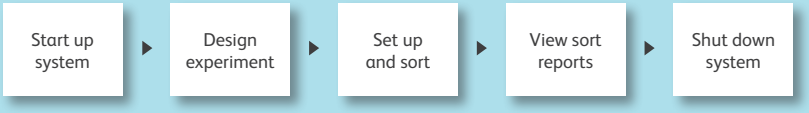
BD FACSMelody™ Cell Sorter Quick Reference Guide

This guide contains instructions for using the BD FACSMelody™ cell sorter with BD FACSChorus™ software version 1.1 and later.

Workflow Overview

The following figure shows the daily cell sorter workflow when using the BD FACSMelody system.



Start up system

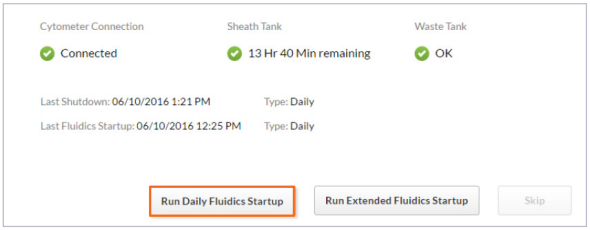
Check fluids

1. Fill the sheath tank with sterile 1X phosphate-buffered saline (PBS).
2. Empty the waste tank and add approximately 1 L of undiluted bleach or a sufficient amount so that 10% of the total volume is bleach.

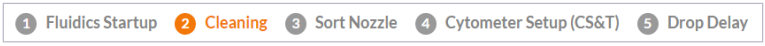
Fluidics startup

1. Press the power button on the front of the cell sorter unit.
2. Start BD FACSChorus™ software by clicking the shortcut on the desktop and log in. The software has been designed with guided, simple, task-oriented screens. There are numbered tabs across the top of the workspace to indicate the order or workflow where information needs to be added.

3. Once the system has connected, click **Run Daily Fluidics Startup**.



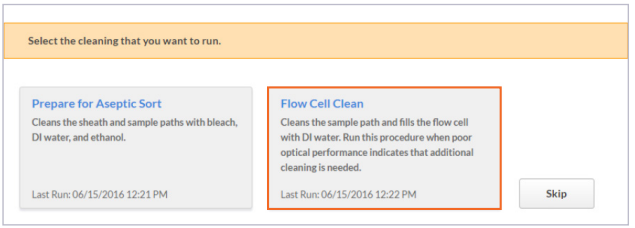
4. Follow the prompts on the screen for each numbered step.
5. After fluidics startup is complete, click **Continue** to see the cleaning options.



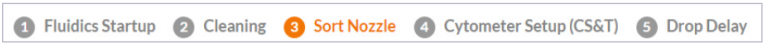
Cleaning

Performing a flow cell clean is recommended at the end of the day. It is an optional step before sorting.

1. Click **Flow Cell Clean** or **Skip**. If you are performing an aseptic sort, click **Prepare for Aseptic Sort**.
2. Follow the prompts for each numbered step of the cleaning procedure.
3. After cleaning is complete, click **Continue** to insert the sort nozzle.



Sort nozzle

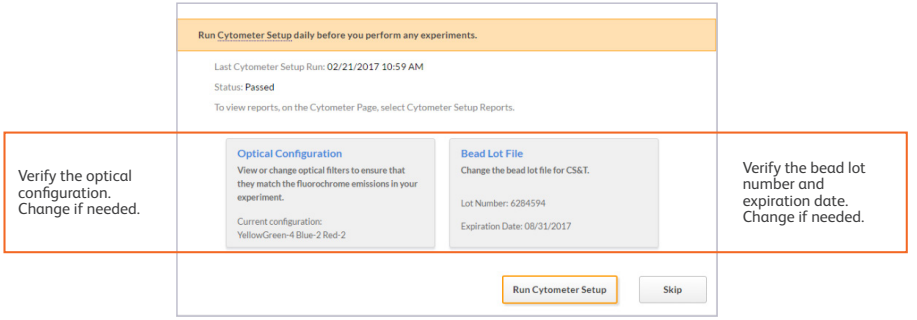


1. Insert the sort nozzle straight into the bottom of the flow cell cuvette with “TOP” facing up. Turn the nozzle-locking lever clockwise to the 12:00 position, and click **Continue**.

Instrument and sort quality control

We recommend running Cytometer Setup and Drop Delay daily before performing any experiments.

