Olympus FV1000 MPE Imaging Protocol

1) *System Startup*

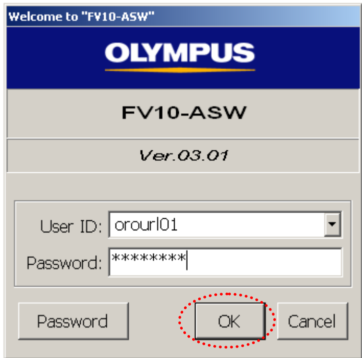
☞ Please note our sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.

☞ **Follow each step** of the startup poster.

☞ **Log into** the computer with your user account.

☞ **Open** the FluoView software from the desktop:

☞ On the following screen, **enter your unique user name and password**, and click **OK**.

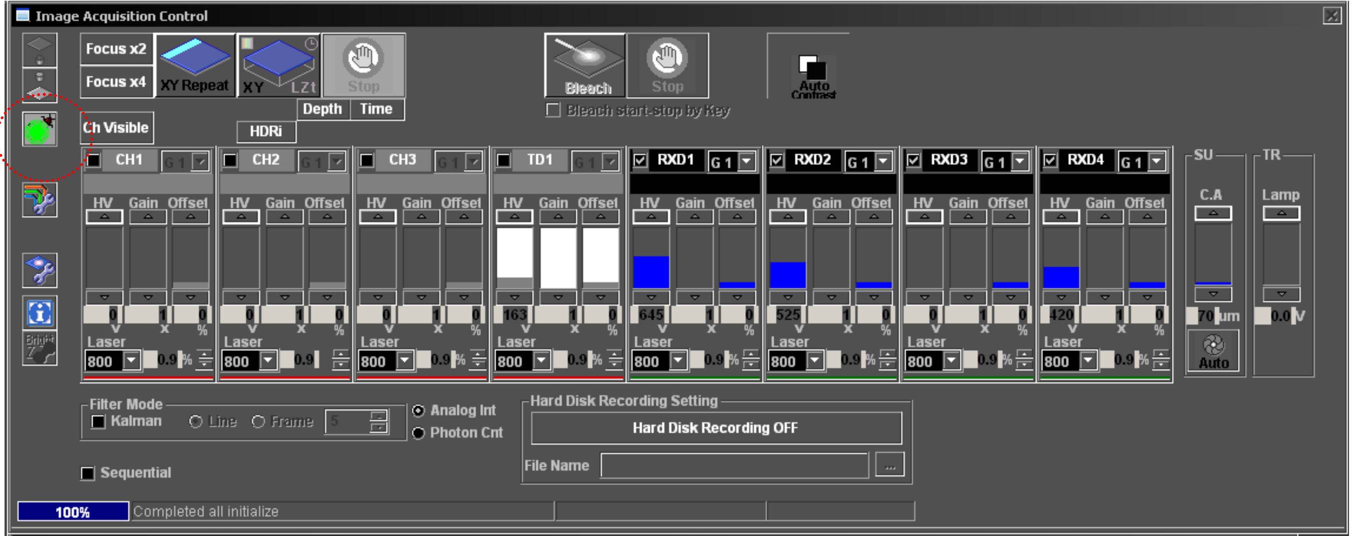


2) *Lens Cleaning*

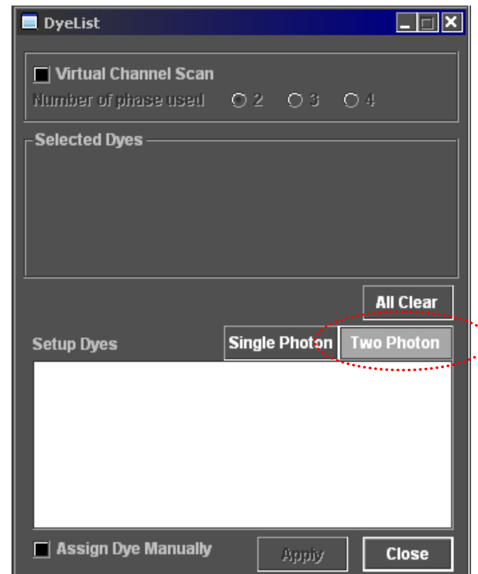
☞ Please **clean all of the lenses** you intend to use **before and after** your session. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

3) *Software control*

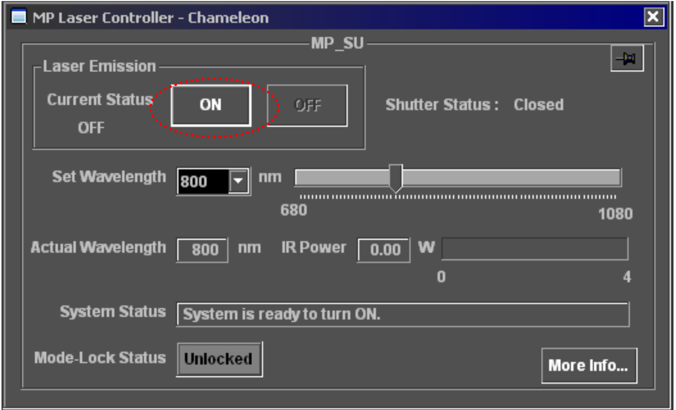
☞ Upon initialization of the software, click on the Dye List menu .

