

ImageXpress Pico

Automated Cell Imaging System
with CellReporterXpress Software Version 2.7

User Guide

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Safety Information

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

It is important to confirm that everyone involved with the operation of the instrument has:

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

Always remember that the key to safety is operating the instrument with care.



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

Warnings, Cautions, Notes, and Tips

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

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

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



WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed. The warning symbol can vary depending on the warning. The definition of the symbol is included in the text of the warning.



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








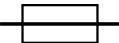





Note: A note calls attention to significant information.










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

Symbols on Instrument Labels

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

Symbol	Indication
	Indicates that the product documentation must be consulted.
	Indicates a potential lifting hazard. To prevent injury, use a minimum of two people to lift the instrument. See Lifting Hazard on page 11 for details on the weight of the instrument.
	Indicates a potential pinch hazard.
	Indicates a potential heat hazard.
	Indicates a rotating parts hazard.
	Indicates the location of a fuse.
	Indicates the instrument serial number.
	Indicates the instrument manufacture date.
	Indicates that you should consult the instructions for use.
	Indicates CSA certification.
	Indicates European technology conformity.

Symbol	Indication
	Indicates compliance with Australian radio communication requirements.
 	Indicates compliance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. 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.
	Indicates the environmental friendly use period for China RoHS. The symbol may indicate the number of years in the use period.
	Indicates that there is an authorized representative in the European community.
	Indicates the instrument manufacturer.
	Indicates the manufacturer catalog number.

Protective Housing

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



WARNING! Do not manually open the top door, open the protective housing, or attempt to gain access to the interior of the instrument. These actions can damage the instrument components and result in hazardous exposure to LED light, hot surfaces, moving parts, or high voltage.

Electrical Safety

To prevent electrically related injuries and property damage, inspect all electrical equipment before use and immediately report all electrical deficiencies. Contact Molecular Devices Technical Support to service equipment that requires the removal of covers or panels.



WARNING! HIGH VOLTAGE. Within the instrument is the potential of an electrical shock hazard existing from a high-voltage source. Read and understand all safety instructions before you install, maintain, or service the instrument.

Do not remove the instrument covers. To prevent electrical shock, use the supplied power cords only and connect to a properly grounded wall outlet.

To ensure sufficient ventilation and provide access for disconnecting power from the instrument, maintain a clearance of 20 cm to 30 cm (7.9 in. to 11.8 in.) at the rear of the instrument.

If the instrument does not power on, you may need to replace the instrument fuses. See [Replacing Fuses on page 9](#) for details.

Replacing Fuses

If the instrument does not power on when you press the Power button, confirm that the power cord is securely connected to a functioning power outlet and to the power port on the rear of the instrument.

If the power fails while the instrument was running, verify that the power cord is not loose or disconnected and that power to the power outlet is functioning properly.

If these checks fail to remedy the loss of power, replace the fuses. See [Instrument Specifications on page 81](#) for fuse details.



CAUTION! Do not touch or loosen screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void the warranty.

The fuses are located in the fuse carrier, which is below the power port at the rear of the instrument.



WARNING! HIGH VOLTAGE Always power off the instrument and disconnect the power cord from the main power source before you perform a maintenance procedure that requires removal of a panel or cover or disassembly of an interior instrument component.

To replace fuses:

1. If the instrument is powered on, press the Power button on the front of the instrument to turn off the power.

When the status light on the Power button is amber, the instrument is fully powered off.



Note: If the ImageXpress Pico system doesn't respond, you can perform a hard shutdown by holding the Power button for five seconds.

2. Disconnect the power cord from the power port.
3. Gently press on the carrier-release tab (below the power port) with a small flat-head screwdriver, and pull the fuse carrier to remove it from the instrument.
4. Gently pull the old fuses from the carrier by hand.
5. Gently place the new fuses into the carrier.
6. Press the fuse carrier into the instrument until the carrier snaps into place.
7. Connect the power cord into the power port.
8. Press the Power button to power on the instrument.

When the light on the Power button is green, the instrument is fully powered on.



Note: If the instrument still does not power on after replacing fuses, contact Molecular Devices Technical Support. See [Obtaining Support on page 20](#) for details.

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



WARNING! LIFTING HAZARD. The ImageXpress Pico system weighs approximately 38 kg (84 lb). Use great care when lifting or moving the instrument. To prevent injury, use a minimum of two people to lift the instrument.



CAUTION!

- Always lift the instrument to move it. Do not slide or push the instrument. Sliding or pushing can damage the feet on the bottom of the instrument.
 - Moving the instrument can damage sensitive parts and disrupt optical alignments. When transporting the instrument, use the original packaging and shipping box to properly secure the instrument. Your warranty 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. When working with potentially hazardous liquids, take applicable safety precautions, such as wearing safety glasses and protective clothing.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials.
- Use a compressed gas supply in a well-ventilated area. The instrument is not air-tight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures your safety officer defines to maintain a safe work environment, such as the use of an automatic warning system.
- Observe the applicable cautionary procedures as defined by your safety officer when using flammable solvents in or near an instrument that is powered on.
- Observe the applicable cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.



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

Chapter 1: ImageXpress Pico Automated Cell Imaging System

1

The ImageXpress® Pico Automated Cell Imaging System is an all-in-one platform for automatically acquiring and analyzing images from fluorescently labeled biological samples in plates and slides. It enables you to increase the throughput of your image acquisition and analysis, allowing you to gain insights in minutes. With the addition of a modular option, the system provides environmental control for live cell 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, which allows complete control of the instrument configuration.

When used in combination with the CellReporterXpress® Image Acquisition and Analysis Software, which features powerful image analysis capabilities and more than 25 available predefined experimental protocols, the ImageXpress Pico system becomes an extremely flexible device, ideally suited for user-defined, automated assays.

Key components of the instrument include the following:

- Built-in, internal light source comprised of six high-powered LEDs enables very high sensitivity fluorescent imaging. Additional LED-based light sources allow transmitted light and overview imaging.
- High-sensitivity, 5-megapixel CMOS camera.
- Hardware-based autofocus system with precision motorized Z-stage focus.
- High-transmission fluorescence imaging optics with world-class chromatic aberration correction, resolution, and image flatness.
- Secondary camera provides overview imaging for slides.
- Precision motorized sample (X-Y) stage.
- High-quality Leica objectives in a six-position turret.
- Filter cubes in a six-position turret.
- Motorized selection of stage position, filter cubes, and objectives.
- Temperature control up to 40°C (104°F) for live cell imaging.
- Optional environmental control system, which enables you to regulate humidity, CO₂, and O₂ inside the environmental control cassette for multi-day, live-cell, time-lapse imaging.
- Operation and configuration control by the integrated CellReporterXpress software.

ImageXpress Pico System Features

Illumination System

Light Source

The ImageXpress Pico system light source is comprised of six high-powered LEDs with a rated lifetime of more than 20,000 hours. It has an excitation spectrum ranging from ultraviolet to red. There are no user-replaceable parts in the light source.

Filter Cube Turret

The six-position filter cube turret uses Leica filter cubes, which contain filters that are adapted and matched to the ImageXpress Pico system. Additional filter cubes are available exclusively from Molecular Devices.

See [Filter Cubes on page 87](#) for details.

Objective (Z) Stage

The Z stage position features a resolution of better than 0.25 μm .

The ImageXpress Pico system uses Fluotar objectives manufactured by Leica Microsystems. The selected objective lens focuses excitation light onto the sample and collects fluorescent light emitted by the sample.

One or more objectives is included with your initial purchase of the ImageXpress Pico system. After that, you can order additional compatible objectives exclusively from Molecular Devices. The following objectives are compatible with the ImageXpress Pico system:

Magnification	Numerical Aperture
4x	0.13
10x	0.32
20x	0.40
40x	0.60
63x	0.70



CAUTION! To prevent damaging both the instrument and your sample, do not use any other objectives with the ImageXpress Pico system.

See [Objectives on page 87](#) for details.

Motorized Objective Turret

The ImageXpress Pico system includes a six-position objective turret.

Sample (X-Y) Stage

Plate Holder



The plate holder is designed for imaging multi-well microplates in standard ANSI (SBS) formats with plastic or glass bottoms and standard footprint dimensions. It can also be used with the optional dish holder, which holds up to six 35 mm (1.38 in.) dishes. Optimal image quality depends on plate flatness, well bottom thickness, and optical clarity.

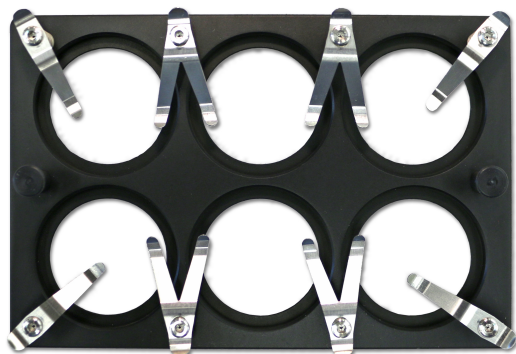
A spring-loaded, mechanical clamp holds the plate or dish holder securely in the plate holder. The clamp automatically opens when the sample stage moves to the load/eject position; it automatically closes when the sample stage moves into position for imaging.

Slide Holder



The slide holder is designed for imaging up to four standard microscope slides of 25 mm x 75 mm (1 in. x 3 in.).

Dish Holder



The optional dish holder is used with the plate holder. It is designed for imaging up to six standard culture dishes of 35 mm (1.38 in.) in diameter.

LED Autofocus

The ImageXpress Pico system uses a high-powered LED to autofocus the sample. For certain assays (particularly those with thin plates and low magnifications), software autofocus is also available.

CellReporterXpress Software Features

The CellReporterXpress Image Acquisition and Analysis Software is the user interface for the ImageXpress Pico system. You will use the CellReporterXpress software to work with the ImageXpress Pico system and control all its functions.

The CellReporterXpress software integrates image acquisition and analysis into a unified workflow. Along with the ImageXpress Pico system, the CellReporterXpress software streamlines automated imaging to offer a simplified solution for scaling up microscopy. Its features include:

- A web-based interface that runs on many browsers, including those found on iPads and Android tablets.
- Over 25 available predefined experimental protocols.
- High-powered analysis tools equivalent to those found in desktop applications.
- Easy-to-manage data with no requirement to configure a database.
- A simplified user interface that is easy to learn and easy to use.



Note: See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details on using the CellReporterXpress software.

Theory of Operation

The ImageXpress Pico system uses the following components and functions:

- [Fluorescence Imaging, see below](#)
- [Objective Lenses, see page 18](#)
- [Filter Cubes, see page 19](#)

Fluorescence Imaging

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

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

Objective Lenses

The ImageXpress Pico system can be configured with high-quality Leica Fluotar objectives. Five objectives are currently available.

You can identify the magnification of an objective by the color band:

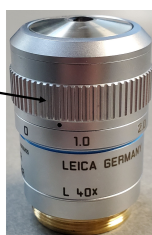
Objective Magnification	Color Band
4x	Red
10x	Yellow
20x	Green
40x	Light Blue
63x	Dark Blue



Color Band on
4x Objective

The 40x objective and 63x objective have application-optimized correction collars to compensate for well bottom thickness or coverslip thickness. The collars have a range of 0 mm to 2 mm correction. Changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very dependent on properly setting these collars.

Correction Collar
on 40x Objective



The settings to be used depend on the well bottom thickness of the plate or the coverslip thickness on the 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 by the plate specifications from the plate manufacturer.

See [Objectives on page 87](#) for details.

Filter Cubes

In the ImageXpress Pico system, the filter cube contains the excitation and emission filters and the dichroic mirror. These optical components are essential parts of an epi-illumination fluorescence imaging system in which the illumination and imaging optical paths overlap at the objective lens.

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.

A dichroic mirror is a specially designed beam splitter that transmits light above a certain cutoff wavelength and reflects light at shorter wavelengths. In the illumination path, the dichroic mirror reflects shorter wavelengths from the light source up through the objective onto the specimen.

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, to restrict the wavelength range.

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

Obtaining Support

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

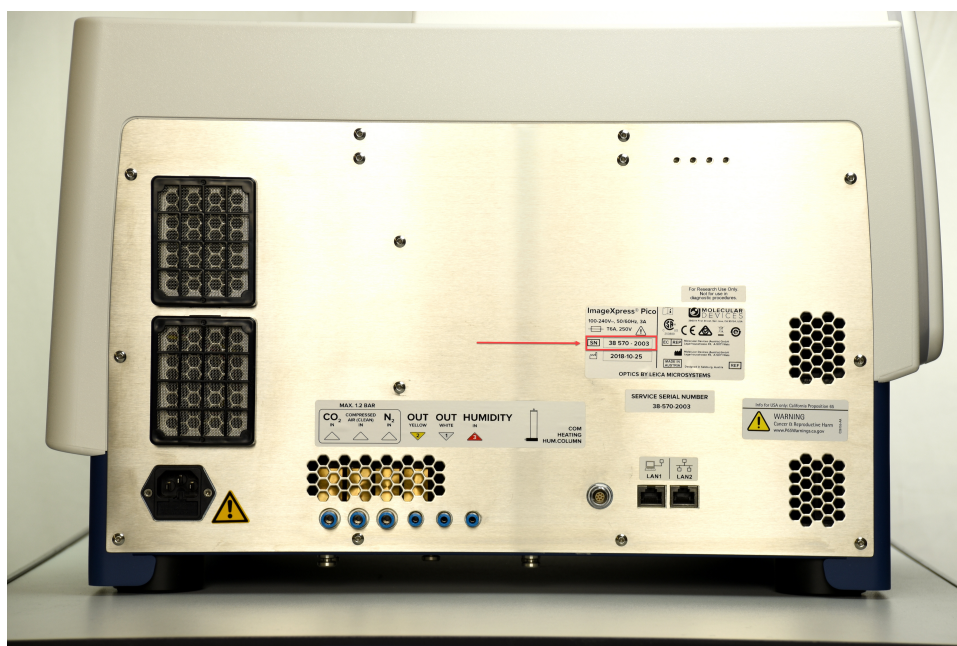
Our Support website, www.moleculardevices.com/service-support, describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

You can contact Molecular Devices Technical Support by submitting a support request through the Molecular Devices Knowledge Base at mdc.custhelp.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

You will need the instrument serial number. The serial number is located on the back panel of the instrument.



