SONY

SP6800

Spectral Analyzer

Sony Biotechnology Inc.

SP6800 Spectral Analyzer

The Sony SP6800 Spectral Analyzer uses spectral technology to optimize sensitivity and enhance dim signal detection by collecting photons from 420nm to 800nm. Spectral technology also simplifies multicolor panel design, by eliminating band-pass filters and conventional compensation matrices while delivering better data and simplifying visualization for the study of heterogeneous populations.

Novel global standardization mode automatically sets the system to a master specification with a single click. This capability eliminates instrument variability from day to day and across multiple instruments for greater reliability.

Advanced electronics and patented optical technologies bring simplicity to Spectral Analysis workflows. Sony's patented Flowpoint™ core stability and tracking system and automated QC ensure the highest resolution possible of target populations.

The system features easy to use software that automates alignment and laser delay with set up wizards and simplified voltage settings. Each system includes FCS Express™ software in addition to Sony analysis packages to offer the highest flexibility in analysis.

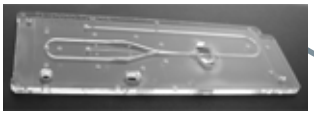
- **Spectral analysis technology optimizes sensitivity while simplifying application design and workflow.**
- **Enhances dim signal detection for better visualization of rare populations, fluorescent proteins, and fluorochromes excited by multiple lasers.**
- **Novel global standardization mode automatically sets the system to a master specification with a single click.**
- **Easy to use software features include automated alignment and laser delay via set up wizards, easy acquisition, and flexible analysis using both Sony and FCS Express software included with every system.**

SP6800 System Overview

The SP6800 spectral flow analyzer improves sensitivity and simplifies application design, workflow, and analysis over conventional flow cytometers. This is achieved using spectral analysis technology, advanced electronics, and patented optical technologies. These capabilities, unique to Sony Biotechnology systems, allow experienced and novice flow cytometrists to achieve greater flexibility for panel design and more accurate visualization of results.

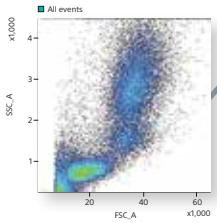
The 405nm, 488nm and 638nm excitation lasers are positioned to reduce fluorescent noise. They enable the system to support 16 or more fluorescent parameters.

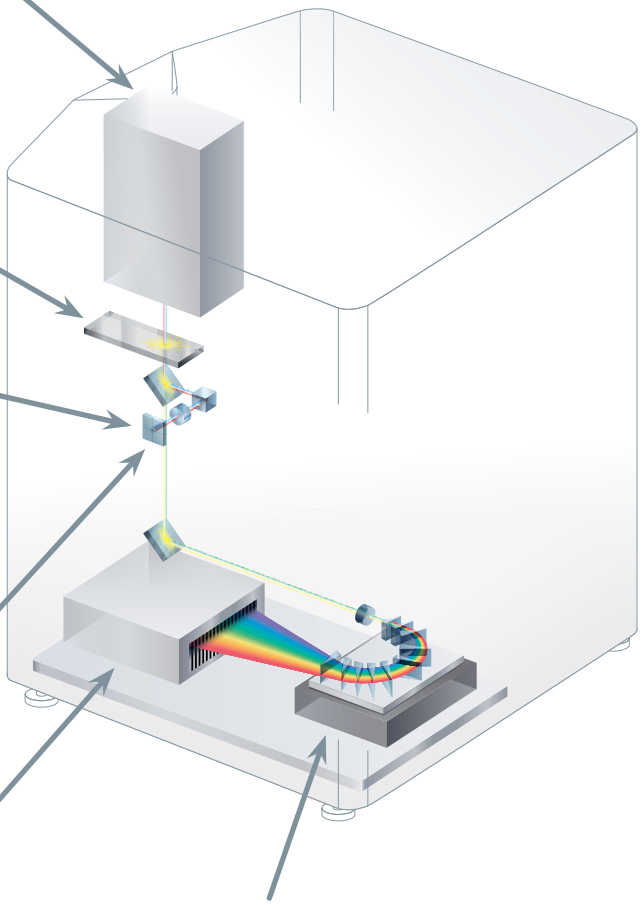
Microfluidics flow cell chip maximizes signal with auto positioning to guarantee high sensitivity. Made of durable plastic with an embedded quartz cuvette, the chip is easy to replace when needed.



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 visualizes core stability and enables the highest resolution.

Scatter analysis
Forward and Side Scatter parameters to allow relative size and complexity measurements.





Emitted light is directed through through a 32 Channel PMT that produces 66 data points of signal detection to analyze emitted photons from 420nm to 800 nm to ensure accurate visualization.

A unique prism collection system
Delivers light through 10 consecutive prisms allowing optimal signal separation while minimizing light loss.

Software

The SP6800 software is easy to learn and use. It guides researchers from set-up to panel design, acquisition, analysis, and shutdown.

User preferences allow users and administrators set up options for overall instrument operation and experiment set up to facilitate use and unattended operation 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 display 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.

Standardization

Standardization mode sets the system to a master specification to eliminate variability in a single instrument or among instruments located across sites. This unique capability allows experiments performed on any Sony cell analyzer to produce highly reliable, accurate, and reproducible results. This function also eliminates setup subjectivity for collaborative or long term studies with different operators or experience levels across sites.

When engaged, standardization mode sets the SP6800 to predetermined global settings that eliminate instrument variability.

Spectral Library

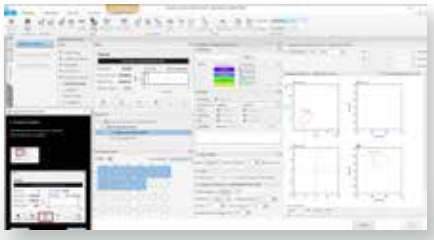
The Spectral Library lets users create a personal reagent library that simplifies experiment creation and saves time. An Acquisition Wizard assists the users with step by step instructions to acquire and analyze single positive controls for your spectral library. Once acquired the spectral reference for that reagent including the spectral index are available for future use. Information from the spectral library is available to users with a simple click improving accuracy and streamline panel design.

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. To increase sample acquisition to the cuvette, a variable booster lets users set acquisition speed from low (33ul/min) normal or high (250ul/min) offering flexibility.



