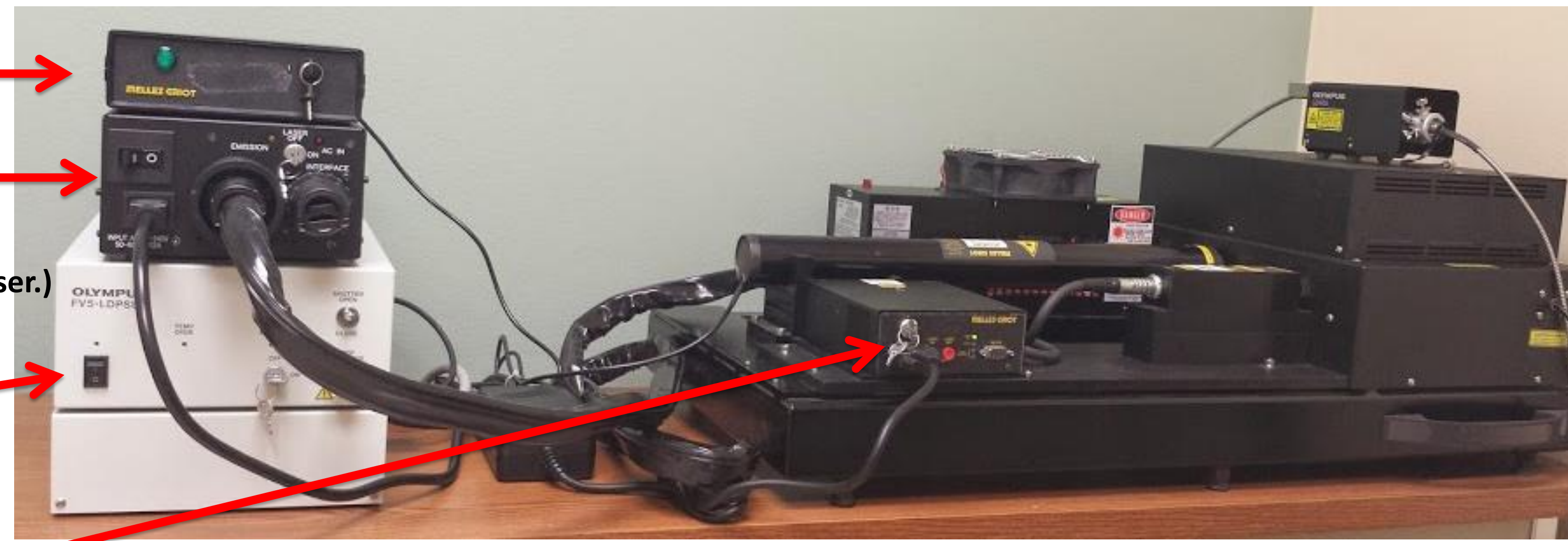


Starting the Olympus Confocal Microscope

1. Turn on ONLY the lasers you will be using.

- **633nm (635 nm, 20 mW)**
- **Argon multi line (458nm, 488nm, 514nm: Total 30 mW)**
 1. Set the power switch to ON. (This starts the fan of the laser.)
 2. Turn the key to the ON position (turn clockwise).
- **405nm: 50 mW (DAPI, HOESTCH)**
Set power switch On, laser emission key always ON and Shutter toggle always OPEN
- **561nm (includes 594nm) HeNe(G) laser (543 nm, 1 mW)**



To ensure stable laser light output, the warm-up period should be 10 minutes or more when using the Argon laser power supply and 30 minutes or more when using the Helium-Neon Green or Red laser power supply.

2. Turn on the modules

- Stage controller (PRIOR)**
- Mercury Lamp power**
(RECORD the hrs on the excel sheet!)
(life on the bulb 200hrs)
- System controller/IX2-UCB**
(microscope "brain")
- FV power supply unit**
(remember to also turn the key ON)
(Galvo control boards, scanning mirrors and gratings, Real time controller- Olympus "brain")
- Turn ON computer**



Starting the FLUOVIEW Software

1. Login- **Olympus**
2. Password-**fluoview**
3. Fill out the user log sheet- Date, User Name, PI, Project ID, UV light time (on the desktop)
4. Double click the **FV icon** found on desktop. (It takes 20 to 30 seconds to fully initialize the hardware. Do not click on anything during this time)
5. Click once on **Start System** to begin FV1000 software initialization.

