

# ImageXpress® Nano

## **Automated Imaging System**

With CellReporterXpress Software

**User Guide** 



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#### **Safety Information**

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

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



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

#### Warnings, Cautions, Notes, and Tips

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

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

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

WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed. Some warnings can have a different symbol on the left, such as electric shock, biohazard, and laser light warnings. The definition of the symbol is included in the text of the warning.



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



**Note:** A note calls attention to significant information.

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

#### Symbols on Instrument Labels

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

#### Table S-1: Instrument Label Alert Symbols

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

#### **Before Operating the Instrument**

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

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

#### **Protective Housing and Safety Interlocks**

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

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



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

#### Safety Interlock Failure

If the focusing laser stays on when the automated top door is open, it is unsafe to continue using the instrument due to a safety interlock failure. Contact Molecular Devices Support immediately. See Obtaining Support on page 53.

#### **Non-Interlocked Doors and Panels**

The side access doors are not interlocked. These doors do not provide access to exposure by the laser light source. Some moving parts exist inside these doors. See Moving Parts Safety on page 9.

The instrument has several panels that are intended for use by field service personnel only, and are not interlocked. All service panels are secured to the protective housing with screws and require a special tool to remove.



WARNING! If you are instructed to remove non-interlocked panels, make sure that the instrument is powered OFF and the power cable is unplugged. Never operate this instrument with any covers or panels removed. Do not attempt to access the service-only areas inside the instrument when the power cable is connected.

#### **Laser Safety**



WARNING! LASER LIGHT. This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol appears in this guide, follow the specific safety information related to the symbol.

The ImageXpress Nano System is rated a Class 1 Laser Product because it houses a laser module, and the laser light cannot be accessed under normal use. The autofocus system uses a Class 3b high-power laser that the operator cannot and must not attempt to access.

#### Table S-2: Embedded Laser Module Specifications

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

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

The operator or the service engineer is not exposed to radiation from the laser module during operation, maintenance, or service. If the top panel is removed for service, the laser beam remains safely contained within the optical system until it passes through the microscope objective, which diverges the beam and renders incident power levels below Class 1 ( $1 \text{ mW/cm}^2$ ).



WARNING! LASER LIGHT. Operate the instrument only when all the doors and panels of the instrument are in place and closed.

#### Light Source Safety

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

The ImageXpress Nano System light source is a solid-state source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source.

#### **Electrical Safety**

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

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

WARNING! The ImageXpress System is an Equipment Class 1 product that relies on protective earth grounding for safe operation. Any interruption of the protective earth ground conductor, inside or outside the instrument, or disconnection of the protective earth ground terminal can result in personal injury.

WARNING! Do not position the equipment so that it is difficult to operate the power switch on the front of the ImageXpress Systems Power and Options Controller.



WARNING! HIGH VOLTAGE. Do not operate the external light source with the external light source housing open. Do not open the external light source housing with the light source powered on.

#### ImageXpress Systems Power and Options Controller

A single power cable connects the instrument to the external ImageXpress Systems Power and Options Controller. The external power controller has an input voltage rating of 100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum. The power controller contains no user-serviceable parts.

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

#### **Fuses and Circuit Protection**

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

#### **Moving Parts Safety**

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

To prevent injury:

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

WARNING! Do not attempt to access the interior of the instrument unless specifically instructed to do so. The moving parts inside the instrument can cause injury. Do not operate the instrument with any covers or panels removed.

Note: Observe all warnings and cautions listed for all external devices attached to or in use during the operation of the instrument. See the applicable user guide for the operating and safety procedures of that device.

#### Lifting Hazard



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

#### **Chemical and Biological Safety**

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

- Handle infectious samples based on good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original containers of solutions before their use.

- Dispose of all waste solutions based on the waste disposal procedures of your facility.
- Operate the instrument in accordance with the instructions outlined in this guide, and take all the required precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. Therefore, take applicable safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials.
- Observe the applicable cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the applicable cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.



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

#### **Cleaning and Maintenance Safety**

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

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

- Contact the applicable Chemical and Biological Safety personnel.
- Review the Chemical and Biological Safety information contained in this guide. See Chemical and Biological Safety on page 9 for details.

Perform only the maintenance tasks described in this guide. Any other maintenance tasks must be done by qualified Molecular Devices personnel only.



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

For approved cleaning and maintenance procedures, see Maintenance on page 45.





