17 April, 2023 Research Flow Cytometry Core Issue 8

Quarterly Newsletter

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Information Provided by your CCHMC RFCC

MFI and Optimal Voltage Settings for Increased Sensitivity, Rigor and Reproducibility

An important step in flow cytometry is setting the proper voltages on the photomultiplier tubes (PMTs) to detect the fluorescence in the samples. If the PMT voltage is too low, dim fluorescent signals will be indistinguishable from noise and cannot be resolved. If the voltage is set too high, bright fluorescent signals will fall out of the linear range of detection and will be inaccurate. An optimal PMT voltage maximizes the separation between negative and positive populations and provides the best resolution of populations in your experiment.

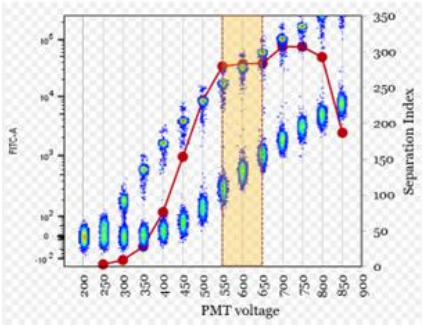


Figure1. Voltration experiment results using a mixture of negative and FITC-labeled beads to optimize PMT voltage. (UTH, 2008, PMT Voltage Optimization.)

To optimize the voltage settings and maximize assay performance, a stained sample or multicolored beads are run at increasing voltages on each detector. This is a voltage walk, voltration, or voltage titration. A separation index between positive and negative populations is calculated at each setting (Figure 1, red line). The optimal setting is the minimum voltage at which the separation index reaches a plateau (Figure 1, yellow highlight).

Optimized voltage settings for all BD sorters and the Bigfoot in the RFCC have been determined using human PBMCs. We use hard-dyed rainbow beads to reproduce the same target median fluorescence intensity (MFI) value each day to correct for expected variations in instrument performance. This eliminates the need to manually adjust voltages.

Increasing the voltages above the target MFI increases the spreading of the population that could then encroach on a population of interest (Figure 2 & 3). Calculating the optimal voltages for each instrument and targeting the MFI for each experiment eliminates inconsistencies due to personal biases and variations between sorts done on different days or by different lab members. Optimal voltage selection is essential for the best panel performance, and the most reliable, reproducible results.

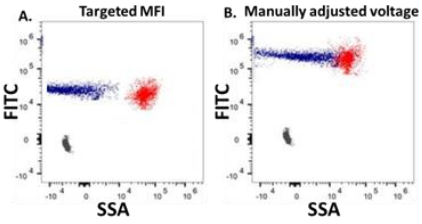
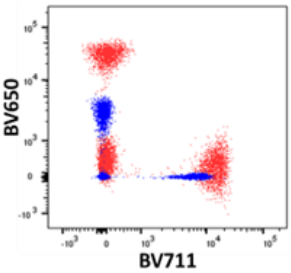


*Figure 2. Spreading occurs at voltages above the target MFI. **A.** At the target MFI, the single positive (blue) and double positive populations (red) are separated. **B.** As the voltage for FITC increases, the spreading increases and it is not possible to separate the double positive population from the single positive population.*

Figure 3. There is more spreading of the red populations obtained with CST settings on the Fusion, compared to the blue populations obtained with optimized settings using target MFIs.



Note for Aurora: Optimized gain settings for human PBMCs are built in and are automatically applied when "Cytek assay settings" is chosen.

Core Updates

Updated Policies and Forms

We have updated policies and forms on the RFCC website. Please take note and share with team members.

- **RFCC Analyzers & Sorters Billing Policies**
- **RFCC Cell Sorting Policies**
- **Training Requests:** Please see our website for training requirements and Stratocore for requesting *Analyzer Training* and *Cell Sorter Training/Consultation* under the request tab on Stratocore
- **Estimating Sort Time document.**

Bigfoot Sorter

The Bigfoot sorter, Jet-in-Air sorter, is open for **staff assisted** and autonomous sorting. This sorter is gentler on delicate or vulnerable cells. The Bigfoot has the ability to do large panels, sort 6 populations, use tubes or plates.

Analyzer Shut Down

Please check the Stratocore schedule while cleaning the instrument after your experiment to know if you need to shut down or leave it on. Others may sign up or cancel requests on Stratocore while you are running your experiment.

If you sign up or cancel time for an analyzer on the same day on Stratocore, please consider contacting the user that is utilizing the instrument before you. Inform them to not shut down the instrument and to keep it running for you; or you have cancelled, and they will need to shut down the instrument if no one else signs up.

RFCC Staff News

Congratulations Kenneth Quayle

His abstract has been chosen for an oral presentation at the upcoming CYTO conference in Canada. Also, Ken has become an American Society for Clinical Pathology (ASCP) Certified Specialist in Cytometry.

Dates to Note

Apr 19, 9-10am, S6.125: High Parameter meeting. Andrew Cox, MD, PhD, "Confirmation of scRNAseq "hits" by Spectral Flow Cytometry: analytical challenges."
Apr 26, 1-2pm, S6.125: ORVCA meeting. Lois Kaminski, PhD. Slingshot Biosciences: "Revolutionary on Demand Cellular Controls: Cell Mimics that Match your Targets."
May 17, 9-10am, S10.130: High Parameter meeting
May 20-24: CYTO 2023. Montreal, Quebec, Canada
June21, 9-10am, S6.125: High Parameter meeting.
June 28, 1-2pm, S6.125: ORVCA meeting