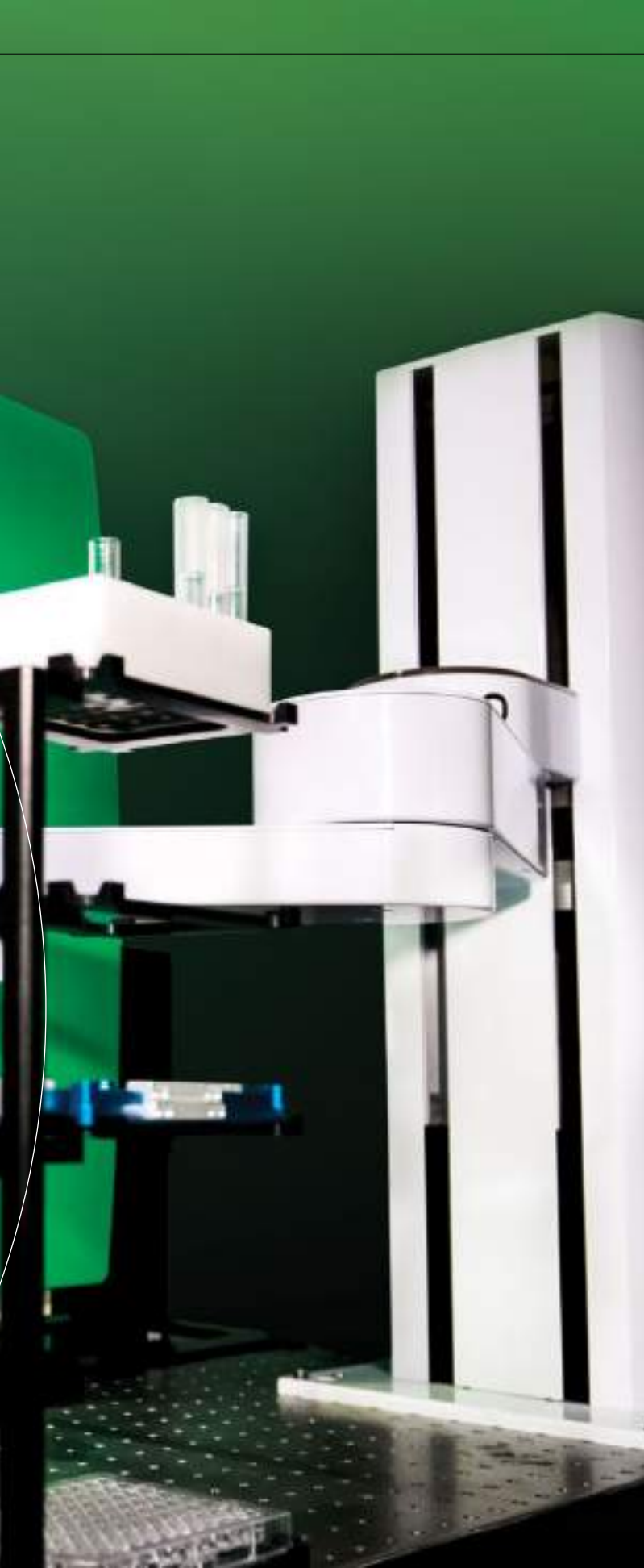


ZE5 Cell Analyzer

Quite Possibly the Only Cell Analyzer You Will Ever Need







A Cell Analyzer Made for Modern Laboratories

Combining speed, automation, performance, and flexibility, the ZE5 Cell Analyzer is the long-awaited solution for modern laboratories. It changes the questions you may have and provides answers you can trust.

- **Switch between tubes and plates with no hardware exchange** — integrated sample loader, accommodating both tube racks and plates, with onboard sample mixing and temperature control provides unparalleled flexibility in handling samples
- **Maximize throughput and productivity using automation** — device-agnostic application programming interface (API) and external fluidics upgrades let you scale up your high-throughput screening with 24/7 operation
- **Power your research with the ability to run high-complexity samples** — up to 5 spatially separated lasers, 27 fluorescence detectors, and a small-particle detector offer ultimate flexibility for your assays
- **Analyze a 96-well plate in less than 15 min or a 384-well plate in less than 60 min*** — high-velocity flow cell and high-speed electronics enable fast acquisition with virtually no aborts and provide higher resolution data in less time
- **Transform manual setup and maintenance procedures into vacation mode** — onboard quality control (QC) beads, bulk fluidics and cleaner, scheduled startup and automated shutdown, and smart designs enable worry-free operation

* For a 96-well plate, 12 min when sampling 10 μl at 2.5 $\mu\text{l}/\text{sec}$ in High-Throughput Mode with 0.25 sec outside/1 sec inside probe wash and 5 sec agitation at column 6. For a 384-well plate, 50 min when sampling 6 μl at 2.5 $\mu\text{l}/\text{sec}$ in High-Throughput Mode with 0.25 sec outside/1 sec inside probe wash and 5 sec agitation at every other column.

High-Throughput Screening

When you need to analyze thousands of samples before moving forward in drug discovery, your low-throughput cytometer just won't cut it. An automation-ready cell analyzer catering to all your high-throughput needs is the ultimate solution.

Fast and Reliable Screening

- Run 96-well plates in <15 min or 384-well plates in <60 min in High-Throughput Mode
- Analyze up to 100,000 events/sec without data loss
- Ensure low carryover while maintaining speed with a flying collar wash station
- Enable assay miniaturization by allowing sampling from extra small volumes

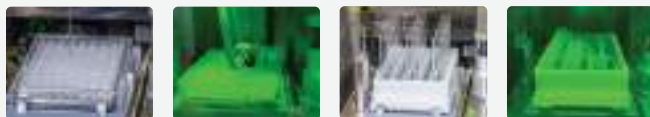
Automation-Friendly and Scale-Up Ready

- Interface with the laboratory information management system and laboratory robotic system through the device-agnostic API
- Choose a turnkey automation system or select your own integration partner for full customization
- Extend operation time to 24/7 with external large-capacity fluidics upgrades

Efficient and Flexible Sample Handling

- Switch between tube racks and plates seamlessly without hardware exchange
- Maintain sample integrity during long screens with onboard temperature control and orbital agitation
- Enable return of unused samples with the bidirectional sample pump*
- Sample with minimal dead volume using intuitive, crash-proof probe calibration

* For non-high-throughput modes only.



