

# 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: FV10-A5W 3.1
- ☞ On the following screen, **enter your unique user name and password**, and click **OK**.

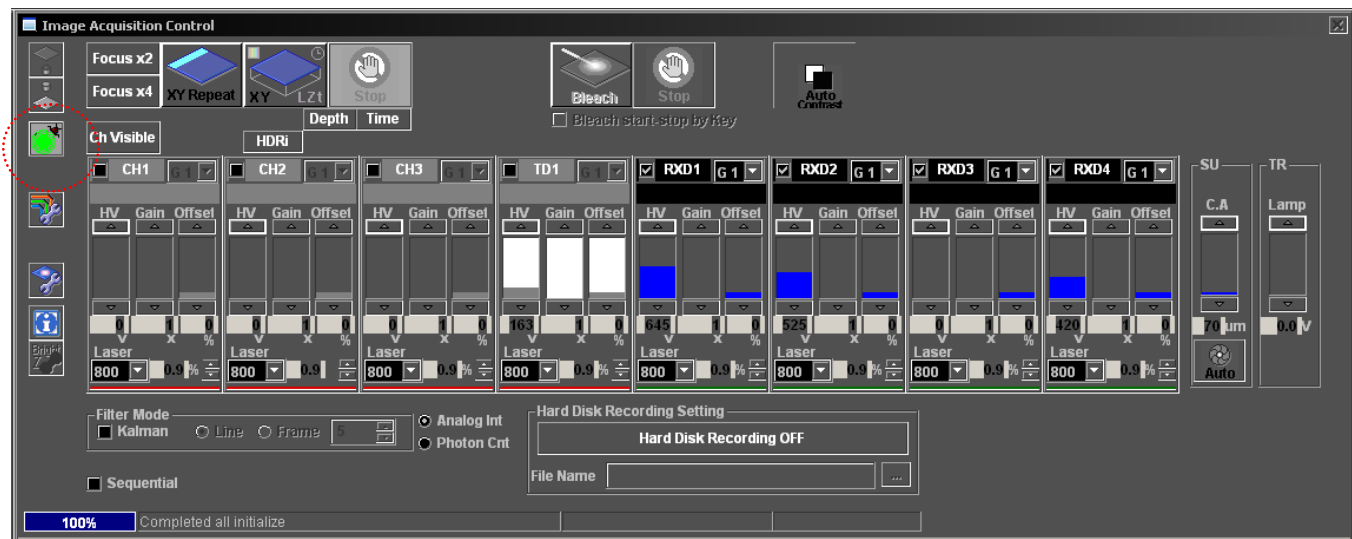


## 2) Lens Cleaning

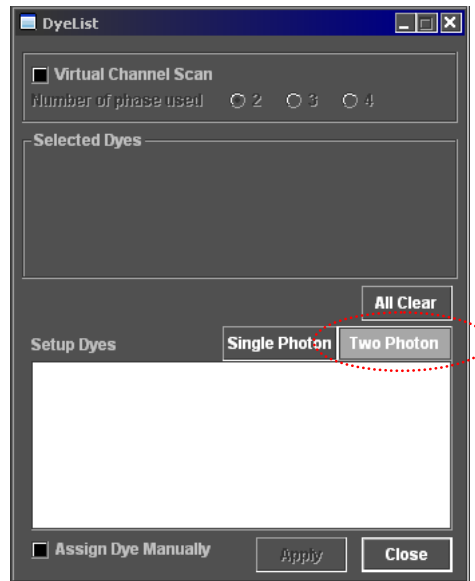
- ☞ Please **clean all of the lenses** you intend to use **before and after** your session. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

## 3) Software control

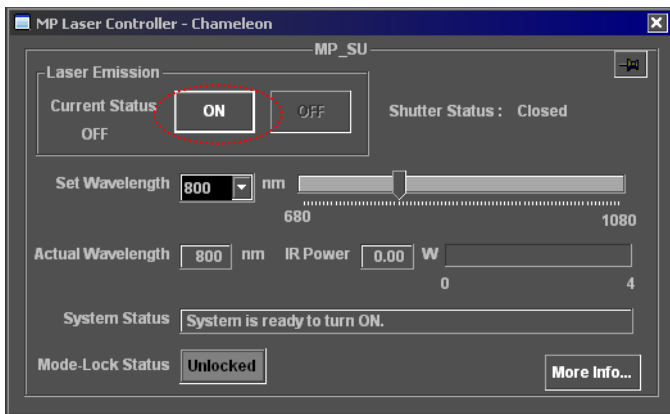
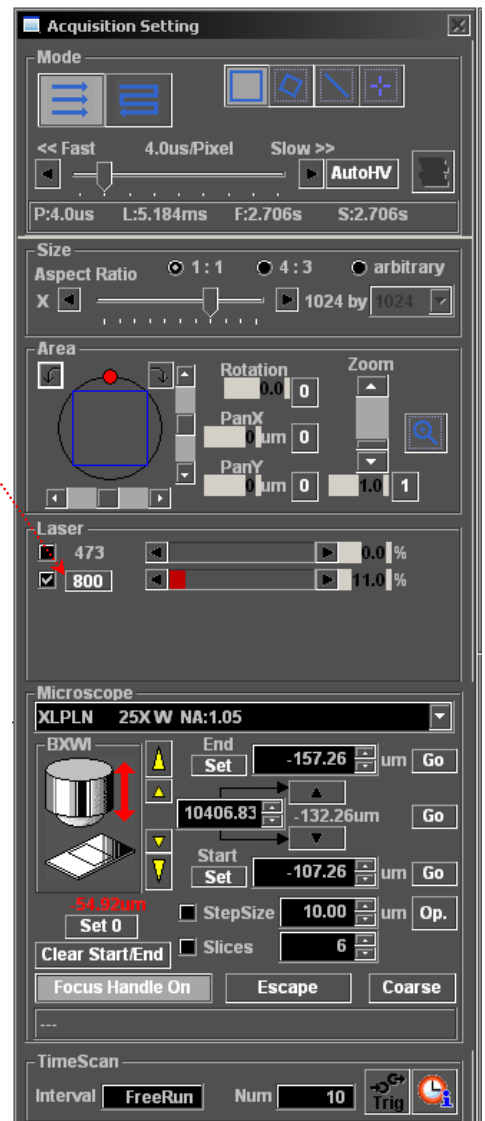
- ☞ Upon initialization of the software, click on the Dye List menu .



