CYTEK

TRANSCEND THE CONVENTIONAL

Cytek® Aurora
Fluorochrome Selection Guidelines
4 Laser 16V-14B-10YG-8R

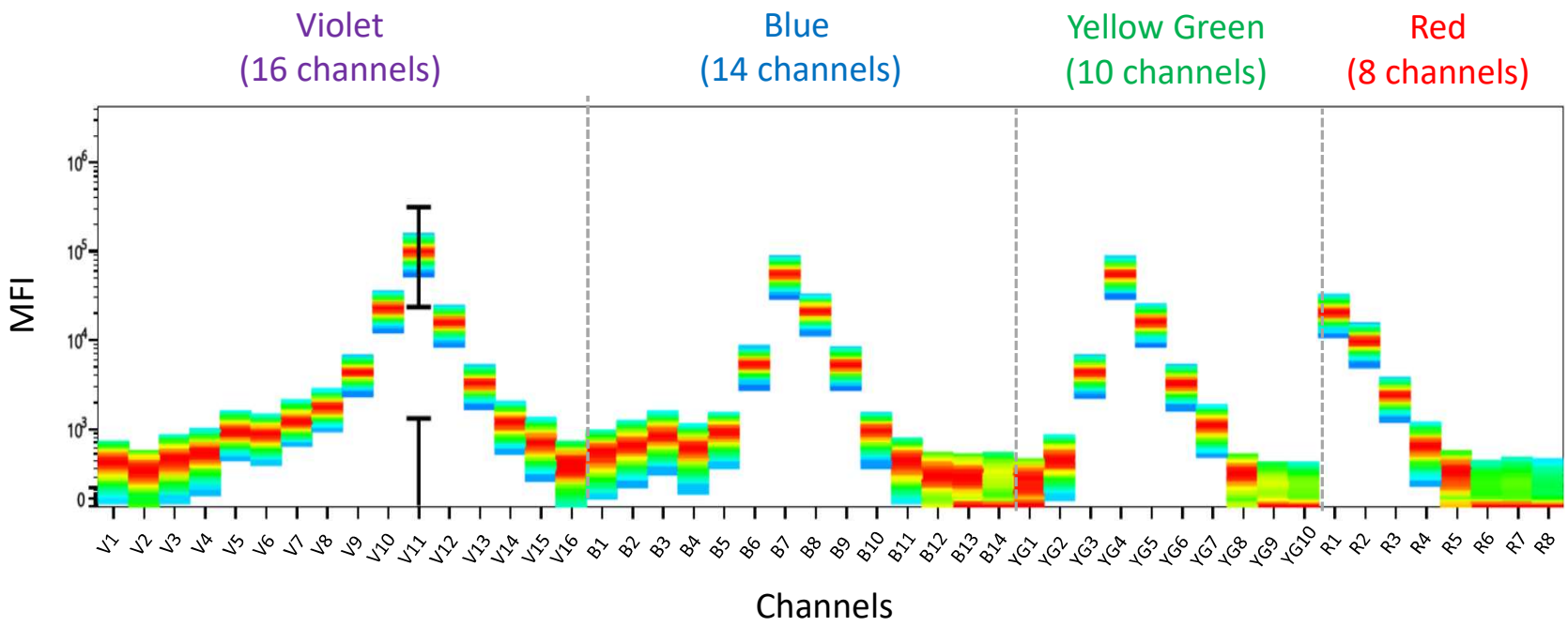
N9-20018 Rev. A

Fluorochrome Signatures

Dyes can be used in combination if they have a unique spectrum signatures.

Look for dyes with unique spectra and consider spread introduced by the dyes when designing multicolor panels (see slide 27).

How to Read Full Spectrum Fluorochrome Signatures

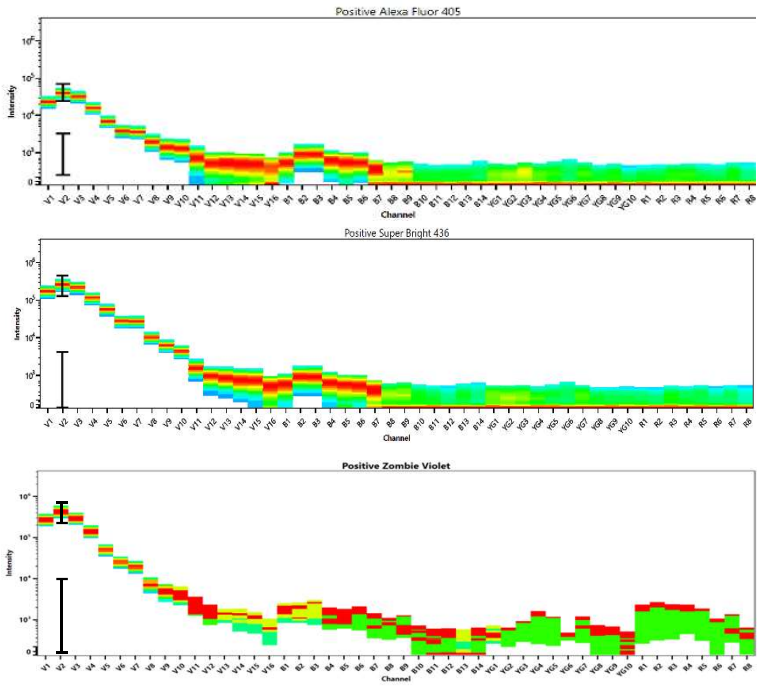


This dye is excited by all 4 lasers. The peak channel (indicated by the black bar) is in channel V11, and it has secondary emission in channels B7, YG4 and R1. Based on this information, expect this dye to introduce spread into dyes emitting at similar wavelengths.

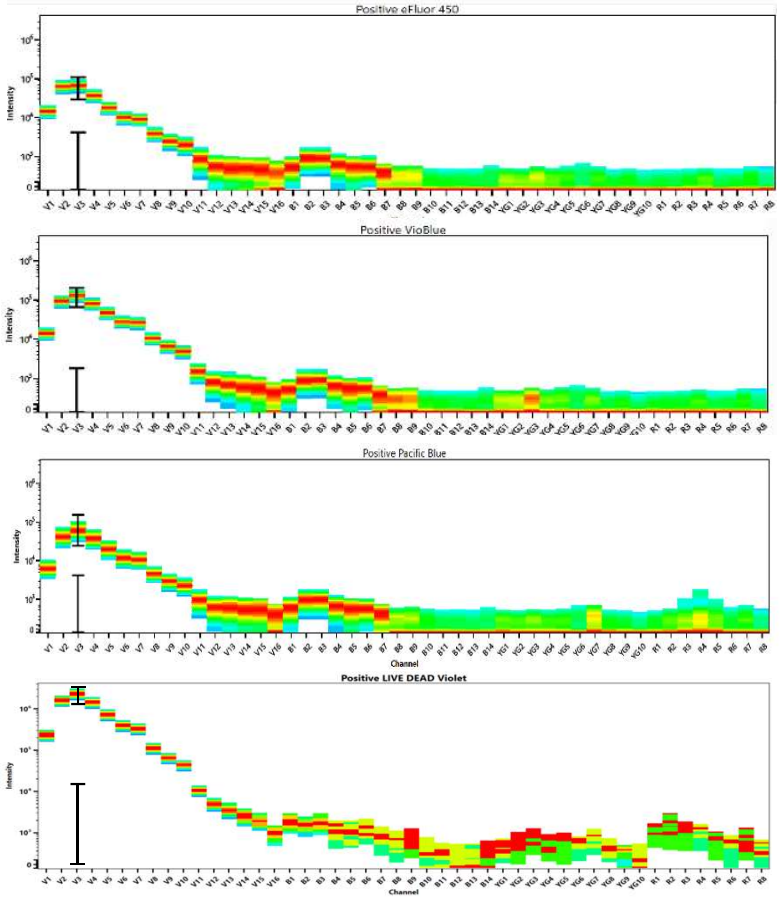
Dyes Primarily Excited by the Violet Laser

Violet Laser Excitable Dyes with Similar Signatures (1 of 3)

