## History

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Change made</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-18120-00</td>
<td>7/2016</td>
<td>Initial release</td>
</tr>
<tr>
<td>23-18120-01</td>
<td>4/2017</td>
<td>Added yellow-green laser, index sorting and optional filters</td>
</tr>
</tbody>
</table>
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Introduction

This chapter covers the following topics:

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

In this guide

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

This guide includes:

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

Search function

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

To view bookmarks and navigate to a section, click the bookmark icon in the upper right corner of the PDF window.
The following table lists the available documents for the BD FACS Melody Cell Sorter.

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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Caution Symbol" /></td>
<td>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.</td>
</tr>
<tr>
<td><img src="image" alt="Biological Hazard Symbol" /></td>
<td>Biological hazard</td>
</tr>
<tr>
<td><img src="image" alt="Electrical Hazard Symbol" /></td>
<td>Electrical hazard</td>
</tr>
<tr>
<td><img src="image" alt="Laser Hazard Symbol" /></td>
<td>Laser hazard</td>
</tr>
<tr>
<td><img src="image" alt="Mechanical Hazard Pinch Points Symbol" /></td>
<td>Mechanical hazard, pinch points</td>
</tr>
<tr>
<td><img src="image" alt="Lifting Hazard Symbol" /></td>
<td>Lifting hazard</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction</strong></td>
<td>This section describes how to get technical support.</td>
</tr>
</tbody>
</table>
| **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:  
  2. Select BD FACSMelody > Products > Instruments > BD FACSMelody Consumables.  
  3. Select the materials to order. |
About the system

This chapter covers the following topics:

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

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

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

The following drawing displays the typical layout of the system on a table.
Chapter 2: About the 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.

<table>
<thead>
<tr>
<th>No</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BD FACSMelody cell sorter</td>
</tr>
<tr>
<td>2</td>
<td>Waste tank</td>
</tr>
<tr>
<td>3</td>
<td>Sheath tank</td>
</tr>
<tr>
<td>4</td>
<td>Monitor</td>
</tr>
<tr>
<td>5</td>
<td>Keyboard and mouse</td>
</tr>
<tr>
<td>6</td>
<td>Computer</td>
</tr>
<tr>
<td>7</td>
<td>Electronics box</td>
</tr>
</tbody>
</table>

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

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

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

**BD FACSMelody cell sorter**

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

The three laser configurations provide the ability to analyze up to 9 colors (11 parameters). The heptagon detector array takes the guesswork out of changing filters and ensures that the correct filters and mirrors are installed.
Several hardware options and upgrades can be used to customize the system for different applications.

<table>
<thead>
<tr>
<th><strong>BD FACSChorus software</strong></th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Workstation</strong></td>
<td>The BD FACSMelody system includes the HP® desktop computer with the Microsoft® Windows® 10 operating system installed, a wireless keyboard and mouse, and a HP® 23-in. monitor.</td>
</tr>
<tr>
<td><strong>Sheath and waste tanks</strong></td>
<td>The system comes with a stainless steel 10-L sheath tank and a polypropylene 10-L waste tank. Note: Do not place the fluidic tanks at a level that is higher than the cell sorter because this can cause incorrect pressure and increase the sheath flow rate. Keep the fluidic tanks in the same location that they were placed in during installation.</td>
</tr>
<tr>
<td><strong>Beads, reagents, and assays</strong></td>
<td>BD CS&amp;T RUO beads are used to check the instrument performance and automatically make adjustments, ensuring consistent values from day to day and experiment to experiment. BD FACS Accudrop beads are used to automatically set an accurate drop delay value. The Accudrop laser is aligned with the center and side (sorting) streams. BD FACSChorus software optimizes the drop delay by sorting the Accudrop beads and identifying a drop delay value that yields the most particles in the side stream and the fewest in the center stream. BD FC beads are used as compensation controls to set up normalized spillover values which are valid for 60 days.</td>
</tr>
</tbody>
</table>
Chapter 2: About the system

Supported sort collection devices

The BD FACSMelody system supports the following sort collection devices:

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

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

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

Options

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

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

Instrument overview

Introduction

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

Main components

The instrument includes the lasers, sample injection chamber, sort chamber, and sample holders. The following image shows some BD FACSMelody cell sorter details.
<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample line access cover</td>
</tr>
<tr>
<td>2</td>
<td>Sample viewing window</td>
</tr>
<tr>
<td>3</td>
<td>Power on/off switch</td>
</tr>
<tr>
<td>4</td>
<td>Sample access door</td>
</tr>
<tr>
<td>5</td>
<td>Sample loading port</td>
</tr>
<tr>
<td>6</td>
<td>Sort collection chamber</td>
</tr>
<tr>
<td>7</td>
<td>Sort block door</td>
</tr>
<tr>
<td>8</td>
<td>Flow cell access door</td>
</tr>
<tr>
<td>9</td>
<td>Sort collection chamber door</td>
</tr>
</tbody>
</table>
Optical components

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

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

<table>
<thead>
<tr>
<th>No</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trigon detector</td>
</tr>
<tr>
<td>2</td>
<td>Holder for filters</td>
</tr>
<tr>
<td>3</td>
<td>Heptagon detector</td>
</tr>
<tr>
<td>4</td>
<td>Heptagon detector</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>No</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heptagon</td>
</tr>
<tr>
<td>2</td>
<td>Handle</td>
</tr>
<tr>
<td>3</td>
<td>Optical filter</td>
</tr>
<tr>
<td>4</td>
<td>ID chip</td>
</tr>
</tbody>
</table>

Location of lasers

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

## Introduction

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

<table>
<thead>
<tr>
<th>Number of lasers</th>
<th>Number of colors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (blue)</td>
<td>2-color (2-0)</td>
</tr>
<tr>
<td>1 (blue)</td>
<td>4-color (4-0)</td>
</tr>
<tr>
<td>2 (blue, yellow-green)</td>
<td>4-color (2-2)</td>
</tr>
<tr>
<td>2 (blue, red)</td>
<td>6-color (4-2)</td>
</tr>
<tr>
<td>2 (blue, yellow-green)</td>
<td>6-color (2-4)</td>
</tr>
<tr>
<td>2 (blue, violet)</td>
<td>6-color (3-3)</td>
</tr>
<tr>
<td>3 (blue, red, violet)</td>
<td>6-color (2-2-2)</td>
</tr>
<tr>
<td>3 (blue, red, yellow-green)</td>
<td>8-color (2-2-4)</td>
</tr>
<tr>
<td>3 (blue, violet, yellow-green)</td>
<td>8-color (2-2-4)</td>
</tr>
<tr>
<td>3 (blue, red, violet)</td>
<td>9-color (4-2-3)</td>
</tr>
</tbody>
</table>

## Configuration details

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

- B = blue
- R = red
- V = violet
- YG = yellow-green
One laser 2 color

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/488 nm</td>
<td>A</td>
<td>PE, PI</td>
<td>560LP</td>
<td>586/42</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>FITC, GFP, BD Horizon Brilliant™ Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: GFP</td>
<td>510/10</td>
<td>510/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Side scatter (SSC)</td>
<td>ND10a</td>
<td>488/15</td>
</tr>
</tbody>
</table>

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

One laser 4 color

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/488 nm</td>
<td>A</td>
<td>PE-Cy™7</td>
<td>552LP</td>
<td>783/56</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>PerCP, PerCP-Cy™5.5, 7-AAD, BD Horizon Brilliant™ Blue 700</td>
<td>665LP</td>
<td>700/54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: BD Horizon PE-CF594, PE-Texas Red®</td>
<td>605LP</td>
<td>613/18</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>PE, PI</td>
<td>560LP</td>
<td>586/42</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>Side scatter (SSC)</td>
<td>ND10a</td>
<td>488/15</td>
</tr>
</tbody>
</table>

a. There is a 10% neutral density filter installed in front of the SSC filter.
### Two-laser system (2B-2YG configuration)

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/488 nm</td>
<td>A</td>
<td>PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700</td>
<td>665LP</td>
<td>700/54</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Optional: GFP</td>
<td>510/10</td>
<td>510/10</td>
</tr>
<tr>
<td>Yellow-green/561 nm</td>
<td>A</td>
<td>mCherry, BD Horizon™ PE-CF594, PE-Texas Red®, PI</td>
<td>605LP</td>
<td>613/18</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>PE, DsRed</td>
<td>582LP</td>
<td>582/15</td>
</tr>
</tbody>
</table>

*a. There is a 10% neutral density filter installed in front of the SSC filter.*
### Two-laser system (4B-2R configuration)

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/ 488 nm</td>
<td>A</td>
<td>PE-Cy7</td>
<td>752LP</td>
<td>783/56</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700</td>
<td>665LP</td>
<td>700/54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: BD Horizon PE-CF594, PE-Texas Red®</td>
<td>605LP</td>
<td>613/18</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>PE, PI</td>
<td>560LP</td>
<td>586/42</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: GFP</td>
<td>510/10</td>
<td>510/10</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>SSC</td>
<td>ND10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>488/15</td>
</tr>
</tbody>
</table>

| 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 |

<sup>a</sup> There is a 10% neutral density filter installed in front of the SSC filter.

