

StarBright Dyes:- Build Bigger, Better Panels with Superior Dyes Excitable by the Ultraviolet, Violet, Blue, and Yellow lasers.



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StarBright Dyes from Bio-Rad, have been specifically designed for multicolor flow cytometry with researchers' needs in mind. StarBright Dyes address the common pain points in flow cytometry such as brightness, broad emission spectra, staining consistency, and ease-of-use, solving issues of signal resolution when constructing complex panels.

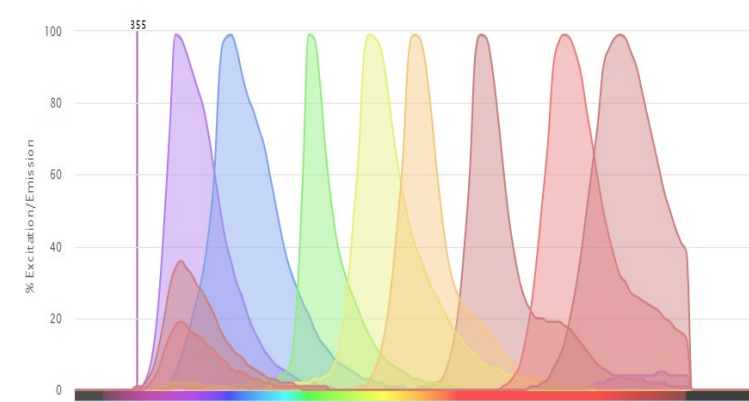
We present a preview of our latest additions to the StarBright range – the StarBright Blue (SBB) and StarBright Yellow (SBY) Dyes. StarBright Blue Dyes are bright and allow an expansion of dyes compatible with the 488 nm laser, whereas StarBright Yellow Dyes are optimally excited by the 561 nm laser, with reduced excitation from the 488 nm laser, making large panel design easier using both the 488 nm and 561 nm laser.

In this study, we show that when StarBright Blue and StarBright Yellow Dyes are combined with other members of the StarBright Dye range and traditional fluorescent dyes, large immunophenotyping panels can be constructed allowing identification of many peripheral blood subsets, without the requirement of special staining buffers. StarBright Dyes can be fixed in both PFA-based and alcohol-based fixatives, and this combined with their ability to be pre-mixed, means the flexibility of StarBright Dyes makes them ideal for the creation of new panels and expanding existing panels.

