

The Use of New StarBright Dyes in Spectral Flow Cytometry



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StarBright Dyes from Bio-Rad are proprietary, bright, fluorescent nanoparticles excitable by the 355 nm, 405 nm and 488 nm lasers, specifically developed for flow cytometry using the ZE5 Cell Analyzer. Here we show how their unique spectral characteristics make these dyes ideal for use in full spectrum flow cytometry, providing more choice and new dye combinations in multicolor panel building. In a 27-color panel, identifying T cell, B cell, and myeloid lineages and subsets, we demonstrate StarBright Dyes can be used to create a high parameter immunophenotyping panel. We included combinations of StarBright Dyes and other dyes, including those that have similar excitation and emission maxima, such as StarBright Violet 515 (SBV515) and Brilliant Violet 510 (BV510), but have spectral profiles that are sufficiently unique to allow accurate unmixing.

Materials and Methods

Staining conditions: Red blood cell lysed human peripheral blood was blocked with 10% human serum and stained with Live/Dead Fixable Blue (Thermo Fisher Scientific). After washing and resuspending, cells were stained with a cocktail containing 26 antibodies in BD Brilliant Stain Buffer (BD), or a single antibody for compensation control tubes. Cells were stained in a 96-well plate for 1 hour at room temperature (RT), washed three times, and resuspended in FACS Buffer.

Staining panel: Antibodies used in the panel are shown in Table 1. All antibodies were titrated to determine the optimal staining concentration prior to use.

Data collection and analysis: Data for these studies were collected on a 4-laser (16UV-16V-16B-10R) Aurora Spectral Analyzer (Cytek). Analysis was performed using Spectroflo (Cytek) and FCS Express 7 (De Novo) Software.

Table 1. Antibodies used in a 27-multiplex panel. A combination of prelaunch StarBright Dye conjugated antibodies (blue), catalog StarBright Dye conjugated antibodies (red), and antibodies conjugated to other fluorophores were used. Unless stated the antibodies were from Bio-Rad.

Target	Fluorophore	Catalog number	Target	Fluorophore	Catalog number
CD3	SBUV400	MCA463SBUV400	CD25	SBV610	MCA2127SBV610
CD19	SBUV445	MCA1940SBUV445	CD27	SBV670	MCA755SBV670
CD4	SBUV510	MCA1267SBUV510	CD10	SBV710	MCA1556SBV710
CD28	SBUV575	MCA709SBUV575	TCR γ d	BV711	331411 (BioLegend)
HLA DR DQ DP	BUV615	751197 (BD)	CD14	SBV790	MCA1568SBV790
CD56	BV421	318327 (BioLegend)	CD57	FITC	MCA1305F
CD11b	Pacific Blue	MCA511PB	CD45RO	SBB700	MCA461SBB700
CD45	SBV440	MCA875SBV440	CD20	PerCP-Cy5.5	302325 (BioLegend)
CD8	SBV475	MCA1226SBV475	CD16	PE	MCA2537PE
IgD	BV510	348219 (BioLegend)	CD38	PE-A750	MCA1019P750
CD2	SBV515	MCA1194SBV515	CD45RA	A700	MCA88A700
CD33	SBV570	MCA1271SBV570	CD127	A647	HCA145A647
CD11c	BV605	301635 (BioLegend)	CD40	APC-Cy7	334323 (BioLegend)

