Agilent

Trusted Answers

Agilent NovoCyte Opteon Spectral Flow Cytometer

Full spectrum, full insight: NovoCyte evolved

NovoCyte Opteon Spectral Flow Cytometer

Introducing the Agilent NovoCyte Opteon, our cutting-edge spectral flow cytometry solution designed to revolutionize your cell analysis research. Featuring up to five lasers and 73 detectors, NovoCyte Opteon meets your needs for increasingly sophisticated, large panel flow cytometry assays. With a proprietary optical design, advanced electronics, and data processing algorithms for optimized signal collection, NovoCyte Opteon delivers data with high sensitivity and resolution.

The wide dynamic range in both fluorescence detection and particle size measurement streamlines your experimental workflow. Onboard temperature control for both lasers and photodetectors provide superior performance and stability for high quality data under different ambient environments. Real-time monitoring of instrument status ensures reliable and uninterrupted data acquisition at varied high-and-low sampling rates. The intuitive, industry-leading Agilent NovoExpress software features flexible reference control setups and autofluorescence (AF) subtraction capability, setting a new standard for an exceptional user experience in flow data acquisition, analysis, and reporting.

Elevate your research with NovoCyte Opteon's high-throughput automation, ready for seamless integration into your laboratory instrument ecosystem and to redefine your scientific horizons.

Revolutionize your cell analysis research

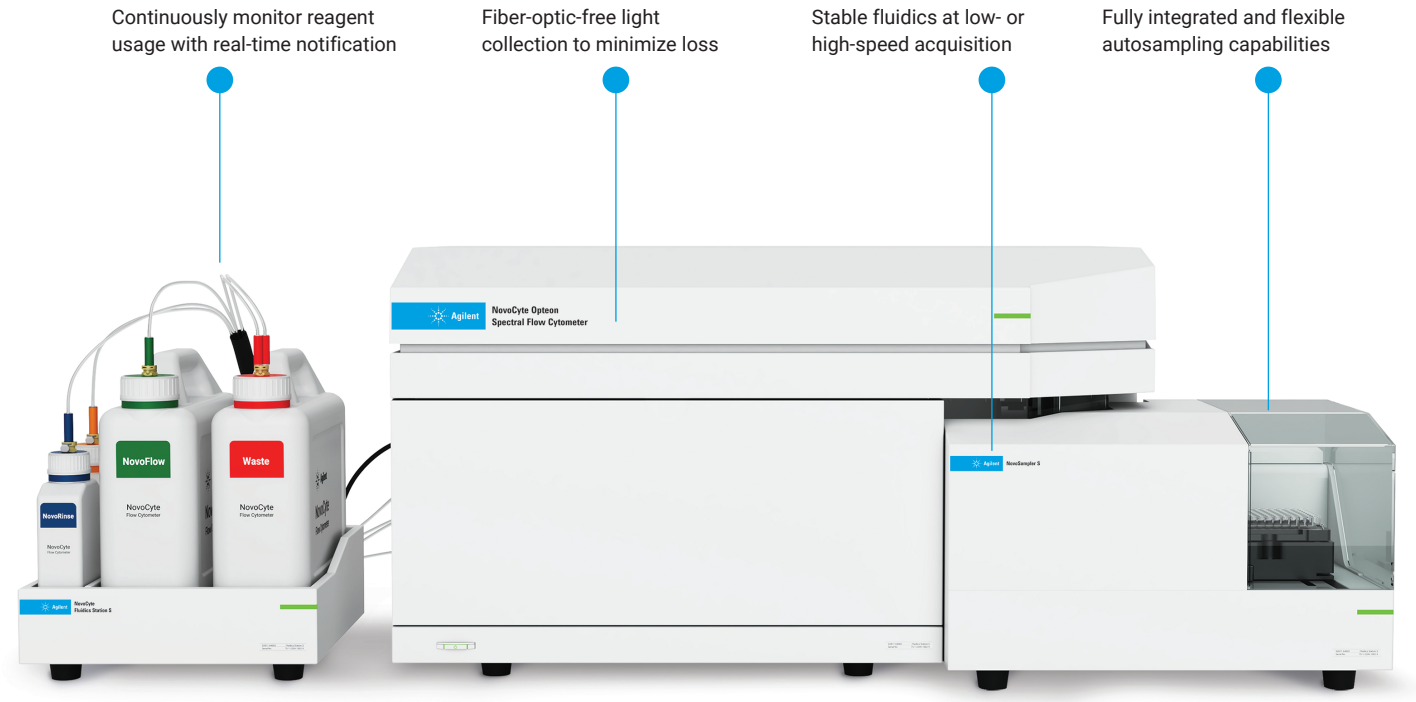
Beyond limits: Armed with up to five lasers, 73 detectors, and a demonstrated 45-color panel, NovoCyte Opteon is your gateway to multidimensional cell analysis.

Exceptional performance: An innovative optics design, advanced electronics, and signal processing algorithms deliver data with high sensitivity, high resolution, and wider dynamic range. From rare cell populations to subtle variations, no detail escapes your scrutiny.

Reliability embodied: Embedded fluidics sensors ensure the delivery of consistent data regardless of experimental conditions. It's more than an instrument; it's a partner in your scientific journey.

Software mastery: Your analytical companion, the NovoExpress software, features expanded powerful functionalities like reference control setup and autofluorescence subtraction while maintaining its legacy of intuitiveness and ease-of-use. Learn swiftly, analyze deeply, report easily.

Automation awaits: Compatible with a variety of labware, NovoCyte Opteon is ready to be automated for high-throughput, walkaway workflows.



Discover more, together

Up to five lasers, 73 detectors

Panel of 45 colors, demonstrated

Data with high sensitivity, high resolution

Innovative optics design

- Free-space optics to maximize signal collection
- Low-noise, precision electronics and advanced data processing algorithms
- 7-log dynamic range to detect and display dim and bright fluorescent signals and simplifying experimental setup workflow
- Dual-laser particle detection for a wide particle size dynamic range

High instrument reliability

- Onboard temperature control for minimizing data fluctuation due to temperature variation
- Stable fluidics for consistent data acquisition at high and low sampling rates
- On-board electronics and fluidics circuitry for real-time instrument status monitoring

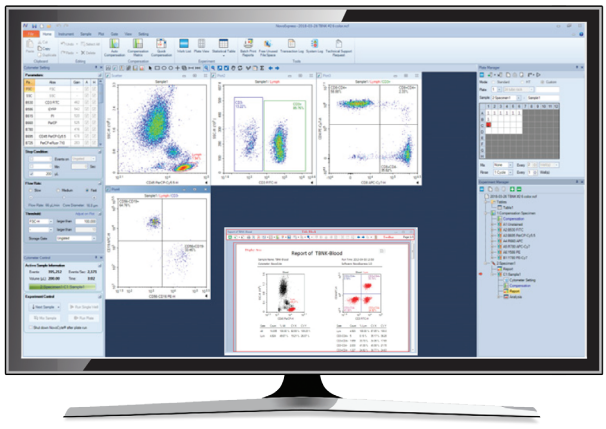
Powerful and intuitive NovoExpress software

- Flexible and simple multiple reference control setup
- Capable of applying a reference control spectrum, measured at different detector gain settings
- Enables AF subtraction with powerful, multipopulation options for an easy and flexible workflow
- Easy to learn or train on the software

Automation ready

- Lab automation-friendly with an open architecture and a developer-ready API

Streamline your sample acquisition, data analysis, and reporting. The latest edition of our industry-leading NovoExpress software provides an exceptional user experience.



