ImageXpress HCS.ai

High-Content Screening System

with MetaXpress Acquire Software Version 2025.1.1

User Guide

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MOLECULAR DEVICES

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Safety Information

This section describes the safe use of the ImageXpress® HCS.ai High-Content Screening System. Safety includes an understanding of the information in this guide, the safety labels on the system, and the precautions that you must follow before and during operation of the system.

When using the ImageXpress HCS.ai system, always observe Good Laboratory Practices (GLP).

It is important to confirm that everyone involved with the operation of the ImageXpress HCS.ai system has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the system.
- Read and understood all Safety Data Sheets (SDS) for all materials being used.
- Read and understood all system documentation, including all danger, warning, and caution statements.

Always remember that the key to safety is operating the system with care. The safety of personnel and equipment can only be ensured if you strictly observe all safety instructions and safety-related warnings.

DANGER! If the ImageXpress HCS.ai system is used in any manner not specified by Molecular Devices, the protection provided by the system may be impaired.

Intended Use

The ImageXpress HCS.ai High-Content Screening System—when used with the MetaXpress® Acquire Image Acquisition Software—is an integrated cellular imaging system designed for rapid, automated screening of fluorescently labeled biological samples in microplates as well as label-free imaging using transmitted light.

DANGER! If the ImageXpress HCS.ai system is used in any manner not specified by Molecular Devices, the protection provided by the system may be impaired.

Note: The ImageXpress HCS.ai system is for research use only. It is not for use in diagnostic procedures.

Safety Symbols in This Guide

All safety symbols in this guide are framed by a triangle. An exclamation mark is used for most safety symbols. Other symbols can warn of specific hazards, such as biohazard, electrical, or laser safety warnings.

When danger, warning, or caution statements appear in this guide, ensure you follow the related safety information.

The following safety symbols may appear in this guide:

DANGER! A danger statement indicates a situation or operation that could cause serious personal injury or death if precautions are not followed. The danger symbol can vary depending on the hazard. The definition of the symbol is included in the text of the statement.

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

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

In addition, the following informational symbols appear in this guide:

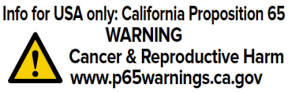
Note: A note calls attention to significant information.

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

Safety Labels on the System

Each safety label found on the ImageXpress HCS.ai system contains an alert symbol to indicate the potential safety hazard. The following table lists the alert symbols that can be found on the instrument and the water immersion controller.

Symbol	Indication
	Indicates that the product documentation must be consulted.
	Indicates a potential heat hazard.
	Indicates a potential lifting hazard. To prevent injury, use a minimum of two people to lift the instrument. See System Specifications on page 73 for details on the weight of the instrument.
	Indicates CSA certification.

Symbol	Indication
	Indicates European technology conformity.
	Indicates United Kingdom technology conformity.
	Indicates Korean technology conformity.
	Indicates compliance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. You must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.
	Indicates the environmental friendly use period for China RoHS. The symbol may indicate the number of years in the use period.
	Indicates that there is an authorized representative in the European community.
	Indicates the instrument manufacturer.
	Indicates the instrument manufacture date.
	Indicates compliance with California Proposition 65, which requires businesses to warn Californians about significant exposures to chemicals that cause cancer, birth defects, or other reproductive harm.

Note: See the documentation for the external light source for details on the safety labels on the light source.

Protective Housing and Safety Interlocks

The ImageXpress HCS.ai system features a protective outer housing and interlocks, which are designed to protect you from exposure to light, hot surfaces, moving parts, and high voltage.

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

DANGER! Do not override any interlocks, 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 laser light, hot surfaces, moving parts, or high voltage.

Safety Interlock Failure

If the external light source or the focusing LED stays on when the top door of the instrument is open, it is unsafe to continue using the instrument due to a safety interlock failure. Contact Molecular Devices Technical Support immediately. See Obtaining Support on page 27 for details.

Non-Interlocked Panels

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.

Light Source Safety

The ImageXpress HCS.ai system is equipped with an external light source, which is connected to the instrument with a light guide. Depending on the model, the light source is one of the following:

- The Advanced Gen1 model uses a Lumencor laser light source.
- The Advanced Gen2 model uses a Pavilion Integration Corporation (PIC) laser light source.
- The Confocal and Widefield models use an LED light source.

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 HCS.ai Advanced system is rated a Class 1 laser product.

The external light source for the ImageXpress HCS.ai Advanced system is a Class 4 (with Gen1) or Class 3B (with Gen2) high-power laser product.

Note: The ImageXpress HCS.ai Widefield system and ImageXpress HCS.ai Confocal system both use an LED light source and do not contain any laser products. See LED Safety on page 11 for details.

Never attempt to open the instrument or access the high-power laser product. Installation and service is to be performed by a Molecular Devices Field Service Engineer only.

The Class 4 (with Gen1) or Class 3B (with Gen2) laser product is designed and tested in accordance with IEC 61010-1, UL 61010-1, CAN/CSA-C22.2 No. 61010-1, EN 61010-1, and IEC/EN 60825-1

Note:

- For Gen1, see lumencor.com/products/celesta-light-engines for complete details on the external laser light source.
- For Gen2, see pavilionintegration.com/products-solutions/lapis-integrated-engines/lapis-integrated-high-power-multi-laser-engine for complete details on the external laser light source.

Item	Description
Emitted Wavelength	405 nm - 748 nm
Maximum Output Power	Approx, 800 mW
Laser Class (Gen1)	Class 4
Laser Class (Gen2)	Class 3B

The ImageXpress HCS.ai Advanced system instrument is equipped with a redundant laser safety system. When samples are being loaded or unloaded, hardware interlocks prevent the laser modules from powering on until the instrument top door is closed.

DANGER! VISIBLE AND/OR INVISIBLE LASER RADIATION. Do not operate the external light source when housing is open and interlocks are defeated. Avoid eye or skin exposure to direct or scattered radiation.

DANGER!

- A Class 4 or Class 3B laser beam can cause materials to smolder or burn, especially at close range. Dark materials, which absorb heat, and materials such as plastic, paper, and fabric can easily be burned by visible laser beams.
- Fumes produced when laser radiation vaporizes or burns a target material—whether metallic, organic, or biologic—may be hazardous.
- To prevent damaging materials at close range in a widefield acquisition, avoid setting an exposure time of more than 10 seconds. In addition, avoid exposing any single site for longer than 10 seconds. (For example, if you are acquiring a Z Series with 10 planes, avoid setting an exposure time of over 1 second.)
- To avoid unintentional operation of the Class 4 or Class 3B laser beam, always power off the external light source when not in use.

WARNING! LASER LIGHT.

- Do not attempt to repair or adjust the Class 4 or Class 3B high-power laser product. Removing the top panel, safety interlocks, external light source, and microscope objective and then looking into the Class 4 or Class 3B laser beam can cause severe eye injury and blindness.
- Operate the instrument only when all the doors and panels of the instrument are in place and closed.

LED Safety

The ImageXpress HCS.ai Confocal system and Widefield system uses a solid-state LED light source. There are no user-replaceable parts in the LED light source. The light source uses an electronic shutter to control the exposure of the sample to excitation light, which helps to minimize sample degradation and photobleaching.

Electrical Safety

To prevent electrical-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. See Obtaining Support on page 27 for details.

To ensure sufficient ventilation and allow access to the power, light source, and gas supply connections, the instrument requires at least 25.4 cm (10 in.) of clearance on the left side.

We recommend that you power off the instrument when it will not be used for an extended period of time.

WARNING!

- **The ImageXpress HCS.ai 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—or disconnection of the protective earth ground terminal can result in personal injury.**
- **Do not block the power switches on the left side of the instrument or on the external light source.**

WARNING! HIGH VOLTAGE. Do not operate the external light source with the external light source housing open. With the ImageXpress HCS.ai Advanced system, never attempt to open or tamper with the laser light source housing due to the extreme hazard of the Class 4 or Class 3B high-power laser product.

Moving Parts Safety

The ImageXpress HCS.ai system contains moving parts that can cause injury. Under normal conditions, the system 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.
- Avoid contact with the top door of the instrument when it is opening or closing.
- Some user-controlled moving parts exist inside the instrument. When performing maintenance operations, avoid contact with parts that are in motion.

WARNING! Do not attempt to access the interior of the system unless specifically instructed to do so by Molecular Devices Technical Support. In addition, do not operate the system with any covers or panels removed. These actions can damage system components and cause injury.

Note: Observe all safety statements for any attached external device during the operation of the system. For details on the operating and safety procedures of an external device, see the documentation for that device.

Lifting Hazard

WARNING! LIFTING HAZARD. The ImageXpress HCS.ai instrument weighs approximately 109 kg (240 lb). Use great care when lifting or moving the instrument. To prevent injury, do not attempt to lift or move the instrument without assistance.

CAUTION!

- Do not slide or push the instrument. Sliding or pushing can damage the feet on the bottom of the instrument.
- Moving the instrument can damage sensitive parts and disrupt optical alignments. When transporting the instrument, use the original packaging and shipping box to properly secure the instrument. Your warranty does not cover problems caused during or as a result of shipment or relocation.

Chemical and Biological Safety

WARNING! BIOHAZARD. Normal operation of the system can involve the use of materials that are toxic, flammable, or otherwise biologically harmful.

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

When using materials with the ImageXpress HCS.ai system that are toxic, flammable, or otherwise biologically harmful, 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 in accordance with your lab's waste disposal procedures.
- Operate the system in accordance with the instructions outlined in this guide, and take all the required precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. When working with potentially hazardous liquids, take applicable safety precautions, such as wearing safety glasses and protective clothing.
- Use compressed gas supplies in a well-ventilated area. The instrument is not airtight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures defined by your safety officer to maintain a safe work environment, such as the use of an automatic warning system.
- Observe the applicable cautionary procedures defined by your safety officer when using toxic, pathological, or radioactive materials.
- Observe the applicable cautionary procedures defined by your safety officer when using flammable solvents in or near that is powered on.

WARNING! BIOHAZARD. You are responsible for decontaminating all system components before returning 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.

Before returning parts, contact Molecular Devices Technical Support if you have questions about decontamination. See Obtaining Support on page 27 for details.

Environmental Control System Safety

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

WARNING!

- **Do not operate the environmental control system with substances or under conditions that can cause a risk of explosion, implosion, or the release of gases.**
- **Use compressed gas supplies in a well-ventilated area. The instrument is not airtight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures defined by your safety officer to maintain a safe work environment, such as the use of an automatic warning system.**
- **Use only the gases described in this guide, which are CO_2, N_2, and compressed air. NEVER attempt to connect a pure O_2 tank or any other unsupported gas supply to the instrument.**

WARNING! BIOHAZARD. You are responsible for decontaminating all system components before returning 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.

Before returning parts, contact Molecular Devices Technical Support if you have questions about decontamination. See Obtaining Support on page 27 for details.

CAUTION!

- The environmental control system includes heated tubing that controls the temperature of the gas flow. Some part of the instrument can reach temperatures of up to 40°C (104°F). Avoid touching the temperature-controlled parts of the system.
- To prevent damage to the instrument, do not allow the gas pressure to exceed 2.1 bar (30 psi).
- With multiple gas supplies, use the same gas pressure for each gas supply.
- Do not use the instrument with hazardous substances.
- If you use any substances or materials that pose a risk of infection, you are responsible for applying best practices when handling these materials.
- If the CO_2 port or the N_2 port is not connected to a gas supply, use a blind plug (included) to close it off.

See Using the Environmental Control System on page 59 for details on using the environmental control system.

Water Immersion Safety

Before using the optional water immersion system, review the following information to ensure proper operation and to reduce the risk of damage to the instrument.

CAUTION!

- Do not remove or change the water immersion objectives that are installed within the instrument. A Molecular Devices Field Service Engineer must install and remove water immersion objectives.
- Do not touch the leak detection sensor, which is the brass color ring around the top of the water immersion objective below the cap.
- Do not place the water immersion controller on top of the instrument or near electronics. The water immersion controller is not watertight. Water will not be contained within the controller if water spills into the water immersion controller.
- We recommend that the base of the water immersion controller be level with the instrument.
- Do not move the water immersion controller after installation by the Molecular Devices Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. Changing the position of the water immersion controller can negatively affect the calibration.
- Do not attempt to remove the water immersion controller cover. Except for the water bottles, the controller does not contain any user-serviceable parts.
- We recommend that the base of the water immersion controller be level with the instrument.
- Do not move the water immersion controller after installation by the Molecular Devices Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. Changing the position of the water immersion controller can negatively affect the calibration.

Chapter 1: ImageXpress HCS.ai High-Content Screening System

The ImageXpress® HCS.ai High-Content Screening System is available in the following configurations :

Model	Light Source	Acquisition Mode
Advanced	Laser, 7-channels	Widefield and Confocal with 4 disk options
Confocal	LED, 5-channels	Widefield and Confocal with 2 disk options
Widefield	LED, 5-channels	Widefield

The following options are available for all models:

- **Environmental Control System**: The optional environmental control system uses a sealing ring on top of the sample plate and the instrument top door above the plate. Together, these form a small, sealed volume. Humidified gas (CO_2, N_2, and compressed air) is supplied into this small volume to form the specified environment. In addition, the system controls the temperature around the plate.
- **Water Immersion System**: The optional water immersion system dispenses small amounts of water to form a bolus the top of a water immersion objective as images are acquired. By matching the refractive index of the sample, the water immersion objective can produce images that are of higher quality than can be attained with an air objective. For example, with a water objective, a reduction in light refraction improves the geometric accuracy during acquisition. Better light collection results in images with brighter intensity at lower exposure times. Images are sharper and clearer, and spheres are more discernible.
- **Magnification Changer**: With the optional magnification changer, the instrument can be equipped with an additional tube lens with 1.5 magnification. You can easily select which tube lens is used for an acquisition. With the 1.5x tube lens selected, the magnification value of the acquired image is 1.5 times the value of the objective. For example, with a 40x objective and the 1.5x tube lens selected, the magnification value of the acquired image will be 60x.
- **Fast Camera Connection**: CoaXpress camera connection is optional. USB camera connection is standard.

System Features

Illumination System: Excitation

The ImageXpress HCS.ai system is equipped with an external light source, which is connected to the instrument with a light guide. Depending on the model, the light source is one of the following:

- The Advanced Gen1 model uses a Lumencor laser light source.
- The Advanced Gen2 model uses a Pavilion Integration Corporation (PIC) laser light source.
- The Confocal and Widefield models use an LED light source.

