

# 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 load **default template** or 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. **Compensate** data if needed. Data can always be recompensed in IDEAS post acquisition.
  - A.) Load the first single color compensation control sample.
  - B.) Open the compensation manager using the icon in the workspace tool bar.
  - C.) Press the "Collect Control and Compute Values" button.
  - D.) When prompted, "Add Dye Coefficients to Matrix," click OK.
  - E.) Continue with steps A-C for each fluorochrome used in the experiment, this will automatically build the correct compensation values for your experiment.
  - F.) Close the window and the new matrix will be applied to all subsequent data.
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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The screenshot displays the ImageStream Mark-II software interface. At the top, there are control panels for Sample (Load, Return), Acquisition (Start, Stop, Pause), and File Acquisition (Custom Filename, Collect, Channels). The main area shows a multi-panel view of cells across 12 channels. On the right, there are several plots: a histogram of Normalized Frequency vs Intensity\_MC\_CH07, a scatter plot of Intensity\_MC\_CH1 vs Intensity\_MC\_CH2, a scatter plot of Intensity\_MC\_CH02 vs Intensity\_MC\_CH03, and a histogram of Aspect Ratio (M1) vs Area\_M01. The bottom right corner shows the Amnis logo and part of EMD Millipore.

Numbered callouts point to specific interface elements:

- 3: Start/Stop buttons
- 5, 11: File Acquisition section
- 6: Sample Load/Return buttons
- 7, 8: Acquisition Start/Stop buttons
- 9: Channel selection buttons (Channel 1-12)
- 10: Illumination settings (405, 488, 642, 785 nm)
- 12: Main image display area
- 15: Histogram and scatter plots