

### **FV3000 Manual**

If you are the first user of the day start from step 1.

If the system was already on, start from Step 3.

- 1) Ensure the following switches are switched on in this sequence:

**Switch 1**-Master for the system.

**Switch beside Switch 1**-LED light for fluorescence observation through eye piece.

Switch 1 should be switched on 24/7, do not switch off. They are located behind the computer.



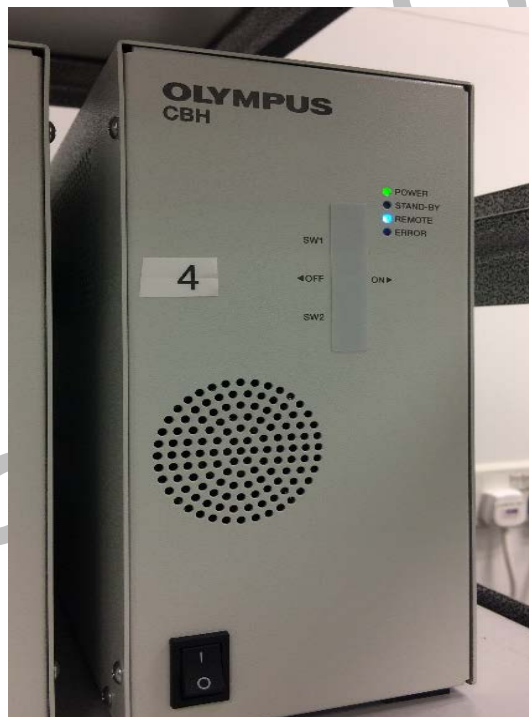
**Switch 2**-CPU of the computer



**Switch 3-** For motorized stage



**Switch 4-** CBH control box

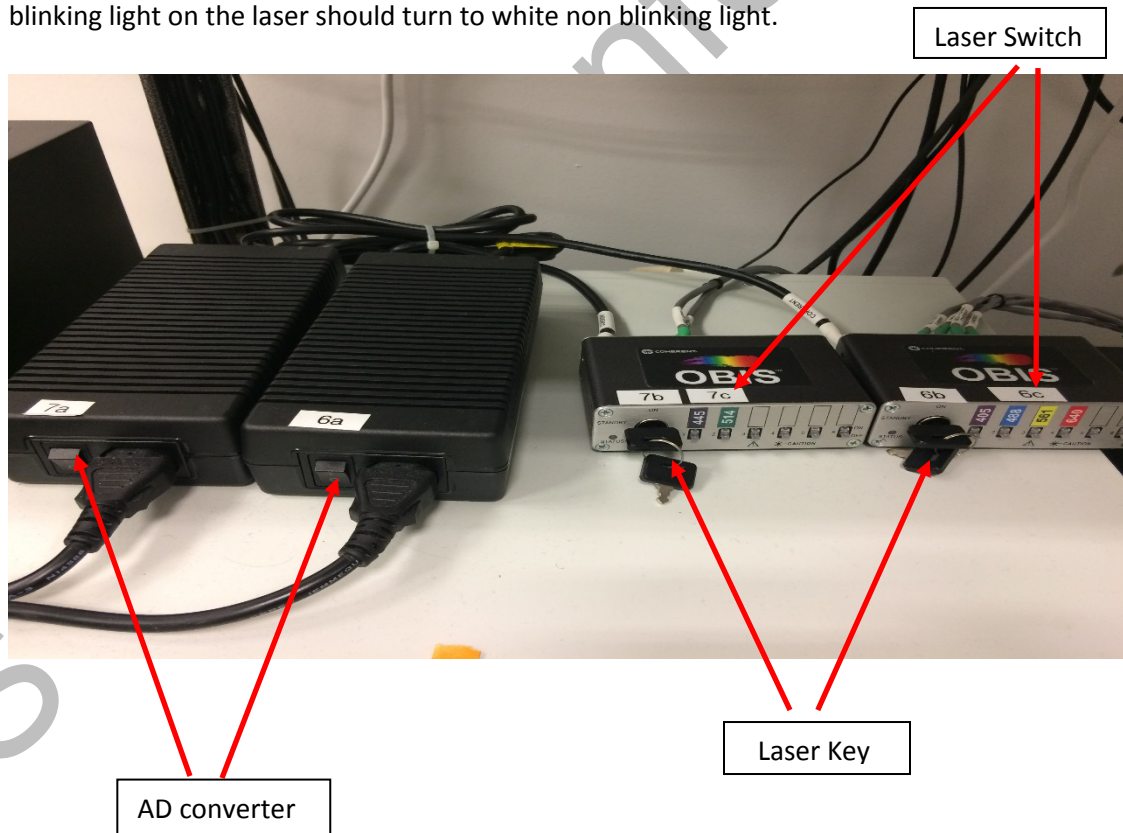


**Switch 5**-Touch panel controller. The 'on' button is at the back of the controller.



**Switch 6a,6b,6c,7a,7b,7c**- 6 Laserlines (switch on in this order).

Switch on only wavelengths of your interest. To know if the lasers are ready to use, the green blinking light on the laser should turn to white non blinking light.

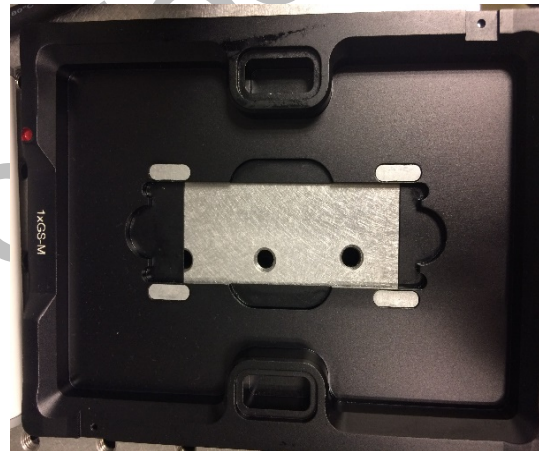


**Switch 8 and 9**-temperature and CO<sub>2</sub> regulator respectively. Switch on them if you are using live cells. And also use the correct stage adaptor.

