TITLE: **Operation of BD FACSMelody**	
NUMBER: **SOP B+labs 01 BD FACSMelody**	**CHANGE CONTROL:**
	REVISED BY : Bob Rovinsky **DATE:** 5/10/24

1 PURPOSE:

The purpose of this procedure is to define proper guidelines for the safe use of the BD FACSMelody.

2 SCOPE:

The procedure applies to all personnel who operate and maintain the BD FACSMelody.

3 RESPONSIBILITY:

These procedures must be followed by all personnel use the BD FACSMelody.

4 REFERENCES & ATTACHMENTS:

Attachment A:	Training Requirements
Attachment B:	Training Resources
Attachment C:	BD FACSMelody Quick Reference Guide
Attachment D:	BD FACSMelody Users Guide
Reference:	B+labs EHS Manual

5 PROCEDURE:

5.1 **Safety:**

5.1.1 All personnel will follow all requirements outlined in the B+labs EHS Manual when utilizing the BD FACS Melody.

5.2 **Training:**

5.2.1 All personnel who utilize the BD FACSMelody shall complete all required training prior to using the unit.

5.2.1.1 Required Training:

5.2.1.1.1 All operators must complete all requisite training. (Attachment A)

5.2.1.1.2 All operators must complete B+labs provided orientation and practical training.

5.2.1.2 Optional/Additional Training: If operators desire additional training, resources are available from BD. (Attachment B). Some are fee for service. Any fees incurred are the responsibility of the user.

5.2.1.3 Remedial Training

5.2.1.3.1 If users fail to comply with operating procedures, retraining shall be required.

5.3 **Operation**:

5.3.1.1 Access to BD FACSMelody

5.3.1.1.1 Complete required training.

5.3.1.1.2 After completing required training, email Tim Lutz tim.lutz@blabscira.com confirming that the training has been completed.

5.3.1.1.3 Upon successful completion of requisite training, individual users will be given a unique ID for temporary system access.

5.3.1.1.4 Users will then be given an orientation by B+labs personnel.

5.3.1.1.5 Users must then pass an operations practical administered by B+labs personnel.

5.3.1.1.6 Upon successful completion of the operations practical, full access will be given to operate the unit.

5.3.1.2 Operators shall follow procedures as stated in the following:

5.3.1.2.1 FACSMelody Quick Reference Guide (Attachment C)

5.3.1.2.2 BD FACSMelody Users Guide (Attachment D)

5.3.1.2.3 Operators shall not perform any functions indicated in RED as shown in the Quick Reference Guide or Users Manual.

5.3.1.2.3.1 If operators fail to comply with established operating procedures, individual user privileges may be revoked and/or remedial training will be required.

5.3.1.3 Contact B+labs personnel regarding any operational concerns.

Attachment A – Training Requirements

BD FACSMelody

All users must review all information contained in the links below.

Self-Paced Training:

- E-Learning Courses
  - BD FACSMelody™ Cell Sorter Overview Course: https://static.bdbiosciences.com/training/facsmelody-cell-sorter-overview/index.html?_gl=1*z8yb0d*_ga*MTQ3NjUzNDg0NS4xNzE0NjYyOTkw*_ga_LL7XK30S8W*MTcxNDc0MzY5MC43LjEuMTcxNDc0NDE1OC42MC4wLjA.#/

- Videos, BD FACSMelody™ Cell Sorter
  - Getting Started: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6234979045001
  - System Startup: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6234982854001
  - Design Experiment: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6235345051001
  - View Data: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6235336768001
  - Set Up & Sort: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6234985199001
  - Daily Shutdown: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6235109587001
  - Loading the Collection Devices: https://players.brightcove.net/81909694001/smS3oTdDk_default/index.html?videoId=6235335553001

Attachment B – Training Resources

For additional instructor-led training or specialist help:

- Remote consulting services
  - Topic help if subject is something we are able to cover
  - Usually for 2 to 4 hours, min 2 hours, $368 per hour, P#654857
- On Site Training
  - Two options
    - At Your Facility, $5,198 per day, up to two operators, one day needed for this model
    - Virtual, $2,310 per day, up to two operators, one day needed for this model
      - **Virtual Training** is hands-on, instructor-led training in your lab, in front of your instrument, connected via web meeting app [Zoom or MS Teams] on a laptop with webcam, and sharing video with a trainer for interactions and attendance. The maximum number of hands-on operators for this training is two. This will require a pre-meeting at least three weeks before the start of training to verify instrument performance and a tech check for web connectivity.
- **Contact:**
  - Training Support - traininginfo@bd.com
    - Tel: 877.232.8995, prompt 5
  - **Wayland Quon**
    Sr. Customer Education Coordinator, Education Services
    - Wayland.Quon@bd.com
      135 North McCarthy Blvd, 2nd Floor, Milpitas, CA 95035, US
      t: 877.232.8995, prompt 5 / **c:** 408.728.4776 / direct: 408.708.0659
      www.bdbiosciences.com/learn
      www.linkedin.com/showcase/bdbiosciences/
  - Research Application - ResearchApplications@bd.com

Note: Fees are subject to change.

Attachment C – BD FACSMelody Quick Reference Guide

BD FACSMelody™ System Quick Reference Guide

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e-Learning: bdbiosciences.com/en-us/learn/training/self-paced-courses#e-learning-courses

Videos: bdbiosciences.com/en-us/learn/campaigns/facsmelody-cell-sorter-training-videos#Introduction

User's Guide: BD FACSChorus™ Software Help menu

BD

BD FACSMelody™ System Quick Reference Guide

This reference guide contains instructions for using the BD FACSMelody™ Cell Sorter with BD FACSChorus™ 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.

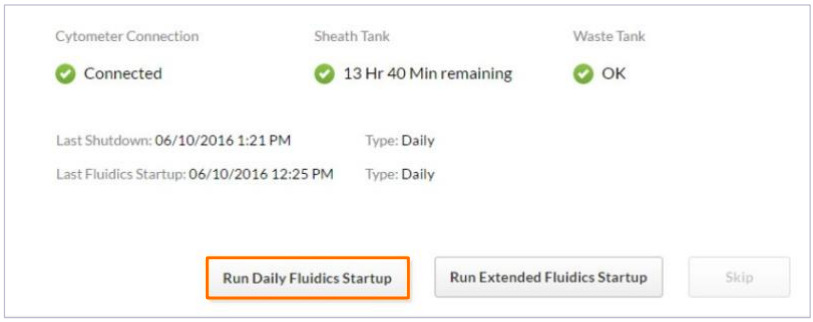
Start up system

Check fluids – STOP: See B+labs Staff

1. Fill the sheath tank to the weld line with sterile 1X phosphate-buffered saline (PBS).
   Note: Keep the sheath tank in the same location. If the location or elevation of the sheath tank is changed, it could affect the flow rate calibration.
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 – STOP: See B+labs Staff

1. Verify that air is being supplied to the instrument via the compressor or house air source.
2. Press the power button on the front of the cell sorter unit.
3. Start BD FACSChorus™ Software by double-clicking the shortcut on the desktop and logging 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.
4. Once the system has connected, click **Run Daily Fluidics Startup**.

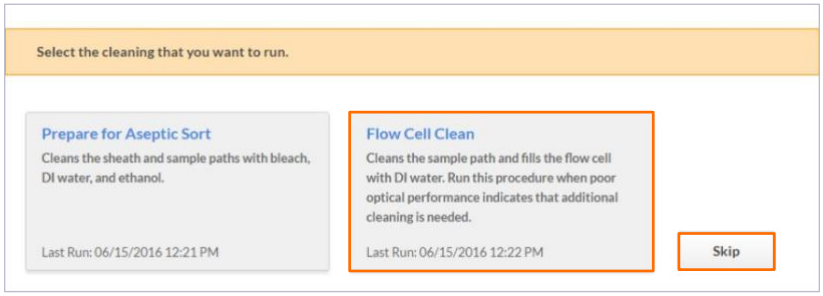


5. Follow the prompts on the screen for each numbered step. Be sure to insert the closed-loop nozzle with the O-ring facing up.
6. After fluidics startup is complete, click **Continue** to see the cleaning options.

Cleaning – STOP: See B+labs Staff

Select the type of cleaning that you want to run. **Note:** This step can be skipped however we recommend that you perform the Flow Cell Clean Procedure after each startup.

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 – STOP: See B+labs Staff

1. 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**.

Instrument and sort quality control –Cytometer Setup can be skipped if both CS&T RUO Beads and AccuDrop Beads have been run today

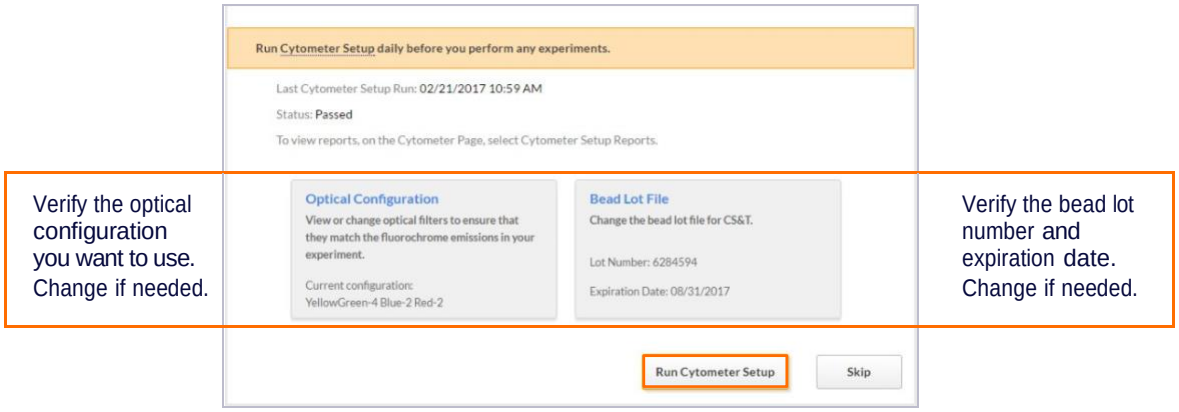
We recommend running Cytometer Setup (CS&T) and Drop Delay daily before performing any experiments.

1. Prepare a tube of BD® CS&T RUO Beads according to the package directions. **Note:** Do not dilute the beads with water.
    - Select sample tube
    - Add 500 uL phosphate-buffer saline (PBS)

    - Vortex CS&T RUO beads vial for 5-10 seconds

- Add 2 drops CS&T beads (store remainder in refrigerator)
- Vortex Tube for 5-10 seconds

2. Click **Run Cytometer Setup**.



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.
   - Vortext Accudrop RUO bead vial

   - Select sample tube
   - Dilute 1 drop Accudrop RUO beads in 0.5 mL phosphate-buffer Saline (PBS) in sample tube

   - Vortex sample tube

5. 

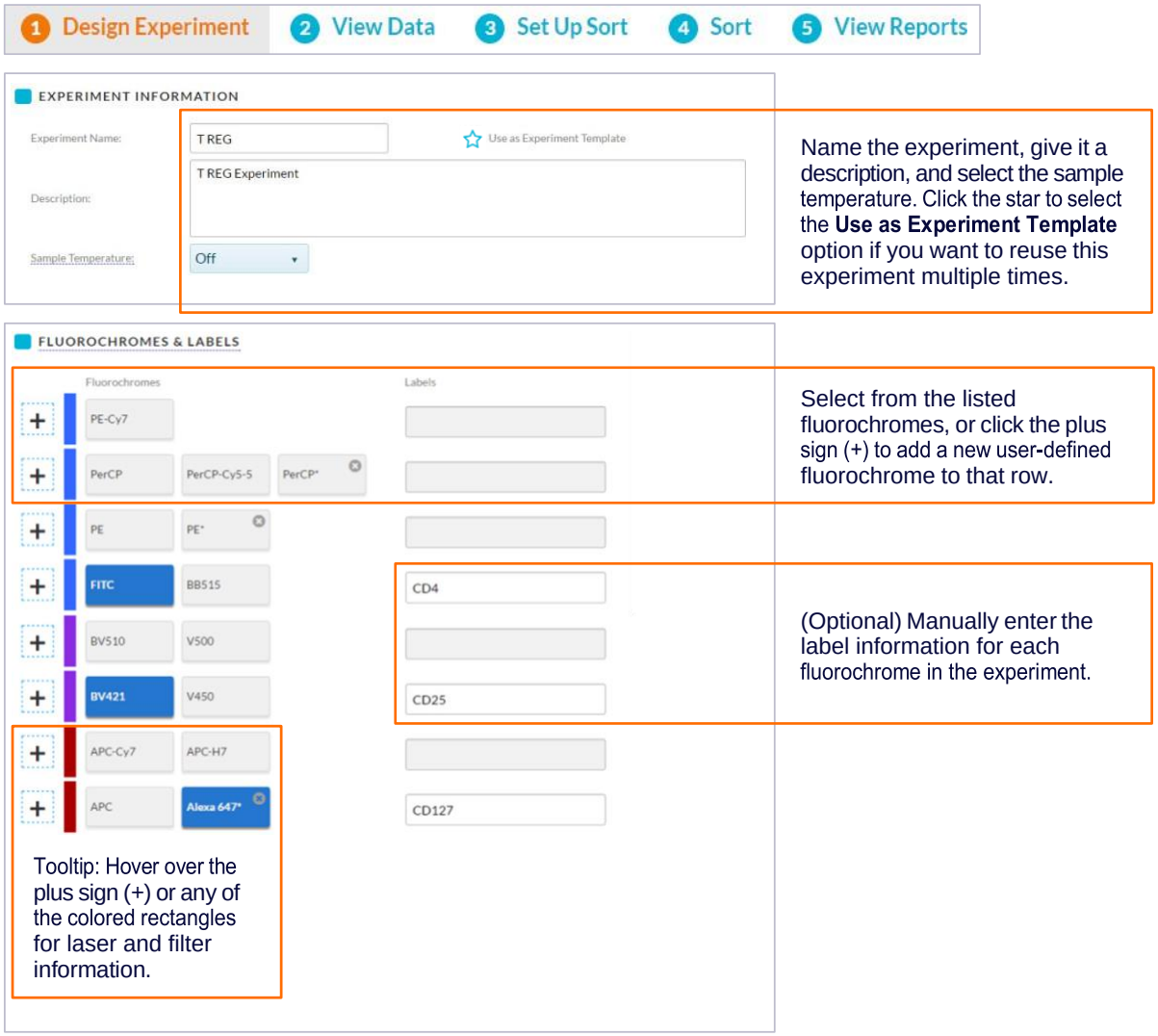
   - Load the tube and follow the prompts.

Create experiment

Experiments are used to define and refine the parameters for data acquisition and sorting.

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.



View data

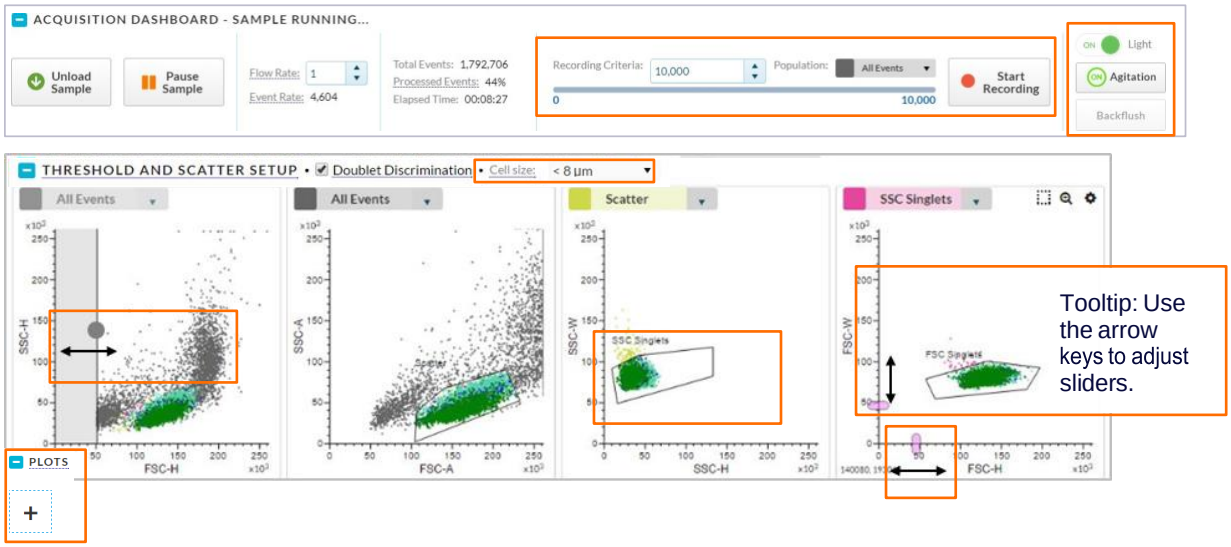
The selections on the View Data tab determine the layout of the experiment data. Optimize the threshold and scatter setup, then collect a pre-sort data file.

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.



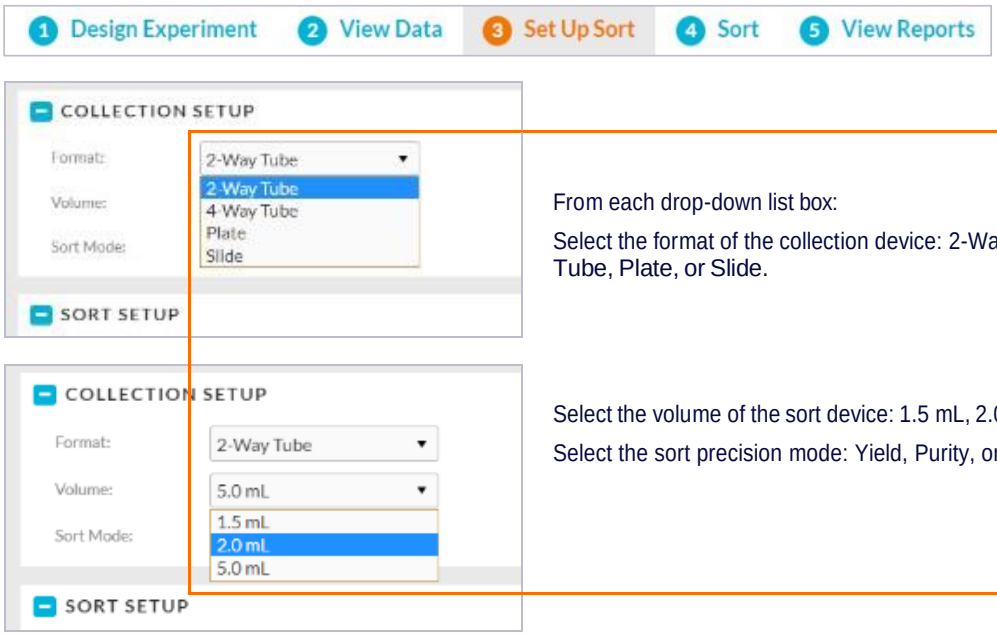
5. (Optional) If you are running your own compensation controls, click **Update Compensation** and follow the guided prompts. Otherwise, the stored spillover values will be used.

6. Select the Recording Criteria 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.



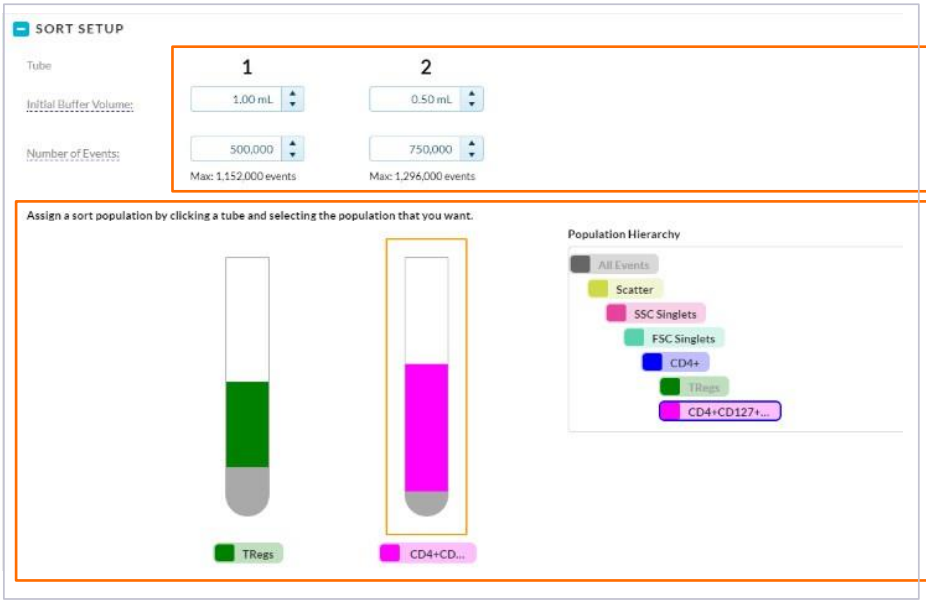
From each drop-down list box:

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 sort population from the Population Hierarchy.

Tubes: Four tubes view

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.

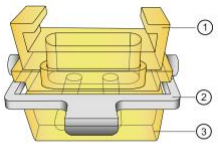
Assign the sort wells by clicking each well, dragging across a group of wells, clicking the letter or number for a row or column, or clicking **Select All.** You can also select non-contiguous wells by using Ctrl+click.

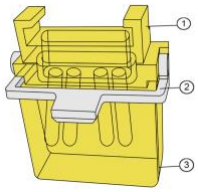
Sort

1. Click the **Sort** tab.

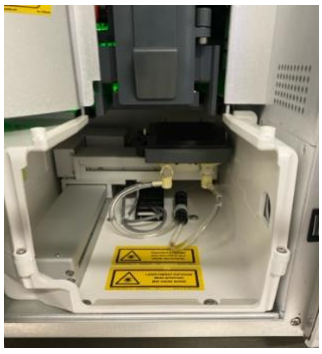
2. Insert the collection tubes into the appropriate tube holder.