#### Chapter 1: Introduction to the ImageXpress Nano System



The ImageXpress<sup>®</sup> Nano Automated Imaging System from Molecular Devices is an integrated cellular imaging and analysis system that is designed for rapid, automated screening of fluorescently labeled biological samples in microplates. With the addition of modular options, the system provides environmental control for live cell imaging and uses transmitted light capability for label-free imaging.

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

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

Key components of the instrument include the following:

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



#### ImageXpress Nano System Instrument Features

Figure 1-1: ImageXpress Nano System Components, Without Options, With the CellReporterXpress Computer Connected Directly to the Instrument



Figure 1-2: ImageXpress Nano System Components, Without Options, With the Instrument and the CellReporterXpress Computer Connected to a Network

#### **Illumination System: Excitation**

#### **Light Source**

The ImageXpress Nano System light source is a solid-state source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source.

	_	

**Note:** The ImageXpress Nano System solid-state light source is limited to an excitation spectrum ranging between 380 nm and 680 nm.

#### **Illumination Optics**

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

#### **Filter Cube Changer**

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

#### **Objective (Z) Stage**

#### **Motorized Z Stage**

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

#### **Objectives**

The standard objectives are Nikon CFI60 series. The selected objective lens focuses excitation light onto the sample, and collects fluorescence light emitted by the sample. See Compatible Objectives, see page 59.

#### **Motorized Objective Changer**

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

#### Sample (X-Y) Stage

#### Sample

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

#### **Plate Holder and Plate Clamp**

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

#### **Motorized X-Y Stage**

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

#### **Autofocus Laser**

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

#### **Electronics**

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

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

#### **Theory of Operation**



#### Figure 1-3: ImageXpress Nano System Optical Path

The ImageXpress Nano System uses the following components and functions:

- Fluorescence Imaging, see page 17
- Excitation and Emission Filters, see page 18
- Objective Lenses, see page 19

#### **Fluorescence Imaging**

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

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

#### **Excitation and Emission Filters**

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

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

#### **Dichroic Mirror**

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

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

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

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

#### **Dichroic Transmission Spectrum**

An ideal dichroic mirror would have an infinitely sharp cut-off. That is, it would have unity transmittance coefficient at wavelengths longer than the cut-off, and zero transmittance (and therefore unity reflectance in a non-absorbing dichroic mirror) at shorter wavelengths. In practice, the characteristic transmission spectrum for a dichroic looks similar to the graph in Figure 1-4.



#### Figure 1-4: Example of a transmission spectrum of a dichroic mirror

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

#### **Objective Lenses**

The ImageXpress Nano System can be configured at the time of purchase with up to 4 of the high-quality Nikon objectives listed in Compatible Objectives on page 59.

**Note:** Extra-long working distance (ELWD) objectives have adjustable spherical-aberration correction collars for imaging through thick substrates such as most microplates. For details on how to calculate and set their correct values, see Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives on page 51.

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

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

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

#### Chapter 2: Using the ImageXpress Nano System



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

- Starting the System on page 21
- Understanding Status Lights on page 24
- Acquiring Data on page 24
- Analyzing Data on page 24
- Maintaining the Instrument on page 24
- Shutting Down the System on page 25

#### Starting the System

The section describes how to power on the instrument and how to start the CellReporterXpress.

- Powering On the Instrument on page 22
- Logging In to the Software on page 23

#### **Powering On the Instrument**

To power on the instrument:

1. Ensure the Ethernet cable is connected to the back of the instrument and connected either directly to the host computer or remotely connected to the network.



- 2. Ensure that the power cords for the instrument and the light source are connected to the ImageXpress Systems Power and Options Controller.
- 3. On the light source, verify that the **power** switch is in the **1** (on) position and the **light** switch is in the **0** (off) position.



4. On the ImageXpress Systems Power and Options Controller, move the **Instrument** switch to the **ON** position.

**Note:** Turning on the **Instrument** switch also turns on the light source.

#### Logging In to the Software

The ImageXpress Nano System requires a host computer to run the CellReporterXpress Software. All acquisition and analysis operations are performed in the CellReporterXpress Software.

	Note: If you provided your own host computer to use with the
_	ImageXpress Nano System, this procedure assumes that you have properly installed
	the CellReporterXpress Software. See the CellReporterXpress Installation Guide for
	details.

To log in to the CellReporterXpress Software on the host computer:

- 1. Do one of the following to display the CellReporterXpress Log In screen:
  - Double-click the **MD.CellReporterXpress** icon on your desktop.
  - Click Start > Molecular Devices > MD.CellReporterXpress.

