

Standard Operation Procedure (SOP)

Two-Photon Microscope General Imaging

| | |
|----------------------------|---|
| Equipment Location: | 458 Stemmler Hall |
| Original document created: | 10/18/04 |
| Document updated: | 12/10/04 |
| Director : | Phil Haydon (pghaydon@mail.med.upenn.edu) |
| Safety Trainer (ST) : | Hajime Takano (htakano@mail.med.upenn.edu) |
| Requirement: | Read this document. All users need to be trained by ST. All users need to read Prairie Ultima User Manual page 1-48. |
| Potential Hazard: | Laser Hazard (Class1 / Class 4)* |
| Safety Precaution: | IR laser is invisible. Do not stare the laser beam path without the proper safety goggle*. Do not expose skin to the laser beam path. |
| Protect equipment: | Do not expose room light to PMT with high voltage turned on. Make sure cooling water circulator is on before turning on laser. * |
| Emergency Contact: | Hajime Takano (267-693-7384) Phil Haydon (610-246-6013) |

Ultima Two-Photon Microscope

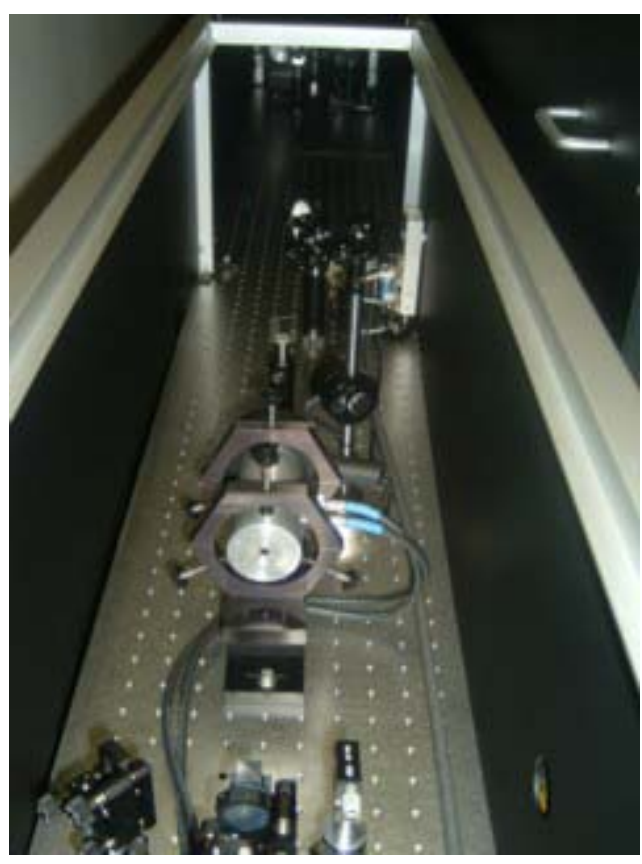
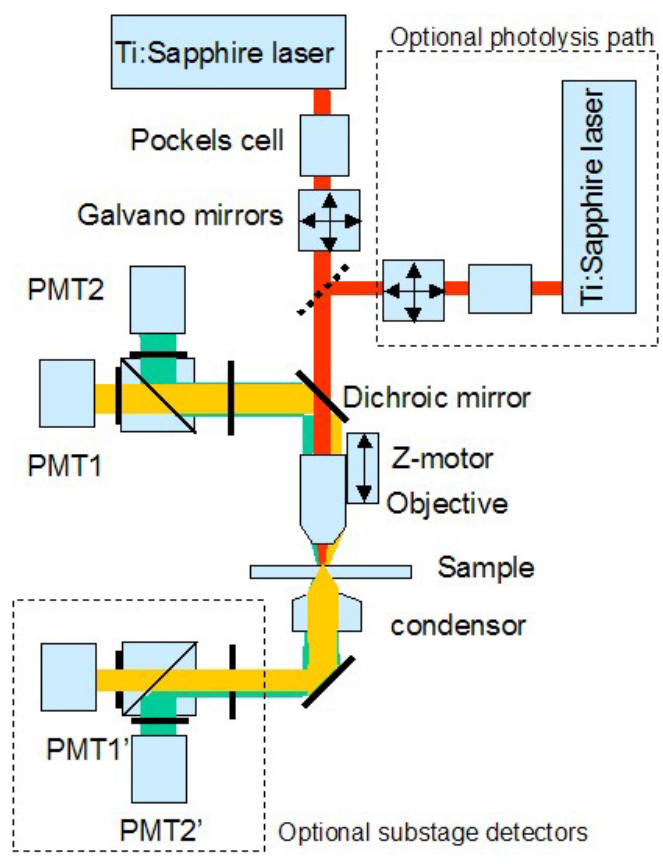
A multi-photon, or two-photon, microscope uses similar instrumentation to a laser scanning confocal microscope: A laser source for sample excitation, a scanhead with galvanometer controlled mirrors (or acousto-optic deflectors) to scan the excitation beam, and photomultiplier tubes to detect fluorescent signals. However, the confocal image differs in that optical sectioning is obtained using excitation in multi-photon microscopy whereas in confocal microscopy it is achieved using the emission pinhole.

