ImageXpress® Pico

Automated Cell Imaging System

User Guide

MOLECULAR DEVICES

5063667 B
June 2018

Product manufactured by Molecular Devices, LLC.
3860 N. First Street, San Jose, California, 95134, United States of America.
Molecular Devices, LLC is ISO 9001 registered.

Contents

Safety Information

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

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

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

Warnings, Cautions, Notes, and Tips

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

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

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

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

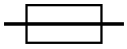
CAUTION! A caution indicates a situation or operation that could cause damage to the instrument or loss of data if correct procedures are not followed.

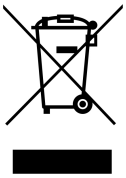
Note: A note calls attention to significant information.

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. For information about the weight of the instrument,
	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.
	This symbol on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. It indicates that you must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.
	Indicates the environmental friendly use period.
	Indicates that there is an authorized representative in the European community.
	Indicates the instrument manufacturer.
	Indicates the manufacturer catalog number.

Before Operating the Instrument

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

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

Protective Housing

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 plate 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 20 cm to 30 cm (7.9 in. to 11.8 in.) gap between the rear of the instrument and the wall.

Power off the instrument when not in use.

If the instrument does not power on, you may need to replace the instrument fuses. See Replacing Fuses on page 8 for details.

Replacing Fuses

If the instrument does not seem to get power after 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 failed 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. You can obtain replacement fuses from Molecular Devices. See Replacement Parts and Optional Extras on page 67 for details on fuse specifications and part numbers.

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 part of the power outlet on 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. Press and hold the **Power** button to power off the instrument.
2. Disconnect the power cord from the power port.
3. Use a small flat-head screwdriver to gently press on the carrier-release tab and then 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.

Note: If the instrument still does not power on after you change the 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! Moving the instrument can damage sensitize 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. Therefore, take applicable safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials.
- Observe the applicable cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the applicable cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

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

Cleaning and Maintenance

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

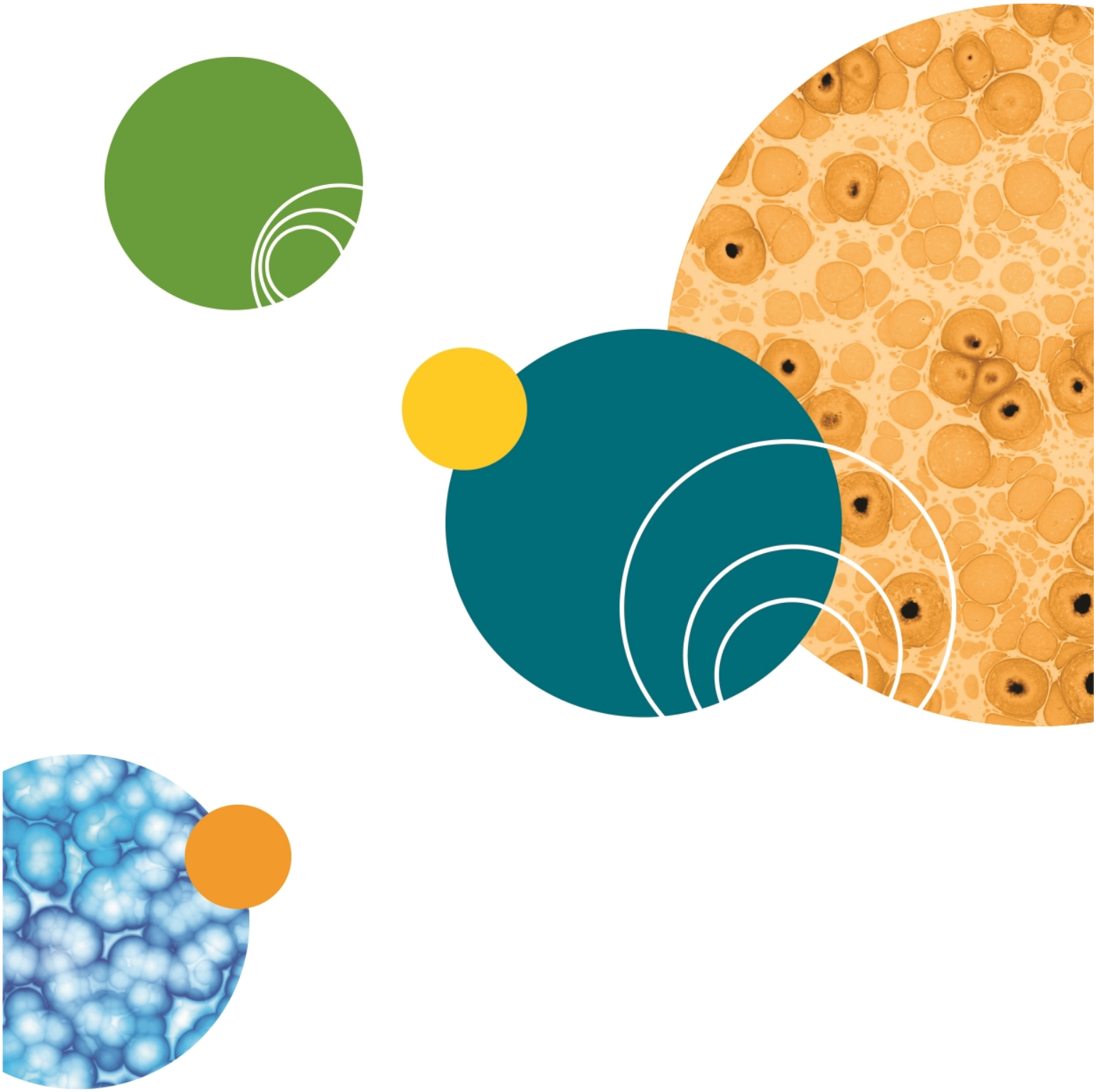
Do the following before you clean equipment that has been exposed to hazardous material:

- Contact the applicable Chemical and Biological Safety personnel.
- Review the Chemical and Biological Safety information contained in this guide. See Chemical and Biological Safety on page 10 for details.

Perform only the maintenance tasks described in this guide. Any other maintenance tasks must be done by qualified Molecular Devices personnel only. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year.

WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before you return parts to Molecular Devices for repair. Molecular Devices does not accept items that have not been decontaminated where it is applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

For approved cleaning and maintenance procedures, see Maintenance on page 41.



Chapter 1: ImageXpress Pico Automated Cell Imaging System

1

The ImageXpress® Pico Automated Cell Imaging System by Molecular Devices® is an affordable 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.

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

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

Key components of the instrument include the following:

- Built-in, internal light source comprised of four high-powered LEDs enables very high sensitivity fluorescent imaging. Additional LED-based light sources allow transmitted light and planetary view 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.
- Planetary view 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 for live cell imaging at 6°C (11°F) above ambient up to 40°C (104° F).
- 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 four 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 Cube Specifications on page 65 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	Color Band
4x	0.13	Red
10x	0.32	Yellow
20x	0.40	Light Green
40x	0.60	Light Blue
63x	0.70	Dark Blue

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

See Compatible Objectives on page 63 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 scanning multi-well microplates in standard ANSI (SBS) formats with plastic or glass bottoms. It can accommodate other plate formats that have standard microplate footprint dimensions. For example, glass slides can be imaged using a slide adapter included in the accessory kit. Optimal image quality depends on plate flatness, well bottom thickness, and optical clarity.

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

Slide Holder

The slide holder holds four standard microscope slides of 25 mm x 75 mm (1 in. x 3 in.).

LED Autofocus

The ImageXpress Pico System uses a high-powered LED to autofocus the sample. For higher magnifications, you can select an additional image-based autofocus.

CellReporterXpress Software Features

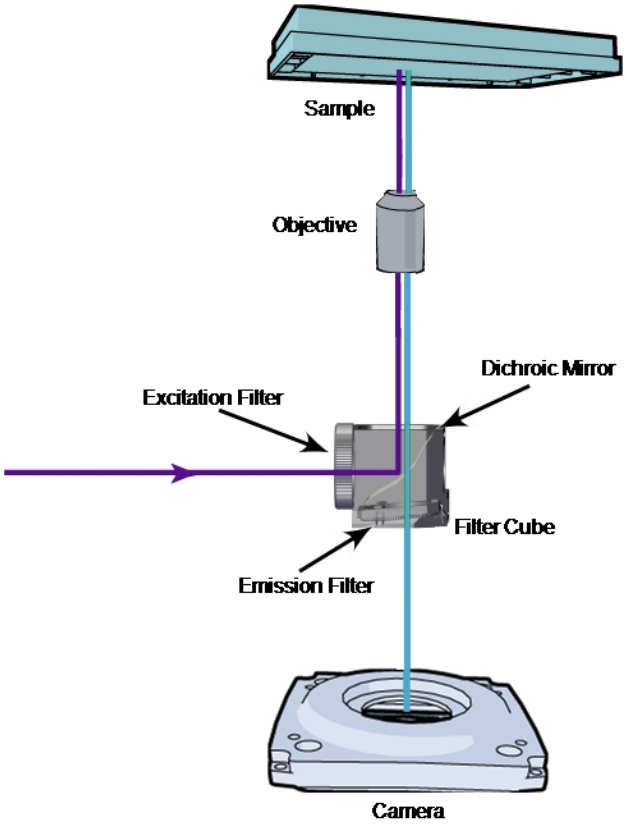
The CellReporterXpress Image Acquisition and Analysis Software by Molecular Devices is the user interface for the ImageXpress Pico System. You will use the CellReporterXpress Software to work with the ImageXpress Pico System and control all its functions.