- Select collection device: 2 tubes, 4 tubes, plate, slide
- For tube collection
  - Place collection tubes in holder

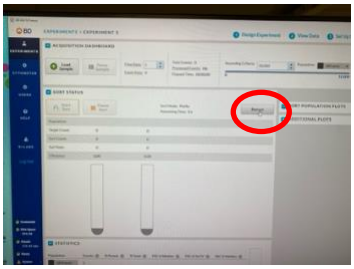




    - Raise metal bar
    - Remove top section
    - Select 2 or 4 tube tube holder
    - Insert collection tubes into lower section
    - Place upper section on lower section
    - Fasten together by lowering metal arm
  - Open sort collection chamber



  - On Sort tab screen, click Retract

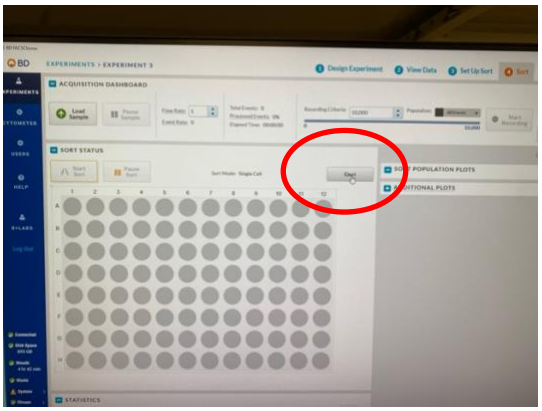


- Remove Splash Shield (if installed)

- Insert sort tube collection device into the sort collection chamber

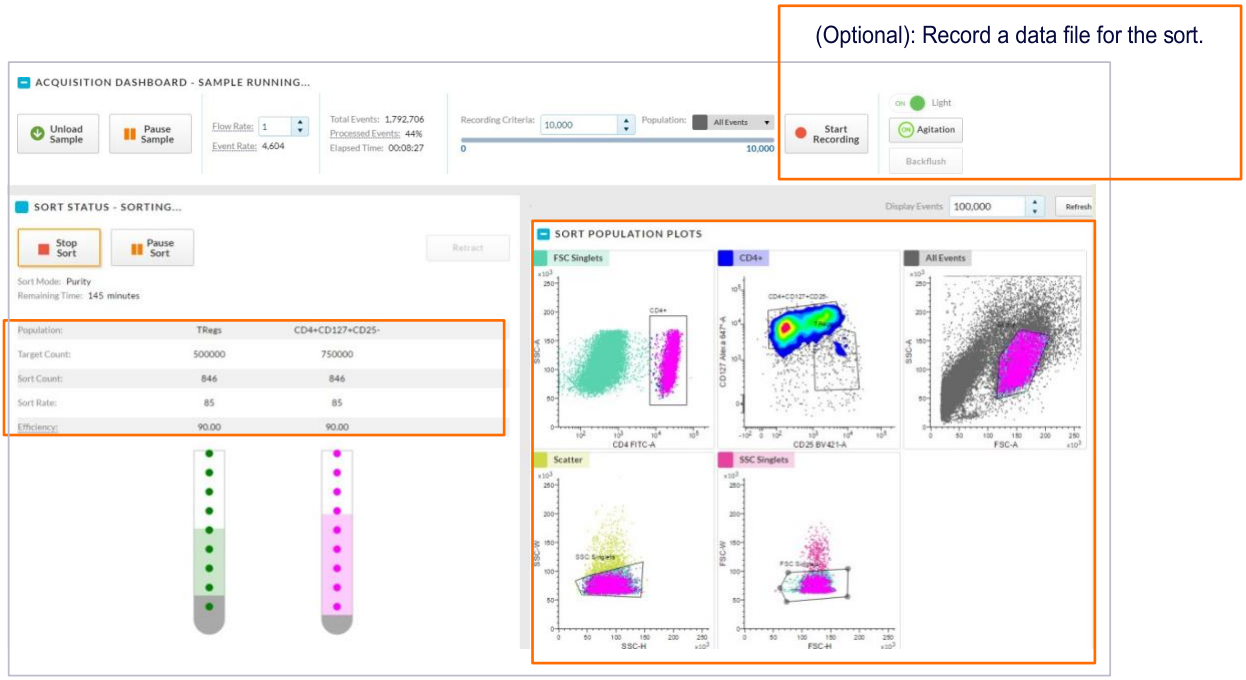
- For plate collection
  - Install Splash Shield
    - Remove tube collection device (if installed)
    - Attach splash shield to underside of sort block

  - On Sort tab screen, click Eject



- Load plate on tray with A1 positioned front left

3. Click **Start Sort**.
4. Monitor the sort by viewing the sort status and sort population plots.

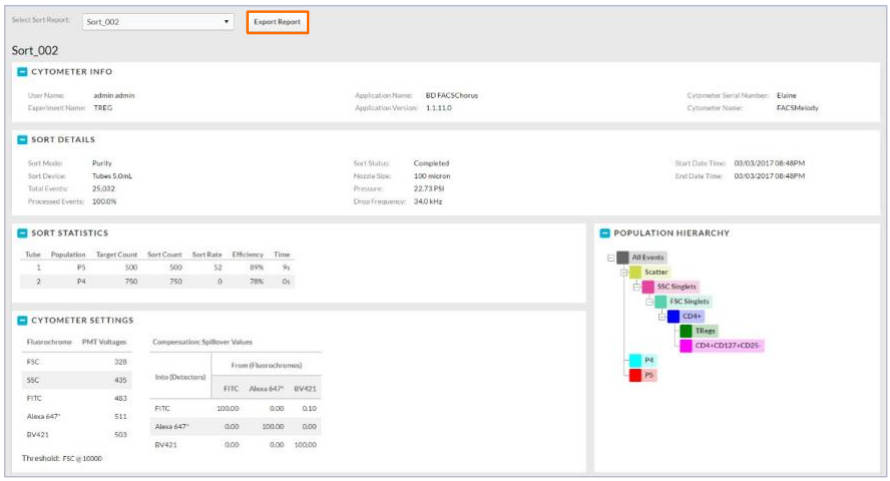


View sort reports

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

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

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


Select Sort Report:
Sort_002
Export Report
Sort_002
CYTOMETER INFO
User Name: admin admin
Experiment Name: TREG
Application Name: BD FACSChorus
Application Version: 1.1.11.0
Cytometer Serial Number: Elaine
Cytometer Name: FACSMelody
SORT DETAILS
Sort Mode: Purity
Sort Device: Tubes 5.0mL
Total Events: 25,032
Processed Events: 100.0%
Sort Status: Completed
Nozzle Size: 100 micron
Pressure: 22.73 PSI
Drop Frequency: 34.0 kHz
Start Date Time: 03/03/2017 08:48PM
End Date Time: 03/03/2017 08:48PM
SORT STATISTICS
Tube Population Target Count Sort Count Sort Rate Efficiency Time
1 P5 500 500 52 89% 9s
2 P4 750 750 0 78% 0s
CYTOMETER SETTINGS
Fluorochrome PMT Voltages
FSC 328
SSC 435
FITC 483
Alexa 647* 511
BV421 503
Compensation: Spillover Values
Into (Detectors)
From (Fluorochromes)
FITC Alexa 647* BV421
FITC 100.00 0.00 0.10
Alexa 647* 0.00 100.00 0.00
BV421 0.00 0.00 100.00
Threshold: FSC @ 10000
POPULATION HIERARCHY
All Events
Scatter
SSC Singlets
FSC Singlets
CD4+
TRegs
CD4+CD127+CD25-
P4
P5

Clean the Sample Line

This procedure cleans the sample line with a bleach solution. We recommend that you perform this at the end of your experiment and inbetween users.

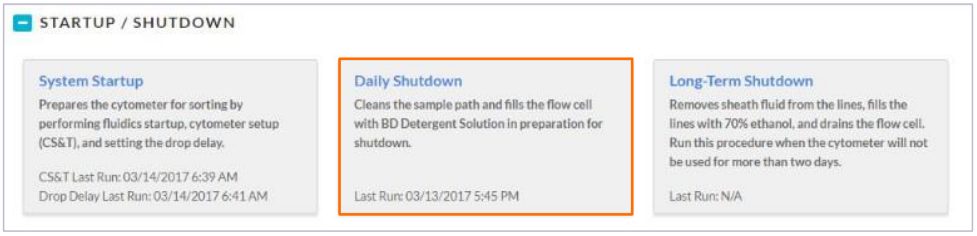
1. Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
2. From the View Data tab, click **Load Sample**.
3. After approximately 5 minutes, click **Unload Sample**.
4. Load a tube containing 3 mL of DI water* onto the sample loading port.
5. Repeat steps 2 and 3.

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

Shut down system – STOP: See B+labs Staff

You will be given an option to perform either Daily Shutdown or Long-Term Shutdown upon logging out or closing the application. You can also access these procedures 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** or **Long-Term Shutdown** option.
3. Follow the prompts on the screen for each numbered step.



4. Power off the cytometer unit.

Troubleshooting tips

STOP: See B+labs Staff - BD FACSChorus™ 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.	Steam 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	Steam 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	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. 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 to clear a possible sample line blockage. 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 fluid levels in the sample tube. If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.
	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	Clean the flow cell. Confirm by checking that 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 fluid levels in the sample tube. 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. If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.
	Sample is not mixed properly	Resuspend the sample. Turn on or increase the sample agitation rate.
	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 FACSChorus™ 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-µm nozzle. In general, the nozzle orifice should be at least 5 times the average particle size in the sort sample. See Shapiro H. *Practical Flow Cytometry*. 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.

This material is for training purposes.
Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.
23-23298(01)

BD Life Sciences, 2350 Qume Drive, San Jose, California 95131 USA

bdbiosciences.com

Attachment D – BD FACSMelody Users Guide

BD FACSMelody™ Cell Sorter User's Guide

For Research Use Only CE

23-18120-01
4/2017

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History

Revision	Date	Change made
23-18120-00	7/2016	Initial release
23-18120-01	4/2017	Added yellow-green laser, index sorting and optional filters

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1

Introduction

This chapter covers the following topics:

- About this guide (page 10)
- Safety symbols (page 12)
- Technical support (page 13)

About this guide

In this guide

This guide provides information for setting up and running the BD FACSMelody™ system using a typical workflow. In addition to becoming familiar with the instructions outlined in the guide, operators should receive the appropriate training on the BD FACSMelody cell sorter before operating the system.

This guide includes:

- Information about system hardware and components, a basic overview of BD FACSMelody system, and instructions about preparing the system for use.
- Instructions for performing quality control, basic acquisition, sorting, and analysis of your data.
- Instructions for maintaining the system and information about the available system options.

Search function

To search for a keyword in this guide, click **Ctrl+F**. The keyword search bar displays.

To view bookmarks and navigate to a section, click the bookmark icon in the upper right corner of the PDF window.

Additional documentation

The following table lists the available documents for the BD FACSMelody Cell Sorter.

Document	Description
BD FACSMelody™ Cell Sorter Site Preparation Guide	Provides the site requirements. Read this guide before the system is installed.
BD FACSMelody™ Cell Sorter Safety and Limitations Guide	Provides safety guidance and system limitations. Read this guide before running the system.
BD FACSMelody™ Cell Sorter Quick Reference Guide	Provides information for using the instrument. Read this guide before running the system.
BD™ CS&T RUO Beads technical data sheet	Provides instructions on preparing the BD CS&T RUO beads for quality control.
BD™ FC Beads technical data sheet	Provides instructions on preparing the BD™ FC beads for compensation control.
BD FACS™ Accudrop technical data sheet	Provides instructions on preparing the BD FACS™ Accudrop beads for calculating drop delay.
Information Security Guidelines	Provides recommendations regarding the security of the BD Biosciences workstations.

Safety symbols

Safety symbols

The following table lists the safety symbols used in this guide to alert you to potential hazards. For a complete description of all safety hazards, see the *BD FACSMelody Cell Sorter Safety and Limitations Guide*.

Symbol[a]	Meaning
	Caution. Indicates the need for the user to consult the user's guide for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the device itself.
	Biological hazard
	Electrical hazard
	Laser hazard
	Mechanical hazard, pinch points
	Lifting hazard

a. Although these symbols appear in color on the instrument, they might be printed in black and white; their meaning remains unchanged.

Technical support

Introduction

This section describes how to get technical support.

Contacting technical support

If assistance is required, contact a BD Biosciences technical support representative or supplier. Visit our website, bdbiosciences.com, for up-to-date contact information.

When contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number
- Any error messages
- Details of recent system performance

Ordering replacement parts

To order replacement parts:

1. Go to bdbiosciences.com.
2. Select **BD FACSMelody** > **Products** > **Instruments** > **BD FACSMelody Consumables**.
3. Select the materials to order.

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About the system

This chapter covers the following topics:

- System overview (page 16)
- Instrument overview (page 19)
- Optical components (page 21)
- Instrument configurations (page 23)
- Changing optical configurations (page 33)
- Fluidic components (page 35)
- BD FACSChorus software (page 38)

System overview

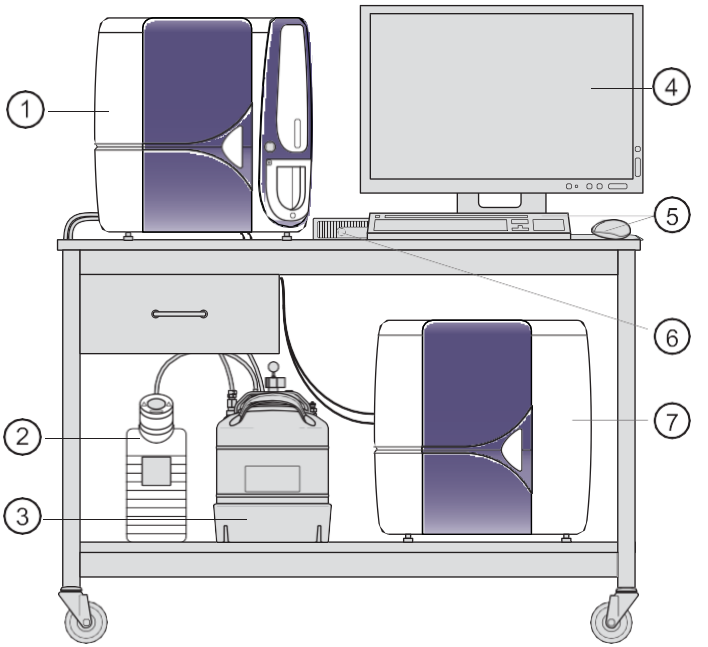
Introduction

This topic provides an overview of the BD FACSMelody system and a description of the main components.

About the system

The BD FACSMelody system includes the BD FACSMelody cell sorter, sheath and waste tanks, workstation with accessories, and BD FACSChorus™ software. All of these components combine to create an integrated system designed for use in a wide variety of research applications.

The following drawing displays the typical layout of the system on a table.



No	Description
1	BD FACSMelody cell sorter
2	Waste tank
3	Sheath tank
4	Monitor
5	Keyboard and mouse
6	Computer
7	Electronics box

The BD FACSMelody system acquires, sorts, and analyzes particles or cells in a liquid suspension. Antibodies to specific cell proteins are labeled with a fluorescent dye and incubated with the cell suspension. The suspension flows through the cell sorter and is interrogated by a laser which excites the fluorescent antibodies and fluorescent cells.

The fluorescence is captured, cells are sorted based on specified criteria, and the resulting data is analyzed to reveal information about the cells. This technique can be used in diverse research areas such as stem cell development, cell signaling pathways, and HIV.

Quality control performance, tracking, and reporting are streamlined and automated.

BD FACSMelody cell sorter

The BD FACSMelody cell sorter is a compact benchtop research cell sorter. The pressure-driven fluidics along with a uniquely designed flow cell and sample injection tube provide reliability and good signal resolution.

The three laser configurations provide the ability to analyze up to 9 colors (11 parameters). The heptagon detector array takes the guesswork out of changing filters and ensures that the correct filters and mirrors are installed.

Several hardware options and upgrades can be used to customize the system for different applications.

BD FACSChorus software

BD FACSChorus software is used to operate the instrument, acquire and sort samples, and analyze the data. The software is designed with guided, easy-to-use screens that include embedded text and instructions. The software controls and continuously monitors the status of the instrument.

Workstation

The BD FACSMelody system includes the HP® desktop computer with the Microsoft®Windows®10 operating system installed, a wireless keyboard and mouse, and a HP® 23-in. monitor.

Sheath and waste tanks

The system comes with a stainless steel 10-L sheath tank and a polypropylene 10-L waste tank.

Note: Do not place the fluidic tanks at a level that is higher than the cell sorter because this can cause incorrect pressure and increase the sheath flow rate. Keep the fluidic tanks in the same location that they were placed in during installation.

Beads, reagents, and assays

BD CS&T RUO beads are used to check the instrument performance and automatically make adjustments, ensuring consistent values from day to day and experiment to experiment.

BD FACS Accudrop beads are used to automatically set an accurate drop delay value. The Accudrop laser is aligned with the center and side (sorting) streams. BD FACSChorus software optimizes the drop delay by sorting the Accudrop beads and identifying a drop delay value that yields the most particles in the side stream and the fewest in the center stream.

BD FC beads are used as compensation controls to set up normalized spillover values which are valid for 60 days.

Supported sort collection devices

The BD FACSMelody system supports the following sort collection devices:

- 1.5- and 2.0-mL tubes
- 5.0-mL tube

The following sort devices are available when the optional automated stage is installed:

- Microscope slide: 27 wells (3 x 9 grid)
- 6-, 24-, 48-, 96-, and 384-well plates
- 96-well PCR tube strip

Options

Optional accessories that can be used with the BD FACSMelody cell sorter include:

- Sample temperature control
- Aerosol management option (AMO)
- Biological safety cabinet (BSC)
- Remote diagnostics with Assurity Linc™
- Plate sorting, using the optional automated stage

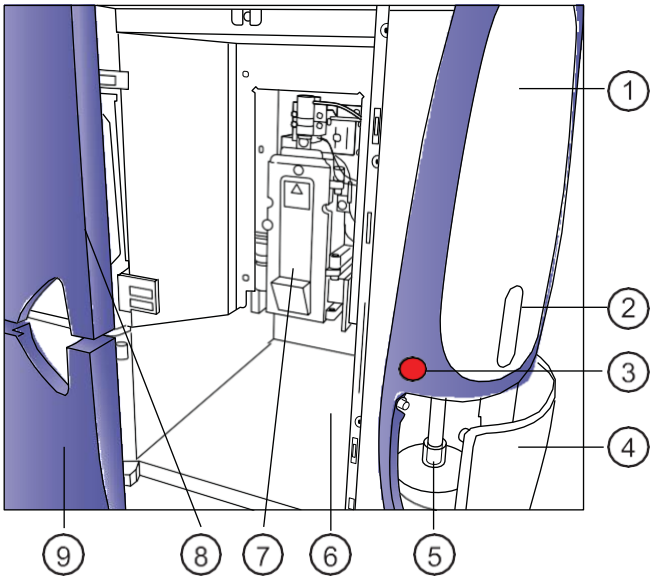
Instrument overview

Introduction

The BD FACSMelody cell sorter consists of three subsystems (fluidics, optics, and electronics) that are located in two cubes, with two tanks for sheath and waste. The two cubes are connected with two electrical umbilical cables, one fluidic umbilical cable and one fiber optics cable.

Main components

The instrument includes the lasers, sample injection chamber, sort chamber, and sample holders. The following image shows some BD FACSMelody cell sorter details.



No.	Description
1	Sample line access cover
2	Sample viewing window
3	Power on/off switch
4	Sample access door
5	Sample loading port
6	Sort collection chamber
7	Sort block door
8	Flow cell access door
9	Sort collection chamber door

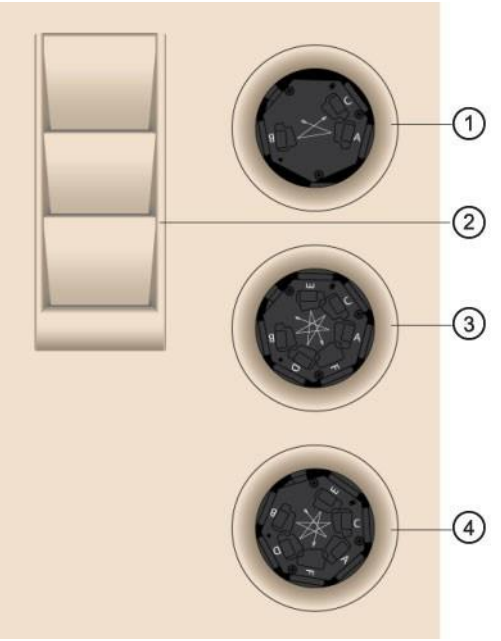
Optical components

Introduction

This topic describes the optical components, including the detectors and the filter holders.

Location of optical components

The optical compartment is located on the front of the electronics box, behind the front door. The arrays for the lasers are accessible when the door is open. The following figure shows the locations of the optical components in a 3-laser instrument.



No	Description
1	Trigon detector
2	Holder for filters
3	Heptagon detector
4	Heptagon detector

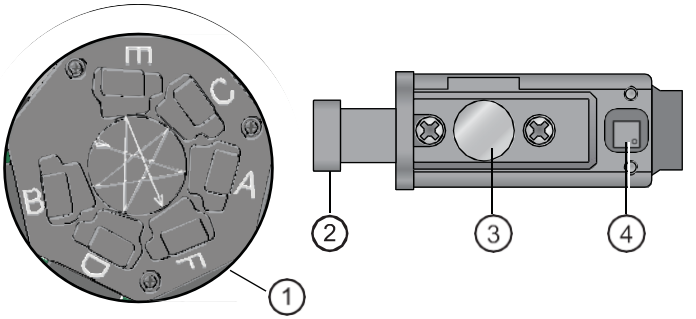
Detector arrays

The detector arrays contain the filters, mirrors, and photomultiplier tubes (PMTs) for each laser. There is a separate detector array for each laser.

Filter holders

Each channel in a detector array has a removable filter holder that contains a bandpass filter and a dichroic mirror for that channel. The filter holder has an ID chip that identifies the holder to the system so the software can confirm that the correct filter holder is in place.

The following figure shows a heptagon and a filter holder.



No	Description
1	Heptagon
2	Handle
3	Optical filter
4	ID chip

Location of lasers

The system lasers and beam-steering optical components are located at the top of the instrument, under the top cover.

Instrument configurations

Introduction

The BD FACSMelody system can include one, two, or three lasers. It is available in the following configurations.

Number of lasers	Number of colors
1(blue)	2-color (2-0)
1(blue)	4-color (4-0)
2 (blue, yellow-green)	4-color (2-2)
2 (blue, red)	6-color (4-2)
2 (blue, yellow-green)	6-color (2-4)
2 (blue, violet)	6-color (3-3)
3 (blue, red, violet)	6-color (2-2-2)
3 (blue, red, yellow-green)	8-color (2-2-4)
3 (blue, violet, yellow-green)	8-color (2-2-4)
3 (blue, red, violet)	9-color (4-2-3)

Configuration details

The following tables show the setup for the different detector arrays for each configuration. The description of the abbreviations is as follows:

- B = blue
- R = red
- V = violet
- YG = yellow-green

One laser 2 color

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE, PI	560LP	586/42
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15

a. There is a 10% neutral density filter installed in front of the SSC filter.

One laser 4 color

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy™7	552LP	783/56
	B	PerCP, PerCP-Cy™5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: BD Horizon PE-CF594, PE-Texas Red®	605LP	613/18
	C	PE, PI	560LP	586/42
	D	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	E	Side scatter (SSC)	ND10[a]	488/15

a. There is a 10% neutral density filter installed in front of the SSC filter.

Two-laser system (2B-2YG configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700	665LP	700/54
	B	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Yellow-green/ 561 nm	A	mCherry, BD Horizon™ PE-CF594, PE-Texas Red®, PI	605LP	613/18
	B	PE, DsRed	582LP	582/15

a. There is a 10% neutral density filter installed in front of the SSC filter.

Two-laser system (4B-2R configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700	665LP	700/54
		Optional: BD Horizon PE-CF594, PE-Texas Red®	605LP	613/18
	C	PE, PI	560LP	586/42
	D	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	E	SSC	ND10[a]	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Optional: Alexa Fluor® 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor® 647	660/10	660/10

a. There is a 10% neutral density filter installed in front of the SSC filter.