The Prairie Technologies Ultima multiphoton microscope is similar in many respects to other available systems, but differs in that it contains two-independent pathways of excitation, one for multiphoton imaging and the other for multiphoton uncaging. Because this system is additionally integrated with electrophysiology a diversity of applications are possible with our systems. For example, the fully configured system is able to perform 4-D imaging, to integrate imaging with photolysis, and to integrate 4-D imaging with photolysis and electrophysiology.

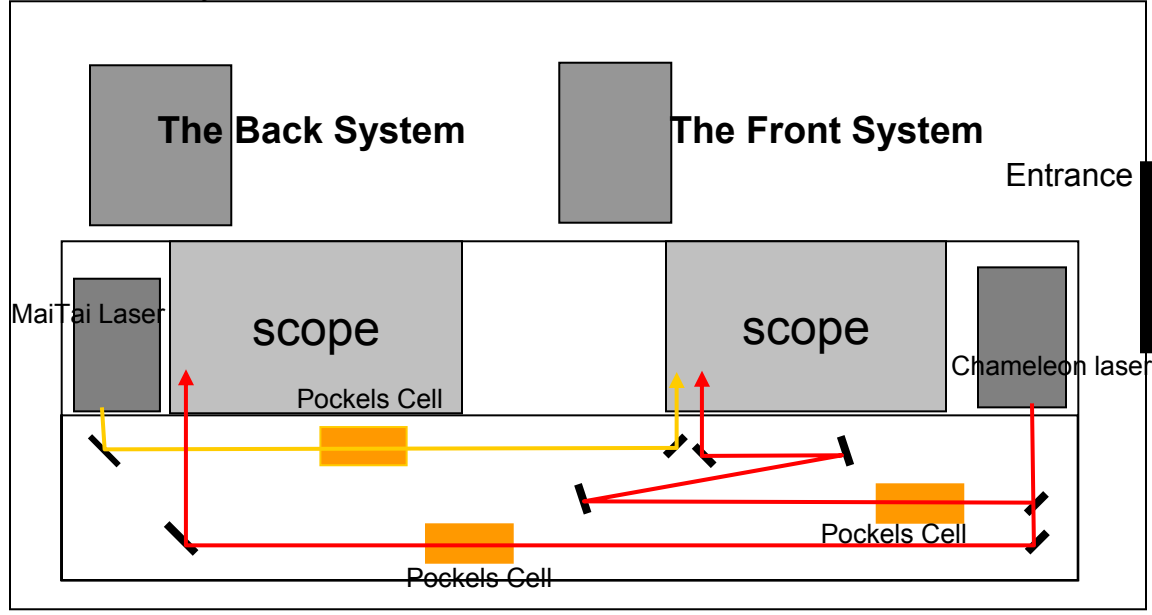
One of our Prairie Technologies Ultima systems attached to an Olympus BX-51 fixed stage microscope is shown in the attached figure.