LOG IN	
1 mld	
<b>a</b>	
Remember me	
LOG IN	

#### Figure 2-1: CellReporterXpress Log In screen

- 2. On the CellReporterXpress Log In screen, in the Login field, enter your user name.
- 3. In the **Password** field, enter your password.

**Note:** Use the Windows system user name and password to log in to the CellReporterXpress Software.

4. Click LOG IN.

-

**Note:** Depending on how your system is configured, it can take up to 5 minutes after powering on the instrument for the CellReporterXpress Software to detect it and display it in the Available Acquisition Devices list.

#### **Understanding Status Lights**

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

Fable 2-1: Status Indicator Colors			
Color	Instrument Status		
Orange	The instrument is powered on, but is not ready to use. This status typically occurs at startup (during initialization) and at shutdown.		
Blue	The instrument is powered on, connected to the software, and ready to use.		
Green	The instrument is acquiring data.		
Red	The instrument is in an error state or cannot communicate with the software. Restart the instrument.		

#### Acquiring Data

The acquisition workflow involves configuring settings, acquiring images, and storing plate data in a database. All acquisition operations are performed in the CellReporterXpress Software.

See Preparing For Acquisition on page 33 for details on getting started. See the Help in the CellReporterXpress Software for details on acquiring data.

#### **Analyzing Data**

The analysis workflow consists of enhancing and analyzing acquired plate data. All analysis operations are performed in the CellReporterXpress Software.

See the Help in the CellReporterXpress Software for details on analyzing data.

#### Maintaining the Instrument

User maintenance tasks for the ImageXpress Nano System include cleaning objectives and cleaning the instrument. See Maintenance on page 45 for details.

Perform only the maintenance tasks described in this guide. Any other maintenance tasks must be done by qualified Molecular Devices personnel only. See Obtaining Support on page 53 for details.

#### Shutting Down the System

To shut down an ImageXpress Nano 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.



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. On the ImageXpress Systems Power and Options Controller, move the **Instrument** switch to the **OFF** position.



**Note:** Turning off the **Instrument** switch also turns off the light source.



**CAUTION!** Wait at least 30 seconds before restarting the system.





#### **Chapter 3: System Installation**



The ImageXpress Nano System ships fully configured, and is installed at your site by a Molecular Devices field service engineer. The base system includes an imaging unit and accessory kit. The host computer and monitor are optional purchases through Molecular Devices. See Computer Specifications on page 29. See Table 3-1 for kit contents.

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



## Figure 3-1: ImageXpress Nano System Components, Without Options, With the CellReporterXpress Computer Connected Directly to the Instrument

- Power supply to the instrument
- Liquid light guide from the external light source to the instrument
- USB 2.0 from the light source to the instrument
- Ethernet from the instrument to the computer
- Ethernet from the computer to the network



## Figure 3-2: ImageXpress Nano System Components Without Options, With the CellReporterXpress Host Computer Connected to the Instrument through a Network

- Power supply to the instrument
- Liquid light guide from the external light source to the instrument
- USB 2.0 from the light source to the instrument
- Ethernet from the instrument to the network
- Ethernet from the computer to the network

Accessory	Description
Bead plate	TetraSpeck <sup>™</sup> fluorescent microspheres test plate
Hex keys	1/16", 0.05"
Slide holder	Single
Calibration slides	Spatial (GP-2) Red (GP-7) Green (GP-8) Blue (GP-9) Yellow (GP-11)
Shading correction plates	Fluorescent Green Fluorescent Red Fluorescent Pink
Storage box	Empty accessory box for miscellaneous storage use

#### Table 3-1: Accessory Kit Contents

#### **Computer Specifications**

For the base model of the ImageXpress Nano System, you provide your own host computer and monitor as your host interface with the CellReporterXpress Image Acquisition and Analysis Software. Optionally, contact your Molecular Devices representative to buy the host computer and monitor through Molecular Devices.

#### **Host Computer**

The host computer functions as a server.

Your host computer must meet the following minimum specifications:

Item	Minimum Requirement	Notes
Operating System	Microsoft Windows 10 (64-bit)	Pro, Enterprise, and Education editions are supported.
CPU Speed	2.4 GHz	
Logical Processors	10	Logical processors are virtual processing units on the CPU that can function as additional physical processing units. 10 logical processors supports 4 concurrent analyses. For each additional concurrent analysis that you want to perform, add 2 logical processors.

