StarBright Yellow, 561 nm Excitable Dyes, Superior Alternative to PE and PE-tandems with Improved Stability and Better Spectral Properties

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A well-designed panel with optimal fluorophore combinations is a critical step when generating multiplexing data. Best practice for panel design ensures that fluorophores are chosen which utilize all the available laser lines, thereby minimizing spillover and spread. However, this is difficult to achieve for the 561 nm (yellow) laser as the choice of fluorophores is limited. Phycoerythrin (PE) and PE-tandems are commonly used, but they are not optimal for all assays.

Antibodies conjugated to StarBright™ Yellow Dyes from Bio-Rad offer an alternative. They are bright, with high stain index values (Figure 1) with reduced 488 nm (blue) cross laser excitation than PE and PE-tandems (Figure 2). Here we show multiplexing panels, using the yellow and blue lasers simultaneously, that incorporate antibodies conjugated to StarBright Yellow Dyes or to PE/PE-tandems. The StarBright Yellow Dye panel has less compensation and spread, resulting in improved population resolution and easier identification of cell populations (Figure 3).

They have less nonspecific binding to monocytes than tandem dyes containing cyanine (Figure 4) and unlike PE and PE-tandem dyes, StarBright Yellow Dyes can be fixed in both alcohol and paraformaldehyde-based fixatives with no change in spectral profile, therefore suitable for a wider range of assays (Figure 5).

These features make StarBright Yellow Dyes a superior choice over common alternatives, resulting in improved reproducibility and panels with reduced nonspecific binding and low spread, generating data with highly resolved cell populations.

Bright Dyes

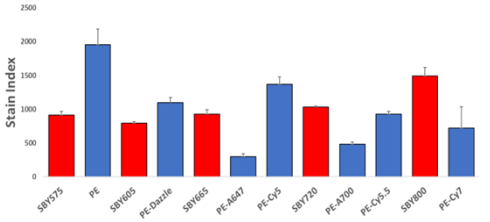

Fig. 1. Stain index values of Mouse Anti-Human CD4 conjugated to StarBright Yellow Dyes (red) and other fluorescent dyes (blue). Red blood cell lysed human peripheral blood was stained with fluorescently labeled Mouse Anti-Human CD4 and acquired on a ZE5 Cell Analyzer (Bio-Rad). Cells were gated on live, single cell lymphocytes and the stain index calculated. Data shown as 3 donors +/- SD.

Reduced Cross-laser Excitation and Improved Cell Resolution

StarBright Dyes are designed to have narrower excitation and emission profiles compared to alternative fluorophores, for example SBY575 has less cross laser excitation than PE (Figure 2). This results in a reduction of spread and improved cell resolution (Figure 3).

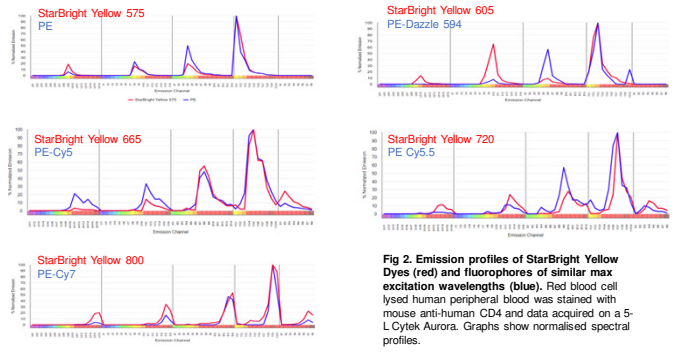

Fig 2. Emission profiles of StarBright Yellow Dyes (red) and fluorophores of similar max excitation wavelengths (blue). Red blood cell lysed human peripheral blood was stained with mouse anti-human CD4 and data acquired on a 5-L Cytek Aurora. Graphs show normalised spectral profiles.

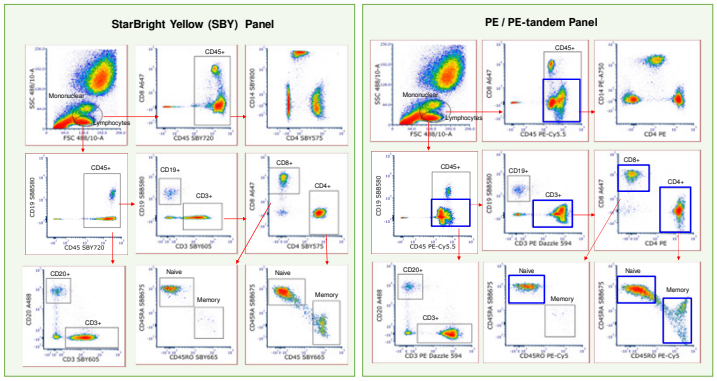

Table 1. Bio-Rad antibodies used for StarBright Dye and PE/PE-tandem panels.