Documentation

Review the product documentation on the Molecular Devices Knowledge Base at mdc.custhelp.com, including installation guides and user guides. In addition, online Help is available within the CellReporterXpress software. Press **F1** to access Help for the current page.

Additional Resources

Web-based microscopy courses:

- www.leica-microsystems.com/science-lab
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The Molecular Probes Handbook offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

- www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

Product Documentation

The following guides are available on the Molecular Devices Knowledge Base at mdc.custhelp.com:

- *CellReporterXpress Installation Guide*
- *CellReporterXpress Release Notes*
- *CellReporterXpress User Guide*
- *ImageXpress Pico Pre-Installation Guide*
- *ImageXpress Pico Installation Guide*
- *ImageXpress Pico User Guide*
- *ImageXpress Pico Calibration Kit Guide*

In addition, the CellReporterXpress software includes context-sensitive Help that you can access from within the software. Just press the **F1** key from within the software to view Help for the current page.



Tip: We recommend that you review the documentation before installing or using the ImageXpress Pico system or the CellReporterXpress software.

About This Guide

This guide is intended for the scientist using the ImageXpress Pico system. It describes the basic functionality and use of the instrument, which is controlled using the CellReporterXpress software.

Use this guide along with the *CellReporterXpress Help* and the *CellReporterXpress User Guide*.

The information in this guide is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at mdc.custhelp.com for the most up-to-date information.

This section provides an overview of the start-to-finish workflow for using the ImageXpress Pico system, including:

- [Starting the System, see page 24](#)
- [Understanding the Status Light, see page 26](#)
- [Acquiring Data, see page 32](#)
- [Analyzing Data, see page 37](#)
- [Maintaining the Instrument, see page 37](#)
- [Shutting Down the System, see page 38](#)



Note: The procedures in this section assume that the ImageXpress Pico system and the CellReporterXpress software have been installed and properly configured. See the *ImageXpress Pico Installation Guide*, the *CellReporterXpress Installation Guide*, and the *CellReporterXpress Installation Guide* for details.

Starting the System

Use the following procedures to power on the ImageXpress Pico system and log in to the CellReporterXpress software.

- [Powering On the Instrument, see below](#)
- [Powering On the Host Computer, see below](#)
- [Logging In to the Software, see page 25](#)

Powering On the Instrument

Press the Power button on the front of the instrument.



When the light on the Power button is green, the instrument is fully powered on.

Powering On the Host Computer

Power on the host computer and monitor, and log in to Windows.

Logging In to the Software





Note: If you have just powered on or restarted the host computer, wait five minutes before logging in. This allows time for the CellReporterXpress software services to start.

To log in to the CellReporterXpress software:

1. On the host computer, do one of the following to display the CellReporterXpress Log In screen:



- On the desktop, double-click  **MD.CellReporterXpress**.
- Click **Start > Molecular Devices > MD.CellReporterXpress**.

2. In the  **Login** field, enter the user name for a Windows account on the host computer.
3. In the  **Password** field, enter the required password for the Windows account.
4. Click **LOG IN**.

See the *CellReporterXpress User Guide* for details on logging in to the software from a client workstation.

Understanding the Status Light

The light on the button at the top right of the instrument provides information about the instrument status.



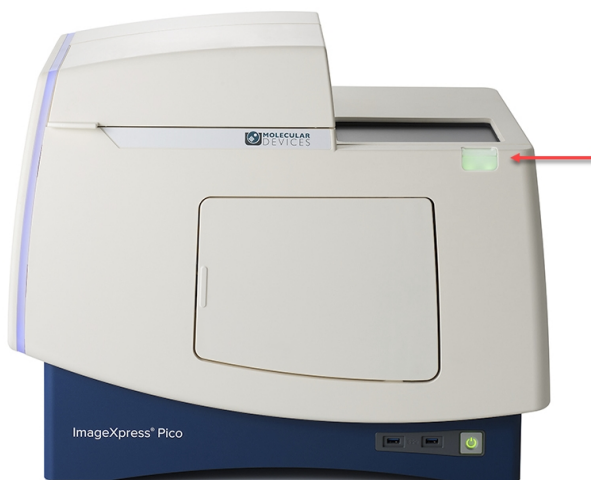
Color	Instrument Status
Green	The instrument is powered on, connected to the software, and ready to use.
Yellow	The instrument is busy (for data acquisition, overview images, or live preview).
Red	The instrument is in an error state or cannot communicate with the software. Restart the instrument. If you are performing a firmware update, confirm that you are using a USB 3 flash drive before restarting the instrument. If the status light remains red, contact Molecular Devices Technical Support.
Blue	The instrument is performing a firmware update.

Operating the Top Door

Open the top door to insert the plate holder or slide holder into the sample stage. You can operate the top door from the instrument or from the CellReporterXpress software.


Opening the Top Door from the Instrument

With the top door closed, press the button at the top right of the instrument.



Opening the Top Door from the Software

To open the top door from the CellReporterXpress software:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. In the **Available Acquisition Devices** list, select the instrument.

3. Click  **Open Plate Door**.


Closing the Top Door from the Instrument

To close the top door from the instrument:

With the top door open, press the button at the top right of the instrument.

Closing the Top Door from the Software

To close the top door from the CellReporterXpress software:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. In the **Available Acquisition Devices** list, select the instrument.

3. Click  **Close Plate Door**.

Inserting a Labware Holder

Open the top door of the instrument to insert a labware holder in the sample stage. The ImageXpress Pico System includes a plate holder for standard multi-well microplates and a 4-slide holder for standard microscope slides. An optional 6-dish holder for standard 35 mm culture dishes is also available. See [Accessories on page 88](#) for details on the items available from Molecular Devices for the ImageXpress Pico system.

The sample stage has an **A1** label to indicate its top left corner.



Inserting the Plate Holder

Like the sample stage, the plate holder also has an **A1** label to indicate its top left corner.



To insert the plate holder:

1. Press the button at the top right of the instrument to open the top door.
2. If the sample stage already contains a labware holder, remove it.
3. Confirm that the spring-loaded clamp on the plate holder is in the open position (as shown).



Note: Use the locking pin on the plate holder to ensure the spring-loaded clamp is in the open position.

4. With the **A1** label at the top left, insert the plate holder in the sample stage.



Note: The pin at the bottom right of the plate holder should fit into the small recess in the sample stage.

5. Confirm that the plate holder is seated flat in the sample stage.
6. Press the button at the top right of the instrument to close the top door.



CAUTION! To prevent from spilling liquid inside the instrument, always use a lid on the plate. Spilling liquid can damage the instrument.

Inserting the Slide Holder

Like the sample stage, the slide holder also has an **A1** label to indicate its top left corner.



To insert the slide holder:

1. Press the button at the top right of the instrument to open the top door.
2. If the sample stage already contains a labware holder, remove it.
3. With the **A1** label at the top left (as shown), insert the slide holder in the sample stage.

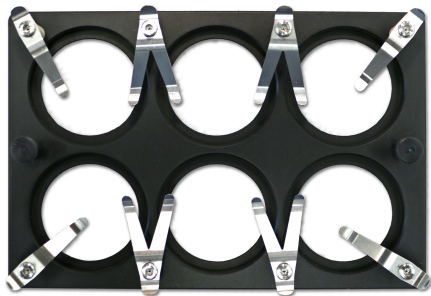


Note: The pin at the bottom right of the slide holder should fit into the small recess in the sample stage.

4. Confirm that the slide holder is seated flat in the sample stage.
5. Press the button at the top right of the instrument to close the top door.

Inserting the Dish Holder

The optional dish holder fits inside the plate holder in the sample stage. It can be inserted in either direction, so there is no label on it.



To insert the dish holder:

1. Insert the plate holder in the instrument. See [Inserting the Plate Holder on page 29](#) for details.
2. If the top door is closed, press the button at the top right of the instrument to open the top door.
3. Insert the dish holder in the plate holder.
4. Confirm that the dish holder is seated flat in the plate holder.
5. Press the button at the top right of the instrument to close the top door.

When you insert dishes in the dish holder, use the clamps to hold each dish in place.



CAUTION! To prevent from spilling liquid inside the instrument, always use a lid on the dish. Spilling liquid can damage the instrument.

Acquiring Data

Data acquisition includes configuring acquisition settings, acquiring images, and storing the acquired data. All data acquisition operations are performed using the CellReporterXpress software. See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details on acquiring data.

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.

Assay Design

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

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

Selection of Different Fluorochromes

Typical high-content and high-throughput assays include one or more fluorochromes, such as fluorescent proteins, antibody-based stains, and chemical-based stains. You should include a nuclear stain (such as DAPI) to help identify cells during image analysis. If the assay involves movement of a protein of interest to or from a particular cellular compartment or organelle, it can also be helpful to include a probe specific to that cellular compartment or organelle.

Individual fluorochromes have unique characteristics that help determine their best use. Use probes that provide bright, specific staining and have excitation and emission spectra suitable for the filter sets in the instrument. For experiments that use multiple stains, select fluorophores that have enough spectral separation. Some fluorochromes provide brighter intensities and require a shorter exposure time, while others do not bleach as quickly and allow a longer exposure time. There also might be toxicity issues with some cell types or bleed-through issues between pairs of fluorochromes. Consider these factors when you choose a fluorochrome.



Note: It might be possible to identify cells using transmitted light images instead of fluorescence.

Cell-Based Assays

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

Plates or Slides

Multi-well plates offer several advantages for high-content screening:

- The well layout is consistent from one plate to another.
- Plates are easier to handle during sample preparation and imaging.
- Plates facilitate scaling up to a larger screen.

Some assays, such as imaging of tissue sections, may require the use of slides.

The ImageXpress Pico system includes a plate holder and a 4-slide holder. The CellReporterXpress software includes separate workflows for plates and slides.

Plate Selection

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

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

- [Plate Format, see page 34](#)
- [Plate Material, see page 34](#)
- [Fluorescence Background, see page 34](#)
- [Bottom Thickness, see page 34](#)
- [Batch-to-Batch Consistency, see page 34](#)

Plate Format

To determine if the plate format is compatible with your assay, consider the following:

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

Plate Material

The composition of the material of the bottom of the plate needs to be of optical quality; if not, your acquired images can be degraded.

The ideal plate for imaging features black well sides and a single-piece, thin, glass bottom, which provides optimal autofocus performance and image quality. A plastic-bottom plate will also work well as long as the variability in the bottom thickness is not too great. Especially with a high-magnification objective, you will likely notice significant differences in clarity between a standard plastic plate, an optically clear plastic plate, and a glass-bottom plate.

Verify that the cells in your assay are compatible with the plate material. Some cells may adhere to and perform better on plastic. Given the wrong surface, some cells may fail to bind and will exhibit unusual behavior, such as rounding up or migrating to the edges of a well. In some cases, coating the plates or using pre-coated plates can be beneficial.

Fluorescence Background

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

Bottom Thickness

Compare the thickness of the plate bottom with the working distance of the objective lens to ensure that it is compatible. In general, objectives with higher numerical aperture (NA) tend to require thin-bottomed plates. Plates with a bottom thickness comparable to a standard coverslip (0.17 mm) work well with all supported objectives.



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

Batch-to-Batch Consistency

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

Sample Preparation

There are many variables involved in sample preparation. It is best to test these variables during the assay optimization phase before you prepare multiple plates for screening.

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

Cell Density

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

Final Buffer or Media

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

Using an embedding medium with a refractive index close to the refractive index of the plastic or the glass will interfere with the LED autofocus. In such cases, we recommend that you use the software-based autofocus. See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details.

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

Plate Handling and Storage

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

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





Adding External Temporary Storage

During acquisition, the ImageXpress Pico system stores images in its internal temporary data storage. Once acquisition is successfully completed, the images are moved from the internal temporary data storage to the available data storage.

For some acquisitions, such as one with several time points, the internal temporary storage may be insufficient, and a validation error will occur. In these cases, add external temporary storage to the instrument. You can use any USB 3 data storage device for external temporary storage, including a flash drive or a hard disk drive. The storage device should be empty and free from viruses.



