Transfer of a 33-color flow cytometry immunophenotyping panel through transfer of application settings from the BD FACSymphony™ A5 SE Cell Analyzer to the BD FACSymphony™ S6 SE Cell Sorter

Ludovic Monheim[1], Moen Sen[2], Michael Rietsma[2], Stephanie Widmann[3] and Aaron Tyznik[3]

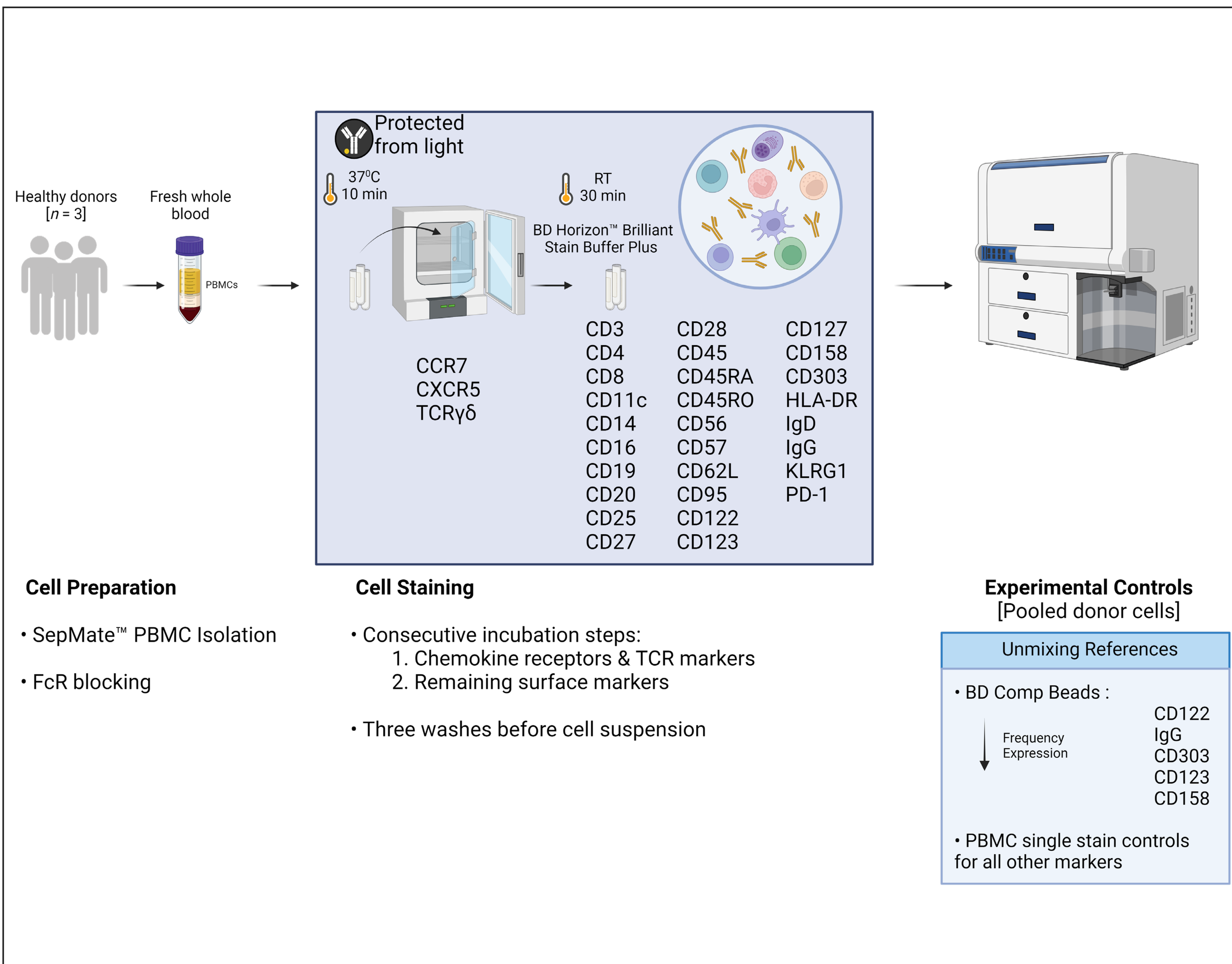
[1]BD Biosciences, Erembodegem, Belgium; [2] BD Biosciences, 2350 Qume Drive, San Jose, CA 95131 ; [2]BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121

