Novel, Violet Fluorescent Nanoparticles for Immunophenotyping Using Flow Cytometry

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Abstract

A novel series of fluorescent nanoparticles with violet excitation have been developed for use with flow cytometry antibodies. These exceptionally bright, photostable dyes also have narrow emission spectra, making them ideal for inclusion in high-parameter multicolor panels. For this study, StarBright Violet 440, 515, 610, 670, 710, and 790 Dyes were conjugated to human B-cell, T-cell, and Treg marker monoclonal antibodies and used to immunophenotype human peripheral blood mononuclear cells (PBMCs) in an 8-color panel. Tests with this panel were conducted in different staining buffers and compared to a panel with dyes having similar spectra and the same clonality. The new StarBright Dye multicolor panel showed better resolution of Treg populations and reliable identification of the different T- and B-cell populations across different stain buffers and conditions, which makes the StarBright Violet Dyes an excellent addition to the field of high-parameter flow cytometry.

Materials and Methods

Healthy human peripheral blood mononuclear cells (PBMCs) (AllCells) were stained with VivaFix 353/442 Viability Dye for 30 min at room temperature. Cells were washed twice in Dulbecco's phosphate-buffered saline (DPBS) and then plated in a 96-well plate at 0.5 million cells/well. Antibody staining mixtures were prepared according to Table 1 in BD Horizon Brilliant Stain Buffer (for BD fluorophores), DPBS, and house-made stain buffer (DPBS + 3% FBS + 0.09% NaN_3), at concentrations previously determined by antibody titration. Cells were stained with antibody mixes for 1 hr at 4°C in the dark. After incubation, cells were washed with the respective buffers, and resuspended. Samples were analyzed on the ZE5 Cell Analyzer. Data were analyzed and plots generated using FCS Express 7 Software.

Table 1. 8-color StarBright Dyes and Brilliant Violet conjugated flow cytometry panels immunophenotyping T-, B-, and Treg cell markers.

Target (clone)	EmFilter	Bio-Rad Dye	BD Dye (Cat#)
CD3 (UCHT1)	460/22	StarBright Violet 440	Pacific Blue (#558117)
CD4 (RPA-T4)	625/24	StarBright Violet 610	Brilliant Violet 605 (#562658)
CD8 (RPA-T8)	720/60	StarBright Violet 710	Brilliant Violet 711 (#563677)
CD19 (HIB19)	670/30	StarBright Violet 670	Brilliant Violet 650 (#740568)
CD20 (2H7)	525/20	StarBright Violet 515	Brilliant Violet 510 (#563067)
CD25 (M-A251)	750LP	StarBright Violet 790	Brilliant Violet 786 (#563701)
CD127 (11592)	670/30	Alexa Fluor 647* (#HCA145A647)	Alexa Fluor 647 (Bio-Rad #HCA145A647)
Viability Dye	447/60	VivaFix 353/442 (Bio-Rad #135111)	

*CD127 (AbD11590) is not currently available conjugated to StarBright Violet Dye.

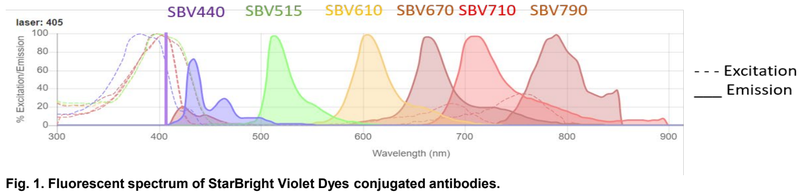
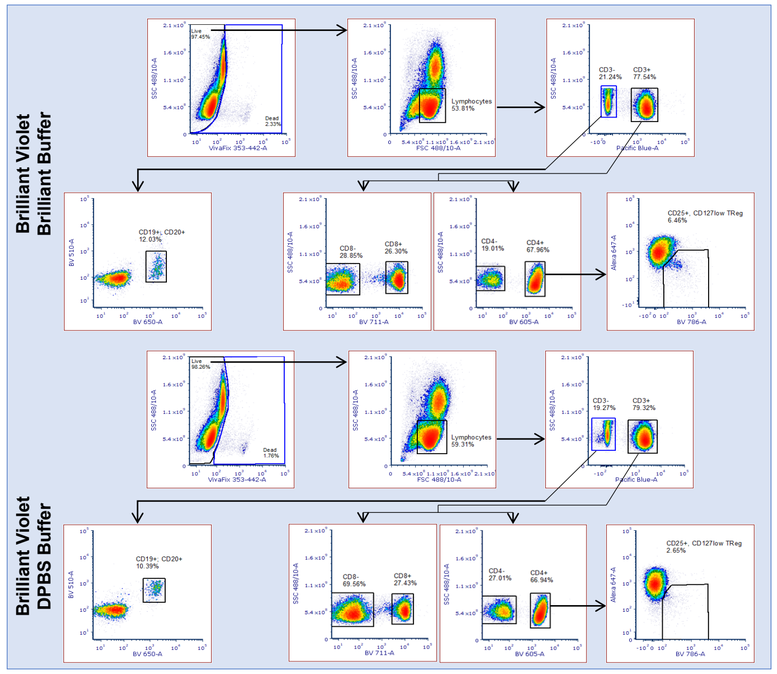


Fig. 1. Fluorescent spectrum of StarBright Violet Dyes conjugated antibodies.