Every Fluorochrome Tells a Story

The ability of spectral flow cytometry to measure multiple parameters at once allows for a more comprehensive characterization of immune cells and subsets, advancing the discovery, development, and testing of biomarkers. Simultaneously, large color panels using 40 or more fluorophores have allowed for deep immunophenotyping of major cell subsets in human peripheral blood.

We developed a 45-color spectral flow cytometry immunophenotyping panel to quantitate peripheral immune subset frequencies, protein expression, activation markers, exhaustion markers, and differentiation markers expressed on the major innate and adaptive immune cell types in healthy peripheral blood mononuclear cells (PBMCs).

Common surface markers expressed by each population were used to determine population frequencies. Further characterization of each population was performed using markers associated with cell activation and proliferation (CD69, HLA-DR, CD38), cell exhaustion and senescence (PD1, CD223, CD57), cell differentiation (CCR7, CD27, CD28, CD45RA, CD45RO, CD127), and cell plasticity or migration potential (CXCR3, CCR6, CCR5, CXCR5). This in-depth analysis was made possible using the Agilent NovoCyte Opteon spectral cytometer.

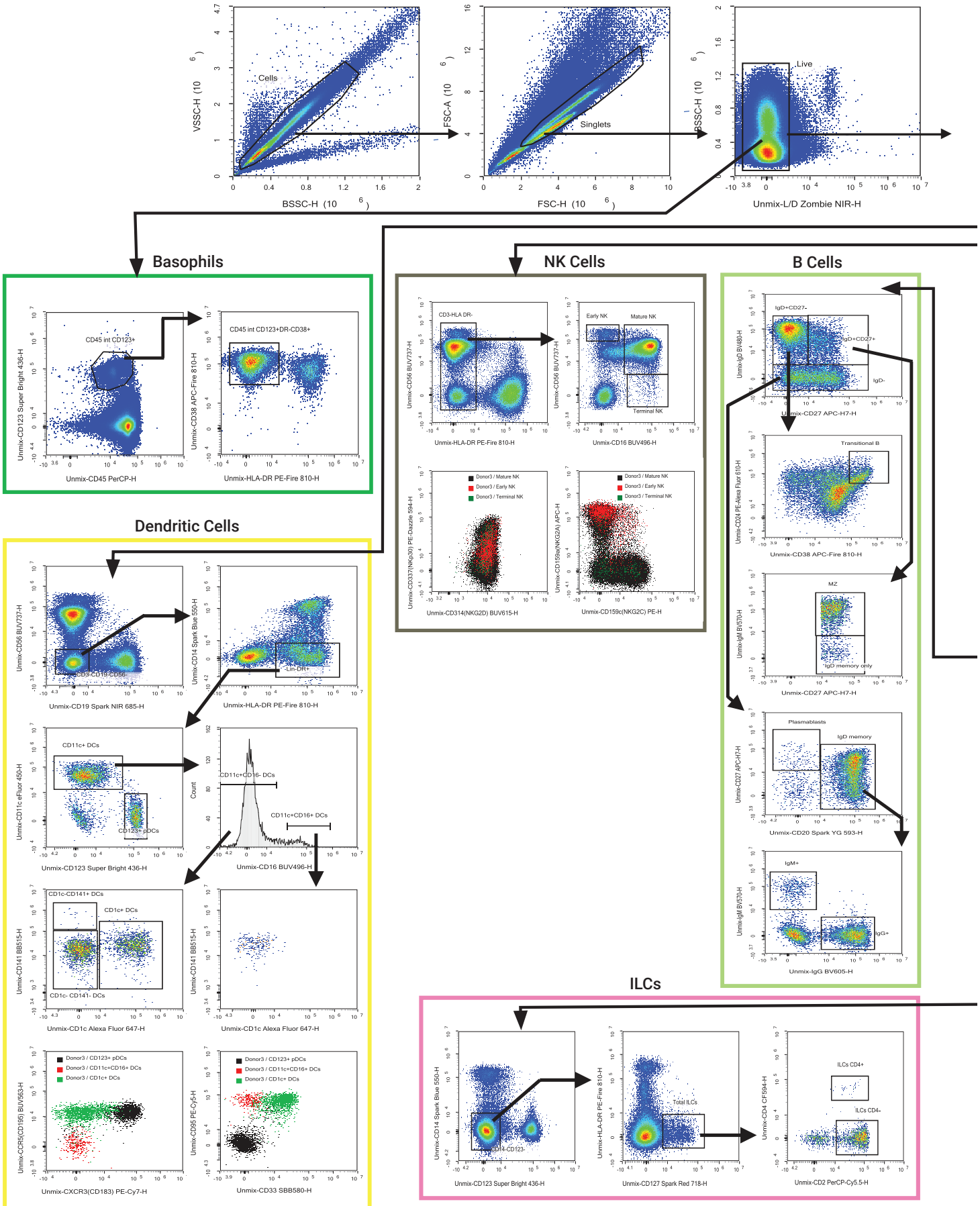
Specificity	Fluorochrome
CD159c (NKG2C)	PE
CD20	Spark YG 593
CD337 (Nkp30)	PE-Dazzle 594
CD4	CF594
CD24	PE-Alexa Fluor 610
CD95 (FAS)	PE-Cy5
CD25	PE-Alexa Fluor 700
CXCR3 (CD183)	PE-Cy7
HLA-DR	PE-Fire 810

