

BD FACSMelody™ Cell Sorter User's Guide

For Research Use Only 

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Regulatory information

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense. Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits. This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations. Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.

History

Revision	Date	Change made
23-18120-00	7/2016	Initial release

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Introduction

This chapter covers the following topics:

- [About this guide \(page 8\)](#)
- [Safety symbols \(page 10\)](#)
- [Technical support \(page 11\)](#)

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
<i>BD FACSMelody™ Cell Sorter Site Preparation Guide</i>	Provides the site requirements. Read this guide before the system the system is installed.
<i>BD FACSMelody™ Cell Sorter Safety and Limitations Guide</i>	Provides safety guidance and system limitations. Read this guide before running the system.
<i>BD FACSMelody™ Cell Sorter Quick Reference Guide</i>	Provides information for using the instrument. Read this guide before running the system.
<i>BD™ CS&T RUO Beads technical data sheet</i>	Provides instructions on preparing the BD CS&T RUO beads for quality control.
<i>BD™ FC Beads technical data sheet</i>	Provides instructions on preparing the BD FC beads for compensation control.
<i>BD FACS™ Accudrop technical data sheet</i>	Provides instructions on preparing the BD FACS Accudrop beads for calculating drop delay.

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 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 **Instruments > Research > Cell Sorters > BD FACSMelody > BD FACSMelody Consumable**.
 3. Select the materials to order.
-

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About the system

This chapter covers the following topics:

- [System overview \(page 14\)](#)
- [Instrument overview \(page 17\)](#)
- [Optical components \(page 19\)](#)
- [Fluidic components \(page 26\)](#)
- [BD FACSCorus software \(page 29\)](#)

System overview

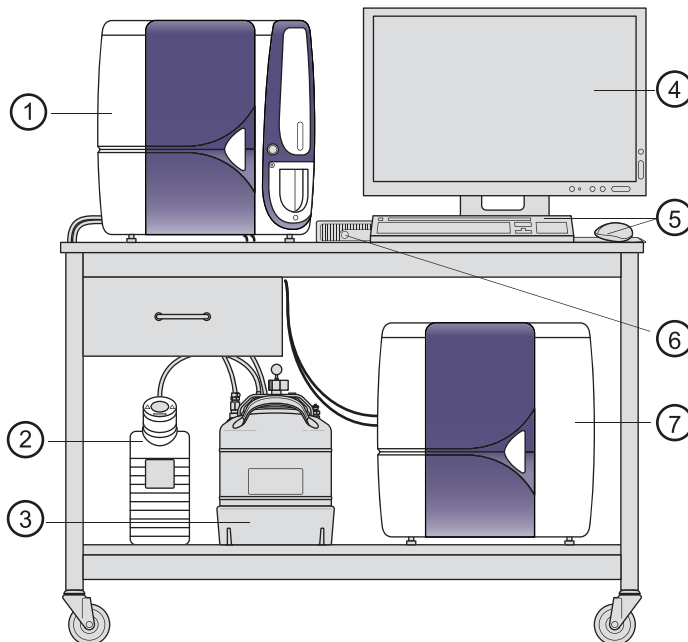
Introduction

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 FACSCorus™ 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 nine (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 FACSCorus software

BD FACSCorus 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 FACSCorus 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)
 - Plate sorting, using the optional automated stage
-

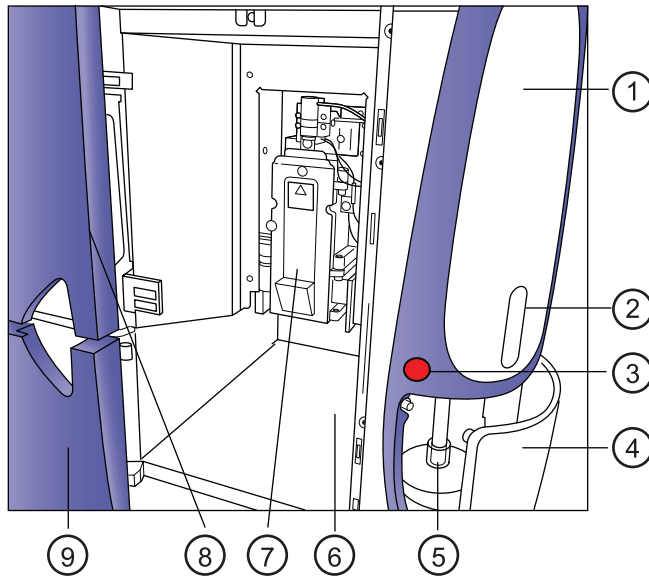
Instrument overview

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

Configurations

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	2-color (2-0)
1	4-color (4-0)
2 (blue, red)	6-color (4-2)
2 (blue, violet)	6-color (3-3)
3 (blue, red, violet)	6-color (2-2-2)
3 (blue, red, violet)	9-color (4-2-3)

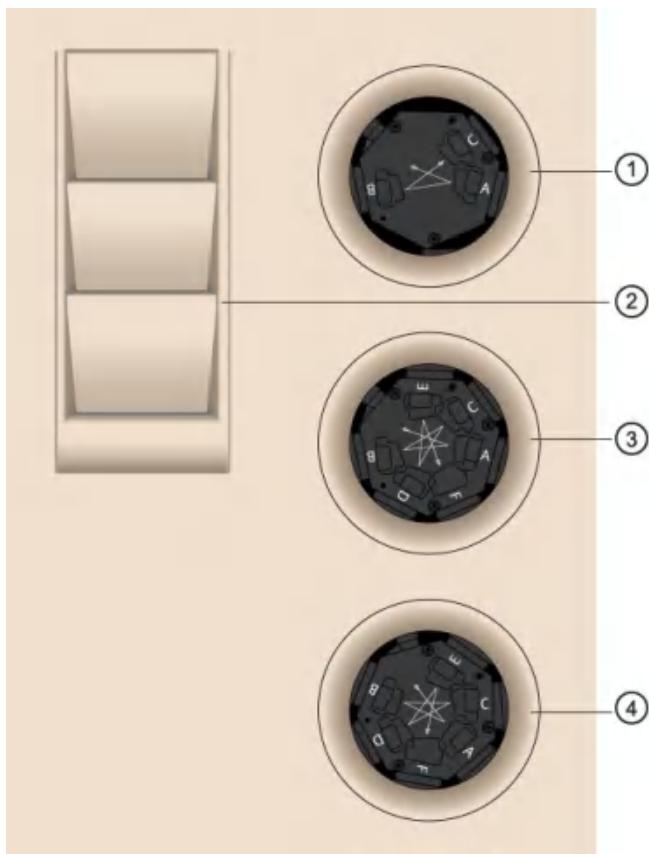
Optical components

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	Red 640-nm trigon detector
2	Holder for filters
3	Blue 488-nm heptagon detector
4	Violet 405-nm heptagon detector

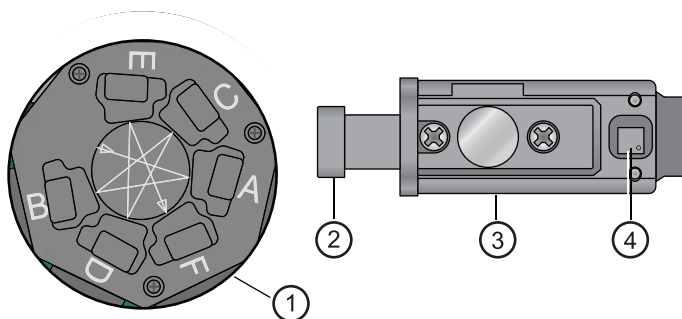
Detector arrays

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	Filter holder
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.