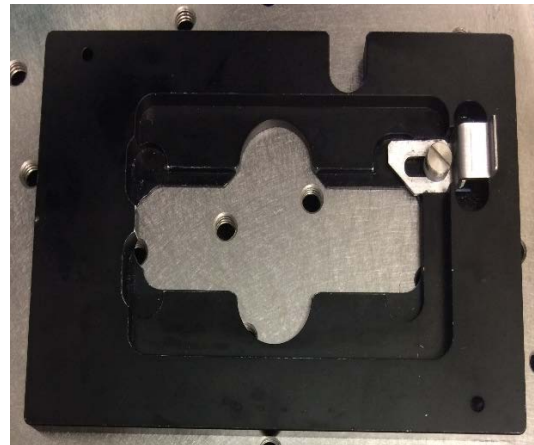
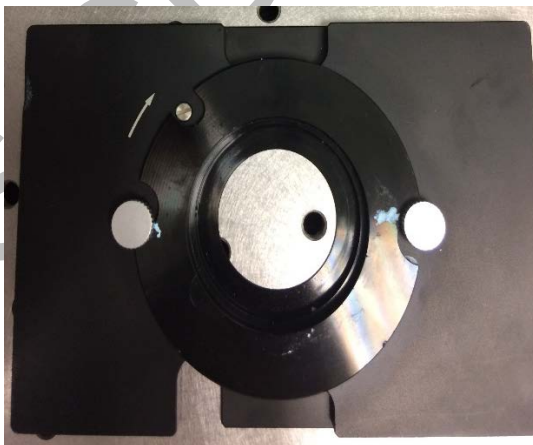


- 1) Turn anti-clock wise to open the valve of the gas cylinder.
- 2) Check the outlet pressure gauge to be at the black-dot-marked position, five minutes after you turn on the gas cylinder.
- 3) Otherwise, use pressure adjustment knob to bring the outlet pressure to the marked position.

***Stage adaptor for live and fixed specimens (Okolab)***



***Stage adaptor for fixed specimens (Olympus)***





**Switch 10**-Light for the microscope box.



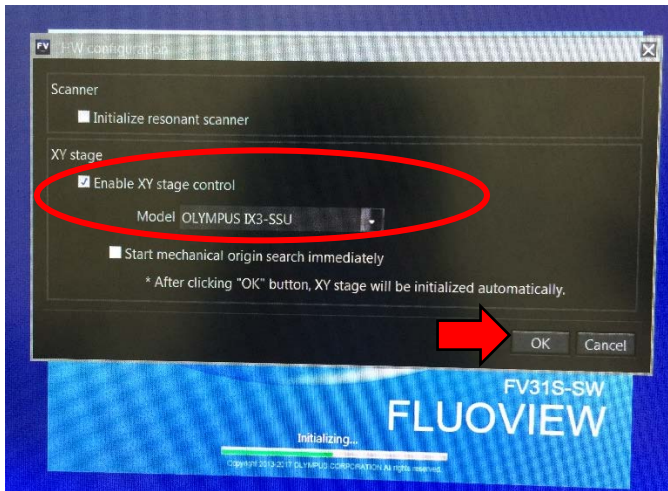
- 2) Log in the computer using password 'olympus' (all small letters).



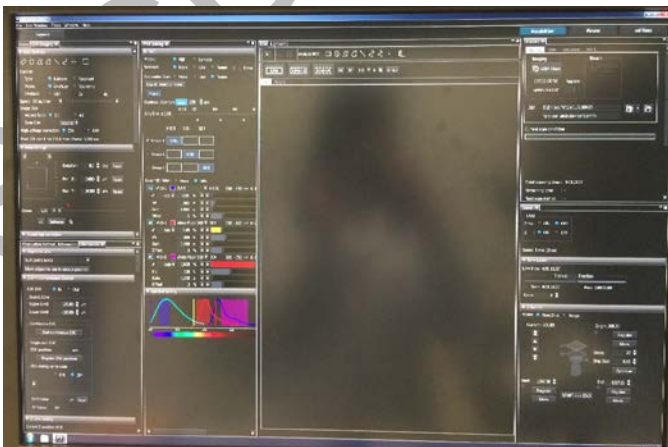
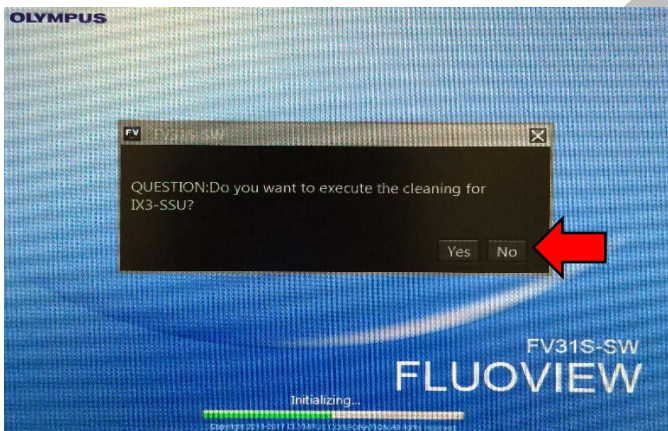
- 3) Click FV31S-SW to load the software.



