User Guide
for ImageXpress®
Automated Cellular Imaging System

Molecular Devices Corporation
Sunnyvale, California 94089

Part # 2500-0176 Rev. A
Copyright

© Copyright 2006, Molecular Devices Corporation. All rights reserved. No part of this publication may be reproduced, transmitted, transcribed, stored in a retrieval system, or translated into any language or computer language, in any form or by any means, electronic, mechanical, magnetic, optical, chemical, manual, or otherwise, without the prior written permission of Molecular Devices Corporation, 1311 Orleans Drive, Sunnyvale, California, 94089, United States of America.

Disclaimer

Molecular Devices Corporation reserves the right to change its products and services at any time to incorporate technological developments. This user guide is subject to change without notice.

Although this user guide has been prepared with every precaution to ensure accuracy, Molecular Devices Corporation assumes no liability for any errors or omissions, nor for any damages resulting from the application or use of this information.

Trademarks

ImageXpress\textsuperscript{MICRO} is a registered trademark of Molecular Devices Corporation.

MetaXpress is a trademark of Molecular Devices Corporation.

All other trademarks are the property of their respective owners.
## Table of Contents

**Chapter 1: Introduction** ................................................................................................................... 1  
  ImageXpress<sup>M</sup> Overview ........................................................................................................ 1  
  System Installation ......................................................................................................................... 2  
  About this Guide ............................................................................................................................ 3  

**Chapter 2: Imaging System Hardware** ....................................................................................... 5  
  Illumination System—Excitation ..................................................................................................... 5  
    Light source ................................................................................................................................. 5  
    Cold mirror .................................................................................................................................. 5  
    Light guide ................................................................................................................................... 5  
    Illumination optics ...................................................................................................................... 5  
    Shutter ......................................................................................................................................... 6  
    Filter cube changer .................................................................................................................... 6  
  Objective (Z) Stage ......................................................................................................................... 6  
    Motorized Z stage ....................................................................................................................... 6  
    Objectives .................................................................................................................................... 6  
    Motorized objective changer ...................................................................................................... 6  
  Sample (X-Y) Stage ....................................................................................................................... 6  
    Sample ......................................................................................................................................... 6  
    Plate holder and plate clamp ....................................................................................................... 7  
    Motorized X-Y stage .................................................................................................................... 7  
  Autofocus Laser (optional) .......................................................................................................... 7  
    Autofocus laser ........................................................................................................................... 7  
  Imaging System—Emission ........................................................................................................... 7
Chapter 3: Basic Operational Theory .............................................................. 9
Fluorescence Imaging .................................................................................. 9
Excitation and Emission Filters .................................................................... 9
Dichroic Mirror .............................................................................................. 10
Dichroic Transmission Spectrum ................................................................. 11
Objective lenses ............................................................................................ 13

Chapter 4: User Procedures ........................................................................ 15
User Safety Instructions .................................................................................. 15
Powering On/Off the ImageXpress MICRO .................................................. 16
  Powering ON ............................................................................................. 16
  Powering OFF ........................................................................................... 16
Light Source .................................................................................................... 17
  Changing the lamp .................................................................................... 17
  Changing the light source fuse .................................................................. 17
Filters Cubes ................................................................................................... 17
  Changing filter cubes ................................................................................. 17
Objectives ........................................................................................................ 19
  Correct objective placement ..................................................................... 20
  Changing objectives ................................................................................... 20
  Cleaning objectives ................................................................................. 21
  Adjusting spherical aberration correction collar on ELWD objectives ...... 22
  Using oil-immersion objectives ............................................................... 23
Cleaning the ImageXpress MICRO .............................................................. 24
  Decontaminating the ImageXpress MICRO instrument ......................... 24

Chapter 5: Robotic Plate Handling (Optional) .............................................. 27
CRS Catalyst Express Robot: Hardware ......................................................... 27
CRS Catalyst Express Robot User Procedures ............................................. 28
  Loading plates onto the CRS Catalyst Express Robot ......................... 28
Appendix A: Technical Assistance ................................................................. 31
Appendix B: Important Safety Information.................................................. 33
Appendix C: Site Requirements.................................................................... 41
Index............................................................................................................. 43
Chapter 1: Introduction

ImageXpress™ Overview

ImageXpress™ from Molecular Devices is an integrated cellular imaging and analysis system that is designed for rapid, automated screening of fluorescently labeled biological samples in microplates. The ImageXpress™ system comprises:

- Imaging hardware.
- MetaXpress instrument control and image analysis software.

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

When used in combination with the powerful imaging capabilities of the MetaXpress software (see the MetaXpress ImageXpress™ System Administration Guide), the instrument becomes an extremely flexible and programmable device, ideally suited for user-defined high-speed automated assays. Key features of the instrument include the following:

- External 300 W Xenon light source connected via liquid light guide.
- User-specified **CCD camera**.
- Fast **laser autofocus** system (optional) with precision motorized Z (focus) stage.
- **Image-based autofocus**.
- Precision motorized **X-Y (sample) stage**.
- High-quality user-changeable **Nikon objectives** in a four-position linear selector.
- User-changeable **filter cubes** in a five-position slider.
- Selectable **binning modes** to decrease exposure time and increase throughput.
- Motorized selection of stage position, filter cubes, and objectives with **asynchronous operation**.
- Diffraction-limited imaging optics for fluorescence emission wavelength range **400 nm to 750 nm**.
- Operation and configuration by integrated **MetaXpress software**.
- **Plate-handling robot** (optional) with automated barcode reader.