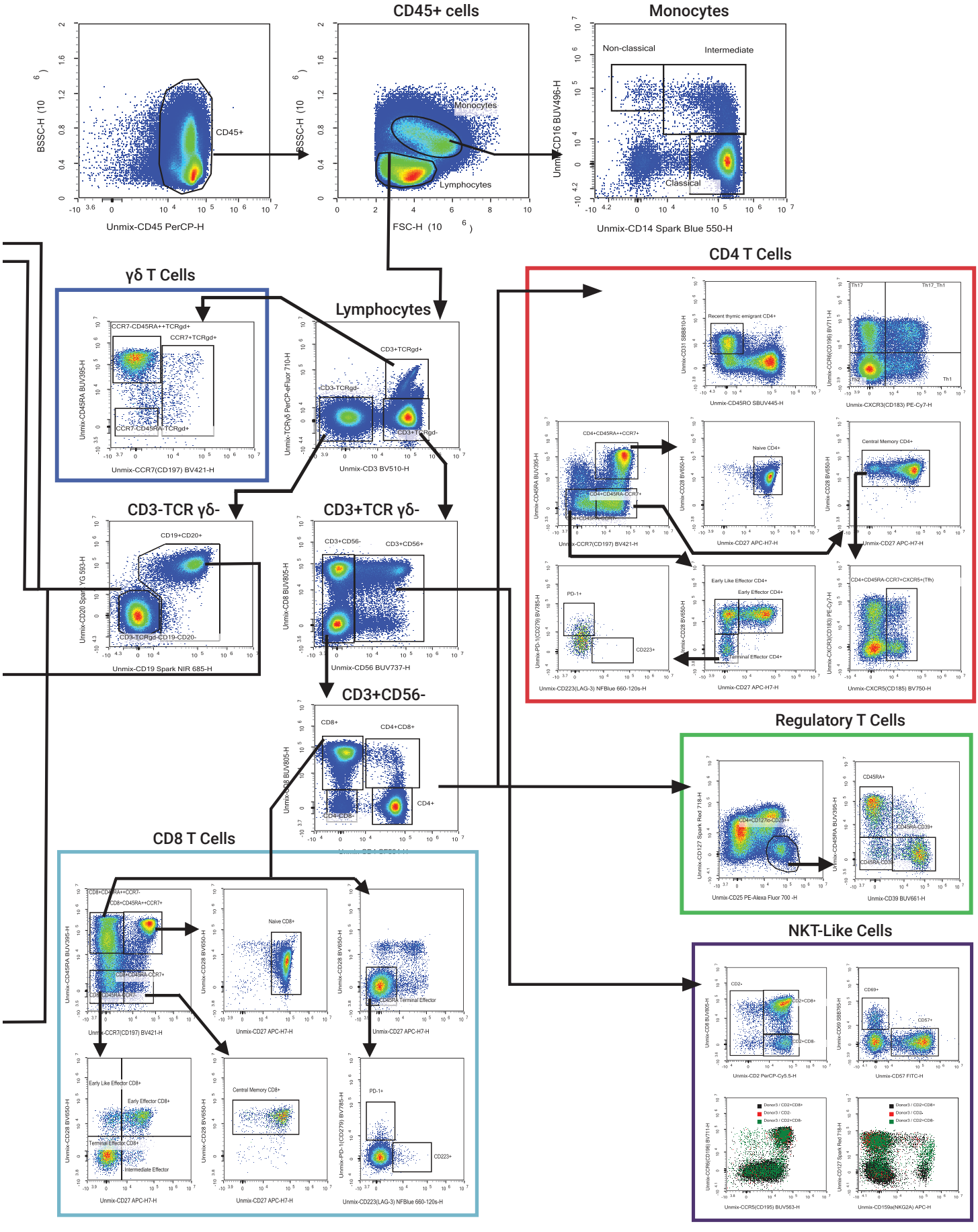
Specificity	Fluorochrome
CD141	BB515
CD57	FITC
CD14	Spark Blue 550
CD33	StarBright Blue 580
CD223 (LAG-3)	NovaFluor Blue 660/120S
CD45	PerCP
CD2	PerCP-Cy5.5
TCRγδ	PerCP-eFluor 710
CD69	StarBright Blue 765
CD31	StarBright Blue 810

Specificity	Fluorochrome
CCR7 (CD197)	BV421
CD123	Super Bright 436
CD11c	eFluor 450
IgD	BV480
CD3	BV510
IgM	BV570
IgG	BV605
CD28	BV650
CCR6 (CD196)	BV711
CXCR5 (CD185)	BV750
PD-1 (CD279)	BV785

Specificity	Fluorochrome
CD45RA	BUV395
CD45RO	StarBright UltraViolet 445
CD16	BUV496
CCR5 (CD195)	BUV563
CD314 (NKG2D)	BUV615
CD39	BUV661
CD56	BUV737
CD8	BUV805

Specificity	Fluorochrome
CD159a (NKG2A)	APC
CD1c	Alexa Fluor 647
CD19	Spark NIR 685
CD127	Spark Red 718
Viability	Zombie NIR
CD27	APC-H7
CD38	APC-Fire 810


Cells
Singlets
Live
VSSC-H (10 6)
BSSC-H (10 6)
FSC-A (10 6)
FSC-H (10 6)
SSC-A (10 6)
Unmix-L/D Zombie NIR-H
Basophils
CD45 int CD123+
CD45 int CD123+DR-CD38+
Unmix-CD123 Super Bright 436-H
Unmix-CD45 PerCP-H
Unmix-CD38 APC-Fire 810-H
Unmix-HLA-DR PE-Fire 810-H
NK Cells
CD3-HLA DR-
Early NK
Mature NK
Terminal NK
Unmix-CD56 BUV737-H
Unmix-HLA-DR PE-Fire 810-H
Unmix-CD16 BUV496-H
Donor3 / Mature NK
Donor3 / Early NK
Donor3 / Terminal NK
Unmix-CD337(NKp30) PE-Dazzle 594-H
Unmix-CD314(NKG2D) BUV615-H
Unmix-CD159a(NKG2A) APC-H
Unmix-CD159c(NKG2C) PE-H
B Cells
IgD+CD27-
IgD+CD27+
IgD-
Unmix-IgD BV480-H
Unmix-CD27 APC-H7-H
Transitional B
Unmix-CD24 PE-Alexa Fluor 610-H
Unmix-CD38 APC-Fire 810-H
MZ
IgD memory only
Unmix-IgM BV570-H
Plasmablasts
IgD memory
Unmix-CD27 APC-H7-H
Unmix-CD20 Spark YG 593-H
IgM+
IgG+
Unmix-IgG BV605-H
Dendritic Cells
CD3-CD19-CD56-
HLA-DR+
Unmix-CD19 Spark NIR 685-H
Unmix-CD14 Spark Blue 550-H
CD11c+ DCs
CD123+ pDCs
CD11c+CD16- DCs
CD11c+CD16+ DCs
Count
Unmix-CD11c eFluor 450-H
Unmix-CD123 Super Bright 436-H
Unmix-CD16 BUV496-H
CD1c-CD141+ DCs
CD1c+ DCs
CD1c- CD141- DCs
Unmix-CD141 BB515-H
Unmix-CD1c Alexa Fluor 647-H
Donor3 / CD123+ pDCs
Donor3 / CD11c+CD16+ DCs
Donor3 / CD1c+ DCs
Unmix-CCR5(CD195) BUV563-H
Unmix-CXCR3(CD183) PE-Cy7-H
Unmix-CD95 PE-Cy5-H
Unmix-CD33 SBB580-H
ILCs
CD14-CD123-
Total ILCs
ILCs CD4+
ILCs CD4-
Unmix-CD123 Super Bright 436-H
Unmix-CD14 Spark Blue 550-H
Unmix-HLA-DR PE-Fire 810-H
Unmix-CD127 Spark Red 718-H
Unmix-CD4 CF594-H
Unmix-CD2 PerCP-Cy5.5-H

