6

Sorting

You can program BD FACSDiva™ software to sort a specified number of particles from multiple populations into a variety of sorting devices including tubes, plates, and slides. Up to four defined populations can be sorted into each tube, allowing up to 24 populations to be sorted at one time.

The following topics are covered in this chapter:

Setting up for sorting

In general, do the following to set up for a sorting experiment. Each step is explained in more detail in previous or subsequent sections.

1. Start up the cytometer and the computer.

   See Cytometer startup (page 88).
2. Install the appropriate size nozzle.
3. Select a new sort setup mode, if needed, and select an appropriate cytometer configuration.

   To change your current sort setup mode, select a nozzle size from the **Sort > Sort Setup** menu.

   For more information, see Sort setup (page 70).
4. Install a sample line filter, if needed.

   See Installing or removing a sample line filter (page 159).
5. Check the laser delay for your sheath pressure and particle size.
   - To set the laser delay automatically using the CS&T module, see Checking cytometer performance (page 99).
   - To set the laser delay manually, see Manual adjustment of laser delay (page 116).
6. If you have the AMO, turn it on at 20%.

   See Operating the BD® Aerosol Management Option (page 193).
7. Optimize cytometer settings for the sample to be sorted.

   See Data collection (page 108).
8. Install the required collection device and set up the side streams.

   See Setting up for bulk sorting (page 120) or Setting up for sorting onto a plate or slide (page 132).
9. Calculate the drop delay.

   See Determining the drop delay – automatic method (page 126).
10. Use gating tools and subsetting methods to define the population(s) of interest.

    Examples of gating analyses can be found in Analyzing data (page 114).
11. Define a sort layout for the tube containing the defined sort populations and proceed with sorting.

    See Sorting (page 127).

Setting up for bulk sorting

This section describes how to set up the streams for two-, four-, or six-way sorting. For sorting using the ACDU option, see Setting up for sorting onto a plate or slide (page 132).

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

To set up for bulk sorting:

1. Install collection tubes in the appropriate collection device.

   Collection tube holders are available for 1-mL microtubes, 1.5-mL Eppendorf® tubes, 12 × 75-mm tubes, and 15-mL centrifuge tubes. For compatible tubes, see Labware (page 190).

2. Install the collection tube holder onto the cytometer.
   a. Close the sort block door and open the sort collection chamber door, if needed. The sort block door must be closed to install the tube holder.
   b. Slide the holder into the slotted fittings below the sort aspirator drawer, then close the sort collection chamber door.

Before installing the collection tube holder, ensure that an O-ring is installed in the groove between the two sections of the tube holder. The O-ring minimizes the chance of aerosols escaping. It can be found in the accessory kit (Catalog No. 337897).

3. Turn on the deflection plates.

   Click the **Voltage** button in the **Side Stream** window. The voltage warning light illuminates, indicating that the plates are charged.

A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the plate voltage is on. The plates remain energized even when the sort block door is open. Do not set the plate voltage over 6,000V.

   Make sure the center stream image does not move after the plates are turned on. Major movement of the center stream could indicate that the plates or the area around the plates needs cleaning.

4. Click the **Test Sort** button and optimize the side streams.

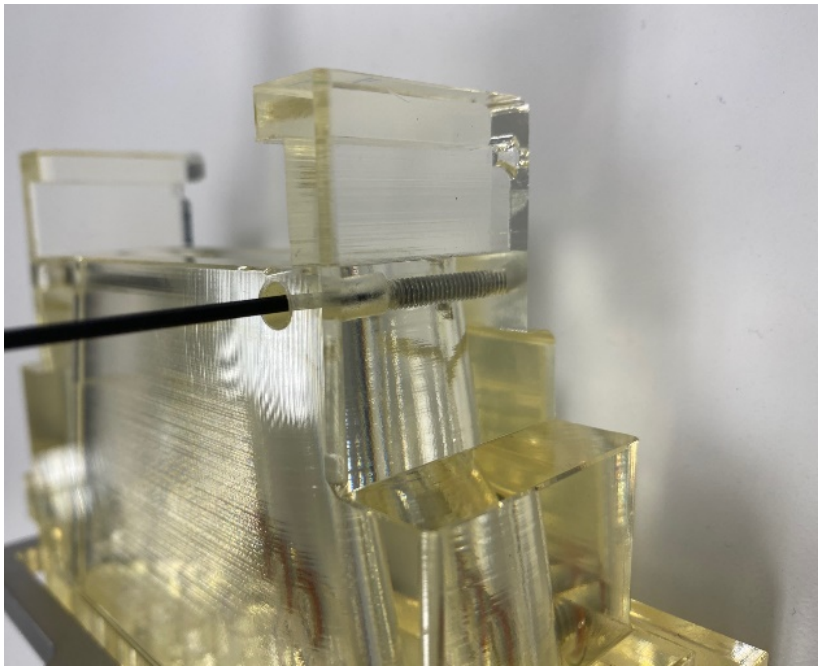
   Adjust the voltage sliders to view the required number of streams. If necessary, adjust the plate voltage to achieve deflection in the outermost tubes. Do not exceed a plate voltage of 6000V.

   If you cannot see a stream image or the image is dim, adjust the micrometer dial on the diode laser to better view the streams.

5. Adjust the 2nd, 3rd, and 4th Drop settings to tighten the center stream and fine-tune the side streams, if needed.

   Generally, the sort setup mode provides good starting values for these settings. Adjust the values only if needed to optimize the streams.

6. Open the aspirator drawer and aim the side stream(s) into each collection tube.
   a. In the **Side Stream** window, click the **Waste Drawer** button to open the drawer.
   b. Open the sort block door and aim each side stream along the horizontal axis into the tube as you adjust the corresponding slider in the **Side Stream** window.
   c. The 6-way sort collection device also includes set screws on either side of the device to center the streams along the front-to-back axis. Adjust the set screws using a 1/16 hex key from the accessory kit.