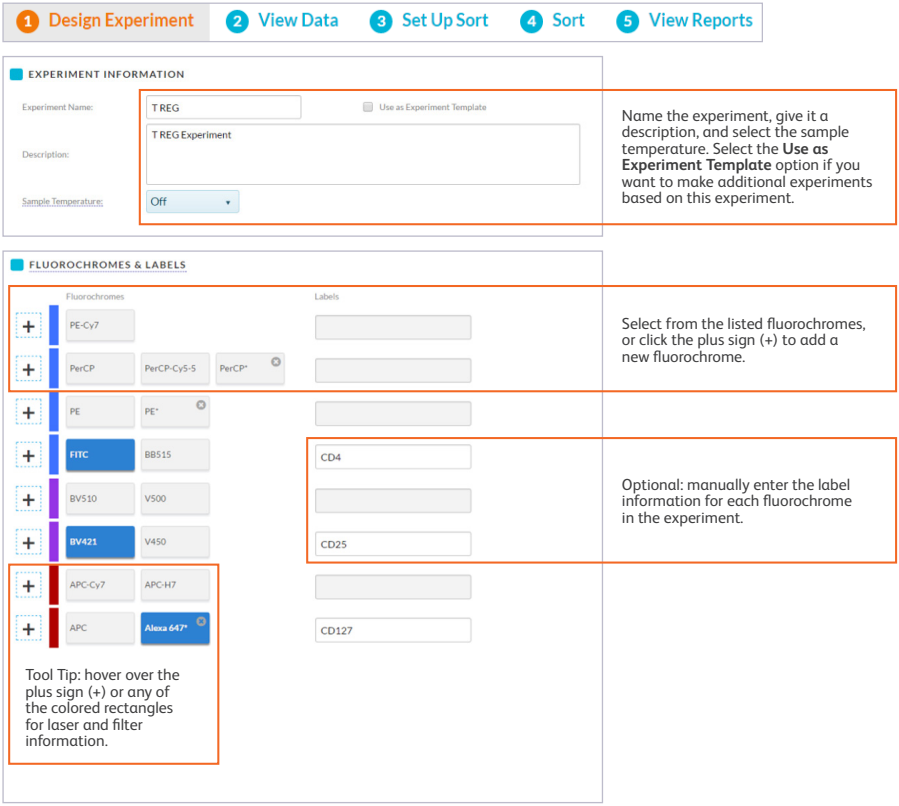
1. Prepare a tube of BD™ CS&T RUO beads by following the package directions for the BD FACSMelody cell sorter.



2. Click **Run Cytometer Setup**.
3. Load the tube and follow the prompts.
4. After CS&T has completed successfully, prepare the BD FACS™ Accudrop beads according to the package directions and click **Continue** to run Drop Delay.
5. Load the tube and follow the prompts.

Experiments

1. Click **New Experiment** and provide the experiment's information. You can also select and duplicate an existing experiment from the experiment list.



View data

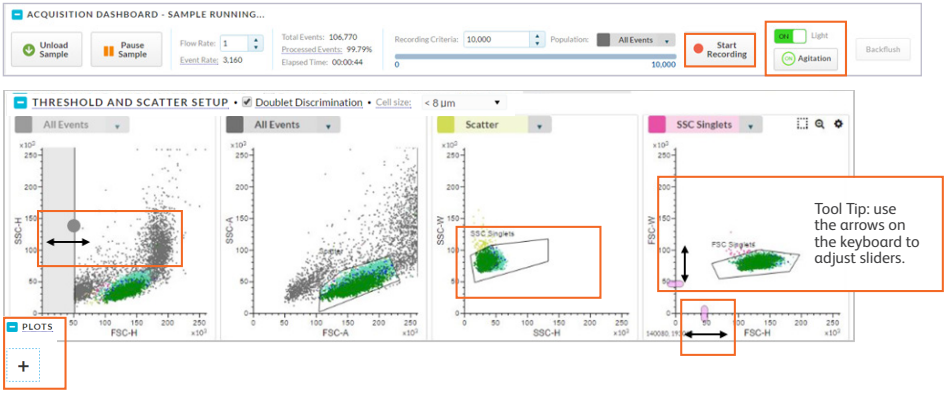
Optimize the threshold and scatter setup, then collect a pre-sort data file.

1. Click the **View Data** tab.

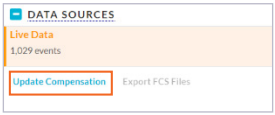
2. On the Acquisition dashboard, click **Load Sample** and adjust the flow rate as needed.
Optional: turn on the sample injection chamber light and agitation option.

3. Select the cell size and use the sliders along the plot axis to adjust the live data cytometer threshold and PMT voltage.

4. Adjust the gates on any plot as needed and select the population to display in the plot. Click **Plots (+)** to create additional plots as needed to define your population(s) of interest.



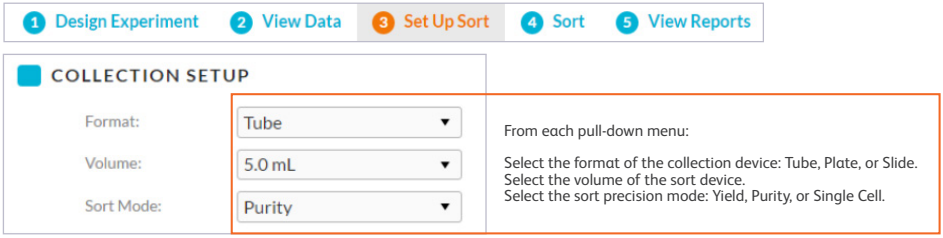
5. **Optional:** If you are running your own compensation controls, click **Update Compensation** and follow the guided prompts. Otherwise, the default system compensation values will be used.



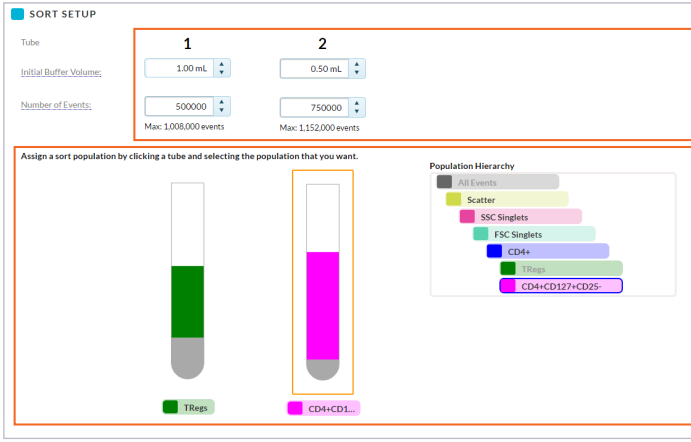
6. Select the Recording Criteria and click **Start Recording** on the Acquisition dashboard to collect a pre-sort data file.

Set up sort

1. Click the **Set Up Sort** tab.



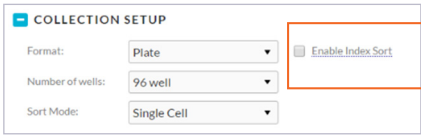
Tubes



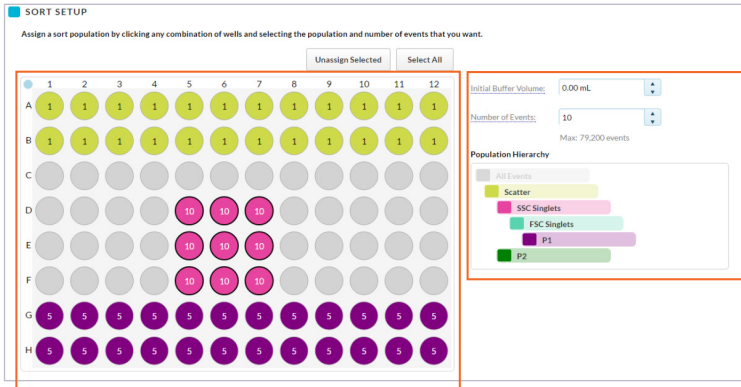
Select the initial buffer volume and the number of target events to be sorted into each tube(s).

Assign the sort population by clicking a tube and selecting the sort population from the **Population Hierarchy**.

Plates and slides



(Optional) Select **Enable Index Sort** to perform an index sort on plates or slides.



Select the initial buffer volume (plates) or additive (slides) and the number of target events to be sorted into each well.

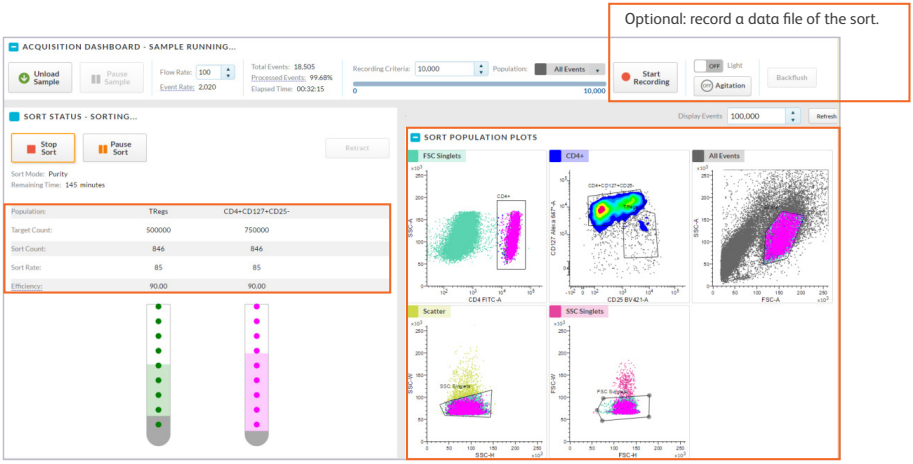
Select the sort population from the **Population Hierarchy**.

Assign the sort wells by clicking each well, dragging across a group of wells, clicking the letter or number for a row or column, or clicking **Select All**. You can also select non-contiguous wells by using Ctrl+click.

Sort

1. Click the **Sort** tab.

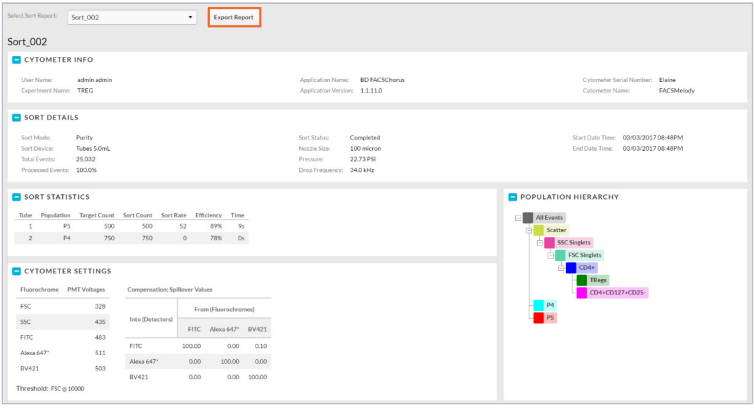
2. Insert the collection tubes into the appropriate tube holder.
3. Click **Start Sort**.
4. Monitor the sort by viewing the sort status and sort population plots.



View reports

1. Click the **View Reports** tab.

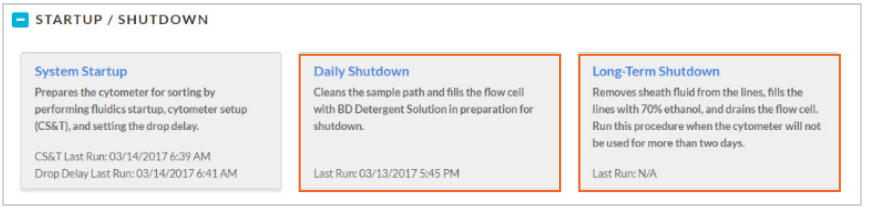
2. View the information and click **Export Report**.



Shut down system

You will be given an option to perform either Daily Shutdown or Long-Term Shutdown upon logging out or closing the application. You may also access these procedures through the Cytometer menu.

1. Click **Cytometer** from the navigation bar.
2. Click the **Daily Shutdown** or **Long-Term Shutdown** option.
3. Follow the prompts on the screen for each numbered step.



Troubleshooting tips

See the Troubleshooting chapter in the *BD FACSMelody Cell Sorter User's Guide* for a complete list of topics and recommended solutions.

Observation	Possible causes	Recommended solutions
The stream has stopped or will not start. Stream error is displayed in the Status window.	Stream disruption due to air bubbles or unstable air pressure	Check that all fluid and air connectors are properly connected to the sheath and waste tanks. Make sure that the sheath-tank lid gasket is seated properly. Purge the sheath filter. Restart the stream by loading a sample.
	Sort nozzle is clogged	Sonicate the sort nozzle. Reinsert the nozzle and start the stream by loading a sample.
	Dirty strobe lens or upper camera window	Stop the stream and clean the strobe lens window and the upper camera window. Restart the stream by loading a sample.
No events seen in plots after clicking Load Sample or Start Sort.	Laser shutter is engaged	Make sure that the flow cell access door is closed.
Problems with Cytometer Setup function	Baseline or performance check failed	Prepare a new CS&T sample with the proper concentration as instructed in the product insert. Clean the flow cell. See Cleaning the flow cell in the *BD FACSMelody Cell Sorter User's Guide*. Close the sort block door and the flow cell door properly.
Problems with Drop Delay function	Sort block door is not closed	Close the sort block door properly. Clean the lower camera and Accudrop laser window.
	Event rate is too low or too high	Prepare a new Accudrop sample with the proper concentration as instructed in the technical data sheet.
	Debris on lower camera or Accudrop window	See Cleaning the Accudrop laser window and lower cameral window in the *BD FACSMelody Cell Sorter User's Guide*.

Additional resources

Visit bdbiosciences.com and select Support > Training to view e-Learning videos. Additional information can also be found in the User's Guide accessible from the BD FACSChorus software Help menu.

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