Easily switch between tubes, 96-well plates, and 384-well plates with no hardware changes using the integrated sample loader.

High-Throughput Enabling Software

- Save time with protocol and whole-plate sample name import options
- Export data files (FCS 3.1) and statistics using multiple export options
- Visualize target regions with Hit Detection and Heat Map features
- Monitor sample population shifting with the Track Region function



Hit Detection: A rapid visualization of the target region during screening. Wells containing the hit region are colored green, non-hit wells blue.



The Green Button Go System from Biosero, Inc. is one of the integration solutions for the ZE5 Cell Analyzer, fully customizable to automate your workflow and accelerate your discovery.

Multicolor Immunophenotyping

Getting the most information from every single sample is crucial for your research and discovery. Splitting samples to analyze a large number of targets in separate panels is no longer a viable option.

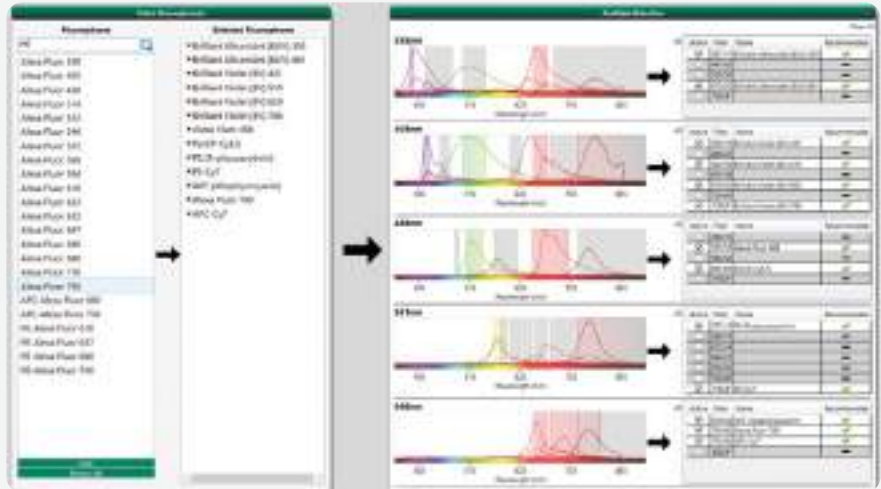
By combining superior optical capabilities with intuitive panel design and autocompensation tools, the ZE5 Cell Analyzer turns high complexity into a simple reality.

High-Complexity Immunophenotyping Simplified

- Provides up to 5 spatially-separated Coherent OBIS lasers and 30 Hamamatsu photomultiplier tube (PMT) detectors
- Detect up to 27 different colors in a single panel
- Validate optical filters automatically with the ZE5-EYE
- Build panels easily with Everest Software's Fluorophore Selector and built-in Spectra Viewer
- Get publication-quality, presentation-ready results with FCS Express Flow Cytometry Software, bundled with new ZE5 Cell Analyzer sales
- Select from thousands of flow cytometry antibodies available from Bio-Rad. Use StarBright Blue Dye-labeled antibodies to boost your resolution. Visit bio-rad-antibodies.com for more information



Up to five spatially separated lasers with water cooling system to maintain beam stability.



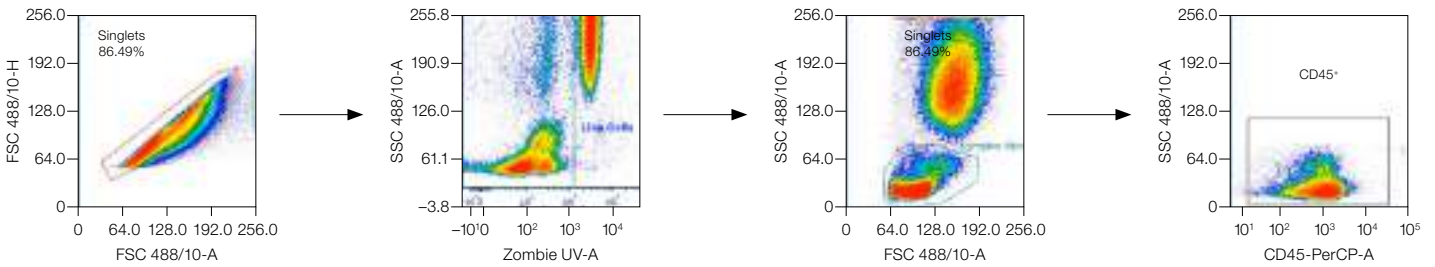
Everest Software allows users to easily select fluorophores and view their spectral overlaps side by side for comprehensive experiment and panel building.

