SONY

ID7000™

Spectral Cell Analyzer

Sony Biotechnology Inc.

STATUS

ERROR

WASH

POWER

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ID7000™ Spectral Cell Analyzer

Shaping the Landscape of Spectral Flow Cytometry

The ID7000™ Spectral Cell Analyzer is a state-of-the-art system blending intuitive workflows with advanced features, making it a powerful tool for high-parameter cell analysis.

Reliability Meets Capability – The ID7000 can be configured with up to **7 lasers and 186 detectors,** the most of any flow cytometer available today. Laser choices range from deep ultraviolet (320 nm) to infrared (808 nm), enabling the system's detection capability to support expansion of multicolor panels as well as future fluorochrome development. Researchers can perform experiments using 44 colors or more, **limited only by fluorochrome availability.**

Intuitive Software Workflows – Ease of use is one of the core ideologies behind the ID7000, which is why the system software is equipped with novel features that provide researchers their results quicker than ever before.

Automated Setup and QC – Ensures the system is performing optimally. Levey-Jennings plots can be generated to monitor system performance over time.

Spectral Reference Library – Eliminates the need for single-color controls to be acquired for every new experiment, saving valuable reagents and increasing efficiency.

Spectral Reference Adjuster – Allows for any unmixing errors to be corrected without having to re-run single-color controls.

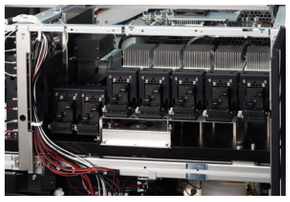
Autofluorescence Finder – Enables autofluorescence to be identified and used as a color in the unmixing matrix, resulting in improved data resolution.

Advanced AutoSampler – Included in the base configuration of the ID7000, the versatile AutoSampler enables true walkaway operation and automatic clog detection, and supports a large variety of sample formats.

The ID7000 is an excellent fit across multiple research areas, providing endless opportunities to unlock deeper scientific insights.

Exploring the ID7000's Innovative Technologies

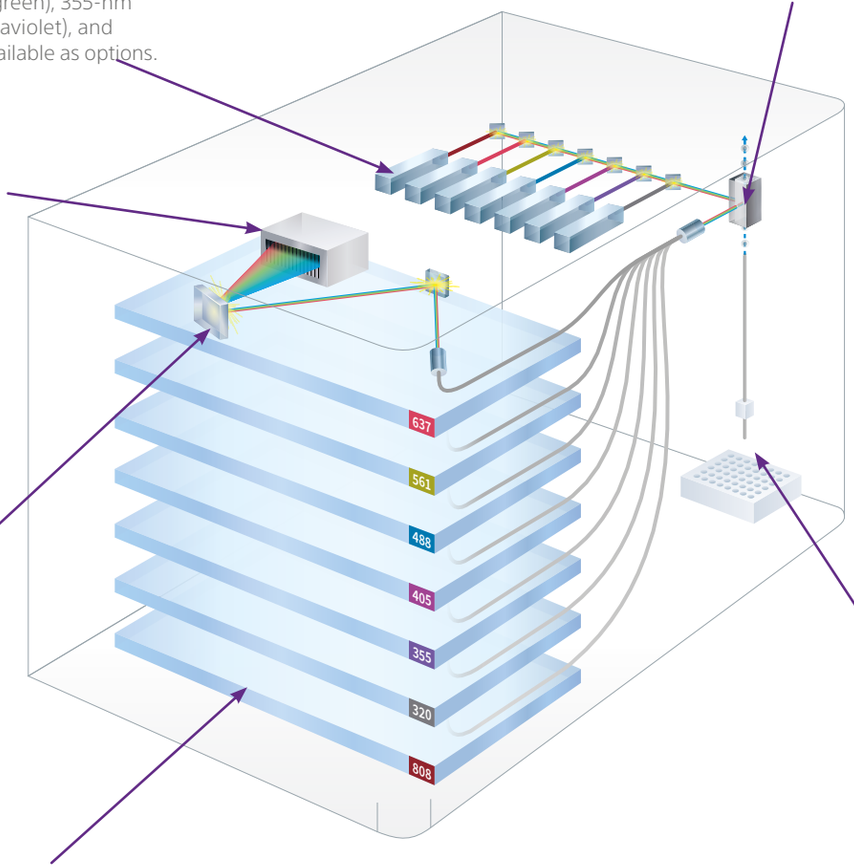
- **Advanced optical design** provides best-in-class data so that researchers can collect the most information from every sample.
- **Signal processing technologies** reduce electronic noise and are central to achieving high sensitivity in a flow cytometer.
- **Excitation and detection optics** allow users to select even the most spectrally similar fluorochromes and resolve signals with confidence.
- **As a pioneer in spectral technology,** Sony has unlocked the full potential of spectral analysis by providing an unparalleled number of lasers and PMT detectors.



Supports up to seven spatially separated lasers. The 488-nm (blue), 637-nm (red), and 405-nm (violet) lasers are standard on all systems. The 561-nm (yellow-green), 355-nm (ultraviolet), 320-nm (deep ultraviolet), and 808-nm (infrared) lasers are available as options.

Optimized flow cell design for highest data quality.

Up to 186 detectors including IR-sensitive PMTs in a 7-laser configuration.

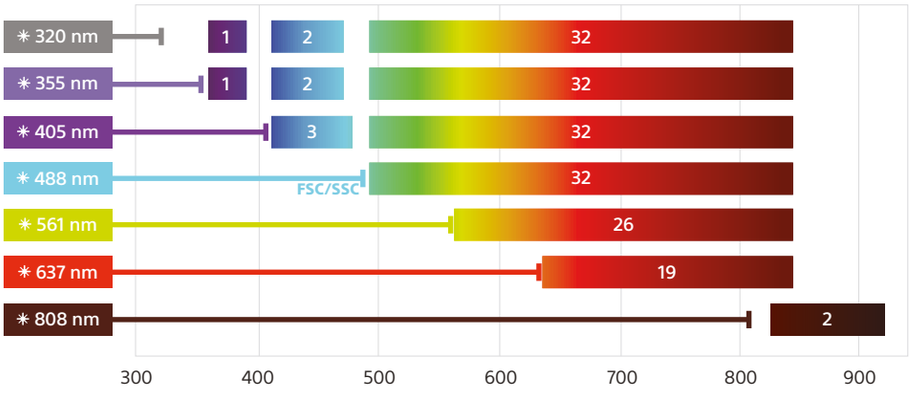


A newly designed light diffraction grating maximizes photon capture from 494 to 844 nm by distributing light evenly across the surface of each 32-channel PMT array, providing a wider angle of incidence and ~10 nm of resolution across the entire detector array.

The modular design of the detection decks allows each deck to be installed independently, providing the flexibility to perform field upgrades.

The standard AutoSampler provides onboard mixing and sample cooling capabilities to simplify operation and ensure accurate results.

Optics



The detection capacity of the ID7000 Spectral Cell Analyzer spans from 360 nm to 920 nm in the 7-laser configuration. The signal is captured using a combination of 32-channel PMT arrays and individual PMTs. The signal above 800 nm is detected with specialized InGaAs PMTs to ensure data quality.

- **Excitation Optics –** Optimized for superior fluorochrome excitation and emission, ensuring clear resolution across the detector array to detect dim and rare markers.
- **Configurations Suitable for Any Laboratory's Specific Needs –** The ID7000 is equipped with three standard excitation lasers found across all available configurations: the 405-nm (violet), 488-nm (blue), and 637-nm (red) lasers. Optional 561-nm (yellow-green), 355-nm (ultraviolet), 320-nm (deep ultraviolet), or 808-nm (infrared) lasers support a wide variety of applications.
- **Expanded Detector Availability Empowers Discovery –** Photomultiplier tubes (PMTs) have been chosen for their high signal-to-noise performance to capture the most photons, leading to higher resolution and lower background, which enhances dim signal detection for superior visualization of rare populations.
- **Spectral fingerprints** are generated when PMTs (32-channel, single-channel, and single-channel IR-sensitive) collect emitted light from 360 nm to 920 nm. Photon capture across the entire spectrum is maximized by a light diffraction grating based collection system, which distributes light evenly across the surface of each 32-channel PMT array. This new, robust system minimizes light loss and captures the entire spectral profile without data stitching.

