

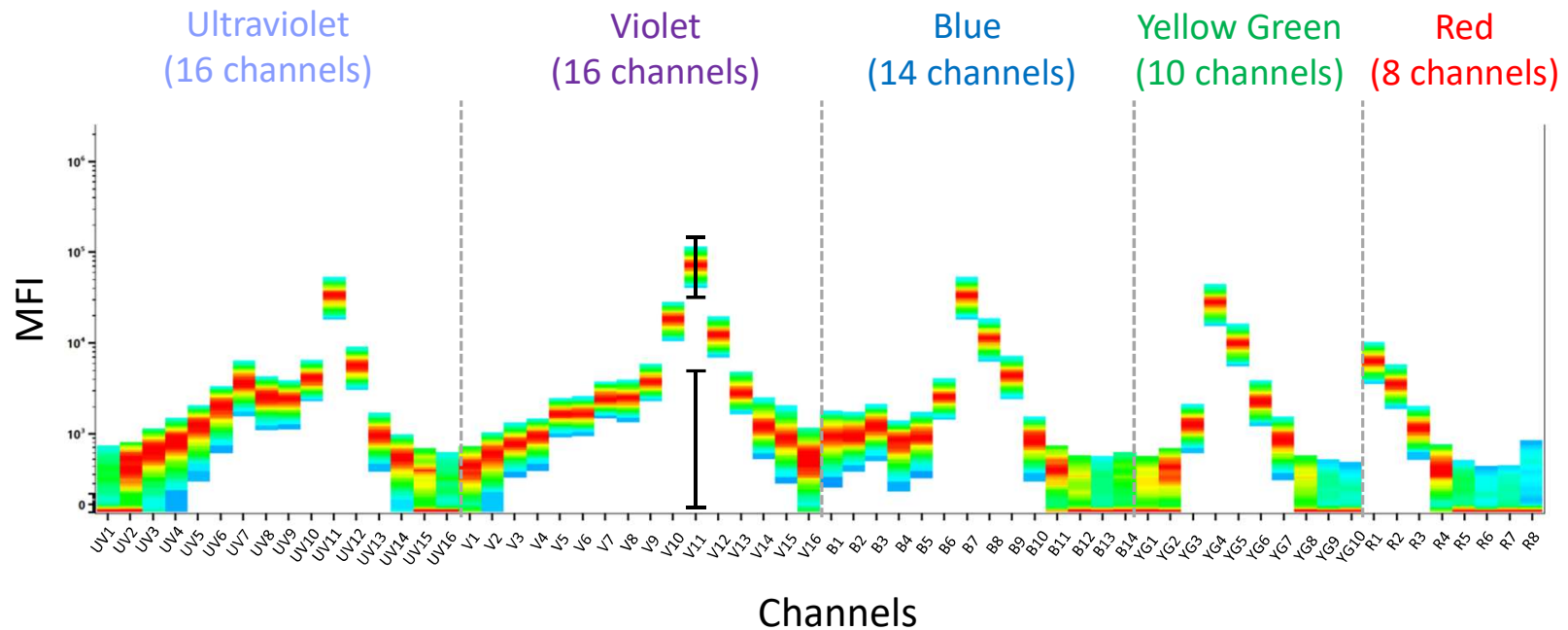
Cytek<sup>®</sup> Aurora  
Fluorochrome Selection Guidelines  
5 Laser 16UV-16V-14B-10YG-8R

# Fluorochrome Signatures

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

Look for dyes with unique spectra and consider spread introduced by the dyes when designing multicolor panels (see slide 29).

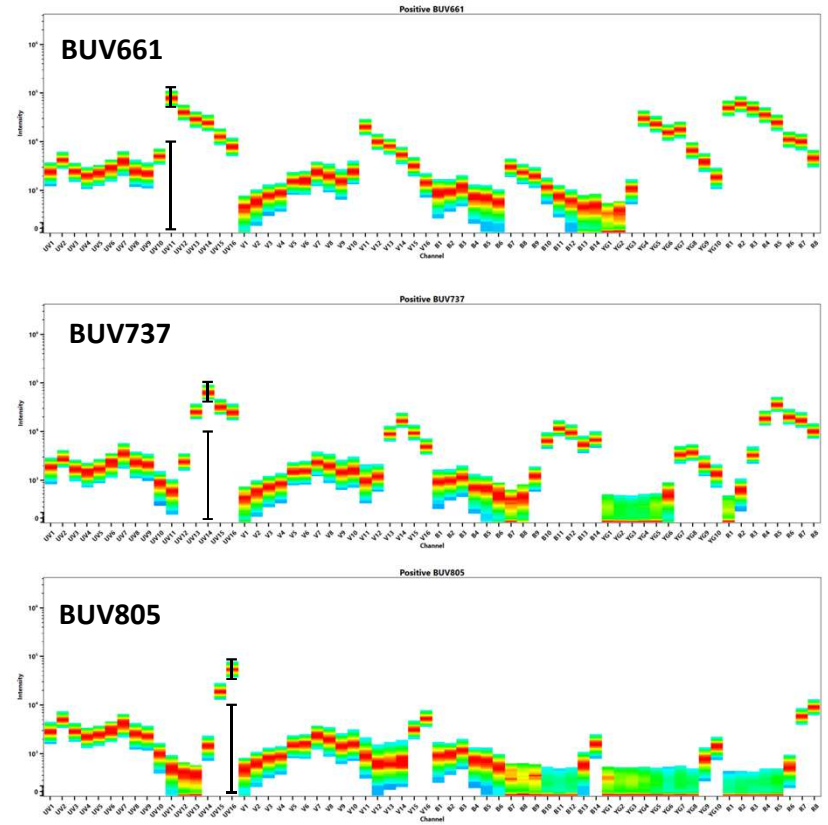
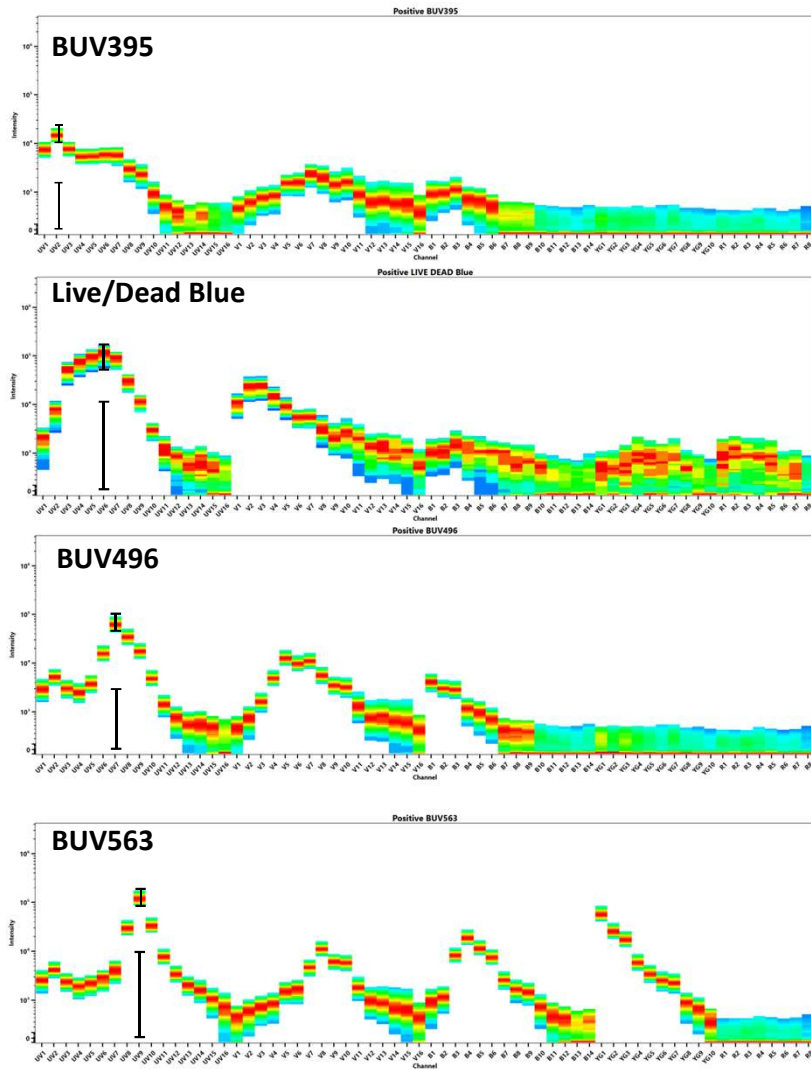
# How to Read Full Spectrum Fluorochrome Signatures



*This dye is excited by all 5 lasers. The peak channel (indicated by the black bar) is in channel V11, and it has secondary emission in channels UV11, 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 Ultraviolet Laser

