ImageXpress® Nano

Automated Imaging System

With MetaXpress Software

User Guide

MOLECULAR DEVICES

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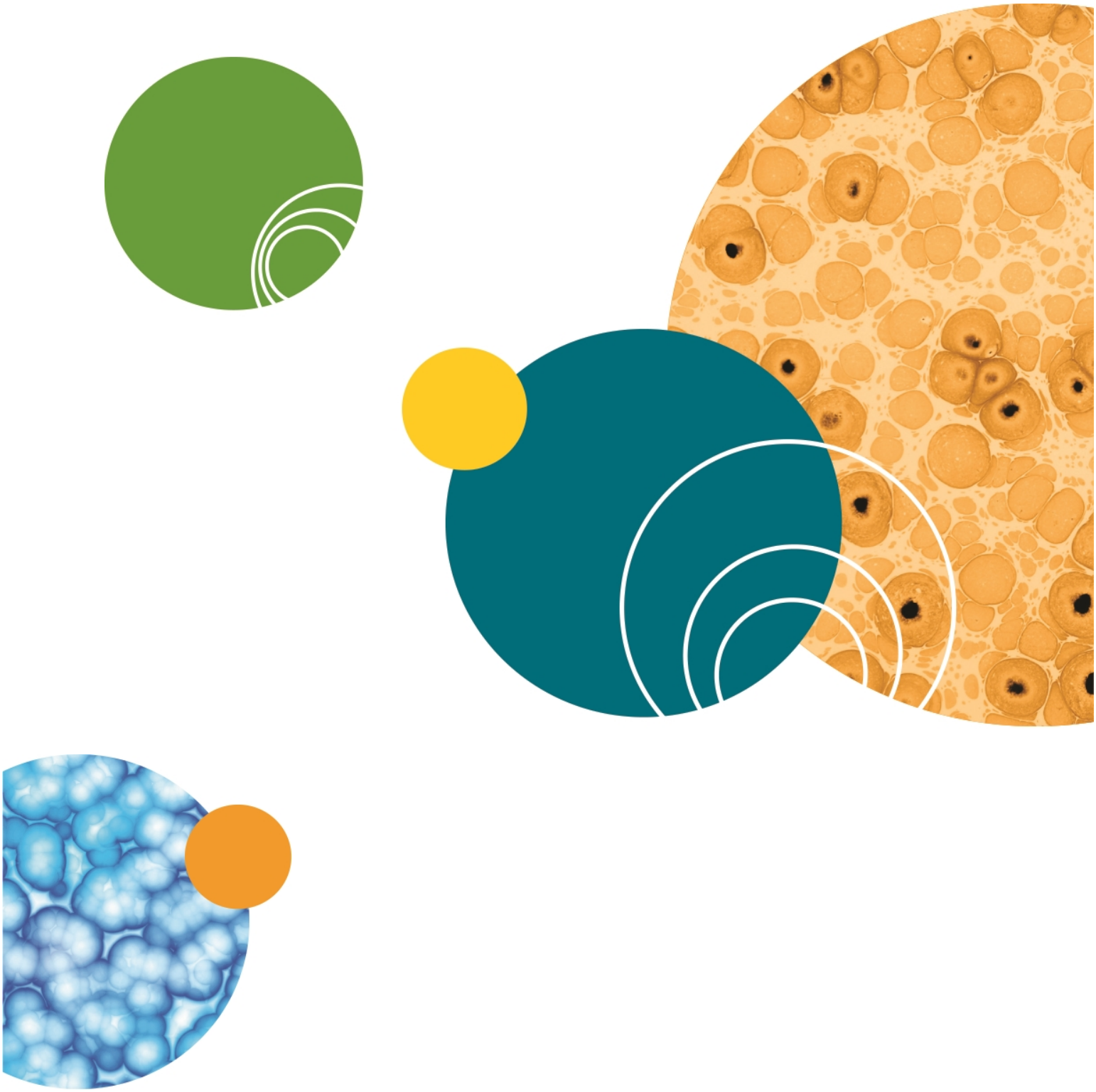
Product manufactured by Molecular Devices, LLC.
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Contents



Safety 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 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 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 149.

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 10.

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.

Table S-2: Embedded Laser Module Specifications

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

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. Operate the instrument only when all the doors and panels of the instrument are in place and closed.

Light Source Safety

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

The ImageXpress Nano System light source 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.

Electrical Safety

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

Molecular Devices recommends 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.

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.

Fuses and Circuit Protection

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

Moving Parts Safety

The instrument contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts.

To prevent injury:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.

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

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 11 for details.

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only.

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 141.

Chapter 1: Introduction to the ImageXpress Nano System

1

The ImageXpress® Nano Automated 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 and uses transmitted light capability for label-free imaging.

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

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

Key components of the instrument include the following:

- External solid-state white light source connected by a liquid light guide
- A CMOS camera
- Laser autofocus system with precision motorized Z (focus) stage
- Image-based autofocus
- Precision motorized X-Y (sample) stage
- High-quality, Nikon objectives in a four-position linear selector
- Filter cubes in a five-position slider
- Motorized selection of stage position, and objectives with asynchronous operation
- High-transmission fluorescence imaging optics with chromatic aberration correction, resolution, and image flatness
- Operation and configuration control by the integrated MetaXpress Software
- Optional expansion solutions available for environmental control and transmitted light

ImageXpress Nano System Instrument Features

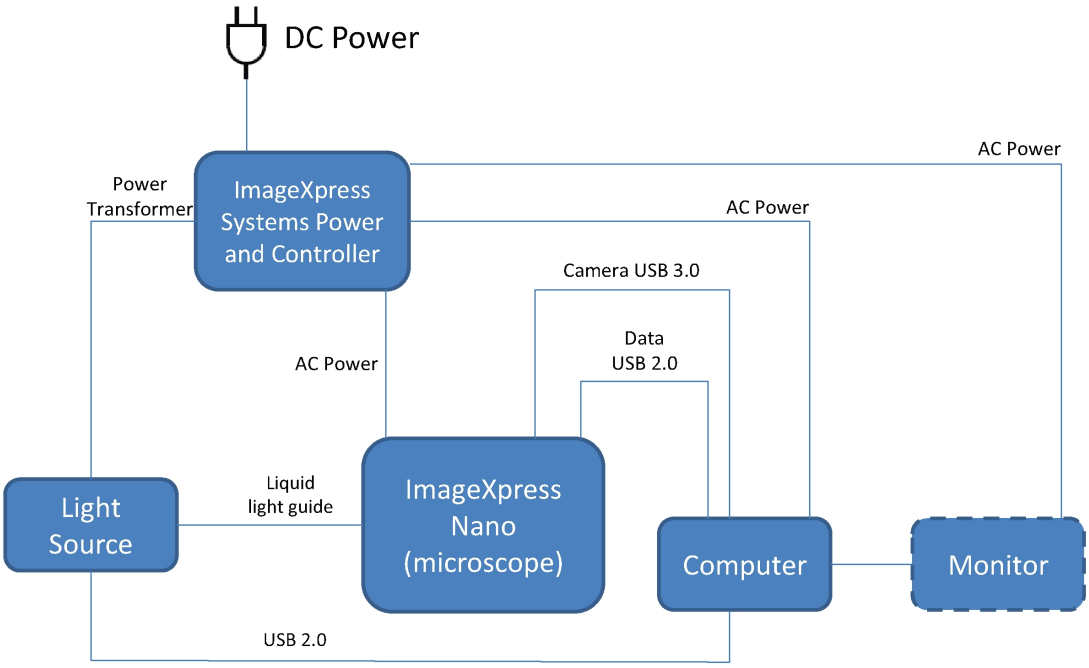


Figure 1-1: ImageXpress Nano System Components Without Options Using MetaXpress® Software

Illumination System: Excitation

Light Source

The ImageXpress Nano System light source 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.

Note: The ImageXpress Nano System solid-state light source is limited to an excitation spectrum ranging 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).

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. Molecular Devices offers a wide range of objectives to suit your experimental needs. See Compatible Objectives, see page 159.

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.

Electronics

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

- External ImageXpress Systems Power and Options Controller and cables
- External solid state light source, fiber, and cables

MetaXpress Software Features

Use the MetaXpress Software with the ImageXpress Nano 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 43 and Configuring Plate Acquisition Options on page 49.
- 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 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

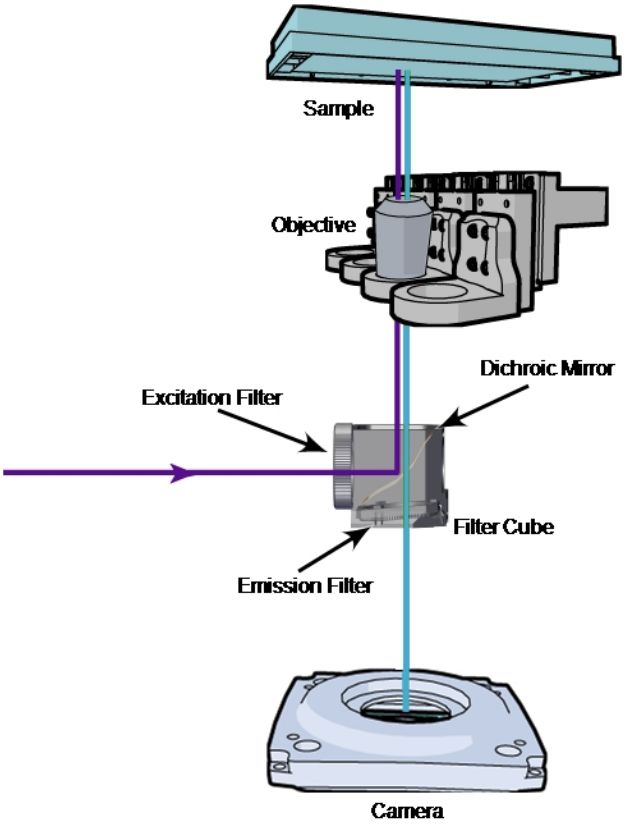


Figure 1-2: ImageXpress Nano System Optical Path

The ImageXpress Nano System uses the following components and functions:

- Fluorescence Imaging, see page 19
- Excitation and Emission Filters, see page 20
- Objective Lenses, see page 21

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

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

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

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

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

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.

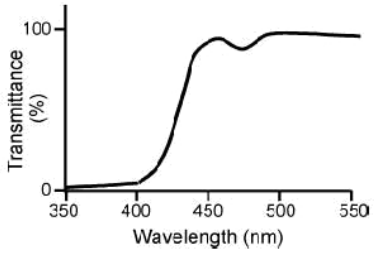


Figure 1-3: Example of a transmission spectrum of a dichroic mirror

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 Nano System can be configured at the time of purchase with up to 4 of the high quality Nikon objectives listed in Compatible Objectives on page 159.

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.

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. 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 Nano System

2

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

Starting the System

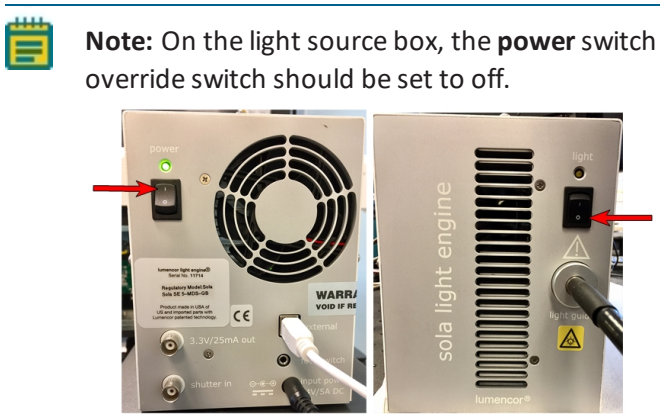
The following procedures explain how to safely power on the instrument and computer and how to start and log in to the MetaXpress Software.

Powering On the Instrument

Before starting the software, you must power on the instrument properly using the following procedure.

To power on the ImageXpress Nano System:

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.



Note: On the light source box, the **power** switch should be set to on and the **light** override switch should be set to off.

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.

Starting the Software

This procedure assumes that your ImageXpress Nano 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 Nano 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**.

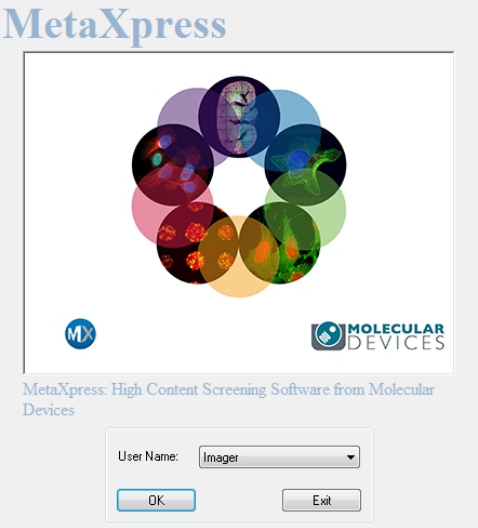


Figure 2-1: MetaXpress Software login dialog

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

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

The MetaXpress Software starts and initializes the various components of the ImageXpress Nano 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 151.

Status Indicator Lights

The status light on the front of the ImageXpress Nano System illuminates with colors that provide information about the instrument status.

Table 2-1: Status Indicator Colors

Color	Instrument Status
Orange	The instrument is powered on without software control.

Table 2-1: Status Indicator Colors (continued)

Color	Instrument Status
Blue	The instrument is powered on with software control and is ready to use.
Green	The instrument is acquiring data.
Red	The instrument is in an error state or cannot communicate with the software. You must reboot the instrument.

If required, you can turn off the status indicator lights. See Turning off the Status Indicator Lights on page 149.

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 43
- Configuring Plate Acquisition Options on page 49

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 Nano System for cleaning objectives, and cleaning the instrument as described in Maintenance on page 141.

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See Obtaining Support on page 149.

Chapter 3: System Installation

3

The ImageXpress Nano 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 Nano System instrument connects to the host computer during installation.

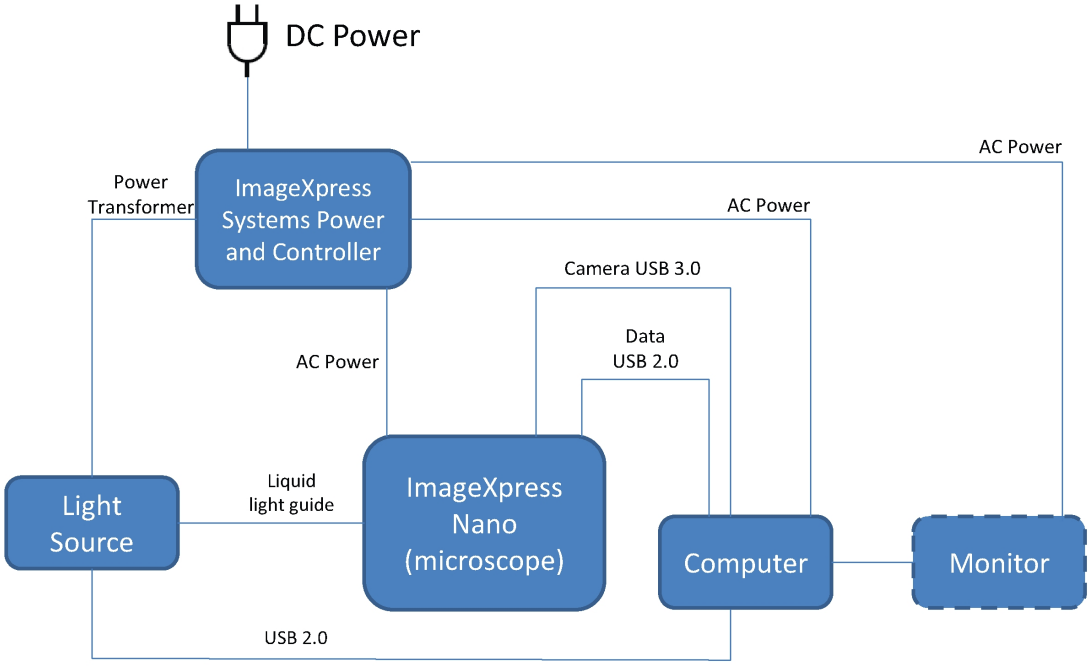


Figure 3-1: ImageXpress Nano System Components Without Options Using MetaXpress® Software

- Power supply to the instrument
- Liquid light guide from the external light source to the instrument
- USB 2.0 from the light source to the instrument
- USB 2.0 from the instrument to the computer for data
- USB 3.0 from the instrument to the computer for camera output

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

Verifying Device Settings in the Meta Imaging Series Administrator Software

This procedure ensures that the ImageXpress Nano 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 , 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**.

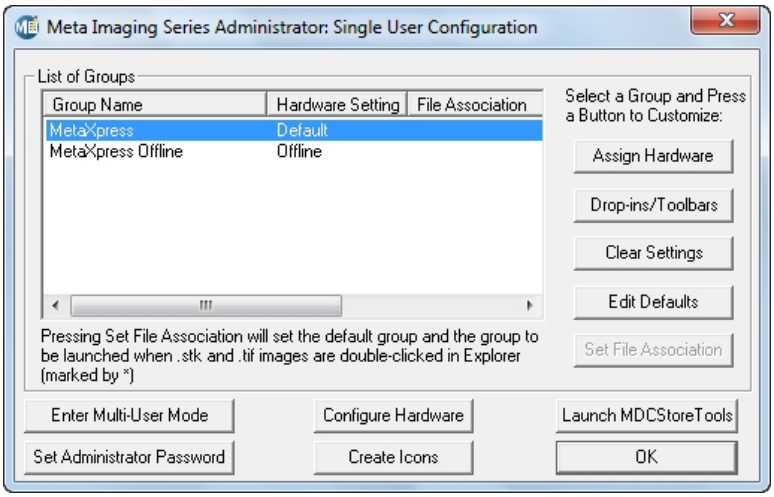


Figure 3-2: Meta Imaging Series Administrator Software home dialog

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.

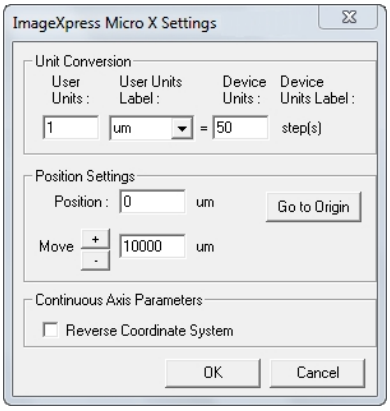


Figure 3-4: ImageXpress Micro X Settings dialog

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.

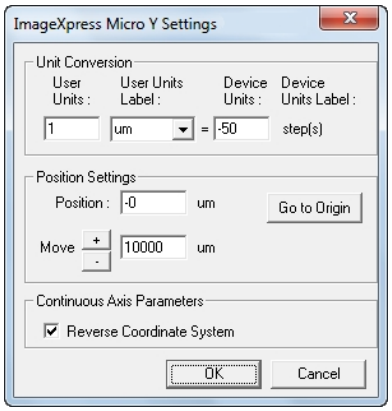


Figure 3-5: ImageXpress Micro Y Settings dialog

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.

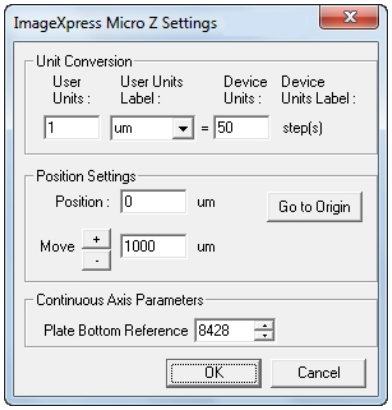


Figure 3-6: ImageXpress Micro Z Settings dialog

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.

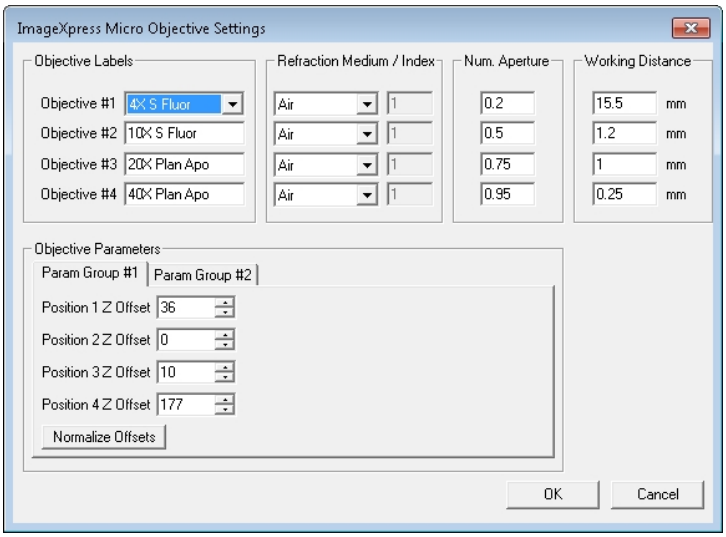


Figure 3-7: ImageXpress Micro Objective Settings dialog

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.
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.