StarBright UltraViolet, Violet, Blue, and Yellow Dyes Emission Spectra

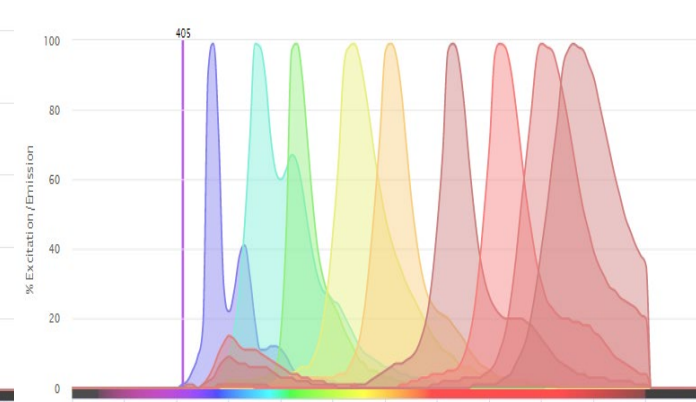
A. StarBright UltraViolet (SBUV) Dyes

SBUV400 SBUV510 SBUV605 SBUV740
SBUV445 SBUV575 SBUV665 SBUV795



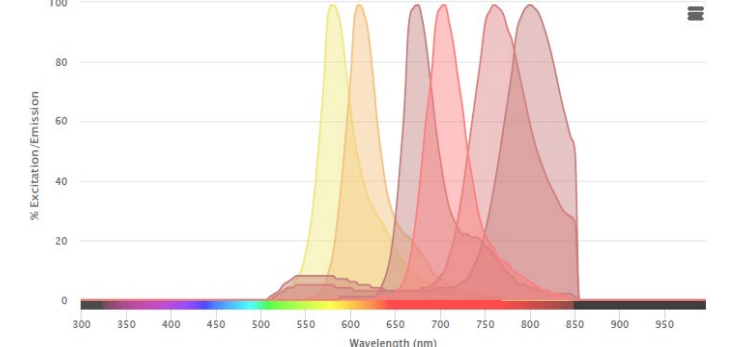
B. StarBright Violet (SBV) Dyes

SBV475 SBV570 SBV670 SBV760
SBV440 SBV515 SBV610 SBV710 SBV790



C. StarBright Blue (SBB) Dyes

SBB615 SBB700 SBB810
SBB580 SBB675 SBB765



C. StarBright Yellow (SBY) Dyes

SBY575 SBY665 SBY800
SBY605 SBY720

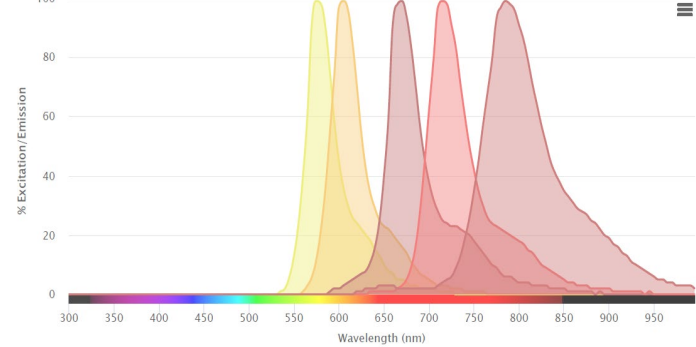


Fig. 1. Excitation and emission spectra for StarBright Dyes. StarBright Dyes excitable by A, the 355 nm laser B, the 405 nm C, the 488 nm laser, or D, the 561 nm laser. Dyes labeled in red will be available later this year.

Materials and Methods

Staining conditions: red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were incubated with a cocktail containing 22 antibodies 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 panel: antibodies used in the panel are shown in Table 1. All antibodies 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. 300,000 cells were acquired for the multiplex panel and 60,000 cells for the single stained controls. Analysis was performed using FCS Express 7 (De Novo Software) Software.

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23 color immunophenotyping panel including StarBright UltraViolet, Violet, Blue, and Yellow Dyes

StarBright Dyes were used successfully in immunophenotyping panels identifying T cell, B cell, monocyte, and granulocyte lineages. Various subsets of these lineages can be clearly distinguished.

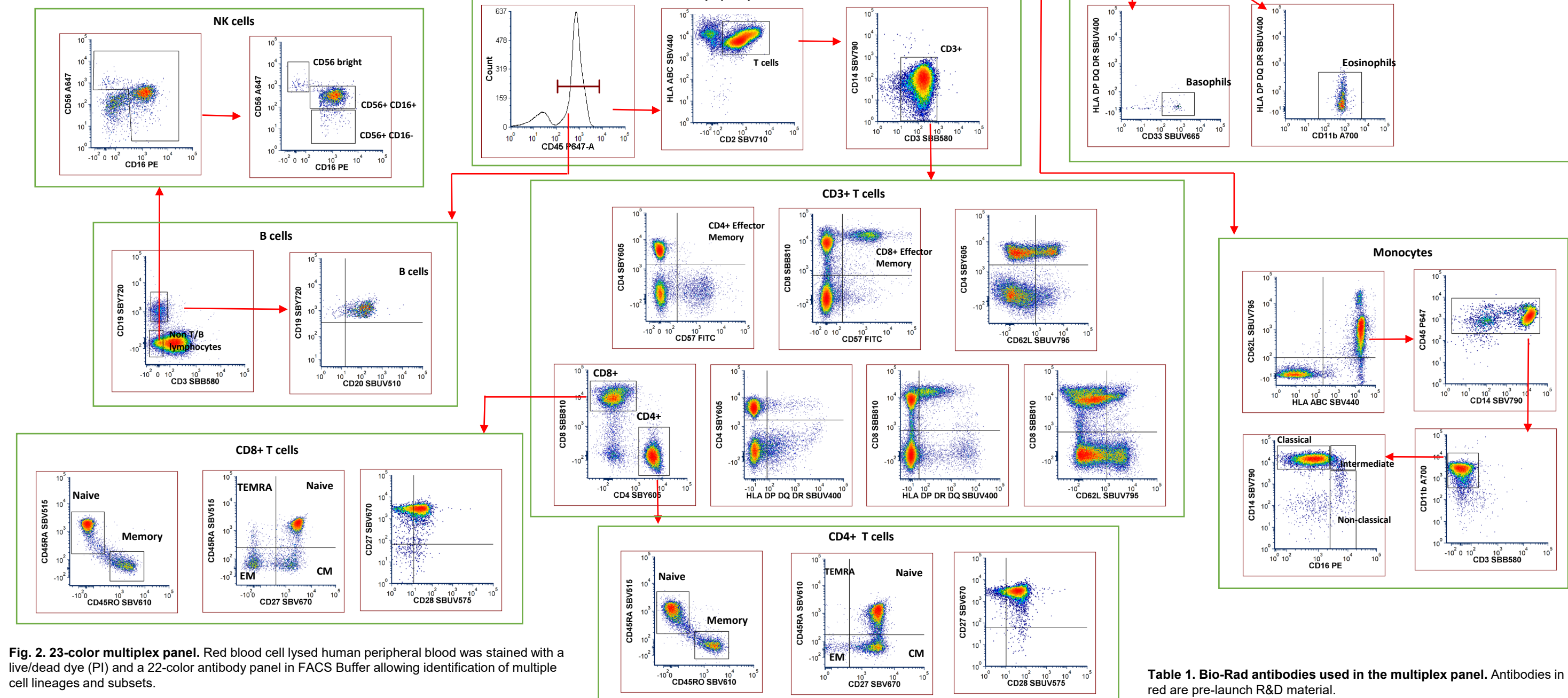
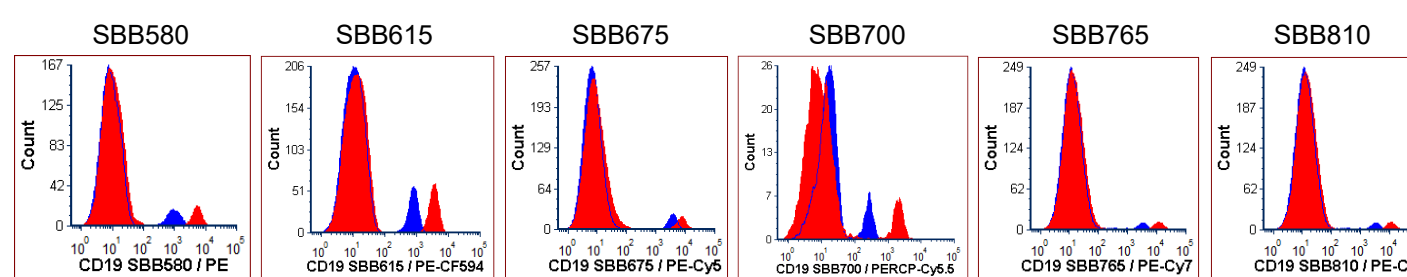


