Laboratory for Infectious Disease Research (LIDR)
Immunology Services Facility
University of Missouri, Columbia, MO

High-speed, cutting-edge spectral sorting and analysis

The Bigfoot Spectral Cell Sorter provides power, safety, performance, and flexibility for your lab

Please contact Dr. Jeff Whyte (whytej@missouri.edu) for more information or to discuss potential projects with the Bigfoot Cell Sorter at the MU LIDR.

Discover the cutting-edge Bigfoot Spectral Cell Sorter

The Invitrogen™ Bigfoot Spectral Cell Sorter with Sasquatch Software (SQS) provides the power, safety, performance, and flexibility for your lab today and into the future.

With a custom-designed, integrated Class II biocontainment cabinet, the Bigfoot Spectral Cell Sorter provides safety and protection without compromising high-parameter sorter performance. Multitube input paired with 18-way virtual sorting and integrated temperature control gives flexibility for all your applications.

With a stand-alone footprint, no ancillary requirements, and high-speed electronics enabling >100,000 eps acquisition and >70,000 eps sorting and hot-swap fluidics, the Bigfoot Spectral Cell Sorter provides unmatched performance in any lab space.

The **MU Laboratory for Infectious Disease Research (LIDR)** Bigfoot Spectral Cell Sorter is equipped with 5 lasers (355, 405, 488, 561, and 640 nm excitation wavelengths), 405 nm side scatter detection for increased resolution of small particles, and capture of up to 48 fluorescence channels providing the versatility for both standard fluorescence detection and spectral unmixing.

SQS provides quick start-up, automated calibration, and accurate quality control combined with an experiment designer, intuitive interface, and efficient shutdown, allowing the system to be easy-to-use while reducing downtime. Remote access capability, system health information, and email notifications save time and streamline your workflow.
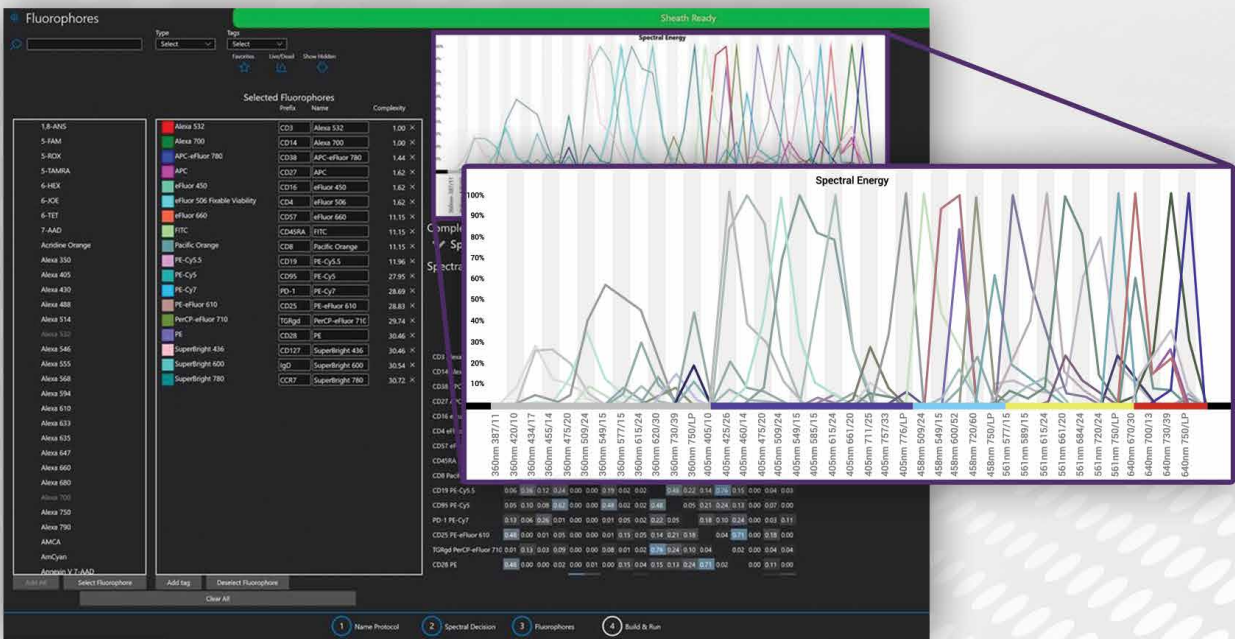
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Sorting and analysis

The first-of-its-kind Bigfoot Spectral Cell Sorter allows both spectral sorting and spectral analysis on cell populations.

- **Configurable**—the high-end configurations of the Bigfoot Spectral Cell Sorter take advantage of the large number of lasers and detectors and allow spectral unmixing for both analysis and sorting

- **Simple**—the experiment wizard assists the user in running controls, identifying potential issues, and applying the unmixing algorithms to produce high-quality data

- **Flexible**—the Bigfoot Spectral Cell Sorter allows both acquisition and sorting on spectrally unmixed data or conventionally compensated data, streamlining the workflow from panel development to high-speed sorting

High speed

Configurable, quick, and calibrated, the high throughput of the Bigfoot Spectral Cell Sorter is in a league of its own: up to 10x faster than the competition.

