Compensation: Choosing between Automatic Compensation and FlowJo

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Written: 1/4/18

Automatic compensation on the machine with BD FACSDiva

- Pros
  - It's automatic!
  - You can look at your samples while they're running because they're compensated
  - **Best if you aren't familiar with compensating**: if you set your voltages incorrectly, there's an error message
  - **Quick for panels with only a few fluorophores**
  - Can apply compensation from other experiments
- Cons
  - you can't use a mixture of cells and beads for compensating
    - "negative" peaks all come from the unstained sample – negative and positive peaks must come from the same type, either all cells or all beads
    - If you're using GFP+ cells as a control, you can't use automatic compensation unless all of your single stains are cells
  - If you want to manually fix or adjust the compensation, it can be more difficult
  - If you want to check that the automatic compensation is correct, it's more difficult and time consuming to do than in FlowJo
  - Takes more time on the machine, which you are being charged for!

NOTE: It is possible to change FACSDiva's compensation in FlowJo.

Compensating in FlowJo – PREFERRED METHOD

- Pros
  - Provides similar results to BD FACSDiva
  - More flexibility than FACSDiva
    - Can easily check that the calculated compensation is correct
    - Can manually adjust the compensation after the automatic calculation
    - Can use a mixture of beads and cells to calculate compensation
  - Spend less time running samples on the machine (it's cheaper and you can take as long as you want in FlowJo!)
  - Can apply compensation from other experiments
- Cons
  - **You have to know what you're doing**: if you set the voltages wrong, then your entire experiment could be unusable
  - You can't interpret the data is it's running because it's uncompensated when you're collecting
    - If you are nervous about running your experiment uncompensated, you could try to collect some cells, export the data, and look at the sample compensated in FlowJo before continuing to run all of your samples (quickest if you can just apply a pre-calculated compensation to the sample)