StarBright Dyes: Improved Panel Design with New StarBright Blue, StarBright Yellow, and StarBright Red Dyes

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StarBright™ Blue (SBB), StarBright Yellow (SBY), and StarBright Red (SBR) Dyes are the latest bright dyes designed for flow cytometry from Bio-Rad™. They expand the range of StarBright Dyes, which also includes StarBright UltraViolet (SBUV) and StarBright Violet (SBV) Dyes, across all five common laser lines. These superior dyes deliver tuneable brightness and spectral properties, greater stability, minimal background staining, improved lot-to-lot reproducibility, and spectral consistency.

The SBB and SBR Dyes are bright and allow an expansion in the number of dyes excited by the 488 nm and 640 nm lasers, respectively. SBY Dyes are bright, true 561 nm excitable dyes with reduced excitation from the 488 nm laser.

Data shown here from the five laser ZE5 Cell Analyzer, demonstrate the benefits of using new StarBright Dyes in multiplexing panels. Firstly, we show how StarBright Dyes from the entire StarBright Dye range, alongside some traditional fluorescent dyes, can be used in a multiplex panel, allowing the identification of many peripheral blood subsets with high resolution and without the requirement of special staining buffers. In addition, we show, in a smaller panel, that SBY Dyes improve the resolution of cell populations, compared to using traditional 561 nm excitable dyes, by reducing compensation and spreading.

Bright with reduced spillover, multiplexing compatible with no requirement for a special buffer, and high stability make StarBright Dyes perfect for all flow cytometry experiments regardless of panel size and protocol.

StarBright Blue, Yellow, and Red Dyes Emission Spectra

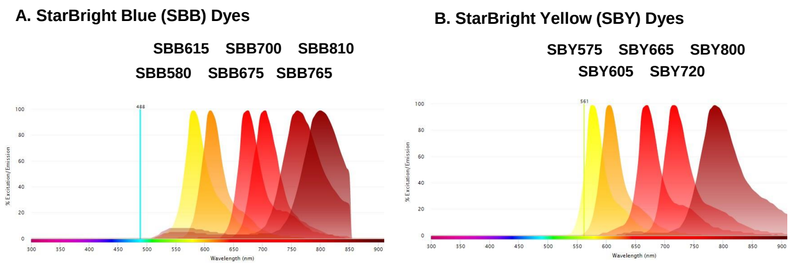

Fig. 1. Emission spectra for StarBright Dyes. StarBright Dyes excitable by **A,** the 488 nm laser **B,** the 561 nm **C,** the 640 nm laser. Dyes labeled in red will be available later this year.

Materials and Methods

Staining conditions. Red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were incubated 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, washed 3X in FACS buffer (PBS + 1% BSA) and resuspended in FACS Buffer. Propidium Iodide (PI) (#1351101, Bio-Rad) was added 5 min prior to acquisition.

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

Data collection and analysis. Cells were acquired on a 5-laser, 30-parameter ZE5 Cell Analyzer with option A, 355 nm laser upgrade (Bio-Rad). 300,000 cells were acquired for the multiplex panels and 60,000 cells for the single stained controls. Analysis was performed using FCS Express 7 Software. (De Novo Software).

27-Color Immunophenotyping Panel Including StarBright UltraViolet, Violet, Blue, Yellow, and Red Dyes

An immunophenotyping panel containing 22 StarBright Dyes, and other common fluorophores, was successfully acquired without the use of a special buffer. Major T cell, B cell, monocyte, and granulocyte lineages were identifiable. Various subsets within these lineages can also be clearly distinguished.

