NovoCyte™ Flow Cytometer

Application Note No. 7

ACEA Biosciences, Inc.

Immunophenotypic analysis of human blood leukocyte subsets using a 13-color antibody panel on the NovoCyte System

Identification and analysis of specific sub-populations of cells is one of the many essential uses of flow cytometry, especially in terms of its ability to multiplex and identify several markers in one sample. As such, immunophenotyping of blood samples is one of the more popular uses of multi-color flow cytometry. In certain applications, using multiple markers simultaneously for immunophenotyping is more powerful and efficient than using multiple samples with fewer markers. However, to accomplish this task, what is needed is a powerful flow cytometer that has the ability to both detect and analyze the plethora of colors that is utilized in such an experiment. Here we demonstrate immunophenotypic analysis of a human blood sample utilizing 13 fluorescent markers on the NovoCyte 3000, a compact benchtop flow cytometer equipped with 3 lasers. It is critical in such an involved experiment to optimize the antibody concentration and to include the proper controls. In this staining, subsets of T cells, B cells, and other leukocytes were identified and analyzed using the NovoExpress acquisition and analysis software. The data clearly demonstrate that using the NovoCyte benchtop flow cytometer allows for performance of high-quality, complex flow cytometry with a 13-color antibody panel.
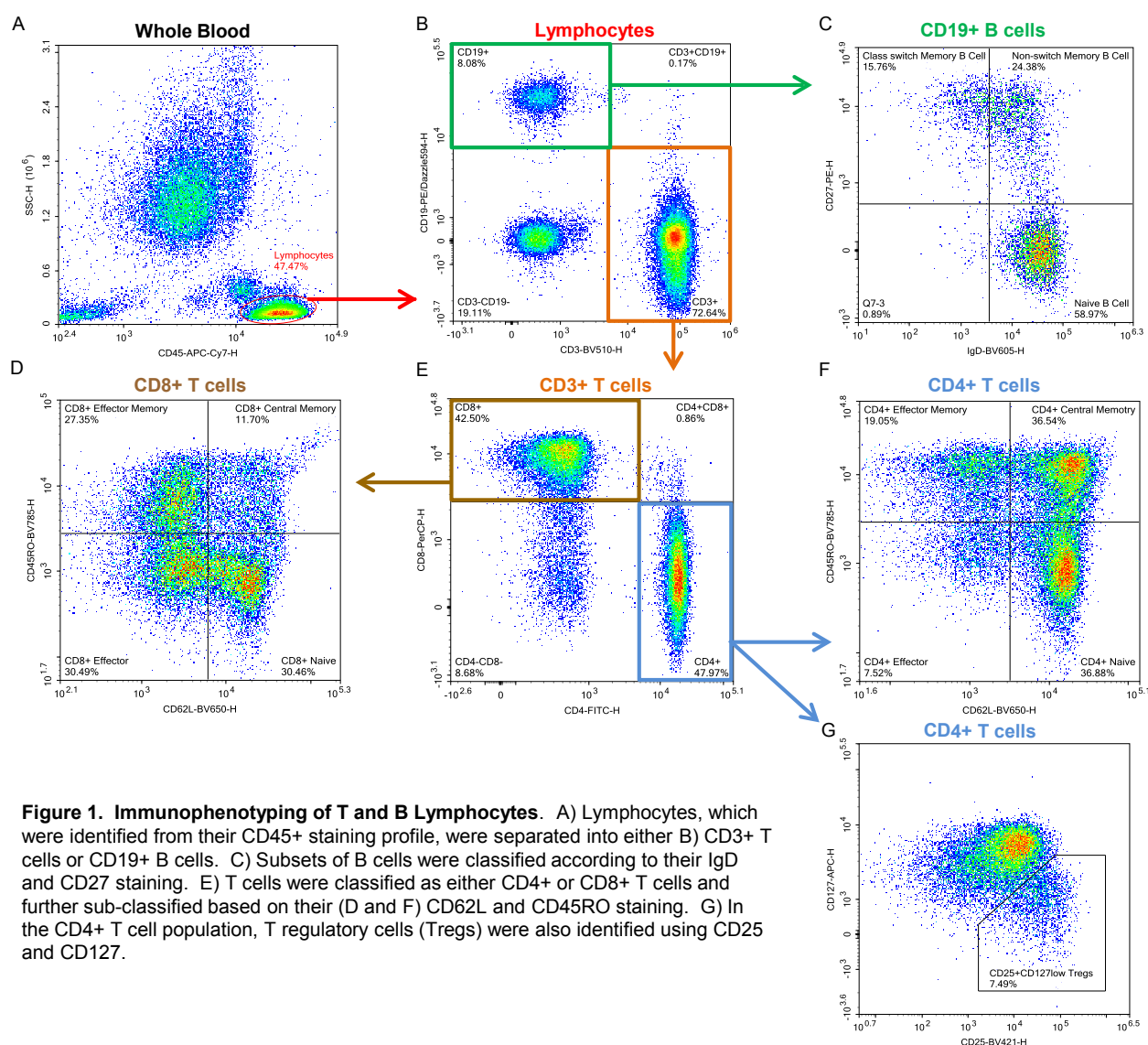