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



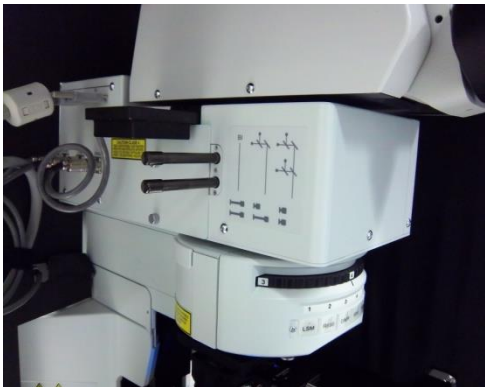
- ☞ In the Acquisition Setting panel, click **800** to open the MP Laser Controller.
- ☞ Ensure the MP laser is set to 800 nm, and turn the laser emission **ON**.



- ☞ 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*

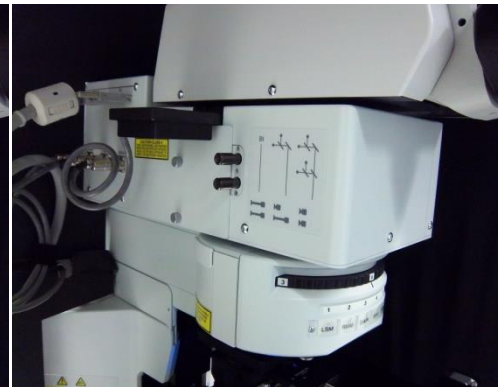
- ☞ **Load your slide** upside down and take care for the following issues:
  - **Select and inspect each slide or dish.**
    - If it is dirty, gently clean with a KimWipe and/or cotton swab. You should do this with all your samples before you come.
    - Carefully rest the slide or dish onto the stage, 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*

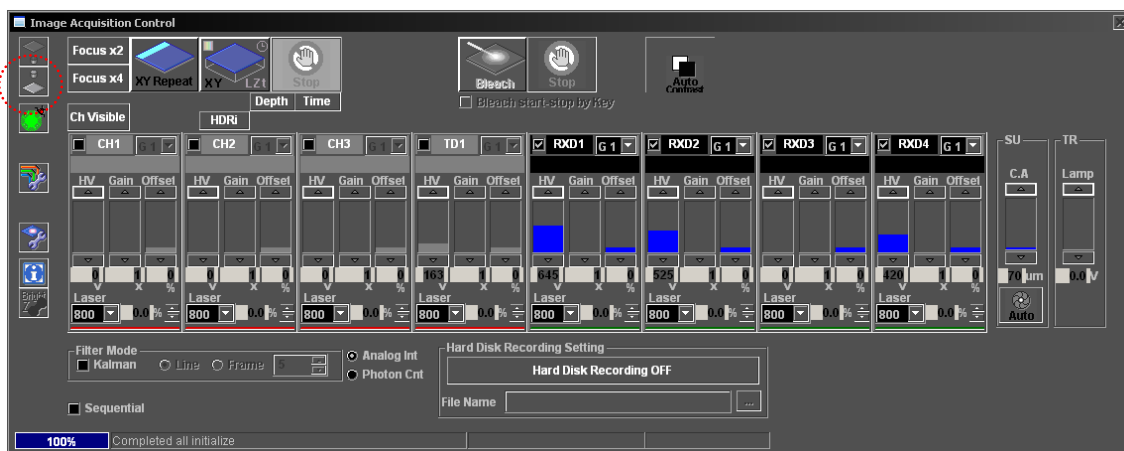


*Light to detectors, SDM570 engaged*

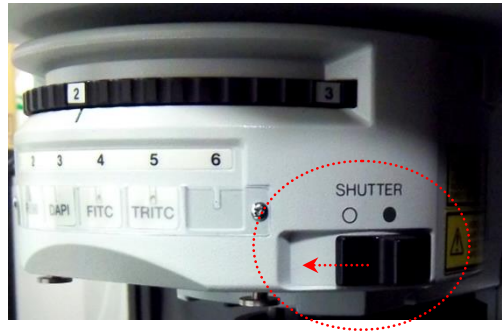
- Move the filterwheel to view the desired fluorescent signal.
  - 3 - DAPI
  - 4 - FITC
  - 5 - TRITC



- 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.



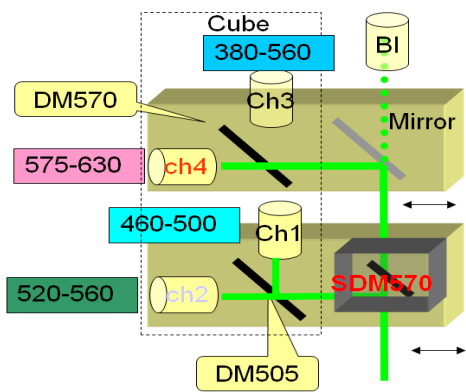
☞ **Check all of your fluorophores** in your specimen. You need to have an idea of what to expect before you begin.