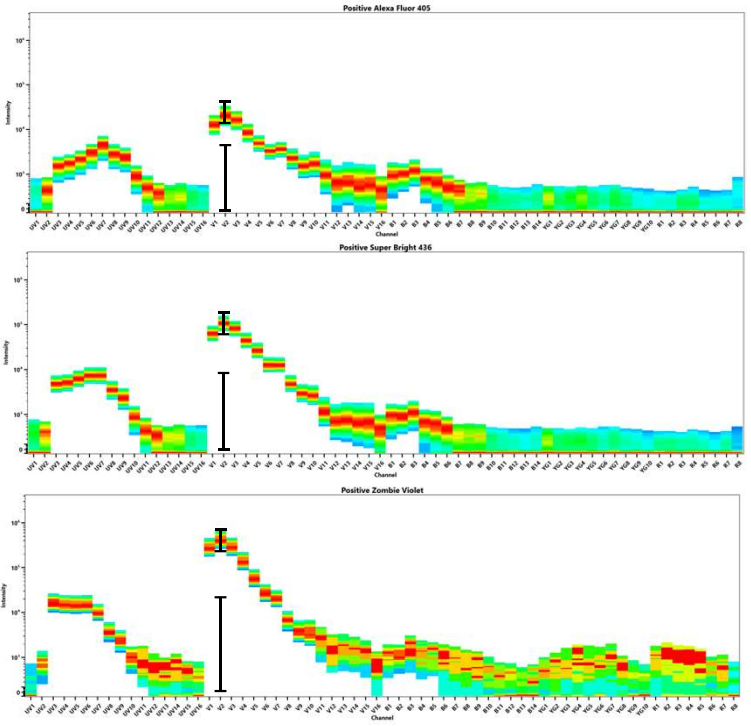
# Ultraviolet Laser Excitable Dyes with Unique Signatures



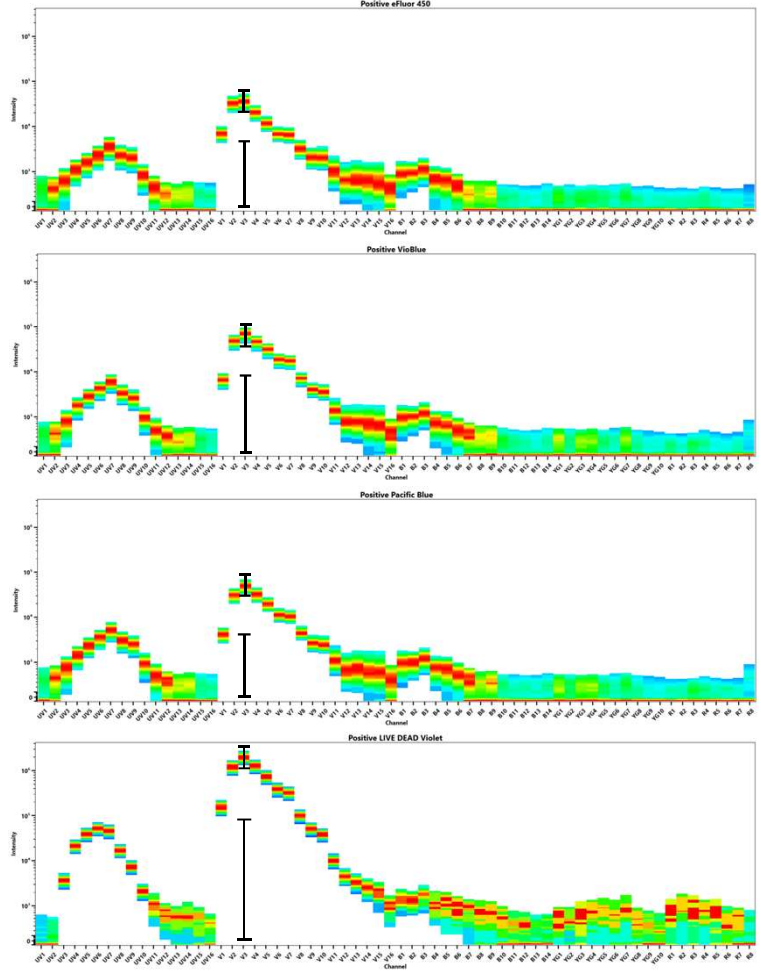
# Dyes Primarily Excited by the Violet Laser

