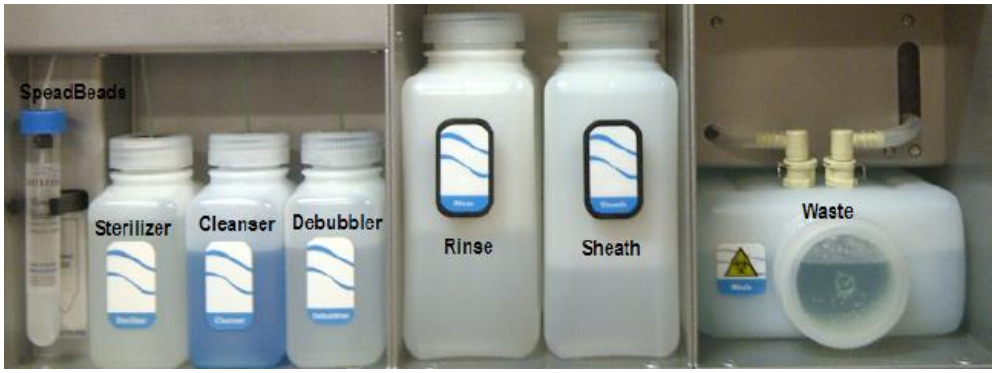
Table of Contents

1. Fluidics



- ***SpeedBeads***: monitor and synchronize the flow of the sample and maintain focus and core tracking
- ***Sterilizer*** = FACSClean
- ***Cleanser*** = Coulter Clenz
- ***Debubbler*** = 70% Isopropyl alcohol
- ***Rinse*** = Deionized Water (diH_2O)
- ***Sheath*** = 1X PBS (Phosphate-buffered saline), 0.1 nm filtered

2. Startup

- First Person of the day will ***Startup-*** Initializes fluidics by flushing sheath and loading beads ~14min
- Select "Run ***ASSIST*** (**A**utomated **S**uite of **S**ystemwide **I**mage**S**tream **T**ests) after initialization" Calibration and testing using SpeedBeads ~20min
- If instrument is already started, ***Start Fluidics*** to ensure Focus and Centering is valid using the SpeedBeads

3. Acquisition

- File > ***Load default template*** or experiment template.
- ***File Acquisition***: set path and number of objects to collect
    a. Always save to the Desktop initially then copy to the J: Drive ONYEN folder
- ***Illumination***: turn on the appropriate lasers.
    a. Begin with all lasers at max output (hover over input box to view range of power available per laser)
    b. Keep SSC power between 3-5 mV

- ***Magnification***: select the 20X, 40X or 60X objective.
  a. If viewing internal components of the cell, need to run 60X objective
  b. 20X= 1 um/pixel
  c. 40X= 0.5 um/pixel
  d. 60X= 0.3 um/pixel
- ***Fluidics***: Select appropriate Speed/sensitivity for your experiment
  a. If viewing internal components of the cell, need to run on Lo speed/Hi sensitivity
- Click ***Load*** and place a fully-stained sample on the uptake port.
  a. Note 15 uL is the minimum amount of liquid uptake
- Adjust the laser powers to maximize signal and prevent saturation.
  a. Use *Raw Max Pixel_MC_ChX by Area_MChX* to determine saturation. Max output is 4000.
  b. Be careful to closely look at axis labels as the scales will automatically update. This can be overridden by R-click Graph> Graph properties> Scaling> Manual > Maximum= 4000 (make sure scale is linear for Raw Max Pixel)
- Create plots and gates to identify the cells to collect:
  a. Cells in Focus: Gradient RMS_M01_Ch01
  b. Singlets: Area_M01 vs. Aspect Ratio_M01
  c. Phenotyping (signal intensity): use Intensity_MC_ChX
- ***Compensation*** > Create Matrix… or click on the Wizards icon () and follow the prompts.
  a. Note: Single stained samples must be collected WITHOUT brightfield/SSC channels
  b. Collect at least 1000 positive events
    i. Compensation wizard is looking for ONLY positive events, so it is preferable to save only events in the positive gate defined.
  c. Run PBS between samples if worried about residual sample
  d. Run DNA dye last
  e. Beads are not sufficient to compensate, must use cells
- Collect all experimental samples (return the remaining sample).
- File > Save Template.

4. Shutdown

- Between users:
  a. FACSClean (3 minutes)
  b. diH_2O (3 minutes)
- Last user
  a. Click ***Shutdown*** (sterilizes the instrument ~43min)
  b. Select "Shutdown after sterilize" (powers off all system components)
  c. Do not exit program

Note: Sample concentration $1x10^7$-$1x10^8$/mL.
Note: BF usually is set to ch1&9, DF (ch6 – SSC) should be between 3-5 mW, and single cells are visualized with a BF Area vs. Aspect Ratio.
Note: If a sample without DNA dye follows a sample with DNA dye, Load FACSClean followed by 1X PBS for a minute each.

5. Data Analysis Tips

- Sample Export File Types:
  - ***a) .rif = raw image file***
  - b) .ctm = compensation matrix
  - c) .cif = compensated image file
  - ***d) .daf = data analysis file***
  - e) .ast = tempate file
    - i) Use for batch Analysis
- F1 in IDEAS will bring up user manual; type to search

6. References

- ImageStreamX ® System Software User's Manual Version Mark II, January 2013.

7. Revisions

SOP Version Number	Date	Tracked Changes (clearly list changes made & why)	Employee
UT SOP004.2	1/25/2023	General Updates throughout to aid in user training	Ayrianna Woody