

# Cytek® cFluor® Dyes and Reagents

## High Parameter Enablers™ Empower Full Spectrum Profiling™

Cytek® Aurora, Aurora CS, and Northern Lights™ flow cytometers deliver powerful cell analysis and sorting capabilities by leveraging Full Spectrum Profiling™ (FSP™). While developing a 40-color panel, published as OMIP-069 (Cytometry, 97: 1044-1051) in 2020, one of the main challenges was to find enough unique fluorochromes and reagents that were commercially available. Since then, our goal as a leader in high resolution high parameter flow cytometry has been to find new fluorochromes and conjugates that would empower FSP™ users.

To unleash the power of Cytek® full spectrum flow cytometers, the Cytek® team has extensively screened and hand selected fluorochromes ideal for high quality data generation on Cytek® systems. This team of experts also identified and developed new, spectrally unique fluorochromes which enable higher parameter FSP™ panels by taking advantage of areas of the spectrum not utilized by previously available dyes. These new fluorochromes are called High Parameter Enablers™ (HPE™).

In a world of ever expanding fluorochrome options, our field teams listened to users who expressed frustration at the time commitment needed to understand the basic characteristics of new fluorophores. What is the primary laser excitation? Can this dye be used if I don't have the primary laser? What is the peak emission? To alleviate this barrier of entrance to new Cytek® reagents, our fluorochromes are given an intuitive naming strategy based on the laser(s) which most efficiently excite the fluorochrome and its maximum emission wavelength, e.g., cFluor® B532 is excited by the Blue (488 nm) laser and has a maximum emission of 532 nm.

HPEs enable the expansion of your existing panels because they work well alongside other fluorochromes widely used in flow cytometry and compliment the full Cytek® cFluor® catalog of products. We have currently identified, and made commercially available, seven HPEs. These are cFluor® V610, cFluor® B532, cFluor® YG584, cFluor® YG610, cFluor® BYG710, cFluor® BYG750, and cFluor® R720. The graph below shows the normalized emission profile for each of these dyes.

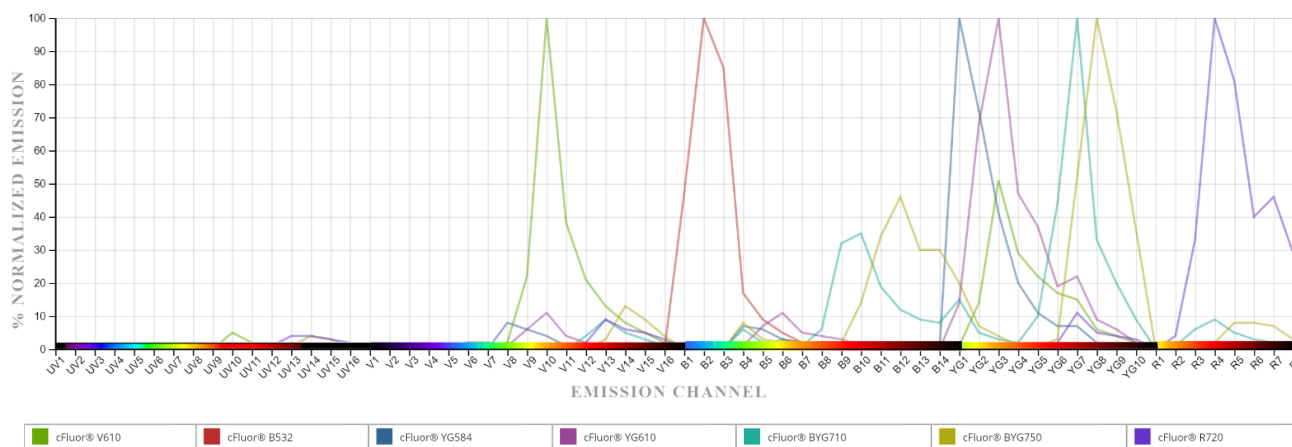


Figure 1: Normalized full spectrum profiles of seven Cytek® cFluor® HPE™ fluorochromes.

In addition to providing antibodies conjugated to HPEs and other cFluors, which are comparable to existing dyes, Cytek® has developed ready-to-use cFluor® kits to provide turnkey solutions for your immunoprofiling needs. Both the Cytek® 25-Color Immunoprofiling Assay and the 14-Color cFluor® Immunoprofiling Kit are useful tools to enumerate and characterize the major human immune cell subsets in blood. The cFluor® TBMNK 8-Color Kit provides a robust base panel which can be easily expanded to meet the needs of even the most in-depth immunological panel. Cytek® is committed to developing more individual reagents and optimized ready-to-use cFluor® reagent kits to jump start your research projects.

**cFluor® HPEs: Advantages and Sample Data**

**Expanding Violet Laser Options With cFluor® V610**

cFluor® V610 is a new HPE fluorochrome which can be used either as a lower spread alternative to BV605 or Super Bright™ 600, or in combination with these fluorochromes to add another parameter to the violet laser. Thanks to its unique spectral characteristics and intermediate brightness (Table 1 and Figure 2) cFluor® V610 is an ideal fluorochrome for intermediate density antigens. When applying appropriate panel design techniques, cFluor® V610 can be used in combination with either BV605 or Super Bright™ 600 (Figure 3), even on co-expressed markers.

