



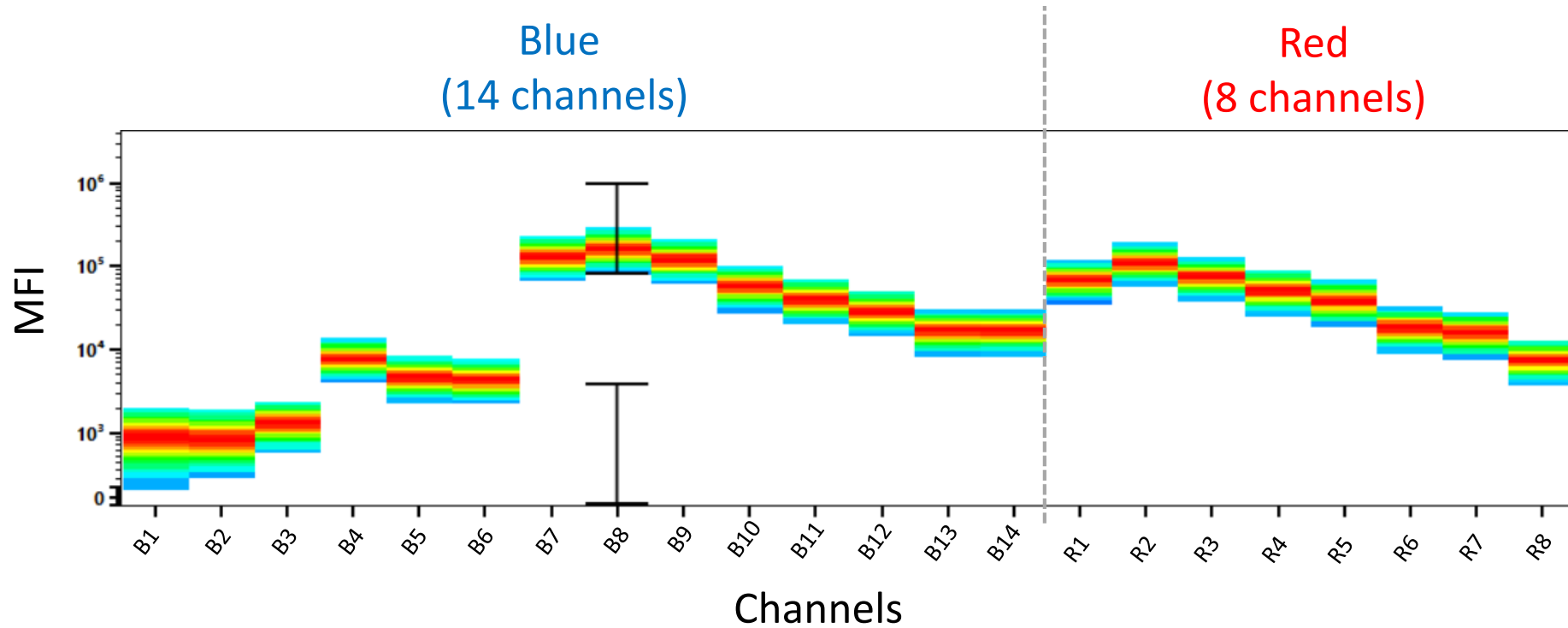
# Cytek® Aurora Fluorochrome Selection Guidelines 2-Laser 14B-8R

# 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 16).

# How to Read Full Spectrum Fluorochrome Signatures

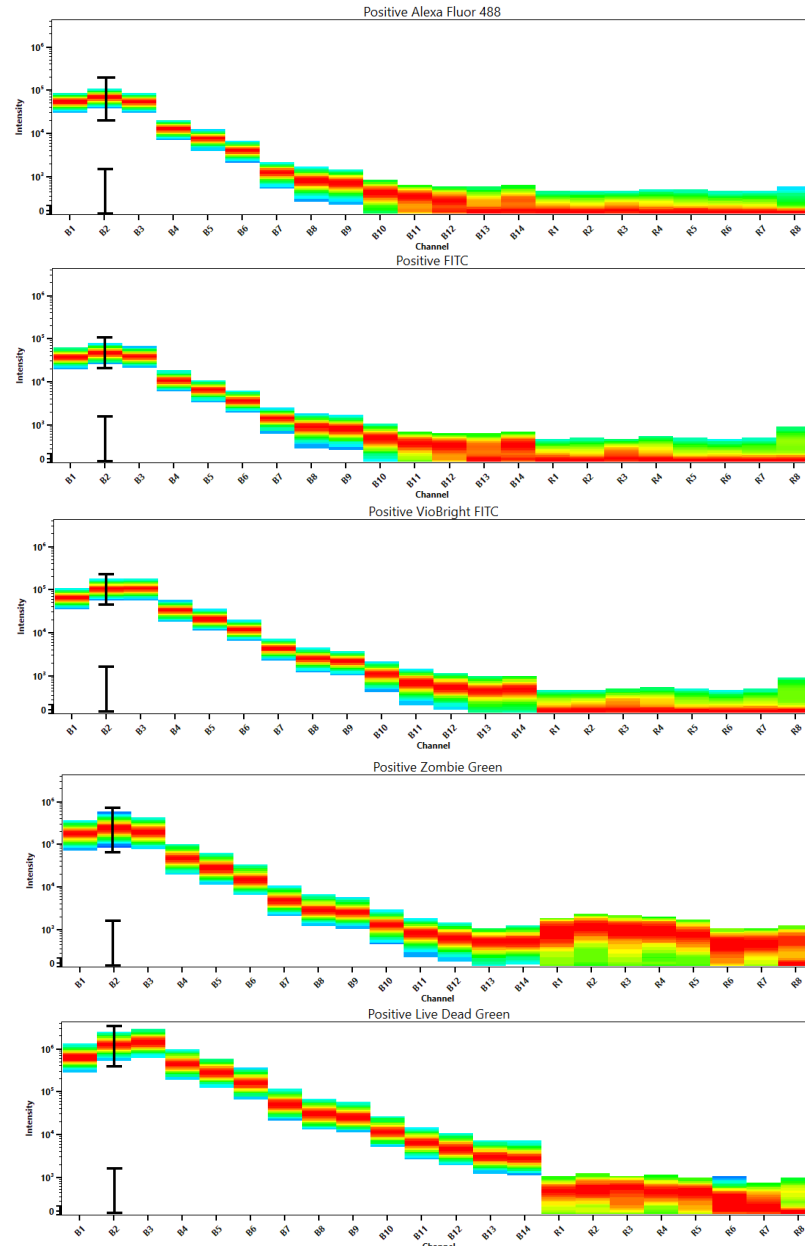


*This dye is excited by both lasers. The peak channel (indicated by the black bar) is in channel B8, and it has secondary emission in channel R2. Based on this information, expect this dye to introduce spread into dyes emitting at similar wavelengths.*