- 4) Ensure that XY stage control is ticked, and the model is Olympus IX3-SSU, click 'OK' to proceed.  
FYI: Resonant scanner allows high scan speed, but you do not get to choose the scan size.



- 5) Click 'No' for this step, it is for staff maintenance. The software will launch shortly after.

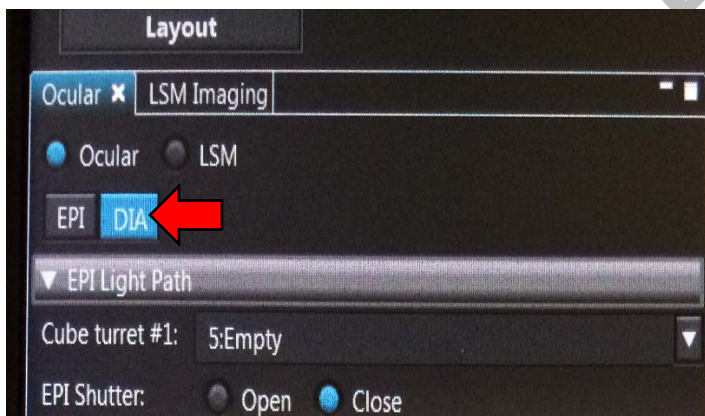


## 6) Direct observation through eye piece-Transmitted light (Using halogen Lamp)

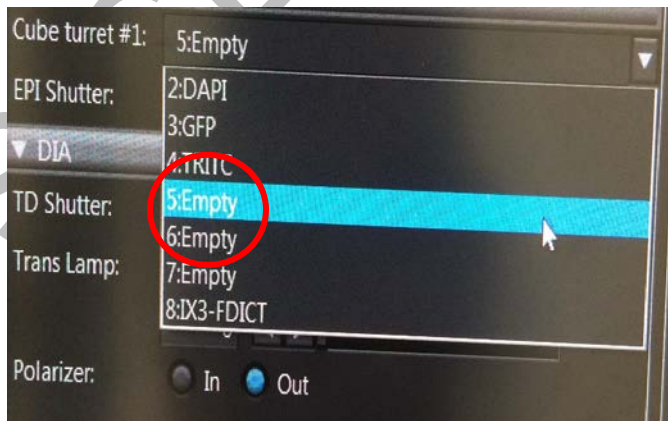
6.1) In the Software, under Ocular tab, select [Ocular].



6.2) Tap [DIA] button

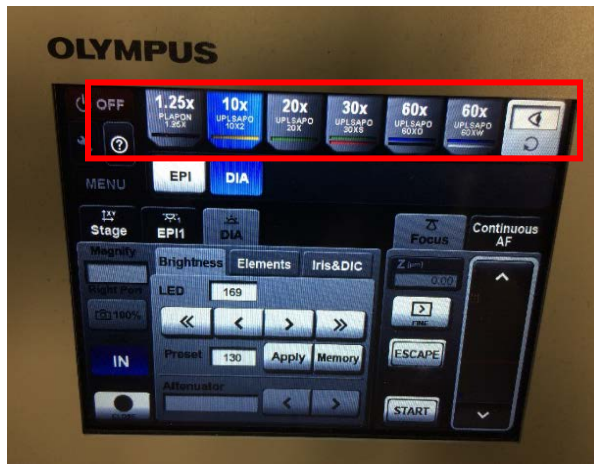


6.3) Select any [Empty] from the dropdown list for cube turret #1.





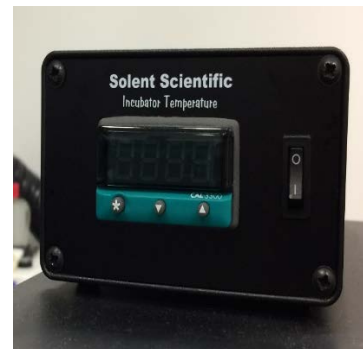
6.4) From the touch panel controller engage the objective len of your interest.



Apply the correct Immersion oil to it. (Objective len 1.25X, 10X and 20X are dry lens, 30XS uses silicon Olympus oil in green bottle, 60XO uses normal Olympus oil in blue bottle and 60XW uses distilled water).



Make sure the correction collar of the objective is turned to 0.17mm as shown below, and use 0.17mm coverslip. By default the correction collar will be turned to 0.17 for 23°C, but if you are doing live cells, you should turn it to 0.17 under 37°C. Switched on the device below and wait for 30 mins for it to warm up if you are doing live cells.

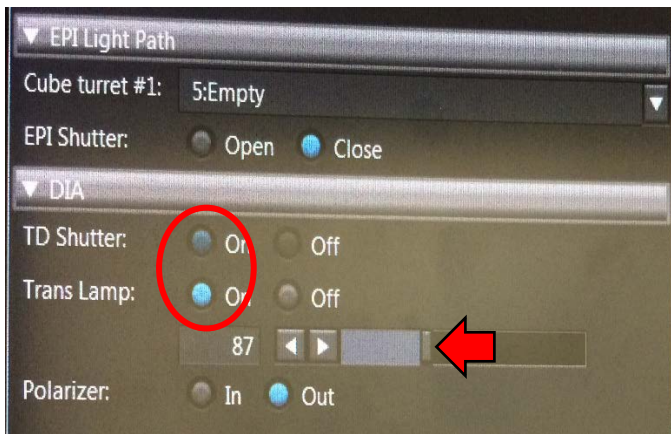




6.5) Place the slide on the stage with your specimen facing downwards to the objective lens.



6.6) Under Trans Lamp, select [ON], and use the slider to adjust the brightness.