Item	Minimum Requirement	Notes
RAM	12 GB	12 GB of RAM supports 4 concurrent analyses. For each additional concurrent analysis that you want to perform, add 2 GB of memory.

#### **Client Devices**

The ImageXpress Nano System uses the web browser-based CellReporterXpress as an interface. The device you use to run the browser-based software is the client device. In a standalone configuration, the host computer functions as the client device. A client device can be a desktop computer, a laptop computer, an iPad tablet, and an Android tablet. Tablets must have a 9" screen or larger.

The following web browser specifications are required:

Operating System	Minimum Browser Version
Microsoft Windows	Google Chrome 60 (64-bit)
Apple Macintosh OS 10.12 or higher	Google Chrome 60 or Apple Safari 11.0
Apple iOS 10.3.3 or higher	Apple Safari
Google Android	Google Chrome 59
Linux	Google Chrome 60

#### **External Hard Drives**

\*

The ImageXpress Nano System includes a USB 3.0 port on the bottom right of the front of the instrument. The port can be used to extend the temporary storage space with a thumb drive or an external portable hard drive for image acquisition. There is enough temporary storage space in the instrument to image in one experiment approximately 30 384-well microplates with one region selection per well.

**CAUTION!** Never unplug an external portable hard drive while an experiment is processing.

**Tip:** To ensure data safety, before unplugging an external hard drive, in the CellReporterXpress, select **Devices**, select the instrument from the **Available** 

Acquisition Devices list, and then click Restart Device. When the status indicator

shows Offline, unplug the hard drive.



Figure 3-3: ImageXpress Nano Instrument Front USB Port





#### **Chapter 4: Preparing For Acquisition**



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

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

- Assay Design on page 33
- Plate Selection on page 34
- Sample Preparation on page 36
- Instrument Maintenance on page 37

#### **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, including fluorescent proteins, antibody-based stains, and chemical-based stains. In general, Molecular Devices recommends including a nuclear stain (such as Hoechst or DAPI) to help identify cells during image analysis. If the assay involves movement of a protein of interest to or from a particular cellular compartment or organelle, it can also be helpful to include a probe specific to that cellular compartment or organelle. If you are planning on using CellReporterXpress Software to analyze your data, review the requirements of that module. Individual fluorochromes have unique characteristics that help determine their best use. Use probes that provide bright, specific staining and that have excitation and emission spectra suitable for the filter sets in your ImageXpress System. For experiments using multiple stains, select fluorophores that have sufficient spectral separation. Some fluorochromes provide brighter intensities and require a shorter exposure time, while others do not bleach as quickly and allow a longer exposure time. There also might be toxicity issues with some cell types or bleed-through issues between pairs of fluorochromes. Consider these factors when choosing a fluorochrome.

**Note:** If your ImageXpress System has the transmitted light option, then it might be possible to identify cells using transmitted light images instead of fluorescence.

#### **Cell-Based Assays**

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

#### **Plates or Slides**

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

#### **Plate Selection**

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

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

- Plate Format, see page 35
- Plate Material, see page 35
- Fluorescence Background, see page 35
- Bottom Thickness, see page 35

- Plate Flatness or Reproducibility of the Z-Pattern, see page 36
- Batch-to-Batch Consistency, see page 36

#### **Plate Format**

Determine if the plate format is compatible with your assay.

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

#### **Plate Material**

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

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

#### **Fluorescence Background**

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

#### **Bottom Thickness**

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

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

#### Plate Flatness or Reproducibility of the Z-Pattern

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

#### **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 36
- Fixation and Staining Conditions, see page 36
- Final Buffer or Media, see page 37
- Plate Handling and Storage, see page 37

#### **Cell Density**

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

#### **Fixation and Staining Conditions**

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

#### **Final Buffer or Media**

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

#### **Plate Handling and Storage**

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

#### **Instrument Maintenance**

For best performance, your should have regular preventive maintenance (PM) services. In between PM services, the system administrator can clean dust off the optics as needed (see Maintenance on page 45). Wear gloves when handling any optical components to avoid contaminating them with dirt or skin oils.