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

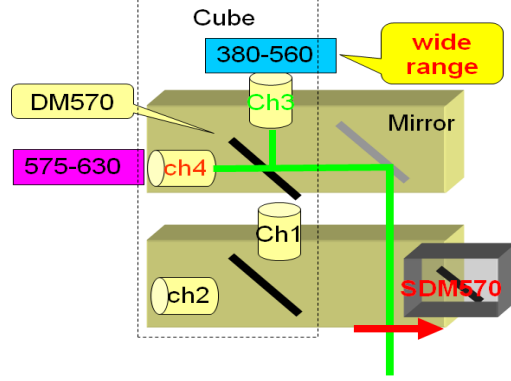
5) *Setting up your experiment*

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

## 4CH NDD FILTER: FV10-MRCYR/XR



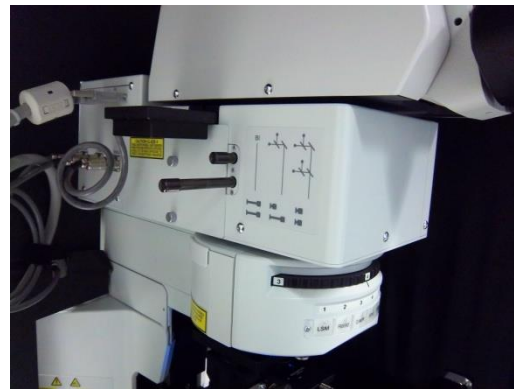
**SDM570 engaged**  
➤ Ch1(CFP), Ch2(YFP), Ch4(R)



**SDM570 disengaged**  
➤ Ch3(wide band DAPI or G) , Ch4 (R)

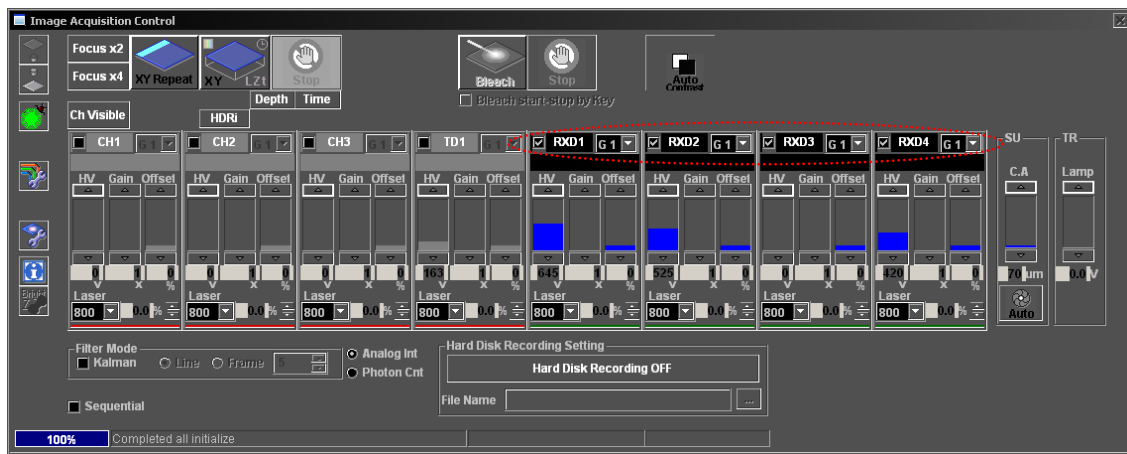


*Light to detectors, SDM570 engaged*  
RXD1 (CFP), RXD2 (YFP), and RXD4 (RFP) available.



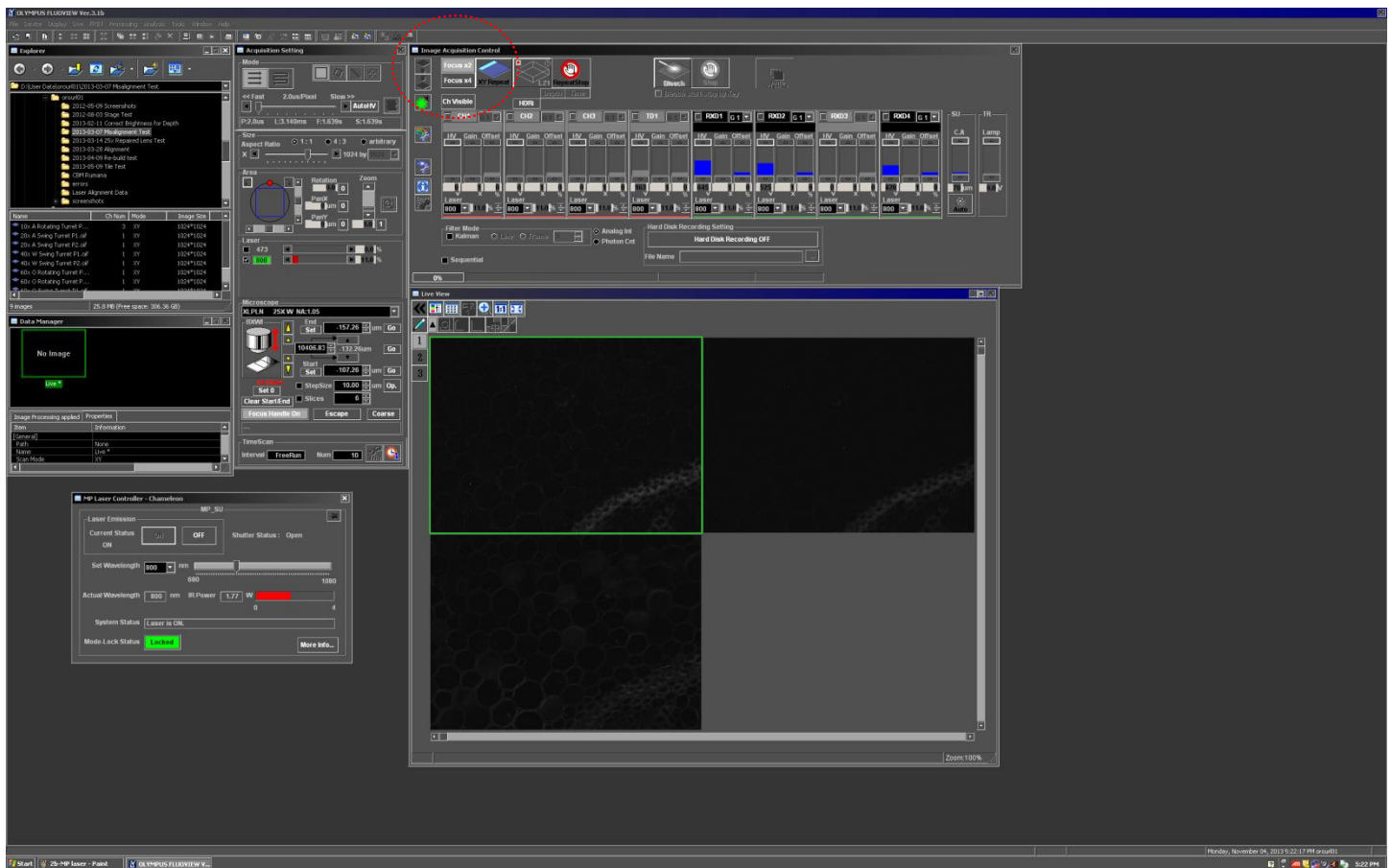
*Light to detectors, SDM570 disengaged*  
RXD3 (wide DAPI-GFP) and RXD4 (RFP) available.