System Shutdown

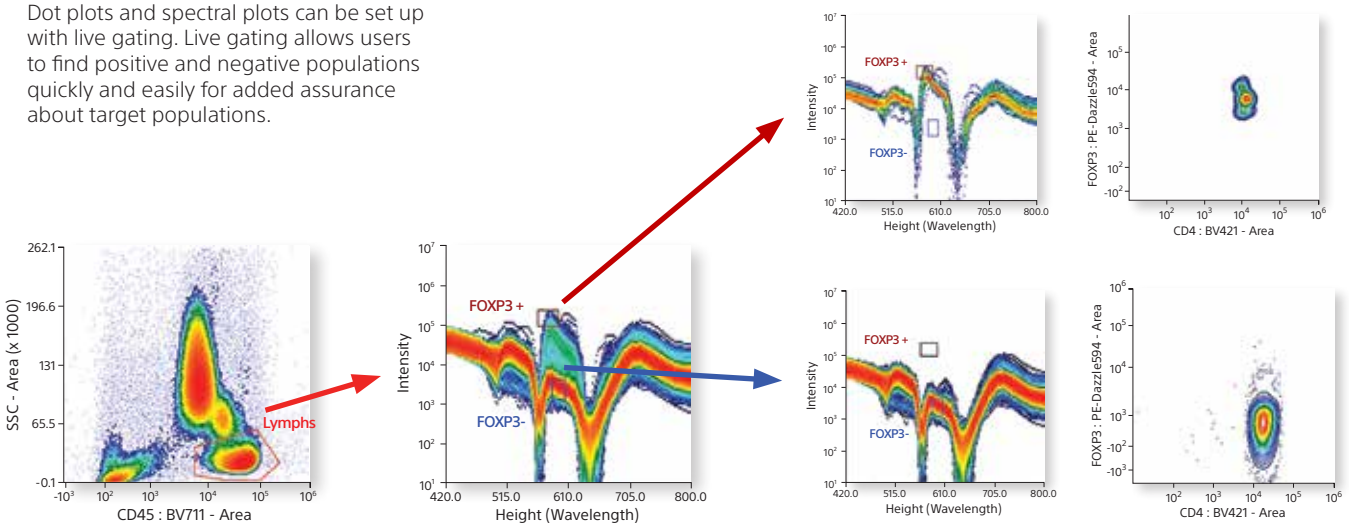
On shutdown, the SP6800 software guides the user though pre –shutdown cleaning and shuts down the instrument automatically. Software wizards are also available to guide users through Bleach Cleaning and Rinse procedures.

FCS Express from De Novo Software

Spectral Analyzers from Sony include FCS Express, from De Novo Software. FCS Express offers a range of new analysis tools from live gating to batch analysis. Native support for Sony Spectral data files to enable sophisticated data transformations and visualizations such as spectral overlays, tSNE, Spade, and heat maps.

Live Gating

Dot plots and spectral plots can be set up with live gating. Live gating allows users to find positive and negative populations quickly and easily for added assurance about target populations.



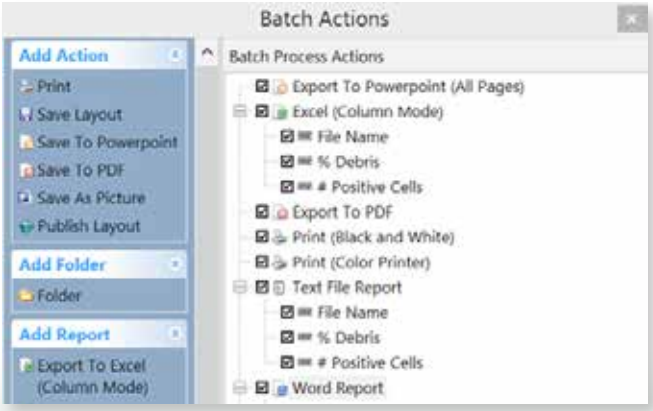
Flexible Analysis

Flexible data analysis can include integrated spreadsheets, custom calculations, charting and regression analysis.

	FITC	PE	PerCP-Cy5.5	PE-Cy7
M1	56.58	44.16	15.18	22.08
M2	1818.84	1718.1	506.46	311.88
M3	14735.64	14209.86	4364.94	2644.08
M4	41860.92	40173.18	14921.94	10414.86
M5	105084.24	101125.02	54574.86	46011.96
M6	202089.95	204531.17	195759.91	198312.21

Batch and Report

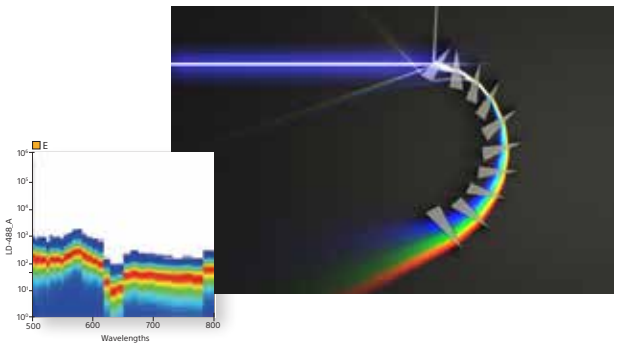
Batch analysis lets you process any number of samples with one click. To support presentation, data can be exported directly to PowerPoint, PDF, and Excel.



Spectral Analysis Technology

Spectral analysis technology is the foundation of the SP6800 system. Spectral flow cytometry streamlines workflow and yields better data by keeping all the light collected. In conventional systems, overlapping fluorescence is subtracted using color compensation, so less light is collected. Instead, spectral flow cytometers sum the fluorescence together and then use unmixing to mathematically separate the colors. This powerful capability also simplifies workflow including panel design, and improves visualization of autofluorescence.

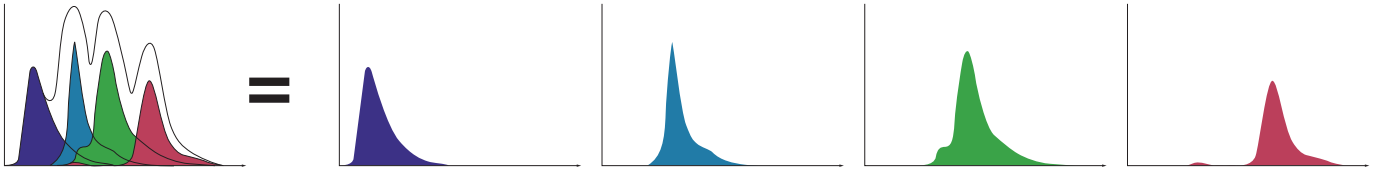
A unique prism collection system delivers emitted light to a 32 channel PMT. This produces 66 data points of signal detection for fluorescence and bright auto fluorescence to achieve accurate visualizations of fluorescent populations. This lets researchers see the complete spectral fingerprint of each fluorochrome from 420nm to 800nm.



Visualization of fluorescent cell populations using the analyzer's unique prism collection system.

Spectral Unmixing

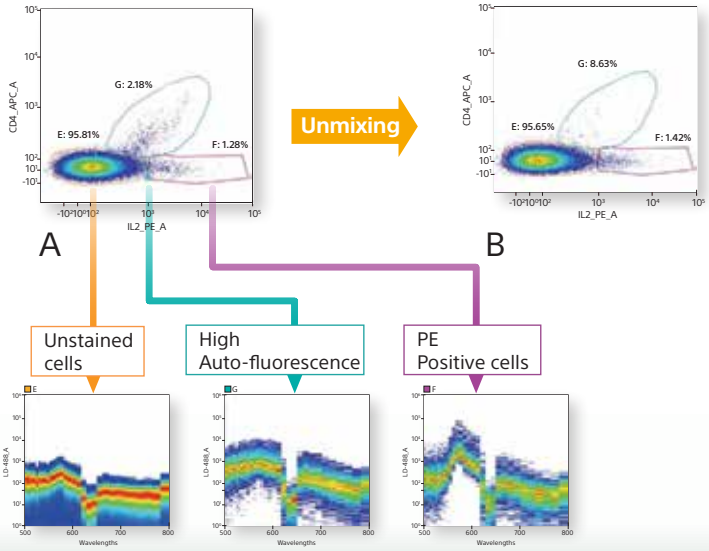
A powerful capability of spectral technology is Unmixing. This allows researchers to separate fluorophores into pure signals that measure the quantity of each fluorophore at each pixel to more accurately measure data for analysis.



