SONY

SA3800

Spectral Analyzer

Sony Biotechnology Inc.

SA3800 Spectral Analyzer

The Sony SA3800 spectral cell analyzer incorporates advanced electronics, patented optical technologies and many points of automation to deliver true workflow simplicity.

Automation is present across all SA3800 analyzer operations, from instrument start up to quality control, acquisition, analysis, and system maintenance. Software wizards guide users through procedures across the workflow that also makes the system approachable to flow cytometry users.

Spectral technology in the SA3800 optimizes sensitivity and enhances dim signal detection by collecting photons from 410nm to 800nm. This simplifies multicolor application design, workflow and analysis for experienced and novice users by eliminating the need for numerous filters and complex instrument configurations.

Spectral technology and automation across the workflow, make the SA3800 an excellent fit for facilities looking for a capable and easy-to-use analyzer for cellular acquisition and analysis.

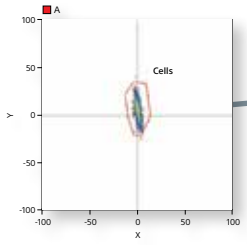
- **Uses spectral analysis technology to optimize sensitivity while simplifying application design and workflow.**
- **Enhances dim signal detection for better visualization of rare populations, fluorescent proteins and fluorochromes excited by multiple lasers.**
- **Features automation across the workflow including automated alignment and a software calibration wizard to simplify operation and improve the reliability of results.**

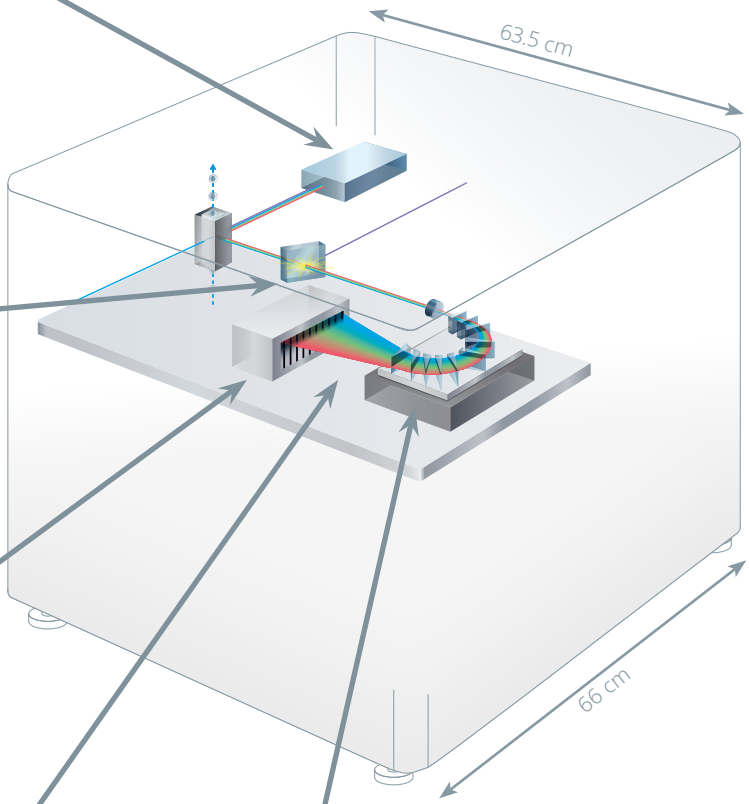
SA3800 System Overview

The SA3800 spectral analyzer improves sensitivity and simplifies application design, workflow and analysis over conventional flow cytometers. This is achieved using spectral analysis technology, automation throughout the system, advanced electronics and patented optical technologies. Unique to Sony Biotechnology systems.

Supports up to 4 lasers including the 488nm (blue) excitation laser that is standard in all systems. The 405nm (violet), 561nm (yellow-green,) and 638nm (red) lasers are available as options.

The Flowpoint™ detection system precisely tracks the core stream shape and position in the flow cell as well as the cross-sectional position of each passing particle to provide highly reliable measurements. This patented technology ensures core stability and enables the highest resolution.

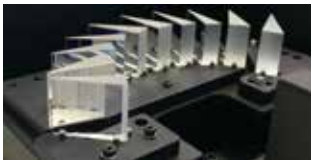




Emitted light is collected by a 32 Channel PMT producing a spectral fingerprint from the collected 32 data points of signal detection.

Microlens array maximizes photon capture by refocusing light from the prism onto the 32 channel PMT array.

A unique prism collection system delivers light through 10 consecutive prisms allowing optimal signal with minimal loss of light.



Software

The SA3800 software guides researchers though common workflow from set-up to acquisition, analysis and maintenance procedures.

System Start Up

At start up Align Check and Performance QC wizards check instrument calibrations, using beads to ensure the instrument is operating optimally. On screen instructions guide the user through procedures, then displays progress and report results. The performance report displays MESF, Q, and B values to describe real-time fluorescence detection performance. If desired, Align check and performance QC reports can be displayed in historical context.

System Shutdown

On shutdown, the SA3800 software guides the user though pre –shutdown cleaning and shuts down the instrument automatically. Software wizards are also available to guide users through Bleach Cleaning and Rinse procedures.