To add external temporary storage for a protocol:

1. Connect a USB 3 data storage device to the USB storage port on the front of the instrument.
2. In the CellReporterXpress software, on the **Home** page, click  **Acquisition**.
3. Click the protocol card for which you want to add external temporary storage.
4. In the **Available Acquisition Devices** list, select the instrument.
5. Click  **Run Protocol**.
6. On the right side of the screen, click  **Storage**.
7. In the **Available Temporary Storage on Device** list, select the data storage device.
8. Click  **Save Protocol**.
9. Click **Save Protocol**.

Analyzing Data

All data analysis includes segmenting and analyzing acquired data. All data analysis operations are performed using the CellReporterXpress software.

See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details on analyzing data.




Maintaining the Instrument

User maintenance tasks for the ImageXpress Pico system include cleaning the instrument and the objectives. See [Maintaining the ImageXpress Pico System on page 57](#) for details.

Molecular Devices offers fixed-price service plans to provide comprehensive coverage for your instrument by our global network of factory-trained engineers. Service plans include annual preventive maintenance, which ensure that your instrument is running at peak performance and meets operational specifications. For details on service plans and other support options, go to www.moleculardevices.com/service-support.

Shutting Down the System

To shut down the ImageXpress Pico system:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. In the **Available Acquisition Devices** list, select the instrument.
3. Click  **Shutdown Device**.
4. If needed, do the following to end the CellReporterXpress session on the host computer:
 - a. Click  **Log Out**.
 - b. Click **OK**.



Note: You may want to continue the CellReporterXpress session to continue analysis or if you have other instruments or remote clients connected.

5. Press the Power button on the front of the instrument.
When the status light on the Power button is amber, the instrument is fully powered off.



Note: If the ImageXpress Pico system doesn't respond, you can perform a hard shutdown by holding the Power button for five seconds.



CAUTION! After shutting down the system, wait at least 30 seconds before restarting the system.

The optional environmental control system enables you to control the environment of your sample in a plate. You can regulate temperature, humidity, CO₂, and O₂ inside the environmental control cassette to do the following:

- Keep cells alive for several days, growing at a rate comparable to that expected with a standard cell culture incubator. This enables you to perform experiments using multi-day, live-cell, time-lapse imaging.
- Reduce the oxygen inside the environmental control cassette. This enables you to perform hypoxia experiments.



Note: Environmental control is fully supported for plate and dish acquisitions only. For slide acquisitions, only temperature control is available; humidity, CO₂, and O₂ control is not available.

The environmental control system offers the following controls:

Component	Set Range	Notes
CO ₂	1% to 15%	
Humidity	85%	Humidity level is fixed.
O ₂	1% to 15% and ambient	
Temperature	25°C to 40°C (77°F to 104°F)	Environmental control cassette is not required. Minimum achievable temperature setting is: 6°C (11°F) above ambient without cassette. 8°C (14°F) above ambient with cassette.



Note:

- To achieve the minimum temperature setting of 25°C (77°F) without the environmental control cassette, the room temperature can be no higher than 19°C (66.2°F).
- To achieve the minimum temperature setting of 25°C (77°F) with the environmental control cassette, the room temperature can be no higher than 17°C (62.5°F).

If your instrument does not include the environmental control system, contact your Molecular Devices representative to discuss adding this optional feature to your instrument.

Environmental Control System Warnings and Cautions

Take note of the following when using the environmental control system:



WARNING!

- Do not operate the environmental control system with substances, or under conditions, which can cause a risk of explosion, implosion, or the release of gases.
- Use a compressed gas supply in a well-ventilated area. The instrument is not air-tight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures your safety officer defines to maintain a safe work environment, such as the use of an automatic warning system.
- Use only the compressed gas supplies described in this guide, which are CO₂, N₂, and compressed air. Do not attempt to connect a pure O₂ tank or any other gas supply to the instrument.



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.



CAUTION!

- The environmental control system includes heated tubing that controls the temperature of the gas flow. Some accessible parts of the tubing and the humidifying column can reach temperatures of up to 50°C (122°F). Avoid touching the temperature-controlled parts of the system.
- Do not use the instrument with hazardous substances.
- If you use any substances or materials that pose a risk of infections with the instrument, it is your responsibility to apply best practices when handling these materials.
- If the CO₂ port or the N₂ port is not connected to a gas cylinder, use a blind plug (included) to close it off.

See [Using the Environmental Control System on page 44](#) for details on other cautions and important information on using the environmental control system.

Environmental Control System Hardware

The environmental control system uses a magnetically sealed cassette that contains the sample labware. Humidified gas (CO₂, N₂, and compressed air) is supplied into the cassette to form the specified environment. Temperature is controlled within the cassette.

The environmental control system consists of the following hardware subsystems:

- **Temperature Control:** Regulates the temperature at 8°C (14°F) above ambient within a range of 25°C to 40°C (77°F to 104°F).



Note: Without the optional environmental control system, it is still possible to regulate temperature inside the instrument at 6°C (11°F) above ambient within a range of 25°C to 40°C (77°F to 104°F).

- **Gas Control:** Provides CO₂, N₂, and compressed air to control the gas concentration inside the environmental control cassette. CO₂ is typically supplied from a gas cylinder. N₂ can be supplied from gas cylinders or a lab gas line. Compressed air can be supplied from a gas cylinder, a lab gas line, or an oil-free air pump or compressor. Gas pressure to the instrument must be regulated within the range of 0.8 bar to 1.2 bar (11.6 psi to 17.4 psi). The ideal setting is 1.0 bar (14.5 psi).
- **Humidity Control:** Provides 85% humidity by bubbling gases through the humidifying column to minimize evaporation from the sample labware over the duration of a time-lapse experiment.

This section contains information on the following:

- [Items Provided by Molecular Devices, see page 42](#)
- [Items Provided by You, see page 43](#)

Items Provided by Molecular Devices

The following hardware components are included with the environmental control system:

- **Environmental Control Cassette:** Holds the labware during acquisition. The cassette is magnetically sealed to maintain the environment and is compatible with most plate and dish formats from 6-well to 384-well with a plate bottom clearance of at least 1.3 mm (0.05 in.) and a maximum height (including the lid) of 23 mm (0.9 in.). Note that the standard height for plates is 14.35 mm \pm 0.25 mm (0.5650 in. \pm 0.0098 in.).



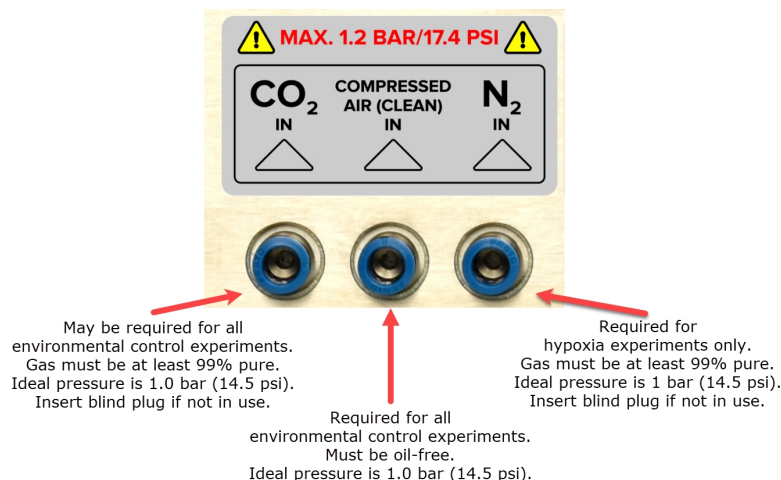
Note: Plate bottom clearance is defined by subtracting well depth and bottom thickness from plate height, as follows:

$$\text{Clearance} = \text{Plate Height} - \text{Well Depth} - \text{Bottom Thickness}$$

- **Environmental Sensors:** Provides accurate readings of temperature, humidity, CO₂, and O₂.
- **Environmental Control Tray:** Attaches to the back of the instrument to hold the humidifying column and the tubing/wiring.
- **Humidifying Column:** Humidifies the mixed gas. Part of the gas mixture is guided through the column, which contains a water reservoir. The water inside the column is warmed for higher humidity uptake.
- **Humidifying Column Tubing/Wiring:** Connects the humidifying column to the instrument.
- **Gas Supply Tubing:** Molecular Devices provides 10 m (32.8 ft) of tubing to connect the instrument to the regulator. If this is not sufficient, you must provide an appropriate length of 4 mm I.D. / 6 mm O.D. polyurethane tubing.
- **Push-to-Connect Fittings:** Attaches to the gas supply regulator or lab gas line to enable easy connections to the instrument.
- **Blind Plugs:** Closes off the CO₂ port, the N₂ port, or both ports when not in use.
- **Humidifying Column Stoppers:** Seals the access port on the humidifying column.
- **Plate Skirt Height Adapter:** Raises the plate inside the environmental control cassette.

Items Provided by You

You must provide the following items for the environmental control system:



- **Required Gas Supplies:** The following gas supplies are required for all experiments using environmental control:
 - **Compressed Air Source:** Supplies pressurized air from a cylinder, a lab gas line, or an oil-free air pump or compressor.
 - **Pressurized CO₂ Source:** Supplies pressurized CO₂ from a cylinder. Gas must be at least 99% pure.



Note: CO₂ is used to regulate the pH of cell culture media for mammalian cells. If you are using an organic buffer solution (for example, HEPES) to regulate the pH of your media, then a CO₂ source may not be required.

- **Optional Gas Supply:** The following gas supply is required only for hypoxia experiments:
 - **Pressurized N₂ Source:** Supplies pressurized N₂ from a cylinder. Gas must be at least 99% pure. Required for hypoxia experiments; otherwise optional.
- **Pressure regulators and Fittings:** Delivers gases from the cylinders to the instrument. Gas pressure to the instrument must be regulated within the range of 0.8 bar to 1.2 bar (11.6 psi to 17.4 psi). The ideal setting is 1.0 bar (14.5 psi). See [Environmental Control System Gas Regulator Details on page 89](#) for details.
- **Gas Supply Tubing:** Molecular Devices provides 10 m (32.8 ft) of tubing to connect the instrument to the regulator. If this is not sufficient, you must provide an appropriate length of 4 mm I.D. / 6 mm O.D. polyurethane tubing.
- **Teflon Tape and Hose Clamps:** Secures tubing and regulator fittings.
- **Ultrapure Water (18 Mohm•cm):** Maintains humidity inside the environmental control cassette. The humidifying column can hold up to 130 ml (4.4 oz) of ultrapure water and must be refilled before the level drops to 50 ml (1.7 oz).

Using the Environmental Control System

This section provides details on setting up and using the environmental control system, including the following:

- [Setting Up the Environmental Control System, see below](#)
- [Connecting and Disconnecting Tubing, see page 48](#)
- [Filling the Humidifying Column, see page 49](#)
- [Loading the Environmental Control Cassette, see page 51](#)
- [Using the Plate Skirt Height Adapter, see page 53](#)
- [Setting Environmental Control Parameters, see page 54](#)

Setting Up the Environmental Control System

To set up the environmental control system:

1. Confirm that the valves on the gas cylinders are completely shut off and that no gas is flowing.
2. If the instrument is powered on, press the Power button on the front of the instrument to turn off the power.

When the status light on the Power button is amber, the instrument is fully powered off.



Note: If the ImageXpress Pico system doesn't respond, you can perform a hard shutdown by holding the Power button for five seconds.

3. On the environmental control tray, loosen the large screw to remove the top piece from the tray.
4. On the bottom piece of the environmental control tray, slide the tab under the instrument so it engages the two screws.



5. Insert the humidifying column in the environmental control tray.



6. Do the following to connect the humidifying column:



Note: See [Connecting and Disconnecting Tubing on page 48](#) for details on connecting tubing.

- a. Align the red dots to connect the electronics cable to the lower port on the humidifying column.



- b. Connect the tubing with the blue band to blue port on the humidifying column.
c. Connect the tubing with the red band to red port on the humidifying column.



7. Do the following to connect the humidifying column to the instrument:
 - a. Connect the tubing with the white 1 band to the **OUT WHITE 1** port.
 - b. Connect the tubing with the yellow 2 band to the **OUT YELLOW 2** port.
 - c. Connect the tubing with the red 3 band to the **HUMIDITY IN 3** port.
 - d. Connect the electronics cable to the **COM HEATING HUM COLUMN** port.
8. Do the following to connect the gas cylinder to the instrument:
 - a. Connect the tubing from the CO₂ source to the **CO₂ IN** port.
 - b. Connect the tubing from the compressed air source to the **COMPRESSED AIR (CLEAN) IN** port.
 - c. If you will be performing hypoxia experiments, connect the tubing from the N₂ source to the **N₂ IN** port.



CAUTION! For any port not connected to a gas cylinder, close off the port with one of the included blind plugs (as shown below).



9. Replace the top piece of the environmental control tray and tighten the screw.



Connecting and Disconnecting Tubing

As part of working with the environmental control system, you must connect and disconnect tubing.

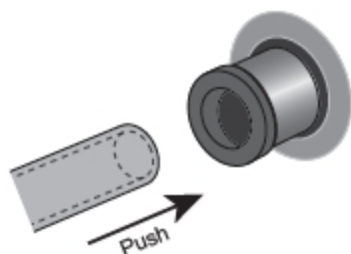


WARNING! Do not connect or disconnect tubing when there is pressure from the gas cylinder.

