

Discrimination of spectrally overlapping fluorophores using Sony Corporation's (SP6800) Spectral Analyzer

Abstract

A maximum of seven colors can be separated using traditional 2-laser flow cytometers, and it is required to have the necessary band pass filters to separate these fluorochromes. In addition, these systems cannot differentiate between spectrally overlapping fluorophores such as PerCP and PerCP/Cy5.5; or FITC, PE and Alexa Fluor 514° (A514). SP6800, a novel spectral analyzer from Sony Corporation, uses an innovative optics system that collects all emitted light and eliminates the need for band pass filters. We performed whole blood staining using a panel of 7-10 markers, which included significantly overlapping fluorochromes PerCP and PerCP/Cy5.5, and A514, PE and FITC and analyzed it on the SP6800. We were able to clearly identify CD56⁺ (PerCP/Cy5.5), CD19⁺ (PerCP), and CD3⁺ (A514) cells. Further comparison of a 7-color panel consisting of CD3 FITC, CD56 PE, CD19 PerCP, CD8 PE/Cy7, CD16 APC, CD4 Alexa Fluor 700° and CD62L APC/Cy7 on the BD LSRFortessa™ system and SP6800 gave similar results. Our data show that the SP6800 can perform all the analysis similar to a traditional cytometer with the added feature of separating spectrally overlapping fluorophores.

Introduction

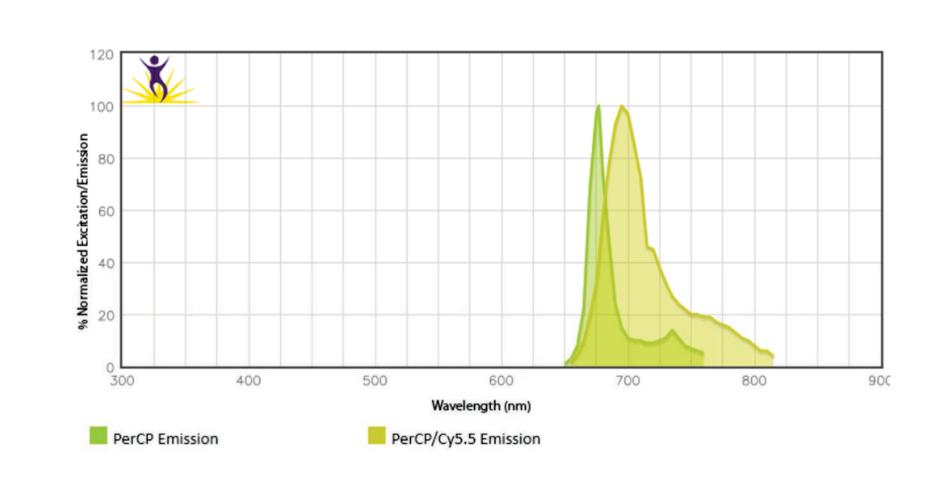
Conventional flow cytometers employ photomultiplier tubes (PMTs), detection filters and mirrors to capture emitted light. The ability to analyze increased numbers of parameters using such systems is limited to some extent by the availability of appropriate filters, and the emitted light needs to be far enough apart to be able to separate the different spectra.

Sony Corporation's new Spectral Flow Cytometer, SP6800 uses a new algorithm to measure and identify multiple closely emitting fluorophores. The algorithm assigns a fingerprint to the fluorescence spectrum and then identifies individual fluorescence emissions. This method allows the analysis of multi-color samples without the need for "traditional" compensation.