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

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/ 488 nm</td>
<td>A</td>
<td>PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700</td>
<td>665LP</td>
<td>700/54</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: GFP</td>
<td>510/10</td>
<td>510/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Side scatter (SSC)</td>
<td>ND10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>488/15</td>
</tr>
</tbody>
</table>
### Two-laser system (3B-3V configuration)

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PE-Cy7</td>
<td></td>
<td>752LP</td>
<td>783/56</td>
</tr>
<tr>
<td>B</td>
<td>PE-Cy™5, PE-Cy™5.5</td>
<td></td>
<td>665LP</td>
<td>697/58</td>
</tr>
<tr>
<td>C</td>
<td>mCherry, BD Horizon PE-CF594, PE-Texas Red®, PI</td>
<td></td>
<td>605LP</td>
<td>613/18</td>
</tr>
<tr>
<td>D</td>
<td>PE, DsRed</td>
<td></td>
<td>582/15</td>
<td>582/15</td>
</tr>
</tbody>
</table>

*There is a 10% neutral density filter installed in front of the SSC filter.*
### Three-laser system (2B-2R-2V configuration)

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

a. There is a 10% neutral density filter installed in front of the SSC filter.
Three laser system
(2B-2R-4YG configuration)

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

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

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/488 nm</td>
<td>A</td>
<td>PerCP, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700</td>
<td>665LP</td>
<td>700/54</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: GFP</td>
<td>510/10</td>
<td>510/10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Side scatter (SSC)</td>
<td>ND10a</td>
<td>488/15</td>
</tr>
<tr>
<td>Red/640 nm</td>
<td>A</td>
<td>APC-Cy7, APC-H7</td>
<td>752LP</td>
<td>783/56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: Alexa Fluor® 700, APC-R700</td>
<td>705LP</td>
<td>720/30</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>APC, Alexa Fluor® 647</td>
<td>660/10</td>
<td>660/10</td>
</tr>
</tbody>
</table>
Three laser system  
(2B-2V-4YG configuration)

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

a. There is a 10% neutral density filter installed in front of the SSC filter.
### Laser Detector Primary fluorochrome Mirror Filter

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

a. There is a 10% neutral density filter installed in front of the SSC filter.
### Three laser system (4B-2R-3V configuration)

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Primary fluorochrome</th>
<th>Mirror</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue/488 nm</td>
<td>A</td>
<td>PE-Cy7</td>
<td>752LP</td>
<td>783/56</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>PerCP, PerCP-Cy5, PerCP-Cy5.5, 7-AAD, BD Horizon Brilliant Blue 700</td>
<td>665LP</td>
<td>700/54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: BD Horizon PE-CF594, PE-Texas Red®</td>
<td>605LP</td>
<td>613/18</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>PE, PI</td>
<td>560LP</td>
<td>586/42</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>FITC, GFP, BD Horizon Brilliant Blue 515, Alexa Fluor® 488</td>
<td>507LP</td>
<td>527/32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optional: GFP</td>
<td>510/10</td>
<td>510/10</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>Side scatter (SSC)</td>
<td>ND10a</td>
<td>488/15</td>
</tr>
<tr>
<td>Red/640 nm</td>
<td>A</td>
<td>APC-Cy7, APC-H7</td>
<td>752LP</td>
<td>783/56</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Alexa Fluor® 700, APC-R700</td>
<td>705LP</td>
<td>720/30</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>APC, Alexa Fluor® 647</td>
<td>660/10</td>
<td>660/10</td>
</tr>
</tbody>
</table>
Changing optical configurations

Introduction

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

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

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

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

To change the optical configuration:

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

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

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

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

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

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

---

**Fluidic components**

**Introduction**

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

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

**Sample injection chamber**

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

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

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

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample tube</td>
</tr>
<tr>
<td>2</td>
<td>Tube holder</td>
</tr>
<tr>
<td>3</td>
<td>Sample loading port</td>
</tr>
</tbody>
</table>
Nozzle

The 100-µm integrated sort nozzle is available along with a closed-loop nozzle for use in cleaning and shutdown procedures. The sort nozzle is keyed to a fixed position at the lower end of the cuvette.
Because the sort nozzle is below the interrogation point, optical alignment is not affected when the nozzle is changed.

---

**BD FACSChorus software**

**Introduction**

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

**Screen design**

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

Status information is displayed on the bottom of the navigation bar. Instructional text and tips are displayed when you hover over screen elements.
Chapter 2: About the system

System status indicator

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

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Navigation bar</td>
</tr>
<tr>
<td>2</td>
<td>Status indicators</td>
</tr>
<tr>
<td>3</td>
<td>Guided workflow</td>
</tr>
<tr>
<td>4</td>
<td>Data entry fields</td>
</tr>
</tbody>
</table>
If problems occur with the configuration of the cytometer, information about the problem and the solution is provided next to the Configuration status indicator. However, this kind of information is not always provided for problems related to Fluidics.
Startup, Cytometer Setup, or Drop Delay. The following table provides this information based on the color of the status indicator.

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

This chapter covers the following topics:

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

About system startup

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

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

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

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

Connection status

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

Fluidics startup

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

- Run Daily Fluidic Startup
- Run Extended Fluidic Startup

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

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

Cleaning

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

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

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

Sort nozzle

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

Cytometer setup—run BD CS&T RUO beads

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

• Measuring baseline takes longer to complete than performance. It occurs:
  – At installation
  – Every 90 days (for the same configuration and bead lot)
  – After preventative maintenance and major service procedures
  – When a new bead lot files is selected

• We recommend measuring performance daily at the start of the day to update settings and track the instrument performance. However, measuring performance daily is an optional step.

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

All CS&T data is specific to a set configuration. Before proceeding, verify that the displayed configuration is the desired configuration.
If you need to update the configuration, see Changing optical configurations (page 33).

**Drop delay–run BD FACS Accudrop beads**

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

**Required materials**

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Supplied by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk fluids</td>
<td>Sterile phosphate buffered saline (PBS)</td>
<td>User</td>
</tr>
<tr>
<td></td>
<td>Bleach (for the waste tank)</td>
<td>User</td>
</tr>
<tr>
<td></td>
<td>Deionized (DI) water</td>
<td>User</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>User</td>
</tr>
<tr>
<td>Setup beads</td>
<td>BD CS&amp;T RUO beads with bead lot file</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>BD FC beads</td>
<td>BD</td>
</tr>
<tr>
<td>Accudrop beads</td>
<td>BD FACS Accudrop beads</td>
<td>BD</td>
</tr>
<tr>
<td>BD Cleaning solution</td>
<td>Solution for cleaning</td>
<td>BD</td>
</tr>
</tbody>
</table>

**Fluidics startup**

**Introduction**

Selecting a fluidic startup option depends on how the system was shut down.
– If no shutdown was performed, then a fluidics startup is not required (not recommended).
– If a daily shutdown was performed, then you can select to perform either a daily or extended start-up.
– If an extended shutdown was performed, then you must perform an extended start-up.

Procedure

To perform a daily or extended fluidics startup:

1. Press the Power button on the front of the instrument.
2. Start the BD FACStorus software and log in.
3. On the opening screen, select either Run Daily Fluidics Startup or Run Extended Fluidics Startup.
   The daily or extended fluidics startup dialog opens with four tasks that need to be completed.
   – Daily fluidics startup or extended fluidics startup depending on the selection
   – Insert the closed-loop nozzle
   – Check the sheath and waste tanks
   – Start the sheath filter purge and prime the stream
   Note: For the extended fluidics startup, ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters. See Preparing new fluid filters (page 146).
4. Follow the prompts to complete the tasks displayed on the screen.
   A green check mark is displayed to indicate successful completion of each task.
   If there are issues, follow the instructions on the error message to troubleshoot the issue.
5. When all of the tasks are complete, click Close.
   The system returns to the opening screen.
6. Click Continue to view the cleaning options.
7. Click **Skip** if no cleaning is needed.

---

**Cleaning**

**Introduction**

Two cleaning options are available:

- Prepare for Aseptic Sort.
- Flow Cell Clean.

**Prepare for aseptic sort**

To prepare for aseptic sort:

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

---

**Prepare for Aseptic Sort**

- **Prepare for Aseptic Sort**
  - **Note:** Ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters.

See [Preparing new fluid filters (page 146)](#).
2. Click Close to return to the Cleaning dialog.

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

### Flow cell clean

To prepare for flow cell clean:

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

2. Click Close to return to the Cleaning dialog.

### More information

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

### About CS&T reports

**Introduction**

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

**Viewing a CS&T report**

To view a CS&T report:

1. Click CYTOMETER on the navigation bar.

2. In the OTHER panel, select Cytometer Setup Reports.
**Report Description**  The sections of the reports are described as follows.

<table>
<thead>
<tr>
<th>Report section</th>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summary</strong></td>
<td>Status</td>
<td>Indicates pass or fail.</td>
</tr>
<tr>
<td></td>
<td>Report type</td>
<td>Indicates Performance or Baseline report.</td>
</tr>
<tr>
<td></td>
<td>Cytometer name and serial number</td>
<td>Provides the name and serial number of the instrument.</td>
</tr>
<tr>
<td></td>
<td>Software</td>
<td>Indicates the version of the software being used.</td>
</tr>
<tr>
<td></td>
<td>Bead lot ID</td>
<td>Indicates which bead lot was used.</td>
</tr>
<tr>
<td></td>
<td>Nozzle size</td>
<td>Indicates the size of the nozzle.</td>
</tr>
<tr>
<td></td>
<td>Configuration</td>
<td>Indicates the instrument configuration.</td>
</tr>
<tr>
<td><strong>Detector settings</strong></td>
<td><strong>Detector</strong></td>
<td><strong>Name</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Filter</strong></td>
<td><strong>Description of wavelengths transmitted</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Mirror</strong></td>
<td><strong>Name of the mirror used with the detector</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Position</strong></td>
<td><strong>Location of the filter holder with mirror</strong></td>
</tr>
<tr>
<td></td>
<td><strong>PMT voltage</strong></td>
<td><strong>Measured PMT voltage</strong></td>
</tr>
<tr>
<td><strong>Bright beads</strong></td>
<td><strong>Median</strong></td>
<td><strong>Median fluorescence intensity (MFI) value of the specific bead</strong></td>
</tr>
<tr>
<td></td>
<td><strong>%rCV</strong></td>
<td><strong>Percent robust coefficient of variation of the bright beads</strong></td>
</tr>
<tr>
<td><strong>Linearity</strong></td>
<td><strong>Min channel</strong></td>
<td><strong>Minimum value for the acceptable linear range of the detector</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Max channel</strong></td>
<td><strong>Maximum value for the acceptable linear range of the detector</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Qr</strong></td>
<td><strong>Relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector</strong></td>
</tr>
</tbody>
</table>
Chapter 3: System startup and shutdown

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.

