

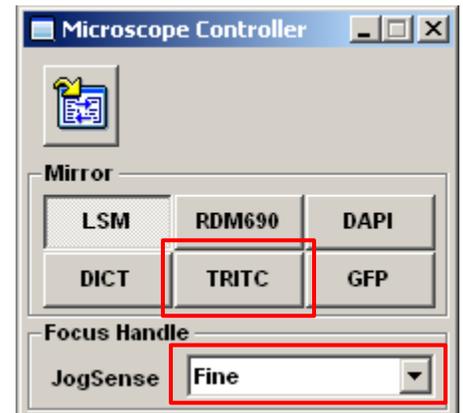
Olympus FV1000 MPE Basic User Guide

Finding Sample by Epi-Fluorescence

1. Turn on the system and open the software
 - Use the user name and passwords provided to you
2. Ask the technician to install the desired objective (60xdry, 25xW, 20xO, 60xO, 100xO)
3. Place sample underneath objective (coverslip/sample up)
 - Apply proper viewing solution
 - Use the focus knob to touch the lens to the solution
4. Pull both sliders on the left side of the microscope out
 - Top—Mirror IN/OUT selector
 - Bottom—Spectral DM IN/OUT selector



5. Select the microscope controller  icon from the main tool bar to open the Microscope controller window.
 - Click the dye you wish to view
 - This is also where you can see what speed the focusing knob is currently set to.
 - To toggle between course and fine you can either use the drop down menu here or push the bottom left corner button (F/C) on the focus knob controller



6. Push the  icon to turn the epi-fluorescent light on
7. Turn off the epi-fluorescent light by pushing the  icon again

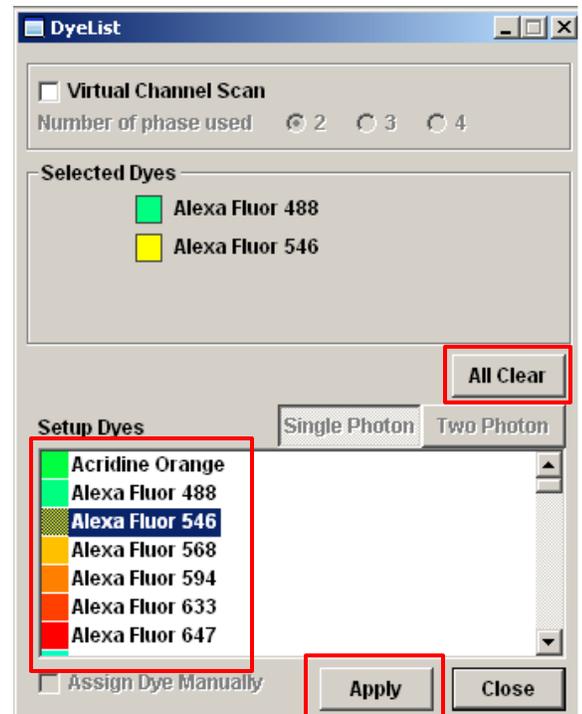
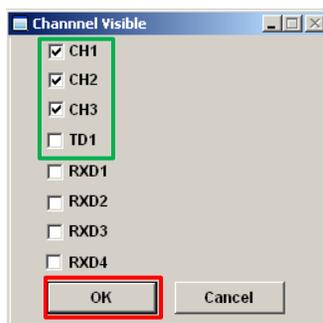
Configuring the Software

Single Photon Images

1. Both sliders on the left should be pulled out

2. Open the Dye list by clicking the  icon
 - Clear the list
 - Double click the desired dyes
 - Select Apply

If you can't see the dye's channel, select Ch Visible and check the desired channel.



