



**HKU
Med**

LKS Faculty of Medicine
Centre for PanorOmic Sciences
香港大學泛組學科研中心

Imaging and Flow Cytometry Core

Imaging and Flow Cytometry Core Centre for PanorOmic Sciences

Olympus FV1200MPE-RS Hybrid Multiphoton System Standard Operation Protocol

Imaging and Flow Cytometry Core




Olympus FV1200MPE-RS Hybrid Multiphoton System Standard Operation Protocol Basic Operation

Turn on system

Please sign on the log sheet before switching on system.

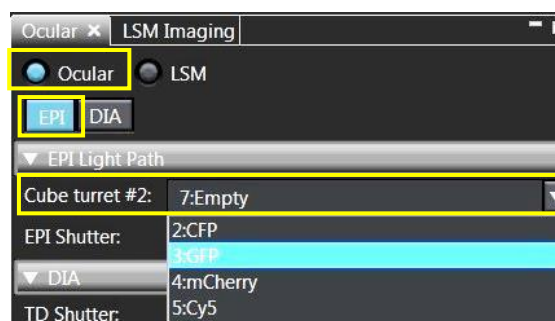
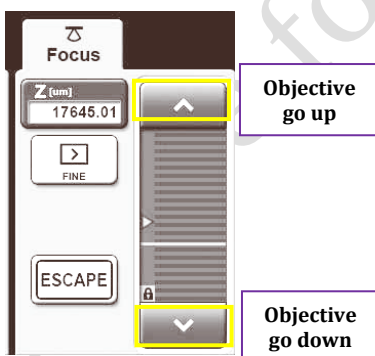
- Turn on Chameleon laser by turning the Laser Key 90° clockwise to horizontal position (if only one laser needs to be used, please only turn on laser 1)
- Turn on computer and log in with “User” ①
- Turn on power supply Unit FV30-PSU ② (please wait for about 10 sec before go to next step)
- Turn on power switch of Main scanner ③ (please wait for about 10 sec before go to next step)
- Turn on power switch of SIM scanner ④
- Turn on fluorescent lamp illuminator ⑤ (the switch is at the left back of the box, shown by →), wait for 3sec, press the “ON/OFF button at the front panel
- Turn on microscope power ⑥
- Turn on the touch panel controller ⑦ (the switch is at the right back of the box, shown by →)



Setting the microscope

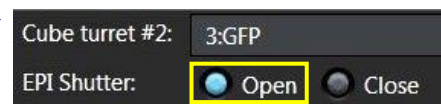
- Double click the “FV30S-SW” icon  on the desktop to initialize the software, it may take 1-2min for software and hardware connection.
- Keep pressing  on touch screen to move the objective up
- Select the objective lens manually and engage it in the light path 
- Put your sample on the stage
- Select **Ocular** to set the microscope for direct observation via the eyepieces
- Click on **EPI** for fluorescence observation
- Select the filter for fluorescence observation in the drop down list of **Cube Turrent #2**.



Pull the handle out or push it in to select objective



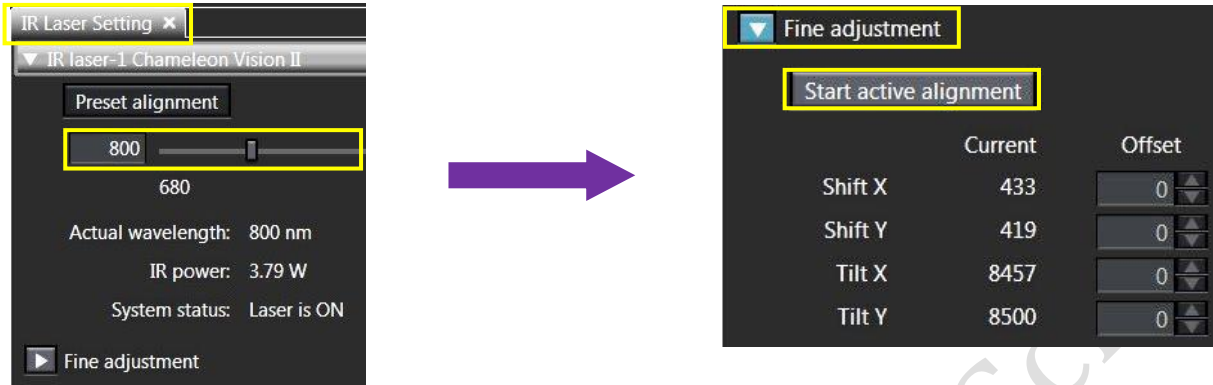
- Put water on the sample if water objective will be used.
- Keep pressing  on touch screen to move the objective close to the sample
- Click **Open** in **EPI shutter** to open shutter if necessary 
- Focus the sample with Corse/fine focus knob
- Locate the view of interest through stage control knob