Experiment Creation

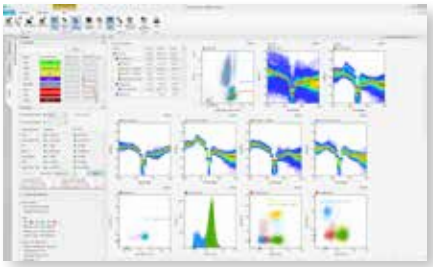
Experiments can be created using a template, an existing experiment, a single stained- or multicolor assay- in the Create Experiment window. Users can point and click to choose (or edit) an existing experiment and can easily select templates for, wells or plates when creating a new experiment. A setup Assay Wizard guides users through the creation of a single-stained or multicolor assay simplifying experiment creation.

Acquisition Functions and Analysis

All acquisition functions, including instrument settings are controlled from the Acquisition Window. Worksheet tools let users choose how the data is displayed- (such as plot types), and customize for their analysis needs. Plots and statistics provide real-time information during acquisition. After acquisition, further operations (grouping, editing, and customizing) can be performed with the data in the Analysis Window.

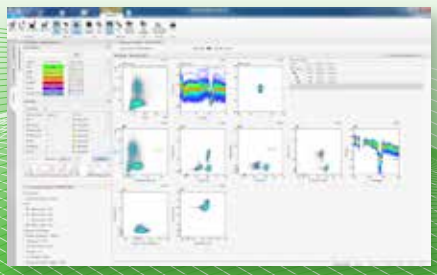
Fluorescence Unmixing

A powerful capability of spectral technology is Fluorescence Unmixing that lets researchers separate fluorophores into pure signals that measure the quantity if each fluorophore at each pixel to more accurately measure data for analysis. This also lets researchers separate the spectra of fluorophores masked by autofluorescence by extracting it from the signal and creating it into a unique fluorescent parameter. With a clear signal for each color channel unaffected by overlapping signals (spillover) and autofluorencence, spectral analysis yields better, unbiased data for analysis.



Spectrum Reference Library

To simplify unmixing, SA3800 software includes a Spectrum Reference Library that includes the published fluorescence emission curves for many common fluorochromes. The library can also be customized with new fluorochromes registered by the user. Using simple point and click to create a reference spectrum including positive and negative controls for each fluorochrome is easily achieved. Resulting in a Stain Index for each fluorochrome in the library. Once created, reference spectrums can be saved and reused.



Standardization

The SA3800's Standardization mode can be selected by users to set multiple instruments in a lab or on instruments located across multiple sites to a master specification to eliminate instrument variability among instruments. This unique capability allows experiments performed on any standardized SA3800s to produce highly reliable, accurate and reproducible results for collaborative and long term studies. This also eliminates setup subjectivity when working with different operators or experience levels across sites.

System Automation

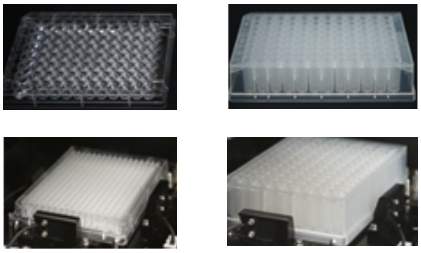
Automation is present across the workflow to simplify operation and ensure accurate results. The system supports a wide variety of standard and deep well plates, and 12x75mm 5ml tubes in the tube loader.

Novel 3D AutoSampler Technology

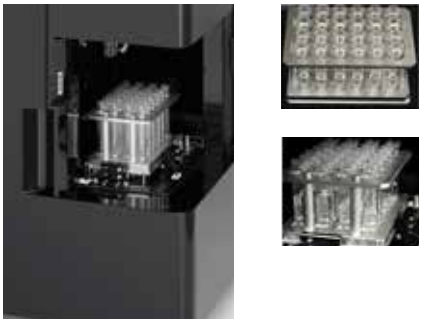
The novel 3D AutoSampler uses a fixed probe and moves the plate in horizontal and vertical directions to minimize sample-to-sample cross contamination and speed cleaning.

Sensors on the probe enable the system to accurately move the sample to the probe, calculate height of the tube or plate and automatically recoil the probe if it touches the container or base plate surface. This unique design minimizes sample-to-sample cross-contamination, reduces clogs and speeds cleaning over conventional two-dimensional sampling that must use longer tubing to reach samples.

Plate motion uses a fixed probe and moves the plate (and sample) in horizontal and vertical directions.



The SA3800 supports standard height and 96- and 384-well plates with round, flat, v- and conical shapes in addition to 96-well half deep and bottom plates.