Alexa Fluor 405, SuperBright 436 and Zombie Violet

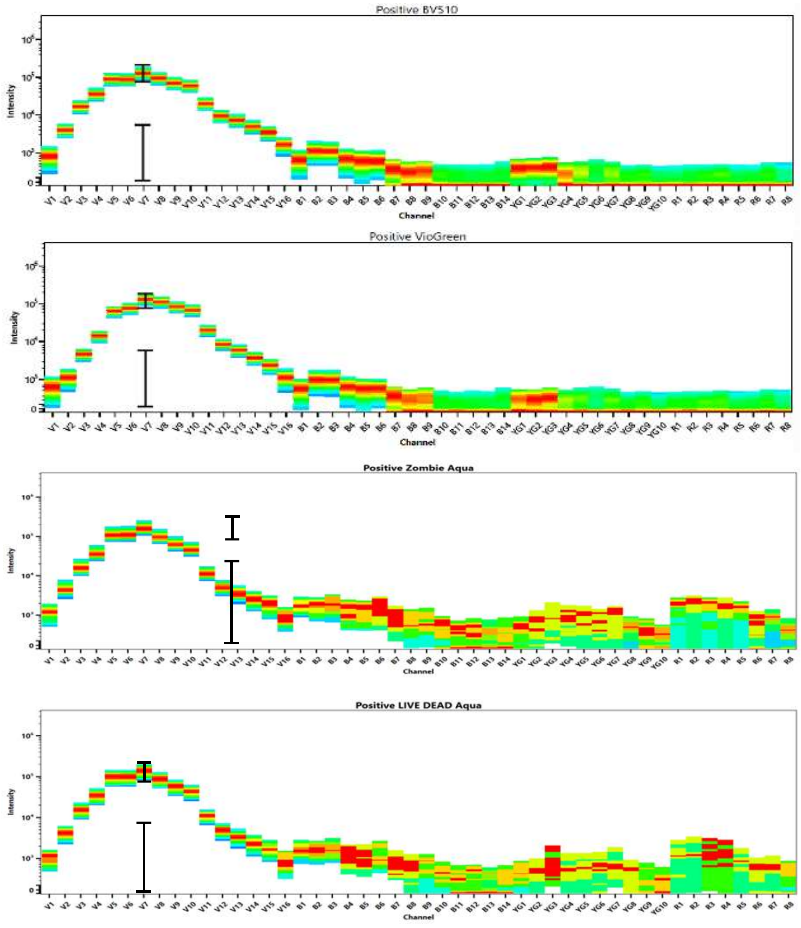


eFluor 450, VioBlue, Pacific Blue and Live/Dead Violet

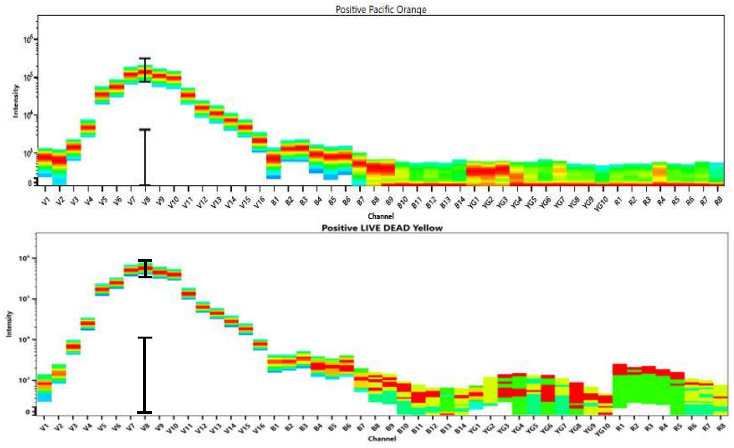


Violet Laser Excitable Dyes with Similar Signatures (2 of 3)

BV510, VioGreen, Zombie Aqua and Live/Dead Aqua

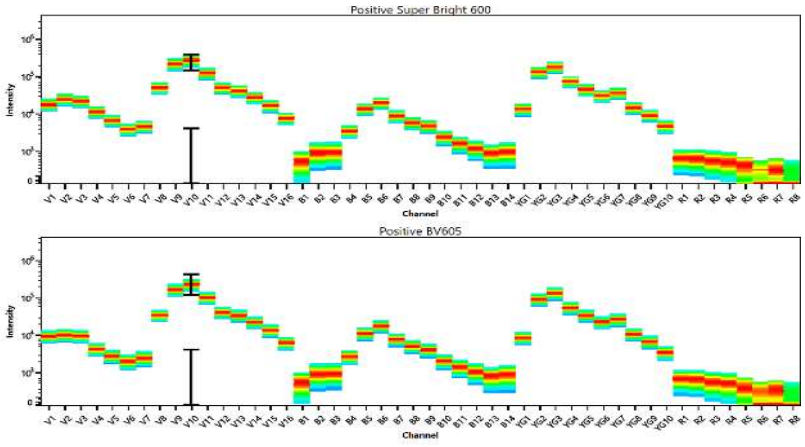


Pacific Orange and Live/Dead Yellow

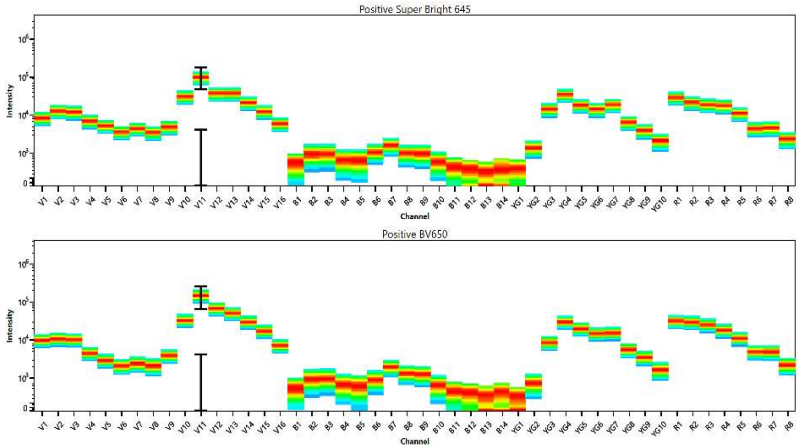


Violet Laser Excitable Dyes with Similar Signatures (3 of 3)