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

Alexa Fluor 405, Super Bright 436 and Zombie Violet

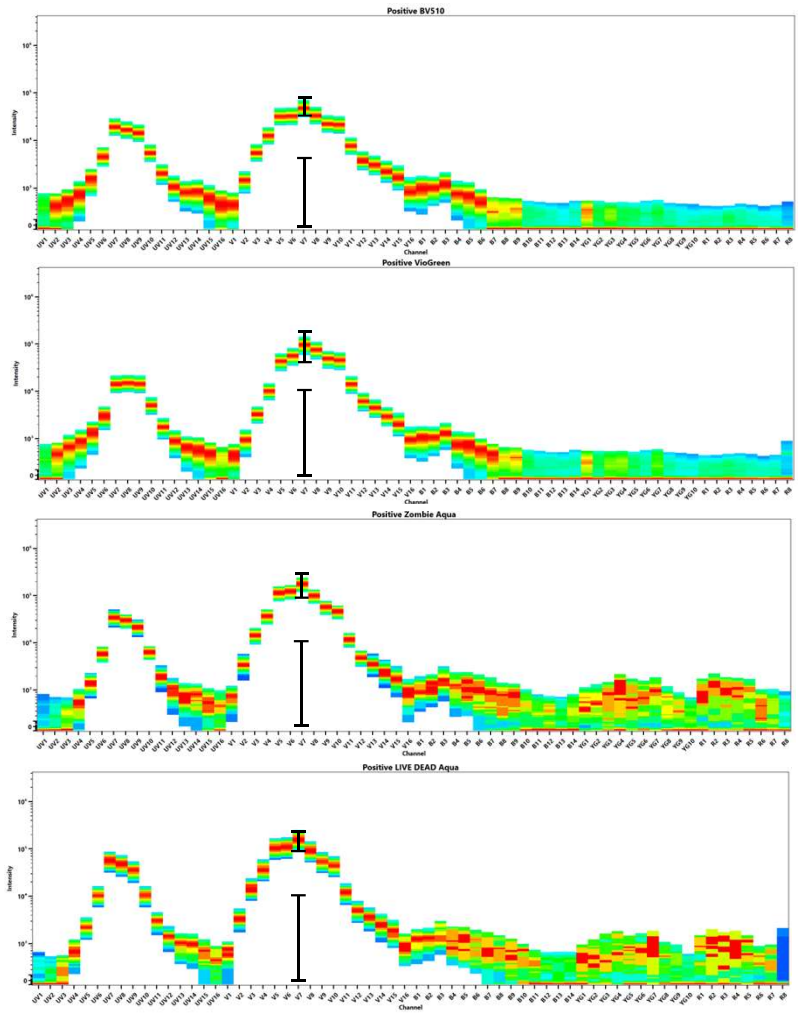


eFluor 450, VioBlue, Pacific Blue and Live/Dead Violet

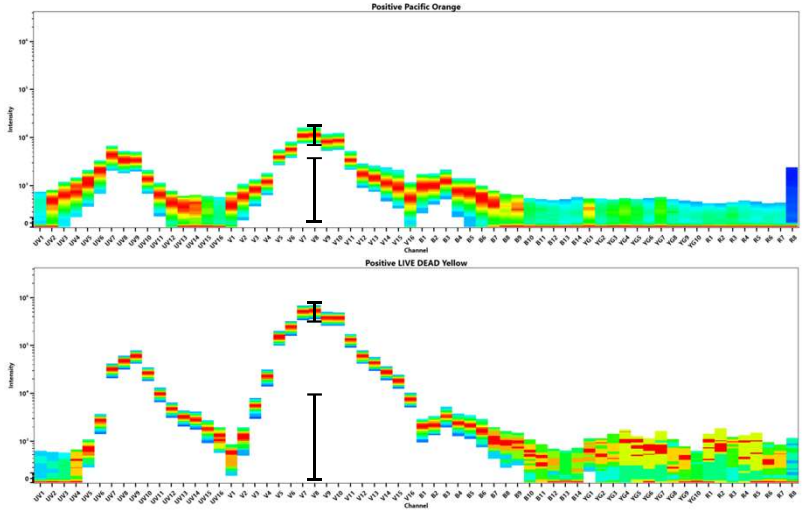


# Violet Laser Excitable Dyes with Similar Signatures (2 of 3)

BV510, VioGreen, Zombie Aqua and Live/Dead Aqua



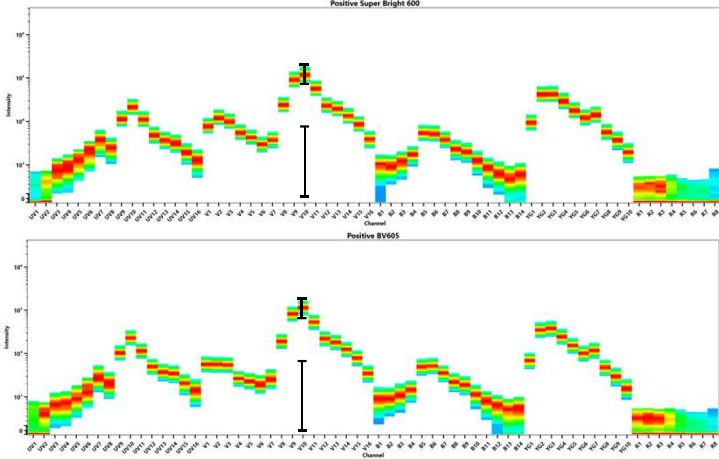
Pacific Orange and Live/Dead Yellow



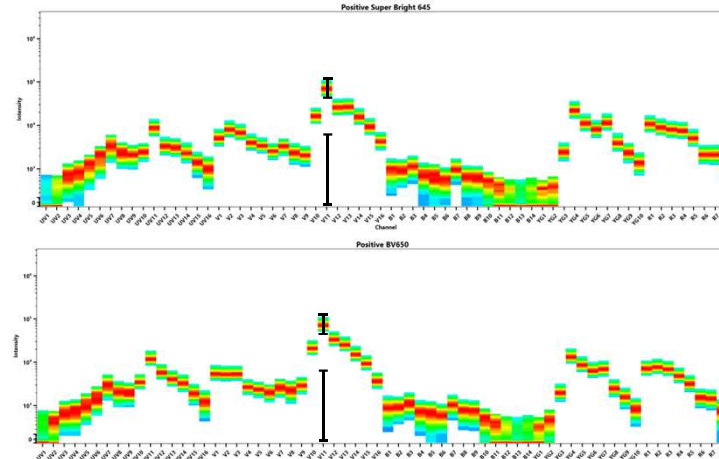


# Violet Laser Excitable Dyes with Similar Signatures (3 of 3)

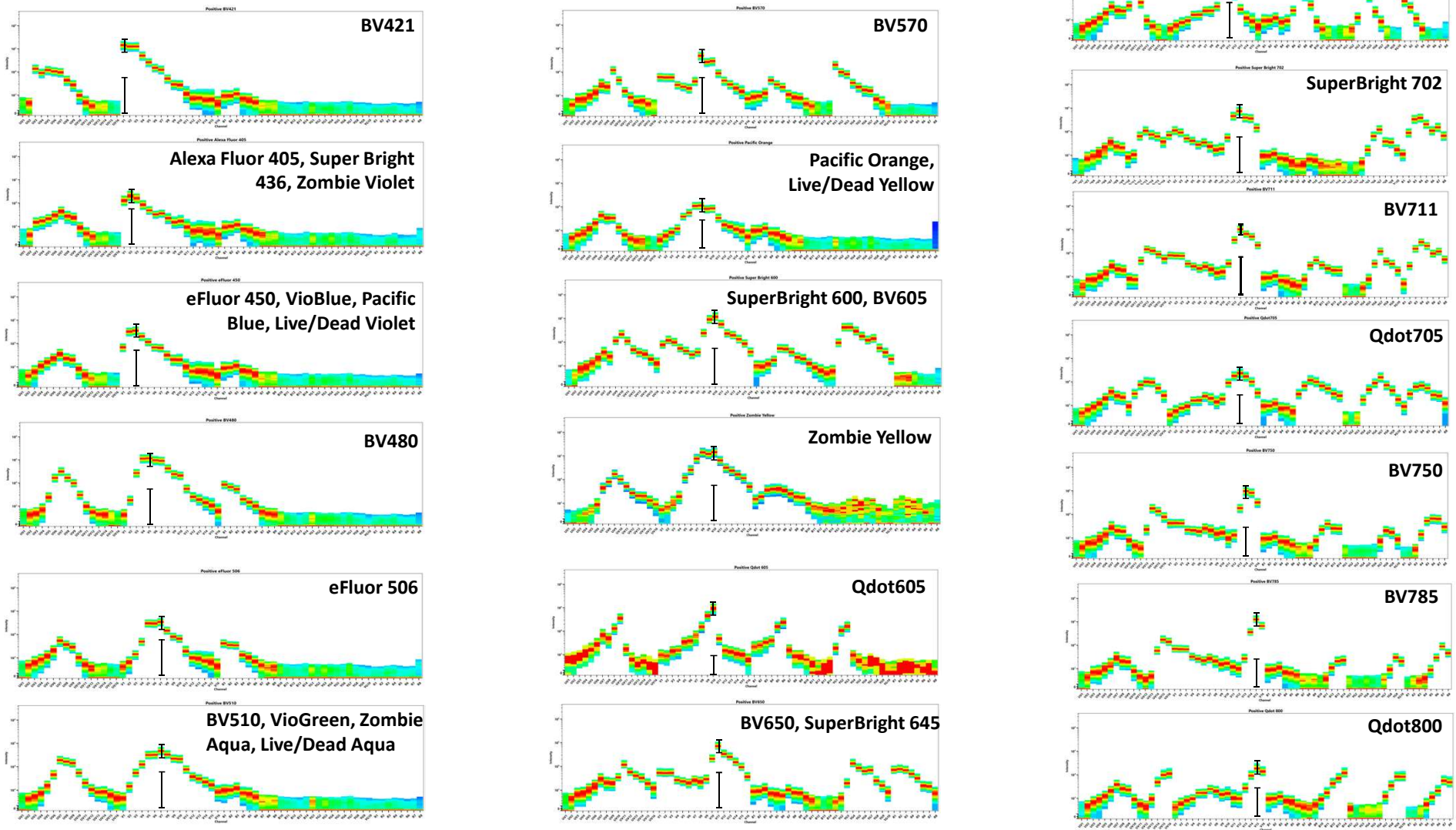
Super Bright 600 and BV605



Super Bright 645 and BV650



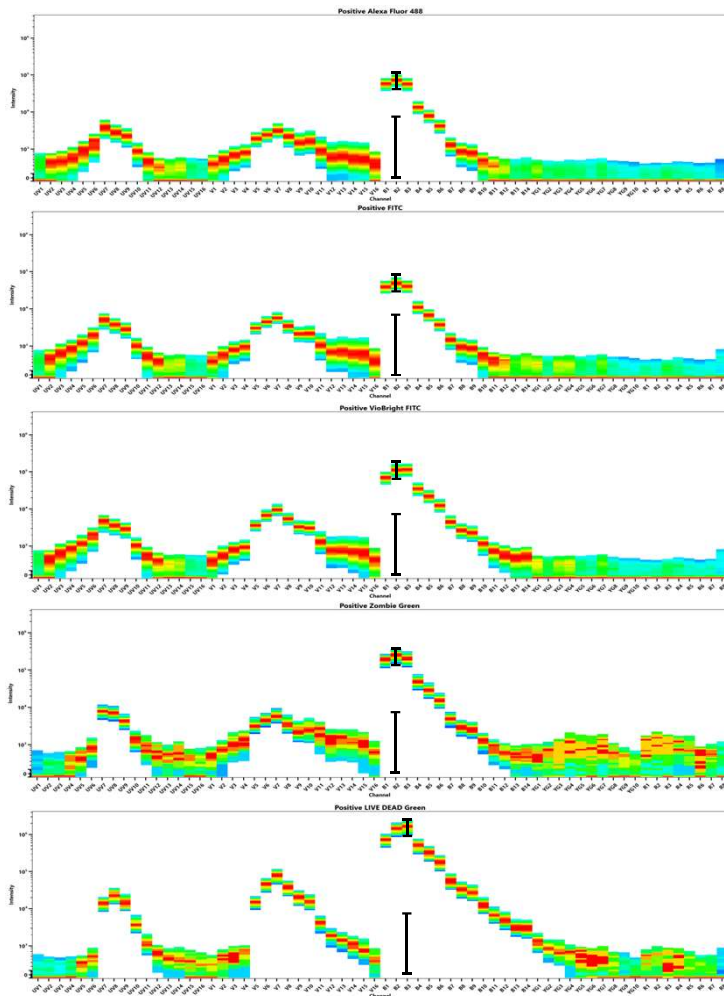
# Violet Laser Excitable Dyes with Unique Signatures