A seal must be formed between the top of the device and the bottom of the sort block. If there is a gap, the front-to-back adjustment is incorrect.

d. When you are satisfied with the side stream deflection, close the sort block door.

7. Click the **Voltage** button to turn off the deflection plates.
8. Attempt to optimize drop delay using the automatic method first, then if necessary use the manual method. For details, see the following procedures:
   - Determining the drop delay – automatic method (page 126)
   - Determining the drop delay – manual method (page 123)

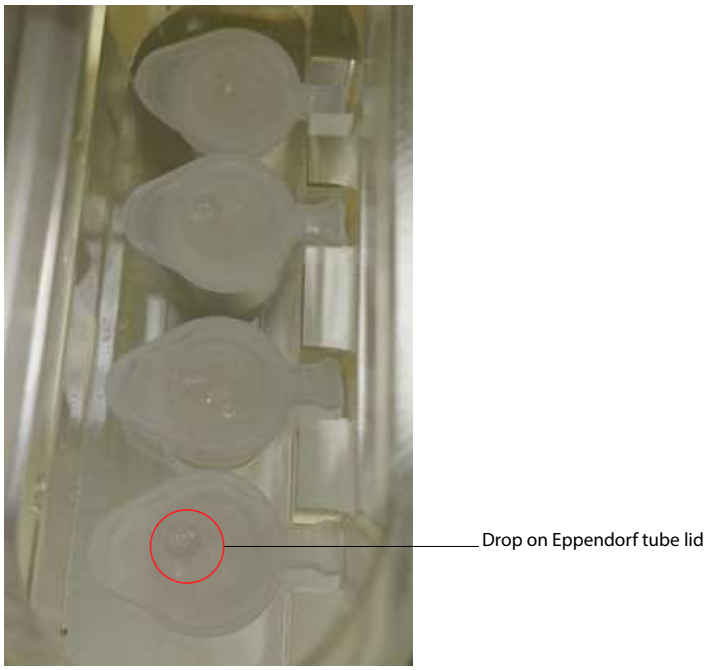
Using the 1.5-mL side stream alignment tool

If you are using 1.5-mL Eppendorf tubes, use six tubes of ethanol to align the side streams and verify drop placement.

1. Set the optimal breakoff.
2. Ensure that the center stream is aligned with the center of the aspirator drawer.
3. With the aspirator drawer closed (retracted), position the alignment tool in the sort block.
4. Turn on the deflection plates.
5. Click **Test Sort**, then adjust sliders to align each side stream to the corresponding notch on the alignment tool.

   Note: To prevent the stream fluid from pooling or splashing, place a wipe or paper towel inside the sample collection chamber.
6. Turn off the deflection plates.
7. Carefully remove the alignment tool.
8. To verify stream alignment, place four capped Eppendorf tubes inside the Eppendorf collection tube holder and insert the tube holder into the sort collection chamber.
9. Turn on the deflection plates.
10. Quickly click **Test Sort** twice to deposit a drop on the Eppendorf tube lids.

11. Verify that the drops are deposited on the center of each Eppendorf tube lid.



Note: For future reference, record the slider values or take a screen shot of the stream setup window.

Determining the drop delay – manual method

BD FACS™ Accudrop technology is used to determine the optimal drop-delay setting for your sorting application. For more information, see Drop-Delay Overview (page 49).

There are two methods for determining the drop delay.

- **Manual drop delay**. Using the standard method as described in the following sections.
- **Auto drop delay**. Using an automated algorithm method. See Determining the drop delay – automatic method (page 126).

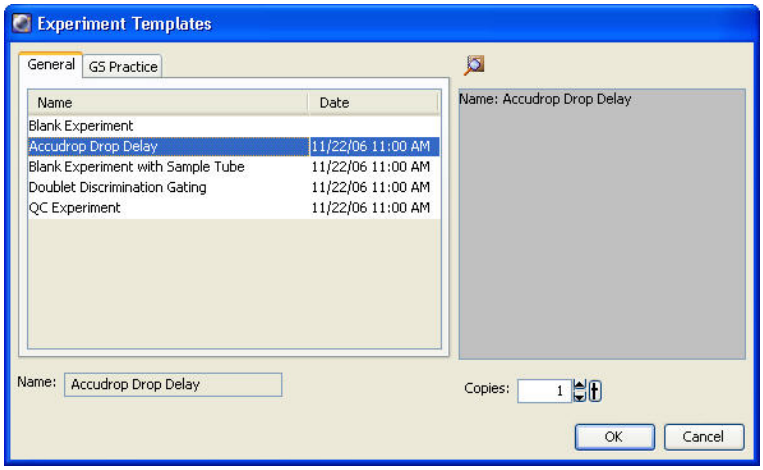
Note: Before beginning these procedures, make sure the stream is stable and the Sweet Spot is on.

Setting up the experiment

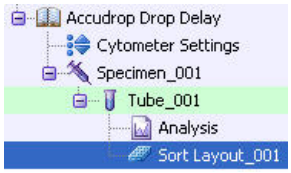
This section describes how to set the drop delay using the Accudrop experiment template. Because no data is recorded, the experiment can be reused as often as you like.

1. Create an experiment from the Accudrop Drop Delay template.

   Select **Experiment > New Experiment**. Select the **Accudrop Drop Delay** experiment and click **OK**.



2. Expand **Specimen_001** and **Tube_001**.
3. Set the current tube pointer to **Tube_001**.
4. Open the sort layout by double-clicking it.



