



Stanford Melody User Guide



Training Links:

<https://www.bdbiosciences.com/en-ch/learn/training/self-paced-courses#e-learning-courses>

Videos:

<https://www.bdbiosciences.com/en-ch/learn/campaigns/facsmelody-cell-sorter-training-videos#Introduction>

Users Guide: BD FACSCorus Software Help Menu

BD FACSMelody™ System Quick Reference Guide

This reference guide contains instructions for using the BD FACSMelody™ Cell Sorter with BD FACSCorus™ Software version 3.0. See the appropriate section in the user's guide for more detailed information.

Workflow Overview

The following shows a typical workflow when using the BD FACSMelody™ System.



Start-up system

Press the power button on the front of the cell sorter unit.

Log in the computer with username: facsuser and password: BDIS.

Before you start

- 1 Fill the sheath tank to the weld line with sheath fluid (DILUENT 2). Do not overfill the tank it can cause failure of the CST. Do not over tighten the wing nut fixture on the top of the sheath tank.

Note: Keep the sheath tank in cart.

- 2 Empty the waste tank and add approximately 500 ml of undiluted bleach or enough so that 10% of the total volume is bleach. The waste is emptied at 50% full do not let the waste tank overflow.

Note: Always check fluids before you start up the machine and at the end of your run.

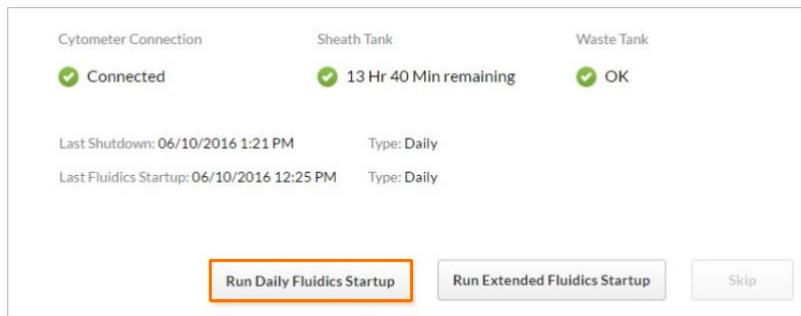
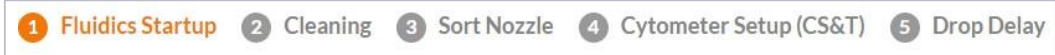
- 3 Aerosol Management System must be used for sorting or analysis for maximum safety. Turn on the main power button on the back of the evacuator just above power cord. Press the POWER button on the membrane panel of the evacuator. Ensure that the suction control rate is set to 20%. (Do not set the suction control rate above 20%; higher rates could affect the stability of the side streams.) Verify that the filter flow gauge reads between 1 and 2.4 inches.
- 4 If you need to use the chiller, please turn it on now since it takes a few minutes to cool.
- 5 Move the deflection plates out with the removal tool, clean the deflection plates with Cavicide-wet kimwipe. Wipe the sample loading port, sort block chamber, sort collection chamber with Cavicide-wet paper towels.
- 6 If the machine hasn't gone through daily fluidic startup procedure, follow page 2-3 to run daily fluidic startup.
- 7 If the machine has been started up but the stream is turned off and the closed-loop nozzle is in the nozzle insertion, login Chorus software > replace the closed-loop nozzle with the sort nozzle > turn on the stream in the stream window > click Experiment navigator to create a new experiment.

Note: If sorting is needed after you turn the stream back on, Running Drop Delay is required. Click Cytometer navigator > choose System Startup > hit Skip until you see the option of Run Drop Delay > load a tube of Accudrop Beads and hit Run Drop Delay > create your new experiment.

If aseptic sorting is needed, please follow the lower session in page 3 to prepare for aseptic sorting.

Fluidics startup

- 1 The air is being supplied to the instrument via house air- a gauge is located on the wall.
- 2 Start BD FACSCorus™ Software by double-clicking the shortcut on the desktop and logging in. Expand the window so you can see the start button functions on the right.
- 3 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.
- 4 Once the system has connected, click **Run Daily Fluidics Startup**.



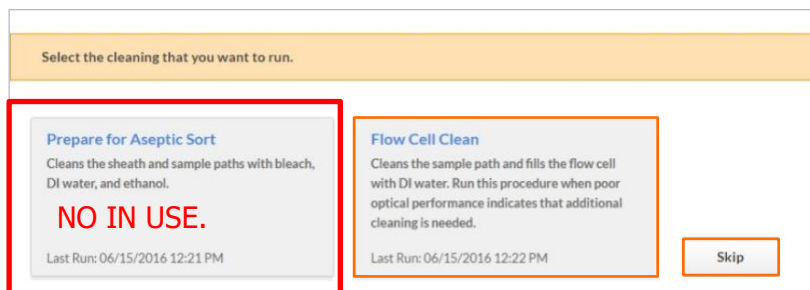
- 5 Follow the prompts on the screen for each numbered step. Be sure to insert the closed-loop nozzle with the O-ring facing up. After fluidics startup is complete, click **Continue** to see the cleaning options.