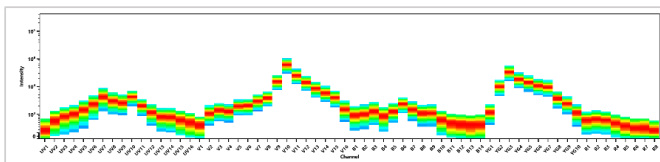
NAME	CHEMISTRY	CONSIDERATIONS	SPECTRA
cFluor® V610	Small Molecule	Spectrally unique. Compatible with BV605 or Super Bright™ 600	

Table 1: cFluor® V610 details

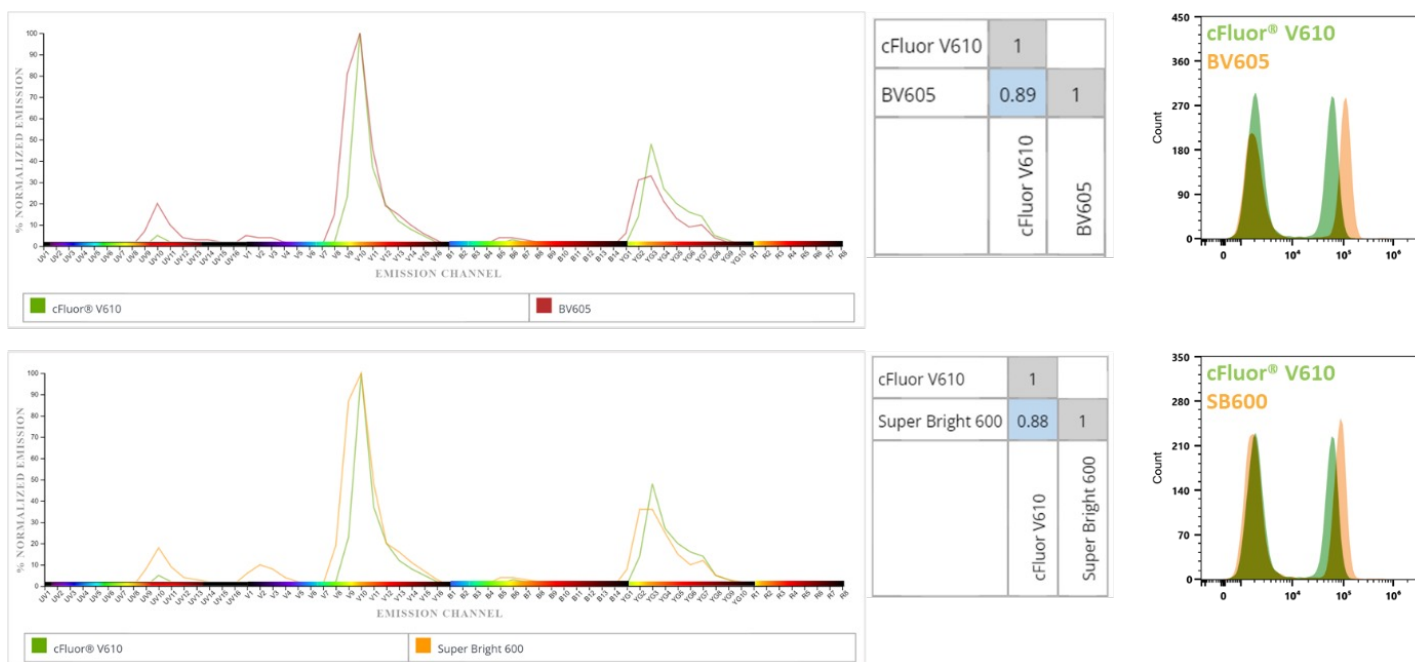
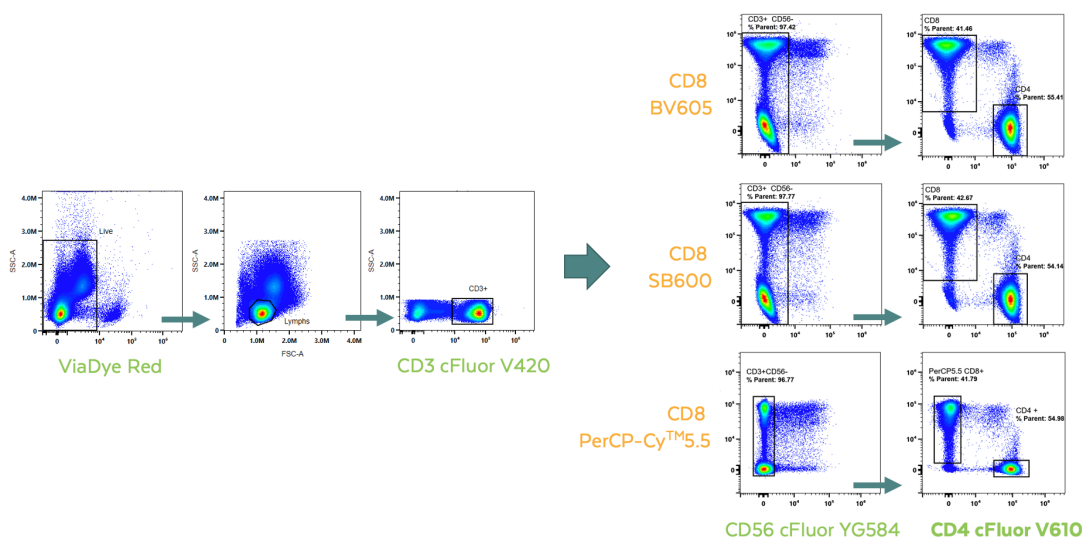