External Light Source

Note: The warranty on the light source will be voided if you attempt to open the light source for any reason. For service, contact Molecular Devices.

The ImageXpress HCS.ai Confocal system and Widefield system uses a solid-state LED light source. There are no user-replaceable parts in the LED light source. The external light source uses an electronic shutter to control the exposure of the sample to excitation light. This helps to minimize sample degradation and photobleaching.

With the ImageXpress HCS.ai Advanced system, the external light source is a Class 4 (with Gen1) or Class 3B (with Gen2) high-power laser product. There are no user-replaceable parts in the solid-state light source.

DANGER! See Laser Safety on page 10 for details on the Class 4 or Class 3B high-power laser product and specific warning and danger statements for working with the external laser light source.

Light Source Filter Wavelength Ranges

The following table shows the ImageXpress HCS.ai system light source excitation spectra for each filter with the laser and LED light source:

Filter	Laser Light Source (Advanced Model Gen 1 and Gen 2)	LED Light Source (Confocal and Widefield Models)
DAPI	386/42 nm	378/52 nm
FITC	486/14 nm	474/27 nm
TRITC	550/21 nm	534/36 nm
TX RED	578/21 nm	575/25 nm
CY5	638/14 nm	635/31 nm
CY7	740/29 nm	n/a
CFP	389/38 nm	389/38 nm
YFP	509/22 nm	475/35 nm

Illumination Optics

The output end of the fiber optic light guide is imaged onto the sample by a set of internal optics and the objective to provide bright, uniform illumination of the specimen over a wide field of view.

Filter Changer

The 10-position emission filter wheel uses standard, commercially available filters. Molecular Devices offers and recommends Semrock filters, based on ImageXpress HCS.ai system validation testing.

The 4-position excitation filter wheel uses a unique frame. You must order the excitation filters from Molecular Devices.

Objective (Z) Stage

Motorized Z Stage

A linear encoder that features better than 20 nm resolution monitors the Z stage position.

Objectives

The standard objectives are Nikon CFI60 series. The objective lens focuses excitation light onto the sample and collects light that the sample emits. See Compatible Objectives on page 78.

CAUTION! You must contact Molecular Devices Technical Support to schedule a Field Service Engineer to install or remove an objective.

Motorized Objective Changer

The instrument includes a 6-position objective changer. Only the selected objective moves up and down when you change the position.

Sample (X-Y) Stage

Sample

The plate holder is designed to scan 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, you can use the glass slide adapter (included in the accessory kit) to image glass slides. 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

A linear encoder that features better than 20 nm resolution monitors the X-Y stage position.

LED Autofocus

The ImageXpress HCS.ai system uses an LED to autofocus the image. The red (690 nm) diode LED projects spot onto the sample. Reflections of this spot from the bottom of the plate and the plate-sample interface are imaged by a dedicated, fast-focus sensor. The spot acts as a reference for autofocus.

Imaging System: Emission

Tube Lens

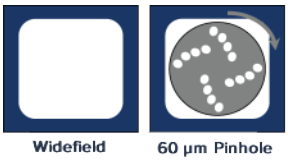
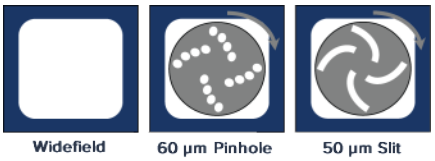
The tube lens collects collimated light from the objective and focuses it onto the detector plane of the camera. The emission wavelength range is 400 nm to 750 nm.

Camera

The ImageXpress HCS.ai system uses a scientific CMOS camera with a 2304 × 2304 image sensor format (6.5 × 6.5 µm pixel size).

Confocal Disks

Three confocal disk box configurations are available for the ImageXpress HCS.ai Advanced and ImageXpress HCS.ai Confocal system. For the confocal performance details with different objectives, see Compatible Objectives on page 78.

Disk	Part Number	Use
	5314337	Confocal Single Disk Unit, including: • 60 µm pinhole disk for basic confocal imaging • Widefield for non-confocal imaging
	5302336	Confocal Screening Option Dual Disk Unit, including: • 60 µm pinhole disk for basic confocal imaging • 50 µm slit disk for high-throughput confocal imaging • Widefield for non-confocal imaging
	5306812	Confocal Deep Tissue Disk Option Dual Disk Unit, including: • 50/250 µm pinhole disk for high-sensitivity in deep tissues • 50/500 µm pinhole disk for high-resolution in deep tissues • Widefield for non-confocal imaging

Electronics

Depending on the model and optional features, the ImageXpress HCS.ai system can include the following components:

- ImageXpress HCS.ai instrument
- Workstation computer, with monitor and cables
- Solid-state laser or LED light source, with fiber-optic light guide and cables
- Water immersion controller with serial cable to instrument and USB 2.0 cable to workstation computer.
- USB 3.0 cable for workstation computer to instrument (camera control) and instrument to workstation computer (data)
- CoaXpress or USB 3.0 cable for internal camera to workstation computer

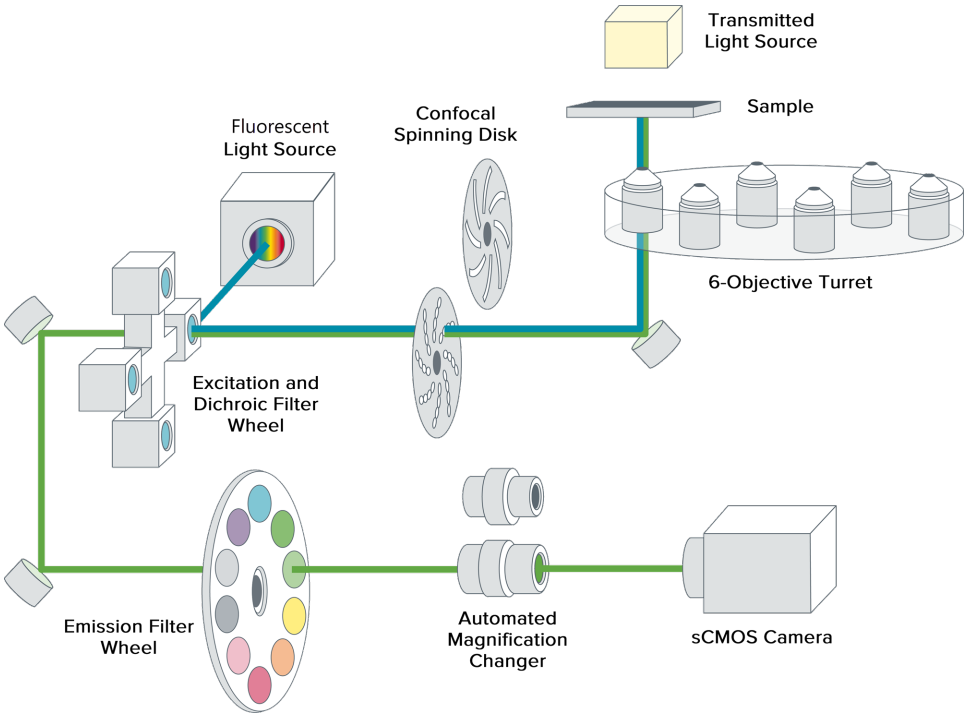
Software Features

The ImageXpress HCS.ai system includes the following software products from Molecular Devices:

- The MetaXpress® Acquire Image Acquisition Software is your interface to the instrument. Use it to configure the settings to design a custom protocol for your acquisition.
- The IN Carta® Image Analysis Software provides powerful analytics for advanced phenotypic classification and 3D image analysis. It delivers robust, quantitative results from complex biological images and datasets using advanced AI technology.

Both software products run on the workstation computer that is part of the ImageXpress HCS.ai system.

Theory of Operation



The ImageXpress HCS.ai system uses the following components and functions:

- Fluorescence Imaging, see page 23
- Confocal Imaging, see page 23
- Excitation and Emission Filters, see page 24
- Dichroic Mirror, see page 25
- Objective Lenses, see page 26

Fluorescence Imaging

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

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

Confocal Imaging

You can use the ImageXpress HCS.ai Advanced system and the ImageXpress HCS.ai Confocal system to perform confocal imaging.

In standard widefield microscopy, fluorescent objects above or below the focal plane are seen as out-of-focus objects and increase the background fluorescence. Confocal microscopy uses point-source illumination and a pinhole aperture ahead of the detector to reject out-of-plane signals.

The main advantage of confocal imaging is the rejection of photons from out-of-focus portions of the sample and the resulting improved resolution and reduced background. By changing the size and spacing of the pinholes in front of the detector you can achieve a suitable balance between the degree of confocality and the sensitivity and speed of the instrument. A smaller pinhole gives better axial resolution, while a larger pinhole increases the collection efficiency resulting in higher sensitivity.

Excitation and Emission Filters

In the ImageXpress HCS.ai system, excitation filters are located within the dichroic mirror inside the filter cubes on a controllable wheel. Emission filters are located on a separate controllable wheel.

To selectively excite one fluorophore more intensely than another or to minimize excitation channel crosstalk, it is necessary to provide illumination that contains only photons with a wavelength range that matches the excitation spectrum of the target fluorophore. A bandpass filter in the illumination optical path (called the excitation filter, since it filters the excitation light) restricts the illumination spectrum to a narrow range of wavelengths. Bandpass filters can be one of the following types:

- Single-bandpass filter: Allows one wavelength range to transmit and are optimized for a single fluorophore.
- Multi-bandpass filter: Allows several wavelength ranges to transmit allowing a single filter to be used with multiple fluorophores.

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 be one of the following types:

- Multi-bandpass filters: Maximizes speed and flexibility for use with multiple fluorophores
- Single-bandpass filter: Maximizes specificity
- Longpass filter: Maximizes the amount of emission light collected

Dichroic Mirror

In the ImageXpress HCS.ai system, framed dichroic mirrors are in a four-position filter cube wheel.

A dichroic mirror is a specially designed beam splitter that transmits light within a certain wavelength range and reflects light within a different wavelength range. 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.

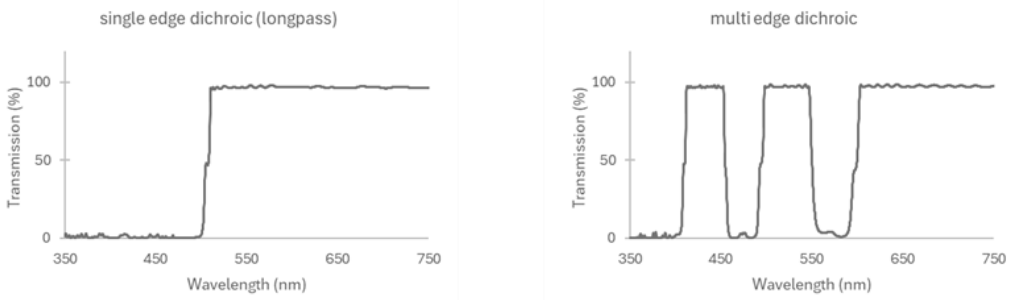
In the illumination path, the dichroic mirror reflects specific wavelengths from the light source up through the objective onto the specimen.

In the imaging optical path, some 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 within the range (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 you handle 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 single-pass mirror) at shorter wavelengths. It is also possible to have both single-edge dichroic mirrors, which have a single transition between reflectance and transmission, and multi-edge dichroic mirrors, which have two or more transitions from high reflectance to high transmission in a single mirror. In practice, the characteristic transmission spectrum for single- and multi-edge dichroic mirrors resembles the following graphs.



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 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 HCS.ai system can be configured with up to six, high-quality Nikon objectives. Two of the six objectives can be water-immersion objectives. See Compatible Objectives on page 78 for details.

Some objectives (for example, the 40x S Plan Fluor ELWD) have correction collars for adjusting to the thickness of the glass cover slip or plate bottom. Adjust the correction collars using the physical thickness of the plate bottom or through optimization of image quality. See Adjusting an Objective Correction Collar on page 41 for details.

Objectives are classified according to optical correction, flatness of field, numerical aperture, and working distance. When choosing objectives to use with the system, it is important to consider the types of plates and type of assay you plan to image. The plate material (plastic or glass) and thickness are major considerations when you choose an objective. Another important note is that generally the more optically corrected an objective, the greater the number of lens elements it contains, with correspondingly reduced light transmission, especially in the UV spectrum. The levels of correction in ascending order are as follows:

- Achromatic
- Fluor
- Plan Fluor
- Plan Apo

CAUTION! You must contact Molecular Devices Technical Support to schedule a Field Service Engineer to install or remove an objective.

For detailed information on objectives, see the Nikon web site at www.nikon.com.

Obtaining Support

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

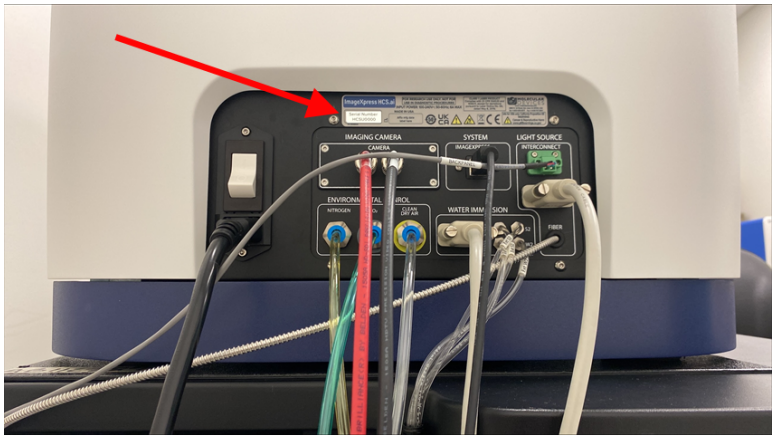
Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety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

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

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

To expedite support, be prepared to provide the instrument serial number and the software activation code. In the MetaXpress Acquire software, go to the Home page and click **About**. The **About** dialog displays the activation code and the instrument serial number. The serial number can also be seen above the ports on the left side of the instrument.



Documentation

Review the product documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com. In addition, online Help is available within the MetaXpress Acquire software. Press **F1** to access Help for the current page.

Training

Molecular Devices provides training on the general operation of the ImageXpress HCS.ai system at the time of installation. Contact Molecular Devices Technical Support for details on training after installation.

