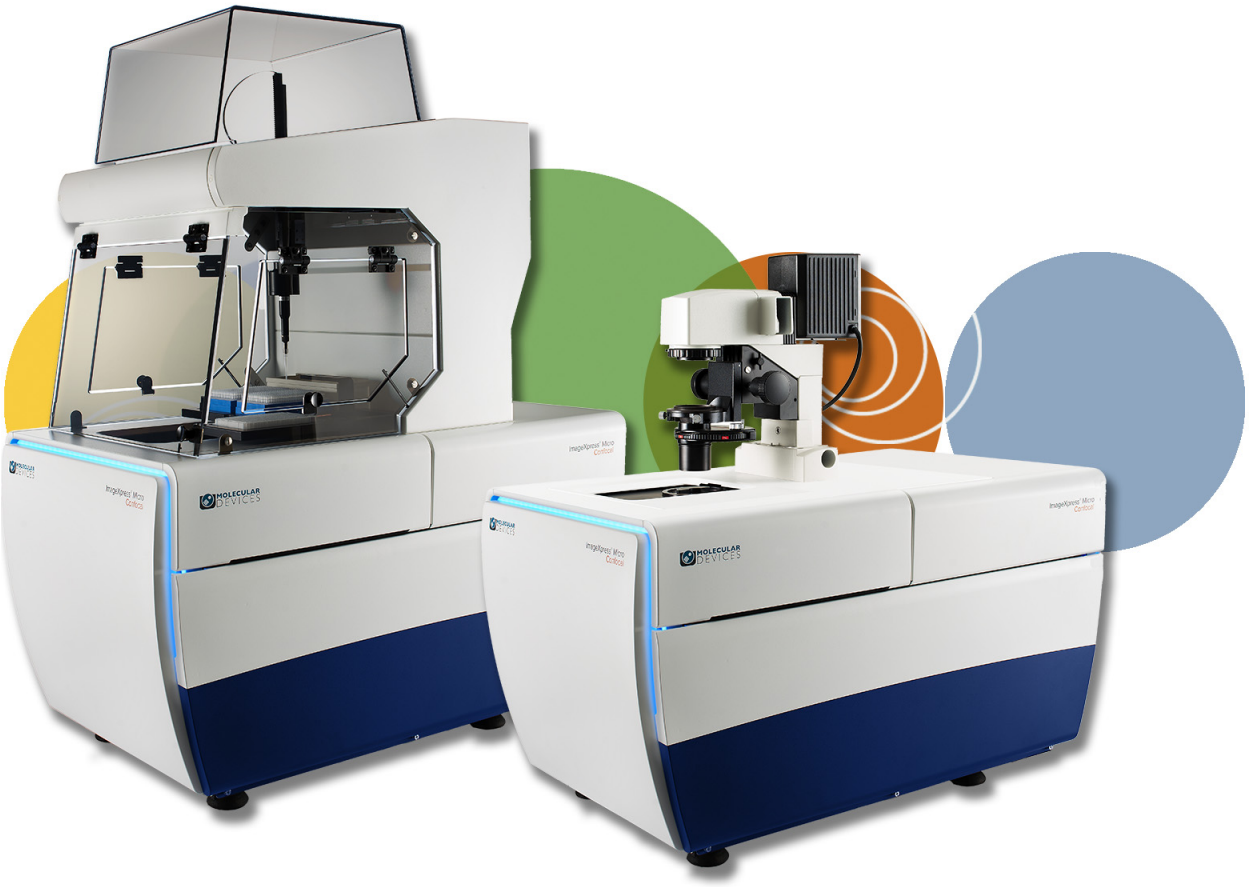

ImageXpress® Micro

High Content Imaging System

Options

User Guide

MOLECULAR DEVICES

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September 2015

Product manufactured by Molecular Devices, LLC.

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

Molecular Devices, LLC is ISO 9001 registered.

Contents

Preface

Who this User Guide is For

This user guide is written for those who want to use an ImageXpress® Micro High Content Imaging System with any of the following optional components:

- ImageXpress Environment Control option
- ImageXpress Fluidics option
- ImageXpress Transmitted Light option

The ImageXpress Environment Control option, ImageXpress Fluidics option, and ImageXpress Transmitted Light option described in this guide are available for only the ImageXpress Micro High Content Imaging System.

Conventions

In this user guide, the following typographical conventions are used.

WARNING! A warning indicates an operation that can cause personal injury if precautions are not followed.

CAUTION! Indicates an operation that can cause damage to the instrument, device, or data, if the precautions are not followed.

Note: Provides essential information for the completion of a procedure.

Tip: Provides useful information that helps apply the techniques and procedures in the text to your specific needs, and can also provide shortcuts. The information in a tip is not essential to the completion of a procedure.

MetaXpress Software

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

- The acquisition workflow involves configuring settings, acquiring images, and storing plate data in a database.
- The analysis workflow consists of processing, enhancing, and analyzing acquired plate data. See the *MetaXpress High Content Image Acquisition & Analysis Software Analysis Guide* included on the MetaXpress Software installation USB flash drive.

Simplified Menu Structure

An optional simplified menu structure can be installed to reduce the number of top-level menus 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**.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, http://www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Part of effective communication with Molecular Devices is determining the channels of support for the ImageXpress Micro System, including the MetaXpress Software. Molecular Devices provides a wide range of support:

- Documentation: Check the guides that are included on the installation media and the help that is available within the MetaXpress Software. Help for an active dialog can be accessed by pressing F1 on your keyboard.
- Online knowledge base: The knowledge base has links to technical notes, software upgrades, newsletters, user guides, and other resources. Visit the Molecular Devices Support web page at http://www.moleculardevices.com/support and follow the links to the knowledge base.
- MetaMorph Software forum: This forum has information on journal scripts and custom modules, and has links to videos and webinars that can help you troubleshoot problems and be more productive using the software. Visit the forum at metamorph.moleculardevices.com/forum.

- Technical Support:

  Phone: Contact Technical Support at (800)-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

  Online: Visit http://www.moleculardevices.com/support and follow the links in the knowledge base to the Technical Support Request Form to send an email to a group of experienced Technical Support representatives.

  Please have the system ID number, system serial number, software version number, and the name of the system owner available when you call.

  - To find your system ID number, in the MetaXpress Software, click **Help > About MetaXpress**. The About dialog displays your system ID number.
  - The system serial number is located on your instrument.
- Additional support resources include:
  - Nikon web-based microscopy course: http://www.microscopyu.com
  - The Molecular Probes Handbook: http://www.lifetechnologies.com/us/en/home/references/molecular-probes-the-handbook.html
    This resource offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis.
  - The following sites offer filter information:
    - http://www.chroma.com
    - http://www.semrock.com
    - http://www.omegafilters.com

Environment Control Option Operations

1

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

The ImageXpress Environment Control option can be installed together with either the Fluidics option or the Transmitted Light option.

See also:

- Fluidics Option Operations on page 31
- Transmitted Light Option Operations on page 55

This chapter contains the following sections:

Environment Control Hardware

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

- **Temperature control** within the upper half of the base instrument. Warm air is provided from the Environmental Control Option Controller or the Systems Power & Options Controller through an air hose. Feedback from temperature sensors installed near the plate maintains the temperature. The temperature can be controlled within a range of 30°C to 40°C, when the ambient room temperature is 22°C or lower.
- **Carbon dioxide** is provided from a customer-supplied tank of pre-mixed 5% CO_2 and 95% air. The tank regulator must be set between 15 PSI and 20 PSI. The Environmental Control Option Controller or the Systems Power & 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 conducts 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 Environment Control option installation:

- **ImageXpress Micro Environmental Control Option Controller** or the **ImageXpress Micro Systems Power & Options Controller**. See Figure 1-1 on page 11 and Figure 1-2 on page 11.
- **Warm air hose and carbon dioxide tubing**. See Figure 1-3 on page 12 and Figure 1-4 on page 12.
- **Temperature sensors**. To ensure accurate readings, the sensors are located near the sample plate.
- **Water reservoir**. See Figure 1-5 on page 13.
- **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 1-6 on page 13.

Figure 1-1: Environmental Control Option Controller used for the ImageXpress Micro Standard and XLS Systems

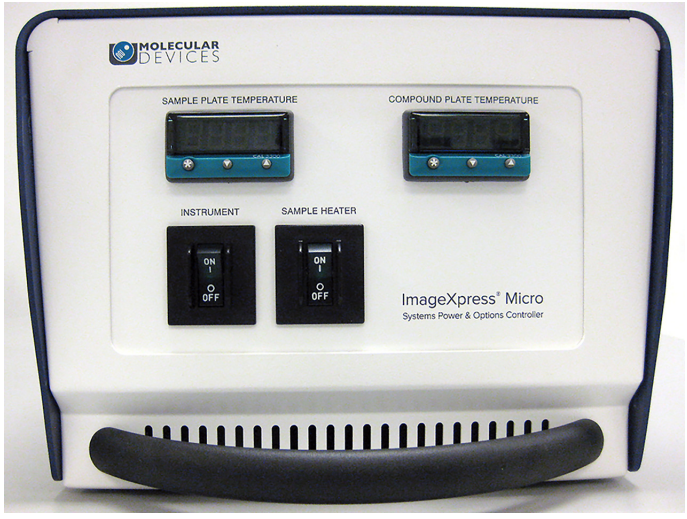


Figure 1-2: Systems Power & Options Controller used for the ImageXpress Micro Confocal System

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

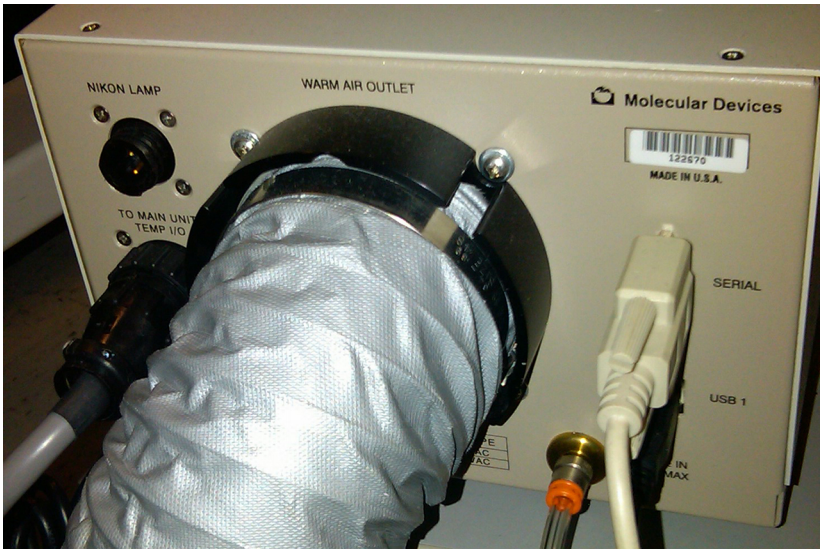


Figure 1-3: Environment Control option warm air hose and carbon dioxide tubing on the back of the Environmental Control Option Controller

Figure 1-4: Environment Control option warm air hose and carbon dioxide tubing on the back of the ImageXpress Micro Standard System

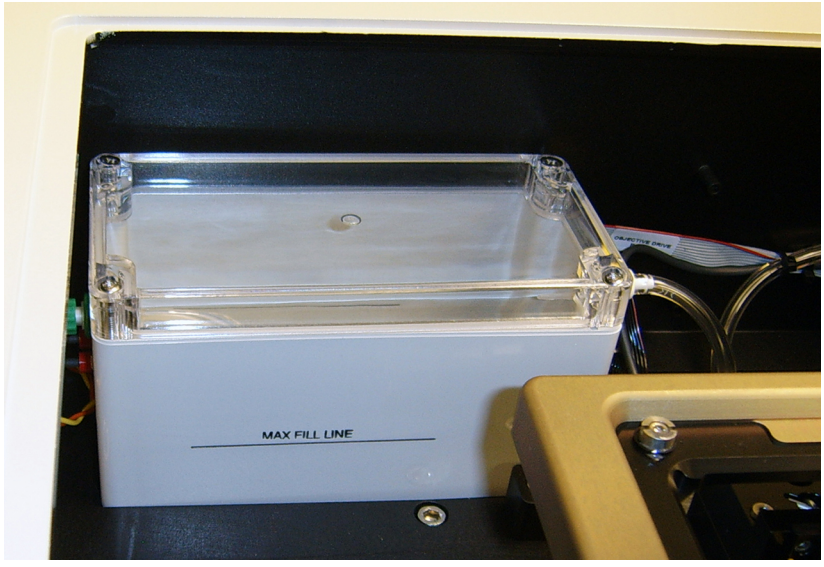


Figure 1-5: Environment Control option water reservoir

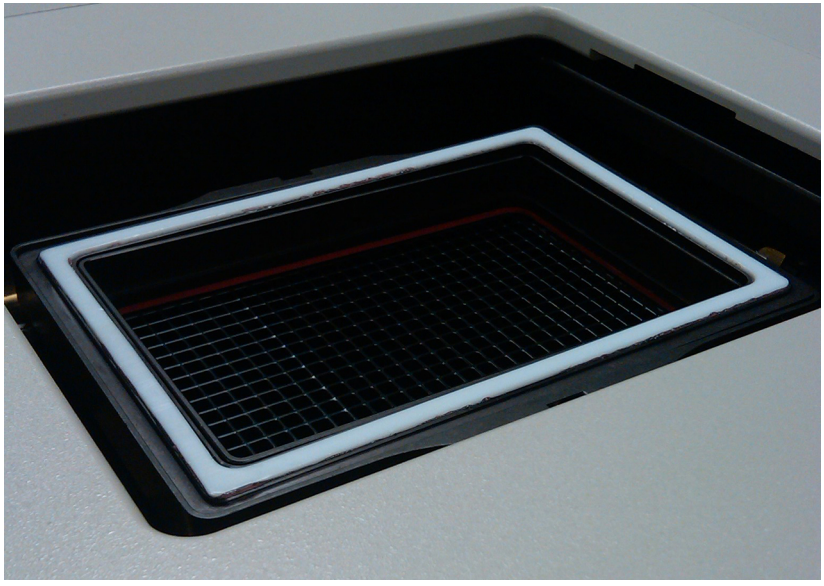


Figure 1-6: Environment Control option live-cell sealing ring

Items to Be Provided by the Customer

The customer must provide the following items for the Environment 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 Environmental Control Option Controller or the Systems Power & Options Controller.
- Microwell plates 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

An FSE (Field Service Engineer) installs the ImageXpress Environment Control option. After installation, environmental control can be set up for experiments.

