Capability of spectral flow cytometry using fluorochromes with highly overlapping spectra in conjunction

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Introduction

Full spectrum flow cytometers use a large number of detectors with narrow bandpath filters to measure the full spectrum emission of every fluorochrome across multiple lasers to create a more detailed signature for each fluorochrome. Compared to conventional flow cytometry which uses compensation to correct for fluorescence spillover, spectral flow cytometry uses a process called spectral unmixing to resolve each fluorochrome. Spectral unmixing uses a mathematical algorithm to decompose the spectral signature of a multicolor sample into a set of fluorochromes and their corresponding abundances, based on the unique spectral signature of each fluorochrome. Through this approach, fluorochromes with near-identical peak emissions but different off-peak emission characteristics can be distinguished and used in conjunction in one panel.

Herein, the capability of distinguishing spectrum similar fluorochromes were demonstrated using the Agilent NovoCyte Opteon spectral flow cytometer.

Results and Discussions

FITC combined with fluorochromes of varying degrees of similarity

FITC used in conjunction with fluorochromes of varying degrees of similarity are compared using the NovoCyte Opteon spectral flow cytometer. As the similarity decreases, the fluorescent spreading error becomes smaller and the resolution increases. When FITC was used in conjunction with BB515, the Similarity Index is 0.99, indicating the spectra of these two dyes are almost the same. Even with such high similarity, their signals still can be resolved.

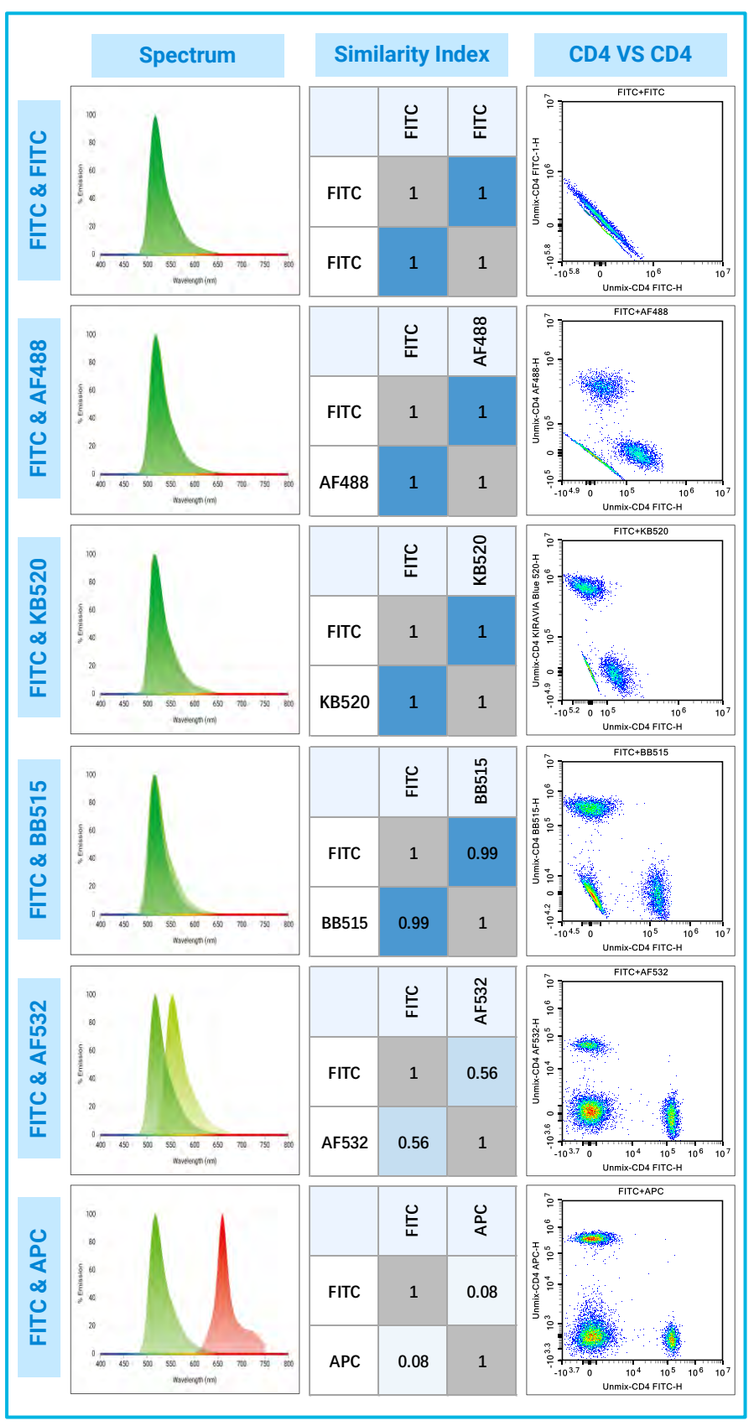

Figure 1. FITC combined with fluorochromes of varying degrees of similarity. PBMC were single stained with CD3 PE-Cy7 and FITC, Alexa Fluor 488, KIRAVIA Blue 520, Brilliant Blue 515, Alexa Fluor 532, or APC-conjugated CD4 antibody. CD3 PE-Cy7/CD4 FITC-stained PBMC samples were mixed with samples labeled with CD3 PE-Cy7 and different fluorochrome-conjugated CD4 antibodies correspondingly and analyzed on a five-laser NovoCyte Opteon spectral flow cytometer.