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





#### **Chapter 5: Optional Expansion Solutions**



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

#### • Transmitted Light Options, see page 39

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

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

#### **Transmitted Light Options**

Operational information is included for the following:

- Transmitted Light Hardware on page 39
- Replacing the Transmitted Light Bulb on page 42

#### **Transmitted Light Hardware**

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

- White light lamp (halogen). The software controls the lamp power. The halogen lamp has a limited life and can be replaced. See Replacing the Transmitted Light Bulb on page 42.
- **Transmitted Light shutter**. Controlled by the software. The shutter protects the sample from the transmitted light when not imaging. The shutter window maintains the environment of the sample. See Figure 5-3.
- Hinge. Increases access to the plate if necessary. See Figure 5-4.

ImageXpress Nano Automated Imaging System with CellReporterXpress Software User Guide



Figure 5-1: ImageXpress Nano System with the Transmitted Light option



Figure 5-2: ImageXpress System with Transmitted Light option (detailed view)



Figure 5-3: Transmitted Light shutter



Figure 5-4: Hinge-release knob and hinge

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

#### **Transmitted Light Experiments**

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

To run a Transmitted Light experiment

1. Load a suitable plate.

The plate must be unlidded, have a clear lid, or have a clear plate seal on the top. Fill microplate wells at least half-way full with liquid.

**CAUTION!** Condensation on a lid or plate seal can negatively affect image quality.

- In the CellReporterXpress, select a protocol using transmitted light, or select a generic protocol.
- 3. Verify that one of the wavelengths is defined as Transmitted Light.

**Tip:** Typically, it is recommended to use this as your first wavelength.

- 4. Configure the remaining settings as usual.
- 5. Run the acquisition.

#### **Replacing the Transmitted Light Bulb**

The ImageXpress Transmitted Light option uses a replaceable 12 volt halogen light bulb. See Replacement Parts and Optional Extras on page 63.

To replace the light bulb:

- 1. Unplug the power cord from the controller box.
- 2. Remove the set screw that secures the cover on top of the tower using a 3 mm hex wrench.



Figure 5-5: Set screw location

3. Remove cover by lifting it upward.



Figure 5-6: Cover removal

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



Figure 5-7: Bulb and lever

5. Put on cotton gloves to handle the bulb.



**CAUTION!** Never touch the bulb with bare hands.

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



#### Figure 5-8: Press lever and remove bulb

- 7. Insert the new bulb with the correct rating and specifications.
- 8. Replace cover and secure it with the set screw.
- 9. Plug in the controller box.

#### **Chapter 6: Maintenance**



Perform only the maintenance tasks described in this guide. Any other maintenance tasks must be done by qualified Molecular Devices personnel only. See Obtaining Support on page 53 for details.

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 for details.

#### **Maintenance Precautions**

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

- Some procedures require that the power supply for the instrument is turned OFF and that the power cable is unplugged before doing the service or maintenance procedure.
- Some procedures may require that you disconnect the USB connection to the hardware server (host) PC and turn off any attached peripherals.
- Access only the user-serviceable components inside the instrument as described in the section. Avoid contact with other components as they can be damaged or knocked out of alignment.

**CAUTION!** Do not touch the autofocus laser.

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

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

- Preventive Maintenance, see page 46
- Cleaning the Instrument, see page 46
- Light Source Maintenance, see page 48
- Objective Maintenance, see page 48

WARNING! Service or maintenance procedures other than those specified in this guide can be done only by Molecular Devices qualified personnel. When service is required, contact Molecular Devices technical support.

#### **Preventive Maintenance**

To ensure optimal operation of the instrument, 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.

#### **Cleaning the Instrument**

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 turn the power switch off and disconnect the power cord from the main power source before using liquids to clean the instrument.
- Wipe up all spills immediately.
- Do not use any cleaning agents other than those recommended in this section without first contacting Molecular Devices Technical Support. See Obtaining Support on page 53.
- Do not use ultraviolet light for sterilization, as this can damage plastic components.
- Do not use any organic solvents.
- To prevent damaging internal components, do not pour or squirt water or alcohol directly onto the instrument.

#### **Cleaning the ImageXpress Nano Instrument**

The following procedure is designed to clean the plate-loading region of the instrument without damaging the internal components of the imaging system.

Before cleaning the instrument, read and follow the Maintenance Precautions on page 45.

The following cleaning procedure is compatible with disinfectant wipes, such as Kimwipes wipers with 70% ethanol.

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

To clean your ImageXpress Nano instrument:



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

- 1. Log in to the CellReporterXpress Software.
- 2. On the **Home** page, click **Devices**.
- 3. Do the following to open the plate loading door and move the stage to the loading position:
  - a. On the **Devices** screen, in the **Available Acquisition Devices** list, select the instrument.
  - b. On the right side of the screen, click 😂 Open Plate Door.
- 4. Click Shutdown Device.
- 5. If needed, do the following to end the CellReporterXpress session on the host computer.