The CellReporterXpress Software integrates image acquisition and analysis into a unified workflow. Along with the imaging device, the CellReporterXpress Software is part of a system that 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 page 18
- Excitation and Emission Filters, see page 18
- Dichroic Mirror, see page 19
- Objective Lenses, see page 20

Fluorescence Imaging

Fluorescence is a property of certain classes of molecules (fluorochromes, fluorescent proteins, or dyes) in which photons of a specific wavelength are absorbed (excitation), and, as a result, 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 active probe molecules, so that application of the combined dye/probe molecule (fluorophore) to the specimen highlights the specific substances or regions to which the probe is targeted.

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

Excitation and Emission Filters

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

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

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

Dichroic Mirror

In the ImageXpress Pico System, the dichroic mirror is in a filter cube.

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

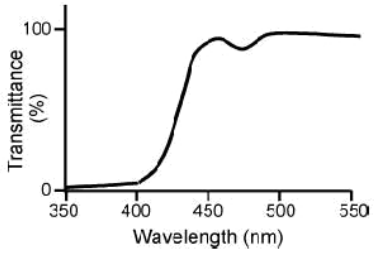
In the illumination path, the dichroic mirror reflects shorter wavelengths from the light source up through the objective onto the specimen.

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

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

Dichroic Transmission Spectrum

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



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

Objective Lenses

The ImageXpress 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
4x	Red
10x	Yellow
20x	Green
40x	Light Blue
63x	Dark Blue

The 40x objective and 63x objective have application-optimized correction collars (CORR) to compensate for external influences such as 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.

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 Compatible Objectives on page 63 for details.

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/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, submit a request to Molecular Devices Technical Support.

Documentation

Review the product documentation on the Knowledge Base, including installation guides and user guides. In addition, online Help is available within the CellReporterXpress Software. Press **F1** to access Help for the active screen.

Technical Support

You can contact Molecular Devices Technical Support by phone or submit a support request through the Knowledge Base. To find regional support contact information, visit www.moleculardevices.com/contact.

You will need the instrument serial number and the software system ID.

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

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

Product Documentation

The following guides are available in the Knowledge Base on the Molecular Devices Support website at www.moleculardevices.com/support:

- *CellReporterXpress Installation Guide*
- *CellReporterXpress IT Configuration Guide*
- *CellReporterXpress User Guide*
- *ImageXpress Pico Installation Guide*
- *ImageXpress Pico User 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: Molecular Devices recommends 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. Molecular Devices recommends that you review the guide on the Knowledge Base for the most up-to-date information.

Chapter 2: Using the ImageXpress Pico System

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

Note: The procedures in this section assume that the ImageXpress Pico System including the CellReporterXpress Software have been installed and properly configured. For details, see the ImageXpress Pico Installation Guide, the CellReporterXpress Installation Guide, and the CellReporterXpress IT Configuration Guide.

Starting the System

Use the following procedures to safely power on the instrument and start the CellReporterXpress Software.

Powering On the Instrument

To power on the instrument:

1. Ensure the Ethernet cable is connected properly:
   - For a standalone configuration, where the instrument is connected directly to the host computer, connect the Ethernet cable to the **LAN1** port on the back of the instrument.
   - For a network configuration or server configuration, where the instrument is connected to a network, connect the Ethernet to the **LAN2** port on the back of the instrument.

   CAUTION! The network connections between the host computer, imaging device, remote clients, and external computers require that all firewalls and routers be configured to allow data transfer between all applicable ports. Without communication across these ports, the instrument cannot connect to the software. See the *CellReporterXpress IT Configuration Guide* for details.

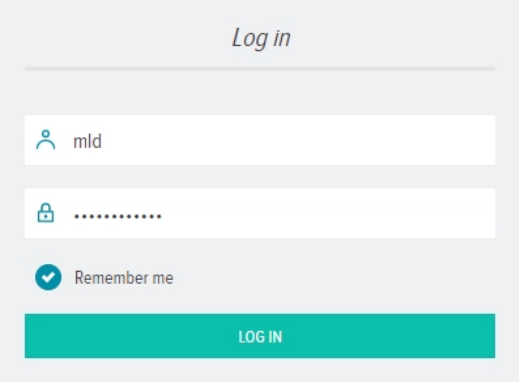
2. Press the **Power** button on the front of the instrument.

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

Logging In to the Software

To log in to the CellReporterXpress Software:

1. 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**.

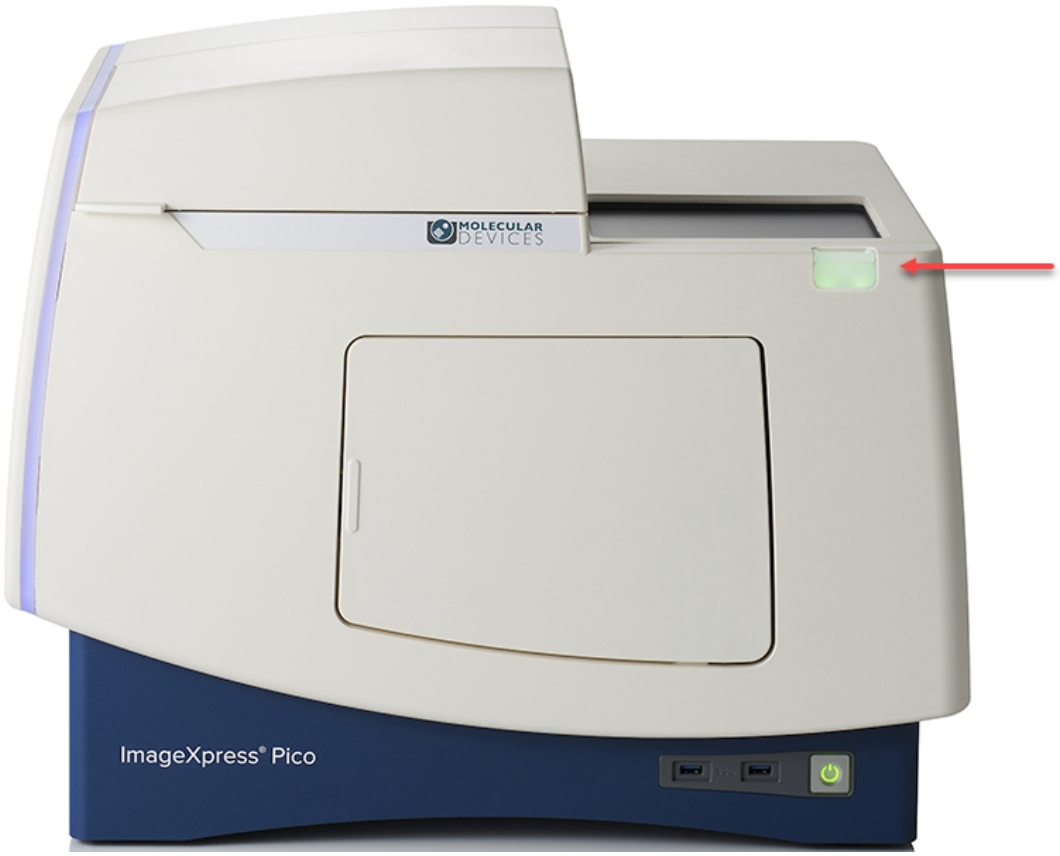


2. In the **Login** field, enter the Windows system user name.
3. In the **Password** field, enter the Windows system password.
4. Click **LOG IN**.

Note: The CellReporterXpress Software uses the Windows login credentials of the host computer to authenticate users. If the host computer does not maintain a constant connection to the network, Molecular Devices recommends that user accounts be Local accounts (and not Roaming or Domain accounts). If Domain accounts are required, the Host computer should remain connected to the domain network at all times.

Understanding the Status Light

The status light on the ImageXpress Pico System illuminates to provide information about the instrument status.



