Advancing Multiplexing: StarBright™ Spectral Dyes for Enhanced Spectral Cytometry

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Full spectrum cytometry is a powerful tool, with the ability to generate data using larger and more intricate panels than conventional flow cytometry. However, panels are often restricted by a shortage of suitable dyes. Many currently available fluorophores are dim, have poor stability, lack distinct emission wavelengths, or have excessive spectral overlap, limiting panel optimization and affecting data quality. Bio-Rad's StarBright Dye range now includes 36 different fluorophores, following the introduction of four new StarBright Dyes. These four dyes were designed specifically for spectral cytometry with unique peak emission profiles. Their distinct narrow emission spectra make it possible to build larger panels as well as enhancing existing ones.

We show data to demonstrate that these innovative dyes can replace suboptimal fluorophores, reducing spectral overlap and spreading, to improve data quality.

New Spectral StarBright Dye Range

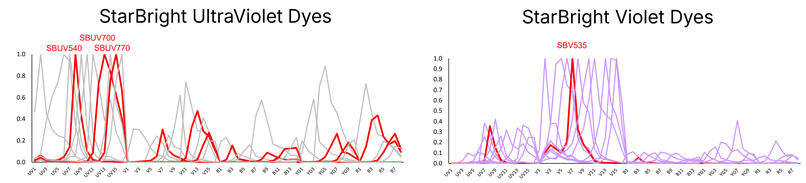


Fig. 1. Spectral profiles of StarBright UltraViolet and Violet Dyes. Emission profiles of Mouse Anti-Human CD4 (MCA1267) StarBright UltraViolet and Violet–conjugated antibodies were generated on a 5-L Cytek Aurora (Cytek Biosciences). New spectral dyes are highlighted in red.

Materials and Methods

Staining. Human PBMCs were stained with VivaFix 353/442 (#1351111, Bio-Rad Laboratories). After washing and resuspending, cells were blocked with Seroblock (#BUF070, Bio-Rad Laboratories) and stained with a single antibody or antibody panel (Table 1) in Brilliant Buffer Plus (#566385, Waters Biosciences). Cells were stained in a 96-well plate for 1 hr at room temperature (RT), washed 3X, and cells were acquired on a 5-laser Cytek Aurora. All antibodies were titrated to determine the optimal staining concentration prior to use.

Data collection and analysis. Data were analyzed using FCS Express 7 (De Novo Software by Dotmatics).

A. Standard Panel

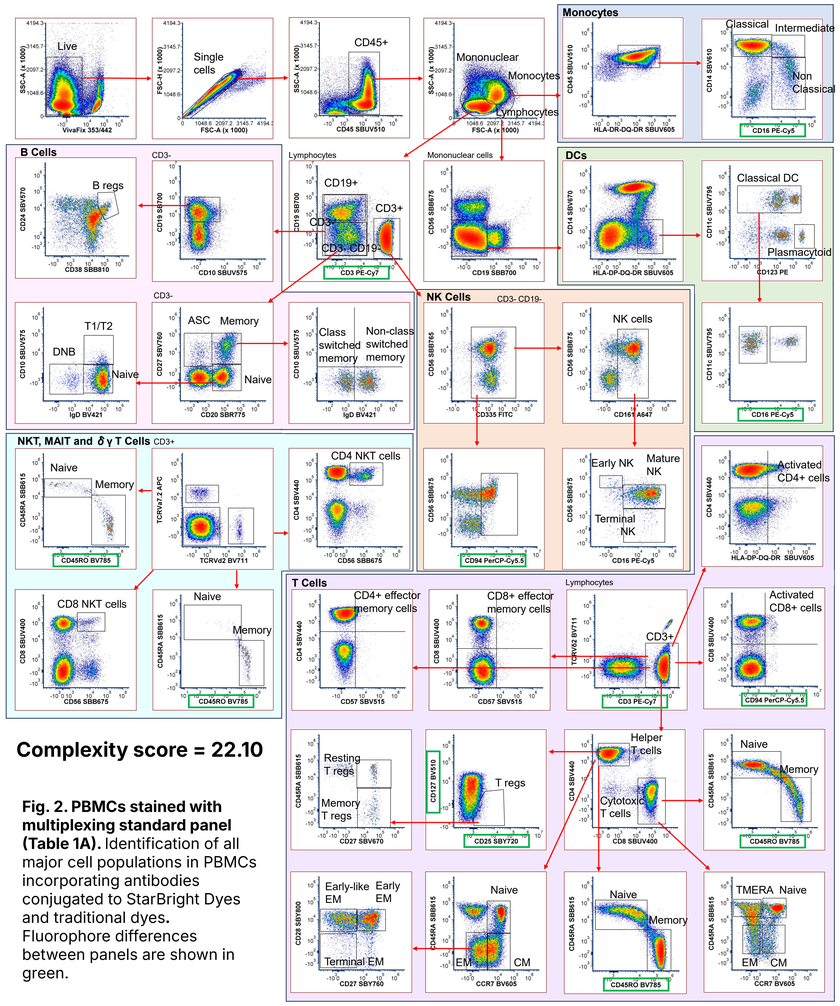
Fluorophore	Peak Channel	Target	Catalog Number
SBUV400	UV2	CD8	MCA1226SBUV400
SBUV510	UV7	CD45	MCA87SBUV510
SBUV575	UV9	CD10	MCA1556SBUV575
SBUV605	UV10	HLA DP DQ DR	MCA477SBUV605
SBUV795	UV16	CD11c	MCA2087SBUV795
BV421	V2	IgD	BioLegend, 348225
SBV440	V3	CD4	MCA1267SBV440
SBV515	V6	CD57	MCA1305*
BV510	**V7**	**CD127**	**BioLegend, 351331**
SBV570	V9	CD24	MCA1379SBV570
BV605	V10	CCR7	BioLegend, 353223
SBV670	V11	CD14	MCA1568SBV670
BV711	V12	TCRγδ	BioLegend, 331411
SBV760	V14	CD27	MCA755SBV760
BV785	**V15**	**CD45RO**	**BioLegend, 304233**
FITC	B2	CD335	MCA4646F
SBB615	B5	CD45RA	MCA88SBB615
SBB675	B8	CD56	TZA062SBB675
SBB700	B9	CD19	MCA1940SBB700
PERCP Cy5.5	**B10**	**CD94**	**BioLegend, 305514**
SBB810	B14	CD38	MCA1019SBB810
PE	Y1	CD123	MCA1263PE
PE-Cy5	**Y5**	**CD16**	**BioLegend, 360735**
SBY720	**Y7**	**CD25**	**MCA2127SBY720**
PE-Cy7	**Y9**	**CD3**	**BioLegend, 300419**
SBY800	Y10	CD28	MCA709SBY800
APC	R1	TCRV2α	BioLegend, 351707
A647	R3	CD161	MCA1855A647
SBR775	R7	CD20	MCA1710SBR775