You may also adjust the brightness by tapping the [DIA] tab and the [Brightness] tab from the touch panel controller.

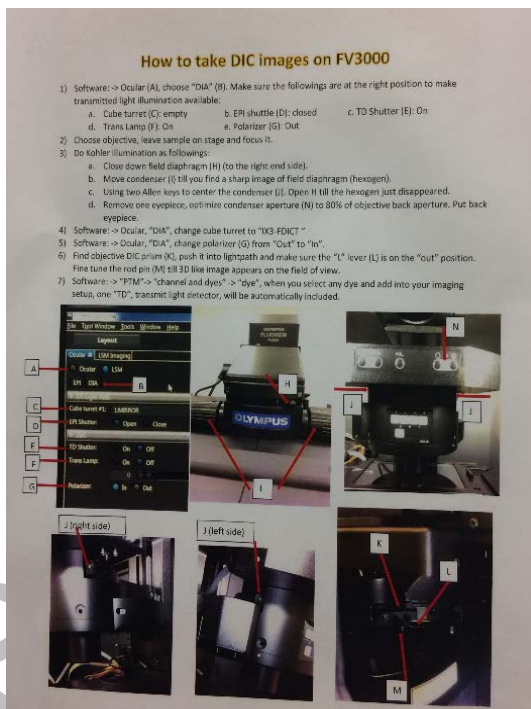


6.7) Look into the eyepiece, rotate the focusing knob of U-MCZ to bring the specimen into focus.

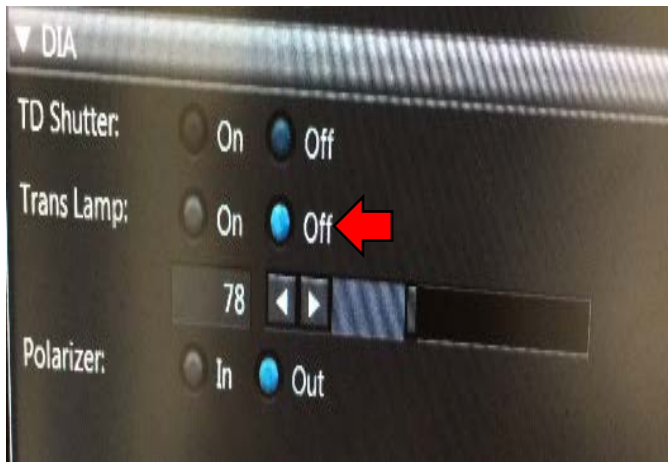


Focusing knobs

6.8) Once your specimen is brought into focus, perform Kohler illumination if your main emphasis is Bright field images. Steps for carrying out Kohler illumination and Transmitted DIC is pasted on the wall as shown.



6.9) To finish transmitted light observation, select [OFF] under Trans Lamp



7) Direct observation through eye piece-Florescence Light (LED lamp)

7.1) Under the Ocular tab select [EPI].



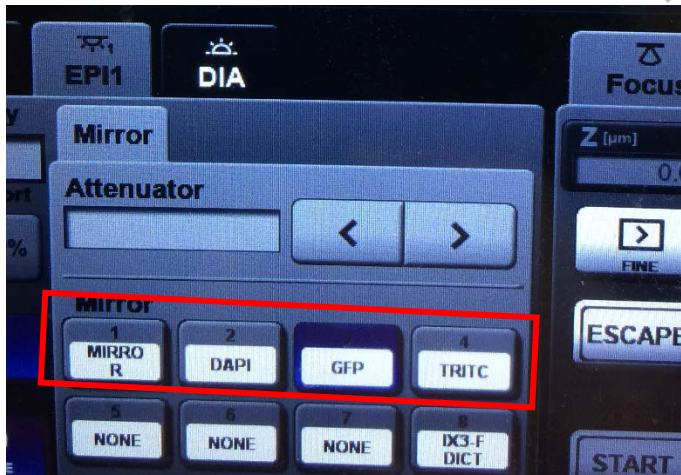
7.2) Under EPI Shutter, select [OPEN].



7.3) You can select the mirror unit from the dropdown list for cube turret #1.

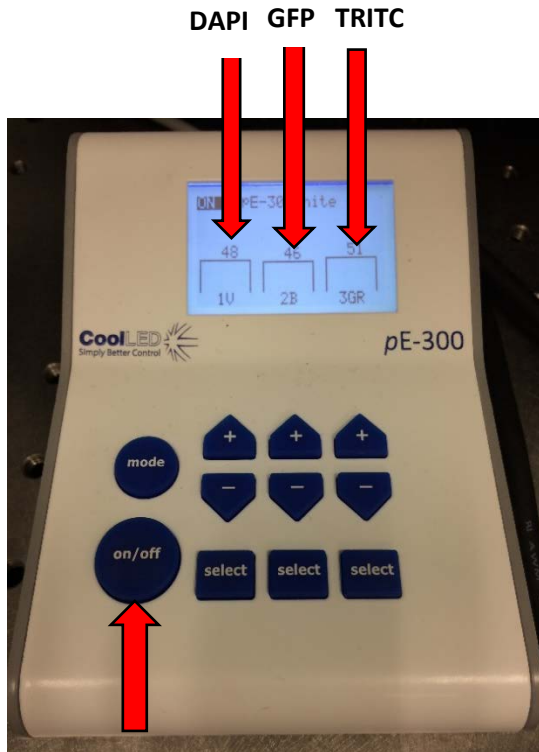


You can also select the mirror unit suitable for the fluorescent probe for observation on the touch panel controller.





7.4) Switch on LED light and select the corresponding LED signal from the Cool LED device.

