Flow Post-its
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Spectral Flow Cytometry: Behind the "filters"

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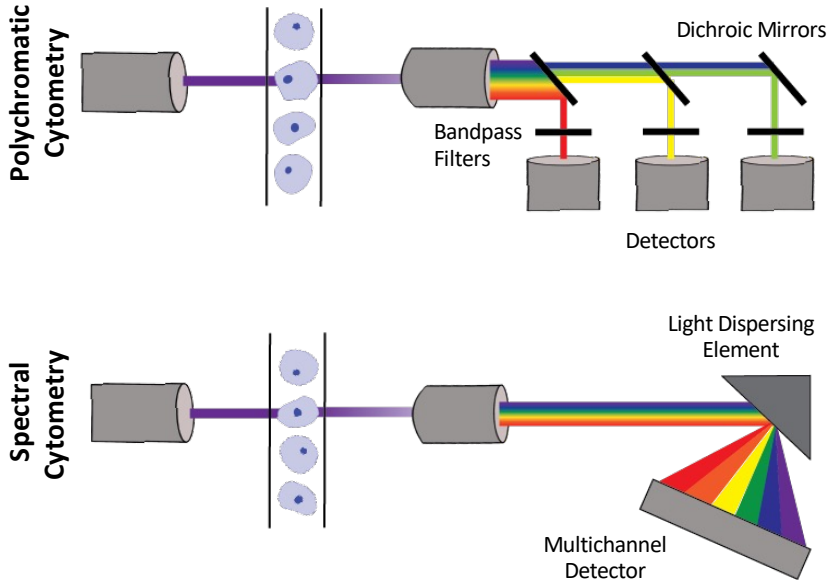
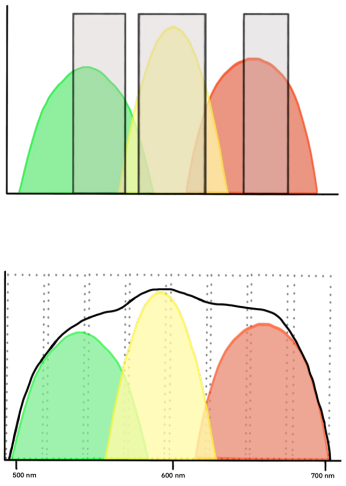
For decades, traditional polychromatic cell analysis by flow cytometry has used a combination of filters and mirrors to detect only a fraction of the emission spectrum (color) of each fluorochrome. In recent years, a few commercially available instruments have introduced a different approach that collects the entire spectrum of each fluorochrome and quantifies their abundance by using an unmixing algorithm.

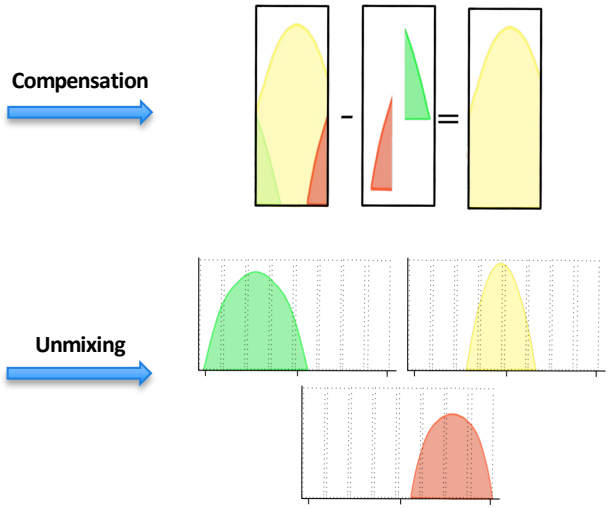
(A) Representative optical layout of polychromatic (traditional) instruments with each detector measuring a fraction of the emission spectrum of a given fluor, and a spectral flow cytometer with multichannel detectors that measure the entire spectrum of every fluor.



(B) In a polychromatic flow cytometer light of each fluor is partially captured using bandpass filters, whereas a spectral instrument will measure the full spectrum of each fluor.



(C) In both approaches, single stained controls are used to calculate fluor abundance. In polychromatic flow cytometry, fluor abundance is determined using compensation to remove the signal of the spillover fluors, whereas spectral cytometry uses unmixing to estimate the amount of each fluor in the cell.



Collecting the entire spectrum of each fluorochrome provides some advantages compared to polychromatic flow cytometry:

1) Ability to detect and distinguish fluorochromes with very similar emission spectra in the same sample (e.g., FITC and GFP)
2) Adding any new dye or fluorescent protein to a panel without having to change optical filters
3) Measuring the autofluorescence spectrum and using it as a parameter to identify new unlabeled cell populations or improve resolution between negative and positive signals by removing the contribution of autofluorescence.