BD FACSMelody™ Cell Sorter User's Guide

23-18120(04)
2022-05

CE For Research Use Only

Laser safety information

Class 1 Laser Product.

Regulatory information

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

FCC information

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

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 Réglement sur le matériel brouilleur du Canada.

History

Revision	Date	Change made
23-18120-00	2016-07	Initial release
23-18120-01	2017-04	Added yellow-green laser, index sorting and optional filters
23-18120-02	2018-06	Added automated stage alignment
23-18120-03	2020-02	Added four-way sort Added additional maintenance procedures and troubleshooting information for the sample line

Revision	Date	Change made
23-18120(04)	2022-05	Added the Backup and Restore utility and additional information about managing data on the workstation Updated images to reflect some modified screens Added detailed information about the BD aerosol management option (AMO) Added information about plot parameters and bi-exponential scaling Added additional information about processes and maintenance procedures Added additional information and graphics for ease of use Added additional learning resources Added a new safety symbol for potential injury Removed the safety symbol for heavy lifting Added more entries to startup toubleshooting topic.

Contents

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Introduction

This chapter covers the following topics:

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.

There is also a series of training videos on your workstation (along with this user's guide) to help you with routine maintenance tasks and troubleshooting steps.

This guide includes:

- Information about system hardware and components, a basic overview of the 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/Resource	Description
BD FACSMelody™ Cell Sorter Site Preparation Guide	Provides the site requirements. Read this guide before the system is installed.
BD FACSMelody™ Cell Sorter Safety and Limitations Guide	Provides safety guidance and system limitations. Read this guide before running the system.
BD FACSMelody™ Cell Sorter Quick Reference Guide	Provides information for using the instrument. Read this guide before running the system.
BD® CS&T RUO Beads technical data sheet	Provides instructions on preparing the BD® CS&T RUO Beads for quality control.
BD® FC Beads technical data sheet	Provides instructions on preparing the BD® FC Beads for compensation control.
BD FACS™ Accudrop technical data sheet	Provides instructions on preparing the BD FACS™ Accudrop Beads for calculating drop delay.
Information Security Guidelines	Provides recommendations regarding the security of the workstation. Refer to the BD FACSMelody™ product page on bdbiosciences.com to find the document corresponding to your workstation.
BD FACSMelody™ video tutorials	Provide a set of graphical, step-by-step instructions on various maintenance tasks and troubleshooting steps.

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
	Hand crush/Force from side hazard
[a] Although these symbols appear in color on the instrument, they might be printed in black and white. Their meaning remains unchanged.	

Technical support

Introduction

This section describes how to get technical support.

Contacting technical support

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

When contacting BD Biosciences, have the following information available:

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

Ordering replacement parts

To order replacement parts:

1. Go to bdbiosciences.com.
2. Select **Home > Instruments** > **Research Instruments > Research Cell Sorters > BD FACSMelody™ > Products > Instruments > BD FACSMelody™ Consumables**.
3. Select the materials to order.

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About the system

This chapter covers the following topics:

System overview

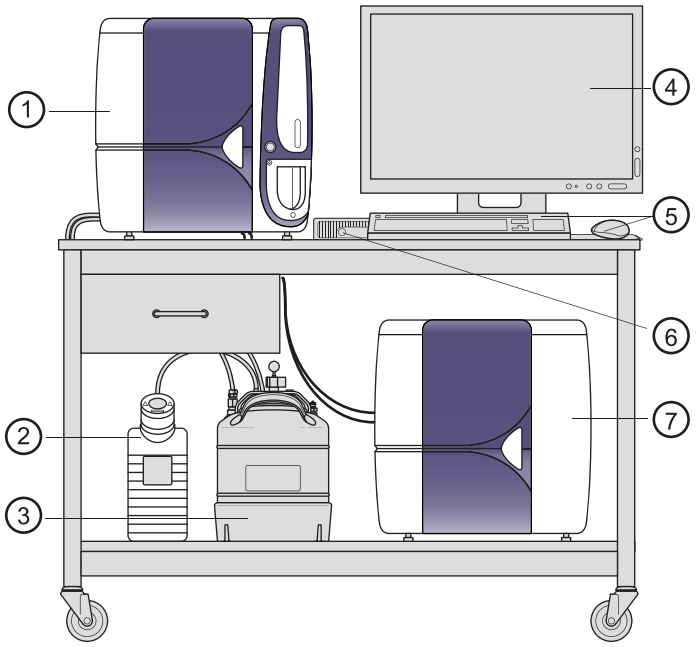
Introduction

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

About the system

The BD FACSMelody™ system includes the BD FACSMelody™ cell sorter, sheath and waste tanks, workstation with accessories, and BD FACSChorus™ software, together with beads and reagents. 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	Cytometer unit[a]
2	Waste tank
3	Sheath tank
4	Monitor
5	Keyboard and mouse
6	Computer
7	Electronics unit

[a] For an illustration of the cytometer unit (or cell sorter), see Main components (page 19).

Only use a waste or sheath tank that is provided with the BD FACSMelody™ system.

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.

Configurations are available with up to 3 lasers and provide the ability to analyze up to 9 colors (11 parameters). The heptagon detector array takes the guesswork out of changing filters and ensures that the correct filters and mirrors are installed.

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

BD FACSChorus™ software

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

Workstation

The BD FACSMelody™ system includes a desktop computer with the Microsoft® Windows® 10 operating system installed, a wireless keyboard and mouse, and a 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: Keep the fluidic tanks in the same location that they were placed in during installation. Placing the fluidic tanks at a higher level than the instrument can cause incorrect pressure and increase the sheath flow rate.

Beads, reagents, and assays

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

BD FACS™ Accudrop Beads are used to automatically set an accurate drop delay value. The Accudrop laser is aligned with the center and side (sorting) streams. BD FACSChorus™ software optimizes the drop delay by

sorting the 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. BD research assays are available as predefined modules that can be used with standard BD reagents to support a wide range of applications. Worksheets with plots and gates are already set up for acquisition, sorting, and analysis. The research assays can also be used as a starting point for creating customized experiments and assays.

Supported sort collection devices

The BD FACSMelody™ system supports two- and four-way sorting on the following sort collection devices:

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

An automated stage that sorts into multiwell plates and microscope slides is available as an option. The following slides and plates are supported:

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

Options

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

- Air compressor
- BD FACSMelody™ Temperature Control
- Aerosol management option (AMO)
- Biological safety cabinet (BSC)
- Remote diagnostics with BD Assurity Linc™ software
- Plate sorting, using the optional automated stage

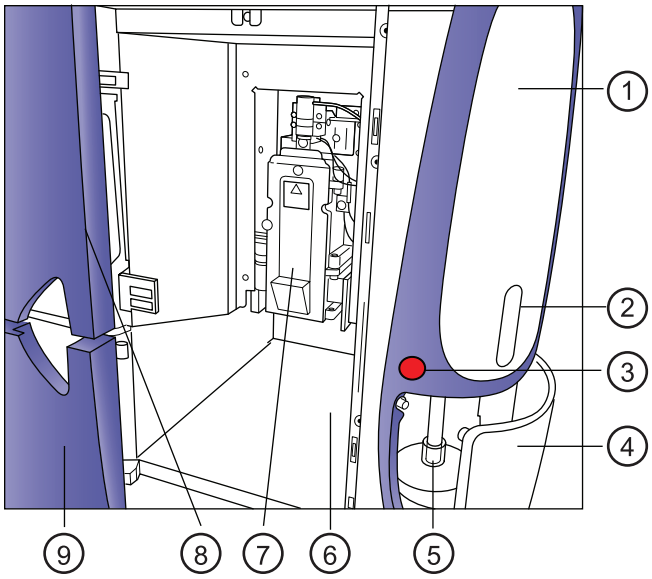
Instrument overview

Introduction

The BD FACSMelody™ cell sorter consists of three subsystems (fluidics, optics, and electronics) that are located in two units, with one tank for sheath fluid and one tank for waste. The two units are connected with two umbilical cables which contain fluidic, electrical, and fiber optic cables.

Main components

The following image shows details of the cytometer unit.



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

BD FACSChorus™ software

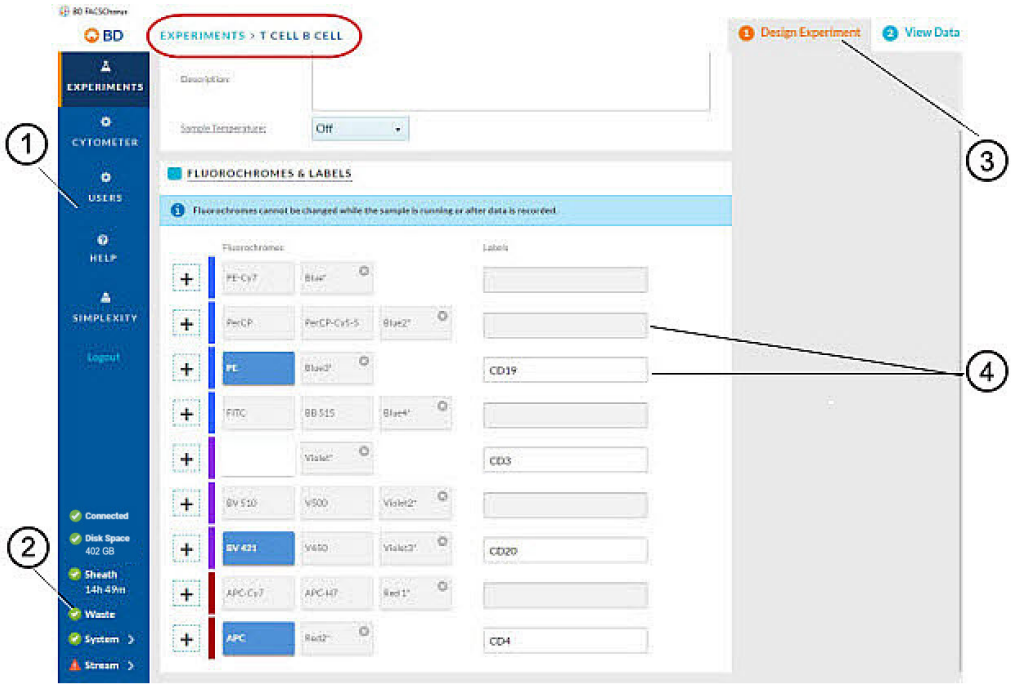
Introduction

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

Screen design

The screen includes a navigation bar on the left with a list of tasks. Selecting a task on the navigation bar displays 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 the assigned order, or can be skipped.

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

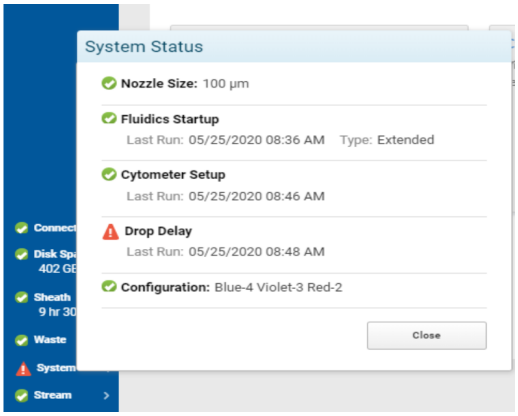


No.	Description
1	Navigation bar
2	Status indicators
3	Guided workflow
4	Data entry fields

System status indicator

The System status indicator on the navigation bar is an aggregate of five categories: Nozzle Size, Fluidics Startup, Cytometer Setup, Drop Delay, and Configuration. The System status indicator shows the highest alert level that was flagged across all five 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 displays and provides the details for the five categories.



If problems occur with the configuration of the instrument, 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 Nozzle Size, 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
Nozzle Size	Red	The sort nozzle is not detected.	Ensure that sort nozzle is inserted and the O-ring is facing up.
Fluidics Startup	Red	System startup has not been performed for more than 24 hours.	On the Cytometer page, select System Startup, and then select a Fluidics Startup option.
Cytometer Setup	Red	The last Cytometer Setup failed. Cytometer Setup must be performed due to a change in the optical configuration. 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 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.	On the Cytometer page, select System Startup and complete the Drop Delay.
	Yellow	Drop Delay has not been performed for more than 24 hours.	

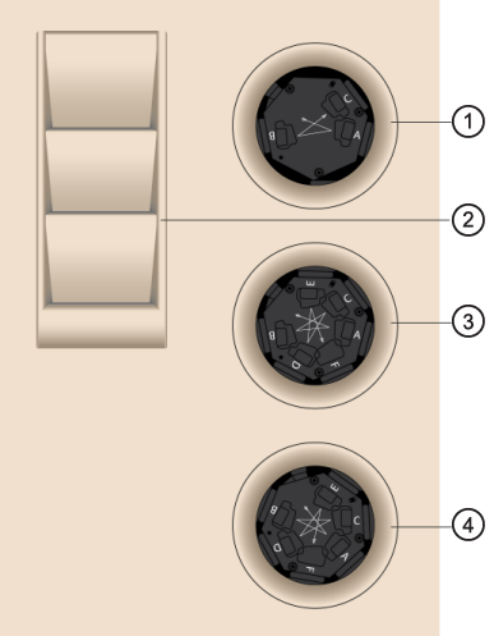
Optical components

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 unit, behind the filter access door. The detectors are accessible when the door is open. The following figure shows the locations of the optical components in a 3-laser instrument.



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

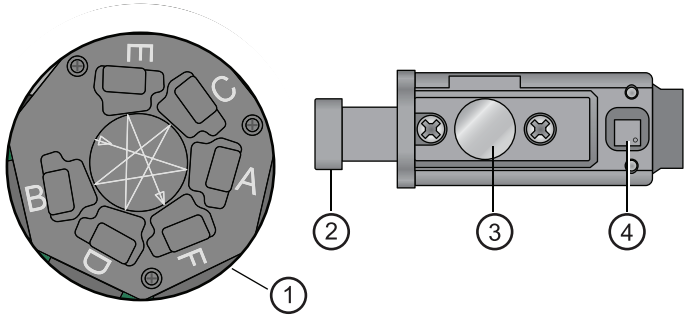
Detector arrays

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

Filter holders

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

The following figure shows a heptagon and a filter holder.



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

Location of lasers

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

Optical configurations

Introduction

The BD FACSMelody™ instrument can be configured with one, two, or three lasers. The following combinations of lasers and colors are available:

Number of lasers	Number of colors
1(blue)	2-color
1(blue)	4-color
2 (blue, yellow-green)	4-color (2-2)
2 (blue, red)	6-color (4-2)
2 (blue, yellow-green)	6-color (2-4)

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

Optical configuration details

The following tables show the optical configurations for the different detector arrays for one-laser, two-laser, and three-laser instruments. The description of the abbreviations is as follows:

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

One laser 2 color

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE, PI	560LP	586/42
		Optional: YFP	535LP	545/20
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15

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

One laser 4 color

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PerCP, PerCP-Cy™5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™	605LP	613/18
	C	PE, PI	560LP	586/42
		Optional: YFP	535LP	545/20
	D	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	E	Side scatter (SSC)	ND10[a]	488/15

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: YFP	535LP	545/20
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™	605LP	613/18
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Yellow-green/ 561 nm	A	mCherry, BD Horizon™ PE-CF594, PE-Texas Red™, PI	605LP	613/18
	B	PE, DsRed	582LP	582/15

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™	605LP	613/18
	C	PE, PI	560LP	586/42
		Optional: YFP	535LP	545/20
	D	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	E	SSC	ND10[a]	488/15
Red/640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Optional: Alexa Fluor™ 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor™ 647	660/10	660/10

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: YFP	535LP	545/20
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™		613/18
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Yellow-green/561 nm	A	PE-Cy7	752LP	783/56
	B	PE-Cy5, PE-Cy5.5	665LP	697/58
	C	mCherry, BD Horizon™ PE-CF594, PE-Texas Red™, PI	605LP	613/18
	D	PE, DsRed	582/15	582/15

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PE, PI	560LP	586/42
		Optional: YFP	535LP	545/20
	C	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	D	Side scatter (SSC)	ND10[a]	488/15
Violet/405 nm	A	BD Horizon Brilliant™ Violet 786	755LP	None
	B	BD Horizon™ V500, BD Horizon Brilliant™ Violet 510, AmCyan, CFP	500LP	528/45
	C	BD Horizon™ V450, Pacific Blue™, DAPI, BD Horizon™ Violet Proliferation Dye 450, BD Horizon™ Fixable Viability Stain 450, BD Horizon Brilliant™ Violet 421	448/45	None

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE, PI	560LP	586/42
		Optional: YFP	535LP	545/20
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Red/640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Optional: Alexa Fluor™ 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor™ 647	660/10	660/10
Violet/405 nm	A	BD Horizon Brilliant™ Violet 786	755LP	None
		Optional: BD Horizon Brilliant™ V500, BD Horizon Brilliant™ Violet 510, AmCyan	500LP	528/45
	B	BD Horizon™ V450, Pacific Blue™, DAPI, BD Horizon™ Violet Proliferation Dye 450, BD Horizon™ Fixable Viability Stain 450, BD Horizon Brilliant™ Violet 421	448/45	None

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: YFP	535LP	545/20
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™	605LP	613/18
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Red/ 640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Optional: Alexa Fluor™ 700, APC-R700	705LP	720/30
	B	APC, Alexa Fluor™ 647	660/10	660/10
Yellow-green/ 561 nm	A	PE-Cy7	752LP	783/56
	B	PE-Cy5, PE-Cy5.5	665LP	697/58
	C	mCherry, BD Horizon™ PE-CF594, PE-Texas Red, PI	605LP	613/18
	D	PE, DsRed	582LP	582/15

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: YFP	535LP	545/20
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™	605LP	613/18
	B	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	C	Side scatter (SSC)	ND10[a]	488/15
Violet/405 nm	A	BD Horizon™ V500,BD Horizon Brilliant™ Violet 510, AmCyan, CFP	500LP	528/45
		Optional: BD Horizon Brilliant™ Violet 786	755LP	None
	B	BD Horizon™ V450, Pacific Blue™, DAPI, BD Horizon™ Violet Proliferaton Dye 450, BD Horizon™ Fixable Viability Stain 450, BD Horizon Brilliant™ Violet 421	448/45	None

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

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

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

Laser	Detector	Primary fluorochrome	Mirror	Filter
Blue/ 488 nm	A	PE-Cy7	752LP	783/56
	B	PE-Cy5, PerCP, PerCP-Cy5, 7-AAD, BD Horizon Brilliant™ Blue 700	665LP	700/54
		Optional: BD Horizon™ PE-CF594, PE-Texas Red™	605LP	613/18
	C	PE, PI	560LP	586/42
		Optional: YFP	535LP	545/20
	D	FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor™ 488	507LP	527/32
		Optional: GFP	510/10	510/10
	E	Side scatter (SSC)	ND10[a]	488/15
Red/640 nm	A	APC-Cy7, APC-H7	752LP	783/56
		Optional: Alexa Fluor™ 700, APC-R700	705LP	720/30
	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, CFP	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.

Changing optical configurations

Introduction

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

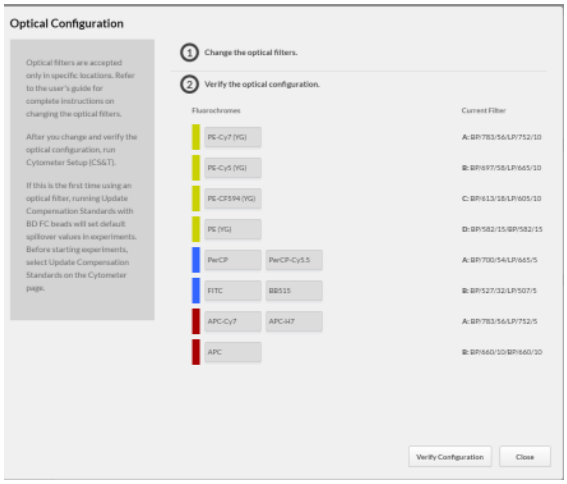
We recommend that you always verify that the new configuration is the configuration you want to use. When you select a different configuration for the first time, you must run Cytometer Setup to create a baseline for that configuration. A new baseline is determined automatically by the system the first time an optical configuration is used, and every 60 days thereafter.

Procedure

Do not attempt to open the sample loading door while a sample is being loaded and run. A load sample pin locks while a sample is being processed. Pulling on the door could damage the door or locking mechanism.

To change the optical configuration:

1. On the **Cytometer** page, select **Optical Configuration**.



2. Follow the instructions on the dialog.
3. Click **Verify Configuration** to verify that the configuration shown is the configuration you want use.

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

   The system indicates if the new configuration is invalid with an error message.
4. Run Cytometer Setup using a fresh sample of BD® CS&T RUO Beads for the new configuration.

   Note: Prepare the CS&T sample with the proper concentration of sterile phosphate buffered saline (PBS) as instructed in the product insert. Do not dilute BD® CS&T RUO Beads with water.
5. (Optional) For a new configuration, you can use normalized spillover values from BD® FC Beads for compensation. First, run BD® CS&T RUO Beads in the Update Compensation Standards workflow. After completing the Update Compensation Standards workflow, samples that are run in a new experiment will have compensated data. There will be no change to existing experiments.

6. (Optional) Create a label for the flurochrome. First, open a new or existing experiment. Find the new filter and add the flurochrome label. If the new flurochrome filter changes the configuration, load BD® FC Beads to create spillover values on the new filter and configuration.

Fluidic components

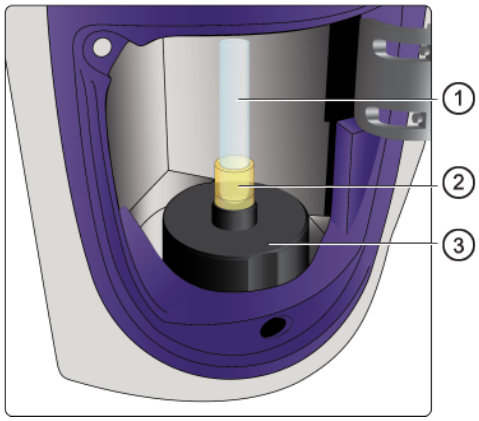
Introduction

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 chamber, flow cell, closed-loop and sort nozzles, sort block, and the sort collection chamber.

