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

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BIO-RAD

Bio-Rad is expanding the StarBright Dye range with novel, bright dyes excitable by the 488 nm laser (StarBright Blue Dyes) or 561 nm laser (StarBright Yellow Dyes). These dyes have been developed for use with flow cytometry antibodies and designed to give exceptional brightness with narrow excitation and emission profiles, high stability, and to be used with common staining buffers.

In this study, we show data from StarBright Dyes that are currently on sale, and novel StarBright Dyes that will be launched later this year that are conjugated to flow validated mouse anti-human antibodies. The data show that StarBright Dyes are bright with excellent spectral properties, making them ideal for inclusion in multicolor immunophenotyping panels, allowing multiplexing expansion across the 488 nm and 561 nm lasers.

StarBright Blue (SBB) and Yellow (SBY) Dyes Emission Spectra

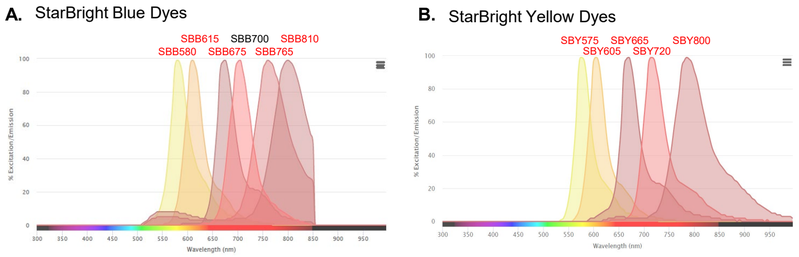


Fig. 1. Excitation and emission spectra for StarBright Dyes. StarBright Dyes excitable by **A,** the 488 nm laser or **B,** the 561 nm laser. Dyes labeled in red will be available later this year.

Materials and Methods

Staining conditions: red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were stained with a single antibody or antibody panel in a 96-well plate for 1 hr at room temperature (RT), washed 3x in FACS Buffer (PBS + 1% BSA) and resuspended in FACS Buffer prior to acquisition. Alternative staining buffers, as indicated in Figure 3, were also used. All antibodies were titrated to determine the optimal staining concentration prior to use.

Staining panel: antibodies used in the panels are shown in Table 1.

Cell fixation: fixation was required after final wash cells were fixed in Bio-Rad Fixation Buffer (Cat. #BUF071) or 2% paraformaldehyde for 20 min at RT in the dark or with 100% ice-cold MeOH at 4°C for 30 min. Cells were washed in FACS Buffer before acquisition.

Data collection and analysis: data for these studies were collected on a five-laser, 30-parameter ZE5 Cell Analyzer. 150,000 cells were collected for the multiplex panels and 60,000 cells for the single stained cells. Analysis was performed using FCS Express 7 Software (De Novo) or FlowJo10 Software (BD) .

Table 1. Bio-Rad reagents used in the multiplex panels using StarBright Blue or Yellow Dyes. A combination of prelaunch StarBright Dye conjugated antibodies (in red), catalog StarBright Dye conjugated antibodies, and other Bio-Rad catalog reagents were used.

StarBright Blue Dye Panel

Target	Fluorescent Dyes	Catalog number
CD3	SBB810	N/A
CD4	SBUV400	MCA1267SBUV400
CD8	SBB675	N/A
CD19	SBB580	N/A
CD45RA	SBUV795	MCA88SBUV795
CD45RO	SBV440	MCA461SBV440
CD14	SBV710	MCA1568SBV710
CD16	A700	MCA2537A700
CD56	A647	MCA2693A647
CD57	FITC	MCA1305F
L/D	DAPI	1351303

StarBright Yellow Dye Panel

Target	Fluorescent Dyes	Catalog number
CD3	SBY800	N/A
CD4	SBY575	N/A
CD8	SBY665	N/A
CD19	SBY605	N/A
CD45RA	SBUV400	MCA88SBUV400
CD45RO	SBV515	MCA461SBV515
CD14	SBV710	MCA1568SBV710
CD16	A700	MCA2537A700
CD56	A647	MCA2693A647
CD57	FITC	MCA1305F
L/D	DAPI	1351303