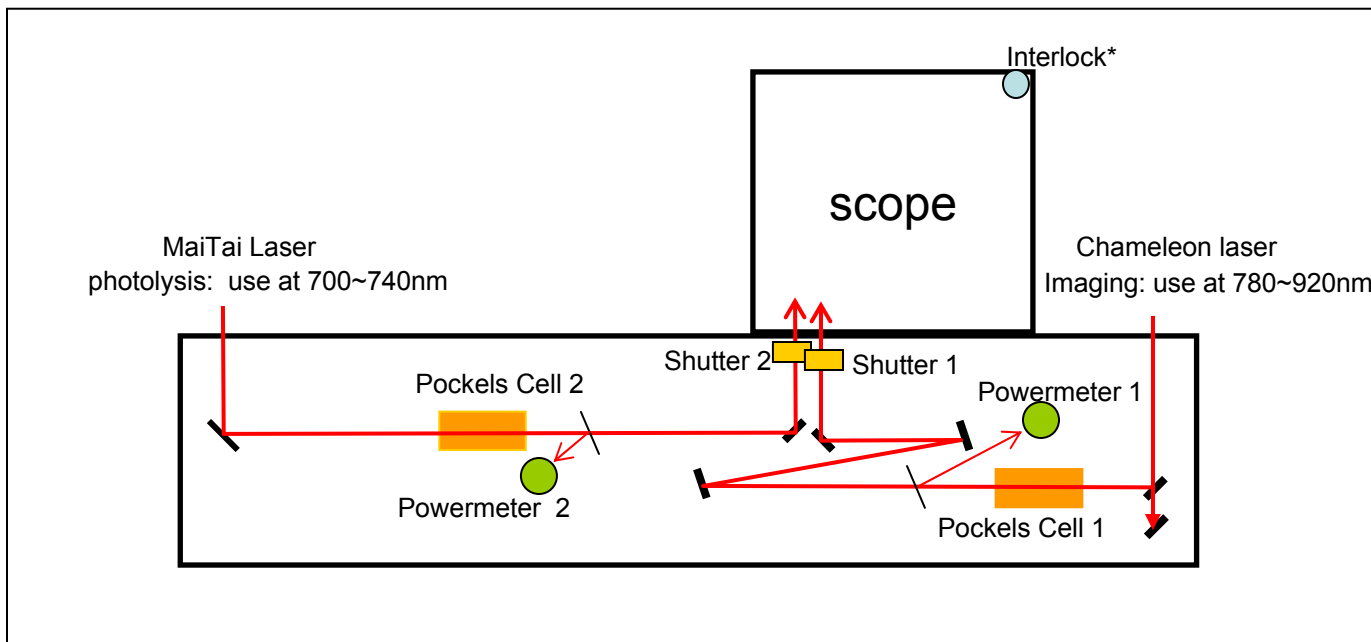
Prairie Technologies Ultima Two-photon Microscope with Olympus BX61WI motorized upright microscope.



Room 458 Layout



The Front System Configuration



***Note: Shutter 1 and shutter 2 open only when sliding door is closed (interlock system).**

Note: Front System and Back System share the Chameleon Laser for Imaging. When using two systems at the same time, only one wavelength can be used. Laser power can be independently adjusted with two independent Pockels cells. MaiTai laser is for photolysis in the Front System.

The Front System

Standard Startup Procedure

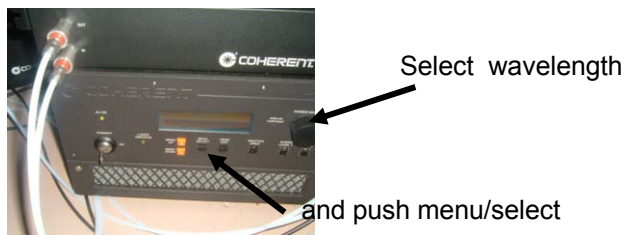
1. Check the log book. Sign in. (Name, Lab, Time)



2. Make sure cooling water circulator is on and operated at 25°C. (just check. Do not touch anything)



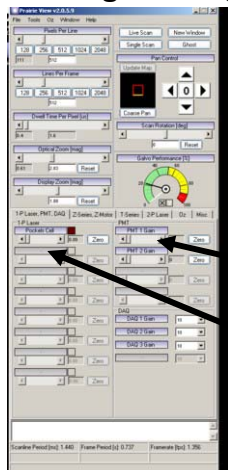
3. Turn the laser key from stand-by position to on position. Wait until it shows "ready".
4. Adjust the dial to change the wavelength, and push menu/select button. If you do not know the wavelength to use, start from 850nm.



5. Turn on the machines (including X-cite lamp if needed) and the computer. Run PrairieView software.



1. Set PMT high voltage to 0, Pockels cell controller (PrairieView software) to 0.



PMT high voltage controller

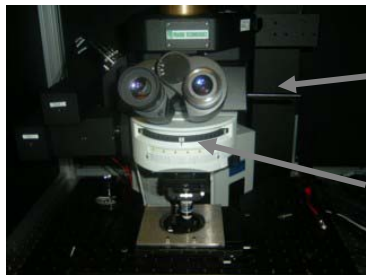
Pockels cell controller

2. Put sample onto the stage. Check the sample with bright field or epi-fluorescence.



3. Turn off the room light.

4. Turn the filter cube wheel to position 1 and pull out the path-selection bar. Close Iris of X-cite light source if using.