Sample chamber

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



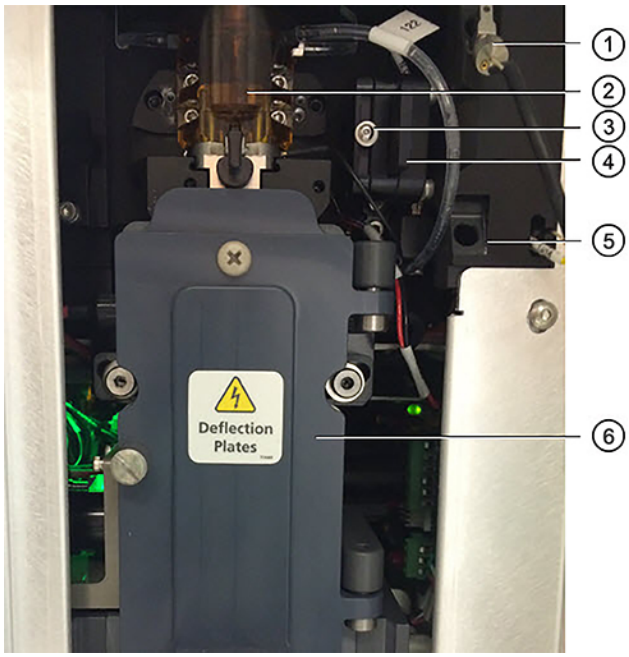
No.	Description
1	Sample tube
2	Sample input holder
3	Sample loading port

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 fibers.

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	Obscuration bar
4	Forward scatter (FSC) detector with neutral density filter
5	Nozzle holder
6	Sort block

Do not attempt to adjust the obscuration bar until you have received training.

Sort 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.

3

System startup and shutdown

This chapter covers the following topics:

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.

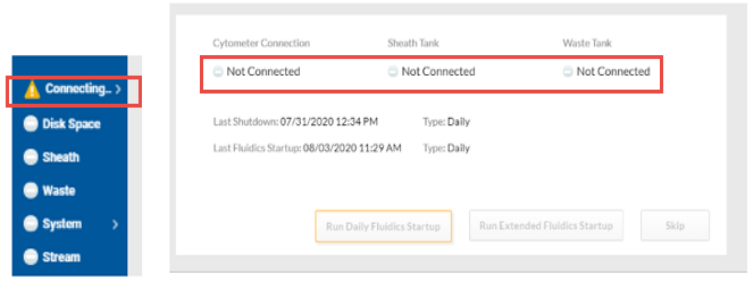
When the system is turned on, it automatically displays the connection status.

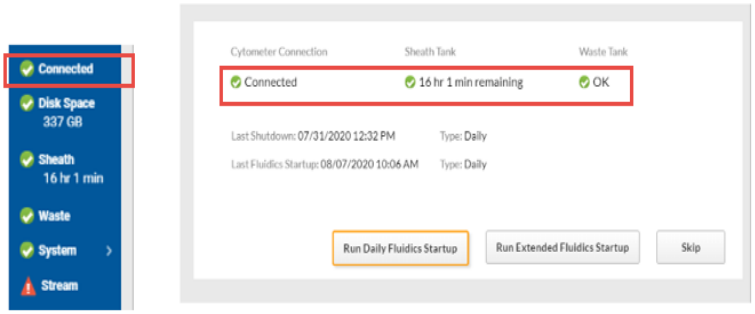
The tasks on the following tabs are not automatic and require user action.

- 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 in the System Startup workflow and displays a dialog with a green status (Connected) or a gray status (Not Connected). The System status indicator displays a yellow status (Connecting) progress indicator to indicate that actions are being completed in the background, or green (Connected) indicator after connection is established.





Fluidics startup

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

- Run Daily Fluidics Startup
- Run Extended Fluidics Startup

Selecting the appropriate Fluidics Startup depends on how the system was shut down.

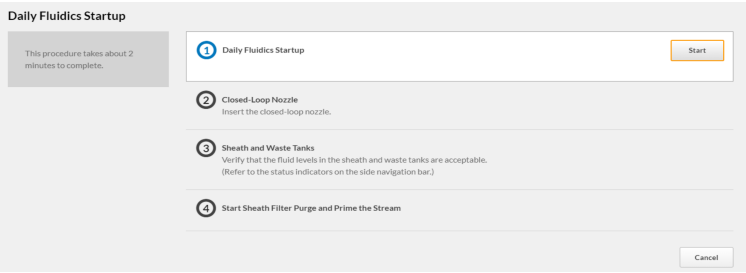
- If a Daily Shutdown was performed, then you can select to perform either a daily or extended startup.
- If a Long-Term shutdown was performed or the system was not used for more than 2 days, then you must perform an extended startup.

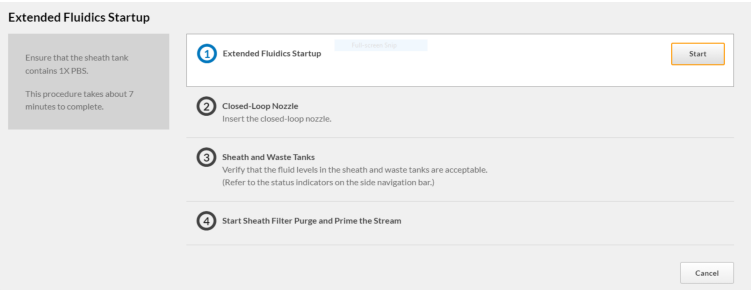
To perform a daily or extended fluidics startup:

1. Ensure that the air supply or compressor is turned on, that the waste tank is empty, and the sheath tank is full.

   Note: We recommend that you fill the sheath tank at the end of each usage of the system to ensure that it is full at the time of system startup.
2. Press the Power button on the front of the instrument.
3. Turn on the computer, start BD FACSChorus™ software and log in.
4. In the opening screen, select either **Run Daily Fluidics Startup** or **Run Extended Fluidics Startup**. The daily or extended fluidics startup dialog displays with four tasks that need to be completed.
   - Daily fluidics startup or extended fluidics startup depending on the selection.
   - Insert the closed-loop nozzle with the O-ring facing up.
   - Ensure that the sheath tank is full and the waste tank is empty.
   - Start the sheath filter purge and prime the stream.

   Note: When performing an extended fluidics startup because of a previous longterm shutdown, ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters. You must replace the ethanol filter with the sheath filter before starting the extended fluidics startup. See Preparing new fluid filters (page 123).
5. Follow the prompts to complete the tasks displayed on the screen.





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

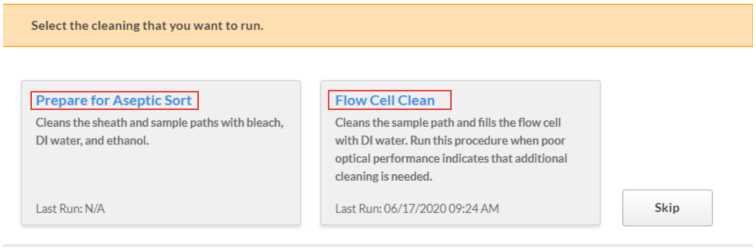
If there are issues, follow the instructions on the error message to troubleshoot the issue. For additional troubleshooting information, see Troubleshooting overview (page 138).

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

   The system returns to the opening screen.
7. Click **Continue** to view the cleaning options.

Cleaning

Two cleaning options are available as shown in the following image.



- Prepare for Aseptic Sort cleans the sheath and sample paths with 10% bleach, DI water, and 70% ethanol. Use this option when you need to sort under sterile conditions. See Preparing for aseptic sort (page 99).
- Flow Cell Clean cleans the sample path and fills the flow cell with DI water. See Cleaning the flow cell (page 98).

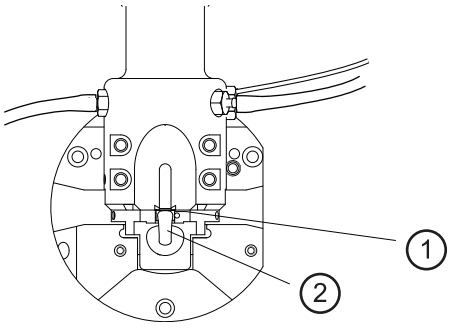
  Note: We recommend that you perform the Flow Cell Clean procedure after each startup. If you need to sort using sterile conditions, use Prepare for Aseptic Sort. See Preparing for aseptic sort (page 99).

Sort nozzle

The system displays a dialog to insert the sort nozzle. The O-ring must be facing up. For more details on how to insert the nozzle, see Changing the nozzle (page 94). After you insert the sort nozzle, the system moves to Cytometer Setup tasks.

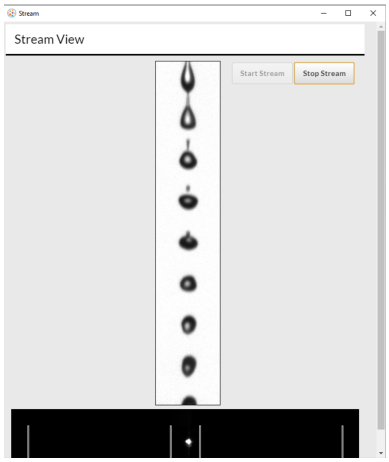
To open the sort nozzle:

1. Remove the closed-loop 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.



2. Insert the sort nozzle into the bottom of the flow cell cuvette (with the orange O-ring and top side facing up) and push it gently all the way forward until it stops.
3. Turn the nozzle-locking lever clockwise to the 12:00 position.
4. Click **Continue**.

   The stream should automatically start.
5. Click the stream status indicator to open the Stream View window to confirm that the stream is forming.



Cytometer Setup– run BD® CS&T RUO Beads

BD® CS&T RUO Beads are used to measure the daily performance capability of the instrument as compared to a baseline.

Note: Do not delete the BD® CS&T RUO Beads bead lot file.

- We recommend running Cytometer Setup at the start of the day to update settings and track instrument performance.
- Before running Cytometer Setup:
  - Prepare a fresh CS&T sample with the proper concentration of sterile PBS as instructed in the product insert.

    Note: Do not dilute BD® CS&T RUO Beads with water.

- Verify that the optical configuration for your experiment is correct. All CS&T data is specific to a set optical configuration. If you need to update the configuration, see Changing optical configurations (page 30).

  Note: For optimal performance, wait for the stream to stabilize (status indicator green) before running CS&T.

- Baseline measurement takes longer to complete than Cytometer Setup. Baseline measurement occurs automatically:
  - At installation
  - Every 60 days (for the same optical configuration and bead lot)
  - When a new bead lot file is selected
  - At the first Cytometer Setup after changing the optical configuration.

  BD Service personnel might create a new baseline manually as part of preventative maintenance or major service procedures.

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

All CS&T data is specific to a set optical configuration. Before proceeding, verify that the displayed configuration is the configuration you want to use. If you need to update the configuration, see Changing optical configurations (page 30).

Drop delay–run BD FACS™ Accudrop Beads

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

Whenever working with BD FACS™ Accudrop Beads, prepare a fresh sample with the proper concentration of sterile PBS as instructed in the technical data sheet.

Note: Do not dilute BD FACS™ Accudrop Beads with water.

Required materials

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

Item	Description	Supplied by
Bulk fluids	Sterile PBS	User
	Bleach (for the waste tank)	User
	Deionized (DI) water	User
	Ethanol	User
Setup beads	BD® CS&T RUO Beads with bead lot file BD® FC Beads	BD

Item	Description	Supplied by
Accudrop Beads	BD FACS™ Accudrop Beads	BD
1.5% BD Detergent Solution	Made from BD® Detergent Solution Concentrate, 15 mL diluted to make 1 L total	BD

About CS&T reports

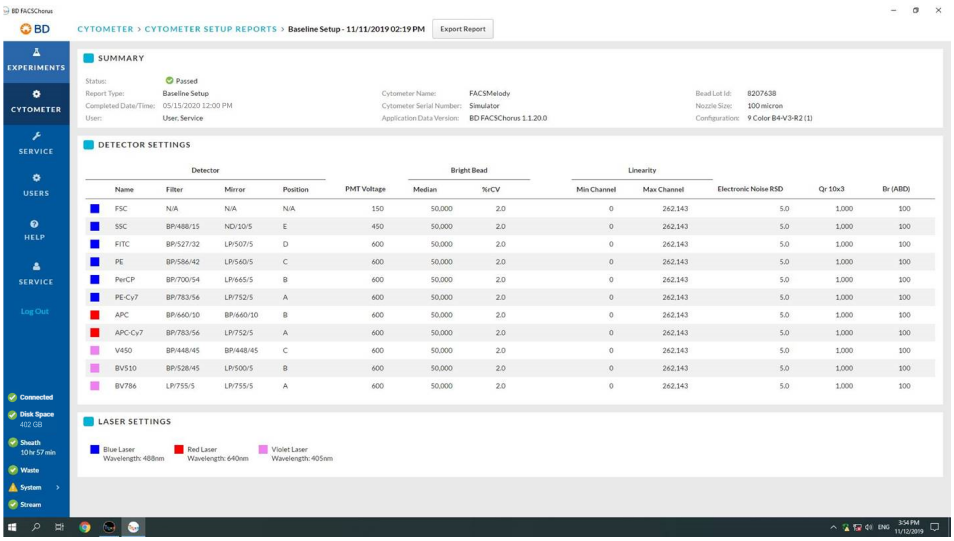
Introduction

CS&T reports contain information about the system, detector settings, lasers, and setup bead lots. Reports are generated each time a baseline and performance check is performed.

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
	Completed Date/Time	Indicates the date and time Cytometer Setup was run
	User	Indicates which user did the performance check
	Configuration	Indicates the optical configuration
Detector settings		
Detector	Name	Name of the detector
	Filter	Description of wavelengths transmitted
	Mirror	Name of the mirror used with the detector
	Position	Location of the filter holder with mirror
	PMT voltage	Measured PMT voltage
Bright beads	Median	Median fluorescence intensity (MFI) value of the specific bead
	%rCV	Percent robust coefficient of variation of the bright beads
Linearity	Min channel	Minimum value for the acceptable linear range of the detector
	Max channel	Maximum value for the acceptable linear range of the detector
	Qr	Relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector. In the Baseline setup report, Electronic noise RSD is also included.
	Br (ABD)	Relative optical background signal, used for tracking the optical background noise levels in a detector
Laser settings		Each laser type and wavelength is shown here.

Editing your user profile

Introduction

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

Procedure

To edit your user profile:

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

Adding, editing, or deleting user accounts

Introduction

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

About this task

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

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

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

Procedure

To add, edit, or delete a user:

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

Shutting down the system

Introduction

From the Cytometer page, you can perform either a daily or a long-term shutdown.

When performing a long-term shutdown, only use tanks that are provided with the BD FACSMelody™ system.

Before you begin

For the daily shutdown, you need at least 3 mL of 1.5% BD Detergent Solution.

For the long-term shutdown procedure, you need 3 mL of DI water, at least 2.5 L of 70% ethanol and a fluid filter used solely for ethanol. Do not use the ethanol filter for other fluids.

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 94)
- Filling the sheath tank (page 108)
- Changing the fluid filter (page 110)
- Emptying the waste tank (page 107)

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.

Procedure

To shut down the system:

1. On the **Cytometer** page, select the shutdown procedure that you want to run.
2. Complete the steps in the wizard.
3. Power off the cytometer unit.

Never mix BD Detergent Solution and bleach because they can create dangerous fumes.

Always run a tube of DI water after running bleach on the cell sorter.

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4

Experiment

This chapter covers the following topics:

Experiment overview

About experiments

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

Experiment parameters are defined by filling in the data fields or making selections on each tabbed workspace. Saved experiments can be used as templates for later experiments. If you save the experiment as a template, you can change the parameters later. However, experiments created from the template before the parameters were changed are not affected.

Experiments are saved and displayed in a list in the Experiments workspace. Saved experiments can be opened and updated as needed. Once data has been recorded, the experiment parameters cannot change. If you want to use different parameters, then you can create a new experiment by either using the experiment as a template, or by duplicating the experiment without data.

Creating an experiment

Introduction

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

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

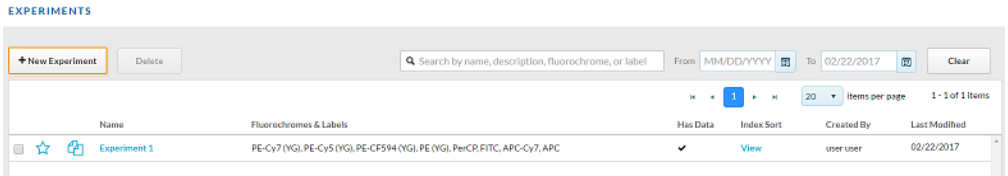
- Design experiment
- View data
- Set up sort
- Sort
- View reports

Creating an experiment

To create an experiment:

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

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

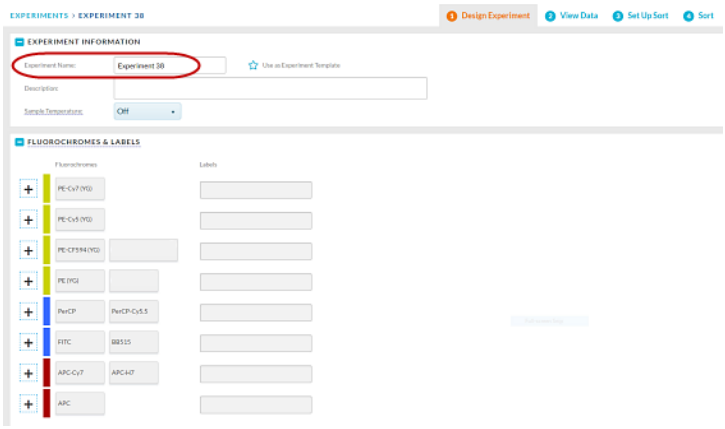


2. Click **+ New Experiment**.

The new experiment dialog displays with the option to start with a blank experiment or with an experiment template if there are existing experiments.

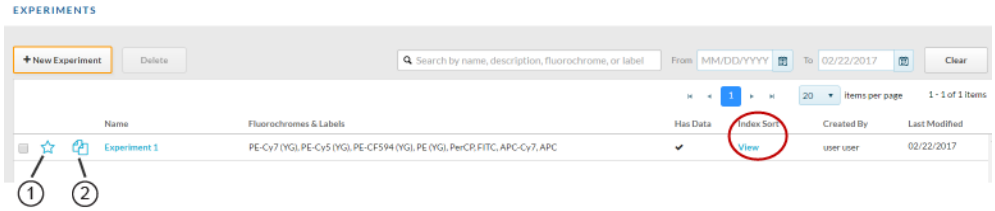
3. Select **Blank Experiment**.

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



Starting with an existing experiment

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



Experiment template option (1)

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

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

Duplicate experiment without data (2)

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

In both cases:

- You can use an experiment that has an index sort (red circle).
- Compensation values are transferred from the existing experiment or template to the new experiment.

Designing an experiment

Introduction

Designing the experiment entails defining the parameters of the experiment, such as the name, sample temperature, and fluorochromes being used. In the Fluorochromes & Labels section, there is one row of fluorochromes for each available detector channel of the optical configuration. Each row contains a plus sign (+), a colored rectangle, and one or more fluorochromes. The plus sign (+) allows for additional user-defined fluorochromes to be created for the experiment. The colored rectangle identifies the laser that excites the fluorochrome. Laser and filter information for each detector channel are displayed when the cursor hovers over the colored rectangle. Default fluorochromes are listed in the columns to the right of the colored rectangles. User-defined fluorochromes appear to the right of the default fluorochromes. User-defined fluorochromes are displayed in the table with an asterisk (*).

Fluorochromes to be used in the experiment must be selected here in order to be an available parameter when creating plots or histograms in the experiment.The system displays a list of default fluorochromes.

Some of the default fluorochromes have spillover values created by running BD FC Beads. If you would like to maintain those values, see the Update Compensation Standards workflow on the Cytometer page.

Some default fluorochromes might not have spillover values and require that you run controls. User-defined fluorochromes do not have default spillover values applied. You can add new fluorochromes and create spillover values by running controls. See Updating compensation standards (page 54).

Procedure

To design the experiment:

1. Follow the prompts on the screen and fill in the data fields as necessary.
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.

   Note: Sample Temperature controls the temperature of the sample chamber but not the temperature of the sorted sample. Temperature control for collection devices is available as an optional feature. See Using the sample temperature control option (page 68)
5. Select one or more fluorochromes from the list by clicking the rectangle corresponding to the fluorochrome you want to use. New user-defined fluorochromes can be created for the experiment by clicking the plus sign (+) beside any fluorochrome to add a fluorochrome to the row, then selecting the new fluorochrome. (Only one fluorochrome can be selected from each row.)
6. (Optional) Hover over any of the colored rectangles to display the laser and filter information.

Defining view data

Introduction

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

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

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

You can also move the slider in the top right of each section to increase or decrease the plot size. This will allow you to view more or fewer.

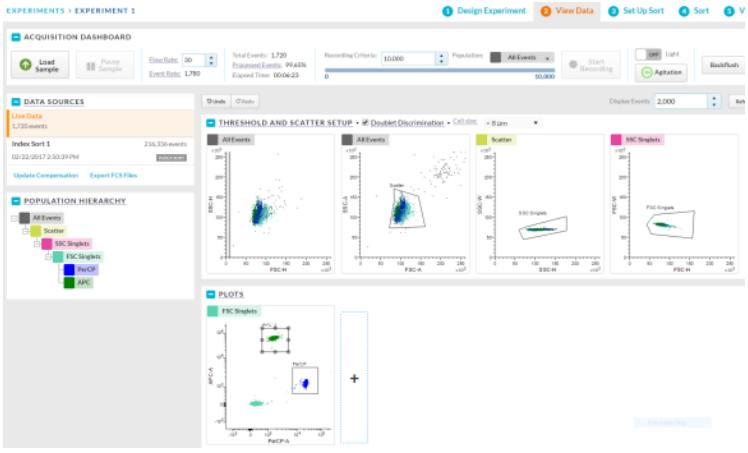
To make a PDF or print the plots, stats, or population hierarchy in the View data page, use Ctrl+P.

Procedure

Do not attempt to open the sample loading door while a sample is being loaded and run. A load sample pin locks while a sample is being processed. Pulling on the door could damage the door or locking mechanism.

