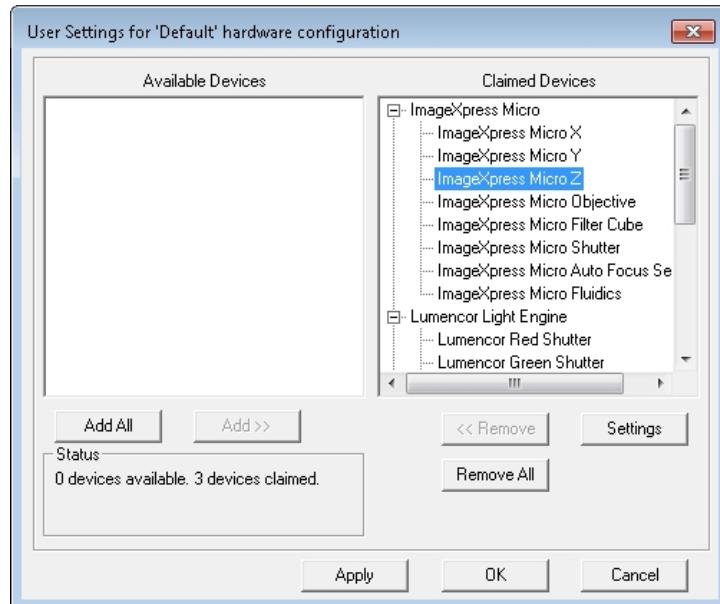


Figure 3-7: User Settings Dialog with ImageXpress Micro Z Selection

16. In the Claimed Devices list, select **ImageXpress Micro Z** and then click **Settings** to display the ImageXpress Micro Z setting dialog.

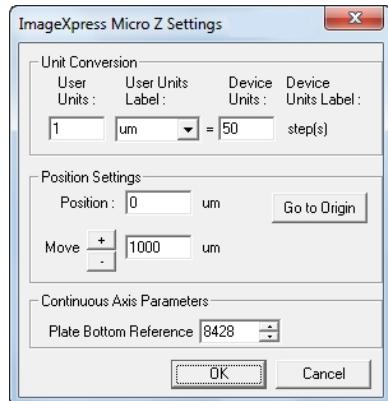


Figure 3-8: ImageXpress Micro Z Settings Dialog

17. Verify that the value in the **Device Units** field is 50.
18. In the **Move** field, increase the step size to 1000 μm .



Note: Do not change the Plate Bottom Reference value.

19. Click **[+]** and **[-]** 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** to display the User Settings dialog.
22. In the Claimed Devices list, select **ImageXpress Micro Objective** and then click **Settings** to display the ImageXpress Micro Objective Settings dialog.

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

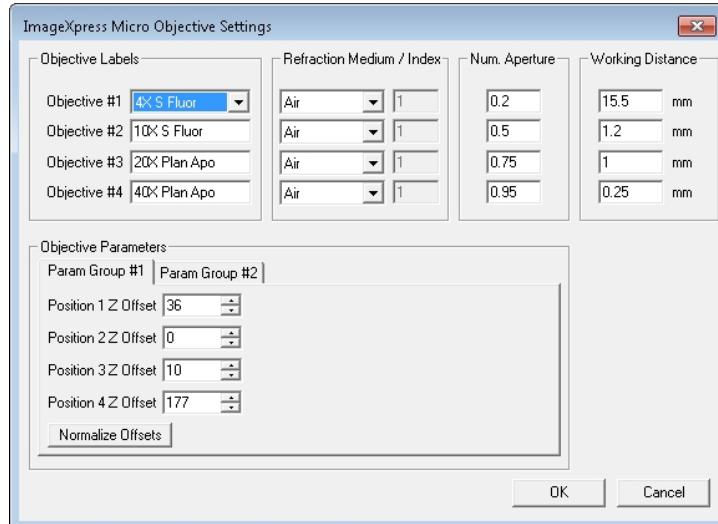


Figure 3-9: ImageXpress Micro Objective Settings Dialog

23. Confirm that the values in the **Objective Labels** fields, the **Refraction Medium/Index** fields, the **Num Aperture** fields, and the **Working Distance** fields match each objective in the system.
24. In the Objective Parameters section, select the **Param Group #1** tab that 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.
26. Select the **Param Group #2** tab.
27. Click **Open Control Dialog** to display the Control - ImageXpress Micro Objective dialog.

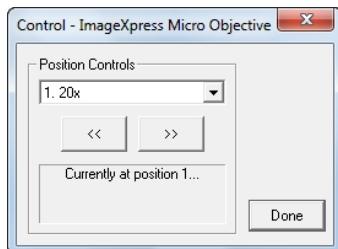


Figure 3-10: Control > ImageXpress Micro Objective Dialog

28. Click **<<** and **>>** and confirm that the objective changer moves appropriately.
29. Click **Done** to display the ImageXpress Micro Objective Settings dialog.
30. Click **OK**.
31. If you attach a plate handling robot to the instrument, confirm those settings. See [Verifying External Control Settings on page 265](#).
32. Click **OK** to display the Configure Hardware dialog and proceed to the next topic.

Verifying Camera Settings in the Meta Imaging Series Administrator Software

To verify the installation of the correct version of the ImageXpress Micro Confocal System camera driver:

1. If needed, power on the ImageXpress Micro Confocal System and open the Meta Imaging Series Administrator Software to the Configure Hardware dialog.
2. In the Configure Hardware dialog, click **Configure Acquisition** to display the Configure Acquisition dialog.
3. The **Installed Drivers** field should contain one driver. If no driver displays, or if more than one driver displays, contact Molecular Devices support to determine which driver is for your system.
4. In the **Installed Drivers** field, select the driver.
5. Click **Configure** to display the Camera Driver dialog.
6. 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 36](#).

Verifying and Backing Up Settings in the MetaXpress Software

After you confirm hardware settings in the Meta Imaging Series Administrator Software, check the settings in the MetaXpress Software.

See the following topics:

- [Verifying Magnification Settings, see below](#)
- [Verifying Illumination Settings on page 37](#)
- [Verifying Calibration Settings on page 38](#)
- [Verifying the Laser Autofocus Sensor on page 40](#)
- [Verifying the Plate Reference Point \(A1 Center\) on page 41](#)
- [Verifying Plate Types on page 43](#)
- [Confirming Laser Autofocus Settings for Plate Files on page 44](#)
- [Verifying Shading Correction Files-Legacy on page 45](#)

Each procedure provides instructions to back up the settings so that you can restore the settings in case they are lost.

 **Tip:** Press F1 in the MetaXpress Software to view the software application help.

Verifying Magnification Settings

Use the Configure Magnification dialog to confirm magnification settings for the ImageXpress Micro Confocal System objectives before you use the system.

To verify the magnification settings:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Devices > Configure Magnification** to display the Configure Magnification dialog.

From the standard menu, click **Devices > Configure Magnification** to display the Configure Magnification dialog.

2. In the Setting section, verify there is an ImageXpress Micro Objective and select the **ImageXpress Micro Objective** check box.

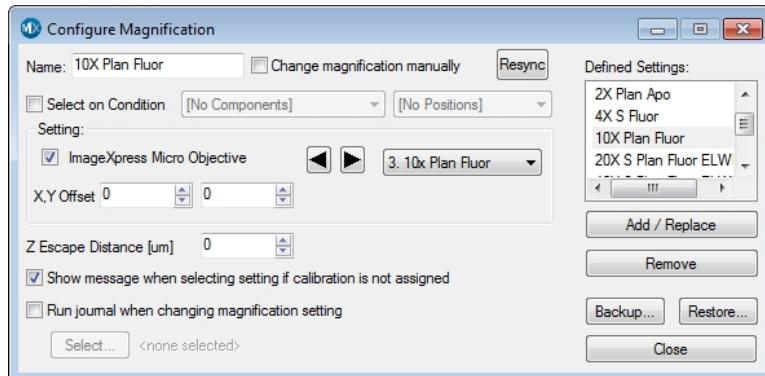


Figure 3-11: Configure Magnification Dialog

3. Confirm that the **Defined Settings** field lists each objective in the system.
4. Click **Backup** to display the Backup All Magnification Settings dialog.
5. In the **File Name** field, enter the name of the backup .mgs file.
6. Navigate to where the backup file is to be stored and click **Save**.
7. Click **Close**.

Verifying Illumination Settings

Use the Configure Illumination dialog to confirm illumination settings for the ImageXpress Micro Confocal System objectives before you use the system.

To verify the illumination settings:

1. In the MetaXpress Software:
 - From the simplified menu, click **Control > Devices > Configure Illumination** to display the Configure Illumination dialog.
 - From the standard menu, click **Devices > Configure Illumination** to display the Configure Illumination dialog.

2. In the **Defined Settings** list, ensure that the correct illuminations display.

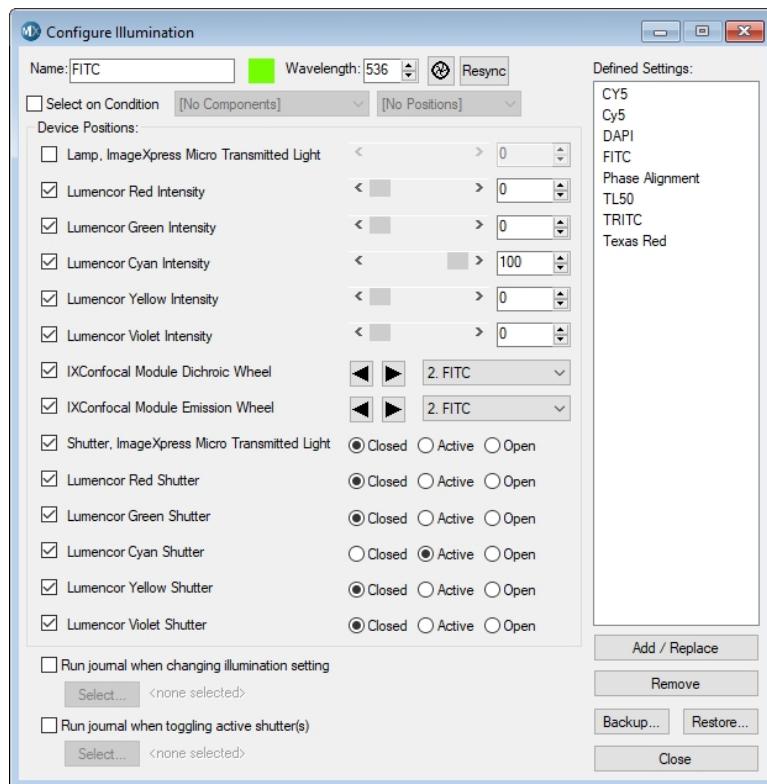


Figure 3-12: Configure Illumination Dialog

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



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

4. Set up other illumination settings, if needed. The value in the **Wavelength** field should match the center wavelength for the emission filter.
5. Click **Backup** to display the Backup All Illumination Settings dialog.
6. In the **File Name** field, enter the name of the backup .ils file.
7. Navigate to where the backup file is to be stored and click **Save**.
8. Click **Restore** and select the file to restore the settings, if needed.
9. Click **Close**.

Verifying Calibration Settings

Use the Calibrate Distances dialog to verify calibration distances.

To verify the calibration settings:

1. In the MetaXpress Software:
From the simplified menu, click **Measure > Distances > Calibrate Distances** to display the Calibrate Distances dialog.
From the standard menu, click **Measure > Calibrate Distances** to display the Calibrate Distances dialog.

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

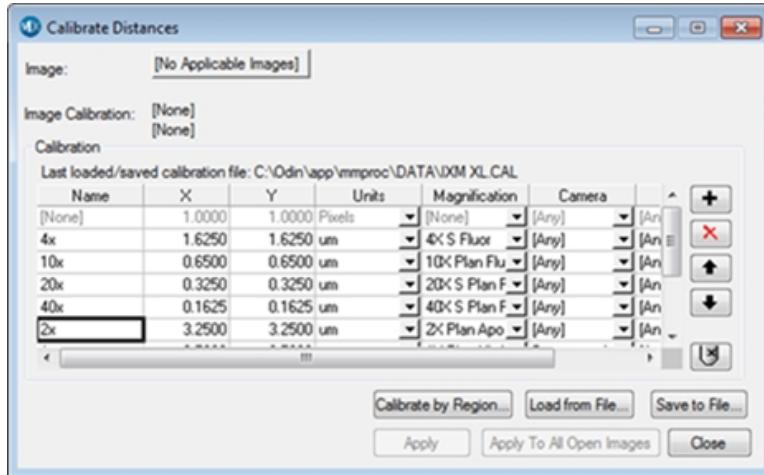


Figure 3-13: Calibrate Distances Dialog

3. Make sure the appropriate magnification setting displays for each calibration.



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

You can use the following estimated values for ImageXpress Micro Confocal System calibration settings:



Note: To measure pixel sizes more accurately, in the IX Taskbar, click System Maintenance and then click Measure Pixel Sizes. If the IX Taskbar is not installed, contact Technical Support.

Table 3-2: Estimated Calibration Settings

Objective Magnification	Unbinned Pixel Size (um)	Width (mm)	Height (mm)	Area (mm ²)
1x	6.8313	13.9905	13.9905	195.7342
2x	3.4157	6.9953	6.9953	48.9335
4x	1.7078	3.4976	3.4976	12.2334
10x	0.6831	1.3991	1.3991	1.9573
20x	0.3416	0.6995	0.6995	0.4893
40x	0.1708	0.3498	0.3498	0.1223
60x	0.1139	0.2332	0.2332	0.0544
100x	0.0683	0.1399	0.1399	0.0196

4. Click **Save to File** to display the Save Spatial Calibrations dialog.
5. In the **File Name** field, enter the name of the backup .cal file.
6. Navigate to where the backup file is to be stored and click **Save**.
7. Click **Close** in the Calibrate Distances dialog.

Verifying the Laser Autofocus Sensor

This procedure uses a bead plate to verify that the laser autofocus sensor responds.

To verify that the Laser Autofocus sensor responds:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.

From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.

2. Select the **Configure** tab.

3. On the Configure tab, select the **Acquisition** tab.

4. Select the **Enable Laser Based Focusing** check box.

5. Select the **Objective and Camera** tab.

6. Click the **Magnification** drop-down and select **10x**.

7. Select the **Plate** tab.

8. Click the **Plate Name** drop-down and select **Bead Plate IXM-C**.

9. Click **Eject Plate**.

10. Load the Tetra Speck bead plate and click **Load Plate**.

11. On the image of the plate, right-click well **A1** to move the stage to that well.

12. On the Configure tab, select the **Autofocus** tab.

13. Click **Configure Laser Settings** to display the Configure Laser Autofocus Settings dialog.

14. Click **Preview Pass** to display a Preview Pass image window with a graph of focus intensities compared to the Z-position. You want to see graphs of sharp peaks in red and green.

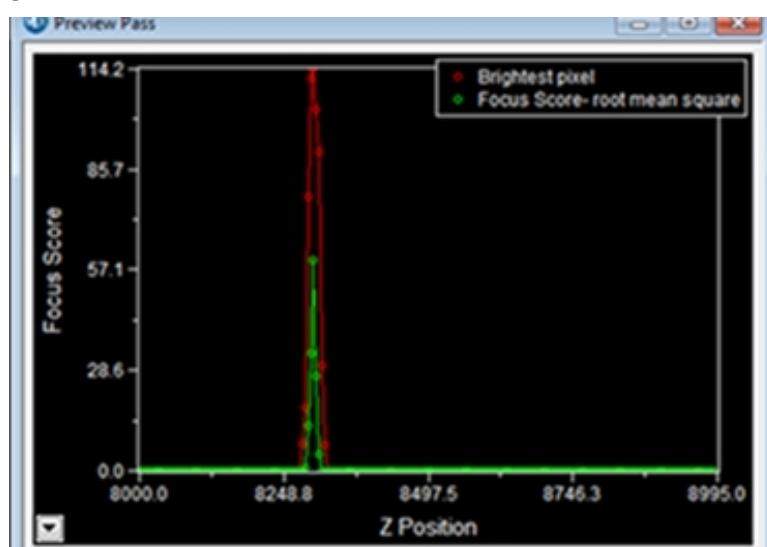


Figure 3-14: Preview Pass Image Window

15. In the Preview Pass image window, ensure that there is at least one peak.
 - If the Preview Pass image window shows at least one peak, the Laser Auto Focus Sensor is enabled and functional.
 - If the Preview Pass image 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, in the **Exposure** row, select the **Override Exposure** check box, increase the value, and click **Preview Pass** again.
 - If the Preview Pass image window still does not show a peak, click **Laser Autofocus Wizard** and then follow the instructions or use the software application help.
 - If the Preview Pass image window still does not show a peak, contact Molecular Devices Technical Support and report the issue. See [Obtaining Support on page 244](#). For more information, see [Confirming Laser Autofocus Settings for Plate Files on page 44](#).
16. Close the Preview Pass image window.
17. Click **Close** in the Configure Laser Autofocus Settings dialog.
18. In the Plate Acquisition Setup dialog, click **Eject Plate**.
19. Remove the Tetra Speck bead plate and click **Load Plate**.
20. Click **Close**.

Verifying the Plate Reference Point (A1 Center)

This procedure requires the metal slide holder plate that ships with the instrument to verify that the plate reference point is in the center of well A1.

To verify the plate reference point is properly set:

1. In the MetaXpress Software:
From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.
From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.
2. Select the **Configure** tab.
3. On the Configure tab, select the **Objective and Camera** tab.
4. Click the **Magnification** drop-down and select the lowest power objective (max. 10x).
5. In the **Camera Binning** field, enter 1.
6. Click the **Acquisition Mode** drop-down and select **Widefield**.
7. Select the **Plate** tab.
8. Click the **Plate Name** drop-down and select **96 Wells (8x12)**.
9. Select the **Sites to Visit** tab.
10. Under **Site Options**, select **Single site**.
11. Select the **W1** tab below the **Acquisition > Wavelengths** tab.
12. Click the **Illumination** drop-down and select **FITC**.



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

13. In the **Exposure** field, enter **100** milliseconds (ms).
14. From the simplified menu and the standard menu, click **Screening > Plate Acquisition and Control** to display the Plate Acquisition and Control dialog.

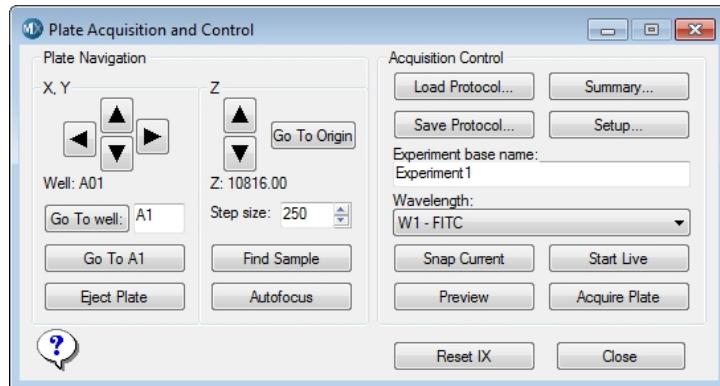


Figure 3-15: Plate Acquisition and Control Dialog

15. Click the **Wavelength** drop-down and select **W1 - FITC**.
16. In the **Step Size** field, enter **250**.
17. Click **Eject Plate** to open the top door.
18. Load the metal slide holder plate and ensure the notch in the plate is in the A1 position on the stage. There is an integrated pinhole at the A1 well position.



Figure 3-16: Loaded Metal Slide Holder Plate with Integrated A1 Well Pinhole

19. Click **Load Plate** to close the top door.
20. Click **Go to A1** to move the stage to the A1 position.
21. Click **Start Live** to display a Live image window.

22. If you use a 4x objective, click the **Z** control arrows to step the Z-motor (reducing the step size as you get closer to focus) until the A1 pinhole comes into focus, and then verify that the hole is visually centered in the field of view.

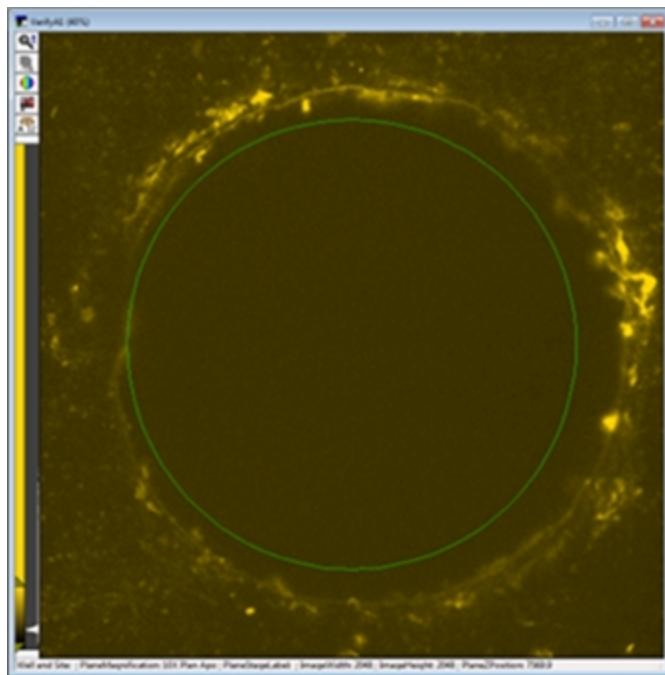


Figure 3-17: A1 Pinhole in Focus at 10X

If the A1 pinhole is not centered, or if you cannot find the hole, contact Molecular Devices Technical Support. See [Obtaining Support on page 244](#).

23. Click **F2: Stop** to close the Live image window.
24. Click **Eject Plate**.
25. Remove the metal slide holder plate and then click **Load Plate**.
26. Click **Close** in the Plate Acquisition and Control dialog.
27. Click **Close** in the Plate Acquisition Setup dialog.

Verifying Plate Types

Use this procedure to verify that the plate type files included with the software are available from the Plate Acquisition Setup dialog.

To verify the plate types were installed:

1. In the MetaXpress Software:
 - From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.
 - From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.
2. Select the **Configure** tab.
3. On the Configure tab, select the **Plate** tab.

4. Click the **Plate Name** drop-down to display the list of plate types.



Figure 3-18: Available Plate Types

- If there are several custom plate types in the list, you are finished.
 - If only **96 Wells (8x12)**, **384 Wells (16x24)**, and **Custom** display, then continue with this procedure to load the preconfigured plate type files.
5. Insert the MetaXpress Software Suite flash drive into the computer.
 6. When the MetaXpress Software installation window displays, click **Explore Installation Folders/Files**.
 7. In Windows Explorer, open the **Plates** folder to view the plate type files (.plt).
 8. Select the plate type files to be available in the software (Shift+Click or Ctrl+Click to multi select).
 9. Right-click and select **Copy** to copy the files to the computer clipboard.
 10. Navigate to **C:\MX6\Plates** (or the Plates folder in a custom installation) then right-click and select **Paste** to have the files appear in the **Plate Name** drop-down list in the software.



Note:

- To refresh the plate list in Plate Acquisition Setup, close and reopen the dialog box.
- The plate files are read-only. Follow steps 11-14 to turn off the read-only attribute.

11. In the **Plates** folder on the computer (not the flash drive), select all the copied files.
12. Right-click and select **Properties** to display the Properties dialog.
13. Select the **General** tab and clear the **Read Only** check box.
14. Click **OK**.

Confirming Laser Autofocus Settings for Plate Files

Before you use a plate file, confirm that the laser autofocus settings are optimized for the plate.



Note: If you use an objective with a correction collar, ensure that the correction collar is set appropriately for the plate. See [Adjusting Objective Correction Collars on page 221](#).

The laser autofocus system moves the vertical (Z-axis) position of the objective to a point where the bottom of the well is in focus before the software acquires each site during plate acquisition.



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.

To confirm the laser autofocus settings:

1. Prepare the plate to test by putting water or buffer in several of the wells.
2. In the MetaXpress Software:
 - From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.
 - From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.
3. Click **Eject Plate**.
4. Place the test plate in position and then click **Load Plate**.
5. Select the **Configure** tab.
6. On the **Configure** tab, select the **Plates** tab.
7. Click the **Plate Name** drop-down and select the plate for which to configure laser settings.
8. On the **Objective and Camera** tab, select a magnification.
9. On the **Acquisition** tab, select the **Enable Laser Based Focusing** check box.
10. Use the navigation tools at the top of the Plate Acquisition Setup dialog to move to an appropriate well and site.
11. On the **Autofocus** tab, click **Configure Laser Settings** to display the Configure Autofocus Settings dialog.



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

12. Click **Find Sample** to display the Plate Acquisition report. 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, use the Laser Autofocus Wizard to optimize the laser autofocus settings.

13. Click **OK** in the Plate Acquisition report.
14. Click **Close** in the Configure Laser Autofocus Settings dialog.

Verifying Shading Correction Files-Legacy



Note: This procedure is required if you select the Legacy Correction check box for one or more wavelengths in an acquisition protocol.

If you select Legacy Correction as the Shading Correction method for an acquisition wavelength, you need the shading correction image files for each magnification and illumination setting. You then use these files to generate the files again whenever you replace an objective or filter set, , or whenever you replace the light guide. See [Updating Shading Correction Settings-Legacy on page 240](#).

To verify that the shading correction image files are available for the Legacy Correction setting:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.

From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.

2. Select the **Configure** tab.

3. On the **Configure** tab, select the **Acquisition** tab.

4. Click **Directory for Stored Correction Images** to display the Browse for Folder dialog. The default location for stored correction images is **C:\Shading Images**.

To ensure that the file names are formatted correctly:

1. Use Windows Explorer to navigate to the **C:\Shading Images** folder, or the location you define.
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

This section provides general guidelines to consider before you acquire plate data. These guidelines help ensure that the images you acquire are the best possible quality.

Consider the following criteria to obtain the best possible fluorescence image quality:

- [Assay Design, see below](#)
- [Plate Selection on page 48](#)
- [Sample Preparation on page 50](#)
- [Plate Acquisition Settings on page 51](#)
- [Instrument Maintenance on page 55](#)

Assay Design

When you design a high-content screening assay, you should consider the downstream image analysis steps. Despite the image enhancement tools and options available in the software, it is difficult to analyze a poor-quality image. Starting with quality images helps ensure that the 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 you run a large-scale screen.

Selection of Different Fluorochromes

Typical high-content and high-throughput assays include one or more fluorochromes, such as fluorescent proteins, antibody-based stains, and chemical-based stains. You should include a nuclear stain (such as Hoechst or DAPI) to help identify cells during image analysis. If the assay involves movement of a protein of interest to or from a particular cellular compartment or organelle, it can also be helpful to include a probe specific to that cellular compartment or organelle. If you plan to use 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 have excitation and emission spectra suitable for the filter sets in the instrument. For experiments that use multiple stains, select fluorophores that have enough spectral separation. Some fluorochromes provide brighter intensities and require a shorter exposure time, while others do not bleach as quickly and allow a longer exposure time. There also might be toxicity issues with some cell types or bleed-through issues between pairs of fluorochromes. Consider these factors when you choose a fluorochrome.



Note: If the ImageXpress System has the transmitted light option, then it might be possible to identify cells using transmitted light images instead of fluorescence.

Cell-Based Assays

The most important consideration when you select 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. 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. Uniformly staining thick samples for confocal imaging can be difficult.

Organism-Based Assays

You can use the ImageXpress Micro Confocal System to image whole organisms, such as nematodes or zebrafish. Treat the organisms with anesthetic, use a gel preparation, or both to immobilize the organisms for best results.

Homogeneous Assays

Homogeneous assays are ones where the unbound or unreacted stains are not washed off before acquiring images. Confocal imaging reduces background fluorescence from out of focus sample volume which improves signal-to-background ratios and allows for homogeneous assays that would not work when widefield imaging.

Plates or Slides

You should use 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 facilitates scaling up to a larger screen. Some assays, such as imaging of tissue sections, may require the use of slides. A slide holder is provided in the accessory kit, and there are software tools available to streamline the slide-imaging workflow.

Plate Selection

The plate type you use can have a significant impact on image quality. You should assess various plates for their compatibility with your assay and use plates of only one brand from a single manufacturer. Mixing various plate types from different manufacturers could introduce unknown variables and contribute to the creation of flawed data.

Consider the following factors when you select plates for your assay:

- [Plate Format on page 49](#)
- [Plate Material on page 49](#)
- [Fluorescence Background on page 49](#)
- [Bottom Thickness on page 49](#)
- [Plate Flatness or Reproducibility of the Z-Pattern on page 49](#)
- [Plate Skirt on page 50](#)
- [Batch-to-Batch Consistency on page 50](#)
- [Robot Compatibility on page 50](#)

Plate Format

Determine if the plate format is compatible with your assay.

- 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 to pipette into and wash the plates?

Plate Material

The composition of the material of the bottom of the plate needs to be of optical quality; if not, the images can be degraded. For fluorescence imaging, plates with black well sides and a single-piece, clear bottom usually perform best. Plastic-bottom plates are generally more uneven and distort light more than glass-bottom plates. When you use an objective with a high magnification, 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. There can be up to a five times difference in auto-fluorescence among plates from different manufacturers.

Bottom Thickness

You should compare the thickness of the plate bottom with the working distance of the objective lens to ensure that it is compatible. In general, objectives with higher numerical aperture (NA) tend to require thin-bottomed plates. Objectives with application-optimized correction collars 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 all supported objectives.



Note: Plates with ultra-thin bottoms or very thick bottoms can be more uneven and can cause focusing issues. For best results, you should use an imaging-quality plate with a bottom thickness between 0.15 mm and 0.7 mm.

Plate Flatness or Reproducibility of the Z-Pattern

A flat plate is faster to scan than an uneven plate. 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

With short working distance objectives, imaging the edge and corner wells can cause the objective to bump into the plate skirt, which requires you to omit the outer wells from the experiment. Some manufacturers offer low-skirt or no-skirt options that allow you to use all the wells in the plate with a short working distance air objective.

Batch-to-Batch Consistency

Some plate manufacturers are more consistent in producing plates than others. If parameters such as the plate-bottom thickness vary from batch to batch, you must optimize the plate settings for each batch.

Robot Compatibility

If you use 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 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 during the assay optimization phase before you prepare multiple plates for screening.

The topics in this section describe items to consider for imaging assays.

Cell Density

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

Fixation and Staining Conditions

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

Final Buffer or Media

To reduce background in fluorescent images, ensure that the buffer or media in which the cells are left is free of fluorescent components, such as Phenol Red. This is most important for widefield assays. Several manufacturers offer media that is optimized for imaging.

You should not use solutions with a high percentage of glycerol, such as mounting media. Glycerol can interfere with the laser autofocus, and the high viscosity can cause pipetting difficulties which results in air bubbles.

A low volume of liquid can interfere with the laser autofocus and with transmitted light images. Make sure that wells are at least halfway full. Avoid letting cells dry out while the plate sits for an extended time before imaging.

Plate Handling and Storage

Since the laser autofocus measures the reflection from the bottom of the plate or from within the sample, dust particles, dirt, fingerprints, and scratches interfere with the reflection and affect the autofocus performance. If the bottom of the plate becomes dirty, clean it with a lens tissue and an optical cleaning solution or 70% ethanol to improve autofocus.

You should store plates in the dark, and fixed plates should generally be stored at 4°C (39°F). An opaque plate seal can be helpful. Avoid condensation of air humidity on the bottom of plates. Before imaging, allow chill-stored plates to return to room temperature.

Plate Acquisition Settings

You make most plate acquisition settings in the Plate Acquisition Setup dialog in the MetaXpress Software. The settings are dependent on the content and distribution of the samples, as well as the requirements of the experiment.

To help you determine the settings, use the following topics as a checklist.

- [Magnification, see below](#)
- [Correction Collars on page 52](#)
- [Binning on page 52](#)
- [Widefield vs. Confocal Imaging Mode on page 52](#)
- [Site Selection on page 52](#)
- [Shading Correction on page 53](#)
- [Autofocus on page 53](#)
- [Illumination Settings on page 53](#)
- [Exposure Time on page 54](#)
- [Z Series Acquisition on page 55](#)

Magnification

The magnification setting depends on the type of information you want to obtain. Generally, 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. To count cells, a 4x objective is often suitable. A 10X PA objective is often used to acquire spheroid images for morphology analysis. 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 you match the plate-bottom thickness 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. Molecular Devices furnishes Plan Apo Lambda objectives that allow acquisition of more of the plate.

Correction Collars

If you use an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you use. See [Adjusting Objective Correction Collars on page 221](#).

Binning

Another method to increase the signal-to-noise ratio (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. Use binning 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 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 depends 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 might 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 might also be needed to obtain suitable images of thick samples.

Site Selection

Use multiple sites in a single well to acquire images from a greater area of the well. If you select the Single Site option on the Sites to Visit tab, the software acquires an image for only a single site located in the center of the well. Use the Fixed Number of Sites option to acquire separate images within a given well, and those images can be of contiguous or distributed areas.

Use the stitch command to assemble the smaller separate images into a single large image. Use this to retain 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 be used to include specific areas of the wells in the plate, while at the same time excluding other areas of the well. For example, the center of the well might exhibit a pipetting artifact from an automated pipettor, or the cells might 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. The ImageXpress Micro Confocal System is designed to minimize shading effects and provides a shading correction feature within the software. This is recommended for assays where comparison of individual cell intensities is critical, such as a cell cycle assay.

Autofocus

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

Illumination Settings

You select the 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. The 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 the experiment. It uses dichroic mirrors to separate the excitation wavelength from the emission wavelength. Specific filter sets are designed for use with specific stains. See [Table 8-1](#).

Exposure Time

An appropriate exposure time for each wavelength is crucial for acquisition and analysis. Click Auto Expose on one of the Wavelength tabs in the Plate Acquisition Setup dialog to provide a good starting point and then adjust the exposure time so that 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 image quality. If you expect the intensity 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.

Signal-to-Noise (S/N) is the ratio of the signal from the foreground objects over the noise from the background variation, signal variation, and other forms of noise from the system. S/N is an important leading indicator of how difficult it will be for the software to accurately discriminate important features in an image from the background. If the S/N is large (for example, >10), then there is high confidence that the software can distinguish foreground pixels from background pixels in the image. If the S/N is relatively small (for example, <2), then it is more difficult for the software to distinguish between objects in the foreground and background for accurate count, morphology, area, signal intensity, and other measurements.

To increase S/N, consider using one of the following methods:

- Increase the image exposure. Longer exposures provide higher signal and higher background in an image, so changing the exposure alone might not increase S/N as expected and should be carefully measured before using long exposures (>1 second). Longer exposures can cause photo bleaching damage and saturate the camera. Intensity measurements of an overexposed image are not accurate, and you should avoid generating these images. One exception to this rule is when you are interested in extremely faint features of the 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.
- Decrease background variation by lowering background levels from the sample, system, or other sources. This could be achieved by changing to confocal imaging with longer exposures, lowering the sample background, or changing the consumable type.
- Average images. In the composite of averaged images, random noise in the background should follow the central limit theorem and therefore lower pixel-to-pixel variation in object foreground and background pixels. With smoother background variation, the S/N will increase. The number of images to be averaged will depend on the sources of variation and stability of the sample (in other words, the amount of photobleaching or photodamage).

Z Series Acquisition

The nature of your sample material can be 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, you can save individual planes or collapse planes into a single 2D projection image.

In Transmitted Light, collecting z planes of cells growing throughout a 3D gel matrix cannot give an accurate image of all planes because the transmitted light must pass completely through the sample from top to bottom so that objects high in the sample will block the light to planes below it.

Instrument Maintenance

For best performance, your ImageXpress Micro Confocal System should have regular preventive maintenance services. In between preventative maintenance services, your system administrator can clean dust off the optics as needed. Wear gloves when you handle any optical components to avoid contaminating them with dirt or skin oils. See [Maintenance on page 193](#).

The basic design of the ImageXpress Micro Confocal System light source and light path within the instrument help ensure that the light that reaches the 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: Configuring Plate Acquisition Options

Before you configure an experiment, it is important to become familiar with the configuration tools in the MetaXpress® Software. The foundation of the MetaXpress Software is the MetaMorph® Microscopy Automation and Image Analysis Software. The MetaMorph Software contains numerous dialogs for image acquisition, processing, and analysis. The MetaXpress Software adds database integration and tools to control the ImageXpress Micro Confocal System and to acquire and analyze microplates.



Note: Beginning with Version 6.0 of the MetaXpress Software, the well and site navigation functions that were available 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 you should use the Plate Acquisition Setup dialog.

The following sections explain the Plate Acquisition Setup dialog where you configure and run screening experiments:

- [Accessing the Plate Acquisition Setup Dialog on page 58](#)
- [Plate Acquisition Setup Dialog Layout on page 59](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 63](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 64](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 69](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 82](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 110](#)
- [Viewing the Summary Panel on page 116](#)
- [Saving Plate Acquisition Protocols on page 117](#)
- [Running Plate Acquisition Protocols on page 117](#)

Accessing the Plate Acquisition Setup Dialog

The MetaXpress Software displays menus along the top of the screen. The Screening menu provides access to the dialogs that allow you to configure protocols and acquire images. The Screening menu also provides access to plate configuration dialogs including the Plate Acquisition Setup dialog.

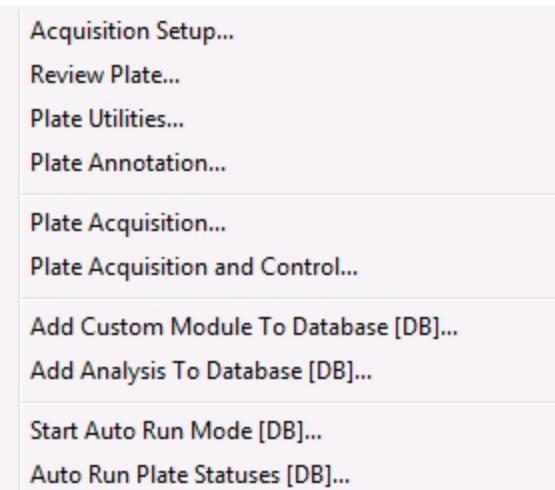


Figure 5-1: Simplified Screening Menu

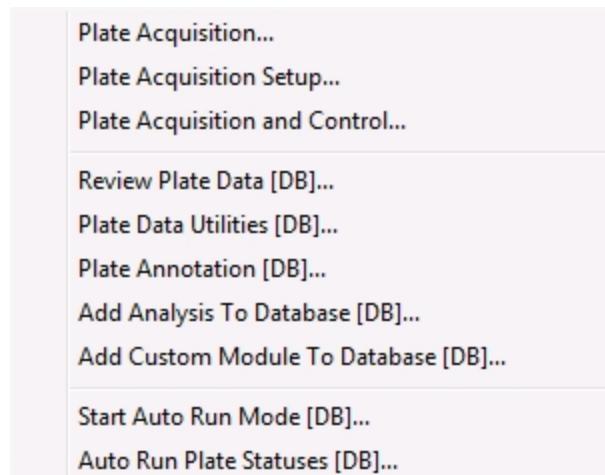


Figure 5-2: Standard Screening Menu

Plate Acquisition Setup Dialog Layout

Use the Plate Acquisition Setup dialog to create protocols for acquiring images from plates.

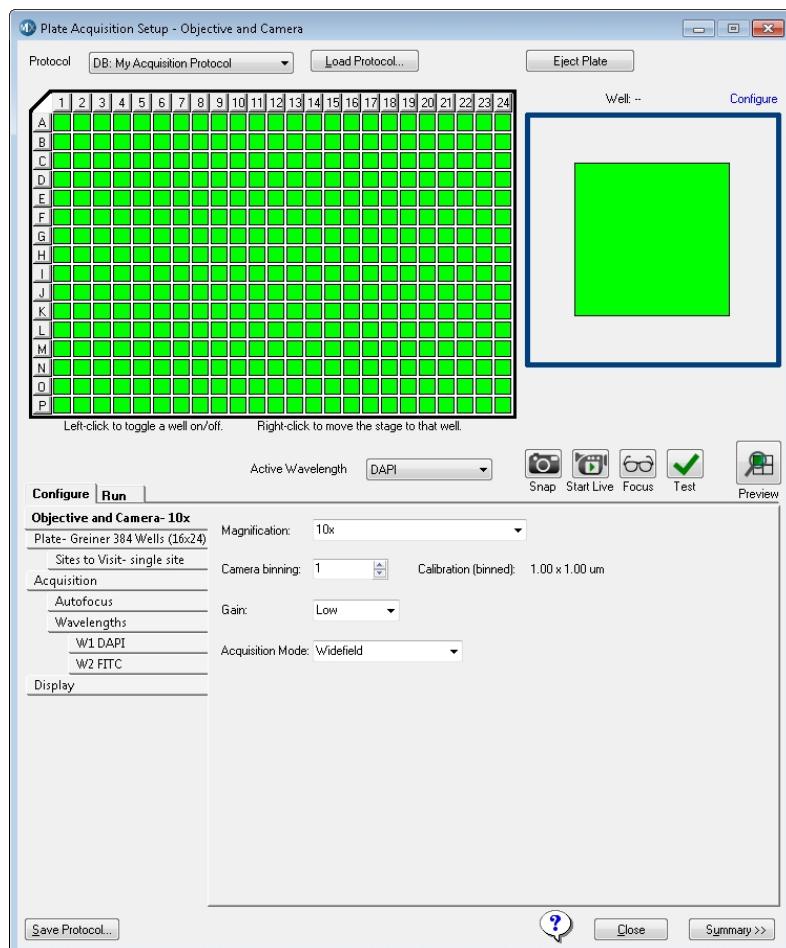


Figure 5-3: Plate Acquisition Setup Dialog

The Plate Acquisition Setup dialog provides three primary functions:

- Use the **Plate Map** and **Site Map** area that displays the plate layout to control some of the movable physical components of the ImageXpress Micro Confocal System.
- Use the **Configure** tab to set up plate acquisition protocols.
- Use the **Run** tab to define the acquisition control to run a plate acquisition according to the protocol. See [Running Plate Acquisition Protocols on page 117](#).

Plate Map and Site Map

The Plate Map area in the upper left is a graphical representation of the plate type you select.

The following functionality is available above the plate map:

- Use the **Protocol** drop-down to select from the eight most recently used plate acquisition protocols.
- Use the **Load Protocol** button to select a protocol that you saved from a previous session for plate acquisition during the current session.
- The **Eject Plate/Load Plate** button switches between the following two options:
 - **Eject Plate** - Opens the top door and plate clamp to load or remove a plate.



Note: The plate clamp closes within 2 minutes.

- **Load Plate** - Closes the top door and the plate clamp so that the instrument can acquire the plate.
- Use the **Configure** link to select the Sites to Visit tab below to assist with site selection.

When you open the Plate Acquisition Setup dialog for the first time, all applicable wells in the plate map display green and are selected for data acquisition. When the plate dimensions, plate skirt, or objective prevents acquisition from the wells at the edge of the plate, the affected wells are blocked, and are surrounded by a gray rectangle. You cannot select blocked wells or acquire an image from blocked wells.



Figure 5-4: Plate Map

Use the Plate Map to select the wells to acquire:

- To exclude wells from the image acquisition, click on a well, click a row or column header, or drag the cursor over a section of wells. Excluded wells display in gray.
- To include or exclude all wells, click the upper left corner triangle.
- To move the stage to a specific well, right-click a well.

The Site Map in the upper right represents the number of sites in each well and the position of the sites to acquire for each well. Use the Site Map to control some of the movable physical components of the ImageXpress Micro Confocal System and to configure the sites to acquire. Sites you select to acquire display green and sites you choose to not acquire display gray.

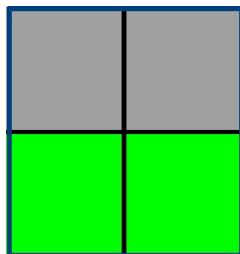


Figure 5-5: Site Map

Below the Plate Map and the Site Map, wavelength tools provide shortcuts to various functions related to the acquisition wavelength. Each icon performs the action at the well where the stage is currently located and in the wavelength that currently displays in the Active Wavelength field.



- The **WI (Water Immersion) Status** icon displays when your instrument has the Water Immersion option and you select a water immersion objective. Click the icon to display the Water Immersion Control dialog which provides the status of the water immersion system and basic water immersion maintenance steps. See [Water Immersion Option on page 184](#).
- Use the **Active Wavelength** drop-down to select an acquisition wavelength.
- Use the  **Snap** icon to acquire an image without performing (or re-performing) an autofocus.
- Use the  **Start Live** icon to start and stop Live mode.



CAUTION! Using Live mode for extended periods of time increases the risk of photo bleaching the site.

-
- Use the  **Focus** icon to perform the Autofocus routine and then acquire an image.
 - Use the  **Test** icon to perform the Autofocus routine and then acquire a Z series and present the corresponding 2D projection when you set up a z acquisition.
 - Use the  **Preview** icon to perform the Autofocus routine and then acquire a Z series at each of the wavelengths you set up in the protocol and present the corresponding 2D projection when you set up a Z acquisition.
-



Note: There are a few cases where configuring a plate acquisition protocol with the Active Wavelength is applicable. These cases are discussed where appropriate in this section. These tools are primarily used when you set up plate acquisition.

Run Tab

After you create the protocol on the Configure tab, use the Run tab to acquire a plate using the protocol. See [Running Plate Acquisition Protocols on page 117](#).

Configure Tab

Use the Configure tab to configure a plate acquisition protocol. The Configure tab is organized in a “top-to-bottom” tab structure that runs down the left side to guide you through the process of setting up the protocol configuration. Each tab is dedicated to a specific function or setting. The tabs dynamically update according to the options you select and the number of wavelengths you acquire.

The following topics describe each tab on the Configure tab:

- [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 63](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 64](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 69](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 82](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 110](#)
- **Save Protocol** displays the Save Acquisition Protocol dialog where you save the plate acquisition settings as a protocol. See [Saving Plate Acquisition Protocols on page 117](#).
- **Summary** displays the Summary panel that displays a summary of the current acquisition settings. See [Viewing the Summary Panel on page 116](#).

Red or yellow indicators might display. Yellow indicates that an optional field is not filled in or another minor error that will not stop plate acquisition. Red indicates that a required field is either not filled in or contains invalid data that you must change before acquisition can occur.

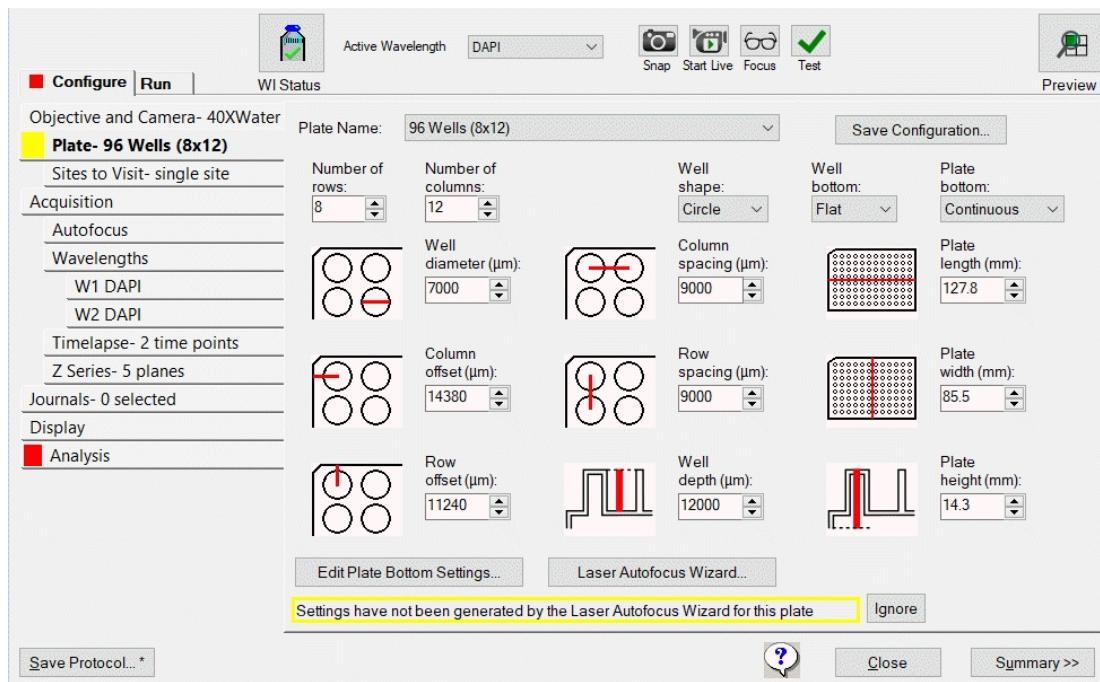


Figure 5-6: Plate Acquisition Setup Dialog with Warning Icons

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

Use the Objective and Camera tab to set the objective magnification, the camera binning, and the acquisition mode for a protocol. Use these settings to improve either the image acquisition speed or the image quality.

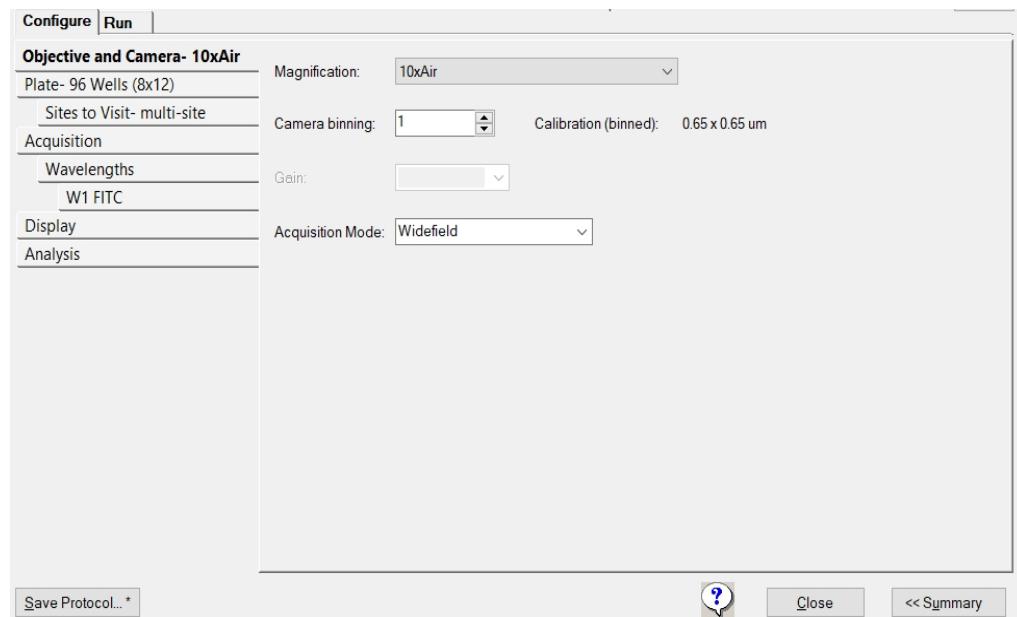


Figure 5-7: Objective and Camera Tab on the Configure Tab

Table 5-1: Configure Tab, Objective and Camera Tab Configuration Options

Option	Description
Magnification	Selects the magnification setting to assign X and Y offset values (parcentricity) and a Z offset (parfocality) to a specific objective. When you select an objective, the objective physically moves to the acquisition position. Note: Define the magnifications in the Configure Magnification dialog. See Verifying Magnification Settings on page 36 . You must assign a calibration to each magnification setting. See Verifying Calibration Settings on page 38 .
Camera Binning	Specifies the binning value to apply to the camera. Binning combines the output of adjacent pixels in square multiples. For example, a camera binning value of 1 is 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. Note: If sufficient light is available, lower camera binning increases the image resolution, whereas 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.
Acquisition Mode	Specifies widefield or confocal imaging mode options, which are dependent on the instrument configuration. Note: An entire acquisition is either widefield or confocal. You cannot change the acquisition mode from timepoint to timepoint or from wavelength to wavelength.

Configuring Objective and Camera Options



Tip: As you configure an acquisition protocol, you can open the Summary panel to view the current values for the protocol settings. See [Viewing the Summary Panel on page 116](#).

To define objective and camera settings for a protocol:

1. Select the **Objective and Camera** tab.
2. Click the **Magnification** drop-down and select the setting of the objective to use for the protocol.



Note: If your system includes the Water Immersion option, when you select a water immersion objective the WI Status icon displays. When you switch from a water immersion objective to an air objective, the system drains the water and the software opens the instrument top door so that you can dry the bottom of the plate before using the air objective.