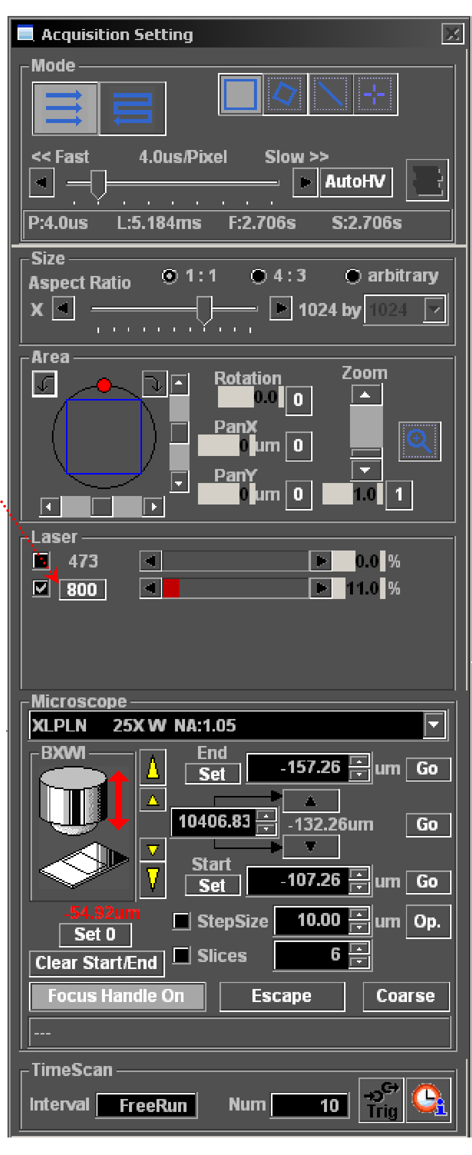


- Within the Dye List, select **Two Photon** and click **Close**.



- In the Acquisition Setting panel, click **800** to open the MP Laser Controller.
- Ensure the MP laser is set to 800 nm, and turn the laser emission **ON**.





- The laser's Mode-Lock Status is shown at the bottom of the controller menu. When the MP laser is ready for imaging, the status will change from **Unlocked** to **Locked**. This could take up to 5 minutes.
- Once the MP laser is locked, tune to your desired wavelength.
- In the Acquisition Setting menu (right) ensure the correct lens is selected from the Microscope dropdown. If the desired lens does not appear in the dropdown, see facility staff.

4) *Microscope control*

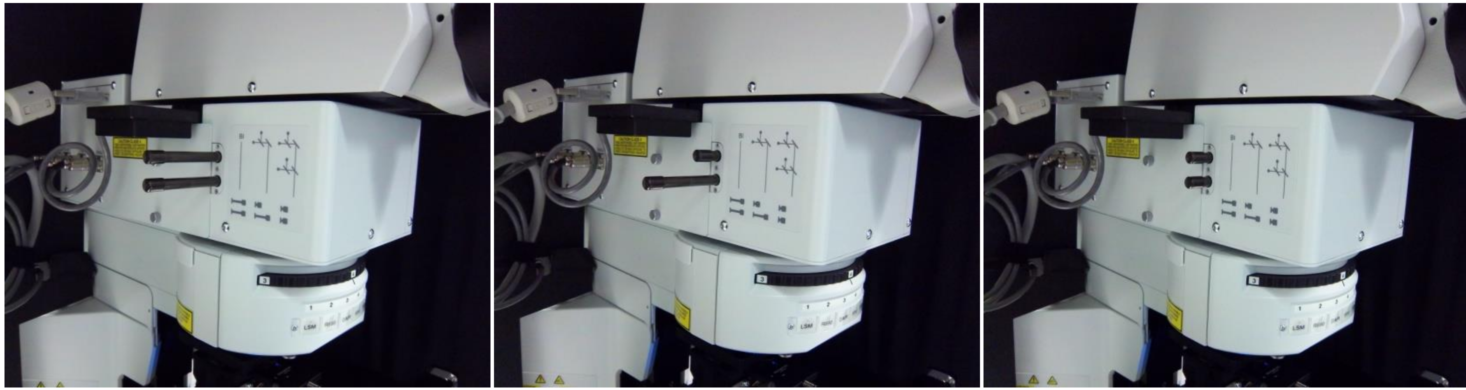
☞ **Load your slide** upside down and take care for the following issues:

- **Select and inspect each slide or dish.**
  - If it is dirty, gently clean with a KimWipe and/or cotton swab. You should do this with all your samples before you come.
  - Carefully rest the slide or dish onto the stage, and adjust the stage adapter to fit accordingly.

☞ Use the stage movement control joystick to position your specimen under the lens.

☞ To focus your sample:

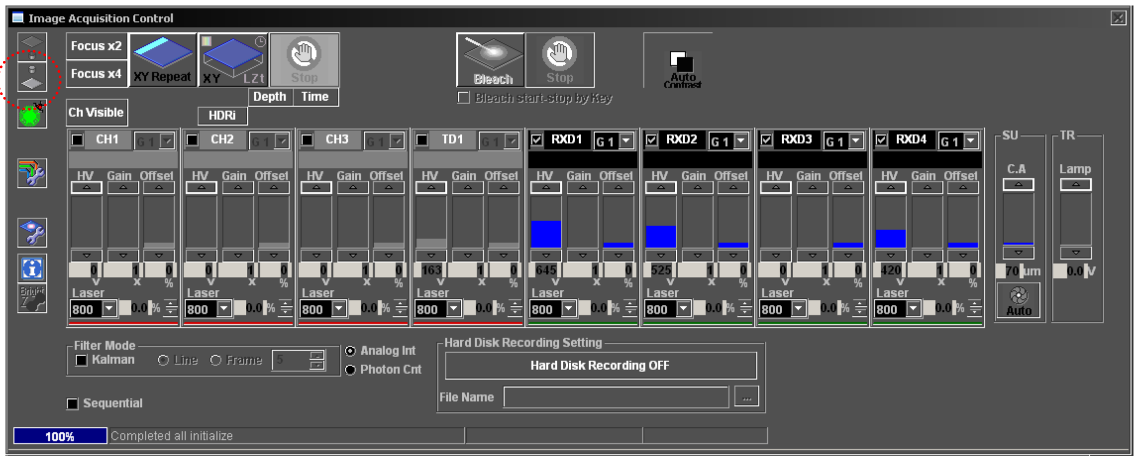
- Pull both sliders out on the top left of the microscope. These sliders have three configurations:



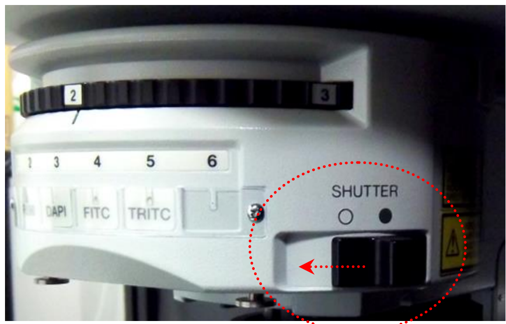
Light to oculars | *Light to detectors, SDM570 disengaged* | *Light to detectors, SDM570 engaged*

- Move the filterwheel to view the desired fluorescent signal.
  - 3 – DAPI
  - 4 – FITC
  - 5 – TRITC

- Turn on the EPI lamp from the Image Acquisition Control menu.



- Open the reflected light shutter on the lower right side of the filterwheel.



☞ Focus on your sample and center your region of interest.

- Note the following controls in this diagram.

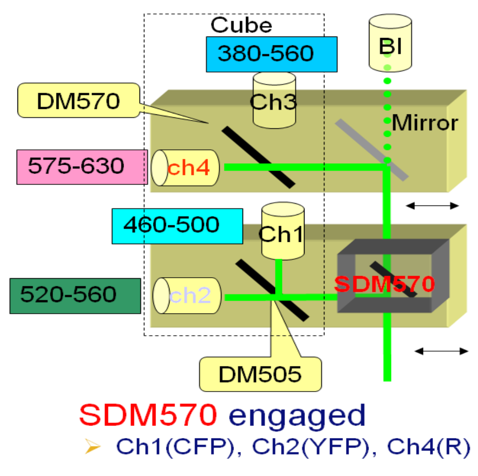
☞ **Check all of your fluorophores** in your specimen. You need to have an idea of what to expect before you begin.

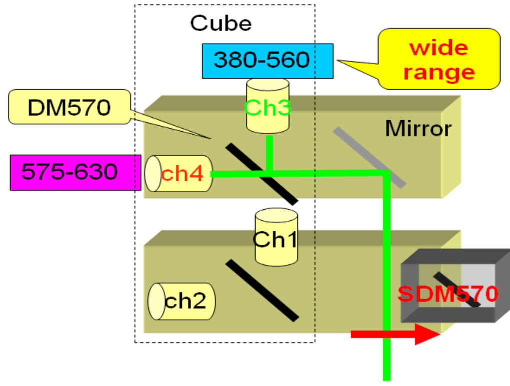
☞ Close the reflected light shutter and turn the **EPI** lamp off to prevent photobleaching of your sample.

5) *Setting up your experiment*

☞ Examine the following schematic to determine the appropriate configuration for your samples:

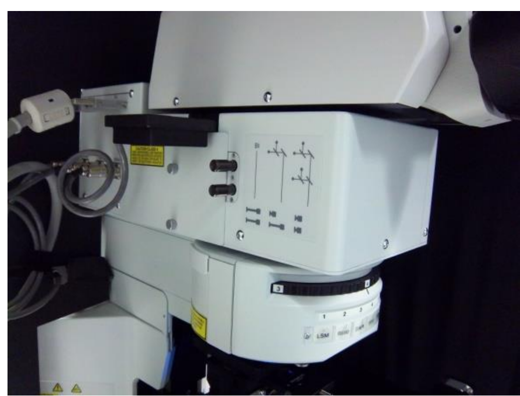
4CH NDD FILTER: FV10-MRCYR/XR



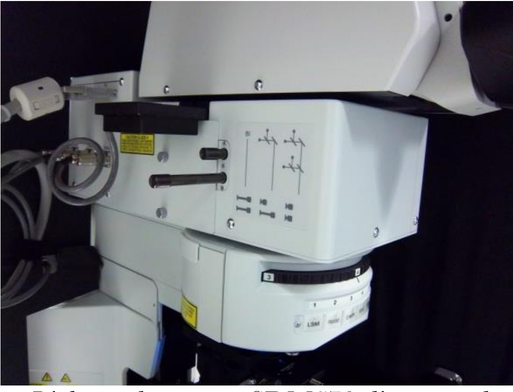


SDM570 disengaged

➢ Ch3(wide band DAPI or G) , Ch4 (R)

