TBNK Panel on the SA3800 Spectral Analyzer

TBNK (B-Cell, T-Cell, and natural killer cells) panels are frequently used to examine major leukocyte populations. All of these populations are important for normal immune function. With the ability to analyze more fluorochromes, the SA3800 allows additional subsets to be defined making the most of your precious samples. In this example two additional markers were added to the traditional panel, CD14 (monocyte marker) and CD7 (lymphocyte marker). The NK cell population is traditionally defined by a heterogeneous expression of the CD16 and CD56 population. The addition of CD7 helps identify the T cell subset of the activated NK population.1 In this experiment a TBNK panel was run on a Sony SA3800 spectral analyzer. The spectral analyzer has several advantages over a traditional flow cytometer.

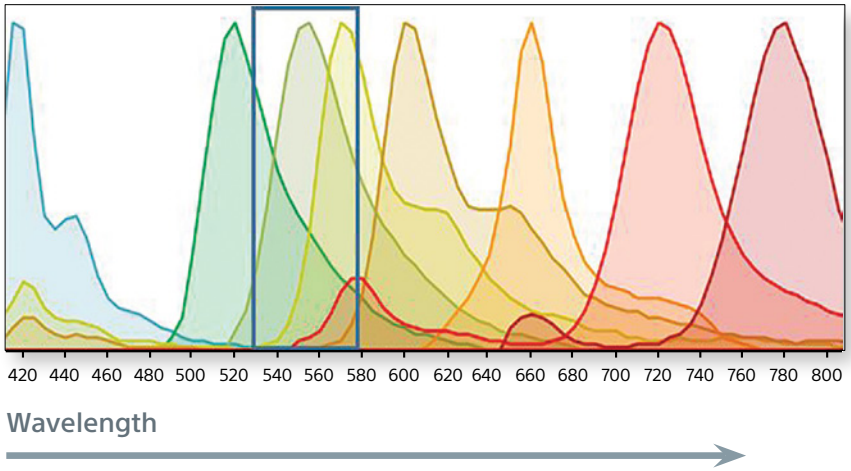
- *PMT layout and spectral unmixing allow the detection of overlapping fluorochromes*
- *Enhancement of dim signal detection for better visualization of populations*
- *Complete data views that enable the viewing of the entire panel in a single graph*

The SA3800 software creates traditional bivariant dot plots in addition to spectral plots. In this example traditional analysis will be presented first and then combined with spectral analysis to emphasize the clarity that spectral plots offer to complex data analysis.

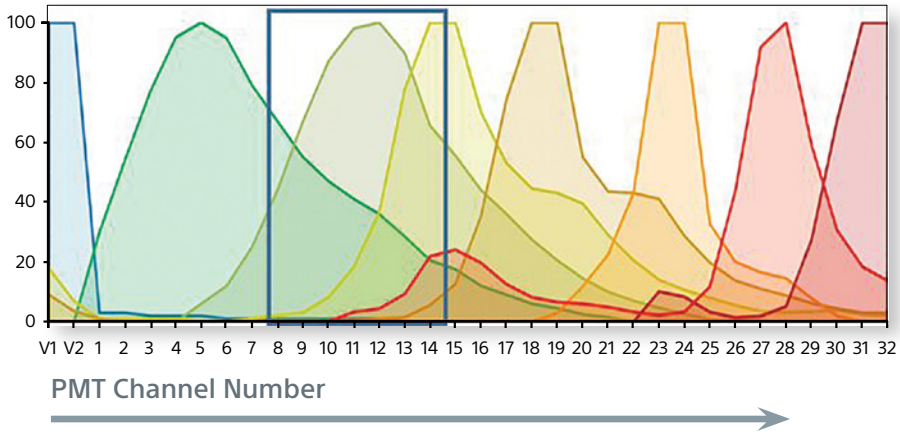
TBNK Panel	
CD16/56	BV421
CD3	FITC
CD45	Alexa 532
CD8	BV570
CD19	BV605
CD7	APC
CD4	PE-Alexa 700
CD14	APC-Cy7

Specificity	Fluorochrome	Purpose/Identifies	Cat. No.
CD3	FITC	All T-cells	2186530
CD4	PE-AlexaFluor® 700	CD4 helper T-cell	N/A
CD8	Brilliant Violet 570™	CD8 effector T-cell	2105190
CD19	Brilliant Violet 605™	B-cells	2111220
CD16	Brilliant Violet 421™	NK-cells	2110190
CD7	APC	NK cell subset	2315540
CD14	APC-Cy™7	Monocytes	2109100
CD45	Alexa Fluor® 532	Gating/Leukocytes	N/A