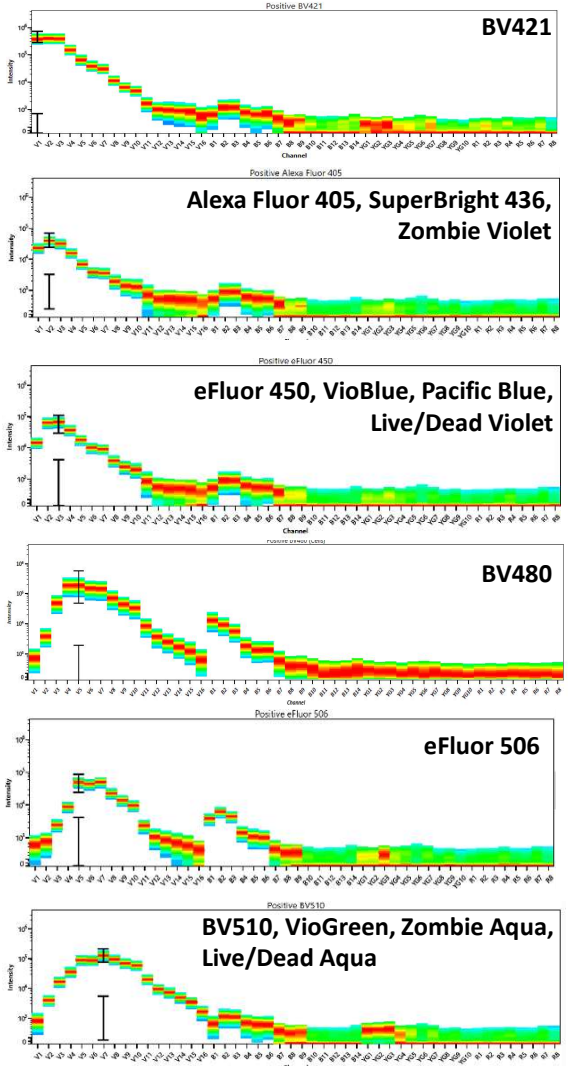
Super Bright 600 and BV605

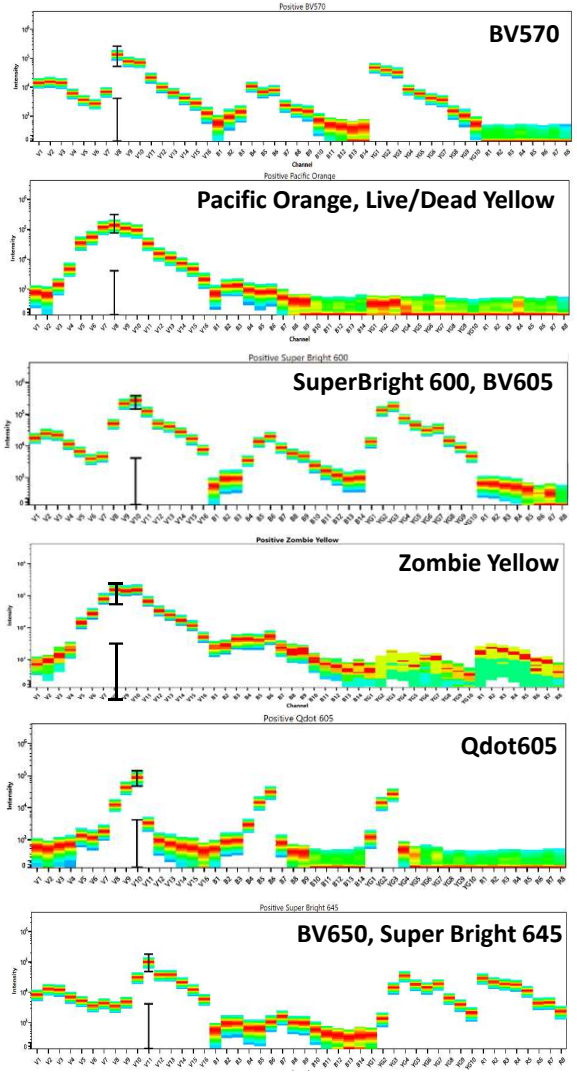


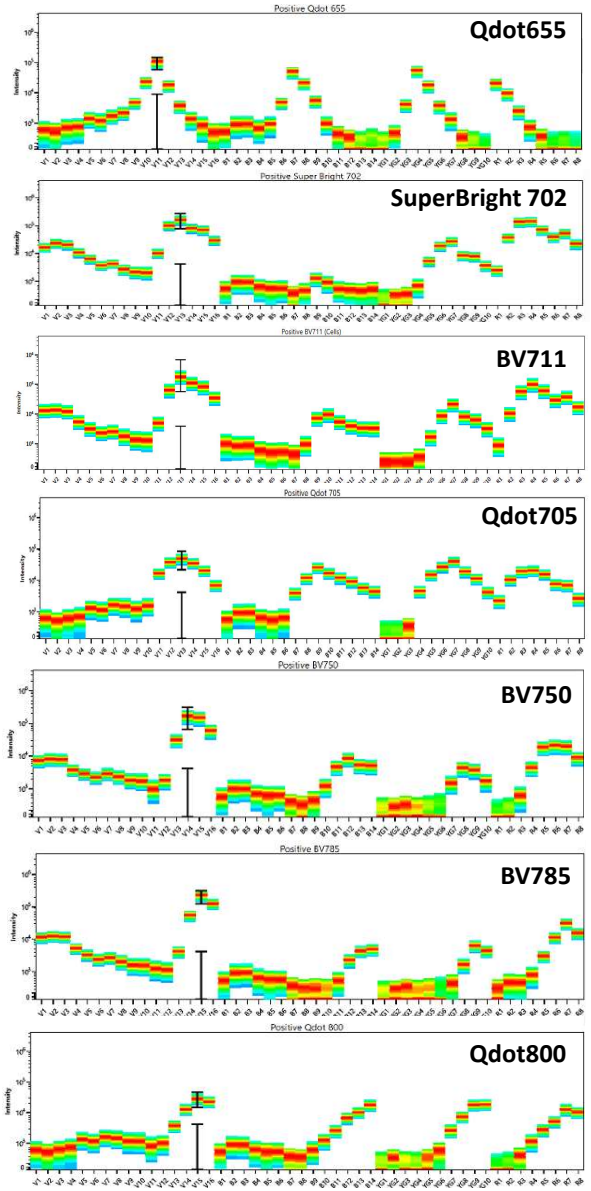
Super Bright 645 andBV650



Violet Laser Excitable Dyes with Unique Signatures



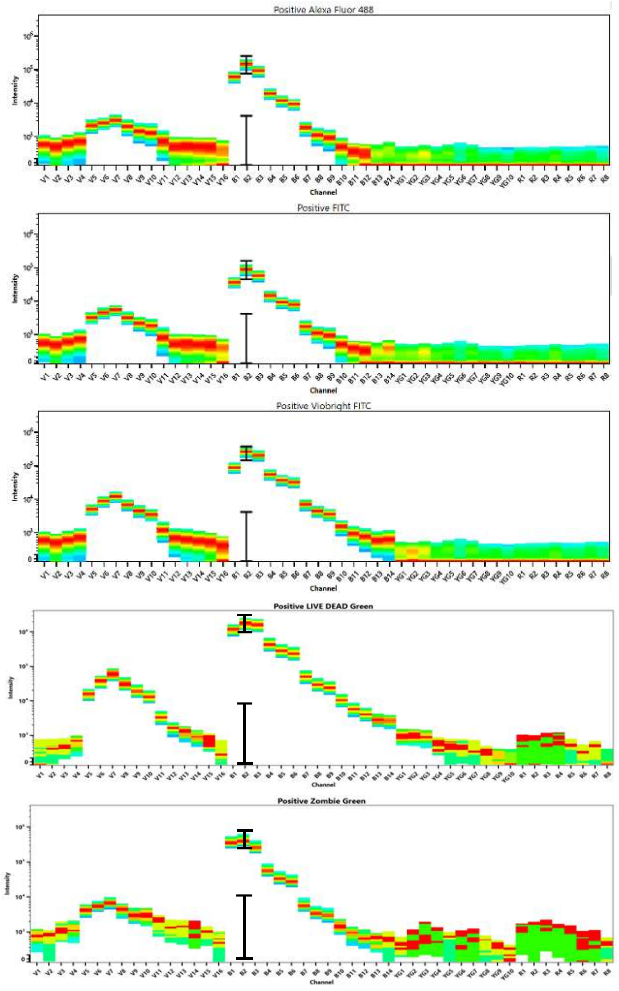




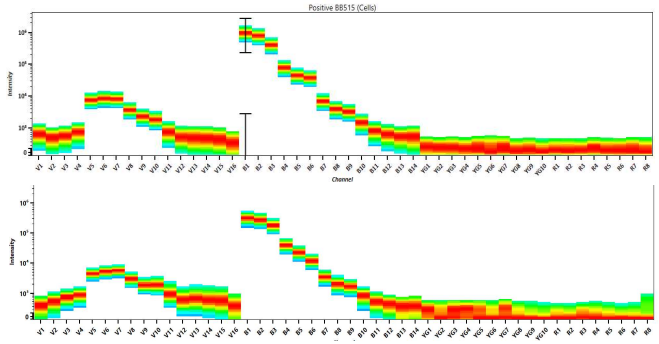
Dyes Primarily Excited by the Blue Laser

Blue Laser Excitable Dyes with Similar Signatures

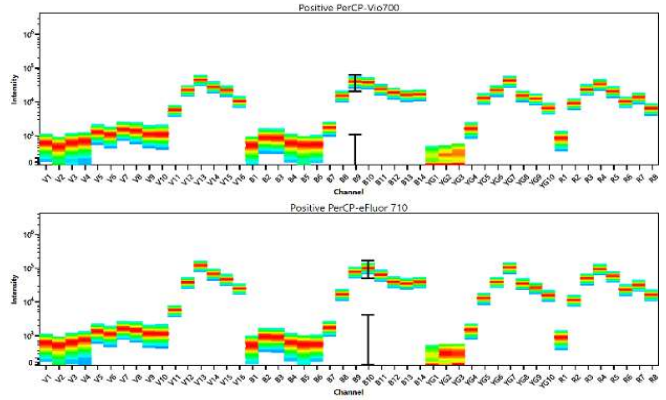
Alexa Fluor 488, FITC, VioBright FITC, Zombie Green and Live Dead Green



