CYTEK®

TRANSCEND THE CONVENTIONAL

Introduction to Spectral Cytometry

Kate Pilkington

Objectives

- Conventional vs Spectral
- Generating Full Spectrum Signatures
- Benefits of Full Spectrum Profiling™
- Understanding Unmixing

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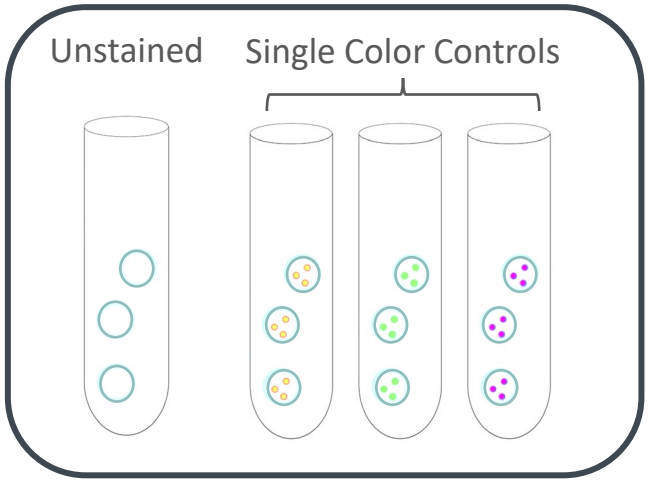
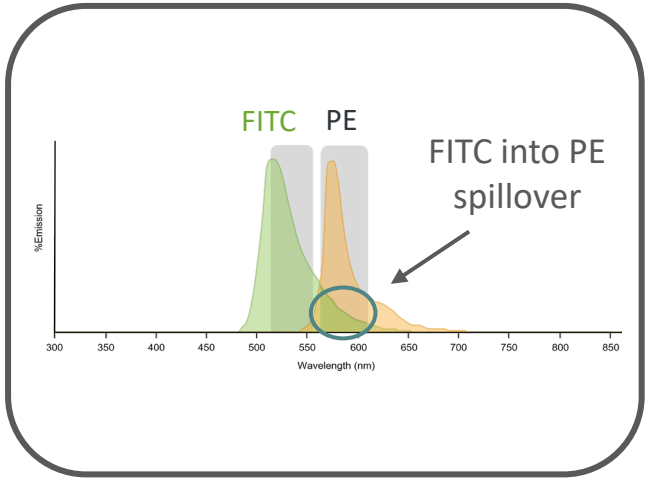
Conventional vs. Full Spectrum: Similarities