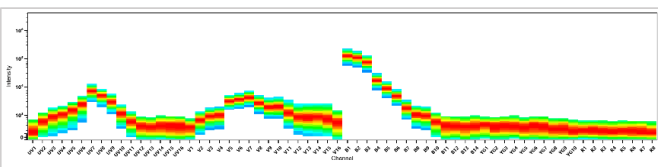
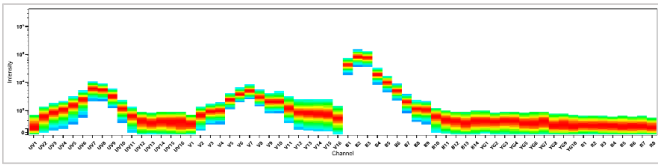
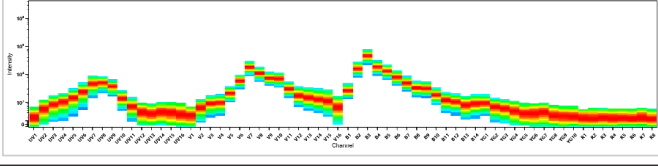
Figure 2: Cytek® cFluor® V610 compared to other similar fluorochromes. Comparison of cFluor® V610 normalized spectra, Similarity Index™, and brightness to BV605™ and Super Bright™ 600.



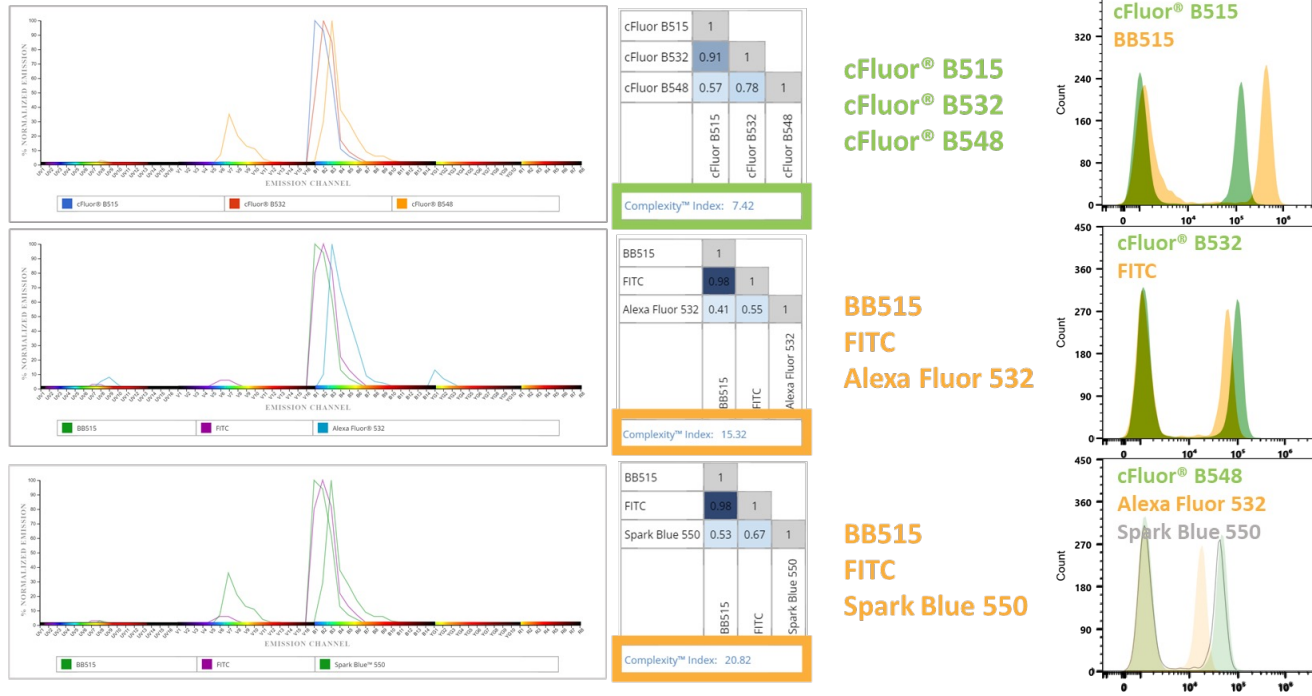
**Figure 3:** cFluor® V610 is spectrally unique and works well with BV605 or Super Bright™ 600. Dead cells were excluded using ViaDye™ Red, T cells were gated using CD3 cFluor® V420, and NK Cells were excluded using CD56 cFluor® YG584. Within the T cells, CD4 cFluor® V610 is shown against CD8 on three different fluorochromes to compare population identification and resolution when using fluorochromes similar (BV605 and Super Bright™ 600) or different (PerCP-Cy5.5) to cFluor V610. In all cases, each population can be clearly identified, with similar percentages of positive cells across all gated populations.

