Titrating antibodies

How it works

Slapping together cells and antibody isn't ideal when performing measurements on a sensitive machine like a flow cytometer. Your day-to-day results will vary and this practice is likely to compromise the conclusion of the experiment.

The easiest way to optimise a flow experiment is to perform a titration of the assay's antibodies for a given cell number. **You only need to do this ONCE for a given antibody:cell-type**. You can use this information as a starting point when performing the assay on another cell type.

Consequence of skipping

Single colour assays

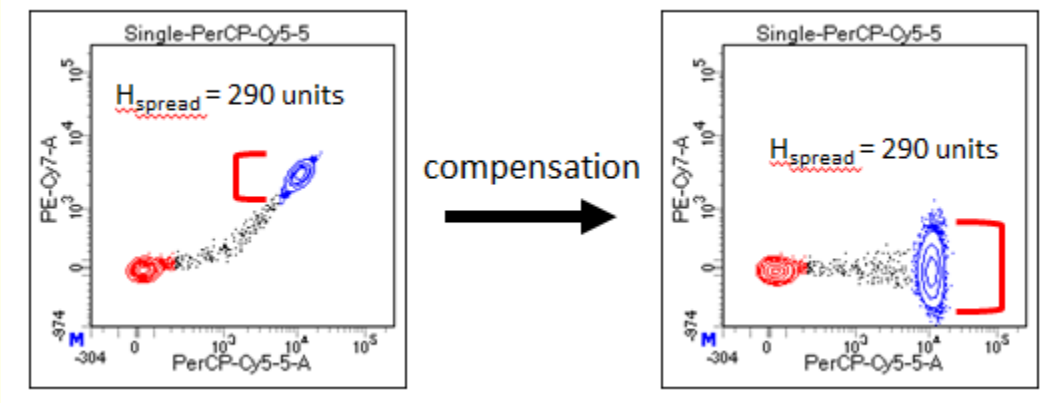
- Can rescue sample by adjusting voltage for high, **but not low** signals.
- Enhancing voltages to correct dim staining will also increase background fluorescence

Multi-colour assays

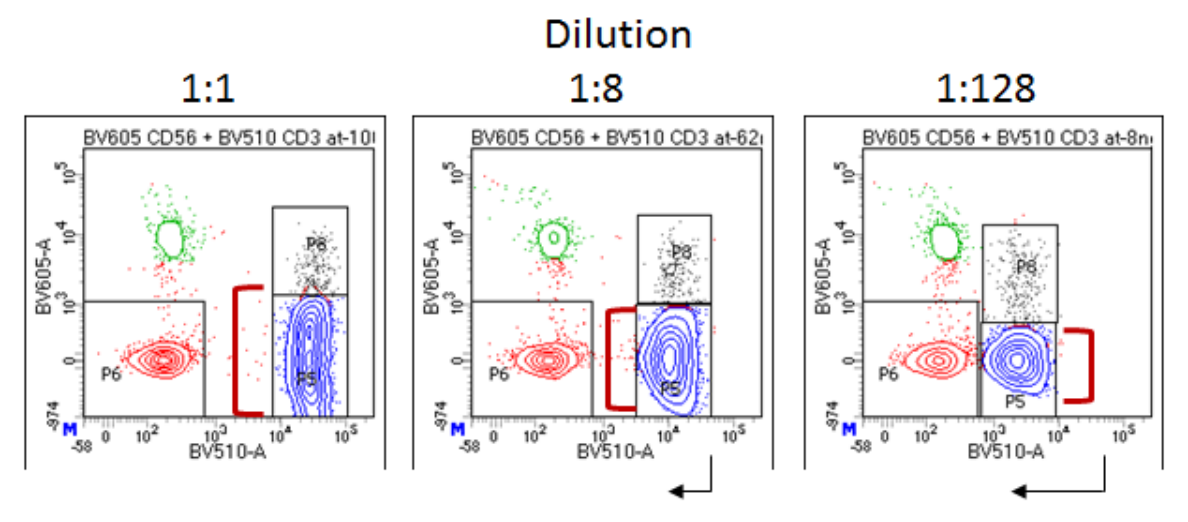
- Proprietary assay
  - following the manufacture's protocol should be enough – they did the optimisation for you
- Homebrew assay
  - /!\ You can not adjust voltages for a multicolour assay after you setup your compensation controls.
  - High risk that discrete cell populations aren't discernible in your assay
    - Suboptimal (dim) staining: positive cells spill into negative cells
    - "over" staining: increase data-spread after compensation > negative cells spill into positive cells
    - FMO controls can help to show spread effects, but prevention (or spread minimisation) can be better than a cure.

Next page; all about spread.
Then, flowchart.

Spread



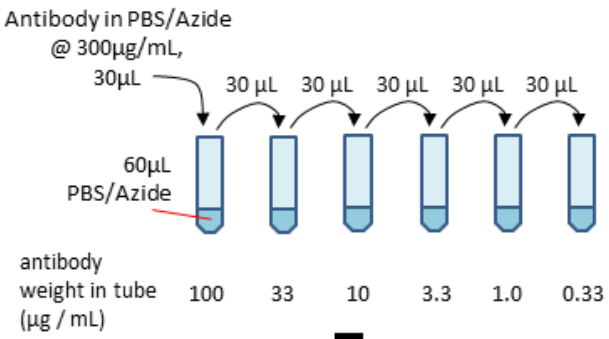
As seen in the data above, the data spread (H-spread) retains the same magnitude after compensation is applied. This may cause issues for dim positive populations overlapping with the neg-population.



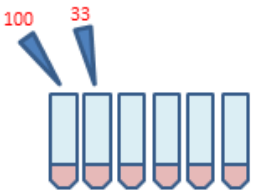
Titration can reduce H-spread and make it easier to distinguish dim populations.

Data kindly prodived by Andrew Lim (Becton Dickinson)

Next page, flow chart



Add an equi-number of cells, 50µL ($4x10^6$/mL) into 6 tubes (prepared ready for staining)

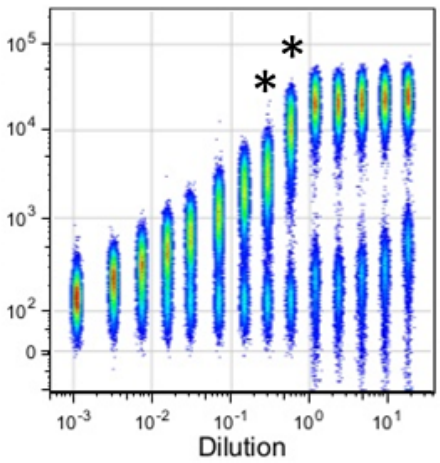


Add 10µL of the antibody dilution in to a tube.

Wash cells in 3mL PBS/azide

Resuspend in 200-500 µL volume

Run samples on cytometer



This mock data shows the ideal antibody titration (asterisks).

Largest separation without increasing background.

You would consider the left-asterisk dilution when trying to reduce spread in complex assays.

Flow data from:
Pratip Chattopadhyay, CYTO 2015