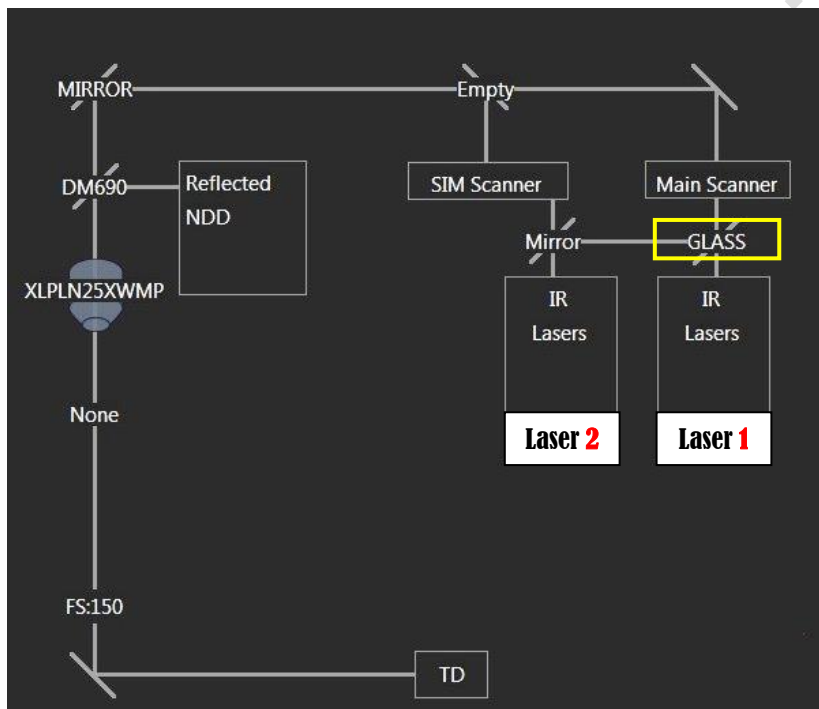
Imaging and Flow Cytometry Core

Configuring the beam path and laser

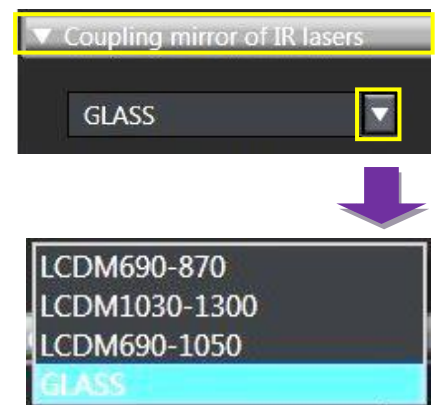
- In **IR Laser Setting** Tool Window, set the wavelength of the IR laser to be used.
- Press the button of **Fine adjustment**, and press the button **Start active alignment** which will align the laser to optimal position.



- Click on the **Lightpath** button to open the **Lightpath** window.