- **Configurable**—the Bigfoot Spectral Cell Sorter has user-configurable sort output holders for 1.5, 5, 15, and 50 mL tubes, microwell plates up to 1,536 wells, microscope slides, and even 10x™ chips with integrated temperature control (4–37°C) providing maximum versatility

- **Virtual sorting**—with standard six-way sorting and virtual 18-way sorting, the Bigfoot Spectral Cell Sorter can be used to separate multiple sort populations—from a single sample or different samples—for walk-away sorting

- **Calibration**—with built-in stream calibration and drop delay as well as media detection, sort setup is simplified for users

- **High throughput**—with sort rates >70,000 eps, sorting is fast and configurable. From four-way sorting into 96-well plates, eight-way sorting into 384-well plates, or straight-down sorting into 1,536-well plates, the speed and recovery of the Bigfoot Spectral Cell Sorter should exceed your expectations.

- **Integrated**—from built-in media detection cameras to volume tracking of sorted samples, the integrated features of the Bigfoot Spectral Cell Sorter reduce common errors with cell sorting

- **Jet-in-air**—the Bigfoot Spectral Cell Sorter's jet-in-air sensing, gentle with fragile cells, enables the usage of a wide range of nozzle tip sizes while taking advantage of high sort speed and yield

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Class II

With a custom-designed Class II biocontainment cabinet, the Bigfoot Spectral Cell Sorter enables both safety and sample protection, and is ergonomically optimized for sorting workflows.
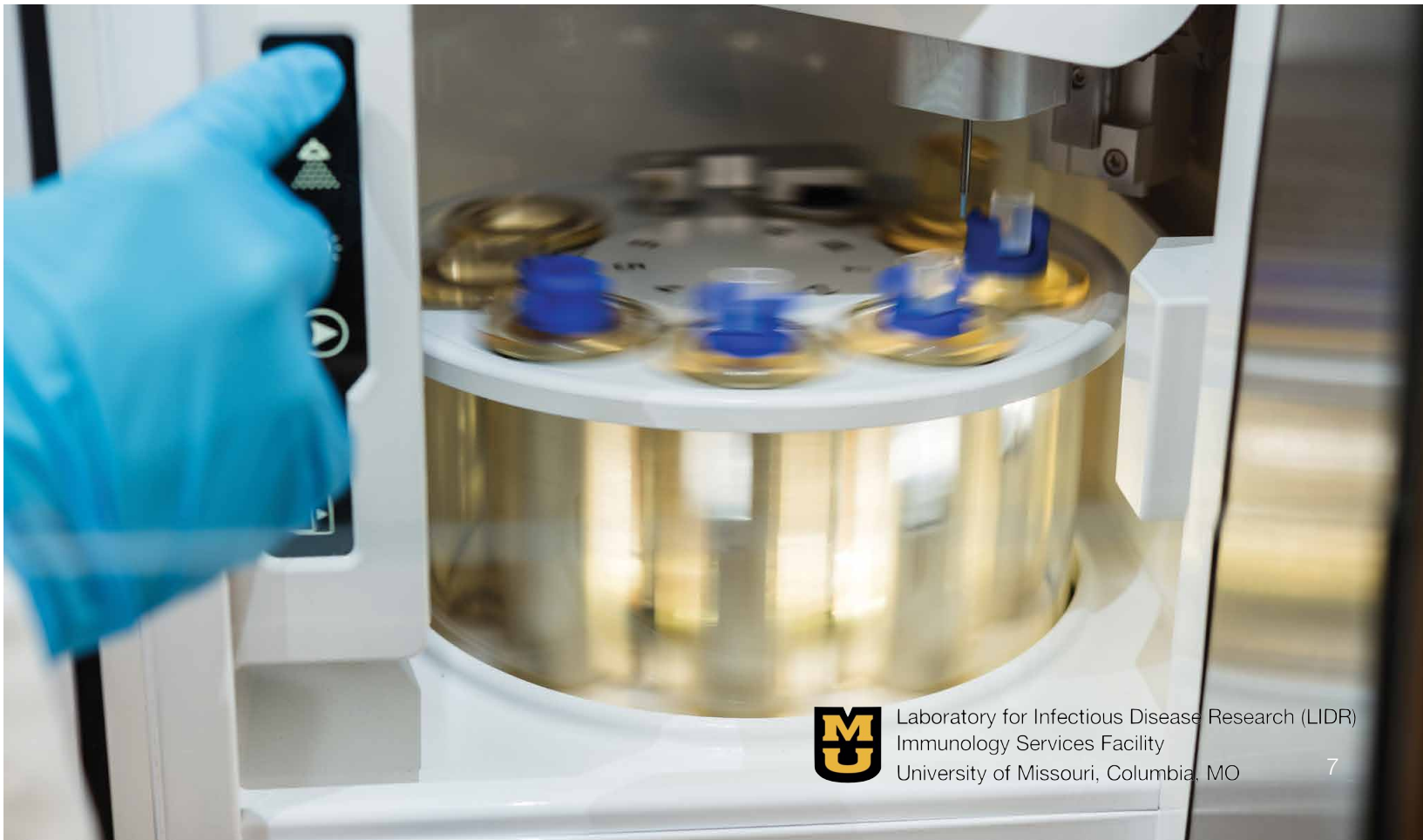
- **Adjustable**—the adjustable sash maintains containment while allowing easy access to the nozzle and sample/sort areas, providing constant user protection