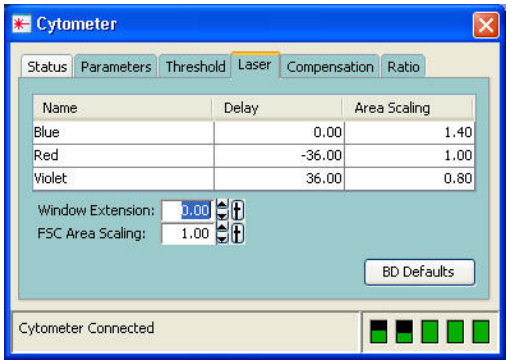
Using manual drop delay

This section describes the manual method of optimizing the drop delay.

1. Load a tube filled with a suspension of Accudrop Beads (approximately one to two drops of beads in 0.5 mL of PBS).

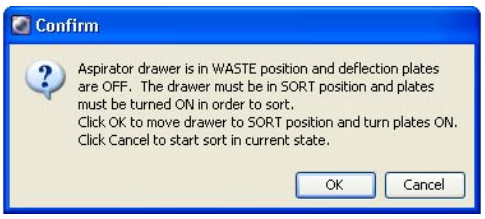
Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. Keep your hands and clothing away from the loading port when a tube is loading or unloading. Do not place objects underneath the loading port.

2. Open the **Parameters** tab of the **Cytometer** window and adjust the FSC and SSC values until events are on-scale. A typical value of FSC is ~500V and of SSC is ~250V.
3. In the **Laser** tab of the **Cytometer** window, set the window extension to zero.



4. Adjust the flow rate to achieve an event rate of 1,000–3,000 events per second.
5. Turn on the voltage in the **Side Stream** window. Click **Sort** in the **Sort Layout** window.

6. Click **Cancel** at the **Confirm** dialog.



There is no need to collect the beads. When the drawer is closed, the beads are sorted to waste.

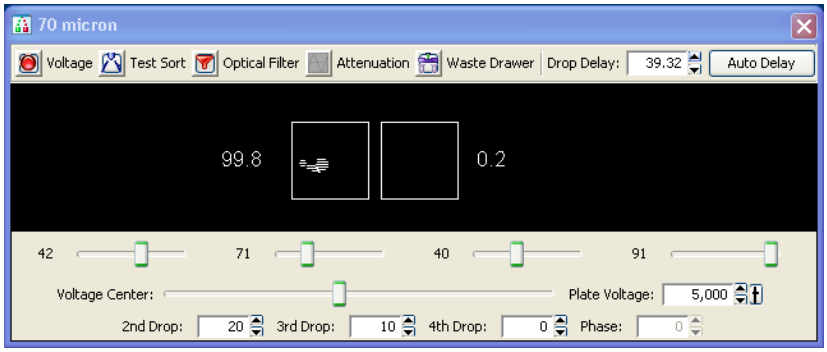
7. Adjust the micrometer dial to obtain the brightest bead spot on the center stream.
8. Click the **Optical Filter** button in the **Side Stream** window.

   This control moves the emission filter that allows you to view the Accudrop Beads in front of the lower camera. When the control is clicked, the image switches from a raw image to a processed (digitized) image. The two boxes indicate the region of the image where the left and center stream intensities are calculated during image processing. The numbers shown are percentages of the total intensity.

   If the left side stream is not completely contained in the left region, adjust the voltage slider to place the stream in the center of the region.
9. Verify that the sort precision mode is set to **Initial**.

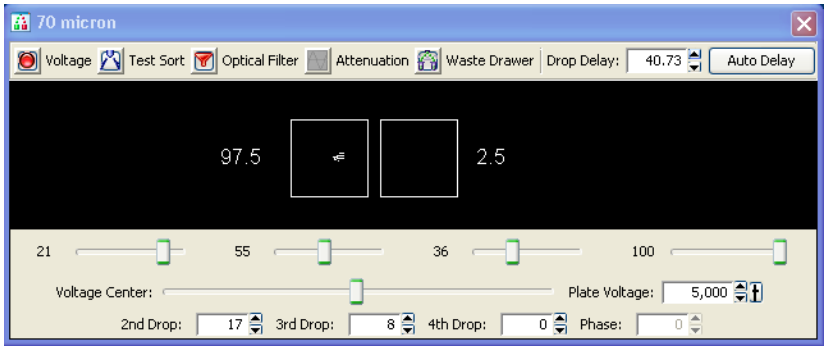
   See Sort Precision Modes (page 52) for more information.
10. Optimize the drop delay.

    Adjust the drop-delay value in 1-drop increments (Ctrl+click arrow control) to achieve close to 100% intensity in the left side stream. Wait a few seconds after each click for a complete response to the delay change.



11. In the **Sort Layout** window, change the precision mode to **Fine Tune**.
12. Optimize the drop delay.

    Adjust the drop-delay value in 0.03-drop increments (click the arrow control) until the left side stream intensity is greater than or equal to 90%. Wait a few seconds after each click for a complete response to the delay change.



13. Click the **Optical Filter** button to move the emission filter away from the camera.
14. Reset the window extension to its original setting (typically 2).
15. Turn off the deflection plates.

Determining the drop delay – automatic method

The auto drop delay feature automates setting the drop delay to get optimized results from sorting. You should use this method after you have a good understanding of how drop delay works in general. See Using manual drop delay (page 124) for more information.

Overview of auto drop delay

Auto drop delay works best when the sort system is stable, and assumes that Accudrop Beads are used. When the process is started, the auto drop algorithm uses several passes to find best drop delay possible under the current conditions of the system. Coarse passes are used to find the initial drop delay within 2 drops of the ideal. The coarse passes are faster than the fine-tune passes. Fine-tune passes are used to locate the ideal drop delay value considering the current conditions.

Using auto drop delay

1. Set up an experiment for drop delay as described in Setting up the experiment (page 123).
2. Load a tube filled with a suspension of Accudrop Beads (approximately 2 drops of beads in 0.5 mL of PBS).

   Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. Keep your hands and clothing away from the loading port when a tube is loading or unloading. Do not place objects underneath the loading port.

