ImageXpress® Micro 4

Widefield High Content Imaging System

User Guide

MOLECULAR DEVICES

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For research use only. Not for use in diagnostic procedures.

Patents: http://www.moleculardevices.com/productpatents

Product manufactured by Molecular Devices, LLC.
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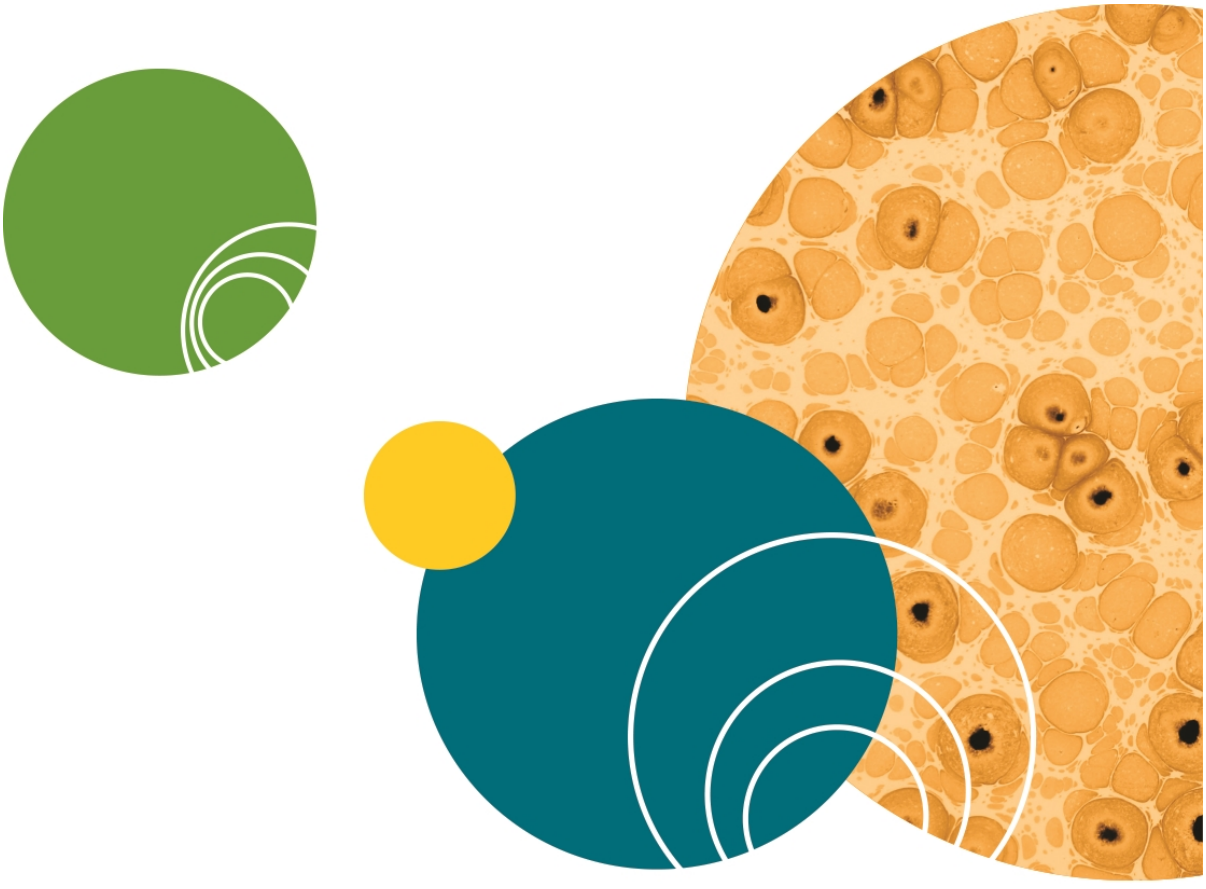
Molecular Devices, LLC is ISO 9001 registered.

Contents



Safety Information

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

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

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

Warnings, Cautions, Notes, and Tips

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

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

The following user-attention statements can be displayed in the text of Molecular Devices user documentation. Each statement implies a particular 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. Some warnings can have a different symbol on the left, such as electric shock, biohazard, and laser light warnings. The definition of the symbol is included in the text of the warning.

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

Note: A note calls attention to significant information.

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

Symbols on Instrument Labels

Each safety label 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.

Table S-1: Instrument Label Alert Symbols

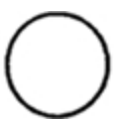
Symbol	Indication
	This symbol indicates that the product documentation must be consulted.
	This symbol indicates a potential laser hazard. See Laser Safety on page 10.
	This symbol indicates a potential lifting hazard. For information about the weight of the instrument,
	This symbol indicates a potential pinch hazard.
	This symbol on the power switch indicates power on.
	This symbol on the power switch indicates power off.

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

Symbol	Indication
	This symbol on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. It indicates that you must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Before Operating the Instrument

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

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

Protective Housing and Safety Interlocks

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

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

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

Safety Interlock Failure

If the focusing laser stays on when the automated top door is open, it is unsafe to continue using the instrument due to a safety interlock failure. Contact Molecular Devices Support immediately. See Obtaining Support on page 309.

Non-Interlocked Doors and Panels

The side access doors are not interlocked. These doors do not provide access to exposure by the laser light source. Some moving parts exist inside these doors. See Moving Parts Safety on page 14.

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-2: Embedded Laser Module Specifications

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

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

The operator or the service engineer is not exposed to radiation from the laser module during operation, maintenance, or service. If the top panel is removed for service, the laser beam remains safely contained within the optical system until it passes through the microscope objective, which diverges the beam and renders incident power levels below Class 1 (1 mW/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.

- The light source for the standard model of the ImageXpress Micro System has a fuse-protected, 300 W Xenon lamp. Xenon lamps have a limited lifetime of approximately 500 hours and need to be replaced upon failure. The Xenon lamp and the fuse are user-replaceable parts. See Light Source Maintenance on page 258.
- The light source for the ImageXpress Micro XL System, the ImageXpress Micro XLS System, and the ImageXpress Micro 4 System is a solid-state source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source.

WARNING! BURN HAZARD. If a Xenon lamp requires replacement, let the lamp cool for at least 30 minutes. The lamp generates an extreme amount of heat and attempting to remove the lamp immediately after use can cause injury.

Liquid Light Guide

The ImageXpress Micro System, ImageXpress Micro XL System, ImageXpress Micro XLS System, and ImageXpress Micro 4 System use a liquid light guide between the external light source and the instrument. The light source generates a very bright light. The Standard model of the instrument uses a high-powered Xenon lamp that emits infrared and ultraviolet radiation that can cause significant skin burns and eye damage.

WARNING! To prevent skin burns and eye damage, do not remove the light guide from the instrument or the light source when the lamp is powered on.

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 of equipment that requires the removal of covers or panels.

Molecular Devices recommends that you power off the instrument when it is not in use.

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.

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

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.

Power Supply

For the ImageXpress Micro System, ImageXpress Micro XL System, and ImageXpress Micro XLS System, a single power cable connects the instrument to the external power supply. The power supply for the ImageXpress Micro System, the ImageXpress Micro XL System, and the ImageXpress Micro XLS System has an input voltage range of AC 100 V to 240 V, 50/60 Hz, 2 A.

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

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

The light source for the standard model of the ImageXpress Micro System has a fuse-protected, 300 W Xenon lamp. If the fuse fails, disconnect the power cord from the light source and follow the instruction on how to replace the fuse. See Maintenance on page 255.

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

In the ImageXpress Micro 4 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. The interlocks and protective housing are designed so that you cannot access the moving parts during a scan.

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.

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 System, ImageXpress Micro XL System and ImageXpress Micro XLS System weighs approximately 82 kg (180 lbs). Do not attempt to lift or move the instrument without assistance.

WARNING! LIFTING HAZARD The ImageXpress Micro 4 System weighs approximately 104 kg (230 lbs). Do not attempt to lift or move the instrument without assistance.

CAUTION! Moving the instrument can disrupt sensitive optical alignments. Molecular Devices recommends that you contact Technical Support to schedule a Field Service engineer to help with moving your instrument. Your warranty or service contract does not cover problems caused during or as a result of shipment or relocation.

Chemical and Biological Safety

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

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

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

Cleaning and Maintenance Safety

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

Do the following before 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 15 for details.

Perform only the maintenance tasks described in this guide. Any other maintenance tasks are to be performed by qualified Molecular Devices personnel only. See Obtaining Support on page 309.

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 255.

Chapter 1: Introduction to the ImageXpress Micro System

The ImageXpress® Micro Widefield 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 Xenon light source connected by a liquid light guide in the standard ImageXpress Micro System
- External white light source connected by a liquid light guide in the ImageXpress Micro XL System, ImageXpress Micro XLS System, and ImageXpress Micro 4 System
- User-specified camera
  - A cooled CCD camera in the standard ImageXpress Micro System
  - A scientific CMOS camera in the ImageXpress Micro XL System, ImageXpress Micro XLS System, and ImageXpress Micro 4 System
- 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 filter cubes in a five-position slider
- Selectable binning modes to decrease exposure time and increase throughput

- Motorized selection of stage position, filter cubes and objectives with asynchronous operation
- High-transmission fluorescence imaging optics with world-class chromatic aberration correction, resolution, and image flatness
- Operation and configuration control by the integrated MetaXpress Software
- Optional expansion solutions available for environmental control, phase contrast transmitted light, and fluidics
- Optional plate-handling robot with an automated barcode reader

ImageXpress Micro System Instrument Features

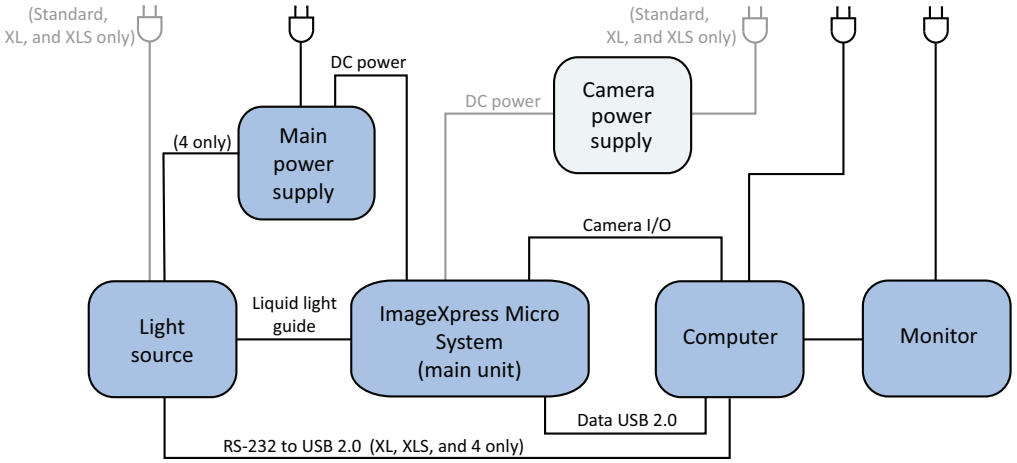


Figure 1-1: All ImageXpress Micro Systems Widefield Components Without Options

Illumination System: Excitation

Light Source

- The light source for the standard model of the ImageXpress Micro System has a fuse-protected, 300 W Xenon lamp. Xenon lamps have a limited lifetime of approximately 500 hours and need to be replaced upon failure. The Xenon lamp assembly and the fuse are user-replaceable parts.
- The light source for the ImageXpress Micro XL System, the ImageXpress Micro XLS System, and the ImageXpress Micro 4 System is a solid-state source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source.

Cold Mirror

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

Note: In the XL, XLS and 4 models of the ImageXpress Micro System, the solid-state light source is limited to between 380 nm and 680 nm.

Illumination Optics

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

Shutter (standard model only)

In the standard model of the ImageXpress Micro System, a solenoid-activated mechanical shutter controls the exposure of the sample to excitation light from the Xenon lamp. This helps to minimize sample degradation and photobleaching. The shutter has an expected lifetime of 1 million cycles.

Filter Cube Changer

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

Objective (Z) Stage

Motorized Z Stage

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

Objectives

The standard objectives are Nikon CFI60 series. The selected objective lens focuses excitation light onto the sample, and collects fluorescence light emitted by the sample. See Compatible Objectives, see page 319.

Motorized Objective Changer

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

Sample (X-Y) Stage

Sample

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

Plate Holder and Plate Clamp

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

Motorized X-Y Stage

The X-Y stage position is monitored using a linear encoder that features better than 100 nm resolution.

Autofocus Laser

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

Imaging System: Emission

Tube Lens

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

Camera

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

The ImageXpress Micro XL System, ImageXpress Micro XLS System, and the ImageXpress Micro 4 System use a scientific CMOS camera. This camera has a 2560 × 2160 image sensor format (6.5 × 6.5 µm pixel size), and has a peak quantum efficiency of 60% at 550 nm. The center 2160 x 2160 pixels are extracted during plate acquisition to ensure adequate illumination uniformity.

Electronics

Without optional equipment, the ImageXpress Micro System includes the following additional components:

- External ImageXpress Systems Power and Options Controller and cables
- USB 2.0 port and cable to computer for device control
- Camera cabling to camera board in computer
- External solid state light source, fiber, and cables

MetaXpress Software Features

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

- The acquisition workflow involves configuring settings, acquiring images, and storing plate data in a database. See Preparing For Acquisition on page 59 and Configuring Plate Acquisition Options on page 69.
- The analysis workflow consists of processing, enhancing, and analyzing acquired plate data. See the *MetaXpress High-Content Image Acquisition and Analysis Software Analysis Guide* included on the MetaXpress Software Suite installation USB flash drive, or the application help available when using the MetaXpress Software.

Simplified Menu Structure

New ImageXpress Micro Systems ship with the simplified menu installed, otherwise as an option, the simplified menu structure can be installed to reduce the number of top-level menus in the MetaXpress Software. All the features of the software are available in this reorganized menu structure.

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

You can use the **Menu Map** in the **Help** menu to help you find the locations of features in the simplified menu structure. The **Menu Map** is available only after the simplified menu installation.

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

   The simplified menu path appears to the right of the desired feature in the menu.
4. Click the menu path in the software window to access the desired feature.

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

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

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

Administrator Tasks

Most of the procedures in this guide are for general users. However, Molecular Devices recommends that you identify one or more users as advanced users or system administrators. The responsibilities of the system administrator vary from site to site. Variables include the number of users on the system, the type of database used, and the type of work done. Some common MetaXpress Software system administrator tasks include:

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

Theory of Operation

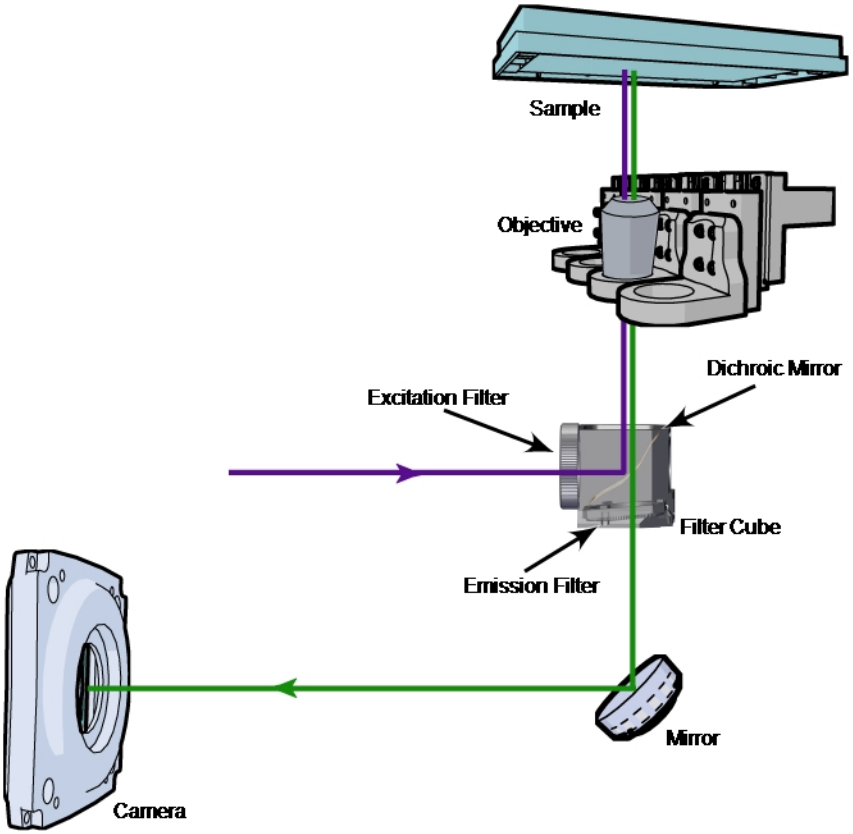


Figure 1-2: Optical Path

The ImageXpress Micro System uses the following components and functions:

- Fluorescence Imaging, see page 24
- Excitation and Emission Filters, see page 24
- Objective Lenses, see page 26

Fluorescence Imaging

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

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

Excitation and Emission Filters

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

To selectively excite one fluorophore more intensely than another, or to minimize excitation channel crosstalk, it is necessary to provide illumination containing only photons with a wavelength range matched to the absorbance (excitation) spectrum of the target dye. A bandpass filter in the illumination optical path (called the excitation filter, since it filters the excitation light) is used to restrict the illumination spectrum to a narrow range of wavelengths.

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

Dichroic Mirror

In the ImageXpress Micro System, the dichroic mirror is in a filter cube, as shown in 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 onto the sample as well as collect the emitted fluorescent light to form the image.

In the illumination path, the dichroic mirror reflects shorter wavelengths from the light source up through the objective onto the specimen.

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

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

All of the optics in the filter cube are interference filters made by depositing a number of thin film coatings on a glass support. They are delicate and easily damaged components. Use care when handling these components.

Dichroic Transmission Spectrum

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

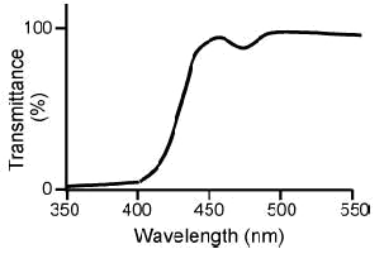


Figure 1-3: Example of a transmission spectrum of a dichroic mirror

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

Objective Lenses

The ImageXpress Micro System can be configured with any of the high-quality Nikon objectives listed in Compatible Objectives on page 319.

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

Note: Extra-long working distance (ELWD) objectives have adjustable spherical-aberration correction collars for imaging through thick substrates such as most microplates. For details on how to calculate and set their correct values, see Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 278.

Several of the other objectives (such as, 40x Super Plan Fluor ELWD) also have correction collars for adjustment according to the thickness of the glass cover slip or thin plate bottom being used. Setting these collars should be done using the physical thickness of the plate bottom or through optimization of image quality.

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

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

Chapter 2: Using the ImageXpress Micro System

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

Starting the System

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

Powering On the Instrument

Powering on the system depends on which model of instrument you are using.

ImageXpress Micro 4 System

To power on the ImageXpress Micro 4 System:

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

1. Ensure that the power cords for the instrument and the light source are connected to the ImageXpress 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 has started and Windows is running, log in to Windows using the User Name and Password combination provided for you by your system administrator.

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

5. Start the MetaXpress Software. See Starting the Software on page 28.

ImageXpress Micro System Including XL and XLS Systems

To power on the ImageXpress Micro System including XL and XLS systems:

1. Ensure that the power cords for the instrument and the light source power supplies are connected to a 100 VAC to 120 VAC or a 220 VAC to 240 VAC power source.
2. Turn on the power switch on the light source power supply unit.

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

3. Wait for the light source to stabilize before acquiring images.
   - In the Standard model of the ImageXpress Micro System, the Xenon lamp takes 15 to 20 minutes to stabilize.
   - In the XL, and the XLS models of the ImageXpress Micro System, the solid-state light source takes less than a minute to stabilize.
4. Turn on the power switch on the front of the ImageXpress Micro System power supply unit.
5. Ensure that the camera power supply is plugged in and, if required, turned on.
6. Turn on the power to the host computer and the monitor.
7. After the computer has started and Windows is running, log in to Windows using the User Name and Password combination provided for you by your system administrator.

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

8. Start the MetaXpress Software. See Starting the Software on page 28.

Starting the Software

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

Note: If you encounter or observe actions or results that are inconsistent with your expected results when using the ImageXpress Micro System and your MetaXpress Software, contact your system administrator before continuing your experiment.

To start the MetaXpress Software:

1. Double-click the MetaXpress icon on your desktop or click **Start > All Programs > MetaXpress > MetaXpress**.
2. In the MetaXpress Software login dialog, from the **User Name** drop-down list, select the user name to use and then click **OK**.

Figure 2-1: MetaXpress Software login dialog

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

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

Figure 2-2: Welcome to MetaXpress dialog

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

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

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

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

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

If the error message continues after the recommended troubleshooting, use AxoTrace and contact Molecular Devices Technical Support. See Logging AxoTrace Software Messages to a .txt File on page 312.

Acquiring Data

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

For detailed information, see the following sections:

- Preparing For Acquisition on page 59
- Configuring Plate Acquisition Options on page 69
- Running Plate Acquisitions on page 143

Analyzing Data

The analysis workflow consists of processing and analyzing acquired plate data. See the *MetaXpress High-Content Image Acquisition and Analysis Software Analysis Guide* included on the MetaXpress Software Suite installation USB flash drive, or when using the MetaXpress Software, view the help by selecting **Help > Help Topics**, or pressing **F1** on your keyboard.

Maintaining the Instrument

Specific user-level maintenance can be done on the ImageXpress Micro System for changing the lamp and fuse, changing filter cubes, changing and cleaning objectives, and cleaning the instrument as described in Maintenance on page 255.

Perform only the maintenance tasks described in this guide. Any other maintenance tasks are to be performed by qualified Molecular Devices personnel only. See Obtaining Support on page 309.

Shutting Down the System

Shutting down your system depends on the instrument model.

ImageXpress Micro System, ImageXpress Micro XL System, or ImageXpress Micro XLS System

To shut down an ImageXpress Micro System, ImageXpress Micro XL System, or ImageXpress Micro XLS System:

1. Exit the MetaXpress Software.

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

4. Turn off the power to the light source unit.

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

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

ImageXpress Micro 4 System

To shut down an ImageXpress Micro 4 System:

1. Exit the MetaXpress Software.

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

Chapter 3: System Installation and Testing

3

The ImageXpress Micro System ships fully configured, and is installed at your site by a Molecular Devices field service engineer. The base system includes the imaging unit, the host computer, and accessory kit.

The ImageXpress Micro 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. See Figure 3-1.

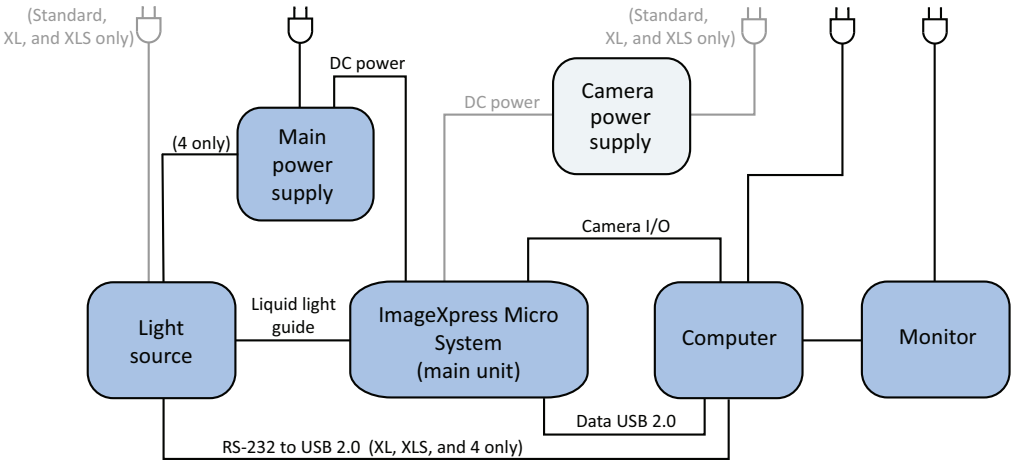


Figure 3-1: ImageXpress Micro System XL, XLS and the ImageXpress Micro 4 System Components Without Options

- Power supply to the instrument
- Liquid light guide from the external light source to the instrument
- USB 2.0 data cable from the instrument to the host computer
- RS-232 from the light source to the host computer
- Cable from the instrument camera to the computer
- Camera power supply cable to the instrument

Table 3-1: Accessory Kit Contents

Accessory	Description
Bead plate	TetraSpeck™ fluorescent microspheres test plate
Hex keys	1/16", 0.05"
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
Storage box	Empty accessory box for miscellaneous storage use

If you need to re-install the MetaXpress Software, or install it on a new computer, please see the *MetaXpress High-Content Image Acquisition and Analysis Software Suite Installation and Update Guide* included on the MetaXpress Software Suite installation USB flash drive, or contact Technical Support. See Obtaining Support on page 309.

Verifying Device Settings in the Meta Imaging Series Administrator Software

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

Note: Molecular Devices recommends running verification tests without a sample plate loaded on the stage.

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

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

1. Follow the procedure described in Powering On the Instrument on page 27, but do not start the MetaXpress Software.
2. Click **Start > All Programs > MetaXpress 6 > Meta Imaging Series Administrator**.
3. In the **Meta Imaging Series Administrator**, from **List of Groups**, select **MetaXpress**.

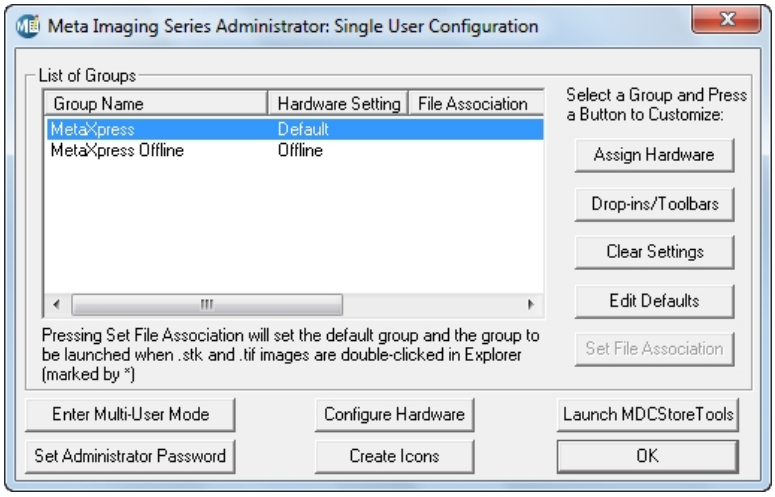


Figure 3-2: Meta Imaging Series Administrator Software home dialog

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

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

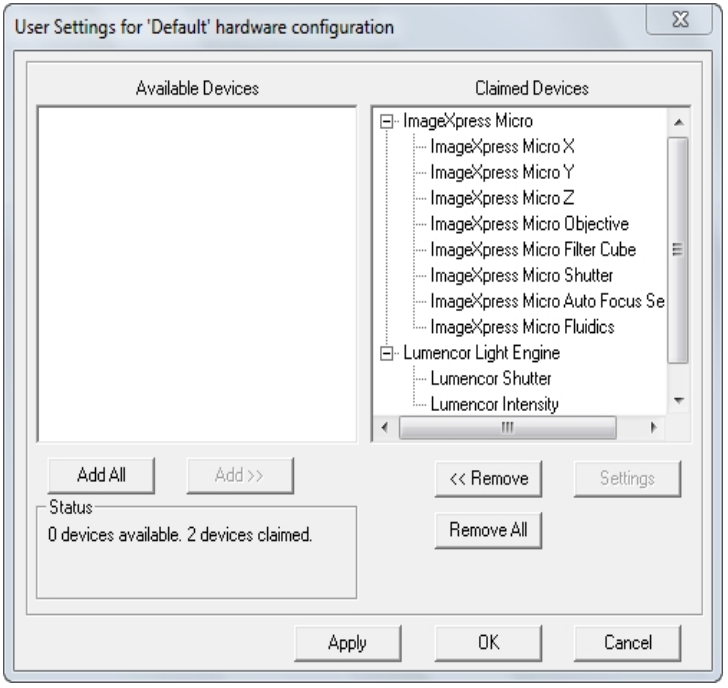


Figure 3-3: User Settings dialog

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

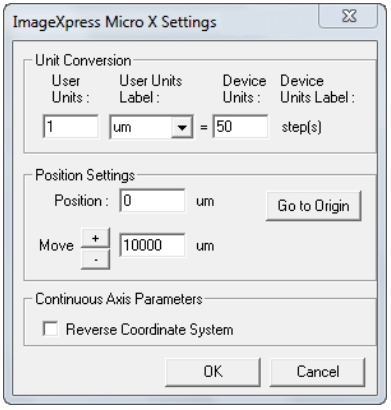


Figure 3-4: ImageXpress Micro X Settings dialog

8. Ensure that the **Reverse Coordinate System** check box is not selected.

9. Click the **+** and **–** buttons and confirm that the stage responds to the control.
10. In the **Move** field, change the step size back to 10 µm and click **OK**.
11. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Y** and then click **Settings**.
12. In the **ImageXpress Micro Y Settings** dialog, in the **Move** field, increase the step size to 10,000 µm.

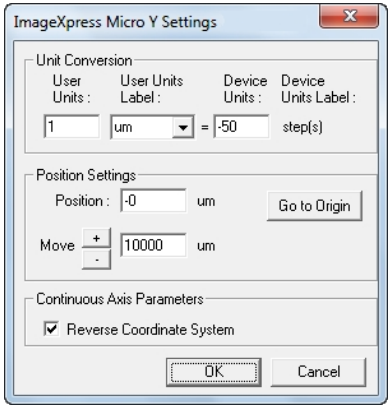


Figure 3-5: ImageXpress Micro Y Settings dialog

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

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

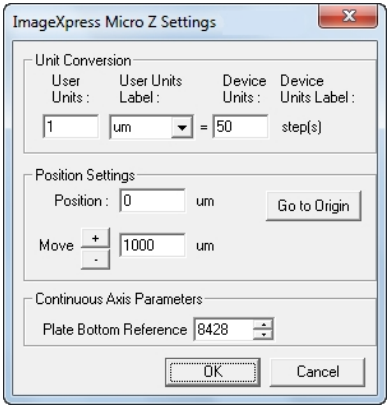


Figure 3-6: ImageXpress Micro Z Settings dialog

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

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

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

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

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

    Position 1 is the position on the right if you are facing the filter cube access door at the front of the instrument.

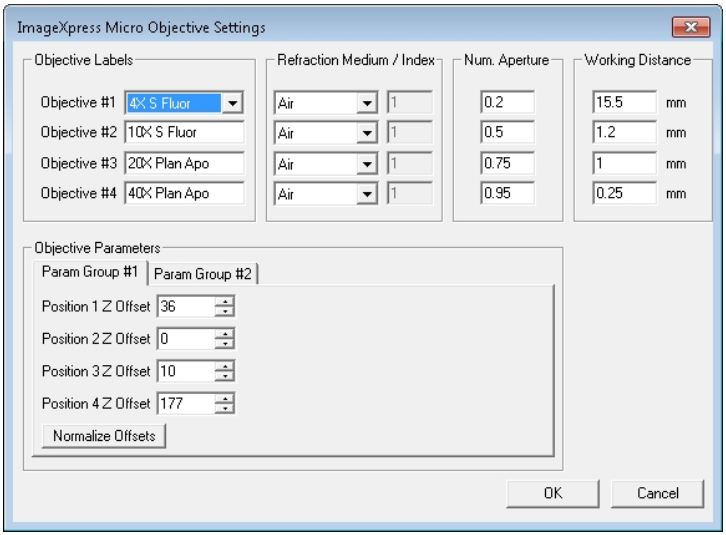


Figure 3-7: ImageXpress Micro Objective Settings dialog

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

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

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

Note: If you need to determine the offset values, see Configuring Parfocality after Changing Objectives on page 284.

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

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

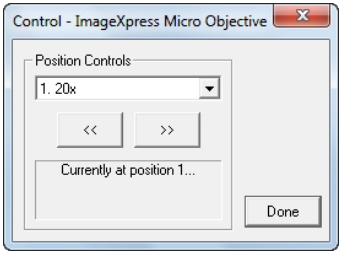


Figure 3-8: Control > ImageXpress Micro Objective dialog

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

> **Note:** If you have a plate handling robot attached to the ImageXpress Micro System, confirm those settings as well. For information, see Verifying External Control Settings on page 333.

31. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Filter Cube** and then click **Settings**.
32. In the **ImageXpress Micro Filter Cube Settings** dialog, confirm that the filter sets listed in the **Filter Labels** field are correct.

    Position 1 is the position closest to you if you are facing the filter cube access door at the front of the instrument.

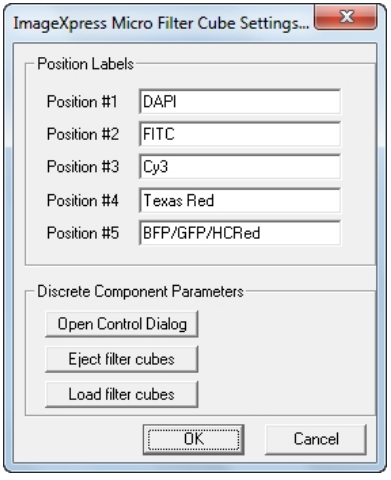


Figure 3-9: ImageXpress Micro Filter Cube Settings dialog

33. In the **ImageXpress Micro Filter Cube Settings** dialog, click **Open Control Dialog**.
34. In the **Control > ImageXpressMicro Filter Cube Settings** dialog, click the arrow buttons to confirm that the filter cube is responding to the program.

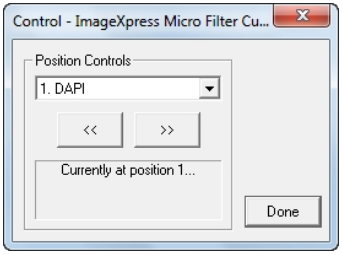


Figure 3-10: Control > ImageXpress Micro Filter Cube dialog

35. Click **Done**.
36. In the **Control > ImageXpress Micro Filter Cube Settings** dialog, click **OK**.
37. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Shutter** and then click **Settings**.
38. For Standard ImageXpress Micro System users, continue to step 39.

    For ImageXpress Micro XL System, ImageXpress Micro XLS System, or ImageXpress Micro 4 System users, skip to step 43.
39. In the **ImageXpress Micro Shutter Settings** dialog, confirm that the **Open Delay** and **Close Delay** fields are both set to 20 milliseconds.

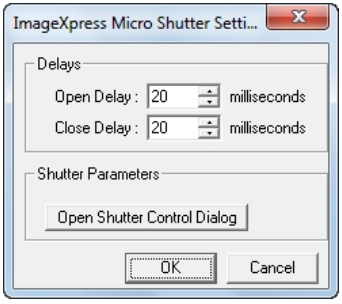


Figure 3-11: ImageXpress Micro Shutter Settings dialog

40. Click **Open Shutter Control Dialog**.

41. In the **Control - ImageXpress Micro Shutter** dialog, click **Toggle** to confirm that the shutter is responding.

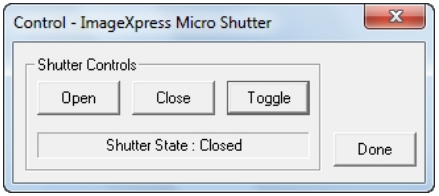


Figure 3-12: Control > ImageXpress Micro Shutter dialog

42. Click **Done**.
43. In the **ImageXpress Micro Shutter Settings**, click **OK**.
44. Click **OK** to close the **User Settings** dialog.

Verifying Camera Settings in the Meta Imaging Series Administrator Software

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

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

   If a driver is not listed in the **Installed Drivers** column, or if more than one driver is listed, contact Molecular Devices support to determine which driver is required for your system.
4. In the **Installed Drivers** column, select the appropriate driver.
5. Click **Configure** to query the camera.
6. In the camera driver dialog, ensure that the camera is available in channel 1.

   This confirms that the camera is responsive.

   If the camera was not queried successfully, ensure that the cabling is correct from the ImageXpress Micro System main unit to the computer and that the camera power supply is plugged in and, if required, turned on.
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 43.

Verifying and Backing Up Settings in the MetaXpress Software

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

- Verifying Magnification Settings on page 44
- Verifying Illumination Settings on page 45
- Verifying Calibration Settings on page 46
- Verifying the Laser Autofocus Sensor on page 48
- Verifying the Plate Reference Point (A1 Center) on page 50
- Verifying Plate Types on page 53
- Confirming Laser Autofocus Settings for Plate Files on page 55
- Verifying Shading Correction Files-Legacy on page 56

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

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

Verifying Magnification Settings

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

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

   In the simplified menu structure, click **Control > Devices > Configure Magnification**.
3. In the **Configure Magnification** dialog, in the **Setting** section, verify that there is an **ImageXpress Micro Objective** setting and that the check box is selected.

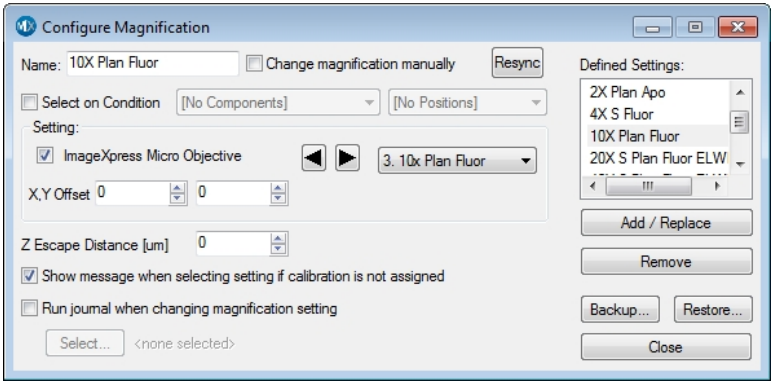


Figure 3-13: The Configure Magnification dialog

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

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

Verifying Illumination Settings

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

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

   In the simplified menu structure, click **Control > Devices > Configure Illumination**.
3. In the **Configure Illumination** dialog, in the **Device Positions** section, ensure that **ImageXpress Micro Filter Cube** is selected.

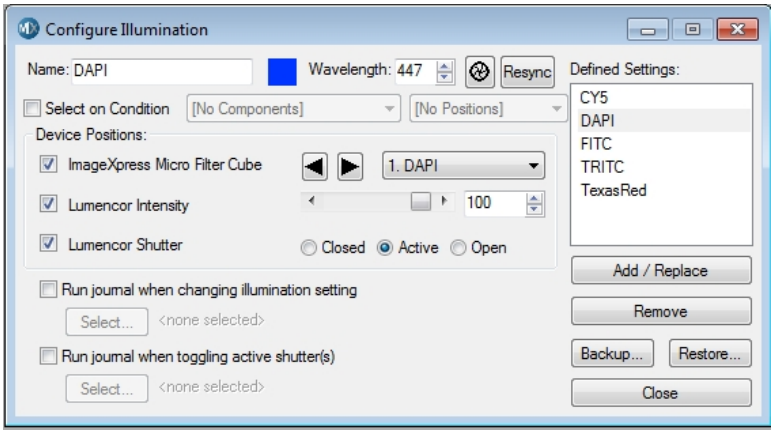


Figure 3-14: The Configure Illumination dialog

4. Ensure that the appropriate shutter is selected as **Active** for each filter set.
5. In the **Defined Settings** list, ensure that the correct illuminations are listed.
6. Set up other illumination settings if needed.

   The value in the **Wavelength** field should match the center wavelength for the emission filter.
7. Click **Backup**.
8. In the **Backup All Illumination Settings** dialog, select a name and location for the backup and then click **Save**.

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

Verifying Calibration Settings

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Measure > Calibrate Distances**.

   In the simplified menu structure, click **Measure > Distances > Calibrate Distances**.
3. In the **Calibrate Distances** dialog, confirm that there are calibration settings in the dialog that match the objective settings from the **Configure Magnification** dialog.

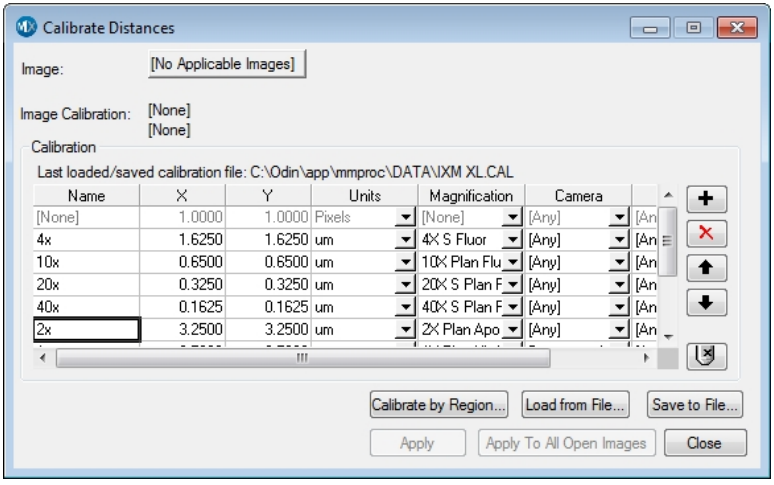


Figure 3-15: The Calibrate Distances dialog

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

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

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

Note: For more information on creating calibration settings, with the **Calibration Distances** dialog open, press **F1** to view the MetaXpress Software application help.
To measure pixel sizes more accurately, in the **IXM Taskbar**, click **System Maintenance** and then click **Measure Pixel Sizes**.
If **IXM Taskbar** is not installed, contact Technical Support.

Table 3-2: Standard ImageXpress Micro System Estimated Calibration Settings

Objective Magnification	Estimated Calibration
1x	6.45 µm/pixel
2x	3.225 µm/pixel
4x	1.6125 µm/pixel
10x	0.645 µm/pixel
20x	0.3225 µm/pixel
40x	0.16125 µm/pixel
60x	0.1075 µm/pixel
100x	0.0645 µm/pixel

Table 3-3: ImageXpress Micro XL System, ImageXpress Micro XLS System, ImageXpress Micro 4 System Estimated Calibration Settings

Objective Magnification	Estimated Calibration
1x	6.50 µm/pixel
2x	3.25 µm/pixel
4x	1.63 µm/pixel
10x	0.65 µm/pixel

Table 3-3: ImageXpress Micro XL System, ImageXpress Micro XLS System, ImageXpress Micro 4 System Estimated Calibration Settings (continued)

Objective Magnification	Estimated Calibration
20x	0.33 µm/pixel
40x	0.16 µm/pixel
60x	0.12 µm/pixel
100x	0.065 µm/pixel

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

Verifying the Laser Autofocus Sensor

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

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.

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

15. In the **Configure Laser Autofocus Settings** dialog, click **Preview Pass**.

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

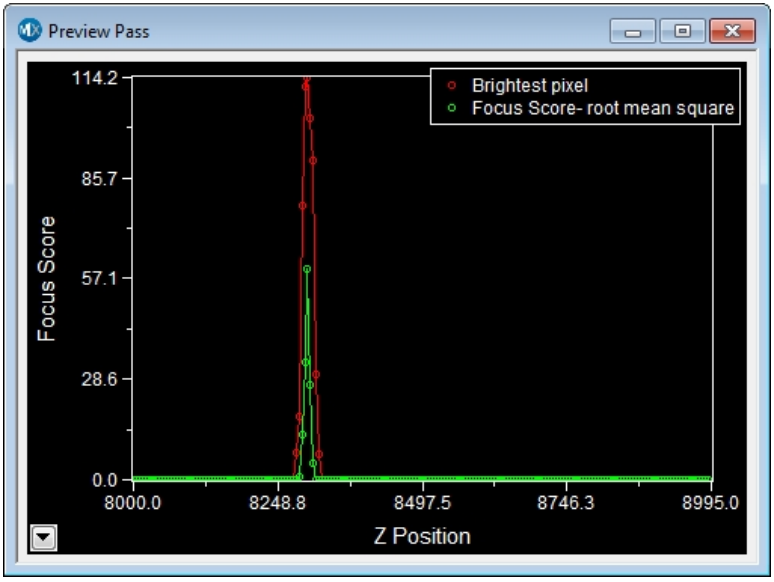


Figure 3-16: The Preview Pass window

16. In the **Preview Pass** window, ensure that there is at least one peak.
    - If the **Preview Pass** window shows at least one peak, the Laser Auto Focus Sensor is enabled and functional.
    - If the **Preview Pass** window does not show any peaks, ensure that the plate is properly seated. Then, in the **Configure Laser Autofocus Settings** dialog in the **Preview Pass** section, select **Exposure > Override exposure**, increase the value, and click **Preview Pass** again.
    - If the **Preview Pass** window still does not show a peak, click **Laser Autofocus Wizard**. Then, in the **Plate Acquire LAF Setup Wizard**, follow the on-screen instructions. For more information while using the **Laser Autofocus Wizard**, you can view the software help by pressing **F1** on your keyboard.
    - If the **Preview Pass** window still does not show a peak, contact Molecular Devices Technical Support and report the issue. See Obtaining Support on page 309.

    For more information on the **Preview Pass** window, see Confirming Laser Autofocus Settings for Plate Files on page 55.
17. Close the **Preview Pass** window.
18. In the **Configure Laser Autofocus Settings** dialog, click **Close**.

19. In the **Plate Acquisition Setup** dialog, click **Eject Plate**.
20. Remove the Tetra Speck bead plate and then click **Load Plate**.
21. Click **Close**.

Verifying the Plate Reference Point (A1 Center)

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

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.

   In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Objective and Camera** tab.
5. From the **Magnification** list, select the lowest power objective. 10x is the highest that should be used.
6. In the **Camera Binning** field, type or select **1**.
7. If you are using the ImageXpress Micro System, from the **Gain** list, select **2**.

   Note: There is no **Gain** field in the MetaXpress Software for use with the ImageXpress Micro 4 System, nor the ImageXpress Micro XLS System.
8. Click the **Plate** tab.
9. From the **Plate name** list, select **96 Wells (8x12)**.
10. Click the **Sites to Visit** tab.
11. Under **Site Options**, select **Single site**.
12. Under the **Acquisition > Wavelengths** tab, click the **W1** tab.
13. From the **Illumination setting** list tab, select **FITC**.

    Note: Filter cubes other than FITC are acceptable to use, but the contrast might not be as high as with the FITC. Exposure times vary significantly depending on your light source and filter cube choice.
14. In the **Exposure** field, type or select **100** milliseconds (ms).
15. Click the **Screening > Plate Acquisition and Control**.

16. In the **Plate Acquisition and Control** dialog, from the **Wavelength** list, select **W1 - FITC**.

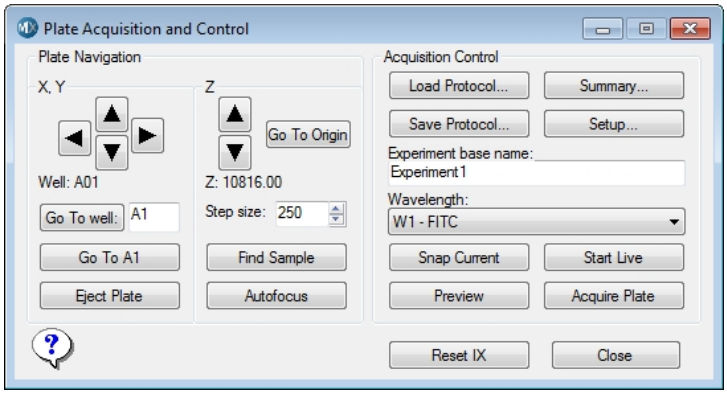


Figure 3-17: The Plate Acquisition and Control dialog

17. In the **Step Size** field, type **250**.
18. Click **Eject Plate** to open the top door.
19. 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.

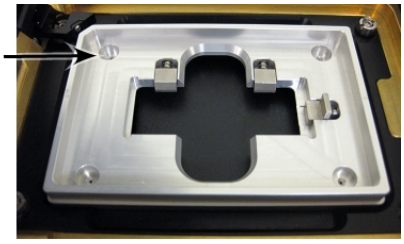


Figure 3-18: Loaded metal slide holder plate with integrated A1 well pinhole

20. Click **Load Plate** to close the top door.
21. Click **Go To A1** to move the stage to the A1 position.
22. Click **Start Live** to open a live image window.

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

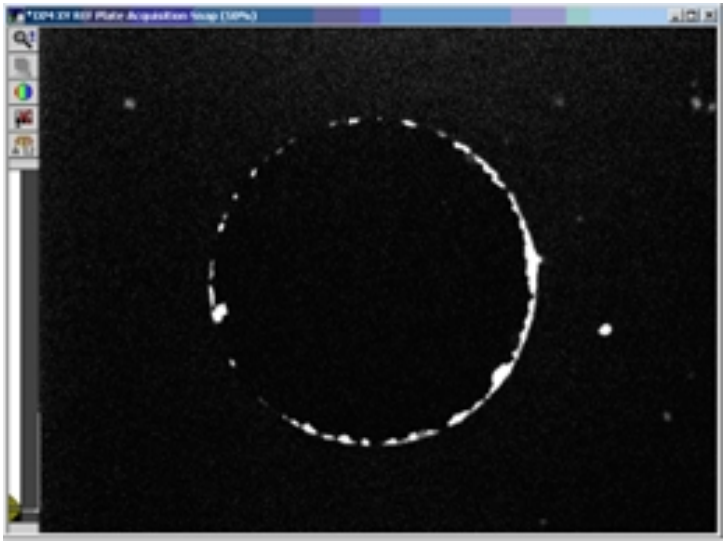


Figure 3-19: The A1 Pinhole in focus at 4X

If the A1 pinhole is not centered, or if you cannot find the hole, contact Molecular Devices Technical Support. See Obtaining Support on page 309.

24. Standard ImageXpress Micro System users only, if your lowest magnification objective is greater than 4x, do the following:
    a. Move the stage up until you are close to focus and then left until you see the edge of the pinhole.
    b. Align the left side of the hole with the left side of the image window and record the stage X position.
    c. Move the stage to the right until you see the edge of the pinhole.
    d. Align the right side of the hole with the right side of the image window and record the stage X position.
    e. Calculate the horizontal center of the reference point.
    f. Move the stage left and then up until you see the top edge of the pinhole.
    g. Align the top of the hole with the top of the image window and record the stage Y position.
    h. Move the stage to down until you see the edge of the pinhole.
    i. Align the bottom of the hole with the bottom of the image window and record the stage Y position.
    j. Calculate the vertical center of the reference point.
    k. Compare the calculated stage position with the position of the stage when you click **Go To A1**.
25. Click **F2: Stop** to stop the live image.
26. Click **Eject Plate**.
27. Remove the metal slide holder plate and then click **Load Plate**.
28. Click **Close**.
29. In the **Plate Acquisition Setup** dialog, click **Close**.

Verifying Plate Types

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.

   In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Plates** tab.

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

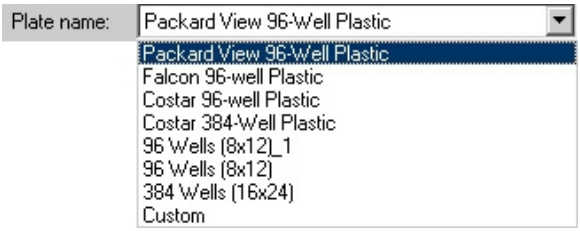


Figure 3-20: Available plate types

   - If there are several custom plate types available in the list, then you are finished with this procedure.
   - If only **96 Wells (8x12)**, **384 Wells (16x24)**, and **Custom** are listed, then continue with this procedure to load the preconfigured plate type files.

6. Insert the MetaXpress Software Suite installation USB flash drive into the computer.
7. When the MetaXpress Software installation window appears, click **Explore Installation Folders/Files**.
8. In Windows Explorer, open the **Plates** folder to view the preconfigured plate type files (.plt).
9. Select the plate type files that you need to be available in the MetaXpress Software.
   - To select adjacent files, click the first file and then hold down the **SHIFT** key and click the last file.
   - To select non-adjacent files, hold down the **CTRL** key and click the files.
10. Place a copy of the selected files in the **Plates** folder in your MetaXpress installation folder.

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

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

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

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

Confirming Laser Autofocus Settings for Plate Files

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

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

To confirm the laser autofocus settings:

1. Prepare the plate that you are going to test by putting water or buffer similar to the buffer you will be using for real experiments in several of the wells.
2. Open the MetaXpress Software and log in to the database.
3. Click **Screening > Plate Acquisition Setup**.

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

    Note: If the **Configure Laser Settings** button is highlighted in red, the autofocus was not configured for this plate and objective configuration. To correct this, run the **Laser Autofocus Wizard**.
13. Click **Find Sample**, and verify that the focus status reports **Focus Found** and that the resulting focus position is appropriate for the plate.

    Note: If the focus status reports **Focus Not Found**, or if the **Fine z-position** result is wrong, optimize the laser autofocus settings using the **Laser Autofocus Wizard**
14. When finished, close the focus status report dialog and the **Configure Laser Autofocus Settings** dialog.

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

Note: If you are using an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you are using. For information on configuring the correction collar, see Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 278.

Verifying Shading Correction Files-Legacy

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

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

Note: For information about creating **Legacy Correction** shading correction image files, see Updating Shading Correction Settings-Legacy on page 304.

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.

   In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Acquisition** tab.
5. During your initial on-site system installation, the shading correction image files are configured to be found in the **C:\Shading Images** folder.

   To change the default image location, click **Directory for Stored Correction Images**, and then in the **Browse for Folder** dialog, select the new location.

