LS&E
Infrastructure Center

Date modified: October 2025 Date of the tutorial: _____

ImageStream Tutorial Checklist

Working in the flow unit:

- No food or beverages are allowed except for closed bottles
- The workstation should be left clean and organized
- Ensure you take your belongings, such as articles, protocols, experiment layout, etc.
- Instructions on the reservation system and working with the KIOSK
- REGISTRATION FORM, Reservation and registration, Safety Instructions, Shadow protocol.

Kiosk

☐ Sign in before starting work (verify budget)

☐ Sign out after finishing work

☐ If you have a tutorial, sign up for both sessions

☐ Turn off the **stream** if the next user has more than 2 hours until their session

☐ Verify that the next user is coming (after working hours)

Machine

☐ Fluids: Sterilizer (0.5% bleach), Cleanser (coulter clenz), Debbubler (70% isopropyl alcohol)

Rinse (DDW), Sheath (PBSX1), Beads (SpeedBead)

☐ Waste –make sure it's not full before turning on the machine

☐ Turn on/off procedure (server, ImageStream, pc, Inspire)

☐ Cleaning and calibrating the machine

☐ If the staff is available, we can power on the machine and run calibration, it's the researcher

Responsibility to notify us before coming to the FACS unit!

Inspire

☐ Experiment duplication (template)

☐ Sample handling (volume, load, and return), Acquisition (counting and recording)

☐ Internal beads exclusion

☐ Channel to record and eliminate, Brightfield

☐ Compensation– Mandatory - the ImageStream is co-linear

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- ☐ Adjust laser voltages
- ☐ Magnification (X20, X40, X60)
- ☐ Graphs -Area, Intensity, Aspect ratio, Gradient RMS
- ☐ Gates (creating, renaming, modifying)
- ☐ Record mode (storage gate and stopping gate)
- ☐ Backup data, deleting files on the desktop
- ☐ Optional for big experiment copy to external HDD (Deepfreeze)
- ☐ Cheat sheet - explanation

Analysis – IDEAS (schedule a tutorial or time with the staff)

- ☐ File types and usage
- ☐ Wizards
- ☐ Export data and access data
- ☐ Mask and Features
- ☐ Help file (mainly for analysis)

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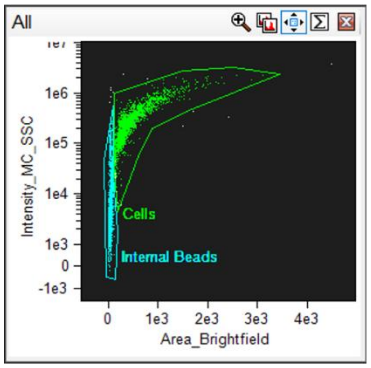
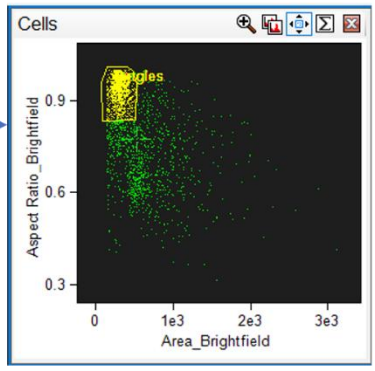
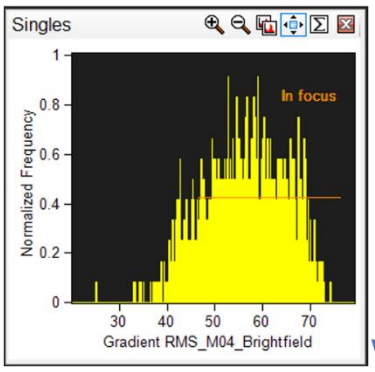
Basic gating strategy (cells)



