

Standard Operating Procedures

(V1_4/7/16)

Safe Working Practices for Leica Laser Micro Dissection Microscope

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I. General Information

The Leica Laser Micro Dissection microscope (LMD) is located on the 4th floor Room 1416 of the Biomedical Research Center. The LMD scope will be available for usage by untrained users during regular business hours (9:00 AM to 5:00 PM) and for trained users 24 hours. Live cell dissection will be available for trained users from 10:00 AM to 5:00 PM. Trained users will be granted after-hours card access to BRC1416 upon approval of the core management, Dr. Muralidharan Jayaraman. Consumables (slides, cap, tubes, and plates) are provided by the users.

In consultation with the Environmental Health & Safety office (EHSO), it was determined that the laboratory could be approved/used as a modified Biosafety Level 2 (BSL-2) facility. It is considered modified BSL-2 due to the absence of an immediately available emergency eyewash. The primary factors enabling this classification are:

- Only fixed tissues and tissue sections from human/animal sources are brought into the laboratory.
- All sample prep should be completed in the user's laboratory prior to arriving in the facility.
- The laboratory is maintained under negative pressure at all times.
- Laboratory door is always closed, and gloves and lab coats must be worn when handling samples or operating the instrument.

II. Facility Orientation and Training

Stephenson Cancer Center (SCC) facility staff are responsible for maintenance and daily operations and provides training and orientation for the LMD system. All appointment scheduling is done using the iLabs calendar system that is administered by the OUHSC Core Facilities (ouhsc.corefacilities.org). If the researcher does not have an existing iLab account, requests can be made to the OUHSC Core Facilities at Cytometry-

Support@ouhsc.edu. Accounts will be approved as quickly as possible but it is recommended to request account registration a minimum of 24 hours prior to training, if at all possible. Training reservations on the system should be coordinated with the SCC core facility contact, Dr. Muralidharan Jayaraman. After training, except for regular minimal day-to-day operation questions, the researcher is responsible for their experiment. Further expert questions are to be directed to the Leica application scientists.

New LMD users are required to take a one-on-one, 90 min, hands-on training before unassisted use of instrumentation. Exposure procedures and emergency response are discussed with users during this initial training and each user is provided with a copy of this document. After training, each user will be required to provide signed confirmation of receipt and understanding of the content within the SOP document. Furthermore, copies of all SOPs are available in the laboratory and on the OUHSC Core Facility website (<http://research.ouhsc.edu/core-facilities/>) so that they can be easily accessed at any time. Questions are encouraged at any time via email. If there are any operational changes, this SOP will be modified accordingly.

III. Startup Procedures (more detailed procedures are also included in a separate document)*

1. Check to make sure that the work area is clear of all unnecessary clutter and any sharp objects are in safe locations.
2. Boot up the computer, if not on.
3. Turn on the laser power supply unit (behind the power supply)
4. Turn on Laser key.
5. Turn on microscope control box.
6. Turn on EL6000 light source, if needed.
7. Launch LMD7 software.
8. Sample preparation is done in their respective lab. Slides with samples are placed on the microscope for brightfield or fluorescent viewing and dissection.
9. Load your collector set up.
10. Staff will assist users for building new experiment settings during training appointments only.
11. **Users are to report any suspected damage to the instrument prior to running. If there is any damage found after usage, you may be held accountable!**

IV. Shutdown Procedures

1. If you acquire pictures during dissection, store them on the /Desktop/Users/'Lab_name' folder. Export your data to an **encrypted storage device** from the user's folder on the desktop. **Encrypted storage devices that have been scanned for viruses are required for use on all core facility computers.**
2. Check the iLab web calendar to see if there is anyone using the instrument for the day.

- a. **If you are the last user- you are required to correctly shutdown the LMD system.**
 - i. **Unload collector to pick up your samples and load back into its place.**
 - ii. **Unload slides from slide holder and load the holder back in place on the microscope. All glass slides should be placed in a leak-proof sharps container. Plastic slides can be placed in biohazard bag.**
 - iii. Close the LMD7 software.
 - iv. Turn off the EL6000, if turned on.
 - v. Turn off the Microscope control box.
 - vi. Turn off the Laser key.
 - vii. Turn off the Laser power supply unit (behind the power supply)
- b. **If you are not the last user- leave everything turned on.**
3. Wipe all surfaces and objectives with 70% ethanol.
4. Discard all sample material into the biohazard container or package this material as needed for safe transport. Wipe down the container used for transportation after handling it with gloves.
5. Wipe down counter top with 70% ETOH.
6. All user and staff must remove gloves, then sanitize hands before leaving the laboratory with hand sanitizer provided in the room or touching other non-instrument items in the lab (phone, scheduling computer, door handles). SCC core facility staff will maintain the biohazard and sharp waste containers. It will remove, autoclave and properly dispose accumulated biohazard and sharp wastes, as required.