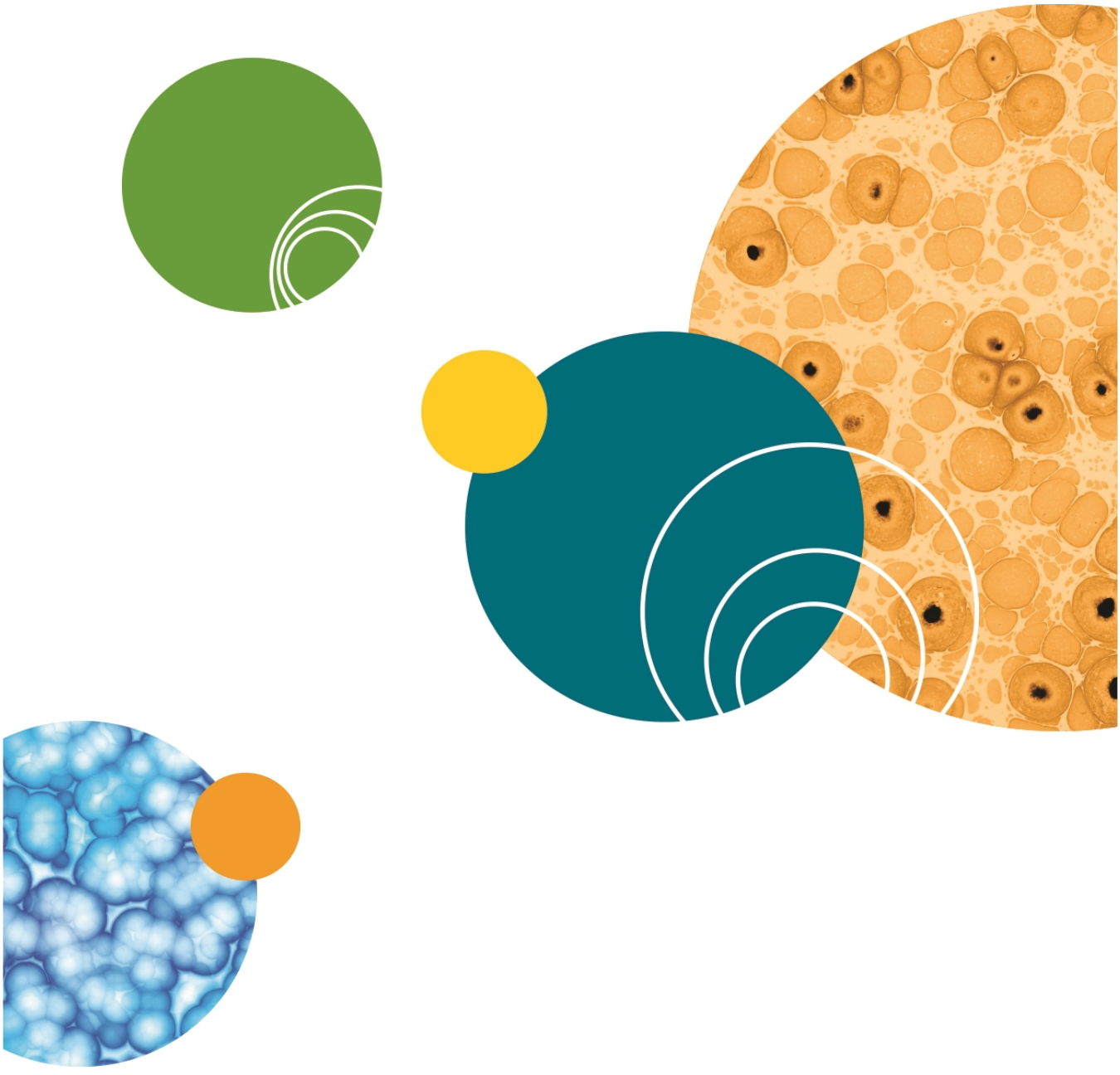
To ensure that properly named shading correction files exist for each objective and filter set combination, do the following:

1. Use Windows Explorer to navigate to the **C:\Shading Images** folder, or to another location if you have changed the default.
2. Locate the shading correction files.
3. Ensure that the file names are formatted correctly and that a file exists for each objective and wavelength.

   Name shading correction images in the following format:

   - `shading_<objective setting>_<wavelength>.tif`

     For example: `shading_4x Plan Apo_DAPI.tif`



Chapter 4: Preparing For Acquisition

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

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

Assay Design

Evaluating your Experiment Requirements

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

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

Selection of Different Fluorochromes

Typical high-content assays include one or more fluorochromes, including fluorescent proteins, antibody-based stains, and chemical-based stains. In general, Molecular Devices recommends including 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 are planning on using a standard MetaXpress Software module to analyze your data, review the requirements of that module.

Individual fluorochromes have unique characteristics that help determine their best use. Use probes that provide bright, specific staining and that have excitation and emission spectra suitable for the filter sets in your ImageXpress System. For experiments using multiple stains, select fluorophores that have sufficient spectral separation. Some fluorochromes provide brighter intensities and require a shorter exposure time, while others do not bleach as quickly and 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 choosing a fluorochrome.

Note: If your 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 selecting cells for a high-content assay is whether they are compatible with the biology being studied. The assay should give a robust response with clear distinction between positive and negative phenotypes. In addition, it is important to select a source of cells where it is possible to obtain consistent results from batch to batch, whether they are primary cells or cell lines, and whether they are transfected or not.

Plates or Slides

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

Plate Selection

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

In addition to availability and cost, consider the following factors when selecting plates for your assay:

- Plate Format, see page 61
- Plate Material, see page 61

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 for pipetting into and washing the plates?

Plate Material

The composition of the material of the bottom of the microplate needs to be of optical quality, or the images can be degraded. For fluorescence imaging, microplates with black well sides and a single-piece clear bottom usually work best. Plastic-bottomed plates are generally more uneven and distort light more than glass-bottomed plates. When using high magnifications, there are significant differences in clarity between standard plastic plates, optically clear plastic plates, and glass bottom plates.

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

Fluorescence Background

There is a large difference in auto-fluorescence between glass and plastic. Also, there can be up to a five times difference in auto-fluorescence among plates from different manufacturers.

Bottom Thickness

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

Note: Plates with ultra-thin bottoms or very thick bottoms can be more uneven, possibly causing focusing issues. For best results, Molecular Devices recommends using imaging-quality plates with 0.15 mm–0.7 mm bottom thickness.

Plate Flatness or Reproducibility of the Z-Pattern

A flat plate is faster to scan than an uneven plate because the autofocus search range can be made smaller. The reproducibility of a plate allows you to set tighter focus ranges specifically for that plate type. This reduces the amount of focusing needed and speeds up acquisition. The major component in plate flatness is the variation from a well to a neighboring well.

Plate Skirt

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

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, the plate settings must be optimized for each batch.

Robot Compatibility

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

Sample Preparation

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

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

- Cell Density, see page 63
- Fixation and Staining Conditions, see page 63
- Final Buffer or Media, see page 63
- Plate Handling and Storage, see page 64

Cell Density

Cell density can affect the performance of the cells as well as downstream image analysis. If the cells are very sparse, you might need to acquire many sites in order to have a sufficient 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 washing 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 optimizing the assay, it is also helpful to test a range of antibody and stain concentrations to determine the best conditions for your cells.

Final Buffer or Media

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

Plate Handling and Storage

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

Plate Acquisition Settings

Nearly all settings for plate acquisition are made in the MetaXpress Software in the **Plate Acquisition Setup** dialog. The settings that you choose are dependent on the content and distribution of your samples, as well as the requirements of your experiment.

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

- Magnification, see page 64
- Correction Collars, see page 65
- Binning, see page 65
- Widefield vs. Confocal Imaging Mode, see page 65
- Site Selection, see page 66
- Shading Correction, see page 66
- Autofocus, see page 66
- Illumination Settings, see page 67
- Exposure Time, see page 67
- Z Series Acquisition, see page 67

Magnification

The magnification setting depends on the type of information you want to get. 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. Generally, for counting cells, a 4x objective is suitable. For translocation, a 20x or 40x objective can be appropriate. Counting or localizing small organelles might require objectives above 40x.

Other considerations include the numerical aperture (NA) of the objective, the working distance, and the optical corrections built into the objective. With the magnification constant, brightness is proportional to the square of NA. Higher NA objectives also produce a sharper picture when used with plate-bottom thickness matched to the objective correction thickness, typically 170 µm. Unfortunately, higher NA objectives, such as a 40X Plan Apo, fail to reach the outer rows and columns of some multi-well plates because of the plate skirt height.

Correction Collars

If you are using an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you are using. For information on configuring the correction collar, see Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 278.

Binning

Another method of increasing 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. Binning is often used to decrease the exposure time dramatically while maintaining the same signal intensity. Another positive feature of binning is that it produces smaller images that require less storage space and are analyzed more quickly. Some assays benefit greatly from binning, while others will require unbinned images.

Widefield vs. Confocal Imaging Mode

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

Therefore, if it is important to measure the total intensity of a particular fluorophore in cells, it 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

Using multiple sites in a single well lets you to acquire images from a greater area of the well. If you select the **Single Site** option on the **Sites to Visit** tab, an image is acquired for only a single site located in the center of the well. The **Fixed number of sites** option lets the MetaXpress Software acquire separate images within a given well, and those images can be of contiguous or distributed areas.

Using the stitch command, you can assemble the smaller separate images into a single large image. This capability lets you 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 also be used to include specific areas of the wells in your experiment data, while at the same time excluding other areas of the well. For example, the center of the well 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. Your ImageXpress Micro System is designed to minimize shading effects. If needed, use the available shading correction within the software. This is especially recommended for assays where comparison of individual cell intensities is critical, such as a cell cycle assay.

Autofocus

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

Illumination Settings

Select the appropriate illumination setting for your assay. The Semrock website at www.semrock.com is a good resource to help you determine the best filters to use with your fluorophores.

Exposure Time

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

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

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

Z Series Acquisition

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

Instrument Maintenance

For best performance, your ImageXpress Micro System should have regular preventive maintenance (PM) services. In between PM services, the system administrator can clean dust off the optics as needed (see Maintenance on page 255). In addition, the liquid light guide providing light from the light source to the instrument degrades over time and must be replaced on a regular basis. Wear gloves when handling any optical components to avoid contaminating them with dirt or skin oils.

The basic design of the ImageXpress Micro System light source and light path within the imager help ensure that the light reaching your sample is the best possible quality. If you find that the light quality has become degraded, contact your system administrator or your Molecular Devices representative to correct the problem.

Chapter 5: Configuring Plate Acquisition Options

Before configuring an experiment, it is important to become familiar with the configuration tools available in the MetaXpress® Software. The foundation of the MetaXpress Software is the MetaMorph® Microscopy Automation and Image Analysis Software. The MetaMorph Software contains numerous dialogs for image acquisition, processing, and acquisition. The MetaXpress Software adds database integration and tools for controlling the ImageXpress Micro System and for acquiring and analyzing microplates.

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

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

Note: For more information about the other dialogs that you can use to acquire plate data, specifically the **Plate Acquisition** and **Plate Acquisition and Control** dialogs, see Running Plate Acquisitions on page 143.

The following topics are included in this section:

Accessing the Plate Acquisition Setup Dialog

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

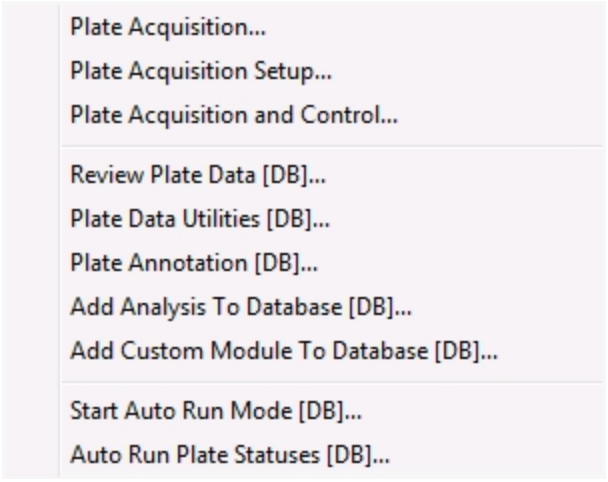


Figure 5-1: Default Screening Menu

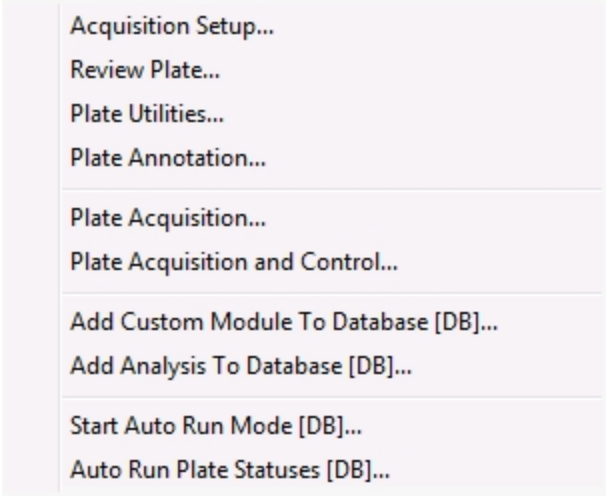


Figure 5-2: Simplified Screening Menu

Plate Acquisition Setup Dialog Layout

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

- **Plate Navigation** that provides manual control of some of the movable physical components of the ImageXpress Micro System.
- Configuration control on the **Configure** tab for setting up plate acquisition protocols.
- **Acquisition Control** on the **Run** tab for doing a plate acquisition according to a selected protocol.

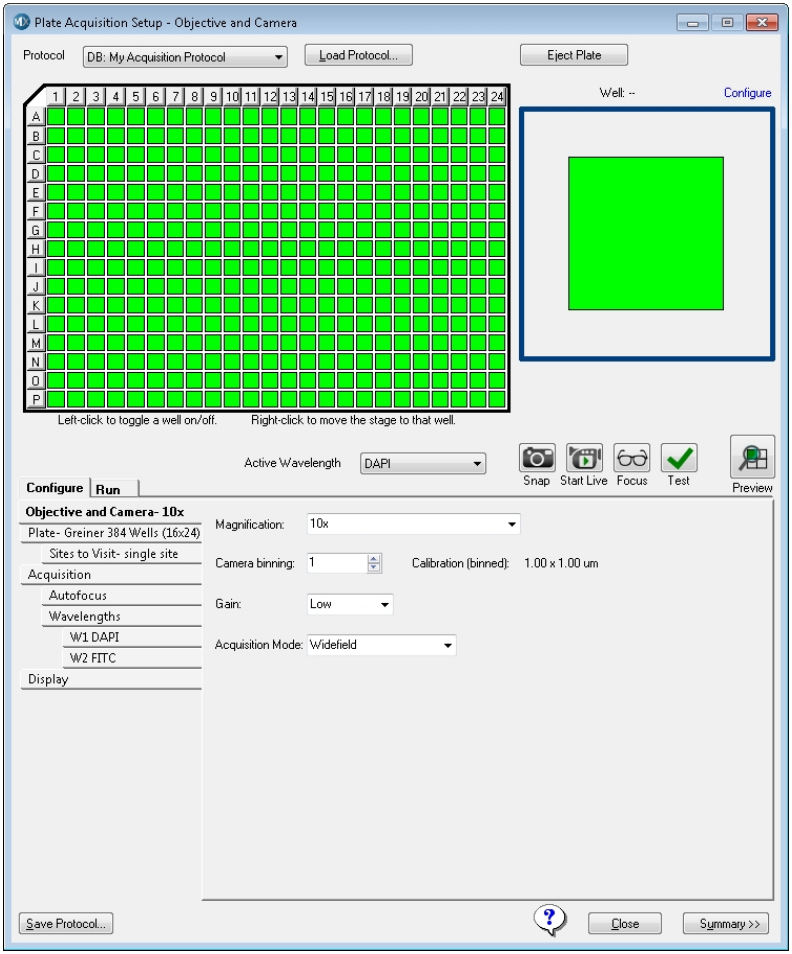


Figure 5-3: Plate Acquisition Setup Dialog

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

- **Protocol** provides a list of the eight most recently used plate acquisition protocols.
- **Load Protocol** button opens the **Load Plate Acquisition Protocol** dialog in which you select a protocol for plate acquisition.
- **Eject Plate/Load Plate** button switches between the following two options
  - **Eject Plate** opens the top door for loading or removing a plate.
  - **Load Plate** closes the top door so that the plate can be acquired.
- **Plate map** (upper left) is a graphical representation of the type of plate for which you are configuring the wells. The graphic is interactive to provide manual control for moving a stage to a specific well. By default, when the **Plate Acquisition Setup** dialog opens the first time, and before any protocols are configured and loaded, all of the wells in the plate map are selected for data acquisition. Selected wells in the plate map are displayed in bright green.

  To disable any data acquisition selection, click on the specific well in the graphic, click and drag over a section of wells, or click the row or column header. Disabled wells in the plate map are displayed in gray.

  To enable or disable all wells, click the upper left corner triangle.

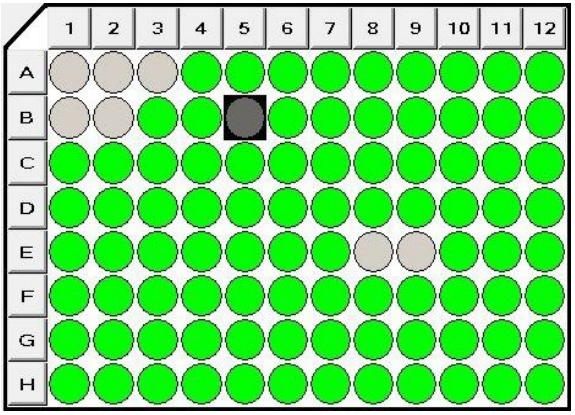


Figure 5-4: Plate Acquisition Setup Dialog, Plate map

- **Site map** (upper right) is a graphical representation of the number of sites in each well and the position of the sites that are to be acquired for each well. The graphic is interactive to provide manual control for some of the movable physical components of the ImageXpress Micro System. You use this interactive graphic to configure the sites that are to be acquired. Selected sites in the map are highlighted in bright green. Disabled sites in the map are highlighted in gray.

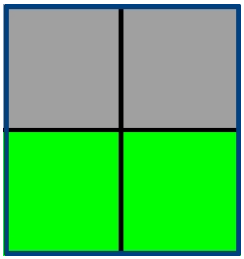


Figure 5-5: Plate Acquisition Setup dialog, Site map

- **Active Wavelength** field displays the currently selected acquisition wavelength.
- **Active Wavelength** tools provide shortcuts to various functions that can be done for the currently selected acquisition wavelength.

Figure 5-6: Active Wavelength tools

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

- **Configure tab** provides the necessary options for configuring a plate acquisition protocol. The **Configure** tab is organized in a “top-to-bottom” tab structure that runs down the left side of the dialog and is designed to guide you through the process of setting up your protocol configuration in a logical order. Each tab is dedicated to a specific type of function or setting. The tabs are dynamically updated according to the options that you have selected and the number of wavelengths that you are acquiring. See
  - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76
  - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78
  - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83
  - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99
  - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137
- **Run tab** includes essential options for acquiring a plate using a selected protocol.
- **Save Protocol** opens the **Save Acquisition Protocol** dialog in which you can save the current plate acquisition settings as a protocol. See Saving a Plate Acquisition Protocol on page 142.

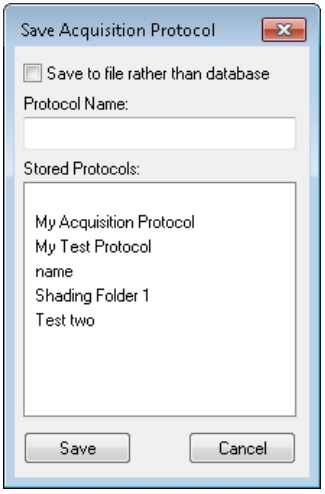


Figure 5-7: Save Acquisition Protocol dialog

- **Summary** button opens the **Summary** panel that displays a summary of all the current acquisition settings. See Viewing the Summary Panel on page 141.

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

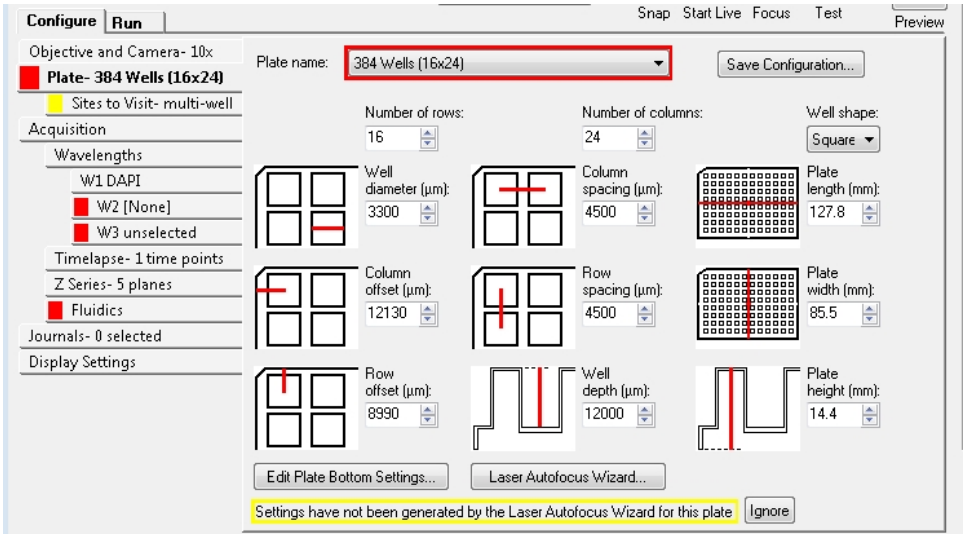


Figure 5-8: Plate Acquisition Setup Dialog with Warning Icons

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

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

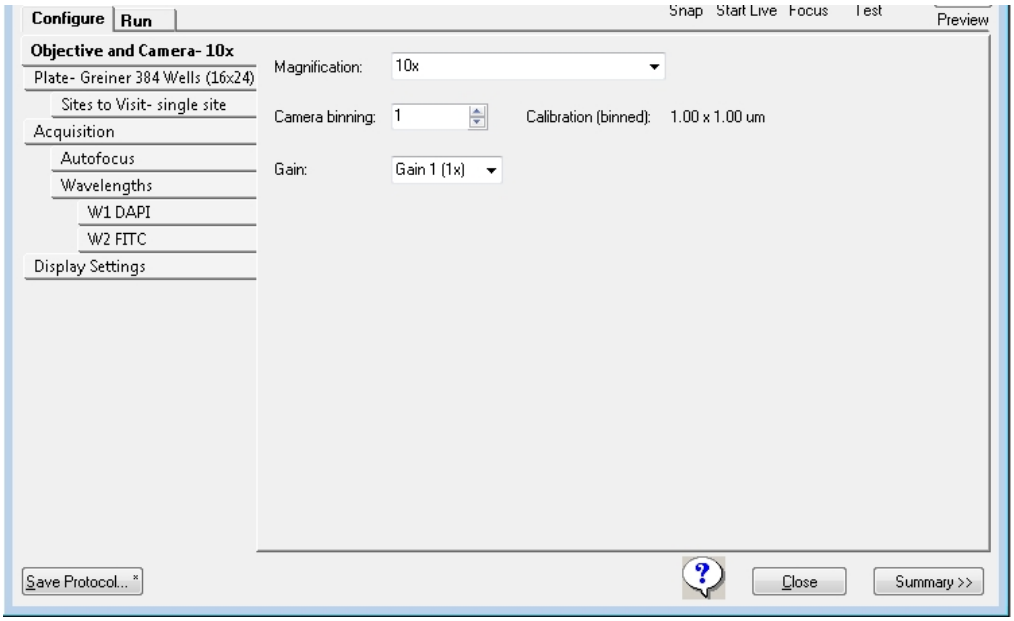


Figure 5-9: Plate Acquisition Setup dialog: Configure tab, Objective and Camera tab

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

Option	Description
Magnification	Selects the magnification setting for the protocol. Magnification settings assign X and Y offset values (parcentricity) and a Z offset (parfocality) to a specific objective. When you select a specific objective, the objective is physically moved to the acquisition position. **Note:** The available magnifications are defined with the **Configure Magnification** dialog. See Verifying Magnification Settings on page 44. You must assign a calibration to each magnification setting. See Verifying Calibration Settings on page 46.

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

Option	Description
Camera binning	Specifies the binning value that is to be applied to the camera. Binning combines the output of adjacent pixels in square multiples. For example, a camera binning value of 1 is only one pixel, a binning value of 2 combines 2x2 or four pixels in a square, a binning value of 3 combines 3x3 or nine pixels in a square, and so on. This reduces the image file size and resolution, 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.
Camera gain	Specifies the amplification that is to be applied to the camera output.

Configuring Objective and Camera Options

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

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

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

4. If applicable, select the amount of gain.
5. When you are finished configuring the acquisition protocol, then continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue with the next steps to further optimize your protocol settings. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Plate Acquisition Setup Dialog: Configure Tab, Plate Tab

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

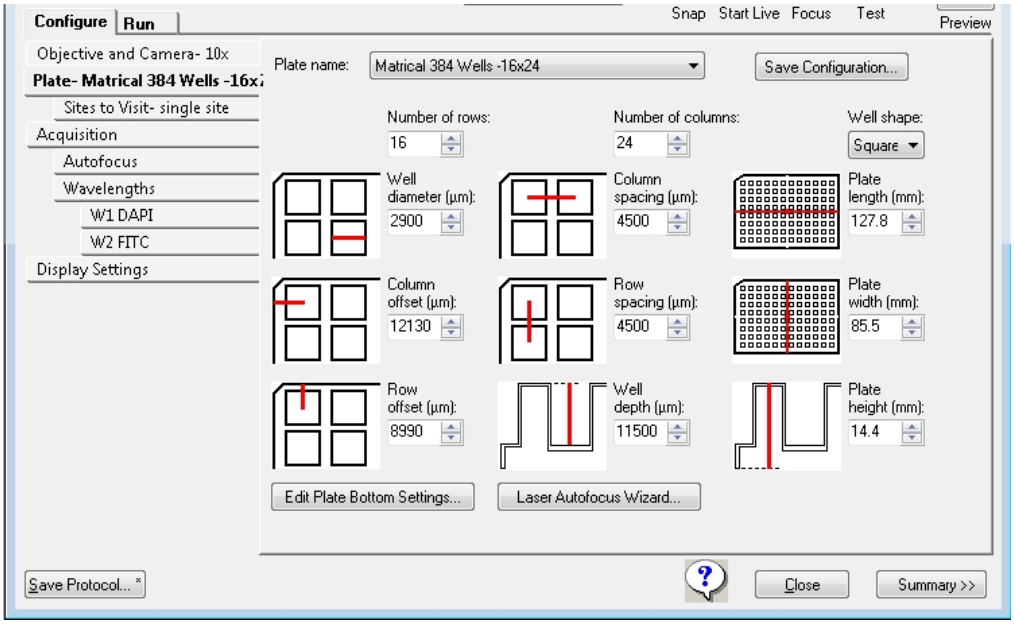


Figure 5-10: Plate Acquisition Setup dialog: Configure tab, Plate tab

Note: The MetaXpress Software installation flash drive comes with a variety of common plate types already configured. From the MetaXpress Software flash drive, find the **Plates** folder for plate files. Also see: Verifying Plate Types on page 53.

Table 5-2: Plate Settings

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

Table 5-2: Plate Settings (continued)

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

Considering Plate Dimensions

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

- Bottom thickness is a value in µm that is the average 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 the objective. These values are not equivalent to the physical thickness measurements that the plate manufacturer provides. The optical thickness is calculated by the software 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. Also, 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 are required for each objective.

Note: The **Laser Autofocus Wizard** calculates measurements as accurately as possible. Some manual verification and adjustment of the settings might be necessary to optimize the results, particularly for thin-bottom plates. For detailed information about the settings that the wizard calculates, see the “Configure Laser Autofocus Settings Dialog Options” topic in the MetaXpress Software help. With the Laser Autofocus wizard open, press **F1** to access the help. If you need more assistance, contact Technical Support. See Obtaining Support on page 309.

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 309.

Configuring the Dimensions for a new Custom Plate Configuration

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

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

1. Choose the appropriate size for your custom plate.
2. Click **Save Configuration**.
3. In the **Plate Acquisition - Save Configuration** dialog, type the name for your custom plate configuration, and then click **Save**.
4. In the **Well shape** field, select the well shape for the plate.
5. Complete the remaining configuration fields as required. See Table 5-2: Plate Settings on page 79.

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

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

   Note: If needed, adjust any objectives with correction collars to match the plate bottom thickness. See Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 278.

10. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
    - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
    - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
    - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
    - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

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

You configure the number of sites that are to be acquired for each well in a plate acquisition protocol on the **Sites to Visit** tab. You can acquire a single site per well, or multiple sites per well. The number of sites that you can acquire in a well depends on the size of the well, the objective magnification, the distribution of sample material in the well, the type of plate, and the fluid content of the well.

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

Note: You use the plate map and site map that are at the top of the **Plate Acquisition Setup** dialog to select and move to the wells and sites that are to be analyzed in a plate acquisition protocol.

Also See:

- Acquiring a Single Site in Each Well on page 83
- Acquiring a Fixed Number of Sites in Each Well on page 86
- Configuring Adaptive Acquisition for Well Sites on page 89
- Configuring a Multi-Well Acquisition on page 97

Acquiring a Single Site in Each Well

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

Tip: To acquire a single off-center site, you should select **Fixed number of sites**, configure the site layout, and then deselect the unwanted sites. See Acquiring a Fixed Number of Sites in Each Well on page 86.

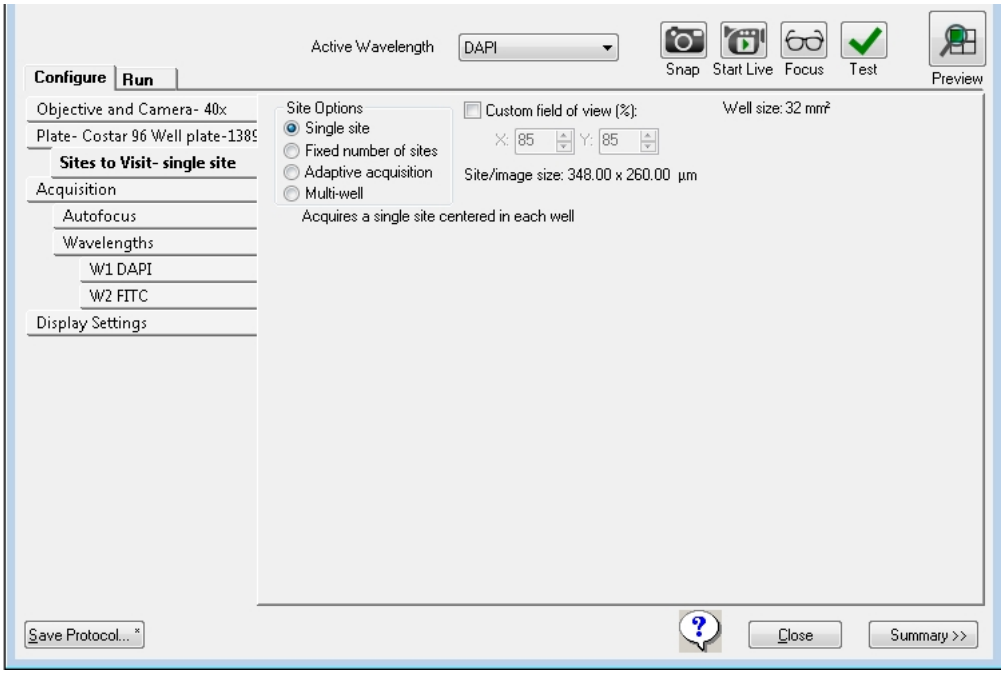


Figure 5-11: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Single site option selected

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

1. Open the **Sites to Visit** tab.
2. Select **Single Site**.

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

Note: The full field of view for the camera is 100 percent (100% in the **X** field and 100% in the **Y** field). So, for example, if the full field of view width is 1000 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.

Note: The **Custom Field of View** feature is compatible with full-size shading correction images when using **Legacy Correction**. See Correcting Image Shading on Acquired Images—Legacy on page 118.

Tip: This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include the area outside the well boundary.
For **Multi-well** acquisitions, use the full field of view, because the **Custom Field of View** value severely reduces the amount from each well acquired.

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

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

5. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Acquiring a Fixed Number of Sites in Each Well

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

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

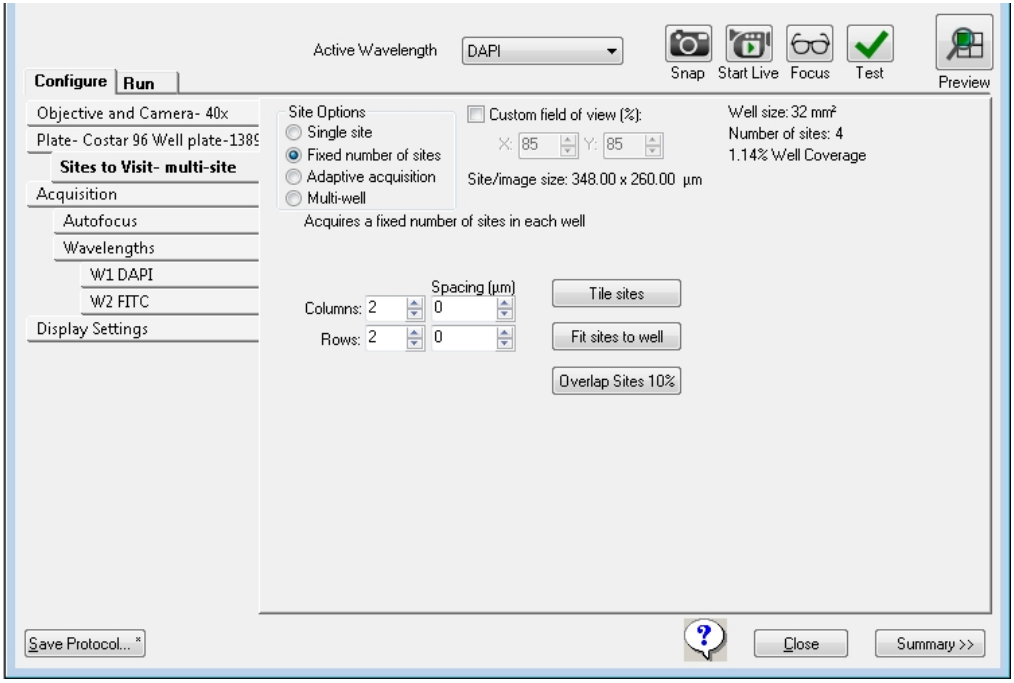


Figure 5-12: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Fixed number of sites option selected

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

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

   Note: The full field of view for the camera is 100 percent (100% in the **X** field and 100% in the **Y** field). So, for example, if the full field of view width is 1000 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.

   Note: The **Custom Field of View** feature is compatible with full-size shading correction images when using **Legacy Correction**. See Correcting Image Shading on Acquired Images—Legacy on page 118.

   Tip: This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include the area outside the well boundary.
   For **Multi-well** acquisitions, use the full field of view, because the **Custom Field of View** value severely reduces the amount from each well acquired.

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

   Note: The maximum allowable values for these fields are **Columns: 45** and **Rows: 35**.

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

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

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

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

   Note: You can configure automated data acquisitions for the wells as part of a protocol. See Running Plate Acquisitions on page 143.

7. Optionally, do one of the following as needed:
   - To manually adjust the spacing between adjacent sites, where the default value is zero, or no spacing, enter the X and Y values for the spacing in the **Spacing (µm)** fields.
   - To manually adjust the spacing between adjacent sites so that the sites overlap, enter negative X and Y values for the spacing in the **Spacing (µm)** fields.

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

   - To automatically adjust the spacing between adjacent sites so that there is zero spacing between them, click **Tile sites**. The X and Y spacing values are set to zero.
   - To automatically adjust the spacing between the adjacent sites to the maximum allowed value based on the selected plate type, click **Fit sites to well**. The X and Y spacing values are updated accordingly.
   - To automatically overlap the sites by 10%, click **Overlap Sites 10%**. The X and Y spacing values are updated to the appropriate negative values based on the selected plate type.
8. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Configuring Adaptive Acquisition for Well Sites

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

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

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

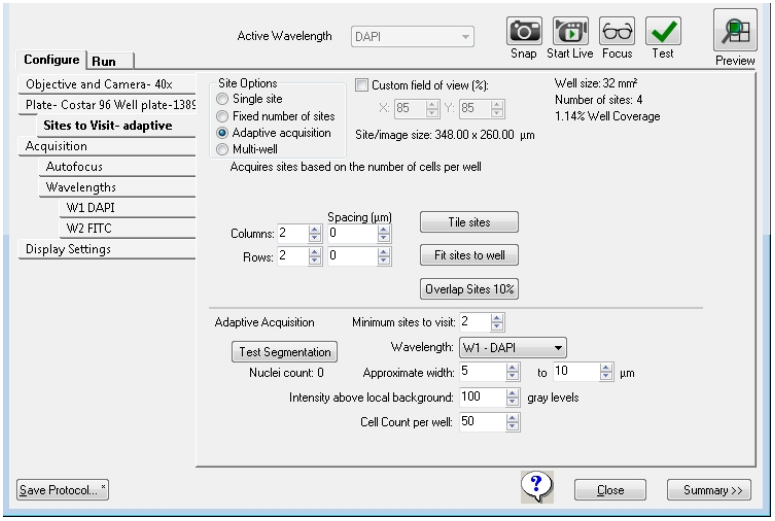


Figure 5-13: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Adaptive Acquisition option selected

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

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

   Note: The full field of view for the camera is 100 percent (100% in the **X** field and 100% in the **Y** field). So, for example, if the full field of view width is 1000 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.

   Note: The **Custom Field of View** feature is compatible with full-size shading correction images when using **Legacy Correction**. See Correcting Image Shading on Acquired Images—Legacy on page 118.

   Tip: This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include the area outside the well boundary.
   For **Multi-well** acquisitions, use the full field of view, because the **Custom Field of View** value severely reduces the amount from each well acquired.

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

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

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

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

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

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

   Note: You can configure automated data acquisitions for the wells as part of a protocol. See Running Plate Acquisitions on page 143.

7. Optionally, do one of the following as needed:
   - To manually adjust the spacing between adjacent sites, where the default value is zero, or no spacing, enter the X and Y values for the spacing in the **Spacing (µm)** fields.
   - To manually adjust the spacing between adjacent sites so that the sites overlap, enter negative X and Y values for the spacing in the **Spacing (µm)** fields.

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

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

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

Table 5-3: Adaptive Acquisition Settings

Setting	Description
Minimum sites to visit	The minimum number of sites to visit in a well. The MetaXpress Software acquires at least this minimum number of sites and then it continues to acquire sites until the total number of cells counted per well value as specified in the **Cell count per well** field is reached.
Wavelength	The wavelength that is used to differentiate nuclei in the source image. **Note:** For the most efficient acquisition, this wavelength should be the same as the wavelength that is designated as being the first to be acquired. See Specifying the Number of Acquisition Wavelengths on page 106.
Approximate width	The approximate minimum width and the approximate maximum width of the nuclei that are expected to be detected. **Note:** Nuclei patterns in the source image that fall below this range are considered as noise. The algorithm might split larger objects that are above the maximum width into smaller objects, which can affect the cell count.
Intensity above local background gray levels	The intensity threshold of nuclei in the source image compared to the neighboring background values. This setting controls the sensitivity of detection. **Note:** For information about setting the intensity threshold, see Calculating the Intensity Above Local Background Gray Levels Value on page 96.
Note: The **Approximate width** and the **Intensity above local background gray levels** work together. A nucleus is determined by an object size and relative intensity above the background.	
Cell count per well	The total number of nuclei in each well that the MetaXpress Software must acquire before stopping acquisition of the well. **Note:** The MetaXpress Software always acquires the minimum number of sites, even if the **Cell count per well** can be acquired with a fewer number of sites. As the **Adaptive Acquisition** mode runs, a real-time count of the cells in the currently selected well is displayed as the images are acquired. **Adaptive Acquisition** stops acquiring when the maximum number of sites that have been defined is reached, even if the specified cell count is not acquired.

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

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

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

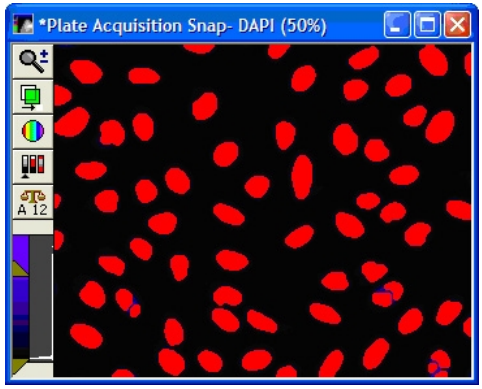


Figure 5-14: An example of Test Segmentation results in the Adaptive Acquisition mode. The red shows the segmentation overlaid on the original DAPI image.

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

10. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
    - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
    - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
    - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
    - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Calculating the Intensity Above Local Background Gray Levels Value

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

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

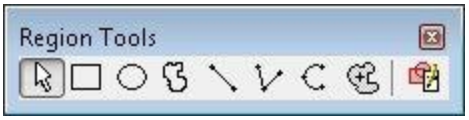


Figure 5-15: Region tools on the Region toolbar

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

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

Figure 5-16: Gray level indicators

5. Position your mouse pointer just to the outside of the object and make note of the gray level value that is displayed in the status bar.

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

> **Tip:** Instead of doing Step 4 and Step 5, you can draw a line region across a cell and its local background, and then use the **Linescan** tool that is available from the **Measure** menu to see more exact intensity values. For more information about this tool, see the MetaXpress Software application help.

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

   Enter this value into the **Intensity above local background** field in this dialog.

Configuring a Multi-Well Acquisition

To acquire high density plates faster, you can run a multi-well acquisition. A multi-well acquisition uses the size of the camera field of view to acquire several wells simultaneously while taking a single image, thereby reducing plate acquisition time. The MetaXpress Software automatically calculates the number of wells in which a site can be simultaneously acquired. This value depends on the plate well density and the magnification that is configured for the protocol. Common configurations for multi-well acquisitions include a 1536-well plate with a 4x magnification objective or a 384-well plate with a 2x magnification objective.

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

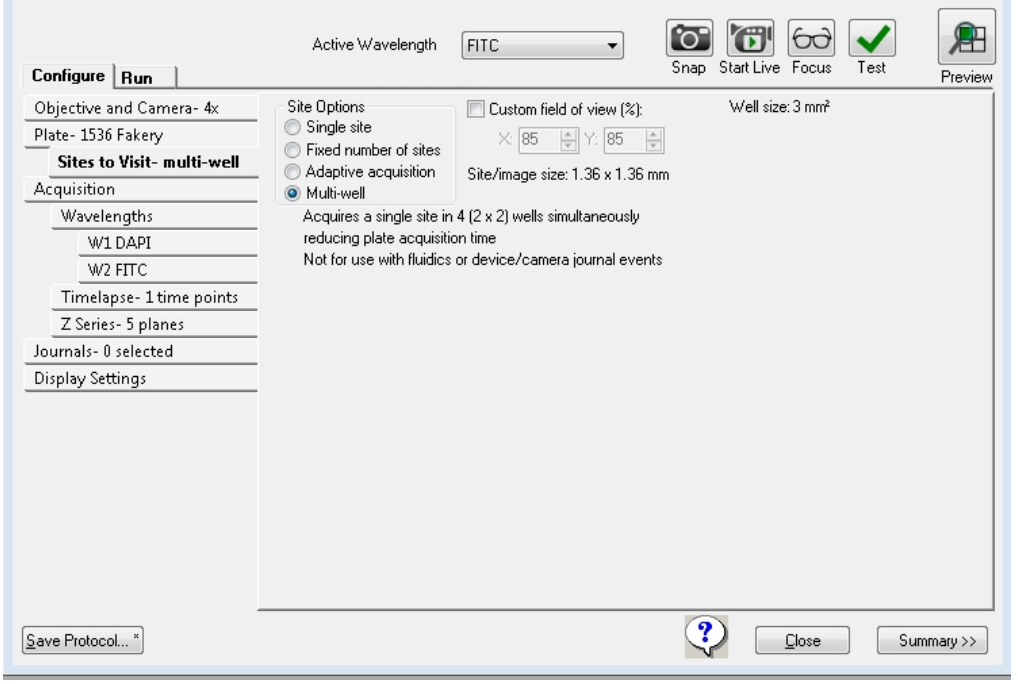


Figure 5-17: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Multi-well option selected

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

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

Note: The full field of view for the camera is 100 percent (100% in the **X** field and 100% in the **Y** field). So, for example, if the full field of view width is 1000 µm, then to acquire a width of 500 µm, enter **50** in the **X** field.

Note: The **Custom Field of View** feature is compatible with full-size shading correction images when using **Legacy Correction**. See Correcting Image Shading on Acquired Images—Legacy on page 118.

Tip: This feature is particularly useful for acquiring images from an unevenly illuminated full field, or for acquiring images that include the area outside the well boundary.
For **Multi-well** acquisitions, use the full field of view, because the **Custom Field of View** value severely reduces the amount from each well acquired.

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

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

5. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab

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

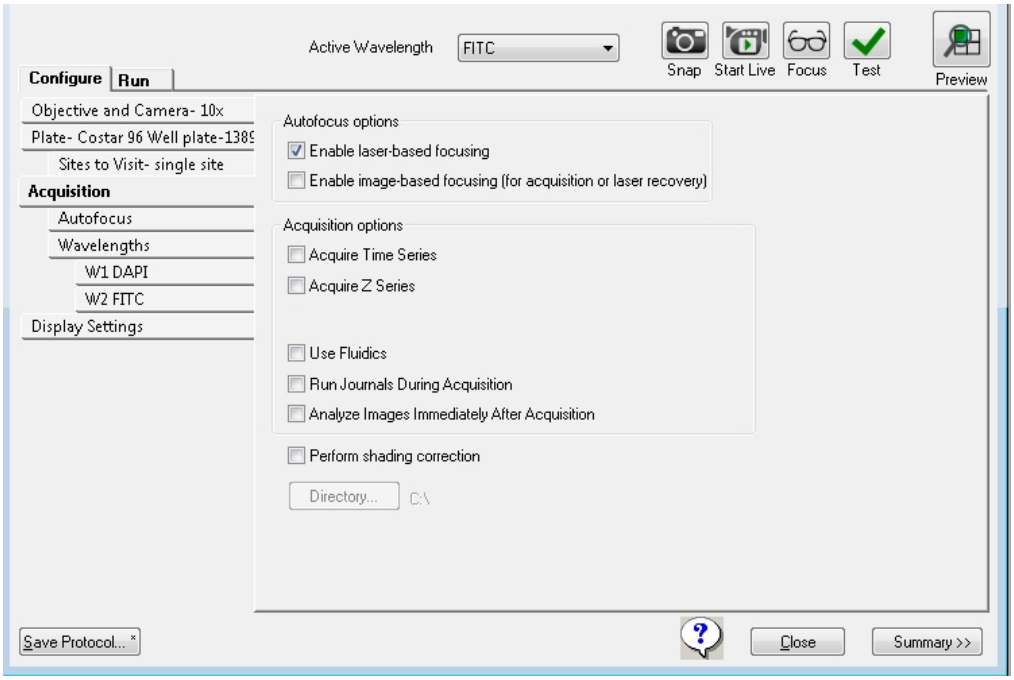


Figure 5-18: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

See:

- Configuring Autofocus Options on page 100
- Specifying the Number of Acquisition Wavelengths on page 106
- Configuring Series Acquisition Options on page 120
- Configuring Journals to Run During Acquisition on page 129
- Configuring Post-Acquisition Analysis Options on page 133

Note: To configure fluidic stations, select **Use Fluidics** to enable the **Fluidics** tab. For information about configuring fluidics stations and properties, see Optional Expansion Solutions on page 175, or the MetaXpress Software help topic “Fluidics Events.”

Configuring Autofocus Options

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

- **Laser-based Focusing** is generally set to find the bottom of the well, and then moves the objective a specified distance up from the well bottom. This method is generally the fastest and does not cause photo damage to your wells. However, this method might not

be sufficient if the distance above the bottom of the well varies in your sample. Thumbprints or scratches at the bottom of the plate affect focus performance.

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

Certain types of samples can benefit from using both **Image-based Focusing** and **Laser-based Focusing**, including live organisms, suspension cells, tissue samples, and assays where the best focus position varies with the phenotype. When you select both focus options, the **Laser-based Focusing** is used move the selected objective to a specified position above the bottom of the well, and the **Image-based Focusing** is used to fine tune the focus.

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

To configure autofocus options, use the following procedure:

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

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

3. Click **Autofocus**.

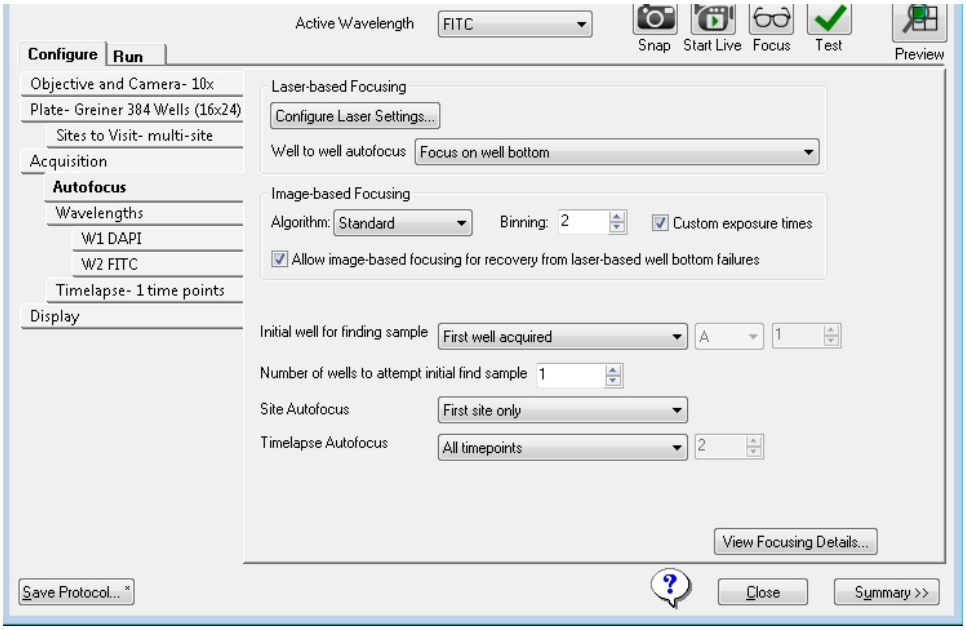


Figure 5-19: Plate Acquisition Setup dialog: Configure tab, Autofocus tab

4. Configure the **Autofocus options**.

Table 5-4: Autofocus options

Options	Description
Laser-based Focusing is available only if **Enable laser-based focusing** is selected on the **Acquisition** tab.	
Configure Laser Autofocus Settings	Opens the **Configure Laser Autofocus Settings** dialog. The settings for this dialog will have been calculated by running the **Laser Autofocus Wizard**. If the wizard has not been run for a selected plate and objective, then the dialog will not contain any values. You can modify the settings that the wizard has calculated for this dialog. **Note:** The MetaXpress Software help topic "Configure Laser Autofocus Settings - Dialog Options" provides detailed information about the settings that the wizard calculates. Press **F1** with the dialog open to access the help. For tips about using the **Laser Autofocus Wizard**, contact Technical Support for assistance. See Obtaining Support on page 309.
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	The default value and 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 values recorded in the **Edit Plate Bottom Settings** option on the **Plate** tab to determine the focus range.
Focus on plate bottom, then offset by bottom thickness	Offsets the focus laser by the **Bottom thickness** of the plate from the plate configuration. Select this option if you are using any of the following: – A thin-bottom plate with 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
Focus on plate and well bottom	The laser focuses on both the plate bottom and the well bottom at every well. Recommended setting for plates with extreme bottom variation.

Table 5-4: Autofocus options (continued)

Options	Description
Image-based Focusing	
Algorithm: includes the following focusing algorithm options.	
Standard	Default algorithm, based on a standard group of settings including a normal camera signal level.
Low Signal	Based on a set of values designed to compensate for a low signal level on the camera, and pixel intensities that are brighter when slightly out of focus.
Camera configuration options:	
Binning	Sets the binning used by the camera during **Auto Focus** and **Show Live**. Horizontal and vertical binning are always set to the same value.
Custom exposure times	For individual wavelengths during autofocus. If this option is not selected, then the exposure time is calculated based on autofocus binning and acquisition exposure time. If this option is selected and you select either or both **Laser and Image** or **Laser with Image Recovery** for the first acquisition wavelength (W1), 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 106.
Allow image-based focusing for recovery from laser-based well bottom failures	Enabled only if both **Enable laser-based focusing** and **Enable image-based focusing** are selected. Use **Image-based focusing** only if **Laser-based focusing** cannot find the plate or well bottom. The image-based recovery search is centered on an estimated well bottom offset position. The estimated position is calculated by using the plate bottom position that was found during the last successful laser autofocus attempt, and then adding 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

Table 5-4: Autofocus options (continued)

Options	Description
Specific well	Finds the sample autofocus using a well that you specify. **Note:** After you select this option, you must specify the well row and 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 your sample is already in focus. **Note:** Select this option when using an oil-immersion objective, and you must manually find the focus before starting the plate acquisition.
Number of wells to attempt initial find sample	Enabled only if the **First well acquired** option is selected. The first well in which a sample is found is the well that is used for autofocusing. The default value is one. **Note:** If you are using a robot for automated plate loading, set this value to three or greater.
Site Autofocus is enabled only if **Fixed number of sites** or **Adaptive Acquisition** is selected on the **Sites to Visit** tab. This section includes several options for configuring site-to-site autofocus.	
First site only	Autofocuses in the top-left site in the well.
Center of well only	Autofocuses in the center of the well. **Note:** Select this option when the magnification is low and the sites are relatively close together.
All sites	Autofocuses for each site. **Note:** Select this option with higher magnification, when the sites are spread far apart, or there is extreme variation in the plate bottom.
Timelapse Autofocus is enabled only if **Acquire Time Series** is selected on the **Acquisition** tab. See Configuring Series Acquisition Options on page 120.	
First timepoint only	Use for fast kinetic acquisitions.
All timepoints	Use for long timelapse acquisitions.