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



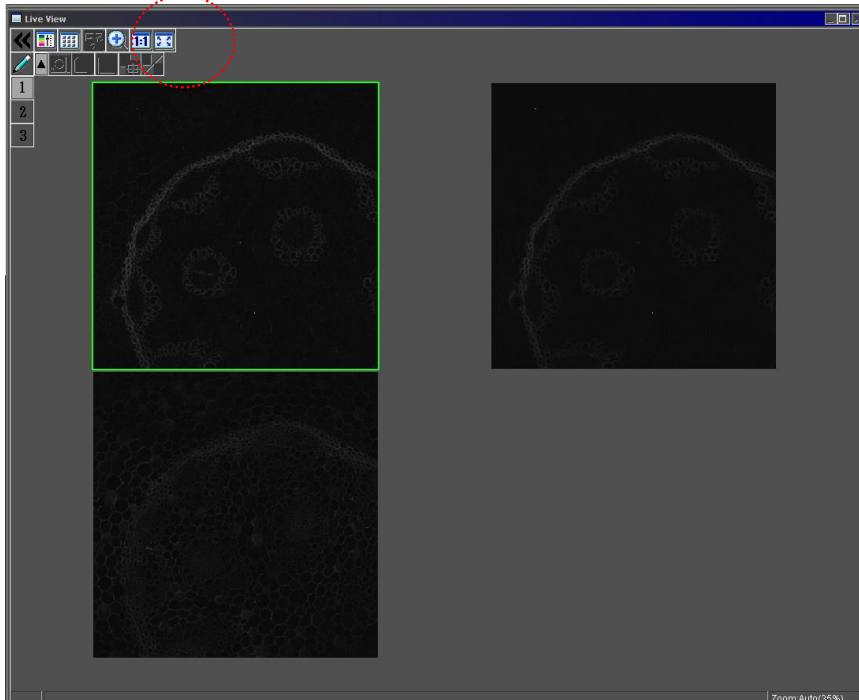
## 6) *The imaging process*

- ☞ 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.

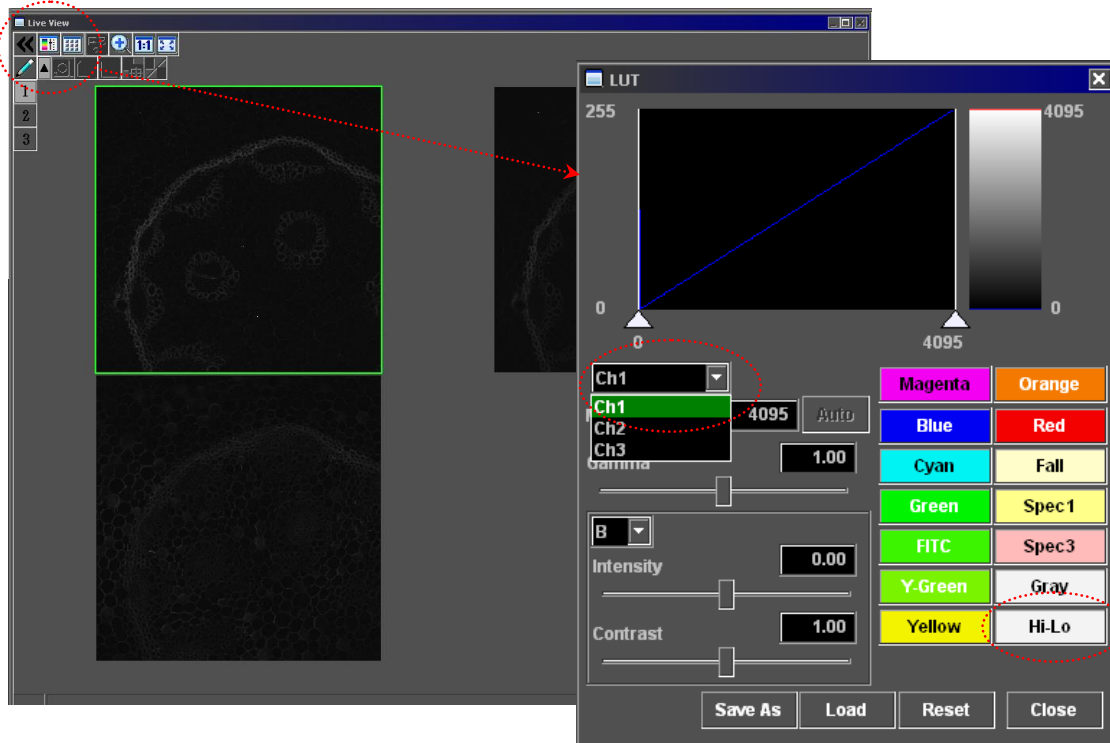


*Convallaria rhizome test slide, Leica Microsystems*

☞ 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.