Harnessing the Power of Spectral Data

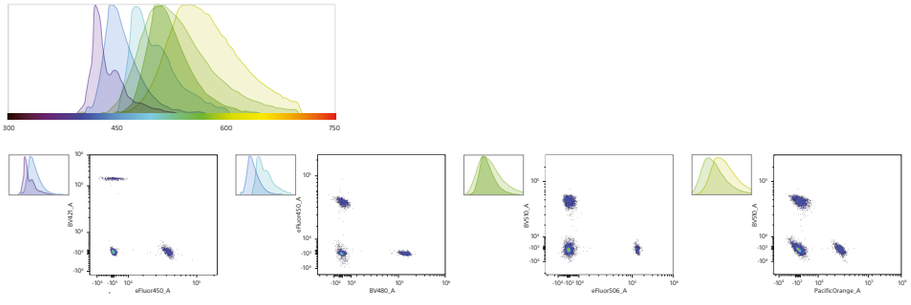
The ID7000 Spectral Cell Analyzer builds on Sony's experience with spectral analysis and simplifies many operations, even for complex experiments. With the true signal for each fluorochrome unaffected by autofluorescence or subjective adjustment of spillover, spectral analysis yields cleaner, unbiased data for every experiment.

Spectral Unmixing

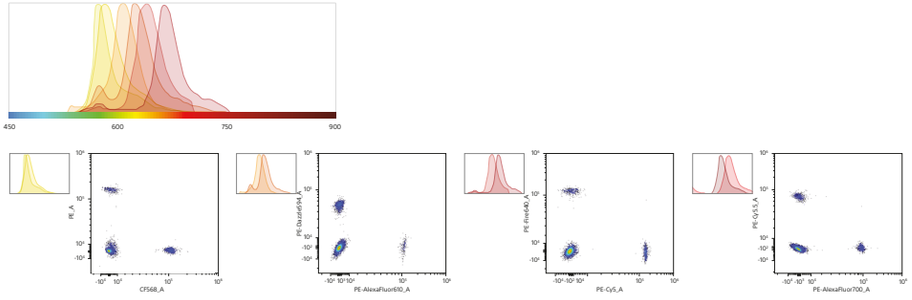
In spectral analysis, signals from all detected channels are used to create one spectral emission signal, regardless of the number of fluorochromes analyzed. Unmixing, a powerful capability, then separates fluorophores into pure signals that measure the intensity of each fluorophore at each wavelength to more accurately measure data for analysis. The ID7000 uses the WLSM (Weighted Least Squares Method) fluorescence unmixing algorithm to separate the individual spectral fingerprints and enable scientists to analyze dim and rare phenotypic marker expression.

Unmixing delivers a more comprehensive picture of rare populations while decreasing the complexities associated with working with fluorescent proteins and fluorochromes excited by multiple lasers. Overall, spectral technology simplifies multicolor panel design by eliminating the use of bandpass filters and highly subjective, conventional compensation matrixes.

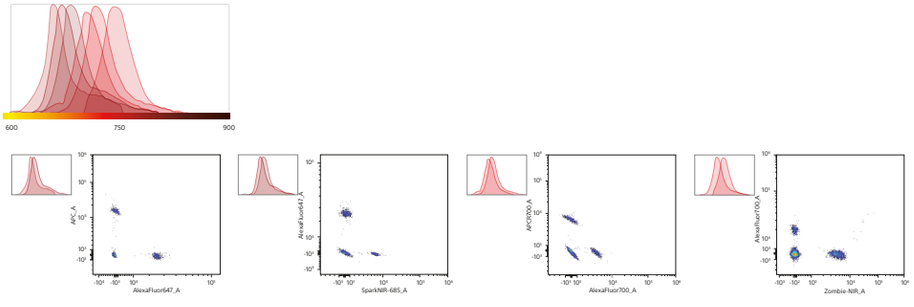
A. Multiple fluorochromes with overlapping emission profiles excited by the 405-nm laser



B. Multiple fluorochromes with overlapping emission profiles excited by the 561-nm laser



C. Multiple fluorochromes with overlapping emission profiles excited by the 637-nm laser



Compensation beads were stained with three different sets of fluorescent antibodies, and excited by the 405-nm, 561-nm, and 637-nm lasers as described. Data was analyzed on the ID7000 Spectral Cell Analyzer.

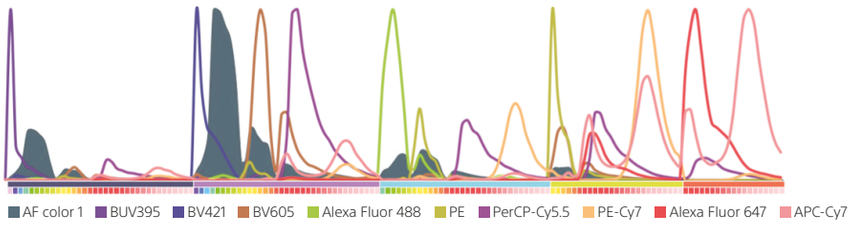
A. For the fluorochromes BV421/ eFluor™ 450/ BV480/ BV510/ eFluor™ 506/ Pacific Orange™ excited by the 405-nm laser, distinctly resolved emission peaks and populations were observed.

B. For the fluorochromes PE/ CF® 568/ PE/Dazzle™ 594/ PE-Alexa Fluor® 610/ PE/FIRE™ 640/ PECy™5/ PE-Cy™5.5/ PE-Alexa Fluor® 700 excited by the 561-nm laser, distinctly resolved emission peaks and populations were observed.

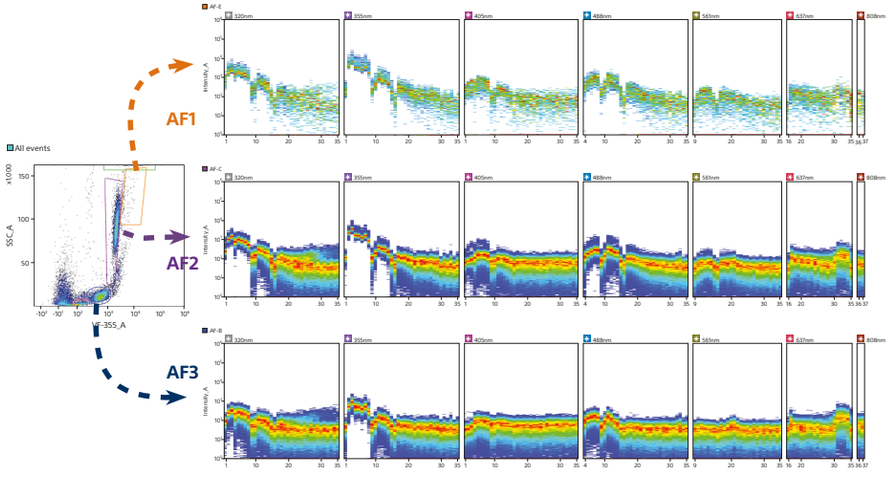
C. For the fluorochromes APC/ Alexa Fluor® 647/ Spark NIR™ 685/ APC-R700™/ Alexa Fluor® 700/ Zombie NIR™ excited by the 637-nm laser, distinctly resolved emission peaks and populations were observed.