To define view data:

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



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

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

3. Use the **Data Sources** panel to select data files to view in the plots, export data files, or update compensation.
4. View the Population Hierarchy to determine how the populations relate to each other. The positions of the populations in the hierarchy can be rearranged by dragging and dropping.

   All populations are a subset of the All Events population.

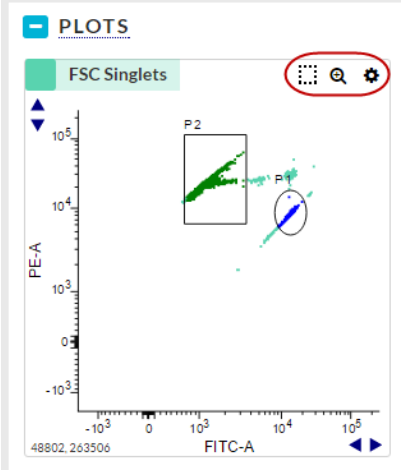
5. Use the plots to set the acquisition threshold, change voltages, and create gates.

   There are four plots by default: Two All Events plots, a Scatter plot, and an SSC singlets plot. The first All Events plot is for defining the threshold and the second is for defining the scatter. The other plots are doublet discrimination plots to help define singlets and eliminate doublets.

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

   a. To set the threshold for data acquisition, hover over the first default plot to make the threshold marker display. Move the marker along the axis to adjust the threshold and remove the low-end debris from the plot. To change the threshold parameter, click the x-axis of the threshold plot and select the parameter you want.
   b. To change the voltage of a parameter, load a sample, then hover over a plot to make the voltage sliders display. Move the sliders along the axis to adjust the voltage or use the up-down and left-right arrows on your keyboard to the adjust the voltages of y- and x-axes respectively.
   c. Use the Cell size selector to scale the area to the height measurement on all plots of the experiment.
   d. To delete a plot, change the population displayed, or change the type of plot, select the plot properties on the upper-left of the plot, or the plot options (gear) icon, update settings as necessary, and click **OK**.
   e. To add additional dot plots, a contour map, histogram, or density plot, click the plus **(+)** button on the right side of the Plots panel and select a new plot. Plots can be enlarged by hovering over a plot and clicking the expand plot (magnifying glass) icon.
   f. To rescale plots to see events with additonal data and improve resolution, select Bi-exponential from the plot parameters and adjust the scaling by clicking the up or down arrow. Digital data can have events with negative values and bi-exponentially scaling allows you to see events below the axis.

6. Create, move, modify, and delete gates on the plots as needed. Hover over the plot to make the icons display. You can also zoom in by double-clicking on the plot or using the scroll bar as you hover over the plot.



   a. To create a new gate, click the square icon with the dotted line, select a shape from the list, and then draw an area on the plot.
   b. To move the gate, select the gate and drag to a new location.
   c. To modify the gate, select the vertices on the gate and drag to a different location.
   d. To delete a gate, select the population in the population hierarchy that is created by the gate, and select **X** to delete.

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

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

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

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

Calculating compensation

Introduction

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

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

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

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

We recommend updating FC bead compensation standards every 60 days.

About compensation calculations

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

Using stored FC Beads values

Blank experiments or experiments created through a template or duplicate without data function that have no pre-existing experimental compensation controls use normalized spillover values for certain default fluorochromes from the last time BD FC Beads were run to calculate compensation. The system compensation values will be applied to all supported default fluorochromes selected in new experiments.

Using user-defined control values

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

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

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

Note: In all cases, if an experiment was created from an experiment template, or by duplicating an existing experiment without data, then all compensation values are transferred to the new experiment.

Before you begin

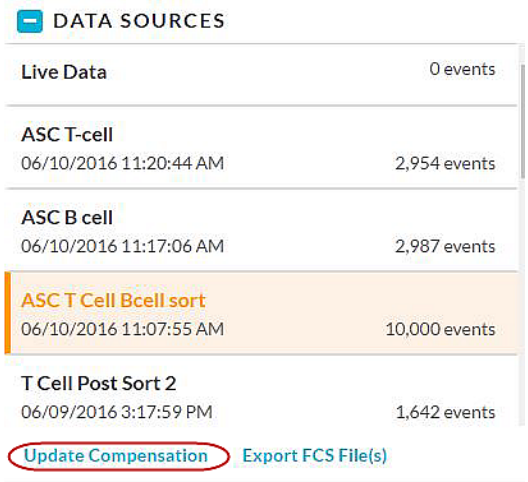
Complete these tasks before you update compensation.

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

Procedure

To update compensation using your own controls for your experiment:

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

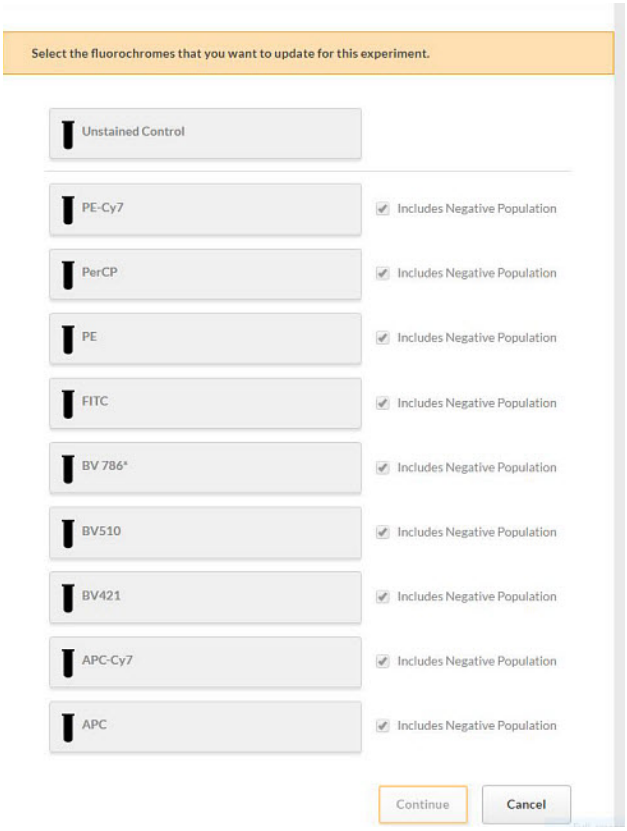


   The Update Compensation dialog displays.

   Note: If the optical configuration has changed, you will not be able to update the compensation. You will need to create a new configuration and compensation matrix. See Changing optical configurations (page 30)

2. Select the fluorochromes you want to update.

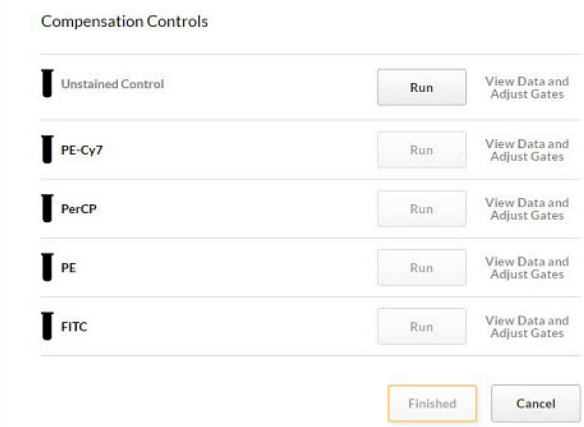
Only the fluorochromes that were selected in the Design Experiment tab are displayed.



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

   Note the following:

   - If you have a separate unstained control, then select the Unstained Control box on the top of the page and run it first before the single-color controls.
   - If you have a separate unstained control, ensure that the appropriate Includes Negative Population checkboxes are cleared, then run the unstained control first before the single-color controls.
   - If your single-color controls have stained and unstained populations, then skip running the unstained control. Ensure that the appropriate Includes Negative Population checkboxes are selected.
   - The selected tubes are displayed on the next tab.



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

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

Updating compensation standards

Introduction

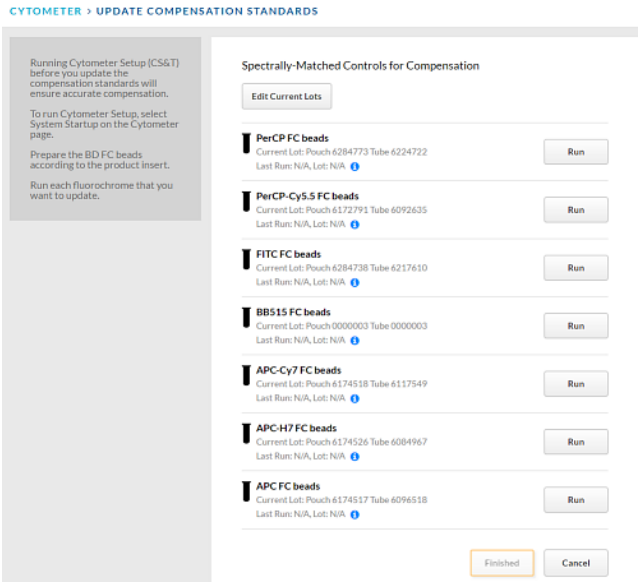
Updates to the standard fluorochrome spectral references for compensation are needed for calculating spillover values at different voltages and setting default spillover values in experiments. Run this procedure with BD FC Beads every 60 days to ensure accuracy.

Procedure

To update the system compensation standards:

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

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



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

3. Verify that the FC bead lot number matches the BD® FC Beadsyou are going to use.
4. (Optional) If needed, select **Edit Current Lots** to select from the available lots with associated bead lot files to ensure accurate compensation calculations.

   If the lot is not shown, the FC bead lot files can be updated by going to the BD Biosciences website.
5. Visit the BD Biosciences website and download the BD FACSChorus™ FC bead lot updater from the Bead Lot Files tab of the BD FACSMelody™ Resources page. You need administrative privileges to run the updater.
6. Prepare the BD FC Beads according to the product insert.
7. Follow the prompts on the screen to run each fluorochrome.
8. Click **Finished** when you are done.

Viewing the compensation matrix

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

Setting up sorting

Introduction

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

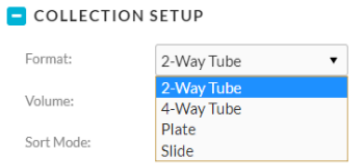
Procedure

To set up the sort operation:

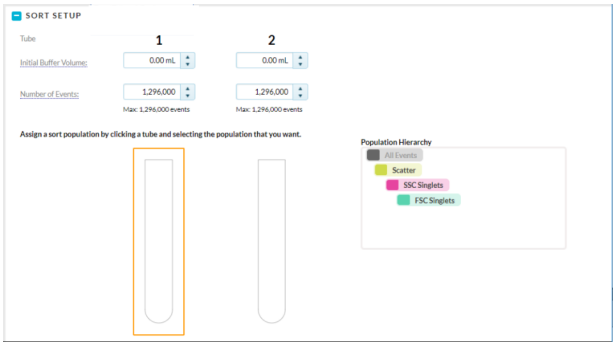
1. Click the **Set Up Sort** tab.
2. In the **Collection Setup** panel, select the details for the sort operation, such as:
   - **Format**, which refers to the collection device (tube, plate, or slide)

     The tube format allows you to use either a 2-way or 4-way sort collection.
   - **Volume**, which refers to the volume of the tube collection device as illustrated below:

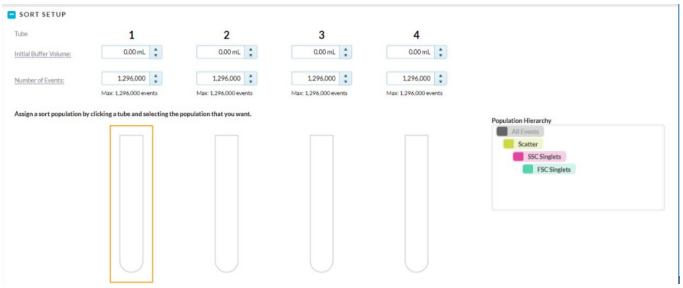
     The Volume operation allows you to perform a two-way or four-way sort on tubes.



     If you select a two-tube collection device, only two tubes display in the Sort Setup area.



If you select a four-tube collection device, four tubes display in the Sort Setup area.



If you use only two tubes after selecting four-way sort from the menu selection and a four-way tube holder is attached, you must use the inner two tubes.

Do not use a two-way holder while doing a four-way sort. This will create sample loss and, potentially, will cause a biological hazard spill.

- **Sort Mode**, with the following options:

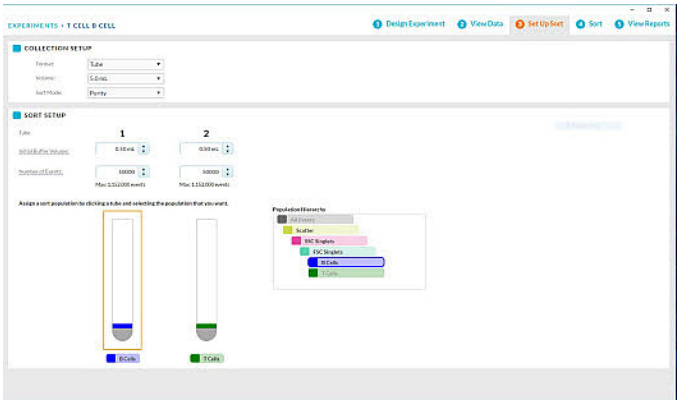
Sort mode	Description
Yield	This is used to obtain a high yield of the cell of interest, but not necessarily a pure population of the cells. In this case, a droplet containing two cells will be sorted as long as one of them is a cell of interest. More than one cell could be sorted into a single well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

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

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

4. In the **Sort Setup** panel, define how the population will be sorted.
   - If using tubes, select the tube and then select the populations of interest from the population hierarchy.

     The tube assumes the color of the selected population.



   - If using a plate or slide, select the wells of the plate or slide and then select the populations of interest from the population hierarchy. The population selected can be from up to and including the eighth level of a hierarchy.

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

   You may specify a buffer volume of up to half the volume of the collection device.
6. Select the number of events to sort into each tube or well. The index sort has a maximum number of 100 events per well or puddle.

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

Selecting wells

To select specific wells:

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

The screen indicates the location of the selected populations.

Loading collection devices

About this topic

The BD FACSMelody™ system supports two- and four-way sorting. Each type of sorter includes 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:

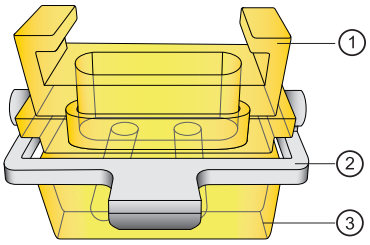
- Microscope slide: 27 wells (3 x 9 grid)
- 6-, 24-, 48-, 96-, and 384-well plates
- PCR tray: 96 well

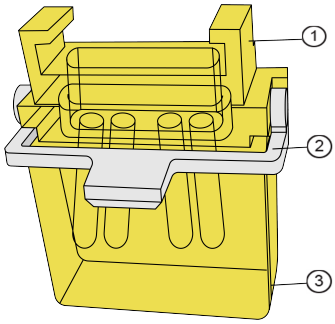
All instrument surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning instrument surfaces. Wear suitable protective clothing, eyewear, and gloves.

Loading tubes

There is a two- and four-way holder for the 1.5- and 2.0-mL tubes and a two- and four-way holder for the 5.0-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.





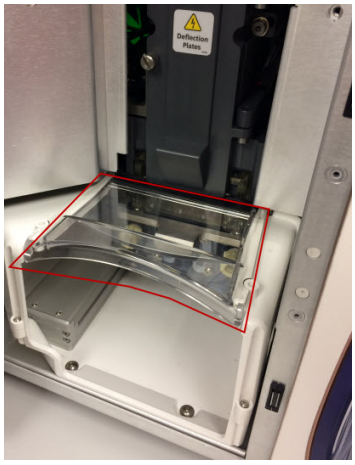
No.	Description
1	Adapter
2	Handle
3	Tube holder

If you use only two tubes after selecting four-way sort from the menu selection and a four-way tube holder is attached, you must use the inner two tubes.

Do not use a two-way holder while doing a four-way sort. This will create sample loss and, potentially, will cause a biological hazard spill.

To load the tube holder:

1. Insert the collection tubes into the appropriate tube holder.
2. Open the sort collection chamber door.
3. Use the metal handle to guide the adapter into place.
4. Attach the adapter to the instrument, below the sort block.
5. Install the aerosol shield (red polygon) to keep any aerosols contained in the sort collection chamber.



6. Close the sort collection chamber door.

Installing the splash shield

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

To install the splash shield:

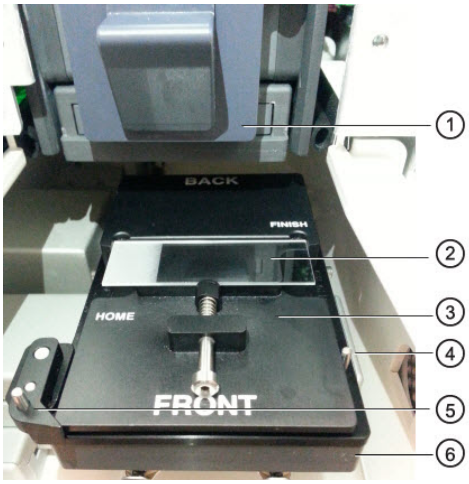
1. Remove the adapter for the tube sort.
2. Attach the splash shield to the underside of the sort block, pushing it back until it clicks into place.

Loading microscope slides on the automated stage

The automated stage consists of a flat platform, guiding pins, a locking lever, and ports to connect to the sample temperature control option. 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 specified 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** on the **Sort** tab in the software to bring the stage forward.
4. Open the sort collection chamber door.
5. Place the slide holder on the stage, touching the guiding pins in the back and right of the automated stage, with the locking lever in the front.



No.	Description
1	Sort block
2	Slide

No.	Description
3	Slide holder
4	Guiding pin
5	Locking lever
6	Automated stage platform

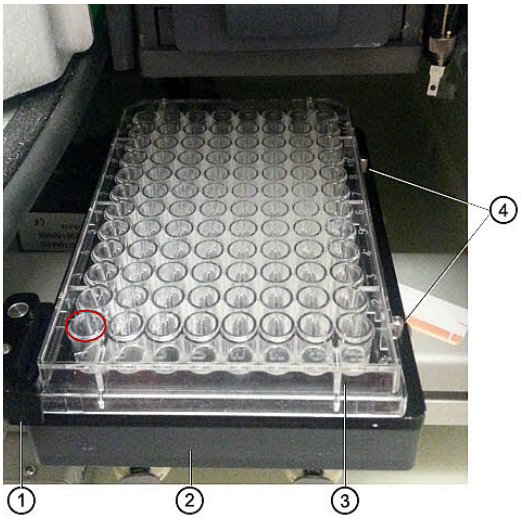
6. Install the aerosol shield to keep any aerosols contained in the sort collection chamber.
7. Close the sort collection chamber door.

Loading plates on the automated stage

Pins on the right side and back of the automated stage are used to position the plate correctly onto the platform. The locking lever on the front left holds the plate in the specified location.

To load plates onto the automated stage:

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



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

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

Sorting

Introduction

Samples are sorted based on the selections in the Set Up Sort tab. Samples can be sorted into tubes, plates, or onto slides. However, index sorting uses only plates and slides, not tubes.

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

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

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

Procedure

To run the sort operation:

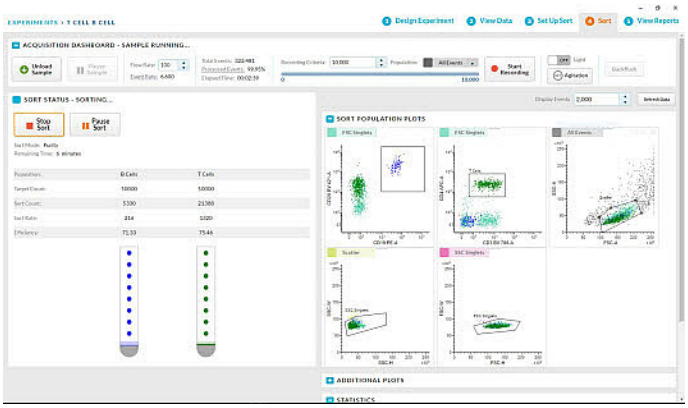
1. Click the **Sort** tab.
2. Click **Load Sample**.
3. (Optional) Click **Start recording**.

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

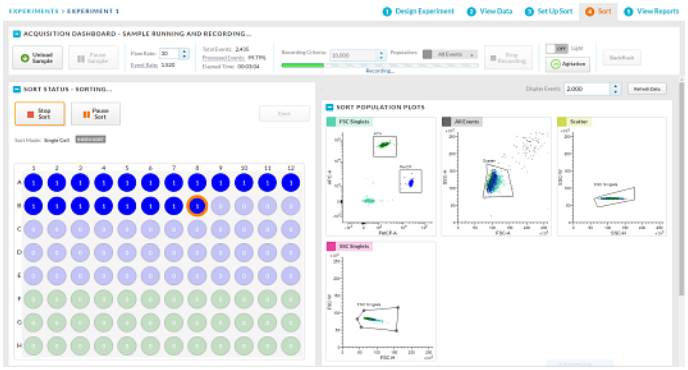
   Regions or markers defining sort populations can be adjusted here as needed.
4. Click **Start Sort**.

   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 Set Up Sort tab.

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



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



5. (Optional) Additional plots with gates other than the sort gates are shown in the Additional plots panel.
6. View the statistics and edit as necessary.

When the sort has completed successfully, a Sort Completed dialog displays with an option to name the sort. The name will be used to reference the Sort Report and, if selected, the Index Sort recording associated with the sort. See Index sorting (page 63) and Reviewing index sort data (page 64).

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:
  - Replace the sorted collection device with a new one of the same type (plate, tube, or slide).
  - Click **Start Sort**.

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

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

Index sorting

Introduction

Index sorting allows you to sort cells onto a plate or slide and indexes the well or slide location to the collected parameters for that cell. You can use this function to ensure that a sorted cell with a specific phenotype has been sorted. Index sorting is useful in characterizing subpopulations of phenotypically similar events using post-sort genetic, chemical, and/or metabolic applications.

Index Sort allows the sorting of up to 100 events per well or location in plates or slides using single-cell sort mode. When performing an Index Sort, a recording is automatically made of the entire sort so that the data is

available for post-sort analysis. Select the Index Sort function only when there is sufficient free hard drive space.

