Introducing StarBright Dyes, Novel Ultra Violet, Violet, and Blue Laser Excitable Fluorescent Nanoparticles Suitable for Immunophenotyping in Flow Cytometry

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StarBright Dyes from Bio-Rad are proprietary, bright, fluorescent nanoparticles, developed for flow cytometry using the ZE5 Cell Analyzer. These photostable dyes have been designed to give exceptional brightness with narrow excitation and emission profiles, and to be used with common staining buffers. They are also ideal for large multicolor immunophenotyping panels.

In this study, StarBright UltraViolet 400, 510, 575, and 605, StarBright Violet 440, 515, 610, 670, 710, and 790, and StarBright Blue 700 were conjugated to antibodies and combined with traditional fluorophore conjugated antibodies, in an 18-color flow cytometry panel, enabling detection of B cell, T cell, and myeloid cell subsets in human peripheral blood using the ZE5 Cell Analyzer.

Materials and Methods

Staining conditions: Red blood cell lysed human peripheral blood was blocked with 10% human serum and stained with VivaFix 583/603 Cell Viability Assay (Bio-Rad). After washing and resuspending in FACS Buffer (PBS + 1% BSA) or BD Brilliant Stain Buffer (BD), cells were incubated with a cocktail containing 17 antibodies or a single antibody, for compensation control tubes. Cells were stained in a 96-well plate for 1 hr at room temperature (RT), washed three times, and resuspended in FACS Buffer prior to acquisition.

Staining 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: Data for these studies were collected on a 5-laser, 30-parameter ZE5 Cell Analyzer. 600,000 cells were collected for the multiplex panel and 60,000 cells for the single stained controls. Analysis was performed using FCS Express Software (De Novo).

Table 1. Bio-Rad reagents used in the multiplex panel. A combination of prelaunch StarBright Dye conjugated antibodies, catalog StarBright Dye conjugated antibodies, and other Bio-Rad catalog reagents were used.

Target	Fluorophore	Catalog number	Target	Fluorophore	Catalog number
CD3	SBUV400	N/A	CD14	SBV790	N/A
CD4	SBUV510	N/A	CD57	FITC	MCA1305F
CD28	SBUV575	N/A	CD10	SBB700	MCA1556SBB700
CD19	SBUV605	N/A	CD20	PE	MCA1710PE
CD25	SBV440	MCA2127SBV440	CD45	PE-A647	MCA87P647
CD45RA	SBV515	MCA88SBV515	CD38	P750	MCA1019P750
CD45RO	SBV610	MCA461SBV610	CD127	A647	HCA145A647
CD27	SBV670	MCA775SBV670	CD16	A700	MCA2537A700
CD8	SBV710	MCA1226SBV710	L/D	Vivafix 583/603	1351117

StarBright Dye Emission Spectra

StarBright Dyes have narrow emission spectra and minimal spillover (Figure 1).

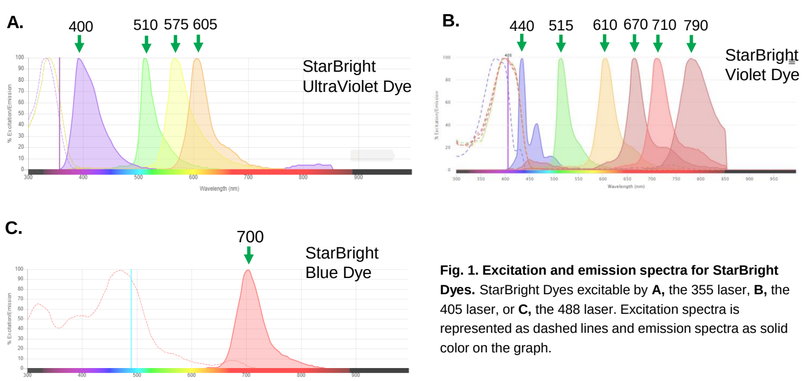

Fig. 1. Excitation and emission spectra for StarBright Dyes. StarBright Dyes excitable by **A,** the 355 laser, **B,** the 405 laser, or **C,** the 488 laser. Excitation spectra is represented as dashed lines and emission spectra as solid color on the graph.