Internal beads exclusion



Doublets discrimination

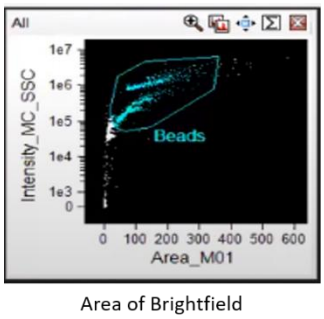


Then continue gating according to your desired dyes and fluorochrome

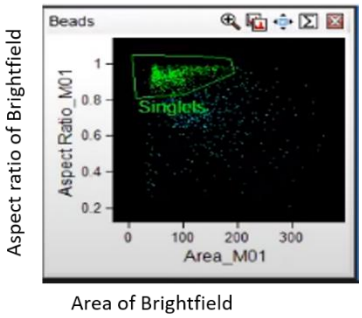
ImageStram cheat sheet

*For compensation turn OFF brightfield and SSC, a prefix of _noBF will be added to your files

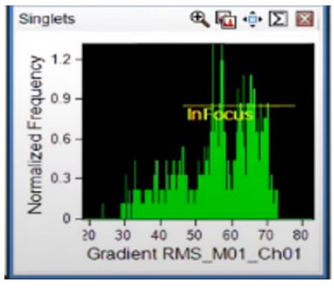
Basic gating strategy (Beads)



Area of Brightfield



Area of Brightfield



Gradient RMS of Brightfield

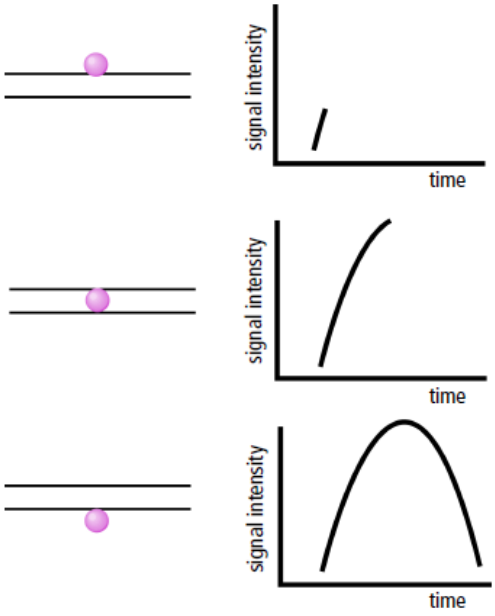
Internal beads exclusion

Doublets discrimination

Then continue gating according to your desired dyes and fluorochrome

LS&E Flow Team

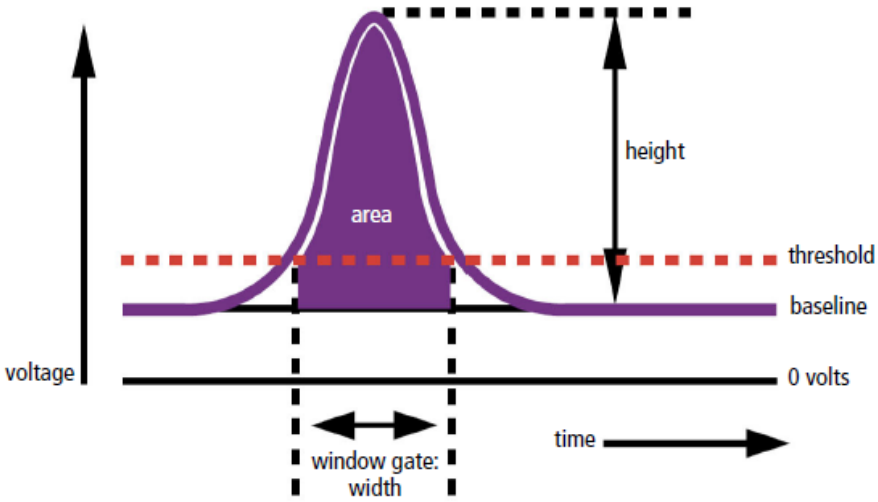
Figure A-10 Anatomy of a pulse



Pulse Measurements

The pulse processors measure pulses by three characteristics: height, area, and width.

Figure A-11 Pulse measurements



- Pulse height is the maximum digitized intensity measured for the pulse.
- Pulse area is an integration of the digitized measures over time.
- Pulse width calculates: $\frac{\text{area}}{\text{height}} \times 64{,}000$