Setting up for index sorting

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

Review the instructions in these sections:

- Designing an experiment (page 48)
- Defining view data (page 49)
- Calculating compensation (page 51)
- Sorting (page 62)

Procedure

To perform an index sort:

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

4. Select the fields and enter values on the **Sort Setup** tab to define the sort populations and well locations for the sort.
5. Select the **Sort** tab, and follow the instructions to start the sort operation.

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

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

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

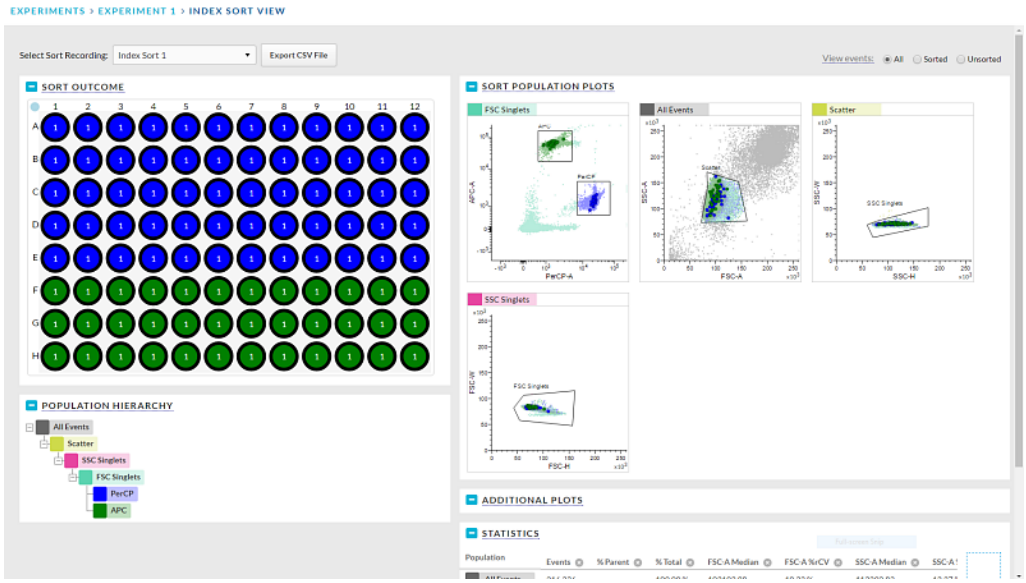
Reviewing index sort data

Introduction

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

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

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



Procedure

To review the Index Sort data:

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

   The Index Sort View page displays. You can perform several actions on this page.
2. Click **All events**, **Sorted**, or **Unsorted** to filter out events.
3. When **All events** or **Sorted** is selected, you can:
   a. Select a well in the plate and see the corresponding events for that well highlighted in the plots.
   b. Select events in the plot and see the corresponding wells for those events highlighted.

      The black outline surrounding a well indicates that all events in the selected region of the plot fall within those wells. The gray outline indicates that the outlined wells contain events that were not included in the selected region of the plot.
   c. View the statistics for all wells in the plate.
   d. Add new statistic by clicking the (+) button.
4. Use **Unsorted f**or statistical purposes only.

Viewing reports

Introduction

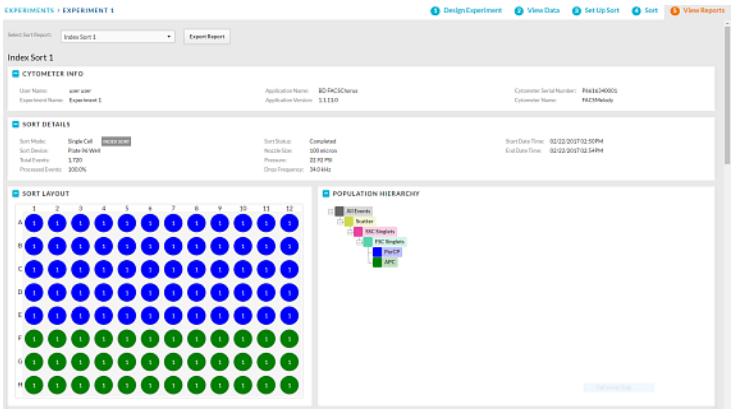
A sort report summarizing the results of the sort is displayed on the View Reports tab when sorting is complete. No sort report is generated until the sort is complete. A sort is considered complete if the sorting criteria are met, the sort is manually stopped, or the sort is stopped automatically due to a clog, empty sample tube, or other similar issues. You can export the report from this tab.

Procedure

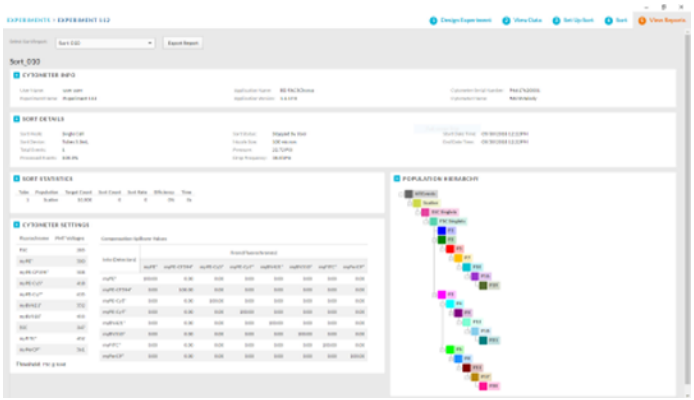
To view and export the report:

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

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



2. Select the report to view or export from the **Select Sort Report** list.
3. Click **Export Report.** A Save As screen will appear. Confirm the file name and location and click Save to export a PDF of the report to the selected location.



4. Open the PDF report and click **Print** to print the PDF report.

5

System options

This chapter covers the following topics:

Overview

Introduction

Several options are provided with the BD FACSMelody™ cell sorter:

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

Using the sample temperature control option

Introduction

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

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

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

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

Setting up the water bath

To set up the water bath for temperature control:

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

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

Note: Because the water flow direction is controlled by the water bath pump, the ports on the cytometer unit are bidirectional. The input and output hoses from the water bath can be connected to either port on the cytometer unit.

6. Fill the water bath with distilled water containing 0.1 g/L of sodium carbonate.

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

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

7. Plug in the water bath power cord.

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

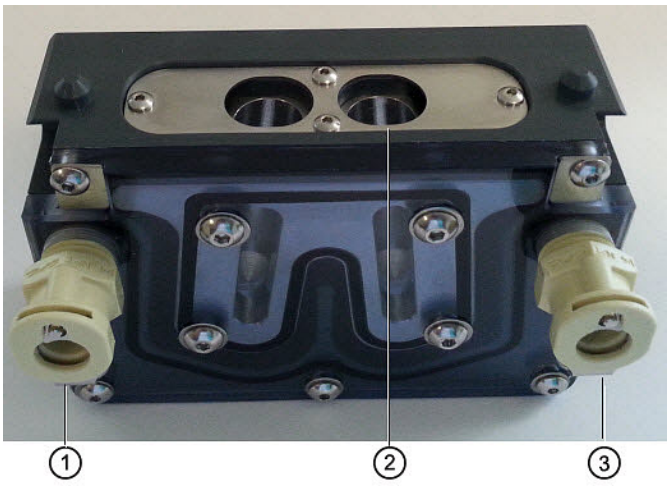
Tube holder setup

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- and four-way sorting.

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



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

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

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

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

4. Attach the tube holder to the adapter.

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

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

b. Push the tube holder all the way in.

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

Automated stage setup

To install the temperature control on the automated stage:

1. Install the splash shield on the sort block of the cytometer unit, 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, eye wear, and gloves.

2. Do the following:
   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.
3. In the **Sort** tab, click the **Eject** button to bring the stage to the front.
   a. Open an experiment, if one is not already open. In the **Set Up Sort** tab, select any plate or slide format in the **Collection Setup** section.
   b. In the **Sort** tab, click the **Eject** button to move the stage to the front of the sort collection chamber.
4. 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.
5. Install the appropriate collection device on the stage.
6. 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 BD® Aerosol Management Option

Introduction

The BD® 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 collection chamber, preventing the aerosols from being circulated back through the chamber. The AMO is intended as a standalone option. The biological safety cabinet (BSC) option comes equipped with a built-in aerosol management system. The AMO has settings for evacuation mode: 20%, 40%, 60%, 80%, and 100%.

Note: The AMO is For Research Use Only. It is not for use in diagnostic or therapeutic procedures.

Note: In all the AMO related procedures and maintenance steps, the sort access door in the instructions refers to the sort collection chamber door in the associated images.

This section covers the following topics:

- BD® Aerosol Management Option components (page 72)
- Operating the BD Aerosol Management Option (page 73)
- Maintenance (page 76)
- Upgrades for the AMO (page 82)
- AMO Specifications (page 82)

Using the AMO

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

- Ensure that the vacuum hose lines from the AMO are connected to the port on the rear of the cytometer unit. Turn on the AMO.
- Operate the AMO at 20% during sorting.
- Operate the AMO at 40% or higher for 1 minute after clearing a clog, and before opening the flow cell access and sort collection chamber doors.
- Verify that the filter flow gauge reads less than 2.4 inches of H_2O.

  Note: For a new filter, the gauge should read 1.0–2.0 inches of H_2O. As the filter is used, the reading will increase. If the gauge reads 2.4 inches of H_2O or greater, replace the filter. You may need to replace the filter sooner based on your usage or application. For more information on safety issues related to cytometry, in particular cell sorting, see isac-net.org. Also see Replacing the ULPA filter (page 77).
- Turn off the AMO when you have completed your work.

BD® Aerosol Management Option components

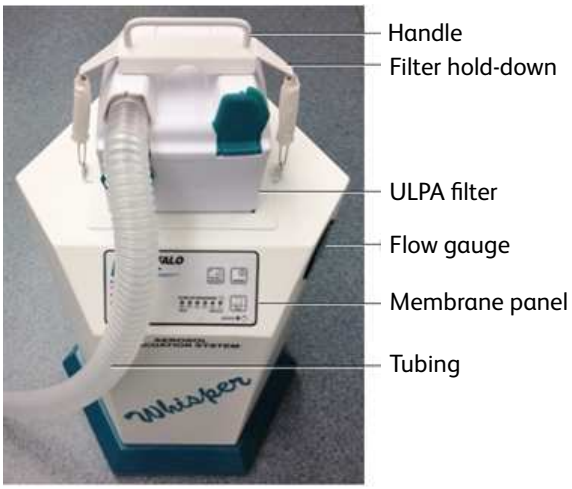
The BD® Aerosol Management Option (AMO) includes the following:

- An evacuator to generate negative pressure
- An ULPA filter to trap particles, with attached tubing that connects the evacuator to the instrument
- An air filter for the sort access door
- A hydrophobic filter on the sort bloc door
- A hinged cover on the sample injection chamber

The AMO does not eliminate the health risks of working with biohazardous material and must be used in conjunction with good laboratory practice.

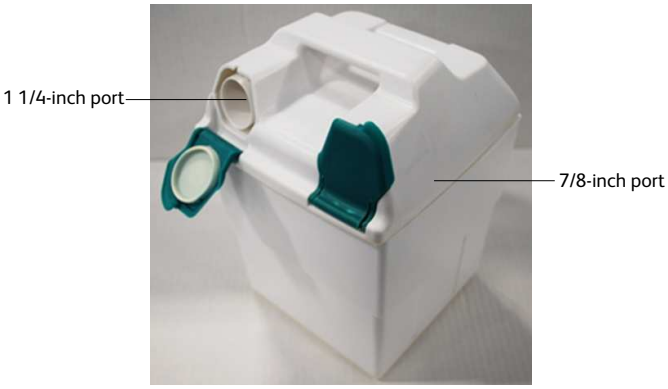
AMO evacuator

The AMO evacuator holds the ULPA filter and attached tubing. Air flow is controlled using push-buttons within a membrane panel on the front of the unit. The evacuator sits on caster wheels for easy maneuverability. It can be moved using the handle attached to the unit.



ULPA filter

The ULPA filter used in the BD AMO captures and retains 99.9995% of all particles down to and including particles 0.12 microns in size.



Operating the BD Aerosol Management Option

This section covers these topics:

Starting up the evacuator

Before starting up the evacuator, make sure that:

- The ULPA filter is completely seated against the bottom of the evacuator filter well.
- One end of the tubing is securely attached to the 1 1/4-inch (left) port on the ULPA filter.
  Note: Ensure that the 7/8-inch (right) port is closed.
- The other end of the tubing is attached to the manifold located in the connection panel on the back of the cytometer.

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

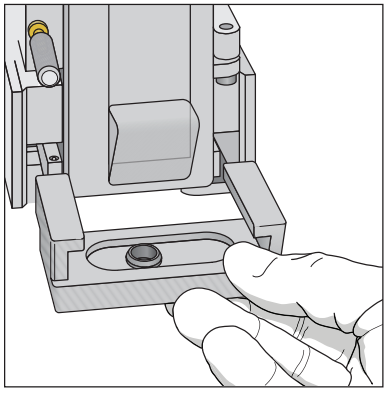
To start up the evacuator:

Install the splash shield or the tube holder below the aspirator drawer. See Installing the splash shield (page 60). The splash shield is required for sorting into a multiwell plate or onto a slide.

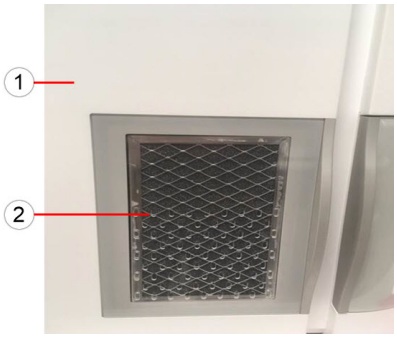
To install the splash shield:

1. Close the sort block door and open the sort collection chamber door, if needed.

   The sort block door must be closed to open the collection chamber door.
2. Remove the tube holder, if one is installed.
3. Insert the splash shield into the slotted fittings below the sort aspirator drawer.
4. Push the splash shield all the way in.



5. Ensure that an air filter (2) is installed in the sort access door (1).



The filter traps airborne dust that could clog the ULPA filter. The filter should be changed every 6 months. See Replacing the air filter (page 81)

6. Close the sort access door.

   Note: The sort collection chamber door must be closed for the evacuator to generate negative pressure in the chamber.

7. Switch on the main power switch (1) on the back of the evacuator.

8. Press the power button on the membrane panel of the evacuator.

9. Press the up or down arrow button to set the suction control rate to 20%.

   Each time either arrow button is pressed, the suction will increase or decrease by 10%. When two lights are lit on the suction control indicator, the actual air flow is the value between the two illuminated percentages.

Do not set the suction control rate above 20%. Higher rates could affect the stability of the side streams.

10. Verify that the filter flow gauge reads less than 2.4 inches of H_2O.

    For a new filter, the gauge should read 1.0-2.0 inches of H_2O. As the filter is used, the reading will increase. If the gauge reads 2.4 inches of H_2O or greater, replace the filter. See Replacing the ULPA filter (page 77).

New filter

Used filter

Setting up the AMO for sorting

To set up for sorting, ensure that you know how to do the following procedures:

- System startup (page 34)
- Starting up the evacuator (page 73)
- Setting up sorting (page 55)

Note: Always start up the evacuator before setting up for sorting. If you start up the evacuator after sort setup is complete, you will need to repeat the setup procedure.

Responding to a nozzle clog during a sort with the AMO

If the stream is disturbed during the sort (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until you clear the clog. In the event of a nozzle clog, do not open the sort access door or access the sort tubes before following this procedure.

Cell sorters that use droplet generation methods, such as the BD FACSMelody™, can produce aerosols around the sample stream. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door and the sort collection chamber door closed during sorting. Follow these steps to stop sample flow and evacuate potential aerosols before opening the sort collection chamber door.

To clear a clogged nozzle on a system with the AMO:

1. If the stream has not already shut down automatically, turn off the stream. See Stopping or restarting the stream (page 90).

   This will shut off the stream, unload the sample, and close the aspirator drawer.
2. Increase the air evacuation rate on the AMO unit to 100%.
3. Open the aspirator drawer using software controls.
4. Wait at least 3 minutes.

   This procedure will minimize aerosols from the sort collection chamber.
5. Close the aspirator drawer.
6. Turn on the stream. See Stopping or restarting the stream (page 90).

   If the clog is removed, the stream will appear similar to as it appeared before the clog.
7. If the clog is not cleared, turn the stream on and off several times to see if the clog will clear itself.
8. If the clog is not removed, turn the stream off and clean the flow cell, followed by turning the stream on to see if the clog has cleared. See Cleaning the flow cell (page 98) and Stopping or restarting the stream (page 90).
9. Wait for at least 3 minutes before proceeding.

10. Open the aspirator drawer and evacuate for at least 60 seconds before closing the aspirator drawer again.
11. You can now access the sort collection chamber and remove the sort collection device.

    Note: When removing collection tubes, be aware that the outside of the tube is potentially contaminated. Use alcohol swabs or bleach to wipe the outsides of tubes.
12. (Optional) If it is necessary to change nozzles or remove a clog from a nozzle, see Changing the nozzle (page 94).
13. With stream turned off, open the sort block door and dry the deflection plates and surfaces as needed.
14. Set the AMO unit back to 20% vacuum.
15. Make sure that all chamber doors are closed and restart the stream.

Turning off the evacuator

Turn off the evacuator after you have finished running biohazardous samples.

To turn off the evacuator:

1. Place the system in standby by pressing the power button on the membrane panel of the evacuator.
2. Switch off the main power switch (1) on the back of the evacuator.

Maintenance

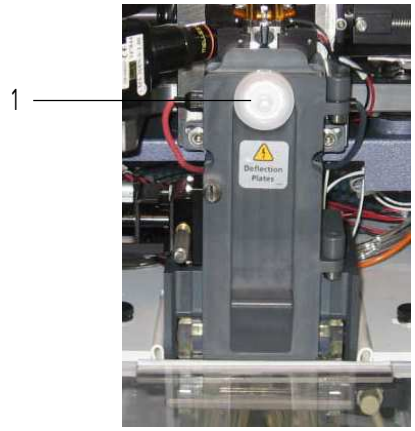
Use the following guidelines to ensure optimal performance of the BD® Aerosol Management Option :

- Change the ULPA filter and attached tubing when the flow gauge indicator is >2.4 at a 20% flow setting or when the red filter life indicator LED is blinking. See Replacing the ULPA filter (page 77).
- Two spare filters and replacement tubing are included with the AMO. To order additional replacement kits, contact your local BD Biosciences representative.

All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle the ULPA filter and attached tubing as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

- Change the air filter in the sort access door every 6 months. The filter traps airborne dust that could clog the ULPA filter. Regular replacement of the air filter will extend the life of your ULPA filter. See Replacing the ULPA filter (page 77) and Replacing the air filter (page 81).
- Do not touch the Filter Life Reset button during normal operation. Doing so could shut down the evacuator and prevent the collection of aerosols.
- Do not disconnect the tubing from the instrument manifold outlet or the ULPA filter unless you are changing the filter. Repeated removal and reattachment of the tubing could loosen the connection and disrupt airflow.

- To ensure optimal airflow, keep the tubing free of kinks and away from sharp or heavy objects. Do not crush or puncture the tubing. Ensure that the tubing is securely attached at both ends before turning on the evacuator power.
- Keep the sort collection chamber free of potentially obstructive debris, such as Kimwipes® or disposable pipets.
- Replace the hydrophobic filter (1) on the sort block door every six months.



Replacing the ULPA filter

Replace the filter when either of the following conditions occur:

- The filter-flow gauge reads 2.4 inches of H_2O or greater at 20% suction.
- The red filter life indicator LED is blinking.

Note: When only the red LED light is illuminated (but is not blinking), you have approximately 1 hour of filter life remaining. If the red light comes on during sorting, the filter will not stop working. Replace the filter as soon as possible when the red light starts blinking.

To replace the ULPA filter:

1. Turn off the evacuator main power and disconnect the electrical plug.

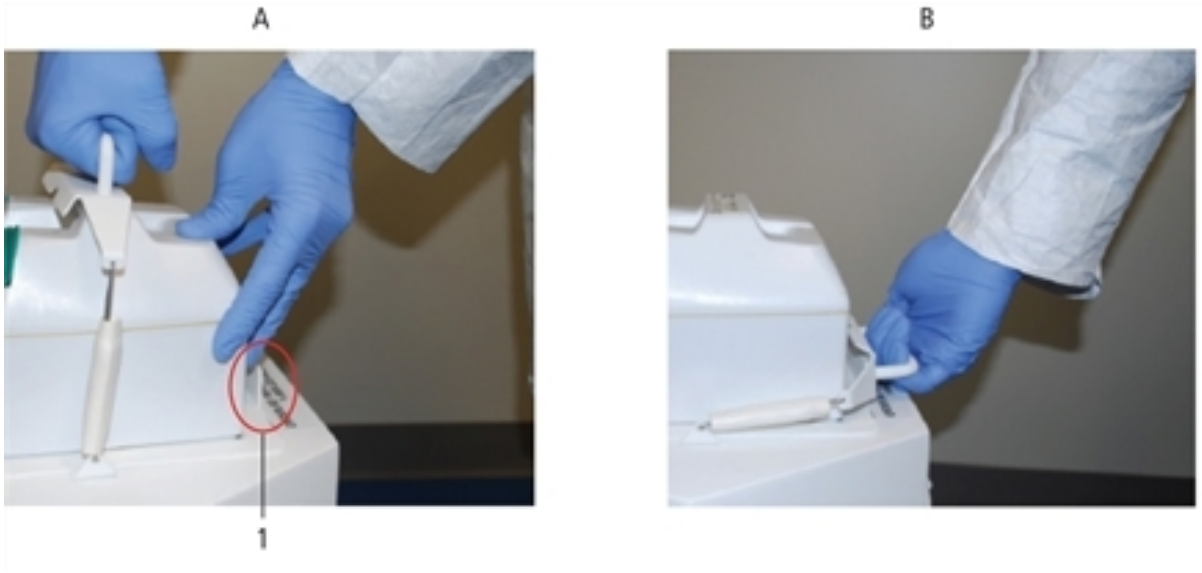
To prevent potential shock, always turn off the evacuator main power and disconnect the electrical plug from the power source before installing or removing any filter.