Connecting Tubing

To connect tubing:

1. Confirm that the pressure from the gas cylinder is turned off.
2. Push the tubing in to the port to connect it.

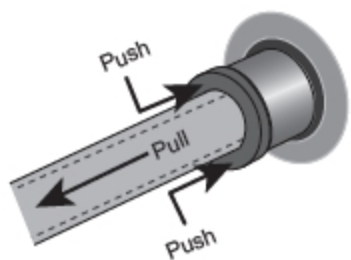


3. After inserting the tubing, gently pull the tubing to confirm it is seated.

Disconnecting Tubing

To disconnect tubing:

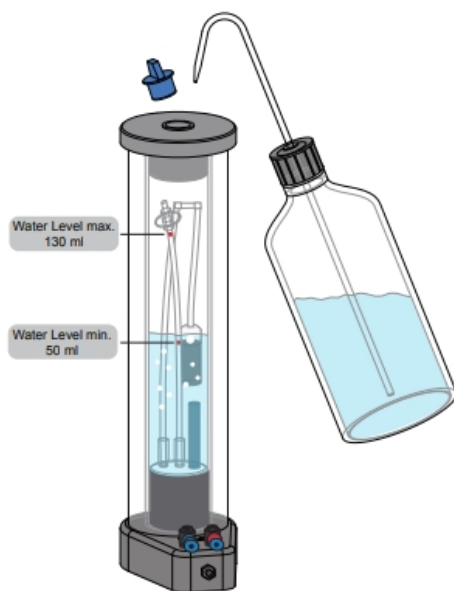
1. Confirm that the pressure from the gas cylinder is turned off.
2. Push in the outer ring as you pull the tubing.



Filling the Humidifying Column

The humidifying column uses ultrapure water to add humidity to the air flow supplied to the environmental control cassette.

The humidifying column holds 130 ml (4.4 oz) of ultrapure water, which is enough to continuously provide humidity for up to three or four days. When the water level approaches the minimum indicator (approximately one-third full), add 18 Mohm•cm ultrapure water to reach the maximum level.



**CAUTION!**

- Do not operate humidity level control if the water level is below the minimum indicator. Operating without enough water can damage the instrument and the humidifying column.
 - Before and during an experiment with humidity level control, check the water level in the humidifying column and refill as needed. (Be aware that refilling the humidifying column during an experiment can reduce the humidity level for several minutes.)
 - Use only 18 Mohm•cm ultrapure water to fill the humidifying column.
 - It can be easy to overfill the humidifying column. When filling the humidifying column, use care not to fill beyond the maximum indicator.
 - Confirm that the rubber stopper is firmly seated in the humidifying column. A loose stopper can allow gas leakage and other environmental control system issues.
 - If you need to empty the humidifying column, be sure to disconnect all tubing/wiring before you begin. Trying to empty the humidifying column with the tubing/wiring connected can damage the instrument and the humidifying column. See [Connecting and Disconnecting Tubing on page 48](#) for details.
-



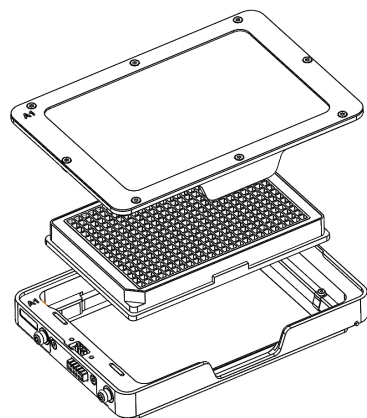
Note: If the humidifying column does run dry, restart the instrument after refilling the column.

To fill the humidifying column:

1. Remove the rubber stopper from the top of the humidifying column.
2. Fill the humidifying column with 18 Mohm•cm ultrapure water to the maximum indicator (approximately two-thirds full). At the maximum indicator, the humidifying column holds 130 ml (4.4 oz).
3. Replace the rubber stopper at the top of the humidifying column.

Loading the Environmental Control Cassette

The environmental control cassette is a magnetically sealed, glass enclosure that holds the plate or dishes in the instrument.



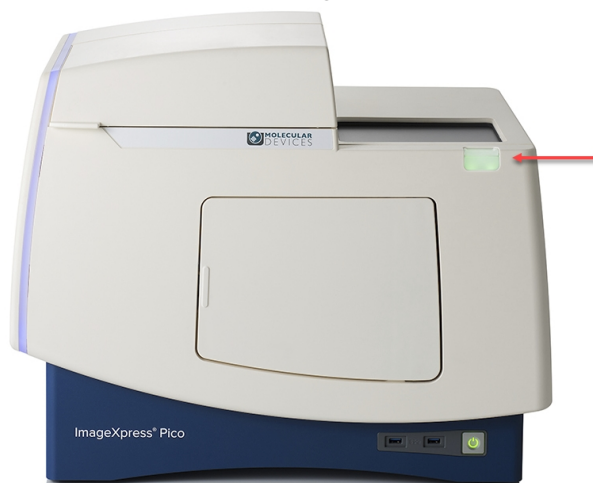
Like the sample stage, both the environmental control cassette and lid also have an **A1** label to indicate their top left corners.



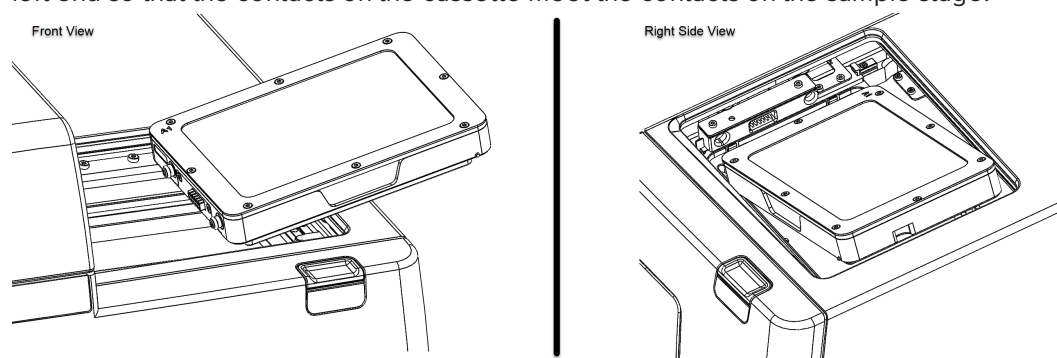
Note: Do not touch the glass parts of the environmental control cassette.

To load the environmental control cassette:

1. If the instrument is powered off, press the Power button on the front of the instrument to turn on the power. See [Powering On the Instrument on page 24](#) for details.
2. Press the button at the top right of the instrument to open the top door.



3. If the sample stage already contains a labware holder, remove it.
4. Insert the environmental control cassette (with nothing inside) in the sample stage from the left end so that the contacts on the cassette meet the contacts on the sample stage.



5. Confirm that the environmental control cassette is firmly seated.



Note: The top door may not close if the cassette is not seated properly.

6. Lift the top of the environmental control cassette and insert the plate containing your samples.

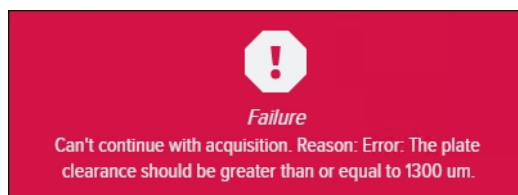


Note: The 4-slide holder is not compatible with the environmental control cassette.

7. Replace the top of the environmental control cassette.
8. Press the button at the top right of the instrument to close the top door.

Using the Plate Skirt Height Adapter

If you are using the environmental control cassette and autofocus fails, the CellReporterXpress software may display the following message:

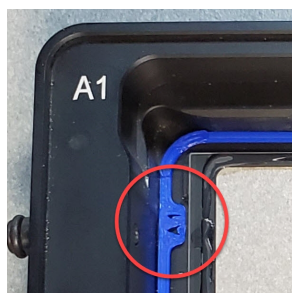


In this case, you may need to insert the plate skirt height adapter.



The plate skirt height adapter sits inside the environmental control cassette to raise the plate, which can help autofocus succeed. When you use the adapter, you must select a labware specification that adds 1.5 mm to the actual **Plate Height** value. See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details on adding and selecting a labware specification.

Like the cassette, the adapter also has an **A1** label to indicate its top left corner.



To use the plate skirt height adapter:

1. Press the button at the top right of the instrument to open the top door.
2. Lift the top of the environmental control cassette and remove the plate containing your samples.
3. With the **A1** label at the top left, insert the plate skirt height adapter in the cassette.
4. Confirm that the adapter is seated flat in the cassette.
5. Re-insert the plate containing your samples in the environmental control cassette.
6. Press the button at the top right of the instrument to close the top door.

When you set up an acquisition with the plate skirt height adapter in the optional environmental control cassette, select a plate format that adds 1.5 mm to the actual **Plate Height** value. See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details on adding and selecting a labware specification.

Setting Environmental Control Parameters

You can set environmental control parameters (including target values and notifications) on the Sensors tab in Devices mode in the CellReporterXpress software. You can also specify environmental sensors to be monitored during an experiment on the Device Sensors page in Acquisition mode.

See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details on setting environmental control parameters.

Maintaining the Environmental Control System

User maintenance tasks for the environmental control system include running air dry and cleaning the humidifying column. See [Environmental Control System Maintenance on page 76](#) for details.

Troubleshooting the Environmental Control System

If you experience an issue with the environmental control system, do the following:

- Confirm that the top of the environmental control cassette is placed correctly (that is, not reversed) and is firmly seated.
- Confirm that the environmental control cassette is loaded properly in the sample stage and is firmly seated. See [Loading the Environmental Control Cassette on page 51](#) for details.
- Confirm that you are using a compatible regulator (which must be a pressure regulator, not a flow regulator). See [Environmental Control System Gas Regulator Details on page 89](#) for details.
- Confirm that the pressure is set correctly. Gas pressure to the instrument must be regulated within the range of 0.8 bar to 1.2 bar (11.6 psi to 17.4 psi). The ideal setting is 1.0 bar (14.5 psi).
- Confirm that you are using the correct gas supplies. Do not substitute another gas supply (such as O₂ or CO₂) for compressed air.

If the issue persists, review the following troubleshooting tips before contacting Molecular Devices Technical Support.

Unused Ports Are Not Fully Closed Off

Typical Symptom: Used gases (such as CO₂) do not reach target set point.

Recommended Actions:

- If an N₂ supply is connected, but not in use, disconnect the N₂ supply and use a blind plug. See [Connecting and Disconnecting Tubing on page 48](#) for details.
- Confirm that blind plugs are securely inserted in the unused port. The following image shows two blind plugs in use (indicated by arrows). If you are regulating CO₂ level, use a blind plug for the N₂ input port only.



Incorrect Set Point Configured

Typical Symptom: Gas levels are unstable.

Recommended Action:

- Confirm that the target level is within the supported range. For example, CO₂ should be set only within the range of 1% and 15%.

Insufficient Water in the Humidifying Column

Typical Symptom: The instrument is unable to consistently achieve a humidity level of between 75% and 95%.



CAUTION! Do not operate humidity level control if the water level is below the minimum indicator. Operating without enough water can damage the instrument and the humidifying column.

Recommended Action:

- Confirm that there is a sufficient amount of ultrapure water in the humidifying column. See [Filling the Humidifying Column on page 49](#) for details.

Water in the Lines

Typical Symptom: Humidity level spikes suddenly.

Recommended Action:

- Run the air dry maintenance. See [Running Air Dry on page 76](#) for details. Note that running air dry can take 60 minutes or more to complete.

This section describes the user maintenance tasks for the ImageXpress Pico system.

Molecular Devices offers fixed-price service plans to provide comprehensive coverage for your instrument by our global network of factory-trained engineers. Service plans include annual preventive maintenance, which ensure that your instrument is running at peak performance and meets operational specifications. For details on service plans and other support options, go to www.moleculardevices.com/service-support.

Before you operate the instrument or perform maintenance, make sure you are familiar with the safety information in this guide. See [Safety Information on page 5](#) for details.

General Maintenance Precautions

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

- Some maintenance procedures require that the instrument be powered off and the power cable be unplugged.
- Some maintenance procedures require that you disconnect the Ethernet cable to the network or host PC and turn off any attached peripherals.
- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.

The following topics describe the maintenance procedures you can perform:

- [Routine Maintenance, see page 58](#)
- [Instrument Maintenance, see page 58](#)
- [Objective Maintenance, see page 61](#)
- [Filter Cube Maintenance, see page 71](#)
- [Environmental Control System Maintenance, see page 76](#)



CAUTION! Maintenance procedures other than those specified in this guide must be performed by Molecular Devices. When service is required, contact Molecular Devices technical support.

Routine Maintenance

To ensure optimal operation of the instrument, perform the following routine maintenance procedures as needed:

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

Instrument Maintenance



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.