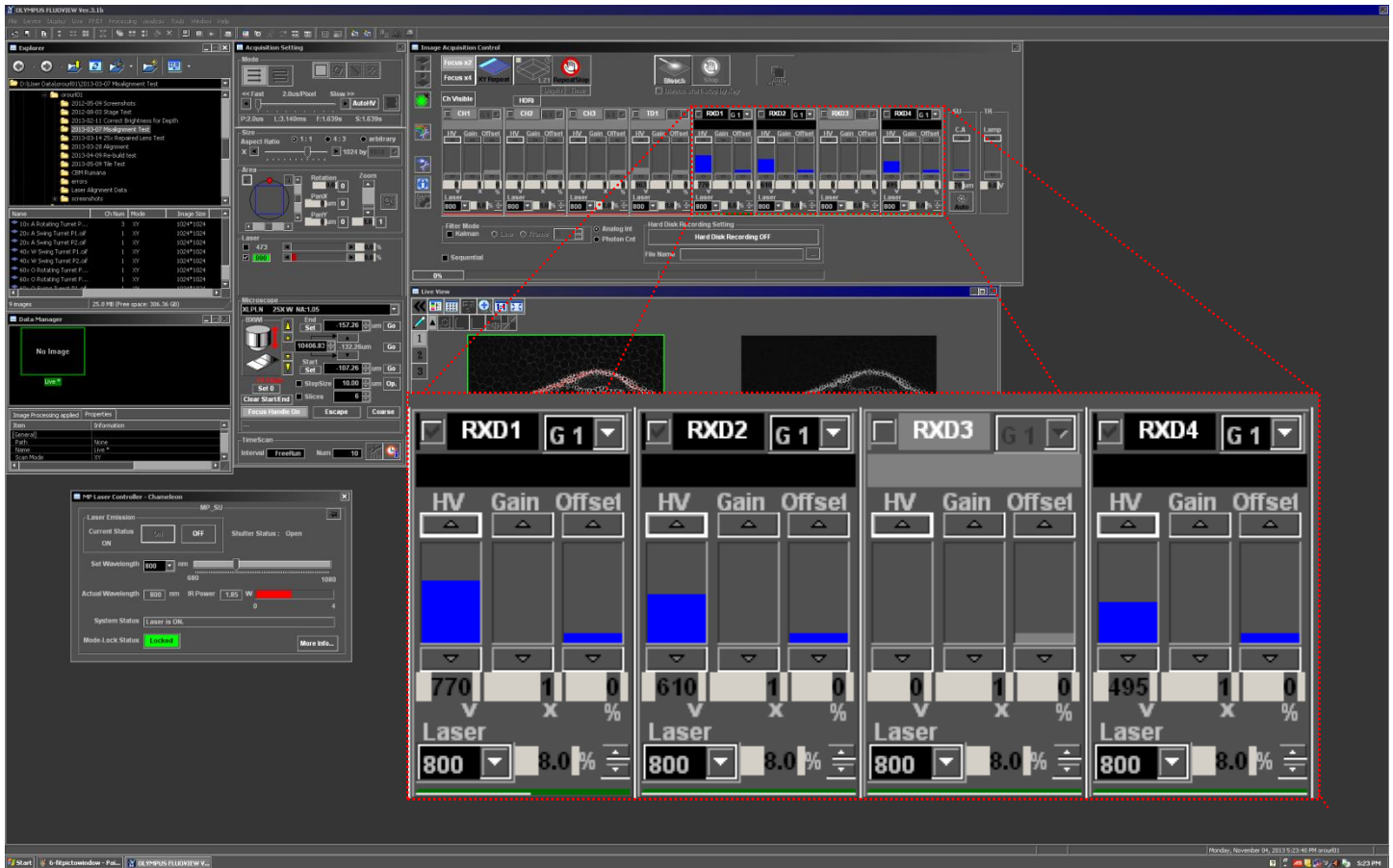
- ☞ Click **RepeatStop** at the top of the Image Acquisition Control menu to stop the fast scan.



- ☞ 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.