Setting the Temperature

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

To set the temperature

1. On the front of the Environmental Control Option Controller or the Systems Power & Options Controller, view the current sample plate temperature.

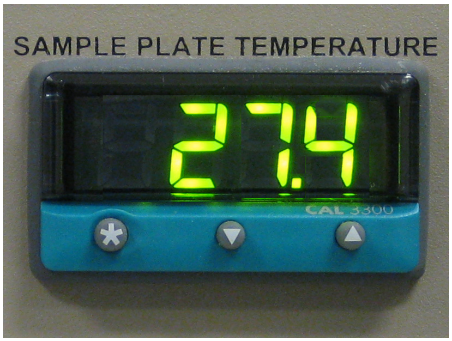


Figure 1-7: Sample Plate Temperature control interface

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

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

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

Setting Up the Water Reservoir

Air supplied to the environmental enclosure flows through the water reservoir to make sure the air has a high degree of humidity.

Tools required for setup:

- Syringe
- Deionized Water (preferably sterilized)

To set up the water reservoir

1. In the MetaXpress software, click **Screening** > **Plate acquisition and Control** > **Eject Plate** to open the top door of the instrument.
2. Remove the insert that surrounds the top door.
3. Disconnect the CO_2/air tubing from the luer lock on the rear of the instrument.
4. Insert a syringe filled with deionized water into the luer lock.
5. View the water reservoir through the open top panel.

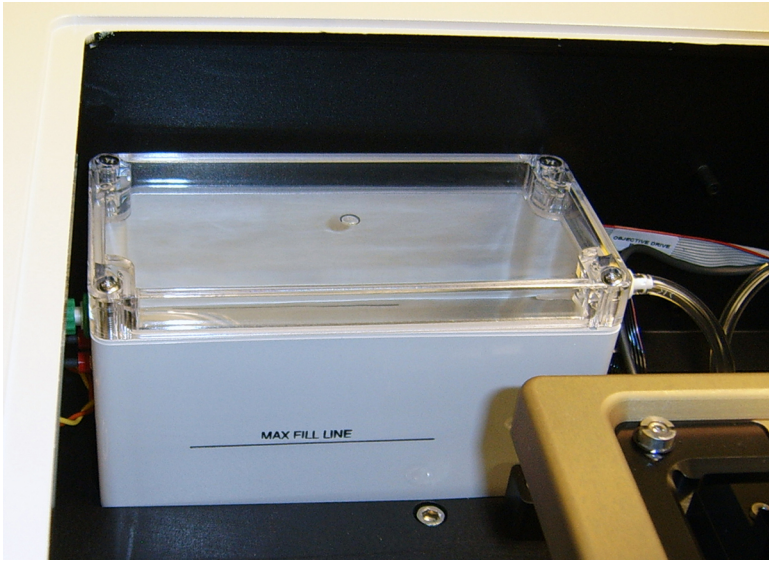


Figure 1-8: Environment Control option water reservoir

6. Dispense the deionized water from the syringe into the water reservoir until the water reaches the fill line.
7. Remove the syringe from the luer lock.
8. Reconnect the CO_2/air tubing to the luer lock.
9. Replace the insert that surrounds the top door.
10. In the MetaXpress software, click **Screening** > **Plate acquisition and Control** > **Load Plate** to close the top door of the instrument.

Setting Up the Carbon Dioxide

A pre-mixed CO_2 tank with a regulator must be set up and connected to the carbon dioxide controller. The carbon dioxide controller then controls the flow rate of carbon dioxide 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 15.

To set up the carbon dioxide

1. Connect the tubing from the pre-mixed CO_2 tank to the Environmental Control Option Controller or the Systems Power & Options Controller.
2. Verify that the tubing from the Environmental Control Option Controller or the Systems Power & Options Controller to the instrument is connected.
3. Connect the regulator to the CO_2 tank.
4. Turn on the CO_2 regulator to approximately 15 PSI to 20 PSI.
5. From the top of the ImageXpress Micro System, lift the insert surrounding the top door and remove it. Keep the top door of the instrument closed.
6. Verify that there is a steady flow of bubbles in the water reservoir.
7. Replace the insert surrounding the top door.
8. Check the environmental control settings to verify that the CO_2 pressure is OK. See Environment Control Software on page 21.

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 18.

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 and Control** > **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.

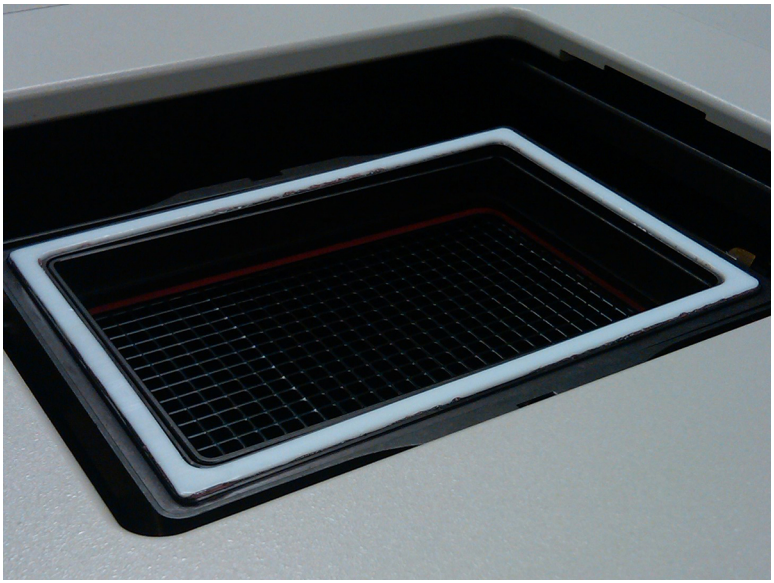


Figure 1-9: Environment Control option live-cell sealing ring

4. In the MetaXpress software, click **Screening** > **Plate acquisition and Control** > **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:

- Allow the instrument to warm up for at least two hours.
- 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.

  The current temperature in the chamber is displayed on the front of the controller.
- 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.

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.

Environment Control Software

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

To check the Environment Control settings

1. In the MetaXpress software, click **Devices > Environment Control.**

   In the simplified menu, click **Control > ImageXpress > Environment Control**.

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

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

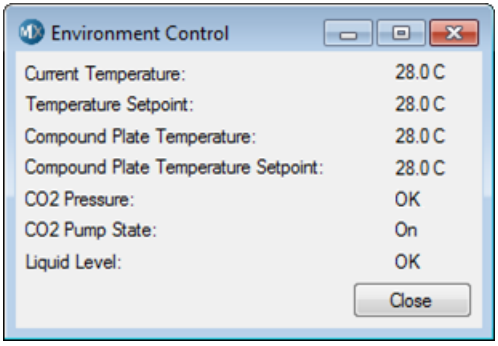


Figure 1-10: Environment Control dialog

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

However, the MetaXpress Software does not control the parameters, such as the level of CO_2 or temperature. These parameters are controlled directly through their hardware devices, such as the temperature controller on the Environmental Control Option Controller or the Systems Power & Options Controller (Figure 1-1 on page 11 and Figure 1-2 on page 11) and the regulator on the carbon dioxide tank.

Environmental Parameters in Image Information

The MetaXpress Software records environmental information when images are captured as shown in Figure 1-11 on page 22.

Note: There are wire sensors in the system to detect water, but the software does not record this as an image annotation.

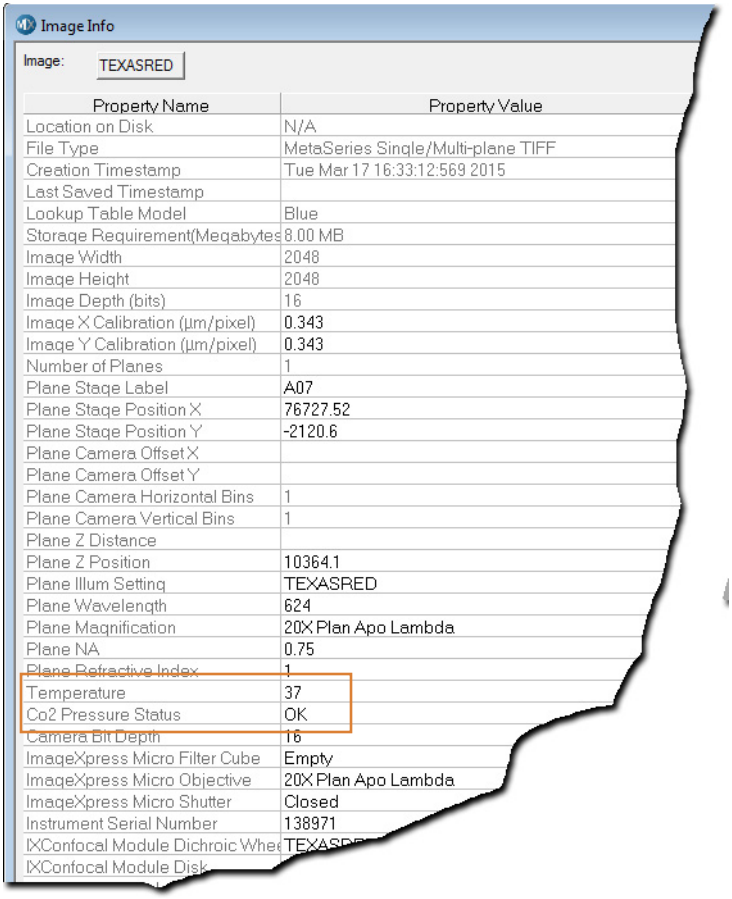


Figure 1-11: Image information annotation with environmental parameters

Doing Timelapse Experiments

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

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

To Set Up a Timelapse Experiment

1. In the MetaXpress software, click **Screening** > **Plate Acquisition Setup**.
2. In the **Plate Acquisition Setup** dialog, in the **Configure** tab, open the Acquisition tab. See Figure 1-12.

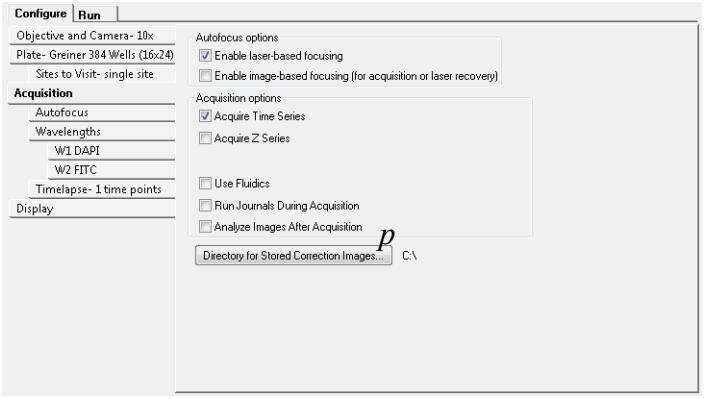


Figure 1-12: Acquire Time Series

3. Select **Acquire Time Series**.

4. Under **Configure**, click **Timelapse** to configure timelapse acquisition options. See Figure 1-13 on page 24.

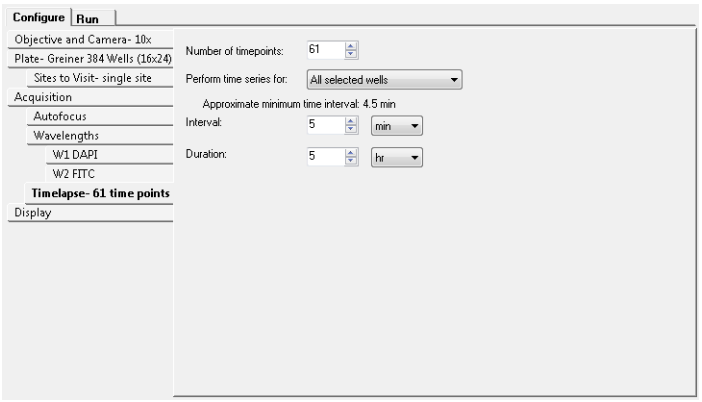


Figure 1-13: Timelapse tab

5. In the **Number of time points** field, specify the number of time points to use. Fluidic events can be associated with these time points. See Doing Fluidics Experiments on page 43.
6. Click **Perform time series for** and select one of the following methods for acquiring a series of timelapse images, corresponding to different types of experiments:
   - **All selected wells** is used for long timelapse experiments. The system images all wells at the first time-point, then all wells at the second time point, and so on.
   - **One well then the next** is used for a fast kinetic experiment. The system images all time points on well 1, then moves to well 2 and images all time points, and so on.
   - **One row then the next or one column then the next** is suitable for an experiment using manual pipetting.

