ImageStream Mark-II Operation Quick Start Guide

To begin normal operation of ISX- MkII:

1. Power up the system and launch the **ISX** application.
2. Check to be sure the buffer containers are full and the waste tank is empty.
3. Select **Startup** and the instrument will flush the system and load sheath in ~12min*.*
4. In the Calibrations view, press **Start all calibrations and tests**. Calibration takes ~10min and when all tests pass the ASSIST button turns green and the system is ready to run for the day.
5. Select file and load **default template** or an experiment template from the File menu.
6. Press **Load** and, load an aliquot of a sample with each fluorochrome present.
7. In the Illumination section, turn on the appropriate **lasers** for each fluorochrome in the experiment**.**
8. Adjust the laser power to maximize brightness and **prevent saturation**.
9. Create dot plots and **regions** to identify the cells to collect, or collect all events.
10. Set the **acquisition parameters** including file name, destination folder, number of events and population(s) to collect.
11. Chose **file format**, either .rif (IDEAS), .fcs (FACS software) or both in the file drop down.
12. Collect **compensation** control files with manual settings (*brightfield and 785 OFF and all channels enabled*) or by using the compensation wizard and following the prompts.
13. Continue collecting all experiment files using consistent instrument settings *(In general brightfield will be in channels 1 and 9, SSC ~40mw in channel 6, and the cells to collect R1 using brightfield area vs. aspect ratio to identify single cells).*
14. Save an experiment **template** by selecting Save Template from the File menu.
15. Shut the system off by pressing the **Shutdown** button. The system will sterilize itself in ~40min.

The ImageStream MkII is a class 1 laser instrument.

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