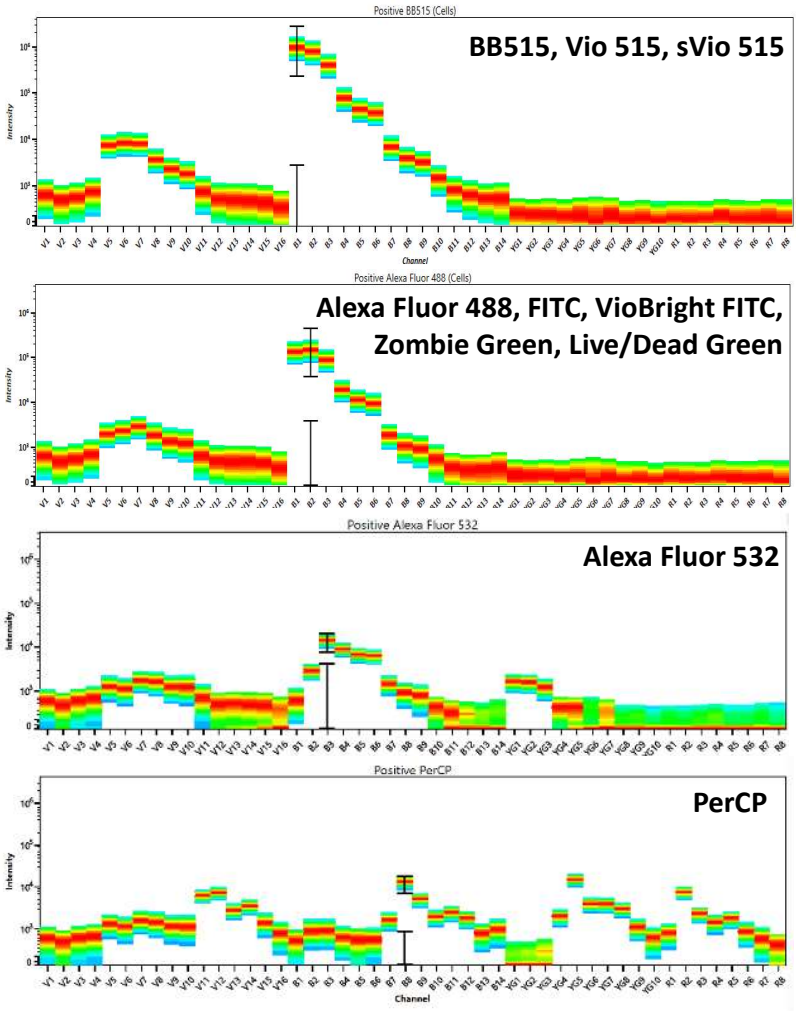
BB515, Vio 515

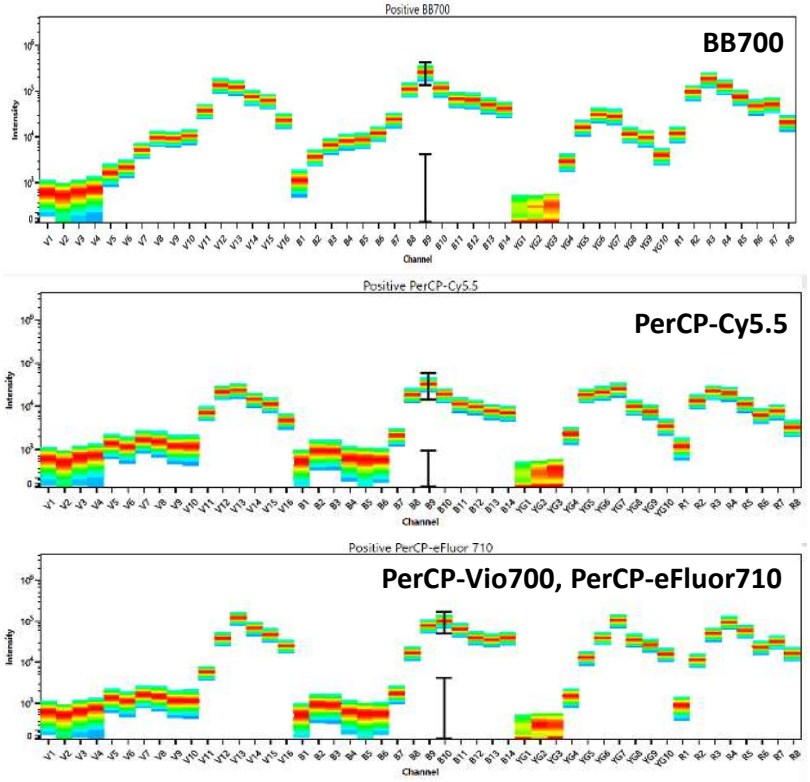


PerCP-Vio 710 and PerCP-eFluor 710



Blue Laser Excitable Dyes with Unique Signatures

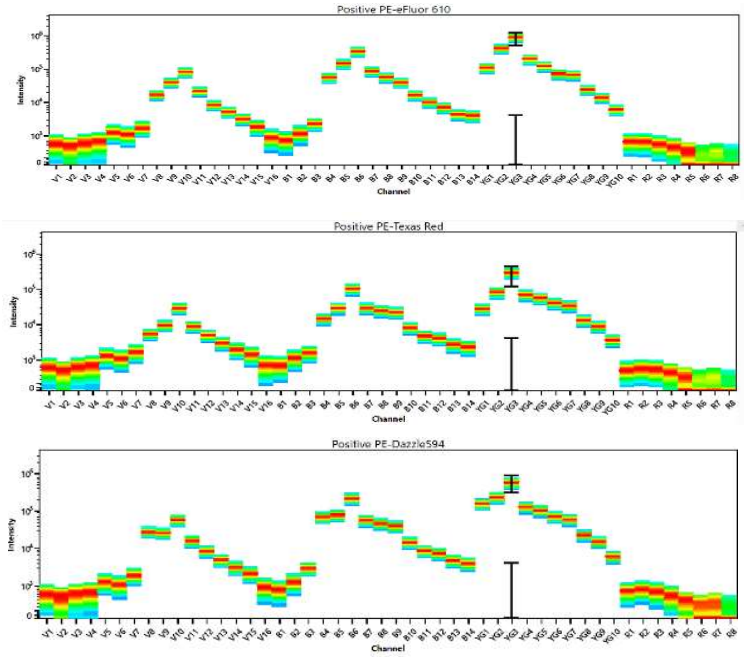




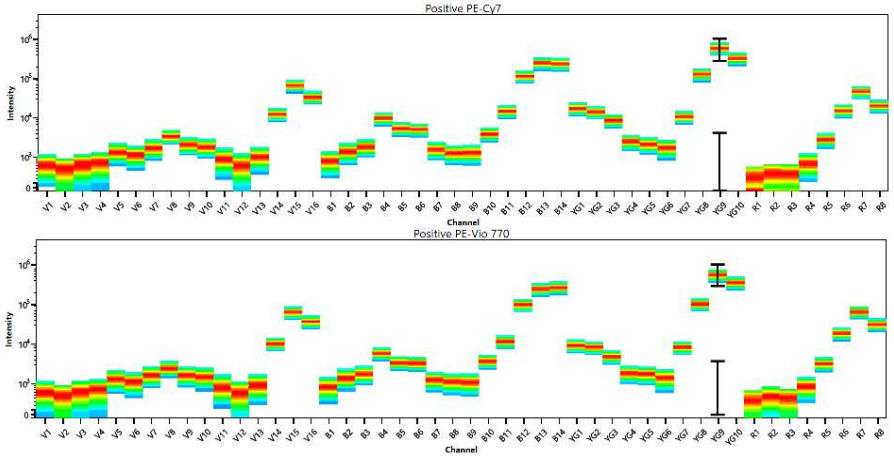
Dyes Primarily Excited by the Yellow Green Laser

Yellow Green Laser Excitable Dyes with Similar Signatures

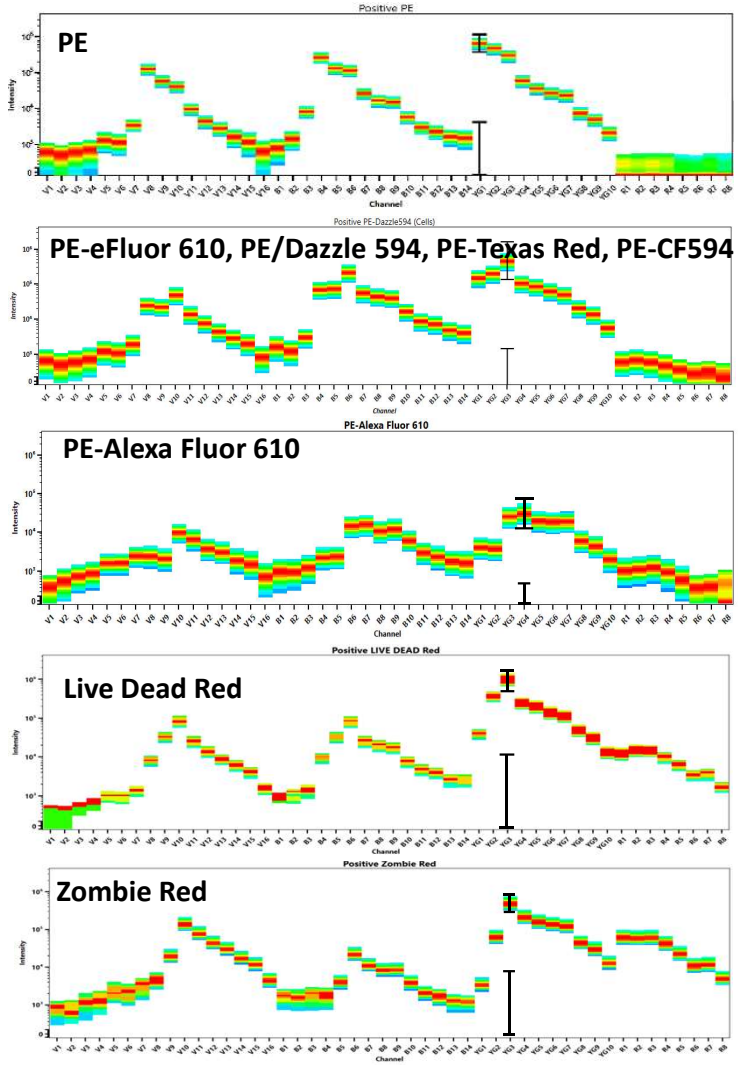
PE-eFluor 610, PE/Dazzle 594, PE-Texas Red