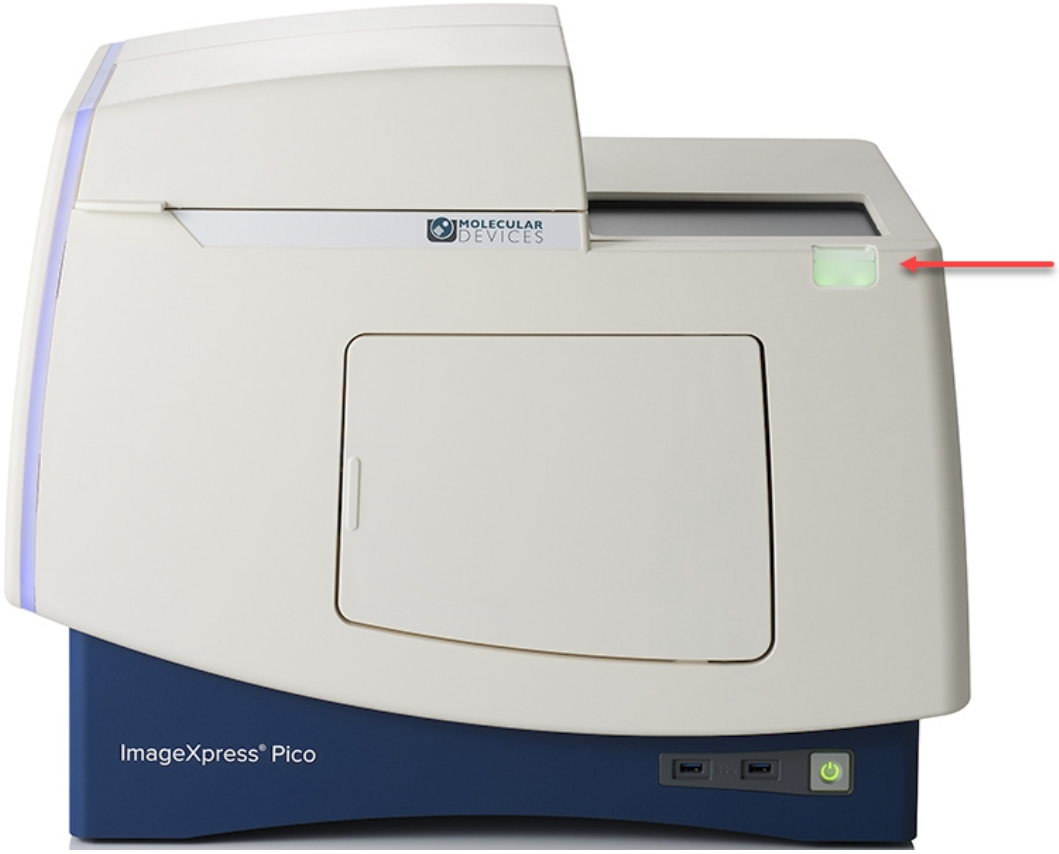
Color	Instrument Status
Green	The instrument is powered on, connected to the software, and ready to use.
Blue	The instrument is performing a firmware update.
Yellow	The instrument is acquiring data.
Red	The instrument is in an error state or cannot communicate with the software. Restart the instrument.

Operating the Sample Door

Open the sample door to insert the plate holder or slide holder into the sample (X-Y) stage. You can operate the sample door from the instrument or from the CellReporterXpress Software.

Opening the Sample Door from the Instrument

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



Opening the Sample Door from the Software

To open the sample 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 Sample Door from the Instrument

To close the sample door from the instrument:

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

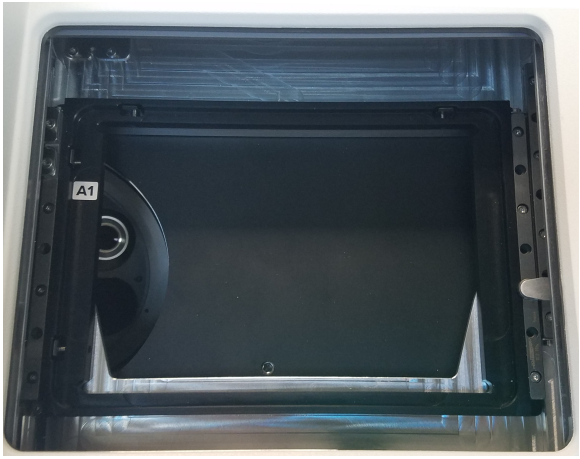
Closing the Sample Door from the Software

To close the sample 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 the Plate Holder or Slide Holder

Open the sample door on the top of the instrument to insert the plate holder or slide holder in the sample (X-Y) stage. The sample (X-Y) stage has an **A1** label to indicate its top left corner.



Inserting a Plate Holder

Like the sample (X-Y) 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 sample door.
2. If the sample (X-Y) stage already contains a plate holder or slide holder, remove it.
3. With the **A1** label at the top left and the spring-loaded clamp in the open position (as shown), insert the plate holder in the sample (X-Y) stage.

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

4. Confirm that the plate holder is seated flat in the sample (X-Y) stage.
5. Press the button at the top right of the instrument to close the sample door.

Inserting a Slide Holder

Like the sample (X-Y) 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 sample door.
2. If the sample (X-Y) stage already contains a plate holder or slide holder, remove it.
3. With the **A1** label at the top left (as shown), insert the slide holder in the sample (X-Y) stage.
4. Confirm that the slide holder is seated flat in the sample (X-Y) stage.
5. Press the button at the top right of the instrument to close the sample door.

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

Evaluating your Experiment Requirements

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

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

Selection of Different Fluorochromes

Typical high-content assays include one or more fluorochromes, such as fluorescent proteins, antibody-based stains, and chemical-based stains. In general, Molecular Devices recommends including 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 your instrument. For experiments using multiple stains, select fluorophores that have sufficient spectral separation. Some fluorochromes provide brighter intensities and require a shorter exposure time, while others do not bleach as quickly and allow a longer exposure time. There also might be toxicity issues with some cell types or bleed-through issues between pairs of fluorochromes. Consider these factors when choosing a fluorochrome.

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

Cell-Based Assays

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

Plates or Slides

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

Plate Selection

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

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

- Plate Format, see page 33
- Plate Material, see page 34
- Fluorescence Background, see page 34
- Bottom Thickness, see page 34
- Batch-to-Batch Consistency, see page 34

Plate Format

Determine if the plate format is compatible with your assay.

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

Plate Material

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

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

Fluorescence Background

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

Bottom Thickness

The thickness of the plate bottom should be compared with the working distance of the objective lens to be used to ensure that it is compatible. In general, objectives with higher numerical aperture (NA) tend to require thin-bottomed plates. 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, possibly causing focusing issues. For best results, Molecular Devices recommends using 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, the plate settings must be optimized for each batch.

Sample Preparation

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

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

- Cell Density, see page 35
- Fixation and Staining Conditions, see page 35
- Final Buffer or Media, see page 35
- Plate Handling and Storage, see page 36

Cell Density

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

Fixation and Staining Conditions

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

Final Buffer or Media

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

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

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

Plate Handling and Storage

Since the LED autofocus measures the reflection from the bottom of the plate or from within the sample, dust particles, dirt, fingerprints, and scratches interfere with the reflection and affect the autofocus performance. To improve the autofocus, clean the bottom of the plate with a lens tissue and an optical cleaning solution.

Plates should be stored 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 timepoints, the internal temporary storage be insufficient, and a validation error will occur. In these cases, add external temporary storage to the instrument.

You can use any USB 3.0 or higher data storage device for external temporary storage, including a flash drive or a hard disk drive.

To add external temporary storage for a protocol:

1. Connect a 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 Maintenance on page 41 for details.

Perform only the maintenance tasks described in this guide. Any other maintenance tasks must be done by qualified Molecular Devices personnel only. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year. See Obtaining Support on page 20 for details.

Shutting Down the System

To shut down the ImageXpress Pico System:

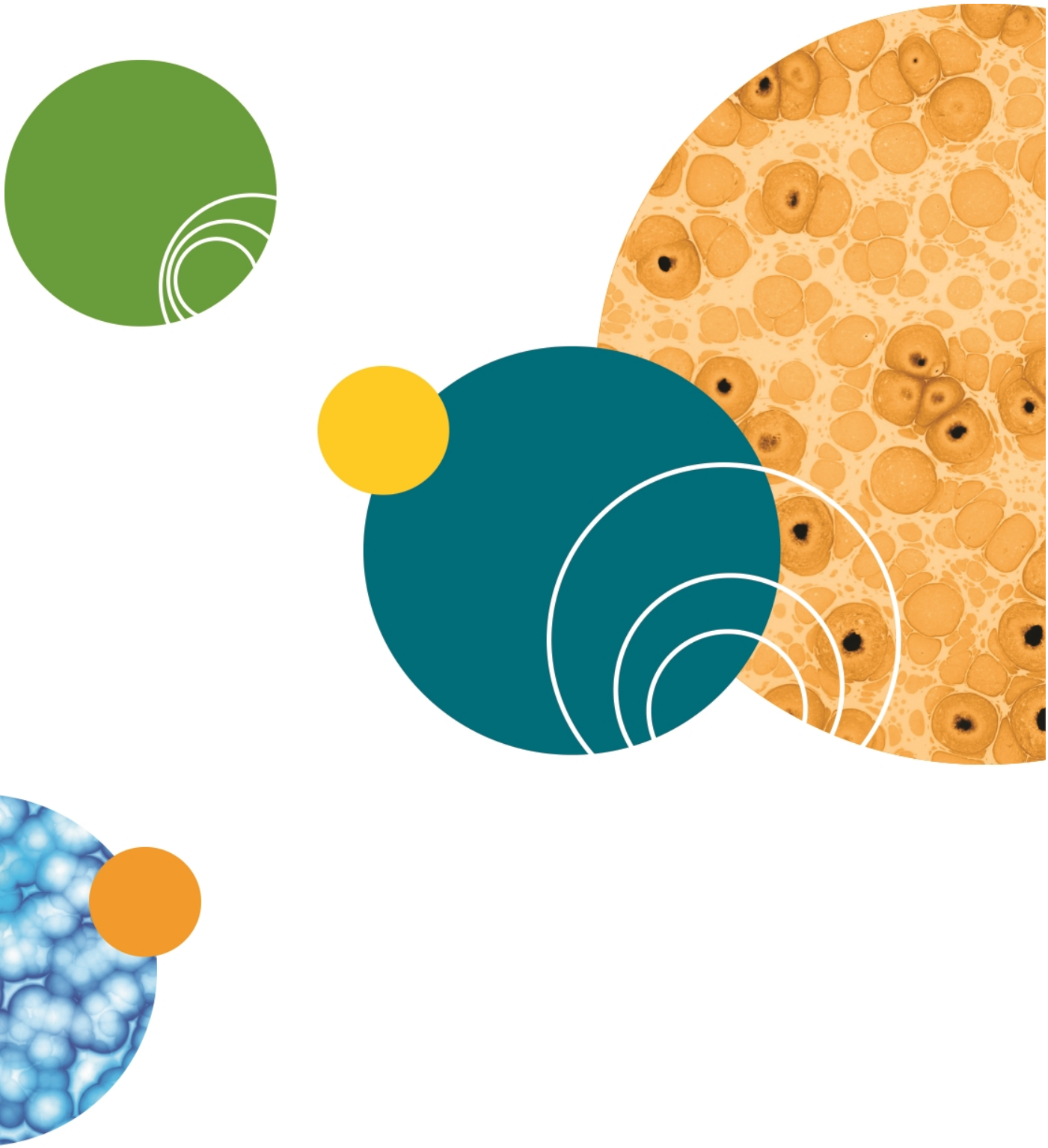
1. In the CellReporterXpress Software, on the Home Page, click  **Devices**.
2. In the **Available Acquisition Devices** list, select the instrument.
3. Click  **Shutdown Device**.
4. If needed, do the following to end the CellReporterXpress session on the host computer:
   a. Click  **Log Out**.
  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

Note: If the ImageXpress Pico System hangs or doesn't respond, you can perform a hard shutdown by holding the Power button on the front of the instrument for five seconds.

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



Chapter 3: Maintenance

3

Perform only the maintenance tasks described in this guide. Any other maintenance tasks must be done by qualified Molecular Devices personnel only. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year. See Obtaining Support on page 20.

Before operating the instrument or performing maintenance operations, make sure you are familiar with the safety information in this guide. See Safety Information on page 5.

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 enclosure as described in the procedure. 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 place access door and the maintenance door closed unless you are performing maintenance tasks.
- Ensure that all components and access doors are closed before starting the instrument.

The following topics describe the maintenance procedures you can perform:

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.

Preventive Maintenance

To ensure optimal operation of the instrument, perform the following preventive 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 error messages displayed by the software.
- Power off the instrument when not in use.

Instrument Maintenance

Observe the following general tips when 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.

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

- 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, always wipe the surface dry with a lint-free cloth.

Cleaning the Instrument

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 Maintenance on page 41.

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 **Plate Door** button at the top right of the instrument to open the plate door.
3. Confirm that no sample is loaded.
4. Press the **Power** button on the front of the instrument to power off the instrument.
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 plate holder and stage area.
    d. Use forceps wrapped with 70% ethanol disinfectant wipes to wipe the perimeter of the plate holder and stage area again.
    e. Use a fresh damp wipe to clean the stage area underneath and around the plate loading area.

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

    f. Use a fresh 70% ethanol disinfectant wipe to clean the stage area 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 **Plate Door** button at the top right of the instrument to close the plate door.

Objective Maintenance

Objective maintenance steps include the following:

- Installing an Objective, see page 45
- Calibrating an Objective, see page 47
- Adjusting a Correction Collar, see page 48
- Cleaning an Objective, see page 51

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

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

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

CAUTION! You must replace objectives in their original positions.

Tip: Molecular Devices recommends removing and maintaining only one objective at a time.

Installing an Objective

Before installing an objective, review the General Maintenance Precautions under Maintenance on page 41.

In addition, observe the following when handling an objective:

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

CAUTION! 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	Color Band
1	4x	Red
2	10x	Yellow
3	20x	Green
4	empty	n/a
5	40x or 63x	Light Blue or Dark Blue
6	empty	n/a

Note: Depending on how your ImageXpress Pico System is configured, you may not have all the objectives.

Note: The 40x objective and the 63x objective cannot be installed in the instrument simultaneously.

To install an objective:

1. In the CellReporterXpress Software, on the Home Page, click  **Devices**.
2. In the **Available Acquisition Devices** list, click  **Show Device Options** to expand the details for the device where you want to install an objective.
3. Click the **Objectives** tab.
4. In the tile for the objective you want to install, click **Component Exchange**.
5. Click the **Choose Objective** dropdown and select the objective you want to install.
6. Click **Open Maintenance Door**.
7. If an objective is already installed in the slot, remove it 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 objective in the slot by gently turning it clockwise.

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

9. Close the maintenance door.
10. In the CellReporterXpress Software, click **Close Maintenance Door**.
11. Click **Close**.

After you install a new objective, you may need to calibrate it. See Calibrating an Objective on page 47 for details.

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

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 ships with any after-sales objective purchase, includes the following items:

- Slide holder
- Stage micrometer slide
- Pink plastic slide
- Red plastic slide
- Bead slide
- Blank glass slide

To calibrate an objective:

1. In the CellReporterXpress Software, on the Home Page, click  **Devices**.
2. In the **Available Acquisition Devices** list, click  **Show Device Options** to expand the details for the device where you want to calibrate an objective.
3. Click the **Objectives** tab.
4. In the tile for the objective you want to calibrate, click **Objective Calibration**.
5. Follow the on-screen instructions to complete the calibration.

Adjusting a Correction Collar

The 40x objective and 63x objective have application-optimized correction collars (CORR) to compensate for external influences such as 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.

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: If the thickness of the intended plate, slide, or coverslip is out of the range of the correction collar, it should not be used with the selected objective.

Before adjusting the correction collar on an objective, review the General Maintenance Precautions under Maintenance on page 41.

In addition, observe the following when handling an objective:

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

CAUTION! 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.

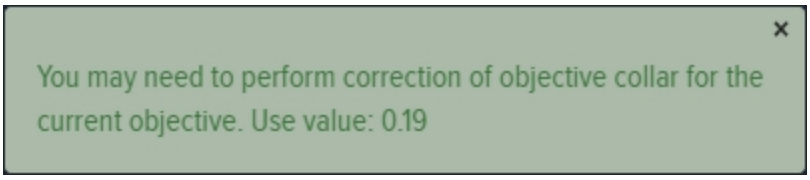
Adjusting the Correction Collar for a Plate

To adjust a correction collar for 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 **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 

Plate Format.
7. In the **Plate Format** list, select the plate.

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 CellReporterXpress Software displays the recommended setting for the correction collar based on the thickness of the plate bottom, slide, or coverslip.



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 objective door opens.
13. If needed, loosen or remove 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 or removed the objective, insert it back in its original slot in the turret or tighten it by gently turning it clockwise.

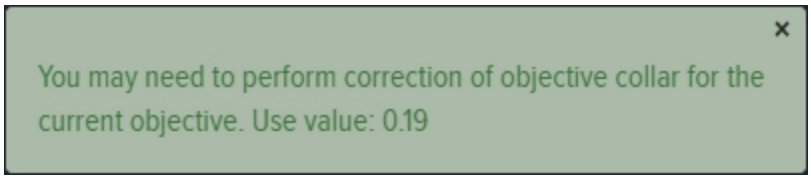
> **Note:** When installing the objective, take care to avoid changing the correction collar setting.

16. Close the objective door.
17. Click **OK**.
18. Test the correction collar setting by examining the image quality of some test snaps.
19. If the image quality is not satisfactory, repeat these steps to re-adjust the correction collar.

Adjusting the Correction Collar for a Slide

To adjust a 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 CellReporterXpress Software displays the recommended setting for the correction collar based on the thickness of the plate bottom, slide, or coverslip.

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 objective door opens.
13. If needed, loosen or remove 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 or removed the objective, insert it back in its original slot in the turret or tighten it by gently turning it clockwise.

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

16. Close the objective door.
17. Click **OK**.
18. Test the correction collar setting by examining the image quality of some test snaps.
19. If the image quality is not satisfactory, repeat these steps to re-adjust the correction collar.

