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 (SGS) 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.

SGS 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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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.

**Sorting and analysis**

High throughput

- **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.

- **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 speed**

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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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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

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

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

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.

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

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

Support

- **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
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 BV421, CD8 APC, CD16 BV510, CD56 PE, CD19 PE-Cy5™, 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.

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8-peak bead resolution in key channels

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

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