CAUTION! If you do not dry the plate bottom, you run the risk of getting water on the air objective and potentially into the instrument.

3. In the **Camera Binning** field, enter the value to apply to the camera.



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

4. Click the **Acquisition Mode** drop-down and select the acquisition mode to use (dependent on the instrument configuration).
5. When you finish configuring the acquisition protocol, continue to [Saving Plate Acquisition Protocols on page 117](#). Otherwise, continue with the next steps to further optimize the protocol settings.

See the following topics:

- [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab, see below](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 69](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 82](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 110](#)

Plate Acquisition Setup Dialog: Configure Tab, Plate Tab

Use the Plate tab to configure the plate dimensions to accurately control the X, Y, and Z movements of the ImageXpress Micro Confocal System. Define accurate plate dimensions to prevent the instrument from making potentially hazardous movements. The plate dimensions you define also ensure that laser-based autofocusing is as accurate as possible.

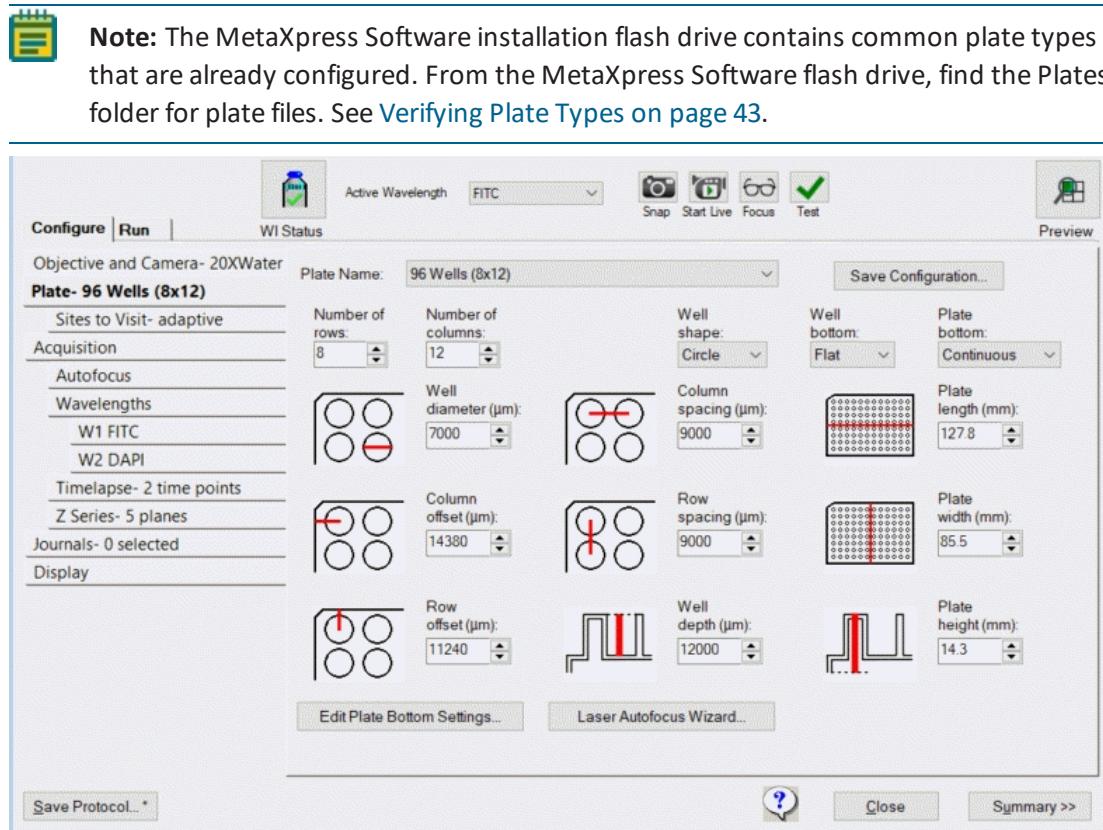


Figure 5-8: Plate Tab on the Configure Tab

Table 5-2: Plate Settings

Option	Description
Plate Name	Specifies the plate type for the protocol. You can select a configuration or you can create a new plate configuration. The fields display values for the plate type you select. If the plate type is not available in the Plate Name list, you can enter the manufacturer's plate specifications. Note: Plate files are stored in the folder C:\MX\plates Note: The manufacturer generally cannot provide values for the plate bottom settings, including optical thickness, which is not the same as physical thickness, and bottom variation. You must run the Laser Autofocus Wizard to measure the values to ensure proper focus. See Considering Plate Dimensions on page 67 .
Save Configuration	Displays the Save Configuration dialog where you name and save a new plate configuration based on the plate type values.
Number of Rows	Indicates the number of rows for the plate type.
Number of Columns	Indicates the number of columns for the plate type.
Well Shape	Indicates the shape of the well, either Circle or Square .
Well Bottom	Indicates the shape of the well bottom, either Flat or Round .

Table 5-2: Plate Settings (continued)

Option	Description
Plate Bottom	Indicates the shape of the plate bottom, either Continuous or Segmented . Continuous indicates the plate bottom is smooth and Segmented indicates the plate bottom is not smooth such as U-bottom plates or plates with channels.
Well Diameter	Specifies the diameter of the well in μm . Note: The wells for many plate types have a slight conical shape. If you create a new plate configuration, enter the diameter of the bottom of the well, not the top.
Column Spacing	Specifies the spacing in μm between each well on the X axis. Note: Generally, this value should be the same for both the X and Y axis; however, if you create a new plate configuration, you can enter 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 create a new plate configuration, you can enter different values, if needed.
Plate Width	Specifies the Plate width in mm. The ANSI standard is 85.5 mm.
Row Offset	Specifies the distance in μm between the center of well A01 and the top edge of the plate.
Well Depth	Specifies the well depth in μm . Note: The correct Well Depth value is required for autofocusing and fluidics events.
Plate Height	Specifies the plate height in mm. Note: The correct Plate Height value is required for autofocusing and fluidics events.
Edit Plate Bottom Settings	Displays the Configure Plate Bottom Settings dialog where you adjust plate bottom settings. CAUTION! This dialog is intended primarily for informational and diagnostic purposes. 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	Displays the Laser Autofocus Wizard which guides you step by step through the process of having the software calculate plate bottom dimensions and the focus laser exposure times that are required for each objective with the plate type.

Considering Plate Dimensions

Even if you select an existing plate type configuration, you may need to use the Laser Autofocus Wizard to verify the accuracy of the bottom thickness and the four different variation measurements for the plate.

Bottom thickness is a value in μm that is the average optical thickness for the well bottom.

The four different measurements include 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 an air objective. These values are not equivalent to the physical thickness measurements that the plate manufacturer provides. The software calculates the optical thickness by dividing the physical thickness of the plate bottom by the refractive index of the material of which it is composed.

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. Plate manufacturers can change plate parameters without changing plate names. The Laser Autofocus Wizard walks you through the steps to automatically calculate plate bottom dimensions as well as the exposure times that each objective requires.



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. See the application help or contact Technical Support. See [Obtaining Support on page 244](#).



CAUTION! Do not use the Laser Autofocus Wizard for slides because this feature is not compatible with slides. If you need assistance imaging slides, contact Technical Support. See [Obtaining Support on page 244](#).

Configuring the Dimensions for New Plates

Use the Plate tab to define the dimensions for a new custom plate. If the plate is 96-well or 384-well format, select a similar plate template, and modify the values as needed. If the plate is any other size, you must specify all values for the plate.

To configure the dimensions for a new plate:

1. Select the **Plate** tab.
2. Click the **Plate Name** drop-down and select a plate that is similar to the one you want to configure.
3. Click **Save Configuration** to display the Plate Acquisition - Save Configuration dialog.
4. In the **Plate Configuration Name** field, enter the name of the plate and click **Save**.



Tip: The drop-down list of plates displays in reverse alphabetical order. The default plate names begin with the name of the plate manufacturer. You may want to start the plate name with the name of the manufacturer to make it easier to find.

5. Click the **Well Shape** drop-down and select the well shape.
6. Complete the remaining configuration fields as required. See [Table 5-2: Plate Settings on page 65](#).



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.

7. Click **Laser Autofocus Wizard** and follow the instructions to calculate plate bottom measurements.
8. If needed, edit the **Well Depth** field and the **Plate Height** field.
9. Click **Save Configuration** to display the Plate Acquisition - Save Configuration dialog.
10. Click **Save** to display a confirmation that you want to overwrite the file you created earlier. Click **Yes** to save the plate.
11. If needed, adjust objectives with correction collars to match the plate bottom thickness. See [Adjusting Objective Correction Collars on page 221](#).
12. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#). Otherwise, continue to any other configuration as needed.

See the following topics:

- [Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 69](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 82](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 110](#)

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

Use the Sites to Visit tab to configure the number of sites to acquire for each well in a plate acquisition protocol. 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 tabs dynamically update according to the options you select. The site map, which is a graphical representation of the sites that you configure, dynamically updates based on the configuration options you select and the values you specify for the options.



Note: Use the plate map and site map at the top of the Plate Acquisition Setup dialog to select and move to the wells and sites to analyze in the plate acquisition protocol.

The following topics describe these procedures:

- [Acquiring a Single Site in Each Well, see below](#)
- [Acquiring a Fixed Number of Sites in Each Well on page 71](#)
- [Configuring Adaptive Acquisition for Well Sites on page 73](#)
- [Configuring Multi-Well Acquisitions on page 80](#)

Acquiring a Single Site in Each Well

Use the Sites to Visit tab to acquire a single site in each well. 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 71](#).

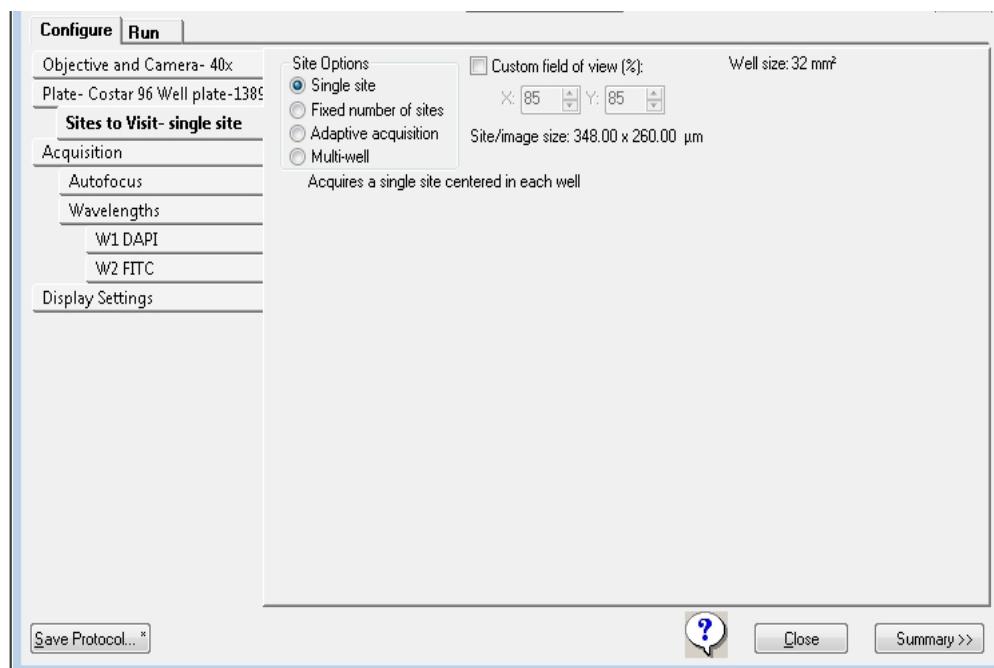


Figure 5-9: Configure Tab, Sites to Visit Tab, Single Site Option

To acquire a single site in each well:

1. Select the **Sites to Visit** tab.
2. Select the **Single Site** Site Option.
3. Select the **Custom Field of View (%)** check box to specify a percentage of the field of view for the camera to acquire, and then enter the **X** (width) value and **Y** (height) value. This is useful to acquire images from an unevenly illuminated full field or to acquire images that include the area outside the well boundary.



Note: The full field of view for the camera is 100 percent (100% in the X field and 100% in the Y field). 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: Custom field of view is compatible with full-size shading correction images when you use Legacy Correction. See [Using Legacy Correction Image Shading on page 98](#).

4. Use the plate map to specify the wells for data acquisition. Active wells are green and inactive wells are gray. When the plate dimensions, plate skirt, and/or objective prevents acquisition from the wells at the edge of the plate, the affected wells are blocked, and display surrounded by a gray rectangle.
 - To exclude a single well, click on a green well. The well turns gray and the acquisition will skip the well. To include the well, click the well again. The well turns green.
 - To exclude a column of wells, click on the number header. The column of wells turns gray and the acquisition will skip the well. To include the column, click the column header again. The wells turn green.
 - To exclude a row of wells, click on the letter header. The row of wells turns gray and the acquisition will skip the well. To include the row, click the letter header again. The wells turn green.
 - To exclude a contiguous group of wells, drag the red outline across a group of wells. The wells turn gray. To include the wells, drag the red outline across the group of wells again. The wells turn green.
 - To exclude or include all wells in the entire plate, click the white triangle in the upper-left corner of the plate map.



Note: You can preselect the wells for the acquisition as part of the automated protocol, or you can select the wells when you run the protocol.

5. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#). Otherwise, continue to any other configuration as needed.

See the following topics:

- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 82](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 110](#)

Acquiring a Fixed Number of Sites in Each Well

Use the Sites to Visit tab to acquire a fixed number of sites in each well.

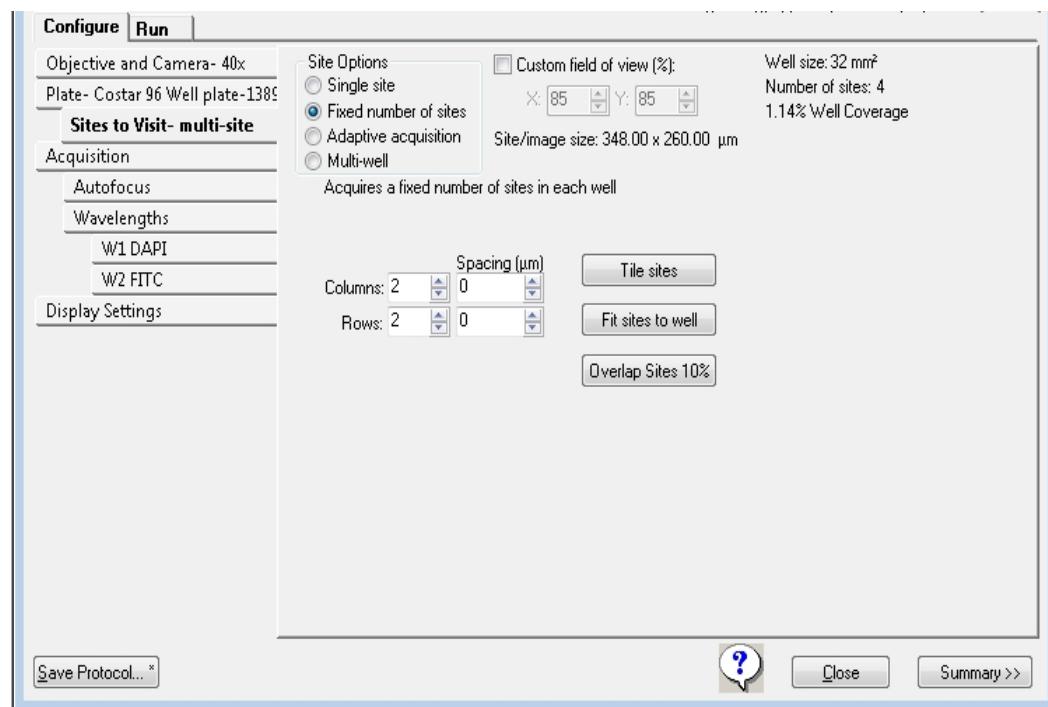


Figure 5-10: Configure Tab, Sites to Visit Tab, Fixed Number of Sites Option

To acquire a fixed number of sites in each well:

1. Select the **Sites to Visit** tab.
2. Select the **Fixed Number of Sites** Site Option.
3. Select the **Custom Field of View (%)** check box to specify a percentage of the field of view for the camera to acquire, and then enter the **X** (width) value and **Y** (height) value. This is useful to acquire images from an unevenly illuminated full field or to acquire images that include the area outside the well boundary.



Note: The full field of view for the camera is 100 percent (100% in the X field and 100% in the Y field). 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: Custom field of view is compatible with full-size shading correction images when you use Legacy Correction. See [Using Legacy Correction Image Shading on page 98](#).

4. In the **Columns** field and the **Rows** field, enter the maximum number of sites to visit. For example, Columns = 2 and Rows = 2 acquires up to four sites, Columns = 3 and Rows = 3 acquires up to nine sites.



Note: Maximum allowable values: Columns 45 and Rows 35.

5. Use the plate map to specify the wells for data acquisition. Active wells are green and inactive wells are gray. When the plate dimensions, plate skirt, and/or objective prevents acquisition from the wells at the edge of the plate, the affected wells are blocked, and display surrounded by a gray rectangle.
 - To exclude a single well, click on a green well. The well turns gray and the acquisition will skip the well. To include the well, click the well again. The well turns green.
 - To exclude a column of wells, click on the number header. The column of wells turns gray and the acquisition will skip the well. To include the column, click the column header again. The wells turn green.
 - To exclude a row of wells, click on the letter header. The row of wells turns gray and the acquisition will skip the well. To include the row, click the letter header again. The wells turn green.
 - To exclude a contiguous group of wells, drag the red outline across a group of wells. The wells turn gray. To include the wells, drag the red outline across the group of wells again. The wells turn green.
 - To exclude or include all wells in the entire plate, click the white triangle in the upper-left corner of the plate map.



Note: You can preselect the wells for the acquisition as part of the automated protocol, or you can select the wells when you run the protocol.

6. Use the site map to specify a sub-set of sites within a well to acquire.
 - To turn a site off, click it. The site turns gray and data will not be acquired for the site. To turn the site back on, click the site again. The site turns green.
 - To turn off a contiguous group of sites, drag the cursor across the sites. To turn the sites back on, drag the cursor across the sites again.



Note: The site controls apply to all sites in all wells at the same time. The software acquires data for the same sites in all wells in the plate.



Note: You can configure automated data acquisitions for the wells as part of a protocol.

7. To adjust spacing, do one of the following:

- To manually increase the spacing between adjacent sites, enter positive numbers in the **Spacing** fields or to overlap the sites, enter negative numbers in the **Spacing** fields.



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

- Click **Tile Sites** to automatically adjust the spacing between adjacent sites so that there is zero spacing between sites.
- Click **Fit Sites to Well** to automatically adjust the spacing between the adjacent sites to the maximum allowed value based on the plate type.
- Click **Overlap Sites 10%** to automatically overlap the sites by 10%. This is generally recommended if you plan to stitch sites together later.



Note: Do not click Overlap Sites 10% for Z series acquisitions.

8. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#). Otherwise, continue to any other configuration as needed.

See the following topics:

- [Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 82](#)
- [Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 110](#)

Configuring Adaptive Acquisition for Well Sites

The Sites to Visit tab Adaptive Acquisition option is a computational algorithm that counts the number of cells on the fly during sample acquisition. This increases the chances of collecting valid data in every well. The number of sites the software acquires per well is based on the number of cells per well.



Note: Adaptive Acquisition 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.

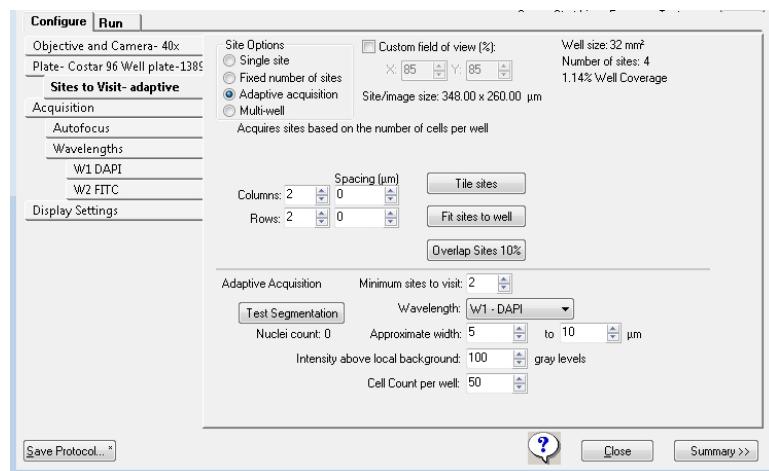


Figure 5-11: Configure Tab, Sites to Visit Tab, Adaptive Acquisition Option

To configure adaptive acquisition:

1. Select the **Sites to Visit** tab.
2. Select the **Adaptive Acquisition** Site Option.
3. Select the **Custom Field of View (%)** check box to specify a percentage of the field of view for the camera to acquire, and then enter the **X** (width) value and **Y** (height) value. This is useful to acquire images from an unevenly illuminated full field or to acquire images that include the area outside the well boundary.



Note: The full field of view for the camera is 100 percent (100% in the X field and 100% in the Y field). 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: Custom field of view is compatible with full-size shading correction images when you use Legacy Correction. See [Using Legacy Correction Image Shading on page 98](#).

4. In the **Columns** field and the **Rows** field, enter the maximum number of sites to visit. For example, Columns = 2 and Rows = 2 acquires up to four sites, Columns = 3 and Rows = 3 acquires up to nine sites.



Note: Maximum allowable values: Columns 45 and Rows 35.

5. Use the plate map to specify the wells for data acquisition. Active wells are green and inactive wells are gray. When the plate dimensions, plate skirt, and/or objective prevents acquisition from the wells at the edge of the plate, the affected wells are blocked, and display surrounded by a gray rectangle.
 - To exclude a single well, click on a green well. The well turns gray and the acquisition will skip the well. To include the well, click the well again. The well turns green.
 - To exclude a column of wells, click on the number header. The column of wells turns gray and the acquisition will skip the well. To include the column, click the column header again. The wells turn green.
 - To exclude a row of wells, click on the letter header. The row of wells turns gray and the acquisition will skip the well. To include the row, click the letter header again. The wells turn green.
 - To exclude a contiguous group of wells, drag the red outline across a group of wells. The wells turn gray. To include the wells, drag the red outline across the group of wells again. The wells turn green.
 - To exclude or include all wells in the entire plate, click the white triangle in the upper-left corner of the plate map.



Note: You can preselect the wells for the acquisition as part of the automated protocol, or you can select the wells when you run the protocol.

6. Use the site map to specify a sub-set of sites within a well to acquire.
 - To turn a site off, click it. The site turns gray and data will not be acquired for the site. To turn the site back on, click the site again. The site turns green.
 - To turn off a contiguous group of sites, drag the cursor across the sites. To turn the sites back on, drag the cursor across the sites again.



Note: The site controls apply to all sites in all wells at the same time. The software acquires data for the same sites in all wells in the plate.



Note: You can configure automated data acquisitions for the wells as part of a protocol.

7. To adjust spacing, do one of the following:

- To manually increase the spacing between adjacent sites, enter positive numbers in the **Spacing** fields or to overlap the sites, enter negative numbers in the **Spacing** fields.



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

- Click **Tile Sites** to automatically adjust the spacing between adjacent sites so that there is zero spacing between sites.
- Click **Fit Sites to Well** to automatically adjust the spacing between the adjacent sites to the maximum allowed value based on the plate type.
- Click **Overlap Sites 10%** to automatically overlap the sites by 10%. This is generally recommended if you plan to stitch sites together later.



Note: Do not click Overlap Sites 10% for Z series acquisitions.

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 software acquires at least this minimum number of sites and then it continues to acquire sites until it reaches the total number of cells counted per well. Note: The maximum number of sites to visit is the number of sites you select in the Site Map.
Wavelength	The wavelength to use to differentiate nuclei in the source image. Note: For the most efficient acquisition, this wavelength should be the same as the wavelength that you designate to be the first to acquire. See Specifying the Number of Acquisition Wavelengths on page 88 .
Approximate Width	The approximate minimum width and the approximate maximum width of the nuclei that you expect to detect. 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. See Calculating the Intensity Above Local Background Gray Levels on page 79 . Note: The Approximate Width and the Intensity Above Local Background Gray Levels work together. A nucleus is determined by object size and relative intensity above background.
Cell Count Per Well	The total number of nuclei in each well that the software must acquire before stopping acquisition of the well. Note: The 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 well displays as the images are acquired. Adaptive Acquisition stops acquiring when the maximum number of sites is reached, even if the cell count is not acquired.

9. To determine if the acquisition settings are appropriate to differentiate nuclei, do the following:
 - a. Select a well and site to review your settings.
 - b. Click **Focus** to bring the sample into focus.



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

- c. 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 display.

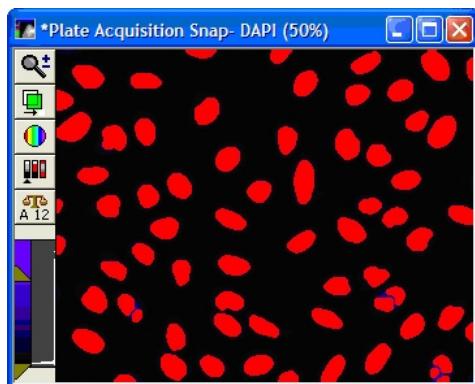


Figure 5-12: Example of Test Segmentation Results in the Adaptive Acquisition Mode. The red shows the segmentation overlaid on the original DAPI image.

- d. Repeat the previous steps until your test results are satisfactory.

Calculating the Intensity Above Local Background Gray Levels

The Sites to Visit tab Adaptive Acquisition option is a computational algorithm that analyzes the number of cells on the fly during sample acquisition. This increases the chances of collecting valid data in every well. The number of sites the software acquires per well is based on the number of cells per well.

To calculate the intensity above local background gray levels value:

1. If the Region Tools toolbar does not display:

From the simplified menu, click **Measure > Regions > Regions Tools**.

From the standard menu, click **Regions > Regions Tools**.

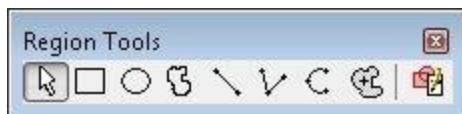


Figure 5-13: Region Tools Toolbar

2. Click .
3. Open the source image.
4. Move the cursor over the image and watch the status bar at the bottom of the software window to note the X, Y coordinates and the gray level value of the pixel that is under the cursor. The X and Y coordinates are enclosed in parentheses and the gray level value displays to the right. For example, (48, 158) -> 18000 indicates that 18000 is the average gray level value of the pixel.
5. Locate one of the dimmest objects (for example, a nucleus, if applicable) that you want the software to detect.
6. Position the cursor on the dimmest part of the object and make note of the gray level value that displays in the status bar.



Figure 5-14: Status Bar Gray Level Indicators

7. Position the cursor just to the outside of the object and make note of the gray level value that displays 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: Draw a line region across a cell and its local background, and then use the Linescan tool to see more exact intensity values. To access the Linescan tool, click **Measure > Intensities > Linescan**.

8. Subtract the gray level value of the background from the gray level value of the object and enter one quarter to one half of this value in the **Intensity Above Local Background** field.

9. To determine if the acquisition settings are appropriate to differentiate nuclei, do the following:
 - a. Select a well and site to review your settings.
 - b. Click **Focus** to bring the sample into focus.



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

- c. 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 display.
10. Repeat the previous steps until your test results are satisfactory.

Configuring Multi-Well Acquisitions

Run a multi-well acquisition to acquire high density plates faster. 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 software automatically calculates the number of wells in which a site can be simultaneously acquired. This value depends on the plate well density and the magnification that you configure 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 you do not select might be exposed to excitation light from imaging neighboring selected wells. However, the software does not save images for the wells that you do not select.

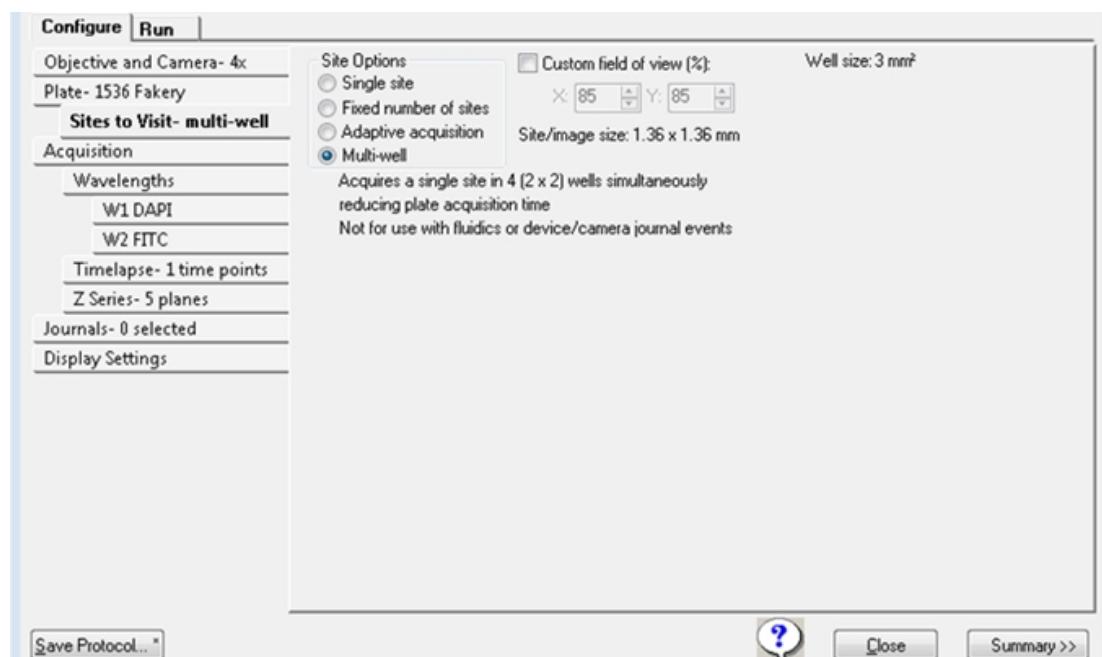


Figure 5-15: Configure Tab, Sites to Visit Tab, Multi-well Option



Note: Multi-well acquisition should not be combined with Fluidics experiments.

To run a multi-well acquisition:

1. Select the **Sites to Visit** tab.
2. Select the **Multi-well** Site Option.
3. Leave the **Custom Field of View (%)** check box clear. For multi-well acquisitions, always use the full field of view.
4. Use the plate map to specify the wells for data acquisition. Active wells are green and inactive wells are gray. When the plate dimensions, plate skirt, and/or objective prevents acquisition from the wells at the edge of the plate, the affected wells are blocked, and display surrounded by a gray rectangle.
 - To exclude a single well, click on a green well. The well turns gray and the acquisition will skip the well. To include the well, click the well again. The well turns green.
 - To exclude a column of wells, click on the number header. The column of wells turns gray and the acquisition will skip the well. To include the column, click the column header again. The wells turn green.
 - To exclude a row of wells, click on the letter header. The row of wells turns gray and the acquisition will skip the well. To include the row, click the letter header again. The wells turn green.
 - To exclude a contiguous group of wells, drag the red outline across a group of wells. The wells turn gray. To include the wells, drag the red outline across the group of wells again. The wells turn green.
 - To exclude or include all wells in the entire plate, click the white triangle in the upper-left corner of the plate map.



Note: You can preselect the wells for the acquisition as part of the automated protocol, or you can select the wells when you run the protocol.

5. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#). Otherwise, continue to any other configuration as needed.

Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab

Use the Acquisition tab to specify the autofocus options and additional acquisition options for the protocol. Most acquisition options you select add an additional tab to the Configure tab.

See the following topics:

- [Configuring Autofocus Options on page 83](#)
- [Specifying the Number of Acquisition Wavelengths on page 88](#)
- [Configuring Series Acquisition Options on page 99](#)
- [Configuring Journals to Run During Acquisition on page 108](#)
- [Configuring Post-Acquisition Analysis Options on page 113](#)



Note: To configure fluidic stations, select the Use Fluidics check box to add a Fluidics tab to the dialog. See [Expansion Solution Options on page 129](#), or the software application help.

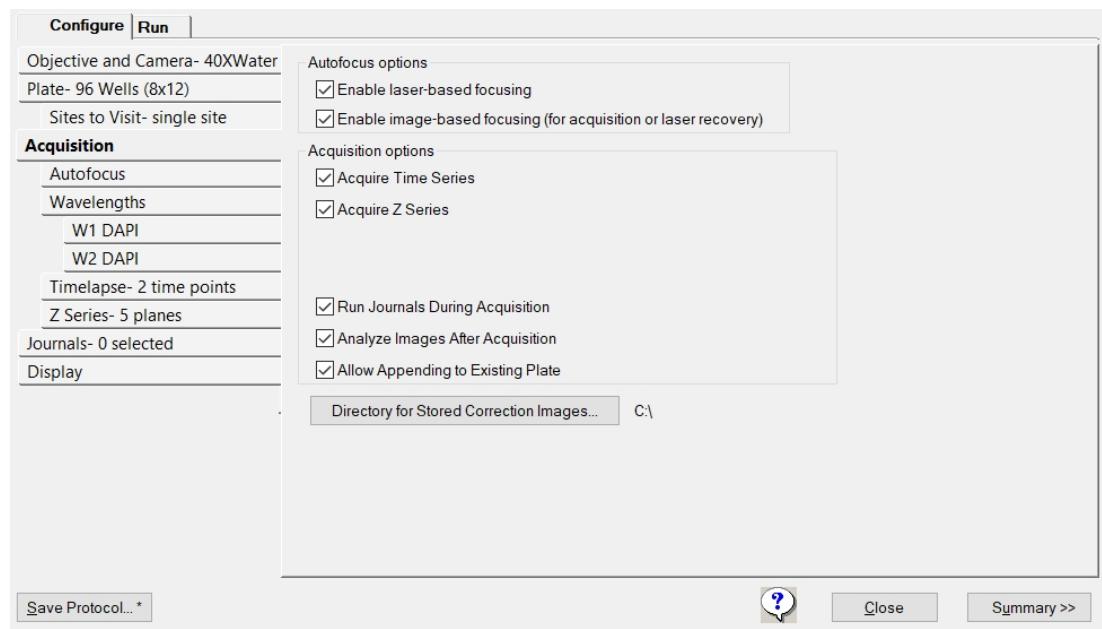


Figure 5-16: Acquisition Tab on the Configure Tab

Configuring Autofocus Options

The Acquisition tab provides two autofocus options:

- **Laser Based Focusing** is generally set to find the bottom of the well, and then moves the objective a distance up from the well bottom. This method is the fastest and does not cause photo damage to your wells. 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 a contrast-based 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 autofocus options for a plate acquisition protocol. Except for oil-immersion objectives, Molecular Devices recommends primarily using Laser Based Focusing. For oil-immersion objectives, use only Image Based Focusing.

Some 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 focus options, the software uses Laser Based Focusing to move the objective to a position above the bottom of the well, and uses the Image Based Focusing to fine tune the focus.

To configure autofocus options:

1. Select the **Acquisition** tab.
2. Under **Autofocus Options**, select one or both options.
 - **Enable Laser Based Focusing**
 - **Enable Image Based Focusing (for Acquisition or Laser Recovery)** for complex samples with variations in distance between the surface of the plate and the sample.

3. Select the **Autofocus** tab.

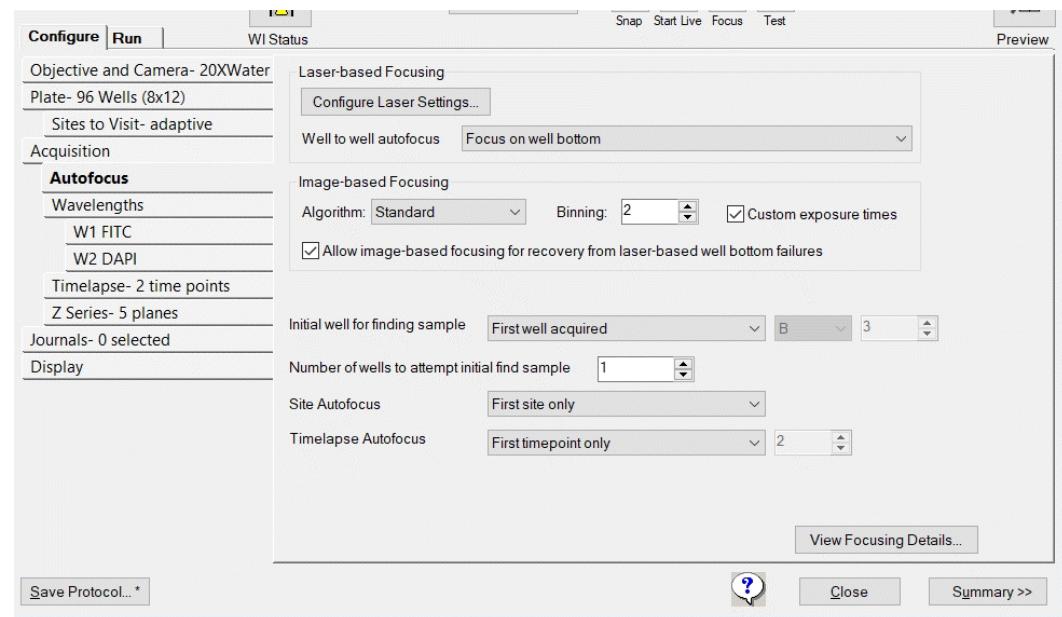


Figure 5-17: Autofocus Tab on the Configure Tab

4. Configure the Autofocus options.

Table 5-4: Autofocus Options

Options	Description
Laser Based Focusing is available when you select the Enable Laser Based Focusing check box on the Acquisition tab.	
Configure Laser Autofocus Settings	<p>Displays the Configure Laser Autofocus Settings dialog. Run the Laser Autofocus Wizard to calculate the settings for this dialog. If you have not run the wizard for the plate and objective, the dialog contains no values. You can modify the settings that the wizard calculates for this dialog.</p> <p>Note: The software application help provides detailed information about the settings that the wizard calculates. For tips about how to use the Laser Autofocus Wizard, contact Technical Support. See Obtaining Support on page 244.</p>
Well to Well autofocus adjusts the focus as the acquisition moves from well to well. Note: Chamber slides with wells can use any of these options.	
Focus on Well Bottom	<p>The recommended option for most plate acquisition protocols. 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 values you enter in the Edit Plate Bottom Settings from the Plate tab to determine the focus range.</p>
Focus on Plate Bottom, Then Offset by Bottom Thickness	<p>Offsets the focus laser by the Bottom Thickness of the plate from the plate configuration. Select this option if you use 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 setting is generally used for magnifications of 4x and below. – A slide – Multi-well acquisition – An ultra-thin plate (< 0.1 mm physical thickness) with any objective.
Focus on Plate and Well Bottom	<p>The laser focuses on both the plate bottom and the well bottom at every well. Recommended setting for plates with extreme bottom variation, such as a thin bottom plate when the measured variations are more than 30% of the bottom thickness.</p>
Image Based Focusing is available when you select the Enable Image-Based Focusing check box on the Acquisition tab.	
Algorithm includes the following focusing algorithm options:	
Standard	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.

Table 5-4: Autofocus Options (continued)

Options	Description
Custom Exposure Times	For individual wavelengths during autofocus. If you do not select this option, then the software calculates the exposure time based on autofocus binning and acquisition exposure time. If you select this option and you select either or both Laser and Image or Laser with Image Recovery for the first acquisition wavelength (W1), or Image Based for any 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 88 .
Allow Image Based Focusing for Recovery from Laser Based Well Bottom Failures	Enabled only if you select both Enable Laser Based Focusing and Enable Image Based Focusing. Use Image Based Focusing only if Laser Based Focusing cannot find the plate or well bottom. The image-based recovery search is centered on an estimated well bottom offset position. The software calculates the estimated position by using the plate bottom position that was found during the last successful laser autofocus attempt and adds the plate bottom thickness and post laser offset values to this plate bottom thickness value.
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. Note: After you select this option, you must specify the well row and column 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 the sample is already in focus. Note: Select this option when you use an oil-immersion objective, and you must manually find the focus before you start the plate acquisition.
Number of Wells to Attempt Initial Find Sample	Enabled only if you select the First Well Acquired option. The first well in which a sample is found is the well the software uses for autofocusing. The default value is one. Note: If you use a robot for automated plate loading, set this value to three or greater.
Site Autofocus is enabled only if you select Fixed Number of Sites or Adaptive Acquisition on the Sites to Visit tab. This section includes several options to configure site-to-site autofocus.	
First Site Only	Autofocuses in the top-left site in the well.

Table 5-4: Autofocus Options (continued)

Options	Description
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; also select this option for round-bottom (U-bottom) plates.
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 if you select Acquire Time Series on the Acquisition tab. See Configuring Series Acquisition Options on page 99 .	
First Timepoint Only	Use for fast kinetic acquisitions.
All Timepoints	Use for long time lapse acquisitions.
Every N th Timepoint	Set a value that is less than the number of time points. See Configuring Continuous Time Series Acquisitions on page 100 .
View Focusing Details	Displays the Auto Focus Details dialog that displays the current autofocus parameters. This information is 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.

- When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#). Otherwise, continue to any other configuration as needed.

Specifying the Number of Acquisition Wavelengths

Use the Wavelengths tab to specify the total number of acquisition wavelengths for an experiment. You must specify at least one acquisition wavelength and you can specify up to eight. When you specify more than one wavelength, subtabs display below the Wavelength tab where you configure exposure time, autofocus, and time lapse settings for each acquisition wavelength. Red indicates that you have yet to configure the acquisition wavelengths.

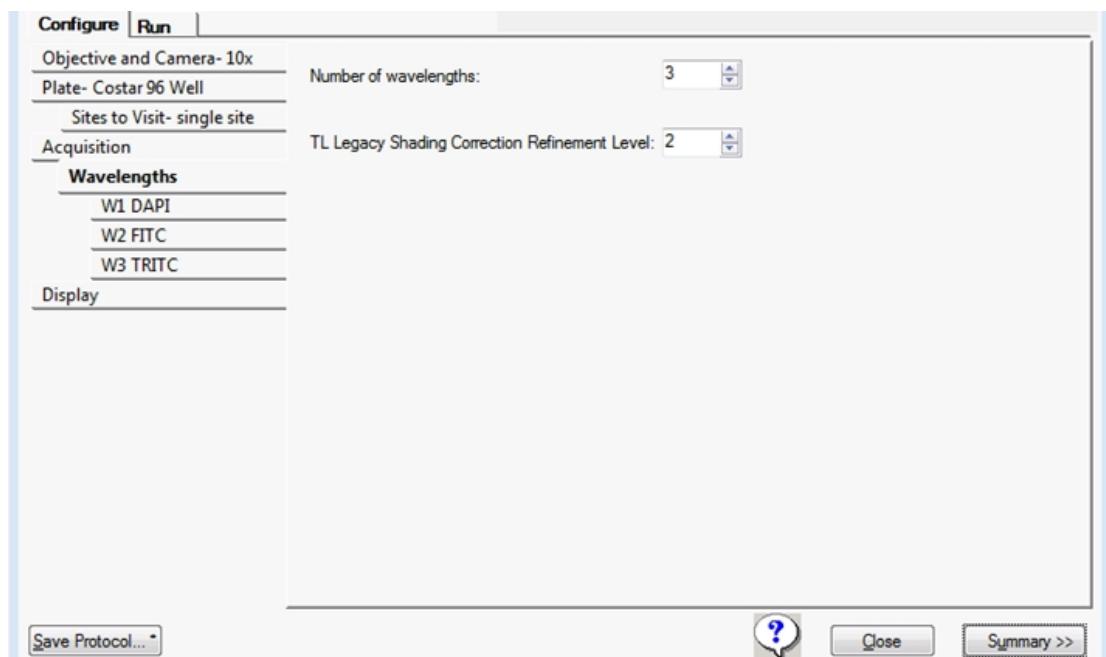


Figure 5-18: Wavelength Tab with Three Wavelengths

Tip: As you configure an acquisition protocol, you can open the Summary panel to view the current values for the protocol settings. See [Viewing the Summary Panel on page 116](#).

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

1. Select the **Wavelengths** tab.
2. In the **Number of Wavelengths** field, specify the total number of wavelengths to be used for acquisition, where the minimum value is one and the maximum value is eight.
3. In the **TL Legacy Shading Correction Refinement Level** field, enter the refinement level. The software applies this option if you select Autocorrect for TL Legacy for Shading Correction for one or more acquisition wavelengths. The larger the value for the refinement level, the flatter the transmitted light image will be. Increasing this value slows the acquisition routine. The single value that you enter applies to each acquisition wavelength for which you select Auto Correction for TL Legacy as the shading correction method. See [Shading Correction Options on page 95](#).
4. Select the individual W# subtabs to configure each acquisition wavelength. See [Configuring Acquisition Wavelengths on page 89](#).

Configuring Acquisition Wavelengths

Use the W# tabs below the Wavelength tab to configure acquisition wavelengths. The sequence 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.

To configure each individual wavelength:

1. Select the **W1** tab.
2. Click the **Illumination** drop-down and select the illumination setting; for example, DAPI. The selection name displays in the Active Wavelength field under the plate map and on the individual tab.

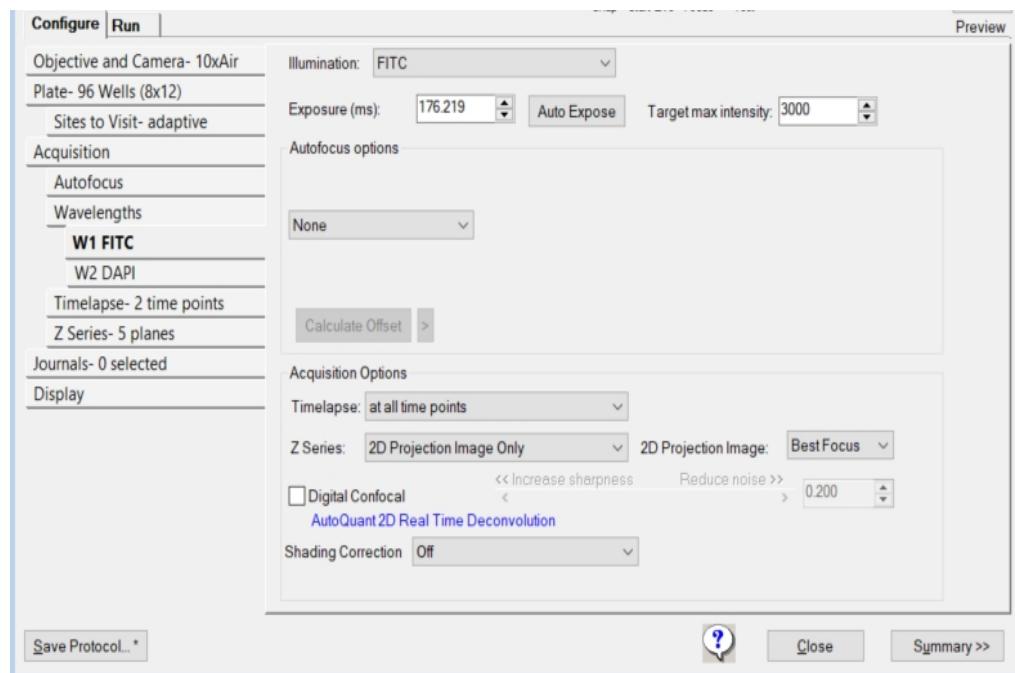


Figure 5-19: W1 Wavelength Tab

3. Configure the remaining acquisition wavelength options, where **Offset (μm)** is the Z motor offset distance for each wavelength. For the first wavelength, if you use laser based focusing, then the offset is the distance between the bottom of the well and the in-focus plane. If you use 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 Setting Options

Options	Description
Bright Sample	If the sample has a high intensity, but a low (1 ms to 7 ms) exposure time, select this to reduce the light power for the wavelength and prevent saturation of the sample. Adjust the exposure time to control the precision of the sample brightness. Note: This option is only available for certain instrument configurations.
Exposure (ms)	Specifies the exposure time in milliseconds for the wavelength. Either enter a value or click Auto Expose to automatically determine an exposure time. Use an in-focus image to determine exposure times. Note: Check both the positive and the negative controls. If you use a dim sample to set the exposure time, a bright sample might become saturated.
Auto Expose	Click to automatically set the exposure time required to obtain the Target Max Intensity. If Auto Expose results in a 1 ms to 5 ms value to achieve this intensity, the software prompts you to activate Bright Sample mode to prevent saturation of the sample. Click Yes to turn on Bright Sample mode and rerun Auto Expose or click No to leave all settings as-is. Note: Auto Expose is a manual tool to help configure the plate. This does not enable automatic exposure adjustments 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: In general, the recommended target intensity value for the brightest control sample is 50-75% of the maximum gray level that is possible with your camera driver.
Autofocus Options specify the type of autofocus to use when acquiring images. The available options depend on the acquisition options you select on the Acquisition tab.	
None	No autofocus is done. If you select this option, no other autofocus configuration options are available.
Laser with Z-Offset	Displays if you select Enable Laser Based Focusing on the Acquisition tab. If selected, 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 calculate the first (W1) wavelength offset.

Table 5-5: Acquisition Wavelength Setting Options (continued)

Options	Description
Calculate Offset icon (>)	<p>Click > to display the options to calculate the post-laser offset value.</p> <p>Use Z Stack allows the capture of multiple Z plane images so that you can select the best in-focus plane.</p> <p>Note: Without Use Z Stack, the software carries out an image autofocus and determines the best in-focus plane.</p> <p>Custom Range determines the total search range and step size to calculate the offset.</p> <p>Note: Without Custom Range, the software uses the default values, based on the objective, for the Calculate Offset using either Z stack or image autofocus.</p>
Laser and Image	<p>Displays for the first acquisition wavelength (W1) and only if you select Enable Laser Based Focusing and Enable Image Based Focusing on the Acquisition tab. If selected, the laser autofocuses first, and then image-based focus fine tunes the focus. You must specify values for the following:</p> <p>Image Based Range +/- μm specifies the search range for the image-based portions of autofocus.</p> <p>Max. Step specifies the maximum step size of a single Z move in μm that is required to get the correct focus position. This setting depends on the objective. Because the focus peak is narrower, use a smaller step size with higher NA objectives.</p> <p>If you select Custom Exposure Time on the Autofocus tab, with Laser and Image selected, specify a value for the following:</p> <p>Exposure (ms) specifies the exposure time in milliseconds for the acquisition wavelength when autofocusing.</p>

Table 5-5: Acquisition Wavelength Setting Options (continued)

Options	Description
Laser with Image Recovery	<p>Displays for the first acquisition (W1) wavelength and only if you select Enable Laser Based Focusing and Enable Image Based Focusing on the Acquisition tab and you select Allow Image Based Focusing for Recovery from Laser Based Well Bottom Failures on the Autofocus tab.</p> <p>When selected, specify the following options:</p> <p>Image Based Range +/- μm specifies the range to use for the image-based portions of autofocusing.</p> <p>Note: 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 to use to get the correct focus position. This setting is dependent on the objective. Because the focus peak is narrower, use a smaller step size with higher NA objectives.</p> <p>Note: Smaller step sizes 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 you select Custom Exposure Time on the Autofocus tab, and you select Laser with Image Recovery, specify a value for the following:</p> <p>Exposure (ms) specifies the exposure time in ms to use for the acquisition wavelength when autofocusing.</p>
Z-Offset from W1	<p>Available 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. Select this option to move the W1 focus position to the offset. Specify the post-laser offset in μm in the Offset field or click Calculate Offset to calculate the offset for all subsequent wavelengths.</p>

Table 5-5: Acquisition Wavelength Setting Options (continued)

Options	Description
Image Based	<p>Available for the second or greater acquisition wavelengths and if you select Enable Image Based Focusing on the Acquisition tab. Specify the Z-Offset from W1 in μm in the Offset field or click Calculate Offset to calculate the offset to use for all subsequent wavelengths. If you select this option, specify the following options:</p> <p>Image Based Range +/- μm specifies the range to use for the image-based portions of autofocusing.</p> <p>Note: 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 to use to attain the correct focus position. This setting is dependent on the objective. Use a smaller step size with higher NA objectives because the focus peak is narrower.</p> <p>Note: Smaller step sizes 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 you select Custom Exposure Times on the Autofocus tab, and you select Image Based, specify a value for the following:</p> <p>Exposure (ms) specifies the exposure time in milliseconds to use for the wavelength when autofocusing.</p>
	<p>Acquisition Options display depending on the Series option you select under Acquisition Options on the Acquisition tab.</p>
Acquire Time Series allows Timelapse	<p>Specifies the image collection intervals to use for the wavelength.</p> <p>At All Time Points allows acquisition of an image using the wavelength at each time point in the experiment.</p> <p>At Start of Experiment acquires an image using the wavelength at the first time point only.</p> <p>At Start/End of Experiment acquires an image using the wavelength at the first time point and the last time point for the experiment.</p> <p>Every Nth Timepoint acquires an image using the wavelength at the time point interval, for example, every 5th time point, beginning with the first time point for the experiment.</p>
Acquire Z Series allows Z Series	<p>Single Plane acquires a single plane based on the autofocus options for the wavelength, as if the Acquire Z Series option were disabled. This is used in a multi-wavelength acquisition when the Z Series acquisition is not required for the wavelength.</p> <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 generates a projection image; in this case all individual Z plane images as well as the projection are saved. This option is available if you clear Acquire Time Series and you select Acquire Z Series on the Acquisition tab.</p>

Table 5-5: Acquisition Wavelength Setting Options (continued)

Options	Description
2D Projection Image	<p>Available for all Z Series acquisition options other than Single Plane to indicate how the 2D projection image is generated.</p> <p>Best Focus calculates the regions of best focus in an image stack on a pixel-by-pixel basis. Similar to the Maximum setting, this setting uses the best focused pixels from the Z stack to create the projection image.</p> <p>Maximum is recommended 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 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 output to the resulting image.</p> <p>Sum is the intensities of the pixels in the stack planes 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 image capacity, then a message opens that indicates that the maximum possible intensity of 65535 was exceeded. The software cuts the pixels off at this value as a result.</p>
Digital Confocal 	<p>Available if the system includes the Digital Confocal feature. Select to do on-the-fly deconvolution-based image sharpening. Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.</p> <p>Note: For information about the Digital Confocal option, log in to the Molecular Devices Knowledge Base at mdc.custhelp.com, and search for Digital Confocal.</p>

Table 5-6: Shading Correction Options

Options	Description	Notes
Off	No shading correction or background subtraction is done.	<ul style="list-style-type: none"> - Not recommended for montages. - Not recommended for assays where intensity of individual objects will be compared across the field of view.
Auto Correction for FL	Combines FL Shading and FL Subtraction. Two reference images are created and used, one for the shading and one for the subtraction.	<ul style="list-style-type: none"> - Not recommended for Transmitted Light images. - Not recommended for time lapse assays of dim samples. - Not recommended for Z-stack assays where 3D reconstruction is used. - Not recommended for use with oil objectives. - Low magnification objectives may produce an artifact as it may catch the edge of the plate in the reference image. - Incorrect instrument configuration could produce a stage error. - You should use the Auto Expose tool when you optimize exposure times or temporarily disable the shading correction if you use snaps. Turn back on to run Plate Acquisition. - The subtraction portion of this correction is not compatible with water immersion objectives. A message displays but acquisition can continue and the shading portion is still applied.