23-Color Immunophenotyping Panel of Human PBMCs

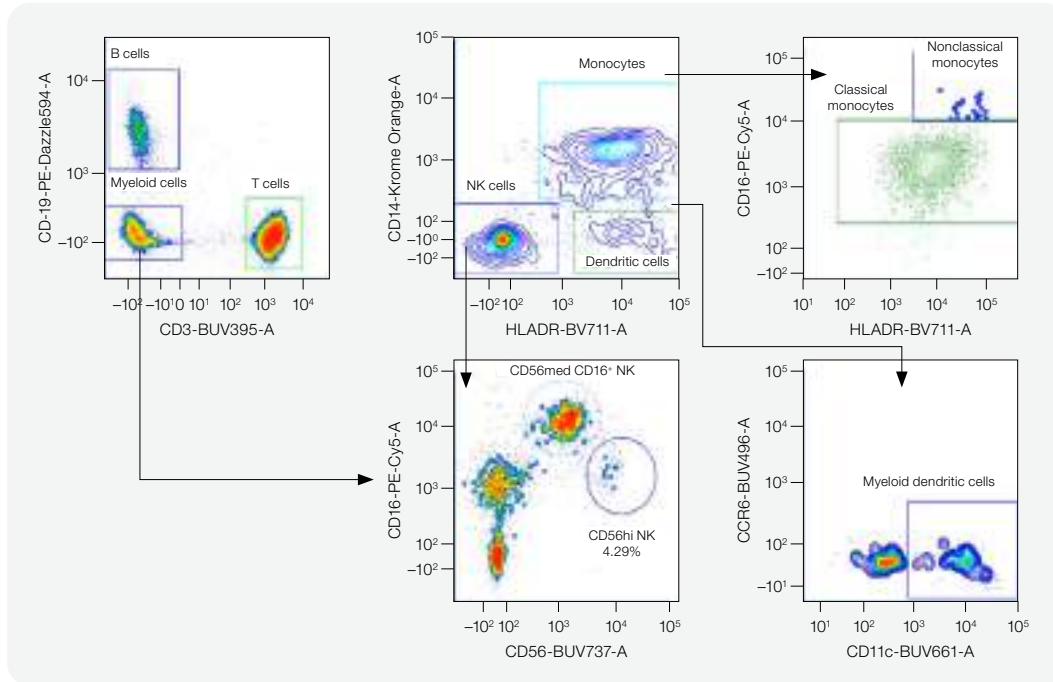
355 nm Filter	Target	Fluorophore	405 nm Filter	Target	Fluorophore	488 nm Filter	Target	Fluorophore	561 nm Filter	Target	Fluorophore	640 nm Filter	Target	Fluorophore
387/11	CD3	BUV395	420/10	CD8	BV421	525/35	CD45RA	FITC	577/15	CD27	PE	670/30	PD1	APC
447/60	Viability	Zombie UV	460/22	CD57	Pacific Blue	593/52	—	—	589/15	—	—	720/60	CD127	APC-AF700
525/50	CCR6	BUV496	525/50	CD14	Krome Orange	670/30*	CD45	PerCP	615/24	CD19	PE-Dazzle594	775/50	CD28	APC-AF750
670/30	CD11c	BUV661	615/24	CD25	BV605	710/20*	CD4	PerCP-eFluor710	640/20	—	—	800/LP	—	—
700/LP	CD56	BUV737	670/30	CD95	BV650				670/30	CD16	PE-Cy5			
			720/60	HLA-DR	BV711				720/60	CD45RO	PE-Cy5.5			
			750/LP	CD38	BV786				750/LP	CCR7	PE-Cy7			

* Customized filters.