### Improving Data Quality Using HPE™ cFluor® B532 and Other cFluor® Blue Laser Dyes

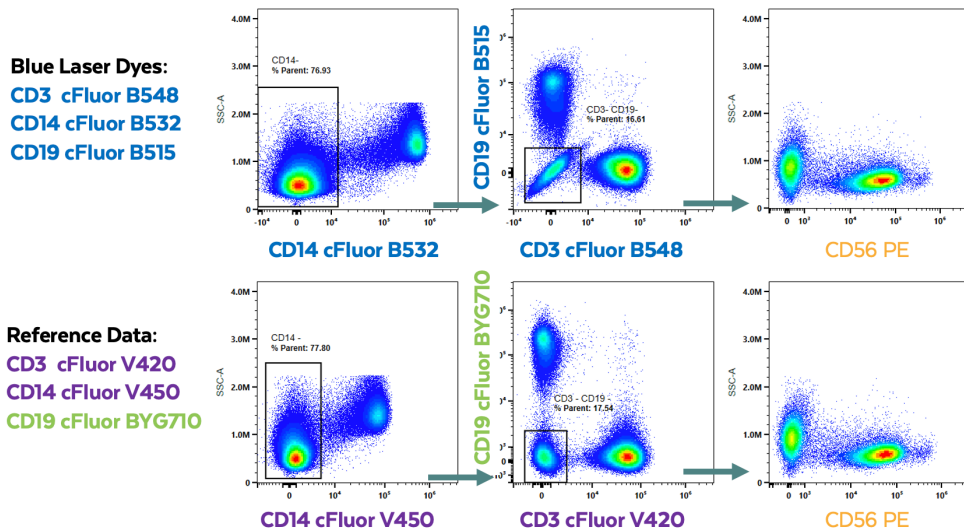
cFluor® B532 is a new HPE™ fluorochrome which is spectrally unique and improves data quality when utilizing the first three detectors of the Blue laser (Table 2). In the 40-color panel used in OMIP-069, BB515, FITC, and Spark Blue™ 550 were used to reach this unprecedented achievement. These three fluorochromes, however, introduce considerable spread and complexity into the panel. When used in combination on non-co-expressed markers, cFluor® B515, cFluor® B532, and cFluor® B548 have lower similarity and complexity to other combinations of fluorochromes used in this region of spectrum (Figures 4 and 5). In turn, this leads to reduced spread, improved resolution, and higher quality data.

NAME	CHEMISTRY	CONSIDERATIONS	SPECTRA
cFluor® B515	Small Molecule	Similar to BB515	
cFluor® B532	Small Molecule	Spectrally unique	
cFluor® V548	Small Molecule	Similar to Spark Blue™ 550	

**Table 2:** Cytek® Blue laser cFluor® details



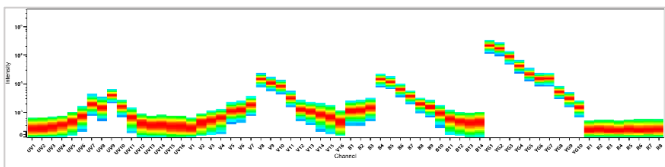
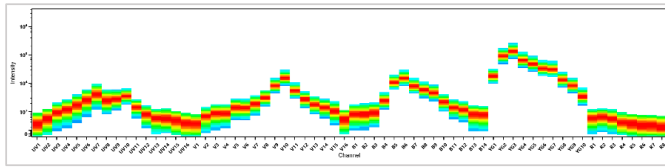
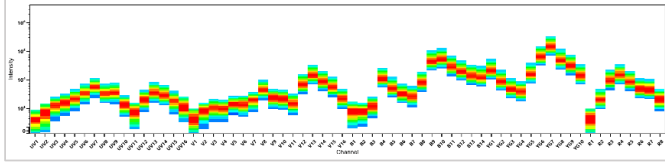
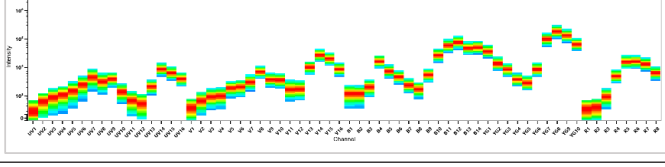
**Figure 4: Cytek® Blue laser cFluors® compared to other similar fluorochromes.** Comparison of cFluor® B515, cFluor® B532, and cFluor® B548 normalized spectra, Similarity Index™, and brightness to BB515, FITC, Alexa Fluor™ 532, and Spark Blue™ 550. Note that the combination of three cFluors® have lower overall similarity and a much lower Complexity Index™ compared to the other combinations of traditional fluorochromes.



