StarBright Dyes: Expanding the Size of Multicolor Spectral Panels with Superior Dyes Excitable by the Ultraviolet, Violet, Blue, Yellow, and Red Lasers.

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The recently launched StarBright™ Dyes from Bio-Rad™ are bright dyes with unique spectral profiles, designed for multicolor flow cytometry with researchers' needs in mind. They are easy-to-use, as they do not require any special buffer, work with all fixatives, can be premixed, show minimal background staining, and are highly reproducible.

The range of StarBright Dyes is expanding with our latest additions, the StarBright Yellow (SBY) and StarBright Red (SBR) Dyes. Here we present a preview of our new dyes on a five-laser spectral cell analyzer. SBY and SBR Dyes were combined with other members of the StarBright Dye range, polymer dyes, and traditional fluorescent dyes in a 43-color immunophenotyping panel, and multiple human peripheral blood subsets were identified. In addition, we highlight some examples of novel dye combinations, which can be used to expand the number of dyes in a spectral panel and therefore increase the markers identified from one sample.

StarBright Dyes exhibit minimal spectral changes when fixed in both PFA-based and alcohol-based fixatives, or when used with common staining buffers. Overall, these benefits make them ideal for inclusion in multicolor spectral flow cytometry panels, enabling improved resolution of cell populations.

StarBright Dye Spectral Profiles

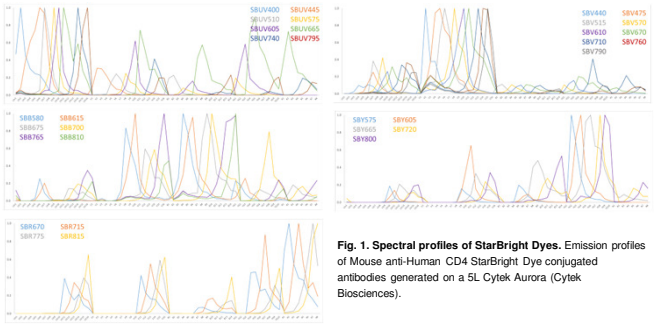


Fig. 1. Spectral profiles of StarBright Dyes. Emission profiles of Mouse anti-Human CD4 StarBright Dye conjugated antibodies generated on a 5L Cytek Aurora (Cytek Biosciences).

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 an antibody panel in FACS buffer (PBS + 1% BSA) or a single antibody for compensation control tubes. Cells were stained in a 96-well plate for 1 hr at room temperature (RT), washed 3X, and fixed in 2% paraformaldehyde before resuspending in phosphate buffered saline (PBS)
Staining panel. All antibodies were titrated to determine the optimal staining concentration prior to use. Antibodies used in the 43-color panel are shown in Table 1.
Data collection and analysis. Cells were acquired on a 5-laser Aurora Spectral Analyzer (Cytek Biosciences). Analysis was performed using SpectroFlo (Cytek Biosciences) and FCS Express 7 (De Novo Software).

Table 1. Bio-Rad antibodies used in the multiplex panel.

Fluorescent Dyes	Target	Catalog Number	Fluorescent Dyes	Target	Catalog Number
SBUV400	CD11c	Coming soon	FITC	CD57	N/A
SBUV445	HLA ABC	MCA81SBUV445	SBB580	CD4	MCA1267SBB580
BUV496	CCR7	N/A	SBB615	CD31	MCA1738SBB615
SBUV510	CD45	MCA87SBUV510	SBB675	CD195	Coming soon
SBUV575	CD28	MCA709SBUV575	SBB700	CD11b	Coming soon
SBUV605	HLA DP DQ DR	MCA477SBUV605	BB700	PD1	N/A
SBUV665	CD163	Coming soon	SBB765	CD62L	MCA1076SBB765
SBUV740	CD105	MCA1557SBUV740	SBB810	CD3	MCA463SBB810
SBUV795	CD10	MCA1556SBUV795	PE	CD16	MCA2537PE
PB	CD63	N/A	SBY575	CD20	MCA1710SBY575
BV421	CD56	N/A	PE Dazzle 594	CD123	N/A
SBV440	CD24	Coming soon	PE-Cy7	CD127	N/A
SBV475	CD45RO	MCA461SBV475	SBY665	CD25	MCA2127SBY665
BV510	IgD	N/A	SBY720	CD45RA	MCA88SBY720
SBV570	CD33	MCA1271SBV570	SBY800	CD2	MCA1194SBY800
BV605	TIGIT	N/A	APC	TCRVa7.2	N/A
SBV670	CD40	Coming soon	A647	CD161	MCA1855A647
BV711	TCRγD	N/A	SBR715	CD19	Coming soon
SBV710	CD27	Coming soon	APC-Cy7	CD1c	N/A
SBV760	CD38	MCA1019SBV760	APC-Fire810	HLA DR	N/A
SBV790	CD14	MCA1568SBV790	SBR815	CD8	Coming soon

APC, allophycocyanin; AXXX, Alexa Fluor; BV, Brilliant violet; BUV, Brilliant ultra violet; CY7, cyanine7; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBR, StarBright Red; SBV, StarBright Violet; SBY, StarBright Yellow.