7. If applicable, select additional options on the **Autofocus** tab. See Figure 1-14 on page 25. These options include:
   - **First timepoint only**: Recommended for fast kinetic experiments.
   - **All timepoints**: Recommended for long timelapse experiments.
   - **Every Nth timepoint**: Offers the flexibility to autofocus regularly during a timelapse experiment.

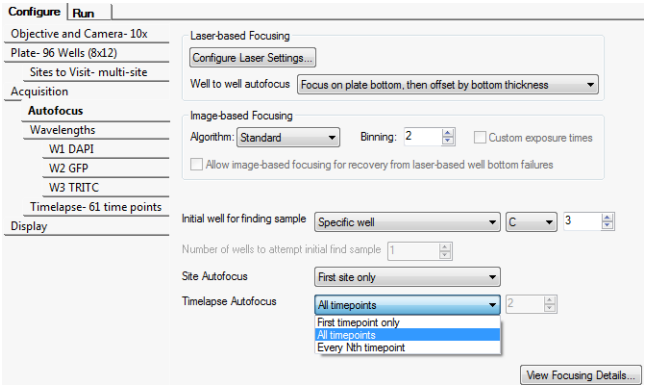


Figure 1-14: Timelapse Autofocus options

8. If applicable, select additional options on the Wavelength tabs. **Wavelength** tabs provide options for acquiring individual wavelength images during a timelapse experiment. See Figure 1-15 on page 26.

   These options include:

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

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

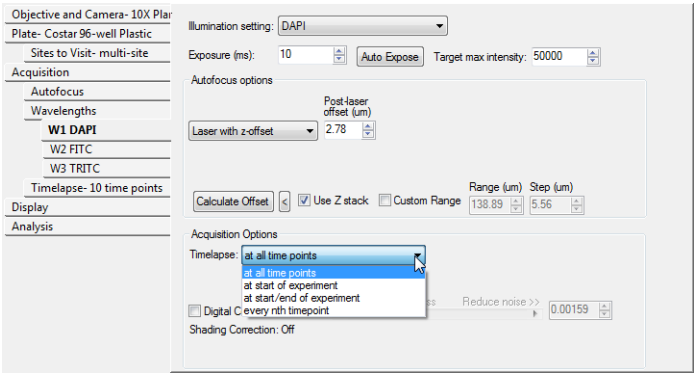


Figure 1-15: The **Timelapse Acquisition** field on the **Wavelength 1** tab

Reviewing Timelapse Data

As with other ImageXpress System data, timelapse data is displayed in the MetaXpress software Review Plate Data dialog.

To review the timelapse data

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

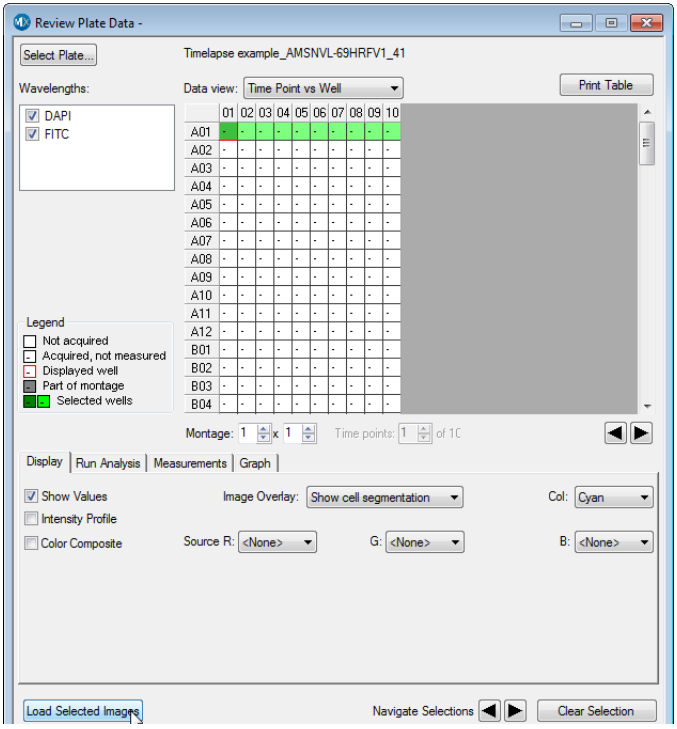


Figure 1-16: Review Plate Data dialog

2. Click **Select Plate**.
3. In the **Select Plate for Review** dialog, select a plate, and then click **Select**.
4. In the **Data view** list, click **Time vs Well**. The montage displays thumbnails of each time point.
5. To create a stack for viewing as a movie, right-click on one well to select it.

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

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

6. Click **Load Selected Images**.

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

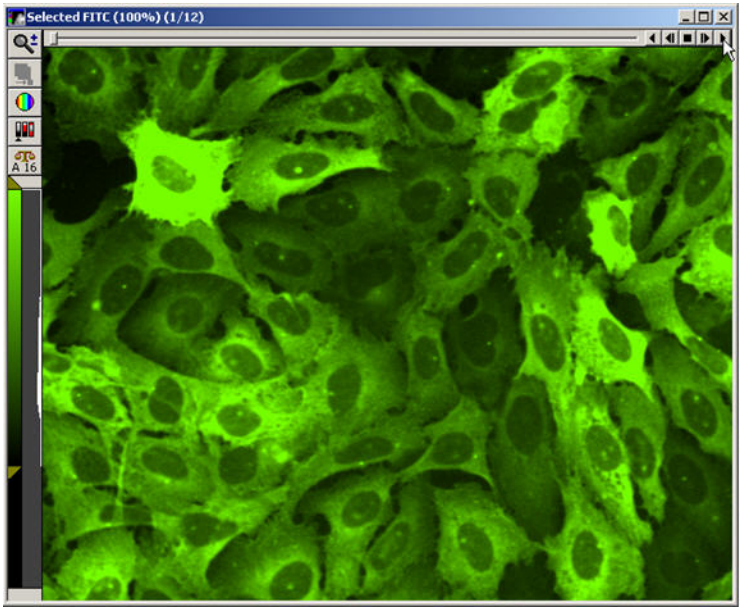


Figure 1-17: Stack of images with navigation buttons above the image pane

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

To make a movie

1. Before you begin, load timelapse images and create a stack. See Reviewing Timelapse Data on page 27.
2. Select the image window of the stack on the MetaXpress Software desktop.
3. In the **MetaXpress** software, click **Stack > Make Movie**.

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

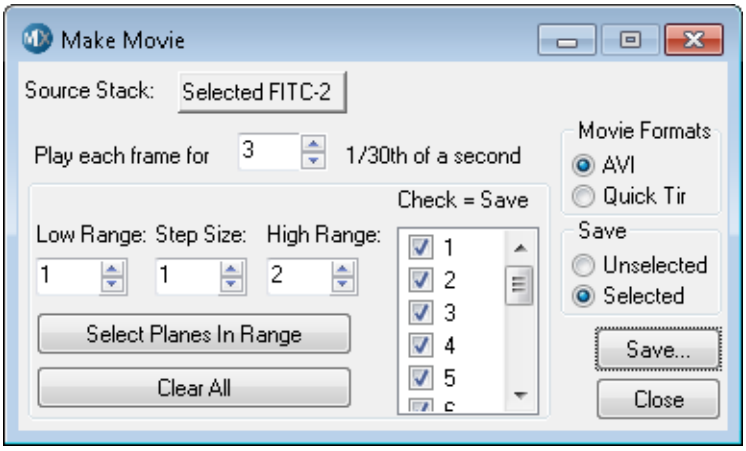
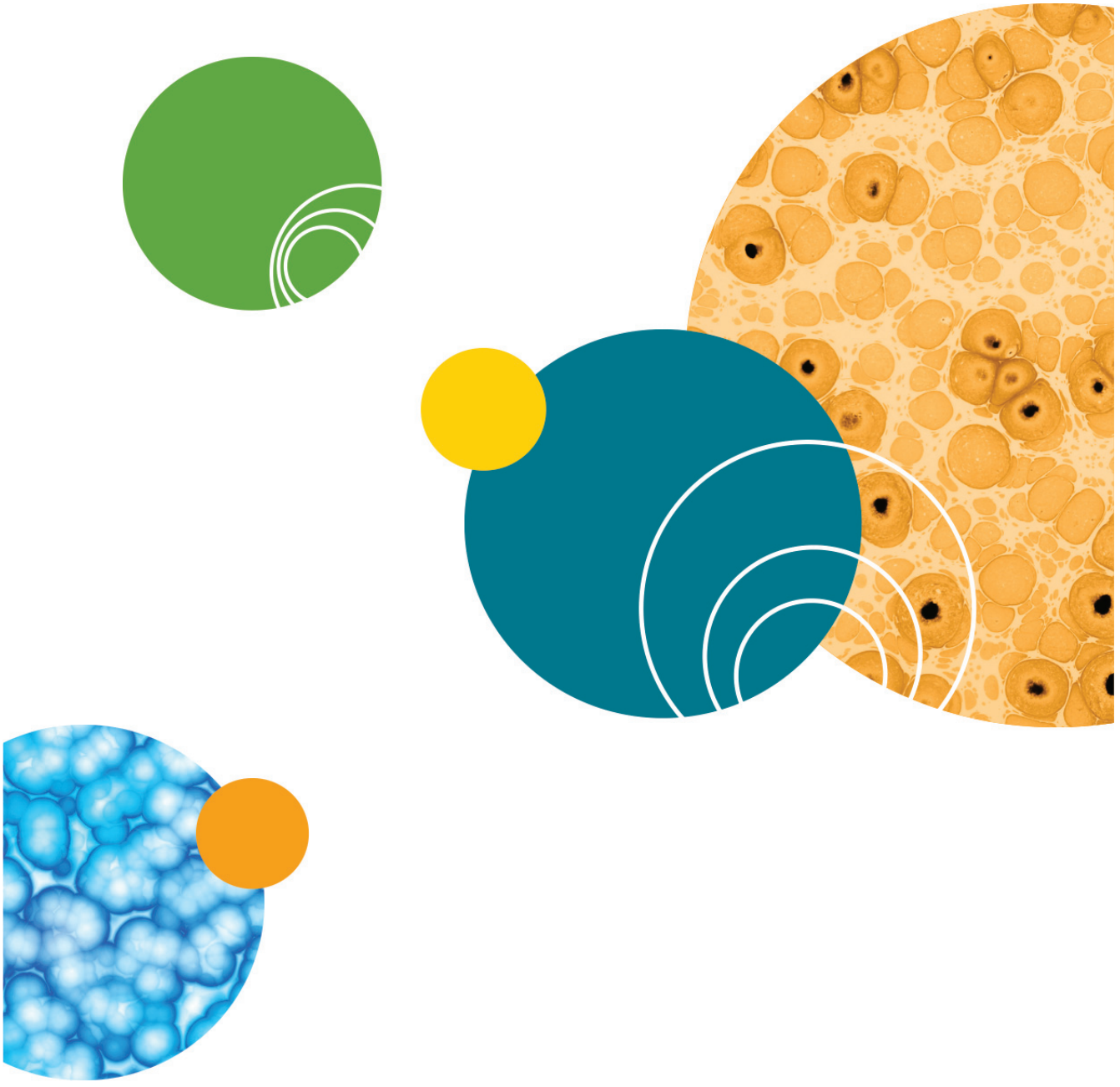


Figure 1-18: Make Movie dialog

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

   For information about the available options, view the application help by pressing F1 while viewing the dialog.
5. Click **Save**.



Fluidics Option Operations

2

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

The robot is capable of dispensing volumes between 3 µL and 200 µL with an accuracy of ±5% or ±1 µL.

The ImageXpress Fluidics option must be installed with the Environment Control option. It is not compatible with the Transmitted Light option.

See Also:

- Environment Control Option Operations on page 9.

This chapter contains the following sections:

Fluidics Hardware

The ImageXpress Fluidics option consists of the following hardware components. See Figure 2-1.

- **Single-channel pipettor**. Used to transfer fluid between two compound/media plates and the sample plate.
- **Shutter**. Allows the system to maintain the environment when not actively pipetting.
- **Plate heaters**. Used to heat the compound plates. These heat a little higher than the displayed temperature, which represents the temperature of the liquid in the well.
- **Tip adapters and tip rack positions**. Compatible with FLIPR® Tetra 96 tips (Part 9000-0761, 50 racks/case) or 384 tips (Part 9000-0763, 50 racks/case).