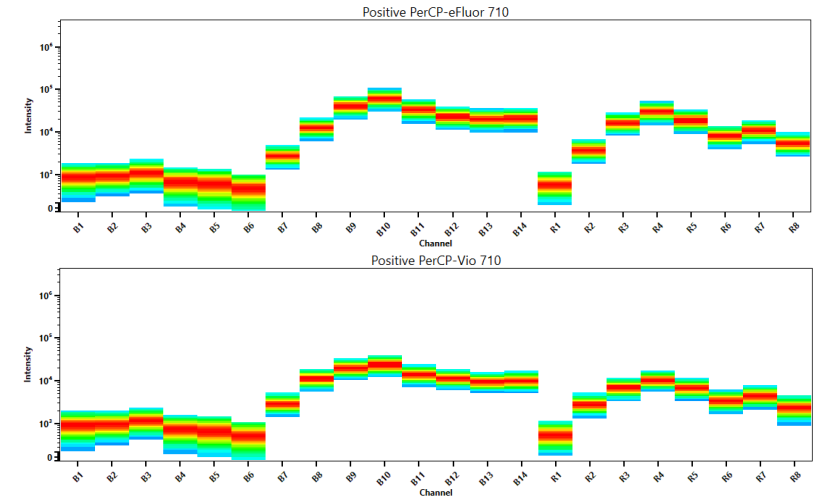
# Dyes Primarily Excited by the Blue Laser

# Blue Laser Excitable Dyes with Similar Signatures (1 of 2)

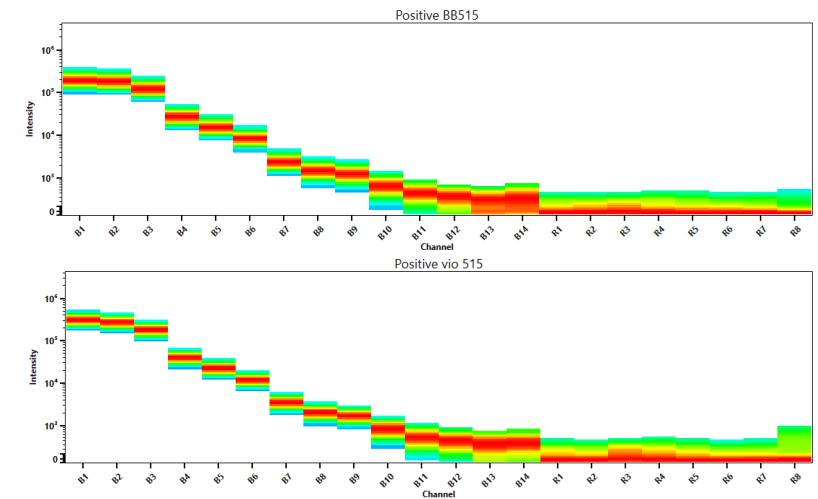
Alexa Fluor 488, FITC, VioBright FITC, Zombie Green and Live Dead Green