Run controls to define fluorescent signal on the cytometer



Account for spectral overlap with a mathematical calculation



Run multicolor samples with calculation applied

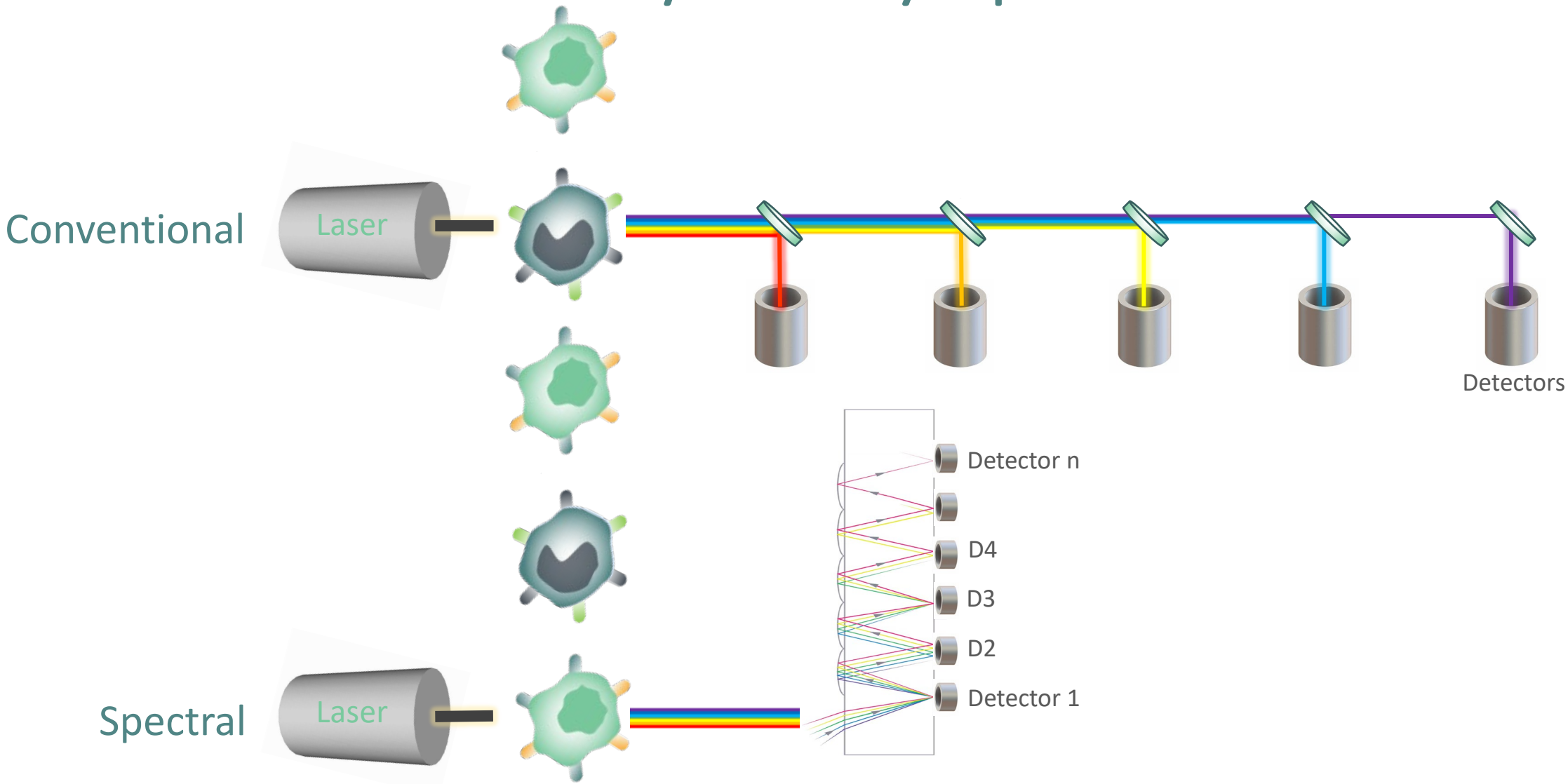
Conventional Flow Cytometry Terminology

Compensation Controls → Input → Compensation → Output → Compensated data

Full Spectrum Flow Cytometry Terminology

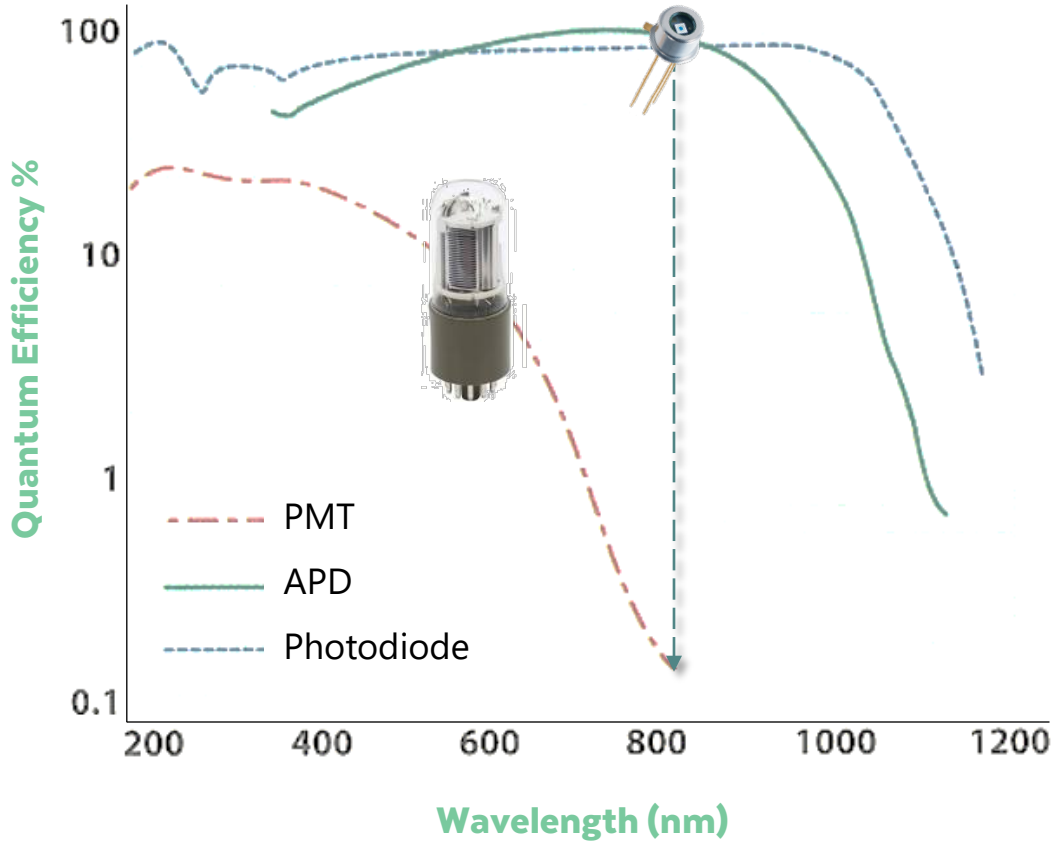
Reference Controls → Input → Spectral Unmixing → Output → Unmixed data

Flow Cytometry Optics



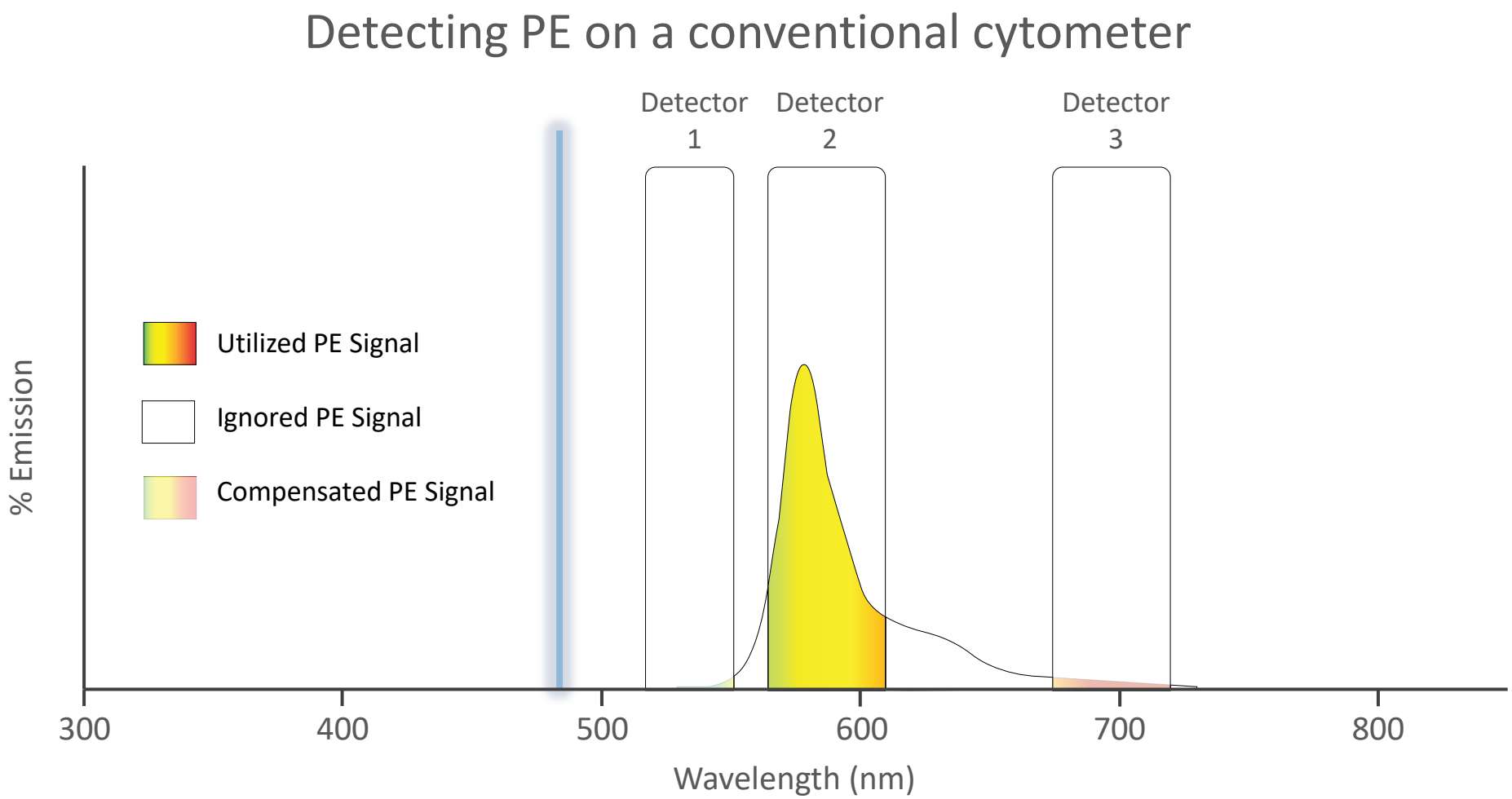
Why APDs?

