FV3000 Training Guide

Micro and Nano Imaging Facility

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Rev	Description	Date	Author
AA	Initial Document Generation	05/01/19	AC
AB	Updated Training Procedures for COVID	02/25/20	AC
AC	Updated Operating and Sample Loading Protocol	05/25/21	AC
AD	Updated new COVID Policies	06/04/21	AC

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Before Turning the System On

1.) Open up the enclosure and check to make sure the 1.25x is selected. Check to make sure there is no debris around the turret.

If the objective in place is not the 1.25x objective or if the area around the turret is not filled with debris,

a. Notify MNI administration.
b. Clean/remove any debris from around the turret
c. Gently rotate the objective turret until the 1.25x objective is in the active objective.

2.) Open the front access panel on the environmental enclosure box and move the translation stage by hand from end to end 4x in x axis. Repeat for the y axis. This helps evenly distribute the lubricants in the motorized stage and ensures proper operation.

3.) Use a small flashlight or the light from your mobile device and inspect the first lens element of the objective for any films or debris. If the objective is dirty, use LENS TISSUE paper and methanol to gently clean the lens element. **DO NOT WIPE WITH DRY SWABS OR LENS TISSUE AS THIS SCRATCHES TO THE LENS COATING.** Wear proper PPE when handling methanol as it is known to be hazardous to your health. For more information on how to properly clean objectives, please visit https://www.youtube.com/watch?v=Tz4Dy5D6kdw or Google search for a YouTube video on How to clean an Objective Lens.

4.) Please remember to reset system to 1.25x objective after use

5.) Please acknowledge MNI and S10 Grant # in any papers or publications.

The specific wordage is available on the instrument webpage and also included below.

"Research reported in this publication was supported by the Boston University Micro and Nano Imaging Facility and the Office of the Director, National Institutes of Health of the National Institutes of Health under award Number S10OD024993. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health."

COVID-19 Policies

As of June 14th, 2021, COVID related cleaning and occupancy restrictions have been rescinded per BU policy. For the most complete and up-to-date policies, users are asked to refer to the BECF website for details.

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System Startup/Shutdown

Confocal Hardware

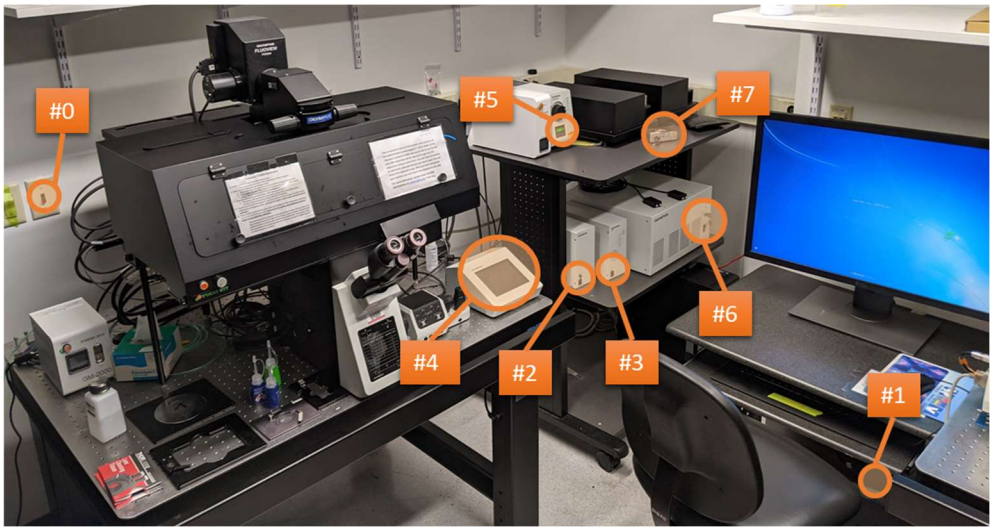

Figure 1. Instrument numbers and location.

Please use the figure above and the instructions below to power on the various confocal hardware components. If you are unsure, please contact an MNI administrator or your lab's designated power user for assistance.

1.) Turn on #0, which is the big red button/switch on the wall behind the instrument. This is the laser in use switch that turns on the AC power outlet to the lasers and the laser in use signs located outside the imaging bay and the facility.