<table>
<thead>
<tr>
<th>Report section</th>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Br (ABD)</td>
<td>Relative optical background signal, used for tracking the optical background noise levels in a detector</td>
</tr>
<tr>
<td>Laser settings</td>
<td></td>
<td>Lasers used when the performance check or baseline was completed</td>
</tr>
</tbody>
</table>
When you delete a user, any experiments, data records, sort reports, or fluorochromes created by the user are also deleted.

**Procedure**

To add, edit, or delete a user:

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

---

**Shutting down the system**

**Introduction**

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

**Before you begin**

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

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 111)
- Filling the sheath tank (page 128)
- Changing the fluid filter (page 131)
- Emptying the waste tank (page 126)

**About this task**

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

For the long-term shutdown procedure, ensure that you designate each fluid filter to one type of fluid and that you do not interchange the filters.
**Note:** If you log out or exit (close) the software without performing a shutdown procedure, the software prompts you to perform a shutdown procedure.

---

**Procedure**

To shut down the system:

1. On the Cytometer page, select the shutdown procedure that you want to run.

2. Complete the steps in the wizard.

3. Run the daily shutdown procedure with a 1.5% BD Detergent Solution.

4. Leave the 1.5% BD Detergent Solution in the flow cell.

**Caution!** Never mix BD Detergent Solution and bleach in the same tube because they can create dangerous fumes.
This chapter covers the following topics:

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

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

Experiment parameters are defined by filling in the data fields or making selections on each tabbed workspace. Saved experiments can be used as templates for later experiments. If you save the experiment as a template, you can change the parameters later. However, the base experiment will still carry over.

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

Creating an experiment

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

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

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

To create an experiment:

1. Select Experiments on the left navigation bar.

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

2. Click + New Experiment.

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

3. Select Blank Experiment.

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

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

### Experiment template option (1)
- Use the experiment template option if you plan to reuse a previously created experiment. An experiment template retains the setup and system settings of the original experiment but does not retain any data or sort report files. When an experiment template is used to create an experiment, voltage settings will be updated based on the last CS&T setup to maintain the optimal setup for your sample.

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

### Duplicate experiment without data (2)
- Use the duplicate experiment without data option to create a new experiment that contains all the setup and system settings of the saved experiment without any data. When the duplicate without data option is used to create an experiment, voltage settings will be updated based on the last CS&T setup to maintain the optimal setup for your sample.

In both cases:
- You can select an experiment that has an index sort (red circle).
- Compensation values are transferred from the existing experiment or template to the new experiment.
Designing an experiment

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

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

**Procedure**

To design the experiment:

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

2. Enter a name in the **Experiment Name** field.

3. (Optional) Select the **Use as an experiment template** checkbox, if you want to reuse this experiment multiple times.

4. (Optional) Select **Sample Temperature** to run the experiment at a defined sample temperature.

   This controls the sample input temperature and not the temperature of the sorted samples.

5. Select one or more fluorochromes from the list or click the plus sign (+) beside any fluorochrome to add a new user defined fluorochrome to that row.

   Only one fluorochrome can be selected from a row. Each row of fluorochromes represents one available detector channel in the optical configuration. Additional user defined fluorochromes that are added to a specific detector channel are indicated with an asterisk. User defined fluorochromes do not have default compensation applied.
6. (Optional) Hover over any of the colored rectangles to display the laser and filter information.

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

### Defining view data

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

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

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

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

To define view data:

1. Click the View Data tab.

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

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

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

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

   All populations are a subset of the All Events population.

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

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

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

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

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

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

e. To add additional dot plots, a contour map, histogram, or density plot, click the plus (+) button on the right side of the Plots panel and select a new plot. Plots can be enlarged by hovering over a plot and clicking the zoom icon.
6. Create, move, modify, and delete gates on the plots as needed. Hover over the plot to make the icons display.

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

7. View the Statistics panel at the bottom of the screen.
The statistics panel displays data for each population in the population hierarchy.

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

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

Calculating compensation

Introduction

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

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

After completing the Update Compensation Standards workflow, then samples that are run in a new experiment will have compensated data. There will be no change to existing experiments.
If the Update Compensation workflow is completed in an experiment, then new samples that are run in the experiment will use the updated values. Additionally, the updated values will apply if the experiment is used as a template or duplicated without data.

We recommend updating FC bead compensation every 60 days.

About compensation calculations

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

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

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

Using a combination of stored FC bead values and user-defined control values
When using user-defined single-color compensation controls (beads or cells) to overwrite the FC bead or existing user-defined spillover values in an experiment, not all fluorochromes need to be updated. To calculate compensation, the system uses the existing FC bead spillover values for the fluorochromes that were not updated, and the new user-defined values for fluorochromes that were updated.
Note: In all cases, if an experiment was created from an existing or experiment template, then all compensation values are transferred to the new experiment.

**Before you begin**

Complete these tasks before you start to update compensation.

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

**Procedure**

To update compensation using your own controls for your experiment:

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

   ![](DATA SOURCES.png)

   The Update Compensation dialog opens.

2. Select the fluorochromes you want to update.
Only the fluorochromes that were selected in the Design Experiment tab are displayed.

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

Note the following:

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

4. Load the appropriate tube, select the tube in the dialog, and then click Run. Adjust gates as needed.
5. Repeat step 4 for each tube.
6. Follow the prompts on the screen until all of the tubes are done.
7. Adjust gates as needed after running all of the controls before compensation is calculated and applied.
8. Click Finished when you are done to apply the compensation.
   When the tubes are done, the system displays the list of tubes in the Data Sources panel.
9. Continue setting up the rest of the experiment parameters.
Updating compensation standards

Introduction

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

Procedure

To update the system compensation standards:

1. Click Cytometer on the navigation pane.

2. Select Update Compensation Standards.

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

The system displays the lot numbers for the pouch and tube for reference.
3. Verify that the FC bead lot number matches the FC beads you are going to use.

4. (Optional) If needed, edit the lot numbers to select from the available lots with associated bead lot files to ensure accurate compensation calculations.

   If the lot is not shown, the FC bead lot files may be updated by going to the BD Biosciences website.

5. (Optional) To update the available BD FC bead lots, visit the BD Biosciences website and download the BD FACSMelody FC bead lot installer from the BD FACSMelody Resources page. You need administrative privileges to run the installer.

6. Prepare the BD FC beads according to the product insert.

7. Follow the prompts on the screen to run each fluorochrome.

8. Click Finished when you are done.

---

### Viewing the compensation matrix

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

---

### Setting up sorting

#### Introduction

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

#### Procedure

To set up the sort operation:

1. Click the Set Up Sort tab.

2. Select the details for the sort operation, such as:
   - **Format**, which refers to the collection device (tube, plate, or slide)
• **Volume**, which refers to the volume of the collection device

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

<table>
<thead>
<tr>
<th>Sort mode</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>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.</td>
</tr>
<tr>
<td>Purity</td>
<td>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.</td>
</tr>
<tr>
<td>Single cell</td>
<td>This is used to obtain a single cell in each well, containing only a defined cell of interest.</td>
</tr>
</tbody>
</table>

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

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

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

   • If using tubes, select the tube and then select the populations of interest from the population hierarchy.
The tube assumes the color of the selected population.

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

5. Select the initial buffer volume that will be added to each tube or well before sorting.
   
   You may specify a buffer volume of up to half the volume of the collection device.

6. Select the number of events to sort into each tube or well. The index sort has a maximum number of 100 events/well.
This information will display on the screen in the tubes and will display if you hover over the well.

**Selecting wells**

To select specific wells:

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

The screen indicates the location of the selected populations.

---

**Loading collection devices**

**About this topic**

The BD FACSMelody system supports the following sort collection devices:

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adapter</td>
</tr>
<tr>
<td>2</td>
<td>Handle</td>
</tr>
<tr>
<td>3</td>
<td>Tube holder</td>
</tr>
</tbody>
</table>

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

6. Close the sort collection chamber door.

**Installing the splash shield**

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

To install the splash shield:

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

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

**To load the slide into the instrument:**

1. Verify that the splash shield is in place.
2. Attach the slide to the slide holder.
3. Select **Eject** in the software to bring the stage forward.
4. Open the sort collection chamber door.
5. Place the slide holder on the stage, touching the guiding pins in the back and the locking lever in the front.

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sort block</td>
</tr>
<tr>
<td>2</td>
<td>Slide</td>
</tr>
<tr>
<td>3</td>
<td>Slide holder</td>
</tr>
<tr>
<td>4</td>
<td>Guiding pin</td>
</tr>
<tr>
<td>5</td>
<td>Locking lever</td>
</tr>
<tr>
<td>6</td>
<td>Stage platform</td>
</tr>
</tbody>
</table>

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

7. Close the sort collection chamber door.
Loading plates on the automated stage

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

To load plates onto the automated stage:
1. Verify that the splash shield is in place.
2. Select Eject in the software to bring the stage forward.
3. Open the sort collection chamber door.
4. Load the plate so that well A1 (red circle) is on the front left of the stage platform. Verify that the plate is touching the guiding pins at the back and the locking lever is set.
5. Install the aerosol shield to keep any aerosols contained in the sort collection chamber.

