

General remarks

Independent usage of the ImageStream Mark II requires an introduction by the staff of the Cytometry Facility.

Questions / reporting technical problems:



Feedback form

Emergencies:

☎ 044 635 53 36

☎ 044 635 02 06

Biosafety Note

Instrument and computer must be left so they are safe to be touched without gloves by the next user!

Task for the 1st user of the day

- The first user of the day is responsible to check the fluid levels inside the machine.
 - a. empty the waste tank to the sink (the liquid has been already decontaminated).
 - b. check levels of all tanks to be at least half full and refill if necessary (stocks can be found on the shelf right to the instrument).
 - c. check Speed Beads and replace with stocks from fridge in hallway if fill level is below 1 cm.

Starting up the ImageStream

- Power on the ImageStream and the 2 computers below the machine.
- Log on with your core login account and password (e.g. John Doe → j.doe).
- Start ISX Software and DO NOT close the opening windows.
- Press startup button including ASSIST. This procedure takes up to 45 minutes. If assist fails please report the red flagged modules using our feedback form.

Running the ImageStream

- The first tube to be loaded into the machine should be a water sample (just return after loading).
- Samples: prepare at least 20µl sample volume (~50 x 10⁶ cells/ml) in 1.5 ml Eppendorf tubes. Avoid air bubbles!
- Turn on all lasers you will need for recording your panel (do not switch on additional lasers because this may reduce the signal detection of your dyes of interest).
- Load sample and adjust signal brightness by adjusting laser power (make sure you avoid saturated = cyan pixels or events with RawMaxPixel intensity at 4096).
- Record single stained controls for every fluorophore in your panel (even if you only have one fluorochrome because spillover can be detected in brightfield channels).
- Save your data as rif-files according to our data saving rules. Locally stored data will be deleted without further notice.

Shutting down

- Wash the sample line by loading a tube with dH₂O (can be returned directly after loading).
- Click the “Shutdown” button and confirm the following dialog in the ISX software.

Turn around page for instructions on how to record single stains for compensation →

Manually recording single stains for compensation

Note: The ISX wizard for compensation runs very slowly and will fail if the sample has low concentration. To avoid any problems, we recommend recording the single stains manually by following workflow...

- Use identical experiment settings (objective, EDF, flow speed) as for the actual experiment.
- Turn on all lasers you will need for your panel and adjust laser powers to have maximal resolution but not saturate pixels in the individual channels. If you run the experiment for the first time you should check the fully stained sample and single stained controls for saturated pixels and adjust laser powers accordingly.
- Turn on recording of all channels (if some channels have been inactivated before).
- Switch OFF Brightfield illumination (via drop down menu).
- Switch OFF SSC (785 laser).
- Gate on positive events in the RawMaxPixel plots (negative events are not required and should be excluded to reduce data size).
- Record 500-1000 positive events (ensure you have set the correct recording gate).
- Repeat previous step for all single stained controls of your panel.

Changing back to regular recording conditions

- Turn ON Brightfield illumination (via drop down menu).
- Turn ON SSC (785 laser).
- Inactivate channels you are not using in your panel to reduce the size of the dataset.
- Exclude speed beads and debris from your recording by gating on your cells of interest in a dot plot typically set to Brightfield Area vs. Brightfield Aspect Ratio (usually in Ch1).