Results



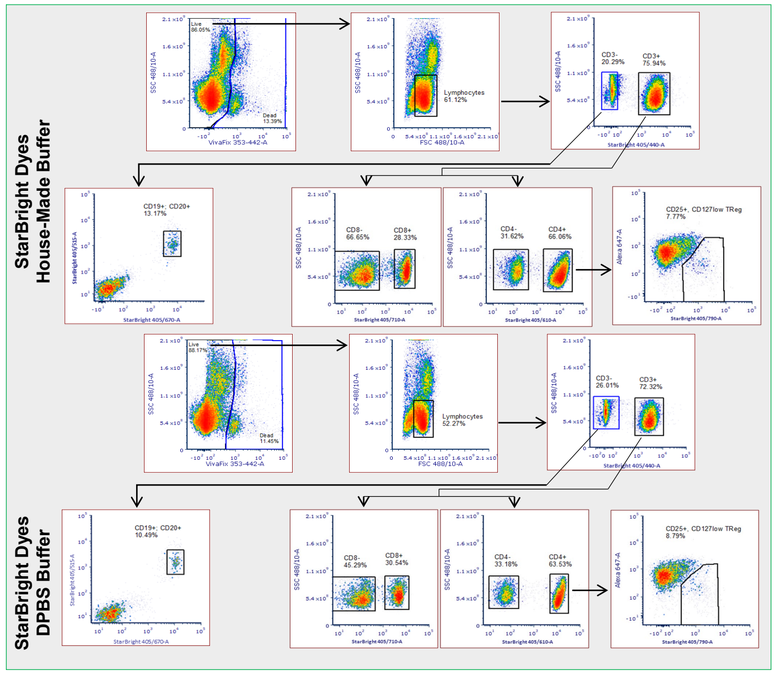


Fig. 2. Lymphocyte population was isolated after VivaFix 353/442 Assay viability staining and gated for $CD3^-$ and $CD3^+$ cells. From the $CD3^-$ gated cells, we looked at $CD19^+/CD20^+$ B-cells. From $CD3^+$ gated cells, we were able to characterize $CD8^+$ and $CD4^+$ T-cell populations. From $CD4^+$ T-cells, we were then able to gate the $CD25^+$, CD127 low-expressing Treg cells.

Comparison of %Treg cells in Brilliant Violet vs StarBright Dyes panels.

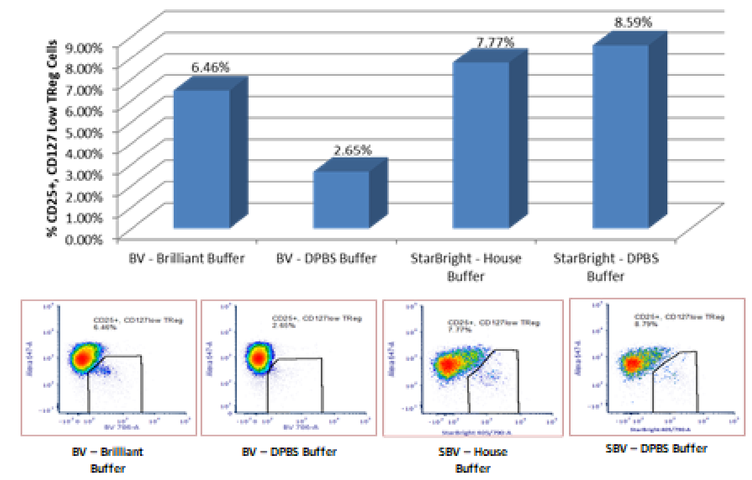
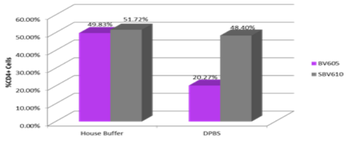


Fig. 3. Percent of $CD4^+CD25^+CD127$ low Treg cells detected with the StarBright and Brilliant Violet panels in the different staining buffers. SBV, StarBright Violet Dyes; BV, Brilliant Violet.

Comparison of $\%CD4^+$ cells between BV605 and SBV610 at optimal titration and between different buffers.



Comparison of SI values at optimal titrations between BV605 and SBV610 between different buffers.

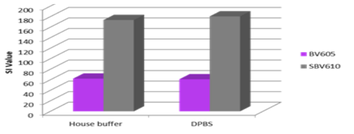


Fig. 4. StarBright Dyes retained consistent $\%CD4^+$ cells in different buffers and higher SI values, showing they are brighter than Brilliant Violet flow antibodies.

Conclusion

- StarBright Violet Dye conjugated antibodies work well in different staining buffers, allowing for more flexibility during staining, and are brighter than Brilliant Violet Dyes.
- StarBright Violet Dye conjugated antibodies show improved performance, compared with Brilliant Violet conjugated antibodies, in identifying smaller cell populations like $CD4^+CD25^+CD127$ low Treg cells in different staining buffers.

References

Macker H T JP McCoy and R Nussenblatt (2012). Standardizing immunophenotyping for the human immunology project. Nat. Rev. Immunol. 12, 191–200.