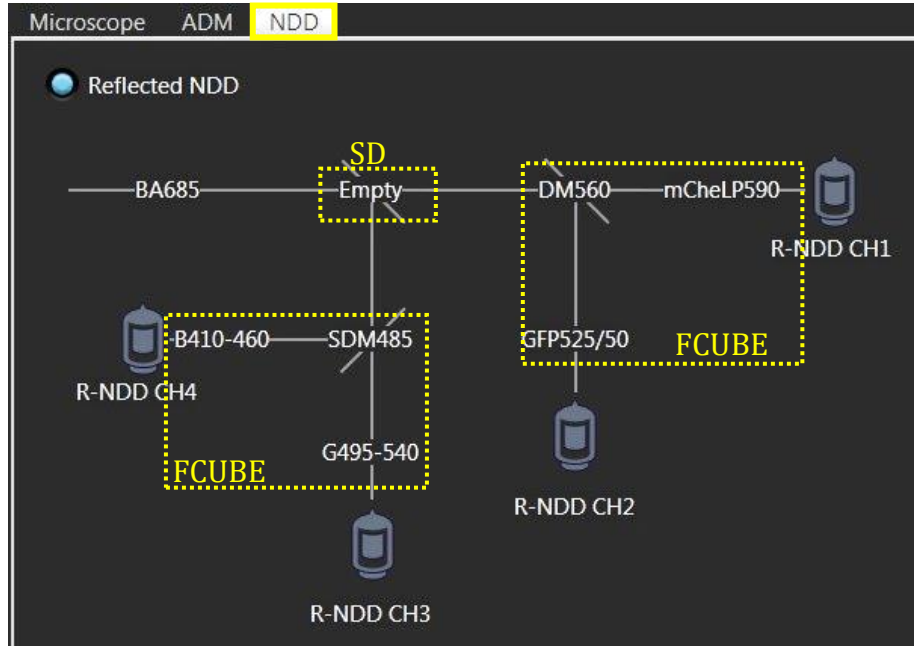


- Select coupling mirror in **Coupling mirror of IR laser** tool window depends on the wavelength to be used.
- Chose "Glass" in "Coupling mirror of IR lasers" if only laser 1 is in use.
- Chose "LCDM690-870" in "Coupling mirror of IR lasers" for two laser lines with Laser 1 $\geq 920\text{nm}$ and Laser 2 $\leq 870\text{nm}$
- Chose "LCDM 1030-1300" in "Coupling mirror of IR lasers" for two laser lines with Laser 1 $\leq 940\text{nm}$ and Laser 2 ≥ 1030



Imaging and Flow Cytometry Core

- Check NDD filters in NDD panel of Lightpath



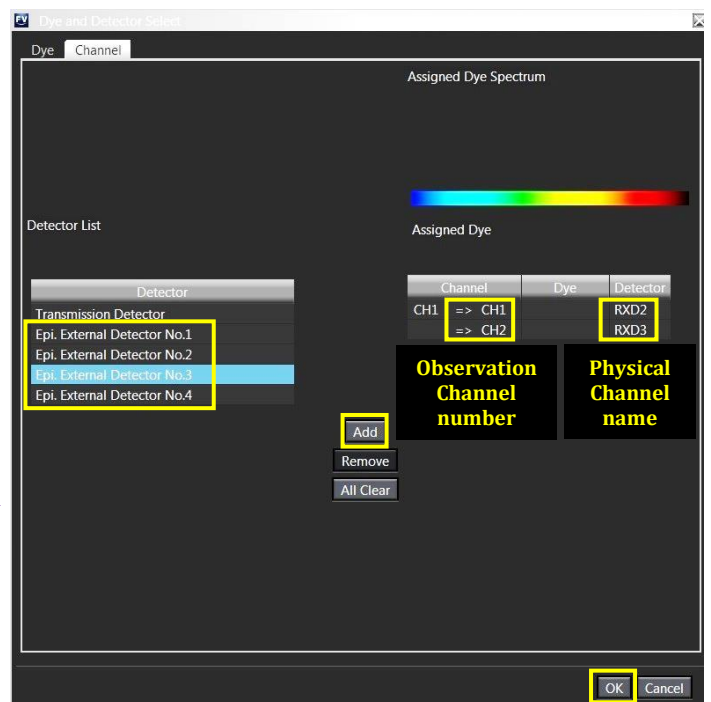
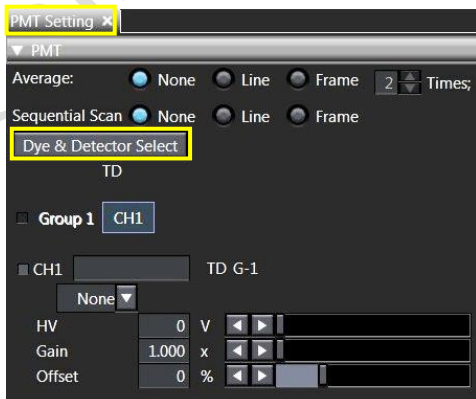
SDM	
SDM570	>570nm pass to Ch1/2, <570nm reflect to Ch3/4
Empty	All pass to Ch1/2
Mirror	All reflect to Ch3/4