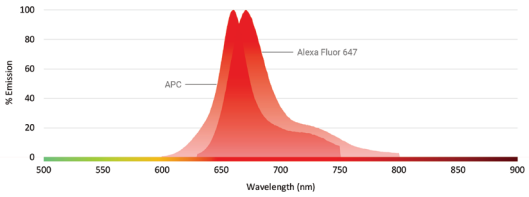

CD45+ cells
Monocytes
CD45+
Monocytes
Non-classical
Intermediate
Classical
Lymphocytes
Unmix-CD45 PerCP-H
FSC-H (10 6)
Unmix-CD14 Spark Blue 550-H
γδ T Cells
Lymphocytes
CD4 T Cells
CD3-TCR γδ-
CD3+TCR γδ-
CD3+CD56-
Regulatory T Cells
CD8 T Cells
NKT-Like Cells

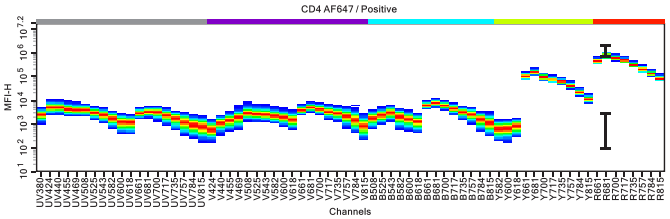
Elevate your data

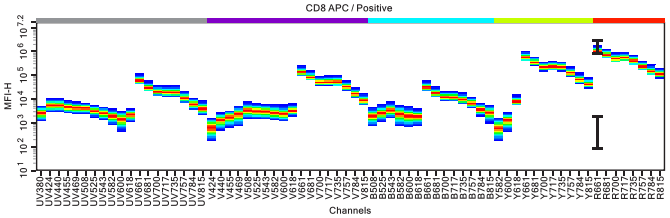
Challenging dye combinations

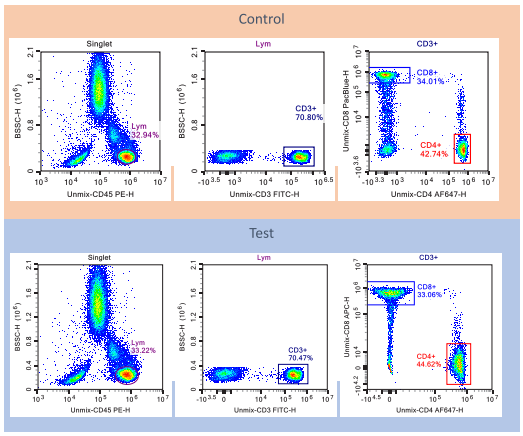
When designing panels for spectral flow cytometry, it's essential to consider the unique spectral signatures of each fluorochrome. Spectral flow cytometers exploit the inherent emission pattern of each fluorescent molecule, allowing discrimination of similar fluorochromes. For instance, allophycocyanin (APC) and Alexa Fluor 647 are now compatible when analyzed on a spectral flow cytometer, despite their similar emission profiles, which is not feasible by a conventional flow cytometer.

Control	Test
CD45 PE	CD45 PE
CD3 FITC	CD3 FITC
CD4 AF647	CD4 AF647
CD8 PB	CD8 APC

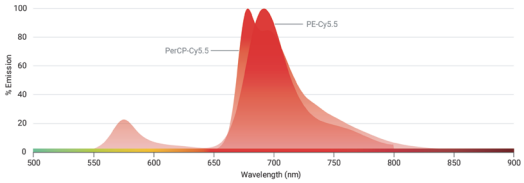


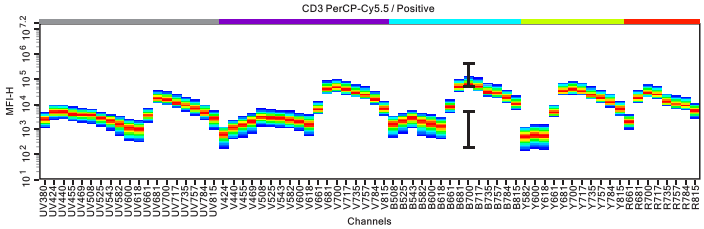


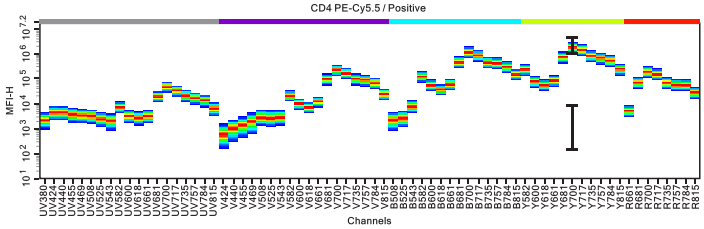


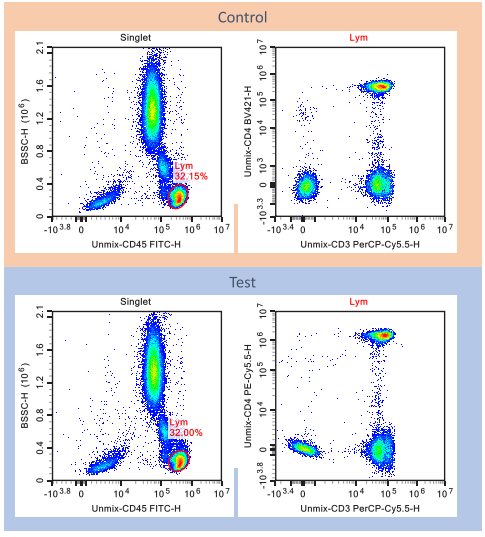


Control	Test
CD3 PerCP-Cy5.5	CD3 PerCP-Cy5.5
CD4 BV421	CD4 PE-Cy5.5
CD45 FITC	CD45 FITC