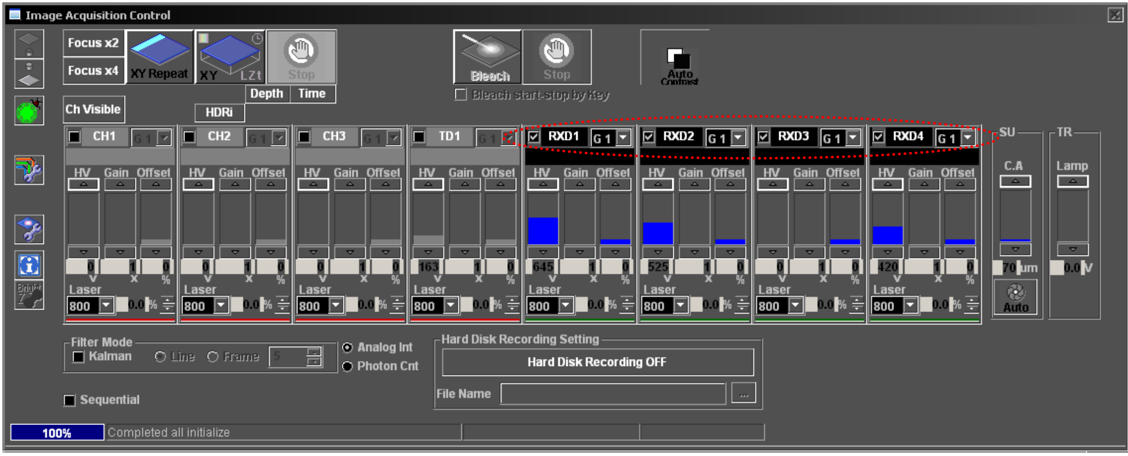


Light to detectors, SDM570 engaged
RXD1 (CFP), RXD2 (YFP), and RXD4 (RFP) available.



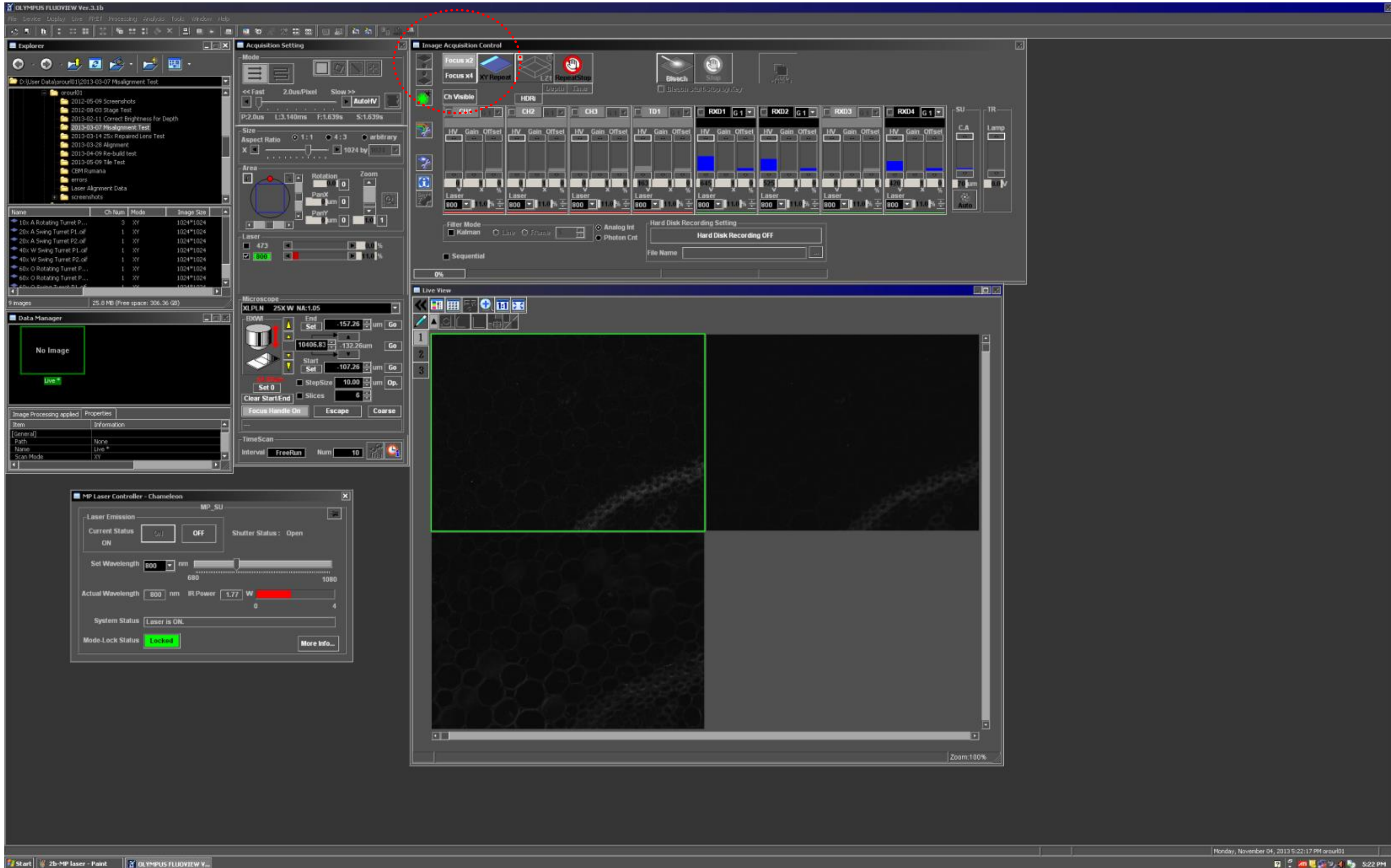
Light to detectors, SDM570 disengaged
RXD3 (wide DAPI-GFP) and RXD4 (RFP) available.