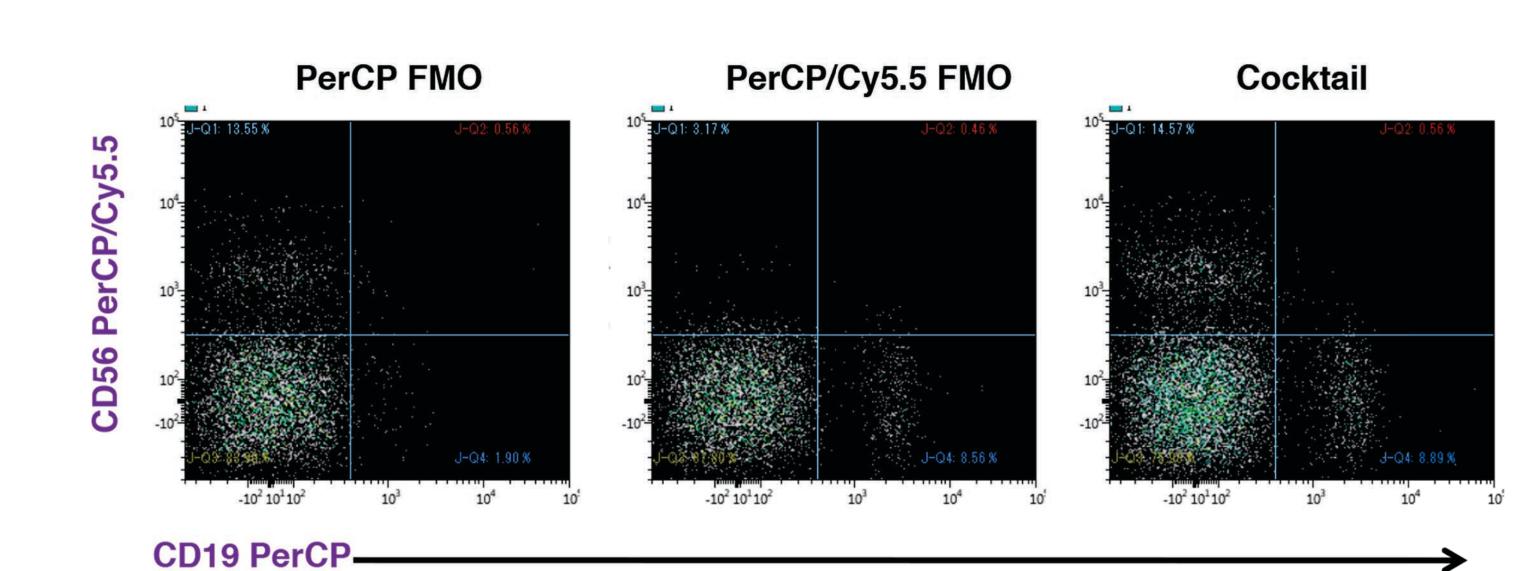
MATERIALS AND METHODS

To test the ability of the SP6800 to discriminate spectrally overlapping fluorophores, we stained whole blood with CD4 FITC, CD3 Alexa Fluor® 514, CD15 PE, CD19 PerCP, CD56 PerCP/Cy5.5 and CD45 PE/Cy7 or with a cocktail consisting of CCR7 FITC, CD4 Alexa Fluor® 514, CD3 Alexa Fluor® 532, CD16 PE, CD19 PerCP, CD45RA PerCP/Cy5.5, CD2 APC, CD14 Alexa Fluor® 700 and CD45 APC/Cy7. Peripheral blood mononuclear cells (PBMCs) from normal donors were stained using a 7-color cocktail consisting of Helios Alexa Fluor® 488, CD39 PE, CD8 PerCP, CD127 PE/Cy7, Foxp3 Alexa Fluor® 647, CD4 Alexa Fluor® 700 and CD25 APC/Cy7 and acquired on a BD LSRFortessa™ or Sony Corporation's SP6800 to determine whether Sony Corporation's SP6800 could identify cellular subsets similar to traditional flow cytometers.

Figure 1. SP6800 can differentiate spectrally overlapping conjugates — CD19 PerCP+ and CD56 PerCP/Cy5.5+ cells can be separated effectively using Sony Corporation's SP6800