3. Select the **scan speed** you'd like to use by moving the scroll bar to the desired speed

4. Select **image size** by moving the scroll bar to the desired dimension (in pixels)

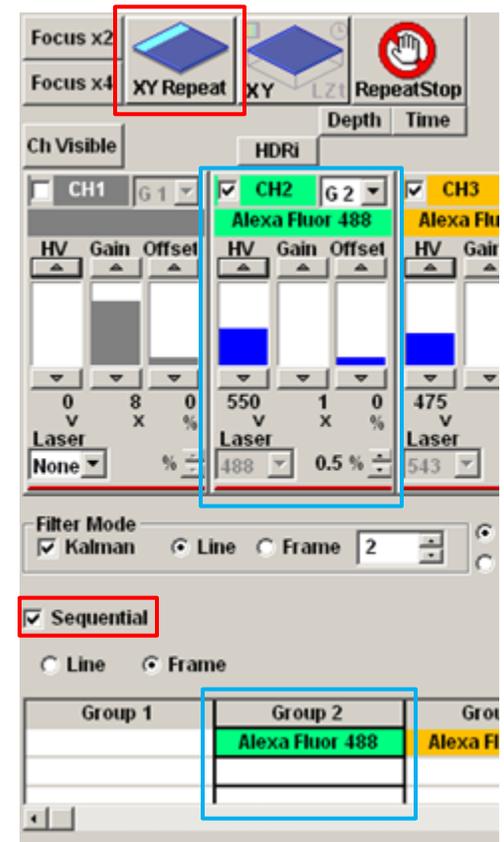
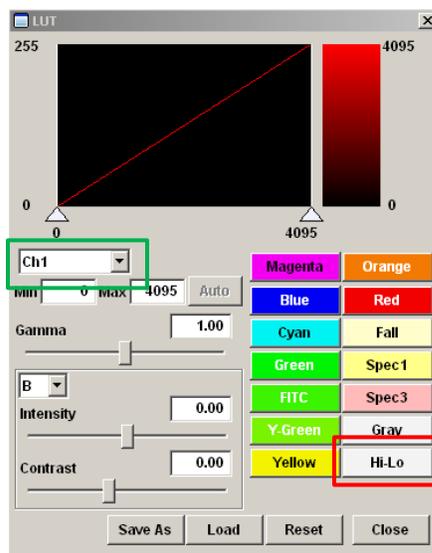
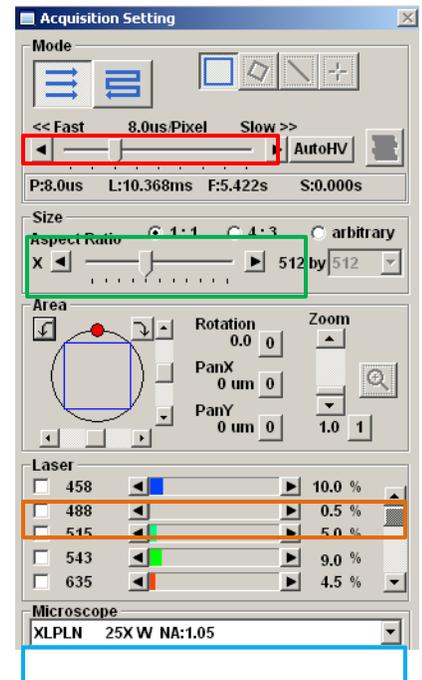
5. Select desired **lasers**

- Check the box and input desired output percentage

6. Make sure the software indicates the correct **objective** matches the objective loaded onto the turret

7. Focus image & adjust image as desired

- Turn on **Sequential**
 - By clicking on a single color in the columns listed as "Groups" beneath you are able to adjust one color at a time without having multiple lasers on
- Select **XY Repeat** to put the image into the Live View window
- Use the **PMT bars** to increase/decrease the sensitivity as desired
- Turn on the **Hi-Lo** in the look up table by holding Ctrl+H or selecting the Look Up Table icon  from the Live View window
 - Use the drop box to select the **channel** to which you're applying the LUT
 - Adjust the PMT bars depending on saturation
 - Red indicates oversaturation
 - Blue indicates under saturation
 - Turn off the Hi-Lo by selecting the color you'd like the channel to appear



8. Acquire your final picture by selecting XY 

9. Save your final picture

- File → Save
 - OIF: Creates "a folder that contains a 16-bit TIFF" and "an accessory file" which can not be opened separately from each other)
 - OIB file: Creates the OIF format files in a single file