- **Tip stripper**. Used for ejecting tips.
- **Waste bin**. Used to contain ejected tips and discarded liquid.
- **Doors with safety interlocks**. Prevents doors from being opened during operation.

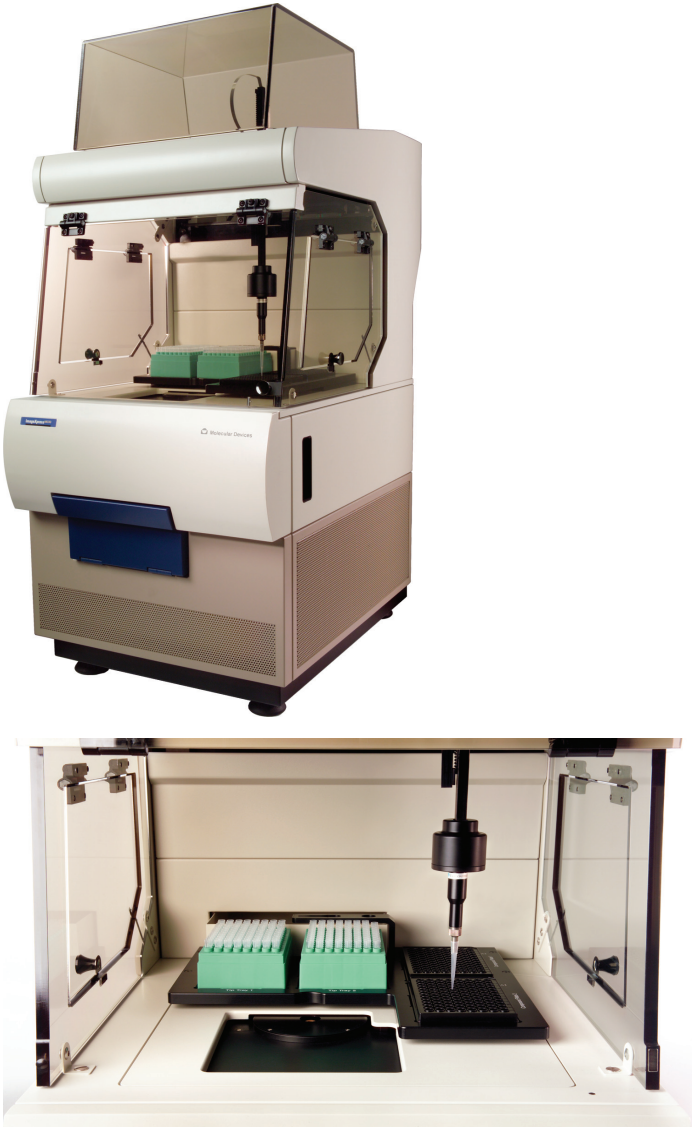


Figure 2-1: Fluidics hardware

Items to Be Provided by the Customer

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

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

Tips

The ImageXpress Fluidics option is compatible with FLIPR Tetra 96 or 384 tips.

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

Plates

The tip and compound plate region inside the environmental enclosure can accommodate up to two compound plates. Molecular Devices recommends using standard 96-well or 384-well plates. U-bottom and V-bottom plates are beneficial when withdrawing and dispensing very small volumes of compound.

Setting Up Fluidics Hardware

The FSE (Field Service Engineer) installs the ImageXpress Fluidics option. After installation, fluidics can be set up for experiments.

To set up the fluidics hardware

1. Load the tip racks. For proper fit and alignment, insert the right side first, and then press the left side down into place.
2. Change the tip adapter as appropriate. The tip adapter should be screwed on firmly but not over-tightened. If the tip adapter is not properly attached, it might not match the fluidics robot calibration.
3. Load the compound plates. For proper fit and alignment, insert the right side first, and then press the left side down into place.
4. Set up environmental control as needed, and allow the compound-plate temperature to reach equilibrium. See Setting Up Environmental Control on page 14.

5. If required, empty the waste bin.
6. Load an unlidded plate with the live-cell sealing ring on the top. See Loading the Sample Plate on page 18.

To Configure Fluidics in Meta Imaging Series Administrator

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

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

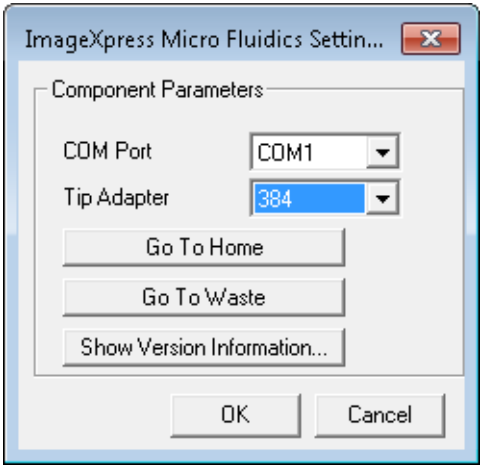


Figure 2-2: ImageXpress Micro Fluidics Settings dialog

5. In the **ImageXpress Micro Fluidics Settings** dialog, If applicable, change the tip adapter or the COM port.

Configuring Fluidics Software (Stations and Properties)

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

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

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

To configure fluidics software

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

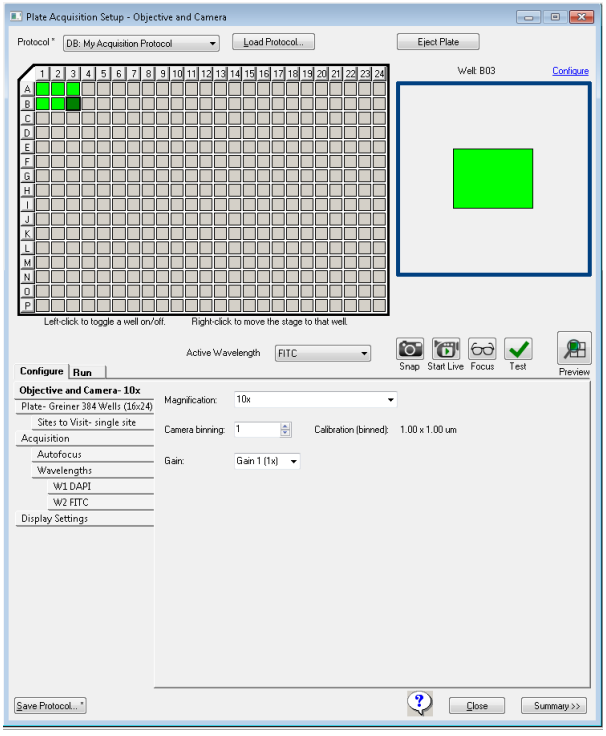


Figure 2-3: Plate Acquisition Setup dialog

2. On the **Plate Acquisition Setup** dialog, on the **Configure** tab, open the **Acquisition** tab.

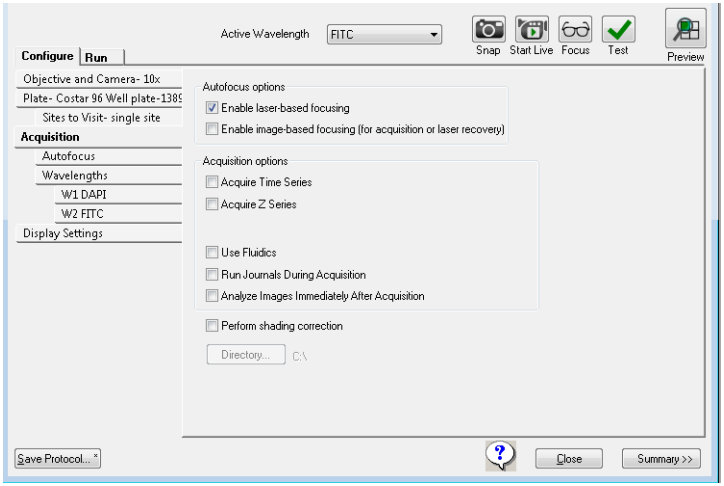


Figure 2-4: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

3. Under **Acquisition**, select **Use Fluidics**.
4. Open the **Fluidics** tab.

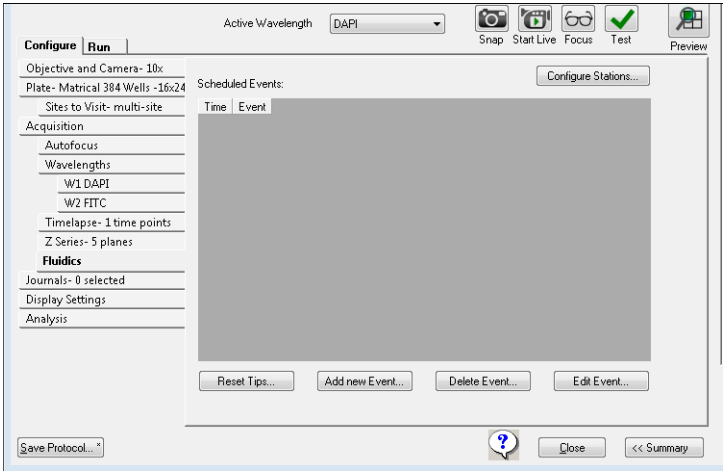


Figure 2-5: Plate Acquisition Setup dialog, Configure tab: Fluidics tab

5. Click **Configure Stations**.

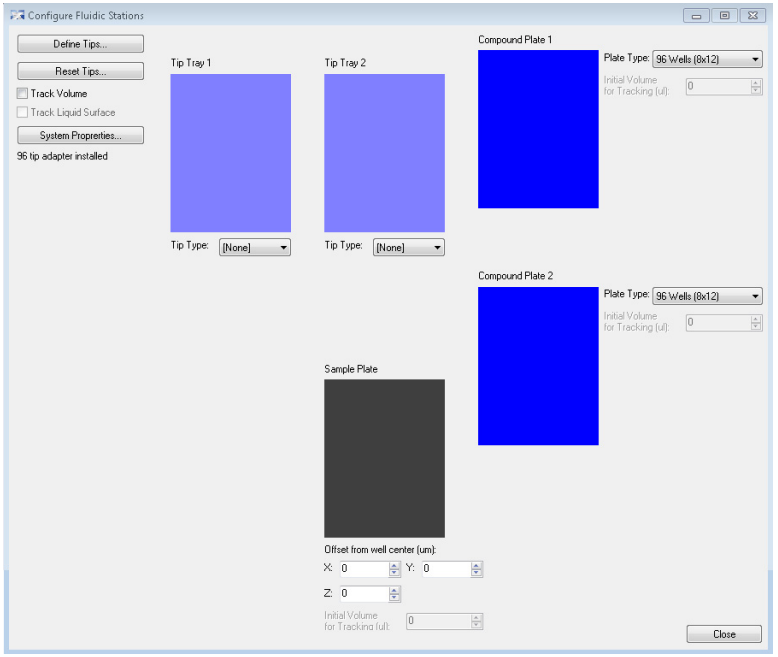


Figure 2-6: Configure Fluidics Stations dialog

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

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

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

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

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

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

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

9. If applicable, select **Track Volume**, and then type a value for the **Initial Volume for Tracking (µl)** for each plate.
10. Do one of the following:
    - If you selected **Track Volume**, then select **Track Liquid Surface,** and then go to Step 11.
    - Optionally, select **Track Volume,** select **Track Liquid Surface,** clear **Track Volume,** and then go to Step 11.
    - Otherwise, go to Step 13.

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

11. Click **System Properties**.

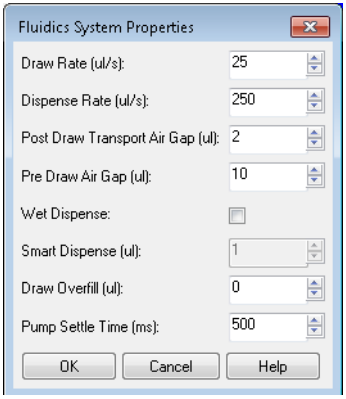


Figure 2-7: Fluidics System Properties dialog

12. On the **Fluidics System Properties** dialog, leave the default values for the fluidic system properties set as-is, or modify any values as needed.

Table 2-1: Fluidic Systems Property values

Option	Description
Draw Rate (µl/s)	The rate at which the pump draws fluid.
Dispense Rate (µl/s)	The rate at which the pump dispenses fluid.
Post Draw Transport Air Gap (µl)	The volume of air that the pump draws after it draws fluid but before the pump arm moves. This air prevents fluid from dripping during pump arm movement. Select this option for fluids such as DMSO.
Pre Draw Air Gap (µl)	The volume of air that the pump draws before drawing fluid. This is used to push remaining drops of fluid out of the tip when dispensing.
Wet Dispense	Specifies whether the tip should be immersed in the fluid during dispense operations at the imaging location. When enabled, if **Track Liquid Surface** is also selected. then dispensing occurs near the liquid surface; otherwise, the dispensing occurs near the bottom of the well.
Smart Dispense (µl)	Enabled only if **Wet Dispense** is selected. The volume that the pump dispenses beyond the requested fluid dispense volume. The additional volume is subtracted from the **Pre Draw Air Gap** volume. The **Smart Dispense** volume must be less than or equal to the **Pre Draw Air Gap** volume.
Draw Overfill (µl)	The volume of fluid that the pump draws in addition to the requested volume of fluid. You can use **Draw Overfill** to increase dispense accuracy when drawing from compound plates. **Note: Draw Overfill** is not applicable for any other plate type.
Pump Settle Time (ms)	The time delay that is used to pause movement of the pump arm after a pump draw or dispense operation to overcome hysteresis. For most operations, 100 ms is the recommended value.