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

Observe the following general tips when cleaning the instrument:

- Always power off the instrument and disconnect the power cord from the main power source before using liquids to clean the instrument.
- Wipe up all spills immediately.
- Periodically clean the outside surfaces of the instrument using a cloth or sponge that has been lightly dampened with water.
- Do not use any cleaning agents other than those recommended in this section without first contacting Molecular Devices Technical Support. See [Obtaining Support on page 20](#).
- Do not use ultraviolet light for sterilization, as this can damage plastic components.
- To prevent damaging internal components, do not pour or squirt water or alcohol directly onto the instrument.
- After cleaning the instrument with a liquid, wipe the surface dry with a lint-free cloth.

Cleaning the Instrument



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

Use this procedure to clean the plate-loading region of the instrument without damaging the internal components of the imaging system.



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

Before cleaning the instrument, review the General Maintenance Precautions under [Maintaining the ImageXpress Pico System on page 57](#).

The following cleaning procedure is compatible with disinfectant wipes with 70% ethanol.



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

To clean the ImageXpress Pico system:

1. Confirm that the maintenance door on the front of the instrument is closed.
2. Press the button at the top right of the instrument to open the top door.
3. Confirm that no sample is loaded.
4. Press the Power button on the front of the instrument.

When the status light on the Power button is amber, the instrument is fully powered off.



Note: If the ImageXpress Pico system doesn't respond, you can perform a hard shutdown by holding the Power button for five seconds.

5. Do the following with gloved hands:
 - a. Use a damp wipe to wipe down the entire outer surface of the instrument.
 - b. Use a 70% ethanol disinfectant wipe to wipe down the entire outer surface again.
 - c. Use forceps wrapped with damp wipes to gently wipe the perimeter of the sample (X-Y) stage.
 - d. Use forceps wrapped with 70% ethanol disinfectant wipes to wipe the perimeter of the sample stage.
 - e. Use a fresh damp wipe to clean the sample stage underneath and around the loading area.



CAUTION! Do not manually move the stage. Manually moving the stage can damage the instrument.

- f. Use a fresh 70% ethanol disinfectant wipe to clean the sample stage underneath and around the plate loading area again.
6. Wait a few minutes for the alcohol to evaporate.
7. Press the Power button on the front of the instrument.
8. Press the button at the top right of the instrument to close the top door.

Objective Maintenance

Objective maintenance procedures include the following:

- Installing an Objective, see page 62
- Calibrating an Objective, see page 66
- Adjusting an Objective Correction Collar, see page 67
- Cleaning an Objective, see page 70

You can identify the magnification of an objective by the color band:

Objective Magnification	Color Band
4x	Red
10x	Yellow
20x	Green
40x	Light Blue
63x	Dark Blue



Color Band on
4x Objective

The standard objectives in the ImageXpress Pico system are configured and calibrated by Molecular Devices when the instrument is delivered.



CAUTION! Replace objectives in their original positions.



Tip: We recommend removing and maintaining only one objective at a time.

Installing an Objective

Before installing an objective, review the General Maintenance Precautions under [Maintaining the ImageXpress Pico System](#) on page 57.



CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

Molecular Devices precalibrates the objectives to specific slots in the turret. You must install the objectives as follows:

Slot	Objective Magnification
1	4x
2	10x
3	20x
4	empty
5	40x or 63x
6	empty






Note:

- Depending on how your ImageXpress Pico system is configured, you may not have all the objectives.
- The 40x objective and the 63x objective cannot be installed in the instrument simultaneously.
- The 63x objective cannot be installed when the environmental control cassette is loaded.

Installing an Objective in an Empty Slot

You must install objectives in specific slots in the turret. See [Installing an Objective on page 62](#) for details.

To install an objective in an empty slot:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. Click  **Show Device Options** to expand the details for the instrument where you want to install an objective.
3. Click the **Objectives** tab.
4. In the tile for the objective slot where you want to install, click  **Component Exchange**.
5. Click the **Choose Objective** drop-down list box, and select the objective you want to install.
6. Click **Open Maintenance Door**.
7. Install the objective in the slot by gently turning it clockwise.






CAUTION! Retain the objective case. When not installed in the instrument, an objective should always be stored in its case.

8. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
9. Click **Apply**.

Replacing an Objective (Different Magnification)

You must install objectives in specific slots in the turret. See [Installing an Objective on page 62](#) for details. Because of this, the only reason to replace an objective with another objective of a different magnification is when you are swapping the 40x objective and the 63x objective in slot 5.

To replace an objective with an objective of a different magnification:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. Click  **Show Device Options** to expand the details for the instrument where you want to replace an objective.
3. Click the **Objectives** tab.
4. In the tile for objective slot **5**, click  **Component Exchange**.
5. Click the **Choose Objective** drop-down list box, and select the objective you want to install.
6. Click **Open Maintenance Door**.
7. Remove the objective to be replaced from the instrument by gently turning it counterclockwise.



CAUTION! When not installed in the instrument, an objective should always be stored in its case.

8. Install the replacement objective in the slot by gently turning it clockwise.



CAUTION! Retain the objective case for future storage needs. When not installed in the instrument, an objective should always be stored in its case.




9. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
10. Click **Apply**.

After you replace an objective, you may need to calibrate it. See [Calibrating an Objective on page 66](#) for details.

Replacing an Objective (Same Magnification)

You must install objectives in specific slots in the turret. See [Installing an Objective on page 62](#) for details. The typical reason to replace an objective with another objective of the same magnification is when you are replacing a damaged objective.

To replace an objective with an objective of the same magnification:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. Click  **Show Device Options** to expand the details for the instrument where you want to replace an objective.
3. Click the **Objectives** tab.
4. In the tile for the objective slot where you want to install, click  **Component Exchange**.
5. Click the **Choose Objective** drop-down list box, and select **None**.
6. Click **Open Maintenance Door**.
7. Remove the objective to be replaced from the instrument by gently turning it counterclockwise.



CAUTION! When not installed in the instrument, an objective should always be stored in its case.

8. Install the replacement objective in the slot by gently turning it clockwise.



CAUTION! Retain the objective case for future storage needs. When not installed in the instrument, an objective should always be stored in its case.

9. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
10. Click **Close**.

After you replace an objective with another objective, you may need to calibrate it. See [Calibrating an Objective on page 66](#) for details.



Note: If you re-install the same objective (after cleaning it, for example), you typically do not need to calibrate it.

Calibrating an Objective

After you install a new objective, you may need to calibrate it. **Molecular Devices precalibrates the objectives included with the initial purchase of the instrument. You must calibrate any objectives purchased after that time.** A calibration kit, which is included with any after-sales objective purchase, includes the following:


- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

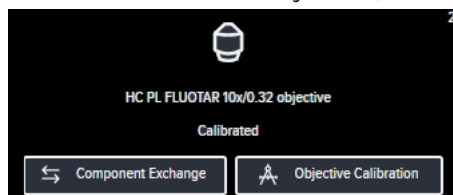
You will need to provide the following item for the calibration process:

- 4-Slide Holder (5068503)

To calibrate an objective:




1. In the CellReporterXpress software, on the **Home** page, click **Devices**.
2. Click  **Show Device Options** to expand the details for the device where you want to calibrate an objective.
3. Click the **Objectives** tab.
4. Note the status of the objective, which is indicated in the tile.



- If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
- If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.



Note: An exception to this is when you replace an objective with another objective of the same magnification. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

5. In the tile for the objective you want to calibrate, click  **Objective Calibration**.
6. Follow the on-screen instructions to complete the calibration.



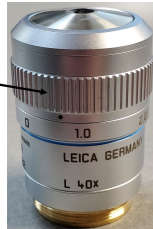
Tip:

- In the **Choose Slide Holder** drop-down list box, select **4 Slide Holder**.
- Insert the slides face down in the slide holder (that is, printed side down).
- When each step completes, click **Next** to continue to the next step.

Adjusting an Objective Correction Collar

The 40x objective and 63x objective have application-optimized correction collars to compensate for well bottom thickness or coverslip thickness. The collars have a range of 0 mm to 2 mm correction. Changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very dependent on properly setting these collars.

Correction Collar
on 40x Objective



The settings to be used depend on the well bottom thickness of the plate or the coverslip thickness on the 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 by the plate specifications from the plate manufacturer.



Note: Do not use a plate, slide, or coverslip with a thickness that is out of the range of the correction collar for the selected objective.

Before adjusting an objective correction collar, review the General Maintenance Precautions under [Maintaining the ImageXpress Pico System on page 57](#).







CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

You would typically adjust a correction collar as part of setting up an acquisition.

Adjusting an Objective Correction Collar with a Plate



To adjust an objective correction collar with a plate:

1. In the CellReporterXpress software, on the **Home** page, click  **Acquisition**.
 2. Click **Add Protocol**.
 3. Click **New Plate Acquisition**.
 4. In the **Acquisition Devices** list, select the instrument.
 5. On the left side of the screen under **Steps**, click  **Acquisition Settings**.
 6. On the right side of the screen under **Tools**, click  **Plate Format**.
 7. In the **Plate Format** list, select the plate format.
 8. On the right side of the screen under **Tools**, click  **Objectives**.
 9. In the **Objectives** list, select the objective.
- If a correction collar adjustment is required, the software displays the recommended setting.

You may need to perform correction of objective collar for the current objective. Use value: 0.19



Note: When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom.

10. On the left side of the screen under **Steps**, click  **Acquisition Device**.
11. On the right side of the screen, click  **Set Up for Adjustment of Objective Collar**.
12. Click **OK**.
The top door opens.
13. If needed, loosen the objective from the instrument by gently turning it counterclockwise.
14. Rotate the correction collar to its new setting.



Tip: You might need a flashlight to see the markings for the graduated scale on the barrel and its current setting.

15. If you loosened the objective, tighten it by gently turning it clockwise.




Note: When tightening the objective, take care to avoid changing the correction collar setting.


16. Close the top door.
17. Click **OK**.


Adjusting an Objective Correction Collar for a Slide

To adjust an objective correction collar for a slide:

1. In the CellReporterXpress software, on the **Home** page, click  **Acquisition**.
2. Click **Add Protocol**.
3. Click **New Slide Acquisition**.
4. In the **Available Acquisition Devices** list, select the instrument.

5. On the left side of the screen under **Steps**, click  **Acquisition Settings**.

6. On the right side of the screen under **Tools**, click  **Slide Format**.
7. In the **Slide Format** list, select the slide format.

8. On the right side of the screen under **Tools**, click  **Objectives**.

9. In the **Objectives** list, select the objective.

If a correction collar adjustment is required, the software displays the recommended setting.

You may need to perform correction of objective collar for the current objective. Use value: 0.19

10. On the left side of the screen under **Steps**, click  **Acquisition Device**.
11. On the right side of the screen, click  **Set Up for Adjustment of Objective Collar**.

12. Click **OK**.

The top door opens.

13. If needed, loosen the objective from the instrument by gently turning it counterclockwise.
14. Rotate the correction collar to its new setting.



Tip: You might need a flashlight to see the markings for the graduated scale on the barrel and its current setting.

15. If you loosened the objective, tighten it by gently turning it clockwise.



Note: When tightening the objective, take care to avoid changing the correction collar setting.

16. Close the top door.
17. Click **OK**.

Cleaning an Objective

If debris and contaminants collect on an objective lens, you can clean it. Before cleaning an objective, review the General Maintenance Precautions under [Maintaining the ImageXpress Pico System on page 57](#).




CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.
- Do not use a product that disperses aerosol propellants or fluid onto the lens surface, such as canned compressed air.
- Do not use a wipe that can leave lint, such as Kimwipes.

To clean an objective:



1. In the CellReporterXpress software, on the **Home** page, click **Devices**.
2. In the **Available Acquisition Devices** list, select the instrument.
3. Click the **Objectives** tab.
4. In the tile for the objective you want to clean, click  **Component Exchange**.
5. Click the **Choose Objectives** drop-down list box, and select **None**.
6. Click **Open Maintenance Door**.
7. Remove the objective from the instrument by gently turning it counterclockwise.
8. Place the objective on a secure surface away from the instrument.
9. Use a bulb duster to carefully blow dust contaminants off the objective.
10. Use lens paper to gently wipe the objective free of contaminants.



Tip: If needed, you can use a 100% methanol lens cleaner. Refer to Leica for details on preferred cleansing solvent and procedure.

11. If needed, wait a few minutes for the methanol to evaporate.
12. Install the objective back its original slot in the turret by gently turning it clockwise.



Tip: If the objective has a correction collar, make sure that the collar is at the correct setting when reinstalling it.

13. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
14. Click **Close**.

After cleaning an objective, you may want to acquire a sample image. If image quality is degraded, repeat this procedure.

Filter Cube Maintenance

Filter cube maintenance procedures include the following:

- [Installing a Filter Cube, see below](#)
- [Calibrating a Filter Cube, see page 75](#)

Installing a Filter Cube

Before installing a filter cube, review the General Maintenance Precautions under [Maintaining the ImageXpress Pico System on page 57](#).



CAUTION! To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.

The following filter cubes are available:

- DAPI
- FITC
- TRITC
- Cy5
- Texas Red
- CFP






Note:

- Depending on how you have configured your ImageXpress Pico system, you may not have received all the filter cubes.
 - Do not install a filter cube in slot 6 of the turret. Slot 6 must be empty (in both the instrument and the software).
-

Installing a Filter Cube in an Empty Slot

To install a filter cube in an empty slot:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. Click  **Show Device Options** to expand the details for the device where you want to install a filter cube.
3. Click the **Filters** tab.
4. In the tile for the filter cube slot where you want to install, click  **Component Exchange**.