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

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.

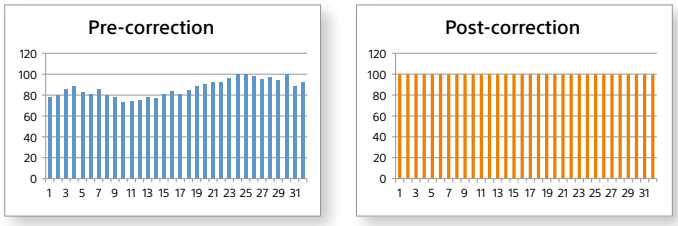
This also lets researchers separate the spectra of fluorophores masked by autofluorescence by extracting it from the signal and creating it into a unique fluorescent parameter. With a clear signal for each color channel unaffected by overlapping signals (spillover) and autofluorencence, spectral analysis yields better, unbiased data for analysis.



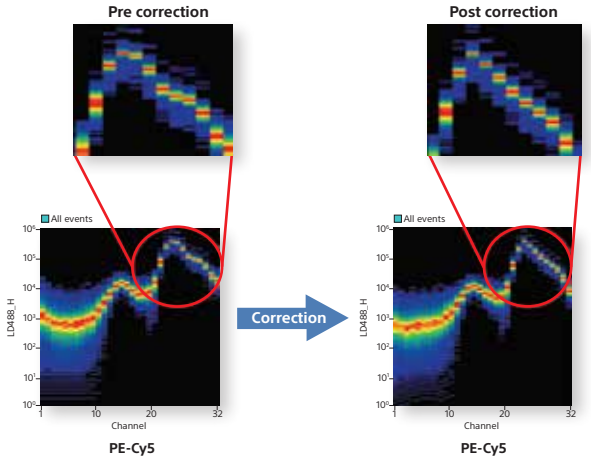
Spectral analysis reduces false-positives and delivers more accurate analysis over conventional flow cytometry. Mouse splenocytes were stained with CD4 APC and anti-IL-2 PE. ***A.*** *In this conventional density plot it is unclear if the light-blue region is a dim PE, weak double positive, or non-specific 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.*

Uniform measurement of Fluorescent Emissions

A correction system adjusts the offset and sensitivity of each channel of the 32 channel PMT to ensure a uniform and accurate measurement of fluorescent emission from 420nm to 800nm. The corrective system brings a standardization to all 32 PMTs ensuring the user is getting the most reliable data with the ease of adjusting only one voltage. This saves time over conventional flow cytometry operation where users must calibrate each individual PMT.



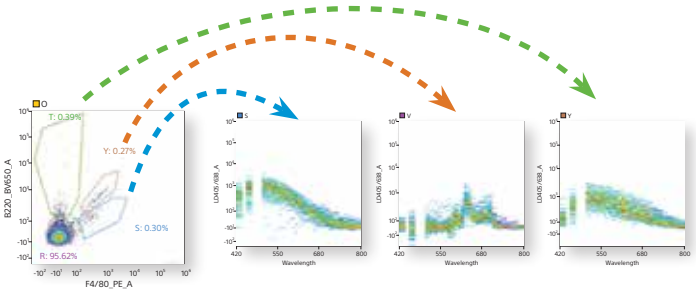
Pre and Post Correction Profile. Each graph illustrates pre and post correction in the SP6800 32 channel PMT. The correction improves accuracy of spectral visualization.



Spectral emission of PE Cy5 pre and post correction. Corrections support accurate unmixing of closely overlapping fluorescence spectra.

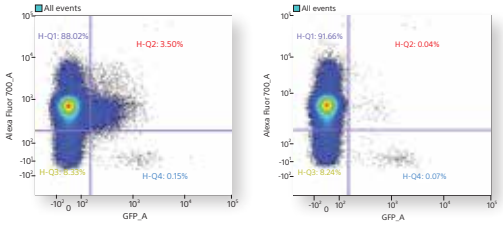
Subtracting Auto-Fluorescence Improves Visualization

In conventional flow cytometry cellular auto-fluorescence produced by pyridine (NAD/NADH), flavin (FMN, FAD), and other intracellular oxidative reactions can cause fluorescent signal contamination of other fluorescent markers. Other common sources of auto-fluorescence include cell fixation and permeabilization. Using spectral technology, auto-fluorescent spectral fingerprints can be subtracted to allow researchers to see the true fluorescent population.



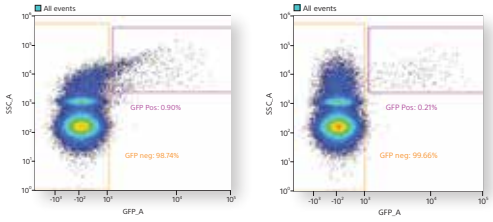
Unstained mouse splenocytes were analyzed with the SP6800 revealing three distinct auto-fluorescent populations. Using the spectral fingerprints obtained in analysis, the appropriate auto-fluorescence can be removed, increasing the precision and quality of results.

A. With Auto-fluorescence **B.** Without Auto-fluorescence



Activated T Cells with GFP and Alexa Fluor 700

C. With Auto-fluorescence **D.** Without Auto-fluorescence

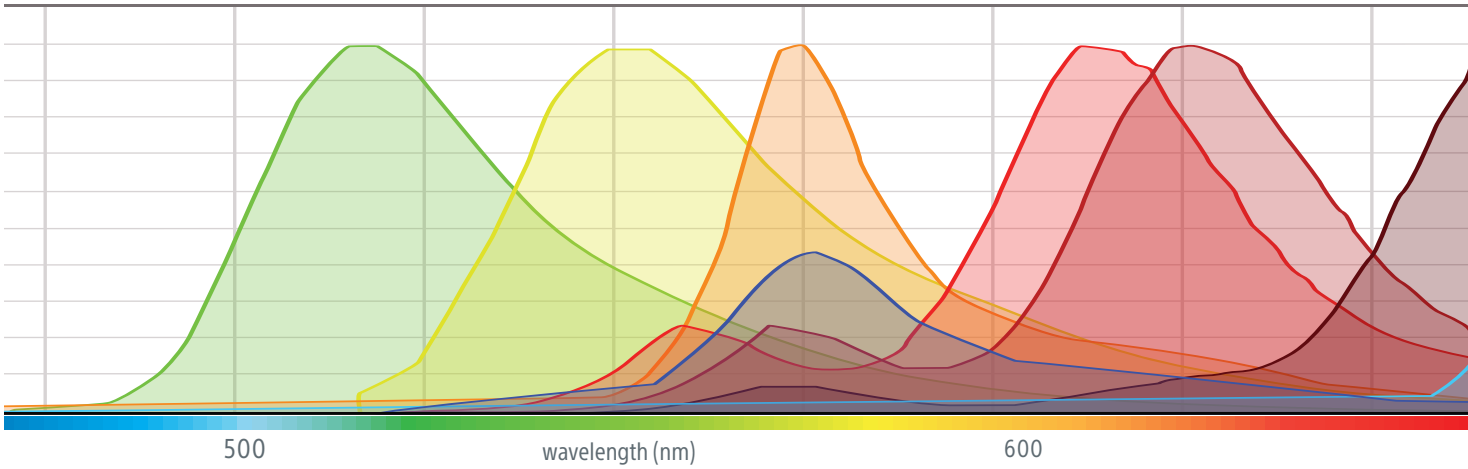


Liposomes with GFP

*Auto-fluorescence can result in the appearance of additional cell populations leading to erroneous data interpretation. **A.** T cells expressing GFP and stained with an antibody conjugated to Alexa Fluor® 700 were analyzed with the SP6800. A double positive population is present in the uncorrected density plot. **B.** This double positive population disappears when the auto-fluorescent spectral fingerprint is subtracted. **C.** Liposomes from cells expressing GFP were analyzed. **D.** This uncovered a small positive GFP population after auto-fluorescence was subtracted.*