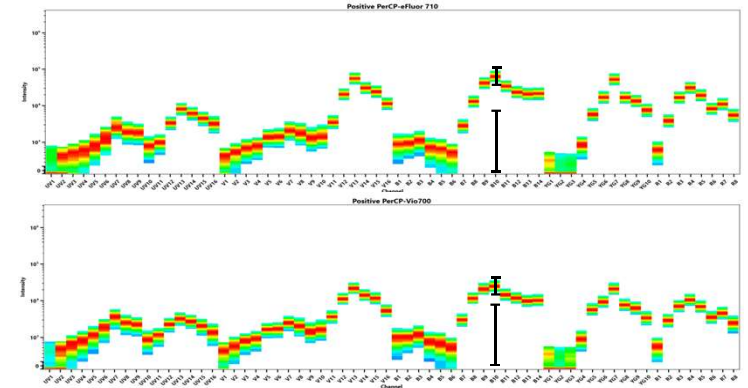
# Dyes Primarily Excited by the Blue Laser

# Blue Laser Excitable Dyes with Similar Signatures

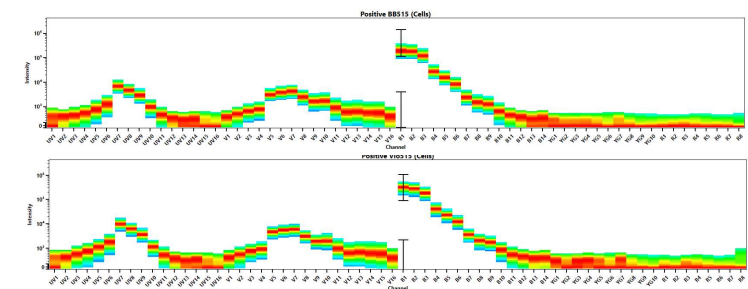
Alexa Fluor 488, FITC, VioBright FITC, Zombie Green and Live Dead Green



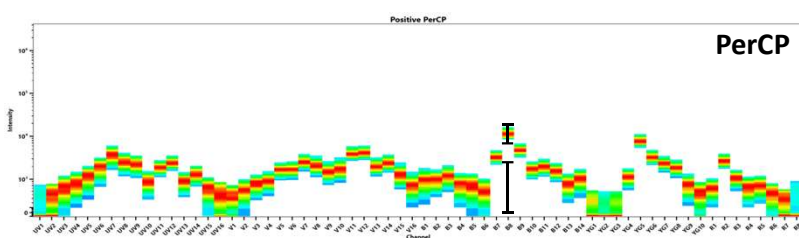
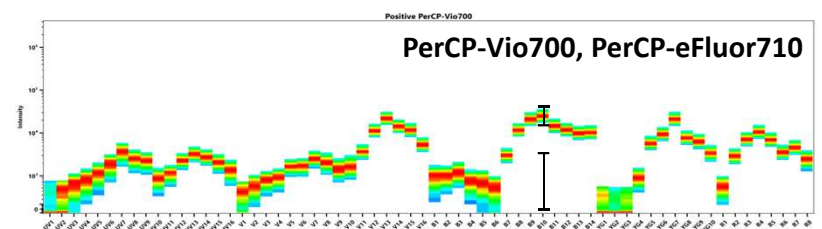
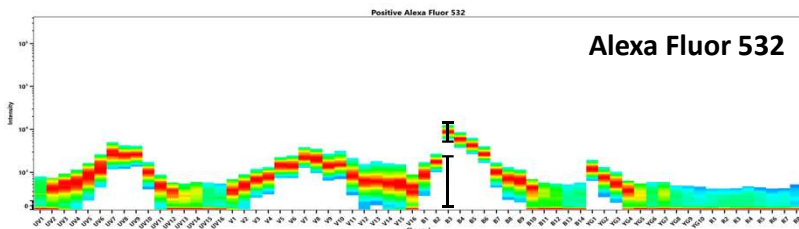
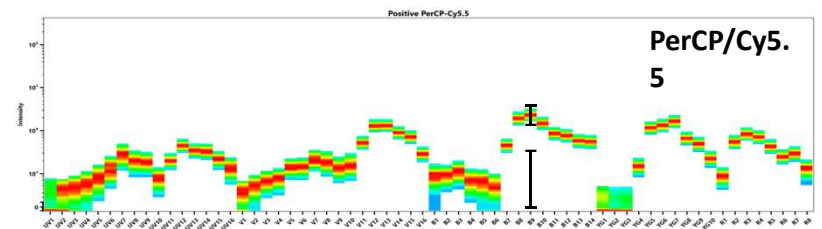
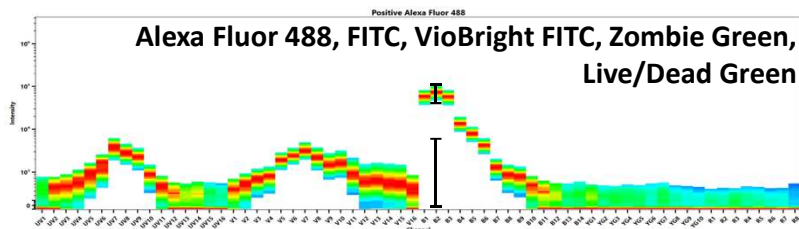
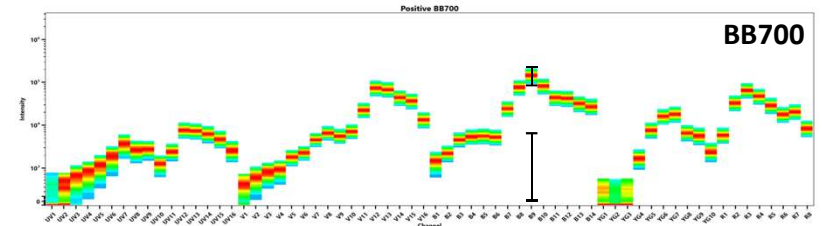
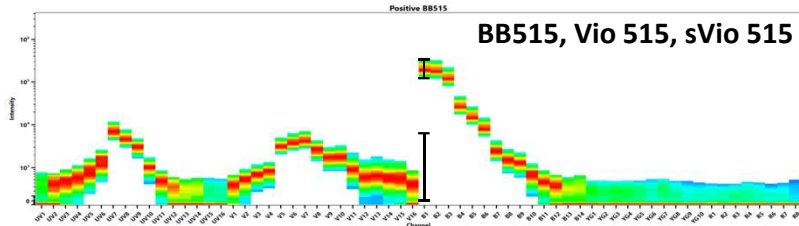
PerCP-eFluor 710 and PerCP-Vio 710



BB515 and Vio515



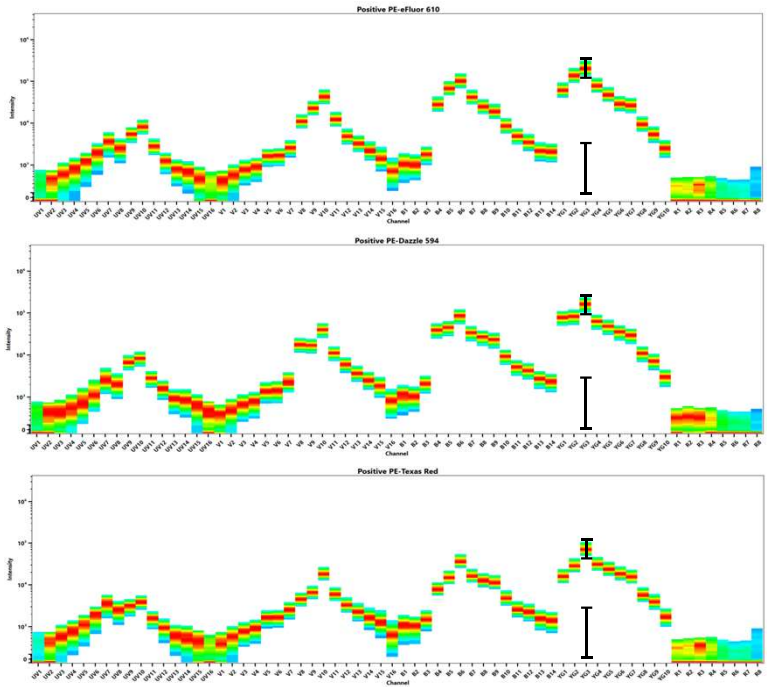
# Blue Laser Excitable Dyes with Unique Signatures