Additional Resources

Web-based microscopy courses:

- www.microscopyu.com
- www.ibiology.org/online-biology-courses/microscopy-series/

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

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

Filter information:

- www.semrock.com
- www.chroma.com
- www.omegafilters.com

About This Guide

This guide is intended for the scientist using the ImageXpress HCS.ai system. It describes the system, how to use it, and how to maintain it.

Use this guide along with the *MetaXpress Acquire Help.*

The information in this guide is valid for MetaXpress Acquire software version 2025.1.1 and is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at support.moleculardevices.com for the most up-to-date information.

Product Documentation

The following guides are available on the Molecular Devices Knowledge Base at support.moleculardevices.com:

- *ImageXpress HCS.ai Product Safety Guide*
- *ImageXpress HCS.ai Pre-Installation Guide*
- *ImageXpress HCS.ai System User Guide*
- *ImageXpress HCS.ai Software Update Guide*

A hard copy of the *ImageXpress HCS.ai Product Safety Guide* is also included in the package with a new system.

In addition, the MetaXpress Acquire software and the IN Carta software both include context-sensitive Help that you can access from within the software. Just press the **F1** key or click the **Help** icon to view Help for the current page.

WARNING! We recommend that you review the documentation before using the ImageXpress HCS.ai system.

Chapter 2: ImageXpress HCS.ai High-Content Screening System Overview

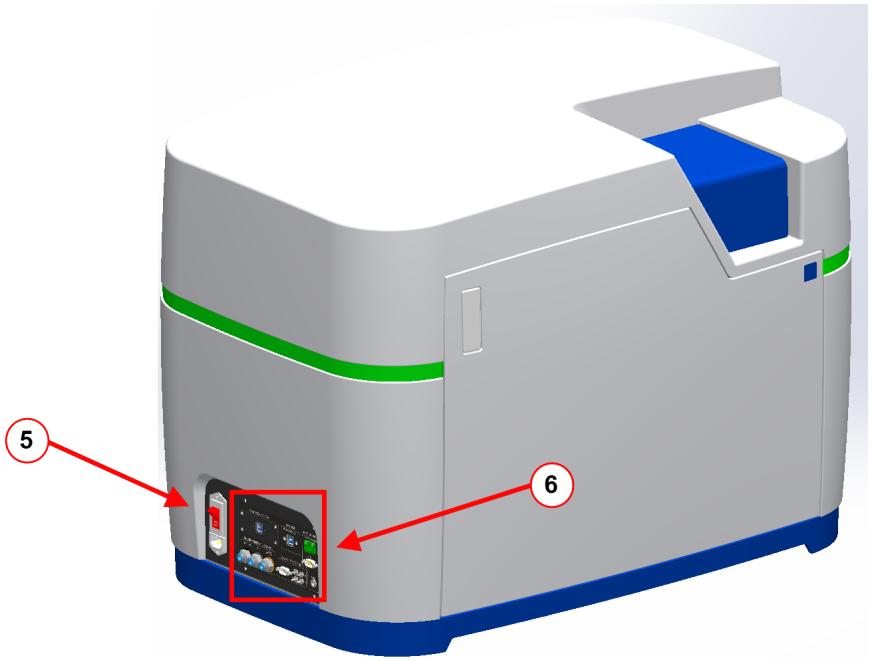
2

The section includes the following topics:

Front View

Item	Description	For Details
1	Front Door Opener	Filling the Environmental Control Reservoir on page 71
2	System Status Light	Understanding the System Status Lights on page 39
3	Top Door	Loading a Plate on page 40 Adjusting an Objective Correction Collar on page 41
4	Top Door Button	

Left Side View



Item	Description	For Details
5	Power Switch	Powering On the Instrument on page 37
6	Connection Ports	Either CoaXpress or USB. See Connection Ports on page 31 for details.

Connection Ports

Connection Ports with CoaXpress connector (inset shows available USB connector)

The following ports are available:

- **Imaging Camera**: Camera connection from workstation computer. Shown above with CoaXpress connector; inset shows available USB connector.
- **System**: Camera control connection from workstation computer.
- **Light Source**: **Fiber** is light guide connection to the laser light source (Advanced model) or LED light source (Confocal and Widefield models). On the Advanced model only, **TTL** is a serial communication connection to the laser light source and **Interlock** is the interlock connector to the light source. On the Confocal and Widefield models, **TTL** and **Interlock** are unused.
- **Environmental Control**: Gas connections to the N_2, CO_2, and compressed air supplies. Any port not connected to a gas supply must be closed off the port with one of the included blind plugs.
- **Water Immersion**: Serial communication connection to the water immersion controller. **S1**, **S2**, **W1**, **W2** are connections to the source and waste bottles in the water immersion control.

Chapter 3: Using the ImageXpress HCS.ai High-Content Screening System

The section includes the following topics:

Starting the System

To start the ImageXpress HCS.ai system, power on the components in the order described in this section:

Tip:

- After starting the ImageXpress HCS.ai system, the system is ready to acquire images. For best results, however, which may be required for high-precision imaging, we recommend waiting up to an hour before acquiring images.
- For best results, shut down the MetaXpress Acquire software when you are not using it.

Powering On the Workstation Computer

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

The default Windows username is `moldev`. The first time you start the workstation computer, you are prompted to establish a password.

CAUTION! We recommend that you make a note of the new password. Molecular Devices Technical Support will not be able to help you recover a forgotten password.

After you log in to Windows, you can set up accounts for additional Windows users as needed.

Powering On the Light Source

After powering on the workstation computer, power on the light source.

- The Advanced Gen1 model uses a Lumencor laser light source. See Powering On the Lumencor Laser Light Source on page 34 for details.
- The Advanced Gen2 model uses a Pavilion Integration Corporation (PIC) laser light source. See Powering On the PIC Laser Light Source on page 35 for details.
- The Confocal model and Widefield model use an LED light source. See Powering On the LED Light Source on page 36 for details.

Powering On the Lumencor Laser Light Source

The Advanced Gen1 model uses a Lumencor laser light source.

To power on the Lumencor laser light source for the first time:

Note: The Molecular Devices Field Service Engineer will typically power on the light source for the first time during installation.

1. Confirm that one end of the light guide is inserted and secured with the set screw.
2. Confirm that the other end of the light guide is connected to the instrument.
3. Confirm that the external gate jumper is inserted in the **External Gate** port.
4. Confirm that the interlock jumper is inserted in the **Remote Interlock** port (on the rear of the light source).
5. Insert the control key in the **Key Control** slot.
6. Turn the control key to the **ON** position.
7. Connect the isolated DC power supply to the light source.
8. Connect the AC power cord to the DC power supply.
9. When the DC power supply is energized, the power button (top right) lights.

Note: You do not need to press the power button. When the power button is lit, the laser light source is powered on.

10. Wait 30 to 45 seconds for the initiation sequence to complete.

Note: Do not press any buttons or connect anything to the light source during the initiation sequence.

When the initiation sequence completes, the status display flashes **LUMENCOR** and then shows the current IP address, internal temperature, and fan status for the light source. The light source fan runs at HI for about 2 seconds and then shuts off. At this point, the light source is ready for use.

To power on the Lumencor light source after the first time:

1. Press the power button (top right).
2. Wait 30 to 45 seconds for the initiation sequence to complete.

Note: Do not press any buttons or insert any plugs during the initiation sequence.

When the initiation sequence completes, the status display flashes **LUMENCOR** and then shows the current IP address, internal temperature, and fan status for the light source. The light source fan runs at HI for about 2 seconds and then shuts off. At this point, the light source is ready for use.

Powering On the PIC Laser Light Source

The Advanced Gen2 model uses a Pavilion Integration Corporation (PIC) laser light source.

Front View

Rear View

To power on the PIC laser light source for the first time:

Note: The Molecular Devices Field Service Engineer will typically power on the light source for the first time during installation.

1. Confirm that one end of the light guide is inserted and secured with the set screw.
2. Confirm that the other end of the light guide is connected to the instrument.
3. Confirm that the interlock jumper is inserted in the **Interlock** port (on the rear).
4. Insert the control key in the **Laser** slot.
5. Turn the control key clockwise to the **ON** position.
6. Connect the isolated DC power supply to the light source.
7. Connect the AC power cord to the DC power supply.
8. Set the power switch on the AC to DC converter to the **ON** position (I).

   Note: When the AC to DC converter is powered on, its internal fan starts.

9. Press **Power On/Off**.

   Note: The **Power On/Off** button turns green and the fan starts.

10. Wait 1 minute for the initiation sequence to complete.

    Note: Do not press any buttons or connect anything to the light source during the initiation sequence.

When the initiation sequence completes, the **READY** light turns green. At this point, the light source is ready for use.

To power on the PIC light source after the first time:

1. Press **Power On/Off**.

   Note: The **Power On/Off** button turns green and the fans start.

2. Wait 1 minute for the initiation sequence to complete.

   Note: Do not press any buttons or connect anything to the light source during the initiation sequence.

When the initiation sequence completes, the **READY** light turns green. At this point, the light source is ready for use.

Powering On the LED Light Source

Front View

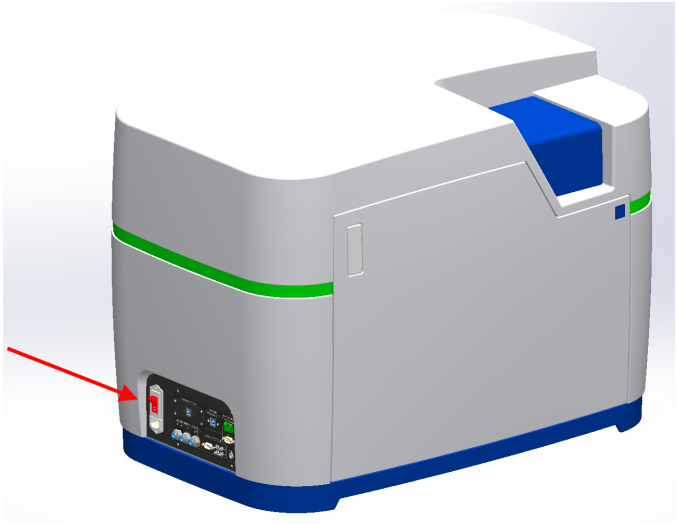
Rear View

To power on the LED light source, switch the Power button to the **On** position.

1. On the front of the light source, confirm that one end of the light guide is inserted and secured.
2. Confirm that the other end of the light guide is connected to the instrument.
3. On the rear of the light source, confirm that the power cord is connected to the **Power** port.
4. Turn the **Power** switch to the **ON** position.

Powering On the Instrument

After powering on the light source, power on the instrument. On the left side of the instrument, set the power switch to **On**.



When the status light is yellow—indicating that the instrument is powered on, but software is not connected—continue to the next section to start the software. See Starting the MetaXpress Acquire Software on page 38 for details.

Starting the MetaXpress Acquire Software

After powering on the instrument, start the MetaXpress Acquire software.

To start to the MetaXpress Acquire software:

1. On the workstation computer, do one of the following to start the software:
   - On the desktop, double-click **MetaXpress Acquire Launcher**.
   - Click **Start** > **MetaXpress Acquire Launcher**.
2. If you are prompted to accept the software license terms, do the following:
   a. Review the software license terms.
   b. To prevent the software license terms from appearing again, select the **Do not show this license agreement in the future** checkbox.
   c. If you accept the software license terms, click **Accept**.

Note: If you do not accept the license terms, you cannot use the software.

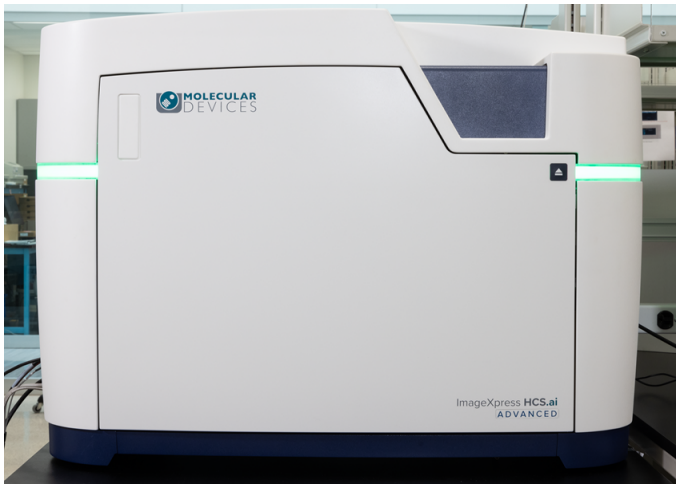
The MetaXpress Acquire software opens, and the **Home** page displays.

Tip:

- After starting the ImageXpress HCS.ai system, the system is ready to acquire images. For best results, however, which may be required for high-precision imaging, we recommend waiting up to an hour before acquiring images.
- For best results, shut down the MetaXpress Acquire software when you are not using it.

Understanding the System Status Lights

The light band around of the instrument provides information about the instrument status.



Status	Summary	Description
Blue	Ready	Instrument is powered on, and software is connected.
Green	In Use	One of the following: • Instrument is acquiring or saving images. • Instrument is waiting to acquire the next time point.
Yellow	Not Ready	Instrument is not ready due to one of the following: • Instrument is powered on, but software is not connected. • Interlock activated (top door, front door, or rear door is open).
Yellow (flashing)	Wait	Instrument is initializing after powering on and cannot open the top door. The top door will open after initialization is complete.
Red	Error	Instrument is in an error state due to one of the following issues and requires attention. • Communication error. • Water Immersion error (source bottle empty, waste bottle full, invalid pressure). In the software, use the status buttons at the bottom left of the screen to troubleshoot.
Red (flashing)	Error	Instrument is in an error state due to a water leak and requires immediate attention. In the software, click **Water Immersion** at the bottom left of the screen for troubleshooting details.

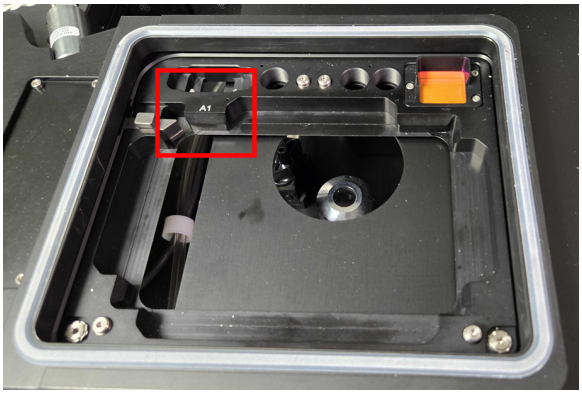
Note: If the status light remains red after troubleshooting, contact Molecular Devices Technical Support. See Obtaining Support on page 27 for details.