6. Close the sort collection chamber door.

### Sorting

**Introduction**

Samples are sorted based on the selections in the Set Up Sort tab. Samples can be sorted into tubes, plates, or onto slides. However, index sorting uses only plates and slides, no tubes.
Index sorting allows you to sort single cells into a plate or onto a slide and indexes the well or slide location to the collected parameters for that cell. You can index sort up to 100 events per well or location in a plate or slide using the single cell sort mode.

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

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

**Procedure**

**To run the sort operation:**

1. Click the *Sort* tab.

2. Click *Load Sample*.

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

4. Click *Start Sort*.

   The selections of collection device, number of events, sort mode, and sort populations displayed in the Sort Status panel reflect the selections made in the View Data and Set Up Sort tabs.
When performing a tube sort, the following is an example of what you might see:

![Image of tube sort]

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

![Image of plate sort]

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

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

<table>
<thead>
<tr>
<th>Using multiple collection devices</th>
<th>An experiment can contain multiple collection devices. If using multiple collection devices, follow this guidance:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• If the additional collection devices use the same sort parameters:</td>
<td></td>
</tr>
<tr>
<td>a. Replace the sorted collection device with a new one of the same type (plate, tube, or slide).</td>
<td></td>
</tr>
<tr>
<td>b. Click <strong>Start Sort</strong>.</td>
<td></td>
</tr>
<tr>
<td>The system begins sorting into the new collection device using the previous settings.</td>
<td></td>
</tr>
<tr>
<td>• If the additional collection devices use different sort parameters:</td>
<td></td>
</tr>
<tr>
<td>a. Replace the sorted collection device with a new one of a different type.</td>
<td></td>
</tr>
<tr>
<td>b. Click <strong>Eject</strong> or <strong>Retract</strong> to move the stage forward or back respectively.</td>
<td></td>
</tr>
<tr>
<td>c. Click <strong>Set Up Sort</strong> and modify the selections as needed.</td>
<td></td>
</tr>
<tr>
<td>d. Click the <strong>Sort</strong> tab and click <strong>Start Sort</strong>.</td>
<td></td>
</tr>
<tr>
<td>The system starts a new sort into the new collection devices using the new sort selections.</td>
<td></td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>

**Index sorting**

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

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

**Setting up for index sorting**

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

Review the instructions in these sections:

- Designing an experiment (page 59)
- Defining view data (page 60)
- Calculating compensation (page 64)
- Sorting (page 79)

**Procedure**

To perform an Index Sort:

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

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

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

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

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

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

Introduction

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

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

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

To review the Index Sort data:

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

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

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

3. When All events or Sorted is selected, you can:
   a. Select a well in the plate and see the corresponding events for that well highlighted in the plots.
   b. Select events in the plot and see the corresponding well for those events highlighted.

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

   c. View the statistics for all wells in the plate.
   d. Add new statistic by clicking the (+) button.

4. Use Unsorted for statistical purposes only.

Viewing reports

Introduction

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

To view and export the report:

1. Click the View Reports tab.

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

2. Select the report to export from the Select Sort Report list.
3. Click **Export Report > Location** to export a PDF of the report to the selected location.

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

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

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

Introduction

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

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

Using the sample temperature control

Introduction

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

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

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

Keep the sort block door and the sort collection chamber door closed during sorting.
Setting up the water bath

To set up the water bath:

1. Remove the threaded plug from the output port on the water bath.

2. Ensure that the drain cock on the back of the water bath is closed by turning it fully clockwise.

3. Set the pump outflow to maximum by turning the knob fully counterclockwise.
   
   Remove the top cover to access the knob, which is located inside the water bath toward the back. See the operating instructions supplied with the water bath for additional details on this process. This is referred to as position 1.

4. Connect the clear tubing end of the insulated hoses to the input and output ports on the water bath. Slide the tubing over the hose barbs and twist gently while installing to get the tubing completely over the barbs.

5. Connect the insulated hoses from the recirculating water bath to the ports on the right side of the cell sorter base.
   
   **Note:** Because the water flow direction is controlled by the water bath pump, the ports on the cell sorter base are bi-directional. The input and output hoses from the water bath can be connected to either port on the cell sorter base.

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

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

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

7. Plug in the water bath power cord.

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

To install the temperature control tube holder:

1. Place collection tubes in the temperature control tube holder.
   
   Tube holders are available for 5-mL, 1.5-, and 2.0-mL Eppendorf tubes for two-way sorting.

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

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

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

4. Attach the tube holder to the adapter.

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

   If you need to remove the tubing, push in the metal locking tab as you pull the tubing out of the port.
This is the same adapter that is used with the non-temperature controlled tube holders.

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

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

---

**Setting up the stage**

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

**To set up the stage:**

1. Install the splash shield below the aspirator drawer.

   ![Splash Shield Image]

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

   a. Remove the tube holder, if one is installed.
   b. Insert the splash shield into the slotted fittings below the sort aspirator drawer. Push the splash shield all the way in.

2. In the Set Up Sort tab, click the Eject button to bring the stage to the front.
a. Open an experiment, if one is not already open, and create a sort layout for any of the plates.

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

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

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

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

4. Install the appropriate collection device on the stage.

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

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

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.

<table>
<thead>
<tr>
<th>Required Sample Temp (°C)</th>
<th>Water Bath Setting (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>37</td>
<td>37.5</td>
</tr>
<tr>
<td>42</td>
<td>43.2</td>
</tr>
</tbody>
</table>
3. Wait at least 90 minutes to allow the recirculating water to reach the required temperature.

---

**Working with the aerosol management option**

**Introduction**

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

**Using the AMO**

To use the AMO:

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

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

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

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


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

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inspection certificate</td>
</tr>
<tr>
<td>2</td>
<td>Gauge indicating the air flow</td>
</tr>
<tr>
<td>3</td>
<td>Power, lights, alarm, aerosol management and blower controls with indicator lights</td>
</tr>
<tr>
<td>4</td>
<td>Sash that should remain at the at the correct level (10 inches) for proper operation</td>
</tr>
<tr>
<td>5</td>
<td>BD FACSMelody cell sorter</td>
</tr>
</tbody>
</table>
Using the BD FACSMelody cell sorter in the BSC is very similar to using it without the BSC. The main change is maintenance of the environment in the BSC. The BSC comes equipped with a built-in AMO. The AMO hose is attached to the back of the instrument, and the AMO is controlled on the BSC console.

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Electronics box</td>
</tr>
<tr>
<td>7</td>
<td>Waste tank</td>
</tr>
<tr>
<td>8</td>
<td>Sheath tank</td>
</tr>
</tbody>
</table>
Using BD Assurity Linc software

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

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

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

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

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

Procedure
To grant access to a BD technical support representative:
1. Ensure that your workstation is connected to the internet.
2. Contact your local BD technical support representative.

If a remote session is required, the BD representative will initiate a session through a secure link.
A dialog opens once the connection is established.

3. Acknowledge the request.

The BD representative can now assist you.
This chapter covers the following topics:

- Maintenance overview (page 103)
- Stopping the stream (page 106)
- Lubricating the sample injection chamber O-ring (page 108)
- Precautions for handling nozzles (page 110)
- Changing the nozzle (page 111)
- Cleaning the sort nozzle (page 112)
- Cleaning the closed-loop nozzle (page 113)
- Replacing the sort nozzle seal temporarily (page 114)
- Aligning the waste aspirator drawer to the stream (page 115)
- Cleaning the flow cell (page 118)
- Cleaning the optical filters (page 119)
- Cleaning the Accudrop laser window and the lower camera window (page 120)
- Cleaning the strobe lens window and upper camera window (page 122)
- Cleaning the deflection plates (page 124)
- Removing or installing the FSC neutral density filter (page 125)
- Emptying the waste tank (page 126)
- Filling the sheath tank (page 128)
- Changing the fluid filter (page 131)
- Purging the sheath filter (page 133)
- Backflushing the sample line (page 133)
- Replacing the sample line (page 134)
- Replacing the sample line filter (page 140)
- Removing the sheath probe (page 142)
- Preparing for aseptic sort (page 144)
- Preparing new fluid filters (page 146)
Maintenance overview