Axxx, Alexa Fluor; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet

StarBright Blue and Yellow Dyes Exceptional Brightness

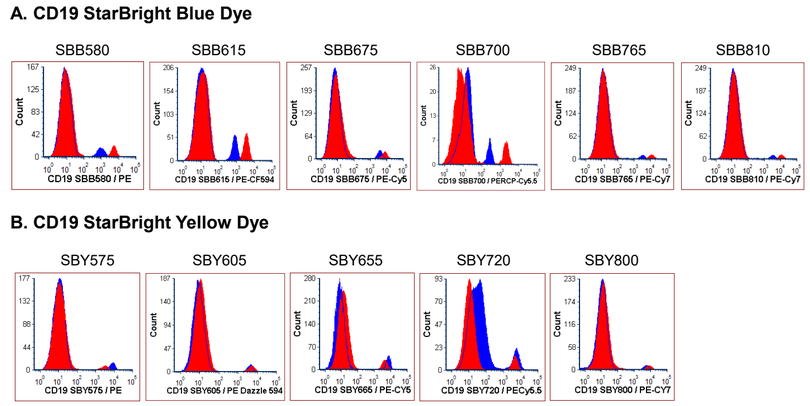


Fig. 2. Histograms showing staining with anti-CD19 StarBright Dyes and conventional dyes. Red blood cell lysed human peripheral blood from several donors was stained with anti-CD19 StarBright Dyes excitable by **A,** the 488 nm laser or **B,** the 561 nm laser. Cells were gated on live, single cells. Red histograms show StarBright Dye and blue histograms is a dye detected in the same filter on the ZE5 Cell Analyzer.

StarBright Blue and Yellow Dyes are Compatible with Multiple Staining Buffers

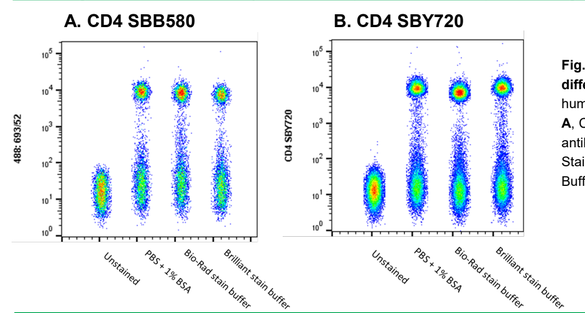


Fig. 3. Plots showing CD4 staining in different buffers. Red blood cell lysed human peripheral blood was stained with **A**, CD4 SBB580 or **B**, CD4 SBY720 antibodies in PBS + 1% BSA, Bio-Rad Stain Buffer (BUF073), or BD Brilliant Stain Buffer.

StarBright Blue and Yellow Dyes are Fixable with No Loss of Performance

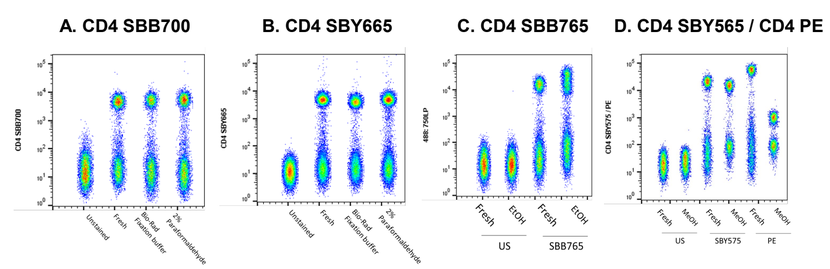


Fig. 4. Plots showing CD4 staining following fixation. Human peripheral blood was stained with CD4 antibodies and then fixed with Bio-Rad fixation buffer (#BUF071), 2% paraformaldehyde (**A, B**), 70% EtOH (**C**), or 100% MeOH (**D**).

StarBright Yellow Dyes Are Designed to Exhibit Low 488 nm Cross-Laser Excitation

StarBright Yellow Dyes have been designed to have reduced cross-laser excitation by the 488 nm laser compared to PE and PE-tandem dyes detected in the same filter.