Table 5-6: Shading Correction Options (continued)

Options	Description	Notes
FL Subtraction Only	<p>Background subtraction is performed on the images. No shading correction is performed. Background subtraction removes stray light that is unrelated to the sample. The system collects a reference image for each wavelength at the start of the plate acquisition and then subtracts this image from the acquired images.</p>	<ul style="list-style-type: none"> - Not recommended for transmitted light images. - Not recommended for time lapse assays of dim samples. - Not recommended for Z-stack assays where 3D reconstruction is used. - Not recommended for use with oil objectives. - Low magnification objectives may produce an artifact as it may catch the edge of the plate in the reference image. - Incorrect instrument configuration could produce a stage error. - You should use the Auto Expose tool when you optimize exposure times or temporarily disable the shading correction if you use snaps. Turn back on to run Plate Acquisition. - Subtraction is not available for water immersion objectives.
FL Shading Only	<p>Images are corrected for shading automatically. The shading correction is tuned for fluorescence and it adjusts for uneven illumination of the sample. An in-focus reference image for each wavelength is collected at the beginning of the plate acquisition. This reference image is used to calculate the shading pattern per wavelength and then applied over the plate. The same reference image is used for all time points in a time series.</p>	<ul style="list-style-type: none"> - Recommended for most fluorescent assays. - Not recommended for transmitted light images. - Use if Auto Correction for FL is producing an artifact from catching the edge of the plate in the reference image.

Table 5-6: Shading Correction Options (continued)

Options	Description	Notes
Auto Correction for TL	<p>This is a shading algorithm tuned for transmitted light but can be used for fluorescence. It does shading correction but not background subtraction. This algorithm is faster than the Auto Correction for TL Legacy algorithm, and does not rely on the TL Legacy Shading Correction Refinement Level on the Wavelengths tab. The shading correction is calculated individually for each image. No reference image is used.</p>	<ul style="list-style-type: none"> - Not recommended for montages. - Not recommended for Z-stack assays. - Not recommended for time lapse assays.
Auto Correction for TL Legacy	<p>Images are corrected for shading automatically, without a need for reference images. The shading correction is tuned for transmitted light, and it adjusts for uneven illumination of the sample. The default value for the TL Shading Correction Refinement Level (Wavelengths tab) is 2. The higher the refinement level, the flatter the transmitted light will be, but the longer the correction will take. The shading correction is calculated individually for each image. No reference image is used.</p>	<ul style="list-style-type: none"> - Not recommended for fluorescent images. - Not recommended for montages. - Not recommended for Z-stack assays. - Not recommended for time lapse assays.

Table 5-6: Shading Correction Options (continued)

Options	Description	Notes
Legacy Correction	<p>Images are corrected for shading using reference images specific to objective/filter combinations (and possibly other optics, such as confocal disk). The reference images must be created in advance by the user. They are typically stored in C:\Shading Images but may be stored elsewhere on the computer.</p> <p>Can be used for either fluorescence or transmitted light. See Using Legacy Correction Image Shading on page 98.</p> <p>Note: In general, the automatic methods are recommended over the Legacy Correction method because correction is based on the current state of the system and is not subject to variation over time.</p>	<ul style="list-style-type: none"> - Recommended for montages. - Not recommended for multi-site imaging of plates in transmitted light, particularly at low magnification, since the meniscus causes site-specific illumination. - Shading reference images must be updated when there are changes to the optics, such as the light source or the liquid light guide. - May be inconvenient to create reference images with oil objectives.

Using Legacy Correction Image Shading



Note: This section is applicable if you select Legacy Correction as the shading correction method for an acquisition wavelength. See [Configuring Acquisition Wavelengths on page 89](#).

Before you do legacy shading correction, acquire the images for each acquisition wavelength and save the images to a stored correction images directory. The default location is C:\Shading Images folder. See [Verifying Shading Correction Files-Legacy on page 45](#).

The file name for a shading correction image must include 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 240](#).

If you acquire an image with the full field of view, 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 64](#).



Note: If a shading correction image cannot be found for the combination of Magnification, Acquisition Mode, and Illumination settings in the directory, no shading correction is done for the wavelength.

To change the default directory location for shading correction images:

1. Select the **Acquisition** tab.
2. Click **Directory for Stored Correction Images** to display the Browse for Folder dialog.

3. Select the new location.
4. Click **OK**.

Configuring Series Acquisition Options

The Acquisition tab provides two series acquisition options (you can select one or both):

- **Acquire Time Series** acquires images at multiple time points. When you select this option, specify the set of images to acquire at each time point.
- **Acquire Z Series** acquires individual optical sections (planes) in sequence through a sample that can produce a 3-D image of the sample. When you select this option, specify the number of steps and the step size to move through the sample.

To configure a series acquisition:

1. Select the **Acquisition** tab.

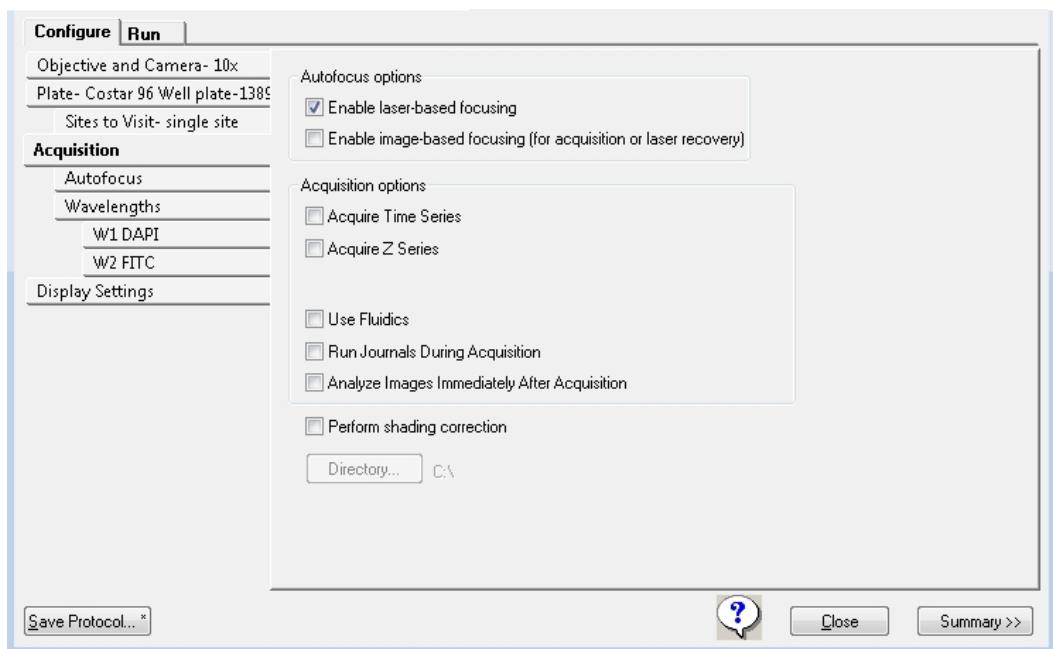


Figure 5-20: Acquisition Tab on the Configure Tab

2. Under **Acquisition Options**, select one or both of the following:
 - Select **Acquire Time Series** and continue to [Configuring Continuous Time Series Acquisitions on page 100](#).
 - Select **Acquire Z Series** and continue to [Configuring Z Series Acquisition Options on page 104](#).

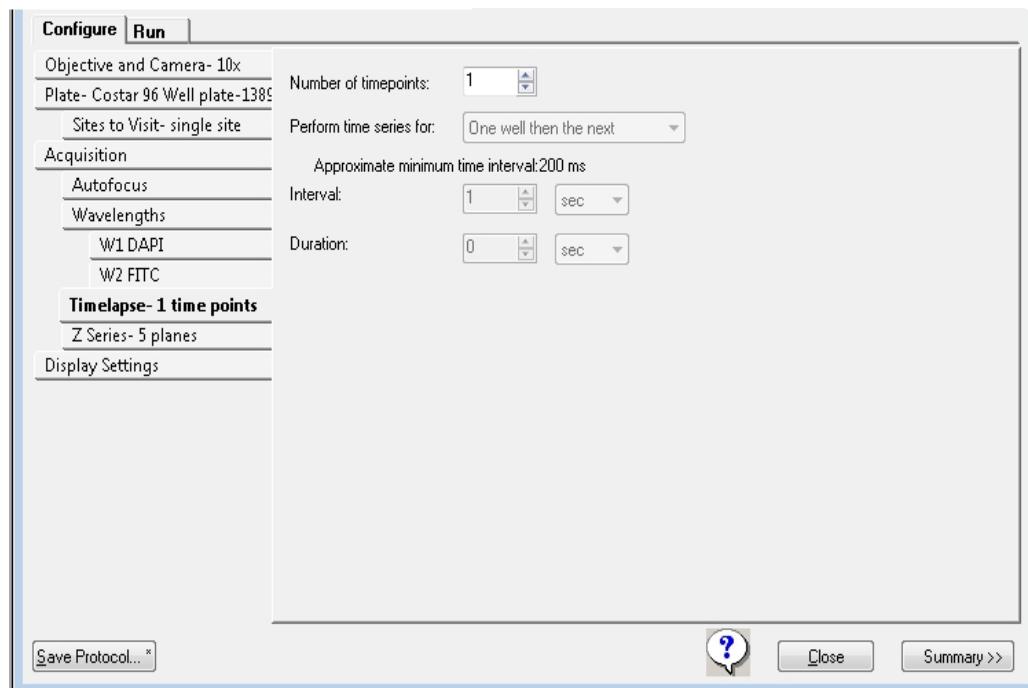
Configuring Continuous Time Series Acquisitions

Use the Timelapse tab to configure time series acquisitions. There are two types of time series acquisitions. This topic describes continuous time series acquisition.

For discontinuous time series acquisition, see [Configuring Discontinuous Time Series Acquisitions on page 102](#).

To configure a continuous time series acquisition:

1. Select the **Timelapse** tab.



2. Configure the time series acquisition options.

Options	Description
Number of Timepoints	Specifies the total number of time points that are to acquire. If you change this value, the software calculates the Number of Timepoints and the Interval to update the Duration field.
	Perform Time Series For is available when the Number of Timepoints is greater than 1. It specifies the loop order for the software to use to acquire images at multiple time points and defines the time interval and total length of the time lapse acquisition routine.
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 images that are in one row of wells at each time point. After the software collects the series, the next row is acquired. Note: This option requires a longer time interval because the software acquires all wells in a row.
One Column Then the Next	Collects all the images in one column of wells at each time point. After the software collects the series, the next column is acquired. Note: This option requires a longer time interval because the software acquires all wells in a column.
All Selected Wells	Acquires all wells at each time point in the acquisition routine. Note: This option requires a longer time interval because the software acquires all wells in the plate.
	Note: The Timelapse Autofocus setting on the Autofocus tab determines any refocusing the software does after the images for the first time point are acquired. See Configuring Autofocus Options on page 83 .

3. In the **Interval** field, enter the amount of time between the start of an acquisition at one timepoint and the start of an acquisition at the next time point. Click the corresponding drop-down and select the unit of time measure.



Note: The software calculates the Approximate Minimum Time Interval value 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, the next time point starts as soon as the previous time point finishes.



Note: The software multiplies the Number of Timepoints by the Interval to determine the Duration value. If you change the Number of Timepoints or the Interval, the Duration field automatically updates.

4. In the **Duration** field, enter the duration value.



Note: The time unit for the Duration does not have to be the same as the time unit for the Interval. If you change the Duration, the Number of Timepoints automatically updates.

5. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#).

Configuring Discontinuous Time Series Acquisitions

With discontinuous time lapse acquisition, you can return the sample plate to the incubator in between time points, and you can read other plates in the interim.

Before adding time points to an experiment, verify the following:

- Folder names, plate names, and bar codes match. If one of these does not match, the plate acquires separately as a new plate.
- The protocol matches the previous protocol using the same plate.

To start a discontinuous time lapse acquisition:

1. Select the **Configure** tab.
2. Configure or select a protocol.



CAUTION! Inappropriate protocols cause errors. See [Error Handling on page 103](#).

3. Select the **Acquisition** tab.
4. Select the **Enable Laser Based Focusing** check box. (Molecular Devices recommends selecting this option, but it is not required.)
5. For certain samples, select the **Enable Image Based Focusing** check box.
6. Select the **Acquire Time Series** check box to acquire a continuous time lapse series of time points within the discontinuous time lapse experiment with the plate left in the instrument.
7. For Z series experiments, select the **Acquire Z Series** check box.

8. Avoid running Fluidics experiments with discontinuous acquisition.
9. If you select the **Acquire Z-Series** check box, select **2D Projection Image Only** on each W# wavelength tab.



Tip: Do not select both Z Series and 2D Projection Image.

-
10. If you select the **Acquire Time Series** check box, select the **Timelapse** tab and do the following to add continuous time points:
 - a. Click the **Perform Time Series For** drop-down:
 - Select **One Well Then the Next** for fast kinetic time lapse in a single well (or site). The software collects all time points in one well before moving to the next well.
 - Select **All Selected Wells** for long-term time lapse experiments. The software acquires all wells during each time point.
 - b. In the **Number of Timepoints** field, enter the number of time points.
 - c. In the **Interval** field, enter the time between the beginning of one time point and the beginning of the next. Select the time unit of measure from the drop-down.
 - d. In the **Duration** field, enter the total time of experiment. Select the time unit of measure from the drop-down. This is equivalent to Interval x Number of time points.



Note: Time points you define here acquire in a continuous acquisition and you can append the time points to a discontinuous time lapse experiment.

-
11. Select the **Run** tab.
 12. In the **Folder Name** field, enter the folder name.
 13. In the **Barcode** field, enter the code. See [Entering Barcodes on page 104](#).



Note: You must use a barcode to append subsequent time points.

-
14. Click **Acquire Plate** to start the plate acquisition.



Note: The next time point you acquire will be appended to the previous plate reads as long as you match the Folder Name, Barcode, and Plate Name. In addition, most of the protocol parameters must remain the same or an error will result.

Error Handling

With discontinuous time lapse acquisition, if you select an inappropriate protocol, the following messages can display, and you cannot append to the plate:

- Site selection has changed.
- Plate type and/or well selection changed.
- Wavelength selection has changed.
- Z-Series option set to save all Z planes.

Entering Barcodes

Enter the barcode manually or with a barcode scanner. Use alphanumeric characters. The Barcode field clears after every acquisition.

To reuse a manually entered barcode:

1. When you create a protocol, enter the barcode to recall in the **Description** field.
2. Copy the **Description** field barcode and paste it in the **Barcode** field.

Configuring Z Series Acquisition Options

Use the Z Series tab to configure Z series options.

To configure Z Series options:

1. Select the **Z Series** tab.

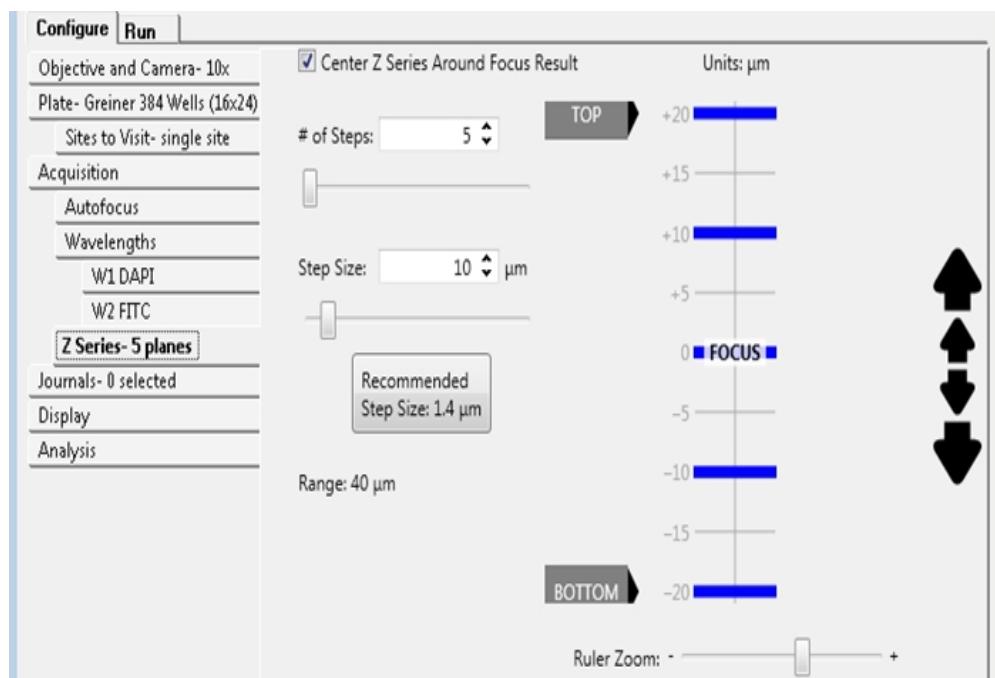


Figure 5-21: Z Series Tab on the Configure Tab

*** Tip:** The blue bars represent the planes to acquire for each site imaged. The software calculates the distance between these bars based on the value in the Step Size field.

2. Configure the Z series acquisition options.

Table 5-7: Z series Acquisition Options

Option	Description
# of Steps	The number of planes at a given site or well position for which to take images. For example, if set to 12, the software takes 12 images along the Z axis of the sample. Do the following to adjust this value: –Enter a value in the field. –Click the Up or Down arrows in the field. –Drag the slider below the field.
Step Size	The distance in μm between the image planes in the Z series. Do the following to adjust this value: –Enter a value in the field. –Click the Up or Down arrows in the field. –Drag the slider below the field. Note: Based on the numerical aperture (NA) setting for the objective, the software automatically calculates a recommended step size provides the best detail for the Z series. Click the Recommended Step Size message to automatically set the Step Size to this value.
Note: As you adjust one or both values, the software automatically calculates the range, which is the overall image depth from top to bottom of the image sample and displays this value below the step size.	

- 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 the following:

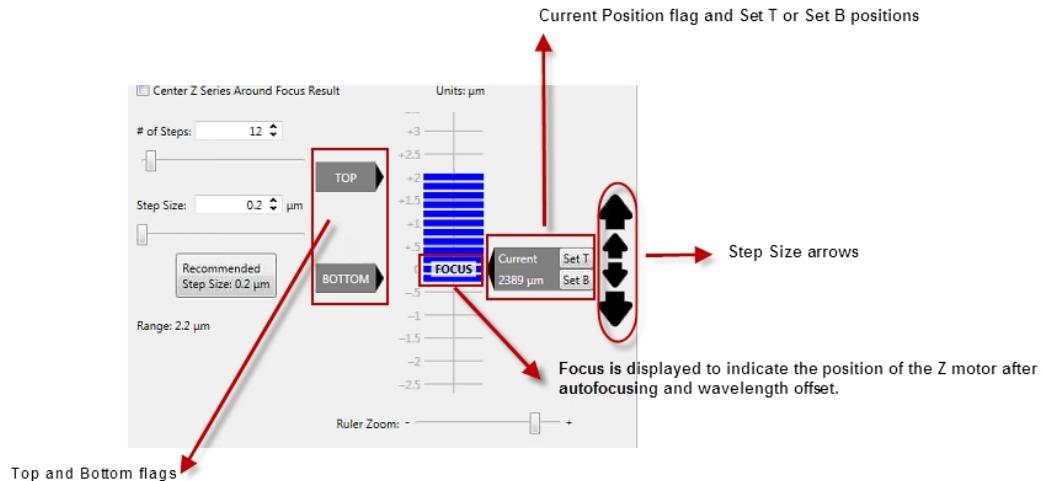


Figure 5-22: Z Series Diagram

- Drag the **Ruler Zoom** slider (below the Z series diagram) or use the mouse scroll wheel to zoom in or zoom out on the diagram.
- Focus** displays on the Z series diagram to indicate the position of the Z motor after autofocusing and wavelength offsets are applied. Do the following to modify the Z stack acquisition settings:
 - 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** flag or the **Bottom** flag to set the top and bottom positions for the Z series acquisition relative to the Focus position. As you adjust these values, the software automatically calculates the values for the # of Steps field and the Range field. The position in μm to which you move a flag temporarily displays on the flag.
- Select the **Center Z Series Around Focus Result** check box to evenly space the Z series planes above and below the Focus position. When you drag a **Top** or **Bottom** flag on the Z series diagram to set the position for the Z series acquisition, the software automatically moves the other position by the same total distance in μm.
- Clear the **Center Z Series Around Focus Result** check box and click **Set B** to designate the bottom Z plane to be acquired at each site.

- 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.



Note: If the Current Position flag does not display, 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. Use this approach to interactively visualize 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.



Tip: Live mode is more responsive when binning is set to 2 or higher.

- 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.) When you are in Live mode, when you click on different planes in the Z series diagram, the image in the Snap window updates accordingly.



Tip: In Test mode, when you capture a Z stack, an image window displays. Click and drag the slider bar at the top of the dialog to scroll through the Z stack images.

In Start Live mode, only the current plane displays. To change the current plane view, click on the step markers in the ruler on the Z tab.

- To move the Z motor up or down one step at a time by the recommended Step Size, click the small step arrows and . To move the Z motor up or down one step at a time by the defined Step Size, click the large step size arrows to the right of the Z series diagram. Click and hold a step size arrow to continuously move the Z motor.

4. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#).

Configuring Journals to Run During Acquisition

Use the Journals tab to configure journals to run during different stages of the acquisition. You can assign one journal configuration per acquisition step.

1. Select the **Acquisition** tab.
2. Select the **Run Journals During Acquisition** check box to add a Journals tab to the Acquisition tab.
3. Select the **Journals** tab.
4. Select the check box for each **Acquisition Step** at which to run a journal.

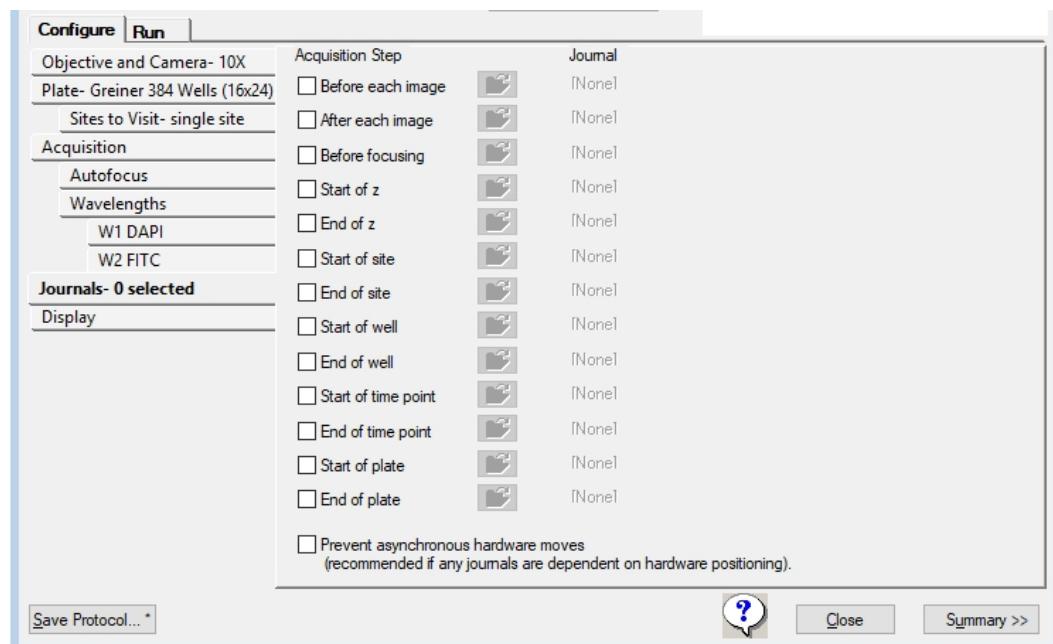


Figure 5-23: Journals Tab, Acquisition Step Selection

Table 5-8: Journal Acquisition Steps

Option	Description
Before Each Image	Runs during the acquisition loop, after the illumination is set and focusing is complete.
After Each Image	Runs during the acquisition loop, after the shutter closes and before images save.
Before Focusing	Runs during the acquisition loop, just before the focus step begins.
Start of Z	Runs before a Z series is acquired. 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 you configure to use the Z series.

Table 5-8: Journal Acquisition Steps (continued)

Option	Description
End of Z	Runs after a Z series acquisition completes. 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 you configure to use the Z series.
Start of Site	Runs during the acquisition loop, before any images are acquired from each site.
End of Site	Runs during the acquisition loop, after all images have been acquired from each site.
Start of Well	Runs during the acquisition loop, at the beginning of each well, before any images are acquired from a well.
End of Well	Runs during the acquisition loop, at the end of each well, after all images have been acquired from a well.
Start of Time Point	Runs during the acquisition loop, at the beginning of each time point, before any images are acquired for each time point.
End of Time Point	Runs during the acquisition loop, at the end of each time point, after all images have been acquired for each time point.
Start of Plate	Runs before the initial find sample step is performed. Cannot be used to modify the Find Sample location.
End of Plate	Runs after the last image acquisition for a plate is complete.
Prevent Asynchronous Hardware Moves	(Optional) Select this option only if a journal moves hardware, such as changing filters, or moving focus. This option ensures that the journals run correctly. Note: Do not select this option without the use of a journal because it can slow the acquisition.

5. Click  for each acquisition step with a selected check box to display the Select Plate Acquisition Journal dialog.

6. Select a journal file.

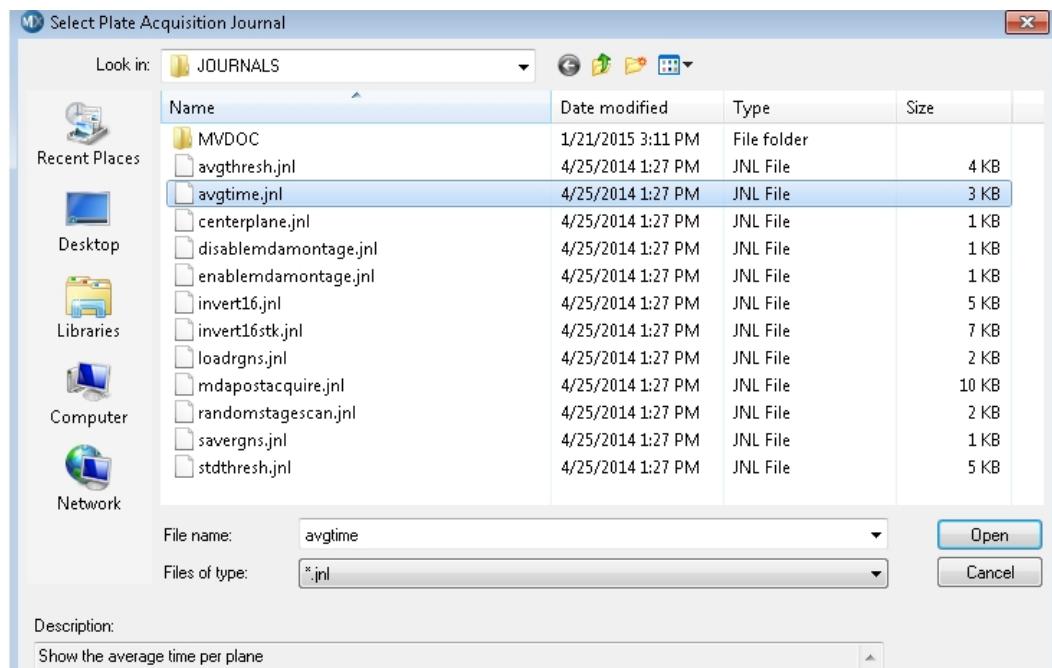


Figure 5-24: Select Plate Acquisition Journal Dialog

7. Click **Open** to display the Journal tab.
8. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#).

Plate Acquisition Setup Dialog: Configure Tab, Display Tab



Note: Use the Active Wavelength Preview tool to generate a preview of an acquired image for each wavelength.

Use the Display tab to 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. 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.



Note: Display settings save with the acquisition protocol.

1. Select the **Display** tab.

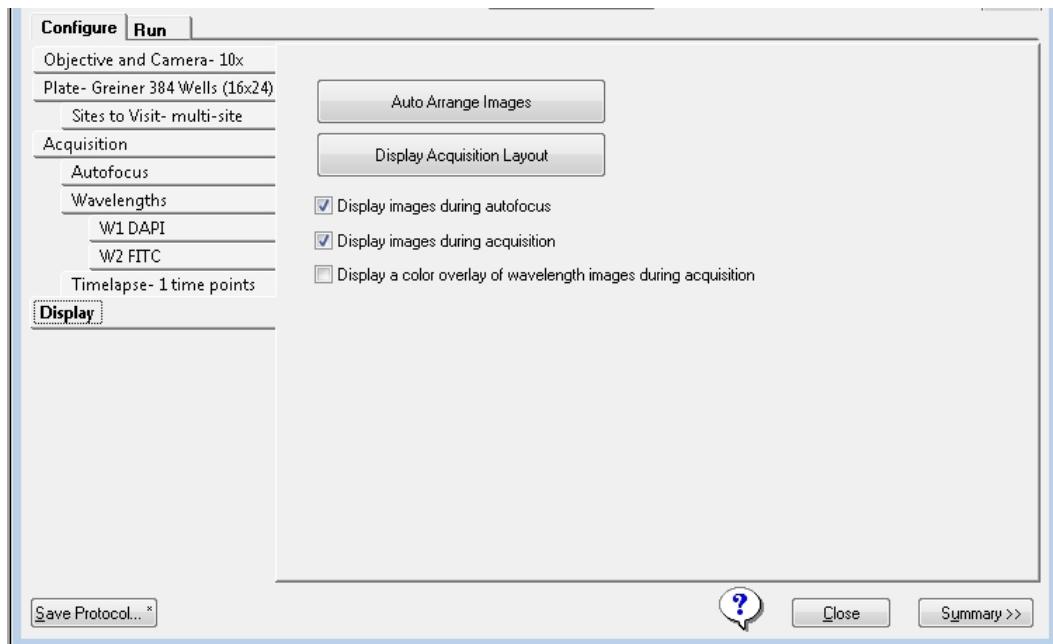


Figure 5-25: Display Tab on the Configure Tab

2. Click **Auto Arrange Images** to use the default settings for image display and for the dialogs that appear during acquisition. The software auto scales images and arranges them in the default layout with an unobstructed status dialog.
3. Click **Display Acquisition Layout** to display the current settings for image display and the Plate Acquisition Status dialog that indicates where dialogs will appear during acquisition. See [Previewing the Acquisition Layout on page 112](#).
4. Select the **Display Images During Autofocus** check box to display the images that the software acquires during image autofocus.
5. Select the **Display Images During Acquisition** check box to display each image as it is acquired.
6. Select the **Display a Color Overlay of Wavelength Images During Acquisition** check box to display a color composite of the images the software captures for each wavelength the software acquires at each site.
7. When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#).

Previewing the Acquisition Layout

Use the Plate Acquisition Status dialog to preview the display settings.

To preview display settings:

1. Select the **Display** tab.
2. Click **Display Acquisition Layout**. Individual image windows display where the image will appear for each wavelength and the Plate Acquisition Status dialog displays where any acquisition related dialog will appear during acquisition.

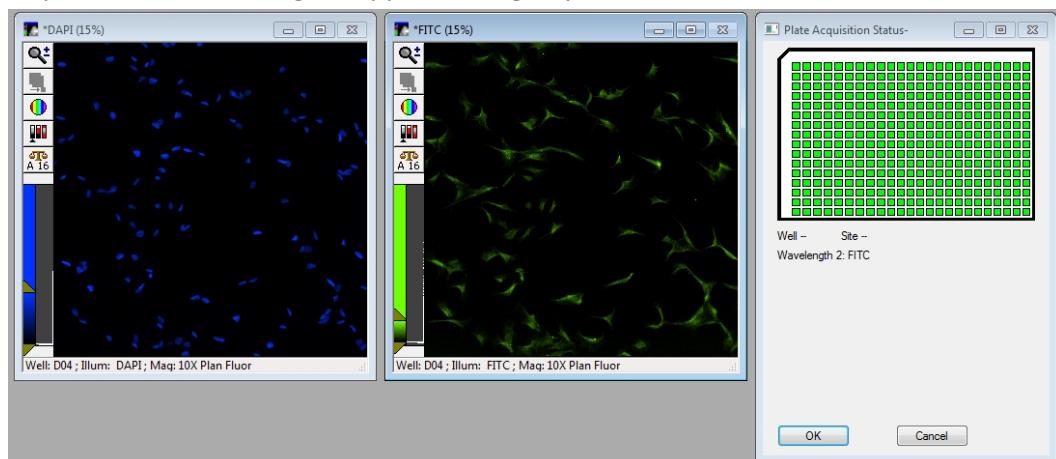


Figure 5-26: Two Wavelength Image Windows and Display Acquisition Status Dialog



Note: If the image windows do not display, click the Active Wavelength Preview to display an image window for each wavelength.

3. Leave the images in the default layout or do any of the following:
 - Rearrange the position of the image windows.
 - Change the size of the image windows.
 - Use the icons in the image windows to change the size, scaling, or look up table.
4. Click **OK** to save the changes. Display settings save with the acquisition protocol.



Note: Adjustments you make to images display when you use the active wavelength tools, like Snap or Focus to change the display settings.

Configuring Post-Acquisition Analysis Options

Use the Analysis tab to select the analysis to run on a plate after the acquisition completes. If you select this option, the software adds the analysis job 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 assays and settings files from any application module, custom module, or journal assay that you save to the MDCStore database.

To configure post-acquisition analysis:

1. Select the **Acquisition** tab.
2. Select the **Analyze Images Immediately After Acquisition** check box to add an Analysis tab to the Configuration tab.
3. Select the **Analysis** tab.



Note: The options that display on the Analysis tab depend on the series acquisition mode check boxes you select on the Acquisition tab (Acquire Time Series or Acquire Z Series) as well as the image to acquire and retain: Single Plane, D Projection Image Only, or Z Series and Projection Image.

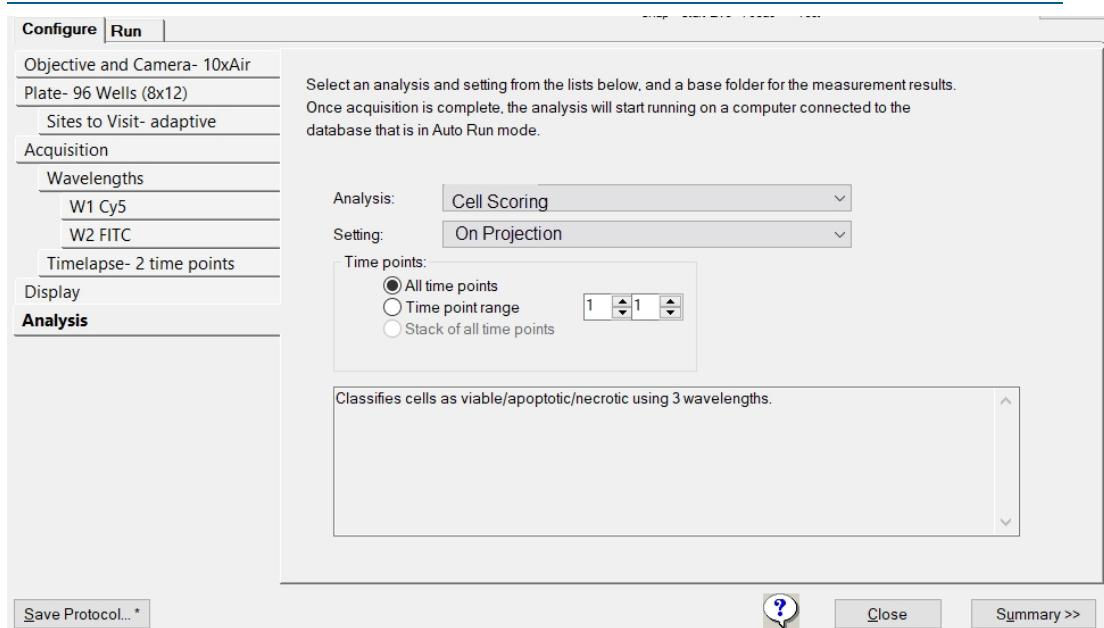


Figure 5-27: Analysis Tab (Time Series)

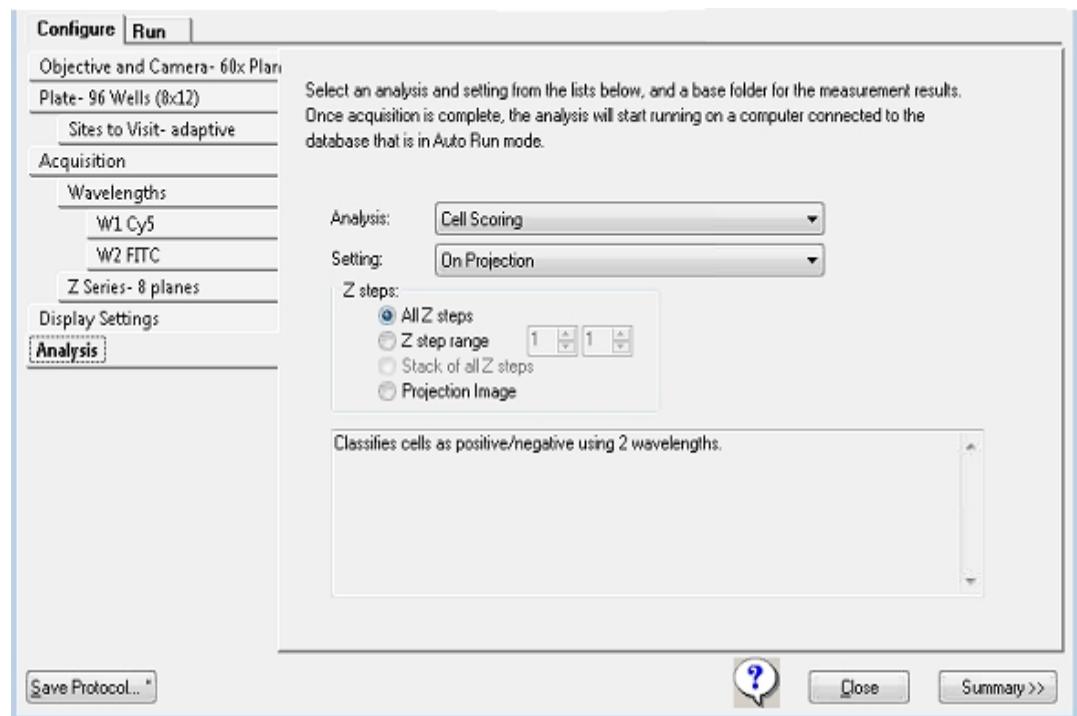


Figure 5-28: Analysis Tab (Z Series and 2D Projection Image Only)

- Click the **Analysis** drop-down and select the analysis protocol to run post-acquisition.



Note: This list includes any application modules, custom modules, or journal assays that you have saved to the MDCStore database. The list of assays is the same list that is available on the Run Analysis tab in the Review Plate Data dialog. Only application modules and custom modules are compatible with the MetaXpress PowerCore Software. Journal-based analysis can only be run within the MetaXpress Software.

- Click the **Setting** drop-down and select the settings file for this module or journal assay.



Note: The list includes all setting files that you save to the MDCStore database.

- Select the acquisition time points for which to run the analysis.

Table 5-9: Time Point Options - Time Series Acquisition

Option	Description
All Time Points	Analyze all acquisition time points.
Time Point Range	Analyze the acquisition time points that fall within the range.
Stack of All Time Points	Available if the 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	Analyze all acquisition time points.
Time Point Range	Analyze the acquisition time points that fall within the range.
Stack of All Time Points	Available if the 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	Analyze all captured Z planes.
Z Step Range	Analyze the Z plane images that fall within the range.
Stack of All Z Steps	Available if the analysis is a journal.
Projection Image	Analyze only the projection image.

- When you finish configuring the protocol, continue to [Saving Plate Acquisition Protocols on page 117](#).

Viewing the Summary Panel

The Summary panel displays to the right of the Plate Acquisition Setup dialog to list a summary of the current acquisition settings.

To view the summary panel:

- At the bottom of the Plate Acquisition Setup dialog, click **Summary >>** to display the Summary panel.

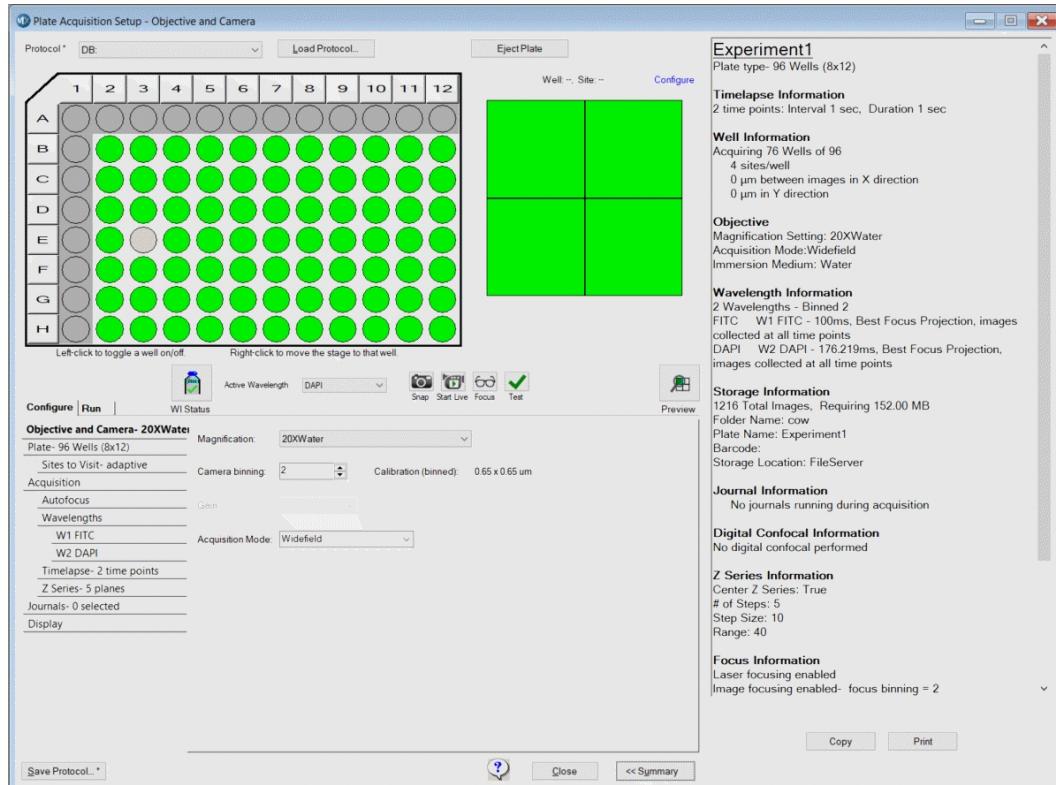


Figure 5-29: Plate Acquisition Setup Dialog with Open Summary Panel

- Click **Copy** to copy the contents to the computer clipboard from where you can paste the contents into another program, such as Microsoft Word.
- Click **Print** to print the summary.
- You can leave the summary open while you configure the acquisition protocol or click **<< Summary** to close it.

Saving Plate Acquisition Protocols

You can save a plate acquisition protocol to the database or to a file. If you plan to integrate your ImageXpress Micro System with a robot for automated plate loading, you must save the protocol to a file.

Saving Plate Acquisition Protocols to the Database

To save a plate acquisition protocol to the database:

1. Click **Save Protocol** to display the Save Acquisition Protocol dialog.
2. In the **Protocol Name** field, enter the name for the protocol.
3. Click **Save**.

You can now acquire plate data with the saved protocol.

Saving Plate Acquisition Protocols to a File

Plate acquisition protocol files have a .hst file type that you cannot change.

To save a plate acquisition protocol to a file:

1. Click **Save Protocol** to display the Save Acquisition Protocol dialog.
2. Select the **Save to File Rather Than Database** check box.
3. Click **Save** to display the Plate Acquisition Setting dialog.
4. Navigate to the location to save the file.
5. In the **File Name** field, enter the name for the protocol.
6. Click **Save**.

You can now acquire plate data with the saved protocol.

Running Plate Acquisition Protocols

Use the Run tab in the Plate Acquisition Setup dialog to run the plate acquisition protocol.

After you load a protocol, configure the wells and sites for which to acquire a data, and verify that the current acquisition settings are appropriate, you can run the protocol and acquire the plate data.

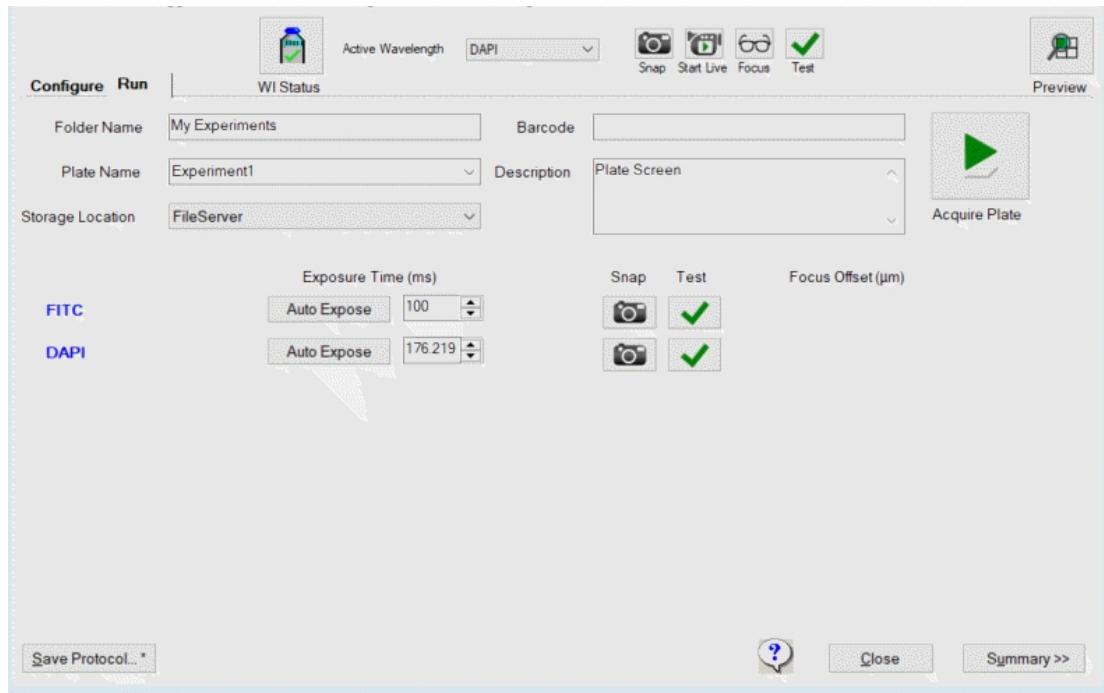


Figure 5-30: Plate Acquisition Setup Dialog, Run Tab

To run a plate acquisition protocol:

1. Select the **Run** tab.
2. In the **Folder Name** field, enter the name of the folder in which to store the plate acquisition data.



Note: Names become part of the image file paths. If the overall path length is too long, it causes errors.

3. In the **Plate Name** field, enter the name/id of the plate from which to acquire data.



Note: Names become part of the image file paths. If the overall path length is too long, it causes errors.

4. In the **Barcode** field, you can enter the bar code manually or use the bar code scanner to automatically enter the bar code. To avoid accidental reuse of a barcode, the barcode does not save with the plate acquisition settings.



Note: If you use a robotic plate handler with an integrated barcode reader, the software populates the barcode field.

5. In the **Description** field, enter the description of the experiment.

6. Do the following to make last minute tweaks as needed:

- Click the blue wavelength name to display the corresponding W# Wavelength tab where you adjust wavelength settings. See [Configuring Acquisition Wavelengths on page 89](#).
- Click **Auto Expose** to automatically set the exposure time required to obtain the Target Max Intensity.
- In the **Exposure Time** field, enter the exposure time for the wavelength.
- - Click to snap an image.
- - Click to test your settings.

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

The Plate Acquisition Setup dialog closes. The Plate Acquisition Status dialog displays an image of the plate wells that have been acquired (outlined in black) and the well that is currently being acquired (outlined in orange). The well/site ID for the well that is currently being acquired displays below this graphic. The software acquires wells in a serpentine fashion, going down one column, and then up the next column. After acquisition begins, the estimated time remaining displays. An image displays briefly for each acquisition wavelength in a standalone image window. After the last image is acquired and saved, the Plate Acquisition Status dialog closes and the Plate Acquisition Setup dialog displays.

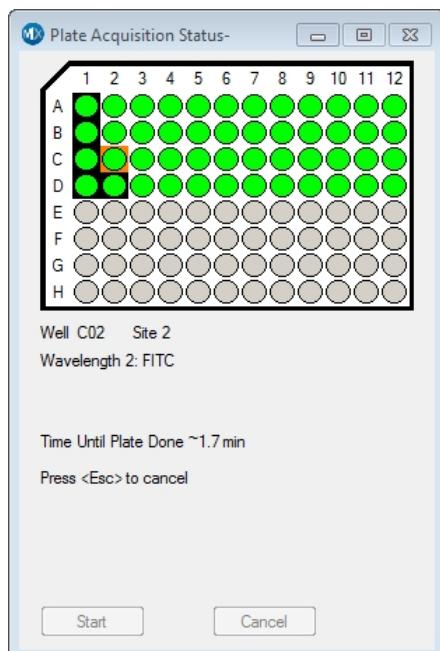


Figure 5-31: Plate Acquisition Status dialog



Chapter 6: Customizing the MetaXpress Software

A powerful feature of the MetaXpress Software is the ability to customize the operation of the software for individual users or groups of users. Various 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, see below](#)
- [Custom Toolbars and Taskbars on page 124](#)
- [Default Paths for Data on page 127](#)



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 flash drive.

