



CYTEK
TRANSCEND THE CONVENTIONAL

Cytek Aurora™ Fluorochrome Selection Guidelines for 5 Laser Systems (UV-V-B-YG-R)

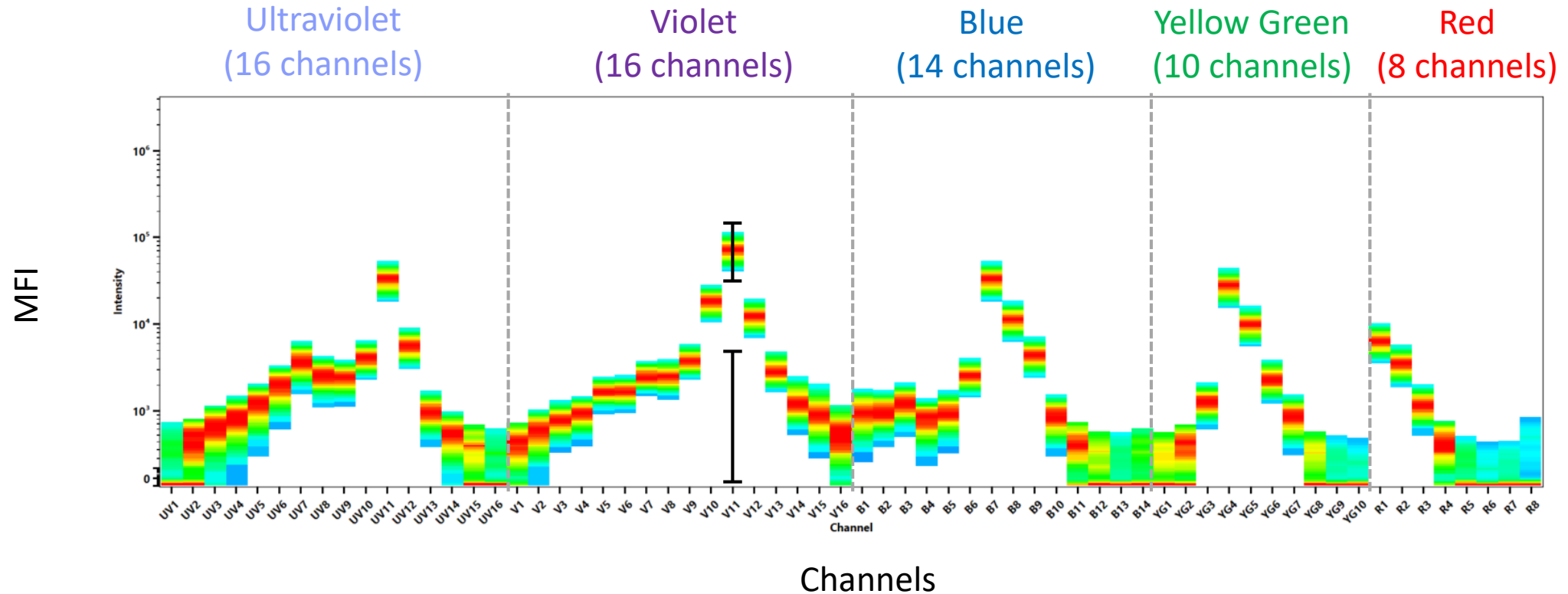
Last Updated: March 2019

Fluorochrome Signatures

Dyes can be used in combination as long as they have a unique spectrum signature.

When designing a multicolor panel, you should look for dyes with unique spectra and also consider spread introduced by the dyes (see slide 28).

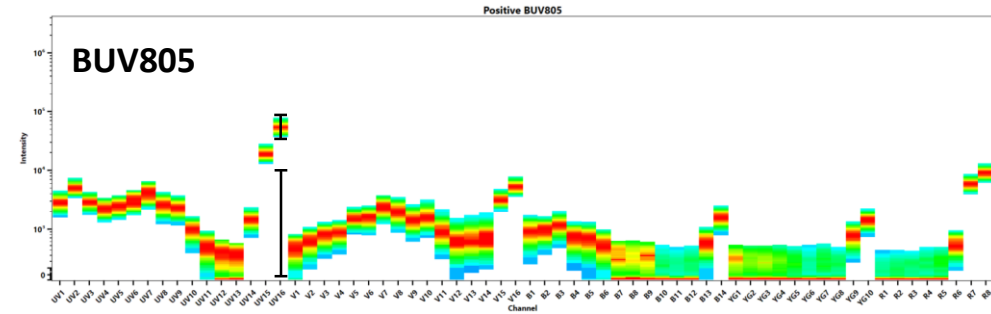
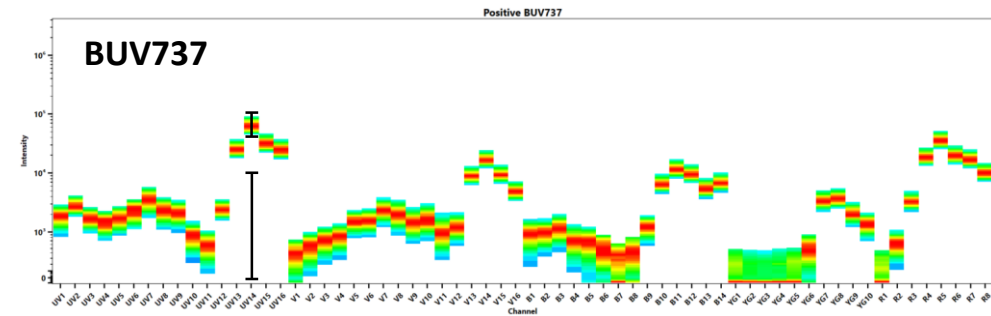
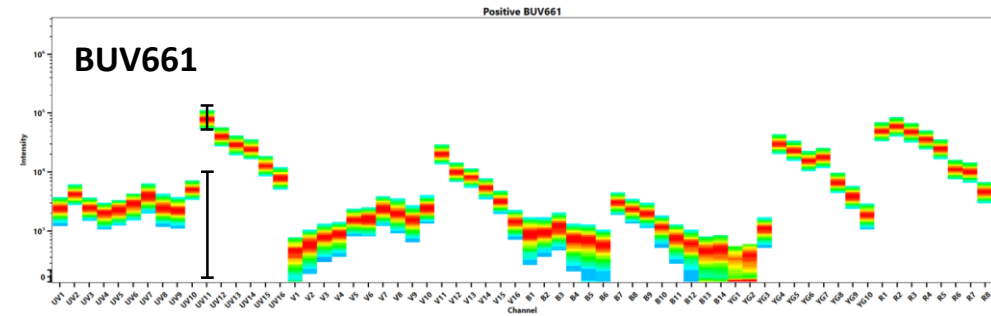
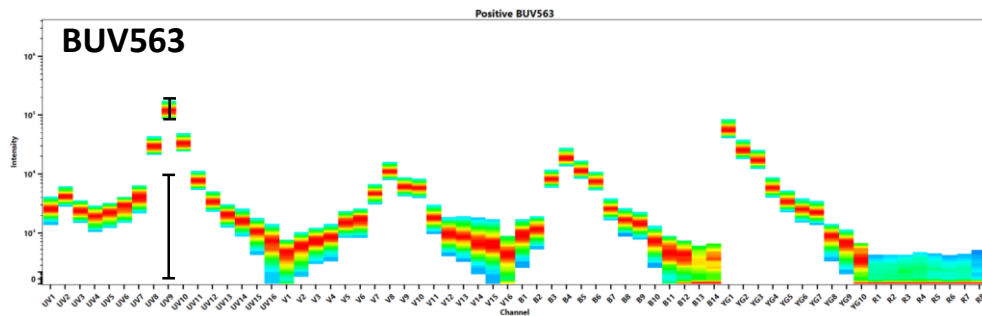
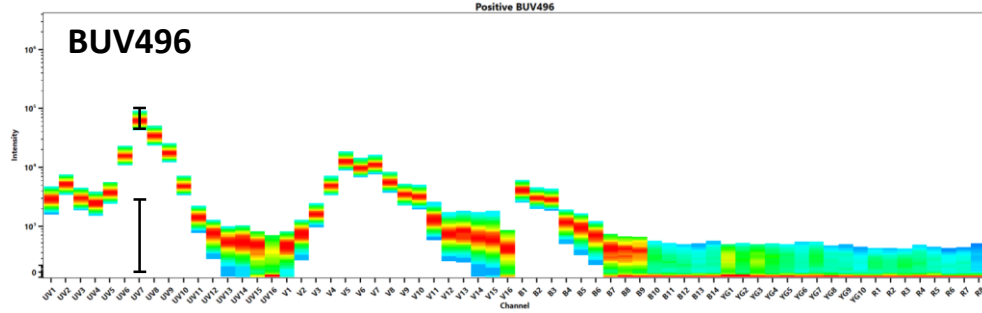
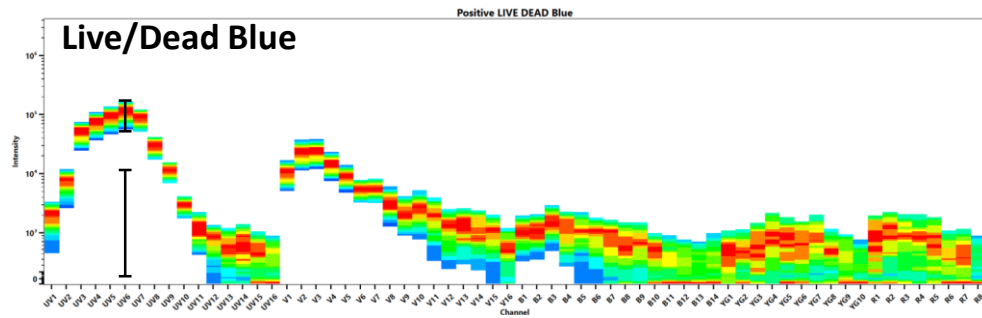
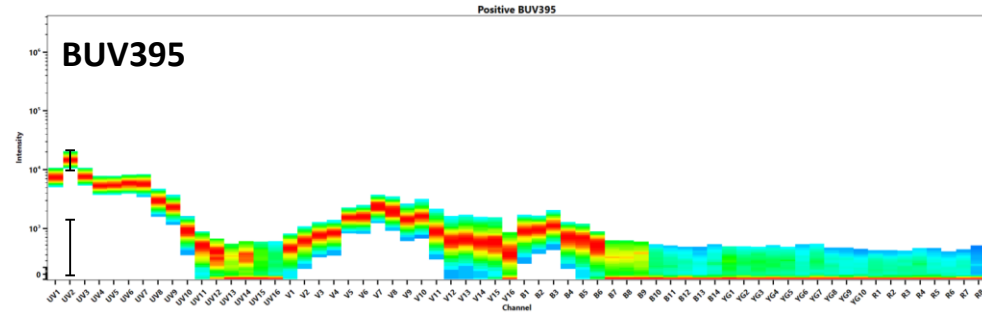
How to Read Full Spectrum Fluorochrome Signatures



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

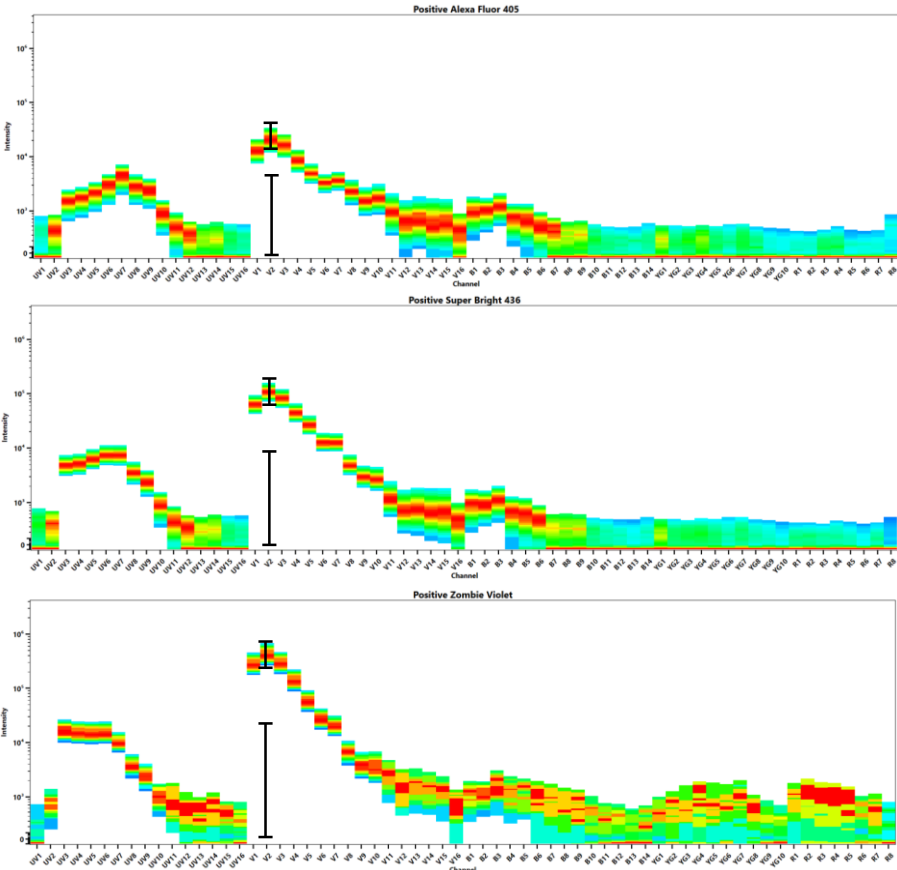
Ultraviolet Laser Excitable Dyes with Unique Signatures