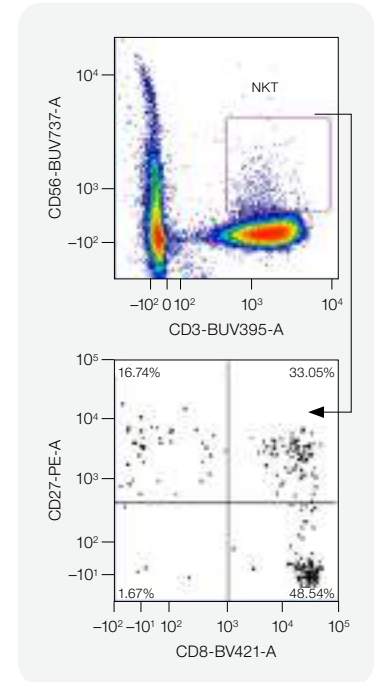
Live Singlet PBMCs



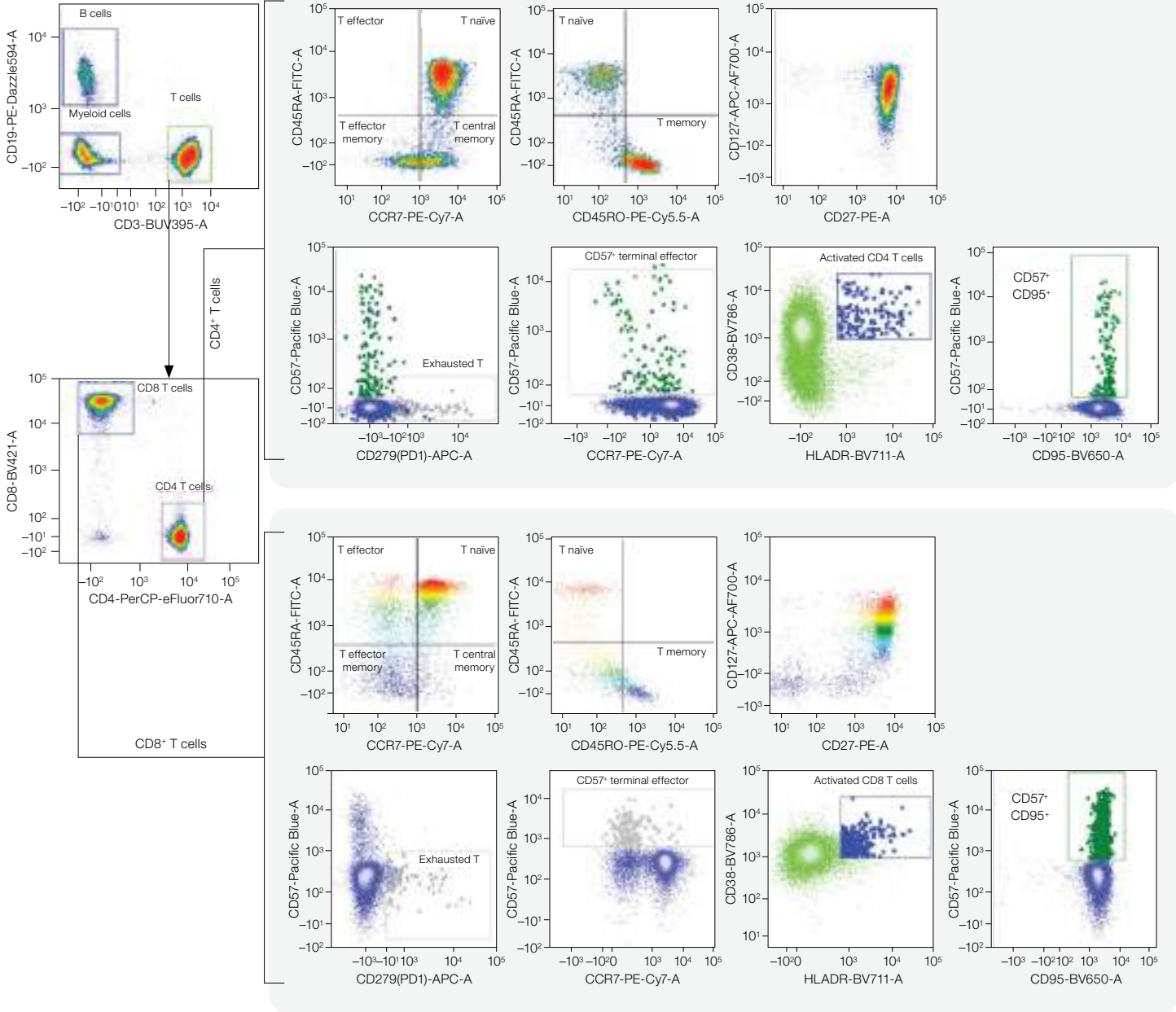
Myeloid Cells



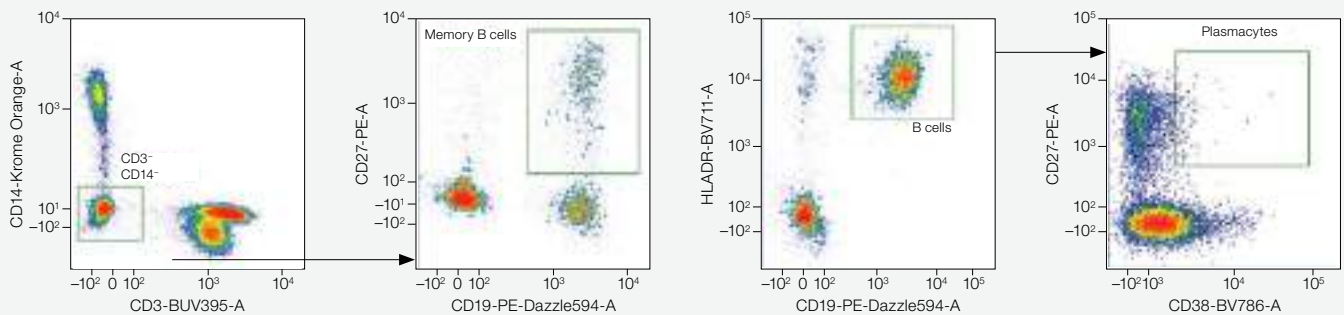
NKT Cells



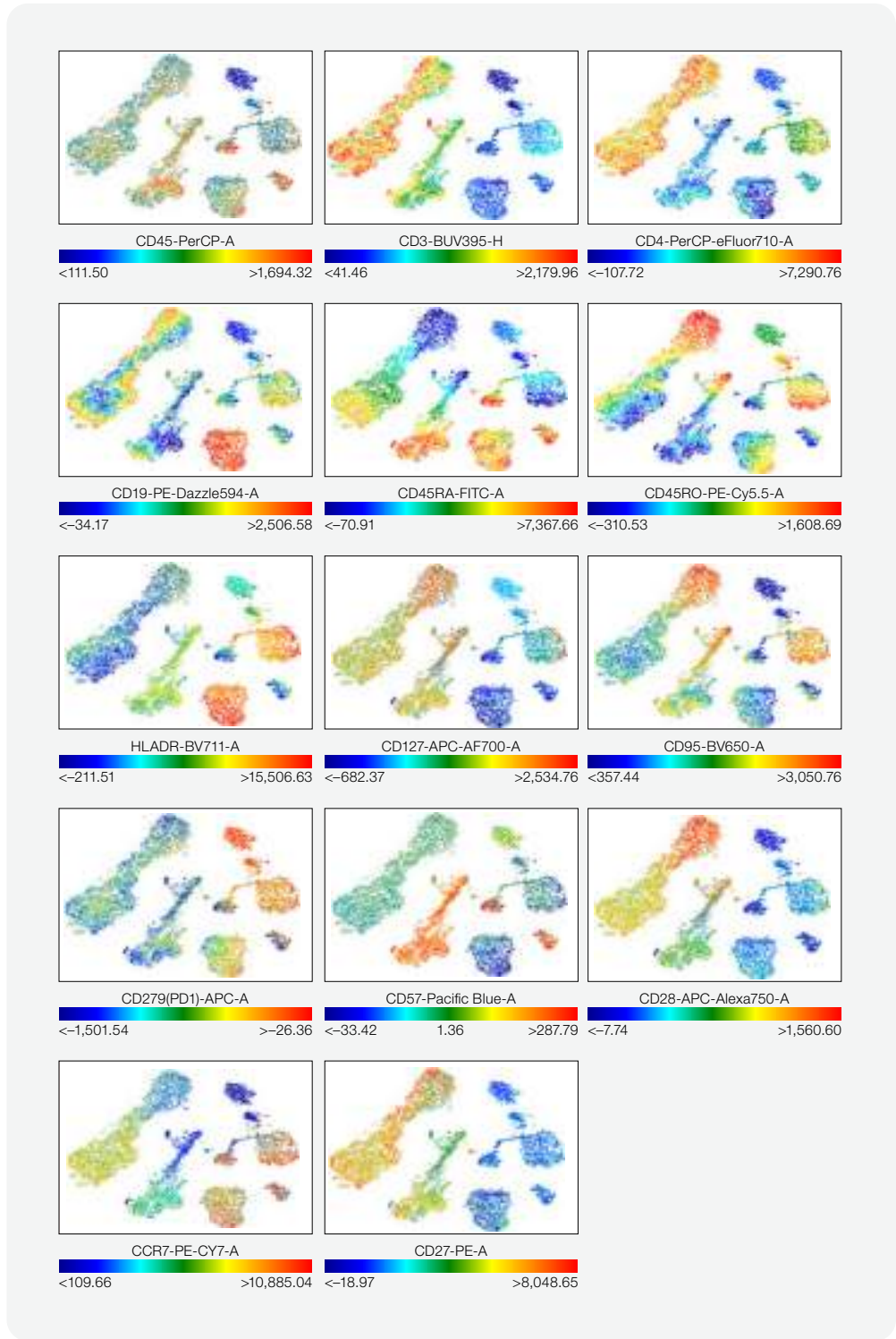
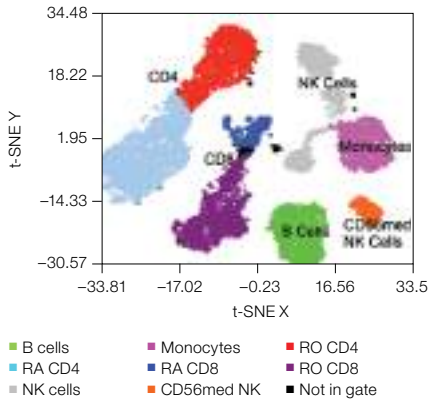
T Cells



