Bigfoot Spectral Cell Sorter

Multi-sample loader offers improved efficiency, increased safety, and fast performance for sorting and analysis

Introduction

The multi-sample loader on the Invitrogen™ Bigfoot Spectral Cell Sorter is the first of its kind among commercially available cell sorters, offering more functionality than many existing flow cytometry analyzers.

The 6-position multi-sample loader allows the controls for a 5- to 10-color experiment to be loaded in one or two sets. The multi-tube loading capability is especially efficient for studying 20 or more fluorescence parameters, which is increasingly common in flow cytometry.

All sample-related subsystems, including the multi-sample loader, are contained inside the fully integrated biosafety cabinet (BSC) for optimal safety, efficiency, and performance.

A multitude of laboratory and research needs are met with one instrument, because the Bigfoot Spectral Cell Sorter combines a multi-sample loader, an integrated BSC, and a high-performance cell sorter in one flexible and powerful system.

Bigfoot Spectral Cell Sorter as an analyzer

Although the Bigfoot Spectral Cell Sorter is a high-end cell sorter, it also offers more functionality than many flow cytometry analyzers, making the Bigfoot Spectral Cell Sorter a strategic choice for facilities that require both types of instruments. The multi-sample loader includes 8 stations on a circular platform. The bead and wash stations are static locations, and there are 6 flexible locations for sample input (Figure 1).

Figure 1. The multi-sample loader on the Bigfoot Spectral Cell Sorter is shown with the sample probe above the wash station.

Bead station

The bead location is dedicated to calibration beads, and has a specially designed cap and on-board agitation. The user inserts a vial of premixed calibration beads into the bead station approximately once a month, and the instrument uses the calibration beads to automatically perform the daily QC protocol, including alignment and drop delay measurements. Unlike other instruments on the market, the Bigfoot Spectral Cell Sorter does not require the user to retrieve calibration beads from the refrigerator, dilute, and prepare a tube for daily setup.

Wash station

The location of the wash station is fixed on the loader. This station makes use of on-board water and cleaners to wash the outside of the probe between samples, while simultaneously backflushing the entire sample path, requiring no user interaction. The entire wash process is accomplished in 8 seconds and results in extremely low sample carryover.

Sample stations

Users can load up to 6 samples at once. All positions have temperature control (4°C–37°C), and an agitation speed is specifically optimized for the installed tube type. Tubes can be individually illuminated to view the remaining sample, and the lights can be turned off to prevent sample degradation.

When a sample's volume runs low, a reliable end-of-sample bubble detector responds within 50 milliseconds to stop the sample. This feature enables the instrument to acquire the entire sample, wash the probe, and move to the next tube without user interaction, and allows the user to walk away from the instrument during acquisition. Sample-to-sample acquisition is accomplished in less than 20 seconds, including the 8-second wash at the dedicated wash station.

Flexible sample input tube types

In addition to the bead and wash locations, the loader features 6 interchangeable sample input locations that can accommodate any combination of 1.5 mL, 5 mL, and 15 mL tubes (Figure 2). The loader configuration is easy to change, and the instrument automatically detects the current adapter. Unlike with other instruments, the user does not have to interact with software to identify new configurations.

Each tube holder has a color-coded top to easily identify the loader configuration, and enables the instrument to automatically identify tube types. A camera detects the color-coded tube holder and the instrument automatically adjusts to the appropriate probe height, identifies the configuration of the loader, and adapts to user preferences.

A precise closing mechanism lowers and seals over the tube holder to create the pressurized environment required to consistently deliver the sample to the interrogation point.

Probe awareness, crash protection, and safety

In response to common user pain points, such as accidentally leaving a cap on a tube while attempting to run the instrument and thereby damaging the probe, the Bigfoot Spectral Cell Sorter includes independent interference sensors that quickly detect obstructions, so probe movement can be stopped before a serious collision can occur. This feature dramatically reduces the need to replace the sample probe. The light curtain safety feature is an additional measure that prevents the loader from moving if the user's hands are near moving parts while interacting with the loader.

Figure 2. Sample input tube adapters for 1.5 mL (white), 5 mL (blue), and 15 mL (red) tubes.

Bigfoot Spectral Cell Sorter as a sorter

The loader characteristics that make the Bigfoot Spectral Cell Sorter an excellent analyzer lay the foundation to be a superior cell sorter.

Additional features with sorting

In addition to previously described functionality, such as the ability to load 6 control tubes at a time, flexible tube type options, probe crash awareness, and more, the multi-sample loader offers additional improvements for cell sorting applications.