1. **High QE in red region**
2. **High sensitivity for far red dyes**



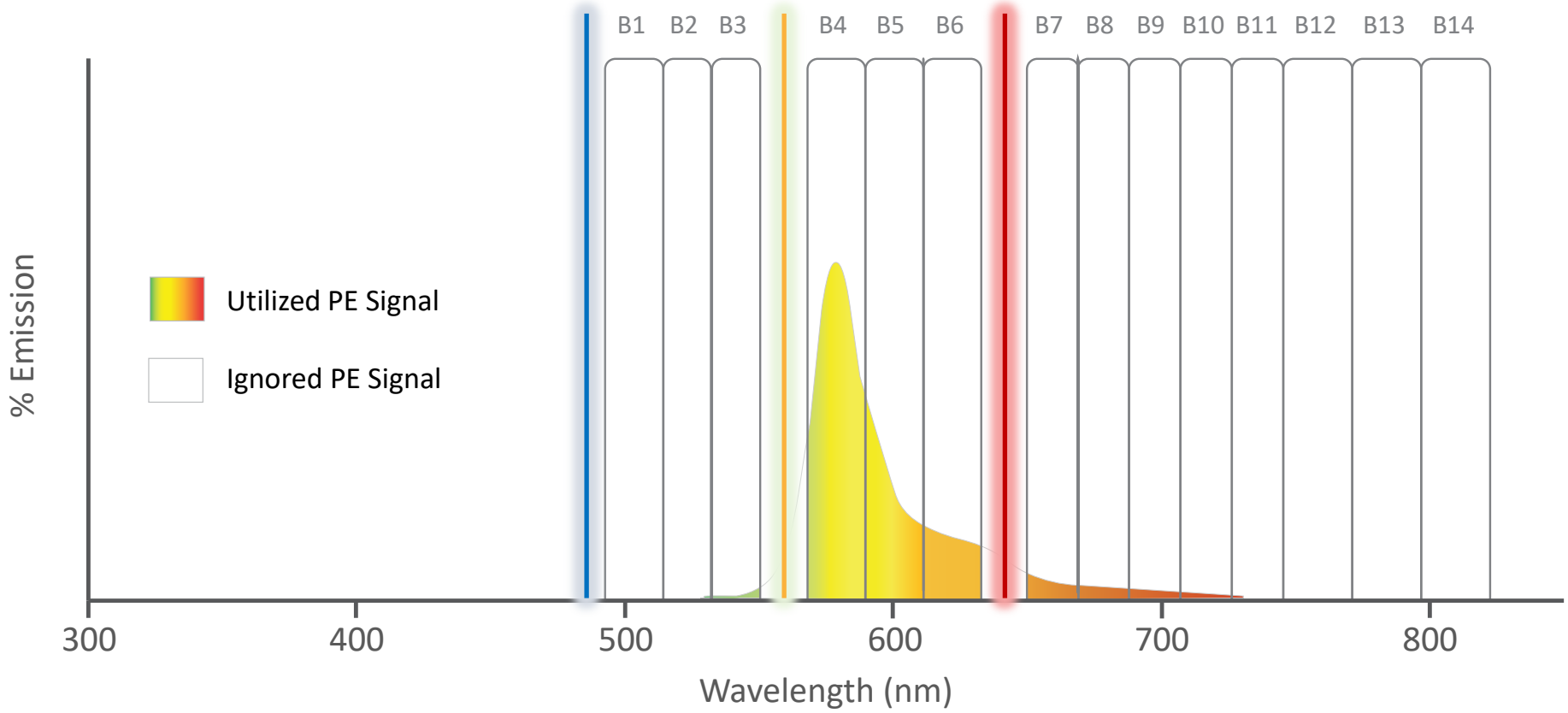
Data from Hamamatsu Photonics

Conventional vs. Full Spectrum: Differences

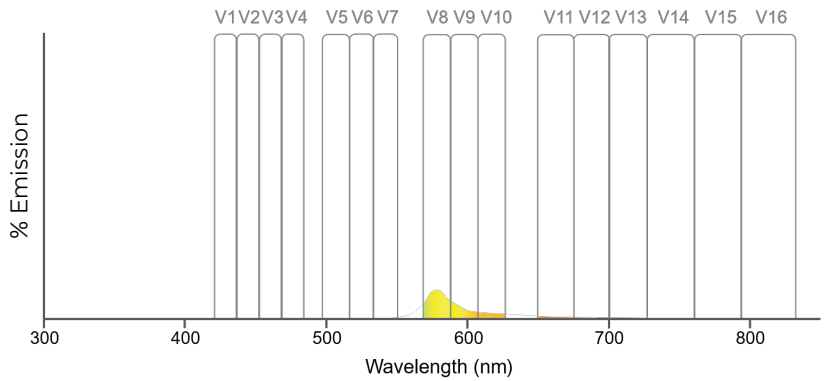


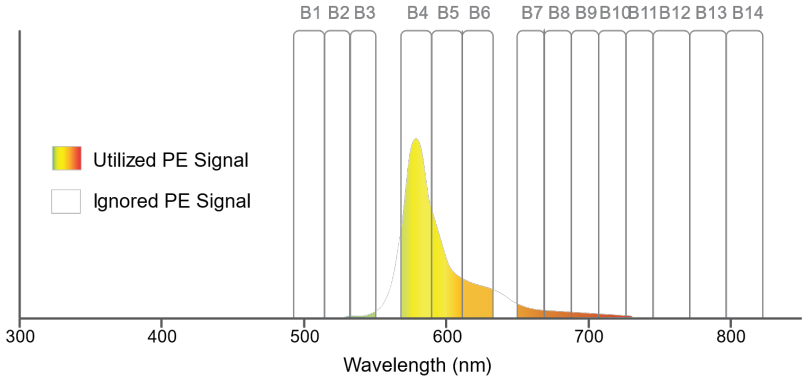
Conventional vs. Full Spectrum: Differences