Note: Do not install a filter cube in slot 6 of the turret. Slot 6 must be empty (in both the instrument and the software).

5. Click the **Choose Filter** drop-down list box, and select the filter cube you want to install.
6. Click **Open Maintenance Door**.
7. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
8. Install the filter cube in the slot by gently pushing it into the slot until it “snaps” into place.








CAUTION! Retain the filter cube packaging. When not installed in the instrument, a filter cube should always be stored in its original packaging.

9. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
10. Click **Apply**.

Replacing a Filter Cube (Different Wavelength)

To replace a filter cube with a filter cube of a different wavelength:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
 2. Click  **Show Device Options** to expand the details for the device where you want to replace a filter cube.
 3. Click the **Filters** tab.
 4. In the tile for the filter cube slot where you want to install, click  **Component Exchange**.
 5. Click the **Choose Filter** drop-down list box, and select the filter cube you want to install.
 6. Click **Open Maintenance Door**.
 7. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
 8. Remove the filter cube to be replaced from the instrument by gently pulling it toward you.
-
-  **CAUTION!** When not installed in the instrument, a filter cube should always be stored in its original packaging.
-
9. Install the filter cube in the slot by gently pushing it into the slot until it “snaps” into place.
-
-  **CAUTION!** Retain the filter cube packaging for future storage needs. When not installed in the instrument, a filter cube should always be stored in its original packaging.
-
10. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
 11. Click **Apply**.

After you replace a filter cube, you may need to calibrate it. See [Calibrating a Filter Cube on page 75](#) for details.








Note: If you re-install the same filter cube (after cleaning it, for example), you do not need to calibrate it.

Replacing a Filter Cube (Same Wavelength)

The typical reason to replace a filter cube with another filter cube of the same wavelength is when you are replacing a damaged filter cube.

To replace a filter cube with a filter cube of the same wavelength:

1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
 2. Click  **Show Device Options** to expand the details for the device where you want to replace a filter cube.
 3. Click the **Filters** tab.
 4. In the tile for the filter cube slot where you want to replace, click  **Component Exchange**.
 5. Click the **Choose Filter** drop-down list box, and select **None**.
 6. Click **Open Maintenance Door**.
 7. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
 8. Remove the filter cube to be replaced from the instrument by gently pulling it toward you.
-
-  **CAUTION!** When not installed in the instrument, a filter cube should always be stored in its original packaging.
-
9. Install the filter cube in the slot by gently pushing it into the slot until it “snaps” into place.
-
-  **CAUTION!** Retain the filter cube packaging for future storage needs. When not installed in the instrument, a filter cube should always be stored in its original packaging.
-
10. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
 11. Click **Close**.

After you replace a filter cube, you may need to calibrate it. See [Calibrating a Filter Cube on page 75](#) for details.



Note: If you re-install the same filter cube (after cleaning it, for example), you do not need to calibrate it.

Calibrating a Filter Cube

After you install a new filter cube, you may need to calibrate it. **Molecular Devices precalibrates the filter cubes included with the initial purchase of the instrument. You must calibrate any filter cubes purchased after that time.** A calibration kit, which is included with any after-sales filter cube purchase, includes the following:


- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

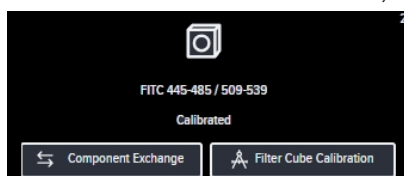
You will need to provide the following item for the calibration process:

- 4-Slide Holder (5068503)

To calibrate a filter cube:




1. In the CellReporterXpress software, on the **Home** page, click **Devices**.
2. Click  **Show Device Options** to expand the details for the device where you want to calibrate a filter cube.
3. Click the **Filters** tab.
4. Note the status of the filter cube, which is indicated in the tile.



- If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
- If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.



Note: An exception to this is when you replace a filter cube with the same type of filter cube. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

5. In the tile for the filter cube you want to calibrate, click  **Filter Cube Calibration**.
6. Follow the on-screen instructions to complete the calibration.



Tip:

- In the **Choose Slide Holder** drop-down list box, select **4 Slide Holder**.
- Insert the slides face down in the slide holder (that is, printed side down).
- When each step completes, click **Next** to continue to the next step.

Environmental Control System Maintenance

Environmental control system maintenance procedures include the following:

- [Running Air Dry, see below](#)
- [Cleaning the Environmental Control Cassette on page 77](#)
- [Cleaning the Outside of the Humidifying Column, see page 78](#)
- [Cleaning the Inside of the Humidifying Column and Tubing, see page 79](#)

Running Air Dry

If your instrument includes the optional environmental control system, you can run air dry to clear any condensation that builds up inside the system. Condensation in the environmental control system can prevent the instrument from consistently achieving a humidity level of between 75% and 95%.





Note:

- Air dry can take 60 minutes or more. During that time, the instrument will be busy.
- Humidity control is not used during air dry, so the process runs successfully both with and without water in the humidifying column. If you already have water in the column, we recommend that you leave it there while running air dry.
- Before packing the instrument for transport, run air dry.
- Before running air dry, load the environmental control cassette in the instrument. Confirm that there is no sample in the cassette.

While air dry is in progress, you can monitor the status on the Monitor page in the CellReporterXpress software. See the *CellReporterXpress Help* or the *CellReporterXpress User Guide* for details.

To run air dry:



1. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
2. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to run air dry.
3. Click the **Maintenance** tab.
4. Click **Air Dry**.
5. Click **Start Air Dry**.
6. Click **Finish**.

The wizard closes and air dry runs.

Cleaning the Environmental Control Cassette

If debris and contaminants collect on the environmental control cassette, you can clean it. Before cleaning the cassette, review the General Maintenance Precautions under [Maintaining the ImageXpress Pico System on page 57](#).

In addition, observe the following when handling the environmental control cassette:

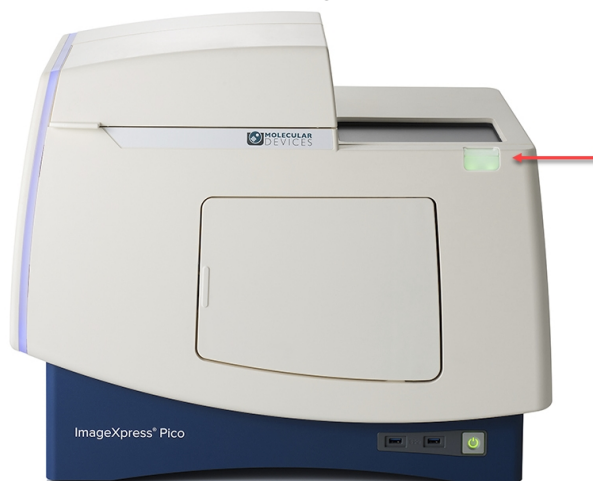


CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling the environmental control cassette.
- Do not use a product that disperses aerosol propellants or fluid onto the cassette surface, such as canned compressed air.
- Do not use a wipe that can leave lint, such as Kimwipes.

To clean the environmental control cassette:

1. Press the button at the top right of the instrument to open the top door.



2. If needed, lift the top of the environmental control cassette and remove the plate.
3. Remove the bottom of the environmental control cassette from the instrument by lifting from the right end.
4. Press the button at the top right of the instrument to close the top door.
5. Place the environmental control cassette on a secure surface away from the instrument.
6. Use a bulb duster to carefully blow dust contaminants off the cassette.
7. Use lens paper to gently wipe the objective free of contaminants.



Tip: If needed, you can use isopropanol. If any streaks are left that degrade image quality, use 100% methanol lens cleaner.

8. If needed, wait a few minutes for the isopropanol or methanol to evaporate.

After cleaning the environmental control cassette, you may want to acquire a sample image. If image quality is degraded, repeat this procedure.

Cleaning the Outside of the Humidifying Column

If needed, you can clean the outside of the humidifying column.

To clean the outside of the humidifying column:

1. If the instrument is powered on, press the Power button on the front of the instrument to turn off the power.

When the status light on the Power button is amber, the instrument is fully powered off.



Note: If the ImageXpress Pico system doesn't respond, you can perform a hard shutdown by holding the Power button for five seconds.

2. Disconnect all tubing/wiring from the humidifying column. See [Connecting and Disconnecting Tubing on page 48](#) for details.
3. Wait approximately 5 minutes to allow the humidifying column to cool.
4. Using a dry cloth or a cloth dampened with one of the following, wipe the outside of the humidifying column.
 - Ultrapure water (18 Mohm•cm)
 - Common lab disinfection solutions that are based on quaternary ammonium compounds (for example, Barrycidal 36 or Pharmacidal)
 - 70% ethanol or isopropanol



CAUTION!

- Other than 70% ethanol or isopropanol, do not use any other organic solvents to wipe the outside of the humidifying column.
- Do not expose the humidifying column to ethanol or isopropanol for an extended period, as this can damage the humidifying column.

Cleaning the Inside of the Humidifying Column and Tubing

Microbial contamination inside the humidifying column and tubing is typically not a problem since ultrapure water and light-starved environments (that is, microscopy rooms) do not support microbe growth. If, however, contamination occurs, you can clean the inside of the humidifying column and tubing.



CAUTION! Do not use anti-microbial agents to clean the humidifying column. Anti-microbial agents can be transported throughout the environmental control system as aerosol and contaminate the specimen.

To clean the inside of the humidifying column and tubing:

1. If the humidifying column is not empty, do the following:
 - a. Disconnect all tubing/wiring from the humidifying column. See [Connecting and Disconnecting Tubing on page 48](#) for details.
 - b. Wait approximately 5 minutes to allow the humidifying column to cool.
 - c. Empty the humidifying column.
 - d. Reconnect all tubing/wiring.
 2. Create 100 ml (3.38 oz.) of 3% hydrogen peroxide solution by mixing the following:
 - 90 ml (3.04 oz.) of 18 Mohm-cm ultrapure water
 - 10 ml (0.34 oz.) of 30% hydrogen peroxide solution
 3. Fill the humidifying column with the 3% hydrogen peroxide solution.
 4. Replace the rubber stopper at the top of the humidifying column.
-









CAUTION! Confirm that the stopper is firmly seated in the humidifying column.

5. Load the environmental control cassette. See [Loading the Environmental Control Cassette on page 51](#) for details.
-



Note: Do not insert a plate in the environmental control cassette. The environmental control cassette should be empty for this procedure.



6. In the CellReporterXpress software, on the **Home** page, click  **Devices**.
7. Click  **Show Device Options** to expand the details for the instrument where you want to clean the inside of the humidifying column.
8. Click the **Sensors** tab.
9. In the **Humidity** row, under **Component State**, click  **Start Regulation**.
The indicator in the **State** field enables to show that humidity control is on.
10. Do the following to set temperature control to 37°C (98.6°F):
 - a. In the **Temperature** row, under **Component State**, in the **Target** field, enter 37.
 - b. Click  **Start Regulation**.
The indicator in the **State** field enables to show that temperature control is on.
11. Confirm that both CO₂ level control and O₂ level control are off.
12. Wait at least one hour as decontamination progresses.
13. After waiting at least one hour, on the **Sensors** tab, in the **Humidity** row, under **Component State**, click  **Stop Regulation**.
14. In the **Temperature** row, under **Component State**, click  **Stop Regulation**.
15. Remove the rubber stopper from the top of the humidifying column.
16. Empty the 3% hydrogen peroxide solution from the humidifying column.
17. Rinse the humidifying column with ultrapure water at least once.
18. Refill the humidifying column with ultrapure water. See [Filling the Humidifying Column on page 49](#) for details.
19. Run air dry. See [Running Air Dry on page 76](#) for details.



Appendix A: Instrument Specifications

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



WARNING!

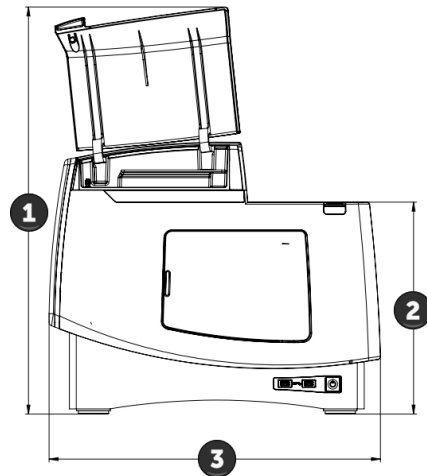
- If the instrument is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.
- The ImageXpress Pico system is an Equipment Class 1 product that relies on protective earth grounding for safe operation. Any interruption of the protective earth ground conductor, inside or outside the instrument, or disconnection of the protective earth ground terminal can result in personal injury.