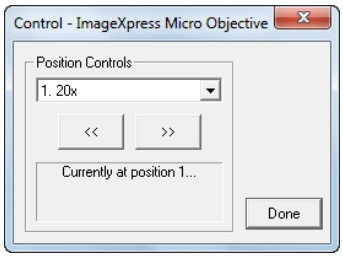


Figure 3-8: Control > ImageXpress Micro Objective dialog

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 Nano System, confirm those settings as well. For information, see Verifying External Control Settings.

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

Verifying Camera Settings in the Meta Imaging Series Administrator Software

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

1. With the powered on, make sure that the Meta Imaging Series Administrator Software is open to the **Configure Hardware** dialog.
2. In the **Configure Hardware** dialog, click **Configure Acquisition**.
3. In the **Configure Acquisition** dialog, ensure that a driver is listed in the **Installed Drivers** column.
   If a driver is not listed in the **Installed Drivers** column, or if more than one driver is listed, contact Molecular Devices support to determine which driver is required for your system.
4. In the **Installed Drivers** column, select the appropriate driver.
5. Click **Configure** to query the camera.
6. In the camera driver dialog, ensure that the camera is available in channel 1.
   This confirms that the camera is responsive.
7. Click **OK** to close the camera driver dialog.
8. Click **OK** to close the **Configure Acquisition** dialog.
9. Click **OK** to close the **Configure Hardware** dialog.
10. Click **OK** to close the **Meta Imaging Series Administrator**.

11. Verify and back up settings in the MetaXpress Software. See Verifying and Backing Up Settings in the MetaXpress Software on page 33.

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 34
- Verifying Calibration Settings on page 34
- Verifying the Laser Autofocus Sensor on page 36
- Verifying the Plate Reference Point (A1 Center) on page 38
- Verifying Plate Types on page 39
- Confirming Laser Autofocus Settings for Plate Files on page 41
- Verifying Shading Correction Files-Legacy on page 42

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 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.

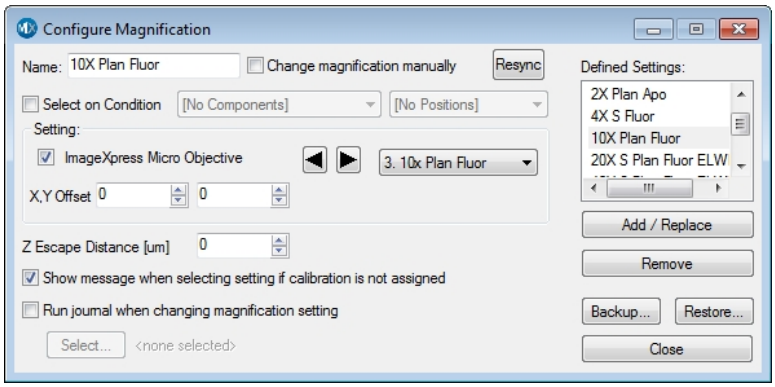


Figure 3-9: The Configure Magnification dialog

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 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.

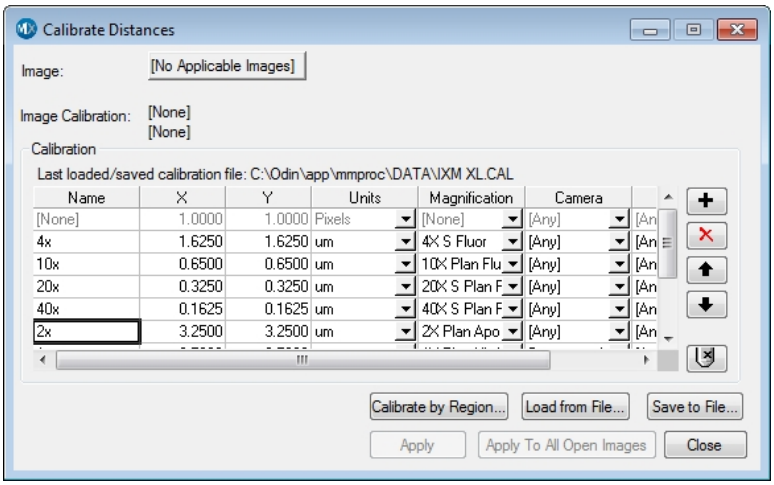


Figure 3-10: The Calibrate Distances dialog

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 Nano 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: Estimated Calibration Settings

Objective Magnification	Estimated Calibration
2x	3.317 µm/pixel
4x	1.659 µm/pixel
10x	0.663 µm/pixel
20x	0.332 µm/pixel
40x	0.166 µm/pixel
60x	0.111 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.

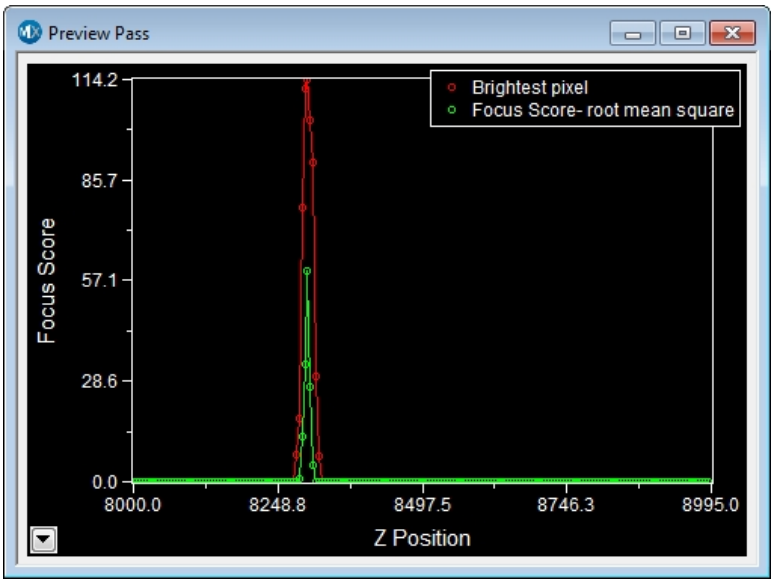


Figure 3-11: The Preview Pass window

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 149.

    For more information on the **Preview Pass** window, see Confirming Laser Autofocus Settings for Plate Files on page 41.
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 .

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

1. Open the MetaXpress Software and log in to the database.
2. Click **Screening > Plate Acquisition Setup**.
   In the simplified menu structure, click **Screening > Acquisition Setup**.
3. In the **Plate Acquisition Setup** dialog, click the **Configure** tab.
4. In the **Configure** tab, click the **Objective and Camera** tab.
5. From the **Magnification** list, select the lowest power objective. 10x is the highest that should be used.
6. In the **Camera Binning** field, type or select **1**.
7. Click the **Plate** tab.
8. From the **Plate name** list, select **96 Wells (8x12)**.
9. Click the **Sites to Visit** tab.
10. Under **Site Options**, select **Single site**.
11. Under the **Acquisition > Wavelengths** tab, click the **W1** tab.
12. From the **Illumination setting** list tab, select **FITC**.
13. In the **Exposure** field, type or select **100** milliseconds (ms).
14. Click the **Screening > Plate Acquisition and Control**.
15. In the **Plate Acquisition and Control** dialog, from the **Wavelength** list, select **W1 - FITC**.

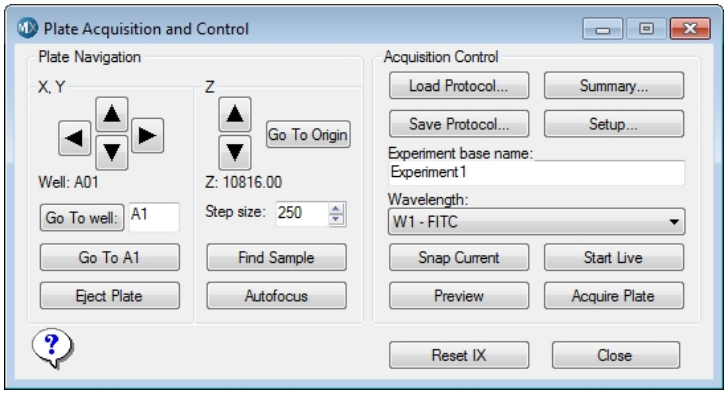


Figure 3-12: The Plate Acquisition and Control dialog

16. In the **Step Size** field, type **250**.

17. Click **Eject Plate** to open the top door.
18. Load the metal slide holder plate, and ensure the notch in the plate is in the A1 position on the stage. There is an integrated pinhole at the A1 well position.

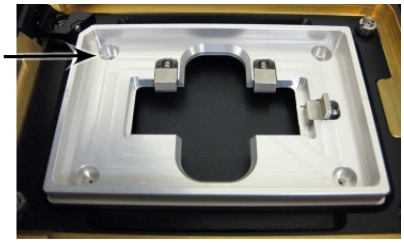


Figure 3-13: Loaded metal slide holder plate with integrated A1 well pinhole

19. Click **Load Plate** to close the top door.
20. Click **Go To A1** to move the stage to the A1 position.
21. Click **Start Live** to open a live image window.
22. If you are using a 4x objective, click the **Z** control arrows to step the Z-motor (reducing the step size as you get closer to focus if needed) until the A1 pinhole comes into focus, and then verify that the hole is visually centered in the field of view.
    If the A1 pinhole is not centered, or if you cannot find the hole, contact Molecular Devices Technical Support. See Obtaining Support on page 149.
23. Click **F2: Stop** to stop the live image.
24. Click **Eject Plate**.
25. Remove the metal slide holder plate and then click **Load Plate**.
26. Click **Close**.
27. 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.

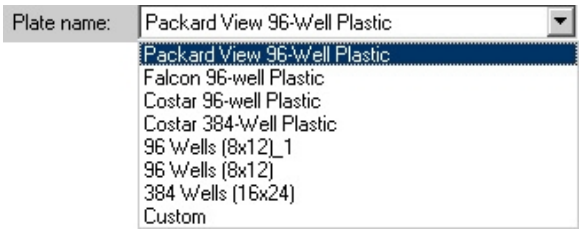


Figure 3-14: Available plate types

   - 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 143.

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 .

Note: For information about creating **Legacy Correction** shading correction image files, see Updating Shading Correction Settings-Legacy on page 145.

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.

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 with identification of the cells during image analysis. If the assay involves movement of a protein of interest to or from a particular cellular compartment or organelle, it 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.

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 45
- Plate Material, see page 45
- Fluorescence Background, see page 45
- Bottom Thickness, see page 45
- Plate Flatness or Reproducibility of the Z-Pattern, see page 45
- Plate Skirt
- Batch-to-Batch Consistency, see page 46
- Robot Compatibility

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 the 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 5X difference in auto-fluorescence among plates from different manufacturers.

Bottom Thickness

The thickness of the plate bottom should be compared with the working distance of the objective lens to be used to ensure that it is compatible. In general, objectives with higher numerical aperture (NA) tend to require thin-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.

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.

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 46
- Fixation and Staining Conditions, see page 46
- Final Buffer or Media, see page 46
- Plate Handling and Storage, see page 47

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.

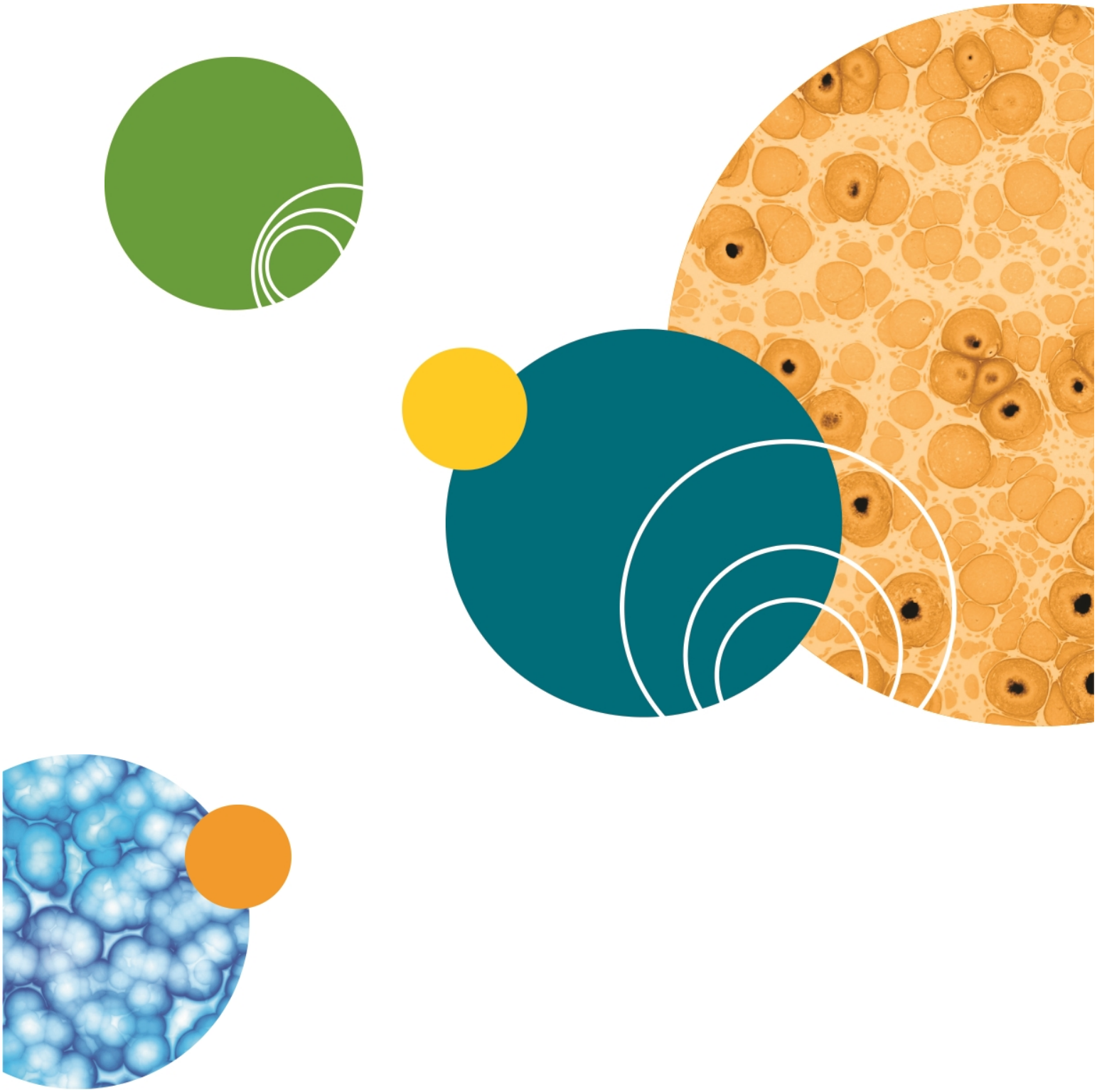
Plate Handling and Storage

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.

Instrument Maintenance

For best performance, your 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 141). Wear gloves when handling any optical components to avoid contaminating them with dirt or skin oils.

The basic design of the 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

5

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 Nano 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.

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.

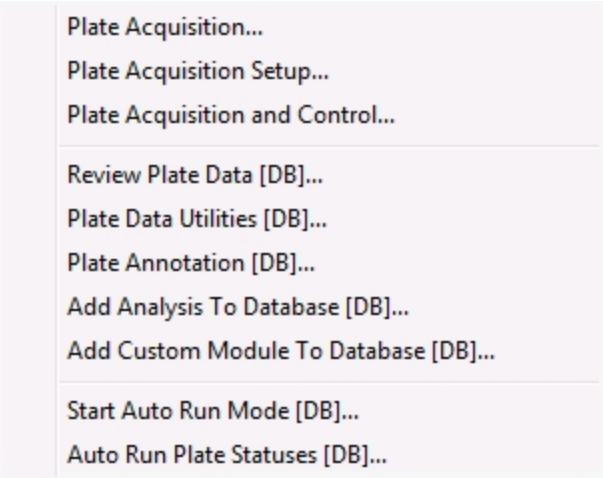


Figure 5-1: Default Screening Menu

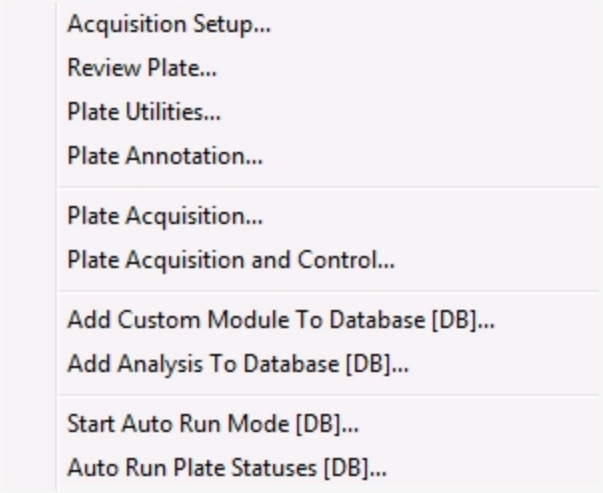


Figure 5-2: Simplified Screening Menu

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 Nano 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.

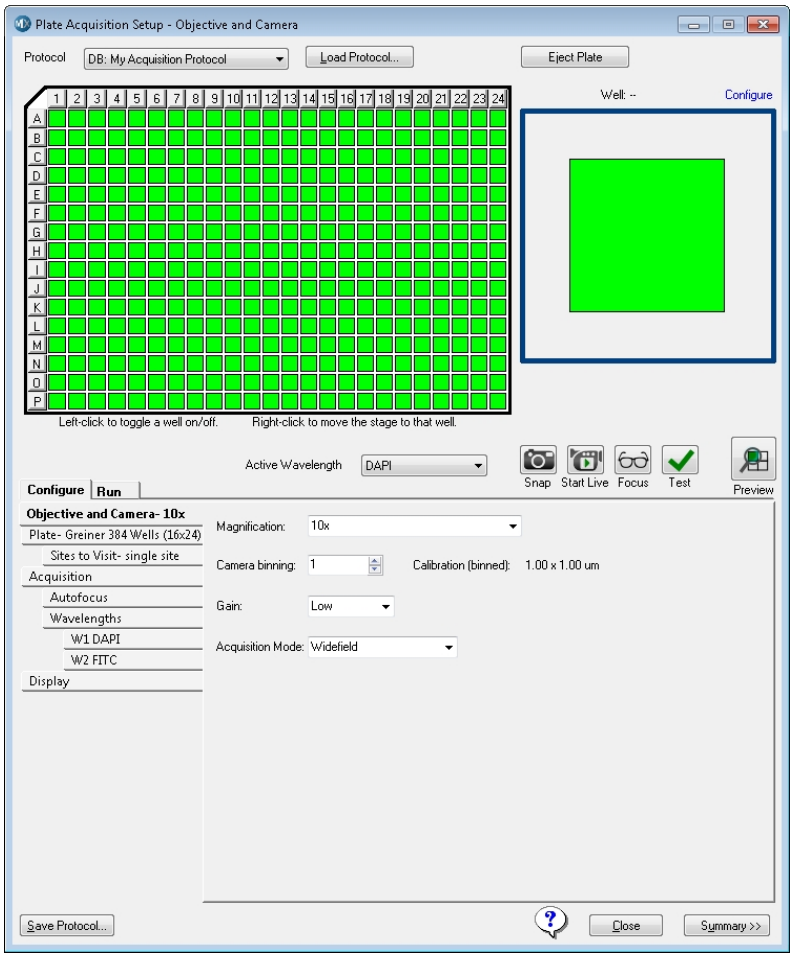


Figure 5-3: Plate Acquisition Setup Dialog

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.

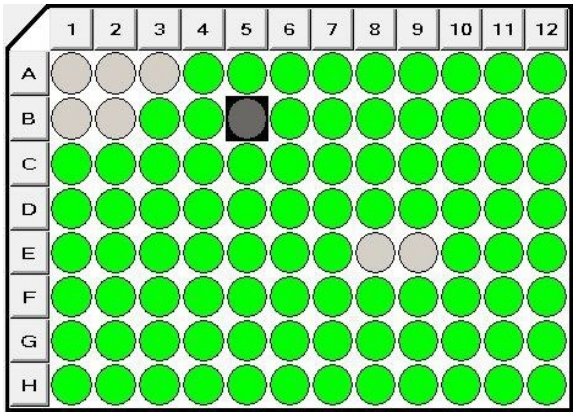


Figure 5-4: Plate Acquisition Setup Dialog, Plate map

- **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 . 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.

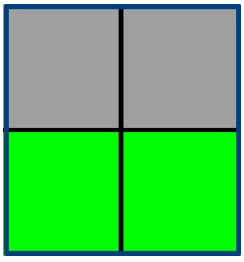


Figure 5-5: Plate Acquisition Setup dialog, Site map

- **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 55
  - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56
  - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61
  - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75
  - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107
- **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 112.

