

Quantitative Immunophenotyping on the FlowSight

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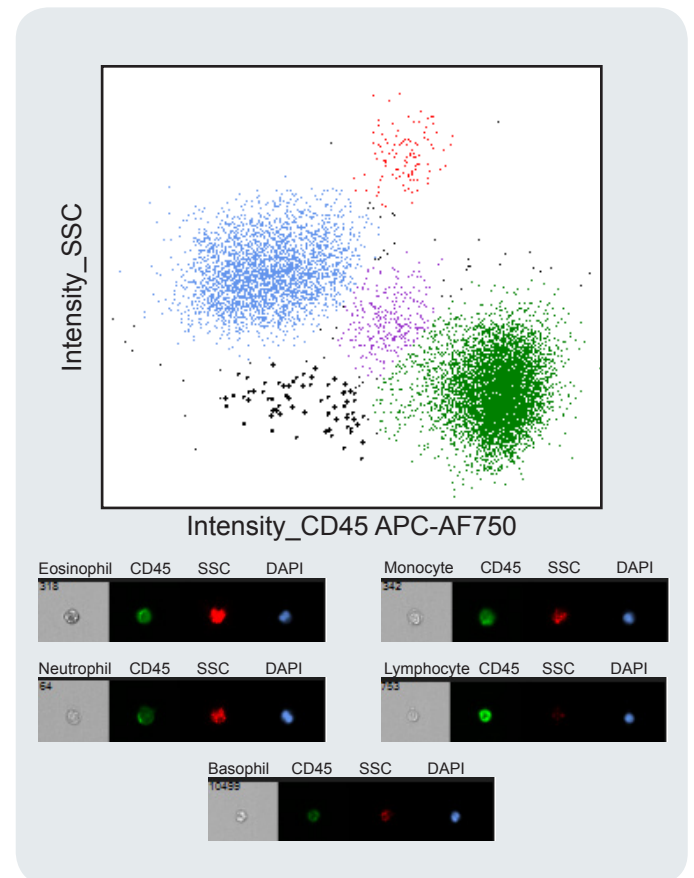
Abstract

Multiparametric classification of a heterogeneous mixture of cells is a cornerstone of blood analysis and requires cytometers with high photonic sensitivity and multiple detection parameters to suitably classify subsets of cells. For example, to discriminate leukocyte subsets whole blood or PBMC samples can be stained with CD45, CD3 can be used to identify T-cells, CD4 for helper T cells, CD19 for B lymphocytes and CD14 for monocytes. Additional markers can be used to define other subsets and also to distinguish phases of activation and differentiation. With imaging flow cytometry, remaining detection parameters can also be used for functional assays, including the assessment of intracellular signaling events, phagocytosis of foreign particles, apoptosis or autophagy. The FlowSight is a 12 channel imaging system that allows for the combination of multiparametric immunophenotyping with a variety of functional assays. The system generates a 20x image of every cell in the sample, allowing for visual verification of not only the health and type of the cell, but of the location of intracellular probes as well.

Figure 1.

Blood Classification using CD45 and SSC

A five part differential of blood cells can be achieved on the basis of CD45 surface staining and laser side scattering characteristics. In this example whole blood was harvested into a heparinized vacutainer and stained with APC-AF750 anti-CD45. After lysing RBC, the sample was fixed, permeabilized and counterstained with DAPI to label the nuclei. Data was collected on the FlowSight using 405nm and 642nm laser excitation, plus 785 nm scatter illumination, allowing discrimination of five whole blood leukocyte populations on the basis of CD45 and SSC intensity, including: lymphocytes (green), monocytes (purple), neutrophils (blue), eosinophils (red), and basophils (black). The images below the plot show the brightfield CD45, SSC and DAPI image of a single cell from each of the five major populations.