Cleaning



Perform the Flow Cell Clean Procedure after each startup.

- 1 Click **Flow Cell Clean**.
- 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 Fluidics Startup
- 2 Cleaning
- 3 Sort Nozzle
- 4 Cytometer Setup (CS&T)
- 5 Drop Delay

Evaluate the nozzle under the microscope before inserting, sonicate if needed. Insert the sort nozzle straight into the bottom of the flow cell cuvette with the orange O-ring and "TOP" facing up. Turn the nozzle-locking lever clockwise to the 12:00 position and click **Continue**.
Door to sort chamber must be closed for system to work properly.

Instrument and sort quality control

We recommend running Cytometer Setup (CS&T) and drop delay daily before performing any experiments.

- 1 Fluidics Startup
- 2 Cleaning
- 3 Sort Nozzle
- 4 Cytometer Setup (CS&T)
- 5 Drop Delay

- 1 Prepare a tube of BD® CS&T RUO Beads according to directions: add 500ul sheath fluid to 2 drops vortexed CST RUO beads. **Note: There are different beads for different machines in the fridge. Please be sure to select correct QC beads for your instrument.** Do not dilute the beads with water, use sheath fluid.
- 2 Click **Run Cytometer Setup**.

Verify the optical configuration you want to use. Change if needed.

Verify the bead lot number and expiration date. Change if needed.

- 3 Load the tube and follow the prompts.
- 4 After the CS&T process has completed successfully, prepare the BD FACS™ Accudrop RUO Beads according to the package directions and click **Continue** to run Drop Delay. **Note: Do not dilute the beads with water, use sheath fluid. Add 1 drop of vortexed Accudrop beads into 1ml of sheath fluid.**
- 5 Load the tube and follow the prompts. **Note: Drop delay should be re-run when the stream is turned off and then turned back on.**

- 1 Fluidics Startup
- 2 Cleaning
- 3 Sort Nozzle
- 4 Cytometer Setup (CS&T)
- 5 Drop Delay

If aseptic sorting is needed, follow the steps below to clean the sample line:

- 1 Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
- 2 From Experiment > create a new experiment or click an existing experiment > click the **View Data** tab, click **Load Sample**, set the flow rate to 20. After approximately 10 minutes, click **Unload Sample**.
- 3 Load a tube containing 3mL of FACSrinse solution onto the sample loading port. Click **Load Sample**. After 1 minutes, click **Unload Sample**.
- 4 Load a tube containing 3mL of sterile DI water onto the sample loading port. Click **Load Sample**, after 5 minutes, hit **Unload Sample**.
- 5 Click **Backflush**, in the pop-out window, hit **Start**, when finish, close this window.

Note: It is very important to always run a tube of DI water after running bleach on the cell sorter.

Create experiment

Experiments are used to define and refine the parameters for data acquisition and sorting. Experiments can only be seen by the user who created them.

Design experiment

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

1 Design Experiment 2 View Data 3 Set Up Sort 4 Sort 5 View Reports

EXPERIMENT INFORMATION

Experiment Name: ☆ Use as Experiment Template

Description:

Sample Temperature:

Name the experiment, give it a description, and select the sample temperature. Click the star to select the **Use as Experiment Template** option if you want to reuse this experiment multiple times.

FLUOROCHROMES & LABELS

Fluorochromes	Labels
<input type="checkbox"/> PE-Cy7	<input type="text"/>
<input type="checkbox"/> PerCP PerCP-Cy5-5 PerCP*	<input type="text"/>
<input type="checkbox"/> PE PE*	<input type="text"/>
<input type="checkbox"/> FITC BB515	<input type="text" value="CD4"/>
<input type="checkbox"/> BV510 V500	<input type="text"/>
<input type="checkbox"/> BV421 V450	<input type="text" value="CD25"/>
<input type="checkbox"/> APC-Cy7 APC-H7	<input type="text"/>
<input type="checkbox"/> APC Alexa 647*	<input type="text" value="CD127"/>

Select from the listed fluorochromes or click the plus sign (+) to add a new user-defined fluorochrome to that row.

(Optional) Manually enter the label information for each fluorochrome in the experiment.

Tooltip: Hover over the plus sign (+) or any of the colored rectangles for laser and filter information.