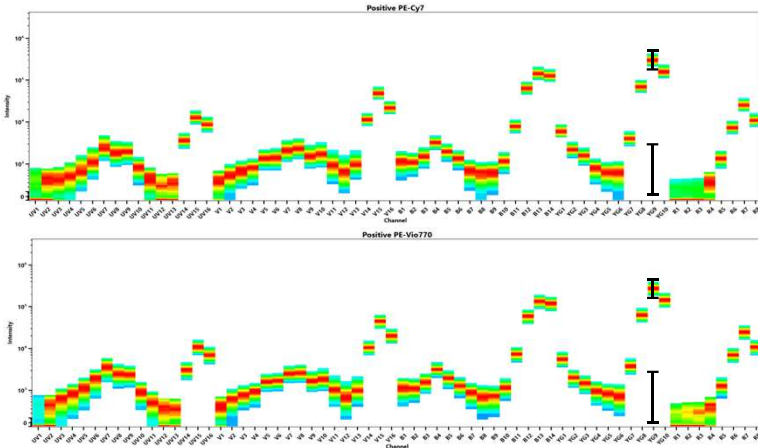
# Dyes Primarily Excited by the Yellow Green Laser

# Yellow Green Laser Excitable Dyes with Similar Signatures

PE-eFluor 610, PE/Dazzle 594, PE-CF594, PE-Texas Red

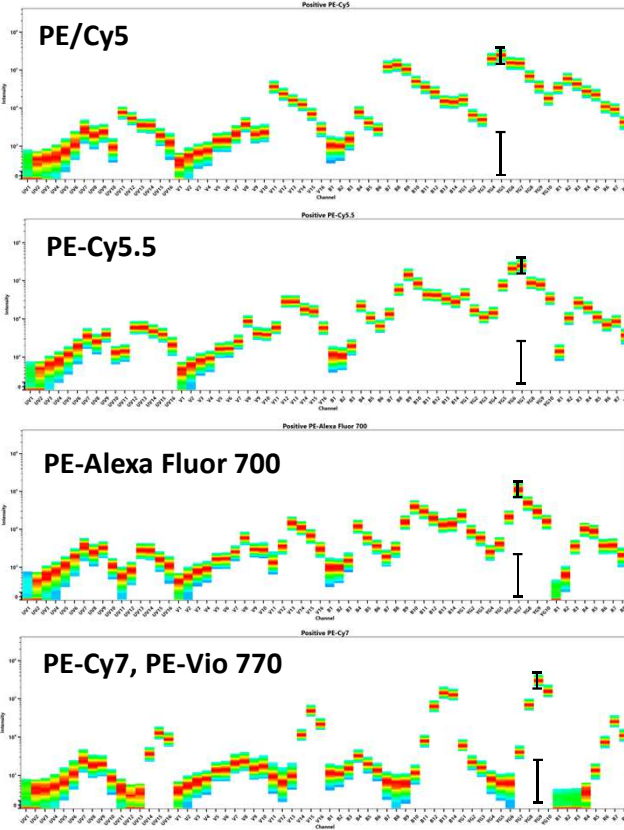
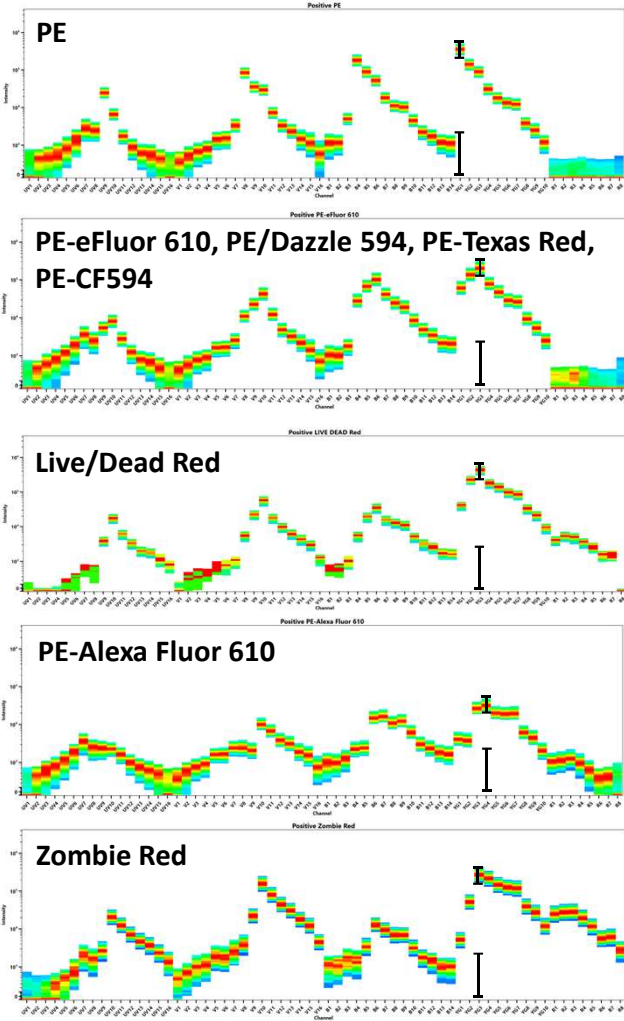