Cleaning an Objective

You can clean an objective lens to remove debris or contaminants they may collect.

Before cleaning an objective, review the General Maintenance Precautions under Maintenance on page 41.

In addition, observe the following when handling an objective:

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

CAUTION! With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

CAUTION! Do not use a product that disperses aerosol propellants or fluid onto the lens surface, such as canned compressed air.

CAUTION! Do not use a wipe that can leave lint on an objective or filter cube, 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  **Exchange objective <Slot x>**.
5. Click the **Choose Objectives** drop down and select the objective you want to clean.

 **CAUTION!** Be sure to select the same objective that is currently installed.

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.

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

11. If needed, wait a few minutes for the alcohol to evaporate.
12. Insert 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. Close the maintenance door.
14. In the CellReporterXpress Software, click **Close Maintenance Door**.
15. Click **Close**.

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

Filter Cube Maintenance

Filter cube maintenance steps include the following:

- Installing a Filter Cube, see page 53
- Calibrating a Filter Cube, see page 55

Installing a Filter Cube

Before installing a filter cube, review the General Maintenance Precautions under Maintenance on page 41.

In addition, observe the following when handling a filter cube:

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

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

Slot	Filter Cube
1	DAPI
2	FITC
3	TRITC
4	Cy5
5	empty
6	empty

To install a filter cube:

1. In the CellReporterXpress Software, on the Home Page, click  **Devices**.
2. In the **Available Acquisition Devices** list, 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 you want to install, click **Component Exchange**.
5. Click the **Choose Filter** dropdown 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. If a filter cube is already installed in the slot, remove it 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.

   Tip: The filter cube should "snap" into place.

10. Close the maintenance door.
11. In the CellReporterXpress Software, click **Close Maintenance Door**.
12. Click **Close**.

After you install a filter, you may need to calibrate it. See Calibrating a Filter Cube on page 55 for details.

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.

Calibrating a Filter Cube

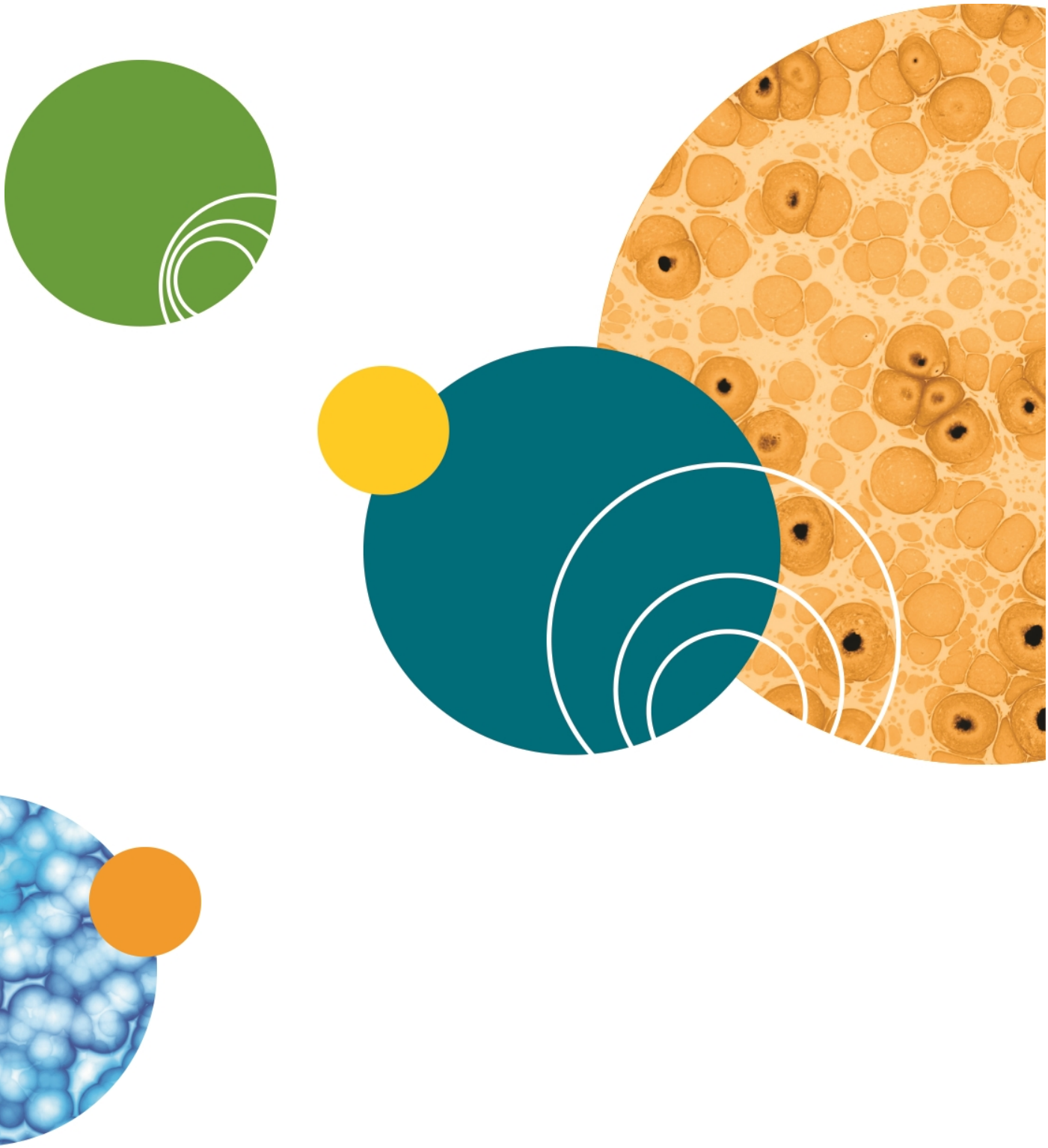
After you install a new filter cube, you may need to calibrate it. **Molecular Devices precalibrates the filter cubes included with the initial purchase of the instrument. You must calibrate any filter cubes purchased after that time.**

A calibration kit, which ships with any after-sales filter cube purchase, includes the following items:

- Slide holder
- Pink plastic slide
- Red plastic slide
- Blank glass slide
- Bead slide

To calibrate a filter cube:

1. In the CellReporterXpress Software, on the Home Page, click  **Devices**.
2. In the **Available Acquisition Devices** list, 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. In the tile for the filter cube you want to calibrate, click **Filter Cube Calibration**.
5. Follow the on-screen instructions to complete the calibration.



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.

WARNING! 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.

WARNING! Do not position the instrument so that it is difficult to operate the power switch on the front.

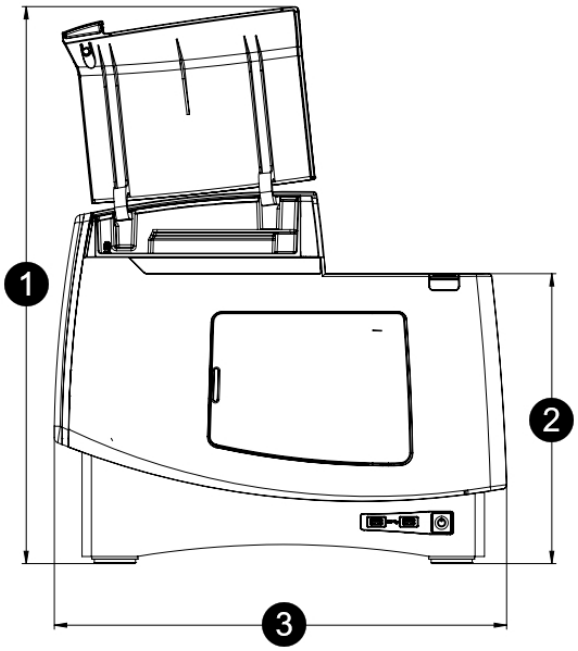
Item	Description
Operating Environment	Indoor use only
System Power	100 VAC to 240 VAC, 50/60 Hz, 1.6 A nominal at 115 V, 200 W maximum
Fuse	Glass Tube 5x20 mm T6A250V
Dimensions	Width: 55.1 cm (21.7 in.) Height: 45.3 cm (17.83 in.) Depth: 42.5 cm (16.73 in.)
Front Clearance (for maintenance door)	19.5 cm (7.68 in.)
Rear Clearance (space between the rear of the instrument and the wall 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	38 kg (83.8 lb.)
Sample Formats (microplates)	ANSI/SLAS compliant Number of Wells: 6, 12, 24, 48, 96, 384 Maximum Height, including lid: 22 mm (0.87 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)
Temperature Control	6°C (11°F) above ambient up to 40°C (104°F)
Ambient Operating Conditions	Temperature: 18°C to 30°C (59°F to 86°F) Temperature (recommended when using temperature control): 20°C to 24°C (68°F to 75°F) 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% relative humidity (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

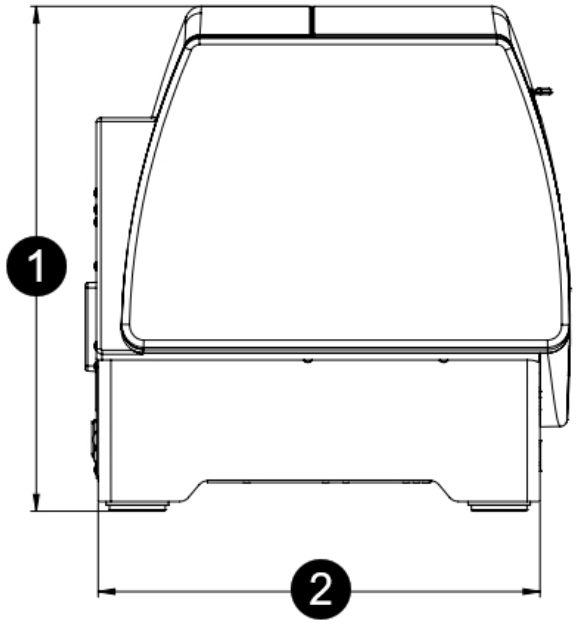
Front View

Item	Description
1	67.8 cm (26.69 in.)
2	35.3 cm (13.90 in.)
3	55.1 cm (21.68 in.)