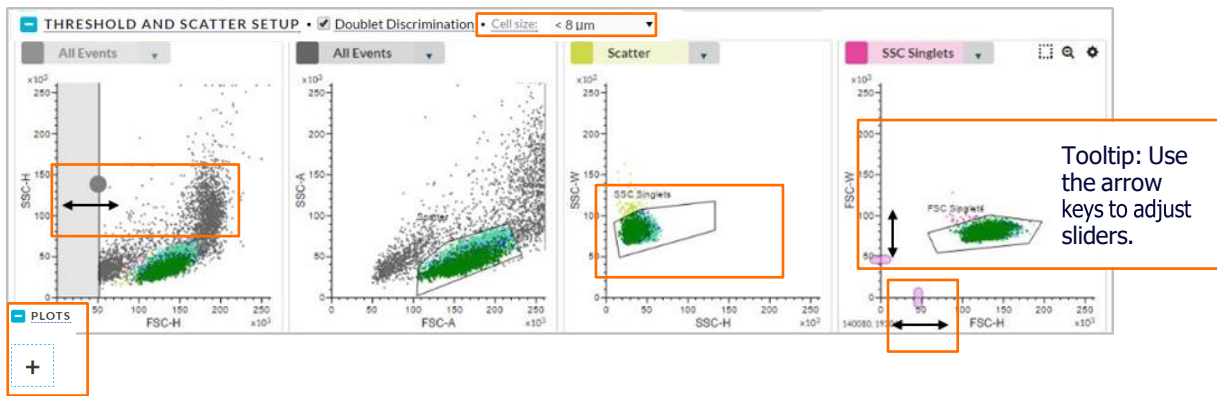
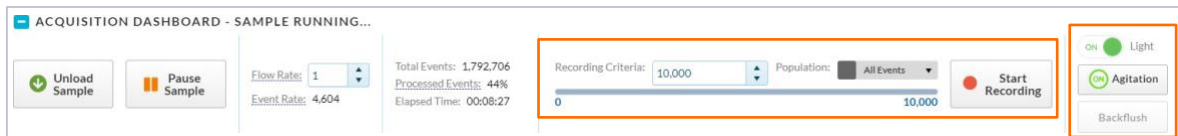
View data

The selections on the View Data tab determine the layout of the experiment data. Optimize the threshold and scatter setup, then collect a pre-sort data file.

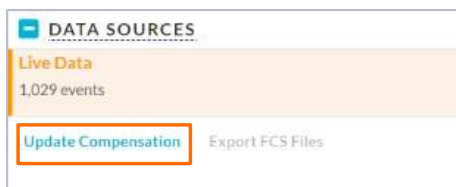
- 1 Click the **View Data** tab.



- 2 On the Acquisition dashboard, click **Load Sample** and adjust the flow rate as needed. (Optional) **Turn on the sample 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 Before running your compensation controls check your controls to **be sure the scatter and flow rates are set properly**, click **Update Compensation** and follow the guided prompts.

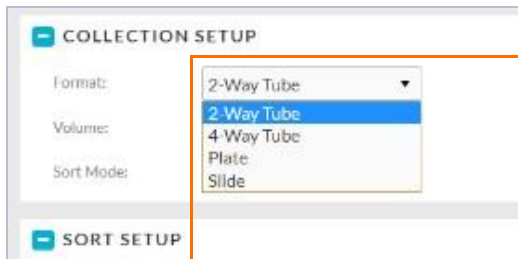


- 6 Select the Recording Criteria for your collection and click **Start Recording** on the acquisition dashboard to collect a pre-sort FCS data file.

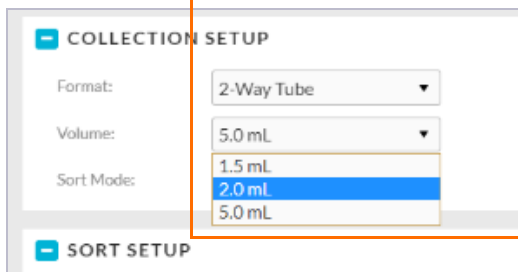
Set up and sort

The selections on the Set-Up Sort tab determine the collection device and the populations in the sample to be sorted.

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

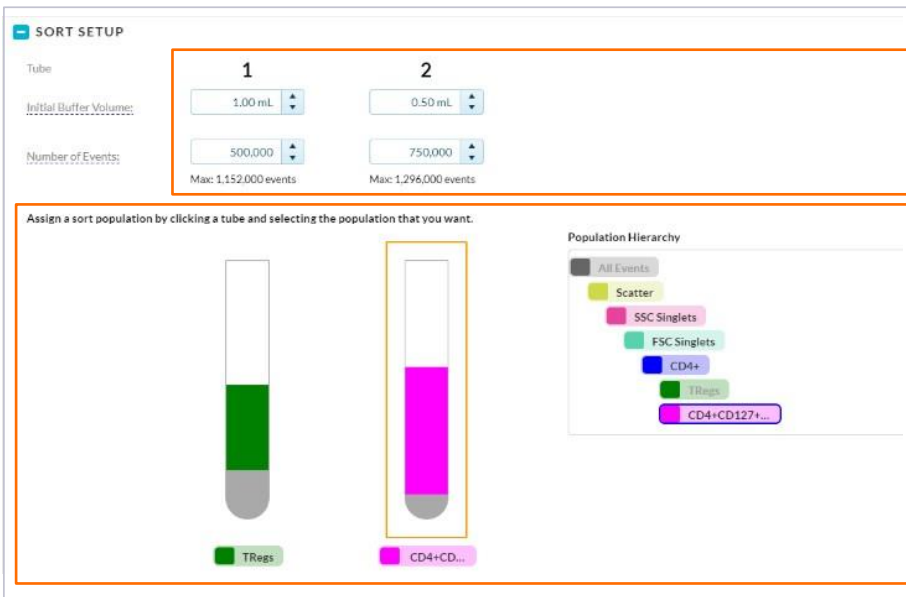


From each drop-down list box:
Select the format of the collection device: 2-Way Tube, 4-Way Tube, Plate, or Slide.



