Quick Start Guide: IS^X Operation

ImageStream^X Operation Quick Start Guide

To begin normal operation of the ImageStream^X:

1. Power up ImageStream^X and launch **INSPIRE™**.
2. Select **Initialize Fluidics**. At the end of this script SpeedBeads should be running.
3. In the file menu, choose **Load Default Template** or manually create. ***
4. In the **Image Gallery** view menu, select **ALL**.
5. Press **Run Setup** to start imaging the beads.
6. Adjust **Core Tracking** to center images laterally (if necessary).
7. Select **Brightfield (BF)** channel. For dual camera systems, BF should be set to channels 1 and 9. For one camera systems, put BF in a channel not used for fluorescence. Click **Set Intensity** to set at **800 counts** (if necessary).
8. Wait until the **Flow Speed CV** is consistently less than **0.2%**.
9. In the **ASSIST** tab, click **Start All** to run calibrations and tests.
10. Press **Flush Lock and Load (FLL)**, and load the *brightest sample* in the experiment, that fluoresces with each fluorochrome used. It's critical that you run this sample first to establish the instrument settings and then DO NOT change them for the entire experiment.
11. In file menu, choose **Open Template** if an experimental template exists, or manually create.
12. Turn on each laser used in the experiment and set the **Laser Power** so each fluorochrome has max pixel values between 100 and 4000 counts, as measured in the dot plots.
13. Select **EDF** collection if desired.
14. Set **Cell Classification** criteria, to eliminate collection of unwanted objects.
15. Enter the **File Name**, **Destination Folder**, set **Sequence #** to **1** and the **Number of Events** to acquire.
16. Click **Run Acquire** to collect and save the first experiment data file.
17. Once acquisition finishes, click **FLL** and load the **next sample**. Make sure that if you are using a DNA Dye that each subsequent sample contains that dye.
18. Press **FLL** and **Comp Settings**, run the **first compensation control**. **Note**: Comp settings turn off **BF** and **SSC**, and enable all channels to be collected. These settings are critical.
19. Reset **Cell Classification** criteria for each compensation control.
20. Using **FLL**, continue collecting the compensation controls with **BF** and **SSC** **off**.
21. If finished for the day, choose **Sterilize System** from the instrument menu.

****Critical Settings:***
BF** in **Ch4** (or **Ch1&9** for dual camera system) @ **800 counts**; **785 Ex laser** @ **2mW**; Ch1-12 **stage setting** = **256**; **Percent Beads** = **100**, **Diameter** = **6um**, **Velocity** =**60mm/sec**, **camera mag**=**40x

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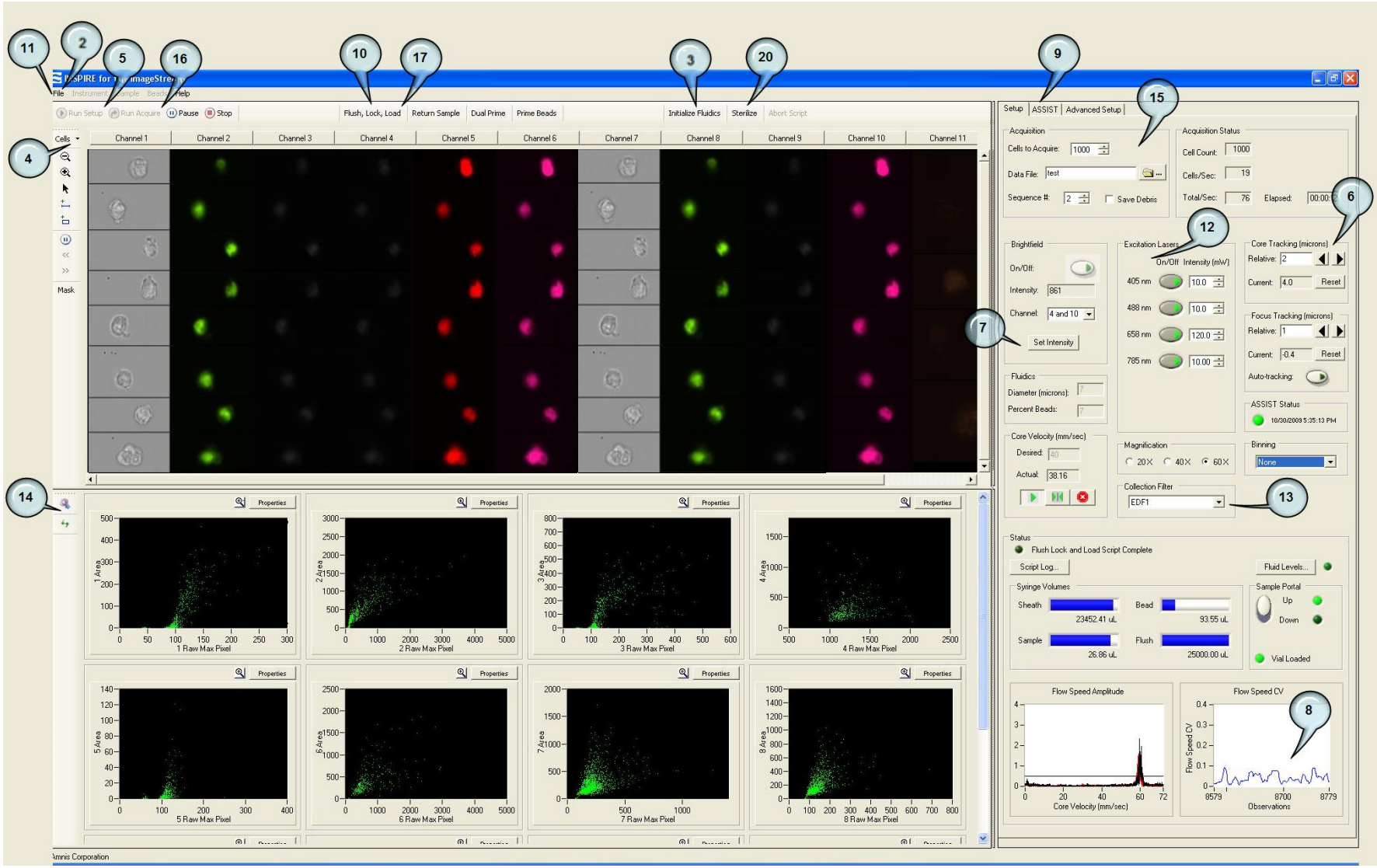

1. Launch **INSPIRE**
2. Click **Initialize Fluidics**
3. **Load Default Template**
4. select **ALL** in **Image Gallery**
5. Press **Run SetUp**
6. Adjust **Core Tracking**
7. Set **Brightfield (BF)**. Click **Set Intensity**
8. **Monitor Flow Speed CV**
9. In the **ASSIST** tab, click **Start All**
10. **Flush Lock and Load (FLL)** to **Load sample**
11. **Open Template**, if applicable
12. Set the **Laser Power**
13. Select **EDF** collection if desired
14. Set **Cell Classification** criteria
15. **File Name**, **Destination Folder**, **Sequence #, Number of Events**
16. Click **Run Acquire**
17. Click **FLL** to **Load next sample**
20. If finished for the day, choose **Sterilize System**

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