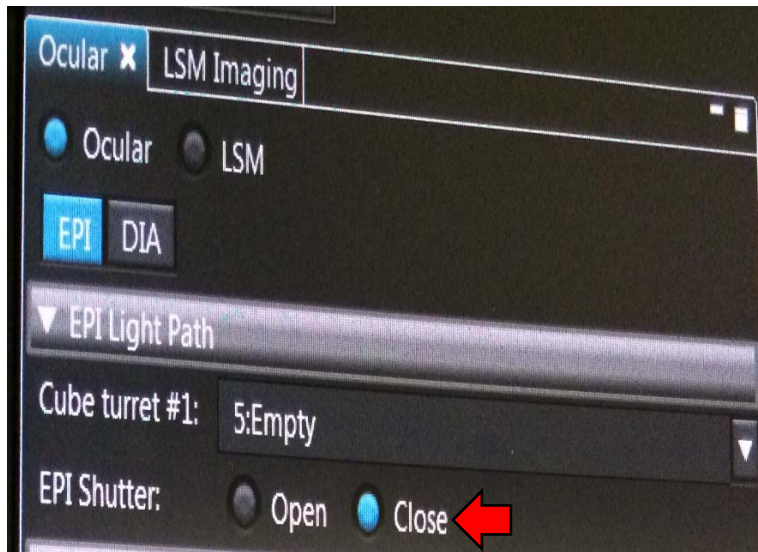


7.5) Look into the eyepiece, rotate the focusing knob of U-MCZ to bring the specimen into focus. Move stage using stage translation knob and you may click on the button for “Fine”/”Coarse” stage movement.



Focusing knobs

7.6) To finish Fluorescence light observation, under EPI Shutter, select [CLOSE]



### LSM observation structure of software screen

#### Ocular Tool Window:

This field is used to select either visual observation or LSM observation

#### PMT Setting Tool Window:

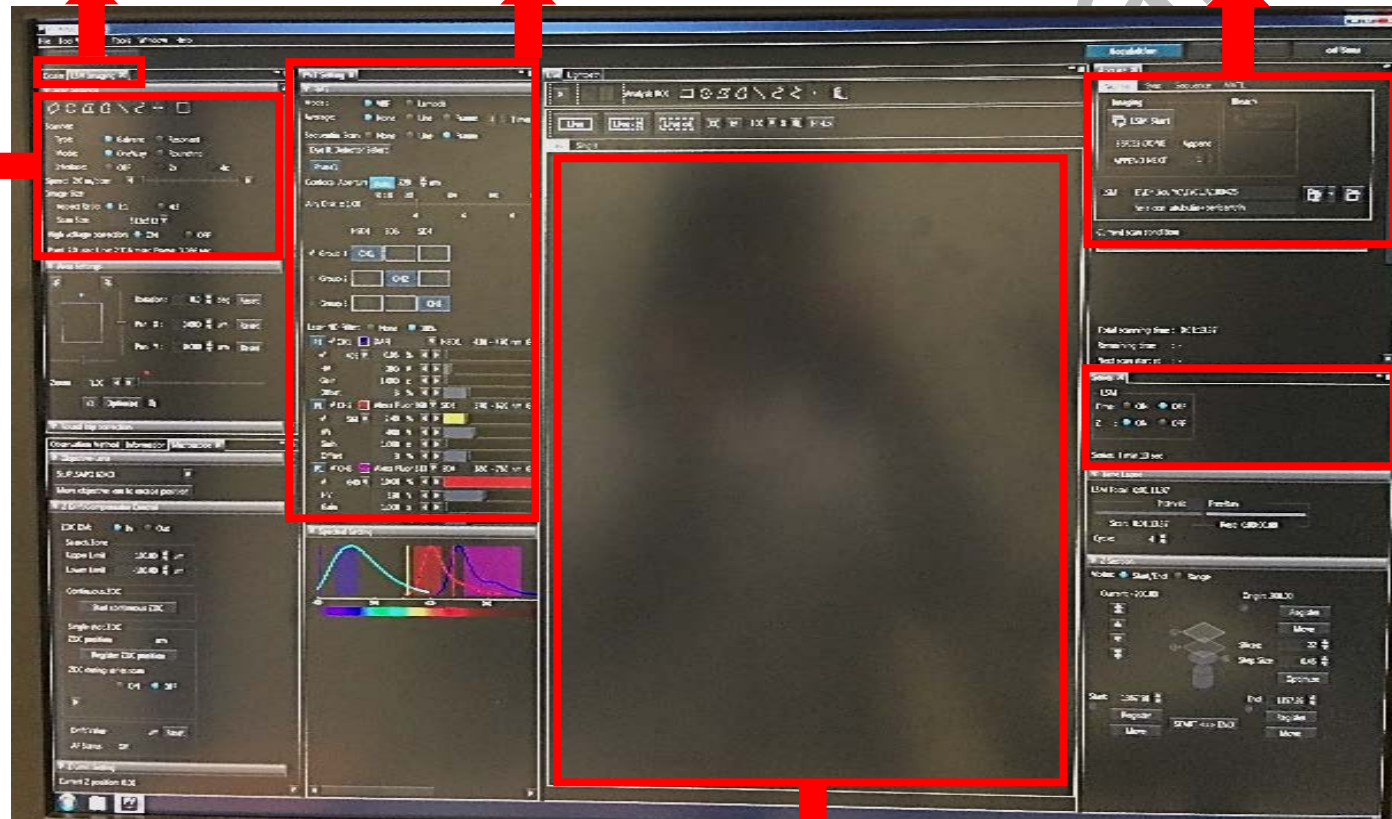
This field is used to set the image channels. The laser intensity or the detector sensitivity is adjusted per each channel.