☞ Enable the corresponding channels within the Image Acquisition control menu:



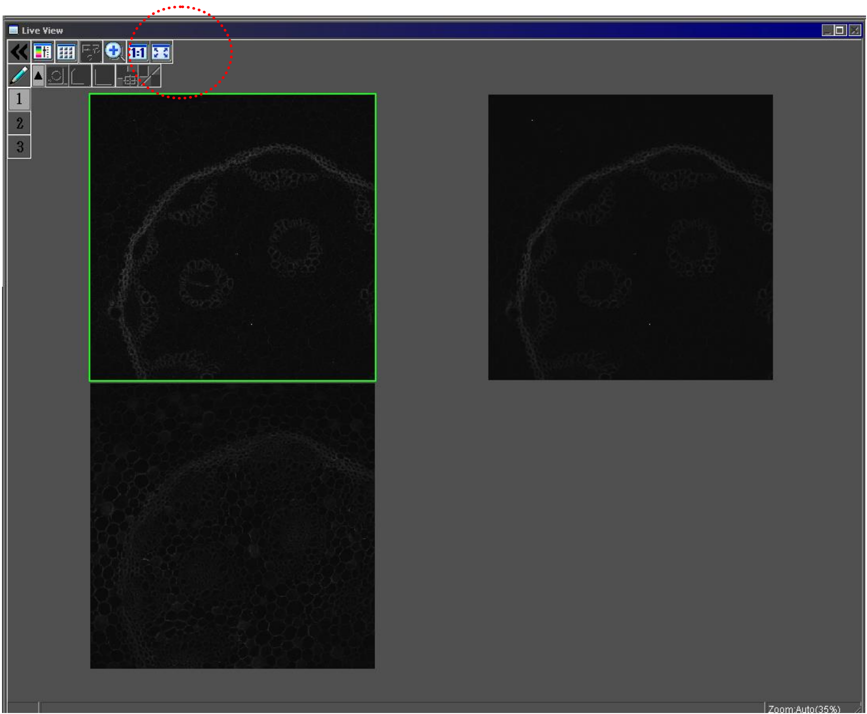
6) *The imaging process*

- ☞ Rotate the filterwheel to position 2, R690.
- ☞ Push in one or both sliders on the top left of the microscope, depending on the configuration chosen above.
- ☞ Open the reflected light shutter.
- ☞ Ensure the microscope is covered and room lights turned off to minimize ambient light exposure to the detectors.
- ☞ Click Focus x2 to view a fast scan of your sample.

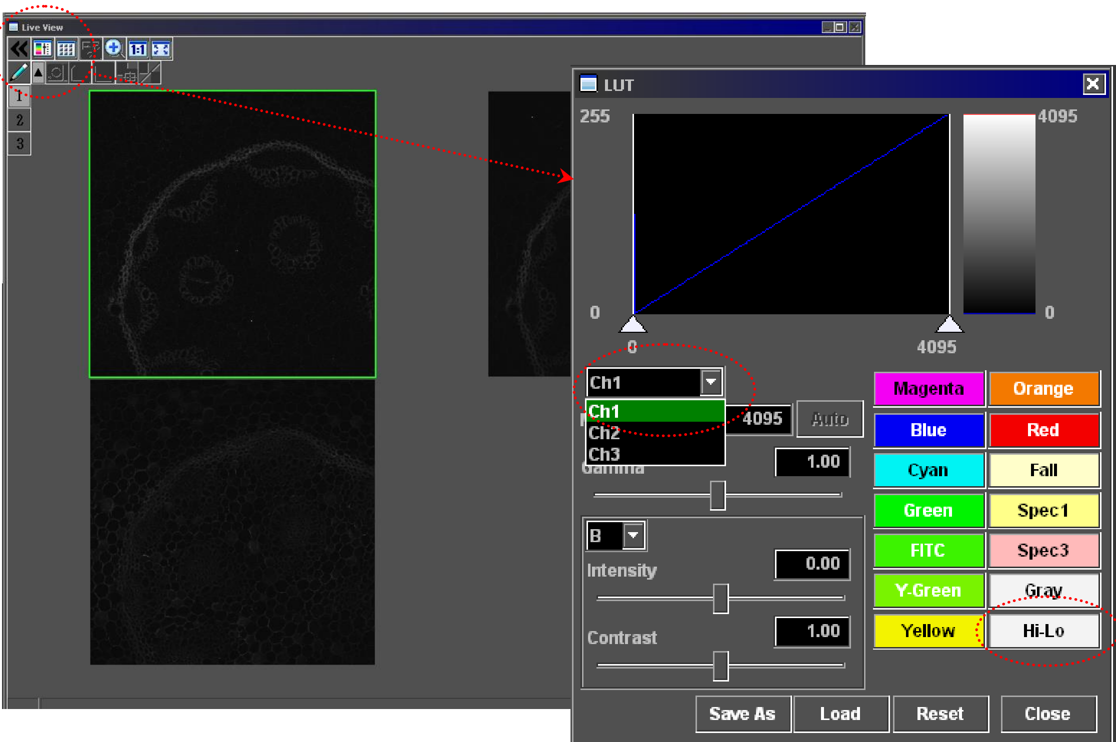


Convallaria rhizome test slide, Leica Microsystems

- In the Live View menu, click to show the full image frame.



- Click the LUT button to change the color display options.



- Select the Hi-Lo setting for each active channel, ie. select Ch1 and click Hi-Lo, select Ch2 and click Hi-Lo…
- Close the LUT menu.

