

Abstract

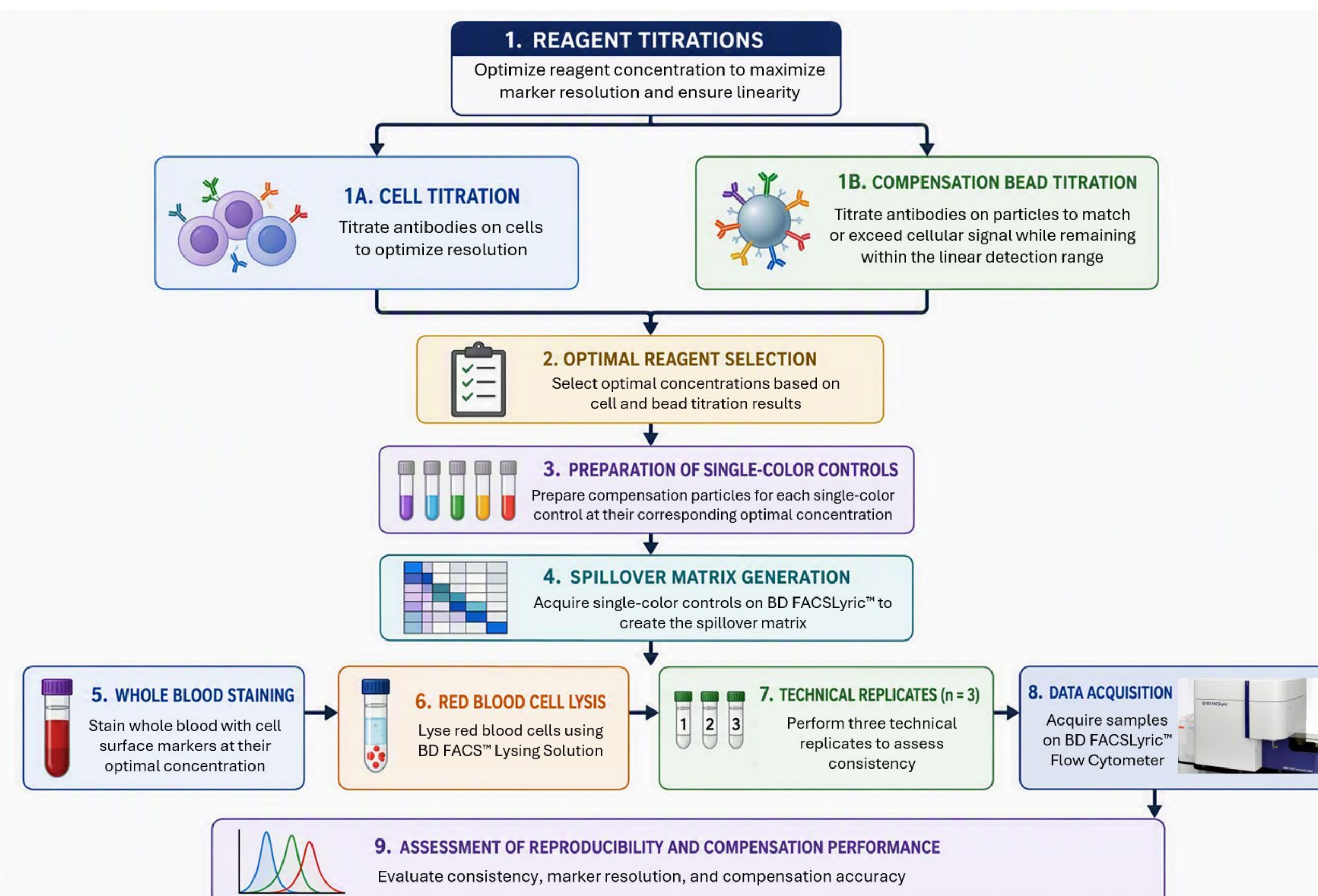
Introduction: Reproducibility is essential in flow cytometry to ensure consistent results across experiments and laboratories, yet variability in staining and compensation can influence data quality and interpretation. Common compensation approaches rely on biological samples, which can introduce variability and may not be feasible when sample availability is limited. Here, we present a robust 12-color immunophenotyping panel on the BD FACSLyric™ System using BD™ SpectraComp™ XT Unmixing and Compensation Particles in combination with BD Horizon™ Real Dyes.