Abstract

In the rapidly advancing field of flow cytometry, cross standardization of flow cytometers remains a challenge. Cross standardization ensures consistency of data generated on separate cytometers. In this study, we demonstrate the transfer of application settings from the BD FACSymphony™ A5 SE Cell Analyzer to the BD FACSymphony™ S6 SE Cell Sorter, enabling convenient transfer of a 33-color panel between two spectral-enabled flow cytometers.

First, the BD FACSymphony™ A5 SE Cell Analyzer and the BD FACSymphony™ S6 SE Cell Sorter were checked for performance and characterized using the BD® Cytometer Setup and Tracking (CS&T) System. Second, both instruments were set up with optimized voltage values obtained from a voltration analysis. Third, Median Fluorescence Intensity (MFI) from mid-peak rainbow fluorescent particles was recorded for each of the 48 PMT detectors on the BD FACSymphony™ A5 SE Cell Analyzer. Next, new application settings were created on the BD FACSymphony™ S6 SE Cell Sorter with voltages set to match the MFI values from the BD FACSymphony™ A5 SE Cell Analyzer. Finally, CD4 samples were recorded on each instrument for confirmation of similar MFI values. Following cross standardization, the 33 -color immunophenotyping panel designed on the BD FACSymphony™ A5 SE Cell Analyzer was directly transferred to the matched BD FACSymphony™ S6 SE Cell Sorter, facilitating the physical isolation of target cell populations of interest.

Methods



Fresh human PBMCs were derived from whole blood obtained from healthy donors. PBMCs were isolated by Ficoll™ gradient centrifugation and resuspended in DPBS (1X). The staining solution comprised a cocktail of antibodies as per the specified test volumes (Table 1) suspended in BD Horizon™ Brilliant Stain Buffer Plus. Cells were stained by first prestaining the freshly isolated PBMCs with antibodies against TCRγδ, CD185 and CD197 for 10 min at 37 ºC. The antibody cocktail was then added to the prestained peripheral blood mononuclear cell (PBMCs) and the mixture was incubated for 30 min at room temperature in the dark. Single-stained controls were processed in parallel with the full panel. Cells were washed twice using cold BSA wash buffer (PBS and 0.01% NaN3). Cells were resuspended in wash buffer and stored on ice, protected from light, until acquisition. Viability dye 7-AAD was added at the specified test volume 5 min before acquisition on the flow cytometer. Optimized gain settings were derived for the BD FACSymphony™ A5 SE Cell Analyzer to generate Application Settings. Application Settings were then applied to maintain consistent fluorescence intensity values across experiments.

Results

1 Instrument configuration and reagent selection

Leukocyte

CD45

T cell	B cell	NK cell	Dendritic cell	Monocyte
CD3, CD4, CD8, CD25, CD27, CD28, CD45RA, CD45RO, CD57, CD62L, CD95, CD127, CCR7, CXCR5, PD-1, KLRG1, TCRγδ	CD19, CD20, CD27, IgD, IgG	CD16, CD56, CD57, CD122, CD158	CD11c, CD123, CD303, HLA-DR	CD14

Laser	Marker	Fluorochrome	Catalog #	Clone	Compensation Control	Test size (µl or µg /test)
UV	CD45RA	BUV395	740315	5H9	PBMCs	0.06 µg
	Auto F	Cells			PBMCs	
	CD45RO	BUV496	749888	UCHL1	PBMCs	0.5 µg
	CD16	BUV563	748851	3G8	PBMCs	0.5 µg
	CD185/CXCR5	BUV661	741559	RF8B2	PBMCs	0.06 µg
	CD28	BUV737	612815	CD28.2	PBMCs	0.25 µg
	CD127	BUV805	748486	HIL-7R-M21	PBMCs	0.5 µg
Violet	CD25	BV421	567485	BC96	PBMCs	2.5 µl
	CD20	V450	561164	L27	PBMCs	2.5 µl
	CD62L	BV480	566174	DREG-56	PBMCs	0.125 µg
	CD122/ IL2-RB	BV510	747741	Mik-β3	Beads	2.5 µl
	IgG	BV605	563246	G18-145	Beads	2.5 µl
	CD56	BV650	564057	NCAM16.2	PBMCs	0.125 ug
	CD303	BV711	748002	V24-785	Beads	1.25 µl
	KLRG1	BV750	753692	Z7-205.rMAb	PBMCs	1.25 µl
	CD123	BV786	564196	7G3	Beads	0.125 µg
Blue	CD57	FITC	555619	NK-1	PBMCs	0.03 µg
	CD4	RB545	569183	SK3	PBMCs	0.06 µg
	CD11c	BB630-P2	Custom	B-LY6	PBMCs	0.5 µg
	CD27	BB660-P2	Custom	M-T271	PBMCs	0.0125 µg
	CD45	PerCP	340665	2D1	PBMCs	10 µl
	CD279/PD-1	BB700	566460	EH12.1	PBMCs	2.5 µl
	CD3	BB755-P	Custom	UCHT1	PBMCs	0.025 µg
	CCR7/CD197	RB780	568748	2-L1-A	PBMCs	1 µg
Yellow Green	CD19	PE	555413	HIB19	PBMCs	5 µl
	CD158	RY586	753232	HP-MA4	Beads	0.125 µg
	CD14	PE-CF594	562335	MφP9	PBMCs	0.125 µg
	CD95	PE-Cy5	559773	DX2	PBMCs	20 µl
	7-AAD		559925		PBMCs (10% heat killed)	5 µl
	TCRγδ	PE-Cy7	655434	11F2	PBMCs	1.25 µl
Red	HLA-DR	APC	559868	TU36	PBMCs	20 µl
	IgD	R718	567993	IA6-2	PBMCs	0.125 µg
	CD8	APC-H7	641409	SK1	PBMCs	0.03 µg

Table 1. Instrument configuration and reagent selection. This 33-color panel allows identification and sorting of specific human (PBMC) populations. Careful evaluation of single-color controls identified superior performance in spectral unmixing using BD® CompBeads Particles in place of cell based controls for five colors (BV510, BV605, BV711, BV786 and RY586) in this panel.

2 Identification of classical human immune cell subsets circulating in human blood

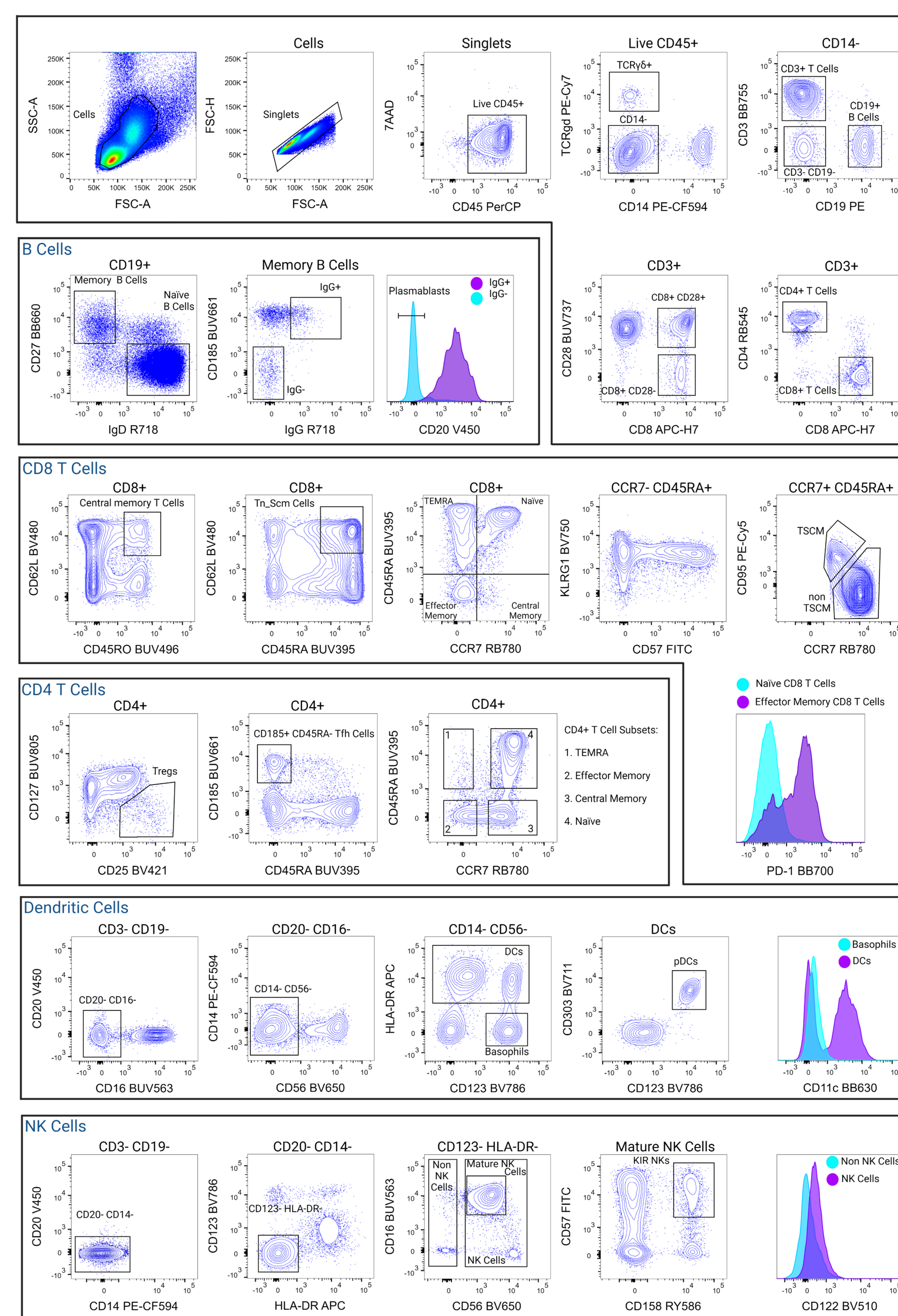
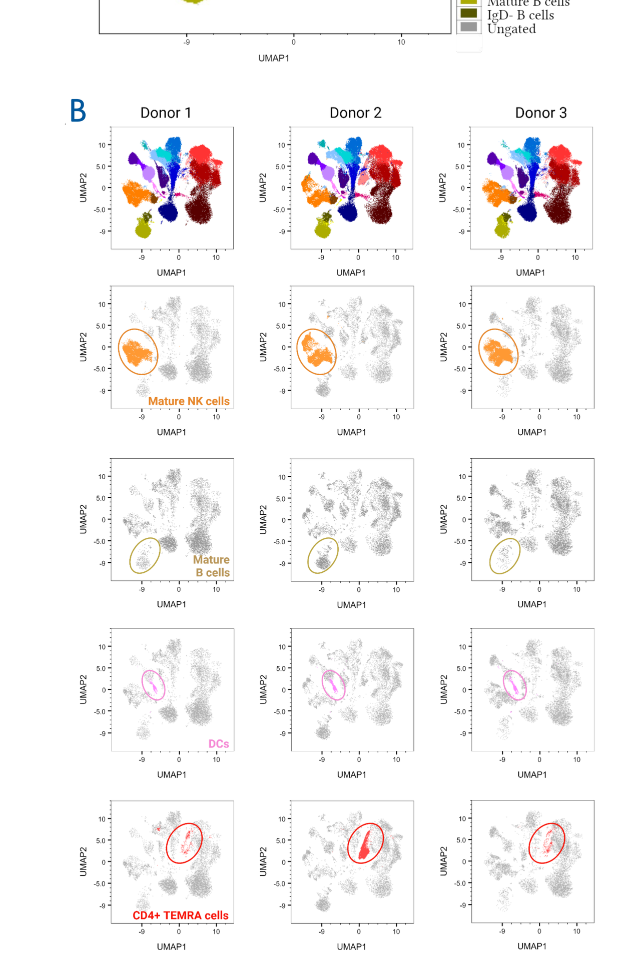


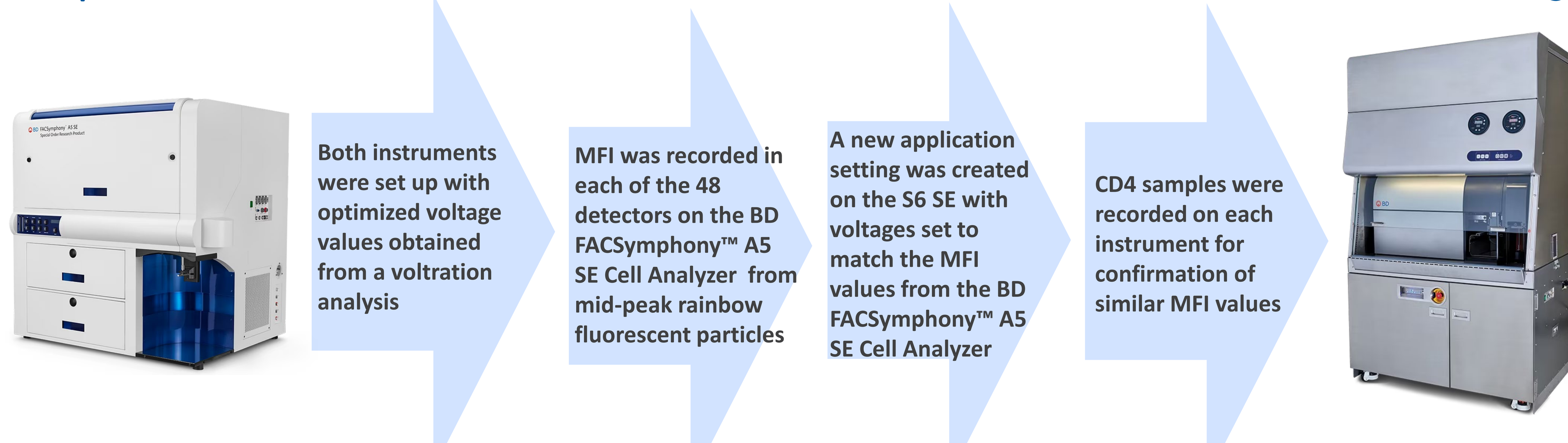
Figure 2. T cells, B cells, DCs and NK cells were gated on CD45+ cells by excluding 7AAD+ inviable cells and doublets. B cells were identified as CD19+ followed by identification of Naïve/Mature CD27-IgD+ B cells. Plasmablasts (CD27+ CD20-) were identified by assessing IgD- IgG- CD185- B cells. Classical T cells were identified as either CD4+, CD8+ or TCRγδ+, followed by identification of well-characterized T cell subsets based on the expression of CD62L and CD45RA or CD45RO (central memory and TN/SCM naïve and stem cell memory T cells), CD45RA and CCR7 (naïve, central memory, effector memory and terminally differentiated effector memory cells), CD95 and CCR7 (stem memory T cells TSCM), CD127 and CD25 (Tregs), and CD185 and CD45RA (T follicular helper cells). PD1 expression was assessed in CD8+ naïve and effector memory cells, and KLRG1 expression was assessed in CD8+ TEMRA cells. Classical DCs were identified as CD3- CD19- CD20- CD16- CD14- CD56- HLA-DR+, followed by identification of the pDC subset exclusively as CD303+CD123+. Basophils were identified as CD3- CD19- CD20- CD16- CD14- CD56- HLA-DR- CD123+. NK cells were identified as CD3- CD19- CD20- CD14- CD123- HLA-DR-. Mature and immature NK cells were distinguished based on the expression of CD16 and CD56 followed by identification of KIR-NK cells as CD57+CD158+ mature NK cells. NK cells and non-NK cells were assessed for the expression of CD122.

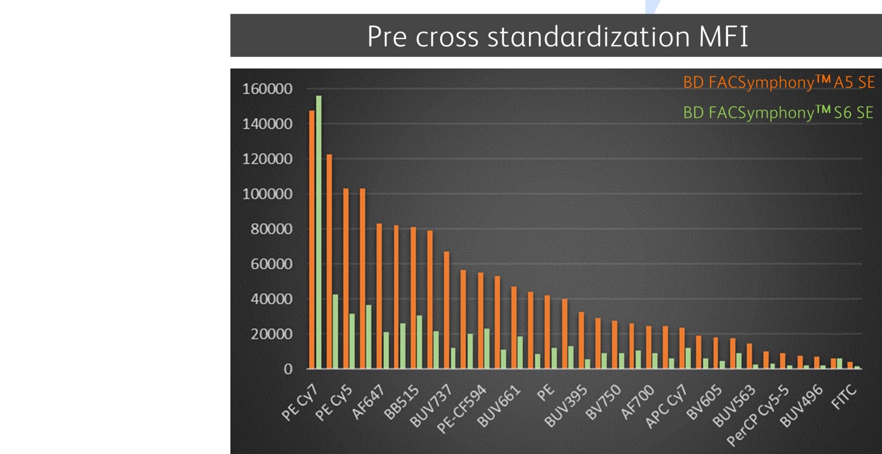
Results

3 High-dimensional UMAP analysis of multiparameter flow cytometry data for identification of donor variable cell subsets for cell sorting



4 Instrument Standardization





Post cross standardization MFI

5 Transfer of the 33 color panel from the BD FACSymphony™ A5 SE Cell Analyzer to the BD FACSymphony™ S6 SE Cell Sorter for cell isolation

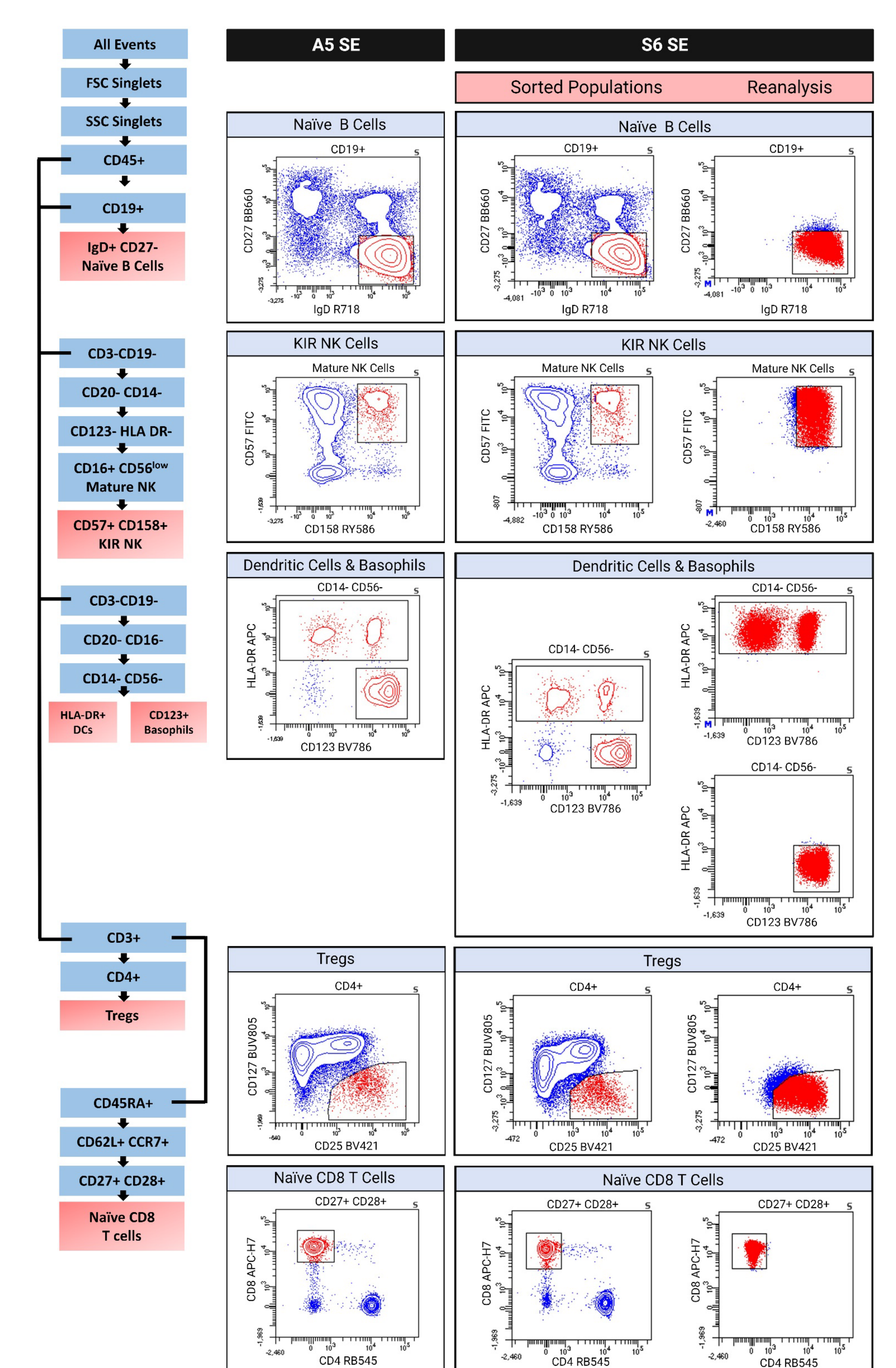


Figure 3A. Algorithmic analysis of multiparameter flow cytometry data was conducted using dimensionality reduction with UMAP and unsupervised clustering with FlowSOM* on concatenated CD45+ cells from n=3 donors. Clusters were annotated using the ClusterExplorer and Marker Enrichment Model tools and thereafter color-coded.

Figure 3B: UMAPs highlighted variations in the frequency of specific cell populations among the three donors.

Figure 4. MFI from CD4 samples recorded on the BD FACSymphony™ A5 SE Cell Analyzer and the BD FACSymphony™ S6 SE Cell Sorter prior to (left) and post (right) cross standardization.

Figure 5. Following cross-standardization, the panel was transferred to the BD FACSymphony™ S6 SE Cell Sorter. Representative data from the same sample demonstrate the comparable phenotypes of cell populations analyzed on the BD FACSymphony™ S6 SE

Cell Sorter. Reanalysis of the sorted target populations, on the BD FACSymphony™ S6 SE Cell Sorter, reveals fidelity to the sort regions as gated on the parent population.

Conclusions

- Panel Design: We described a 33-color spectral panel that provides resolution of specific T, B, NK and DC cell populations in human healthy PBMC donor samples.
- Data analysis: High-dimensional UMAP analysis of multiparameter flow cytometry data allowed identification of donor variable cell subsets.
- Spectral unmixing: Live spectral unmixing of multiparameter flow cytometry panel allowed identification of cell subsets for cell isolation.

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