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

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Maintenance task</th>
<th>When to perform</th>
</tr>
</thead>
<tbody>
<tr>
<td>External surfaces</td>
<td>Cleaning external surfaces (page 106)</td>
<td>As needed</td>
</tr>
<tr>
<td>Sample loading area</td>
<td>Lubricating the sample injection chamber O-ring (page 108)</td>
<td>As needed or when sample loading fails</td>
</tr>
<tr>
<td>Nozzle and flow cell</td>
<td>Changing the nozzle (page 111)</td>
<td>As needed</td>
</tr>
<tr>
<td></td>
<td>Cleaning the sort nozzle (page 112)</td>
<td>When you see indications of clogging</td>
</tr>
<tr>
<td></td>
<td>Cleaning the closed-loop nozzle (page 113)</td>
<td>When you see indications of salt buildup or clogging</td>
</tr>
<tr>
<td></td>
<td>Replacing the sort nozzle seal temporarily (page 114)</td>
<td>If the seal is lost or damaged and you do not have a replacement nozzle</td>
</tr>
<tr>
<td></td>
<td>Aligning the waste aspirator drawer to the stream (page 115)</td>
<td>If you install a sort nozzle that is new or different from the one that came with the instrument</td>
</tr>
<tr>
<td></td>
<td>Cleaning the flow cell (page 118)</td>
<td>As needed</td>
</tr>
<tr>
<td>Category</td>
<td>Maintenance task</td>
<td>When to perform</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Optics</strong></td>
<td>Cleaning the optical filters (page 119)</td>
<td>As needed</td>
</tr>
<tr>
<td></td>
<td>Cleaning the Accudrop laser window and the lower camera window (page 120)</td>
<td>When the software is unable to set drop delay, or when the software is unable to verify the side streams when sorting is started</td>
</tr>
<tr>
<td></td>
<td>Cleaning the strobe lens window and upper camera window (page 122)</td>
<td>When smudges appear in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed</td>
</tr>
<tr>
<td></td>
<td>Cleaning the deflection plates (page 124)</td>
<td>When you have trouble viewing the side stream</td>
</tr>
<tr>
<td></td>
<td>Removing or installing the FSC neutral density filter (page 125)</td>
<td>As needed</td>
</tr>
<tr>
<td>Category</td>
<td>Maintenance task</td>
<td>When to perform</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Fluidics</td>
<td>Emptying the waste tank (page 126)</td>
<td>As indicated by the software and whenever you fill the sheath tank</td>
</tr>
<tr>
<td></td>
<td>Replacing the waste filter cap</td>
<td>Monthly</td>
</tr>
<tr>
<td></td>
<td>Filling the sheath tank (page 128)</td>
<td>Daily or as needed</td>
</tr>
<tr>
<td></td>
<td>Changing the fluid filter (page 131)</td>
<td>Every 3 months or as needed</td>
</tr>
<tr>
<td></td>
<td>Purging the sheath filter (page 133)</td>
<td>After you install a new sheath filter and whenever you observe problems with the stream</td>
</tr>
<tr>
<td></td>
<td>Backflushing the sample line (page 133)</td>
<td>When you observe sample carryover, or after you run samples with adherent cells or dye</td>
</tr>
<tr>
<td></td>
<td>Replacing the sample line (page 134)</td>
<td>Every 4–6 months or when decreased event rates indicate that the sample line might be clogged</td>
</tr>
<tr>
<td></td>
<td>Replacing the sample line filter (page 140)</td>
<td>When decreased event rates indicate that the sample line might be clogged</td>
</tr>
<tr>
<td></td>
<td>Removing the sheath probe (page 142)</td>
<td>As needed, before you autoclave the sheath tank</td>
</tr>
<tr>
<td></td>
<td>Preparing for aseptic sort (page 144)</td>
<td>Before you start an aseptic sort</td>
</tr>
</tbody>
</table>
Stopping the stream

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

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

Procedure
To stop the stream:
1. Open the Stream View window by clicking the Stream status indicator in the navigation bar.

2. In the Stream View window, click Stop Stream.

3. To restart the stream, load a tube in the Experiments workspace using the Acquisition Dashboard on the View Data or Sort tab.

Cleaning external surfaces

Introduction
All cytometer surfaces that have been exposed to sheath fluid should be cleaned to prevent salt buildup.
Before you begin
If the stream is on, turn it off. See Stopping the stream (page 106).

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

About this task
The following surfaces should be inspected and cleaned when necessary:

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

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

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

Select any of the following options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean the salt buildup off the surface of the flow cell cuvette</td>
<td>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.</td>
</tr>
<tr>
<td>Clean the salt buildup off the surface of the closed-loop nozzle</td>
<td>Remove the closed-loop nozzle and wipe it thoroughly with water. Ensure that it is dry before you reinstall it.</td>
</tr>
<tr>
<td>Clean the salt buildup off the remaining surfaces</td>
<td>Wipe the surfaces with a cloth dampened with water followed by 70% ethanol.</td>
</tr>
<tr>
<td>Decontaminate the surfaces</td>
<td>Wipe the surfaces with a cloth dampened with 10% bleach solution followed by DI water.</td>
</tr>
</tbody>
</table>

**Lubricating the sample injection chamber O-ring**

**Introduction**

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

**Procedure**

To lubricate the sample injection chamber O-ring:

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

   Ensure that the loading port is empty.
2. On the Cytometer page, select Replace Sample Line Filter, and then click Start. (Do not complete the remaining steps of the wizard.)

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

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

5. Exit the wizard without completing it by clicking Cancel.
## Precautions for handling nozzles

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

<table>
<thead>
<tr>
<th>Precaution</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always use the closed-loop nozzle for cleaning and shutdown procedures.</td>
<td>To keep the flow cell clean and reduce salt buildup and clogs. A clean flow cell provides improved sensitivity and higher performance.</td>
</tr>
<tr>
<td>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.</td>
<td>To prevent the seal from coming loose and falling out.</td>
</tr>
<tr>
<td>Always insert and remove nozzles straight in and straight out.</td>
<td>To reduce wear and tear on the seal.</td>
</tr>
<tr>
<td>Always grasp the closed-loop nozzle by the metal nut, not the tubing, wire, or plastic sleeve.</td>
<td>To prevent wear and tear on the tubing and wire. They might become detached with repeated mishandling.</td>
</tr>
<tr>
<td>Do not expose nozzles to strong base solutions such as Contrad® 70.</td>
<td>To prevent the seal from coming loose and falling out. Any contact with such solutions might damage the seal.</td>
</tr>
<tr>
<td>Do not wipe the surface of the seal with anything.</td>
<td>To prevent damage to the seal that could result in leaking.</td>
</tr>
</tbody>
</table>
Chapter 6: Maintenance

Changing the nozzle

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

About this task

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

Procedure

To change the nozzle:

1. Ensure that the stream is turned off, and then open the flow cell access door.

2. Remove the closed-loop or sort nozzle (1) from the flow cell by turning the nozzle-locking lever (2) counterclockwise to the 9:00 position and then pulling the nozzle straight out.

3. Insert the new closed-loop or sort nozzle into the flow cell (with the top side facing up) and push it gently all the way forward until it stops.

4. Turn the nozzle-locking lever clockwise to the 12:00 position.

5. If the nozzle you installed is a sort nozzle that is new or different from the one that came with the instrument, align the
waste aspirator drawer to the stream to ensure proper sort setup. See Aligning the waste aspirator drawer to the stream (page 115).

---

**Cleaning the sort nozzle**

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

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

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

![Unclogged nozzle tip](image)

**About this task**

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

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

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

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

4. Reinsert the nozzle into the flow cell.

---

**Cleaning the closed-loop nozzle**

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

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

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

      Handle the nut with the connected wire and tubing carefully to prevent kinking or detachment. Make sure that the ferrule stays on the tubing.
Note: If the ferrule is old or shows any sign of damage, replace it. A ferrule that is damaged or used for too long can become stuck.

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

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

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

5. Close the flow cell access door.

---

**Replacing the sort nozzle seal temporarily**

**Introduction**

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

**Procedure**

To replace the sort nozzle seal temporarily:

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

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

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

   **Note:** Do not wipe the nozzle with anything because it could leave fibers or other contamination, or dislodge the O-ring.
3. Using the magnifier in the accessory kit, inspect the nozzle to verify that the O-ring is installed all the way into the groove.

---

**Aligning the waste aspirator drawer to the stream**

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

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

**About this task**

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

**Procedure**
To align the waste aspirator drawer to the stream:
1. Open the flow cell access door.
2. Loosen the screws on both sides of the sort block door by using a hex wrench.

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

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

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

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

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

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

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

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

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

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

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

Before you begin
Ensure that you know how to do the following procedures:

- Changing the nozzle (page 111)
- Emptying the waste tank (page 126)
- Filling the sheath tank (page 128)

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

Procedure
To clean the flow cell:

1. On the Cytometer page, select Flow Cell Clean, and then click Start.

2. Complete the steps in the wizard.

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

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

Cleaning the optical filters

Introduction

You should inspect and clean the optical filters as needed.

About this task

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

Procedure

To clean the optical filters:

1. Open the filter access door on the electronics box.

2. Wrap a triangular section of lens paper around the cotton end of a cotton swab, and then moisten and seal the end with a few drops of alcohol.

3. Holding the cotton swab in a horizontal position, gently rub any spots on the filter surface and wipe clean.

4. Allow the solvent to evaporate and check the filter surface for streaks.

5. Inspect the 1/4-inch-diameter section in the center of the filter on both sides for scratches.

Filters are coated with different dielectrics that can get scratched. If you see scratches, replace the filter.
6. Insert the cleaned filter into the heptagon trigon, and make sure the filters are pushed all the way in.

Cleaning the Accudrop laser window and the lower camera window

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

About this task

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

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

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

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

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

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

5. Wipe the windows with lens paper or a soft, lint-free cloth soaked with DI water, and then dry the windows.

6. Close the sort block door, the flow cell access door, and the sort collection chamber door.
Cleaning the strobe lens window and upper camera window

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

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

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

![Stream views](image)

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

Procedure
To clean the strobe lens window and upper camera window:
1. Ensure that the stream is turned off. See Stopping the stream (page 106).
2. Open the flow cell access door and the sort collection chamber door.

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

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

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

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

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

7. Click the Stream status indicator and check that the camera image is clean in the Stream View window.
8. Close the sort block door, the flow cell access door, and the sort collection chamber door.

---

**Cleaning the deflection plates**

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

**Before you begin**
You need the deflection plate removal tool from the accessory kit.

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

**Procedure**
To remove the deflection plates:
1. Ensure that the stream is turned off. See Stopping the stream (page 106).
2. Open the flow cell access door and the sort collection chamber door.
3. Ensure that the red warning light next to the sort block door is off, indicating that the deflection plates are off.
4. Open the sort block door by turning the thumbscrew on the front of it.

5. Hold your thumb on the plate (or use your other hand), and then pull the deflection plates out carefully so that they do not fall.

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

Removing or installing the FSC neutral density filter

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

About this task
Note: The ND filter must be installed when you run Drop Delay.
Procedure  

To remove or install the FSC ND filter:
1. Open the flow cell access door.
2. Loosen the set screw on the top of the FSC ND filter assembly with a 1.5-mm Allen wrench.
3. Perform one of the following:
   a. Remove the FSC ND filter from the slot.
   b. Install the new filter by sliding it into the slot with the label facing the flow cell, as shown in the preceding picture.
4. Close the flow cell access door.