2.) Turn on #1, which is the computer. Usually, the computer can be left on but with the user logged off the system.

3.) Turn on #2 and #3. These are the motor control units for the microscope. They are located on second shelf of the mobile rack.

4.) Turn on #4. This is the hand controller for the instrument. Turn on by pressing the button on the back of the unit. **When turn it off, press the "off" on the screen, then push the button on the back to turn off.**

5.) When #4 has finished powering on, press "Start Operation".

6.) Open up the front cover of the environmental chamber if it is not already open. Locate the XY control knobs that moves the motorized microscope stage. Check to make sure motion is set to course.

7.) Turn the X knob and visually inspect that the stage is moving parallel to the wall. Repeat for the Y knob and make sure it is moving perpendicular to the wall.

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If the stage is not moving or you are experiencing problems controlling the stage in the software, please power off #1 through #4, wait for one minute, and then power them back on.

8.) Turn on #5 by pressing and holding "on/off" button for 1 sec. This is the epi-burner lamp. The blue light will show that it is on. The knob on the front adjusts the power out of the unit into the microscope. This is the light source you will use to visualize your sample when looking through the binocular. To turn off, press and hold the on/off button for 3-5 seconds and then release. **Leave on at least 30mins before turning off or leave on until end of imaging session and then turn off. There will be a 300 second count down timer. During this time, you will not be able to restart the lamp.**

9.) Turn on #6. This is the laser control unit. **Turn the button on and key it on. It is very important to make sure that you turn the key to the "ON" position.** Otherwise, the laser fans don't turn on and they overheat and kill the laser. To turn off, turn key to off position and turn off button.

10.) Key on #7. The LED should turn green. Turn on the switch for the laser that you will be using to image. **Please remember to turn off the lasers and key off the switch after use.**

Inserting and Removing a Sample

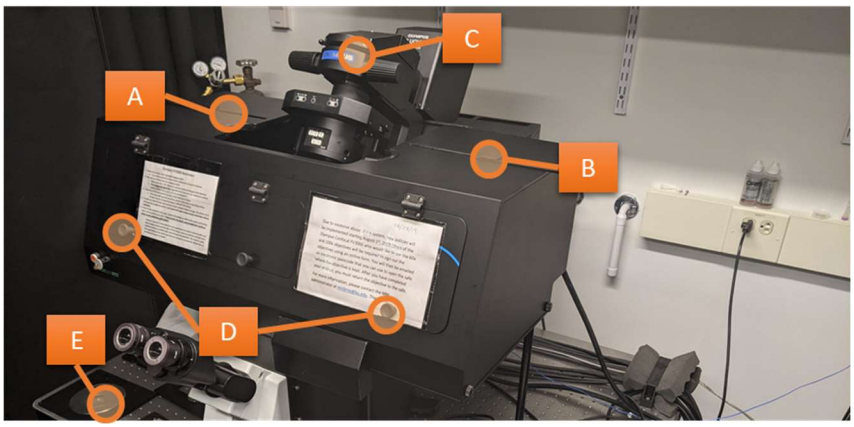


Figure 2. Top of environmental enclosure opened and condenser arm tilted back.

1.) Slide the rectangular bars on the top of the environmental enclosure to the left and right side (A and B). The object is a black rectangle on the top of the box. When closing the box, inspect around the edges of the baffle and the condenser arm to ensure a proper fit. If there is a gap, turn the condenser knobs 1/8 of a turn clockwise and try closing the baffles again.

2.) Push gently on the mark "OLYMPUS" logo on the condenser arm (C) until it starts tilting backward.

3.) Open the front access panel by lifting the left or right-most circular knobs (D). The center knob opens a viewport and only used when you wish to look inside the enclosure.

4.) Insert the desired microscope stage inset. These insets are located to the left of the microscope and on the optical table. (E)

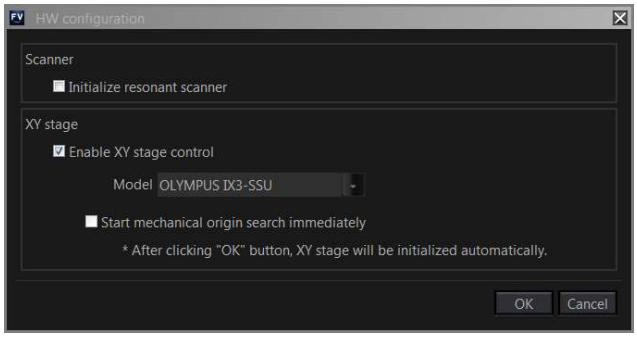
FluoVIEW FV3000 Software

Turning on software

1.) Log into the computer using your BU Kerberos username and password

2.) Open up Olympus software **FV31S-SW**. We recommend you pin this item onto your windows taskbar for easy access.