Managing Autofluorescence

Spectral analysis, while allowing researchers to see the full emission signal without using bandpass filters, also enables autofluorescence to be handled as a separate color. In conventional flow cytometry, cellular autofluorescence produced by pyridine (NAD/NADH), flavin (FMN, FAD), and other intracellular oxidative reactions can interfere with signals of other fluorescent markers. Other common sources of autofluorescence include cell fixation and permeabilization. Spectral technology subtracts one or more autofluorescent spectral fingerprints to allow researchers to see the true fluorescent populations.

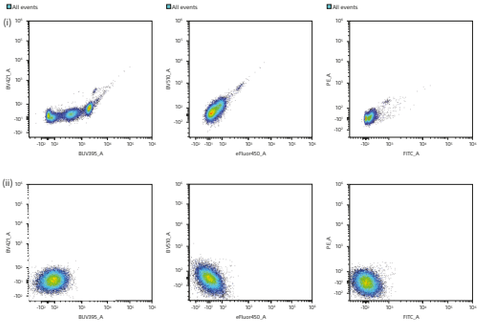


Spectral emission curves from a sample stained with nine fluorochromes are shown, as analyzed with a 5-laser ID7000 system. The cellular autofluorescence spectral curve can be seen along with the fluorescence spectral curves due to the nine fluorochromes used. The ability to distinguish the cellular fluorescence contribution independently from the signal due to the fluorochromes yields unbiased data.



An unstained lysed whole blood sample was analyzed with the ID7000 Spectral Cell Analyzer to understand the contribution of cellular autofluorescence.

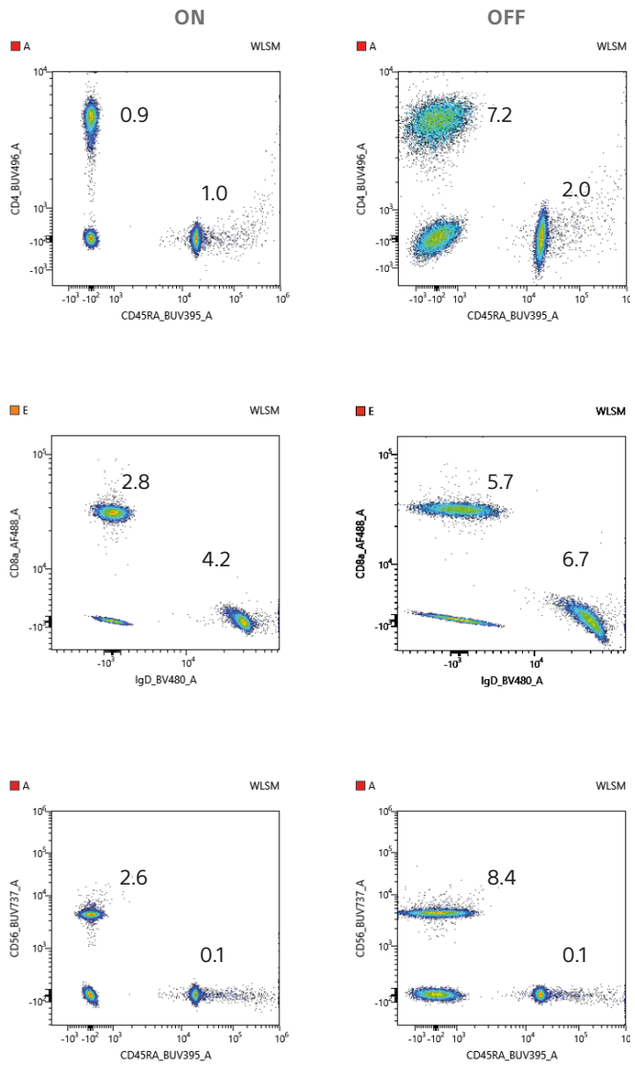
A. This figure shows spectral ribbon displays when studying the gated populations of lymphocytes, granulocytes, and eosinophils. Removing the signal due to this autofluorescence by using spectral fingerprints obtained in the analysis increased the precision and quality of results.



B. This figure shows the parametric display for the same samples. In the first row (i), the plots show presence of additional spurious populations as a result of intrinsic cellular autofluorescence. In the second row (ii), the autofluorescence subtraction was applied to remove contributions of AF1, AF2, and AF3, and the spurious populations were eliminated.

Increase Panel Design Flexibility with the Deep UV (320nm) Laser

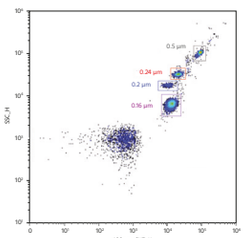
- **Enhance Panel Design Flexibility –** The Deep UV (320-nm) laser provides an additional 35 channels from 360 nm to 840 nm. The low noise and consistent beam shape of this laser enhance the ID7000's optical capability.
- **Reduced Spillover Spread –** In addition to the extra channels available with the Deep UV laser, just having it on without an assigned fluorochrome during acquisition of 41-color and 50-color panels improves spillover spread and discrimination of cell subsets.



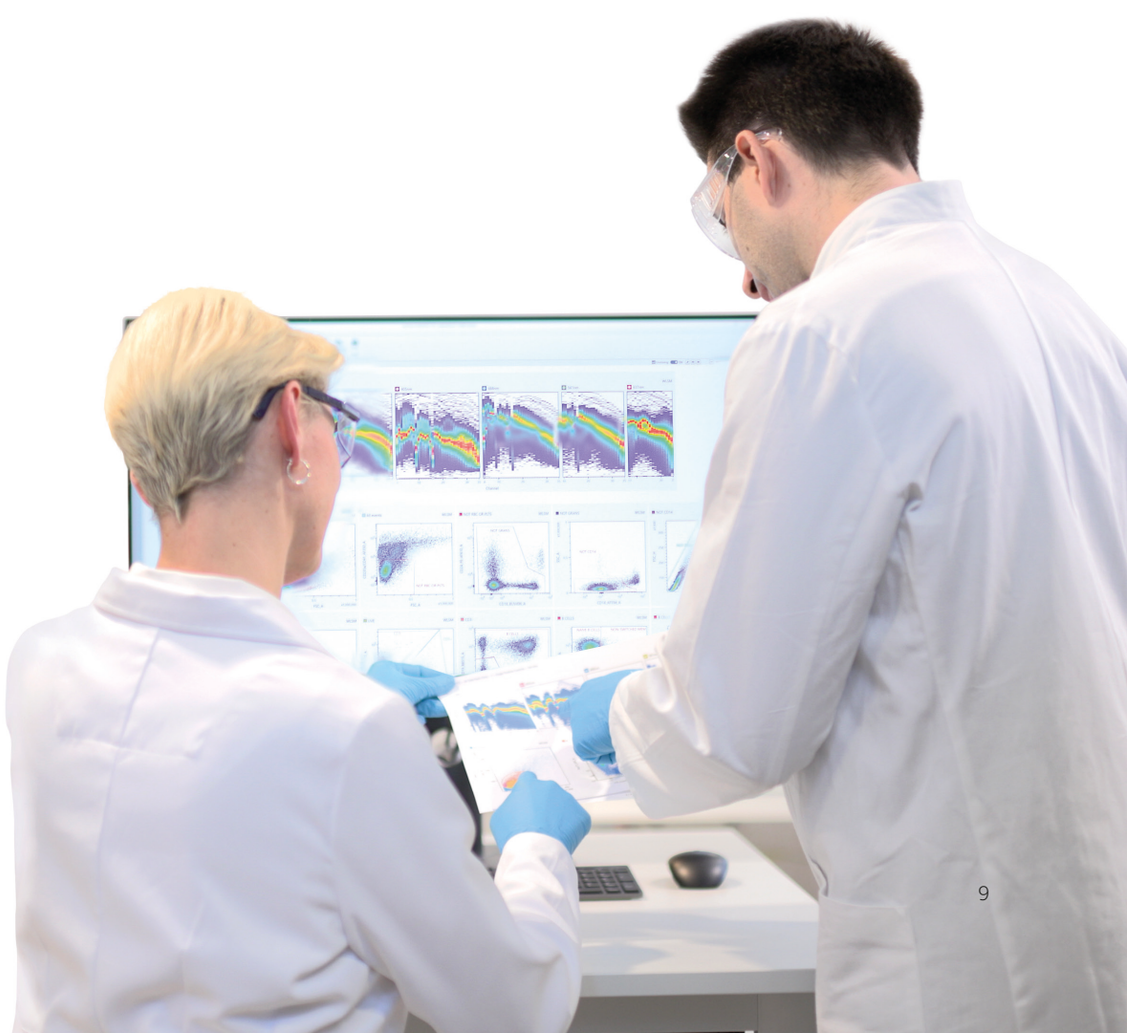
Shown in the plots is the effect of turning on the Deep UV (320-nm) laser (left) and leaving it off (right) in a high-parameter panel. Increased resolution can be observed when the Deep UV laser was turned on during acquisition.