**System Installation**

The ImageXpress<sup>Micro</sup> is shipped fully configured, and is installed at your site by a Molecular Devices field service engineer. The base system includes the imaging unit, host computer, and toolkit (includes a slide holder, three calibration slides, hex wrenches for changing filter cubes, and a TetraSpeck™ bead plate).

The ImageXpress<sup>Micro</sup> host computer is shipped with the MetaXpress software already installed, and the instrument is connected to the host computer during installation. There are four main connections, excluding power cords:

- Power supply to the ImageXpress<sup>Micro</sup>.
- Light guide from the external lamp to the ImageXpress<sup>Micro</sup>.
- USB 2.0 data cable from the ImageXpress<sup>Micro</sup> to the host computer.
Introduction

- Cable from the CCD camera to the computer.

The optional Photometrics CoolSNAP™HQ camera requires a dedicated power supply and associated power supply cable to the ImageXpress MICRO.

If you need to install or re-install the MetaXpress software on a new computer, please see the MetaXpress ImageXpress MICRO System Administration Guide, or contact Technical Support.

About this Guide

This User’s Guide is intended to document the technical features and specifications of the ImageXpress MICRO system hardware, as well as assist the user in the procedures of equipment installation and configuration, maintenance and troubleshooting. If you are a new user of the ImageXpress MICRO imaging system, we recommend that you take some time to familiarize yourself with the instrument using this document as a reference.

Other valuable information is contained in the MetaXpress ImageXpress MICRO System Administration Guide. We recommend that you consult it in conjunction with this guide.

Here is a brief summary of the contents of this User’s Guide:

- Please read the Important Safety Information at the end of this Guide to avoid injury to yourself or damage to your equipment.
- Chapter 1 is an introduction to the ImageXpress MICRO, and includes a checklist of parts and system set-up.
- Chapter 2 contains descriptions of many of the major components of the ImageXpress MICRO imaging hardware.
- Chapter 3 briefly outlines the theoretical concepts involved in the instrument’s operation.
- Chapter 4 outlines instructions for the care and maintenance of the ImageXpress MICRO instrument, and describes how to access and replace user-serviceable and user-changeable components.
• Chapter 5 describes the optional robotic plate-handling hardware and user procedures.

• The Appendices include details regarding Technical Support, warranty and repair issues, safety information, and site requirements for installation of the ImageXpressMICRO hardware.
Chapter 2: Imaging System Hardware

Illumination System—Excitation

**Light source**

ImageXpress\textsuperscript{MICRO} has an external light source Xenon arc lamp (300 W), which provides continuous, high-intensity broadband illumination. The long-life lamp has a minimum expected lifetime of 500 hours.

**Cold mirror**

The light source incorporates a cold mirror which prevents light with wavelengths longer than 675 nm from reaching the sample, minimizing sample heating and stress on optical components.

**Light guide**

A liquid light guide couples the light from the light source to the illumination optics in the ImageXpress\textsuperscript{MICRO} system unit. The lamp and its housing, constituting the light source assembly, are precisely aligned with the light guide during manufacture, and require no further position adjustment.

**Illumination optics**

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

**Shutter**

A solenoid-activated mechanical shutter controls the exposure of the sample to excitation light to minimize sample degradation and photobleaching.

**Filter cube changer**

The 5-position filter cube changer takes standard Nikon TE2000 filter cubes. ImageXpress\textsuperscript{MICRO} uses Semrock filters.

**Objective (Z) Stage**

**Motorized Z stage**

The Z stage is controlled by a linear encoder and has better than 100 nm resolution.

**Objectives**

Standard objectives are Nikon CFI60 series. The selected objective lens focuses excitation light onto the sample, and collects fluorescence light emitted by the sample. Molecular Devices offers a wide range of objectives to suit your experimental needs.

**Motorized objective changer**

There is a 4-position objective changer. Only the selected objective moves up and down in Z.

**Sample (X-Y) Stage**

**Sample**

The plate holder is designed for scanning multiwell plastic and glass bottom microplates in standard ANSI (SBS) formats, but can accommodate other plate formats that have standard microplate footprint dimensions. For example, glass slides can be imaged using a slide adapter included in
the tool kit. Optimal image quality depends on plate flatness 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/unload position, and automatically closes when the X-Y stage moves the plate into position for imaging.

**Motorized X-Y stage**

The X-Y stage is controlled by a linear encoder and has better than 100 nm resolution.

**Autofocus Laser (optional)**

**Autofocus laser**

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

**Imaging System—Emission**

**Tube lens**

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

**CCD camera**

ImageXpressMICRO has been optimized for use with the Roper Scientific / Photometrics CoolSNAPHQ or CoolSNAPES cameras.