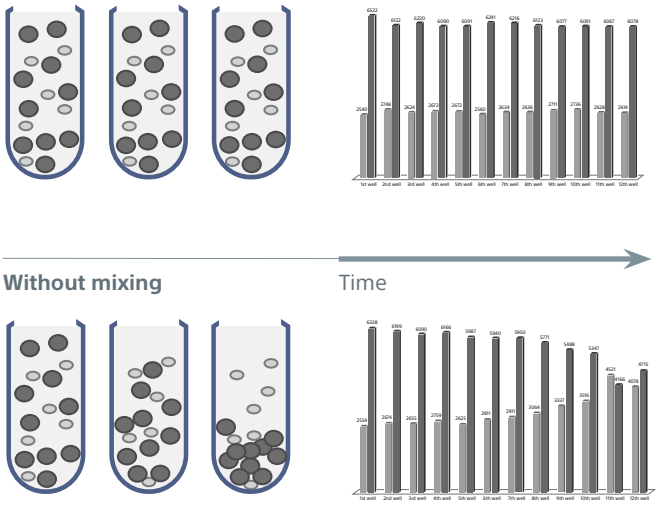
Tube Loader supports 5ml 12x75mm tubes.

Mixing Automation and Cooling Plate

The 3D AutoSampler mixing function ensures consistent sampling throughout the acquisition of 96-, or 384-well samples. Software optimizes settings for each plate type. Importantly, the mixing function maintains the integrity and heterogeneity of samples ensuring that all particles are properly suspended for consistent results.

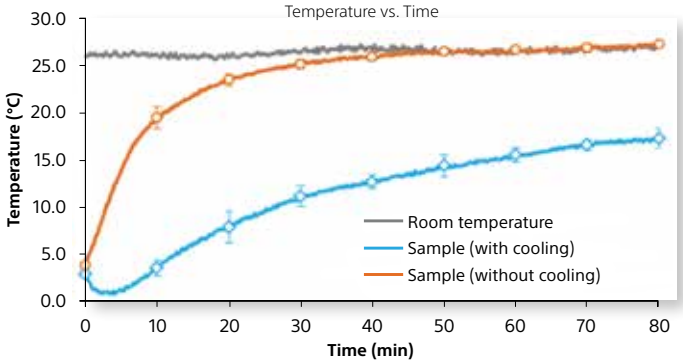
A cooling plate controls the surface of the 3D base to further reduce variability and inhibit sample degradation over time.

SA3800 3D AutoSampler mixing



This figure illustrates how cell heterogeneity is maintained using the system's 3D mixing function. Without mixing larger cells settle in heterogeneous samples, as shown in the lower illustration. Graph data describes how the sample integrity is maintained throughout plate acquisition from well 1-96 to deliver more complete, consistent results using 3 µm and10 µm beads.

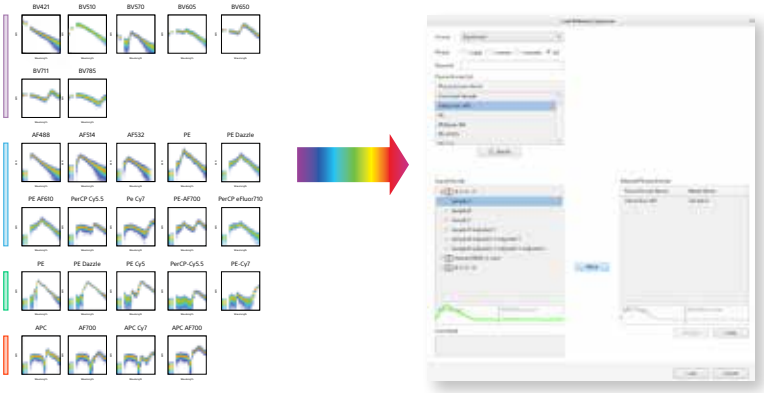
Temperature controls



The graph describes the impact of the cooling plate on sample temperature during a 80 minute plate acquisition.

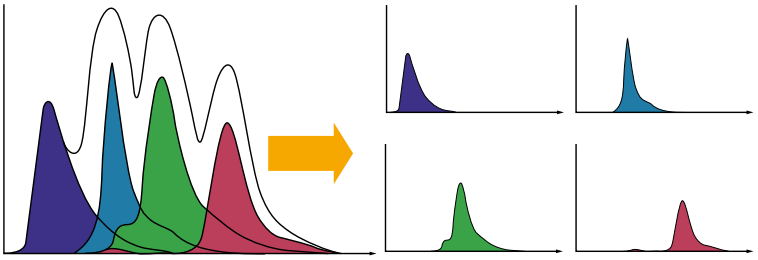
Spectral Library

Spectral technology eliminates the need for bandpass filters and conventional compensation matrices to allow greater flexibility in multicolor application panel design. This lets researchers save single positive fluorochromes in a Spectral Library to easily import them into current and future experiments.