Table 5-4: Autofocus options (continued)

Options	Description
Every Nth timepoint	Set a value that is less than the number of specified timepoints. See Configuring Time Series Acquisition Options on page 122.
View Focusing Details	Opens the **Auto Focus Details** dialog, which lists the current autofocus parameters. This information can be useful for diagnostic purposes when troubleshooting focusing issues. Click **Copy** to copy the parameters to the clipboard, and then paste them into a third party application, such as Microsoft Word.

5. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Specifying the Number of Acquisition Wavelengths

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

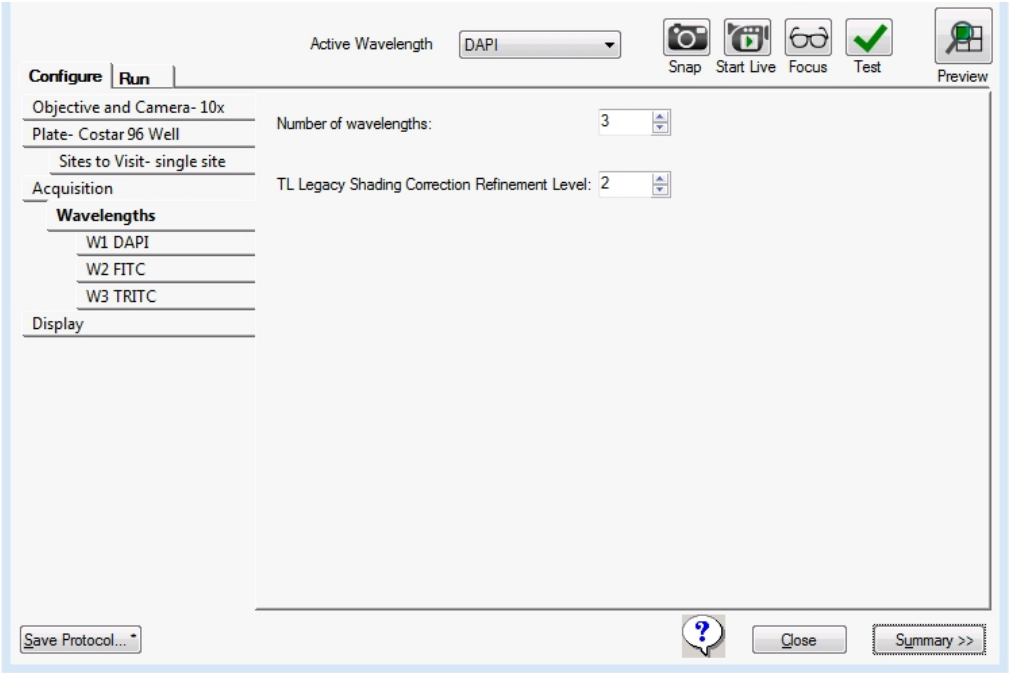


Figure 5-20: Plate Acquisition Setup dialog: Configure tab, Wavelength tab: Three placeholder Wavelength tabs

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

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

1. Click the **Wavelengths** tab.

Note: TL Legacy Shading Correction Refinement Level is always displayed on the **Wavelengths** tab. The default value is two. This option is applied only if **Autocorrect for TL Legacy** is selected 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, however increasing this value will slow the acquisition routine. The single value that is specified here applies to all acquisition wavelengths for which **Auto Correction for TL Legacy** is selected as the shading correction method. See Shading Correction on page 117.

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

Configuring an Acquisition Wavelength

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

For each individual wavelength placeholder tab, do the following in the order listed:

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

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

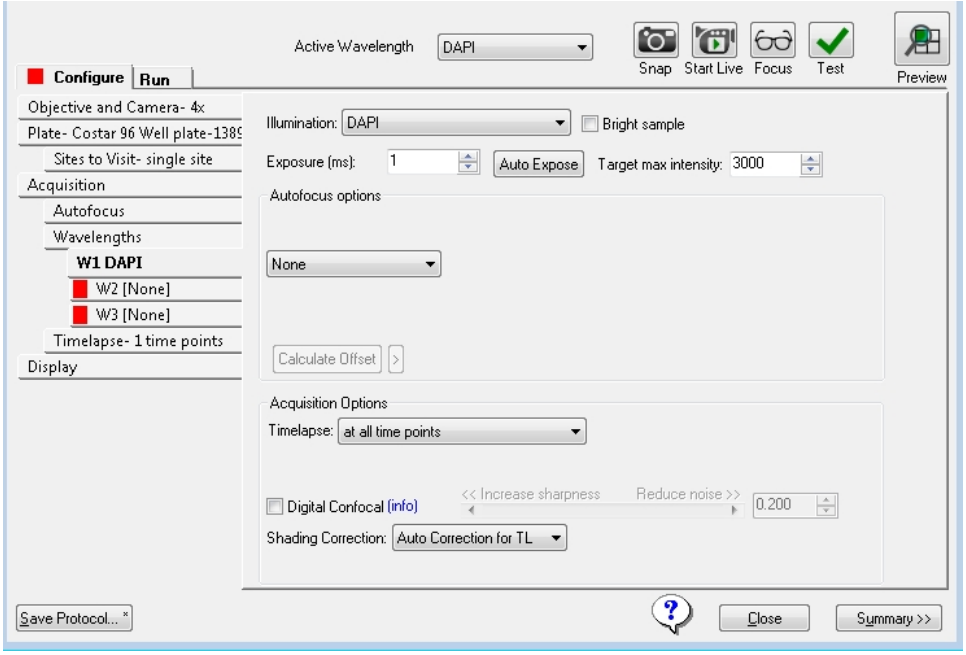


Figure 5-21: Plate Acquisition Setup dialog: Configure tab, Wavelength tab: Setting placeholder Wavelength tab

3. Configure the remaining acquisition wavelength options, where:

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

Table 5-5: Acquisition Wavelength Setting Options

Options	Description
Bright Sample	If your sample has a high intensity, but a low (1 ms to 7 ms) exposure time, then select this option to reduce the light power for the selected acquisition wavelength and prevent saturation of the sample. After you select this option, adjust your exposure time accordingly to control the precision of the sample brightness.
Exposure (ms)	Specifies the exposure time in milliseconds for the active wavelength. You can type a value in this field or click **Auto Expose** to automatically determine an exposure time. Exposure times should be determined using an in-focus image. **Note:** When you are configuring exposure times, check both the positive and the negative controls. If you use a dim sample to set the exposure time, then a bright sample might become saturated.
Auto Expose	Click to automatically set the exposure time that is required to obtain the **Target max intensity**. If **Auto Expose** results in a 1 ms to 5 ms value to achieve this intensity, then you are automatically prompted to activate **Bright Sample** mode to prevent saturation of your sample. Click **Yes** to turn on **Bright Sample** mode and automatically rerun **Auto Expose**, or click **No** to leave all settings as-is. **Note:** Auto Expose is a manual tool to help configure the plate. This feature 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:** The recommended target intensity value is 75% of the maximum gray level that is possible with your camera driver.

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

Options	Description
Autofocus options specify the type of autofocus that is to be used when acquiring images. The available options depend on the acquisition options that are selected on the **Acquisition** tab.	
None	No autofocusing is done. If you select this option, then no other autofocusing configuration options are available.
Laser with Z-offset	Displayed as an option only if **Enable laser-based focusing** is selected on the **Acquisition** tab. If selected, then the **Laser-based Focusing** is used based on the settings that the **Laser Autofocus Wizard** calculates, or as configured using **Configure Laser Settings** on the **Acquisition** tab. Specify the **Post-laser offset** value in µm, or click **Calculate Offset** to automatically calculate the first (W1) wavelength offset.
Calculate Offset icon (>)	Click **>** to display the options that are used for calculating the post-laser offset value. **Use Z stack** enables the capture of multiple Z plane images so that you can select the best in-focus plane. **Note:** Without **Use Z stack**, MetaXpress Software carries out an image autofocus and determines the best in-focus plane. **Custom Range** determines the total search range and step size for calculating the offset. **Note:** Without **Custom Range**, the default values, based on the current objective, are used for the **Calculate Offset** tool using either Z stack or image autofocus.

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

Options	Description
Laser And Image	Displayed as an option for only the first acquisition wavelength (W1) and only if **Enable laser-based focusing** and **Enable image-based focusing** are selected on the **Acquisition** tab. If selected, the laser autofocuses first, and then image-based focusing fine tunes the focus. You must also specify values for the following: **Image-based range +/- µm** specifies the search range for the image-based portions of autofocusing. **Max. step** specifies the maximum step size of a single Z move in µm that is required to get the correct focus position. This setting depends on the objective that is used. Because the focus peak is narrower, use a smaller step size with higher NA objectives. If **Custom exposure time** is selected on the **Autofocus** tab, then with **Laser and image** selected, you must also specify values for the following: **Exposure (ms)** specifies the exposure time in milliseconds for the acquisition wavelength when autofocusing. **Gain** sets the sensitivity of the camera when autofocusing. The **Gain** option is not available for every camera type.

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

Options	Description
Laser with Image Recovery	Displayed only for the first acquisition (W1) wavelength and only if **Enable laser-based focusing** and **Enable image-based focusing** are selected on the **Acquisition** tab and **Allow image-based focusing for recovery from laser-based well bottom failures** is selected on the **Autofocus** tab. When selected, you must also specify the following options: **Image-based range +/- µm** specifies the range that is to be used for the image-based portions of autofocusing. **Note:** You should adjust the range based on the sample variability. A larger range requires a longer time to focus. **Max. step** specifies the maximum step size in µm of a single Z move that is to be used to get the correct focus position. This setting is dependent on the objective that is used. Because the focus peak is narrower, use a smaller step size with higher NA objectives. **Note:** Smaller step sizes generally require more steps to arrive at the final focus position. Increasing the number of image autofocus steps increases the chances of photobleaching or phototoxicity. If **Custom exposure time** is selected on the **Autofocus** tab, then with **Laser with Image Recovery** selected, you must also specify values for the following: **Exposure (ms)** specifies the exposure time in ms that is to be used for the acquisition wavelength when autofocusing. **Gain** sets the sensitivity of the camera when autofocusing. The **Gain** option is not available for every camera type.
Z-offset from W1	Available only for the second or later (W2, W3, and so on) acquisition wavelengths. The offset for W1 must be calculated before you can set this value. Selecting this option moves the W1 focus position to the specified offset. Specify the post-laser offset in µm in the **Offset** field, or click **Calculate Offset** to automatically calculate the offset for all subsequent acquisition wavelengths.

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

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

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

Options	Description
Acquire Time Series enables **Timelapse**	Specifies the image collection intervals to use for the selected wavelength. **at all time points** is the default value. This setting enables acquisition of an image using the selected wavelength at each time point in the experiment. **at start of experiment** acquires an image using the selected wavelength at the first time point only. **at start/end of experiment** acquires an image using the selected wavelength at the first time point and the last time point for the experiment. **every nth timepoint** acquires an image using the selected wavelength at the indicated time point interval, for example, every 5th time point, beginning with the first time point for the experiment.
Acquire Z Series enables **Z Series**	**Single plane** acquires only a single plane based on the autofocus options for the selected wavelength, just as if the Acquire Z Series option were disabled. It is used in a multi-wavelength acquisition when the Z Series acquisition is not required for the selected wavelength. **2D Projection Image Only** acquires the Z Series, and a single projection image, which is saved. **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 only available if **Acquire Time Series** is cleared and **Acquire Z Series** is selected in the **Acquisition** tab.

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

Options	Description
2D Projection Image	Available for all Z Series acquisition options other than **Single Plane** indicates how the resulting 2D projection image is generated. **Best Focus** is the default value. The MetaXpress Software estimates the regions of best focus in the image stack to within one-tenth pixel accuracy along the Z axis. Two resolution grid sizes are used to enhance the criterion of focus through the stack. **Maximum** is recommended only for fluorescence. For each corresponding pixel position in the images, the pixel that has the highest intensity value out of all the planes is determined, and this is the value that is output to the resulting image. **Minimum** is recommended only for transmitted light. For each corresponding pixel position in the images, the pixel that has the lowest intensity value out of all the planes is determined, and this is the value that is output to the resulting image. **Sum** is the intensities of the pixels in the stack planes are added for each corresponding pixel position, and this is the value that is output to the resulting image. **Note:** If the sum overflows the 16-bit image capacity, then a warning message opens that indicates that the maximum possible intensity of 65535 was exceeded. The MetaXpress Software cuts the pixels off at this value as a result.
Digital Confocal	Enabled only if your organization has purchased the optional **Digital Confocal** feature. Select to do on-the-fly deconvolution-based image sharpening. **Note:** For information about the Digital Confocal option, go to http://www.moleculardevices.com/support, click on the **Knowledge Base** link, and search for “Digital Confocal.”

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

Options	Description
Shading Correction	Indicates whether shading correction has been enabled for the acquisition wavelength. Options are the following, where FL represents fluorescence and TL represents transmitted light: **Off** indicates that no shading correction is done. **Auto Correction for FL** is the most complete approach. It does both a shading correction and a background subtraction. Background subtraction removes stray light that is unrelated to the light that is emitted by the sample. The shading correction is tuned for fluorescence and adjusts for uneven illumination of the sample. The shading reference is generated once and applied over all samples. **FL Subtraction Only** does the background subtraction but not shading correction. Use this method at high magnification, or when there is a concern about the algorithm being able to correctly determine background, which can occur if the fluorescent target takes up the majority of the field of view. For example, in a scenario in which images were being taken of a tissue sample and the sample of interest covered the entire field of view, then the shading algorithm might interpret some of the signal as shading and slightly over-correct the image. **FL Shading Only** does only shading correction. It does not do background subtraction. The shading correction is tuned for fluorescence. The shading reference is generated once and applied over all samples. Recommended selection. Use this method primarily for oil immersion objectives where subtraction should not be done because it would require the breaking of the oil droplet. **Auto Correction for TL** is a shading algorithm for transmitted light images that has faster performance and improves the flatness compared to **Auto Correction for TL Legacy**. It does shading correction but not background subtraction. This option does not use the **TL Legacy Shading Correction Refinement Level** on the **Wavelengths** tab. The shading reference is collected at every site. **Auto Correction for TL Legacy** is a shading algorithm that is specifically tuned for transmitted light instead of fluorescence. It does shading correction but not background subtraction. If you select this option, then you must set the **TL Legacy Shading Correction Refinement Level** on the **Wavelengths** tab. The shading correction uses this value to flatten the images as they are captured. The shading reference is collected at every site. See Specifying the Number of Acquisition Wavelengths on page 106.

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

Options	Description
Shading Correction (continued)	**Legacy Correction** is an option that was also available in earlier versions of the MetaXpress Software. This option requires the use of preset magnification, illumination, and acquisition mode reference images that are located in a specified directory. See Correcting Image Shading on Acquired Images—Legacy on page 118. **Note:** The new methods are recommended over the **Legacy Correction** method. With the new methods, correction is always based on the current state of the system and is not subject to variation over time.

CAUTION! On an instrument with the LED Transmitted Light option installed, for non-Transmitted Light assays, Molecular Devices recommends using a black plate cover during imaging to reduce background, especially when acquiring long exposure time fluorescent images.

4. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Correcting Image Shading on Acquired Images—Legacy

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

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

The file name for a shading correction image must always include the exact matches for the **Magnification** and **Illumination** settings, for example, *Shading_10X Plan Fluor_DAPI.tif*. See Updating Shading Correction Settings-Legacy on page 304.

Tip: If a shading correction image was acquired with the full field of view, then you can use the **Custom Field of View** feature on the **Plate** tab with shading correction. See Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.

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

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

1. Click **Acquisition**.

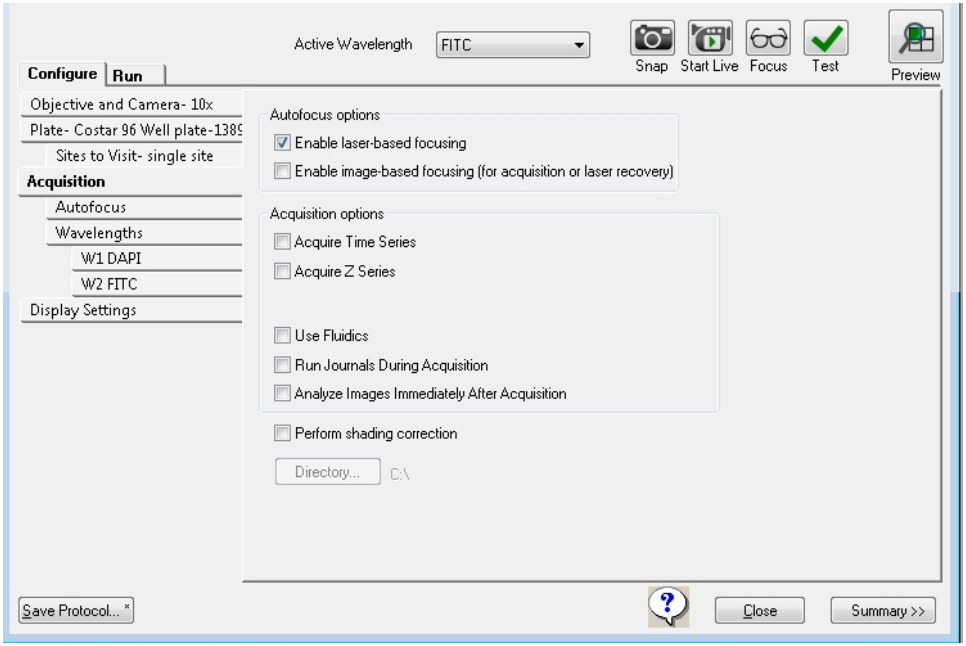


Figure 5-22: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

2. Click **Directory for Stored Correction Images**, and then in the **Browse for Folder** dialog, select the new location.

3. Click **OK** to close the dialog and return to the **Acquisition** tab.
4. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Configuring Series Acquisition Options

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

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

You can select one or both of these options.

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

1. Click **Acquisition**.

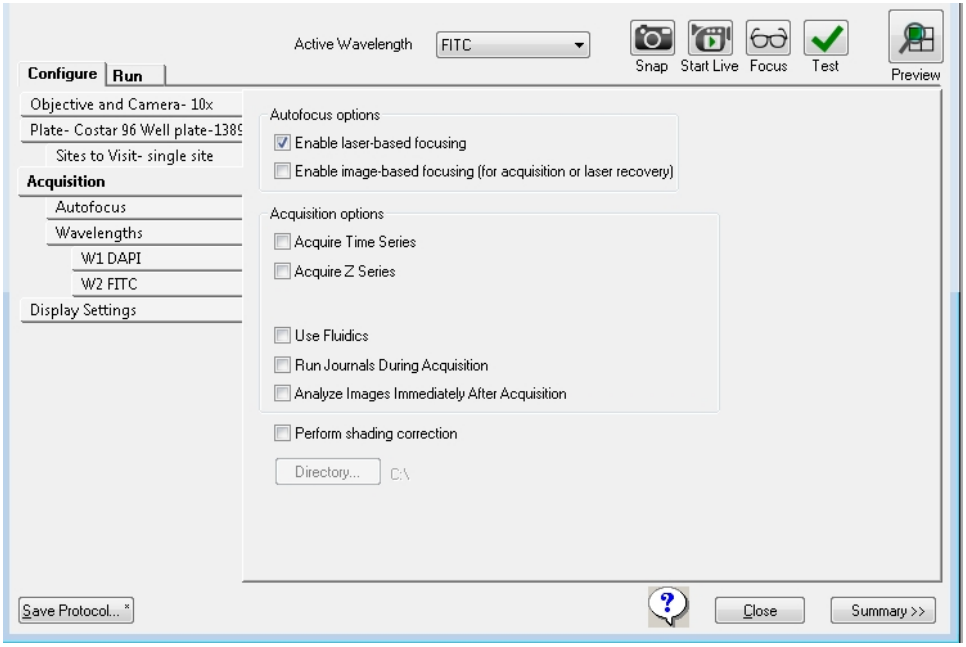


Figure 5-23: Acquisition Setup dialog, Configure tab, Acquisition tab, Acquisition options, Series options

2. Under **Acquisition options**, select one or both of the following:
   - **Acquire Time Series**
   - **Acquire Z Series**
3. Continue according to your selection:
   - For **Acquire Time Series**, continue to Configuring Time Series Acquisition Options on page 122.
   - For **Acquire Z Series**, continue to Configuring Z Series Acquisition Options on page 125.

Configuring Time Series Acquisition Options

1. Click **Timelapse**.

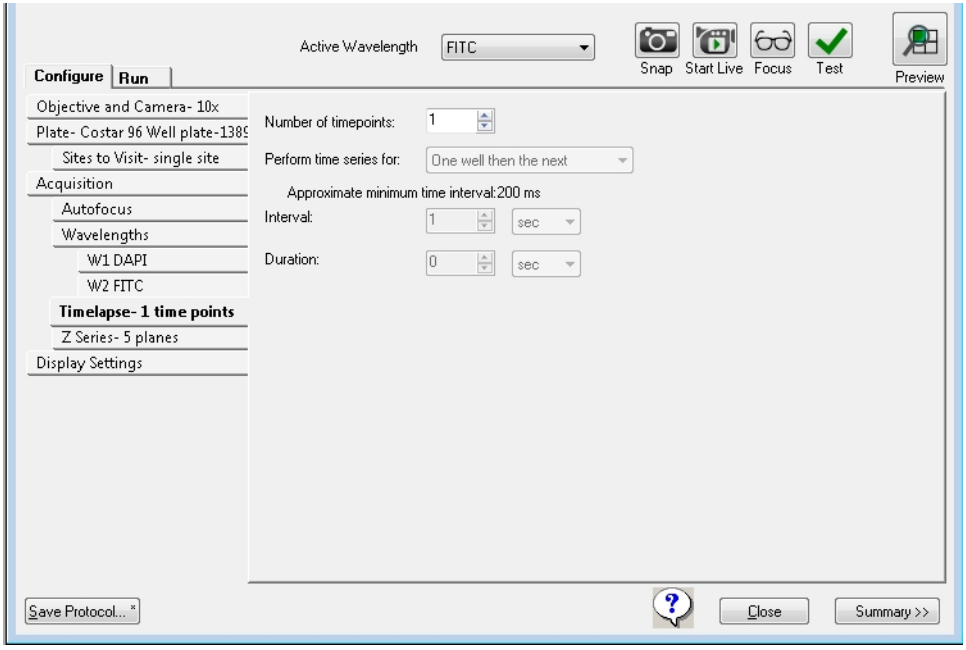


Figure 5-24: Plate Acquisition Setup dialog, Configure tab, Timelapse tab

2. Configure the time series acquisition options.

Table 5-6: Times series acquisition options

Options	Description
Number of timepoints	Specifies the total number of time points that are to be acquired. The default value is one. If you change this value, then the **Duration** field is automatically updated by calculating the **Duration** from the **Number of time points** and the **Interval**.
Perform time series for is enabled only when the value for the **Number of time points** is > 1. It specifies the loop order that is to be used for acquiring images at multiple time points and also 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 the images that are in one row of wells at each time point. After the series is collected, the next row is acquired. **Note:** This option requires a longer time interval because all the wells in a row are acquired.
One column then the next	Collects all the images in one column of wells at each time point. After the series is collected, the next column is acquired. **Note:** This option requires a longer time interval because all the wells in a column are acquired.
All selected wells	Specifies that all selected wells will be acquired at each time point in the acquisition routine. **Note:** This option requires a longer time interval because all the wells in the plate are acquired.
Note: Any refocusing that is done after the images for the first timepoint are acquired is determined by the **Timelapse Autofocus** setting that is specified on the **Autofocus** tab. See Configuring Autofocus Options on page 100.	

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

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

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

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

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

5. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Configuring Z Series Acquisition Options

1. Click **Z Series**.

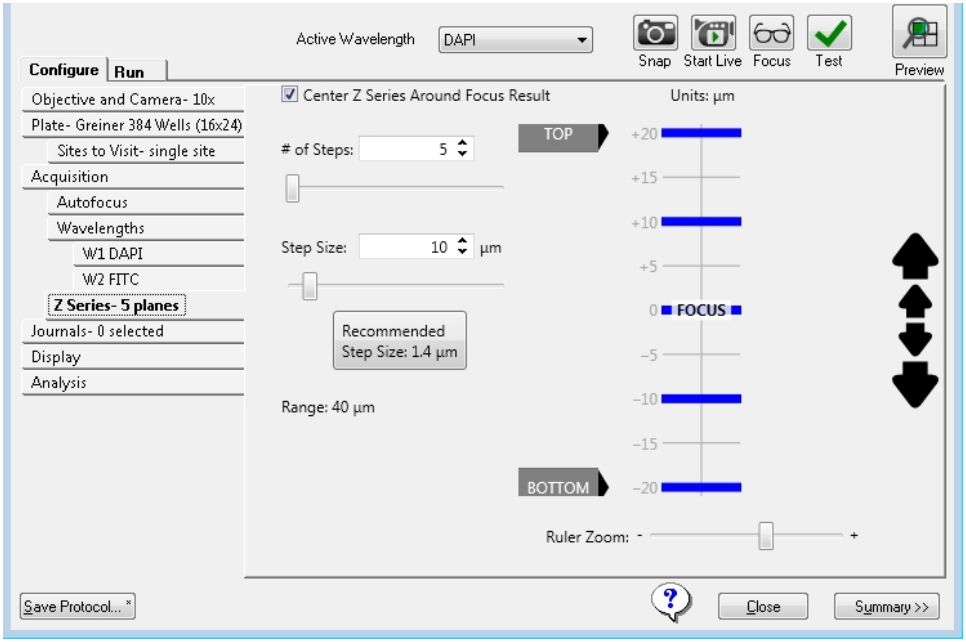


Figure 5-25: Plate Acquisition Setup dialog: Configure tab, Z Series tab showing the Z Series diagram

Tip: The blue horizontal bars displayed on the Z series ruler diagram represent the planes that will be acquired for each site imaged. The distance between these bars is calculated based upon the value entered 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 images are to be taken. For example, if set to 12, which is the default value, then 12 images will be taken along the Z axis of the sample. You can do the following to adjust this value: –Manually type a value in the field. –Click the Up or Down arrows in the field. –Drag the slider bar that is displayed below the field.
Step Size	The distance in µm between the image planes in the Z series. You can do the following to adjust this value: –Manually type a value in the field. –Click the Up or Down arrows in the field. –Drag the slider bar that is displayed below the field. **Note:** Based on the numerical aperture (NA) setting for the objective, the MetaXpress Software automatically calculates a recommended Step Size that would provide the best detail for your Z series. You can click this **Recommended Step Size** message to automatically set the **Step Size** to this recommended value.
Note: As you adjust one or both of these values, then the MetaXpress Software automatically calculates the **Range**, which is the overall image depth from top to bottom of the image sample, and displays this value below the **Step Size** on the tab.	

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

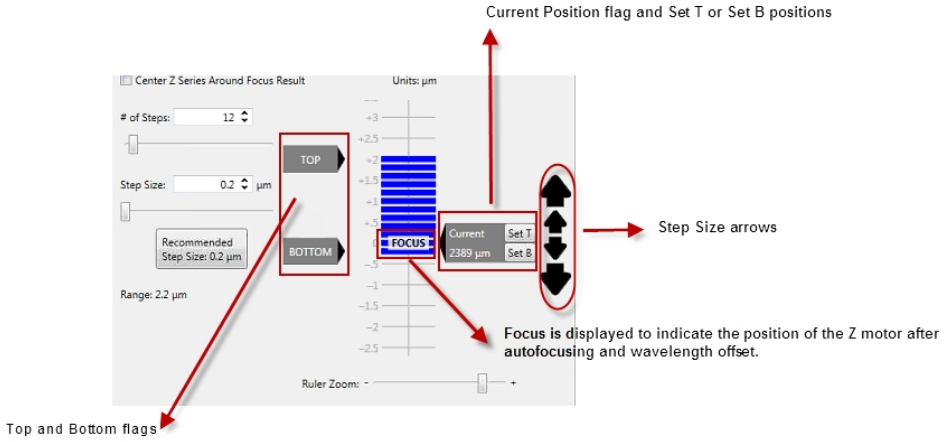


Figure 5-26: Z Series diagram

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

  Tip: Alternatively, you can zoom in and out on the ruler by placing your cursor over the ruler and using the scroll wheel on your mouse.

- **Focus** is displayed on the Z series diagram to indicate the position of the Z motor after autofocusing and wavelength offsets are applied. You can modify the Z stack acquisition settings using the following steps:
  - Click the **Focus** active wavelength tool to place the Z motor at the **Focus** indicator in the Z series diagram and display its current position in µm. The ruler markings indicate the distance in µm above and below the **Focus** position.
  - Drag the **Top** or **Bottom** flags to manually set the top and bottom positions for the Z series acquisition relative to the **Focus** position. As you manually adjust one or both of these values, the MetaXpress Software automatically calculates and displays the appropriate values for the **# of Steps** and the **Range**. The position in µm to which a flag is moved is temporarily displayed on the flag.
- Select **Center Z Series Around Focus Result** to evenly space the Z series planes above and below the **Focus** position. Now, when you drag a **Top** or **Bottom** flag on the Z series diagram to manually set the position for the Z series acquisition, the MetaXpress Software automatically moves the other position by exactly the same total distance in µm.

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

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

  Tip: Use this function with the **Start Live** active wavelength tool. Click **Start Live**, and then with the **Focus** set, click and drag the **Current Position** flag to view the different positions in an image in real time. Using this approach you can 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.

- 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 is updated accordingly.

  Tip: In **Test** mode, when you capture a Z stack, an image window appears. Click and drag the slider bar at the top of the dialog to scroll through the Z stack images.
  Using **Start Live** mode only shows the current selected plane. 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 size arrows, which are the small black vertical arrows to the right of the Z series diagram. To move the Z motor up or down one step at a time by the defined **Step Size**, click the large step size arrows, which are the large black vertical arrows to the right of the Z series diagram. If you click and hold a step size arrow, then the Z motor is moved continuously.

4. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Configuring Journals to Run During Acquisition

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

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

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

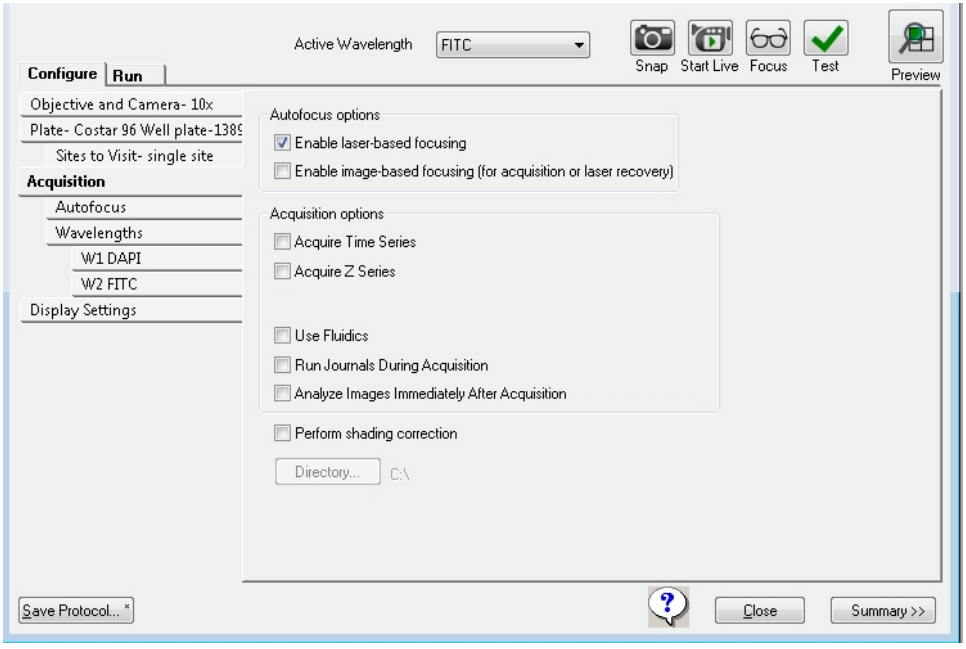


Figure 5-27: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

3. Click **Journals**.

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

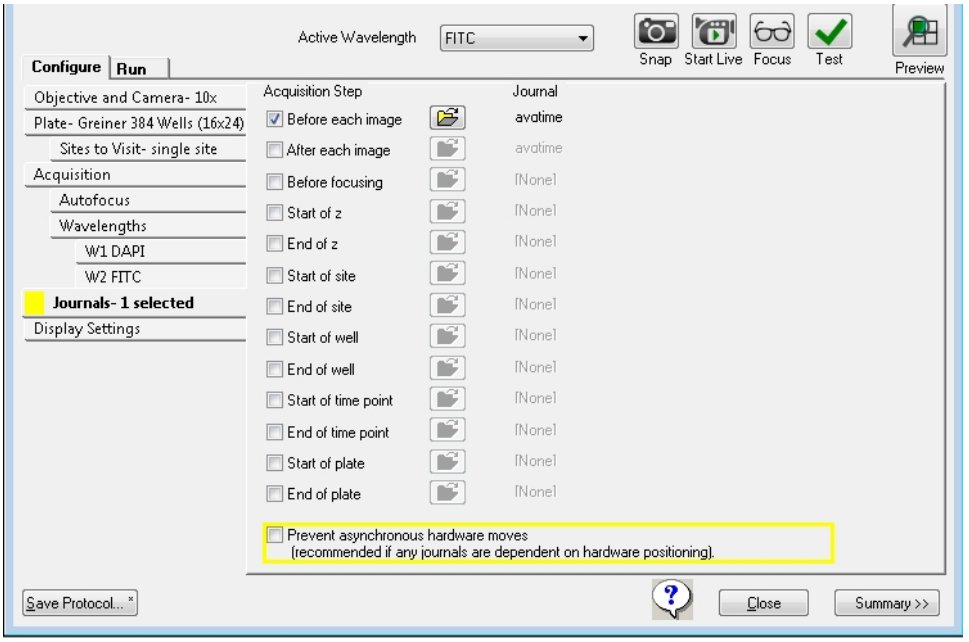


Figure 5-28: Plate Acquisition Setup dialog: Journals tab, Acquisition Step Selection and Loaded Journal

Table 5-8: Journal acquisition steps

Option	Description
Before each image	Runs only during the acquisition loop, after the illumination is set and focusing is complete.
After each image	Runs only during the acquisition loop, after the shutter is closed and before images are saved.
Before focusing	Runs only during the acquisition loop, just before the focus 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 is configured to use the Z series.

Table 5-8: Journal acquisition steps (continued)

Option	Description
End of z	Runs after a Z series acquisition is completed. The journal runs when the Z motor is at the highest step in the series and after any projection images are generated. The journal runs once for each wavelength that is configured to use the Z series.
Start of site	Runs only during the acquisition loop, before any images are acquired from each site.
End of site	Runs only during the acquisition loop, after all images have been acquired from each site.
Start of well	Runs only during the acquisition loop, at the beginning of each well, before any images are acquired from a well.
End of well	Runs only during the acquisition loop, at the end of each well, after all images have been acquired from a well.
Start of time point	Runs only during the acquisition loop, at the beginning of each time point, before any images are acquired for each time point.
End of time point	Runs only during the acquisition loop, at the end of each time point, after all images have been acquired for each time point.
Start of plate	Runs after the stage is moved to the find sample position, but before the find sample step is performed.
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 selected 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 the folder icon for the selected acquisition step.

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

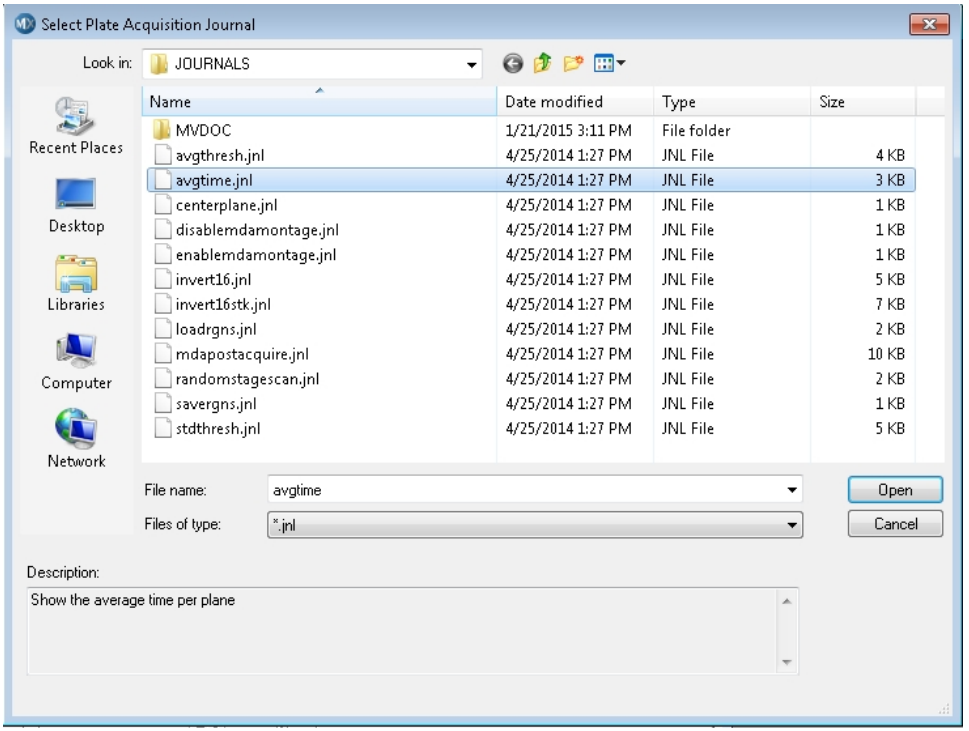


Figure 5-29: Select Plate Acquisition Journal dialog

7. Click **Open** to close the **Select Plate Acquisition Journal** dialog and return to the **Journal** dialog.
8. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Configuring Post-Acquisition Analysis Options

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

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

1. Click **Acquisition**.

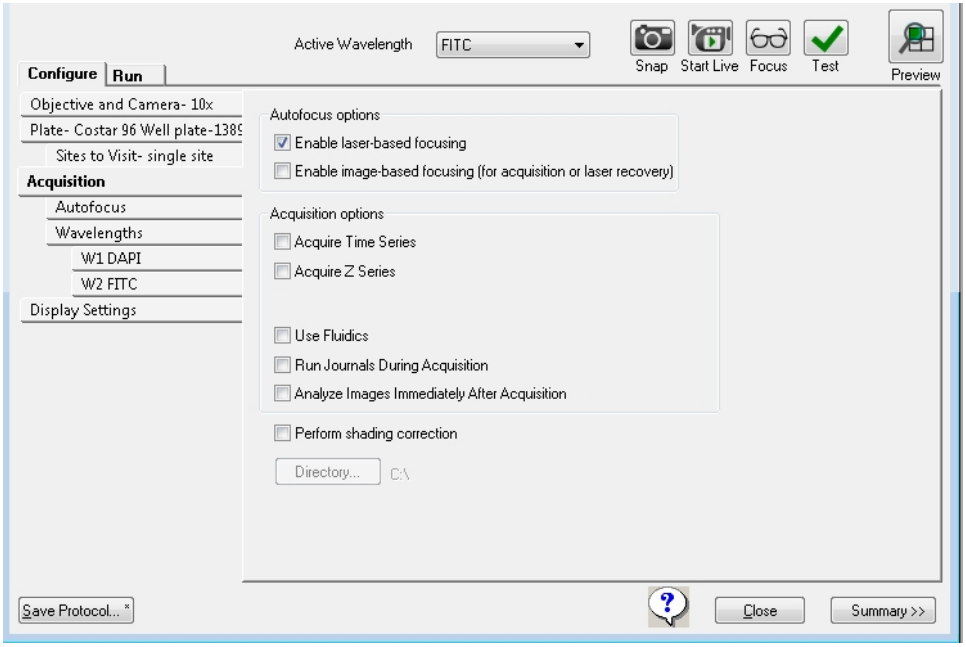


Figure 5-30: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

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

3. Click **Analysis**.

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

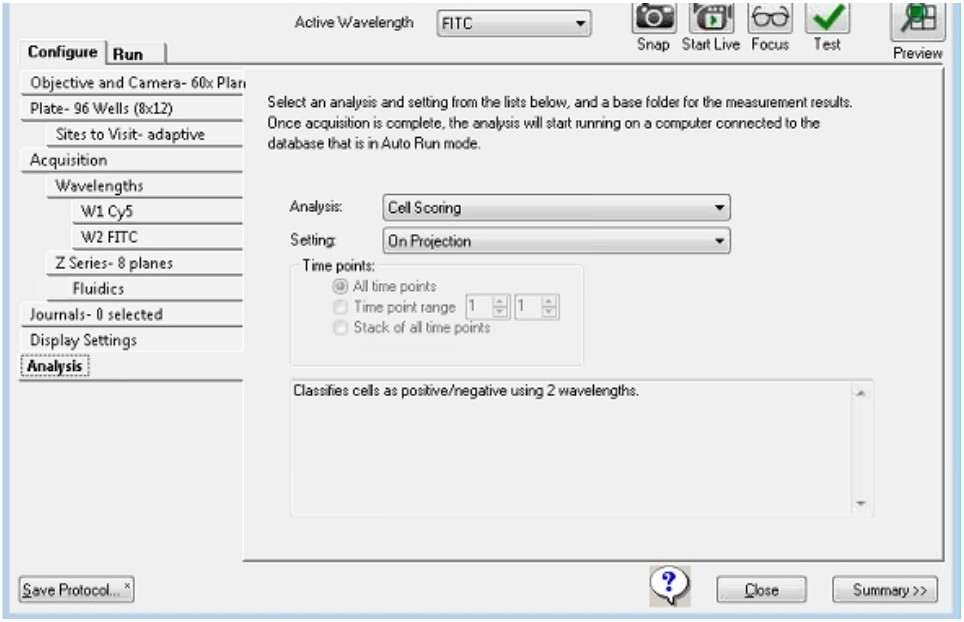


Figure 5-31: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Time Series)

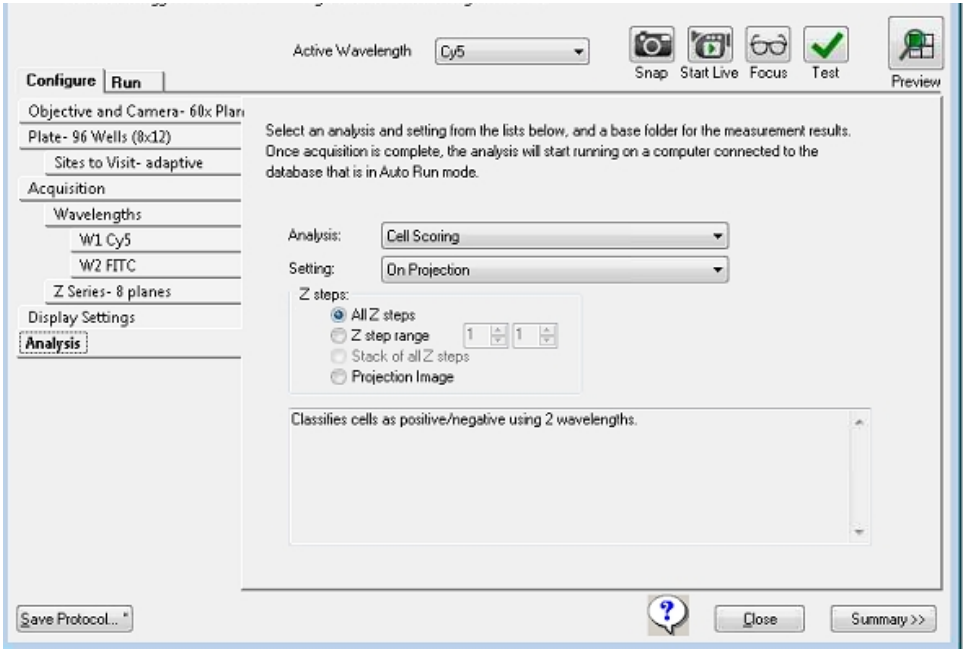


Figure 5-32: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Z Series and 2D Projection Image Only)

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

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

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

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

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

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

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

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

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

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

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

7. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 137.

Plate Acquisition Setup Dialog: Configure Tab, Display Tab

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

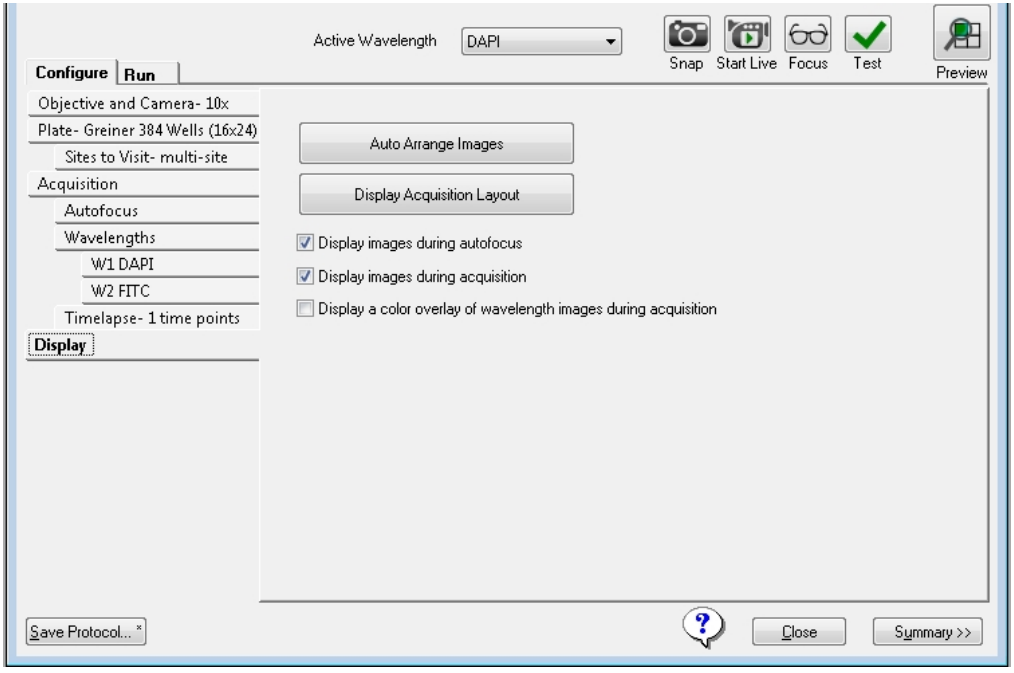


Figure 5-33: Plate Acquisition Setup dialog: Configure tab, Display tab

For a description of the options on this tab, see Table 5-12: Display Settings on page 138.

Table 5-12: Display Settings

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

Configuring the Display Settings for an Acquisition Protocol

Tip: As you are configuring an acquisition protocol, you can open the **Summary** panel and leave it open to view the current values for all of the protocol settings. See Viewing the Summary Panel on page 141.

1. Click **Display**.
2. To use the MetaXpress Software default settings for displaying images during acquisition, click **Auto Arrange Images**.
3. Optionally, select or clear any or all the image preview options:
   - **Display images during autofocus**
   - **Display images during acquisition**
   - **Display a color overlay of wavelength images during acquisition**
4. To display the current settings for displaying images and dialogs during acquisition, click **Display Acquisition Layout**.

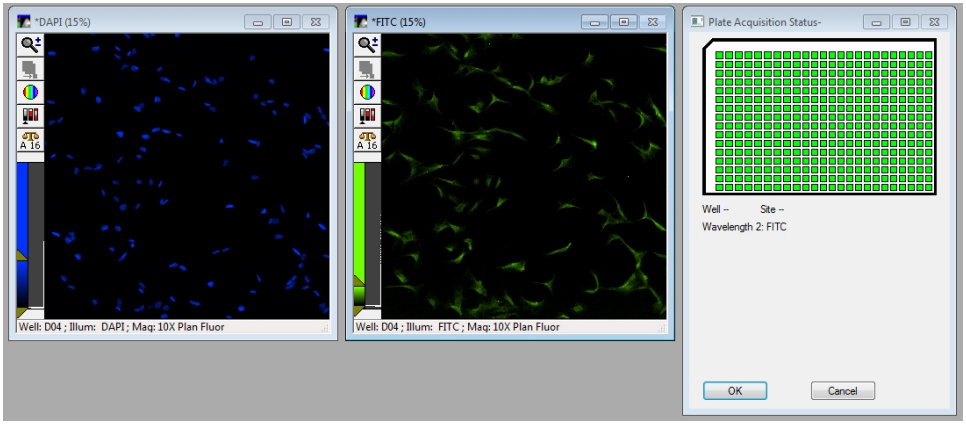


Figure 5-34: Display Settings tab, Display Acquisition Layout Settings

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

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

- Rearrange the position of the image windows.
- Change the size of the image windows.
- Use the **Preview** window tools to change the size, scaling, or LUT for an image.

6. To save the changes, click **OK** or to revert to the previous settings, click **Cancel** . Display settings are saved with the acquisition protocol.

   Note: Adjustments to images displayed as a result of the active wavelength tools, like Snap or Focus, change the display settings.

7. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 142. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 83.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 99.

Viewing the Summary Panel

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

1. At the bottom of the **Plate Acquisition Setup** dialog, click **Summary >>** to open the panel.
2. Optionally, do any or all of the following as needed:
   - Use the scroll bar to the right of the **Summary** panel to scroll up and down through the content.
   - Click **Copy** to copy the contents to your clipboard, and paste the contents into another program, such as Microsoft Word.
   - Click **Print** to send the contents to a selected printer.

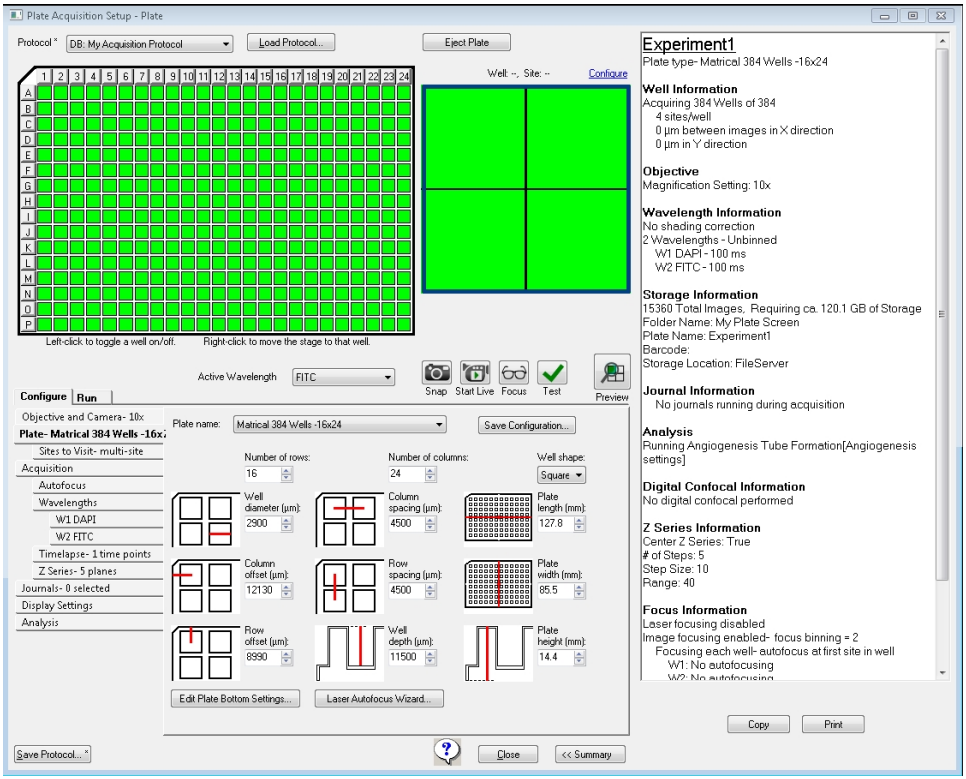


Figure 5-35: Plate Acquisition Setup dialog with open Summary panel

3. When you are done using the **Summary** panel, you can leave it open during the entire process of configuring an acquisition protocol, or you can click **<< Summary** to close it.

Saving a Plate Acquisition Protocol

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

Saving a Plate Acquisition Protocol to a Database

1. Click **Save Protocol**.
2. On the **Save Acquisition Protocol** dialog, in the **Protocol Name** field, type a name for the protocol.
3. Click **Save**.

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

Saving a Plate Acquisition Protocol to a File

When you save a protocol to a file, the file type is .HTS and must not be changed. The default location for saving a protocol is C:\MX6\HTSSTATE, but you can select a different location.

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

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

Chapter 6: Running Plate Acquisitions

In the MetaXpress Software, you can run a plate acquisition from any of the following locations:

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

Prior to MetaXpress Software Version 6.0, all the acquisition functions were spread between the **Plate Acquisition** dialog and the **Plate Acquisition and Control** dialog. As of MetaXpress Software Version 6.0, the acquisition functions are also available in a consolidated layout from the **Plate Acquisition Setup** dialog.

In this section, for historical purposes, using the **Plate Acquisition** dialog and the **Plate Acquisition and Control** dialog to do specific functions for acquiring plates is still documented, and functional, but for convenience and efficiency, Molecular Devices recommends that you use only the **Plate Acquisition Setup** dialog. See Configuring Plate Acquisition Options on page 69.

Note: If you are using a robotic plate handler to load your plates, you initiate acquisition from within the environment of the software that controls the plate handler device. See Robotic Plate Handling on page 333.