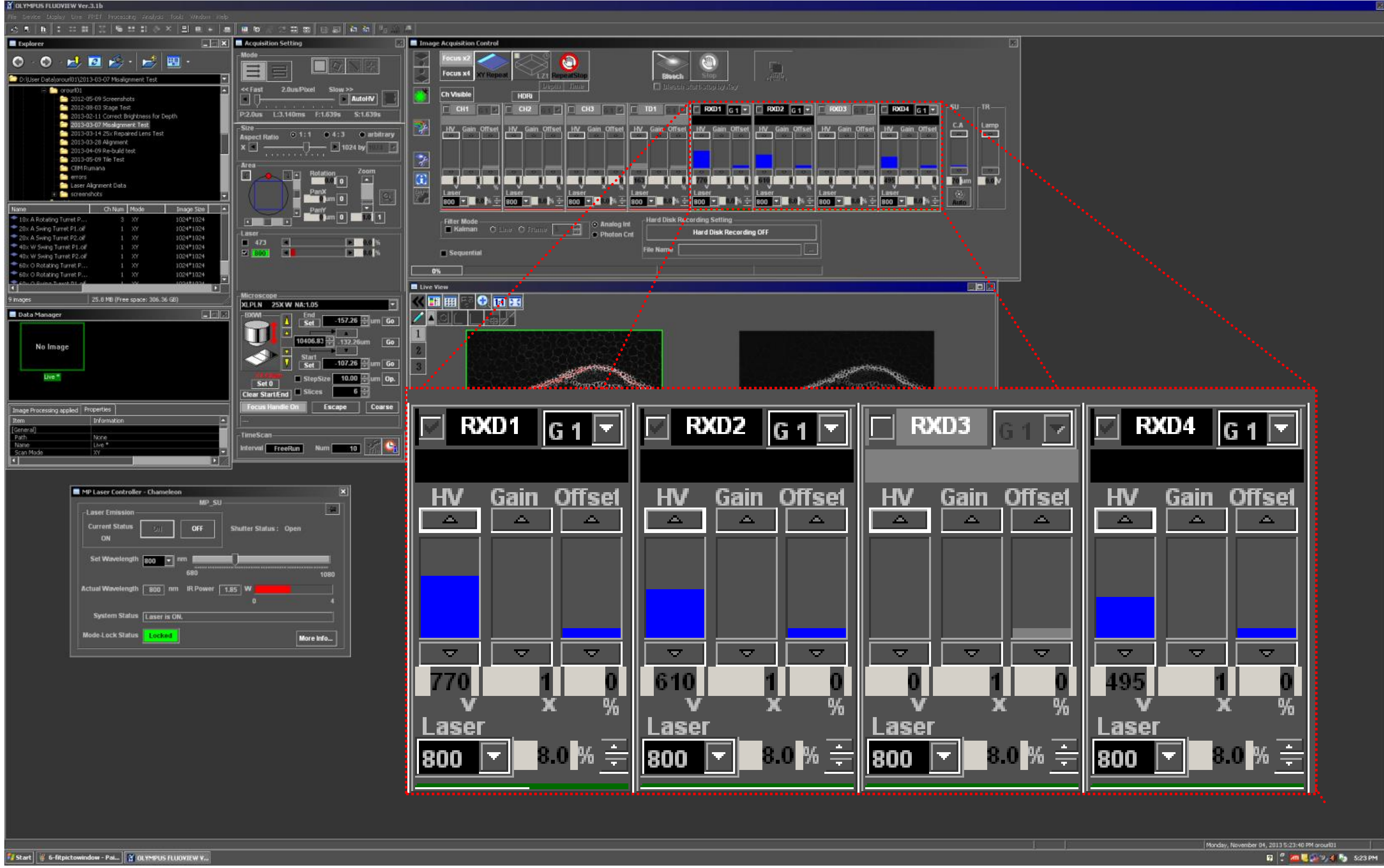
☞ Slowly increase the MP laser power until your signal is visible. Note: increasing laser power too high can damage your sample!

☞ Use the focus control knob to find the brightest focal plane in your specimen of interest.

☞ Click RepeatStop at the top of the Image Acquisition Control menu to stop the fast scan.

☞ Click XY Repeat to view a slow scan of your sample. This scan will be brighter, and is representative of what your final image will look like.

☞ Adjust the HV (high voltage) on each individual channel to maximize the intensity of your signal while avoiding saturation (red pixels). There should be only a few red pixels in each channel.

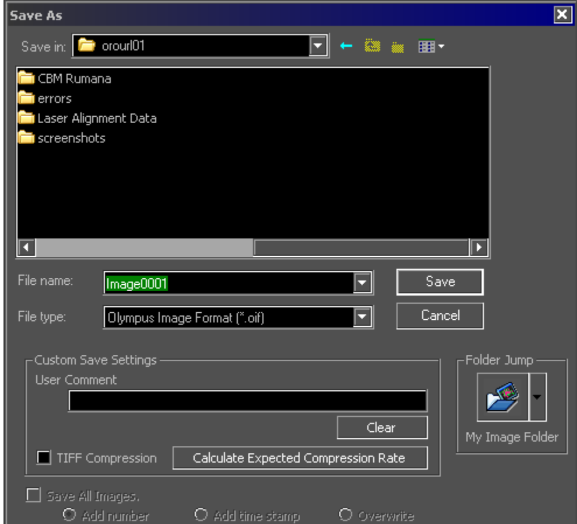


☞ Click RepeatStop at the top of the Image Acquisition Control menu to stop the slow scan.

☞ Click XY LZt to acquire your image.

☞ To save the acquired images, right click within the image window, and select 'Save As…'

☞ Save all your files as type '.oif' – this will save both the meta data as well as a folder of tif images.