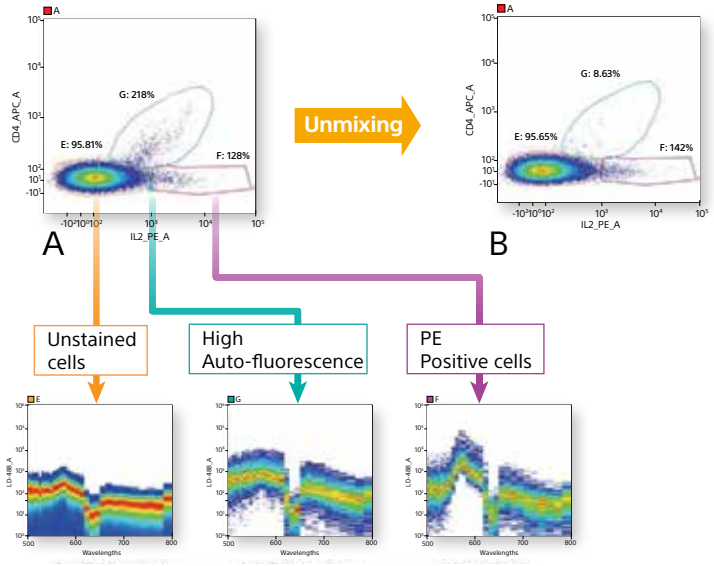


Spectral Unmixing

Spectral unmixing separates each spectral fingerprint to better visualize each fluorochrome marker. Unlike conventional filtering where overlapping signals are lost, spectral unmixing captures the photons emitted from 420nm to 800nm. In doing so it enhances dim signal detection for better visualization of rare populations, fluorescent proteins and fluorochromes excited by multiple lasers.



Spectral Unmixing separates each spectral fingerprint for complete and optimal visualization of fluorochomes.



Spectral analysis reduces false-positives and delivers more accurate analysis over conventional flow cytometry. Mouse splenocytes were stained with CD4 APC and anti-IL-2 PE. ***A.*** *In this conventional density plot it is unclear if the light-blue region is a dim PE, weak double positive, or non-specific binding.* ***B.*** *Using Spectral analysis the spectral data of each region is compared against the Spectral Library to unmix the sample. This reveals the light blue region is high auto-fluorescence. Representative data collected on SP6800.*

Sample Data

Using spectral unmixing the SA3800 allows maximum flexibility for fluorochrome selection without compromising results. The panel below and the spectral data on the following pages demonstrate the flexibility of the SA3800 system. Most importantly, results are comparable to those obtained using conventional systems with optimized fluorochromes.

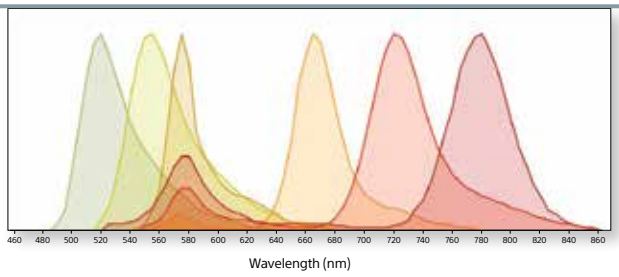
Phenotyping of B-Cells, Effector T-Cells, Helper T-Cells, and Monocytes Using a Single Blue Laser

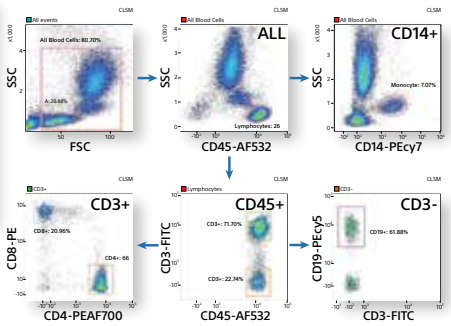
In this panel all lymphocytes were identified by staining with CD45. From the CD45+ population, B-cells (CD19), monocytes (CD14) and T-cells (CD3) were identified. The T-cell population was further analyzed to determine relative percentages of effector T-cells (CD8) and helper T-cells (CD4). The percentages of cells obtained were comparable to data obtained from conventional flow cytometers using multiple lasers (data not shown).

In contrast to conventional flow cytometry systems, the SA3800 can utilize at least 6 fluorochromes off the blue laser. Applying spectral unmixing, even highly over-lapping fluorochromes such as Alexa Fluor 532, FITC and PE can be distinguished.

488 Laser

Specificity	Fluorochrome
CD3	FITC
CD45	Alexa Fluor® 532
CD8	PE
CD19	PE-Cy™5
CD4	PE-Alexa Fluor® 700
CD14	PE-Cy™7





Fixed whole blood cells were stained with the panel described above and analyzed on an SA3800 using the blue (488 nm) laser. Populations were gated on forward (FSC) and side scatter (SSC) then CD45+. From that population several subsets were identified as indicated.