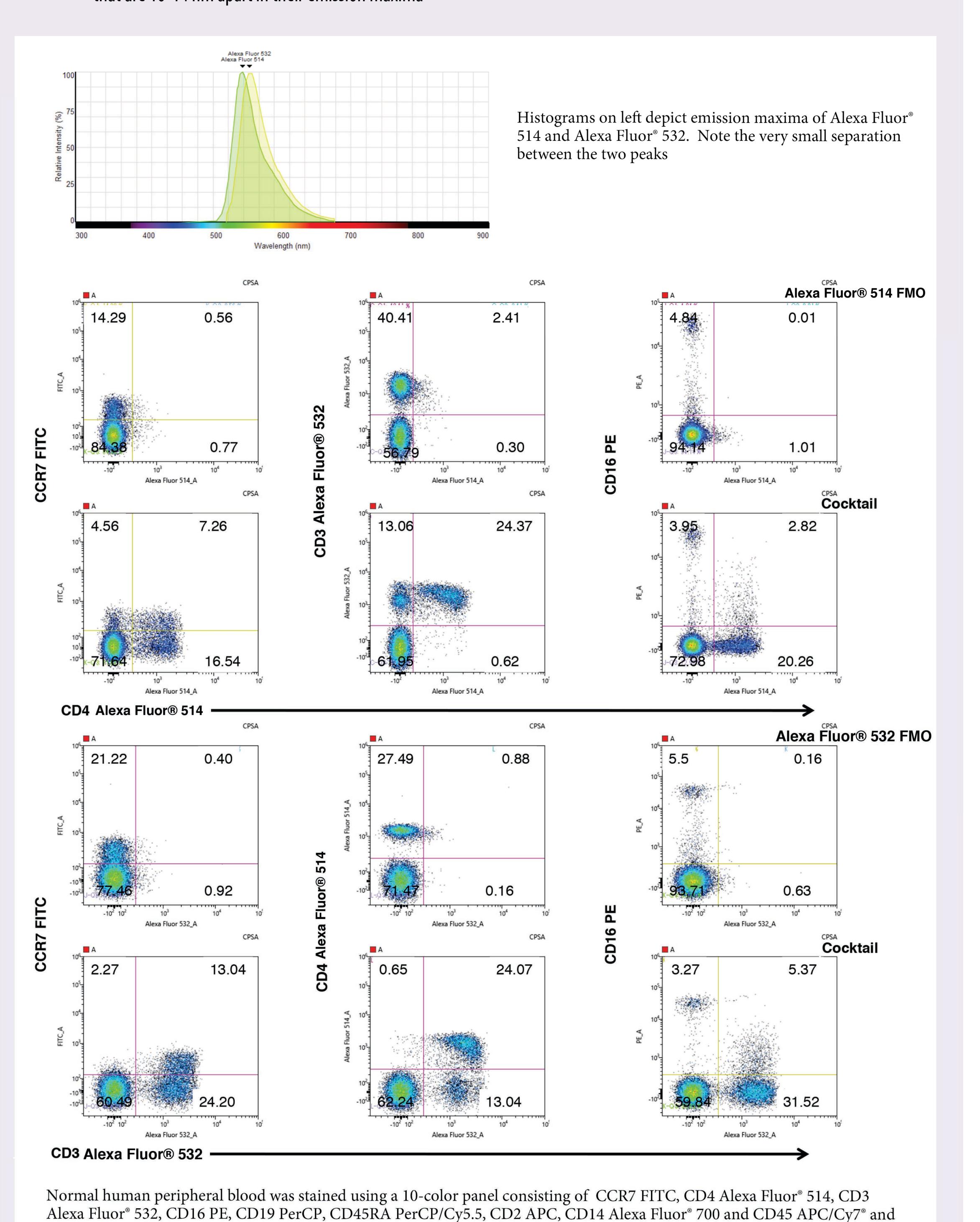
Histograms depict emission maxima of PerCP (677nm) and PerCP/Cy5.5 (690nm).



Normal human peripheral blood was stained using a 5-color panel consisting of CD4 FITC, CD3 Alexa Fluor® 514, CD15 PE, CD19 PerCP, CD56 PerCP/Cy5.5 and CD45 PE/Cy7 and acquired on the SP6800. Left panel – PerCP/Cy5.5 FMO, middle panel – PerCP/Cy5.5 FMO and right panel - cocktail with all five antibodies.