Users and Groups in the Meta Imaging Series Administrator Software

Use the Meta Imaging Series Administrator Software commands 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 allows you to select hardware settings and configure drop-ins and toolbars for groups that have already been created.
- Multi-User mode, where you can create new groups, add users to different groups, and define hardware settings for groups.

As a System Administrator, you can work within Multi-User mode to create groups and users.

The software installation includes predefined groups and hardware settings. The number of groups depends on the configuration of your system. Use the Multiple User Configuration dialog in the Meta Imaging Series Administrator Software to manage these settings.

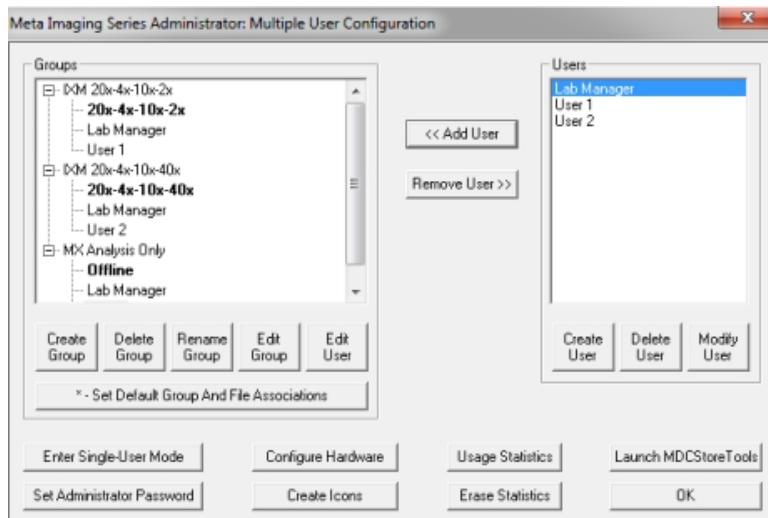


Figure 6-1: Multiple User Configuration Dialog

This example shows a system with the following groups defined:

- **IXM 20x-4x-10x-2x**: This group has access to a particular set of objectives.
- **IXM 20x-4x-10x-40x**: This is a group has access to a particular set of objectives.
- **MX Analysis Only**: This group can perform analysis in the MetaXpress Software when the instrument is powered off.

To allow a group to use the hardware and software settings you create for the 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 does not include hardware settings and is useful for analysis of acquired images. Since the offline group does not include hardware settings, it does not attempt to establish communication with the other MetaXpress Software components. This lets the application start faster, and lets you run the software without turning on any hardware.



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

To create an offline MetaXpress Software group in the Meta Imaging Series Administrator Software:

1. Click **Start > Programs > MetaXpress** and then right-click **Meta Imaging Series Administrator** and select **Run as Administrator** to start the MetaXpress Meta Imaging Series Administrator Software.
2. If the program opens in Single User Configuration mode, click **Enter Multi-User Mode**.
3. Click **Create Group** to display the Create Group dialog.

4. In the **Group Name** field, enter a group name, such as **MetaXpress Offline**.

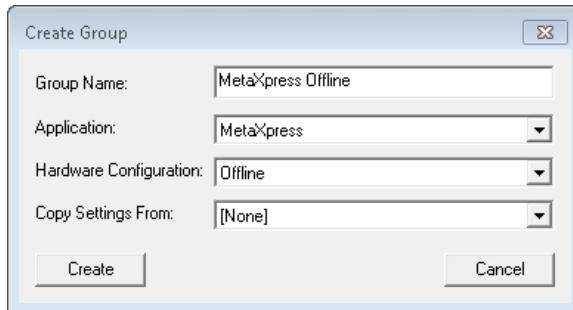


Figure 6-2: Create Group Dialog

5. Click the **Application** drop-down and select **MetaXpress**.
6. Click the **Hardware Configuration** drop-down and select **Offline**.
7. Click the **Copy Settings From** drop-down and 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 to make it available. Alternatively, you can return to Single User mode, then the new group works without adding users.

13. To avoid error messages, select the offline group, click **Edit Group**, then click **Drop-Ins/Toolbars** and disable the **plateacquire** drop-in.
14. Click **OK** to exit the Meta Imaging Series Administrator Software.

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

After you create the MetaXpress Offline group and add users with the previous procedure, use the Create Icons command to create icons for the new group. This command installs shortcuts for new groups to the MetaXpress folder on the Windows desktop. You can then copy these shortcuts to the Windows desktop. This allows users to choose which software configuration to start from the desktop.

To create and add group icons to the Windows desktop:

1. Click **Start > Programs > MetaXpress** and then right-click **Meta Imaging Series Administrator** and select **Run as Administrator** to start the MetaXpress Meta Imaging Series Administrator Software.
2. Click **Create Icons**.
3. Click **OK** to exit the Meta Imaging Series Administrator Software.
4. Double-click the **MetaXpress 6.x** shortcut on the desktop.

5. In the **MetaXpress 6.x** folder, confirm that a shortcut for the group you create is listed. See [Creating an Offline Version of the MetaXpress Software on page 122](#).
6. Right-click the shortcut (for example, **MetaXpress Offline**) and 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 configure groups, you can create or modify custom toolbars and taskbars to include specific combinations of tools and commands.

Customizing Toolbars

Use the Configure Drop-ins/Toolbars command to add menu commands to toolbars, move commands from one toolbar to another, and add journals to or remove journals from toolbars.

To customize the toolbars:

1. Click **Start > Programs > MetaXpress** and then right-click **Meta Imaging Series Administrator** and select **Run as Administrator** to start the MetaXpress Meta Imaging Series Administrator Software.
2. In the Meta Imaging Series Administrator > List of Groups, select a Group Name.
3. Depending on the user mode:
 - a. Single User Mode: Click **Drop-ins/Toolbars** to display the Configure Drop-ins/Toolbars dialog.
 - b. Multi-User Mode: In the Edit Group dialog, click **Drop-ins/Toolbars** to display the Configure Drop-ins/Toolbars dialog.
4. Select the **Toolbars** tab.
5. Clear the **Use Default Toolbars** check box.
6. Select a **Commands** option:
 - Select **Menus** to add menu commands to toolbars.
 - Select **Toolbars** to add toolbar commands to other toolbars.
 - Select **Journals** to add journals to a toolbar or to create new Journal toolbars.
7. To add a command to a toolbar, drag the command from the left pane to the toolbar folder in the right pane.
8. Click **OK** in the Configure Drop-ins/Toolbars dialog.
9. Click **Yes** in the message to confirm that you want the users in the group to use the modified configuration.
10. Click **OK** in the Edit Groups dialog.
11. Click **OK** to exit the Meta Imaging Series Administrator Software.

The toolbars display the next time you start the MetaXpress Software.

Creating Taskbars

Use Taskbars to access frequently used commands and journals. Each toolbar can have up to 48 buttons in a configuration of rows and columns. You can mix and match journals, commands, or other toolbars within the same toolbar. Create toolbars that combine commands and journals that are specific to your experiments.

To create and load a toolbar:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Journal > Toolbars > Create Toolbar** to display the Toolbar Editor dialog and the New Toolbar dialog.

From the standard menu, click **Journal > Toolbars > Create Toolbar** to display the Toolbar Editor dialog and the New Toolbar dialog.

2. Position them so that you can see both the dialogs at the same time.
3. Drag the edges of the New Toolbar dialog to until the dialog displays the number of rows and columns for the toolbar.
4. In the New Toolbar dialog, click into a cell to make it active and then drag the right or left edge to define the width of the buttons in the toolbar.
5. In the Toolbar Editor dialog, select a **Category** option:
 - Select **Function** to add a software function to the toolbar.
 - Select **Journal** to add a journal to the toolbar.
 - Select **Toolbar** to add a link to another toolbar to the toolbar.



Note: For Journal or Toolbar, the directory names display in square brackets.

Double-click a directory name to display the files in that directory or double-click [...] to go up one level in the directory structure.

6. Locate the item to add to the toolbar and double-click it to add the item to the active button in the New Toolbar dialog.

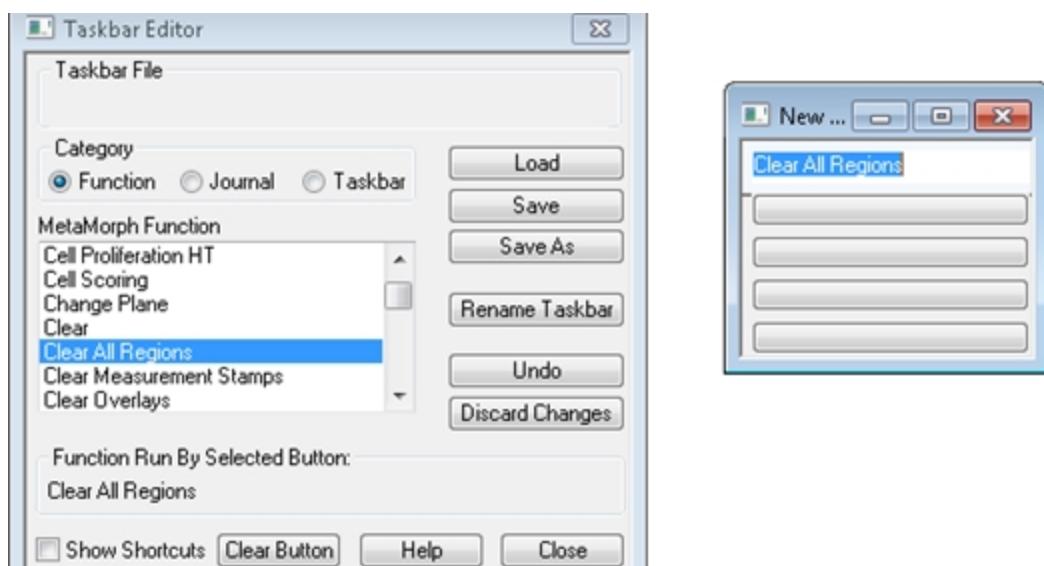


Figure 6-3: Add Functions to a Toolbar

7. Repeat to select a blank button in the New Taskbar dialog then double-click the item in the list in the Taskbar Editor dialog to add the task to the taskbar.
8. If necessary, click **Undo** to undo the last action or click **Clear Button** to clear an item from the active button.
9. Click **Rename Taskbar** to display the Rename Taskbar dialog where you change the name of the taskbar.
 - a. In the **Taskbar Title** field, enter the new name.
 - b. Click **OK** to return to the Taskbar Editor dialog. The title bar in what was the New Taskbar dialog now displays the taskbar name.
10. In the Taskbar Editor dialog, click **Save** to display the Save As dialog where you save the taskbar.
 - a. In the File Name field, enter the name of the taskbar .jtb file.
 - b. Navigate to the location to save the taskbar file.
 - c. Click **Save** to display the Taskbar Editor dialog.
11. Click **Close** in the Taskbar Editor dialog. Both dialogs close.
12. To use a taskbar:

From the simplified menu, click **Control > Journal > Taskbars > Load Taskbar** to display the Select a Taskbar dialog.
From the standard menu, click **Journal > Taskbars > Load Taskbar** to display the Select a Taskbar dialog.
13. Navigate to the and select the taskbar .jtb file.

14. Click **Open**.



Tip: When you use taskbars, press F4 to display the last used taskbar.

Default Paths for Data

Use the Configure Default Paths dialog 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:** The 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.

Change the following data file types to point their file paths to the data drive:

- Log files
- Memory lists
- Calibrations
- Illumination Settings
- Magnification Settings

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



Note: The MetaXpress Software protocol saves to the database by default.



Note: You should make monthly backups of the Data and HTS State files.

To edit the default data paths for a group:

1. In the MetaXpress Software.
2. From the simplified menu and the standard menu, click **Edit > Configure Default Paths** to display the Configure Default Paths dialog.
3. Select the item to modify.

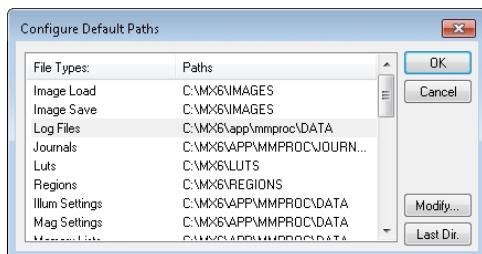


Figure 6-4: Configure Default Paths Dialog

4. Click **Modify** to display the Browse for Folder dialog.
 - a. Select the folder to use as the default path or click **New** to create a new folder.
 - b. Click **OK** in the Browse for Folder dialog.
5. Click **OK** in the Configure Default Paths dialog.



Chapter 7: Expansion Solution Options

You can upgrade the ImageXpress System to include the following expansion solution options:

- [Environmental Control Option, see below](#)
- [Fluidics Option on page 150](#)
- [Transmitted Light Option on page 167](#)
- [Water Immersion Option on page 184](#)

Contact your Molecular Devices representative to discuss adding expansion solution options to your system.

Environmental Control Option

The ImageXpress Environmental Control option is designed to maintain an environment for living cells to allow multi-day, live-cell, time lapse imaging. Temperature, carbon dioxide, and humidity can all be maintained within the sample plate so that cells can be kept alive for many days, growing at a rate comparable to that expected in a standard cell culture incubator. In addition to offering kinetic and time lapse imaging capabilities, the environmental enclosure can accommodate a single-channel, fluidics robot for delivering compounds during experimentation.

The ImageXpress Environmental Control option can be installed together with the ImageXpress Fluidics option, the ImageXpress Transmitted Light option, or the ImageXpress Water Immersion option.

This section includes the following topics:

- [Environmental Control Hardware, see below](#)
- [Setting Up Environmental Control on page 132](#)
- [Environmental Control Software on page 140](#)
- [Performing Time Lapse Experiments on page 141](#)
- [Cleaning Environmental Control Components on page 146](#)

Environmental Control Hardware

The ImageXpress Environmental Control option consists of a sealing ring on top of the sample plate and a top door above the plate that together forms a small, sealed volume. Humidified carbon dioxide is sourced into this small volume to form the required environment above the plate. Temperature is controlled within the upper half of the instrument.

The ImageXpress Environmental Control option consists of the following hardware subsystems:

- **Temperature control** within the upper half of the base instrument. Warm air is provided from the ImageXpress Systems Power and Options Controller through an air hose. Feedback from temperature sensors installed near the plate maintains the temperature. The temperature inside the microplate chamber can be maintained at 8°C above ambient to 40°C.
- **Carbon dioxide** is provided from a customer-supplied tank of pre-mixed 5% CO₂ and 95% air. The tank regulator must be set between 15 PSI and 20 PSI. The ImageXpress Systems Power and Options Controller controls the flow to the space above the plate, maintained by the live-cell sealing ring. If a plate is ejected and loaded, the system does a purge cycle automatically.
- **Humidity** is passively provided by bubbling the carbon dioxide through a water reservoir, minimizing evaporation from the sample plate over the duration of a time lapse experiment.

Items Included in the Installation

The following hardware components are included in an ImageXpress Environmental Control option installation:

- **ImageXpress Systems Power and Options Controller.** See [Figure 7-1](#).
- **Warm air hose and carbon dioxide tubing.** See [Figure 7-2](#) and [Figure 7-3](#).
- **Temperature sensors.** To ensure accurate readings, the sensors are located near the sample plate.
- **Water reservoir.** See [Figure 7-4](#).
- **Live-cell sealing ring.** Compatible with 96-well and 384-well standard height plates. The standard height for these plates is 14.35 mm ± 0.25 mm (0.5650 inches ± 0.0098 inches). See [Figure 7-5](#).



Figure 7-1: ImageXpress Systems Power and Options Controller for the ImageXpress Micro Confocal System



Note: The Compound Plate Temperature setting is available when the Fluidics option is installed.

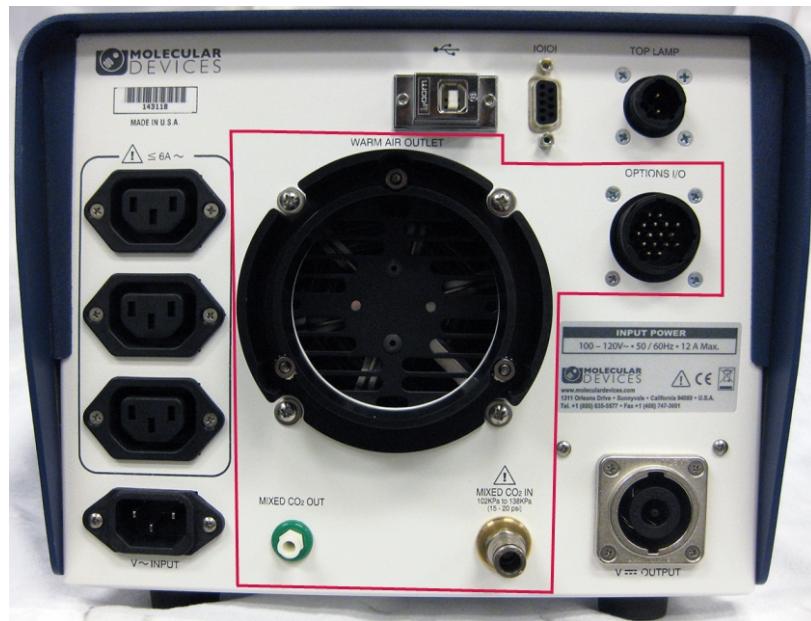


Figure 7-2: Environment Control Connections on the Back of the ImageXpress Systems Power and Options Controller



Figure 7-3: Environment Control Warm Air Hose and Carbon Dioxide Tube on the Back of the ImageXpress Micro Confocal System



Figure 7-4: Environment Control Water Reservoir

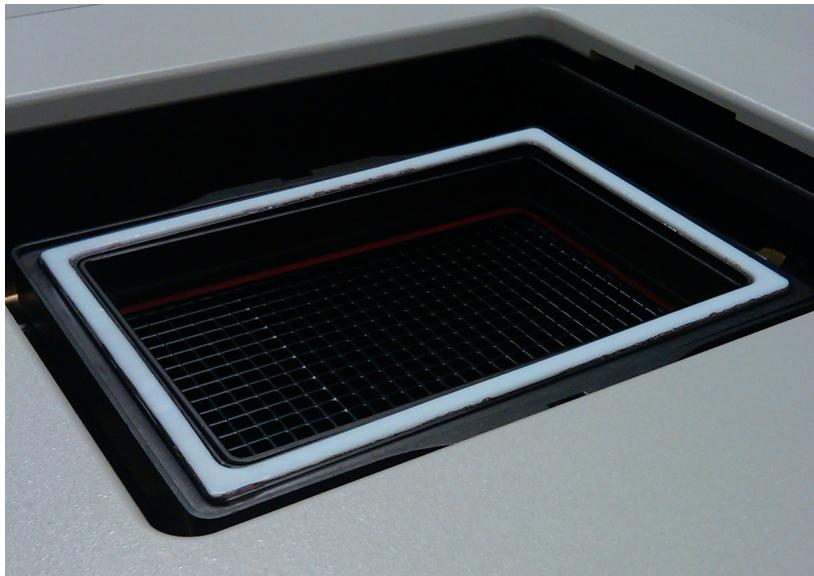


Figure 7-5: Environment Control Live-cell Sealing Ring

Items to be Provided by the Customer

The customer must provide the following items for the ImageXpress Environmental Control option installation:

- A tank of pre-mixed 5% CO₂ and 95% air.
- The regulator, fittings, and tubing required to deliver 15 to 20 PSI carbon dioxide from the tank to the ImageXpress Systems Power and Options Controller. For more information, refer to the *ImageXpress Micro Confocal Pre-Installation Guide*.
- Microplates with standard ANSI height. Most 96-well and 384-well plates are standard height. The standard height for these plates is: 14.35 mm ± 0.25 mm (0.5650 inches ± 0.0098 inches).
- Deionized water to maintain humidity.

Setting Up Environmental Control

A Molecular Devices Field Service Engineer installs the ImageXpress Environmental Control option. After installation, environmental control can be set up for experiments.

Setting the Temperature

The temperature controller is calibrated before the instrument ships from the factory. Use the external temperature controller to set the temperature that you want the environmental enclosure to maintain.

To set the temperature:

1. On the front of the ImageXpress Systems Power and Options Controller, view the current sample plate temperature.



Figure 7-6: Sample Plate Temperature Control Interface

2. To view the temperature set point, press ★.
3. To increase the temperature set point, press ★ and ▲ .
4. To decrease the temperature set point, press ★ and ▼ .

After you release ★, the current temperature in the chamber is displayed.



Note: For the ImageXpress Micro Confocal System, you can set the temperature with the Instrument power switch in the On position. To regulate the temperature in the chamber, the Sample Heater power switch must be also in the On position.

Setting Up the Water Reservoir

The purpose of the water reservoir is to add humidity to the air flow supplied to the environmental enclosure.

Use the following procedure to fill the water reservoir outside of the instrument. You can fill the water reservoir inside of the instrument. See [Filling the Water Reservoir Inside the Instrument on page 135](#).

Requirements:

- Phillips screwdriver
- 250 mL Deionized Water (preferably sterilized)

To fill the water reservoir outside the instrument:

1. In the MetaXpress Software:
From the simplified menu, click **Screening >Acquisition Setup > Eject Plate** to open the top door of the instrument.
From the standard menu, click **Screening > Plate Acquisition Setup > Eject Plate** to open the top door of the instrument.
2. Exit the MetaXpress Software and turn off the instrument at the main power switch, which is located on the external power supply.

3. Remove the top access panel surrounding the top door and open the upper hinged side door for more access.

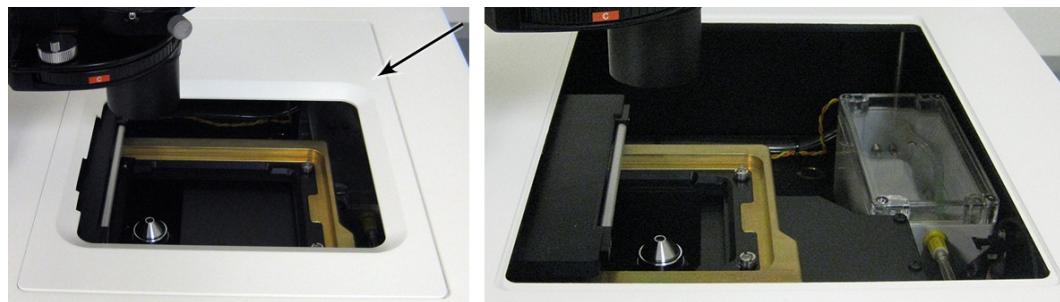


Figure 7-7: Top Access Panel Around the Top Door On and Off

4. Locate the water reservoir through the top opening.

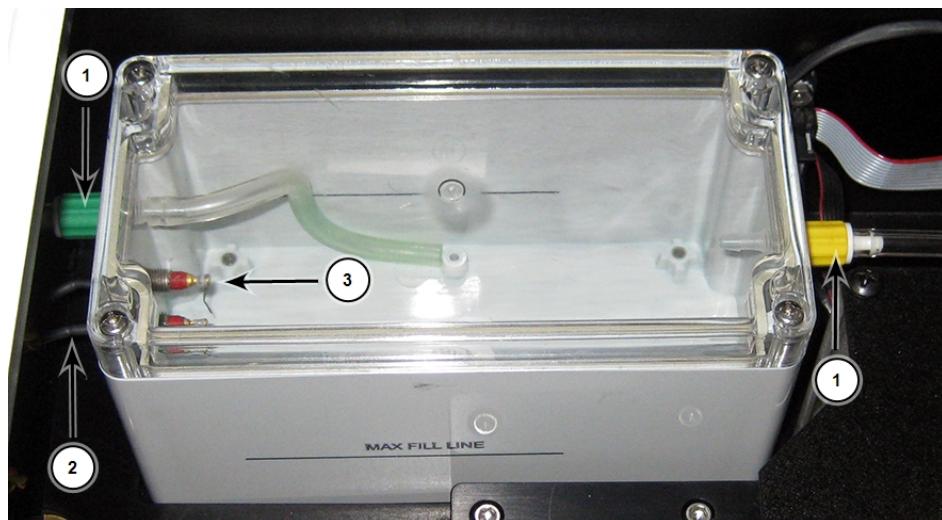


Table 7-1: Environment Control Water Reservoir

Item	Description
1	Luer Locks
2	Water Sensors Wires
3	Water Sensor

5. Disconnect the CO₂/air tubing from the luer locks on both side of the water reservoir.
6. Unplug the sensors from the green luer lock side of the water reservoir and remove the water reservoir from the instrument.
7. Use a Phillips screwdriver to loosen the four screws securing the reservoir lid.
8. Remove the lid.
9. Fill the reservoir with deionized water to the **Max Fill Line** (250 mL).
10. Replace the lid, tighten the screws, and then place the reservoir back in the instrument.
11. Reconnect the wires to the sensors on the green luer lock side. The color of the sensor wire is irrelevant when connecting to the sensor socket. Either color of sensor wire can connect to either sensor socket.

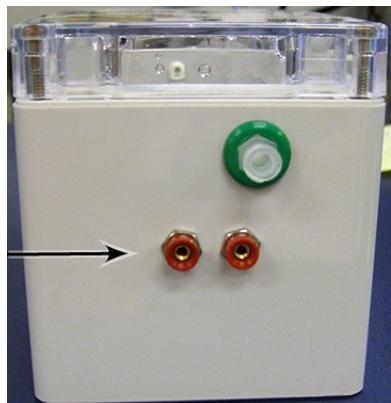


Figure 7-8: Water Level Sensor Wire Sockets

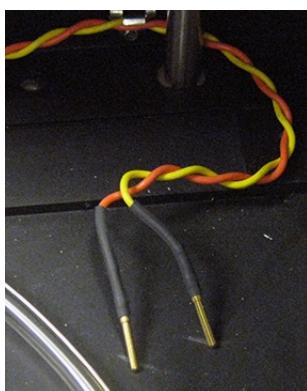


Figure 7-9: Water Level Sensor Wires Inside the Instrument

12. Reconnect the CO₂/air tubing to the luer locks on both sides of the water reservoir.
13. Replace the top access panel surrounding the top door and close the upper hinged side door. See [Figure 7-7](#).
14. Start the instrument.
15. In the MetaXpress Software:
From the simplified menu, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.
From the standard menu, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

Filling the Water Reservoir Inside the Instrument

Requirements:

- Syringe
- 250 mL Deionized Water (preferably sterilized)

To fill the water reservoir inside the instrument:

1. In the MetaXpress Software:
From the simplified menu, click **Screening > Acquisition Setup > Eject Plate** to open the top door of the instrument.
From the standard menu, click **Screening > Plate Acquisition Setup > Eject Plate** to open the top door of the instrument.

2. Exit the MetaXpress Software and turn off the instrument at the main power switch, which is located on the external power supply.
3. Remove the top access panel surrounding the top door and open the upper hinged side door for more access.

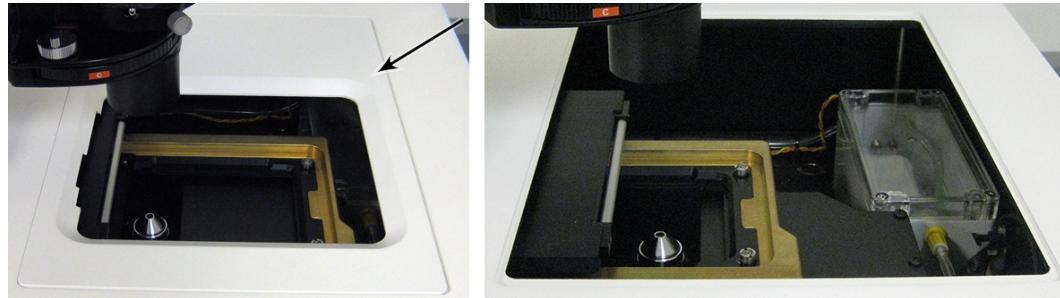


Figure 7-10: Access Panel Around the Top Door On and Off

4. Locate the water reservoir through the top opening.



Figure 7-11: ImageXpress Environmental Control Option Water Reservoir

5. Open the upper hinged side door for more access.
6. Disconnect the CO₂/air tubing from the green luer lock on the side of the water reservoir.
7. Insert a syringe filled with deionized water through the green luer lock.
8. Dispense the deionized water from the syringe into the water reservoir until the water reaches the **Max Fill Line** (250 mL).
9. Remove the syringe from the green luer lock.
10. Reconnect the CO₂/air tubing to the green luer lock.
11. Replace the top access panel surrounding the top door and close the upper hinged side door. See [Figure 7-10](#).
12. Start the instrument.
13. In the MetaXpress Software:
From the simplified menu, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.
From the standard menu, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

Setting Up the Carbon Dioxide Tank

A pre-mixed CO₂ tank with a regulator must be set up and connected to the CO₂ inlet on the Options Controller. The CO₂ Options Controller then controls the flow rate of CO₂ delivered to the water reservoir within the environmental enclosure.

Before you begin, make sure the water reservoir is set up. See [Setting Up the Water Reservoir on page 133](#).

To set up the CO₂:

1. Connect the regulator to the CO₂ tank.
2. Connect the tubing from the pre-mixed CO₂ tank regulator to the ImageXpress Systems Power and Options Controller.
3. Verify that the tubing from the ImageXpress Systems Power and Options Controller to the instrument is connected.



Figure 7-12: CO₂ Tube Connection on the Rear of the Instrument

4. Turn on the CO₂ regulator to approximately 15 PSI to 20 PSI.
5. From the top of the instrument, lift the top access panel surrounding the top door and remove it. Keep the top door of the instrument closed.



Figure 7-13: Top Access Panel Around the Top Door On and Off

6. Verify that there is a steady flow of bubbles in the water reservoir.
7. Replace the top access panel surrounding the top door.
8. Check the environmental control settings to verify that the CO₂ pressure is OK. See [Environmental Control Software on page 140](#).

After Setting Up the Carbon Dioxide

Load an unlidded plate with the live-cell sealing ring on top. If there are concerns about contamination, a breathable seal can be used over the top of the plate.



Note: Before you do imaging experiments, wait for the system and plate to reach equilibrium. Allow at least two hours for the system and 30 minutes for the plate. Because focus settings and offsets change with temperature, you might need to optimize them after the system and plate have reached equilibrium.

Loading the Sample Plate

You must use the live-cell sealing ring to maintain the CO₂ flow to the sample plate. The sealing ring helps to contain the inputted air directly over the cells and maintains the proper CO₂ and temperature levels.



WARNING! BIOHAZARD. Wear gloves when handling sample plates.



Note: To ensure that it is at the proper temperature, before you load the sample plate, make sure that the live-cell sealing ring is in the system or in the incubator.

To load the sample plate:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup > Eject Plate** to open the top door of the instrument.

From the standard menu, click **Screening > Plate Acquisition Setup > Eject Plate** to open the top door of the instrument.

2. Insert the sample plate into the stage and then remove the lid from the plate. If there are concerns about contamination, a breathable seal can be used over the top of the plate.

3. Place the live-cell sealing ring directly on top of the plate, making sure that it fits securely onto the plate.

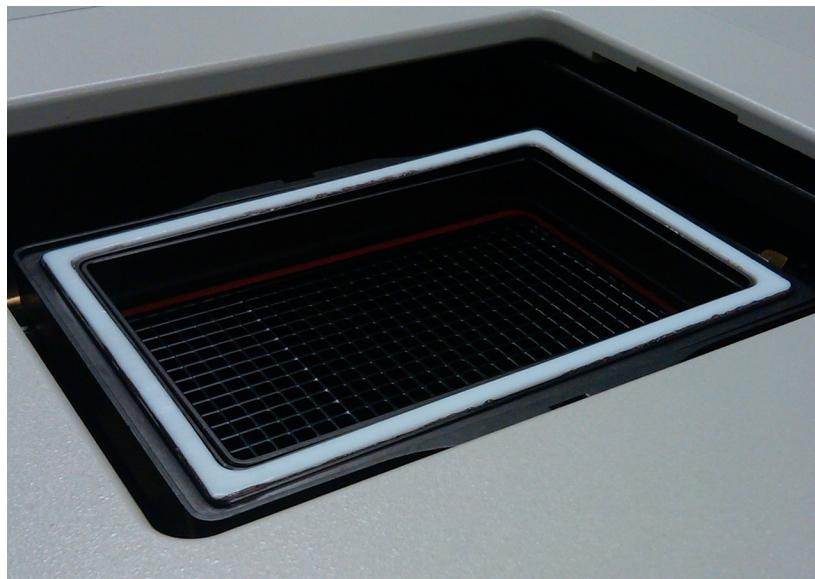


Figure 7-14: Environment Control Live-cell Sealing Ring

4. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.

From the standard menu, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.



Note: Temperature fluctuations in the plate and its surroundings cause the plate and its cells to shift in X, Y, and Z direction. To minimize these temperature fluctuations, take the following precautions.

Before You Run an Experiment

- Check the water reservoir level. Refill as needed.
- Check the CO₂ supply. Refill as needed.
- Allow the instrument to warm up for at least two hours. The current temperature in the chamber is displayed on the front of the controller.
- Make sure the plate sealing ring is at 37°C before use, either by keeping it inside the instrument or inside an incubator. Incorrect sealing-ring temperature can cause temperature fluctuation.
- Wait 30 minutes to 1 hour for the plate to reach equilibrium. You can use a journal to set this waiting period.
- If there is interstitial space between wells, pipette deionized water or media into these areas. This helps to increase the thermal mass of the plate and reduces overall evaporation.
- Fill all unused wells with deionized water or buffer.



Note: Due to the changes in Z-height over time, you might need to adjust the plate focus parameters in the MetaXpress Software occasionally. To do so, open the MetaXpress Software, and then click **Screening > Plate Acquisition Setup > Plate tab > Edit Plate Bottom Settings**. Increase the adjacent well max variation to accommodate the added Z-height variation introduced by the fluctuation of temperature.

When setting laser autofocus options, make sure that the instrument and plate temperatures are at equilibrium. Room-temperature laser autofocus settings are not ideal when the system is used at higher temperatures.

Environmental Control Software

The MetaXpress Software controls the ImageXpress instrument screening and monitors the system's environmental parameters. You can check Environment Control settings and connections in the MetaXpress Software.

To check the Environment Control settings:

1. In the MetaXpress Software:

From the simplified menu, click **Control > ImageXpress > Environment Control** to display the Environment Control dialog.

From the standard menu, click **Devices > Environment Control** to display the Environment Control dialog.

2. View:

- Current Temperature (Celsius).
- Temperature Setpoint
- Carbon Dioxide Pressure (Low or OK)

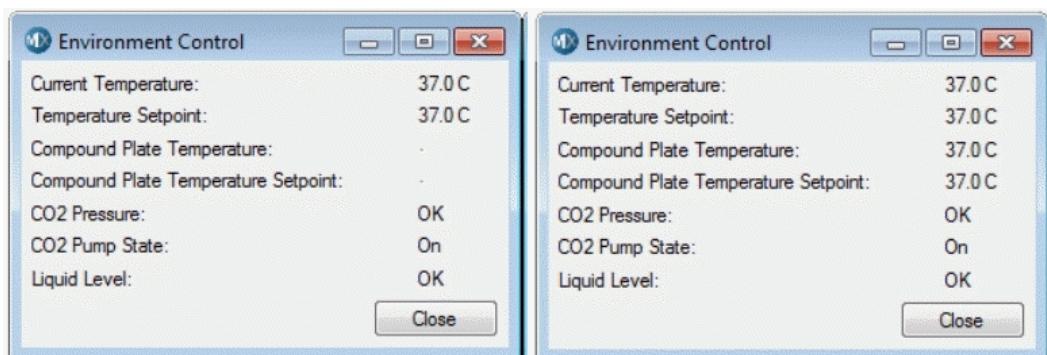


Figure 7-15: Environment Control Dialog Without Fluidics and With Fluidics

When the top door of the ImageXpress instrument is opened, the MetaXpress Software triggers the environment control to increase the flow rate of the carbon dioxide to flood the chamber with humidified and carbon dioxide-controlled air. When a plate is loaded and the top door of the ImageXpress instrument is closed, the system transiently purges the carbon dioxide at a higher flow rate to flood the chamber with humidified and carbon dioxide-controlled air.

The MetaXpress Software does not control the environmental control parameters, such as the level of CO₂ or temperature. These parameters are controlled directly through their hardware devices, such as the regulator on the carbon dioxide tank and the temperature controller on the ImageXpress Systems Power and Options Controller. See [Figure 7-1](#).

Performing Time Lapse Experiments

The ImageXpress Environmental Control option incubates live cells, enabling imaging experiments to be performed over hours or days. Wells can be imaged repeatedly at fixed time intervals, and movies can be constructed from a series of images.



Note: In time lapse acquisitions, the time starts when the first well is acquired. If the acquisition time exceeds the time specified for the time lapse experiment, the system proceeds as fast as possible. As a result, the time between time points might not match the time specified, but timestamps on images are accurate.



Note: In time lapse acquisitions, the image save option for the Z option is to save the 2D projection images.

To set up a time lapse experiment:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.

From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.

2. On the Configure tab, select the **Acquisition** tab.

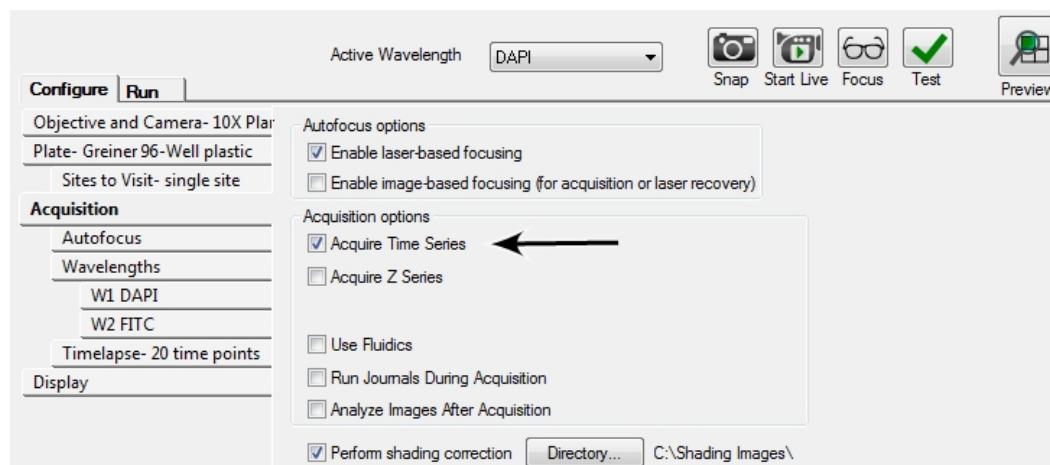


Figure 7-16: Plate Acquisition Setup Dialog, Acquisition Tab

3. Select the **Acquire Time Series** check box to add a Timelapse tab to the dialog.

4. Select the **Timelapse** tab.

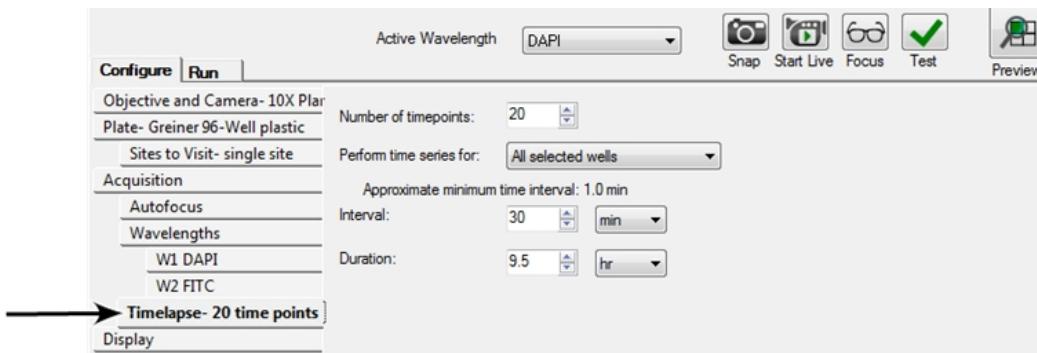


Figure 7-17: Plate Acquisition Setup Dialog, Timelapse Tab

5. In the **Number of time points** field, enter the number of time points. Fluidic events can be associated with the time points. See [Performing Fluidics Experiments on page 160](#).
6. Click the **Perform Time Series For** drop-down:
 - Select **All Selected Wells** for long time lapse experiments. The system images all wells at the first time point, then all wells at the second time point, and so on.
 - Select **One Well Then the Next** for fast-kinetic experiments. The system images all time points on well 1, then moves to well 2 and images all time points, and so on.
 - Select **One Row Then the Next** or **One Column Then the Next** for experiments that use manual pipetting.
7. If applicable, select the **Autofocus** tab.

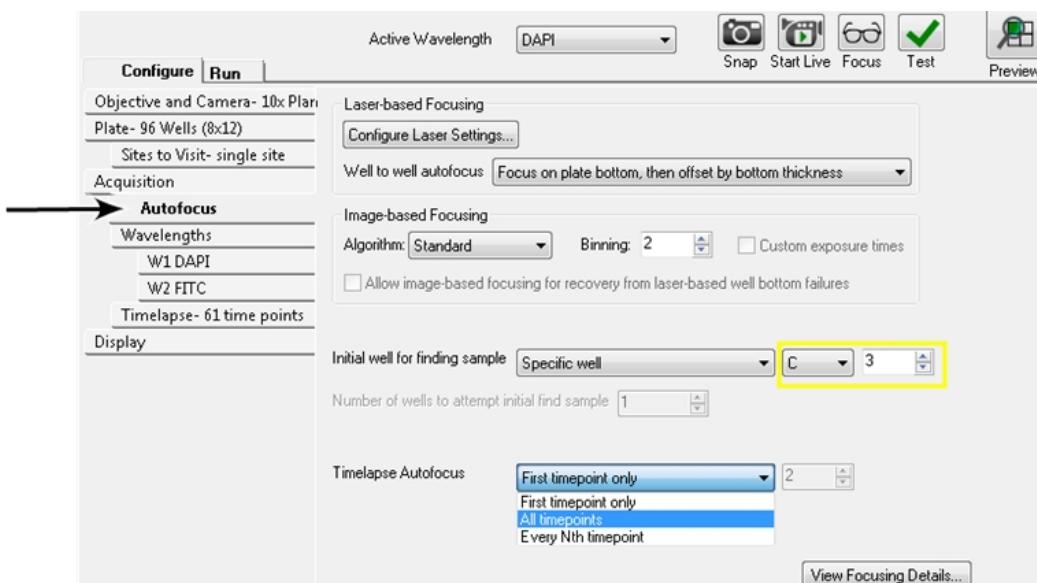


Figure 7-18: Plate Acquisition Setup Dialog, Autofocus Tab

8. Click the **Timelapse Autofocus** drop-down:
 - Select **First Timepoint Only** for fast-kinetic experiments.
 - Select **All Timepoints** for long time lapse experiments.
 - Select **Every Nth Timepoint** to autofocus regularly during a time lapse experiment.

9. If applicable, select each **W# Wavelength** tabs.

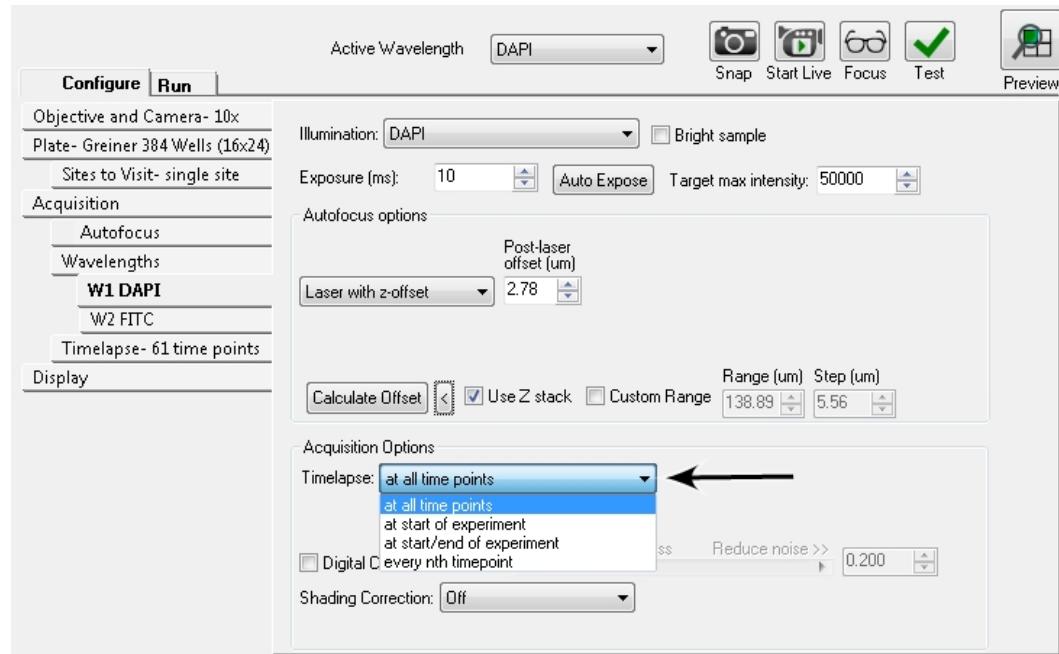


Figure 7-19: Plate Acquisition Setup Dialog, Wavelength 1 Tab

10. Click the **Timelapse** drop-down:

- Select **At All Time Points** to acquire all time points for the wavelength.
- Select **At Start of Experiment** to acquire only the first time point for the wavelength.
- Select **At Start/End of Experiment** to acquire only the first and last time points for the wavelength.
- Select **Every Nth Timepoint** to acquire time lapse images only for every nth time point for the wavelength.

If you do not image a wavelength at every time point, the most recent image acquired for that wavelength is saved in the database for that time point.

Reviewing Time Lapse Data

As with other ImageXpress System data, time lapse data displays in the Review Plate Data dialog.

To review the time lapse data:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Review Plate** to display the Review Plate Data dialog.

From the standard menu, click **Screening > Review Plate Data** to display the Review Plate Data dialog.

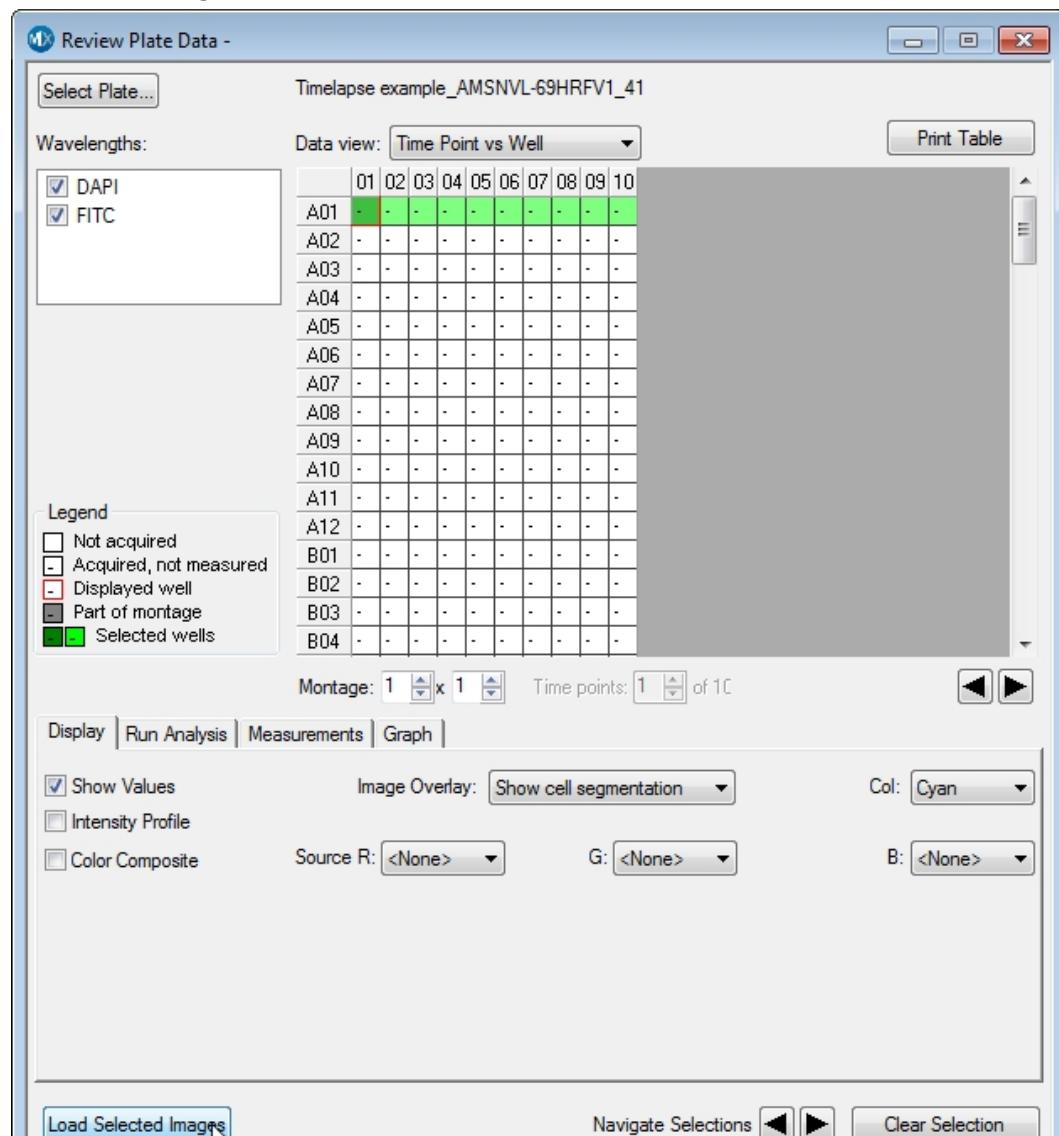


Figure 7-20: Review Plate Data Dialog

2. Click **Select Plate** to display the Select Plate for Review dialog.
3. Select a plate and click **Select**.

4. In the Data View list, click **Time vs Well** to displays thumbnails of each time point in the montage.
5. To create a stack to view as a movie, right-click on one well to select it. The well changes color.
6. Click **Clear Selection** at the bottom of the dialog to clear the selection, if needed.
7. Click **Load Selected Images**.
8. In the image window, use the navigation buttons above the image pane to view each time point as a plane in the stack.

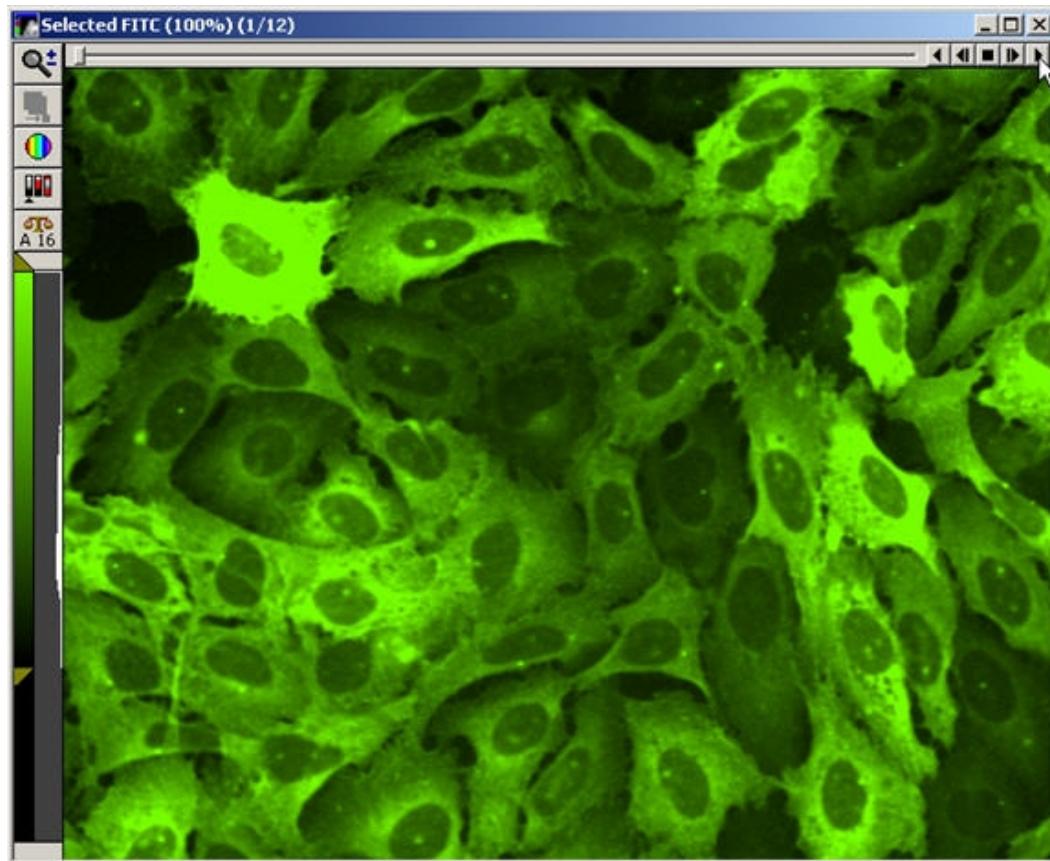


Figure 7-21: Stack of Images with Navigation Buttons Above the Image Pane



Note: In a time lapse experiment, the time starts when the first well is acquired. If the acquisition time exceeds the time you enter for the time lapse experiment, the system proceeds as fast as possible. As a result, the time between time points might not match the time you enter, but the timestamps on images are accurate.

