OLYMPUS FLUOVIEW 300 CONFOCAL MICROSCOPE: TURNING ON/OFF THE MICROSCOPE

1. **Turn ON microscope in this order:**
   a. Turn on the computer if not already on. **Password = fluoview**
   b. Turn on lasers in this order: (they need to warm up for ~15 min before imaging)
      i. Turn on two toggle switches (2 small black boxes)
      ii. AC power on (I)
      iii. Turn key parallel to the top boxes (turn it clockwise)
   c. Turn on objective lens control box (bottom Left box)
   d. Turn on mercury lamp (box on top of objective control on Left)
      **** Note: once the mercury lamp is turned on and then turned off, you must wait 15 min before turning it back on again!!!!**
   e. Turn on mixer box (on bottom Middle)
   f. Turn on light switch (Left of computer)
   g. Start software – double click on the Fluoview Microscope Icon on the desktop. The software will not load properly unless the microscope is ON.

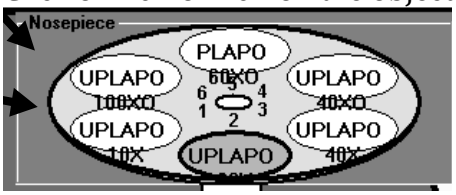
2. Load sample onto glass slide if you haven't done so already (i.e. make a wet mount)

3. Drop the stage by pressing the **TOP** green button on either side of the front of the microscope to LOWER the stage

4. Open the Microscope Control by clicking on the  icon in the software (upper toolbar). A picture of the side-view of the microscope will appear

5. Make sure the Shutter is **closed** while you are getting set up. Click the shutter icon to raise and lower shutter to block lasers  (open)  (closed)

6. Click on 10X or 20X on the objective wheel on the screen.



7. Place your slide on the stage and push the **TOP** green button on the front side of the microscope again to RAISE the stage

8. Focus sample and Acquire image. **See Imaging Procedures Manual.**
   (Contact Amanda (x5088) or David (x5075) for training or assistance)

9. **When finished acquiring image(s):**
   a. Close shutter to block laser beam (on microscope diagram in software)
   b. Drop stage (push TOP button)
   c. Collect slides
   d. BLOT 40x or 60x oil obj. lens with **lens paper** only to remove oil!!
   e. Go back to 10X or 20X by clicking icon on screen
   f. Raise stage (push top button) – make sure you do this or software won't load properly!!
   g. **Close the software** (discard all files that weren't saved unless you want to save them)

10. **Turn off the microscope in this order:**
   a. Turn objective control box off (bottom Left)
   b. Turn mercury lamp off (box on top of objective control)

      **** Note: once the mercury lamp is turned on and then turned off, you must wait 15 min before turning it back on again!!!!**

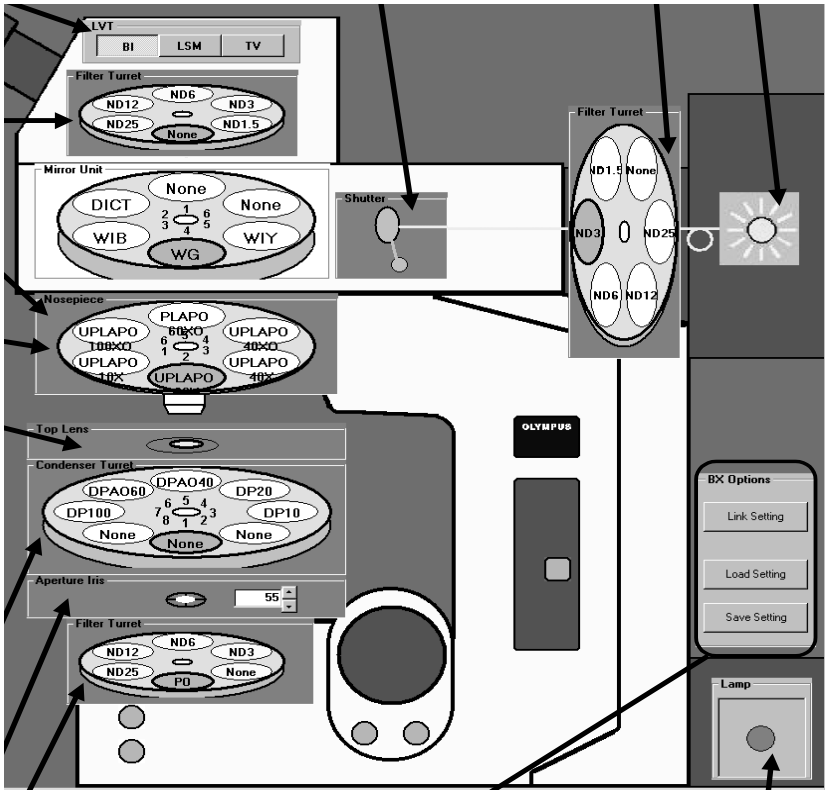
   c. Turn off the mixer box (bottom Right)
   d. Turn off light (Left of computer)
   e. Switch laser boxes off (turn toggle switches to O)
   f. Turn key perpendicular to top boxes (turn counter-clockwise)
   g. Turn AC power off (O)
   h. Turn off computer monitor and/or shutdown computer
   i. Have a nice day! ☺

OLYMPUS FLUOVIEW 300 CONFOCAL MICROSCOPE: BASIC IMAGING PROCEDURES

Once your samples are loaded on the microscope, make sure you are at a LOW objective **(4x, 10x, or 20x)** to find your specimen and then acquire a Z-stack.