Methods: Whole blood from a healthy donor was stained with cell surface markers, followed by red blood cell lysis using BD FACS™ Lysing Solution. Three technical replicates were stained and analyzed to assess consistency. Reagent titrations were performed on cells to optimize marker resolution and on compensation particles to ensure signal intensity matched or exceeded cellular signal while remaining within the linear detection range. This dual titration approach improved compensation accuracy and reduced inter-assay variability.

Results: The panel enabled identification of major immune subsets, including monocytes, B cells, NK cells, NKT cells, CD4⁺ and CD8⁺ T cells, and T cell memory subsets. Consistent population frequencies and resolution were observed across replicates, demonstrating reproducible performance. Dual titration of cells and compensation particles supported accurate compensation, robust signal separation, and reduced variability.

Conclusion: This workflow enables reproducible immune profiling from whole blood using minimal sample volume. The use of BD™ SpectraComp™ XT Unmixing and Compensation Particles in place of biological compensation samples demonstrated reproducible performance under the study conditions, while reducing donor-dependent variability and supporting consistent assay setup in sample-limited research settings.

Methods

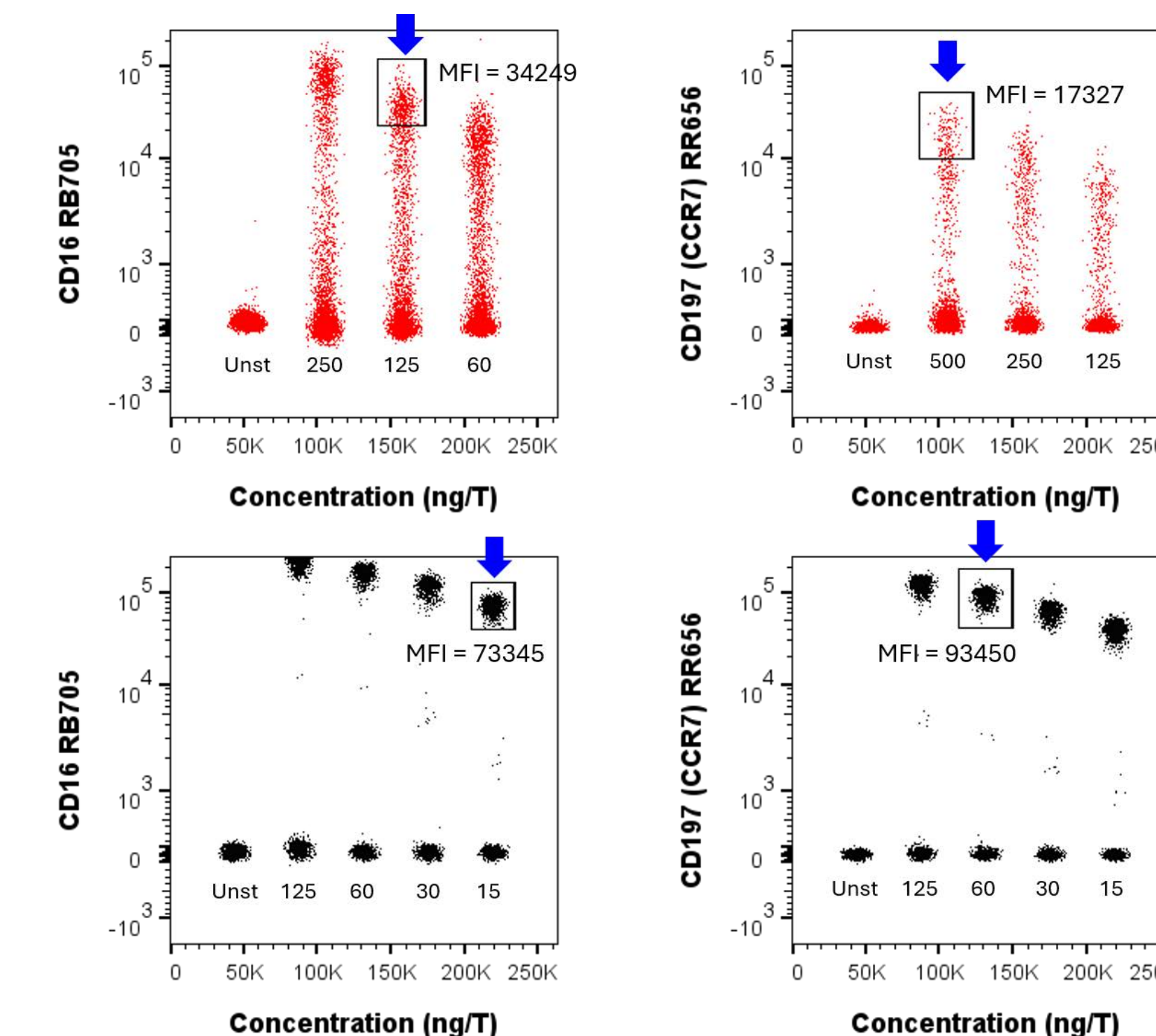


Marker	Clone	Fluorochrome	Cat #
CD45	HI30	V500	560777
CD3	UCHT1	RB502	574470
CD4	SK3	R718	566930
CD8	RPA-T8	RB575	573344
CD45RA	HI100	RB780	569081
CD197	2-L1-A	RR656	574479
CD27	M-T271	BR421	562513
CD19	HI819	APC-H7	560727
CD20	Obinutuzumab297.rmb	BV711	758007
CD56	NCAM16.2	RV828	572642
CD16	3G8	RB705	570243
CD14	6D3.rMab	BV605	752931

Overview of the workflow used for antibody titration, panel optimization, and reproducibility assessment using BD™ SpectraComp™ XT Unmixing and Compensation Particles and lysed whole blood samples on the BD FACSLyric™ platform (left).

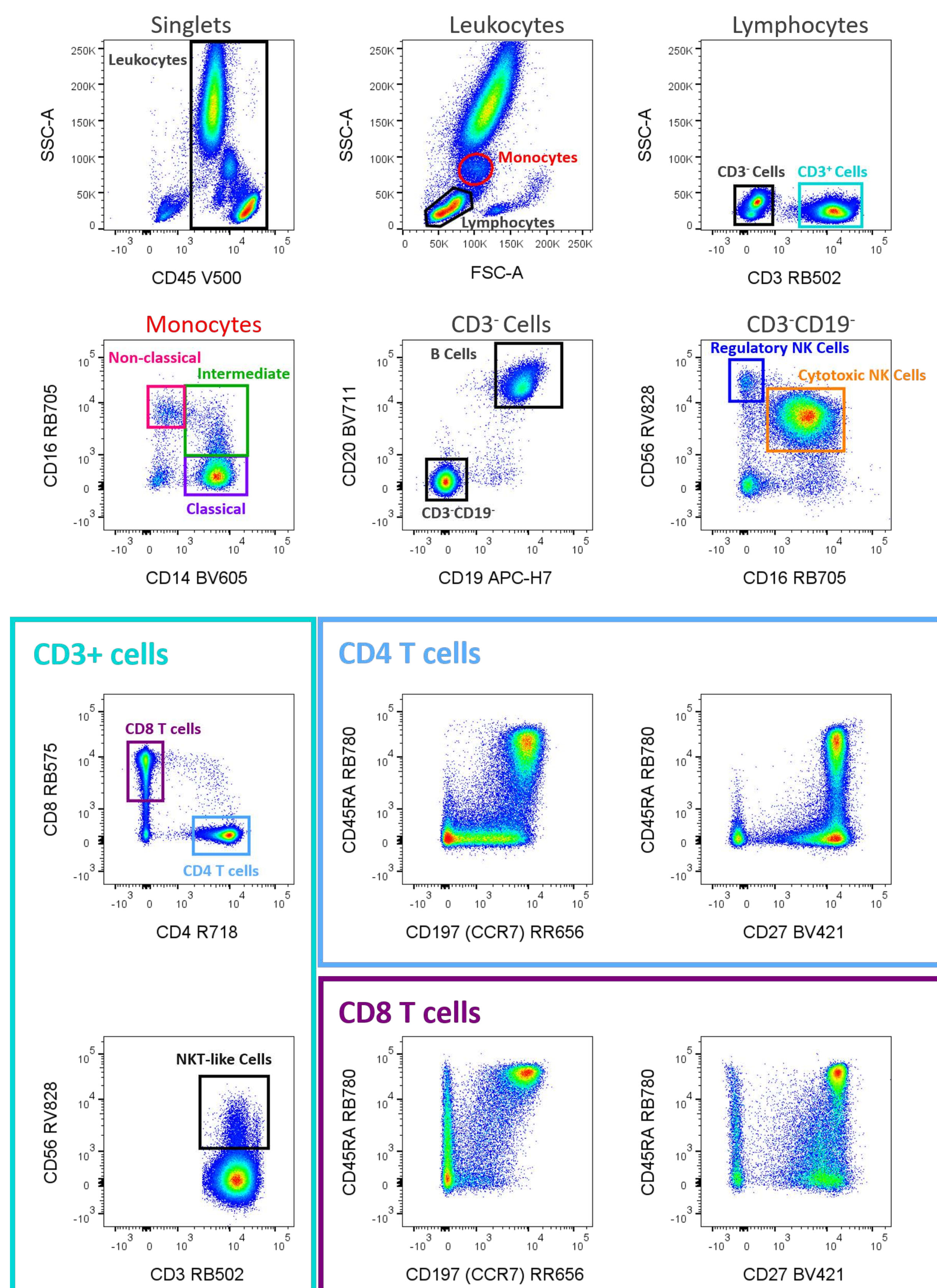
Table showing Antibody reagents used in the 12-color flow cytometry panel (right).

