27-Marker High-Sensitivity Experiments on a 3-Laser CytoFLEX

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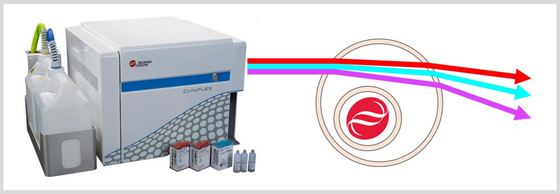
Introduction

With the increasing complexity of scientific investigations, occasionally the numbers of biomarkers desired for a flow cytometry panel may press the limits of a flow cytometer. Or, one may simply desire to segregate as many immunophenotyping markers as possible to specific channels in order to leave other channels open for important antibodies of interest, such as cell signaling and activation markers. While a small number of channels, and thus detectors, on a flow cytometer can be a limitation, a large number does not necessarily confer a great advantage: the maximum number of biomarkers that can be analyzed by a flow cytometer is not actually limited to the number of channels.

In this poster, we demonstrate how advanced users of flow cytometry may overcome the limitation of channels on a flow cytometer. The number of markers that can be used with a conventional flow cytometer can be increased by simply stacking biomarkers that do not co-label the populations of interest. When the markers are not on the same cell, no compensation or deconvolution is required to parse the signals between multiple markers within each channel; the parent populations simply need to be gated in other channels.

Materials and Methods

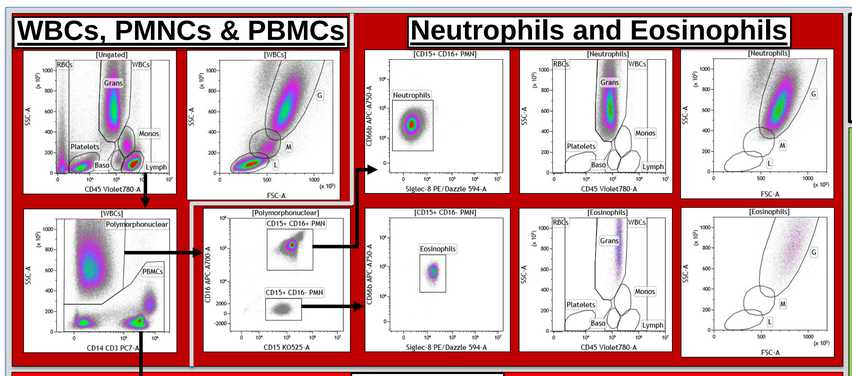
Item	Catalog #	Vendor
VersaLyse	A09777	Beckman Coulter
VersaComp	B22804	Beckman Coulter
3-Laser CytoFLEX V-B-R	B53000	Beckman Coulter
Kaluza Software v1.5a	A82959	Beckman Coulter