18-Color Immunophenotyping Panel

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

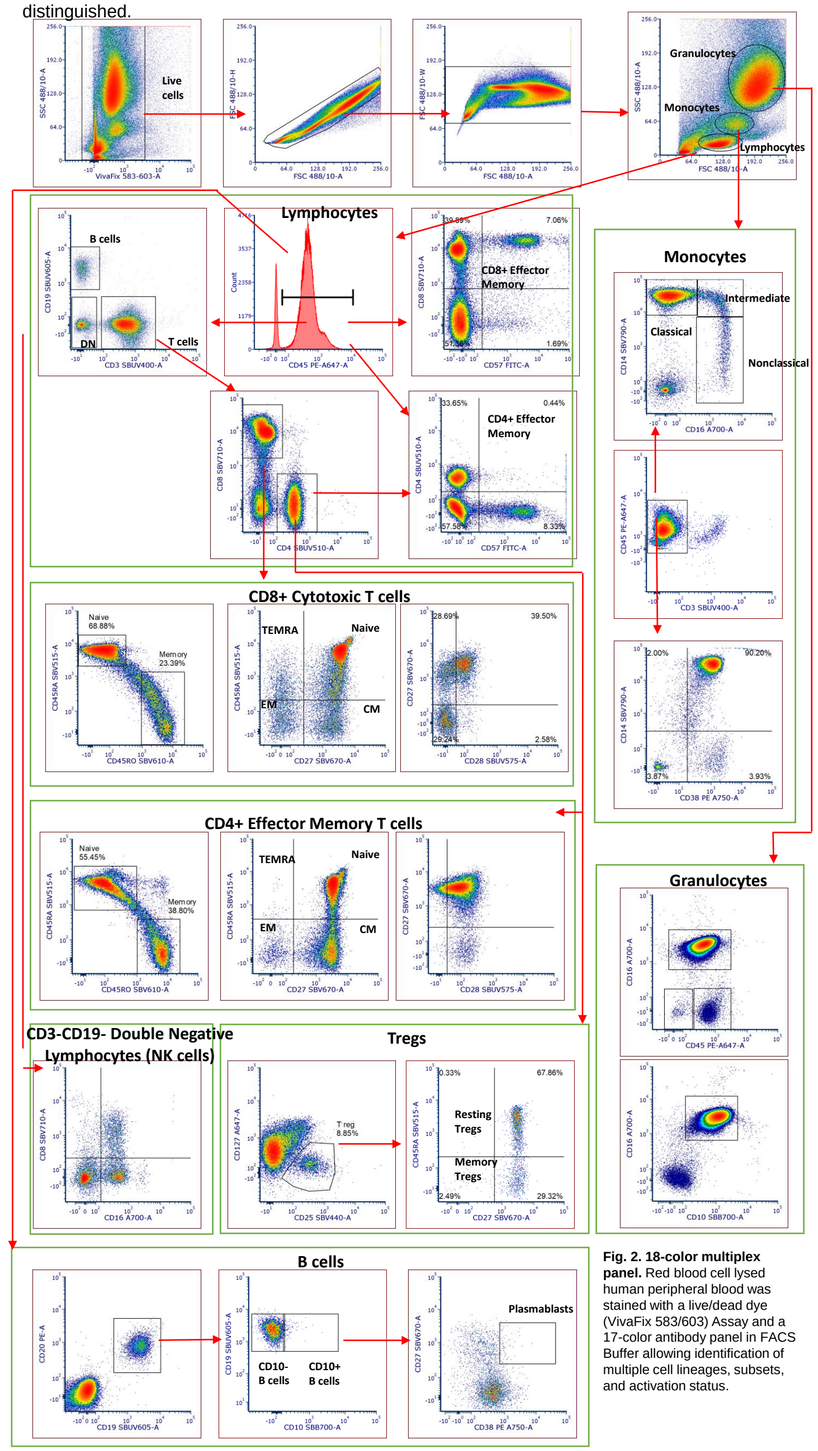

Fig. 2. 18-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye (VivaFix 583/603) Assay and a 17-color antibody panel in FACS Buffer allowing identification of multiple cell lineages, subsets, and activation status.

Compensation and Spreading

StarBright Dyes can be used successfully in combination with other StarBright Dyes and traditional fluorophores with minimal amounts of compensation and spreading as shown in Figures 3 and 4.

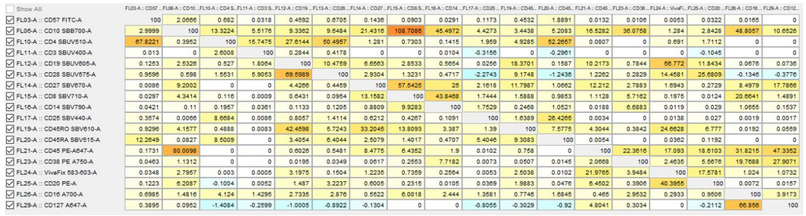

Fig. 3. Compensation matrix.

Fig. 4. Spreading matrix.

StarBright Dyes Can Be Stained in Multiplexing Panels Using Common Buffers

The 18-color panel was stained in different staining buffers with no significant difference in staining pattern (Figure 5). StarBright Dyes can be used together, without the requirement of a special staining buffer, and can also be easily incorporated into existing panels where a special buffer is required.

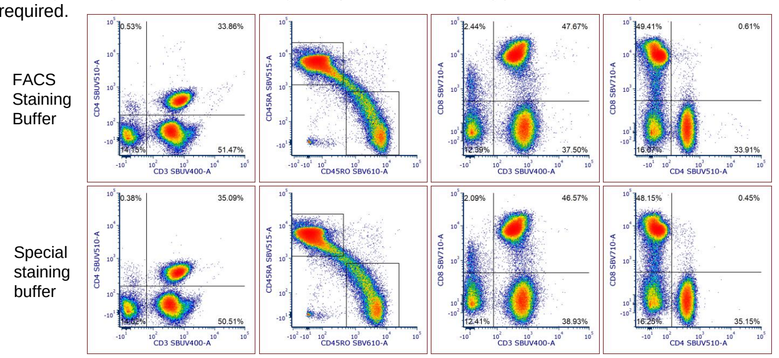

Fig. 5. Two color plots of two StarBright Dyes conjugated antibodies from the 18-color panel. Human blood stained in FACS Staining Buffer (PBS + 1% BSA) or special buffer (BD Brilliant Stain Buffer). Gated on live, single cell lymphocytes.

Conclusions

- StarBright Dyes excited by the 355, 405, and 488 lasers offer a bright dye with narrow excitation and emission spectra
- StarBright Dyes can be used in combination with other fluorophores in large multiplex panels, without the requirement for a special buffer
- The brightness and spectral characteristics of the StarBright Dyes allow for clear resolution of rare cell populations, such as Treg populations and low-density antigens
- StarBright Dyes are an excellent choice for inclusion in multicolor panels for flow cytometry