

Yale School of Medicine

Flow Cytometry Facility

ImageStreamXmk^{II}® Training Manual



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Dear Amnis ImageStreamXmk^{II} Users,

The Amnis ImageStreamXmk^{II} is supported by Shared Instrument Grant No. 1-S10-RR-026526-01, thanks to which researchers have opportunity to implement that system into their research.

Please remember to cite the Shared Instrument Grant No. 1-S10-RR-026526-01 in your publications.

Thank you,

Flow Core Facility Staff

Image StreamXmk^{II} Usage Policies

Training and Access:

1. All new users, regardless of previous ImageStreamX system experience, must take appropriate training with Ashraf Khalil on the proper use of the instruments and Core policies.
2. Once training is completed users will be able to book their times through www.yaleflowscheduling.internal.yale.edu
3. Please check the website for current fee for using the ImageStreamXmk^{II} system.
4. ALL SAMPLES MUST BE FIXED BEFORE RUNNING.
5. If a user is not going to be able to make a scheduled time, they must delete the event from yaleflowscheduling.internal.yale.edu at least 3 hours before the scheduled time to avoid being charged.
6. Users will be charged for the greater of time scheduled or actual time used on the Amnis.
7. Individuals who do not show up for a scheduled time while the instrument is running will be charged for the scheduled time.
8. Using someone else's login information will result in a loss of Core facility privileges for two weeks. The user who scheduled time with someone else's account information, as well as the individuals who lent their password will both have their Core facility privileges suspended. It is important that you do not share your user ID and password with anyone. This rule will allow for more fair allocation of instrument time, will prevent untrained users from having access to the machines, and will prevent billing time using someone's else's account. If you need to have a new password issued and/or need training, please contact Ashraf at ashraf.khalil@yale.edu or call 203-785-7958.

Maintenance and Care of Machine:

9. Facility staff will Initialize and run ASSIST M-F if reservations are in YFS for the day prior to 9am. Weekend and after-hours users should follow ImageStreamXmk^{II} Operation Quick Start Guide in order to initialize the system and run ASSIST.
10. Description on how to run ASSIST is included in ImageStreamXmk^{II} Operation Quick Start Guide.
11. ASSIST must be run at the start of the day. In order to check if ASSIST was run for the day, go to the instrument menu and select Calibrate with Assist. This will allow you to see if the calibrations were run.
12. A failed calibration or test is flagged with a red box. If a calibration or test fails, run the calibration or test individually and if it fails again call or email Ashraf Khalil.
13. Sample preparation; make sure the sample concentration does not exceed $1-2 \times 10^7$ cells per ml.
14. Sample filtration; make sure that your sample is filtered through a 40 micron filter; if you are running large cells or you are looking for cells interaction you can use a 70 micron filter. If sample aggregation is a problem, use an anti-clumping buffer such as EDTA or Accumax prior to fixation.
- 15. Make sure your samples are fixed (i.e. 1% PFA for 20 minutes).**
16. After running your samples run 10% bleach to remove all traces of debris and cells from a sample line.
 - Select **Load** from the instrument control panel, when prompted place tube with 50 μ l 10% bleach and click OK.
 - Run 10% bleach for 5min.
 - Select **Load**, remove empty tube and select OK for “Please remove tube” message.
 - When asked to place tube with 50 μ l of the next sample, select cancel.
 - Select OK for the error message.
 - Turn off all the lasers
 - Empty Waste Container
17. INSPIRE will alert the user if a fluid needs to be refilled. All ImageStreamXmk^{II} fluidics refills are located on the wire shelves in the ImageStreamXmk^{II} room. A replacement tube of Speed Beads is kept on the top shelf in the small fridge near the sink in 617. Please alert Ashraf if you replace speed beads or if supplies are running low.
18. If you are the last user of the day, choose Shutdown from the instrument control panel. This procedure sterilizes the system and leaves it with pumps empty and water in the fluidic lines. The instrument will shut off after sterilization is complete.
19. To report any problems with the ImageStreamXmk^{II} System, please first try to contact Ashraf Khalil (5-7958) directly or by email.
20. Serious abuse of the ImageStreamX System and the above rules will be penalized. Violation of rule 8, 12, 15- 18 will not be tolerated. We can and will track who leaves the machines in improper conditions.

Usage Policy Violations and Associated Fines

Level I Violations:

- 1: Failure to run ASSIST calibration and tests.
- 2: Using someone login information
- 3: Leaving ImageStreamX System on without performing “Sterilize System”.
- 4: Altering the Machines without Authorization.
- 5: Any Gross Abuse of the Machines

Level I Fines:

First Violation: \$100 Fine

Second Violation: \$250 Fine

Third Violation: \$350 Fine and FACS Facility Privileges Suspended For 2 Weeks

Level II Violations:

- 1: Not Emptying the Waste Container.
- 2: Not Filling the Sheath Fluid Container.
- 3: Not Filling the Speed Beads Tube.