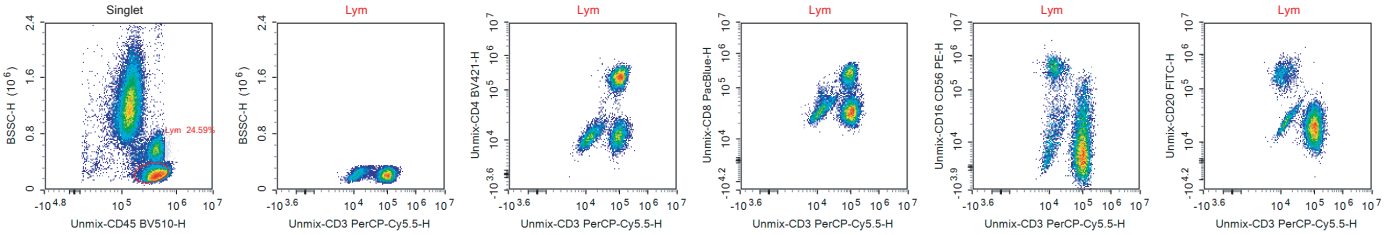
Autofluorescence removal

Improved resolution and more accurate unmixing

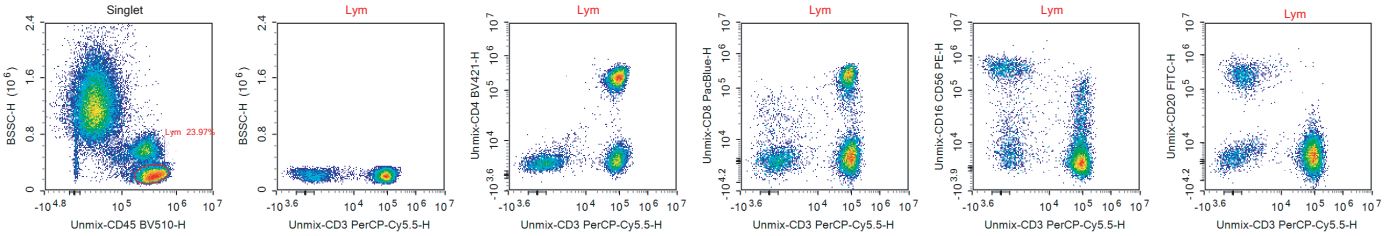
Autofluorescence (AF) subtraction allows for improved resolution of specific cellular populations of interest. Applying AF subtraction to individual cell populations in a mixed-cell sample allows for enhanced resolution and improved sensitivity for analysis, and the NovoExpress software makes AF subtraction simple and versatile.

Specificity	Fluorochrome
CD45	BV510
CD3	PerCP-Cy5.5
CD4	BV421
CD8	Pacific Blue
CD20	FITC
CD16+CD56	PE

Unmixing **without** AF subtraction



Unmixing **with** AF subtraction



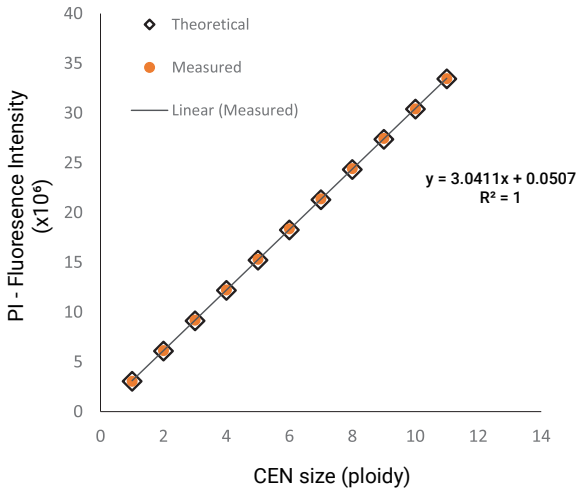
Sample: *Full-stained CD-Chex plus (Streck, Cat#213323)*

- **What is cellular AF subtraction?** It refers to the intrinsic fluorescence emitted by cells due to endogenous molecules (such as NADH, flavins, and lipofuscin).
- **How does cellular AF subtraction affect flow cytometry?** AF subtraction can mask or interfere with the fluorescence signals from fluorescent markers used in flow cytometry, especially in larger and more granular cells.
- **How does spectral flow cytometry address AF subtraction?** Spectral flow cytometry treats AF subtraction as an additional, distinct spectral component. By unmixing the spectral data, the influence of AF subtraction on the detection and quantification of fluorescent markers used in the assay can be removed, enabling accurate analysis and identification of cell populations. This enhances the resolution of low-expressing targets and dim levels of fluorescence signals.

AF subtraction is critical for obtaining high-quality data, improving sensitivity, and enhancing the accuracy of spectral flow cytometry analyses.

8 pk bead data: sensitivity

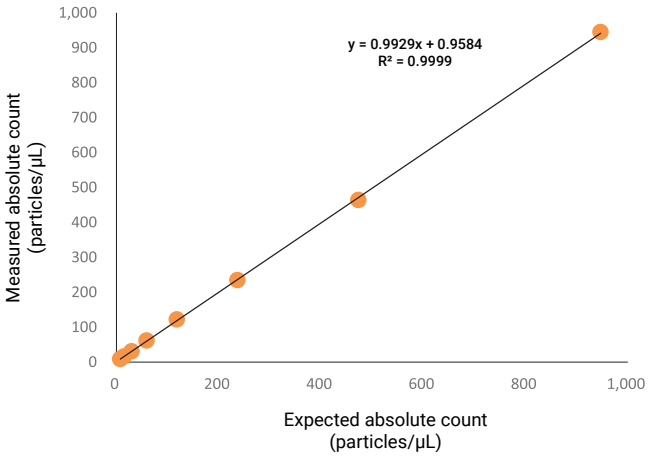
Data Precision You Can Trust



Fluorescence linearity by CEN

The optical and electronic subsystems are the result of state-of-the-art engineering. This design enables the NovoCyte Opteon spectral flow cytometer to deliver a highly linear signal response detection for all channels across a wide dynamic range.

To demonstrate the detection linearity, Chicken Erythrocyte Nuclei (CEN) ploidy was measured in relation to the mean fluorescence intensity of Propidium Iodide (PI) staining.