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.

6. On the ImageXpress Systems Power and Options Controller, move the **Instrument** switch to the **OFF** position.

**Note:** Turning off the **Instrument** switch also turns off the light source.

- 7. Ensure the filter cube access door is closed and no sample is loaded.
- 8. With gloved hands, use a damp wipe to wipe down the entire outer surface including side panels and top panels of the instrument.
- 9. Use an alcohol wipe or a disinfectant wipe and go over the entire surface again.
- 10. To gently wipe the perimeter of the plate holder and stage area, where a plate would be loaded, use forceps wrapped with damp wipes.
- 11. To go over the plate holder and stage area again, use forceps wrapped with alcohol wipes or disinfectant wipes.

12. To clean the stage area underneath and around the plate loading area, use a fresh damp wipe.



**Tip:** The stage moves freely without power. To clean the plate holder and stage area, you can slide the stage around.

- 13. Use a fresh alcohol wipe or a disinfectant wipe and go over the stage area underneath and around the plate loading region again.
- 14. Wait a few minutes for the alcohol to evaporate.
- 15. On the external ImageXpress Systems Power and Options Controller for your ImageXpress System, power ON the instrument. See Powering On the Instrument on page 22.
- 16. Log in to the CellReporterXpress Software.
- 17. If the top door stays open after restarting the instrument, do the following to close the door:
  - a. On the **Home** page, click **Market Devices**.
  - b. On the **Devices** screen, in the **Available Acquisition Devices** list, select the instrument.
  - c. On the right side of the screen, click Close Plate Door.

#### **Light Source Maintenance**

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



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

The external light source for the ImageXpress Nano System is a solid-state light source that has a rated lifetime of more than 10,000 hours. There are no user-replaceable parts in this light source. When the light source needs replacing, contact Molecular Devices. See Obtaining Support on page 53.

#### **Objective Maintenance**

You can clean an objective that is installed in the instrument or remove the objective from the instrument for cleaning. See Cleaning an Objective on page 49.

You can identify the magnification of the objectives installed inside your instrument by the following color bands:

- Red—4x
- Yellow—10x
- Green—20x
- Light blue—40x
- Dark blue—60x

#### **Correct Objective Placement**

Molecular Devices configures your instrument with any objective with a correction collar installed in the outer positions 1 and 4. The outer positions provide easier access through one of the side panels for adjusting the correction collar.



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

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**Note:** Molecular Devices recommends that you leave the reference objective in place and replace only the other objectives. The reference objective is typically a 10x objective and is typically installed in position 3.

#### **Cleaning an Objective**

If debris or contaminants have collected on an objective, follow these instructions for cleaning the objective lens.

Before cleaning an objective, read and follow the Maintenance Precautions on page 45.



**CAUTION!** To prevent skin oils from damaging the optical coatings, Molecular Devices recommends wearing powder-free disposable gloves during the following procedure.

To clean an ImageXpress Nano objective:

- 1. Within the CellReporterXpress, click **Devices**.
- 2. Click Shutdown Device.



**Note:** When using the ImageXpress Nano System the Shutdown Device function only safely disconnects the software from the instrument.

3. Power off the ImageXpress Systems Power and Options Controller.

**Tip:** With the instrument power off, the stage and the objective changer move freely.

- 4. Determine where the objective is located.
  - If the objective is in one of the center positions, access it from the top, remove any plate or slide holder on the stage.
- 5. Locate the objective that you want to clean.



WARNING! Beware of the free-moving stage. It slides around loosely when the instrument is powered off.

If needed, loosen the objective inside the instrument or remove the objective from the instrument.



**Tip:** You might need to use a flashlight to view the markings.

6. Place the objective on a secure surface, away from the instrument, and use a bulb duster to carefully to blow dust contaminants off the objective.



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

7. Use lens paper, and if necessary, lens cleaner like 100% methanol, to gently wipe the objective free of contaminants.

For the preferred cleansing solvent and procedure, see the information from the objective manufacturer.



**CAUTION!** Do not use Kimwipes wipes or similar lint-leaving products to wipe a lens.

8. After cleaning your objective, replace it inside the instrument into the same slot from which you removed it.

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

- 9. After replacing the objective, do one of the following:
  - If you accessed the objective from the side, close the upper, hinged side panel door.
  - If you accessed the objective from the top, then replace the plate or slide holder on the stage.
- 10. Power on the ImageXpress Systems Power and Options Controller.