The CoolSNAPHQ is a 12-bit system that uses a 1392 × 1040-element CCD (6.45 × 6.45-μm pixel pitch), and has peak quantum efficiency greater than 60%.
Electronics

The ImageXpress\textsuperscript{MICRO} instrument also contains the following components:

- External power supply and cable.
- USB 2.0 port and cable to computer for device control.
- Camera cable to camera PCI board in computer.
- Optional power cable for the CoolSNAP$^\text{HQ}$ camera.
Chapter 3: Basic Operational Theory

Fluorescence Imaging

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

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

Excitation and Emission Filters

In the ImageXpressMICRO system the excitation and emission filters are located in a filter cube.
In order to selectively excite one fluorophore more intensely than another, or in other words, to minimize excitation channel crosstalk, it is necessary to provide illumination containing only photons with a wavelength range matched to the target dye’s absorbance (excitation) spectrum. If the primary illumination source provided is broadband, such as the Xenon arc lamp in the ImageXpress<sup>Micro</sup> system, a bandpass filter in the illumination optical path (called the excitation filter, since it filters the excitation light) is used to restrict the illumination spectrum to a narrow range of wavelengths.

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

**Dichroic Mirror**

In the ImageXpress<sup>Micro</sup> system the dichroic mirror is in a filter cube.

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

In the illumination path, the dichroic mirror reflects shorter wavelengths from the light source up through the objective onto the specimen, as shown in Figure 1:
Figure 1. Dichroic mirror creates two light pathways.

Incident light from the illumination source that is longer wavelength than the cutoff is transmitted to a beam dump that absorbs and diffuses the waste light to prevent it from entering the imaging optical path.

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

Dichroic mirrors are interference filters made by depositing a number of thin film coatings on a glass support. Dichroics need to be kept thin for high image quality, so the supporting glass is quite fragile, and generally the film coating is not protected with a cover glass. This means that unprotected dichroics are delicate and easily damaged components, and so care must be taken when handling them.

Dichroic Transmission Spectrum

An ideal dichroic mirror would have an infinitely sharp cut-off: that is, it would have unity transmittance coefficient at wavelengths longer than the cut-off, and zero transmittance (and therefore unity reflectance in a non-
absorbing dichroic mirror) shorter wavelengths. In practice, the characteristic transmission spectrum for a dichroic looks something like the figure below:

![Figure 2. Example of a transmission spectrum of a dichroic.](image)

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

The ImageXpress\textsuperscript{Micro} can be configured with any of the following high quality Nikon objectives:

<table>
<thead>
<tr>
<th>Objective</th>
<th>NA</th>
<th>WD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFI PLAN FLUOR 4X</td>
<td>0.13</td>
<td>17.10</td>
</tr>
<tr>
<td>CFI PLAN APO 4X</td>
<td>0.20</td>
<td>15.70</td>
</tr>
<tr>
<td>CFI SUPER FLUOR 10X</td>
<td>0.50</td>
<td>1.20</td>
</tr>
<tr>
<td>CFI PLAN FLUOR ELWD 20X C correction collar 0–2 mm</td>
<td>0.45</td>
<td>7.40</td>
</tr>
<tr>
<td>CFI PLAN FLUOR ELWD 40X C correction collar 0–2 mm</td>
<td>0.60</td>
<td>2.7–3.7</td>
</tr>
<tr>
<td>CFI PLAN FLUOR 10X</td>
<td>0.30</td>
<td>16.0</td>
</tr>
<tr>
<td>CFI SUPER FLUOR 20X</td>
<td>0.75</td>
<td>1.0</td>
</tr>
<tr>
<td>CFI-PLAN APO 40X correction collar, Spring load</td>
<td>0.95</td>
<td>0.12–0.16</td>
</tr>
<tr>
<td>CFI SUPER FLUOR 40X 0.11–0.23 correction collar, Spring load</td>
<td>0.90</td>
<td>0.30</td>
</tr>
<tr>
<td>CFI PLAN FLUOR ELWD 60X C correction collar 0.5–1.5mm</td>
<td>0.70</td>
<td>1.1–2.1</td>
</tr>
<tr>
<td>CFI PLAN FLUOR 60X correction collar, Spring load</td>
<td>0.85</td>
<td>0.44–0.48</td>
</tr>
<tr>
<td>CFI PLAN FLUOR 100X Dry with correction collar 0.14–0.2, Spring load</td>
<td>0.95</td>
<td>0.30</td>
</tr>
<tr>
<td>CFI PLAN FLUOR 40X OIL Spring load</td>
<td>1.30</td>
<td>0.20</td>
</tr>
<tr>
<td>CFI PLAN APO 60X A OIL Spring load, Lock-up</td>
<td>1.40</td>
<td>0.21</td>
</tr>
<tr>
<td>CFI PLAN FLUOR 100X OIL Spring load, Lock-up</td>
<td>1.30</td>
<td>0.20</td>
</tr>
</tbody>
</table>

If the objective you want to use is not listed, please contact us to verify compatibility with the ImageXpress\textsuperscript{Micro} system.

Note that the extra-long working distance (ELWD) objectives have adjustable spherical-aberration correction collars for imaging through thick substrates such as most microplates. Please see User Procedures in this manual for details on how to calculate and set their correct values.

Several of the other objectives (e.g., CFI SUPER FLUOR 40X) also have correction collars for adjustment according to the thickness of the glass cover slip being used. Setting of these collars should be done according to
the cover slip manufacturer’s specifications or through optimization of image quality.