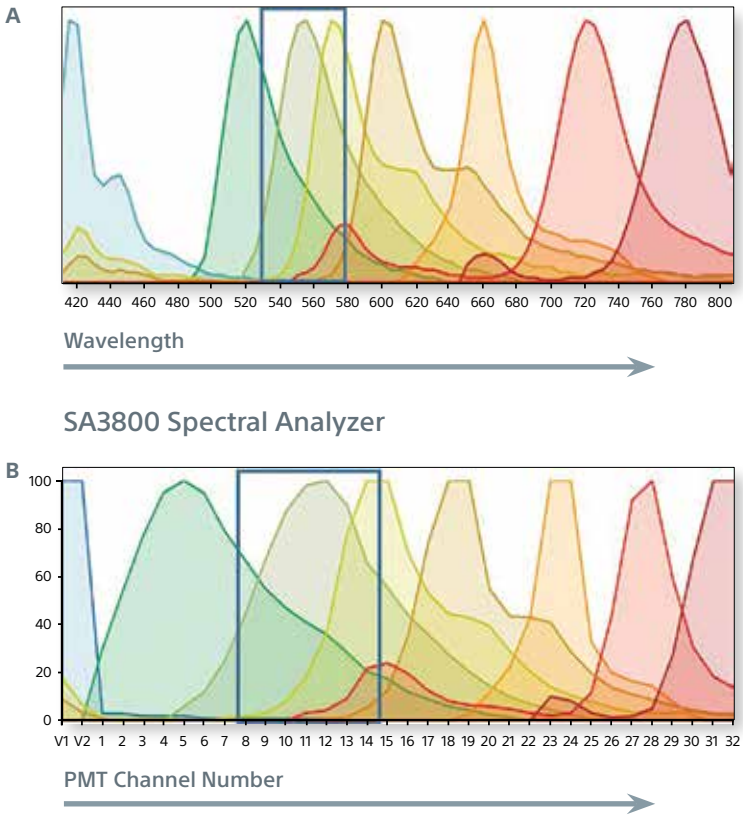
TBNK Panel

TBNK (T-Cell, B-Cell, and natural killer cells) panels are frequently used to examine major leukocyte populations important for normal immune function. In this panel two additional markers were added to the traditional panel, CD14 (a monocyte marker) and CD7 (a lymphocyte marker).

CD7, identifies a population of activated NK cells that is negative for CD56. The NK cell population is traditionally defined by CD16 and CD56 expression, but is heterogeneous. The addition of CD7 further defines the NK population. CD7 is also expressed on T-cell subsets, but not on B-cells.

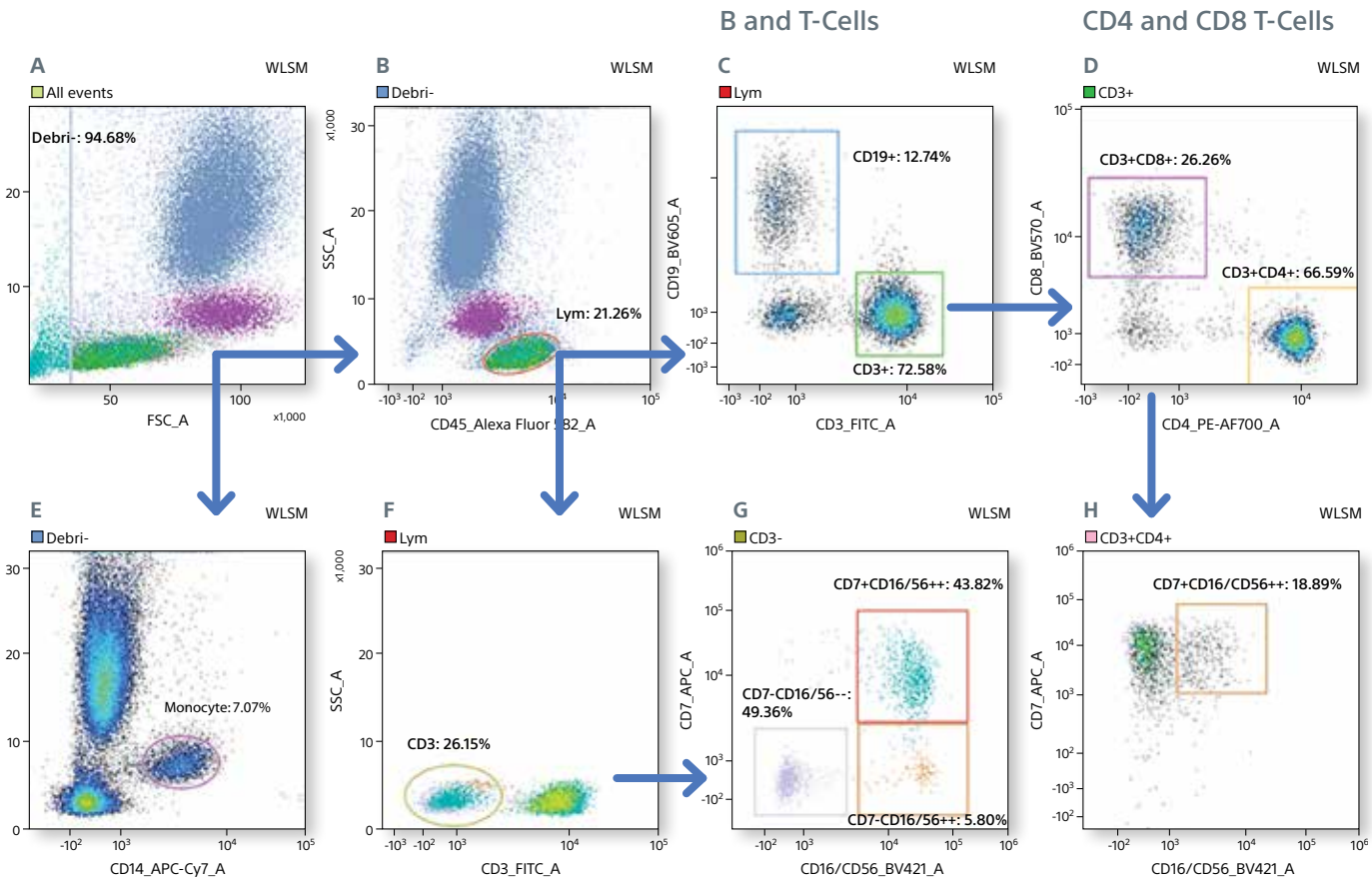
Data is presented in traditional 2X2 dot plots in addition to spectral plots to illustrate the value of spectral visualization for analyzing complex data.