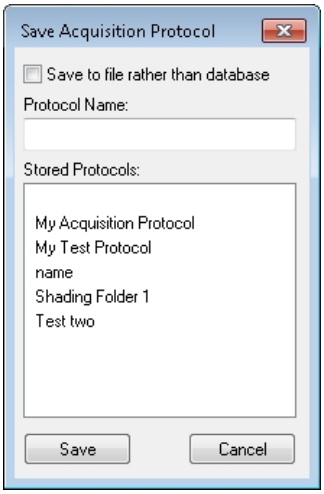


Figure 5-7: Save Acquisition Protocol dialog

- **Summary** button opens the **Summary** panel that displays a summary of all the current acquisition settings. See Viewing the Summary Panel on page 111.

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.

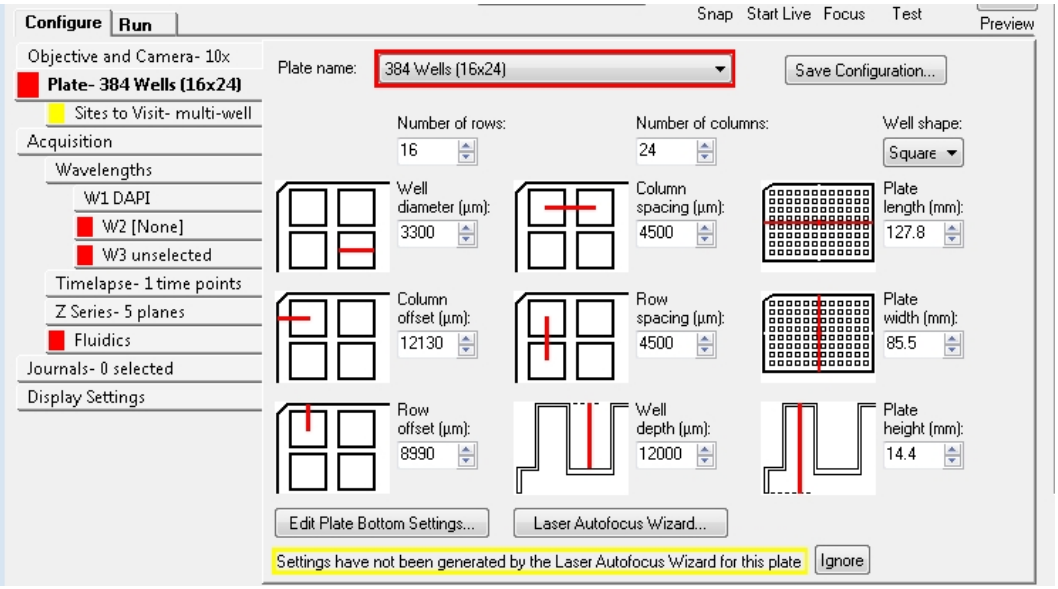


Figure 5-8: Plate Acquisition Setup Dialog with Warning Icons

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

You set the objective magnification, the camera binning, 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.

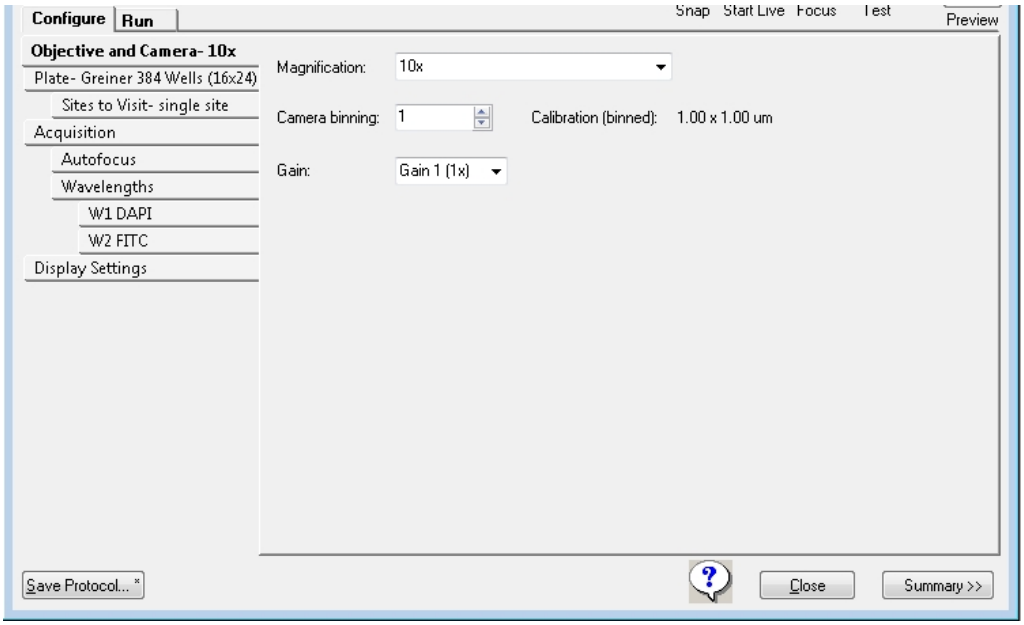


Figure 5-9: Plate Acquisition Setup dialog: Configure tab, Objective and Camera tab

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 34. You must assign a calibration to each magnification setting. See Verifying Calibration Settings on page 34.
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.

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

Option	Description
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.

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 112. Otherwise, continue with the next steps to further optimize your protocol settings. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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 Nano 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.

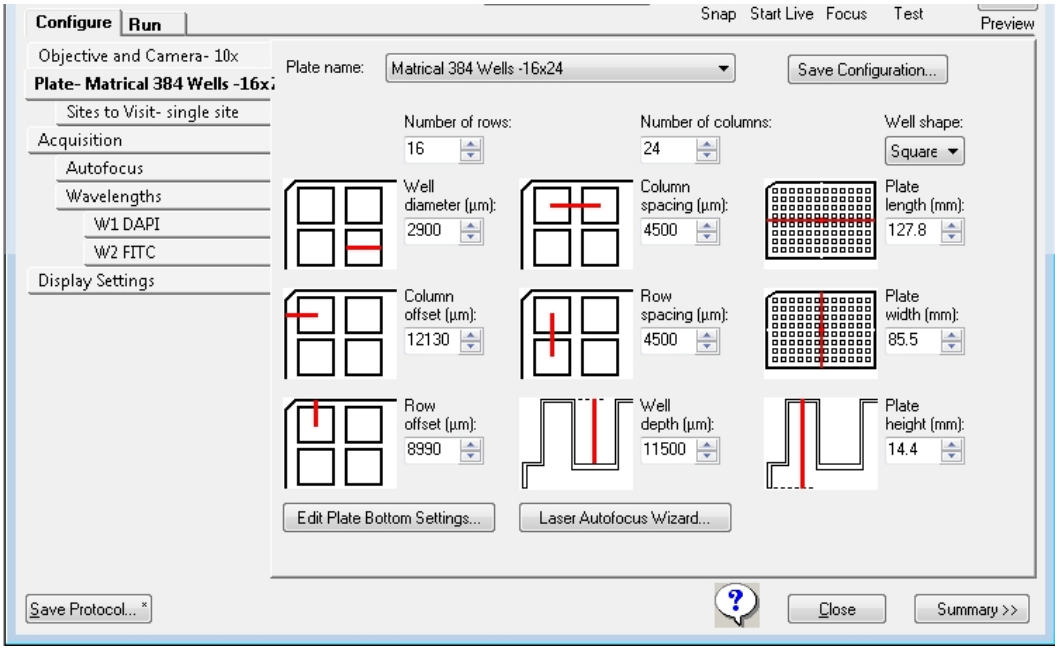


Figure 5-10: Plate Acquisition Setup dialog: Configure tab, Plate tab

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 39.

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 59.
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**.

Table 5-2: Plate Settings (continued)

Option	Description
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.
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.
Plate height	Specifies the plate height in mm. **Note:** The correct **Plate height** value is required for autofocusing.
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 149.

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 149.

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.

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 57.

   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 143.

10. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 112. Otherwise, continue to any other configuration as needed. See:
    - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
    - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
    - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.
    - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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 61
- Acquiring a Fixed Number of Sites in Each Well on page 63
- Configuring Adaptive Acquisition for Well Sites on page 66
- Configuring a Multi-Well Acquisition on page 73

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 63.

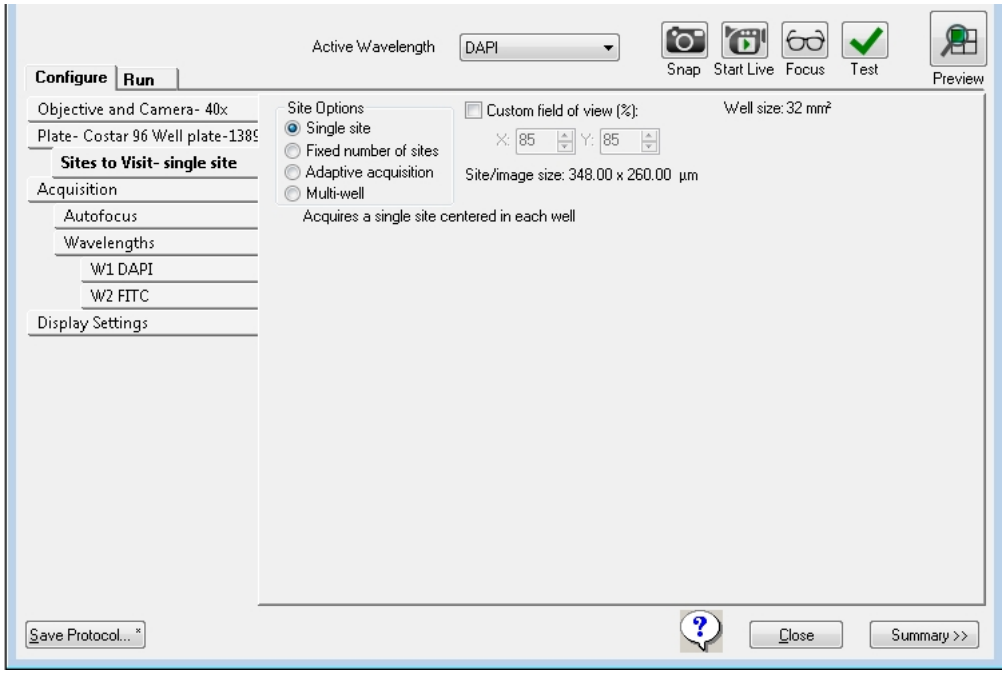


Figure 5-11: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Single site option selected

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.

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**.

   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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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.

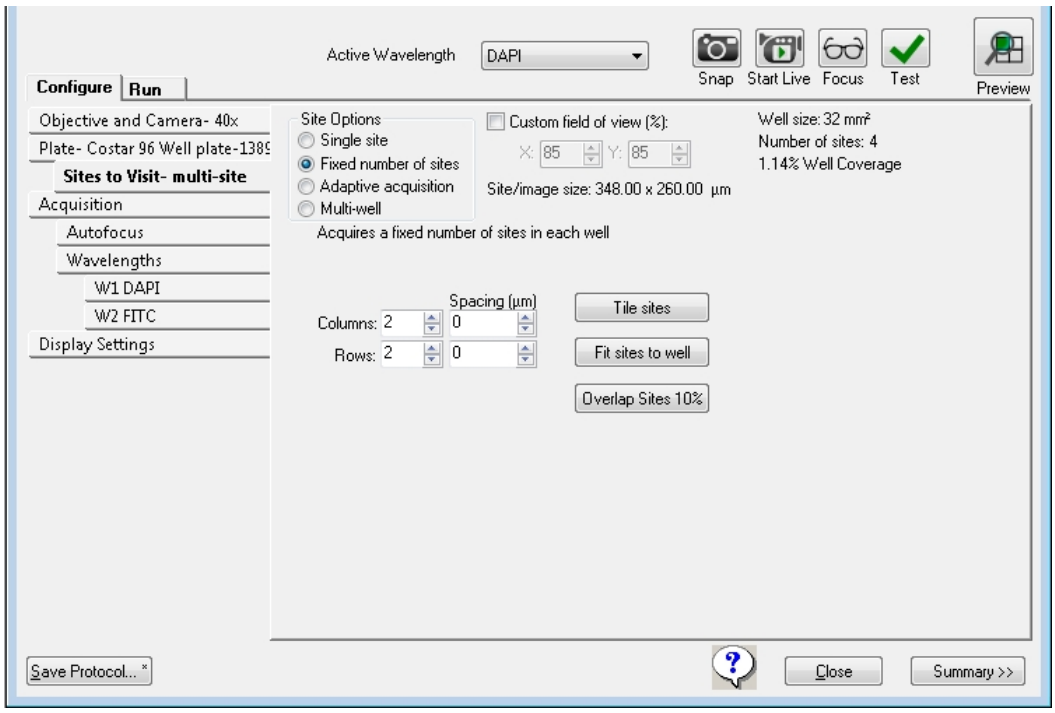


Figure 5-12: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Fixed number of sites option selected

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**.

   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. .

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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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.

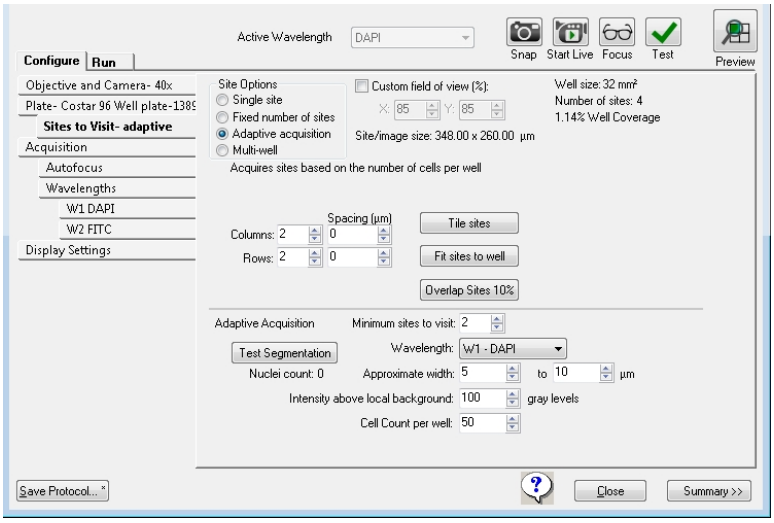


Figure 5-13: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Adaptive Acquisition option selected

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**.

   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. .

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 80.
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 72.
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.

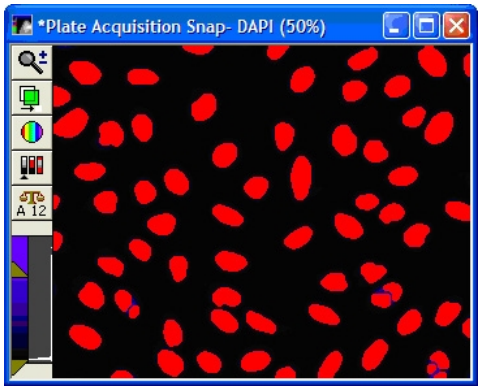
     

     Figure 5-14: An example of Test Segmentation results in the Adaptive Acquisition mode. The red shows the segmentation overlaid on the original DAPI image.

   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 112. Otherwise, continue to any other configuration as needed. See:
    - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
    - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
    - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.
    - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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:

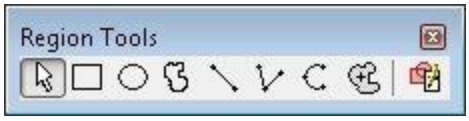


Figure 5-15: Region tools on the Region toolbar

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.

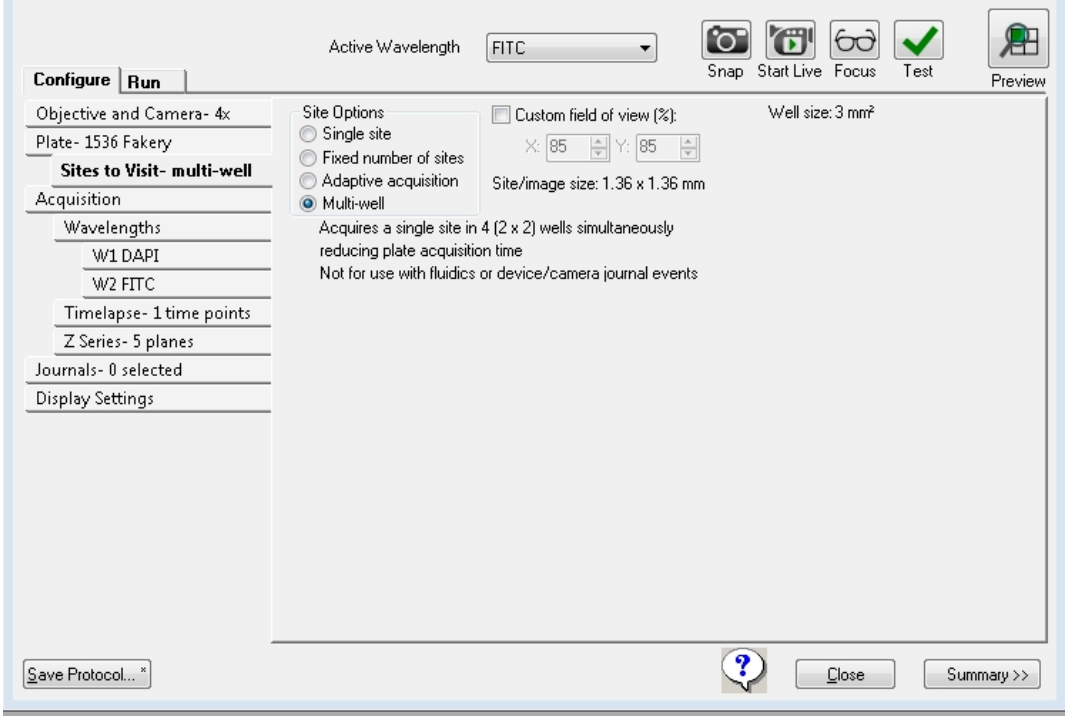


Figure 5-17: Plate Acquisition Setup dialog: Configure tab, Sites to Visit tab, Multi-well option selected

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.

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**.

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 112. Otherwise, continue to any other configuration as needed. See:

- Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
- Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
- Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.
- Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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 **Run Journals During Acquisition**. Depending on the options that you select, other tabs with additional configuration options can be displayed.

See:

- Configuring Autofocus Options on page 75
- Specifying the Number of Acquisition Wavelengths on page 80
- Configuring Series Acquisition Options on page 91
- Configuring Journals to Run During Acquisition on page 99
- Configuring Post-Acquisition Analysis Options on page 103

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. Molecular Devices recommends primarily using **Laser-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.

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**.

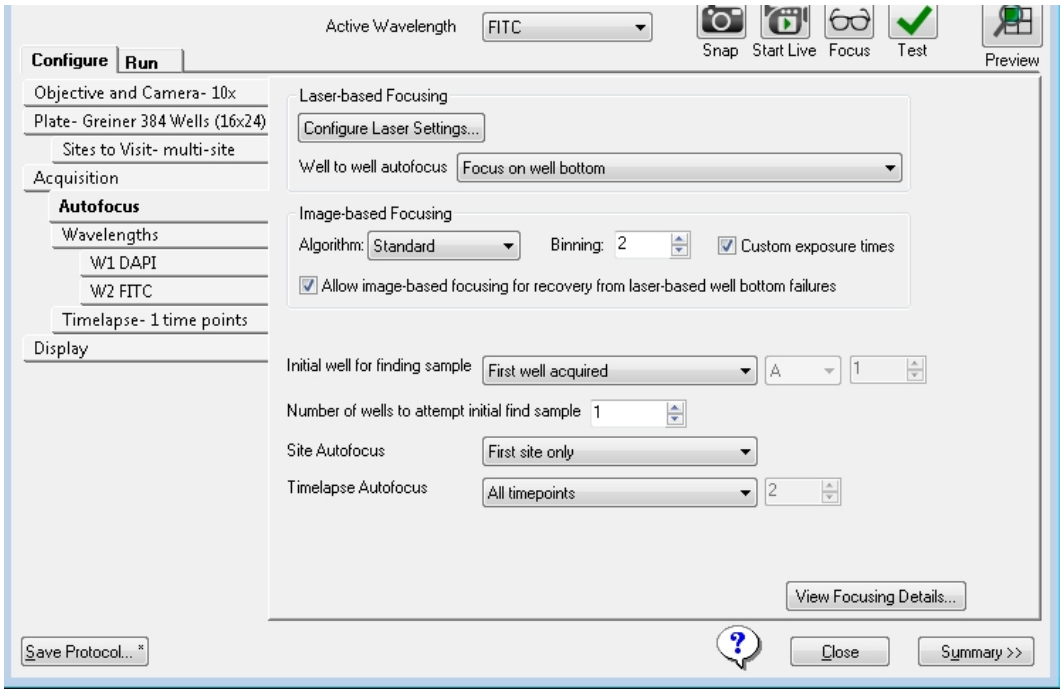


Figure 5-18: Plate Acquisition Setup dialog: Configure tab, Autofocus tab

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 149.
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.
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:	

Table 5-4: Autofocus options (continued)

Options	Description
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 80.
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
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.
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.	