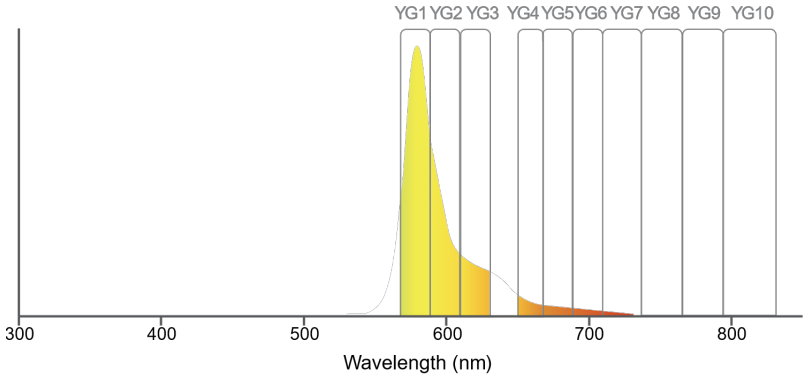
Detecting PE on a Cytek® System



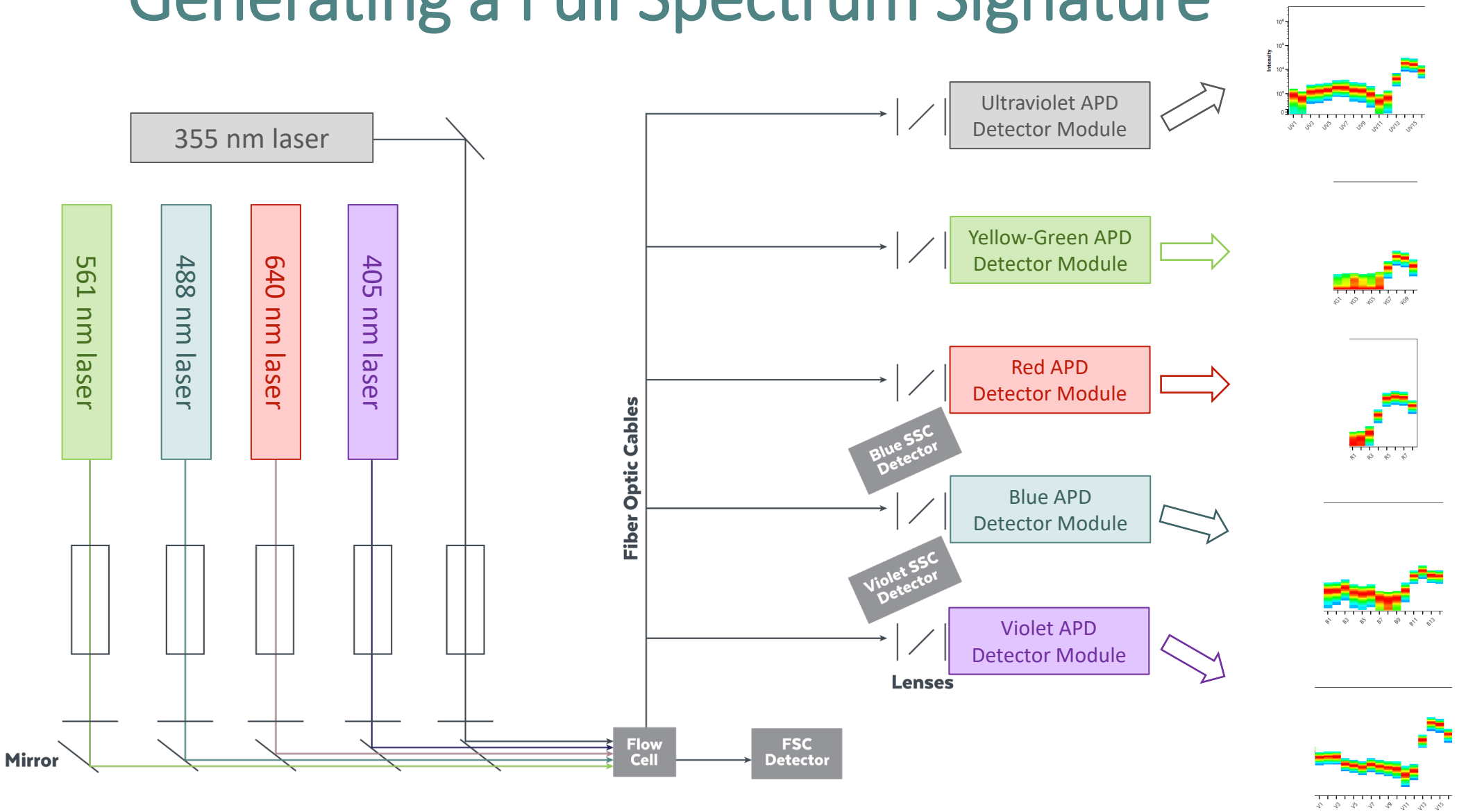
Full Spectrum Profiling™



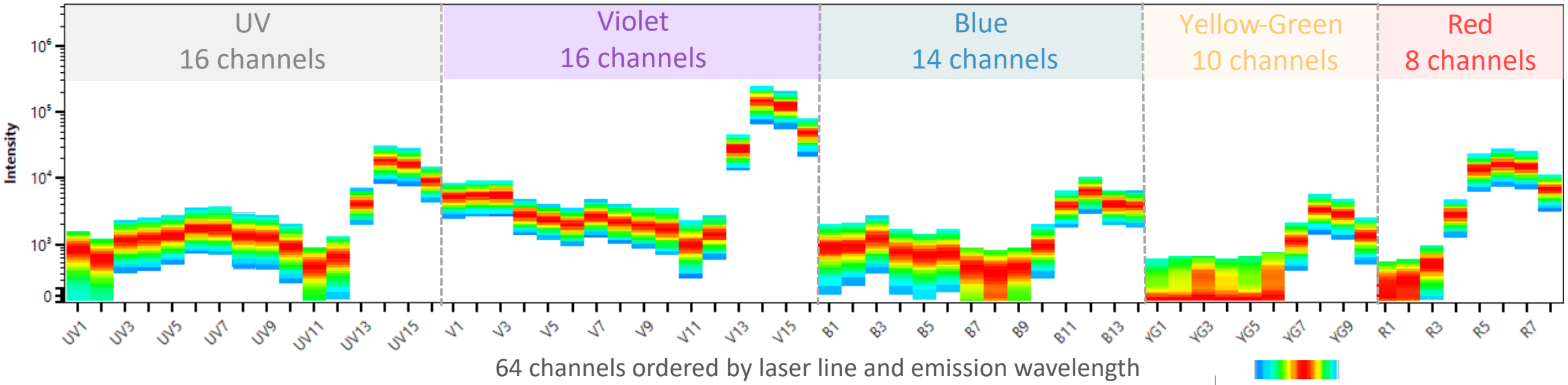




Generating a Full Spectrum Signature

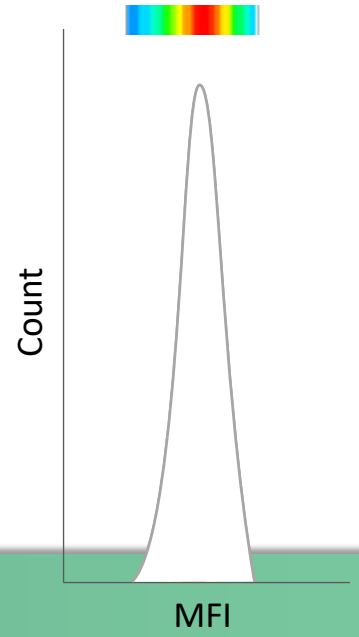


Building a Full Spectrum Signature