2. Disconnect the tubing from the bulkhead fitting.

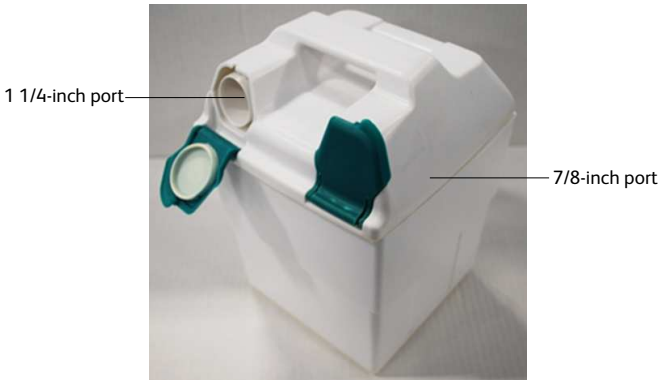
   The manifold is located in the connection panel on the back of the cytometer.

3. Remove the spring-loaded filter hold-down.

   While pushing down on the filter, pull up on the spring-loaded handle (A), and guide the handle over the top of the filter and behind the metal plate (1) in the back of the evacuator (B).



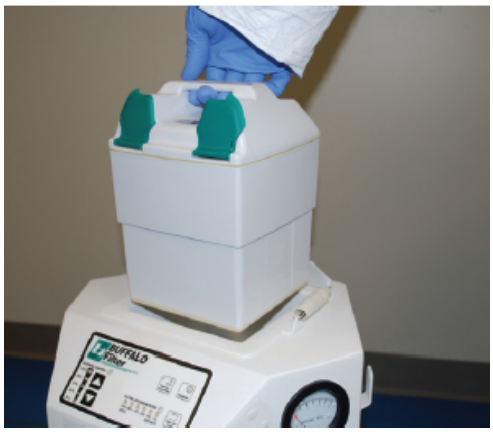
The preceding photos show the discontinued ULPA filter. The new ULPA filter has two ports (1 1/4-inch and 7/8-inch).



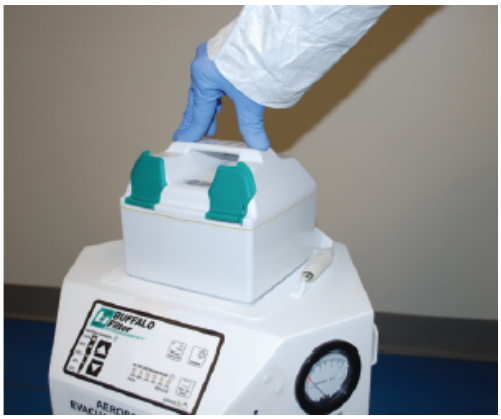
4. Lift off the ULPA filter and attached tubing from the evacuator and dispose of both the filter and the tubing.

All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle the ULPA filter, attached tubing, and all instrument hardware as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

5. Insert the new ULPA filter into the evacuator filter well.



6. Push down on the filter to ensure that it is seated against the bottom of the filter chamber.



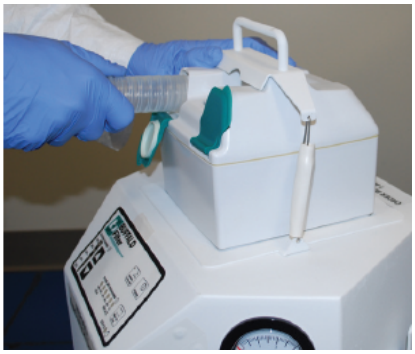
Note: For optimal evacuation of aerosols, the filter must be completely seated in the evacuator filter well.

7. Lift the spring-loaded filter hold-down and place it on top of the filter.
8. Press and hold the Filter Life Reset button on the membrane panel for 5–10 seconds.

All of the green, amber, and red LED lights will turn off, and then on. Hold down the button until the 100% indicator light is lit. This resets the 180-hour filter life clock.

Note: As the life of the filter is exhausted, the indicator lights will go out, starting from 100%. When only the red LED light is illuminated (but is not blinking), you have approximately 1 hour of filter life remaining.

9. Connect one end of the replacement tubing to the 1 1/4-inch (left) port on the ULPA filter, and the other end to the tubing manifold on the back of the cytometer.



Ensure that the cover on the 7/8-inch (right) port is closed.

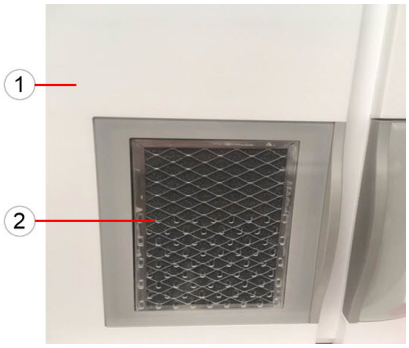
Note: For optimal evacuation of aerosols, ensure that the tubing is securely connected at both ends.

10. Connect the evacuator power plug to the power source.

Replacing the air filter

To extend the life of your ULPA filter, we recommend that you replace the air filter every 6 months. The filter traps airborne dust that could clog the ULPA filter. Regular replacement of the air filter will extend the life of your ULPA filter.

The air filter (2) is located inside the sort access door (1).



To replace the air filter:

1. Open the sort access door.

   The air filter is held in place by a repositionable adhesive.

2. Unscrew the screw at the top of the cover.
3. Pull off the air filter from the inside of the plastic panel.

4. Grasp the filter and then carefully slide it out of the door.

Note: Bag the air filter immediately to avoid exposure to biohazardous agents.

All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle the ULPA filter, attached tubing, and all instrument hardware as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

5. Install a new air filter in the door.
6. Slide the new filter in from the right.
7. Make sure to install the filter with the grid side facing out so that it is visible through the closed door.
8. Firmly press the new air filter against the inside of the clear plastic panel. Ensure that all the vent holes in the clean panel are covered by the filter.

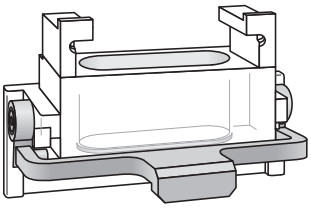
Grid side faces out

Non-grid side faces in

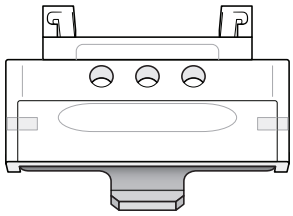
9. Reinstall the cover and tighten the screw.

Upgrades for the AMO

Upgrade kits are available for the AMO to improve the efficiency of the system to clear aerosols that can be produced during the sorting process. The components include a new universal tube holder top section with three holes and a new sort block door with a hydrophobic filter.



Universal top (front view)



Universal top (back view)

Contact BD Customer Support for information on the upgrade kits. See Technical support (page 12).

AMO Specifications

Specifications for the BD® Aerosol Management Option are as follows.

Evacuator

- Greater than or equal to 7 CFM (ft^3/min) normal operation rate (20% suction control)
- Greater than or equal to 30 CFM boost evacuation (100% suction control)
- <35 lb unpacked weight

ULPA filter module

- VLSI grade
- Traps particles greater than or equal to 0.12 µm
- Three-stage filtration (pre-filter, ULPA, post-filter)
- Particulate removal efficiency >99.9995%

Air filter

- Filter medium is an open-cell polyurethane foam
- Medium specially coated for improved fire retardation and fungi resistance
- High dust-trapping capacity, low air resistance
- Can be used in a wide variety of climatic conditions
- Rated UL 94 HF-1

Working with the biological safety cabinet

Introduction

The biological safety cabinet (BSC) is a custom Baker SterilGARD® 403A-HE Optimax Class II clean air and containment enclosure for the BD FACSMelody™ system. 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.

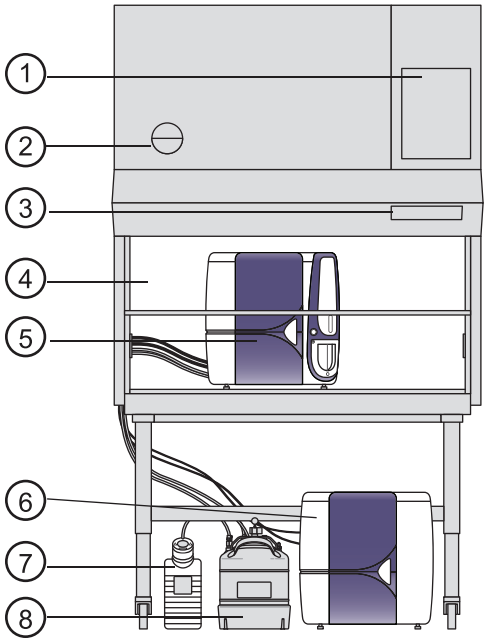
Recommendations

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	Cytometer unit
6	Electronics unit
7	Waste tank
8	Sheath tank

Using the BD FACSMelody™ cell sorter in the BSC

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

To work with the BSC:

1. Turn on the blower and keep it on.
2. Set the sash to the correct height (10 in.).
3. Verify that the laminar air flow is working.
4. Turn on the AMO to Low.
5. Verify that there are no alarms.
6. Operate the instrument per the instructions.

7. Operate the AMO at High for 1 minute after completing a sort or clearing a clog, and before opening the flow cell access and sort collection chamber doors.

Using BD Assurity Linc™ software

Introduction

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

About BD Assurity Linc™ software

BD Assurity Linc™ software is a highly secure remote systems management service that connects BD instruments to BD technical support personnel using Secure Socket Layer/Advanced Encryption Standard encryption. Using the BD Assurity Linc™ Agent, BD support personnel can securely access your workstation through an enterprise server and diagnose problems remotely. This direct connection to your workstation allows the BD support personnel to rapidly troubleshoot and assist you with system questions or problems. It also ensures quick real-time resolution of many issues. You must grant access to the instrument to enable this remote diagnostics feature.

Description of functionality

BD Assurity Linc™ software can continually monitor the health of your instrument and automatically communicate any changes to the BD Technical Support server using the instrument and BD FACSChorus™ log files. Because software monitors and stores cytometer performance data, key data is already available for diagnosis by BD when problems or questions arise, This can help the BD Customer Support Representative speed up diagnostic troubleshooting efforts.

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

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

Procedure

To grant access to a BD technical support representative:

1. Ensure that your workstation is connected to the internet.
2. Contact your local BD technical support representative.

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

   A dialog displays once the connection is established.
3. Acknowledge the request.

   The BD representative can now assist you.

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6

Maintenance

This chapter covers the following topics:

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 91)	As needed
Sample loading area	Lubricating the sample chamber O-ring (page 92)	As needed or when sample loading fails
Nozzle and flow cell	Changing the nozzle (page 94)	As needed
	Cleaning the sort nozzle (page 95)	When you see indications of clogging
	Cleaning the closed-loop nozzle (page 95)	When you see indications of salt buildup or clogging
	Replacing the sort nozzle seal temporarily (page 96)	If the seal is lost or damaged and you do not have a replacement nozzle
	Aligning the waste aspirator drawer to the stream (page 97)	If you install a sort nozzle that is new or different from the one that came with the instrument
	Cleaning the flow cell (page 98)	As needed
	Preparing for aseptic sort (page 99)	For sorting under sterile conditions
Optics	Cleaning the optical filters (page 102)	As needed
	Cleaning the Accudrop laser window and the lower camera window (page 102)	When the software is unable to set drop delay, or when the software is unable to verify the side streams when sorting has started
	Cleaning the strobe lens window and upper camera window (page 103)	When smudges appear in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed
	Cleaning the deflection plates (page 105)	When you have trouble viewing the side stream
	Removing or installing the FSC neutral density filter (page 106)	As needed

Category	Maintenance task	When to perform
Fluidics	Emptying the waste tank (page 107)	As indicated by the software and whenever you fill the sheath tank
	Replacing the waste filter cap	Monthly
	Filling the sheath tank (page 108)	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 114)	Every 4–6 months or when decreased event rates indicate that the sample line might be clogged
	Replacing the sample line filter (page 119)	When decreased event rates indicate that the sample line might be clogged
	Removing the sheath probe (page 121)	As needed, before you autoclave the sheath tank
	Preparing new fluid filters (page 123)	Before you start an aseptic sort
Fluidics	Aligning the automated stage (page 123)	After replacing a damaged sort nozzle, when using a sheath fluid other than PBS, or whenever it is especially important that each drop falls in the exact center of the well

Stopping or restarting the stream

Introduction

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

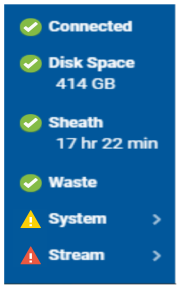
About this task

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

Procedure

To stop or restart the stream:

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



2. In the **Stream View** window, click **Stop Stream**.
3. To restart the stream, do one of the following:
   - Load a tube in the **Experiments** workspace using the Acquisition Dashboard on the **View Data** or **Sort** tab.
   - Click **Start Stream** in the **Stream View** window.

Cleaning external surfaces

Introduction

All instrument surfaces that have been exposed to sheath fluid should be cleaned to prevent salt buildup. For optimal performance, we recommend that you clean surfaces daily.

Before you begin

If the stream is on, turn it off. See Stopping or restarting the stream (page 90).

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 94).

About this task

The following surfaces should be inspected and cleaned when necessary:

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

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

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

Procedure

To clean the external surfaces, select any of the following options:

Option	Description
Clean the salt buildup off the surface of the flow cell cuvette	Remove the nozzle and use a cotton swab moistened with water around the opening and the inside of the bottom of the cuvette where the nozzle is inserted.
Clean the salt buildup off the surface of the closed-loop nozzle	Remove the closed-loop nozzle and wipe it thoroughly with water. Ensure that it is dry before you reinstall it.
Clean the salt buildup off the remaining surfaces	Wipe the surfaces with a cloth dampened with water followed by 70% ethanol.
Clean the waste aspirator drawer	Flush the waste drawer compartments using a bottle of DI water just before turning off the stream or system shutdown (so that it is still aspirating) to prevent clogging of the waste drawer with salt, cells, or beads).
Decontaminate the surfaces	Wipe the surfaces with a cloth dampened with 10% bleach solution followed by DI water.

Lubricating the sample chamber O-ring

Introduction

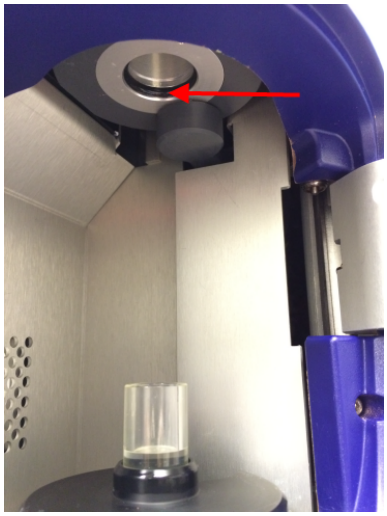
The sample chamber contains an O-ring at the opening at the bottom of the chamber. You should lubricate this O-ring as needed to maintain the proper operation of the sample chamber.

Procedure

To lubricate the sample chamber O-ring:

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

   Ensure that the loading port is empty.
2. On the **Cytometer** page, select **Replace Sample Line Filter**, and then click **Start**. (Do not complete the remaining steps of the wizard.)
3. When the step 1 is completed, reach inside the sample chamber and check to see if the O-ring is dry.



4. If the O-ring is dry, apply a small amount of O-ring lubricant to the O-ring.
5. Exit the wizard without completing it by clicking **Cancel**.

Precautions for handling nozzles

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

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

Changing the nozzle

Introduction

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

About this task

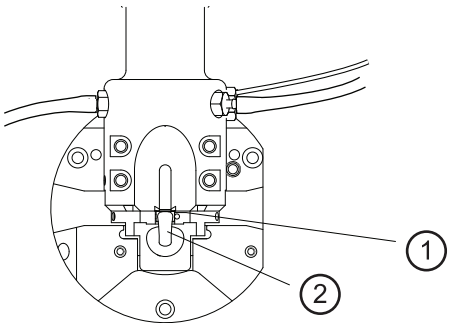
Ensure that the nozzle is clean. See Cleaning the sort nozzle (page 95) or Cleaning the closed-loop nozzle (page 95) as applicable.

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, eye wear, and gloves.

Procedure

To change the nozzle:

1. Ensure that the stream is turned off, and then open the flow cell access door. See Stopping or restarting the stream (page 90).
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 bottom of the flow cell cuvette (with the orange O-ring and 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 you installed a new sort nozzle or one that is different from the nozzle 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 97).

Cleaning the sort nozzle

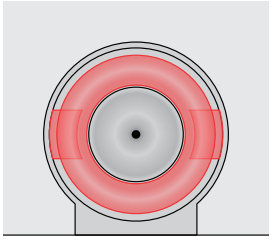
Introduction

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 94).

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

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, eye wear, and gloves.

Procedure

To clean the sort nozzle:

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

   Note: Do not use bleach, Contrad, or any strong detergents to clean the nozzle. See Precautions for handling nozzles (page 93).
3. Allow the nozzle to air dry for a few minutes.
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 94).

Procedure

To clean the closed-loop nozzle:

1. Remove the closed-loop nozzle from the flow cell.
2. Remove salt buildup on the surface of the nozzle by wiping it thoroughly with water and letting it air dry.
3. Clean and remove clogs from the nozzle by doing the following steps:
   a. Remove the nozzle by holding the nut on the side of the nozzle and unscrewing the nozzle.

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

      Note: If the ferrule is old or shows any sign of damage, replace it. A ferrule that is damaged or used for too long can become stuck.
   b. Sonicate the nozzle in a tube containing DI water, ensuring that it is fully submerged. Repeat as needed until the nozzle is clean.
   c. Make sure that the ferrule is on the tubing, and then screw the closed-loop nozzle back onto the nut.

      Make sure that the waste line has not come loose from the nozzle.
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. Allow the nozzle to air dry for a few minutes.
3. 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.

4. 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 the stream is off, turn it on by clicking **Start Stream** in the **Stream View** window.

If the stream does not start, or if shuts off quickly after starting, you might need to:

- Empty the waste tank or fill the sheath tank. See Emptying the waste tank (page 107) and Filling the sheath tank (page 108).
- Check for debris in the nozzle tip by examining the nozzle tip under a microscope and cleaning the nozzle if necessary. See Cleaning the sort nozzle (page 95).

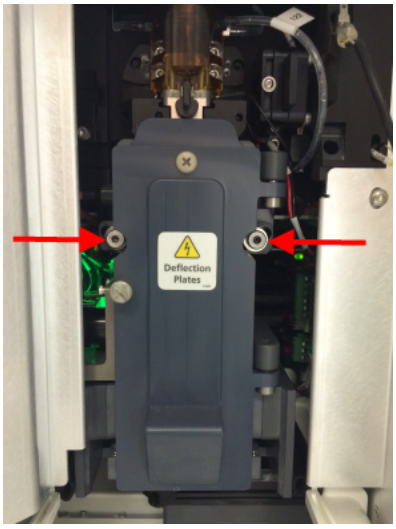
About this task

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

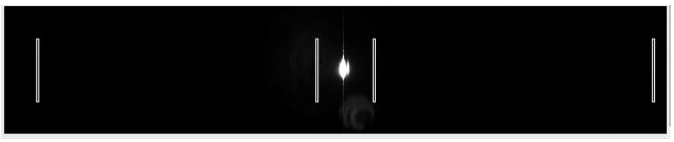
Procedure

To align the waste aspirator drawer to the stream:

1. Open the flow cell access door.
2. Loosen the screws on both sides of the sort block door by using a hex wrench.



3. In the software, open the **Stream View** window by clicking the **Stream** status indicator in the navigation bar.
4. Gently nudge the sort block to the left or right until the stream displays in the middle between the two hash marks of the lower stream view. (The hash marks represent the edges of the waste aspirator.)



5. Slowly tighten the screws, ensuring that the stream position in the drawer does not move.
6. 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 1.5% BD Detergent Solution every time you perform a daily shutdown procedure. You can use Flow Cell Clean separately whenever additional cleaning is needed. In cases where debris builds up in the flow cell as indicated by high CVs, clean the flow cell with 1.5% BD Detergent Solution rather than DI water.

Before you begin

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 94)
- Emptying the waste tank (page 107)
- Filling the sheath tank (page 108)

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 91).

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

Cleaning with sterile DI water

To clean the flow cell with sterile DI water:

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

Cleaning with BD Detergent Solution

This procedure "scrubs" the flow cell by running Flow Cell Clean several times, with detergent, air, and DI water. Use this procedure in cases where debris builds up in the flow cell as indicated by high CVs in the CS&T report.

To scrub the flow cell with detergent:

1. On the **Cytometer** page, select **Flow Cell Clean**. Follow the instructions on the wizard, but substitute 1.5% BD Detergent Solution for DI water.
2. Place an empty sample tube in the sample chamber and run **Flow Cell Clean** again. This will create bubbles that scrub the inside of the sample path and flow cell.
3. Wait 5 minutes, then run **Flow Cell Clean** three additional times with sterile DI water to rinse the detergent thoroughly from the flow cell.

Preparing for aseptic sort

Introduction

The Prepare for Aseptic Sort procedure cleans the flow cell and the entire system of any potential contaminants. You should run this procedure when you want to sort under sterile conditions.

Before you begin

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 94)
- Filling the sheath tank (page 108)
- Changing the fluid filter (page 110)
- Emptying the waste tank (page 107)
- Removing the sheath probe (page 121)
- Preparing new fluid filters (page 123)
- Shutting down the system (page 43)
- Fluidics startup (page 35)

You need the following items:

- Four fluid filters, one for each type of fluid
- At least 2.5 L of 10% bleach solution
- At least 2.5 L of DI water
- At least 2.5 L of 70% ethanol solution
- At least 2.5 L of sheath fluid (1X PBS)
- Sterile DI water for rinsing

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

About this task

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

Be sure to allow sufficient time to autoclave the sheath tank and allow it to come to room temperature before you add the sterile sheath fluid.

If you have two sheath tanks, you can autoclave one tank while you use the other to wash with bleach, sterile DI water, and ethanol. See 2-tank method (page 100). Contact your BD representative for information on purchasing an additional tank.

