



Sony Biotechnology Inc.



### SA3800 Spectral Analyzer

The Sony SA3800 is a personal cell analyzer with spectral technology that simplifies application design and enables high throughput testing.

Spectral technology in the SA3800 optimizes sensitivity and enhances dim signal detection by collecting photons from 420nm to 800nm. Spectral technology simplifies multicolor application design, workflow and analysis for experienced and novice users by eliminating the need for numerous filters and complex instrument configurations.

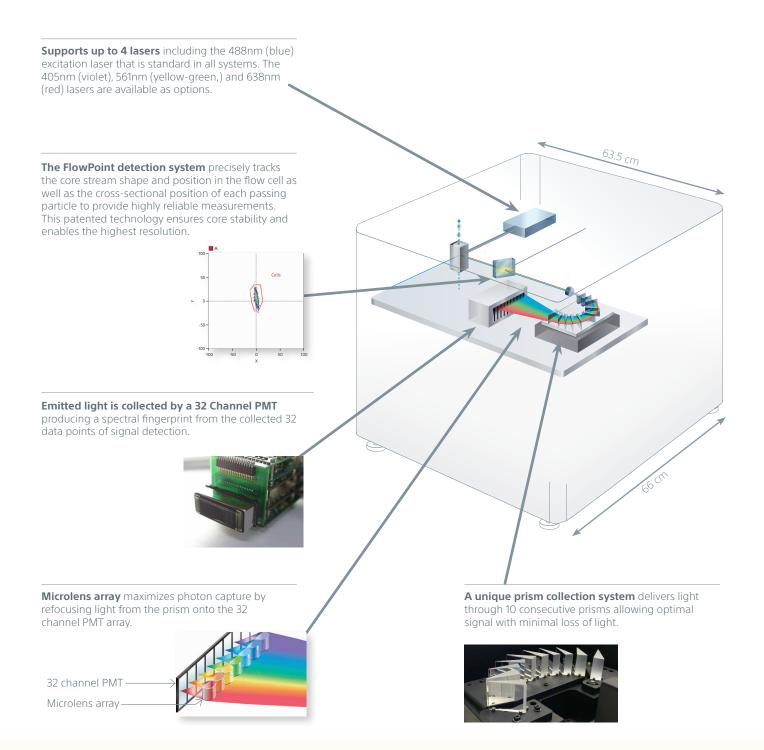
Automation is present across all SA3800 analyzer operations, from instrument start up to QC, acquisition, analysis, and system maintenance. For example, the system's 3D AutoSampler moves the plate in horizontal and vertical directions to eliminate sample-to-sample cross contamination. Reusable control data lets labs save time by reusing common workflows for new experiment set up and repetitive testing common in longitudinal studies.

Advanced electronics, patented optical technologies and many points of automation leverage Sony consumer electronics expertise to deliver true simplicity to the SA3800 workflow. Together these qualities make the SA3800 an excellent fit for facilities looking for an affordable, easy-to-use and capable 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. These capabilities, unique to Sony Biotechnology systems, allow experienced and novice flow cytometrists to achieve more accurate data visualization and greater flexibility for panel design.

### Software

SA3800 software is designed to be simple and intuitive. From set-up to acquisition and analysis many procedures are automated to simplify workflow.

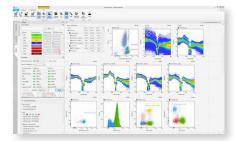
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*Figure 1.* SA3800 wizard and plate overview simplifies set up and acquisition.

The calibration wizard simplifies set-up with step-by-step automation. The plate summary view and controls further simplify set-up for even the most complex sample layouts. **Figure 2.** Acquisition software lets researchers monitor status.

Acquisition software lets researchers monitor the status and progress of acquisition from a dashboard view. A single voltage adjustment is needed to set all 32 PMTs.



**Figure 3.** Flexible analysis enables spectral visualization of gated populations for improved accuracy.

The software also allows flexibility with analysis, to ensure the highest accuracy of results. Populations can be gated and visualized spectrally to ensure populations are accurately defined.

## 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 introduced by the SA3800 uses a fixed probe and moves the plate in horizontal and vertical directions to reduce clogs, 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. Two-dimensional sampling used in conventional flow cytometers is limited to horizontal motion for sampling and, as a result, use longer tubing to reach samples which results in clogs, higher carryover and increased cleaning time.



**Figure 4.** Plate motion uses a fixed probe and moves the plate (and sample) in horizontal and vertical directions.







*Figure 5.* The SA3800 supports standard height 384- and 96- well plates with round, flat, *v*- and conical shapes in addition to 96-well half deep and deep bottom plates.





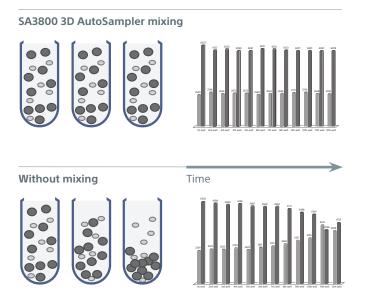


*Figure 6. Tube Loader supports 5ml* 12x75mm tubes.

### **Mixing Automation and Cooling Plate**

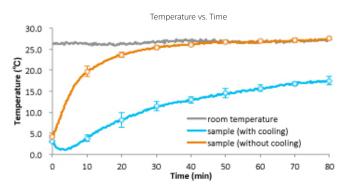
The autoloader mixing function ensures consistent sampling throughout the acquisition of 96, or 384 samples. Settings are optimized for each plate type. Importantly, this function maintains the integrity and heterogeneity of samples ensuring that all particles are properly suspended for consistent results.