Fluorochrome Panel Guide

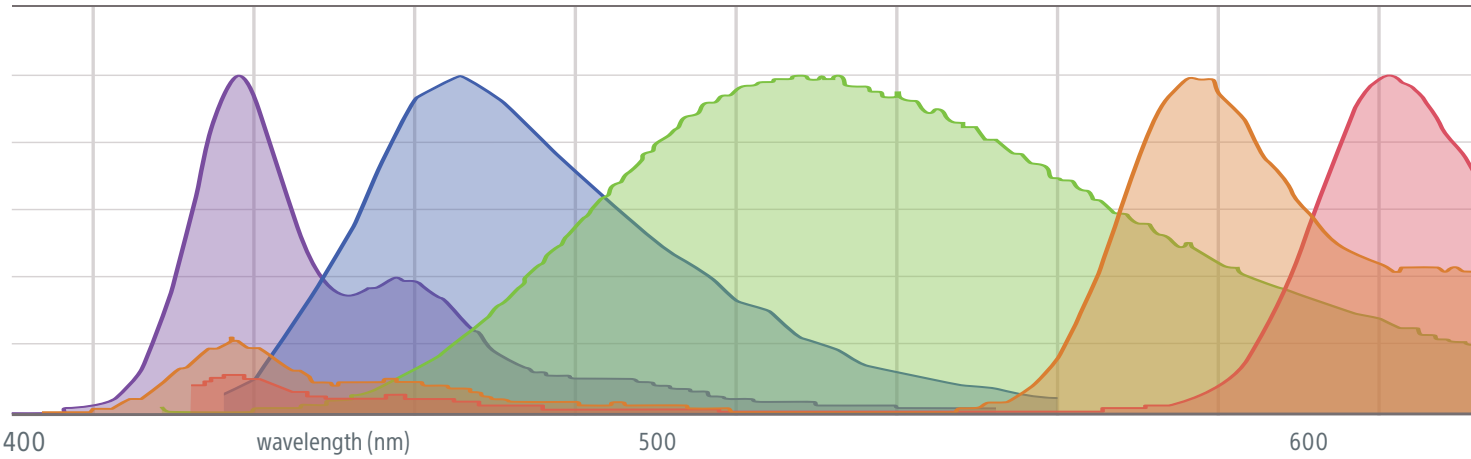
Application data dye options and combinations. Select one from each group.



Group 1	Group 2	Group 3	Group 4
Alexa Fluor® 488; 488nm	Alexa Fluor 532; 488nm	PE; 488nm	PE Dazzle™ 594; 488nm
Brilliant™ Blue 515; 488nm	Alexa Fluor 514; 488nm		PE Texas Red®; 488nm
FITC; 488nm			

Laser

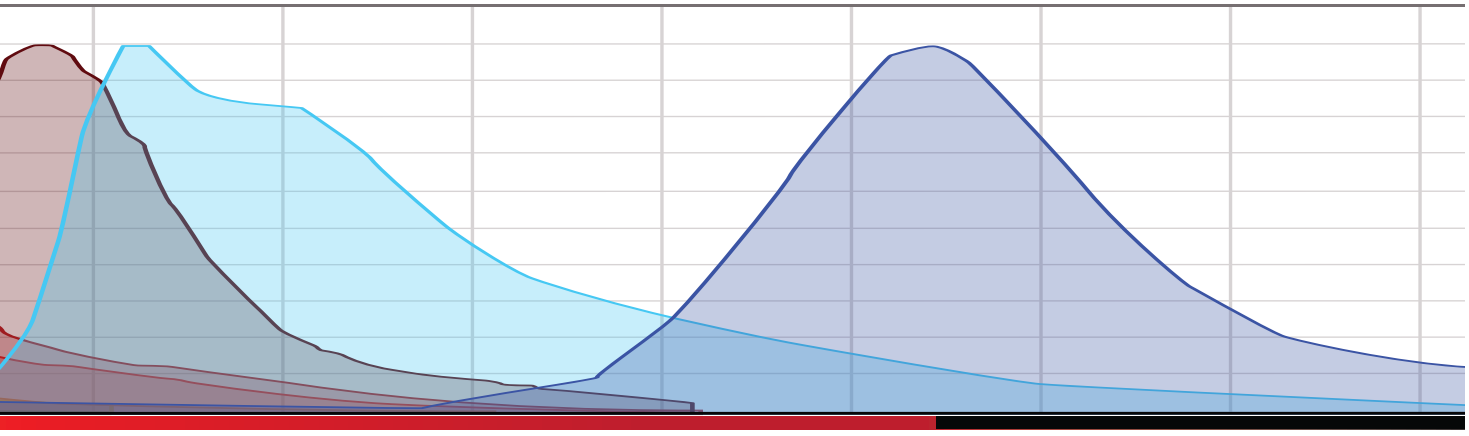
- 488nm



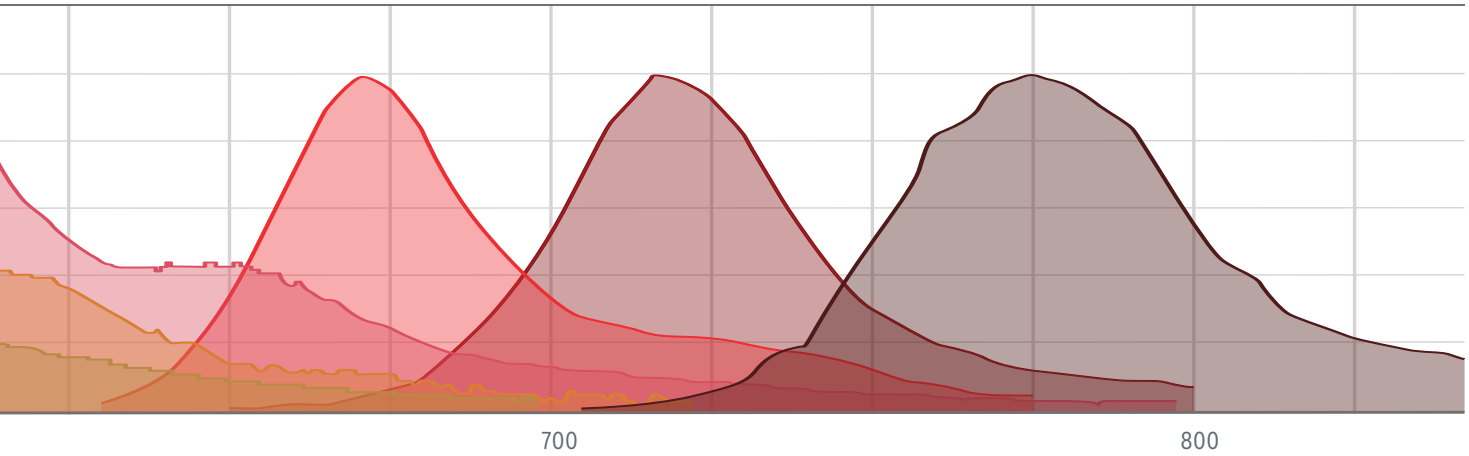
Group 9	Group 10	Group 11	Group 12
Brilliant Violet™ 421; 405nm	Pacific Blue™; 405nm	Brilliant Violet 510™; 405nm	Brilliant Violet 570™; 405nm
Alexa Fluor 405; 405nm	Horizon™ V450; 405nm	AmCyan; 405nm	Pacific Orange™; 405nm
		Horizon V500; 405nm	
		Krome Orange™; 405nm	