Select the volume of the sort device: 1.5 mL, 2.0 mL, or 5.0 mL. Select the sort precision mode: Yield, Purity, or Single Cell.

Tubes: Two tubes view



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

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

Tubes: Four tubes view

Sort Setup

Tube

1	2	3	4
Initial Buffer Volume: 0.00 mL	0.00 mL	0.00 mL	0.00 mL
Number of Events: 1,296,000 Max: 1,296,000 events	1,296,000 Max: 1,296,000 events	1,296,000 Max: 1,296,000 events	1,296,000 Max: 1,296,000 events

Assign a sort population by clicking a tube and selecting the population that you want.

Population Hierarchy

- All Events
- Scatter
- SSC Singlets
- FSC Singlets
- Combined
- P1
- FITC
- Unstained
- APC
- PerCP

Plates and slides

Note For sorting into plates, insert the splash guard, eject the ACU. To retract the ACU, please go back to Set Up Sort session, change the format to tube mode, then go to Sort session, hit Retract in Sort Status panel.

Collection Setup

Format: Plate

Number of wells: 96 well

Sort Mode: Single Cell

Enable Index Sort

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

Sort Setup

Assign a sort population by clicking any combination of wells and selecting the population and number of events that you want.

Unassign Selected | Select All

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C												
D					10	10	10					
E					10	10	10					
F					10	10	10					
G	5	5	5	5	5	5	5	5	5	5	5	5
H	5	5	5	5	5	5	5	5	5	5	5	5

Initial Buffer Volume: 0.00 mL

Number of Events: 10
Max: 79,200 events

Population Hierarchy

- All Events
- Scatter
- SSC Singlets
- FSC Singlets
- P1
- P2

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.

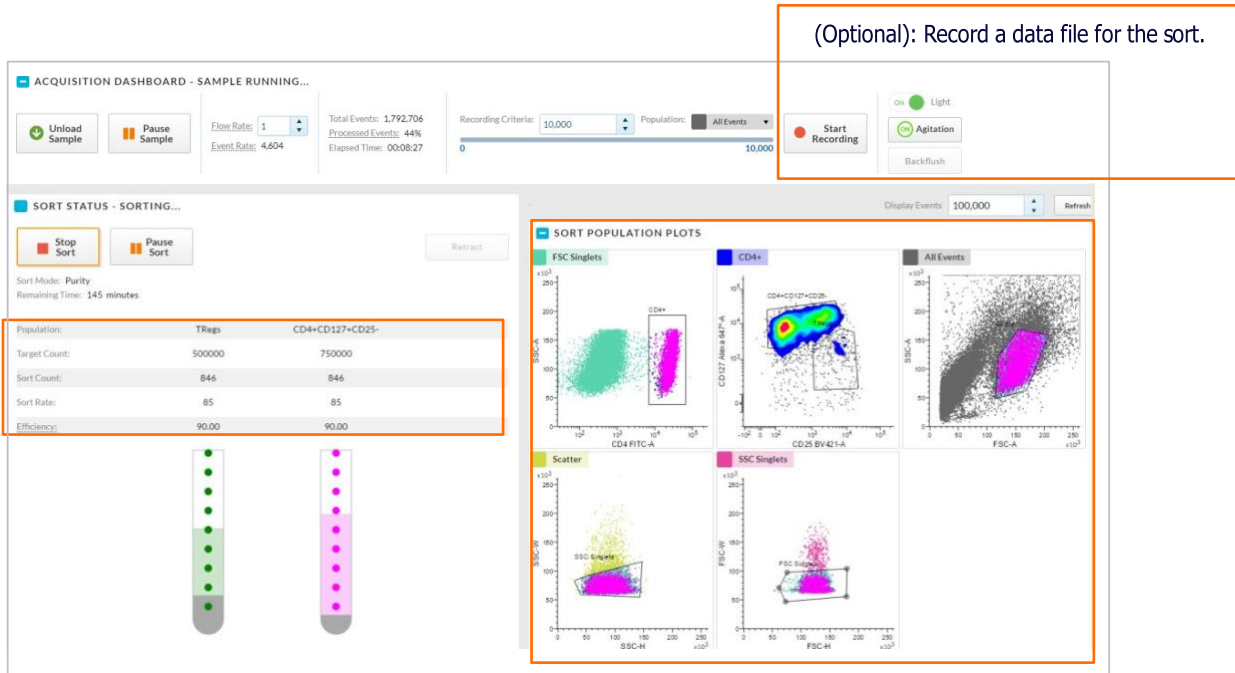
Assign the sort wells by clicking each well, dragging across a group of wells, clicking the letter or number for a row or column, or clicking **Select All**. You can also select non-contiguous wells by using **Ctrl+click**.