Small Particle Detection Provides Excellent Resolution

- With the rapidly increasing need to understand disease mechanisms and develop targeted therapies, small particle detection plays a critical role. This technology enables researchers to delve into the intricacies of cellular biology, uncovering subtle differences in cell populations, identifying rare events, and exploring phenomena at a molecular level.
- Analysis of cellular exosomes, viruses, and other subcellular particles are all applications supported by small particle detection.
- The ability to resolve particles as small as 160 nm is critical for separating rare populations from noise.

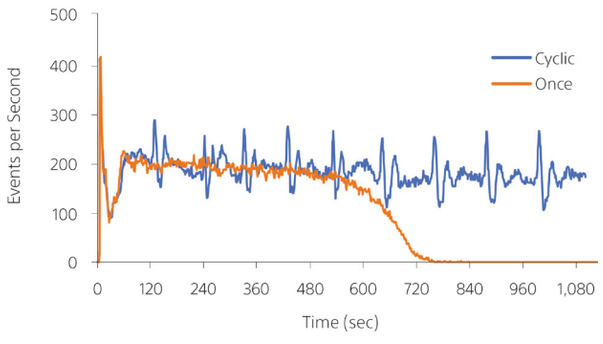


Small Particle Detection
The ID7000 can detect small particles. The figure shows how a mix of fluorescent beads of varied diameters available as a commercial product (Megamix, Biocytex), was analyzed to clearly resolve the 160-nm beads from all other beads and separate the signal from noise. Analysis used SSC and channel 7 of the 32-channel PMT array on the blue laser deck.

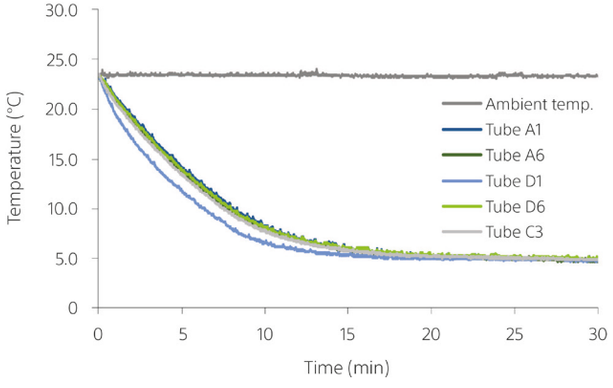


Automated Sample Acquisition Increases Efficiency

- **Walk-away Automation with a Reliable AutoSampler –** The advanced AutoSampler was meticulously designed with efficiency of day-to-day operation in mind. At the heart of every ID7000, the best-in-class AutoSampler supports a wide variety of sample formats:
  - 5-mL (12 x 75-mm) tube racks of 24 tubes
  - 96-well standard height U/V/Flat-bottom plates
  - 96-well half-deep, deep plates
  - 384-well standard flat bottom plates
- **Temperature Regulation –** Samples can be cooled to 4°C (39°F) using a Peltier element to prevent signal degradation and reduce variability.
- **Sample Agitation –** Individual tubes or wells can be agitated at the beginning of acquisition, intermittently, or continuously, enabling stable, long-duration acquisition without sample settling.
- **Low Dead Volume Mode –** This mode allows you to get the most information from precious samples, with a minimum volume of 3–10 µL using plates.

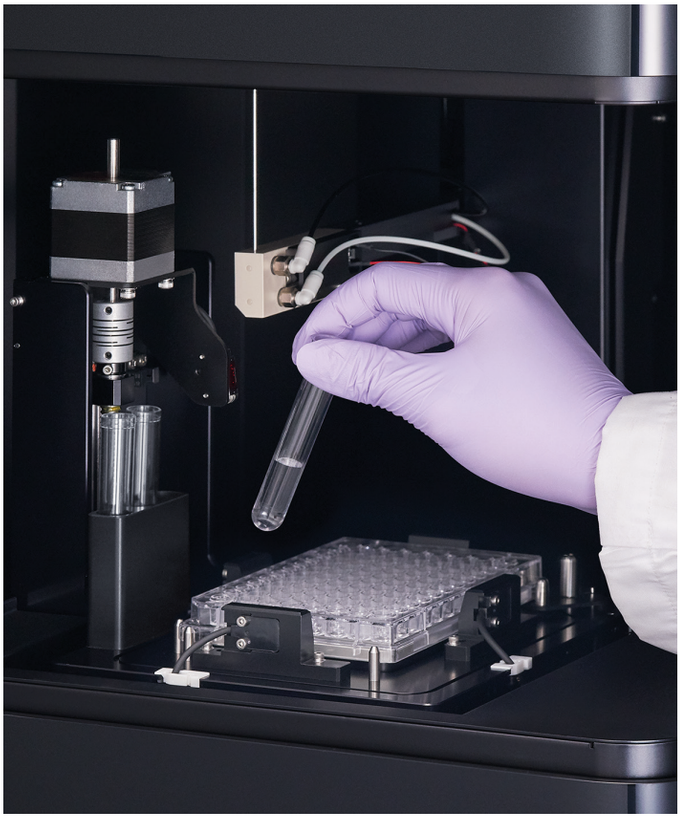


AutoSampler Agitation Modes
The ID7000 sample agitation feature is designed to keep large particles in suspension. The figure shows data with 30-µm particles when applying the initial mixing mode (Once) and the Cyclic mixing mode (Cyclic). The particles remained in suspension for 10 minutes when using the initial mixing mode, before there was an observed reduction in event rate. The Cyclic mixing mode, when used, continually mixed the sample throughout the entire acquisition, resulting in a consistent event rate with no settling.



5-mL Tube Rack Sample Cooling Over Time
The graph shows how sample temperature is maintained in the ID7000 AutoSampler when using a 24-position tube rack. Temperature measurement for tubes at multiple positions indicate that they maintained cooling over time when used in a 23°C ambient environment.

- **Automatic Clog Detection –** With the Event Check functionality, air bubbles or clogs are automatically detected, and the system will pause acquisition to clean itself. Auto Acquire preferences can be set to automatically resume acquisition after recovering from a clog, or to stop acquisition entirely.
- **Minimal Carryover –** The Dual Probe Wash option incorporates a cleaning mechanism that moves up and down to clean the inside and outside of the sample nozzle, for carryover >0.1%.
- **Automated Shutdown –** The AutoSampler is equipped with an extra loading station that can hold up to three 5-mL tubes. These tubes can be loaded with QC particles to streamline system QC, or wash and cleaning solution when choosing the automated shutdown option.
- **High-Throughput Automation –** The Automation Bundle supports high-throughput screening. This field-upgradable option enables the ID7000 to be equipped with an automated door and integrates a plate-handling arm that can load plates into the AutoSampler from microplate stacks.

