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

- 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.