Configuring Settings (Ex: Double dyeing color XY)

1. Under the **Image Acquisition Control Window** Click on the 3rd button below
2. Click on **DyeList** to open a drop-down window with a **set up dyes** list of common dyes and fluorophores in the database. Choose your dyes.
3. Select you dyes of interest by double-clicking each and pressing **Apply**
The software will automatically configure the parameters to appropriate settings for the dyes
4. Click **Close** button to close the **DyeList**.
5. Under the **Image Acquisition Controller**
(Choose the configuration that best fits your sample)
 - * Click- **Sequential** (multi-track) (Note: sequential scan will take longest to acquire, but will cut down on potential bleed-through between channels)
 - * **Line-** fast
 - * **Frame-** slow

Labels in the screenshots include: Scan Mode, Scan Speed, No. of Pixels, Zoom & Rotation, Laser output, Lambda Scan, Objective lens, Z-stack, Time Scan, Spectral Setting, Focus x2= fast continuous scanning, Bright field Light, Fluorescence Light, Lambda scan, Z- Series, Time course, XY Repeat= scan continuously with set scan speed, XY: acquire your image (snap), Stop Scanning, Confocal aperture, Halogen Lamp Adj., Auto Pinhole Setting, Sequential: Two tracks/ two channels (less bleed through), Select one channel at a time for adjusting.

Finding Your Specimen

1. Load your sample "upside down" onto the stage of the microscope.
2. Click **Image Acquisition**
To view the fluorescent image through the eyepiece, select a filter for viewing the fluorescence (blue, green, red) the shutter will automatically turn on (WU, NIBA, WIG, CY5)
3. In the key pad you can select the objectives . **Be careful & read the touchpad.! Do not put oil onto an air or water objective!!**
4. Focus sample . You can switch from coarse to fine focus by pressing the **C/F button**
5. After focusing to desired objective **close shutter off** by clicking (set to "not pressed")

Acquiring a Preliminary Image

1. Under the same window- If doing multi-track, its easiest to optimize ONE at a time by √ the left corner box
2. Set **Pinhole** to 1 Airy unit (click AUTO button)
3. Press the XY Repeat scan buttons to start the scanning procedure and then user can adjust parameters and acquire an image. (set the scan speed- 2us to scan faster and AutoHV)
4. Alternatively, change the **HV** (never above 700) to increase or decrease the detector sensitivity
5. Adjust focus as needed and adjust **Gain- for saturation**, red pixels (default is 1) and **Offset- for background**, blue pixels (default is 0)
6. Check intensity mode by **Ctrl H** and this will show how much saturation (red) or background (blue) your image has. Press XY Repeat and adjust your parameters.

Acquiring Your Final Image (Single XY Plane)

Publication Parameters:

Scan mode: line
Frame size: 1024 x 1024
Scan speed (pixel dwell time) : 12.5us/Pixel
No. of Pixels: 1:1

1. Click **XY** button to acquire your final image.
2. Save Image: OIB files

* Once you are satisfied that each channel's detector is set optimally to the range of your image, you can create your final image by checking all the channel boxes on the top left corner.

*Recommended: reduce noise by slowing the scan speed and averaging a number of lines.

Shut down of software and system

Please first check the online calendar to see if there is another user coming in up to an hour after you, and call to confirm they are still coming (especially if it is near the end of the day). **If there is another user coming in, close all your files but do NOT exit the FV1000 program, and clean used oil/water slides, but leave everything else ON!!**

1. Move the objective to the empty position
2. **Argon laser-** Turn off only if you are the last user for the day.
IF NOT, leave the argon laser running for the next user.
 - Turn the **key** to OFF position
 - **WAIT FOR 10 MINUTES FOR THE LASER TO COOL BEFORE PROCEEDING!**
Feel the fan on the back of the LASOS box. It needs to be off.
 - This helps to cool the Ar laser and extend its useful life.
 - Set the power switch to OFF (after cool down)
3. **405 nm diode lasers-** If used, turn key off and then turn off switch
4. **633 HeNe lasers-** Turn off only if you are the last user for the day
5. **561 HeNe lasers-** Turn off only if you are the last user for the day
 - First push laser OFF button (Red color) then turn off key
6. Turn off Prior stage controller box
7. Turn off Mercury Burner Power Supply Unit (first log the life time of the bulb on the spreadsheet)
8. Turn off Microscope Control Box IX2-UCB
9. Turn off Main confocal controller
 - First turn off the key and then the Switch
10. Backup your data →D:FV10-ASW\User\Guest\your folder
(Important!! We do NOT store any converted images (Tiff,Jpegs.) ONLY oib files!!)
11. Log off your time in the user log sheet and note any problems encountered
12. Clean any oil or water slides that were used but DO NOT clean OBJECTIVES only if you are properly trained for oil (Please talk Monica)
13. Cover the microscope if you are the last user! Thank you! ☺