

StarBright Violet Dyes: Superior Staining With StarBright Dyes.



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StarBright Dyes are exceptionally bright fluorescent nanoparticles with narrow excitation and emission characteristics. The brightness allows better resolution of rare populations and low-density antigens, while the optimized excitation and emission profiles the flexibility to fit into any multicolor flow cytometry panel. They are compatible with most staining protocols, work in virtually all buffers with no loss in performance, and do not require any special buffers when combined with other StarBright Dyes, organic fluorophores, protein-based fluorophores, and polymer dyes. Resistant to photobleaching, stable without loss of performance over time, and fixable, StarBright Dyes allow you to build bigger and better panels with ease and high lot-to-lot reproducibility.

To assess the performance of StarBright Dyes, 12-color flow cytometry panels containing multiple Brilliant Violet (BV) or Super Bright (SB) Dyes were directly compared with a StarBright Dye containing panel. The ability to resolve multiple populations, the spillover, and the spreading of populations were assessed.

Materials and Methods

Staining conditions: red blood cell lysed human peripheral blood was blocked with 10% human serum and stained with VivaFix 355/442 Cell Viability Assay (Bio-Rad). After washing and resuspending in a standard FACS buffer (PBS + 1% BSA) or Brilliant Stain Buffer (BD), cells were incubated with a cocktail containing 11 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.

Multiplex panel: antibodies and fluorescent formats used in the panels are shown in Table 1. All antibodies were titrated to determine the optimal staining concentration prior to use. The panel containing StarBright Dyes only consisted of 11 colors as no suitable equivalent of StarBright Violet 515 Dye (SBV515) or BV510 was available in the range. Where possible, all clones were matched.

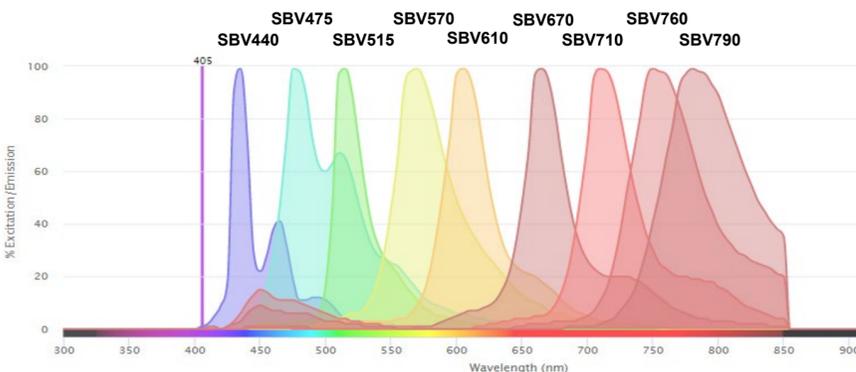
Data collection and analysis: data for these studies were collected on a five laser, 30 parameter ZE5 Cell Analyzer. 100,000 cells were collected for the multiplex panel and 60,000 cells for the single stained controls. Analysis was performed using FCS Express Software (De Novo). StarBright Violet 440 Dye and Super Bright 436 emissions were collected using a 460/22 filter. Brilliant Violet 421 emission was collected using a 420/10 filter.

Table 1. Antibodies used in the three panels.