13. Click **OK** to close the **Fluidics System Properties** dialog and return to the **Configure Fluidics Stations** dialog.
14. If applicable, click **Define Tips** and in the **Define Tips** dialog, define additional tip types, and then click **Save**.

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

15. After you have completed a dispensing cycle, to indicate that you have reloaded tip racks and plates, click **Reset Tips,** and on the **Reset Tips/Liquid Levels** dialog, click **Reset** for the appropriate tip trays and plates, and then click **Close**.

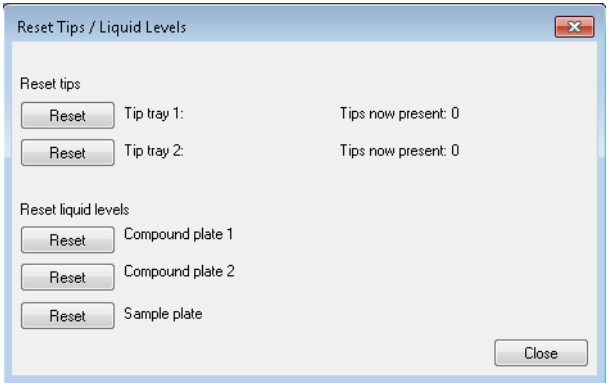


Figure 2-8: Reset Tips/Liquid Levels dialog

Manual Fluidics Control

Do the following procedure to control the fluidics station manually.

Tip: The fluidics interface enables you to transfer fluid from one well of the compound plate to a corresponding well in the sample plate. If you need to transfer fluid between non-corresponding wells, or if different actions need to be performed for different wells, record these actions in a journal (custom routine) to accomplish this. See To record the steps in a journal on page 42. You can add journals as events when you run an experiment. See To configure a fluidics experiment on page 43.

To control fluidics manually

1. In the MetaXpress software, click **Devices > Fluidic Control**.

   In the simplified menu, click **Control > ImageXpress > Fluidic Control**.

2. In the **Fluidic Control** dialog, specify the action you would like to do. You can manually pick up and eject tips, draw and dispense fluid, and mix.

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

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

3. Click **Go**.

   The fluid is drawn from the plate.

4. In the **Action** list, click **Dispense**, and then click **Go** to add the fluid to the plate.

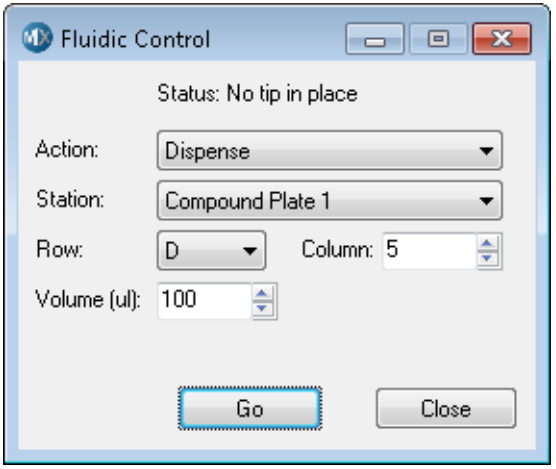


Figure 2-9: Fluidic Control dialog

To record the steps in a journal

1. In the MetaXpress software, on the **Journal** menu, click **Start recording**.
2. Do the steps that you want to record. See To control fluidics manually on page 41.
3. Click **Journal > Stop recording**, and then save the journal file.
4. To modify the journal, click **Journal > Edit Journal**, and then select the journal to be edited. See Figure 2-10 on page 42.

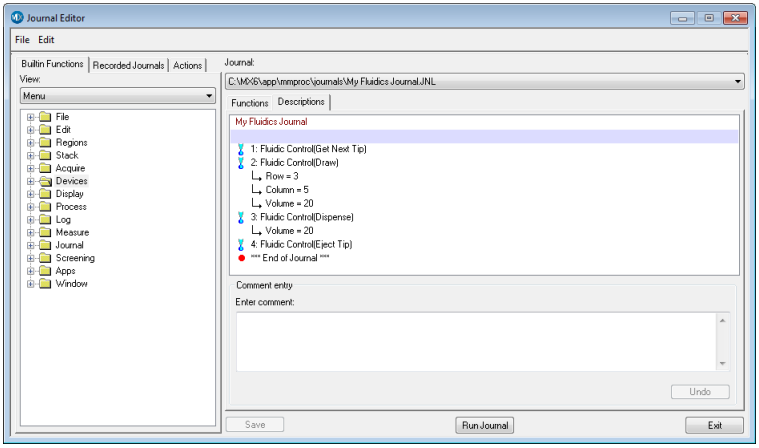


Figure 2-10: Example Journal using manual fluidics control

Doing Fluidics Experiments

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

Do the following procedure to configure a fluidics experiment.

To configure a fluidics experiment

1. In the MetaXpress software, click **Screening > Plate Acquisition Setup**.
2. On the **Plate Acquisition Setup** dialog, on the **Configure** tab, open the **Acquisition** tab.

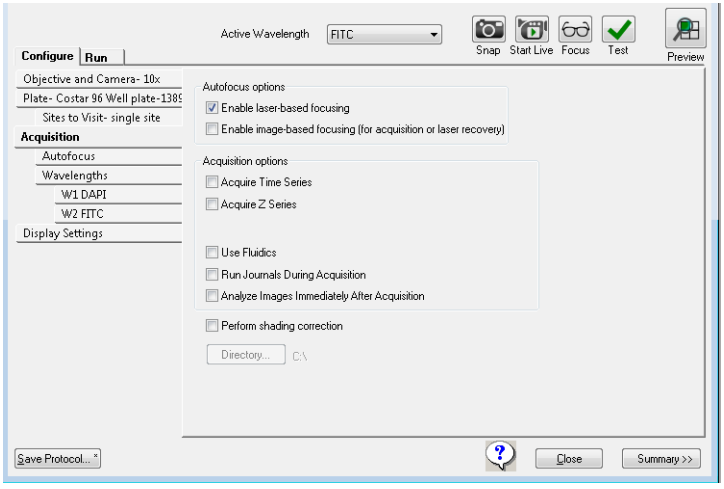


Figure 2-11: Plate Acquisition Setup dialog: Configure tab, Acquisition tab

3. Under **Configure**, click **Fluidics** to schedule fluidics events. See Figure 2-12.

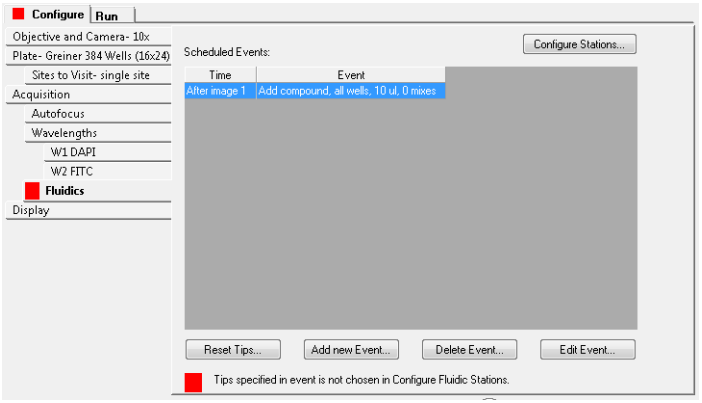


Figure 2-12: Adding events to a fluidics experiment

4. Click **Add new Event** to add an event to the **Scheduled Events** field.
5. In the **Fluidic Event** dialog, specify the time point at which you want the event to occur, and then specify whether the event should occur before or after the time point. See Figure 2-13.

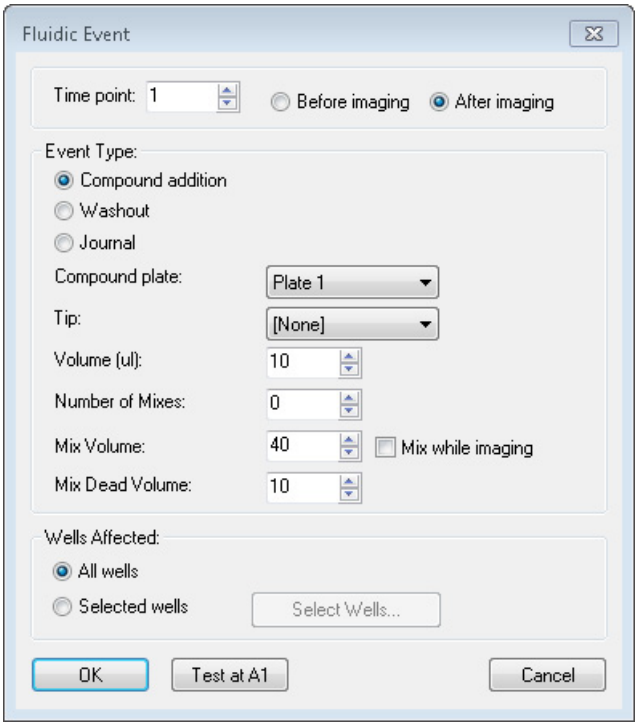


Figure 2-13: Time point setting in Fluidic Event dialog

For example, if you select time point 5 and select **Before imaging**, then the fluidic event occurs before the time point. If you select **After imaging**, then the fluidic event occurs after the time point. See Figure 2-14 on page 45.

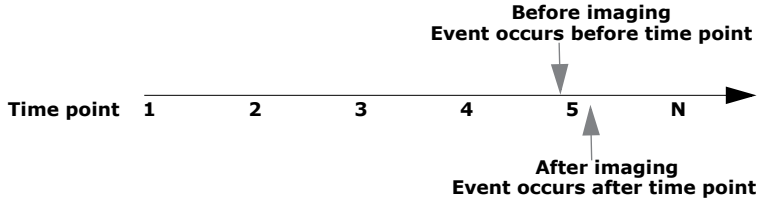


Figure 2-14: Setting events to occur before and after a time point

Note: The number of time points is set on the Timelapse tab. See Doing Timelapse Experiments on page 23.

6. In the Fluidic Event dialog, select the event type and its properties. Event types include:
   - **Compound addition**: A Compound addition event, shown in Figure 2-15 on page 46, draws liquid from the compound plate and adds it to the sample plate. There is a one-to-one mapping between the wells on the compound plate and the sample plate. That is, compound is removed from a well on the compound plate and applied to the well in the same position on the sample plate (for example, A1 to A1, A2 to A2). When you are running mixes that consist of more than a single cycle, **Mix Dead Volume** specifies an amount of fluid that is to remain in the tip and not to be returned to the well during intermediate repeated cycles of draws and dispense. For more information about compound addition settings, see the MetaXpress Software application help (press F1 while viewing the dialog).

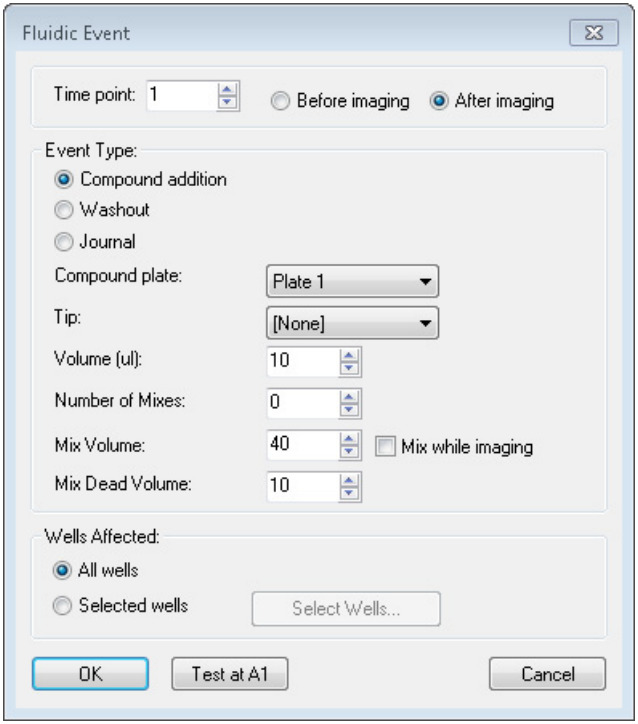


Figure 2-15: Fluidic Event dialog Compound addition event

- **Washout**: A Washout event removes liquid from the sample plate and replaces it with liquid from the compound plate. There is a one-to-one mapping between the wells as shown in Figure 2-16.

  An exchange consists of a washout event in which fluid is removed from the sample plate and discarded, and fresh media or fluid is placed in the well. If you want the system to discard the old tip after removing and discarding the fluid from the sample plate, select **New tip each exchange**. For more information, see the software Help (press F1 while viewing the dialog).