B. Optimized Panel

Fluorophore	Peak Channel	Target	Catalog Number*
SBUV400	UV2	CD8	MCA1226SBUV400
SBUV510	UV7	CD45	MCA87SBUV510
SBUV540	**UV8**	**CD16**	**MCA5665SBUV540**
SBUV575	UV9	CD10	MCA1556SBUV575
SBUV605	UV10	HLA DP DQ DR	MCA477SBUV605
SBUV700	**UV13**	**CD94**	**MCA2254SBUV700**
SBUV770	**UV15**	**CD3**	**MCA463SBUV770**
SBUV795	UV16	CD11c	MCA2087SBUV795
BV421	V2	IgD	BioLegend, 348225
SBV440	V3	CD4	MCA1267SBV440
SBV515	V6	CD57	MCA1305*
SBV535	**V7**	**CD127**	**HCA402SBV535**
SBV570	V9	CD24	MCA1379SBV570
BV605	V10	CCR7	BioLegend, 353223
SBV670	V11	CD14	MCA1568SBV670
BV711	V12	TCRγδ	BioLegend, 331411
SBV760	V14	CD27	MCA755SBV760
SBV790	**V16**	**CD25**	**MCA2127SBV790**
FITC	B2	CD335	MCA4646F
SBB615	B5	CD45RA	MCA88SBB615
SBB675	B8	CD56	TZA062SBB675
SBB700	B9	CD19	MCA1940SBB700
SBB810	B14	CD38	MCA1019SBB810
PE	Y1	CD123	MCA1263PE
SBY720	**Y7**	**CD45RO**	**MCA461SBY720**
SBY800	Y10	CD28	MCA709SBY800
APC	R1	TCRV2α	BioLegend, 351707
A647	R3	CD161	MCA1855A647
SBR775	R7	CD20	MCA1710SBR775

Table 1. Bio-Rad antibodies used in the multiplex panel. All antibodies are from Bio-Rad Laboratories unless stated. Antibodies in bold and highlighted in gray are different between the two panels. * Conjugated to SBV515 using TrailBlazer™ Tag (#12020038) and TrailBlazer StarBright Dye SBV515 Label Kits (#12020041).

