

Standard Operating Procedures

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Safe Working Practices for Leica SP2 Microscope

Table of Contents

- I. General Facility Information**
- II. Facility Orientation and Training**
- III. Startup Procedures**
- IV. Shutdown Procedures**
- V. Spill Procedures**
- VI. Rules for Safe use of all Microscopes**
- VII. Exposure to Biohazardous Material**

I. General Facility Information

The OUHSC Image and Flow Cytometry Laboratory is located on the 3rd floor Room 1317 of the Biomedical Research Center with the main office located on the 1st floor Room 1106. Regular business hours are between 9:00 AM to 5:00 PM Monday through Friday, but after-hours card access to facilities may be granted for experienced users upon request. Facility doors are locked from 5:00 PM through 8:00 AM. The laboratory is approved at Biosafety Level 2 (BSL-2) with restriction regarding anything higher than BSL-2. If needed, the addition of reagents to live cells can be done in the facility within the biosafety cabinet. All other 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

Facility staff provides training and orientation for all instrumentation. New users are required to take a one-on-one, 2 hours, hands-on training on the relevant instrumentation. Exposure procedures and emergency response are discussed with our users during training. Exemption from hands-on instrument training is given to users who are only signing up for assisted appointments but lab personnel will still go over safety guidelines and will provide each user with a copy of this document. Additionally, we will provide each user with a copy of the SOPs and will require a signed confirmation of receipt and content. Furthermore, copies of all SOPs are available in the laboratory and on the facility website so that they can be easily accessed at any time. Questions are encouraged at any time.

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. Turn on the microscope at the Leica power source on the floor.
3. Turn on the scanner, all the fans, and all lasers needed on the desktop.
4. Turn on the Excite mercury lamp on the floor.
5. Log on to the computer (PI:user with no password) and start the Leica software(expect a five minute delay for the software to initialize).
6. Load the settings as described in the operation specific guide.
7. Staff will assist users for building new experiment settings during assisted appointments only.
8. **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. Export your data to an **encrypted storage device** from the user's images folder shortcut on the desktop. **Encrypted storage devices that have been scanned for viruses are required for use on all core facility computers.**
2. Check the 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 Leica SP2!**
 - i. **Wait 5 minutes before turning off the laser cooling fans allow the PC power to remain on** (red switch on the far right).
 - ii. Close the program and turn off the scanner, all of the lasers, and the Excite lamp.
 - b. **If you are not the last user-** log out, but 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 or ConFLIKT.
6. All user and staff must remove gloves, then wash and sanitize hands before leaving the laboratory or touching other non-instrument items in the lab (i.e. phone, scheduling computer, doorhandles, etc.).

V. Spill Procedures

Microscope surfaces are wiped with 70% ETOH or ConFLIKT, left on surface for 5 minutes followed by a water rinse with deionized water. Large spills on microscope working surfaces are cleaned with 70% ETOH or ConFLIKT. We require all users to follow guidelines in our spill protocol when working at our facility. Spill kits are provided in the laboratory. The 70% ETOH or ConFLIKT is provided on all bench-tops. PPE (gloves) and other items used for clean up should be properly disposed of in biohazard containers after cleaning of a spill.

VI. Rules for Safe Use of all Microscopes:

The OUHSC Core Facility for Flow Cytometry and Imaging operates as 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. 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 and during the handling of all samples within the Flow Cytometry and Imaging Facility as there are significant chemical hazard risks involved with many of the stains and fixation methods used for most samples used within this facility.

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.

No eating, drinking, smoking, or applying cosmetics in the Flow Cytometry and Imaging Facility.

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.

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 2C
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

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.