Flow Post-its
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Cross Laser Excitation

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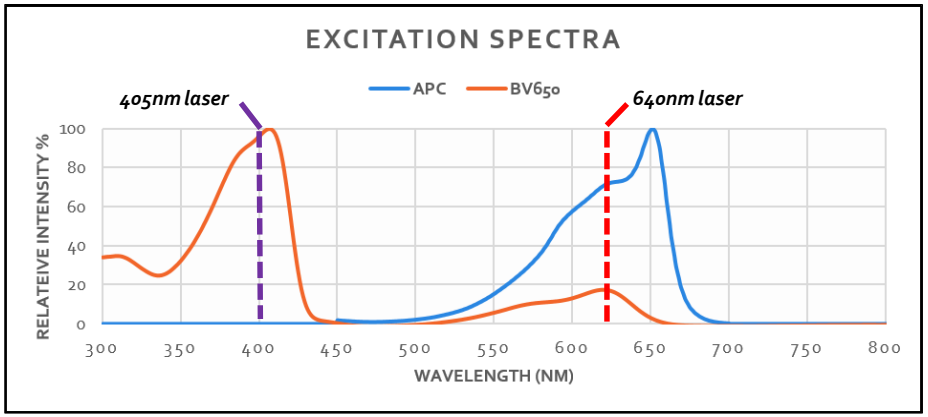
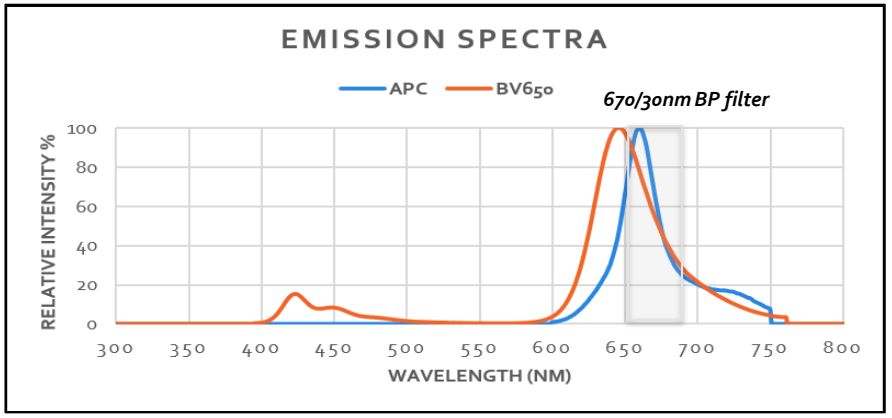
Fluorochromes used in flow cytometry are excited by light across a range of wavelengths. Most modern flow cytometers are equipped with **multiple lasers** to enable excitation of fluorochromes by **different wavelengths of light**. Although fluorochromes can be maximally excited by different wavelength lasers, **cross laser excitation can occur,** causing fluorescence spillover.



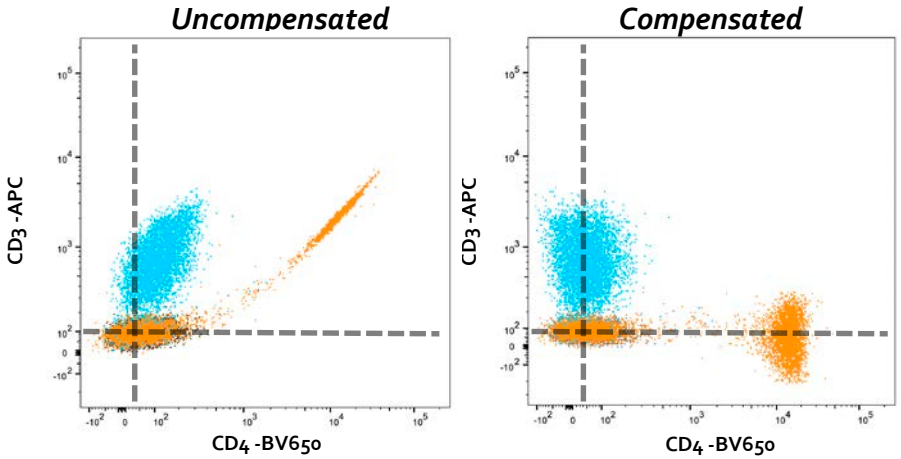


Cross Laser Excitation: A Case Study

Excitation Spectra: Plotted excitation spectra of APC and BV650. Based on this plot, APC is not expected to be excited by the 405 nm laser. However, many manufacturers do not show the complete spectra, which in the case of APC starts at 450 nm not allowing us to see the 405 nm excitation.

***Emission Spectra*:** Plotted theoretical emission spectra of APC and BV650. Emitted light from these two fluors is largely overlapping and the bandpass filter used for detection is similar for both fluorochromes.

The Data: Overlays of unstained, APC & BV650 single color controls. Uncompensated plot shows **cross laser excitation** of both fluorochromes and the subsequent spillover into the secondary detectors, revealing BV650 and APC are both excited by the 405 and 640nm lasers and emit in similar wavelengths, showing **spillover correction is necessary**.



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https://fccf.mskcc.org
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