11. In the software, on the Acquisition Device page, for the selected Available Acquisition

**Devices**, when the Online icon is green, click Close Plate Door.

Tip: If the Available Acquisition Devices icon stays Offline, click Restart Device.

12. Test the objective cleanliness by examining the image quality of acquired images. If the quality has degraded, re-clean the objective by repeating this procedure.

# Adjusting the Spherical-Aberration Correction Collar on ELWD Objectives

The ELWD (extra long working distance) Nikon objectives that can be supplied with your ImageXpress Nano System have adjustable correction collars that minimize spherical aberration in the image of the specimen. The collars have a range of 0 mm to 2 mm correction, and changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very much dependent on properly setting these collars.

**Note:** Some other objectives (non-ELWD) can also have correction collars. The range can vary depending on the objective.

The settings to be used depend on the thickness of the microplate well or slide on which the specimen is mounted. In general, set the correction collar for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined by the plate specifications from the plate manufacturer.



\*

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

Before adjusting the correction collar on an objective, read and follow the Maintenance Precautions on page 45.



**CAUTION!** To prevent skin oils from damaging the optical coatings, Molecular Devices recommends wearing powder-free disposable gloves during the following procedure.

- 1. Within the CellReporterXpress Software, within Acquisition> Protocols, open a protocol that uses the objective.
- 2. On the **Acquisition Device** page, click **Open Plate Door**.
- 3. Click Prepare Objective Collar for Adjustment.

4. Click Shutdown Device.

**Note:** When using the ImageXpress Nano System the Shutdown Device function only safely disconnects the software from the instrument.

5. Power off the ImageXpress Systems Power and Options Controller.



- 6. Determine where the objective is located.
  - If the objective is in one of the center positions, access it from the top, remove any plate or slide holder on the stage.
- Locate the correction collar on the objective that you want to adjust. To locate the correction collar, if needed, loosen the objective inside the instrument or remove the objective from the instrument.



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

8. Rotate the correction collar to its new setting.

The new setting value provided by the CellReporterXpress corresponds to the physical bottom thickness of the plate or slide with coverslip.

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You may need to perform correction of objective collar for the current objective. Use value: 0.635

#### Figure 6-1: Example of the collar value from CellReporterXpress

If needed, replace the objective in to the instrument or tighten the objective inside the instrument.



**CAUTION!** When replacing an objective, avoid accidentally changing the collar setting.

- 9. After adjusting, do one of the following:
  - If you accessed the objective from the side, close the upper, hinged side panel door.
  - If you accessed the objective from the top, then replace the plate or slide holder.
- 10. Power on the ImageXpress Systems Power and Options Controller.

11. In the software, on the Acquisition Device page, for the selected Available Acquisition

Devices, when the Online icon is green, click Close Plate Door.

Tip: If the Available Acquisition Devices icon stays Offline, click Restart Device.

- 12. Click Finish Adjustment of Objective Collar.
- 13. Test the correction collar setting by examining the image quality of acquired images. If the quality has degraded, re-adjust the correction collar by repeating this procedure.

#### **Obtaining Support**

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

Our support website, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, click **Request Support** to submit a request to our technical support representatives.

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

To find regional support contact information, visit www.moleculardevices.com/contact.

Molecular Devices provides a wide range of support:

- Documentation: Check the guides that are included on the installation media and the Help that is available within the CellReporterXpress Software.
- Online Knowledge Base: The Knowledge Base contains links to technical notes, software upgrades, newsletters, user guides, and other resources. Visit the Molecular Devices Support website at www.moleculardevices.com/support and follow the links to the Knowledge Base.

 Technical Support: You can contact Molecular Devices Technical Support by phone or submit a support request through the Knowledge Base. In either case, you will need the instrument serial number, software version number, and the name of the system owner. The serial number is located on the back panel of the instrument.

Figure 6-2: Serial Number location on the back of the ImageXpress Pico System



#### Figure 6-3: Serial Number location on the back of the ImageXpress Nano System

- Additional Resources:
  - Web-based microscopy courses:
    - http://www.microscopyu.com
    - http://www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopyshort-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:
    - http://www.lifetechnologies.com/us/en/home/references/molecular-probesthe-handbook.html
  - Filter information:
    - http://www.semrock.com
    - http://www.chroma.com
    - http://www.omegafilters.com



The instrument must be installed on a level and stable surface. For additional specifications, refer to the *Pre-Installation Guide*.