Results and Discussions

Examples of spectrum similar fluorochrome combinations

Several panels containing fluorochromes with similar spectra were designed and compared in parallel with panels containing the same markers but conjugated to fluorochromes with different spectra. The spectrum-similar fluorochrome combinations include BV421/Pacific Blue, Qdot 705/BV711, BB515/FITC, APC/Alexa Fluor 647, PerCP-Cy5.5/PerCP-eFluor 710, and PerCP-Cy5.5/PE-Cy5.5. Fluorochromes in these combinations can be resolved when used in conjunction with each other and the subpopulation results are not affected. However, the spillover spreading of spectrum-similar fluorochromes cannot be eliminated.

EDTA anticoagulated human peripheral blood was stained. Red blood cells were lysed. Afterward, the samples were washed and analyzed on a five-laser NovoCyte Opteon flow cytometer.

Spectral density plots generated by the NovoCyte Opteon flow cytometer and theoretical spectral plots are shown in the figures.

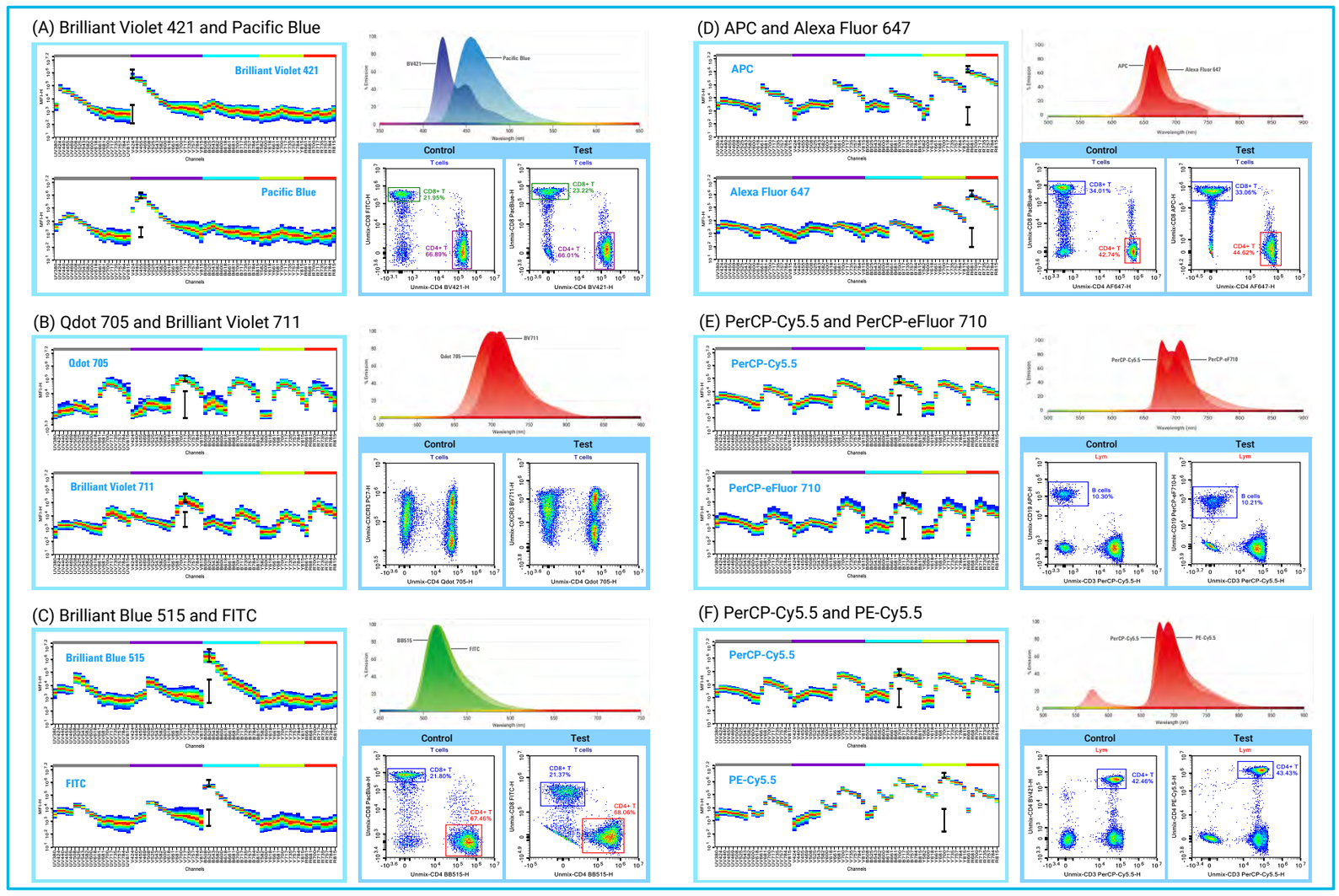

Figure 2. The detection of spectrum-similar fluorochrome combinations. The combinations include BV421/Pacific Blue(A), Qdot 705/BV711(B), BB515/FITC(C), APC/Alexa Fluor 647(D), PerCP-Cy5.5/PerCP-eFluor 710(E), and PerCP-Cy5.5/PE-Cy5.5(F). Control: no spectrum-similar fluorochromes included; Test: spectrum similar fluorochromes included.