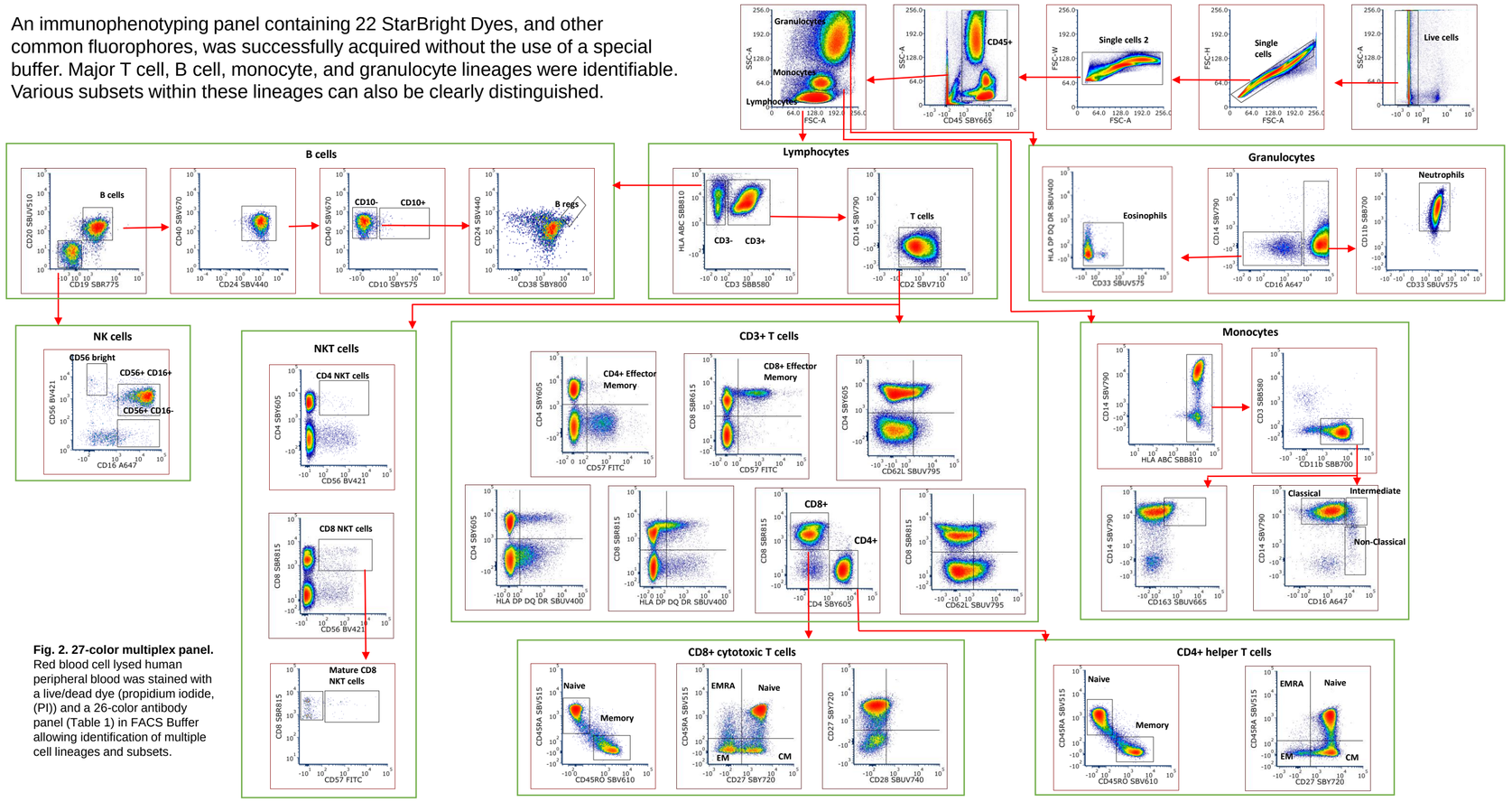

Fig. 2. 27-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye (propidium iodide, (PI)) and a 26-color antibody panel (Table 1) in FACS Buffer allowing identification of multiple cell lineages and subsets.

Reduced Spread with StarBright Yellow Dyes Compared to Traditional 561 nm Dyes

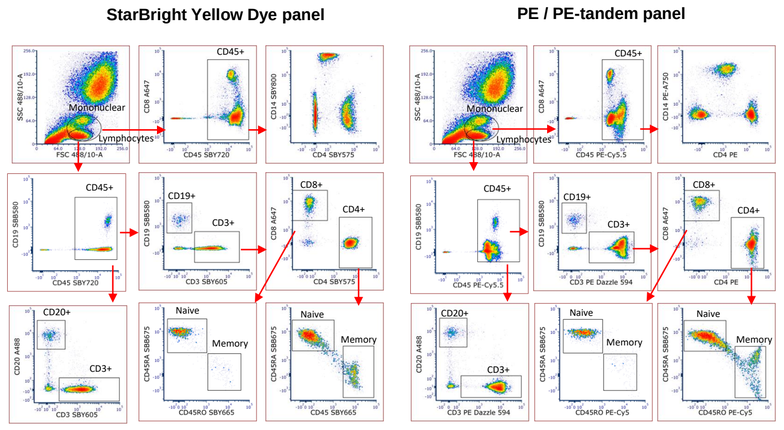

Figure 3. StarBright Dye panel shows reduced spreading and improved cell resolution over a comparison panel. Red cell lysed human peripheral blood was stained with two antibody panels using StarBright Yellow Dyes or PE and PE-tandem dyes that emit at a similar wavelength in combination with Alexa Fluor 488 (A488), SBB580, SBB675, and Alexa Fluor 647 (A647). Cells were acquired on a ZE5 Cell Analyzer

Table 1. Bio-Rad antibodies used in the 27-color panel.

Fluorescent Dyes	Target	Catalog Number
SBUV400	HLA DP DQ DR	MCA477SBUV400
SBUV510	CD20	MCA1710SBUV510
SBUV575	CD33	MCA1271SBUV575
SBUV665	CD163	Coming soon
SBUV740	CD28	MCA709SBUV740
SBUV795	CD62L	MCA1076SBUV795
BV421	CD56	N/A
SBV440	CD24	Coming soon
SBV515	CD45RA	MCA88SBV515
SBV610	CD45RO	MCA461SBV610
SBV670	CD40	Coming soon
SBV710	CD2	MCA1194SBV710
SBV790	CD14	MCA1568SBV790
FITC	CD57	MCA1305F

Fluorescent Dyes	Target	Catalog Number
SBB580	CD3	MCA463SBB580
SBB700	CD11b	Coming soon
SBB810	HLA ABC	MCA81SBB810
SBY575	CD10	MCA1556SBY575
SBY605	CD4	MCA1267SBY605
SBY665	CD45	MCA87SBY665
SBY720	CD27	MCA755SBY720
SBY800	CD38	MCA1019SBY800
A647	CD16	MCA5665A647
A700	CD31	MCA1738A700
SBR775	CD19	Coming soon
SBR815	CD8	Coming soon
PI	L/D	1351101

Conclusions

- The StarBright Dye range is expanding to include dyes excited by the 488, 561 and
- 640 nm lasers, offering bright dyes with narrow excitation and emission spectra (Figure 1)
- StarBright Dyes can be used together in multiplexing panels without the requirement for a special buffer (Figure 2)
- Starbright Yellow Dyes improve the resolution of cell populations, compared to using traditional 561 nm excitable dyes in a panel (Figure 3)
- **StarBright Dyes are an excellent choice for inclusion in multiplexing panels**

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