- **Ease of access**—only parts such as the nozzle, sample, and sort chamber are enclosed; the electronics, lasers, and optics remain outside the containment zone, improving service access and temperature regulation

- **Integrated**—dedicated sort-area aerosol management system (AMS), HEPA filter, and integrated software communication provide containment warnings and increased flow to maintain protection if a clog is detected

- **Organized**—built-in sample vortex, plate storage area, tube rack, and biohazard bag streamline workflow

- **Compliant**—the system has been verified to be functionally equivalent to the personnel and product protection standards for a Class II Type A2 biosafety cabinet per NSF International Standard 49

- **Certified**—an airflow/containment certification protocol is provided, giving guidance to your internal biohood certifier

Efficient

- **6 input positions**—the Bigfoot Spectral Cell Sorter enables sampling from 1.5, 5, and 15 mL tubes with automatic tube-type sensing and built-in crash detection

- **Integrated wash station and on-board calibration beads**—the integrated wash station reduces carryover, and on-board calibration beads allow programmable, automated start-up and calibration

- **Built-in agitation and temperature control**—using the built-in agitation and temperature control (4–37°C), the Bigfoot Spectral Cell Sorter maintains the integrity of your samples from start to finish

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Powerful

With custom electronics, firmware, and software designed specifically for high-performance sorting, the Bigfoot Spectral Cell Sorter has the power and flexibility to tackle your application challenges.

- **Accurate**—proprietary electronics simultaneously collect high dynamic range data for measured peak, area, and width for every channel to accurately characterize your sample

- **High-end performance**—the massively parallel, pipelined architecture eliminates hard aborts and allows complex, high-color experiments with up to a 60 x 60 compensation matrix, or spectral unmixing without limiting instrument performance

- **Zero dead time**—dynamic window extension ensures full data collection for every sample

- **Automatic laser delay**—without user interaction, the electronics automatically configures the optimal laser delay for different nozzle sizes and pressures

- **Sort logic**—flexible configuration allows for independent sort logic setups built from 64 total bivariate gates of up to 512 x 512 resolution, along with multiple modes for purity, enrichment, and drop envelopes

Stable

- **Gentle with fragile cells**—jet-in-air sensing is gentle with fragile cells, maximizing viability with a wide range of nozzle tip sizes while taking advantage of high sort speeds and yield

- **5-axis automated stream alignment**—ease of use is improved and short- to long-term variability reduced with automated 5-axis stream alignment and QC

- **Precise droplet monitoring**—integrated control systems ensure optimal droplet formation persistence and position stabilization over time, ensuring accurate drop delay maintenance through the day

- **Simplified tip swapping**—designed with ease of use in mind, the nozzle storage station and swap tip wizard simplify the workflow and help reduce user errors during setup

- **Reduced dead volume**—built-in bubble detection notifies the user and automatically stops sample, reducing dead volume

- **50–150 μm nozzle tips**—supports 50, 70, 100, 120, and 150 μm nozzle tips with varying sheath pressures
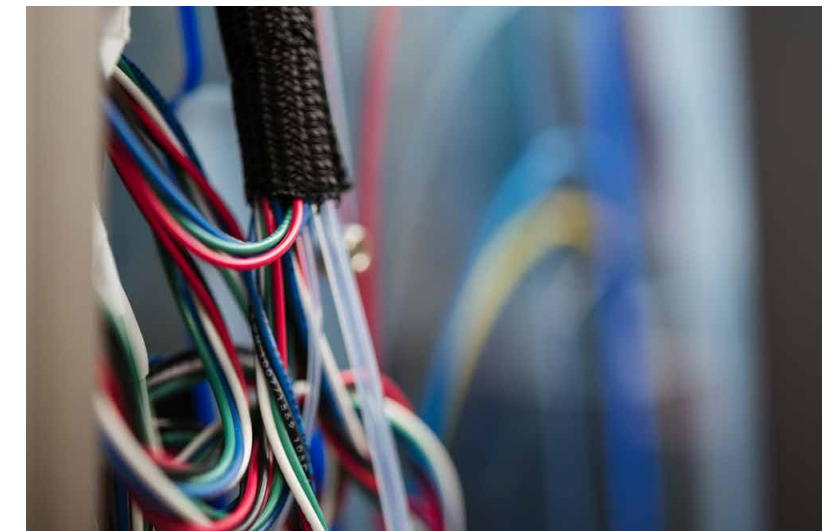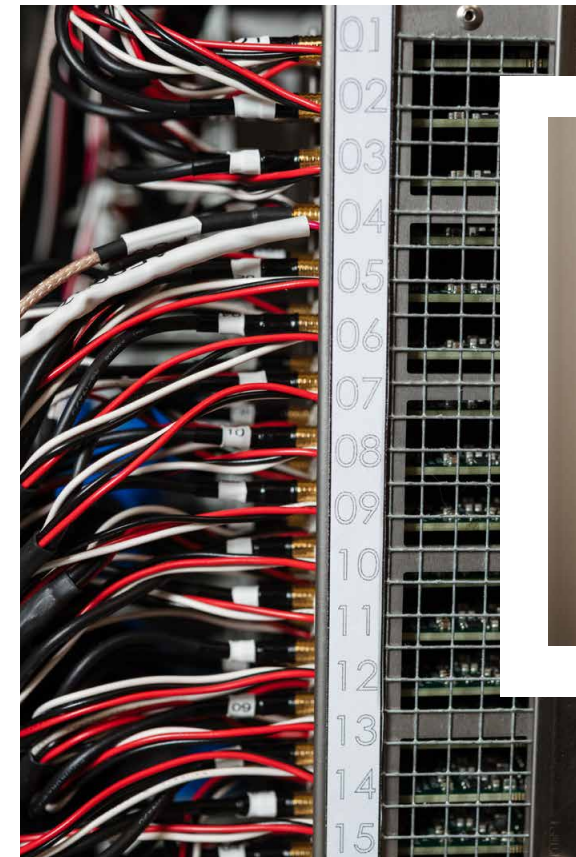
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99 cm