If you have only one tank, you will need to halt the procedure after washing with bleach, DI water and ethanol to autoclave the tank. See 1-tank method (page 101).

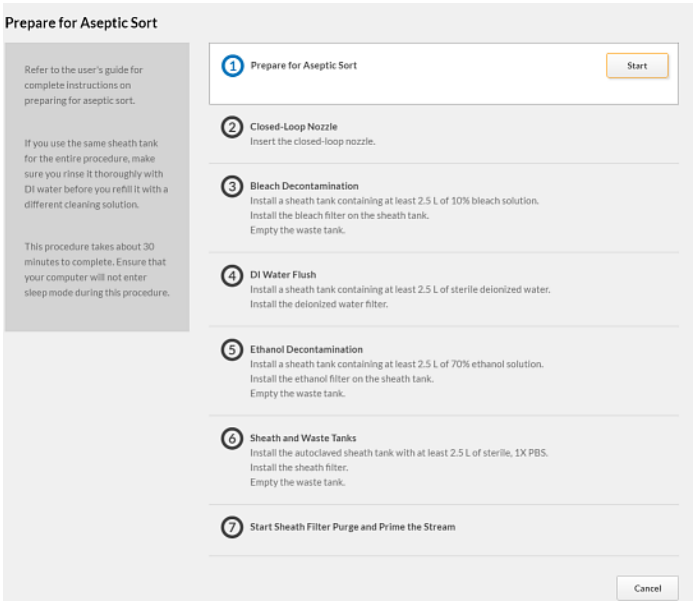
2-tank method

To prepare for aseptic sort when two tanks are available:

1. Set optimal conditions by doing the following:
   a. Depending how long your system has been shut down, run either an extended fluidics startup or a daily fluidics startup. See Preparing new fluid filters (page 123)
   b. Perform a Flow Cell Clean. See Cleaning the flow cell (page 98)
2. Sterilize one sheath tank:
   a. Remove the sheath probe along with the attached sheath sensor cable. See Removing the sheath probe (page 121)

      The cable is marked with a DO NOT AUTOCLAVE label.
   b. Autoclave the sheath tank (with the gauge attached) according to your organization's protocols. BD recommends autoclaving at 125 °C and 15 psig for 30 minutes with a 7.5-minute warmup and shutdown cycle.
3. Use the second tank to 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.
4. Select **Prepare for Aseptic Sort**.
   A wizard displays.



5. Select number one (1), and then click **Start**. Verify that the closed-loop nozzle is inserted (2).
6. Follow the steps of the wizard to wash with bleach (3), sterile DI water (4), and 70% ethanol (5).

Be sure to wash the tank with sterile DI water and to empty the waste tank between solutions.

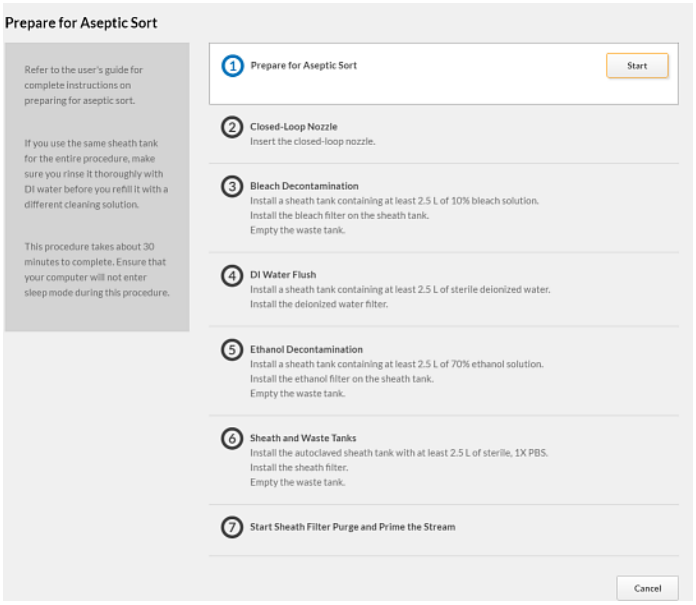
7. Complete Steps 6 and 7 of the wizard using the autoclaved sheath tank, sheath filter, and sterile sheath fluid.

1-tank method

To prepare for aseptic sort when only one tank is available:

1. Set optiomal conditions by doing the following:
   a. Depending how long your system has been shut down, run either an extended fluidics startup or a daily fluidics startup. See Preparing new fluid filters (page 123)
   b. Perform a Flow Cell Clean. See Cleaning the flow cell (page 98)
2. On the **Cytometer** page in the software, select **System Startup**.
3. 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.
4. Select **Prepare for Aseptic Sort**. A wizard displays.



5. Select number one (1), and then click **Start**. Verify that the closed-loop nozzle is inserted (2).
6. Follow the steps of the wizard to wash with bleach (3), sterile DI water (4), and 70% ethanol (5).

   Be sure to wash the tank with sterile DI water and to empty the waste tank between solutions.
7. When Step 5 has completed, click **Cancel** to exit the wizard.
8. Perform a Flow Cell Clean or Daily Shutdown procedure using a sterile sample tube containing 3 mL of sterile DI water. Remove the sheath tank and empty the waste tank.
9. Sterilize the sheath tank:
   a. Remove the sheath probe. See Removing the sheath probe (page 121).
   b. Remove the sheath sensor cable.

      The cable is marked with a DO NOT AUTOCLAVE label.

c. Autoclave the sheath tank (with the gauge attached) according to your organization's protocols. BD recommends autoclaving at 125 °C and 15 psig for 30 minutes with a 7.5-minute warmup and shutdown cycle.
d. Allow sufficient time for the tank to autoclave and cool to room temperature before proceeding.

10. Start the instrument and perform an extended fluidics startup using the autoclaved sheath tank and sterile sheath fluid. This step replaces Steps 6 and 7 of the wizard.

Cleaning the optical filters

Introduction

You should inspect and clean the optical filters as needed.

About this task

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 unit.
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 or trigon, and make sure the filters are pushed all the way in.

Cleaning the Accudrop laser window and the lower camera window

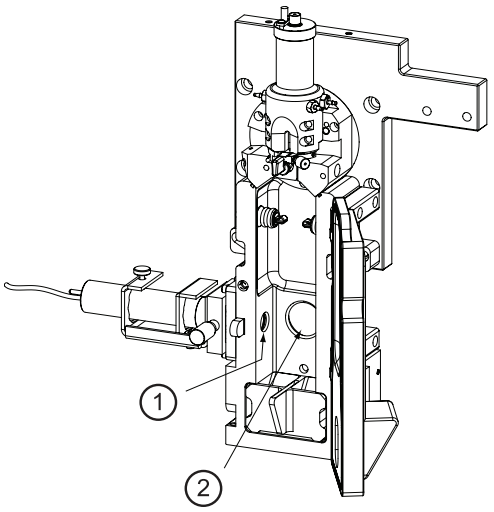
Introduction

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

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 or restarting the stream (page 90).
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 windows 1 and 2 (shown earlier) 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

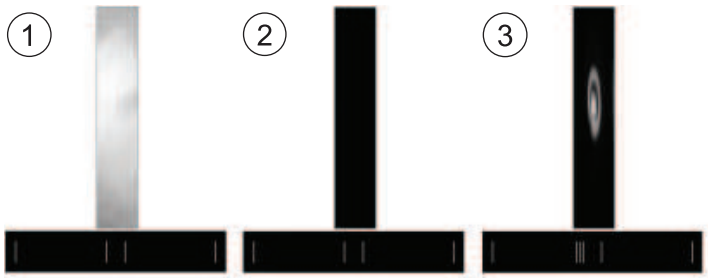
Introduction

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 displays.

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

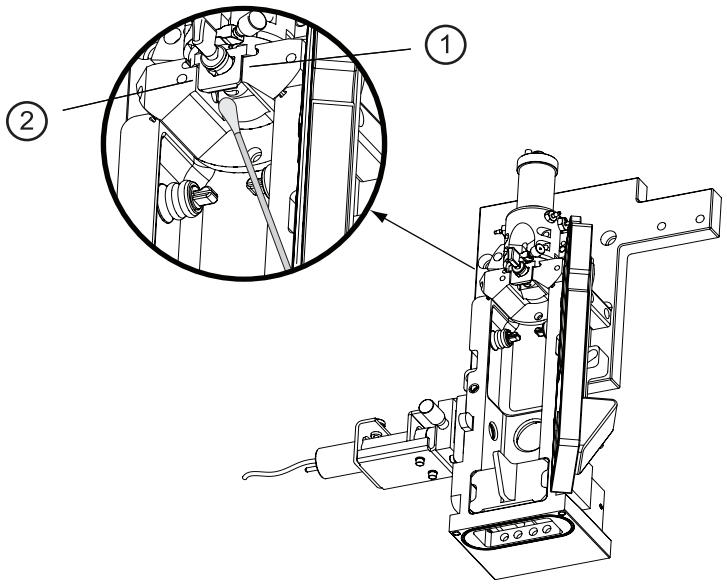
Do not touch the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. A 12,000-volt potential exists between the deflection plates when they are on. The plates remain energized even when the sort block door is open.

Procedure

To clean the strobe lens window and upper camera window:

1. Ensure that the stream is turned off. See Stopping or restarting the stream (page 90).
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 block.



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

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 or restarting the stream (page 90).
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. The 1.5 and 2.0 ND filters are helpful for applications involving large particles.

About this task

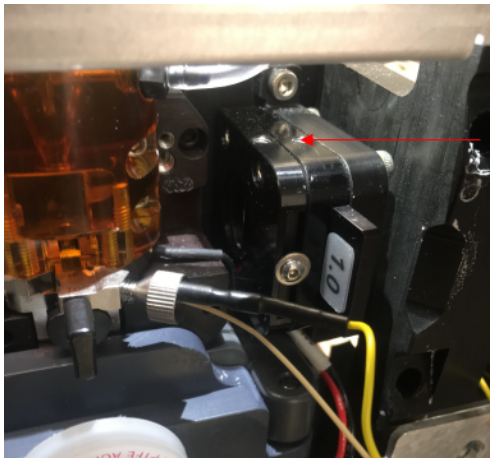
Note: The 1.0 ND filter must be installed when you run Drop Delay.

Procedure

To remove or install the FSC ND filter:

1. Remove the top cover of the sorter unit by loosening the hex screw on the front and lifting off the cover.
2. Open the flow cell access door.
3. Loosen the set screw on the top of the FSC ND filter assembly with a 1.5-mm Allen wrench.

Do not touch the obscuration bar screw.



4. Perform one of the following:
   a. Remove the FSC ND filter from the slot.
   b. Install the new filter by sliding it into the slot with the label facing the flow cell, as shown in the preceding picture.
5. 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. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

Procedure

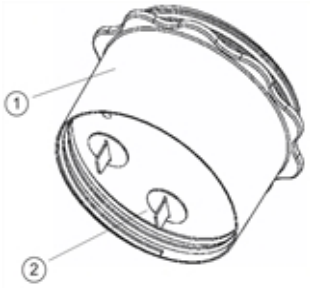
To empty the waste tank:

1. Turn off the stream. See Stopping or restarting the stream (page 90).
2. Disconnect the sensor and fluid lines from the waste tank.

   Note: The waste tank can become pressurized when the instrument 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.

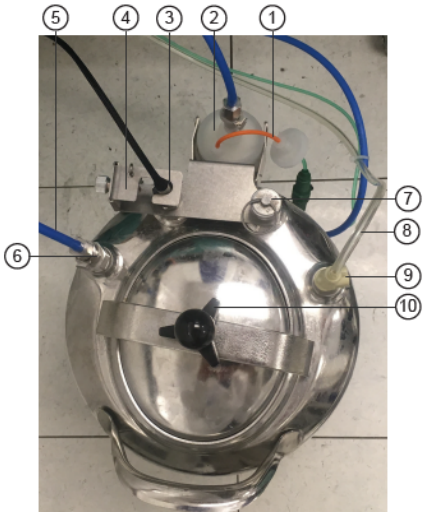
Filling the sheath tank

Introduction

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

About this task

The following illustration shows the sheath tank and connectors.



No.	Description
1	Fluid filter purge line
2	Fluid filter
3	Sheath sensor
4	Containment device
5	Fluid line
6	Fluid connector
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 or restarting the stream (page 90).
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. The weld line is indicated by the arrow in the following figure.

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.
9. Purge the sheath filter. See 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.

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

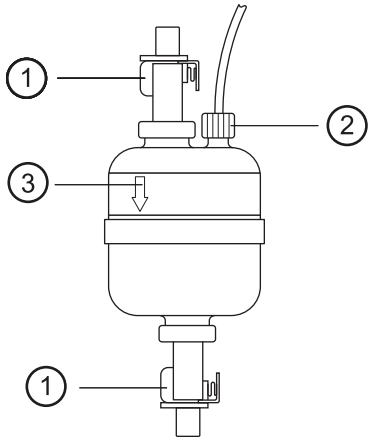
Spare filters are included with the accessory kit.

See Preparing new fluid filters (page 123) before you start the procedure.

Procedure

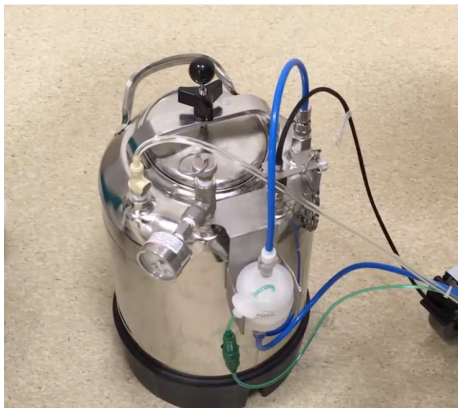
To change the fluid filter:

1. Turn off the stream. See Stopping or restarting the stream (page 90).
2. Disconnect the air line from the sheath tank.
3. Pull up on the ring of the pressure relief valve to release the pressure from the tank. Verify that all of the pressure is released by pulling up a second time.
4. Disconnect the filter by pressing the metal tabs on each end.



No.	Description
1	Tabs
2	Filter purge line nut
3	Direction of flow

5. Disconnect the filter purge line by unscrewing the nut.
6. Write the current date on the filter so that you will know when to replace it.
7. Reconnect the filter purge line to the new filter.



8. Use the arrows on the filters that indicate the direction of the flow through the filter and replace the filter with a new one in the same orientation.
9. Reconnect the air line to the tank and check for leaks when the pressure is turned on.
10. Purge the sheath filter. See 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 fill the sheath tank, install a new sheath filter or 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 chamber is automatically flushed inside and out with sheath fluid to eliminate potential sample carryover. You can perform additional backflush cleaning whenever you observe sample carryover, or after running samples with adherent cells or dye.

Procedure

To backflush the sample line:

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

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

Cleaning the sample line

Introduction

You should clean the sample line with bleach at the end of your experiment.

Procedure

Do not attempt to open the sample loading door while a sample is being loaded and run. A load sample pin locks while a sample is being processed. Pulling on the door could damage the door or locking mechanism.

To clean the sample line:

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

We recommend that you clean the sample line after each experiment.

Always run a tube of DI water after running bleach on the cell sorter.

Clearing a sample line blockage

Introduction

Buildup can occur in the sample line if your instrument has been unused for an extended period of time. Indications of buildup include seeing no events or no decrease of fluid levels in the sample tube when cleaning the flow cell. Follow this procedure to ensure removal of any clogs or buildup from the sample line, which will improve performance until it is time to replace the sample line.

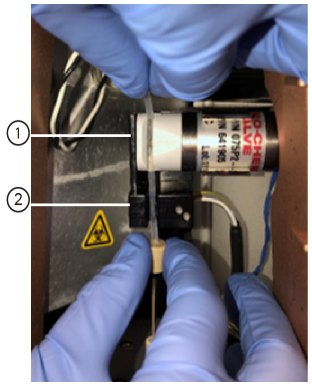
Before you begin

You need the 2.5-mm Allen wrench from the accessory kit.

Procedure

To clear the sample line:

1. On the **Cytometer** page, select **Replace Sample Line**, and then click **Start** on the wizard.
   Running the wizard opens the slot for the pinch valve and allows for easy handling of the sample line.



2. Pull the sample line tubing out of the slot in the pinch valve (1) and bubble detector (2) at the top of the sample chamber.

Do not allow kinks to form in the sample line during removal or insertion.

All biological specimens and materials can transmit potentially fatal infection. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eye wear, and gloves.

3. Gently massage the clear portion of the tubing between your fingers to break any blockage in the line.
4. Make sure the portion of the sample line below the bubble detector is not bent. Then gently push the sample line back into the bubble detector. The sample line should be seated firmly against the back of the bubble detector.
5. Push the pinch valve tubing back into the pinch valve. If necessary, ensure that the tubing is firmly seated.
6. Proceed to the end of the wizard. This allows the pinch valve to close properly.

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 kinked or clogged.

Before you begin

You need the following items from the accessory kit:

- Replacement sample line assembly
- 2.5-mm Allen wrench

About this task

Use sterile forceps to handle the sample line filter.

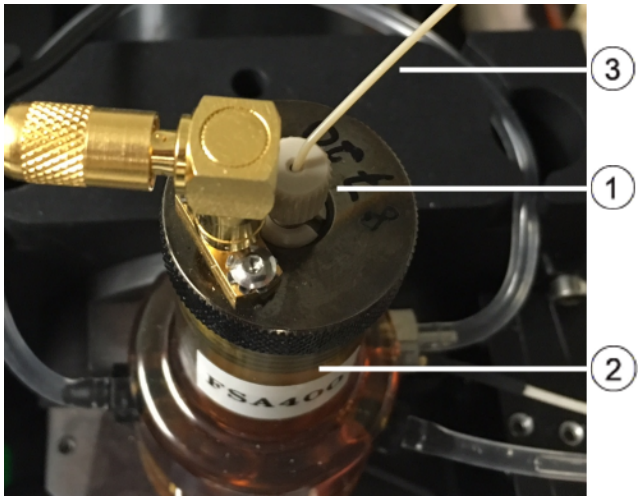
All biological specimens and materials can transmit potentially fatal infection. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eye wear, and gloves.

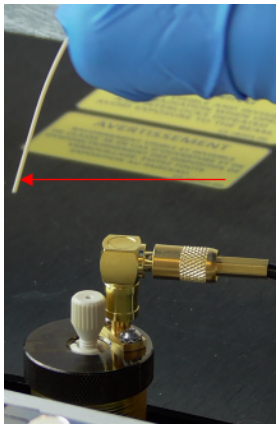
Procedure

To replace the sample line:

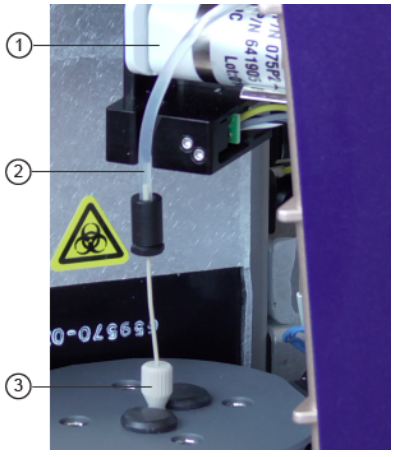
1. On the **Cytometer** page, select **Replace Sample Line**, and then click **Start** on the wizard.
2. Remove the sample line:
   a. Remove the sample line access door and open the flow cell access door.
   b. Remove the top cover of the sorter unit by loosening the hex screw on the front and lifting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

   Note: If the cover is bolted down and cannot be lifted, call BD Biosciences technical support for assistance.
   c. Loosen the fitting (1) at the top of the flow cell (2) and slowly pull the sample line (3) out. Ensure that the fitting remains in the top of the flow cell.

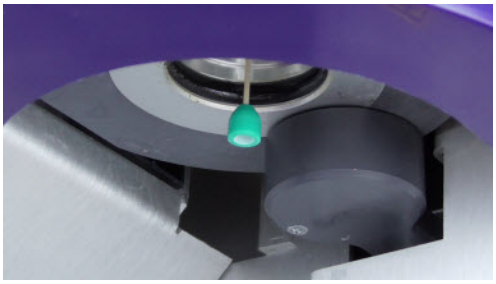




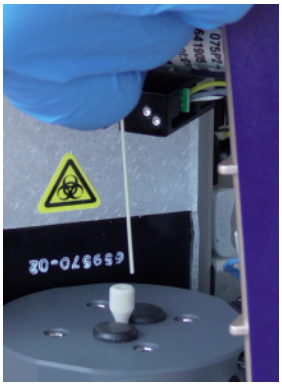
d. Pull the tubing (2) out of the slot in the pinch valve (1) at the top of the sample chamber, and loosen the fitting (3) that connects the tubing to the chamber.



e. If a filter is installed on the sample line, remove it by pushing the sample line down so that the end is inside the sample chamber. Use forceps to remove the filter.



f. Slowly pull the sample line out from the top of the sample chamber and ensure that the fitting remains in the top of the sample chamber.

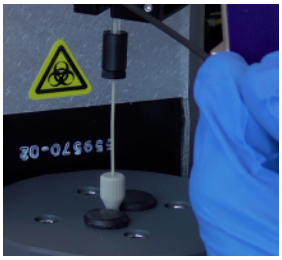


g. Discard the sample line tubing.

3. Install the new sample line tubing:

Do not allow kinks to form in the sample line during insertion.

a. Insert the sample line tubing into the fitting on the top of the sample chamber.



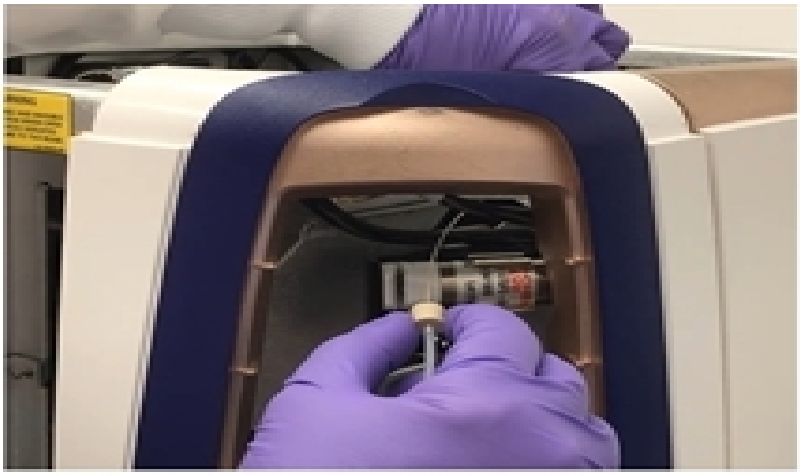
b. Push the tubing from the top until it is in the middle of the sample viewing window.

c. Tighten the fitting on the top of the sample chamber, leaving the fitting loose enough so that the sample line height can still be adjusted.