PerCP-eFluor 710 and PerCP-Vio 710

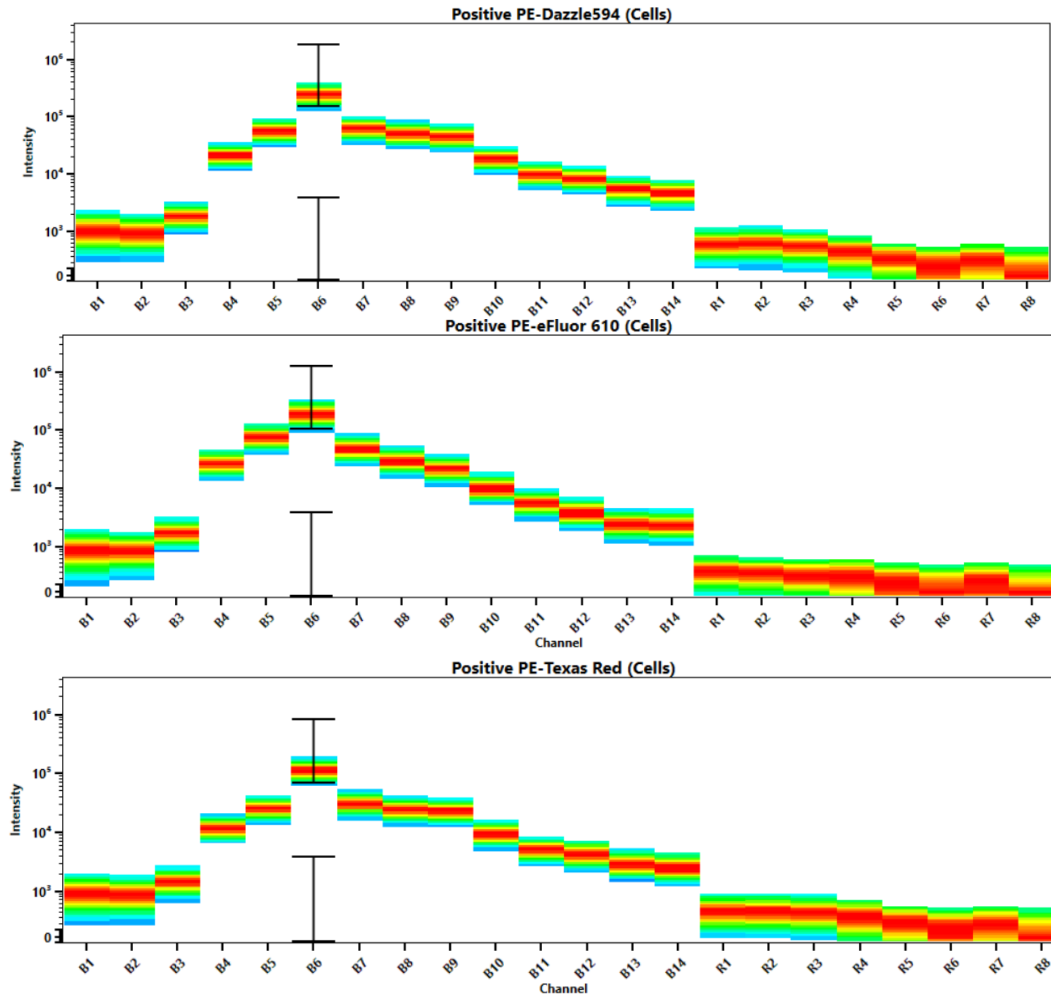


BB515, Vio515 and sVio 515

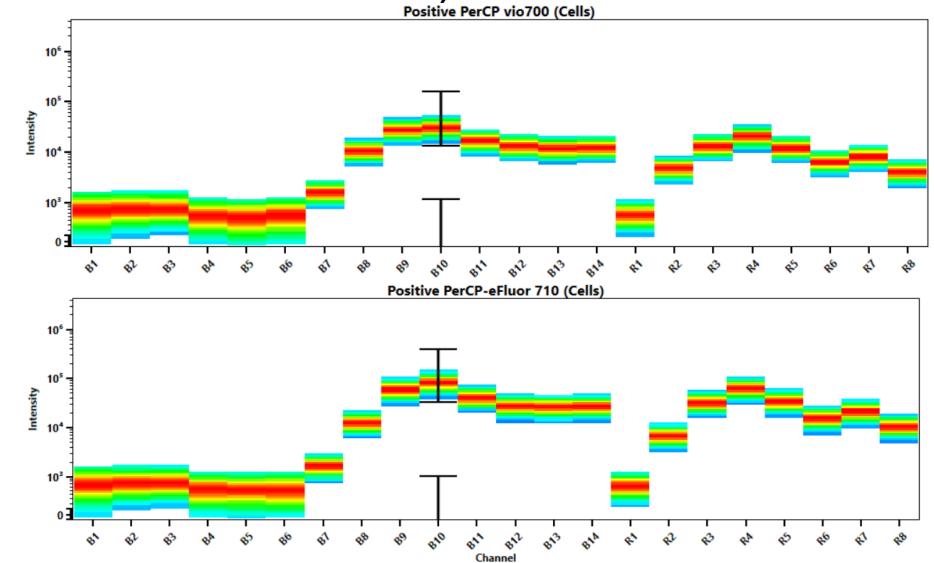


# Blue Laser Excitable Dyes with Similar Signatures (2 of 2)

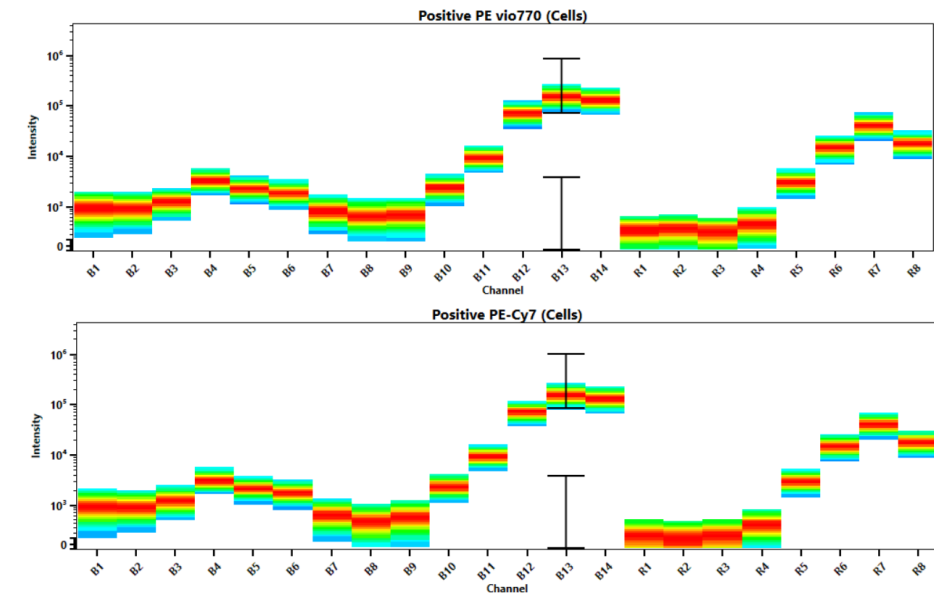
PE/Dazzle 594, PE-eFluor 610, PE-Texas Red