177 cm

99 cm

BIGFOOT

Convenient storage is provided for adapters, spare parts, and tubes to help reduce cluttered lab space.

No external support components are needed. Goodbye external water baths, vacuum pumps, and compressors.

Hot-swappable bulk-fluid bottles allow for a full shift of operation, or for continuous operation by changing during a sort. Optional kits are available for connecting to house DI and waste to reduce the need to fill and empty bulk-fluid tanks.

Adjustable monitor and keyboard arms provide ergonomically optimized flexibility over multiple heights and positions.

On-board cleaner, decontamination, and sheath concentrate bottles allow for automated sheath mixing as well as system rinsing and cleaning without manual intervention.
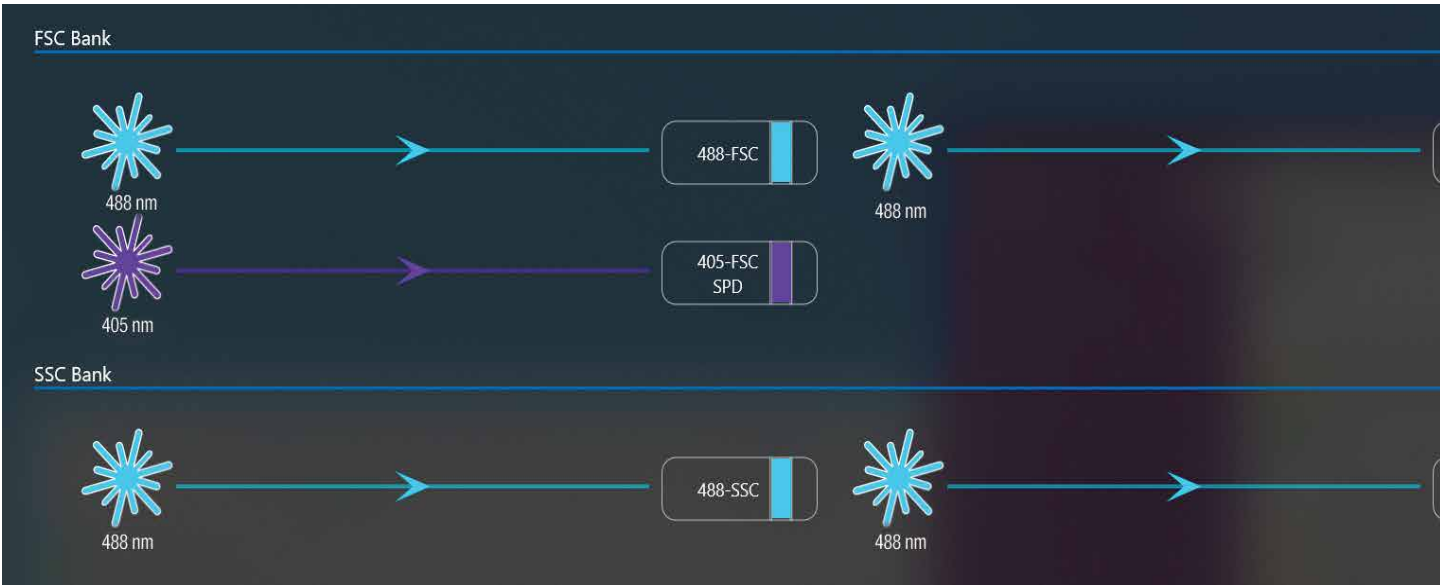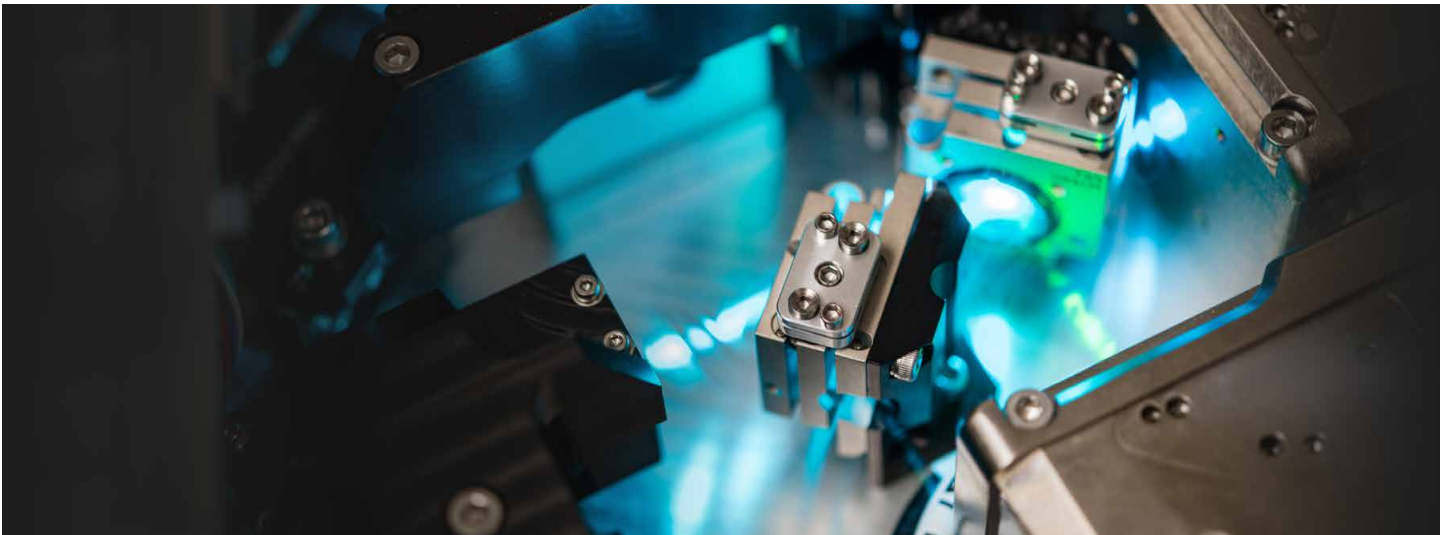
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Flexible

