StarBright Dyes — Making Panel Design Easier For Everyone

S. Sanderson and M. Blundell

StarBright™ Dyes are a range of new fluorescent dyes developed by Bio-Rad designed for use in multicolor flow cytometry. They address the common challenges faced when constructing panels, which are described below. These bright dyes have the additional benefit of a unique full spectral profile, making them ideal for inclusion in immunophenotyping panels for both conventional and spectral flow cytometry. Data shown here reveal how the benefits of StarBright Dyes can help make panel design easier for everyone.

Challenges in Panel Design		Solution
Cells hard to detect as not all dyes are bright	→	Bright dyes
High levels of compensation and spreading	→	Narrow excitation and emission
Amending workflow to incorporate special buffers	→	Dyes that work in any buffer
Errors introduced when assembling panels	→	Premixing large panels for later use
Inconsistent performance with beads or when fixed	→	Consistent dyes that generate reproducible data

Bright dyes

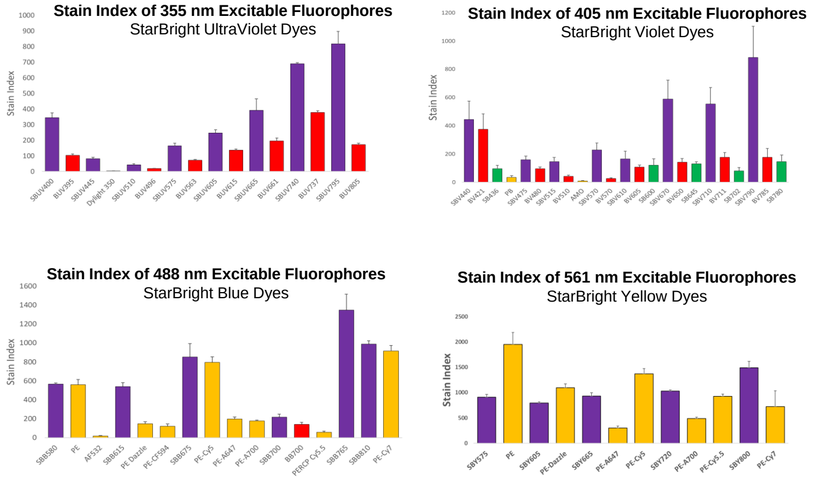

Figure 1. Stain Index values of Mouse Anti-Human CD4 conjugated to StarBright Dyes (purple) and other fluorescent dyes excited by the 355, 405, 488 or, 561 nm laser. Red cell lysed human peripheral blood was stained with fluorescently labeled Mouse Anti-Human CD4 and acquired on the ZE5 Cell Analyzer (Bio-Rad). Cells were gated on live, single cell lymphocytes and the stain index calculated. Data shown as 3 donors +/- SD. Purple, StarBright Dyes; red, Brilliant dyes; green, SuperBright dyes; yellow, non-polymer dyes.

Fixable in PFA or Alcohol with No Loss of Performance

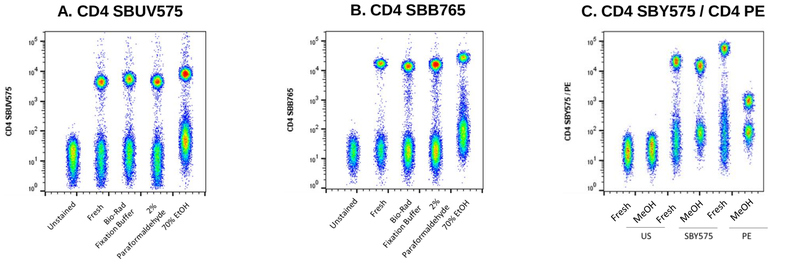

Figure 2. StarBright Dye compatibility with fixation reagents, including paraformaldehyde and alcohol-based fixatives. Red cell lysed human peripheral blood was stained with Mouse Anti-Human CD4 (MCA1267) antibodies and then acquired on a ZE5 Cell Analyzer before and after fixation with Bio-Rad Fixation Buffer (#BUF071), 2% paraformaldehyde, 70% EtOH **(A, B)** or 100% MeOH (**C**).

