INSPIRE® Quick Start Guide

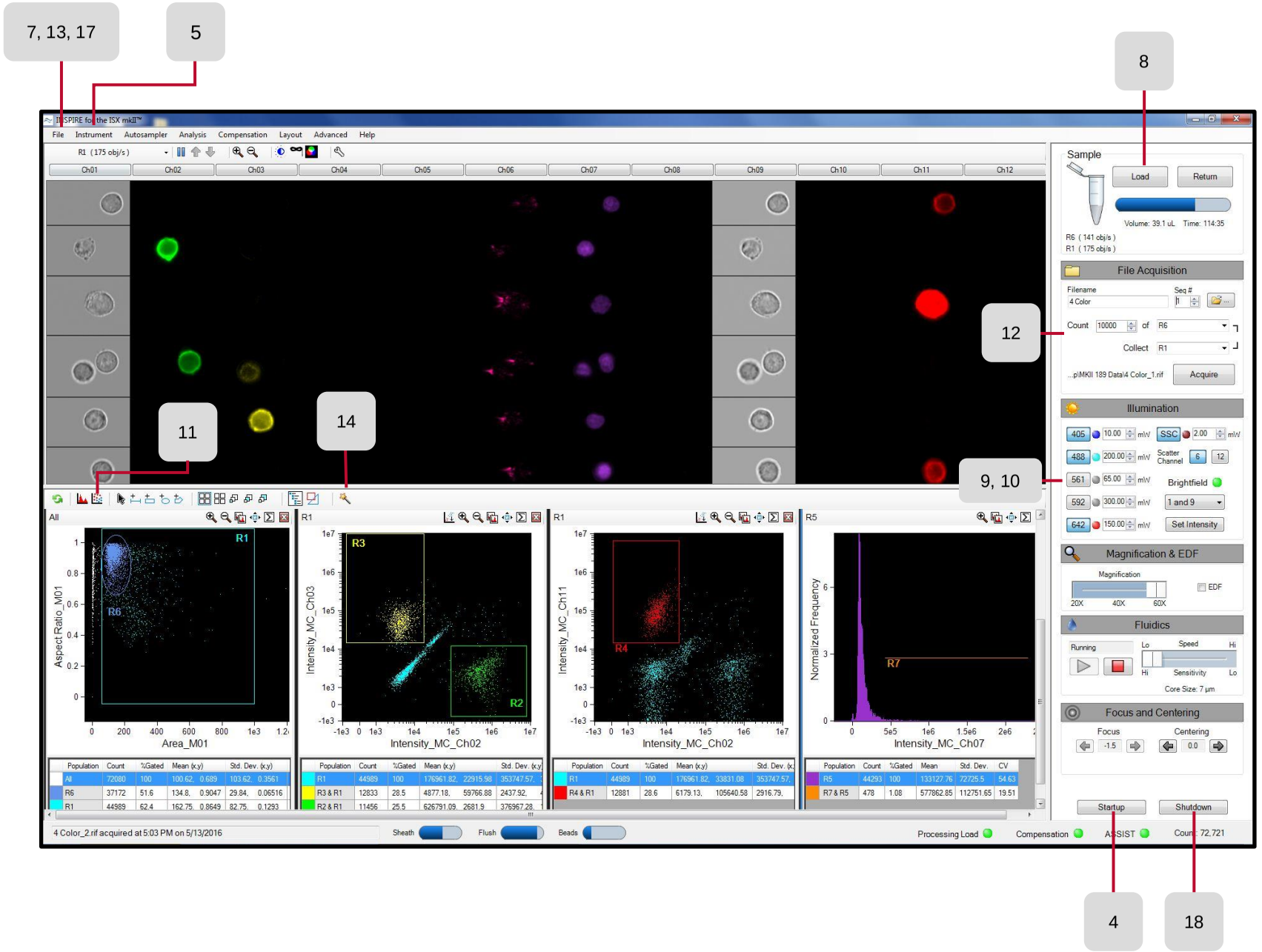
Amnis® ImageStream®X MK II Operation

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

1. Power up the system (1 instrument, 2 large computer, 3 small computer)
2. Launch the **ISX** 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 SpeedBeads and 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.

Luminex
complexity simplified.

780-07541-01, Rev. B



780-07541-01, Rev. B