Table 5-4: Autofocus options (continued)

Options	Description
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 91.	
First timepoint only	Use for fast kinetic acquisitions.
All timepoints	Use for long timelapse acquisitions.
Every Nth timepoint	Set a value that is less than the number of specified timepoints. See Configuring Time Series Acquisition Options on page 92.
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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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 5-19.

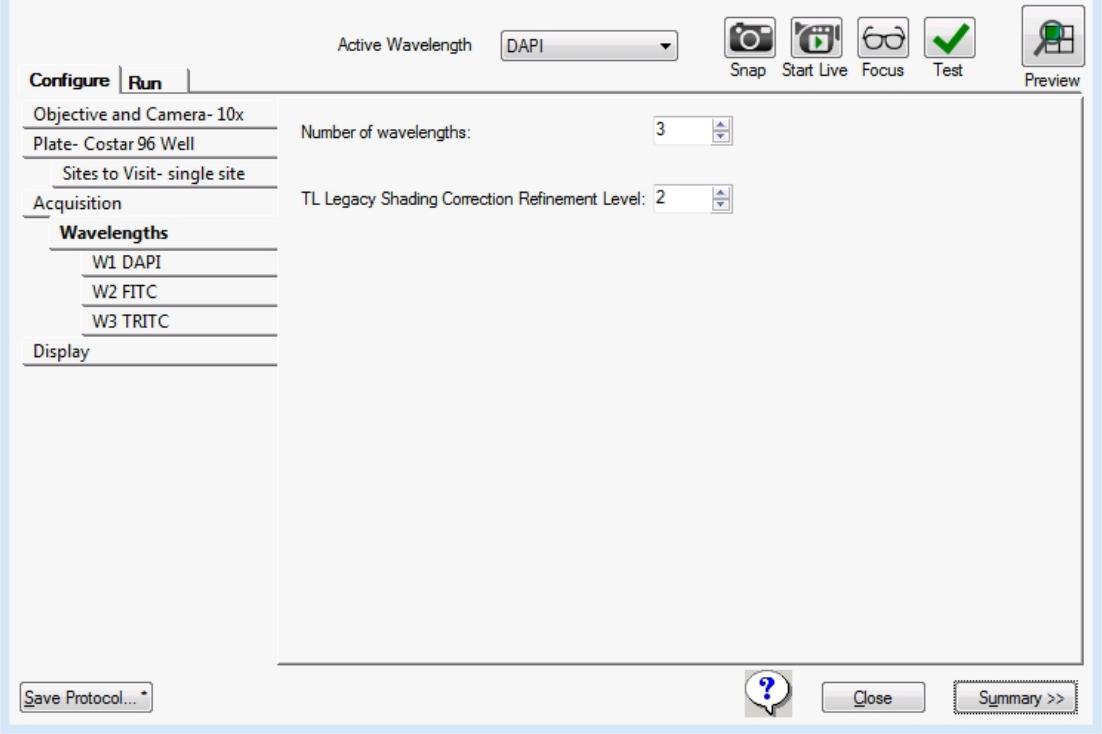


Figure 5-19: Plate Acquisition Setup dialog: Configure tab, Wavelength tab: Three placeholder Wavelength tabs

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.

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 88.

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 82.

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.

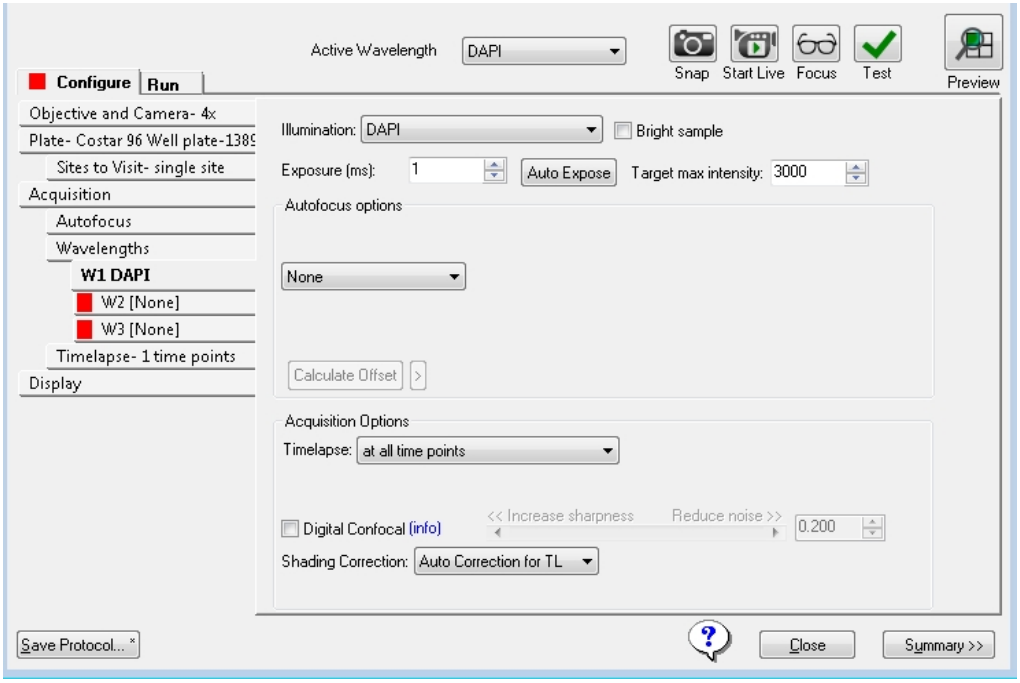


Figure 5-20: Plate Acquisition Setup dialog: Configure tab, Wavelength tab: Setting placeholder Wavelength tab

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. For the ImageXpress Nano System, the optimal value should be approximately 3300.
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.

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

Options	Description
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.
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.

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.
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.
Acquisition options are displayed depending on the **Series** option selected under **Acquisition options** on the **Acquisition** tab.	
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.

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

Options	Description
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.
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. **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 80. **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 89. **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.

4. If you are done configuring your protocol, continue to Saving a Plate Acquisition Protocol on page 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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 82.

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 42.

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 56.

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

1. Click **Acquisition**.

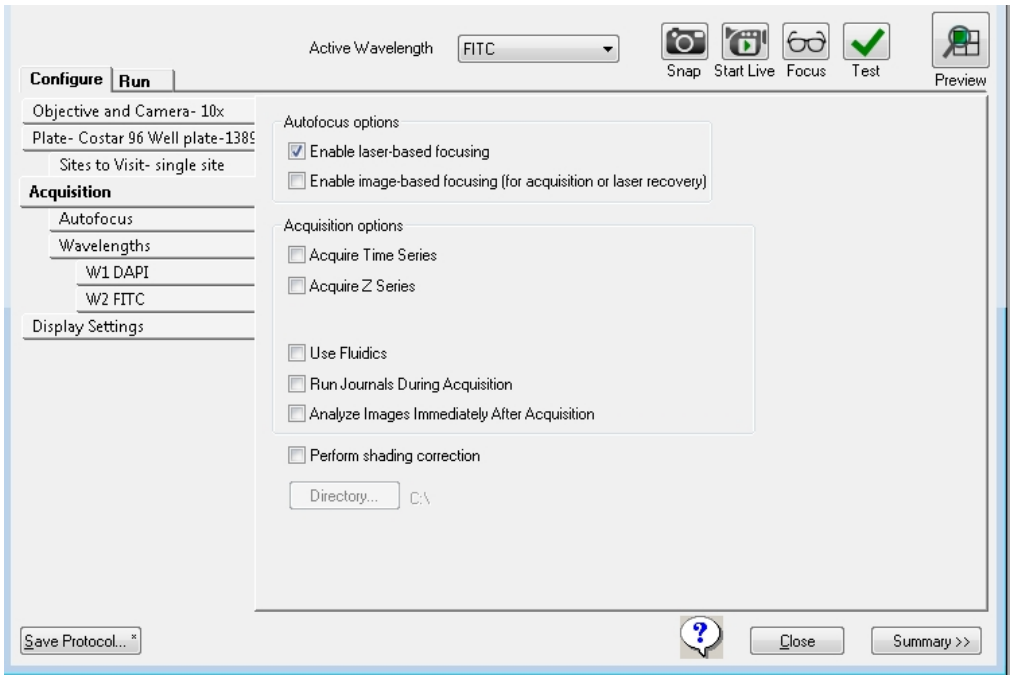


Figure 5-21: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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.

1. Click **Acquisition**.

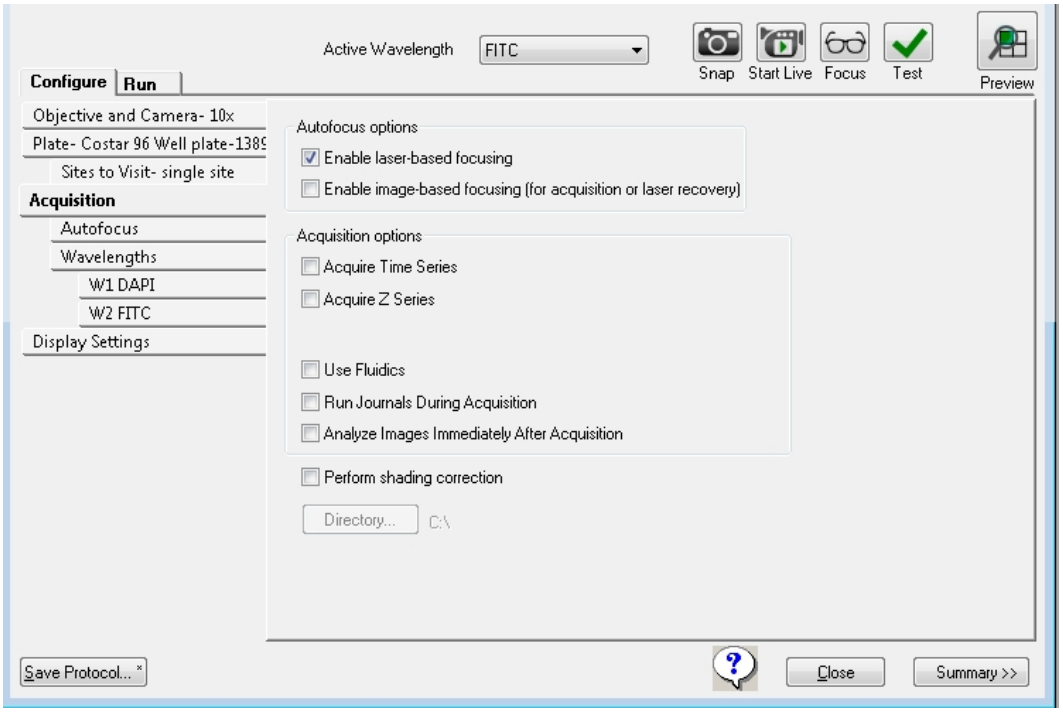


Figure 5-22: Acquisition Setup dialog, Configure tab, Acquisition tab, Acquisition options, Series options

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 92.
   - For **Acquire Z Series**, continue to Configuring Z Series Acquisition Options on page 95.

Configuring Time Series Acquisition Options

1. Click **Timelapse**.

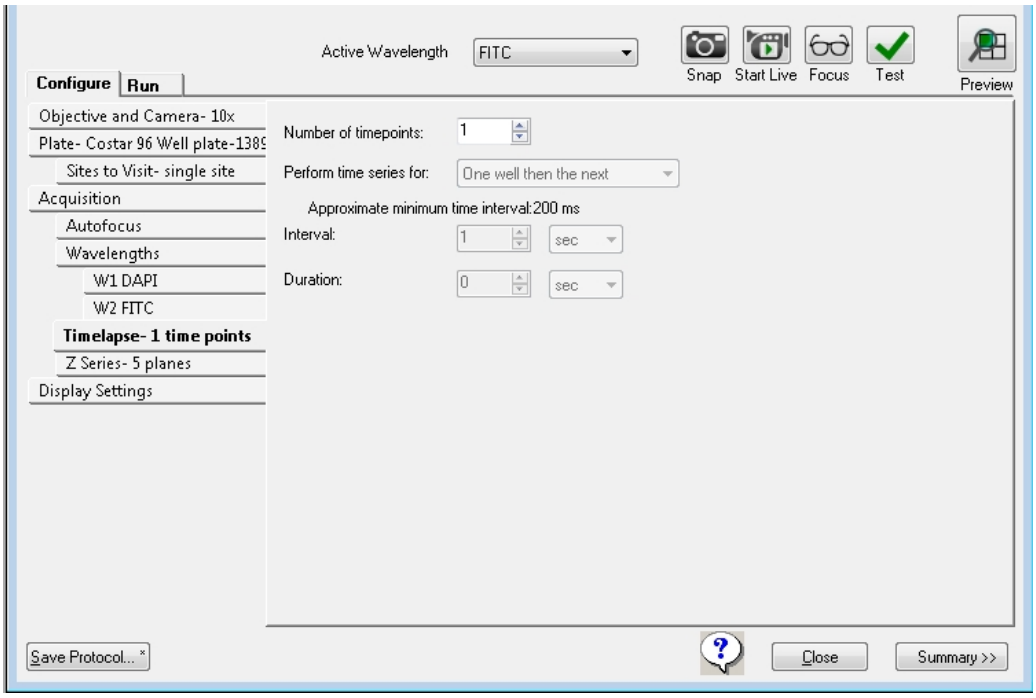


Figure 5-23: Plate Acquisition Setup dialog, Configure tab, Timelapse tab

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 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 75.	

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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

Configuring Z Series Acquisition Options

1. Click **Z Series**.

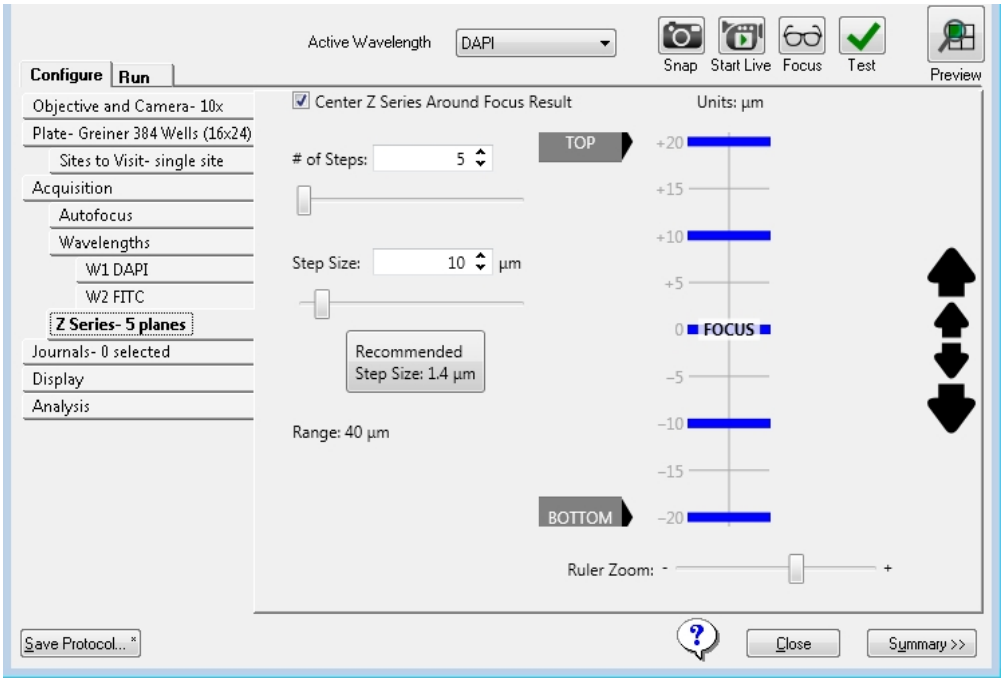


Figure 5-24: Plate Acquisition Setup dialog: Configure tab, Z Series tab showing the Z Series diagram

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:

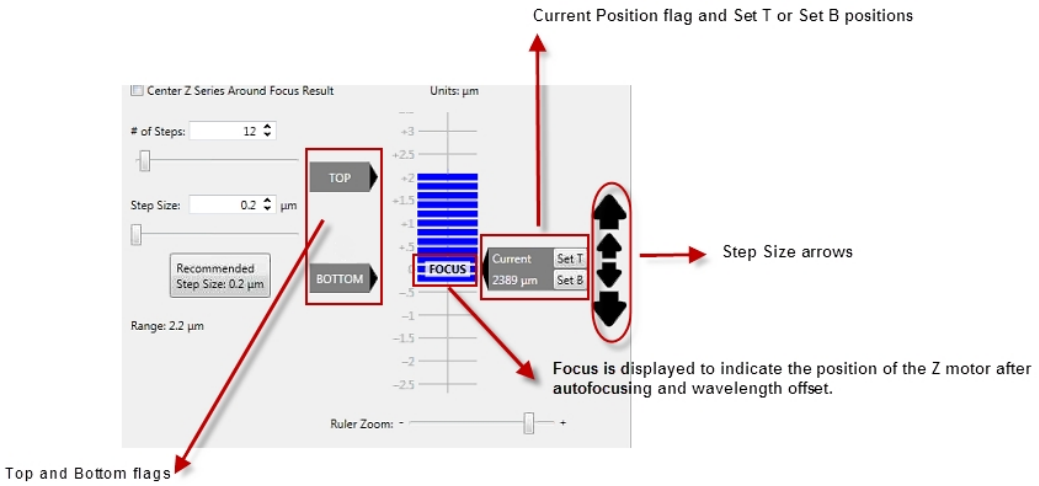


Figure 5-25: Z Series diagram

- 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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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.

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

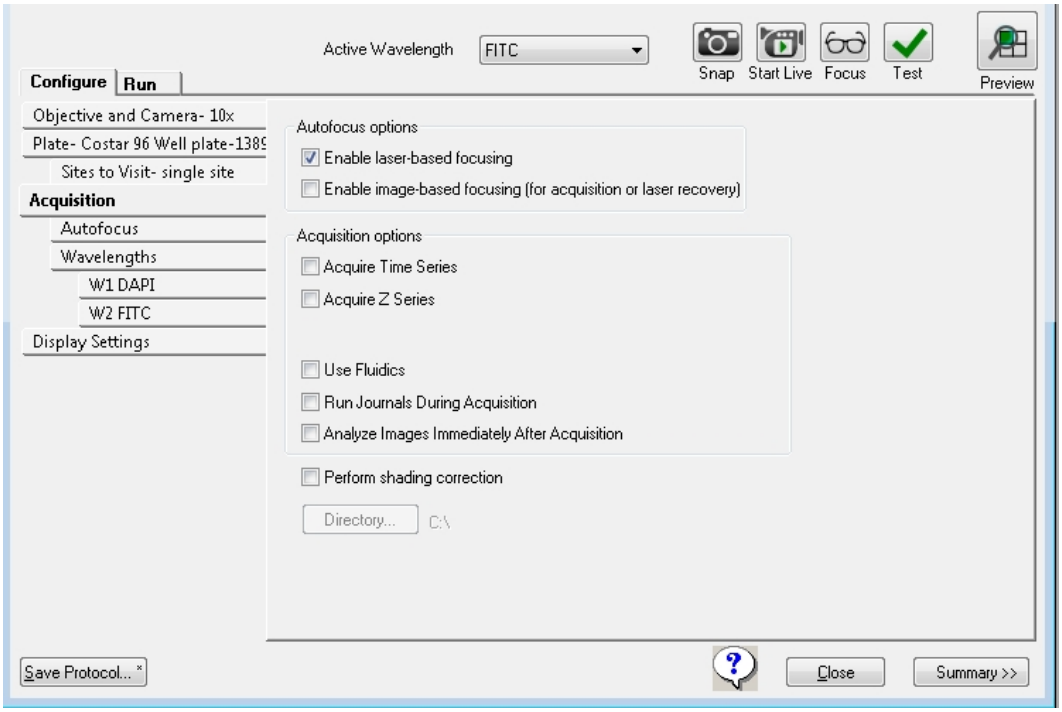


Figure 5-26: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

3. Click **Journals**.

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

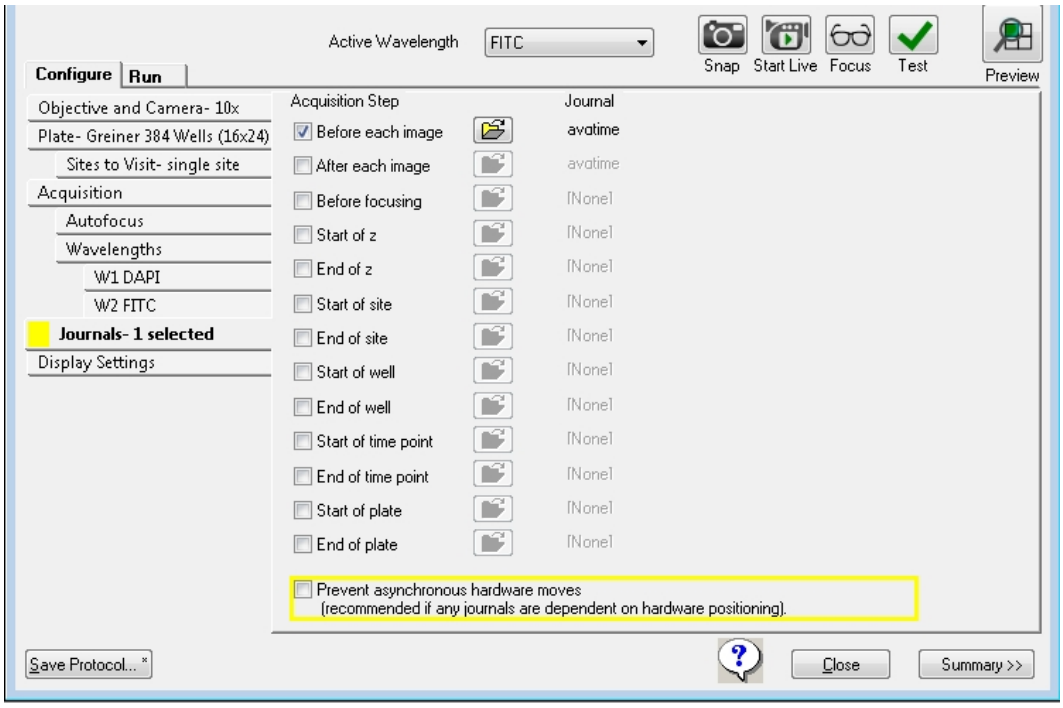


Figure 5-27: Plate Acquisition Setup dialog: Journals tab, Acquisition Step Selection and Loaded Journal

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.
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.