Reduced Spillover and Spreading

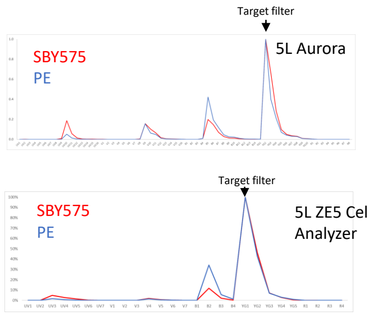

Figure 3. Reduced spillover of StarBright Yellow (SBY) 575 (red) compared to PE (blue) into off target filters. Human peripheral blood was stained with CD4 antibodies and acquired on a ZE5 Cell Analyzer and a 5L Cytek Aurora (Cytek Biosciences). Signal into all the filters was measured. Arrow denotes reduced spillover off the 488 nm laser from SBY575.

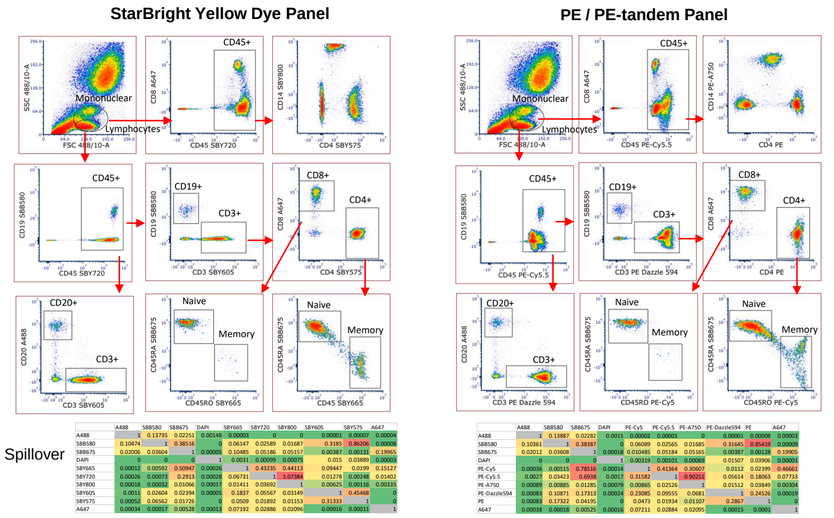

Table 1. Antibodies used in the multicolor comparison panel.

Laser: Filter	Marker	StarBright panel	PE and PE tandem panel
355: 509/25	L/D	DAPI	DAPI
488: 525/35	CD20	MCA1710 A488	MCA1710 A488
488: 593/52	CD19	MCA1940 SBB580	MCA1940 SBB580
488: 692/80	CD45RA	MCA88 SBB675	MCA88 SBB675
561: 583/30	CD4	MCA1267 SBY575	PE
561: 615/24	CD3	MCA463 SBY605	PE-Dazzle 594
561: 670/30	CD45RO	MCA461 SBY665	MCA461C PE-Cy5
561: 726/30	CD45	MCA87 SBY720	MCA87 PECY5.5
561: 750LP	CD14	MCA1568 SBY800	MCA1568 P750
640: 670/30	CD8	MCA1226 A647	MCA1226 A647

Figure 4. StarBright Dye panel shows reduced spillover and spreading over comparison panel. Red cell lysed human peripheral blood was stained with antibody panels shown in Table 1 and acquired on a ZE5 Cell Analyzer. Data were analyzed using FCS Express 7.

Reproducible in Any Buffer within Lot and Lot-to-Lot

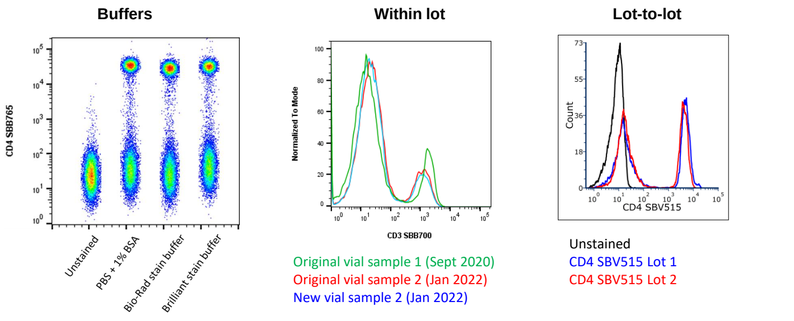

Figure 5. StarBright Dyes compatibility with all staining buffers and staining consistency. Red cell lysed human peripheral blood was stained with antibodies conjugated to StarBright Dyes and acquired on a ZE5 Cell Analyzer.

