Expanding the Size of Multicolor Panels with Ease with New StarBright Blue and StarBright Yellow Dyes

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The recently launched StarBright™ Dyes from Bio-Rad are bright, stable, exhibit high lot-to-lot reproducibility, and spectral consistency. They are specifically designed for multicolor flow cytometry with researchers needs in mind. They address the common pain points in flow such as brightness, broad emission spectra, staining consistency, and ease-of-use, solving issues of signal resolution when constructing complex panels. These bright dyes have the additional benefit of a unique full spectral profile, making them ideal for inclusion in spectral flow cytometry, when creating multicolor panels.

Here we present data from our newest additions to the StarBright Dye range, the StarBright Blue and StarBright Yellow Dyes on a five-laser spectral cell analyzer (Aurora, Cytek Biosciences). Both dye series are bright and allow expansion across the 488 nm and 561 nm lasers giving more choice for all panel sizes but are particularly useful when large panel design is required.

In this study, we show when StarBright Blue (SBB) and StarBright Yellow (SBY) Dyes are combined with other members of the StarBright Dye range, polymer dyes, and traditional fluorescent dyes, large immunophenotyping panels can be constructed which can be successfully unmixed due to the low complexity index. In addition, novel dye combinations can be identified by increasing the number of dyes and therefore markers identified from one sample.

StarBright Blue and Yellow Dye Spectral Profiles

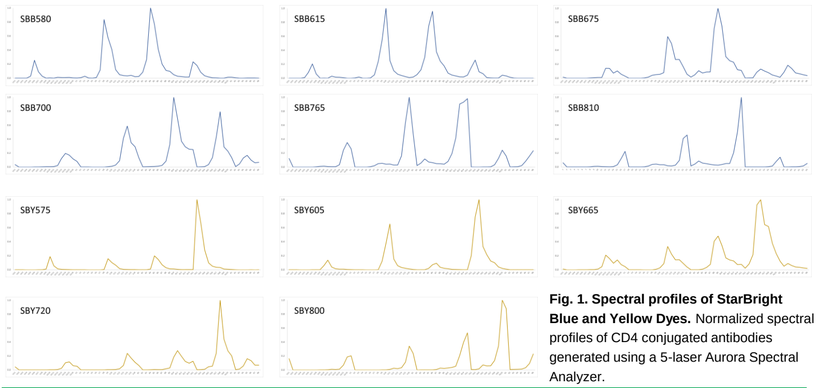

Fig. 1. Spectral profiles of StarBright Blue and Yellow Dyes. Normalized spectral profiles of CD4 conjugated antibodies generated using a 5-laser Aurora Spectral Analyzer.

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 in Brilliant Stain Buffer (BD Biosciences) or PBS + 1% BSA, with an antibody panel 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 resuspended in FACS Buffer.
Staining panel. All antibodies were titrated to determine the optimal staining concentration prior to use. Antibodies used in the 38-color panel are shown in Table 1.
Data collection and analysis. Cells were acquired on a 5-laser Aurora Spectral Analyzer. Analysis was performed using SpectroFlo (Cytek Biosciences) and FCS Express 7 (De Novo Software).

Table 1. Antibodies used in the 38-color multiplex panel.

Fluorescent Dye	Target	Bio-Rad catalog number
SBV400	CD11c	Coming soon
SBV445	HLA ABC	MCA81SBV445
SBV510	CD31	MCA1738SBV510
SBV575	CD28	MCA709SBV575
SBV605	HLA DP DQ DR	MCA477SBV605
SBV665	CD163	Coming soon
SBV740	CD105	MCA1557SBV740
SBV795	CD10	MCA1556SBV795
PB	CD11b	MCA711PB
BV421	CD56	N/A
SBV440	CD24	Coming soon
SBV475	CD45RO	MCA461SBV475
BV510	IgD	N/A
SBV515	CD2	MCA1194SBV515
SBV570	CD33	MCA1271SBV570
SBV610	CD27	MCA755SBV610
SBV670	CD40	Coming soon
BV711	TCRγD	N/A
SBV760	CD38	MCA1019SBV760

Fluorescent Dye	Target	Bio-Rad catalog number
SBV790	CD14	MCA1568SBV790
FITC	CD57	N/A
SBB580	CD4	MCA1267SBB580
SBB615	CD19	MCA1940SBB615
SBB675	CD195	Coming soon
PerCP	CD45	MCA87PERCP
SBB700	CD11b	Coming soon
SBB765	CD62L	MCA1076SBB765
SBB810	CD3	MCA463SBB810
PE	CD16	MCA2537PE
SBY575	CD20	MCA1710SBY575
SBY605	CD25	MCA2127SBY605
PE-Cy5	CD127	N/A
SBY720	CD45RA	MCA888SBY720
SBY800	CD8	MCA1226SBY800
APC	CD63	MCA2142APC
A647	CD161	MCA1855A647
A700	CD9	MCA469A700

38-Color Aurora Multiplexing Panel Containing 26 StarBright Dyes

Large immunophenotyping panel identifying multiple human peripheral blood subsets.