Table 5-8: Journal acquisition steps (continued)

Option	Description
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.

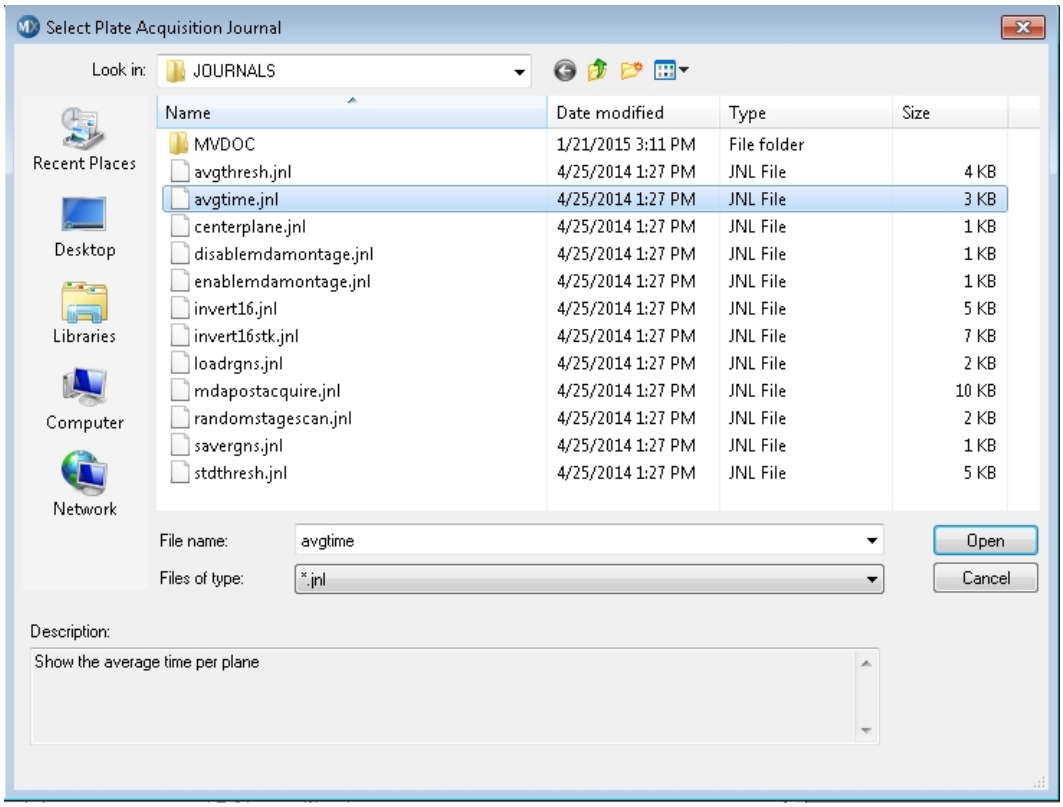


Figure 5-28: Select Plate Acquisition Journal dialog

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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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.

1. Click **Acquisition**.

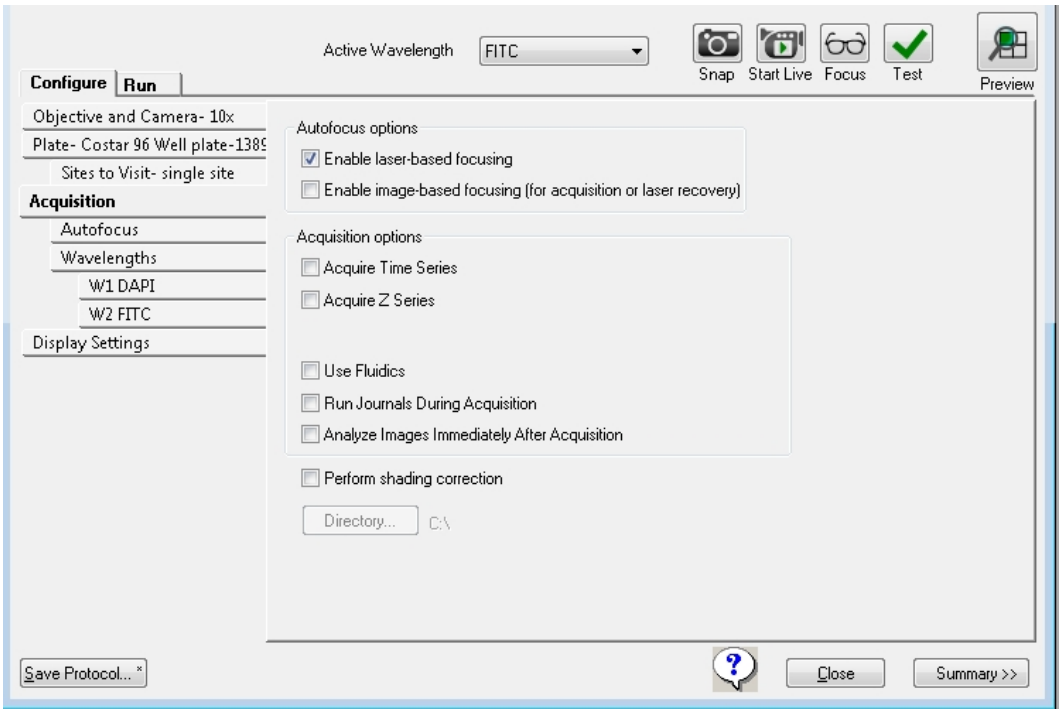


Figure 5-29: Plate Acquisition Setup dialog, Configure tab, Acquisition tab

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**.

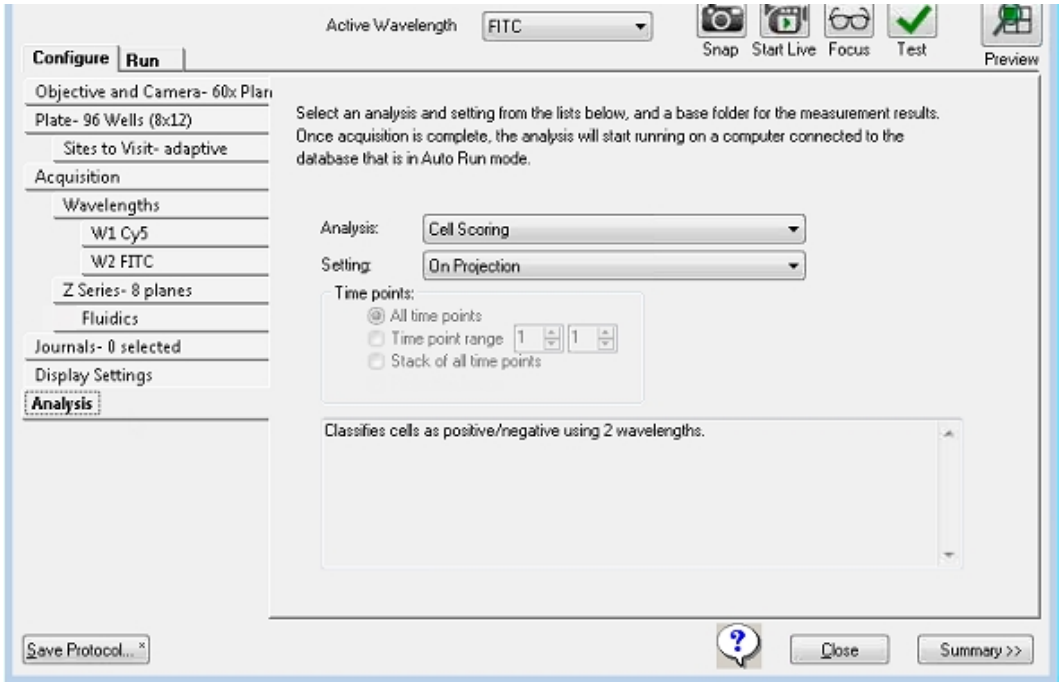


Figure 5-30: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Time Series)

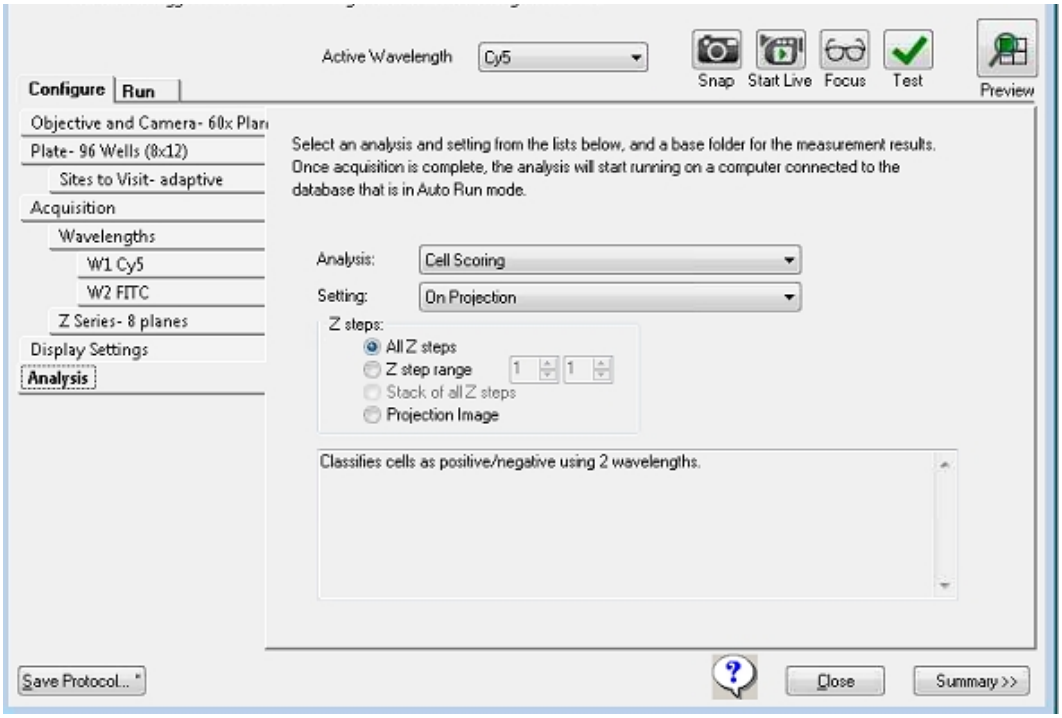


Figure 5-31: Plate Acquisition Setup dialog: Configure tab, Analysis tab (Z Series and 2D Projection Image Only)

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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Display Tab on page 107.

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.

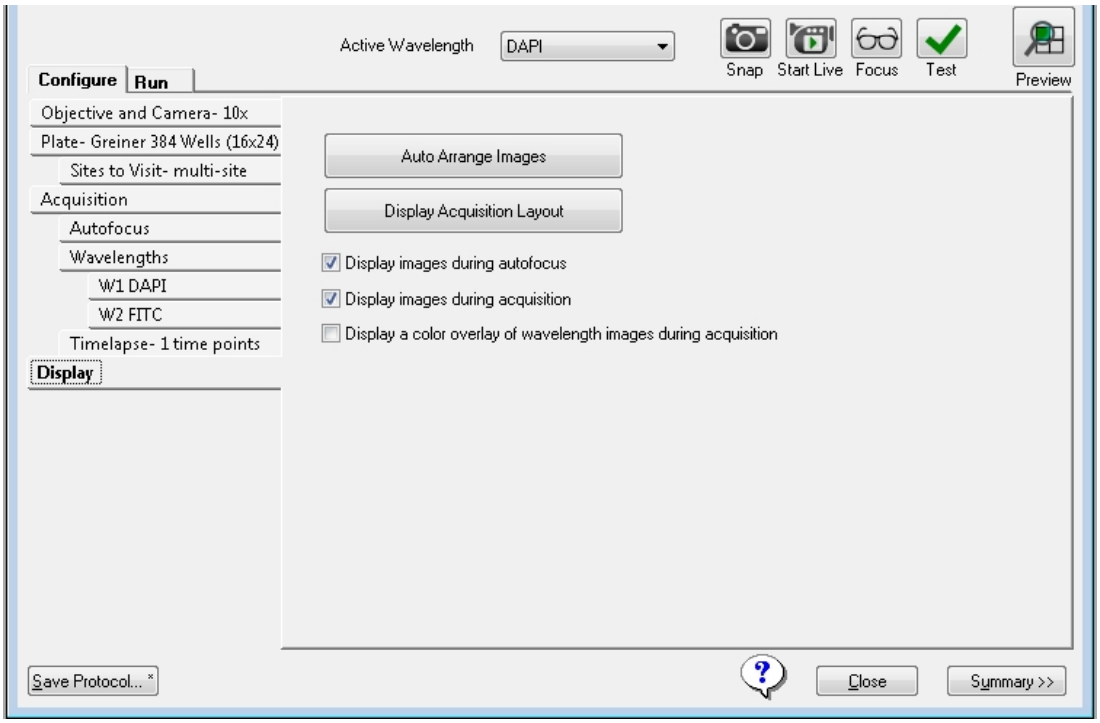


Figure 5-32: Plate Acquisition Setup dialog: Configure tab, Display tab

For a description of the options on this tab, see Table 5-12: Display Settings on page 108.

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.

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**.

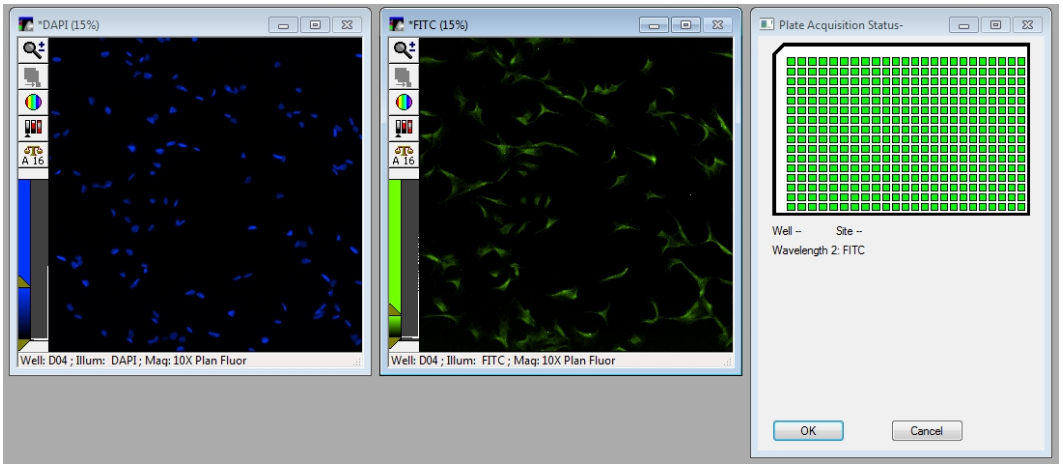


Figure 5-33: Display Settings tab, Display Acquisition Layout Settings

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 112. Otherwise, continue to any other configuration as needed. See:
   - Plate Acquisition Setup Dialog: Configure Tab, Objective and Camera Tab on page 55.
   - Plate Acquisition Setup Dialog: Configure Tab, Plate Tab on page 56.
   - Plate Acquisition Setup Dialog: Configure Tab, Sites to Visit Tab on page 61.
   - Plate Acquisition Setup Dialog: Configure Tab, Acquisition Tab on page 75.

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.

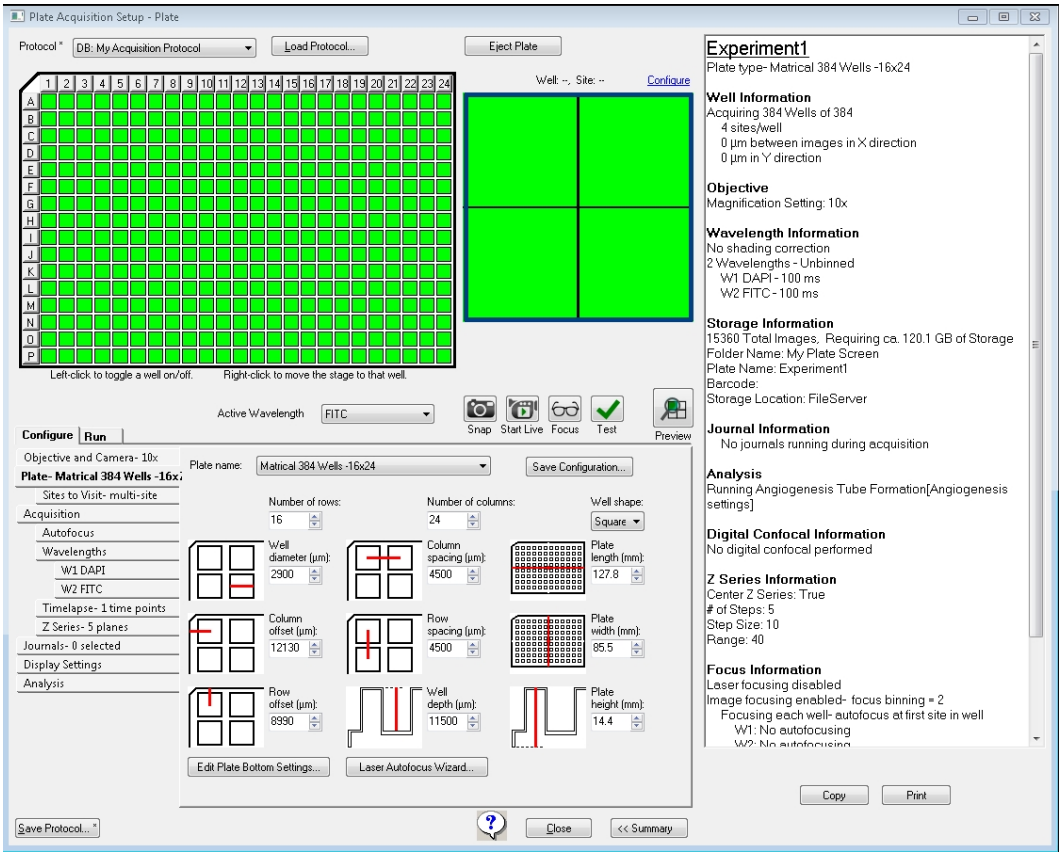


Figure 5-34: Plate Acquisition Setup dialog with open Summary panel

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 Nano 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: Customizing the MetaXpress Software

6

A powerful feature of the MetaXpress Software is the ability to customize the operation of the software for individual users or groups of users. Various objectives and workflows call for customized settings. 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 113
- Custom Toolbars and Taskbars, see page 116
- Default Paths for Data, see page 119

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 17.

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 Nano System ships with a number of groups and hardware settings predefined. The number of groups depends on the configuration of your system. Figure 6-1 shows the **Multiple User Configuration** window in the Meta Imaging Series Administrator Software.

