

## Bigfoot Cell Sorter Quick Start Guide

DM00109 Rev. C, Approved 3/22/2022

Refer to the Bigfoot User Guide in the Help section of SQ Software (SQS) for detailed instructions on how to use the Bigfoot instrument and software.

### Instrument Startup

1. Open SQS and log in.
2. Check system status. Ensure that the instrument is powered on and connected to the operating computer. Fill the bulk fluids containers and empty the waste containers if necessary. Note that the fluid containers can be exchanged during operation.
3. Click the **Startup** icon.



### Automated Quality Control with Bead Alignment 2.0

The quality control procedure should be run every day to ensure optimal system performance. The system performs filter validation, tests the measurement event rate and makes adjustments, adjusts voltages to bring the populations to channel 256, tunes laser delay, and tests the initial alignment. If the instrument meets the CV and voltage specs at this point the system will not adjust the nozzle. However, if initial alignment reveals the setup does not meet specifications, it performs a full X/Y grid sweep that will take approximately 15 minutes while the instrument fully characterizes the optimal signal for each laser. When the full grid sweep is complete, the system performs a sub grid sweep to determine the optimal position for all laser paths. After the instrument has found the optimal nozzle position, it always returns to this optimal point unless a new nozzle position is saved. Note: The event rate during this process is monitored by software to help conserve beads.

1. Perform **Startup**.
2. Click **Full QC**. Note: Automated QC can be run before or after you log in to the software. The test passes when all parameters are within range. Note: You can also run QC manually. See the Bigfoot User Guide for details.



### Home Screen

After logging into the software, you have the following choices:

- **New Sort** – Experiment Builder provides the steps to set up a new spectral or compensation experiment.
- **Quick Run** – Enables all parameters and brings you to the workspace to set up and run experiments manually. Note: The QC protocol is accessed through the Quick Run Flex Controls.
- **Resume** – Resumes the last protocol that was used in the workspace.
- **Open Protocol** – Opens a file manager and allows you to select an existing experiment.

## Temperature Control

We recommend setting sample temperature to the desired run temperature before running your experiment.

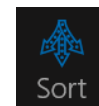
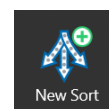
## Loader Tube Holders

Ensure that all tube holders (with the red, blue, or white tops) are full seated in the loader positions. Align the pin and slot and turn the holder clockwise until it clicks into place.

## New Sort

To set up a **New Sort**

1. Click the **New Sort** button.
2. Assign the experiment a name in the **Name** screen and click the **Next** arrow in the lower-right side of the screen.
3. Select compensation or spectral unmixing if applicable.
4. In the **Fluorophores** screen, double-click fluorophore names to add them to the experiment and select sample control options if desired. Click the **Next** arrow.
5. Place samples into the loader in the order shown in the samples panel.
6. In the workspace, run the negative sample and adjust settings as desired.
7. Record control samples and use the auto-compensation wizard if selected to remove spillover.
8. Set up plots in the primary group for sorting. Create regions and assign them as sort regions by right clicking in the region and clicking the Sort toggle button to on.
9. From the **Flex Controls**, click the **Sort** icon and assign output media, sort directions, modes, and limits.
10. Run a small amount of the sample to be sorted and adjust regions/gates as needed.
11. Click the **Set Sort Logic** button and review the Sort Pre-Check screen. Ensure the correct media is placed in the sort output area and the streams are deflected correctly. Press the **Check Streams Deflection** buttons to confirm. Run **Adjust Deflections** if needed. You can manually adjust droplet spacing and stream deflection individually to increase efficiency.
12. Click **Start Sort** to begin sorting.



## Quick Run

To set up a **Quick Run**:

1. Click **Quick Run** in the main screen. The workspace opens with all lasers and detectors are enabled by default.
2. Configure the workspace by manually creating plots and regions.
3. Acquire samples and set up sorting if desired.



Quick Run

## Acquiring Data from a Loader Position

Acquire from the loader if you are running one, or only a few, samples.

1. After setting up the workspace, select the sample to be run and verify the position in the sample list. Set the **Event Limit** if desired.
2. Place the sample to be acquired in the assigned position.
3. Click the **Run** button to acquire the sample.
4. Adjust settings and regions.
5. When you are ready to save data, click the **Record** button.
6. Acquisition will stop automatically if an event limit was set. If not, click the **Stop** button when you are finished.



Run



Record

## Analysis

After acquiring samples, you can analyze the data in the workspace.

To analyze data immediately after acquisition that is still present in the workspace:

1. Click on the play button next to the desired run in the sample list. Data will be loaded into the selected group.

To analyze data that has been saved but is no longer present in the workspace:

1. Click the **+** button in the **FCS Files** panel.
2. Select the desired FCS files.
3. Once the FCS files are loaded, assign a group from the drop-down list, and click the **Play** button to load the data into the selected plots.
4. Create plots and regions as necessary for analysis.

## Shutdown

1. Click the **Shutdown** button on the Home screen or on the Login screen.
2. Schedule automatic startup if desired.
3. The system automatically depressurizes the fluidics, rinses the system with cleaner, turns off all the lasers, docks the nozzle at the wash station and enters a shutdown/low airflow state until **Startup** is initiated.



## System Support

For service and support, please contact the manufacturer at:

345 East Mountain Ave  
 Fort Collins, CO 80524 USA  
 +1 970 295 4570

<http://www.thermofisher.com>