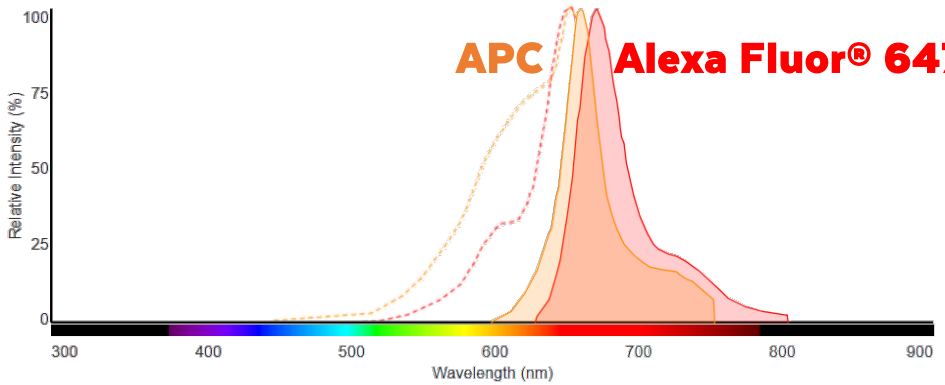


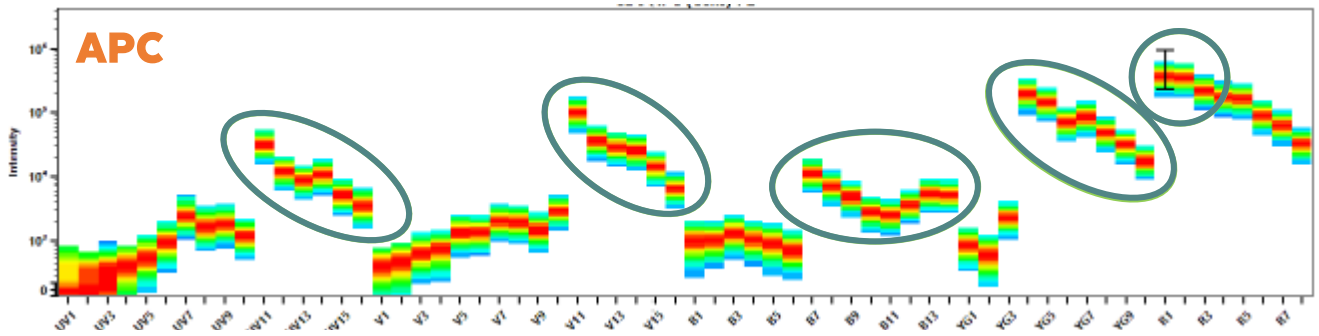
64 channels ordered by laser line and emission wavelength

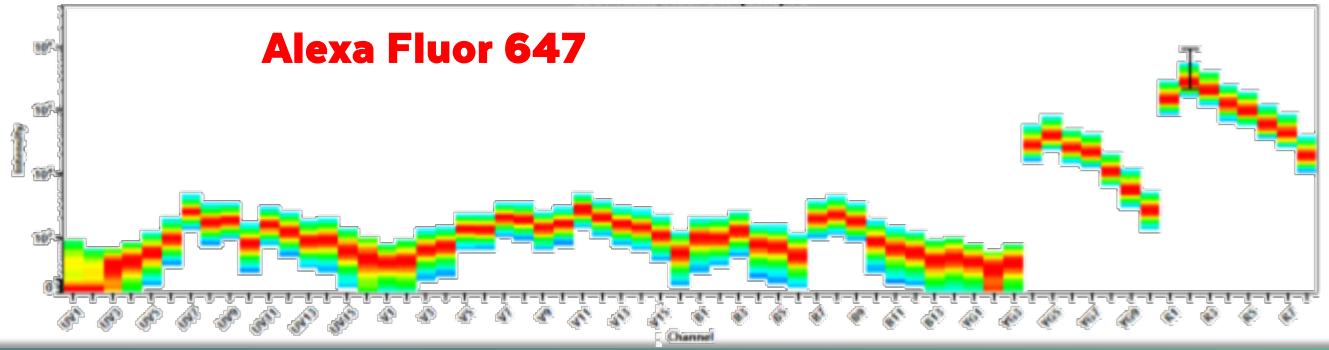
The signals are captured from each of the different modules and stitched together to create a single spectral signature