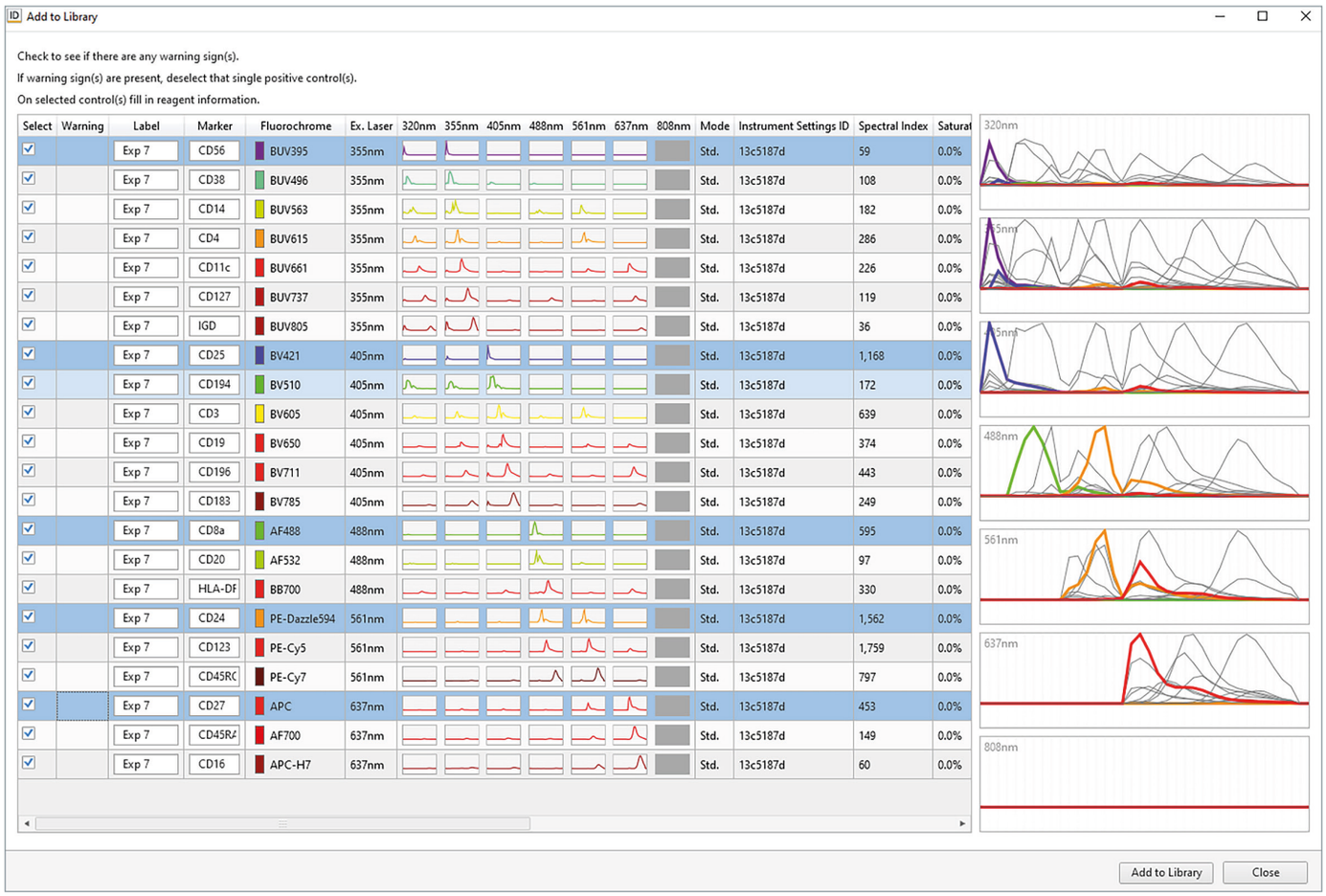


The ID7000 AutoSampler includes an extra tube station that accommodates up to three 12 x 75-mm (5-mL) tubes that can be used to support automated QC, system cleaning, and automatic shutdown workflows.

Close-up of the Dual Probe Wash, which can be programmed with multiple cleaning modes for flexibility in sample acquisition.

A Workhorse System Suitable for Any Core Lab

- **User-Friendly System Software –** The ID7000 system software accommodates users at all experience levels. Newer users can take advantage of a guided mode that simplifies system operation, while more experienced users can dive straight into their workflows in expert mode to run and analyze experiments.
- **User-Specific Preferences –** Busy core labs will benefit from the ability of administrators and users to specify options for overall instrument operation and experimental setup.
- **Automated Setup and QC –** Setting up the ID7000 for a day of operation is a simple process. After loading the appropriate QC beads into the extra tube station in the AutoSampler, Align Check and Performance QC wizards check instrument calibrations, ensuring the instrument is operating optimally. QC results can be shared or archived, and Levey-Jennings plots can be generated to keep track of instrument performance (such as linearity and MESF) over time.
- **Time Savings with the Spectral Reference Library –** The need for constantly re-recording single-color controls is a thing of the past. Beads or cells can be used as single-color controls and results added to the Spectral Reference Library after spectral unmixing for use in future experiments. Users can create personal or shared reagent libraries, which simplifies the already easy process of experiment creation.



The Spectral Reference Library makes it easy to add and reuse reference spectra across experiments. Reference spectra can be stored with identifying information such as lot number, experiment name, and marker name to streamline experiment workflows.

Intuitive Workflows for Acquisition and Analysis

The workflow after performing Automated Setup and QC is streamlined:

1. Select the Experiment Design Wizard.
2. Add all desired fluorochromes to the experiment. This can be done in a few different ways:
   - **a**. Choose the fluorochromes manually from a list.
   - **b.** Use existing fluorochromes from a previously run experiment in the Spectral Reference Library.
   - **c.** Import an Excel® file containing fluorochromes and marker names.
3. Select the plate or tube type and designate the locations of single-color controls.
4. Acquire single-color controls and set gates around the positive populations.
5. Calculate the unmixing matrix in the Unmixing Settings window.
   - **a.** Identify and correct any unmixing errors using the Spectral Reference Adjuster.
   - **b.** After the unmixing matrix has been calculated, save the spectral references to the Spectral Reference Library for future use.
6. Acquire fully stained samples.