The optical platform of the Bigfoot Spectral Cell Sorter adapts to your needs with flexible laser options and optimized filter sets.

- **Flexible**—the Bigfoot Spectral Cell Sorter offers free space excitation of up to nine lasers into seven pinholes ranging from 349 nm to 785 nm, allowing flexible wavelength selection for your multicolor experiments

- **Stable**—integrated beam shaping and short path lengths maintain optical stability day to day

- **Configurable**—with up to 60 detectors, the Bigfoot Spectral Cell Sorter will adapt to your multicolor applications while still allowing optical filter changes for future needs

- **Multiple scatter options**—multiple scatter options, including small particle scatter detection and depolarization, offer the ability to fully equip your system for your needs

- **Smart**—integrated detection-path checking is provided, with a broadband LED source to confirm detection performance and optical configuration
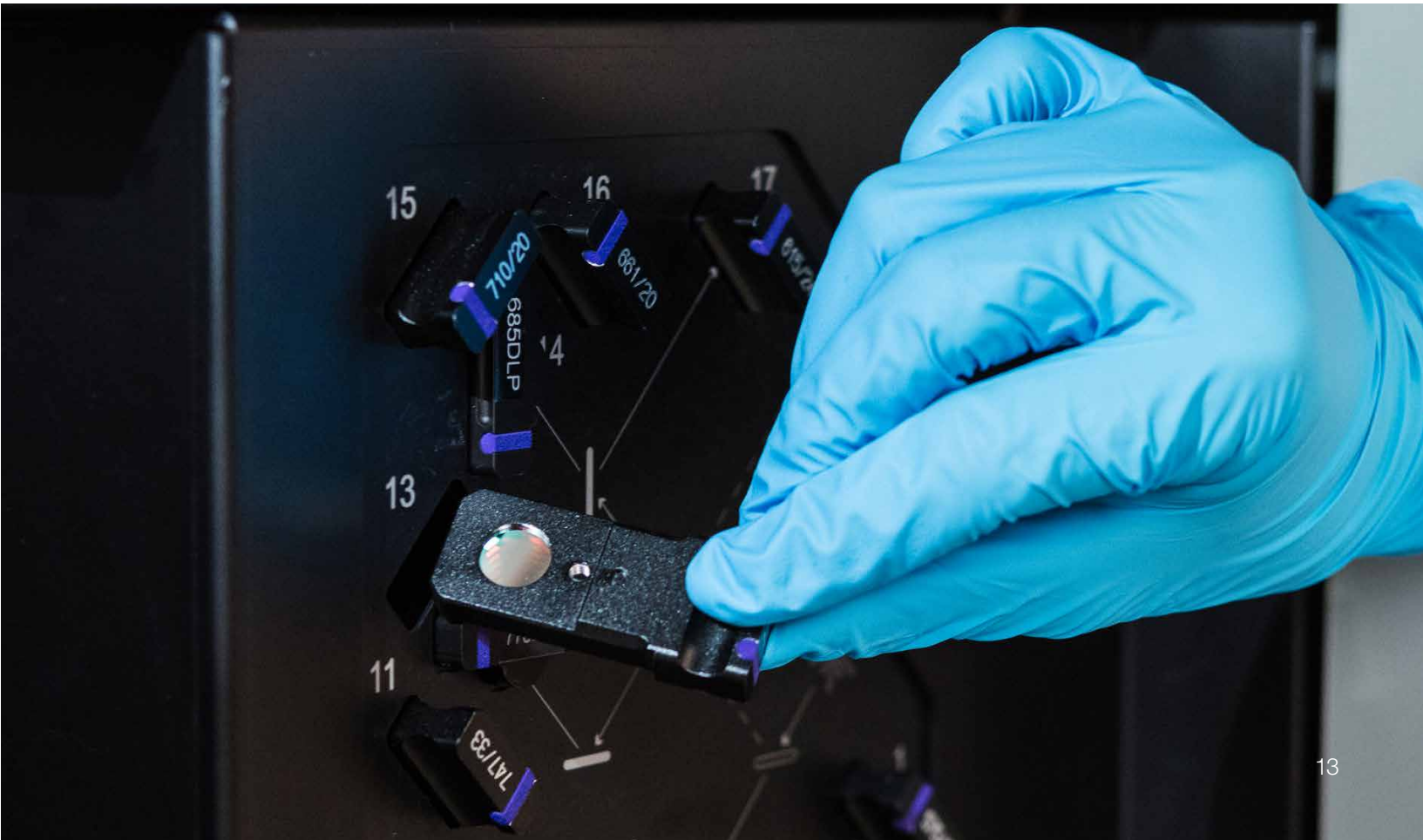

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Intuitive

Support

SQS utilizes a clean user interface with intuitive workflow and integrated features, allowing simplified acquisition, analysis, and sorting.

- **Automated**—start-up, quality control, and drop delay processes are all automated and let you program the system to be ready when you are

- **Advanced fluorochrome selector**—helps with design for both standard and spectral experiments, and warns of potential conflicts

- **Remote monitoring**—system health monitoring provides status and control, giving you the freedom to walk away from the system while still monitoring experiments and to start-up or shutdown remotely

- **High speed**—Acquire and store files at >100,000 eps up to 100 million events per FCS file with all parameters enabled, reducing rare event detection time

- **Full reporting capabilities**—with reports such as system usage, quality control, trending, and sort summary, information is presented clearly for further analysis

- **Compatibility**—generate FCS files that are compatible with other analysis software packages

- **Analysis features**—features including overlays, color-gating, back-gating, index sorting, and ratio plots give you added capability to display and publish data

- **System health**—with multiple cameras and sensors throughout, the Bigfoot Spectral Cell Sorter provides feedback on all subsystems and logs status for proactive troubleshooting

- **Launcher**—along with ensuring the proper initialization and launch of SQS, the launcher also provides the most secure method of installing and updating instrument software

- **Remote support**—cloud-based instrument reporting allows our knowledgeable team to support your system with the fastest response, increasing your uptime

- **Email notifications**—manage email notifications to help save time and streamline your workflow, helping you to support your end users