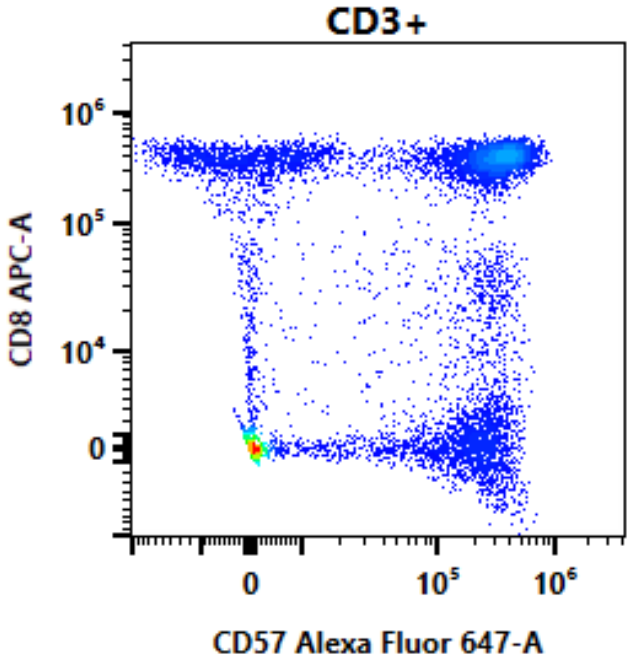
Use of Highly Overlapping Dyes







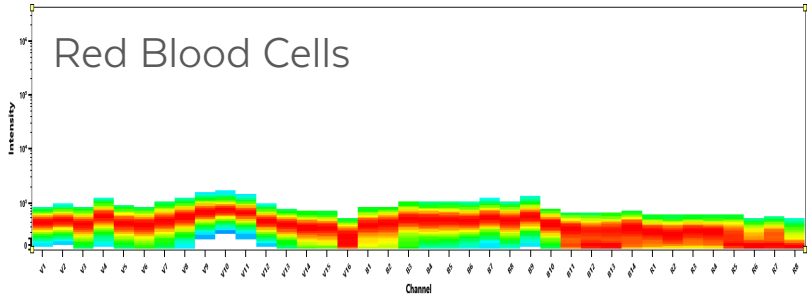
Plot gated on singlet lymphocytes

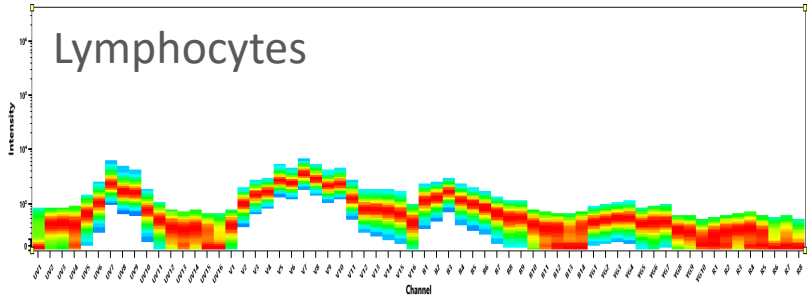


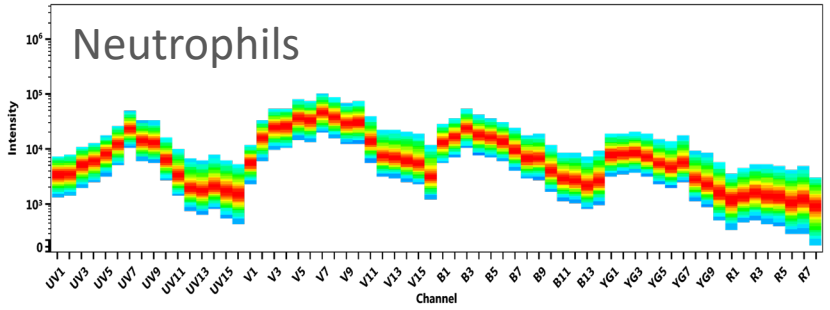
Fluorochromes with highly overlapping emission spectra can be used effectively on co-expressed markers

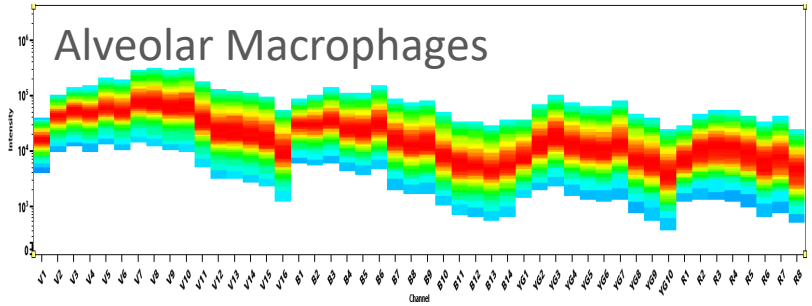
FSP™ Technology Easily Defines Autofluorescence

Autofluorescence is the native emission of light that comes from cellular components observed in unstained cells





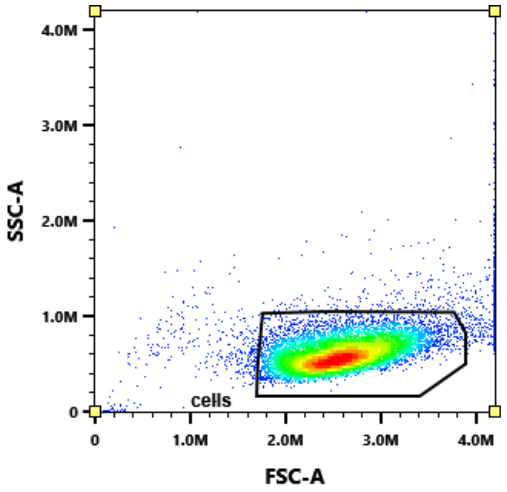


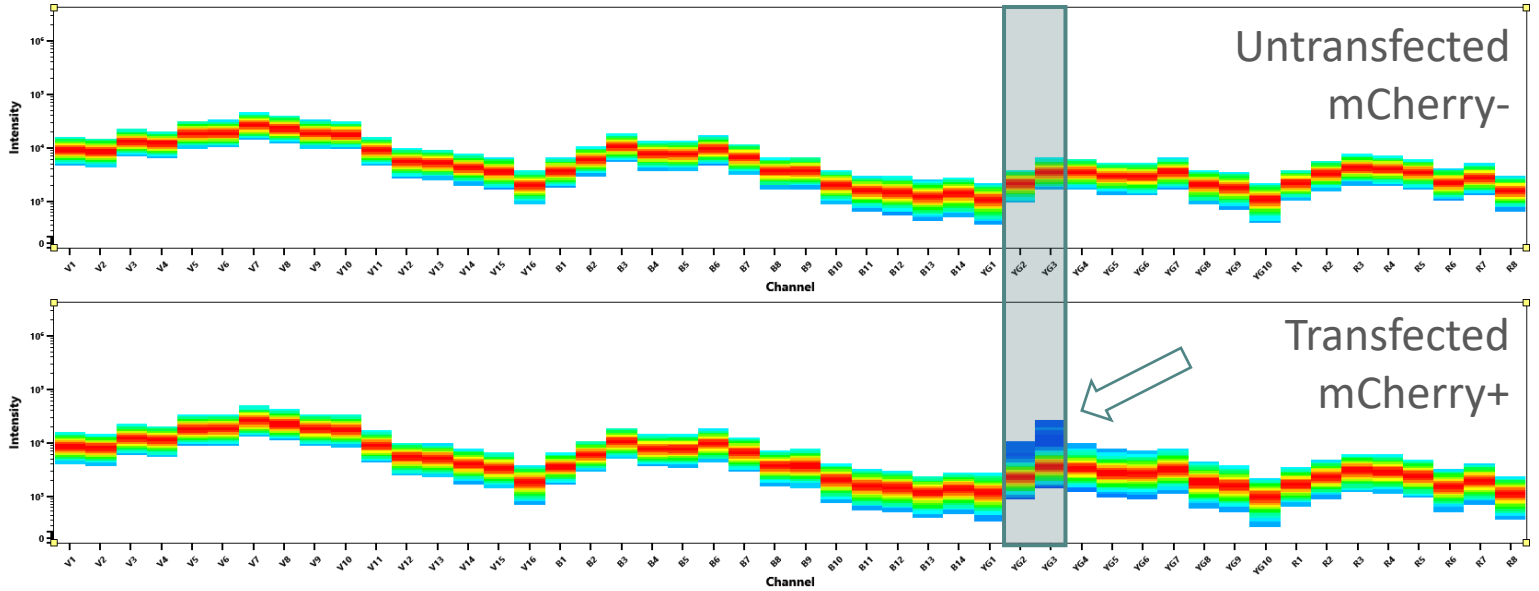


Cytek® Full Spectrum Profiling™ Technology can extract autofluorescence and potentially improve marker resolution

