Flexible Multicolor Panel Design using the MA900 Cell Sorter

The MA900 from Sony meets the demands of most sorting applications, supporting up to 4 lasers, 12 fluorescence parameters, and 4-way sorting. Powerful, modern technologies built into the MA900 system dramatically simplify operation to make sorting less subjective and improve reliability. The optical design of the MA900 features a free-form PMT layout which expands fluorochrome choices without the need to reconfigure the system for each application (Table 1).

In this application note, we highlight the use of the MA900 cell sorter configured with 2 lasers (488 nm and 405 nm) for analysis and sorting of a 10-color immunophenotyping panel. Human peripheral blood mononuclear cells (PBMCs) were stained with reagents for analysis of common T-cell, B-cell, NK-cell, and monocyte subsets. These subsets were identified using different gating strategies, and sort performance was evaluated by performing 4-way sorting of CD4+ T cells, CD8+ T cells, NK cells, and B cells, followed by post-sort analysis.

Sorter A (3 laser)		MA900 (2 laser)	
488 nm	Alexa Fluor® 488	488 nm	Alexa Fluor® 488
	PE		PE
	PerCP-Cy5.5		PerCP-Cy5.5
	PE-Texas Red®		PE/Dazzle
	PE-Cy7	405 nm	BV421
405 nm	Horizon V450		BV510
	Horizon V450		BV605
638 nm	APC		BV650
	Alexa Fluor 700		BV711
	APC-Cy7		BV750

Table 1. Transferring a 10-color panel from 3-laser Sorter A to the 2-laser MA900