Pull out the kob.

Position 1



iris

5. Close the sliding front panel of the light box. (now interlock is on)



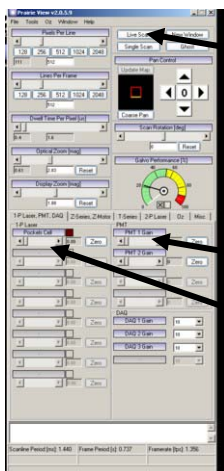
Interlock system

1. Open laser shutter from the laser controller.



Shutter open/close

2. Click live scan. Increase PMT high voltage to 650-700. The image window should show some kind of noise (non-zero value). If there is no signal, consult with ST.



Live Scan

PMT high Voltage to 650-700.

Pockels cell controller

3. Gradually increase the pockels cell controller (Ultima software) until image shows up.
4. Adjust PMT power, pockels cell controller (Ultima Software), Image averaging, Dwell time (start with $4\mu\text{s}$), Optical zoom Refer to Prairie Manual for more detail.
5. Check the power meter value and write it down to **log book** along with the pockels cell voltage, wavelength of the laser, and laser power shown in the controller.

Power (mW)



Typically several mW

Power (mW)



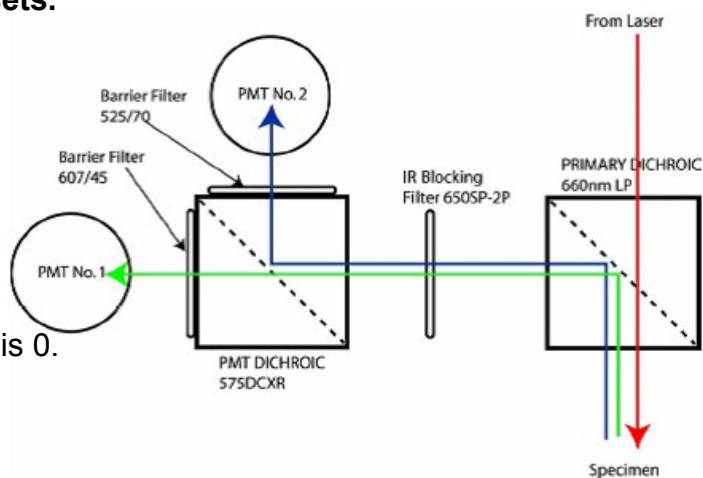
Typically 1000~1400mW

Standard Closing Down Procedure

- Position filter cube wheel to 2-6 (not 1). Push in the path selection bar.
- Set PMT high voltage to 0. Pockels controller (software) to zero.
- Close the program.
- Close laser shutter. Turn the laser key to the stand-by position.
- **Describe the experiment, performance of scope, time of usage on the log book. Clean up and do not leave anything around the scope.**



Note: Installing/changing filter sets.



- Make sure PMT high voltage is 0.
- Remove the magnetic holder.



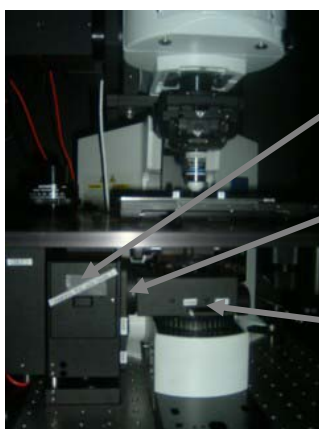
- Filter sets are stored in a plastic container in the plastic drawer.



- Carefully install the filter cube on the holder and remount to the scope.

NOTE: Substage Detector

- The Front System equips substage detector in addition to the standard upper detectors. Currently, however, the substage detector manual shutters are in close position, and PMT high voltage is not turned on. Assuming zero signal is added to the upper PMT detector signal.
 - When substage detectors are needed, follow these instruction carefully. You need to read the Prairie Ultima Manual in addition to this instruction.
1. There are two manual shutters. To open the shutter under the substage condenser, turn the lever to the right. (Right: open, left "close) To open the manual shutter by the detector, push the lever down. (Up: close, Down: Open).
Note: Do not expose strong light to the detectors.