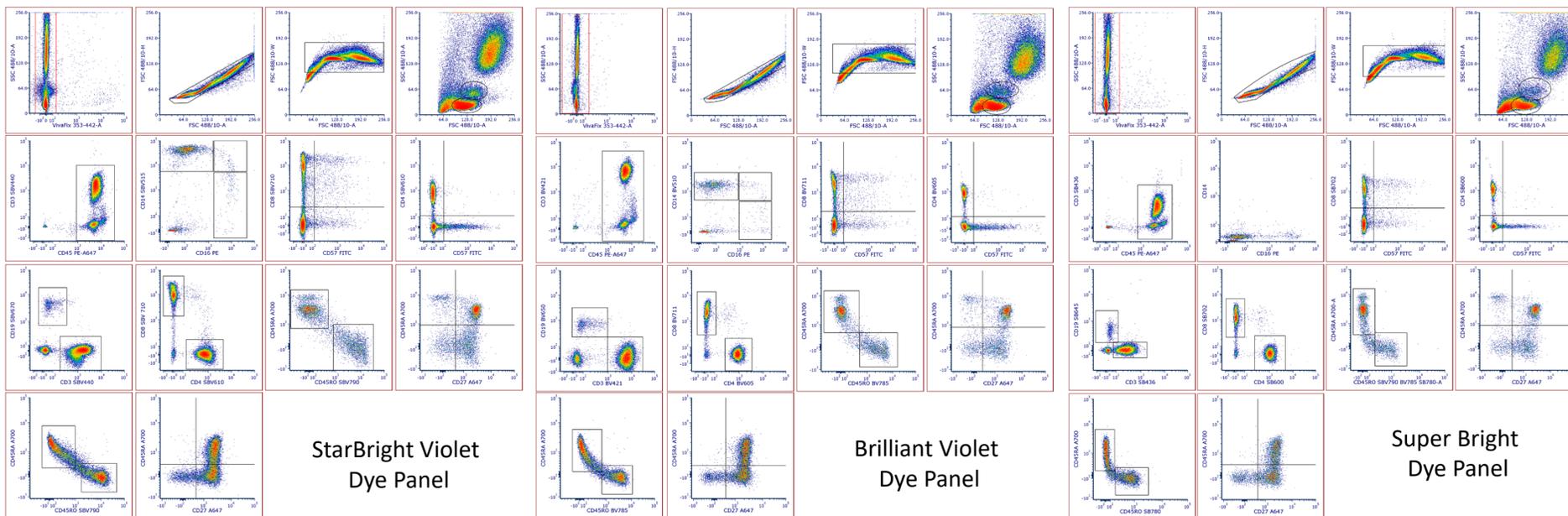
Marker	Catalog # (SBV Panel)	StarBright Dye	Brilliant Violet	Super Bright
CD3	MCA463SBV440	SBV440	BV421	SB436
CD14	MCA1568SBV515	SBV515	BV510	N/A
CD4	MCA1267SBV610	SBV610	BV605	SB600
CD19	MCA1940SBV670	SBV670	BV650	SB645
CD8	MCA1226SBV710	SBV710	BV711	SB702
CD45RO	MVA461SBV790	SBV790	BV785	SB780
CD57	MCA1305F	FITC	FITC	FITC
CD16	MCA2357PE	PE	PE	PE
CD45	MCA87P647	PE-A647	PE-A647	PE-A647
CD27	MCA755A647	A647	A647	A647
CD45RA	MCA88A700	A700	A700	A700
L/D	1351111	VivaFix 355/442	VivaFix 355/442	VivaFix 355/442

Axxx, Alexa Fluor; BV, Brilliant Violet; FITC, fluorescein isothiocyanate; L/D, live/dead; PE, phycoerythrin; SB, Super Bright; SBV, StarBright Violet.

StarBright Violet Dye Emission Spectra



Violet Dye Comparison of 12 Color Immunophenotyping Panels on Human Peripheral Blood