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

For detailed information on objectives, please see the Nikon website (www.nikon.com).
Chapter 4: User Procedures

⚠️ User Safety Instructions

To avoid personal injury or damage to your equipment during user service and maintenance, it is important to strictly observe the safety information outlined immediately below:

- Ensure that the power supply for the ImageXpress\textsuperscript{MICRO} instrument is turned OFF and the power cable unplugged. If the MetaXpress software is running, first exit the program before turning off the instrument.

- Disconnect the USB connection to the hardware server (host) PC, and turn off any attached peripherals, such as the robot plate-loading arm.

- Access ONLY the user-serviceable components inside the enclosure—avoid contact with other components as they may be damaged or knocked out of alignment.

**Caution:** Be sure not to touch the autofocus laser.

- Keep liquids, vapor and dust well away from the interior of the instrument. Do not attempt to clean inside the enclosure.

- Do not leave the interlocked access panels open for extended periods of time.

- Ensure all components and access panels are replaced before restarting the instrument.
Powering ON/OFF the ImageXpress\textsuperscript{MICRO}

**Powering ON**

1. Turn the external lamp ON with the power switch located on the light source.
2. Wait at least 15–20 minutes for the Xenon lamp to stabilize before acquiring images.
3. Turn the ImageXpress\textsuperscript{MICRO} instrument ON with the main power switch that is located on the external power supply.
4. If the system is configured with a CoolSNAP\textsuperscript{HQ} camera, turn on the camera with the power switch located on the camera’s power supply.
5. Turn the host computer ON.
6. Refer to the MetaXpress ImageXpress\textsuperscript{MICRO} System Administration Guide for instructions on starting and running the MetaXpress software.

**Powering OFF**

1. Exit the MetaXpress software and any applications being used with the ImageXpress\textsuperscript{MICRO} instrument or associated hardware.
2. Turn the ImageXpress\textsuperscript{MICRO} instrument OFF at the main power switch that is located on the external power supply.
3. Turn the external lamp OFF with the power switch located on the light source.
4. If the system is configured with a CoolSNAP\textsuperscript{HQ} camera, turn off the camera with the power switch located on the camera’s power supply.

Do not power cycle the light source too frequently. It is better to leave the lamp on for a short while when it is not being used than to turn it on and off frequently. If the lamp is unused for a longer period of time, turn it off.
Light Source

Changing the lamp

When it is time to replace the lamp in the ImageXpress\textsuperscript{MICRO} light source, be aware that the whole lamp assembly (bulb and heat sink) must be replaced, and not the bulb alone.

Please contact Molecular Devices Technical Support for instructions.

Changing the light source fuse

The ImageXpress\textsuperscript{MICRO} light source ships with a spare fuse.

If you need to change the fuse in the light source, please contact Molecular Devices Technical Support for instructions.

Filters Cubes

If you decide to replace or add to any of the optical components in the factory-standard ImageXpress\textsuperscript{MICRO} instrument, there are two procedures that need to be completed:

- Changing the component within the instrument.
- Updating the software to reflect the new hardware configuration.

The following instructions outline how to change the hardware components only. For instructions on updating the software, please refer to the MetaXpress ImageXpress\textsuperscript{MICRO} System Administration Guide.

Changing filter cubes

The ImageXpress\textsuperscript{MICRO} instrument’s filter cubes are mounted in a five-position slider within the instrument enclosure. Filter cubes are delicate components, and special care is required when handling them. Please follow these outlined instructions for adding or replacing a filter cube.

You can leave the lamp powered on during this procedure.

⚠️ Caution: We advise wearing powder-free gloves during the following procedure to prevent skin oils from damaging optical coatings. Read and follow the User Safety Instructions at the beginning of this chapter for safe user-service procedures.
1. Open the Meta Imaging Series Administrator program from the Windows Start menu.
2. Select Configure Hardware.
3. Select Configure Devices.
5. Click on Settings.
6. Click the Eject Filter Cubes button.
7. Close the Meta Imaging Series Administrator program.
8. Power OFF the ImageXpressMICRO instrument at the main power switch, which is located on the external power supply.
9. Open the door on the front of the instrument that allows access to the filter cube changer.
10. Pull up on the front latch to release the filter cube cassette from the filter cube changer.

![Image of filter cube changer]

**Figure 3.** Removing filter cubes.

12. Remove the filter cube cassette.
13. Loosen the hex screw that holds the filter cube in place, and remove the cube.
14. Slide the new cube into place and lightly tighten the hex screw.
15. Place a finger underneath the ejected filter cube changer in the instrument to hold it in place. Carefully line up the filter cube cassette with the changer, and push the filter cube cassette back into place until the latch engages.

⚠️ Caution: If you feel resistance while replacing the filter cube cassette, do not proceed. Remove it and recheck to make sure that it is lined up correctly with the changer.

16. Power ON the ImageXpress Micro instrument at the main power switch, which is located on the external power supply.
17. Open the Meta Imaging Series Administrator program from the Windows Start menu.
18. Select Configure Hardware.
21. Click on Settings.
22. Click the Load Filter Cubes button.
23. Follow the procedures described in the MetaXpress ImageXpress Micro System Administration Guide to specify the new filter cube. The position closest to the door is position 1.
24. Close the Meta Imaging Series Administrator program.

