Starting up the System

1. Check the temperature on the laser (under the table). It should read 21.0˚C. If it does not, alert someone in the imaging center and do not proceed.
2. On the rack to the left of the table – turn on switches to power up the microscope components:
    a. OLYMPUS power box on bottom shelf
    b. OLYMPUS FV10MP-LCU (1st shelf)
    c. OLYMPUS CBH (second shelf)
    d. OLYMPUS U-HGLGPS (second shelf) – this is optional. Turn this on if you would like to use epifluorescence to locate your specimen.
3. Turn on the touchscreen computer that sits to the right of the microscope. The switch is on the back right corner. Wait for this to boot and touch the "start operation" button that appears. This must be open before you launch the software.
4. Log into ILAB with your full musc.edu user name and the ILAB password. It is not your MUSC password.
5. Click on the FluoView software: FV10-ASW 4.2
    a. A "guest" window will appear. No password is needed. Click through it.
    b. The software should initialize. Be patient, it is testing the communication between the microscope and the software.

OLYMPUS FLUOVIEW Ver.4.2 Software

Under the Acquisition Setting Window.

1. Click on the button under **Laser.**
   The MP Laser Controller window should appear. Turn the laser ON. IT will take ~7 minutes for the laser to warm up. When the laser is ready, the box showing the wavelength will turn green and the mode-lock status will say "Locked" in a green box. Change the wavelength to what is needed for your experiments. Use the slider or type in the % laser power. The laser level will vary with the wavelength, but I would recommend starting at 2% or less.
2. Under **MODE**: Choose a scan speed
3. Under **SIZE**: Choose an aspect ratio and image size
4. Under **AREA**: here you can select the zoom and rotate the view of the image
5. Under **Microscope**: Here you can select the objective, depth and position of z-stacks and the interval in time-lapse imaging.
    a. Z-stack: It can be useful to set 0 before beginning to set up a z-stack.
    b. Focus to the top of your sample and "set" it
    c. Focus to the bottom of your sample and "set" it
    d. You can type in the z-interval or the number of z-slices
6. Under **Time Scan**: here you can set the time lapse interval. Here you can type in the interval of the time lapse and the number of iterations.

Under the Image Acquisition Control Window

1. Top ICON, column on the left. The square with the dot above it: this turns on the light to the microscope. It will appear yellow when you click on it.
2. Second ICON, column on the Left. The square with the dot above it: this turns on the epifluorescence to the microscope and will appear turquoise/green when you click on it.
3. Third ICON, column on the left. Green bulb. Unused.
4. Fourth Icon, rainbow/wrench. It shows the current light path. It will give you no information about the filter cube positions.

5. Fifth Icon, the “i”. This will open a window that will give you a synopsis of your imaging parameters – such as the pixel and image size, the scan speed and z-interval.
6. Sixth Icon, “bright Z”. This will open a window in which you can set a laser brightness correction for depth if necessary.
7. Top of the window. Focus x2 and Focus x4. Use these buttons for scanning as you set up a z-stack or focus. They will scan as fast as possible with a 512x512 resolution.
8. Tope of the window: XY Repeat. This button will scan with the acquisition setting you have selected – it will continue scanning until you stop it.
9. Top of the window: XY button. This will acquire a single scan with your image and stop.
    a. Clicking on the “depth” and the “time” buttons will turn this single scan button into a button that will allow you to acquire a z-stack or time lapse image.
10. Selecting channels. Each channel has 3 sliders: HV, the voltage for the detector, Gain and Offset. Use these to increase the sensitivity of each channel and maximize the signal without increasing the noise. It is not recommended that you use the Gain setting. Remember that by increasing the offset you are changing the true black level. It is possible to essentially erase low pixel intensities from the image if the offset is set too high. Similarly, increasing the Detector voltage (HV) too much will yield an image with saturated pixels.
    a. At any time, if you wish to see a visual representation of the offset and saturation, press CTRL+H. Saturated pixels appear RED and true black pixels appear BLUE.
    b. There are 5 channels that are available:
        - TD1 -- brightfield
        - RXD1
        - RXD2
        - GaAsP1
        - GaAsP2.
    c. The RXD1s and GaAsPs are the detectors for fluorescence. The manual sliders and filter cubes determine what it is that they are able to detect. The light path and available channels are determined by which filter cubes are in or out of the light path and at which position. See accompanying diagram.
    d. Using “sequential” (bottom left corner)
        i. all channels will be acquired simultaneously and using the same laser wavelength and settings unless sequential is checked
        ii. checking “sequential” allows you to use different laser power for each channel and different laser wavelengths
            1. a window will appear that will also allow you to group individual channels together – those that are imaged using the same wavelength and power, for example, should be grouped together.
    e. Kalman – filter mode. You can chose to average multiple scans either line by line or frame by frame
    f. HARD DISK RECORDING. For most all scans except single scans, the computer will alert you that it must use hard disk recording. When selected, each frame is saved as a file and the computer does not rely on RAM during acquisition. This is the preferred and necessary method to save your date when collecting time lapse, multiposition and z-stack images.
        i. Clicking “ON” will bring up a directory – there you should select the folder into which the files will be saved. Give the folder and your file a name. the software will build on the name stem that you provide (name-0001, name-002, etc.)
    g. TR – transmitted light. You can select the intensity of the lamp here.

Touchscreen computer

When using the microscope, it can be convenient to select the objective, turn on and off the light and select the epifluorescence filter cubes here. In general I like to use the FluoView software to do most things. They are supposed to work seamlessly together, but sometimes I feel they do not.

Sliders on the microscope – selecting filters.

There are 4 sliders on the microscope. See diagram.

1. Slider 1. The slider on the microscope base itself that moves in and out of the microscope. Use this to place the filter in for the 2photon, and pull it out so that you can see your specimen through the microscope. This is the easiest one to forget – if you clearly see your sample through the eyepieces, but cannot detect anything with the 2 photon, this is the most likely culprit. Make sure it is IN for image acquisition.
2. Slider 2. This is the slider for selecting the light that is diverted to PMTs 1 and 2
3. Slider 3. This is the slider for selecting the light that is diverted to PMT 2 and all the GaAsPs.
4. Slider 4. This is the slider for selecting whether GaAsP1 will receive any light, or if it will all be diverted to GaAsP 2.

SHUTTING DOWN THE SYSTEM

1. To be safe, click on the wavelength button on the laser window and turn the laser OFF before shutting down the software. If the software crashes, the laser may not turn off. You'll need to relaunch the software to shut off the laser.
2. Close the software. If you have open windows, or have adjusted open images, the software will ask you if you would like to save each one.
3. Log out of Ilab.
4. Turn off the touchscreen computer – click off, wait for the Olympus screen saver window to appear and then click the off button on the back right side of the touchscreen computer.
5. Turn of the 3 or 4 switches controlling the microscope power boxes on the rack.

Olympus 1200 Light Path Diagram