UCMERCED

Stem Cell Instrumentation Foundry

SOP for Flow Cytometer Standardization

Date Last Modified: 2/19/2020

Author: David Gravano

Purpose

Instrument standardization is a critical process to ensure experimental reproducibility and data reliability. SCIF daily quality assurance (CS&T for FACSDiva instruments) ensures instruments are operating within specifications, but does not guarantee fluorescent readings will be comparable between all your experiments. In order to make sure your fluorescent values are comparable between experiments and rule out the instrument as a source of experimental variability the SCIF provides fluorescent beads that should be run by the user following this SOP.

Materials provided by the SCIF – Spherotech mid-range rainbow fluorescent particles – 1 peak

Procedure

1) Set up your experiment with all proper voltage parameters for your samples. Do compensation, etc. Run your samples and record them as normal. This experiment will serve as the baseline.
2) Once all samples are run, turn off compensation for the next tubes you are about to record (uncheck compensation in the compensation tab). Select QC beads to utilize (or use beads provided by SCIF) and note the LOT#. It's recommend to use our mid-range fluorescent beads or a multi-peak bead that will have a peak near the fluorescent intensity of your samples. Make sure compensation is turned off before recording anything at this point.
3) Create a new Global worksheet and rename it Standardization QC so you can record and track the beads in every subsequent experiment. Gate on the beads in a FSC vs SSC plot and generate a histogram for each fluorescent channel. Note, the beads may be off scale for SSC as they do have very high SSC relative to cells. If using multipeak beads, gating on a bead fluorescent peak near 10^5 is recommended. Make sure to note which peak it is. For example, if using 8-peak beads make sure you know which peak is your target population. This may be different for each channel. Using mid-range single-peak beads the SCIF provides highly simplifies this process.
4) To determine your baseline target fluorescence values for each parameter in your experiment, record the sample while running on low flow rate. Record at least 5000 beads. Create a statistics view (Populations → Create Statistics View) to document the mode fluorescence intensity value of the mid-range beads in each channel (geometric mean and/or median can also be used, but mode is very good at determining the bead peak value). To edit the statistics view window and show the stat you want, right click and select "Edit Statistics View". This is all you have to do for the first experiment and it will establish a reference point for the instrument for future experiments.

5) When setting up your next experiment, you will have your target mode fluorescence intensities from your baseline experiment. Duplicate your first experiment without data. Note the mode target values from your baseline experiment. We suggest making a printout of these values to reference every time you perform an experiment. Make sure compensation is turned off for these QC tubes. Under the first tube of this experiment, run the beads, and record the “preset” tube (before any voltage adjustments. These would have the exact voltages as your first experiment). Check these “preset” mode fluorescence intensity values to the baseline target values for each channel. If they are off by more than 10% you’ll need to adjust voltages. It is handy to calculate the allowable range (eg. +/- 10% of baseline) for quick reference.
6) For the next tube, adjust the voltages on FSC, SSC, and the appropriate fluorescent channels to match the baseline target values. Once set, record a “match Baseline” tube after you hit the mode fluorescence target values. Under your compensation controls, make sure the voltage settings match those of the tube you just recorded on all parameters. The machine has been standardized at this point. Turn compensation back on and move on to performing compensation as usual. Note, if your mode intensities are off by more than 10% and the instrument needs adjustment, please notify the SCIF. This could indicate an issue with the instrument or be reflective of a recent change with the instrument such as laser alignment. Also note, this does not control for variability in your data due to pipetting, antibody dilution, cell concentration. It only removes variability due to changes in the cytometer.
7) Run all your samples. When finished, record a tube of the beads (compensation turned off) as a final “post” tube with the settings at the end of the experiment, so you can check to see if the machine experienced any potential drift or issues during the run. Remember to make sure compensation is turned off for any QC tube involving the beads.
8) If you are running out of a lot of beads, you can reset your mode intensity target values by first normalizing the machine to the baseline values following the steps above with the old beads. Make sure compensation is turned off. Once machine is normalized to baseline, record and note the new lot of beads under “reset target values” tube and document the new target mode fluorescence intensity values for the peak of interest. For any future experiment, you can use these new target values with the new beads to standardize the machine accordingly back to its original baseline.