3. Adjust the flow rate to achieve these values of events per second:

   70 micron = 1,000 to 3,000
   85 micron = 800 to 2,000
   100 micron = 600 to 1,500
   130 micron = 400 to 1,200

   Note: If this cannot be achieved using a flow rate setting between 1 and 5, adjust the bead concentration.
4. Turn on the voltage in the **Side Stream** window. Click **Sort** in the **Sort Layout** window.
5. Click **Cancel** in the **Confirm** dialog.

   There is no need to collect the beads. When the drawer is closed, the beads are sorted to waste.
6. Adjust the micrometer dial to obtain the brightest bead spot on the center stream.
7. Click the **Auto Delay** button in the **Side Stream** window.

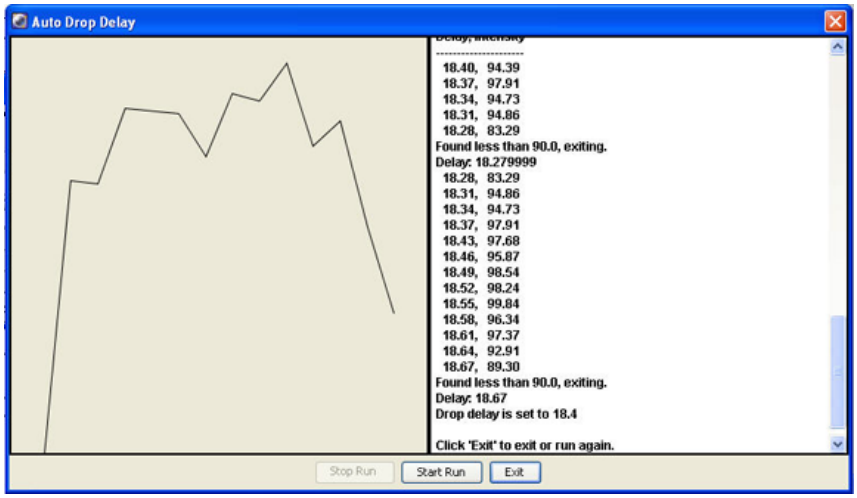
A dialog opens, similar to that shown in the figure that following this procedure. The graph in the left pane of the dialog represents brightness of the stream (Y axis) versus drop delay (X axis). Typically the graph should have a flat portion, with several small peaks in either direction (up or down) or one prominent peak.

Note: If the original drop delay value was close to the appropriate value prior to starting auto delay, then you may not get a graph displayed because few data points were required to determine the optimal drop delay.

8. Select **Start Run** in the **Auto Drop Delay** dialog.
9. Monitor the **Auto Drop Delay** dialog for progress.

   A message is displayed when the process is completed.

   Note: If the sort is stopped during algorithm execution (either by user action or because the system detected a failure), the run will be stopped with an appropriate message. Start the sort again, verify that the stream is stable and the sort is not pausing, and re-run the auto drop delay.



Sorting

Before beginning the sort, do the following:

1. Perform the steps outlined in Setting up for sorting (page 120).
2. Use gating tools and subsetting methods to define the population(s) of interest.

   Examples of gating analysis can be found in Analyzing data (page 114).

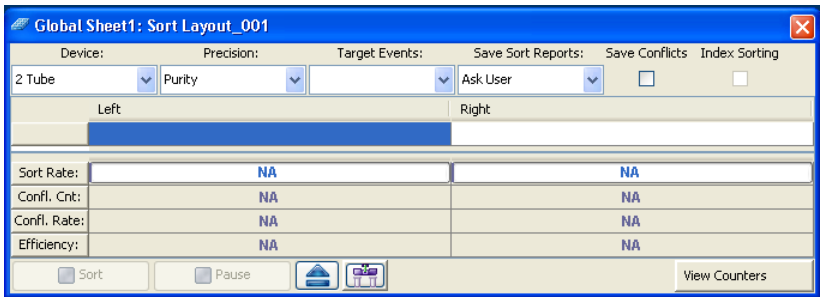
   Note: Gates drawn on a biexponential scale can be used for sorting. However, the cytometer will sort on a log scale. Therefore, a gate that crosses the zero boundaries will sort all events below zero into that gated population. This can cause a variance between the sort results and the statistical results in the software. If the gate is completely below zero on a biexponential plot, no events will be sorted.

   Snap-to gates cannot be used for sort gates.

Setting up the experiment

Note: When more than one drop is deflected in the same direction, residual charge from the first drop degrades the quality of the side streams. Thus, when sorting more than one stream to a side or sorting into small wells where precise deflection is required, use the 4-Way Purity mode or select a mode with a yield mask of zero. For more information, see Yield Mask (page 50).

1. Create a new sort layout by clicking the **New Sort Layout** button on the **Browser** toolbar.
   By default, the 2-Tube Sort Layout is displayed.
2. Make appropriate entries in the **Sort Layout** window.



   - Select the collection device from the **Device** menu.
   - Change the sort precision mode to **Purity** (two tubes), **4-Way Purity** (four tubes), or **Single Cell** (plate or slide).
   - Enter the number of target events by selecting a value from the menu or entering a number in the field.
   - Select a **Save Sort Reports** option: **Save None**, **Save All**, or **Ask User**. See Setting up a sort layout (page 73).
   - Select the **Save Conflicts** checkbox if you are using a 2- or 4-tube sort layout and want to save conflicts.
   - Select the **Index Sorting** checkbox if you are doing an index sort. See Index sorting (page 136).
   - Select the sort location field(s) to be sorted into. Select multiple fields by dragging the mouse. Select a row or column by clicking the row or column header.
   - Add the required population(s) to each sort location field.
   - To display fewer counters in the **Sort Layout** window, click the **View Counters** button and clear a menu option. The corresponding counter is hidden. (Only counters with a checkmark next to the name are displayed.)