Objectives

If you decide to add or replace any of the optical components in the factory-standard ImageXpress Micro system, there are two parts to the procedure:

- Changing the component within the instrument.
- Updating the software to reflect the new hardware configuration.
The following instructions outline how to change the hardware components only. For instructions on updating the software, please refer to the MetaXpress ImageXpress<sup>MICRO</sup> System Administration Guide.

⚠️ Caution: Read the section “Correct Objective Placement” before installation or replacing an objective.

Correct objective placement

Molecular Devices recommends that users place the ELWD objectives (20X, 40X, 60X) or any other objective with a correction collar in one of the two outer positions (1 or 4) so that the correction collar can be accessed from one of the side panels.

Changing objectives

The ImageXpress<sup>MICRO</sup> instrument’s objectives are mounted in a four-position linear selector. Objectives are very delicate components, and special care is required when handling them. Objectives can be added or replaced by following these steps.

You can leave the lamp powered on during this procedure.

⚠️ Caution: We advise wearing powder-free gloves during the following procedures to prevent skin oils from damaging optical coatings.

1. Read and follow the User Safety Instructions at the beginning of this chapter for safe user-service procedures.

2. Read and follow the previous section outlining Correct Objective Placement.

3. In MetaXpress or the Meta Imaging Series Administrator software move the objective changer to an appropriate position for installing the objective. Selecting position 1 (the position on the right) moves the objectives to the left, and selecting position 4 (the position on the left) moves the objectives to the right.

4. Exit MetaXpress software and turn off the ImageXpress<sup>MICRO</sup> instrument at the main power switch, which is located on the instrument’s external power supply.

5. Place the new objective in its protective casing on a clean work area surface near the front of the ImageXpress<sup>MICRO</sup> instrument.
6. While moving objectives in and out, beware of the free-moving stage (it slides around loosely when the instrument is powered off). This may be a hazard to the objective in your hand.

7. Remove the left or right (as appropriate) side panel of the instrument by grasping the handle, pulling it away from the instrument, and supporting the back of the door with your other hand.

8. Reach in and unscrew any objective you want to remove. To access position 2, you will need to remove the objective in position 1. To access position 3, you will need to remove the objective in position 4.

9. Set the objective’s correction collar, if applicable.

10. Reach in and screw in the objective.

11. Replace the side door by aligning the tabs at the back of the door, and then snapping the front of the door into place.

12. Turn ON the ImageXpressMICRO instrument at the main power switch, which is located on the instrument’s external power supply.

13. Follow the procedures described in the MetaXpress ImageXpressMICRO System Administration Guide to specify the new objective within the software.

**Cleaning objectives**

In the event that debris or contaminants have collected on an objective, follow these instructions for cleaning the objective lens:

1. Read and follow the User Safety Instructions at the beginning of this chapter for safe user-service procedures.

2. In MetaXpress or the Meta Imaging Series Administrator software select the desired objective.

3. Open the top door.

4. Exit MetaXpress software and turn off the ImageXpressMICRO instrument at the main power switch, which is located on the instrument’s external power supply.

5. To remove dust, use compressed air to blow dust contaminants off objectives.
Caution:

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

6. To wipe the objective free of contaminants, use lens paper and solvent of choice. If unsure which solvent to use, consult the objective manufacturer for preferred cleansing solvent and procedure.

Caution: Do not use Kimwipes® to wipe a lens.

Adjusting the spherical-aberration correction collar on ELWD objectives

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

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

- Obtaining the plate specifications from the plate manufacturer.
- Smashing a spare plate and using calipers to measure the thickness.
- Measuring the optical thickness with the laser autofocus and multiplying it by the refractive index (1.59 for polystyrene; 1.52 for glass).

Once you have determined the thickness of your plate or slide, follow these steps to adjust a given correction collar:

1. Read and follow the User Safety Instructions at the beginning of this chapter for safe user-service procedures.
2. Follow the steps in **Changing Objectives** (above) for accessing the objective selector and lenses. If you put these objectives in the outer two positions, they can be accessed from the two side doors, which can be removed by hand.

3. Locate the correction collar on the objective that you want to adjust. Note the graduated scale on the barrel and its current setting. You may have to use a flashlight to view the markings.

4. Rotate the correction collar to its new setting.

5. Securely close the access doors.

6. Test the correction collar setting by examining the image quality of acquired images. If the quality has degraded, re-adjust the correction collar.

**Using oil-immersion objectives**

Oil-immersion objectives may be used with the ImageXpress\textsuperscript{MICRO} for research-mode imaging. Please consult with your sales representative for ordering information.

To apply oil to the objective:

1. Eject the plate to open the top door.

2. Remove any plates in the system.

3. Sparingly add oil to the top of the appropriate objective using a dropper bottle.

4. Insert the sample, either with a thin glass coverslip or in a microplate with a thin glass bottom (oil-immersion objectives are not compatible with plastic microplates).

In the MetaXpress software, slowly step up the objective until you are near to focus. Please note that oil-immersion objectives are not recommended for scanning entire microplates.

