StarBright Dyes — Build Bigger Better Panels with Superior Violet and Ultraviolet Laser Excitable Dyes

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BIO-RAD

StarBright UltraViolet (SBUV) and StarBright Violet (SBV) Dyes are the brightest ultraviolet and violet laser excitable fluorescent dyes specifically developed for flow cytometry. Their narrow excitation and emission spectra, despite their increased brightness, do not cause loss of resolution due to excessive spillover and spreading. StarBright Dyes can be premixed, fixed, and have no requirement for a special buffer when multiplexing, providing researchers with the flexibility to build bigger, better panels.

In this study, we demonstrate the brightness and show the flexibility of StarBright Dyes by comparing freshly made and premixed antibody cocktails from Bio-Rad antibodies to immunophenotype human peripheral blood.

StarBright UltraViolet and Violet Dyes Emission Spectra

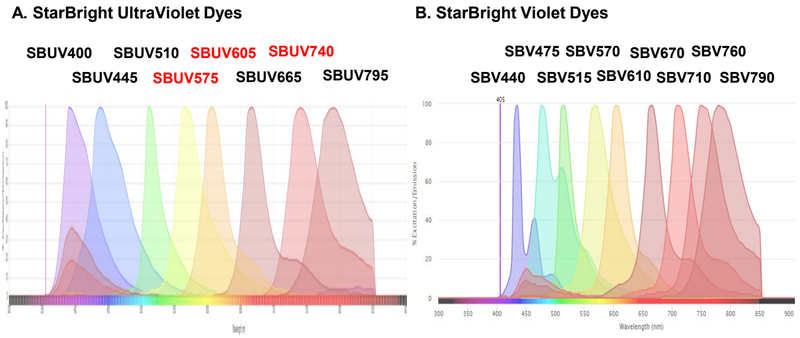

Fig. 1. Emission spectra for StarBright UltraViolet and Violet Dyes. StarBright Dyes excitable by **A,** the 355 nm laser, or **B,** the 405 nm laser. Dyes labeled in red will be available soon.

Materials and Methods

Staining conditions for multiplex panels: red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were incubated with a cocktail containing 11 freshly made antibodies or prepared 11 days previously or a single antibody, for compensation control tubes. Cells were stained in a 96-well plate for 1 hr at room temperature (RT), washed 3 times (3X) in FACS Buffer (PBS + 1% BSA) and resuspended in FACS Buffer. DRAQ7 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.

Staining conditions for stain index data: red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were stained in a 96-well plate for 1 hr at RT in the dark with a mouse anti-human CD4 antibody, washed 3x in FACS buffer, and resuspended in FACS Buffer. DRAQ7 was added 5 min prior to acquisition. 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 with the option of a 355 nm laser upgrade or standard configuration. 150,000 cells were collected for the multiplex panels and 60,000 cells for the single stained controls and CD4 samples. Analysis was performed using FCS Express 7 Software (De Novo) .

Table 1. Bio-Rad reagents used in the multiplex panel. Antibody in red is pre-launch R&D material.

Target	Fluorescent Dyes	Catalog Number	Target	Fluorescent Dyes	Catalog Number
CD20	SBUV400	MCA1710SBUV400	CD33	SBV515	MCA1271SBV515
CD4	SBUV510	MCA1267SBUV510	CD45RA	SBV610	MCA88SBV610
CD3	SBUV605	MCA463SBUV606	CD8	SBV670	MCA1226SBV670
CD19	SBUV665	MCA1940SBUV665	CD14	SBV710	MCA1568SBV710
CD45RO	SBUV795	MCA461SBUV795	HLA DP DQ DR	SBV790	MCA477SBV790
CD10	SBV440	MCA1556SBV440	L/D	DRAQ7	N/A

StarBright UltraViolet and Violet Dyes Can Be Used Simultaneously in a Multiplexing Panel without Special Buffers Using Premixed Antibodies

