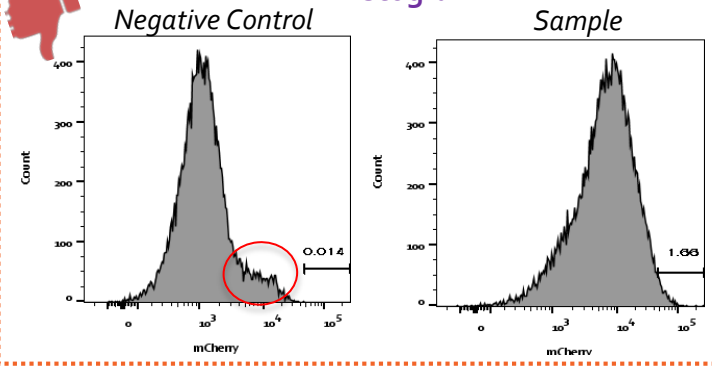
Flow Post-its
Kathy Daniels

Histograms or Dot Plots?

June 2021

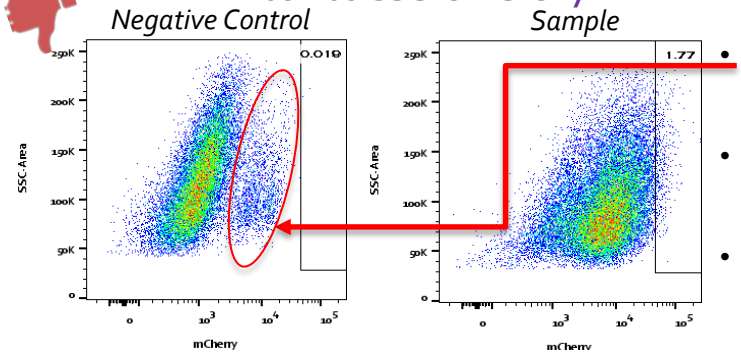
Gating strategies are used in flow cytometry experiments in order to **identify populations** of interest by including or excluding subsets. The choice of **detectors** used to acquire along with the **plot types** used to display the data may have an impact on identifying populations.

Histogram



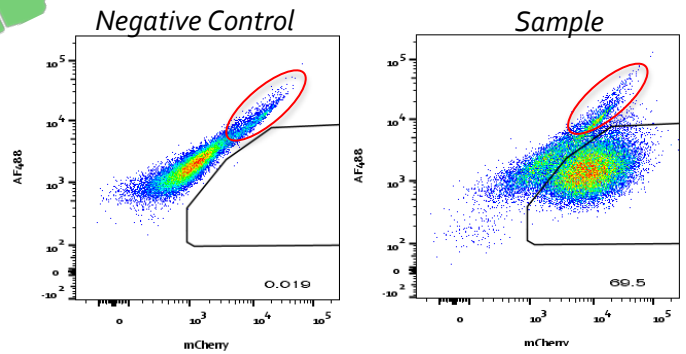
- Univariate display of data.
- *Unable to resolve distinct populations.*
- Using the gate set on negative control, only 1.66% of cells are considered positive.

Dot Plot: SSC vs mCherry



- Bivariate display of side scatter vs mCherry.
- Highly autofluorescent population seen in negative control.
- Unable to resolve autofluorescence from positive cells.
- Using the gate set on negative control, only 1.77% of cells are positive.

Dot Plot: AF488 vs mCherry



- Bivariate display of AF488 vs mCherry
- AF488 detector left open (488nm excitation, 530/30 bandpass filter) as it is an optimal channel to detect autofluorescence.
- Positive gate was set on the negative control taking into account the highly autofluorescent population.
- Using this plot and gate, the **mCherry positive cells are clearly resolved** from the autofluorescence only cells and a more accurate population percentage of 69.5% is observed.

In the example shown above, the same negative control and experimental sample is shown using different options to display the data. The **choice of plots and detectors have a huge impact on identifying of populations**. By utilizing detectors off of the UV, Violet and Blue laser lines that collect light between 400-600nm, we can account for high autofluorescence cells to better identify populations of interest.

We thank Dr. Tobiloba Oni for providing these fcs files for educational purposes.

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