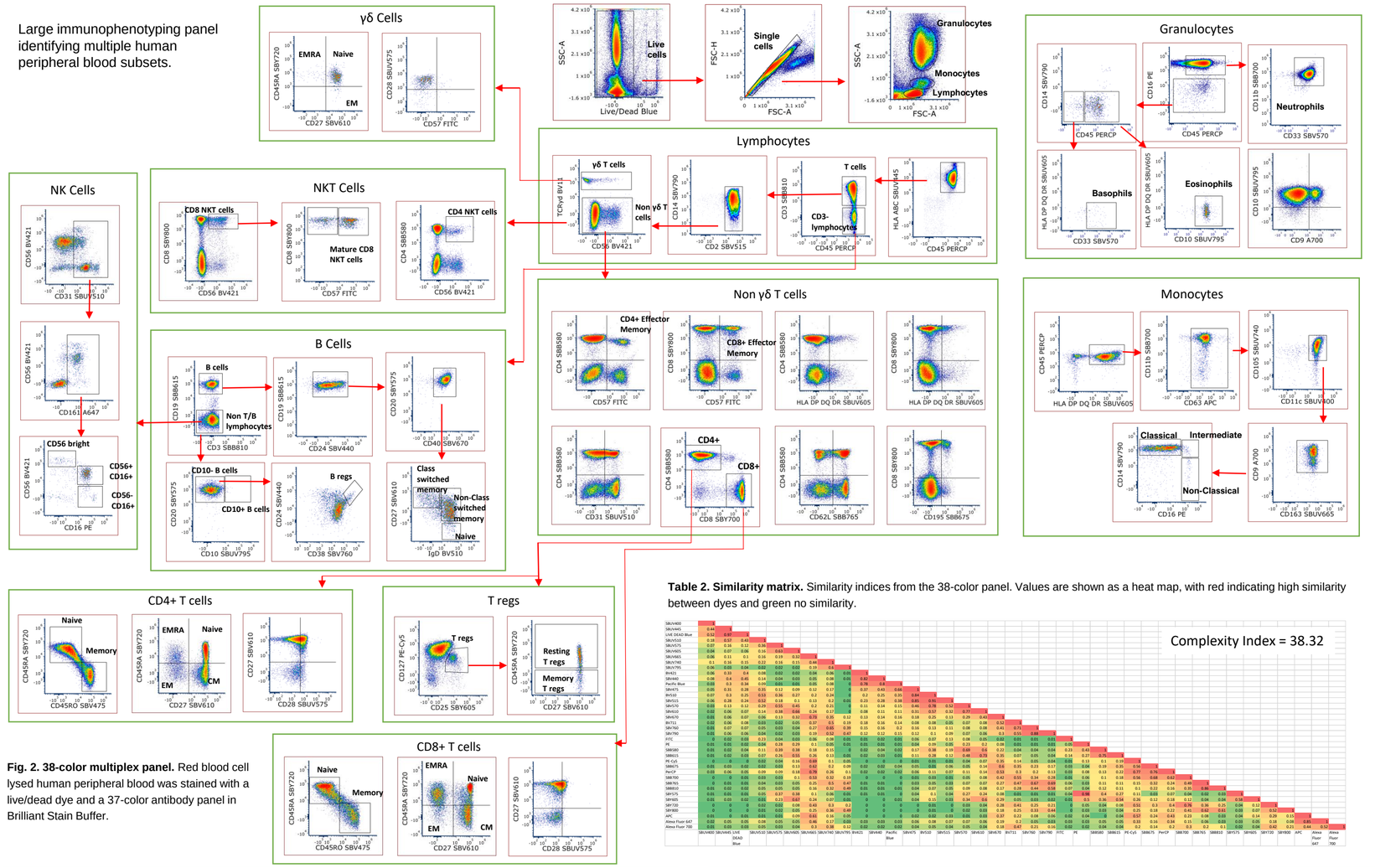

Fig. 2. 38-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye and a 37-color antibody panel in Brilliant Stain Buffer.

Table 2. Similarity matrix. Similarity indices from the 38-color panel. Values are shown as a heat map, with red indicating high similarity between dyes and green no similarity.

Novel StarBright Dye combinations

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

Figure 3 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.

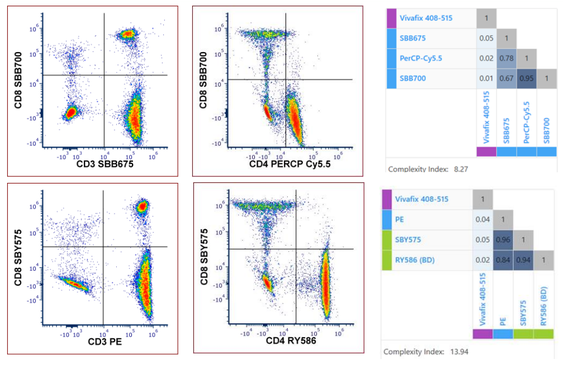

Fig. 3. Small 4-color panels including dyes with similar maximum excitation and emission wavelengths. Red blood cell lysed human peripheral blood was stained with a live/dead dye and a three-antibody panel in PBS + 1% BSA.

Conclusions

- StarBright Dyes are bright dyes with unique spectra (Figure 1)
- StarBright Blue and Yellow Dyes can be combined with other fluorophores in a high parameter multiplexing panel on a 5-laser Aurora (Figure 2)
- The unique spectra of StarBright Dyes enable novel combinations to be used (Figure 3) despite high similarity scores with careful panel design, they provide increased flexibility and choice
- StarBright Dyes work effectivity in Brilliant Stain Buffer (Figure 2), but there is no requirement for a special buffer, they work in all buffers tested including PBS + 1% BSA (Figure 3)
- StarBright Dyes can be used to generate large spectral panels with a low complexity index resulting in accurate unmixing, low spreading with clear separation and easy identification of populations

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