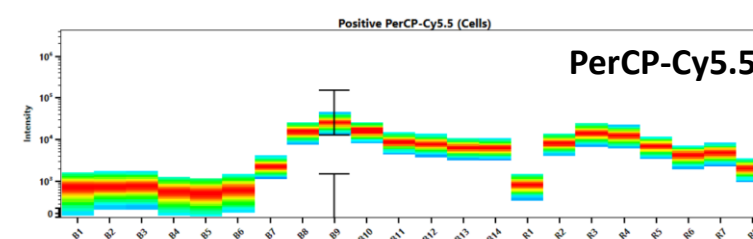
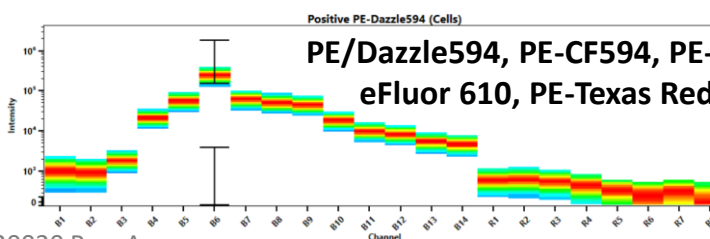
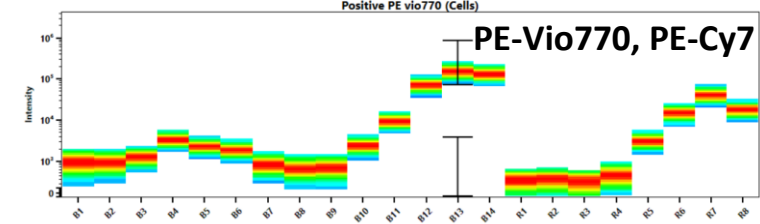
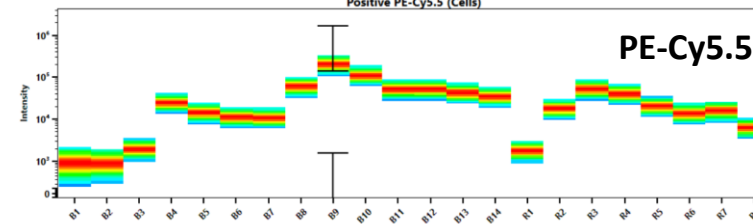
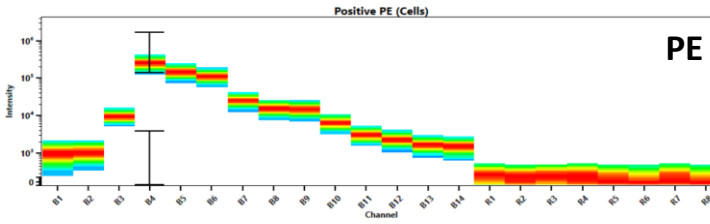
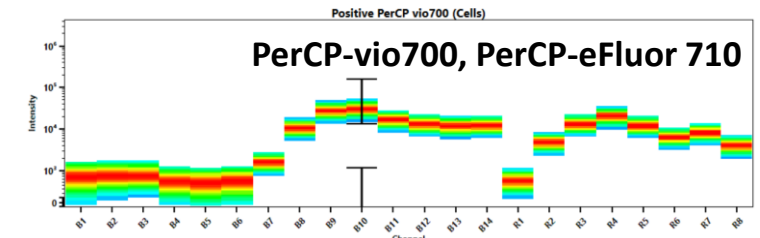
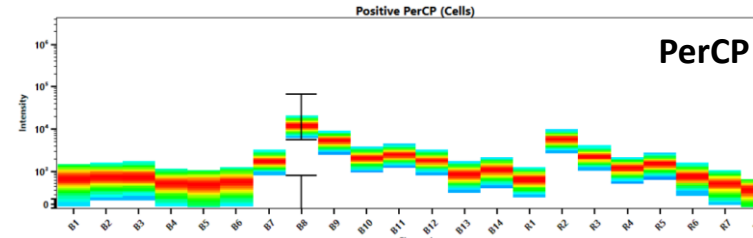
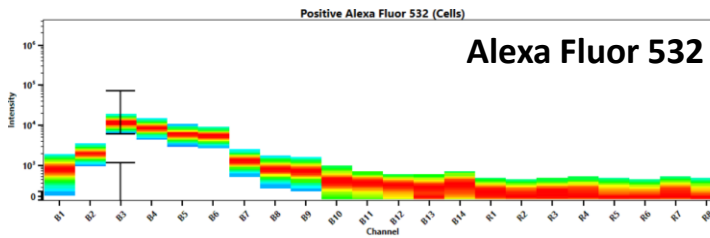
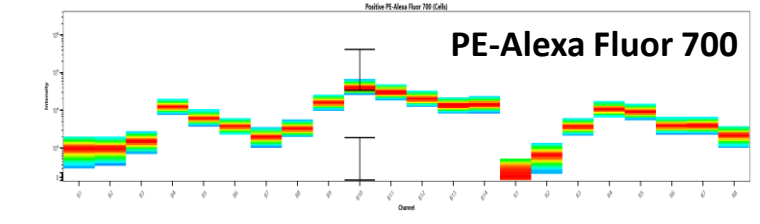
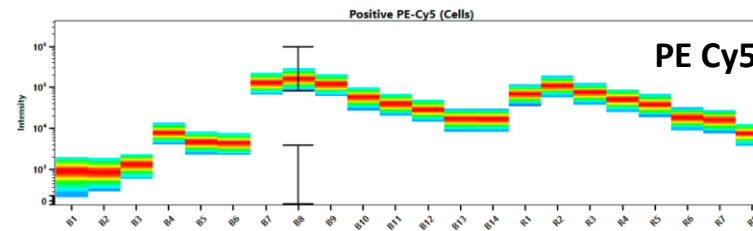
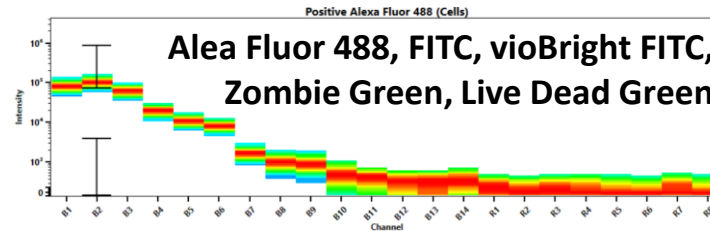
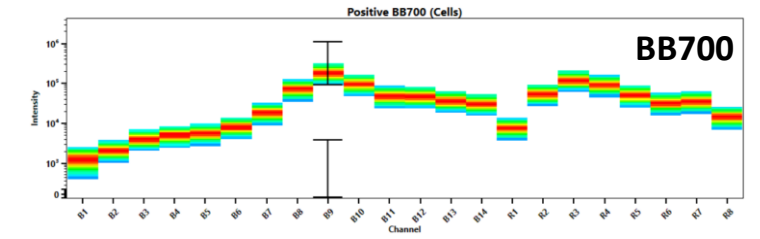
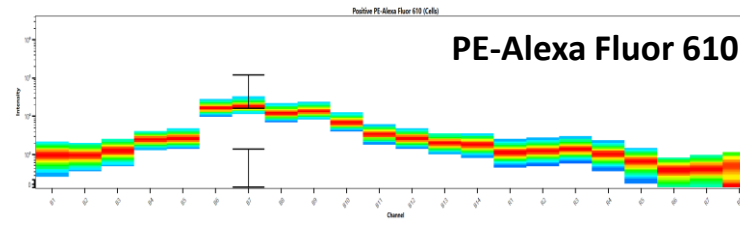
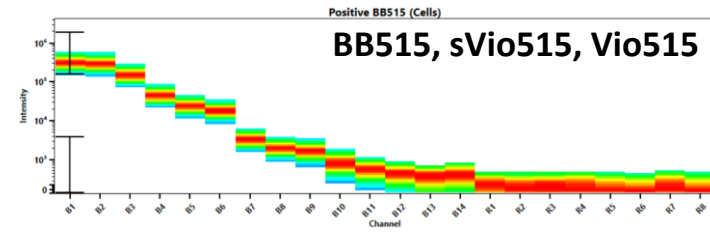
PerCP-Vio700, PerCP-eFluor 710