Default detector array setup

The following tables show the default setup for the different detector arrays.

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
	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	665LP	700/54
	C	PE, PI	560LP	586/42
	D	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
	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
(4-2 configuration)

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PerCP, PerCP-Cy5.5, 7-AAD	665LP	700/54
	C	PE, PI	560LP	586/42
	D	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
	E	SSC	ND10 ^a	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
	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
(3-3 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
	D	Side scatter (SSC)	ND10 ^a	488/15

Laser	Detector	Primary fluorochrome	Mirror	Filter
Violet/ 405 nm	A	BD Horizon Brilliant™ Violet 786	755LP	755LP
	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
(2-2-2
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
	C	Side scatter (SSC)	ND10 ^a	488/15

Lasers	Detector	Primary fluorochrome	Mirror	Filter
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
	B	APC, Alexa Fluor® 647	660/10	660/10
Violet/ 405 nm	A	BD Horizon Brilliant Violet 786	755LP	None
	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
(4-2-3
configuration)

Laser	Detector	Primary fluoro-chrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PerCP, PerCP-Cy5, PerCP-Cy5.5, 7-AAD	665LP	700/54
	C	PE, PI	560LP	586/42
	D	FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488	507LP	527/32
	E	Side scatter (SSC)	ND10 ^a	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
	B	APC, Alexa Fluor® 647	660/10	660/10
Violet/ 405 nm	A	BD Horizon Brilliant Violet 786	755LP	None
	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.

Fluidic components

Introduction

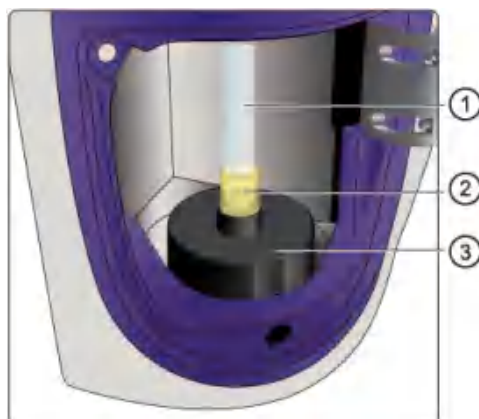
Laser light is focused on the sample core stream within the flow cell. Fluorescent molecules excited by the different laser

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 window to observe the loaded tube.



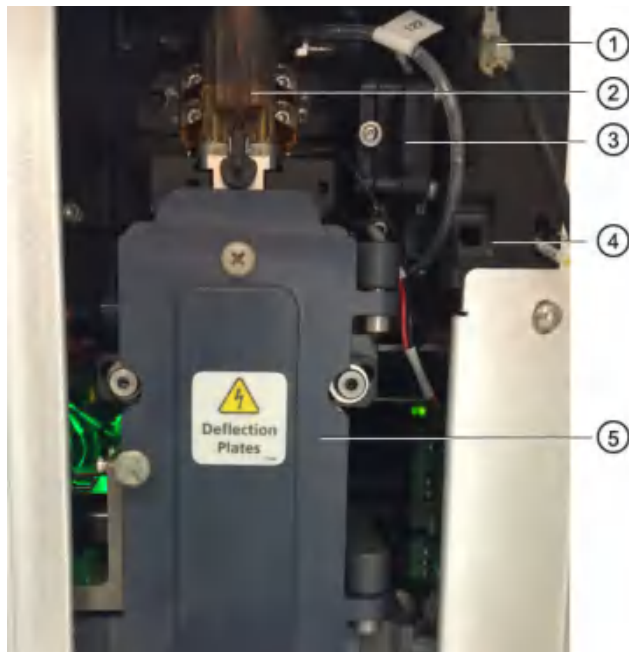
No.	Description
1	Sample tube
2	Tube holder
3	Sample loading port

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

No.	Description
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 FACSCorus software

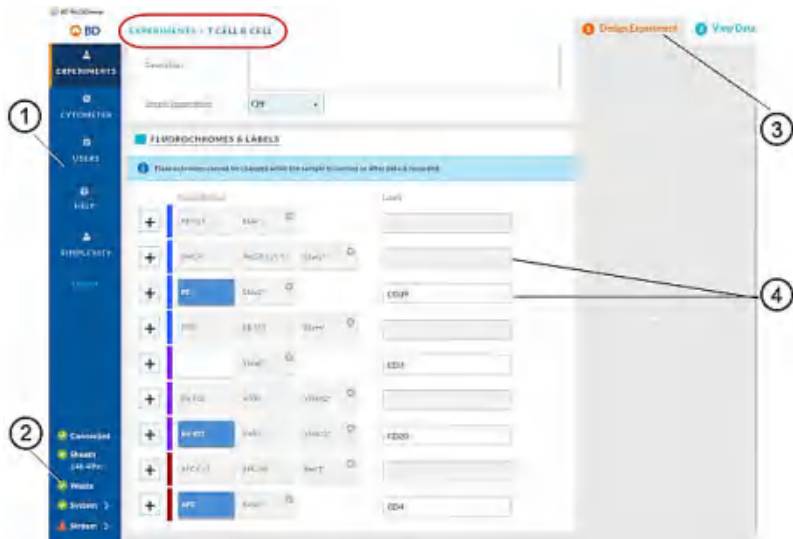
Introduction

BD FACSCorus 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.

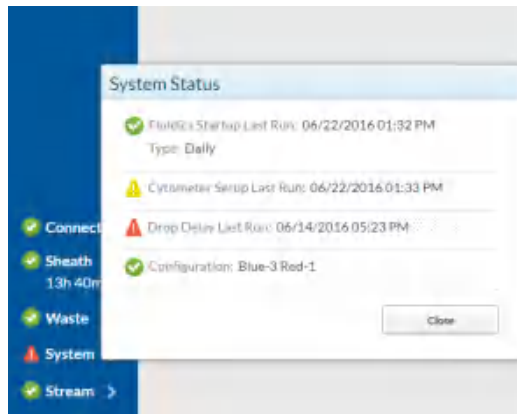


No.	Description
1	Navigation bar
2	Status indicators
3	Guided workflow
4	Data entry fields

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.	

3

System startup and shutdown

This chapter covers the following topics:

- [System startup \(page 34\)](#)
- [Fluidics startup \(page 36\)](#)
- [Cleaning \(page 37\)](#)
- [About CS&T reports \(page 38\)](#)
- [Editing your user profile \(page 40\)](#)
- [Adding, editing, or deleting user accounts \(page 41\)](#)
- [Shutting down the system \(page 41\)](#)

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 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

These are optional steps that can be skipped depending on whether the procedure followed on the last shutdown included a cleaning step. The last shutdown procedure is indicated on the screen.

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 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 6 months (for the same configuration and bead lot)
 - After preventative maintenance and major service procedures
- 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.

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 FACSCorus 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
Setup beads	BD CS&T RUO beads with bead lot file BD FC beads	BD
Accudrop beads	BD FACS Accudrop beads	BD

Fluidics startup

Procedure

To perform a daily or extended fluidics startup:

1. Press the Power button on the front of the instrument.
2. Start the BD FACSCorus 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.

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 cleaning** if no cleaning is needed.
-

Cleaning

Introduction

This procedure can be skipped if a cleaning was done as part of the shutdown procedure. 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 following tasks.