2. Turn on the PMT high voltage. The switch is located the far left side of the PMT controller. (Bottom controller)
3. Adjust the knob manually to change PMT high voltage (typically 700~800).
4. Substage PMT signal is simply added to the upper PMT signal. (Assuming zero signal is added to the upper PMT detector signal when PMT high voltage is off)
5. Use the same filter setup as the upper detector. (no filter or with Prairie filter)
6. Closing down: Close two shutters, Turn of the high voltage switch.

Note: Photolysis

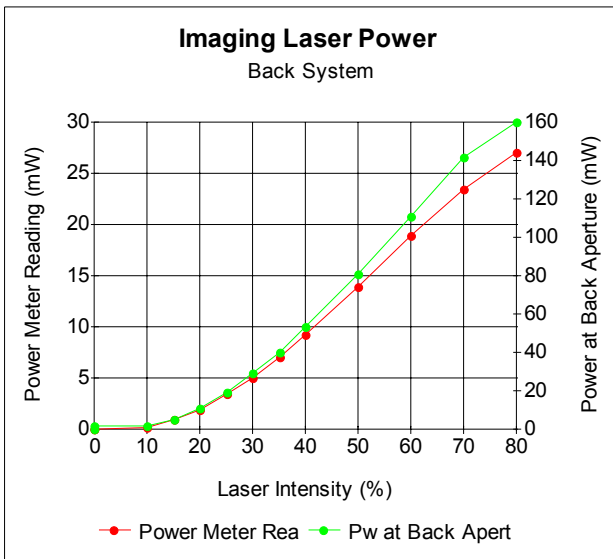
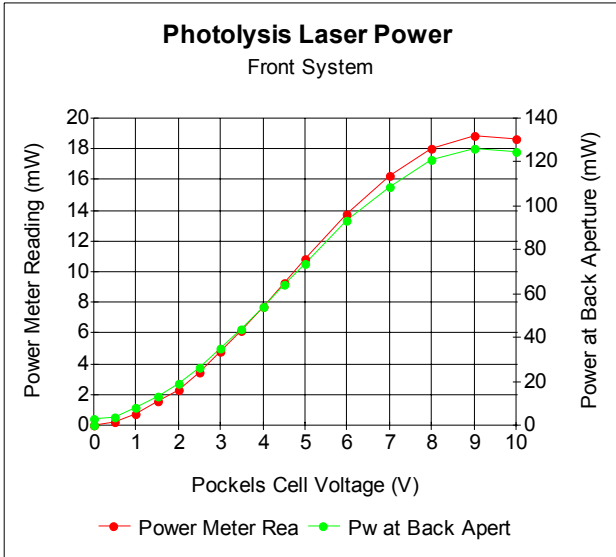
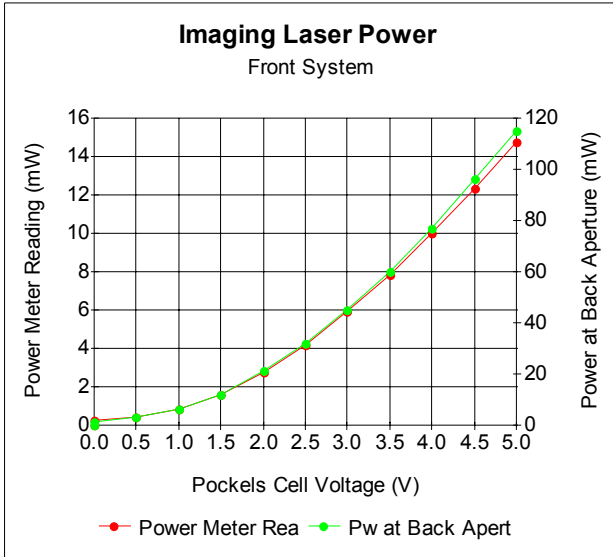
- Read the Standard Operation Procedure for photolysis/imaging manual and need to be separately trained by ST.