Results (1) Dual Titration Strategy



Representative bead and LWB titrations. Blue arrows indicate the selected optimal concentrations. For LWB (red plots), concentrations were chosen to maximize population resolution while maintaining on-scale signal. For beads (black plots), concentrations were selected to achieve signal intensity equal to or greater than that observed in cells while remaining on scale. Gates denote the populations used for MFI calculation. Unst = Unstained sample.

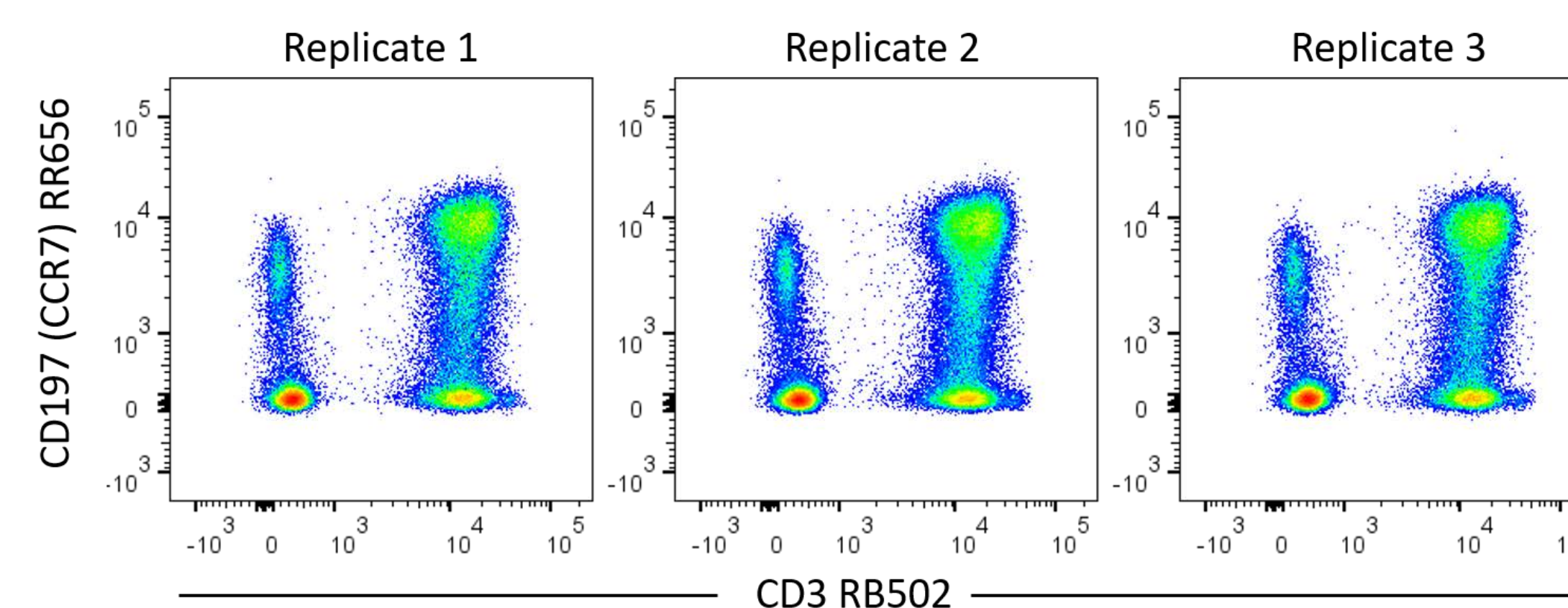
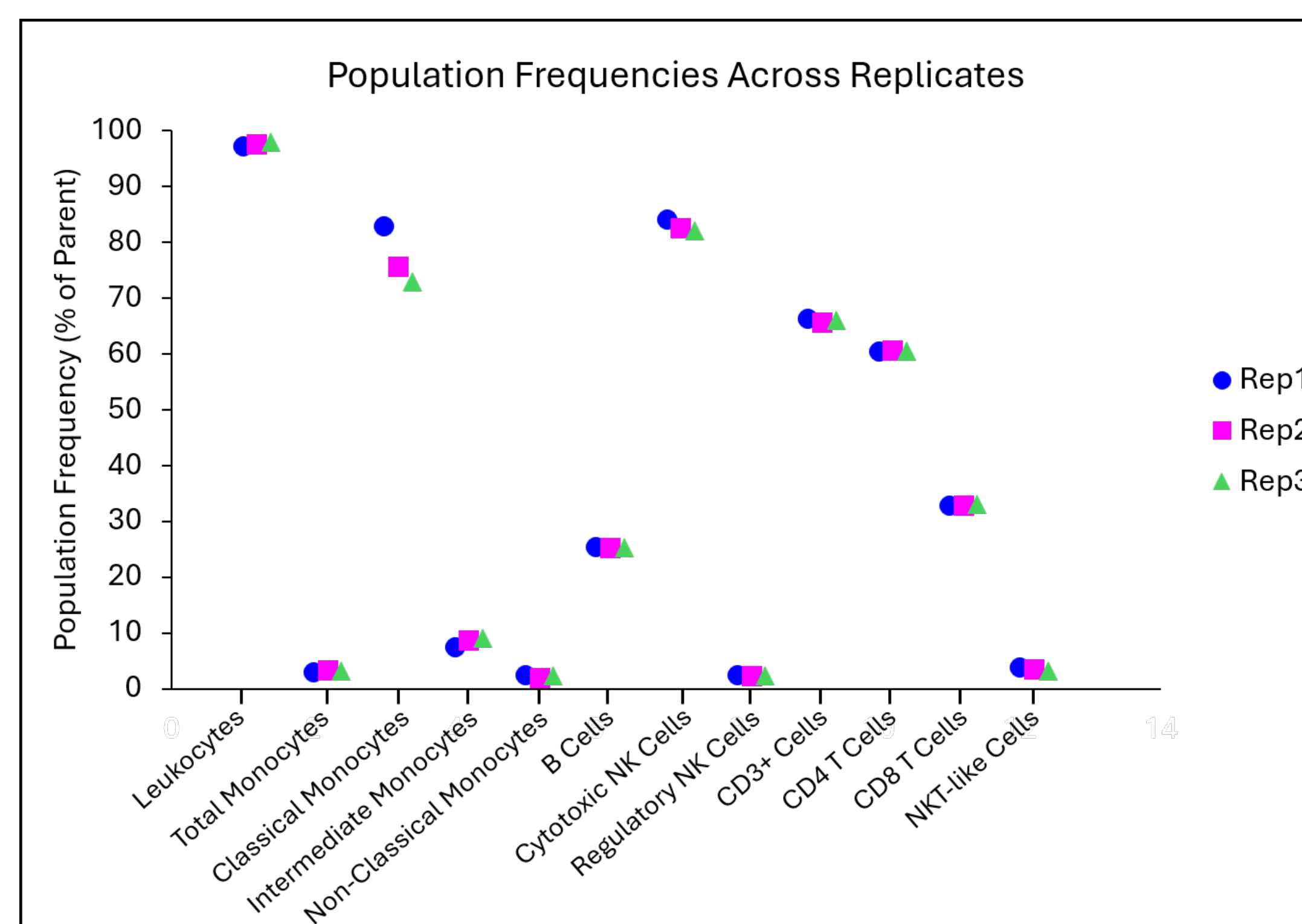
Results (3) Panel Performance