PE-Vio770, PE-Cy7



# Blue Laser Excitable Dyes with Unique Signatures

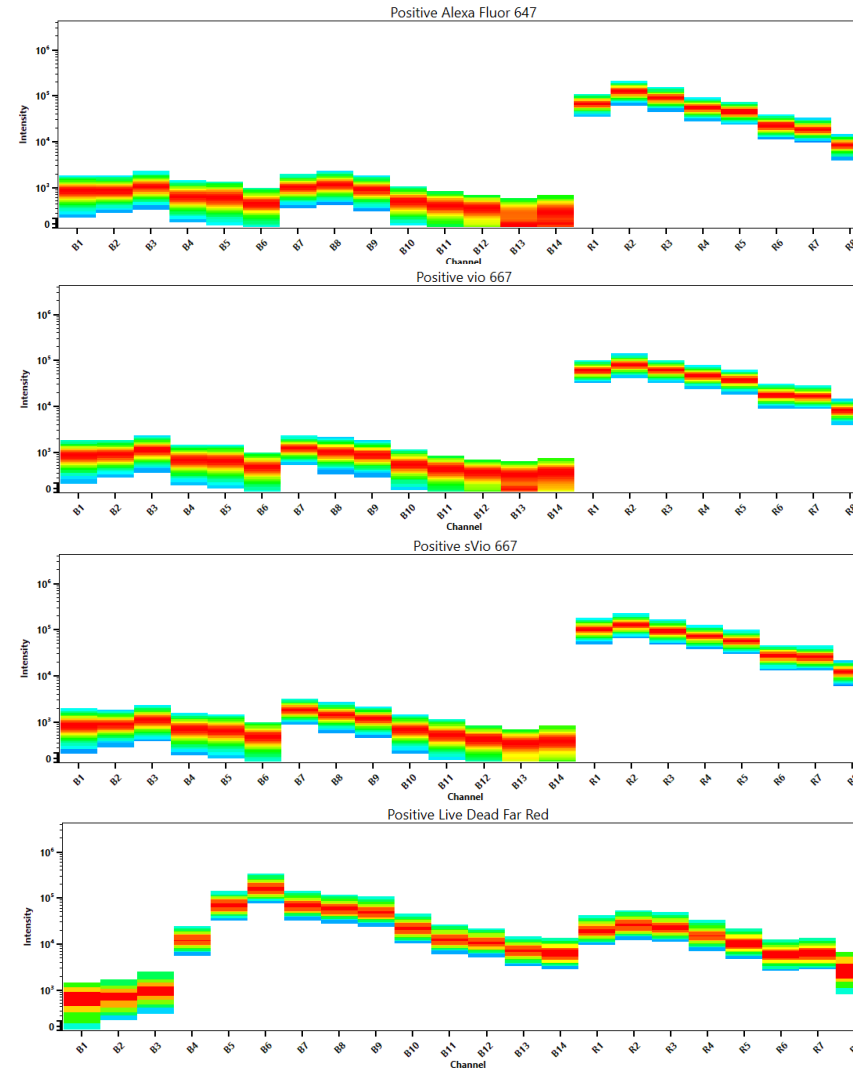


# Dyes Primarily Excited by the Red Laser



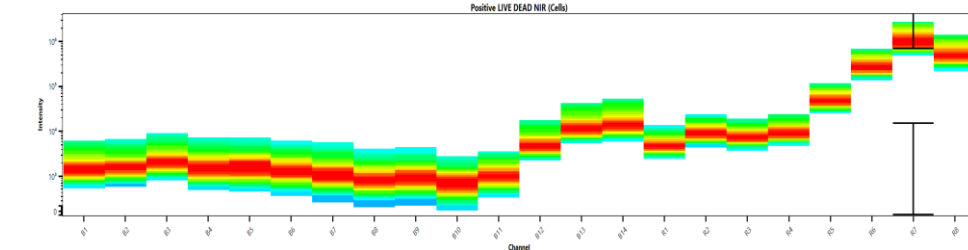
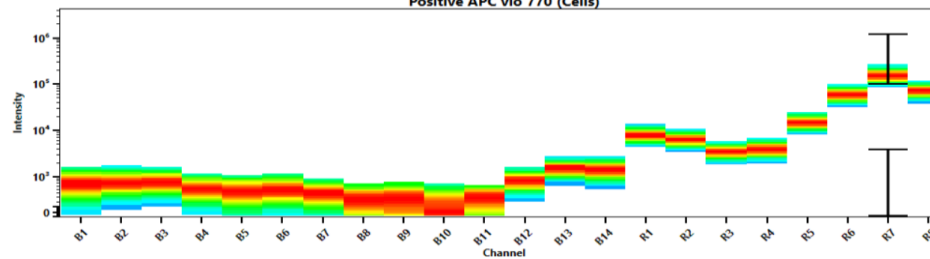
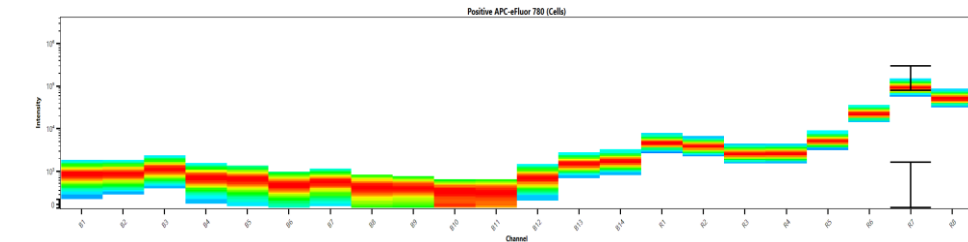
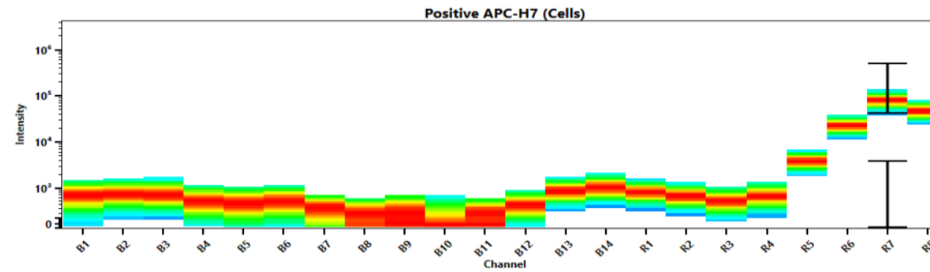
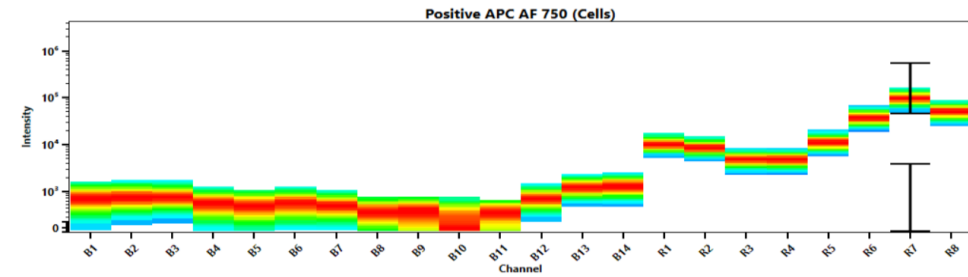
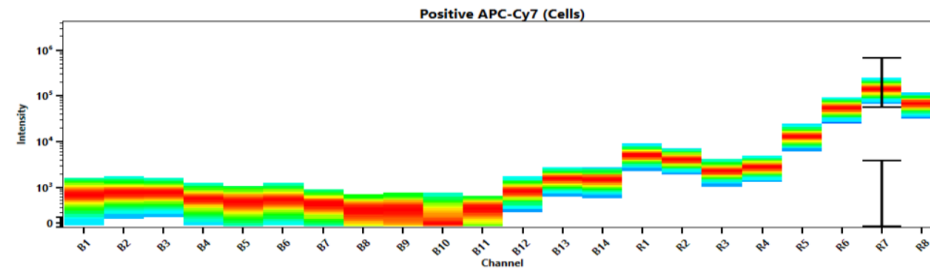
# Red Laser Excitable Dyes with Similar Signatures (1 of 2)