Item	Description
Operating Environment	Indoor use only
System Power	100 VAC to 240 VAC, 50/60 Hz, 1.6 A nominal at 115 V, 200 W maximum
Fuses	5 mm x 20 mm 6 A MEDIUM 250 V UL CSA ceramic tube
Dimensions	<i>without environmental control system</i> Width: 55.1 cm (21.7 in.) Height: 45.3 cm (17.8 in.) Depth: 42.5 cm (16.7 in.)
	<i>with environmental control system</i> Width: 62.9 cm (24.7 in.) Height: 45.3 cm (17.8 in.) Depth: 51.6 cm (20.3 in.)
Front Clearance (for maintenance door)	19.5 cm (7.68 in.)
Rear Clearance (for ventilation and cable connections)	20 cm to 30 cm (7.9 in. to 11.8 in.)
Top Clearance	23.1 cm (9.1 in.)
Weight	<i>without environmental control system</i> 38 kg (83.8 lb)
	<i>with environmental control system</i> 40 kg (88.2 lb)
Sample Formats, dishes (optional)	Standard culture dishes, 35 mm (1.38 in.)
Sample Formats, microplates	ANSI/SLAS compliant Number of Wells: 6, 12, 24, 48, 96, 384 Maximum Height, including lid: 23 mm (0.9 in.)
Sample Formats, slides	Standard microscope slides, 25 mm x 75 mm (1 in. x 3 in.)

Item	Description
Reading Modes	Fluorescence Transmitted Light (Brightfield) Colorimetric
Objectives	4x 10x 20x (optional) 40x (optional) 63x (optional)
Fluorescence Color Channels	FITC DAPI (optional) TRITC (optional) CY5 (optional) Texas Red (optional) CFP (optional)
Environmental Control	CO ₂ : 1% to 15% (optional) O ₂ : 1% to 15% (optional) Humidity: 85% (optional)
Temperature Control	<i>without environmental control cassette</i> 6°C (41°F) above ambient up to 40°C (104°F)
	<i>with environmental control cassette</i> 8°C (46°F) above ambient up to 40°C (104°F)
Ambient Operating Conditions	Temperature (general use without environmental control): 18°C to 30°C (64°F to 86°F) Temperature (when using temperature control): 18°C to 28°C (64°F to 82°F); ambient temperature must be at least 8°C (46°F) below the target temperature Relative Humidity: 20% to 75% (non-condensing)
Ambient Storage Conditions	Temperature: -20°C to +60°C (-4°F to 140°F) Relative Humidity: 15% to 75% (non-condensing)
Altitude Restrictions	Up to 2000 m (6,562 ft)
IEC Installation Category	II
Pollution Degree	2
Data Connection	Two (2) Ethernet ports
IEC Ingress Protection	IP20

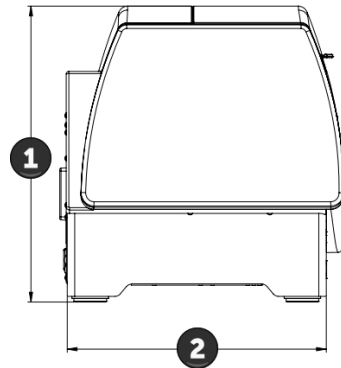
Instrument Dimensions without Environmental Control System

Front View



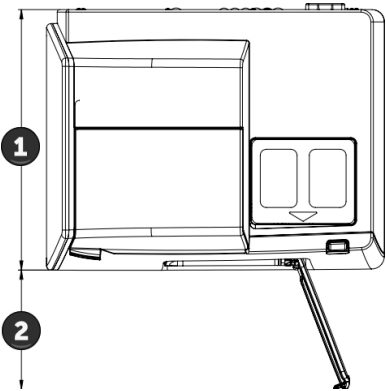
Item	Description
1	67.8 cm (26.7 in.)
2	35.3 cm (13.9 in.)
3	55.1 cm (21.7 in.)

Side View



Item	Description
1	45.3 cm (17.8 in.)
2	39.7 cm (15.6 in.)

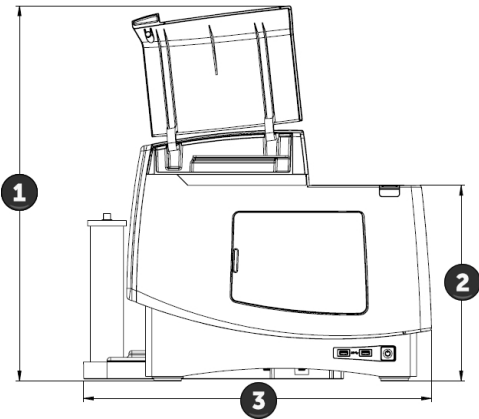
Top View



Item	Description
1	42.5 cm (16.7 in.)
2	19.5 cm (7.7 in.)

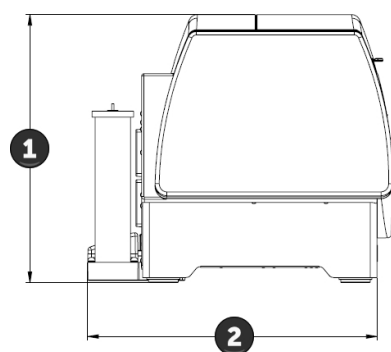
Instrument Dimensions with Environmental Control System

Front View



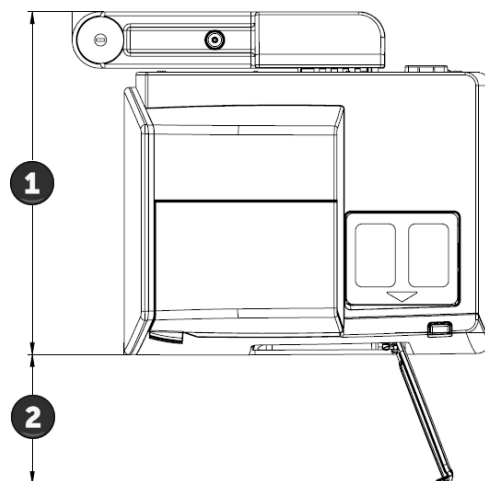
Item	Description
1	67.8 cm (26.7 in.)
2	35.3 cm (13.9 in.)
3	62.9 cm (24.8 in.)

Side View



Item	Description
1	45.3 cm (17.8 in.)
2	49.0 cm (19.3 in.)

Top View



Item	Description
1	51.6 cm (20.3 in.)
2	19.5 cm (7.7 in.)



Appendix B: Optional Parts and Accessories

This section describes the items available from Molecular Devices for the ImageXpress Pico system, including the following:

- Objectives, see below
- Filter Cubes, see below
- Accessories, see page 88

See [Obtaining Support on page 20](#) for details on contacting Molecular Devices.

Objectives

The following Leica objectives, which are available from Molecular Devices, are compatible with the ImageXpress Pico system:

Objective	Part Number	Mag.	Numerical Aperture (NA)	Working Distance	Corr. Collar
PL FLUOTAR 4x/0.13	5064345	4x	0.13	17.0 mm	No
HC PL FLUOTAR 10x/0.32	5064346	10x	0.32	11.13 mm	No
HC PL FLUOTAR 20x/0.40	5064347	20x	0.4	7.45 mm	No
HC PL FLUOTAR L 40x/0.60 CORR	5064348	40x	0.6	3.0 mm	Yes
HC PL FLUOTAR L 63x/0.70 CORR	5064349	63x	0.7	2.0 mm	Yes



CAUTION! To prevent damaging both the instrument and your sample, do not use any other objectives with the ImageXpress Pico system.

Filter Cubes

The following filter cubes, which are available from Molecular Devices, are compatible with the ImageXpress Pico system:

Filter	Part Number	Excitation	Emission	Dichroic
DAPI	5064350	370/40 nm	450/60 nm	410 nm
FITC	5064351	465/40 nm	525/30 nm	500 nm
TRITC	5064352	530/45 nm	594/40 nm	560 nm
Cy5	5064353	630/40 nm	695/45 nm	655 nm
Texas Red	5069939	560/50 nm	645/75 nm	595 nm
CFP	5069940	400/30 nm	480/40 nm	475 nm

Accessories

The following accessories, which are available from Molecular Devices, are compatible with the ImageXpress Pico system.

Base System Accessories

Item	Part No.
4-Slide Holder	5068503
Plate Holder	5068504
6-Dish Holder for 35 mm Dishes	5077007
Calibration Kit	5068505
Fluorescent Bead Plate	1-5590-0010
Two (2) CAT6 Ethernet Cables, 2 m (6.6 ft)	5052189
Power Cord for USA/Canada, 2.29 m (7.5 ft)	4400-0002
Power Cord for Europe, 1.96 m (6.4 ft)	4400-0036
Power Cord for China, 2.50 m (8.2 ft)	4400-0276
Fuses, 5 mm x 20 mm 6 A MEDIUM 250 V UL CSA ceramic tube	5082137

Workstation and Monitor Bundle Accessories

Item	Part No.
Workstation and Monitor Bundle	5058414

Environmental Control System Accessories

Item	Part No.
Environmental Control Cassette	5070105
Humidifying Column	5070110
Humidifying Column Tubing/Wiring	5070108
Two (2) Humidifying Column Stoppers	5070107
Gas Supply Tubing, 10 m (32.8 ft)	5070103
Push-to-Connect Straight Fitting 6 mm O.D. - 1/4" NPT Male	5075610
Two (2) Blind Plugs	5070106
Three (3) Plate Skirt Height Adapters	5077006



Appendix C: Environmental Control System Gas Regulator Details

If you are using the optional environmental control system, you must provide the gas supplies, along with the pressure regulators required to connect the instrument to the gas supply.

Unlike other imaging systems from Molecular Devices, the ImageXpress Pico system allows independent control of both CO₂ and O₂ levels. As a result, you may need to connect up to three separate gas supplies to the system. The connections required to connect the instrument are dependent on whether the gas is supplied from a compressed gas cylinder, a house air supply, or an air compressor. The ImageXpress Pico system can use the following gases:

- CO₂, which is typically supplied from a gas cylinder.
- N₂, which can be supplied from gas cylinders or a lab gas line.
- Compressed air, which can be supplied from a gas cylinder, a lab gas line, or an oil-free air pump or compressor.

In all cases, the gases must be oil-free and at least 99% pure.



Note:

- All applications of the environmental control system require sources of compressed air and CO₂. These make it possible to enrich the CO₂ gas environment above ambient levels.
 - An N₂ source is required for hypoxia experiments, where O₂ levels are to be depleted to sub-ambient levels. If you are planning on performing hypoxia experiments, all three gases—compressed air, CO₂ and N₂—are required.
-

Using a Gas Cylinder

Gas pressure to the instrument must be regulated within the range of 0.8 bar to 1.2 bar (11.6 psi to 17.4 psi). The ideal setting is 1.0 bar (14.5 psi). A two-stage regulator, as described below, is required to step-down and regulate the pressure from the gas cylinder.

Gas	Regulator Type	Fitting Size	Fitting Picture
CO ₂	CGA 320	13/16"	
N ₂	CGA 580	15/16"	
Compressed Air	CGA 590	15/16"	



Note:

- CGA 580 and CGA 590 have identical fittings, but CGA 580 is designed for non-oxidizing gases (N₂), while CGA 590 is for oxidizing gases (compressed air).
- The regulator type is often stamped on the end of the regulator on the side that will connect to the cylinder.
- We recommend that the delivery pressure range of the regulator be 4.14 bar (60 psig) or less, which will enable you to easily set the pressure to the required level.

Two-stage regulators are available from several vendors, including:

- Matheson (www.mathesongas.com)
- Airgas (www.airgas.com)
- VWR (vwr.com)

Connecting the Regulator to the Instrument

Molecular Devices provides the following:

- Three (3) ¼" NPT male to 6mm O.D. push-to-connect fittings
- 10 m (32.8 ft) of 4 mm I.D. / 6 mm O.D. polyurethane tubing

You may need to provide a ¼" x ¼" NPT female-to-female fitting (available from vendors such as Anderson Metals).



Note: A typical gas regulator outputs to a ¼" NPT fitting, which can be either male or female.

To connect the regulator to the instrument:

1. If your regulator outputs to a male fitting, connect a ¼" x ¼" NPT female-to-female fitting to the regulator output.
2. Connect the provided ¼" NPT male to 6mm O.D. Push-to-Connect fitting.
3. Connect an appropriate length of the provided 4 mm I.D. / 6 mm O.D. polyurethane tubing.
4. Connect the tubing to the instrument. See [Connecting and Disconnecting Tubing on page 48](#) for details.

Using an Oil-Free Lab Gas Line

Some laboratories are equipped to supply gas from a house gas line, where compressed air and/or N₂ are supplied from hose barb connections. In many cases, house gas lines output is greater than 1.2 bar (17.4 psi), which is the maximum allowed pressure for the instrument. In this case, a single-stage, line pressure regulator is required.



WARNING! All gases from the lab gas line must be oil-free. Failure to supply oil-free gases to the ImageXpress Pico system may damage the instrument and void the warranty.



Note: We recommend that the delivery pressure range of the regulator be 4.14 bar (60 psig) or less, which will enable you to easily set the pressure to the required level.

Gas regulators for a house gas line are available from several vendors, including:

- Matheson (www.mathesongas.com)
- Airgas (www.airgas.com)
- VWR (vwr.com)

Connecting the Lab Gas Line to the Instrument



Note: In some cases, the hose barb connection for the lab gas line may be removable. If so, you may want to remove it to connect the line pressure directly to the output.