Loading a Plate

To load a plate in the sample stage:

1. Press the button on the front of the instrument to open the top door.

2. Note that the sample stage has an **A1** label to indicate its top left corner.



3. With the **A1** well at the top left, place the plate in the sample stage.

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

Note: Do not twist or rotate the plate in the sample stage.

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

Acquiring Images

See the *MetaXpress Acquire Help* in the software for details on creating and running an acquisition protocol.

Adjusting an Objective Correction Collar

The ELWD (extra-long working distance) air objectives and the water immersion objectives have an adjustable correction collar that minimizes spherical aberration in the image of the specimen. The collar adjustment range varies depending on the objective. Change the range setting to adjust the distances between components inside the objective barrel. Image quality and resolution are very much dependent on properly setting the objective collar.

The setting depends on the thickness of the plate containing the specimen. In general, set the correction collar for the physical thickness of the plate that you use for the acquisition.

You can determine the physical thickness in one of the following ways:

- Get the plate specifications from the plate manufacturer.
- Break a spare plate and use calipers to measure the thickness.
- Using the **Measure** function in the **Plate Specifications** dialog in the MetaXpress Acquire software.

After you determine the thickness of the plate, you can adjust the correction collar.

CAUTION! If the thickness of the plate is out of the range of the correction collar for an objective, you will likely not be able to achieve good focus with that objective.

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

To adjust an objective correction collar:

1. In the MetaXpress Acquire software, on the Home page, click **Acquire**.
2. On the left side of the screen, click **Configure**.
3. On the **Configure** page, click the **Objective** drop-down, and select the objective you want to adjust.
4. Click **Access Objective**.
   The top door opens, and the objective turret moves the objective to the front.
5. Rotate the correction collar to its new setting.
6. In the **Access Objective** dialog, click **OK**.
7. Test the correction collar setting by examining the image quality of acquired images.
   If the quality has degraded, repeat these steps to continue adjusting the correction collar.

Chapter 4: Preparing for Acquisition

This section provides general guidelines to consider before you acquire plate data. These guidelines help ensure that the images you acquire are the best possible quality.

Consider the following criteria to obtain the best possible fluorescence image quality:

Assay Design

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

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

Selection of Different Fluorochromes

Typical high-content and high-throughput assays include one or more fluorochromes, such as fluorescent proteins, antibody-based stains, and chemical-based stains. You should include a nuclear stain (such as 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.

Individual fluorochromes have unique characteristics that help determine their best use. Use probes that provide bright, specific staining and have excitation and emission spectra suitable for the filter sets in the instrument. For experiments that use multiple stains, select fluorophores that have enough spectral separation. See Filter Specifications on page 81 for details on the wavelength ranges for each filter. Some fluorochromes provide brighter intensities and require a shorter exposure time, while others do not bleach as quickly and allow a longer exposure time. There also might be toxicity issues with some cell types or bleed-through issues between pairs of fluorochromes. Consider these factors when you choose a fluorochrome.

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

Cell-Based Assays

The most important consideration when you select cells for a high-content assay is whether they are compatible with the biology being studied. The assay should give a robust response with clear distinction between positive and negative phenotypes. It is important to select a source of cells where it is possible to obtain consistent results from batch to batch, whether they are primary cells or cell lines, and whether they are transfected or not. Compared to widefield imaging, confocal imaging provides higher-quality images of thick samples, 3D cell masses, or tissues. Uniformly staining thick samples for confocal imaging can be difficult.

Organism-Based Assays

You can use the ImageXpress HCS.ai system to image whole organisms, such as nematodes or zebrafish. Treat the organisms with anesthetic, use a gel preparation, or both to immobilize the organisms for best results.

Homogeneous Assays

Homogeneous assays are ones where the unbound or unreacted stains are not washed off before acquiring images. Confocal imaging reduces background fluorescence from out of focus sample volume which improves signal-to-background ratios and allows for homogeneous assays that would not work when widefield imaging.

Plates

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

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

Plate Selection

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

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

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

Plate Format

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

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

Plate Material

WARNING! The ImageXpress HCS.ai Advanced instrument uses a Class 4 (with Gen1) or Class 3B (with Gen2) high-power laser light source, which can cause materials to smolder or burn, especially at close range. Dark materials, which absorb heat, and materials such as plastic, paper, and fabric can easily be burned by visible laser beams. See Laser Safety on page 10 for details on the Class 4 or Class 3B high-power laser product and specific warning and danger statements for working with the external laser light source.

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

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

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

Fluorescence Background

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

Bottom Thickness

Compare the thickness of the plate bottom with the working distance of the objective lens to ensure that it is compatible. In general, objectives with higher numerical aperture (NA) tend to require thin-bottomed plates. Objectives with application-optimized correction collars 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 all supported objectives.

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

Plate Flatness or Reproducibility of the Z-Pattern

A flat plate is faster to scan than an uneven plate. 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.

Plate Skirt

With short working distance objectives, imaging the edge and corner wells can cause the objective to bump into the plate skirt, which requires you to omit the outer wells from the experiment. Some manufacturers offer low-skirt or no-skirt options that allow you to use all the wells in the plate with a short working distance air objective.

Batch-to-Batch Consistency

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

Robot Compatibility

If you use a plate-loading robot, ensure that the plates can easily be held by the robot grippers. Some types of plates do not work well with the grippers supplied with the robot and require custom grippers to work correctly. If one or more plate types do not work with your robot grippers, contact Molecular Devices for assistance. Also make sure that there is a consistent location to affix a barcode on the side of the plate.

Sample Preparation

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

This section describes considerations for imaging assays.

Cell Density

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

Fixation and Staining Conditions

Fixation, permeabilization, and wash 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 you optimize the assay, it is helpful to test a range of antibody and stain concentrations to determine the best conditions for the 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. This is most important for widefield assays. Several manufacturers offer media that is optimized for imaging.

You should not use solutions with a high percentage of glycerol, such as mounting media. Glycerol can interfere with the LED autofocus, and the high viscosity can cause pipetting difficulties which results in air bubbles.

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

Plate Handling and Storage

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

Store plates in the dark. Store fixed plates 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 chilled plates to return to room temperature.

Acquisition Settings

Set up your acquisition in the MetaXpress Acquire software. The settings are dependent on the content and distribution of the samples, as well as the requirements of the experiment.

Available settings include the following:

Magnification

The magnification setting depends on the type of information you want to obtain. Generally, higher magnification gives higher resolution of the objects of interest, but it produces a smaller field of view, which requires more sites to give the equivalent number of cells.

- For whole organism imaging or cell counting, a 4x objective is often suitable.
- For spheroid image acquisition and morphology analysis, a 10x Plan Apo objective is often used.
- For a nuclear translocation assay, a 20x or 40x objective can be appropriate.
- For counting or localizing subcellular organelles, an objective of at least 40x or a water-immersion objective might be required.

Other considerations include the numerical aperture (NA) of the objective, the working distance, and the optical corrections built into the objective. With the magnification constant, brightness is proportional to the square of NA. Higher NA objectives also produce a sharper picture when you match the plate-bottom thickness to the objective correction thickness, typically 170 µm. Unfortunately, some higher magnification objectives with high NA values may fail to reach the outer rows and columns of some multi-well plates because of the plate skirt height. Use plates with low skirt heights to maximize well access.

Automated Tube Lens

The ImageXpress HCS.ai instrument includes a tube lens with 1x magnification, so the magnification value of the acquired image is equal to the value of the objective.

With the optional magnification changer, the instrument can be equipped with an additional tube lens with 1.5 magnification. You can easily select which tube lens is used for an acquisition. With the 1.5x tube lens selected, the magnification value of the acquired image is 1.5 times the value of the objective. For example, with a 40x objective and the 1.5x tube lens selected, the magnification value of the acquired image will be 60x.

Correction Collars

If you use an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you use.

Binning

One method to increase the signal is to bin the pixels from the camera. Binning combines the electrons from adjacent pixels to create the effect of a single, larger pixel. Binning increases the signal at the expense of decreased resolution. Use binning to decrease the exposure time dramatically while maintaining the same signal intensity. Another positive feature of binning is that it produces smaller images that require less storage space and are analyzed more quickly. Some assays benefit greatly from binning, while others require unbinned images.

Widefield Imaging vs. Confocal Imaging

Widefield imaging includes fluorescence from parts of the sample that are above or below the focal plane. Confocal imaging excludes fluorescence from parts of the sample that are out of focus. The depth of focus depends on the optics, particularly the objective lens, and the degree of confocality depends on the size and shape of the hole that is used. Small pinholes achieve higher confocality and better sectioning capability than widefield, at the expense of reduced light intensities.

Therefore, if it is important to measure the total intensity of a particular fluorophore in cells, it might be preferable to use widefield imaging. Widefield imaging also tends to perform better with cells that are flat. If it is important to measure colocalization between two markers, particularly in a thick sample, then confocal imaging is recommended. In some cases, collecting a Z-series of multiple focal planes might also be needed to obtain suitable images of thick samples.

Site Selection

When you acquire a single site, the MetaXpress Acquire software acquires one image at the center of the well. To increase the coverage of the well, you can acquire multiple sites. With multiple sites, you acquire multiple images, one for each site. Multiple sites can be contiguous areas or distributed throughout the well.

Use stitching to assemble the smaller separate images into a single large image. Use this to retain image resolution while increasing the image area of coverage. The sites you select are used during the entire experiment.

Sites can be used to include specific areas of the wells in the plate, while at the same time excluding other areas of the well. For example, the center of the well might exhibit a pipetting artifact from an automated pipettor, or the cells might clump more at the edges of the wells.

The optional QuickID™ targeted acquisition enables you to create protocols that perform an initial acquisition and analysis to identify objects of interest. These objects can then be selectively imaged in a follow-up acquisition, increasing acquisition speed and efficiency. For example, you could use targeted acquisition to identify the location of organoids in a multi-well plate using widefield mode at low magnification (perhaps 4x) and then image these identified organoids using confocal mode at high magnification with Z-stack acquisition (perhaps 20x).

For details on QuickID targeted acquisition, including links to application notes, see www.moleculardevices.com/technology/quickid-targeted-image-acquisition.

Shading Correction

All microscopes exhibit some degree of illumination variation, or shading, across the field of view. Shading is an artifact that can come from the objective, optics, light source, or background light from the room. The ImageXpress HCS.ai system is designed to minimize shading effects and provides a shading correction feature within the software. This is recommended for assays where comparison of individual cell intensities is critical, such as a cell cycle assay.

Autofocus

The ImageXpress HCS.ai system includes an advanced LED autofocus system. LED autofocus is fast, reliable, does not photo bleach the sample, and is not dependent on the quality of the staining. Most experiments require only LED autofocus with a defined Z-offset for each wavelength. Some assays, such as those that use whole organisms or suspension cells, might require some image-based autofocus in addition to the LED autofocus. You define LED autofocus settings for each plate type and objective. The Measure Plate function helps optimize these settings. If you see frequent focus failures, contact your system administrator or Molecular Devices representative.

Illumination Settings

You select the illumination setting for your assay. The Semrock website (www.semrock.com) is a good resource to help you determine the best filters to use with your fluorophores.

The ImageXpress HCS.ai system uses an excitation filter wheel and separate filter wheel to select the corresponding emission wavelength filtration for the experiment. It uses dichroic mirrors to separate the excitation wavelength from the emission wavelength. Specific filter sets are designed for use with specific stains.

Exposure Time

An appropriate exposure time for each wavelength is crucial for acquisition and analysis. After focusing on the sample, click **Auto Expose** on the **Channels** page to provide a good starting point and then adjust the exposure time so that the grayscale intensity within a cell is at least three times the intensity of the background. If the background comes from out-of-plane fluorescence, consider using confocal imaging to improve the image quality. If you expect the intensity to vary with the phenotype, adjust the exposure time with both positive and negative controls so that the bright samples are not saturating and the dim samples are still visible.

Signal-to-Noise (S/N) is the ratio of the signal from the foreground objects over the noise from the background variation, signal variation, and other forms of noise from the system. S/N is an important leading indicator of how difficult it will be for the software to accurately discriminate important features in an image from the background. If the S/N is large (for example, greater than 10), then there is high confidence that the software can distinguish foreground pixels from background pixels in the image. If the S/N is relatively small (for example, less than 2), then it is more difficult for the software to distinguish between objects in the foreground and background for accurate count, morphology, area, signal intensity, and other measurements.

To increase S/N, consider using one of the following methods:

- Increase the image exposure. Longer exposures provide higher signal and higher background in an image, so changing the exposure alone might not increase S/N as expected and should be carefully measured before using long exposures (>1 second). Longer exposures can cause photo bleaching damage and saturate the camera. Intensity measurements of an overexposed image are not accurate, and you should avoid generating these images. One exception to this rule is when you are interested in extremely faint features of the sample that are otherwise not visible. A good example of this is overexposure of a neurite image where you are not interested in the bright cell body, but you are interested in the weakly stained outgrowths. Longer exposure times also increase the overall plate acquisition time.
- Decrease background variation by lowering background levels from the sample, system, or other sources. This could be achieved by changing to confocal imaging with longer exposures, lowering the sample background, or changing the consumable type.

Z Series Acquisition

The nature of your sample material can be very dense or thin. Dense sample material requires more light and might require collection of multiple focal planes in a Z Series. Depending on the analysis needs, you can save individual planes or collapse planes into a single 2D projection image.

In Transmitted Light, collecting z planes of cells growing throughout a 3D gel matrix cannot give an accurate image of all planes because the transmitted light must pass completely through the sample from top to bottom so that objects high in the sample will block the light to planes below it.