Making Time Lapse Movies

To make a movie:

1. Before you begin, load time lapse images and create a stack.
2. Select the image window of the stack on the MetaXpress Software desktop.
3. In the MetaXpress Software:

From the simplified menu, click **Edit > Stack > Make Movie** to display the Make Movie dialog.

From the standard menu, click **Stack > Make Movie** to display the Make Movie dialog.

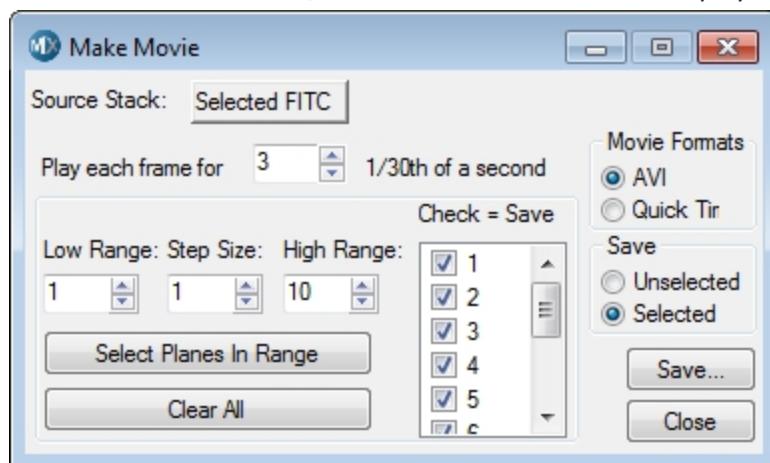


Figure 7-22: Make Movie Dialog

4. Select the options. For information about the options, see the application help.
5. Click **Save**.

Cleaning Environmental Control Components

It is important to regularly clean the environmental enclosure, including specific environmental components. Components can come in contact with biological, chemical, and toxic agents. Therefore, all cleaning procedures should be handled with care. Molecular Devices recommends that you always wear powder-free gloves when you access the internal components of the enclosure. See [Maintenance on page 193](#).



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

Cleaning Carbon Dioxide Tubing

The tubing within the environmental enclosure should be cleaned only if, during a visual inspection, moisture has collected in the tubing or if contamination is seen in the tubing.

There are two tubing sections associated with CO₂ delivery. One runs from the Options Controller located outside the enclosure to the water reservoir within the enclosure, and the other one exits the water reservoir and delivers the CO₂ to the stage (plate) area.



Figure 7-23: CO₂ Tubing Connections on the Water Reservoir

Item	Description
1	Input tube connection
2	Output tube connection



CAUTION! Do not let the second stretch of tubing that leaves the water reservoir to the stage area come into contact with the water in the reservoir.

When you replace or clean tubing, make sure that the exit tubing from the reservoir is significantly above the water surface. If at any time, you can see moisture within the tubing, disconnect both ends and use compressed air to dry the inside of the tubing. Make sure to reconnect all tubing before you run the instrument.

The tubing that runs from the Options Controller to the enclosure has an in-line filter installed near the end closest to the controller. When you reconnect this tubing make sure that the in-line filter is in the correct position.

To clean any portion of the tubing:

1. Take note of how the tubing is connected, and then disconnect the portion of the tubing that is contaminated and remove it from the environmental enclosure.
2. Use 70% isopropanol to flush the interior of the tubing.
3. Use compressed air to dry the interior of the tubing.
4. Reconnect tubing in the environmental enclosure and ensure connections are tight.
5. Confirm that CO₂ is delivered to the water reservoir by visually confirming that bubbles move through the reservoir. See [Setting Up the Carbon Dioxide Tank on page 137](#).

Cleaning the Water Reservoir

Clean the water reservoir on an as needed basis when you see dirt or mold in the reservoir.

Requirements:

- Phillips screwdriver
- 70% Ethanol
- 250 mL Deionized Water (preferably sterilized)

To clean the water reservoir:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup > Eject Plate** to open the top door of the instrument.

From the standard menu, click **Screening > Plate Acquisition and Control > Eject Plate** to open the top door of the instrument.

2. Exit the MetaXpress Software and turn off the instrument at the main power switch, which is located on the external power supply.
3. Remove the top access panel surrounding the top door and open the upper hinged side door for more access.

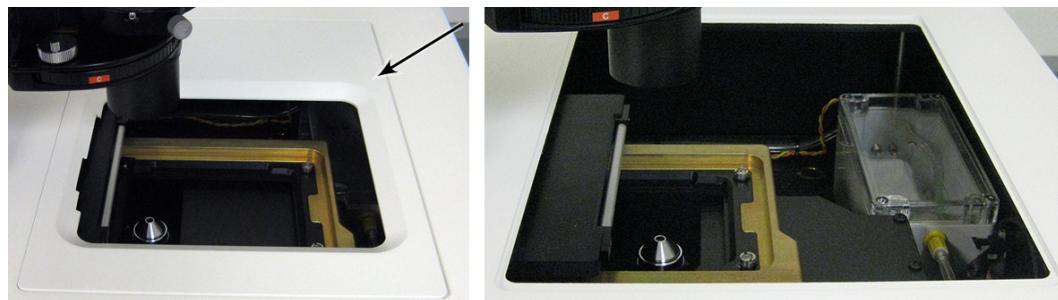


Figure 7-24: Top Access Panel Around the Top Door On and Off

4. Disconnect the CO₂/air tubing from the luer locks on both sides of the water reservoir.
5. Unplug the sensors from the green luer lock side of the water reservoir and remove the water reservoir from the instrument.
6. Use a Phillips screwdriver to loosen the four screws securing the reservoir lid.
7. Remove the lid.
8. Dispose of the water, and then rinse the reservoir with 70% ethanol.
9. Rinse the reservoir with deionized water to remove the ethanol.
10. Fill the reservoir with deionized water to the **Max Fill Line** (250 mL).
11. Replace the lid, tighten the screws, and then place the reservoir back in the instrument.
12. Reconnect the wires to the sensors on the green luer lock side.



Note: The color of the sensor wire is irrelevant when connecting to the sensor socket. Either color of sensor wire can connect to either sensor socket.

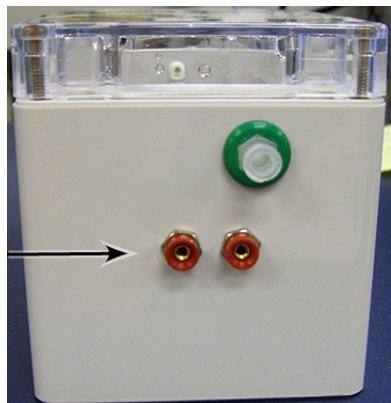


Figure 7-25: Water Level Sensor Wire Sockets

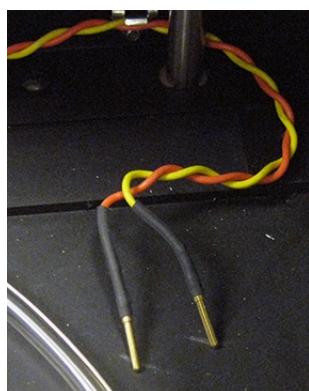


Figure 7-26: Water Level Sensor Wires Inside the Instrument

13. Reconnect the CO₂/air tubing to the luer locks on both sides of the water reservoir.
14. Replace the top access panel surrounding the top door and close the upper hinged side door. See [Figure 7-24](#).
15. Start the instrument.
16. In the MetaXpress Software:
From the simplified menu, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.
From the standard menu, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

Fluidics Option

The ImageXpress Fluidics option consists of a single-channel, fluidics robot. This robot picks up tips from a tip box and draws fluid from and delivers fluid to a given well on the compound and sample plates. For the sample plate, the solenoid-operated plate shutter opens briefly, allowing the robot access to a given well without compromising the environmental control.

The robot is capable of dispensing volumes between 3 μL and 200 μL with an accuracy of $\pm 5\%$ or $\pm 1 \mu\text{L}$, whichever is greater.

The ImageXpress Fluidics option can be installed together with the ImageXpress Environmental Control option.

This section includes the following topics:

- [Fluidics Hardware, see below](#)
- [Setting Up Fluidics Hardware on page 152](#)
- [Configuring Fluidics Software \(Stations and Properties\) on page 153](#)
- [Performing Fluidics Experiments on page 160](#)
- [Preventing Evaporation from Compound Plates on page 165](#)
- [Cleaning Fluidics Components on page 166](#)

Fluidics Hardware

The ImageXpress Fluidics option consists of the following hardware components. See [Figure 7-27](#) and [Figure 7-28](#).

- **Single-channel pipettor.** Used to transfer fluid between two compound/media plates and the sample plate.
- **Shutter.** Allows the system to maintain the environment when not actively pipetting.
- **Plate heaters.** Used to heat the compound plates. These heat a little higher than the displayed temperature, which represents the temperature of the liquid in the well.
- **Tip adapters and tip rack positions.** Compatible with the FLIPR® Tetra High Throughput Cellular Screening System 96 tips (Part 9000-0761, 50 racks/case) or 384 tips (Part 9000-0763, 50 racks/case).
- **Tip stripper.** Used for ejecting tips.
- **Waste bin.** Used to contain ejected tips and discarded liquid.
- **Doors with safety interlocks.** Prevents doors from being opened during operation.



Figure 7-27: ImageXpress Micro Confocal System with ImageXpress Fluidics option

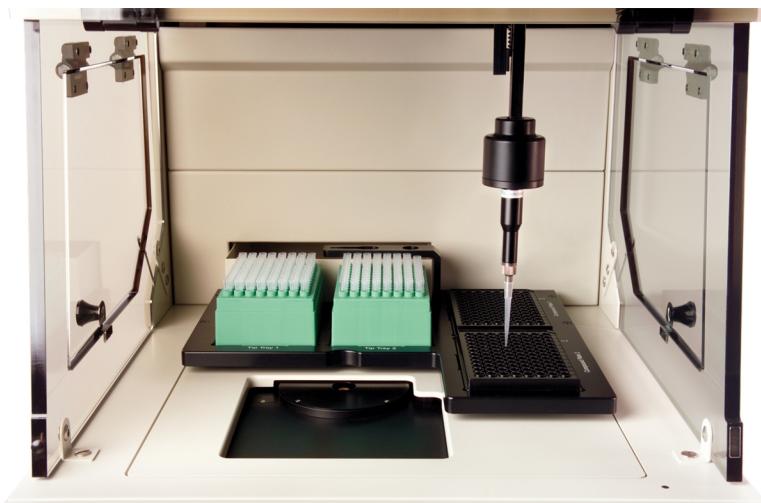


Figure 7-28: ImageXpress Fluidics option close up

Items to Be Provided by the Customer

The customer must provide the following items (in addition to the Environment Control option items):

- ANSI standard compound plates.
- FLIPR Tetra High Throughput Cellular Screening System 96 tips (Part 9000-0761, 50 racks/case) or 384 tips (Part 9000-0763, 50 racks/case). Tips can be ordered from Molecular Devices.
- Absorbent pad for liquid waste disposal.

Tips

The ImageXpress Fluidics option is compatible with the FLIPR Tetra High Throughput Cellular Screening System 96 or 384 tips.

After compounds are dispensed, tips are disposed into the waste disposal tray located at the back of the environmental enclosure.

Plates

The tip and compound plate region inside the environmental enclosure can accommodate up to two compound plates. You should use standard 96-well or 384-well plates. U-bottom and V-bottom plates are beneficial when you withdraw and dispense very small volumes of compound.

Setting Up Fluidics Hardware

A Molecular Devices Field Service Engineer installs the ImageXpress Fluidics option. After installation, fluidics can be set up for experiments.

To set up the fluidics hardware:

1. Load the tip racks. For proper fit and alignment, insert the right side first, and then press the left side down into place.
2. Change the tip adapter as appropriate. The tip adapter should be screwed on firmly but not over-tightened. If the tip adapter is not properly attached, it might not match the fluidics robot calibration.
3. Load the compound plates. For proper fit and alignment, insert the right side first, and then press the left side down into place.
4. Set up environmental control and allow the compound-plate temperature to reach equilibrium. See [Setting Up Environmental Control on page 132](#).
5. If required, empty the waste bin. If your experiment involves disposal of liquid, place an absorbent pad in the waste bin. See [Cleaning the Waste Disposal Box and the Plate Sealing Ring on page 166](#).
6. Load an unlidded plate with the live-cell sealing ring on the top. See [Loading the Sample Plate on page 138](#).

If you change tips (that is, from a 96-well to a 384-well tip or from a 384-well to a 96-well tip), you need to configure the fluidics as described in the following procedure.

To configure fluidics in MetaXpress Meta Imaging Series Administrator Software:

1. Click **Start > Programs > MetaXpress** and then right-click **Meta Imaging Series Administrator** and select **Run as Administrator** to start the MetaXpress Meta Imaging Series Administrator Software.
2. Click **Configure Hardware** to display the Configure Hardware dialog.
3. Select the correct hardware configuration and click **Configure Devices** to display the User Settings for Hardware Configuration dialog.
4. In the Claimed Devices list, select **ImageXpress Micro Fluidics**, and then click **Settings** to display the ImageXpress Micro Fluidics Settings dialog.

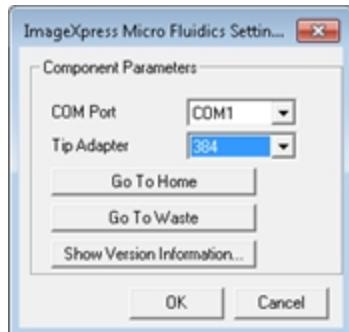


Figure 7-29: ImageXpress Micro Fluidics Settings Dialog

5. If applicable, change the tip adapter.



Note: In rare situations, it is necessary to change the COM port.

Configuring Fluidics Software (Stations and Properties)

To use the ImageXpress Fluidics option, you must configure fluidics stations and properties.

The Configure Fluidics Stations functions are available:

- From the simplified menu, click **Control > ImageXpress > Configure Fluidics Stations**
- From the standard menu, click **Devices > Configure Fluidic Stations**
- In the **Plate Acquisition Setup** dialog, on the **Fluidics** tab, from the **Configure Stations** option.

This section details the Plate Acquisition Setup > Fluidics > Configure location, but the procedures are the same if you access the functions from the menu.

To configure fluidics software:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.

From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.

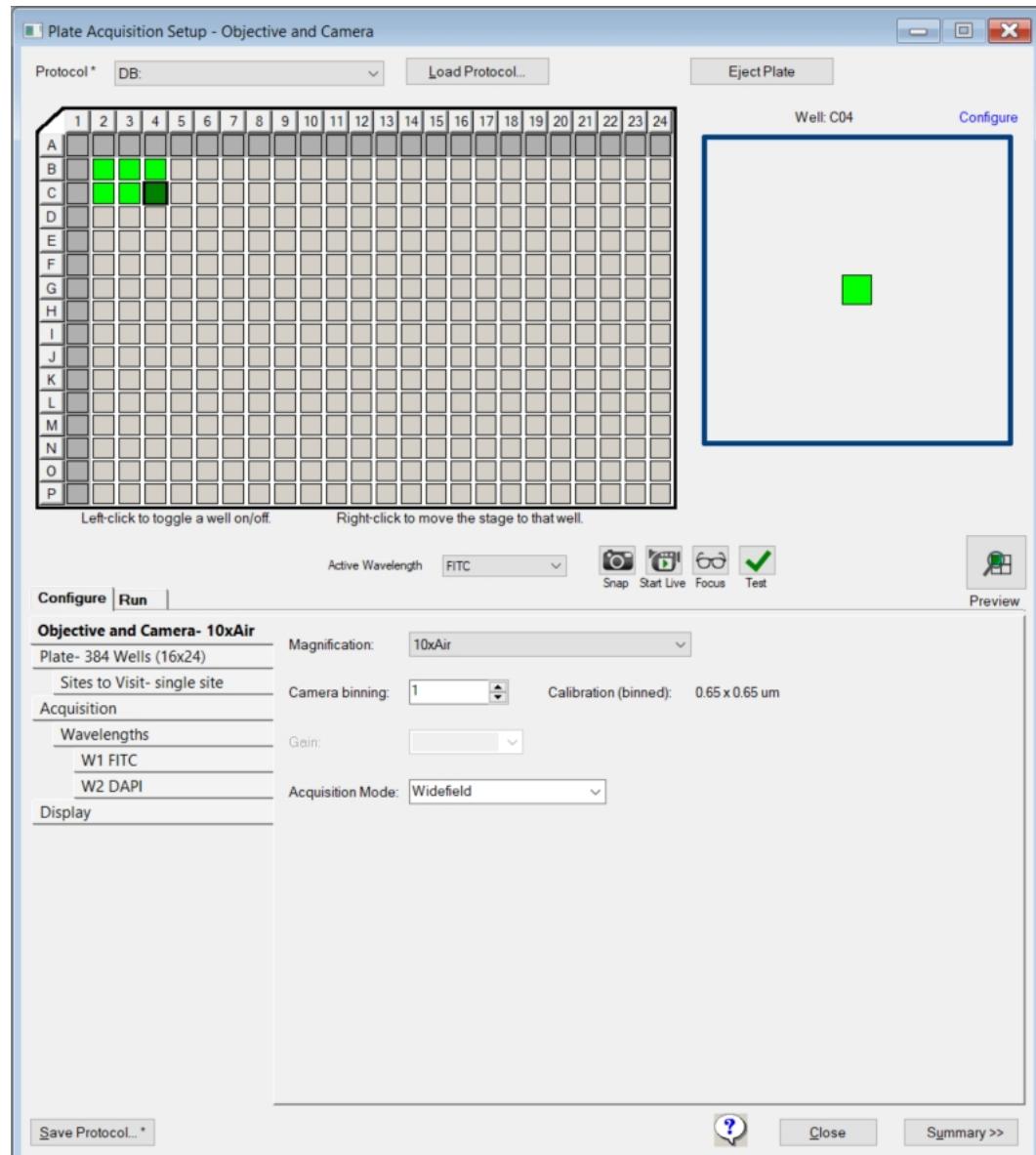


Figure 7-30: Plate Acquisition Setup Dialog

2. On the Configure tab, select the **Acquisition** tab.

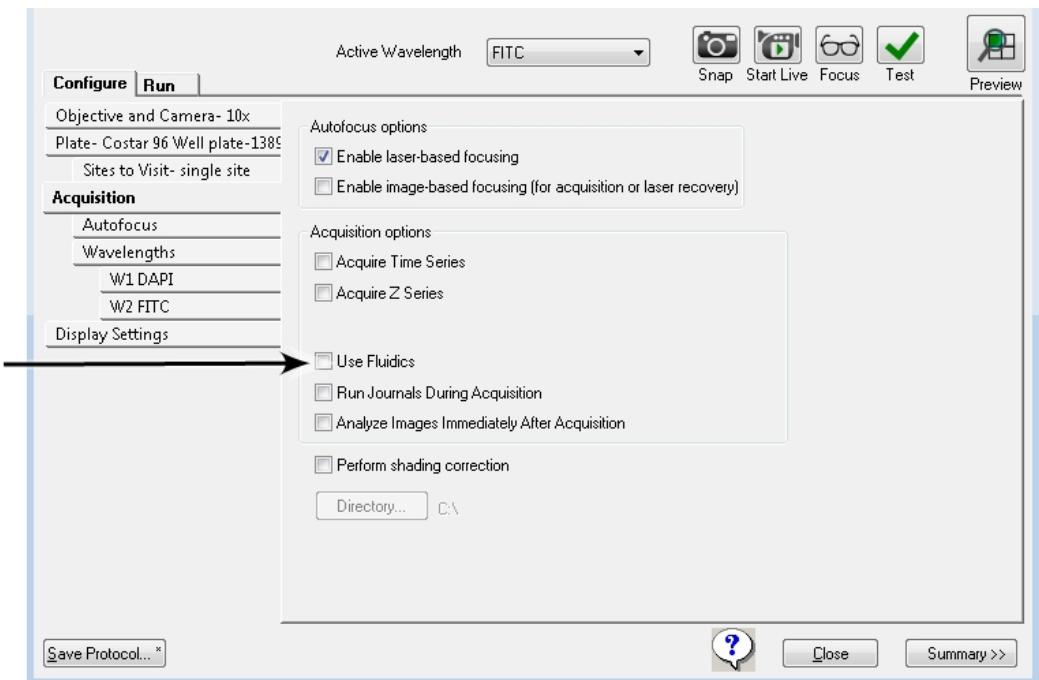


Figure 7-31: Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab

3. Select the **Use Fluidics** check box to add a Fluidics tab to the dialog.
 4. Select the **Fluidics** tab.

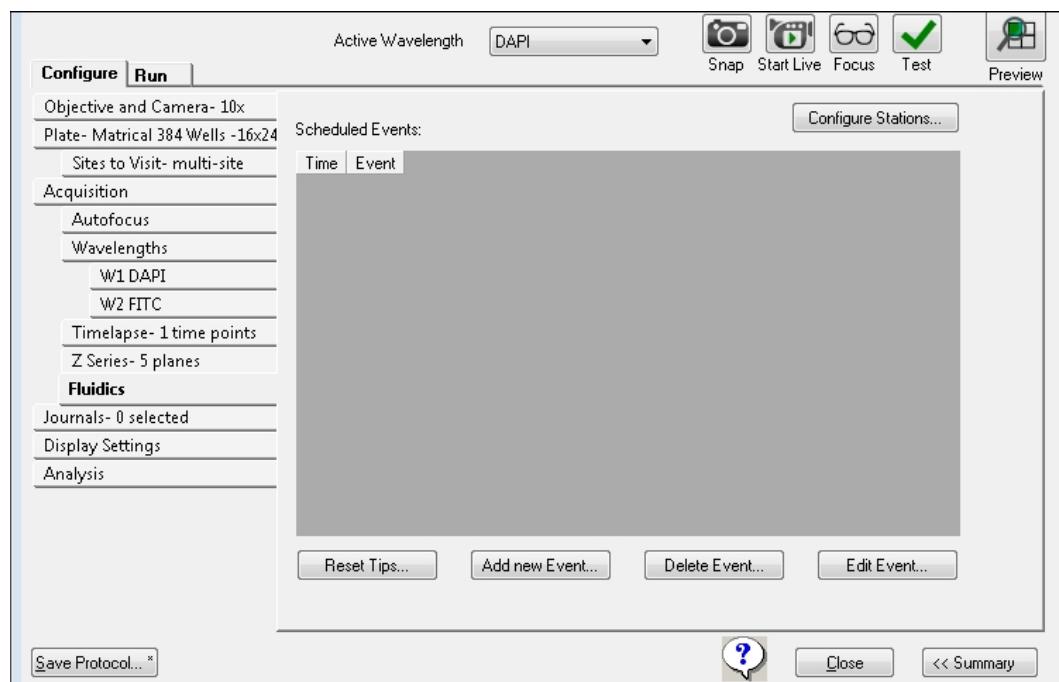


Figure 7-32: Plate Acquisition Setup Dialog, Configure Tab: Fluidics Tab

- Click **Configure Stations** to display the Configure Fluidic Stations dialog.

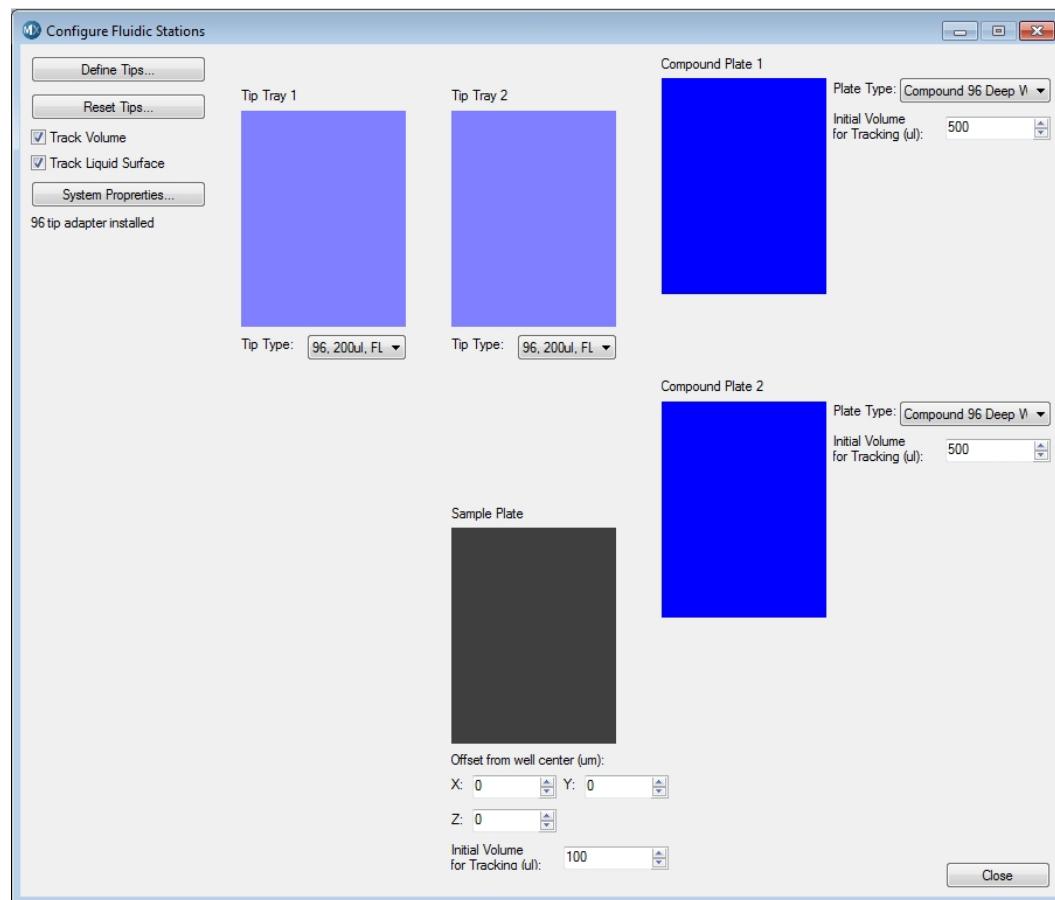


Figure 7-33: Configure Fluidic Stations Dialog

- For each Tip Tray, click the **Tip Type** drop-down and select a tip type.



Note: The system comes pre-configured with settings for the FLIPR Tetra system 96 and 384 tips.

- For each Compound Plate, click the **Plate Type** drop-down and select a plate type.



Note: You can use only standard 96-well or 384-well plates for fluidics.

- If applicable, for the Sample Plate, enter a value for **X**, **Y**, and **Z** offsets from the well center (in µm) for the sample plate.

Enter a positive Z offset to have the pipettor sample higher than normal. Enter a negative Z offset to have the pipettor sample lower than normal.



CAUTION! An incorrect X, Y, or Z offset can cause the pipette tip to crash into the sample plate.

- If applicable, select the **Track Volume** check box and in the **Initial Volume for Tracking** field enter the initial volume for each plate.

10. Do one of the following:

- If you select the **Track Volume** check box, select the **Track Liquid Surface** check box, and then go to Step 11.
- Optionally, select the **Track Volume** check box, select the **Track Liquid Surface** check box, clear the **Track Volume** check box, and then go to Step 11.
- Otherwise, go to Step 13.



Note: The Track Liquid Surface check box is used in conjunction with the Wet Dispense feature. If you select the Track Liquid Surface check box, then wet dispensing is done near the liquid surface.

11. Click **System Properties** to display the Fluidics System Properties dialog.

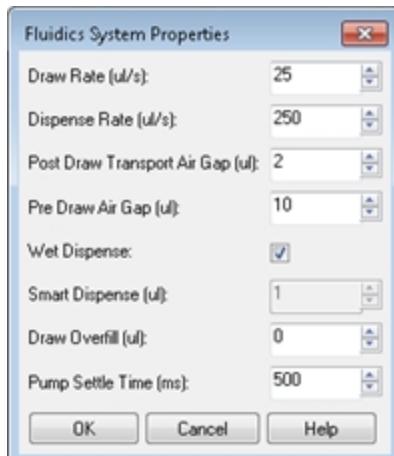


Figure 7-34: Fluidics System Properties Dialog

12. Leave the default values for the fluidics system properties set as-is, or modify any values as needed.

Table 7-2: Fluidic Systems Property Values

Option	Description
Draw Rate ($\mu\text{l}/\text{s}$)	The rate at which the pump draws fluid.
Dispense Rate ($\mu\text{l}/\text{s}$)	The rate at which the pump dispenses fluid.
Post Draw Transport Air Gap (μl)	The volume of air that the pump draws after it draws fluid but before the pump arm moves. This air prevents fluid from dripping during pump arm movement. Select this option for fluids such as DMSO.
Pre-Draw Air Gap (μl)	The volume of air that the pump draws before drawing fluid. This pushes remaining drops of fluid out of the tip when dispensing.
Wet Dispense	Specifies whether the tip should be immersed in the fluid during dispense operations at the imaging location. When enabled, if you select Track Liquid Surface, then dispensing occurs near the liquid surface; otherwise, the dispensing occurs near the bottom of the well.
Smart Dispense (μl)	Enabled when you select Wet Dispense. The volume that the pump dispenses beyond the requested fluid dispense volume. The additional volume is subtracted from the Pre-Draw Air Gap volume. The Smart Dispense volume must be less than or equal to the Pre-Draw Air Gap volume.
Draw Overfill (μl)	The volume of fluid that the pump draws in addition to the requested volume of fluid. You can use Draw Overfill to increase dispense accuracy when drawing from compound plates. Note: The software does not apply Draw Overfill to the sample plate.
Pump Settle Time (ms)	The time delay the software uses to pause movement of the pump arm after a pump draw or dispense operation to overcome hysteresis. For most operations, 500 ms is the default value.

13. Click **OK** to display to the Configure Fluidics Stations dialog.
14. If applicable, click **Define Tips** to display the Define Tips dialog where you define additional tip types, and then click **Save**.



CAUTION! Improper tip definitions can cause the pipette tip to crash into the sample plate. Molecular Devices recommends that you do not make changes to the standard tip definitions.

15. After you complete a dispensing cycle, to indicate that you have reloaded tip racks and plates, click **Reset Tips** to display the Reset Tips/Liquid Levels dialog. Click **Reset** for each tip tray and plate, and then click **Close**.

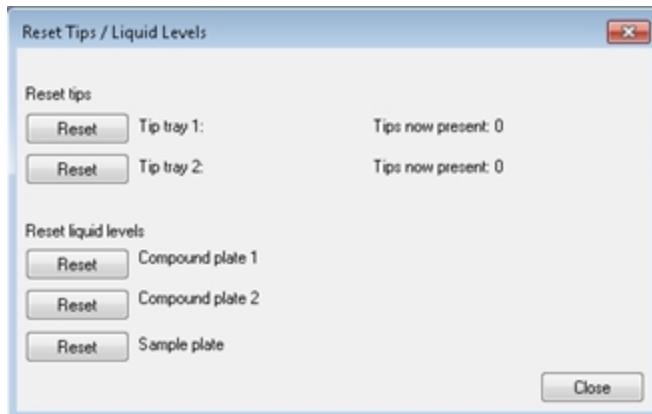


Figure 7-35: Reset Tips/Liquid Levels Dialog

Manual Fluidics Control

Use the fluidics interface to transfer fluid from one well of the compound plate to a corresponding well in the sample plate. If you need to transfer fluid between non-corresponding wells, or if different actions need to be performed for different wells, record these actions in a journal (custom routine) to accomplish this.

To control fluidics manually:

1. In the MetaXpress Software:

From the simplified menu, click **Control > ImageXpress > Fluidic Control** to display the Fluidic Control dialog.

From the standard menu, click **Devices > Fluidic Control** to display the Fluidic Control dialog.

2. Click the **Action** drop-down and select the action, then click the remaining drop-downs to define the steps in the action to do. You can manually pick up and eject tips, draw and dispense fluid, and mix.

For example, to remove 20 μ l from well A01 in the compound plate and add it to the sample plate, specify the following:

- **Action:** Draw
- **Station:** Compound Plate 1
- **Row:** A
- **Column:** 1
- **Volume (μ l):** 20

3. Click **Go** to draw the fluid from the plate.
4. Click the **Action** drop-down and select **Dispense**, and then click **Go** to add the fluid to the plate.

To record the steps in a journal:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Journal > Start Recording**.

From the standard menu, click **Journal > Start Recording**.

2. Do the steps that you want to record. See [To control fluidics manually: on page 159](#).

3. From the simplified menu, click **Control > Journal > Stop Recording** and then save the journal file.

From the standard menu, click **Journal > Stop recording** and then save the journal file.

4. To modify the journal:

From the simplified menu, click **Control > Journal > Edit Journal** and then select the journal to edit.

From the standard menu, click **Journal > Edit Journal** and then select the journal to edit.

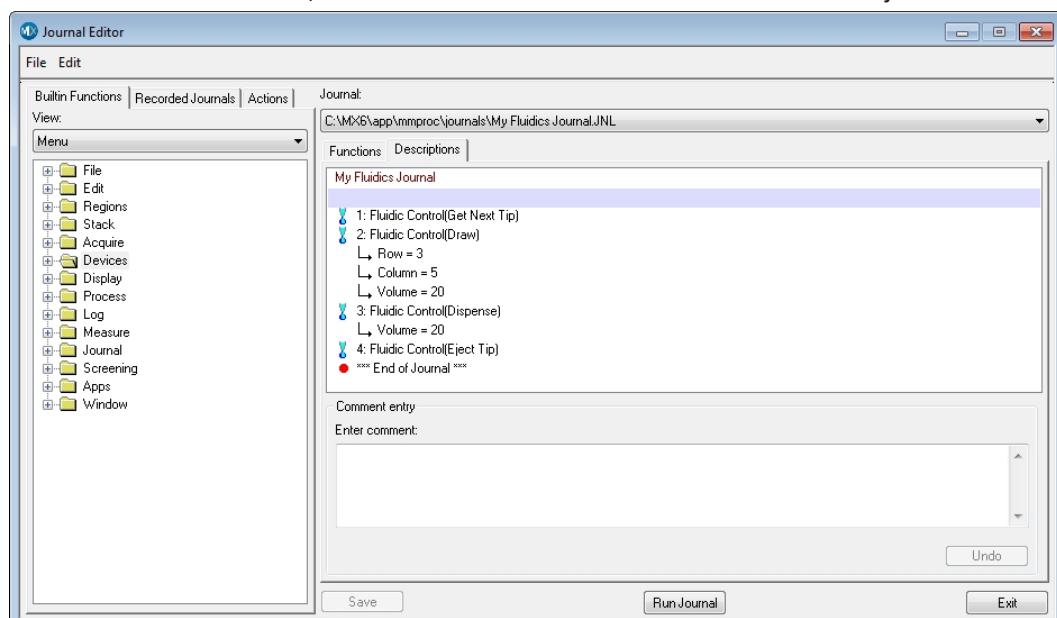


Figure 7-36: Example Journal Using Manual Fluidics Control

Performing Fluidics Experiments

Fluidics experiments are defined by adding events to the Scheduled Events list. You can add three types of events: Compound Addition, Washout, and Journal. Fluidics events can be performed on all wells being imaged, or on a subset of wells. For example, you can add different amounts of compound to different wells or treat wells for different amounts of time.

To configure a fluidics experiment:

1. In the MetaXpress Software:

From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.

From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.

2. On the Configure tab, select the **Acquisition** tab.

3. Select the **Use Fluidics** check box to add a Fluidics tab to the dialog.



Figure 7-37: Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab

4. Select the **Fluidics** tab to schedule fluidics events.

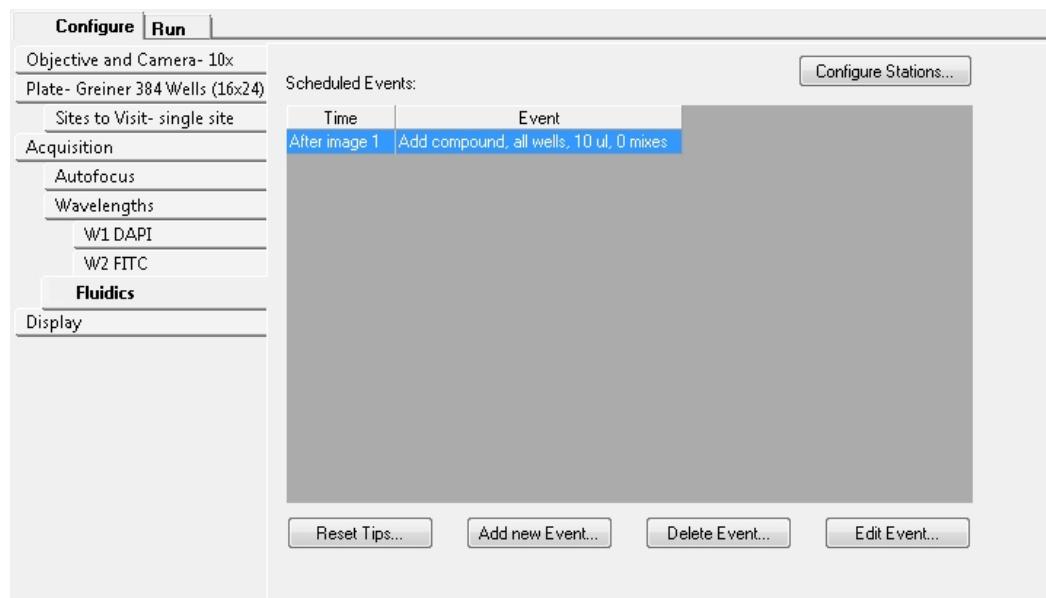


Figure 7-38: Adding Events to a Fluidics Experiment

5. Click **Add New Event** to display the Fluidic Event dialog.

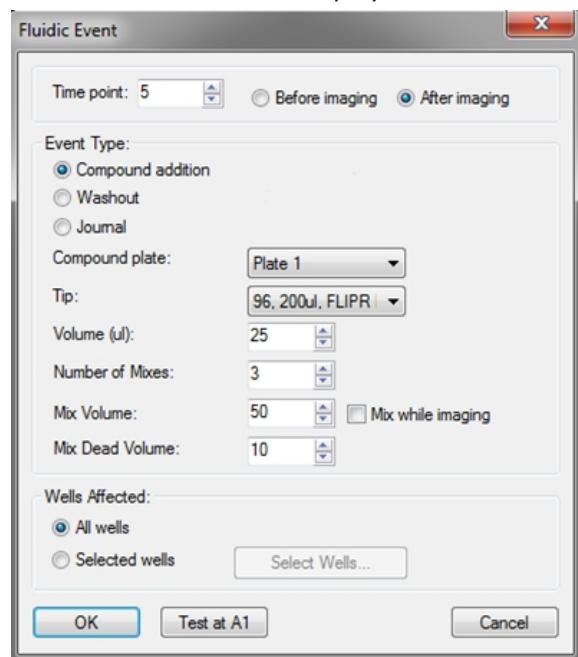


Figure 7-39: Time Point Setting in Fluidic Event Dialog

6. In the **Time Point** field, enter the time point at which the event is to occur, and then select either **Before Imaging** or select **After Imaging** to indicate whether the event should occur before or after the time point.
For example, if you select time point 5 and select Before Imaging, then the fluidic event occurs before the time point. If you select After Imaging, then the fluidic event occurs after the time point.

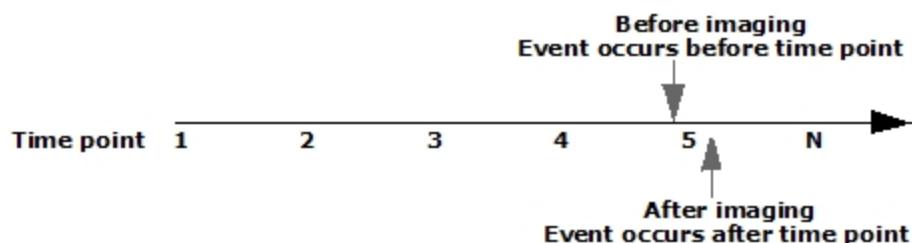


Figure 7-40: Setting Events to Occur Before and After a Time Point



Note: Set the number of time points on the Timelapse tab. See [Performing Time Lapse Experiments on page 141](#).

7. Select an **Event Type** option:

- Select **Compound Addition** to draw liquid from the compound plate and add it to the sample plate. There is a one-to-one mapping between the wells on the compound plate and the sample plate. Compound is removed from a well on the compound plate and applied to the well in the same position on the sample plate (for example, A1 to A1, A2 to A2). When you run mixes that consist of more than a single cycle, **Mix Dead Volume** specifies the amount of fluid to remain in the tip and not to be returned to the well during intermediate repeated cycles of draws and dispense.

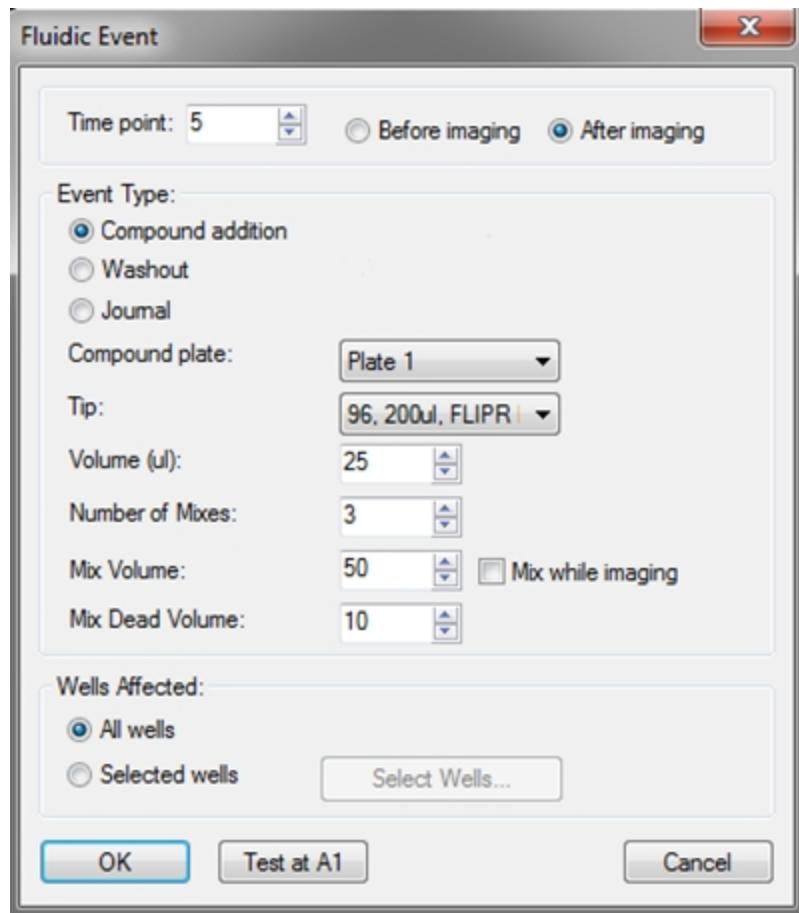


Figure 7-41: Fluidic Event Dialog Compound Addition Event

- Select **Washout** to remove liquid from the sample plate and replace it with liquid from the compound plate. There is a one-to-one mapping between the wells. An exchange consists of a washout event in which fluid is removed from the sample plate and discarded and fresh media or fluid is placed in the well. To have the system discard the tip after removing and discarding the fluid from the sample plate, select the **New Tip Each Exchange** check box.

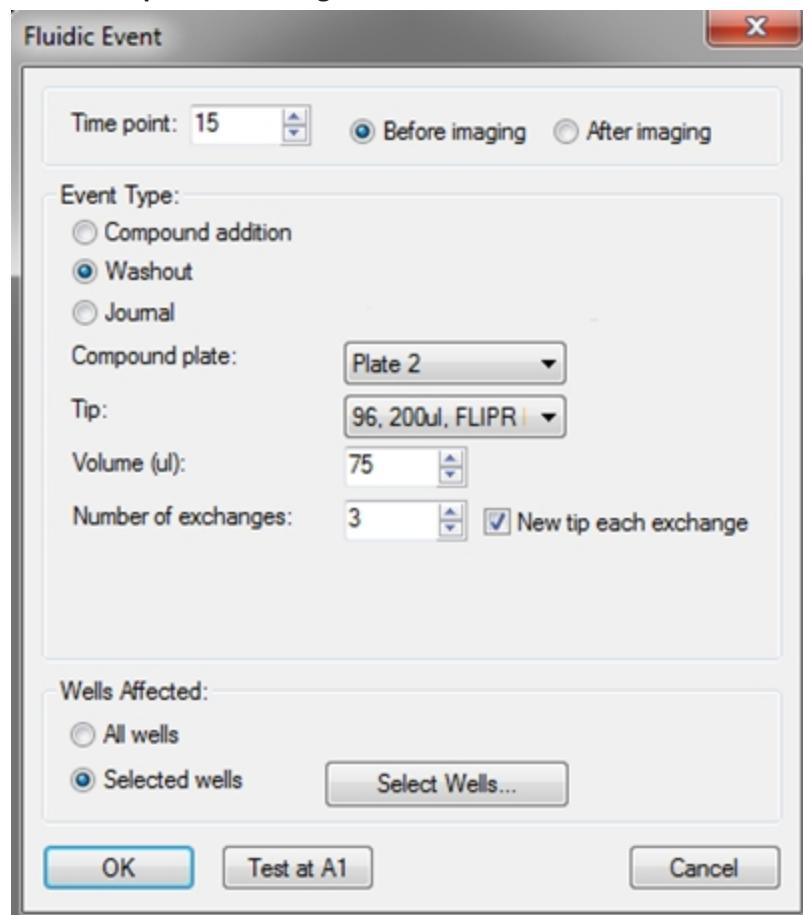


Figure 7-42: Fluidic Event Dialog Washout Event

- Select **Journal** for special cases or custom protocols (for example, experiments that do not use a one-to-one mapping of wells or that draw once and dispense multiple times).

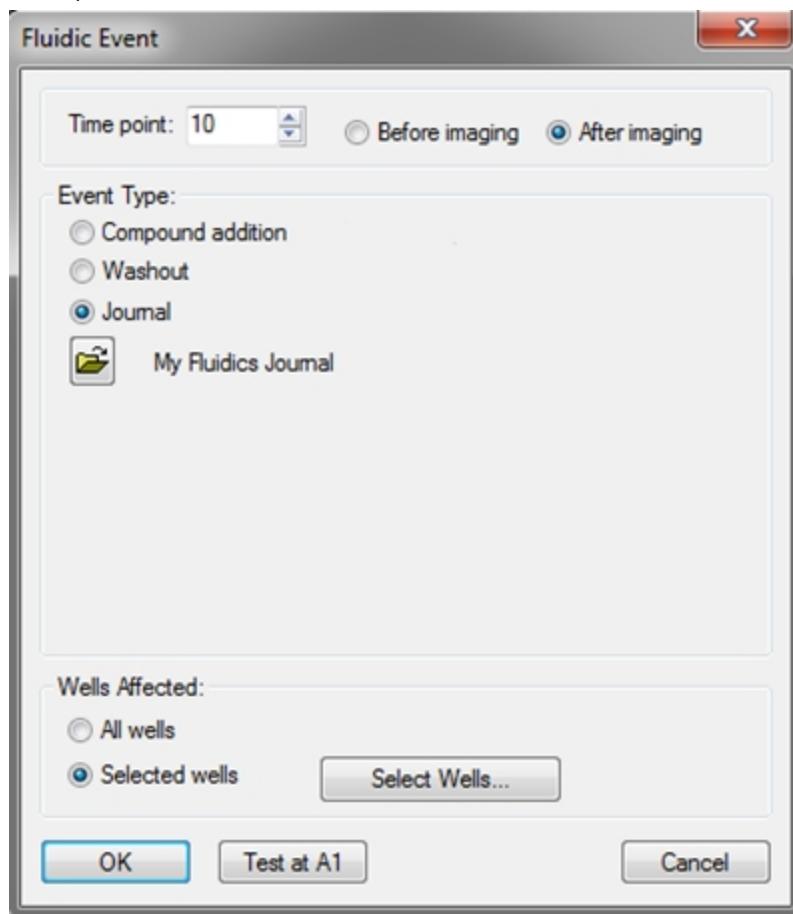


Figure 7-43: Fluidic Event Dialog Journal Event

8. Click **OK**.

Preventing Evaporation from Compound Plates

Incubation at elevated temperatures, such as 37°C, causes rapid evaporation of water from aqueous compound preparations in storage plates. DMSO-based preparations are hygroscopic and absorb water from the surrounding air. To minimize these problems, seal the compound storage plates with aluminum foil seals before placing them in the environmental chamber. Foil seals can be pierced during the experiment by the pipette tips on the fluidics robot, only exposing individual wells after use.

Seals generally adhere better to plates with raised rings around the wells than to plates with a smooth surface. Evaporation is greater from the outer wells of a microplate, both from micro-environmental variations, and because of poorer adhesion at the outer edges of the seal. It might be preferable to omit these wells from experiments with long incubation periods. It is also useful to set up the fluidics plate configuration on an equivalent, unsealed plate before trying to pipette from a sealed plate.

Evaluated Seals

Molecular Devices has evaluated both heat-applied and pressure-applied seals with a variety of polypropylene and polystyrene plates. One example is listed here. Other manufacturers might provide similar performance.

Heat-Applied Seals

Tomtec heat-applied aluminum foil seals

- Part numbers: Tomtec AutoSeal instrument (710-100) and foil seal material (AS-3)
- For specifications, ordering information, pricing, and sealing instructions, contact Tomtec (www.tomtec.com)
- Features
 - Automated robotic plate sealing
 - Suitable for polypropylene plates
 - Easily punctured by the fluidics robot using FLIPR Tetra pipette tips
 - Compatible with DMSO
 - Easily peeled from the plate

Cleaning Fluidics Components

It is important to regularly clean the environmental enclosure, including specific fluidics components. Components can come in contact with biological, chemical, and toxic agents. Therefore, all cleaning procedures should be handled with care. Molecular Devices recommends that you always wear powder-free gloves when you access the internal components of the enclosure. See [Maintenance on page 193](#).



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

Cleaning the Waste Disposal Box and the Plate Sealing Ring

Proper cleaning of the waste disposal box and the plate sealing ring is highly dependent on the type of waste being disposed of and whether the box is being used with a protective liner. These components can come into direct contact with unlidded sample plates, compounds, and media. Molecular Devices strongly recommends that you regularly clean between experiments.