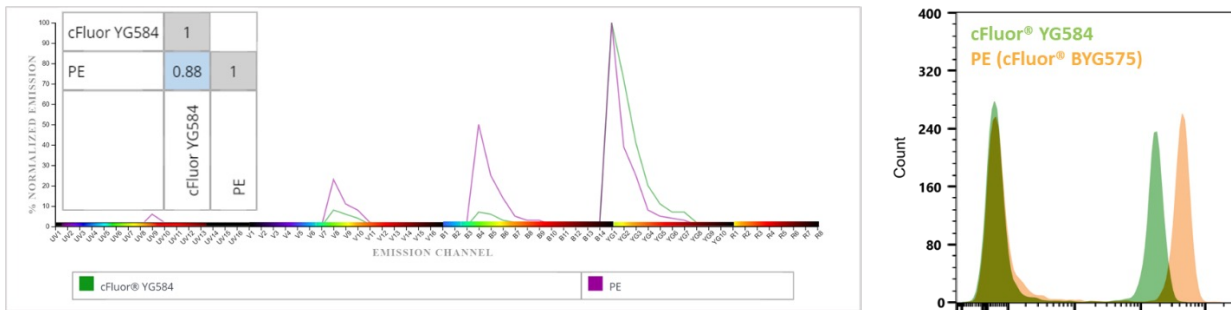
**Figure 5: cFluor® B532 is spectrally unique and works well in combination with cFluor® B515 and cFluor® B548 when used on non-co-expressed markers.** Data was collected on a 5L Cytek® Aurora system running CD3, CD14, and CD19 using either cFluor® B548, cFluor® B532, and cFluor® B515 or cFluor® V420, cFluor® V450, and cFluor® BYG710 as a comparison reference data to visualize and quantify the impact of using these three cFluors® together in the same panel. Cells were first gated as CD14- before comparing CD3 vs. CD19 to isolate the CD3-CD19- cells which were further evaluated for expression of CD56. The frequency and resolution of all populations were comparable between panels and all expected populations were easily gated. Autofluorescence extraction was enabled to improve separation of the positive and negative populations.

### cFluor® HPE™ Yellow-Green and Blue-Yellow-Green Dyes

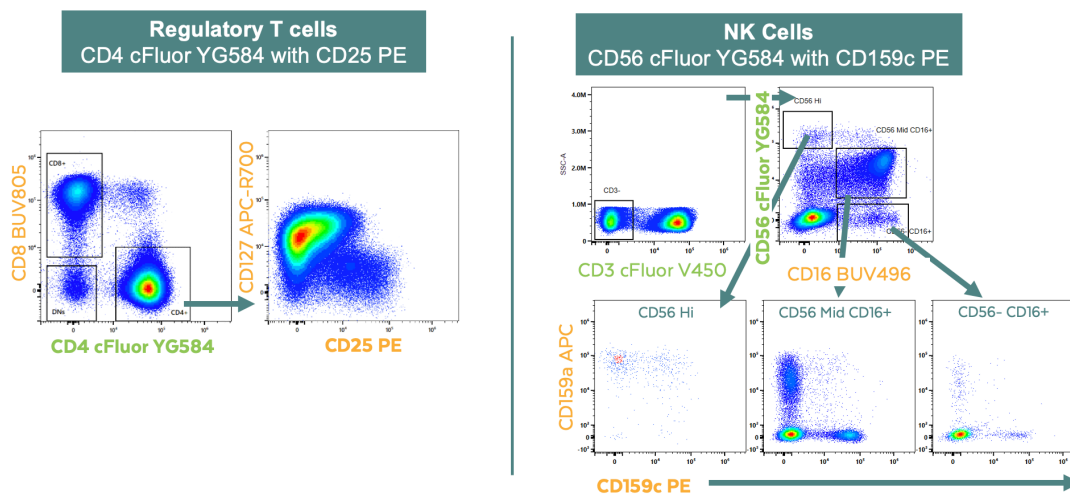
cFluor® YG584, cFluor® YG610, and cFluor® BYG750 are new HPE™ fluorochromes (Table 3). These are each very bright fluorochromes, and when incorporated following appropriate panel design techniques, can be used to add up to three additional parameters to Cytek® instruments with a Yellow-Green laser, and 1 additional parameter in instruments that have a Blue laser and no Yellow-Green laser. cFluor® YG584 was first used in the 40-color panel introduced in OMIP-069 and can be used in combination with PE (cFluor® BYG575), a highly similar fluorochrome, on co-expressed markers, such as CD4 and CD25 (Figures 6 and 7).

NAME	CHEMISTRY	CONSIDERATIONS	SPECTRA
cFluor® YG584	Small Molecule	Spectrally unique. Compatible with PE	
cFluor® YG610	Small Molecule	Spectrally unique. Compatible with BYG610	
cFluor® BYG710	Tandem	Very bright alternative to PE/Fire 700	
cFluor® BYG750	Tandem	Spectrally unique. Compatible with PE-Cy7	

**Table 3:** Cytek® Yellow-Green and Blue-Yellow-Green laser cFluor® details.



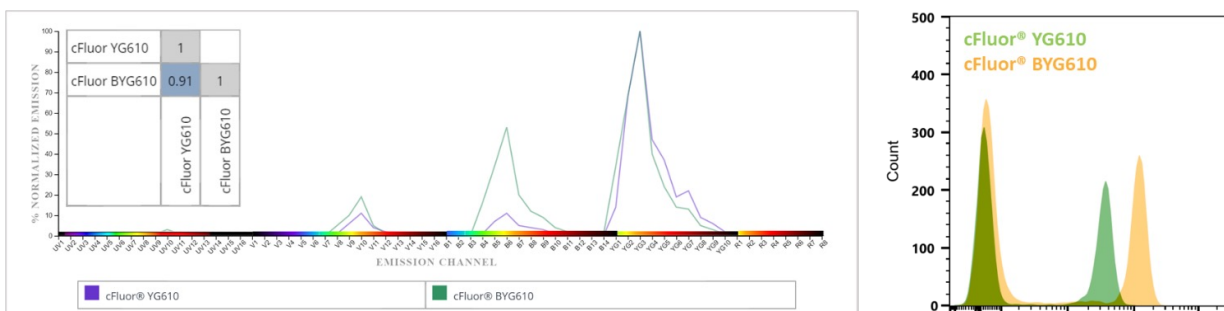
**Figure 6:** Cytek® cFluor® YG584 compared to PE. Comparison of cFluor® YG584 normalized spectra, Similarity Index™, and brightness to PE.