When you are done with the oil-immersion objective, eject the plate, remove the sample, and clean the top of the objective with a piece of lens paper.
Cleaning the ImageXpress\textsuperscript{MICRO}

In the event that your instrument needs service or preventative maintenance, it is necessary first to clean and decontaminate the instrument. The following protocol is designed to destroy biohazardous material specifically within the plate-loading region without damaging the internal components of the imaging system. To prevent damaging the instrument, please read and follow these precautionary guidelines carefully:

- In order to protect the ImageXpress\textsuperscript{MICRO} optics and electronics, do not remove the front panels of instrument during the cleaning procedure.
- Do not use any cleaning agents other than those recommended below without first contacting Molecular Devices Technical Support.
- Do not use ultraviolet light for sterilization, as this may damage plastic components.
- Do not use any organic solvents without first consulting Technical Support.
- Do not pour or squirt water or alcohol directly onto the instrument, to prevent damaging internal components.

For any decontamination procedure, we recommend that you wear gloves. In general, we recommend using disinfectant wipes (or Kimwipes\textsuperscript{®} with 70% ethanol) to remove biological agents. To remove non-viable biohazardous agents (carcinogens, toxins) we recommend first wiping the contaminated area with a damp wipe, followed by a second wipe with 100% ethanol to speed drying. With any aqueous cleaning agent, be sure not to use so much that excess liquid flows down into the instrument.

You can leave the lamp powered on during this procedure.

Decontaminating the ImageXpress\textsuperscript{MICRO} instrument

1. Read and follow the User Safety Instructions at the beginning of this chapter for safe user-service procedures.

2. Open the door in MetaXpress software. Exit MetaXpress software and turn off the ImageXpress\textsuperscript{MICRO} instrument at the main power switch, which is located on the instrument’s external power supply.
3. Ensure that the side panels have not been removed, the filter cube access door is closed, and no sample is loaded.

4. With gloved hands, use a damp wipe to wipe down the entire outer surface including side panels and top panels of the instrument. Then, use an alcohol wipe or a disinfectant wipe and go over the entire surface again.

5. Now, use forceps wrapped with Kimwipes® to gently wipe the perimeter of the plate/stage region where a plate would normally be loaded. Wipe with damp Kimwipes® first, then with an alcohol or disinfectant wipe.

6. The stage is freely moving without power, so to decontaminate the plate/stage region underneath where the plate is loaded, you can open the door and slide the stage around.

7. Use a fresh damp wipe to wipe down the stage area underneath and around the plate loading region, followed by wiping with an alcohol wipe or disinfectant wipe.
Chapter 5: Robotic Plate Handling (Optional)

If your ImageXpress\textsuperscript{MICRO} has the robotic plate handler integrated with the base imaging system, there are additional hardware and user procedures to become familiar with. The robotic plate handler is integrated with the ImageXpress\textsuperscript{MICRO} system in such a way that plates can be scanned for barcodes, loaded onto the imaging system where images are acquired and then returned to a home location.

If at any point you would like to upgrade your system with the robotic plate handler, contact Molecular Devices sales support.

**CRS Catalyst Express Robot: Hardware**

The CRS robotic plate handler is easily integrated with the ImageXpress\textsuperscript{MICRO} imaging system. The Robot step-by-step protocol is as follows, with specific components highlighted in the following figure:

1. Fetch a microplate from a hotel shelf.
2. Scan the microplate barcode.
3. Load the microplate onto the ImageXpress\textsuperscript{MICRO}.
4. Acquire images.
5. Unload the microplate from the ImageXpress\textsuperscript{MICRO}.
6. Return the microplate to the original hotel shelf.
CRS Catalyst Express Robot User Procedures

Loading plates onto the CRS Catalyst Express robot

In order to use the CRS Catalyst Express robot to scan barcodes and to load and unload plates onto the ImageXpress\textsuperscript{MICRO} instrument, you must properly load your plates onto the hotels of the robotic plate handler. If the plates are not loaded in the correct orientation, two errors will occur:

1. The barcode will not be scanned.
2. Well position A1 will not be correctly located in the front left corner of the plate-loading region on the ImageXpress\textsuperscript{MICRO} instrument.

The following steps will prevent these errors:

1. If you plan to scan barcodes, properly affix the barcode labels before loading plates onto the robotic plate handler. We recommend that labels are located on the front left side of the plate and they must be consistently located in the same position on all plates being scanned. For example:

![Figure 4. Boxed region shows barcode affixed on front of microplate.](image)

Note: We suggest using labels with a minimum line width of no less than 7.5 mm, preferably 10 mm. For recommendations on compatible barcode vendors, please contact Molecular Devices Technical Support.
2. The CRS Catalyst Express has three vertical racks ("hotels") onto which you can load microplates. The directions that follow assume you are positioned in front of the robot as pictured immediately below. The hotel numbers (1–3) are at the front of the robot.

![Figure 5](image1.png)

Figure 5. Numbers mark the three hotel racks where microplates are loaded.

3. Plates need to be loaded into the hotels with the barcodes facing the front of the robot.

![Figure 6](image2.png)

Figure 6. Box shows barcode centrally located on the microplate, which should be facing toward the front of the hotel.