To clean both components:

1. Remove components from the environmental enclosure.
2. Use one of the following cleaning techniques depending on the severity of contamination of the box:
 - Damp wipe followed by a disinfectant wipe (70% ethanol)
 - Exposure to UV by placement within a tissue culture hood

Transmitted Light Option

The ImageXpress Transmitted Light option is designed for brightfield and phase-contrast transmitted-light imaging. In general terms, transmitted light is used to describe microscopy in which light is transmitted from a source on one side of the specimen, with the objective on the other side. Typically, the light is first passed through a condenser so high illumination is focused on the specimen. For more information on phase contrast microscopy, visit the Nikon website:

www.microscopyu.com/articles/phasecontrast/phasmicroscopy.html

This section includes the following topics:

- [Transmitted Light Hardware, see below](#)
- [Transmitted Light Software Configuration on page 169](#)
- [Setting Up Koehler Illumination on page 173](#)
- [Transmitted Light Phase Ring Alignment on page 175](#)
- [Performing Transmitted Light Experiments on page 181](#)
- [Replacing the Transmitted Light Bulb on page 181](#)

Transmitted Light Hardware

The ImageXpress Transmitted Light option consists of the following hardware components.

- **Nikon phase optics.** Allows either phase contrast or brightfield imaging. See [Figure 7-44](#) and [Figure 7-45](#).
- **Phase contrast objectives.** Must be selected for phase contrast imaging.
- **White light lamp (halogen).** The software controls the white light lamp (halogen) power. The lamp bulb has a limited life and can be replaced. See [Replacing the Transmitted Light Bulb on page 181](#).



WARNING! The surface of the halogen lamp gets very hot during operation and remains hot for up to 20 minutes after operation. Do not touch the lamp surface while it is hot.

-
- **Transmitted Light shutter.** Controlled by the software. The shutter protects the sample from the transmitted light when not imaging. The shutter window maintains the environment of the sample. See [Figure 7-46](#).
 - **Phase alignment lens.** Located in the emission filter wheel and is placed in position using the MetaXpress Software for the ImageXpress Micro Confocal System.
 - **Hinge.** Allows robotic access to the plate if necessary. See [Figure 7-47](#).
 - **ImageXpress Systems Power and Options Controller.** Used to turn the option on and off and control the illumination intensity using the MetaXpress Software. It is shared with the ImageXpress Environmental Control option when both options are installed.



Figure 7-44: ImageXpress Micro Confocal System with the Transmitted Light Option

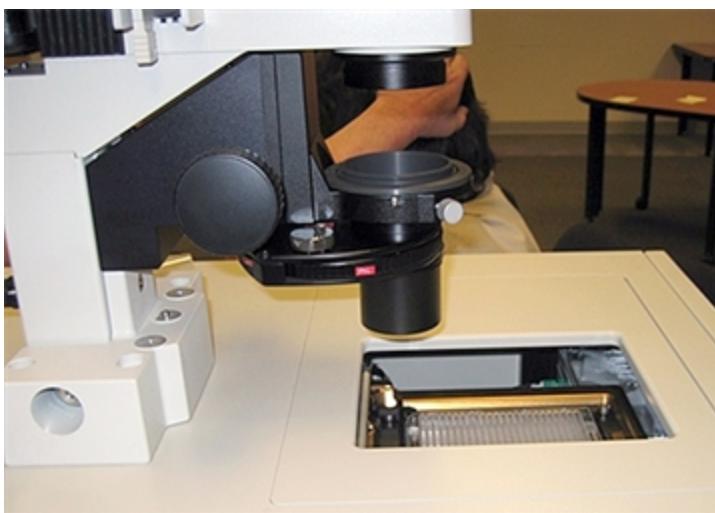


Figure 7-45: ImageXpress System with Transmitted Light Option (Detailed View)

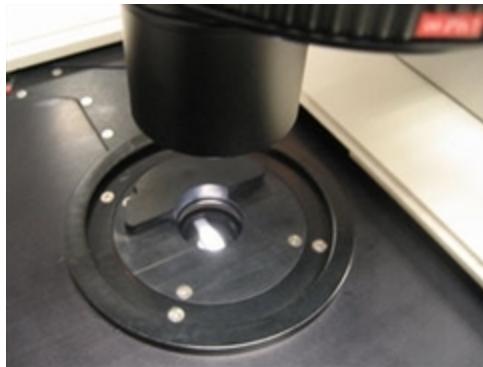


Figure 7-46: Transmitted Light shutter

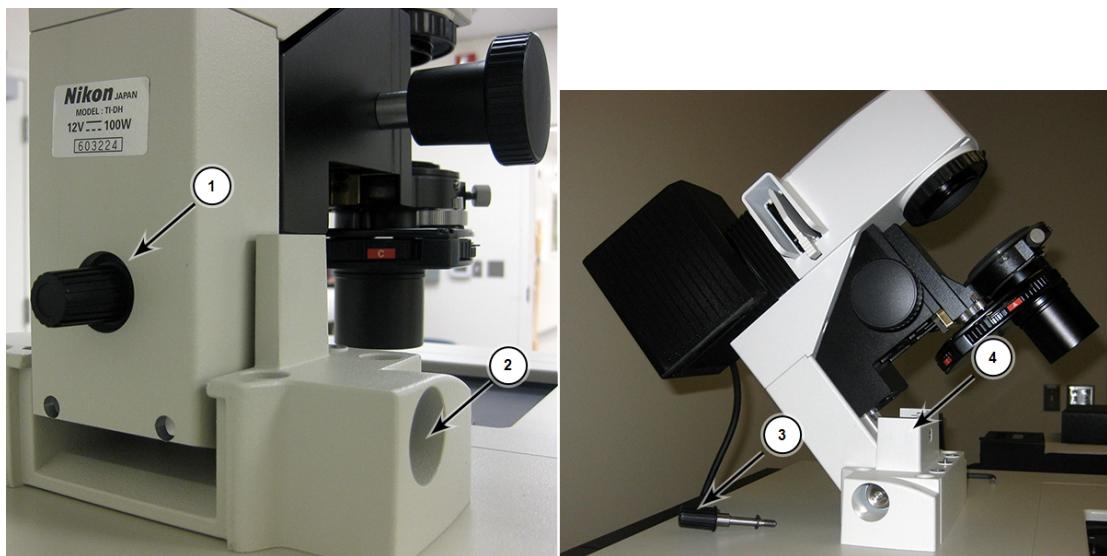


Figure 7-47: Hinge Release Knob and Hinge

Item	Description
1	Hinge release knob
2	Hinge
3	Hinge release knob
4	Assembly tipped back with hinge release knob unscrewed

Transmitted Light Software Configuration

You can create illumination settings to turn the lamp on and off. Do the following procedure to create illumination settings named **Transmitted Lamp Off** and **Transmitted Light**, and to set a level for the lamp illumination.



Tip: Do not select illumination settings that automatically turn the transmitted light on and off or adjust the lamp power during acquisition. Doing so is time consuming and can damage the lamp.

To configure transmitted light settings in the MetaXpress Software:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Devices > Configure Illumination** to display the Configure Illumination dialog.

From the standard menu, click **Devices > Configure Illumination** to display the Configure Illumination dialog.

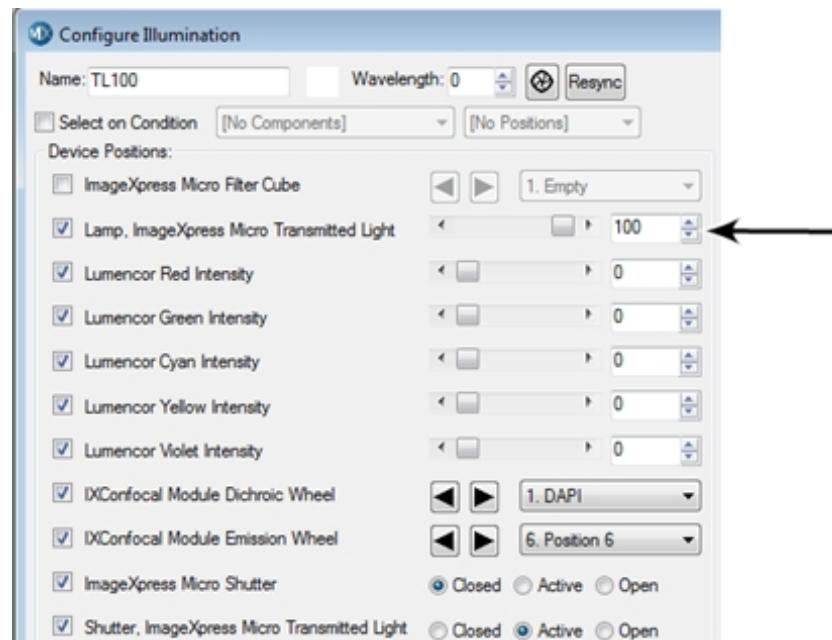


Figure 7-48: Configure Illumination Dialog: Lamp On

2. In the Name field, enter **TL Off**.

3. Select the **Lamp, ImageXpress Micro Transmitted Light** check box and set the **Lamp** illumination to zero.

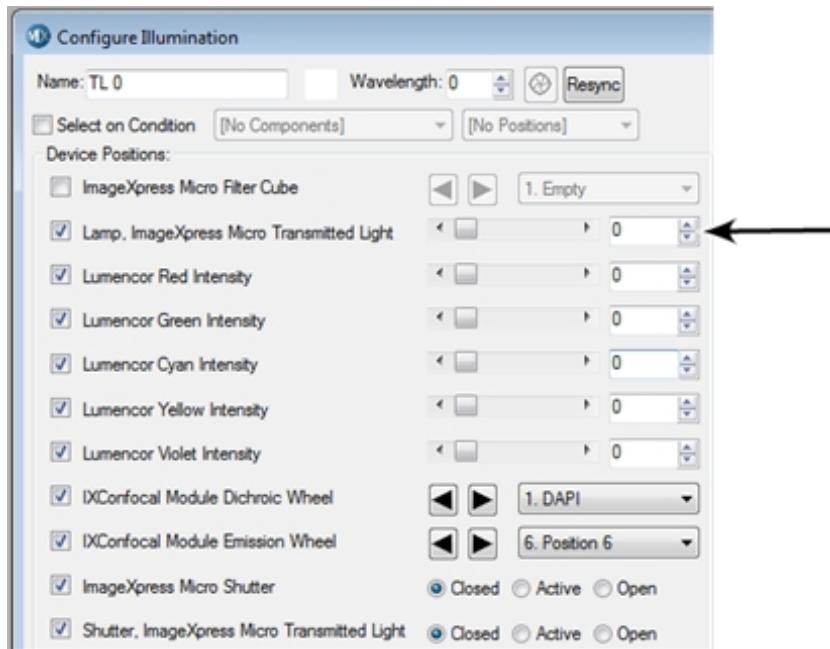


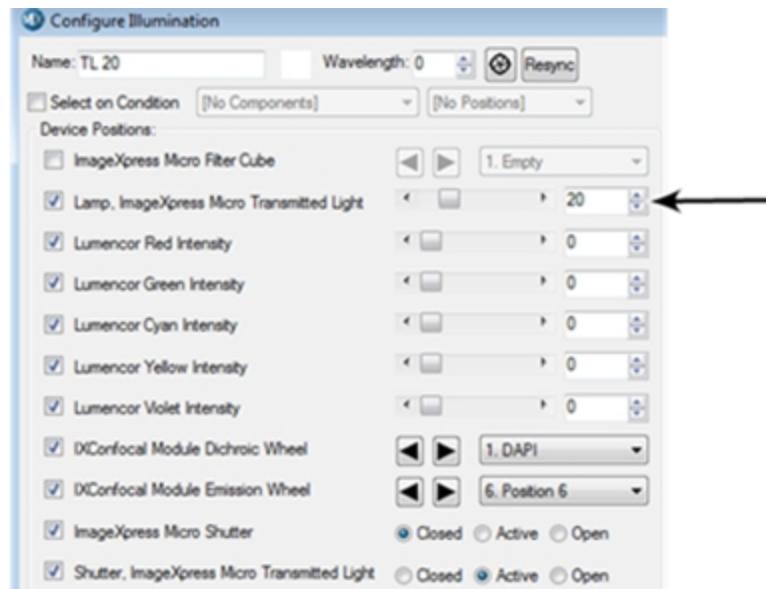
Figure 7-49: Configure Illumination Dialog: Transmitted Lamp Off

4. In the Name field, enter **TL**.
5. Select the **IXConfocal Module Dichroic Wheel**, click the corresponding drop-down and select **DAPI**.
6. Select the **IXConfocal Module Emission Wheel**, click the corresponding drop-down and select **Empty**, usually **Position 7**, or **FITC**.
7. Select the **Lamp, ImageXpress Micro Transmitted Light** check box, and set lamp illumination as a percentage of full power. Adjust this setting to meet the experiment requirements.

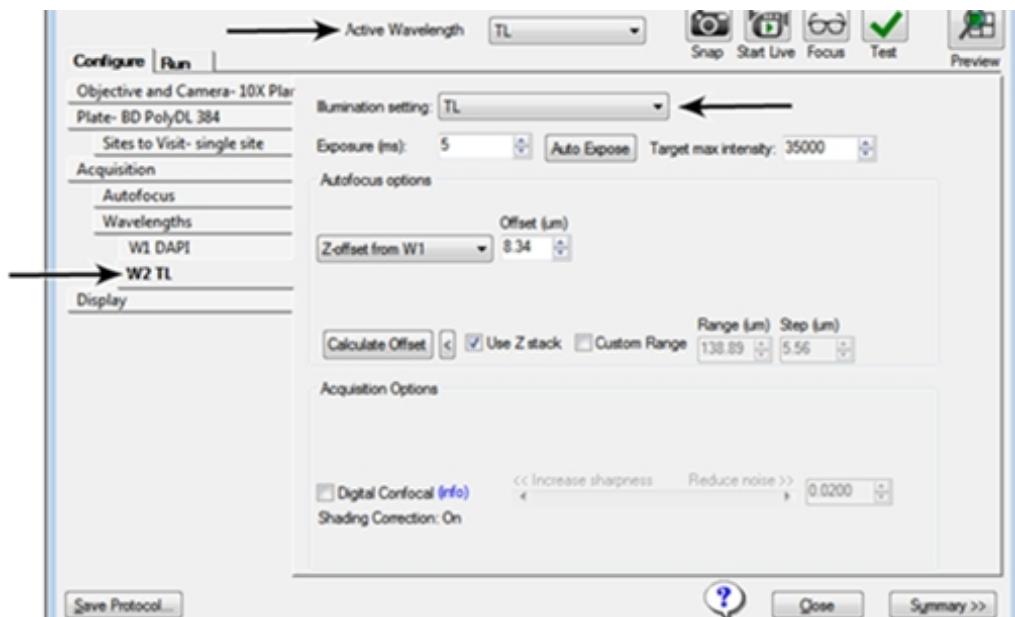


Tip: Set the lamp power low enough that at least a 10 ms exposure is required when imaging the sample.

The example sets the lamp power to 20% of maximum.

**Figure 7-50: Configure Illumination Dialog: Transmitted Light**

TL now displays as an option in the Plate Acquisition Setup dialog from the Active Wavelength drop-down and on each W# Wavelength tab from the Illumination Setting drop-down.

**Figure 7-51: Transmitted Light Wavelength Setting in Protocol Configuration**

Setting Up Koehler Illumination

To ensure high-quality illumination with brightfield or phase contrast imaging, set up the Transmitted Light option for Koehler illumination. Do this alignment anytime there is concern about the quality of the transmitted light images or when changing plate type or slide type. Since the objectives are parfocal, repeating this procedure after switching objectives is unnecessary.

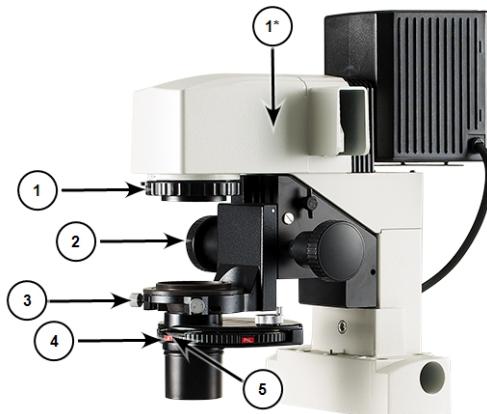


Table 7-3: ImageXpress Transmitted Light Overview (actual model may vary)

Item	Description
1	Field Aperture Diaphragm Control (* can be located here on older Transmitted Light models)
2	Condenser Focus Control
3	Condenser Adjustment Screws (Aperture Lateral Adjustment Control)
4	Condenser Turret
5	Field Stop Slider (Condenser Aperture Diaphragm Control)



Note: For the ImageXpress Micro Confocal System, make sure that the instrument is in widefield mode before setting up Koehler illumination.

To set up the ImageXpress Transmitted Light option for Koehler Illumination:

1. Load the sample (multi-well plate or slide).
2. Select a low magnification objective such as the 10x Plan Apo or a 4x objective.
3. Rotate the condenser turret (4) to select the **A** position (at the front). Open the condenser aperture diaphragm by moving the field stop slider (5) above the **A** to the right.
4. In the MetaXpress Software:
 - From the simplified menu, click **Screening > Acquisition Setup** to display the Plate Acquisition Setup dialog.
 - From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.
5. Move the stage to a well or slide area where there is visible sample.

6. Select one of the available Transmitted Light illumination settings.
7. Use laser-based autofocus, image-based autofocus, or live mode to focus on the sample.
8. Click **Start Live**.
9. Close the field aperture diaphragm (1) most of the way by moving the lever left or down if you have the older model (1*).
10. Adjust the condenser height with the condenser focus control (2) until a bright polygon surrounded by sharp dark edges is visible.



Figure 7-52: Condenser Height Focus Adjustment Comparison

11. If you cannot detect sharp edges, open the field aperture diaphragm (1) further, and try adjusting the condenser height again.
12. Center the white polygon with the condenser adjustment screws (3), also known as field diaphragm adjustment screws.
13. Open the field aperture diaphragm (1) by moving the lever right, or up if you have the older model (1*), until the whole field is illuminated, and the dark edges are just outside of the field of view. Opening this too far can increase glare in the images.
14. Close the condenser aperture diaphragm slightly by moving the field stop slider (5) above the A to the left, to optimize the sharpness and contrast of the image.
15. Click **Stop Live**.
16. Click **Focus** to confirm proper illumination. If you see dark edges in your field of view, repeat step 13.

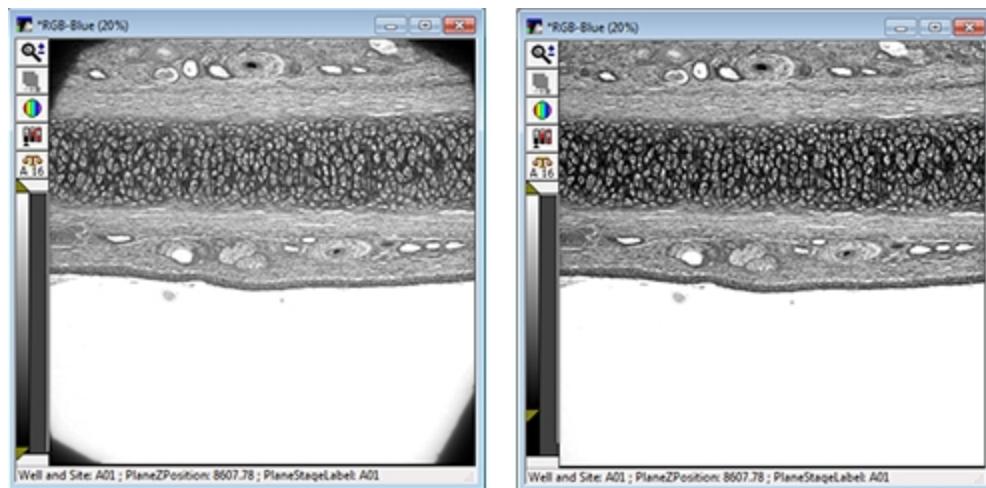


Figure 7-53: Adjustment of the Field Aperture Diaphragm Comparison

Transmitted Light Phase Ring Alignment

The FSE (Field Service Engineer) performs phase ring alignment when installing the ImageXpress Transmitted Light option. After the phase ring is aligned, the microscope generally holds its position for objective changes. The alignment should be checked periodically, especially when you see degradation of phase contrast image quality.

Phase ring alignment requires a special lens to be placed in position before starting the phase ring adjustment procedure.

On the ImageXpress Micro Confocal System, the phase ring adjustment lens is configured in the emission wheel Position 8 and is only used during the phase ring adjustment process using the MetaXpress Software. Molecular Devices recommends leaving the phase ring adjustment lens installed in the emission wheel Position 8.

To use the phase ring adjustment lens on the ImageXpress Micro Confocal System :

1. In MetaXpress Software:

From the simplified menu, click **Control > Devices > Device Control** to display the Device Control dialog.

From the standard menu, click **Devices > Device Control** to display the Device Control dialog.

2. Click **Component Control** to display the Compound Control dialog.
3. Click the Component drop-down and select the **IXConfocal Module Emission Wheel**.
4. Click the **Position** drop-down and select **Position 8**.
5. Click **Close** to display the Device Control dialog.
6. Click **Close**.

To align the phase ring:



Note: For the ImageXpress Micro Confocal System, make sure that the instrument is in widefield mode before aligning the phase ring.

1. Make sure that the phase ring adjustment lens is in place.

On the ImageXpress Micro Confocal System, the phase ring adjustment lens is configured in the emission wheel Position 8 and is only used during the phase ring adjustment process using the MetaXpress Software. Molecular Devices recommends leaving the phase ring adjustment lens installed in the emission wheel Position 8.

2. Rotate the condenser turret so that the A position is selected (at the front).



Figure 7-54: Condenser Turret with Position A at the Front

3. If Koehler illumination has not been set up, turn the adjustment ring, which is located below the arm of the Transmitted Light tower, to the middle of the aperture range.

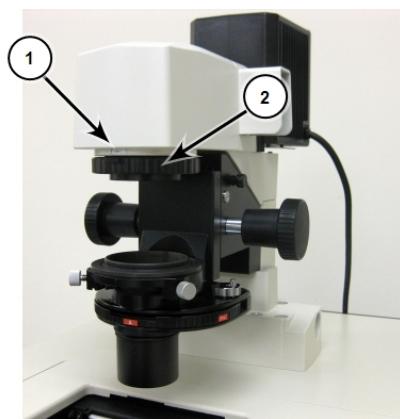


Figure 7-55: Aperture Stop Position for Phase Ring Alignment

Item	Description
1	Label indicating aperture range
2	Field aperture diaphragm control

4. If Koehler illumination has not been set up, move the field-stop slider, located just above the A label on the condenser turret, all the way to the right.



Figure 7-56: Field Stop Slider

5. Select the diffusion filter or place some lens paper or lint-free wipes on top of the condenser lens to serve as a diffuser. If necessary, weigh them down slightly to keep them from blowing away.
6. In the MetaXpress Software, select the desired phase contrast objective.
7. Set the focus (Z position) to approximately 8000 µm.
If necessary, you can adjust this later.
8. Select the **Transmitted Light Illumination** setting.
9. In the **Configure Illumination** dialog, to select the phase ring alignment lens, change the **IXConfocal Module Emission Filter** to **Position 8**.
10. With no sample present, snap an image.

11. If necessary, adjust the illumination power level and the exposure time to obtain a visible but not saturated image. The image of the phase ring might be off-center. This is acceptable if most of the image is visible.



Tip: If the phase ring is not visible at all, contact technical support.

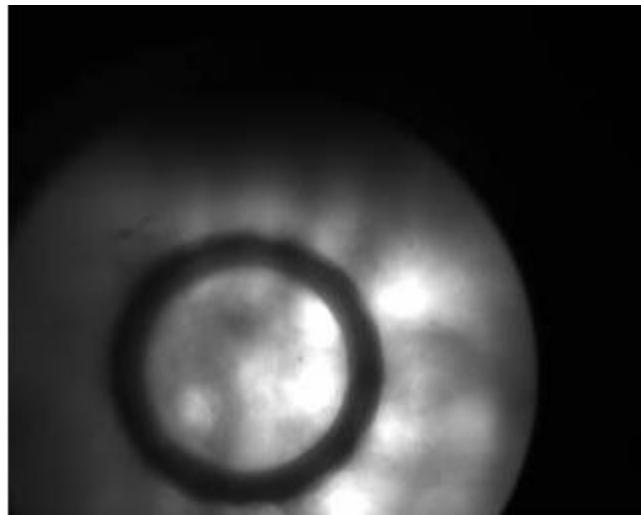


Figure 7-57: Out of Focus Image of Phase Contrast Ring

12. Adjust the position of the Z-stage to obtain a reasonably sharp image of the objective phase ring.



Figure 7-58: In Focus Image of Phase Contrast Ring

13. Use the region tools in the MetaXpress Software to mark the position of the phase ring. Use the rectangular region tool to make sure that you capture both horizontal and vertical axes.

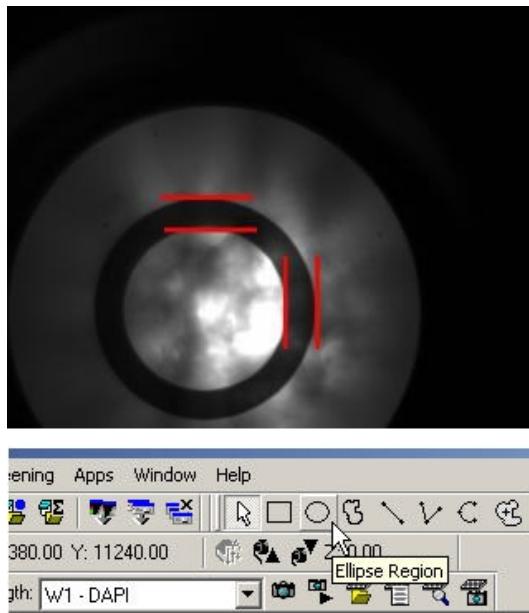


Figure 7-59: Marking the Position of the Phase Ring

14. In the MetaXpress Software:
From the simplified menu, click Measure > Regions > Save Region to save the marked positions.
From the standard menu, click **Regions > Save Regions** to save the marked positions.
15. Manually rotate the condenser turret to the annulus for the phase objective (PhL for 4x, Ph1 for 10x and 20x, Ph2 for 40x).
The annulus is at the front of the condenser turret.
Phase contrast objectives typically have their specifications indicated in green letters on the outer barrel. These specifications also include the matching annulus designation (for example, PhL, Ph1, Ph2).

16. Snap an image to show the condenser annulus ring. You might need to adjust the exposure time.

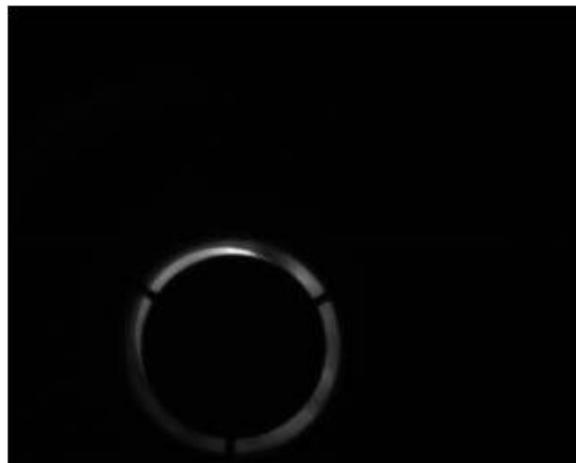


Figure 7-60: Image of Condenser Ring with the Loaded Region

17. In the MetaXpress Software, in either the Acquire dialog or the Plate Acquisition and Control dialog, click **Live Mode**.
18. From the simplified menu, click **Measure > Regions > Load Regions** to load in the saved regions.
From the standard menu, click **Regions > Load Regions** to load in the saved regions.
19. Using the condenser annulus centering knobs located on the right top and left top surface of the condenser turret, center the condenser annulus ring with the objective phase ring.
20. Lock down the annulus centering knobs.



Figure 7-61: Centering the Condenser Annulus Ring with the Objective Phase Ring

21. Adjust the height of the Transmitted Light condenser with the condenser height adjustment knob until a sharp image is obtained. If required, adjust the lamp power or exposure level.

22. In the MetaXpress Software, click **Stop Live**.

For the ImageXpress Micro Confocal System, the lens you use for phase ring adjustment automatically moves out of the optical path when you select an emission wavelength for the acquisition.

23. In the Configure Illumination dialog, verify that the **IXConfocal Module Emission Filter** setting is restored to its normal setting, such as **Empty**.

Performing Transmitted Light Experiments

Do the following procedure if you are running an experiment with Transmitted Light as one of the selected wavelengths.

Transmitted light acquisition is compatible with the confocal modes in the ImageXpress Micro Confocal System.

To run a Transmitted Light experiment:

1. If you run a phase-contrast experiment, make sure to select a phase-contrast objective. Phase-contrast objectives are not required for brightfield experiments.
2. Make sure the annulus on the Transmitted Light arm matches the objective, and set it to **A** for brightfield experiments.
3. Load a suitable plate. The plate must be unlidded, have a clear lid, or have a clear plate seal on the top. Fill wells at least half-way full of liquid.



CAUTION! Condensation on a lid or plate seal can negatively affect image quality.

4. In the Configure Illumination dialog, select an Illumination setting to turn on the Transmitted Lamp .
5. In the MetaXpress Software:
From the simplified menu, click **Screening > Plate Acquisition** to display the Plate Acquisition Setup dialog.
From the standard menu, click **Screening > Plate Acquisition Setup** to display the Plate Acquisition Setup dialog.
6. Define one of the wavelengths as Transmitted Light.
7. Configure the remaining settings as usual.
8. Run the acquisition.



Tip: When the transmitted light unit is not in use, in the Configure Illumination dialog, select the Transmitted Lamp Off setting to increase the life of the halogen lamp.

Replacing the Transmitted Light Bulb

The ImageXpress Transmitted Light option uses a replaceable 12-volt halogen light bulb. See [Replacement Parts and Optional Extras on page 261](#).



WARNING! The surface of the halogen lamp gets very hot during operation. Do not touch the lamp surface while it is hot. Wait at least 20 minutes after operation to allow the lamp to cool before you begin this procedure.

To replace the transmitted light bulb:

1. Unplug the power cord from the controller box.
2. Remove the set screw that secures the cover on top of the tower using a 3 mm hex wrench.



Figure 7-62: Set Screw Location

3. Remove cover by lifting it upward.



Figure 7-63: Cover Removal

4. Face the light source from the rear and identify the bulb and the lever.

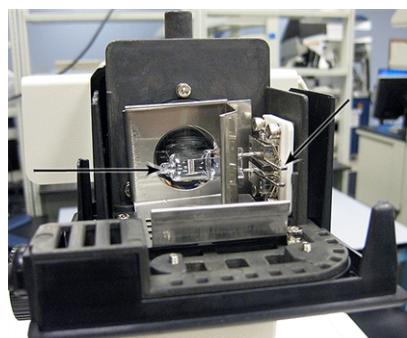


Figure 7-64: Bulb and Lever

5. Put on cotton gloves to handle the bulb.



CAUTION! Never touch the bulb with bare hands.

6. Press the lever towards the right and pull the bulb out towards the left. Make note of the location where the pin of the bulb was inserted.

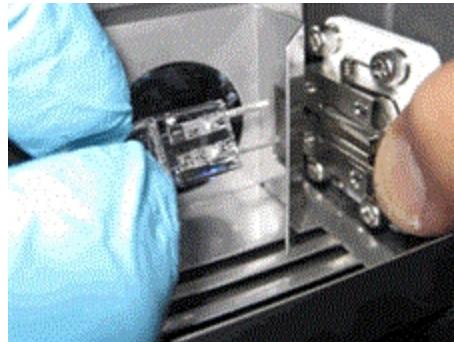


Figure 7-65: Press Lever and Remove Bulb

7. Insert the new bulb with the correct rating and specifications.
8. Replace cover and secure it with the set screw.
9. Plug in the controller box.

Water Immersion Option

The ImageXpress Water Immersion option dispenses small amounts of water onto the top of a water immersion objective as images are acquired. By matching the refractive index of the sample, the water immersion objective can produce images that are of higher quality than can be attained with air objectives. For example, with a water objective, a reduction in light refraction improves the geometric accuracy during acquisition. Better light collection results in images with brighter intensity at lower exposure times. Images are sharper and clearer, and spheres are more discernible.



Note: The ImageXpress Water Immersion option is compatible with the Environmental Control, Transmitted Light, and automated plate loading options.

This section includes the following topics:

- [Water Immersion Safety, see below](#)
- [Water Immersion Hardware Components on page 185](#)
- [Performing Experiments with the Water Immersion Option on page 187](#)
- [Cleaning Water Immersion Components on page 191](#)
- [Troubleshooting Water Immersion Issues on page 191](#)

Water Immersion Safety

Before using the ImageXpress Water Immersion option, review the following information to ensure proper operation and to reduce the risk of damage to the instrument.



CAUTION!

- Do not remove or change the water immersion objectives that are installed within the instrument. A Molecular Devices Field Service Engineer must install and remove water immersion objectives.
- Do not touch the leak detection sensor, which is the brass color ring around the top of the objective below the cap.
- Do not place the water immersion controller on top of the instrument or near electronics. The water immersion controller is not watertight. Water will not be contained within the controller if water spills into the water immersion controller.
- Do not move the water immersion controller to a location that is above or below where it is installed by the Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. If you change the elevation of the water immersion controller, you can negatively affect the calibration.
- Do not attempt to remove the water immersion controller cover. With the exception of the water bottles, the controller does not contain any user-serviceable parts.
- Use only distilled water in the source water bottle. Deionized water can be used, but is not required. Do not use tap water, and do not reuse the water that is in the waste bottle.

Water Immersion Hardware Components

The Water Immersion option consists of the following hardware components:

- [Water Immersion Objectives, see below](#)
- [Water Immersion Controller on page 186](#)

Water Immersion Objectives



CAUTION! Do not remove or change the water immersion objectives that are installed within the instrument. A Molecular Devices Field Service Engineer must install and remove water immersion objectives.

The Water Immersion option can include up to two of the water immersion objectives listed in the following table.

Table 7-4: Water Immersion Objective Specifications

Water Immersion Objective	Numerical Aperture (NA)	Working Distance	Correction Collar Range
20X	0.95	0.95mm	0.11mm to 0.23 mm
40X	1.15	0.59mm	0.15mm to 0.19mm
60X	1.2	0.28mm	0.15mm to 0.18mm

The water immersion objectives must be located in the first and/or fourth objective slots.

Water immersion objectives have correction collars that are similar to the correction collars on air objectives. The objectives also have a cap that allows the creation of a bolus (bubble of water) above the objective and that directs water to a drain. The drain maintains a vacuum to draw water from the objective to a waste water bottle.



CAUTION! Do not touch the leak detection sensor, which is the brass color ring around the top of the objective below the cap.

Water Immersion Controller



CAUTION! Do not remove the water immersion controller cover. With the exception of the water bottles, the controller does not contain any user-serviceable parts.

The water immersion controller consists of three main components.

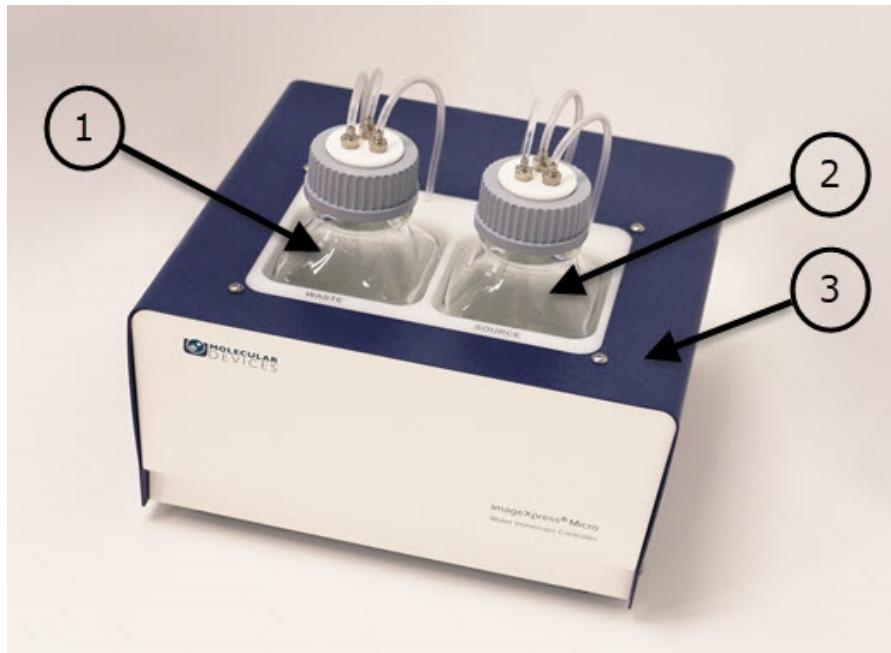


Figure 7-66: Water Immersion Components

Table 7-5: Water Immersion Component Descriptions

Item	Description
1	Waste water bottle (500 mL), which contains used water that is drained from the objective. The system warns you when the water level in the recovery bottle reaches 400 mL and may halt an acquisition in progress if needed.
2	Source water bottle (500 mL), which contains distilled water to be dispensed to the objective during image acquisition. The system warns you when the water level in the source bottle reaches 100 mL and may halt an acquisition in progress if needed.
3	Housing, which contains the pump, valves, and sensors: the diaphragm pump delivers water and vacuums recovered water; pressure, vacuum, and level sensors monitor system status; four valves control flow states; and, the relief valve maintains constant pressure.



CAUTION! Do not move the water immersion controller to a location that is above or below where it is installed by the Molecular Devices Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. If you change the elevation of the water immersion controller, you can negatively affect the calibration.

Performing Experiments with the Water Immersion Option

The process for acquiring images with water immersion objectives is essentially the same as the process for acquiring images with air objectives. This section describes the additional steps for using water immersion objectives:

- [Inspecting, Refilling, and Emptying the Water Bottles, see below](#)
- [Setting Up Plate Acquisition to Use a Water Immersion Objective on page 188](#)
- [Confirming Water Immersion Status on page 190](#)

Inspecting, Refilling, and Emptying the Water Bottles

Before you acquire images with a water immersion objective, ensure that:

- The source bottle has enough water for the experiment.
- The waste bottle has enough space for the water that will be drained from the water immersion objective.
- The bottle caps are screwed on tightly.

To refill or empty a water bottle:

1. Unscrew the bottle cap and, while holding the cap and tubing, remove the bottle from the water immersion controller.
2. Drape the tubing and cap over the front of the water immersion controller (to prevent the tubing and cap from coming into contact with the table surface or other potential contaminants).
3. Fill or empty the bottle.



CAUTION! Use only distilled water in the source water bottle. Deionized water can be used, but is not required. Do not use tap water and do not reuse the water that is in the waste bottle.



Note: When filling the source bottle, add water slowly. Adding water too quickly can cause air bubbles to form.

4. Insert the tubing into the bottle and then put the bottle back into the controller.
5. Tighten the bottle cap.
6. In the software:
 - a. If the Plate Acquisition Setup dialog is not already open, click **Screening > Acquisition Setup**.
 - b. In the Plate Acquisition Setup dialog, click  **WI Status**.
The Water Immersion Control dialog opens.
 - c. Click **Refill/Empty Bottle**.
The Bottle Maintenance dialog opens.
 - d. Click **Test Vacuum** and **Test Pressure** to ensure the bottles are properly sealed.

The water immersion system has multiple leak detectors and pressure sensors to determine if there is unintended water loss. When a sensor is triggered, the system automatically shuts off the pump and closes the valves to prevent damage to the instrument. For more information, see [Troubleshooting Water Immersion Issues on page 191](#).

Setting Up Plate Acquisition to Use a Water Immersion Objective

In the MetaXpress software, you configure an acquisition using a water immersion objective the same way that you would configure an acquisition using an air objective. When using a water immersion objective, the Plate Acquisition Setup dialog provides:

- A water immersion status icon beneath the plate map.
- The installed water immersion objectives in the list of available magnifications on the Objective and Camera tab.

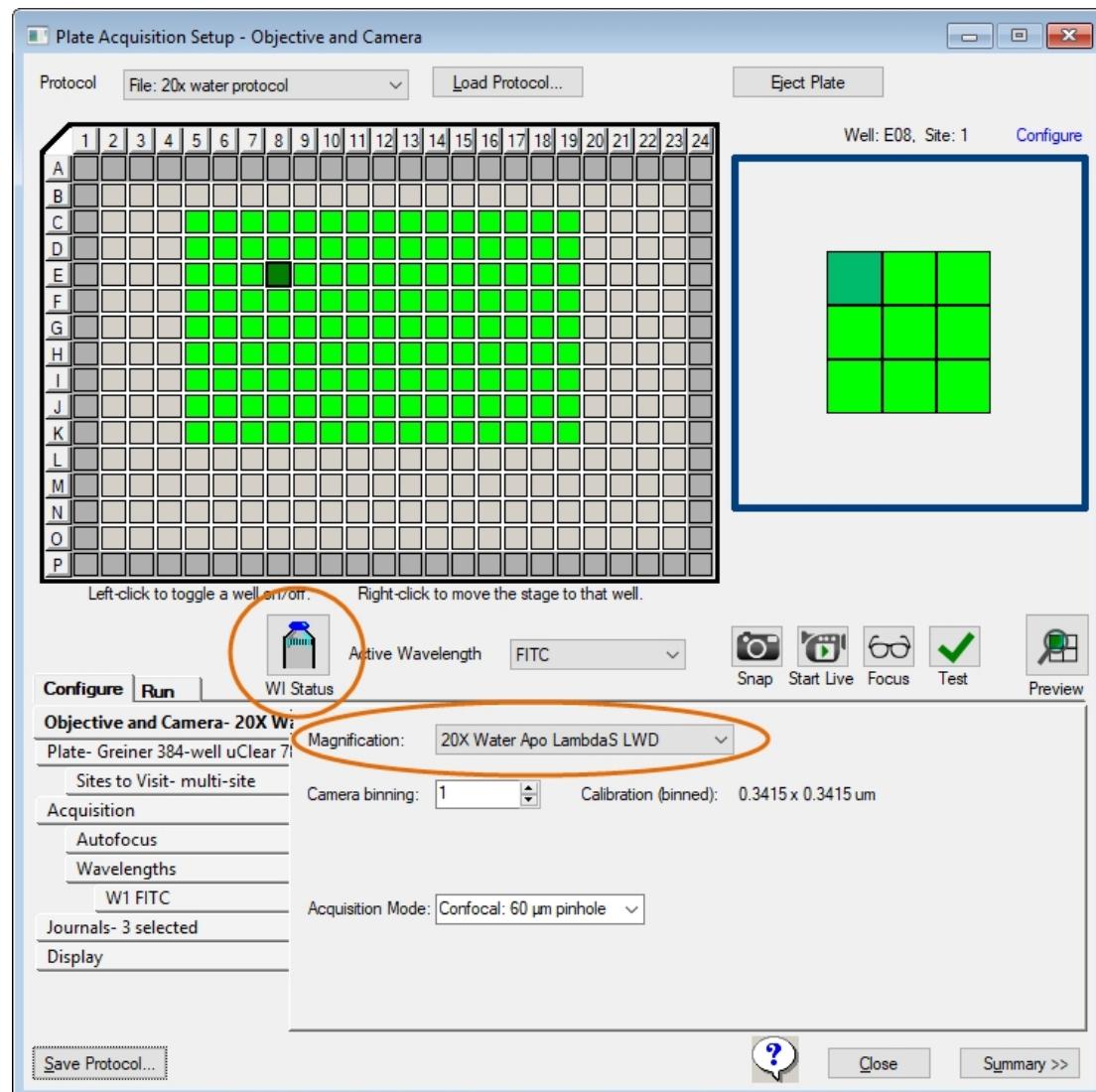


Figure 7-67: Plate Acquisition Setup Dialog with Water Immersion Option

The following table describes how the WI Status icon indicates the status of the water immersion system.

Table 7-6: WI Status descriptions

Icon	System Status
	The water immersion system and settings are appropriately configured.
	When you begin an image acquisition, the icon displays a green check mark to indicate that the water immersion system is initializing (the status bar at the bottom of the software window displays the status of this process). Once initialization is complete, the system automatically proceeds to acquire images and the icon continues to display a green check mark.
	An error is preventing image acquisition. See the error message that displays in red in the software status bar at the bottom of the software window. This type of warning can indicate, for example, that the cap is not tightly screwed on one of the water bottles, that the source bottle is empty, or that the waste bottle is full.

On certain plates, outer wells might be blocked from acquisition to prevent collision with the water immersion objective.



Note: The Shading Correction feature (on the Wavelengths tab in the Plate Acquisition Setup dialog) is not fully compatible with water immersion objectives. The Auto Correction for FL setting is partially compatible; the shading portion is compatible with water immersion objectives, but the subtraction portion is not. A message will display, but acquisition can continue, and the shading portion is still applied. The FL Subtraction Only setting is not compatible with water immersion objectives.

If you switch objectives, the system drains the water from the water immersion objective currently in use.

- If you switch to a water immersion objective, then the system automatically dispenses water above that objective before the first acquisition.
- If you switch to an air objective, then the system opens the instrument top door and prompts you to dry the bottom of the plate before you proceed to acquire subsequent images.



CAUTION! If you switch from a water immersion objective to an air objective and plan to acquire images using the same plate, you must dry the plate bottom to prevent water damage to the air objective and the instrument.

Confirming Water Immersion Status

The software automates most water immersion activity. The Water Immersion Control dialog is provided primarily for informational purposes.

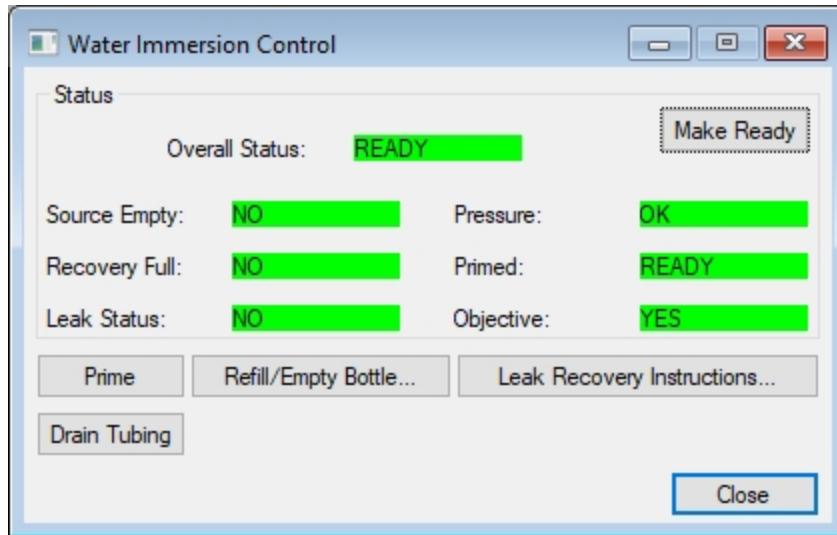


Figure 7-68: Water Immersion Control Dialog

To open the Water Immersion Control dialog, do one of the following:

- In the Plate Acquisition Setup dialog, click  **WI Status**.
- From the simplified menu, click **Control > ImageXpress > Water Immersion Control**.
- From the standard menu, click **Devices > Water Immersion Control**.

The Water Immersion Control dialog provides the following buttons:

- **Make Ready** - Performs procedures that prepare the water immersion system for acquisition.



Note: You do not need to click Make Ready before acquiring images. Once you begin an acquisition, the system automatically performs these prerequisite procedures.

- **Prime** - Fills the tubing between the source bottle and the objective with water and builds pressure in the source bottle so that water will dispense at a constant rate.
- **Refill/Empty Bottle** - Provides instructions for refilling the source bottle and emptying the waste bottle.
- **Leak Recovery Instructions** - Provides instructions for managing a water leak; these instructions will display automatically if a leak is preventing water from being dispensed to the water immersion objective.
- **Drain Tubing** - Clears the tubing of water.

Cleaning Water Immersion Components

Components can come in contact with biological, chemical, and toxic agents. Therefore, all cleaning procedures should be handled with care. For additional information about cleaning the system, see [Maintenance on page 193](#).

- Replace the distilled water in the source bottle if the water immersion system has been unused for a significant period of time.
- Do not reuse the water from the waste bottle due to potential contaminants from the bottom of plates.
- Use a lens cloth to gently wipe the top of a water immersion objective.
- Wipe the exterior of the source bottle and the waste bottle before you place the bottles back into the water immersion controller.
- If you plan to store the instrument, you plan to move the instrument, or you do not intend to use the water immersion system for a long period of time, contact Molecular Devices for instructions.
- Do not access or clean the interior of the water immersion controller other than the area that holds the bottles.

Troubleshooting Water Immersion Issues

If you encounter an issue with the water immersion system, try performing one or more of the procedures below before contacting Molecular Devices.



If the WI Status icon is not displayed on the Plate Acquisition Setup dialog, verify in the Meta Imaging Series Administrator application that you are using the correct hardware profile.

If the system is preventing plate acquisition, confirm that the plate settings are accurate and that the plate is compatible with the objective.

If the system is unable to focus on the plate, follow these steps:

1. Review the plate settings.
2. Visually inspect the water bolus to ensure that it is formed properly on top of the objective.
3. Run the Laser Autofocus Wizard.

If a message indicates that a leak has been detected, follow these steps:



Note: To access leak recovery instructions in the software, on the Plate Acquisition

Setup dialog, click **WI Status**



to display the Water Immersion Control dialog and

then click **Leak Recovery Instructions**.

-
1. Eject the plate.
 2. Move the stage so that you can see the objective.
 3. Use a lens cleaning tissue to soak up water that is on the top and sides of the water immersion objective. Look for water that may have gone over the edge of the objective or elsewhere in the system; dry any water found, provided that you can reach it safely.

4. Check the water immersion tubing to ensure it is not pinched or kinked.
5. Tighten the bottle caps. If the Leak Recovery Instructions dialog is not already open in



the software, on the Plate Acquisition Setup dialog, click **WI Status** to display the Water Immersion Control dialog.

- a. To test the vacuum seal, click **Test Vacuum**.
- b. To test that the vacuum line is not clogged, click **Test Vacuum Line**.
- c. To test that water is dispensed correctly, click **Test Dispense**.

If a message continues to indicate an error or issues persist, contact Molecular Devices.

Chapter 8: Maintenance

Perform only the maintenance tasks described in this guide. Contact Molecular Devices to inspect and perform preventive maintenance service on the instrument each year.

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

If you need to re-install the MetaXpress Software or install the software on a new computer, see the *MetaXpress High-Content Image Acquisition and Analysis Software Suite Installation and Update Guide* included on the MetaXpress Software Suite flash drive or contact Technical Support. See [Obtaining Support on page 244](#).

Maintenance Precautions

To avoid personal injury or damage to the instrument during maintenance procedures, observe the following precautions:

- Some procedures require that the instrument power supply is turned OFF and that the power cable is unplugged before you do the maintenance procedure.
If the MetaXpress Software is running, exit the program before you turn off the instrument.
- Some procedures may require that you disconnect the connection between the instrument and the computer and turn off any attached peripherals, such as the robot plate-loading arm.
- 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 you start the instrument.
- Access only the user-serviceable components inside the instrument as described in the section. Avoid contact with other components as they can be damaged or knocked out of alignment.



CAUTION! Do not touch the autofocus laser.

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

- [Routine Maintenance on page 194](#)
- [Cleaning the Instrument on page 194](#)
- [Light Source Maintenance on page 196](#)
- [Replacing the Confocal Disk Box on page 196](#)
- [Creating Hardware Profiles on page 202](#)
- [Filter Maintenance on page 205](#)
- [Objective Maintenance on page 218](#)
- [Updating Shading Correction Settings-Legacy on page 240](#)
- [Turning Off the Status Indicator Lights on page 244](#)



CAUTION! Maintenance procedures other than those specified in this guide must be performed by Molecular Devices. When service is required, contact Molecular Devices technical support.

Routine Maintenance

To ensure optimal operation of the instrument, perform the following routine maintenance procedures as needed:

- Wipe off visible dust from exterior surfaces 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 software error messages.

Cleaning the Instrument

Observe the following general tips when you clean the instrument:



WARNING! BIOHAZARD. Always wear gloves when you operate 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 you use liquids to clean the instrument.
- Wipe up all spills immediately.
- Periodically clean the outside surfaces of the instrument using a lint-free cloth that has been lightly dampened with a 70% ethanol or 0.5% bleach solution.
- If needed, clean the microplate carrier by placing a lint-free cloth that has been lightly dampened with a 70% ethanol or 0.5% bleach solution on the mechanism and letting it soak for approximately 30 minutes.
- If a bleach solution has been used, wipe the instrument using a lint-free cloth that has been lightly dampened with water to remove the bleach residue.
- Do not use any cleaning agents other than those recommended in this section without first contacting Molecular Devices Technical Support. See [Obtaining Support on page 244](#).
- Do not use ultraviolet light for sterilization, as this can damage plastic components, except as noted in [Expansion Solution Options on page 129](#).
- Do not use organic solvents.
- To prevent damaging internal components, do not pour or squirt water or alcohol directly onto the instrument.