#### Acquire Tool Window:

This field is used to acquire the images.

#### LSM Imaging Tool Window:

This field is used to set the scan method for image acquisition. Scanner selection, scan area, scan speed or etc. is set in this field.



#### Series Tool Window:

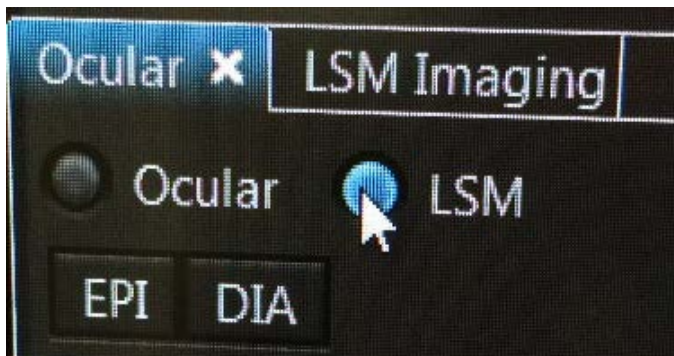
This field is used to set the Z series and T series image acquisition.

#### Live Window:

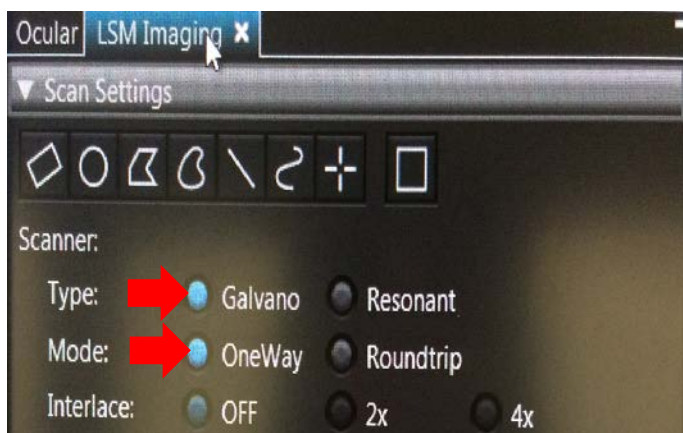
This field displays the images by repeat scan.

## 8) Acquiring Image using Laser

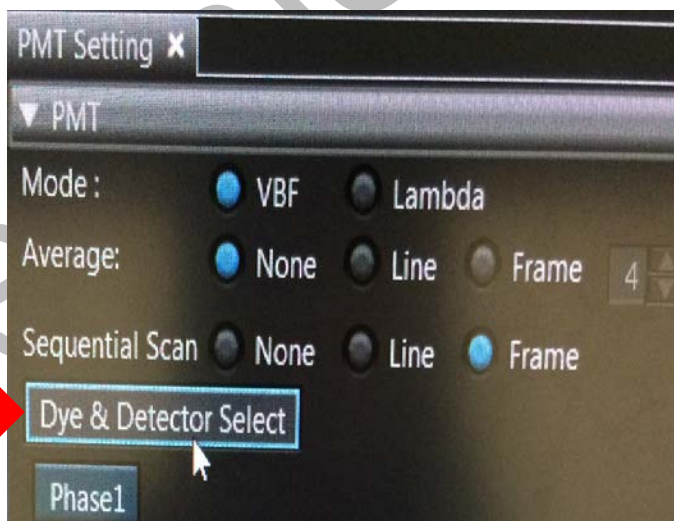
8.1) Select "LSM" in [Ocular] Tool window.



8.2) Select LSM Imaging tab. Under LSM Imaging Tool Window, select Galvano under [Type] for high resolution scanning, Resonant for high speed scanning, and select OneWay under [Mode].



8.3) Press the [Dye & Detector Select] button on PMT Setting Tool Window.

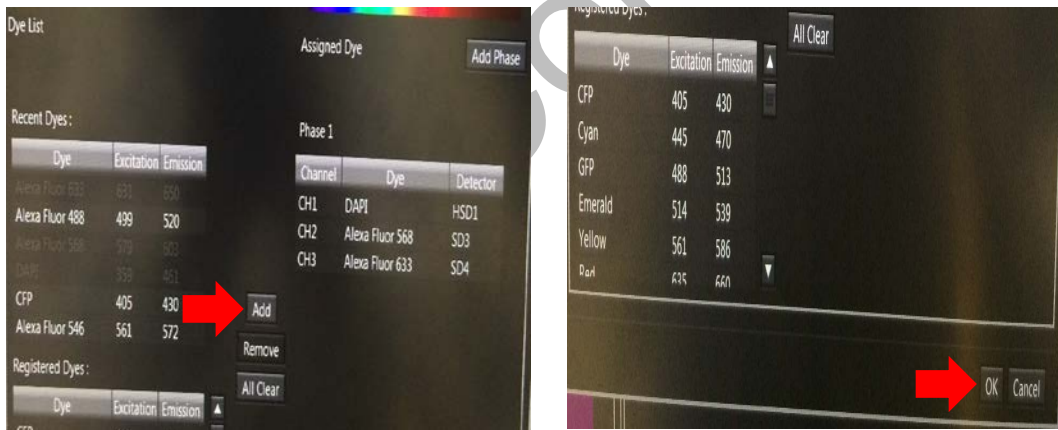




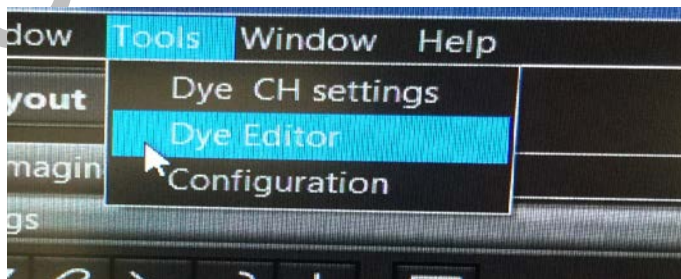
8.4) Select the [Dye] tab in the [Dye & Detector Select] dialog box.