StarBright Dye Spectral Profiles

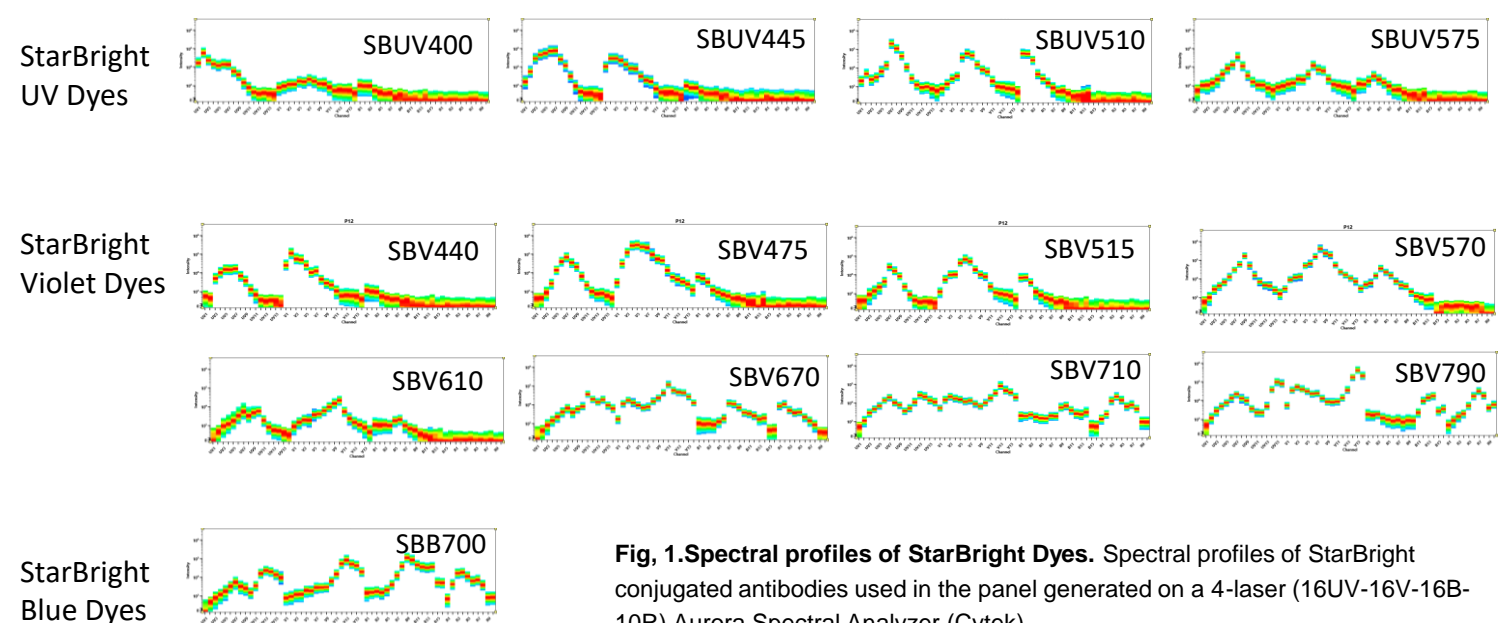
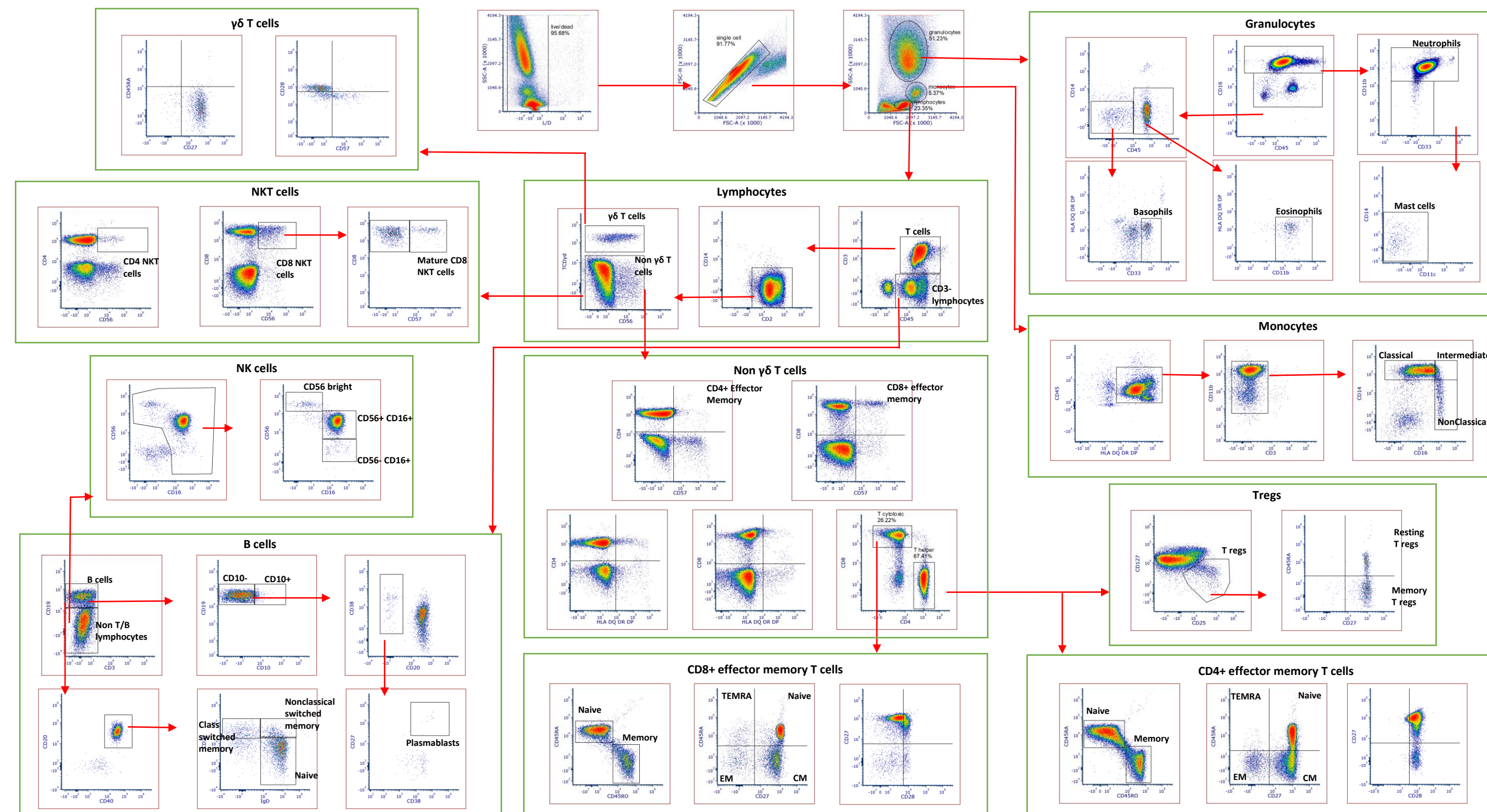


