Flow Post-its
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Autofluorescence and Compensation

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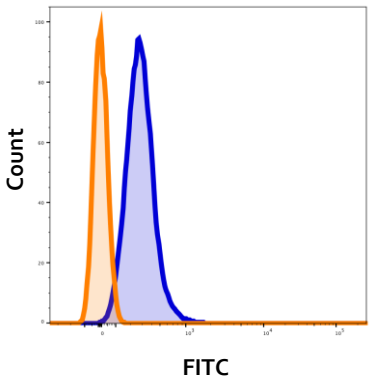
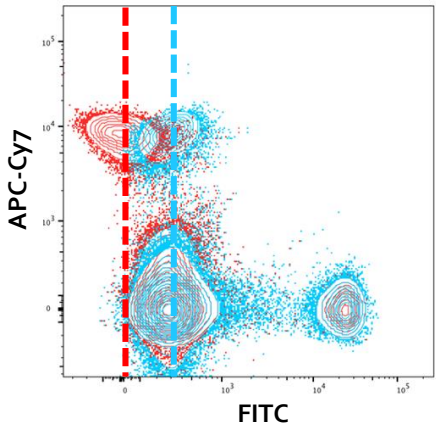
When carrying out **compensation** or **unmixing** in multicolor Flow Cytometry experiments, the use of the appropriate controls is critical. Both beads and cells are often used in these corrections for fluorescence spillover, often times in combination. It is necessary in these cases to have the appropriate **autofluorescence** referenced for each single color for acquisition and analysis softwares to accurately correct for spillover.



Overlay of **unstained cells** and **unstained beads** show a clear difference in autofluoresence.

Example to the right depicts an experiment where FITC single color was beads and the APC-Cy7 single color was cells. Compensation calculated with an incorrect **universal negative** of unstained cells for all controls is in red. In blue, the correct **sample specific autofluorescence** was used for each single color. **SSI** was used to determine success of compensation results for experimental sample.

Secondary Stain Index
Correct negative -0.02
Incorrect negative -1.08



Helpful Hints

- Fixation/permeabilization can have an impact on autofluorescence. Control cells and beads should be treated the same as your experimental samples to account for this. (*Exception*: Brilliant or Super Bright staining buffers should not be used on compensation beads.)
- Different cells can have different autofluorescence signatures. The same considerations should be taken when using multiple cell types as single color controls.

Important Note

The calculation itself was correct in both cases. As seen here, the calculation correctly matched the spillover channel MFI to the negative control that it was referenced to.