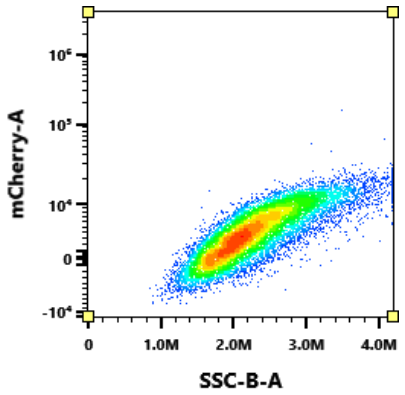
Benefits of Autofluorescence Extraction

HeLa human cells were transfected with a vector carrying an mCherry reporter

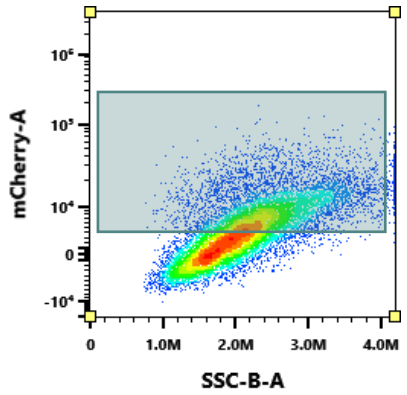




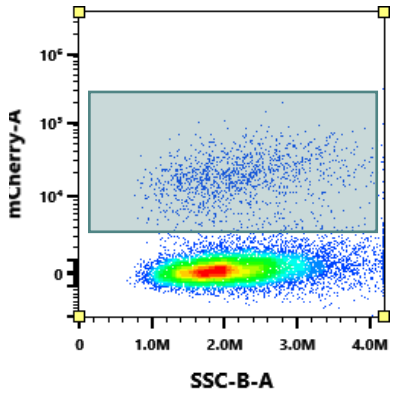
Untransfected Without Autofluorescence Extraction



Transfected Without Autofluorescence Extraction

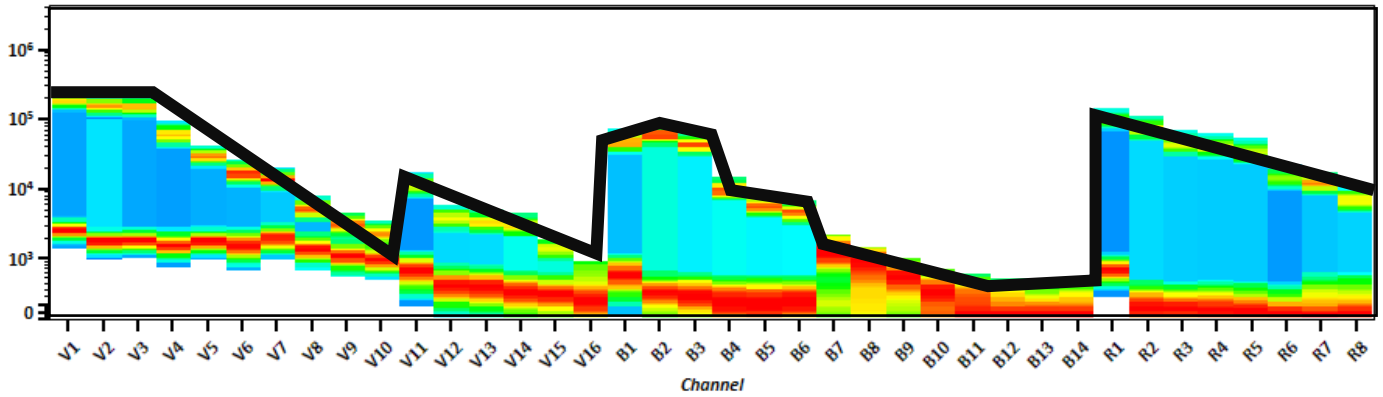


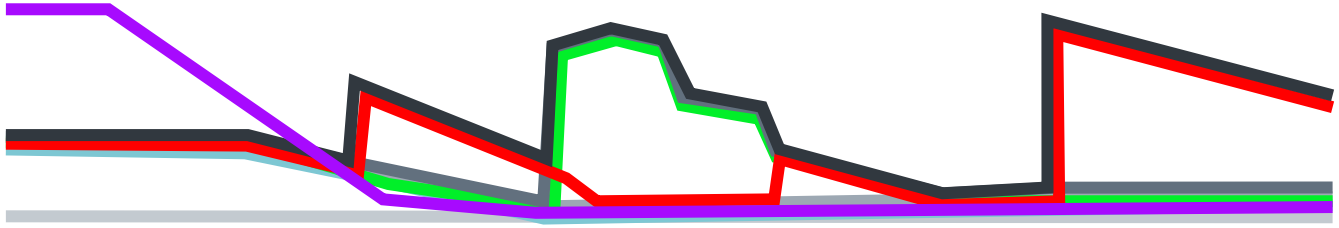
Transfected With Autofluorescence Extraction



Spectral Unmixing - Ordinary Least Squares (OLS)

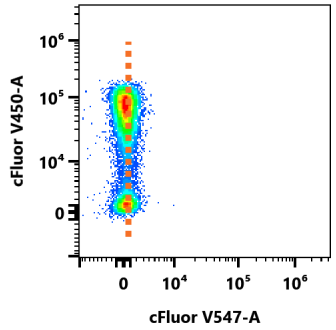
The spectral unmixing algorithm uses the provided controls to calculate the contribution of each fluorophore in the multicolor assay.