Two-laser system (2B-4YG configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700	665LP	700/54
	B	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15

Laser	Detector	Primary fluorochrome	Mirror	Filter
Yellow-green/ 561 nm	A	PE-Cy7	752LP	783/56
	B	PE-Cy™5, PE-Cy™5.5	665LP	697/58
	C	mCherry, BD Horizon PE-CF594, PE-Texas Red®, PI	605LP	613/18
	D	PE, DsRed	582/15	582/15

a. There is a 10% neutral density filter installed in front of the SSC filter.

Two-laser system (3B-3V configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PE, PI	560LP	586/42
	C	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	D	Side scatter (SSC)	ND10[a]	488/15

Laser	Detector	Primary fluorochrome	Mirror	Filter
Violet/ 405 nm	A	BD Horizon Brilliant™ Violet 786	755LP	None
		Optional: BD Horizon™ V500, BD Horizon Brilliant™ Violet 510, AmCyan	500LP	528/45
	B	BD Horizon™ V500, BD Horizon Brilliant™ Violet 510, AmCyan	500LP	528/45
	C	BD Horizon™ V450, Pacific Blue™, DAPI, BD Horizon™ Violet Proliferation Dye 450, BD Horizon™ Fixable Viability Stain 450, BD Horizon Brilliant™ Violet 421	448/45	None

a. There is a 10% neutral density filter installed in front of the SSC filter.

Three-laser system (2B-2R-2V configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE, PI	560LP	586/42
	B	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Alexa Fluor® 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor® 647	660/10	660/10

Laser	Detector	Primary fluorochrome	Mirror	Filter
Violet/ 405 nm	A	BD Horizon Brilliant Violet 786	755LP	None
		Optional: BD Horizon™ V500, BD Horizon Brilliant™ Violet 510, AmCyan	500LP	528/45
	B	BD Horizon V450, Pacific Blue, DAPI, BD Horizon Violet Proliferation Dye 450, BD Horizon Fixable Viability Stain 450, BD Horizon Brilliant Violet 421	448/45	None

a. There is a 10% neutral density filter installed in front of the SSC filter.

Three laser system (2B-2R-4YG configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700	665LP	700/54
	B	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Optional: Alexa Fluor® 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor® 647	660/10	660/10

Laser	Detector	Primary fluorochrome	Mirror	Filter
Yellow-green/ 561 nm	A	PE-Cy7	752LP	783/56
	B	PE-Cy5, PE-Cy5.5	665LP	697/58
	C	mCherry, BD Horizon PE-CF594, PE-Texas Red®, PI	605LP	613/18
	D	PE, DsRed	582LP	582/15

a. There is a 10% neutral density filter installed in front of the SSC filter.

Three laser system (2B-2V-4YG configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700	665LP	700/54
	B	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15

Laser	Detector	Primary fluorochrome	Mirror	Filter
Violet/ 405 nm	A	BD Horizon V500, BD Horizon™ Brilliant Violet 510, AmCyan, CFP	500LP	528/45
		Optional: BD Horizon™ Brilliant Violet 786	755LP	None
	B	BD Horizon V450, Pacific Blue, DAPI, BD Horizon Violet Proliferaton Dye 450, BD Horizon Fixable Viability Stain 450, BD Horizon Brilliant Violet 421	448/45	None
Yellow-green/ 561 nm	A	PE-Cy7	752LP	783/56
	B	PE-Cy5, PE-Cy5.5	665LP	697/58
	C	mCherry, BD Horizon PE-CF594, PE-Texas Red®, PI	605LP	613/18
	D	PE, DsRed	582LP	582/15

a. There is a 10% neutral density filter installed in front of the SSC filter.

Three laser system (4B-2R-3V configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PerCP, PerCP-Cy5, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700	665LP	700/54
		Optional: BD Horizon PE-CF594, PE-Texas Red®	605LP	613/18
	C	PE, PI	560LP	586/42
	D	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
		Optional: GFP	510/10	510/10
	E	Side scatter (SSC)	ND10[a]	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Alexa Fluor® 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor® 647	660/10	660/10

Laser	Detector	Primary fluorochrome	Mirror	Filter
Violet/ 405 nm	A	BD Horizon Brilliant Violet 786	755LP	None
		Optional: BD Horizon V500, BD Horizon Brilliant Violet 510, AmCyan	500LP	528/45
	B	BD Horizon V500, BD Horizon Brilliant Violet 510, AmCyan	500LP	528/45
		BD Horizon Brilliant Violet 786	755LP	None
	C	BD Horizon V450, Pacific Blue, DAPI, BD Horizon Violet Proliferation Dye 450, BD Horizon Fixable Viability Stain 450, BD Horizon Brilliant Violet 421	448/45	None

a. There is a 10% neutral density filter installed in front of the SSC filter.

Changing optical configurations

Introduction

Optical configurations can be customized by using optional mirror/filter combinations. See the preceding configuration tables to determine the positions where the optional filters will be accepted.

When a new configuration is created, a new baseline must be performed with CS&T beads. If a filter is changed and the created configuration matches an existing configuration, then a new baseline is not required. We recommend that you always verify that the new configuration is your desired configuration.

Procedure **To change the optical configuration:**

1. Follow the System startup wizard.
2. Click **Continue** and then **Skip**.
3. Insert the sort nozzle and click **Continue**.
4. Select **Optical Configuration**.

5. Follow the instructions on the dialog.
6. Click **Verify Configuration** to verify that the configuration shown is the desired configuration.

   The system indicates if the new configuration is valid with a success message.

The system indicates if the new configuration is invalid with an error message.

7. Run a new baseline using CS&T beads for the new configuration.

8. (Optional) For a new configuration, you can use normalized spillover values from FC beads for compensation. First, run CS&T beads in the Update Compensation Standards workflow and then run the desired FC beads before you run experiments.

Note: You can also access this function from the Cytometer page.

Fluidic components

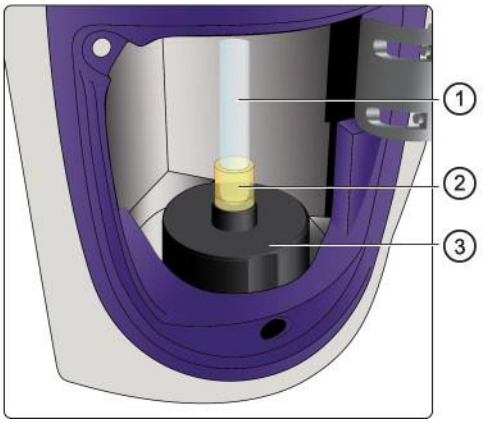
Introduction

Laser light is focused on the sample core stream within the flow cell. Fluorescent molecules excited by the different laser wavelengths are detected by the optics and analyzed by the electronics. Particles are then either sorted into a collection device within the sort collection chamber or transported to the waste tank.

The fluidic components include the sheath and waste tanks, sample injection chamber, tube holders, flow cell, closed-loop and sort nozzles, sort block, and the sort collection chamber.

Sample injection chamber

The sample injection chamber is the location at which tubes are loaded into the system. Use the sample viewing window to observe the loaded tube.



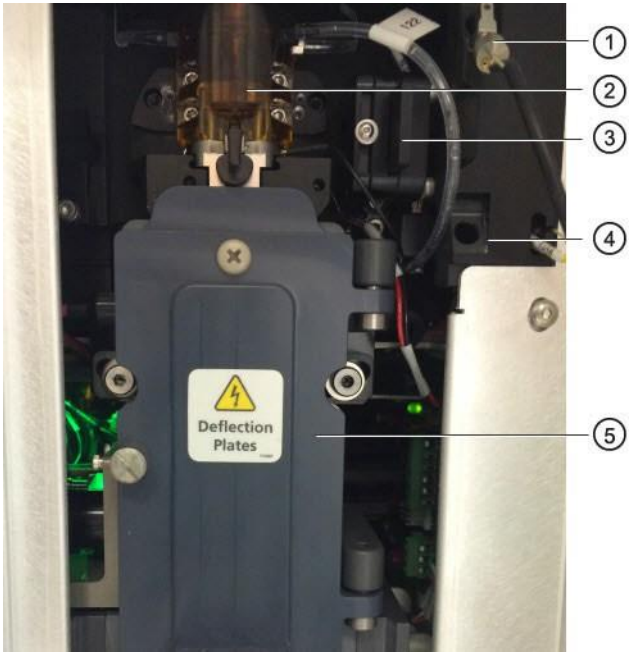
No.	Description
1	Sample tube
2	Tube holder
3	Sample loading port

Flow cell

The flow cell is located above the sort block. Within the flow cell, hydrodynamic focusing forces particles through the cuvette in a single-file stream, where laser light intercepts the stream at the sample interrogation point.

The unique flow cell design permits particles to flow through the cuvette at a low velocity, allowing longer exposure to laser energy. The cuvette is gel-coupled to the fluorescence objective lens to transmit the greatest amount of emitted light from the interrogation point to the collection optics.

After passing through the cuvette, the stream is accelerated as it enters the nozzle tip, where the drop drive breaks the stream into droplets for sorting.



No.	Description
1	Nozzle holder with closed loop nozzle
2	Flow cell
3	Forward scatter detector with neutral density filter
4	Nozzle holder
5	Sort block

Nozzle

The 100-µm integrated sort nozzle is available along with a closed-loop nozzle for use in cleaning and shutdown procedures. The sort nozzle is keyed to a fixed position at the lower end of the cuvette.

Because the sort nozzle is below the interrogation point, optical alignment is not affected when the nozzle is changed.

BD FACSChorus software

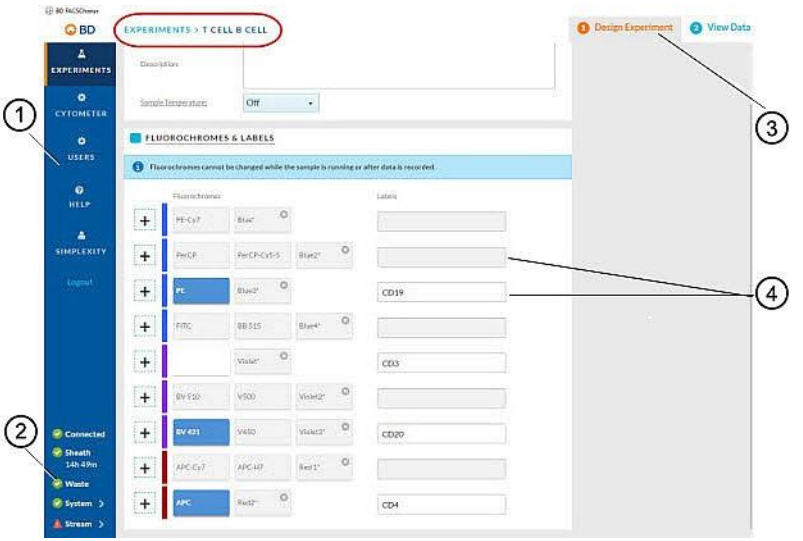
Introduction

BD FACSChorus software runs the BD FACSMelody instrument. The software has been designed with guided, simple, task-oriented screens.

Screen design

The screen includes a navigation bar on the left with a list of tasks. Selecting a task on the navigation bar opens a workspace on the right. There are numbered tabs across the top of the workspace to indicate the order or workflow for performing tasks. During startup, the tabs must be completed in the assigned order. However, when creating an experiment, the tabs can be selected in any order.

Status information is displayed on the bottom of the navigation bar. Instructional text and tips are displayed when you hover over screen elements.

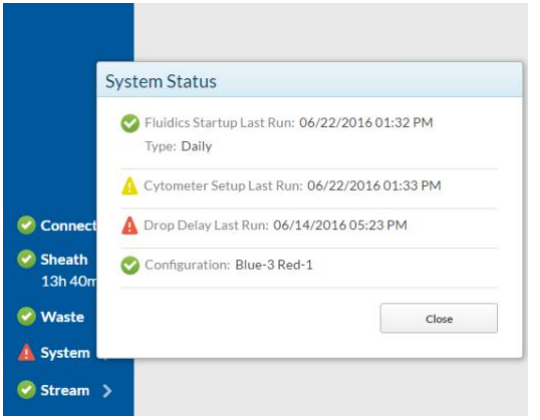


No.	Description
1	Navigation bar
2	Status indicators
3	Guided workflow
4	Data entry fields

System status indicator

The System status indicator on the navigation bar is an aggregate of four categories: Fluidics Startup, Cytometer Setup, Drop Delay, and Configuration. The System status indicator shows the highest alert level that was flagged across all four categories. For example, if two of the categories are green, one is yellow, and one is red, the System status indicator will be red.

You can click the System status indicator to see the details. The System Status window opens and provides the details for the four categories.



If problems occur with the configuration of the cytometer, information about the problem and the solution is provided next to the Configuration status indicator. However, this kind of information is not always provided for problems related to Fluidics

Startup, Cytometer Setup, or Drop Delay. The following table provides this information based on the color of the status indicator.

Status Indicator	Icon Color	Problem	Solution
Fluidics Startup	Red	System startup has not been performed for more than 24 hours.	On the Cytometer page, select System Startup, and then select Extended Fluidics Startup.
	Yellow	Not applicable. No Yellow status exists for Fluidics Startup.	
Cytometer Setup	Red	No results exist for when Cytometer Setup was last performed. (Rare)	On the Cytometer page, select System Startup and complete the Cytometer Setup (CS&T).
	Yellow	Cytometer Setup must be performed due to a change in the optical configuration.	
		Cytometer Setup has not been performed for more than 24 hours, or it failed the last time it was performed.	
Drop Delay	Red	The last Drop Delay failed, or it must be performed again due to a change in the optical configuration.	On the Cytometer page, select System Startup and complete the Drop Delay.
	Yellow	Drop Delay has not been performed for more than 24 hours.	

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3

System startup and shutdown

This chapter covers the following topics:

- System startup (page 44)
- Fluidics startup (page 46)
- Cleaning (page 48)
- About CS&T reports (page 49)
- Editing your user profile (page 51)
- Adding, editing, or deleting user accounts (page 51)
- Shutting down the system (page 52)

System startup

About system startup

The BD FACSMelody cell sorter startup process has been automated to quickly provide a ready-to-use system with a stable stream. The instrument is designed so that either the computer or the cell sorter can be turned on first. Alerts and instructions are displayed to indicate the status of the instrument.

When the system is turned on, it automatically displays the connection status.

The tasks on the following tabs are not automatic and require user action.

- Fluidics startup
- Cleaning
- Sort nozzle
- Cytometer setup–run BD CS&T RUO beads
- Drop delay–run BD FACS Accudrop beads

Connection status

During startup, the system monitors the connection between the instrument and the computer and displays a dialog with a green status (connected) or a gray status (not connected). The system also displays a yellow status (connecting) progress indicator to indicate that actions are being completed in the background.

Fluidics startup

Once the system has connected, the fluidic pumps are automatically started. There are two choices for running the fluidic startup:

- Run Daily Fluidic Startup
- Run Extended Fluidic Startup

Selecting the appropriate start depends on how the system was shut down.

  - If no shutdown was performed, then a fluidics startup is not required (not recommended).

  - If a daily shut down was performed, then you can select to perform either a daily or extended startup.
  - If an extended shut down was performed, then you must perform an extended startup.

Cleaning

There are two cleaning options that are available. These are:

- Prepare for Aseptic Sort which cleans the sheath and sample paths with 10% bleach, DI water, and 70% ethanol.
- Flow Cell Clean which cleans the sample path and fills the flow cell with DI water.

We recommend running the flow cell clean between samples or different operators. This is an optional step that can be skipped.

Sort nozzle

The system displays a dialog to insert the sort nozzle. When completed, the system moves to cytometer setup tasks.

Cytometer setup–run BD CS&T RUO beads

BD CS&T RUO beads are used to measure the baseline and daily performance capability of the instrument.

- Measuring baseline takes longer to complete than performance. It occurs:
  - At installation
  - Every 90 days (for the same configuration and bead lot)
  - After preventative maintenance and major service procedures
  - When a new bead lot files is selected
- We recommend measuring performance daily at the start of the day to update settings and track the instrument performance. However, measuring performance daily is an optional step.

The system displays a progress bar and unloads the tube of BD CS&T RUO beads after the completion dialog is displayed. A CS&T report is also generated at this time.

All CS&T data is specific to a set configuration. Before proceeding, verify that the displayed configuration is the desired configuration.

If you need to update the configuration, see Changing optical configurations (page 33).

Drop delay–run BD FACS Accudrop beads

BD FACS Accudrop beads are used to automatically set an accurate drop delay value. The Accudrop laser is aligned with the center and side (sorting) streams. BD FACSChorus software optimizes the drop delay by sorting Accudrop beads and identifying a drop delay value that yields the most particles in the side stream and the fewest in the center stream. This is an optional daily task.

Required materials

The following table describes the required materials for the operation of the system.

Item	Description	Supplied by
Bulk fluids	Sterile phosphate buffered saline (PBS)	User
	Bleach (for the waste tank)	User
	Deionized (DI) water	User
	Ethanol	User
Setup beads	BD CS&T RUO beads with bead lot file BD FC beads	BD
Accudrop beads	BD FACS Accudrop beads	BD
BD Cleaning solution	Solution for cleaning	BD

Fluidics startup

Introduction

Selecting a fluidic startup option depends on how the system was shut down.

- If no shutdown was performed, then a fluidics startup is not required (not recommended).
- If a daily shut down was performed, then you can select to perform either a daily or extended start-up.
- If an extended shut down was performed, then you must perform an extended start-up.

Procedure **To perform a daily or extended fluidics startup:**

1. Press the Power button on the front of the instrument.
2. Start the BD FACSChorus software and log in.
3. On the opening screen, select either **Run Daily Fluidics Startup** or **Run Extended Fluidics Startup**.

   The daily or extended fluidics startup dialog opens with four tasks that need to be completed.

   - Daily fluidics startup or extended fluidics startup depending on the selection
   - Insert the closed-loop nozzle
   - Check the sheath and waste tanks
   - Start the sheath filter purge and prime the stream

   Note: For the extended fluidics startup, ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters. See Preparing new fluid filters (page 146).

4. Follow the prompts to complete the tasks displayed on the screen.

   A green check mark is displayed to indicate successful completion of each task.

   If there are issues, follow the instructions on the error message to troubleshoot the issue.

5. When all of the tasks are complete, click **Close**.

   The system returns to the opening screen.

6. Click **Continue** to view the cleaning options.

7. Click **Skip** if no cleaning is needed.

Cleaning

Introduction

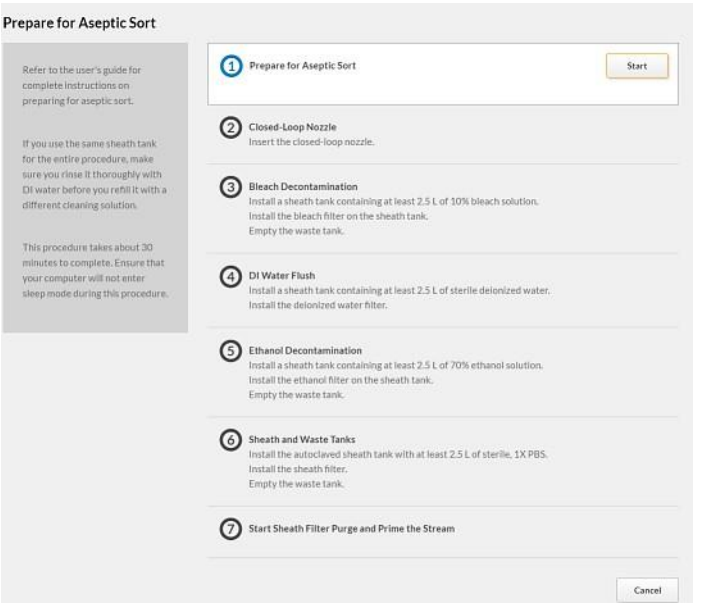
Two cleaning options are available:

- Prepare for Aseptic Sort.
- Flow Cell Clean.

Prepare for aseptic sort

To prepare for aseptic sort:

1. Follow the prompts on the screen to complete the tasks.



Note: Ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters.

See Preparing new fluid filters (page 146).

2. Click **Close** to return to the **Cleaning** dialog.

Caution! All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning cytometer surfaces. Wear suitable protective clothing, eyewear, and gloves.

Flow cell clean

To prepare for flow cell clean:

1. Follow the prompts on the screen to complete the tasks.
   a. Perform a flow cell clean with 1.5% BD Detergent Solution.
   b. Place an empty sample tube in the sample chamber and run another flow cell clean. This will create bubbles in the flow cell.
   c. Perform flow cell clean three (3) additional times with DI water to rinse the detergent thoroughly from the flow cell.
2. Click **Close** to return to the **Cleaning** dialog.

More information

- Preparing for aseptic sort (page 144)
- Cleaning the flow cell (page 118)

About CS&T reports

Introduction

CS&T reports contain information about the system, detector settings, lasers, setup bead lots, and instrument settings. Reports are generated after the completion of a baseline and performance check. A report is generated each time a procedure is completed.

Viewing a CS&T report

To view a CS&T report:

1. Click **CYTOMETER** on the navigation bar.
2. In the **OTHER** panel, select **Cytometer Setup Reports**.

Report Description The sections of the reports are described as follows.

Report section	Field	Description
Summary	Status	Indicates pass or fail.
	Report type	Indicates Performance or Baseline report.
	Cytometer name and serial number	Provides the name and serial number of the instrument.
	Software	Indicates the version of the software being used.
	Bead lot ID	Indicates which bead lot was used.
	Nozzle size	Indicates the size of the nozzle.
	Configuration	Indicates the instrument configuration.
Detector settings		
Detector	Name	Name for the detector
	Filter	Description of wavelengths transmitted
	Mirror	Name of the mirror used with the detector
	Position	Location of the filter holder with mirror
	PMT voltage	Measured PMT voltage
Bright beads	Median	Median fluorescence intensity (MFI) value of the specific bead
	%rCV	Percent robust coefficient of variation of the bright beads
Linearity	Min channel	Minimum value for the acceptable linear range of the detector
	Max channel	Maximum value for the acceptable linear range of the detector
	Qr	Relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector

Report section	Field	Description
	Br (ABD)	Relative optical background signal, used for tracking the optical background noise levels in a detector
Laser settings		Lasers used when the performance check or baseline was completed

Editing your user profile

Introduction You can change your password and your name in your user profile.

Procedure

To edit your user profile:

1. Click your name on the navigation bar.
2. Make the changes that you want, and then click **Save**.

Adding, editing, or deleting user accounts

Introduction If you have an Admin account, you can add, edit, or delete user accounts on the User Management page. You can also lock or unlock a user account.

About this task

When you create a new user, you can select a User role or an Admin role for the user. Select the Admin role only if you want the user to be able to add, edit, or delete accounts.

You can lock a user account if you want to revoke a user's access to the system without deleting the account. To lock or unlock an account, edit the user account and select this option under Account Status.

