Tier III User 2P Start-Up & Shut-Down Procedures

Note: Please turn on the 2P system at least 30 minutes (>1 hr preferred) prior to your experiment allow the laser and scanners to warm up.

Two Photon Microscope Front View
Tier III User 2P Start-Up & Shut-Down Procedures

Two Photon Microscope Rear View
Start-Up Procedures

1. **Ti:Sapphire Laser systems**: Turn the power switch key clockwise from STNDBY to ON for Laser #1 as shown below. If Laser #2 is needed, turn it on as well. Check the front panel for any error messages and report them promptly. Be sure the status is "OK" before you start your experiment.

2. **Laser Chiller units**: Check that the laser chiller unit display reads **20 ± 1°C**. If not, carefully unscrew the white plastic cap and check for the coolant level and verify that there is adequate flow (liquid surface should be moving). If the coolant is low or flow is not visible, please inform the IVIC staff promptly. **Chillers should always be left on to protect the laser systems from overheating.**
Note: Before powering on the electronics control box, make sure PMT switch is OFF (Kill PMTs), the upper turret set to position 5 and the lower turret set to 2.

3. Turn on the electronic components on the computer rack starting from the top and working towards the bottom. Conventional switch labels are: "O" = OFF, "I" = ON

3.1. **Uniblitz Shutter Controller**: The power switch for the shutter controller is on the back. Also, please toggle the shutters to the normal close (NC) position (green light should be off on front panel).

3.2. **Electronics Control box (Scanner and PMT power)**: Please turn on the power switch on the left as shown below and make sure the PMT Kill switch on the right is depressed (push bottom half down).
3.3. **Pockel's Cell Power Supply:** Turn on both Pockel's cell power supplies even if only one laser is being used. This is to prevent unintentional exposure of a non powered up attenuator to the laser. The front knobs are the bias voltages and should only be adjusted by the IVIC staff.

3.4. **Warner Temperature Controller Unit:** Turn on the unit using the power switch on the right. Also, make sure the individual channel heater switches (blue toggles) are in the middle "off" position until chambers and inline heater are set up and ready to use.

3.5. **Prior OptiScan stage controller:** Turn on the unit using the switch on the front panel as shown below.
3.6. **Bright Field Illuminator (Olympus TH4-100):** If bright field illumination is necessary to find your sample then turn on the power supply as shown below. There is a second switch, a green button, located next to the light intensity knob that can be used to turn the light off and on during experiments. Please make sure this light is turned off before rotating the turrets to position 6 and 1 and activating the PMTs for imaging.

4. **Acquisition Computer Software (Always leave computer power on)**

4.1. **Open PowerCal software.** Close the error message (NIDAQ library not found) by clicking "OK". Under the Laser Power Tab, set the special modes to Potentiometer Power Control. Minimize the window.

4.2. **Open ImageWarp Software.** Open one of your script file and paste in the folder address where data will be written. Check to make sure the Red channel is set properly to 1 or 2 in the script. Save script with updated changes.
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Shut-Down Procedures

This document covers the two photon microscope laser system shut-down only. Please refer to Critical 2P Operating Procedures for objective care and cleaning. For animal care after imaging is done, please refer to the IVIC Policies and Procedures.

1. Close ImageWarp and PowerCal Software but leave the computer and UPS running at the end of your experiments.
2. Turn the laser power switch counter clockwise from ON to STNDBY. Please leave the MRU units and power strips running. It they are powered down it may take several days for the laser cavity to be purged and for the system to function at full capacity again.
3. Using the same order described in Start-Up Procedures Step 3, shut down the two photon microscope by switching the power switch to OFF position. Please do not turn off chillers. Chillers should always be left on to protect the laser systems from overheating.
4. Make sure PMT switch is OFF and the upper turret is set to position 5 and the lower turret set to 2.