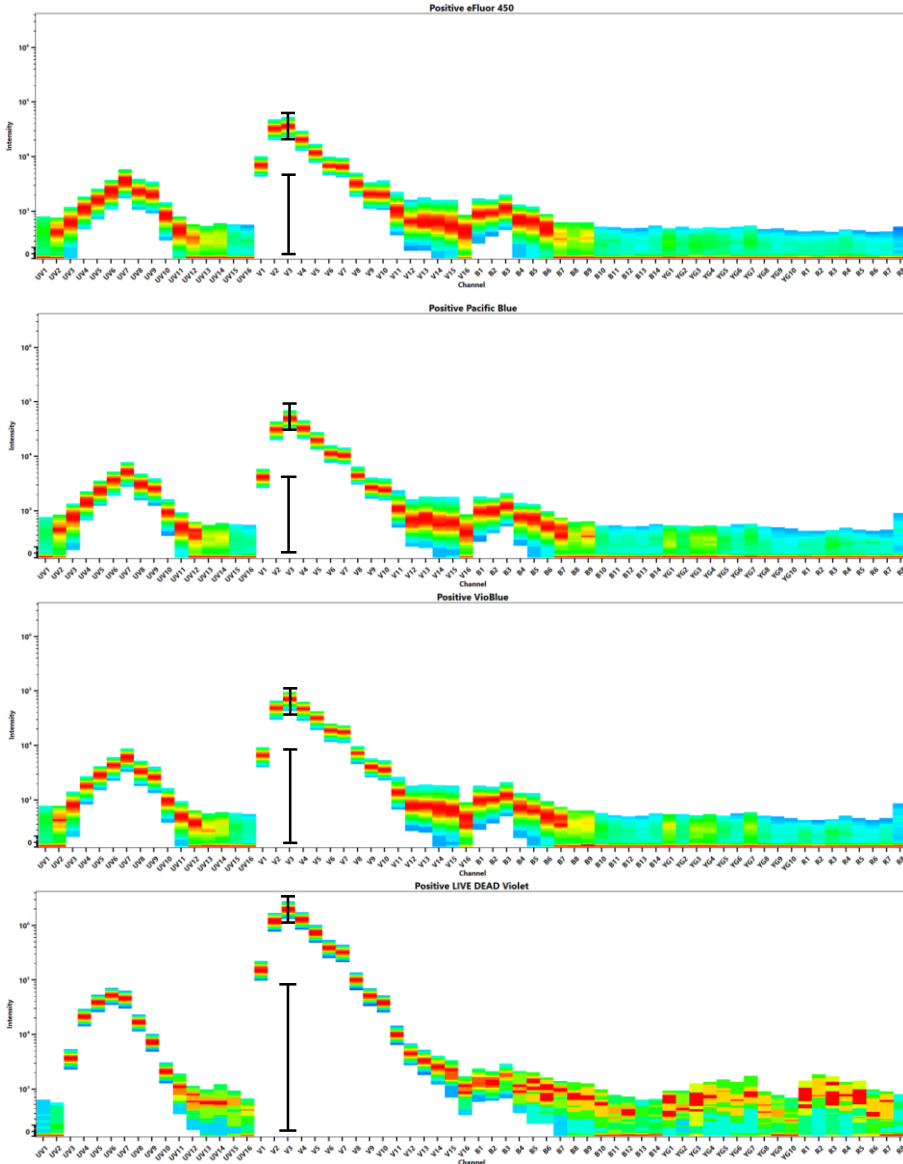
Dyes Primarily Excited by the Violet Laser

Violet Laser Excitable Dyes with Similar Signatures

Alexa Fluor 405, Super Bright 436 and Zombie Violet

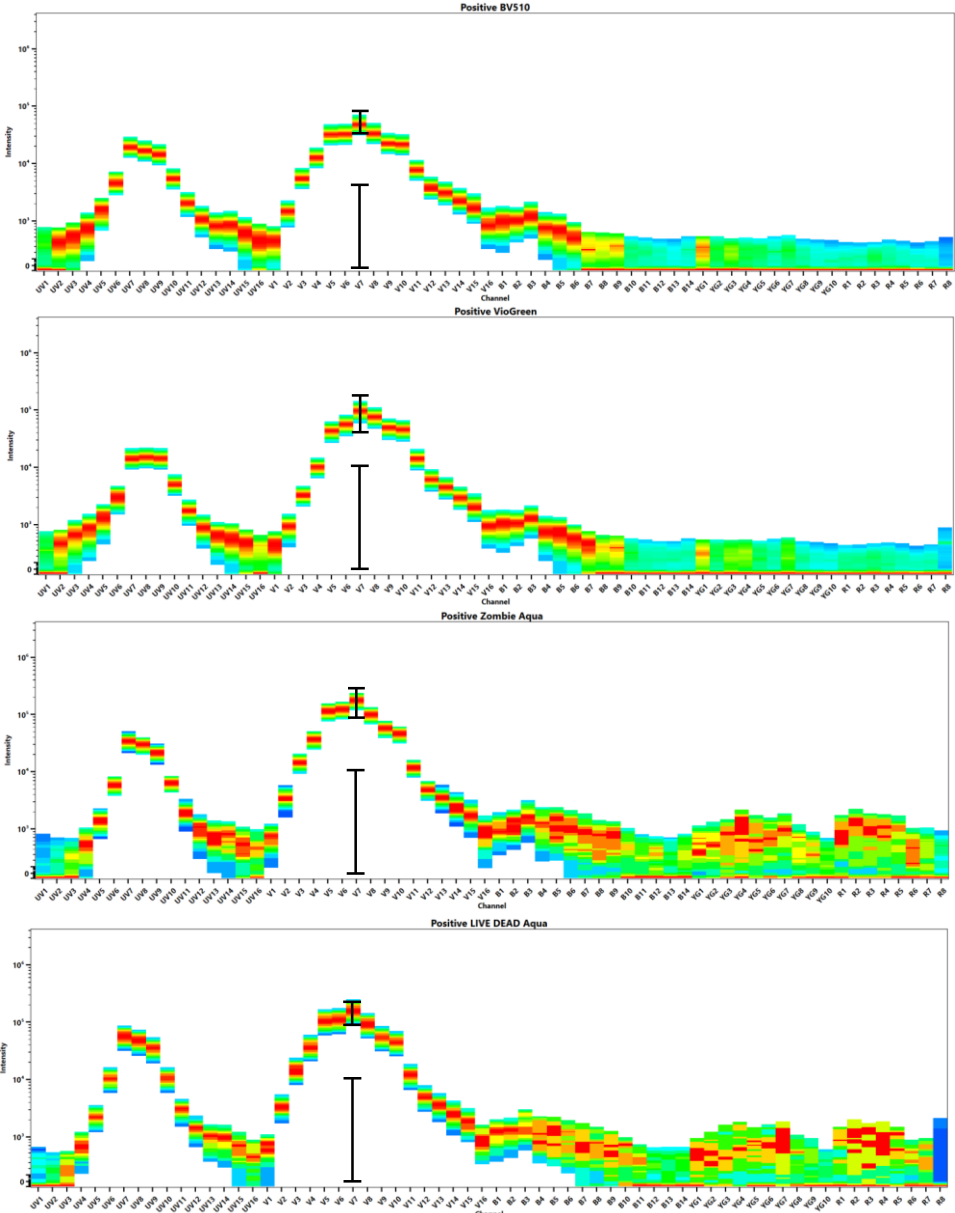


eFluor 450, VioBlue, Pacific Blue and Live/Dead Violet

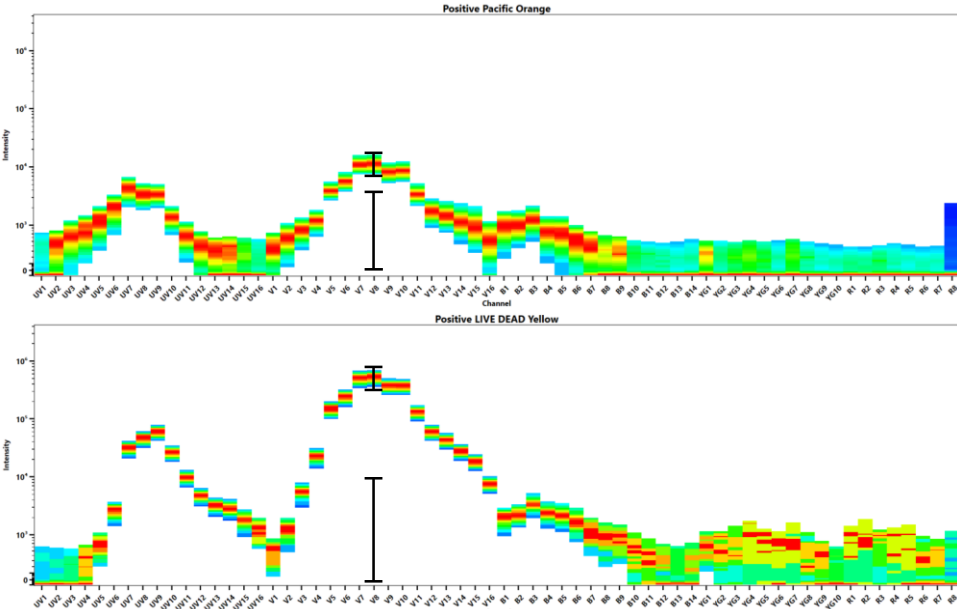


Violet Laser Excitable Dyes with Similar Signatures

BV510, VioGreen, Zombie Aqua and Live/Dead Aqua

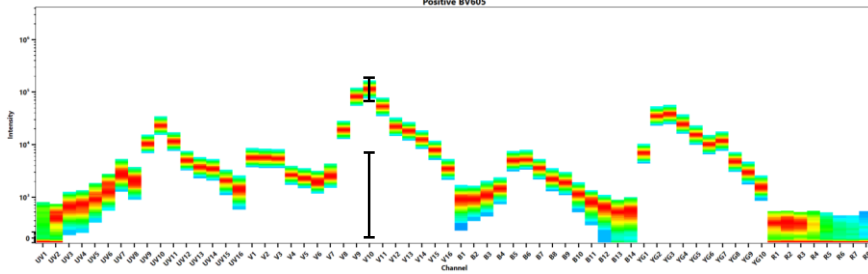
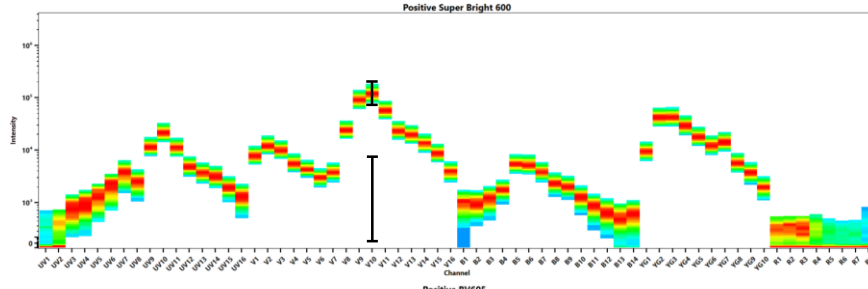