8.5) Select "Dye" to be observed from [Dye List] and click the [Add] button. Or you can drag and drop the selected "Dye" to the observation channel list. After setting all channels, press the [OK] button.



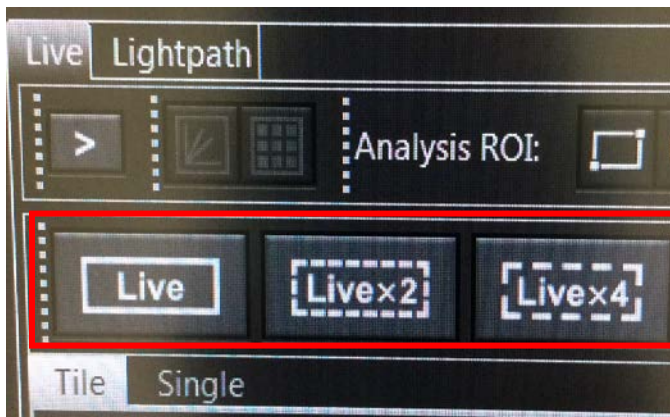
If the "Dye" you want to observe is not in the list, create "Dye" or select "Dye" with similar "Excitation" and "Emission" wavelength instead. You can create "Dye" in [Dye Editor] dialog box displayed when you select [Dye Editor] in the [Tool] menu.



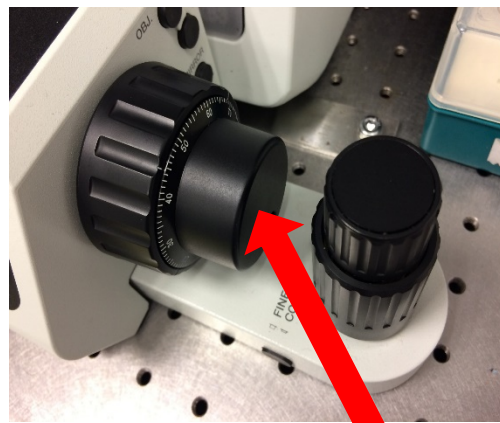
8.6) On the [PMT Setting Tool Window], tick only the checkbox of your main dye.



8.7) Press the [Live] button on the Live Window. Pressing the [Livex2] button or the [Livex4] button will display the live image whose repeat interval is reduced by subtractive scan. This function is useful when searching the cells or observing with moving the focus.



8.8) Apply some laser intensity (%) to your main dye, and rotate the fine knob until you get a bright Image of your specimen on the computer screen.



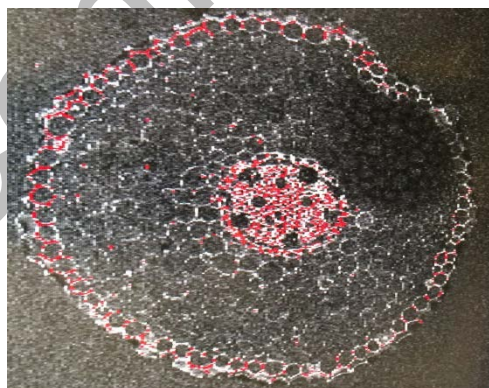
Fine Knob

## 8.9) Next adjust the Laser Intensity (%), HV and offset.

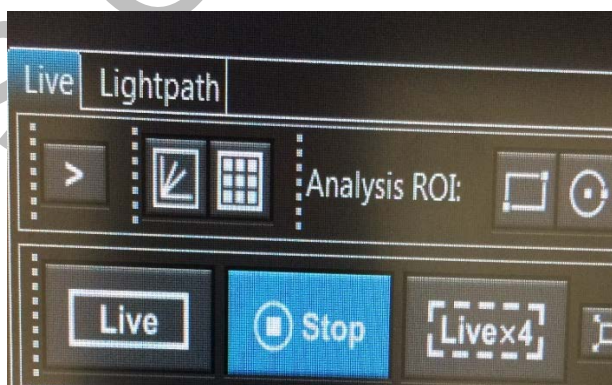
Note: The higher the HV, the stronger the signal, but image will be noisy if HV exceed 600 for High Sensitivity Detector (HSD), and 700 for Sensitivity Detector (SD). On the other hand, the higher laser intensity (%), the stronger the signal but your specimen will photobleach. Hence you will need to toggle with these 2 parameters to get a nice image.



To know if your image is saturated, select [Hi Lo], red pixel means saturation. Also, adjust offset to get some blue pixel. The image is ideal when you see a little of both blue and red pixels.

