

Laser Safety Standard Operating Procedure

Forward:

This procedure shall be reviewed annually by all persons who use Class 3B & 4 lasers or laser systems listed in this SOP. This procedure shall also be reviewed every two years by the Permittee or Laboratory Laser Safety Supervisor (LSS) to ensure it reflects the most current conditions. Changes in the operating procedure shall be forwarded to EHS – Laser Safety for review and approval.

Laboratory Information:

Laboratory PI Name:	<u>Laser Safety Staff</u>	Date:	<u>9/11/2023</u>
Department:	<u>EHS Laser Safety</u>	Revision #:	<u>2</u>
Building & Room #:	<u>ECG 1.200</u>	Author:	<u>Matthew Kennington</u>

Contact Information:

Laboratory LSS:	<u>John Snow</u>	Phone #:	<u>512-471-2042</u>
University LSO:	<u>DeWayne Holcomb</u>	Phone #:	<u>512-471-2038</u>
Maintenance/Repair:	<u>Facilities Services</u>	Phone #:	<u>512-471-2020</u>

Medical Emergencies

1. Call 911 for medical emergencies and shut down all laser operations.
2. Notify the Laboratory LSS and University LSO of all laser-related injuries and near misses as soon as possible.

Laser Description: Describe the laser(s) setup and how it is used including general beam parameters, optics, and equipment. Include a diagram or picture with the beam path depicted. This may be included as an attachment if necessary.

Confocal microscope used to image cell microstructures and modified proteins with four laser sources at 405nm, 488nm, 561nm, 637nm. The laser sources are enclosed in a laser combiner box with four separate fiber optic cables transmitting the laser light to the microscope from the combiner box. Laser power is reduced through the microscope system that results in <10mW laser power output from the objective at the sample stage. Users are instructed to set light source intensity slider is at 50% or less to ensure safe operation without protective eyewear. Damaged or disconnected fiber optic cables pose the greatest exposure hazard and should be checked for damage and secure connections prior to powering the device on. Computer software controls an automatic shutter that prevents the user from using the eyepiece while the laser sources are active. Users should still verify that lasers are inactive prior to using the eyepiece to view sample.

Laser Safety Standard Operating Procedure

Laser Parameters: Complete the table below using the operating conditions (power output, pulse energy, duration, etc.) of the laser. If more than one laser is used, copy and paste this table to complete the laser parameters for the other lasers. Laser eyewear is to be inspected by the user for lens applicability and integrity prior to each use.

Make:	Coherent	Wavelength (nm):	405
Model:		Power Output (W):	100 mW (max); < 5mW (operational)
Serial Number:	OBIS LM-405-100	Beam Diameter (mm):	.8
Class:	3B	Beam Divergence (1/e ²) (mrad):	<1
Cont. or Pulsed:	Continuous Wave	Duration (ns) & Rate (Hz):	N/A
Eyewear Make:	N/A - Exempt	Eyewear Wavelength:	N/A - Exempt
Eyewear Model:	N/A - Exempt	Eyewear Optical Density:	N/A - Exempt

Make:	Coherent	Wavelength (nm):	488
Model:		Power Output (W):	150 mW (max); < 5mW (operational)
Serial Number:	OBIS LM-488-150	Beam Diameter (mm):	.7
Class:	3B	Beam Divergence (1/e ²) (mrad):	<1.2
Cont. or Pulsed:	Continuous Wave	Duration (ns) & Rate (Hz):	N/A
Eyewear Make:	N/A - Exempt	Eyewear Wavelength:	N/A - Exempt
Eyewear Model:	N/A - Exempt	Eyewear Optical Density:	N/A - Exempt

Make:	Coherent	Wavelength (nm):	561
Model:		Power Output (W):	100 mW (max); < 5mW (operational)
Serial Number:	OBIS LM-561-100	Beam Diameter (mm):	.7
Class:	3B	Beam Divergence (1/e ²) (mrad):	<1.2
Cont. or Pulsed:	Continuous Wave	Duration (ns) & Rate (Hz):	N/A
Eyewear Make:	N/A - Exempt	Eyewear Wavelength:	N/A - Exempt
Eyewear Model:	N/A - Exempt	Eyewear Optical Density:	N/A - Exempt

Make:	Coherent	Wavelength (nm):	637
Model:		Power Output (W):	140 mW (max) < 5mW (operational)
Serial Number:	OBIS LM-637-140	Beam Diameter (mm):	.7
Class:	3B	Beam Divergence (1/e ²) (mrad):	<1.3
Cont. or Pulsed:	Continuous Wave	Duration (ns) & Rate (Hz):	N/A
Eyewear Make:	N/A - Exempt	Eyewear Wavelength:	N/A - Exempt
Eyewear Model:	N/A - Exempt	Eyewear Optical Density:	N/A - Exempt