Pacific Orange and Live/Dead Yellow

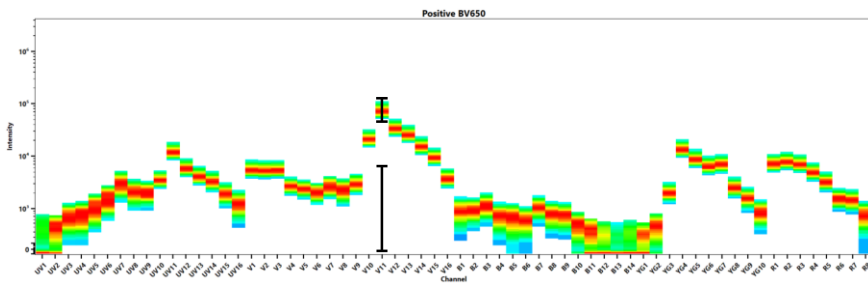
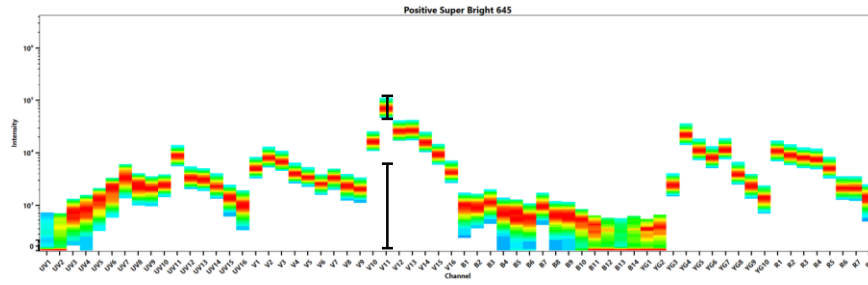


Violet Laser Excitable Dyes with Similar Signatures

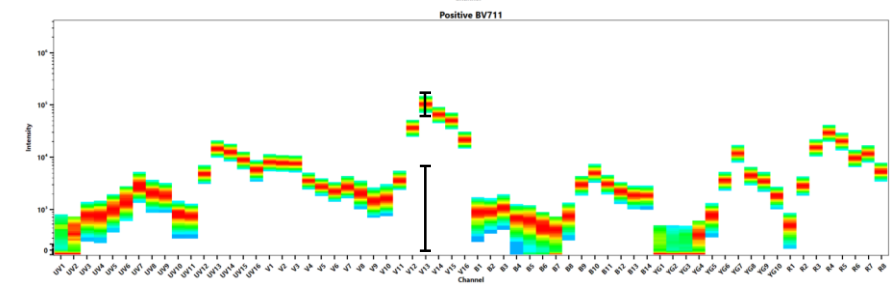
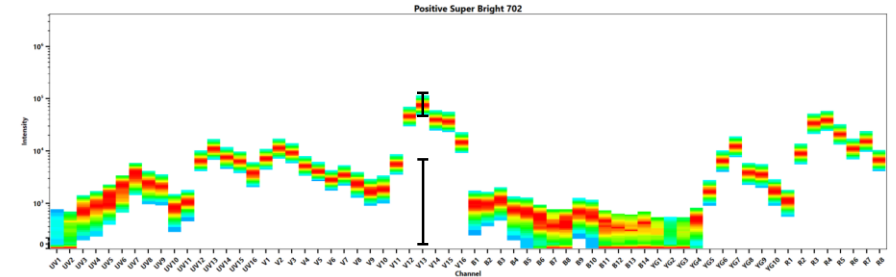
Super Bright 600 and BV605



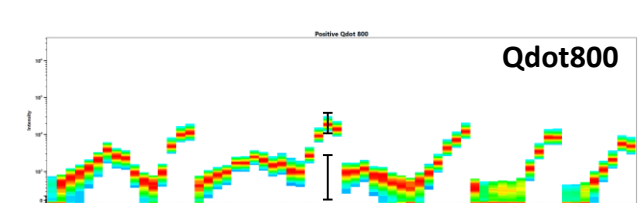
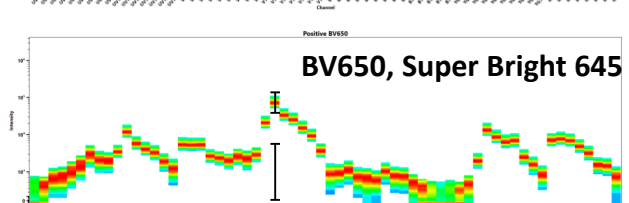
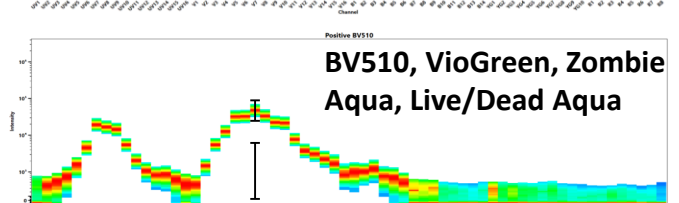
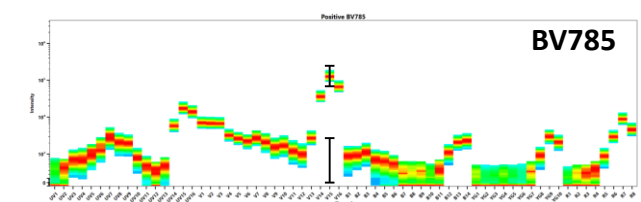
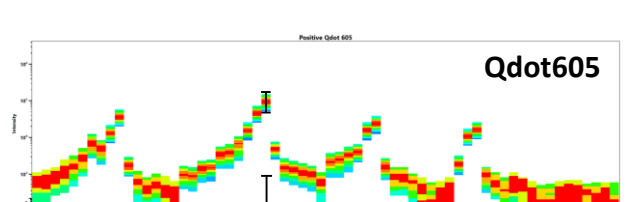
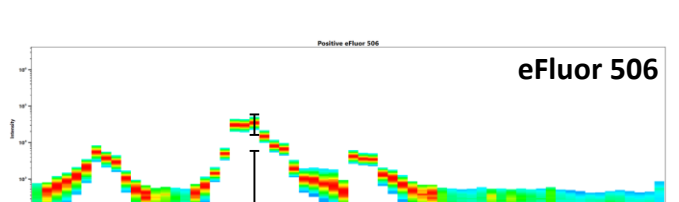
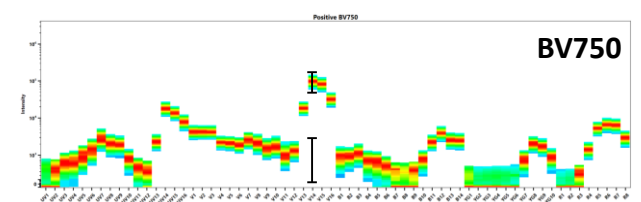
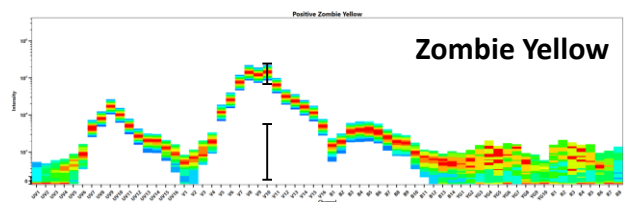
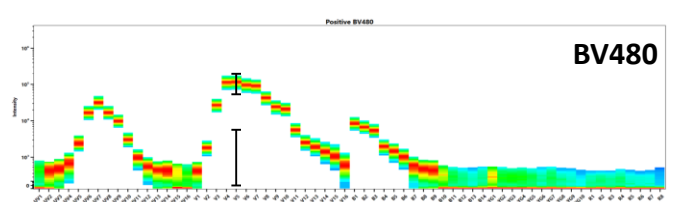
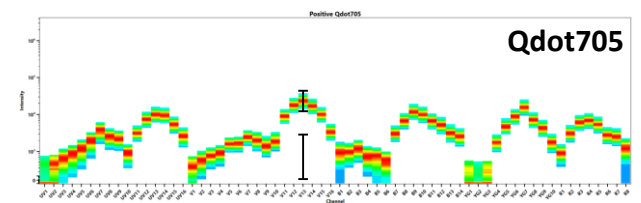
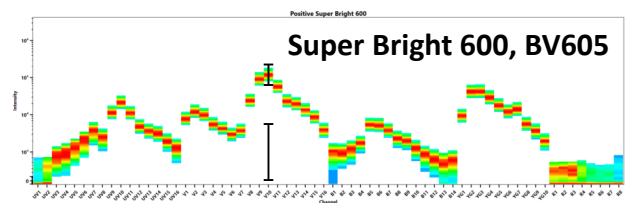
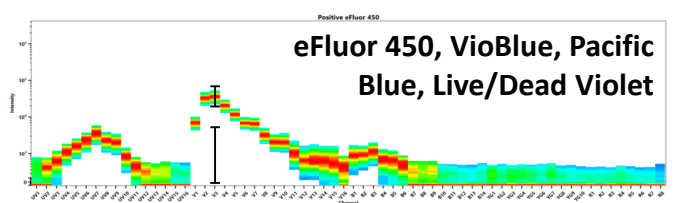
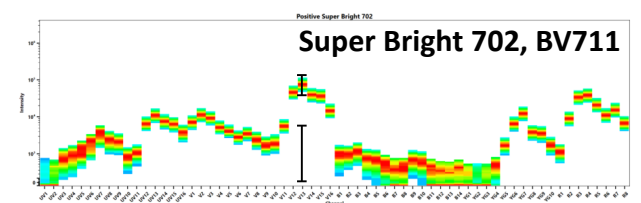
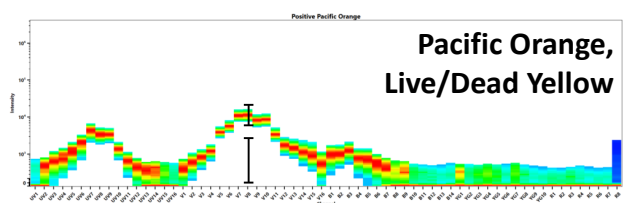
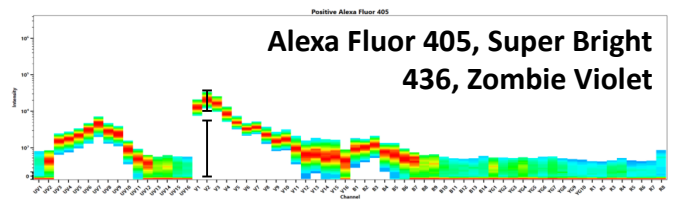
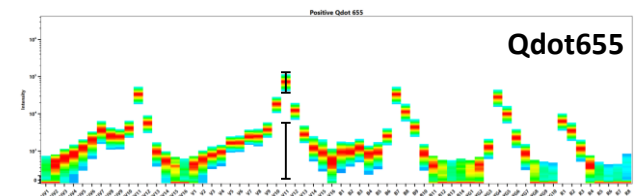
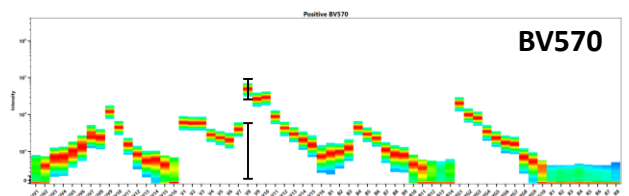
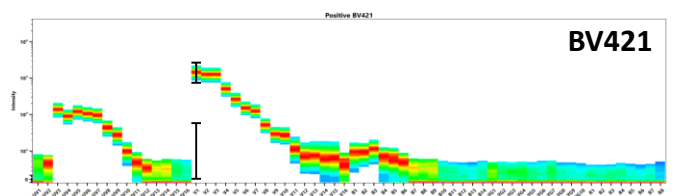
Super Bright 645 and BV650



