THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

AMNIS IMAGESTREAM X

USE AND MAINTENANCE

PURPOSE

The purpose of this procedure is to ensure proper and consistent use and maintenance of the Amnis ImageStream^X imaging flow cytometer including tasks such as startup, quality control, data acquisition, shutdown, and troubleshooting.

SCOPE

Compliance with this procedure is the responsibility of all employees and users of the Advanced Cytometry & Sorting Facility at South Campus's Amnis ImageStream^X imaging flow cytometer.

DEFINITIONS

PBS: Phosphate Buffered Saline.

diH_2O: Deionized water.

MATERIALS AND REAGENTS

- Amnis ImageStream^X imaging flow cytometer.
- PBS.
- 10% bleach.
- diH_2O.
- Coulter Clenz.
- 70% isoproanol.
- SpeedBead ImageStream^X system calibration reagent.
- Tubes

PROCEDURE

1.0 Start Up

1.1. Open the front panel of the instrument and check that the rinse bottle is full of diH_2O, the sheath bottle is full of 1X 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, and SpeedBeads are loaded, and the waste bottle is empty.

1.2. Press the green power button inside 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 or if the instrument will be off for two or more weeks.

1.3. Turn on the Dell Precision (CPU is on the floor) and Dell Optiplex computers (below the monitor). Log in using the user name “Amnis” and password “is100”.

1.4. Double click the ISX icon (INSPIRE software) to open the acquisition software.

2.0 Quality Control

2.1. Once the instrument power is ON, computers are connected and ISX Icon (INSPIRE software) is open, click “Startup” tab in the lower right corner of the software to initialize the instrument and fluidics startup.

2.2. Once the startup is initialized, check if the “Assist” box is checked. If not, click the box and activate the “Assist” so Assist is automatically run after the instrument initialization.

2.3. When the calibration tests and Assist pass, return to the “Setup” tab. If Assist fails on some of the tasks, you can re-run Assist individually to that particular task. If it fails again, call instrument service.

2.4. For additional help with calibration please see the Troubleshooting section of this SOP.

3.0 Data Acquisition

3.1. Open an existing template in the software by going to File > Open Template. A template can be created from scratch or modified using an existing template.

3.2. Click “Load” on the top of the software. The loader will be released, 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 15 µl.

3.3. Choose the objective under “Magnification”.

3.4. Ensure that the speed is set to Low during experiment set up.

3.5. Turn on each laser being used for the experiment by selecting them in Excitation Lasers on the right. Adjust laser power if needed according to the brightness of the sample.

Note: All fluorochromes excited by a laser will be affected when laser power is altered. Ex: Adjustment of the 561 nm laser power to change brightness of PE will also affect brightness of PE-Cy5.

Note: Be careful not to saturate the Raw Max Pixels of any fluorochrome when adjusting laser power.

3.6. Select the Brightfield channel in the Illumination tab on the right.

Note: By default Brightfield is detected in channels 1 and 9, but it can be changed to channels 2 and 8, channels 4 and 10, channels 6 and 12, or OFF.

3.7. Under the Setup tab, 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}.

3.8. Click “Acquire” to begin saving the events. You can also select the saving gate and storage gate.

3.9. 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.

3.10. Repeat the previous two steps for every samples you have.

Note: If “Load” is selected instead of “Return”, the remaining sample will be flushed to waste.

3.11. 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. Recommendation is to run the comp control through wizard.

3.12. 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 diH2O and running for one minute.

4.0 Shutdown

4.1. Ensure that the rinse bottle is full of diH_2O, 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. Empty the waste bottle and refill it with 160 ml of bleach.

4.2. Ensure that there are no tubes in the uptake port.

4.3. Click “Sterilize”. This procedure is an automated shutdown 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.

4.4. If the instrument will be unused for more than two weeks, replace the bead vial with a vial of diH_2O, and click “Initialize”. Then from the main toolbar select File > Exit and shutdown instrument.

5.0 Troubleshooting

5.1. If there are connectivity issues, turn off the instrument and computers, wait for few minutes, and then restart the instrument and computers.

5.2. 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 diH2O. If the problem persists, go to Maintenance > Debubble to try to dislodge the clog or bubble.

5.3. If any other problem occur, call instrument service

RELATED PROCEDURES

None.

REFERENCES

Amnis Corporation. “INSPIRE™ ImageStream^{X} System Software User’s Manual”. Version 4.0. July 2010.

REVISION HISTORY

VERSION	ACTION	DATE	INITIALS
1.0	Initial release	05-03-2017	VP
1.1	Modification/Review	04-16-2020	VP/SS