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



**Figure 7.** This figure illustrates how cell heterogeneity is maintained using the SA3800's 3D mixing technology. In the absence of mixing, larger cells settle in heterogeneous samples. Data presented to the right, shows  $3\mu m$  and  $10\mu m$  beads; the SA3800 maintained sample integrity throughout plate acquisition from well 1-96 to deliver more complete, consistent results.

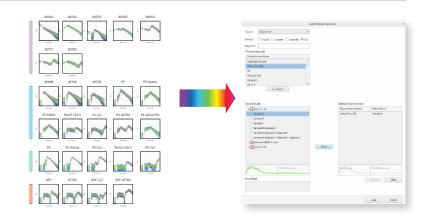
#### Temperature controls



*Figure 8.* The SA3800 cooling unit lets researchers keep samples cooled below room temperature throughout the duration of 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.

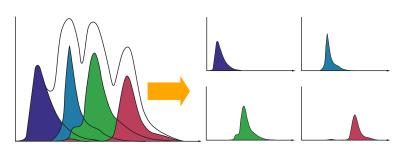
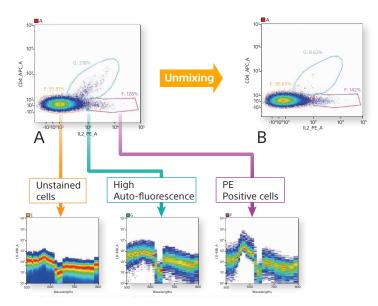


Figure 13. Spectral Unmixing separates each spectral fingerprint for complete and optimal visualization of fluorochomes.



**Figure 14.** 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 antibody conjugated to 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

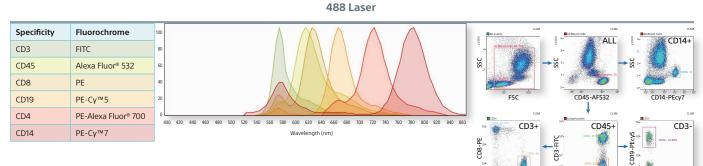
Using spectral unmixing the SA3800 allows maximum flexibility for fluorochrome selection without compromising results. The panel below and the spectral data on the following pages demonstrate the flexibility of the SA3800 system. Most importantly, results are comparable to those obtained using conventional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

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

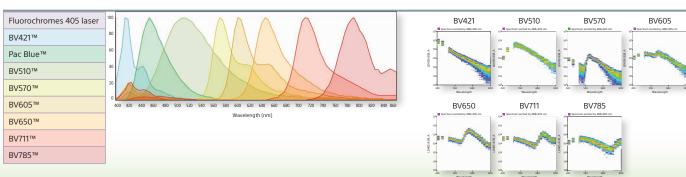
In this panel described in the table below, all lymphocytes were identified by staining with CD45. From the CD45+ population, B-cells (CD19), monocytes (CD14) and T-cells (CD3) were identified. The T-cell population was further analyzed to determine relative percentages of effector T-cells (CD8) and 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.

CD4-PEAF700



**Figure 16.** 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 (FCS) and side scatter (SSC) then CD45+. From that population several subsets were identified as indicated.

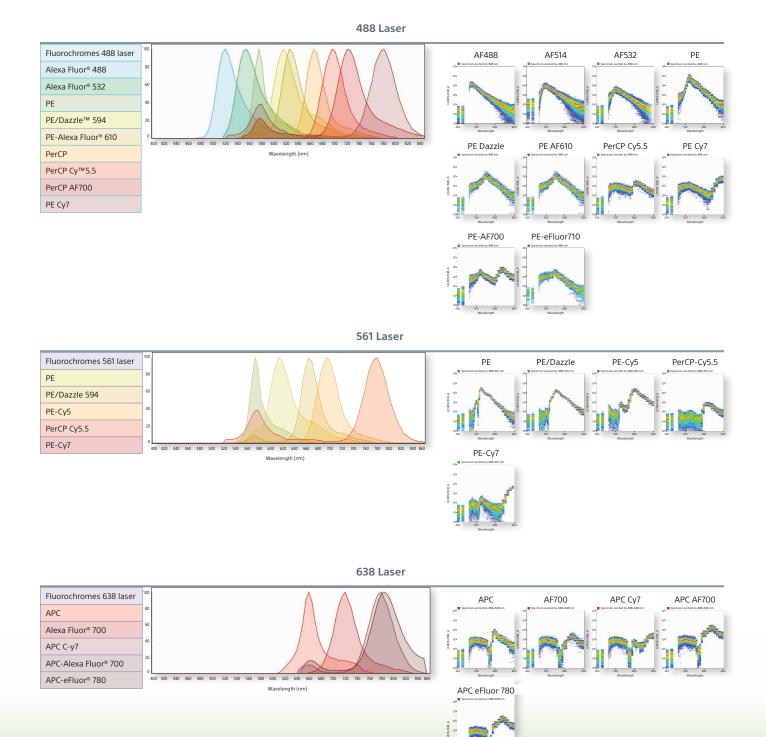


405 Laser

CD3-FITC

CD45-AF532

# Sample Data



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# Specifications

	ltem	Specifications			
	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)			
Optics /Performance	AD converter resolution	A/D 50MHz 16bit/20-bit effective resolution			
	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)			
	Sample volume rate	Two levels (Low, Normal)			
Fluidics	Cleaning	Auto Probe Cleaning, Priming, Shutdown-cleaning, Cuvette back flushing			
	Waste/DIW tank	Both tanks are 2L			
	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			
3D Autoloader	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)			
	Dimensions	W: 660 mm x H: 674 mm x D: 635 mm (SA3800 main body)			
Ancillary	Weight	95kg (AutoSampler model, does not include external tank holder)			
	Power Consumption	350 W Max			

# AutoSampler model

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-SA3800FA	4	488, 405, 638, 561
LE-SA3800GA	3	488, 405, 561

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