Fig. 1. Spectral profiles of StarBright Dyes. Spectral profiles of StarBright conjugated antibodies used in the panel generated on a 4-laser (16UV-16V-16B-10R) Aurora Spectral Analyzer (Cytek).

27-Color Immunophenotyping Panel



Similarity Matrix

Table 2. Similarity matrix. Similarity indices from the 27-color panel and overall complexity index value of 70.32.

	SBUV400	SBUV445	SBUV510	SBUV575	BV421	SBV440	SBV475	SBV515	SBV570	SBV610	SBV670	SBV710	SBV790	FITC	PE	PerCP-Cy5.5	PE-A750	Alexa Fluor 488	Alexa Fluor 700	APC-Cy7	
SBUV400	1	0.37	0.42	0.13	0.06	0.05	0.04	0.06	0.01	0.03	0.04	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
SBUV445	0.37	1	0.06	0.54	0.17	0.08	0.37	0.47	0.37	0.33	0.29	0.27	0.09	0.06	0.04	0.06	0.1	0.03	0.06	0.02	0.03
LIVE DEAD Blue	0.42	0.54	1	0.43	0.13	0.07	0.37	0.45	0.34	0.26	0.22	0.2	0.06	0.05	0.03	0.06	0.09	0.03	0.05	0.02	0.02
SBUV510	0.13	0.06	0.43	1	0.35	0.1	0.08	0.13	0.12	0.38	0.52	0.55	0.24	0.08	0.12	0.05	0.06	0.01	0.03	0.36	0.09
SBUV575	0.06	0.17	0.13	0.35	1	0.45	0.03	0.04	0.04	0.16	0.4	0.2	0.63	0.31	0.39	0.17	0.08	0.03	0.03	0.36	0.03
SBV421	0.05	0.08	0.07	0.1	0.45	1	0.02	0.02	0.02	0.06	0.18	0.06	0.27	0.43	0.45	0.34	0.16	0.08	0.05	0.01	0.15
SBV440	0.04	0.37	0.37	0.08	0.03	0.02	1	0.84	0.78	0.37	0.19	0.21	0.07	0.08	0.07	0.12	0.18	0.09	0.11	0.01	0.02
SBV475	0.06	0.47	0.45	0.13	0.04	0.02	0.84	1	0.85	0.46	0.25	0.26	0.08	0.07	0.08	0.13	0.2	0.08	0.12	0.01	0.02
Pacific Blue	0.01	0.37	0.34	0.12	0.04	0.02	0.78	0.85	1	0.66	0.36	0.38	0.12	0.07	0.11	0.16	0.24	0.07	0.15	0.01	0.03
SBV475	0.03	0.33	0.26	0.38	0.16	0.06	0.37	0.46	0.64	1	0.24	0.25	0.43	0.17	0.31	0.18	0.21	0.05	0.12	0.01	0.01
SBV510	0.04	0.29	0.22	0.52	0.8	0.18	0.19	0.25	0.38	0.44	1	0.8	0.78	0.39	0.37	0.27	0.2	0.06	0.1	0.06	0.28
SBV515	0.03	0.27	0.2	0.55	0.2	0.06	0.21	0.26	0.38	0.45	0.8	1	0.45	0.14	0.31	0.13	0.15	0.03	0.09	0.14	0.13
SBV570	0.01	0.09	0.06	0.24	0.81	0.27	0.07	0.08	0.12	0.41	0.74	0.45	1	0.61	0.77	0.32	0.14	0.05	0.06	0.53	0.04
BV605	0.01	0.06	0.05	0.08	0.31	0.43	0.08	0.07	0.17	0.39	0.14	0.61	0.61	1	0.94	0.65	0.28	0.19	0.12	0.02	0.32
SBV610	0	0.04	0.03	0.12	0.39	0.45	0.07	0.08	0.11	0.31	0.57	0.31	0.31	0.94	1	0.48	0.17	0.07	0.06	0.04	0.41
SBV670	0.01	0.06	0.06	0.05	0.17	0.34	0.12	0.13	0.16	0.18	0.27	0.32	0.32	0.65	0.48	1	0.63	0.52	0.33	0.02	0.17