Volumetric absolute counting

Direct absolute cell counts make reference beads obsolete

The NovoCyte Opteon uses a high-accuracy syringe pump to directly control the sample and provide accurate, absolute count results in every run. Why use reference beads when you don't need them?

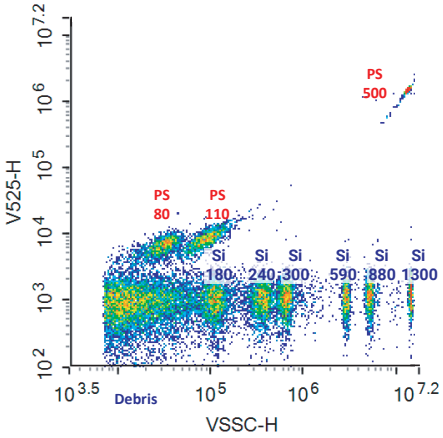
- The volumetric absolute count is determined for each and every sample automatically
- Complicated calibration of the fluidics system is not required
- Expensive reference beads are not required

Dual SSC detection with a wide particle detection range

Detect small and large particles in the same sample

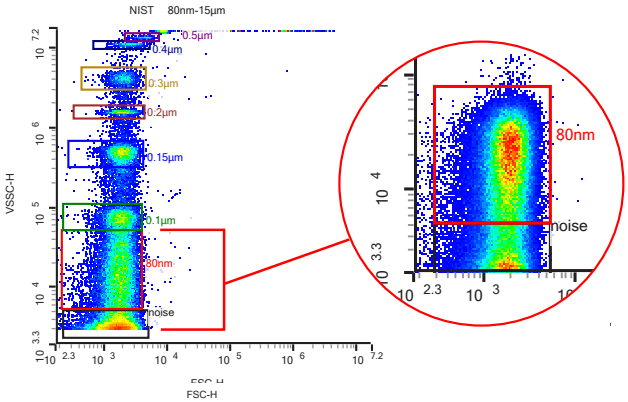
The NovoCyte Opteon forward scatter/side scatter (FSC/SSC) detection optics and signal processing electronics have been optimized with dual 488 nm-SSC (B-SSC) and 405 nm-SSC (V-SSC). These optimizations resolve particles as small as 80 nm without adjusting the settings to detect larger cells in the same sample. With this high-resolution, platelets, bacteria, and various sub-micron particles can be readily identified and analyzed alongside your cellular subsets.

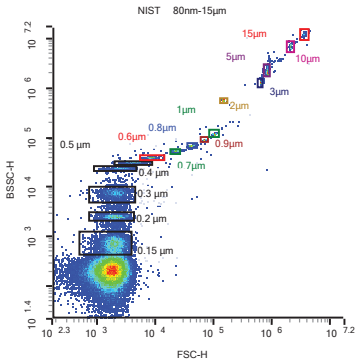
ApogeeMix beads



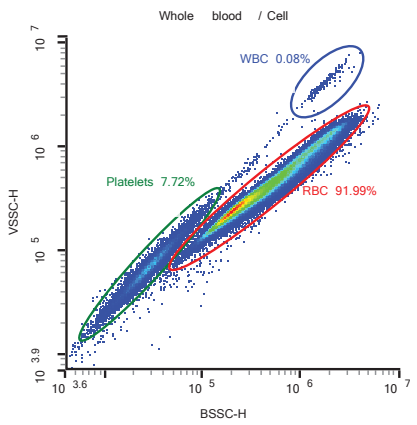
ApogeeMix beads, a mixture of polystyrene fluorescent and silica beads, were analyzed using V-SSC and fluorescence. NIST Traceable Particle Size Standards were analyzed using either V-SSC for smaller particles and B-SSC for larger particles.

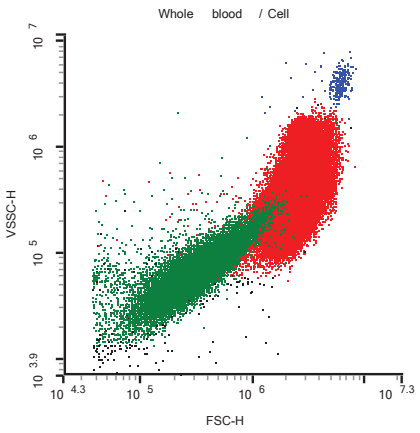
NIST beads





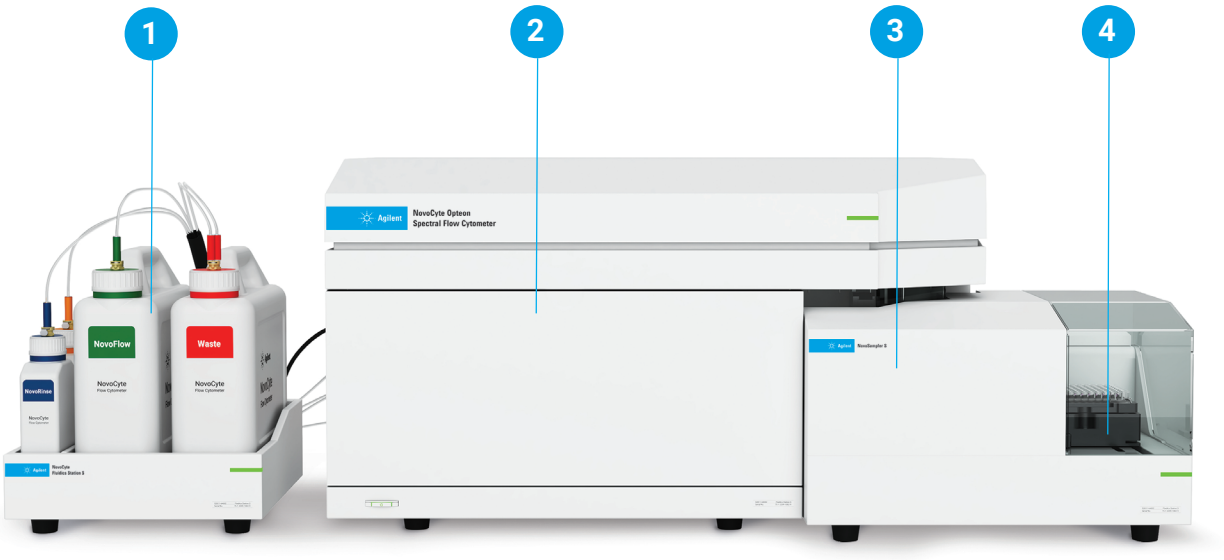
Dual SSC to separate WBCs from RBCs





Dual SSC with V-SSC and B-SSC are used to separate whole blood into platelets, red blood cells, and white blood cells without the need for any lysis protocols.
Note: Optimization may be required.