Plate Acquisition Setup Dialog

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

Note: The procedures in this section are organized to guide you through the process of running a plate acquisition protocol in the most logical order, but you can do the procedures in the order that works best for you.

Loading a Plate Acquisition Protocol

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

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

2. Click **Load Protocol**.
3. From the **Load Plate Acquisition Protocol** dialog, do one of the following:

Table 6-1: Load Plate Acquisition Protocol Dialog Options

Option	Steps
To load a protocol from the database	Select a protocol from the list, and click **Load from DB**.
To load a protocol from a file	Click **Load From File**, select a protocol (.HTS) file, and then click **Open**.
To load a protocol from a previously acquired plate	Click **Load From Plate**, use the **Select Plate** dialog to locate the plate in the database, then click **Select**.
To delete a protocol from the database	Select a protocol from the list, and click **Delete From DB**.
To export a protocol from the database	Select a protocol from the list, and click **Export to File**. Select an appropriate folder and file name, then click **Save**.

4. Click **Eject Plate**, to open the door, and then load the plate for acquisition.
5. Click **Load Plate** to close the door.
6. After you have loaded a plate acquisition protocol, if the protocol specifies the correct wells and sites for your acquisition, continue to Verifying the Acquisition Settings on page 147. Otherwise, continue to (Optional) Configuring the Wells and Sites for the Data Acquisition on page 144.

(Optional) Configuring the Wells and Sites for the Data Acquisition

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

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

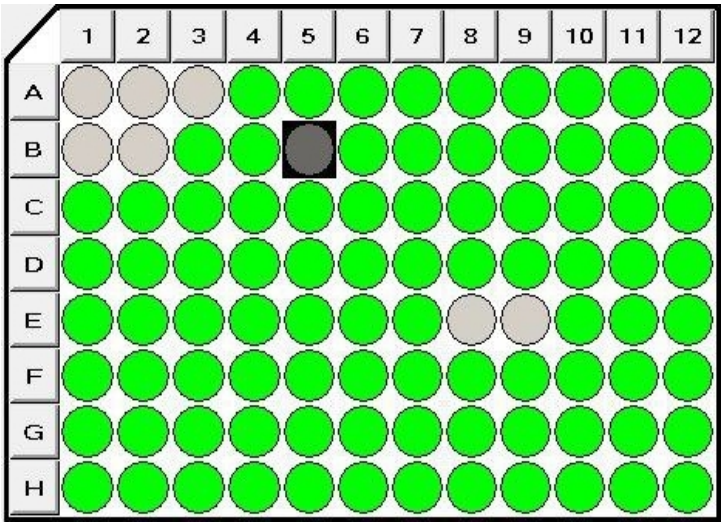


Figure 6-1: Plate Acquisition Setup dialog, Plate map

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

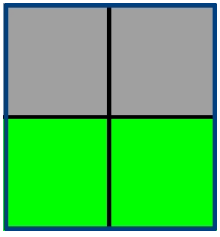


Figure 6-2: Plate Acquisition Setup dialog, Site map

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

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

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

Verifying the Acquisition Settings

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

Figure 6-3: Wavelength selection and interactive Wavelength tools

Note: Note: The **Snap** and **Test** functions for an acquisition wavelength are also available on the Run tab. See Figure 6-4.

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

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

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

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

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

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

3. Click **Run**.

Note: The **Exposure Time** value and the **Focus Offset** value that are displayed for each acquisition wavelength at the bottom of the Run tab are the values that you specified for the wavelength during configuration of the loaded acquisition protocol. You can modify these values for an acquisition wavelength here, or from the individual wavelength tabs. See Specifying the Number of Acquisition Wavelengths on page 106.

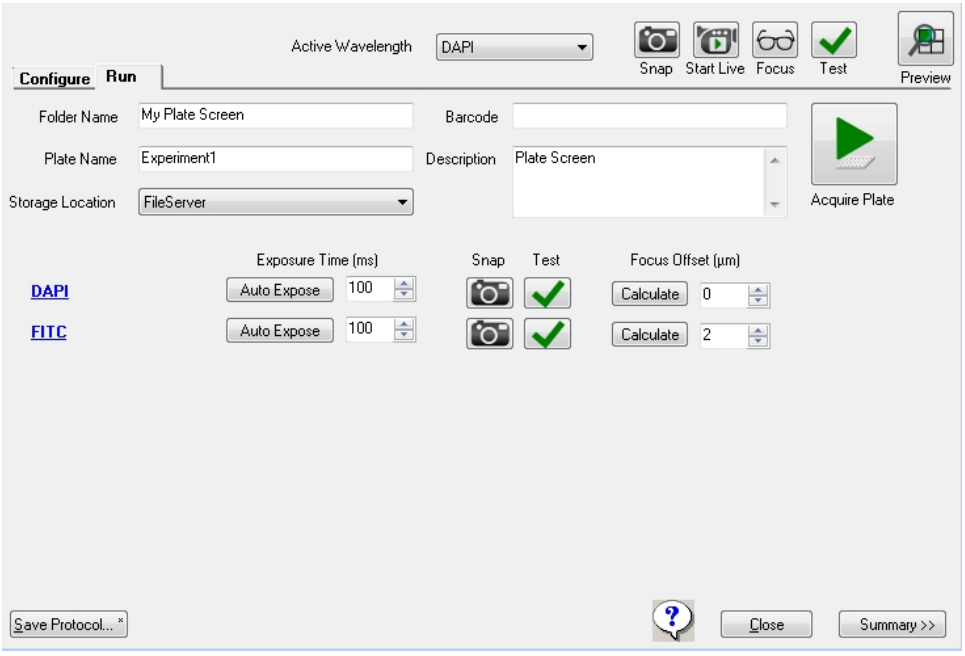


Figure 6-4: Plate Acquisition Setup dialog, Run tab

4. Verify your acquisition settings.

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

   Table 6-2: Verifying Acquisition Settings

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

5. If you are satisfied with the results, then continue to Running a Plate Acquisition Protocol on page 150; otherwise, modify your acquisition settings and repeat this procedure as needed until you are satisfied with the results, and then continue to Running a Plate Acquisition Protocol on page 150.

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

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

Running a Plate Acquisition Protocol

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

1. Enter the information for your experiment.

Table 6-3: Experiment Information

Item	Description
Folder Name	Recommended The name of the folder in which the plate acquisition data is to be stored. **CAUTION:** Names become part of the image file paths, and if the overall path length is too long it causes errors.
Plate Name	Required The name/id of the plate for which data is being acquired. **CAUTION:** Names become part of the image file paths, and if the overall path length is too long it causes errors.
Storage Location	Required The location in which the folder that contains the acquisition data is to be saved. Your Administrator configures the available options.
Barcode	Optional You can enter the value manually, or automatically using a hand-held barcode scanner. **Note:** To avoid accidental reuse of a barcode, the barcode does not save with the plate acquisition settings and protocol. **Note:** If the instrument is being used with a robotic plate handler, then the automation software populates the barcode value as appropriate.
Description	Optional A description of the experiment that you are running to acquire the plate data.

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

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

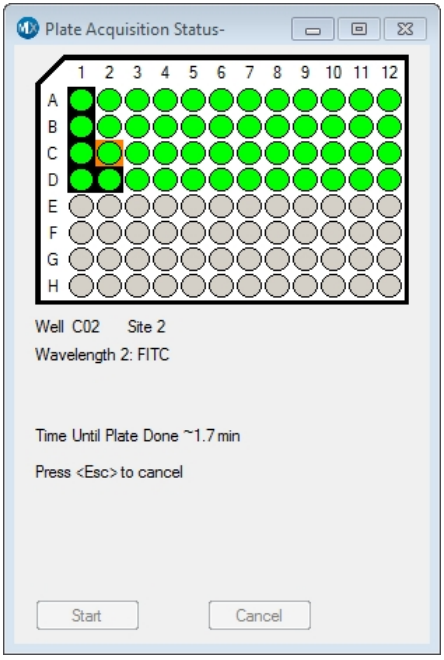


Figure 6-5: Plate Acquisition Status dialog

Plate Acquisition Dialog

The **Plate Acquisition** dialog has the least number of controls and options compared to the other three locations from which you can initiate plate acquisition.

You can do only the following:

- Type a **Plate name**.
- Select and load the **Protocol** file to use for the acquisition.
- View a **Summary** of the acquisition settings.
- **Acquire Plate**

Using the Plate Acquisition Dialog

Use the **Plate Acquisition** dialog to quickly start acquiring plates using any of the protocols defined in the **Plate Acquisition Setup** command. You can also view a **Summary** of the current protocol and change the **Plate name** from this dialog.

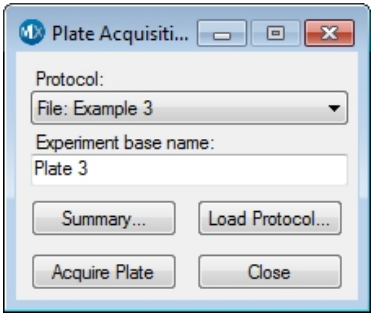


Figure 6-6: Plate Acquisition dialog

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

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

   The **Plate Acquisition** dialog appears.
2. Ensure that a plate is in place on the microscope stage.
3. Select a **Protocol** file to use from the **Protocol** field.
4. To change the experiment name, type a name in the **Experiment Base Name** field.

5. Click **Summary** to view details about the current protocol.

> **Note:** If the correct protocol is already selected on the **Protocol** drop-down list when the **Plate Acquisition** dialog opens, then you can skip to Step 8.

6. Click **Load Protocol** to open the **Load Plate Acquisition Protocol** dialog.

Figure 6-7: Load Plate Acquisition Protocol dialog

7. From the **Load Plate Acquisition Protocol** dialog, do one of the following:

Table 6-4: Load Plate Acquisition Protocol Dialog Options

Option	Steps
To load a protocol from the database	Select a protocol from the list, and click **Load from DB**.
To load a protocol from a file	Click **Load From File**, select a protocol (.HTS) file, and then click **Open**.
To load a protocol from a previously acquired plate	Click **Load From Plate**, use the **Select Plate** dialog to locate the plate in the database, then click **Select**.
To delete a protocol from the database	Select a protocol from the list, and click **Delete From DB**.
To export a protocol from the database	Select a protocol from the list, and click **Export to File**. Select an appropriate folder and file name, then click **Save**.

8. Click **Acquire Plate** to acquire images from a plate based on the current protocol.
9. Click **Close** to exit the dialog.

Plate Acquisition and Control Dialog

At the beginning of the process, while designing your experiment, you will most likely need to make changes to the X, Y, and Z positions to verify correct plate and focus alignment. The **Plate Acquisition and Control** dialog provides controls that enable you to simplify and expedite this process. You can also use this dialog as a starting point for configuring the **Plate Acquisition Setup** dialog. Click **Setup** to open the **Plate Acquisition Setup** dialog. Other controls on this dialog enable you to initiate **Autofocus**, specify the experiment base name, **Load Protocol**, **Save Protocol**, display the **Summary**, open a **Live** window, open a **Preview** window, **Snap** an image, and acquire a plate. For additional information about this dialog, see the following topics or refer to the application help available in the MetaXpress Software.

Plate Acquisition and Control Dialog Layout

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

Note: Most of the tools available in the Plate Acquisition and Control dialog are also available in the **Plate Acquisition** toolbar. To display the **Plate Acquisition** toolbar, click **Window>Toolbars>Plate Acquisition**.

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

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

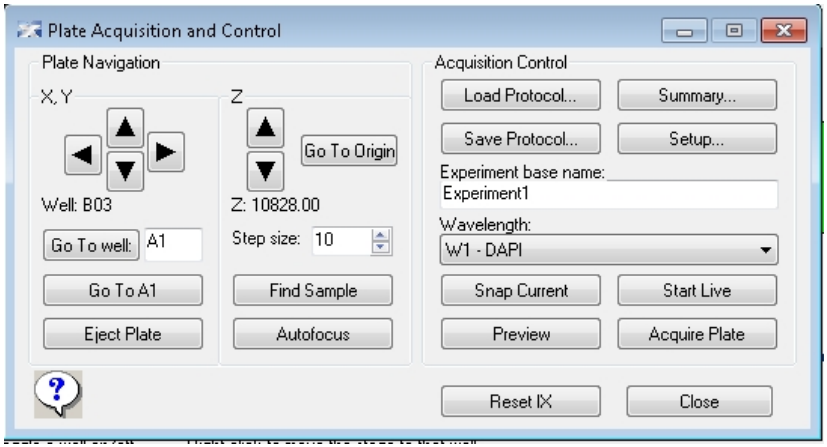


Figure 6-8: Plate Acquisition and Control dialog

Table 6-5: Plate Acquisition and Control Dialog Controls

Option	Description
Plate Navigation:	
X, Y Controls	Moves the stage in increments of one well in the direction of the selected arrow button.
Well	Indicates the well currently in position for image acquisition.
Site	Indicates a site within a specific well that is currently in position for image acquisition.
Go to well	Moves the stage to the well number that you type into the Go to well box
Go To A1	Moves the stage to the A1 position.
Z Controls	Moves the Z-motor in one-step increments in the direction of the selected arrow button. The step size is set in the Step Size field.
Go to Origin	Moves the Z-motor to the 0 focus position. **Note:** Molecular Devices recommends not resetting the origin.
Step size	Sets the size of the individual focus in µm increments using the Z control arrows. Maximum step size is 1000 µm.

Table 6-5: Plate Acquisition and Control Dialog Controls (continued)

Option	Description
Find Sample	Performs a large range auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire.
Autofocus	Performs autofocus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup dialog.
Acquisition Control:	
Load Protocol	Loads the selected protocol from an existing screening protocol file. Protocol files are stored either in the database or on the file system. When you click **Load Protocol**, the **Load Plate Acquisition Protocol** dialog appears. Click **Load from File**, **Load from Plate**, or **Load from DB** to load your selected protocol. The **Load Protocol** function is identical to the **Load Protocol** option in the **Plate Acquisition Setup** dialog. Additional available protocol options are **Delete From DB** and **Export to File**.
Summary	Lists the current settings selected for your acquisition, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well. The Summary function is identical to the Summary tab in the Plate Acquisition Setup dialog.
Save Protocol	Saves the current protocol to a file on the local hard drive or to the database. When you click **Save Protocol**, the **Save Acquisition Protocol** dialog appears. Type the name of a new protocol file that you want to create, or select a listed protocol file name to overwrite an existing protocol file.
Setup	Opens the Plate Acquisition Setup dialog and enables you to change acquisition settings.
Plate name	Defines the plate name.
Wavelength	Selects the wavelength to use for your snap or live image.

Table 6-5: Plate Acquisition and Control Dialog Controls (continued)

Option	Description
Snap Current	Acquires a single image of the currently in place well at the current settings for stage (XY-position), focus (Z-position), wavelength, well, site, and exposure.
Show Live	Continuously acquires images based on the current settings, and updates the image as settings are changed.
Preview	Previews the current display and exposure settings by opening the Plate Acquisition Status dialog and autofocusing and acquiring an image for each wavelength. After all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog and changing the size, scaling, and LUT of images. These new display settings save and continue during acquisition.
Acquire Plate	Starts the plate acquisition using the current protocol.
Reset IX Micro	Reinitializes the ImageXpress Micro instrument.
Close	Closes the dialog.

Using the Plate Acquisition and Control Dialog

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

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

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

   OR

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

5. Select a step size in the **Step size** field. Use a large value for a movement or a small value for fine movement.
6. Click **Go to Origin** to move the Z focus motor to the 0 position.
7. Click **Find Sample** to initiate the Find Sample focusing routine on the current well.
8. Click **Autofocus** to autofocus on the current well using the autofocus option for the wavelength selected in the **Wavelength** field.
9. Click **Load Protocol** to open the **Load Plate Acquisition Protocol** dialog.
10. From the **Load Plate Acquisition Protocol** dialog, do one of the following:
Table 6-6: Load Plate Acquisition Protocol Dialog Options

Option	Steps
To load a protocol from the database	Select a protocol from the list, and click **Load from DB**.
To load a protocol from a file	Click **Load From File**, select a protocol (.HTS) file, and then click **Open**.
To load a protocol from a previously acquired plate	Click **Load From Plate**, use the **Select Plate** dialog to locate the plate in the database, then click **Select**.
To delete a protocol from the database	Select a protocol from the list, and click **Delete From DB**.
To export a protocol from the database	Select a protocol from the list, and click **Export to File**. Select an appropriate folder and file name, then click **Save**.

11. Click **Summary** to open the **Screen Summary** dialog and view your current settings.
12. Click **Setup** to open the **Plate Acquisition Setup** dialog and change your acquisition settings.
13. Click the **Wavelength** field to select a wavelength that has been defined for the current setting in the **Plate Acquisition Setup** dialog.
14. Click **Snap Current** to acquire a single image with the current settings.
15. Click **Show Live** to acquire images in live mode so you can manually focus the microscope.

Note: Live mode can lead to photobleaching.

16. Click **Preview** to open the **Plate Acquisition Status** dialog and an example image window for each wavelength. During this time, you can adjust the display of images and windows so that they will be appropriately sized and positioned for acquisition.
17. Click **Acquire Plate** to acquire images.

18. Click **Close** to exit the dialog.

Acquisition Progress Dialogs

You can observe and determine for your plate acquisition, the following:

- **Screen Summary**
- **Plate Acquisition Status**

Using the Screen Summary Dialog

The **Screen Summary** dialog shows a complete summary list of all of the experiment settings. The **Summary** button is available from the **Plate Acquisition Setup** dialog, the **Plate Acquisition** dialog, and the **Plate Acquisition and Control** dialog.

To view the **Screen Summary** dialog, click the **Summary** button.

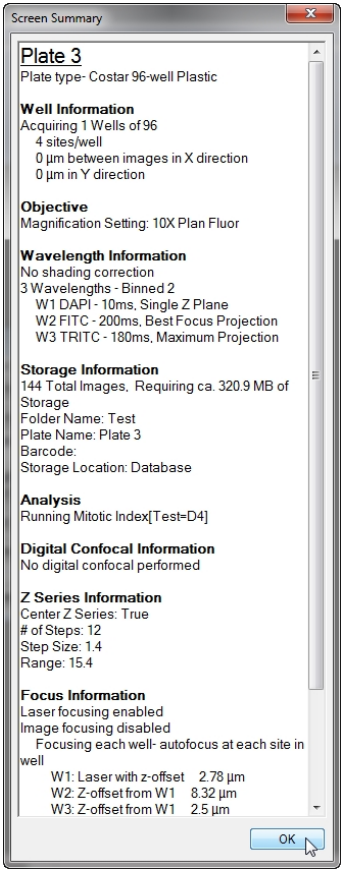


Figure 6-9: Screen Summary dialog

Using the Plate Acquisition Status Dialog

The **Plate Acquisition Status** dialog shows the progress of your plate acquisition by indicating the wells not scheduled for acquisition and wells scheduled for acquisition, completed well acquisitions, and the well currently being acquired.

To view the **Plate Acquisition Status** dialog, click **Screening > Plate Acquisition and Control > Preview**.

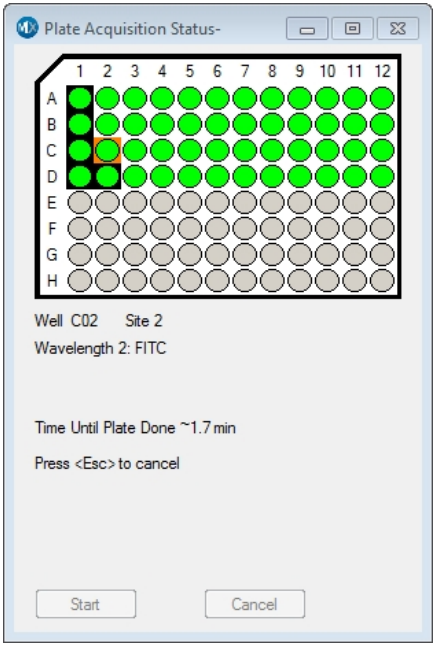


Figure 6-10: Plate Acquisition Status Dialog

Table 6-7: Plate Acquisition Status Dialog Status Indicators

Color	Description
White	Wells not scheduled for acquisition
Green	Wells scheduled for acquisition
Green with Black outline box	Wells that have completed acquisition
Green with Orange outline box	Well is currently being acquired **Note:** During a timelapse acquisition there can be multiple wells highlighted in orange.

Plate Acquisition Toolbar

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

If the **Plate Acquisition toolbar** is not visible, click **Window > Toolbars > Plate Acquisition** from the default menu, or click **Control > Window > Toolbars > Plate Acquisition** from the simplified menu.

Figure 6-11: Plate Acquisition toolbar

Table 6-8: Plate Acquisition Toolbar Buttons

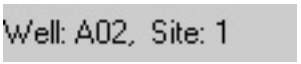
Button Icon	Description
	Moves the stage up in one-well increments
	Moves the stage down in one-well increments
	Moves the stage right in one-well increments
	Moves the stage left in one-well increments
	Moves the stage forward in one-site increments
	Moves the stage backward in one-site increments
	Moves the stage to the load/eject position
	Current well and/or site position
	Moves the Z position (focus) upward in single step increments

Table 6-8: Plate Acquisition Toolbar Buttons (continued)

Button Icon	Description
	Moves the Z position (focus) downward in single step increments
	Performs a very coarse auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire.
	Performs auto focus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup tool
	Selects the wavelength to use for the snap or live image
	Acquires a single image of the currently in place well at the current settings for stage (XY-position), focus (z-position), wavelength, well, site, and exposure
	Show Live continuously acquires images based on the current settings, and updates the image as settings are changed
	Loads the selected protocol from an existing protocol file. Protocols are stored either in the database or on the file system. When you click Load Protocol, the Load Plate Acquisition Protocol dialog appears.
	Screen Summary lists the current settings selected for your acquisition including, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well

Table 6-8: Plate Acquisition Toolbar Buttons (continued)

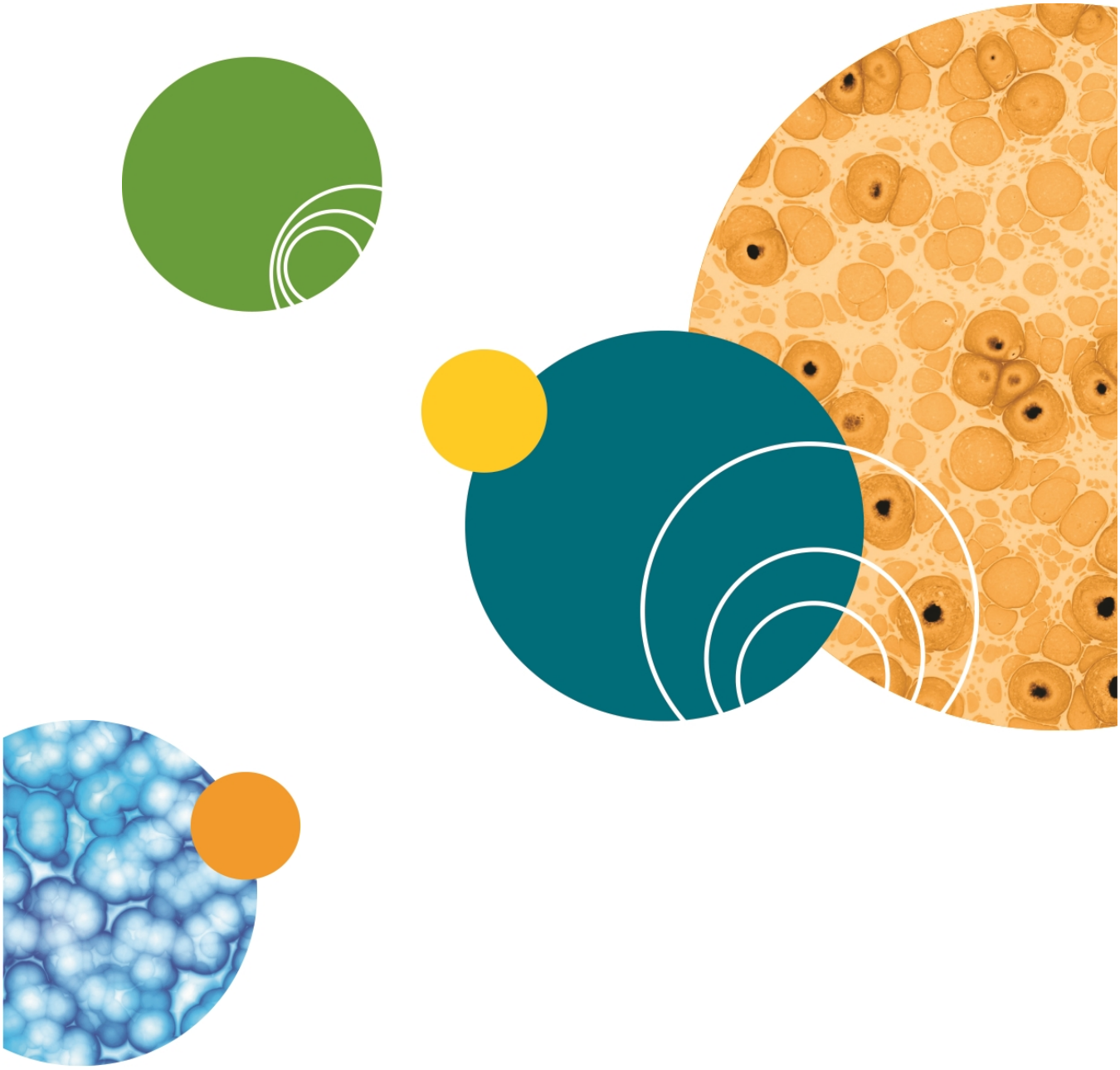
Button Icon	Description
	Previews the current display and exposure settings by opening the Plate Acquisition Status dialog and autofocusing and acquiring an image for each wavelength. After all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog and changing the size, scaling, and LUT of images. These new settings will used during acquisition
	Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Setup dialog

Acquiring a Multi-Well Plate

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

To acquire a multi-well plate:

1. Configure and save your settings file using the **Plate Acquisition Setup** dialog.
2. Use the **Plate Acquisition and Control** dialog or toolbar to do the following:
   a. Load your settings file and review the settings using the **Summary >>** button.
   b. Confirm your settings if needed using the available tools.
   c. Enter a **name**.
   d. Start the acquisition. During acquisition, the acquired images are saved into the image file server, managed by the database.
3. If you want to make any changes to the stage or Z Position, or snap an image to test the current settings before starting the acquisition, use the **Plate Acquisition and Control** dialog or the **Plate Acquisition** toolbar to perform these and other tasks.
4. Perform any post-acquisition analysis using the **Review Plate Data** or **Plate Data Utilities** dialogs. This can be configured to start automatically from the **Plate Acquisition Setup** dialog.



Chapter 7: 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 page 165
- Custom Toolbars and Taskbars, see page 168
- Default Paths for Data, see page 172

An optional simplified menu structure can be installed to reduce the number of top-level menus in the MetaXpress Software. See Simplified Menu Structure on page 21.

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

Users and Groups in the Meta Imaging Series Administrator Software

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

- Single-User mode, where the Administrator enables you to select hardware settings and configure drop-ins and toolbars for groups that have already been created.
- Multi-User mode, where you 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 for your MetaXpress Software.

Your ImageXpress Micro System ships with a number of groups and hardware settings predefined. The number of groups depends on the configuration of your system. Figure 7-1 shows the **Multiple User Configuration** window in the Meta Imaging Series Administrator Software.

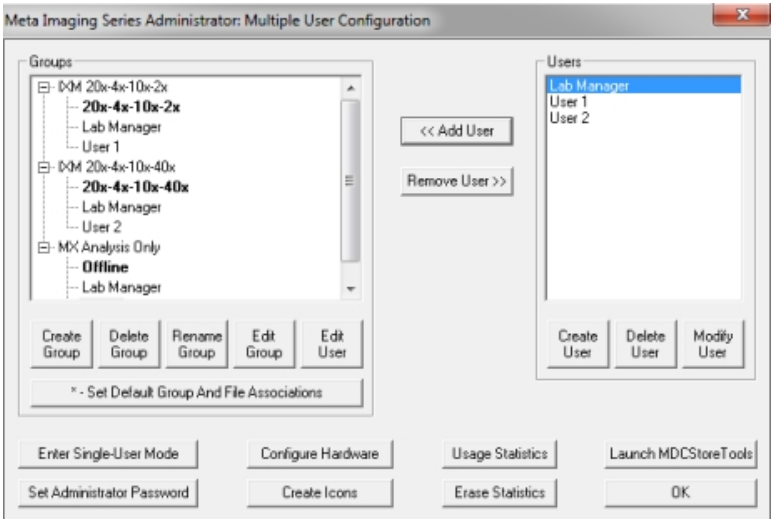


Figure 7-1: Example of the Multiple User Configuration window

This example shows a system with the following groups defined:

- **IXM 20x-4x-10x-2x**: This is the ImageXpress Micro System configured with a particular set of objectives.
- **IXM 20x-4x-10x-40x**: This is the ImageXpress Micro System configured with a particular set of objectives.
- **MX Analysis Only**: This group is configured for analysis only to run the MetaXpress Software with the instrument powered off.

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

Creating an Offline Version of the MetaXpress Software

Molecular Devices recommends creating an offline group for MetaXpress Software users. This offline group 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.

Use the following procedure to create an offline MetaXpress Software group in the Meta Imaging Series Administrator Software:

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

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator**.

   Right-click on the application name and select **Run as administrator** when running the Meta Imaging Series Administrator Software.
2. In the Meta Imaging Series Administrator Software, if the program opens in **Single User Configuration** mode, click **Enter Multi-User Mode**.
3. Click **Create Group**.
4. In the **Create Group** dialog, in the **Group Name** field, type a group name, such as **MetaXpress Offline**.

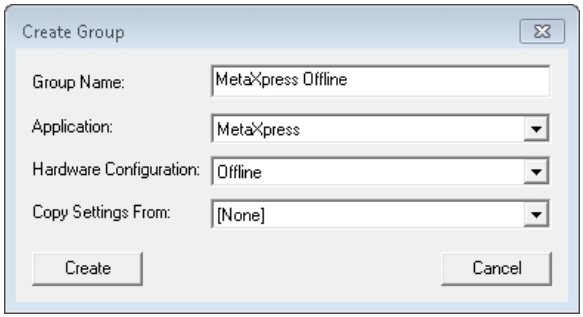


Figure 7-2: Create Group dialog

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

Note: You must add at least one user to the new offline group 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 creating the **MetaXpress Offline** group and adding users with the previous procedure, use the **Create Icons** command to create icons for the new group. This command installs shortcuts for any new groups to the **MetaXpress** folder on the Windows desktop. These shortcuts can then be copied directly to the Windows desktop. This lets users choose which software configuration to start from the desktop.

Use the following procedure 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 your desktop.
5. In the **MetaXpress 6.x** folder, confirm that a shortcut for the group that was created in Creating an Offline Version of the MetaXpress Software on page 166 is listed.
6. Right-click the shortcut (for example, **MetaXpress Offline**), and then select **Send To > Desktop** to create the shortcut on the desktop.
7. Continue to add shortcuts to the desktop, as needed.
8. Double-click the new desktop shortcut to open that instance of the software.

Custom Toolbars and Taskbars

After you have groups configured, you can create or modify custom toolbars and taskbars to include specific combinations of tools and commands.

Customizing Toolbars

With the **Configure Drop-ins/Toolbars** command, you can add menu commands to toolbars, move commands from one tool bar to another, and add journals to or remove journals from toolbars.

Use the following procedure to customize the toolbars:

1. Click **Start > 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. Select a **Group Name** from **Meta Imaging Series Administrator > List of Groups**.
3. Depending on which User Mode you are in:
   a. Single User Mode: Click **Drop-ins/Toolbars**.
   b. Multi-User Mode: In the **Edit Group** dialog, click **Drop-ins/Toolbars**.
4. In the **Configure Drop-ins/Toolbars** dialog, click the **Toolbars** tab.
5. If it is selected, deselect the **Use default toolbars** check box.
6. In the **Commands** section, select the item you want to add to the toolbar:
   - Select **Menus** to add menu commands to toolbars.
   - Select **Toolbars** to add toolbar commands to other toolbars.
   - Select **Journals** to add journals to any toolbar or to create new Journal toolbars.
7. To add any command to a toolbar, drag the command from the left pane to the appropriate toolbar folder in the right pane.

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

8. Click **OK** when finished.
9. Click **Yes** in the message to confirm that you want the users in the group to use the modified configuration.
10. Click **OK** to close the **Edit Groups** dialog.
11. Click **OK** to exit the Meta Imaging Series Administrator Software.

The modified toolbars will be available the next time you start the corresponding version of the MetaXpress Software.

Creating Taskbars

You can create custom taskbars directly in the MetaXpress Software. Taskbars are a convenient way to access frequently used commands and journals. Each taskbar can have up to 48 buttons in a configuration of rows and columns of your choosing. You can mix and match journals, commands, or other taskbars within the same taskbar. Molecular Devices recommends creating taskbars that combine commands and journals specific to your experiments.

Tip: When using taskbars, to show the last used taskbar, press **F4**.

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

1. Start the MetaXpress Software.
2. Click **Journal > Taskbars > Create Taskbar**.

   In the simplified menu, click **Control > Journal > Taskbars > Create Taskbar**.
3. When the **Taskbar Editor** dialog and **New Taskbar** window open, position them so that you can see both the dialog and the window at the same time.
4. Define the number of rows and columns for the taskbar by dragging the edges of the **New Taskbar** window until the desired number of rows and columns appear in the window.
5. Define the width of all the buttons in the taskbar by dragging the right or left edge of the active button until the buttons are the desired width.
6. Under **Category**, select the type of item you want to add to the taskbar:
   - Select **Function** to add a software function to the taskbar.
   - Select **Journal** to add a journal to the taskbar.
   - Select **Taskbar** to add a link to another taskbar to the taskbar.

Note: If you select **Journal** or **Taskbar** as the **Category**, the directory names are displayed in square brackets in the list. Double-click a directory name to display the appropriate files in that directory, or double-click the double period **[..]** to go up one level in the directory structure.

7. When you have located the item you want to add to the taskbar, double-click its entry in the list to add it to the active button in the taskbar as shown in Figure 7-3.

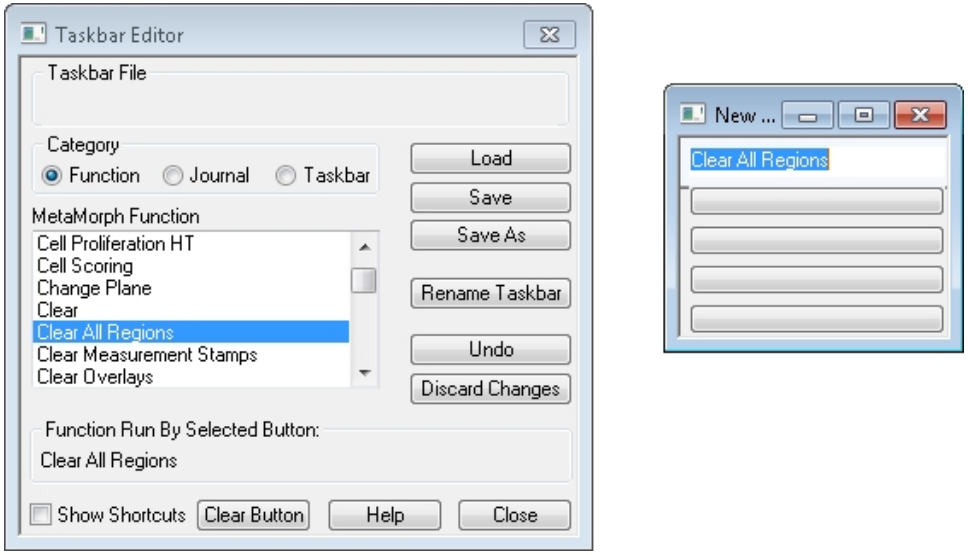


Figure 7-3: Adding a function to a taskbar

8. Continue to add items to the taskbar by clicking a blank button, selecting the **Category**, and double-clicking the entry in the list.
9. If necessary, click **Undo** to undo the last action or click **Clear Button** to clear an item from the active button.
10. To give the task bar a descriptive name, click **Rename Taskbar**.
11. In the **Rename Taskbar** dialog, in the **Taskbar Title** field, type the new name.
12. Click **OK**.

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

Default Paths for Data

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

- **Default Data Paths**: Your system computer has a dedicated hard drive partition for data. The default data file paths for each user should all point to this data drive. For example: D:\Data\Username.

  The following data file types should have their file paths changed to point to the data drive:

  - Log files
  - Memory lists
  - Calibrations
  - Illumination Settings
  - Magnification Settings
- **Default HTS State Path**: The MetaXpress Software protocol file path should also point to the data drive. For example: D:\MX\HTSSTATE\.

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

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

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

1. Start the MetaXpress Software.
2. Click **Edit > Configure Default Paths**.

3. In the **Configure Default Paths** dialog, select the item with the default file path you want to modify.

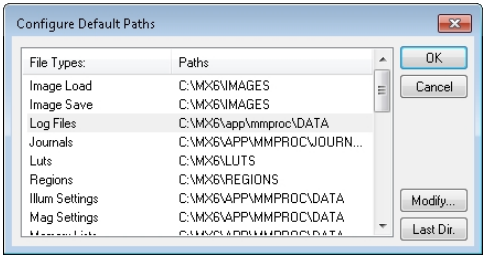
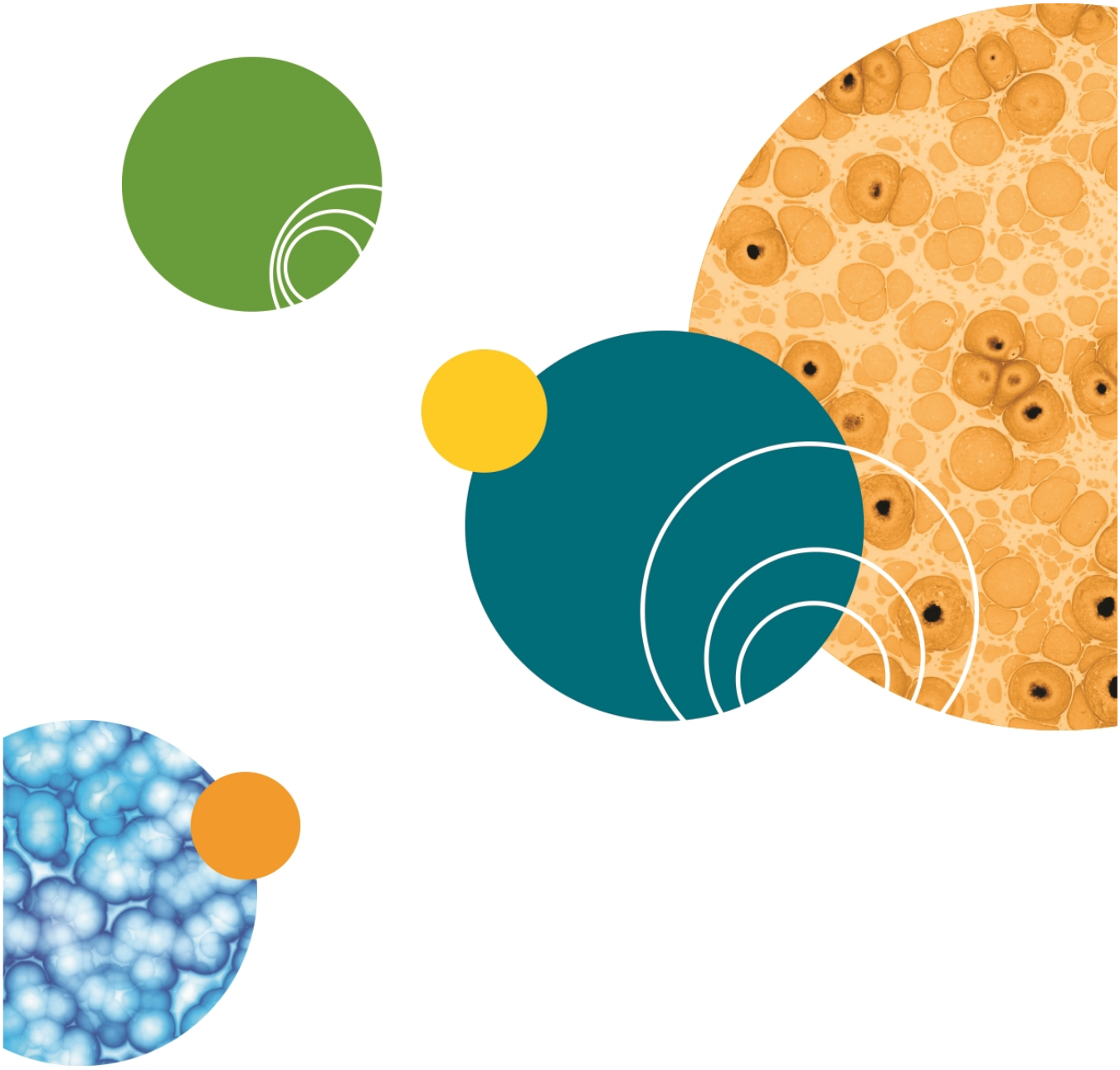


Figure 7-4: Configure Default Paths dialog

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



Chapter 8: Optional Expansion Solutions

Users of an ImageXpress System can upgrade to include the following optional expansion solutions:

- Environmental Control Options, see page 175
- Fluidics Options, see page 202
- Transmitted Light Options, see page 224
- LED Transmitted Light Options, see page 249

Contact your Molecular Devices representative to discuss adding appropriate optional expansion solutions to your system.

For systems factory-equipped with any of these expansion solution options, the following sections provide operating procedures for each option.

Environmental Control Options

The ImageXpress Environmental Control option is designed to maintain an environment for living cells to enable multi-day, live-cell, timelapse 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 timelapse 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 LED Transmitted Light option.

See also:

- Fluidics Options, see page 202
- Transmitted Light Options, see page 224
- LED Transmitted Light Options, see page 249

Operational information is included for the following:

- Environmental Control Hardware, see page 176
- Setting Up Environmental Control, see page 181
- Environmental Control Software, see page 191
- Doing Timelapse Experiments, see page 192
- Cleaning Environmental Control Components, see page 198

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 Environmental Control Option 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% CO_2 and 95% air. The tank regulator must be set between 15 PSI and 20 PSI. The Environmental Control Option 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 timelapse experiment.

Items Included in the Installation

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

- **Environmental Control Option Controller**. See Figure 8-1 and Figure 8-2.
- **Warm air hose** and **carbon dioxide tubing**. See Figure 8-5 and Figure 8-6.
- **Temperature sensors**. To ensure accurate readings, the sensors are located near the sample plate.
- **Water reservoir**. See Figure 8-7.
- **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 8-8.

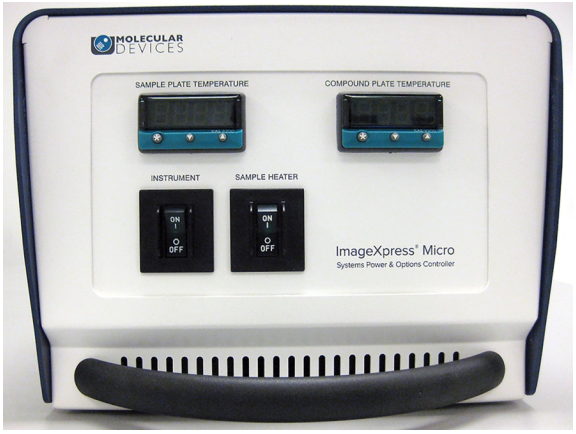


Figure 8-1: ImageXpress Systems Power and Options Controller used for the ImageXpress Micro 4 System

Figure 8-2: Environmental Control Option Controller used for the ImageXpress Standard, XL, and XLS Systems

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

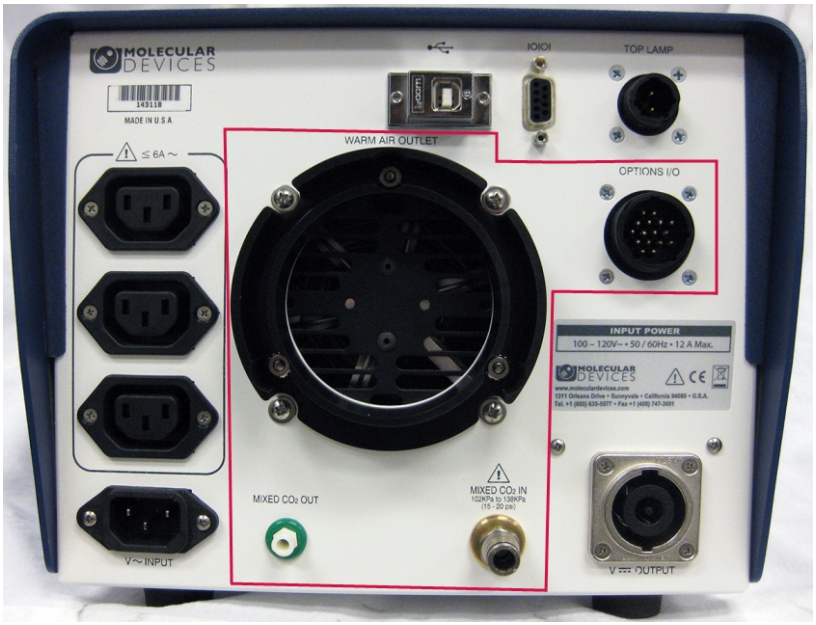


Figure 8-3: Environment Control option connections on the back of the ImageXpress Systems Power and Options Controller used for the ImageXpress Micro 4 System

Figure 8-4: Environment Control option warm air hose and carbon dioxide tubing on the back of the ImageXpress Micro 4 System

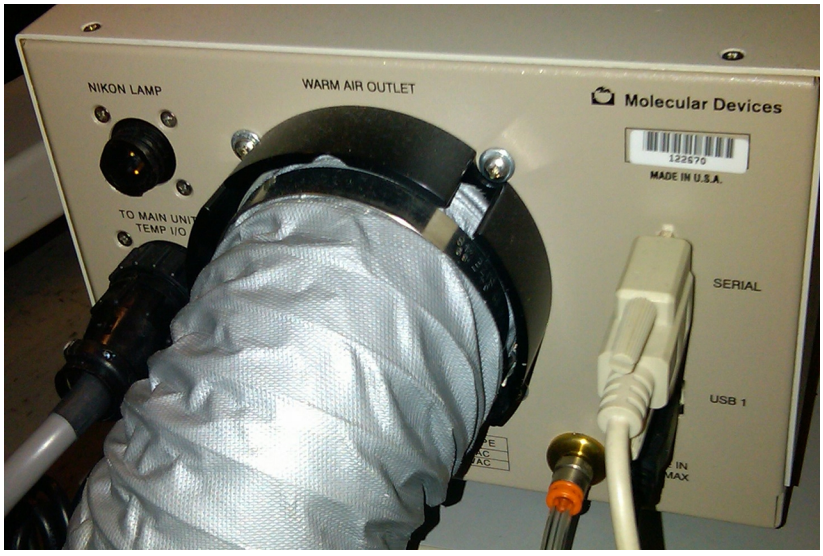


Figure 8-5: Environment Control option warm air hose and carbon dioxide tubing on the back of the Environmental Control Option Controller used for the ImageXpress Standard, XL, and XLS Systems

Figure 8-6: Environment Control option warm air hose and carbon dioxide tubing on the back of the ImageXpress Micro Standard System

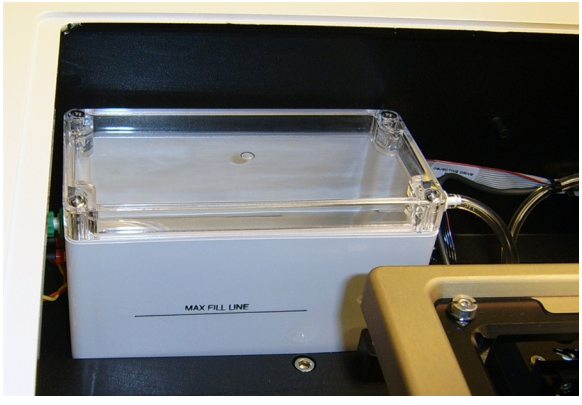


Figure 8-7: Environment Control option water reservoir

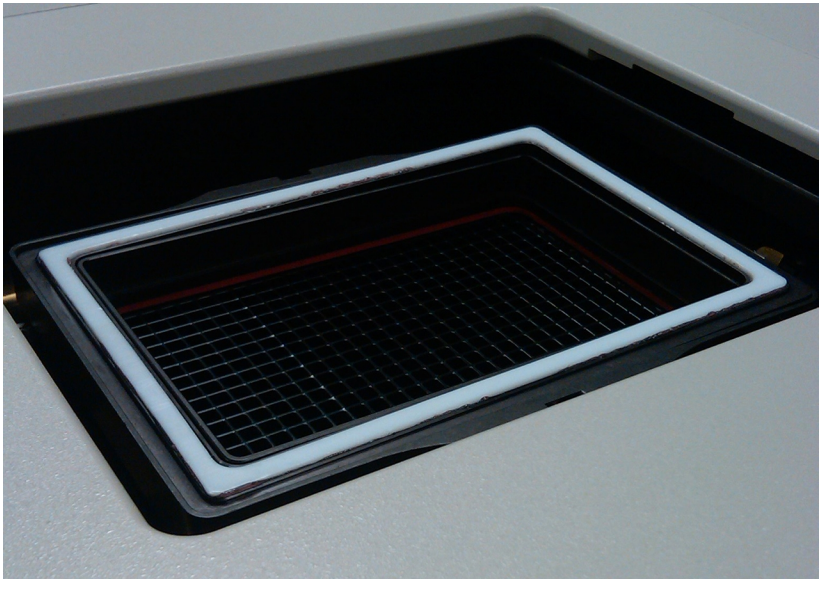


Figure 8-8: Environment Control option live-cell sealing ring

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% CO_2 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 the ImageXpress Micro 4 System, or the Environmental Control Option Controller for the ImageXpress Micro XLS System.
- 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 FSE (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 Environmental Control Option Controller, view the current sample plate temperature.

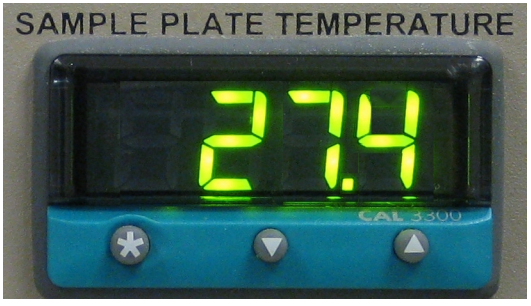


Figure 8-9: Sample Plate Temperature control interface

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.

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. Alternatively, to fill the water reservoir inside of the instrument, see Filling the Water Reservoir Inside the Instrument on page 185.

Requirements:

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

To fill the water reservoir outside the instrument:

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

   In the Simplified Menu Structure, click **Screening >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 (Figure 8-10), and remove the side panel for more access.

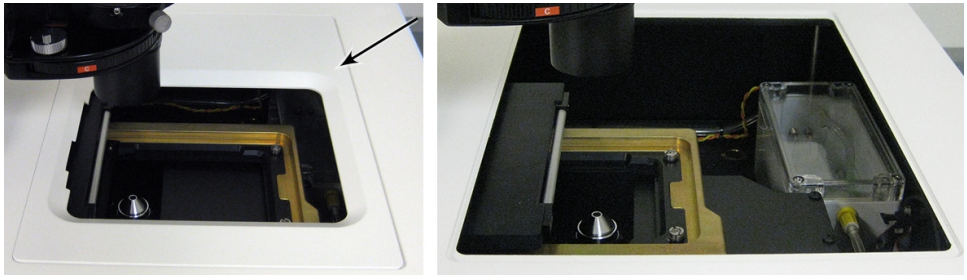


Figure 8-10: Top access panel around the top door on and off

4. Locate the water reservoir through the top opening.

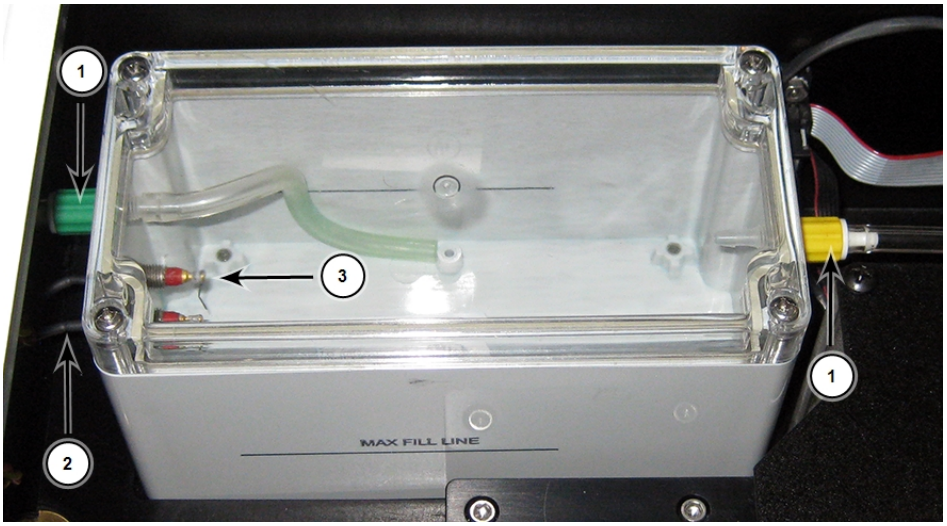


Table 8-1: Environment Control Option Water Reservoir

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

5. Disconnect the CO_2/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.

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.

