

Standard Operation Procedures

(v6 5/1/17)

Safe Working Practices for Flow Cytometry on the Nikon TE2000

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 restrictions regarding anything higher than BSL-2. Staining procedures, which do not require washing steps (i.e. live/dead stain) may be completed at the flow facility in the biosafety cabinet. 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 and operating the Nikon TE2000

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 hour, hands-on training on the relevant instruments. 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. Additionally, we will provide each user with a copy of the SOP 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. Training on the FACSCalibur instrument includes basic instrument operation and analysis as well as all relevant safety procedures, spill

management, and decontamination. The SOP acknowledgement form must be signed and dated for all instruments before use.

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

1. Check to make sure the work area is clear of all unnecessary clutter, and any sharp objects are in safe locations.
2. Rotate the coarse focus knob so that the objective turret is in at the lower limit (Do not force beyond the limits).
3. Turn on the camera on the left side of the microscope.
4. Turn on the mercury lamp if fluorescence is needed.
5. Start the Metamorph program. Then, load the task bar as described in the operation specific manual.
6. Make sure that exposure and threshold settings are correct.
7. Staff will assist users for building new experiments 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 Nikon images folder shortcut. **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 Nikon TE2000!**
 - i. Turn off the program before turning off the microscope.
 - ii. Turn off lamps, camera, and the main microscope switch (red).
 - b. **If you are not the last user-** log out. Leave everything turned on.
3. Wipe all microscope surfaces and objectives with 70% ethanol.
4. Discard all sample material into the biohazard container or package this material as needed for safe transport back to your own laboratory. Wipe down counter tops with 70% ETOH or ConFLIKT.
5. Wipe down the container used for transportation after handling it with gloves.
6. All users and staff must remove gloves and lab coats, then wash their 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 or on other lab surfaces are cleaned with 70% ETOH or ConFLIKT left on surface for 20 minutes with a soaked, wet towel. Spill kits are provided in the laboratory. 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 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 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.