Sort

- 1 Click the **Sort** tab.
- 2 Insert the collection tubes into the appropriate tube holder. Click **Start Sort**.

Note Use the Flow Rate 1 to start sorting. It's recommended that the Flow Rate is no more than 20 to maintain good purity and efficiency.

- 3 Monitor the sort by viewing the sort status and sort population plots.



- 4 Once complete sorting, unload sample tube > follow the AMO operation guide to clear aerosol > set unit on 100% for 2 minutes before opening the chamber, take out collection tubes.
- 5 Prepare a post sort analysis tube of a sorted population to check the purity of your sort if desired, run a sample line back flush from the cleaning modes before loading your sample.

Note If the nozzle is clogged during experiment, follow page 11 to response.

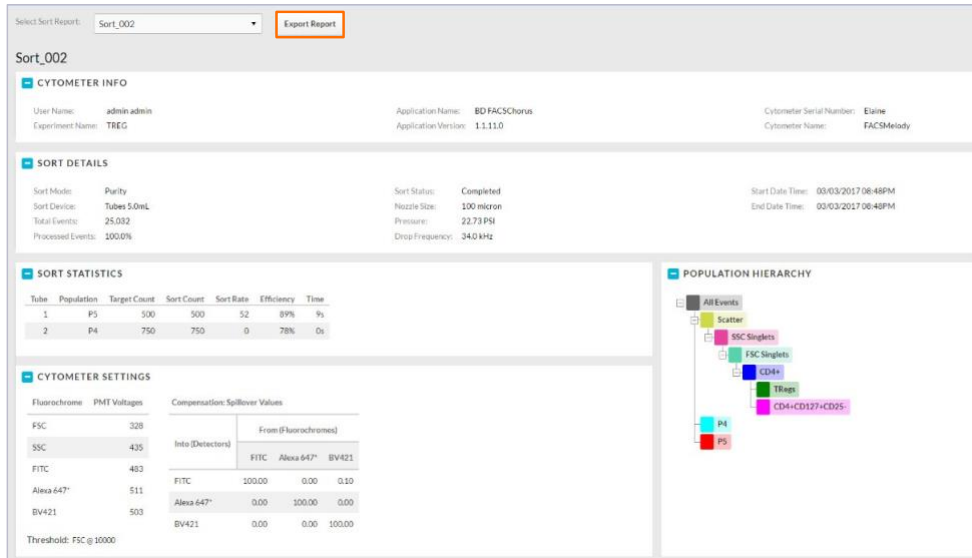
View sort reports

A sort report summarizing the results of the sort is displayed on the View Reports tab when sorting is complete.

This is where you can view the parameter voltages and compensation matrix, you are not able to adjust the compensation matrix on this system

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

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



Clean the Sample Line

- 1 This procedure cleans the sample line with a bleach solution. You are required to run this at the end of your experiment. Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
- 2 From the View Data tab, click **Load Sample**, set the flow rate to 20. After approximately 10 minutes, click Unload Sample.
- 3 Load a tube containing 3mL of FACSrinse solution onto the sample loading port. Click **Load Sample**. After 1 minutes, click **Unload Sample**.
- 4 Load a tube containing 3 mL of DI water onto the sample loading port. From the View Data tab, click **Load Sample**, after 5 minutes, hit **Unload Sample**.

Note: It is very important to always run a tube of DI water after running bleach on the cell sorter.

- 5 If you are not the last user, turn off the stream, clean the flowcell with a tube of sterile DI water (go to Cytometer navigator, choose Flow Cell Clean, follow the prompts), log out of the software.

If you are the last user of the day, or if the gap between you and the next user is over 4 hours, shut down the machine as follows.

Shut down system

You will perform Daily Shutdown upon logging out or closing the application. You can also access this procedure through the Cytometer menu. Note: Only use tanks that are provided with the BD FACSMelody™ System.

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

STARTUP / SHUTDOWN

System Startup

Prepares the cytometer for sorting by performing fluidics startup, cytometer setup (CSS T), and setting the drop delay.

CSST Last Run: 03/14/2017 6:39 AM
Drop Delay Last Run: 03/14/2017 6:41 AM

Daily Shutdown

Cleans the sample path and fills the flow cell with BD Detergent Solution in preparation for shutdown.

Last Run: 03/13/2017 5:45 PM

Long-Term Shutdown

Removes sheath fluid from the lines, fills the lines with 70% ethanol, and drains the flow cell. Run this procedure when the cytometer will not be used for more than two days.

Last Run: N/A

- 4 Power off the cytometer unit.