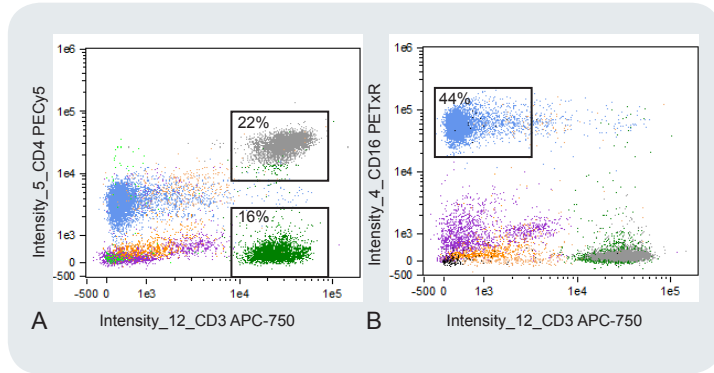
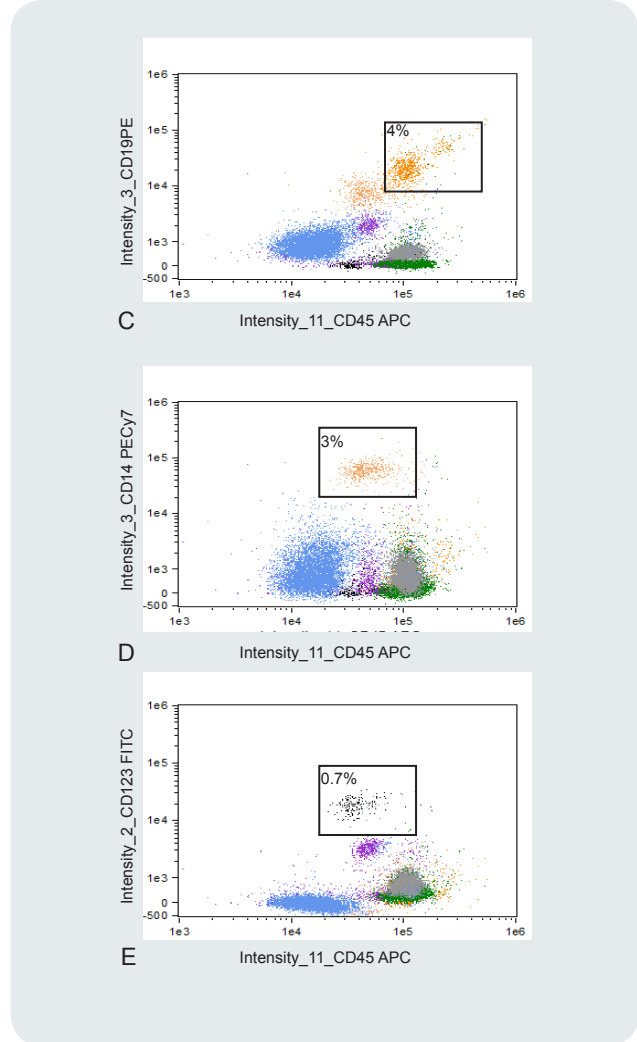


Figure 2.
8 Color Immunophenotyping

Immunophenotyping is commonly used to identify blood cell subpopulations. Up to 10 colors of fluorescence can be used on the FlowSight and in this example we used CD45, CD14, CD16, CD19, CD3, CD4, and CD123 as well as DAPI to look at nuclear morphology. Using a serial gating scheme, we identified several subpopulations, including: (A) CD3+ T cells, CD4+ helper T cells; (B) CD16+ granulocytes; (C) CD19+ B cells; (D) CD14+ monocytes; (E) CD123+ pDC/basophils.

Conclusions

The high fluorescence sensitivity of the FlowSight, along with its dedicated scatter laser, facilitate the classification of whole blood lysates using CD45 and SSC measurements. The system accommodates up to 10 fluorescent dyes for detailed immunophenotyping in conjunction with functional assays.



FlowSight Specifications

Physical Characteristics

17.7 W x 18.3 H x 14.7 D inches (450mm x 465mm x 635mm)

135 lbs. (61kg)

Instrument Capabilities

Images per cell	Up to 12
Event rate	2,000 cells per second
Image modes	Brightfield, SSC, Fluorescence
Automation	Startup, Calibration, Shutdown

Excitation Sources

LASER (NM)	EXAMPLE DYES
405	DAPI, Pacific Blue, eFluor 450, +
488	FITC, PE, PE-TxRed, ECD, PerCP +
561	PE, AlexaFluor 546, Cy3, +
642	AlexaFluor 647, APC, APC-Cy7 +