Alexa Fluor 647, Vio 667, sVio 667, Live Dead Far Red

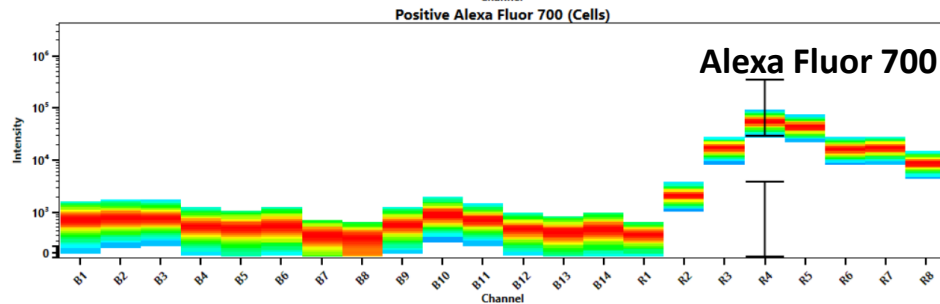
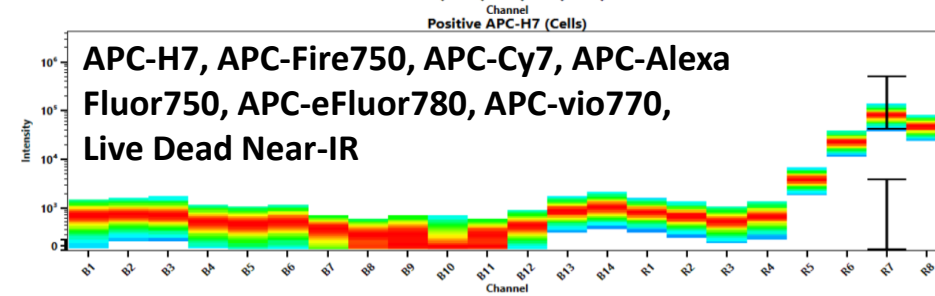
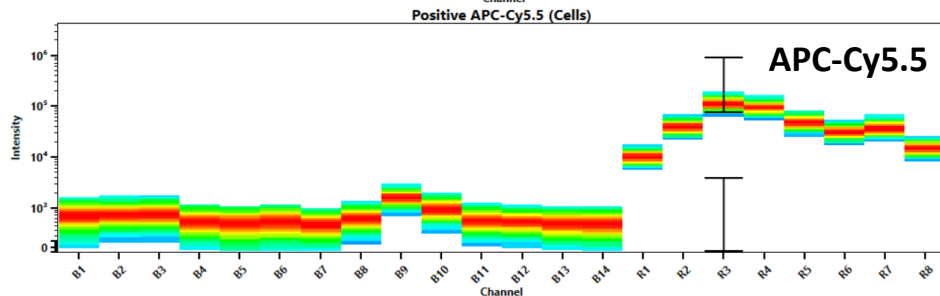
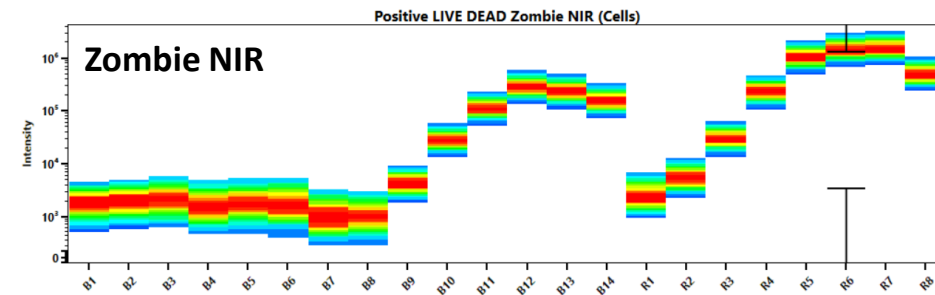
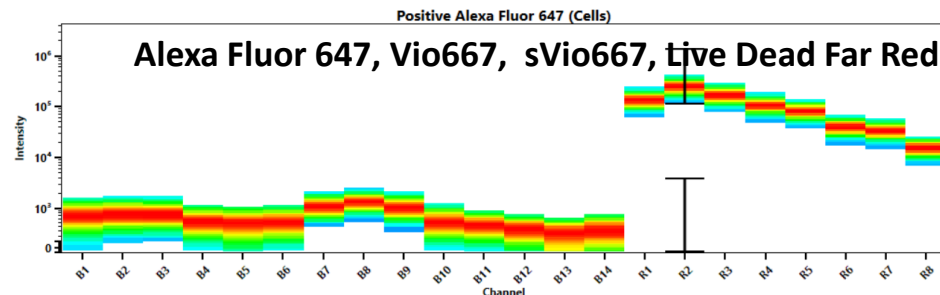
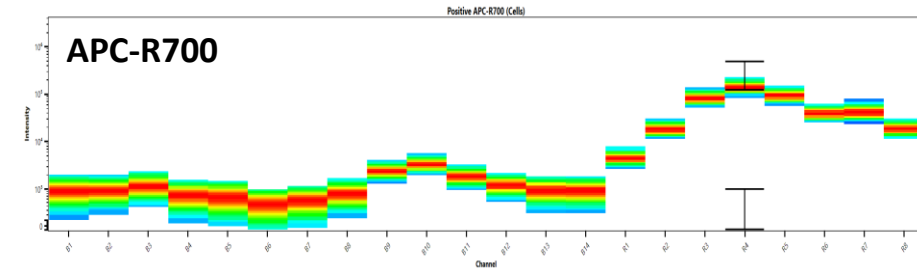
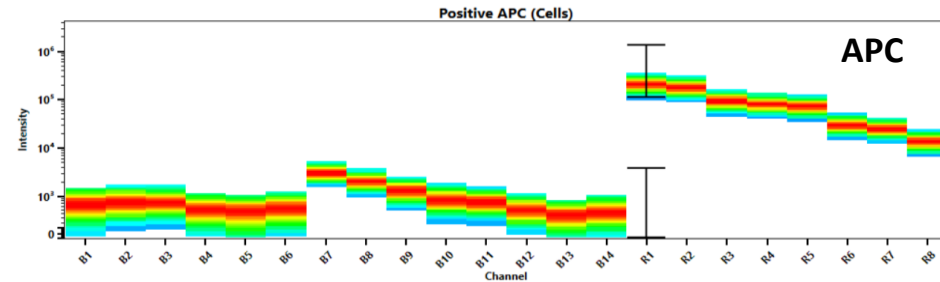