A Traditional Flow Cytometer

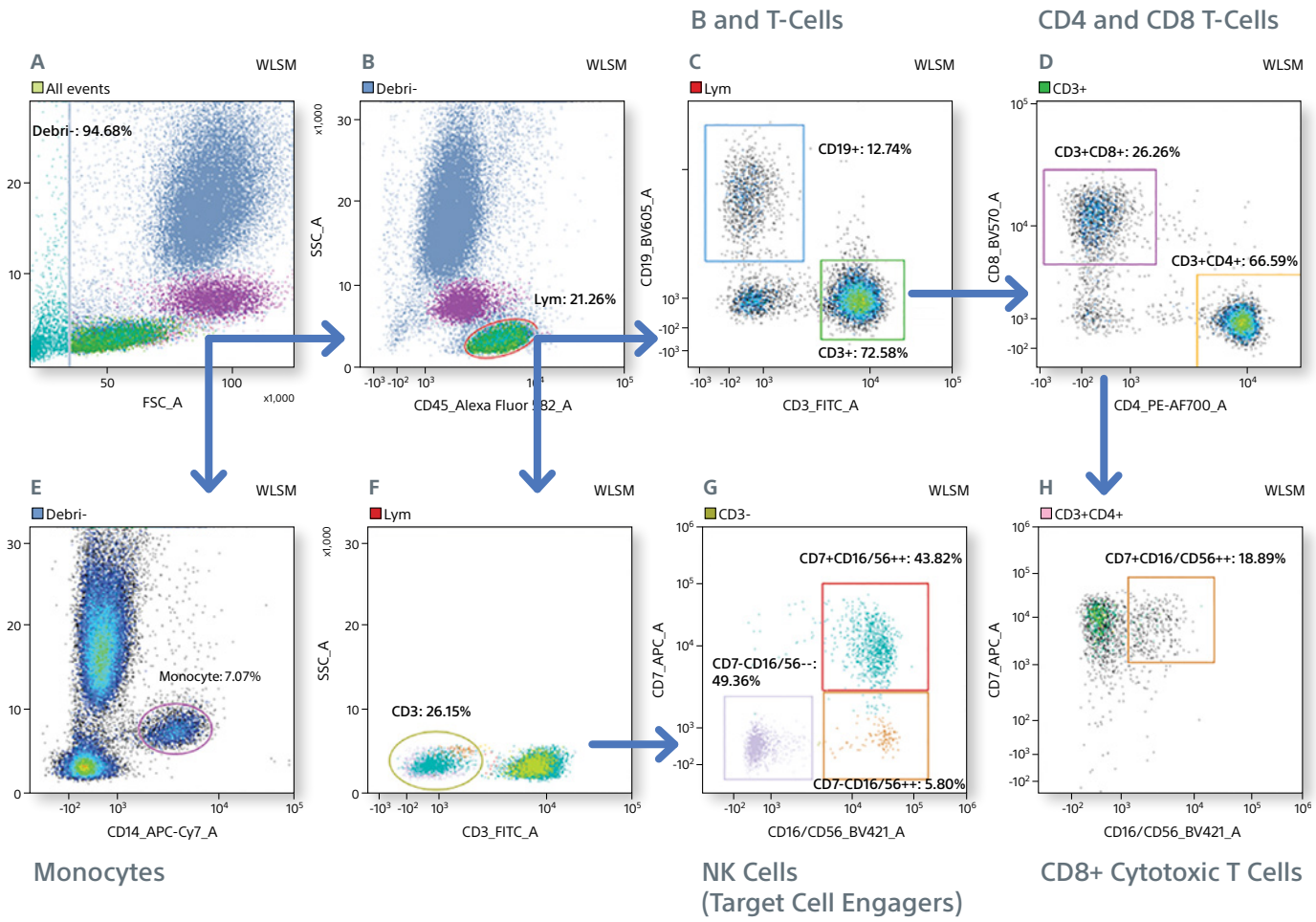


B SA3800 Spectral Analyzer



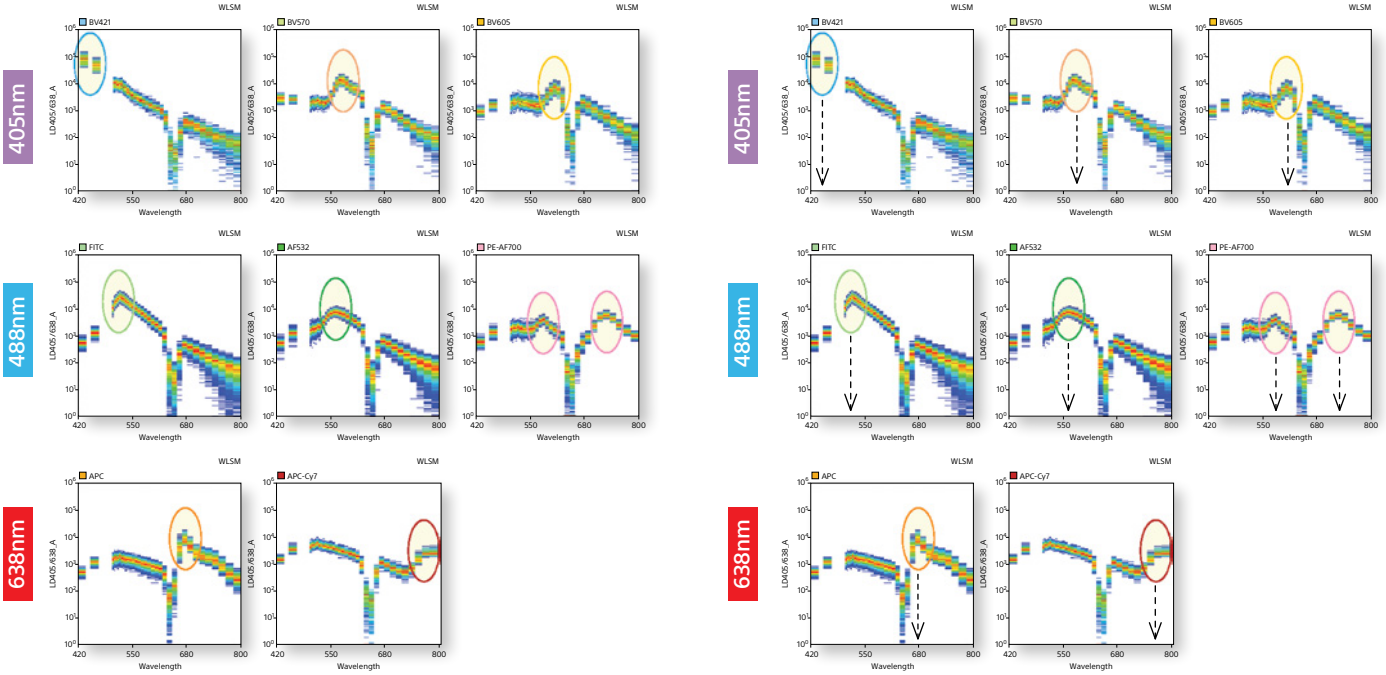
*Spectral profiles of fluorochromes used in the TBNK panel (above) by wavelength (**A**) and channel number (**B**). The SA3800 has 2 PMTs and one 32 channel PMT array. The PMT array was engineered to enable the separation of fluorophores such as FITC, Alexa Fluor® 532 and BV570. The separation of such overlapping combinations is further enhanced through spectral unmixing software.*

Normal human PBMCs were stained with the TBNK panel and analyzed on the SA3800.