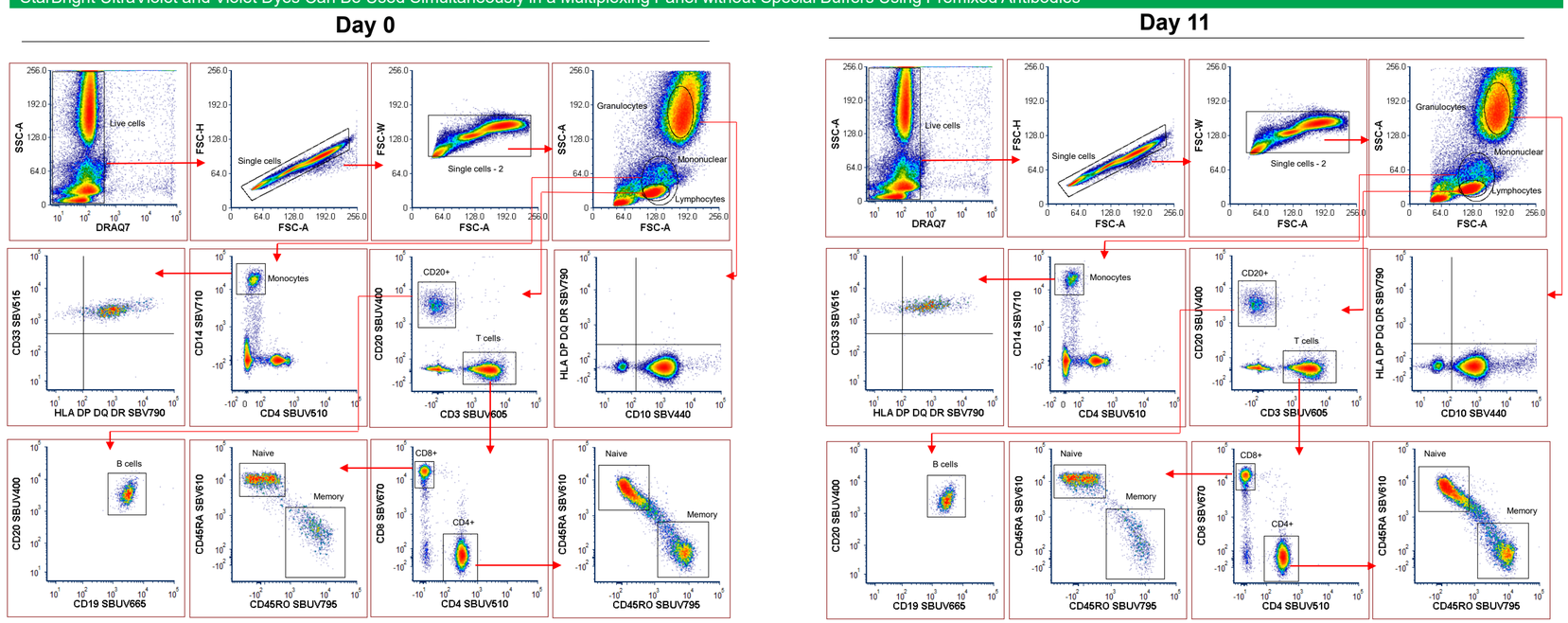

Fig. 2. 12-color multiplex panel. Red blood cell lysed human peripheral blood was stained with an 11-color antibody panel, fresh or premixed 11 days previously, and a live/dead dye (DRAQ7) allowing for identification of multiple cell lineages and subsets.

StarBright UltraViolet and Violet Dyes were used successfully in an immunophenotyping panel without the need for a special buffer. Antibodies can be premixed beforehand for at least 11 days, with no reduction in resolution of populations compared to a fresh antibody mix. Cell percentages remained the same for both antibody mixes. For example in the plots CD3+, CD4+, CD8+, and CD20+ percentage positive cells were 73.12%, 71.24%, 22.58%, and 8.26% for fresh compared to 72.98%, 70.36%, 23.19%, and 7.71% for premixed antibodies.

StarBright UltraViolet and Violet Dyes Have Exceptional Brightness

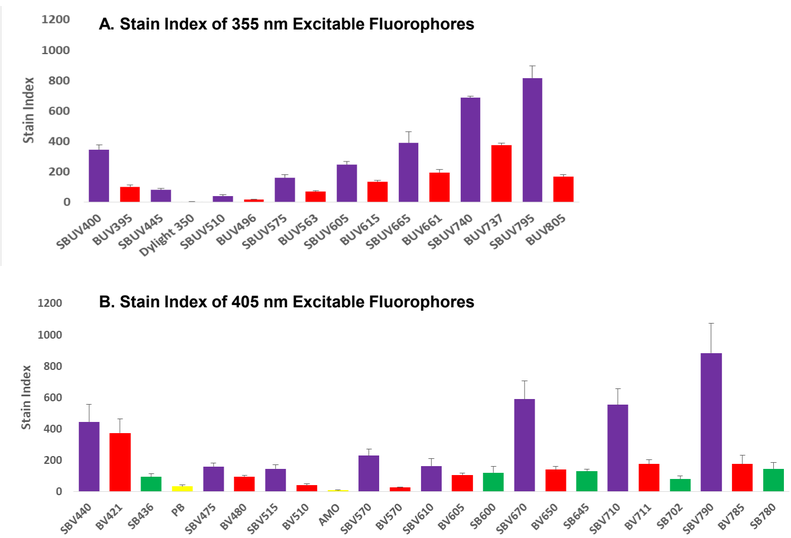

Fig. 3. Stain Index values for StarBright UltraViolet (A) and Violet Dyes (B). Stain Index values for mouse anti-human CD4 staining of red blood cell lysed human peripheral blood, gated on live, single cell lymphocytes. Data acquired on a ZE5 Cell Analyzer. Data shown 3 donors +/- SD.