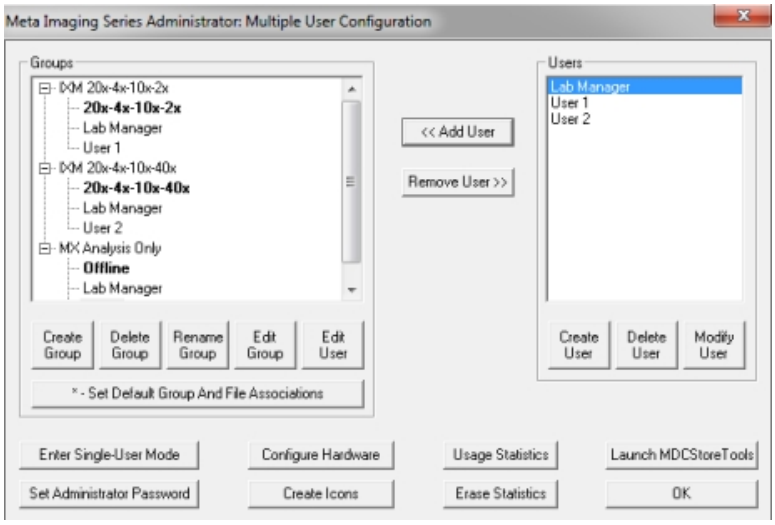


Figure 6-1: Example of the Multiple User Configuration window

This example shows a system with the following groups defined:

- **IXM 20x-4x-10x-2x**: This is the ImageXpress Nano 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**.

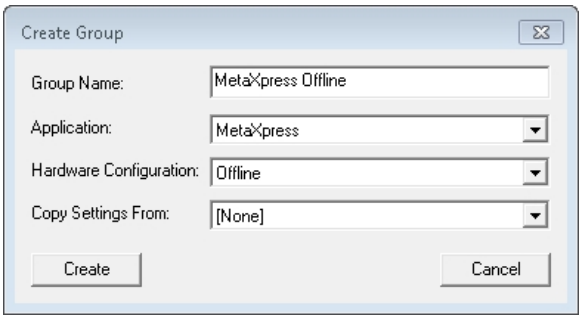


Figure 6-2: Create Group dialog

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

> **Note:** You must add at least one user to the new offline group 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 114 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 6-3.

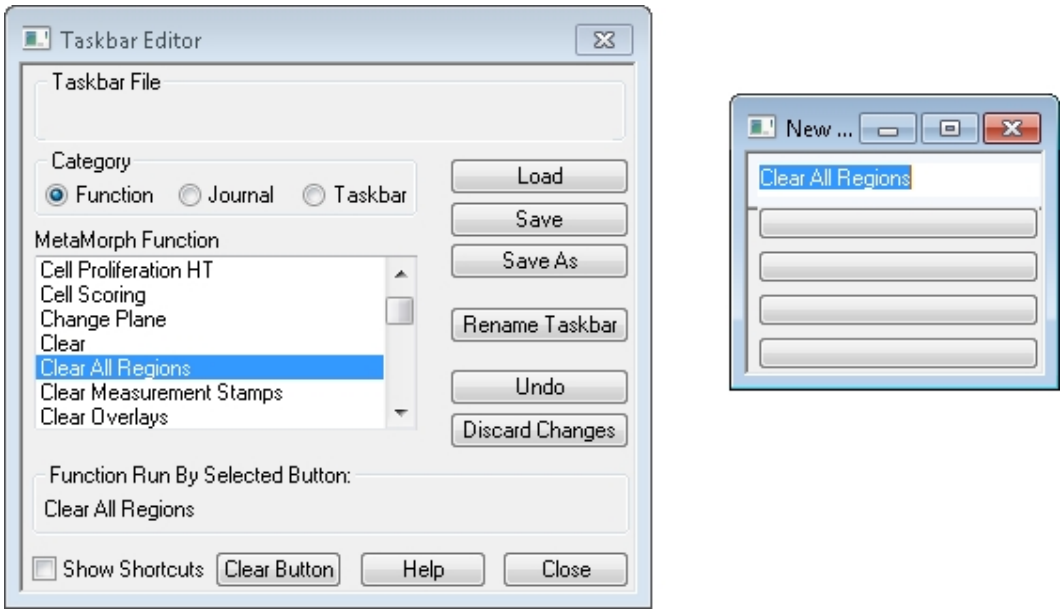


Figure 6-3: Adding a function to a taskbar

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

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

Default Paths for Data

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

- **Default Data Paths**: Your system computer has a dedicated hard drive partition for data. The default data file paths for each user should all point to this data drive. For example: D:\Data\Username.
  The following data file types should have their file paths changed to point to the data drive:
  - Log files
  - Memory lists
  - Calibrations
  - Illumination Settings
  - Magnification Settings
- **Default HTS State Path**: The MetaXpress Software 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.

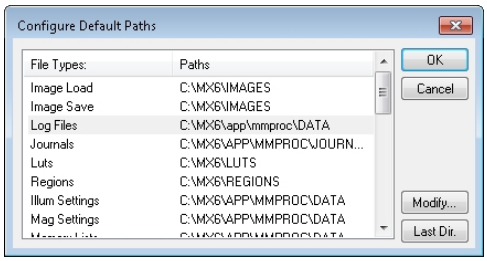


Figure 6-4: Configure Default Paths dialog

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

Chapter 7: Optional Expansion Solutions

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

- Environmental Control Options, see page 121
- Transmitted Light Options, see page 134

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.

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

See also:

- Transmitted Light Options, see page 134

Operational information is included for the following:

- Environmental Control Hardware, see page 121
- Setting Up Environmental Control, see page 125
- Environmental Control Software
- Cleaning Environmental Control Components, see page 133

Environmental Control Hardware

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

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

- **Temperature control** within the upper half of the base instrument. Warm air is provided from the ImageXpress Systems Power and Options Controller through an air hose. Feedback from temperature sensors installed near the plate maintains the temperature.

The temperature inside the microplate chamber can be maintained at 8°C above ambient to 40°C.

- **Carbon dioxide** is provided from a customer-supplied tank of pre-mixed 5% CO_2 and 95% air. The tank regulator must be set between 15 PSI and 20 PSI. The ImageXpress Systems Power and Options Controller controls the flow to the space above the plate, maintained by the live-cell sealing ring. If a plate is ejected and loaded, the system does a purge cycle automatically.
- **Humidity** is passively provided by bubbling the carbon dioxide through a water reservoir, minimizing evaporation from the sample plate over the duration of a timelapse experiment.

Items Included in the Installation

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

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

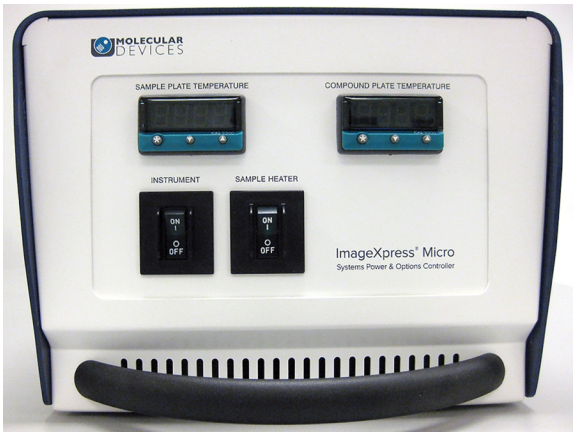


Figure 7-1: ImageXpress Systems Power and Options Controller used for the ImageXpress Nano System

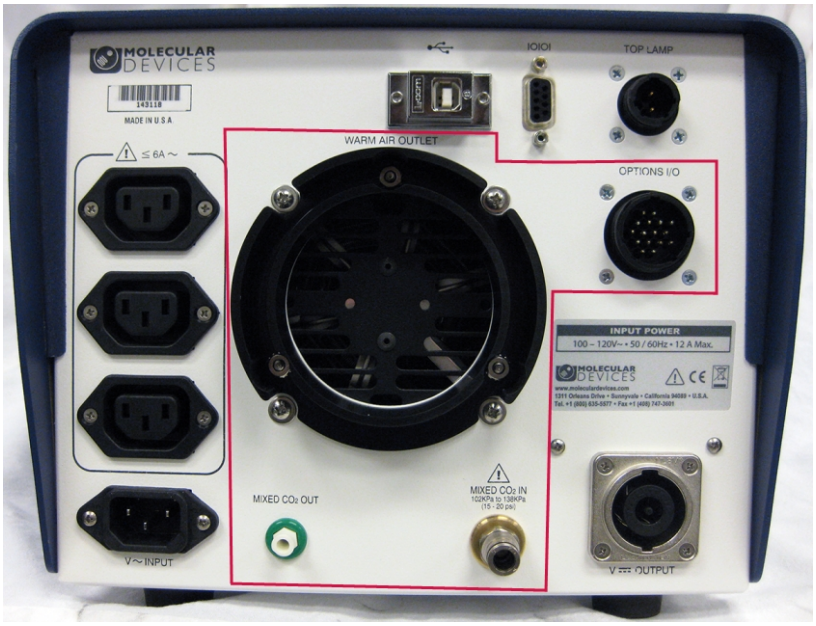


Figure 7-2: Environment Control option connections on the back of the ImageXpress Systems Power and Options Controller used for the ImageXpress Nano System

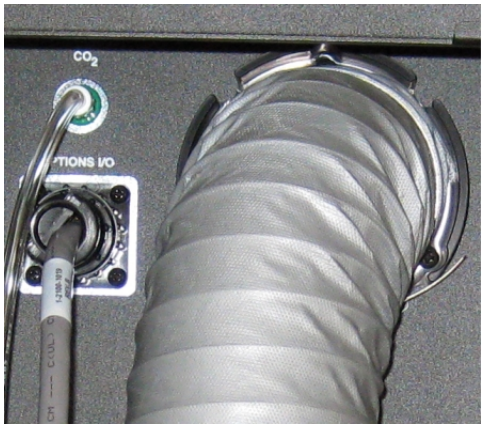


Figure 7-3: Environment Control option warm air hose and carbon dioxide tubing on the back of the ImageXpress Nano System

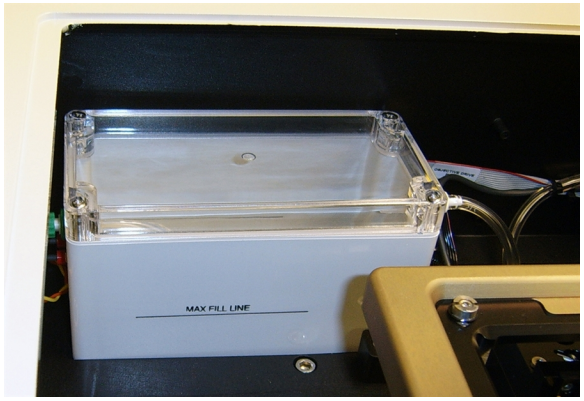


Figure 7-4: Environment Control option water reservoir

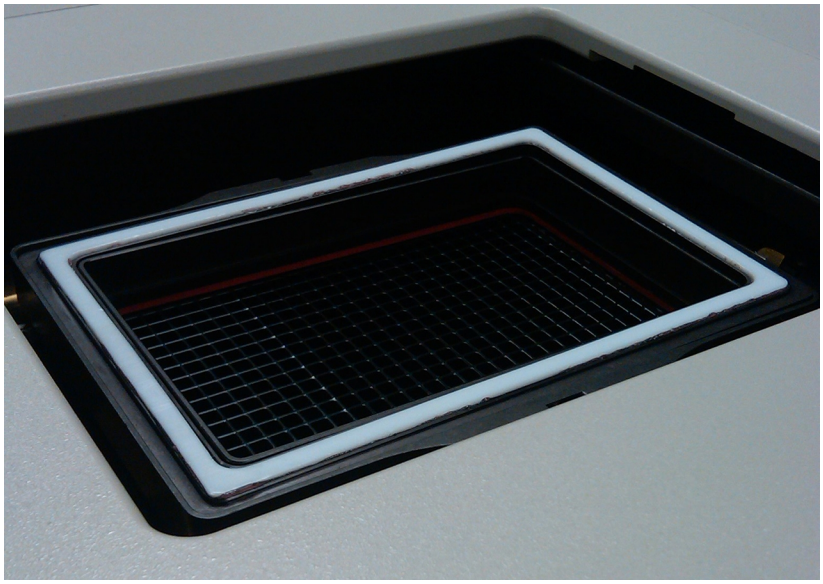


Figure 7-5: Environment Control option live-cell sealing ring

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 Nano 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 , view the current sample plate temperature.

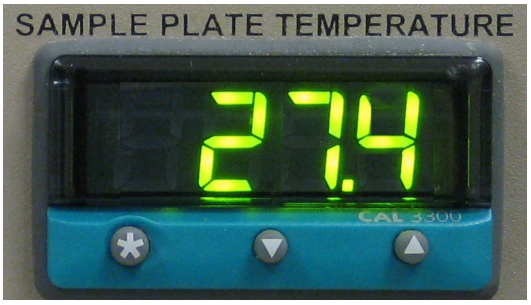


Figure 7-6: Sample Plate Temperature control interface

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

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

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 129.

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 7-7), and for more access.

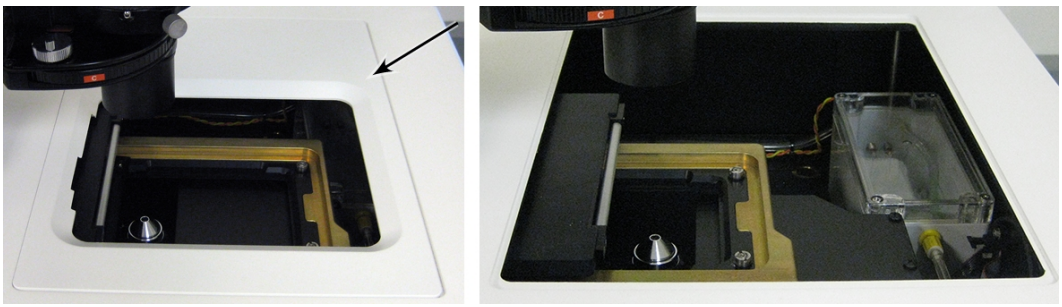


Figure 7-7: Top access panel around the top door on and off

4. Locate the water reservoir through the top opening.

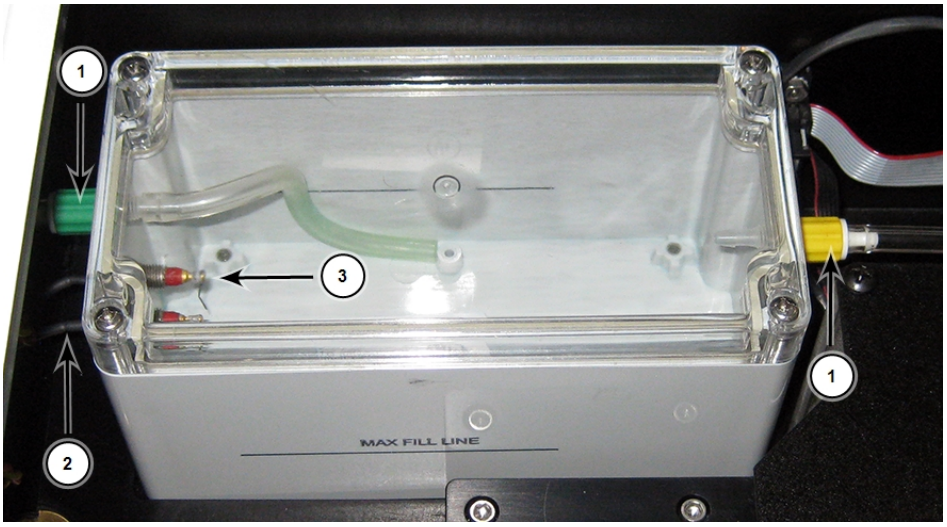


Table 7-1: Environment Control Option Water Reservoir

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.

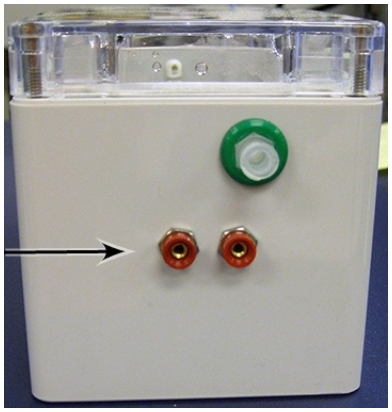


Figure 7-8: Water level sensor wire sockets

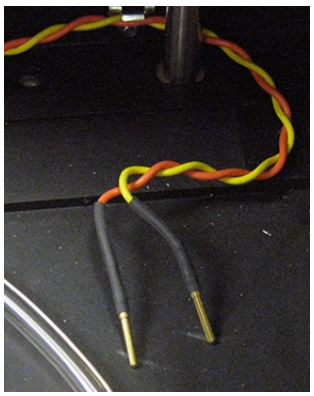


Figure 7-9: Water level sensor wires inside the instrument

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 . See Figure 7-7.
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 7-10), and for more access.

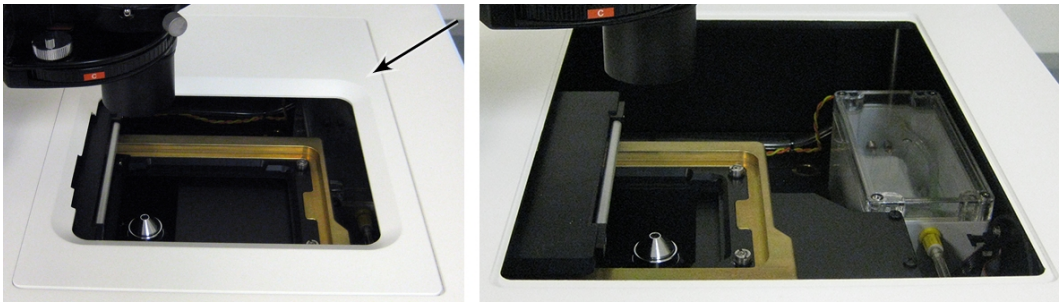


Figure 7-10: Access panel around the top door on and off

4. Locate the water reservoir through the top opening.

Figure 7-11: ImageXpress Environmental Control option water reservoir

5. 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 . See Figure 7-10.
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 126.

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 .
3. Verify that the tubing from the 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.

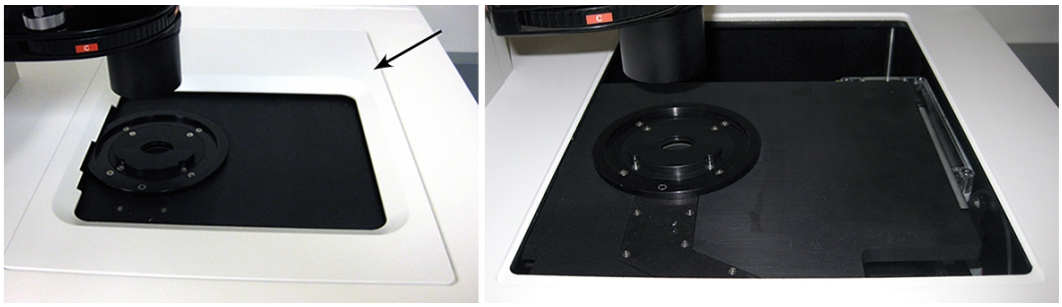


Figure 7-12: Top access panel around the top door on and off

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.

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 131.

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.

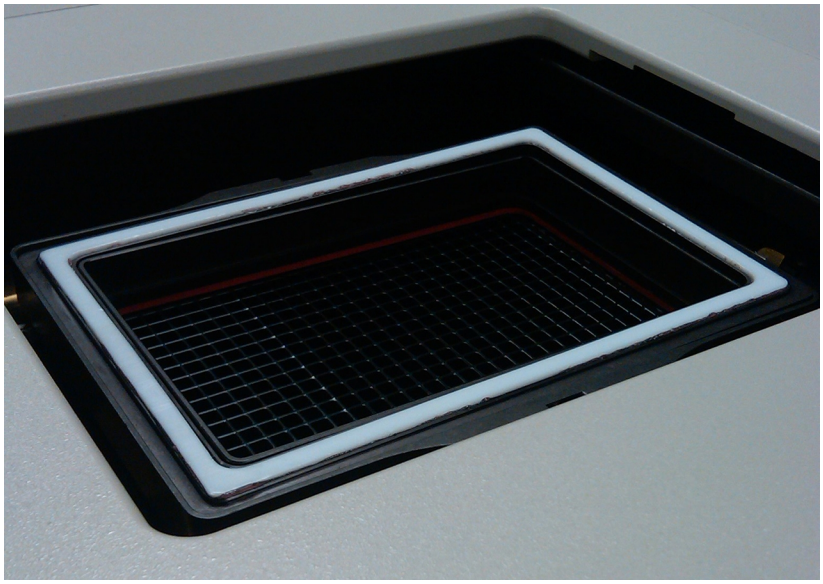


Figure 7-13: Environment Control option live-cell sealing ring

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.

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 141.

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

Environmental Control components to be cleaned include:

- Cleaning Carbon Dioxide Tubing
- Cleaning the Water Reservoir

Transmitted Light Options

Operational information is included for the following:

Transmitted Light Hardware

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

- **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 138.
- **Transmitted Light shutter**. Controlled by the software. The shutter protects the sample from the transmitted light when not imaging. The shutter window maintains the environment of the sample. See Figure 7-16.
- **Hinge**.Increases access to the plate if necessary. See Figure 7-17.