Laser

- 405nm
- 638nm



Group 5	Group 6	Group 7	Group 8
PE Alexa Fluor 610; 488nm	PE Cy5™; 488nm	PerCP Cy5.5; 488nm	PE Cy7; 488nm
	PerCP eFluor 710; 488nm	PE Cy5.5; 488nm	PE Vio®770; 488nm
	PerCP; 488nm		
	PE Alexa Fluor 700; 488nm		



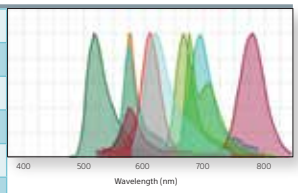
Group 13	Group 14	Group 15	Group 16
Brilliant Violet 605™; 405nm	Brilliant Violet 650™; 405nm	Brilliant Violet 711™; 405nm	Brilliant Violet 785™; 405nm
Qdot® 605; 405nm	Qdot 655; 405nm	Qdot 705; 405nm	Qdot 800; 405nm
eVolve™ 605; 405nm	eFluor 660; 405nm	Alexa Fluor 700; 638nm	APC Cy7; 638nm
	Alexa Fluor 647; 638nm	APC Cy5.5; 638nm	APC Alexa 750; 638nm
	APC; 638nm		APC eFluor 780; 638nm
	Cy5; 638nm		

16 color panel on Human Peripheral Blood

Application data using 2 lasers

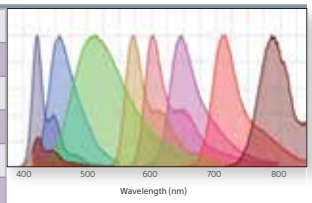
488nm Laser

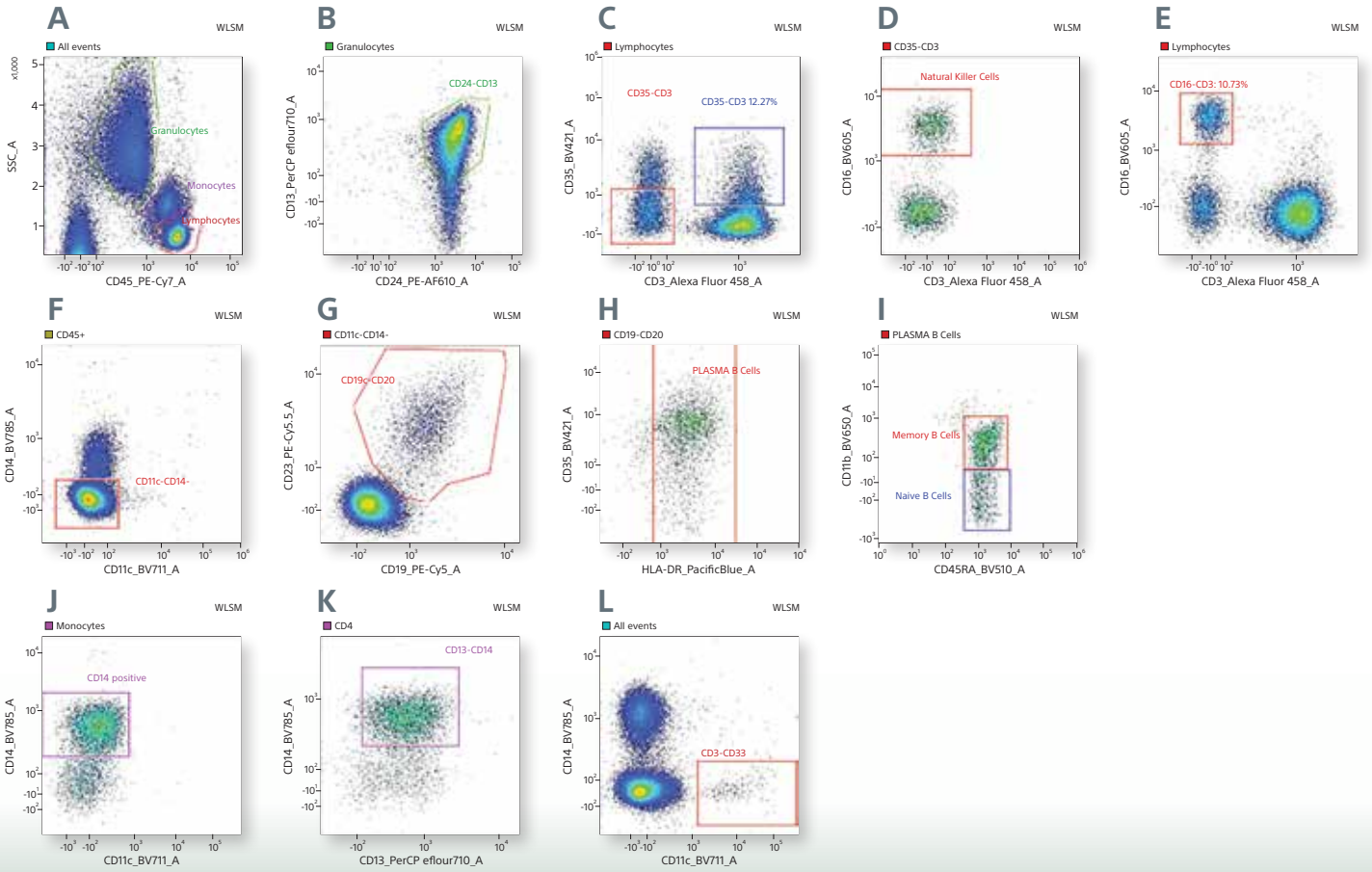
CD3	Alexa Fluor 488
CD8	PE
CD4	PE Dazzle 594
CD24	PE Alexa Fluor 610
CD19	PE Cy5
CD20	PE Cy5.5
CD13	PerCP efluor® 710
CD45	PE Cy7



405nm Laser

HLA-DR	Pacific Blue
CD38	BV421
CD45RA	BV510
CD33	BV570
CD16	BV605
CD11b	BV650
CD11c	BV711
CD14	BV785





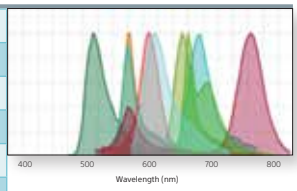
Examples of the resolution of several important cell populations using only two lasers with the SP6800. Clear population resolution is obtained with highly overlapping fluorochomes such as PE-Cy5/ PE-Cy5.5 (G) and Pacific Blue/BV421 (H).