Do not over-tighten the fitting and do not use tools. Over-tightening the fitting can kink or damage the tubing.

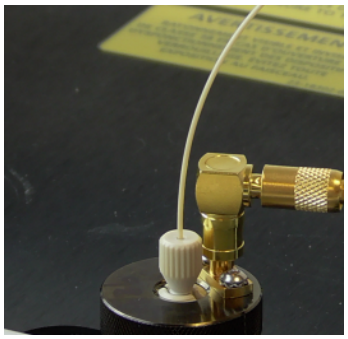
d. Route the sample line tubing to the flow cell.

e. With one hand, remove the new sample line from the protective sleeve and, with the other hand, carefully route the end of the sample line up through and under the chassis.



Make sure to route the sample line under the metal bar and gently pull it toward the top of the flow cell.

f. Insert the end of the sample line tubing into the fitting at the top of the flow cell until the tubing stops.



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

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

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

h. Click **Continue** on Step 2 of the wizard.

4. Complete the Sample Line Height step according to the instructions in Step 3 of the wizard and click **Continue**.

   Ensure that the sample line touches the bottom of the tube freely but without bowing or bending the line. If you need to adjust the length, loosen the fitting on top of the sample chamber, make the adjustment, and tighten the fitting again.

5. Complete the Fittings step according to the instructions in Step 4 of the wizard.
6. Follow Step 5 of the wizard to push the pinch valve tubing back into the pinch valve. If necessary, ensure that the tubing is firmly seated by pressing it with an Allen wrench.

7. Click **Continue**.
8. Click **Close**.
9. Load a tube of DI water and perform a Flow Cell Clean. See Cleaning the flow cell (page 98).
10. Check for leaks by ensuring that no fluid is observed on or around the fittings.
11. Check that fluid is flowing into the sample line by confirming that the level of DI water in the tube is lower after the Flow Cell Clean.
12. Replace the sample line access door and the top cover of the sorter unit, then close the flow cell access door.

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 filter might be clogged.

About this task

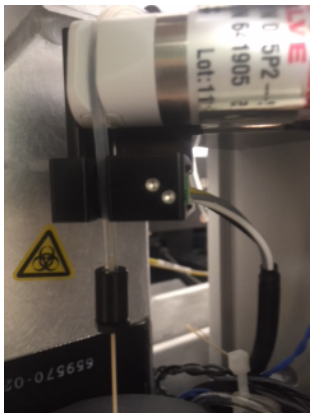
Use sterile forceps to handle the sample line filter.

All biological specimens and materials can transmit potentially fatal infection. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eye wear, and gloves.

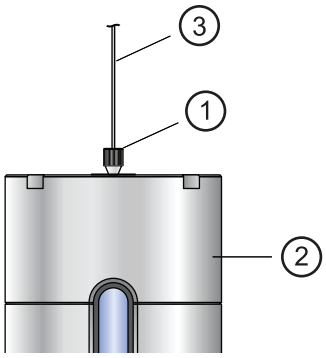
Procedure

To replace the sample line filter:

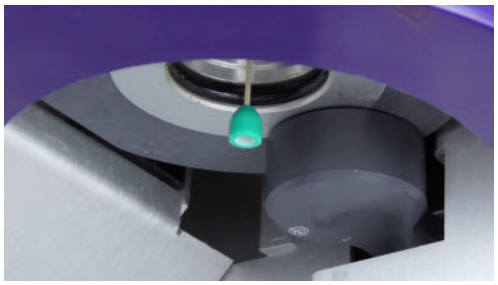
1. On the **Cytometer** page, select **Replace Sample Line Filter**, and then click **Start**.
2. Complete the Sample Line Filter step on the wizard:
   a. Remove the sample line access door from the sorter unit.
   b. Pull the pinch valve tubing out of the slot in the pinch valve.



   c. Loosen the sample line fitting (1) at the top of the sample 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 chamber.
   e. If a sample line filter is already installed on the sample line, remove it by pushing the sample line down so that the end is below the chamber and you can use forceps to remove the filter.



   f. Install the new sample line filter by sliding it onto the end of the sample line using sterile forceps.
   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 fitting on top of the sample chamber, adjust the length, and tighten the fitting again.

4. Complete the **Sample Line Fitting** step according to the instructions in the wizard.
5. Push the pinch valve tubing back into the pinch valve according to the instructions in the wizard.

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

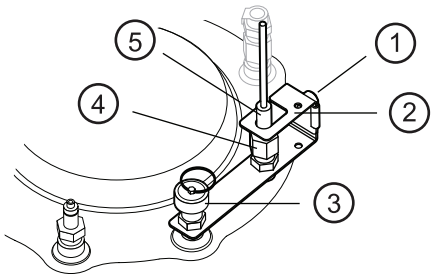
Note: Do not autoclave the sheath probe. It is not designed to withstand the conditions of autoclaving.

Procedure

To remove the sheath probe:

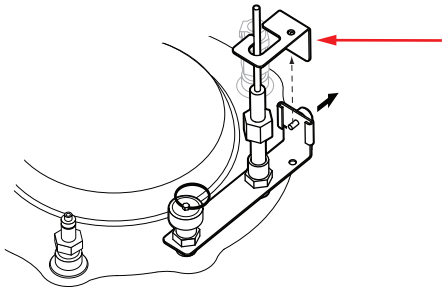
1. Turn off the stream. See Stopping or restarting the stream (page 90).
2. Disconnect the air line from the sheath tank.
3. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.
4. Loosen the nut at the top of the sheath probe with a wrench and vent the sheath pressure again if the tank is still pressurized.

   Note: The nut could slide down the length of cable when you disconnect the sheath probe. Check the length of the cable when you are ready to reconnect the sheath probe.

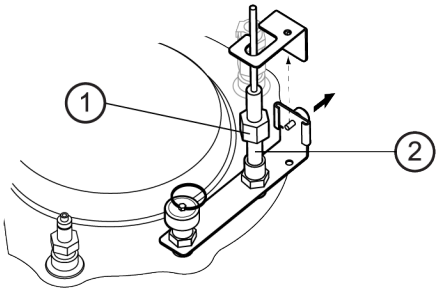


No.	Description
1	Thumbscrew
2	Containment device
3	Pressure relief valve
4	11/16-inch nut
5	Sheath probe

5. Loosen the thumbscrew on the containment device.
6. Pull the top section of the containment device straight up and out of the bottom section.



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

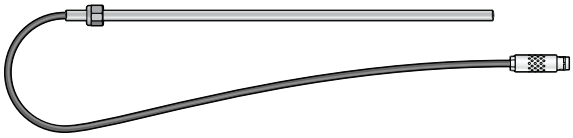


No.	Description
1	11/16-inch nut
2	Press fitting

If the press fitting becomes loose, do not use the sheath sensor. It could dislodge and cause bodily injury.

If you find a loose press fitting, contact your BD service representative for assistance.

8. Decontaminate the sheath probe using 70% ethanol.



Preparing new fluid filters

Introduction

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

Before you begin

Ensure that you know how to perform the following procedures:

- Filling the sheath tank (page 108)
- Changing the fluid filter (page 110)
- Purging the sheath filter (page 112)

Procedure

To prepare new fluid filters:

1. Empty the sheath tank, rinse with DI water and refill with DI water. See Filling the sheath tank (page 108).
2. Remove the cap from the new filter and save it for reuse.
3. Change the fluidic filter to the new filter. See Changing the fluid filter (page 110).
4. In the BD FACSChorus™ software, select **Cytometer > Sheath filter purge** and select **Start**.
5. Repeat step 4 three times to ensure all of the air is removed from the fluid filter.
6. Change the fluid filter to prepare another new fluid filter, or install the appropriate filter for the next workflow.
7. Replace the cap on the newly prepared fluid filter and store at room temperature.
8. Empty the sheath tank and fill with appropriate fluid for the next workflow.

Aligning the automated stage

Introduction

Proper alignment of the automated stage ensures that each sorted drop falls into the exact center of each well of a plate or slide.

About this task

We recommend that you test and adjust the stage alignment in the following situations:

- The amount of sample is limited.
- The volume in the collection device is very small.
- You are using a 384-well plate.
- You are using a sheath fluid other than PBS.
- You are using a different sort nozzle.

Note: If you use more than one nozzle with your instrument, you need to run the alignment procedure for each nozzle prior to use.

Before you begin

Ensure the following:

- The deflection plates are clean. See Cleaning the deflection plates (page 105).
- The sort nozzle is inserted. See Changing the nozzle (page 94).
- The splash shield is in place. See Installing the splash shield (page 60)
- The waste aspirator drawer is aligned to the stream. See Aligning the waste aspirator drawer to the stream (page 97).
- The stream has started and the Stream Status indicator is green. See Stopping or restarting the stream (page 90).

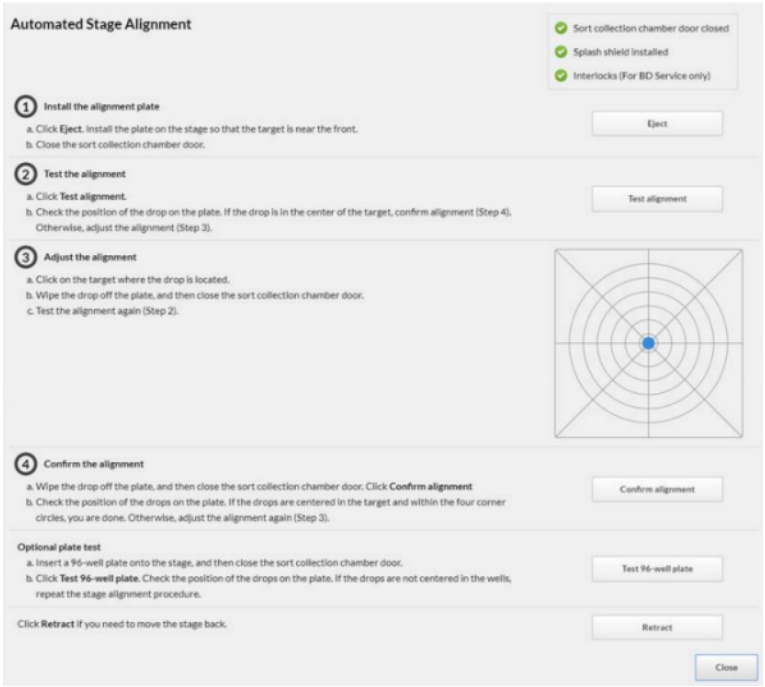
This procedure requires the alignment plate, which is supplied with the automated stage option:

Procedure

To check automated stage alignment and adjust if necessary:

1. Ensure that the sort collection chamber door is closed.
2. On the workstation, click **Cytometer > Other > Automated Stage Alignment**.

   A dialog displays:



3. Follow the instructions on the dialog to align the automated stage and optionally perform the plate test.

   Note: Always close the sort collection chamber door before you perform any command that moves the automated stage.

   Note: To see the location of the drop, you might need to temporarily remove the alignment plate from the instrument. Click **Retract** to move the stage back.

   Note: If you are using nonstandard plates, you might need to click the target at a position slightly offset from the center to center the drop in the well.

Managing your database

Introduction

This topic describes the tasks involved in managing your database. The BD FACSChorus™ Backup and Restore utility allows you to back up and restore your database.

About the Backup and Restore utility

Use the Backup and Restore utility to back up or restore all data that is stored in the BD FACSChorus™ software database, along with all FCS files located in the software-defined directories.

With this utility, you can create a single backup set which contains a backup of the database, along with experiment FCS files. The utility maintains backup sets indefinitely (as long as you are backing up and restoring using the same software version and you have not modified the backed up data). It displays the amount of free disk space on the workstation. You can discard existing backup sets to free up space.

You can also use any backup set to restore the database and FCS files. When you restore, you erase any new data created since the backup was created.

The backup location

You can place backup data on the workstation or on an external storage device. For expediency, we recommend that you first back up the data to the workstation and then move it to an external source to free up space on the workstation. However, if you have a significant amount of data, you might need to back up directly to an external device.

Note: You must restore data from the same location you used during the backup process.

Note: You might experience some latency when backing up data to an external device. The amount of time depends on the speed of external device you use as well as the connection type.

Before you begin

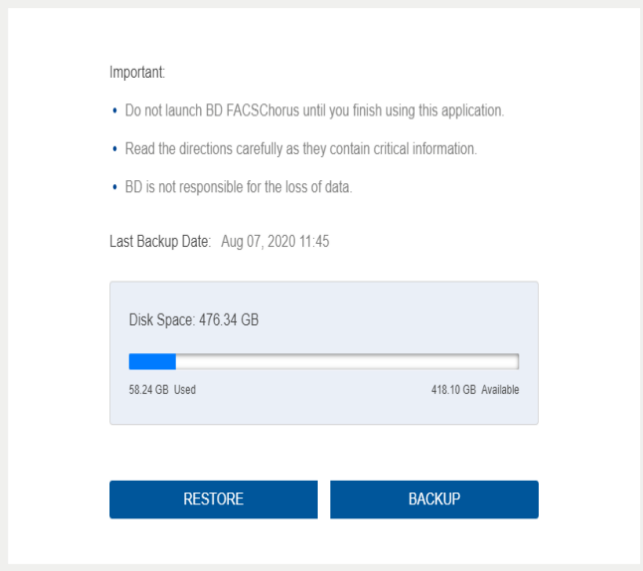
Make sure the BD FACSChorus™ software is closed.

Creating a new backup set

To create a new backup set:

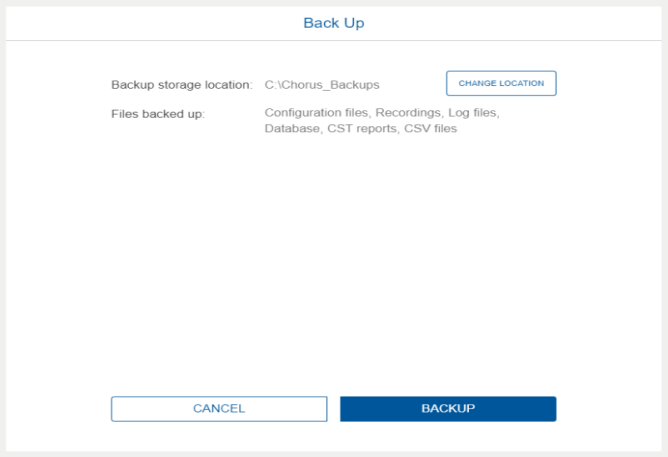
1. Start the BD FACSChorus™ Backup and Restore from the icon on the desktop.

2. Click **BACKUP**.

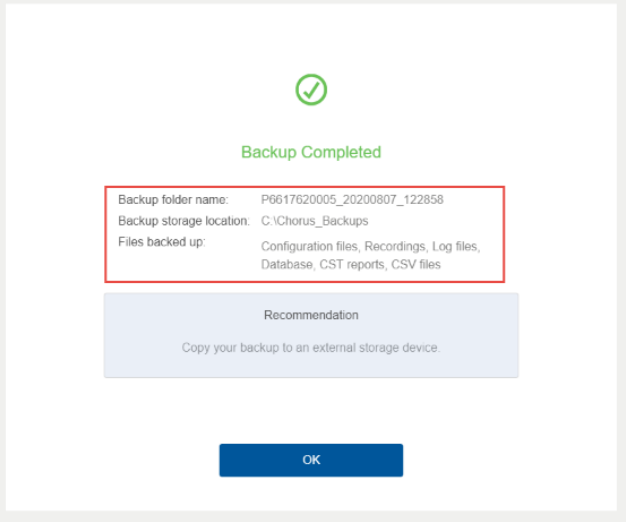


The Back Up window opens, indicating the amount of disk space.

3. Verify that at least 250 GB disk space is available and click **BACKUP**.



The backup process starts and displays a progress bar. A completion dialog is displayed and indicates success or failure. If the backup succeeds, the timestamp of the new backup is provided.



If the backup fails, the reason is indicated. For example, if the amount of space required is greater than the amount available, the software prompts you to free up additional space and try again.

Note: We recommend leaving the Backup storage location set to the default location of C:\Chorus_Backups on the local workstation. After the backup process is complete, the backup folder can be retrieved from this location.

Note: Backups saved on the local workstation can later be moved to an external storage device.

4. After creating the backup folder, we recommend that you copy your backup to an external storage device.

   Do not change the contents of a backup folder. Otherwise, will not be able to restore the backup using the Backup and Restore utility.

5. Click **OK** to return to the first screen of the utility or close the window.

Maintaining data integrity

To be able to restore your backed up data, you must maintain the integrity of your the data. Certain changes are allowed while others will comprise the data integrity. The following table shows which changes are

supported as well as changes that are not supported:

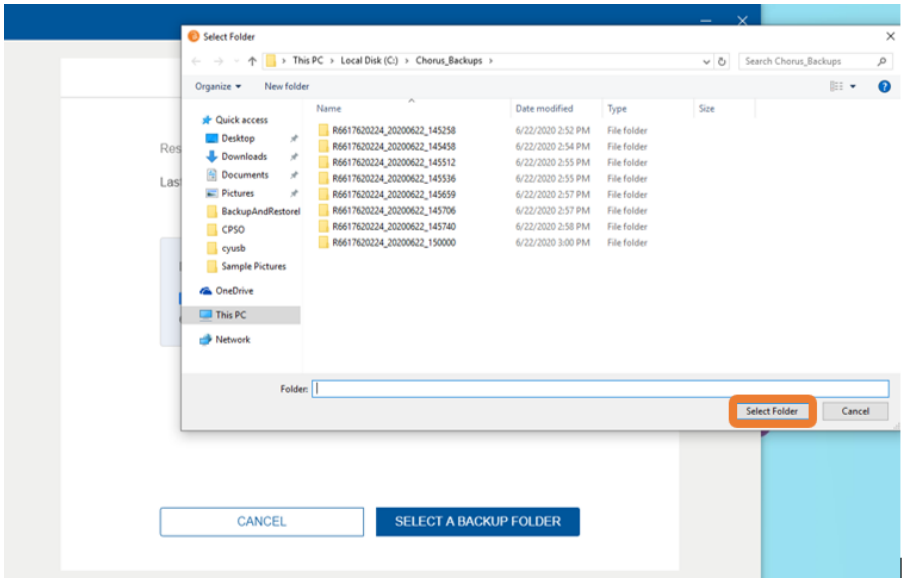
	Action
Allowed	Change the name of the backup folder
	Move backup files between the local workstation and other external sources
Not Allowed	Change the name of any folders or files inside the backup folder
	Change or delete the contents of the files contained in the backup folder

Restoring a backup set

To restore a backup set:

1. Click the BD FACSChorus™ Backup and Restore utility icon on the desktop.

2. Click **RESTORE**.
3. Click **SELECT A BACKUP FOLDER**.
4. Go to the backup folder you want to restore and click **SELECT FOLDER**.



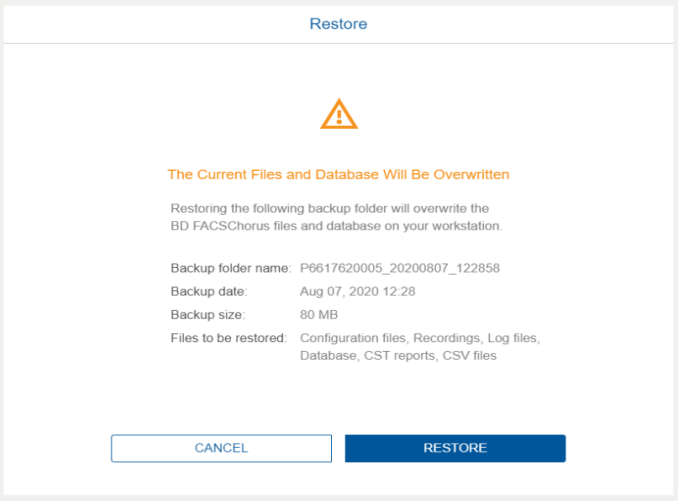
Note: We recommend that you restore from a folder that is on the local workstation. If the backup was copied to an external drive, copy the folder back to the workstation before completing the restore process. By default, backups are saved to and restored from C:\Chorus_Backups.

The files are displayed with the timestamp of the selected backup set and the required disk space.

A confirmation dialog is displayed. The restore process begins and displays a progress bar. A completion dialog is displayed and indicates success or failure.

5. Click **RESTORE**.

   If the estimated space required is greater than the amount available, the system prompts you to free up additional space and try again.



   Note: This process will remove any previous backed up files from this location.

6. Click **OK** to return to the first screen of the utility or close the window.

Maintaining your workstation data

Experiments that are no longer being used should be removed or saved to an external source. We recommend that you check at least once per month, or more frequently as defined by lab validated procedures. Ideally, you should maintain 40 percent of your hard drive as free disk space. This will allow the software to perform optimally.

Experiments should be archived to a known location, preferably not on the hard drive of the BD FACSMelody™ workstation.

Procedure

To archive experiments not actively in use:

1. Perform a BD FACSChorus™ database backup and export it to a known location, preferably not on the hard drive of the BD FACSMelody™ workstation.
2. Navigate to the Manage Experiments workspace.
3. Select the experiments you would like to archive.
4. In the File menu, select **Export Experiments** > **With Data** to export the selected experiments to the desired known location.
5. Confirm that the files were successfully exported.
6. In the Edit menu, select **Delete** to delete the selected experiments that have been exported.
7. Exit the BD FACSChorus™ application.

8. To show hidden files and folders in Windows 10:
   a. In the Search box, type **Show hidden files and folders**, then press **Enter**.
   b. In the View tab, select **Show hidden files and folders**, then click **OK**.
9. Navigate to C:\ProgramData\BD\FACSChorus\Experiments\Deleted Tubes and delete the contents of that directory.
10. Perform a database backup again and export it to a known location, preferably not on the hard drive of the BD FACSMelody™ workstation.