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

- Prepare for Aseptic Sort
- Closed Loop Nozzle

- Bleach Decontamination
 - DI Water Flush
 - Ethanol Decontamination
 - Sheath and Waste Tanks
 - Start Sheath Filter Purge and Prime the Stream
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 following tasks:
 - Flow Cell Clean
 - Closed Loop Nozzle
 - Sheath and Waste Tanks
 - DI water
 2. Click **Close** to return to the **Cleaning** dialog.
-

More information

- [Preparing for aseptic sort \(page 122\)](#)
 - [Cleaning the flow cell \(page 97\)](#)
-

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

Report section	Field	Description
	Max channel	Maximum value for the acceptable linear range of the detector
	Electronic noise RSD	Robust standard deviation (rSD) of the electronic noise in the particular detector; used to predict the minimum acceptable signal levels required for the best attainable resolution and sensitivity for the system
	Qr	Relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector
	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	<p>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.</p> <p>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.</p> <p>When you delete a user, any experiments, data records, sort reports, or fluorochromes created by the user are also deleted.</p>
Procedure	<p>To add, edit, or delete a user:</p> <ol style="list-style-type: none">1. Click USERS.2. Do any of the following actions:<ul style="list-style-type: none">• 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.
Before you begin	For the long-term shutdown procedure, you need at least 1 L of 70% ethanol and an ethanol filter.

Ensure that you know how to do the following procedures:

- [Changing the nozzle \(page 91\)](#)
 - [Filling the sheath tank \(page 107\)](#)
 - [Changing the fluid filter \(page 110\)](#)
 - [Emptying the waste tank \(page 105\)](#)
-

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.
-

4

Experiment

This chapter covers the following topics:

- [Experiment overview \(page 44\)](#)
- [Creating an experiment \(page 44\)](#)
- [Designing an experiment \(page 47\)](#)
- [Defining view data \(page 47\)](#)
- [Setting up sorting \(page 50\)](#)
- [Calculating compensation \(page 53\)](#)
- [Updating compensation standards \(page 57\)](#)
- [Loading collection devices \(page 59\)](#)
- [Sorting \(page 65\)](#)
- [Viewing reports \(page 68\)](#)

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.

Selections are defined by filling in the data fields or making selections on each tabbed workspace. Experiments can be used as templates for later experiments.

Experiments are saved and displayed in a list in the Experiment workspace. Saved experiments can be opened and updated as needed.

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.

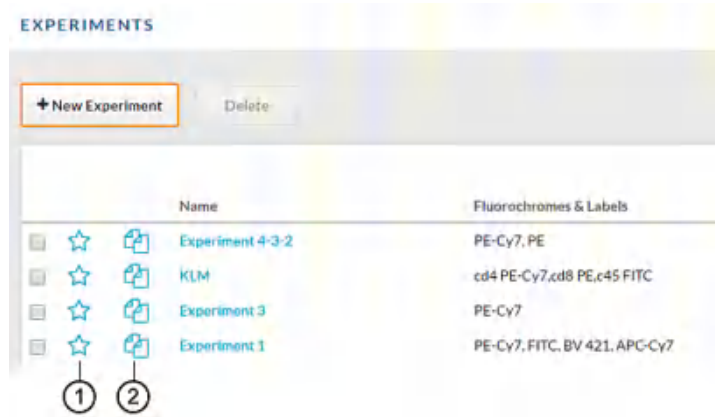
If using a blank experiment, use the tabbed workspaces to define the experiment parameters.

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

Starting with a blank experiment

To create an experiment:

1. Select **Experiment** on the left navigation bar.
2. Click + **New Experiment**.



Experiment template option (1)

- Use the experiment template option if you plan to reuse a previously created experiment multiple times in the future. An experiment template retains the setup and 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 icon to create a new experiment that contains all the setup and settings of the saved experiment without any data. You can only duplicate an experiment once. If you want to duplicate the experiment more than once, use the experiment template option. When duplicate without data 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.

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 that have normalized spillover values from running BD FC beads. You can add new fluorochromes and update 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 fluorochrome to that row. Only one fluorochrome can be selected from a row.
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. Many tools are not visible unless the object is

selected or the mouse is hovering over the object (for example, plot controls and tools and data file options).

To make a pdf or print the plots, stats, or population hierarchy in the view data page, use **Ctrl+P**. Use the Windows Snip-It tool to copy and paste plots, stats, or population hierarchy.

Procedure

To define the 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 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.

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 change the population displayed in the plot, change the type of plot, or delete the plot, select the population filter or the plot options (gear) icon, update your desired settings, and click **OK**.
 - b. 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.
 - c. 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 to adjust the voltages of y- and x-axes.
 - d. To add additional dot plots, a contour map, histogram, or density plot, click the plus (+) button on 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 add more statistics or edit existing statistics, click the plus (+) button on the **Statistics** panel.
- b. Select the statistics from the **Edit Statistics** dialog and click **OK**.

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:

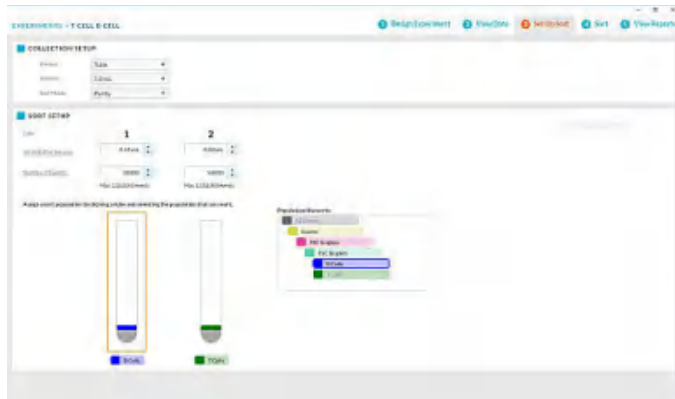
1. Click the **Set Up Sort** tab.
2. Select the details for the sort operation, such as:

- **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. 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.



4. Select the initial buffer volume that will be added to each tube or well before sorting.
5. Select the number of events to sort into each tube or well.

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

Selecting the wells**To select specific wells:**

- To select all wells in the plate, click the **Select All** button.
- 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 non-contiguous wells, use **Ctrl+click** on the required wells.

The screen indicates the location of the selected populations.

Calculating compensation

Introduction

BD FACSCorus 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.

Compensation calculation is based on ABD values, where one ABD is defined as the fluorescence of one antibody bound to a CD4⁺ cell.

We recommend updating FC bead compensation every sixty (60) days.

About compensation calculations

BD FACSCorus software can calculate compensation using stored FC beads spillover values, new 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.

Using new user-defined control values

The default FC bead spillover values in any experiment can be overwritten with user-defined single-color compensation controls (beads or cells) through the update compensation function on the View Data page. The system will use the new values from these controls to calculate compensation.

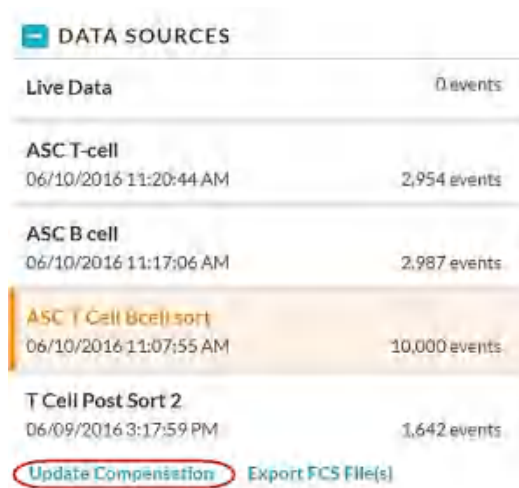
Using a combination of stored FC beads values and new user-defined control values

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

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.