# Red Laser Excitable Dyes with Similar Signatures (2 of 2)

APC-Cy7, APC-H7, APC/Fire750 , APC-Vio770, APC-eFluor780, Live Dead Near-IR



# Red Laser Excitable Dyes with Unique Signatures



# Fluorochrome Peak Channels

| Blue Excited Fluors                                  | Peak Channel |
|--|--------------|
| BB515, sVio515, Vio515                               | B1           |
| Alexa Fluor 488, FITC, VioBright FITC, Zombie Green  | B2           |
| Alexa Fluor 532, Live/Dead Green                     | B3           |
| PE   | B4           |
| PE/Dazzle 594, PE-CF594, PE-eFluor 610, PE-Texas Red | B6           |
| PE-Cy5, PerCP  | B8           |
| PE-Cy5.5, PerCP-Cy5.5, BB700                         | B9           |
| PerCP Vio700, PerCP-eFluor 710                       | B10          |
| PE Vio770, PE-Cy7                                    | B13          |

| Red Excited Fluors   | Peak Channel |
|--|--------------|
| APC  | R1           |
| Alexa Fluor 647, Vio 667, sVio 667, Live/Dead Far Red, eFluor 660                        | R2           |
| APC-Cy5.5  | R3           |
| Alexa Fluor 700, APC-R700  | R4           |
| APC-Alexa 750, APC/Fire 750, APC-Cy7, APC-Vio 770, APC-eFluor 780, APC-H7, Live/Dead NIR | R7           |

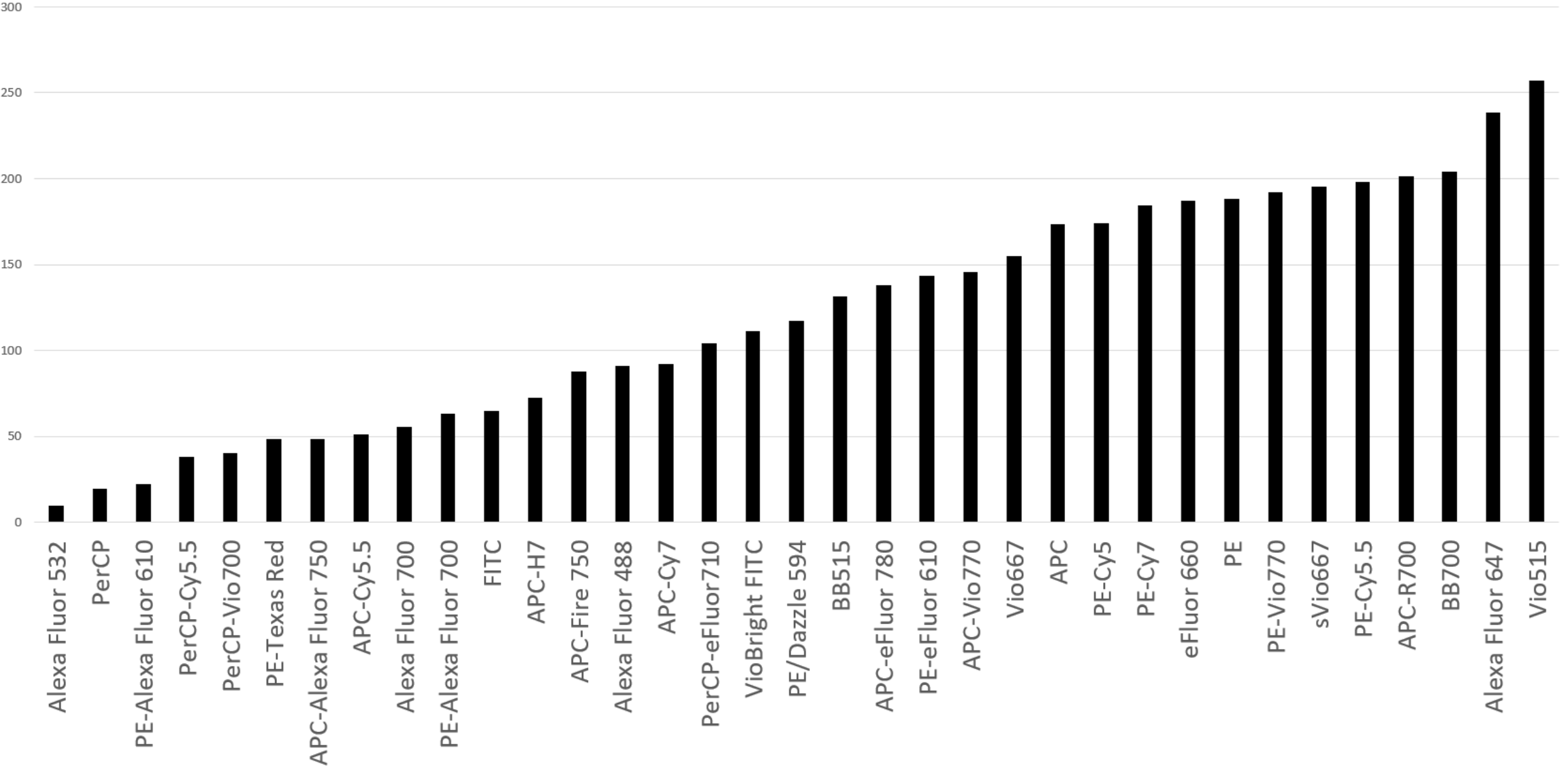
# Example of 13 dyes that can be used in combination