If you need to retrieve one or more of the experiments from the archive, use the Import Experiment functionality to import the desired experiment(s) from the archiving location.

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Technical specifications

This chapter covers the following topics:

Optical specifications

Parameter	Value
Excitation laser	1-laser (blue), 2-color 1-laser (blue), 4-color 2-laser (blue, red), 6-color (4-2) 2-laser (blue, violet), 6-color (3-3) 2 (blue, yellow/green), 4-color (2-2) 2 (blue, yellow/green), 6-color (2-4) 3-laser (blue, red, violet), 6-color (2-2-2) 3-laser (blue, red, violet), 9-color (4-2-3) 3 (blue, violet, yellow/green), 8-color (2-2-4) 3 (blue, red, yellow/green), 8-color (2-2-4)
Laser specifications	Blue laser Wavelength: 488 nm Output power: 20 mW Nominal power: 16 mW Beam spot size: 9 ±3 µm x 67 ±5 µm Red laser Wavelength: 640 nm Output power: 40 mW Nominal power: 36 mW Beam spot size: 9 ±3 µm x 67 ±5 µm Violet laser Wavelength: 405 nm Output power: 40 mW Nominal power: 36 mW Beam spot size: 9 ±3 µm x 67 ±5 µm Yellow-green laser Wavelength: 561 nm Output power: 50 mW Nominal power: 40 mW Beam spot size: 9 ±3 µm x 67 ±5 µm

Parameter	Value
Laser alignment	Fixed and spatially separated alignment of all lasers with the cuvette flow cell
Beam divergence angle (full-angle)	488 nm: <1.2 mrad 640 nm: <1.3 mrad 405 nm: <1.0 mrad 561 nm: <1.2 mrad
Optical coupling	The quartz cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens for optimal light collection efficiency. Numerical aperture: 1.2.
Detection channels	FSC, SSC, and up to nine fluorescence. See Optical configurations (page 23) for more details.
Fluorescence and side scatter detection	Reflective optics with single transmission bandpass filter in front of each PMT High performance customized PMT modules for all fluorescence and SSC channels Light collected by objective lens is delivered by fiber optics to specially designed heptagon or trigon detector arrays.
Stream illumination at 25°C (Accudrop laser)	
Optical power	>18 mW
Lasing wavelength	660 nm
Beam divergence angle (full-angle)	<3.0 mrad

Fluidic specifications

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

Sample input formats

Parameter	Value
Sample input	5.0-mL polystyrene or polypropylene tubes

Collection devices

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

Note: Follow the same procedure for loading a 96-well plate when loading a 96-well PCR tray. To ensure appropriate alignment, place the 96-well PCR tray in a holder (Genemate 96 well PCR Storage Rack #R-7909 from Bioexpress) prior to loading.

Electronic and software specifications

Parameter	Value
Software	BD FACSChorus™ version 1.0 or later
Operating system	Microsoft Windows 10 64-bit for BD FACSChorus™ version 1.3 or later
Monitor	23-inch LCD with a minimum 1,920 x 1,080 resolution
Memory	At least 8 GB RAM
Storage	Hard drive with at least 500 GB free space
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 unit	50.8 x 55.9 x 48.3 cm (20 x 22 x 19 in.)
Biological safety cabinet (optional)	136.5 x 87.6 x 233.4–249.9 cm (53.8 x 34.5 x 91.9–98.4 in.)
Sample temperature control (optional)	42.9 x 91.3 x 47.1 cm (16.90 x 35.95 x 18.55 in.)
Aerosol Management Option	45.21 x 38.1 x 54.61 cm (17.8 x 15 x 21.5 in.)
See the *BD FACSMelody™ Cell Sorter Site Preparation Guide* for additional information on dimensions and clearances.	
Instrument weight	Cytometer unit: 40.75 kg (89.8 lb) Electronics unit: 36.25 kg (79.9 lb)

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8

Troubleshooting

This chapter covers the following topics:

Troubleshooting overview

Introduction

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

The tips in this chapter are designed to help you troubleshoot your experiments. If additional assistance is required, contact your local BD Biosciences technical support representative. See Technical support (page 12).

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

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 95).
	Salt buildup in the nozzle location between the flow cell and the locking lever	Clean the area to remove the salt buildup.
Error starting stream after inserting sort nozzle or loading sample	Sheath tank low or empty, or waste tank full or almost full	Fill the sheath tank to the maximum level or empty the waste tank. See Filling the sheath tank (page 108) or Emptying the waste tank (page 107).
	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 94). Make sure the nozzle is dry.
	Dirty strobe lens or upper camera window	Clean the lens and the window as described in Cleaning the strobe lens window and upper camera window (page 103).
	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 95). If the nozzle seems damaged, replace it. See Changing the nozzle (page 94) and Replacing the sort nozzle seal temporarily (page 96). Restart the stream. See Stopping or restarting the stream (page 90).
	Debris in flow cell	Scrub the flow cell. See Cleaning the flow cell (page 98).
Error starting stream after inserting sort nozzle or loading sample	Air in sheath line or filter	Stop and restart the stream. See Stopping or restarting the stream (page 90). Purge the sheath filter. See Purging the sheath filter (page 112). Run daily fluidics startup. See About CS&T reports (page 40).
	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 or compressor is on and the pressure is between 80 and 95 psi. Verify that the sheath tank lid is sealed properly.
	Residual ethanol in fluidic lines	Run extended fluidics startup.
	Sheath filter orientation is incorrect.	Change the orientation of the filter. See Changing the fluid filter (page 110).

Observation	Possible Causes	Recommended Solutions
BD FACS™ Accudrop laser scan fails to locate stream after change to 100 µm nozzle	Stream is unable to focus or software fails to detect that the nozzle was changed.	1. Check stream (see solutions for Error starting stream after inserting sort nozzle or loading sample). 2. If necessary, adjust sort block so that the stream is in the center of the waste aspirator. See Aligning the waste aspirator drawer to the stream (page 97). 3. Restart the workstation to trigger software detection of the new nozzle.
Lower (stream) camera does not show laser/stream	Stream is unable to focus or software fails to detect that the nozzle was changed.	1. Check stream (see solutions for Error starting stream after inserting sort nozzle or loading sample). 2. If necessary, adjust sort block so that the stream is in the center of the waste aspirator. See Aligning the waste aspirator drawer to the stream (page 97). 3. Restart the workstation to trigger software detection of the new nozzle.
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 94).
	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 95). If the nozzle seems damaged, replace it. See Changing the nozzle (page 94) and Replacing the sort nozzle seal temporarily (page 96). Restart the stream. See Stopping or restarting the stream (page 90).
	New sort nozzle was inserted.	If you are using a new nozzle, the sort block might need to be repositioned to align with the stream. See Aligning the waste aspirator drawer to the stream (page 97).
	Air bubbles in flow cell	Stop and restart the stream to remove bubbles. See Stopping or restarting the stream (page 90).
	Ethanol or other cleaning solution in flow cell Dirty flow cell	Scrub the flow cell. See Cleaning the flow cell (page 98).
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.

Observation	Possible Causes	Recommended Solutions
Problems with Cytometer Setup function	Baseline or performance check failed, or stopped before completing	Prepare a new CS&T sample with the proper concentration of sterile PBS as instructed in the product insert. **Note:** Do not dilute BD® CS&T RUO Beads with water. Close the sort block door and the flow access door properly. Clean the flow cell. See Cleaning the flow cell (page 98). Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. See Clearing a sample line blockage (page 113). If sample flow seems to be blocked, then backflush the sample line several times to clear the block. See Backflushing the sample line (page 112). Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. See Replacing the sample line filter (page 119). Perform clean flow cell again and check that the fluid levels in the sample tube have decreased.
Problems with Cytometer Setup function	Beads not on scale Low event rate or zero event rate	Prepare a new CS&T sample with the proper concentration of sterile PBS as instructed in the product insert. **Note:** Do not dilute BD® CS&T RUO Beads with water. Close the sort block door and the flow access door properly. Turn off the stream and remove, sonicate, and reinsert the nozzle. See Stopping or restarting the stream (page 90) and Cleaning the sort nozzle (page 95). Clean the flow cell. See Cleaning the flow cell (page 98). Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. See Clearing a sample line blockage (page 113). If sample flow seems to be blocked, then backflush the sample line several times to clear the block. See Backflushing the sample line (page 112). Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. See Replacing the sample line filter (page 119). Perform clean flow cell again and check that the fluid levels in the sample tube have decreased.

Observation	Possible Causes	Recommended Solutions
Problems with Drop Delay function	Sort block door is not closed	Close the sort block door properly.
	Flow cell access door is open	Close the flow cell access door properly
	Event rate is too low or too high	Prepare a new Accudrop sample with the proper concentration of sterile PBS as instructed in the technical data sheet. **Note:** Do not dilute BD FACS™ Accudrop Beads with water. Clean the flow cell. See Cleaning the flow cell (page 98). Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. See Clearing a sample line blockage (page 113). If sample flow seems to be blocked, backflush the sample line several times to clear the block. See Backflushing the sample line (page 112). Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. See Replacing the sample line filter (page 119). Clean the flow cell again and check fluid levels in the sample tube. If sample flow continues to be blocked, replace the sample line. See Replacing the sample line (page 114). Clean the flow cell again and check fluid levels in the sample tube
	Debris on lower camera or Accudrop window	Clean the lower camera and Accudrop laser window. See Cleaning the Accudrop laser window and the lower camera window (page 102).

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 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.

Observation	Possible Causes	Recommended Solutions
No events in plots or events don't update in plots after clicking Load Sample	Sample line or sample line filter is clogged	Clean the flow cell. See Cleaning the flow cell (page 98). Confirm by checking that fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. See Clearing a sample line blockage (page 113). If sample flow seems to be blocked, backflush the sample line several times to clear the block. See Backflushing the sample line (page 112). Clean the flow cell again and check fluid levels in the sample tube. If sample flow continues to be blocked, change the sample line filter. See Replacing the sample line filter (page 119). Clean the flow cell again and check fluid levels in the sample tube. If sample flow continues to be blocked, replace the sample line. See Replacing the sample line (page 114). Clean the flow cell again and check fluid levels in the sample tube.
	Sample is not mixed properly	Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 49).
	Threshold is not set to correct parameter	Set the threshold to the correct parameter for your application. See Defining view data (page 49).
	Threshold setting is too low or too high	Adjust the threshold setting. See Defining view data (page 49).
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 49).
	Threshold setting is too low or too high	Adjust the threshold setting. See Defining view data (page 49).
	Events left out of a gate	When drawing a gate, make sure that events on the axes are included.
	Cell size is set incorrectly	Ensure that the setting for the cell size is appropriate for your sample. See Defining view data (page 49).
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.

Observation	Possible Causes	Recommended Solutions
Erratic event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 49).
	Sheath tank is low	Fill the sheath tank. See Filling the sheath tank (page 108).
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sample chamber O-ring is worn	Contact your BD Biosciences service engineer.
Unexpectedly high event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 49).
	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 49).
	Sample is too concentrated	Dilute the sample.
	Flow rate is too high	Decrease the flow rate.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and then load the sample again. Scrub the flow cell. See Cleaning the flow cell (page 98).
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 49).
	Sample is too dilute	Concentrate the sample.
	Threshold setting is too high	Adjust the threshold setting. See Defining view data (page 49).
	Sample line assembly or sample line filter installed incorrectly	Verify the sample line assembly or sample line filter installation. See Replacing the sample line (page 114) and Replacing the sample line filter (page 119).

Observation	Possible Causes	Recommended Solutions
Unexpectedly low event rate	Sample line is clogged or kinked	If visible kinks are found in the sample line, replace the sample line assembly. See Replacing the sample line (page 114). If visible kinks are not found in the sample line, clean the flow cell. See Cleaning the flow cell (page 98). Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. See Clearing a sample line blockage (page 113). If sample flow seems to be blocked, backflush the sample line several times to clear the block. See Backflushing the sample line (page 112). Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. See Replacing the sample line filter (page 119). Clean the flow cell again and check that the fluid levels in the sample tube have decreased.
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 49) and Calculating compensation (page 51).
	Flow rate is too high	Decrease the flow rate.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and then load the sample again.
	Debris in flow cell or nozzle	Scrub the flow cell. See Cleaning the flow cell (page 98). Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 95).
	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 49).
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.

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 49).
	Dead cells or debris in sample	Examine the sample under a microscope to determine the source of the debris. Adjust sample preparation if needed.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sheath filter needs to be replaced	Replace the sheath filter. See Changing the fluid filter (page 110).
Processed events are <90%	Threshold setting is too low	Adjust the threshold setting. See Defining view data (page 49)..
	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 49).
Sample loading failed	Sample chamber O-ring is dry and causing chamber to stick	Lubricate the sample chamber O-ring. See Lubricating the sample chamber O-ring (page 92).

Sorting troubleshooting

Observation	Possible Causes	Recommended Solutions
Stream turns off unexpectedly	Nozzle clog detected or debris in nozzle	Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 95).
	Debris in flow cell	Scrub the flow cell. See Cleaning the flow cell (page 98).
	Sheath tank empty or waste tank full	Empty the waste tank or fill the sheath tank. See Emptying the waste tank (page 107) and Filling the sheath tank (page 108).

Observation	Possible Causes	Recommended Solutions
Unable to start sort	BD FACSChorus™ software cannot locate the side streams	Clean the lower camera window. See Cleaning the Accudrop laser window and the lower camera window (page 102). Close the sort block door properly. When using four-way sort, wait for a few minutes to allow the Accudrop to find the four streams. If the streams are still not found, clean the nozzle. See Cleaning the sort nozzle (page 95) and Changing the nozzle (page 94). Also, clean the deflection plates. See Cleaning the deflection plates (page 105).
	Salt bridge	Clean the deflection plates and the area around and behind the plates. See Cleaning the deflection plates (page 105).
Arcing between deflection plates	Salt bridge	Clean the deflection plates and the area around and behind the plates. See Cleaning the deflection plates (page 105).
Fanning around center or side streams	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. See Changing the nozzle (page 94).
	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 95). If the nozzle seems damaged, replace it. See Changing the nozzle (page 94) and Replacing the sort nozzle seal temporarily (page 96). Restart the stream. See Stopping or restarting the stream (page 90).
	Dirty deflection plates	Clean the deflection plates. See Cleaning the deflection plates (page 105).
	Particles too big for sort nozzle	Verify that the particle size is appropriate for the 100-µm nozzle. In general, the nozzle orifice should be at least 5 times the average particle size in the sort sample. See Shapiro H. *Practical Flow Cytometry*. Fourth Edition. New York, NY: John Wiley and Sons; 2003:263.
Low sort efficiency	Event rate is too high for drop frequency	Decrease the flow rate.
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements. See Setting up sorting (page 55).
	Gating conflict	Verify the gating hierarchy.
Erratic sort rate	Flow rate is too high	Decrease the flow rate.

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 55).
	Incorrect logic in population hierarchy	Verify the gating hierarchy. Do not assign conflicting gates (for example, parent population in Tube 1, child population in Tube 2).
Plate sorting failure	Splash shield not installed	Install the splash shield.
	Sort collection chamber door is open	Close the sort collection chamber door.
	Automated stage does not move	Close the access doors, then restart the instrument and workstation.
Unable to sort into targeted well in plate	Debris on deflection plates	Clean deflection plates. See Cleaning the deflection plates (page 105).
	Waste aspirator drawer not aligned to stream	Align the waste aspirator drawer. See Aligning the waste aspirator drawer to the stream (page 97).
	Automated stage improperly aligned	Align the stage. See Aligning the automated stage (page 123). If the problem cannot be resolved by aligning the automated stage, contact your BD service representative for assistance.

Electronics troubleshooting

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

Backup-and-restore troubleshooting

Observation/Error Message	Possible Cause	Recommended Solution
Invalid Backup Folder Selected for Restore: The backup folder selected is invalid.	• The backup folder is no longer in the location specified within the Backup and Restore utility. • The backup folder has been deleted. • The backup folder contents have been modified.	Verify that the folder is still in file path selected in the utility. Do not modify the contents of any backup folder. If content has been modified, the folder will not be able to be restored.
Incompatible Backup Folder: The selected backup folder is not compatible with the current BD FACChorus version on this workstation.	The backup folder was created using a different version of BD FACSChorus™.	You will not be able to restore a backup saved from a previous version of BD FACSChorus™ software. (For example, a backup created using BD FACSChorus™ 2.0 software cannot be restored to BD FACSChorus™ 3.0 software).
There is not enough disk space - Free up some disk space.	There is not enough space on the disk to perform the restore.	Remove some files from the local workstation to create enough space to restore the backup folder.
FACSChorus is Running, please shut down FACSChorus before attempting a backup or restore.	BD FACSChorus™ software is open.	Close the BD FACSChorus™ software and turn off the instrument before using the Backup and Restore utility.
Backup Failed: Format the storage device as NTFS or us an alternative storage location.	An external device with FAT32 format is the backup storage location.	The backup folder is too large to save on the external device with FAT32 format. Reformat the device as NTFS or select an alternative storage location. We recommend that backup folders are saved to the local workstation.
Backup and Restore utility will not open.	Another instance of the Backup and Restore utility is already running.	Check if the Backup and Restore utility is already open. Only one instance of the utility can be open at a time on the workstation.
BD FACSChorus™ will not open correctly after a restore.	The restore process may have been interrupted for some reason such as loss of power to the workstation.	Close the BD FACSChorus™ software and then repeat the restore process using the Backup and Restore utility.

BD® Aerosol Management Option troubleshooting

The tips in this section are provided to help you troubleshoot issues that arise when using the BD® Aerosol Management Option (AMO).

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier. See Technical support (page 12).

If any of the following are observed, assume that the AMO is not evacuating properly, and do not open the doors to the sort chamber.

Control Panel Troubleshooting

Observation	**Possible Cause**	**Recommended Solution**
Evacuator indicator lights off	Evacuator power cord unplugged	Connect the evacuator power cord to the power source.
	Evacuator power switched off	Switch on the evacuator main power. See Starting up the evacuator (page 73).
	Evacuator motor failure	Contact your local BD technical support representative.
	Circuit breaker tripped	Depress the circuit breaker on the rear of the evacuator into its original position.
	Site power failure	Turn off the evacuator power switch and wait for site power to be restored.
Evacuator indicator lights pulsing	Erratic power source	Plug the power cord into a different outlet.
Arrow keys not responding	Improper operation	Push each button firmly before removing your finger from the control.
	Defective membrane panel	Contact your local BD technical support representative.
Red filter-life indicator light on	Approaching 180 hours of filter use	Monitor the light. When it blinks, change the filter.
	Filter life not reset after filter change	After changing the filter, press and hold the Filter Life Reset button until the 100% indicator light is lit.
Red filter-life indicator blinking	Filter used over 180 hours	Replace the filter.

Filter Flow Gauge Troubleshooting

Observation	Possible Cause	Recommended Solution
Zero reading on filter flow gauge	Power off	Press the power button on the membrane panel of the evacuator.
	Filter defective	Replace the filter.
	Filter improperly seated in evacuator	Re-seat the filter with the evacuator power off.
	Tubing loose or not connected	Ensure that the tubing is securely connected below the sort chamber and to the filter module.
	Tubing kinked or damaged	Inspect the tubing for kinks or punctures. Replace the tubing, if needed.
	Wrong tubing type or part	Ensure that the correct type of tubing is in use.
Erratic reading on filter flow gauge	Defective filter	Replace the filter.
	Filter improperly seated in evacuator	Re-seat the filter with the evacuator power off.
Off-scale reading on filter flow gauge	Tubing or sort collection chamber obstructed	Inspect the tubing for kinks or punctures. Replace the tubing, if needed. Check for obstructions in the sort collection chamber. Remove any obstruction.
	Filter clogged or saturated	Replace the ULPA filter. See Replacing the ULPA filter (page 77).

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Consumables and supplies

This chapter covers the following topics:

Ordering information

To order spare parts and consumables from BD Biosciences:

- Within the US, call (877) 232-8995.
- Outside the US, contact your local BD Biosciences customer support representative.

Worldwide contact information can be found at bdbiosciences.com

Beads

This topic lists the available beads

Cytometer Setup and Tracking Beads

Bead	Laser	Supplier	Catalog No.
BD® CS&T RUO Beads	• Violet (405 nm) • Blue (488 nm) • Red (640 nm) • Yellow-green (561 nm)	BD Biosciences	• 661414 (50 tests) • 661415 (150 tests)

Fluorescence Compensation (FC) Beads

Bead	Laser	Supplier	Catalog No.
BD® FC Beads BV786	Violet (405 nm)	BD Biosciences	661630
BD® FC Beads BV711	Violet (405 nm)		661629
BD® FC Beads BV605	Violet (405 nm)		661626
BD® FC Beads V500-C	Violet (405 nm)		661624
BD® FC Beads V450	Violet (405 nm)		661623
BD® FC Beads FITC	Blue (488 nm)		661615
BD® FC Beads PerCP	Blue (488 nm)		661618
BD® FC Beads BB515	Blue (488 nm)		661631
BD® FC Beads PE-Cy7	Yellow-green (561 nm)		661617
BD® FC Beads PE	Yellow-green (561 nm)		661616
BD® FC Beads APC-H7	Red (640 nm)		661621
BD® FC Beads APC-Cy7	Red (640 nm)		661622
BD® FC Beads APC	Red (640 nm)		661620

BD FACS™ Accudrop Beads

Bead	Supplier	Catalog Number
BD FACS™ Accudrop Beads	BD Biosciences	661612 (25 tests)

Reagents

Reagent	Supplier	Catalog Number
BD® Detergent Solution Concentrate	BD Biosciences	66058
BD FACSFlow™ Sheath Fluid		342003
Chlorine bleach (5% sodium hypochlorite)	Clorox® or other major supplier (to ensure that the bleach is at the correct concentration and free of particulate matter)	–

Equipment supplies

Item	Supplier	Catalog Number
Waste Tank Caps, pack of 12	BD Biosciences	33885407
Sample Line Assy BD FACSMelody™ Service		66222707
BD FACSMelody™ Nozzle Assy 100 Mn Sq Cuvette		662037
Filter 0.2 µm 440 cm^2 33 mm Capsule PPL		336945

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