

Introduction

- Agilent has developed the NovoCyte Opteon spectral flow cytometer, which consists of up to five lasers (349nm, 405nm, 488nm, 561nm, and 637nm) and 73 detectors (70 fluorescence detection channels, FSC, BSSC, and VSSC).
- Taking an OMIP-069¹¹ panel as a backbone, a 45-color immunophenotyping panel was designed and optimized for the Agilent NovoCyte Opteon spectral flow cytometer.
- Five additional biomarkers were added for broader research applications (Table 1). These biomarkers are CD31, CD45RO, CD69, CD33 and LAG-3. The addition of CD31 and CD45RO enables the identification of recent thymic emigrants (RTEs) within the CD4+ T cells. CD69 is an early activation marker that is upregulated on T cells upon stimulation. LAG-3 enables the analysis of exhausted T cells together with PD-1. CD33 allows the phenotyping of DCs and monocytes.
- Fluorochromes for four markers in OMIP-069 were replaced (Table 1) for optimized results.

Experimental

Information of sample preparation and data analysis

Samples were prepared by sequential staining using PBMCs. Data were analyzed using NovoExpress (Opteon) software (v2.0.0) for 2D plots, and FlowJo software (v10.10.0) for high dimensional analysis (UMAP v4.0.4, FlowSOM v4.0.0).

Marker	Fluorochromes	Marker	Fluorochromes	Marker	Fluorochromes
CD45RA	BUV395	CD141	BB515	CD159a	APC
CD45RO	SBUV445	CD57	FITC	CD1c	AF647
CD16	BUV496	CD14	Spark Blue 550	CD19	SNIR685
CCR5	BUV563	CD33	SBB580	CD127	SR718
CD314	BUV615	CD223	NFB660/120s	Viability	Zombie NIR
CD39	BUV661	CD45	PerCP	CD27	APC-H7
CD56	BUV737	CD2	PerCP-Cy5.5	CD38	APC-Fire 810
CD8	BUV805	TCRγδ	PerCP-eF710		
CCR7	BV421	CD69	SBB765		
CD123	SB436	CD31	SBB810		
CD11c	eF450	CD159c	PE		
IgD	BV480	CD20	Spark YG 593		
CD3	BV510	CD337	PE-Dazzle 594		
IgM	BV570	CD4	CF594		
IgG	BV605	CD24	PE-AF610		
CD28	BV650	CD95	PE-Cy5		
CCR6	BV711	CD25	PE-AF700		
CXCR5	BV750	CXCR3	PE-Cy7		
PD-1	BV785	HLA-DR	PE-Fire 810		

Table1. Antibodies used for 45-color panel.

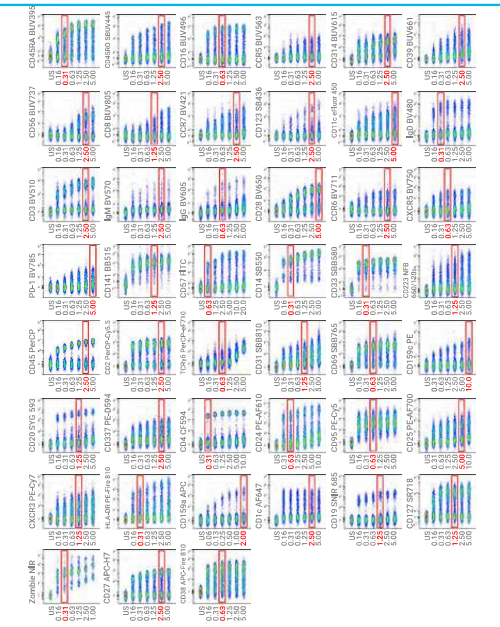


Figure 1. Antibody titrations for the 45-color panel. All antibodies were titrated over six concentrations with a starting concentration ranging from 1 to 20 μL per 100 μL staining volume. The optimal titer was chosen based on the Stain Index values (i.e. choosing a higher Stain Index, while considering a lower titer to minimize the spreading error) and is indicated by the red box in the plot.

Experimental

Spectral signatures and Similarity Index Matrix

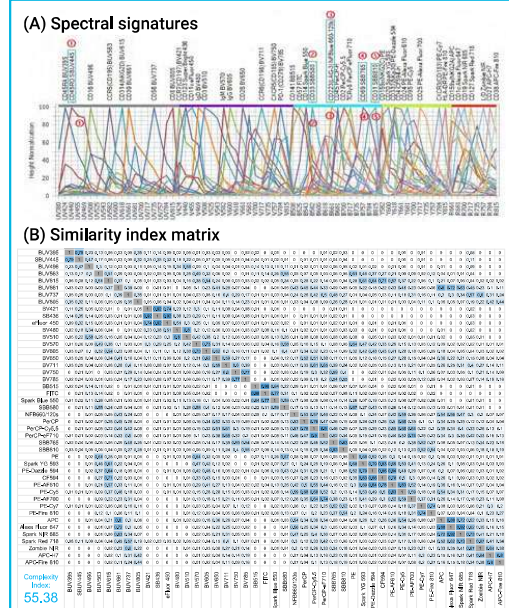


Figure 2. Spectral signatures and Similarity Index Matrix for the 45-color panel on the NovoCyte Opteon™ flow cytometer. (A) Spectral signatures. The five added dyes were filled in spectral gaps between the current repertoire of fluorochromes. (B) Similarity Index Matrix. The Complexity Index is 55.38, just 1.66 higher than 53.72 according to published data¹¹.