Select the fluorochromes that you want to update for this experiment.

<input type="checkbox"/> Unstained Control	
<input checked="" type="checkbox"/> PE	<input checked="" type="checkbox"/> Includes Negative Population
<input checked="" type="checkbox"/> BV 786	<input checked="" type="checkbox"/> Includes Negative Population
<input checked="" type="checkbox"/> BV 421	<input checked="" type="checkbox"/> Includes Negative Population
<input checked="" type="checkbox"/> APC	<input checked="" type="checkbox"/> Includes Negative Population

3. Select the tubes you need and then click **Continue**.
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.

When the tubes are done, the system displays the list of tubes in the Data Sources panel.

8. Continue setting up the rest of the experiment parameters.
-

Updating compensation standards

Introduction

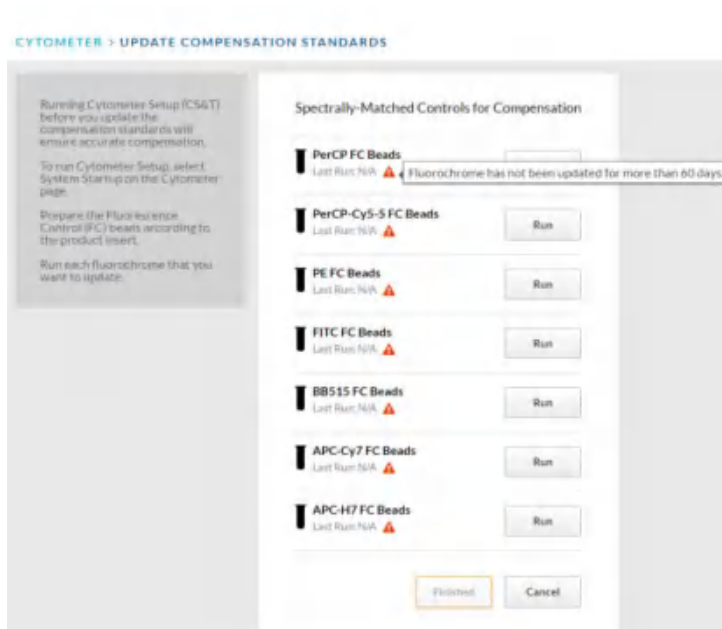
Updates to the standard fluorochrome spectral references for compensation are needed for calculating spillover values at different voltages and setting default spillover values in experiments. Run this procedure to with BD FC beads every 60 days to ensure accuracy.

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 red icons indicate the values that are older than 60 days.



3. Prepare the BD FC beads according to the product insert.
4. Follow the prompts on the screen to run each fluorochrome.
5. 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.

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)

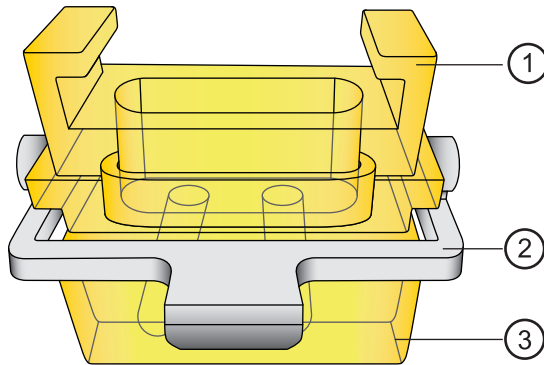


Caution! All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning cytometer surfaces. Wear suitable protective clothing, eyewear, and gloves.

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.

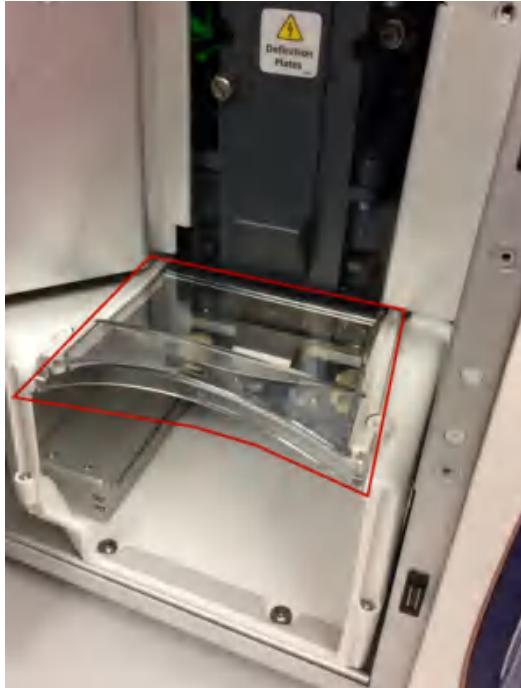


No.	Description
1	Adapter
2	Handle
3	Tube holder

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 onto 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. The splash shield allow the automated sort stage to eject and retract.

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 until it clicks into place.



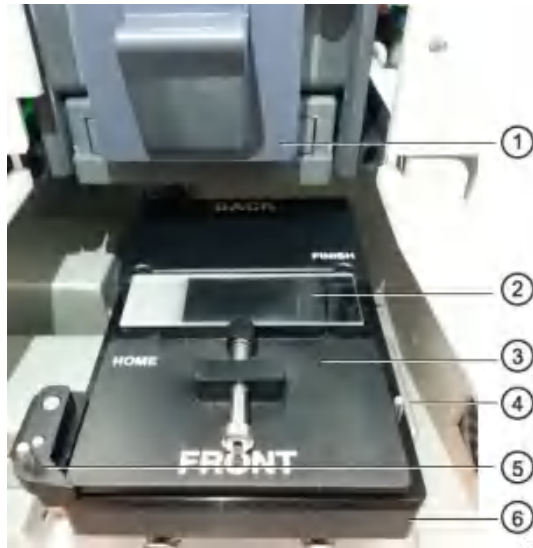
Loading microscope slides on the automated sort stage

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.

- 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

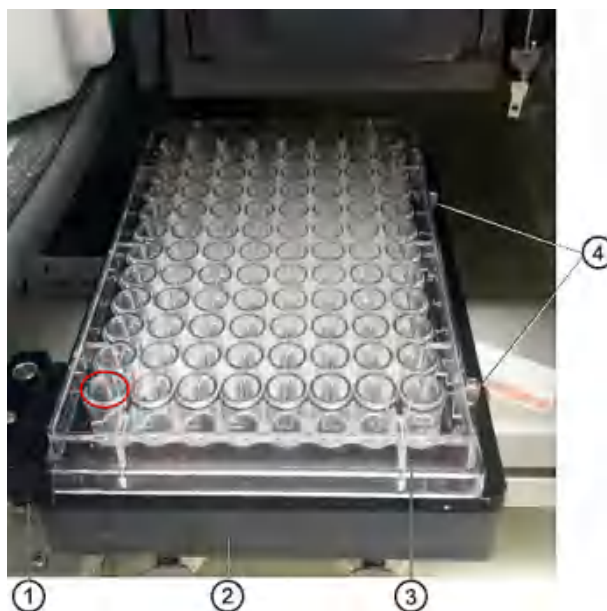
- Select **Retract** to retract the stage into place for sorting.
- Install the aerosol shield to keep any aerosols contained in the sort collection chamber.
- Close the sort collection chamber door.

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 instrument. 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. Click **Retract** to retract the plate into place for sorting.
6. Install the aerosol shield to keep any aerosols contained in the sort collection chamber.
7. 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. The sort

collection chamber door should be kept 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.