B Cells



T-Distributed Stochastic Neighbor Embedding (t-SNE) Analysis

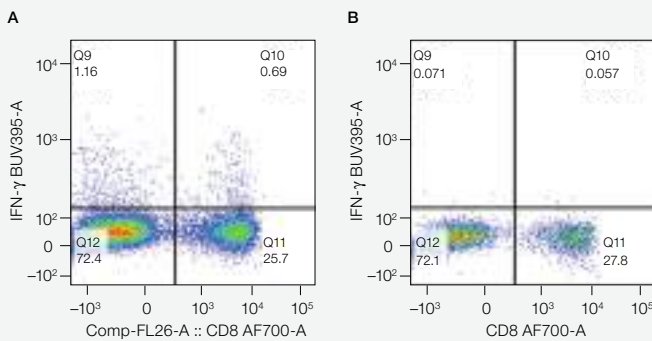


A 23-color immunophenotyping panel on human peripheral blood mononuclear cells (PBMCs) was developed and analyzed on the 5-laser ZE5 Cell Analyzer. The panel identified live singlet PBMCs and myeloid, NKT, T-, and B-cell subsets with activation and differentiation markers. t-SNE plots colored by markers of major subsets are also presented. Data were analyzed with FCS Express 7.04.0014 Software. The experiment was done in collaboration with Dr. Ravi Mylvaganam, Massachusetts General Hospital.

Rare Event Detection

Monitoring cytokine-producing T cells during infection or tracking minimal residual disease in blood requires sampling more than 100 million cells just to characterize a sufficient number of targets. Analyzing so many cells in a slow flow cytometer can take hours. Having to do so for dozens of samples may take days.

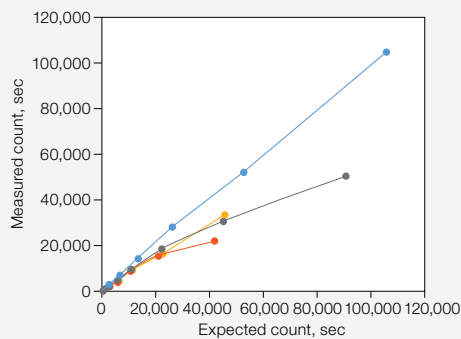
With the ZE5 Cell Analyzer, you can analyze up to 100,000 events per second (eps), which enables you to analyze 100 million cells in just 15 min. The ZE5 Cell Analyzer has a high-velocity flow operating at 8 m/sec, twice the stream velocity of other systems. High-velocity fluidics, sensitive optics, and capable and fast electronics all come together to achieve such a feat.



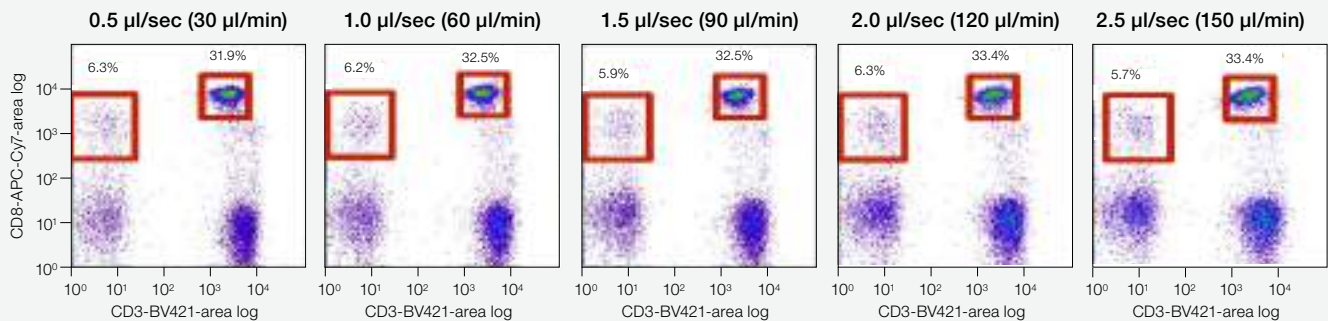
Intracellular cytokine detection. A, identification of rare IFN- γ -producing CD8⁺ T cells (<1% frequency) in nivolumab-stimulated human PBMCs; **B**, fluorescence minus one (FMO) control.

Fast and Sensitive Rare Event Detection

- Ensure every event gets processed, even at high speeds, with powerful 50 MHz data processing and dynamic window extension
- Minimize data spread during high-speed acquisition. With 10 psi sheath pressure and a narrow lumen flow cell, the ZE5 Cell Analyzer maintains a tight sample core that ensures great resolution over a broad range of sample flows
- Get higher resolution and better signal-to-noise ratio with liquid-cooled, high-powered lasers and low-noise, sensitive PMT detectors



The ZE5 Cell Analyzer outperforms other systems as it continues to acquire data into the 100,000 eps range with no data loss. Other systems drop off around 20,000 eps due to data aborts. Serially diluted Dragon Green Beads (Bangs Laboratories, Inc.) at known concentrations were used to determine when the measured count fell off the expected count. ZE5 Cell Analyzer (—); cytometer 1 (—); cytometer 2 (—); cytometer 3 (—). Data courtesy of Karen Helm, University of Colorado, Denver.