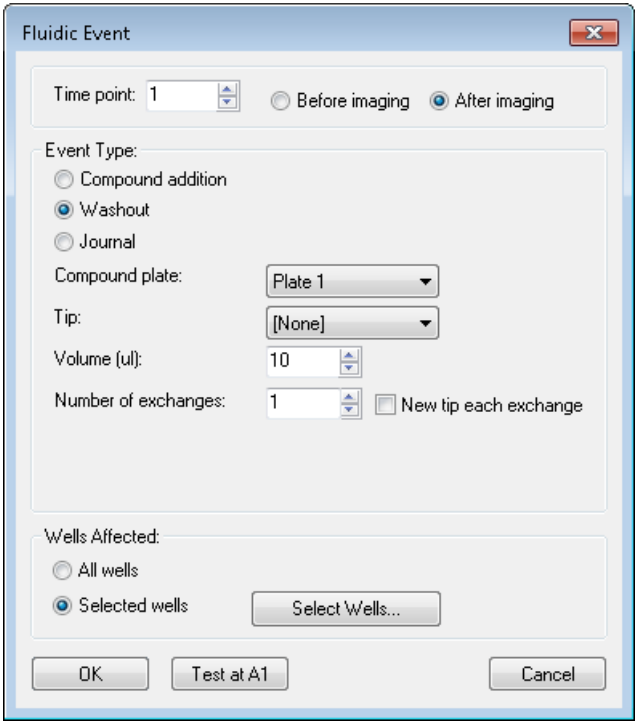


Figure 2-16: Fluidic Event dialog Washout Event

- **Journal**: A Journal event is used for any special cases or custom protocols (for example, experiments not using a one-to-one mapping of wells, or drawing once and dispensing multiple times) as shown in Figure 2-17.

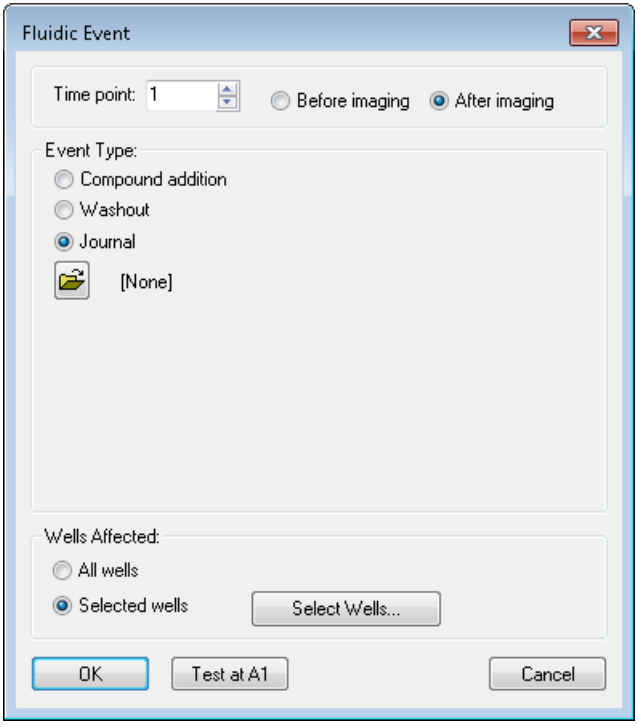


Figure 2-17: Fluidic Event dialog Journal event

7. Click **OK**.

Preventing Evaporation from Compound Plates

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

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

Evaluated Seals

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

Heat-applied seals

Tomtec heat-applied aluminum foil seals

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

Pressure-applied seals

G&L Precision Die Cutting pressure-applied aluminum foil seals

- Part number: G&L Aluminum Microplate Liddings (GL-255)
- For specifications, ordering information, pricing, and sealing instructions, contact G&L Precision (www.glprecision.com)
- Features
  - Manual plate sealing
  - Suitable for polypropylene and polystyrene plates
  - Easily punctured by the fluidics robot using FLIPR Tetra pipette tips
  - Compatible with DMSO

Environment Control and Fluidics Options Maintenance

3

It is important to regularly clean the environmental enclosure, including specific fluidics and 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 the *ImageXpress Micro Widefield High Content Imaging System User Guide* or the *ImageXpress Micro Confocal High Content Imaging System User Guide*.

The following sections provide information on how to clean fluidics components. If you have any further questions about specific cleaning procedures, contact Technical Support. See Obtaining Support on page 7.

This chapter contains the following sections:

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

Carbon Dioxide Tubing

The tubing within the environmental enclosure should be cleaned only if moisture has collected in the tubing or visual inspection of the tubing suggests contamination. There are two tubing sections associated with carbon dioxide delivery. One runs from the carbon dioxide controller located outside the enclosure to the water reservoir within the enclosure, and the other one exits the water reservoir and delivers the carbon dioxide to the stage (plate) area.

Do not let the second stretch of tubing leaving the water reservoir to the stage area come into contact with the water in the reservoir. When replacing or cleaning tubing, make sure that the exit tubing from the reservoir is well above the water's surface. If at any time, you can see moisture within the tubing, disconnect both ends and use compressed air to dry the inside of the tubing. Make sure that you reconnect all tubing.

The tubing that runs from the carbon dioxide controller to the enclosure has an in-line filter installed near the end closest to the controller. When reconnecting this tubing make sure that the in-line filter is in the correct position.

To clean any portion of the tubing

1. Note how the tubing is connected, and then disconnect the portion of the tubing that is contaminated and remove it from the environmental enclosure.
2. Use 70% isopropanol to flush the interior of the tubing.
3. Use compressed air to dry the interior of the tubing.
4. Reconnect the tubing within the environmental enclosure, making sure all connections are tight.
5. Confirm that carbon dioxide is being delivered to the water reservoir by visually confirming that bubbles move through the reservoir. See Setting Up the Carbon Dioxide on page 17.

Cleaning the Water Reservoir

Air supplied to the environmental enclosure flows through the water reservoir, ensuring the air has a high degree of humidity.

To clean the water reservoir

Required tool: Phillips screwdriver

1. In the MetaXpress software, click **Screening** > **Plate acquisition and Control** > **Eject Plate** to open the top door of the instrument.
2. Exit the MetaXpress Software and turn off the ImageXpress Micro instrument at the main power switch, which is located on the instrument's external power supply.
3. Remove the insert surrounding the top door, and open or remove the side panel.
4. Disconnect the air tubing and the sensors from the water reservoir.
5. Remove the reservoir from the instrument.
6. Using a Phillips screwdriver, remove the screws securing the reservoir lid to the instrument.
7. Dispose of the water, and then rinse the reservoir with 70% ethanol.
8. Replace the lid, tighten the screws, and then place the reservoir back in the instrument.
9. Fill the reservoir with deionized water to the fill line. See Setting Up the Water Reservoir on page 15.
10. Reconnect the air tubing and sensors to the water reservoir.
11. Replace the insert surrounding the top door, close or replace the side panel, and close the top door of the instrument.

Waste Disposal Box and Plate Sealing Ring

Proper cleaning of the waste disposal box and the plate sealing ring is highly dependent on the type of waste being disposed of and whether the box is being used with a protective liner. These components can come into direct contact with unlidded sample plates, compounds, and media. Therefore, Molecular Devices strongly recommends that you regularly clean between experiments. Clean both components by first removing them from the environmental enclosure, and then using one of the following cleaning techniques depending on the severity of contamination of the box:

- Damp wipe followed by a disinfectant wipe (70% ethanol)
- Exposure to UV by placement within a tissue culture hood

Transmitted Light Option Operations

4

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

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

The ImageXpress Transmitted Light option can be installed with or without the Environment Control option. It is not compatible with the Fluidics option.

See Also:

- Environment Control Option Operations on page 9

This chapter contains the following sections:

Transmitted Light Hardware

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

- **Nikon phase optics**. Allows either phase contrast or brightfield imaging. See Figure 4-1 on page 57 and Figure 4-2 on page 57.
- **Phase contrast objectives**. Must be selected for phase contrast imaging.
- **White light lamp (halogen)**. The software controls the lamp power.

  The halogen lamp has a limited life and can be replaced. For instructions see the following knowledge base article: http://mdc.custhelp.com/app/answers/detail/a_id/19101/
- **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 4-3 on page 58.
- **Flip-in Lens**. Enables phase ring alignment for the ImageXpress Micro Standard, XL, and XLS Systems. See Transmitted Light Phase Ring Alignment on page 61.

  On the ImageXpress Micro Confocal System, the phase ring alignment lens is in the emission filter wheel and is placed in position using the software.
- **Hinge**. Allows robotic access to the plate if necessary. See Figure 4-4 on page 58.
- **ImageXpress Micro Transmitted Light Option Controller** or the **ImageXpress Micro Systems Power & Options Controller**. Used to turn the option on and off. It is shared with the Environment Control option when both options are installed. See Figure 1-1 on page 11 and Figure 1-2 on page 11.

Figure 4-1: ImageXpress Micro Standard System with the Transmitted Light option

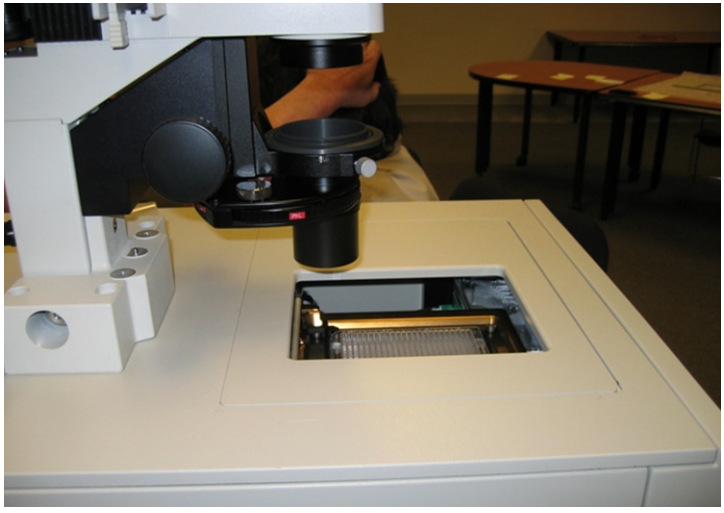


Figure 4-2: ImageXpress Micro Standard System with Transmitted Light option (detailed view)

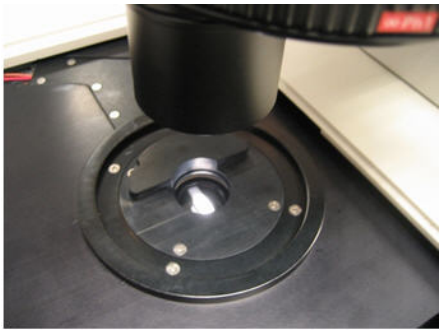


Figure 4-3: Transmitted Light shutter

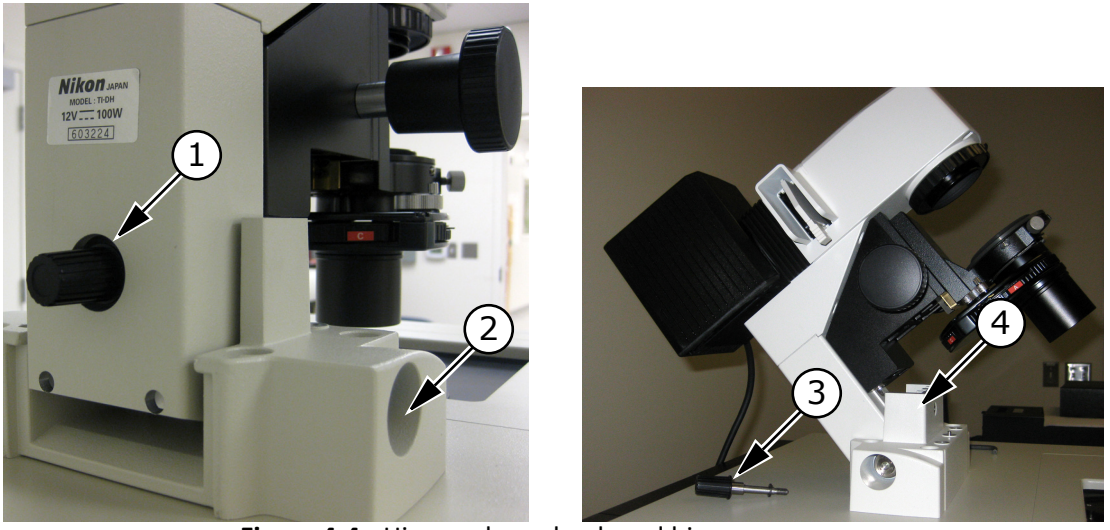


Figure 4-4: Hinge-release knob and hinge

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

Transmitted Light Software Configuration

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

Tip: Do not turn the transmitted light on and off or adjust the lamp power during acquisition. Doing so takes a considerable amount of time and can possibly damage the lamp.