7) *Returning to the microscope*

☞ To return to the microscope to find another field:

- Pull both sliders out on the top left of the microscope.
- Move the filterwheel to position 3, 4, or 5 to view the desired fluorescent signal.
- Turn on the EPI lamp from the Image Acquisition Control menu.
- Open the reflected light shutter on the lower right side of the filterwheel.

☞ Focus on your sample and center your region of interest.

8) *Taking a Z-Stack*

☞ Rotate the filterwheel to position 2, **R690**.

☞ Push in one or both sliders on the top left of the microscope, depending on the configuration chosen above.

☞ Open the reflected light shutter.

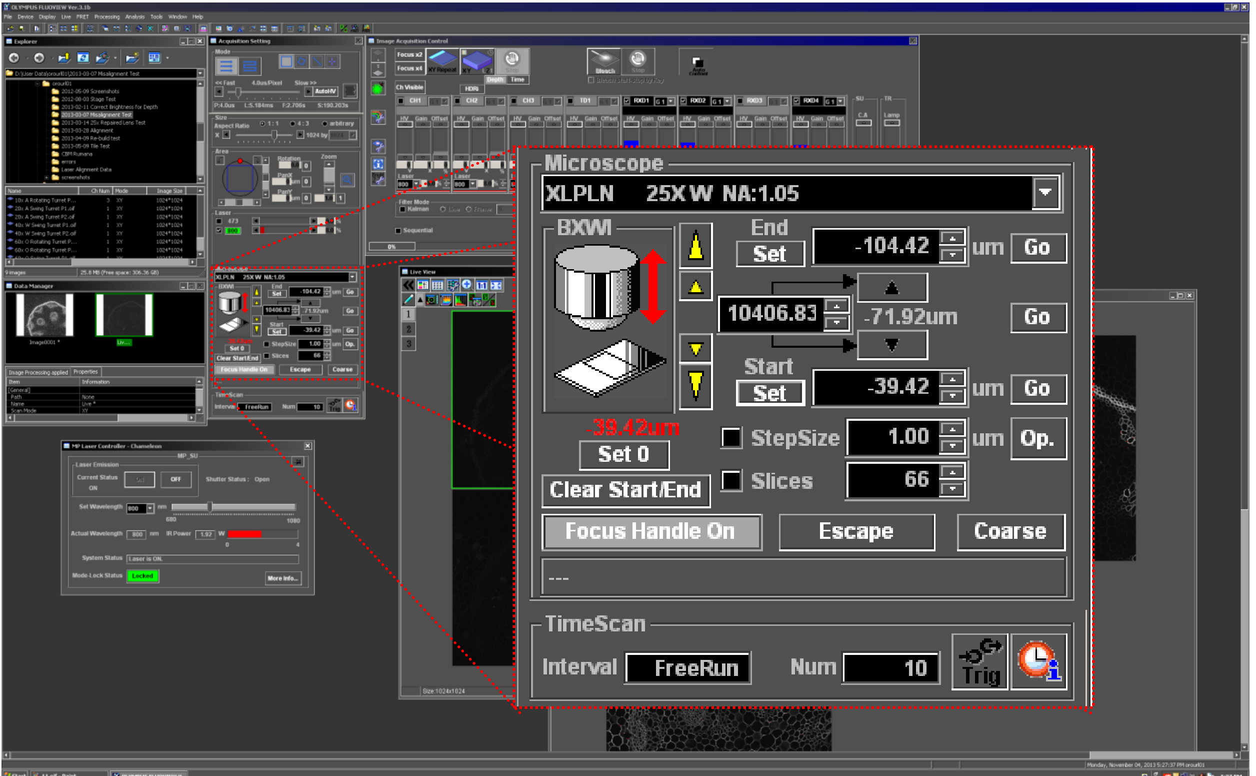
☞ Ensure the microscope is covered and room lights turned off to minimize ambient light exposure to the detectors.

☞ Click Focus x2 to view a fast scan of your sample.

☞ Use the focus knob to search for the brightest focal plane in your specimen. Set the laser power and HV for all your channels.

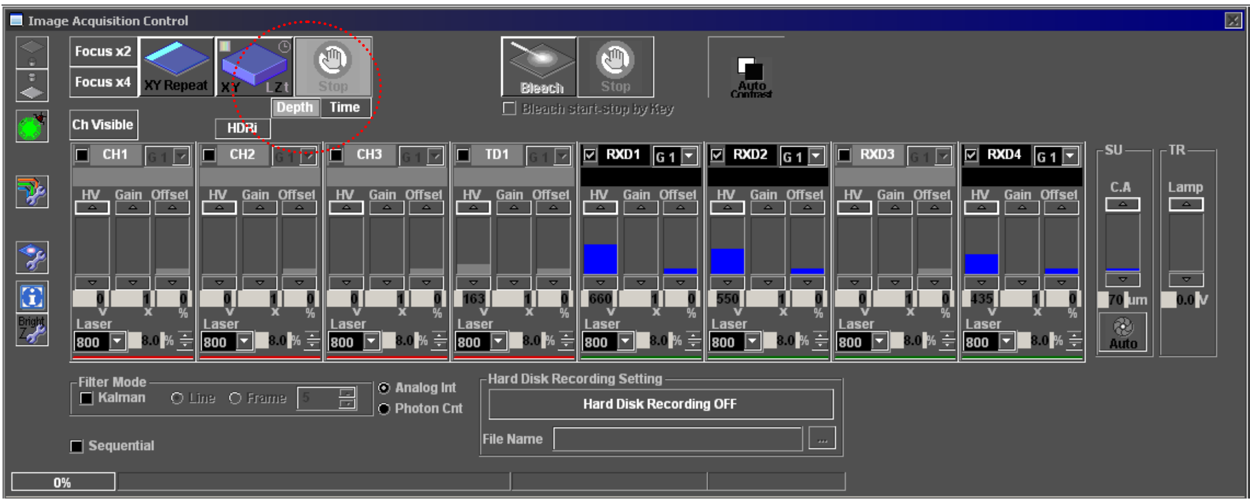
☞ Set your Z-stack limits:

- Use the focal knob to set the focal plane towards the bottom of your specimen (rotate down) and click Start Set.
- Use the focal knob to set the focal plane towards the top of your specimen (rotate up) and click End Set.