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 you clean the instrument, read and follow the [Maintenance Precautions on page 193](#).

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



CAUTION! Never use an autoclave to clean instrument components.



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

1. Start the MetaXpress Software.
2. If the Plate Acquisition toolbar is not visible, click **Window > Toolbars > Plate Acquisition**.
 3. In the Plate Acquisition toolbar, click  to move the stage to the load position.
4. Exit the MetaXpress Software.
5. On the ImageXpress Systems Power and Options Controller for the ImageXpress Micro Confocal System, power OFF the instrument. See [Shutting Down the System on page 28](#).
6. Ensure that no sample is loaded.
7. With gloved hands, use a damp wipe to wipe down the entire outer surface including side panels and top panels of the instrument.
8. Use an alcohol wipe or a disinfectant wipe and go over the entire surface again.
9. Use forceps wrapped with damp wipes to gently wipe the perimeter of the plate holder and stage region where a plate would be loaded.
10. Use forceps wrapped with alcohol wipes or disinfectant wipes to go over the plate holder and stage region again.
11. Use a fresh damp wipe to wipe down the stage area underneath and around the plate loading region.
The stage moves freely without power, so you can slide the stage around to clean the plate holder and stage region underneath where the plate is loaded.
12. Use a fresh alcohol wipe or a disinfectant wipe and go over the stage area underneath and around the plate loading region again.
13. Wait a few minutes for the alcohol to evaporate.
14. On the ImageXpress Systems Power and Options Controller for the ImageXpress Micro Confocal System, power ON the instrument. See [Powering on the Instrument on page 25](#).
15. Start the MetaXpress Software.
16. If the top door stays open after you restart the instrument, in the Plate Acquisition toolbar, click  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. This light source is a solid-state light source that has a rated lifetime of more than 20,000 hours. There are no user-replaceable parts in the light source.



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

Replacing the 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. There are three 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 that contains a 60 µm pinhole disk and a 42 µm pinhole disk. The accessory kit includes the hex keys you use to replace the Confocal disk box.



Figure 8-1: Disk Box Location in the Confocal Module Housing

Before you replace a disk box, read and follow the [Maintenance Precautions on page 193](#).

Basic tools required to replace confocal disk boxes:

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

The following topics describe how to remove and install the Confocal disk box:

- [Removing the Disk Box on page 198](#)
- [Installing the Disk Box on page 199](#)
- [Testing the Disk Box on page 200](#)

Removing the Disk Box

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



Note: You must power off the instrument and the computer.

To remove a disk box:

1. Shut down the system. See [Shutting Down the System on page 28](#).
2. 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 8-1](#).
5. Use the provided 2.5 mm hex key to 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. Use the provided 4 mm hex key to 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.

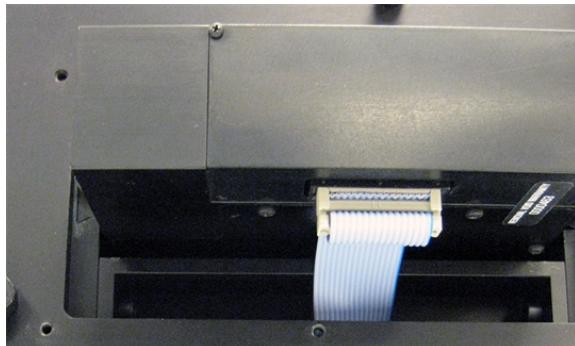


Figure 8-2: Ribbon Cable on the Bottom of the Disk Box

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 the Disk Box



Note: Follow the steps in the previous topic to remove the disk box from the instrument.

To install a disk box:

1. Use bulb duster to blow visible particulates off the disk box lens and window.
Do not use canned or compressed air as this can blow propellant 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 is in the wrong location and you cannot 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.

3. Use the provided 4 mm hex key to tighten the two 4 mm side hex screws on the disk box so that the box is secured from moving in any direction.



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

4. On the bottom of the disk box, plug in the ribbon cable.



Figure 8-3: Ribbon Cable and Cable Socket on the Bottom of the Disk Box

5. Place the disk box cover over the six screw holes and use the provided 2.5 mm hex key to tighten the six 2.5 mm hex screws on the disk box cover enough to secure it on the Confocal module housing.
6. Close the lower hinged side door.
7. Power on the system. See [Powering on the Instrument on page 25](#).
8. When the system powers on, use the MetaXpress Software to perform a functional test of the new disk box. See [Testing the Disk Box on page 200](#).

Testing the Disk Box

Use this procedure to verify Confocal disk box functionality.



Note: Power on the instrument and the computer.

To test a disk box:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Devices > Device Control** to display the Device Control dialog.

From the standard menu, click **Devices > Device Control** to display the Device Control dialog.



Figure 8-4: Device Control Dialog

2. Click the **Magnification** drop-down and select a low magnification.
3. Click **Component Control** to display the Component Control dialog.

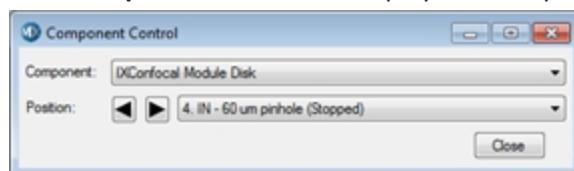


Figure 8-5: Component Control Dialog

4. Click the **Component** drop-down and select **IXConfocal Module Disk**.
5. Click the **Position** drop-down and select **<position #>. IN - <Confocal disk type> (Stopped)**.



Note: Positions for Stopped are even numbers and for Running are odd numbers.

6. In the Plate Acquisition toolbar, click

7. From the simplified menu, click **Control > Acquire > Acquire > Show Live** to display a Live image window.

From the standard menu, click **Acquire > Acquire > Show Live** to display a Live image window.

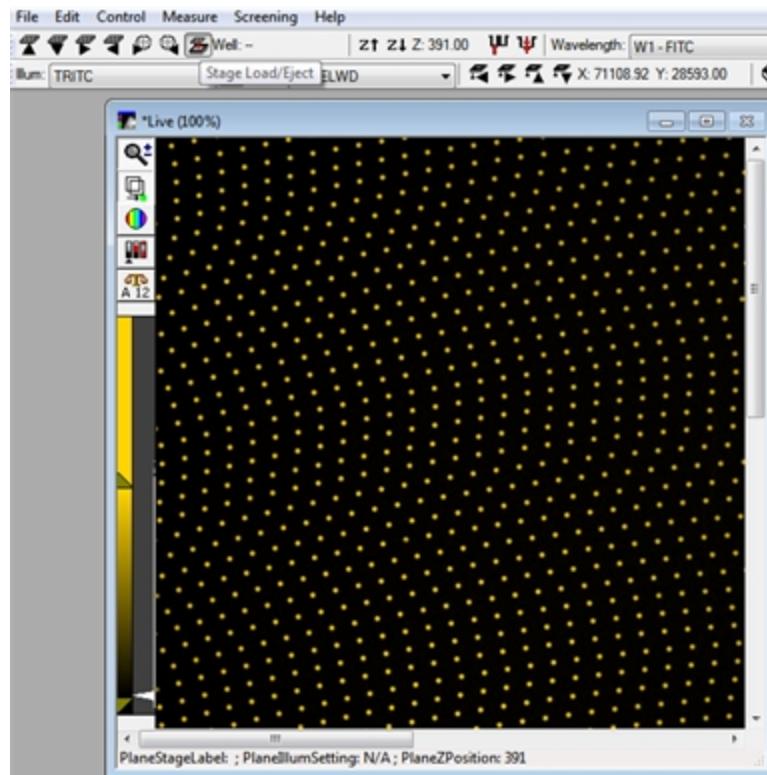


Figure 8-6: Live Image Window for a Stopped Confocal Pinhole Disk Pattern When the Top Door is Open

8. In the Component Control dialog, click the **Component** drop-down and select **Component > IXConfocal Module Disk**.

9. Click the **Position** drop-down and select **<position #>. IN - <Confocal disk type> (Running)**.

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

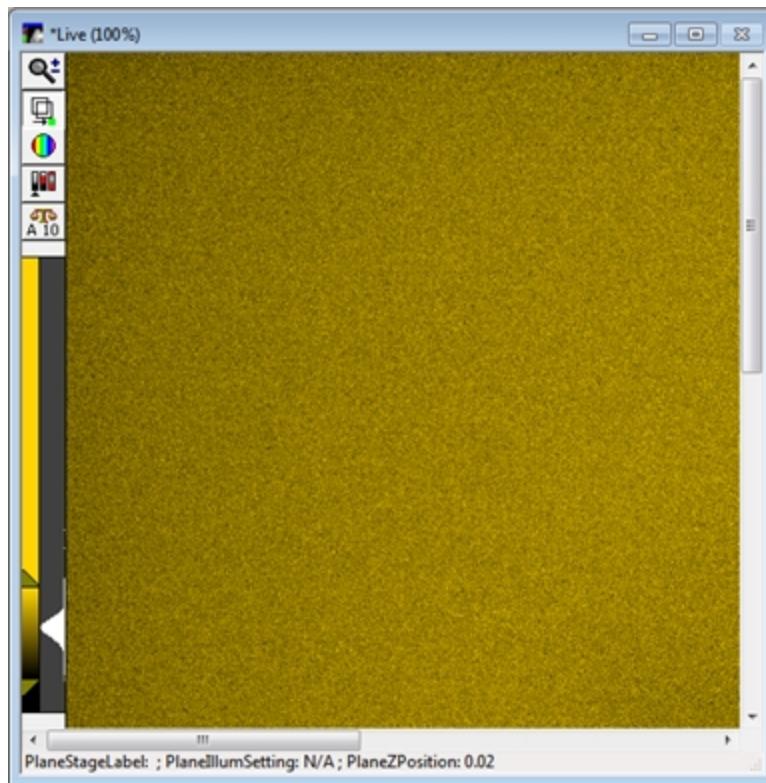


Figure 8-7: Live Window for a Running Confocal Pinhole Disk Pattern



CAUTION! If the Live image window pattern does not change, the confocal disk is not spinning. Before you contact support, verify that the disk box installation is correct. See [Installing the Disk Box on page 199](#).

10. If you have a dual confocal disk box, test the other disk. Go back through this procedure starting at step 2. Otherwise, you are finished with the disk box functionality test.

Creating Hardware Profiles

A hardware profile is a system settings file to which the software refers to identify which hardware and configurations are available for use.

To create a new hardware profile:

1. Click **Start > All Programs > Meta Imaging Series**, and then right-click **Meta Imaging Series Administrator** and select Run as Administrator to start the MetaXpress Meta Imaging Series Administrator Software.

2. In the **List of Groups**, note of the name of the hardware setting that corresponds to the group name you use.

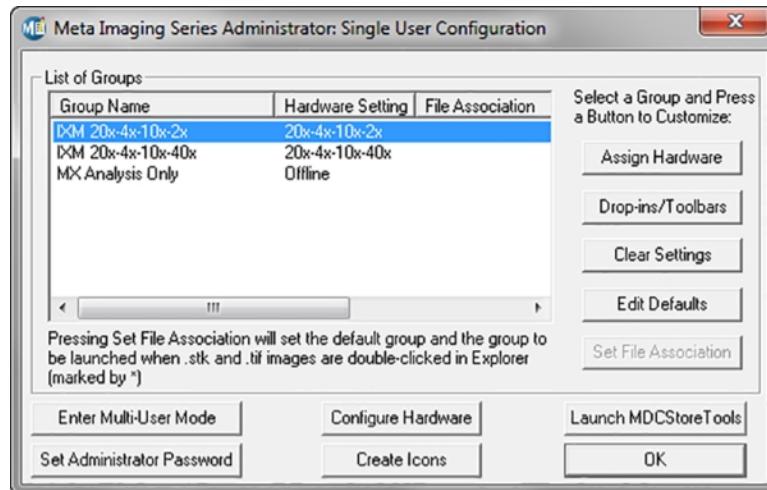


Figure 8-8: Single User Mode

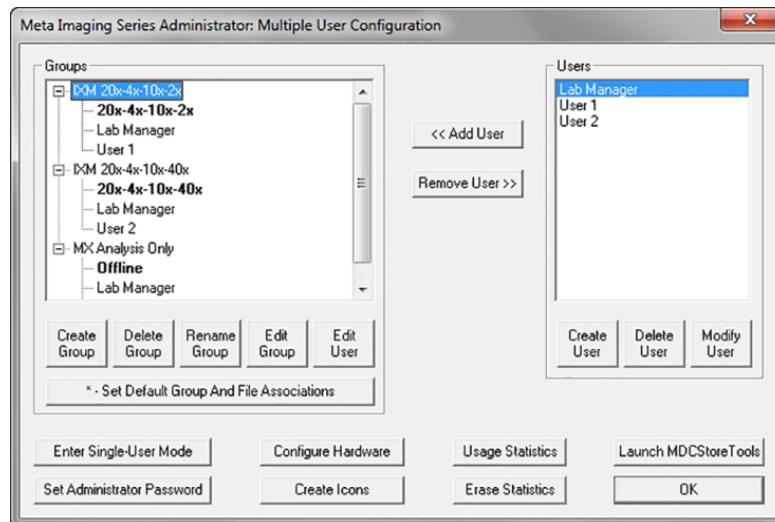


Figure 8-9: Multi User Mode

3. Click **Configure Hardware** to display the Configure Hardware dialog.

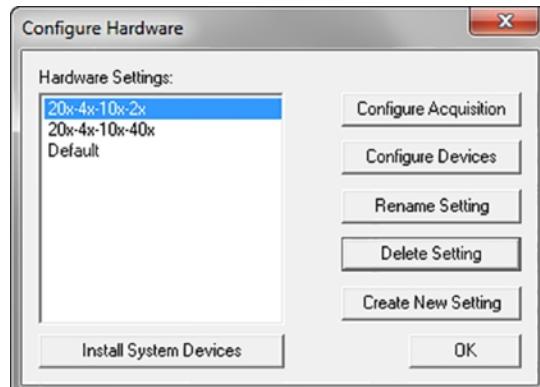


Figure 8-10: Configure Hardware Dialog

4. Click **Create New Setting** to display the Create New Setting dialog.

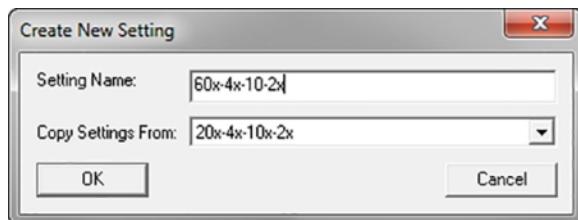


Figure 8-11: Create New Setting dialog

5. In the **Setting Name** field, enter the setting name.
6. Click the **Copy Settings From** drop-down and select the current hardware configuration.
7. Click **OK** to display the Configure Hardware dialog.

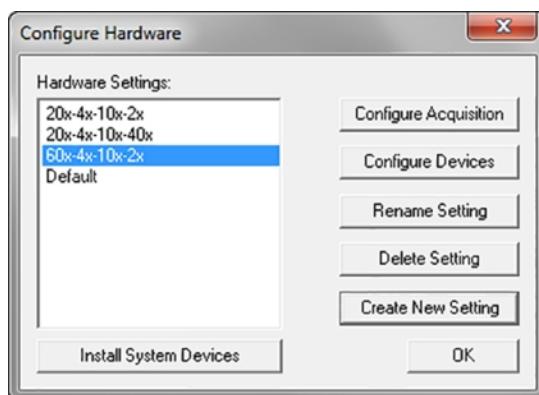


Figure 8-12: Configure Hardware Dialog with New Hardware Setting Listed

8. In the Hardware Settings list, select the new hardware configuration and click **Configure Devices** to display the User Settings dialog.
9. Make the changes to the objectives or filters as needed. See [Editing Objective Settings in the Meta Imaging Series Administrator Software on page 224](#), and see [Editing Filter Settings on page 215](#).
10. Click **OK** to display the Configure Hardware dialog.
11. Click **OK** to display the main Meta Imaging Series Administrator dialog.

Making New Hardware Profile Setting Groups

To make a new group:

1. Click **Enter Multi-User Mode**, copy settings from the existing group, and make sure to select the new hardware configuration. For User and Groups information, see [Users and Groups in the Meta Imaging Series Administrator Software on page 121](#).
2. When you finish creating the group, if you use Single-User Mode, click **Enter Single-User Mode** to prevent errors or missing settings.

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 enter the filter information into the software to reflect the new hardware configuration.

The following topics describe how to change or add filters:

- [Replacing Filters, see below](#)
- [Creating Hardware Profiles on page 202](#)
- [Editing Filter Settings on page 215](#)
- [Editing Illumination Settings on page 217](#)
- [Updating Shading Correction Settings-Legacy on page 240](#)

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. 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 to replace each of the filter types. Filters are delicate components and special care is required when you handle them.



Figure 8-13: Confocal Module Housing with Filters Access Door Open

Before you replace filters, read and follow the [Maintenance Precautions on page 193](#).

The following topics describe the on-instrument filter and mirror removal and installation procedures:

- [Replacing Emission Filters, see below](#)
- [Replacing the Dichroic Mirror on page 210](#)

Replacing Emission Filters

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 you handle them.

Before you replace filters, read and follow the [Maintenance Precautions on page 193](#).

Basic tools required, but not provided, to replace 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, you should wear powder-free gloves during the filter replacement procedures.

Required tools:

- Emission Filter
- Emission Filter Tool

Removing Emission Filters

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: Power on the instrument and the computer.



Tip: If the instrument has the ImageXpress Transmitted Light option installed, leave the phase alignment lens installed in Position 8 of the emission filter wheel.

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** to display the Configure Hardware dialog.
4. In the Hardware Settings list, select a hardware configuration and click **Configure Devices** to display the User Settings dialog.

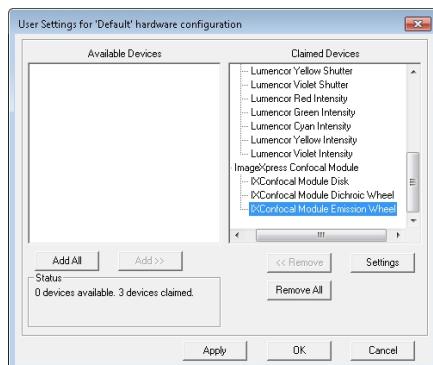


Figure 8-14: User Setting Selection of IXConfocal Module Emission Wheel Settings

5. In the Claimed Devices list, under ImageXpress Confocal Module, select **IXConfocal Module Emission Wheel** and click **Settings** to display the IXConfocal Module Emission Wheel Settings dialog.

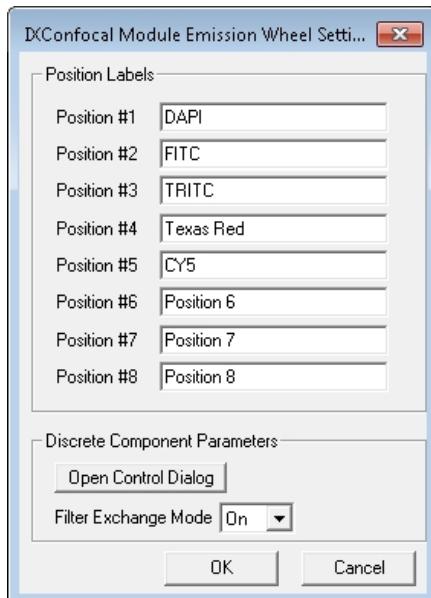


Figure 8-15: IXConfocal Module Emission Wheel Settings Dialog

6. Click the **Filter Exchange Mode** drop-down and select **On**.
7. Click **Open Control Dialog** to display the Control IXConfocal Module Emission Wheel dialog.

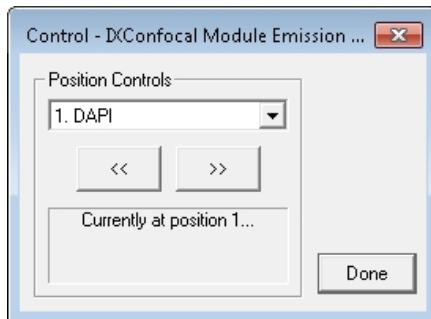


Figure 8-16: IXConfocal Module Emission Wheel Control Dialog

8. Click the **Position Controls** drop-down and select the position of the filter to remove.



CAUTION! To select Position 1, 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.

9. When the wheel moves to the position, click **Done** to display the IXConfocal Module Emission Wheel Settings dialog.
10. Put on powder-free 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 8-17: 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 you can see the rim of the 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 the way into the emission access slot.

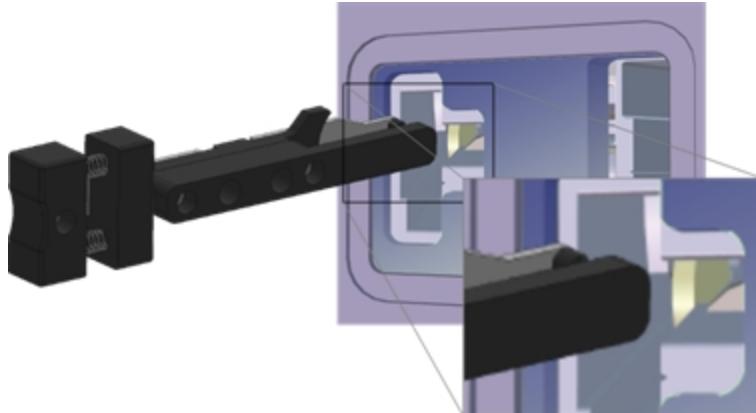


Figure 8-18: Orientation to Insert the Emission Filter Tool

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 the flashlight, place your 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 the next topic to install an emission filter into the now empty wheel slot.

Installing Emission Filters



Note: Follow the steps in the previous topic to remove an emission filter from a wheel slot position.

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.

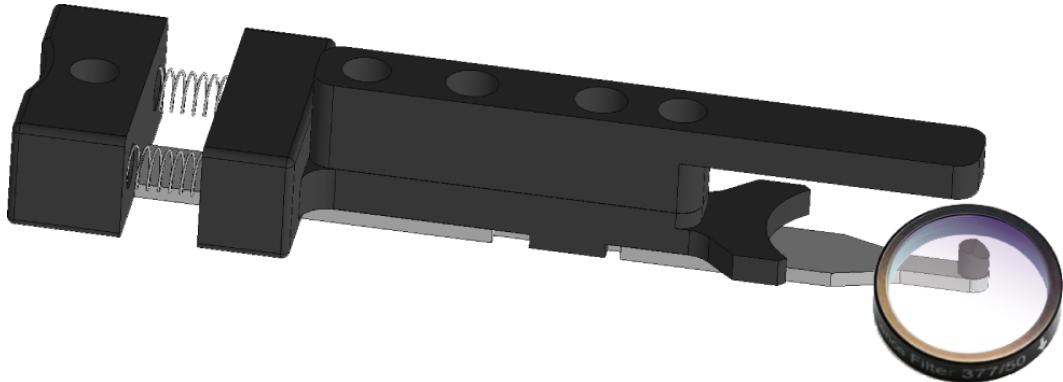


Figure 8-19: Emission Filter Tool and Emission Filter Orientation

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 you removed, in the IXConfocal Module Emission Wheel Settings dialog, in the corresponding **Position Labels** field, enter the new filter name.

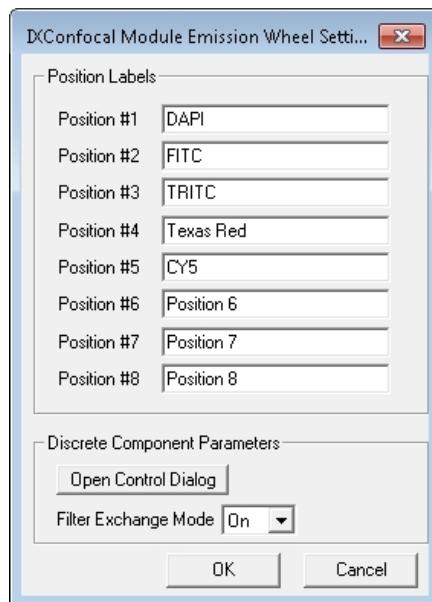


Figure 8-20: IXConfocal Module Emission Wheel Settings Dialog

9. In the IXConfocal Module Emission Wheel Settings dialog, click the **Filter Exchange Mode** drop-down and select **Off**.
10. Click **OK** to display the User Settings dialog.
11. Click **OK** to display the Configure Hardware dialog.
12. Click **OK** to display the Meta Imaging Series Administrator Software.
13. Click **OK** to exit the software.

Replacing the Dichroic Mirror

In the ImageXpress Micro Confocal System, the dichroic mirrors install into a wheel within the instrument enclosure. Mirrors are delicate components, use special care when you handle them.

Before you replace dichroic mirrors, read and follow the [Maintenance Precautions on page 193](#).

Required tools:

- Basic tools required to replace filters. See [Replacing Filters on page 205](#).
- Dichroic mirror in frames
- Dichroic Mirror tool

Removing the 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: Power on the instrument and the computer.

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** to display the main Meta Imaging Series Administrator Software dialog.
3. Click **Configure Hardware** to display the Configure Hardware dialog.
4. In the Hardware Settings list, select a hardware configuration and click **Configure Devices** to display the User Settings dialog.

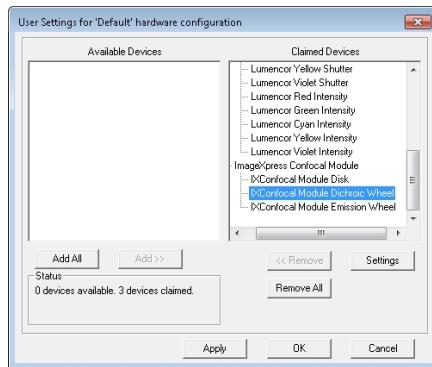


Figure 8-21: User Setting Selection of IXConfocal Module Dichroic Wheel Settings

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

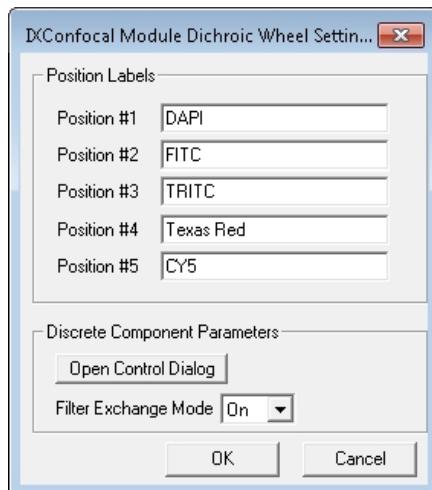


Figure 8-22: IXConfocal Module Dichroic Wheel Settings Dialog

6. Click the **Filter Exchange Mode** drop-down and select **On**.

7. Click **Open Control Dialog** to display the Control IXConfocal Module Dichroic Wheel dialog.

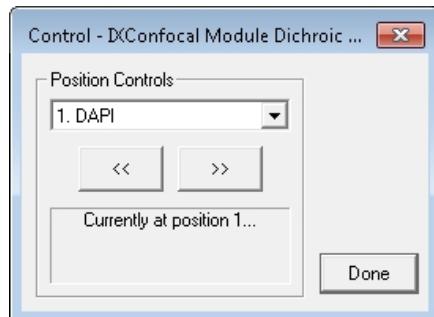


Figure 8-23: IXConfocal Module Dichroic Wheel Control Dialog

8. Click the **Position Controls** drop-down and select the numbered position for the mirror to remove.



CAUTION! To select Position 1, 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.

9. When the wheel moves to the position, click **Done** to display the IXConfocal Module Dichroic Wheel Settings dialog.
10. Put on powder-free 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 8-24: 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 the way into the dichroic mirror access slot.

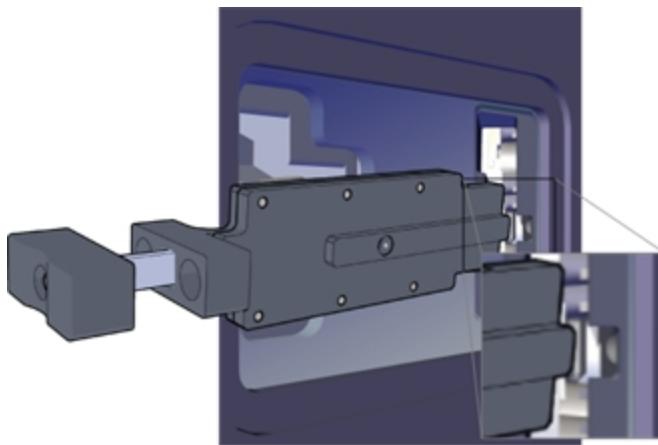


Figure 8-25: Orientation to Insert the Dichroic Mirror Tool

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 the flashlight, place your 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 the next topic to install a dichroic mirror into the now empty wheel slot.

Installing the Dichroic Mirror



Note: Follow the steps in the previous topic to remove the dichroic mirror from a wheel slot position.

To install a dichroic mirror:

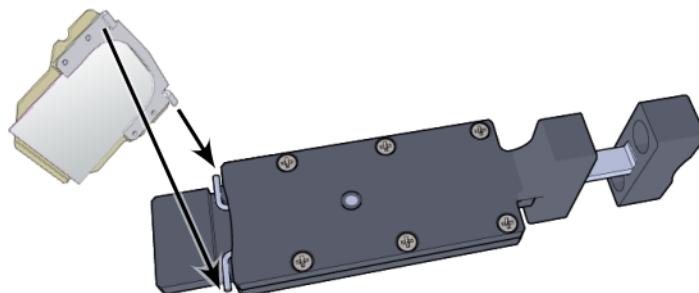


CAUTION! To protect the dichroic mirror, load the dichroic mirror tool with the dichroic mirror over a table or other work surface.

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 faces the camera.

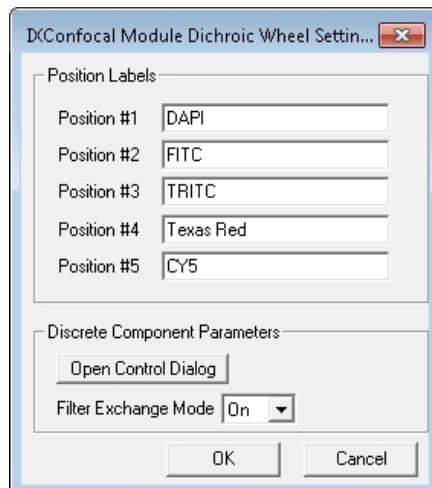
**Figure 8-26: Dichroic Mirror Tool and Dichroic Mirror Installation Orientation**

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. In the IXConfocal Module Dichroic Wheel Settings dialog, in the corresponding **Position Labels** field, enter the filter name.

**Figure 8-27: IXConfocal Module Dichroic Wheel Settings Dialog**

10. In the IXConfocal Module Dichroic Wheel Settings dialog, click the **Filter Exchange Mode** drop-down and select **Off**.
11. Click **OK** to display the User Settings dialog.
12. Click **OK** to display the Configure Hardware dialog.
13. Click **OK** to display the Meta Imaging Series Administrator Software dialog.
14. Click **OK** to exit the software.

Editing 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.

Editing Emission Filter Settings

To edit emission filter settings:

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator** to display the Meta Imaging Series Administrator.
2. In the List of Groups, select **MetaXpress** and click **Configure Hardware** to display the Configure Hardware dialog.
3. In the Hardware Settings list, select a hardware configuration and click **Configure Devices** to display the User Settings dialog.
4. In the Claimed Devices list, under ImageXpress Confocal Module, double-click **IXConfocal Module Emission Wheel** to display the IXConfocal Module Emission Wheel Settings dialog.

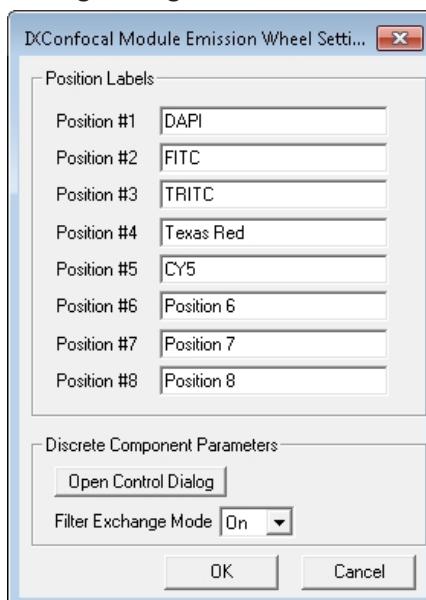


Figure 8-28: IXConfocal Module Emission Wheel Settings Dialog

5. In the **Position Labels** field, enter the filter name.
6. Click **OK** to display the User Settings dialog.
7. Click **OK** to display the Configure Hardware dialog.
8. If multiple hardware configurations are present and are affected by the change, repeat these steps for each hardware configuration.
9. Click **OK** to display the Meta Imaging Series Administrator Software dialog.
10. Click **OK** to exit the software.

Editing Dichroic Mirror Settings

To edit dichroic mirror settings:

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator** to display the Meta Imaging Series Administrator Software.
2. In List of Groups, select **MetaXpress** and click **Configure Hardware** to display the Configure Hardware dialog.
3. In the Hardware Settings list, select a hardware configuration and click **Configure Devices** to display the User Settings dialog.
4. In the Claimed Devices list, under ImageXpress Confocal Module, double-click **IXConfocal Module Dichroic Wheel** to display the IXConfocal Module Dichroic Wheel Settings dialog.

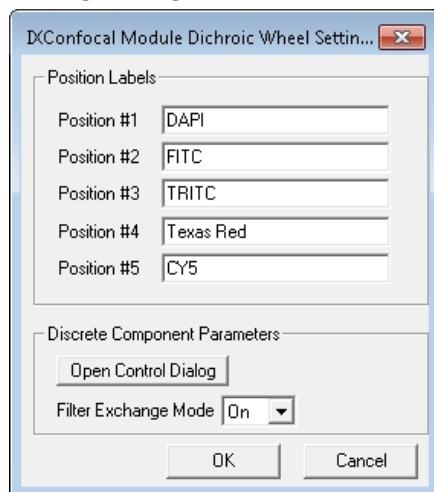


Figure 8-29: IXConfocal Module Dichroic Wheel Settings Dialog

5. In the **Position Labels** field, enter the mirror name.
6. Click **OK** to display the User Settings dialog.
7. Click **OK** to display the Configure Hardware dialog.
8. If multiple hardware configurations are present and are affected by the change, repeat these steps for each hardware configuration.
9. Click **OK** to display the Meta Imaging Series Administrator Software dialog.
10. Click **OK** to exit the software.

Editing Illumination Settings

To edit illumination settings:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Devices > Configure Illumination** to display the Configure Illumination dialog.

From the standard menu, click **Devices > Configure Illumination** to display the Configure Illumination dialog.

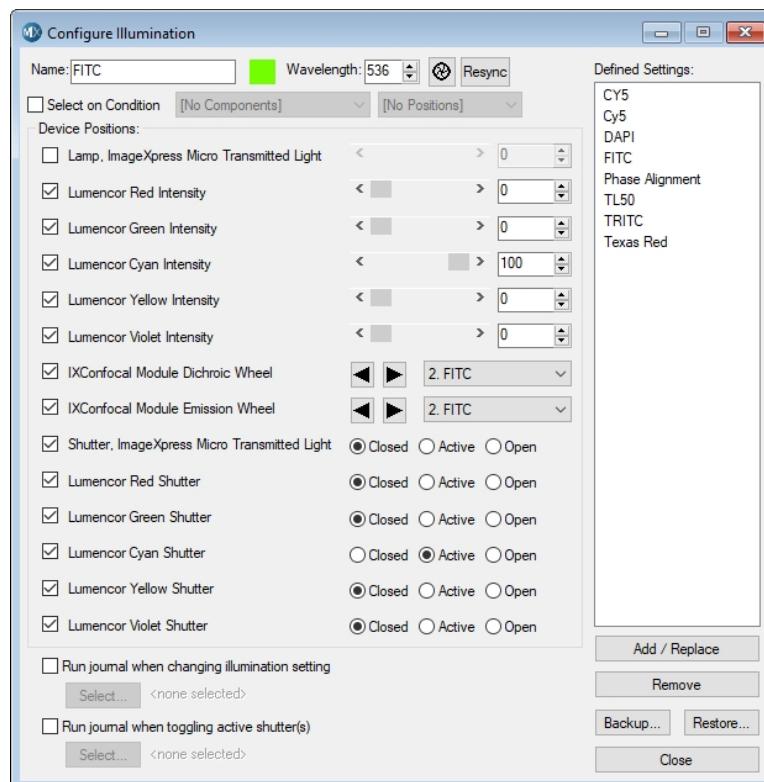


Figure 8-30: Configure Illumination Dialog

2. Ensure that the check box for illumination device you changed is selected in the **Device Positions** area and then select the filter that you installed from the corresponding drop-down.
3. Ensure that the appropriate shutter is selected as **Active** for each filter set.
4. 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.

5. In the **Name** field, enter the name of the new filter cube.
6. In the **Wavelength** field, enter the center emission wavelength of the new filter cube.
7. Click **Add/Replace** to add the setting to the Defined Settings list.

8. If you replace a filter that had an existing setting, select the old setting in the **Defined Settings** list and click **Remove**.
9. Click **Backup** to back up the settings to a file.
10. Click **Close**.

Settings for Common Fluorophores

Table 8-1: Settings for Common Fluorophores

Fluorophore	Shutter Color Channel	Excitation Filter
DAPI, Hoechst	Violet (380 to 420 nm)	377/54 nm
FITC, GFP, EGFP	Cyan (440 to 500 nm)	475/34 nm
Cy3	Green (490 to 550 nm)	531/40 nm or 543/22 nm, contact Molecular Devices Technical Support to verify.
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 System, you must physically change or add the component and then enter the changes in the software to reflect the new hardware configuration.



CAUTION! A Molecular Devices Field Service Engineer must install and remove water immersion objectives. The Water Immersion option supports up to two water immersion objectives and they must be installed in the first and/or fourth objective slots. If you use water immersion objectives, see [Water Immersion Option on page 184](#).

You can clean an objective that is installed in the instrument or remove the objective from the instrument for cleaning. See [Cleaning Objectives on page 219](#).

If you use oil-immersion objectives, see [Using Oil-Immersion Objectives on page 220](#).

The following color bands help you identify the magnification of the objectives:

- Red—4x
- Yellow—10x
- Green—20x
- Light blue—40x
- Dark blue—60x

Correct Objective Placement

Whenever possible, Molecular Devices installs objectives with correction collars in the outer positions 1 and 4. The outer positions provide easier access through the side panels to adjust the correction collar.



Tip: If you change positions of the objectives, you should install ELWD objectives (20X, 40X, 60X) or any objective with a correction collar, in one of the outer positions (1 or 4). See [Adjusting Objective Correction Collars on page 221](#).

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

If the objective you replace was the one you 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 230](#), or contact Molecular Devices technical support. See [Obtaining Support on page 244](#).



Note: Whenever possible, you should leave the reference objective in place and replace the other objectives. The reference objective is typically a 10x objective and is typically installed in position 3.

The following topics describe how to change or add an objective:

- [Creating Hardware Profiles, see page 202](#)
- [Installing Objectives, see page 222](#)
- [Editing Objective Settings in the Meta Imaging Series Administrator Software, see page 224](#)
- [Configuring Parfocality After Changing Objectives, see page 226](#)
- [Determining the Plate Bottom Reference Point After Changing the Reference Objective, see page 230](#)
- [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software, see page 235](#)
- [Updating Magnification Settings, see page 238](#)
- [Updating Shading Correction Settings-Legacy, see page 240](#)

Cleaning Objectives

Clean the objective when debris or contaminants collect on an objective.



CAUTION! You must contact Molecular Devices to schedule a Field Service Engineer to install or remove a water immersion objective.

Before you clean an objective, read and follow the [Maintenance Precautions on page 193](#).



CAUTION! To prevent skin oils from damaging the optical coatings, you should wear powder-free gloves during the following procedure.

To clean an objective:

1. Start the MetaXpress Software.
2. In the MetaXpress Software on the IX Taskbar, click **Adjust Correction Collar** to access the specific objective.
3. If the objective is accessible from the side, open the upper, hinged side panel door of the instrument.
4. If the objective is in one of the center positions, access it from the top. When prompted by the software, remove the plate or slide holder, and then click **Continue**.
5. Place the objective on a secure surface, away from the instrument, and use a bulb duster to carefully 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.

6. 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

You can use oil-immersion objectives with the ImageXpress Micro Confocal System for research-mode imaging. For ordering information, contact your sales representative.



Note: Oil-immersion objectives are not recommended to scan entire plates.

To apply oil to the objective:

1. In the MetaXpress Software from the IX Taskbar, click **Adjust Correction Collar** to access the specific objective. If prompted to select the mode of access, select **From the Top**.
2. When prompted, remove any plates in the instrument.
3. Use a dropper bottle to sparingly add oil to the top of the objective.
4. Insert the sample with either a thin glass coverslip, or in a plate with a thin glass bottom, and then click **Continue**.



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

5. In the Plate Acquisition Setup dialog, select the **Configure** tab then select the **Acquisition** tab.
6. Select the **Enable Image Based Focusing** check box.



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

7. Select the **Autofocus** tab.

8. Click the **Initial Well for Finding Sample** drop-down and select **Skip Find Sample**.



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

9. Slowly step up the objective manually until you are near to focus.
10. When you are done with the oil-immersion objective, click **Adjust Correction Collar** to access the objective again, and use lens paper to clean the top of the objective. If needed, remove the objective for better cleaning. See [Cleaning Objectives on page 219](#).

Adjusting Objective Correction Collars

The ELWD (extra long working distance) Nikon objectives and the water immersion objectives have an adjustable correction collar that minimize spherical aberration in the image of the specimen. The collar adjustment range varies depending on the objective. Change the range setting to adjusts the distances between components inside the objective barrel. Image quality and resolution are very much dependent on properly setting the objective collar.

The setting depends on the thickness of the plate well or slide on which you mount the specimen. In general, set the correction collar for the physical thickness of the plate or slide that you image.

Determine the physical thickness 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 determine the thickness of the plate or slide, you can adjust the correction collar.



CAUTION! If the thickness of the plate, slide, or cover slip is out of the range of the correction collar, you should not use it with the objective.

Before you adjust the correction collar on an objective, read and follow the [Maintenance Precautions on page 193](#).



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

To adjust an objective correction collar:

1. Start the MetaXpress Software.
2. In the IX Taskbar, click **Adjust Correction Collar** to access the objective.
3. If the objective is accessible from the side, open the upper, hinged side panel door of the instrument.
4. If the objective is in one of the center positions, access it from the top.
When prompted by the software, remove the plate or slide holder, and then click **Continue**.

5. Locate the correction collar on the objective.

Loosen or remove an air objective to locate the correction collar, if needed.



CAUTION! You must contact a Molecular Devices Field Service Engineer to remove a water immersion objective.

6. Rotate the correction collar to its new setting. If needed, replace or tighten the air objective.
7. If you access the objective from the side, close the upper, hinged side panel door.
8. Click **Continue**.
9. If you access the objective from the top, then replace the plate or slide holder when prompted, and then click **Continue**.
10. Test the correction collar setting by examining the image quality of acquired images. If the quality has degraded, repeat these steps to re-adjust the correction collar.

Installing Objectives

The objectives in the ImageXpress Micro Confocal System are mounted in a four-position linear selector. Objectives are very delicate components. Use special care when you handle objectives. Also see [Compatible Objectives on page 251](#).

To determine the correct position for the objective, see [Correct Objective Placement on page 219](#).



CAUTION! You must contact Molecular Devices to schedule a Field Service Engineer to install or remove a water immersion objective.

Before you replace an objective, read and follow the [Maintenance Precautions on page 193](#).



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

To install an objective:

1. Start the MetaXpress Software.
2. In the MetaXpress Software move the objective changer to the position to install the objective.
 - Select position 1 (the position on the right) to move the objectives to the left.
 - Select position 4 (the position on the left) to move the objectives to the right.
3. Exit the MetaXpress Software.
4. On the ImageXpress Systems Power and Options Controller for the ImageXpress Micro Confocal System, power OFF the instrument.
5. Place the new objective in its protective case 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.



WARNING! When you move objectives in and out, beware of the free-moving stage. It slides around loosely when the instrument is powered off.



CAUTION! Use caution to prevent damage to the objective in your hand.

7. Reach in and unscrew the objective 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 new 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 Systems Power and Options Controller for the ImageXpress Micro Confocal System, power ON the instrument.
12. Start the Meta Imaging Series Administrator Software.
13. Create a new hardware profile. See [Creating Hardware Profiles on page 202](#).
14. Create a new group and select the new hardware profile. See [Creating an Offline Version of the MetaXpress Software on page 122](#).
15. Create a new group icon. See [Creating MetaXpress Software Group Icons and Adding Them to the Windows Desktop on page 123](#).
16. Exit the Meta Imaging Series Administrator Software.
17. Start the MetaXpress Software using the new group icon.
18. From the simplified menu, click **Control > Devices > Configure Magnification** to display the Configure Magnification dialog.
From the standard menu, click **Devices > Configure Magnification** to display the Configure Magnification dialog.
19. Enter the new objective information. See [Updating Magnification Settings on page 238](#).
20. From the simplified menu, click **Measure > Distances > Calibrate Distances** to display the Calibrate Distances dialog.
From the standard menu, click **Measure > Calibrate Distances** to display the Calibrate Distances dialog.
21. In the Calibrate Distances dialog, verify that the pixel size calibration is set at or close to the default value for this magnification. See [Calibrating Distances on page 239](#).
22. Configure parfocality measurements using the **IX Taskbar** and enter **X,Y Offset** in the Configure Magnification dialog.
If the **IX Taskbar** is unavailable, then use the bead plate to do parfocality measurements. See [Configuring Parfocality After Changing Objectives on page 226](#).
23. Use the **IX Taskbar** to measure pixel sizes, then enter the results in the Calibrate Distances dialog. See [Calibrating Distances on page 239](#).
24. Exit the MetaXpress Software.
25. Start the Meta Imaging Series Administrator Software.

26. Enter the parfocality results (focus values) in the Objectives Settings dialog. See [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 235](#).
27. If you need to measure the plate bottom reference, then enable Maintenance Mode, otherwise, exit the Meta Imaging Series Administrator Software and continue to the next step. See [Finding the Plate Bottom Reference Objective and Reference Point on page 230](#) and [Editing Objective Settings in the Meta Imaging Series Administrator Software on page 224](#).



CAUTION! Unless there is an important reason to change the Plate Bottom Reference Objective after the system is installed, you should leave the Plate Bottom Reference Objective in place at all times. Contact Technical Support for assistance.

28. Start the MetaXpress Software.
29. Verify that the new objective is listed in the **Magnification** list. See [Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 63](#).
30. If needed, create new shading correction files. See [Updating Shading Correction Settings-Legacy on page 240](#).
31. Configure the Laser Auto Focus settings for the new objective with the plates and slides. See [Confirming Laser Autofocus Settings for Plate Files on page 44](#) and [Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 64](#).

Editing Objective Settings in the Meta Imaging Series Administrator Software

To edit objective settings:

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

- Click **Settings** to display the ImageXpress Micro Objective Settings dialog.

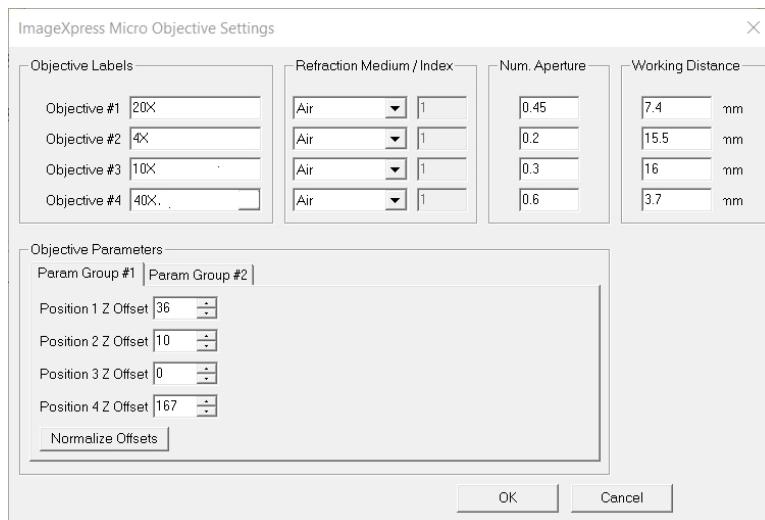


Figure 8-31: ImageXpress Micro Objective Settings Dialog

- In the **Objective #** row for the new objective:
 - In the **Objective Label** field, edit the text, if necessary.
 - Click the **Refractive Medium/Index** drop-down and select the refractive medium and enter the working distance in the text field, if necessary. If the objective has a range, enter the higher number.
 - In the **Num. Aperture** field, enter the numerical aperture for the new objective as is printed on the objective.
 - In the **Working Distance** field, enter the working distance. If it has a range, enter the higher number.



Note: Make a note of these values. You need to enter this information again for the hardware settings.

- Click **OK** in the ImageXpress Micro Objective Settings dialog to display the Install System Devices dialog.
- In the Installed Devices field, select **ImageXpress Micro** and then click **Settings** to display the ImageXpress Micro Settings dialog.
- Select the **Parameter Group #1** tab.
- For a Plate Bottom Reference and XY reference, select the **Maintenance Mode** check box.
- Click **OK** to display the Install System Devices dialog.
- Click **OK** to display the Configure Hardware dialog.



Note: In the next steps, enter the values from earlier in the Configure Devices dialog to ensure that the settings carry over for all hardware profiles.

- In the Hardware Settings list select the hardware settings you use and then click **Configure Devices** to display the User Settings dialog.

15. In the Claimed Devices list select **ImageXpress Micro Objective** and click **Settings** to display the ImageXpress Micro Objective Settings dialog.
16. In the **Objective #** row:
 - a. In the **Objective Labels** field, enter the same information you previously entered.
 - b. Click the **Refractive Medium/Index** drop-down and select the same value you previously entered and enter the same value in the text field.
 - c. In the **Num. Aperture** field, enter the numerical aperture for the new objective with the same information you previously entered.
 - d. In the **Working Distance** field, enter the same working distance you previously entered. If it has a range, enter the higher number.
17. Click **OK** to display the User Settings dialog.
18. Click **OK** to display the Configure Hardware dialog.
19. Click **OK** to display the Meta Imaging Series Administrator dialog.
20. Click **OK** to exit the software.
21. Configure parfocality. See [Configuring Parfocality After Changing Objectives on page 226](#).

Configuring Parfocality After Changing Objectives

To configure parfocality, 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. You can configure parfocality with or without the taskbar.



CAUTION! You must contact Molecular Devices to schedule a Field Service Engineer to install or remove a water immersion objective.

Configuring Parfocality With the Taskbar

If the IX Taskbar is not available, see [Configuring Parfocality Without the Taskbar on page 227](#).

To configure parfocality:

1. In the IX Taskbar, click **System Maintenance**.



Note: If the Main Taskbar is not visible, press **F4**.