1. First start by finding your sample using the transmitted light.
    a. Make sure the **BOTTOM SLIDER** on the top right of microscope is all the way **IN** (or you will see nothing through the oculars). (In=BI (binos); Out=LSM (laser scanning microscope))
    b. Make sure the transmitted light is on (icon in the bottom right of diagram below) and the shutter is closed (blocking laser).
    c. Click on None in the Mirror Unit wheel (filters) on the screen (see diagram below)
    d. Use the XY stage control knobs and fine focus to find and focus on a suitable sample. ****Note:** you can skip this step (finding your specimen with transmitted light) after you've imaged using fluorescence a few times and you know what to look for.

2. Once you have found a suitable specimen/area, you're ready for fluorescence
    a. Click the transmitted light icon to turn off
    b. Click the shutter open
    c. Click on the appropriate filter for your fluorophore (e.g. CY3 for green (TRITC), FITC for blue (FITC), etc.)
3. You should see your specimen fluorescing. If you do not, then make sure you have the correct filter in, make sure you're actually focused on a specimen (confirm using transmitted light). If you still do not see anything, then check your fluorescent labeling protocol.

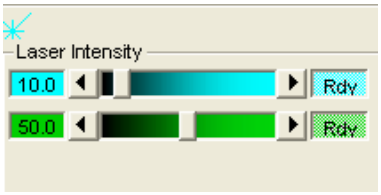


4. **DROP** the stage and choose the desired higher objective (20x dry, 40x oil, 60x oil, or 20x oil). If you are moving to an **oil** objective, then put a drop of oil in the center of your coverslip, click on the oil objective on the screen, and then **RAISE** the stage. **DO NOT put oil on a non-oil objective!!**

5. Observe your specimen through the oculars to make sure it is still in focus and in the field of view. Use the XY stage control to move to a different area if desired.

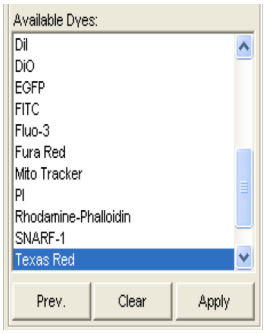
6. **Pull the slider OUT** when you're ready to do a scan.

7. Set up Lasers and Dyes: (sample dependent)
    a. Turn off the lasers you are not using: (all lasers are on by default)
        i. Click on the laser icon on the top toolbar to open the laser window 
        ii. Click the box that says **Rdy** to turn **Off** the lasers you're not using

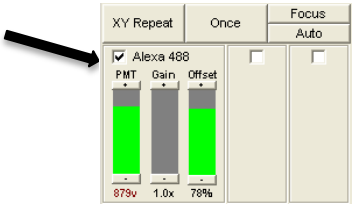


**Note: we have 3 lasers, blue, green and red. Also,
DO NOT CHANGE THE INTENSITIES!

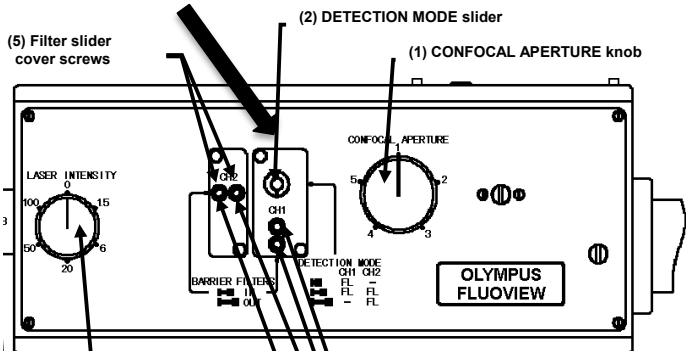
    b. Select the appropriate dye and put it in the appropriate channel
        i. Click the yellow 'ball' icon (acquisition dyes) on the top toolbar 
        ii. Find on your dye from the list on the bottom



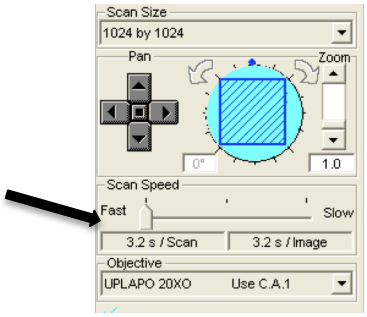
        iii. Click and hold your dye and drag it into the appropriate channel (top left of screen). The Left Channel is for Blue, Middle Channel is Green, Right Channel is Transmitted light. Make sure the correct box(s) is checked.