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



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



5. Refine the gates in the plots in the **Sort Population** panel. These are the plots that were selected in the **View Data** tab.
6. (Optional) If additional plots were added on the View Data tab, click **Additional Plots** to view them.
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).

If using a plate, click **Eject** or **Retract** to move the stage forward and back.
 - b. Click **Start Sort**.

The system continues 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 the same or different type.

If using a plate, click **Eject** or **Retract** to move the stage forward and back.
 - b. Click **Set Up Sort** and modify the selections as needed.
 - c. Click the **Sort** tab and click **Start Sort**.

The system continues sorting into the new collection devices using the new sort selections.

When the sort has completed successfully, a Sort Completed dialog displays.

Viewing reports

Introduction

Data 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. 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. Select **Print** to print a PDF of the report.

- Click **Export Report > Print > 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: Simplexly admin Application Name: BD FACSCorus
 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	PE	APC	BV 421	BV 786	
FSC	163	PE	100.00	0.01	0.00	0.13
SSC	408	APC	0.00	100.00	0.00	0.50
PE	417	BV 421	0.08	0.00	100.00	0.06
APC	510	BV 786	0.00	0.48	79.15	100.00
BV 421	551					
BV 786	495					

Threshold: FSC@51454

POPULATION HIERARCHY

```

All Events
├── Scatter
│   ├── SSC Singlets
│   │   ├── FSC Singlets
│   │   │   └── B Cells
  
```

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

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5

System options

This chapter covers the following topics:

- [Overview \(page 72\)](#)
- [Using the sample temperature control \(page 72\)](#)
- [Working with the aerosol management option \(page 77\)](#)
- [Working with the biological safety cabinet \(BSC\) \(page 77\)](#)

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)
 - 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.

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.
5. Slide the tubing over the hose barbs and twist gently while installing to get the tubing completely over the barbs.
6. 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 multi-directional. The input and output hoses from the water bath can be connected to either port on the cell sorter base.

7. 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.

8. 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.

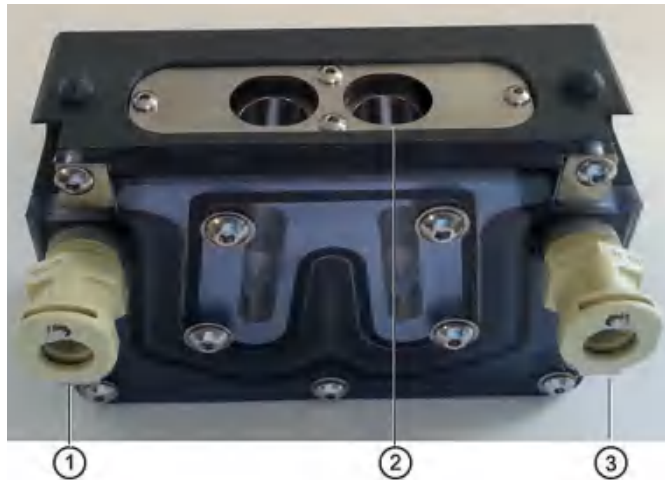
Setting up the tube holder

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 (1 or 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. Insert the collection tubes into the tube holder.
6. 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.
7. 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. 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 tubes.
 - 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 aerosol management option (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 (BSC)

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.

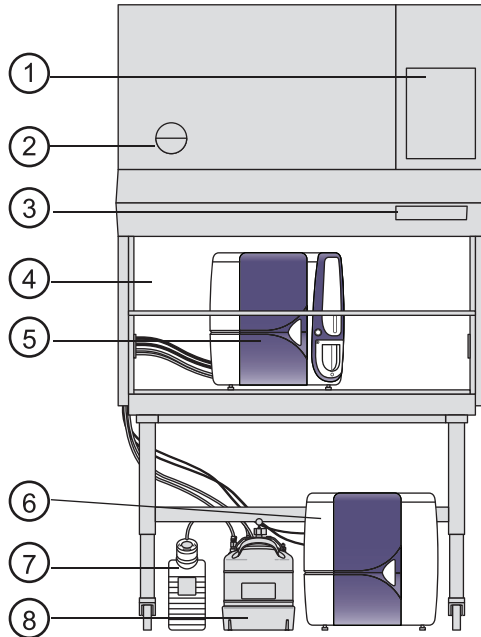
See Baker's SG 403A-HE Optimax Operator's Manual for details on the structure and operation of the cabinet.

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 aerosol management option (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.

6

Maintenance

This chapter covers the following topics:

- [Maintenance overview \(page 83\)](#)
- [Stopping the stream \(page 86\)](#)
- [Lubricating the sample injection chamber O-ring \(page 88\)](#)
- [Precautions for handling nozzles \(page 90\)](#)
- [Changing the nozzle \(page 91\)](#)
- [Cleaning the sort nozzle \(page 92\)](#)
- [Cleaning the closed-loop nozzle \(page 93\)](#)
- [Replacing the sort nozzle seal temporarily \(page 94\)](#)
- [Aligning the waste aspirator drawer to the stream \(page 95\)](#)
- [Cleaning the flow cell \(page 97\)](#)
- [Cleaning the optical filters \(page 98\)](#)
- [Cleaning the Accudrop laser window and the lower camera window \(page 99\)](#)
- [Cleaning the strobe lens window and upper camera window \(page 101\)](#)
- [Cleaning the deflection plates \(page 103\)](#)
- [Removing or installing the FSC neutral density filter \(page 104\)](#)
- [Emptying the waste tank \(page 105\)](#)
- [Filling the sheath tank \(page 107\)](#)

- [Changing the fluid filter \(page 110\)](#)
- [Purging the sheath filter \(page 112\)](#)
- [Backflushing the sample line \(page 112\)](#)
- [Replacing the sample line \(page 113\)](#)
- [Replacing the sample line filter \(page 117\)](#)
- [Removing the sheath probe \(page 120\)](#)
- [Preparing for aseptic sort \(page 122\)](#)

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 86)	As needed
Sample loading area	Lubricating the sample injection chamber O-ring (page 88)	As needed or when sample loading fails
Nozzle and flow cell	Changing the nozzle (page 91)	As needed
	Cleaning the sort nozzle (page 92)	When you see indications of clogging
	Cleaning the closed-loop nozzle (page 93)	When you see indications of salt buildup or clogging
	Replacing the sort nozzle seal temporarily (page 94)	If the seal is lost or damaged and you do not have a replacement
	Aligning the waste aspirator drawer to the stream (page 95)	If you install a sort nozzle that is new or different from the one that came with the instrument
	Cleaning the flow cell (page 97)	As needed

Category	Maintenance task	When to perform
Optics	Cleaning the optical filters (page 98)	As needed
	Cleaning the Accudrop laser window and the lower camera window (page 99)	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 101)	When smudges appear in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed
	Cleaning the deflection plates (page 103)	When you have trouble viewing the side stream
	Removing or installing the FSC neutral density filter (page 104)	As needed

Category	Maintenance task	When to perform
Fluidics	Emptying the waste tank (page 105)	As indicated by the software and whenever you fill the sheath tank
	Replacing the waste filter cap	Monthly
	Filling the sheath tank (page 107)	Daily or as needed
	Changing the fluid filter (page 110)	Every 3 months or as needed
	Purging the sheath filter (page 112)	After you install a new sheath filter and whenever you observe problems with the stream
	Backflushing the sample line (page 112)	When you observe sample carryover, or after you run samples with adherent cells or dye
	Replacing the sample line (page 113)	Every 4–6 months or when decreased event rates indicate that the sample line might be clogged
	Replacing the sample line filter (page 117)	When decreased event rates indicate that the sample line might be clogged
	Removing the sheath probe (page 120)	As needed, before you autoclave the sheath tank
	Preparing for aseptic sort (page 122)	Before you sort

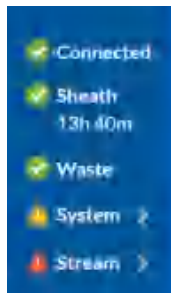
Stopping the stream

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**.