Data Export

Notes:

- a. *Please backup your data by transferring it to the server immediately upon finishing your FACS experiment. This system is in place to ensure that FACS data for each user is backed up and is readily available for each user to access and download it from any location.*
- b. *Note that the computers are not used to store your data. If too much data is left on the database, the Chorus software cannot function properly.*
- c. *Also, the database tends to be fragile, and is prone to corruption. If this happens any data that was not backed up prior to the corruption may be inaccessible. Each user can only keep 2 experiments in the database to refer to, most should be deleted from the database after they are transferred to the Box account and make sure the data has been saved in your own devices. **Saved experiments can be used as templates for later experiments. If you save the experiment as a template, you can change the parameters later. However, experiments created from the template before the parameters were changed are not affected.***

1. In the View Data tab of an Experiment, hit Export All FCS Files in the Data Sources panel to the left.
2. In the pop-out window, browse to "C:\FACSData", hit Save.
3. **Go to C:\FACSData, right-click at your exported experiment to select Extract All, hit OK.**
4. Go to the Desktop or taskbar.
5. Open (double-click) the "Data_Upload" program.
6. A command terminal will open, then a dialogue shows.
7. Follow the on-screen instructions to proceed.
8. When uploading completed, it will show "Finished. Click Exit." at the last line. **DO NOT CLOSE THE PROGRAM EARLY OR TURN OFF THE COMPUTER.** Otherwise, your upload will be cancelled.
9. You will receive an email from a member of the FACS Staff containing a link and password to your data folder on the SoM Box service. Follow the instructions to access and download your data. **This link will be used to access all your future data.**

Responding to A Nozzle Clog During an Experiment

If the stream is disturbed during an experiment due to a clogged nozzle. If sorting, the sort procedure is designed to stop automatically and block the sort tubes. The sort will not restart until you clear the clog. In the event of a nozzle clog, follow the procedure below.

1. Do not open the sort collection door or access the sort tubes before following this procedure.
 2. If the stream has not already shut down automatically, turn off the stream.
 3. Turn the AMO suction control to 100%.
 4. Wait 2 minutes. This procedure will evacuate aerosols from the sort collection chamber.
 5. Recover from the clog:
 - a. Remove the nozzle, soak it in a tube of 70% Ethanol for 5min to disinfect (the nozzle should not be in 70% Ethanol for over 30min, or it will be trashed), then sonicate it in deionized (DI) water to clear the clog.
 - b. Open the sort block door, use Cavicide-wet paper towel to decontaminate the deflection plates, nozzle lever, and other surfaces as necessary and dry.
 - c. Reinsert the nozzle. Start the stream to verify the clog has been cleared.
- NOTE** A drop delay must be rerun whenever the stream is from Off to On to resume a sort.
6. Set the AMO suction control back to 20%.
 7. Close the sort block and sort collection chamber doors.
 8. Continue your experiment.

Troubleshooting tips

BD FACSCorus™ Software provides some troubleshooting instructions when errors are encountered. The tips in this section are focused on errors or troubleshooting that the software is not able to address and designed to help you troubleshoot your experiments. If additional assistance is required, contact your local BD Biosciences technical support representative. See the appropriate section in the user's guide for complete instructions on how to perform the recommended solutions.

Startup troubleshooting

Observation	Possible causes	Recommended solutions
Closed loop nozzle is not detected	Salt buildup on the closed-loop nozzle	Clean the closed loop nozzle.
	Salt buildup in the nozzle location between the flow cell and the locking lever	Clean the area to remove the salt buildup.
Error starting stream after inserting sort nozzle or loading sample	Sheath tank low or empty, or waste tank full or almost full	Fill the sheath tank to the maximum level or empty the waste tank.
	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. Make sure the nozzle is dry.
	Dirty strobe lens or upper camera window	Clean the lens and the window as described in Cleaning the strobe lens window and upper camera window.
	Clogged or damaged sort nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. If the nozzle seems damaged, replace it. Restart the stream.
	Debris in flow cell	Scrub the flow cell.
Error starting stream after inserting sort nozzle or loading sample	Air in sheath line or filter	Stop and restart the stream. Purge the sheath filter. Run daily fluidics startup.
	Dry sheath filter	Purge the sheath filter.
	Air pressure is too low, too high, or variable	Verify that the external air supply or compressor is on and the pressure is between 80 and 95 psi. Verify that the sheath tank lid is sealed properly.
	Residual ethanol in fluidic lines	Run extended fluidics startup.
	Sheath filter orientation is incorrect.	Change the orientation of the filter.