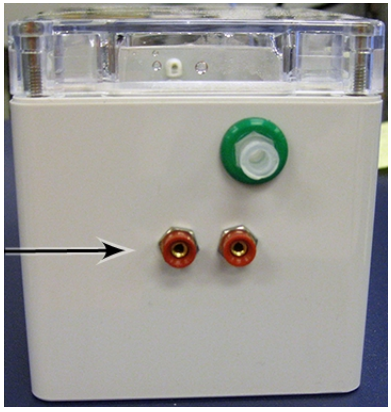


Figure 8-11: Water level sensor wire sockets

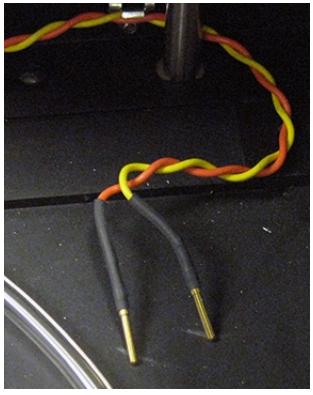


Figure 8-12: Water level sensor wires inside the instrument

12. Reconnect the CO_2/air tubing to the luer locks on both sides of the water reservoir.
13. Replace the top access panel surrounding the top door and replace the side panel. See Figure 8-10.
14. Start the instrument.
15. In the MetaXpress Software, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

    In the Simplified Menu Structure, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.

Filling the Water Reservoir Inside the Instrument

Use the following procedure to fill 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, click **Screening > Plate Acquisition Setup > Eject Plate** to open the top door of the instrument.

   In the Simplified Menu Structure, click **Screening >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 (Figure 8-13), and remove the side panel for more access.

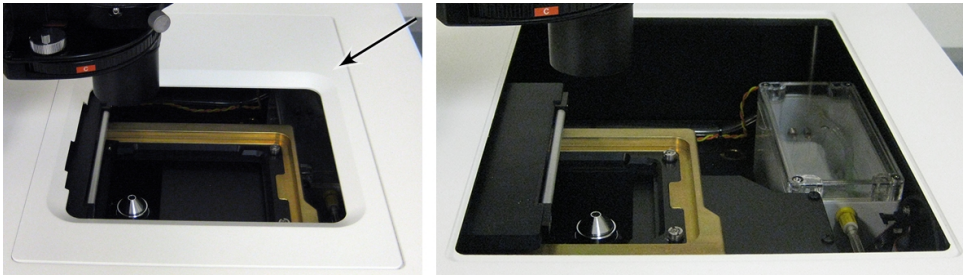


Figure 8-13: Access panel around the top door on and off

4. Locate the water reservoir through the top opening.

Figure 8-14: ImageXpress Environmental Control option water reservoir

5. Remove the side panel for more access.
6. Disconnect the CO_2/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 CO_2/air tubing to the green luer lock.
11. Replace the top access panel surrounding the top door and replace the side panel. See Figure 8-13.
12. Start the instrument.
13. In the MetaXpress Software, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

    In the Simplified Menu Structure, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.

Setting Up the Carbon Dioxide Tank

A pre-mixed CO_2 tank with a regulator must be set up and connected to the CO_2 inlet on the Options Controller. The CO_2 Options Controller then controls the flow rate of CO_2 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 182.

To set up the CO_2:

1. Connect the regulator to the CO_2 tank.
2. Connect the tubing from the pre-mixed CO_2 tank regulator to the Environmental Control Option Controller.
3. Verify that the tubing from the Environmental Control Option Controller to the instrument is connected.
4. Turn on the CO_2 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.

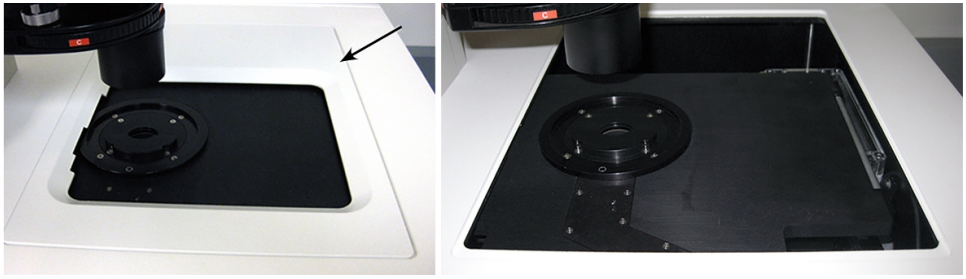


Figure 8-15: Top access panel around the top door on and off

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 CO_2 pressure is OK. See Environmental Control Software on page 191.

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. See Loading the Sample Plate on page 188.

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 CO_2 flow to the sample plate. The sealing ring helps to contain the inputted air directly over the cells and maintains the proper CO_2 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, click **Screening > Plate Acquisition Setup > Eject Plate** to open the top door of the instrument.

   In the Simplified Menu Structure, click **Screening > 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.

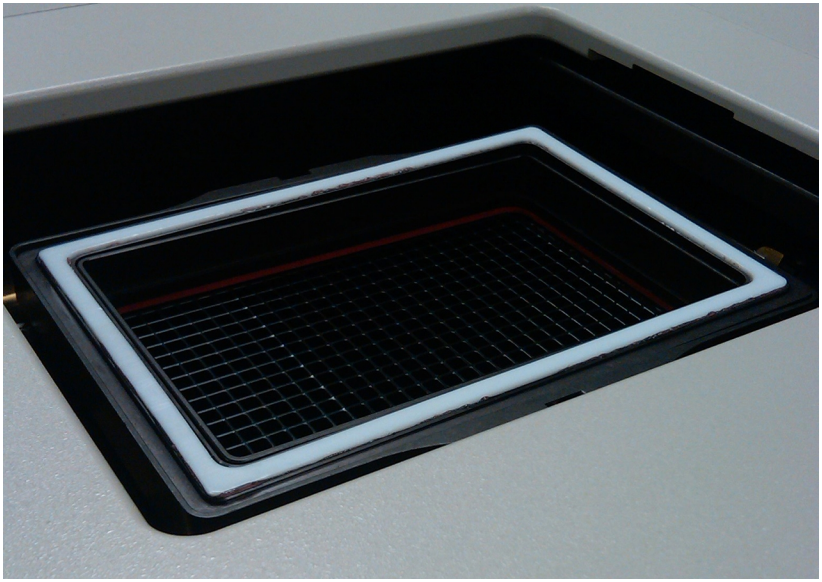


Figure 8-16: Environment Control option live-cell sealing ring

4. In the MetaXpress Software, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

   In the Simplified Menu Structure, click **Screening > 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 CO_2 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 Micro System 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 theMetaXpress Software, click **Devices > Environment Control**.

   In the Simplified Menu Structure, click **Control > ImageXpress > Environment Control**.

   In the **Environment Control** dialog, the **Current Temperature** and the **Temperature Setpoint** values are displayed.

   - Temperature is recorded in degrees Celsius.
   - Carbon dioxide pressure is recorded as **Low** or **OK**.

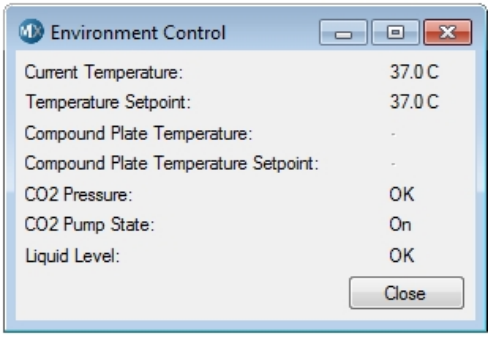


Figure 8-17: Environment Control dialog without fluidics

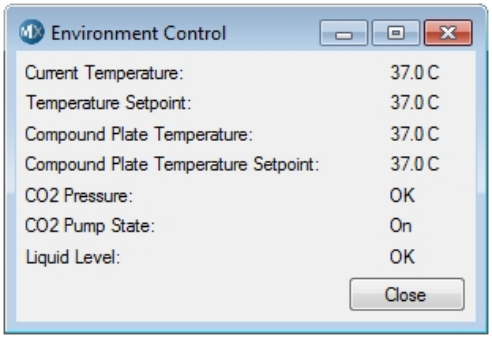


Figure 8-18: Environment Control dialog with fluidics

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 in order 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 in order 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 CO_2 or temperature. These parameters are controlled directly through their hardware devices, such as the temperature controller on the Environmental Control Option Controller (Figure 8-2) and the regulator on the carbon dioxide tank.

Doing Timelapse 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 Timelapse acquisitions, the time starts when the first well is acquired. If the acquisition time exceeds the time specified for the timelapse 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 Timelapse acquisitions, the image save option for the Z option is to save the 2D projection images.

To set up a timelapse experiment:

1. In the MetaXpress Software,click **Screening > Plate Acquisition Setup**.

   In the Simplified Menu Structure, click **Screening > Acquisition Setup**.

2. In the **Plate Acquisition Setup** dialog, in the **Configure** tab, open the **Acquisition** tab. See Figure 8-19.

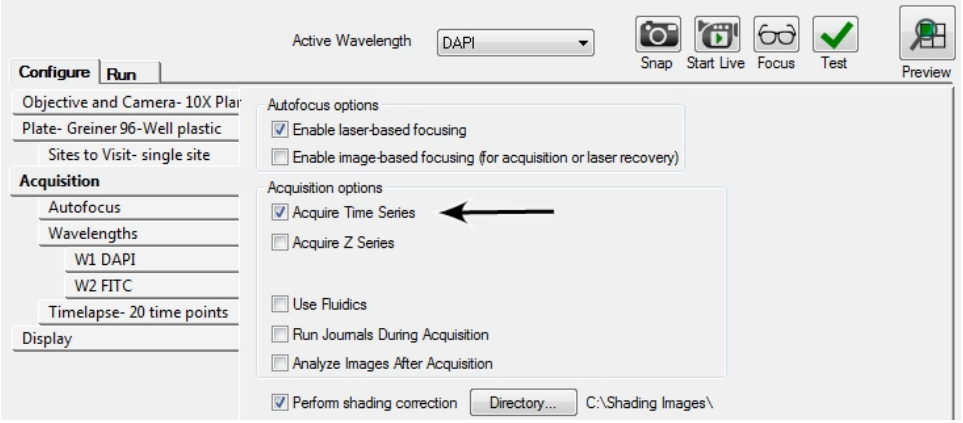


Figure 8-19: Acquire Time Series

3. Select **Acquire Time Series**.
4. Under **Configure**, click **Timelapse** to configure timelapse acquisition options. See Figure 8-20.

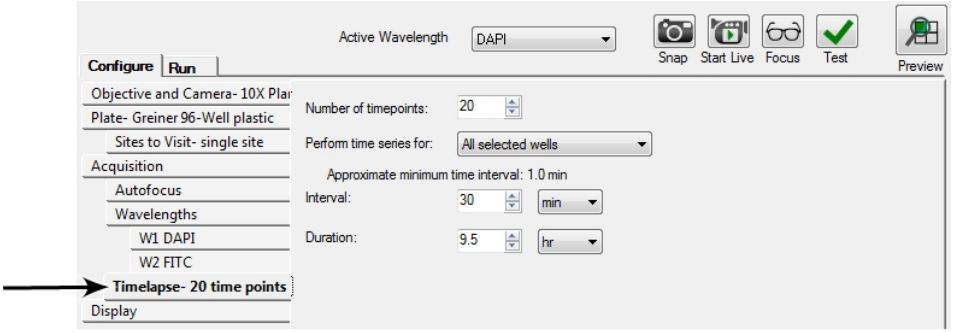


Figure 8-20: Timelapse tab

5. In the **Number of time points** field, specify the number of time points to use. Fluidic events can be associated with these time points. See Doing Fluidics Experiments on page 217.

6. Click **Perform time series for** and select one of the following methods for acquiring a series of timelapse images, corresponding to different types of experiments:
   - **All selected wells** is used for long timelapse experiments. The system images all wells at the first time point, then all wells at the second time point, and so on.
   - **One well then the next** is used for a fast kinetic experiment. The system images all time points on well 1, then moves to well 2 and images all time points, and so on.
   - **One row then the next or one column then the next** is suitable for an experiment using manual pipetting.
7. If applicable, select additional options on the Autofocus tab. See Figure 8-21. These options include:
   - **First timepoint only**: Recommended for fast kinetic experiments.
   - **All timepoints**: Recommended for long timelapse experiments.
   - **Every Nth timepoint**: Offers the flexibility to autofocus regularly during a timelapse experiment.

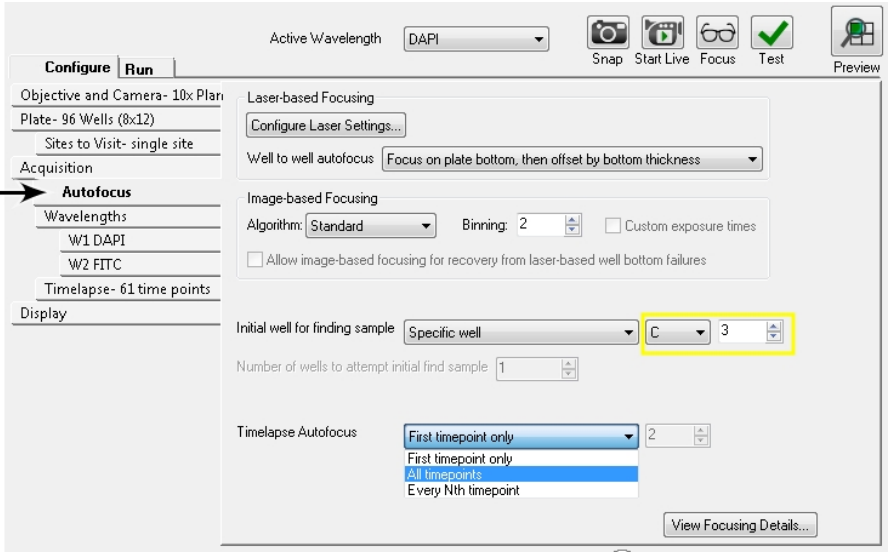


Figure 8-21: Timelapse Autofocus options

8. If applicable, select more options on the Wavelength tabs. **Wavelength** tabs provide options for acquiring individual wavelength images during a timelapse experiment. See Figure 8-22.

   These options include:

   - **At all time points**: The default for timelapse experiments. All time points are acquired for this wavelength.
   - **At start of experiment**: Only the first time point is acquired for this wavelength.
   - **At start/end of experiment**: Only the first and last time points are acquired for this wavelength.
   - **Every nth timepoint**: Acquires timelapse images only for every nth time point for this 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.

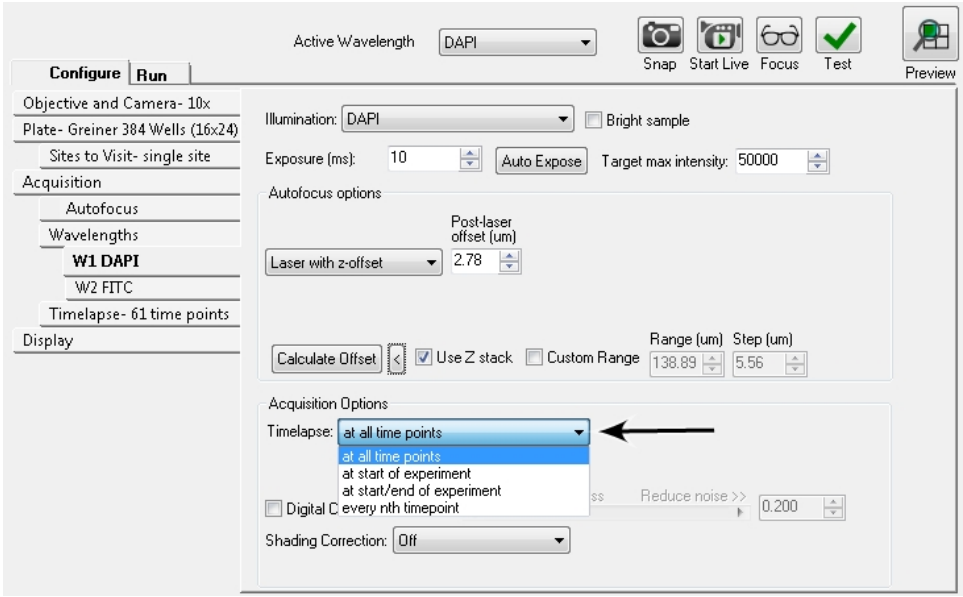


Figure 8-22: The Timelapse Acquisition field on the Wavelength 1 tab

Reviewing Timelapse Data

As with other ImageXpress System data, timelapse data is displayed in the MetaXpress Software **Review Plate Data** dialog. See:

- Reviewing Timelapse Data on page 196
- Making a Timelapse Movie on page 198

Reviewing Timelapse Data

To review the timelapse data:

1. In the MetaXpress Software, click **Screening > Review Plate Data**.

   In the Simplified Menu Structure, click **Screening > Review Plate**.

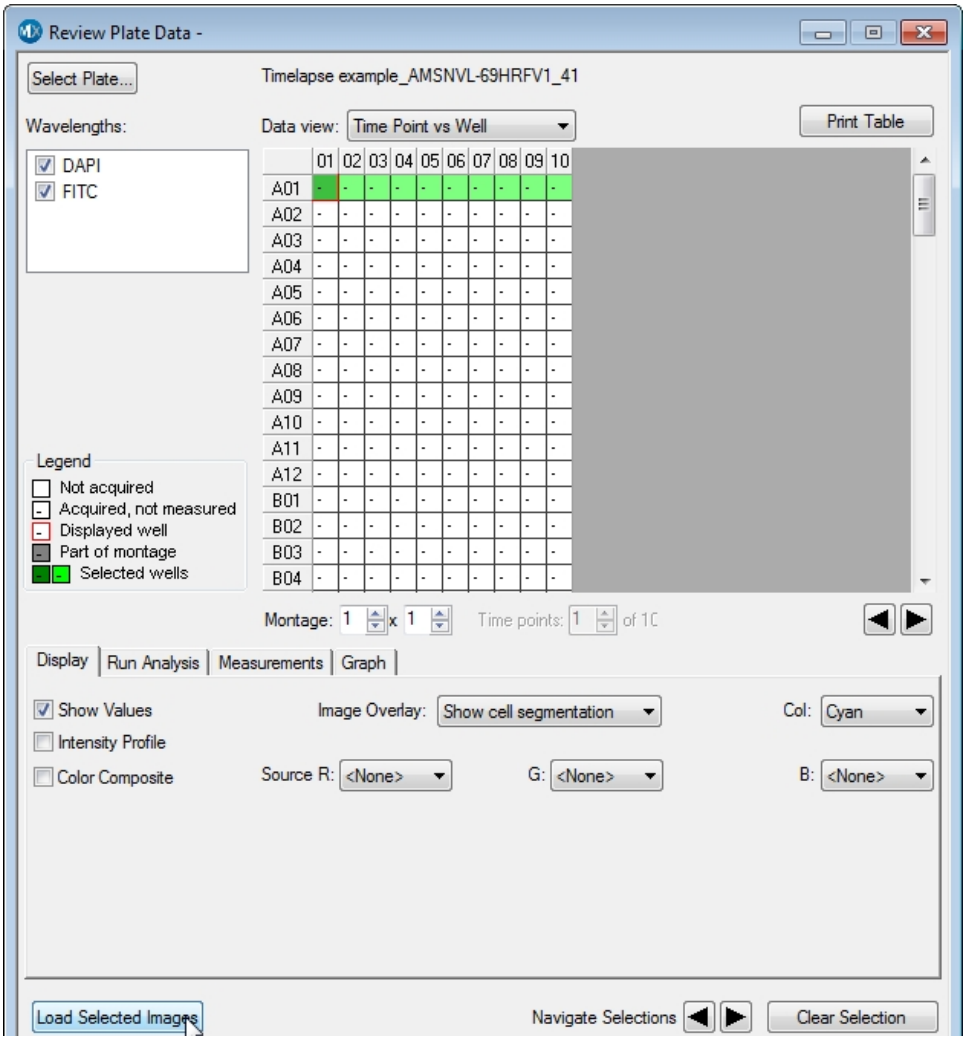


Figure 8-23: Review Plate Data dialog

2. Click **Select Plate**.
3. In the **Select Plate for Review** dialog, select a plate, and then click **Select**.

4. In the **Data view** list, click **Time vs Well**. The montage displays thumbnails of each time point.
5. To create a stack for viewing as a movie, right-click on one well to select it.

   The well changes color to indicate that the well is selected.

> **Note:** To clear the selection, click **Clear Selection** at the bottom of the dialog.

6. Click **Load Selected Images**.

   In the image window, you can view each time point as a plane in the stack using the navigation buttons above the image pane.

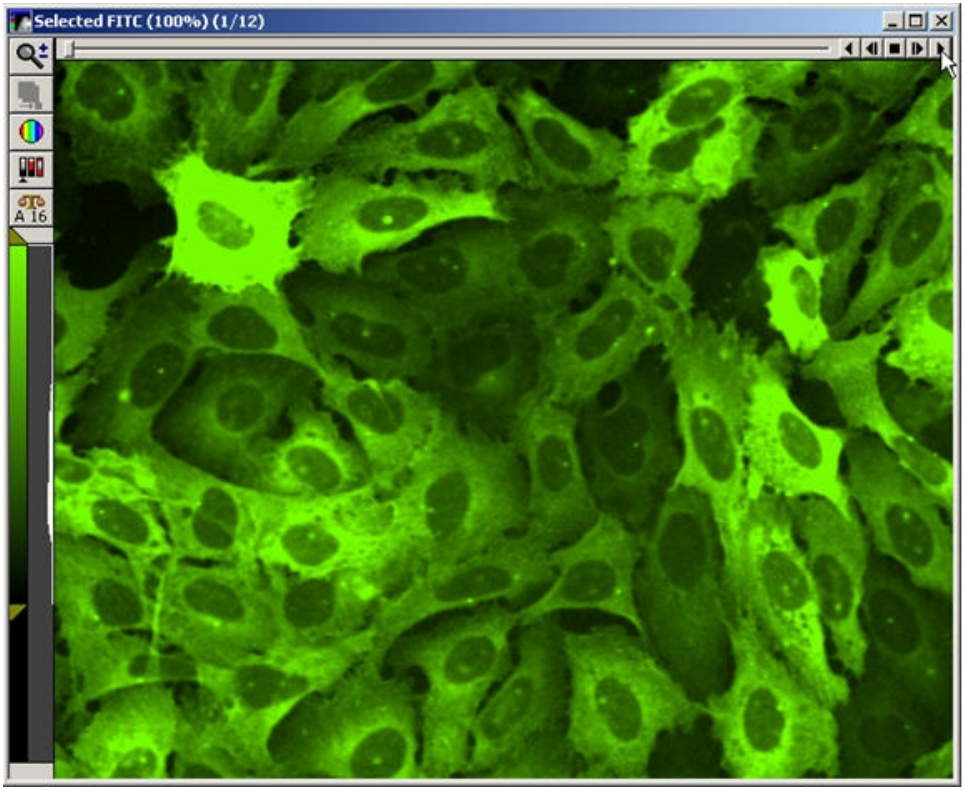


Figure 8-24: Stack of images with navigation buttons above the image pane

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

Making a Timelapse Movie

To make a movie:

1. Before you begin, load timelapse images and create a stack. See Reviewing Timelapse Data on page 195.
2. Select the image window of the stack on the MetaXpress Software desktop.
3. In the MetaXpress Software, click **Stack > Make Movie**.

   In the Simplified Menu Structure, click **Edit > Stack > Make Movie**.

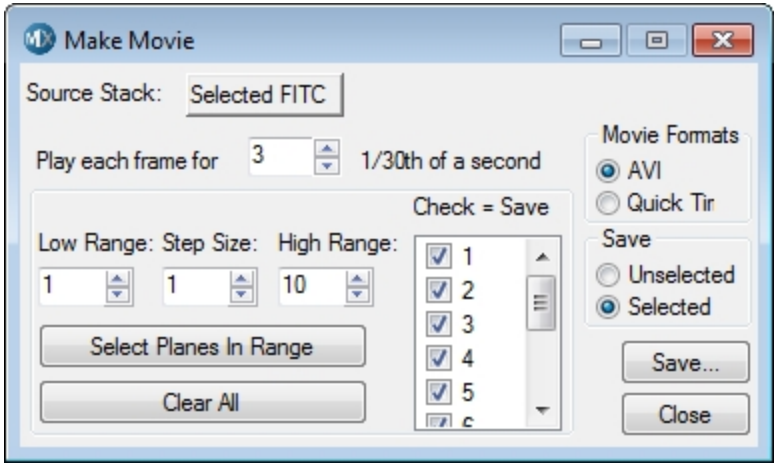


Figure 8-25: Make Movie dialog

4. In the **Make Movie** dialog, select the desired options.

   For information about the available options, view the application help by pressing **F1** while viewing the dialog.
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 wear powder-free gloves at all times when you access the internal components of the enclosure. For additional information about cleaning the system, see Maintenance on page 255.

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

Environmental Control components to be cleaned include:

- Cleaning Carbon Dioxide Tubing on page 199
- Cleaning the Water Reservoir on page 200

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 CO_2 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 CO_2 to the stage (plate) area. See Figure 8-26.

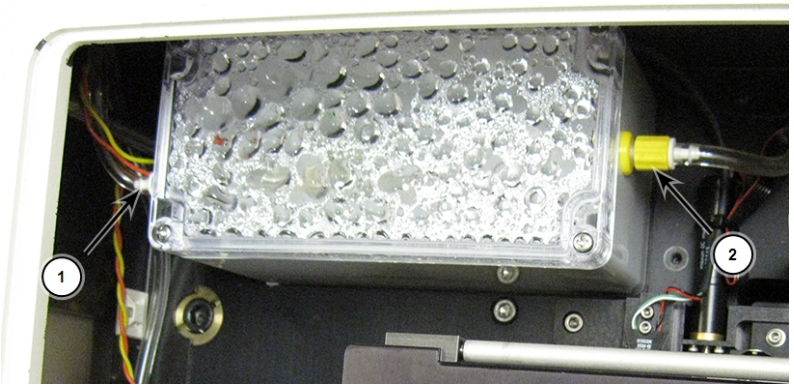


Figure 8-26: CO_2 tubing connections on the water reservoir

Item	Description
1	Input tube connection
2	Output tube connection

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

When replacing or cleaning 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 that you reconnect all tubing before running 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 reconnecting 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 the tubing within the environmental enclosure, making sure all connections are tight.
5. Confirm that CO_2 is being delivered to the water reservoir by visually confirming that bubbles move through the reservoir. See Setting Up the Carbon Dioxide Tank on page 186.

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

   In the Simplified Menu Structure, click **Screening > 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 (Figure 8-27), and remove the side panel for more access.

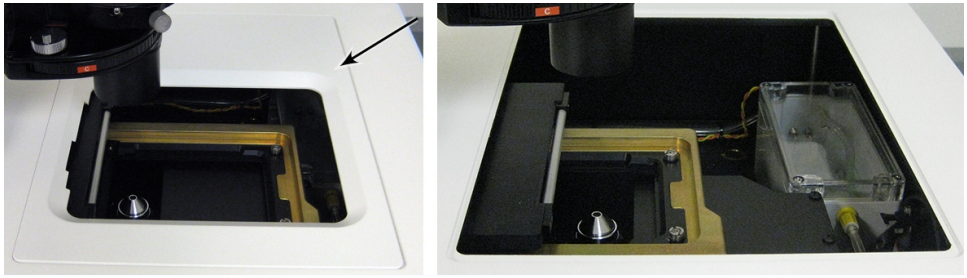


Figure 8-27: Top access panel around the top door on and off

4. Disconnect the CO_2/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. Dispose of the water, and then rinse the reservoir with 70% ethanol.
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.

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.

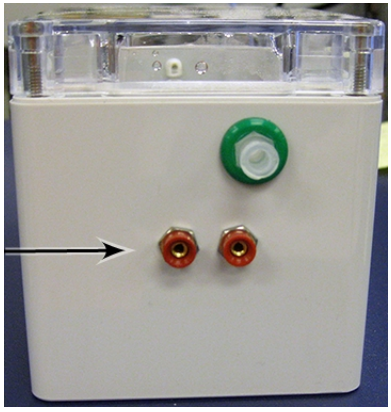


Figure 8-28: Water level sensor wire sockets

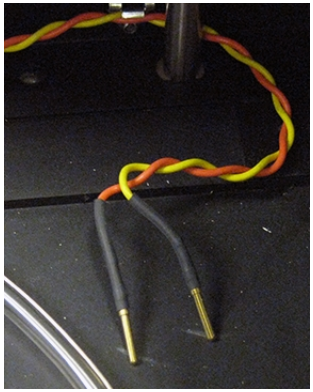


Figure 8-29: Water level sensor wires inside the instrument

12. Reconnect the CO_2/air tubing to the luer locks on both sides of the water reservoir.
13. Replace the top access panel surrounding the top door and replace the side panel. See Figure 8-27.
14. Start the instrument.
15. In the MetaXpress Software, click **Screening > Plate Acquisition Setup > Load Plate** to close the top door of the instrument.

    In the Simplified Menu Structure, click **Screening > Acquisition Setup > Load Plate** to close the top door of the instrument.

Fluidics Options

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 ±5% or ±1 µL, whichever is greater.

The ImageXpress Fluidics option can be installed together with the ImageXpress Environmental Control option. See Environmental Control Options on page 175 for details.

Operational information is included for the following:

- Fluidics Hardware on page 203
- Setting Up Fluidics Hardware on page 206
- Configuring Fluidics Software (Stations and Properties) on page 207

Fluidics Hardware

The ImageXpress Fluidics option consists of the following hardware components. See Figure 8-30, Figure 8-31 and Figure 8-32.

- **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.

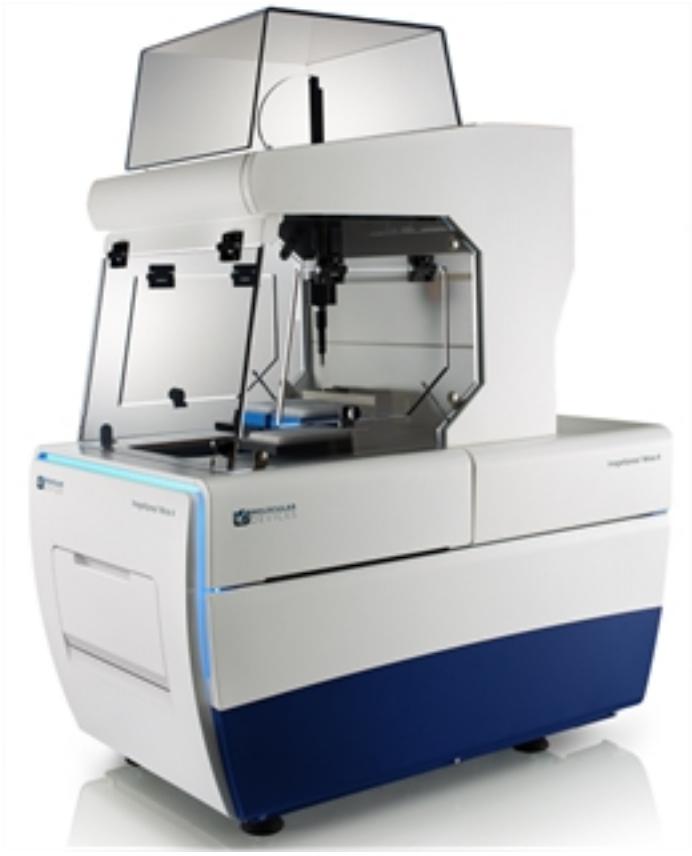


Figure 8-30: ImageXpress Micro 4 System with ImageXpress Fluidics option

Figure 8-31: ImageXpress Micro System with ImageXpress Fluidics option

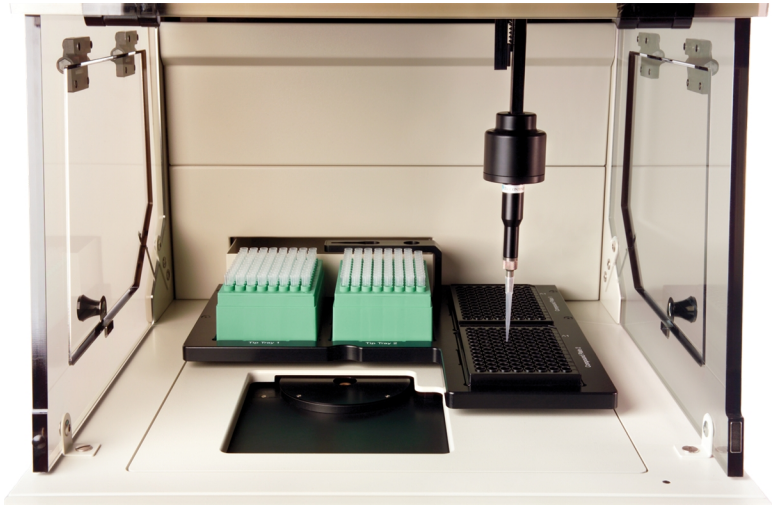


Figure 8-32: ImageXpress Fluidics option close up

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. Molecular Devices recommends using standard 96-well or 384-well plates. U-bottom and V-bottom plates are beneficial when withdrawing and dispensing very small volumes of compound.

Setting Up Fluidics Hardware

A Molecular Devices FSE (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 as needed, and allow the compound-plate temperature to reach equilibrium. See Setting Up Environmental Control on page 181.

5. If required, empty the waste bin.

   If your experiment involves disposal of liquid, place an absorbent pad in the waste bin. Also see Cleaning the Waste Disposal Box and the Plate Sealing Ring on page 224.

6. Load an unlidded plate with the live-cell sealing ring on the top. See Loading the Sample Plate on page 188.

To configure fluidics in MetaXpress Meta Imaging Series Administrator Software:

If you change tips, 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.

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**.
3. In the **Configure Hardware** dialog, select the correct hardware configuration, and then click **Configure Devices**.
4. In the right pane of the **User Settings** for hardware configuration dialog, click **ImageXpress Micro Fluidics**, and then click **Settings**.

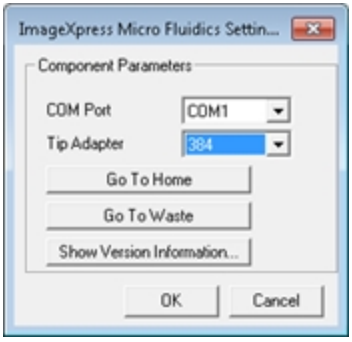


Figure 8-33: ImageXpress Micro Fluidics Settings dialog

5. In the **ImageXpress Micro Fluidics Settings** dialog, 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.

Note: The **Configure Fluidic Stations** functions are available at **Devices > Configure Fluidic Stations**, in the Simplified Menu Structure, **Control > ImageXpress > Configure Fluidics Stations**, and from the **Configure Stations** option from **Plate Acquisition Setup > Configure > Fluidics**.

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, click **Screening > Plate Acquisition Setup**.

   In the Simplified Menu Structure, click **Screening > Acquisition Setup**.

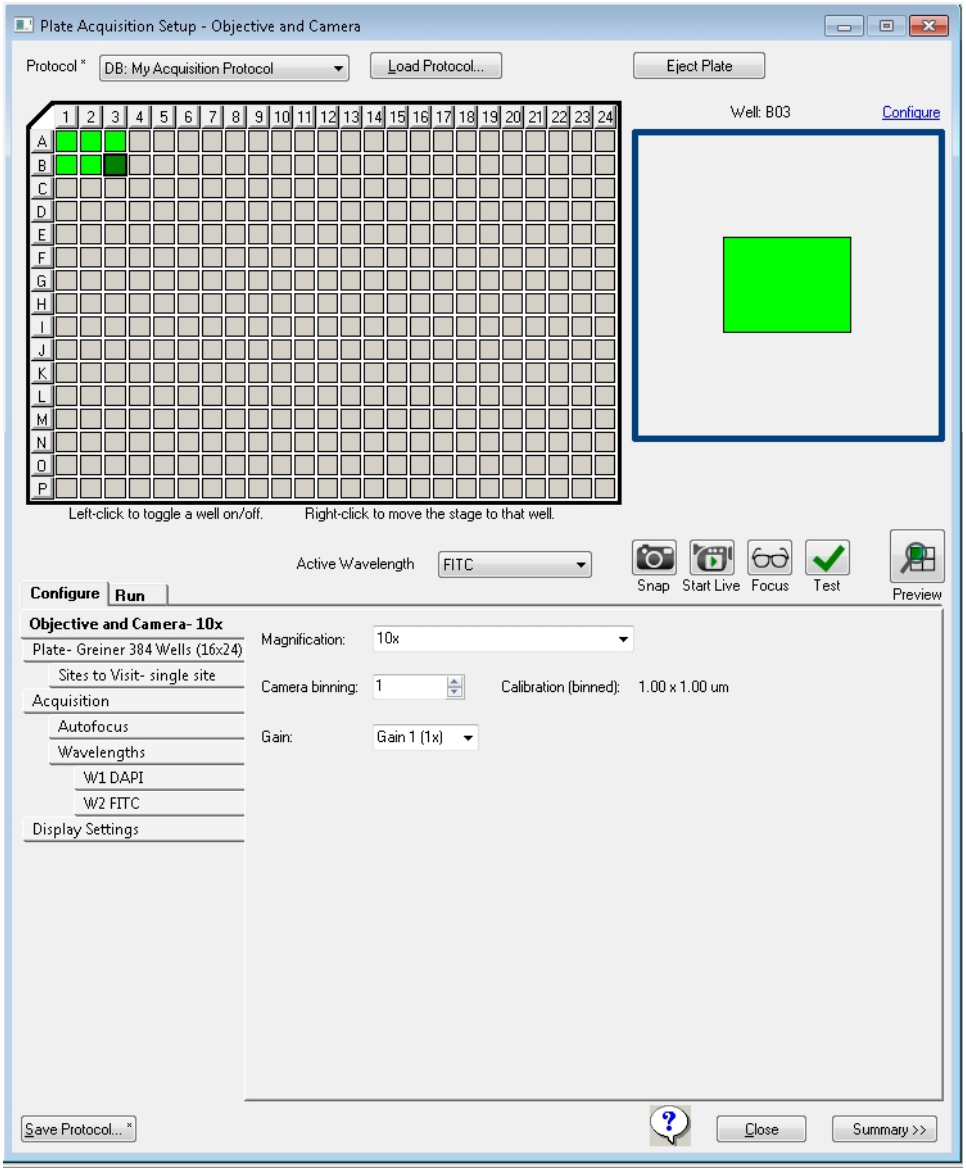


Figure 8-34: Plate Acquisition Setup dialog

2. In the **Plate Acquisition Setup** dialog, select the **Configure > Acquisition** tab.

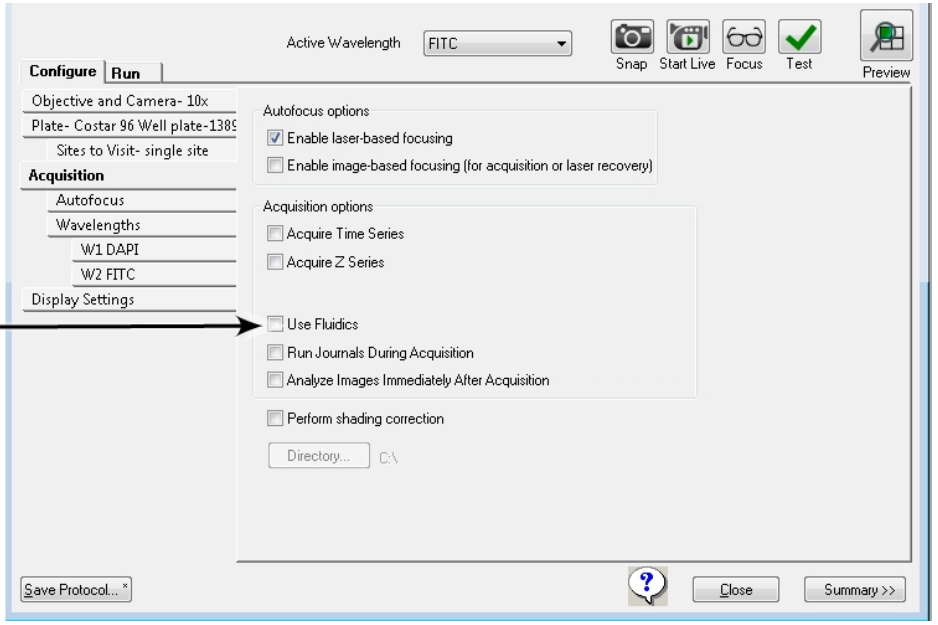


Figure 8-35: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

3. Select **Use Fluidics**.

4. Click on the **Fluidics** tab.

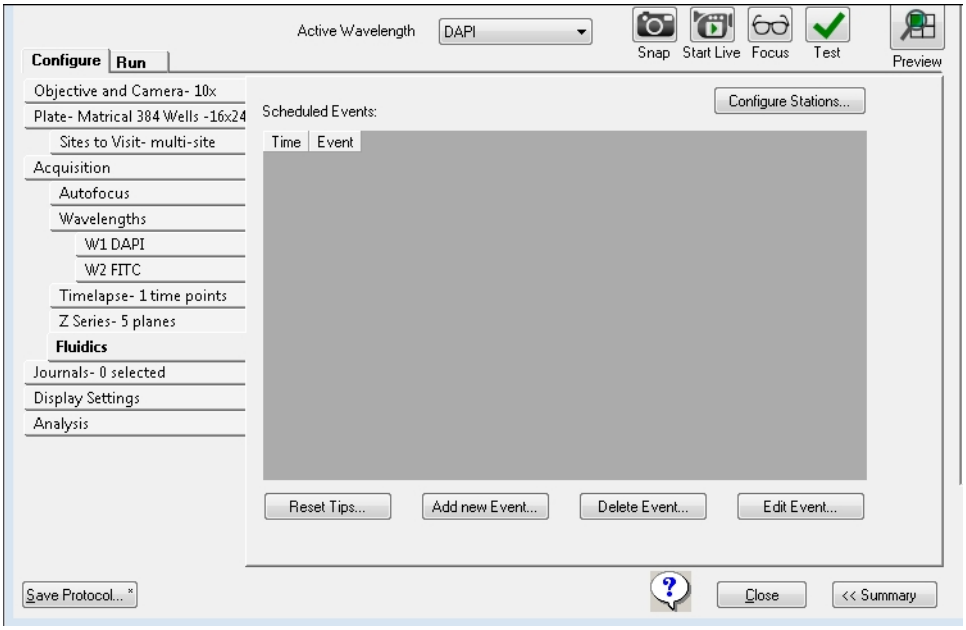


Figure 8-36: Plate Acquisition Setup dialog, Configure tab: Fluidics tab

5. Click **Configure Stations**.

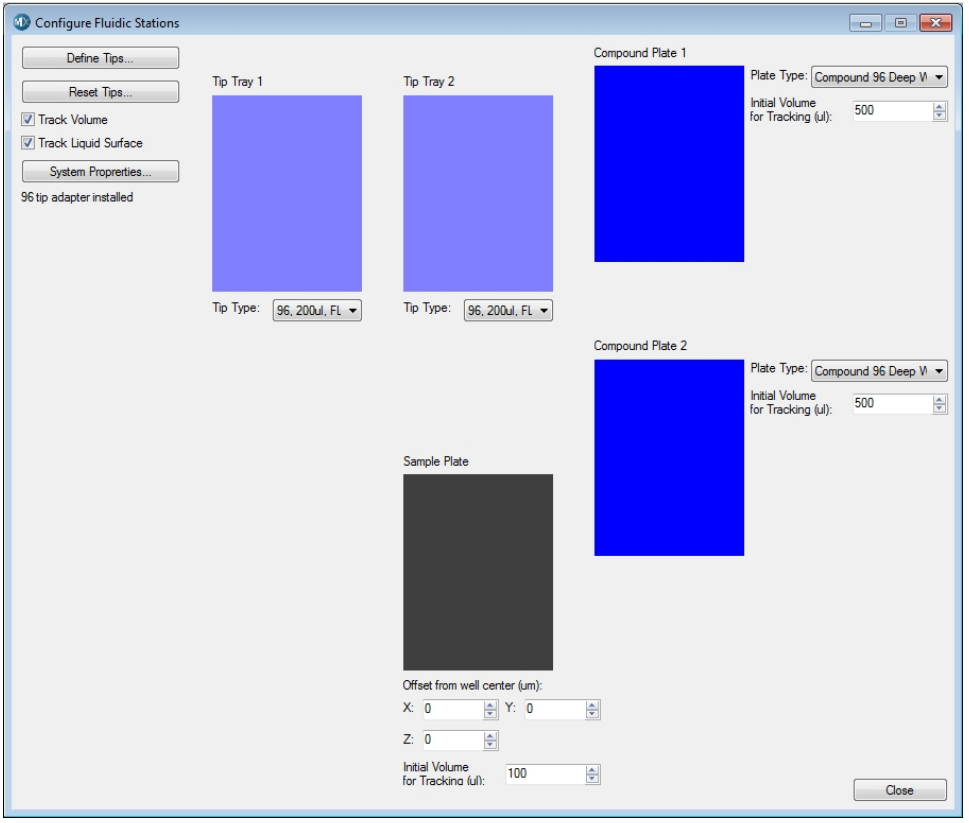


Figure 8-37: Configure Fluidic Stations dialog

6. In the **Configure Fluidics Stations** dialog, for each **Tip Tray**, select a **Tip Type**.

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

7. For each **Compound Plate**, select a **Plate Type**.

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

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

   Type a positive Z offset to have the pipettor sample higher than normal. Type 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.

9. If applicable, select **Track Volume**, and then type a value for the **Initial Volume for Tracking (µl)** for each plate.
10. Do one of the following:
    - If you selected **Track Volume**, then select **Track Liquid Surface**, and then go to Step 11.
    - Optionally, select **Track Volume**, select **Track Liquid Surface**, clear **Track Volume**, and then go to Step 11.
    - Otherwise, go to Step 13.

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

11. Click **System Properties**.

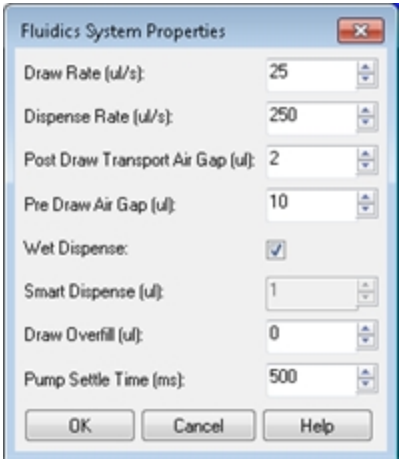


Figure 8-38: Fluidics System Properties dialog

12. On the **Fluidics System Properties** dialog, leave the default values for the fluidics system properties set as-is, or modify any values as needed.

Table 8-2: Fluidic Systems Property values

Option	Description
Draw Rate (µl/s)	The rate at which the pump draws fluid.
Dispense Rate (µl/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 is used to push 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 **Track Liquid Surface** is also selected, then dispensing occurs near the liquid surface; otherwise, the dispensing occurs near the bottom of the well.
Smart Dispense (µl)	Enabled only if **Wet Dispense** is selected. 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**: **Draw Overfill** is not applied to the sample plate.
Pump Settle Time (ms)	The time delay that is used 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 close the **Fluidics System Properties** dialog and return to the **Configure Fluidics Stations** dialog.
14. If applicable, click **Define Tips** and in the **Define Tips** dialog, 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 have completed a dispensing cycle, to indicate that you have reloaded tip racks and plates, click **Reset Tips**, and on the **Reset Tips/Liquid Levels** dialog, click **Reset** for the appropriate tip trays and plates, and then click **Close**.

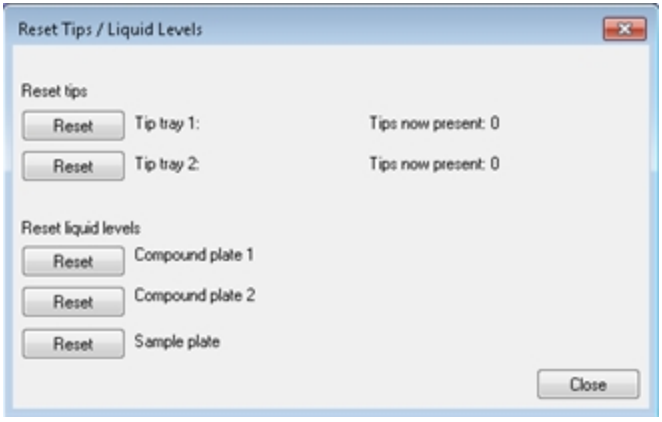


Figure 8-39: Reset Tips/Liquid Levels dialog

Manual Fluidics Control

Do the following procedure to control the fluidics station manually.

Tip: The fluidics interface enables you 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. See To record the steps in a journal on page 216. You can add journals as events when you run an experiment. See To configure a fluidics experiment: on page 217.

To control fluidics manually

1. In the MetaXpress Software, click **Devices > Fluidic Control**.

   In the simplified menu, click **Control > ImageXpress > Fluidic Control**.
2. In the **Fluidic Control** dialog, specify the action you would like to do. You can manually pick up and eject tips, draw and dispense fluid, and mix.

   For example, to remove 20 µl 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 (µl)**: 20
3. Click **Go**.

   The fluid is drawn from the plate.
4. In the **Action** list, click **Dispense**, and then click **Go** to add the fluid to the plate.

To record the steps in a journal

1. In the MetaXpress Software, on the **Journal** menu, click **Start recording**.
2. Do the steps that you want to record. See To control fluidics manually on page 216.
3. Click **Journal > Stop recording**, and then save the journal file.

4. To modify the journal, click **Journal > Edit Journal**, and then select the journal to be edited. See Figure 8-40.

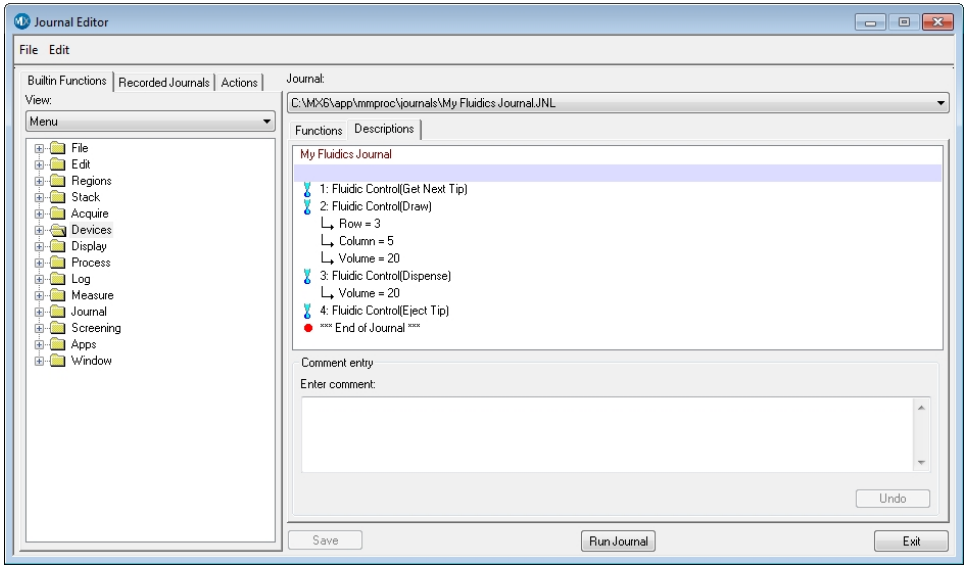


Figure 8-40: Example Journal using manual fluidics control

Doing 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.

Do the following procedure to configure a fluidics experiment.

To configure a fluidics experiment:

1. In the MetaXpress Software, click **Screening > Plate Acquisition Setup**.

   In the Simplified Menu Structure, click **Screening > Acquisition Setup**.
2. In the **Plate Acquisition Setup** dialog, click **Configure > Acquisition**.

3. Select **Use Fluidics**. See Figure 8-41.

Figure 8-41: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

4. Click **Fluidics** to schedule fluidics events. See Figure 8-42.

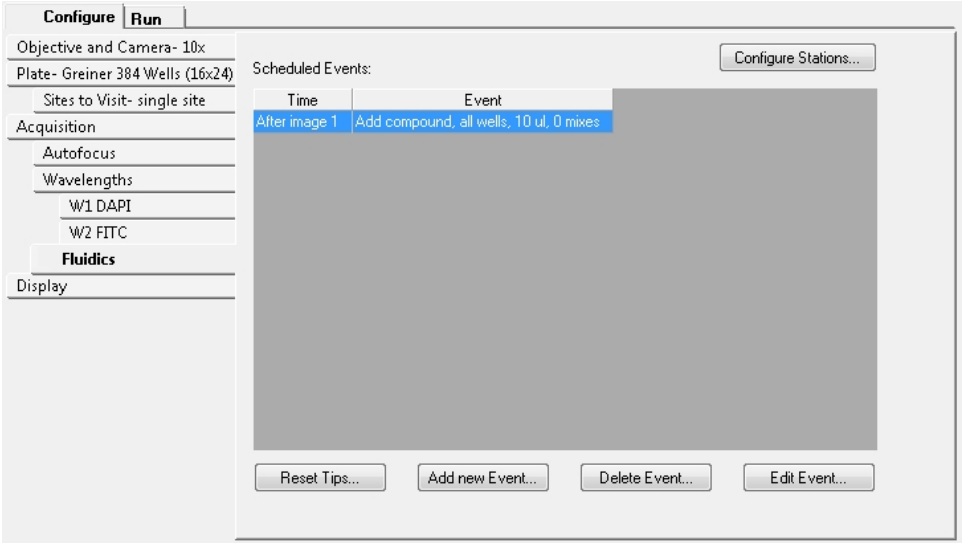


Figure 8-42: Adding events to a fluidics experiment

5. Click **Add new Event** to add an event to the **Scheduled Events** field.

6. In the **Fluidic Event** dialog, specify the time point at which you want the event to occur, and then specify whether the event should occur before or after the time point. See Figure 8-43.