Super Bright 702 and BV711



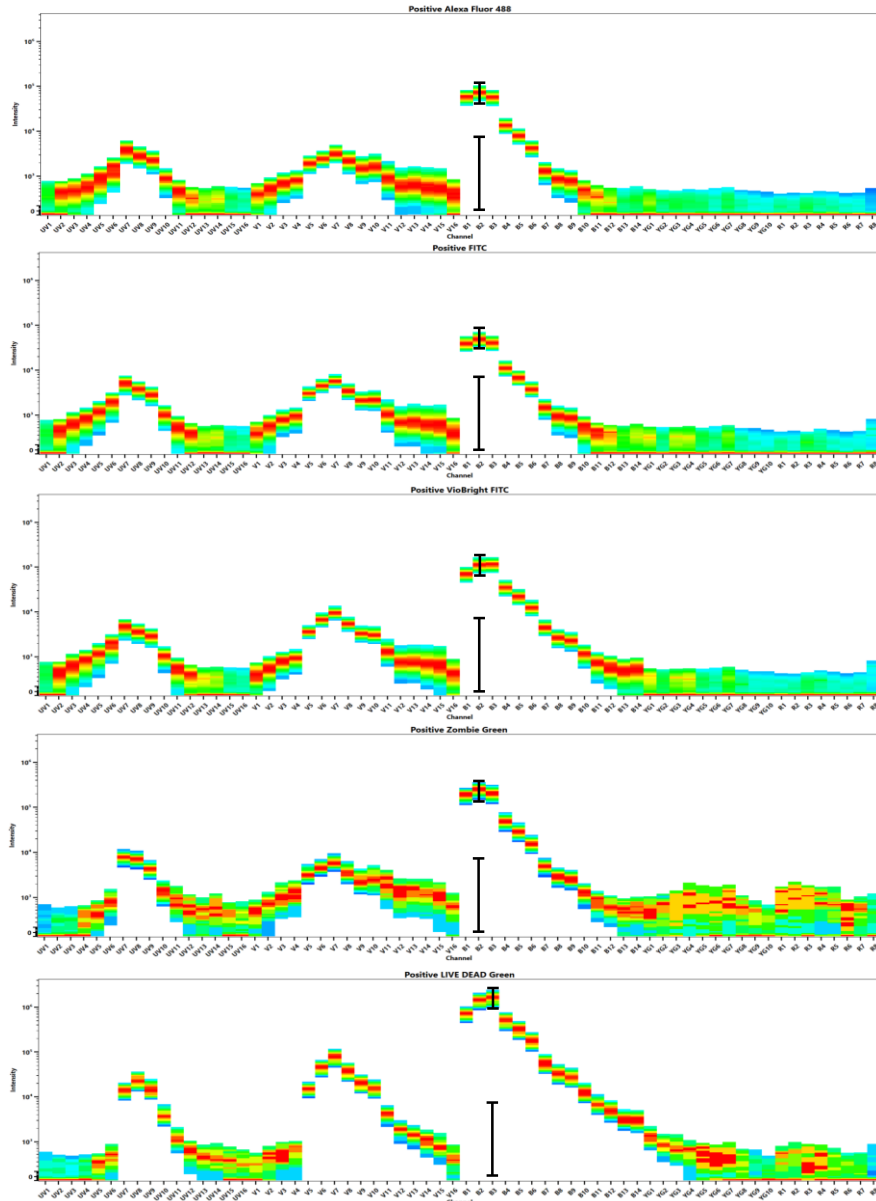
Violet Laser Excitable Dyes with Unique Signatures



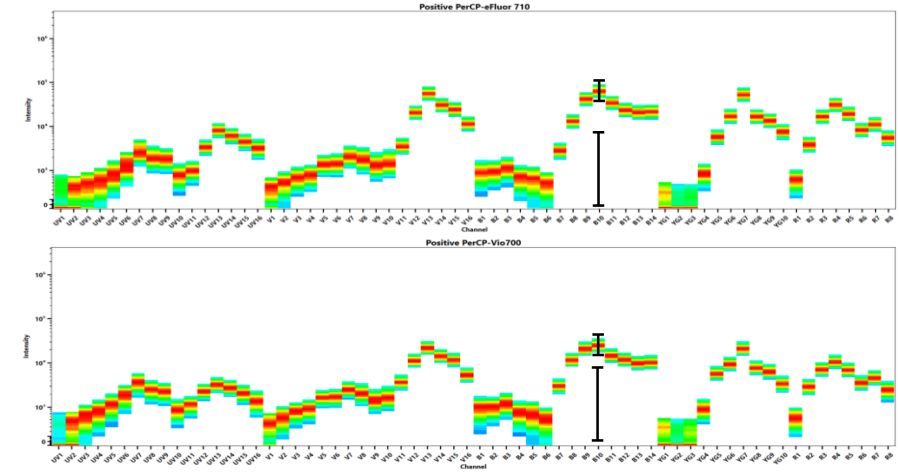
Dyes Primarily Excited by the Blue Laser

Blue Laser Excitable Dyes with Similar Signatures

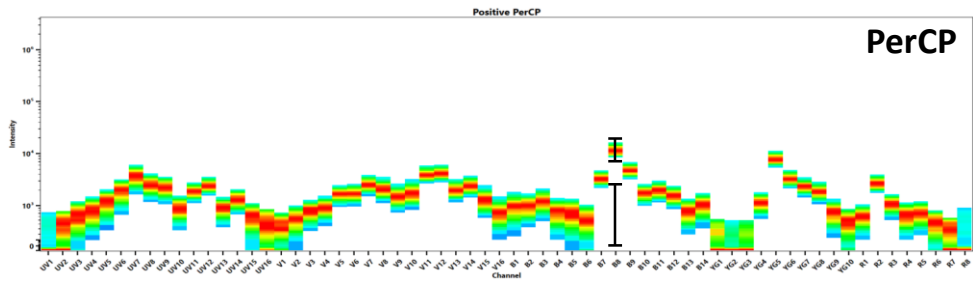
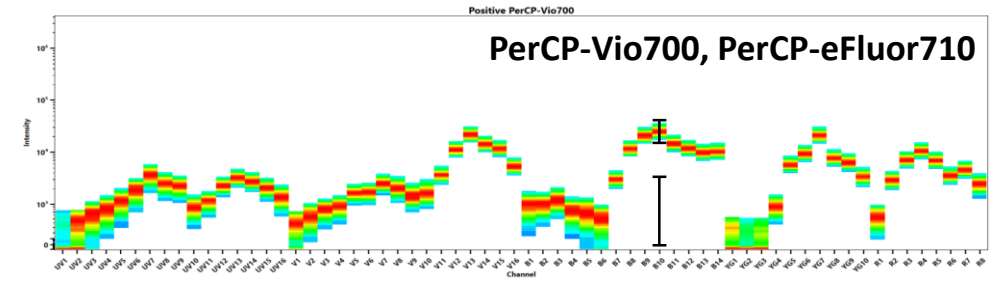
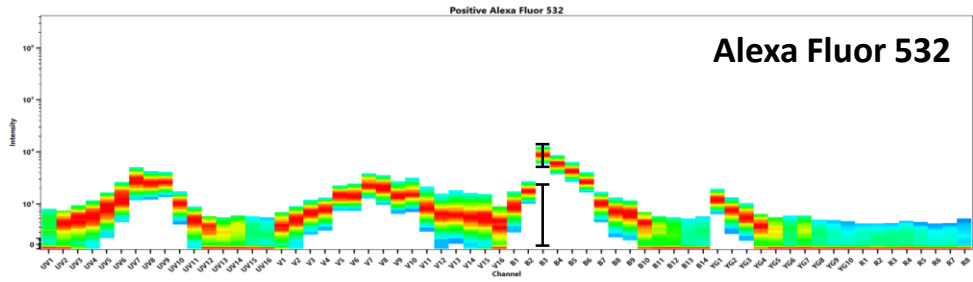
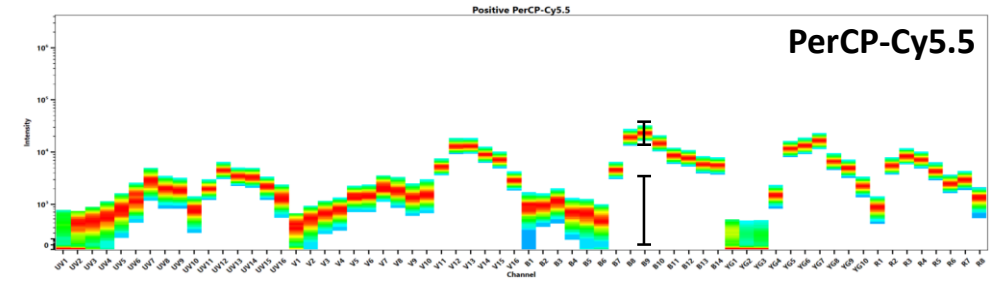
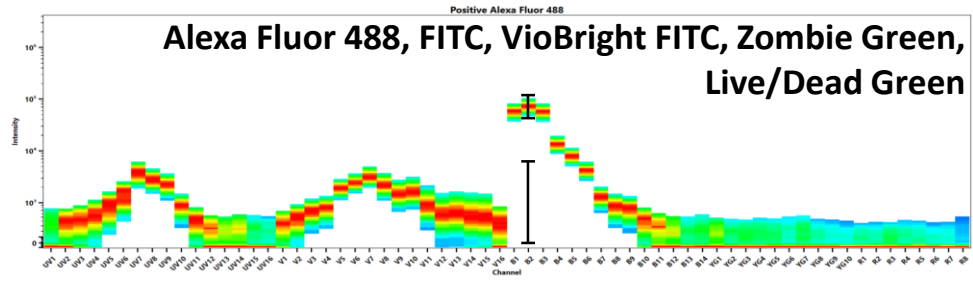
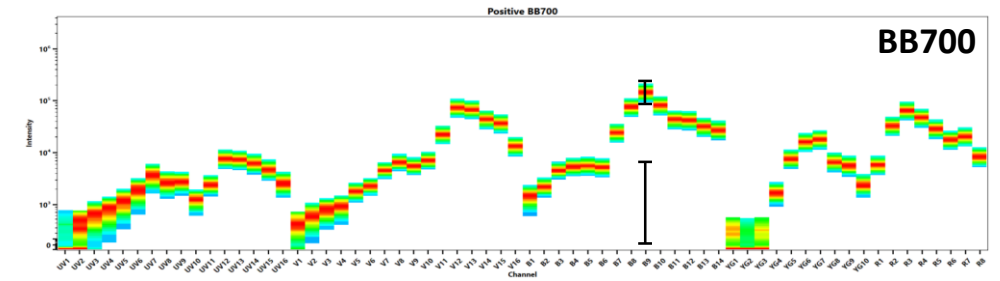
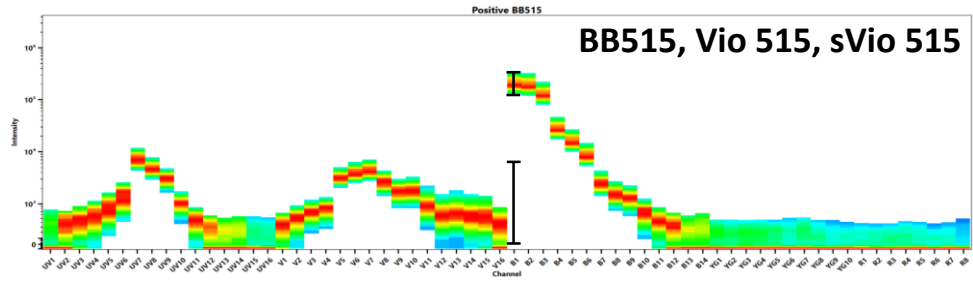
Alexa Fluor 488, FITC, VioBright FITC, Zombie Green and Live Dead Green