PE/Cy7 and PE-Vio 770





# Yellow Green Laser Excitable Dyes with Unique Signatures

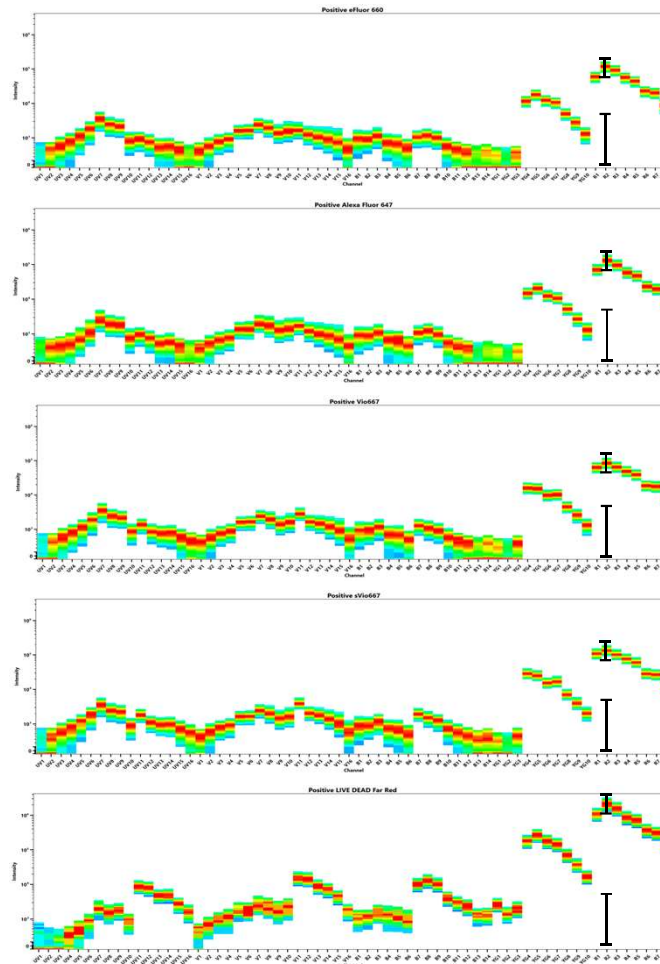




# Dyes Primarily Excited by the Red Laser

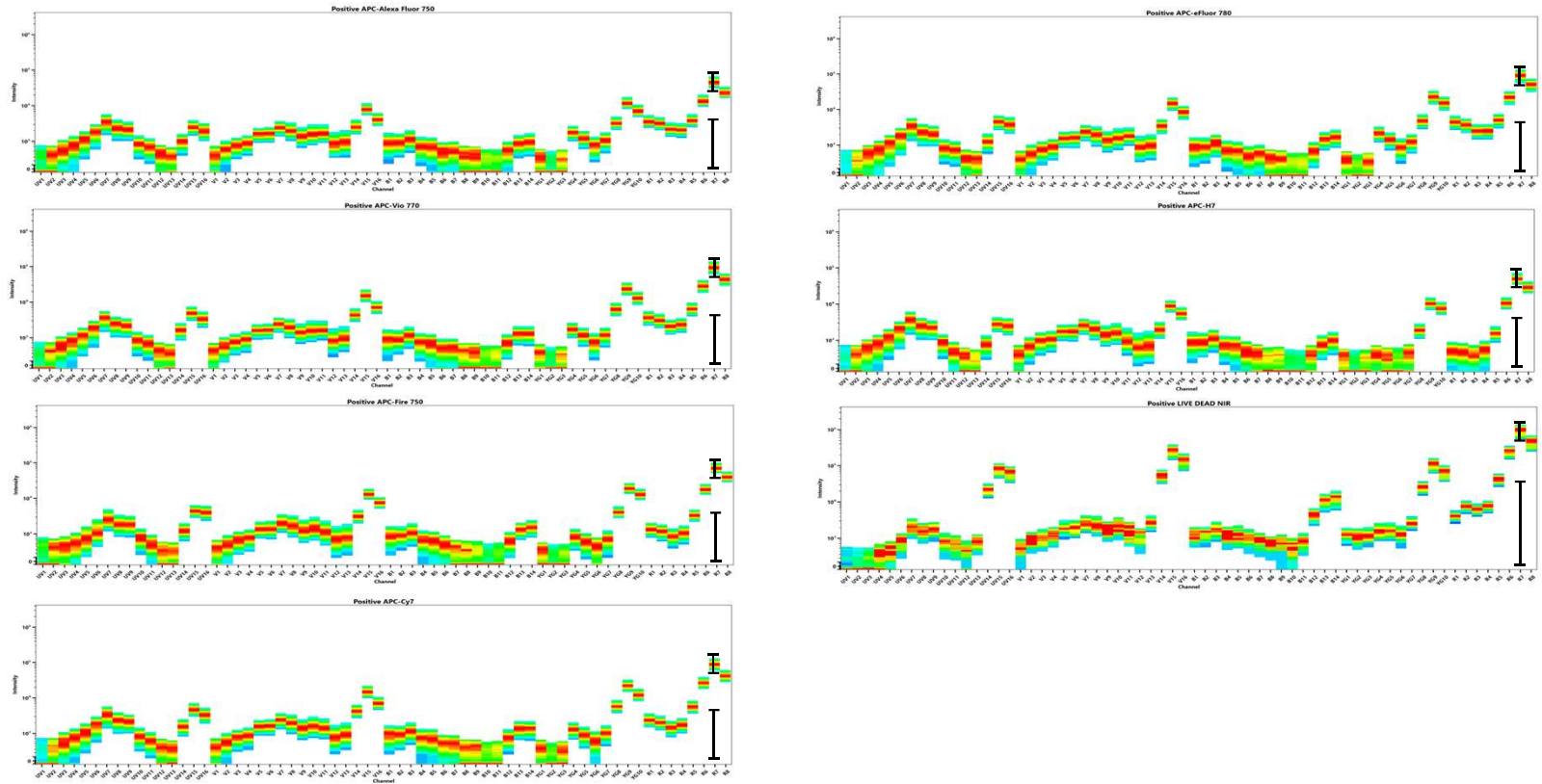
# Red Laser Excitable Dyes with Similar Signatures

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

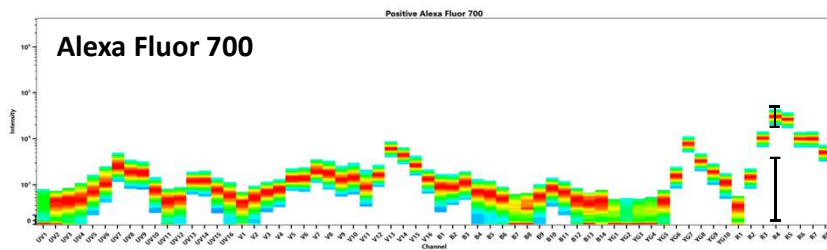
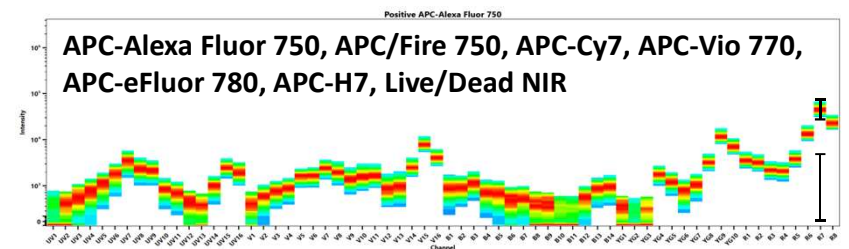
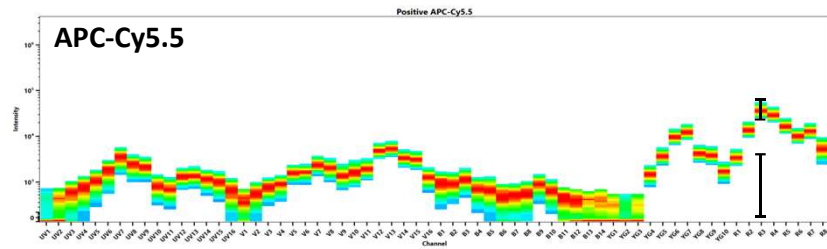
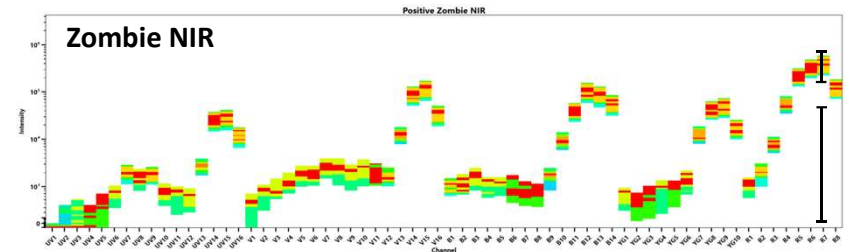
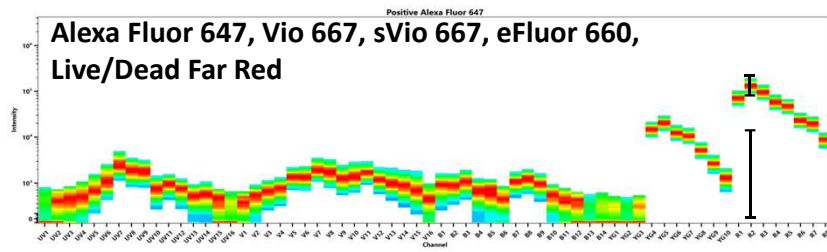
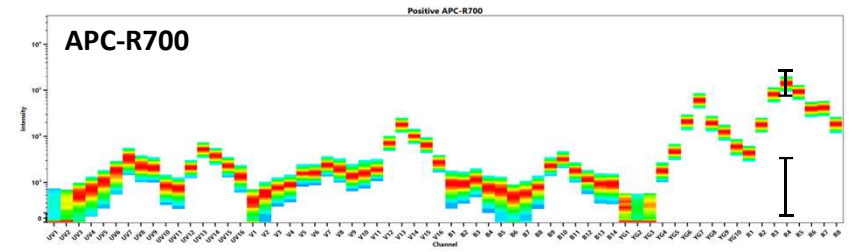
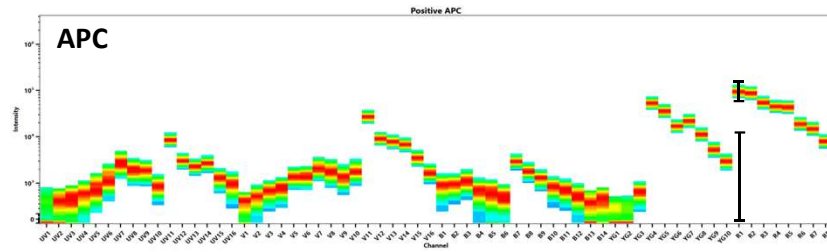