Identification of human monocyte and lymphocyte subsets. Representative analysis of human whole blood from a healthy donor. Total leukocytes were identified by CD45 expression. Monocytes and lymphocytes were separated based on light scatter properties. Monocytes were further classified into classical, intermediate, and non-classical subsets according to CD14 and CD16 expression. Within the lymphocyte gate, cells were divided into CD3⁻ and CD3⁺ populations. CD3⁻ cells were used to identify B cells (CD19⁺CD20⁺), while the CD3⁺CD19⁻ subset was used to define NK cell subsets based on CD56 and CD16 expression. Within the CD3⁺ population, NKT-like, CD4⁺, and CD8⁺ T cells were identified. Expression of CD45RA, CCR7, and CD27 was further evaluated to characterize memory subsets within CD4⁺ and CD8⁺ T cell populations. Samples were acquired on a 3-laser, 12-color BD FACSLyric™ Flow Cytometer. Data analysis was performed using FlowJo™ v10 Software.

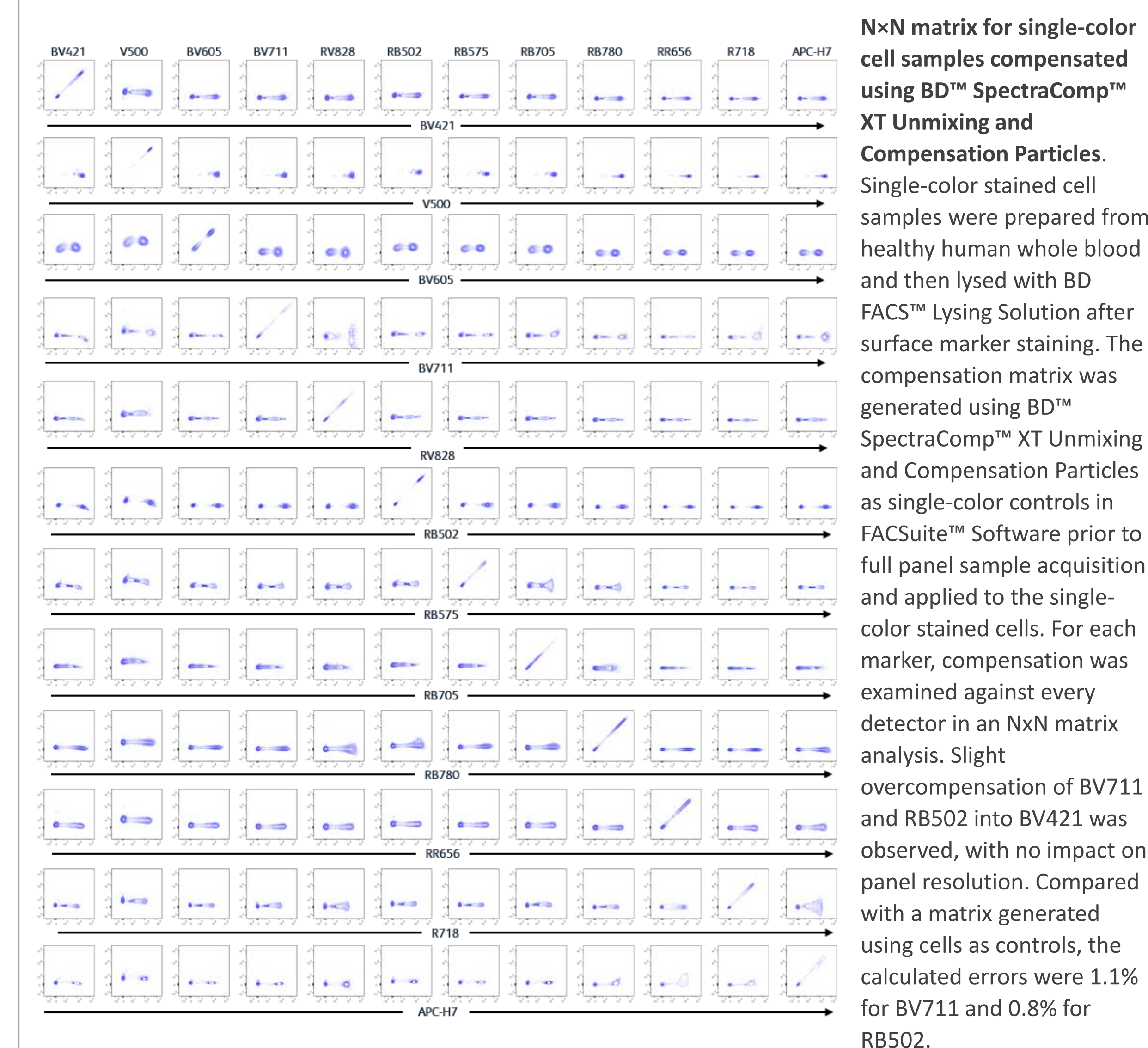
Results (2) Population Frequencies

	Rep1	Rep2	Rep3	Mean	SD	CV
Leukocytes	97.2	97.6	98.0	97.6	0.3	0.3
Monocytes	3.0	3.3	3.3	3.3	0.2	5.8
Classical	82.9	75.7	72.9	76.4	4.5	5.9
Intermediate	7.6	8.6	9.1	8.5	0.6	7.5
Non-Classical	2.6	2.0	2.4	2.6	0.7	26.5
B cells	25.4	25.2	25.2	25.3	0.1	0.4
Cytotoxic NK	84.1	82.5	82.1	83.3	1.2	1.5
Reg NK	2.4	2.4	2.3	2.4	0.0	1.9
CD3+	66.3	65.6	66.0	65.9	0.3	0.4
CD4 T cells	60.5	60.6	60.4	60.5	0.1	0.1
CD8 T cells	32.9	32.9	33.1	33.0	0.1	0.3
NKT cells	3.9	3.5	3.2	3.5	0.3	8.0