V. Spill Procedures

Microscope surfaces are wiped with 70% ETOH, left on surface for 15 minutes followed by a water rinse with deionized water. Large spills on microscope working surfaces are cleaned with 70% ETOH. We require all users to follow guidelines available in the EHSO Laboratory Safety Manual (*Part 13-Spill Control/Emergency Response: Section F-Biological Spills*) when working in the LMD facility. EHSO spill response procedures are available either online at: <https://labman.ouhsc.edu/hbSections.aspx?ID=685> (*Section F-Biological Spills*) or in the hard copy maintained in room 1413

Spill kits are provided both in Room 1413 and Room 1416. The 70% ETOH is provided on the bench-top beside the microscope. PPE (gloves) and other items used for clean up should be properly disposed of in biohazard containers under the bench after cleaning of a spill.

VI. Rules for Safe Use of all Microscopes:

The LMD system operates in a BSL-2 laboratory. The rules below are reviewed and revised as necessary and each facility user is responsible for reviewing them on a regular basis to insure compliance. An emergency eye wash is located at Room 1413, which is located directly across from the lab (due east) approximately 25 feet away. The rules for safe use of BSL-2 materials and instruments exposed to BSL-2 materials can be found on the University IBC webpage using this link:

<http://www.ouhsc.edu/ehso/labman/Section%206%20-%20Biological%20Safety.pdf>

and

<http://www.ouhsc.edu/ehso/policies/IDPolicy2010.pdf.pdf>

Reminder: Gloves and lab coats are to always be worn while operating all instruments or use of instrument computers (if PPE is worn while using computers and the assumption is that glove contamination is inevitable (see statement below), then don't these need to be disinfected/sterilized?) and during the handling of all samples within the lab space as there are hazard risks involved with many of the stains and fixation methods used for most samples.

Never touch door handles or telephone receivers with gloved hands! Contamination of these surfaces pose a danger to unwary users. **Gloves and lab coats must always be assumed to be contaminated** with chemical and biological material. After work, gloves will be disposed in Biohazard box and never touch the door knob with gloves.

Glass slides will be disposed in Sharp container under the bench.

No eating, drinking, smoking, or applying cosmetics in the laboratory.

VII. Exposure to Biohazardous Material

All employees with occupational exposure to human blood, tissues, or cell lines are required to take the online EHSO bloodborne pathogen training course at the time of assignment to tasks where occupational exposure may take place, when changes affect employees' occupational exposure, and at least annually thereafter. The hepatitis B vaccine should be made available to all employees who have occupational exposure to blood or other potentially infectious materials. If an employee sustains an exposure incident (such as a stick with a contaminated needle/scalpel/dental wire or a splash of potentially infectious material in the eye, mouth, mucous membrane, or non-intact skin), the exposed person should immediately:

- A. Clean the wound with soap; flush mucous membranes with water or normal saline solution;
- B. Notify his/her supervisor, designated coordinator, or other designated individual;

- C. Proceed for treatment within 1-2 hours of the exposure (see the OUHSC/OU-Tulsa Infectious Diseases Policy for current recommended treatment locations).
- D. If possible, for laboratory exposures, bring a sample of the source material to the treatment facility for testing.

The ultimate responsibility for reporting exposures, spills, and other biological hazards rests with the Principle Investigators, supervisors, and all employees. Such exposures and hazards need to be reported to supervisors, principle investigators, EHSO, and a medical professional.

Sources: OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030)
OUHSC EHSO Infectious Disease
Policy(<http://www.ouhsc.edu/ehso/policies/IDPolicy2010.pdf.pdf>)

The following facilities are recommended for treatment of occupational injuries or exposures, however employees may choose any health care professional they wish.

Employee Health
OU Physicians Building Suite 4D
825 NE 10th
Oklahoma City, OK
271-9675 (271-WORK)
Hours: 8:00 a.m. – 4:30 p.m. Monday through Friday (Call before going to the clinic)

OU Medical Center Presbyterian Tower Emergency Room
700 NE 13th Street
Oklahoma City, OK
405/271-3667
Hours 4:30 p.m. - 8:00 a.m. Monday - Friday and weekends