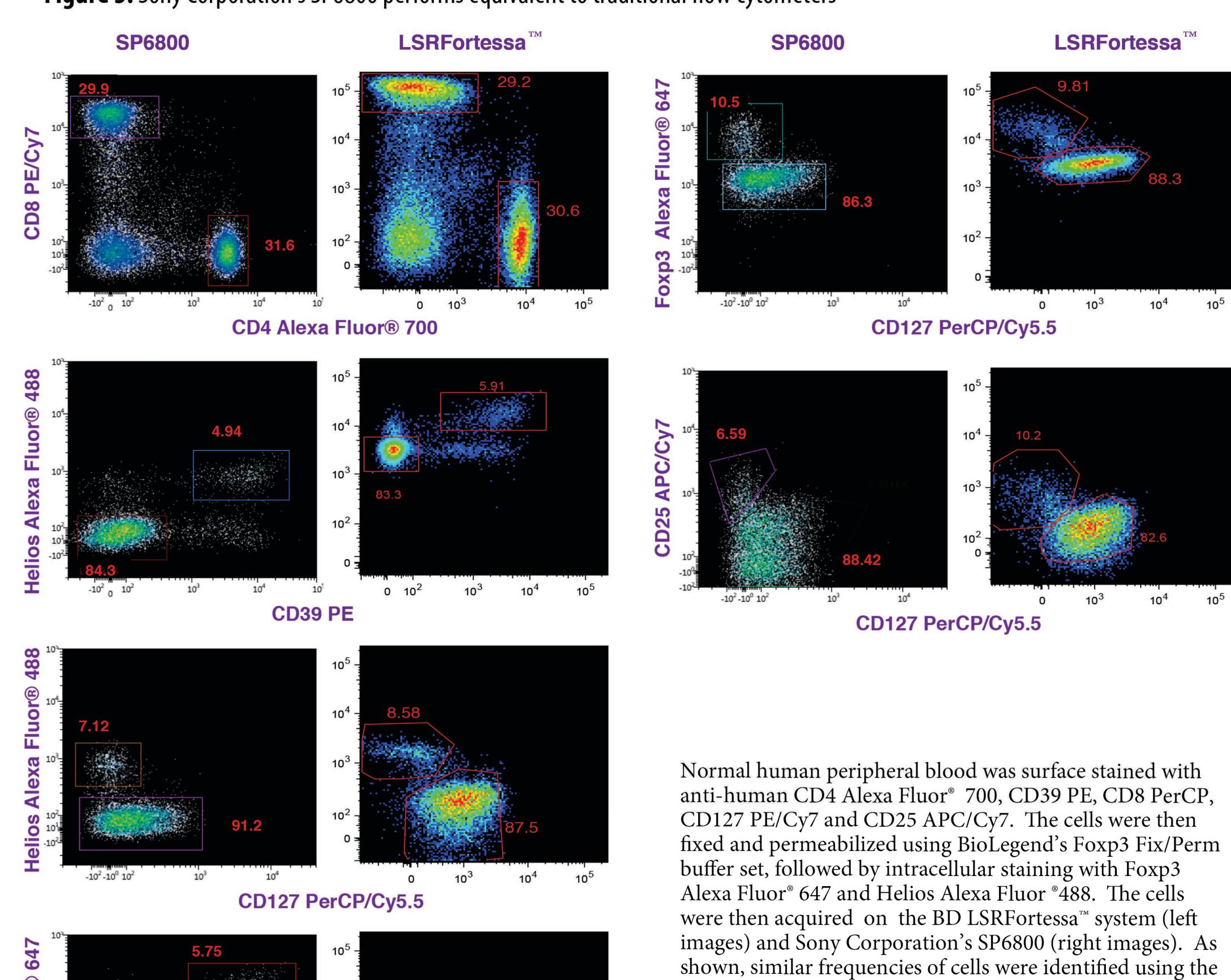
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Figure 2. SP6800's CPSA algorithm discriminates and separates fluorophores (Alexa Fluor® 514 and Alexa Fluor ® 532) that are 10-14 nm apart in their emission maxima



acquired on the SP6800.

Figure 3. Sony Corporation's SP6800 performs equivalent to traditional flow cytometers



CONCLUSIONS

- The SP6800 is an easy-to-use system
- The SP6800 can differentiate between PerCP and PerCP/Cy5.5 two significantly overlapping fluorophores 20 nm apart
- The SP6800 can differentiate between Alexa Fluor® 514 and Alexa Fluor® 532 two significantly overlapping fluorophores that are 10 nm apart

different instrument systems.

• Comparison of a Treg phenotyping panel shows that the SP6800 performs similar to traditional flow cytometers

Alexa Fluor® is a registered trademark of Life Technologies. BD LSRFortessa™ is a trademark of Becton, Dickinson and Company.

CD39 PE