Common Manual for User of Leica DMi8 Confocal Laser Scanning Microscope

This is a simplified version that focuses mainly on routine operations.
For more detailed instructions, please refer to the user manual and the technician (Ms. Crystal CHEUNG, sfcheung@hku.hk)

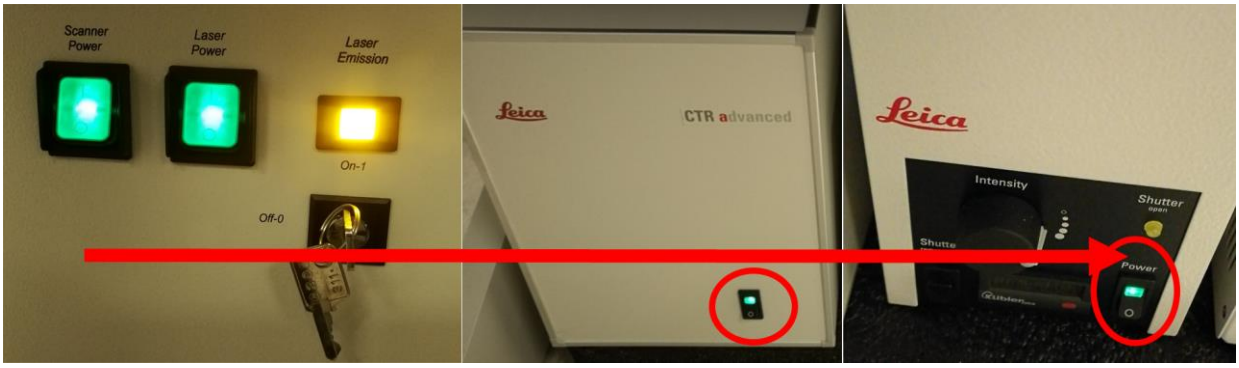
1. Scope

1.1 This document provides operating procedures and requirements to Leica DMi8 Confocal Laser Scanning Microscope.

1.2 **Booking System:** http://store.chemistry.hku.hk:8085/. Each time slot is 1 hour, 1 day advanced booking.
Address: CYM LG 216
Primary Contact: Ms. Crystal CHEUNG
Email: sfcheung@hku.hk

2. Boot Order

2.1 Turn on the Computer, Scanner Power, Laser Power, Laser Emission (key: off 0 ⇒ on 1), CTR advanced, successively.



2.2 Turn on the Fluorescence exciting light source if necessary. (only for fluorescence imaging, NOT needed for confocal imaging)

(**Warning:** The fluorescence exciting light source should not be turned on unless after 30 mins cooling)

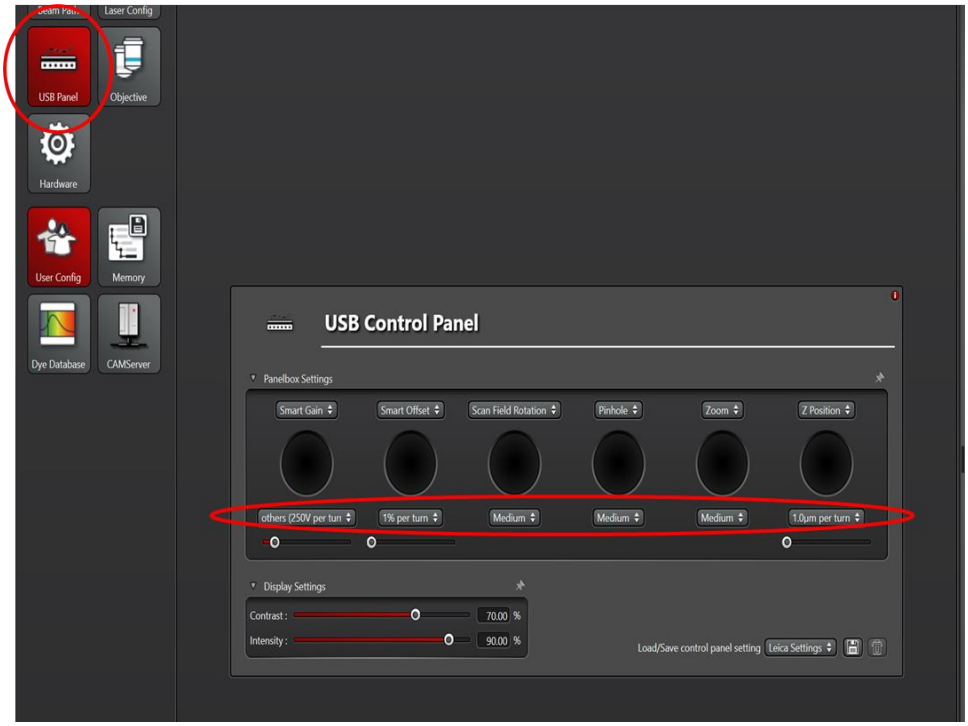
2.3 Start the control software by double-clicking "LAS X" on the desktop.