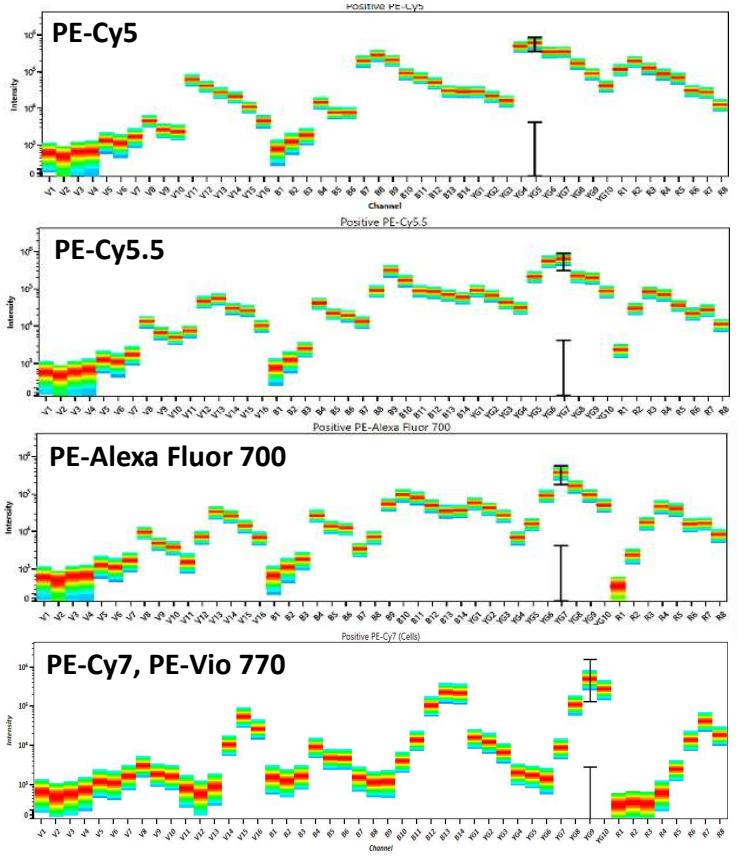


PE/Cy7 and PE-Vio 770



Yellow Green Laser Excitable Dyes with Unique Signatures

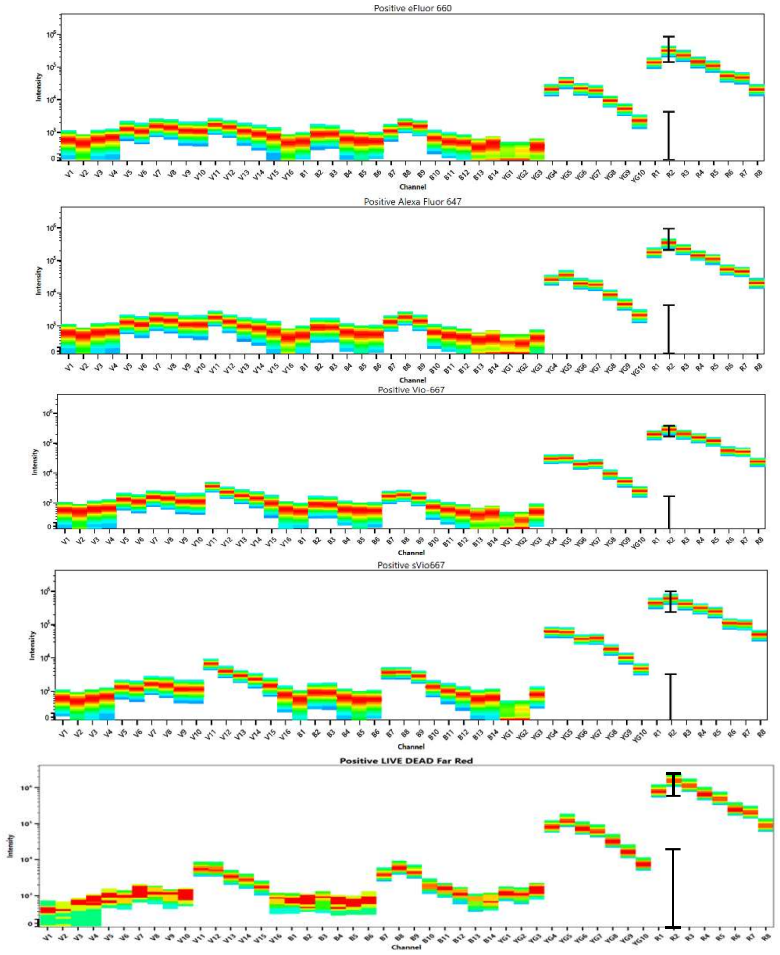




Dyes Primarily Excited by the Red Laser

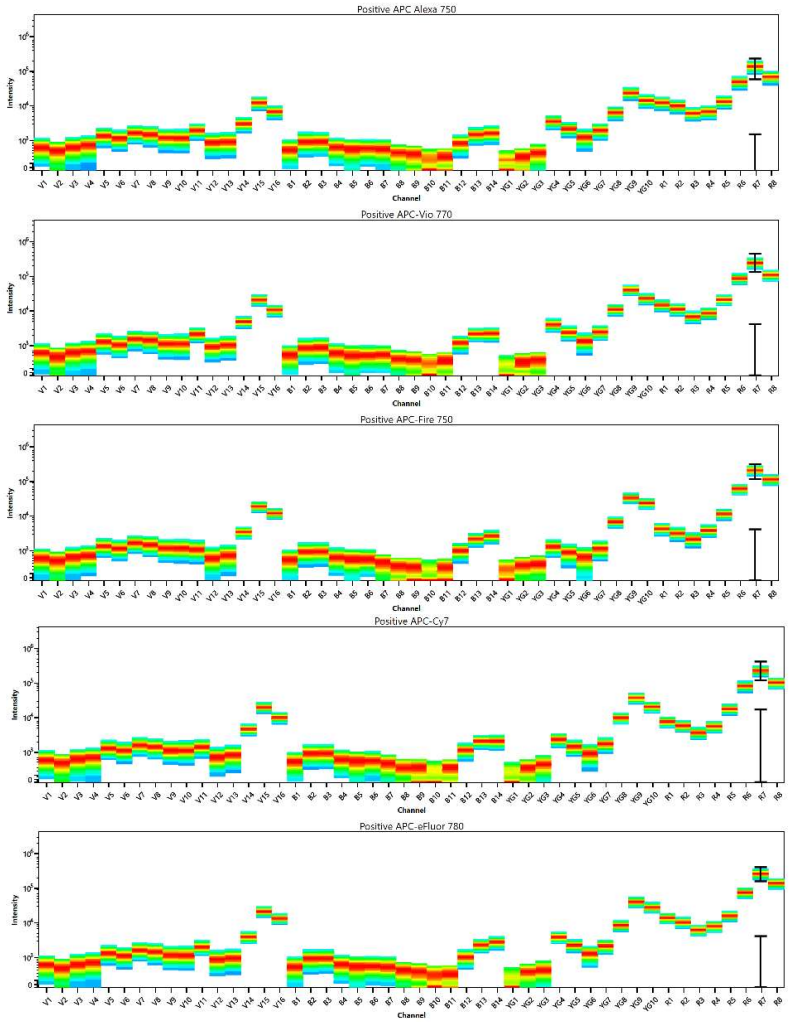
Red Laser Excitable Dyes with Similar Signatures (1 of 2)

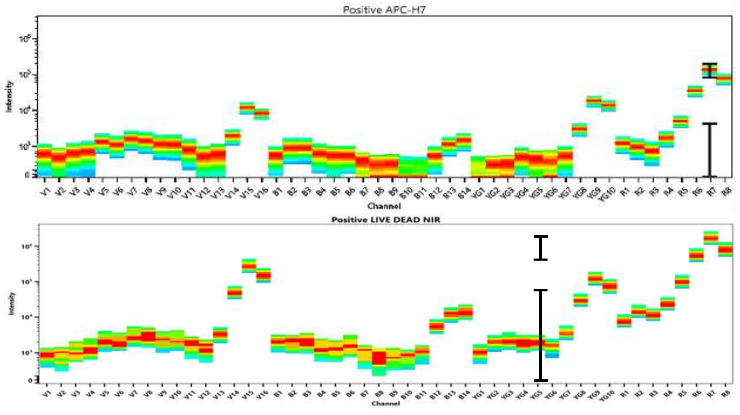
eFluor 660, Alexa Fluor 647, Vio 667, sVio 667 and Live/Dead Far Red



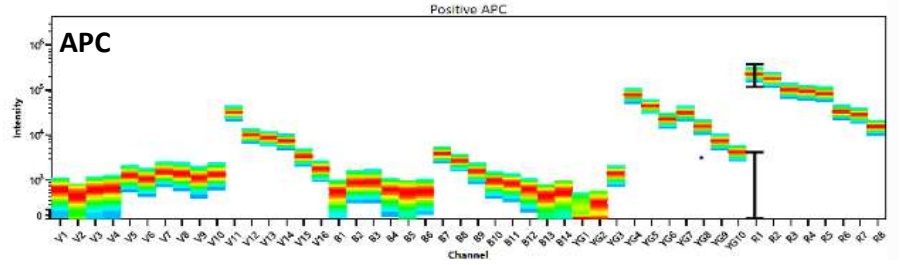
Red Laser Excitable Dyes with Similar Signatures (2 of 2)

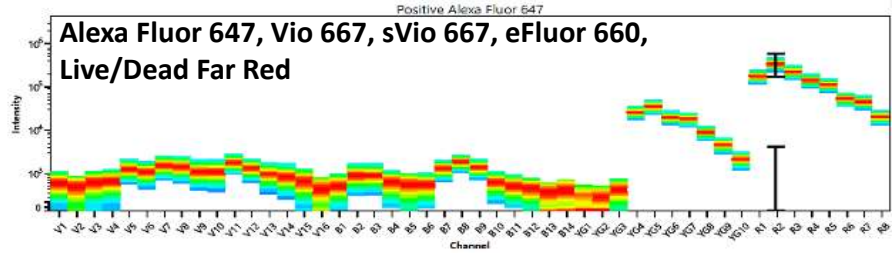
APC-Alexa 750, APC-Vio 770, APC/Fire 750, APC-Cy7, APC-eFluor 780, APC-H7 and Live/Dead NIR