Instrument Maintenance

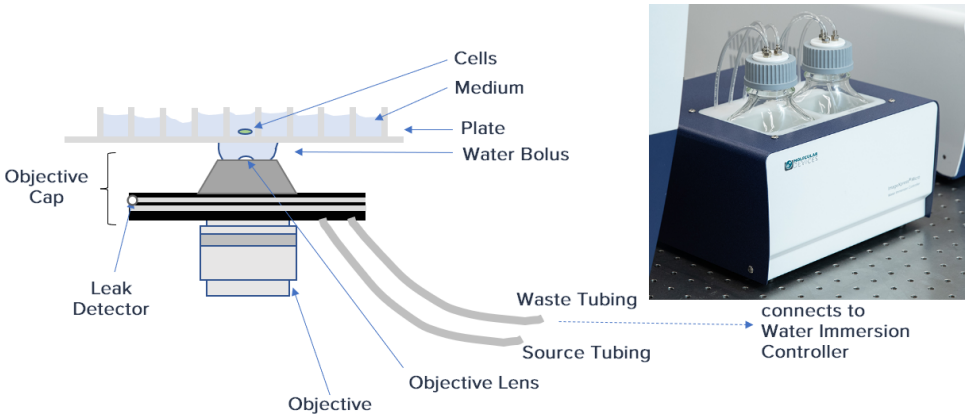
For best performance, your ImageXpress HCS.ai system should have regular preventive maintenance services. In between preventative maintenance services, your system administrator can clean dust off the optics as needed. Wear gloves when you handle any optical components to avoid contaminating them with dirt or skin oils. See Maintaining the System on page 66.

The basic design of the ImageXpress HCS.ai system light source and light path within the instrument help ensure that the light that reaches the 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: Using the Water Immersion System

5

The optional water immersion system dispenses small amounts of water to form a bolus the top of a water immersion objective as images are acquired. By matching the refractive index of the sample, the water immersion objective can produce images that are of higher quality than can be attained with an air objective. For example, with a water objective, a reduction in light refraction improves the geometric accuracy during acquisition. Better light collection results in images with brighter intensity at lower exposure times. Images are sharper and clearer, and spheres are more discernible.



This section describes the following:

Water Immersion Safety

Before using the optional water immersion system, review the following information to ensure proper operation and to reduce the risk of damage to the instrument.

CAUTION!

- Do not remove or change the water immersion objectives that are installed within the instrument. A Molecular Devices Field Service Engineer must install and remove water immersion objectives.
- Do not touch the leak detection sensor, which is the brass color ring around the top of the water immersion objective below the cap.
- Do not place the water immersion controller on top of the instrument or near electronics. The water immersion controller is not watertight. Water will not be contained within the controller if water spills into the water immersion controller.
- We recommend that the base of the water immersion controller be level with the instrument.
- Do not move the water immersion controller after installation by the Molecular Devices Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. Changing the position of the water immersion controller can negatively affect the calibration.
- Do not attempt to remove the water immersion controller cover. Except for the water bottles, the controller does not contain any user-serviceable parts.
- We recommend that the base of the water immersion controller be level with the instrument.
- Do not move the water immersion controller after installation by the Molecular Devices Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. Changing the position of the water immersion controller can negatively affect the calibration.

Water Immersion Hardware Components

The water immersion system consists of the following hardware components:

- Water Immersion Objectives, see below
- Water Immersion Controller, see page 55

Water Immersion Objectives

The water immersion system can include up to two of the water immersion objectives listed in the following table.

Water Immersion Objective	Numerical Aperture (NA)	Working Distance	Correction Collar Range
20x Water Apo Lambda S XC WI	0.95	0.95 mm	0.11 mm to 0.23 mm
40x Water Apo Lambda S XC WI	1.15	0.59 mm	0.15 mm to 0.19 mm
60x Water Plan Apo VC XC WI	1.2	0.28 mm	0.15 mm to 0.18 mm

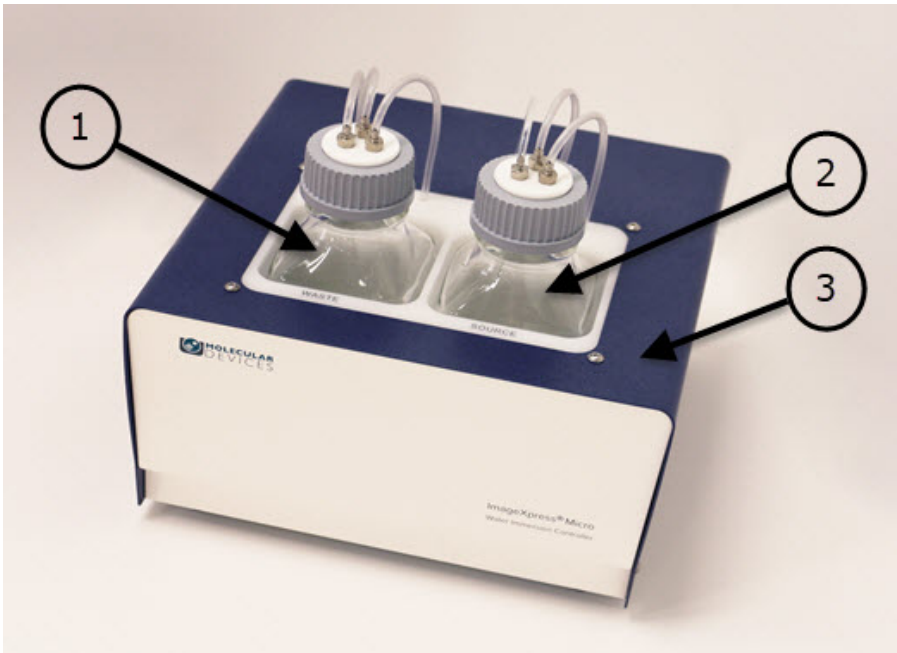
CAUTION! Do not remove or change the water immersion objectives that are installed in the instrument. A Molecular Devices Field Service Engineer must install and remove water immersion objectives.

Water immersion objectives have correction collars that are similar to the correction collars on air objectives. The objectives also have a cap that allows the creation of a bolus (bubble of water) above the objective and that directs water to a drain. The drain maintains a vacuum to draw water from the objective to a waste water bottle.

CAUTION! Do not touch the leak detection sensor, which is the brass color ring around the top of the objective below the cap.

Water Immersion Controller

The water immersion controller consists of three main components.



Item	Description
1	Waste water bottle, which contains up to 500 mL (16.9 oz) of used water drained from the objective. The system warns you when the water level in the waste bottle reaches 400 mL (13.5 oz) and may halt an acquisition in progress, if needed.
2	Source water bottle, which contains up to 500 mL (16.9 oz) of distilled water to be dispensed to the objective during image acquisition. The system warns you when the water level in the source bottle drops to 100 mL (3.4 oz) and may halt an acquisition in progress, if needed.
3	Housing, which contains the pump, valves, and sensors, including: • Diaphragm pump to deliver water and vacuums recovered water • Pressure, vacuum, and level sensors to monitor system status • Four valves to control flow states • Relief valve to maintain constant pressure

CAUTION!

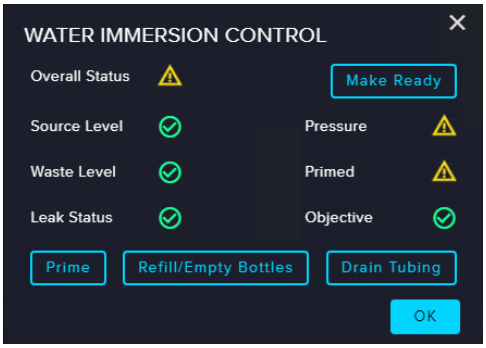
- Do not remove the water immersion controller cover. Exception for the water bottles, the controller does not contain any user-serviceable parts.
- We recommend that the base of the water immersion controller be level with the instrument.
- Do not move the water immersion controller after installation by the Molecular Devices Field Service Engineer. To ensure high-performance acquisition with water immersion, the Field Service Engineer calibrates the water dispense rate. Changing the position of the water immersion controller can negatively affect the calibration.

Using a Water Immersion Objective

In the MetaXpress Acquire software, on the **Configure** page, in the **Objective** field, select a water immersion objective. See Water Immersion Hardware Components on page 54 for a list of water immersion objectives.

Note: On some plates, outer wells may be blocked from acquisition to prevent collision with the water immersion objective.

Check the **Water Immersion Status** icon at the bottom left of the screen. The icon indicates the status of the water immersion system components. If the **Water Immersion Status** displays yellow or red, click the icon to display the **Water Immersion Control** dialog.



The **Water Immersion Control** dialog shows the status of the following components:

- **Overall Status**: Displays the overall status of the water immersion system. If all other statuses are green, the overall status displays green.
- **Source Level**: Displays the status of the source bottle. If the source bottle is missing or if the water level in the source bottle drops to 100 mL (3.4 oz), this status displays red. See Refilling and Emptying the Water Bottles on page 69 for details on refilling the source bottle.
- **Waste Level**: Displays the status of the waste bottle. If the waste bottle is missing or if the water level in the source bottle reaches 400 mL (13.5 oz), this status displays red. See Refilling and Emptying the Water Bottles on page 69 for details on emptying the waste bottle.
- **Leak Status**: Displays leak status of the water immersion objective. If a leak is detected, this status displays red. When this happens, an **Info** icon displays. Click **Info** for details on recovering from a leak.
- **Pressure**: Displays the line pressure status of the water immersion system. If the line pressure is low, this status displays red. If the line pressure is not ready, this status displays yellow. In most cases, click **Make Ready** to pressurize the water immersion system.
- **Primed**: Display the priming status of the water immersion system. Priming fills the lines with water to enable bolus creation. If the water immersion system is not primed, the status displays yellow. In most cases, click **Prime** or **Make Ready** to prime the water immersion system.
- **Objective**: Displays the status of the water immersion objective. If a water immersion objective is not selected, this status displays yellow.

Note: Mouse over any status to see a message about the status.

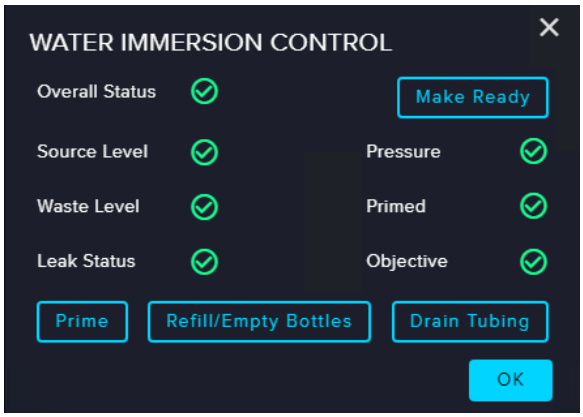
The **Water Immersion Control** dialog includes the following buttons:

- **Make Ready**: Prepare the water immersion system for acquisition.

  Note: You do not need to click **Make Ready** before acquiring images. Once you begin an acquisition, the system automatically prepares the water immersion system for acquisition.

- **Prime**: Fills the tubing between the source bottle and the objective with water and builds pressure in the source bottle so that water will dispense at a constant rate.
- **Refill/Empty Bottle**: Displays instructions for refilling the source bottle and emptying the waste bottle.
- **Drain Tubing**: Clears the tubing of water.
- **OK**: Closes the **Water Immersion Control** dialog.

When all status indicators in the **Water Immersion Status** dialog are green, the water immersion objective is ready.



After Using a Water Immersion Objective

When change back to an air objective after using a water immersion objective, you must dry the plate before continuing. The bottom of the plate may be wet, due to the bolus that was created by the water immersion system. Use lens tissue to dry the bottom of the plate.

Maintaining the Water Immersion System

See Water Immersion System Maintenance on page 68 for details on the water immersion system maintenance steps, including cleaning water immersion system components and refilling/empty the water bottles.

Troubleshooting the Water Immersion System

If you encounter an issue with the water immersion system, try performing one or more of the procedures below before contacting Molecular Devices.

If the system is preventing plate acquisition, confirm that the plate settings are accurate and that the plate is compatible with the objective.

If the system is unable to focus on the plate, follow these steps:

1. Review the plate settings.
2. Open the top to visually inspect the water bolus to ensure that it is formed properly on top of the objective.
3. In the software, go to the **Channels** page and click **Test Autofocus**.

If the system indicates that a leak has been detected, the Leak Status icon in the Water Immersion Control dialog displays red. When this happens, an **Info** icon displays. Click **Info** and follow the on-screen instruction for details on recovering from a leak.

If a message continues to indicate an error or issues persist, contact Molecular Devices Technical Support. See Obtaining Support on page 27 for details.

Chapter 6: Using the Environmental Control System

The optional environmental control system uses a sealing ring on top of the sample plate and the instrument top door above the plate. Together, these form a small, sealed volume. Humidified gas (CO_2, N_2, and compressed air) is supplied into this small volume to form the specified environment. In addition, the system controls the temperature around the plate.

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

Environmental Control System Safety

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

WARNING!

- **Do not operate the environmental control system with substances or under conditions that can cause a risk of explosion, implosion, or the release of gases.**
- **Use compressed gas supplies in a well-ventilated area. The instrument is not airtight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures defined by your safety officer to maintain a safe work environment, such as the use of an automatic warning system.**
- **Use only the gases described in this guide, which are CO_2, N_2, and compressed air. NEVER attempt to connect a pure O_2 tank or any other unsupported gas supply to the instrument.**

WARNING! BIOHAZARD. You are responsible for decontaminating all system components before returning 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.

Before returning parts, contact Molecular Devices Technical Support if you have questions about decontamination. See Obtaining Support on page 27 for details.

CAUTION!

- The environmental control system includes heated tubing that controls the temperature of the gas flow. Some part of the instrument can reach temperatures of up to 40°C (104°F). Avoid touching the temperature-controlled parts of the system.
- To prevent damage to the instrument, do not allow the gas pressure to exceed 2.1 bar (30 psi).
- With multiple gas supplies, use the same gas pressure for each gas supply.
- Do not use the instrument with hazardous substances.
- If you use any substances or materials that pose a risk of infection, you are responsible for applying best practices when handling these materials.
- If the CO_2 port or the N_2 port is not connected to a gas supply, use a blind plug (included) to close it off.

See Using the Environmental Control System on page 59 for details on using the environmental control system.

Environmental Control System Hardware

The environmental control system consists of the following hardware subsystems:

- **Temperature Control**: Regulates the temperature at 5°C (9°F) above ambient within a range of 30°C to 40°C (86°F to 104°F).
- **Gas Control**: Provides CO_2, N_2, and compressed air to control the gas concentration around the plate. CO_2 is typically supplied from a gas cylinder. N_2 can be supplied from gas cylinders or a lab gas line. Compressed air can be supplied from a gas cylinder, a lab gas line, or an oil-free air pump or compressor. Gas pressure to the instrument must be regulated within the range of 1.0 bar and 2.1 bar (15 psi and 30 psi).
- **Humidity Control**: Provides humidity by bubbling gases through the environmental control reservoir to minimize evaporation from the sample over the duration of a time-lapse experiment.