Fig. 2. 23-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye (PI) and a 22-color antibody panel in FACS Buffer allowing identification of multiple cell lineages and subsets.

Table 1. Bio-Rad antibodies used in the multiplex panel. Antibodies in red are pre-launch R&D material.

StarBright Blue and Yellow Dyes Exceptional Brightness

A. CD19 StarBright Blue Dye



B. CD19 StarBright Yellow Dye

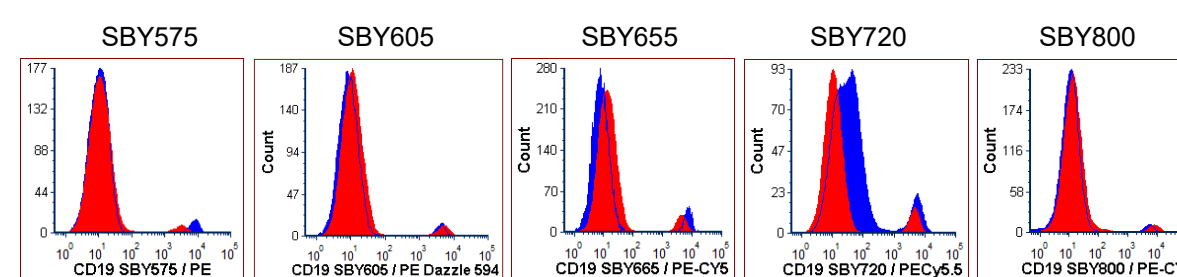


Fig. 3. Histograms showing staining with anti-CD19 StarBright Dyes and conventional dyes. Red blood cell lysed human peripheral blood from several donors was stained with anti-CD19 StarBright Dyes excitable by A, the 488 nm laser or B, the 561 nm laser. Cells were gated on live, single cells. Red histograms show StarBright Dye and blue histograms show a competitor dye detected in the same filter on the ZE5 Cell Analyzer.

| Fluorescent Dyes | Target | Catalog Number | Fluorescent Dyes | Target | Catalog Number |
|------------------|--------------|----------------|------------------|--------|----------------|
| SBUV400 | HLA DP DQ DR | MCA477SBUV400 | FITC | CD57 | MCA1305F |
| SBUV510 | CD20 | MCA1710SBUV510 | SBB580 | CD3 | |
| SBUV575 | CD28 | | SBB700 | CD10 | MCA1556SBB700 |
| SBUV665 | CD33 | MCA1271SBUV665 | SBB810 | CD8 | |
| SBUV795 | CD62L | MCA1076SBUV795 | PE | CD16 | MCA2537PE |
| SBV440 | HLA ABC | MCA81SBV440 | SBY605 | CD4 | |
| SBV515 | CD45RA | MCA88SBV515 | PE-A647 | CD45 | MCA87P647 |
| SBV610 | CD45RO | MCA461SBV610 | SBY720 | CD19 | |
| SBV670 | CD27 | MCA755SBV670 | PE-A750 | CD38 | MCA1019P750 |
| SBV710 | CD2 | MCA1194SBV710 | A647 | CD56 | MCA2693A647 |
| SBV790 | CD14 | MCA1568SBV790 | A700 | CD11b | MCA551A700 |

Conclusions

- The StarBright Dye range is expanding to include dyes excited by the 355 nm, 405 nm, 488 nm, or the 561 nm laser
- New StarBright Dyes excited by the 488 nm, and 561 nm lasers offer a bright dye with narrow excitation and emission spectra (Figure 1 and Figure 3)
- Multiple StarBright Dyes (18) can be used together in multiplexing panels without the requirement for a special buffer (Figure 2)
- StarBright Dyes are an excellent choice for inclusion in multiplexing panels