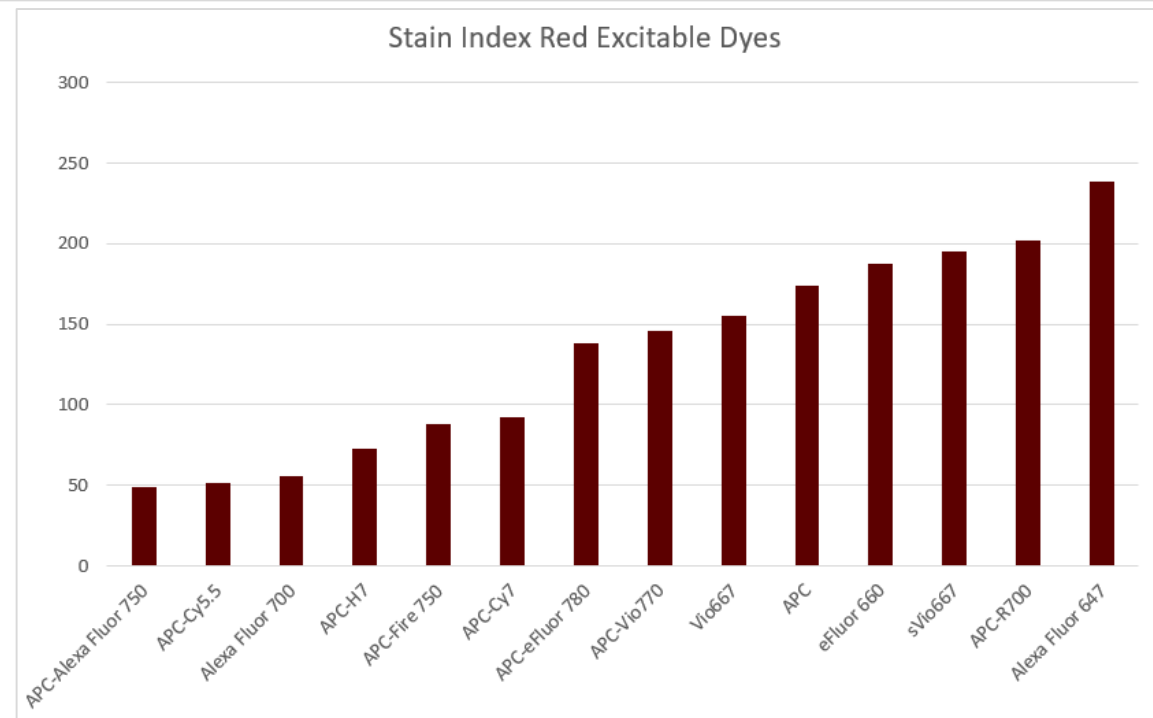
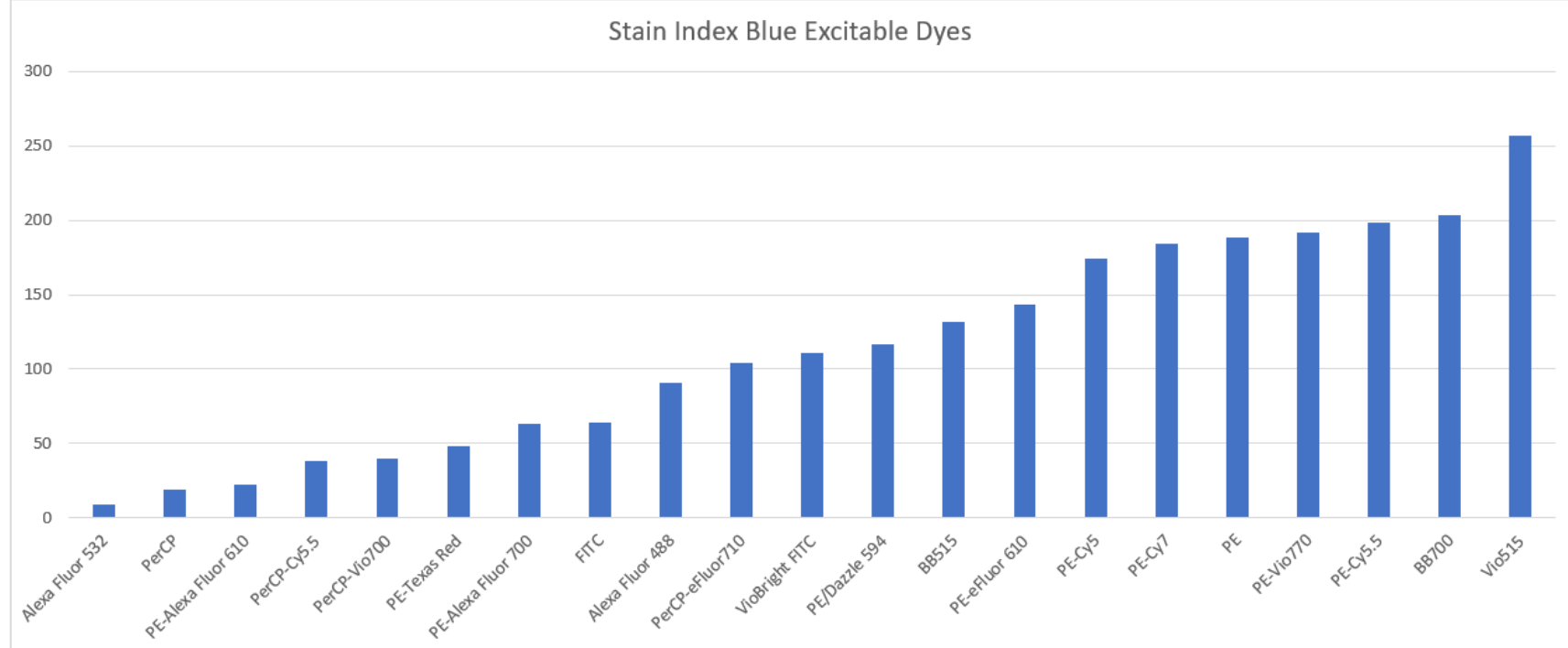
| Fluorophore                 | Fluorophore                |
|-----------------------------|----------------------------|
| BB515                       | APC                        |
| Alexa Fluor 488 or FITC     | Alexa Fluor 647            |
| Alexa Fluor 532             | APC-R700 or AF700          |
| PE                          | APC/Fire 750 or equivalent |
| PE/Dazzle 594 or equivalent |                            |
| PE-Cy5                      |                            |
| PerCP-Cy5.5                 |                            |
| PerCP-eFluor 710            |                            |
| PE-Cy7                      |                            |

# Stain Indexes

Data generated using CD4 staining on human PBMCs

# Stain Index Ranking - 36 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 13 Fluors that can be Used in Combination

|                  | 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 |
|------------------|-------|-----------------|-----------------|-------------|------------------|----|--------------|--------|--------|-----|-----------------|-----------------|--------------|
| 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     |       |                 |                 |             |                  |    |              |        |        |     |                 |                 |              |

*To read this table: fluor in the row impacts the one in the column. Pink means the fluor in that row has spread into the dye in the column (for example BB515 into Alexa Fluor 488). Areas in dark pink are where more attention to panel design is needed.*

# Document Revision History

| Effective Date | Description of Change | Revision | EC No.   |
|----------------|-----------------------|----------|----------|
| 10/21/2019     | Initial Release       | A        | EC-00265 |