Flow Post-its
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Myths: Spillover Values vs. Spread

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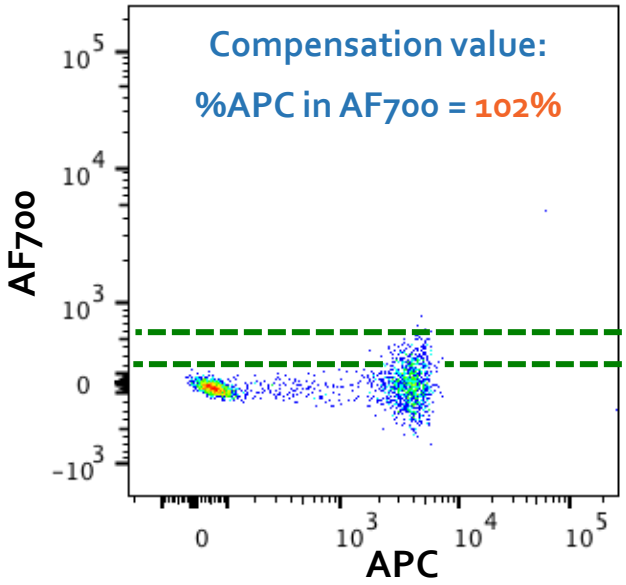
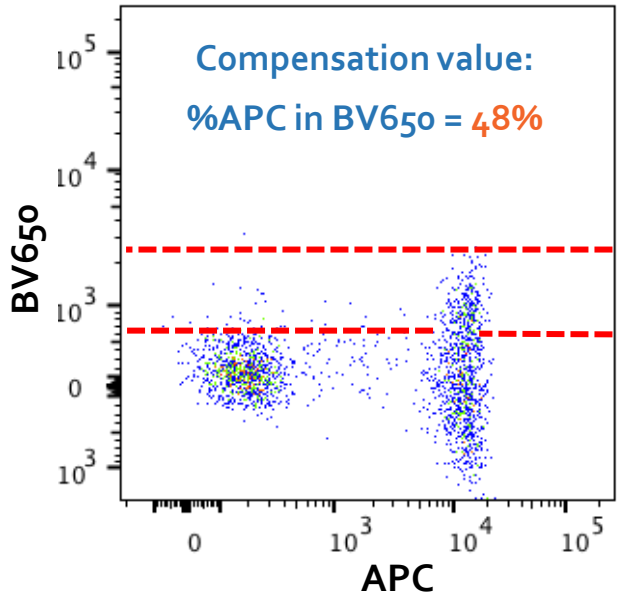
In multicolor Flow Cytometry experiments, **Spillover Spread** translates into the width of the positive population in adjacent detectors, and is one of the most important factors impacting resolution. The amount of spillover spread is commonly misunderstood as being a result of **compensation** or **unmixing**, but this is not the case. Spreading is a result of photon counting error, prior to any processing of the data such as compensation. The spillover value, on the other hand, is a result of amount of overlap and detector gains, among others. Therefore, independent of spread and has no impact on resolution.



When compensated, the **APC** and **AF700** two color experiment shows minimal spread (left). Compensation values exceed 100% and the population resolves well.

Alternatively, an **APC** and **BV650** two color experiment shows a much higher level of spreading (right). Compensation value is significantly lower but there is a much higher spread, consequently reducing the resolution in the secondary BV650 channel.



***Myth*:**

1. The higher the compensation value when correcting for spillover, the larger the spread.
2. Compensation values should always be under 100%

***Flow Fact*:**

1. Spillover spreading is independent of compensation.
2. Compensation values have no impact on resolution. Voltages or gains should not be adjusted to reduce spillover values.

When designing multicolor panels, avoid close or overlapping flurochromes on coexpressing markers as this will introduce high spillover spread. This can reduce the resolution and quality of the data.