3.) As the software is loading, you will see a HW configuration dialog box, like the one below, appear on the screen. Make sure these are the settings for the first dialog box.



4.) Another dialog box will appear that will ask if you would like to cleaning the stage. Select “NO”.

Scan Options

The FV3000 is a very robust instrument that can aid you in your research. Below is a short list and brief description of the system’s imaging capabilities. For a complete list and instructions on how to use these imaging modes, please refer to the official Olympus Quick Start Guide that is on the BU website.

Type	Description
XY	This mode captures a standard XY image
XYZ	This mode steps through a specified Z range and captures an XY image at each Z step. This is also known as a Z stack.
XYT	This mode captures a standard XY image at specified time periods. For long duration imaging, there is an additional option to use Z drift compensation (ZDC) to compensate for Z axis drifting over time.
4D (XYZT)	This mode captures a Z stack at specified time periods. For long duration imaging, there is an additional option to use Z drift compensation (ZDC) to compensate for Z axis drifting over time.

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Multi-Area Time Lapse	This mode enables to you selectively image multiple ROI's, tile smaller ROI's into a larger image, or any combination of the two for all image acquisition modes.
Spectral imaging	This mode enables you to scan the fluorescent emission spectra.

Determining the PMT Settings on the FV3000

After you have configured the scan parameters and selected the dyes you wish to image, you will need to tune the PMT detectors. Below is set of procedures to help you get started in determining the right settings for your scan.

1.) Select a single fluorescent channel. Make all other PMT channels are unchecked.
2.) For the selected channel, identify if the detector is an SD or HSD detector.
    a. For SD detector set HV to 750V. For HSD detectors, set HV to 500V.

Setting	**Value**
Laser Power	0%
HV	750
Gain	1
Offset	4

    b. For HSD detector set HV to 750V. For HSD detectors, set HV to 500V.

Setting	**Value**
Laser Power	0%
HV	500
Gain	1
Offset	4

3.) Enable Hi Lo LUT and set the Laser ND filter to 10%.
4.) Run Live x2 and increment the laser power up very slowly until you see weak/some signals in the image.
5.) Use the fine Z focus knob or the Z focus control in the software to step the objective and bring the image into focus.
6.) Adjust laser power so that some pixels appear red/saturated. **If the image shows a lot of RED in Hi-Low mode, STOP THE SCAN IMMEDIATELY.** Turn the laser power down or set the laser power to 0% and start again.
7.) If you feel that the image is too noisy, you may adjust the HV values lower and readjust the laser power to maximize the image intensity. After you have finished, adjust the offset to 3%.

8.) Repeat for the next fluorescent channel. You do not need to refocus with the next channel because the sample will already be focused. Iterate until you have finished tuning all imaging channels.
9.) If you have multiple phases, select the next phase and repeat.
10.) Turn off the Hi-Lo LUT.
11.) You can choose to save these settings as an observation method. It is located under the observation methods tab.

Microscope Objective Options and Accessories

60x and 100x Oil Objectives

There are two large NA, high magnification oil immersion objectives that you can use with the FV3000. Please contact MNI staff through the online portal to request additional training on how to properly use these objectives. After you have completed training, please remember the following.

Each objective uses specialized oil. **DO NOT MIX THESE OILS.**

The 60x objective uses oil from the blue plastic canister.

The 100x uses oil from the green plastic canister.

The objective reservation form must be completed after you've made the instrument reservation and prior to arriving at the facility to use the instrument. The reservation form is located under the "OTHER RESOURCES" section of the webpage for the instrument.

Objectives Options

The facility has a selection of Olympus objectives that is compatible with the FV3000. Please contact us through the online portal for more information and training.

Live Cell Imaging

The FV3000 is equipped accessories that can maintain temperature, humidity, and CO2 for live cell imaging. Please contact us through the online portal for more information and training.