CD3 → Human whole blood was lysed and stained with CD45-AF488, CD3-BV421, and CD8-APC-Cy7. Twenty thousand events were acquired and data resolution was consistent at increasing flow rates from 0.5 to 2.5 µl/sec.



Top: Onboard, level-monitored deionized (DI) water and waste tanks allowing for 8 hr operation. Bottom: Optional fluidic upgrade, including 20 L DI and waste carboys on level-monitored fluidic carts lasting for 22 hr. Fluidic upgrade option 2, the direct house DI connection is not shown.

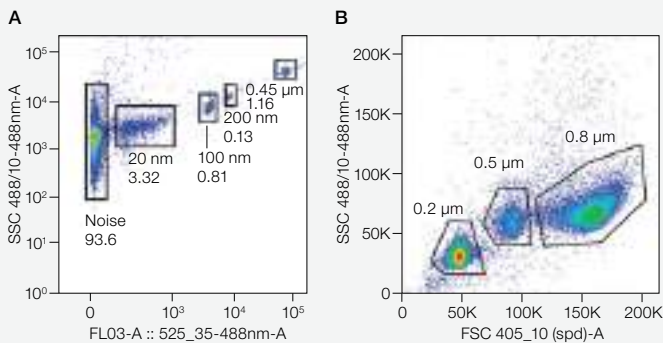
Small Particle Detection

Exosomes and their roles as biomarkers and therapeutic targets are emerging areas of research. Detection of exosomes and microvesicles by flow cytometry can be difficult because resolving these extracellular vesicles (EVs) from electronic noise and sheath impurity is challenging and limited by the optical sensitivity of the cytometer.

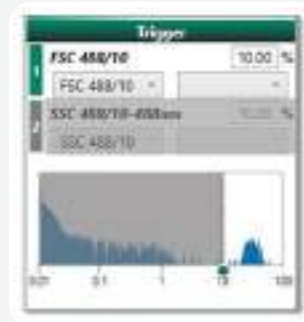
The ZE5 Cell Analyzer was intuitively engineered to accommodate a large range of particle sizes and offers great sensitivity for detection of nanoparticles. With its extremely low noise electronics, dedicated small particle PMT detector, and dual parameter live thresholding, distinguishing small particles from debris and noise is no longer a challenge.

Gain Confidence in Small Particle Detection

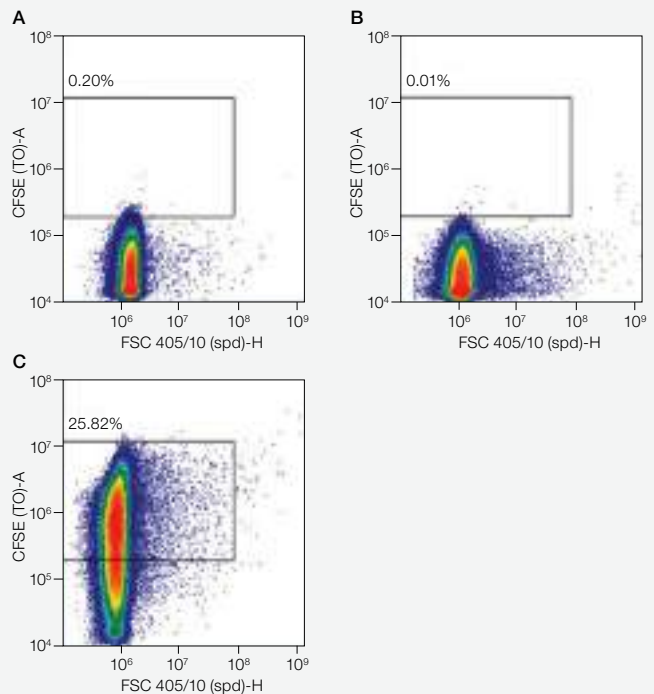
- Resolve 0.1–0.2 μm polystyrene beads with minimal effort
- Eliminate manual setup with the dedicated small particle detector and 0.22 μm inline sheath filter
- Trigger off any 2 parameters (light scatter or fluorescence) for best chance of detection
- Set threshold values with a unique live threshold plot, with visibility to the full range of electronic signals



Microparticle resolution. **A**, resolution of 20, 100, and 200 nm, and 0.45 μm nanoparticles (Flow Cytometry Sub-Micron Particle Size Reference Kit, Thermo Fisher Scientific Inc.) with FITC as trigger; **B**, detection of 0.2, 0.5, and 0.8 μm particles (Submicron Bead Calibration Kit, Bangs Laboratories) with FSC 488/10 as trigger. Data courtesy of Dr. Daniel Mielcarz, Geisel School of Medicine, Dartmouth College.



The live threshold plot of the ZE5 Cell Analyzer allows thresholding from up to two detectors, with the entire range of signals made visible.



Detection of malaria-derived EV cargos using the ZE5 Cell Analyzer.

P. falciparum-derived EVs were purified with a size distribution of 50–120 nm. Protein cargo of the EVs was stained with carboxyfluorescein succinimidyl ester (CFSE) and analyzed on the ZE5 Cell Analyzer. **A**, CFSE only (without EVs); **B**, unstained EVs; **C**, EVs stained with CFSE. Data courtesy of Dr. Ziv Porat, Weizmann Institute of Science, adapted from publication: Dekel E et al. (2020). Antibody-free labeling of malaria-derived extracellular vesicles using flow cytometry. *Biomedicines* 8, 98.

An Efficient Flow Cytometry Core Lab

A busy core lab is a successful core lab. However, being busy means accommodating lots of users and all of their unique needs.

The ZE5 Cell Analyzer was designed to handle a highly flexible workload with minimal effort. The adaptable sample loading platform, built-in checks and balances, and intuitive software ensure that every user gets their way. Level-monitored and optional high-capacity fluid tanks allow the system to run all day long. In addition, startup, cleaning, filter check, and shutdown are all automated to give you a much needed break.