Acquiring Two Photon Images

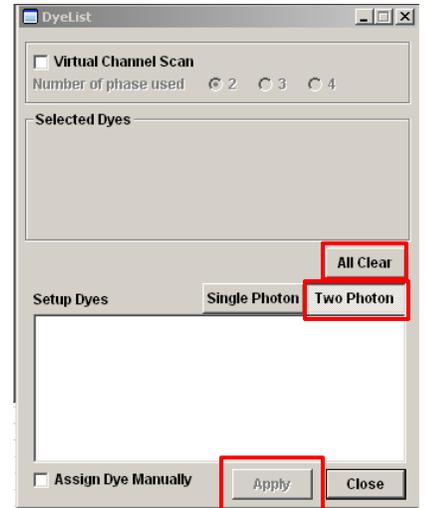
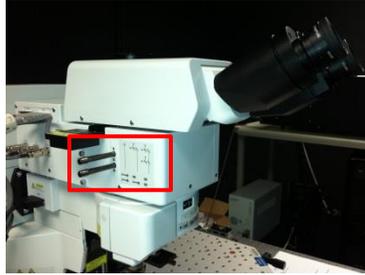
1. The detectors are EXTREMELY sensitive to light. Switch to Darkroom Color screen by clicking the  icon located in the main toolbar. (To switch back to the regular color screen select the  icon.)

2. Open the Dye list by clicking the  icon

- Select All Clear
- Choose Two Photon
- Select Apply

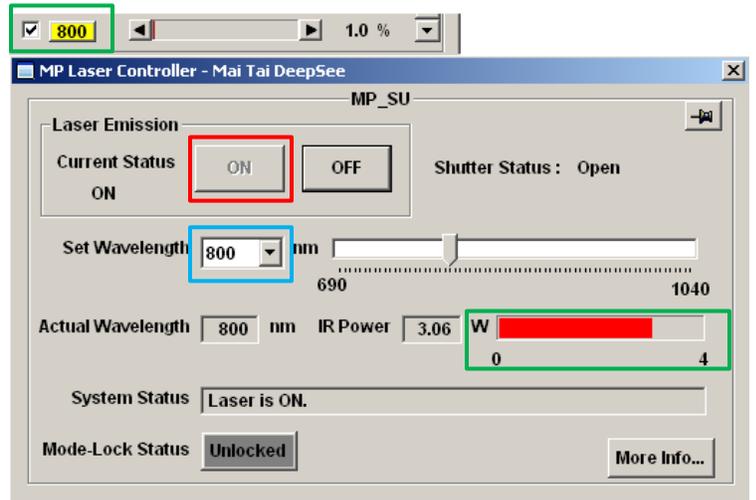
3. Place sliders on the left of the microscope to open the desired filter cubes

- Both IN opens channels:
 - RXD1: Violet (420-460nm)
 - RXD2: Green (495-540nm)
 - RXD4: Red (575-630nm)
- Top IN, Bottom OUT opens channels:
 - RXD3: Long pass (380-560nm)
 - RXD4: Red (575-630nm)