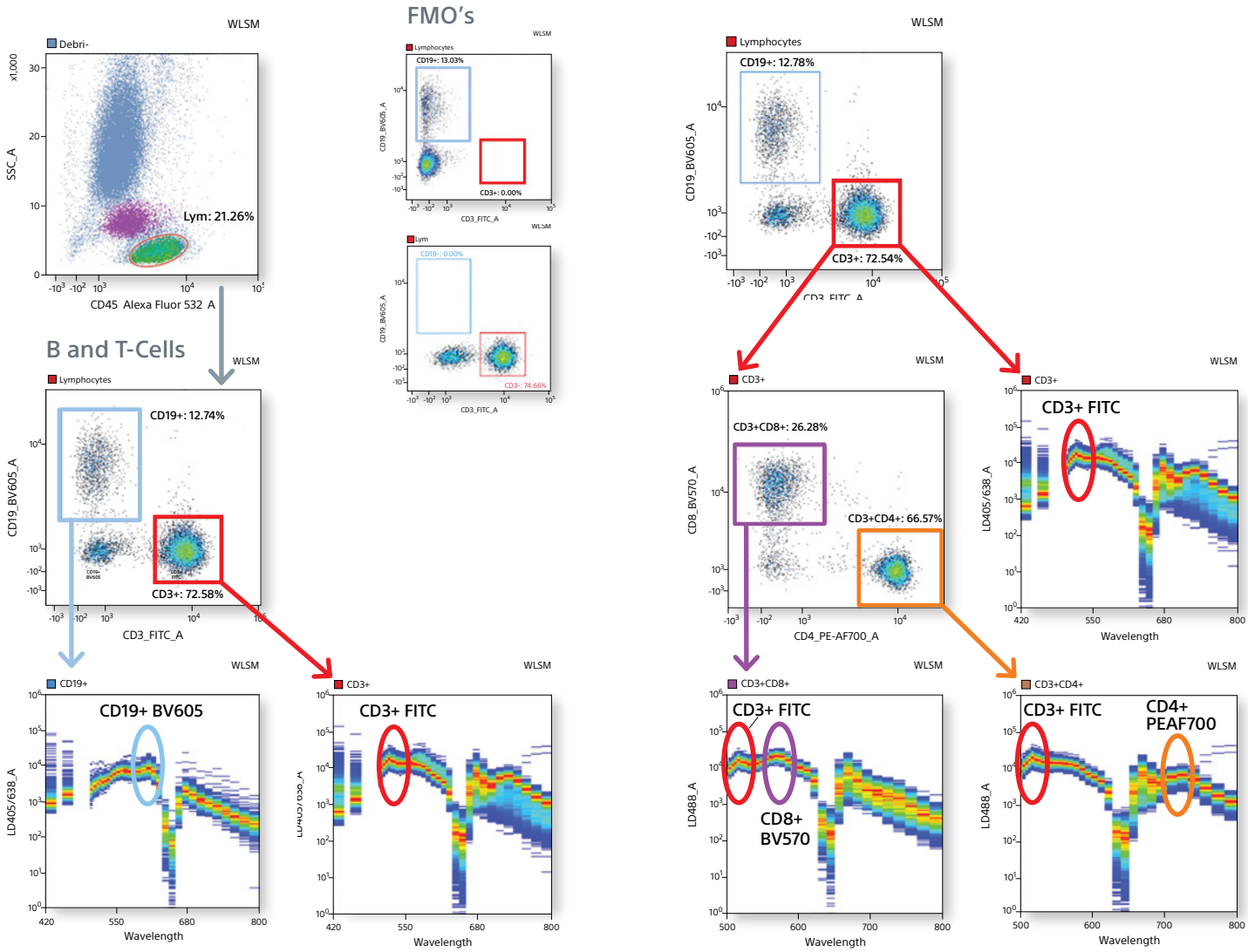


*TBNK data analyzed with traditional dot plots. Forward and side scatter combined with CD45 was used to identify lymphocytes (**B**), from the lymphocyte population B and T cells were identified (**C**). The T-cell population was further divided into CD4 and CD8 T-cells (**D**). From the CD8 T-cell population cytotoxic T-cells were identified using CD7 and CD16/CD56 (**H**). From the forward and side scatter profiles with the addition CD14 (**E**) the percentage of monocytes was determined. To examine the NK cells lymphocytes (**B**) CD3 was used to remove the T-cells (**F**). Figure G shows the population of NK cells.*

Spectral plots represent another method to examine the same data set.
When viewing single color fluorochrome controls, peaks within the fluorochrome signature are easily identified.



Plots highlight the individual fluorochromes within the spectra. Arrows in the plots on the right highlight the fluorochrome peak and its corresponding wavelength. Wavelengths correspond to the expected emission peaks.



BV605 CD19+ B Cells FITC CD3+ T Cells

Dye	Specificity	Purpose
AF532	CD45	Lymphocytes
FITC	CD3	T Lymphocytes
BV605	CD19	B Lymphocytes

Dye	Specificity	Purpose
BV570	CD8	Suppressor/Cytotoxic T Lymphocytes
PE-Alexa Fluor® 700	CD4	Helper/Inducer T Lymphocytes

B-cell and T-cell populations (stained with BV570 and AF532 respectively) are identified through conventional bivariant dot plots. Populations can also be identified by their location in the spectral plot. CD19+ cell populations are represented by the peak at 605nm while T-cells can be seen at the 530 nm peak. CD4 and CD8 T-cells stained with PE-AF700 and BV570 respectively. The CD4 spectral plot (stained with PE-AF700) shows a peak at 700nm for CD4 + cells. The CD8 spectral plot (stained with BV570) shows a peak on the x-axis for CD8+ cells. To improve accuracy, SA3800 software can also be used to verify dot plot gating simply by moving the dot plot gate while observing the excitation peaks in the spectral plot and adjusting the dot plot gate accordingly. Spectral plots can also be gated for deeper analysis, if needed.

Conclusion

Spectral analysis is a new method to examine cell populations. Results of experiments with spectral analysis are comparable or better to that of conventional flow cytometry with some key advantages. Richer data sets can be generated facilitating easy identification of tandem degradation, sample degradation, and staining abnormalities. In addition, with spectral analysis many fluorochromes with overlapping spectra, such as FITC and AF532, can be used together enabling the use of more fluorochromes off the same laser. This allows more data to be generated from limited samples and more flexibility in experimental design.compromising data quality.

[1]Milush, Jeffrey M., et al. "CD56negCD16+ NK cells are activated mature NK cells with impaired effector function during HIV-1 infection." Retrovirology 10.1 (2013): 158.