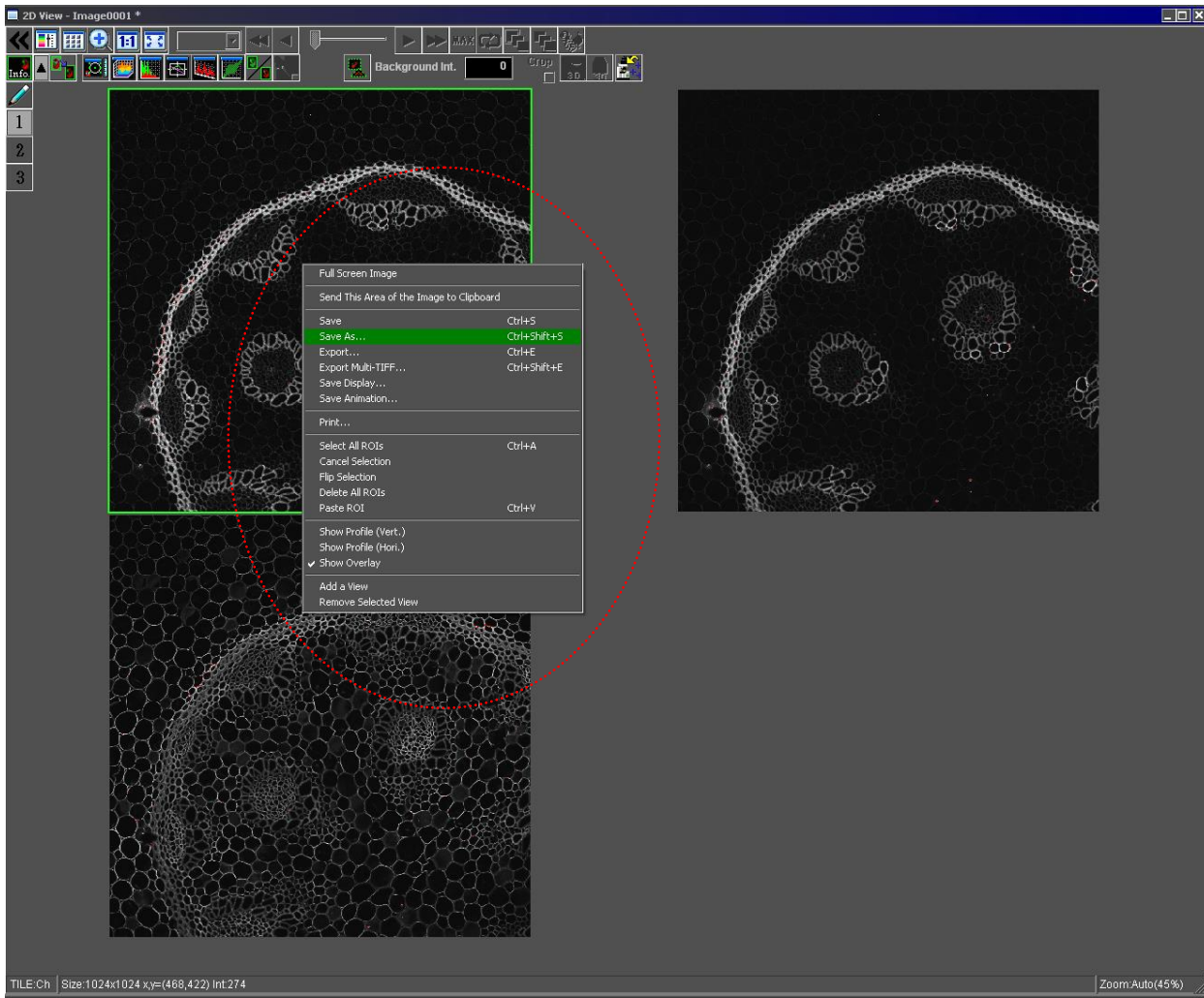
- Adjust the HV (high voltage) on each individual channel to maximize the intensity of your signal while avoiding saturation (red pixels). There should be only a few red pixels in each channel.



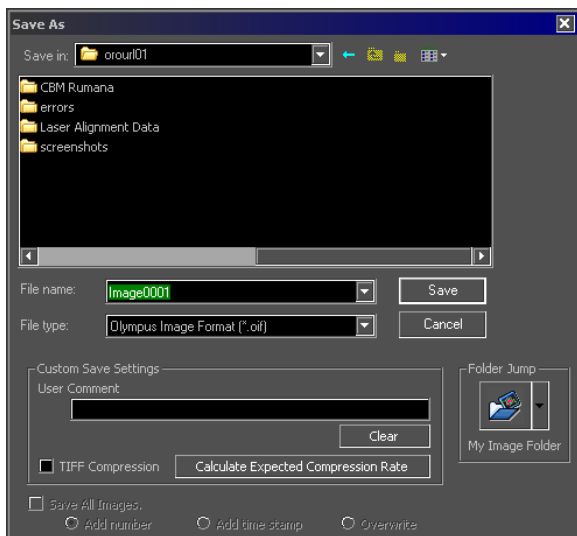
- Click  at the top of the Image Acquisition Control menu to stop the slow scan.

- Click  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.