PerCP-Vio 710 and PerCP-eFluor 710



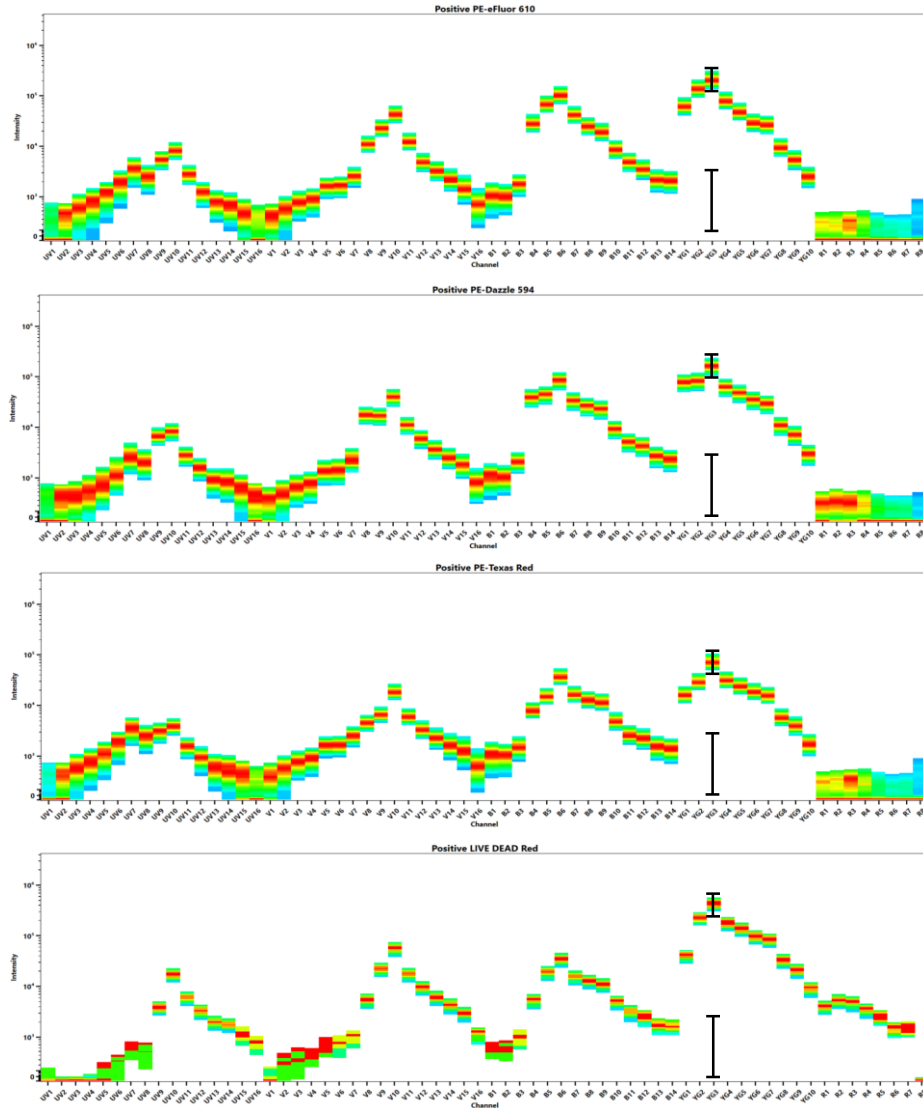
Blue Laser Excitable Dyes with Unique Signatures



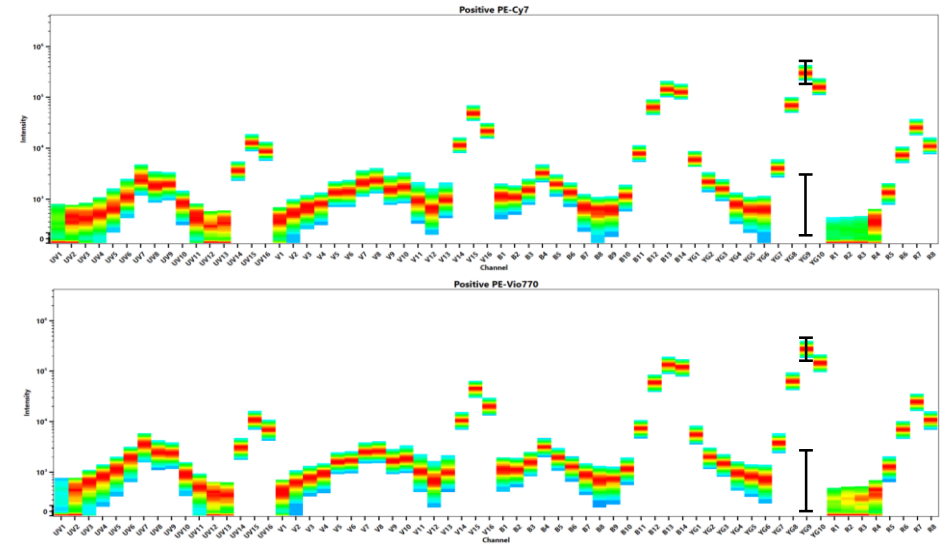
Dyes Primarily Excited by the Yellow Green Laser

Yellow Green Laser Excitable Dyes with Similar Signatures

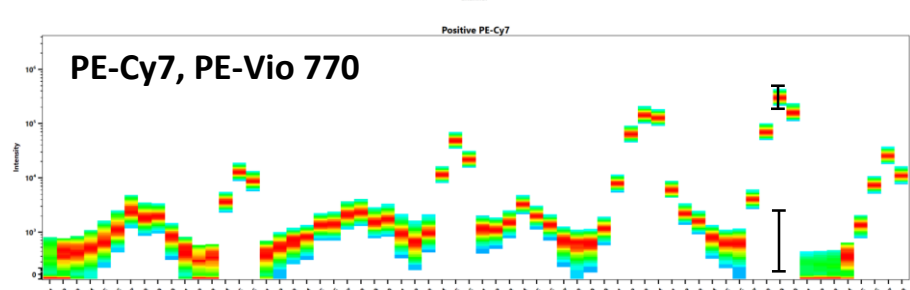
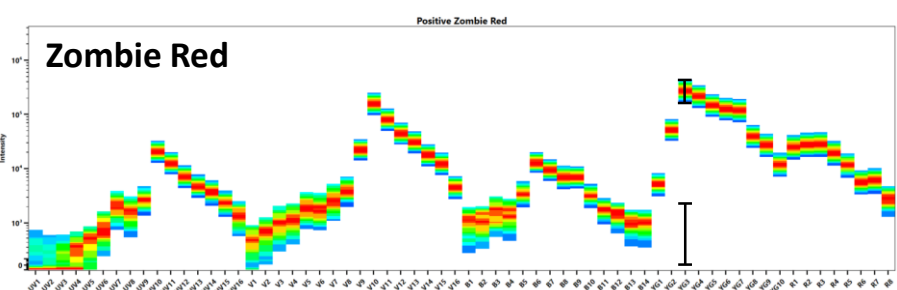
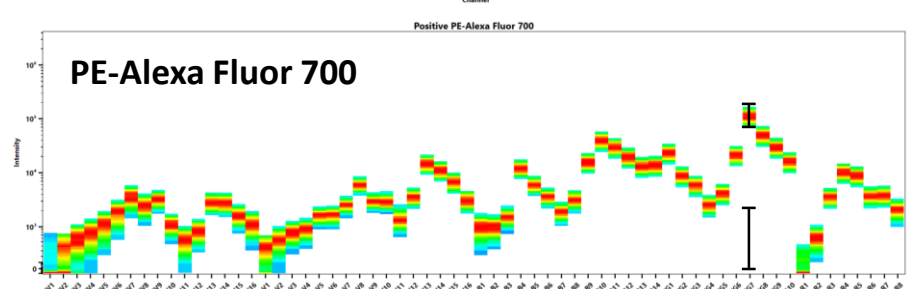
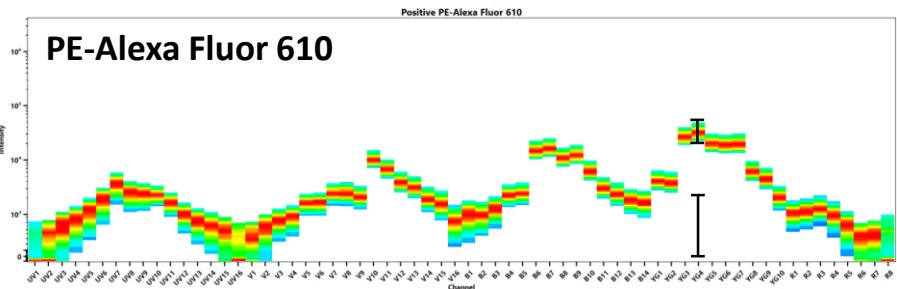
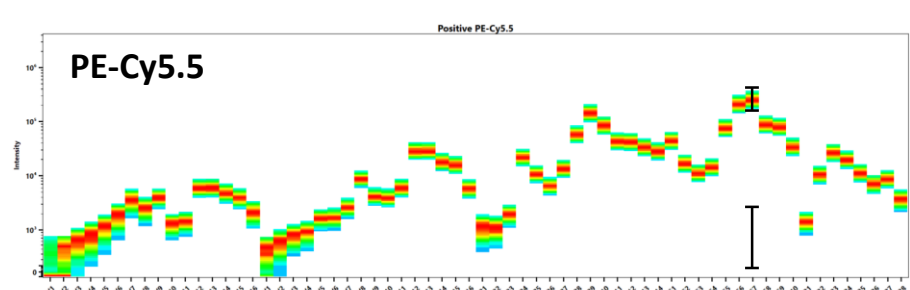
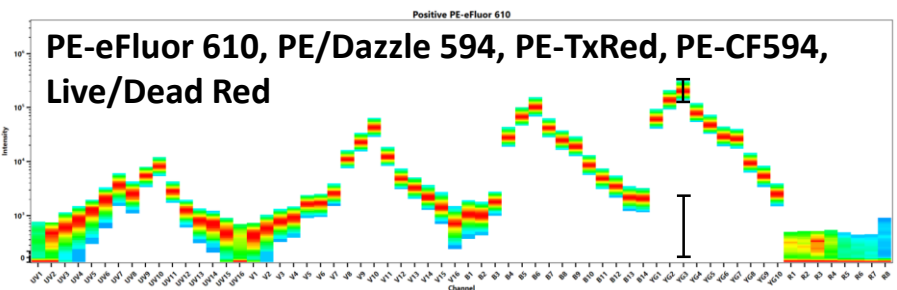
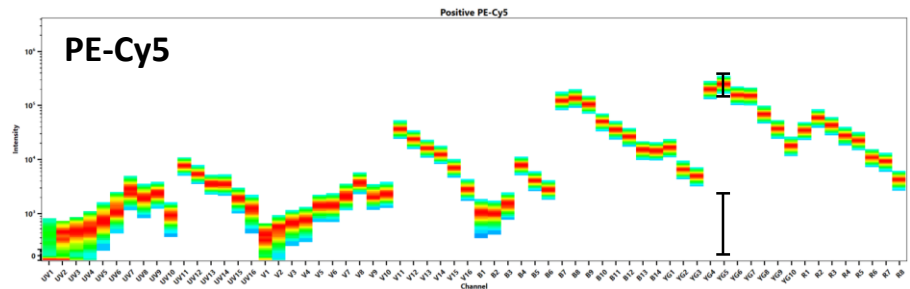
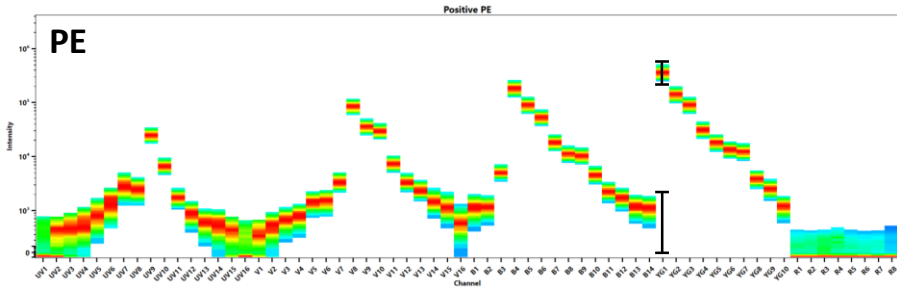
PE-eFluor 610, PE/Dazzle 594, PE-CF594, PE-Texas Red and Live Dead Red