Laser Power Measurements Example 10/13/04

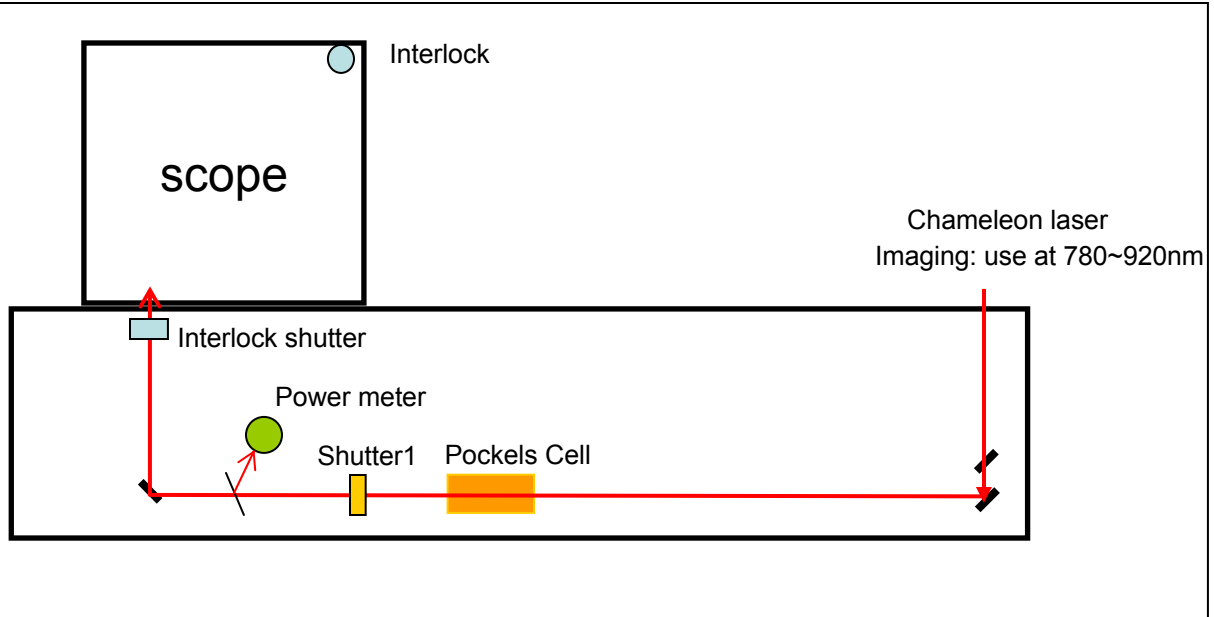
Chameleon (Imaging) 820nm

MaiTai (Photolysis) 720nm

Note: See the log book for updated measurements.



The BACK System Configuration



Note: Shutter 1 open/close independently with the interlock system.

Note: The power meter is located after the shutter 1.

Note: Front System and Back System share the Chameleon Laser for Imaging. When using two systems at the same time, only one wavelength can be used. Laser power can be independently adjusted with two independent Pockels cells. MaiTai laser is for photolysis in the Front System.

The BACK System

Standard Startup Procedure

1. **Sign in the log book.**
2. Make sure cooling water circulator is on (Just look. Do not touch anything)
3. **Turn on the Laser.** Turn the laser key from “standby” position to “on” position. Wait until the display panel shows “ready” (right side).
4. **Select wavelength** to use. Adjust the wavelength by rotating the dial and push “menu/select” button. If you do not know the wavelength to use, start with 850nm.
5. Turn on the computer and hardware if they were off. Turn on mercury lamp if necessary. **Run “two-photon microscopy”.**
6. Set **“PMT high voltage” to “off”, “laser intensity” to 0.**
7. Put sample onto the stage. Check the sample with bright field or epi-fluorescence.
8. Note: Filter Cubes (2: bright field (no cube) 3: GFP 4: EGFP 5: TRITC)
9. (NEW!!) Note: After checking with epi-fluorescence, turn the iris to off position on the EXFO X-Cite system to prevent the unnecessary light leaking into PMT.
 - Turn off the room light.
 - **Turn the filter cube wheel to position (1: Laser) and pull out the “light path selector knob”.**
 - Close the sliding front panel.
 - **Open laser shutter** from the laser controller (hardware).
 - **Check PMT.** Still keep the laser intensity to 0. Click continuous scan. Increase PMT high voltage to 70-80%. The image window should show some noise (non-zero value). If there is no signal, consult with ST.
 - Gradually increase the laser intensity from the software.
 - Adjust PMT high voltage(%), Laser intensity (%), Image averaging, Dwell time (start with 4 μ s), zoom etc...

Standard Closing Down Procedure

- Set PMT high voltage to off. Laser intensity (software) to zero.
- Position filter cube wheel to 2-6 (not 1). Push in the path selector knob.
- Close the program.
- Close laser shutter on the laser controller. Turn the laser key to “standby” position.
- **Describe the experiment, performance of the microscope, time of usage on the log book. Clean up the area. Do not leave anything around the scope.**