Figure 1. Immunophenotyping of T and B Lymphocytes. A) Lymphocytes, which were identified from their CD45+ staining profile, were separated into either B) CD3+ T cells or CD19+ B cells. C) Subsets of B cells were classified according to their IgD and CD27 staining. E) T cells were classified as either CD4+ or CD8+ T cells and further sub-classified based on their (D and F) CD62L and CD45RO staining. G) In the CD4+ T cell population, T regulatory cells (Tregs) were also identified using CD25 and CD127.

A

CD45+ Leukocytes excluding T & B cells
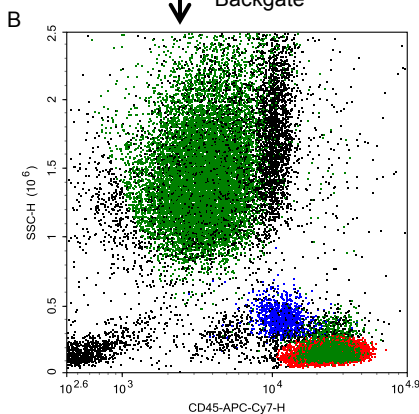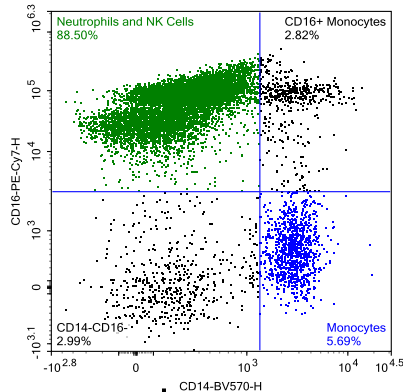

Backgate

B

Figure 2. Immunophenotyping lymphocyte and non-lymphocyte subsets. A) Neutrophils and NK cells were detected by analyzing the leukocytes (CD45+) excluding T and B lymphocytes for CD16+/CD14- staining. Monocytes were detected analyzing the same population, but identified by CD14+ only stain or CD14+/CD16+ double positive staining. B) Selected populations from A were backgated to CD45 vs SSC.

13-color Antibody Panel

Laser	FL channel on NovoCyte	Format	Specificity
405nm	VL1	BV421	CD25
	VL2	BV510	CD3
	VL3	BV570	CD14
	VL4	BV605	IgD
	VL5	BV650	CD62L
	VL6	BV785	CD45RO
488nm	BL1	FITC	CD4
	BL2	PE	CD27
	BL3	PE/Dazzle 594	CD19
	BL4	Per CP	CD8
	BL5	PE-Cy7	CD16
640nm	RL1	APC	CD127
	RL2	APC-Cy7	CD45

Methods

Whole, human blood was stained with the antibody cocktail detailed above for 30 minutes at room temperature in the dark. To remove red blood cells, 2mL of 1X RBC lysis buffer (ACEA Biosciences) was added to blood samples with gentle vortexing and incubated for 15 minutes. Samples were then washed, fixed in 1% paraformaldehyde, and run on the NovoCyte flow cytometer. Color compensation was calculated automatically using the auto compensation feature after acquiring single color compensation bead samples. Fluorescence minus one (FMO) controls were used to ensure accurate gating.

Reference

Maecker TH, McCoy JP, and Nussenblatt R. Nature Immunol. 2012 Feb 17; 12(3): 191–200
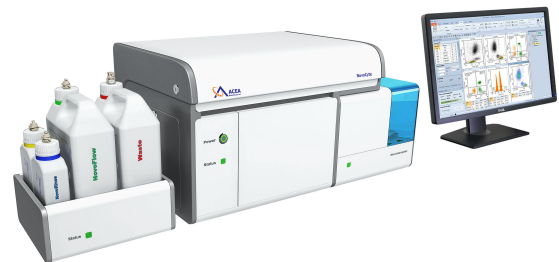
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