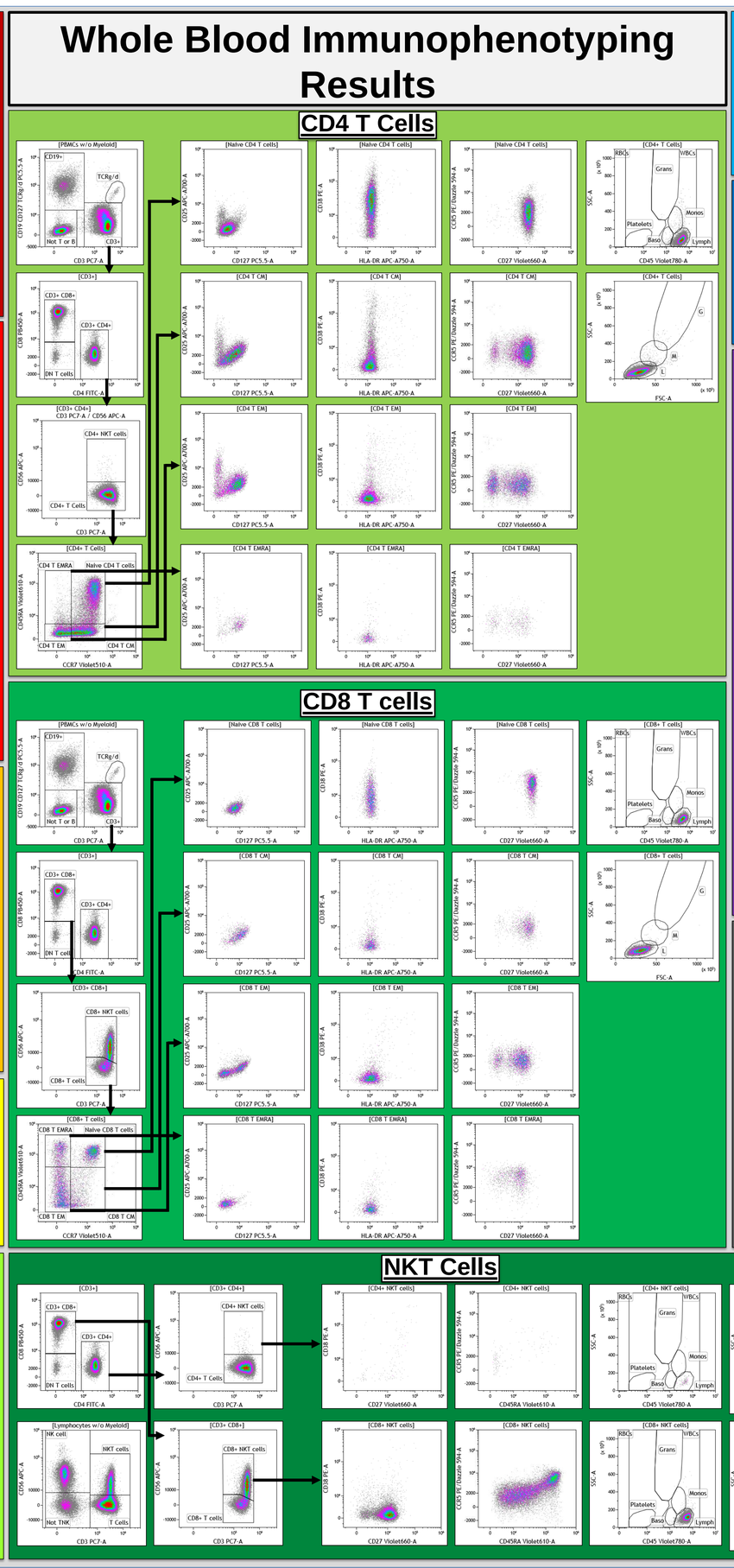


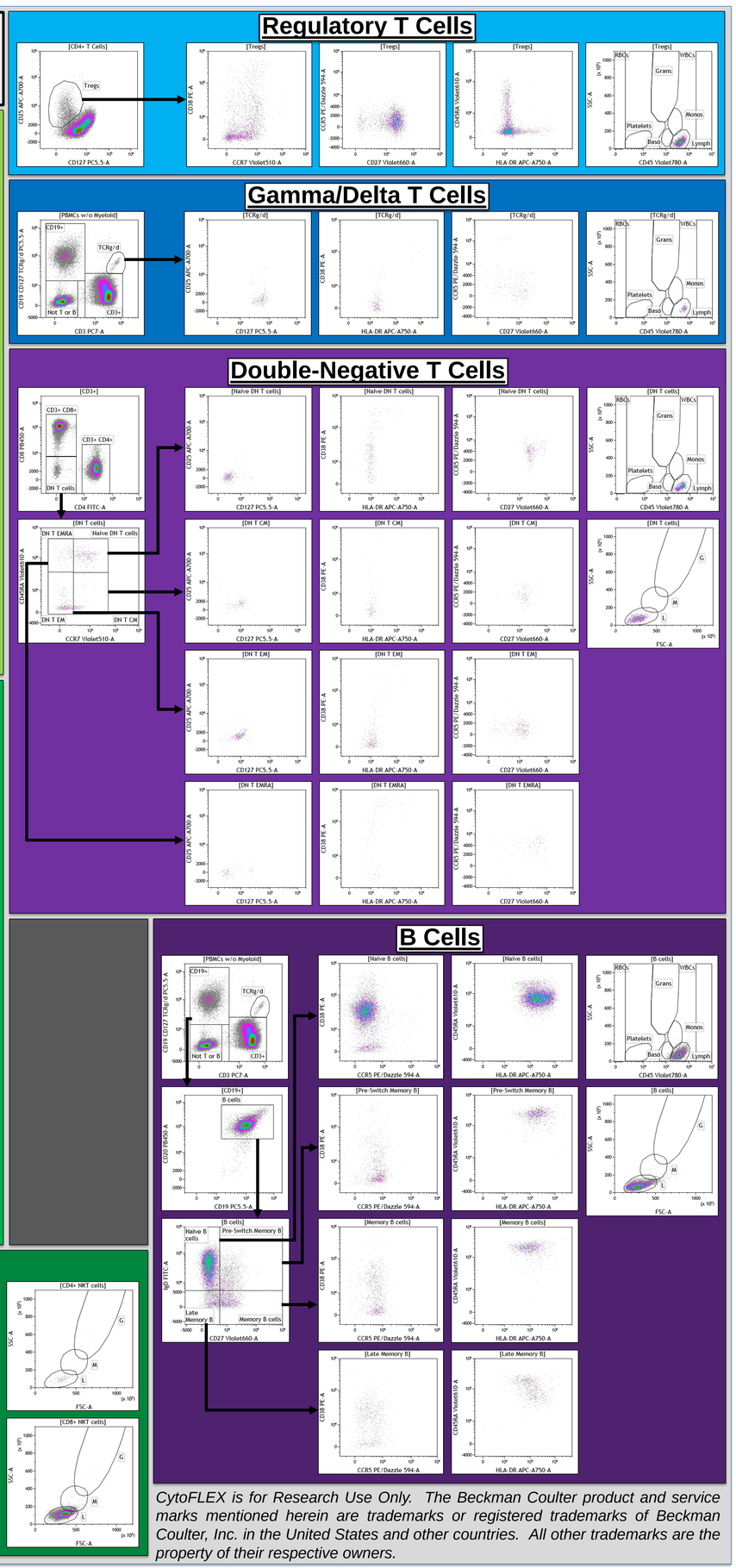
Procedure

1) 100µL of fresh human blood was incubated with a mixture of the antibodies in the table below for 30 min at RT.
2) The sample was lysed with 1mL of VersaFix (i.e., VersaLyse + 0.2% PFA) for 20 min at RT.
3) The sample was washed twice with PBS, and 500K events were acquired on a 3-Laser CytoFLEX.
4) The 13-color compensation panel was setup using VersaComp beads.
5) Following acquisition, the data were analyzed using Kaluza software.

Antibody	CD4	IgD	CD33	CD38	CCR5	Siglec-8	CD64	CD19	CD127	TCRg/d	CD3	CD14	CD56	CD16	CD25	HLA-DR	CD66b	CD8	CD20	CD11c	CD15	CCR7	CD45RA	CD203c	CD27	CD123	CD45
Company	Beckman	Beckman	Beckman	Beckman	Biolegend	Biolegend	Beckman	Beckman	Biolegend	Beckman	Beckman	Beckman	Beckman	Beckman	Beckman	Beckman	Beckman	Beckman	Beckman	BioLegend	Beckman	Biolegend	Biolegend	Biolegend	Biolegend	Biolegend	Biolegend
Catalog #	IM0448U	B30652	IM1135U	IM2371U	359126	347109	A98434	A66328	351322	A99021	6607100	A22331	IM2474U	B20023	A86356	B42021	B08756	A82791	A74777	337226	B01176	353231	304134	324620	302828	306020	304048
Fluorophore	FITC	FITC	FITC	PE	PE/Dazzle 594	PE/Dazzle 594	ECD	PC5.5	PC5.5	PC5.5	PC7	PC7	APC	APC-AF700	APC-AF700	APC-AF750	APC-AF750	Pacific Blue	Pacific Blue	BV421	Krome Orange	BV510	BV605	BV605	BV660	BV660	BV785
B cells																											
NK cells																											
NKT cells																											
CD4 T cells																											
CD8 T cells																											
DN T cells																											
TCRg/d T cells																											
Monocytes																											
Dendritic Cells																											
Basophils																											
Neutrophils																											
Eosinophils																											







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Discussion

Ultimately, it takes a little work to map out the biomarker expressions when stacking antibodies, but such a method increases the potential size of panels that can be acquired by conventional flow cytometry. In addition, this method can be used on a smaller scale to increase the number of immunophenotyping markers that can be segregated into specific channels, leaving other necessary channels open for cell signaling and/or activation markers, which are often only available in a limited selection of fluorophore conjugates.

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