Figure 7-14: ImageXpress Nano System with the Transmitted Light option

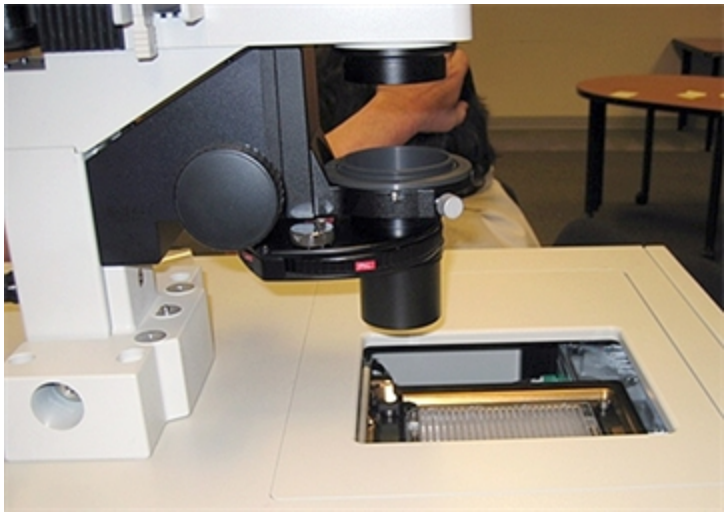


Figure 7-15: ImageXpress System with Transmitted Light option (detailed view)

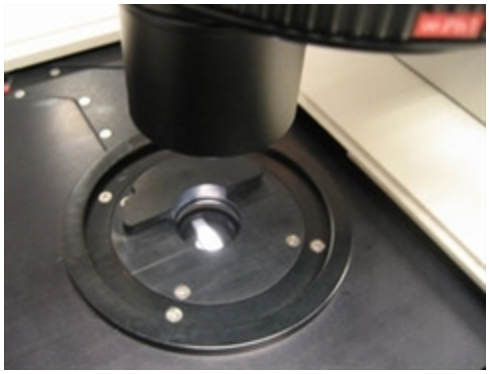


Figure 7-16: Transmitted Light shutter

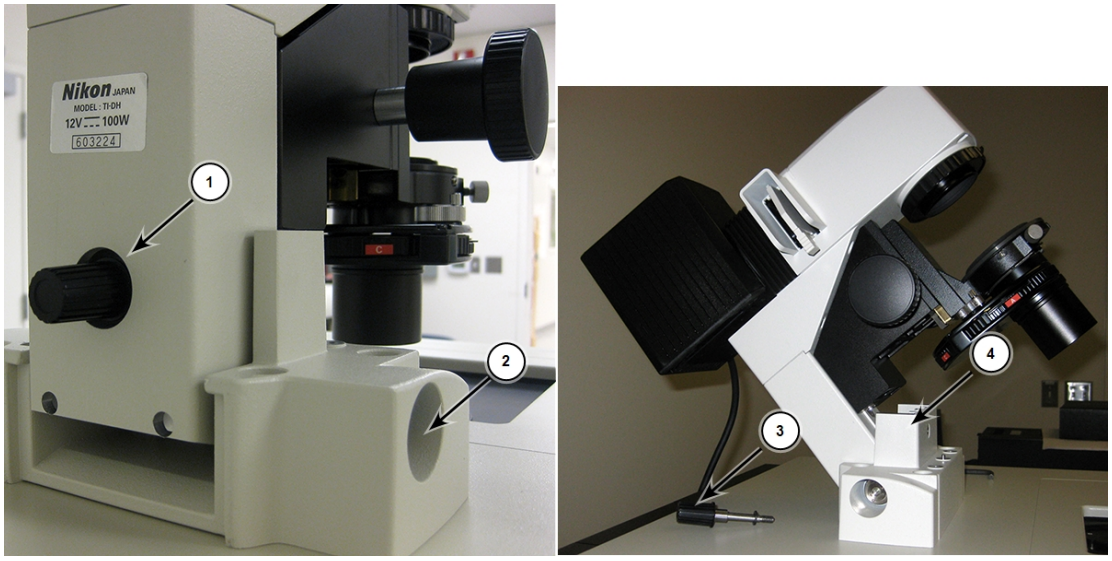


Figure 7-17: Hinge-release knob and hinge

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

Setting up Koehler Illumination

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

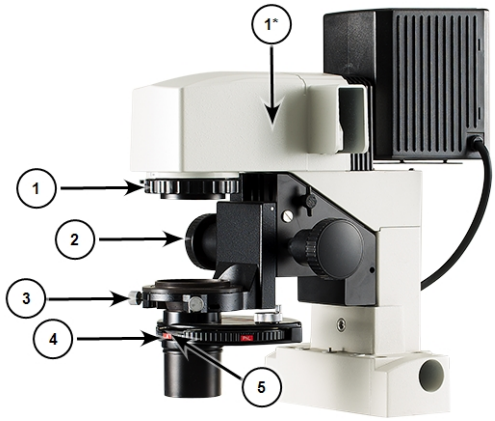


Table 7-2: ImageXpress Transmitted Light option overview

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.
10. Adjust the condenser height with the condenser focus control (2) until a bright polygon surrounded by sharp dark edges is visible.

Figure 7-18: 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.

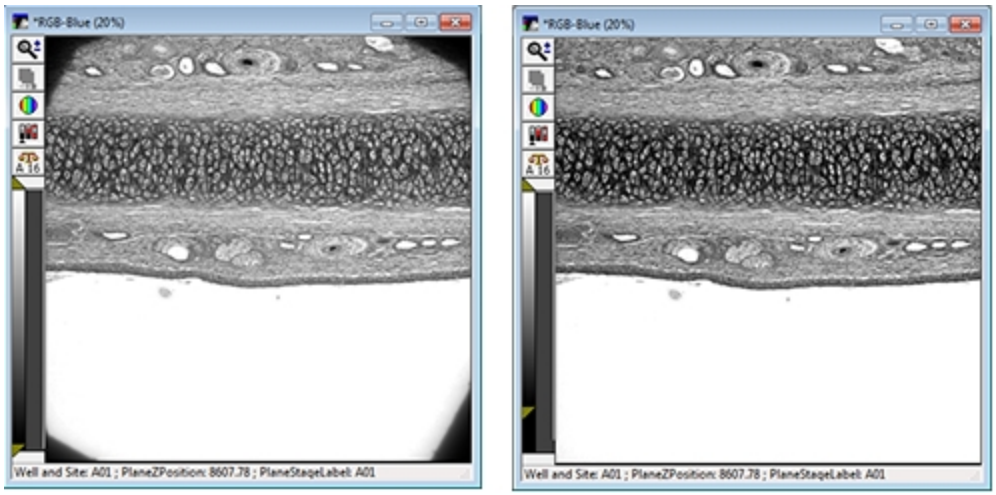


Figure 7-19: Adjustment of the Field Aperture Diaphragm Comparison

The adjustment is now complete.

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 163.

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.

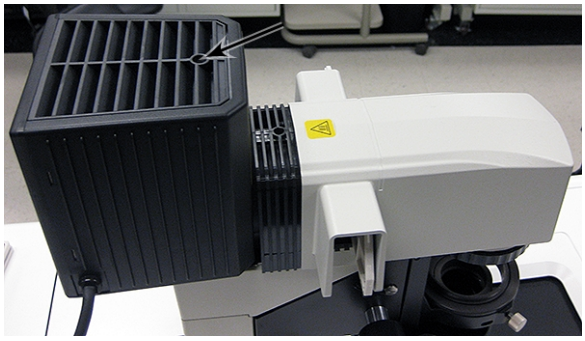


Figure 7-20: Set screw location

3. Remove cover by lifting it upward.

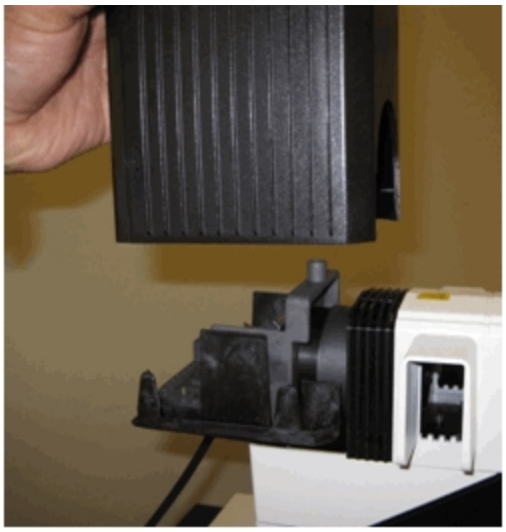


Figure 7-21: Cover removal

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

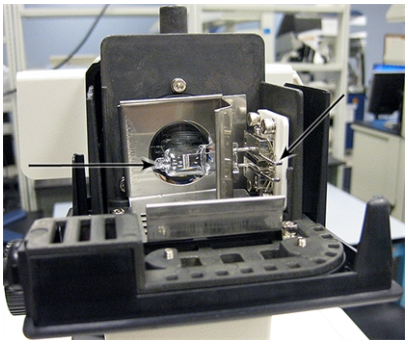


Figure 7-22: Bulb and lever

5. Put on cotton gloves to handle the bulb.

CAUTION! Never touch the bulb with bare hands.

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

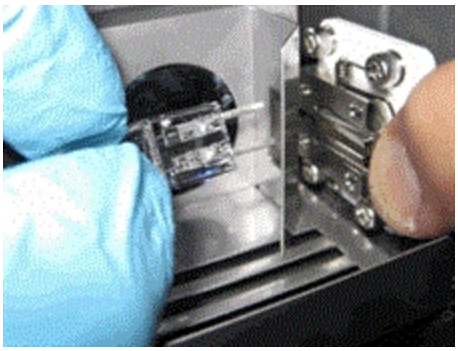


Figure 7-23: Press lever and remove bulb

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.

Chapter 8: Maintenance

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See Obtaining Support on page 149.

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.

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.
- Some procedures require that you disconnect the USB connection to the hardware server (host) PC, and turn off any attached peripherals.
- Access only the user-serviceable components inside the enclosure as described in the procedure. Avoid contact with other components as they can be damaged or knocked out of alignment.

  CAUTION! Do not touch the autofocus laser.

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

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

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.
- 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 149.
- Do not use ultraviolet light for sterilization, as this can damage plastic components.
- Do not use any organic solvents.
- Do not pour or squirt water or alcohol directly onto the instrument, to prevent damaging internal components.

Light Source Maintenance

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

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

The external light source for the ImageXpress Nano System is a solid-state light source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source. When the light source needs replacing, contact Molecular Devices. See Obtaining Support on page 149.

Objective Maintenance

Objective lenses can be cleaned while inside the instrument, or you can remove the objective lenses from the instrument for cleaning. .

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: Objectives must be replaced in their original positions. Molecular Devices recommends removing and maintaining only one objective at a time.

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.

Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives

The ELWD (extra long working distance) Nikon objectives that can be supplied with the 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 141.

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 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**.
4. 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.

5. Rotate the correction collar to its new setting. If needed, replace or tighten the objective.
6. If you accessed the objective from the side, close the upper, hinged side panel door.
7. Click **Continue**.
8. If you accessed the objective from the top, then replace the plate or slide holder when prompted, and then click **Continue**.
9. 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.

Updating Shading Correction Settings-Legacy

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

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

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.

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 146.
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 149.

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 149.

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 149.
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**.

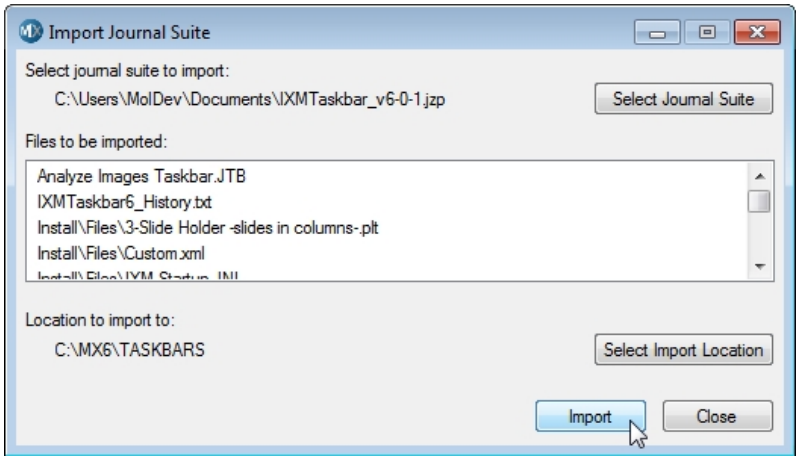


Figure 8-1: Import Journal Suite dialog

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**.

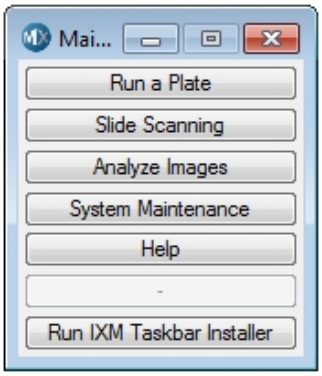


Figure 8-2: The Main Taskbar dialog

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.

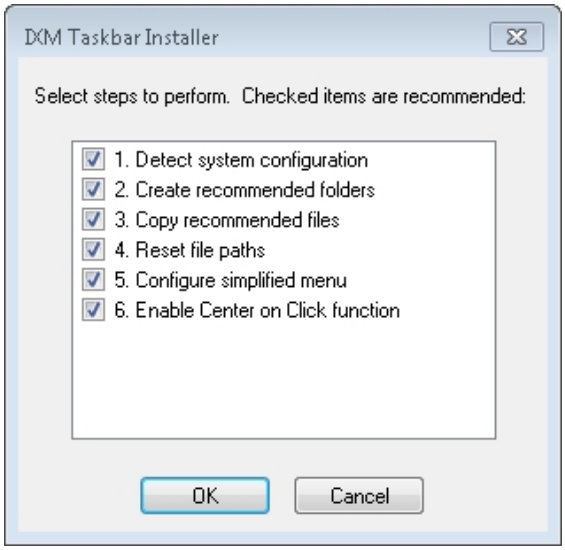


Figure 8-3: IXM Taskbar Installer

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 17.
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 149.

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 status light on the front of the ImageXpress Nano System illuminate with colors that provide information about the instrument status.

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.

- 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.
- For all systems, the serial number is located on the back connector panel of your instrument.

Figure 8-4: Serial Number location on the back of ImageXpress Nano

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 153.

Logging AxoTrace Software Messages to a .txt File

The AxoTrace Software tracks the hardware status of the ImageXpress Nano 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 8-5: 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.

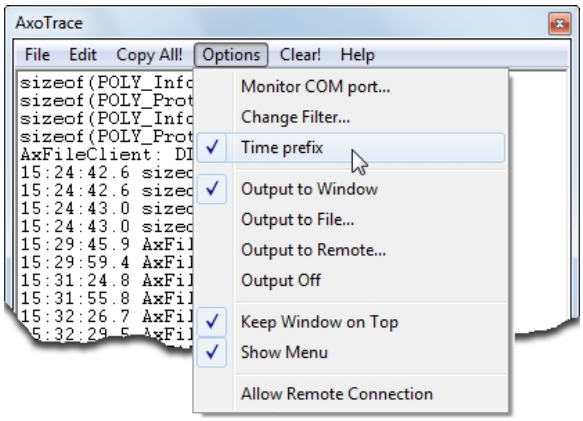


Figure 8-6: AxoTrace Software Options menu

5. Click **File > Exit** to close the AxoTrace window.
6. Start the MetaXpress Software.
7. Turn off the ImageXpress Nano 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 149.

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.

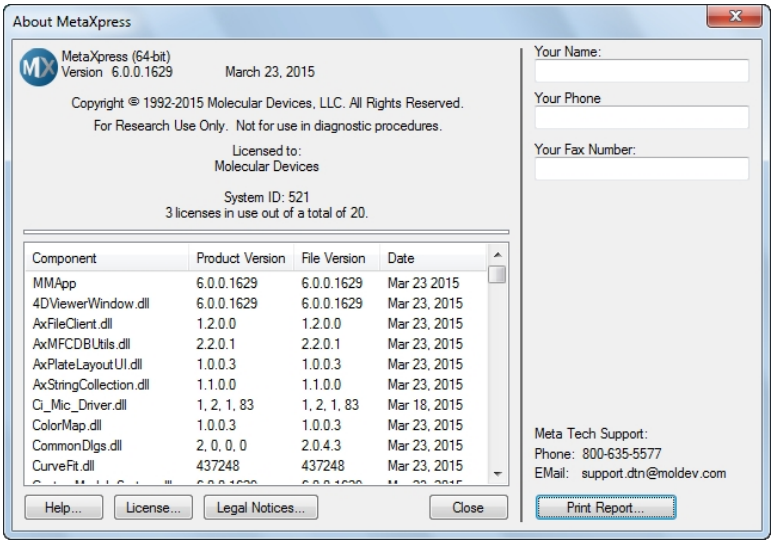
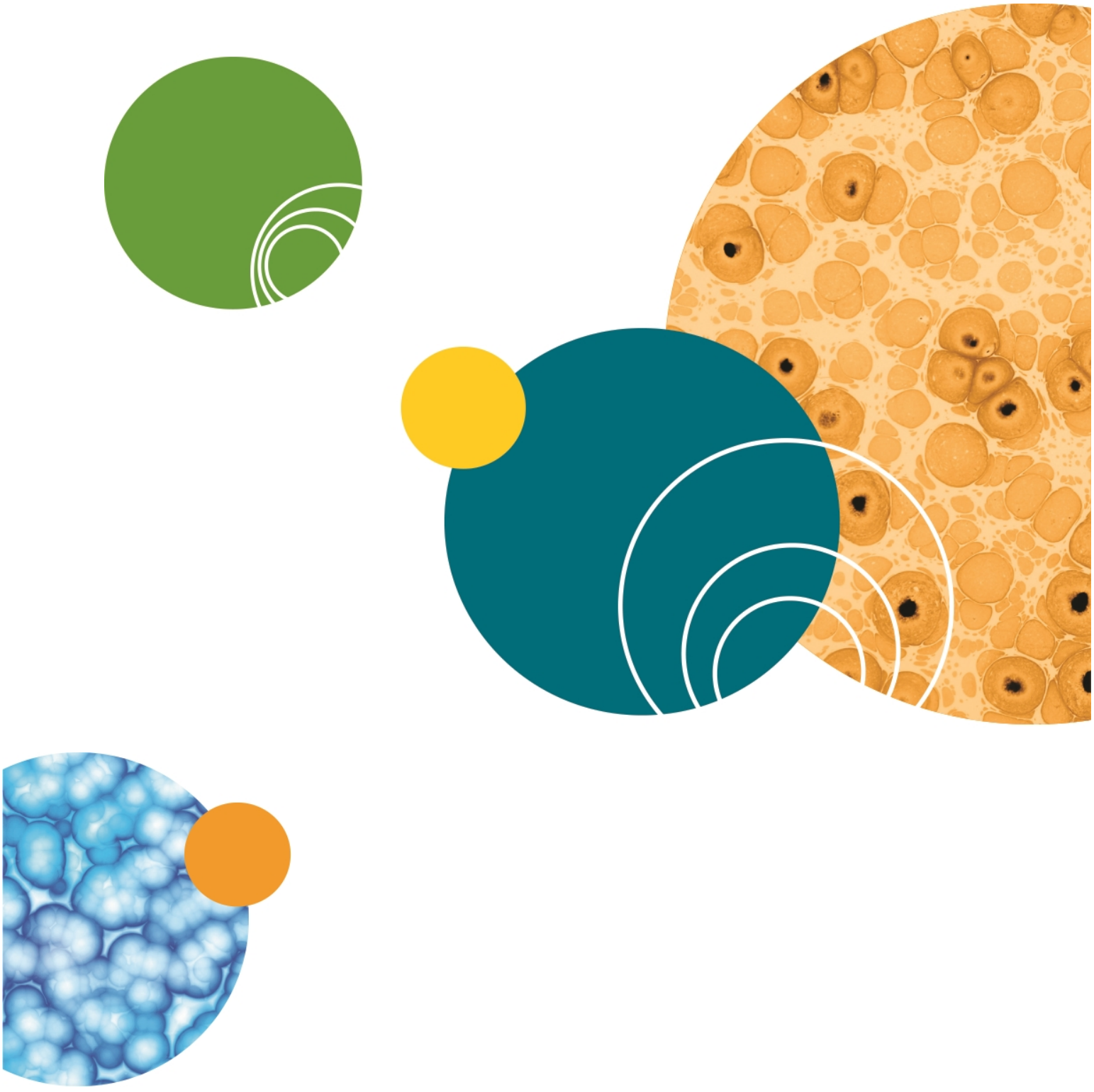


Figure 8-7: About MetaXpress Software

3. Click **Print Report**.
4. In the **Print Setup** dialog, select to print to a PDF file or to a text file and then click **OK**.
5. Send the report file to appropriate Molecular Devices support personnel as an email attachment.



Appendix A: Instrument Specifications

A

The instrument must be installed on a level and stable surface. 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 Nano 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.

