Flow Post-its
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"Recycling" Compensation

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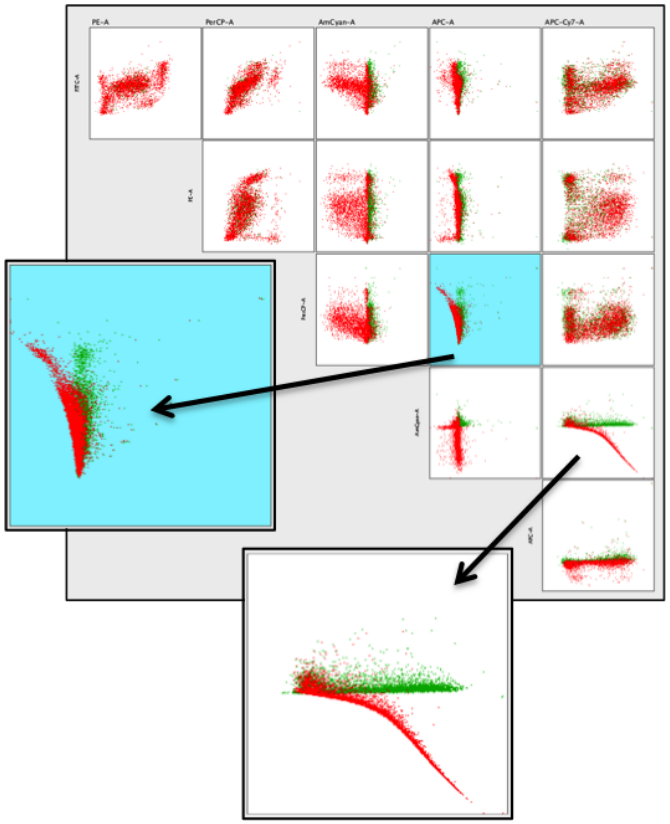
One of the most critical components to a successful flow cytometry experiment is the use of the **appropriate controls**. Single color controls allow for us to compensate or unmix data in order to correct for **spectral overlap,** enabling us to confidently identify our populations of interest. These controls should be freshly made and run at each experiment to account for any changes in the instrument or reagents*. **Reusing old compensation matrices from old experiments will lead to incorrect data**.

Compensation Overlay



Compensation Overlay

- Overlay of **Recycled** and **Same Day** compensation show clear discrepancies. If not corrected, MFI values, population percentages and data interpretation would be incorrect.

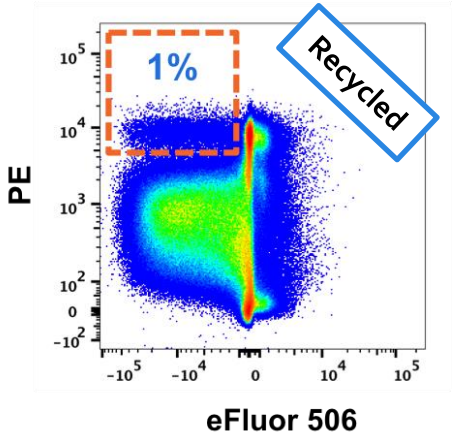
Recycled Compensation

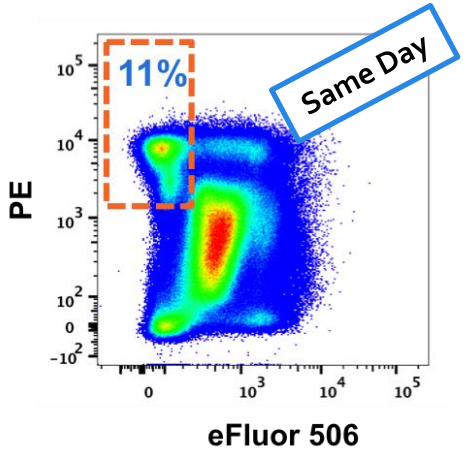
- Extreme negatives seen. This is typically indicative of overcompensation.
- Poor resolution of populations.
- Gated population shows 1%.

Same Day Compensation

- No extreme negatives are noted.
- Resolution of populations shows significant improvement.
- Gated population now shows 11%.

**Instrument drift*:* laser drift or replacement, flow cell degradation, changes in optical filters, etc.
**Reagent drift*:* Tandem dye degradation, experimental signal intensity change (i.e. expression level increases), etc.





Flow Cytometry Core Facility

https://fccf.mskcc.org

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