Starting and monitoring the sort

To start the sort:

1. Open the sort collection chamber door and install the collection tubes, plate, or slide.

   The flow cell access door is equipped with a shutter mechanism that shuts off the laser light when the door is opened. To ensure there is no interruption to data acquisition, do not open the door while sorting or recording.

2. Close the sort collection chamber door.

   Failure to close the sort collection chamber door prevents the evacuator from generating negative pressure in the chamber and could affect drop placement while sorting.

3. Install the sample tube onto the loading port and click **Load**.

   Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. Keep your hands and clothing away from the loading port when a tube is loading or unloading. Do not place objects underneath the loading port.

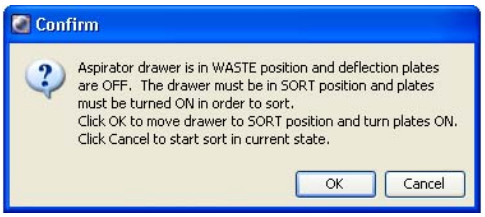
4. Adjust the flow rate.

Sorting results are typically optimized at lower flow rates.

5. (Optional) Turn on the deflection plates and open the aspirator drawer.

   The deflection plates turn off automatically each time a tube is unloaded. If you do not turn them back on before beginning a sort, a dialog is displayed in which you can turn on the plates and open the aspirator drawer by clicking OK.

6. Verify that the current tube pointer is indicating the appropriate tube in the **Browser**, then click **Sort**.
7. Click **OK** if you are prompted to open the aspirator drawer or turn on the deflection plates.

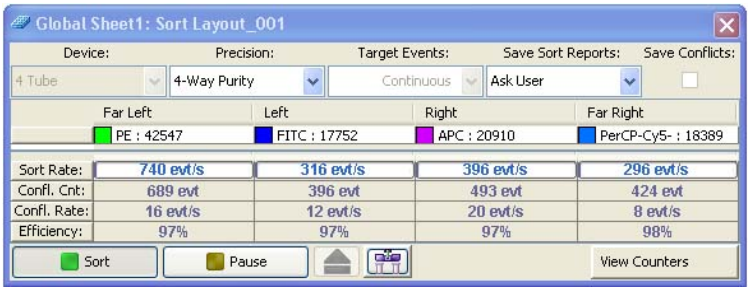


If you click Cancel, sorting will begin with the deflection plates off and the drawer closed. As a result, sort populations will be identified and counted, but no deflection (or sorting) will occur. If you sort with the drawer closed, events will be sorted to waste.

Note: Click **Record Data** to save data for the tube. Acquisition and sorting continue after the required number of events has been recorded.

Sorting continues until the required number of cells has been sorted. Acquisition stops and the drawer closes when sorting is complete. If the number of Target Events is set to Continuous, sorting continues until you manually stop sorting by clicking the Stop Acquiring button in the Dashboard, or the Sort button in the Sort Layout window.

Monitor the sort progress in the Sort Layout window. The number of events sorted into each sort location is displayed in the corresponding field. The sort rate and sort conflict rate are displayed in the corresponding counter fields.



Note: When the Sweet Spot is on, sorting pauses automatically if the Drop 1 or Gap values are out of range. This ensures that sorting occurs only under the proper breakoff conditions. If a more severe problem such as a clog is detected, the stream shuts off and sorting stops. The deflection plates shut off, the aspirator drawer closes, and the sample tube is unloaded.

8. (Optional) You can print the sort report at this time, or open the report later and print it then. You can also export the report.

Stopping and Resuming a Sort

The Stop/Resume function allows you to temporarily stop the sort and still retain the counter values. This is particularly useful when the sample volume is low and you need to refill the tube, or to replace collection tubes.

Replacing a sample tube

1. To stop a sort while it is running, click the **Sort** button in the **Sort Layout** window.
2. If prompted, click **OK** to save the sort report.

   You can set the sort report to save automatically after each sort. See Setting up a sort layout (page 73).
3. Unload the sample tube by clicking **Unload** on the **Acquisition Dashboard**.
4. Refill the sample tube, then click **Load** in the **Acquisition Dashboard**.

   Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. Keep your hands and clothing away from the loading port when a tube is loading or unloading. Do not place objects underneath the loading port.
5. Click the **Resume** button in the **Sort Layout** window to continue sorting.
6. Click **OK** when you are prompted to open the aspirator drawer or turn on the deflection plates.

   The sort counters resume from the value where they stopped. The threshold counter restarts. However, the value is accumulated and the total count is saved in the final sort report.

Replacing the collection tubes

1. To stop a sort while it is running, click the **Sort** button in the **Sort Layout** window.
2. If prompted, click **OK** to save the sort report.

   You can set the sort report to save automatically after each sort. See Setting up a sort layout (page 73).
3. Click **Stop Acquiring** in the **Acquisition Dashboard** to stop the sample flow.
4. Turn off the deflection plates by clicking the **Voltage** button in the **Side Stream** window.
5. Remove the lower section of collection tube holder by lifting up on the handle and pulling the lower section of the holder down and forward.
6. Replace the collection tubes as needed.
7. Reinstall the tube holder and pull down on the handle to secure it in place.
8. Click **Acquire Data** in the **Acquisition Dashboard** to restart the sample flow.
9. Click the **Resume** button in the **Sort Layout** window to continue sorting.
10. Click **OK** when you are prompted to open the aspirator drawer or turn on the deflection plates.

    The sort counters resume from the value where they stopped. The threshold counter restarts. However, the value is accumulated and the total count is saved in the final sort report.

Pausing and resuming a sort

The Pause/Resume function allows you to temporarily pause the sort, and still retain the sort counter values. This is useful when you need to make adjustments to an experiment during a sort. Be aware that the sample continues to flow during a pause.

Note: If you need to replace the sample tube to refill it, or to replace collection tubes, you should stop the sort. See Stopping and Resuming a Sort (page 129).