When you delete a user, any experiments, data records, sort reports, or fluorochromes created by the user are also deleted.

Procedure

To add, edit, or delete a user:

1. Click **USERS**.
2. Do any of the following actions:
   - To add a new user, click **+ New User**.
   - To edit a user, click the name of the user in the list.
   - To delete a user, click the **Delete** icon.

Shutting down the system

Introduction

You can perform a daily shutdown or a long-term shutdown. Both procedures are accessible on the Cytometer page in the software. Long-term shutdown must be performed every six months.

Before you begin

For the long-term shutdown procedure, you need at least 2.5 L of 70% ethanol and an ethanol filter.

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 111)
- Filling the sheath tank (page 120)
- Changing the fluid filter (page 131)
- Emptying the waste tank (page 126)

About this task

If you are shutting down the system for more than two days, select the long-term shutdown procedure. Otherwise, select the daily shutdown procedure.

For the long-term shutdown procedure, ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters.

Note: If you log out or exit (close) the software without performing a shutdown procedure, the software prompts you to perform a shutdown procedure.

Procedure

To shut down the system:

1. On the **Cytometer** page, select the shutdown procedure that you want to run.
2. Complete the steps in the wizard.
3. Run the daily shutdown procedure with a 1.5% BD Detergent Solution.
4. Leave the 1.5% BD Detergent Solution in the flow cell.

Caution! Never mix BD Detergent Solution and bleach in the same tube because they can create dangerous fumes.

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4

Experiment

This chapter covers the following topics:

- Experiment overview (page 56)
- Creating an experiment (page 56)
- Designing an experiment (page 59)
- Defining view data (page 60)
- Calculating compensation (page 64)
- Updating compensation standards (page 69)
- Setting up sorting (page 70)
- Loading collection devices (page 73)
- Sorting (page 79)
- Index sorting (page 82)
- Viewing reports (page 86)

Experiment overview

About experiments

Experiments are used to define and refine the parameters for the sorting operation. An experiment is created for one or more collection devices and associates the settings used during sorting with the recorded data files and saved report. A report is saved for each sort.

Experiment parameters are defined by filling in the data fields or making selections on each tabbed workspace. 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, the base experiment will still carry over.

Experiments are saved and displayed in a list in the Experiment workspace. Saved experiments can be opened and updated as needed. Once data has been recorded, the experiment parameters cannot change. If you want to use different parameters, then a new experiment can be created by using the experiment as a template.

Creating an experiment

Introduction

Experiments can be created using a blank experiment or an experiment template. Available experiment templates are listed when you select New Experiment.

If using a blank experiment, use the tabbed workspaces to define the experiment parameters.

- Design experiment
- View data
- Set up sort
- Sort
- View reports

Creating an experiment

To create an experiment:

1. Select **Experiments** on the left navigation bar.

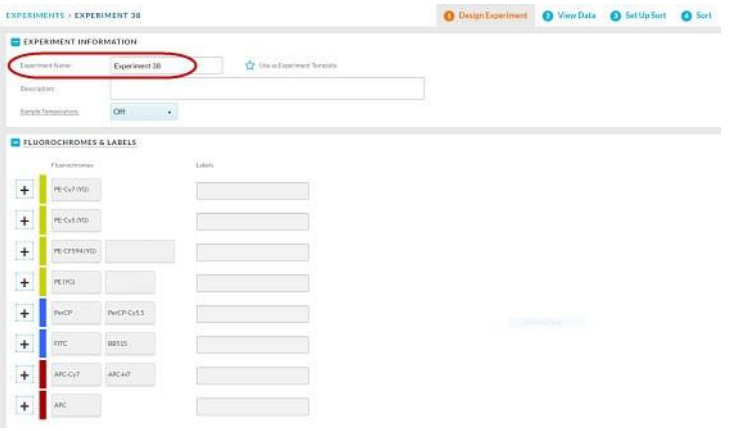
   The experiments page opens with options on how to create the experiment.

2. Click **+ New Experiment**.

   The new experiment dialog opens with the option to start with a blank experiment or with an experiment template if there are existing experiments. The experiment list indicates whether a specific experiment has index sort data available to view.

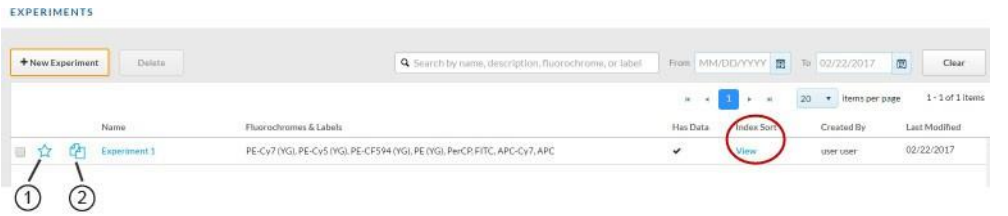
3. Select **Blank Experiment**.

   The new experiment screen opens on the Design Experiment tab with a generic experiment name. Experiment names are automatically generated but can be changed by filling in the Experiment Name field.



Starting with an existing experiment

You can create a new experiment from an existing experiment using one of the following options.



Experiment template option (1)

- Use the experiment template option if you plan to reuse a previously created experiment. An experiment template retains the setup and system settings of the original experiment but does not retain any data or sort report files. When an experiment template is used to create an experiment, voltage settings will be updated based on the last CS&T setup to maintain the optimal setup for your sample.

  Note: Templates are available only to the account in which they were created.

Duplicate experiment without data (2)

- Use the duplicate experiment without data option to create a new experiment that contains all the setup and system settings of the saved experiment without any data. When the duplicate without data option is used to create an experiment, voltage settings will be updated based on the last CS&T setup to maintain the optimal setup for your sample.

In both cases:

  – You can select an experiment that has an index sort (red circle).

  – Compensation values are transferred from the existing experiment or template to the new experiment.

Designing an experiment

Introduction

Designing the experiment entails defining the parameters of the experiment, such as the name, sample input temperature, and fluorochromes being used. The system displays a list of default fluorochromes. Some of the default fluorochromes have normalized spillover values from running BD FC beads. If you would like to maintain those values, see the Update Compensation Standards workflow on the Cytometer page.

Some default fluorochromes might not have normalized spillover values and would need you to run controls. You can add new fluorochromes and create compensation values by running new controls.

Procedure

To design the experiment:

1. Follow the prompts on the screen and fill in the data fields as needed.
2. Enter a name in the **Experiment Name** field.
3. (Optional) Select the **Use as an experiment template** checkbox**,** if you want to reuse this experiment multiple times.
4. (Optional) Select **Sample Temperature** to run the experiment at a defined sample temperature.

   This controls the sample input temperature and not the temperature of the sorted samples.
5. Select one or more fluorochromes from the list or click the plus sign (+) beside any fluorochrome to add a new user defined fluorochrome to that row.

   Only one fluorochrome can be selected from a row. Each row of fluorochromes represents one available detector channel in the optical configuration. Additional user defined fluorochromes that are added to a specific detector channel are indicated with an asterisk. User defined fluorochromes do not have default compensation applied.

6. (Optional) Hover over any of the colored rectangles to display the laser and filter information.

   Note: User-defined fluorochromes are displayed in the table with an asterisk (*).

Defining view data

Introduction

The selections on the View data tab determine the layout of the experiment data. Select or hover over an object to display hidden tools or actions (for example, plot controls and tools and data file options).

Plots are positioned in the order in which they were created. To view more or fewer plots without scrolling, use the following keyboard shortcuts:

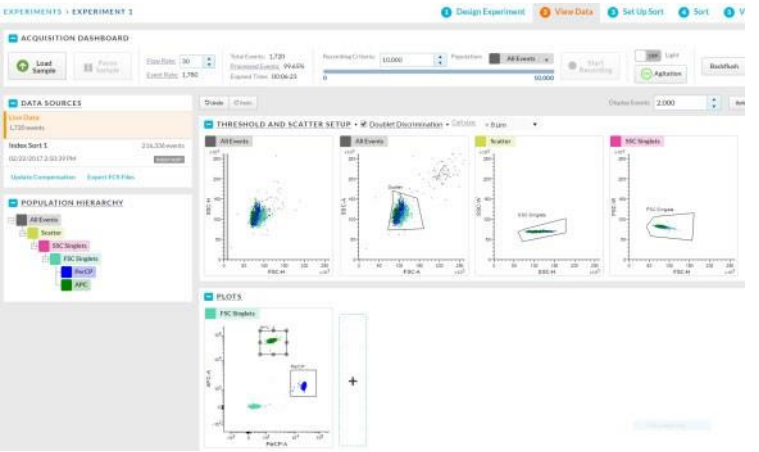
- To zoom in and view fewer plots, press + while holding Ctrl.
- To zoom out and view more plots, press - while holding Ctrl.
- To reset the size back to normal, press 0 while holding Ctrl.

To make a pdf or print the plots, stats, or population hierarchy in the View data page, use Ctrl+P.

Procedure

To define view data:

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



2. Use the **Acquisition Dashboard** panel to load and unload samples, adjust flow rate, agitate samples, run a sample line backflush, control the sample injection chamber light, and record data.

   Samples can be loaded and unloaded as needed. A new FCS file is created when Start Recording is selected and the recording criteria are met, or if the recording is manually stopped.

3. Use the **Data Sources** panel to select data files to view in the plots, export data files, or update compensation.

4. View the Population Hierarchy to determine how the populations relate to each other. The positions of the populations in the hierarchy can be rearranged by dragging and dropping.

   All populations are a subset of the All Events population.

5. Use the plots to set the acquisition threshold, change voltages, and create gates.

There are four plots by default. These are All Events, Scatter, SSC Singlets, and FSC singlets. The first plot is for defining the threshold and the second is for defining the scatter. The third and fourth are doublet discrimination plots to help define the singlets and eliminate doublets from the sort.

You can change the information in the plots or add more plots as needed. Hover over the plot to make the icons display.

a. To set the threshold for data acquisition, hover over the first default plot to make the threshold marker display. Move the marker along the axis to adjust the threshold and remove the low-end debris from the plot. To change the threshold parameter, click the x-axis of the threshold plot and select the desired parameter.

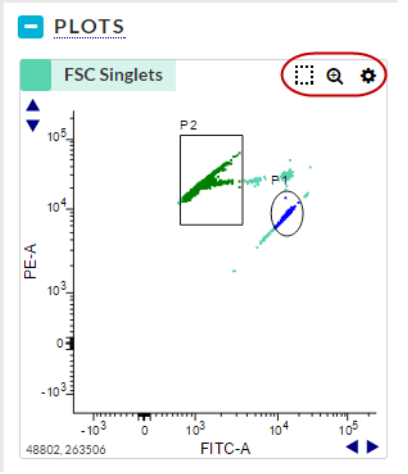
b. To change the voltage of a parameter, hover over a plot to make the voltage sliders display. Move the sliders along the axis to adjust the voltage or use the up-down and left-right arrows on your keyboard to the adjust the voltages of y- and x-axes respectively.

c. Use the Cell size selector to scale the area to the height measurement on the plot.

d. To delete a plot, change the population displayed, or change the type of plot, select the population filter or the plot options (gear) icon, update your desired settings, and click **OK**.

e. To add additional dot plots, a contour map, histogram, or density plot, click the plus **(+)** button on the right side of the Plots panel and select a new plot. Plots can be enlarged by hovering over a plot and clicking the zoom icon.

6. Create, move, modify, and delete gates on the plots as needed. Hover over the plot to make the icons display.



   a. To create a new gate, click the square icon with the dotted line, select a shape from the list, and then draw an area on the plot.

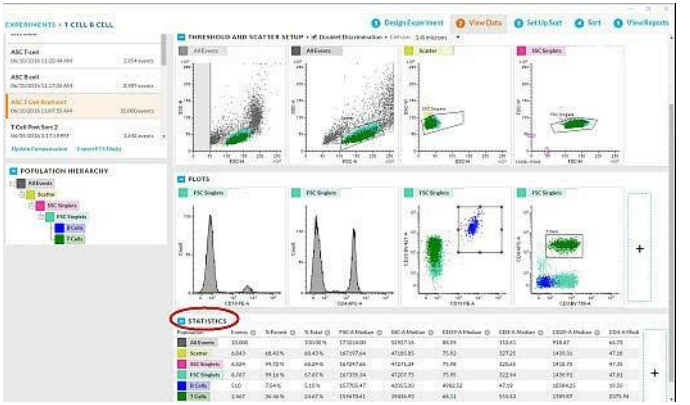
   b. To move the gate, select the gate and drag to a new location.

   c. To modify the gate, select the vertices on the gate and drag to a different location.

   d. To delete a gate, select the population in the population hierarchy that is created by the gate, and select **X** to delete.

7. View the Statistics panel at the bottom of the screen.

The statistics panel displays data for each population in the population hierarchy.



a. To delete, add or modify existing statistics, click the plus **(+)** button on the right side of the Statistics panel.

b. Select the statistics from the **Edit Statistics** dialog and click **OK**.

Calculating compensation

Introduction

BD FACSChorus software calculates compensation using stored normalized spillover values created from fluorescence control (FC) beads which are spectrally matched to a particular dye or fluorochrome, and/or spillover values created from user-defined control samples which are collected in an experiment.

Only samples that are run after compensation has been completed will have compensated data. There will be no compensated data for samples that were run before compensation was completed.

After completing the Update Compensation Standards workflow, then samples that are run in a new experiment will have compensated data. There will be no change to existing experiments.

If the Update Compensation workflow is completed in an experiment, then new samples that are run in the experiment will use the updated values. Additionally, the updated values will apply if the experiment is used as a template or duplicated without data.

We recommend updating FC bead compensation every 60 days.

About compensation calculations

BD FACSChorus software can calculate compensation using stored FC bead spillover values, user-defined control values, or a combination of stored and new control values.

Using stored FC beads values

Blank experiments or experiments created through a template or duplicate with data function that have no pre-existing experimental compensation controls use normalized spillover values from the last time FC beads were run to calculate compensation. The system compensation values will be applied to all new experiments.

Using user-defined control values

You can use user-defined single-color compensation controls (beads or cells) through the Update Compensation function on the View Data page. When this is done in an experiment, the default FC bead spillover values in the current experiment are overwritten. However, the default FC bead spillover values in other experiments will remain unchanged.

Using a combination of stored FC bead values and user-defined control values

When using user-defined single-color compensation controls (beads or cells) to overwrite the FC bead or existing user-defined spillover values in an experiment, not all fluorochromes need to be updated. To calculate compensation, the system uses the existing FC bead spillover values for the fluorochromes that were not updated, and the new user-defined values for fluorochromes that were updated.

Note: In all cases, if an experiment was created from an existing or experiment template, then all compensation values are transferred to the new experiment.

Before you begin

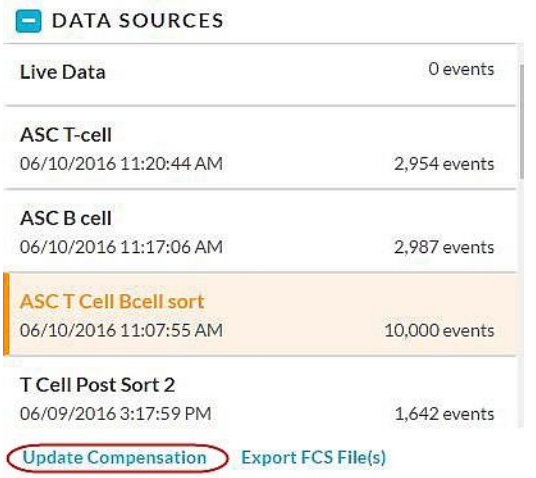
Complete these tasks before you start to update compensation.

- Load a sample in the View Data page.
- Change voltages to have your cells/beads on scale.
- Select **Update compensation** in the Data Sources panel.

Procedure

To update compensation using your own controls for your experiment:

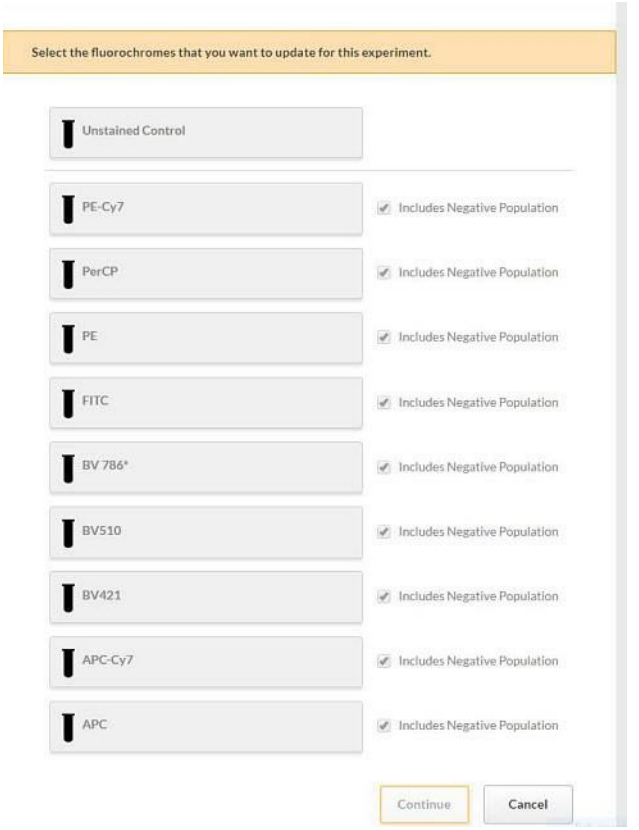
1. Select **Experiment** > **View Data** > **Data Sources** > **Update Compensation**.



   The Update Compensation dialog opens.

2. Select the fluorochromes you want to update.

Only the fluorochromes that were selected in the Design Experiment tab are displayed.



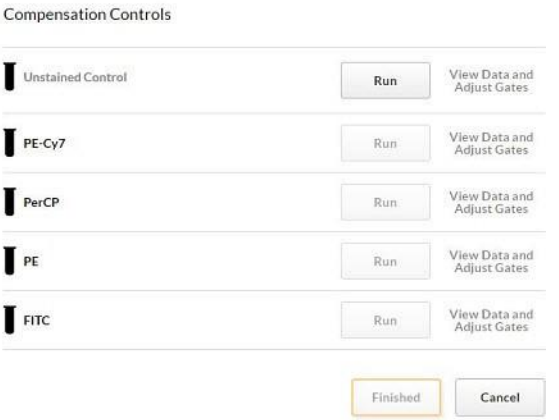
3. Select the parameters you need to update compensation for and then click **Continue**.

   Note the following:

   – If you have a separate unstained control, then run it first before the single-color controls.

   – If you have a separate unstained control, ensure that the appropriate Includes Negative Population boxes are cleared, then run the unstained control first before the single-color controls.

- If your single-color controls have stained and unstained populations, then skip running the unstained control. Ensure that the appropriate Includes Negative Population boxes are selected.
- The selected tubes are displayed on the next tab.



4. Load the appropriate tube, select the tube in the dialog, and then click **Run**. Adjust gates as needed.
5. Repeat step 4 for each tube.
6. Follow the prompts on the screen until all of the tubes are done.
7. Adjust gates as needed after running all of the controls before compensation is calculated and applied.
8. Click **Finished** when you are done to apply the compensation.

   When the tubes are done, the system displays the list of tubes in the Data Sources panel.
9. Continue setting up the rest of the experiment parameters.

Updating compensation standards

Introduction

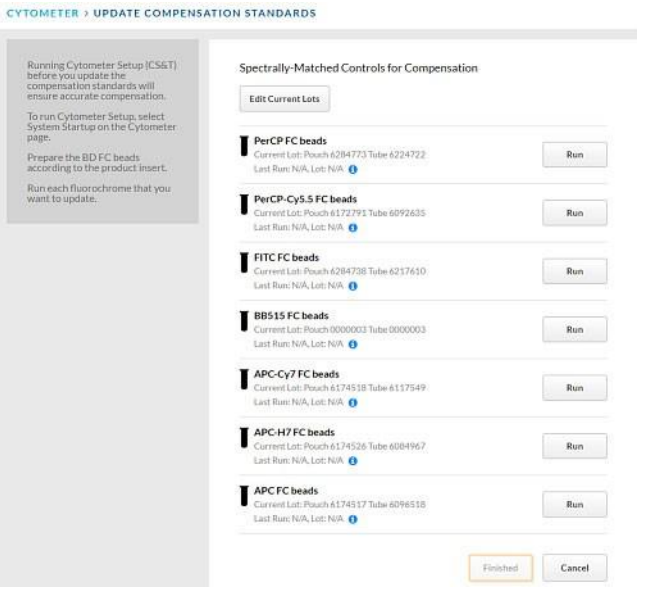
Updates to the standard fluorochrome spectral references for compensation are needed for calculating spillover values at different voltages and setting default spillover values in experiments. Run this procedure with BD FC beads every 60 days to ensure accuracy.

Procedure

To update the system compensation standards:

1. Click **Cytometer** on the navigation pane.
2. Select **Update Compensation Standards**.

The Spectrally-Matched Controls for Compensation dialog opens. The blue icons indicate the values that are older than 60 days.



The system displays the lot numbers for the pouch and tube for reference.

3. Verify that the FC bead lot number matches the FC beads you are going to use.
4. (Optional) If needed, edit the lot numbers to select from the available lots with associated bead lot files to ensure accurate compensation calculations.

   If the lot is not shown, the FC bead lot files may be updated by going to the BD Biosciences website.
5. (Optional) To update the available BD FC bead lots, visit the BD Biosciences website and download the BD FACSMelody FC bead lot installer from the BD FACSMelody Resources page. You need administrative privileges to run the installer.
6. Prepare the BD FC beads according to the product insert.
7. Follow the prompts on the screen to run each fluorochrome.
8. Click **Finished** when you are done.

Viewing the compensation matrix

To view the compensation matrix or the PMT voltage values for your experiment, go to the sort report. If the sort report for that experiment does not exist, perform a sort to display the values.

Setting up sorting

Introduction

The selections on the Set Up Sort tab determine which populations in the sample will be sorted.

Procedure

To set up the sort operation:

1. Click the **Set Up Sort** tab.
2. Select the details for the sort operation, such as:
   - **Format**, which refers to the collection device (tube, plate, or slide)

- **Volume**, which refers to the volume of the collection device
- **Sort Mode**, with the following options:

Sort mode	Description
Yield	This is used to obtain a high yield of the cell of interest, but not necessarily a pure population of the cells. In this case, a droplet containing two cells will be sorted as long as one of them is a cell of interest. More than one cell could be sorted into a single well with this mode.
Purity	This is used to obtain a highly purified cell sample. In this case, a droplet containing two cells will be sorted only if both cells are of interest. Nearly all of the cells in the sorted sample will be the cell of interest. More than one cell could be sorted into a single well using this mode.
Single cell	This is used to obtain a single cell in each well, containing only a defined cell of interest.