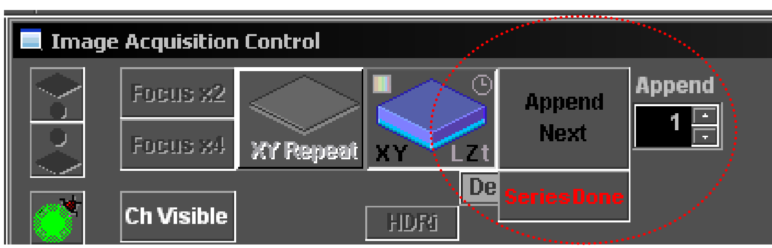


- Click RepeatStop.
- Note the stack size (z-Volume) and number of steps. You may select an optimized Z-interval by clicking Op.. This step is critical for post processing techniques like deconvolution or 3D reconstruction.
- If you don't need that many slices or to have such small Z slice intervals, you may manually input either the StepSize of number of Slices in the selections below the set positions.
- Enable Depth below the XY LZt button within the Image Acquisition Control menu.

  The frame XY LZt will now turn into a volume XY LZt.



- Click XY LZt to acquire your Z-stack.
- When acquisition is complete, you will have the option to either append additional optical slices, or complete the series.
  - To append additional optical slices to the completed Z-stack, enter the number of slices in Append 1 and click Append Next.
  - To complete the series, click SeriesDone.

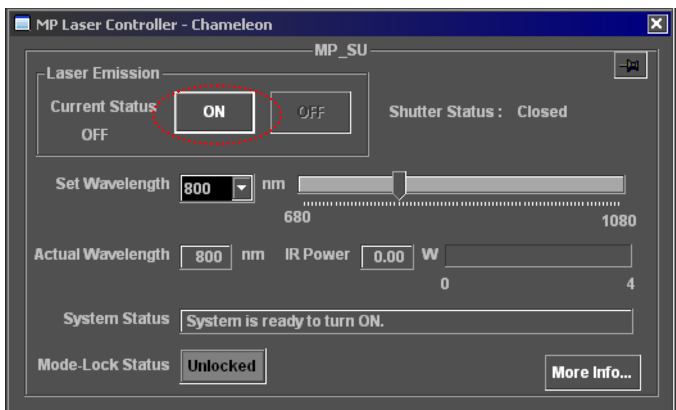


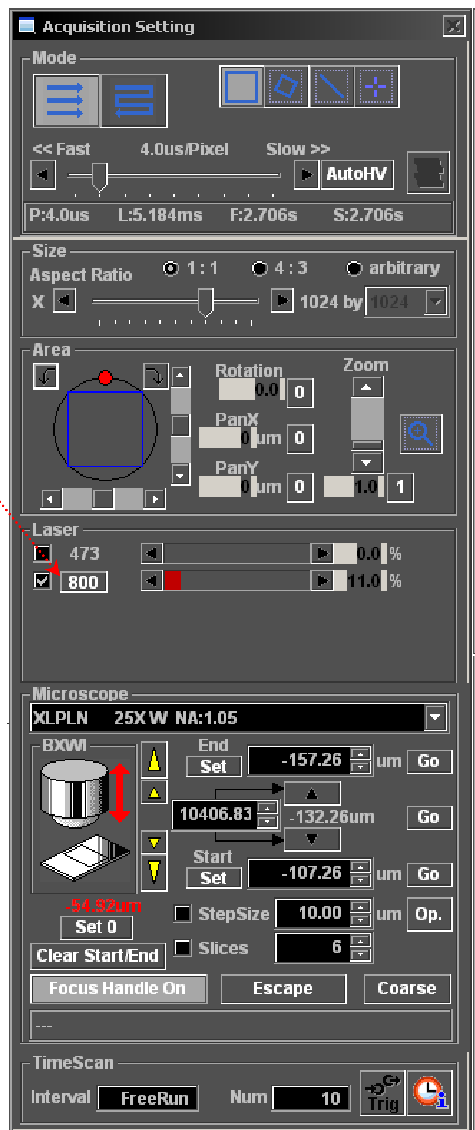
☞ To save the acquired images, right click within the image window, and select 'Save As…'

☞ You can view the maximum intensity projection of the Z-stack by clicking the icon within the 2D View window, and selecting .

9) *System Shutdown*

- At the end of every session, turn off the MP laser.
  - In the Acquisition Setting panel, click 800 to open the MP Laser Controller.
  - Return the MP laser to 800 nm, and turn the laser emission to OFF.





- Before you proceed with the shutdown sequence, please remember to back up all of your data and to clean the dry and oil immersion lenses.
- Sign onto the facility calendar and check for users after your session. If you don't see anything scheduled for less than 2 hours, proceed with the full shutdown sequence shown on the Shutdown poster. Otherwise, close the software and log off your account.
- If you do have to perform a full shutdown, please follow the shutdown poster steps in order.