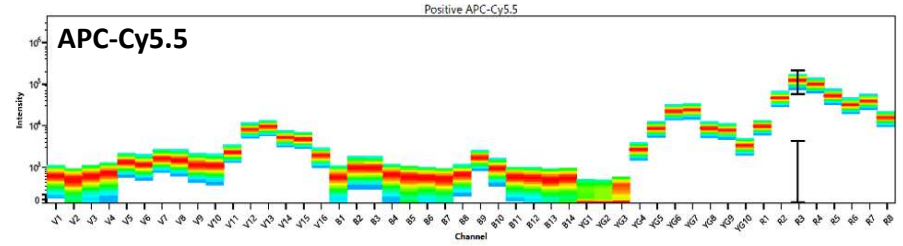


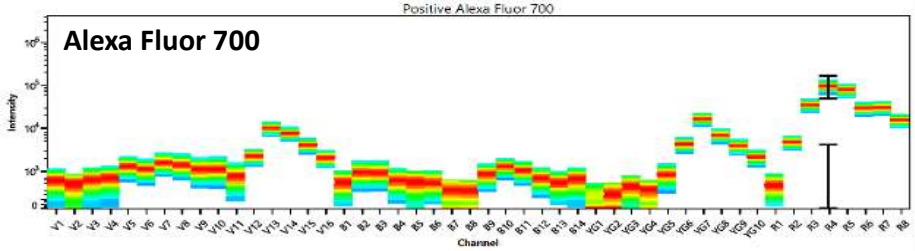


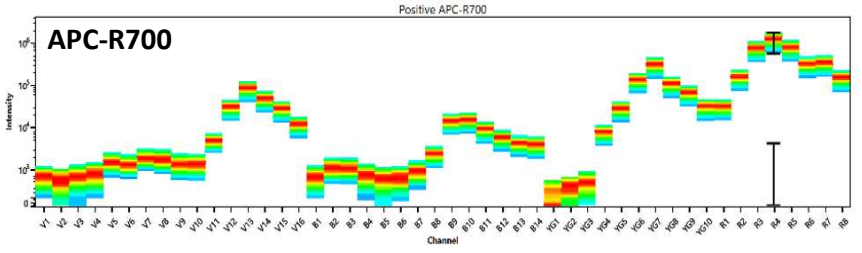
Red Laser Excitable Dyes with Unique Signatures

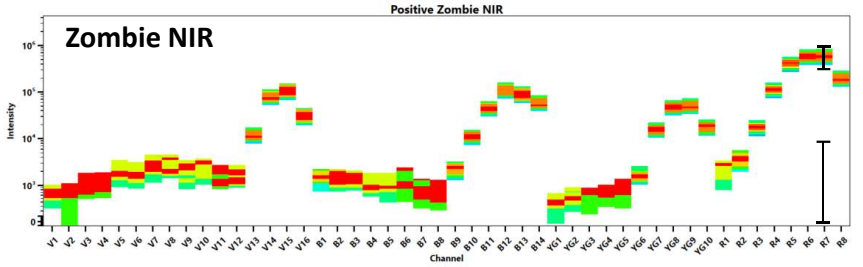


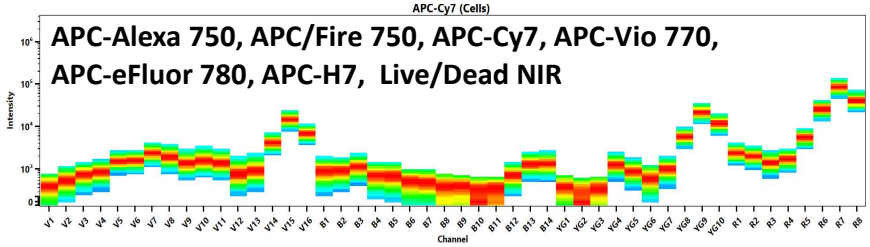






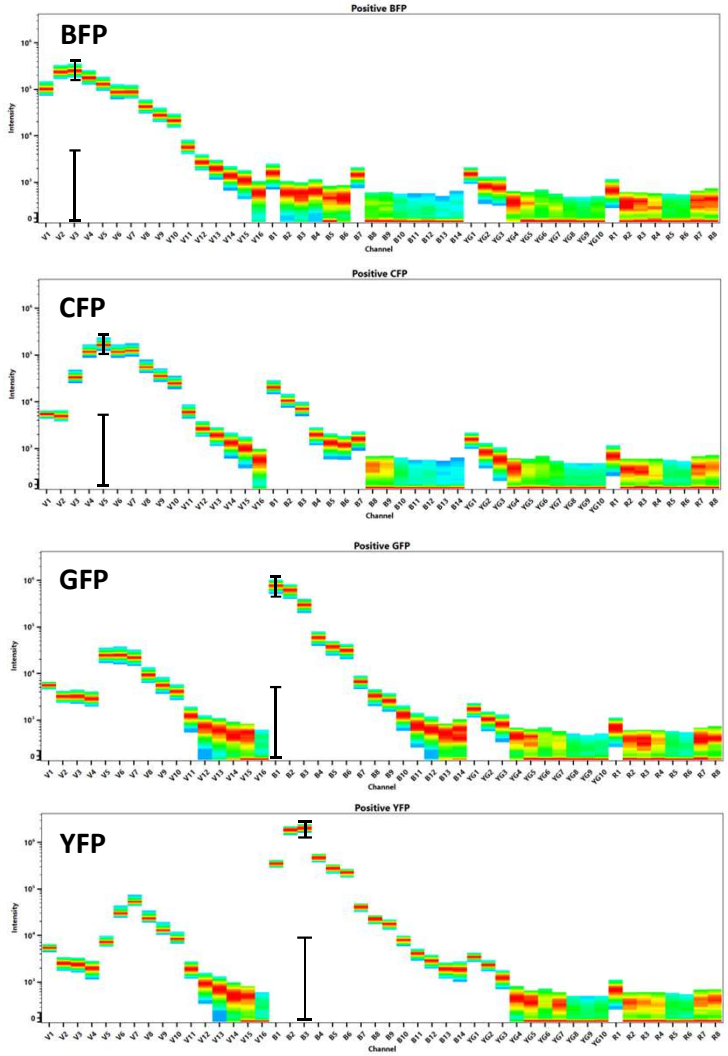


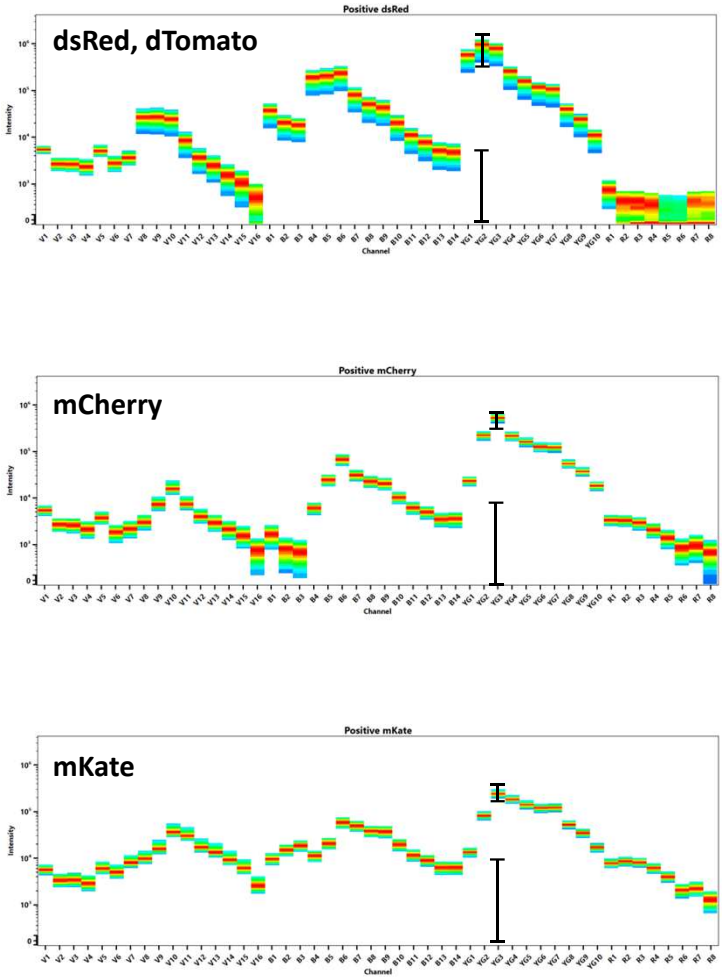




Fluorescent Protein Signatures

Fluorescent Proteins with Unique Signatures





Peak Channels & Possible Combination of Dyes

Fluorochrome Peak Channels

Violet Excited Fluors	Peak Channel
BV421	V1
Alexa 405, SuperBright 436	V2
eFluor450 , VioBlue, Pacific Blue	V3
BV480	V4
eFluor 506	V5
BV510, VioGreen	V7
BV570, Pacific Orange	V8
BV605, SuperBright 600, Qdot 605	V10
BV650, SuperBright 645, Qdot 655	V11
BV711, Super Bright 702, Qdot705	V13
BV750	V14
BV785, BV786, Qdot 800	V15
Blue Excited Fluors	
Vio 515, sVio 515, BB515	B1
Alexa Fluor 488, FITC, VioBright FITC	B2
Alexa Fluor 532	B3
PerCP	B8
PerCP-Cy5.5, BB700	B9
PerCP-eFluor 710, PerCP-Vio 700	B10
Yellow Green Excited Fluors	
PE	YG1
PE/Dazzle 594, PE-CF 594, PE-Texas Red, PE-eFluor 610	YG3
PE-Alexa Fluor 610	YG4
PE/Cy5	YG5
PE-Cy5.5, PE-Alexa Fluor 700	YG7
PE/Cy7, PE-Vio 770	YG9
Red Excited Fluors	
APC	R1
Alexa Fluor 647, Vio 667, sVio 667, eFluor 660	R2
APC-Cy5.5	R3
Alexa Fluor 700, APC-R700	R4
APC-Alexa750, APC/Fire 750, APC-Cy7, APC-Vio 770, APC-eFluor780, APC-H7	R7