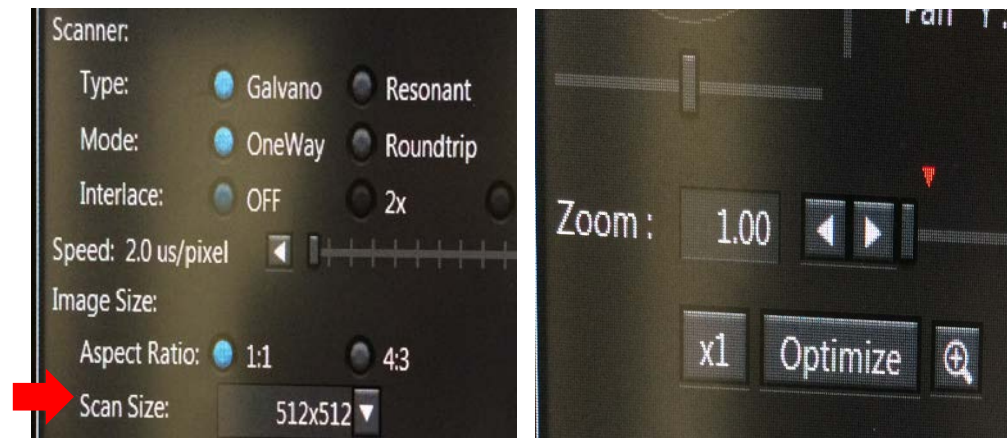


## 8.10) Once you are satisfied with the image, press [Stop] button on the Live Window. The display of the live image is stopped. Repeat step 8.6 to 8.9 for your other dyes, but do not further adjust the focus.



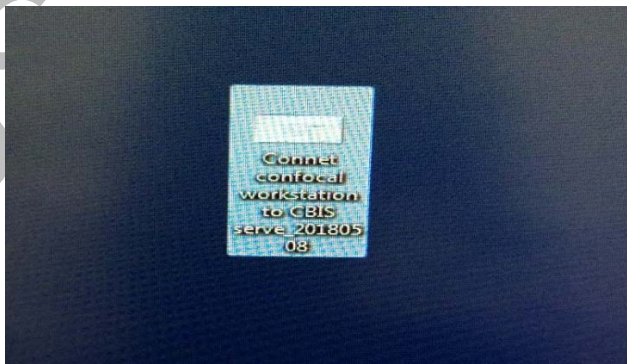


- 8.12) To achieve high resolution image, it is crucial to meet Nyquist Sampling by adjusting the Scan Size and Zoom, such that the actual scanning pixel of X is half of the Optical resolution of X.



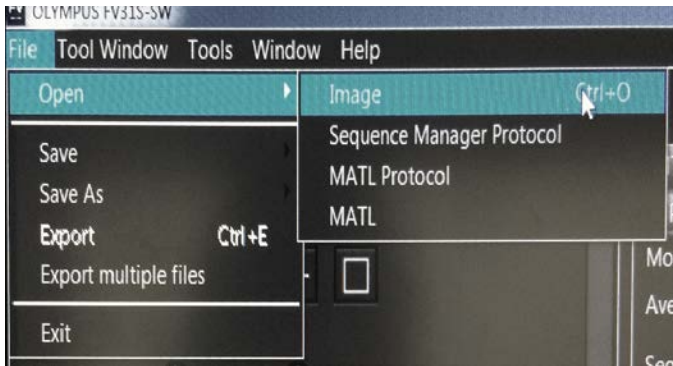
Observation Method		Information x	Microscope
LSM Scanning Information			
Pixel Size			
Actual scanning pixel		Optical resolution	
X:	um/pix	X:	um/pix
Y:	2.4859um/pix	Y:	0.6285um/pix
Z:	0.4600um/pix	Z:	7.8286um/pix

- 8.13) Create a folder under E drive if you don't have one. To take an image select LSM Start, and the image will be saved automatically in your folder.
- 8.14) To retrieve your image file, transfer them to CBIS's server, and retrieve them from the shared computer in the lab. You can refer to the guide that is saved on FV3000 computer for detailed information.

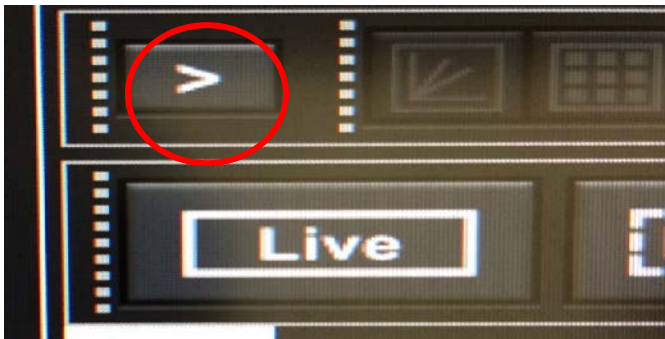




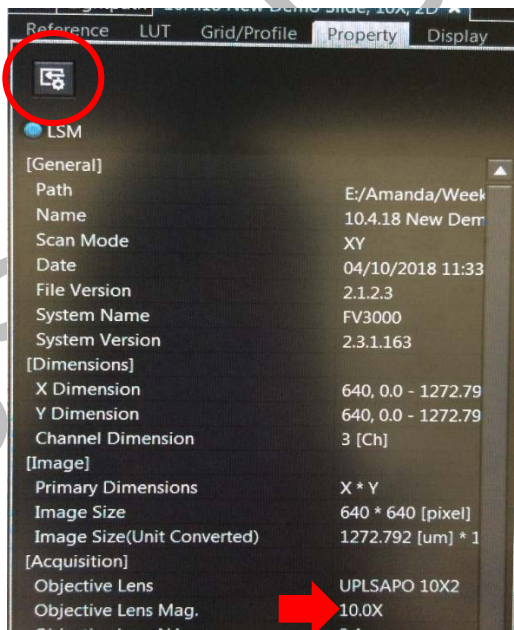
9.1) To reuse your previous settings, go to file -> open-> image. Select the image of your interest.



9.2) Click on your opened image and look for this arrow,



9.3) Look for this logo, click it and all the settings of your previous image can be reused except for the Objective lens, you can find out the magnification you have used previously and manually select it for your current imaging.



- 10) If you are the last user of the day, switch off the system from switch 10 to 1 (in backward sequence).
- 11) Sign out from the logbook.

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