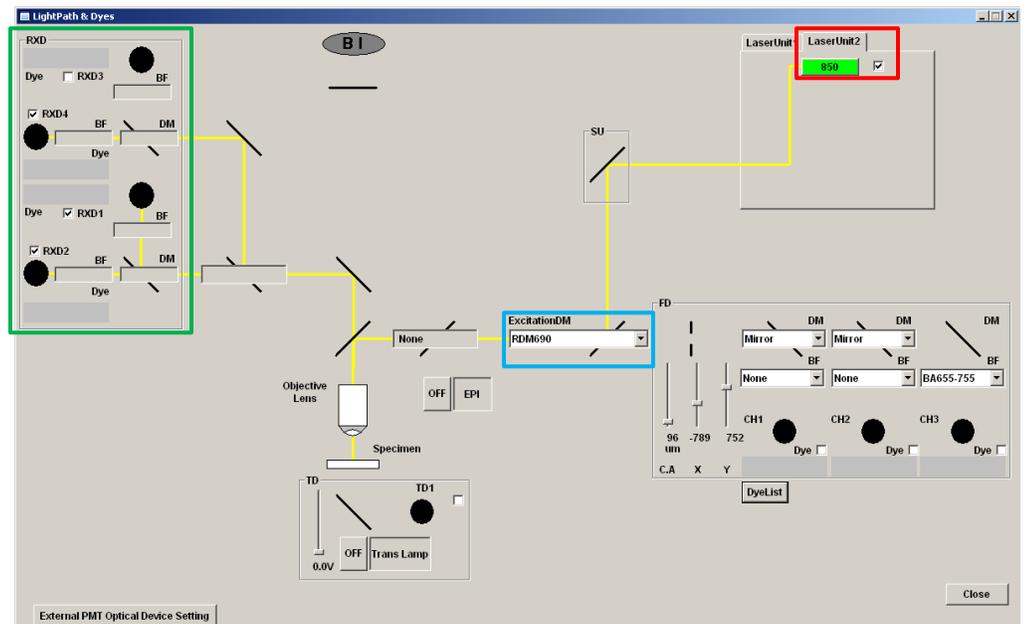
4. Turn on the IR laser

- Check the **IR laser box** and click the number to open the MP Laser Controller
 - Click **ON**
 - The system status will tell when the laser is ready to use (takes about 10 minutes to warm up fully)
 - The **IR Power meter** gives a red bar indicating how many watts are being used
 - Use the drop menu to select the **desired wavelength**



5. Check your light path by clicking the  icon

- **Laser selection** should be IR only
- **RDM690 mirror**
- Check desired **emission channel** boxes to activate
 - **RXD1: Violet** (420-460nm)
 - **RXD2: Green** (495-540nm)
 - **RXD3: Long Pass** (380-560nm)
 - **RXD4: Red** (575-630nm)



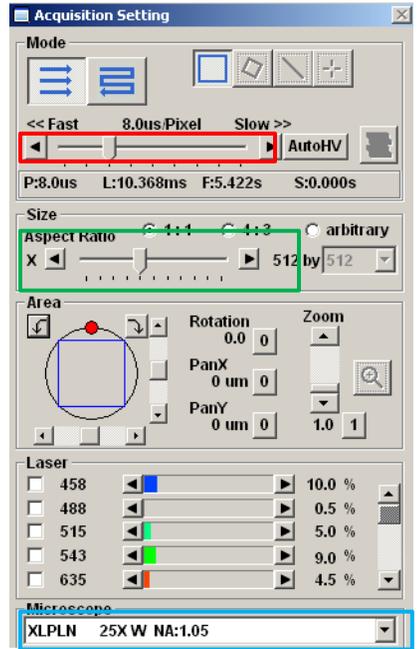
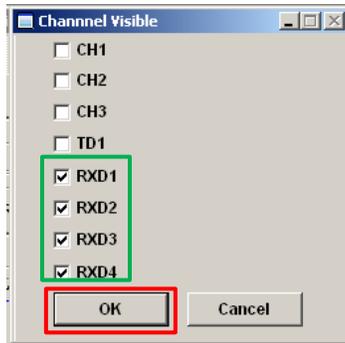
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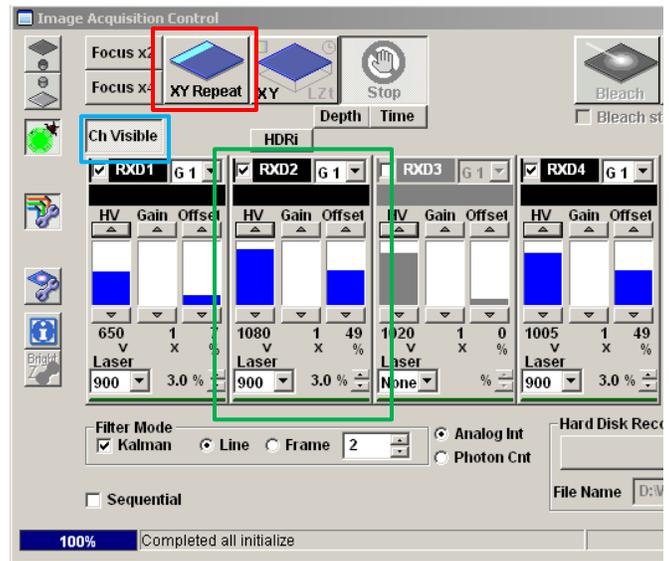
5. Make sure the software indicates the correct **objective** that is loaded onto the turret

6. Focus image & adjust image as desired

- If you do not see the RXD channel you need select **Ch Visible** and check the proper boxes to visualize them



- Select **XY Repeat** to put the image into the Live View window
- Use the **PMT bars** to increase/decrease the sensitivity as desired
 - You may also control the wavelength and output percentage of the laser in this location
 - Note: The wavelength and output percentage is the SAME for all channels simultaneously. Changing this in one channel will affect the intensity in the others as well.*



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