To configure transmitted light settings in the MetaXpress software

1. In the MetaXpress software, click **Devices > Configure Illumination**.

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

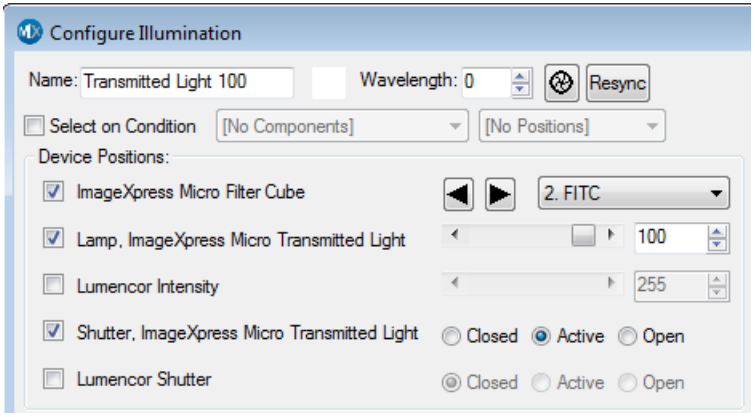


Figure 4-5: Configure Illumination dialog: Lamp On

2. In the Configure Illumination dialog, create an illumination setting named **Transmitted Lamp Off**, select **Lamp, ImageXpress Micro Transmitted Light,** and set the lamp illumination to zero, as shown in Figure 4-6.

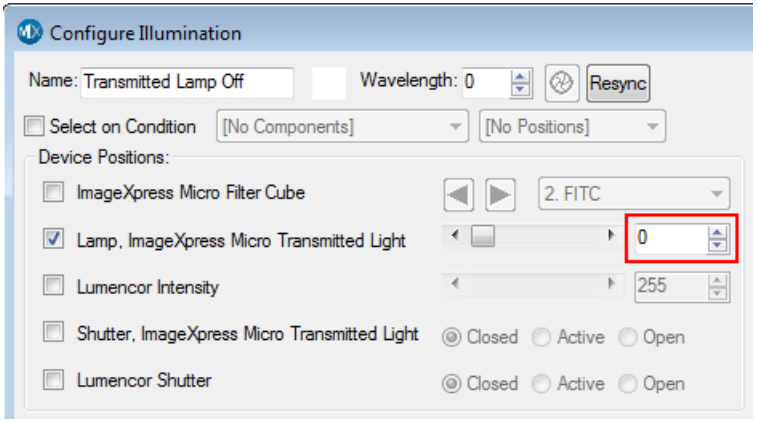


Figure 4-6: Configure Illumination dialog: Transmitted Lamp Off

3. Create an illumination setting named **Transmitted Light**, select **Lamp, ImageXpress Micro Transmitted Light,** and set lamp illumination as a percentage of full power, as shown in Figure 4-7.

   The example sets the lamp power to 20% of maximum. You can adjust this setting to meet the requirements of your experiment.

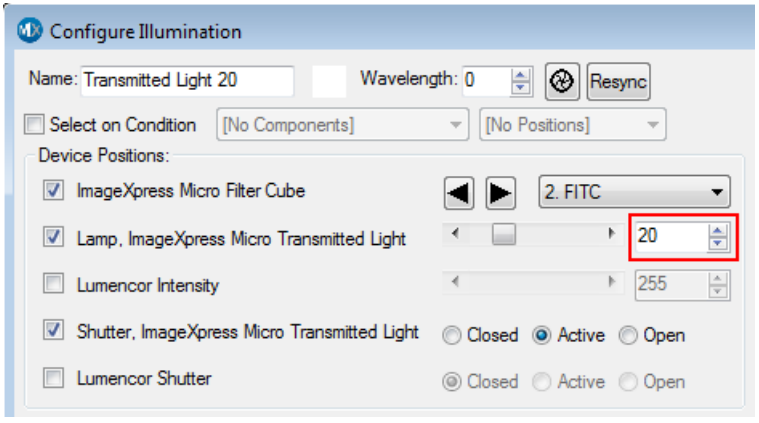


Figure 4-7: Configure Illumination dialog: Transmitted Light

After the settings have been configured, Transmitted Light can be selected as a wavelength in the **Plate Acquisition Setup** dialog.

Transmitted Light Phase Ring Alignment

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

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

- On the ImageXpress Micro Standard and XL Systems, the optics cover is removed and the flip-in lens is manually placed in position for the phase ring adjustment. See To use the flip-in lens on the ImageXpress Micro Standard and XL Systems on page 61.
- On the ImageXpress Micro XLS System, the flip-in lens is manually placed in position for the phase ring adjustment without removing the optics cover. See To use the flip-in lens on the ImageXpress Micro XLS System on page 63.
- On the ImageXpress Micro Confocal System, the phase ring adjustment lens is placed in position for the phase ring adjustment using the Meta Imaging Series Administrator Software. See To use the phase ring adjustment lens on the ImageXpress Micro Confocal System on page 65.

To use the flip-in lens on the ImageXpress Micro Standard and XL Systems

CAUTION! Wear gloves, and do not touch, move, or otherwise damage the mirror to the right of the flip-in lens.

1. Power off the instrument.
2. Use a Phillips screwdriver to remove both of the left side panels of the instrument.
3. Use a 1/16 inch hex key to remove the black optics cover over the lens.

4. Move the flip-in lens into place for the alignment procedure.
   The flip-in lens must be out of the way for any actual imaging.

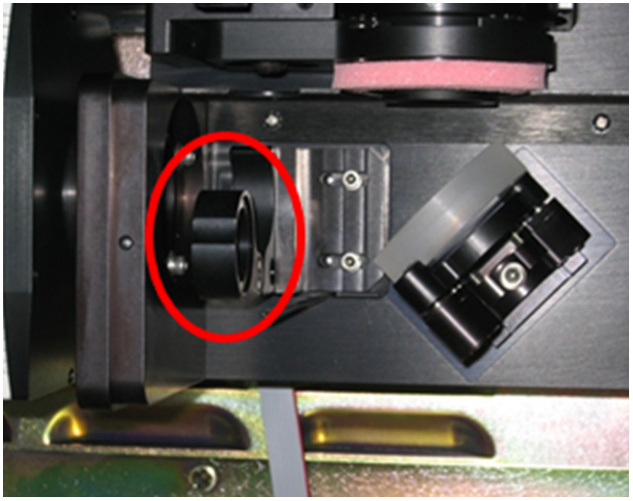


Figure 4-8: Flip-in lens on the ImageXpress Micro Standard and XLS Systems

5. Power on the instrument.
6. Adjust the phase ring. See To align the phase ring on page 67.
7. After the phase ring has been adjusted, power off the instrument.
8. Move the flip-in lens out of the optical path.
9. Use a 1/16 inch hex key to install the black optics cover over the lens.
10. Use a Phillips screwdriver to install both of the left side panels on the instrument.

To use the flip-in lens on the ImageXpress Micro XLS System

1. Power off the instrument.
2. Use a Phillips screwdriver to remove both of the left side panels of the instrument to access the flip-in lens mounted on the outside of the optics cover.

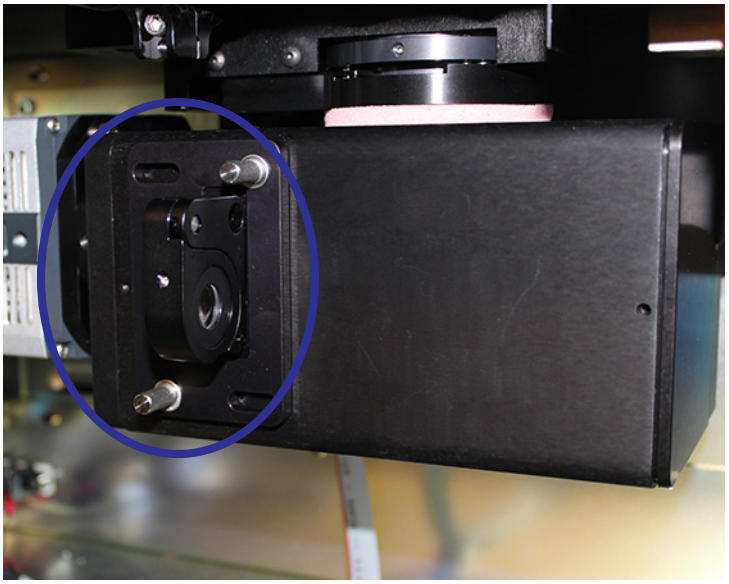


Figure 4-9: Flip-in lens on the ImageXpress Micro XLS System

3. Remove the two thumbscrews and their washers from the flip-in lens mounting plate.

   There is a flat washer and a split-ring washer on each thumbscrew on the flip-in lens assembly.

Figure 4-10: Thumbscrew and washers

4. Remove the flip-in lens assembly from the instrument.
5. Remove the protective cloth bag from the flip-in lens.
6. Move the flip-in lens into it fully extended position.

Figure 4-11: Flip-in lens extended for phase ring alignment

7. Slide the flip-in lens assembly onto the optics cover with the flip-in lens on the inside for the phase ring alignment.
8. Install the washers and tighten the thumbscrews to hold the flip-in lens assembly in position for phase ring alignment.
9. Adjust the phase ring. See To align the phase ring on page 67.
10. After the phase ring has been adjusted, power off the instrument.
11. Remove the two thumbscrews and their washers from the flip-in lens mounting plate.
12. Remove the flip-in lens assembly from the instrument.
13. Move the flip-in lens into it fully closed position.
14. Cover the flip-in lens with the protective cloth bag.
15. Mount the flip-in lens assembly on the optics cover with the closed and covered flip-in lens on the outside.
16. Install the washers and tighten the thumbscrews.
17. Use a Phillips screwdriver to install both of the left side panels on the instrument.

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

1. Exit the MetaXpress Software if it is running.
2. 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.
3. Click **Configure Hardware**.
4. In the **Configure Hardware** dialog, select the correct hardware configuration, and then click **Configure Devices**.
5. In the right pane of the **User Settings for hardware configuration** dialog, click **IXConfocal Module Emission Wheel**, and then click **Settings**.

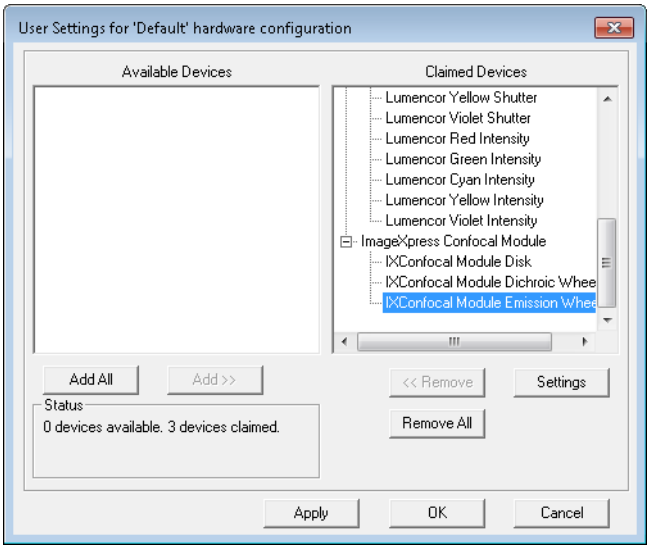


Figure 4-12: User Setting Selection of IXConfocal Module Emission Wheel Settings

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

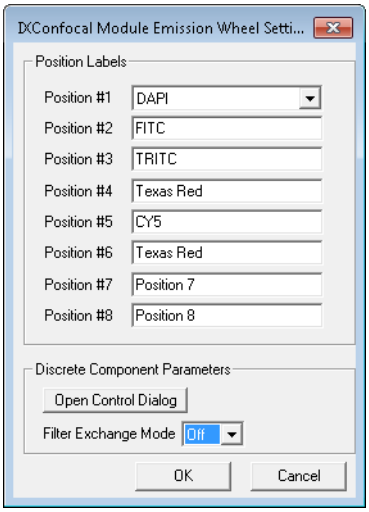


Figure 4-13: The IXConfocal Module Emission Wheel Settings dialog

7. Click **Open Control Dialog**.

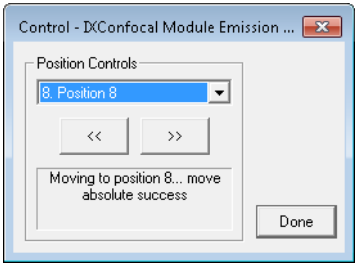


Figure 4-14: IXConfocal Module Emission Wheel Control dialog

8. From the **Position Controls**, select the numbered position for the phase ring adjustment lens.

   By default, the phase ring adjustment lens is installed in position 8.

9. After the wheel moves to the selected position, click Done.
10. Close all open dialogs and exit the MetaXpress Meta Imaging Series Administrator software.

To align the phase ring

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

1. Make sure that the phase ring adjustment lens is in place.
   - On the ImageXpress Micro Standard and XL Systems, the optics cover is removed and the flip-in lens is manually placed in position for the phase ring adjustment. See To use the flip-in lens on the ImageXpress Micro Standard and XL Systems on page 61.
   - On the ImageXpress Micro XLS System, the flip-in lens is manually placed in position for the phase ring adjustment without removing the optics cover. See To use the flip-in lens on the ImageXpress Micro XLS System on page 63.
   - On the ImageXpress Micro Confocal System, the phase ring adjustment lens is placed in position for the phase ring adjustment using the Meta Imaging Series Administrator Software. See To use the phase ring adjustment lens on the ImageXpress Micro Confocal System on page 65.
2. Rotate the condenser turret so that the A position is selected (at the front).

