Flow Post-its
Kathy Daniels

Threshold

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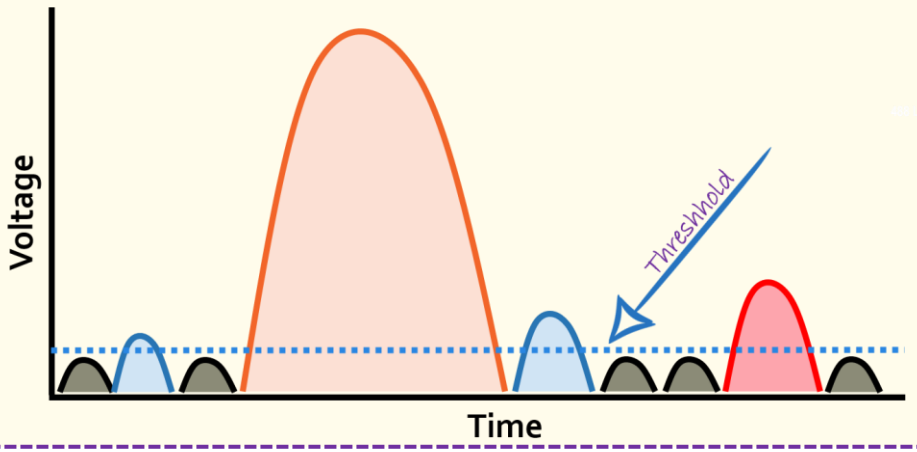
When acquiring data on a flow cytometer, the instrument operator must adjust multiple settings in order to **best resolve** the **populations** of interest. In addition to setting appropriate scatter and fluorescent voltages/gains, **the threshold must be optimized,** dependent on your application of interest, to ensure you are seeing all events of interest and that you will have a successful experimental run.



The ***threshold*** (or trigger) is the minimum voltage pulse signal that must be met in order for an event to be recognized by the cytometer.

Setting the threshold too low can result in a high level of **electronic noise** and unnecessarily large fcs files. A threshold that is too high can prevent you from seeing populations of cells.

Adjusting the threshold so that you can see some level of **debris**, **dying/dead cells** and **cells of interest** is important.

Threshold is typically set on forward scatter, but this can be modified to SSC or fluorescent detectors based on different applications such as small particle detection or cell cycle.

Example to the right shows an example of the impact of increasing threshold (set on forward scatter) on the same sort sample. When set too low (a.), the plot shows a high level of noise and can negatively impact sorting efficiency. When set appropriately (b.) we can still see debris and dead cells in addition to the gated cells of interest. If set too high (c.), it can skew viability percentage and unwanted events can be sorted as they are ignored by the instrument.