PE-Cy7 and PE-Vio 770



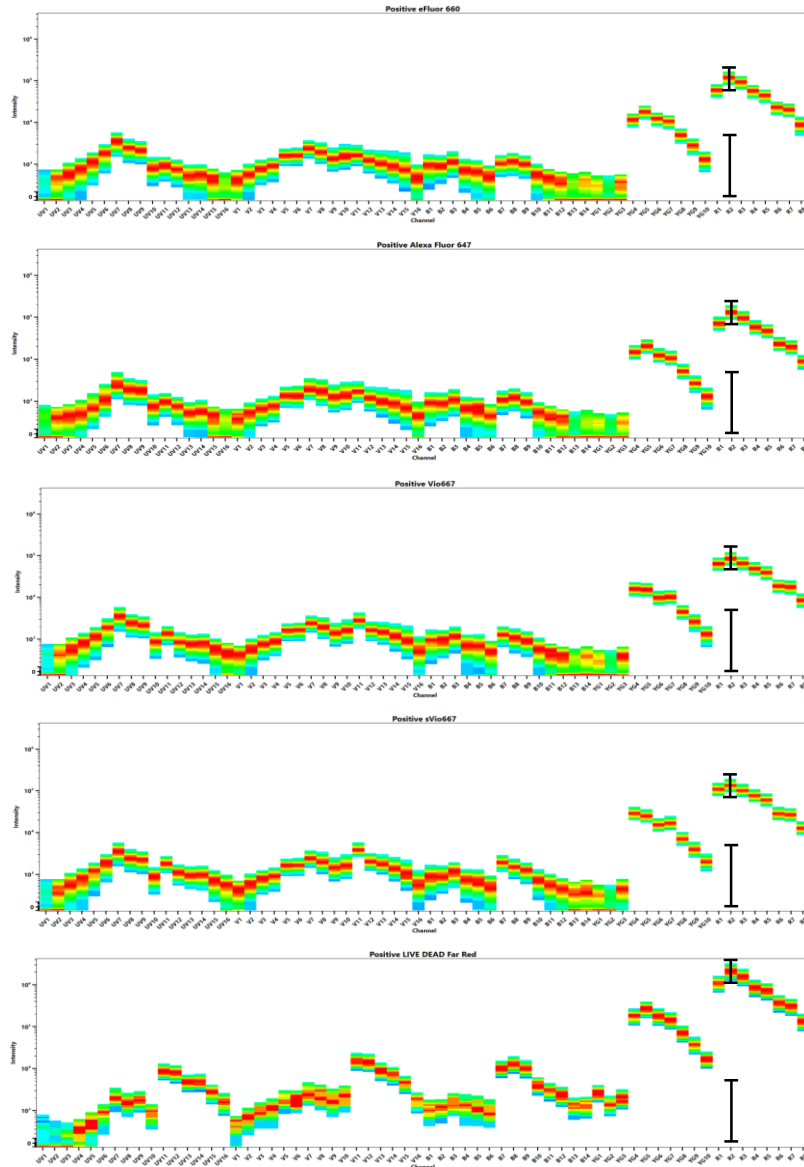
Yellow Green Laser Excitable Dyes with Unique Signatures



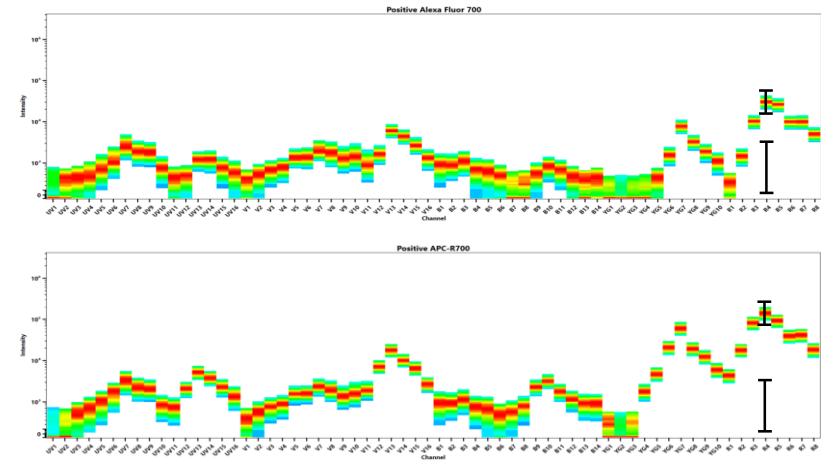
Dyes Primarily Excited by the
Red Laser

Red Laser Excitable Dyes with Similar Signatures

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

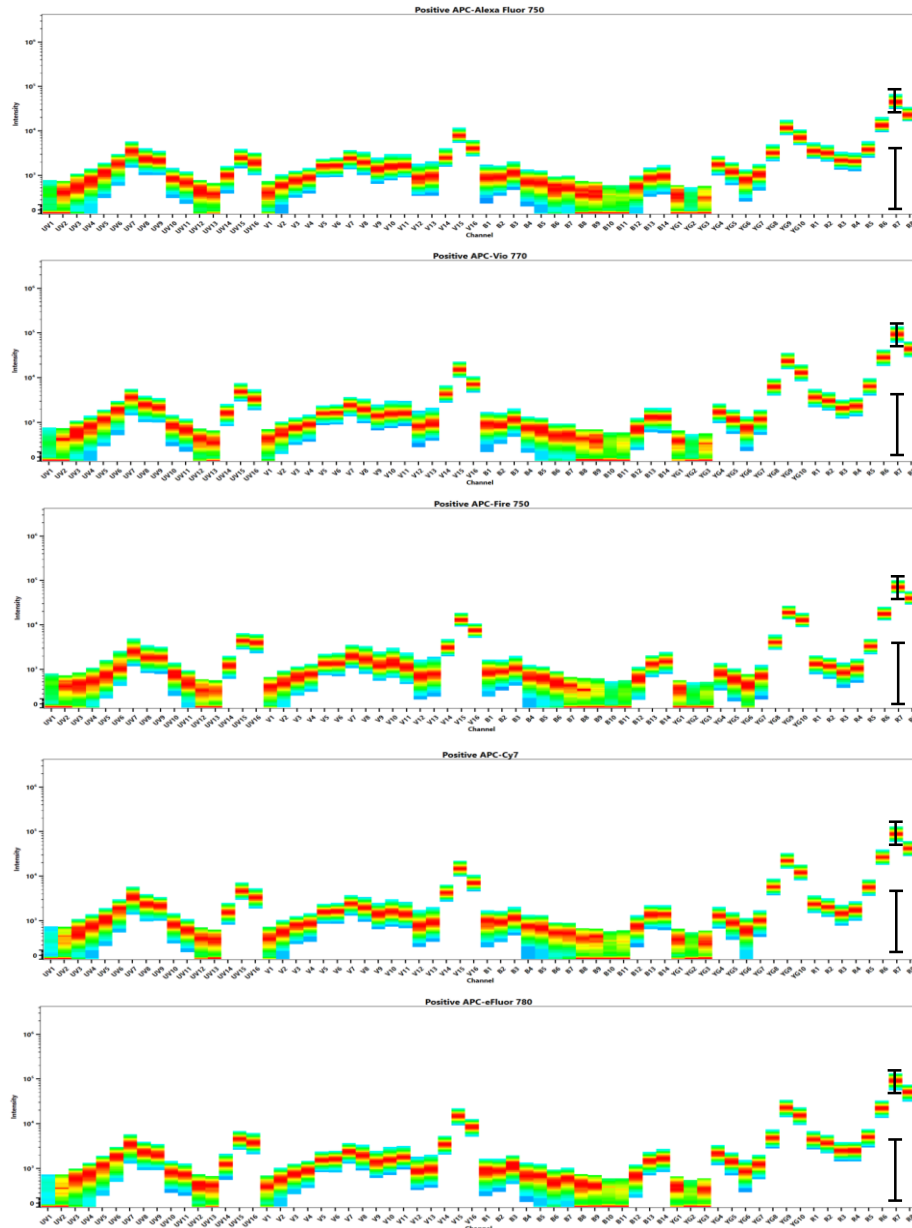


Alexa Fluor 700 and APC-R700

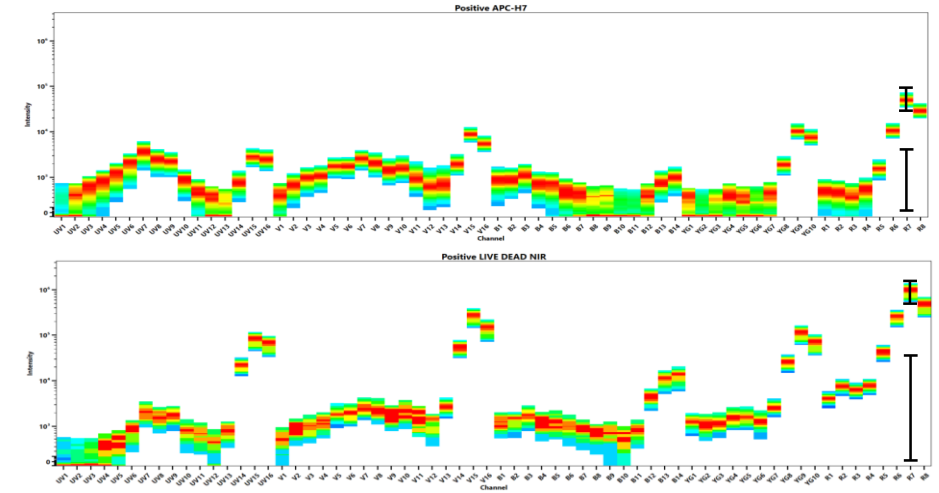


Red Laser Excitable Dyes with Similar Signatures

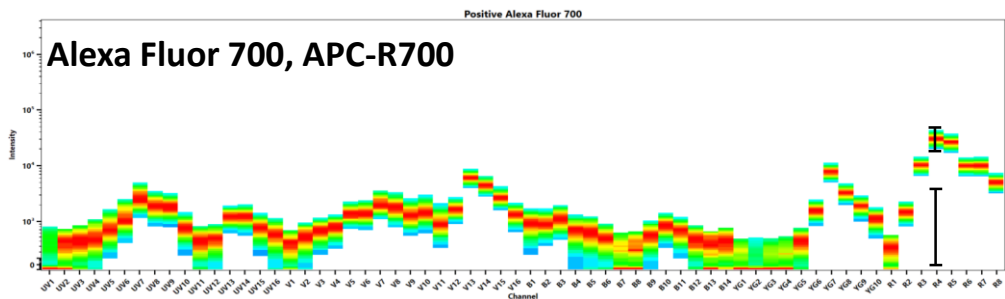
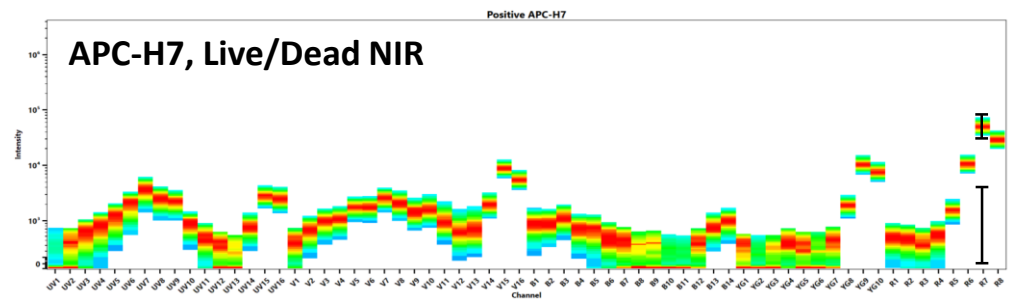
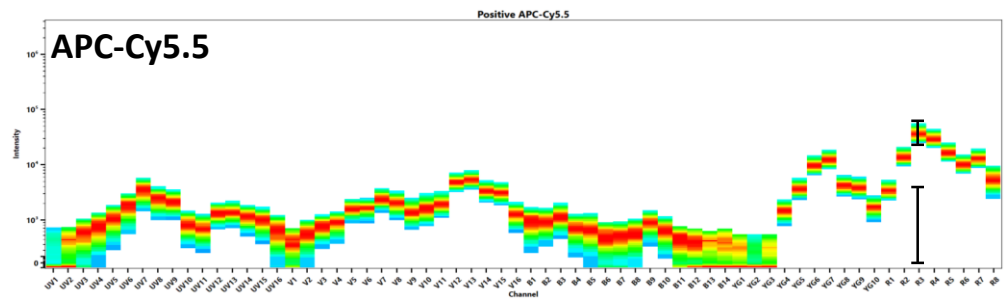
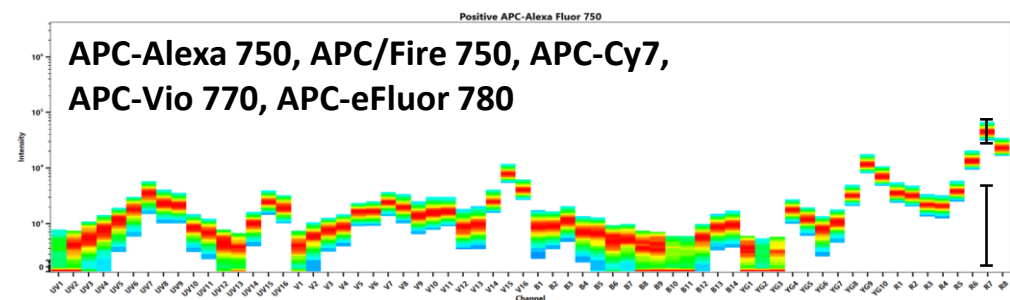
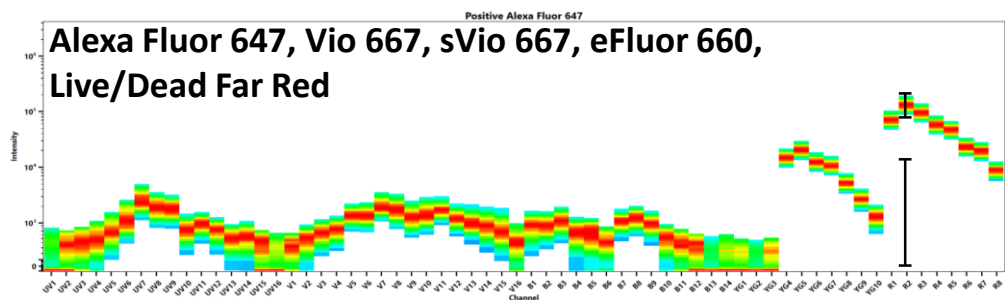
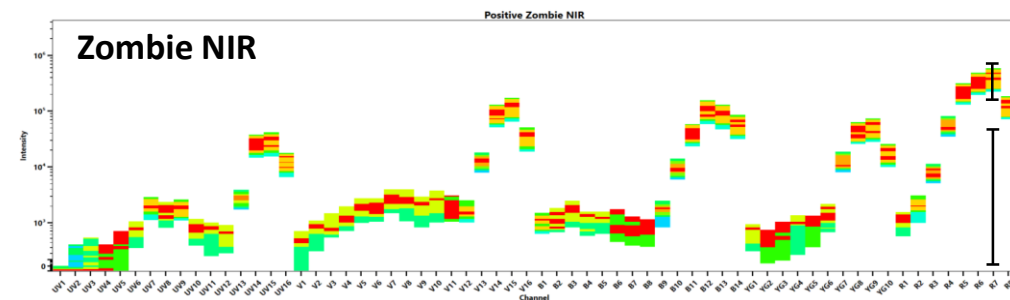
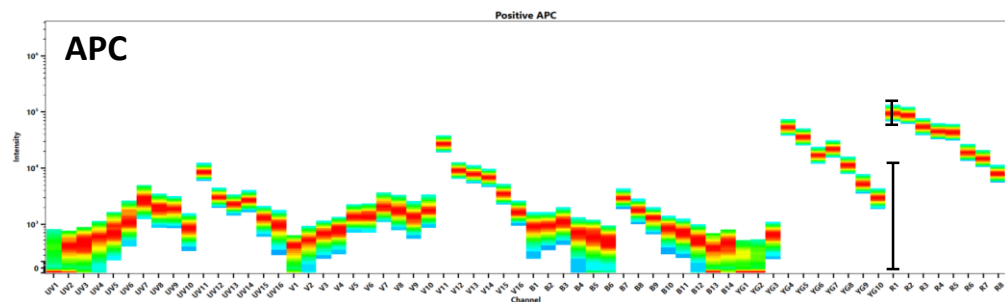
APC-Alexa 750, APC-Vio 770, APC/Fire 750, APC-Cy7 and APC-eFluor 780