16 color stained sample of Human Peripheral Blood

Application data using 3 lasers

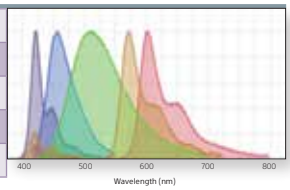
488nm Laser

CD3	Alexa Fluor 488
CD8	PE
CD4	PE Dazzle 594
CD24	PE Alexa Fluor 610
CD19	PE Cy5
CD20	PE Cy5.5
CD13	PerCP efluor 710
CD45	PE Cy7



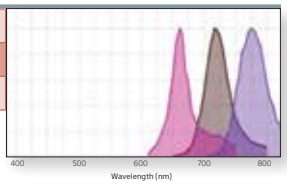
405nm Laser

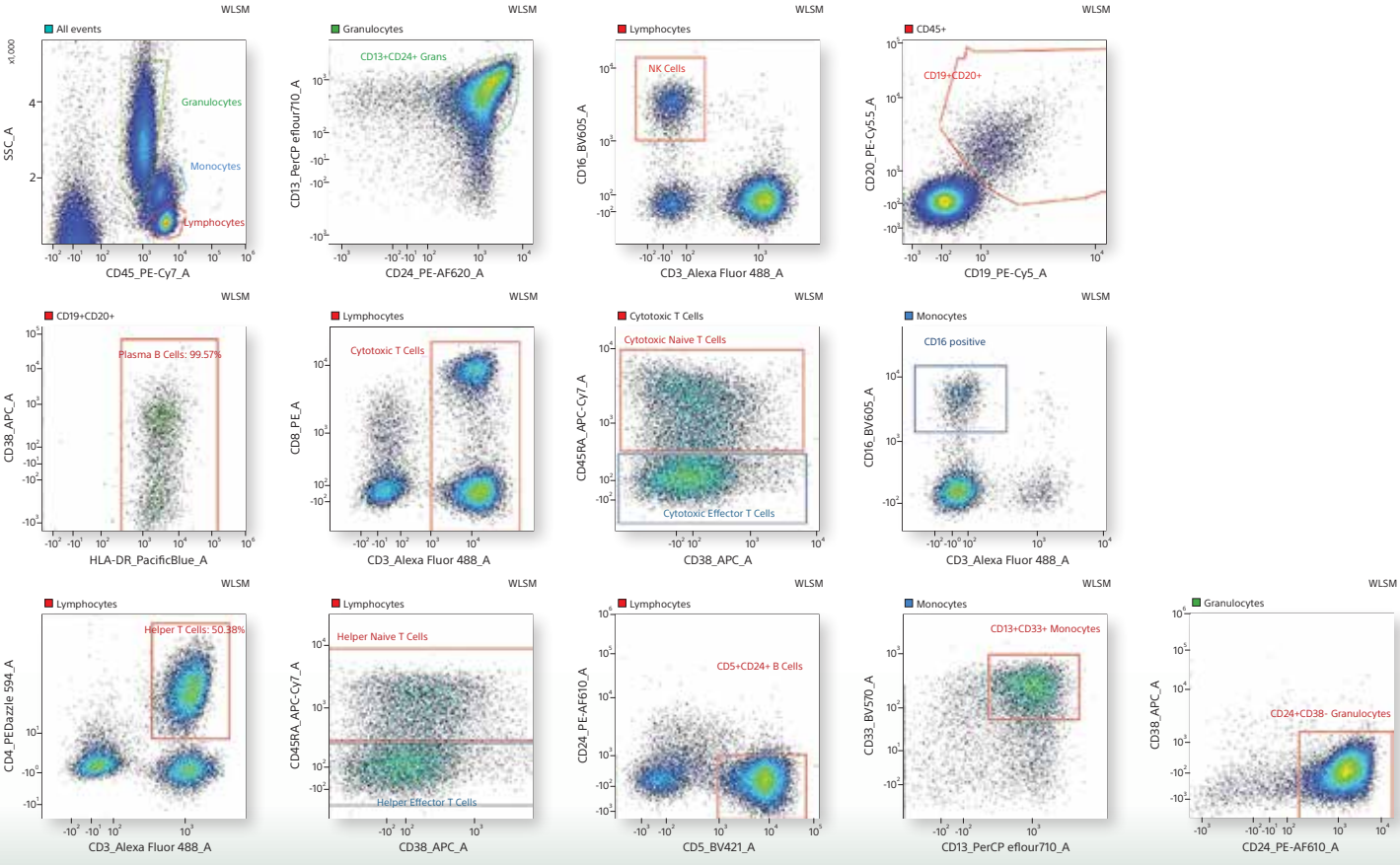
HLA-DR	Pacific Blue
CD5	BV421
CD56	BV510
CD33	BV570
CD16	BV605



638nm Laser

CD38	APC
CD11c	Alexa Fluor 700
CD45RA	APC-Cy7

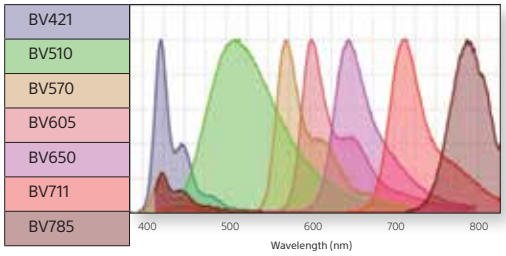


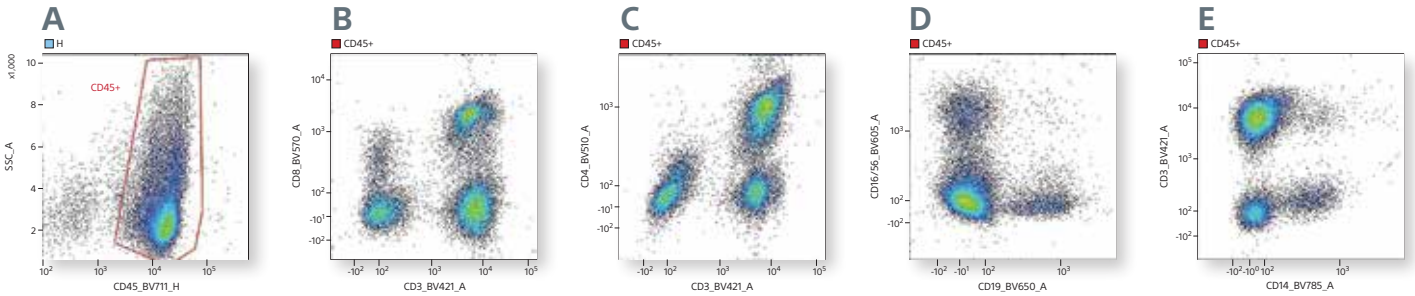


Examples of the detection of several important cell populations using sixteen fluorochromes excited by three lasers. Unlike conventional flow cytometers that can detect up to five off the blue, in this example we demonstrate that with the SP6800 eight fluorochromes can be excited off the blue with clear sample resolution. Even highly overlapping fluorchromes such as PE-Cy5 and PE-Cy5.5 can be resolved.