NDD filter cube list at FCF:

FCUBE	Dichroic mirror	BF for Ch1/Ch3	BF for Ch2/Ch4
Blue/Green	DM485	G 495-540 (Green)	B 410-460 (Blue)
Red/FarRed	DM650	FR 660-750 (FarRed)	R 575-645 (Red)
GFP/mCherry	DM560	mCherry LP590	GFP 525/50
mCherry/Qdot655	DM630	Qdot 655/40	mCherry 600/50
SHG/YFP	DM495	YFP 535/50	SHG 460/50

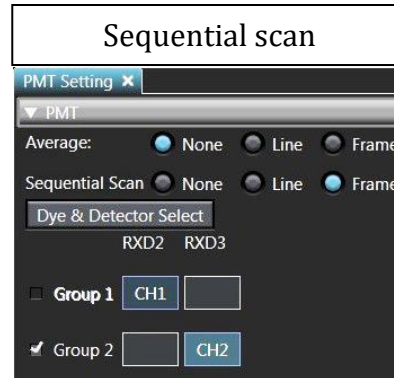
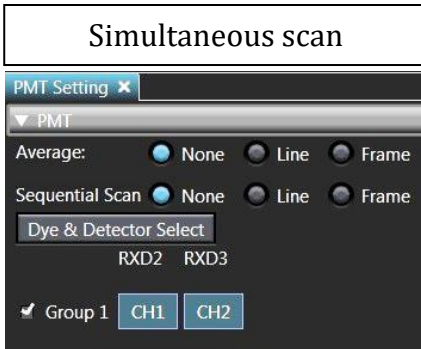
Please be noted that FCUBE filter might be changed based on user's application. Please contact FCF staff if the FCUBE filter in use does not fit your application.

- Press the **Dye & Detector Select** button in **PMT Setting** tool window. The **Dye & Detector Select** dialog box appears
- Select the detector to be assigned to the observation channel in Detector list
- Press the **Add** button. The observation channel number and the abbreviation (physical channel name) of the assigned detector are displayed in the observation channel list.
- After setting all channels, press the button **OK**.



Imaging and Flow Cytometry Core

- If sequential scan needed for multi-channel acquisition, click **Line** or **Frame** in **PMT Setting** tool window

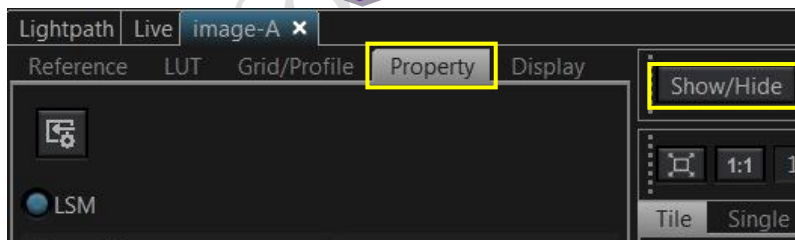
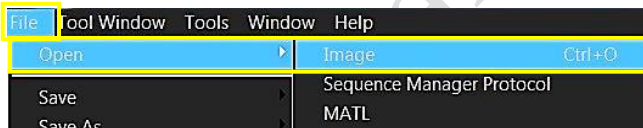


- Simultaneous scan**
 - Use for one or two excitation wavelength
 - Advantage: faster image acquisition
 - Disadvantage: cross talk might be happened between channels

- Sequential scan**
 - Only applicable for two different laser wavelength
 - Advantage: when one group is active, only one detector and one laser line is switched on. This may reduce cross talk
 - Disadvantage: slower image acquisition

Reuse previous acquisition settings

- Select [Open] - [Image] in the [File] menu on the software screen to display the dialog box, and select the image to view.
- Click **Show/Hide** and select **Property** to load information of acquisition settings
- Click to reload previous setting



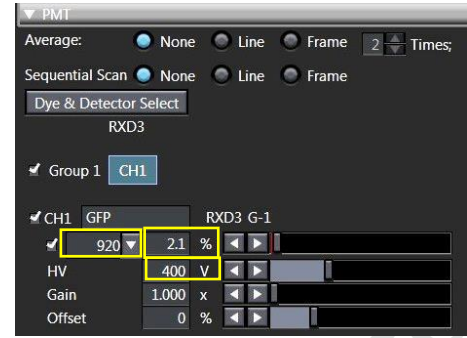
Adjusting the live image

- Click on the **LSM** button to switch to scanning mode, then click **LSM imaging** for scan settings