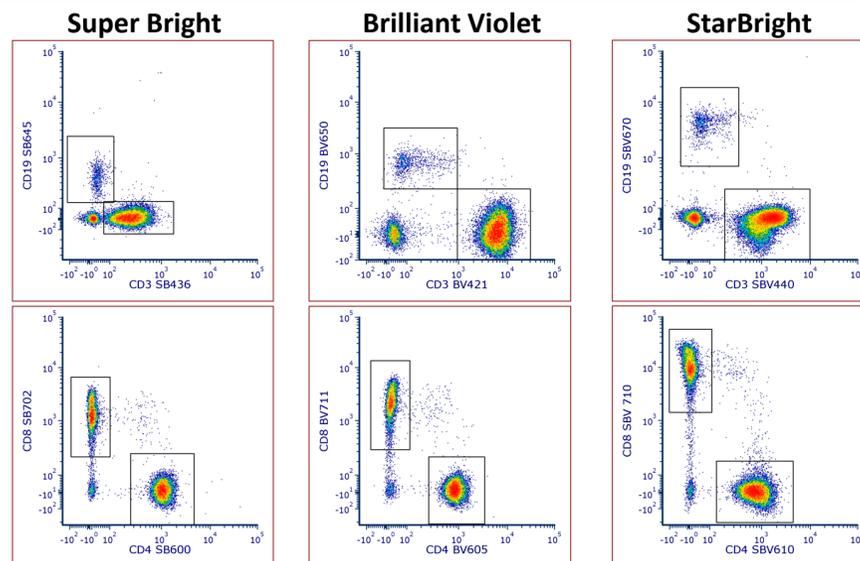
Spillover Matrix

	FL03-A	FL10-A	FL14-A	FL15-A	FL16-A	FL17-A	FL19-A	FL20-A	FL21-A	FL25-A	FL26-A	FL29-A
CD57 FITC-A	1.0	0.00981	0.0	0.0	0.0034	0.00516	0.02349	0.0	0.0	0.0	0.0	0.0
VivaFix 353-442-A	0.0	1.0	0.02818	0.01676	0.00675	2.00781	0.04279	0.49728	0.0071	0.01044	0.00388	0.00468
CD19 SBV710	0.0	0.00016	1.0	0.53378	0.24964	0.02365	0.11322	0.01008	0.17817	0.00448	0.12259	0.23033
CD8 SBV710	0.00003	0.00006	0.14066	1.0	0.48313	0.01723	0.1648	0.00156	0.05366	0.00015	0.20762	0.01916
CD45RO SBV790	0.00044	0.001	0.00423	0.08176	1.0	0.01925	0.00184	0.00812	0.0001	0.0002	0.00084	0.00001
CD3 SBV440	0.00044	0.00815	0.01015	0.00732	0.00017	1.0	0.02211	0.022318	0.0	0.0	0.0	0.0
CD4 SBV610	0.01305	0.01064	0.02446	0.13069	0.0352	0.02135	1.0	0.07267	0.0625	0.01047	0.0011	0.00172
CD14 SBV515	0.1403	0.07602	0.02871	0.01458	0.00001	0.08869	0.11332	1.0	0.00026	0.00375	0.00013	0.00014
CD45 PE-A647-A	0.00034	0.0023	0.026	0.00784	0.00134	0.00003	0.00241	0.00117	1.0	0.3813	0.50002	0.88844
CD16 PE-A	0.0115	0.0008	0.00815	0.00347	0.00058	0.00097	0.01016	0.00872	1.0	0.00007	0.00058	0.00068
CD45RA A700-A	0.0007	0.00041	0.00098	0.00277	0.00185	0.00037	0.00027	0.00049	0.00038	0.00033	1.0	0.02477
CD27 A647-A	0.00272	0.0	0.00163	0.00141	0.00014	0.0	0.00029	0.0	0.06117	0.0	0.69007	1.0

Spreading Matrix

Reference/Spillover	FITC-A	VivaFix 353-442-A	SBV670-A	SBV710-A	SBV790-A	SBV440-A	SBV610-A	SBV515-A	PE-A647-A	PE-A	A700-A	A647-A
FITC-A	1.0	0.102075	0.0	0.0	0.0	0.554846	0.110258	0.0	0.495099	0.127083	0.0	0.0
VivaFix 353-442-A	0.565056	1.0	0.46191	0.429343	0.430075	2.21898	0.453415	0.658855	0.676089	0.786828	0.847531	0.663966
SBV710-A	0.038957	0.000006	1.0	0.206308	0.161635	0.170547	0.391398	0.0858306	0.077905	0.147945	0.844606	0.0
SBV710-A	0.038957	0.000006	1.0	0.206308	0.161635	0.170547	0.391398	0.0858306	0.077905	0.147945	0.844606	0.0
SBV790-A	0.0593883	0.268714	0.085355	0.344648	0.291407	0.116999	0.028231	0.066804	0.031156	0.071853	0.177828	0.092266
SBV440-A	0.196593	0.000006	0.180499	0.170369	0.101848	0.112063	0.259574	0.0	0.0	0.0	0.0	0.0
SBV610-A	0.134807	0.838643	0.761469	0.539322	0.441569	1.288474	0.274473	0.396949	0.423573	0.284006	0.295055	0.0
SBV515-A	1.415732	0.3116	0.223554	0.184007	0.148987	0.632322	0.584451	0.0607751	0.126388	0.074228	0.059272	0.0
PE-A647-A	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737	0.0094737
PE-A	0.071822	0.393452	0.121223	0.073807	0.099277	0.156102	0.130277	0.063223	0.532569	0.0	0.10674	0.286665
A700-A	0.093349	0.093349	0.274844	0.242554	0.091294	0.093349	0.093349	0.093349	0.093349	0.093349	0.093349	0.259589
A647-A	0.136578	0.142736	0.122255	0.12467	0.109999	0.083487	0.081242	0.057799	0.355627	0.0	1.403238	0.0

Highlighted Plots



Conclusions

The 12-color panels enabled the identification of many of the major lymphocyte and monocyte subsets found within peripheral blood: B lymphocytes, T helper, T cytotoxic, and T regulatory cells within the lymphocyte gate. In addition, naïve and memory status, and specific memory subsets were identified such as TEMRA, EM, CM, and co-stimulatory molecule expression. Within the monocyte gate, classical, intermediate, and nonclassical monocytes were observed.

- There were significant improvements in the separation of populations when StarBright Violet (SBV) Dyes were used (see main figure and highlighted plots).
- The superior brightness of SBV Dyes gives improved resolution with the added benefit of reduced spillover and spreading due to the narrow excitation and emission profiles
- Furthermore, with no special buffer required, this staining could be performed in PBS 1% BSA

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