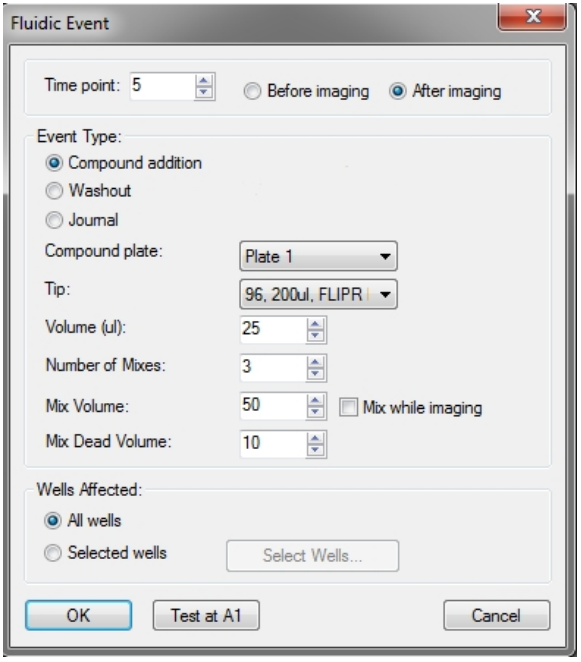


Figure 8-43: Time point setting in Fluidic Event dialog

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. See Figure 8-44.

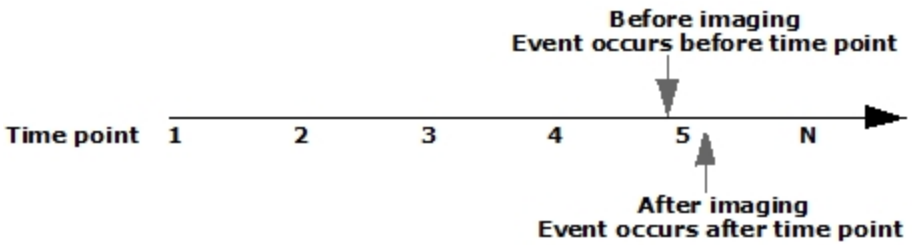


Figure 8-44: Setting events to occur before and after a time point

Note: The number of time points is set on the **Timelapse** tab. See Doing Timelapse Experiments on page 192.

7. In the **Fluidic Event** dialog, select the event type and its properties. Event types include:
   - **Compound addition**: A Compound addition event, shown in Figure 8-45, draws liquid from the compound plate and adds it to the sample plate. There is a one-to-one mapping between the wells on the compound plate and the sample plate. That is, 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 are running mixes that consist of more than a single cycle, **Mix Dead Volume** specifies an amount of fluid that is to remain in the tip and not to be returned to the well during intermediate repeated cycles of draws and dispense. For more information about compound addition settings, see the MetaXpress Software application help (press **F1** while viewing the dialog).

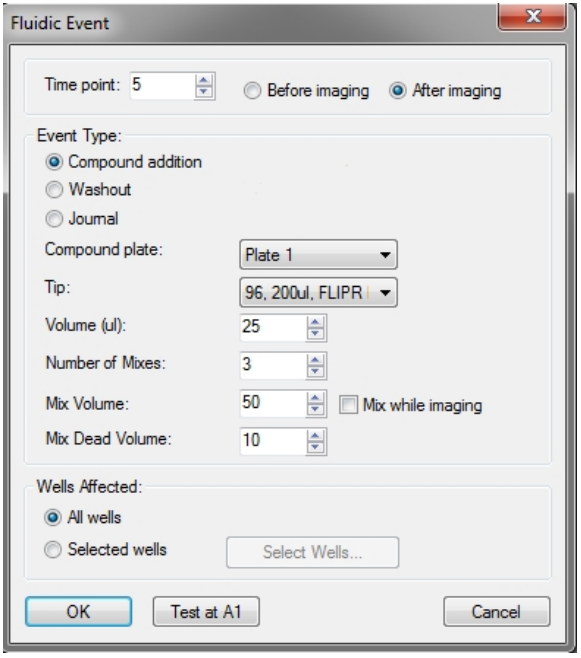


Figure 8-45: Fluidic Event dialog Compound addition event

- **Washout**: A Washout event removes liquid from the sample plate and replaces it with liquid from the compound plate. There is a one-to-one mapping between the wells as shown in Fluidic Event dialog Washout Event on page 221.

  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. If you want the system to discard the old tip after removing and discarding the fluid from the sample plate, select **New tip each exchange**. For more information, see the software Help (press **F1** while viewing the dialog).

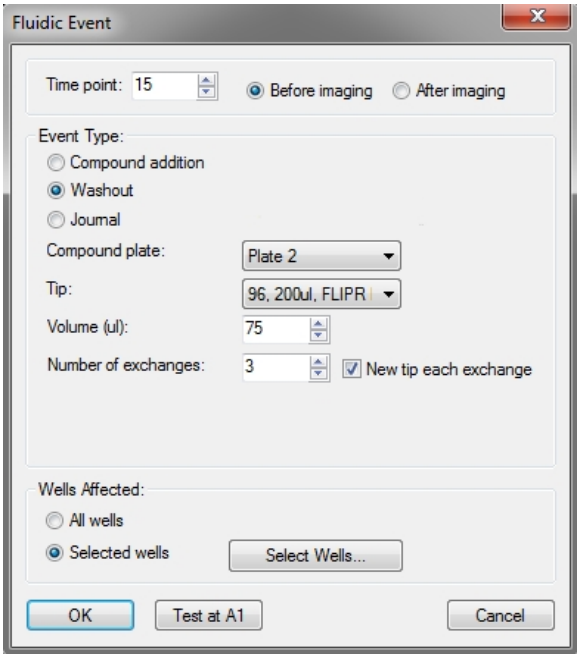


Figure 8-46: Fluidic Event dialog Washout Event

- **Journal**: A Journal event is used for any special cases or custom protocols (for example, experiments not using a one-to-one mapping of wells or drawing once and dispensing multiple times) as shown in Fluidic Event dialog Journal event on page 222.

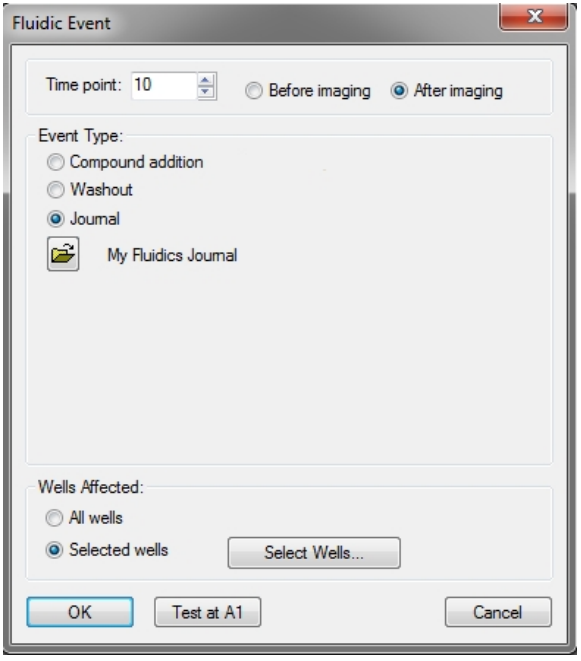


Figure 8-47: Fluidic Event dialog Journal event

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. Two examples are 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

Pressure-Applied Seals

G&L Precision Die Cutting pressure-applied aluminum foil seals

- Part number: G&L Aluminum Microplate Liddings (GL-255)
- For specifications, ordering information, pricing, and sealing instructions, contact G&L Precision (www.glprecision.com)
- Features
  - Manual plate sealing
  - Suitable for polypropylene and polystyrene plates
  - Easily punctured by the fluidics robot using FLIPR Tetra pipette tips
  - Compatible with DMSO

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 wear powder-free gloves at all times when you access the internal components of the enclosure. For additional information about cleaning the system, see Maintenance on page 255.

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

Fluidics components to be cleaned include:

- Cleaning the Waste Disposal Box and the Plate Sealing Ring on page 224

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 Options

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/phasemicroscopy.html

The ImageXpress Transmitted Light option can be installed with or without the ImageXpress Environmental Control option. It is not compatible with the ImageXpress Fluidics option. Doing Transmitted Light experiments with Fluidics requires the ImageXpress LED Transmitted Light option. See LED Transmitted Light Options on page 249.

See also:

- Environmental Control Options on page 175

Operational information is included for the following:

- Transmitted Light Hardware on page 225
- Transmitted Light Software Configuration on page 228
- Setting up Koehler Illumination on page 231
- Transmitted Light Phase Ring Alignment on page 234
- Replacing the Transmitted Light Bulb on page 246

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 8-49Figure 8-48 and Figure 8-50.
- **Phase contrast objectives**. Must be selected for phase contrast imaging.
- **White light lamp (halogen)**. The software controls the lamp power.

  The halogen lamp has a limited life and can be replaced. See Replacing the Transmitted Light Bulb on page 246.
- **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 8-51.
- **Phase Alignment Lens**. Enables phase ring alignment for the ImageXpress Micro System, XL, XLS and 4 Systems. See Transmitted Light Phase Ring Alignment on page 234.
- **Hinge**. Allows robotic access to the plate if necessary. See Figure 8-52.
- **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. See Figure 8-1.

Figure 8-48: ImageXpress Micro 4 System with the Transmitted Light option

Figure 8-49: ImageXpress Micro System with the Transmitted Light option

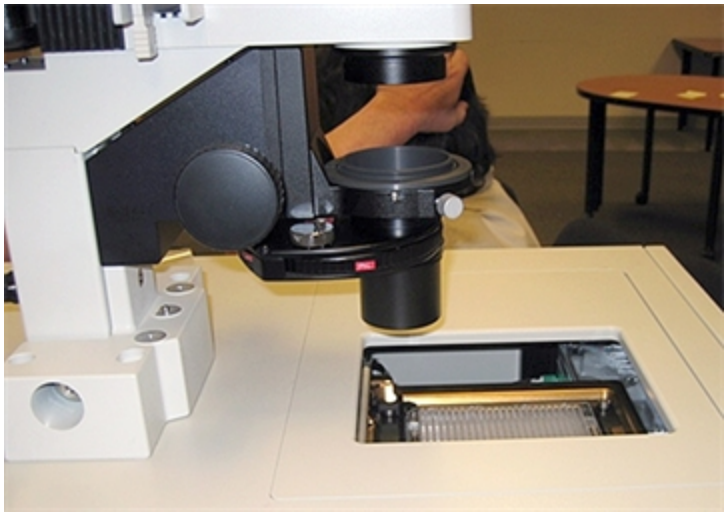


Figure 8-50: ImageXpress System with Transmitted Light option (detailed view)

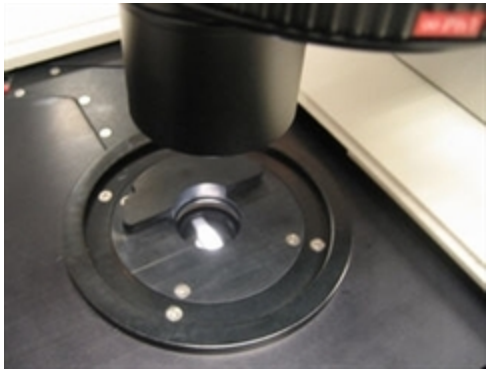


Figure 8-51: Transmitted Light shutter

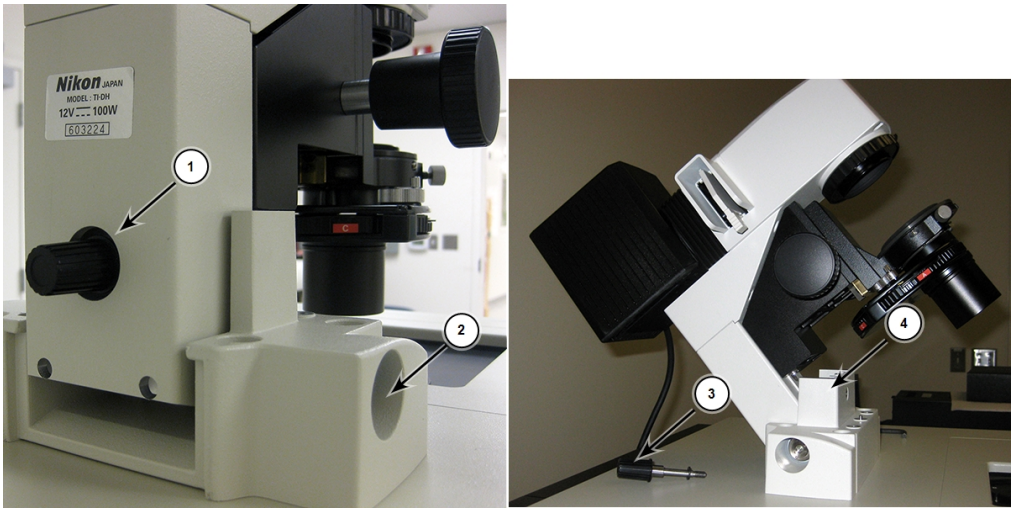


Figure 8-52: Hinge-release knob and hinge

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, click **Devices > Configure Illumination**.

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

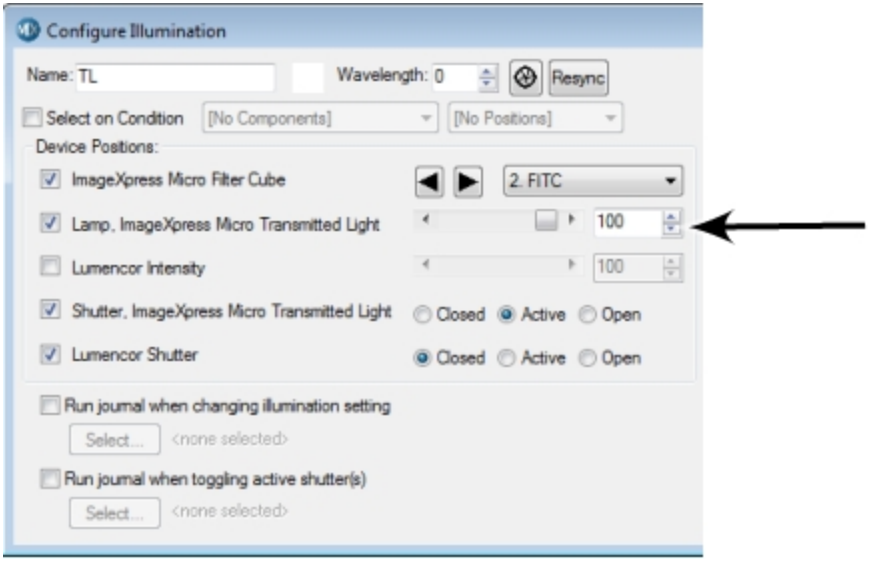


Figure 8-53: Configure Illumination dialog: Lamp On

2. In the **Configure Illumination** dialog, create an illumination setting named **TL Off**, select **Lamp, ImageXpress Micro Transmitted Light**, and set the **Lamp** illumination to zero, as shown in Figure 8-54.

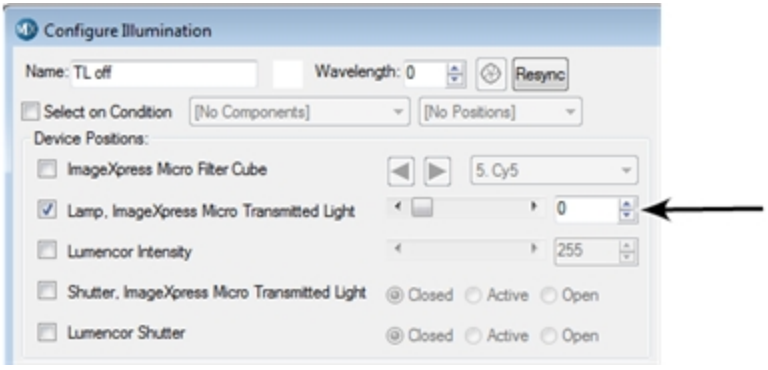


Figure 8-54: Configure Illumination dialog: Transmitted Lamp Off

3. Create an illumination setting named **TL**.
4. For **Device Positions > ImageXpress Filter Cube**, select either **Empty**, or **FITC**

5. Select **Lamp, ImageXpress Micro Transmitted Light**, and set lamp illumination as a percentage of full power, as shown in Figure 8-55. You can adjust this setting to meet the requirements of your experiment.

> **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 8-55).

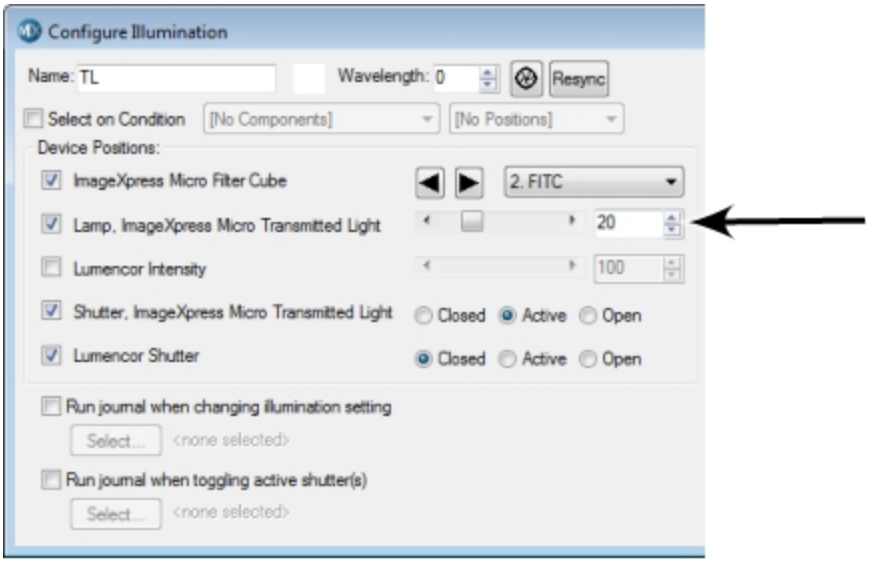


Figure 8-55: Configure Illumination dialog: Transmitted Light

After the settings have been configured, **TL** is selectable as a wavelength in the **Plate Acquisition Setup** dialog.

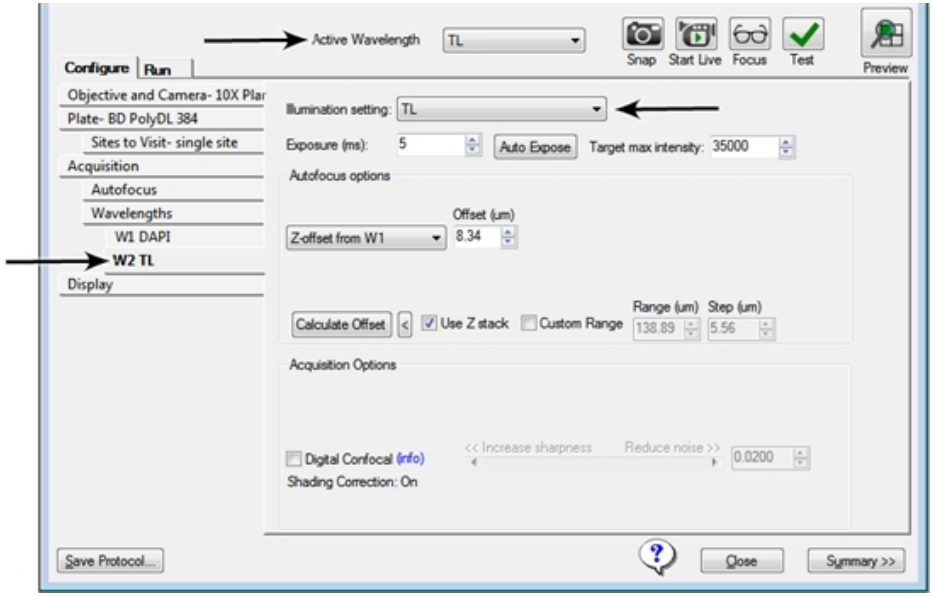


Figure 8-56: Transmitted Light wavelength setting in protocol configuration

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.

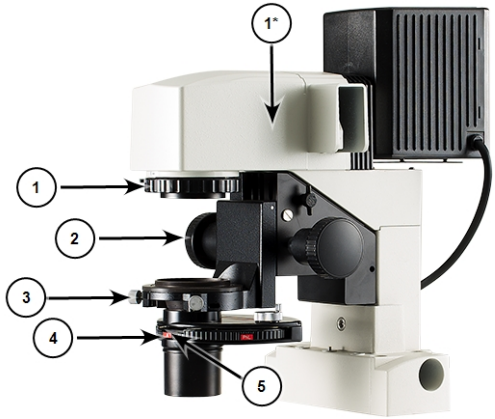


Table 8-3: ImageXpress Transmitted Light option overview

Item	Description
1	Field Aperture Diaphragm Control (* this 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)

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 Fluor or a 4x objective.
3. Rotate the condenser turret (4) so that the **A** position is selected (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, click **Screening > Plate Acquisition Setup**.

   In the simplified menu, click **Screening > Acquisition Setup**.
5. In the **Plate Acquisition Setup** dialog, 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 the 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 8-57: 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 transmitted light image.
15. Click **Stop Live**.

16. Click **Focus** to confirm proper illumination. If you see dark edges in your field of view, repeat step 12.

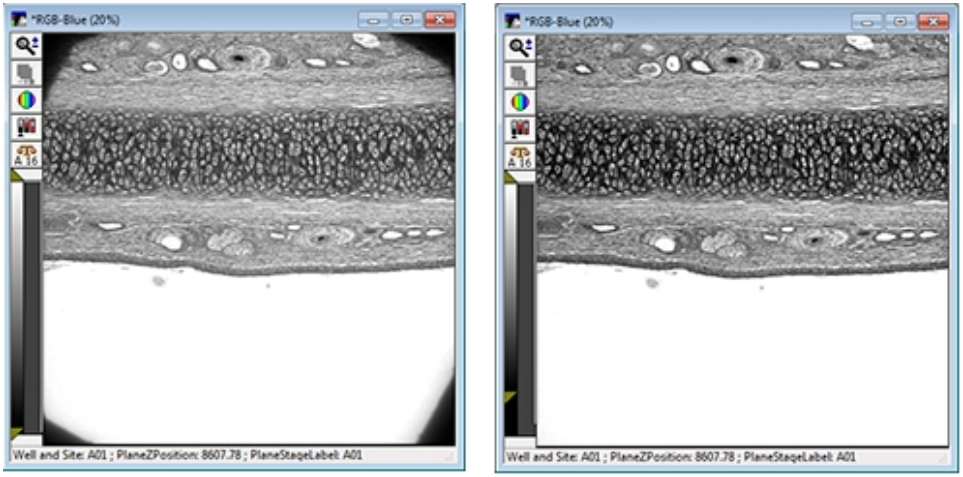


Figure 8-58: Adjustment of the Field Aperture Diaphragm Comparison

The adjustment is now complete.

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 4 System, the phase ring adjustment lens is manually placed in position for the phase ring adjustment. See To use the phase ring adjustment lens on the ImageXpress Micro 4 System: on page 235
- On the ImageXpress Micro System and XL System, the optics cover is removed and the phase ring adjustment lens is manually placed in position for the phase ring adjustment. See To use the phase ring adjustment lens on the ImageXpress Micro System and XL System: on page 236.
- On the ImageXpress Micro XLS System, the phase ring adjustment lens is manually placed in position for the phase ring adjustment without removing the optics cover. See To use the phase ring adjustment lens on the ImageXpress Micro XLS System: on page 238.

To use the phase ring adjustment lens on the ImageXpress Micro 4 System:

1. Power off the instrument.

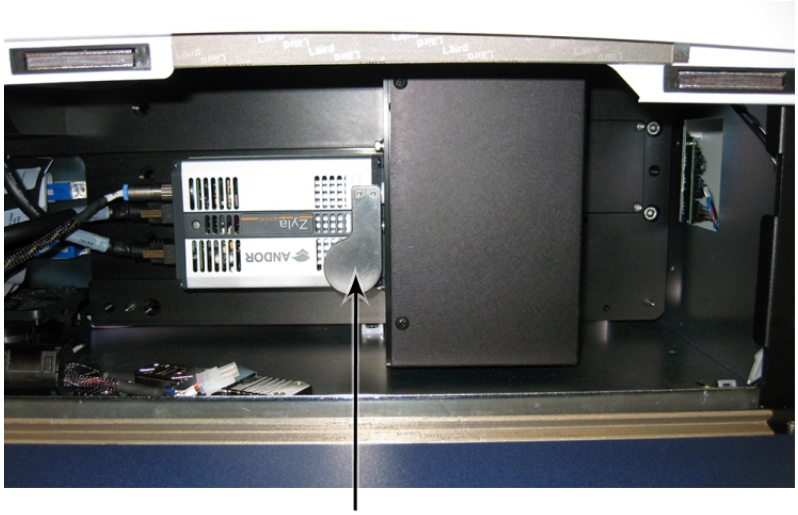


Figure 8-59: Access to the mirror box with the phase ring adjustment lens

2. Open the lower hinged side door to access the black mirror box.

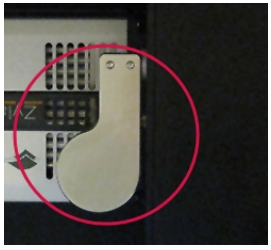


Figure 8-60: Mirror box with the phase ring adjustment lens out of position for alignment

3. Raise the phase ring adjustment lens lever to position the mirror into the optical path for the alignment procedure.

CAUTION! The phase ring adjustment lens must be out of the optical path for any actual imaging.

Figure 8-61: Mirror box with the phase ring adjustment lens in position for alignment

4. Power on the instrument.
5. Adjust the phase ring. See To align the phase ring on page 240.
6. After the phase ring has been adjusted, power off the instrument.
7. Lower the phase ring adjustment lens out of the optical path.
8. Close the lower hinged side door.

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

CAUTION! Wear gloves, and do not touch, move, or otherwise damage the mirror to the right of the phase ring adjustment lens.

1. Power off the instrument.
2. Use a Phillips screwdriver to remove both of the left side panels of the instrument.
3. Use a 1/16 inch hex key to remove the black optics cover over the lens.

4. Move the phase ring adjustment lens into place for the alignment procedure.

CAUTION! The phase ring adjustment lens must be out of the way for any actual imaging.

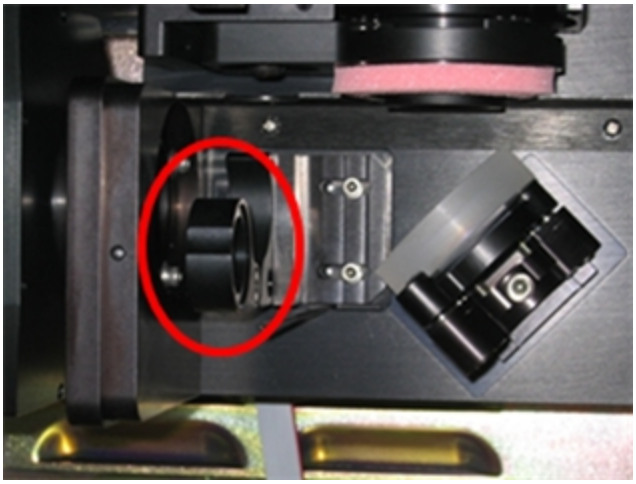


Figure 8-62: Phase ring adjustment lens on the ImageXpress Micro System and XLS System

5. Power on the instrument.
6. Adjust the phase ring. See To align the phase ring on page 240.
7. After the phase ring has been adjusted, power off the instrument.
8. Move the phase ring adjustment lens out of the optical path.
9. Use a 1/16 inch hex key to install the black optics cover over the lens.
10. Use a Phillips screwdriver to install both of the left side panels on the instrument.

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

1. Power off the instrument.
2. Use a Phillips screwdriver to remove both of the left side panels of the instrument to access the phase ring adjustment lens mounted on the outside of the optics cover.

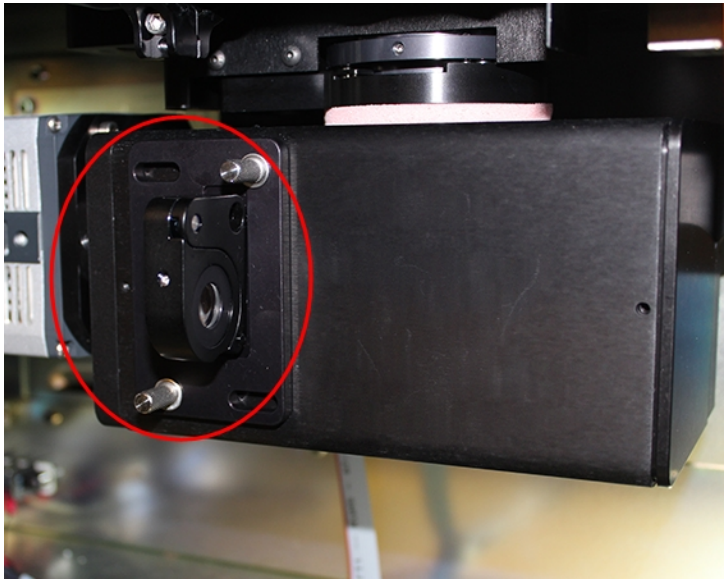


Figure 8-63: Phase Ring Adjustment Lens on the ImageXpress Micro XLS System

3. Remove the two thumbscrews and their washers from the phase ring adjustment lens mounting plate.

   There is a flat washer and a split-ring washer on each thumbscrew on the phase ring adjustment lens assembly.

Figure 8-64: Thumbscrew and washers

4. Remove the phase ring adjustment lens assembly from the instrument.
5. Remove the protective cloth bag from the phase ring adjustment lens.

6. Move the phase ring adjustment lens into it fully extended position.

Figure 8-65: Phase Ring Adjustment Lens Extended for Phase Ring Alignment

7. Slide the phase ring adjustment lens assembly onto the optics cover with the phase ring adjustment lens on the inside for the phase ring alignment.
8. Install the washers and tighten the thumbscrews to hold the phase ring adjustment lens assembly in position for phase ring alignment.
9. Adjust the phase ring. See To align the phase ring on page 240.
10. After the phase ring has been adjusted, power off the instrument.
11. Remove the two thumbscrews and their washers from the phase ring adjustment lens mounting plate.
12. Remove the phase ring adjustment lens assembly from the instrument.
13. Move the phase ring adjustment lens into it fully closed position.
14. Cover the phase ring adjustment lens with the protective cloth bag.
15. Mount the phase ring adjustment lens assembly on the optics cover with the closed and covered phase ring adjustment lens on the outside.
16. Install the washers and tighten the thumbscrews.
17. Use a Phillips screwdriver to install both of the left side panels on the instrument.

To align the phase ring

1. Make sure that the phase ring adjustment lens is in place.
   - On the ImageXpress Micro 4 System System, the phase ring adjustment lens is manually placed in position for the phase ring adjustment. SeeTo use the phase ring adjustment lens on the ImageXpress Micro 4 System: on page 235
   - On the ImageXpress Micro System and XL System, the optics cover is removed and the phase ring adjustment lens is manually placed in position for the phase ring adjustment. SeeTo use the phase ring adjustment lens on the ImageXpress Micro System and XL System: on page 236.
   - On the ImageXpress Micro XLS System, the phase ring adjustment lens is manually placed in position for the phase ring adjustment without removing the optics cover. See To use the phase ring adjustment lens on the ImageXpress Micro XLS System: on page 238.
2. Rotate the condenser turret so that the A position is selected (at the front).

Figure 8-66: 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.

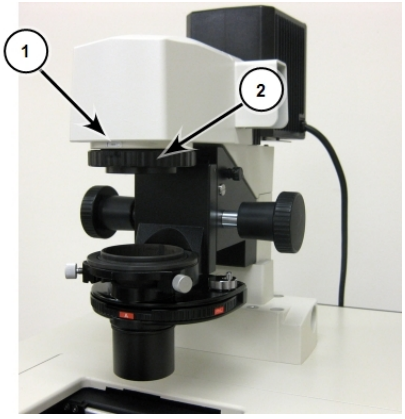


Figure 8-67: Aperture Stop Position for Phase Ring Alignment

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.

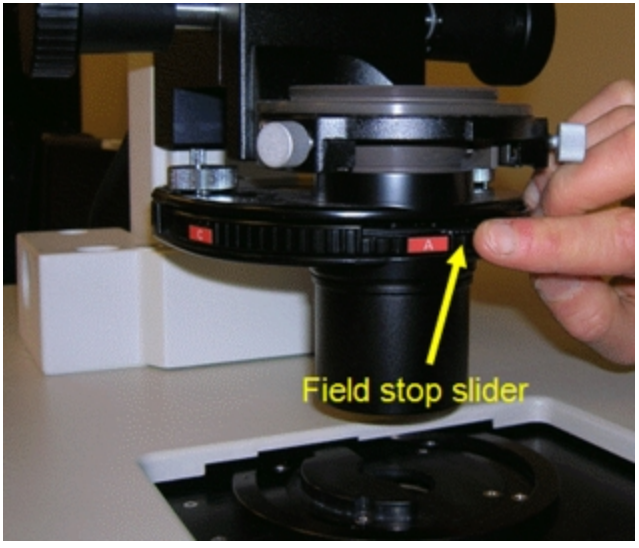


Figure 8-68: Field Stop Slider

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. With no sample present, snap an image.
10. If necessary, adjust the illuminator level and the exposure time to obtain a visible but not saturated image. The image of the phase ring might be off-center. See Figure 8-69. This is acceptable as long as most of the image is visible.

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

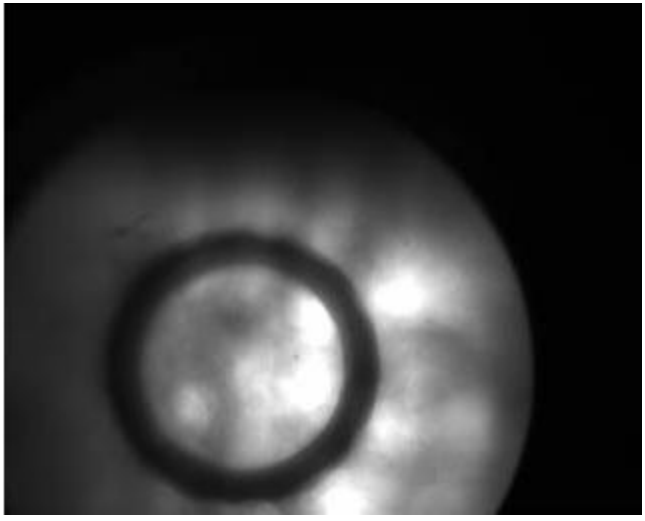


Figure 8-69: Out-of-focus image of phase contrast ring

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

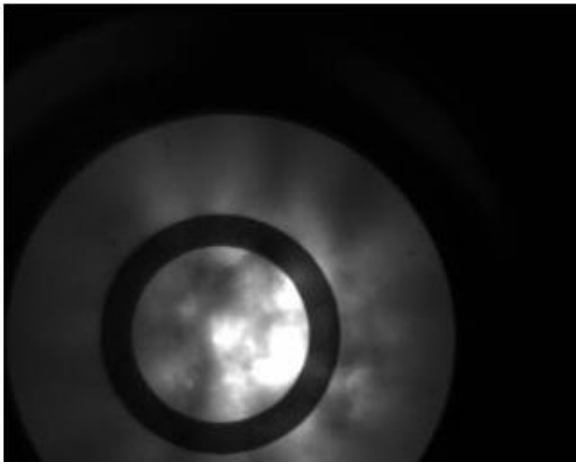


Figure 8-70: In-focus image of phase contrast ring

12. Use the region tools in the MetaXpress Software to mark the position of the phase ring. Make sure that you capture both horizontal and vertical axes. Use either the ellipse region tool or multiple line tools. See Figure 8-71.

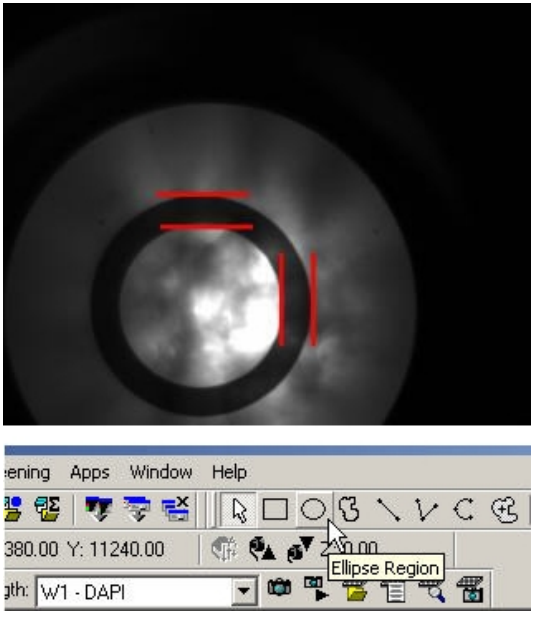


Figure 8-71: Marking the position of the phase ring

13. In the MetaXpress Software, click **Regions > Save Regions** to save the marked positions.

14. Manually rotate the condenser turret to the appropriate annulus for the phase objective (PhL for 4x, Ph1 for 10x and 20x, Ph2 for 40x).

    The selected 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).

15. Snap an image.

    The condenser annulus ring shows. See Figure 8-72. You might need to adjust the exposure time.

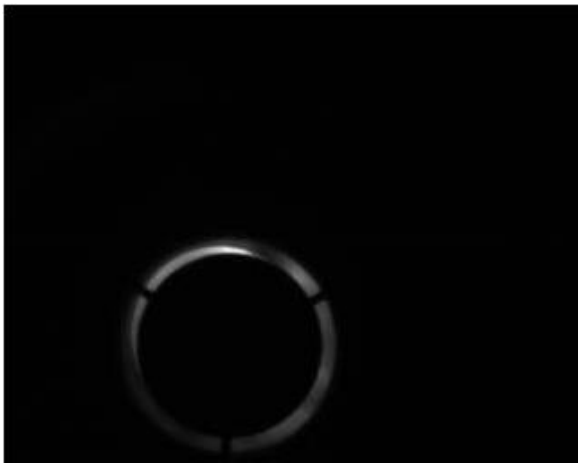


Figure 8-72: Image of condenser annulus ring

16. In the MetaXpress Software, click **Live Mode** in either the **Acquire or Plate Acquisition and Control** dialog.
17. Click **Regions > Load Regions** to load in the saved regions.
18. 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. See Figure 8-73.

19. Lock down the annulus centering knobs.

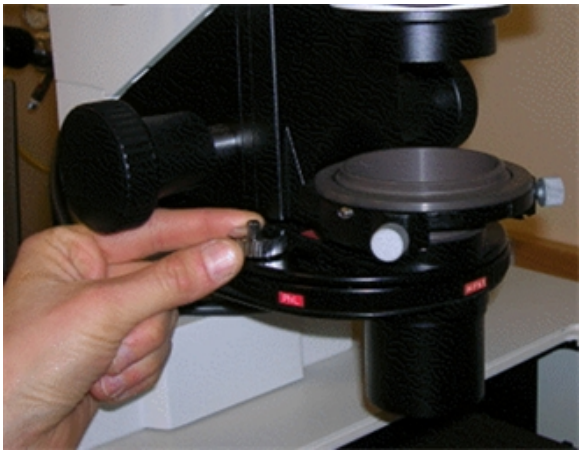


Figure 8-73: Centering the condenser annulus ring with the objective phase ring

20. 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.
21. In the MetaXpress Software, click **Stop Live**. For the ImageXpress Micro 4 System, ImageXpress Micro System, XL, or XLS Systems, move the phase ring adjustment lens out of the optical path.
    - For the ImageXpress Micro 4 System, see To use the phase ring adjustment lens on the ImageXpress Micro 4 System: on page 235.
    - For the ImageXpress Micro System and XL System, see To use the phase ring adjustment lens on the ImageXpress Micro System and XL System: on page 236.
    - For the ImageXpress Micro XLS System, see To use the phase ring adjustment lens on the ImageXpress Micro XLS System: on page 238.

Transmitted Light Experiments

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

To run a Transmitted Light experiment

1. If you are running a phase-contrast experiment, make sure a phase-contrast objective is selected. 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 microplate wells at least half-way full with liquid.

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

4. Turn on the Transmitted Lamp by selecting the appropriate Illumination setting in the **Configure Illumination** dialog.
5. In the MetaXpress Software, click **Screening > Plate Acquisition Setup**.
6. In the **Plate Acquisition Setup** dialog, define one of the wavelengths as Transmitted Light.
7. Configure the remaining settings as usual.
8. Run the acquisition.

Tip: To increase the life of the halogen lamp, turn off the lamp when the transmitted light unit is not in use by selecting the **Transmitted Lamp Off** setting in the **Configure Illumination** dialog.

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 329.

To replace the 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.

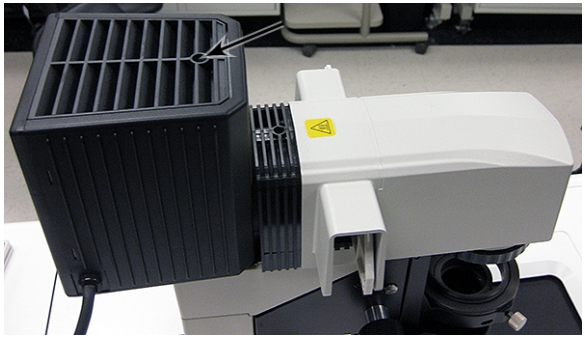


Figure 8-74: Set screw location

3. Remove cover by lifting it upward.

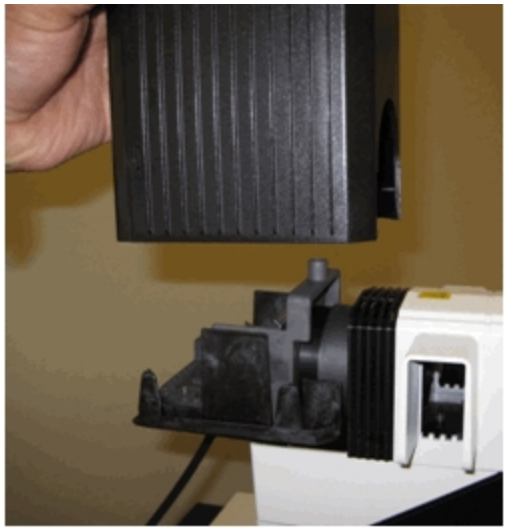


Figure 8-75: Cover removal

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

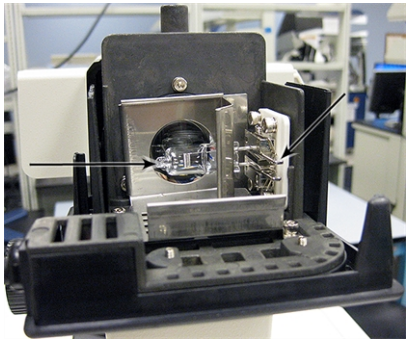


Figure 8-76: Bulb and lever

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.

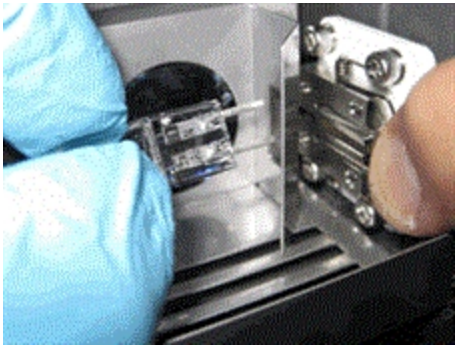


Figure 8-77: Press lever and remove bulb

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.

LED Transmitted Light Options

The ImageXpress LED Transmitted Light option is designed for brightfield imaging only. Brightfield is one of the most widely used types of microscopy illumination techniques and it is ideal for stained samples or any samples that naturally absorb a large amount of visible light.

The ImageXpress LED Transmitted Light option can be installed with the ImageXpress Environmental Control option, and the ImageXpress Fluidics option.

System Compatibility:

- Software: MetaXpress Software version 5.0 and newer
- Instrument: ImageXpress Micro System, ImageXpress Micro XL System, ImageXpress Micro XLS System, and ImageXpress Micro 4 System
- Plates and slides
- 4x to 60x objectives

The ImageXpress LED Transmitted Light option has a few limitations:

- The LED is a red light, which is not compatible with the RGB filter set for colorimetric imaging.
- Phase-contrast experiments cannot be run.
- There can be increased background during long-exposure time fluorescent acquisitions. For non-Transmitted Light assays on an instrument with the LED Transmitted Light option installed, Molecular Devices recommends using a black plate cover during imaging.

See also:

- Environmental Control Options on page 175
- Fluidics Options on page 202

Operational information is included for the following:

- LED Transmitted Light Software Configuration on page 250
- LED Transmitted Light Experiments on page 252

LED Transmitted Light Software Configuration

CAUTION! Do not use the following settings with the Transmitted Light (TL) tower hardware option. See Transmitted Light Software Configuration on page 228.

If they are missing or you if create a new hardware profile, you must create illumination settings to turn the LED transmitted light on and off.

Do the following procedure to create illumination settings named **TL** and **TL Off**. The associated journals require these specific names.

Note: Also required, a setting for each fluorescent filter cube, and for every fluorescent setting, the **Lamp, ImageXpress Micro Transmitted Light** must be selected and set to **0**. See Verifying Illumination Settings on page 45.

To configure LED transmitted light settings in the MetaXpress Software:

1. In the MetaXpress Software, click **Devices > Configure Illumination**.

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

2. In the **Configure Illumination** dialog, create an illumination setting named **TL**, select an **ImageXpress Micro Filter Cube**, select **Lamp, ImageXpress Micro Transmitted Light**, set the lamp illumination to 100, and verify that **Shutter, ImageXpress Micro Transmitted Light** is deselected (Figure 8-78).

   For the filter cube, use the Texas Red cube, or an empty slot.

   CAUTION! Molecular Devices does not reccomend using the ImageXpress LED Transmitted Light option with an alternate filter cube.

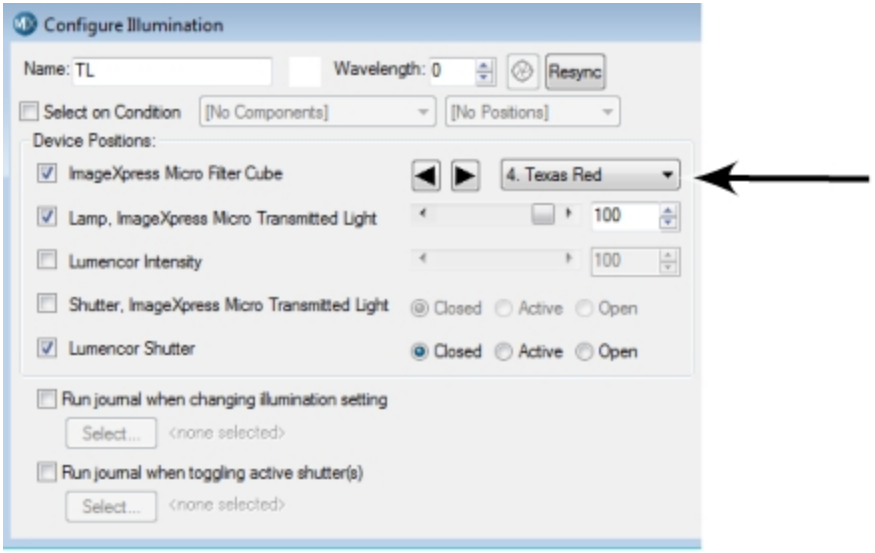


Figure 8-78: Configure Illumination dialog: LED light on

3. In the **Configure Illumination** dialog, create an illumination setting named **TL Off**, select **Lamp, ImageXpress Micro Transmitted Light**, and set the lamp illumination to zero (Figure 8-79).

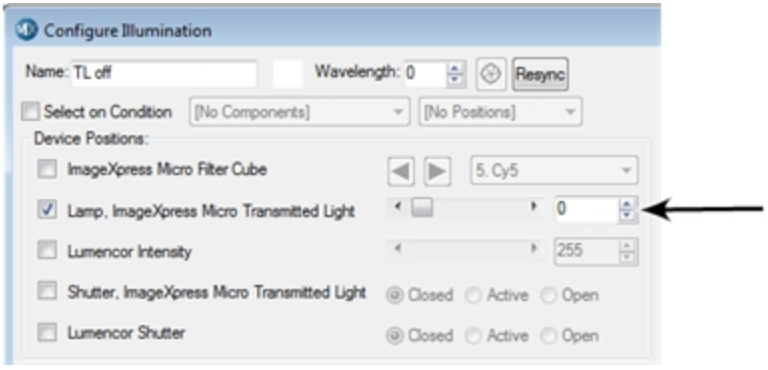


Figure 8-79: Configure Illumination dialog: LED light off

LED Transmitted Light Experiments

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

CAUTION! There can be increased background during long-exposure time fluorescent acquisitions.

To run an LED Transmitted Light experiment:

1. Load a suitable plate.

   The plate must be unlidded, have a clear lid, or have a clear plate seal on the top. Condensation on a lid or plate seal can negatively affect image quality.
2. Turn on the LED Transmitted Light by selecting the appropriate Illumination setting in the **Configure Illumination** dialog. See LED Transmitted Light Software Configuration on page 250.
3. In the MetaXpress Software, click **Screening > Plate Acquisition Setup**.

   CAUTION! On an instrument with the LED Transmitted Light option installed, for non-Transmitted Light assays, Molecular Devices recommends using a black plate cover during imaging to reduce background.
4. In the **Plate Acquisition Setup** dialog, define one of the wavelengths as **Transmitted Light**.
5. Set the appropriate **Journals**. See Using Journals During LED Transmitted Light Plate Acquisitions on page 252.
6. Configure the remaining settings.
7. Run the acquisition.

Using Journals During LED Transmitted Light Plate Acquisitions

Set up the required journal files in the **Journals** tab for use in one of the following types of experiments:

- Fluorescence and LED Transmitted Light on page 253
- Long Timelapse Experiments Using LED Transmitted Light on page 254

Fluorescence and LED Transmitted Light

To control the LED Transmitted Light during experiments that combines fluorescence with the LED Transmitted Light:

1. From the **Journals** tab, select **End of the plate**, and click the folder icon.
2. In the **Select Plate Acquisition Journal** dialog, select the *BrightfieldImaging_EndofPlate.JNL* journal file, and click **Open**.

   Includes the selection of a wavelength with **Lamp, ImageXpress Micro Transmitted Light** off to turn off the LED at the end of the plate acquisition.
3. Unless other journals are being used during acquisition, leave the **Prevent asynchronous hardware moves** option deselected (Figure 8-80).

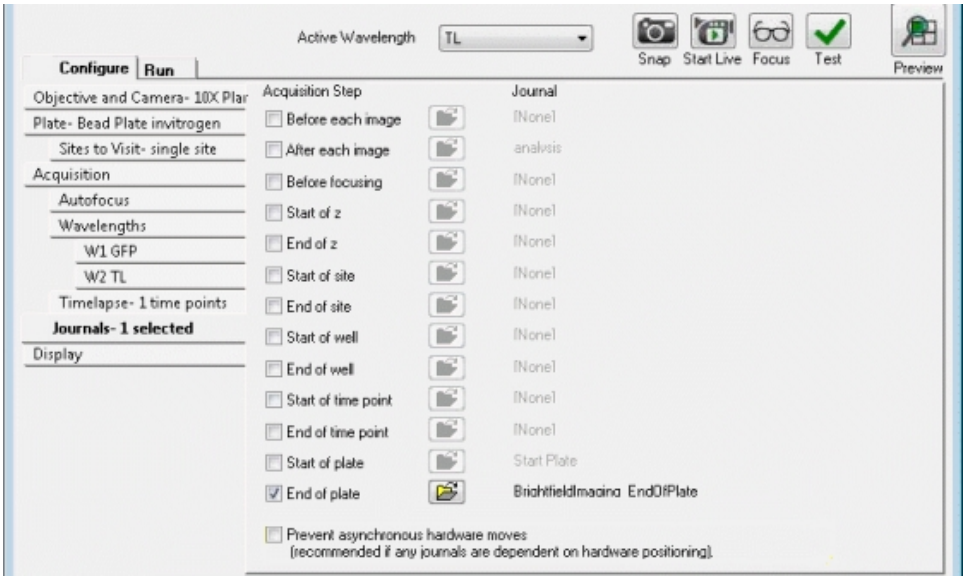


Figure 8-80: Selected acquisition steps and assigned journal for experiments using the LED Transmitted Light

Long Timelapse Experiments Using LED Transmitted Light

CAUTION! There can be increased background during long-exposure time fluorescent acquisitions.

To control the LED Transmitted Light during long timelapse experiments with or without fluorescence:

1. From the **Journals** tab, select **End of time point**, and click the folder icon.
2. In the **Select Plate Acquisition Journal** dialog, select the *Turn off TL_End of Timepoint_revB.JNL* journal file, and click **Open**.
3. Select the **Prevent asynchronous hardware moves** option.
4. Alternatively, in **Wavelengths**, configure a fluorescent wavelength as the last selected wavelength so that the LED Transmitted Light turns off in between time points.