This section contains information on the following:

- Items Provided by Molecular Devices, see below
- Items Provided by You, see page 62

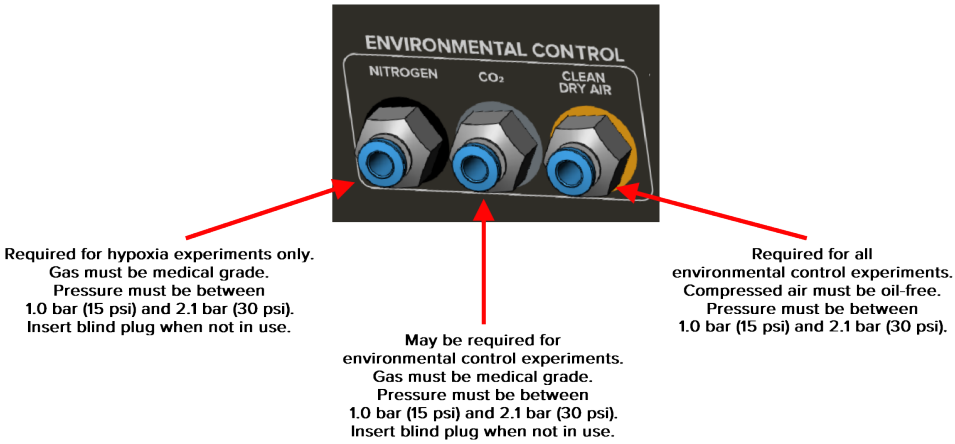
Items Provided by Molecular Devices

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

- **Environmental sensors** to provide accurate readings of temperature, humidity, CO_2, and O_2.
- **Gas Supply Tubing** to connect the gas supplies to the instrument.
- **Push-to-connect fittings** to easily connect the gas supply to the instrument.
- **Blind plugs** to close off the CO_2 port, the N_2 port, or both ports when not in use.

Items Provided by You

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



- **Required Gas Supplies**: The following gas supplies are required for all experiments using environmental control:
  - **Pressurized compressed air** from a gas cylinder, a house gas line, or an oil-free air compressor.
  - **Pressurized, medical-grade CO_2** from a gas cylinder.

    Note: CO_2 is used to regulate the pH of cell culture media for mammalian cells. If you are using an organic buffer solution (for example, HEPES) to regulate the pH of your media, then a CO_2 source may not be required.
- **Optional Gas Supply**: The following gas supply is required only for hypoxia experiments:
  - **Pressurized, medical-grade N_2** from a gas cylinder or a house gas line. Required for hypoxia experiments; otherwise optional.
- **Pressure regulators** to deliver gases to the instrument. Gas pressure to the instrument must be regulated within the range of 1.0 bar and 2.1 bar (15 psi and 30 psi).
- **Gas supply tubing** to connect the instrument to the regulator. If the 10 m (32.8 ft) of tubing provided by Molecular Devices is not sufficient, you must provide an appropriate length of 4 mm I.D. / 6 mm O.D. polyurethane tubing.
- **Teflon tape and hose clamps** to secure the tubing and fittings.
- **Deionized water** to maintain humidity inside the environmental control cassette. The environmental control reservoir holds 330 ml (11.2 oz) of deionized water, which is enough to continuously provide humidity for about 30 days. Refill the environmental control reservoir when the level drops to around ¼ full.

See Environmental Control System Gas Requirements on page 84 for details on the gas supplies and pressure regulators required for the environmental control system.

Setting Up the Environmental Control System

To set up the environmental control system:

1. Confirm that the valves on the gas cylinders are completely shut off and that no gas is flowing.
2. Confirm that the environmental control reservoir contains enough deionized water for your acquisition.

   Note: The reservoir holds 330 mL (11.2 oz) of deionized water, which should be enough to provide humidity for up to one month. We recommend that the reservoir be at least ¼ full.

3. Do the following to connect the gas supplies to the instrument:

   Note: See Connecting and Disconnecting Tubing on page 64 for details on using the push-to-connect connectors.

   a. Connect the tubing from the CO_2 source to the **CO_2** port.
   b. Connect the tubing from the compressed air source to the **CLEAN DRY AIR** port.
   c. If you will be performing hypoxia experiments, connect the tubing from the N_2 source to the **NITROGEN** port.

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

Connecting and Disconnecting Tubing

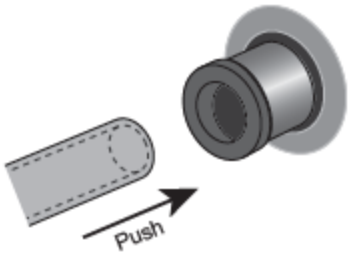
As part of working with the environmental control system, you must connect and disconnect tubing.

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

Connecting Tubing

To connect tubing:

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

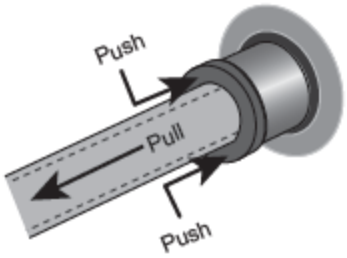


3. After inserting the tubing, gently pull the tubing to confirm it is seated.

Disconnecting Tubing

To disconnect tubing:

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



Setting Environmental Control Parameters

You can set target values for environmental control parameters on the Environmental page in the MetaXpress Acquire software. You can monitor the current status of the environment control system in the Environmental Control dialog.

See the *MetaXpress Acquire Help* for details on setting environmental control parameters.

Maintaining the Environmental Control System

See Environmental Control System Maintenance on page 71 for details on the environmental control system maintenance steps, including filling the environmental control reservoir.

Troubleshooting the Environmental Control System

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

- Confirm that the gas supplies are connected properly to the specified port and that the tubing is not crimped or blocked.
- Confirm that you are using a compatible regulator (which must be a pressure regulator, not a flow control valve). See Environmental Control System Gas Requirements on page 84 for details.
- Confirm that the pressure is set correctly. Gas pressure to the instrument must be regulated within the range of 1.0 bar and 2.1 bar (15 psi and 30 psi).
- Confirm that you are using the correct gas supplies. Do not substitute another gas supply (such as O_2 or CO_2) for compressed air.

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

Unable to Consistently Achieve the Target Gas Level

Possible Reasons:

- Unused N_2 and/or CO_2 ports are not fully closed off.
- Gas supplies are connected, but not turned on (that is, there is no pressure at the port).
- Gas supplies are empty.

Recommended Actions:

- Confirm that blind plugs are securely inserted in the unused port. The following image shows blind plugs inserted in two unused ports (indicated by arrows). If a CO_2 supply is connected without an N_2 supply, insert a blind plug in the N_2 port only.

- If an N_2 supply is connected, but not in use, disconnect the N_2 supply and insert a blind plug in the port. See Connecting and Disconnecting Tubing on page 64 for details.
- Confirm that gas supplies are not empty.

Chapter 7: Maintaining the System

The section includes the following topics:

Routine Maintenance

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

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

Cleaning the System

An important part of system maintenance is keeping the instrument clean, including both the exterior of the instrument and the area around the plate holder. To clean the instrument, use a lint-free wipe that has been lightly dampened with 70% ethanol. Clean the exterior of the instrument periodically. Clean the plate holder area as needed.

Wipe up all spills immediately.

WARNING! BIOHAZARD. To avoid contact with any hazardous or biohazardous materials or fluids, always wear gloves when cleaning the instrument.

CAUTION! Observe the following general tips when you clean the instrument:

- Always turn the power switch off and disconnect the power cord from the main power source before cleaning the instrument.
- 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 27.
- Do not use ultraviolet light to sterilize the instrument. Ultraviolet light can damage plastic components.
- Do not use organic solvents to clean the instrument.
- Do not use an autoclave to clean instrument components.
- To prevent damaging instrument components, do not pour or squirt any liquid (water or alcohol) directly onto or into the instrument.

Note: The procedure in this section is not intended to decontaminate or sterilize the instrument. Contact Molecular Devices Technical Support if you have questions about decontamination. See Obtaining Support on page 27 for details.

To clean the instrument:

1. Press the button on the instrument to open the top door and move the stage to the load position.
2. Shut down the system.
   The stage moves freely without power, enabling you access all surfaces of the plate holder area.
3. Ensure that no sample is loaded.
4. With gloved hands, clean the exterior of the instrument with a 70% ethanol wipe.
5. Using a forceps wrapped with a 70% ethanol wipe, gently clean the perimeter of the plate holder area, including around the stage (where a plate would be loaded).
6. Repeat the previous step with a fresh 70% ethanol wipe.
7. Use a fresh 70% ethanol wipe to clean underneath the stage and around the plate holder area.
8. Repeat the previous step with a fresh 70% ethanol wipe.
9. Wait a few minutes for the ethanol to evaporate.
10. Power ON the instrument. See Starting the System on page 32 for details.
11. Start the MetaXpress Acquire software.
12. If the instrument top door remains open, press the button on the instrument to close the top door.

Light Source Maintenance

See the documentation for the laser or LED light source for details on any required or recommended maintenance.

Water Immersion System Maintenance

The section includes the following topics:

Cleaning Water Immersion Components

Components can come in contact with biological, chemical, and toxic agents. Therefore, all cleaning procedures should be handled with care.

- Replace the distilled water in the source bottle if the water immersion system has been unused for a significant period of time.
- Do not reuse the water from the waste bottle due to potential contaminants from the bottom of plates.
- Use a lens cloth to gently wipe the top of a water immersion objective.
- Wipe the exterior of the source bottle and the waste bottle before you place the bottles back into the water immersion controller.
- Do not access or clean the interior of the water immersion controller other than the area that holds the bottles.
- If you plan to store the instrument, you plan to move the instrument, or you do not intend to use the water immersion system for a long period of time, contact Molecular Devices Technical Support. See Obtaining Support on page 27 for details.

Refilling and Emptying the Water Bottles

Before you acquire images with a water immersion objective, ensure that:

- The source bottle has enough water for the experiment.
- The waste bottle has enough space for the water that will be drained from the water immersion objective.
- The bottle caps are screwed on tightly.

The water immersion system has multiple leak detectors and pressure sensors to determine if there is unintended water loss. When a sensor is triggered, the system automatically shuts off the pump and closes the valves to prevent damage to the instrument. See Troubleshooting the Water Immersion System on page 58 for details.

To refill the source bottle:

1. Unscrew the source bottle cap and, while holding the cap and tubing, remove the bottle from the water immersion controller.
2. Drape the tubing and cap over the front of the water immersion controller (to prevent the tubing and cap from coming into contact with the table surface or other potential contaminants).
3. Fill the source bottle with up to 500 mL (16.9 oz) of distilled water.

   CAUTION!
   - Use only distilled water in the source water bottle. Deionized water can be used, but is not required. Do not use tap water and do not reuse the water from the waste bottle.
   - We recommend that you degassing or sonicating the water to remove air bubbles.
   - When filling the source bottle, add water slowly. Adding water too quickly can cause air bubbles to form.
4. Insert the tubing into the bottle and then replace the source bottle in the water immersion controller.
5. Tighten the bottle cap.
6. In the MetaXpress Acquire software:
   a. At the bottom left of the screen, click  **Water Immersion Status**.
      The **Water Immersion Control** dialog opens.
   b. Click **Refill/Empty Bottles**.
   c. In the **Refill/Empty Bottle** dialog, click **Test Vacuum** and **Test Pressure** to ensure the bottles are properly sealed.

To empty the waste bottle:

1. Unscrew the waste bottle cap and, while holding the cap and tubing, remove the bottle from the water immersion controller.
2. Drape the tubing and cap over the front of the water immersion controller (to prevent the tubing and cap from coming into contact with the table surface or other potential contaminants).
3. Empty the waste bottle and dispose of the used water.

   CAUTION! Do not re-use water from the waste bottle.

4. Insert the tubing into the bottle and then replace the bottle in the controller.
5. Tighten the bottle cap.
6. In the MetaXpress Acquire software:
   a. At the bottom left of the screen, click  **Water Immersion Status**.
      The **Water Immersion Control** dialog opens.
   b. Click **Refill/Empty Bottles**.
   c. In the **Refill/Empty Bottle** dialog, click **Test Vacuum** and **Test Pressure** to ensure the bottles are properly sealed.

Environmental Control System Maintenance

Filling the Environmental Control Reservoir

The environmental control reservoir uses deionized water to add humidity to the air flow around the plate.

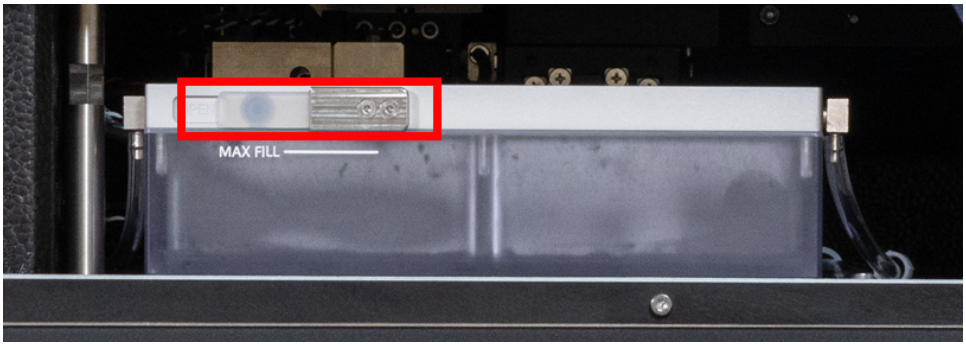
The environmental control reservoir holds 330 ml (11.2 oz) of deionized water, which is enough to continuously provide humidity for about 30 days. Refill the environmental control reservoir when the level drops to around ¼ full.

To fill the environmental control reservoir:

1. Press the instrument front door button to open the front door.

2. Locate the environmental control reservoir above the inner door.

3. Pull back the rubber stopper on the environmental control reservoir .



4. Use a squeeze bottle to fill the environmental control reservoir with deionized water to the maximum indicator. At the maximum indicator, the reservoir holds 330 ml (11.2 oz).

5. Replace the rubber stopper on the environmental control reservoir.
6. Close the front door on the instrument.

Appendix A: System Specifications

WARNING!