Bulk sample sorting

The Bigfoot Spectral Cell Sorter is capable of sorting bulk samples up to 90 mL, using 6 tubes of 15 mL each.

Rapid data visualization

The tube holder design with low air volume provides rapid and stable pressurization for quick response on data plots, reducing the time required to determine the appropriate PMT voltage settings. The speed of sample-to-sample acquisition also allows quick verification of 6 consecutive controls to help ensure quality.

Rapid clog detection protects sorted sample

Clog detection stops the stream and deploys the waste catcher in less than 100 milliseconds. This is significantly faster than user response time, protecting valuable sorted samples from contamination. When the system is in this state, the fan speed on the aerosol management system is increased to remove any aerosolized particles from the sort chamber before it is opened.

Loader efficiency increases safety

Lab personnel in biohazardous environments must work efficiently and use techniques that reduce interactions with hazards to maximize safety. Every time the barrier airflow curtain is breached, the user is at increased risk for an unsafe interaction with pathogens. The Bigfoot Spectral Cell Sorter was specifically designed to minimize this problem. Inside the BSC's laminar airflow barrier there is ample deck space for sample racks and plates as well as access to a built-in sample vortex mixer, tube rack, and biohazard bag. This makes it possible for the user to complete common tasks without breaching the safety barrier multiple times during a normal workflow. The multi-sample loader is contained inside the BSC for optimal safety, efficiency, and performance (Figure 3). The BSC system has been verified to meet personnel and product protection standards functionally equivalent to a Class II Type A2 biosafety cabinet per the National Sanitation Foundation (NSF) Standard 49, Section 3.8.2.1 [1].

Figure 3. The multi-sample loader is inside the BSC.

The Bigfoot Spectral Cell Sorter includes four major innovations to help users limit hazardous contact:

1. The sample loader holds multiple controls or samples, reducing the need for the user to enter and exit the BSC continually for sample loading. The 6 sample input locations on the loader make it possible to perform a 16-color experiment, with 16 single-color controls, one negative control, and one test sample, with only three passes in and out of the BSC.
2. The sort collector accommodates numerous sort tubes to collect multiple sorts from different runs or one long run, reducing the need for the user to enter and exit the BSC to unload sorted samples.
3. Plates can be sorted in less than 20 seconds, so the time the user interacts with the biohazardous environment is reduced.
4. The internal control panel limits the need for the user to reach into and out of the BSC to operate the system, as basic interaction with input and output is typically accomplished without the need to interact with the software.

Service

When a probe or sample line requires replacement, the Bigfoot Spectral Cell Sorter allows easy access to the user-replaceable components safely within the BSC without the need to run a full decontamination protocol.

The design of the Bigfoot Spectral Cell Sorter encloses only potentially pathogen-exposed components and leaves the rest of the instrument accessible to users and service personnel. Interior work surfaces are made with nonporous, durable 304 stainless steel and have rounded corners for easy cleaning.

Demonstration of sample load to acquisition

To demonstrate the performance of the multi-sample loader, one 5 mL tube of DI water was placed in each of the 6 tube holders, and the software was set to acquire all 6 samples. The Bigfoot Service Software was used to record and graph instrument activity as the loader rotated into position, prepared each sample for acquisition, and initiated acquisition (Figure 4). ImageJ Analysis Software [2] was used to analyze the resulting graphs and determine the elapsed time for each stage of the process as reported in Table 1. The data illustrate that the Bigfoot Spectral Cell Sorter is ready to acquire samples on average within 9.89 seconds, excluding the time for rotation to the sample station, which varies with the location of each position.