1. To pause a sort while it is running, click the **Pause** button in the **Sort Layout** window.
2. Make adjustments to the experiment as needed.
3. Click the **Resume** button in the **Sort Layout** window to continue sorting.

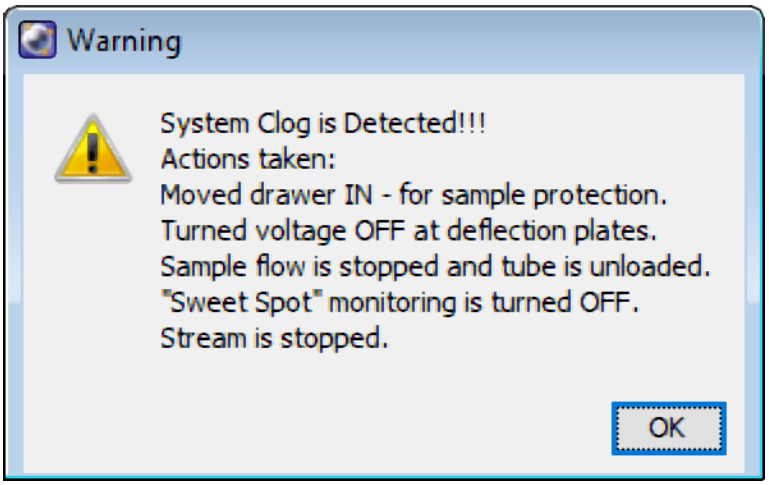
Responding to a nozzle clog during a sort

Follow this procedure if your system does not have a BSC or the Aerosol Management Option (AMO).

If your system:

- Does not have a BSC, but does have the AMO, see Responding to a nozzle clog during a sort with the AMO (page 195).
- Does have a BSC and the Aerosol Management System (AMS), see Responding to a nozzle clog during a sort (page 211).

If the stream is disturbed during the sort (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes (if Sweet Spot is on). If the system detects a clog, it displays the following warning:



The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog, do not open the sort collection chamber door or access the sort tubes before following this procedure.

Cell sorters that use droplet generation methods, such as the BD FACSymphony™ S6 cell sorter, can produce aerosols around the sample stream. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door and the sort collection chamber door closed during sorting. Follow these steps to stop sample flow and evacuate potential aerosols before opening the sort collection chamber door.

To clear a clogged nozzle:

1. If the stream has not already shut down automatically, turn off the stream by clicking the **Stream** button (with a checkmark) at the top of the **Breakoff** window.

   This will shut off the stream, unload the sample, and close the aspirator drawer.
2. Turn on the stream and view the breakoff.

   If the clog is removed, the breakoff will be similar to the breakoff before the clog.
3. If the clog is not cleared, turn the stream on and off several times to see if the clog will clear itself.
4. If the clog is not removed, turn the stream off and perform the Clean Flow Cell procedure with DI water (see Cleaning the flow cell (daily) (page 140)), followed by turning the stream on to see if the clog will be cleared.
5. With the aspirator drawer closed, wait for at least 3 minutes to clear aerosols before opening the sort collection chamber and the sort block door.
6. If it is necessary to change nozzles or remove a clog from a nozzle, see Cleaning the integrated nozzle (page 156).
7. With the stream turned off, open the sort block door and dry the plates and surfaces as needed.

When removing collection tubes, be aware that the outside of the tube is potentially contaminated. Use alcohol swabs or bleach to wipe the outsides of tubes.

8. Make sure that all chamber doors are closed and restart the stream.
9. Perform these tasks if needed:
   - Turn on the Sweet Spot
   - Check the drop delay
   - Check the side stream deflection

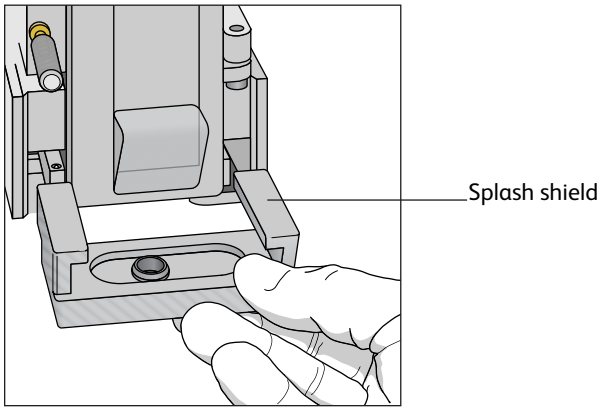
Setting up for sorting onto a plate or slide

The following sections describe how to set up for sorting onto a plate or slide. For general guidelines, see Setting up for sorting (page 120).

Installing the sorting hardware

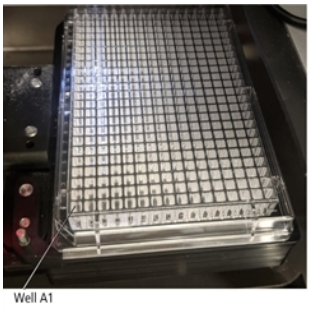
Any cytometer surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling sorting hardware. Wear suitable protective clothing and gloves.

1. Install the splash shield below the aspirator drawer.
   a. Close the sort block door and open the sort collection chamber door, if needed.
      The sort block door must be closed in order to open the collection chamber door.
   b. Remove the tube holder, if one is installed.
   c. Slide the splash shield into the slotted fittings below the sort aspirator drawer and push it all the way in.