Emptying the waste tank

Introduction  

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

Before you begin  

Ensure that you have enough bleach solution to equal 10% of the volume of the waste tank.
Chapter 6: Maintenance

About this task

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

Procedure

To empty the waste tank:
1. Turn off the stream. See Stopping the stream (page 106).
2. Disconnect the sensor and fluid line cap from the waste tank.
   
   **Note:** The waste tank can become pressurized when the cytometer is running. Wait at least 1 minute for the pressure to dissipate before you open the tank.

3. Remove the disposable waste cap (large-sized cap) and attached trap from the tank.
   
   Place the assembly on the bench with the label-side up.

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

4. Empty the waste tank according to your standard laboratory procedures for biohazardous waste.
5. Add approximately 1 L of bleach to the waste tank (10-L container) or a sufficient amount so that 10% of the total volume is bleach.

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

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

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

8. Reconnect the sensor and fluid line.

---

**Filling the sheath tank**

**Introduction**

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

The following illustration shows the sheath tank and connectors.

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluid filter</td>
</tr>
<tr>
<td>2</td>
<td>Fluid filter purge line</td>
</tr>
<tr>
<td>3</td>
<td>Containment device</td>
</tr>
<tr>
<td>4</td>
<td>Sheath sensor</td>
</tr>
<tr>
<td>5</td>
<td>Fluid connector</td>
</tr>
<tr>
<td>6</td>
<td>Fluid line</td>
</tr>
<tr>
<td>7</td>
<td>Pressure relief valve</td>
</tr>
<tr>
<td>8</td>
<td>Air line</td>
</tr>
<tr>
<td>9</td>
<td>Air connector</td>
</tr>
<tr>
<td>10</td>
<td>Cover knob</td>
</tr>
</tbody>
</table>
**Note:** Avoid getting the top of the sheath sensor wet. If the top of the sheath sensor gets wet, wipe it dry. The sheath sensor will not detect the level of sheath fluid accurately if the top of it is wet.

**Procedure**

To fill the sheath tank:

1. Turn off the stream. See Stopping the stream (page 106).
2. Disconnect the air line.
3. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.
4. Unscrew the sheath tank cover knob and remove the cover.
5. Fill the tank with sheath fluid up to the upper weld line on the inside of the tank.

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

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

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

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

8. Connect the air line.

---

**What to do next**

Purging the sheath filter (page 133)

---

**Changing the fluid filter**

**Introduction**

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

---

**About this task**

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

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

Spare filters are included with the accessory kit.

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

To change the fluid filter:

1. Turn off the stream. See Stopping the stream (page 106).

2. Disconnect the air line from the sheath tank.

3. Pull up on the ring of the pressure relief valve to release the pressure from the tank. Verify that all of the pressure is released by pulling up a second time.

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

5. Disconnect the filter purge line by unscrewing the nut.

6. Write the current date on the filter so that you will know when to replace it.

7. Reconnect the filter purge line to the new filter.

8. Use the arrows on the filters that indicate the direction of the flow through the filter; replace the filter with a new one in the same orientation.

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tabs</td>
</tr>
<tr>
<td>2</td>
<td>Filter purge line nut</td>
</tr>
</tbody>
</table>
9. Reconnect the air line to the tank and check for leaks when the pressure is turned on.

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

Purging the sheath filter

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

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

Procedure  To purge the sheath filter:
On the Cytometer page, select Sheath Filter Purge, and then click Start.

Backflushing the sample line

Introduction  After a sample tube is unloaded, the sample line tubing within the sample injection chamber is automatically flushed inside and out with sheath fluid to eliminate potential sample carryover. You can perform additional backflush cleaning whenever you observe sample carryover, or after running samples with adherent cells or dye.
Procedure
To backflush the sample line:
With the stream on, select one of the following options:

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

Replacing the sample line

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

Before you begin
You need the following items from the accessory kit:

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

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

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

### Procedure

To replace the sample line:

1. On the Cytometer page, select Replace Sample Line, and then click Start.

2. Remove the sample line:
   a. Remove the sample line access door and open the flow cell access door.
   b. Remove the top of the cytometer by loosening the hex screws on the front and back and lifting the top off.
c. Unscrew the connecting nut (1) at the top of the flow cell (2) and slowly pull the sample line (3) out.

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

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

e. Slide the ferrule (if it is still attached) and the nut off the end of the sample line.
f. Pull the pinch valve tubing out of the slot in the pinch valve.

![Image of pinch valve and sample line]

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

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

i. Slowly pull the sample line (1) out from the top and ensure that a cone-shaped ferrule (2) is still attached to it.
Note: If the ferrule is left behind in the injection chamber fitting, gently push the tip of the ferrule-removal tool into the top of the ferrule and pull the ferrule straight out.

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

3. Install the new sample line:

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

   ![Caution!] Do not bend the sample line during insertion.

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

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

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

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

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

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

   ![Caution!] Do not bend the sample line during insertion.
f. Insert the pilot tubing (1) into the fitting at the top of the flow cell until tubing stops.

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

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

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

h. In the wizard, click Continue.

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

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

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

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

7. Load a tube of DI water and ensure that there are no leaks.
Replacing the sample line filter

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

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

Procedure
To replace the sample line filter:
1. On the Cytometer page, select Replace Sample Line Filter, and then click Start.
2. Complete the Sample Line Filter step:
   a. Remove the sample line access door from the cytometer.
   b. Pull the pinch valve tubing out of the slot in the pinch valve.
c. Loosen the sample line fitting nut (1) at the top of the injection chamber (2) to allow the sample line (3) to slide freely through the fitting.

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

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

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

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

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

   Push the pinch valve tubing back into the pinch valve.

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

---

**Removing the sheath probe**

**Introduction**

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

**About this task**

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

**Procedure**

To remove the sheath probe:

1. Turn off the stream. See Stopping the stream (page 106).
2. Disconnect the air line from the sheath tank.
3. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.
4. Loosen the nut at the top of the sheath probe with a wrench and vent the sheath pressure again if the tank is still pressurized.

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.
Preparing for aseptic sort

Introduction

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

Before you begin

Ensure that you know how to do the following procedures:

- Changing the nozzle (page 111)
- Filling the sheath tank (page 128)
- Changing the fluid filter (page 131)
- Emptying the waste tank (page 126)
- Removing the sheath probe (page 142)
- Preparing new fluid filters (page 146)

You need the following items:

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

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

8. Decontaminate the sheath probe using 70% ethanol.
About this task

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

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

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

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

Procedure

To prepare for aseptic sort:

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

2. Remove the sheath sensor cable.
   The cable is marked with a DO NOT AUTOCLAVE label.

3. Autoclave the sheath tank (with the gauge attached) according to your organization’s protocols.

4. On the Cytometer page in the software, select System Startup.

5. Select and complete one of the fluidics startup options, or click Skip.
   When the fluidics startup is completed or skipped, you are prompted to select a cleaning option.
6. Select **Prepare for Aseptic Sort**, select number one (1), and then click **Start**.

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

---

### Preparing new fluid filters

**Introduction**

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

**Before you begin**

Ensure that you know how to perform the following procedures:

- Filling the sheath tank (page 128)
- Changing the fluid filter (page 131)
- Purging the sheath filter (page 133)
To prepare new fluid filters:

1. Empty the sheath tank, rinse with DI water and refill with DI water. See Changing the fluid filter (page 131).

2. Remove the cap from the new filter and save it for reuse.

3. Change the fluidic filter to the new filter. See Filling the sheath tank (page 128).

4. In the BD FACSCchorus software, select Cytometer > Sheath filter purge and select Start.

5. Repeat step 4 three times to ensure all of the air is removed from the fluid filter.

6. Change the fluid filter to prepare another new fluid filter, or install the appropriate filter for next desired workflow.

7. Replace the cap on the newly prepared fluid filter and store at room temperature.

8. Empty the sheath tank and fill with appropriate fluid for next desired workflow.
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Technical specifications

This chapter covers the following topics:

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

### Optical specifications

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation laser</td>
<td>• 1-laser (blue), 2-color (2-0)</td>
</tr>
<tr>
<td></td>
<td>• 1-laser (blue), 4-color (4-0)</td>
</tr>
<tr>
<td></td>
<td>• 2-laser (blue, red), 6-color (4-2)</td>
</tr>
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<td></td>
<td>• 2-laser (blue, violet), 6-color (3-3)</td>
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<td>• 3 (blue, violet, yellow/green), 8-color (2-2-4)</td>
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<tr>
<td></td>
<td>• 3 (blue, red, yellow/green)8-color (2-2-4)</td>
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Chapter 7: Technical specifications