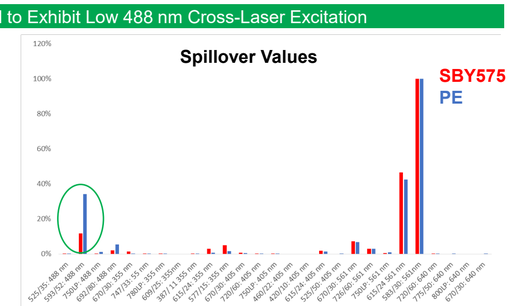
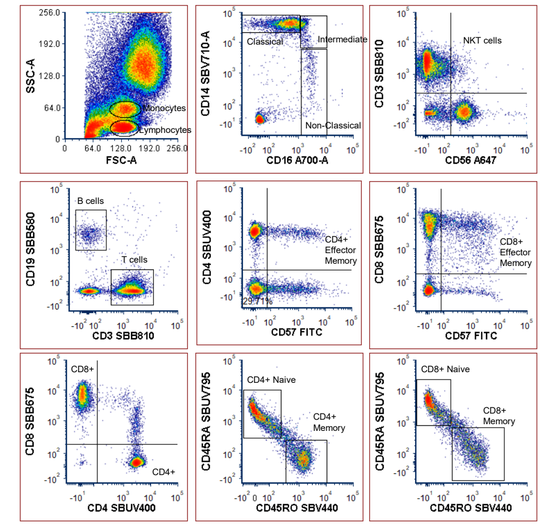


Fig. 5. Spillover of SBY575 (red) and PE (blue) into other filters. Human peripheral blood was stained with CD4 antibodies and acquired on a ZE5 Cell Analyzer. Signal into all the filters was measured.

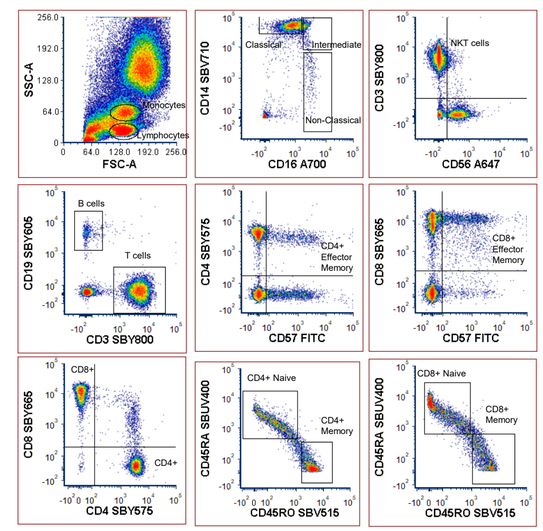
StaBright Blue Dye Panel



StarBright Blue Dyes can be successfully used along with other StarBright Dyes and traditional fluorophores to identify common and rare subsets including naïve and memory status in human peripheral blood.

Fig. 6. Immunophenotyping panel including StarBright Blue Dye conjugated antibodies. Red blood cell lysed human peripheral blood was stained in with 11 antibodies and a L/D marker. Cells were also gated on live, single cells (not shown).

StarBright Yellow Dye Panel



StarBright Yellow Dyes can be used successfully in combination with other StarBright Dyes and traditional fluorophores to identify common and rare subsets, including naïve and memory status in human peripheral blood.

Fig. 7. Immunophenotyping panel including StarBright Yellow Dye conjugated antibodies. Red blood cell lysed human peripheral blood was stained in with 11 antibodies and a L/D marker. Cells were also gated on live, single cells (not shown).

Conclusions

- StarBright Dyes excited by the 488 nm and 561 nm lasers offer a bright dye with narrow excitation and emission spectra (Figure 1, 2, and 5)
- StarBright Blue and Yellow Dyes are compatible with different staining buffers (Figure 3) and fixation reagents (Figure 4)
- StarBright Blue and Yellow Dyes can be used with other fluorophores in multiplex panels, without the requirement for a special buffer (Figure 6 and 7)
- StarBright Dyes are an excellent choice for inclusion in multicolor panels for flow cytometry and allow panel expansion using the 488 nm and 561 nm lasers