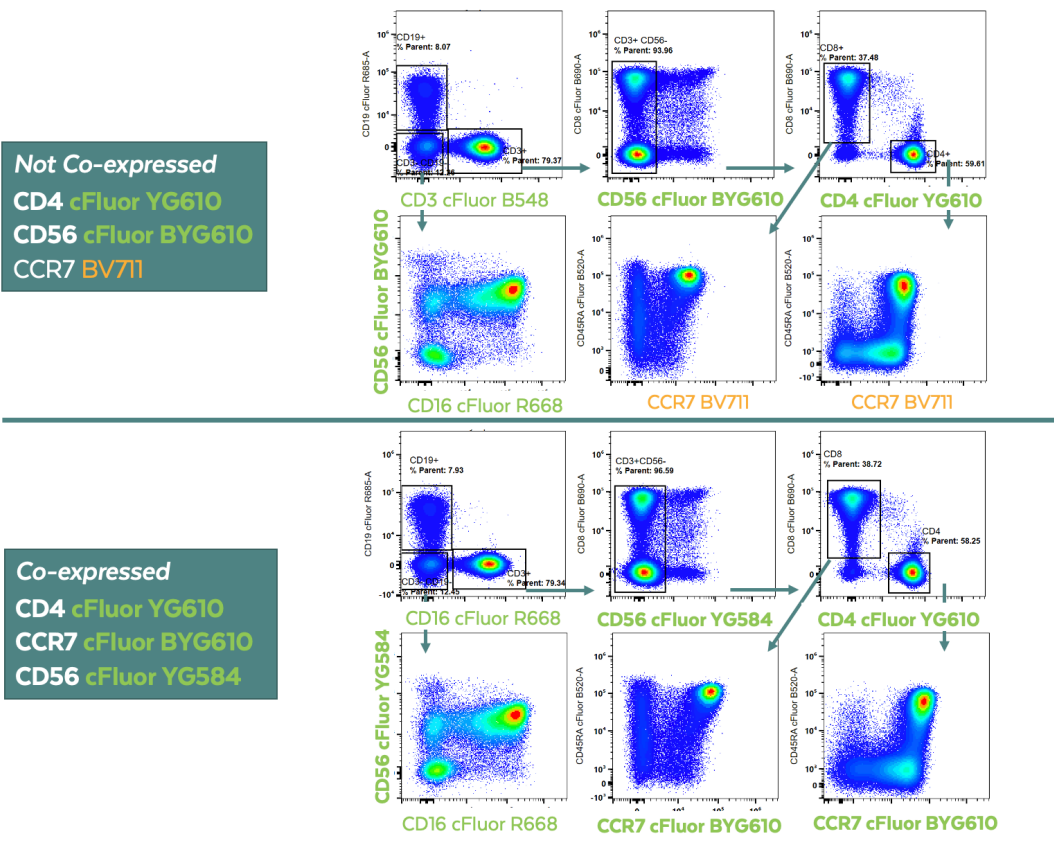
**Figure 7: cFluor® YG584 is spectrally unique and works well in combination with PE.** Data was collected on a 5L Cytek® Aurora system and sample data sets with panels containing cFluor® YG584 and PE (cFluor® BYG575) on co-expressing antigens are shown. Regulatory T cells (Tregs) can be well identified even when cFluor® YG584 and PE are used on co-expressed markers (CD4 and CD25, respectively) (left). In the NK cell data set (right), multiple subsets were identified using CD56 on cFluor® YG584 and CD16 BUV496 on to then evaluate co-expression with CD159a APC and CD159c PE. As with the Treg data, CD159c on PE is clearly resolved even though it is co-expressed with CD56 on a highly similar fluorochrome (cFluor® YG584).

### Combining cFluor® YG610 and cFluor® BYG610 to Grow Your Panels

cFluor® YG610 is a new, spectrally unique HPE™ which can be used in combination with cFluor® BYG610 to expand the use of instruments with a Yellow-Green laser. Although they have a high Similarity Index™ (Figure 8), we have consistently found that these two fluorochromes can successfully be used on co-expressed markers, such as CD4 and CCR7 (Figure 9).