# Red Laser Excitable Dyes with Similar Signatures

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

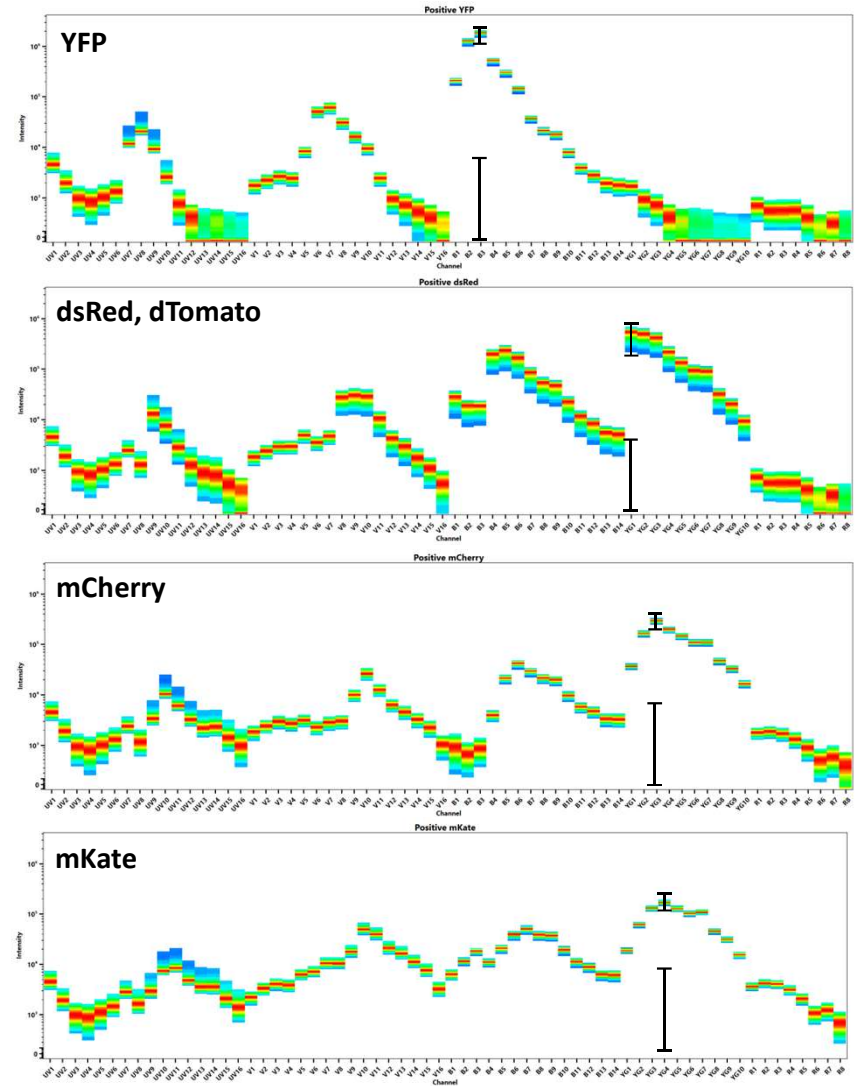
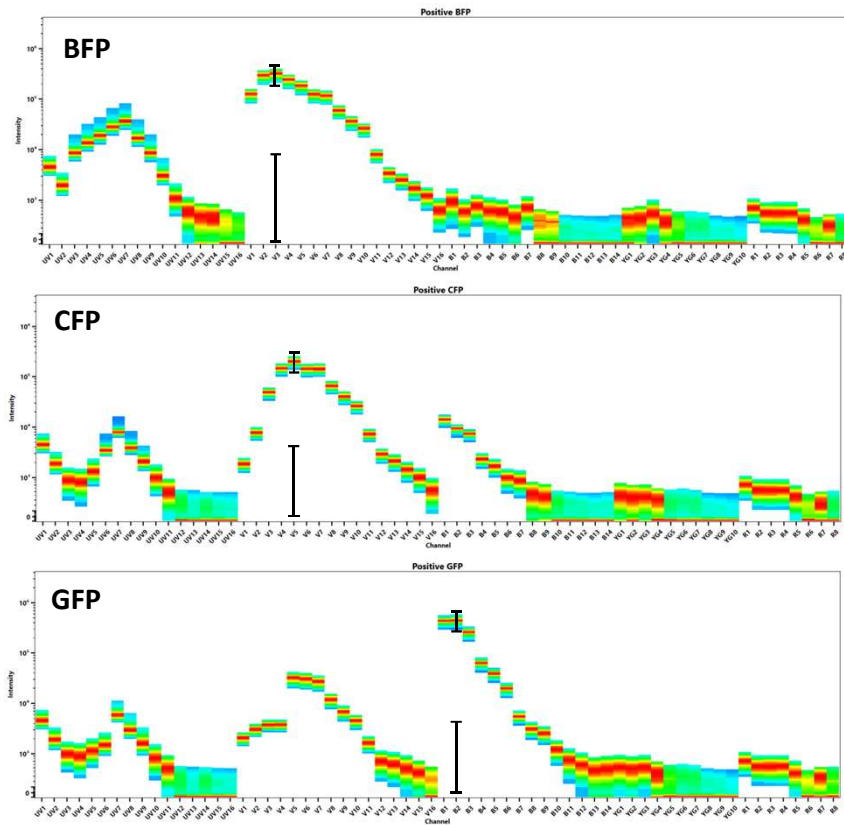


# Red Laser Excitable Dyes with Unique Signatures



# Fluorescent Protein Signatures

# Fluorescent Proteins



# Peak Channels & Possible Combination of Dyes

# Fluorochrome Peak Channels

UV Excited Fluors	Peak Channel
BUV395	UV2
BUV496	UV7
BUV563	UV9
BUV661	UV11
BUV737	UV14
BUV805	UV16
Violet Excited Fluors	Peak Channel
BV421	V1
Alexa Fluor 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, SuperBright 702, Qdot705	V13
BV750	V14
BV785, BV786, Qdot 800	V15
Blue Excited Fluors	Peak Channel
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	Peak Channel
PE	YG1
PE/Dazzle 594, PE-CF594, PE-TexasRed, PE-eFluor 610	YG3
PE-Alexa Fluor 610	YG4
PE/Cy5	YG5
PE-Cy5.5, PE-AlexaFluor 700	YG7
PE/Cy7, PE-Vio 770	YG9
Red Excited Fluors	Peak Channel
APC	R1
Alexa Fluor 647, Vio 667, sVio 667, efluor660	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



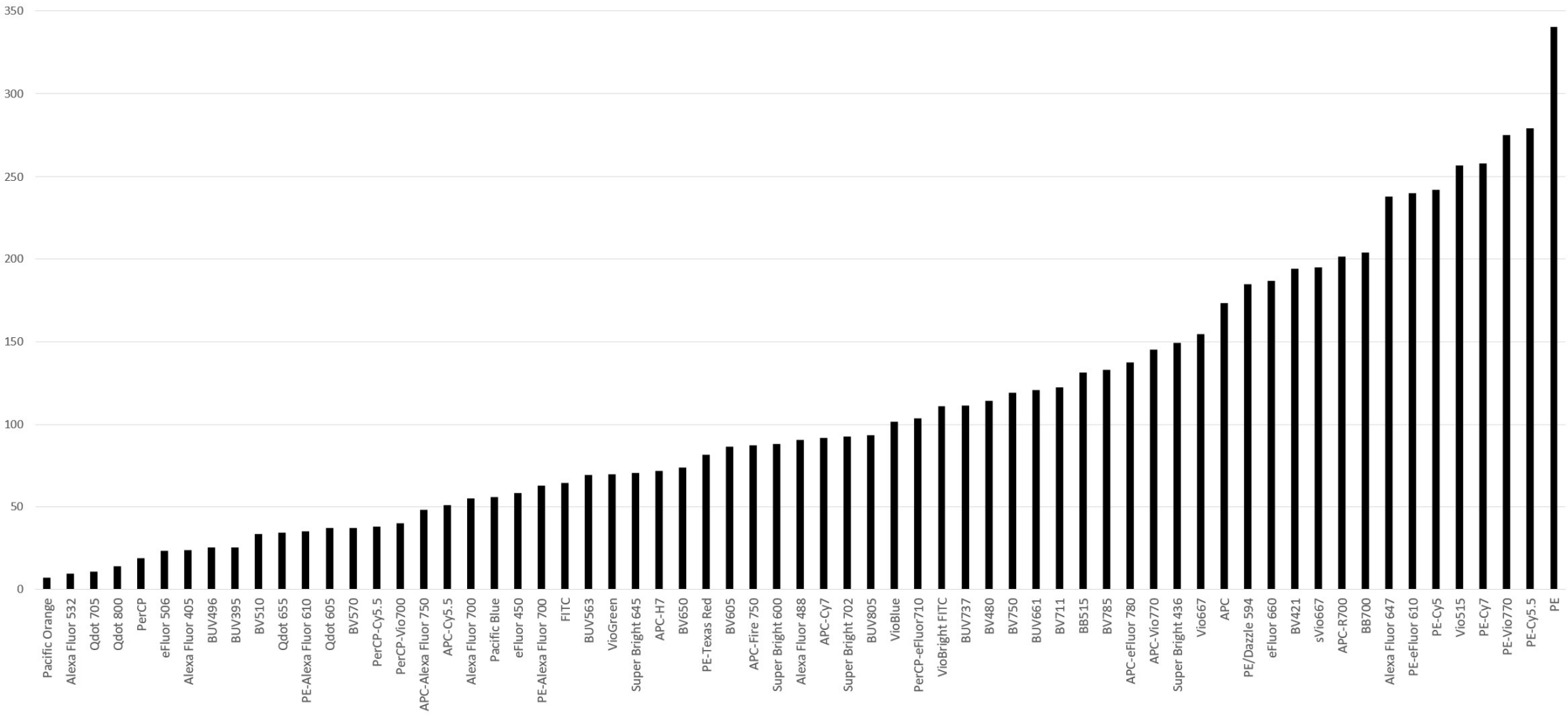
# Example of 30 Dyes that Can Be Used in Combination (CAREFUL PANEL DESIGN IS NEEDED)

UV Excited Fluors	Violet Excited Fluors	Blue Excited Fluors	Yellow Green Excited Fluors	Red Excited Fluors
BUV395	BV421	BB515	PE	APC
BUV496	Super Bright 436	Alexa Fluor 488	PE/Dazzle 594	Alexa Fluor 647
BUV563	efluor450	Alexa Fluor 532	PE/Cy5	APC-R700
BUV661	BV480	PerCP/Cy5.5	PE/Cy7	APC/Fire 750
BUV737	BV510	PerCP-eFluor 710		
BUV805	BV570			
	BV605			
	BV650			
	BV711			
	BV750			
	BV785			