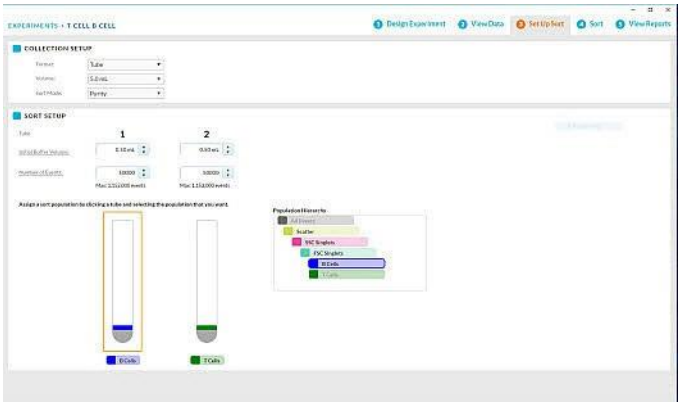
3. (Optional) Select **Enable Index Sort** to perform an index sort on plates or slides.

   Index Sort allows sorting of up to 100 events per well or location in plates or slides using single cell sort mode. A recording is automatically made of the entire sort so that the data is available for post-sort analysis. Select the Index Sort function only when there is sufficient free hard drive space.

4. In the lower panel, define how the population will be sorted.

   - If using tubes, select the tube and then select the populations of interest from the population hierarchy.

The tube assumes the color of the selected population.



- If using a plate or slide, select the wells of the plate or slide and then select the populations of interest from the population hierarchy. The hierarchy can have a maximum of eight levels.

5. Select the initial buffer volume that will be added to each tube or well before sorting.

   You may specify a buffer volume of up to half the volume of the collection device.

6. Select the number of events to sort into each tube or well. The index sort has a maximum number of 100 events/well.

This information will display on the screen in the tubes and will display if you hover over the well.

Selecting wells

To select specific wells:

- To select all wells in the plate, click the **Select All** button, or you may select the circle in the upper-left-hand corner of the plate or slide to select or clear all of the wells.
- To select a row or column, click the letter or number at the beginning of the row or column, respectively.
- To select contiguous wells, select the white space and drag a box around the required wells.
- To select or clear non-contiguous wells, **Ctrl+click** the required wells.

The screen indicates the location of the selected populations.

Loading collection devices

About this topic

The BD FACSMelody system supports the following sort collection devices:

- 1.5- and 2.0-mL tubes
- 5.0-mL tube

The following sort devices are available when the optional automated stage is installed:

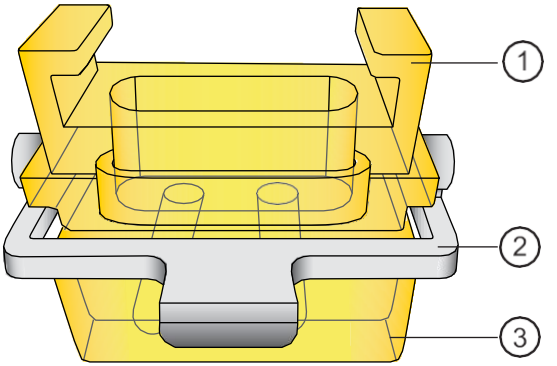
- 6-, 24-, 48-, 96-, and 384-well plates
- 96-well PCR tube strip
- Microscope slide: 27 wells (3 x 9 grid)

Caution! All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning cytometer surfaces. Wear suitable protective clothing, eyewear, and gloves.

Loading tubes

There is one holder for the 1.5- and 2.0-mL tubes and another for the 5-mL tubes. However, the overall construction and handling of the holders is the same.

Each holder snaps together with an adapter to attach to the instrument. The adapter snaps on top of the tube holder.

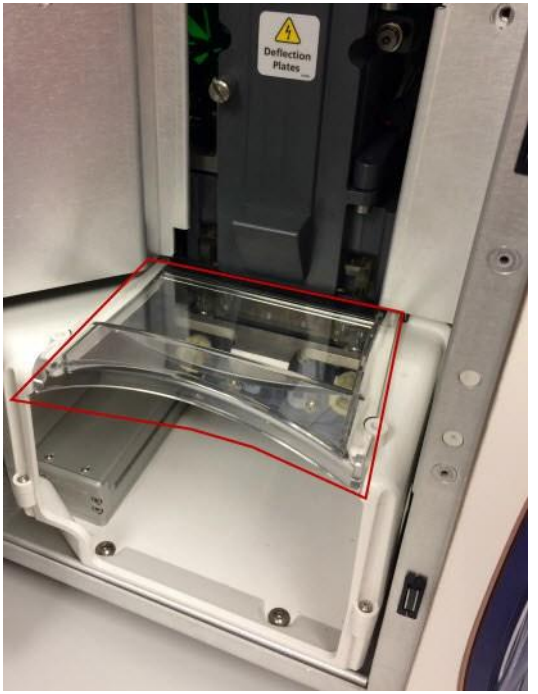


No.	Description
1	Adapter
2	Handle
3	Tube holder

To load the tube holder:

1. Insert the collection tubes into the appropriate tube holder.
2. Open the sort collection chamber door.
3. Use the metal handle to guide the adapter into place.
4. Attach the adapter to the instrument, below the sort block.

5. Install the aerosol shield (red polygon) to keep any aerosols contained in the sort collection chamber.



6. Close the sort collection chamber door.

Installing the splash shield

The splash shield must be in place before you can sort onto a slide or plate.

To install the splash shield:

1. Remove the adapter for the tube sort.

2. Attach the splash shield to the underside of the sort box, pushing it back until it clicks into place.

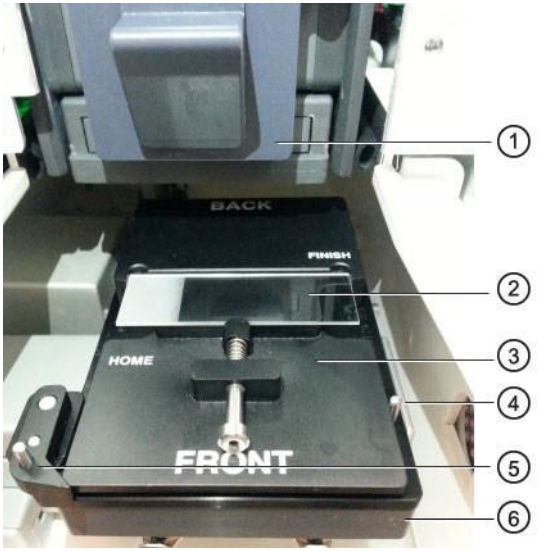
Loading microscope slides on the automated sort stage

The sort stage consists of a flat platform, guiding pins, and a locking lever. The pins on the right side and back are used to position the slide holder correctly onto the platform. The locking lever on the front left holds the slide holder in the desired location.

To load the slide into the instrument:

1. Verify that the splash shield is in place.
2. Attach the slide to the slide holder.
3. Select **Eject** in the software to bring the stage forward.
4. Open the sort collection chamber door.

5. Place the slide holder on the stage, touching the guiding pins in the back and the locking lever in the front.



No.	Description
1	Sort block
2	Slide
3	Slide holder
4	Guiding pin
5	Locking lever
6	Stage platform

6. Install the aerosol shield to keep any aerosols contained in the sort collection chamber.

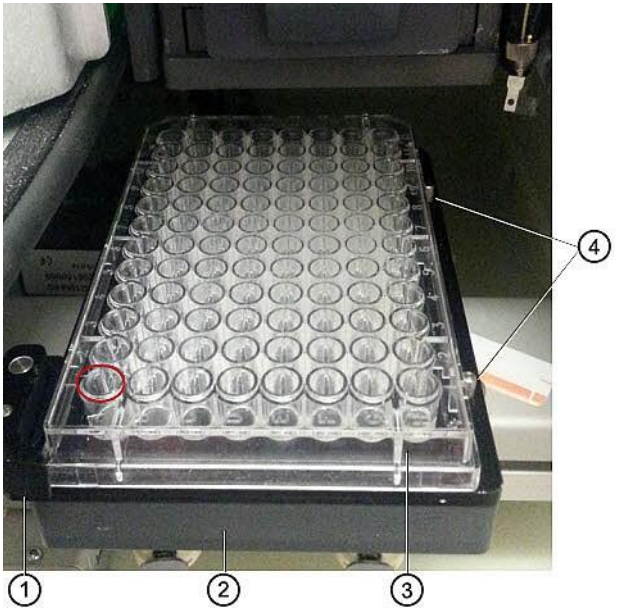
7. Close the sort collection chamber door.

Loading plates on the automated stage

The sort stage consists of a flat platform, guiding pins at the back and sides, and a locking lever. The pins on the right side and back are used to position the plate correctly onto the platform. The locking lever on the front left holds the plate in the desired location.

To load plates onto the automated stage:

1. Verify that the splash shield is in place.
2. Select **Eject** in the software to bring the stage forward.
3. Open the sort collection chamber door.
4. Load the plate so that well A1 (red circle) is on the front left of the stage platform. Verify that the plate is touching the guiding pins at the back and the locking lever is set.



No.	Description
1	Locking lever
2	Stage platform
3	96-well plate
4	Guiding pins

5. Install the aerosol shield to keep any aerosols contained in the sort collection chamber.
6. Close the sort collection chamber door.

Sorting

Introduction

Samples are sorted based on the selections in the Set Up Sort tab. Samples can be sorted into tubes, plates, or onto slides. However, index sorting uses only plates and slides, no tubes.

Index sorting allows you to sort single cells into a plate or onto a slide and indexes the well or slide location to the collected parameters for that cell. You can index sort up to 100 events per well or location in a plate or slide using the single cell sort mode.

On the Sort tab, only plots that contain sort gates are shown in the Sort Population Plots section.

Keep the sort collection chamber door closed when sorting. The door keeps the chamber free of dust and other airborne particles, and seals the chamber during aerosol evacuation for systems equipped with the AMO.

Procedure

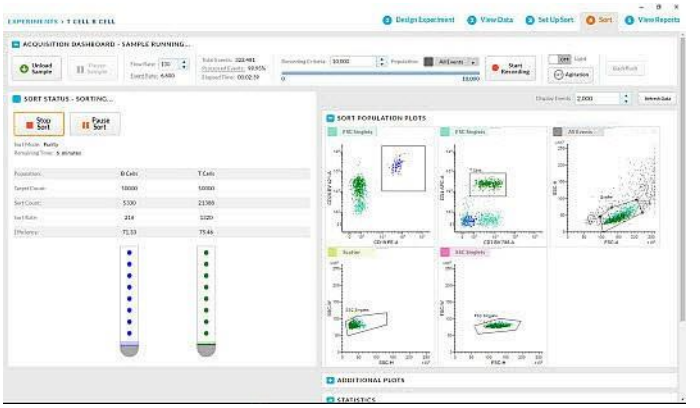
To run the sort operation:

1. Click the **Sort** tab.
2. Click **Load Sample**.
3. (Optional) Click **Start recording**.

   You can also use the Acquisition Dashboard panel to set the event rate, flow rate, light, agitation, recording criteria, and run a backflush.
4. Click **Start Sort**.

   The selections of collection device, number of events, sort mode, and sort populations displayed in the Sort Status panel reflect the selections made in the View Data and Set Up Sort tabs.

When performing a tube sort, the following is an example of what you might see:



When performing a plate sort, the following is an example of what you might see. The well being sorted into has an orange circle.

5. Refine the gates in the plots in the **Sort Population Plots** panel. These are the plots that were selected in the View Data tab. Only plots that contain sort gates are shown in the Sort Population plots.

6. (Optional) Additional plots with gates other than the sort gates are shown in the Additional plots panel.

7. View the statistics and edit as needed.

Using multiple collection devices

An experiment can contain multiple collection devices. If using multiple collection devices, follow this guidance:

- If the additional collection devices use the same sort parameters:
  a. Replace the sorted collection device with a new one of the same type (plate, tube, or slide).
  b. Click **Start Sort.**

  The system begins sorting into the new collection device using the previous settings.
- If the additional collection devices use different sort parameters:
  a. Replace the sorted collection device with a new one of a different type.
  b. Click **Eject** or **Retract** to move the stage forward or back respectively.
  c. Click **Set Up Sort** and modify the selections as needed**.**
  d. Click the **Sort** tab and click **Start Sort.**

     The system starts a new sort into the new collection devices using the new sort selections.

     When the sort has completed successfully, a Sort Completed dialog displays with an option to name the sort. The name will be used to reference the Sort Report and, if selected, the Index Sort recording associated with the sort.

Index sorting

Introduction

Index sorting allows you to sort cells onto a plate or slide and indexes the well or slide location to the collected parameters for that cell. You can use this function to ensure that a sorted cell with a specific phenotype has been sorted. Index sorting is useful in

characterizing subpopulations of phenotypically similar events using post-sort genetic, chemical, and/or metabolic applications.

Index Sort allows the sorting of up to 100 events per well or location in plates or slides using single-cell sort mode. When performing an Index Sort, a recording is automatically made of the entire sort so that the data is available for post-sort analysis. Select the Index Sort function only when there is sufficient free hard drive space.

Setting up for index sorting

Index sorting uses the same steps as required in setting up for a plate or slide sort.

Review the instructions in these sections:

Designing an experiment (page 59)

Defining view data (page 60)

Calculating compensation (page 64)

Sorting (page 79)

Procedure

To perform an Index Sort:

1. Create a new experiment by following the instructions on the Design Experiment tab.
2. Define the experiment parameters on the View Data tab.
3. On the **Setup Sort** tab, under **Collection Setup**:
   - Set **Format** to plate or slide.
   - Set **Number of wells** in the collection device. The maximum number of events per well is 100.

– Select the **Enable Index Sort** checkbox.

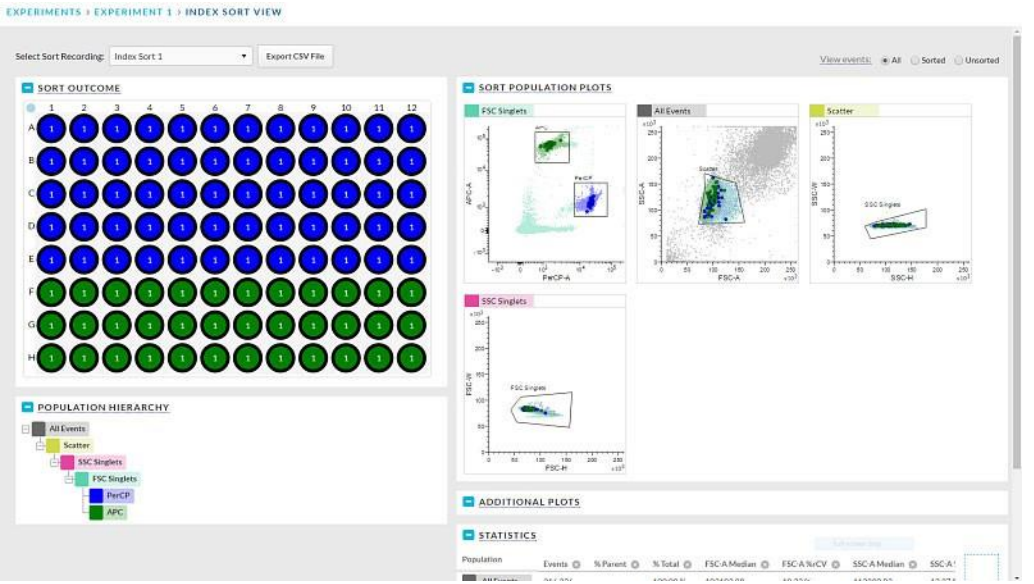
4. Select the fields and enter values on the **Setup Sort** tab to define the wells.

5. Select the **Sort** tab, and follow the instructions to start the sort operation.

   During the index sort, the recording criteria will be grayed out. The system will automatically record the data and the progress bar will match the sort progress on the plate or slide.

   An FCS file is available in the View Data tab for the recording that was made during the index sort.

   You can rename the sort record after the sort operation is complete. The new name is given to the sort recording and will display on the sort report.



Reviewing index sort data

Introduction

Use the Index Sort View page to review the Index Sort data. The Index Sort View page filters events for display into All Events, Sorted, and Unsorted events. The statistics displayed in the view are for the entire plate.

Only plots and gates that were created prior to the index sort are included in the Index Sort View page. If additional plots are desired, a new index sort must be performed to display the additional plots in the Index Sort View. The population hierarchy view is for display only and is not interactive.

The CSV file provides all parameter data for each sorted event. The CSV file format may be automatically imported into the BD Genomics Pinpoint application.

Procedure

To review the Index Sort data:

1. Select the Index Sort experiment of interest from the **Experiment Management** page and click **View** in the **Index Sort** column or select the index icon (grid of nine black squares) in the View Data page.

   The Index Sort view page opens. You can perform several actions on this page.

2. Click **All events**, **Sorted**, or **Unsorted** to filter out the desired events.

3. When **All events** or **Sorted** is selected, you can:

   a. Select a well in the plate and see the corresponding events for that well highlighted in the plots.

   b. Select events in the plot and see the corresponding well for those events highlighted.

      The black outline indicates that all events in the selection region falls within those wells; the gray outline indicates that a subset of the selected events were sorted to those wells.

   c. View the statistics for all wells in the plate.

   d. Add new statistic by clicking the (+) button.

4. Use **Unsorted f**or statistical purposes only.

Viewing reports

Introduction

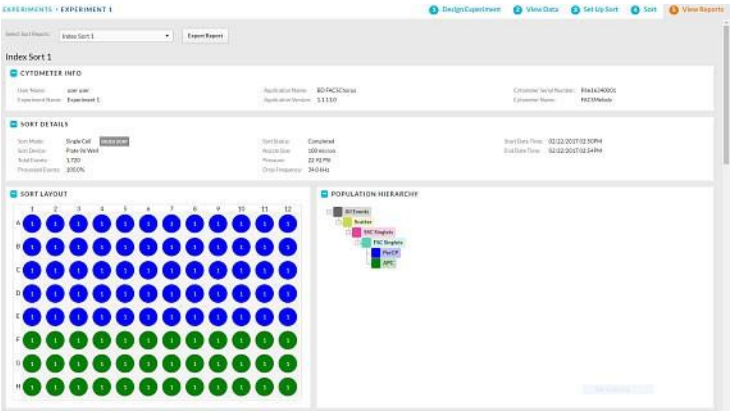
A sort report summarizing the results of the sort is displayed on the View Reports tab when sorting is complete. There is no data if the sort is not complete. A sort is considered complete if the sorting criteria are met, the sort is manually stopped, or the sort is stopped automatically due to a clog, empty sample tube, or other similar items.You can export the report from this tab.

Procedure

To view and export the report:

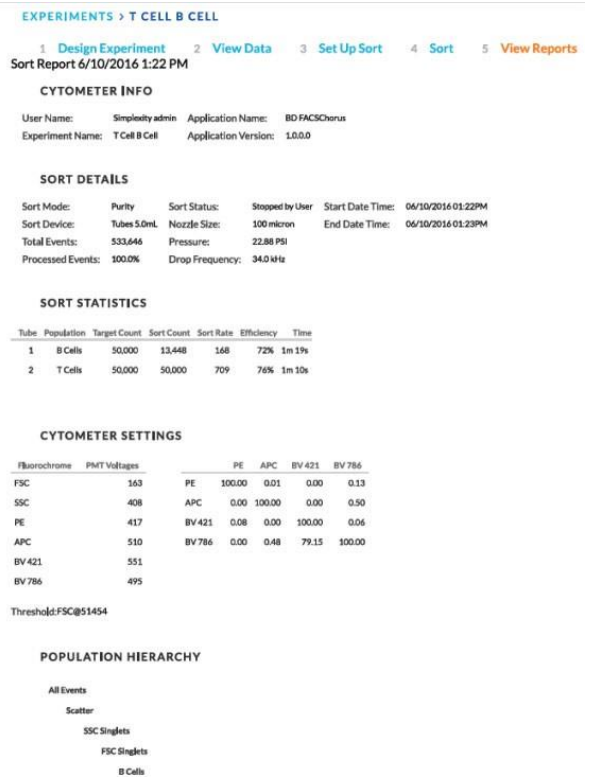
1. Click the **View Reports** tab.

   A report is made for each sort operation. For example, if three plates or three pairs of tubes were used in an experiment, then three reports will be generated.



2. Select the report to export from the **Select Sort Report** list.

3. Click **Export Report** > **Location** to export a PDF of the report to the selected location.



EXPERIMENTS > T CELL B CELL

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

Sort Report 6/10/2016 1:22 PM

CYTOMETER INFO

User Name:	Simplexity admin	Application Name:	BD FACSChorus
Experiment Name:	T Cell B Cell	Application Version:	1.0.0.0

SORT DETAILS

Sort Mode:	Purity	Sort Status:	Stopped by User	Start Date Time:	06/10/2016 01:22PM
Sort Device:	Tubes 5.0mL	Nozzle Size:	100 micron	End Date Time:	06/10/2016 01:23PM
Total Events:	533,646	Pressure:	22.88 PSI		
Processed Events:	100.0%	Drop Frequency:	34.0 kHz		

SORT STATISTICS

Tube	Population	Target Count	Sort Count	Sort Rate	Efficiency	Time
1	B Cells	50,000	13,448	168	72%	1m 19s
2	T Cells	50,000	50,000	709	76%	1m 10s

CYTOMETER SETTINGS

Fluorochrome	PMT Voltages
FSC	163
SSC	408
PE	417
APC	510
BV 421	551
BV 786	495

	PE	APC	BV 421	BV 786
PE	100.00	0.01	0.00	0.13
APC	0.00	100.00	0.00	0.50
BV 421	0.08	0.00	100.00	0.06
BV 786	0.00	0.48	79.15	100.00

Threshold:FSC@51454

POPULATION HIERARCHY

All Events
Scatter
SSC Singlets
FSC Singlets
B Cells

4. Navigate to the **View Data** tab and click **Export All FCS files** to export the saved data files.

5. Open the PDF report and click **Print** to print the PDF report. Individual recordings can also be exported as single FCS files using the export icon in the toolbar for each recording that appears when hovering.

5

System options

This chapter covers the following topics:

- Overview (page 90)
- Using the sample temperature control (page 90)
- Working with the aerosol management option (page 95)
- Working with the biological safety cabinet (page 96)
- Using BD Assurity Linc software (page 99)

Overview

Introduction

There are several options that are available with the BD FACSMelody cell sorter. These options include:

- Sample temperature control
- Aerosol management option (AMO)
- Biological safety cabinet (BSC)
- Remote diagnostics with BD Assurity Linc
- Plate sorting
- Air compressor
- Table for the instrument and workstation

Using the sample temperature control

Introduction

The BD™ sample temperature control option can be used to control the temperature of sorted samples in the BD FACSMelody cell sorter. The BD sample temperature control option includes a recirculating water bath and specially designed collection tube holders with ports for recirculating water.

To ensure that the sample collection device is at the correct temperature, start the water bath (115-V and 110-V models) at least 90 minutes before you start sorting.

Any instrument surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing, eyewear, and gloves.

Keep the sort block door and the sort collection chamber door closed during sorting.

Setting up the water bath

To set up the water bath:

1. Remove the threaded plug from the output port on the water bath.
2. Ensure that the drain cock on the back of the water bath is closed by turning it fully clockwise.
3. Set the pump outflow to maximum by turning the knob fully counterclockwise.