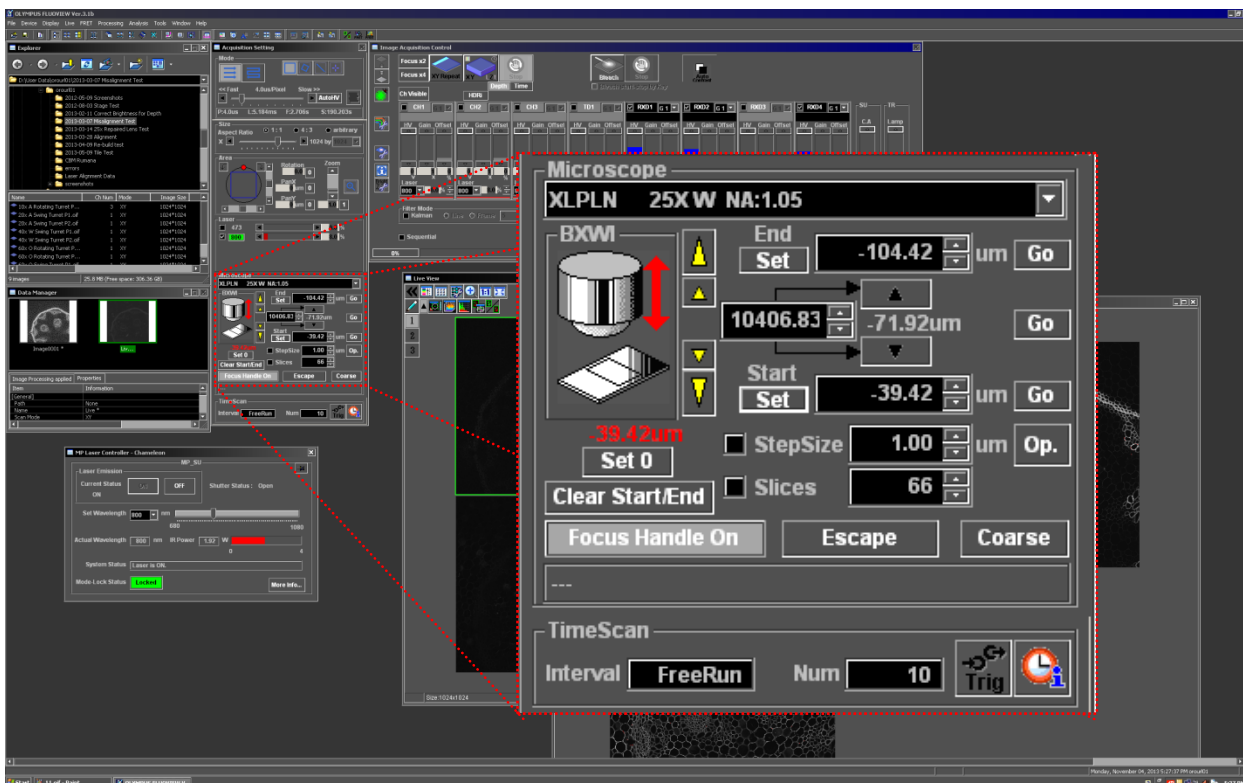


## 7) Returning to the microscope

- ☞ 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**.





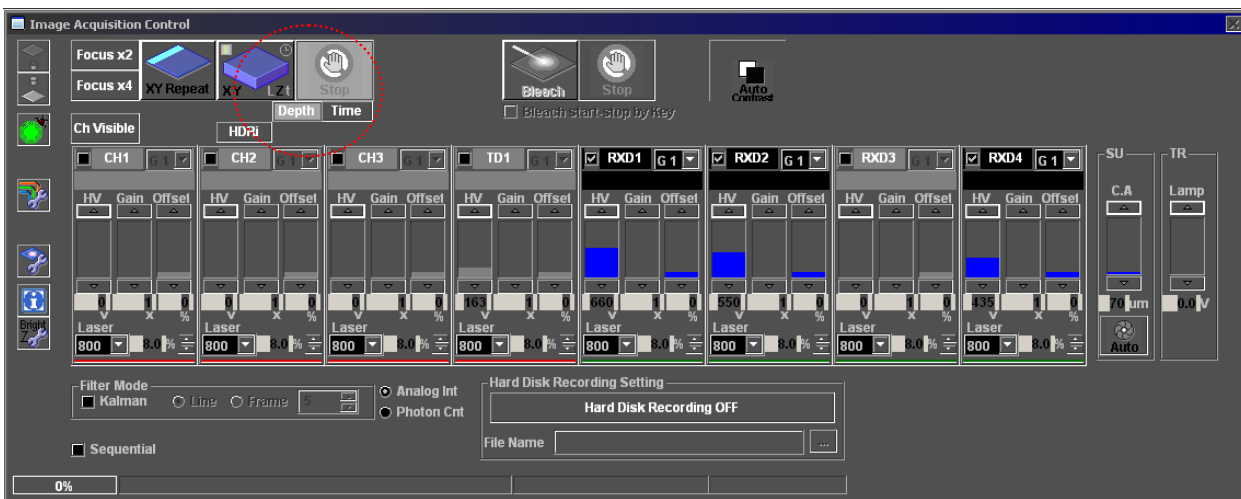
Click .

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  button within the Image Acquisition Control menu.

The frame  will now turn into a volume .



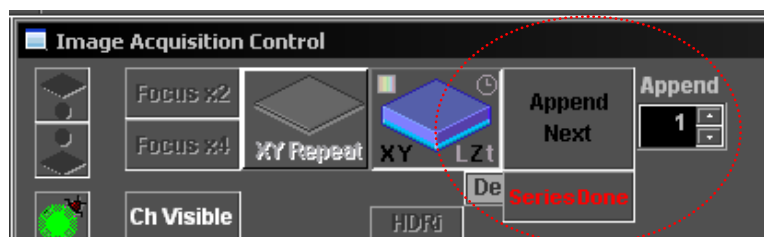
Click  to acquire your Z-stack.

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** and


click **Append Next**.