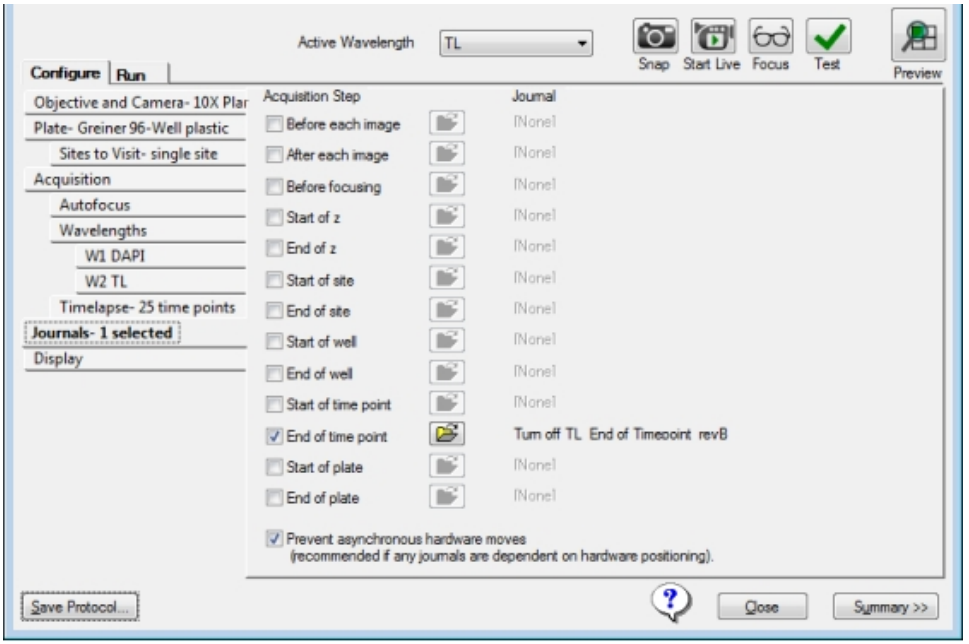


Figure 8-81: Selected acquisition steps and assigned journals for longer timelapse experiments using LED Transmitted Light

Chapter 9: Maintenance

Perform only the maintenance tasks described in this guide. Any other maintenance tasks are to be performed by qualified Molecular Devices personnel only. See Obtaining Support on page 309.

Before you operate the instrument or do maintenance operations, 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 it on a new computer, please see the *MetaXpress High-Content Image Acquisition and Analysis Software Suite Installation and Update Guide* included on the MetaXpress Software Suite installation USB flash drive, or contact Technical Support. See Obtaining Support on page 309.

Safety Precautions

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

- Some procedures require that the power supply for the instrument is turned OFF and that the power cable is unplugged before doing the service or maintenance procedure.

  If the MetaXpress Software is running, exit the program before turning off the instrument.
- Some procedures require that you disconnect the USB connection to the hardware server (host) PC, and turn off any attached peripherals, such as the robot plate-loading arm.
- Access only the user-serviceable components inside the enclosure as described in the procedure. Avoid contact with other components as they can be damaged or knocked out of alignment.

  CAUTION! Do not touch the autofocus laser.
- Do not leave the interlocked access panels open for extended periods of time.
- Ensure that all components and access panels are replaced before starting the instrument.
- To prevent dust from collecting inside the instrument, keep all access doors closed unless you are doing maintenance tasks.
- Ensure that all components and access doors are closed before starting the instrument.

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

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

Preventive Maintenance

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

- 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 error messages displayed by the software.

Power off the instrument when not in use.

Clean the Instrument

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

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

- Wipe up all spills immediately.
- To protect the ImageXpress Micro System optics and electronics, do not remove the front panels of instrument during the cleaning procedure.
- Do not use any cleaning agents other than those recommended in this procedure without first contacting Molecular Devices Technical Support. See Obtaining Support on page 309.
- Do not use ultraviolet light for sterilization, as this can damage plastic components, except as noted in Optional Expansion Solutions on page 175.
- Do not use any organic solvents.
- Do not pour or squirt water or alcohol directly onto the instrument, to prevent damaging internal components.

Cleaning the ImageXpress Micro System Instrument

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

Before cleaning the instrument, read and follow the Safety Precautions on page 255.

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

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

You can leave the lamp powered on during this procedure.

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

1. Start the MetaXpress Software and log in to the database.
2. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.

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

   In the simplified menu, click **Control > Window > Toolbars > Plate Acquisition**.
3. Exit the MetaXpress Software.
4. On the external power supply for the ImageXpress Micro System, power OFF the instrument. See Shutting Down the System.
5. Ensure that the side panels have not been removed, the filter cube access door is closed, and no sample is loaded.

6. With gloved hands, use a damp wipe to wipe down the entire outer surface including side panels and top panels of the instrument.
7. Use an alcohol wipe or a disinfectant wipe and go over the entire surface again.
8. Use forceps wrapped with damp wipes to gently wipe the perimeter of the plate holder and stage region where a plate would normally be loaded.
9. Use forceps wrapped with alcohol wipes or disinfectant wipes to go over the plate holder and stage region again.
10. Use a fresh damp wipe to wipe down the stage area underneath and around the plate loading region.

    The stage is freely moving without power, so to clean the plate holder and stage region underneath where the plate is loaded, you can slide the stage around.
11. Use a fresh alcohol wipe or a disinfectant wipe and go over the stage area underneath and around the plate loading region again.
12. Wait a few minutes for the alcohol to evaporate.
13. On the external power supply for the ImageXpress Micro System, power ON the instrument. See Powering On the Instrument.
14. Start the MetaXpress Software and log in to the database.
15. If the top door stays open after restarting the instrument, in the **Plate Acquisition** toolbar, click **Stage Load/Eject** to close the door.

Light Source Maintenance

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

- The light source for the standard model of the ImageXpress Micro System has a fuse-protected, 300 W Xenon lamp. Xenon lamps have a limited lifetime of approximately 500 hours and need to be replaced upon failure. The Xenon lamp assembly and the fuse are user-replaceable parts.
- The light source for the ImageXpress Micro XL System, the ImageXpress Micro XLS System, and the ImageXpress Micro 4 System is a solid-state source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source.

Replacing the Xenon Lamp Assembly

The light source for the standard model of the ImageXpress Micro System has a fuse-protected, 300 W Xenon lamp. Xenon lamps have a limited lifetime of approximately 500 hours and need to be replaced upon failure.

When replacing the Xenon lamp, the entire assembly must be replaced. The assembly includes the lamp and the heat sink.

If you have an ImageXpress Micro System, when changing your lamp, to prevent the new lamp assembly from overheating, clean the dust that accumulates on the fan vents using compressed air.

Instructions for changing the Xenon lamp are available in the Molecular Devices knowledge base. See www.moleculardevices.com/support.

After replacing the Xenon lamp, see Updating Shading Correction Settings-Legacy on page 304.

WARNING! BURN HAZARD. If a Xenon lamp requires replacement, let the lamp cool for at least 30 minutes. The lamp generates an extreme amount of heat and attempting to remove the lamp immediately after use can cause injury.

Replacing the Light Guide

The ImageXpress Micro System, ImageXpress Micro XL System, ImageXpress Micro XLS System, and ImageXpress Micro 4 System use a liquid light guide between the external light source and the instrument.

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

Instructions for replacing the light guide are available in the Molecular Devices knowledge base. See www.moleculardevices.com/support.

After replacing the liquid light guide, see Updating Shading Correction Settings-Legacy on page 304.

WARNING! To prevent skin burns and eye damage, do not remove the light guide from the instrument or the light source when the lamp is powered on.

Replacing the Solid-State Light Source

The light source for the ImageXpress Micro XL System, the ImageXpress Micro XLS System, and the ImageXpress Micro 4 System is a solid-state source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source.

If the solid-state light source fails, disconnect the power cord from the light source and follow the instruction on how to replace the light source. Instructions for replacing the solid-state light source are available in the Molecular Devices knowledge base. See www.moleculardevices.com/support.

Note: In the ImageXpress Micro XLS System, the external power supply for the solid-state light source provides over-current protection for the light source limited to 7.9 amps maximum. The solid-state light source and power supply contain no user-serviceable parts.

Replacing the Fuse in the Xenon Lamp Light Source

The light source for the standard model of the ImageXpress Micro System has a fuse-protected, 300 W Xenon lamp. A spare fuse ships with this light source.

If the fuse fails, disconnect the power cord from the light source and follow the instruction on how to replace the fuse. Instructions for replacing the fuse are available in the Molecular Devices knowledge base. See www.moleculardevices.com/support.

Creating a New Hardware Profile

A hardware profile is a saved system settings file that the software refers to for identifying which hardware and configurations are available and specified for use.

To create a new hardware profile:

1. Click **Start > All Programs > Meta Imaging Series**, and then right-click **Meta Imaging Series Administrator**
2. In the **Meta Imaging Series Administrator**, from the **List of Groups**, note of the name of the hardware configuration corresponding to the group that you are using

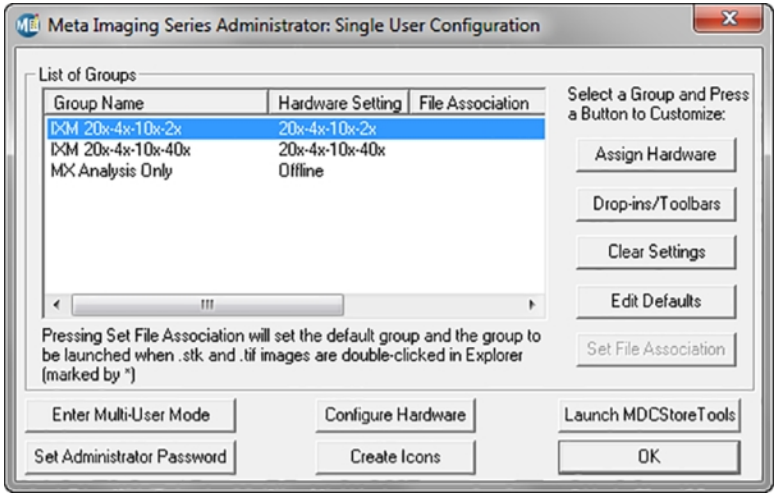


Figure 9-1: Single-user mode

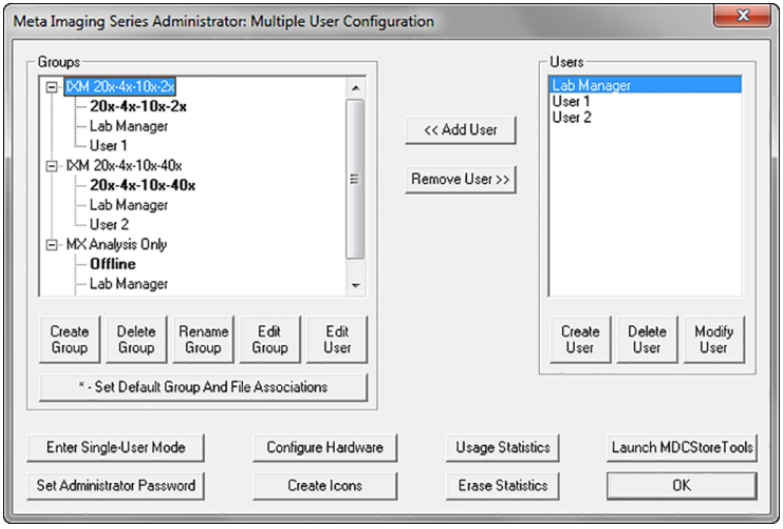


Figure 9-2: Multi-user mode

3. Click **Configure Hardware**.

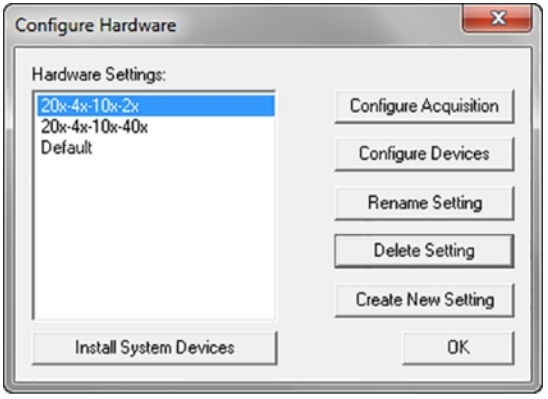


Figure 9-3: Configure Hardware dialog

4. In the **Configure Hardware** dialog, click **Create New Setting**.

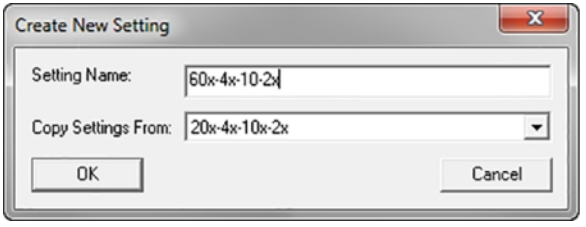


Figure 9-4: Create New Setting dialog

5. In the **Create New Setting** dialog, type a **Setting Name**.
6. In **Copy Settings From**, select your current hardware configuration, then click **OK**.

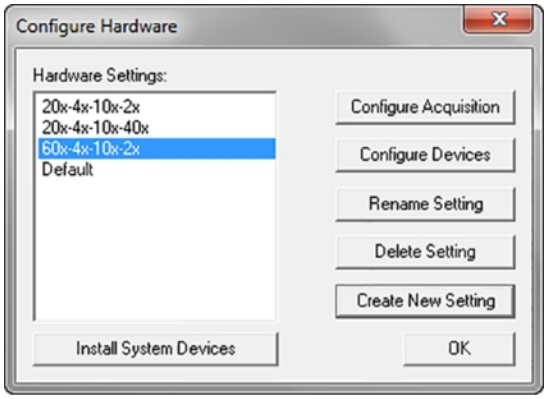


Figure 9-5: Configure Hardware dialog with new hardware setting listed

7. In **List of Hardware Settings**, select the new hardware configuration, then click **Configure Devices**.
8. Make the changes to the objectives or filters as needed. See Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 282, and see Editing the Filter Settings on page 272.
9. Click **OK**, then click **OK** again to save the changes and return to the main **Meta Imaging Series Administrator** dialog.

Making a New Hardware Profile Setting Group

To make a new group:

1. Click **Enter Multi-User Mode**, copy settings from the existing group, and make sure the new hardware configuration is selected. For User and Groups information, see Users and Groups in the Meta Imaging Series Administrator Software on page 165.
2. When you are done creating the group, if you usually use **Single-User Mode**, to prevent errors or missing settings, click **Enter Single-User Mode**.

Filter Maintenance

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

Changing or adding a filter requires the following procedures:

- Accessing Filter Cubes on page 264
- Adding Filter Cubes on page 267
- Removing Filter Cubes on page 268
- Replacing Filter Cubes on page 270
- Editing the Filter Settings on page 272
- Creating a New Hardware Profile on page 261
- Editing the Illumination Settings in the Software on page 274
- Updating Shading Correction Settings-Legacy on page 304

Accessing Filter Cubes

The filter cubes are mounted in a five-position slider within the instrument enclosure. Filter cubes are delicate components, and special care is required when handling them.

Before touching a filter cube, read and follow the Safety Precautions on page 255.

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

Use the following procedure to get to and remove the filter cube cassette to make changes.

The filter cube cassette can be accessed several ways, including:

- Accessing the Filter Cube Cassette Using the Main Taskbar on page 264
- Accessing the Filter Cube Cassette Using the Meta Imaging Series Administrator Software on page 266
- Accessing the Filter Cube Cassette From the MetaXpress Software on page 267

Accessing the Filter Cube Cassette Using the Main Taskbar

To use the Main Taskbar to access the filter cube cassette:

1. From the Main Taskbar, click **System Maintenance**.

Figure 9-6: The Main Taskbar

2. From the System Maintenance Taskbar, click **Eject Filter Cubes**.

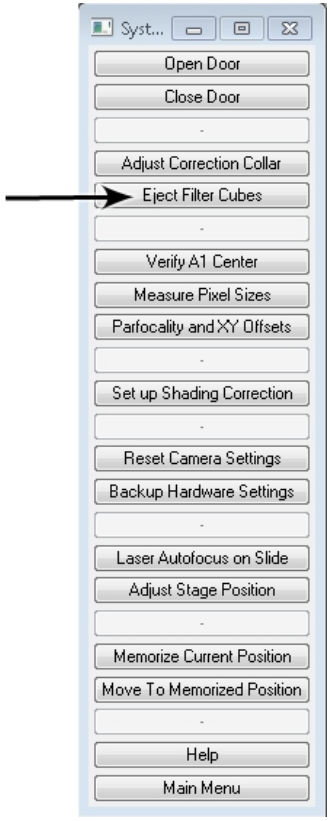


Figure 9-7: The Eject Filter Cube Button on the System Maintenance Task Bar

Accessing the Filter Cube Cassette Using the Meta Imaging Series Administrator Software

To access and remove the filter cube cassette using the Meta Imaging Series Administrator Software:

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. In the **Meta Imaging Series Administrator**, click **Configure Hardware**.
3. Click **Hardware Setting > Configure Devices**.
4. Select **ImageXpress Micro Filter Cube Changer**.
5. Click **Settings**.
6. Click **Eject Filter Cubes**.
7. Close the **Meta Imaging Series Administrator**.
8. On the external power supply of the ImageXpress Micro System, power OFF the instrument.
9. Open the filter-cube access door on the front of the instrument to access the filter cube changer.
10. Pull up on the front latch to release the filter cube cassette from the filter cube changer.

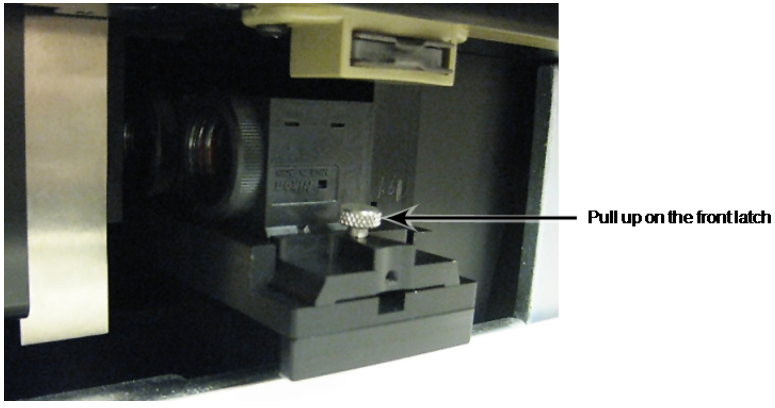


Figure 9-8: Getting to the Filter Cubes

11. Remove the filter cube cassette (Figure 9-9).

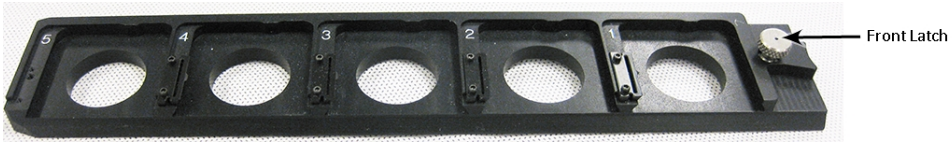


Figure 9-9: Empty Filter Cube Cassette

Accessing the Filter Cube Cassette From the MetaXpress Software

To access the filter cube cassette from the MetaXpress Software:

- Select the filter set.

  The highest wavelength is typically in position 5, like Cy5.

Adding Filter Cubes

The filter cubes are mounted in a five-position slider within the instrument enclosure. Filter cubes are delicate components, and special care is required when handling them.

Before touching a filter cube, read and follow the Safety Precautions on page 255.

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

When adding a filter cube, you are installing a new filter cube in to an empty filter cube cassette slot.

To add a filter cube:

1. Remove the filter cube cassette from the instrument. See Accessing Filter Cubes on page 264.

2. Slide a new filter cube into an empty filter cube cassette slot until it clicks into place (Figure 9-10).

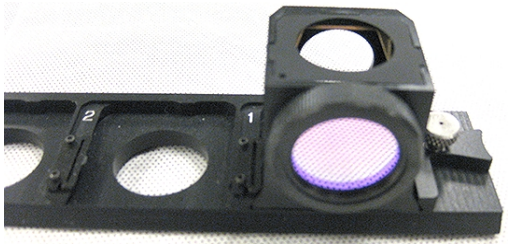


Figure 9-10: Filter Cube Added to Slot 1 of the Filter Cube Cassette

Note: For the Standard ImageXpress Micro System, the ImageXpress Micro XL System, and some ImageXpress Micro XLS Systems, use the 5/64” hex key to tighten the slot, as needed. Do not over-tighten.

3. Re-install the filter cube cassette in to the instrument.
   a. Without touching any of the filter cube lenses, carefully line up the filter cube cassette with the instrument changer track, and then push the filter cube cassette back into place until the latch engages.

      CAUTION! If you feel resistance while replacing the filter cube cassette, stop. Remove the cassette and check to make sure that it is lined up correctly with the changer track.

   b. Close the access door.
4. Update the software to correspond to the new hardware configuration. See Editing the Filter Settings on page 272 and Editing the Illumination Settings in the Software on page 274.

Removing Filter Cubes

The filter cubes are mounted in a five-position slider within the instrument enclosure. Filter cubes are delicate components, and special care is required when handling them.

Before touching a filter cube, read and follow the Safety Precautions on page 255.

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

When removing a filter cube, you are uninstalling an existing filter cube from a filter cube cassette slot.

To replace a filter cube:

1. Remove the filter cube cassette from the instrument. See Accessing Filter Cubes on page 264.
2. Slide the filter cube for removal out of the filter cube cassette slot (Figure 9-11).

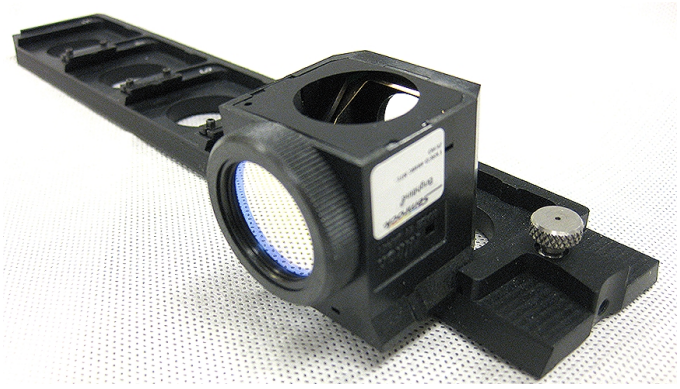


Figure 9-11: Filter Cube Removal from Slot 1 of the Filter Cube Cassette

Note: For the Standard ImageXpress Micro System, the ImageXpress Micro XL System, and some ImageXpress Micro XLS Systems, use the 5/64” hex key to loosen the slot, as needed.

3. Without touching any of the filter cube lenses, carefully line up the filter cube cassette with the instrument changer track, and then push the filter cube cassette back into place until the latch engages.

CAUTION! If you feel resistance while replacing the filter cube cassette, stop. Remove the cassette and check to make sure that it is lined up correctly with the changer track.

4. Re-install the filter cube cassette in to the instrument.
    a. Without touching any of the filter cube lenses, carefully line up the filter cube cassette with the instrument changer track, and then push the filter cube cassette back into place until the latch engages.

    CAUTION! If you feel resistance while replacing the filter cube cassette, stop. Remove the cassette and check to make sure that it is lined up correctly with the changer track.

    b. Close the access door.
5. On the external ImageXpress Systems Power and Options Controller, power ON the instrument.
6. Update the software to correspond to the new hardware configuration. See Editing the Filter Settings on page 272 and Editing the Illumination Settings in the Software on page 274.

Replacing Filter Cubes

The filter cubes are mounted in a five-position slider within the instrument enclosure. Filter cubes are delicate components, and special care is required when handling them.

Before touching a filter cube, read and follow the Safety Precautions on page 255.

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

When replacing a filter cube, you are removing an existing installed filter cube and replacing it with a new filter cube in the same filter cube cassette slot.

To replace a filter cube:

1. Remove the filter cube cassette from the instrument. See Accessing Filter Cubes on page 264.
2. Slide the filter cube to be replaced out of the cassette slot (Figure 9-12).

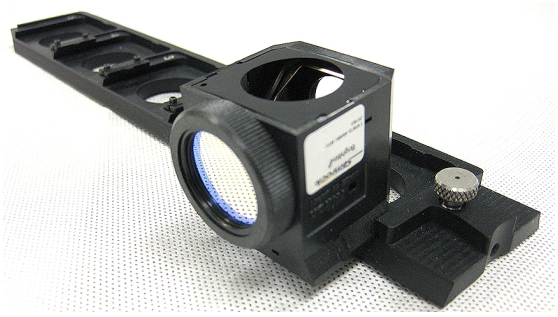


Figure 9-12: Filter Cube in Slot 1 of the Filter Cube Cassette

Note: For the Standard ImageXpress Micro System, the ImageXpress Micro XL System, and some ImageXpress Micro XLS Systems, use the 5/64” hex key to loosen the slot, as needed.

3. Slide the new cube into the vacated cassette slot until it clicks into place (Figure 9-13).

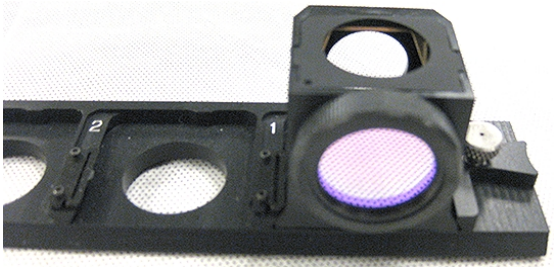


Figure 9-13: Filter Cube in Slot 1 of the Filter Cube Cassette

Note: For the Standard ImageXpress Micro System, the ImageXpress Micro XL System, and the ImageXpress Micro XLS System, use the 5/64” hex key to tighten the slot, as needed.

4. Re-install the filter cube cassette in to the instrument.
   a. Without touching any of the filter cube lenses, carefully line up the filter cube cassette with the instrument changer track, and then push the filter cube cassette back into place until the latch engages.

      CAUTION! If you feel resistance while replacing the filter cube cassette, stop. Remove the cassette and check to make sure that it is lined up correctly with the changer track.

   b. Close the access door.
5. On the external ImageXpress Systems Power and Options Controller, power ON the instrument.
6. Update the software to correspond to the new hardware configuration. See Editing the Filter Settings on page 272 and Editing the Illumination Settings in the Software on page 274.

Editing the Filter Settings

1. Click **Start > All Programs > MetaXpress Meta Imaging Series > Meta Imaging Series Administrator**.
2. Create a new hardware profile. See Creating a New Hardware Profile on page 261.
3. In the **Configure Hardware** dialog, verify the hardware settings selections in the **Hardware Settings** list, and then click **Configure Devices**.
4. In the **User Settings** dialog, select **Claimed Devices >ImageXpress Micro Filter Cube**, and then click **Settings**.

5. In the **ImageXpress Micro Filter Cube Settings** dialog, in the **Position Labels** field matching the filter cube cassette slot, edit the name of the filter cube you are changing.

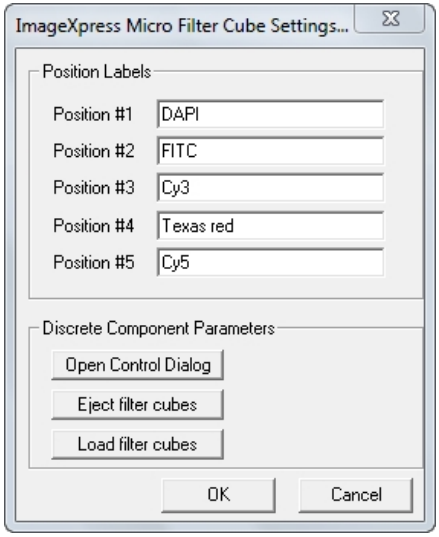


Figure 9-14: The ImageXpress Micro Filter Cube Settings dialog

6. Click **OK** to close the **User Settings** dialog.
7. Click **OK** to close the **Configure Hardware** dialog.
8. Create a new group and select the new hardware profile. See Creating an Offline Version of the MetaXpress Software on page 166.
9. Create a new group icon. See Creating MetaXpress Software Group Icons and Adding Them to the Windows Desktop on page 168.
10. Exit the Meta Imaging Series Administrator Software.
11. Start the MetaXpress Software.
12. Edit the illumination settings in the software. See Editing the Illumination Settings in the Software on page 274.

Editing the Illumination Settings in the Software

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

   In the simplified menu, click **Control > Devices > Configure Illumination**.
3. In the **Configure Illumination** dialog, ensure that the illumination device you changed is selected in the **Device Settings** field and then select the filter that you installed from the corresponding option field.

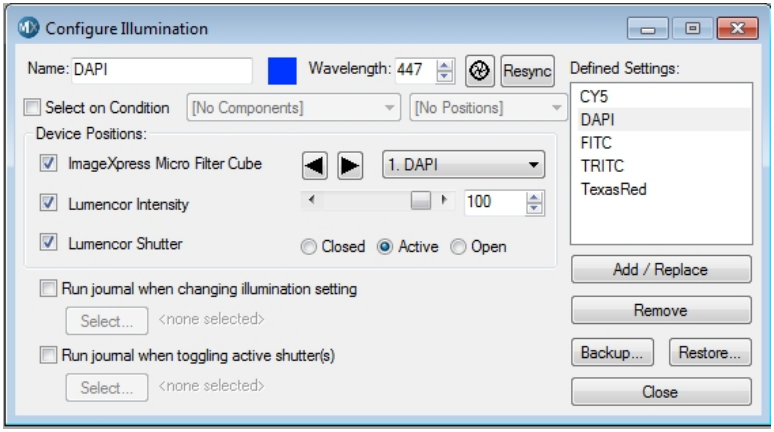


Figure 9-15: The Configure Illumination dialog

4. On the ImageXpress Micro System, select the **ImageXpress Micro Shutter** check box and then select **Active** for the shutter.

   On the ImageXpress Micro 4 System, ImageXpress Micro XLS System, and ImageXpress Micro XL System, click the **Lumencor Shutter** check box, and click **Active**. Also click the **Lumencor Intensity** checkbox, and set the value appropriately, generally to the maximum setting (**100**).

   If the system has a Transmitted Light tower, select the **Shutter, ImageXpress Micro Transmitted Light** check box and select **Closed**.

   If the system has an LED Transmitted Light option, select the **Lamp, ImageXpress Micro Transmitted Light** check box, and set the value to **0**, and then deselect the **Shutter, ImageXpress Micro Transmitted Light** check box.
5. In the **Name** field, type the name of the new filter cube.
6. In the **Wavelength** field, type the center emission wavelength of the new filter cube.
7. Click **Add/Replace** to add this setting to the **Defined Settings** list.

8. If you replaced a filter with an existing setting, select the old setting from the **Defined Settings** field and then click **Remove**.
9. After the settings are updated, click **Backup** to back up the modified settings to a file.
10. Click **Close**.

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 update the software to reflect the new hardware configuration.

Objective lenses can be cleaned while inside the instrument, or you can remove the objective lenses from the instrument for cleaning. See Cleaning an Objective on page 276.

If you are using oil-immersion objectives, see Using Oil-Immersion Objectives on page 277.

You can identify the magnification of the objectives installed inside your instrument by the following color bands:

- Yellow—10x
- Red—4x
- Green—20x
- Light blue—40x
- Dark blue—60x

Correct Objective Placement

By default Molecular Devices configures your instrument with any objective with a correction collar installed in the outer positions 1 and 4. The outer positions provide easier access through one of the side panels for adjusting the correction collar.

Tip: If you change positions of the objectives, Molecular Devices recommends installing ELWD objectives (20X, 40X, 60X) or any objective with a correction collar, in one of the outer positions (1 or 4). See Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 278.

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

If the objective you are replacing was the one used to determine the plate bottom reference point, you also must do the procedures described in Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 289, or contact Molecular Devices technical support. See Obtaining Support on page 309.

Note: Molecular Devices recommends that you leave the reference objective in place and replace only the other objectives. The reference objective is typically a 10x objective and is typically installed in position 3.

Changing or adding an objective can require the following procedures:

- Installing an Objective, see page 281
- Creating a New Hardware Profile, see page 261
- Editing the Objective Settings in the Meta Imaging Series Administrator Software, see page 282
- Configuring Parfocality after Changing Objectives, see page 284
- Determining the Plate Bottom Reference Point after Changing the Reference Objective, see page 289
- Entering the Focus Objective Values in the Meta Imaging Series Administrator Software, see page 297
- Updating Magnification Settings, see page 300
- Updating Shading Correction Settings-Legacy, see page 304

Cleaning an Objective

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

Before cleaning an objective, read and follow the Safety Precautions on page 255.

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

1. Start the MetaXpress Software and log in to the database.
2. In the MetaXpress Software on the IXM 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.

   On an old system, such as the standard ImageXpress Micro System, the ImageXpress Micro XL System, or the ImageXpress Micro XLS System, remove the left or right (as appropriate) side panel of the instrument by grasping the handle, pulling it away from the instrument, and supporting the back of the door with your other hand.
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 to blow dust contaminants off the objective.

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

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

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

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

To apply oil to the objective:

1. In the MetaXpress Software from the IXM 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 appropriate objective.
4. Insert the sample with either a thin glass coverslip, or in a microplate with a thin glass bottom, and then click **Continue**.

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

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

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

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

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

7. Slowly step up the objective manually until you are near to focus.
8. When you are done with the oil-immersion objective, 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 an Objective on page 276.

Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives

The ELWD (extra long working distance) Nikon objectives that can be supplied with the ImageXpress Micro System have adjustable correction collars, that minimize spherical aberration in the image of the specimen. The collars have a range of 0 mm to 2 mm correction, and changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very much dependent on properly setting these collars.

Note: Some other objectives (non-ELWD) can also have correction collars. The range can vary depending on the objective.

The settings to be used depend on the thickness of the microplate well or slide on which the specimen is mounted. In general, set the correction collar for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined in one of the following ways:

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

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

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

Before adjusting the correction collar on an objective, read and follow the Safety Precautions on page 255.

1. Start the MetaXpress Software and log in to the database.
2. In the MetaXpress Software on the IXM 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.

   On an old system, such as the standard ImageXpress Micro System, the ImageXpress Micro XL System, or the ImageXpress Micro XLS System, remove the left or right (as appropriate) side panel of the instrument by grasping the handle, pulling it away from the instrument, and supporting the back of the door with your other hand.
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 that you want to adjust.

   If needed, loosen or remove the objective to locate the correction collar.

   Tip: You might need to use a flashlight to view the markings for the graduated scale on the barrel and its current setting.
6. Rotate the correction collar to its new setting. If needed, replace or tighten the objective.
7. If you accessed the objective from the side, close the upper, hinged side panel door.

   On an old system, such as the standard ImageXpress Micro System, the ImageXpress Micro XL System, or the ImageXpress Micro XLS System, replace the side door by aligning the tabs at the back of the door, and then snapping the front of the door into place.
8. Click **Continue**.
9. If you accessed 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, re-adjust the correction collar by repeating this procedure.

Installing a new Objective

The objectives in the ImageXpress Micro System are mounted in a four-position linear selector. Objectives are very delicate components, and special care is required when handling them. Also see Compatible Objectives on page 319.

To determine the correct position for the objective, see Correct Objective Placement on page 275.

Before installing a new objective, read and follow the Safety Precautions on page 255.

You can leave the lamp in the external light source powered on during this procedure.

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

To add a new objective:

1. Physically install the new objective. See Installing an Objective on page 281.
2. If it is open, exit the MetaXpress Software.
3. Start the Meta Imaging Series Administrator Software.
4. Create a new hardware profile. See Creating a New Hardware Profile on page 261.
5. Create a new group and select the new hardware profile. See Creating an Offline Version of the MetaXpress Software on page 166.
6. Create a new group icon. See Creating MetaXpress Software Group Icons and Adding Them to the Windows Desktop on page 168.
7. Exit the Meta Imaging Series Administrator Software.
8. Start the MetaXpress Software using the new group icon.
9. Go to the **Configure Magnification** dialog and enter the new objective information. See Updating Magnification Settings on page 300.
10. Go to the **Calibrate Distances** dialog and verify that the pixel size calibration is set at or close to the default value for this magnification. See Calibrating Distances on page 301.
11. Configure Parfocality measurements using the **IXM Taskbar** and enter **X,Y Offset** in the **Configure Magnification** dialog.

    If the **IXM Taskbar** is unavailable, then use the bead plate to do Parfocality measurements. See Configuring Parfocality after Changing Objectives on page 284.
12. Use the **IXM Taskbar** to measure pixel sizes, then enter the results in the **Calibrate Distances** dialog. See Calibrating Distances on page 301.
13. Exit the MetaXpress Software.
14. Start the Meta Imaging Series Administrator Software.
15. 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 297.

16. If the Plate Bottom Reference needs to be measured, then enable Maintenance Mode, otherwise, exit the Meta Imaging Series Administrator Software and continue to step 17. See Finding the Plate Bottom Reference Objective and Reference Point on page 290 and Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 282.

CAUTION! Unless there is an important reason to change it after your system is installed, Molecular Devices recommends leaving the Plate Bottom Reference Objective in place at all times. Contact Technical Support for assistance.

17. Start the MetaXpress Software.Verify that the new objective is listed in the **Magnification** list. See Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 76.
18. If needed, create new shading correction files. See Updating Shading Correction Settings-Legacy on page 304.
19. Configure the Laser Autofocus settings for the new objective with the associated plates and slides, as appropriate. See Confirming Laser Autofocus Settings for Plate Files on page 55 and Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 78.

Installing an Objective

The objectives in the ImageXpress Micro System are mounted in a four-position linear selector. Objectives are very delicate components, and special care is required when handling them. Also see Compatible Objectives on page 319.

To determine the correct position for the objective, see Correct Objective Placement on page 275.

Before replacing an objective, read and follow the Safety Precautions on page 255.

You can leave the lamp in the external light source powered on during this procedure.

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

1. Start the MetaXpress Software and log in to the database.
2. In the MetaXpress Software on the IXM taskbar, click **Adjust Correction Collar** to access the specific objective.
3. Place the new objective in its protective casing on a clean work-area surface near the front of the ImageXpress Micro System.

4. If the objective is accessible from the side, open the upper, hinged side panel door of the instrument.

   On an old system, such as the standard ImageXpress Micro System, the ImageXpress Micro XL System, or the ImageXpress Micro XLS System, remove the left or right (as appropriate) side panel of the instrument by grasping the handle, pulling it away from the instrument, and supporting the back of the door with your other hand.
5. Reach in and unscrew the objective you want to remove.
6. Set the correction collar on the new objective, if needed.
7. With the objective in your hand, reach into the instrument and screw the objective into its position.
8. If you accessed the objective from the side, close the upper, hinged side panel door.

   On an old system, such as the standard ImageXpress Micro System, the ImageXpress Micro XL System, or the ImageXpress Micro XLS System, replace the side door by aligning the tabs at the back of the door, and then snapping the front of the door into place.
9. Click **Continue**.
10. If you accessed the objective from the top, then replace the plate or slide holder when prompted, and then click **Continue**.
11. Exit the MetaXpress Software.
12. Start and update the Meta Imaging Series Administrator Software to reflect the new hardware configuration. See Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 282.

Editing the Objective Settings in the Meta Imaging Series Administrator Software

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

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

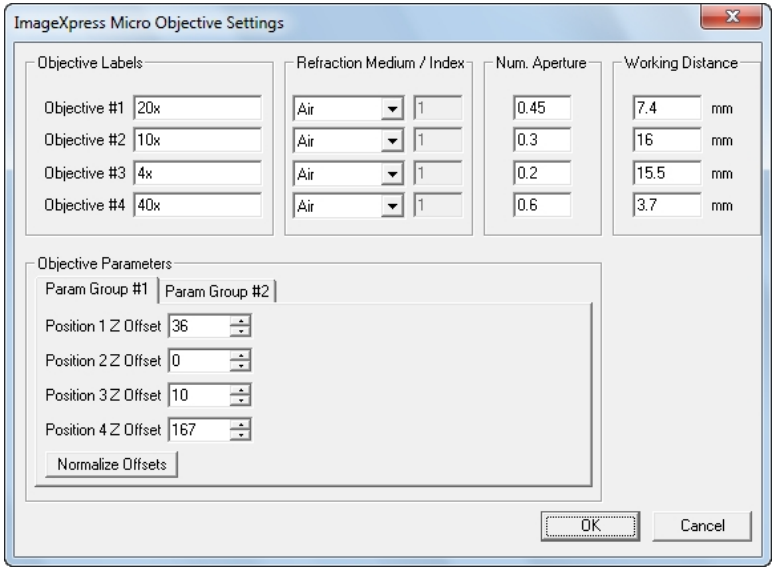


Figure 9-16: ImageXpress Micro Objective Settings Dialog

7. Edit the **Refractive Medium/Index** value, if necessary, and specify the working distance. If it has a range, enter the higher number.
8. In the corresponding **Num. Aperture** field, type the numerical aperture for the new objective as is printed on the objective, then specify the **Working Distance**. If it has a range, enter the higher number.

Note: Make a note of the values you entered in the previous steps. You will need to enter this information again for your specific hardware settings.

9. Click **OK** to close the **ImageXpress Micro Objective Settings** dialog.
10. In the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro** and then click **Settings**.
11. In the **ImageXpress Micro Settings** dialog, ensure that the **Parameter Group #1** tab is active.

    If you are doing a Plate Bottom Reference and XY reference, then also select the **Maintenance Mode** check box.
12. Click **OK** to close the **ImageXpress Micro Settings** dialog.

13. Click **OK** to close the **Install System Devices** dialog.

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

14. In the **Configure Hardware** dialog, ensure that the hardware settings you are using are selected in the **Hardware Settings** list and then click **Configure Devices**.
15. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.
16. In the **ImageXpress Micro Objective Settings** dialog, edit the text in the appropriate **Objective #** field for the new objective with the same information you previously entered.
17. Edit the **Refractive Medium/Index** value with the same information you previously entered.
18. In the corresponding **Num. Aperture** field, type the numerical aperture for the new objective with the same information you previously entered, then specify the **Working Distance**. If it has a range, enter the higher number.
19. Click **OK** to close the **ImageXpress Micro Settings** dialog.
20. Click **OK** to close the **User Settings** dialog.
21. Click **OK** to close the **Configure Hardware** dialog.
22. Close the **Meta Imaging Series Administrator**.
23. Configure parfocality. See Configuring Parfocality after Changing Objectives on page 284.

Configuring Parfocality after Changing Objectives

To configure parfocality, you must use the MetaXpress Software to find valid focus values for each objective, and then enter them into the **MetaXpress Objective** dialog in the Meta Imaging Series Administrator Software. You can configure parfocality with or without the taskbar. See Configure Parfocality With the Taskbar on page 285 and Configure Parfocality Without the Taskbar on page 286. Also see Filter Specifications and Calibration Compatibility on page 327.

Configure Parfocality With the Taskbar

To configure parfocality:

1. On the **IXM Taskbar**, click **System Maintenance**.

> **Note:** If the **Main Taskbar** is not visible, click **F4**.

Figure 9-17: IXM Main Taskbar

2. Click **Parfocality and XY Offsets** and follow the prompts in the dialog boxes that display.

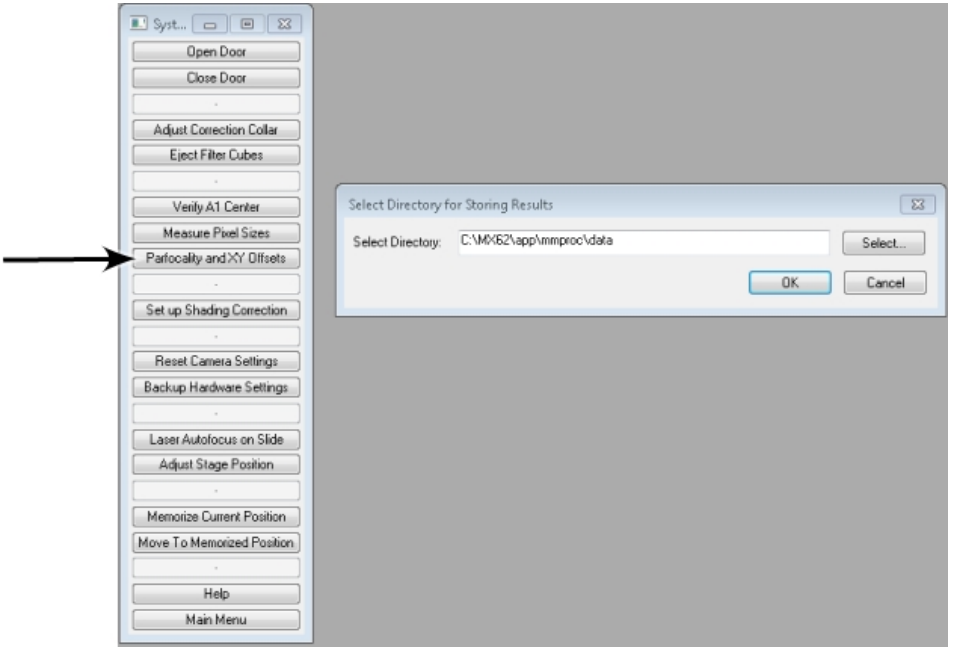


Figure 9-18: System Maintenance Taskbar

Configure Parfocality Without the Taskbar

If the taskbar is unavailable, to configure parfocality:

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

   In the simplified menu, click **Control > Devices > Configure Magnification**.
3. In the **Configure Magnification** dialog, select the **ImageXpress Micro Objective** check box.

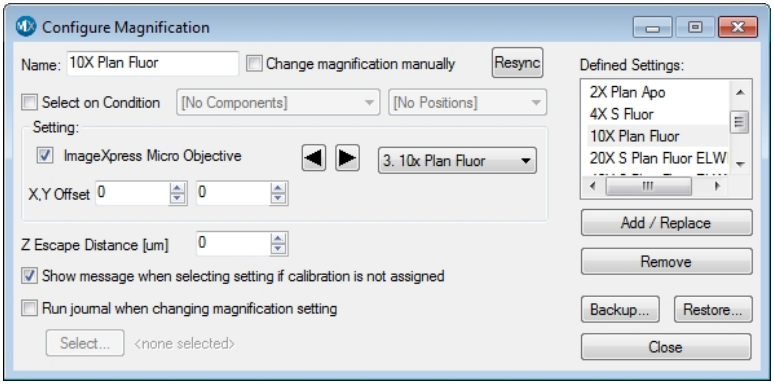


Figure 9-19: The Configure Magnification dialog

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

Figure 9-20: Illum setting in the Device Control toolbar

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

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

6. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.

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

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

7. Load the bead plate that shipped with the instrument on to the stage, and then click **Stage Load/Eject** again to return the stage to its previous position.
8. Click **Acquire > Acquire**.

   In the simplified menu, click **Control > Acquire > Acquire**.
9. In the **Acquire** dialog, click **Show Live** to open a live image window.
10. Click **Devices > Move Stage to Absolute Position**.

    In the simplified menu, click **Control > Devices > Move Stage to Absolute Position**.
11. In the **Move Stage to Absolute Position** dialog, use the **Current Position: X**, **Y**, and **Z** controls to find and focus a sample in the live image window.

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

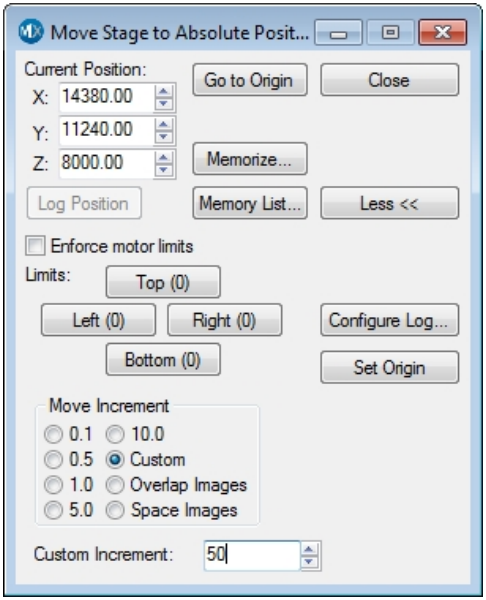


Figure 9-21: Move Stage to Absolute Position dialog

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

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

    Table 9-1: Objective Focus Values

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

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

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

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

14. In the **Move Stage to Absolute Position** dialog, use the **Current Position: Z** control to find and focus a sample in the live image window.

    Since you found the X and Y coordinates of the sample for the first objective, you need to change only the Z position for the other objectives.

15. When the sample is in focus, write down the **Current Position: Z** value.
16. After you have written down the focus values for each objective, in the **Plate Acquisition** toolbar, click **Stop Live**, then click **Stage Load/Eject** to move the stage to the load position.
17. Remove the bead plate, and then click **Stage Load/Eject** again to return the stage to its previous position.
18. Continue updating the software settings:

19.
- If the objective you are changing is the one used to determine the plate bottom reference point, see Determining the Plate Bottom Reference Point after Changing the Reference Objective on page 289.

  Molecular Devices recommends that you leave the reference objective in place and replace only the other objectives.
- If the objective was NOT the one used to determine the plate bottom reference point, exit the MetaXpress Software and see Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 297.

Determining the Plate Bottom Reference Point after Changing the Reference Objective

Note: You do NOT need to do this procedure unless you replaced the reference objective. If you did not replace the reference objective, see Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 297.

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

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

Updating the plate bottom reference point requires two separate procedures:

- Finding the Plate Bottom Reference Objective and Reference Point on page 290
- Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 296

The objective position used for this setup is specified in a line of the system calibration file **MetaXpress.ref**, located in the **Hardware** folder of your root install directory (**C:\MX6\Hardware** by default).

The line is:

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

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

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

Note: Molecular Devices recommends that you contact technical support before attempting these procedures. See Obtaining Support on page 309.

Finding the Plate Bottom Reference Objective and Reference Point

Before starting this procedure, make sure that the MetaXpress Software is in maintenance mode. The software was put in maintenance mode in Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 282.

CAUTION! The plate bottom reference objective position is applied to all hardware profiles.

To find the plate bottom reference objective and reference point:

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

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

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

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

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

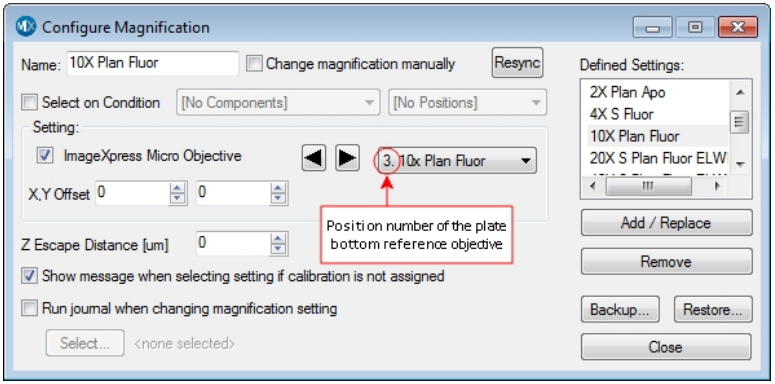


Figure 9-22: The Configure Magnification dialog

7. Write down the position number of the selected objective. You will need to refer to this value in Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 296.

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

Table 9-2: Plate Bottom Reference Objective

Plate Bottom Reference Objective Position

8. Close the **Configure Magnification** dialog.

   If a message appears prompting you to replace the stored setting, click **No**.

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

9. Click **Devices > Stage > Move Stage to Absolute Position**.

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

10. In the **Move Stage to Absolute Position** dialog, in the **Current Position: Z** field, type **6000** and then press **ENTER** to move the Z-motor.
11. In the **Current Position: X** field, type **64900**, and in the **Current Position: Y** field, type **42200**, and then press **ENTER** to move to the approximate center of the plate.
12. Click **Devices > Focus**.

    In the simplified menu, click **Control > Devices > Focus**.

13. In the **Focus** dialog, click **Configure Laser**.

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

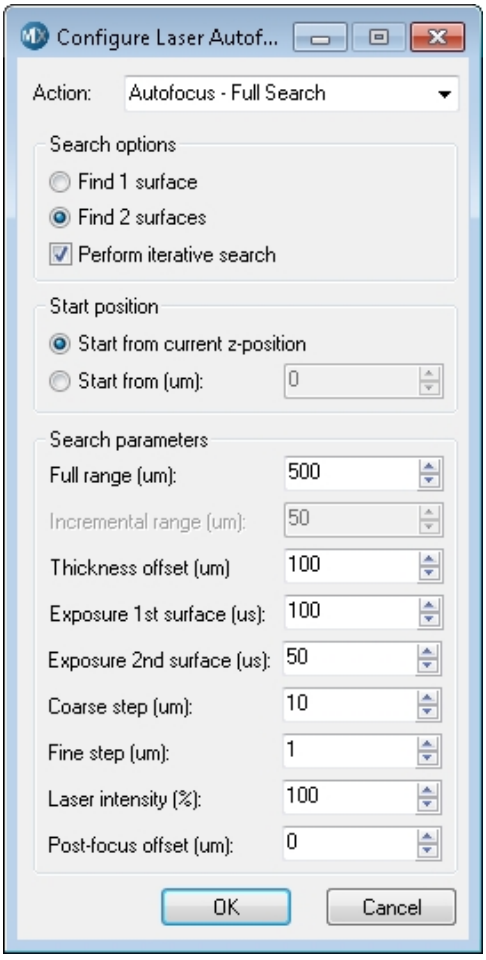


Figure 9-23: Configure Laser Autofocus dialog

15. Set the **Full range** value to **1000** microns (µm).
16. Set the **Laser intensity** value to **100**%.
17. Set the **Exposure 1st surface** value to **10** microseconds (µs).
18. Set the **Coarse step** value to **5** microns (µm).