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 \(page 86\)](#).

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 \(page 91\)](#).

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 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 plate voltage on.

Procedure**To clean the external surfaces:**

Choose 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. Let it 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 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 change the nozzle.

About this task

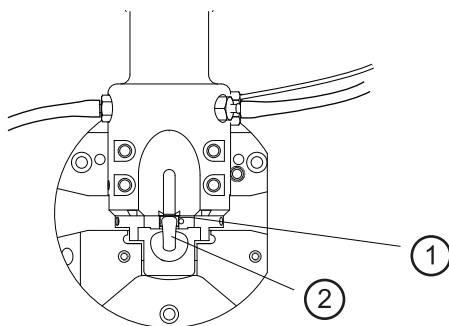


Caution! All biological specimens and materials coming into contact with biological specimens can transmit potentially fatal disease. Handle nozzles as if they are capable of transmitting infection. Wear suitable protective clothing, eyewear, and gloves.

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

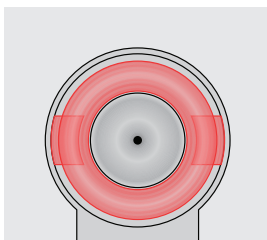
waste aspirator drawer to the stream to ensure proper sort setup. See [Aligning the waste aspirator drawer to the stream \(page 95\)](#).

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 \(page 91\)](#).

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 test tube containing DI water and 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 use lens paper to wipe it.
 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 \(page 91\)](#).

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 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 test tube containing DI water or a mild detergent and 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.

- 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

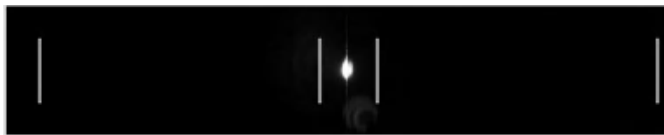
To align the waste aspirator drawer to the stream:

- 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.)



- If you are able to make the stream appear between the two hash marks, slowly tighten the hex screws, ensuring that the stream position does not move. Then close the flow cell access door and the sort chamber door. You are done.
 - If you are unable to make the stream appear between the two hash marks, continue to step 5.
5. Open the sort collection chamber door.

6. Ensure that the red warning light next to the sort block door is off, indicating that the deflection plates are off.
7. Open the sort block door by turning the thumbscrew on the front of it.
8. Gently nudge the sort block to the left or right until the stream hits directly in the center of the waste aspirator drawer.



9. Slowly tighten the screws, ensuring that the stream position in the drawer does not move.
 10. 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 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 \(page 91\)](#)
 - [Emptying the waste tank \(page 105\)](#)
 - [Filling the sheath tank \(page 107\)](#)
-

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 \(page 86\)](#).

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.

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

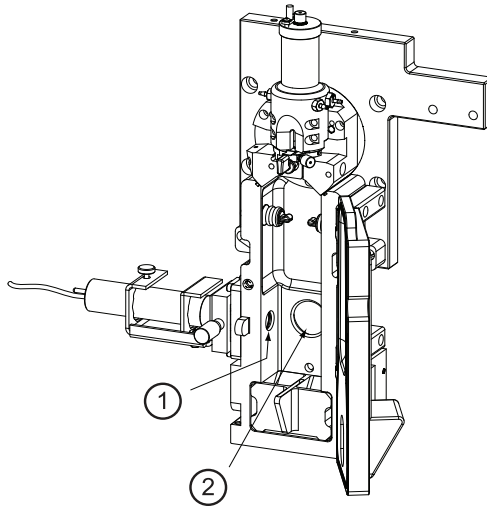
You should clean the Accudrop laser window and the lower camera window when the software is unable to set the drop delay, or when the software is unable to verify the side streams when sorting is started.

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 \(page 86\)](#).
 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

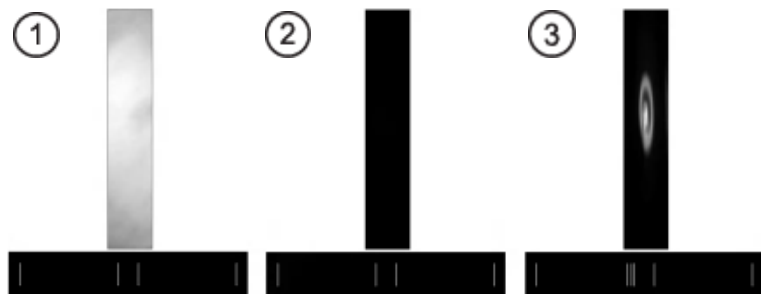
Introduction

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.

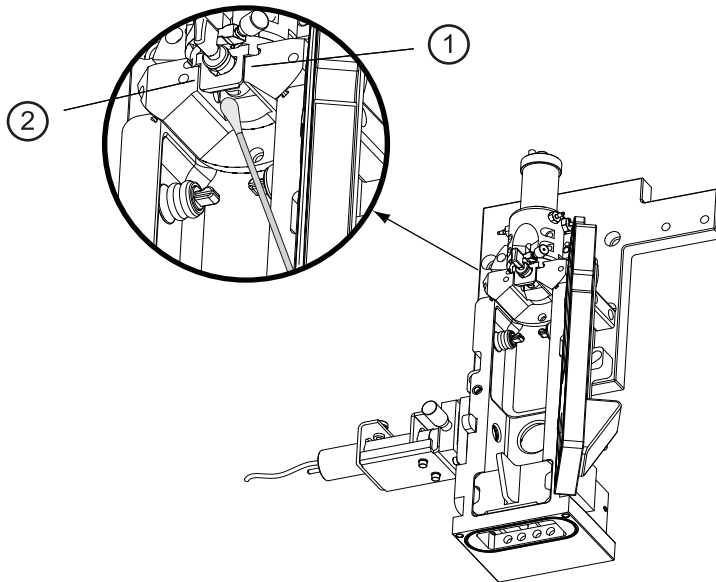
Procedure

To clean the strobe lens window and upper camera window:

1. Ensure that the stream is turned off. See [Stopping the stream \(page 86\)](#).

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 \(page 86\)](#).
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. Choose one of the following options:
 - Remove the FSC ND filter by loosening the set screw and pulling the filter out of the slot.



- Install the filter by sliding it into the slot with the filter side facing forward and the label facing the flow cell. Tighten the set screw.

Note: You can remove the nozzle holder to get better access to the ND filter slot.

3. 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:

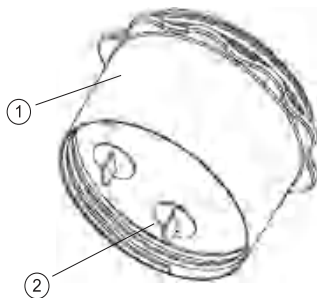
1. Turn off the stream. See [Stopping the stream \(page 86\)](#).
2. Disconnect the sensor and fluid line cap from the waste tank.

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.

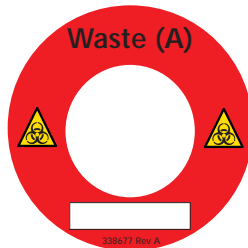
Note: Do not wet the cap on top of the trap (1). If you see liquid inside the trap, remove the drain plug (2) and fully drain the liquid before you replace the plug.