Consistent Performance on Beads and Cells

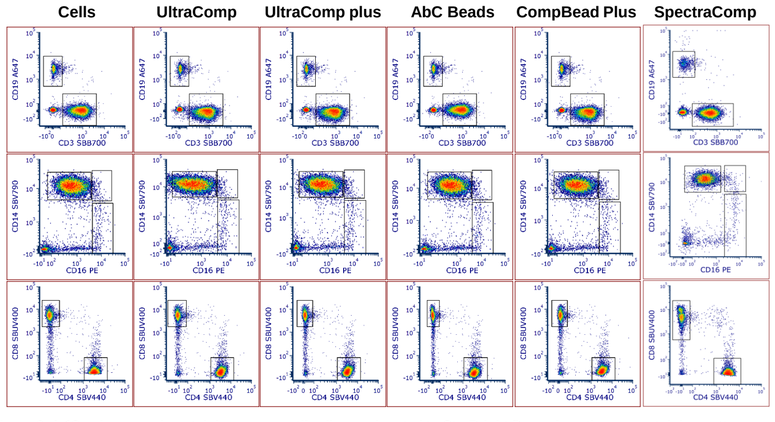

Figure 6. A 12-colour panel containing seven antibodies conjugated to StarBright Dyes show the same staining pattern when single stained cells or compensation beads are used to generate the compensation matrix. Red cell lysed human peripheral blood was stained in PBS + 1% BSA with 11 Bio-Rad antibodies and a L/D marker and acquired on the ZE5 Cell Analyzer. Compensation matrixes were generated using single stained cells or compensation beads and applied to the fully stained samples using FCS Express Software (De Novo).

Premixed Panel Compatibility

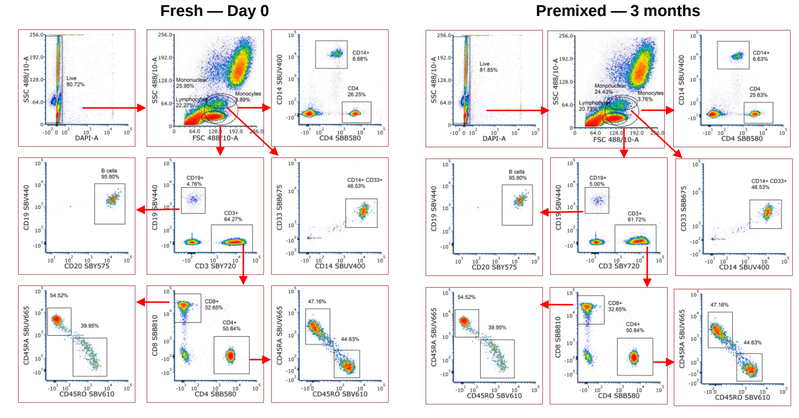

Figure 7. Antibody cocktail prepared in PBS + 1% BSA stored at 4°C for 3 months, compared to a freshly made antibody cocktail. Red cell lysed human peripheral blood was stained with an antibody panel made fresh or stored at 4°C for 3 months and acquired on a ZE5 Cell Analyzer. Data were analyzed using FCS Express 7.

Conclusion

StarBright Dyes help alleviate common challenges in multicolor flow cytometry panels such as brightness, spillover, fixation, and buffer compatibility.

Challenges in Panel Design		Solution	StarBright Dyes
Cells hard to detect as not all dyes are bright	→	Bright dyes	✓
High levels of compensation and spreading	→	Narrow excitation and emission	✓
Amending workflow to incorporate special buffers	→	Dyes that work in any buffer	✓
Errors introduced when assembling panels	→	Premixing large panels for later use	✓
Inconsistent performance with beads or when fixed	→	Consistent dyes that generate reproducible data	✓

Visit us at bio-rad-antibodies.com/StarBright

Bio-Rad Laboratories, Inc. | Life Science Group | 2000 Alfred Nobel Drive | Hercules, CA 94547 USA