Observation	Possible causes	Recommended solutions
Stream not in center of waste aspirator drawer	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle.
	Clogged or damaged sort nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. If the nozzle seems damaged, replace it. Restart the stream.
	New sort nozzle was inserted.	If you are using a new nozzle, the sort block might need to be repositioned to align with the stream.
	Air bubbles in flow cell	Stop and restart the stream to remove bubbles.
	– Ethanol or other cleaning solution in flow cell – Dirty flow cell	Scrub the flow cell.
Prepare for Aseptic Sort fails	Fluid or air lines are detached	Verify that the fluid or air line connections are attached. Push firmly on each line to ensure that it is connected.
Problems with Cytometer Setup function	Baseline or performance check failed, or stopped before completing	Prepare a new CS&T sample with the proper concentration as instructed in the product insert. Close the sort block door and the flow access door properly. Clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, then backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Perform clean flow cell again and check that the fluid levels in the sample tube have decreased.
Problems with Cytometer Setup function	– Beads not on scale – Low event rate or zero event rate	Prepare a new CS&T sample with the proper concentration as instructed in the product insert. Note: Do not dilute BD® CS&T RUO Beads with water. Close the sort block door and the flow access door properly. Turn off the stream and remove, sonicate, and reinsert the nozzle. Clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, then backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Perform clean flow cell again and check that the fluid levels in the sample tube have decreased.
BD FACS™ Accudrop laser scan fails to locate stream after the nozzle is changed.	Stream is unable to focus or software fails to detect that the nozzle was changed.	Check the stream. If necessary, adjust sort block so that the stream is in the center of the waste aspirator. See Aligning the waste aspirator drawer to the stream in the user's guide. Restart the workstation to trigger software detection of the new nozzle.
Lower (stream) camera does not show laser/stream	Stream is unable to focus or software fails to detect that the nozzle was changed.	Check the stream. If necessary, adjust sort block so that the stream is in the center of the waste aspirator. See Aligning the waste aspirator drawer to the stream in the user's guide. Restart the workstation to trigger software detection of the new nozzle.

Acquisition troubleshooting

Observation	Possible causes	Recommended solutions
Problems with Drop Delay function	Sort block door is not closed	Close the sort block door properly.
	Flow cell access door is open	Close the flow cell access door properly.
	Event rate is too low or too high	<p>Prepare a new Accudrop sample with the proper concentration as instructed in the technical data sheet. Note: Do not dilute BD FACS™ Accudrop RUO with water.</p> <p>Clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased.</p> <p>If the fluid levels in the sample tube have not decreased, massage the sample line to clear a possible sample line blockage.</p> <p>If sample flow seems to be blocked, backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased.</p> <p>If sample flow continues to be blocked, change the sample line filter. Clean the flow cell again and check fluid levels in the sample tube.</p> <p>If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.</p>
	Debris on lower camera or Accudrop window	Clean the lower camera and Accudrop laser window.
No events in plots or events don't update in plots after clicking Load Sample	Selected data source is a recorded file	Select the Live Data data source.
	Laser shutter is engaged	Close the flow cell access door properly.
	No sample in the tube	Add sample to the tube or install a new sample tube.
	Sample line or sample line filter is clogged	<p>Clean the flow cell. Confirm by checking that fluid levels in the sample tube have decreased.</p> <p>If the fluid levels in the sample tube have not decreased, massage the sample line.</p> <p>If sample flow seems to be blocked, backflush the sample line several times to clear the block. Clean the flow cell again and check fluid levels in the sample tube.</p> <p>If sample flow continues to be blocked, change the sample line filter. Clean the flow cell again and check fluid levels in the sample tube.</p> <p>If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.</p>
	Sample is not mixed properly	<p>Resuspend the sample.</p> <p>Turn on or increase the sample agitation rate.</p>
	Threshold is not set to correct parameter	Set the threshold to the correct parameter for your application.
Threshold setting is too low or too high	Adjust the threshold setting.	

Observation	Possible causes	Recommended solutions
Unexpected events in plots or fewer events in gated populations than expected	Incorrect logic in population hierarchy	Verify the gating strategy.
	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
	Threshold setting is too low or too high	Adjust the threshold setting.
	Events left out of a gate	When drawing a gate, make sure that events on the axes are included.
	Cell size is set incorrectly	Ensure that the setting for the cell size is appropriate for your sample.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
Erratic event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Sheath tank is low	Fill the sheath tank.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sample chamber O-ring is worn	Contact your BD Biosciences field service engineer.
Unexpectedly high event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Threshold setting is too low	Adjust the threshold setting.
	Sample is too concentrated	Dilute the sample.
	Flow rate is too high	Decrease the flow rate.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and then load the sample again. Scrub the flow cell.
Unexpectedly low event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Sample is too dilute	Concentrate the sample.
	Threshold setting is too high	Adjust the threshold setting.
	Sample line assembly or sample line filter installed incorrectly	Verify the sample line assembly or sample line filter installation.
	Sample line is clogged or kinked	If visible kinks are found in the sample line, replace the sample line assembly. If visible kinks are not found in the sample line, clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Clean the flow cell again and check that the fluid levels in the sample tube have decreased.