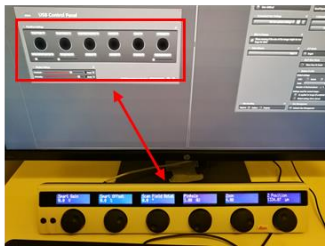
(**Warning:** Do not touch both software and hardware equipment during the initializing process)

2.4 Choose "machine.xlhw" and "DMi8" in Configuration and Microscope, respectively. Then click OK to initialize the system. Turn off "Resonant" if you want to take the high-resolution image; turn it on if you're going to take high frame rate video.

2.5 Click the “Configuration” in the initial interface, then click “Laser Config” to turn on the laser source you need.

You may also need to define the USB Control Panel and its modulation rate. In principle, you are only allowed to change the modulation rate.



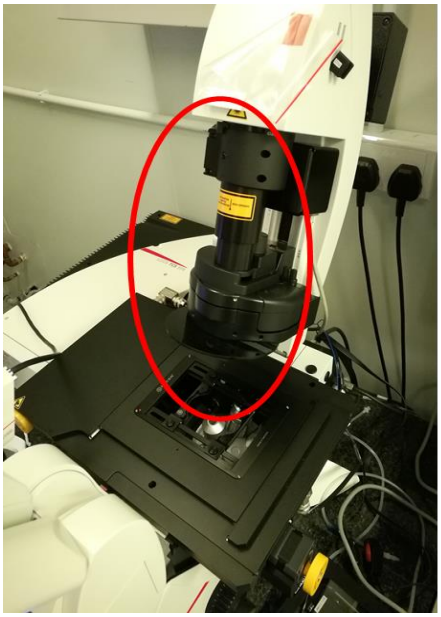


3. Place the sample and microoperation

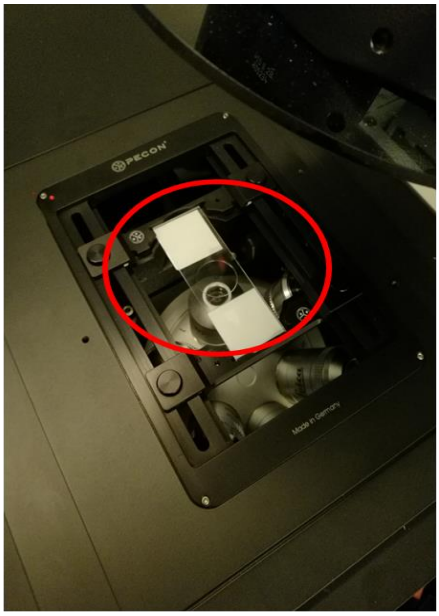
3.1 Choose the Objective on the touch screen. You can also choose the light source and their intensity on the touch screen.

(Warning: dry your sample and the objective if you want to switch from oil lens to dry lens)

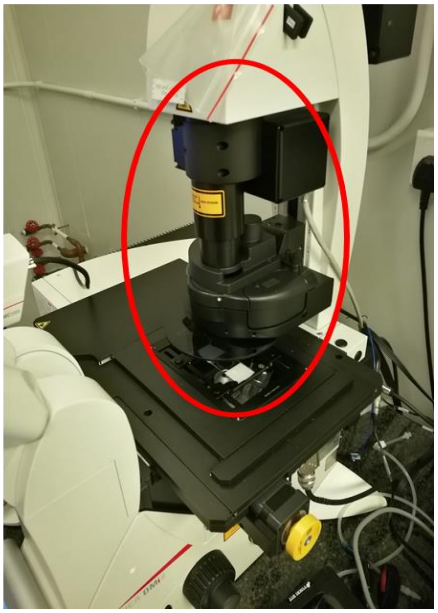
3.2 Hold up the scanning head and place you sample, following by laying down the scanning head.



Hold up scanning head



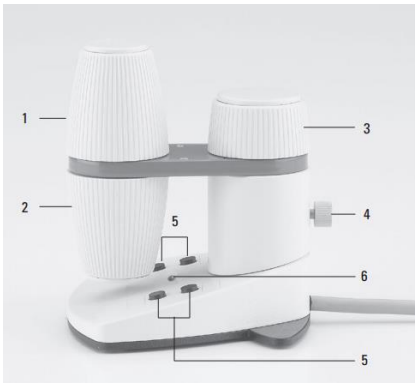
Place the sample



Lay down scanning head

3.3 Find the focal plane by changing the Focusing and locate the interest area by changing the XY axis. Then click “Shutter” to protect your sample.

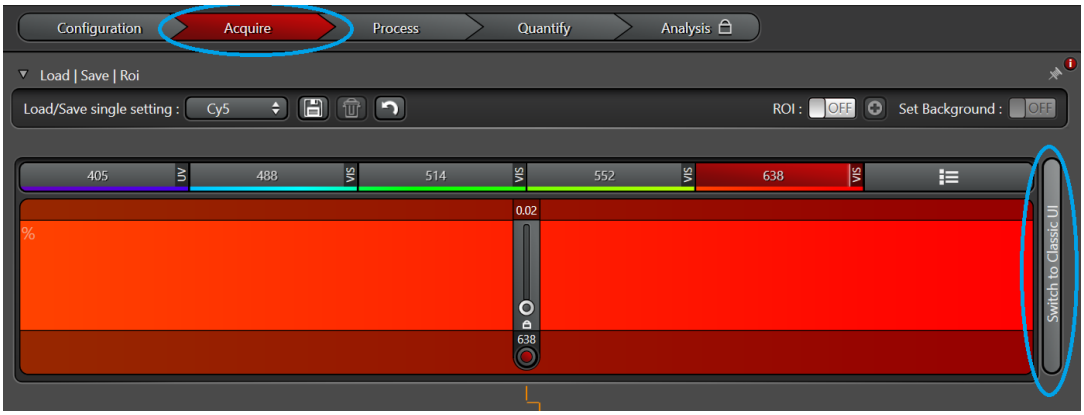
(Warning: **do not observe your sample under laser illumination)**

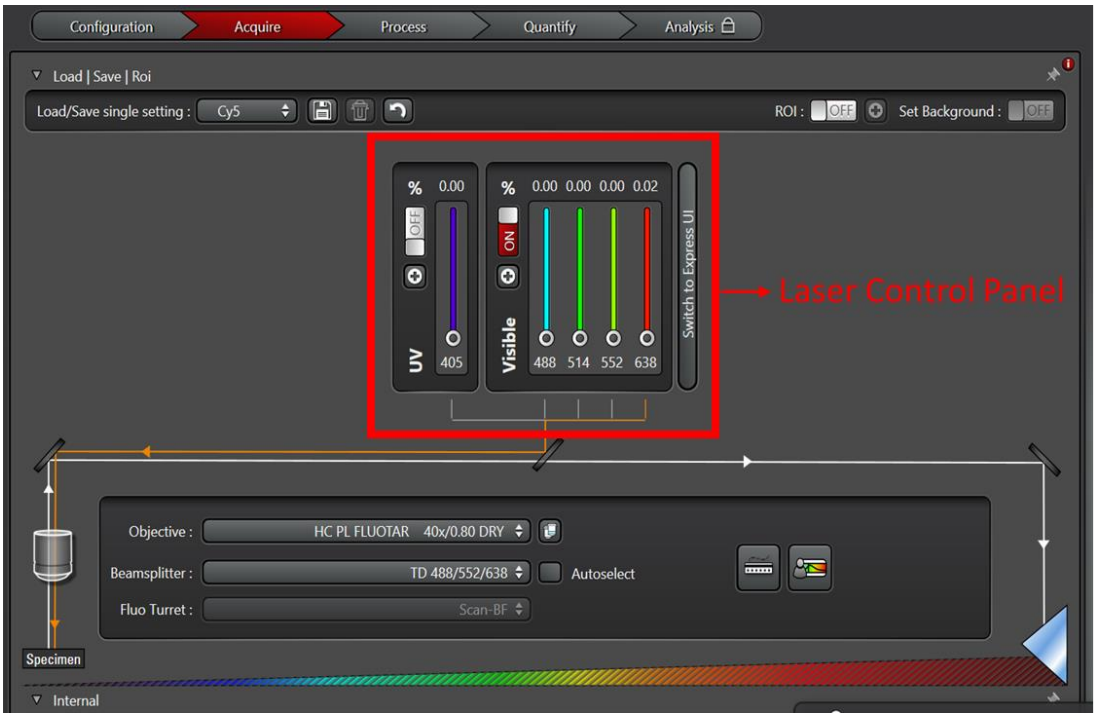


1 Movement in x-direction
2 Movement in y-direction
3 Focus setting
4 Individual setting of the knob height position
5 Variable function keys (preset at the factory)
6 Status LED

4. Image collection

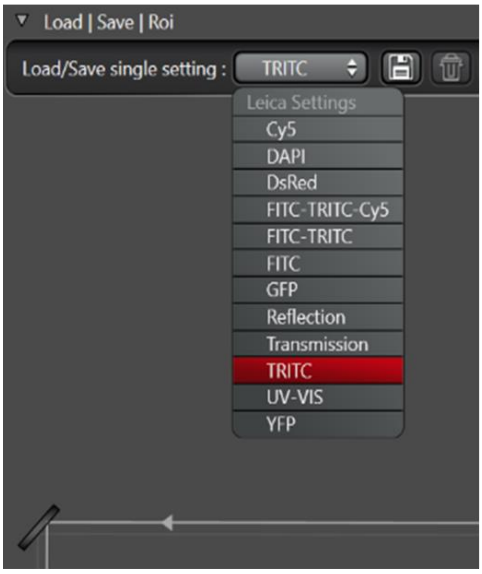
4.1 Click “Switch to Classic UI” in “Acquire” interface to open laser control panel.

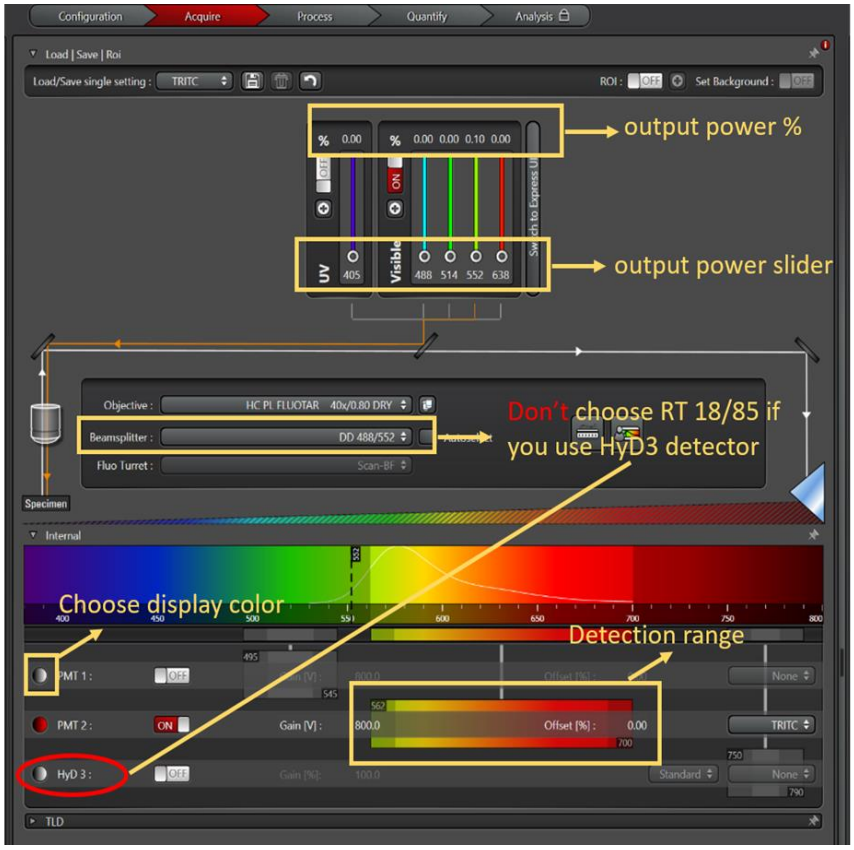




4.2 Click “Load/Save single setting” to choose similar material, the system would auto-switch to the recommended settings. The recommended percent of the output power is lower than or about 1%. You can turn on/off the laser and modulate the intensity slightly of the laser using center roller of the mouse or writing in the percent directly.

(Warning: do not choose RT 18/85 if you want to use HyD3 detector)





4.3 According to your sample, you can change laser source, modulate the output power, choose appropriate objective, beamsplitter, detector (PMT1, PMT2, HyD3 and TLD), gain or offset. The detection range could be modulated by double-clicking to write in or clicking and dragging the mouse. The selection principle for beamsplitter:

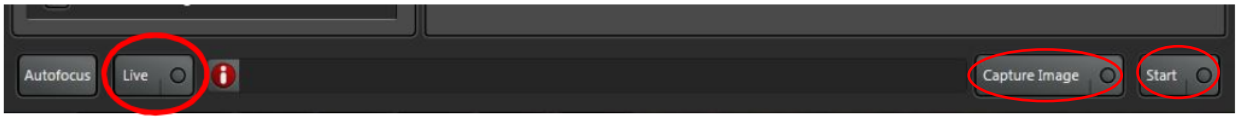
1, 405 nm laser: “Substrate”.

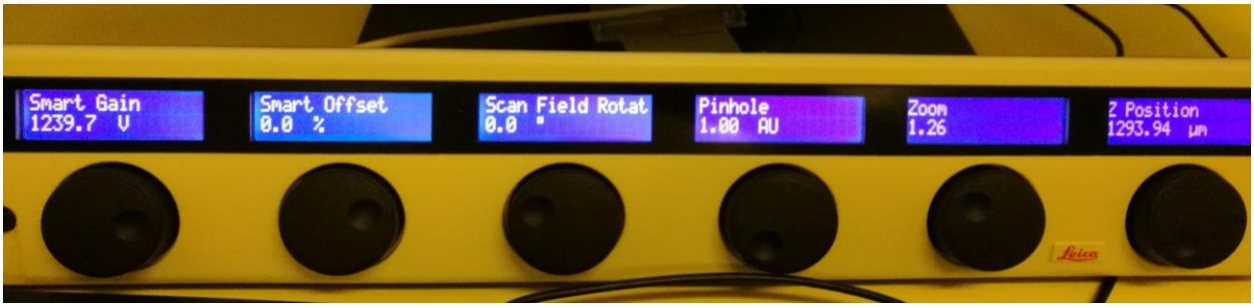
2, 488 nm, 514 nm, 552 nm and 638 nm laser is according to their wavelength. Such as 488 nm laser: “DO 488/552” or “TD 488/552/638”.

3, “RT 15/85” available for all laser with 15% reflection and 85% transmission. It is commonly used to spectral scan

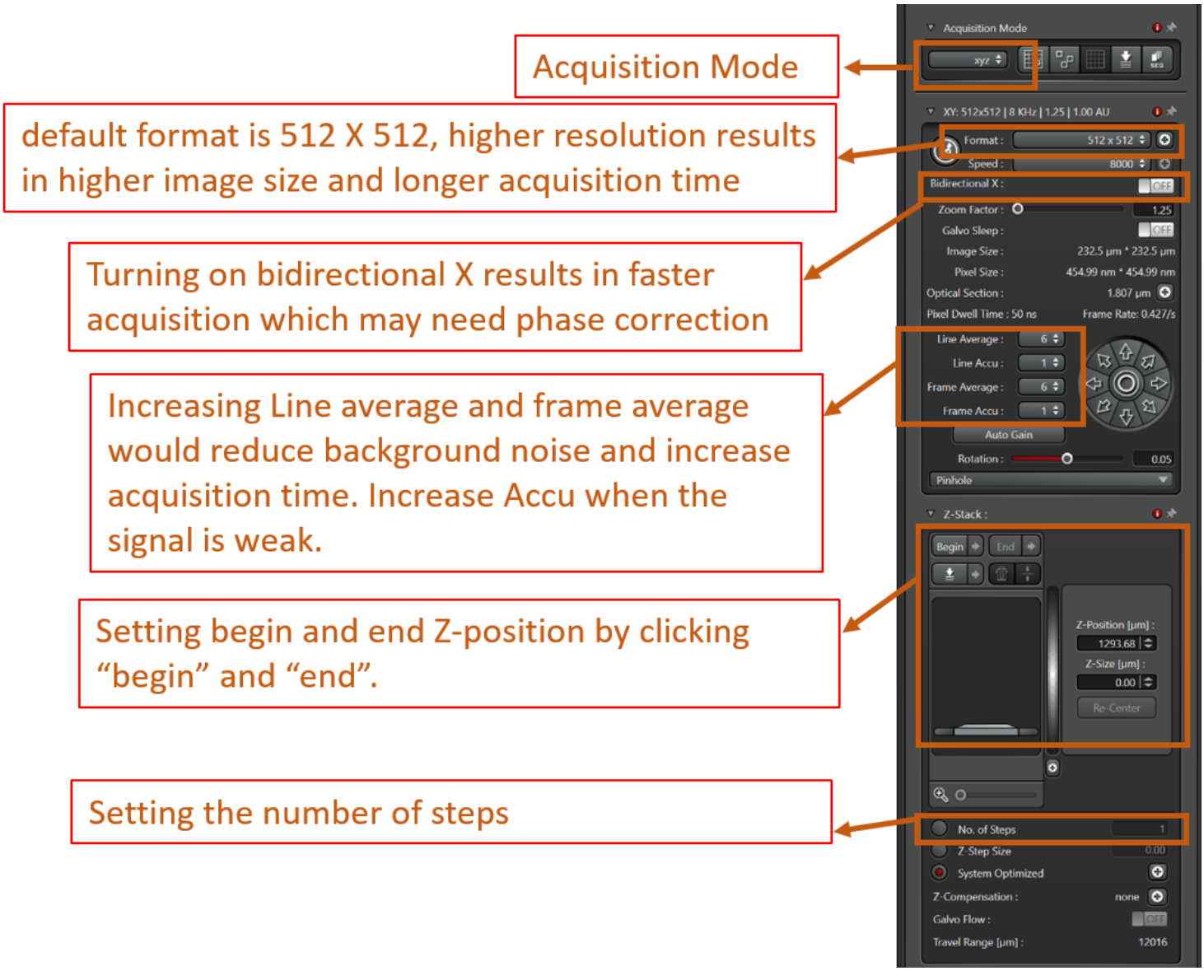
4.4 Choose “Acquisition Mode” (such as xyz, xyt, xyλ, xyzt, xyzλ and xyzλt). The default mode is xyz which offer 3D image acquisition or flat scanning image without define Z-stack.

4.5 Click “Live” to preview the image. You should try to find the focal plane and best display quality by modulating Smart Gain, Smart Offset, Z Position, etc. Then click “Stop” to finish the preview and protect your sample.

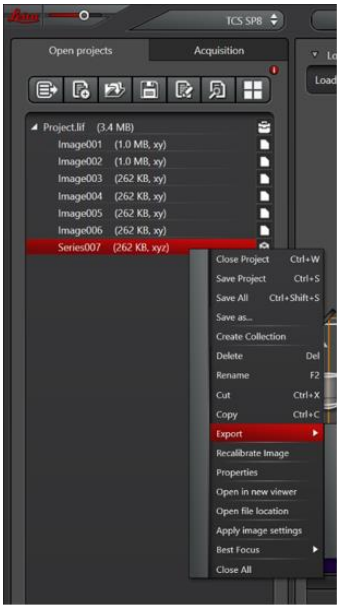


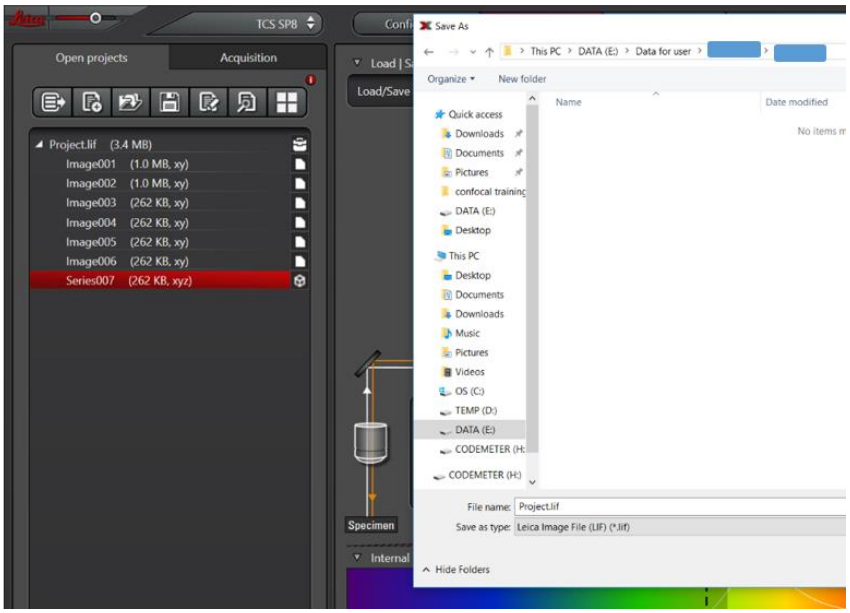


4.6 Setting scanning parameters. Click “Capture Image” to capture single image or “Start” to record a set of images or video.



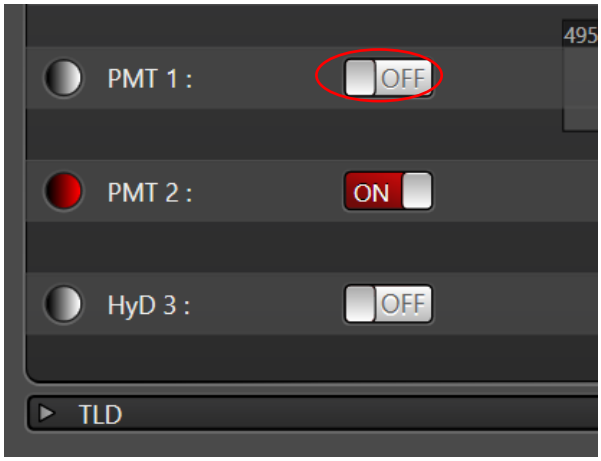
4.7 Click “Open projects” and save your data with format in “E:\Data for user”. The origin file format is *.lif.

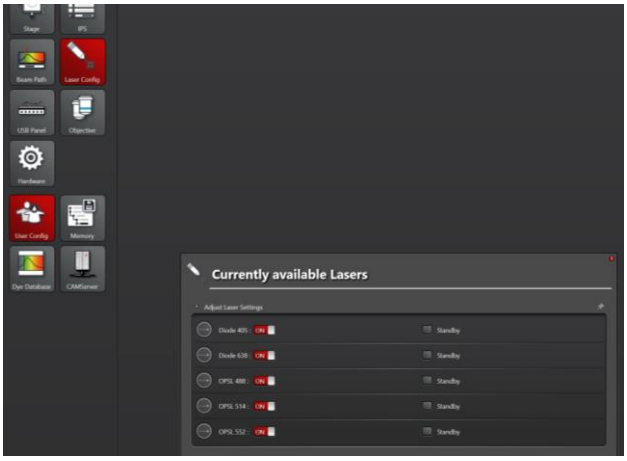




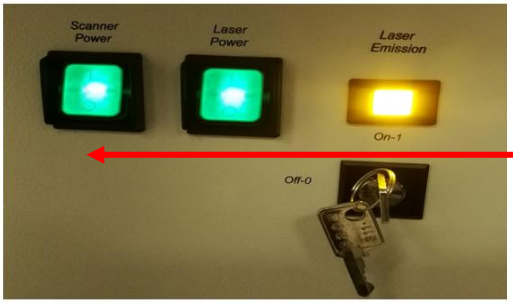
5. Shutdown

5.1 Turn off the detector, laser source and software (LAS X), successively





5.2 The lens turn to 5X. Then turn off fluorescence excited light source, CTR advanced, Laser Emission (key: on 1 ⇒ off 0), Laser Power and Scanner Power, successively.



5.3 Take away your sample, clean the desk, register in notebook and cover the microscope.

Currently, the data can only be copy through your hku email.