   Remove the top cover to access the knob, which is located inside the water bath toward the back. See the operating instructions supplied with the water bath for additional details on this process. This is referred to as position 1.
4. Connect the clear tubing end of the insulated hoses to the input and output ports on the water bath. Slide the tubing over the hose barbs and twist gently while installing to get the tubing completely over the barbs.
5. Connect the insulated hoses from the recirculating water bath to the ports on the right side of the cell sorter base.

   Note: Because the water flow direction is controlled by the water bath pump, the ports on the cell sorter base are bi-directional. The input and output hoses from the water bath can be connected to either port on the cell sorter base.
6. Fill the water bath with distilled water containing 0.1 g/L of sodium carbonate.

   Sodium carbonate helps reduce corrosion. See the water bath manufacturer's documentation for fill levels and other setup information.

   Note: We do not recommend using ethylene glycol (antifreeze) in the water bath.
7. Plug in the water bath power cord.

   Note: Do not start the water bath until after you have connected the recirculating water tubing, as described in the following sections.

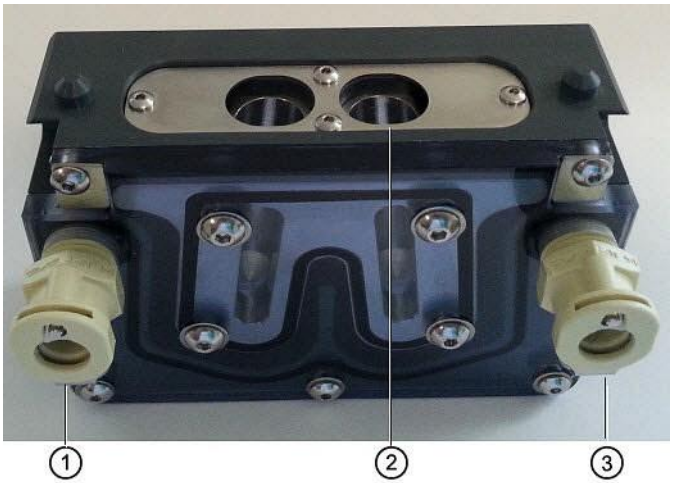
Setting up the tube holder

To install the temperature control tube holder:

1. Place collection tubes in the temperature control tube holder.

   Tube holders are available for 5-mL, 1.5-, and 2.0-mL Eppendorf tubes for two-way sorting.

2. Attach the recirculating water tubing to the tube holder. Push the tubing into the port until the tubing snaps into place.



   Attach the input tubing to the one port (1or 3) and the output tubing to the other port (3 or 1).

3. Place the collection tube in the tube holder (2).

   Any instrument surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing, eyewear, and gloves.

   If you need to remove the tubing, push in the metal locking tab as you pull the tubing out of the port.

4. Attach the tube holder to the adapter.

This is the same adapter that is used with the non-temperature controlled tube holders.

5. Install the tube holder on the instrument.

   a. Remove the current tube holder (if one is installed), and slide the temperature controlled tube holder into the slotted fittings below the sort aspirator drawer.

   b. Push the tube holder all the way in.

6. Close the sort collection chamber door and start the water bath.

Setting up the stage

This section describes how to attach the recirculating water tubing to the automated stage.

To set up the stage:

1. Install the splash shield below the aspirator drawer.

Any instrument surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing, eyewear, and gloves.

   a. Remove the tube holder, if one is installed.

   b. Insert the splash shield into the slotted fittings below the sort aspirator drawer. Push the splash shield all the way in.

2. In the **Set Up Sort** tab, click the **Eject** button to bring the stage to the front.

a. Open an experiment, if one is not already open, and create a sort layout for any of the plates.

b. In the **Sort Layout** view, click the **Eject** button to move the stage to the front of the sort collection chamber.

3. Attach the recirculating water tubing to the stage. Push the tubing into the port until the tubing snaps into place.

   Attach the input tubing to one port on the left side of the stage, and the output tubing to the port on the right side.

   If you need to remove the tubing, push in the metal locking tab as you pull the tubing out of the port.

4. Install the appropriate collection device on the stage.

5. Close the sort collection chamber door and start the water bath.

   Note: To ensure that the sample collection device is at the correct temperature, start the water bath (115-V and 110-V models) at least 90 minutes before you start sorting.

Starting the water bath

To start the water bath:

1. Switch on the main power on the water bath control panel.

2. Use the up or down arrow keys to set the temperature.

   Note: To achieve the required sample temperature, you will need to set the water bath temperature slightly higher or lower. These settings might need adjustment depending on the ambient temperature in your laboratory. We recommend that you calibrate the water bath for your operating environment.

Required Sample Temp (°C)	Water Bath Setting (°C)
4	2
37	37.5
42	43.2

3. Wait at least 90 minutes to allow the recirculating water to reach the required temperature.

Working with the aerosol management option

Introduction

The aerosol management option (AMO) uses a vacuum source to rapidly evacuate aerosolized particles through an ultra-low penetration air (ULPA) filter during routine sorting or acquisition. This unit evacuates aerosols from the sort chamber, preventing the aerosols from being circulated back through the chamber. The AMO is intended as a standalone option. The biological safety cabinet (BSC) option comes equipped with a built-in aerosol management system. The AMO has settings for evacuation mode: 20%, 40%, 60%, 80%, and 100%.

Using the AMO

To use the AMO:

The following are some guidelines for using the AMO with the BD FACSMelody system:

1. Turn on the AMO.
2. Operate the AMO at 20% during sorting.
3. Operate the AMO at 40% or higher for 1 minute after completing a sort or clearing a clog, and before opening the flow cell access and sort chamber doors.
4. Change the filter according to the manufacturer's instructions.
5. Turn off the AMO when you have completed your work.

Working with the biological safety cabinet

Introduction

The biological safety cabinet (BSC) is a custom Baker SterilGARD® 403A-HE Optimax Class II clean air and containment enclosure for the BD FACSMelody Cell Sorter. The unit is designed to protect laboratory personnel from exposure to materials in the BSC, and also protect the materials in the BSC from external contaminants.

Some features of the BSC include vertical laminar airflow with a front access opening, and supply and exhaust air with separate high efficiency particulate air (HEPA) filters. The BSC can be configured to vent to the room or to a house exhaust system.

See Baker's SG 403A-HE Optimax Operator's Manual for details on the structure and operation of the cabinet.

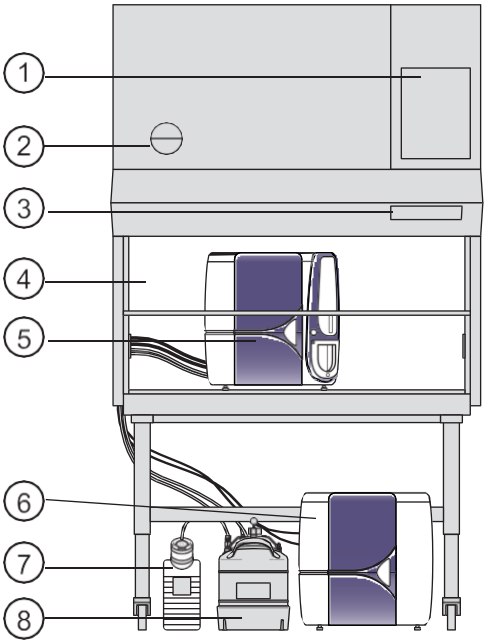
Recommendations

We recommend that you:

- Have the BSC certified by a third party for air flow and for compliance to NSF/ANSI Standard 49 or any other official standard(s) applicable to biological safety cabinets in your country.
- Schedule routine safety audits of the cell sorter and the BSC by a third party safety officer.
- For more information about BSC use, see Appendix A-Primary containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets. In: *Biosafety in Microbiological and Biomedical Laboratories.* Rockville, MD: US Dept of Health and Human Services; 2009. HHS publication (CDC) 21-1112.
- Review Schmid I, Nicholson JKA, Giorgi JV, et al. Biosafety guidelines for sorting of unfixed cells. *Cytometry.* 1997;28:99-117.

BD FACSMelody cell sorter layout in the BSC

The following diagram indicates the layout of the BD FACSMelody cell sorter in the BSC.



No.	Description
1	Inspection certificate
2	Gauge indicating the air flow
3	Power, lights, alarm, aerosol management and blower controls with indicator lights
4	Sash that should remain at the at the correct level (10 inches) for proper operation
5	BD FACSMelody cell sorter

No.	Description
6	Electronics box
7	Waste tank
8	Sheath tank

Using the BD FACSMelody cell sorter in the BSC

Using the BD FACSMelody cell sorter in the BSC is very similar to using it without the BSC. The main change is maintenance of the environment in the BSC. The BSC comes equipped with a built-in AMO. The AMO hose is attached to the back of the instrument, and the AMO is controlled on the BSC console.

To work with the BSC:

1. Turn on the blower and keep it on.
2. Set the sash to the correct height (10 in.).
3. Verify that the laminar air flow is working.
4. Turn on the AMO to Low.
5. Verify that there are no alarms.
6. Operate the instrument per the instructions.
7. Operate the AMO at High for 1 minute after completing a sort or clearing a clog, and before opening the flow cell access and sort chamber doors.

Using BD Assurity Linc software

Introduction

This topic describes how to establish a remote session with a BD technical representative using BD Assurity Linc software.

About BD Assurity Linc software

BD Assurity Linc is a highly secure remote systems management service that connects BD instruments to BD technical support personnel. Using the BD Assurity Linc Agent, BD support personnel can securely access your workstation through an enterprise server and diagnose problems remotely. You must grant access to the instrument to enable this remote diagnostics feature.

Description of functionality

BD Assurity Linc can continually monitor the health of your instrument and automatically communicate any changes to the BD Technical Support server. When problems or questions arise, key data is already available for diagnosis by BD, which can speed up troubleshooting efforts.

With your explicit authorization, the BD support representative can see what you see on-screen, and in many cases, can make adjustments or suggestions that prevent downtime and the need for a service call.

When an on-site visit is needed from a BD Field Service or Technical Application Support engineer, the system logs and alarms can be checked before they leave the BD office, helping to ensure that the right personnel and the right parts are dispatched to your site.

Procedure

To grant access to a BD technical support representative:

1. Ensure that your workstation is connected to the internet.

2. Contact your local BD technical support representative.

    If a remote session is required, the BD representative will initiate a session through a secure link.

A dialog opens once the connection is established.

3. Acknowledge the request.

The BD representative can now assist you.

6

Maintenance

This chapter covers the following topics:

- Maintenance overview (page 103)
- Stopping the stream (page 106)
- Lubricating the sample injection chamber O-ring (page 108)
- Precautions for handling nozzles (page 110)
- Changing the nozzle (page 111)
- Cleaning the sort nozzle (page 112)
- Cleaning the closed-loop nozzle (page 113)
- Replacing the sort nozzle seal temporarily (page 114)
- Aligning the waste aspirator drawer to the stream (page 115)
- Cleaning the flow cell (page 118)
- Cleaning the optical filters (page 119)
- Cleaning the Accudrop laser window and the lower camera window (page 120)
- Cleaning the strobe lens window and upper camera window (page 122)
- Cleaning the deflection plates (page 124)
- Removing or installing the FSC neutral density filter (page 125)
- Emptying the waste tank (page 126)
- Filling the sheath tank (page 128)

- Changing the fluid filter (page 131)
- Purging the sheath filter (page 133)
- Backflushing the sample line (page 133)
- Replacing the sample line (page 134)
- Replacing the sample line filter (page 140)
- Removing the sheath probe (page 142)
- Preparing for aseptic sort (page 144)
- Preparing new fluid filters (page 146)

Maintenance overview

About maintenance tasks

To preserve the reliability of the cell sorter, basic preventive maintenance procedures must be performed.

The following table shows the maintenance procedures and when they should be performed.

Category	Maintenance task	When to perform
External surfaces	Cleaning external surfaces (page 106)	As needed
Sample loading area	Lubricating the sample injection chamber O-ring (page 108)	As needed or when sample loading fails
Nozzle and flow cell	Changing the nozzle (page 111)	As needed
	Cleaning the sort nozzle (page 112)	When you see indications of clogging
	Cleaning the closed-loop nozzle (page 113)	When you see indications of salt buildup or clogging
	Replacing the sort nozzle seal temporarily (page 114)	If the seal is lost or damaged and you do not have a replacement nozzle
	Aligning the waste aspirator drawer to the stream (page 115)	If you install a sort nozzle that is new or different from the one that came with the instrument
	Cleaning the flow cell (page 118)	As needed

Category	Maintenance task	When to perform
Optics	Cleaning the optical filters (page 119)	As needed
	Cleaning the Accudrop laser window and the lower camera window (page 120)	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 (page 122)	When smudges appear in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed
	Cleaning the deflection plates (page 124)	When you have trouble viewing the side stream
	Removing or installing the FSC neutral density filter (page 125)	As needed

Category	Maintenance task	When to perform
Fluidics	Emptying the waste tank (page 126)	As indicated by the software and whenever you fill the sheath tank
	Replacing the waste filter cap	Monthly
	Filling the sheath tank (page 128)	Daily or as needed
	Changing the fluid filter (page 131)	Every 3 months or as needed
	Purging the sheath filter (page 133)	After you install a new sheath filter and whenever you observe problems with the stream
	Backflushing the sample line (page 133)	When you observe sample carryover, or after you run samples with adherent cells or dye
	Replacing the sample line (page 134)	Every 4–6 months or when decreased event rates indicate that the sample line might be clogged
	Replacing the sample line filter (page 140)	When decreased event rates indicate that the sample line might be clogged
	Removing the sheath probe (page 142)	As needed, before you autoclave the sheath tank
	Preparing for aseptic sort (page 144)	Before you start an aseptic sort

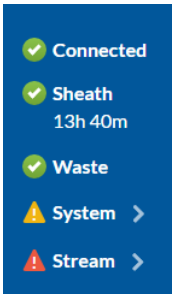
Stopping the stream

Introduction A number of maintenance procedures require the stream to be turned off (stopped). You can stop the stream manually in the Stream View window.

About this task Stopping the stream also turns off the deflection plates if they are on.

Procedure **To stop the stream:**

1. Open the **Stream View** window by clicking the **Stream** status indicator in the navigation bar.



2. In the **Stream View** window, click **Stop Stream**.
3. To restart the stream, load a tube in the **Experiments** workspace using the Acquisition Dashboard on the **View Data** or **Sort** tab.

Cleaning external surfaces

Introduction All cytometer surfaces that have been exposed to sheath fluid should be cleaned to prevent salt buildup.

Before you begin

If the stream is on, turn it off. See Stopping the stream (page 106).

For cleaning the external surfaces of the flow cell cuvette and the closed-loop nozzle, ensure that you know how to remove the nozzle. See Changing the nozzle (page 111).

About this task

The following surfaces should be inspected and cleaned when necessary:

- Deflection plates
- Sample loading port
- Collection devices
- Inside the sort chamber
- Closed-loop nozzle
- Base of the flow cell where the nozzle is inserted
- Inside the sort block

Caution! All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning cytometer surfaces. Wear suitable protective clothing, eyewear, and gloves.

Caution! To prevent shock, turn off the stream (plate voltage) before cleaning on or around the deflection plates. To prevent arcing (sparking), make sure the plates are completely dry before you turn the stream (plate voltage) on.

Procedure **To clean the external surfaces:**

Select any of the following options:

Option	Description
Clean the salt buildup off the surface of the flow cell cuvette	Remove the nozzle and use a cotton swab moistened with water around the opening and the inside of the bottom of the cuvette where the nozzle is inserted.
Clean the salt buildup off the surface of the closed-loop nozzle	Remove the closed-loop nozzle and wipe it thoroughly with water. Ensure that it is dry before you reinstall it.
Clean the salt buildup off the remaining surfaces	Wipe the surfaces with a cloth dampened with water followed by 70% ethanol.
Decontaminate the surfaces	Wipe the surfaces with a cloth dampened with 10% bleach solution followed by DI water.

Lubricating the sample injection chamber O-ring

Introduction The sample injection chamber contains an O-ring at the opening at the bottom of the chamber. You should lubricate this O-ring as needed to maintain the proper operation of the sample injection chamber.

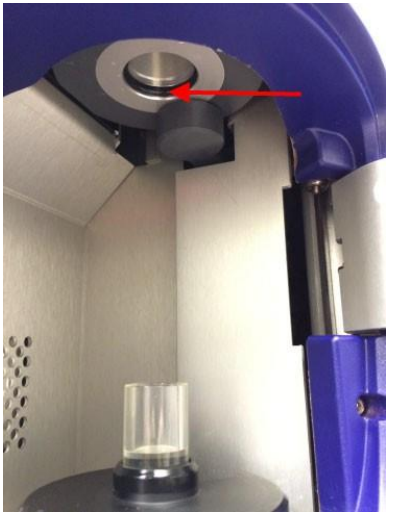
Procedure **To lubricate the sample injection chamber O-ring:**

1. Ensure that the loading port is at the bottom of the sample injection chamber, and then open the sample injection chamber door.

   Ensure that the loading port is empty.

2. On the **Cytometer** page, select **Replace Sample Line Filter**, and then click **Start**. (Do not complete the remaining steps of the wizard.)

3. When the step 1 is completed, reach inside the sample injection chamber and check to see if the O-ring is dry.



4. If the O-ring is dry, apply a small amount of O-ring lubricant to the O-ring.

5. Exit the wizard without completing it by clicking **Cancel**.

Precautions for handling nozzles

In addition to cleaning the nozzles properly, you should follow these precautions when you handle nozzles.

Precaution	Reason
Always use the closed-loop nozzle for cleaning and shutdown procedures.	To keep the flow cell clean and reduce salt buildup and clogs. A clean flow cell provides improved sensitivity and higher performance.
Do not expose nozzles to bleach or detergents for long periods of time. However, you can prepare for the aseptic sort procedure without causing any problems to the O-ring in the nozzle.	To prevent the seal from coming loose and falling out.
Always insert and remove nozzles straight in and straight out.	To reduce wear and tear on the seal.
Always grasp the closed-loop nozzle by the metal nut, not the tubing, wire, or plastic sleeve.	To prevent wear and tear on the tubing and wire. They might become detached with repeated mishandling.
Do not expose nozzles to strong base solutions such as Contrad® 70.	To prevent the seal from coming loose and falling out. Any contact with such solutions might damage the seal.
Do not wipe the surface of the seal with anything.	To prevent damage to the seal that could result in leaking.

Changing the nozzle

Introduction

The sort nozzle is used for sorting, and the closed-loop nozzle is used for cleaning and shutdown procedures. The software prompts you when you need to switch between the nozzles.

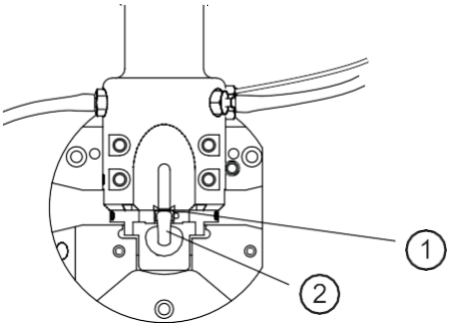
About this task

Caution! All biological specimens and materials coming into contact with biological specimens can transmit potentially fatal disease. Handle nozzles as if they are capable of transmitting infection. Wear suitable protective clothing, eyewear, and gloves.

Procedure

To change the nozzle:

1. Ensure that the stream is turned off, and then open the flow cell access door.
2. Remove the closed-loop or sort nozzle (1) from the flow cell by turning the nozzle-locking lever (2) counterclockwise to the 9:00 position and then pulling the nozzle straight out.



3. Insert the new closed-loop or sort nozzle into the flow cell (with the top side facing up) and push it gently all the way forward until it stops.
4. Turn the nozzle-locking lever clockwise to the 12:00 position.
5. If the nozzle you installed is a sort nozzle that is new or different from the one that came with the instrument, align the

waste aspirator drawer to the stream to ensure proper sort setup. See Aligning the waste aspirator drawer to the stream (page 115).

Cleaning the sort nozzle

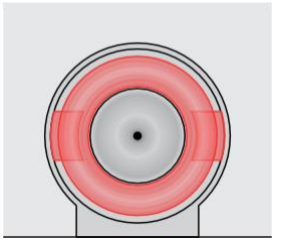
Introduction

You should clean the sort nozzle as needed, or when stream irregularities indicate that the nozzle is clogged.

Before you begin

Ensure that you understand how to change the nozzle. See Changing the nozzle (page 111).

You can check whether the sort nozzle is clogged by examining the opening at the center of the seal area under a microscope. The following illustration shows an unclogged nozzle tip.



About this task

Caution! All biological specimens and materials coming into contact with biological specimens can transmit potentially fatal disease. Handle nozzles as if they are capable of transmitting infection. Wear suitable protective clothing, eyewear, and gloves.

Procedure

To clean the sort nozzle:

1. Remove the sort nozzle from the flow cell.

2. Sonicate the nozzle for approximately 1 minute in a tube containing DI water, ensuring that the nozzle opening is fully submerged. Repeat as needed until the nozzle is clean.

   Note: Do not use bleach, Contrad, or any strong detergents to clean the nozzle.

3. Allow the nozzle to air dry for a few minutes, or dry it gently using lens paper.

4. Reinsert the nozzle into the flow cell.

Cleaning the closed-loop nozzle

Introduction

You should clean the closed-loop nozzle when you see any indications of salt buildup or clogging.

Before you begin

Ensure that you understand how to change the nozzle. See Changing the nozzle (page 111).

Procedure

To clean the closed-loop nozzle:

1. Remove the closed-loop nozzle from the flow cell.

2. Remove salt buildup on the surface of the nozzle by wiping it thoroughly with water and letting it air dry.

3. Clean and remove clogs from the nozzle by doing the following steps:

   a. Remove the nozzle by holding the nut on the side of the nozzle and unscrewing the nozzle.

      Handle the nut with the connected wire and tubing carefully to prevent kinking or detachment. Make sure that the ferrule stays on the tubing.

Note: If the ferrule is old or shows any sign of damage, replace it. A ferrule that is damaged or used for too long can become stuck.

b. Sonicate the nozzle in a tube containing DI water, ensuring that it is fully submerged. Repeat as needed until the nozzle is clean.

c. Make sure that the ferrule is on the tubing, and then screw the closed-loop nozzle back onto the nut.

4. Reinsert the closed-loop nozzle and turn the nozzle-locking lever clockwise to the 12:00 position.

5. Close the flow cell access door.

Replacing the sort nozzle seal temporarily

Introduction

If the original seal on the sort nozzle is lost or damaged, and you do not yet have another sort nozzle, you can use a standard O-ring as a short-term replacement for the sort nozzle seal.

Procedure

To replace the sort nozzle seal temporarily:

1. Ensure that the groove in the nozzle is clean.

   If any part of the seal is still in the nozzle groove, sonicate the nozzle in a bleach solution until the seal comes off, and then rinse the nozzle in DI water.

2. Using the O-ring pick tool or the wooden end of a cotton swab, install the O-ring in the nozzle groove and allow the nozzle to air dry for a few minutes.

