Flow Post-its
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Spillover Values Over 100%

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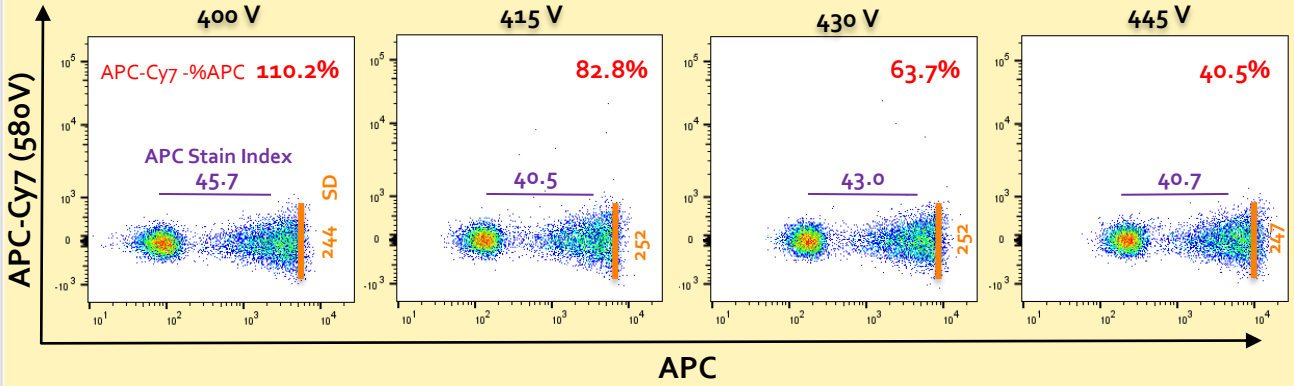
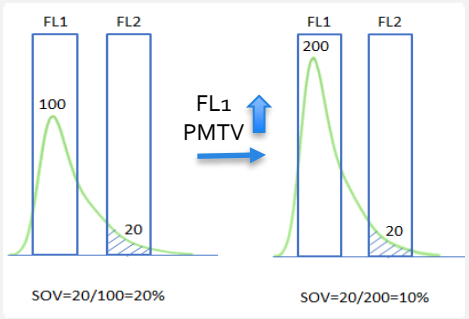
Calculating the compensation matrix is essential when performing a multicolor flow cytometry experiment to correct for fluorescence spillover in each individual channel. There is a misconception that spillover values (SOVs) must be under 100% for "correct" compensation.



In this experiment, samples and single stained controls were acquired at 4 different PMT voltages (PMTVs) for APC: 400 V, 415 V, 430 V and 445 V, with the APC-Cy7 PMTV set at 580 V. All PMTVs are in optimal conditions, i.e., providing maximum resolution.

In conclusion, increasing the primary PMTV for APC does lower the SOV but does not improve the **resolution** or change the **spread in the secondary channel**.

Understanding the concept



$$SOV=\frac{Signal_{FL2}}{Signal_{FL1}}$$

SOVs are determined by the ratio of the measured signal in the secondary channel relative to the primary channel. When The FL1 PMTV increases, only signal amplification in the primary channel (FL1) changes, therefore reducing the relative spillover. If PMTVs are already optimized, there is no impact on resolution or spread.

SOVs are a function of the emission spectra of each fluorochrome, the wavelength range of the filters being used, and the relative gains of each detector. In other words, **SOVs over 100% are not an indication of "bad" compensation,** and the absolute values should not be modified in an attempt to improve the quality of the data**. If anything, attention should be focused on improving resolution and spread, which is a consequence of instrument and reagent performance, as well as optimal panel design.**

References Roederer, M. (2002), doi: 10.1002/0471142956.cy0114s22; Mair F, Tyznik AJ. (2019). doi: 10.1007/978-1-4939-9650-6_1

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