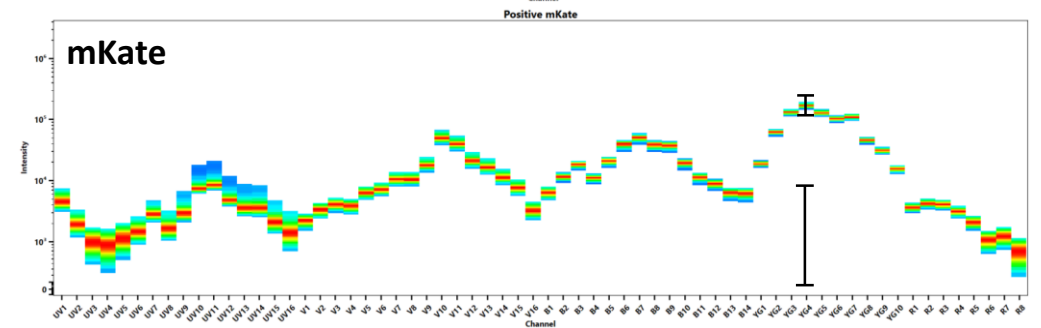
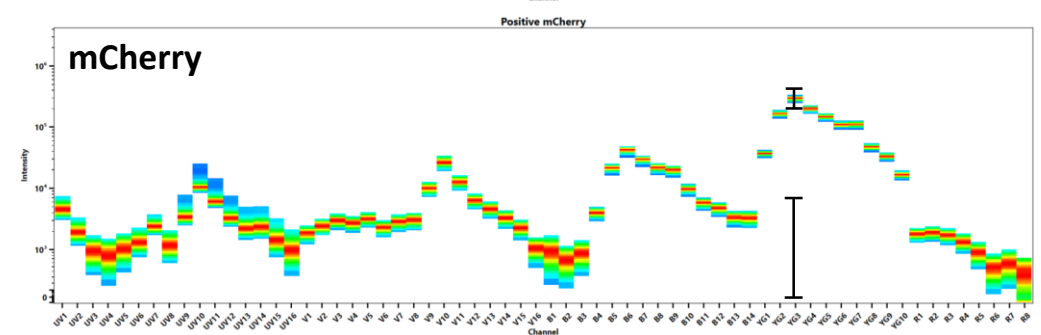
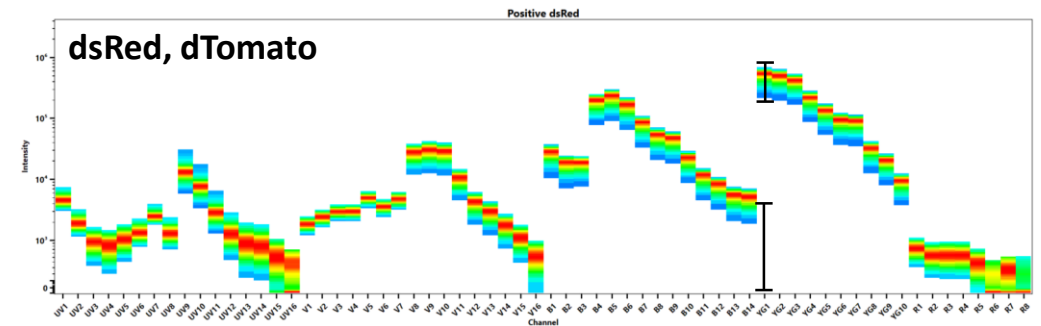
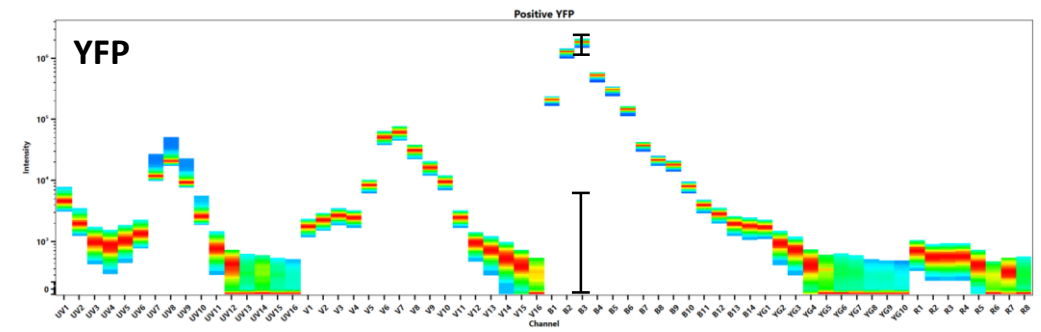
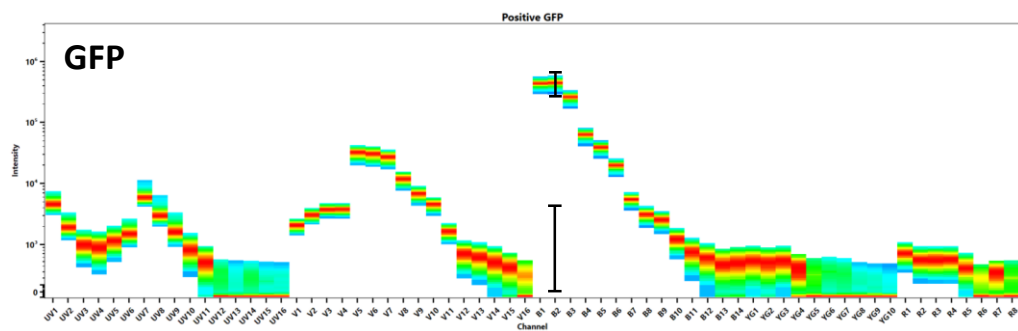
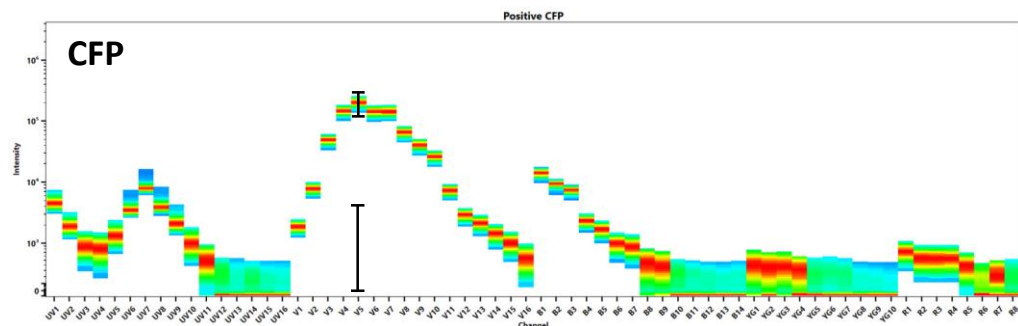
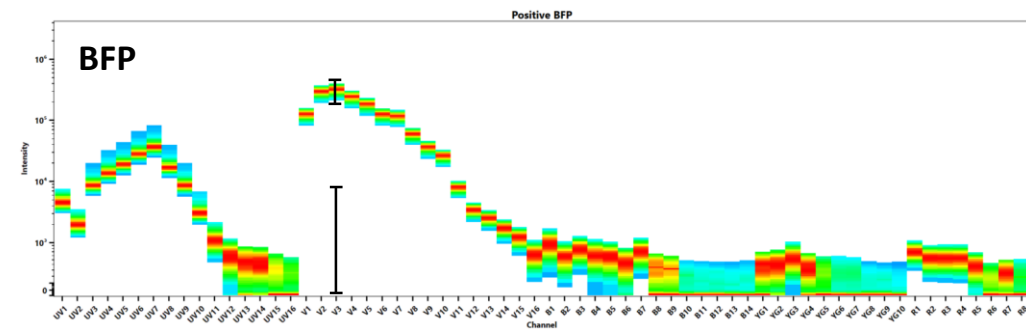
APC-H7 and Live/Dead NIR



Red Laser Excitable Dyes with Unique Signatures



Fluorescent Proteins



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 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 | 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-CF 594, PE-TexasRed, PE-eFluor 610, PE-Alexa Fluor 610 | YG3 |
| PE-Cy5 | YG5 |
| PE-Cy5.5, PE-AlexaFluor 700 | YG7 |
| PE-Cy7, PE-Vio 770 | YG9 |
| Red Excited Fluors | Peak Channel |
| APC | R1 |
| Alexa647, Vio 667, sVio 667, eFluor660 | R2 |
| Alexa 700, APC-R700 | R4 |
| APC-Alexa750, APC/Fire 750, APC-Cy7, APC-Vio 770, APC-eFluor780, APC-H7 | R7 |

