

# Standard Operating Procedures (v4 3/9/20)

## Safe Working Practices for Flow Cytometry on the Imagestream

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### **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. 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 ImageStream.

### **II. Facility Orientation and Training**

Facility staff provides training and orientation for all instrumentation. New users are required to take a one-on-one 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. 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 they can be easily accessed at any time. Training on the Imagestream instrument includes basic instrument operation and analyses, as well as all relevant safety procedures, spill management, and decontamination.

### III. Startup Procedures

1. Open the front panel and check fluid levels: Rinse bottle is full of diH<sub>2</sub>O, the sheath bottle is full PBS, the sterilizer bottle is full of 10% bleach, the cleanser bottle is full of Coulter Clenz, the debubbler bottle is full of 70% isopropanol, SpeedBeads are loaded, and the waste bottle is empty.
2. Press the green power button inside of the front door to turn on the instrument.  
*Note: There is an additional power switch on the back of the instrument that should only be used in an emergency.*
3. Turn on the Dell Precision and Dell Optiplex computers. Log in using “flowlab” and password is “flowlab”.
4. Double click the INSPIRE icon to open the acquisition software.

### IV. Quality Control

1. Click “Startup” to run instrument calibration.
2. Ensure that all test pass with a Green Status before continuing with acquisition.
3. For addition help with calibration please see the Troubleshooting section of this SOP.

### V. Data Acquisition

1. Open an existing template in the software by going to File > Load Template then select the saved template and click open. A new template can be opened to create a template from scratch.
2. Click “Load” on the top of the software, then load the sample tube with all of the fluorochromes being used and the brightest fluorescence.  
*Note: Samples should be loaded in 1.5 ml microcentrifuge tubes.*  
*Note: Samples must have a minimum volume of 20  $\mu$ L.*
3. Ensure that speed is set to Low.
4. Turn on each laser being used for the experiment by selecting them in Excitation Lasers on the right side of the software. Adjust laser power if needed according to the brightness of the sample.
5. Select the Brightfield channel. By default, Brightfield is set at channel 1 and 9. You can change that to 2 and 8, 4 and 10, 5 and 11, 6 and 12 or OFF by selecting the appropriate Brightfield option in the drop down menu under the Illumination tab on right side.
6. Under the File acquisition section enter the number of cells to acquire and the file name. Select Browse and save the files on Desktop >Users > {PI name} > {User Name} {YYYYMMDD}.
7. Click “Acquire” to begin saving events to a file.
8. When acquisition has finished click “Return”. Remove the first sample tube and click “OK”. Load another tube by clicking “Load”, then placing a new tube when the software requests it.  
*Note: If “Load” is clicked instead of “Return”, the remaining sample will be flushed to the waste.*
9. Repeat the previous two steps for as many samples as needed.

10. Once all the samples have been acquired, run single color compensation controls. Single color controls can be run manually by turning off Brightfield, or by using the Auto Compensation Wizard.
11. After all samples and controls have been acquired, clean the instrument by loading a tube with 10% bleach, running for one minute, then loading a sample of diH<sub>2</sub>O and running for one minute.

## **VI. Shutdown**

1. Ensure that the rinse bottle is full of diH<sub>2</sub>O, the sterilizer bottle is full of 10% bleach, the cleanser bottle is full of Coulter Clenz, and the debubbler bottle is full of 70% isopropanol. Add 160 ml of bleach to the waste bottle, wait 15 minutes, and empty the waste.
2. Ensure that there are no tubes in the uptake port.
3. Click “Shutdown”. This procedure is an automated and should take approximately 45 minutes to complete.  
*Note: This is adequate for daily shutdown, and the next step is not necessary. If the instrument will be unused for more than two weeks, continue with the next step.*
4. If the instrument will be unused for more than two weeks, replace the bead vial with a vial of diH<sub>2</sub>O, and click “Initialize”. Then from the main toolbar select File > Exit and shutdown instrument.

## **VII. Troubleshooting**

1. If there are connectivity issues, turn off the instrument and computers, wait for few minutes, and then restart the instrument and computers.
2. If no events are detected when running a sample (including SpeedBeads), the sample line might be clogged or the flow cell might contain a bubble. Wash with 10% bleach then with diH<sub>2</sub>O. If the problem persists, go to Maintenance > Debubble to try to dislodge the clog or bubble.
3. If any other problems occur, call Amnis service.

## **VIII. Spill Procedures**

Flow cytometer surfaces are wiped with 10% bleach, left on surface for 5 minutes, and followed by a water rinse. Large spills on flow cytometer surfaces or on other lab surfaces are cleaned with 10% bleach left on surface for 20 minutes with a soaked wet towel. We require all users to follow guidelines in our spill protocol when working at our facility. Spill kits are provided in the laboratory. The 10% bleach solution (made daily) is provided on all bench-tops. PPE (gloves) is properly disposed of in biohazard containers after cleaning of a spill.

## **IX. Exposure to Biohazardous Material**

All employees with occupational exposure should receive bloodborne pathogen training 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); and
- 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)

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 10<sup>th</sup>

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 13<sup>th</sup> Street

Oklahoma City, OK

405/271-3667

Hours 4:30 p.m. - 8:00 a.m. Monday Through Friday and weekends