- **If the ImageXpress HCS.ai system is used in any manner not specified by Molecular Devices, the protection provided by the system may be impaired.**
- **The ImageXpress HCS.ai 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 system—or disconnection of the protective earth ground terminal can result in personal injury.**

Note: Specifications are subject to change without notice.

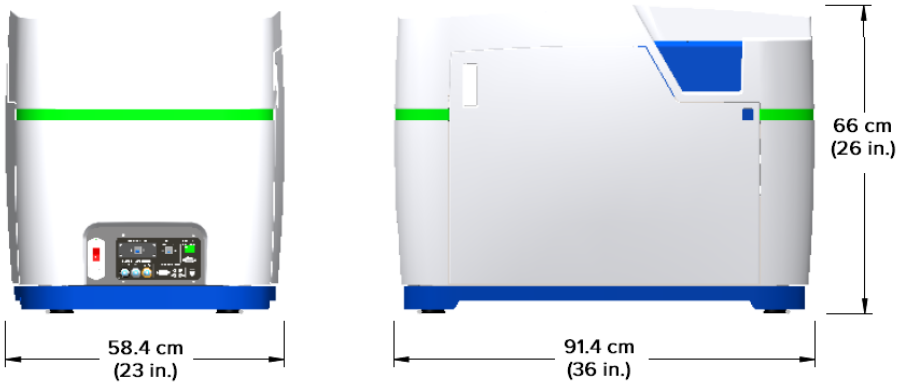
Item	Description
Instrument Dimensions	Width: 91.4 cm (36 in.) Depth: 58.4 cm (23 in.) Height: 66 cm (26 in.)
Instrument Weight	109 kg (240 lb)
Clearance Requirement	Front/Rear: 68.6 cm (27 in.) Left/Right: 25.4 cm (10 in.) Top: 50.8 cm (20 in.)
Input Power Requirement	100-240 VAC, 50/60Hz, 6 amps maximum
System Power Consumption	2,580 W maximum at start; 6 amps
Fuses	none
Light Source Power	One of the following: • Laser (Advanced models): 100-240 VAC, 50/60 Hz ±10%, AC to DC converter included • LED (Confocal and Widefield models): 12 VDC, 102 W, 8.5 amps
Computer Power Input	100 VAC to 240 VAC ±10%, 50/60 Hz, 690 VA, 120 W
Monitor Power Input	100 VAC to 240 VAC ±10%, 50/60 Hz, 40 W
IEC Installation Category	II
IEC Pollution Degree	2
ISM Equipment Class	1
Ingress Protection	IP20
Operating Environment	Indoor use only
Altitude Restriction	Not to exceed 2,000 m (6,562 ft)
Operating Temperature	Permissive ambient temperature: 15°C to 30°C (59°F to 86°F) Recommended ambient temperature: 18°C to 25°C (64.4°F to 77°F)
Operating Relative Humidity	35% to 50% non-condensing
Shipping Temperature	-25°C to 55°C (-13°F to 131°F)

Item	Description
Shipping Relative Humidity	10% to 50% relative humidity (non-condensing)
Supported Microplates	ANSI/SLAS compliant Number of Wells: 6, 24, 96, 384, 1536 Maximum Height, including lid: 23 mm (0.9 in.)
Imager	5.3-megapixel, sCMOS camera 2,304 × 2,304 image sensor format 89.1 frames per second 95% QE
Imaging Modes	Transmitted Light (Brightfield) and one of the following: • Laser light source: Up to 8 Fluorescence channels • LED light source: Up to 7 Fluorescence channels
Acquisition Speed	Typical value of 3.5 min per 96-well plate (1 FOV, 1 channel)
Light Source	One of the following: • Laser (Advanced model): 7 color (401 to 748 nm) • LED (Confocal and Widefield models): 5 color (377 to 632 nm)
Magnification Changer	1x (standard), 1.5x (optional)
Supported Objectives	2x Plan Apo Lambda 4x Plan Apo Lambda D 10x Plan Apo Lambda D 20x Plan Apo Lambda D 20x S Plan Fluor ELWD 20x Water Apo Lambda S XC WI 40x S Plan Fluor ELWD 40x Water Apo Lambda S XC WI 60x Plan Fluor XC 60x S Plan Fluor ELWD 60x Water Plan Apo VC XC WI
Supported Fluorescence Channel Colors	DAPI FITC TRITC Texas Red Cy5 Cy7 (Advanced model only) CFP YFP
Environmental Control	Temperature: 5°C (9°F) above ambient within a range of 30°C to 40°C (86°F to 104°F) O_2: 1% to 21% CO_2: 0% to 15%

Instrument Dimensions

The instrument weighs 109 kg (240 lb) and has the following dimensions:

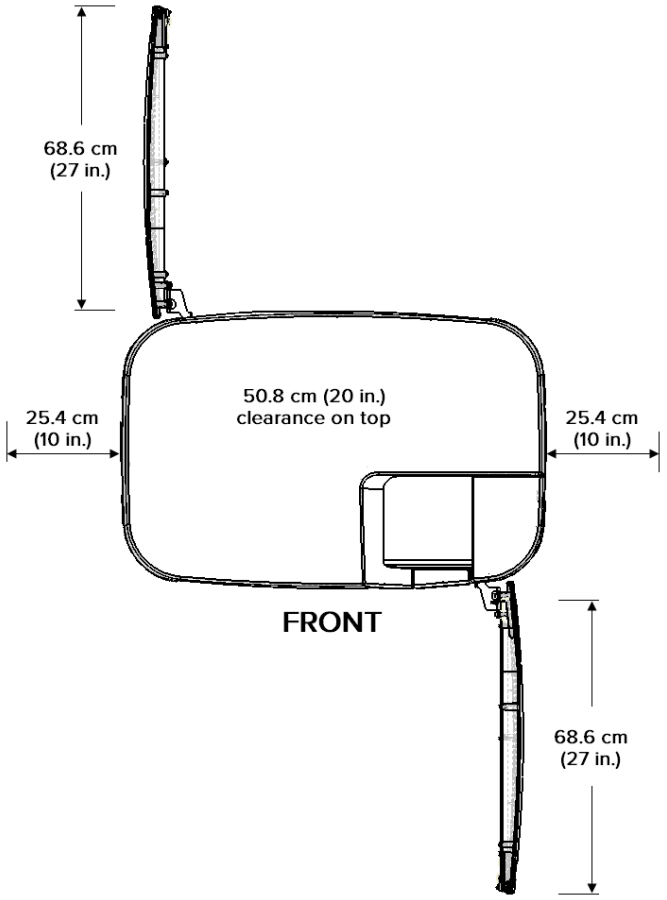
	Width	Depth	Height
Instrument	91.4 cm (36 in.)	58.4 cm (23 in.)	66 cm (26 in.)



Instrument Clearance

On its table, the instrument requires a footprint of 0.5 m^2 (5.75 ft^2). In addition, the instrument requires the following clearances:

Side	Clearance	Purpose
Top	50.8 cm (20 in.)	Load plates and allow access for service.
Front	68.6 cm (27 in.)	Open the front door to access objectives and filters.
Left	25.4 cm (10 in.)	Allow access to power, light source, and gas supply connections.
Rear	68.6 cm (27 in.)	Allow access for service.
Right	25.4 cm (10 in.)	Ventilation.



Note: If you are unable to provide the required clearance, we recommend that you do one of the following to allow the instrument to be moved for maintenance or service:

- Retain and store the rolling cart from the installation crate.
- Use the optional system table, which is designed specifically for the ImageXpress HCS.ai instrument and components. Contact Molecular Devices Technical Support for details. See Obtaining Support on page 27.

Component Dimensions

The dimensions of the ImageXpress HCS.ai system components are as follows:

Component	Width	Depth	Height	Weight
Workstation Computer	19 cm (7.5 in.)	48.3 cm (19 in.)	44.5 cm (17.5 in.)	21.8 kg (48 lb)
Water Immersion Controller	25.4 cm (10 in.)	25.4 cm (10 in.)	25.4 cm (10 in.)	9.1 kg (20 lb)
Laser Light Source (Advanced Gen1 model)	15.2 cm (6 in.)	34.3 cm (13.5 in.)	20.3 cm (8 in.)	9.1 kg (20 lb)
Laser Light Source (Advanced Gen2 model)	19 cm (7.5 in.)	34 cm (13.4 in.)	25 cm (9.8 in.)	13.6 kg (30 lb)
LED Light Source (Widefield and Confocal models)	12.7 cm (5 in.)	19.1 cm (7.5 in.)	19.1 cm (7.5 in.)	3.2 kg (7 lb)
Workstation Monitor 27"	61.2 cm (24.1 in.)	18.5 cm (7.3 in.)	53.6 cm (21.1 in.)	7.3 kg (16 lb)
Workstation Monitor 32"	71.1 cm (28 in.)	23.4 cm (9.2 in.)	62 cm (24.4 in.)	10 kg (22 lb)

Appendix B: Compatible Objectives

The following table details the Nikon objectives that are compatible with the ImageXpress HCS.ai system. It also provides plate compatibility information and theoretical calculation of confocal performance for the objectives you use in the system.

Objective (Part Number)	Refraction Medium	Numerical Aperture	Working Distance	Correction Collar (min - max)	Plate Compatibility
2x Plan Apo Lambda D (5301118)	Air	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 D (5301119)	Air	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 Apo Lambda D (5301120)	Air	0.45	4 mm	---	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
20x Plan Apo Lambda D (5301121)	Air	0.8	0.8 mm	---	Thin bottom (0.17 mm)* Thin bottom (0.17 mm) No Skirt
20x S Plan Fluor ELWD (6500-0108)	Air	0.45	8.2 - 6.9 mm	0 - 2 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
20x Water Apo Lambda S XC WI (5075816)	Water	0.95	0.99 - 0.90 mm	0.11 - 0.23 mm	Thin bottom (0.17 mm)* Thin bottom (0.17 mm) No Skirt
40x S Plan Fluor ELWD (1-6300-0109)	Air	0.6	3.6 - 2.8 mm	0 - 2 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
40x Water Apo Lambda S XC WI (5075817)	Water	1.15	0.61 - 0.59 mm	0.15 - 0.19 mm	Thin bottom (0.17 mm)* Thin bottom (0.17 mm) No Skirt

Objective (Part Number)	Refraction Medium	Numerical Aperture	Working Distance	Correction Collar (min - max)	Plate Compatibility
60x Plan Fluor XC (1-6300-0414)	Air	0.85	0.4 - 0.31 mm	0.11 - 0.23 mm	Thin bottom (0.17 mm)* Thin bottom (0.17 mm) No Skirt
60x S Plan Fluor ELWD (6500-0110)	Air	0.7	2.6 - 1.8 mm	0.1 - 1.3 mm	Thin bottom (0.17 mm) Thin bottom (0.17 mm) No Skirt Thick bottom (0.25 mm to 1 mm)
60x Water Plan Apo VC XC WI (5075818)	Water	1.2	0.31 - 0.28 mm	0.15 - 0.19 mm	Thin bottom (0.17 mm)* Thin bottom (0.17 mm) No Skirt

* = Potential interference with plate skirt when imaging edge wells.

Note: When imaging edge wells with thin-bottom plates, the short working distance of the following objectives can cause interference with the plate skirt:

- 2x Plan Apo Lambda
- 4x Plan Apo Lambda D
- 10x Plan Apo Lambda D
- 20x Plan Apo Lambda D
- 20x S Plan Fluor ELWD
- 20x Water Apo Lambda S XC WI
- 40x S Plan Fluor ELWD
- 40x Water Apo Lambda S XC WI
- 60x Plan Fluor XC
- 60x S Plan Fluor ELWD
- 60x Water Plan Apo VC XC WI

If the software does not automatically block the edge wells, either omit the edge wells or use a plate with a low skirt.

The following table shows the theoretical calculation of confocal performance with compatible objectives:

Objective	60 µm Pinhole Diameter in Airy Units	50 µm Pinhole Diameter in Airy Units	50 µm Slit Width in Airy Units	Optical Thickness using 60 µm Pinhole[1]	Optical Thickness using 50 µm Pinhole[1]
10x Plan Apo Lambda D	4.1 AU	3.4 AU	3.4 AU	19.9 µm	17.0 µm
20x Plan Apo Lambda D	3.7 AU	3.1 AU	3.1 AU	5.6 µm	4.7 µm
20x S Plan Fluor ELWD	2.1 AU	1.7 AU	1.7 AU	11.4 µm	10.1 µm
20x Water Apo Lambda S XC WI	4.4 AU	3.6 AU	3.6 AU	6.2 µm	5.3 µm
40x S Plan Fluor ELWD	1.4 AU	1.1 AU	1.1 AU	4.9 µm	4.5 µm
40x Water Apo Lambda S XC WI	2.6 AU	2.2 AU	2.2 AU	2.7 µm	2.3 µm
60x Plan Fluor XC	1.3 AU	1.1 AU	1.1 AU	2.2 µm	2.0 µm
60x S Plan Fluor ELWD	1.1 AU	0.9 AU	0.9 AU	3.1 µm	2.9 µm
60x Water Plan Apo VC XC WI	1.8 AU	1.5 AU	1.5 AU	1.8 µm	1.6 µm

1 = $FWHM_{axial}$ for pinholes larger than 1 Airy Unit (AU) as set forth by Toomre, et alia.
Go to zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html for details.

Appendix C: Filter Specifications

The following filter cube sets are compatible with the ImageXpress HCS.ai system. All listed filter cube sets are available from Molecular Devices. See Obtaining Support on page 27 for details.