43-color Aurora Multiplexing Panel Using 27 StarBright Dyes

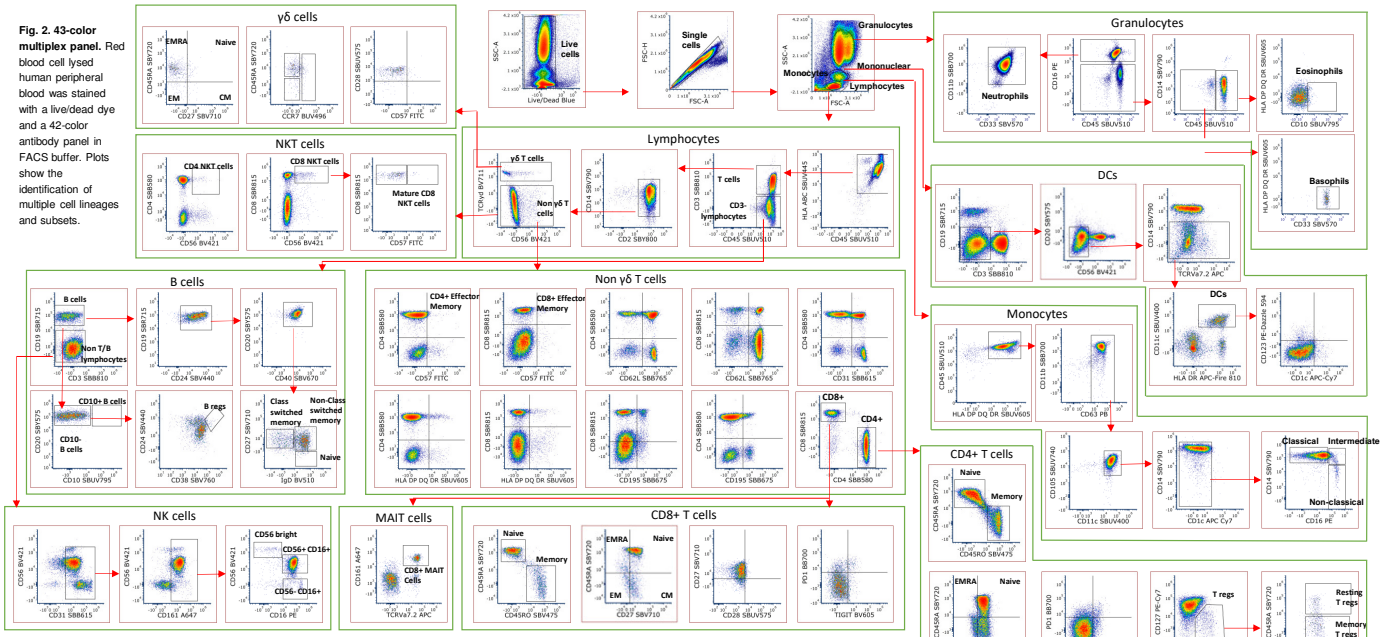
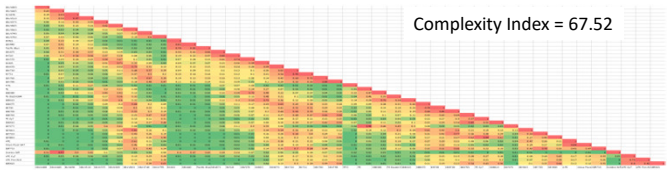


Fig. 2. 43-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye and a 42-color antibody panel in FACS buffer. Plots show the identification of multiple cell lineages and subsets.

Table 2. Similarity matrix. Similarity indices from the 43-color panel.



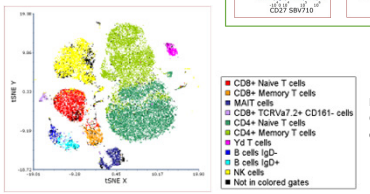


Fig. 3. t-SNE high dimensional data plot. Gated on live, CD45+ lymphocytes showing clusters of major lymphocyte cell populations.

Novel StarBright Yellow and Red Dye Combinations

StarBright Dyes can be used in novel dye combinations, which can't be used together in conventional flow cytometry.

Figure 4 shows two panels using novel combinations of StarBright Dyes with conventional Dyes. Despite high similarity scores and spreading, careful panel design will allow these to be used together in large panels, increasing dye choice.

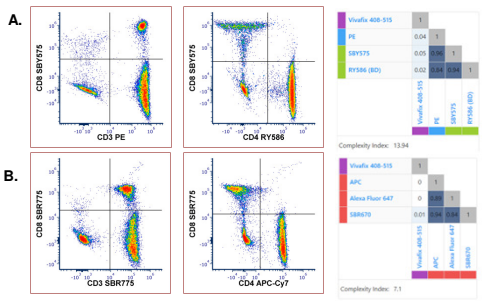


Fig. 4 Small 4-color panels including dyes with similar maximum excitation and emission wavelengths. Red blood cell lysed human peripheral blood was stained with a live/dead dye and a three-antibody panel in PBS + 1% BSA.

Conclusions

- StarBright Dyes are bright dyes with unique spectra (Figure 1)
- StarBright Dyes can be combined with other fluorophores in a high parameter 43-color multiplexing panel, on a 5-laser Aurora (Figures 2 and 3). The panel gave a low complexity index resulting in accurate unmixing, low spreading with easy identification of populations
- The unique spectra of StarBright Dyes enable novel combinations to be used (Figure 4) despite high similarity scores with careful panel design, they provide increased flexibility and choice
- **StarBright Dyes make an excellent choice for inclusion in new and expanding existing spectral panels**

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