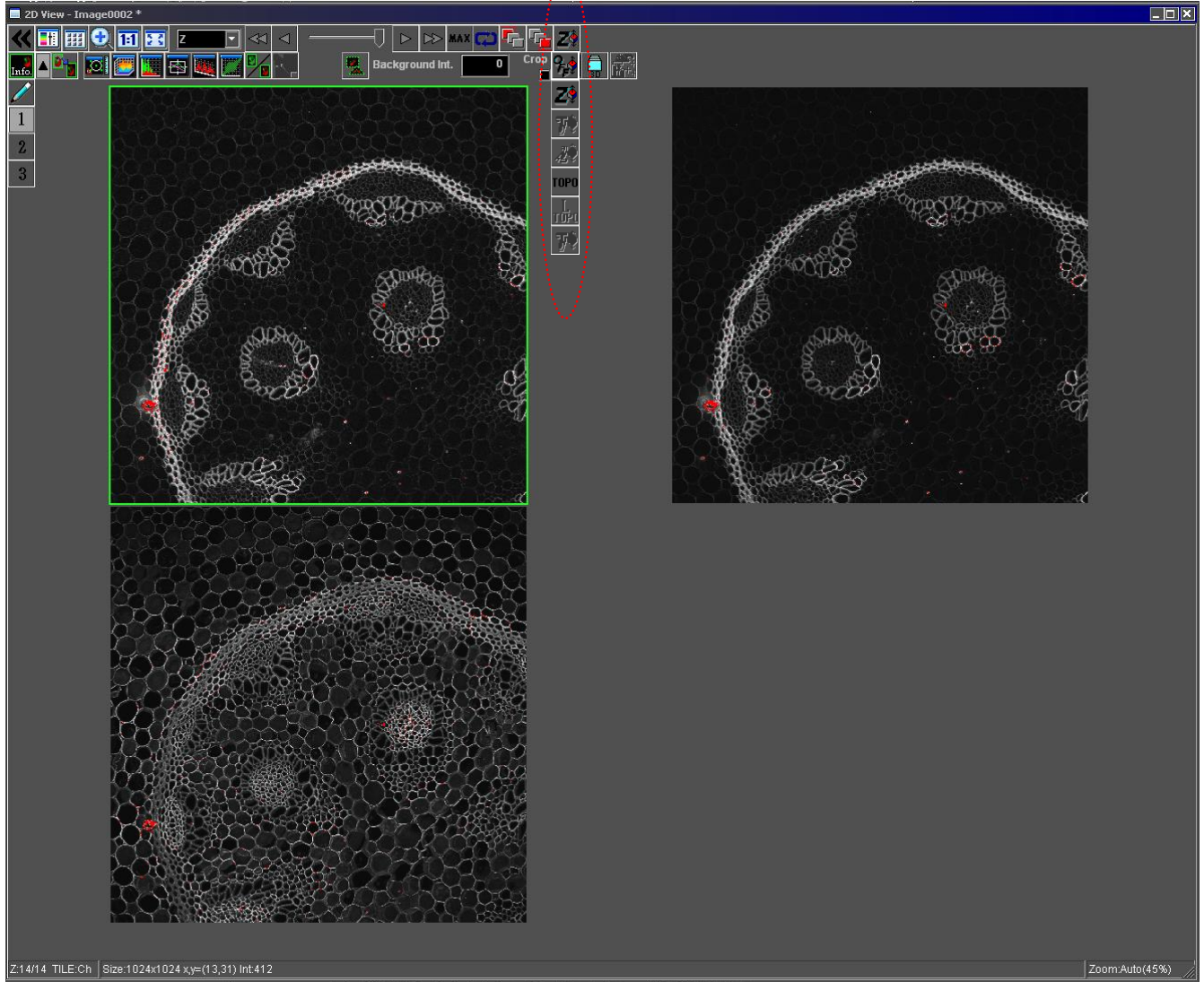
- To complete the series, click **Series Done**.



☞ To save the acquired images, right click within the image window, and select ‘Save As...’

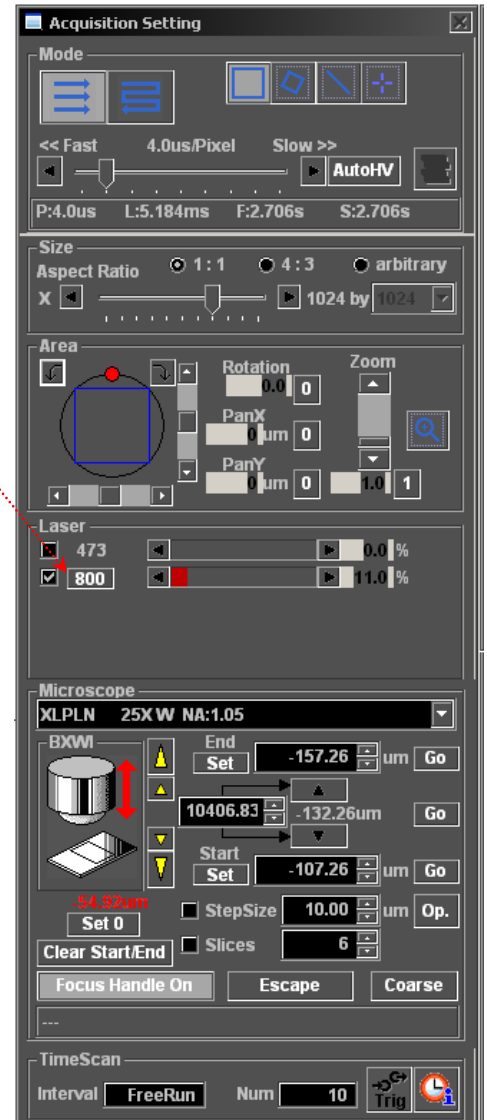
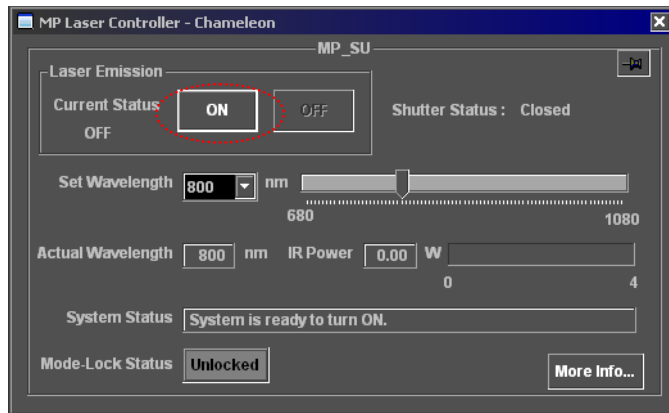
☞ You can view the maximum intensity projection of the Z-stack by clicking the  icon within the 2D View

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.