4. When correctly placed in the hotels, well position A1 is located in the front left corner as viewed from the front of the robot.
5. If your plates are loaded with the barcode facing front and A1 in the front left location, then your plates will be scanned and correctly loaded onto the ImageXpress\textsuperscript{MICRO} plate-loading region.
Appendix A: Technical Assistance

Molecular Devices is committed to providing the highest quality technical support available. To expedite the processing of your problem or query, please follow these steps first:

- If the problem only occurred after recent changes to your hardware or software configuration, restoring your system to the old configuration may help to identify which change caused the problem.

- Try to determine if the problem only occurs with particular configuration files, experiment settings, journals, application modules, or data sets.

- Note your system specifications before contacting Technical Support: instrument model number, PC hardware configuration, MetaXpress software version number and Windows version.

- Carefully note the problem description: the initial (startup) instrument hardware and software settings, then the exact sequence of steps needed to reproduce the problem.
For technical assistance, product comments and feedback, or help to resolve a problem, please visit Molecular Devices Technical Support webpage at www.moleculardevices.com/pages/support or you may reach us by phone at 1-800-635-5577 (US only); elsewhere, contact your local representative.
Appendix B: Important Safety Information

The operator of the ImageXpress\textsuperscript{MICRO} instrument is assumed to be trained in the correct operation of the instrument and the safety issues. Throughout the ImageXpress\textsuperscript{MICRO} User’s Guide, the word “you” refers to this trained operator. Using controls, making adjustments, or performing procedures other than those specified in this guide may result in hazardous exposure to laser light, high voltage, hot surfaces or moving parts. Exposure to these hazards can cause severe or fatal injury.

Safety text used in this guide

Make sure you follow the precautionary statements presented in this guide.

⚠️ Warning: Indicates a possibility of severe or fatal injury to the user or other persons if the precautions are not observed.

⚠️ Caution: Indicates that damage to the instrument, loss of data, or individual injury could occur if the user fails to comply with the advice given.

⚠️ Important: Highlights information that is critical for optimal performance of the system.

💬 Note: Identifies items of general interest.

The protective housing

The protective outer housing and instrument panel interlocks are designed to protect you from exposure to laser light, hot surfaces or moving parts.
⚠️ **Warning:** Do not defeat the interlocks, open the protective housing, or try to gain access to the interior of the instrument through any other openings, unless specifically instructed by one of the User Procedures outlined in this manual. Read each procedure carefully and follow all outlined safety precautions. Incorrectly opening the outer protective housing can damage the instrument components and result in hazardous exposure to laser light, hot surfaces or moving parts.

### Interlock failure

There are three safety interlocks on the automated door. Do not operate this instrument with the door open.

⚠️ **Warning:** Do not disable any of the interlocks. When the automated door is opened, the interlocks trigger both the laser light source and the motion control electronics to turn off to prevent hazards associated with laser emission or moving parts.

If you experience any of the following symptoms, you may have interlock failure:

- The focusing laser stays on after the automated door is opened.
- The sample stage or filter mechanisms continue to move after the automated door is opened.

If this is the case, it is unsafe to continue using the ImageXpress\textsuperscript{MICRO}. Please contact Technical Support immediately (see *Appendix A: Technical Assistance*).

### Non-interlocked panels

There are several other panels on the instrument that are intended for use by field service personnel and are not interlocked. All non-interlocked service panels are secured to the protective housing using screws and require a special tool to remove.

⚠️ **Warning:** Make sure the instrument is powered OFF and power cable unplugged in the event you are instructed to remove non-interlocked panels. Absolutely do not operate or access the interior of this instrument with any covers or panels removed.
**Laser safety**

The ImageXpress\textsuperscript{MICRO} is a Class I laser device. There may be a high power laser (used for auto-focus) embedded within the unit, which the user cannot and should not attempt to access. The embedded laser classification and power follows:

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Power</th>
<th>Divergence</th>
<th>Duration</th>
<th>Embedded Laser’s Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>690 nm</td>
<td>20 mW</td>
<td>&lt; 1 mrad</td>
<td>Fast Pulsing</td>
<td>Class III</td>
</tr>
</tbody>
</table>

Samples can be placed safely in the loading area without exposure to laser power levels above Class I. Even with the top panel removed and the laser beam exposed, the beam passes through a microscope objective and the resulting light is so divergent and diffuse that the measured power is well below Class I.

⚠️ **Warning:** Deliberately removing the top panel and the microscope objective, and staring directly into the laser beam can cause eye injury or blindness.

**Lamp safety**

The ImageXpress\textsuperscript{MICRO} is equipped with an external 300 W Xenon lamp. The lamp has a limited lifetime and will need to be replaced upon failure (see User Procedures within this manual).

⚠️ **Warning:** In the event that the lamp requires replacement, ensure that you have allowed the lamp to cool for at least 30 minutes. The lamp generates an extreme amount of heat and attempting to remove the lamp immediately after use can result in injury.

**Liquid light guide**

The ImageXpress\textsuperscript{MICRO} uses a high-powered Xenon lamp that emits ultraviolet radiation. The infrared and ultraviolet radiation generated by this lamp can cause significant skin burns and eye damage.