Laser Light Source (Advanced model)

Filter	Type	Part Number	Wavelengths
DAPI	Emission	5314884	Emission: 421-462.5 nm (442/42)
	Excitation	5314881	Excitation: 365-407 nm (386/42) Dichroic: 411/505/582/669/763
FITC	Emission	5314885	Emission: 506-534 nm (520/28)
	Excitation	5314881	Excitation: 478.9-493 nm (486/14) Dichroic: 411/505/582/669/763
TRITC	Emission	5314886	Emission: 580-611 nm (595/31)
	Excitation	5314881	Excitation: 540-561 nm (550/21) Dichroic: 411/505/582/669/763
TX RED	Emission	5314873	Emission: 604-644 nm (624/40)
	Excitation	5314882	Excitation: 567.4-588.6 nm (578/21) Dichroic: 459/526/596
CY5	Emission	5314874	Emission: 665-695 nm (680/30)
	Excitation	5314881	Excitation: 631.4-645 nm (638/14) Dichroic: 411/505/582/669/763
CY7	Emission	5314887	Emission: 777.5-809.5 nm (794/32)
	Excitation	5314881	Excitation: 725-753.6 nm (740/29) Dichroic: 411/505/582/669/763
CFP	Emission	5314875	Emission: 467-499 nm (483/32)
	Excitation	5314883	Excitation: 370-408 nm (389/38) Dichroic: FF414
YFP	Emission	5314888	Emission: 533-552.6 nm (543/20)
	Excitation	5314882	Excitation: 497.6-519.5 nm (509/22) Dichroic: 459/526/596

LED Light Source (Confocal model and Widefield model)

Filter	Type	Part Number	Wavelengths
DAPI	Emission	5314870	Emission: 414-450 nm (432/36)
	Excitation	5314866	Excitation: 352-404 nm (378/52) Dichroic: FF409/493/596
FITC	Emission	5314871	Emission: 510-540 nm (525/30)
	Excitation	5314866	Excitation: 461-487.5 nm (474/27) Dichroic: FF409/493/596
TRITC	Emission	5314872	Emission: 570.5-599.5 nm (585/29)
	Excitation	5314867	Excitation: 516-552 nm (534/36) Dichroic: FF560/659
TX RED	Emission	5314873	Emission: 604-644 nm (624/40)
	Excitation	5314866	Excitation: 562.5-587.5 nm (575/25) Dichroic: FF409/493/596
CY5	Emission	5314874	Emission: 665-695 nm (680/30)
	Excitation	5314867	Excitation: 620-651 nm (635/31) Dichroic: FF560/659
CFP	Emission	5314875	Emission: 467-499 nm (483/32)
	Excitation	5314883	Excitation: 370-408 nm (389/38) Dichroic: FF414
YFP	Emission	5314876	Emission: 541.8-576.2 nm (559/34)
	Excitation	5314869	Excitation: 457.3-492.7 nm (475/35) Dichroic: FF506

Appendix D: Optional Parts and Accessories

This section describes the items available from Molecular Devices for the ImageXpress HCS.ai system.

Note: New objectives and filter cube sets must be installed by a Molecular Devices Field Service Engineer.

Objectives

For a list of compatible Nikon objectives, see Compatible Objectives on page 78.

Filter Sets

For a list of compatible filter cube sets, see Filter Specifications on page 81.

Spinning Disks

Part Number	
5314337	Confocal Single Disk Unit, including: • 60 µm pinhole disk for basic confocal imaging • Widefield for non-confocal imaging
5302336	Confocal Screening Option Dual Disk Unit, including: • 60 µm pinhole disk for basic confocal imaging • 50 µm slit disk for high-throughput confocal imaging • Widefield for non-confocal imaging
5306812	Confocal Deep Tissue Disk Option Dual Disk Unit, including: • 50/250 µm pinhole disk for high sensitivity in deep tissues • 50/500 µm pinhole disk for high resolution in deep tissues • Widefield for non-confocal imaging

Environmental Control System Accessories

Part Number	Description
5070106	Blind Plugs
E1004	Breathe Seal for Cell & Tissue Culture, non-sterile, pack of 100
5070103	Gas Supply Tubing, 6 mm O.D, 4 mm I.D, 10 m (32.8 ft)

Plate Accessories

Part Number	Description
5315546	Four-position microscope slide holder with microplate footprint
1-5200-1337	Single-position microscope slide holder with microplate footprint
9500-0027	4 um Bead Plate
R8000	0.5 um Bead Plate

Appendix E: Environmental Control System Gas Requirements

The ImageXpress HCS.ai system is available with the optional environmental control system, which enables you to perform multi-day, live-cell, time-lapse experiments and hypoxia experiments. This section provides the information you need to prepare for the environmental control system, including:

It is important to consider the proper connections and fittings to connect the instrument to the gas supply. The environmental control system allows independent control of both CO_2 and O_2 levels. As a result, you may need to connect up to three separate gas supplies to the system.

The regulators and connections required to connect the instrument are dependent on how the gas is supplied—from a compressed gas cylinder, a lab gas line, or an air compressor.

Required Items

If your ImageXpress HCS.ai system includes the environmental control system, you must provide the following:

- **Required Gas Supplies**: The following gas supplies are required for all experiments using environmental control:
  - **Pressurized compressed air** from a gas cylinder, a house gas line, or an oil-free air compressor.
  - **Pressurized, medical-grade CO_2** from a gas cylinder.

    Note: CO_2 is used to regulate the pH of cell culture media for mammalian cells. If you are using an organic buffer solution (for example, HEPES) to regulate the pH of your media, then a CO_2 source may not be required.
- **Optional Gas Supply**: The following gas supply is required only for hypoxia experiments:
  - **Pressurized, medical-grade N_2** from a gas cylinder or a house gas line. Required for hypoxia experiments; otherwise optional.
- **Pressure regulators** to deliver gases to the instrument. Gas pressure to the instrument must be regulated within the range of 1.0 bar and 2.1 bar (15 psi and 30 psi).
- **Gas supply tubing** to connect the instrument to the regulator. If the 10 m (32.8 ft) of tubing provided by Molecular Devices is not sufficient, you must provide an appropriate length of 4 mm I.D. / 6 mm O.D. polyurethane tubing.
- **Teflon tape and hose clamps** to secure the tubing and fittings.
- **Deionized water** to maintain humidity inside the environmental control cassette. The environmental control reservoir holds 330 ml (11.2 oz) of deionized water, which is enough to continuously provide humidity for about 30 days. Refill the environmental control reservoir when the level drops to around ¼ full.

See Environmental Control System Safety on page 60 for specific warning and caution statements for the environmental control system.

Note:

- All applications of the environmental control system require sources of compressed air and CO_2. These make it possible to enrich the CO_2 gas environment above ambient levels.
- An N_2 source is required for hypoxia experiments, where O_2 levels are to be depleted to sub-ambient levels. If you are planning to perform hypoxia experiments, all three gases—compressed air, CO_2 and N_2—are required.

Supported Gas Supplies

The environmental control system requires that the gas supply be oil-free and medical grade. CO_2 and N_2 are typically supplied from a compressed gas cylinder. Compressed air is often supplied from one of the following:

- Gas cylinder
- Lab gas line
- Oil-free air compressor

The connections required to connect the instrument to the gas source vary based on the gas supply you use. This appendix describes each connection.

Unsupported Gas Supplies

WARNING!

- **Using an unsupported gas supply with the environmental control system may damage the instrument and void the warranty.**
- **Never connect pure O_2 or any other unspecified gas supply to the instrument.**

To avoid damage to the instrument, DO NOT use the following:

- Pre-mixed gas supplies
- N_2 or CO_2 boil-off from a Dewar flask
- N_2 from an N_2 generator
- Any gas supply that is not oil-free
- Any gas supply that is not medical grade
- Any gas supply that cannot be set to supply gas at between 1.0 bar and 2.1 bar (15 psi and 30 psi)

Using a Gas Cylinder

Gas pressure to the instrument must be regulated within the range of 1.0 bar and 2.1 bar (15 psi and 30 psi). A two-stage regulator is required to step down and regulate the pressure from the gas cylinder.

Regulators type designations for gas cylinders vary based on the region. The following table lists examples of region-specific regulator types:

Region	CO_2 Regulator Type	N_2 Regulator Type	Compressed Air Regulator Type
North America	CGA320	CGA580	CGA590
Germany	DIN477-1 Nr.6	DIN 477-1 No.10	DIN477-1 Nr.13
Great Britain	BS 341 No.8	BS 341 No.3 or BS 341 No.30	BS 341 No.3 or BS 341 No.31
Italy	UNI 4406 /UNI2	UNI 4409 / UNI5	UNI 4410 / UNI6
France	ANFOR NF E 29-650/C	ANFOR NF E 29-650/C	ANFOR NF E 29-650/D or ANFOR NF E 29-650/B

Note: The information in the table is not exhaustive and may change without notice.

Tip:

- The regulator type is often stamped on the end of the regulator on the side that will connect to the cylinder.
- Select a cylinder size that meets your needs. Gas within the environmental control system flows at up to 20 l/hr (0.7 ft^3/hr). So, for example, for typical CO_2 regulation at 5% volume, a 10 l (0.35 ft^3) liquid CO_2 cylinder can last at least six months.

Several vendors offer gas regulators, and the one you use is not critical as long as the following conditions are met:

- The regulator type is appropriate for the tank as indicated in the table above.
- The regulator is a two-stage, gas pressure regulator. Be sure that you are using a gas pressure regulator, not a flow control valve.
- The maximum delivery pressure of the regulator is 10 bar (145 psi) or less. We recommend that you use a regulator with a maximum delivery pressure of 4.14 bar (60 psi), which makes it easier to set the required pressure.

CAUTION! To prevent damage to the instrument, do not allow the gas pressure to exceed 2.1 bar (30 psi).

Some examples of regulator vendors in North America are listed in the following table:

Vendor (Website)	Gas	Type	Part Number
McMaster-Carr (www.mcmaster.com)	CO_2	CGA320	7951A67
	N_2	CGA580	7951A62
Airgas (www.airgas.com)	Air	CGA590	Y12244B590-AG
	CO_2	CGA320	Y12244B320-AG
	N_2	CGA580	Y12N245B580-AG
Matheson (store.mathesongas.com)	Air	CGA320	SEQ3121A320
	CO_2	CGA580	SEQ3121A580
	N_2	CGA590	SEQ3121A590

Note: The information in the table is not exhaustive and may change without notice.

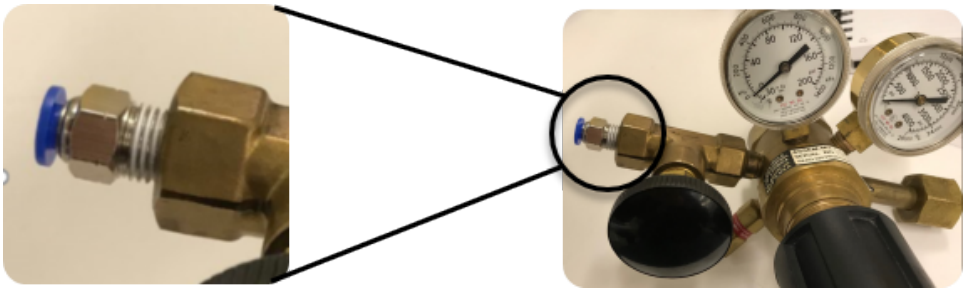
You can also purchase an acceptable regulator from one of the following vendors:

- Air Products (www.airproducts.com)
- Fisher Scientific (www.fishersci.com)
- Linde (www.linde-gas.com)
- VWR (www.vwr.com)

Connecting a Gas Cylinder to the Instrument

Many gas regulators can accommodate a ¼" NPT male fitting. Use Push-to-Connect fittings (¼" NPT male to 6 mm O.D.) to easily connect this type of gas cylinder to the instrument. Attach the fitting to the regulator to easily connect the hose; no other connectors are required.

The following shows an example of a CO_2 regulator with ¼" NPT male to 6 mm O.D. Push-to-Connect fitting:



If your regulator terminates with a ¼" NPT male fitting, you will need to provide a ¼" NPT female to ¼" NPT female connector, as shown below.

You can purchase this fitting from many vendors, including Anderson Metals (part number 56103-04).

Using a Lab Gas Line

Some laboratories are equipped with a gas line to supply compressed air and N_2. The line output is typically greater than 2.1 bar (30 psi), which is the maximum allowed pressure for the environmental control system. In this case, a single-stage line pressure regulator is required.

Several vendors offer single-stage line regulators, and the one you use is not critical as long as the maximum delivery pressure of the regulator is 10 bar (145 psi) or less. We recommend that you use a regulator with a maximum delivery pressure of 4.14 bar (60 psi), which makes it easier to set the required pressure. One example of an acceptable line regulator is the Matheson Model 3470A General Purpose Line Regulator (part number SEQ3473A), which is shown to the right.

Connecting a Lab Gas Line to the Instrument

Many lab gas lines terminate with a hose barb connector, like the one shown to the left.

For a hose barb connector, connect a short piece of tubing and secure it with a hose clamp. Then attach a second hose barb connector and, again, secure it with a hose clamp. Finally, attach a ¼" NPT male to 6 mm O.D. Push-to-Connect fitting. The finished assembly should look like the one shown on the right.

It is also possible that your lab gas line terminates with a line regulator. In this case, attach the ¼" NPT male to 6 mm O.D. Push-to-Connect fitting directly to the line regulator.

To connect a lab gas line, you may need the following:

Manufacturer	Part Number	Description
FasParts	FP126-8B	Hose ID / Hose Barb to ¼" Female NPT FIP FPT Straight Brass Fitting
EDGE INDUSTRIAL	E.I. BARB 53	¼" Hose ID to ¼" Female NPT FNPT Straight Brass Fitting
EDGE INDUSTRIAL	E.I. BARB 58	3/8" Hose ID to ¼" Female NPT FNPT Straight Brass Fitting
Various	---	Hose Clamp

Note: The information in the table is not exhaustive and may change without notice.

Using an Oil-Free Air Compressor

If a lab gas line or a gas cylinder is not available, you can connect an oil-free air compressor to the instrument. This is the least preferred option since air compressors tend to be noisy and a source of vibration.

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

Most oil-free air compressors have an internal regulator. The one you use must be adjustable to between 1.0 bar and 2.1 bar (15 psi and 30 psi). Otherwise, you will need to connect a line regulator as with a lab gas line. See Using a Lab Gas Line on page 89 for details.

Ibidi (ibidi.com) is one example of a vendor of a supported oil-free air compressor.

Connecting a Lab Gas Line to the Instrument

Similar to a lab gas line, most laboratory air compressors terminate with a hose barb connection or a ¼" NPT-style connection.

For a hose barb connector, connect a short piece of tubing and secure it with a hose clamp. Then attach a second hose barb connector and, again, secure it with a hose clamp. Finally, attach a ¼" NPT male to 6 mm O.D. Push-to-Connect fitting. The finished assembly should look like the one shown on the right.

For a ¼" NPT-style connection, attach the ¼" NPT male to 6 mm O.D. Push-to-Connect fitting directly to the line regulator.

Appendix F: Electromagnetic Compatibility

Regulatory for Canada (ICES/NMB-001:2020)

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.

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