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Consistent path lengths, stable optical filter layouts, and highly sensitive PMT detectors optimize detection across the entire spectrum.
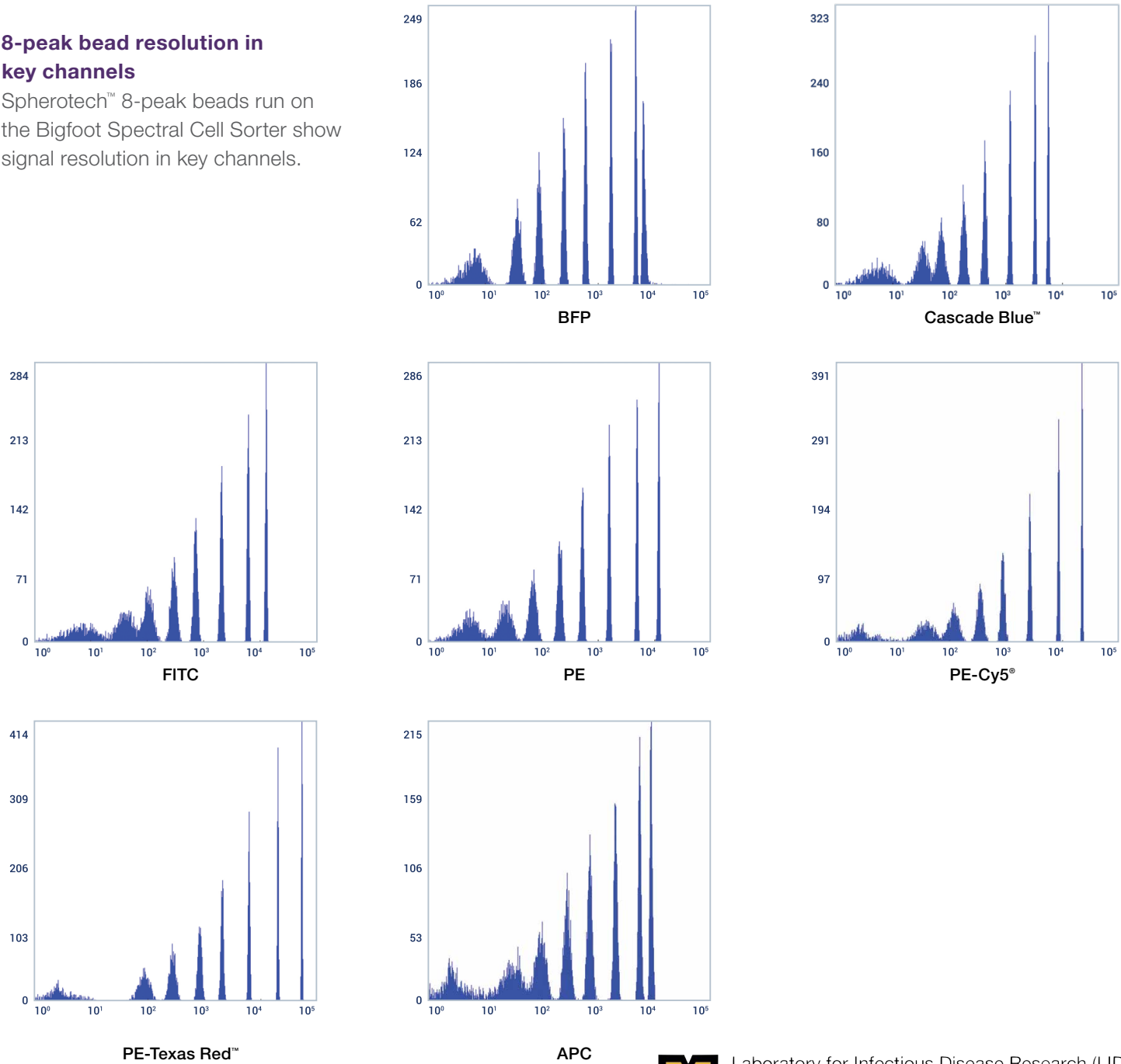
Analysis of human blood

The data below show a multicolor panel run on a nine-laser Bigfoot Spectral Cell Sorter. The panel shown defines T-cell subsets using lysed whole blood