The NovoCyte experience: simplifying your workflow



1) Continuously monitors fluid levels

A fluidic station will sense low sheath fluid or high waste levels, eliminating the need for manual inspection. Fluidics consumption is estimated before each plate run to ensure uninterrupted sample acquisition.

2) Easy startup and shut down

The quick startup, with automated fluidic rinsing, prepares the instrument for your daily use in only minutes. The configurable and automatic pre-scheduled shutdown cleaning procedure thoroughly cleans at a specified time each day to eliminate the hassle of end-of-day manual cleaning.

3) Embedded quality control

Quickly run daily quality control (QC) automatically generate comprehensive QC reports, and conveniently track performance over time with Levey-Jennings plots. The automatic QC test ensures proper performance monitoring not only day-to-day, but also over long-term use.

4) Hassle-free fluidics

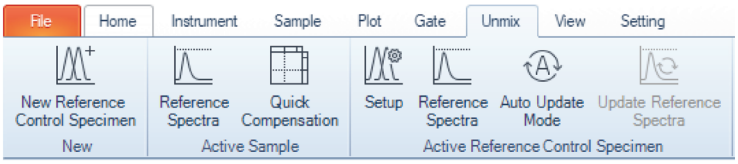
Electronically monitored valves and sensors allow for automatic clog detection and recovery. A feedback control system continuously manages the sheath flow rate to maintain exceptional stability. The embedded self-diagnosis and recovery functions reduce downtime and ensure data quality.

NovoExpress Software:

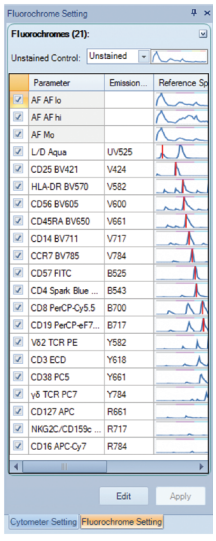
Smart technology, simple solutions

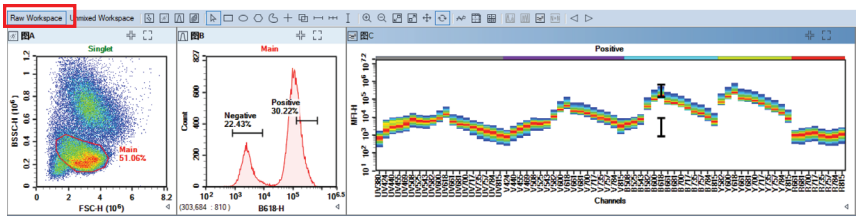
Features of the NovoExpress software:

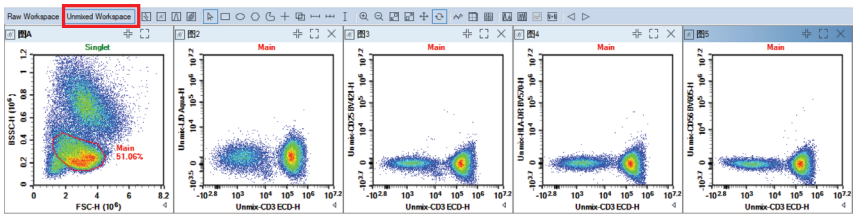
- Built-in fluorochrome reference library (937 fluorochromes covering all commercially available fluorochromes). Easily search and add fluorochromes to the assay panel.
- Linear signal-to-gain relationship allowing the use of different gain settings for reference control and multi-color samples. The previous reference control can be used if applicable.
- Versatile AF subtraction capability. Easy workflow and software functions enable exploration of different AF subtraction populations for unmixing.
- Virtual filters allowing for data analysis using the conventional Compensation Matrix you are already familiar with.
- Powerful analysis tools including Similarity Index Matrix (SIM) and Spillover Spreading Matrix (SSM) to simplify panel design optimization.
- Easy-to-use compensation adjustment tools after unmixing to further refine errors and improve data quality.
- Versatile detector gain adjustment options allow for adjustment either individually, per laser line, or all together to accommodate signal intensity with large panels.
- Live unmixing during data acquisition.

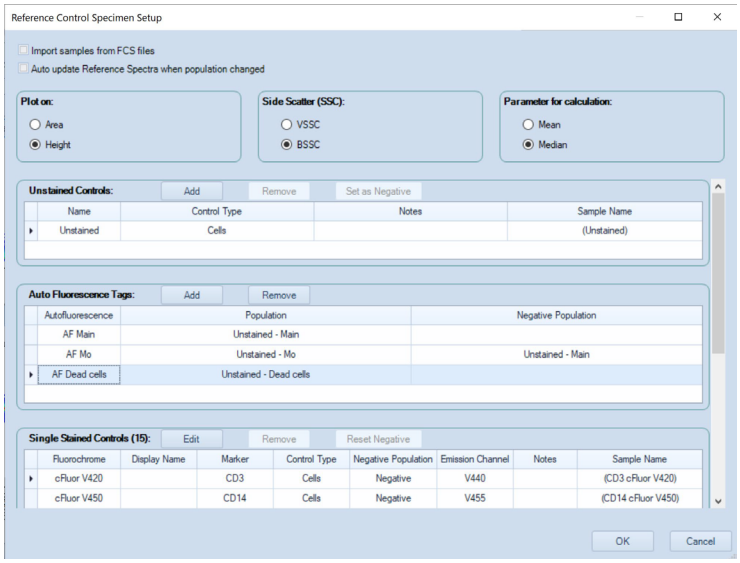


Easily set up and manage reference controls using the NovoExpress software's powerful functions, including live spectral unmixing, versatile AF subtraction, spectral spreading matrices, and fluorochrome libraries.