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.



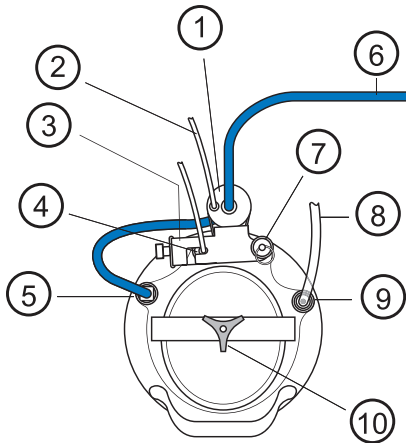
8. Reconnect the sensor and fluid line.
-

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 breakoff 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

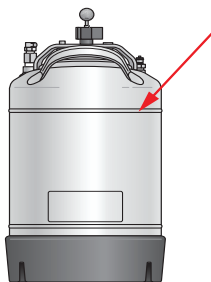
Note: Avoid getting the top of the sheath sensor wet. If the top of the sheath sensor gets wet, wipe it dry. The sheath sensor will not detect the level of sheath fluid accurately if the top of it is wet.

Procedure

To fill the sheath tank:

1. Turn off the stream. See [Stopping the stream \(page 86\)](#).
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 \(page 112\)](#)

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.

When you use the filter as a sheath filter, you should change it every 3 months, or when increased debris in an FSC vs SSC plot indicates that the sheath filter needs to be replaced.

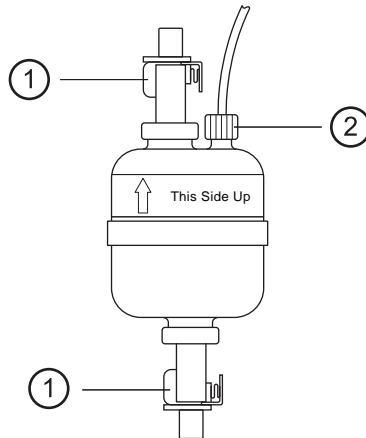
Spare filters are included with the accessory kit.

Procedure

To change the fluid filter:

1. Turn off the stream. See [Stopping the stream \(page 86\)](#).
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.

- Disconnect the filter by pressing the metal tabs on each end.



No.	Description
1	Tabs
2	Filter purge line nut

- Disconnect the filter purge line by unscrewing the nut.
- Observe the direction of the flow through the filter and replace the filter with a new one in the same orientation.
- Reconnect the filter purge line.
- Reconnect the air line and check for leaks when the pressure is turned on.
- Write the current date on the filter so that you will know when to replace it.

What to do next

[Purging the sheath filter \(page 112\)](#)

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:**
Choose 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)



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.

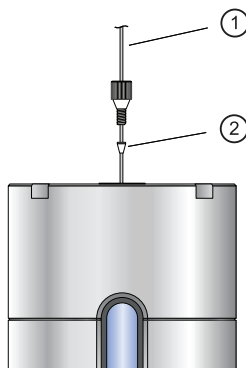
If the ferrule is left behind in the flow cell, gently push the tip of the ferrule-removal tool into the top of the ferrule and pull the ferrule straight out.

- 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.



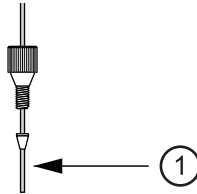
- g. Unscrew the nut at the top of the sample injection chamber.
- h. If a sample line filter is installed on the sample line, remove it by pushing the sample line down so that the end is below the chamber and you can remove the filter.
- i. Slowly pull the sample line (1) out from the top and ensure 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. 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.

- b. 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.

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

Note: If the ferrule is damaged, replace it with a spare from the accessory kit.



Caution! Do not bend the sample line during insertion.

- d. Insert the sample line into the sample injection chamber fitting and push the tubing from the top until it is slightly above the bottom of the chamber viewing window.

- e. 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.

- f. Push the pinch valve tubing back into the pinch valve.
 - g. 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

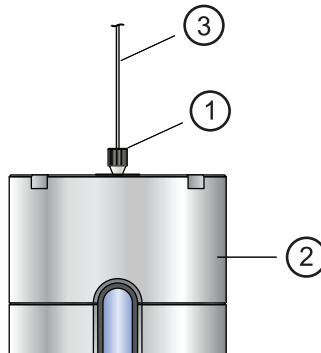
All biological specimens and materials can transmit potentially fatal infection. To prevent exposure to biohazardous agents, add bleach to the waste container (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective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

Procedure**To replace the sample line filter:**

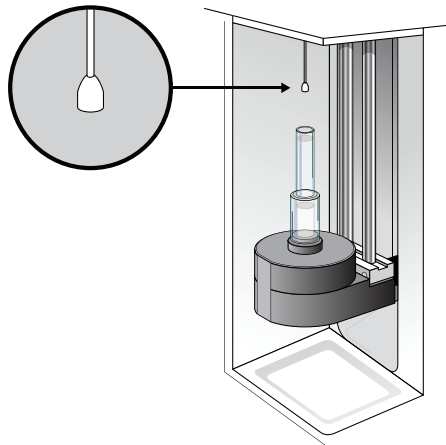
1. On the **Cytometer** page, select **Replace Sample Line Filter**, and then click **Start**.
2. Complete the Sample Line Filter step:
 - a. Remove the sample line access door from the cytometer.
 - b. Pull the pinch valve tubing out of the slot in the pinch valve.



- 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

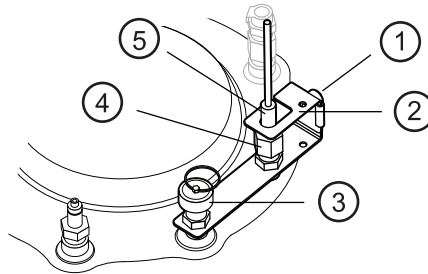
Note: Do not autoclave the sheath probe. It is not designed to withstand the conditions of autoclaving.

Procedure

To remove the sheath probe:

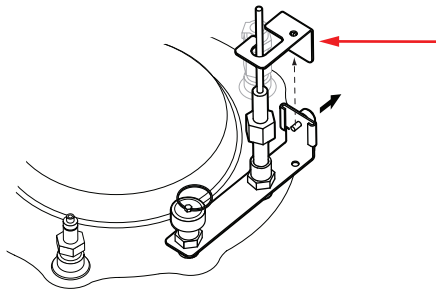
1. Turn off the stream. See [Stopping the stream \(page 86\)](#).
2. Disconnect the air line from the sheath tank.
3. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.

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.



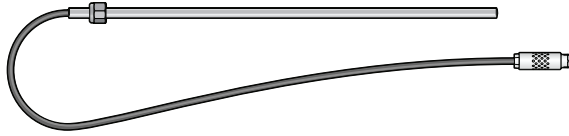
No.	Description
1	Thumbscrew
2	Containment device
3	Pressure relief valve
4	1/16-inch nut
5	Sheath probe

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 1/16-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 \(page 91\)](#)
- [Filling the sheath tank \(page 107\)](#)
- [Changing the fluid filter \(page 110\)](#)
- [Emptying the waste tank \(page 105\)](#)

You need the following items:

- Four fluid filters
- At least 1.75 L of 10% bleach solution
- At least 1.75 L of DI water
- At least 1.75 L of 70% ethanol solution
- At least 2 L of 1X PBS

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 procedure takes 30 minutes to complete.