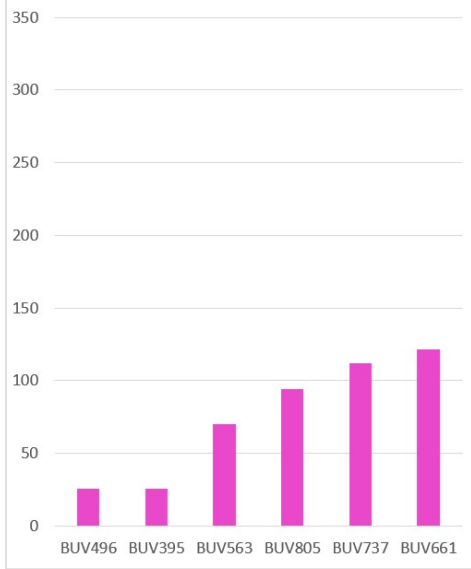
# Stain Indexes

Data generated using CD4 staining in human PBMCs

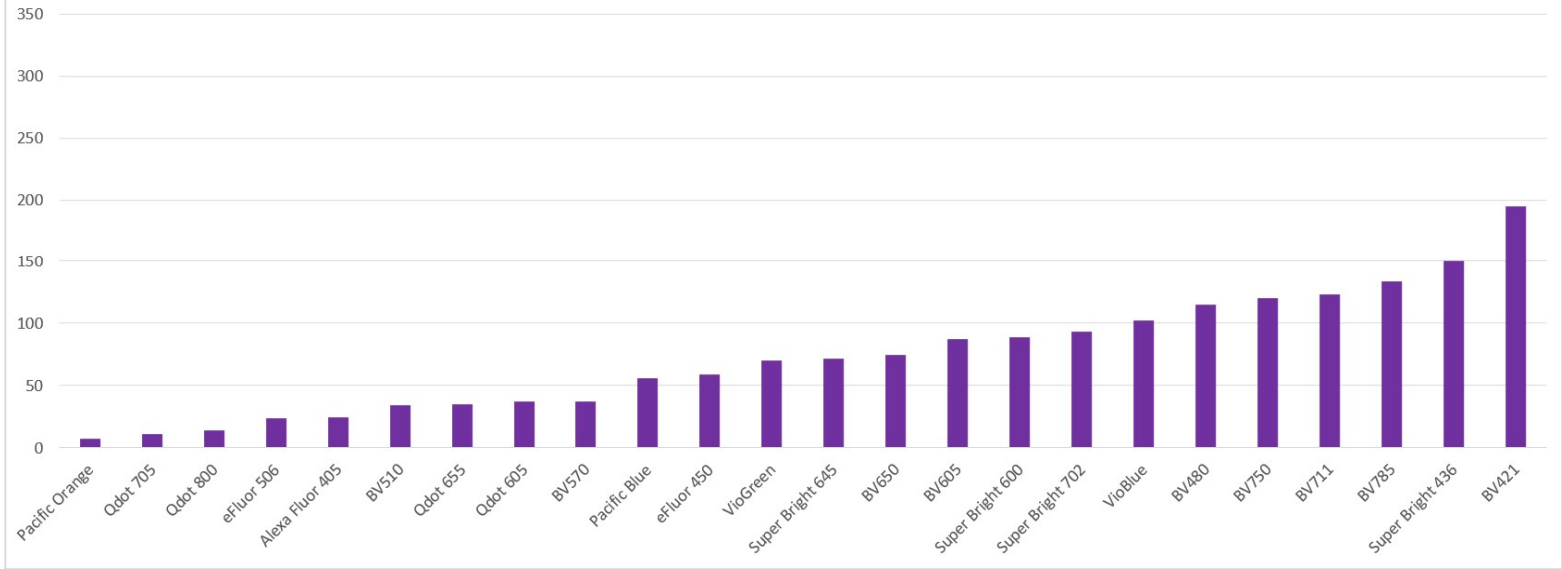
# Stain Index Ranking - 65 Dyes



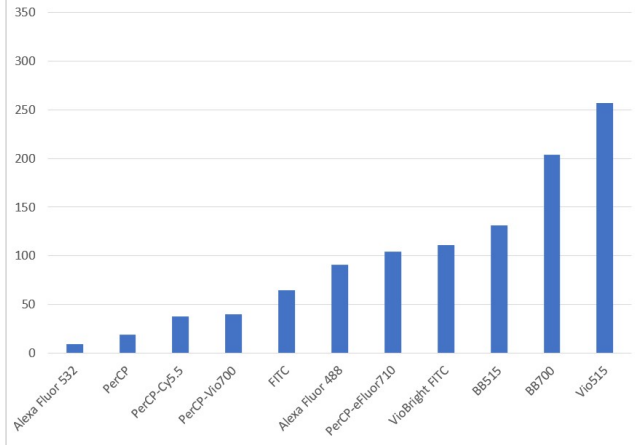
Stain Index UV Excitable Dyes



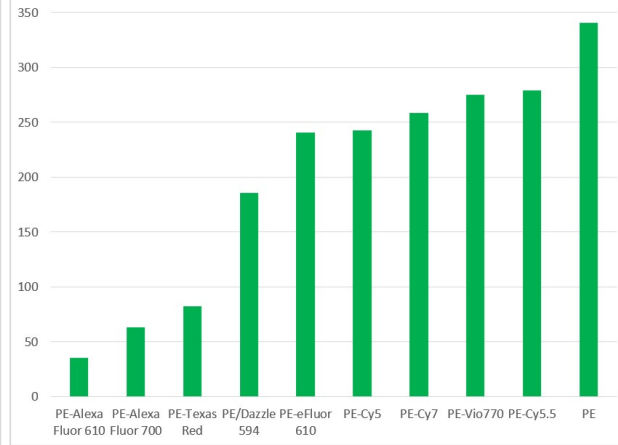
Stain Index Violet Excitable Dyes



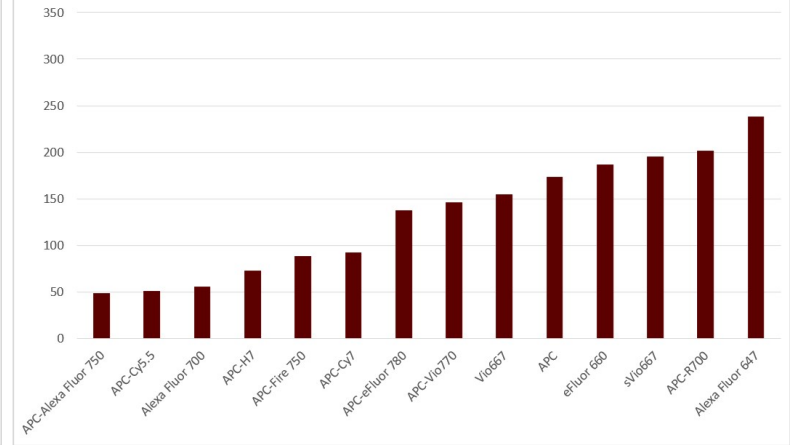
Stain Index Blue Excitable Dyes



Stain Index Yellow Green Excitable Dyes



Stain Index Red Excitable Dyes



# 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 30 Fluors that can be Used in Combination

	BUV395	BUV496	BUV563	BUV661	BUV737	BUV805	BV421	Super Bright 436	eFluor 450	BV480	BV510	BV570	BV605	BV650	BV711	BV750	BV785	BB515	Alexa Fluor 488	Alexa Fluor 532	PerCP-Cy5.5	PerCP-eFluor 710	PE	PE-Dazzle594	PE-Cy5	PE-Cy7	APC	Alexa Fluor 647	Alexa Fluor 700	APC-Fire 750	
BUV395	Black																														
BUV496		Black																													
BUV563			Black																												
BUV661				Black																											
BUV737					Black																										
BUV805						Black																									
BV421							Black																								
Super Bright 436								Black																							
eFluor 450									Black																						
BV480										Black																					
BV510											Black																				
BV570												Black																			
BV605													Black																		
BV650														Black																	
BV711															Black																
BV750																Black															
BV785																	Black														
BB515																		Black													
Alexa Fluor 488																			Black												
Alexa Fluor 532																				Black											
PerCP-Cy5.5																					Black										
PerCP-eFluor 710																						Black									
PE																						Black									
PE-Dazzle594																							Black								
PE-Cy5																								Black							
PE-Cy7																									Black						
APC																										Black					
Alexa Fluor 647																											Black				
Alexa Fluor 700																													Black		
APC-Fire 750																														Black	

*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 PE into AF532). Areas in bright pink and red indicate pairs for which more attention to panel design is needed.*