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



Spectral profiles of fluorochromes used in the TBNK panel by wavelength (A) and channel number (B). The SA3800 detection system is composed of has 2 PMT's (V1 and V2) and one 32 channel PMT array. The PMT array enables separation of highly overlapping fluorophores such as FITC, Alexa Fluor® 532 and BV570. The PMT array is engineered to maximize the number of channels that detect emission from 650nm, making separation of highly overlapping fluorochrome combinations possible. This capability and proprietary unmixing algorithms expand fluorochome choices for panel design.

Normal human PBMCs were stained with the panel listed above and analyzed on the SA3800. Data below demonstrate the predicted populations of cells.



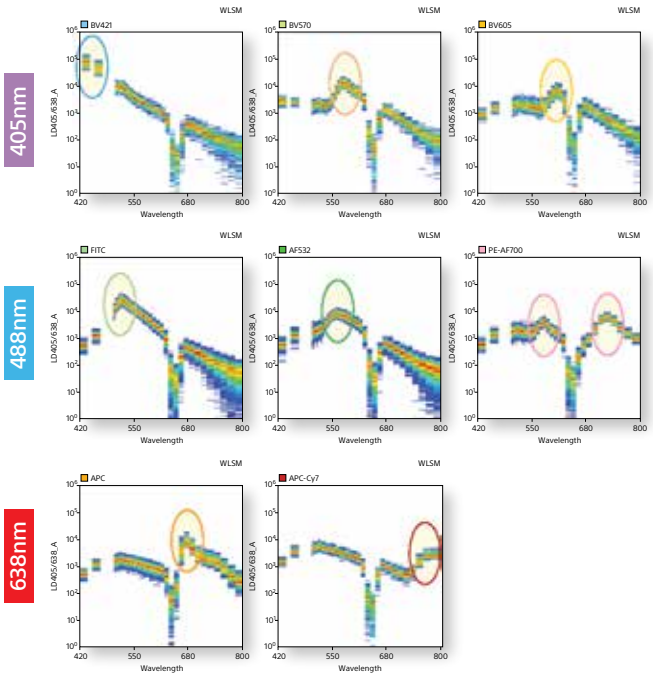
Monocytes

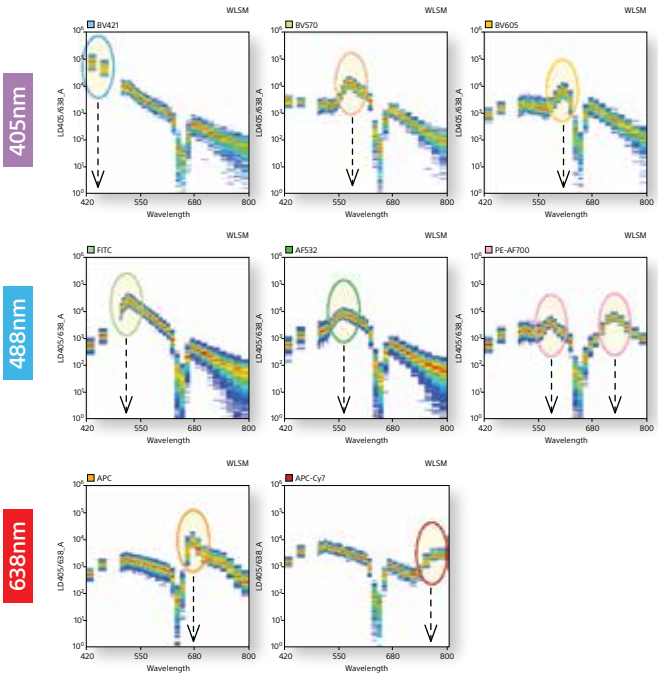
NK Cells (Target Cell Engagers)

CD8+ Cytotoxic T Cells

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.