Application Data: Brilliant Violet Dyes

Spectral Analysis allows multi-laser excited fluorochromes to be run without using a complicated compensation matrix.



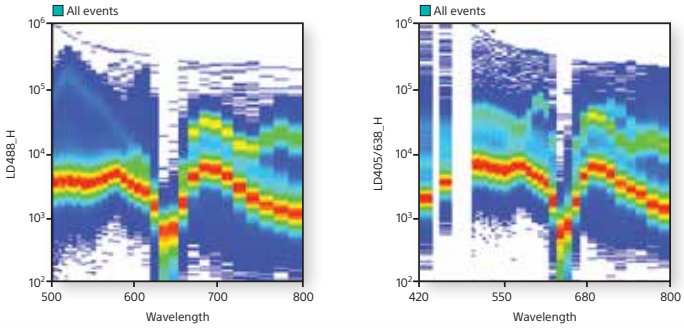


Sample data from Human PBMCs stained with seven markers conjugated to Brilliant Violet dyes offered by Sony Biotechnology. ***D.*** *All populations were clearly resolved including fluorochomes with significant spectral overlap such as BV605 and BV650 (****D).***

12-color Staining of Human Peripheral Blood Leukocytes

Example of Human Peripheral Blood Leukocytes was analyzed on the SP6800 and a BD LSRFortessa conventional flow cytometer. Spectral analysis was able to remove the autofluorescenece and use unmixing to separate each fluorochrome which resulted in clarity and resolution of each population in these density plots.

Marker	Fluorochrome	488nm Laser	405/638nm Laser
CD45RA	Alexa Fluor 488		
TCR-gd	PE		
CD56	PE-Dazzle594		
CD45	PerCP-Cy5.5		
CD3	PE-Cy7		
CCR7	BV421		
CD27	BV510		
CD33	BV570		
CD4	BV605		
CD19	APC		
CD8	Alexa Fluor 700		
HLADR	APC-Cy7		



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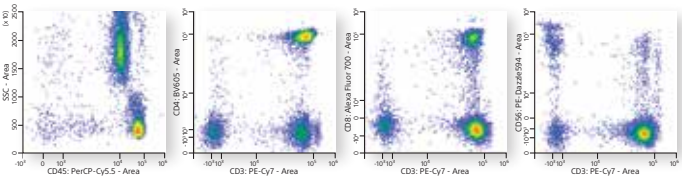
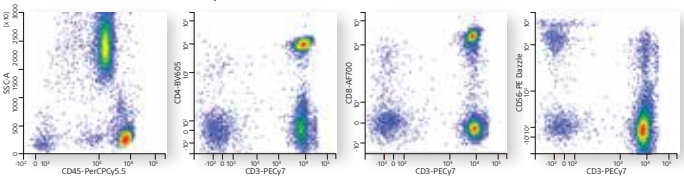
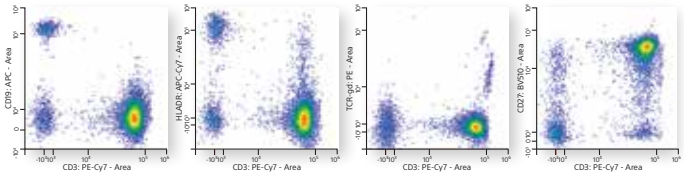


Conventional Flow Cytometer

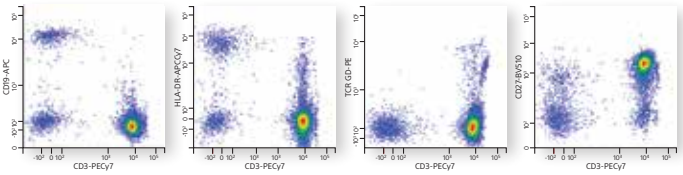


2

Sony SP6800 Spectral Analyzer

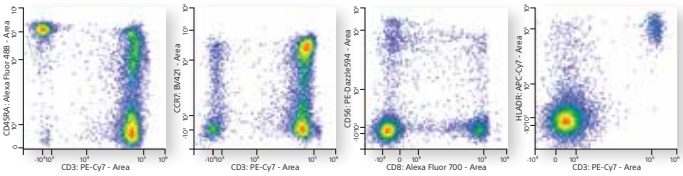


Conventional Flow Cytometer

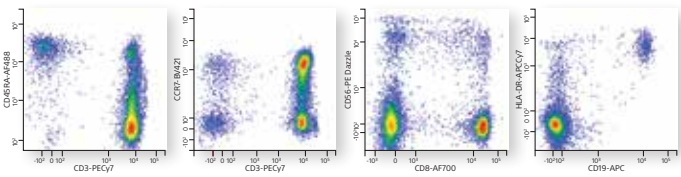


3

Sony SP6800 Spectral Analyzer

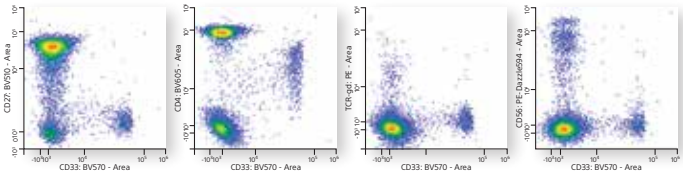


Conventional Flow Cytometer

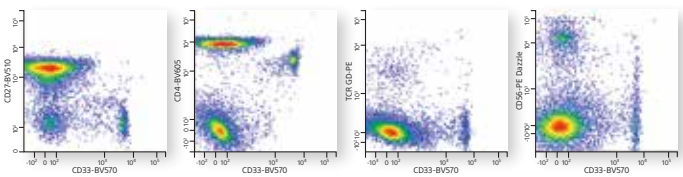


4

Sony SP6800 Spectral Analyzer

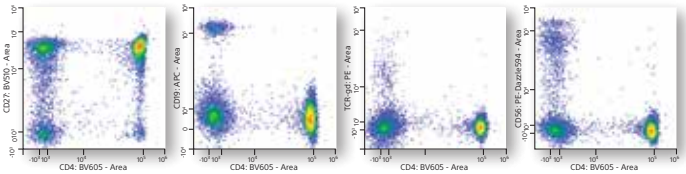


Conventional Flow Cytometer

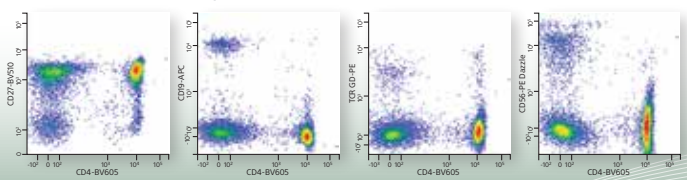


5

Sony SP6800 Spectral Analyzer

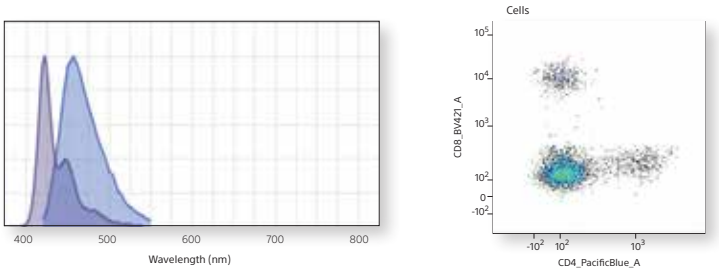


Conventional Flow Cytometer



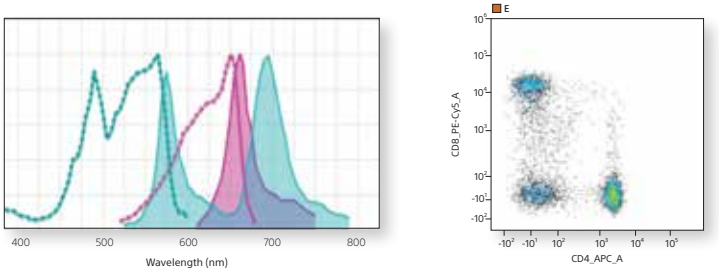
Application Data: Dyes with issues

Common problems in flow cytometry occur when running fluorochromes with emission peaks that are too close to one another, multi-laser excitations, fluorescent proteins, and unstable tandems. The SP6800 is capable of analyzing all of these by looking at all photons from 420nm to 800nm and unmixing each unique spectral fingerprint.