Laser:Filter	Marker	StarBright Dye Panel	PE and PE-tandem Panel
488: 525/35	CD20	A488	A488
488: 593/52	CD19	SBB580	SBB580
488: 692/80	CD45RA	SBB675	SBB675
488:750LP	CD33	SBB810	SBB810
561: 583/30	CD4	SBY575	PE
561: 615/24	CD3	SBY605	PE-Dazzle 594
561: 670/30	CD45RO	SBY665	PE-Cy5
561: 726/30	CD45	SBY720	PE-Cy5.5
561: 750LP	CD14	SBY800	PE-A750
640: 670/30	CD8	A647	A647

Fig. 3. Panels including StarBright Yellow Dyes or alternative PE and PE-tandem dyes with similar excitation and emission max. Red blood cell lysed human peripheral blood was stained with the antibody panels (Table 1) and a L/D dye (DAPI, Bio-Rad #1351303) and acquired on a ZE5 Cell Analyzer. Data were analyzed using FCS Express 7 (De Novo Software by Dotmatics). Cells were gated on live, single cells. Populations with high spread in the PE/PE-tandem panels are highlighted in blue.

Reduced Monocyte Binding

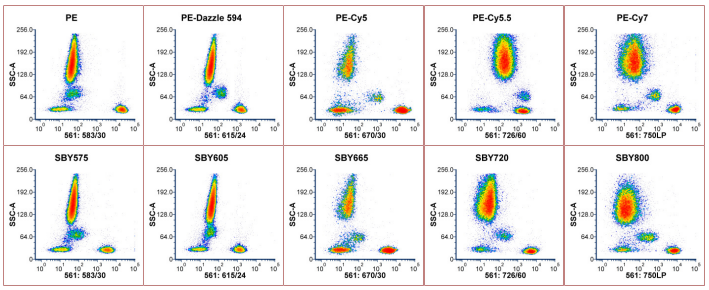

Fig. 4. Binding of mouse anti-human CD3 conjugated to StarBright Yellow Dyes and other fluorescent dyes to human peripheral blood. Red blood cell lysed human peripheral blood was stained with fluorescently labeled mouse anti-human CD3 and acquired on the ZE5 Cell Analyzer. Cells were gated on live, single cells.

Fixable with PFA and Alcohol

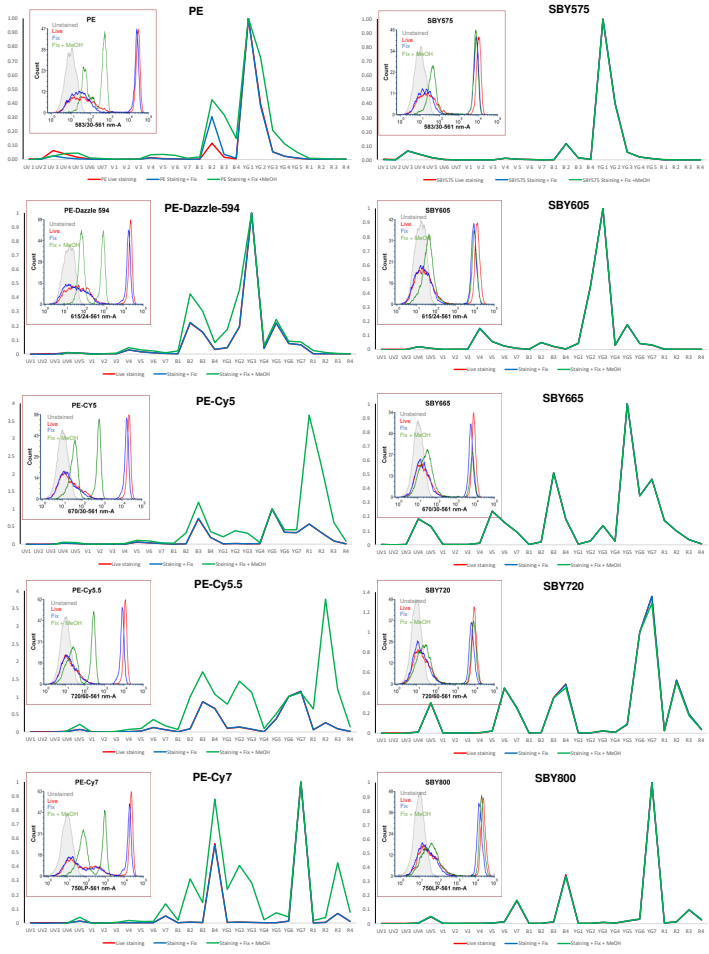

Fig. 5. Effect of fixatives on StarBright Yellow Dye conjugated antibodies compared to PE and PE-tandems. Human PBMCs were stained with mouse anti-human CD4 and acquired on a ZE5 Cell Analyzer before fixation, after fixation in a PFA based fixation buffer (#00-8222-49, Thermo Fisher Scientific) for 15 min and after fixation in a PFA based fixation buffer followed by incubation in 100% MeOH for 30 min. Cells were gated on live, single cell lymphocytes. Histogram overlays and the signal from CD4 positive cells in each filter are shown.

Conclusions

- StarBright Yellow Dyes are five, novel bright dyes that have improved spectral characteristics over PE and PE-tandems due to reduced excitation by the 488 nm laser
- When used in a simple immunophenotyping panel there is reduced spreading compared to a panel containing PE and PE-tandems with minimal reduction of signal due to a lower stain index
- StarBright Yellow Dyes do not exhibit any nonspecific binding on monocyte populations often seen with cyanine dye containing tandems
- StarBright Yellow Dyes can be fixed in PFA and alcohol with minimal loss of signal and no spectral changes unlike PE and PE tandem dyes which lose signal and exhibit severe spectral changes

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