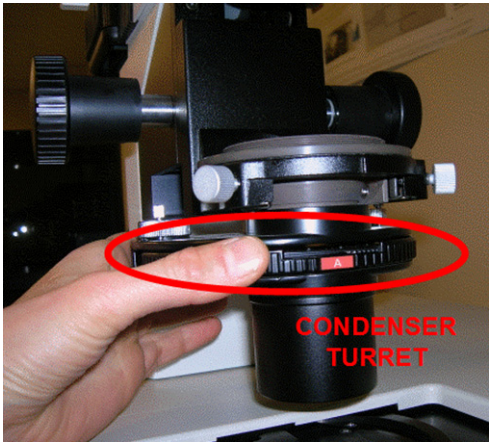


Figure 4-15: Condenser turret with position A at the front

3. Turn the adjustment ring, which is located below the arm of the Transmitted Light tower, to the middle of the aperture range.

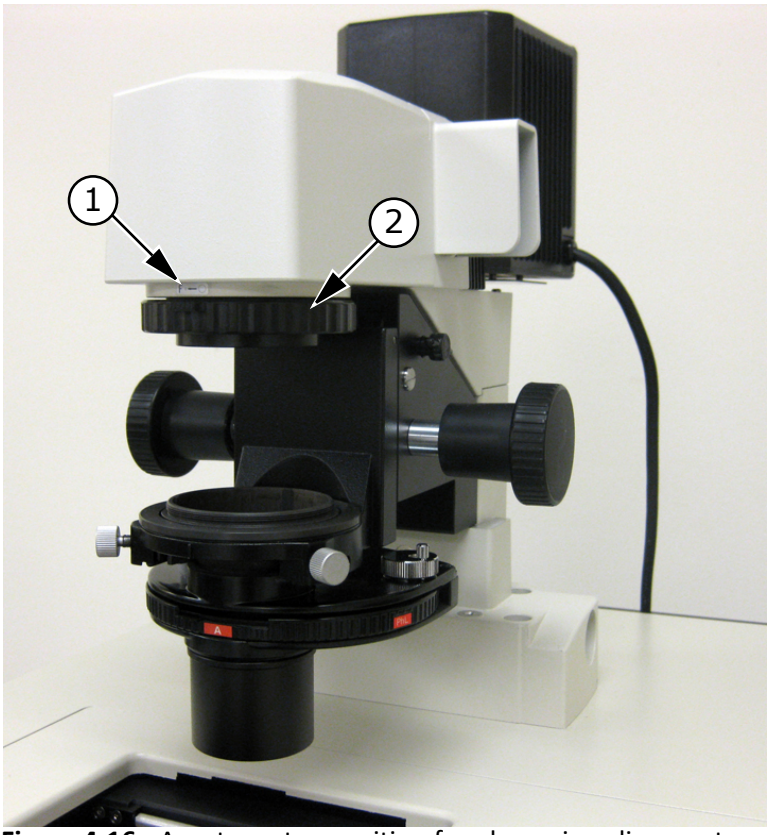


Figure 4-16: Aperture stop position for phase ring alignment

Item	Description
1	Label indicating aperture range
2	Adjustment ring

4. Move the field-stop slider, located just above the **A** label on the condenser turret, all the way to the right.

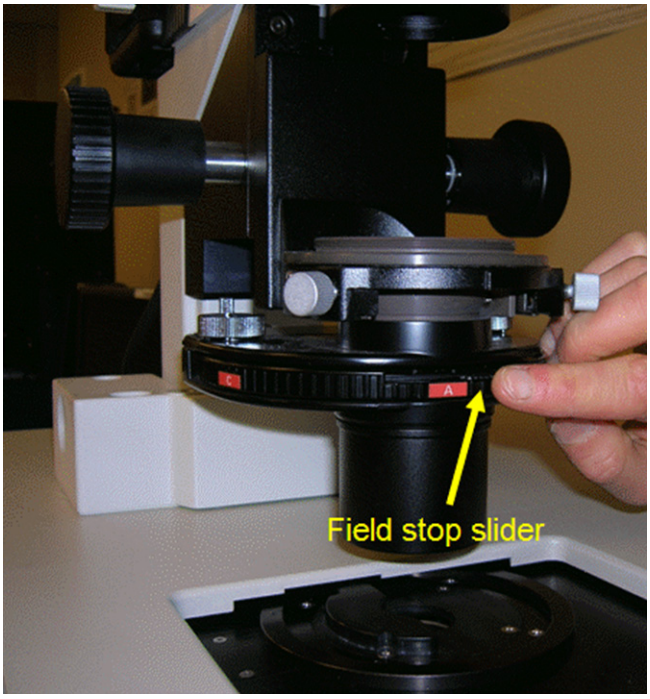


Figure 4-17: Field stop slider

5. Select the diffusion filter, or place some lens paper or lint-free wipes on top of the condenser lens to serve as a diffuser. If necessary, weigh them down slightly to keep them from blowing away.
6. In the MetaXpress software, select the desired phase contrast objective.
7. Set the focus (Z position) to approximately 8000 µm.

   If necessary, you can adjust this later.
8. Select the Transmitted Light illumination setting.
9. With no sample present, snap an image.
10. If necessary, adjust the illuminator level and the exposure time to obtain a visible but not saturated image. The image of the phase ring might be off-center. See Figure 4-18. This is acceptable as long as most of the image is visible.

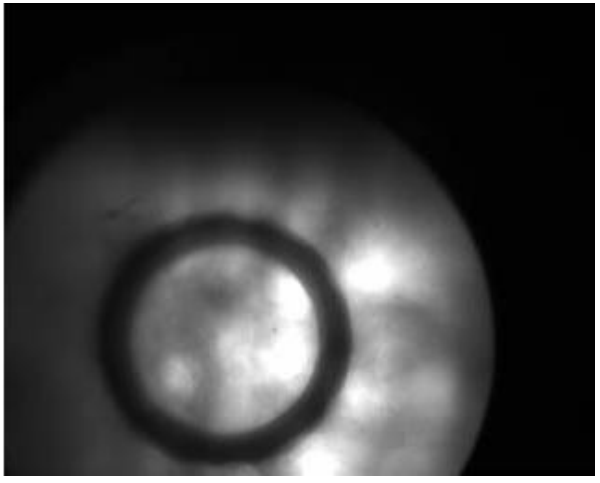


Figure 4-18: Out-of-focus image of phase contrast ring

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

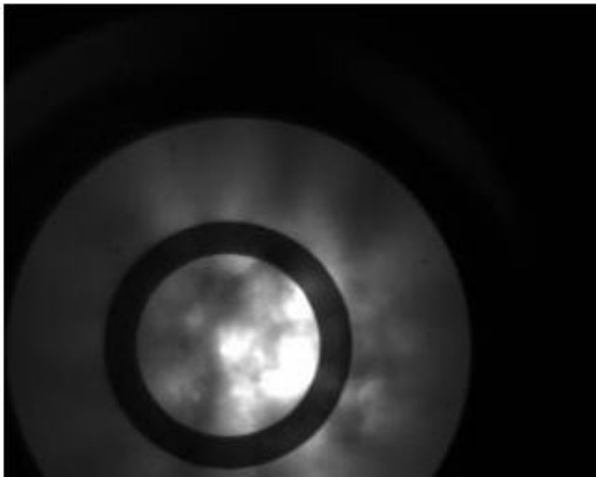


Figure 4-19: In-focus image of phase contrast ring

12. Use the region tools in the MetaXpress software to mark the position of the phase ring. Make sure that you capture both horizontal and vertical axes. Use either the ellipse region tool or multiple line tools. See Figure 4-20.

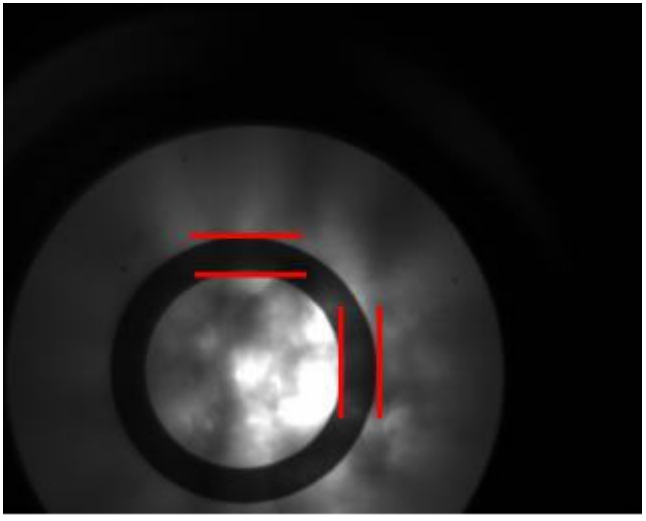


Figure 4-20: Marking the position of the phase ring

13. In the MetaXpress software, click **Regions > Save Regions** to save the marked positions.
14. Manually rotate the condenser turret to the appropriate annulus for the objective (PhL for 4x, Ph1 for 10x and 20x, Ph2 for 40x).

    The selected annulus is at the front of the condenser turret.

    Phase contrast objectives typically have their specifications indicated in green letters on the outer barrel. These specifications also include the matching annulus designation (for example, PhL, Ph1, Ph2).

15. Snap an image.

    The condenser annulus ring shows. See Figure 4-21. You might need to adjust the exposure time.

Figure 4-21: Image of condenser annulus ring

16. In the MetaXpress software, click **Live Mode** in either the **Acquire** or **Plate Acquisition and Control** dialog.
17. Click **Regions > Load Regions** to load in the saved regions.
18. Using the condenser annulus centering knobs located on the right top and left top surface of the condenser turret, center the condenser annulus ring with the objective phase ring. See Figure 4-22.

19. Lock down the annulus centering knobs.

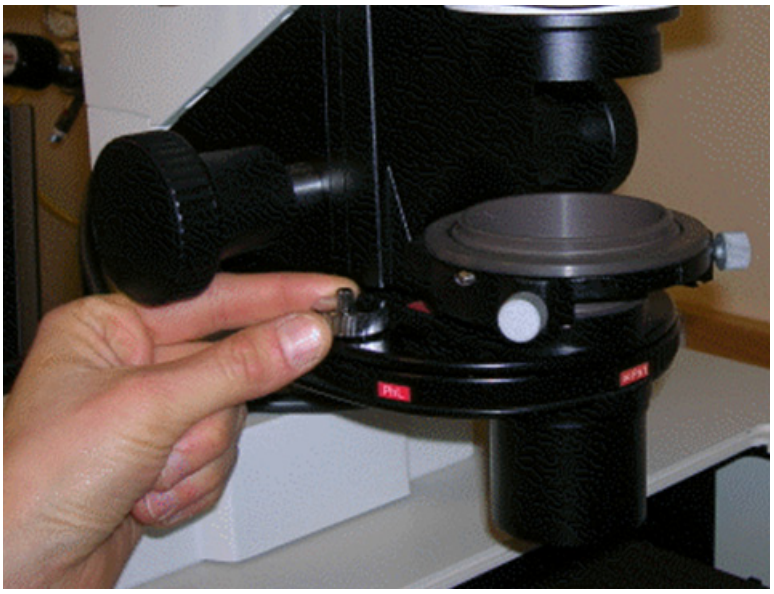


Figure 4-22: Centering the condenser annulus ring with the objective phase ring

20. Adjust the height of the Transmitted Light condenser with the condenser height adjustment knob until a sharp image is obtained. If required, adjust the lamp power or exposure level.
21. In the MetaXpress software, click **Stop Live**.
22. For the ImageXpress Micro Standard, XL, or XLS Systems, move the flip-in lens out of the optical path.
    - For the ImageXpress Micro Standard and XL Systems, see To use the flip-in lens on the ImageXpress Micro Standard and XL Systems on page 61.
    - For the ImageXpress Micro XLS System, see To use the flip-in lens on the ImageXpress Micro XLS System on page 63.

    For the ImageXpress Micro XLS System, the lens used for phase ring adjustment automatically moves out of the optical path when an emission wavelength is selected for your acquisition.

Transmitted Light Experiments

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

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

To run a Transmitted Light experiment

1. If you are running a phase-contrast experiment, make sure a phase-contrast objective is selected. Phase-contrast objectives are not required for brightfield experiments.
2. Make sure the annulus on the Transmitted Light arm matches the objective.
3. Load a suitable plate.

   The plate must be unlidded, have a clear lid, or have a clear plate seal on the top. Condensation on a lid or plate seal can negatively affect image quality.
4. Turn on the Transmitted Lamp by selecting the appropriate Illumination setting in the **Configure Illumination** dialog.
5. In the MetaXpress software, click **Screening > Plate Acquisition Setup**.
6. In the Plate Acquisition Setup dialog, define one of the wavelengths as Transmitted Light.
7. Configure the remaining settings as usual.
8. Run the acquisition.

Tip: To increase the life of the halogen lamp, turn off the lamp when the transmitted light unit is not in use by selecting the **Transmitted Lamp Off** setting in the **Configure Illumination** dialog.

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