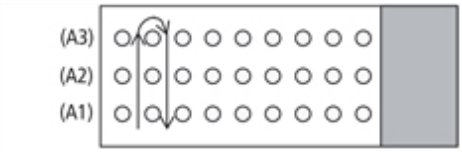
2. Click the **Access Stage** button to bring the ACDU stage to the front.
   a. Open an experiment, if one is not already open, and create a sort layout for any of the tubes.
   b. In the **Sort Layout** window, click the **Access Stage** button to move the stage to the front of the sort collection chamber.
3. Install the appropriate collection device on the stage.
   - If you are sorting into a plate, install the plate with well A1 toward the front of the stage.
   - If you are sorting onto a slide, install the slide-adapter tray with the printed side up. If your slide has a

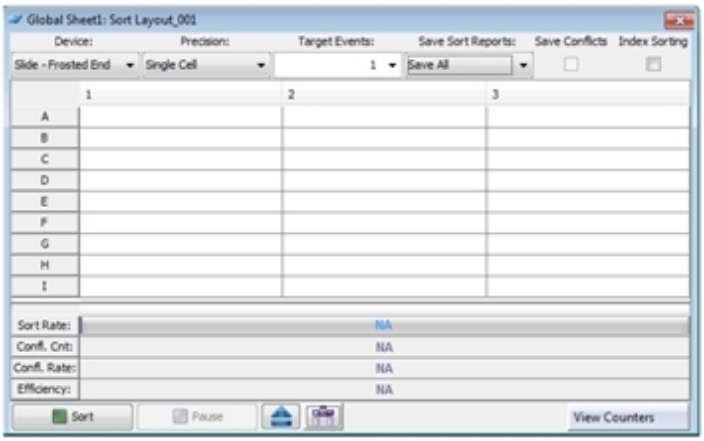
frosted end, place the frosted end to the right.



Sorting starts on the front left corner of the device (A1 location), and proceeds from front to back, and then from left to right, sorting in a serpentine motion. Thus, for a plate, sorting proceeds from well A1–A12, B12–B1, C1–C12, and so on.

When sorting onto a slide, sorting proceeds in rows across the short end of the slide, and in columns along the long end of the slide. Make sure that you set up your sort layout accordingly.





Setting up the stream

This section describes how to optimize side stream deflection and how to adjust the home location.

When sorting onto a plate or slide, the stage is pre-programmed to move a set distance between wells on a plate or spots on a slide. The home location is used as the starting point. The far left stream should hit the center of the well in the top left corner of a plate or the top left corner of a slide at the home location.

Default home location coordinates exist for each standard sort collection device: Falcon® multiwell plates with 6, 24, 48, 96, and 384 wells, and standard or frosted-end slides. For other plate types, you will need to create a custom device. See Creating a custom device (page 135).

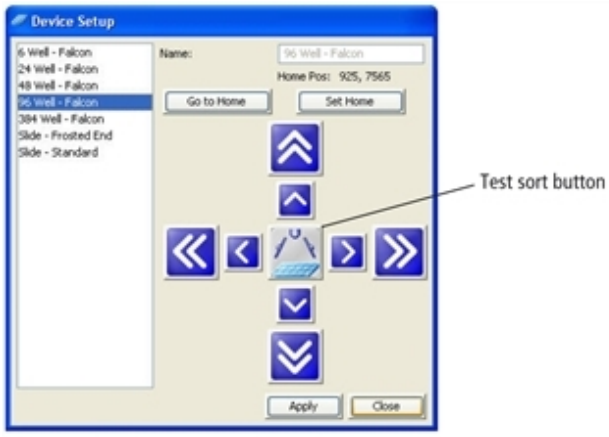
Use the following procedure to verify the home location and adjust it.

1. Turn on the deflection plates.

   Click the **Voltage** button in the **Side Stream** window. The voltage warning light illuminates, indicating that the plates are charged.

Note: Make sure the center stream image does not move after the plates are turned on. Major movement of the center stream could indicate that the plates or the area around the plates needs cleaning. See External cleaning (page 144).

2. Select **Sort > Home Device**.
3. In the **Device Setup** dialog, select the collection device you are using and click **Go to Home**.



The stage moves to the pre-programmed home position.

4. Click the **Test Sort** button and optimize the far left side stream, then click the **Test Sort** button again to turn it off.

   Ensure that the aspirator drawer is open. Adjust the far left slider for minimal deflection of the stream. The stream should be deflected just enough to clear the hole in the splash shield. Do not adjust the other sliders.

   If you cannot see a stream image or the image is dim, adjust the micrometer dial on the diode laser to better view the streams.

5. Double-click the **Test Sort** button to deposit a drop at the home location.

   Note: To ensure consistent drop placement, keep the sort collection door closed when depositing drops on the vessel.

6. Inspect the collection device to see where the drop was deposited.

   If you need to move the stage to the front, close the **Device Setup** dialog and click the **Access Stage** button in the **Sort Layout** window.

7. Wipe the collection device dry and place it back on the tray support.

   If needed, click the **Access Stage** button to send the stage back and select **Sort > Home Position** to access the **Device Setup** dialog again.

8. Adjust the home location, if necessary.

   Click the appropriate arrow buttons to move the tray support as needed. Large arrows move the tray by five steps. Small arrows move the tray by one step.

9. Repeat steps 5 through 8 until the drop is centered appropriately.
10. Click **Set Home**, then **Close**.
11. Click the **Voltage** button to turn off the deflection plates.
12. Optimize the drop delay.