Standardization Mode Drives Reproducibility

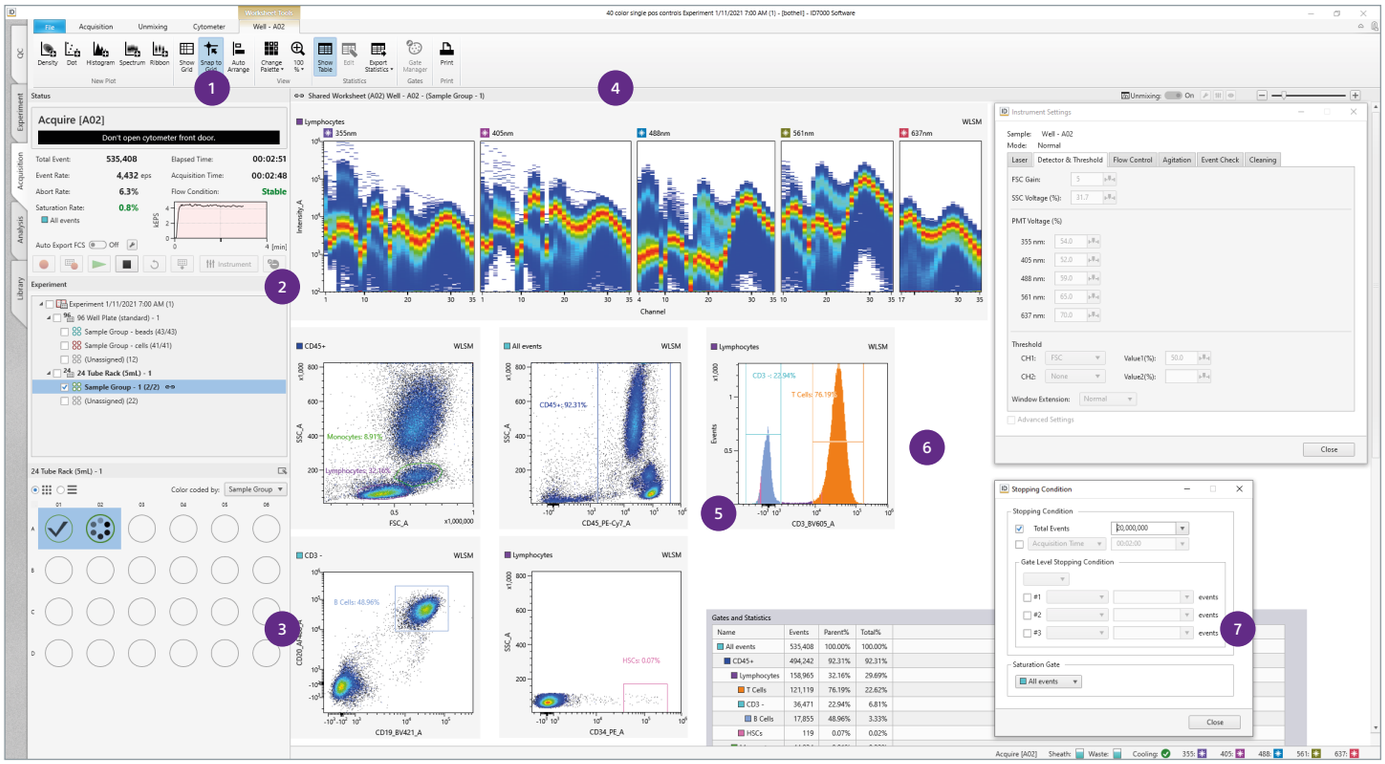
- **Standardization mode** sets the ID7000 to an optimized master specification that allows researchers to maintain instrument settings between experiments and across multiple instruments to support longitudinal studies and cross-site collaboration.
- **How it works:** PMT voltage correlation coefficients are calculated for each laser detection deck during daily QC, resulting in standardized measurements across all lasers, detectors, and instruments.
- In Standardization mode, all PMT array voltages can be adjusted synchronously, or arrays can be adjusted individually for each laser.

Standardization Mode Benefits	
Longitudinal studies – Across single and multiple systems	✓
Cross-site collaboration	✓
Streamlined workflow – Spectral References from the Spectral Reference Library can be reused across experiments	✓
PMT voltages for cells can be different from single-color controls	✓
Compatibility of an optimized panel between the ID7000 and FP7000	✓

Acquisition

Selecting the Acquisition workflow tab displays information required for managing sample acquisition.

The Acquisition workflow tab

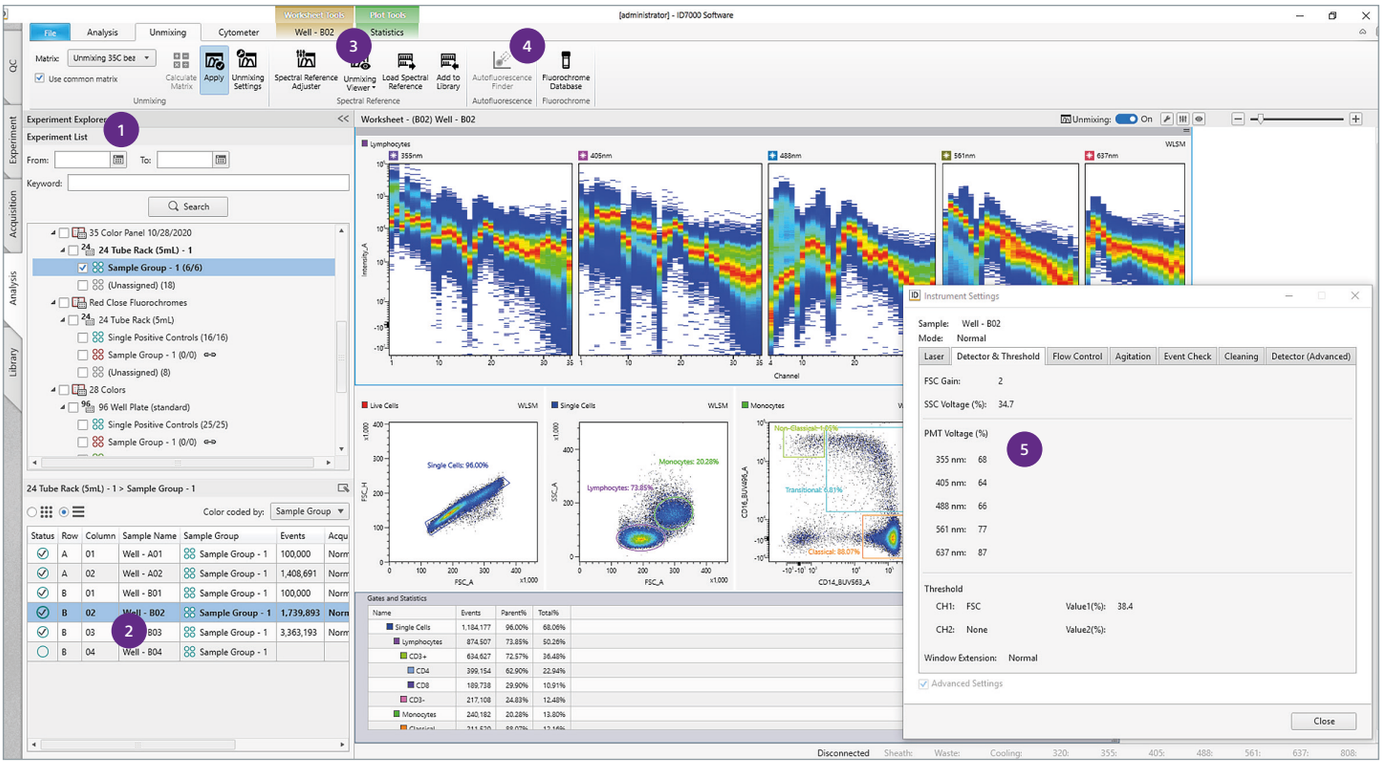


1. **The Acquire/Status Pane** displays parameters for data acquisition and contains the functionalities to manage sample acquisition, including setting up auto export.
2. **The Experiment Pane** displays available experiments and templates, and the organization structure of the experiment.
3. **The Sample Layout (Plate) Panel** displays the visual of the chosen layout for the sample acquisition format. It visually indicates completion of acquisition and inclusion in sample groups.
4. **Workspace** for Plots
5. **Gates and Statistics** displays statistics calculated automatically from acquired data.
6. **The Stopping Condition Window** allows users to specify data acquisition stopping criteria.
7. **A Persistent Footer** allows users to monitor the status of optics (Lasers) and Fluidics (Sheath and Waste).