Figure 8-32: IXM Main Taskbar

2. Click **Parfocality and XY Offsets** and follow the prompts in the dialogs that display.

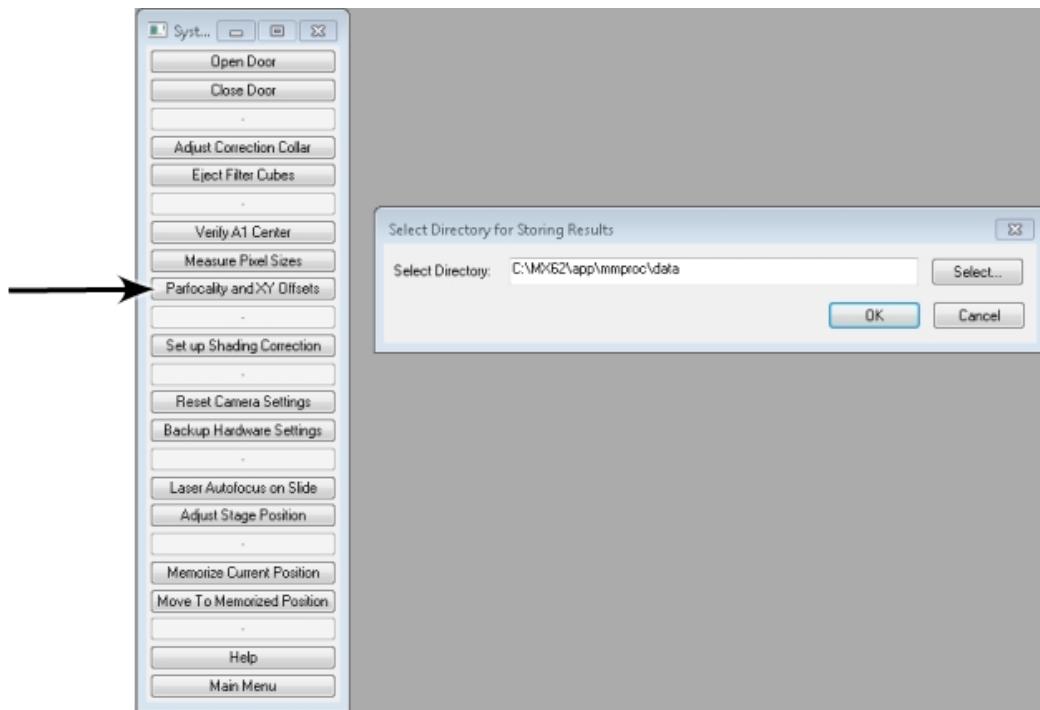


Figure 8-33: System Maintenance Taskbar

Configuring Parfocality Without the Taskbar

To configure parfocality when the taskbar is not available:

1. In the MetaXpress Software:

From the simplified menu, click **Control > Devices > Configure Magnification** to display the Configure Magnification dialog.

From the standard menu, click **Devices > Configure Magnification** to display the Configure Magnification dialog.

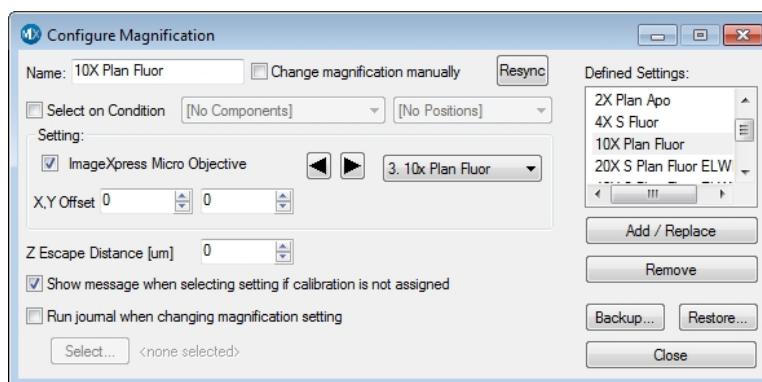


Figure 8-34: Configure Magnification Dialog

2. Select the **ImageXpress Micro Objective** check box.
3. Click the **Setting** drop-down and select the objective with the highest numerical aperture (NA) that is in position #1.

4. If the Device Control toolbar is not visible, click **Window > Toolbars > Device Control**.
5. In the Device Control toolbar, click the **Illum** drop-down and select the **FITC** (or other visible light) illumination setting.



Figure 8-35: Illum Setting in the Device Control Toolbar

6. If the Plate Acquisition toolbar is not visible, click **Window > Toolbars > Plate Acquisition**.



7. In the **Plate Acquisition** toolbar, click to move the stage to the load position.
8. Load the bead plate that ships with the instrument on to the stage
9. Click again to return the stage to its previous position.
10. From the simplified menu, click **Control > Acquire > Acquire** to display the Acquire dialog.
From the standard menu, click **Acquire > Acquire** to display the Acquire dialog.
11. Click **Show Live** to display a Live image window.
12. From the simplified menu, click **Control > Devices > Move Stage to Absolute Position** to display the Move Stage to Absolute Position dialog.
From the standard menu, click **Devices > Move Stage to Absolute Position** to display the Move Stage to Absolute Position dialog.

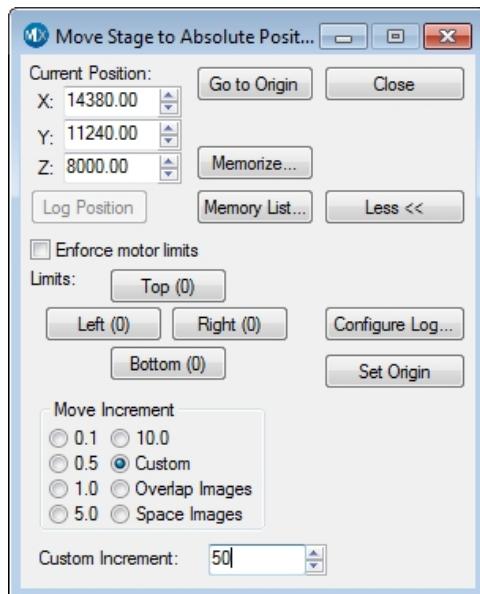


Figure 8-36: Move Stage to Absolute Position Dialog

13. 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.

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

Table 8-2: Objective Focus Values

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

15. In the Configure Magnification dialog, click the **Setting** drop-down and select the objective with the next highest numerical aperture (NA) that is in position #2. Repeat the following steps for each objective 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.

16. 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.
17. When the sample is in focus, write down the **Current Position: Z** value.
18. After you write down the focus values for each objective, in the Plate Acquisition toolbar, click , then click  to move the stage to the load position.
19. Remove the bead plate, and then click  again to return the stage to its previous position.
20. Continue to update the software settings:
- If the objective you change is the one you use to determine the plate bottom reference point, see [Determining the Plate Bottom Reference Point After Changing the Reference Objective on page 230](#). Whenever possible, you should leave the 10x objective in place and replace the other objectives.
 - If the objective is NOT the one you use 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 235](#).

Determining the Plate Bottom Reference Point After Changing the Reference Objective



Note: Do this procedure only if you replace 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 235](#).

The plate bottom reference point is a setting that the MetaXpress Software uses for auto focus. Plate bottom reference is configured before the system is shipped. 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: Whenever possible, you should leave the reference objective in place and replace the other objectives.

There are two separate procedures to update the plate bottom reference point:

- [Finding the Plate Bottom Reference Objective and Reference Point on page 230](#)
- [Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 234](#)



Note: You should contact technical support before you attempt these procedures. See [Obtaining Support on page 244](#).

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 the 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 (between 1 to 4).

If you update the plate bottom reference point, you must update the value in the calibration file.

Finding the Plate Bottom Reference Objective and Reference Point

Before you start this procedure, make sure that the MetaXpress Software is in maintenance mode. See [Editing Objective Settings in the Meta Imaging Series Administrator Software on page 224](#).



CAUTION! The software applies the plate bottom reference objective position to all hardware profiles.

To find the plate bottom reference objective and reference point:

1. Start the MetaXpress Software.
2. If the Plate Acquisition tool bar is not visible, click **Window > Toolbars > Plate Acquisition**.
3. In the Plate Acquisition toolbar, click  to move the stage to the load position.
4. Remove any plate from the stage and load one of the flat-field correction (FFC) plates that shipped with the system.
5. Click  again to return the stage to its previous position.
6. From the simplified menu, click **Control > Devices > Configure Magnification** to display the Configure Magnification dialog.
From the standard menu, click **Devices > Configure Magnification** to display the Configure Magnification dialog.
7. Select the **ImageXpress Micro Objective** check box.
8. Click the corresponding drop-down and select the objective to use as the reference.

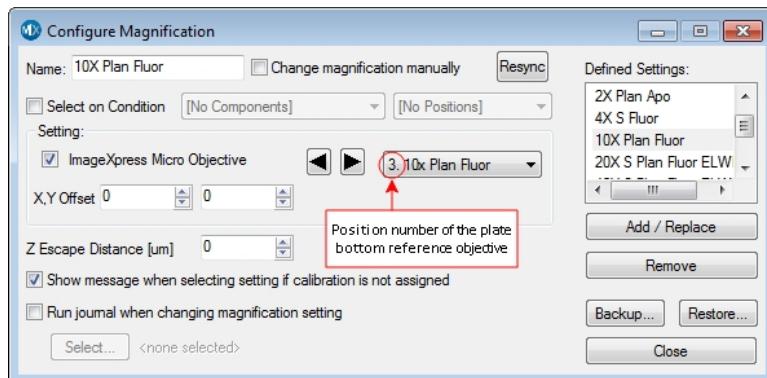


Figure 8-37: Configure Magnification Dialog

9. Write down the position number of the objective in the following table. You will need this number in [Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 234](#).

Table 8-3: Plate Bottom Reference Objective

Plate Bottom Reference Objective Position

10. Click **Close** and in the Replace Stored Settings message, click **No**.
11. From the simplified menu, click **Control > Devices > Move Stage to Absolute Position** to display the Move Stage to Absolute Position dialog.
From the standard menu, click **Devices > Stage > Move Stage to Absolute Position** to display the Move Stage to Absolute Position dialog.
12. In the **Current Position: Z** field, enter **6000** and then press **Enter** to move the Z-motor.
13. In the **Current Position: X** field, enter **64900**.

14. In the **Current Position: Y** field, enter **42200**, and then press **Enter** to move to the approximate center of the plate.
15. From the simplified menu, click **Control > Devices > Focus** to display the Focus dialog. From the standard menu, click **Devices > Focus** to display the Focus dialog.
16. Select the **Auto Focus** tab.
17. Click **Configure Laser** to display the Configure Laser Autofocus dialog.

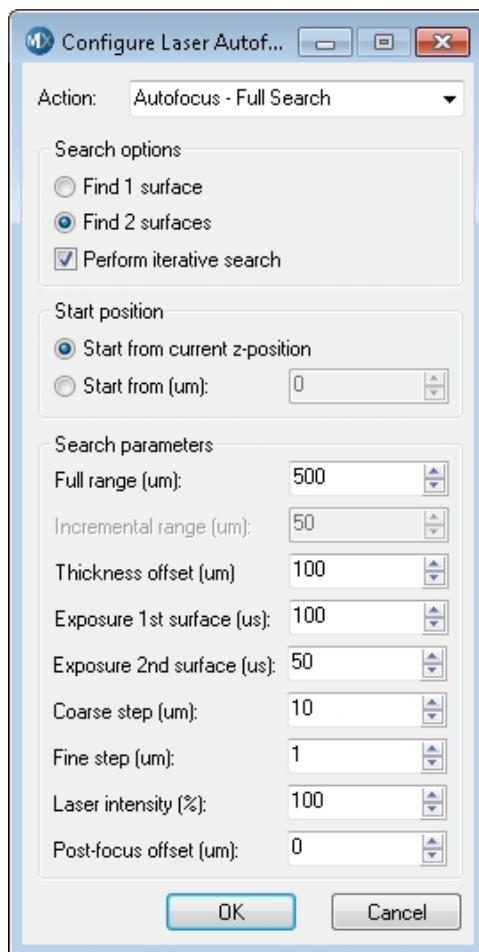


Figure 8-38: Configure Laser Autofocus dialog

18. Select the **Start from Current Z-Position** Start Position option.
19. In the **Full Range** field, enter **1000**.
20. In the **Exposure 1st Surface** field, enter **10**.
21. In the **Coarse Step** field, enter **5**.



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

22. In the **Laser Intensity** field, enter **100**.

23. In the Focus dialog, on the Auto Focus tab, click **Preview Pass** to display a Preview Pass image window that displays a graph of focus intensities compared to Z-position.

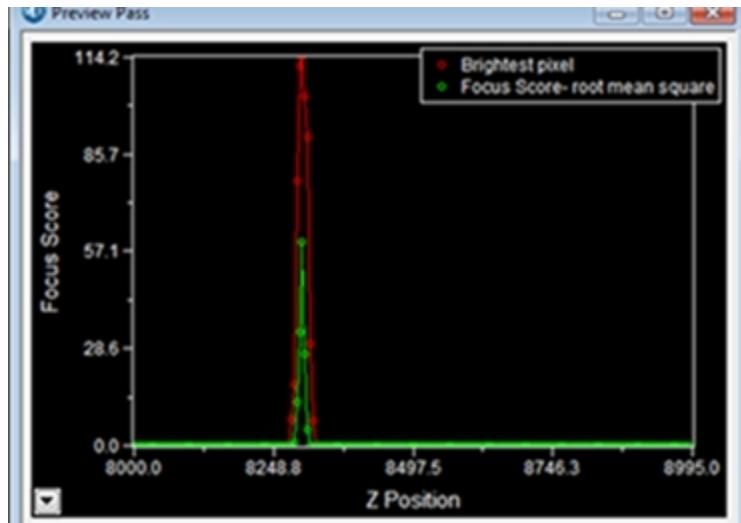


Figure 8-39: Preview Pass Image Window

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:

- In the Focus dialog, increase the **Start Position** value by 1000 and click **Preview Pass**. Repeat until you cover a range between 6000 and 10000.
- If there still is no peak in the graph, move to a new area of the plate and click **Preview Pass**. If no peak after you move twice and cover a z-range from 6000 to 10000, contact Technical Support.

24. In the Preview Pass image window, click the trace line at the top of the green line of the first peak. The graph X, Y coordinates display as a tool tip. The X coordinate is the value for the Z-position, 8300 in the example.

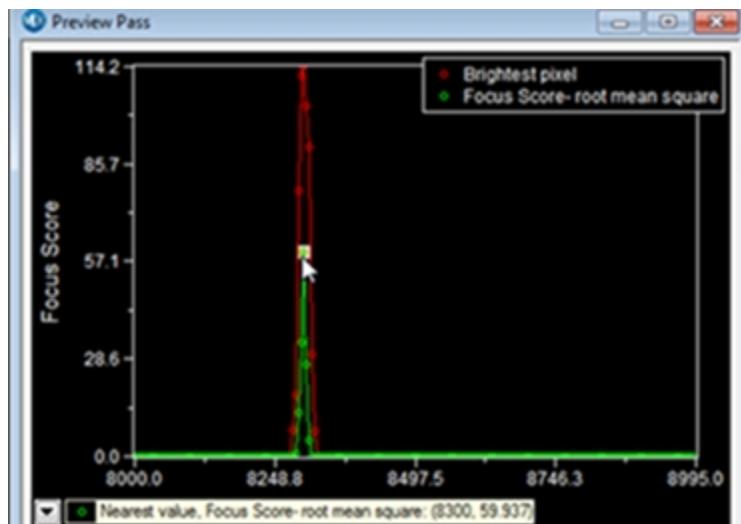


Figure 8-40: Z-Position of Peak

25. Write down the Z-position for the center focus position in the following table.

Table 8-4: Plate Bottom Reference Points

Position	X Value	Y Value	Z Value
Center	64900	42200	
Upper-Left	8900	77200	
Lower-Left	8900	7200	
Upper-Right	120900	77200	
Lower-Right	120900	7200	

26. In the Move Stage to Absolute Position dialog, in the **Current Position: X** field, enter **8900**.
27. In the **Current Position: Y** field, enter **77200** and then press **Enter** to move to the upper-left corner of the plate.
28. In the Focus dialog, on the Autofocus tab, click **Preview Pass** to display a Preview Pass image window.
29. Click the trace line at the top of the green line of the first peak.
30. Write down the Z-position for the upper-left position.
31. Repeat the previous five steps for the other three corners of the plate, using the X and Y values in [Table 8-4](#).
32. 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 235](#).
33. In the Plate Acquisition toolbar, click  to move the stage to the load position.
34. Remove the flat-field correction (FFC) plate, and then click  again to return the stage to its previous position.
35. Exit the MetaXpress Software.
36. Continue to the next topic to edit the **MetaXpress.ref** file.

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



Note: Only do this procedure if you need to complete [Finding the Plate Bottom Reference Objective and Reference Point on page 230](#).

To enter the value for the position of the plate bottom reference objective in the **MetaXpress.ref** configuration file:

1. Click **Start > All Programs > Accessories > Notepad** to open the Notepad application.
2. In Notepad, click **File > Open** to display the Open dialog.

3. Navigate to the **C:\MX6\Hardware** (or the location you used for a custom installation) and select the **MetaXpress.ref** file.



Note: Make sure that the copy of the MetaXpress.ref that you open is in the Hardware folder of the MetaXpress Software installation folder (For example, C:\MX6\Hardware) and NOT in main MetaXpress installation folder.

4. Click **Open** to open the file in Notepad.
5. Find the following line:
[system calibration]
PlateBottomReferenceObjective=X
where **X** is the position of the objective used as the plate bottom reference objective.
6. Replace the number at the end of the line with the objective position you wrote down. See [Table 8-3: Plate Bottom Reference Objective on page 231](#).
7. 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.

8. Exit Notepad.
9. Continue to update the software settings. See [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 235](#).

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

Turn off maintenance mode before you do the steps in this topic. See [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 235](#).

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** to display the Meta Imaging Series Administrator dialog.
2. In the List of Groups, select **MetaXpress**.
3. Click **Configure Hardware** to display the Configure Hardware dialog.
4. Click **Install System Devices** to display the Install System Devices dialog.
5. In the Installed Devices field, select **ImageXpress Micro**.
6. Click **Settings** to display the ImageXpress Micro Settings dialog.
7. Select the **Parameter Group #1** tab.
8. Clear the **Maintenance Mode** check box.
9. Click **OK** to display the Install System Devices dialog.
10. In the Installed Devices field, select **ImageXpress Micro Objective**.

- Click **Settings** to display the ImageXpress Micro Objective Settings dialog.

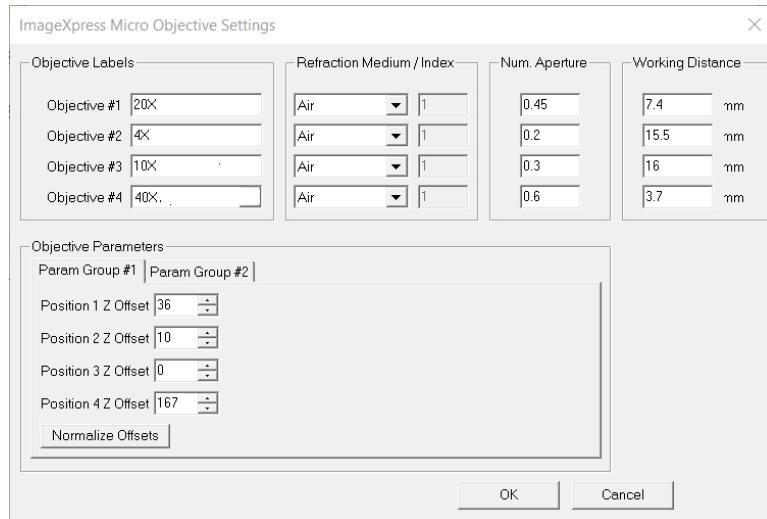


Figure 8-41: ImageXpress Micro Objective Settings Dialog

- In the **Position 1 Z Offset** field, enter the focus value you wrote down for the objective 1. See [Table 8-2: Objective Focus Values on page 229](#).



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.

- In each **Position # Z Offset** field, enter the focus value for the other three objectives.



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

- Do not click Normalize Offsets.
- Click **OK** to display the Install System Devices dialog.
- If you did the steps to [Finding the Plate Bottom Reference Objective and Reference Point on page 230](#):
 - In the Installed Devices field, select **ImageXpress Micro Z**.
 - Click **Settings** to display the ImageXpress Micro Z Settings dialog.
 - In the **Plate Bottom Reference** field, enter the lowest plate bottom reference point Z-position value that you wrote down and circled. See [Table 8-4: Plate Bottom Reference Points on page 234](#).
 - Click **OK** to display the Install System Devices dialog.
- Click **OK** to display the Configure Hardware dialog.



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

18. In the Hardware Settings list, ensure that the hardware settings you use are selected and click **Configure Devices** to display the User Settings dialog.
19. In the Claimed Devices field, select **ImageXpress Micro Objective**.
20. Click **Settings** to display the ImageXpress Micro Objective Settings dialog.
21. In the **Objective Labels, Refraction Medium/ Index, Num. Aperture**, and **Working Distance** fields, enter the values that you entered after you changed the objectives. See [Editing Objective Settings in the Meta Imaging Series Administrator Software on page 224](#).
22. In the **Position 1 Z Offset**, **Position 2 Z Offset**, **Position 3 Z Offset**, and **Position 4 Z Offset** fields, enter the corresponding focus values that you wrote down for objectives 1, 2, 3, and 4. See [Table 8-2: Objective Focus Values on page 229](#).
23. Do not click Normalize Offsets.
24. Click **OK** to display the Install System Devices dialog.
25. If you completed [Finding the Plate Bottom Reference Objective and Reference Point on page 230](#):
 - a. In the Installed Devices field, select **ImageXpress Micro Z**.
 - b. Click **Settings** to display the ImageXpress Micro Z Settings dialog.
 - c. In the **Plate Bottom Reference** field, enter the lowest plate bottom reference point Z-position value that you wrote down and circled. See [Table 8-4: Plate Bottom Reference Points on page 234](#).
 - d. Click **OK** to display the Install System Devices dialog.
26. Click **OK** to display the Configure Hardware dialog.



Note: If you use more than one hardware profile, repeat the previous steps as needed for each affected hardware profile.

-
27. Click **OK** to display the Meta Imaging Series Administrator dialog.
 28. Click **OK** to exit the software.
 29. Update the settings in the MetaXpress Software. See [Updating Magnification Settings on page 238](#).

Updating Magnification Settings

Before you start this procedure, make sure that the MetaXpress Software is NOT in maintenance mode. See [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 235](#).

To update magnification settings:

1. Start the MetaXpress Software.
2. From the simplified menu, click **Control > Devices > Configure Magnification** to display the Configure Magnification dialog.

From the standard menu, click **Devices > Configure Magnification** to display the Configure Magnification dialog.

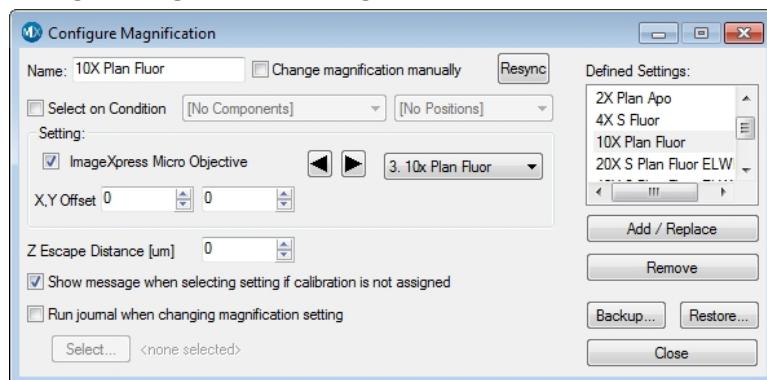


Figure 8-42: The Configure Magnification dialog

3. Select the **ImageXpress Micro Objective** check box.
4. Click the **Setting** drop-down and select the installed objective.
5. In the **Name** field, enter the name for the new objective.
6. Click **Add/Replace** to add the objective to the Defined Settings list.
7. If you replaced an objective with an existing setting, select the old setting in the Defined Settings list and click **Remove**.
8. After the settings update, click **Backup** and save the magnification file.
9. Click **Close**.

See [Calibrating Distances on page 239](#) as needed.

Calibrating Distances

Turn off maintenance mode before you do the steps in this topic. See [Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 235](#).

To calibrate distances:

- From the simplified menu, click **Measure > Distances > Calibrate Distances** to display the Calibrate Distances dialog.

From the standard menu, click **Measure > Calibrate Distances** to display the Calibrate Distances dialog.

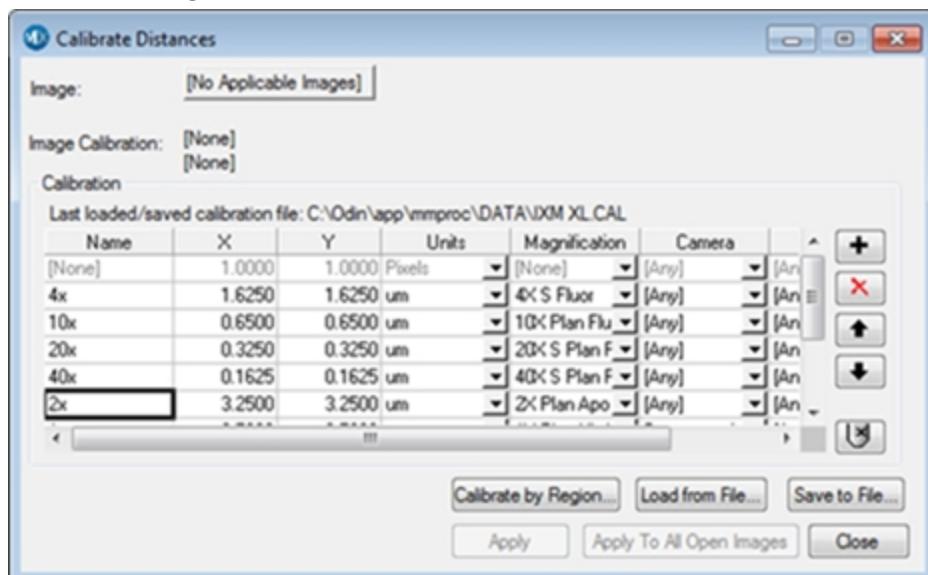


Figure 8-43: Calibrate Distances Dialog

- Click **+** to add a new Calibration row.

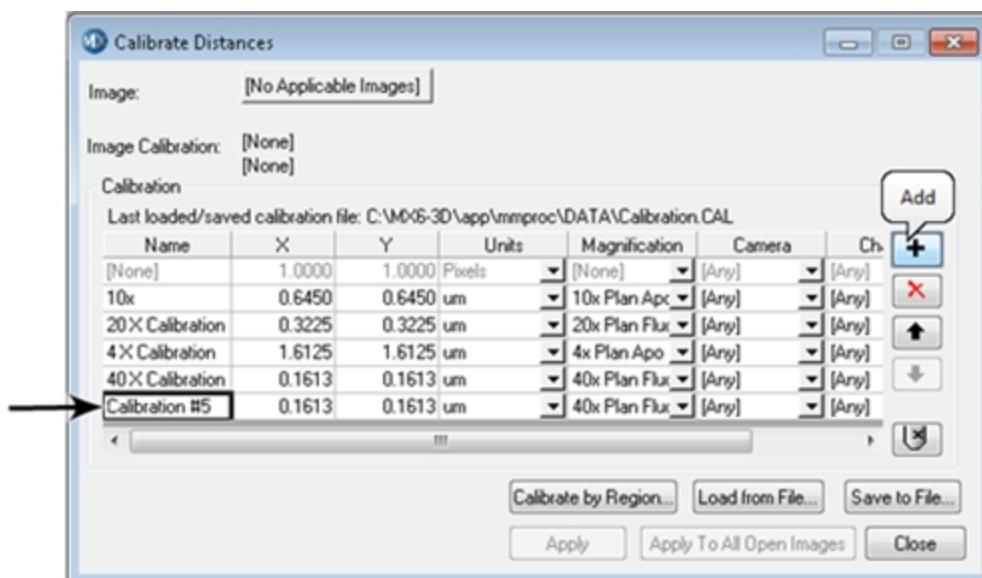


Figure 8-44: Add a New Calibration Row

3. Double-click in the **Name** field, enter the name of the new objective, and then press **Enter**.
4. In the **X** and **Y** fields, enter the calibration values for the new objective. X and Y values must match.

You can use the following estimated values for ImageXpress Micro Confocal System calibration settings:



Note: To measure pixel sizes more accurately, in the IX Taskbar, click System Maintenance and then click Measure Pixel Sizes. If the IX Taskbar is not installed, contact Technical Support.

Table 8-5: Estimated Calibration Settings

Objective Magnification	Unbinned Pixel Size (um)	Width (mm)	Height (mm)	Area (mm ²)
1x	6.8313	13.9905	13.9905	195.7342
2x	3.4157	6.9953	6.9953	48.9335
4x	1.7078	3.4976	3.4976	12.2334
10x	0.6831	1.3991	1.3991	1.9573
20x	0.3416	0.6995	0.6995	0.4893
40x	0.1708	0.3498	0.3498	0.1223
60x	0.1139	0.2332	0.2332	0.0544
100x	0.0683	0.1399	0.1399	0.0196

5. Click **Magnification** drop-down and select the new objective.
6. Click the **Camera** drop-down and select a camera.
7. Click the **Channel** drop-down and select a channel.
8. Click **Save to File** and save the file.
9. Click **Close**.

See [Updating Magnification Settings on page 238](#) as needed.

Updating Shading Correction Settings-Legacy



Note: This procedure is required if you select the Legacy Correction check box for one or more acquisition wavelengths.

If you select Legacy Correction as the Shading Correction method for an acquisition wavelength, you need the shading correction image files for each objective and filter set and then generate the files again whenever you replace an objective or filter, or whenever you replace the light guide.

To generate the shading correction files, you need a shading correction plate that is appropriate for the filter set you use. Shading correction plates are included in the 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 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 the computer:

1. Start the MetaXpress Software.
2. From the simplified menu, click **Control > Journal > Taskbars > Load Taskbar** to display the Select a Taskbar dialog.
From the standard menu, click **Journal > Taskbars > Load Taskbar** to display the Select a Taskbar dialog.
3. Navigate to the **C:\MX6\Taskbars** or to the location of your custom installation.
4. Select the **System Maintenance Taskbar.jtb** file. If the System Maintenance Taskbar.jtb file does not exist, see [Importing the Main Taskbar Journal Suite on page 241](#).
5. Click **Open** to load the taskbar.
6. After you meet all requirements, see [Running the Shading Correction Journal from the Main Taskbar on page 244](#).

Importing the Main Taskbar Journal Suite

The IX Taskbar is a collection of tools that enhance and streamline common imaging workflows.

Do the following procedure only if the Main Taskbar is not installed on the computer. If the Main Taskbar is installed, see [Running the Shading Correction Journal from the Main Taskbar on page 244](#).



Tip: If you plan to create additional groups (or configurations) in the Meta Imaging Series Administrator Software, do this procedure before you create 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 244](#).
2. Download the **IXM_Taskbar_v#.jzp** file to the computer.
3. Start the instrument.
4. Start the MetaXpress Software.

5. From the standard menu, click **Journal > Import Journal Suite** to display the Import Journal Suite dialog.
From the simplified menu, click **Control > Journal > Import Journal Suite** to display the Import Journal Suite dialog.

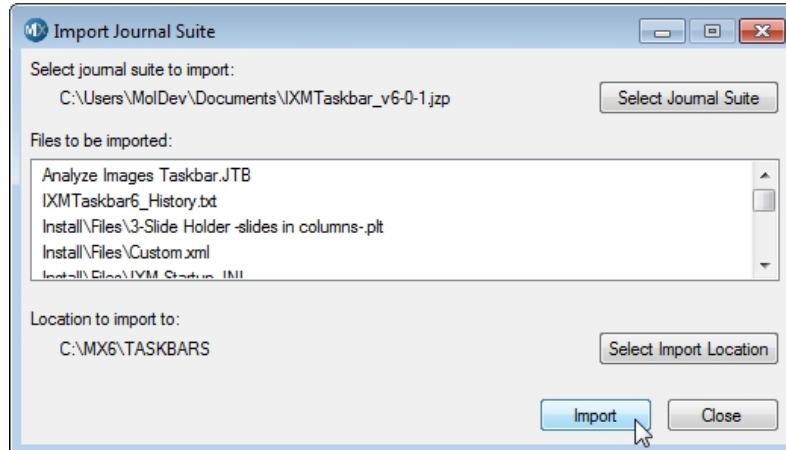


Figure 8-45: Import Journal Suite Dialog

6. Click **Select Journal Suite** to display the Select Import Suite File Name dialog.
7. Navigate to and select the **IXM_Taskbar_v#.jzp** file.
8. Click **Open** to display the Import Journal Suite dialog.
9. Click **Select Import Location** to display the Browse for Folder dialog.
10. Navigate to **C:\MX6\Taskbars** or the location in your custom installation or if it is missing, create a **Taskbars** folder.



Tip: To determine the correct installation folder, right-click the MetaXpress Software icon on the desktop and select **Open File Location**.

-
11. Click **OK** to display the Import journal Suite dialog.
 12. Click **Import**.
 13. Click **Close**.
 14. From the simplified menu, click **Control > Journal > Taskbars > Load Taskbar** to display the Select a Taskbar dialog.
From the standard menu, click **Journal > Taskbars > Load Taskbar** to display the Select a Taskbar dialog.
 15. Navigate to **C:\MX6\Taskbars** or the location in your custom installation.
 16. Select the **Main Taskbar.jtb** file.

17. Click **Open** to display the Main Taskbar dialog.

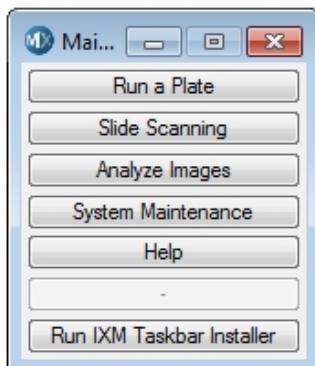


Figure 8-46: Main Taskbar Dialog

18. Click **Run IX Taskbar Installer** to display the IX Taskbar Installer dialog.

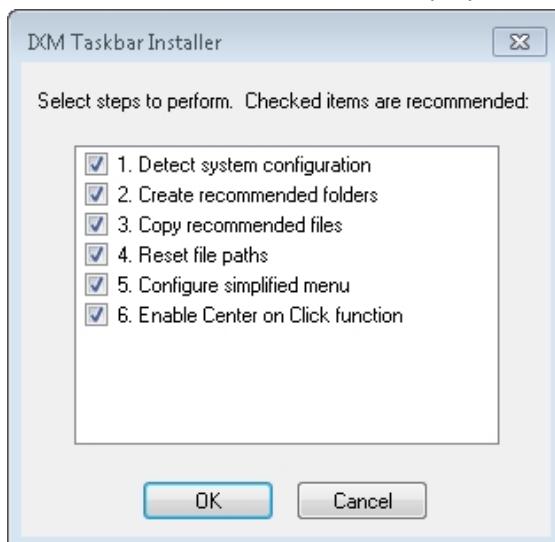


Figure 8-47: IX Taskbar Installer

19. Select the check box for each step to perform.



Note: The check boxes that are pre-selected are the recommended tasks for the configuration.

20. Click **OK**.
21. If the Select Configurations dialog displays, select the current group or select **All Groups** and click **OK**.
You should select **All Groups**.
22. If the Select Users dialog displays, select the current user or select **All Users** and click **OK**.
You should select **All Users**.
23. In the Select Directory for Default Paths dialog, navigate to **C:\MX6** of the main MX6 folder for your custom installation.
24. Click **OK** to display the Configure Menu dialog.

25. Select to **Install** or **Uninstall** the simplified menu.
You should use the simplified menu. See [Simplified Menu Structure on page 19](#).
26. Click **OK**.
27. Exit and restart the MetaXpress Software.

Running the Shading Correction Journal from the Main Taskbar

To run 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.

Turning Off the Status Indicator Lights

The edges of the ImageXpress Micro System illuminate with colors that provide information about the instrument status. See [Understanding Status Lights on page 28](#).

To turn off the instrument status indicator lights from the MetaXpress Software:

1. From the simplified menu, click **Control > Devices >Device Control** to display the Device Control dialog.
From the standard menu, click **Devices >Device Control** to display the Device Control dialog.
2. Select the **Other** tab.
3. Clear the **Enable Microscope LED** check box.
4. Click **Close**.

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 level of technical service.

Our Support website, www.moleculardevices.com/service-support, describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

Technical Support

You can contact Molecular Devices Technical Support by submitting a support request through the Molecular Devices Knowledge Base at mdc.custhelp.com. You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

You will need the instrument serial number and the software system ID. In the MetaXpress Software, click **Help > About MetaXpress**. The **About** dialog displays the system ID. The serial number is located on the back panel of the instrument.



Figure 8-48: Serial Number Location on the Instrument Rear

Newer systems have the serial number automatically displayed in the upper right corner of the computer desktop screen.

Documentation

Review the product documentation on the installation media. In addition, online Help is available within the MetaXpress Software. Press **F1** to access Help for the current dialog.

Additional Resources

Web-based microscopy courses:

- www.microscopyu.com
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The Molecular Probes Handbook offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

- www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

Filter information:

- www.semrock.com
- www.chroma.com
- www.omegafilters.com

Gathering Support Information

Before you contact Molecular Devices for support, you need the following information available to help the Technical Support personnel troubleshoot the problem:

- 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:

- **Plate Acquisition Settings File:** By default, the plate acquisition settings file saves to the database. To save the settings to a file, in the Plate Acquisition Setup dialog, select the Experiment tab. Click Save Protocol and then select Save to File. The settings file saves to the C:\MX6\HTSSTATE folder by default.
- **Journal files:** If you ran a journal when the problem occurred, include copies of the journal files that you used. By default, journal files save in the C:\MX6\app\mmproc\journals folder. If you run journals through the Review Plate Data tab, the journals save in C:\analysis and C:\assay.
- **System Information Report:** This report contains information about system settings and the release levels of the .dll files in the software. For steps to create a System Information report that you can email to Molecular Devices, see [Creating System Information Reports on page 248](#).

Logging AxoTrace Software Messages to .txt Files

The AxoTrace Software tracks the hardware status of the ImageXpress Micro System and logs 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. Do the following 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.

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 8-49: AxoTrace Software Icon in the Windows Taskbar

2. Exit the MetaXpress Software.
3. Double-click the AxoTrace Software icon in the Windows taskbar to display the AxoTrace dialog.

4. Click **Options** and make sure that the **Time Prefix** option displays a check mark.

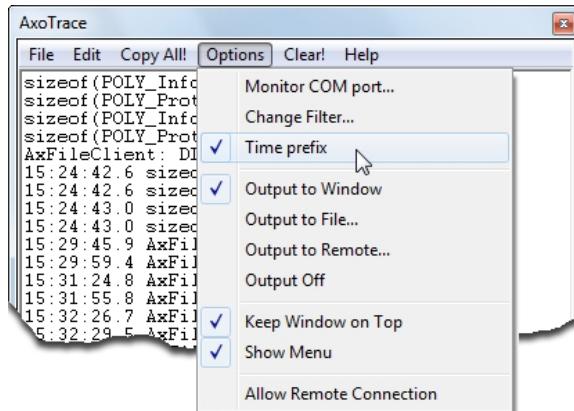


Figure 8-50: AxoTrace Software Options Menu

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.
9. After you reproduce the error, exit the MetaXpress Software.
10. Double-click the AxoTrace Software icon in the taskbar to display the AxoTrace dialog.
11. Click **File > Save As** to display the Save As dialog.
12. In the **Name** field, enter the file name.
13. Navigate to where you want to save the log file.
14. Click **Save** to display the AxoTrace dialog.
15. Click **File > Exit**.
16. Send the AxoTrace Software log as an email attachment to Molecular Devices Technical Support. See [Obtaining Support on page 244](#).

Creating System Information Reports

The System Information Report contains system setting information that is useful to resolve issues.

To create a System Information Report.

1. In the MetaXpress Software, click **Help > About MetaXpress** to display the About MetaXpress dialog.
2. In the fields on the right, enter your contact information.

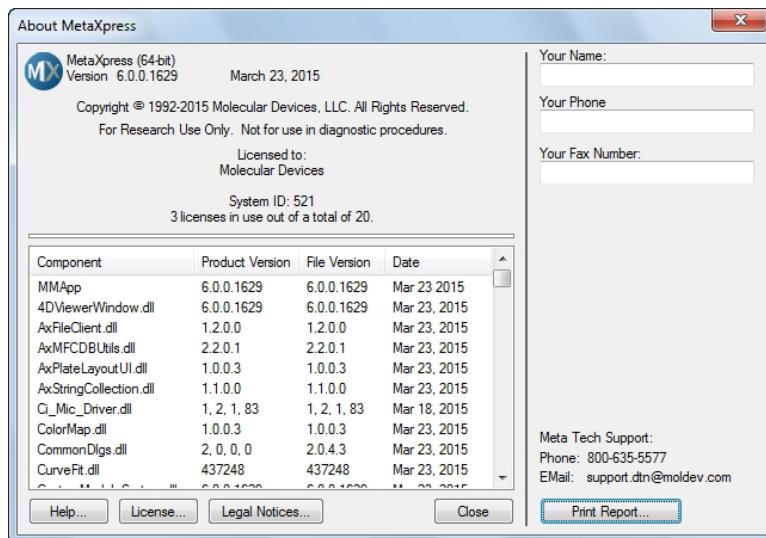


Figure 8-51: About MetaXpress Dialog

3. Click **Print Report** to display the Print Setup dialog.
4. Click the **Name** drop-down and select **Adobe PDF** or to a text file.
5. Click **OK** to display the Save As dialog.
6. In the **Name** field enter the file name.
7. Navigate to the location to save the file.
8. Click **Save** to display the report in .pdf format.
9. Send the report file to the Molecular Devices support personnel as an email attachment.

Appendix A: Instrument Specifications

For additional specifications, refer to the *Pre-Installation Guide*.



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

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	I
Installation category	II
Pollution degree	2
Ingress Protection	IP20

Instrument Dimensions

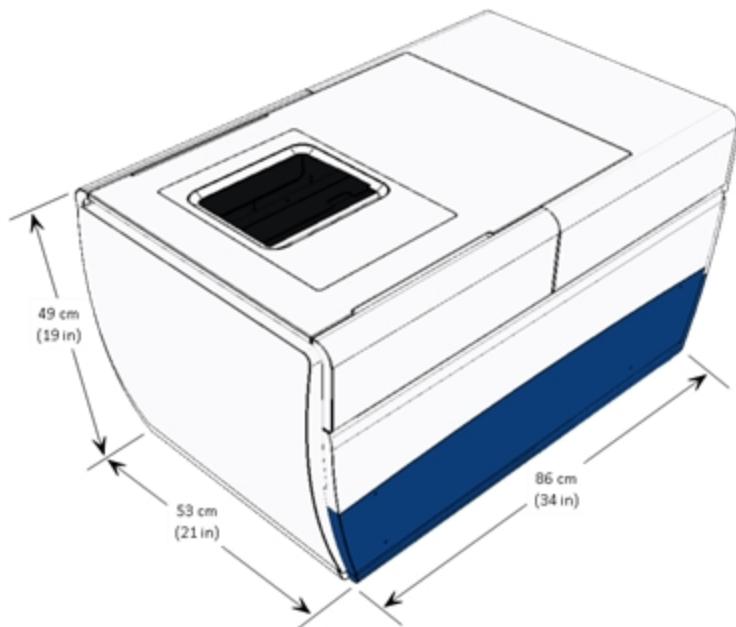


Figure A-1: Instrument Height, Width, and Length

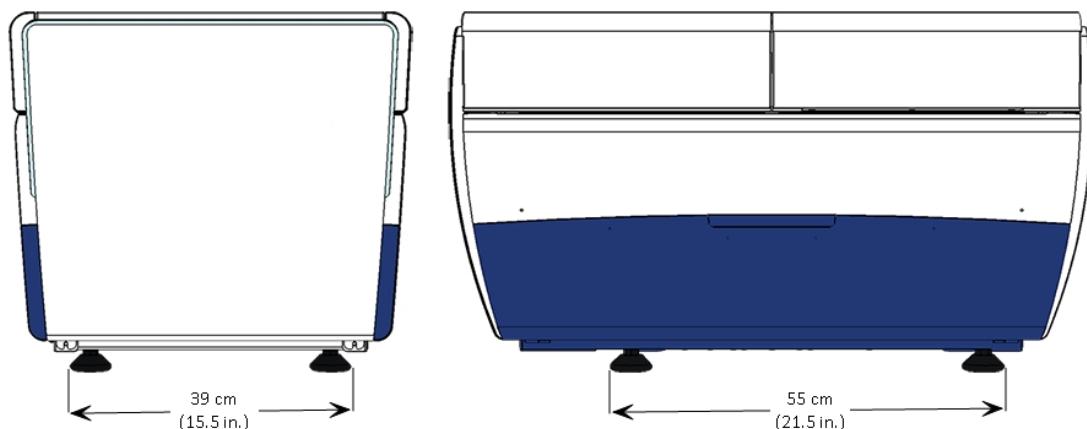


Figure A-2: Distance Between the Outside Edges of the Instrument Feet

Appendix B: Compatible Objectives

The following table details the Nikon objectives that are compatible with the ImageXpress Micro Confocal System. It also provides plate compatibility information and theoretical calculation of confocal performance for the objectives you use in the system.

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System and Settings

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 (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)

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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 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)

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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 (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 , 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

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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	5038915	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 (0.17 mm) No Skirt
60x Plan Apo Lambda 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

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro Confocal System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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)
20xc WI (Water Immersion) CFI Apochromat LWD Lambda S cc 0.11 mm to 0.23 mm	5075816	No	0.95	0.95	Thin bottom (0.17 mm) ¹ , Thin bottom (0.17 mm) No Skirt
40xc WI (Water Immersion) CFI Apochromat LWD Lambda S cc 0.15 mm to 0.19 mm	5075817	No	1.15	0.59	Thin bottom (0.17 mm) ¹ , Thin bottom (0.17 mm) No Skirt
60xc WI (Water Immersion) CFI Plan Apochromat VC cc 0.15 mm to 0.18 mm	5075818	No	1.2	0.28	Thin bottom (0.17 mm) ¹ , Thin bottom (0.17 mm) No Skirt

¹Potential interference with plate skirt when imaging edge wells.

²Image degradation above plate thickness 0.3 mm.

³20x and 40x ELWD image through cover slips, but other objectives give better resolution and shorter exposures.



Note: When you use with thin-bottom plates, the short working distance of 20X S Fluor, 20X Plan Apo, 40X S Fluor, 40X Plan Apo, 40X Plan Fluor Oil, 60X Plan Fluor, 60X Plan Apo Oil, 100X Plan Fluor Oil, 20xc WI CFI Apochromat LWD Lambda S, 40xc WI CFI Apochromat LWD Lambda S, and 60xc WI CFI Plan Apochromat VC objectives can cause interference with the plate skirt when you attempt to image edge wells. If the software does not automatically block the edge wells, you should either omit the edge wells or use a plate with a low skirt.

Table B-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 ELWD ADM	1.4 AU	1.0 AU	1.2 AU	5.1 µm	4.3 µm
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
20xc Water Immersion CFI Apochromat LWD Lambda S	4.58 AU	3.21 AU	3.82 AU	6.50 µm	4.71 µm
40xc Water Immersion CFI Apochromat LWD Lambda S	2.77 AU	1.94 AU	2.31 AU	2.79 µm	2.09 µm

Table B-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
60xc Water Immersion CFI Plan Apochromat VC	1.93AU	1.35 AU	1.61 AU	1.89 μm	1.47 μm

¹ $\text{FWHM}_{\text{axial}}$ for pinholes larger than 1 Airy Unit as set forth by Toomre, et alia.



Appendix C: Filter Specifications and Calibration Compatibility

Filter	Wavelengths	Shading Correction Plate	Calibration Slide
DAPI	Excitation: 377/54 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)
Cy3	Excitation: 531/40 nm or 543/22 nm ¹ 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)

¹Contact Molecular Devices Technical Support to verify.



Appendix D: Replacement Parts and Optional Extras

For an up-to-date list of replacement parts and optional extras, see
www.moleculardevices.com.

Part Number	Description
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 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	Cy3 Filter Set
5039859	Texas Red Filter Set
5037556	Cy5 Filter Set
5042555	Accessory Tool Kit
5039434	Lumencor Fiber-Optic Solid-State Light Source (until April 2020)
5071389	Lumencor Fiber-Optic Solid-State Light Source (from May 2020)
5044996 - 5044999	Custom Emission Filters
5047472 - 5047475	Custom Dichroic Mirrors
1-GP-2	GP-2 Spatial Calibration Slide
5033647	Replacement Solid-State Light Source
1-3335-0005	12 V Halogen Lamp for Nikon Transmitted Light (TL) Tower
2300-2460	ImageXpress Micro Fluidics Tip Adapter, 96-well
2300-2280	ImageXpress Micro Fluidics Tip Adapter, 384-well
E1004	Breathe Seal for Cell & Tissue Culture, non-sterile, pack of 100

Part Number	Description
E1005	Breathe Seal for Cell & Tissue Culture, sterile, packed in tamperproof bags of 25; pack of 50
9000-0761	Cellular Screening System 96 tips (50 racks/case)
9000-0763	Cellular Screening System 384 tips (50 racks/case)
5004555	Immersion oil, 8 cc, MXA20233
1-2600-0865	Replacement Live Cell Plate Sealing Ring
5000284	Robot-friendly Three Slide Holder for Imaging

For a list of compatible Nikon objectives see [Compatible Objectives on page 251](#).

Appendix E: File Privileges for the MetaXpress Software

This appendix describes which directories must be accessible to MetaXpress Software administrators and users. The following information assumes that the MetaXpress Software is installed to the default path of C:\MX6.



Note:

- There are no restrictions as to where the software is installed. You can install the software to C:\Program Files\MX6.
- When the system has multiple configurations and multiple groups not named MetaXpress, substitute the correct group name in the following list of folder paths, as needed.

Software Administrator

Read/write access is required for everything under C:\MX6. The Software Administrator needs to create and periodically modify shading correction images in the C:\Shading Images folder. Because of the potential for a security violation, the System Administrator can accomplish this operation and you do not need to grant the Software Administrator write access to the root directory.

Standard Users

Read Only Access:

- 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 (and the group name is MetaXpress):

- 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
-



CAUTION! Do not delete or rename the *Backup* folder from the computer. At the start of every plate acquisition, the software archives the current configuration and instrument settings to a date-stamped .zip file in the C:\Backup\ folder. If the software configuration or instrument settings get corrupted, you can restore the settings from the backup archives. Contact Molecular Devices Technical Support for assistance.

- C:\Shading Images

If the system is set up for a single user (and the group name is MetaXpress):

- C:\MX6\Groups\MetaXpress

If the system is set up for multiple users (and the group name is MetaXpress):

- C:\MX6\Groups\MetaXpress\Users\Individual user

Appendix F: Robotic Plate Handling

If your ImageXpress System has a robotic plate handler integrated with the base imaging system, there are additional hardware and user procedures. The robotic plate handler is integrated with the ImageXpress 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. Contact Molecular Devices sales or support if you want a robotic plate handler upgrade for your system.

The following is the Robot step-by-step protocol:

1. Fetch a plate from a hotel shelf.
2. Scan the plate barcode.



Note: You should use barcodes, though they are optional.

3. Load the plate onto the ImageXpress System.
4. Acquire images.
5. Unload the plate from the ImageXpress System.
6. Return the plate 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 are enabled in the Meta Imaging Series Administrator Software:

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator** to display the **Meta Imaging Series Administrator** dialog.
2. Click **Configure Hardware** to display the Configure Hardware dialog.
3. Click **Install System Devices** to display the Install Systems Devices dialog.
4. In the Installed Devices list, ensure that **External Control** displays. If not, in the Available Hardware list select **External Control** and click **Install>>**
5. In the Installed Devices list, select **External Control**.
6. Click **Settings** to display the External Control Settings dialog.
7. Select the **Connections Settings** tab.
8. Click the **COM** Port drop-down and select the COM port.
9. Clear the **Operate Device in Emulation Mode** check box.

10. In the Included Components field, verify that **Control** displays. If not, in the Available Components list Select **Control** and click **Add>>**.
11. Click **OK** to display the Install System Devices dialog.
12. Click **Apply**.
13. Click **OK** to display the Configure Hardware dialog.
14. In the Hardware Settings field, select the hardware configuration.
15. Click **Configure Devices** to display the User Settings dialog.
16. In the Claimed Devices list, verify that **External Control** displays. If not, in the Available Hardware list select **External Control** and click **Install>>**.
17. Click **Apply**.
18. Click **OK** to display the Configure Hardware dialog
19. Repeat these steps for each hardware configuration that needs the External Control.
20. Click **OK** to display the Meta Imaging Series Administrator Software dialog.
21. and **OK** to exit the software.
22. Start the MetaXpress Software.
23. Click **Screening** and verify that the **External Control** option is available.

Appendix G: Electromagnetic Compatibility

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 a Class A digital device, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at their own expense. Changes or modifications made to this equipment not expressly approved by the party responsible for compliance may void the FCC authorization to operate this equipment.

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