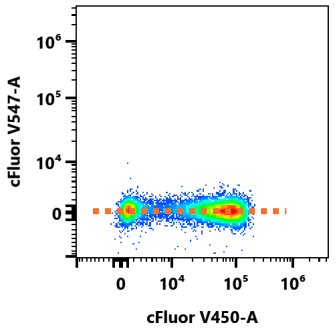




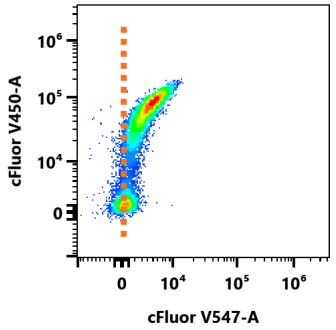
We can think of this as extracting or deconvoluting each component until we have nothing left.

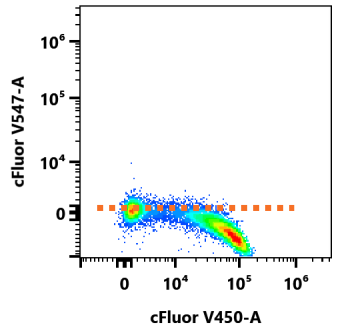
Spectral Unmixing Applied to Data





✓ MFI of the positive matches the MFI of the negative





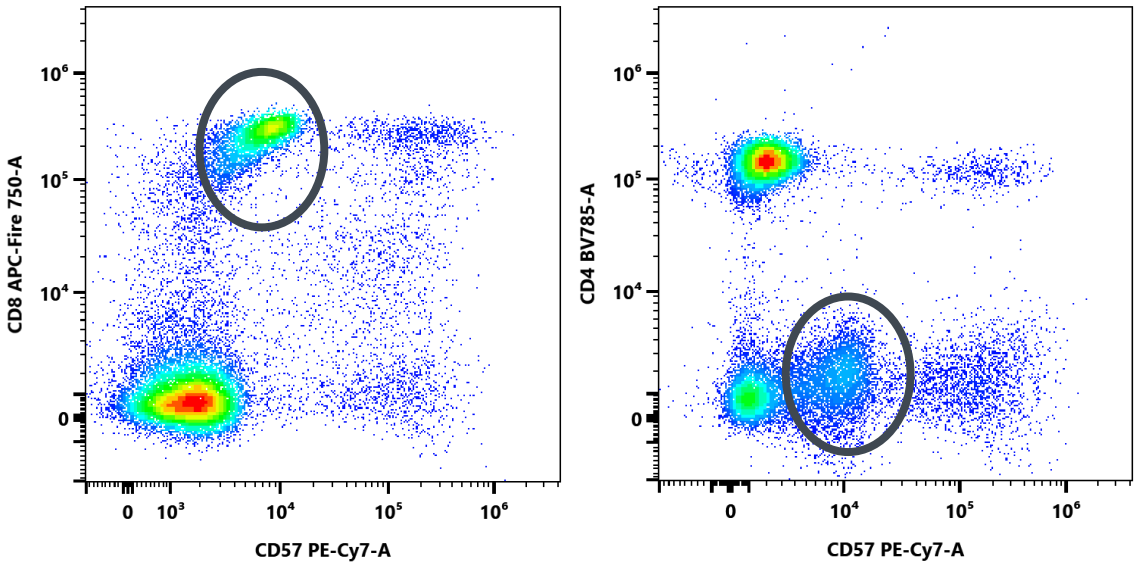
✗ MFI of the positive DOES NOT match the MFI of the negative

Unmixing/compensation errors can be either above or below the negative MFI

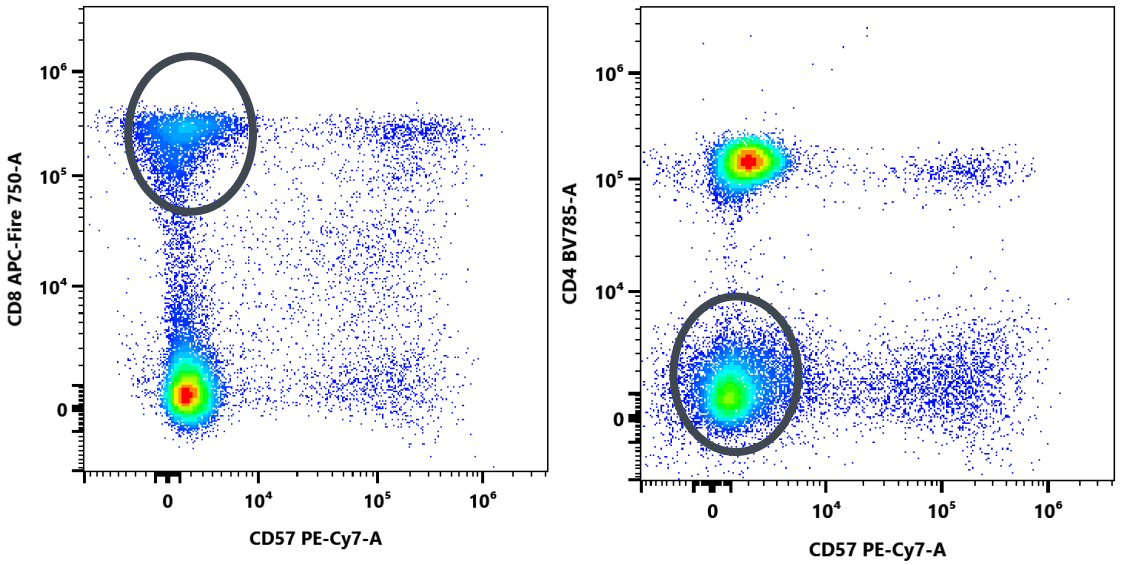
Unmixing/Compensation Errors Lead to Wrong Conclusions

Impacts data accuracy and result interpretation: false populations!

Data WITH Unmixing/Compensation Error



Correct Unmixing/Compensation



Flow Cytometry Applications

RESEARCH

- Cellular Biology
- Immunophenotyping
- Membrane potential
- pH
- Immunology
- Proliferation
- Apoptosis
- Cell cycle
- Gene transfections
- Fluorescent proteins
- Cell signalling
- RNA
- Cell pigments (chlorophyll, phycobillins)
- Multiplexed bead arrays
- Histone acetylation, phosphoflow
- Autophagy
- Cellular senescence, necrosis, viability
- ROS production
- Plant Cytometry
- Marine Cytometry
- Extracellular Vesicles
- Microbiology

CLINICAL

- Pathology and Laboratory Medicine
- Leukemia and Lymphoma
- MRD
- Stem Cell Enumeration
- Autoantibodies
- HIV/AIDS – CD4 Enumeration
- Fetal RBC
- Immunodeficiencies
- Paroxysmal Nocturnal Hemoglobinuria
- Reticulocytes