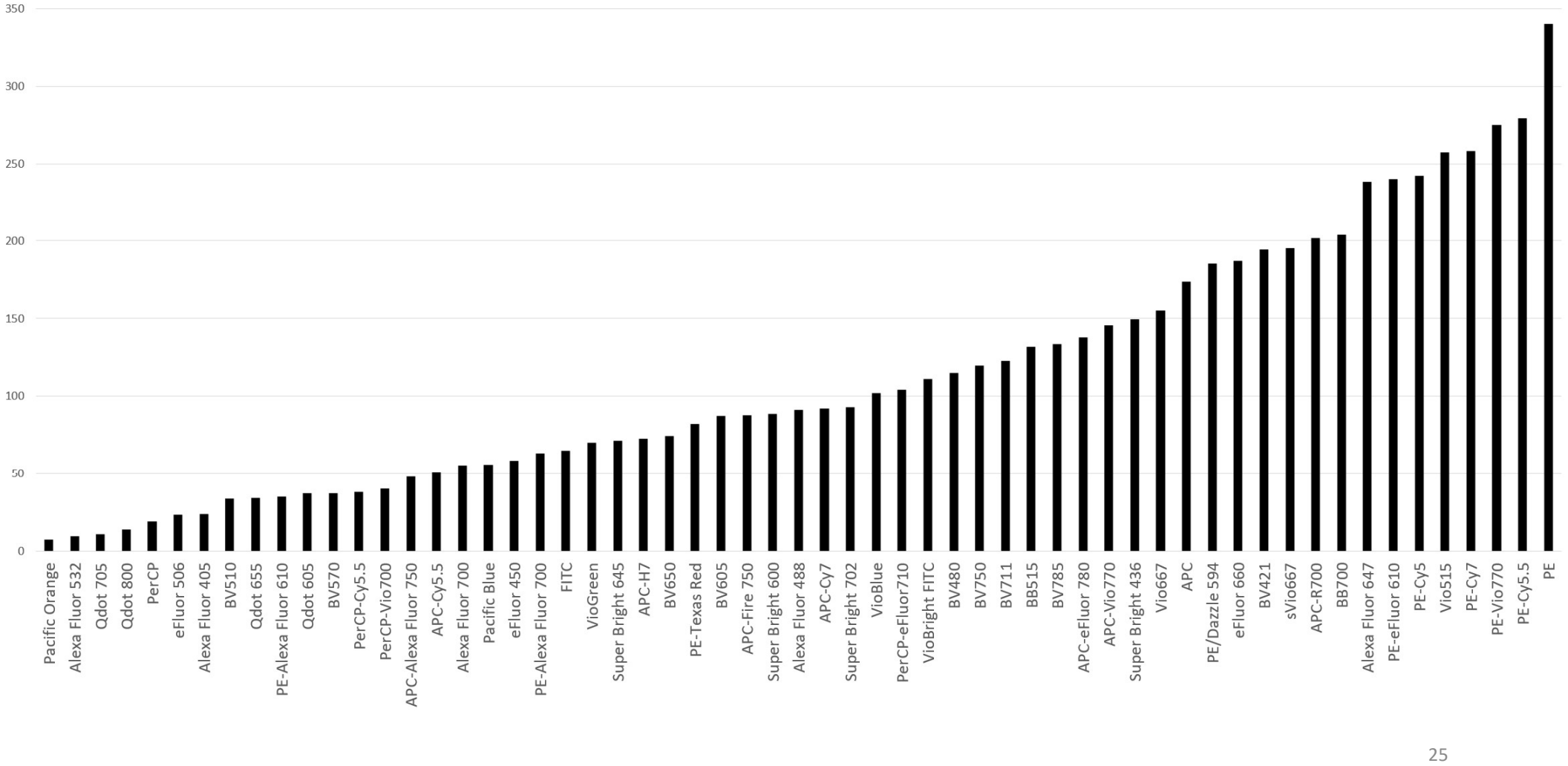
Example of 24 Dyes that Can Be Used in Combination (CAREFUL PANEL DESIGN IS NEEDED)

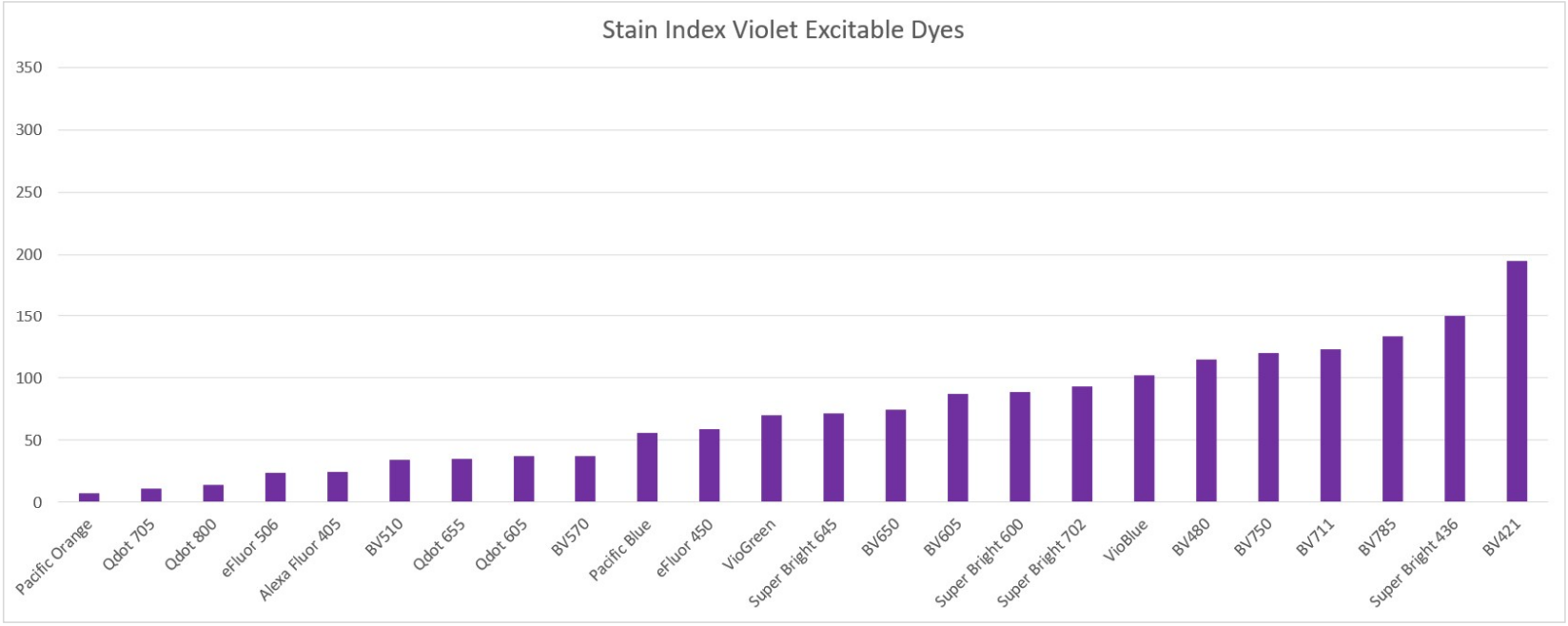
Violet Excited Fluors	Blue Excited Fluors	Yellow Green Excited Fluors	Red Excited Fluors
BV421	BB515	PE	APC
SuperBright 436	Alexa Fluor 488	PE/Dazzle 594	Alexa Flour 647
eFluor450	Alexa Fluor 532	PE/Cy5	APC-R700
BV480	PerCP/Cy5.5	PE/Cy7	APC/Fire750
BV510	PerCP-eFluor 710		
BV570			
BV605			
BV650			
BV711			
BV750			
BV785			