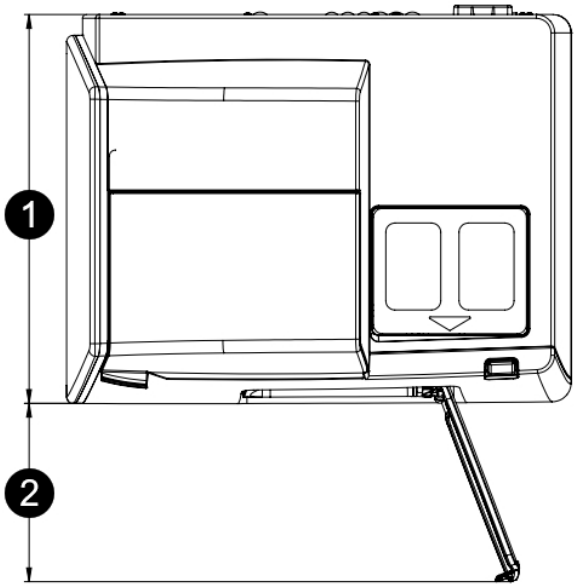
Side View

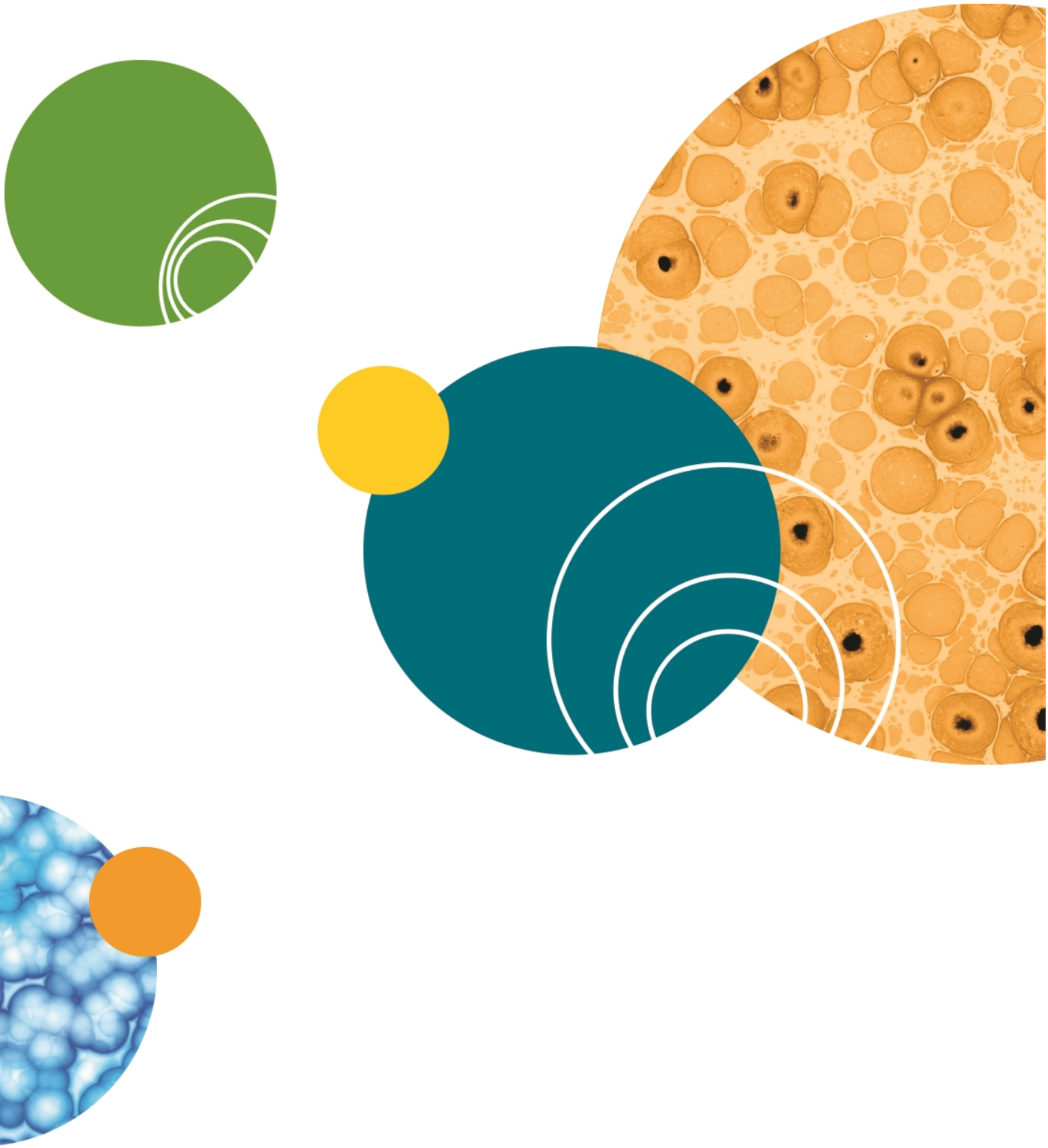
Item	Description
1	45.3 cm (17.83 in.)
2	39.7 cm (15.63 in.)



Top View

Item	Description
1	42.5 cm (16.73 in.)
2	19.5 cm (7.68 in.)