Molecular Devices provides the following:

- Three (3) ¼" NPT male to 6mm O.D. push-to-connect fittings
- 10 m (32.8 ft) of 4 mm I.D. / 6 mm O.D. polyurethane tubing

You must provide the following:

- Appropriate length of 3/8" I.D. or equivalent tubing
- 3/8" hose barb to ¼" Female NPT fitting (available from vendors such as EDGE INDUSTRIAL)
- Hose clamp

To connect the lab gas line to the instrument:

1. Connect the 3/8" I.D. tubing to the hose barb.
2. Connect the 3/8" hose barb to ¼" Female NPT fitting.
3. Connect the provided ¼" NPT male to 6mm O.D. Push-to-Connect fitting.
4. Use a hose clamp to secure the hose barb connections.
5. Connect the tubing to the instrument. See [Connecting and Disconnecting Tubing on page 48](#) for details.

Using an Oil-Free Air Compressor

When neither house air or compressed air from a gas cylinder is available, you can use an oil-free air compressor. An air compressor should require the same connections as those described above for a house air supply. Laboratory air compressors typically have either hose barb connections or 1/4" NPT connections. Since air compressors typically have an internal regulator, a line regulator is unlikely to be needed.



WARNING! The air compressor must be oil-free. This feature is typically noted on the specification sheet from the supplier. Failure to supply oil-free air to the ImageXpress Pico system may damage the instrument and void the warranty.

An oil-free compressor can be purchased from many vendors, including Cole-Parmer (www.coleparmer.com).

Unsupported Gas Supplies

The following gas supplies are not supported for use with the ImageXpress Pico:

- Pre-mixed gas supplies
- N₂ boil-off from a liquid N₂ dewar
- N₂ from a N₂ generator
- Any gas supply that cannot be set to supply gas at between 0.8 bar and 1.2 bar (11.6 psi and 17.4 psi)



Appendix D: Packing the Instrument for Transport

The packaging is designed to protect the instrument during transportation. Before transporting the instrument, carefully pack it in its original shipping box with all packing materials. If needed, contact Molecular Devices for a replacement shipping box.



WARNING! LIFTING HAZARD. To prevent injury, use a minimum of two people to lift the instrument.



CAUTION! Take care to pack the instrument properly for transport. Improper packing may void the warranty.

To pack the instrument:



Note: Before you begin, if your instrument includes the optional environmental control system, run air dry to clear any condensation from the environmental control system. See [Running Air Dry on page 76](#) for details.

1. If needed, do the following to disassemble the environmental control system:
 - a. Confirm that the valves on the tanks are completely shut off and that no gas is flowing.
 - b. On the environmental control tray, loosen the large screw to remove the cover from the tray.
 - c. Disconnect all tubing/wiring from the instrument and the humidifying column.

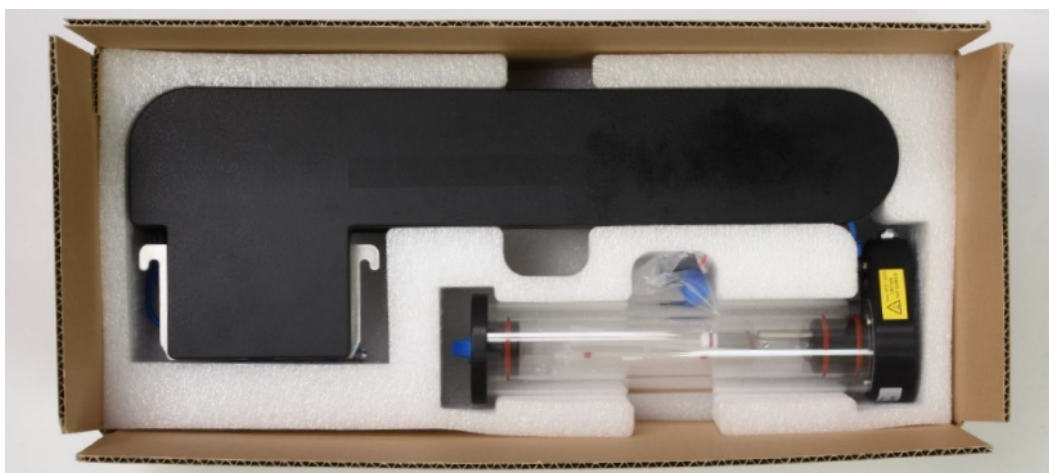


Note: See [Connecting and Disconnecting Tubing on page 48](#) for details on connecting tubing.

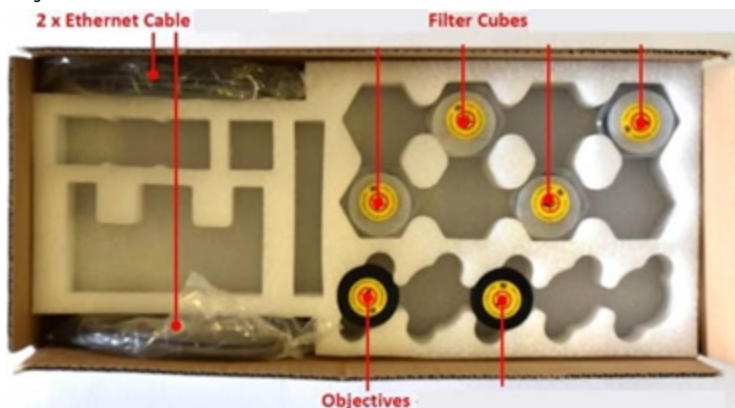
- d. Remove the humidifying column from the environmental control tray.
- e. Empty the humidifying column.
- f. Pull the environmental control tray away from the instrument to remove it.
- g. Pack the tubing/wiring inside the environmental control tray and replace the cover and large screw.



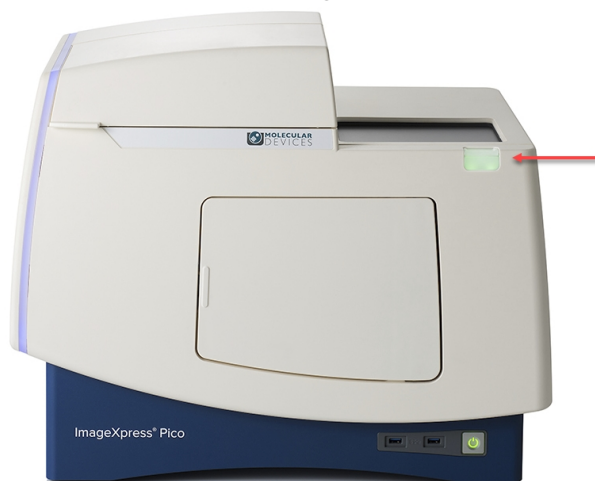
- h. Pack the environmental control system components in their original packaging, as shown.



2. Remove any installed filter cubes and pack them in their original packaging. See [Installing a Filter Cube on page 71](#) for details on removing a filter cube.
3. Remove any installed objectives and pack them in their original packaging, including the objective case. See [Installing an Objective on page 62](#) for details on removing an objective.



4. Press the button at the top right of the instrument to open the top door.

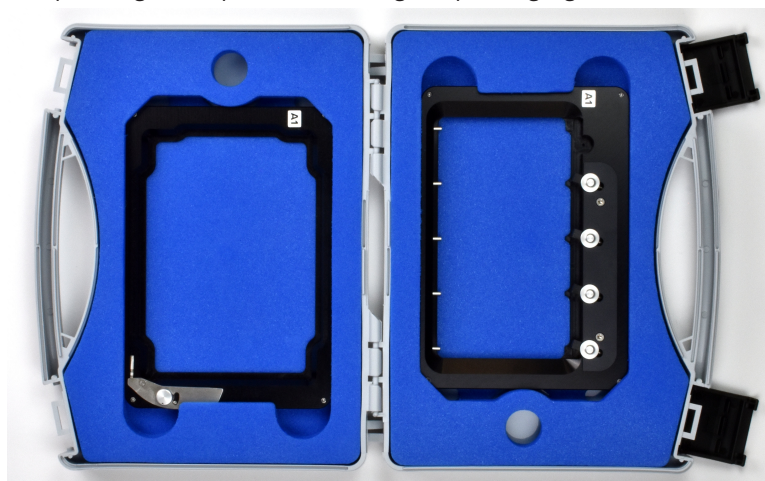


5. Press the Power button on the front of the instrument.
When the status light on the Power button is amber, the instrument is fully powered off.



Note: If the ImageXpress Pico system doesn't respond, you can perform a hard shutdown by holding the Power button for five seconds.

6. Disconnect the power cord and the Ethernet cable(s).
7. With the instrument off and all cables disconnected, manually move the sample stage to the center of the opening.
8. If needed, remove the labware holder or the environmental control cassette from the sample stage and pack it in its original packaging.



9. Do the following to secure the sample stage:
 - a. Insert the foam transport lock in the opening until it is flush with the top surface of the instrument.



CAUTION! Do not transport the instrument without properly inserting the foam transport lock. If needed, contact Molecular Devices for a replacement foam transport lock.

- b. Use shipping tape to secure the foam transport lock to ensure it does not come loose.



10. Replace the instrument in the plastic bag from the original packaging.



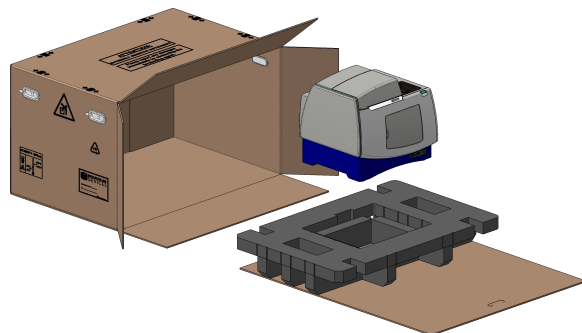
11. With one person on each end, place the instrument in the bottom foam packing as shown below.



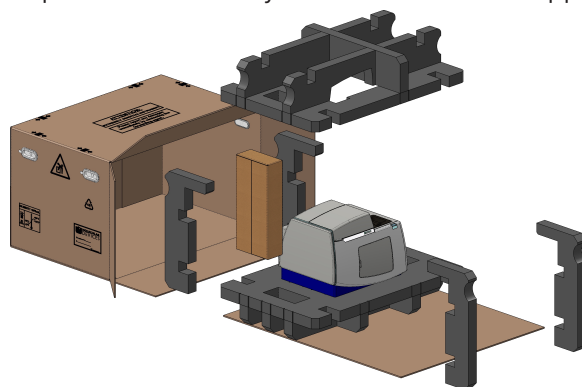
WARNING! LIFTING HAZARD. To prevent injury, use a minimum of two people to lift the instrument.



CAUTION! Keep the instrument upright and level when lifting. Do not tip or shake the instrument.

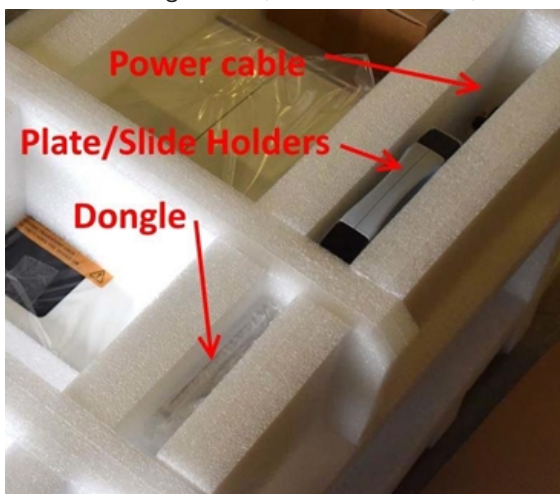


12. Replace the accessory boxes and the foam supports.

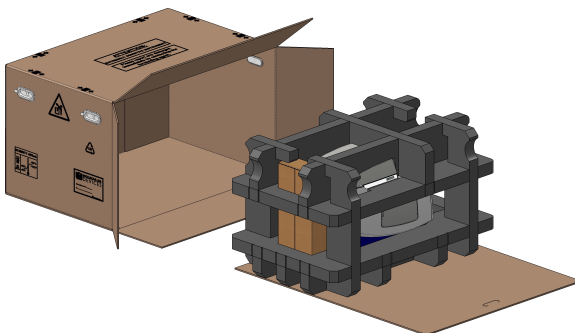


13. Replace the dongle in its case and wrap it in bubble wrap.

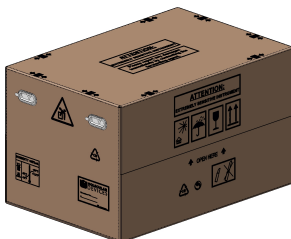
14. Insert the dongle case, the holders case, and the cables between the foam.



15. Slide the instrument on its cardboard base into the box.



16. Seal the box.



17. Place the eight (8) edge protectors on the designated positions on the top edges of the box.



18. With one person on each end, place the box on the pallet.
19. Secure four (4) binding straps around the box and pallet.



CAUTION! Keep the box upright during transport. Do not tip or tilt the box or place it on its side.



Appendix E: Electromagnetic Compatibility

Regulatory for Canada (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est conforme à la norme NMB-001 du Canada.

ISM Equipment Classification (Group 1, Class A)

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

Information to the User (FCC Notice)

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at their own expense. Changes or modifications made to this equipment not expressly approved by the party responsible for compliance may void the FCC authorization to operate this equipment.

Contact Us

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Email: info@moldev.com

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