Instituto de Medicina Molecular Lisboa

IMAGE STREAM TROUBLESHOOTING

Flow Cytometry

1. **No flow speed signal**
    a. System has not been primed Prime beads or dual prime.
    b. Pumps are not running: Prime beads or dual prime.
    c. Pumps are empty If sheath syringe is empty, load sheath, then dual prime. If the bead pump is empty, load beads, then dual prime.
    d. Cell concentration is too low Make sure the sample concentration is between 10^7 (15cells/sec) and 10^8 cells/mL (23cells/sec.).
2. **Flow speed is not stable**
    a. The sample is clumpy or is not at a high concentration Filter and/or concentrate the sample
3. **Unstable fluidics (air or clog in system)**
    a. Air bubble in the flow cell Run the **Purge Bubbles** script. If that does not work, repeat it. If that doesn't work, return your sample and run the **Sterilize** script (Instrument dropdown menu).
    b. Sheath incompatibility with sample Verify you are using the appropriate sheath solution (refer to the Preparing the ImageStream for operation chart). For experiments using beads or containing surfactants, dH2O sheath is optimal. For running cells, Ca^{++}/Mg^{++} free PBS is optimal.
4. **Event rate slows over time**
    a. Cells have settled in the lines Stop and save the acquisition. Turn **Sample Agitation** on if it was off. Run the **Super-agitate Sample** script. Dual prime, re-establish the core
    b. Air bubble in the flow cell Run the **Purge Bubbles** script. If that does not work, repeat it. If that doesn't work, return your sample and run the **Sterilize** script (Instrument dropdown menu).
    c. Sample syringe is empty Flush sample, load the next sample and dual prime.
    d. Sheath syringe is empty Load sheath, the dual prime.
5. **No images**
    a. Camera is not running Click **Run/Setup.** If the camera is already running, click **Stop** to stop the camera, and then click **Run/Setup**.
    b. Image is paused Click Resume
    c. Insufficient Illumination Make sure the excitation laser is turned on and set to the proper intensity setting. Confirm the brightfield lamp is turn on and set to the proper intensity setting.

d. Cells are not centered with objective's field of view Manually find the core stream, track left or right in 10 µm increments to find the core, then manually adjust focus.

6. **Imaging is intermittent or appears frozen and object acquisition rates are erratic**

   a. Sample concentration is low Make sure the sample concentration is between 10^7 and 10^8 cells/mL

   b. The sample is too concentrated The maximum sample concentration is 4-5x10^8 cells/mL, with the recommended concentration 1-10 x10^7 cells/mL

7. **Cell Classifiers are not working**

   a. Cells are classified as debris In the cell detection window either click **Ignore all enabled** or turn all classification parameters off and toggle image view mode to Cells. Set parameters to include desired cell images.

   b. Cells are classified as beads Set the Bead classification parameters (**Advanced - Acquisition** tab) to make sure they are set to include beads but not cells in the Beads view

   c. Image view mode is set to debris Toggle Image view mode to Cells or Beads

8. **Everything is out of focus**

   a. Camera synchronization setting is incorrect Recalibrate the camera synchronization using ASSIST or manually set it to a value that has previously worked (Advanced – Flow Speed tab)

9. **Cross-contamination from previous samples**

   a. DNA dye from previous sample is labeling current sample Run Manual Flush Sample script with 10%bleach.