from a normal donor. Antibodies used are CD45 FITC, CD3 Alexa Fluor™ 532, CD4 BUV395, CD8 APC, CD16 BV510, CD56 PE, CD19 PE-Cy5.5®, CD20 Alexa Fluor™ 700, CD25 PE-eFluor™ 610, CD127 BV421, TCRgd PerCP-eFluor™ 710, CD14 PE-Cy7®, CD45RA BV711, and CD45RO BV786. The data are shown with spectral unmixing and biexponential scaling.
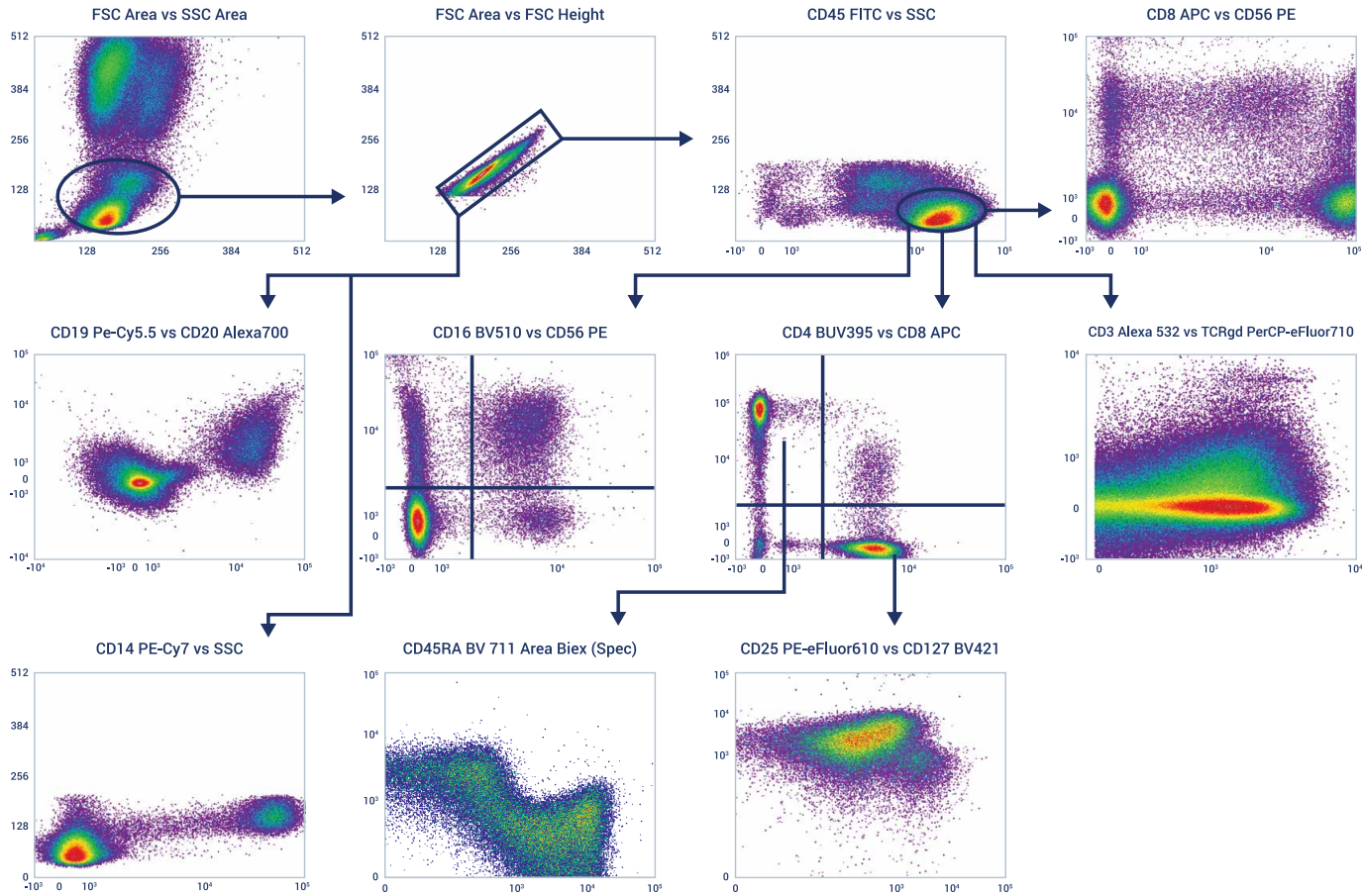
8-peak bead resolution in key channels