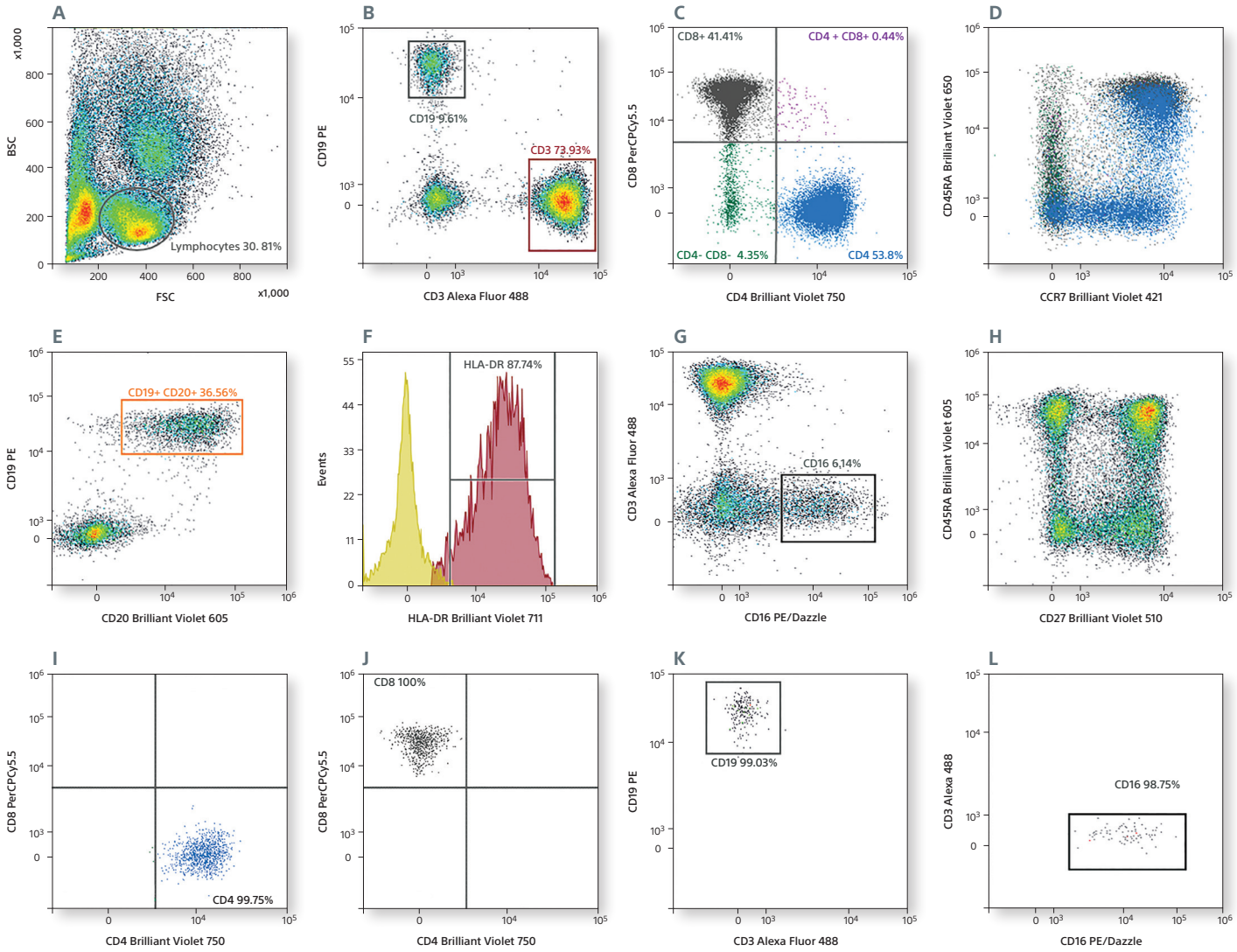
Methods

Whole blood was lysed with RBC Lysis Buffer and stained with antibodies against Alexa Fluor® 488 CD3, PE CD19, PerCP-Cy™5.5 CD8, PE/Dazzle™ CD16, Brilliant Violet 421™ CCR7, Brilliant Violet 510™ CD27, Brilliant Violet 605™ CD20, Brilliant Violet 650™ CD45RA, Brilliant Violet 711™ HLA-DR, and Brilliant Violet 750™ CD4 (Table 2). All reagents were from Sony Biotechnology Inc. Cells were incubated with the antibodies for 20 minutes on ice, washed twice with staining buffer, and resuspended in phosphate buffered saline (PBS) and kept on ice until further analysis.

The MA900 cell sorter was set up with a 100-µm microfluidics sorting chip using the Autocalibration feature on the system. Fluorescence compensation was done with single stained control tubes and the Cell Sorter Software V3.0 to generate the spillover matrix. Using the gating strategy described in the following section, the multicolor tube was analyzed to identify the subpopulations for sorting.

Laser	Filter	Fluorochrome	Antibody
488 nm	525/50	Alexa Fluor® 488	Mouse anti-CD3
	585/30	PE	Mouse anti-CD19
	617/30	PE/Dazzle	Mouse anti-CD16
	695/50	PerCP-Cy5.5	Mouse anti-CD8
	785/60	PE-Cy7	Not used
405 nm	450/50	BV421	Mouse anti-CCR7
	525/50	BV510	Mouse anti-CD27
	585/30	BV570	Not used
	617/30	BV605	Mouse anti-CD20
	665/30	BV650	Mouse anti-CD45RA
	720/60	BV711	Mouse anti-HLA-DR
	785/60	BV750	Mouse anti-CD4

Table 2. MA900 instrument configuration



Data

Stained cells were analyzed on the MA900. Lymphocytes were gated using the scatter gate (A). The CD3+ population (B) was used for gating CD4+ and CD8+ cells (C). CCR7+CD45RA+ lymphocytes are shown in (D). CD19+CD20+ B cells were gated from lymphocytes (E), and the HLADR expression of B cells was analyzed (F). CD16+ NK cells (G) were gated from lymphocytes. CD27 expression of CD45RA+ subsets of lymphocytes was analyzed (H). Using the 4-way sort mode on the MA900 cell sorter, CD4+ and CD8+ T cells, CD19+ B cells, and CD16+ NK cellswere sorted. Post-sort analysis of each sorted population is shown (I–L).

Subset	Pre Sort: % Parent	Post Sort: % Parent
CD4+ T cells	40.67%	99.75%
CD8+ T cells	53.25%	100%
CD19+ B cells	9.82%	99.03%
CD16+ NK cells	3.05%	98.75%

Table 3. Sort performance

Summary

The MA900 cell sorter model (LE-MA900CP) equipped with 2 lasers, 488 nm and 405 nm, analyzed a 10-color immunophenotyping panel. Using hierarchical and boolean gating strategies, 11 distinct subsets of T, B, and NK populations were identified. Of these subsets, four target populations were purified by sorting. Reanalysis of the sorted populations showed that they were isolated at purities higher than 98% and at an efficiency of >80%.

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