Easy User Onboarding and Operation

- Spot potential panel design issues with the built-in Spectra Viewer
- Streamline sample loading through the integrated, versatile sample loader
- Maintain sample integrity during long screens with onboard temperature control and agitation



Set up the Vacation Mode for automatic Startup and Shutdown during prolonged instrument downtime.

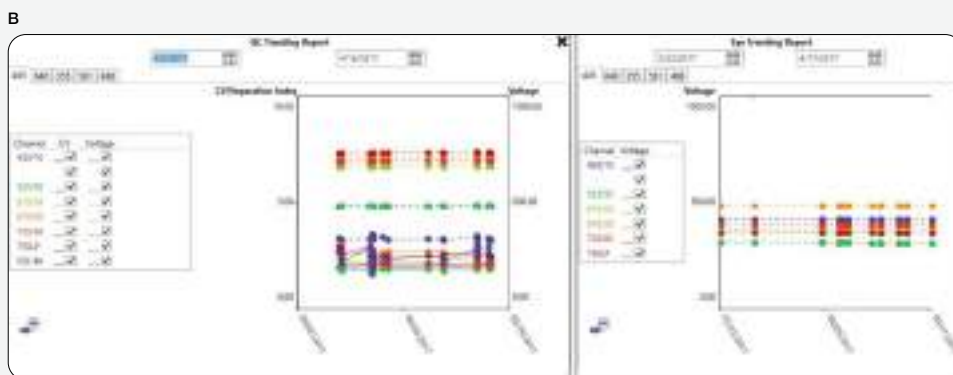
- Save sample using innovative sample pump with minimal dead volume and ability to return unused samples
- Manage multiple users and track login times with User Management and Report functions from Everest Software

Hassle-Free Setup and Maintenance

- Free up your instrument on time with scheduled Startup and automatic Shutdown
- Enjoy hands-free setup with onboard QC beads and an automatic 3.5 min QC process
- Catch incorrect filter placement with the ZE5-EYE
- Enable Vacation Mode for the administrator to set intervals for automatic Startup and Shutdown
- Track the instrument's performance with an array of functionality reports
- Prolong operation time with level-monitored, hot-swappable fluid tanks. Upgrade from 4 to 20 L tanks to extend run time from 8 to 22 hr



Enable Automatic Startup for a future time.



Instrument setup and performance tracking. **A**, onboard QC beads for hands-free QC process; **B**, QC Trending Report and ZE5-EYE Report for instrument performance monitoring.

ZE5 Cell Analyzer Laser and Filter Configuration Guide

5-Laser (27 colors)*			7 Off UV Option A (27 colors)			7 Off UV Option B (27 colors)		
Laser	Filter	Fluorochrome	Laser	Filter	Fluorochrome	Laser	Filter	Fluorochrome
355	387/11	BUV395	355	387/11	BUV395	355	387/11	BUV395
	447/60	AF350, DAPI		509/24	BUV496, DAPI, Zombie UV		460/22	DAPI, Zombie UV, L/D Blue
	525/50	BUV496		577/15	BUV563		509/24	BUV496
	670/30	BUV661		615/24	BUV615		577/15	BUV563
	700LP	BUV737		670/30	BUV661		670/30	BUV661
405	420/10	BV421	405	747/33	BUV737	405	747/33	BUV737
	460/22	SBV440, Pacific Blue		780 LP	BUV805		780 LP	BUV805
	525/50	SBV515, BV510, CFP		420/10	BV 421		420/10	BV421
	615/24	SBV610, BV605		460/22	SBV440, Pacific Blue		460/22	SBV440, Pacific Blue
	670/30	SBV670, BV650		525/50	SBV515, BV510, CFP		525/50	SBV515, BV510, CFP
	720/60	BV711		615/24	SBV610, BV605		615/24	SBV610, BV605
	750LP	BV786		670/30	SBV670, BV650		670/30	SBV670, BV650
488	488/10	SSC	488	720/60	BV711	488	720/60	BV711
	525/35	FITC, GFP, YFP, AF488, BB515		750LP	BV786		750LP	BV786
	593/52	PE, PE-CF594, PE-Dazzle		488/10	SSC		488/10	SSC
	692/80	SBB700, PerCp-Cy5.5, PE-Cy5		525/35	FITC, GFP, AF488, BB515		525/35	FITC, GFP, AF488, BB515
	750LP	BB790, PE-AF750, PE-Cy7		593/52	PE, PE-CF594, PE-Dazzle		593/52	PE, PE-CF594, PE-Dazzle
561	577/15	PE	561	692/80	SBB700, PerCp-Cy5.5, PE-Cy5	561	692/80	SBB700, PerCp-Cy5.5, PE-Cy5
	589/15	DsRed, tdTomato		750LP	BB790, PE-AF750, PE-Cy7		750LP	BB790, PE-AF750, PE-Cy7
	615/24	PE-Dazzle, PE-CF594, mCherry		583/30	PE, tdTomato, DsRed, RFP		583/30	PE, tdTomato, DsRed, RFP
	640/20	mPlum		615/24	PE-TR, PE-CF594, PE-Dazzle594, mCherry		615/24	PE-TR, PE-CF594, PE-Dazzle594, mCherry
	670/30	PE-Cy5, PE-AF647		670/30	PE-AF647, PE-Cy5		670/30	PE-AF647, PE-Cy5
	720/60	PE-Cy5.5		720/60	PE-Cy5.5		720/60	PE-Cy5.5
	750LP	PE-Cy7, PE-AF750		750LP	PE-Cy7, PE-AF750		750LP	PE-Cy7, PE-AF750
640	670/30	APC, AF647	640	670/30	APC, AF647	640	670/30	APC, AF647
	720/60	AF700		720/60	AF700		720/60	AF700
	775/50	APC-Cy7, AF750		775/50	APC-Cy7, AF750		775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810		800LP	AF790, APC/Fire810		800LP	AF790, APC/Fire810

Note: This is not a comprehensive list of fluorophores and dyes that can be used with the ZE5 Cell Analyzer.
 * Eligible for upgrade with 7 detectors off the ultraviolet (UV) laser.