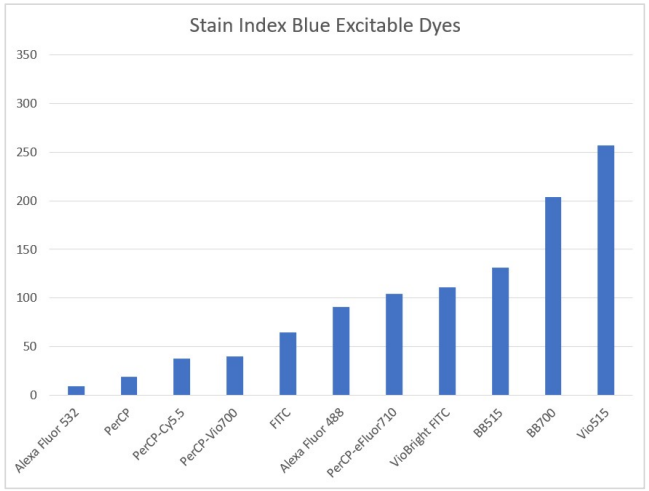
Stain Indexes

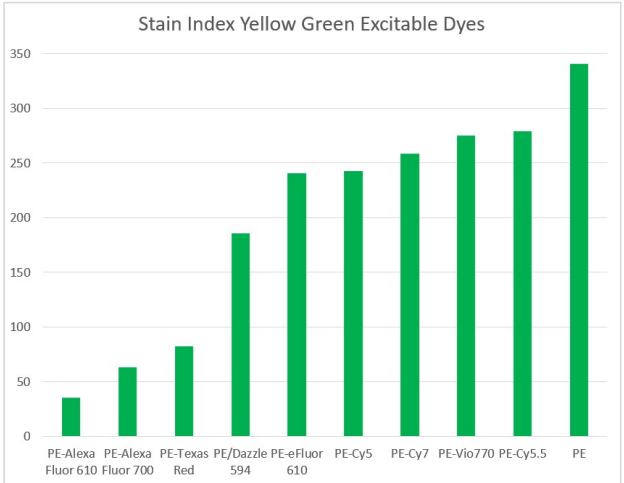
Data generated using CD4 staining in human PBMCs

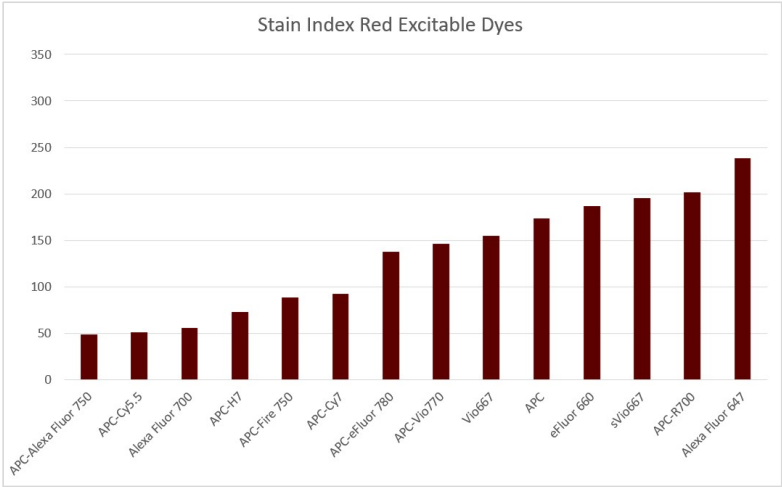
Stain Index Ranking - 59 Dyes




Stain Index Violet Excitable Dyes
350
300
250
200
150
100
50
0
Pacific Orange
Qdot 705
Qdot 800
eFluor 506
Alexa Fluor 405
BV510
Qdot 655
Qdot 605
BV570
Pacific Blue
eFluor 450
VioGreen
Super Bright 645
BV650
BV605
Super Bright 600
Super Bright 702
VioBlue
BV480
BV750
BV711
BV785
Super Bright 436
BV421


Stain Index Blue Excitable Dyes
350
300
250
200
150
100
50
0
Alexa Fluor 532
PerCP
PerCP-Cy5.5
PerCP-Vio700
FITC
Alexa Fluor 488
PerCP-eFluor710
VioBright FITC
BB515
BB700
Vio515


Stain Index Yellow Green Excitable Dyes
350
300
250
200
150
100
50
0
PE-Alexa Fluor 610
PE-Alexa Fluor 700
PE-Texas Red
PE/Dazzle 594
PE-eFluor 610
PE-Cy5
PE-Cy7
PE-Vio770
PE-Cy5.5
PE

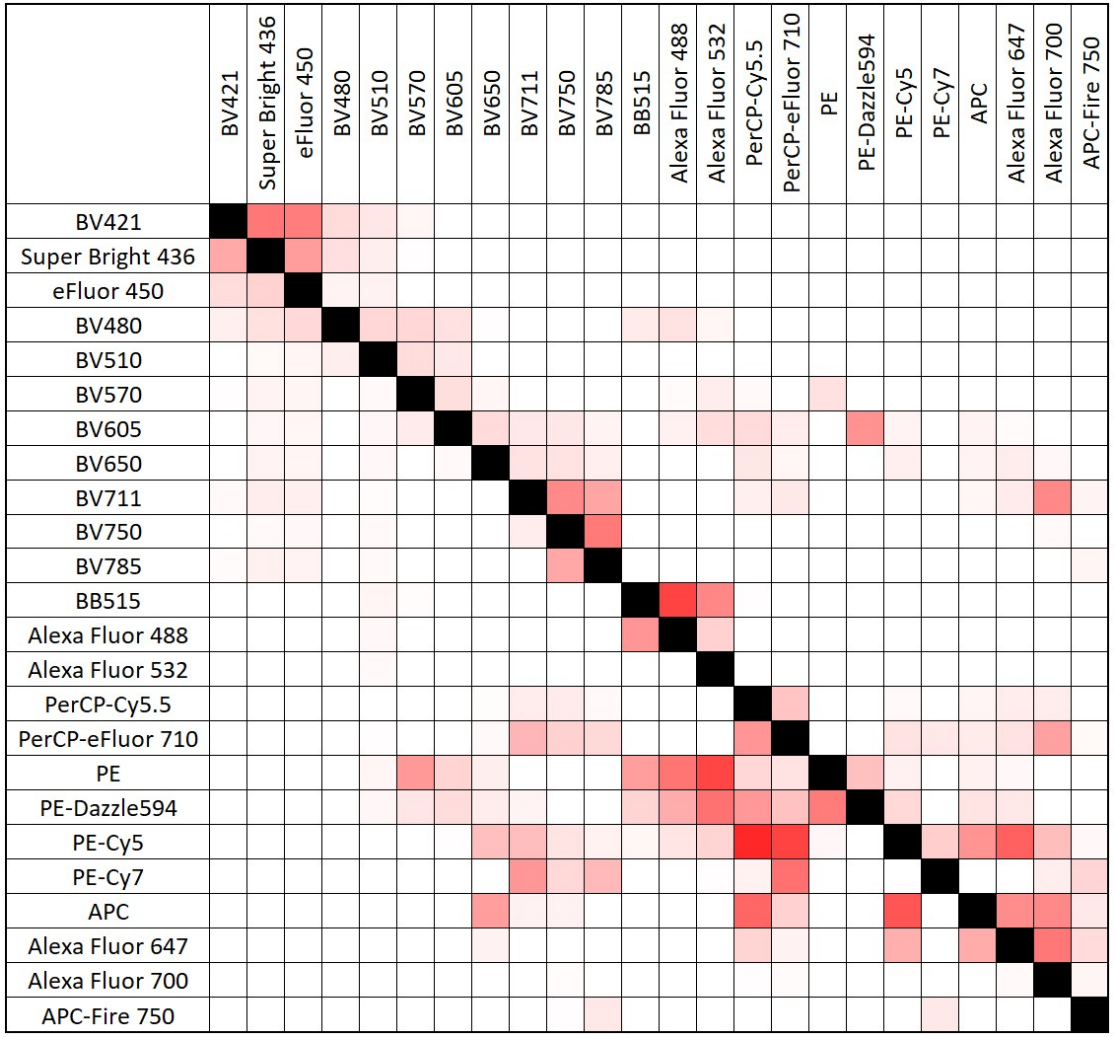

Stain Index Red Excitable Dyes
350
300
250
200
150
100
50
0
APC-Alexa Fluor 750
APC-Cy5.5
Alexa Fluor 700
APC-H7
APC-Fire 750
APC-Cy7
APC-eFluor 780
APC-Vio770
Vio667
APC
eFluor 660
sVio667
APC-R700
Alexa Fluor 647

Cross-Stain Index Matrix

Dyes used in combination need to have unique spectra AND need to be assessed in terms of spread that they introduce to other dyes.

For example PerCP-Cy5.5 and PE-Cy5.5 have distinct signatures, but since both dyes emit in the same wavelength range and significant spread is introduced by PE-Cy5.5, careful panel design is needed when used in combination.

Spread matrix for 24 fluors that can be used in combination



To read this table: spread of fluor in the row impacts resolution of the fluor in the column. Red means the fluor in that row has significant spread into the dye in the column (for example BB515 into AF488). Areas in bright pink and red indicate pairs for which more attention to panel design is needed.