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<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser specifications Blue laser</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>488 nm</td>
</tr>
<tr>
<td>Output power</td>
<td>20mW</td>
</tr>
<tr>
<td>Nominal power</td>
<td>16mW</td>
</tr>
<tr>
<td>Beam spot size</td>
<td>9 ±3 µm x 67 ±5 µm</td>
</tr>
<tr>
<td>Red laser</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>640 nm</td>
</tr>
<tr>
<td>Output power</td>
<td>40mW</td>
</tr>
<tr>
<td>Nominal power</td>
<td>36 mW</td>
</tr>
<tr>
<td>Beam spot size</td>
<td>9 ±3 µm x 67 ±5 µm</td>
</tr>
<tr>
<td>Violet laser</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>405 nm</td>
</tr>
<tr>
<td>Output power</td>
<td>40mW</td>
</tr>
<tr>
<td>Nominal power</td>
<td>36 mW</td>
</tr>
<tr>
<td>Beam spot size</td>
<td>9 ±3 µm x 67 ±5 µm</td>
</tr>
<tr>
<td>Yellow-green laser</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>561 nm</td>
</tr>
<tr>
<td>Output power</td>
<td>50 mW</td>
</tr>
<tr>
<td>Nominal power</td>
<td>40 mW</td>
</tr>
<tr>
<td>Beam spot size</td>
<td>9 ±3 µm x 67 ±5 µm</td>
</tr>
<tr>
<td>Laser alignment</td>
<td>Fixed and spatially separated alignment of all lasers with the cuvette flow cell</td>
</tr>
<tr>
<td>Beam divergence angle (full-angle)</td>
<td>488 nm: &lt;1.2 mrad</td>
</tr>
<tr>
<td></td>
<td>640 nm: &lt;1.3 mrad</td>
</tr>
<tr>
<td></td>
<td>405 nm: &lt;1.0 mrad</td>
</tr>
<tr>
<td></td>
<td>561 nm: &lt;1.2 mrad</td>
</tr>
<tr>
<td>Optical coupling</td>
<td>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.</td>
</tr>
</tbody>
</table>
Detection channels Forward scatter (FSC), side scatter (SSC), and up to nine fluorescence. See the BD FACSMelody Cell Sorter Filter Guide for laser and detection configurations and optical filter specifications. See Instrument configurations (page 23) for more details.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
</table>
| Fluorescence and side scatter detection | • Reflective optics with single transmission bandpass filter in front of each PMT  
• High performance customized PMT modules for all fluorescence and SSC channels  
• Light collected by objective lens is delivered by fiber optics to specially designed heptagon or trigon detector arrays.  
• The cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens (1.2 NA) for optimal collection efficiency. |

See the BD FACSMelody Filter Guide for laser and detection configurations and optical filter specifications.

Stream illumination at 25°C (Accudrop laser)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical power</td>
<td>&gt;18 mw</td>
</tr>
<tr>
<td>Lasing wavelength</td>
<td>660 nm</td>
</tr>
<tr>
<td>Beam divergence angle (full-angle)</td>
<td>&lt;3.0 mrad</td>
</tr>
</tbody>
</table>
### Fluidic specifications

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

### Sample input formats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample input</td>
<td>5.0-mL polystyrene or polypropylene tubes</td>
</tr>
</tbody>
</table>

### Collection devices

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software</td>
<td>BD FACSChorus version 1.0 or later</td>
</tr>
<tr>
<td>Operating system</td>
<td>Microsoft Windows 10 64-bit</td>
</tr>
<tr>
<td>Monitor</td>
<td>23-inch LCD with a minimum 1,920 x 1,080 resolution</td>
</tr>
<tr>
<td>Memory</td>
<td>8 GB RAM</td>
</tr>
<tr>
<td>Storage</td>
<td>500-GB hard drive</td>
</tr>
<tr>
<td>FCS format</td>
<td>FCS 3.1</td>
</tr>
</tbody>
</table>
# Physical specifications

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating temperature</td>
<td>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.</td>
</tr>
<tr>
<td>Humidity</td>
<td>The operating humidity tolerance is between 40% and 60% relative humidity (noncondensing).</td>
</tr>
<tr>
<td><strong>Dimensions (H x W x D)</strong></td>
<td></td>
</tr>
<tr>
<td>Cell sorter</td>
<td>49.5 x 55.9 x 48.3 cm (19.5 x 22 x 19 in.)</td>
</tr>
<tr>
<td>Electronics box</td>
<td>50.8 x 55.9 x 48.3 cm (20 x 22 x 19 in.)</td>
</tr>
<tr>
<td>Biological safety cabinet (optional)</td>
<td>136.5 x 87.6 x 233.4–249.9 cm</td>
</tr>
<tr>
<td></td>
<td>(53.8 x 34.5 x 91.9–98.4 in.)</td>
</tr>
<tr>
<td>Sample input temperature control (optional)</td>
<td>42.9 x 91.3 x 47.1 cm</td>
</tr>
<tr>
<td></td>
<td>(16.90 x 35.95 x 18.55 in.)</td>
</tr>
<tr>
<td>Aerosol Management Option</td>
<td>45.21 x 38.1 x 54.61 cm</td>
</tr>
<tr>
<td></td>
<td>(17.8 x 15 x 21.5 in.)</td>
</tr>
<tr>
<td>See the BD FACS Melody Cell Sorter Site Preparation Guide for additional information on dimensions and clearances.</td>
<td></td>
</tr>
<tr>
<td>Instrument weight</td>
<td>Cell sorter: 40.75 kg (89.8 lb)</td>
</tr>
<tr>
<td></td>
<td>Electronics box: 36.25 kg (79.9 lb)</td>
</tr>
</tbody>
</table>
This chapter covers the following topics:

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

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

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

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

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

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed loop nozzle is not detected</td>
<td>Salt buildup on the closed-loop nozzle</td>
<td>Clean the closed loop nozzle. See Cleaning the closed-loop nozzle (page 113).</td>
</tr>
<tr>
<td></td>
<td>Salt buildup in the nozzle location between the flow cell and the locking lever</td>
<td>Clean the area to remove the salt build up.</td>
</tr>
</tbody>
</table>
## Startup troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error starting stream after inserting sort nozzle</td>
<td>Sheath tank low or empty or waste tank full or almost full</td>
<td>Refill the sheath tank. See Filling the sheath tank (page 128) or Emptying the waste tank (page 126).</td>
</tr>
<tr>
<td></td>
<td>Sort nozzle inserted improperly</td>
<td>Remove the nozzle and ensure that the O-ring is in place. Reinsert the nozzle. See Changing the nozzle (page 111).</td>
</tr>
<tr>
<td></td>
<td>Dirty strobe lens or upper camera window</td>
<td>Clean the lens and the window as described in Cleaning the strobe lens window and upper camera window (page 122).</td>
</tr>
<tr>
<td></td>
<td>Clogged or damaged sort nozzle</td>
<td>Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 112).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If the nozzle appears damaged, replace it. See Changing the nozzle (page 111)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click Experiment &gt; View Data to restart the stream.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Load the tube and select Load Sample to start stream.</td>
</tr>
<tr>
<td>Debris in flow cell</td>
<td>Clean the flow cell.</td>
<td>Clean the flow cell. See Cleaning the flow cell (page 118).</td>
</tr>
</tbody>
</table>
## Startup troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error starting stream after inserting sort nozzle or loading sample</td>
<td>Air in sheath line or filter</td>
<td>Purge the sheath filter. See <a href="#">Purging the sheath filter (page 133)</a>. Run daily fluidics startup. See <a href="#">Fluidics startup (page 46)</a>.</td>
</tr>
<tr>
<td>Dry sheath filter</td>
<td></td>
<td>Purge the sheath filter. See <a href="#">Purging the sheath filter (page 133)</a>.</td>
</tr>
<tr>
<td>Air pressure is too low, too high, or variable</td>
<td></td>
<td>Verify that the external air supply is on and the pressure is between 80 and 95 psi. Verify that the sheath tank lid is sealed properly.</td>
</tr>
<tr>
<td>Residual ethanol in fluidic lines</td>
<td></td>
<td>Run extended fluidics startup.</td>
</tr>
</tbody>
</table>
## Startup troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream not in center of waste aspirator drawer</td>
<td>Sort nozzle inserted improperly</td>
<td>Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. See Changing the nozzle (page 111).</td>
</tr>
</tbody>
</table>
|                                          | Clogged or damaged sort nozzle   | Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope.  
|                                          |                                  | If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 112).       
|                                          |                                  | If the nozzle appears damaged, replace it. See Changing the nozzle (page 111).        
|                                          |                                  | Click Experiment > View Data to restart the stream.                                  
|                                          |                                  | Load the tube and select Load Sample to start stream.                                |
| New sort nozzle was inserted.            | If you are using a new nozzle, the sort block may need to be repositioned to align with the stream. See Changing the nozzle (page 111). |                                                                                      |
| Prepare for aseptic sort fails          | Fluid or air lines are detached  | Verify that the fluid or air line connections are attached. Push firmly on each line to ensure that it is connected. |
## Startup troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Problems with Cytometer Setup function</td>
<td>Baseline or performance check failed</td>
<td>Prepare a new CS&amp;CT sample with the proper concentration as instructed in the product insert.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clean the flow cell. See <a href="#">Cleaning the flow cell (page 118)</a>.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Close the sort block door and the flow cell access door properly.</td>
</tr>
<tr>
<td>Problems with Drop Delay function</td>
<td>Sort block door is not closed</td>
<td>Close the sort block door properly.</td>
</tr>
<tr>
<td></td>
<td>Event rate is too low or too high</td>
<td>Prepare a new Accudrop sample with the proper concentration as instructed in the technical data sheet.</td>
</tr>
<tr>
<td></td>
<td>Debris on lower camera or Accudrop window</td>
<td>Clean the lower camera and Accudrop laser window. See <a href="#">Cleaning the Accudrop laser window and the lower camera window (page 120)</a>.</td>
</tr>
</tbody>
</table>
## Acquisition troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No events in plots or events don't update in plots after clicking Load Sample</td>
<td>Selected data source is a recorded file</td>
<td>Select the Live Data data source.</td>
</tr>
<tr>
<td></td>
<td>Laser shutter is engaged</td>
<td>Close the flow cell access door properly.</td>
</tr>
<tr>
<td></td>
<td>No sample in the tube</td>
<td>Add sample to the tube or install a new sample tube.</td>
</tr>
<tr>
<td></td>
<td>Sample line is clogged</td>
<td>Backflush the sample line. See Backflushing the sample line (page 133).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If that does not work, replace the sample line assembly. See Replacing the sample line (page 134).</td>
</tr>
<tr>
<td></td>
<td>Sample line filter is clogged</td>
<td>Replace the sample line filter. See Replacing the sample line filter (page 140).</td>
</tr>
<tr>
<td></td>
<td>Sample is not mixed properly</td>
<td>Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 60).</td>
</tr>
<tr>
<td></td>
<td>Threshold is not set to correct parameter</td>
<td>Set the threshold to the correct parameter for your application. See Defining view data (page 60).</td>
</tr>
<tr>
<td></td>
<td>Threshold setting is too low or too high</td>
<td>Adjust the threshold setting. See Defining view data (page 60).</td>
</tr>
</tbody>
</table>
**Acquisition troubleshooting (continued)**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexpected events in plots or fewer events in gated populations than expected</td>
<td>Incorrect logic in population hierarchy</td>
<td>Verify the gating strategy.</td>
</tr>
<tr>
<td></td>
<td>Threshold not set to correct parameter</td>
<td>Set the threshold to the correct parameter for your application. See Defining view data (page 60).</td>
</tr>
<tr>
<td></td>
<td>Threshold setting is too low or too high</td>
<td>Adjust the threshold setting. See Defining view data (page 60).</td>
</tr>
<tr>
<td></td>
<td>Events left out of a gate</td>
<td>When drawing a gate, make sure that events on the axes are included.</td>
</tr>
<tr>
<td></td>
<td>Cell size is set incorrectly</td>
<td>Ensure that the setting for the cell size is appropriate for your sample.</td>
</tr>
<tr>
<td></td>
<td>Sample preparation is inadequate</td>
<td>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.</td>
</tr>
</tbody>
</table>
## Acquisition troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic event rate</td>
<td>Sample is not adequately mixed or is aggregated</td>
<td>Filter the sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resuspend the sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Turn on or increase the sample agitation rate. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Sheath tank is low</td>
<td></td>
<td>Fill the sheath tank. See Filling the sheath tank (page 128).</td>
</tr>
<tr>
<td>Sample preparation is inadequate</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Sample injection chamber O-ring is worn</td>
<td></td>
<td>Contact your BD Biosciences service engineer.</td>
</tr>
<tr>
<td>Unexpectedly high event rate</td>
<td>Sample is not adequately mixed or is aggregated</td>
<td>Filter the sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resuspend the sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Turn on or increase the sample agitation rate. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Threshold setting is too low</td>
<td></td>
<td>Adjust the threshold setting. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Sample is too concentrated</td>
<td></td>
<td>Dilute the sample.</td>
</tr>
<tr>
<td>Flow rate is too high</td>
<td></td>
<td>Decrease the flow rate.</td>
</tr>
<tr>
<td>Bubbles in flow cell</td>
<td></td>
<td>Turn off the stream, wait a few seconds, and then load the sample again.</td>
</tr>
</tbody>
</table>
### Acquisition troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexpectedly low event rate</td>
<td>Sample is not adequately mixed or is aggregated</td>
<td>Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Sample is too dilute</td>
<td></td>
<td>Concentrate the sample.</td>
</tr>
<tr>
<td>Threshold setting is too high</td>
<td></td>
<td>Adjust the threshold setting. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Sample line is clogged or kinked</td>
<td></td>
<td>Backflush the sample line. See Backflushing the sample line (page 133).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If that does not work or if visible kinks are found in the sample line, replace the sample line assembly. See Replacing the sample line (page 134).</td>
</tr>
<tr>
<td>Sample line assembly or sample line filter installed incorrectly</td>
<td></td>
<td>Verify the sample line assembly or sample line filter installation. See Replacing the sample line filter (page 140).</td>
</tr>
</tbody>
</table>
## Acquisition troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distorted populations or high CVs</td>
<td>Instrument settings adjusted incorrectly</td>
<td>Optimize the threshold setting, voltage settings, and run user-defined compensation to optimize compensation settings. See Defining view data (page 60) and Calculating compensation (page 64).</td>
</tr>
<tr>
<td>Flow rate is too high</td>
<td>Decrease the flow rate.</td>
<td></td>
</tr>
<tr>
<td>Bubbles in flow cell</td>
<td>Turn off the stream, wait a few seconds, and then load the sample again.</td>
<td></td>
</tr>
<tr>
<td>Debris in flow cell or nozzle</td>
<td>Clean the flow cell.</td>
<td>Clean the flow cell. See Cleaning the flow cell (page 118). Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 112)</td>
</tr>
<tr>
<td>Sample is not adequately mixed or is aggregated</td>
<td>Filter the sample.</td>
<td>Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Sample preparation is inadequate</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>
## Acquisition troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive amount of debris in plots</td>
<td>Threshold setting is too low</td>
<td>Adjust the threshold setting. See Defining view data (page 60).</td>
</tr>
<tr>
<td></td>
<td>Dead cells or debris in sample</td>
<td>Examine the sample under a microscope to determine the source of the debris. Adjust sample preparation if needed.</td>
</tr>
<tr>
<td></td>
<td>Sample preparation is inadequate</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Sheath filter needs to be replaced</td>
<td>Replace the sheath filter. See Changing the fluid filter (page 131).</td>
</tr>
<tr>
<td>Processed events are &lt;90%</td>
<td>Threshold setting is too low</td>
<td>Adjust the threshold setting. See Defining view data (page 60).</td>
</tr>
<tr>
<td></td>
<td>Event rate is too high</td>
<td>Decrease the flow rate.</td>
</tr>
<tr>
<td></td>
<td>Sample is not adequately mixed or is aggregated</td>
<td>Filter the sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resuspend the sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Turn on or increase the sample agitation rate. See Defining view data (page 60).</td>
</tr>
<tr>
<td>Sample injection chamber does not close</td>
<td>Sample injection chamber O-ring is dry and causing chamber to stick</td>
<td>Lubricate the sample injection chamber O-ring. See Lubricating the sample injection chamber O-ring (page 108).</td>
</tr>
</tbody>
</table>
## Sorting troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream turns off unexpectedly</td>
<td>Debris in flow cell or nozzle</td>
<td>Clean the flow cell. See Cleaning the flow cell (page 118).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Remove the nozzle, and examine the nozzle tip under a microscope.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 112).</td>
</tr>
<tr>
<td>Sheath or waste tank full</td>
<td></td>
<td>Empty the waste tank or fill the sheath tank. See Emptying the waste tank (page 126)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and Filling the sheath tank (page 128).</td>
</tr>
<tr>
<td>Unable to start sort</td>
<td>BD FACSChorus software cannot locate the side streams</td>
<td>Clean the lower camera window. See Cleaning the Accudrop laser window and the lower camera window (page 120). Close the sort block door properly.</td>
</tr>
<tr>
<td></td>
<td>Salt bridge</td>
<td>Clean the deflection plates and the area around and behind the plates. See Cleaning the deflection plates (page 124).</td>
</tr>
<tr>
<td></td>
<td>Drop charge cable is loose or missing</td>
<td>Verify that the stream-charging wire is inserted all the way into the barb.</td>
</tr>
<tr>
<td>Arcing between deflection plates</td>
<td>Salt bridge</td>
<td>Clean the deflection plates and the area around and behind the plates. See Cleaning the deflection plates (page 124).</td>
</tr>
</tbody>
</table>
## Sorting troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanning around center or side streams</td>
<td>Sort nozzle inserted improperly</td>
<td>Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. See Changing the nozzle (page 111).</td>
</tr>
<tr>
<td></td>
<td>Clogged or damaged sort nozzle</td>
<td>Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. See Cleaning the sort nozzle (page 112). If the nozzle appears damaged, replace it. See Changing the nozzle (page 111) and Replacing the sort nozzle seal temporarily (page 114). Click Experiment &gt; View Data to restart the stream. Load the tube and select Load Sample to start stream.</td>
</tr>
<tr>
<td>Dirty deflection plates</td>
<td>Clean the deflection plates.</td>
<td>See Cleaning the deflection plates (page 124).</td>
</tr>
<tr>
<td>Incorrect sort mode</td>
<td>Verify that the sort mode is appropriate for your sorting requirements. See Calculating compensation (page 64).</td>
<td></td>
</tr>
<tr>
<td>Particles too big for sort nozzle</td>
<td>Verify that the particle size is appropriate for the 100-um nozzle.</td>
<td></td>
</tr>
<tr>
<td>Low sort efficiency</td>
<td>Event rate is too high for drop frequency</td>
<td>Decrease the flow rate.</td>
</tr>
<tr>
<td></td>
<td>Gating conflict</td>
<td>Verify the gating hierarchy.</td>
</tr>
</tbody>
</table>
### Sorting troubleshooting (continued)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic sort rate</td>
<td>Flow rate is too high</td>
<td>Decrease the flow rate.</td>
</tr>
<tr>
<td>Unexpected sort results</td>
<td>Incorrect drop delay</td>
<td>Run drop delay. See System startup (page 44).</td>
</tr>
<tr>
<td></td>
<td>Incorrect sort mode</td>
<td>Verify that the sort mode is appropriate for your sorting requirements. See Calculating compensation (page 64).</td>
</tr>
<tr>
<td></td>
<td>Incorrect logic in population hierarchy</td>
<td>Verify the gating hierarchy. Do not assign conflicting gates (for example, parent population in Tube 1, child population in Tube 2).</td>
</tr>
<tr>
<td>Plate sorting failure</td>
<td>Splash shield not installed</td>
<td>Install the splash shield.</td>
</tr>
<tr>
<td></td>
<td>Sort chamber door is open</td>
<td>Close the sort chamber door.</td>
</tr>
</tbody>
</table>
## Electronics troubleshooting

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