Procedure

To prepare for aseptic sort:

1. Remove the sheath probe. See [Removing the sheath probe \(page 120\)](#).
2. Autoclave the sheath tank according to your organization's protocols.
3. On the **Cytometer** page in the software, select **System Startup**.
4. 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.

5. Select **Prepare for Aseptic Sort**, and then click **Start**.
 6. Complete the steps in the wizard.
-

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7

Technical specifications

This chapter covers the following topics:

- [Optical specifications \(page 126\)](#)
- [Fluidic specifications \(page 128\)](#)
- [Sample input formats \(page 128\)](#)
- [Collection devices \(page 128\)](#)
- [Electronic and software specifications \(page 129\)](#)
- [Physical specifications \(page 129\)](#)

Technical specifications

Optical specifications

Parameter	Value
Excitation laser	<ul style="list-style-type: none"> • 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) • 3-laser (blue, red, violet), 6-color (2-2-2) • 3-laser (blue, red, violet), 9-color (4-2-3)
Laser specifications	<p>Blue laser</p> <ul style="list-style-type: none"> • Wavelength: 488 nm • Optical power: 16 mW nominal power • Beam spot size: $9 \pm 3 \mu\text{m} \times 67 \pm 5 \mu\text{m}$ <p>Red laser</p> <ul style="list-style-type: none"> • Wavelength: 640 nm • Optical power: 36 mW nominal power • Beam spot size: $9 \pm 3 \mu\text{m} \times 67 \pm 5 \mu\text{m}$ <p>Violet laser</p> <ul style="list-style-type: none"> • Wavelength: 405 nm • Optical power: 36 mW nominal power • Beam spot size: $9 \pm 3 \mu\text{m} \times 67 \pm 5 \mu\text{m}$
Laser alignment	Fixed and spatially separated alignment of all lasers with the cuvette flow cell
Beam divergence angle (full-angle)	<p>488 nm: <1.2 mrad</p> <p>640 nm: <1.3 mrad</p> <p>405 nm: <1.0 mrad</p>

Parameter	Value
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.
Detection channels	Forward scatter (FSC), side scatter (SSC), and up to nine fluorescence. See the BD FACSMelody <i>Filter Guide</i> for laser and detection configurations and optical filter specifications.
Fluorescence and side scatter detection	<ul style="list-style-type: none"> • 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 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 <i>Filter Guide</i> 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 FACSCorus software: 4°C, 22°C, 37°C, and 42°C, or off
Sample agitation:	Adjustable through BD FACSCorus software to keep the sample constantly suspended
Flow cell	Quartz cuvette
Nozzle	100- μ m nozzle is removable and can be sonicated. A registered key-fit position at the bottom of the cuvette provides fixed stream alignment.
Fluidic tanks	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 FACSCorus 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 (53.8 x 34.5 x 91.9–98.4 in.)
Sample input temperature control (optional)	42.9 x 91.3 x 47.1 (16.90 x 35.95 x 18.55 in.)

Parameter	Value
Aerosol Management Option	45.21 x 38.1 x 54.61 17.8 x 15 x 21.5 in.)
See the BD FACSMelody <i>Site Preparation Guide</i> 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)

8

Troubleshooting

This chapter covers the following topics:

- [Troubleshooting overview \(page 132\)](#)
- [Startup troubleshooting \(page 133\)](#)
- [Acquisition troubleshooting \(page 137\)](#)
- [Sorting troubleshooting \(page 143\)](#)
- [Electronics troubleshooting \(page 146\)](#)

Troubleshooting overview

Introduction

BD FACSCorus 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 FACSCorus 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 \(page 11\)](#).

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

- [Startup troubleshooting \(page 133\)](#)
- [Acquisition troubleshooting \(page 137\)](#)
- [Sorting troubleshooting \(page 143\)](#)
- [Electronics troubleshooting \(page 146\)](#)

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 93) .
	Salt buildup in the nozzle location between the flow cell and the locking lever	Clean the area to remove the salt build up.
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 107) or Emptying the waste tank (page 105) .
	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 91) .
	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 101) .
	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 92) . If the nozzle appears damaged, replace it. See Changing the nozzle (page 91) Click Experiment > View Data to restart the stream.
	Debris in flow cell	Clean the flow cell. See Cleaning the flow cell (page 97) .

Startup troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
	Air in sheath line or filter	Purge the sheath filter. See Purging the sheath filter (page 112) . Run daily fluidics startup. See Fluidics startup (page 36) .
	Dry sheath filter	Purge the sheath filter. See Purging the sheath filter (page 112) .
	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 91) .
	Clogged or damaged sort nozzle	<p>Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope.</p> <p>If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 92).</p> <p>If the nozzle appears damaged, replace it. See Changing the nozzle (page 91).</p> <p>Click Experiment > View Data to restart the stream.</p>
	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 91) .
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	<p>Prepare a new CS&T sample with the proper concentration as instructed in the product insert.</p> <p>Clean the flow cell. See Cleaning the flow cell (page 97).</p> <p>Close the sort block door and the flow cell access door properly.</p>

Startup troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
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 99) .

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 112) . If that does not work, replace the sample line assembly. See Replacing the sample line (page 113) .
	Sample line filter is clogged	Replace the sample line filter. See Replacing the sample line filter (page 117) .
	Sample is not mixed properly	Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 47) .
	Threshold is not set to correct parameter	Set the threshold to the correct parameter for your application. See Defining view data (page 47) .
	Threshold setting is too low or too high	Adjust the threshold setting. See Defining view data (page 47) .

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 47) .
	Threshold setting is too low or too high	Adjust the threshold setting. See Defining view data (page 47) .
	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 the setting for the cell size is appropriate for your sample.
	Sample preparation is inadequate	Ensure 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. See Defining view data (page 47) .
	Sheath tank is low	Fill the sheath tank. See Filling the sheath tank (page 107) .
	Sample preparation is inadequate	Ensure 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.

Acquisition troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
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 47) .
	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 47) .
	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 47) .
	Sample is too dilute	Concentrate the sample.
	Threshold setting is too high	Adjust the threshold setting. See Defining view data (page 47) .
	Sample line is clogged or kinked	Backflush the sample line. See Backflushing the sample line (page 112) . 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 113) .
	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 117) .

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 47) and Calculating compensation (page 53) .
	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 97) . Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 92)
	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 47) .
	Sample preparation is inadequate	Ensure 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 47) .
	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 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 110) .
Processed events are <90%	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 47) .
	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 47) .
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 88) .

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 97) . Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 92) .
	Sheath or waste tank full	Empty the waste tank or fill the sheath tank. See Emptying the waste tank (page 105) and Filling the sheath tank (page 107) .
Unable to start sort	BD FACSCorus software cannot locate the side streams	Clean the lower camera window. See Cleaning the Accudrop laser window and the lower camera window (page 99) . 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 103) .
	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 103) .

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. Reinsert the nozzle. See Changing the nozzle (page 91) .
	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 92) . If the nozzle appears damaged, replace it. See Changing the nozzle (page 91) and Replacing the sort nozzle seal temporarily (page 94) . Click Experiment > View Data to restart the stream.
	Dirty deflection plates	Clean the deflection plates. See Cleaning the deflection plates (page 103) .
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements. See Setting up sorting (page 50) .
	Particles too big for sort nozzle	Verify 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.
Erratic sort rate	Flow rate is too high	Decrease the flow rate.

Sorting troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unexpected sort results	Incorrect drop delay	Run drop delay. See System startup (page 34) .
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements. See Setting up sorting (page 50) .
	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 FACSCorus 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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