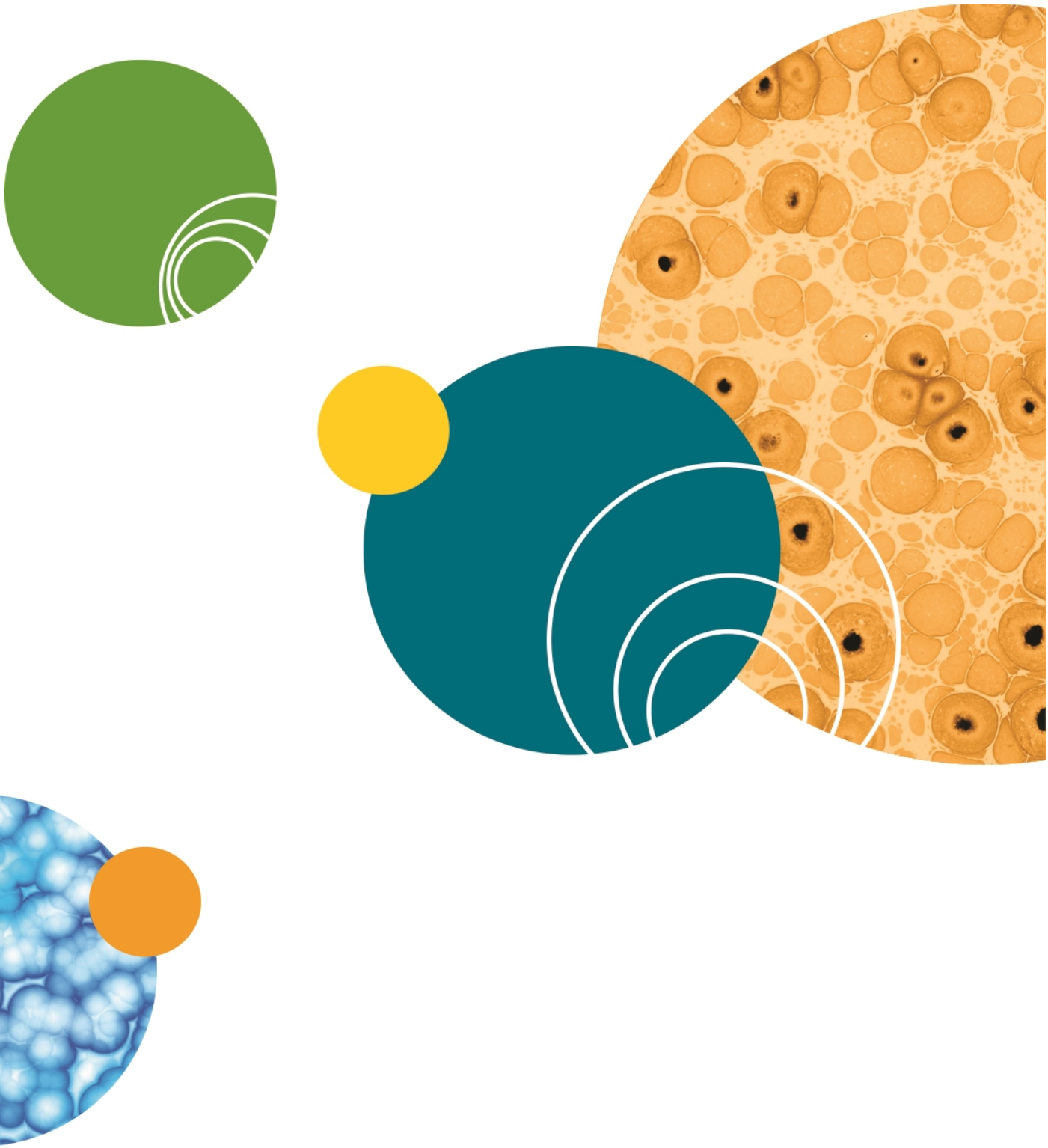
Appendix B: Compatible Objectives

B

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

Objective	Magnification	Numerical Aperture (NA)	Working Distance	Correction Collar
PL FLUOTAR 4x/0.13	4x	0.13	17.0 mm	No
HC PL FLUOTAR 10x/0.32	10x	0.32	11.1 mm	No
HC PL FLUOTAR 20x/0.40	20x	0.4	7.4 mm	No
HC PL FLUOTAR L 40x/0.60 CORR	40x	0.6	3.0 mm	Yes
HC PL FLUOTAR L 63x/0.70 CORR	63x	0.7	2.0 mm	Yes

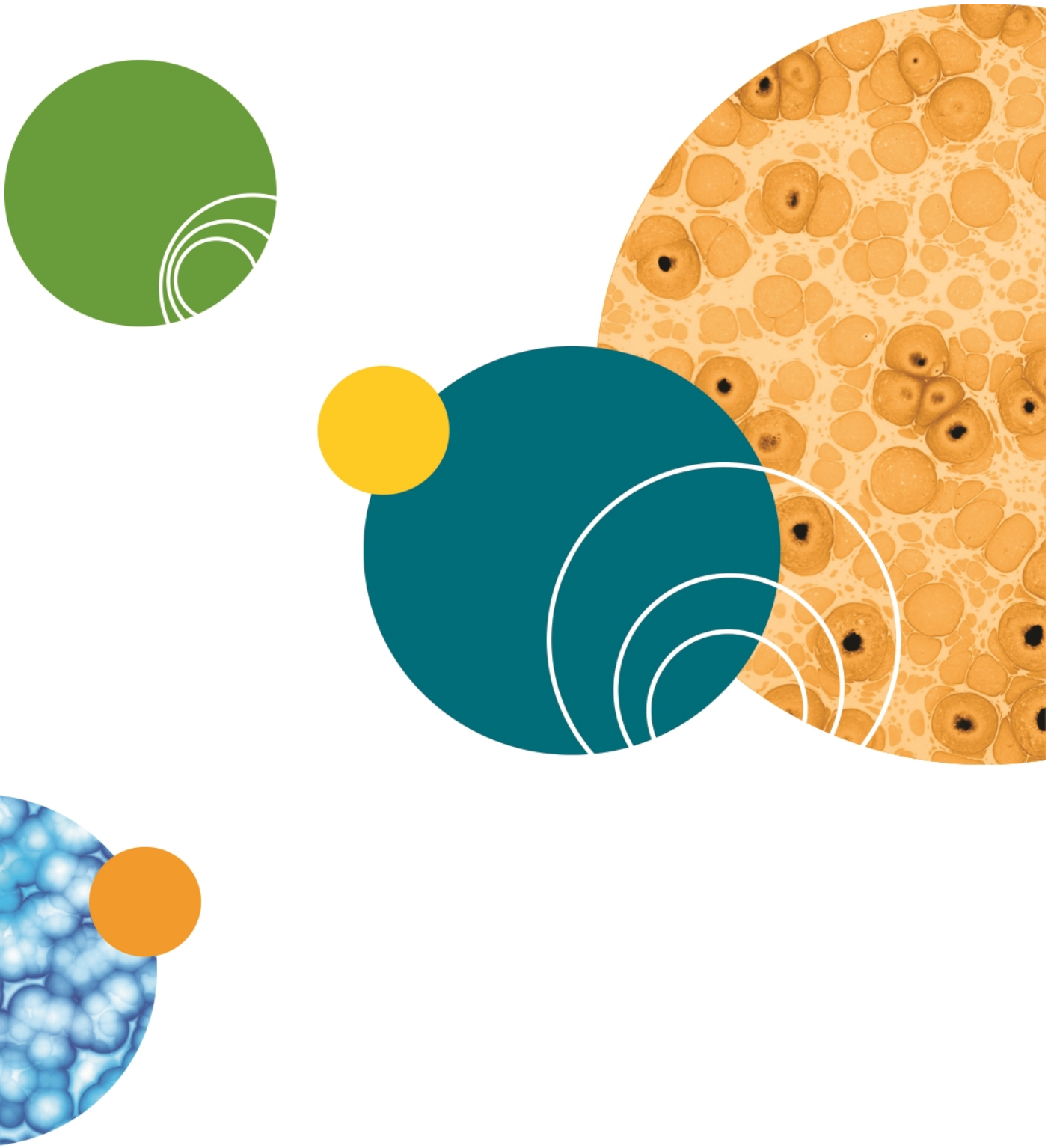
CAUTION! To prevent damaging both the instrument and your samples, do not use any other objectives with the ImageXpress Pico System.



Appendix C: Filter Cube Specifications

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

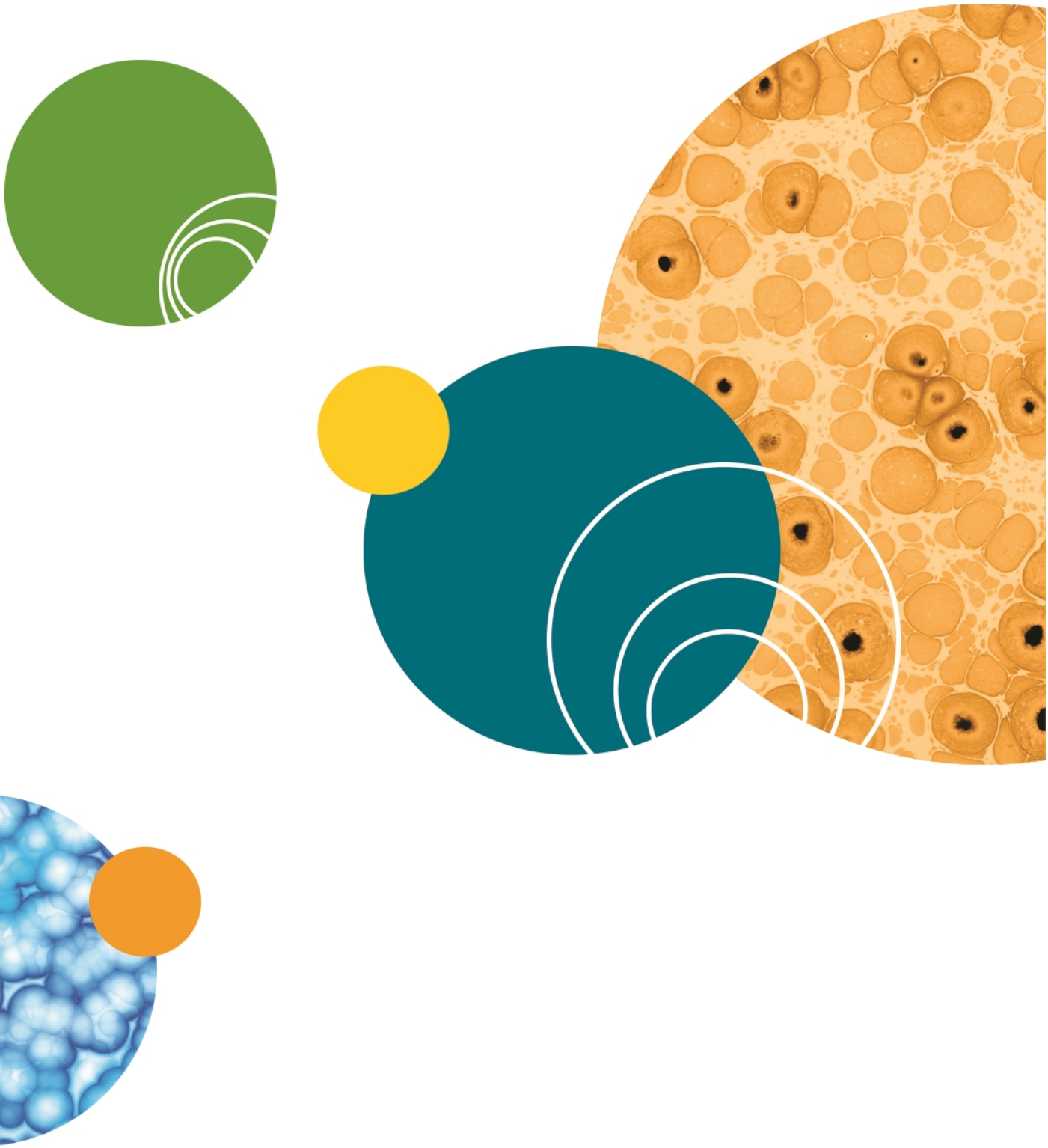
Filter	Wavelengths
DAPI	Excitation: 370/40 nm Emission: 450/60 nm Dichroic: 410 nm
FITC	Excitation: 465/40 nm Emission: 525/30 nm Dichroic: 500 nm
TRITC	Excitation: 530/45 nm Emission: 594/40 nm Dichroic: 560 nm
Cy5	Excitation: 630/40 nm Emission: 695/45 nm Dichroic: 655 nm



Appendix D: Replacement Parts and Optional Extras

For an up-to-date list of replacement parts and optional extras, go to www.moleculardevices.com.