Multiple fluorochrome combinations excited by the 405-nm, 561-nm, or 637-nm lasers

Compensation beads (Cat# CMIgP-30-5H, Spherotech) were stained with different fluorescent antibodies. Those labeled with fluorochromes primarily excited by one laser (violet, yellow, or red laser) were mixed together to simulate multi-color panels and acquired on the Agilent NovoCyte Opteon™ spectral flow cytometer. Not mixed single-stained compensation bead samples were used to generate spectrum signatures for each fluorochrome. The unmixed results are presented here. All populations can be well distinguished.

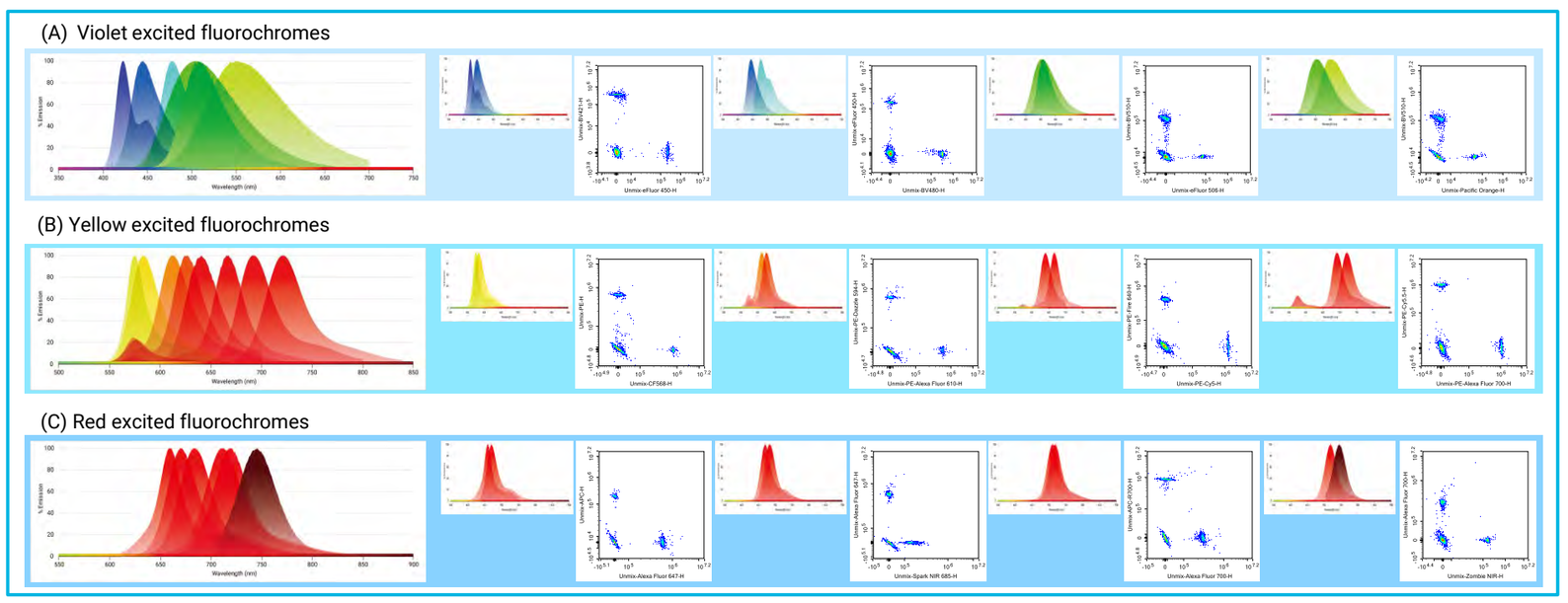

Figure 3. Unmixing of multiple fluorochromes with overlapping emission spectra excited by the 405-nm, 561-nm, or 637-nm lasers. (A) Combination of violet laser excited fluorochromes, BV421, eFluor 450, BV480, BV510, eFluor 506, and Pacific Orange. **(B)** Combination of yellow laser excited fluorochromes, PE, CF568, PE-Dazzle 594, PE-Alexa Fluor 610, PE-Fire 640, PE-Cy5, PE-Cy5.5, and PE-Alexa Fluor 700. **(C)** Combination of red laser excited fluorochromes APC, Alexa Fluor 647, Spark NIR 685, APC-R700, Alexa Fluor 700, and Zombie NIR.

Conclusions

- Fluorochromes with highly overlapping spectra can be used in conjunction using spectral flow cytometry, increasing the flexibility in fluorochrome selection.
- Spillover spreading between these fluorochromes cannot be eliminated and should be considered carefully in panel design .

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