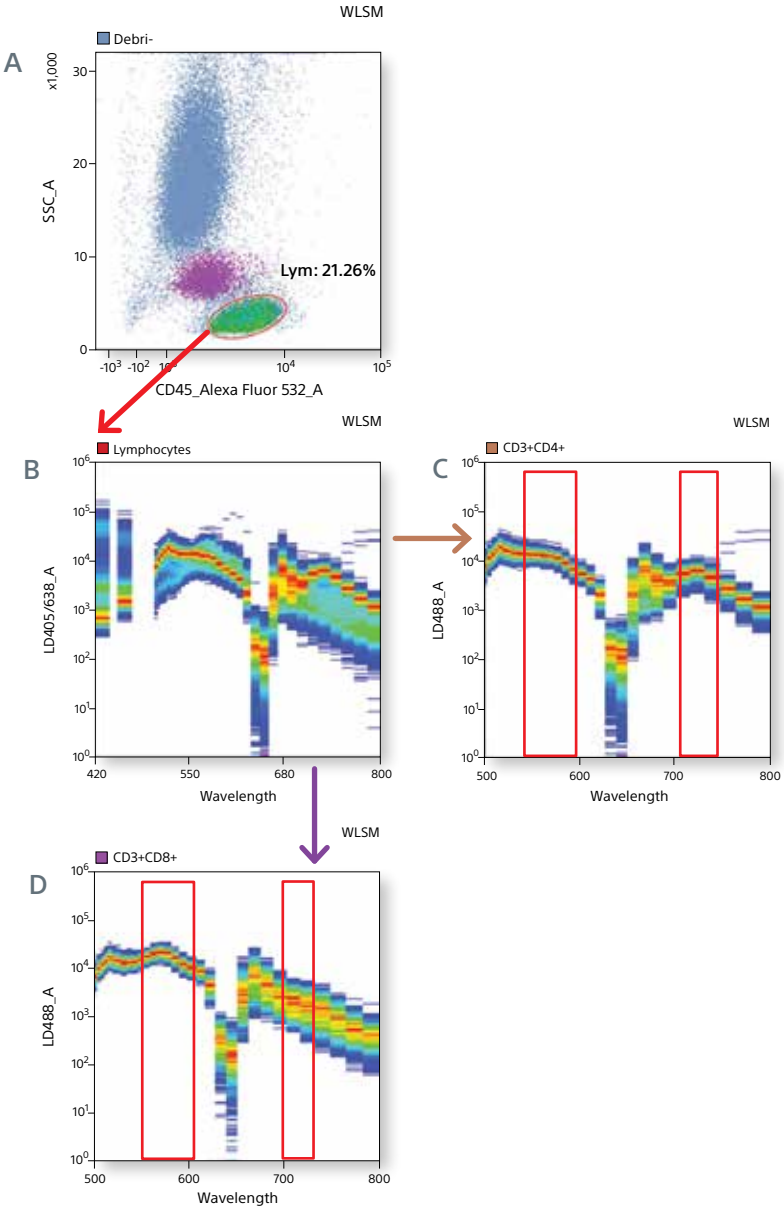
Spectral plots represent another method to examine the same data set. When viewing single color fluorochrome controls, peaks within the fluorochrome signature are easily identified.