   Note: Do not wipe the nozzle with anything because it could leave fibers or other contamination, or dislodge the O-ring.

3. Using the magnifier in the accessory kit, inspect the nozzle to verify that the O-ring is installed all the way into the groove.

Aligning the waste aspirator drawer to the stream

Introduction

The stream and sort setup are optimized for the sort nozzle that was provided with the system. If you install a sort nozzle that is new or different from the one that came with the instrument, you need to realign the aspirator drawer to the stream for proper sort setup.

Before you begin

- If you are sorting, stop sorting by clicking **Stop Sort** on the **Sort** tab. The deflection plates are automatically turned off when the cytometer is not sorting.
- If the stream is off, turn it on by loading a sample or by completing the Sort Nozzle step in the System Startup.

About this task

Caution! Do not touch the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. A 12,000-volt potential exists between the deflection plates when they are on. The plates remain energized even when the sort block door is open.

Procedure

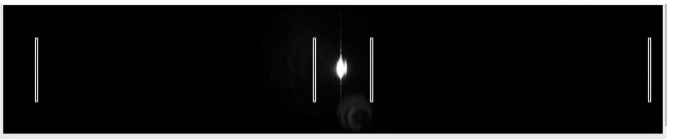
To align the waste aspirator drawer to the stream:

1. Open the flow cell access door.

2. Loosen the screws on both sides of the sort block door by using a hex wrench.

3. In the software, open the **Stream View** window by clicking the **Stream** status indicator in the navigation bar.

4. Gently nudge the sort block to the left or right until the stream appears in the middle between the two hash marks of the lower stream view. (The hash marks represent the edges of the waste aspirator.)



Note: If the stream is still not visible after following the above steps, then follow these additional steps.

5. Loosen the screws on both sides of the sort block door by using a hex wrench.

6. Start the stream with the flow cell access and sort block doors open.

7. Visually align the stream to the center of the aspirator.

8. Click the **Stream** icon on the navigation bar to display the Stream view.

9. Use the **Stream** view to make minor adjustments to the stream until it is centered.

10. Slowly tighten the screws, ensuring that the stream position in the drawer does not move.

11. Close the sort block door, the flow cell access door, and the sort collection chamber door.

Cleaning the flow cell

Introduction

The sample path and flow cell are cleaned with DI water or 1.5% BD Detergent Solution every time you perform a daily shutdown procedure. However, you can also perform this cleaning separately whenever additional cleaning is needed.

Before you begin

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 111)
- Emptying the waste tank (page 126)
- Filling the sheath tank (page 120)

About this task

In addition to cleaning the flow cell, ensure that you clean the area where the nozzle is inserted to prevent salt buildup. If salt buildup exists where the nozzle is inserted, the software might not detect the closed-loop nozzle when it is inserted. See Cleaning external surfaces (page 106).

Procedure

To clean the flow cell:

1. On the **Cytometer** page, select **Flow Cell Clean**, and then click **Start**.
2. Complete the steps in the wizard.

Caution! Never mix BD Detergent Solution and bleach in the same tube because this can create dangerous fumes.

3. (Optional) In cases where there is buildup in the flow cell as indicated by high CVs in the CS&T report:
   a. Perform a flow cell clean with 1.5% BD Detergent Solution.
   b. Place an empty sample tube in the sample chamber and run another flow cell clean. This will create bubbles in the flow cell.

c. Perform flow cell clean three additional times with DI water to rinse the detergent thoroughly from the flow cell.

Cleaning the optical filters

Introduction You should inspect and clean the optical filters as needed.

About this task

Caution! Handle the filters with care to avoid scratching the surfaces and to prevent them from falling out of the holder. To clean the optical filters, use cotton swabs, optical lens paper, and spectral-grade methanol or absolute ethanol in a dropper bottle. Do not use acetone.

Procedure **To clean the optical filters:**

1. Open the filter access door on the electronics box.
2. Wrap a triangular section of lens paper around the cotton end of a cotton swab, and then moisten and seal the end with a few drops of alcohol.
3. Holding the cotton swab in a horizontal position, gently rub any spots on the filter surface and wipe clean.
4. Allow the solvent to evaporate and check the filter surface for streaks.
5. Inspect the 1/4-inch-diameter section in the center of the filter on both sides for scratches.

   Filters are coated with different dielectrics that can get scratched. If you see scratches, replace the filter.

6. Insert the cleaned filter into the heptagon trigon, and make sure the filters are pushed all the way in.

Cleaning the Accudrop laser window and the lower camera window

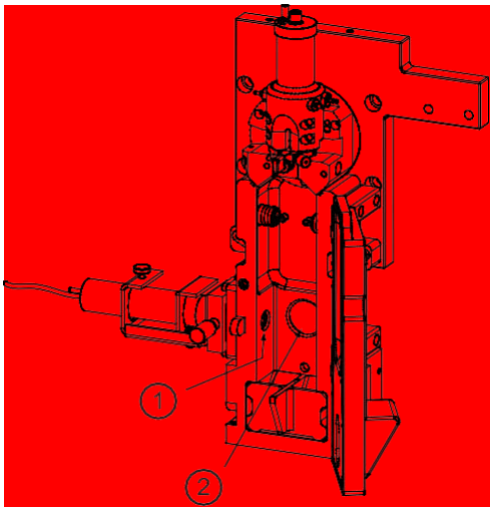
Introduction

You should clean the Accudrop laser window and the lower camera window when the software is unable to set the drop delay, or when the software is unable to verify the side streams when sorting is started.

About this task

Caution! Do not touch the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. A 12,000-volt potential exists between the deflection plates when they are on. The plates remain energized even when the sort block door is open.

The following illustration shows the Accudrop laser window (1) and the lower camera window (2).



Procedure **To clean the Accudrop laser window and lower camera window:**

1. Ensure that the stream is turned off. See Stopping the stream (page 106).
2. Open the flow cell access door and the sort collection chamber door.
3. Ensure that the red warning light next to the sort block door is off, indicating that the deflection plates are off.
4. Open the sort block door by turning the thumbscrew on the front of it.
5. Wipe the windows with lens paper or a soft, lint-free cloth soaked with DI water, and then dry the windows.
6. Close the sort block door, the flow cell access door, and the sort collection chamber door.

Cleaning the strobe lens window and upper camera window

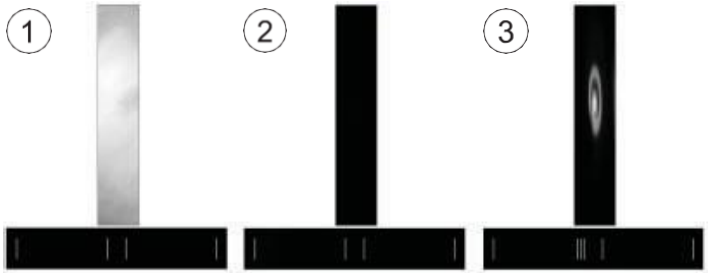
Introduction

You should clean the strobe lens window and the upper camera window if smudges appear in the processed image in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed.

Before you begin

You can check the stream view by clicking the Stream status indicator in the navigation bar. The Stream View window opens.

The following images show a stream view that is clear (1) and two stream views that indicate that the camera and strobe windows need to be cleaned (2, 3).



About this task

Caution! Do not touch the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. A 12,000-volt potential exists between the deflection plates when they are on. The plates remain energized even when the sort block door is open.

Procedure

To clean the strobe lens window and upper camera window:

1. Ensure that the stream is turned off. See Stopping the stream (page 106).

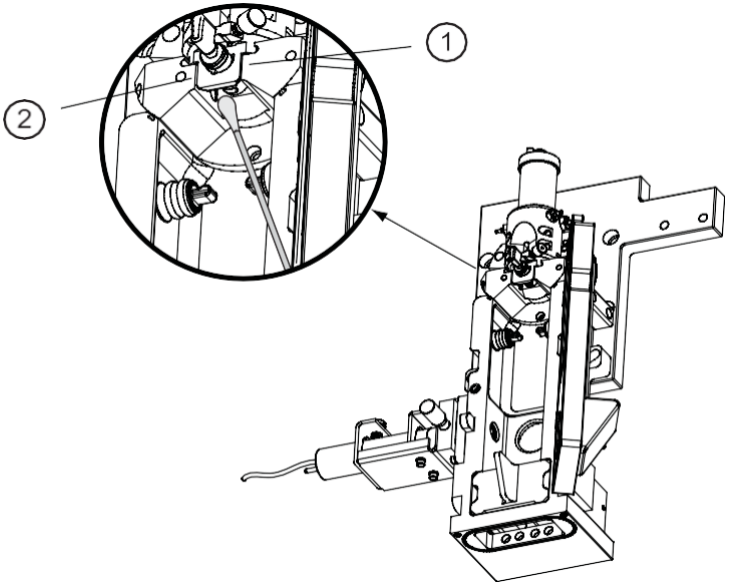
2. Open the flow cell access door and the sort collection chamber door.

3. Ensure that the red warning light next to the sort block door is off, indicating that the deflection plates are off.

4. Open the sort block door by turning the thumbscrew on the front of it.

5. Insert a lint-free cotton swab, or a swab with lens paper wrapped around it, just below the bottom of the flow cell.

   The strobe lens window (1) and the upper camera window (2) are located behind two circular openings on either side of the top of the sort chamber.



6. Gently wipe the upper camera window and the strobe lens (opposite the window) to remove any saline.

7. Click the Stream status indicator and check that the camera image is clean in the Stream View window.

8. Close the sort block door, the flow cell access door, and the sort collection chamber door.

Cleaning the deflection plates

Introduction

Before you clean the deflection plates, you need to remove them by using the deflection plate removal tool. Then you can clean the deflection plates with DI water.

Before you begin

You need the deflection plate removal tool from the accessory kit.

About this task

Caution! Do not touch the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. A 12,000-volt potential exists between the deflection plates when they are on. The plates remain energized even when the sort block door is open.

Procedure

To remove the deflection plates:

1. Ensure that the stream is turned off. See Stopping the stream (page 186).
2. Open the flow cell access door and the sort collection chamber door.
3. Ensure that the red warning light next to the sort block door is off, indicating that the deflection plates are off.

4. Open the sort block door by turning the thumbscrew on the front of it.
5. Hold your thumb on the plate (or use your other hand), and then pull the deflection plates out carefully so that they do not fall.

6. Clean the deflection plates with DI water and allow them to dry before you reinstall them.

Removing or installing the FSC neutral density filter

Introduction

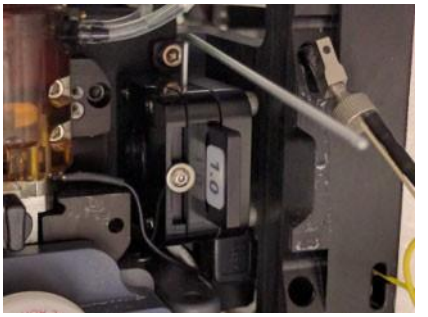
The FSC neutral density (ND) filter decreases the FSC signal and keeps events on scale, which is helpful for applications involving large particles. However, for applications involving small particles, such as bacteria or platelets, you might want to remove the ND filter.

About this task

Note: The ND filter must be installed when you run Drop Delay.

Procedure **To remove or install the FSC ND filter:**

1. Open the flow cell access door.
2. Loosen the set screw on the top of the FSC ND filter assembly with a 1.5-mm Allen wrench.



3. Perform one of the following:
   a. Remove the FSC ND filter from the slot.
   b. Install the new filter by sliding it into the slot with the label facing the flow cell, as shown in the preceding picture.
4. Close the flow cell access door.

Emptying the waste tank

Introduction You should empty the waste tank every time you fill the sheath tank and whenever the software indicates that the container is getting full.

Before you begin Ensure that you have enough bleach solution to equal 10% of the volume of the waste tank.

About this task

All biological specimens and materials can transmit potentially fatal infection. To prevent exposure to biohazardous agents, add bleach to the waste container (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

Procedure

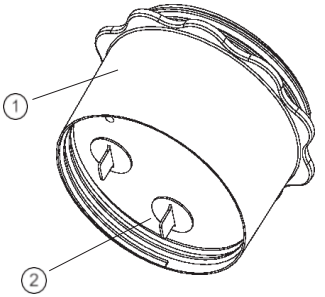
To empty the waste tank:

1. Turn off the stream. See Stopping the stream (page 106).
2. Disconnect the sensor and fluid line cap from the waste tank.

   Note: The waste tank can become pressurized when the cytometer is running. Wait at least 1 minute for the pressure to dissipate before you open the tank.
3. Remove the disposable waste cap (large-sized cap) and attached trap from the tank.

   Place the assembly on the bench with the label-side up.

   Note: Do not wet the cap on top of the trap (1). If you see liquid inside the trap, remove the drain plug (2) and fully drain the liquid before you replace the plug.



4. Empty the waste tank according to your standard laboratory procedures for biohazardous waste.

5. Add approximately 1 L of bleach to the waste tank (10-L container) or a sufficient amount so that 10% of the total volume is bleach.

6. Replace the waste trap and the attached filter cap, and then tighten them by hand until they are closed.

   Note: To prevent over-pressurization during fluidics startup, do not overtighten the trap or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants or adhesives.

7. If one month has passed since you last changed the cap, replace the filter cap with a new one and write the date on it as a reminder.

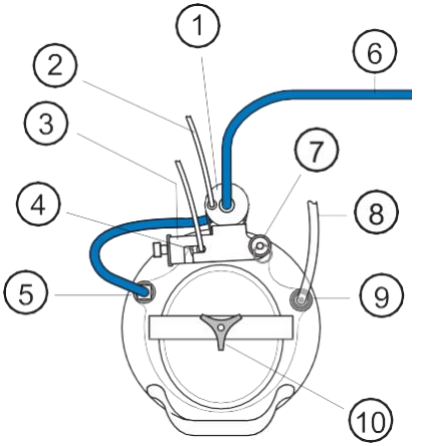
8. Reconnect the sensor and fluid line.

Filling the sheath tank

Introduction

You should fill the sheath tank whenever the software indicates that the sheath fluid level is low. If you continue to run the cytometer when the sheath fluid level is low, the startup times for the break-off stream will increase, and the system will eventually turn off the stream.

About this task The following illustration shows the sheath tank and connectors.



No.	Description
1	Fluid filter
2	Fluid filter purge line
3	Containment device
4	Sheath sensor
5	Fluid connector
6	Fluid line
7	Pressure relief valve
8	Air line
9	Air connector
10	Cover knob

Note: Avoid getting the top of the sheath sensor wet. If the top of the sheath sensor gets wet, wipe it dry. The sheath sensor will not detect the level of sheath fluid accurately if the top of it is wet.

Procedure **To fill the sheath tank:**

1. Turn off the stream. See Stopping the stream (page 105).
2. Disconnect the air line.
3. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.
4. Unscrew the sheath tank cover knob and remove the cover.
5. Fill the tank with sheath fluid up to the upper weld line on the inside of the tank.

   Note: Do not overfill the sheath tank because it can cause incorrect sample flow rates.

6. Replace the cover and tighten the knob.

Make sure the large O-ring on the inside lip of the cover is seated correctly and has not slipped out of position. The tank can leak if the cover is not secured properly.

7. If you removed the sheath tank to refill it, place the tank back in its original position.

   Note: The flow rate is calibrated with the sheath tank. If the location or elevation of the sheath tank is changed, it could affect the flow rate calibration.

8. Connect the air line.

What to do next Purging the sheath filter (page 133)

Changing the fluid filter

Introduction The fluid filter is used to filter sheath fluid, bleach, DI water, or ethanol. You need to change the fluid filter depending on the procedure you are doing and the indications in the software.

About this task **Note:** Before it is installed, a fluid filter can be used as a sheath filter, a bleach filter, a DI water filter, or an ethanol filter. After the filter is installed for one of these purposes, however, you cannot use it for a different purpose. Because of this, you should label each filter so that you know which one to use for each purpose.

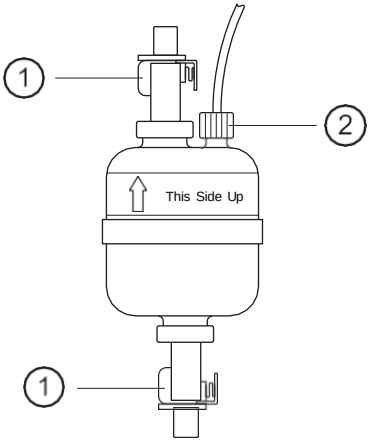
Change the fluid filter every 3 months, or when increased debris in an FSC vs SSC plot indicates that the filter needs to be replaced.

Spare filters are included with the accessory kit.

See Preparing new fluid filters (page 146) before you start the procedure.

Procedure **To change the fluid filter:**

1. Turn off the stream. See Stopping the stream (page 106).
2. Disconnect the air line from the sheath tank.
3. Pull up on the ring of the pressure relief valve to release the pressure from the tank. Verify that all of the pressure is released by pulling up a second time.
4. Disconnect the filter by pressing the metal tabs on each end.



No.	Description
1	Tabs
2	Filter purge line nut

5. Disconnect the filter purge line by unscrewing the nut.
6. Write the current date on the filter so that you will know when to replace it.
7. Reconnect the filter purge line to the new filter.
8. Use the arrows on the filters that indicate the direction of the flow through the filter; replace the filter with a new one in the same orientation.

9. Reconnect the air line to the tank and check for leaks when the pressure is turned on.

What to do next Purging the sheath filter (page 133)

Purging the sheath filter

Introduction Purging the sheath filter helps prevent bubbles from entering the flow cell. The sheath filter is purged as part of the fluidic startup procedures, but you can also purge the sheath filter separately after you install a new sheath filter or whenever you observe problems with the stream.

About this task The sheath filter must be purged when a new filter is installed to remove air from the filter. Multiple purges might be needed to fully remove the air.

Procedure **To purge the sheath filter:**

On the **Cytometer** page, select **Sheath Filter Purge**, and then click **Start**.

Backflushing the sample line

Introduction After a sample tube is unloaded, the sample line tubing within the sample injection chamber is automatically flushed inside and out with sheath fluid to eliminate potential sample carryover. You can perform additional backflush cleaning whenever you observe sample carryover, or after running samples with adherent cells or dye.

Procedure

To backflush the sample line:

With the stream on, select one of the following options:

- In the **Acquisition Dashboard** on the **View Data** tab or the **Sort** tab, click **Backflush**.
- On the **Cytometer** page, select **Sample Line Backflush**, and then click **Start**.

Replacing the sample line

Introduction

You should replace the sample line every 4–6 months, or when decreased event rates indicate that the sample line might be clogged.

Before you begin

You need the following items from the accessory kit:

- Replacement sample line assembly
- Ferrules (to replace ferrules that are old or damaged)
- Ferrule removal tool (if the ferrule drops into the injection chamber fitting)
- 2.5-mm Allen wrench

About this task

Note: Ensure that you replace any ferrule that is old or shows any sign of damage. A ferrule that is damaged or used for too long can become stuck.

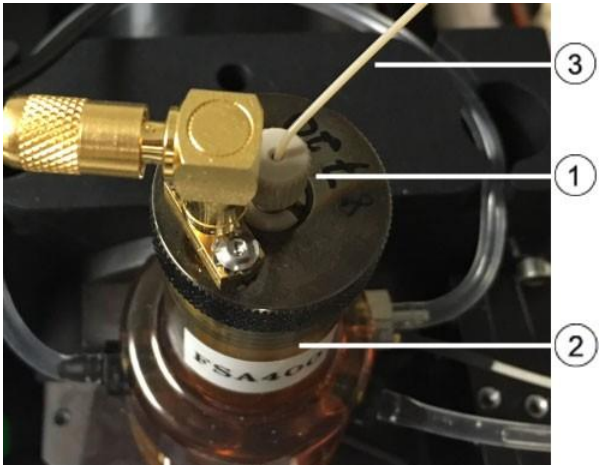
All biological specimens and materials can transmit potentially fatal infection. To prevent exposure to biohazardous agents, add bleach to the waste container (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

Procedure

To replace the sample line:

1. On the **Cytometer page**, select **Replace Sample Line**, and then click **Start**.
2. Remove the sample line:
   a. Remove the sample line access door and open the flow cell access door.
   b. Remove the top of the cytometer by loosening the hex screws on the front and back and lifting the top off.

c. Unscrew the connecting nut (1) at the top of the flow cell (2) and slowly pull the sample line (3) out.

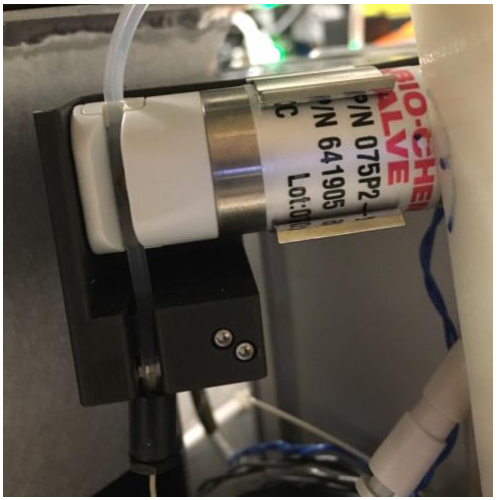


d. Check whether the cone-shaped ferrule is still attached to the sample line.

   If the ferrule is left behind in the flow cell, gently push the tip of the ferrule-removal tool into the top of the ferrule and pull the ferrule straight out.

e. Slide the ferrule (if it is still attached) and the nut off the end of the sample line.

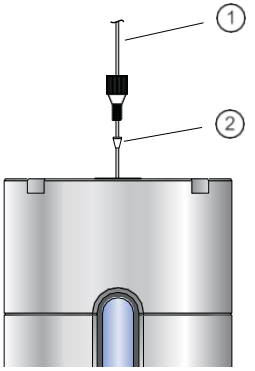
f. Pull the pinch valve tubing out of the slot in the pinch valve.



g. Unscrew the nut at the top of the sample injection chamber.

h. If a sample line filter is installed on the sample line, remove it by pushing the sample line down so that the end is below the chamber and you can remove the filter.

i. Slowly pull the sample line (1) out from the top and ensure that a cone-shaped ferrule (2) is still attached to it.



Note: If the ferrule is left behind in the injection chamber fitting, gently push the tip of the ferrule-removal tool into the top of the ferrule and pull the ferrule straight out.

j. Slide the ferrule (if it is still attached) and the nut off the end of the sample line.

3. Install the new sample line:

a. Slide the nut and then the ferrule onto the shorter section of the new sample tubing.

Caution! Do not bend the sample line during insertion.

b. Insert the sample line into the sample injection chamber fitting and push the tubing from the top until it is in the middle of the chamber viewing window.

Note: Use a flashlight or turn on the chamber light using the **Experiment** > **View data** page.

c. Finger-tighten the nut on the top of the chamber, leaving the nut loose enough so that the sample line height can still be adjusted.

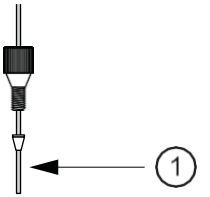
Caution! Do not overtighten the nut and do not use tools. Over-tightening the nut can kink or damage the tubing.

d. Push the pinch valve tubing back into the pinch valve.

e. Slide the nut and then the ferrule onto the longer section of the new sample tubing.