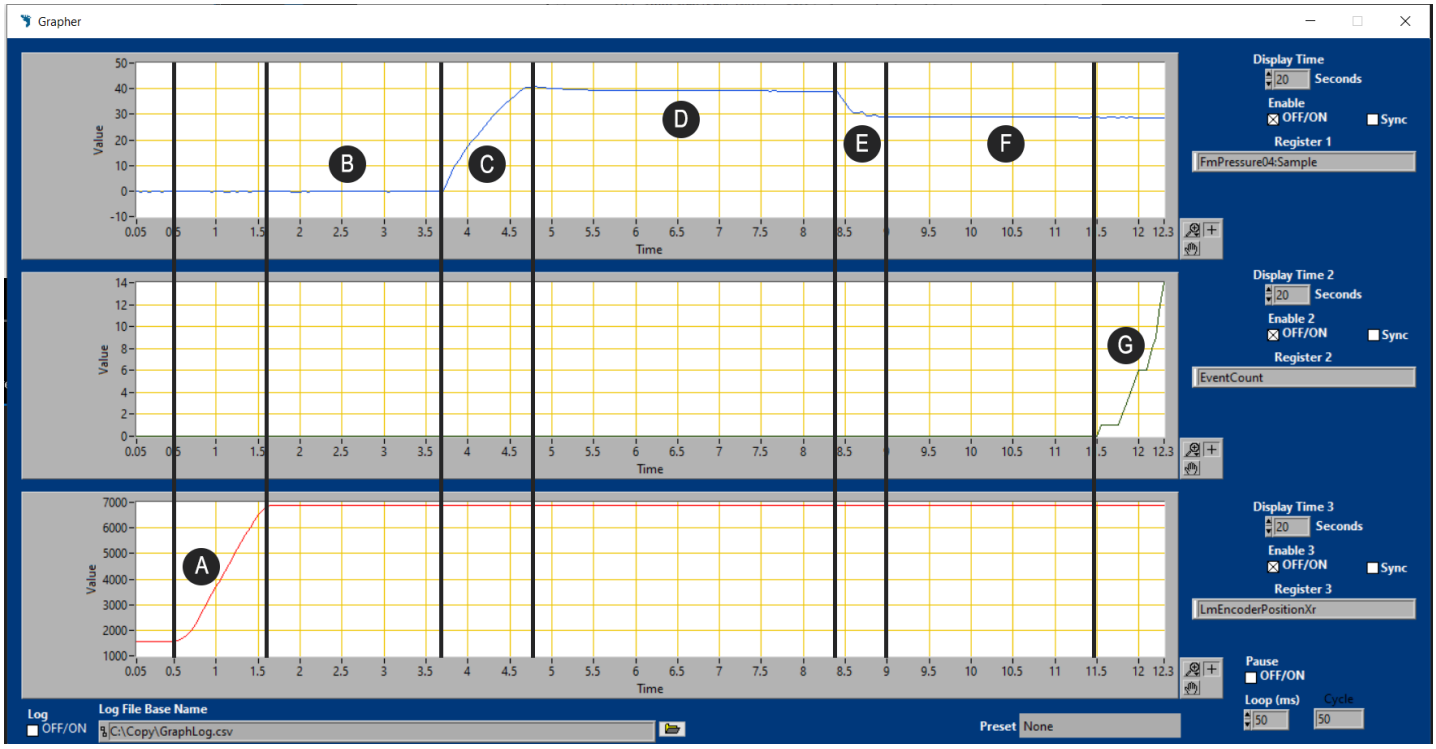

Figure 4. Bigfoot Service Software was used to graph each step in the acquisition process. (A) Rotation to sample station, **(B)** clamp sealing, **(C)** sample chamber pressurization, **(D)** sample boosting, **(E)** lowering pressure to the optimal acquisition pressure, **(F)** stabilizing sample pressure and loading protocol, and **(G)** start acquiring sample.

Table 1. Duration of preparation steps required before sample acquisition on the Bigfoot Spectral Cell Sorter. The time it takes for a position to rotate to the sample station varies depending on its start position. All values in the table represent time in seconds.

Tube position	Rotate to sample station	Clamp/ seal	Pressurize sample chamber	Boost sample	Lower to acquisition pressure	Stabilize pressure & load protocol	Time until acquisition (not including rotation)	Time until acquisition (including rotation)
1	0.54	1.94	1.28	4.29	0.76	1.50	9.77	10.31
2	0.80	1.93	1.38	4.23	0.86	1.36	9.75	10.55
3	0.91	2.05	1.30	4.31	0.83	1.48	9.97	10.88
4	1.11	2.11	1.44	4.19	0.85	1.50	10.08	11.19
5	1.34	2.11	1.27	4.28	0.89	1.33	9.88	11.22
6	1.53	2.01	1.44	4.20	0.86	1.37	9.88	11.41
Avg. time (sec)	1.04	2.02	1.35	4.25	0.84	1.42	9.89	10.93

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Conclusion

Technical ingenuity and user experience innovations come together to make the Bigfoot Spectral Cell Sorter's multi-sample loader a superior option for efficiency, safety, and performance in flow cytometry. The Bigfoot Spectral Cell Sorter helps lab managers maximize resources with the only instrument that provides the functionalities of high-throughput analysis, integrated BSC safety, and world-class sorting capability.

References

1. The NFS Joint Committee on Biosafety Cabinetry. NSF/ANSI 49 - 2018 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. NSF International/American Standards Institute 2019;3.8.2:5-6.
2. ImageJ Image Processing and Analysis in Java. **https://imagej.nih.gov/ij/**

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