Results and Discussions

Gating strategy

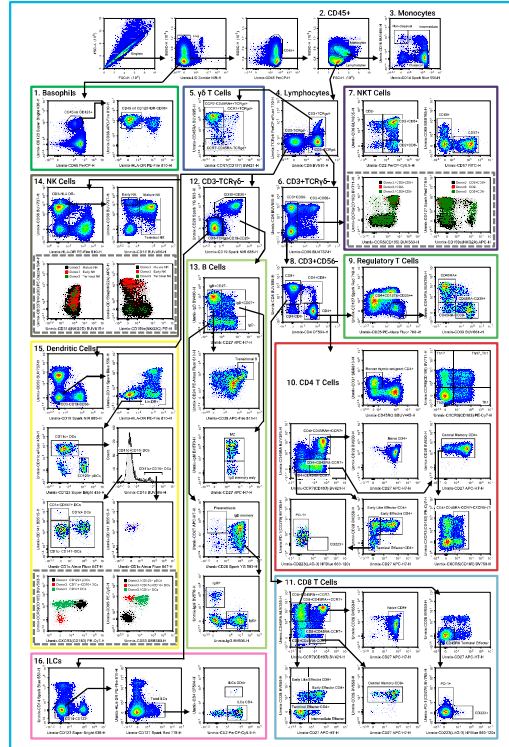


Figure 3. Gating strategy to identify the main immune cell subsets. All leukocyte subsets can be clearly distinguished by manual gating, including (1) Basophils, (2) CD45+ cells, (3) Monocytes, (4) γδ T cells, (5) NKT cells, (6) Tregs, (7) CD4 T cells, (8) CD8 T cells, (9) B cells, (10) NK cells, (11) Dendritic cells, (12) Lymphoid cells (ILCs). All data presented here are derived from frozen PBMCs of one healthy donor.

Results and Discussions

High dimensional analysis

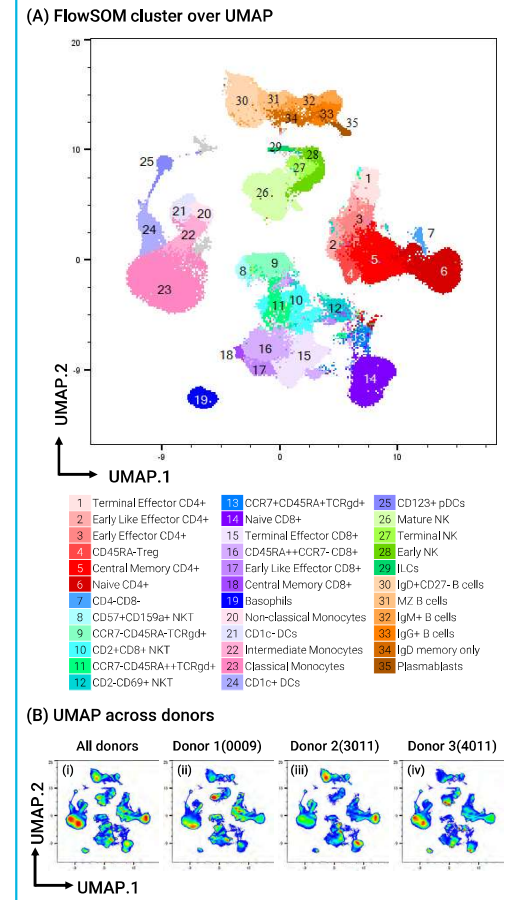


Figure 4. High dimensional analysis. After platelets, debris, doublets and dead cells were excluded, the cells in the live CD45+ gate from each donor are reduced to 500,000 events and concatenated into one file, then UMAP and FlowSOM are performed separately based on all markers except CD45 and live/dead dye. (A) UMAP with FlowSOM cluster overlay. (B) UMAP results across donors: (i) All donors, (ii) Donor 1 (0009), (iii) Donor 2 (3011), and (iv) Donor 3 (4011).

Conclusions

- We successfully added more dyes to the original OMIP-069 panel to be able to detect more biomarkers.
- All immune subsets could still be accurately and clearly distinguished without affecting the dimensionality reduction and clustering result of the original markers even though more highly overlapping fluorochromes were included in this 45-color panel.
- The results demonstrate the performance and high accuracy of the Agilent NovoCyte Opteon as a spectral flow cytometer.
- Researchers have more choices and can easily generate highly complex flow cytometry panels up to 45 colors in a single tube to address their biological questions.

References

[1] Park LM, Lannigan J, Jaimes MC. OMIP-069: 30-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood. Cytometry A 2020;97:1044–1051.

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