Caution! Do not bend the sample line during insertion.

f. Insert the pilot tubing (1) into the fitting at the top of the flow cell until tubing stops.



Caution! Within the cuvette flow cell fitting, make sure that the pilot is seated flush against the pilot depth. Dead volume between the pilot and the pilot depth can lead to sample carryover or leaking.

g. Finger-tighten the nut at the top of the flow cell.

Caution! Do not overtighten the nut and do not use tools. Over-tightening the nut can kink or damage the tubing.

h. In the wizard, click **Continue**.

4. Complete the Sample Line Height step according to the instructions in the wizard.

   Ensure that the sample line does not bow or bend when a tube is loaded. If you need to adjust the length, unscrew the nut on top of the sample injection chamber, adjust the length, and tighten the nut again.

5. Complete the Fittings step according to the instructions in the wizard.

6. Replace the sample line access door and the top of the cytometer, and then close the flow cell access door.

7. Load a tube of DI water and ensure that there are no leaks.

Replacing the sample line filter

Introduction

Sample line filters can be installed on the end of the sample line to filter out large particles from the sample. You should replace the sample line filter when decreased event rates indicate that the sample line might be clogged.

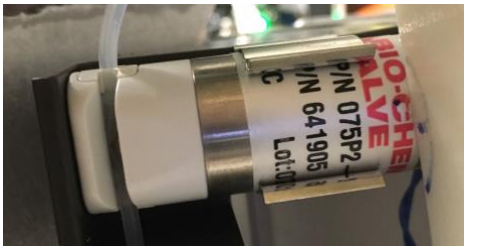
About this task

All biological specimens and materials can transmit potentially fatal infection. To prevent exposure to biohazardous agents, add bleach to the waste container (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

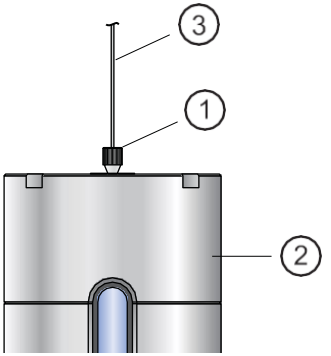
Procedure

To replace the sample line filter:

1. On the **Cytometer** page, select **Replace Sample Line Filter**, and then click **Start**.
2. Complete the Sample Line Filter step:
   a. Remove the sample line access door from the cytometer.
   b. Pull the pinch valve tubing out of the slot in the pinch valve.

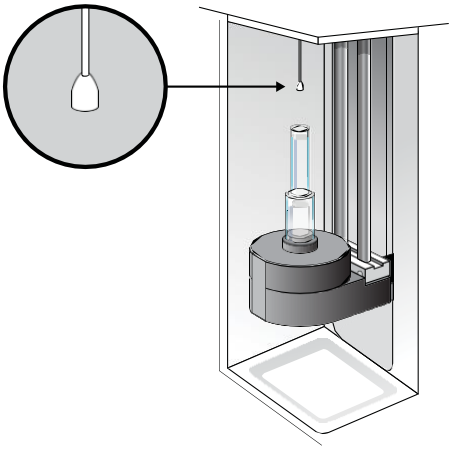


c. Loosen the sample line fitting nut (1) at the top of the injection chamber (2) to allow the sample line (3) to slide freely through the fitting.



d. Push the sample line downward so that the end is below the bottom of the sample injection chamber.

e. If a sample line filter is installed on the sample line, remove it by pulling it off the end.



f. Install the new sample line filter by sliding it onto the end of the sample line.

g. Click **Continue**.

3. Complete the Sample Line Height step according to the instructions in the wizard

   Ensure that the sample line does not bow or bend when a tube is loaded. If you need to adjust the length, unscrew the nut on top of the sample injection chamber, adjust the length, and tighten the nut again.

   Push the pinch valve tubing back into the pinch valve.

4. Complete the Sample Line Fitting step according to the instructions in the wizard, and then close the sample line access door.

Removing the sheath probe

Introduction

The sheath probe must be removed from the sheath tank before you autoclave the tank in preparation for performing the aseptic sort procedure.

About this task

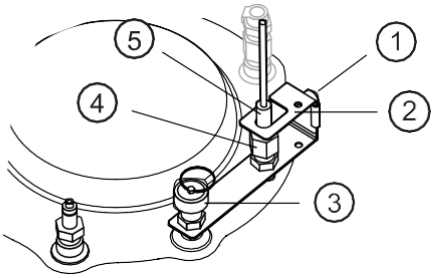
Note: Do not autoclave the sheath probe. It is not designed to withstand the conditions of autoclaving.

Procedure

To remove the sheath probe:

1. Turn off the stream. See Stopping the stream (page 106).
2. Disconnect the air line from the sheath tank.
3. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.

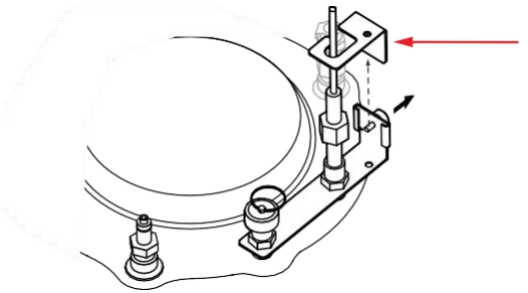
4. Loosen the nut at the top of the sheath probe with a wrench and vent the sheath pressure again if the tank is still pressurized.



No.	Description
1	Thumbscrew
2	Containment device
3	Pressure relief valve
4	11/16-inch nut
5	Sheath probe

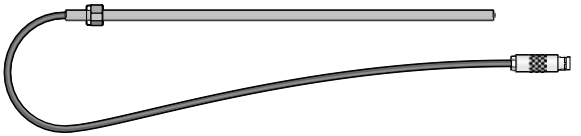
5. Loosen the thumbscrew on the containment device.

6. Pull the top section of the containment device straight up and out of the bottom section.



7. Finish loosening the 11/16-inch nut at the top of the probe and pull the probe straight up and out of the sheath tank.

8. Decontaminate the sheath probe using 70% ethanol.



Preparing for aseptic sort

Introduction

The Prepare for Aseptic Sort procedure cleans the entire system of any potential contaminants. You should run this procedure before you sort.

Before you begin

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 111)
- Filling the sheath tank (page 120)
- Changing the fluid filter (page 131)
- Emptying the waste tank (page 126)
- Removing the sheath probe (page 142)
- Preparing new fluid filters (page 146)

You need the following items:

- Four fluid filters
- At least 2.5 L of 10% bleach solution
- At least 2.5 L of DI water
- At least 2.5L of 70% ethanol solution
- At least 2.5 L of 1X PBS

Do not mix bleach and ethanol. Rinse with DI water in between using solutions.

About this task

Ensure that the fluid filters are designated to one type of fluid and are not interchanged.

If you use the same sheath tank for the entire procedure, make sure you rinse the tank thoroughly with DI water before you refill it with a different solution.

An accessory kit is available that allows you to autoclave a second tank in preparation for an aseptic sort. Contact BD Technical Support for more information.

This sort procedure takes 30 minutes to complete. However, the overall procedure of changing tanks and running rinses takes approximately one hour.

Procedure

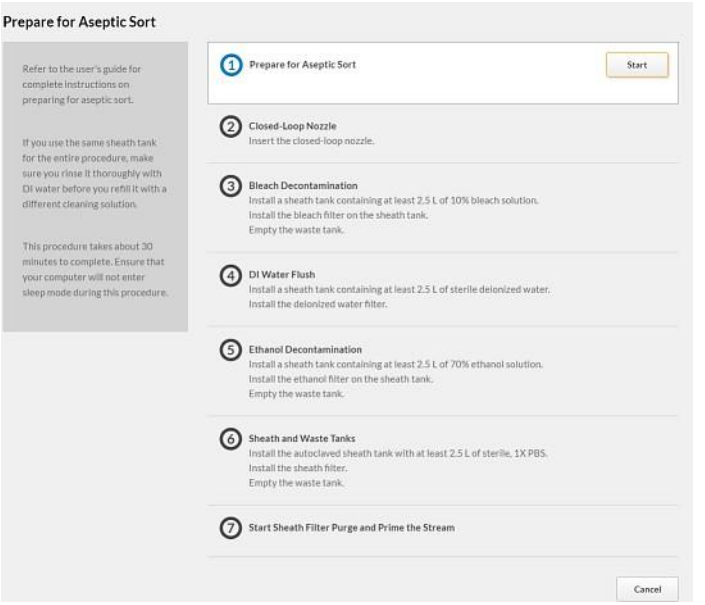
To prepare for aseptic sort:

1. Remove the sheath probe. See Removing the sheath probe (page 142).
2. Remove the sheath sensor cable.

   The cable is marked with a DO NOT AUTOCLAVE label.
3. Autoclave the sheath tank (with the gauge attached) according to your organization's protocols.
4. On the **Cytometer** page in the software, select **System Startup**.
5. Select and complete one of the fluidics startup options, or click **Skip**.

   When the fluidics startup is completed or skipped, you are prompted to select a cleaning option.

6. Select **Prepare for Aseptic Sort**, select number one (1), and then click **Start**.



7. Complete the rest of the steps in the wizard.

Preparing new fluid filters

Introduction

The fluid filter is used to filter sheath fluid, bleach, DI water, or ethanol. Change fluid filters every 3 months or sooner if there are issues with debris. If new dry filters are used during replacement, then trapped air in the filter can create bubbles in the flow cell. To reduce bubbles in the flow cell, wet the filters before use.

Before you begin

Ensure that you know how to perform the following procedures:

- Filling the sheath tank (page 126)
- Changing the fluid filter (page 131)
- Purging the sheath filter (page 133)

Procedure

To prepare new fluid filters:

1. Empty the sheath tank, rinse with DI water and refill with DI water. See Changing the fluid filter (page 131).
2. Remove the cap from the new filter and save it for reuse.
3. Change the fluidic filter to the new filter. See Filling the sheath tank (page 128).
4. In the BD FACSChorus software, select **Cytometer > Sheath filter purge** and select **Start**.
5. Repeat step 4 three times to ensure all of the air is removed from the fluid filter.
6. Change the fluid filter to prepare another new fluid filter, or install the appropriate filter for next desired workflow.
7. Replace the cap on the newly prepared fluid filter and store at room temperature.
8. Empty the sheath tank and fill with appropriate fluid for next desired workflow

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7

Technical specifications

This chapter covers the following topics:

- Optical specifications (page 150)
- Fluidic specifications (page 153)
- Sample input formats (page 153)
- Collection devices (page 153)
- Electronic and software specifications (page 154)
- Physical specifications (page 155)

Technical specifications

Optical specifications

Parameter	Value
Excitation laser	• 1-laser (blue), 2- color (2-0) • 1-laser (blue), 4-color (4-0) • 2-laser (blue, red), 6-color (4-2) • 2-laser (blue, violet), 6-color (3-3) • 2 (blue, yellow/green), 4-color (2-2) • 2 (blue, yellow/green), 6-color (2-4) • 3-laser (blue, red, violet), 6-color (2-2-2) • 3-laser (blue, red, violet), 9-color (4-2-3) • 3 (blue, violet, yellow/green), 8-color (2-2-4) • 3 (blue, red, yellow/green)8-color (2-2-4)

Parameter	Value
Laser specifications	Blue laser • Wavelength: 488 nm • Output power: 20mW • Nominal power: 16mW • Beam spot size: 9 ±3 µm x 67 ±5 µm Red laser • Wavelength: 640 nm • Output power: 40mW • Nominal power: 36 mW • Beam spot size: 9 ±3 µm x 67 ±5 µm Violet laser • Wavelength: 405 nm • Output power: 40mW • Nominal power: 36 mW • Beam spot size: 9 ±3 µm x 67 ±5 µm Yellow-green laser • Wavelength: 561 nm • Output power: 50 mW • Nominal power: 40 mW • Beam spot size: 9 ±3 µm x 67 ±5 µm
Laser alignment	Fixed and spatially separated alignment of all lasers with the cuvette flow cell
Beam divergence angle (full-angle)	488 nm: <1.2 mrad 640 nm: <1.3 mrad 405 nm: <1.0 mrad 561 nm: <1.2 mrad
Optical coupling	The quartz cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens for optimal light collection efficiency. Numerical aperture: 1.2.

Parameter	Value
Detection channels	Forward scatter (FSC), side scatter (SSC), and up to nine fluorescence. See the BD FACSMelody *Cell Sorter Filter Guide* for laser and detection configurations and optical filter specifications. See Instrument configurations (page 23) for more details.
Fluorescence and side scatter detection	• Reflective optics with single transmission bandpass filter in front of each PMT • High performance customized PMT modules for all fluorescence and SSC channels • Light collected by objective lens is delivered by fiber optics to specially designed heptagon or trigon detector arrays. • The cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens (1.2 NA) for optimal collection efficiency.
See the BD FACSMelody *Filter Guide* for laser and detection configurations and optical filter specifications.	
Stream illumination at 25°C (Accudrop laser)	
Optical power	>18 mw
Lasing wavelength	660 nm
Beam divergence angle (full-angle)	<3.0 mrad

Fluidic specifications

Parameter	Value
Temperature control	Adjustable through BD FACSChorus software: 4°C, 22°C, 37°C, and 42°C, or off
Sample agitation:	Adjustable through BD FACSChorus software to keep the sample constantly suspended
Flow cell	Quartz cuvette
Nozzle	100-µm nozzle is removable and can be sonicated. A registered key-fit position at the bottom of the cuvette provides fixed stream alignment.
Fluidic tanks	• Autoclavable 10-L stainless steel sheath container • 10-L polypropylene waste container

Sample input formats

Parameter	Value
Sample input	5.0-mL polystyrene or polypropylene tubes

Collection devices

Parameter	Value
Two-way sorting	1.5-, 2.0- and 5.0-mL tubes
One-way sorting	• Plates: 6-, 24-, 48-, 96-, and 384-well plates • PCR tray: 96 well • Microscope slide: 3 x 9 grid
Temperature control	Water recirculation unit to provide heating or cooling for collection into tube holders, multiwell plates, and slides (optional)

Electronic and software specifications

Parameter	Value
Software	BD FACSChorus version 1.0 or later
Operating system	Microsoft Windows 10 64-bit
Monitor	23-inch LCD with a minimum 1,920 x 1,080 resolution
Memory	8 GB RAM
Storage	500-GB hard drive
FCS format	FCS 3.1

Physical specifications

Parameter	Value
Operating temperature	The instrument has an operating range between 17.5°C (63.5°F) and 27.5°C (81.5°F) on the benchtop and 17.5°C (63.5°F) and 22.5°C (72.5°F) with the BSC option. We recommend that the lab temperature fluctuate less than 5°C within a day for best operation.
Humidity	The operating humidity tolerance is between 40% and 60% relative humidity (noncondensing).
Dimensions (H x W x D)	
Cell sorter	49.5 x 55.9 x 48.3 cm (19.5 x 22 x 19 in.)
Electronics box	50.8 x 55.9 x 48.3 cm (20 x 22 x 19 in.)
Biological safety cabinet (optional)	136.5 x 87.6 x 233.4–249.9 cm (53.8 x 34.5 x 91.9–98.4 in.)
Sample input temperature control (optional)	42.9 x 91.3 x 47.1 cm (16.90 x 35.95 x 18.55 in.)
Aerosol Management Option	45.21 x 38.1 x 54.61 cm (17.8 x 15 x 21.5 in.)
See the BD FACSMelody *Cell Sorter Site Preparation Guide* for additional information on dimensions and clearances.	
Instrument weight	Cell sorter: 40.75 kg (89.8 lb) Electronics box: 36.25 kg (79.9 lb)

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8

Troubleshooting

This chapter covers the following topics:

- Troubleshooting overview (page 158)
- Startup troubleshooting (page 159)
- Acquisition troubleshooting (page 164)
- Sorting troubleshooting (page 170)
- Electronics troubleshooting (page 173)

Troubleshooting overview

Introduction

BD FACSChorus software provides many troubleshooting instructions when errors are encountered. Follow those instructions prior to executing the recommended solutions listed here. Solutions here are focused on errors or troubleshooting that BD FACSChorus software is not able to address.

The tips in this chapter are designed to help you troubleshoot your experiments. If additional assistance is required, contact your local BD Biosciences technical support representative. See Technical support (page 13).

Troubleshooting suggestions in this chapter are grouped under the following headings:

- Startup troubleshooting (page 159)
- Acquisition troubleshooting (page 164)
- Sorting troubleshooting (page 170)
- Electronics troubleshooting (page 173)

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. See Cleaning the closed-loop nozzle (page 113).
	Salt buildup in the nozzle location between the flow cell and the locking lever	Clean the area to remove the salt build up.

Startup troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Error starting stream after inserting sort nozzle or loading sample	Sheath tank low or empty or waste tank full or almost full	Refill the sheath tank. See Filling the sheath tank (page 128) or Emptying the waste tank (page 126).
	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. See Changing the nozzle (page 111).
	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 (page 122).
	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. See Cleaning the sort nozzle (page 113). If the nozzle appears damaged, replace it. See Changing the nozzle (page 111). Click **Experiment** > **View Data** to restart the stream. Load the tube and select **Load Sample** to start stream.
	Debris in flow cell	Clean the flow cell. See Cleaning the flow cell (page 118).

Startup troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Error starting stream after inserting sort nozzle or loading sample	Air in sheath line or filter	Purge the sheath filter. See Purging the sheath filter (page 133). Run daily fluidics startup. See Fluidics startup (page 46).
	Dry sheath filter	Purge the sheath filter. See Purging the sheath filter (page 133).
	Air pressure is too low, too high, or variable	Verify that the external air supply 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.

Startup troubleshooting (continued)

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. See Changing the nozzle (page 111).
	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. See Cleaning the sort nozzle (page 112). If the nozzle appears damaged, replace it. See Changing the nozzle (page 111). Click **Experiment** > **View Data** to restart the stream. Load the tube and select **Load Sample** to start stream.
	New sort nozzle was inserted.	If you are using a new nozzle, the sort block may need to be repositioned to align with the stream. See Changing the nozzle (page 111).
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.

Startup troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
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 (page 118). Close the sort block door and the flow cell access door properly.
Problems with Drop Delay function	Sort block door is not closed	Close the sort block door properly.
	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	Clean the lower camera and Accudrop laser window. See Cleaning the Accudrop laser window and the lower camera window (page 120).

Acquisition troubleshooting

Observation	Possible Causes	Recommended Solutions
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 is clogged	Backflush the sample line. See Backflushing the sample line (page 133). If that does not work, replace the sample line assembly. See Replacing the sample line (page 134).
	Sample line filter is clogged	Replace the sample line filter. See Replacing the sample line filter (page 140).
	Sample is not mixed properly	Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 60).
	Threshold is not set to correct parameter	Set the threshold to the correct parameter for your application. See Defining view data (page 60).
	Threshold setting is too low or too high	Adjust the threshold setting. See Defining view data (page 60).

Acquisition troubleshooting (continued)

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. See Defining view data (page 60).
	Threshold setting is too low or too high	Adjust the threshold setting. See Defining view data (page 60).
	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.

Acquisition troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
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. See Defining view data (page 60).
	Sheath tank is low	Fill the sheath tank. See Filling the sheath tank (page 128).
	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 injection chamber O-ring is worn	Contact your BD Biosciences 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. See Defining view data (page 60).
	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 60).
	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.

Acquisition troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
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. See Defining view data (page 60).
	Sample is too dilute	Concentrate the sample.
	Threshold setting is too high	Adjust the threshold setting. See Defining view data (page 60).
	Sample line is clogged or kinked	Backflush the sample line. See Backflushing the sample line (page 133). If that does not work or if visible kinks are found in the sample line, replace the sample line assembly. See Replacing the sample line (page 134).
	Sample line assembly or sample line filter installed incorrectly	Verify the sample line assembly or sample line filter installation. See Replacing the sample line filter (page 140).

Acquisition troubleshooting (continued)

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. See Defining view data (page 60) and Calculating compensation (page 64).
	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	Clean the flow cell. See Cleaning the flow cell (page 118). Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 112).
	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 60).
	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.

Acquisition troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Excessive amount of debris in plots	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 60).
	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. See Changing the fluid filter (page 131).
Processed events are <90%	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 60).
	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. See Defining view data (page 60).
Sample injection chamber does not close	Sample injection chamber O-ring is dry and causing chamber to stick	Lubricate the sample injection chamber O-ring. See Lubricating the sample injection chamber O-ring (page 105).

Sorting troubleshooting

Observation	Possible Causes	Recommended Solutions
Stream turns off unexpectedly	Debris in flow cell or nozzle	Clean the flow cell. See Cleaning the flow cell (page 118). Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 113).
	Sheath or waste tank full	Empty the waste tank or fill the sheath tank. See Emptying the waste tank (page 126) and Filling the sheath tank (page 125).
Unable to start sort	BD FACSChorus software cannot locate the side streams	Clean the lower camera window. See Cleaning the Accudrop laser window and the lower camera window (page 120). Close the sort block door properly.
	Salt bridge	Clean the deflection plates and the area around and behind the plates. See Cleaning the deflection plates (page 124).
	Drop charge cable is loose or missing	Verify that the stream-charging wire is inserted all the way into the barb.
Arcing between deflection plates	Salt bridge	Clean the deflection plates and the area around and behind the plates. See Cleaning the deflection plates (page 124).

Sorting troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Fanning around center or side streams	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. See Changing the nozzle (page 111).
	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. See Cleaning the sort nozzle (page 112). If the nozzle appears damaged, replace it. See Changing the nozzle (page 111) and Replacing the sort nozzle seal temporarily (page 114). Click **Experiment** > **View Data** to restart the stream. Load the tube and select **Load Sample** to start stream.
	Dirty deflection plates	Clean the deflection plates. See Cleaning the deflection plates (page 124).
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements. See Calculating compensation (page 64).
	Particles too big for sort nozzle	Verify that the particle size is appropriate for the 100-um nozzle.
Low sort efficiency	Event rate is too high for drop frequency	Decrease the flow rate.
	Gating conflict	Verify the gating hierarchy.

Sorting troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Erratic sort rate	Flow rate is too high	Decrease the flow rate.
Unexpected sort results	Incorrect drop delay	Run drop delay. See System startup (page 44).
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements. See Calculating compensation (page 64).
	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 chamber door is open	Close the sort chamber door.

Electronics troubleshooting

Observation	Possible Causes	Recommended Solutions
Cell sorter will not connect	Cell sorter power is off	Turn on the cell sorter main power.
	Communication failure between the workstation and the cell sorter	Exit BD FACSChorus software and restart it. Reset the instrument electronics: switch off the main power, wait 10 seconds until the system is fully depressurized, and then switch the power back on. Restart the computer.
	Ethernet cable between workstation and cell sorter is disconnected	Unplug and then plug in the cable and make sure it is secure.
	IP address changed	Enter the correct IP address. Call BD Biosciences for assistance.

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