FSU | COLLEGE OF MEDICINE

STANDARD OPERATING PROCEDURE

Operation of ImageStreamX mark II

1. Purpose and Policy

The purpose of this document is to provide standard for best practices when using the ImageStreamX mark II (ISX) for imaging flow cytometry experiment.

ISX combines the high throughput performance of flow cytometry with the imagery and functional insights of microscopy. It offers 4 lasers (405nm, 488nm, 561nm, 640nm) and total 12 channels (including 2 for Brightfield).

The following procedures are listed in the ISX system and INSPIRE software User's Guide. This SOP should not be considered to replace the User's Guide or guidance from software.

The ISX can be operated by users if proper training is received and proved by lab staff.

2. Procedure for User

Users are encouraged to discuss with lab staff about panel design, protocol optimization and instrument settings before experiments.

If any issue is found by the user during the experiment, it should be reported to the lab staff immediately.

2.1 Instrument Startup

- Open the front panel, check the level of sterilizer (10% bleach), cleanser (FlowClean, BeckmanCoulter), debubbler (70% Isopropanol), rinse (ddH_2O) and sheath fluid (PBS). Fill the bottle as needed.

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- Empty the waste bottle, and fill with 160 mL 100% bleach.
- Take off the SpeedBeads tube, and sonicate for 5 mins. Mark the solution level and reinstall. Replace it with a new tube if needed.
- Turn on the Linux computer, then the Window computer and instrument (power button on the upper left corner inside the front panel).
- Log in the Amnis account on the computer.
- Start the INSPIRE software and wait for the interface.
- Start the **"STARTUP"** and check **"run ASSIST after initiation"**, wait about 60 mins to complete.

2.2 Experiment

- If FCS files are also required, then check **"Generate FCS file"** under the file tab along with RIF.
- Under the File Acquisition tab, Select **"Browse"** and make a new folder on the desktop, named by date.
- Select Objective lens (20x, 40x, 60x) needed for the experiment under Magnification tab. Select **"High Gain"** for small particles.
- Ensure that the speed is set to **"Low"** during experiment set-up under Fluidics tab.
- Turn on each laser needed for the experiment under Illumination tab.
- Activate the channels required for the experiment.
- Click **"Load"** and wait the sample loader open.
- Run all stained sample first.
- Create histogram plot for focus event, with "Gradient RMS" on Ch01 parameter (Mostly higher than 40).
- Create dot plot for single event from the gate on "focused cells", plot for "Area (X-axis)" and "Aspect Ratio (Y-axis)" on Ch01. Make a gate of on cells showing Aspect Ratio closer to 1.0 (single cells) and with intermediate Area (100-600).

- Create histogram plot for “Raw Max Pixel” for all fluorescent channels from the gate on “focused & single cells”. Adjust laser powers if needed to ensure Raw Max Pixel is less than 4E3 so signals are not saturated.
- Enter the name of sample and number of cells to be acquired from gate.
- Click **“Acquire”** to begin saving the events.
- Click **“Return”** to retrieve the sample. **Note: If “Load” is selected instead of “Return”, the remaining sample will be flushed to waste.**
- Between each sample, run 10% Bleach for one minute followed by running PBS for one minute.
- Run single color compensation controls. Single color controls should be run with Brightfield turning off, SSC laser off and enabling all the channels.
- After all samples, run 10% Bleach for one minute followed by running ddH_2O for one minute.

2.3 Instrument Shutdown

- Check the solution level again and start the “**Shutdown”.** This procedure is an automated shutdown/sterilization and should take approximately 45 mins to complete.
- The computer will be turned off automatically.

2.4 Data Management

- It’s user’s responsibility to keep all the generated data (.rif file, .cif file, .daf file, FSC file, .ctm file, .ast file and all images). The data on the computer may be deleted without notification.
- Experiment data should be only transferred through Flow Lab thumb to the Flow Lab computer and then share with user.

3. Special Procedure for BSL-2 Requirements

Follow this procedure if your sample requires BSL-2. Read and sign the BSL-2 procedures. Notify the lab staff before running the experiment.

3.1 Sample Preparation and Liquid Handling

- Sample preparation should be done in a BSL-2 hood in the user’s lab and transfer to flow lab in a close cap tube.
- The tube cap should keep close all the time when vortex the tube.
- Centrifugation can be performed only with the aerosol-tight caps.

3.2 Decontamination

- After running all the samples, run 10% bleach sample for 10 mins, and proceed to Shutdown procedure as in step 2.3.
- Wipe all the surface, tube loader and bench with 70% alcohol.

3.3 Waste

- Solid waste (tips, tubes) should be submerged in 10% bleach and autoclaved following BSL-2 procedure.
- Leave the waste tank with 10% bleach (final concentration) for at least 30 mins.

4. Procedure for Lab Staff

4.1 Maintenance

- Lab staff should confirm the instrument is shut down at 5 pm on the workday.
- If the instrument will be unused for more than one month, replace the bead vial with a vial of ddH_2O and click **"Initialize"**. Then from the main toolbar select File > **"Exit and shutdown"**.
- Instrument STARTUP should be done at least once a month if not frequently used.
- All the maintenance procedures should be recorded, including septic cleaning, filter change and data backup.

4.2 Data management

- The experiment data on the computer should be backup to the FACS Data drive once a month.
- All experiment data will be deleted if free space on the C drive is less than 30%.

5. References

ISX system and INSPIRE software User's Guide (780-01286-04), IDEAS Manual (780-00959-00) are stored in the folder on the desktop for reference.

Also see the ISAC SRL (https://archive.org/details/@isac_srl) for more protocol and SOPs.

BSL-2 Facility Policies and Procedures.

6. Approvals

The signatures and dates below are required for full SOP approval and implementation.

This SOP was written by:

Li Sun	Director	Li Sun	7/24/2026
Name	Title	Signature	Date

This SOP was reviewed and authorized by:

Li Sun	Director	Li Sun	7/24/2026
Name	Title	Signature	Date