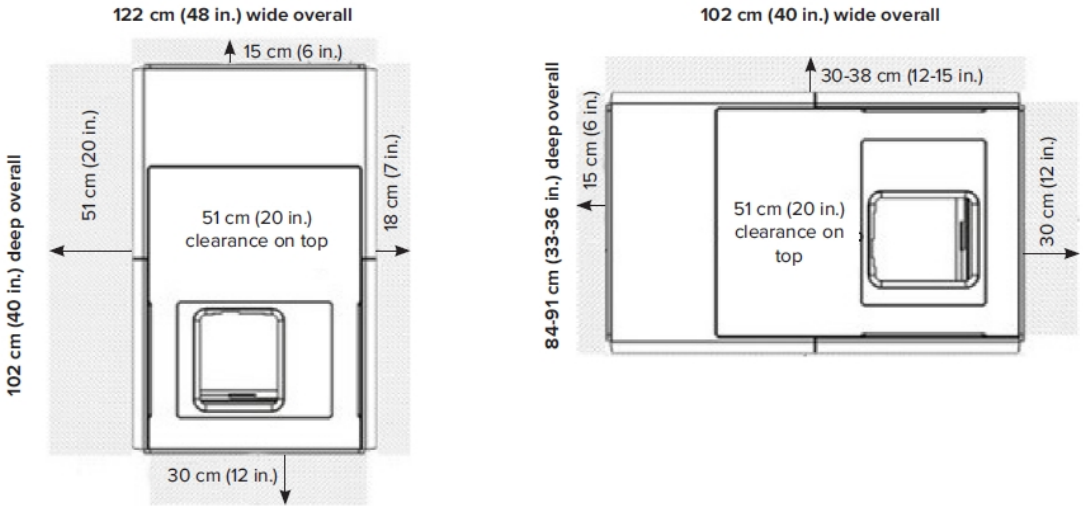


Figure A-1: Front and Sideways Installation Space Requirements

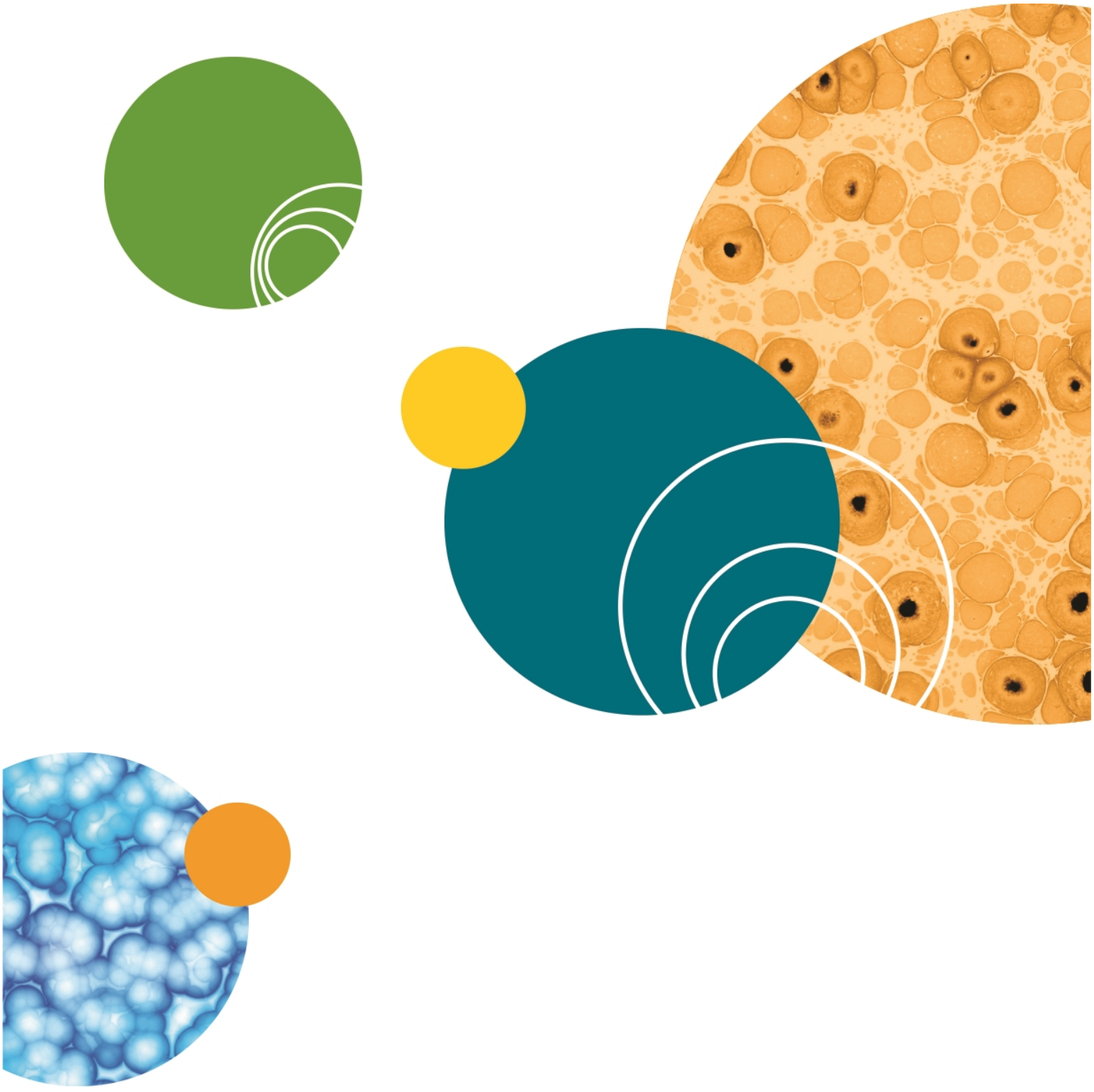
Table A-1: Operational and Environmental Specifications for the Instrument Without Options

Item	Description
Operating environment	Indoor use only
Systems power and options controller input	100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum
Light source power	220 W, 24 V, AC to DC converter included
Computer power input	100 VAC to 240 VAC, 50/60 Hz
Computer monitor power input	100 VAC to 240 VAC, 50/60 Hz
Weight, base unit instrument, no options	93 kg (205 lbs.)
Weight, external light source	7 kg (16 lbs)
Weight, external system power and options controller no options	10 kg (22 lbs)
Weight, computer and monitor	16 kg (35 lbs)
Ambient operating temperature	18°C to 30°C
Humidity restrictions	35% to 50% non-condensing
Altitude restrictions	Not to exceed 2000 m (6562 ft)
ISM Equipment class	1
IEC Installation category	II
IEC Pollution degree	2
IEC Ingress Protection	IP20

Instrument Dimensions

Figure A-2: Instrument Height, Width, and Length

Figure A-3: Distance Between the Outside Edges of the Instrument Feet



Appendix B: Compatible Objectives

B

The following table details the Nikon objectives that are compatible with the ImageXpress Nano System. It also provides microplate compatibility information for the objectives used in the system.

Table B-1: Nikon Objectives Compatible with the ImageXpress Nano System and Settings

Objective Magnification and Type	Molecular Devices Part Number	Numerical Aperture (NA)	Working Distance	Plate Compatibility
2x Plan Apo Lambda	1-6300-0451	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 Plan Apo Lambda	1-6300-0121	0.20	20 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	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)
20x Super Plan Fluor ELWD cc 0 mm to 2 mm	6500-0108	0.45	8.1 mm to 7.0 mm	Thin bottom (0.17 mm)*, 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	0.60	3.7 mm to 2.7 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 Nano System and Settings (continued)

Objective Magnification and Type	Molecular Devices Part Number	Numerical Aperture (NA)	Working Distance	Plate Compatibility
60x Super Plan Fluor ELWD cc 0.1 mm to 1.3 mm	6500-0110	0.70	1.8 mm to 2.62 mm	Thin bottom (0.17 mm)*, Thin bottom (0.17 mm) No Skirt[3] Thick bottom (0.25 mm to 1 mm)

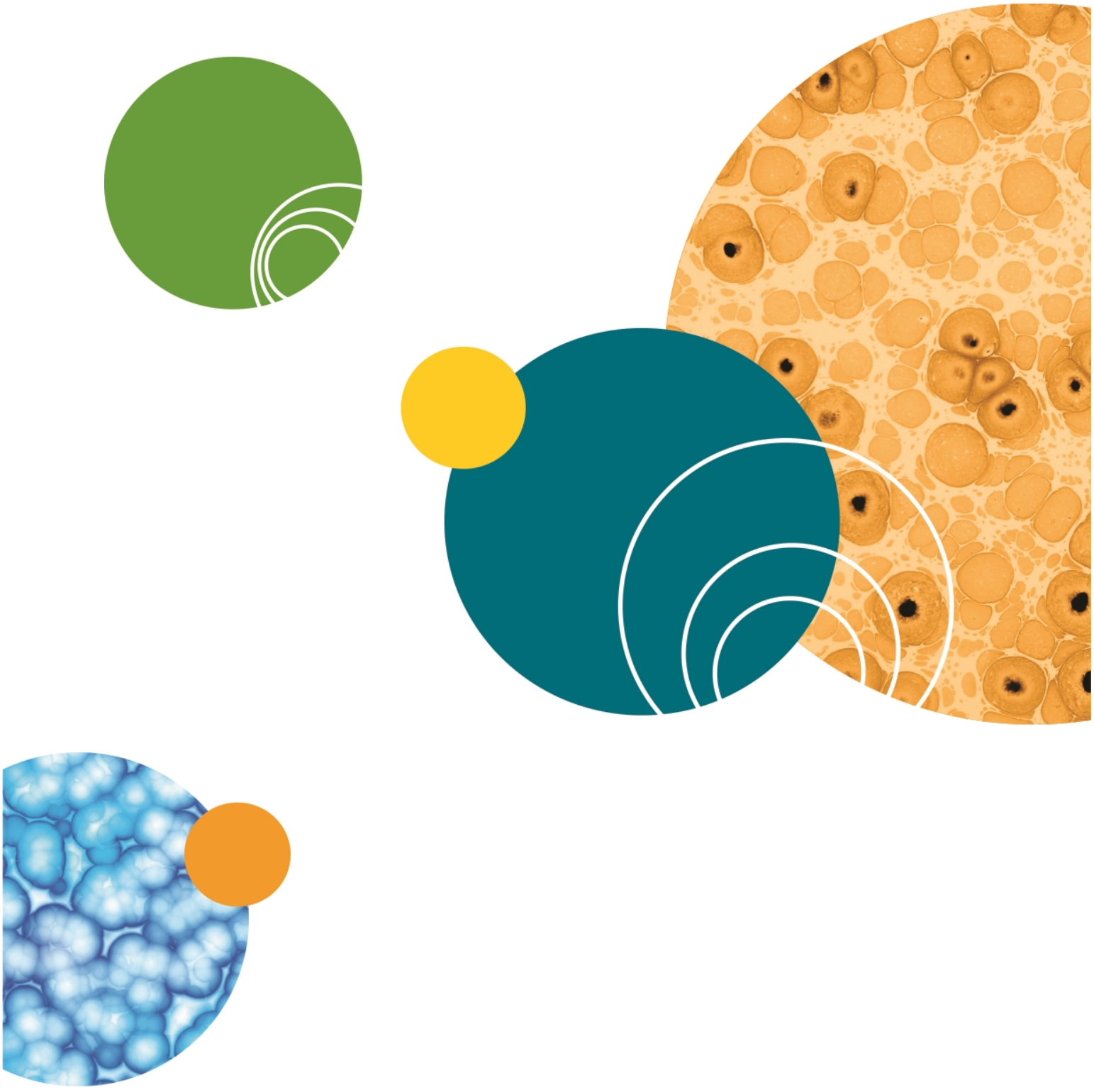
*20x and 40x ELWD image through cover slips, but other objectives give better resolution and shorter exposures.

Appendix C: Filter Specifications

C

Table C-1: Filter Specifications for the ImageXpress Nano System

Filter	Wavelengths	Shading Correction Plate
DAPI	Excitation: 377/50 nm Emission: 447/60 nm Dichroic: 409 nm	Green Pink
CFP	Excitation: 438/24 nm Emission: 483/32 nm Dichroic: 458 nm	Pink
GFP	Excitation: 472/30 nm Emission: 520/35 nm Dichroic: 495 nm	Pink Red
FITC	Excitation: 474/27 nm Emission: 525/45 nm Dichroic: 495 nm	Pink Red
YFP	Excitation: 500/24 nm Emission: 542/27 nm Dichroic: 520 nm	Pink Red
TRITC	Excitation: 543/22 nm Emission: 593/40 nm Dichroic: 562 nm	Red
Cy3	Excitation: 531/40 nm Emission: 593/40 nm Dichroic: 562 nm	Green
Texas Red	Excitation: 562/40 nm Emission: 624/40 nm Dichroic: 593 nm	Red
Cy5	Excitation: 628/40 nm Emission: 692/40 nm Dichroic: 660 nm	Red

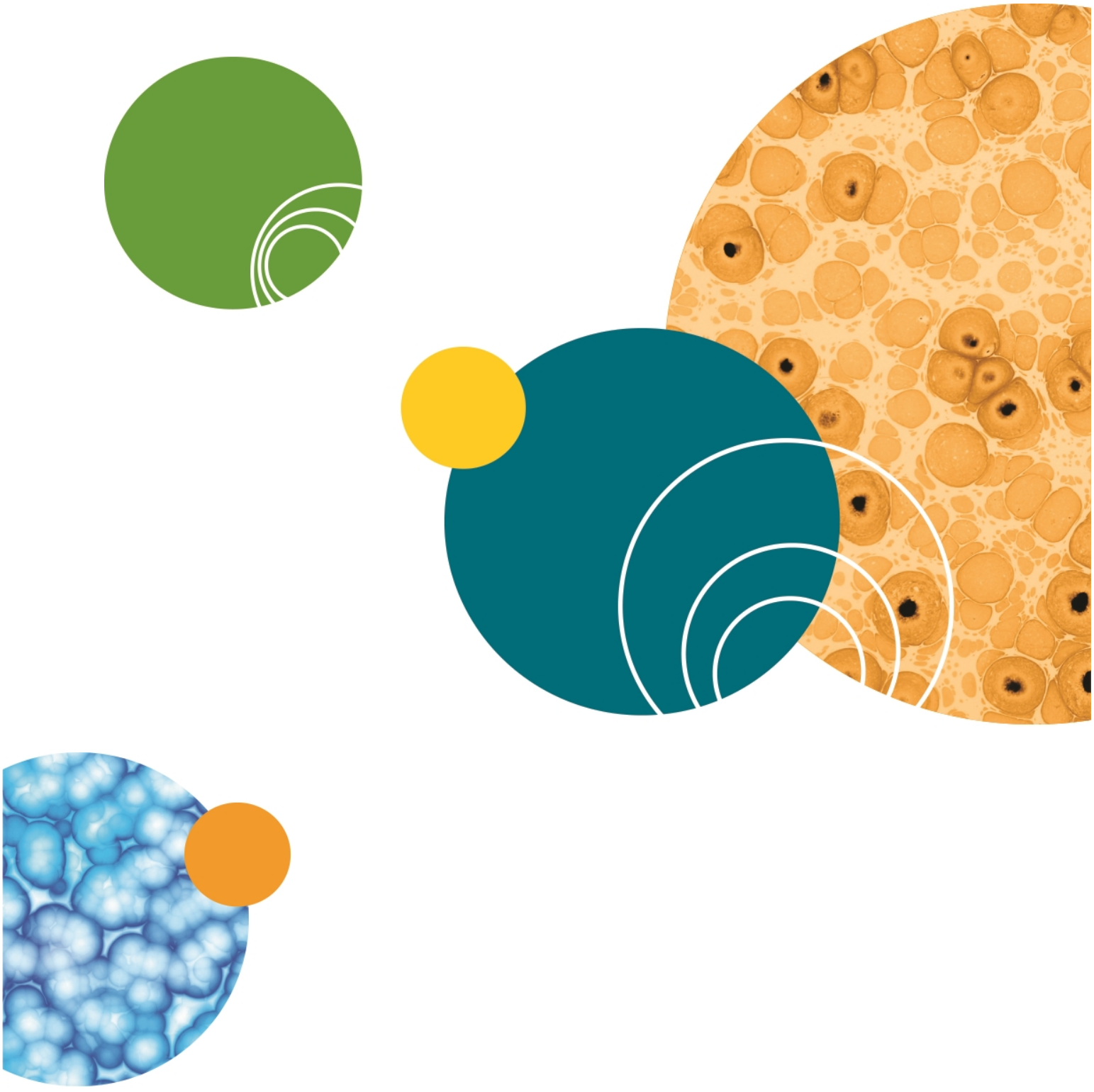


Appendix D: Replacement Parts and Optional Extras

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 Nano System

Part Number	Description
—	For a List of Compatible Nikon Objectives see Compatible Objectives on page 159
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
5048412	Accessory Tool Kit
5033647	Replacement Solid-State Light Source
1-3335-0005	12 V Halogen Lamp for Nikon Transmitted Light (TL) Tower



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: Electromagnetic Compatibility

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)

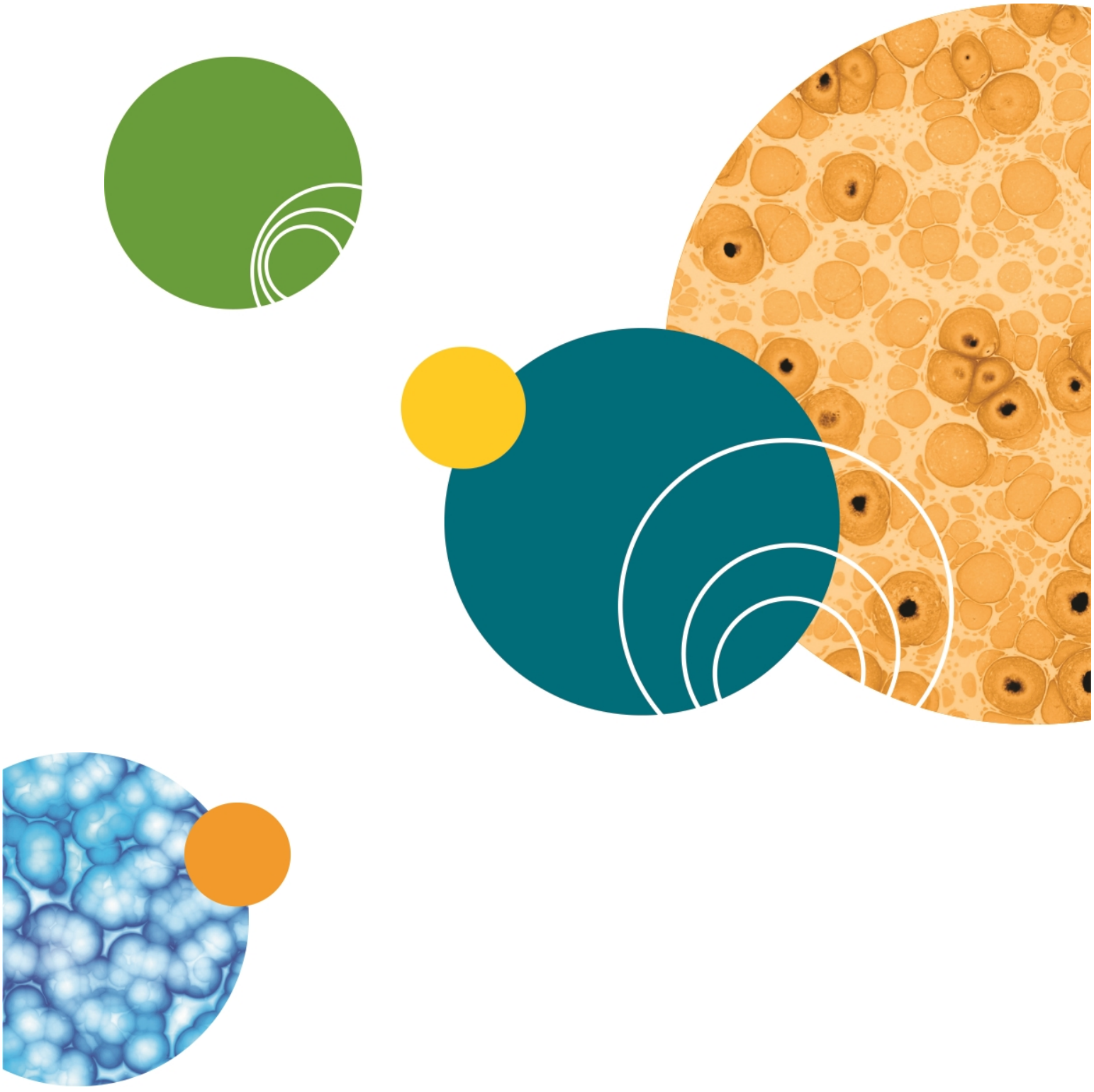
This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power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



Index

A

B

C

D

E

F

H

I

J

K

L

M

R

S

T

W

X

Z

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