SBV710	0.01	0.1	0.09	0.06	0.08	0.16	0.18	0.2	0.24	0.21	0.2	0.15	0.14	0.28	0.17	0.63	1	0.96	0.56	0.01	0.06
BV711	0	0.03	0.03	0.01	0.03	0.08	0.08	0.07	0.05	0.06	0.03	0.05	0.19	0.07	0.52	0.96	1	0.56	0.01	0.03	0.57
SBV790	0	0.06	0.05	0.03	0.03	0.05	0.11	0.12	0.15	0.12	0.1	0.09	0.06	0.12	0.06	0.33	0.56	0.56	1	0.01	0.02
FITC	0.01	0.02	0.02	0.36	0.05	0.01	0.01	0.01	0.01	0.05	0.06	0.14	0.06	0.02	0.04	0.02	0.01	0.01	0.01	1	0.13
PE	0.01	0.03	0.02	0.09	0.36	0.15	0.02	0.02	0.03	0.12	0.28	0.13	0.35	0.32	0.41	0.17	0.06	0.03	0.02	0.13	1
PerCP-Cy5.5	0	0.01	0	0	0.03	0.1	0	0	0	0.01	0.03	0	0.04	0.15	0.07	0.44	0.58	0.57	0.28	0.01	0.08
SBB700	0	0	0	0.01	0.04	0.11	0	0	0	0.01	0.04	0.01	0.08	0.09	0.44	0.64	0.64	0.34	0.02	0.09	0.35
PE-A750	0	0	0	0.01	0.03	0.02	0	0	0	0.01	0.01	0.02	0.01	0.03	0.03	0.07	0.13	0.12	0.24	0.02	0.08
Alexa Fluor 488	0	0	0	0.01	0	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.1	0.18	0.02	0.01	0.01	0.29	0.11	0.03
Alexa Fluor 700	0	0	0	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.12	0.37	0.42	0.12	0.01	0.35	0.22
APC-Cy7	0	0.02	0.02	0.02	0.01	0	0	0	0	0	0.01	0.01	0.01	0.01	0.04	0.14	0.17	0.2	0	0.14	0.09

Complexity Index: 70.32

Fig. 2. 27-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye and a 26-color antibody panel in Brilliant Stain Buffer (BD), allowing identification of multiple cell lineages, subsets, and activation status.

Conclusions

- StarBright Dyes offer an excellent choice for use in full spectrum flow cytometry as well as conventional flow cytometry
- Following panel building best practices avoiding excessive spreading, StarBright Dyes can be used in combination with other fluorophores in large multiplex panels
- StarBright Dyes provide additional options and flexibility when designing high parameter multicolor panels, and their unique signature allows novel dye combinations to be unmixed which are not compatible in conventional flow cytometry

Novel combinations include:

- StarBright Violet (SBV) 440, Pacific Blue and Brilliant Violet (BV) 421
- SBV475, SBV515 and BV510
- StarBright Blue and PerCP-Cy5.5
- SBV610 and BV605
- SBV710 and BV711

- Due to the unique spectral properties of these dyes, we predict multicolor panels containing over 50 fluorescent markers will now be possible

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