Laser Safety Standard Operating Procedure

Laser Safety Program Resources:

EHS has several resources on their website at: <https://ehs.utexas.edu/programs/lasers/> including information regarding laser safety training and program requirements. The Laser Safety Program Manual can also be found here and should be referred to for:

- Lab PI roles and responsibilities
- Laser User roles and responsibilities
- Laser permits and registration
- Program requirements (SOP, Training, etc.)
- PPE requirements (eyewear and inspections)
- Signs and Labeling
- Non-Radiation Hazards
- Procurement and Disposal Requirements

Operating Procedures:

All Class 3B and 4 lasers and laser systems shall have a documented operating procedure that provides the end user the necessary instruction for completing their experiment safely. The operating procedure shall include instructions for all times it is necessary for the laser to be powered on including normal operation, alignments, service, and repairs as applicable. The procedure shall incorporate all safety measures including when to don/doff eyewear, room securement, signs and warning labels, housekeeping, and other control measures identified in the hazard section above. This procedure shall be updated to reflect current operations prior to commencing the experiment.

- A. Initial preparation of lab environment for normal operation (lab security, warning light on, keys, interlocks and guards, identification of personnel, etc.)
 1. Ensure door is closed and unnecessary personnel are not in the room.
 2. Remove cover from microscope.
 3. Ensure that the optical table is stable by pressing down on all four corners of the optical table – air should release. The table should stabilize within a few seconds after pressing the corners down.
 4. Ensure fiber optic cables are connected and not damaged.
 5. Turn on the two power strips on the top of the optical table.
 6. Turn on the Computer and log in.
 7. On the desktop open microscope software.
 8. Wait for warning notifications on the right side of the screen to clear while the camera temperature stabilizes and the lasers warm up.
- B. Target area preparation
 1. Handle the objective lenses with care.
 - i. 20x lens should NEVER have any oil on it and should never touch the glass of the sample.
 - ii. 60x lens should ONLY be used with immersion oil and should be cleaned thoroughly afterward.
 2. Ensure objective lens is sufficiently far from the sample stage to not scratch it with the sample glass.

Laser Safety Standard Operating Procedure

3. Adjust sample stage to sample size.
4. Secure sample to stage.

C. Operation procedures are as follows:

1. Ensure all warning indicators are clear.
2. On the right side of the screen go to “Acquisition Control”
3. Select your protocol
4. Select your sample holder (slide vs. well)
5. Select the active channel
 - i. Laser wavelength
 - ii. Fluorophore
 - iii. Imaging mode
 - iv. Camera used for imaging
6. Adjust objective magnification under “Channel Manager – Global Settings”
7. To locate your sample using the eyepiece select “EYES” on the imaging mode under “Active Channel”.
8. Adjust the laser power, exposure time, and EM Gain for each channel based on your experiment needs. The transmission percentage must be specified to be below 50% to be eye-safe at the microscope aperture. See Channel Manager screenshot below for parameter settings.
9. Click on “Live” to examine your sample.

D. Shutdown procedures for this laser are as follows:

1. Ensure lasers are off.
2. Lower the z position by clicking “Esc. Z” under the “Acquisition Control – Navigation” window.
3. Remove sample and gently clean the objective with provided lens cloth
4. Close the Fusion software and wait until the software has completely shut down. (you will also hear the system shut down)
5. Sign off your user account
6. Turn off the computer.
7. Turn off the two power strips on the optical table
8. Cover the microscope.

E. Alignment procedures (describe the specific steps and settings needed to reduce power before interacting directly with the beam path. For example, shuttering the pump laser, using ND filters, etc.)

1. Users are not authorized to make adjustments to the optical path. The manufacturer shall be contacted if any adjustments to laser associated equipment.

Laser Safety Standard Operating Procedure



Channel Manager Screenshot – Parameter Settings

Laser Safety Standard Operating Procedure

Physical Controls: Describe the physical controls of the laser setup in the condition which the setup is intended to be operated. Edit the comment section as necessary to depict the lab specific controls implemented. EHS will review and approve the described control measures.