Multiplexing Panel Compensation and Spreading

The StarBright UltraViolet and Violet Dyes can be successfully compensated with minimal spreading, as seen in the analysis plots. Some dye combinations have spreading, such as SBUV795 and SBUV710, but best practice and careful panel design means these can be successfully and simultaneously used in a panel on mutually exclusive markers.

Table 2. Compensation (A) and spillover spreading (B) matrices for the panel in Fig. 2, generated using FCS Express Software.

A. Compensation Matrix

	SBUV665	SBUV795	SBUV510	SBUV400	SBUV605	SBV670	SBV710	SBV790	SBV440	SBV610	SBV515	Draq7
SBUV665	1.000	0.228	0.000	0.021	0.015	0.353	0.217	0.087	0.000	0.003	0.000	0.605
SBUV795	0.001	1.000	0.005	0.033	0.004	0.000	0.001	0.040	0.000	0.000	0.000	0.005
SBUV510	0.168	0.086	1.000	0.335	0.347	0.029	0.025	0.012	0.028	0.099	0.686	0.003
SBUV400	0.004	0.002	0.014	1.000	0.004	0.000	0.000	0.000	0.001	0.000	0.000	0.000
SBUV605	0.448	0.041	0.005	0.026	9000	1.000	0.089	0.039	0.009	0.000	0.279	0.001	0.000
SBV670	0.407	0.099	0.001	0.000	0.025	1.000	0.544	0.238	0.015	0.116	0.007	0.103
SBV710	0.043	0.234	0.001	0.003	0.007	0.109	1.000	0.433	0.013	0.016	0.006	0.216
SBV790	0.002	0.438	0.001	0.001	0.000	0.008	0.089	1.000	0.017	0.003	0.008	0.019
SBV440	0.010	0.000	0.058	0.000	0.007	0.007	0.006	0.003	1.000	0.020	0.244	0.000
SBV610	0.101	0.009	0.002	0.000	0.233	0.288	0.118	0.029	0.009	1.000	0.050	0.000
SBV515	0.017	0.002	0.070	0.007	0.038	0.037	0.013	0.001	0.056	0.135	1.000	0.003
Draq7	0.004	0.016	0.001	0.000	0.000	0.003	0.009	0.008	0.002	0.001	0.001	1.000

B. Spillover Spreading Matrix

	SBUV665	SBUV795	SBUV510	SBUV400	SBUV605	SBV670	SBV710	SBV790	SBV440	SBV610	SBV515	Draq7
SBUV665	0.000	1.370	0.050	0.140	0.150	1.890	0.790	0.690	0.000	0.210	0.060	1.230
SBUV795	0.100	0.000	0.190	0.040	0.050	0.100	0.510	0.010	0.030	0.020	0.040	0.200
SBUV510	0.640	1.220	0.000	0.450	0.480	0.620	0.510	0.490	0.160	0.390	1.090	0.460
SBUV400	0.170	0.190	0.090	0.000	0.070	0.040	0.040	0.000	0.050	0.080	0.100	0.100
SBUV605	1.270	0.550	0.080	0.140	0.000	0.650	0.370	0.300	0.000	1.370	0.090	0.670
SBV670	2.280	0.790	0.020	0.050	0.180	0.000	2.910	1.040	0.130	0.500	0.060	0.640
SBV710	1.200	5.730	0.210	0.340	0.000	0.890	0.000	1.120	0.300	0.040	0.110	1.400
SBV790	0.160	3.930	0.050	0.130	0.090	0.250	0.490	0.000	0.130	0.080	0.100	0.330
SBV440	0.000	0.000	0.925	0.090	0.180	0.450	0.000	0.020	0.000	2.990	0.940	0.200
SBV610	0.600	0.290	0.040	0.030	1.490	1.060	0.680	0.380	0.070	0.000	0.310	0.370
SBV515	0.300	0.230	0.120	0.300	0.000	0.520	0.420	0.220	0.230	0.560	0.000	0.190
Draq7	0.150	0.600	0.140	0.000	0.040	0.140	0.170	0.240	0.100	0.000	0.060	0.000

Conclusions

- StarBright Dyes, excited by the 355 nm and 405 nm lasers, offer a bright dye with narrow excitation and emission spectra (Figure 1 and 3)
- StarBright UltraViolet and Violet Dyes can be used in multiplexing panels without the requirement for a special buffer and premixed together for greater than 11 days with no loss in performance (Figure 2)
- StarBright Dyes are an excellent choice for inclusion in multiplexing panels for conventional and spectral flow cytometry

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