Level II Fines:

First Violation: Warning to User and PI Via E-mail

Second Violation: \$100 Fine

Third Violation: \$250

Fine Fourth Violation: \$350 Fine

Biological Safety

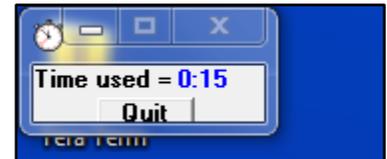
- The Image Stream is rated at BSL1. Samples must be fixed before running, recommended fixation is 1% paraformaldehyde for at least 10 minutes before running the samples on the ImageStreamXmk^{II}. Please see the sample preparation guide for more information.
- The use, containment and disposal of biologically hazardous materials are required to be in accordance with Personnel Protective Equipment Directive 93/95/E and are the responsibility of the end user. Follow all local, state, and federal biohazard-handling regulations for disposal of the contents of the waste reservoir.
- Prevent waste-reservoir overflow by emptying the container when the waste indicator indicates that it is full.
- Run the instruments sterilize routine after each day's use. Note that this procedure has not been proven to result in microbial sterility.
- The Bio Safety Level for the instrument is BSL1.

Image StreamXmk^{II} Users System Login:

Starting the System:

- Power on both computers and the instrument.
- Login to Windows, Password: is100
- Next you'll see white screen with the space for password,
- Type your Yale Flow Scheduling password
- Wait 5 minutes
- Select ISX software icon from the computer desktop
- Once Inspire has launched select Startup button from the instrument control panel to initialize the system and run ASSIST. This can take up to an hour, users can log out and come back after the ASSIST is finished.
- If you **are not the last user for the day** please run 5minutes of bleach and “Load” then cancel. Ensure that all the lasers are off
- Go to **the top left corner** and select “Quit” from open window.

- You should see white screen with space for password.



- If you are **the last user for the day** select *Shutdown* from the Instrument Menu
- Go to **the Control Section** and select “Shutdown”.



- Then select “OK” from the open window to sterilize /shutdown the instrument

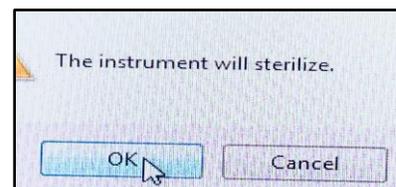


Image StreamXmk^{II} Optics:

The standard 488 nm laser in the Image StreamXmk^{II} is augmented with the following additional lasers: 405, 561, 658 and 785 nm.

The output of each laser can be independently adjusted for greater experimental flexibility and optimal results.

		Excitation Laser (nm)					
Ch	Band (nm)	405	488	561	642	785	Ch
1	430-480	BRIGHTFIELD					1
2	480-560	FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, SpectrumGreen, LysoTrackerGreen, MitoTrackerGreen, QD525*					2
3	560-595	PE, PKH26, Cy3, AF555, DSRed					3
4	595-640	PE, Cy3, AF546, AF555, DyLight549, DyLight594*, PKH26, DSRed, SpectrumOrange, MitoTrackerOrange					4
5	640-745	PE- TexRed* , ECD*, PE-AF610*, 7AAD*, PI*, RFP, QD625*, eFluor625*					5
6	745-800	PE-Cy5*, PE-AF647*, PerCP*, PerCP-Cy5.5*, DRAQ5*, QD705*, eFluor650*					6
7	430-505	DAPI, Hoechst, PacBlue, CascadeBlue, AF405, eFluor405, DyLight405, CFP, LIVE/DEAD Violet					7
8	505-570	PacOrange, CascadeYellow, AF430, QD525*					8
9	570-595	BRIGHTFIELD					9
10	595-640	QD625*, eFluor625*					10
11	640-745	QD705*, eFluor650*					11
12	745-800	QD800*					12
		APC, AF647, AF660, AF680, DRAQ5, Cy5, DyLight649, DyLight680, PE-AF647, PE-Cy5, PerCP, PerCP-Cy5.5					
		APC-Cy7, APC-AF750, APC-eFluor750, Cy7, AF750, DyLight750, eFluor750, PE-Cy7*, PE-AF750*					
		SSC					

Dyes with an * are excited by at least one laser directed to camera1 and another directed to camera2. The channel that the dye will appear brightest in depends on the relative laser powers used. Recommended dyes (based on optimal excitation and detection channels) are in boldface. QD565 and QD585 are not included because their primary fluorescence appears in the Ch9 Brightfield reference image.

Image StreamXmk^{II}: Filtering samples

For better-quality images remove clumps by filtering the sample through 40 or 70 μm mesh before loading onto the ImageStream:

After the final staining step:

1. Wash cells twice with wash buffer
2. Resuspend pellet in 100ul PBS
3. Place mesh square over sample tube opening
4. Pipet entire volume up and through the mesh
5. Transfer filtered cells to 0.6 mL microcentrifuge tubes