Observation	Possible causes	Recommended solutions
Distorted populations or high CVs	Instrument settings adjusted incorrectly	Optimize the threshold setting, voltage settings, and run user-defined compensation to optimize compensation settings.
	Flow rate is too high	Decrease the flow rate.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and then load the sample again.
	Debris in flow cell or nozzle	Scrub the flow cell with BD® Detergent Solution. Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle.
	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sheath filter is more than 3 months old	Replace the sheath filter.
Excessive amount of debris in plots	Threshold setting is too low	Adjust the threshold setting.
	Dead cells or debris in sample	Examine the sample under a microscope to determine the source of the debris. Adjust sample preparation if needed.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sheath filter needs to be replaced	Replace the sheath filter.
Processed events are <90%	Threshold setting is too low	Adjust the threshold setting.
	Event rate is too high	Decrease the flow rate.
	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
Stream turns off unexpectedly	Nozzle clog detected or debris in nozzle	Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle.
	Debris in flow cell	Follow the scrub the flow cell procedure with 1.5% BD® Detergent Solution.
	Sheath tank empty or waste tank full	Empty the waste tank or fill the sheath tank.
Unable to start sort	BD FACSCorus™ software cannot locate the side streams	Clean the lower camera window. Close the sort block door properly. When using four-way sort, wait for a few minutes to allow the Accudrop to find the four streams. If the streams are still not found, clean the nozzle. Also, clean the deflection plates.
	Salt bridge	Clean the deflection plates and the area around and behind the plates.

Observation	Possible causes	Recommended solutions
Arcing between deflection plates	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle.
	Clogged or damaged sort nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. If the nozzle seems damaged, replace it. Restart the stream.
	Dirty deflection plates	Clean the deflection plates.
	Particles too big for sort nozzle	Verify that the particle size is appropriate for the 100- μ m nozzle. In general, the nozzle orifice should be at least 5 times the average particle size in the sort sample. See Shapiro H. <i>Practical Flow Cytometry</i> . Fourth Edition. New York, NY: John Wiley and Sons; 2003:263.
Low sort efficiency	Event rate is too high for drop frequency	Decrease the flow rate.
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.
	Gating conflict	Verify the gating hierarchy.
Erratic sort rate	Flow rate is too high	Decrease the flow rate.
Unexpected sort results	Incorrect drop delay	Run drop delay.
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.
	Incorrect logic in population hierarchy	Verify the gating hierarchy. Do not assign conflicting gates (for example, parent population in Tube 1, child population in Tube 2).
Plate sorting failure	Splash shield not installed	Install the splash shield.
	Sort collection chamber door is open	Close the sort collection chamber door.
	Automated stage does not move	Close the access doors, then restart the instrument and workstation.
Unable to sort into targeted well in plate	Debris on deflection plates	Clean deflection plates.
	Waste aspirator drawer not aligned to stream	Align the waste aspirator drawer.
	Automated stage improperly aligned	Align the stage. If the problem cannot be resolved by aligning the automated stage, contact your BD service representative for assistance.

Electronics troubleshooting

Observation	Possible causes	Recommended solutions
Cell sorter will not connect to workstation	Cell sorter power is off	Turn on the cell sorter main power.
	Ethernet cable between workstation and cell sorter is disconnected	Unplug and then plug in the cable and make sure it is secure.
	IP address or other connectivity information changed.	Call BD Biosciences for assistance.

Maintenance tasks

Category	Task	When to perform
Shutdown	Clean the sample line	At the end of each experiment and between users.
	Daily shutdown	At the end of any given day the system is being used. You can also perform this cleaning separately whenever additional cleaning of the sample path and flow cell is needed.
	Long-term shutdown	Perform every 6 months and when the system will be off for more than 2 days.
Update compensation standards	Update the normalized spillover values	Run this procedure with BD® FC Beads every 60 days.
Nozzle and flow cell	Clean the sort and/or closed-loop nozzle	When you see indications of clogging or salt buildup.
	Clean the flow cell	Perform separately whenever additional cleaning is needed, and in cases where debris builds up in the flow cell as indicated by high CVs in the CS&T report. See procedure for cleaning the flow cell in the user's guide.
	Align the waste aspirator drawer to the stream	If you install a sort nozzle that is new or different from the one that came with the instrument.
Fluidics	Replace the waste filter cap	Monthly.
	Change the fluid filter	Every 3 months or as needed.
	Purge the sheath filter	Perform as a task after installing a new sheath filter and whenever you observe problems with the stream.
	Replace the sample line	Every 4-6 months or when decreased event rates indicate that the sample line might be clogged.
	Backflush the sample line	When you observe sample carryover, or after you run samples with adherent cells or dye.
	Replace the sample line filter	When decreased event rates indicate that the sample line might be clogged.
	Align the automated stage	After replacing a damaged sort nozzle, when using a sheath fluid other than PBS, or whenever it is especially important that each drop falls in the exact center of the well.
Optics	Clean the deflection plates	When you have trouble viewing the side stream or after a clog.
	Clean the Accudrop laser window and the lower camera window	When the software is unable to set drop delay, or when the software is unable to verify the side streams when sorting is started.
	Cleaning the strobe lens window and upper camera window	When smudges appear in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed.