

Standard Operating Procedure (SOP) for Class IV Laser-Based Scanning Multi-Photon Microscope.

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Standard Class IV laser safety procedures are to be followed at all times. The use of this laser-based multiphoton microscope assumes: (1) completion of the laser safety class at <http://www.ehs.ucr.edu/training/online/laser/indexlms.html>; (2) following Laser SOP available in the lab and (3) using appropriate Personal Protective Equipment (PPE) for the operation of a Class IV Ti:Sapphire 690 – 1040 nm femtosecond laser.

The SOP is broken down into four sections: 1. Turning ON Ti:Sapphire Laser; 2. Visual location of samples under a microscope; 3. Multi-photon microscopy imaging; 4. System shut-down procedure.

1. Turning ON Ti:Sapphire Laser

- 1) Examine the laser controller and water chiller displays for any warning messages. If any warnings are present, or you see “Add water” message on the water chiller display alert Prof. Lyubovitsky. Do not proceed to the next step.
- 2) Locate a key that turns on the laser.
- 3) Insert the key into a slot on the laser controller box. Turn clockwise to ON position.
- 4) Turn ON the MaiTai by pushing and holding for several seconds the GREEN ON button until you see the red EMISSION indicator to appear on the top of MaiTai control window.
- 5) Make sure the Pulsing button on the top of control window is green. RS232 interface button should be green as well.
- 6) Make sure that IR power indicator increases to about 2.8 W at 810 nm. It takes about two minutes.
- 7) You can now open a laser shutter located in the middle left of the control window. Hold SHUTTER button for a few seconds. Confirm the shutter is open (radio button on top is Red, and the shutter button is yellow).
- 8) Check the laser power with a power meter. The setting will depend on the sample but generally should be below 150 mW.

LASER IS READY FOR USE.

2. Visual location of samples under a microscope

- a) Gently take out the cube that houses the main dichroic filter (do not touch this filter which look like a silvery metal surface) and place it in a safe location.
- b) Pull out (towards you) a lever in front of you.
- c) Turn on the lamp and observe the light to illuminate your specimen. Increase light intensity if it is not sufficient.
- d) Move objective (by translating the z-focus) to about 1 mm from the surface of the sample.
- e) Looking into the ocular, begin moving the objective away from the sample (clock-wise) until your specimen comes into an acceptable focus.
- f) Turn off the lamp intensity to zero.

THERE SHOULD BE NO LAMP LIGHT ILLUMINATING THE SPECIMEN AT THIS POINT - IT WILL DAMAGE THE PMTs and RENDER THEM UNUSABLE!

3. Multi-photon microscopy imaging

- 1) Turn off all the room lights.
- 2) Turn on the microscope controller box (slender black box).
- 3) Turn on the computer
- 4) Click ThorLabs icon on the desktop to start the program. Make sure the PMTs are off and PMT gains are at zero.
- 5) Click the boxes to ON position for the PMTs that you will use.
- 6) Click Tiling box.
- 7) Click the right-pointing arrow on the top left corner of the data acquisition menu. This will initiate real time scanning.
- 9) Slowly increase gain on the PMTs until you see the image on the computer screen.
- 10) No image? Very slowly adjust the fine focus (manually or through the software). Since the objective is not spring loaded it can get easily damaged at this step.
- 11) When a desired picture is obtained, stop the acquisition and save data. The file format is tiff and can be read with Photoshop or ImageJ.
- 12) Seek additional training with Dr. Lyubovitsky if advanced image acquisition such as for example stacking streaming, tiling, etc. is of the interest.

4. Multiphoton Microscopy Imaging System shut-down procedure

Close data acquisition program.

Shut off data acquisition computer.

Turn off controller.

In the MaiTai control program click button Shutter (the laser will stop lasing at this point). Follow by clicking the OFF button.

Turn the laser off with a key on the controller box.

Remove the key and place it in a laboratory designated box and location.

TRAINING REQUIREMENTS:

Laboratory workers conducting research under this protocol must complete the following training requirements:

- 1) Laser Safety Online Training
- 2) Our Laboratory Safety Training (Binder)
- 3) Individual training with Dr. Lyubovitsky (PPE Hazard Assessment certification)
- 4) Individual training with Dr. Lyubovitsky (Other controls certification - engineering, administrative, work practices)

LABORATORY-SPECIFIC TRAINING

Document any laboratory-specific training below. Attach additional sheets, if necessary.

Training Requirement	Name (Last, First)	Date	Student Signature	Instructor Signature

TRAINING