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

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WARNING! The ImageXpress Nano System is an Equipment Class 1 product that relies on protective earth grounding for safe operation. Any interruption of the protective earth ground conductor, inside or outside the instrument, or disconnection of the protective earth ground terminal can result in personal injury.

WARNING! Do not position the equipment so that it is difficult to operate the power switch on the front of the ImageXpress Systems Power and Options Controller.



Figure A-1: Front and Sideways Installation Space Requirements

Table A-1: O	perational	and Env	vironmental	Specifications	for the	Instrument	Without
Options							

Item	Description
Operating environment	Indoor use only
Systems power and options controller input	100 VAC to 240 VAC, 50/60 Hz, 12 amps maximum
Light source power	220 W, 24 V, AC to DC converter included
Weight, base unit instrument, no options	95 kg (210 lbs)
Weight, external light source	7 kg (16 lbs)
Weight, external system power and options controller no options	10 kg (22 lbs)
Ambient operating temperature	18°C to 30°C
Humidity restrictions	35% to 50% non-condensing
Altitude restrictions	Not to exceed 2000 m (6562 ft)
ISM Equipment class	1
IEC Installation category	II
IEC Pollution degree	2
IEC Ingress Protection	IP20

#### Instrument Dimensions



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



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





### **Appendix B: Compatible Objectives**



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

Table B-1: Nikon	Objectives	Compatible wit	h the ImageXpre	ss Nano System	and Settings

Objective Magnification and Type	Molecular Devices Part Number	Numerical Aperture (NA)	Working Distance	Plate Compatibility
2x Plan Apo Lambda	1-6300-0451	0.10	8.5 mm	Thin bottom (0.17 mm), Thin bottom (0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)
4x Plan Apo Lambda	1-6300-0121	0.20	20 mm	Thin bottom (0.17 mm), Thin bottom (0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)
10x Plan Fluor	1-6300-0190	0.30	16.0 mm	Thin bottom (0.17 mm), Thin bottom (0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)
20x Super Plan Fluor ELWD cc 0 mm to 2 mm	6500-0108	0.45	8.1 mm to 7.0 mm	Thin bottom (0.17 mm) <sup>*</sup> , Thin bottom (0.17 mm) No Skirt <sup>*</sup> , Thick bottom (0.25 mm to 1 mm)
40x Super Plan Fluor ELWD cc 0 mm to 2 mm	6500-0109	0.60	3.7 mm to 2.7 mm	Thin bottom (0.17 mm) <sup>*</sup> , Thin bottom (0.17 mm) No Skirt, Thick bottom (0.25 mm to 1 mm)

ImageXpress Nano Automated Imaging System with CellReporterXpress Software User Guide

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

#### Table B-1: Nikon Objectives Compatible with the ImageXpress Nano System and Settings (continued)

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

## Appendix C: Filter Specifications



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

Table C-1: Fi	ilter Specifi	cations for t	he ImageXpres	s Nano System





### **Appendix D: Replacement Parts and Optional Extras**



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



**Note:** New filter cubes and objectives must be installed by Molecular Devices Field Service Engineers.

Part Number	Description
_	For a List of Compatible Nikon Objectives see Compatible Objectives on page 59
1-6300-0442	DAPI Filter Set
5045173	FITC Filter Set
1-6300-0444	TRITC Filter Set
1-6300-0445	Cy3 Filter Set
1-6300-0446	Cy5 Filter Set
1-6300-0447	CFP Filter Set
1-6300-0448	YFP Filter Set
1-6300-0449	Texas Red Filter Set
1-6300-0450	GFP Filter Set
5048412	Accessory Tool Kit
5033647	Replacement Solid-State Light Source
1-3335-0005	12 V Halogen Lamp for Nikon Transmitted Light (TL) Tower





#### **Appendix E: Electromagnetic Compatibility**



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

This ISM device complies with Canadian ICES-001. Cet appareil ISM est confomre à la norme NMB-001 du Canada.

#### ISM Equipment Classification (Group 1, Class A)

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

#### Information to the User (FCC Notice)

This equipment has been tested and found to comply with the limits for non-consumer ISM equipment, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a non-residential installation. This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instructions, might cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and TV reception. The user is cautioned that changes and modifications made to the equipment without the approval of the manufacturer could void the user's authority to operate this equipment.





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