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

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

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

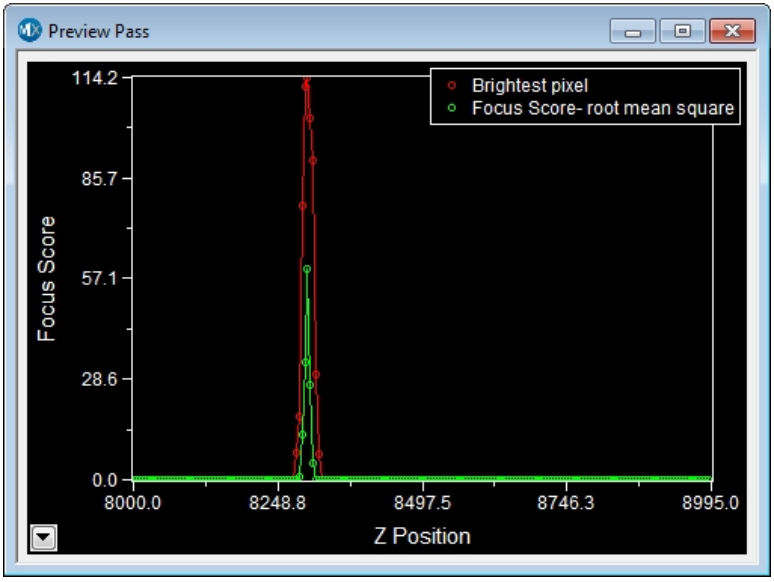


Figure 9-24: Preview Pass window

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

If there is no peak in the graph, try the following:

- In the **Focus** dialog, increase the **Start Position** value by 1000 and click **Preview Pass**. Repeat as needed until you cover a range between 6000 and 10000.
- If there still is no peak in the graph, then try moving to a new area of the FFC plate and click **Preview Pass**. If you still cannot find a peak after moving to a new area of the FFC plate twice and covering a z-range from 6000 to 10000, contact Technical Support. See Obtaining Support on page 309.

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

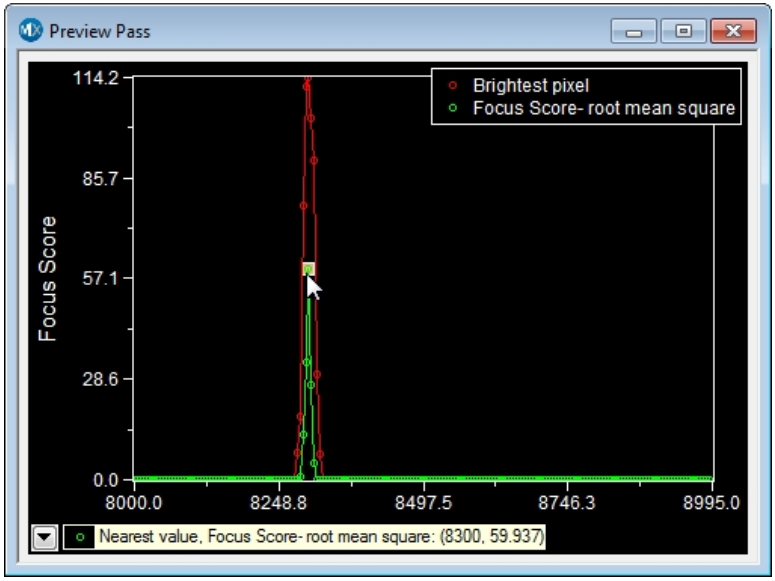


Figure 9-25: Z-position of peak

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

In Figure 9-25, the Z-position value is **8300**.

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

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

Table 9-3: 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	

22. In the **Move Stage to Absolute Position** dialog, in the **Current Position: X** field, type **8900**.

23. In the **Current Position: Y** field, type **77200** and then press **ENTER** to move to the upper-left corner of the plate.
24. In the **Focus** dialog, in the **Autofocus** tab, click **Preview Pass**.
25. In the **Preview Pass** window, click the trace line at the top of the green line of the first peak.
26. Write down the Z-position for the upper-left position.
27. Repeat **Step 21** through **Step 25** for the other three corners of the plate, using the X and Y values in Table 9-3.
28. 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 297.
29. In the **Plate Acquisition** toolbar, click **Stage Load/Eject** to move the stage to the load position.
30. Remove the flat-field correction (FFC) plate, and then click **Stage Load/Eject** again to return the stage to its previous position.
31. Exit the MetaXpress Software.
32. Edit the **MetaXpress.ref** file. See Entering the Plate Bottom Reference Objective Value in the MetaXpress.ref Configuration File on page 296.

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

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

Note: Only do this procedure if you needed to complete Finding the Plate Bottom Reference Objective and Reference Point on page 290.

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

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

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

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

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

   where **X** is the position of the objective used as the plate bottom reference objective.
3. Replace the number at the end of the line with the objective position you wrote down. See Table 9-2: Plate Bottom Reference Objective on page 291.
4. Save the modified file.

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

5. Exit Notepad.
6. Continue updating the software settings. See Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 297.

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

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

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

8. In the **ImageXpress Micro Objective Settings** dialog, in the **Position 1 Z Offset** field, type the focus value that you wrote down for the objective 1. See Table 9-1: Objective Focus Values on page 288.

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

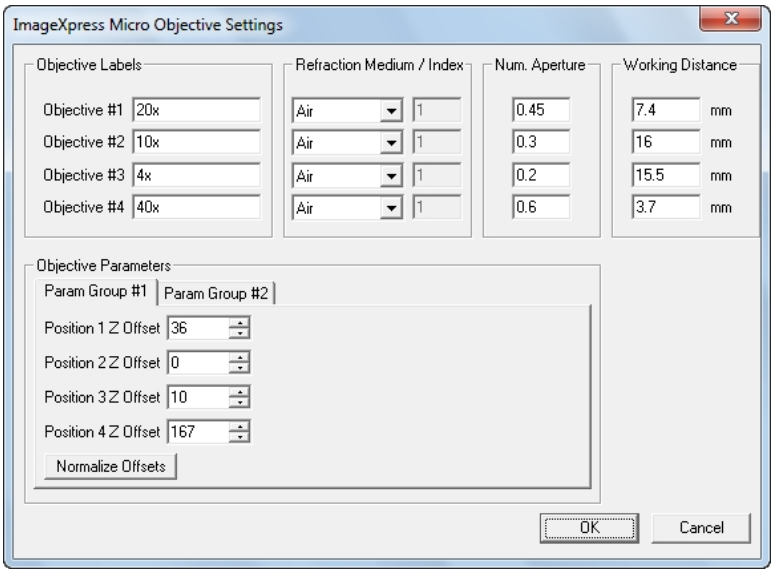


Figure 9-26: ImageXpress Micro Objective Settings Dialog

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

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

10. Click **Normalize Offsets** to calculate the offsets for each Z position.
11. Click **OK** to close the **ImageXpress Micro Objective Settings** dialog.
12. If you completed Finding the Plate Bottom Reference Objective and Reference Point on page 290, then in the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro Z** and then click **Settings**.

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

13. In the **ImageXpress Micro Z Settings** dialog, in the **Plate Bottom Reference** field, type the lowest plate bottom reference point Z-position value that you wrote down and circled. See Table 9-3: Plate Bottom Reference Points on page 294.
14. Click **OK** to close the **ImageXpress Micro Z Settings** dialog.
15. Click **OK** to close the **Install System Devices** dialog.

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

16. In the **Configure Hardware** dialog, ensure that the hardware settings you are using are selected in the **Hardware Settings** list and then click **Configure Devices**.
17. In the **User Settings** dialog, from **Claimed Devices**, select **ImageXpress Micro Objective** and then click **Settings**.
18. In the **ImageXpress Micro Objective Settings** dialog, in the **Objective Labels**, **Refraction Medium/ Index**, **Num. Aperature**, and **Working Distance** fields, type the values that you entered after you changed the objectives. See Editing the Objective Settings in the Meta Imaging Series Administrator Software on page 282.
19. In the **Position 1 Z Offset**, **Position 2 Z Offset**, **Position 3 Z Offset**, and **Position 4 Z Offset** fields, type the corresponding focus values that you wrote down for objectives 1, 2, 3, and 4. See Table 9-1: Objective Focus Values on page 288.
20. Click **Normalize Offsets** to calculate the offsets for each Z position.
21. Click **OK** to close the **ImageXpress Micro Settings** dialog.
22. If you completed Finding the Plate Bottom Reference Objective and Reference Point on page 290, then in the **Install System Devices** dialog, from **Installed Devices**, select **ImageXpress Micro Z** and then click **Settings**.

    If you did not change or replace the reference objective, then go to **Step 25**.
23. In the **ImageXpress Micro Z Settings** dialog, in the **Plate Bottom Reference** field, type the lowest plate bottom reference point Z-position value that you wrote down and circled. See Table 9-3: Plate Bottom Reference Points on page 294.
24. Click **OK** to close the **ImageXpress Micro Z Settings** dialog.
25. Click **OK** to close the **User Settings** dialog.

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

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

28. Update the settings in the MetaXpress Software. See Updating Magnification Settings on page 300.

Updating Magnification Settings

Before starting this procedure, make sure that the MetaXpress Software is NOT in maintenance mode. Maintenance mode was turned off in Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 297.

To update magnification settings:

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

   In the simplified menu, click **Control > Devices > Configure Magnification**.
3. In the **Configure Magnification** dialog, select the **ImageXpress Micro Objective** check box.

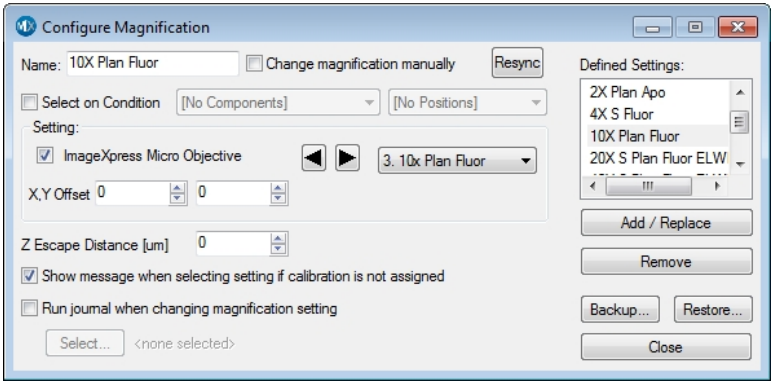


Figure 9-27: The Configure Magnification dialog

4. From the **Setting** drop-down list, select the installed objective.
5. In the **Name** field, type a name for the new objective.
6. Click **Add/Replace** to add this objective to the **Defined Settings** list.
7. If you replaced an objective with an existing setting, select the old setting from the **Defined Settings** field and then click **Remove**.
8. After the settings are updated, click **Backup**.
9. Click **Close** to close **Configure Magnification** dialog.

See Calibrating Distances on page 301 as needed.

Calibrating Distances

Before starting this procedure, make sure that the MetaXpress Software is NOT in maintenance mode. Maintenance mode was turned off in Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 297.

To calibrate distances:

1. Click **Measure > Calibrate Distances**.

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

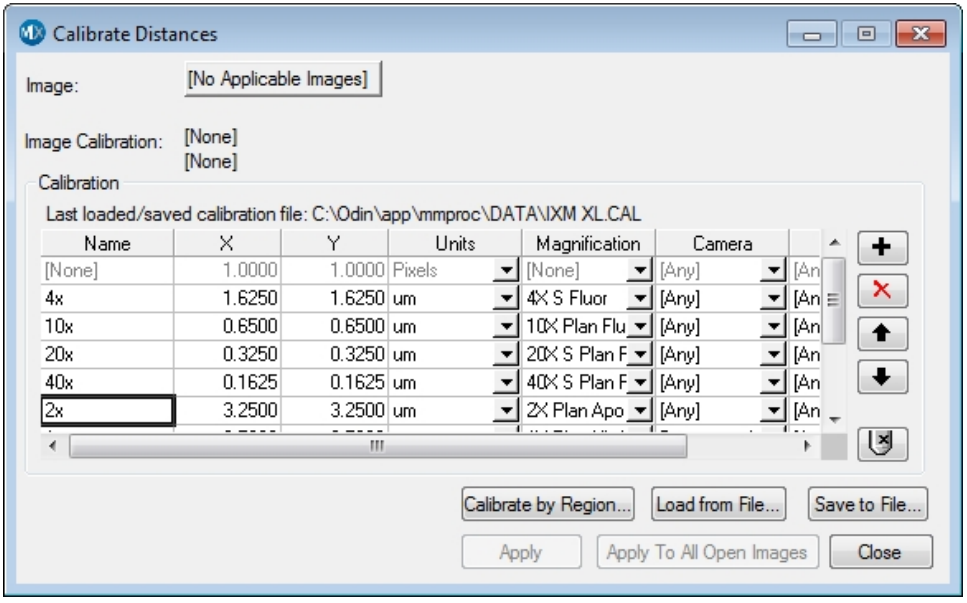


Figure 9-28: Calibrate Distances dialog

2. Click **+** to add a new **Calibration** value.

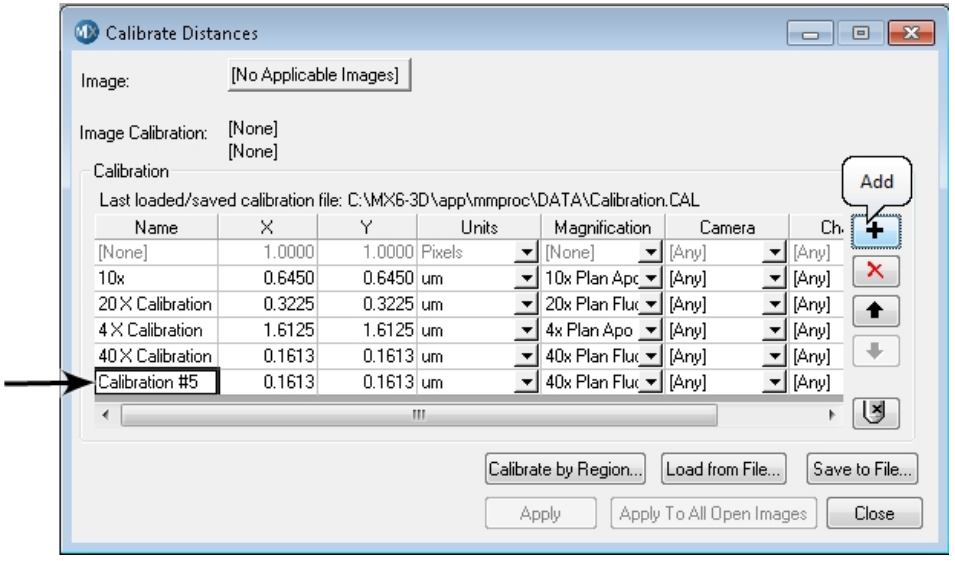


Figure 9-29: Add a new Calibration value

3. In the **Calibrations** field, type the name of your new objective and then press **ENTER**.

4. In the **X** and **Y** fields, type the calibration values for the new objective. X and Y values must match.

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

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

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

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

Table 9-4: Standard ImageXpress Micro System Estimated Calibration Settings

Objective Magnification	Estimated Calibration
1x	6.45 µm/pixel
2x	3.225 µm/pixel
4x	1.6125 µm/pixel
10x	0.645 µm/pixel
20x	0.3225 µm/pixel
40x	0.16125 µm/pixel
60x	0.1075 µm/pixel
100x	0.0645 µm/pixel

Table 9-5: ImageXpress Micro XL System, ImageXpress Micro XLS System, ImageXpress Micro 4 System Estimated Calibration Settings

Objective Magnification	Estimated Calibration
1x	6.50 µm/pixel
2x	3.25 µm/pixel
4x	1.63 µm/pixel
10x	0.65 µm/pixel
20x	0.33 µm/pixel
40x	0.16 µm/pixel

Table 9-5: ImageXpress Micro XL System, ImageXpress Micro XLS System, ImageXpress Micro 4 System Estimated Calibration Settings (continued)

Objective Magnification	Estimated Calibration
60x	0.12 µm/pixel
100x	0.065 µm/pixel

5. In the **Magnification** field, select the new objective.
6. In the **Camera** field, select a camera.
7. In the **Channel** field, select a channel.
8. After the settings are updated, click **Save to File**.
9. Click **Close**.

See Updating Magnification Settings on page 300 as needed.

Updating Shading Correction Settings-Legacy

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

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

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

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

- Editing the Filter Settings, see page 272
- Editing the Illumination Settings in the Software, see page 274

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

- Editing the Objective Settings in the Meta Imaging Series Administrator Software, see page 282
- Configuring Parfocality after Changing Objectives, see page 284
- Determining the Plate Bottom Reference Point after Changing the Reference Objective, see page 289

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

- Entering the Focus Objective Values in the Meta Imaging Series Administrator Software on page 297
- Updating Magnification Settings on page 300

Requirements

Shading Plates

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

Remove the paper backing from each plate before use. Handle the plates by the edges to avoid getting fingerprints on the imaging surface. Never use alcohol or other solvents to clean the plates. You can use compressed air to remove dust from the plates.

To determine which shading plate to use for each filter set, see Filter Specifications and Calibration Compatibility on page 327.

System Maintenance Taskbar

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

1. Start the MetaXpress Software and log in to the database.
2. Click **Journal > Taskbars > Load Taskbar**.

   In the simplified menu, click **Control > Journal > Taskbars > Load Taskbar**.
3. In the **Select a Taskbar** dialog, navigate to the **Taskbars** folder in the MetaXpress Software installation folder.

   The default installation is in **C:\MX6\Taskbars**.
4. Select the **System Maintenance Taskbar.jtb** file.

   If the **System Maintenance Taskbar.jtb** file does not exist on your computer, see Importing the Main Taskbar Journal Suite on page 305.
5. Click **Open** to load the taskbar.

After you have met all the requirements, you can update the shading correction settings. See Running the Shading Correction Journal from the Main Taskbar on page 309.

Importing the Main Taskbar Journal Suite

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

Do the following procedure only if the **Main Taskbar** is not installed on your computer. If the **Main Taskbar** is installed, see Running the Shading Correction Journal from the Main Taskbar on page 309.

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

1. Contact Technical Support to get the journal suite file **IXMTaskbar_v#.jzp**, where **#** is the current version of the software. See Obtaining Support on page 309.
2. Download the **IXM_Taskbar_v#.jzp** file to the workstation computer.
3. Start the instrument.
4. Start the MetaXpress Software and log in to the database.
5. Click **Journal > Import Journal Suite**.

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

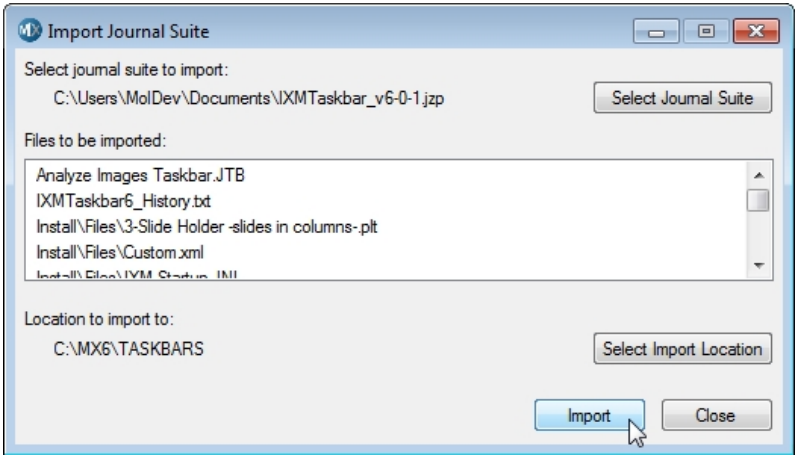


Figure 9-30: Import Journal Suite dialog

6. In the **Import Journal Suite** dialog, click **Select Journal Suite** and locate the **IXM_Taskbar_v#.jzp** file.

   The path to the journal suite file is displayed at the top of the **Import Journal Suite** dialog.

7. Click **Select Import Location** and browse to the **Taskbars** folder within the MetaXpress installation directory.

   The default installation is in **C:\MX6\Taskbars**. If it is missing, create the **Taskbars** folder.

   Tip: To determine the correct installation folder, right-click the MetaXpress Software icon on you desktop and then click **Open file location**.

8. Select the **Taskbars** folder and click **OK**.
9. In the **Import journal Suite** dialog, click **Import**.
10. Click **Close** when done.

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

11. Click **Journal > Taskbars > Load Taskbar**.

    In the simplified menu, click **Control > Journal > Taskbars > Load Taskbar**.

12. In the **Select a Taskbar** dialog, navigate to the **Taskbars** folder in the MetaXpress Software installation folder.

    The default installation is in **C:\MX6\Taskbars**.

13. Select the **Main Taskbar.JTB** file and then click **Open**.

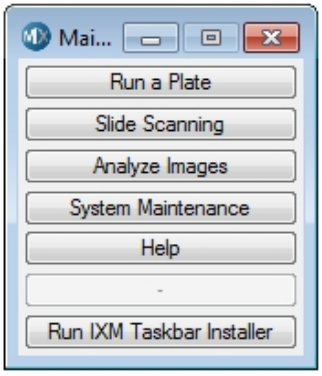


Figure 9-31: The Main Taskbar dialog

14. In the **Main Taskbar**, click **Run IXM Taskbar Installer**.

15. In the MetaXpress Directory, follow the prompts in the subsequent dialogs to confirm that the current version of the MetaXpress Software is installed in the correct directory.

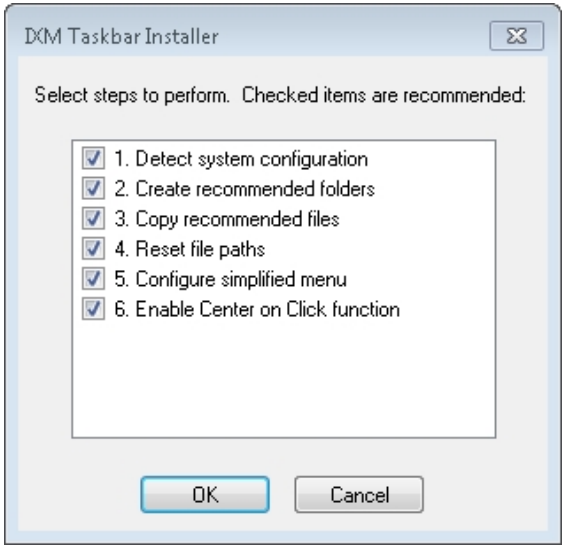


Figure 9-32: IXM Taskbar Installer

Note: The check boxes that are pre-selected in the **IXM Taskbar Installer** dialog are the recommended tasks for your configuration.

16. In the **IXM Taskbar Installer** dialog, click **OK**.
17. If the **Select Configurations** dialog appears, select the current group or **All groups**, and then click **OK**.

    Molecular Devices recommends that you select **All groups**.
18. If the **Select Users** dialog appears, select the current user or **All users**, and then click **OK**.

    Molecular Devices recommends that you select **All users**.
19. In the **Select directory for default paths** dialog, select the main MetaXpress installation directory.

    The default installation is in **C:\MX6**.
20. Click **OK**.
21. In the **Configure Menu** dialog, select to **Install** or **Uninstall** the simplified menu.

    Molecular Devices recommends using the simplified menu. See Simplified Menu Structure on page 21.
22. Click **OK**.
23. Exit and restart the MetaXpress Software.

To run the shading correction journal, see Running the Shading Correction Journal from the Main Taskbar on page 309.

Running the Shading Correction Journal from the Main Taskbar

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

Tip: To show or hide the taskbar that was most recently used, press **F4** on your keyboard.

Turning off the Status Indicator Lights

The edges of the ImageXpress Micro System illuminate with colors that provide information about the instrument status. See Status Indicator Lights.

If required, you can turn off the status indicator lights in the MetaXpress Software from the **Devices >Device Control > Other** tab, or in the simplified menu **Control > Devices >Device Control > Other** tab, and deselect the **Enable Microscope LED** check box.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support website, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you seek, follow the links to the Technical Support Service Request Form to send an email to our technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support at 800-635-5577 (North America only) or +1 408-747-1700. In Europe, call +44 (0) 118 944 8000.

To find regional support contact information, visit www.moleculardevices.com/contact.

Molecular Devices provides a wide range of support:

- Documentation: Check the guides that are included on the installation media and the Help that is available within the MetaXpress Software. Help for an active dialog can be accessed by pressing **F1** on your keyboard.

- Online Knowledge Base: The Knowledge Base has links to technical notes, software upgrades, newsletters, user guides, and other resources. Visit the Molecular Devices Support website at www.moleculardevices.com/support and follow the links to the knowledge base.
- MetaMorph Software forum: This forum has information on journal scripts and custom modules, and has links to videos and webinars that can help you troubleshoot problems and be more productive using the software. Visit the forum at metamorph.moleculardevices.com/forum.
- Technical Support:

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

  Please have the system serial number, software version number, and the name of the system owner available.

  To find your serial number:

  - In the MetaXpress Software, click **Help > About MetaXpress**. The **About** dialog displays your system ID number.
  - Newer systems have the serial number automatically displayed in the upper right corner of the computer desktop screen.
  - For all systems, the serial number is located on the back connector panel of your instrument.

Figure 9-33: Serial Number location on the back of the ImageXpress instrument

- Additional support resources include:
  - Web-based microscopy courses:
    http://www.microscopyu.com
    http://www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html
  - The Molecular Probes Handbook:
    http://www.lifetechnologies.com/us/en/home/references/molecular-probes-the-handbook.html
    This resource offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis.
  - The following sites offer filter information:
    - http://www.semrock.com
    - http://www.chroma.com
    - http://www.omegafilters.com

Gathering Support Information

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

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

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

- **Copy of the Plate Acquisition Settings file:** By default, the plate acquisition settings file is saved to the database. To save the settings to a file, go to the **Experiment** tab of the **Plate Acquisition Setup** dialog, click **Save Protocol**, and then select **Save to file** rather than to the database. The settings file is then saved to the **C:\MX6\HTSSTATE** folder by default.
- **Journal files:** If you were running a journal when the problem occurred, include copies of the journal files that you were using. By default, journal files are saved in the **C:\MX6\app\mmproc\journals** folder. If you are running journals through the review plate data tab, then the journals are saved in **c:\analysis** and **c:\assay**.
- **System Information Report:** This report contains information about many system settings and the release levels of all the.dll files in your currently installed MetaXpress Software. For information on how to create a System Information report to email to Molecular Devices, see Creating a System Information Report on page 314.

Logging AxoTrace Software Messages to a .txt File

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

To log AxoTrace messages to a .txt file:

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

   The AxoTrace Software icon appears in the Windows taskbar.

Figure 9-34: AxoTrace Software icon in the Windows taskbar

2. Exit the MetaXpress Software.
3. Double-click the AxoTrace Software icon in the Windows taskbar.
4. In the AxoTrace window, click **Options** and make sure that the **Time prefix** option is selected.

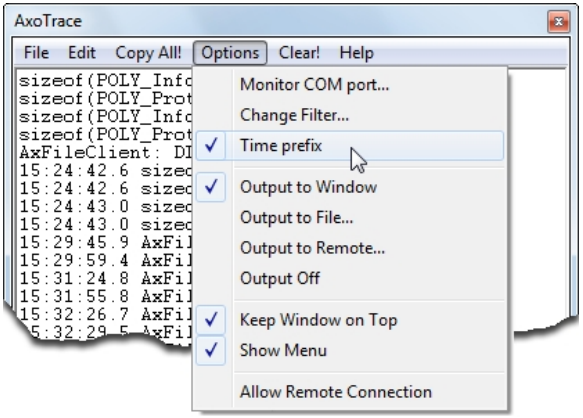


Figure 9-35: AxoTrace Software Options menu

5. Click **File > Exit** to close the AxoTrace window.
6. Start the MetaXpress Software.

7. Turn off the ImageXpress Micro System and then turn it back on.

   The light source can remain on during this step.
8. Reproduce the error, and after the error is reproduced, exit the MetaXpress Software.
9. Double-click the AxoTrace Software icon in the taskbar.
10. In the AxoTrace window, click **File > Save As**.
11. In the **Save As** dialog, in the **Name** field, navigate to the location where you want to save the log file, type the file name (for example, axotrace log.txt), and then click **Save** to save the log file.
12. Click **File > Exit** to close the AxoTrace window.
13. Send the AxoTrace Software log as an email attachment to Molecular Devices Technical Support. See Obtaining Support on page 309.

Creating a System Information Report

Much of the required system setting information can be obtained by creating a System Information Report. You can create this report from the **About MetaXpress** dialog using the following procedure.

1. In the MetaXpress Software, click **Help > About MetaXpress**.
2. In the **About MetaXpress** dialog, in the fields on the right, type your contact information.

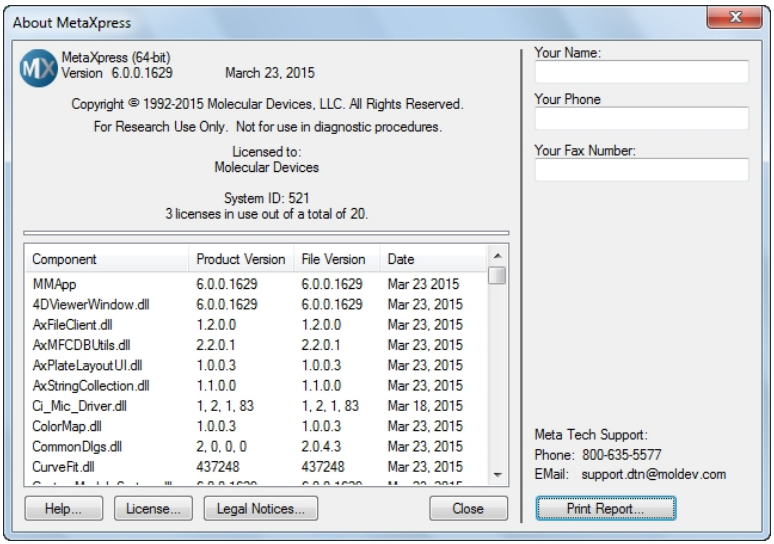


Figure 9-36: About MetaXpress Software

3. Click **Print Report**.
4. In the **Print Setup** dialog, select to print to a PDF file or to a text file and then click **OK**.
5. Send the report file to appropriate Molecular Devices support personnel as an email attachment.

Appendix A: Instrument Specifications

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	**4:** 100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum
Light source power	**4:** 220 W, 24 V, AC to DC converter included **Standard:** 100 to 240 VAC, 50/60 Hz, 360 VA, 300 W **XLS:** 100 to 240 VAC, 50/60 Hz, 288 VA, 2.5 A
Camera power supply power input	**Standard:** 100 to 240 VAC, 50/60 Hz, 240 VA, 2.0 A **XLS:** 100 to 240 VAC, 50/60 Hz, 120 VA, 1.0 A
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	**4:** 104 kg (230 lbs.) **Standard:** 82 kg (180 lbs) **XLS:** 82 kg (180 lbs)
Ambient operating temperature	15°C to 30°C
Humidity restrictions	35% to 50% non-condensing
Altitude restrictions	Not to exceed 2000 m (6562 ft)
Equipment class	1
Installation category	II
Pollution degree	2
Ingress Protection	IP20

Instrument Dimensions

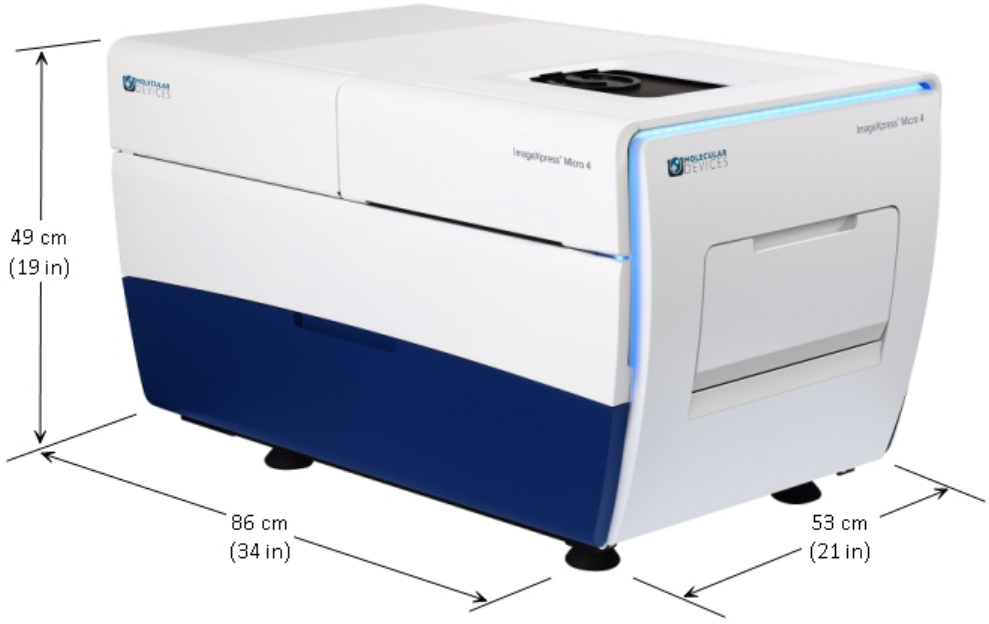


Figure A-1: Instrument Height, Width, and Length

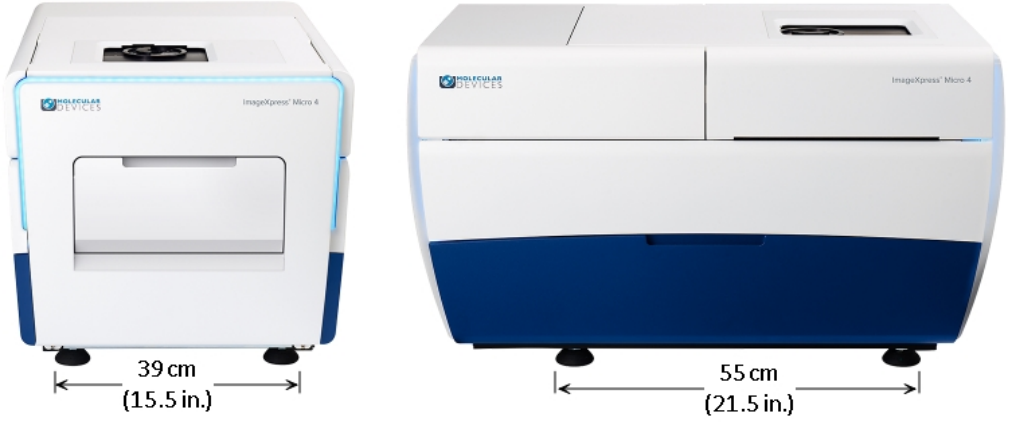


Figure A-2: Distance Between the Outside Edges of the Instrument Feet

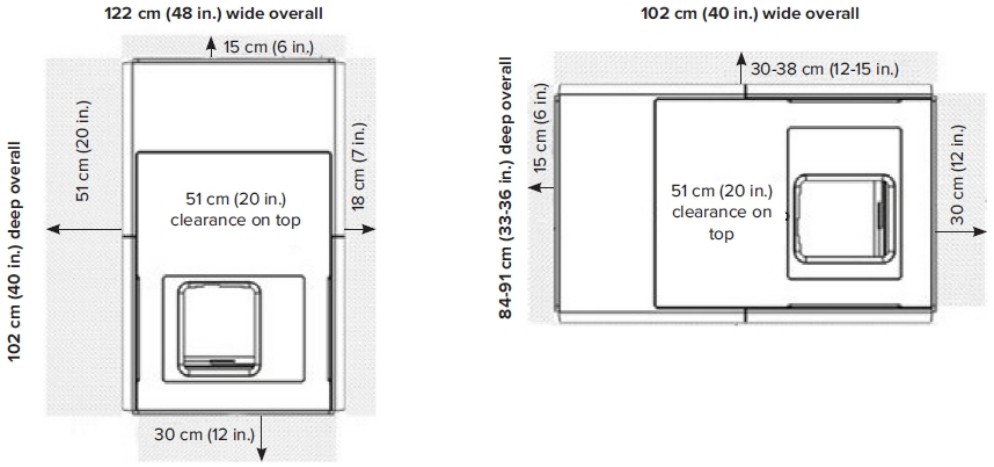


Figure A-3: Front and Sideways Installation Space Requirements

Appendix B: Compatible Objectives

B

The following table details the Nikon objectives that are compatible with the ImageXpress Micro System. It also provides microplate compatibility information for the objectives used in the system.

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro 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)

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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)
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]

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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 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)

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
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
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)

Table B-1: Nikon Objectives Compatible with the ImageXpress Micro System and Settings (continued)

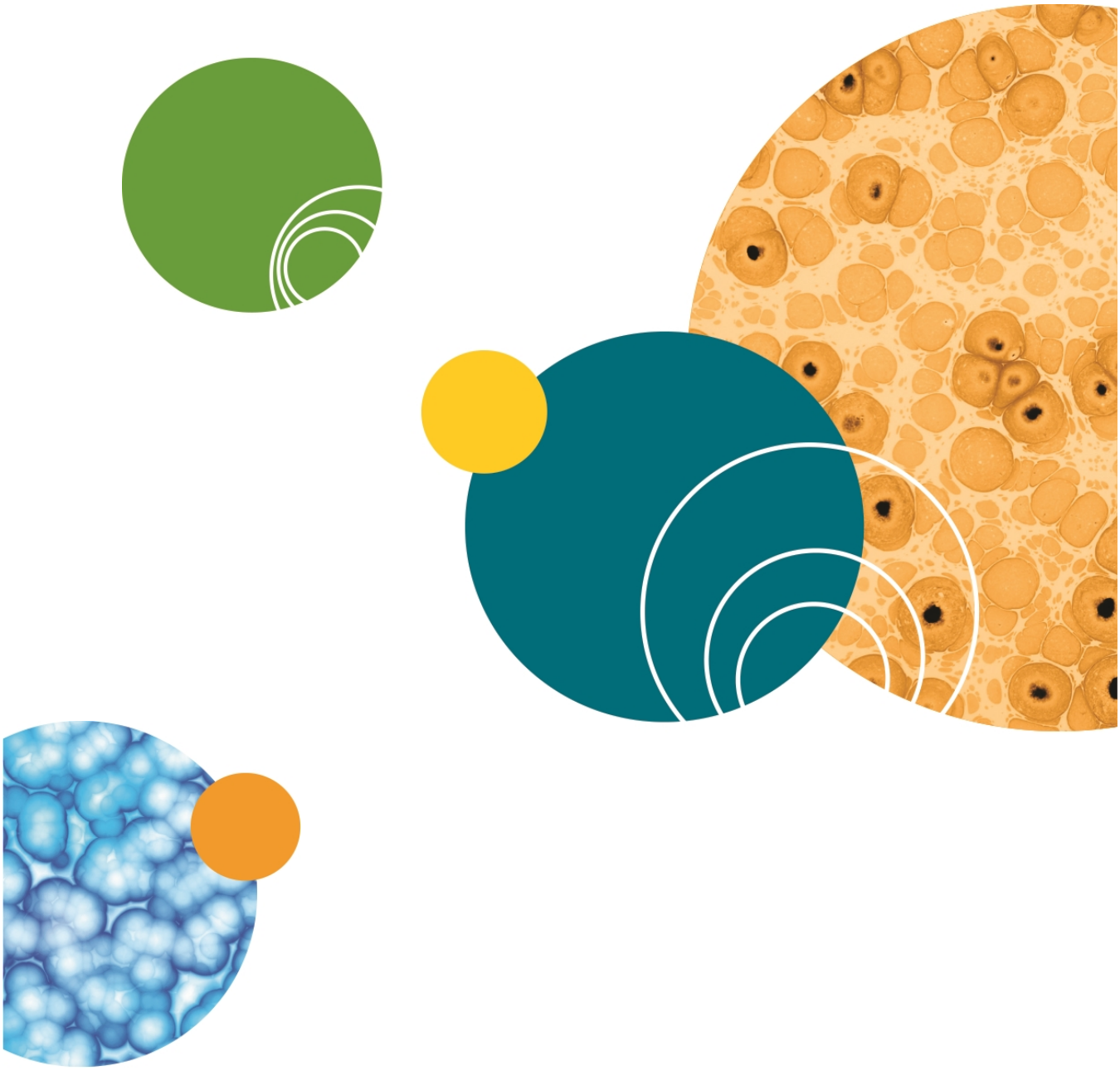
Objective Magnification and Type	Molecular Devices Part Number	Phase Contrast	Numerical Aperture (NA)	Working Distance	Plate Compatibility
60x Plan Apo Lambda	TBD	No	0.95	0.11 mm to 0.21 mm	Thin bottom (0.17 mm)[1], Thin bottom (0.17 mm) No Skirt
60x Plan Fluor	1-6300-0414	No	0.85	0.3 mm	Thin bottom (0.17 mm)[1], Thin bottom (0.17 mm) No Skirt
60x Plan Apo Oil	1.6300-0417	No	1.40	0.13 mm	Thin bottom (0.17 mm)[1], Thin bottom (0.17 mm) No Skirt
60x Plan Fluor ELWD ADL cc 0.1 mm to 1.3 mm	6500-0113	Yes, Ph2	0.70	1.8 mm to 2.62 mm	Thin bottom (0.17 mm)[3], Thin bottom (0.17 mm) No Skirt[3], Thick bottom (0.25 mm to 1 mm)
100x Plan Fluor Oil	1-6300-0418	No	1.30	0.2 mm	Thin bottom (0.17 mm)[1], Thin bottom (0.17 mm) No Skirt
100x CFI L Plan EPI cc 0 mm to 0.7 mm	1-6300-0419	No	0.85	1.2 mm to 0.85 mm	Thin bottom (0.17 mm), Thin bottom (0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)

[1]Potential interference with microplate skirt when imaging edge wells.

[2]Image degradation above microplate thickness 0.3 mm.

[3]20x and 40x ELWD image through cover slips, but other objectives give better resolution and shorter exposures.

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



Appendix C: Filter Specifications and Calibration Compatibility

Table C-1: Filter Specifications and Calibration Compatibility for the ImageXpress Micro 4 System

Filter	Wavelengths	Shading Correction Plate	Calibration Slide
DAPI	Excitation: 377/50 nm Emission: 447/60 nm Dichroic: 409 nm	Green Pink	Green (GP-8)
CFP	Excitation: 438/24 nm Emission: 483/32 nm Dichroic: 458 nm	Pink	Green (GP-8)
GFP	Excitation: 472/30 nm Emission: 520/35 nm Dichroic: 495 nm	Pink Red	Yellow (GP-11)
FITC	Excitation: 474/27 nm Emission: 525/45 nm Dichroic: 495 nm	Pink Red	Yellow (GP-11)
YFP	Excitation: 500/24 nm Emission: 542/27 nm Dichroic: 520 nm	Pink Red	Yellow (GP-11)

Table C-1: Filter Specifications and Calibration Compatibility for the ImageXpress Micro 4 System (continued)

Filter	Wavelengths	Shading Correction Plate	Calibration Slide
TRITC	Excitation: 543/22 nm Emission: 593/40 nm Dichroic: 562 nm	Red	Yellow (GP-11) Red (GP-7)
Cy3	Excitation: 531/40 nm Emission: 593/40 nm Dichroic: 562 nm	Green	Yellow (GP-11) Red (GP-7)
Texas Red	Excitation: 562/40 nm Emission: 624/40 nm Dichroic: 593 nm	Red	Yellow (GP-11) Red (GP-7)
Cy5	Excitation: 628/40 nm Emission: 692/40 nm Dichroic: 660 nm	Red	Blue (GP-9) Red (GP-7)
Fura-2 340x (must be installed adjacent to Fura-2 387x)	Excitation: 340/26 nm Emission: 510/84 nm Dichroic: 409 nm	Pink	Green (GP-8)
Fura-2 387x (must be installed adjacent to Fura-2 340x)	Excitation: 387/11 nm Emission: 510/84 nm Dichroic: 409 nm	Pink	Green (GP-8)
Note: Fura filters are not compatible with a solid-state light source.			

Appendix D: Replacement Parts and Optional Extras

For an up-to-date list of replacement parts and optional extras, see the website at: www.moleculardevices.com

Table D-1: Replacement Parts and Optional Extras for the ImageXpress Micro System (Standard), ImageXpress Micro XLS System, and the ImageXpress Micro 4 System

Part Number	Description
—	For a List of Compatible Nikon Objectives see Compatible Objectives on page 319
5016604	Digital Confocal Option in MetaXpress Software
5025771	RGB Filter Set
1-6300-0410	FURA-2 340X/387X Filter Set for Standard ImageXpress Micro System and ImageXpress Micro XLS System with custom light source (Sutter XL)
1-6300-0442	DAPI Filter Set
5045173	FITC Filter Set
1-6300-0444	TRITC Filter Set
1-6300-0445	Cy3 Filter Set
1-6300-0446	Cy5 Filter Set
1-6300-0447	CFP Filter Set
1-6300-0448	YFP Filter Set
1-6300-0449	Texas Red Filter Set
1-6300-0450	GFP Filter Set
6540-0089	Custom Single Excitation Filter Set Mounted in Nikon TE2000 Cube
6540-0096	Custom Dual Excitation Filter Set Mounted in Nikon TE2000 Cube
6540-0097	Custom Triple Excitation Filter Set Mounted in Nikon TE2000 Cube
6540-0098	Custom Quad Excitation Filter Set Mounted in Nikon TE2000 Cube
1-GP-2	GP-2 Spatial Calibration Slide
5048412	Accessory Tool Kit

Table D-1: Replacement Parts and Optional Extras for the ImageXpress Micro System (Standard), ImageXpress Micro XLS System, and the ImageXpress Micro 4 System (continued)

Part Number	Description
5033647	Replacement Solid-State Light Source
5015677	Replacement Liquid Light Guide for ImageXpress Micro XL System
5016706	5 mm Replacement Solid-State Light Source for ImageXpress Micro XLS System
5015677	Replacement Liquid Light Guide for ImageXpress Micro XLS System
1-3335-0007	Replacement Xenon Lamp, 300 Watt with heatsink for ImageXpress Micro System
1-6300-0386	Replacement Liquid Light Guide for ImageXpress Micro System
1-S2300-1038	ELMCH, Shutter/Light Baffle, ITD for Standard ImageXpress Micro System
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
E1005	Breathe Seal for Cell & Tissue Culture, sterile, packed in tamper proof 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, 8cc, MXA20233
1-2600-0865	Replacement live cell plate sealing ring
5000284	Robot-friendly Three Slide Holder for Imaging

Appendix E: File Privileges for the MetaXpress Software

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

Note: The following information assumes that the MetaXpress Software has been installed to the default path of **C:\MX6**.

Note: There are no restrictions as to where the software is installed. Nothing prevents installing the software to the path of **C:\Program Files\MX6**.

Note: When your system has multiple configurations and multiple groups not named **MetaXpress**, substitute the appropriate group name for the default group name **MetaXpress** as needed in the following list of folder paths.

Software Administrator

Read/write access is required for everything under the **C:\MX6** tree. The Software Administrator also needs to create and periodically modify shading correction images in the **C:\Shading Images** folder. Because this is a possible security violation, this operation can be accomplished by the System Administrator so that the Software Administrator is not given write access to the root directory.

Standard Users

Read Only access needed:

- C:\MX6
- C:\MX6\app\mmproc
- C:\MX6\app\mmproc\Dropins
- C:\MX6\Help - all subdirectories -
- C:\MX6\Groups\

If the system is set up for multiple users:

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

If the system is an acquisition computer:

- C:\MX6\Hardware\
- C:\MX6\Hardware\ - all subdirectories -
- C:\MX6\Plates\
- C:\MX6\Vinput\ - all subdirectories -

Read/Write and Modify Access needed:

- C:\Analysis
- C:\Assay
- C:\Backup

CAUTION! Do not delete or rename the *Backup* folder from your system computer. At the start of every plate acquisition, the MetaXpress Software archives the current configuration and instrument settings to a date-stamped .zip file in the C:\Backup\ folder. If your software configuration or instrument settings get corrupted, you can restore the settings using the backup archives. Contact Molecular Devices Technical Support for assistance.

- C:\Shading Images

If the system is set up for a single user:

- C:\MX6\Groups*MetaXpress*

Note: Or appropriate group name, not necessarily called *MetaXpress*.

If the system is set up for multiple users:

- 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 to become familiar with. 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.

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

A robotic plate handler is easily integrated with the ImageXpress System. The following is the Robot step-by-step protocol:

1. Fetch a microplate from a hotel shelf.
2. Scan the microplate barcode.

   Note: Molecular Devices recommends using barcodes, though they are optional.

3. Load the microplate onto the ImageXpress System.
4. Acquire images.
5. Unload the microplate from the ImageXpress System.
6. Return the microplate to the original hotel shelf.

Verifying External Control Settings

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

Note: The **External Control** module must be purchased and activated on the MetaXpress Software key. If it is not activated, try updating the MetaXpress Software. If it is still not activated, contact your Molecular Devices representative.

To confirm that the **External Control** settings in the Meta Imaging Series Administrator Software are enabled:

1. Click **Start > All Programs > MetaXpress > Meta Imaging Series Administrator**.
2. Click **Configure Hardware**.
3. In the **Configure Hardware** dialog, click **Install System Devices**.

4. In the **Install Systems Devices** dialog, ensure that **External Control** is listed in the **Installed Devices** list.

   If it is not listed, select it from the **Available Hardware** list and click **Install>>**.
5. From the **Installed Devices** list, select **External Control** and click **Settings**.
6. In the **External Control Settings** dialog, click the **Connections Settings** tab.
7. Verify that the correct COM port is selected.
8. Verify that the option **Operate device in emulation mode** is deselected.
9. Verify that **Control** is listed in the **Included components** list.

   If it is not listed, select it from the **Available Hardware** list and click **Install>>**.
10. Click **OK** to return to the **Install System Devices** dialog, then click **Apply** and **OK** to return to the **Configure Hardware** dialog.
11. Select an appropriate hardware configuration from the **Hardware Settings** list and click **Configure Devices**.
12. In the **User Settings** dialog, ensure that **External Control** is listed in the **Claimed Devices** list.

    If it is not listed, select it from the **Available Hardware** list and click **Install>>**.
13. Click **Apply** and **OK** to return to the **Configure Hardware** dialog
14. Repeat steps 11-13 for each hardware configuration that needs the **External Control**.
15. Click **OK** and **OK** to exit the Meta Imaging Series Administrator Software.
16. Start the MetaXpress Software, and select the **Screening** menu to verify that the **External Control** option is available.

Appendix G: Electromagnetic Compatibility

G

Regulatory for Canada (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

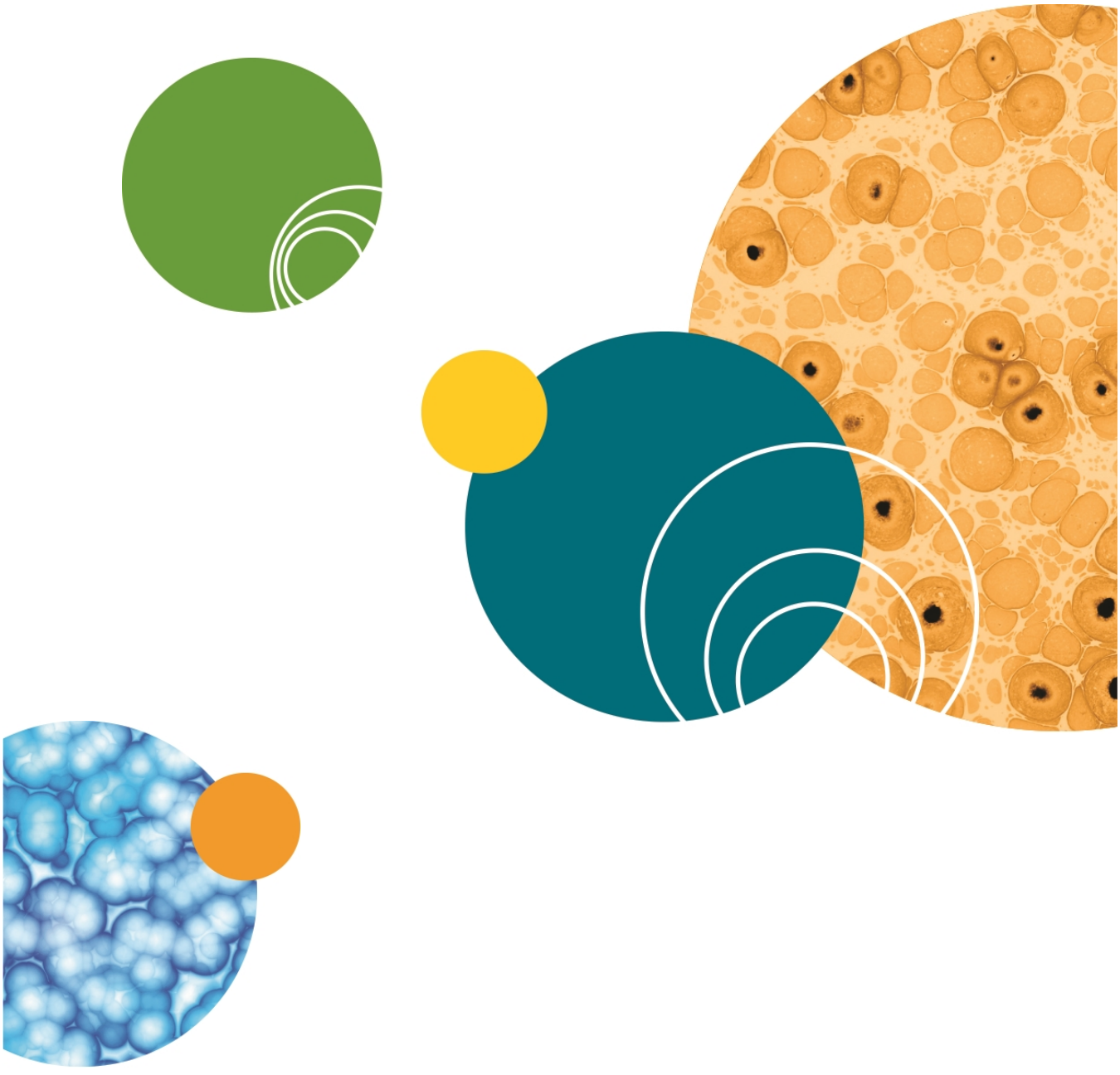
This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power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

Information to the User (FCC Notice)

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

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

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



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