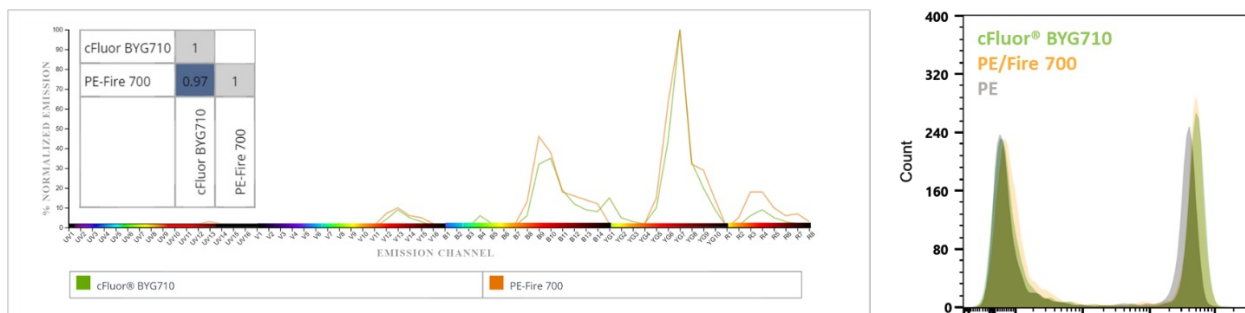
**Figure 8: Cytek® cFluor® YG610 compared to cFluor® BYG610.** Comparison of cFluor® YG610 normalized spectra, Similarity Index™, and brightness to cFluor® BYG610.



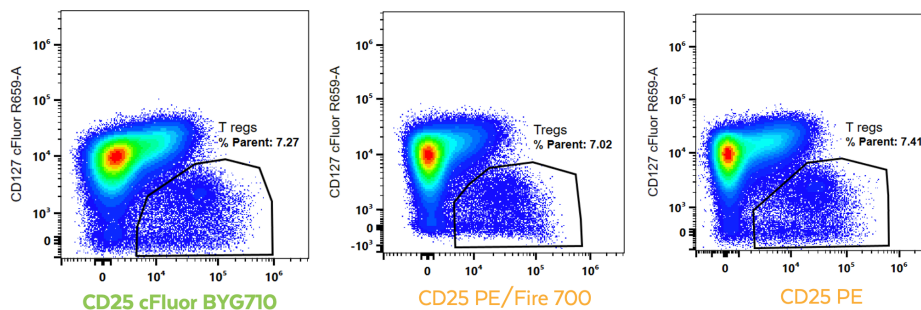
**Figure 9: cFluor® YG610 is spectrally unique and works well in combination with cFluor® BYG610.** Data was collected on a 5L Cytek® Aurora system and sample data sets were run to show the resolution of CCR7 when using highly similar fluorochromes on either non-co-expressed markers (CD56 on cFluor® BYG610 and CD4 on cFluor® YG610, top) or co-expressed markers (CCR7 on cFluor® BYG610 and CD4 on cFluor® YG610, bottom). The data is highly comparable between panels, and no loss in resolution was observed when cFluor® BYG610 and cFluor® YG610 were used on co-expressed markers.

### cFluor® BYG710, A Very Bright Alternative to PE/Fire™ 700

cFluor® BYG710 is an excellent choice for dim tertiary markers as it is a bright tandem dye efficiently excited by both the blue and yellow green lasers. cFluor® BYG710 matches the brightness of PE or PE/Fire™ 700 (Figure 10). Despite having a Similarity Index™ of 0.97 between cFluor® BYG710 and PE/Fire™ 700, which makes them spectrally unique, we do not recommend using them in combination as there is significant amount of spread that is introduced into the panel, resulting in suboptimal data resolution.



**Figure 10: Cytek® cFluor® BYG710 compared to PE/Fire™ 700.** Comparison of cFluor® BYG710 normalized spectra, Similarity Index™, and brightness to PE/Fire 700. Brightness comparison is also shown to PE (right).



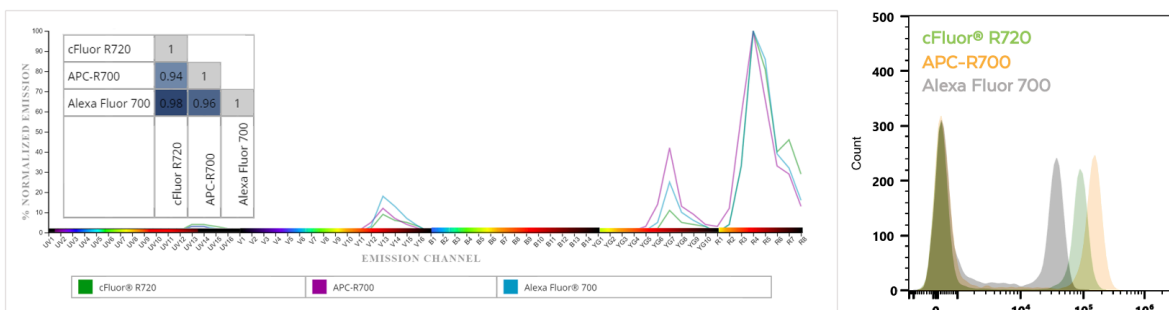
**Figure 11: cFluor® BYG710 is spectrally unique and has similar brightness to PE.** To compare the ability to resolve low-density antigens using cFluor® BYG710, a regulatory T cell panel was developed to compare the resolution when using CD25 on cFluor® BYG710, PE/Fire™ 700, or PE. Plots are gated on live CD3 CD4<sup>+</sup> lymphocytes. As shown, all three fluorochromes performed comparably and no difference was observed in the ability to resolve CD25.

