## **Quick Start Guide**

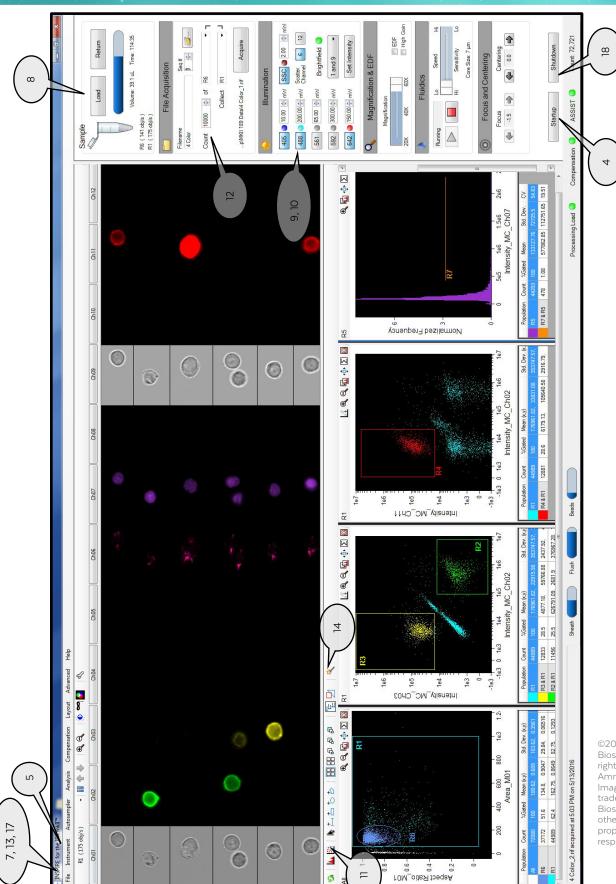
## Cytek® Amnis® ImageStream®X MkII System Operation

## 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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