Analysis

Analysis operations can be performed using the Analysis workflow tab. The key elements of this workspace include:

The Analysis workflow tab

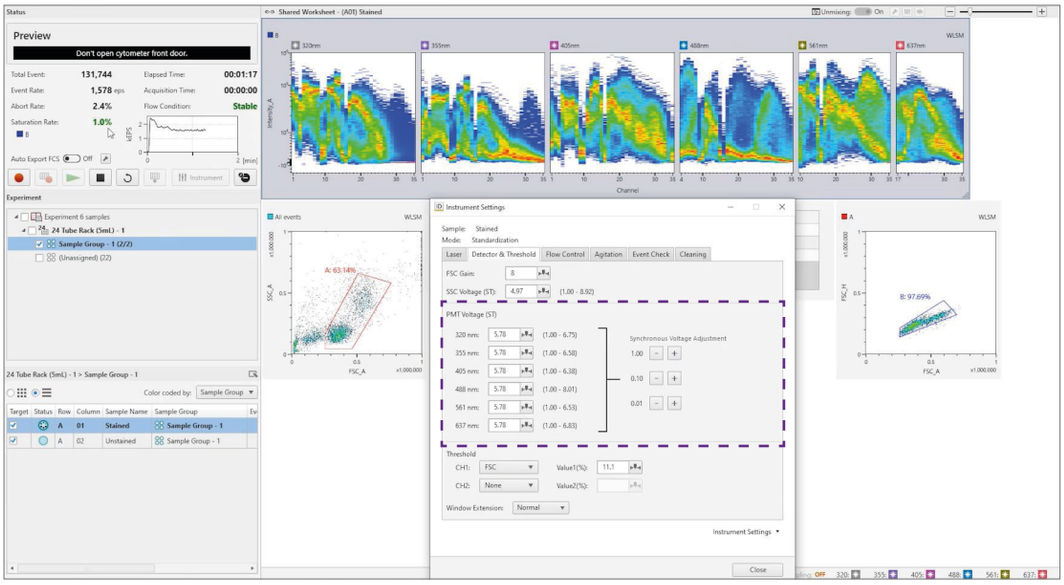


1. **The Experiment Pane** displays experimental data as a list and allows searching by date and keyword.
2. **The Sample Layout** is available on the Plate Pane, where individual samples can be viewed as a list or map and selected to populate the analysis worksheet.
3. **The Spectral Reference Adjuster** is used to optimize unmixing results when needed, by adjusting the spectral signatures of the single-color controls.
4. **The Autofluorescence Finder Wizard** can be used to locate and subtract autofluorescence contribution from the signals of the experimental sample.
5. **The Instrument Settings Window** displays the laser power, voltage, flow rate, agitation mode, and acquisition monitoring settings for each sample.

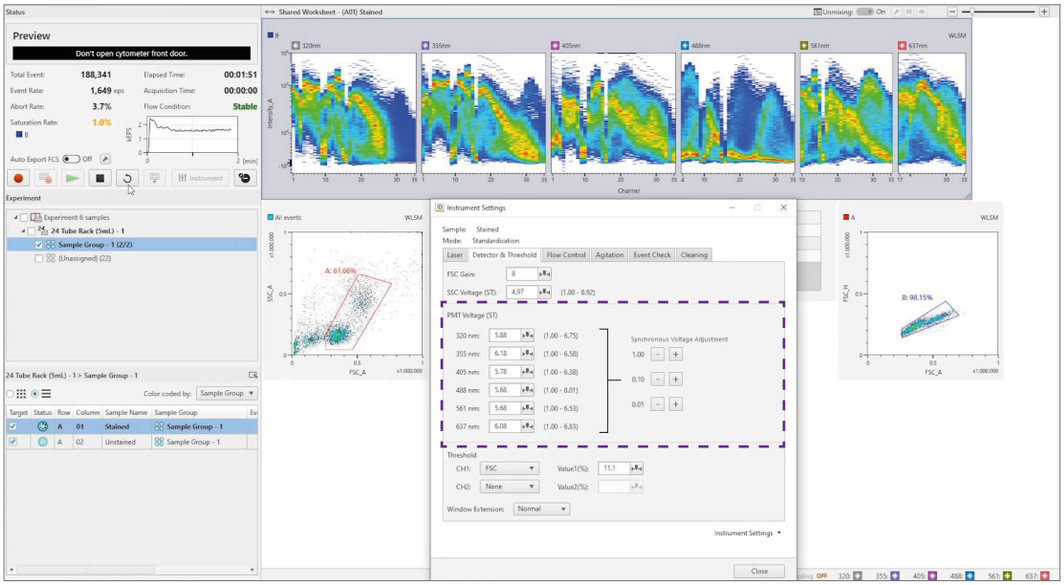
Quickly and Easily Optimize PMT Voltages

The One Max, All Max method was designed as an easy way for users to achieve the highest possible resolution for their data within Standardization mode. The PMT voltages for cells can be changed independently of the voltages of the single-color controls without compromising data or spectral unmixing quality.

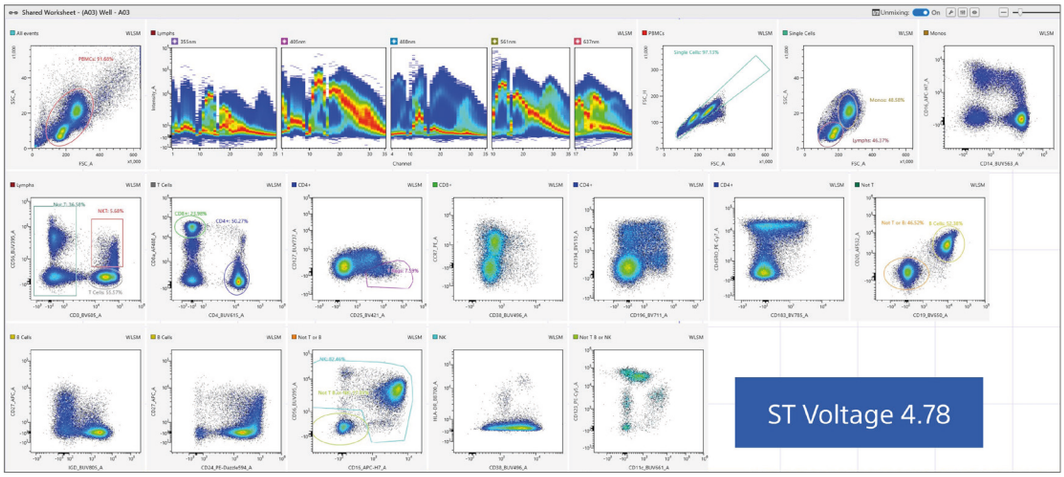
One Max refers to the adjustment of all PMT voltages synchronously until the first maximum emission peak from any detector is near saturation. One Max is ideal when time or sample is limited.



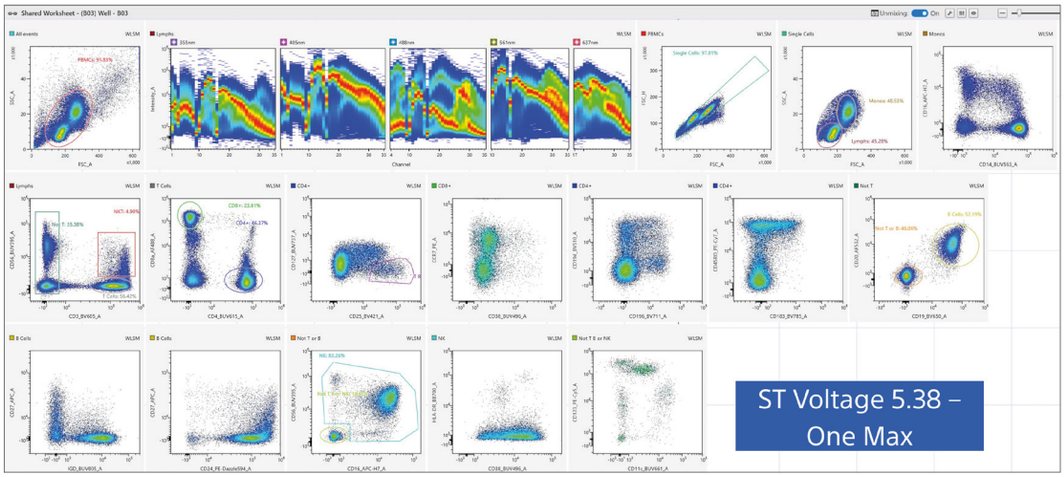
All Max is the adjustment of each detector's voltage individually until the maximum peak in each detector is near saturation. All Max provides the most robust resolution for a sample running on the ID7000.