⚠️ **Warning:** Do not remove the light guide from the instrument or the lamp when the lamp is powered on.
High-voltage hazard

There are no high-voltage electronics found inside the ImageXpress® MICRO. High-ignition voltages do exist inside the external Xenon lamp light-source housing, which can be lethal.

⚠️ **Warning:** Do not operate the light source with the housing open or powered on.

Moving parts

The ImageXpress® MICRO contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts. The interlocks and protective housing are designed so you cannot access the moving parts during a scan.

⚠️ **Warning:** Do not try to gain access to the interior of the instrument unless specifically instructed to do so. The moving parts inside the instrument can cause injury. Do not operate this instrument with covers or panels removed.

Fuses

The Xenon light source contains a fuse. In the event of fuse failure, disconnect the power cord and consult User Procedures for instruction on how to replace the fuses. The instrument power supply contains a circuit-breaker switch with a trip point of 18.75 amps.
Power supply

The ImageXpress\textsuperscript{MICRO} has one cable running from the instrument to the external power supply (input voltage range is from 100 to 240 VAC, 50/60 Hz, 2 A).

⚠️ **Warning:** Make sure this cable is unplugged before accessing any part of the instrument. Failure to do so may result in serious harm.

Lifting hazard

⚠️ **Warning:** The ImageXpress\textsuperscript{MICRO} weighs approximately 180 lbs (82 kg). Do not attempt to lift or move the instrument without assistance. Moving your instrument can disrupt sensitive optical alignments. It is recommended that you call customer support to schedule a Field Service engineer to help with moving your instrument. Your warranty or service contract will not cover problems caused during or as a result of shipment or relocation.

Maintenance and service

User service and maintenance is strictly limited to the procedures outlined in this manual. Access for the majority of these procedures is via the interlocked panels described above. No other user service not outlined
within this manual is permitted. If there is a problem or you have questions, please contact Technical Support.

List of controls

This guide constitutes a list of controls.

⚠️ **Caution:** Use of controls, adjustments or performance of procedures other than those specified within this manual may result in hazardous conditions or injury.

Hazardous material precautions

Use standard laboratory procedures and cautions when working with chemicals.

⚠️ **Caution:** Always follow the manufacturer’s precautions when working with chemicals. Molecular Devices is not responsible or liable for any damages caused by or as a consequence of the use of any hazardous material.

Safety label

If the label becomes illegible or is missing for any reason, please contact Technical Support for a free replacement label. While waiting for a replacement label, copy the label below and attach a copy of the label to the instrument.
Figure 8. Safety label.

Symbol explanations

- Lifting Hazard, Heavy object!
- Attention, Laser radiation!
Appendix C: Site Requirements

The ImageXpress® MICRO is designed to operate indoors under laboratory conditions. For optimal performance, site requirements must be met. As with any precision optical instrument, care should be taken to maintain a low-dust, low-vibration environment. Temperature and humidity extremes may compromise performance.

Environmental Humidity: 5–95% non-condensing.
Altitude: Up to 1.25 miles (2000 m).

Power Requirements: The ImageXpress® MICRO can be directly connected to all international supply voltages. The input voltage range is from 100 to 240 V~ and input frequency range 50 to 60 Hz. No range switching is required. Fluctuations must not exceed ± 10% of the nominal voltage. Use the included IEC power cord to connect the external power supply to a GROUNDED power receptacle that is rated for 15 A. If using a power strip, do not connect the acquisition computer to the same power strip as the instrument and light source.
Power Consumption: ImageXpress<sup>MI</sup> power consumption is 1100 watts for 2–3 seconds at initialization, 800 watts average operating RMS.

Space Requirements: Table or bench top 30 inches (76 cm) deep. There needs to be space below the table for the light source and power supply such that the light guide and power cable can easily reach the back of the instrument.

Rear Clearance: The rear of the instrument should be no closer than 6 inches (15 cm) to a wall.

Weight Requirements: Sufficient to support 180 lbs (82 kg) with minimal vibration.
Index

Abbé Illumination ............... 6
Absorption .............................. 9
Apochromat ............................ 14
Asynchronous .......................... 1
Autofocus ............................... 7
Bandpass ................................. 10
Camera .................................
    CCD .................................. 7
    CCD .................................. 7
Computer System
    Requirements ....................... 5
Connect .................................
    Instrument .......................... 5
    Correction Collar .................... 13, 22
Dichroic .................................. 11
Emission ................................. 9, 10
Epi-Illumination ....................... 1, 10
Excitation ............................... 9, 10
Fluorescence ........................... 1, 6, 10, 11
Fuses ........................................ 36
    Replacing ......................... 17

Hardware Server .................... 2, 15
Host Computer ......................... 2, 15
Illumination ............................. 6
ImageXpress
    Peripherals ........................... 15
    Software ............................. 2, 3, 7, 17, 19

Laser .................................
    Autofocus ............................. 7
    Light Guide ........................... 5
    Longpass ............................. 10
Objective Lens .......................... 6, 10
Objective Turret ........................ 6
Peripherals ............................. 15
Plate Holder ............................. 6
PWM (Pulse Width
    Modulation) ............................ 7
Safety Information .................... 33
Sample ................................. 6
Shutter ................................. 6
Spherical Aberration ............... 22
Telescope ................................. 7