continues

ZE5 Cell Analyzer Laser and Filter Configuration Guide, continued

4-Laser (24 colors)***			3-Laser Option 1 (17 colors)*			3-Laser Option 2 (17 colors)		
Laser	Filter	Fluorochrome	Laser	Filter	Fluorochrome	Laser	Filter	Fluorochrome
405	420/10	BV421	405	420/10	BV421	488	488/10	SSC
	460/22	SBV440, Pacific Blue		460/22	SBV440, Pacific Blue		509/24	FITC, GFP, AF488, BB515
	525/50	SBV515, BV510, CFP		525/50	SBV515, BV510, CFP		549/15	YFP
	615/24	SBV610, BV605		615/24	SBV610, BV605		583/30	PE
	670/30	SBV670, BV650		670/30	SBV670, BV650		615/24	PE-TR, PE-CF594, PE-Dazzle
	720/60	BV711		720/60	BV711		692/80	SBB700, PerCp-Cy5.5, PE-Cy5
	750LP	BV786		750LP	BV786		750LP	BB790, PE-AF750, PE-Cy7
488	488/10	SSC	488	488/10	SSC	561	577/15	PE
	509/24	FITC, GFP, AF488, BB515		509/24	FITC, GFP, AF488, BB515		589/15	DsRed, tdTomato
	549/15	YFP		549/15	YFP		615/24	PE-Dazzle, PE-CF594, mCherry
	583/30	PE		583/30	PE		640/20	mPlum
	615/24	PE-TR, PE-CF594, PE-Dazzle		615/24	PE-TR, PE-CF594, PE-Dazzle		670/30	PE-Cy5, PE-AF647
	692/80	SBB700, PerCp-Cy5.5, PE-Cy5		692/80	SBB700, PerCp-Cy5.5, PE-Cy5		720/60	PE-Cy5.5
	750LP	BB790, PE-AF750, PE-Cy7		750LP	BB790, PE-AF750, PE-Cy7		750LP	PE-Cy7, PE-AF750
561	577/15	PE	640	670/30	APC, AF647	640	670/30	APC, AF647
	589/15	DsRed, tdTomato		720/60	AF700		720/60	AF700
	615/24	PE-Dazzle, PE-CF594, mCherry		775/50	APC-Cy7, AF750		775/50	APC-Cy7, AF750
	640/20	mPlum		800LP	AF790, APC/Fire810		800LP	AF790, APC/Fire810
	670/30	PE-Cy5, PE-AF647						
640	670/30	APC, AF647	640	670/30	APC, AF647	640	670/30	APC, AF647
	720/60	AF700		720/60	AF700		720/60	AF700
	775/50	APC-Cy7, AF750		775/50	APC-Cy7, AF750		775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810		800LP	AF790, APC/Fire810		800LP	AF790, APC/Fire810

Note: This is not a comprehensive list of fluorophores and dyes that can be used with the ZE5 Cell Analyzer.

* Eligible for upgrade with a small particle detector.

** Eligible for upgrade with a UV laser.

Specifications

System

Fluorescence sensitivity	Average MESF: FITC: <70; PE: <50; APC: <40
Forward scatter sensitivity	<0.2 μm FSC resolution with small particle detection module
Loader	Integrated sample loader with agitation and temperature control. 96- and 384-well standard and deep-well plates; 40-tube rack for 5 ml 12 x 75 mm tubes; 24-tube rack for 1.5 ml microcentrifuge tubes; stat tube position for single 5 ml tube
Throughput	<15 min for 96-well plate and <60 min for 384-well plate in High-Throughput Mode

Optics

Excitation	Up to five spatially separated lasers. Standard options include: 355 nm, 50 mW 488 nm, 100 mW 640 nm, 100 mW 405 nm, 100 mW 561 nm, 50 mW
Detection	Up to 30 detectors, including FSC and SSC; optional second FSC detector (for small particle detection)
Cuvette	Fused silica with 145 x 265 μm channel

Electronics

Speed	Up to 100,000 eps with all parameters enabled
Data processing	Simultaneous measured peak, area, and width for every channel. 24-bit data for peak and area. 17-bit data for width with high-resolution linear interpolation at the half height

Fluidics

Sample flow rates	0.025–3.5 $\mu\text{l}/\text{sec}$
Bulk fluids	4 x 4 L bulk fluid tanks on board for sheath and waste. Onboard additive concentrate and cleaner

Installation

Power	100–240 VAC, 50/60 Hz
Dimensions (W x D x H)	29 x 27 x 26 in.; 74 x 69 x 66 cm
Weight	<240 lb; <110 kg
Temperature and humidity	18–28°C; 20–60% RH
Air and vacuum supply	Included, on board

Software

Flow cytometry standard (FCS) format	FCS 3.1
QC	Automated quality control with onboard calibration beads
Acquisition and analysis	Everest Software

Workstation

Dell Precision Workstation	
Processor	Intel Core i7-7700 (Quad Core, 3.6 GHz, 4.2 GHz turbo, 8 MB)
Operating system	Windows 10 Pro
Language pack	English
Graphics	Intel 630 integrated
Memory	DDR4; 32 GB RAM
Networking	Intel Gigabit LAN
Monitor	29 in. with speakers
SSD boot drive	256 GB
HDD data drive	4 TB

Regulatory Compliance

Regulation	CE, Class I (1) laser product Research use only
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**Bio-Rad
Laboratories, Inc.**

Life Science
Group

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Canada 1 905 364 3435 **China** 86 21 6169 8500 **Czech Republic** 00 800 00 24 67 23 **Denmark** 00 800 00 24 67 23 **Finland** 00 800 00 24 67 23
France 00 800 00 24 67 23 **Germany** 00 800 00 24 67 23 **Hong Kong** 852 2789 3300 **Hungary** 00 800 00 24 67 23 **India** 91 124 4029300 **Israel** 0 3 9636050
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Switzerland 00 800 00 24 67 23 **Taiwan** 886 2 2578 7189 **Thailand** 66 2 651 8311 **United Arab Emirates** 36 1 459 6150 **United Kingdom** 00 800 00 24 67 23