Imaging and Flow Cytometry Core

- In **PMT** window, select the laser, set an initial value for laser intensity and HV value
It is suggested to set the laser intensity <5% and HV <800
- Press the **Live** button in the **Live** window. (The button view is changed to **Stop**.) The live image is displayed in display area.
- In **PMT** Window, fine-tune Laser Intensity (%), Sensitivity (HV), Gain and Offset to optimize the image



- Click on **Hi-Lo** button to display saturation pixels. Red = Saturation (maximum). Blue = Zero (minimum).
- Adjust **laser intensity (%)** and **HV** to obtain few red pixels shown on image. Increase **Offset** to introduce blue pixels in background area.
- Click **Stop** to complete the preview of the sample.



* User is advised **not to use too extreme settings on laser intensity and HV** (as **too high laser power will induce bleaching** onto the sample; **sample burning** might be happened if the laser power is too high; and **too high HV will cause significant noise and shorten the shell life of the PMT**).
In any given cases, users **should keep the HV < 800**.

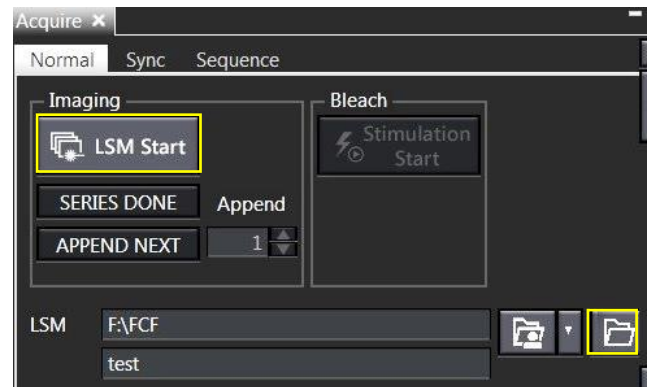
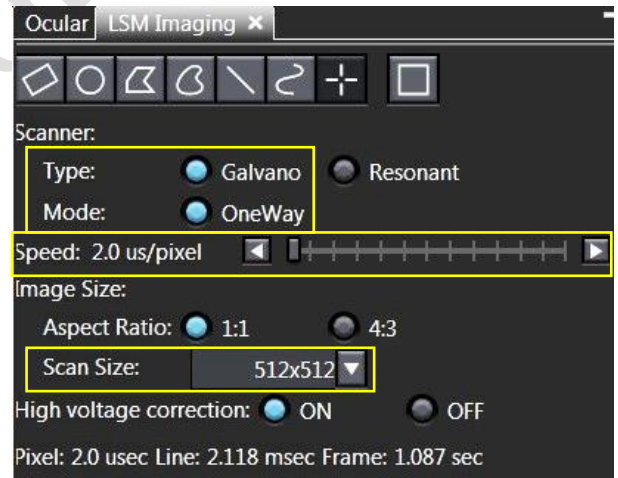
Acquiring an image

- In **LSM Imaging** Tool Window, select the scanner **Type** and **Mode**.

Type	"Galvano" (High definition scan) or "Resonant" (High speed scan)
Mode	"OneWay" (Scan in one direction) or "Roundtrip" (Scan in both directions)



Galvano and **OneWay** is suggested in regular acquisition.

- Use the slider in the **Speed** to adjust the scan speed. Slower speed increases signal/noise ratio of the image.
- Select the **Scan Size** as predefined number of pixels.
- Select the number of **average** in **PMT** tool window. Averaging improves the image by increasing the signal-to-noise ratio.
- In "Acquire" tool window, press the  button and select your folder in **F** drive to save the images. The acquired images are saved automatically.
- Press  to start acquiring the image.