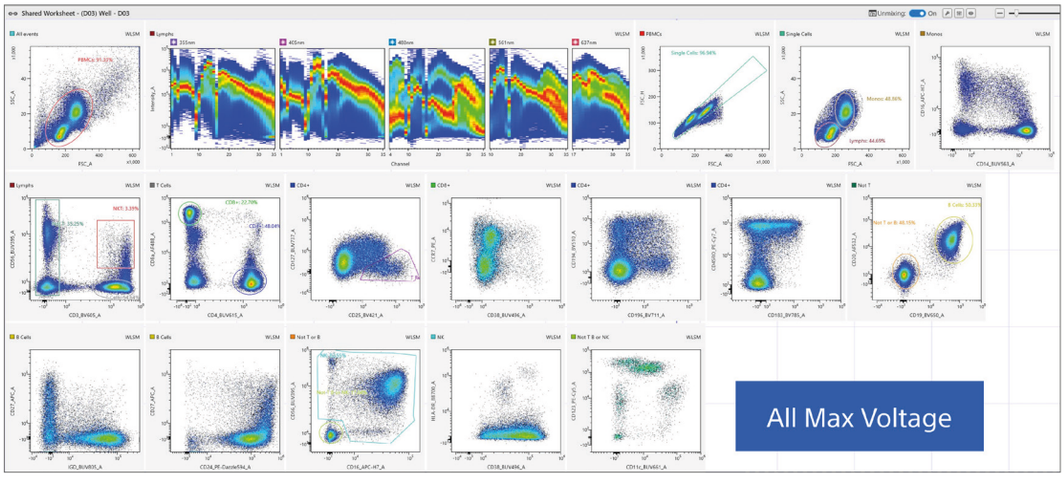
All PMT voltages synchronously set to 4.78 with no lasers reaching saturation.



One Max – PMT voltages synchronously set to 5.38. Saturation can be seen on the 561-nm laser.

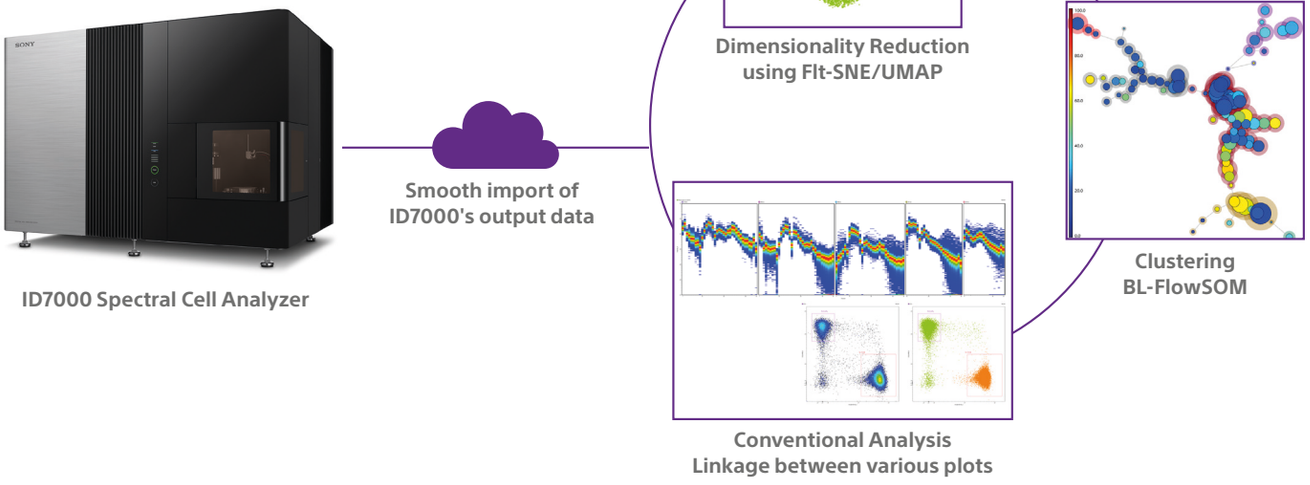


All Max – PMT voltages modified independently so that each laser has reached saturation across all PMT detection decks.



Powerful Analysis Tools to Boost Your ID7000 Workflow

- **The Spectral Flow Analysis (SFA) Life Sciences Cloud Platform** is high-dimensional analysis software designed to give researchers the ability to gather the deepest insights from their data.
- **Superior Speed and Accuracy –** With a graphical user interface matching the ID7000 system software, SFA is user-friendly and can import database files directly from the ID7000 so that advanced analyses can be started immediately.
- **No More Plug-ins –** All of the advanced analysis algorithms within SFA can be used upon downloading the software, so there is no need to download plug-ins.
- **Algorithms for Every Occasion –** SFA is a one-stop shop for advanced analysis workflows. Here is an example of some of the algorithms that exist within the software:
  - **Data cleaning algorithms** for ensuring objectivity: flowAI, flowCut, flowClean, PeacoQC.
  - **Clustering algorithms:** BL-FlowSOM, developed in-house by Sony, improves the FlowSOM algorithm by introducing Batch Learning (BL).
  - **Dimensionality reduction algorithms:** FIt-SNE, UMAP
- **Interactive Analysis –** Conventional plots and outcomes from dimensional reduction/ clustering can be linked.
- **Data Sharing and Management –** Part of the driving force behind SFA is the ability to share and manage data with users across an organization. Collaboration is the key to scientific progress.



Need Support?
Our Team of Experts is Here to Help

- The ID7000 Spectral Cell Analyzer has been developed to provide tools for research scientists who seek to use high-complexity multicolor panels effectively and with confidence to answer questions in various fields of biology. Our service teams go above and beyond to provide support for all flow cytometry needs..
- **Knowledgeable Technical Support –** Our application scientist team can help you get the most out of your ID7000 by providing tips and support for all parts of your experimental workflow.
- **Responsive Service Engineers –** Our field service engineers can assess your system's health or quickly get it back up and running in rare instances when repairs need to be made.
- **Extra Resources at Your Fingertips –** Webinars, technical notes, educational videos, a spectra viewer, and reagents such as our catalog of over 9,000 monoclonal antibodies can all be found on our website: www.sonybiotechnology.com.
- **21 CFR Part 11 Support –** The ID7000 system software is 21 CFR Part 11 compliant and supports researchers working in regulated environments by providing audit trails and eSignatures.

IQ

Our IQ service provides documented verification that the instrument is installed according to our specifications and safety regulations. During the IQ, a trained engineer verifies the latest supported firmware and software versions were installed, verifies instrument setup, checks that physical and environmental safety conditions are met, and provides a signed audit-ready report.

OQ

Our OQ service follows a comprehensive, well-defined protocol to verify the system functions according to preset and validated operational specifications. We update the OQ protocol following each instrument hardware and software release, ensuring your laboratory receives the most up-to-date service.

Laser configurations

Model	No. of lasers, wavelength	No. of detectors*
LE-ID7000A	3LD: 405/488/637	FSC/SSC + 86F
LE-ID7000B	4LD: 405/488/561/637	FSC/SSC + 112F
LE-ID7000C	5LD: 355/405/488/561/637	FSC/SSC + 147F
LE-ID7000D	6LD: 355/405/488/561/637/808	FSC/SSC + 149F
LE-ID7000E	6LD: 320/355/405/488/561/637	FSC/SSC + 182F
LE-ID7000F	7LD: 320/355/405/488/561/637/808	FSC/SSC + 184F

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