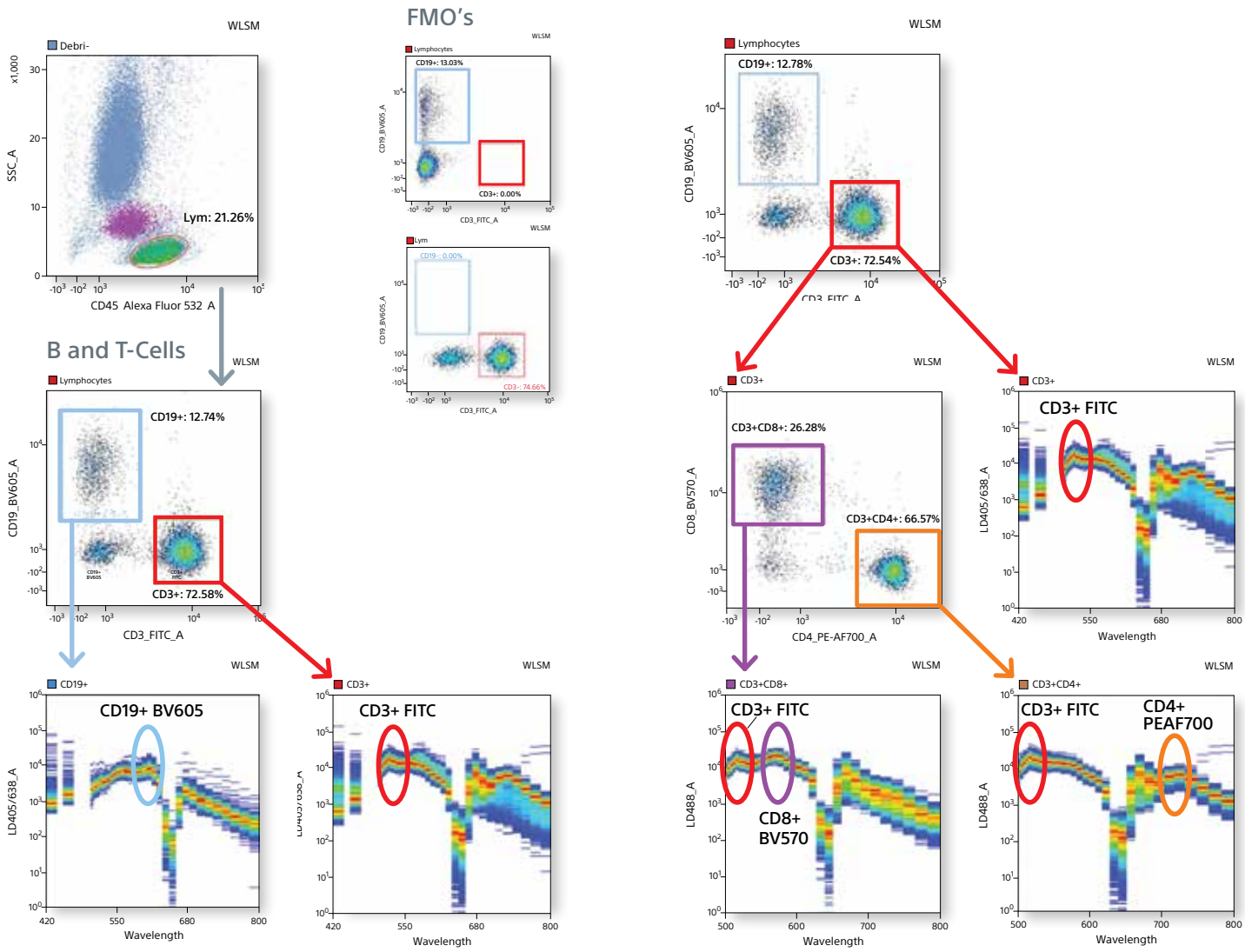


These spectral plots illustrate how spectral technology enables more detailed visualizations to gain a deeper understanding of the biology. Plots on the left highlight the individual fluorochromes within the spectra. Arrows in the plots on the right highlight the fluorochrome peak and its corresponding wavelength. Wavelengths correspond to the expected emission peaks.

Spectral plots contain important "panel level" information. This deeper look at the CD4/CD8 spectral plots illustrates how spectral technology reveals the many types of cells present including CD3+, CD4+, CD3+, and CD8+ with in this heterogeneous mixture.



The SA3800 provides both conventional dot plots and spectral analysis plots to researchers. Spectral plots display full-panel excitation peaks and cell density in a single view. This figure shows unique spectral signatures of CD4+ and CD8+ T cells. Plot B shows the mixed population of lymphocytes that are further characterized in plots C and D that show panel specific signatures of CD4+ and CD8+ populations respectively. The CD4+ cells (C) stained with PE-AF700 and FITC show corresponding peaks on the x-axis at 700nm and 530nm. The CD8+ cells (D) were stained with FITC and BV570 and show peaks at 530nm (as in C) and 570nm.



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

Spectral plots provide additional information over conventional dot plots to improve the accuracy of gating dim populations. This figure shows 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.

Specifications

	Item	Specifications
Optics /Performance	Excitation Options	405nm, 488nm, 561nm, 638nm
	Detection signals	FSC, SSC, FL 32chPMT (500 to 800nm), Violet x2ch PMT (420-500nm)*
	Pulse parameter	Area, Height, Width (all channels)
	Signal resolution	Height 20 bit, Area 32 bit/Sampling frequency: 50MHz
	Flourescence Sensitivity	FITC: 120 MESF; PE: 70 MESF (nominal)
	Flourescence Resolution	CV<3% for the singlet peak of propidium iodide-stained CEN
	Detectable cell size	0.5 µm to 40 µm
	Event rate	20,000 eps (max)
Fluidics	Sample volume rate	Two levels (Low, Normal)
	Cleaning	Auto Probe Cleaning, Priming, Shutdown-cleaning, Cuvette back flushing
	Waste/DIW tank	Both tanks are 2L
Single loader	Single tube	Falcon 5 mL (12 x 75-mn) polystryene/polypropylene
3D AutoSampler	Sample well	96 well plate: standard height Flat/V/U, 96 half deep, 96 deep, 384 standard flat
	Sample tube	Tube rack: 24 Falcon 5 mL (12 x 75-mm) polystyrene/polypropylene
	Sample volume	Tube: Minimum 100uL / Maximum 2000uL 96 well-plate: standard height: 55uL -200uL 384 well-plate: standard height: 40uL -75uL
	Carryover	<0.1% (under high speed/normal mode)
	Measurement speed	96 well plate in 25 minutes* *Acquisition time per well: *2 seconds
	Reagent Stability	Mixing function, sample cooling block (passive)
Ancillary	Dimensions	W: 660 mm x H: 674 mm x D: 635 mm (SA3800 main body)
	Weight	95kg (AutoSampler model, does not include external tank holder)
	Power Consumption	350 W Max

3D AutoSampler

Model	No. of lasers	Laser wavelengths (nm)
LE-SA3800AA	1	488
LE-SA3800BA	2	488, 638
LE-SA3800CA	2	488, 405
LE-SA3800DA	2	488, 561
LE-SA3800EA	3	488, 405, 638
LE-SA3800GA	3	488, 405, 561
LE-SA3800FA	4	488, 405, 638, 561

Single loader model

Model	No. of lasers	Laser wavelengths (nm)
LE-SA3800AS	1	488
LE-SA3800BS	2	488, 638
LE-SA3800CS	2	488, 405
LE-SA3800DS	2	488, 561
LE-SA3800ES	3	488, 405, 638
LE-SA3800GS	3	488, 405, 561
LE-SA3800FS	4	488, 405, 638, 561

** 405 models only.*

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