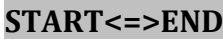

Imaging and Flow Cytometry Core

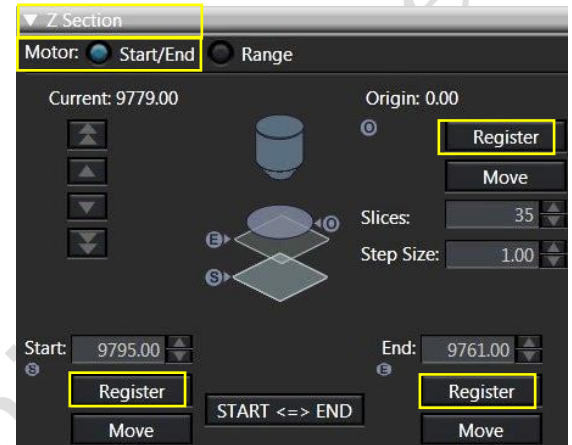
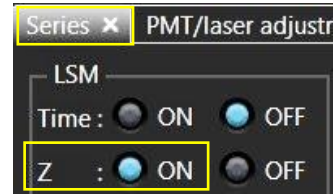
Scanning Z series




- Select **ON** for **Z** in **Series** Tool Window.
- Select "**Start/End**" in **Motor** on **Z Section**.
- Select **Mark First/Last** on the **Z Settings** panel.
- Click  button to have continuously scanning.
- Press the  button in **Origin** at the Z position which is the reference position to acquire the images.

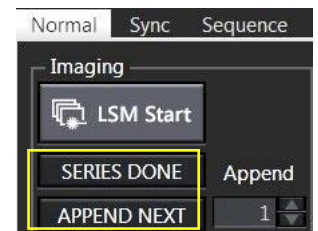
**The distance from the Z position registered in [Origin] to the current Z position is displayed in [Current].*

If you register the top surface of the specimen in [Origin], the depth from the top surface to the observation position is always displayed in [Current], which is very useful.

- Turn the focusing knob gently to locate the start position of the specimen to **Register** start. Then locate the end position of specimen to **Register** end.
- Press the  button to reverse the start position and the end position if necessary.
- Click  to complete the preview of the sample.
- Set "**set size**" for Z-stack scanning. *This digit suggests the distance in um between each slide.*

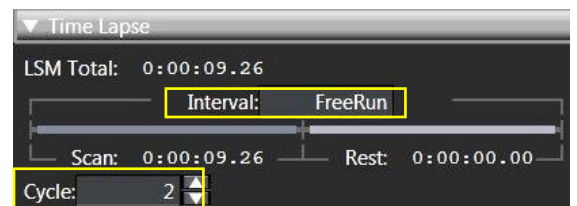


- Click on the  button to start the recording of the Z Series.
- After the image is acquired, pressing the  button allows you to perform the image acquisition repetitively under the same condition. Press the  button to complete the image acquisition.




Setting T series

- Select **ON** for **Time** in **Series** Tool Window.
- Set the interval by input in how many seconds in **Interval**.
**If you attempt to set the time shorter than the time displayed in [Scan] (time taken for acquiring 1 cycle image) in [Interval], "FreeRun" appears. In this case, the interval to acquire the image is the time displayed in [Scan].*



- Set the number of image acquisitions in Cycle.

The time from the start of the T series image acquisition to the end of the T series image acquisition is calculated and displayed in [LSM Total]. Set [Cycle] so that [LSM Total] becomes an appropriate time


- Click on the  button to start the recording of the Z Series.



Imaging and Flow Cytometry Core

Turn off system

Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (1h) after you.

1. Soak the water objective in MilliQ water for 5min
2. Turn off fluorescent lamp illuminator by pressing "ON/OFF" button for **3 seconds**, the illuminator will show "300" to count down for **5min** for cooling down the lamp.
3. Turn off Chameleon laser by turning the laser key 90° anticlockwise to vertical position
4. Turn off power switch of SIM scanner ④
5. Turn off power switch of Main scanner ③
6. Turn off power supply Unit FV30-PSU ②
7. Exit "FV30S-SW" software and upload your data to server
8. Turn off computer ①
9. When the lamp illuminator **5min** count-down finish, turn off the power switch of lamp illuminator ⑤ (the switch is at the left back of the box, shown by →)
10. Keep pressing  on touch screen to move the objective to upper limit, then clean up with lens paper
11. Turn off the touch panel controller ⑦ (the switch is at the right back of the box, shown by →)
12. Turn off microscope power ⑥

***Users are reminded to sign on the log sheet before departure.**