See Compatible Objectives on page 63 for a list of compatible objectives.



Appendix E: 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! When transporting the instrument, warranty claims are void if damage during transport is caused by improper packing.

To pack the instrument:

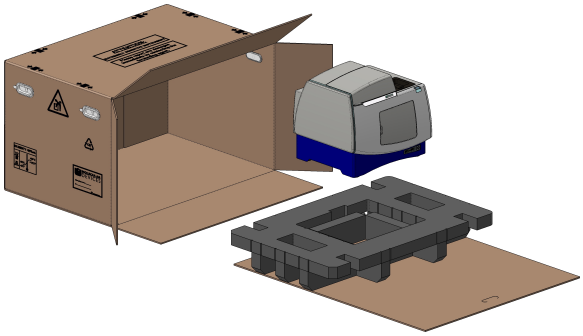
1. Remove the plate holder or the slide holder from the sample (X-Y) stage.
2. Remove any installed filter cubes and pack them in their original packaging. See the *ImageXpress Pico Installation Guide* for details on removing a filter cube.
3. Remove any installed objectives and pack them in their original packaging, including the objective case. See the *ImageXpress Pico Installation Guide* for details on removing an objective.
4. Open the plate door at the top of the instrument.
5. With the instrument off and all cables disconnected, manually move the sample (X-Y) stage to the center of the opening.
6. To secure the sample (X-Y) stage, insert the foam transport lock in the opening until it is flush with the top surface of the instrument.

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

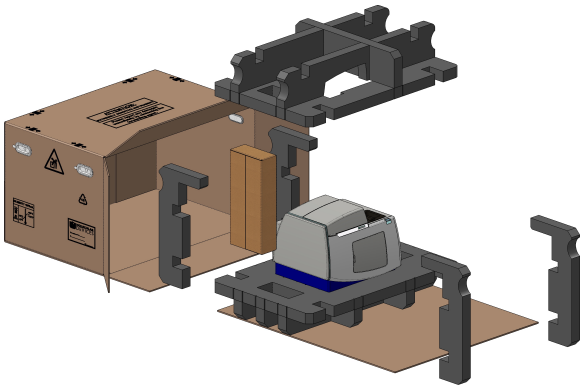
8. With one person on each end, place the instrument in the bottom foam packing as shown below.

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

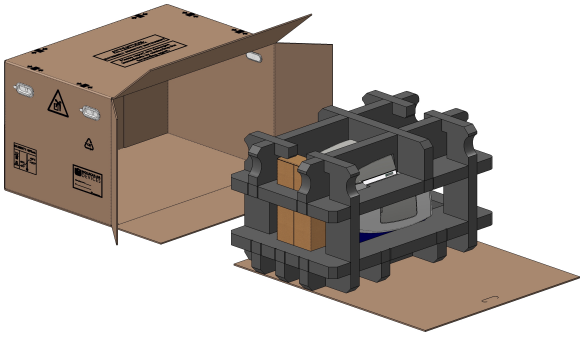
CAUTION! Keep the instrument upright and level when lifting. Do not tip or shake the instrument.



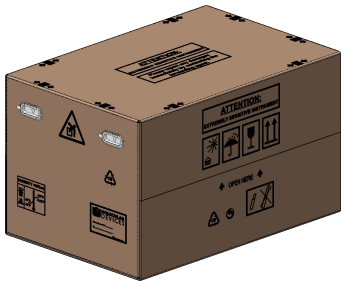
9. Replace the accessory boxes and the foam supports.



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

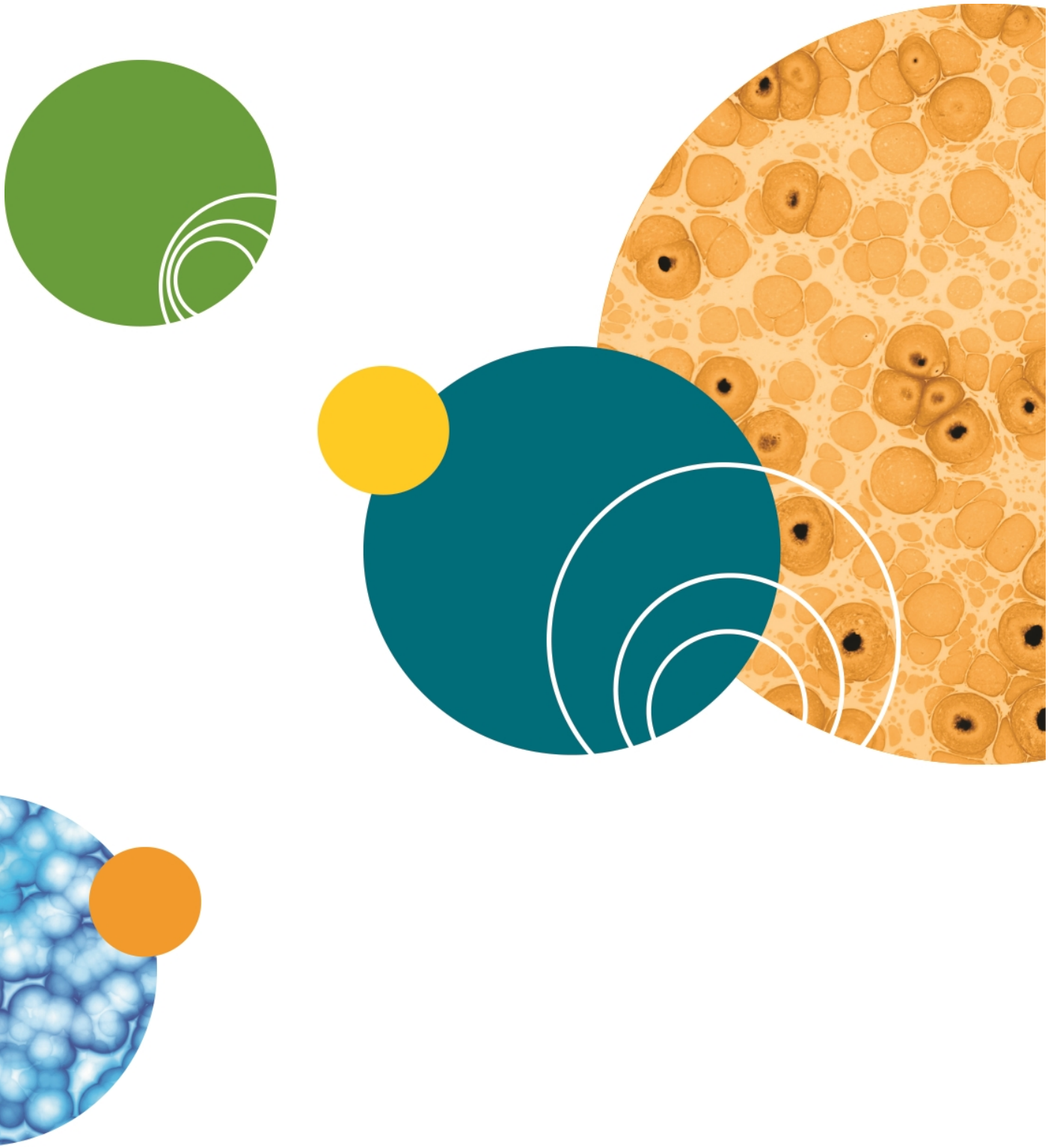


11. Seal the box for transportation.



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

See the *ImageXpress Pico Installation Guide* for details on unpacking the instrument after transport.



Appendix F: Electromagnetic Compatibility

F

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

This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

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

Information to the User (FCC Notice)

This equipment has been tested and found to comply with the limits for 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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Web: moleculardevices.com
Email: info@moldev.com

Visit our website for a current listing of worldwide distributors.

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