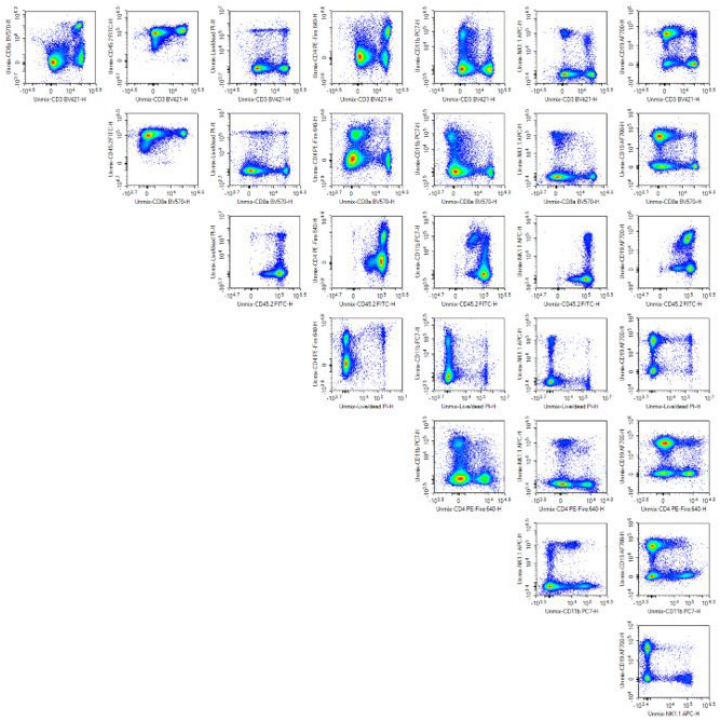








Reference controls are flexible and reusable, enabling easy, user-friendly setup of multiple controls and unstained samples for unmixing so you can see what works best. The simplified workflow allows for experiment optimization.

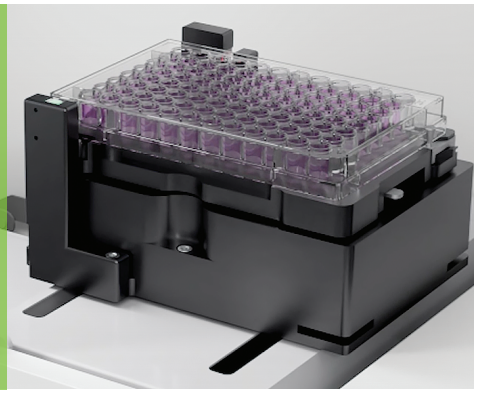


NxN plots

Powerful NxN plots make it easy to check unmixing results and data quality.

Hands Free, Hassle Free

Autosampling with the NovoSampler S



NovoSampler S: 96- and 384-well plate

The Agilent NovoSampler S is an automatic sample loading system for high-throughput and automated sample acquisition. Seamlessly integrated with the NovoCyte Opteon spectral flow cytometer, the NovoSampler S is easy to setup and operate, delivering a high-speed, walk-away automation ready solution.

- Reliable orbital shaking keeps samples in suspension throughout the process; minimize sample carryover
- Fully-automated plate calibration eliminates the needs for manual alignment and calibration
- Versatile loading mode and increased throughput using various sample formats (including, e.g., 40-tube rack, 96/384-well plates)
- Rapid and high-throughput acquisition in as few as 20 minutes for a 96-well plate and under 80 minutes for a 384-well plate
- Lab automation-friendly with an open architecture and developer-ready API

Minimize sample carryover

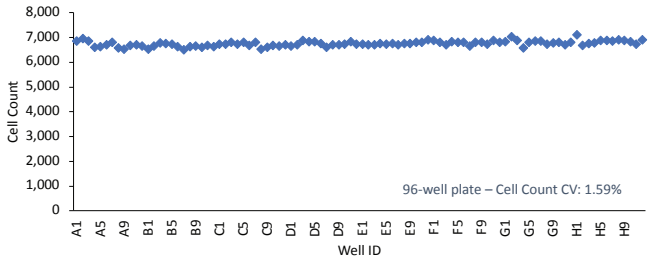
A wide range of flow cytometry applications requires sequential processing and quantitative analysis of sample groups. Minimizing sample carryover is important when acquiring multiple samples and during the analysis of rare events. Carryover from previous samples can substantially affect rare event detection. An automated and customizable washing step after sample acquisition eliminates manual intervention and allows for less than 0.1% sample carryover.

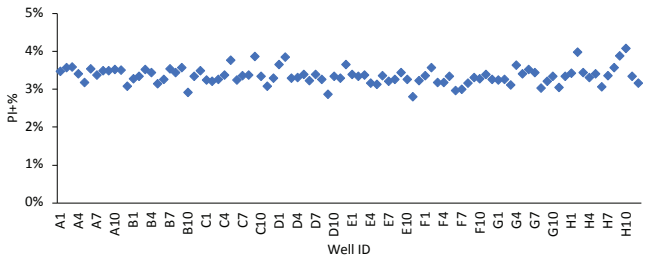
Flexible run time

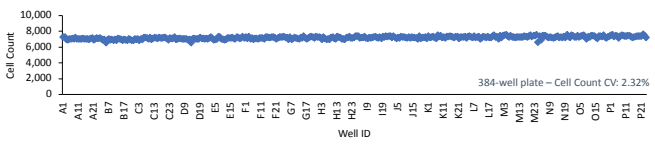
Maximize your productivity with optional large sheath and large waste fluid containers that allow extended sample processing capabilities before replenishment. Up to 15 L of waste capacity ensures 20 hours of continuous instrument operation with large sample batches.

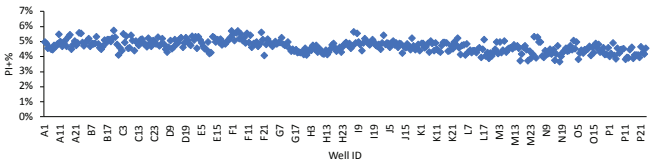
Uniform mixing ensures high reproducibility

The NovoSampler S utilizes the embedded sample mixing settings optimized for different labware and customized options. Easily adjust the mixing speed, duration, and acceleration to optimize mixing efficiency for your sample type.









The NovoSampler S uses orbital shaking to maintain cells in suspension while running the plate and allows for consistent and reproducible results. Shown here are stable cell counts no matter which plate type and with no effect on cell viability.

Configuration Options and Filters: Three-, Four-, and Five-Laser Systems

The NovoCyte Opteon spectral flow cytometer can accomodate three to five lasers, with different configurations available. Providing up to 73 detectors in addition to FSC, B-SSC, and V-SSC NovoCyte Opteon covers the full spectrum to provide high-quality data and the flexibility to run a wide variety of sample types.

Models	Number of Lasers	349 nm (20 mW)	405 nm (130 mW)	488 nm (100 mW)	561 nm (100 mW)	637 nm (120 mW)	Total Number of Detectors
		19	18	14	11	8	
UVBYR	5	√	√	√	√	√	73
UVBR	4	√	√	√		√	62
VBYR	4		√	√	√	√	54
URYB	4	√		√	√	√	54
UVYB	4	√	√	√	√		65
VBR	3		√	√		√	43
VYB	3		√	√	√		46
RYB	3			√	√	√	35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