Check If Applicable:	Control:	Comments:
<input checked="" type="checkbox"/>	Entryway (door) Interlocks or Controls	Entry to the lab is restricted to authorized and properly trained lab personnel only with an active keypad lock. The lab door is to remain closed at all times unless the laser is shutdown and under the direct supervision of an authorized person.
<input type="checkbox"/>	Laser Enclosure Interlocks	Any laser enclosure interlocks will be engineered to fail safe and require manual re-activation if defeated.
<input type="checkbox"/>	Laser Housing Interlocks	Fail-safe or redundant interlocks shall be provided if they can be removed or displaced during operation and still allow access to Class 3B or 4 laser radiation. Warning labels shall be provided near the interlock if it can be defeated or by-passed.
<input checked="" type="checkbox"/>	Emergency Stop	A power strip located on the optical table next to the microscope provides power to the entire system. In the event of an emergency shutdown, the red switch on the power strip shall be used to immediately shutdown the lasers.
<input type="checkbox"/>	Beam Stops	The beam terminates in an aluminum beam block capable of withstanding the heat from the laser setup without degradation.
<input checked="" type="checkbox"/>	Master Switch	The laser is only operable via computer controlled software. The computer is password locked to ensure only authorized individuals have the ability to operate the laser devices.

Hazards & Controls:

Check If Applicable:	Hazard:	Controls:
<input checked="" type="checkbox"/>	Housekeeping	The beam path and surrounding areas will be kept free of clutter and obstructions. Hand clearing of clutter from the optical table and beam area will be performed prior to each laser operation. All optical fibers must be clear of clutter so they can be inspected prior to each use.
<input type="checkbox"/>	High Voltage	The building manager and facilities electrical shop shall be consulted prior to operation/maintenance involving high voltage exposure including any adjustments needed.
<input type="checkbox"/>	Capacitors	Any capacitors will be enclosed within a protective panel during operation and fully discharged prior to maintenance.
<input checked="" type="checkbox"/>	Unenclosed Beam Access to Beam	The beam is contained within the fiber optic cables and microscope until the sample stage. Objective lenses have a high divergence that reduces the nominal hazard zone to several centimeters from the objective optic. Laser intensity is reduced with microscope software to ensure <5mW laser power at the sample stage.

Laser Safety Standard Operating Procedure

<input type="checkbox"/>	Fumes/Vapors	Any fumes/vapors generated during operation will be exhausted through a fume hood or local ventilation apparatus.
<input type="checkbox"/>	Ultraviolet Radiation or Blue Light	Appropriate barriers and PPE to protect skin and eyes from UV and eyes from blue light will be in place upon consultation with EHS if needed. This may include lab coats, eyewear, gloves, face shields or topical sunblock applications.
<input type="checkbox"/>	Compressed Gases	Compressed gases will be properly secured and labeled. Safety caps will be in place for unused cylinders. Flammable and oxidizing cylinders shall be stored at least 20 feet apart unless specifically required for an experiment upon consultation with EHS. OH 204 compressed gas cylinder training from EHS should be taken and is available in UT Learn.
<input type="checkbox"/>	Hazardous Chemicals/Waste	No hazardous waste is expected to be made during ordinary operation. If hazardous waste is generated, training course OH 202 should be taken (available in UT Learn) and all waste properly handled, labeled and stored per EHS guidelines.
<input type="checkbox"/>	Reflective Material in Beam Path	The open beam paths will be kept free of clutter to prevent inadvertent ignition of materials, specular and diffuse reflections, and laser generated airborne contaminants.
<input type="checkbox"/>	Fire	A fire extinguisher is located within a few steps of the table. Laser operators will ensure familiarity with its location and complete FF 205 hands on fire extinguisher training from Fire Prevention Services. Beam blocks will be used to absorb laser energy capable of generating hazardous levels of heat.
<input type="checkbox"/>	Laser at eye level of person sitting or standing	The laser is mounted below the eye level of a person sitting normally. Beam blocks and additional barriers will be used to prevent the cohesive beam from travelling beyond the limits of the optical table.
<input type="checkbox"/>	Infrared Lasers	Invisible lasers will be properly blocked and attenuated. Adequate viewing equipment such as IR viewers, cards, cameras, etc. must be available to the end user to ensure reflections are minimized.
<input checked="" type="checkbox"/>	Correct Eyewear	The microscope has been exempted from laser eyewear use as long as the transmission percentage is set to 50% or below. This results in a sample stage output power below 5mW. Appropriate EHS approved laser eyewear protection with labelling of wavelength and optical density will be present and worn by all lab personnel working in rooms with accessible laser radiation. The eyewear will be made readily available prior to entering a nominal hazard zone at the door or curtain entrance, properly maintained, cleaned, and stored per manufacturers recommendations.
<input checked="" type="checkbox"/>	Secured Laser	Lasers are located in a closed laser combiner that is secured to the optical table. Laser fiber optic cables that run from the combiner to the microscope shall be kept free of clutter and without extreme bends that could damage the fiber.