**Looking for a bright and stable red dye? Try cFluor® R720**

cFluor® R720 is a new HPE™ Red laser dye developed by Cytek® to improve data captured from the Red laser. This new dye provides a brighter alternative to Alexa Fluor™ 700 and a more stable alternative to APC-R700, because it is not a tandem dye (Table 4 and Figure 12). Introducing minimal spread into panels, cFluor® R720 is a great option for intermediate density antigens with substantial co-expression and works well with cFluor® R780 and ViaDye™ Red.

NAME	CHEMISTRY	CONSIDERATIONS	SPECTRA
cFluor® R720	Small Molecule	Brighter than Alexa Fluor™ 700. More stable, alternative to APC-R700	

**Table 4: cFluor® cFluor® R720 details**



**Figure 12: Cytek® cFluor® R720 compared to similar fluorochromes..** Comparison of cFluor® R720 normalized spectra, Similarity Index™, and brightness to APC-R700 and Alexa Fluor™ 700.

Visit [www.cytekbio.com](http://www.cytekbio.com) or contact your local representative to learn more about our latest cFluor fluorochromes and reagents.



# Cytek cFluor Chart

Cytek® cFluor®	1° Excitation Laser	Peak Emission	Peak Channel	Dye With Similar Emission	Chemistry	cFluor Advantages
cFluor® V420	Violet	420 nm	V2	Alexa Fluor® 405	Small molecule	Brighter than Alexa Fluor® 405
cFluor® V450	Violet	450 nm	V3	Pacific Blue™; V450; eFluor® 450; VioBlue®	Small molecule	Brighter than alternative dyes
cFluor® V547	Violet	547 nm	V8	Pacific Orange™; Spark Violet™ 538	Small molecule	Brighter than Pacific Orange™
cFluor® V610	Violet	610 nm	V10	BV605	Small molecule	<b>Spectrally unique</b> , works well with BV605
cFluor® B515	Blue	515 nm	B1	BB515; Vio™ B515; Vio Bright™ B515	Small molecule	Works well with cFluor B532 and cFluor B548
cFluor® B520	Blue	520 nm	B2	FITC; Alexa Fluor® 488; KIRAVIA Blue 520™	Small molecule	
cFluor® B532	Blue	532 nm	B3	Vio Bright™ FITC™	Small molecule	<b>Spectrally unique</b> , works well with cFluor B515
cFluor® B548	Blue	548 nm	B3	Spark Blue™ 550; Alexa Fluor® 532	Small molecule	Works well with cFluor B515, BB515 or Vio Bright® B515
cFluor® B675	Blue	675 nm	B8	PerCP	Protein	
cFluor® B690	Blue	690 nm	B9	PerCP-Cy™5.5 or PerCP/Cyanine5.5; BB700	Tandem	
cFluor® YG584	Yellow-Green	584 nm	YG1	PE	Small molecule	<b>Spectrally unique</b> , works well with PE
cFluor® YG610	Yellow-Green	610 nm	YG3	PE-Dazzle™ 594; PE-CF594; PE-Vio® 615; PE-eFluor® 610; eFluor® 615	Small molecule	<b>Spectrally unique</b> , works well with PE-Dazzle™ 594
cFluor® BYG575	Blue, Yellow-Green	575 nm	YG1	PE	Protein	
cFluor® BYG610	Blue, Yellow-Green	610 nm	B6, YG3	PE-Dazzle™ 594; PE-CF594; PE-Vio® 615; PE-eFluor® 610; eFluor® 615	Tandem	Brighter than PE-Dazzle™ 594
cFluor® BYG667	Blue, Yellow-Green	667 nm	B8, YG5	PE-Cy™5 or PE/Cyanine5	Tandem	
cFluor® BYG710	Blue, Yellow-Green	710 nm	B10, YG7	PE/Fire™ 700; PE-Alexa Fluor® 700	Tandem	SI similar to PE, PE-Cy™5, less spillover into PE-Cy™5
cFluor® BYG750	Blue, Yellow-Green	750 nm	B12, YG8	None	Tandem	<b>Spectrally unique</b> , SI similar to PE
cFluor® BYG781	Blue, Yellow-Green	781 nm	B13, YG9	PE-Cy™7 or PE/Cyanine7; PE-Vio® 770	Tandem	
cFluor® R659	Red	659 nm	R1	APC	Protein	
cFluor® R668	Red	668 nm	R2	Alexa Fluor® 647; eFluor® 660; Cy™5	Small molecule	
cFluor® R685	Red	685 nm	R3	APC-Cy™5.5 or APC/Cyanine5.5; Alexa Fluor® 660; Spark NIR™ 685	Small molecule	Less spillover into YG, Violet, & Alexa Fluor® 700
cFluor® R720	Red	720 nm	R4	Alexa Fluor® 700; APC-R700	Small molecule	<b>Spectrally unique</b> , SI greater than Alexa Fluor® 700
cFluor® R780	Red	780 nm	R7	APC/Fire™ 750; APC-H7; APC-Cy™7 or APC/Cyanine7	Tandem	
cFluor® R840	Red	840 nm	R8	APC/Fire™ 810	Tandem	

cFluor® V547, cFluor® B515, cFluor® R668 and cFluor® R720 are equivalent to CF®405L, CF®488A, CF®647 and CF®700 respectively, manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytek (LICENSEE). The manufacture, use, sale, offer for sale, or import of the product is covered by one or more of the patents or pending applications owned or licensed by Biotium. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.

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