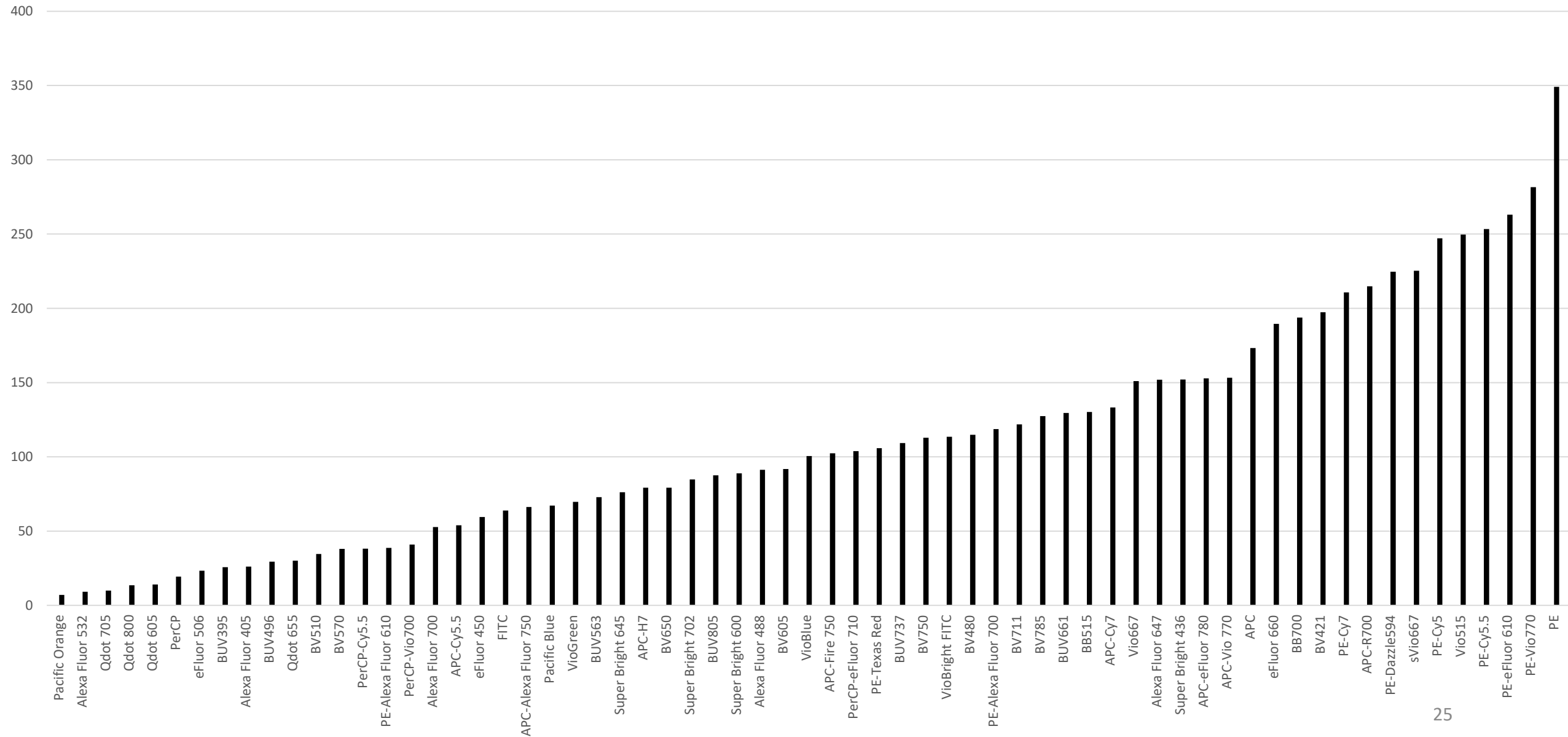
Example of 30 Dyes that Can Be Used in Combination (CAREFUL PANEL DESIGN IS NEEDED)

| 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/Dazzle594 | Alexa647 |
| BUV563 | efluor450 | Alexa Fluor 532 | PE-Cy5 | APC-R700 |
| BUV661 | BV480 | PerCP-Cy5.5 | PE-Cy7 | APC/Fire750 |
| BUV737 | BV510 | PerCP-eFluor 710 | | |
| BUV805 | BV570 | | | |
| | BV605 | | | |
| | BV650 | | | |
| | BV711 | | | |
| | BV750 | | | |
| | BV785 | | | |

Stain Indexes

Data generated using CD4 staining in human PBMCs

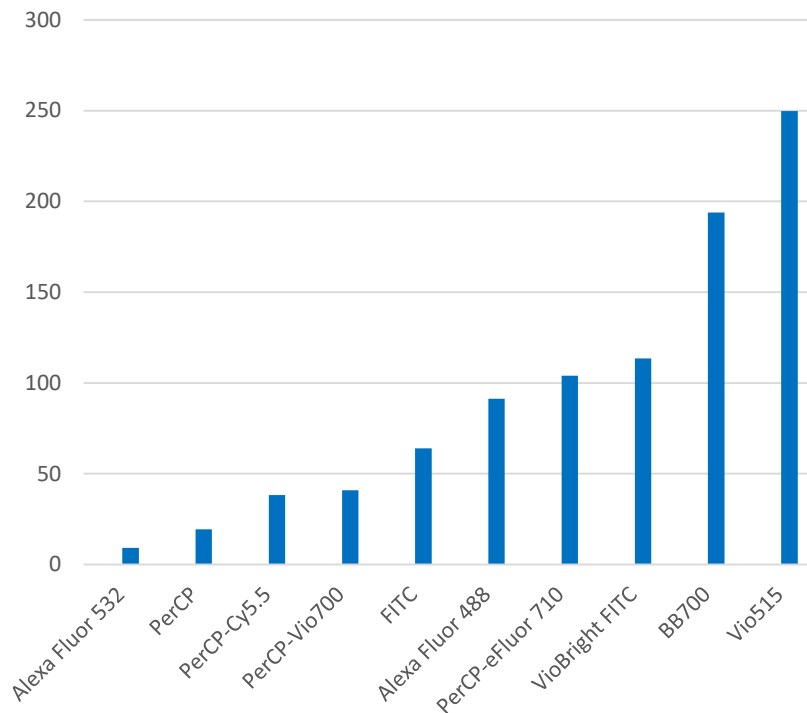
Stain Indexes - 5 Laser System - 65 Dyes



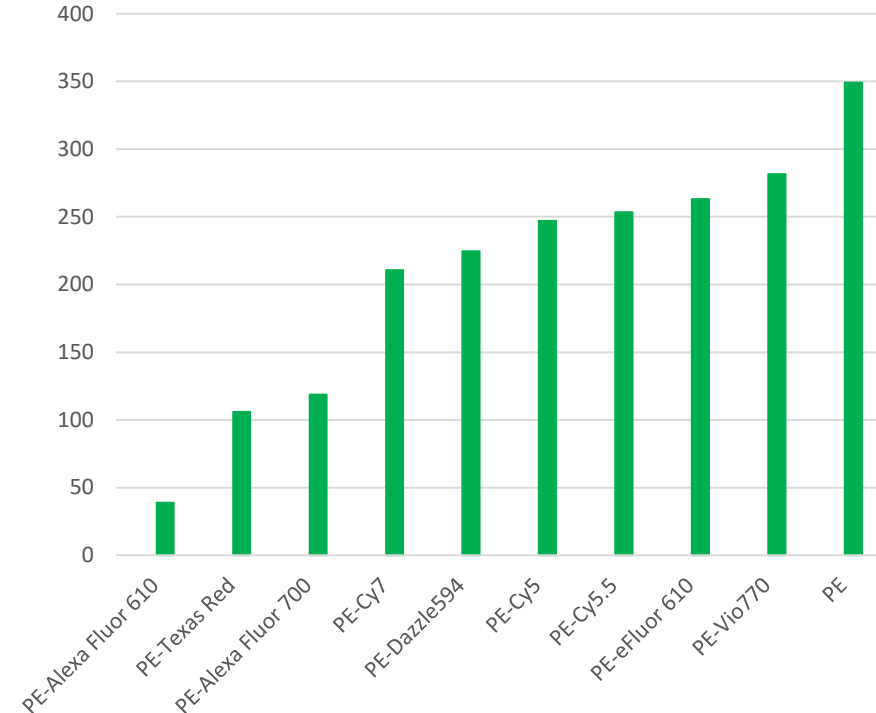
Stain Index UV Excitable Dyes



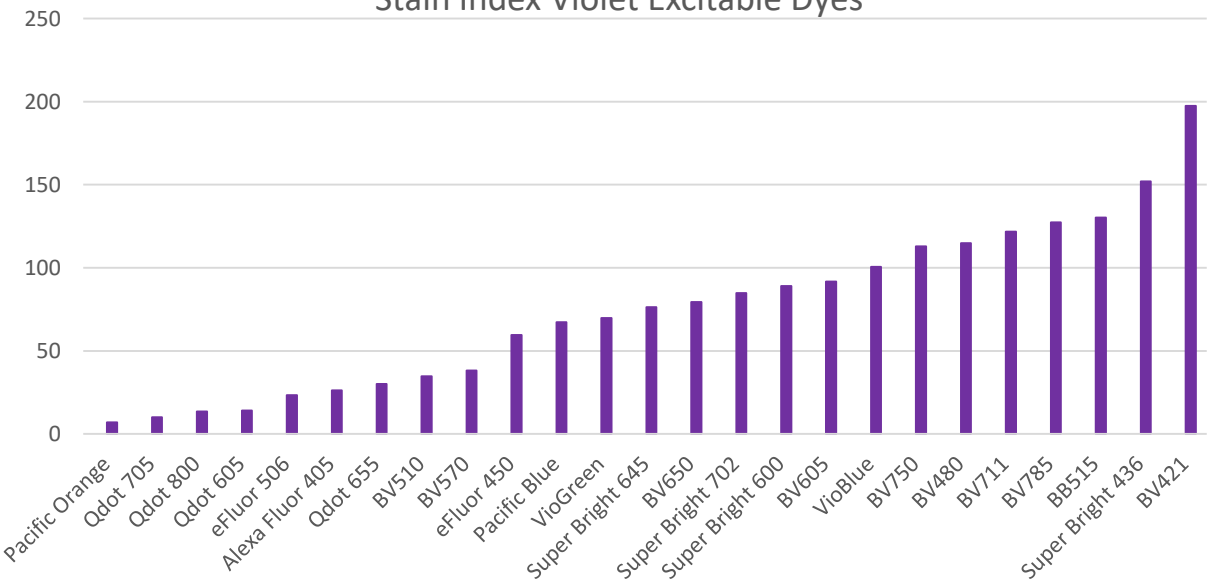
Stain Index Blue Excitable Dyes



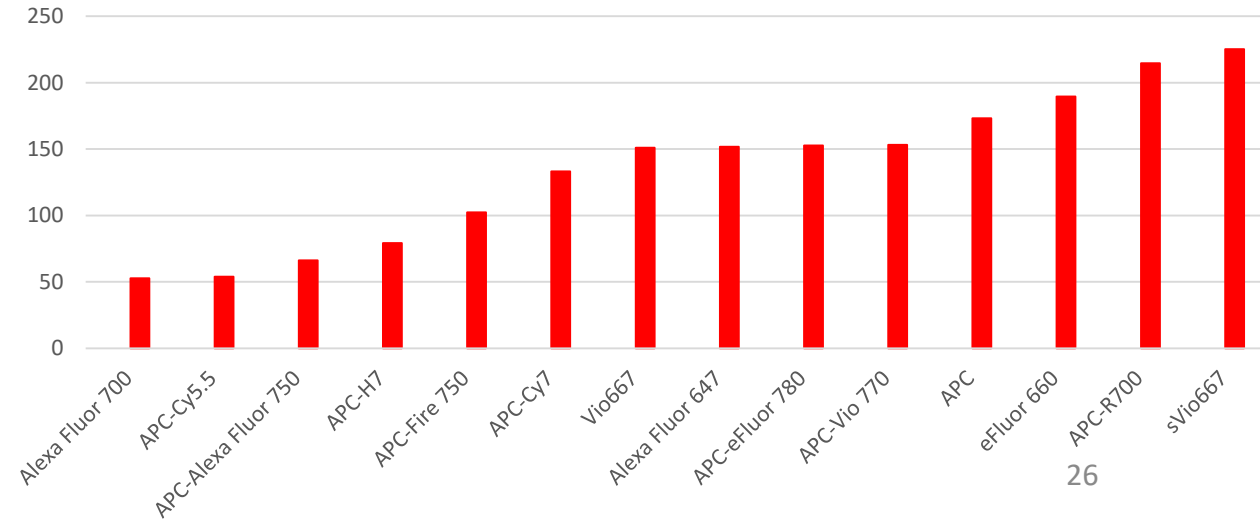
Stain Index Yellow Green Excitable Dyes



Stain Index Violet Excitable Dyes



Stain Index Red Excitable Dyes



Cross-Stain Index Matrix

Dyes used in combination need to have a unique spectrum AND also 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, you will use one or the other (not both!).

Spread matrix for 30 fluors that can be use 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 | APC-R700 | 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 | | |
| APC-R700 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 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.