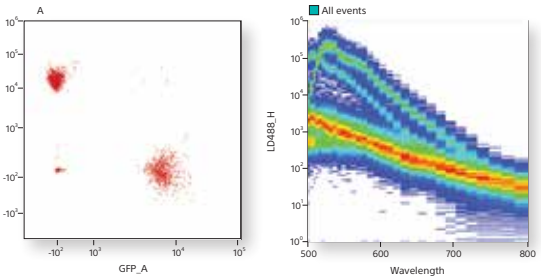


Example 1: *Pacific Blue (457nm emission) and Brilliant Violet 421 (422nm emission)*

APC excitation
APC emission
PE Cy5 excitation
PE Cy5 emission



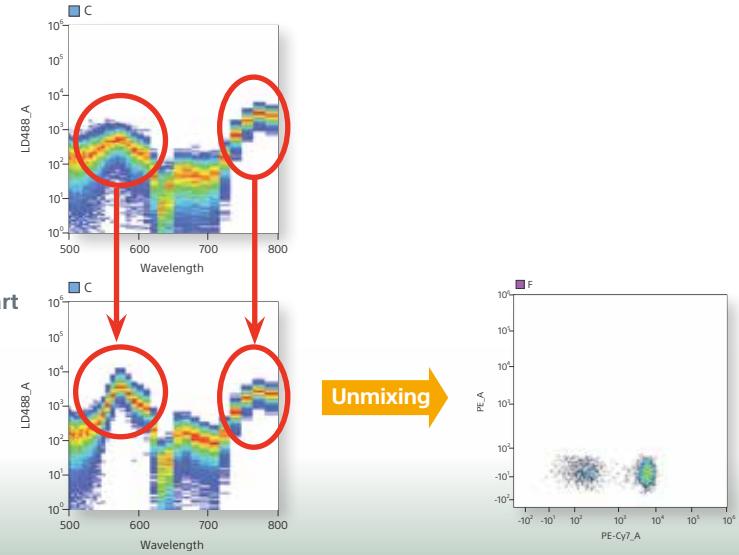
Example 2: *PE Cy5 (excited with 488nm and 638nm lasers) and APC (excited with 638nm laser)*



Example 3: *Fluorescent GFP and YFP need no special bandpass filter set with the SP6800*

Normal PE Cy7

PE Cy7 falling apart



Example 4: *Spectral Analysis unmixes the spectral fingerprint the spectral fingerprint of PE Cy7 tandem to produce excellent resolutions.*

Specifications

Configuration		2 laser model, 488/638	2 laser model, 405/488	3 laser model,405/488/638
Model Number		**LE-SP6800ZB**	**LE-SP6800ZC**	**LE-SP6800ZE**
Optics	**Laser specification**			
	Lasers	Semiconductor 2 laser model 488nm, 638nm	Semiconductor 2 laser model 405/488	Semiconductor 3 laser model, 405/488/638
	Maximum power (at flow cell)	488nm 40mW 638nm 60mW	405nm 60mW 488nm 40mW	405nm 60mW, 488nm 40mW 638nm 60mW
	Irradiation form	2-spot dual-axis irradiation		
	Detection optics			
	Scatter signals	Forward scatter (FSC), Side scatter (SSC)		
	Spectroscopic method	Prism array spectroscopy		
	Fluorescent channels	32 channel PMT (wavelength: 500-800nm)	32 channel PMT (wavelength: 500-800nm) PMT x 2 (420-440nm, 450-470nm)	
System	Signal resolution	Height 20 bit, Area 32 bit Sampling frequency: 50MHz		
	Measurement parameters	64 channels, FSC, SSC, cell position XY	66 channels, FSC, SSC, cell position XY	
	Pulse shape parameters	Height, Area, Width		
	Optical alignment	Automated alignment with Corefinder™ technology		
	Event Rate	10,000eps (standard)/20,000eps (maximum)		
Fluidics	Sample loader type	Single auto-loader		
	Supporting sample tube	5ml polystyrene round tubes		
	Sheath Flow Speed	Low (approx. 3m/s), Mid (approx. 5m/s), High (approx. 10m/s)		
	Detection channel dimensions	200µm x 200µm		
Performance	Fluorescence sensitivity	FITC: 120 MESF / PE: 70 MESF		
	Linearity	FITC R^2 ≧ 0.995 / PE R^2 ≧ 0.995		
	Detection size range	0.5~40µm (beads)		
	Fluorescence detection resolution	488nm Laser/CH16, 638nm Laser/CH27* : ≧2.5% (HPCV)		
Software	Unmixing algorithms	Spectrum method (LSM, WLSM, PSA and Constraint option), Reverse matrix method (Conventional)		
	Autofluorescence	Autofluorescence spectral detection		
	Virtual Filter	Possible to change wavelength region for each fluorochromes in analysis		
	Export Data Format	Flow Cytometry Standard (FCS) 3.0, 3.1		
Main Unit	Dimensions	Main unit: Width 60.0cm x Depth 63.5cm x Height 71.3cm Fluidics cart: Width 78.6cm x Depth 52.1cm x Height 58.0cm		
	Weight	Main unit: approx. 99kg (dried weight)/Fluidics cart: approx. 32kg (dried weight)		
	Air pressure supply	350kPa~450kPa (51psi~65psi)		
	AC Power supply	AC100V 50/60Hz, AC120V 60Hz		
	Power consumption	220W (max)		
	Operating temperature	16-29 degrees Celsius, Room temperature variation: within 5 degrees Celsius		
	Operating humidity	20% to 80% (condensation free)		
	Recommended PC	SP6800 workstation		

** Except for LE-SP6800ZC (This HPCV value is calculated for red laser.)*

North America/International

1730 North First Street
San Jose, CA 95112 U.S.A.
Voice: +1 800-275-5963
FAX: +1 408-352-4130
sales@sonybiotechnology.com
http://www.sonybiotechnology.com

Japan

1-7-1, Konan, Minato-Ku,
Tokyo, 108-0075 Japan
Tel: +81 120-677-010
Fax: +81 120-388-060
sales_Japan@sonybiotechnology.com
http://www.sony.co.jp/LS

Europe

The Heights, Brooklands.
Weybridge, Surrey, KT13 0XW, UK
sales_EU@sonybiotechnology.com

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