Flow Post-its
Kathy Daniels

Optimizing Voltages & Gains

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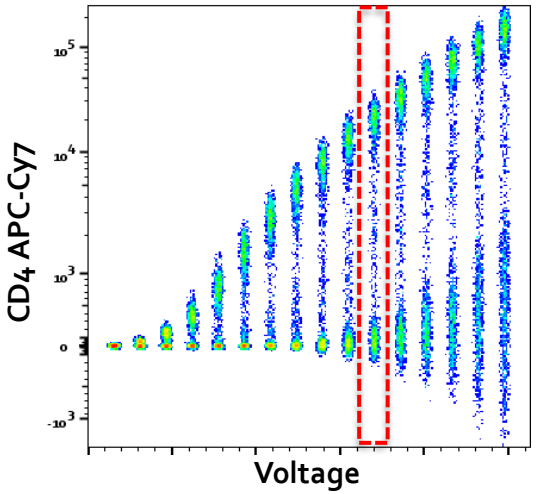
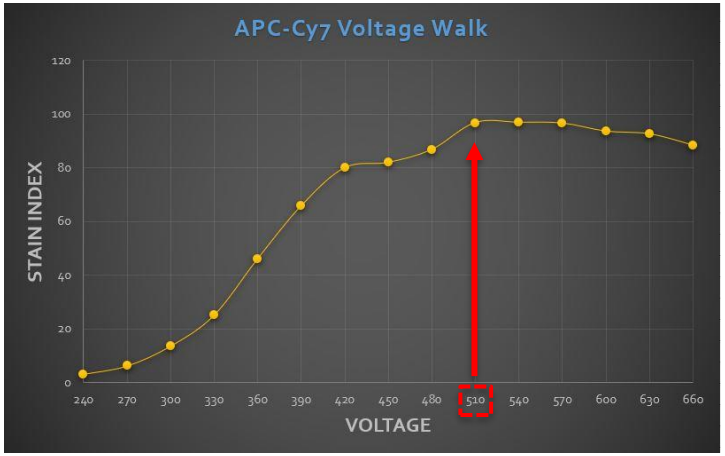
Setting PMT voltages or APD gains appropriately is a critical step for a successful flow cytometry experiment. If voltages/gains are set too low, it can result in loss of resolution of populations, especially for populations that have a very low level of expression. Alternatively, if set too high, populations may be off scale resulting in inaccurate statistics. Carrying out a **voltage/gain walk up** is one method to determine the **minimum PMT voltage or APD gain** that will provide **the best resolution** of populations in your experiment.





Lymphocytes were stained with CD4 APC-Cy7 and ran on a BD Fortessa. FSC and SSC settings remained constant while APC-Cy7 detector was increased in increments of 30V until signal was off scale. Stain Index (SI) was calculated to determine optimal voltage.

At a voltage of 510V, maximum SI was seen. Increasing the voltage does not benefit the data, but reduction of voltage can result in loss of population resolution.

Helpful Hints

- Optimized settings are instrument specific. Cross-instrument standardization will need to be done in order to have comparable settings across multiple instruments.
- Alternative methods to determine optimal starting voltages/gains are available. i.e. "Peak 2 method"
- Voltage walks help ensure that the autofluorescence is above electronic noise for your experimental samples. When working with cell types with differing autofluorescence, experiment specific voltage walks should be done.
- *Suggested Reading:* Mair, F. and Tyznik, A. 2019 doi: 10.1007/978-1-4939-9650-6_1

Stain Index*

$$\frac{MFI_{pos} - MFI_{neg}}{2 * rSD_{neg}}$$

*Calculated using the Stain Index Plugin in FlowJo v.10.7.2

When setting up your flow cytometry experiment, consideration should be taken at the instrument to optimizing the acquisition settings. **Through a voltage walk up and data analysis, the voltage or gain at which the optimal separation of populations occurs can be determined.** Settings will differ across experiments when working with cells that have different levels of autofluorescence, requiring a separate optimization.

Memorial Sloan Kettering Cancer Center
Flow Cytometry Core Facility
https://fccf.mskcc.org
Whitehead Institute