Spherotech™ 8-peak beads run on the Bigfoot Spectral Cell Sorter show signal resolution in key channels.

Flow cytometry gating strategy plots: FSC Area vs SSC Area, FSC Area vs FSC Height, CD45 FITC vs SSC, CD8 APC vs CD56 PE, CD19 Pe-Cy5.5 vs CD20 Alexa700, CD16 BV510 vs CD56 PE, CD4 BUV395 vs CD8 APC, CD3 Alexa 532 vs TCRgd PerCP-eFluor710, CD14 PE-Cy7 vs SSC, CD45RA BV 711 Area Biex (Spec), CD25 PE-eFluor610 vs CD127 BV421.

8-peak bead histograms: BFP, Cascade Blue™, FITC, PE, PE-Cy5®, PE-Texas Red™, APC.

System		
	Sensitivity	<100 MESF for FITC, PE, APC; <0.2 µm FSC resolution with small particle detection module
	Sample input	6 tube input positions for 1.5 mL, 5 mL, or 15 mL tubes; temperature control (4–37°C) and agitation for all positions
	Sort output	Up to six-way sorting into tubes; configurable tube holders include 1.5 mL, 5 mL, 15 mL, and 50 mL adapters. Multiway microwell plate sorting up to 1,536 wells. Temperature control (4–37°C) for all media types.
	Nozzle	70 µm and 100 µm ceramic nozzle tips with adjustable pressure settings
	Biocontainment	Integrated Class II biocontainment cabinet for protection of sample and user. Separate AES for sort chamber evacuation.
Optics		
	Excitation	349 nm 405 nm 488 nm 561 nm 640 nm
	Detection	Up to 48 parameters, including FSC and SSC
Electronics		
	Speed	>100,000 eps acquisition and >70,000 eps sorting with all 48 parameters
	Data processing	• Simultaneous measured peak, area, and width for every channel; 24 bit data for peak and area to maximize dynamic range • True measured width at half-height • Low noise converters and proprietary digital processing reduce channel noise
Fluidics	Bulk fluids	2 x 4 L bulk fluid tanks on board for DI water and waste. 4 L bottle for sheath or sheath concentrate. Two 1 L on-board cleaners. Optional kits available for connecting to house DI and waste.
Installation	Power	100–240VAC 50/60Hz; 800W max
	Dimensions	99 cm x 99 cm x 177 cm (W x D x H)
	Weight	400 kg
Software	Operating system	Windows™ 10 operating system
	Software	Sasquatch Software (SQS)
	FCS format	FCS 3.1
	QC	Automated quality control and drop delay with on-board calibration beads
	Workstation	10-core i9-10900 processor, 32 GB RAM, and 2 TB PCIe SSD
	Monitor	32 in. 4k
Regulatory		CE, Class 1 (1) laser product
		Research Use Only

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