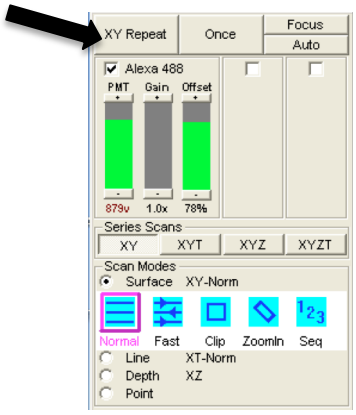
iv. Change the Detection slider on the Confocal: Push the slider all the way IN for dyes with a wavelength shorter than 570nm (e.g. FITC, Alexa488); MIDDLE position for dyes around 570nm (e.g. TRITC) or dual-labeled; OUT position for dyes greater than 570nm (e.g. CY5)



8. Set up Image Acquisition Settings:
   a. Make sure Scan Speed is set on Fast and that your Zoom is at 1.0 (i.e. not zoomed in). Scan size should be 800 x 600 for normal images (1024 x 1024 for publications) , and you should have the appropriate aperture in place.

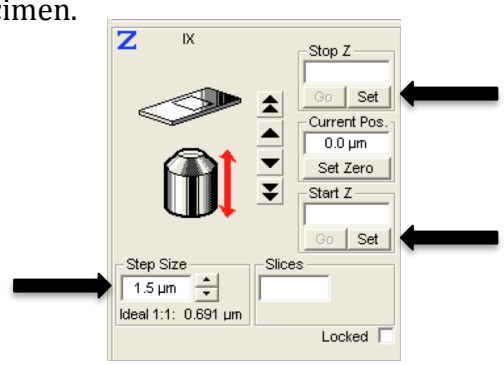


   b. Click on XY Repeat to view your specimen on the computer screeen.



   c. You should see your specimen appear on the screen (it will be displayed as whatever color is selected – you can change the color if desired). You can adjust the image if it is too bright/dim by adjusting the PMT (and, minimally, the Gain/Offset). See Amanda for a tutorial on additional techniques.

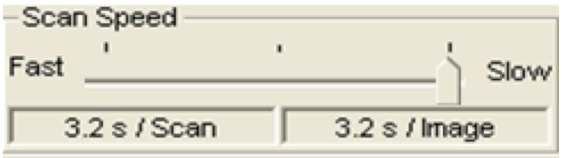
9. Setting your Z-stack:
    a. Use the arrows to scroll through your specimen. The single arrow moves by 0.1um increments with each click, or click and hold to slowly scan through your specimen. The double arrows move by 1.0um with each click, or click and hold to move quickly through your specimen.



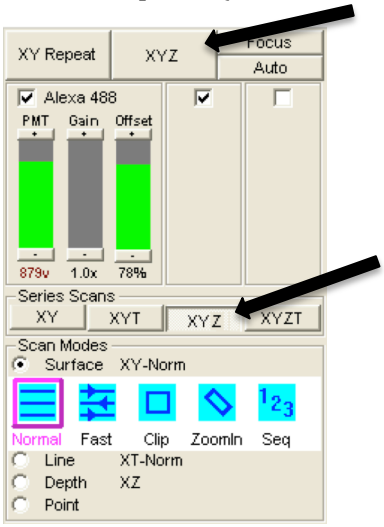
    b. Use the arrows to find the middle of your specimen and click Set Zero.
    c. Use the up arrow to scroll to the top of your specimen (or as far up as you want to image) and click Set on the Stop Z box.
    d. Use the down arrow to scroll to the bottom of your specimen (or as far down as you want to image) and click Set on the Start Z box. (It doesn't matter what you set as the 'start' or the 'stop', just as long as your Z-stack goes from -# to +# around your Zero point.
    e. Set your Step Size according to your specimen. As a general rule, the larger the step size, the quicker the scan, but the less information you get. A larger sample requires a larger step size, and vice versa for a smaller sample (multicellular vs. unicellular). (e.x. Penium samples use 0.3-0.5um step size). You want to aim for approx 25-45 slices for a normal resolution scan.

10. Once your parameters for acquiring a Z-stack are set, you are ready to image. Click Stop Scan on the top Left of screen. 

11. Change your scan speed from Fast to Med. or Slow for Z-stack acquisition.



12. Click on XYZ to pull up the XYZ option (next to XY Repeat), and then click on XYZ on the top middle of the panel.



13. Your scan will begin and you will see your image appear as each slice is being scanned. When the confocal completes the scan, a box will pop up in the top left corner.

Click on the Z icon and click on the bottom Z of the drop-down menu. This will compile all the stacks to form a 3D image of your specimen.

14. If your image is acceptable, click on File I/O → Save Experiment or Save Display (Experiment will save your entire Z-stack as a mulit-tiff file (large!). If you just want to see the final product of the Z-stacks, click Save Display. If you're planning on importing the stack into a 3D rendering software, Save Experiment (and Save Display as well).

15. Click Series Done to go back to do another scan and/or set up another Z-stack.

16. Repeat Steps 1-15 above for additional images. When finished, refer to ON/OFF Procedures for shutting down the confocal.