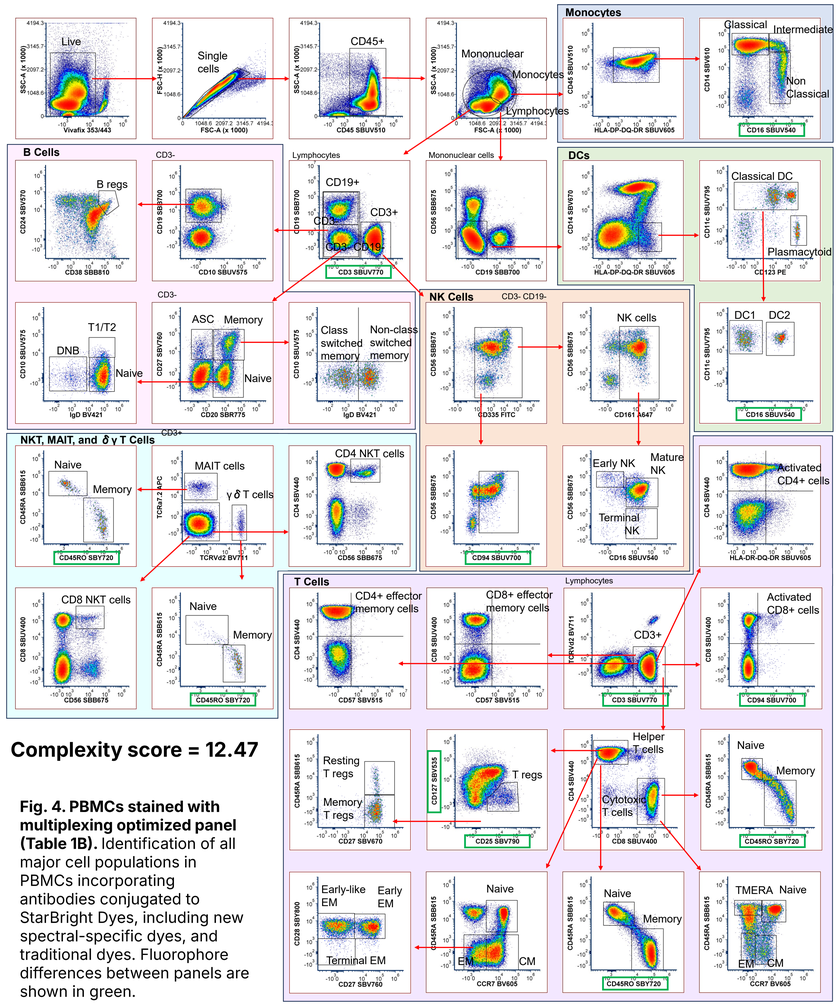
Multiplexing Standard Panel



Complexity score = 22.10

Fig. 2. PBMCs stained with multiplexing standard panel (Table 1A). Identification of all major cell populations in PBMCs incorporating antibodies conjugated to StarBright Dyes and traditional dyes**.** Fluorophore differences between panels are shown in green.

Multiplexing Optimized Panel, Includes Spectral-Specific StarBright Dyes



Complexity score = 12.47

Fig. 4. PBMCs stained with multiplexing optimized panel (Table 1B). Identification of all major cell populations in PBMCs incorporating antibodies conjugated to StarBright Dyes, including new spectral-specific dyes, and traditional dyes. Fluorophore differences between panels are shown in green.

Improvements in Standard Panel

Improved separation, reduced spreading, and clearer population identification were observed following the panel redesign. The updated panel replaces tandem dyes and dyes with high spillover or autofluorescence-like profiles (BV510) with StarBright Dyes, including new spectral-specific formats.

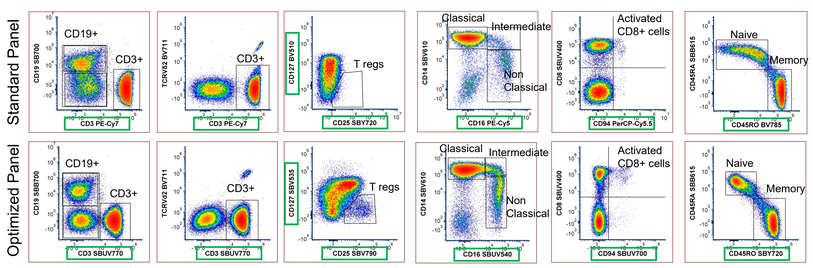


Fig. 3. Panel improvements after the inclusion of StarBright Dye–conjugated antibodies. The top row shows plots from the standard panel, and the second row shows plots from the optimized panel. Fluorophore differences between the panels are shown in green.

Conclusion

Small changes to existing panels, such as incorporating the new spectral-specific StarBright Dyes, can significantly improve panel performance. By replacing fluorophores with high spectral spillover or tandem dyes, we demonstrate reduced spreading, improved population resolution, and lower overall panel complexity.

New spectral-specific StarBright Dyes are bright with unique peak channels that integrate seamlessly with existing fluorophores and offer powerful alternatives to traditional dyes, enabling larger panels and clearer cell identification.

Bulletin Number 3941

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