Reproducibility of population frequencies across technical replicates. Frequencies of leukocyte subsets (% of parent population) were measured in three independently stained and acquired technical replicates (Rep1-Rep3). The table summarizes replicate values, mean, standard deviation (SD), and coefficient of variation (CV) (top), while the dot plot visualizes the consistency of population frequencies across replicates (middle). Representative CD197 (CCR7) versus CD3 pseudocolor plots demonstrate nearly identical population distribution and staining intensity across replicates (bottom). Together, these results indicate low technical variability and robust reproducibility of the panel.

Results (3) Compensation Accuracy Evaluation



Conclusions

- o BD Horizon™ Real Dyes were used in a 12-color immunophenotyping panel that identified major immune cell subsets and T-cell memory populations in whole blood.
- o Consistent population frequencies and marker resolution across technical replicates demonstrated reproducible performance on the BD FACSLyric™ System.
- o Dual titration of cells and BD™ SpectraComp™ XT Unmixing and Compensation Particles supported compensation performance and signal separation under the study conditions.
- o BD™ SpectraComp™ XT Unmixing and Compensation Particles were used in place of biological compensation controls and supported compensation performance under the study conditions, reducing donor-dependent variability and preserving valuable samples.
- o Together, BD Horizon™ Real Dyes and BD™ SpectraComp™ XT Unmixing and Compensation Particles supported a reproducible workflow for immune profiling in sample-limited research settings.