

STANDARD OPERATING PROCEEDURE	
Equipment Name	Sutter Movable Objective multiphoton microscope
Location	LMB, 1N360
Scope	Two photon imaging of Drosophila brains.
Date	10/07/2019

GENERAL

Since microscopy is frequently carried out in low/red light illumination conditions, check that the floor is clear of equipment. Place ancillary equipment on the table top or under the table. Check that there are no trailing leads.

Check that the microscope and table top are clean and unwanted samples have been disposed of.

LASER SAFETY

All users must follow LMB safety policy and local rules. These are described in the Laser Safety Guidelines on the LMB website

http://intranet.lmb.internal/download/research/lasers/Laser_safety_guidelines.docx

The Ti Sapphire laser is a class 4 laser. This therefore has the potential to cause permanent eye and skin damage if misused. The **Sutter Movable Objective multiphoton microscope** has interlocks and mechanical shields to prevent inadvertent exposure to the beam. Never attempt to remove mechanical shielding (instrument covers and beam tubes) or defeat interlocked components of this system. With the exception of the light exiting the objective there are no exposed laser beams in this system.

All users must have attended a laser safety course for class 4 lasers.

All users must have been trained by István Taisz or Greg Jefferis.

Do not attempt to image when the front shielding panel is open.

Do not open the light-safe enclosure for the beam path on the optical table.

Do not remove the blanking plate on the microscope head.

Store the key for the Ti Sapphire laser in the Key Press when not in use.

Before turning the system on.

Visually check that all the laser beam tubes running from the Ti Sapphire laser to the microscope are in position. Any problems contact István Taisz. Do not turn the system on.

Check that there is an objective mounted on the microscope. Do not turn the system on if the objective is missing. Replace the missing objectives first.

Ti:Sapphire Laser Operation.

When not in use the Ti Sapphire laser is left in 'standby'. In this state no laser light is being generated. The Ti:sapphire laser may be operated outside of the Sutter Movable Objective multiphoton microscope control software.

Laser OFF: The key is turned to STANDBY. The front panel displays 'Status:Standby'. Laser power = 0 mW.

Laser ON: The key is turned to ON. The display panel shows the laser wavelength and the laser output power. The laser shutter OPEN button is lit.

Laser beam exiting the objective and sample handling

Upon exiting the objective, the laser light is focussed to a small (~1 micron diameter) spot and raster scanned over the sample. The intensity at focus exceeds maximum permissible exposure levels to the eye and skin. Beyond the focal spot the beam rapidly diverges. The beam is directed downwards from the objective.

Never place your hands (or other parts of your body) in the beam.

Never change the sample when the system is scanning. Hit the 'stop scan' button in software. This also shutters off all lasers.

Never angle the sample such that light may be reflected out sideways from the microscope. Samples must be placed flat on the microscope stage.

Do not use reflective (mirror like) sample holders.

FOR ALIGNMENT

During alignment and servicing operations and whenever any beam shield is removed observe the following additional precautions:

The laser safety warning light shall be switched on

The additional "Open beam laser work in progress" sign shall be posted on the door

The laser shall be operated in "alignment mode" (i.e. low power)

Laser safety goggles are available

For alignment follow instructions as on

http://flybrain.lmb.internal/dokuwiki/doku.php?id=protocols:aligning_the_2p_laser