

Quick Start Guide

Cytek® Amnis® ImageStream®X MkII System Operation

Operation of the ImageStream®X Mk II system with software map on the reverse side:

1. Power up the system (1 instrument, 2 large computer, 3 small computer)
2. Launch the **INSPIRE™** software application.
3. Check to be sure the **buffer containers** are full and the waste tank is empty.
4. Select **Startup** and the instrument will load sheath in ~14min. “Calibrate with Assist” Checked.
5. Or under the Instrument/ Calibrate view, press **Start all calibrations and tests**.
6. When all tests pass, ASSIST light is green and the system is ready to run. **Close view**.
7. Select file and load **default template** or an experiment template from the File menu.
8. Press **Load** and, load an aliquot of a sample with each fluorochrome present.
9. Under Illumination, turn on the appropriate **lasers** for each fluorochrome.
10. Adjust the laser power to maximize brightness and **prevent saturation**.
11. Create dot plots and **regions** to identify the cells to collect, or collect all events. Typically Ch1_Area vs. Ch1_Aspect Ratio is used to eliminate debris, and collect cells.
12. Set the **acquisition parameters**: file name, destination, number of events and region to collect.
13. If needed save .fcs files as well as the image based .rif file.
14. **Compensate** data if needed. Data is normally compensated during **analysis**, however you can select the compensation drop down and load matrix, or create a new one.
 - a. In the wizard’s directory, select the compensation wizard.
 - b. Load the compensation control sample, press next, and verify the channel is correct, next.
 - c. Verify the system finds the correct channel, press next.
 - d. Set the acquisition file name, destination/ population to save, press acquire.
 - e. Press next, and the wizard will cycle back to step B.
 - f. Repeat steps B through E for each compensation control sample. Press exit to save matrix.
15. To manually collect **compensation controls**, turn off Brightfield and SSC, verify all channels are on, and collect 1000 All events (or suitable region) for each compensation control sample.
16. Continue collecting all **experiment files**. *(In general brightfield will be in Ch1 and 9, SSC ~5 mW in Ch6, and the cells to collect R1 using brightfield area vs. aspect ratio to identify single cells).*
17. Save an experiment **template** by selecting Save Template from the File menu.
18. Shut the system off by pressing the **Shutdown** button. The system will sterilize itself in ~40 min.

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The screenshot shows the INSPIRE software interface with the following callouts:

- 4:** Processing Load, Compensation, and ASSIST buttons.
- 5:** File menu and Instrument/Autosampler/Analysis/Compensation/Layout/Advanced/Help sub-menus.
- 7, 13, 17:** Channel tabs (Ch01 to Ch12).
- 8:** Sample area with Load, Return, and Volume/Time indicators.
- 9, 10:** Histograms for Intensity_MC_Ch07.
- 12:** Histograms for Intensity_MC_Ch02.
- 14:** Histograms for Intensity_MC_Ch03 and Intensity_MC_Ch02.
- 18:** Startup, Shutdown, and ASSIST buttons.

The interface includes a top control panel with File Acquisition, Illumination, Magnification & EDF, Fluidics, and Focus and Centering sections. The main display area shows a grid of images and histograms for various channels (Ch01-Ch12). The bottom status bar shows processing load and acquisition details.