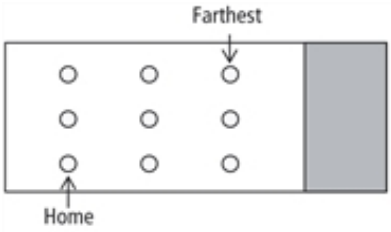
    See one of the following procedures:

    - Determining the drop delay – manual method (page 123)
    - Determining the drop delay – automatic method (page 126)

13. Proceed with Sorting (page 127).

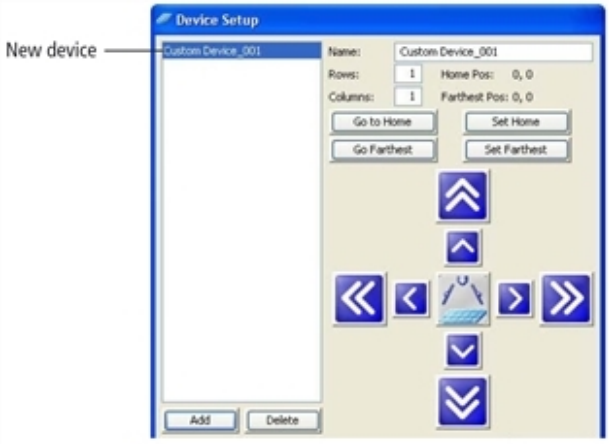
Creating a custom device

You can program the ACDU stage to sort into any grid configuration. Create a custom device by entering the number of rows and columns and setting the home and farthest locations. BD FACSDiva™ software calculates the increment between rows and columns to determine the sort locations. The home and farthest locations for a 96-well plate are A1 and H12, respectively.



1. Select **Sort > Custom Devices**.
2. Click the **Add** button in the **Custom Devices** dialog.

   A new device is added to the list of custom devices. By default, devices are named Custom Device_00*x*, where *x* is the next consecutively numbered device.



3. Select the text in the **Name** field and enter a new name.
4. Enter the number of sort location rows and columns.

   A device can have up to 60 rows and 25 columns.
5. Use the arrow buttons and the **Test Sort** button to set the home location, then click **Set Home**.

   See Setting up the stream (page 133) for details. There are no default values for custom devices, so greater initial adjustment with the arrow buttons is required.
6. Use the same procedure to set the farthest location, then click **Set Farthest**.

   The farthest sort location is the well or spot on the lower-right corner of the collection device.
7. Click **Close**.

   After you set the home and farthest locations, custom devices are listed in the Device menu in the Sort Layout window.

   Note: Once custom devices are defined, you cannot change the number of rows and columns.
8. Click the **Voltage** button to turn off the deflection plates.
9. Proceed with Determining the drop delay – manual method (page 123) and Sorting (page 127).

Deleting a custom device

1. Select **Sort > Custom Devices**.
2. Select the name of the custom device to be deleted in the **Custom Devices** dialog.
3. Click **Delete**.

   The device is deleted from the Custom Device list, but is retained within any sort layouts where it was used.

Index sorting

Index sorting allows you to sort single 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.

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:

- Setting up for sorting (page 120)
- Setting up for sorting onto a plate or slide (page 132)

To perform an index sort:

1. Create a new experiment, using a global worksheet, for the index sort.

   Note: If you want to export data from each well into one CSV file, you must use a global worksheet.
2. Create a new global worksheet.
3. Create the plots and gates needed to define the populations of interest.
4. Create a new sort layout or select an existing sort layout defined for index sorting.
5. Make the following selections in the **Sort Layout** window.
   - Precision = Single cell (recommended setting)
   - Target events = 1
   - Index sorting checkbox = Selected

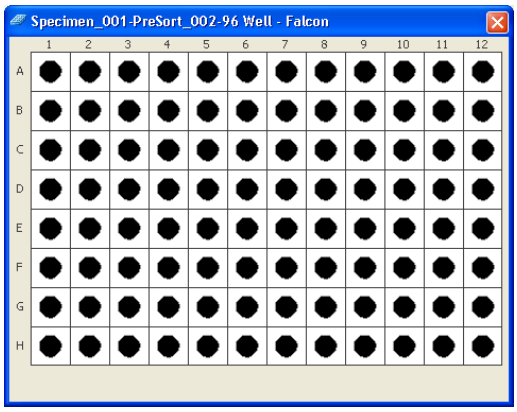
   Make the other selections in the **Sort Layout** window according to your experiment requirements.

6. Click the **Sort** button in the **Sort Layout** window to start the sort.

You must be acquiring sample to start a sort. A new tube is automatically added to the experiment. Upon completion, the new tube will contain the index sort data.

7. When the sort is finished, right-click the new tube in the experiment and select **Index Sorting Analysis**.

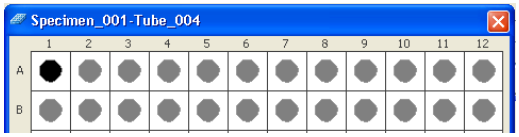
   The Index Sorting Analysis displays.



   The color legend for the wells is shown in the following table.

Color	Indication
Black	Sorted well data is showing in the plots
Gray	Sorted well data is available, but not showing in the plots
White	No sorted events
Red	More than one event sorted into a well

8. Select a well in the Index Sort Analysis to see the event sorted into that well displayed in a plot.



   After selecting an individual well, the other wells in the Index Sort Analysis turn to gray, indicating that they contain data, but are not being displayed.

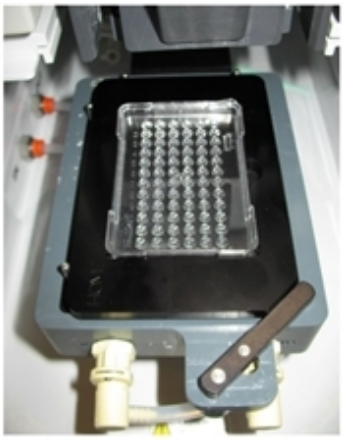
   You can select multiple wells by Shift+clicking or Ctrl+clicking. You can also drag over multiple wells in the Index Sort Analysis to display multiple wells.

9. To increase the size of a dot on a plot for better visibility, open the population hierarchy, select a population, navigate to the **Population Inspector**, and select a larger size.
10. To see the statistics for an index sort, right-click a plot and select **Create Statistics View**.
11. To export the statistics for an index sort as a CSV file, right-click in the statistics view and select **Export Index Sort Statistics**.
12. In the dialog that opens, navigate to the location where you want to save the CSV file and click **Save**.

Terasaki plate adapter

A Terasaki plate adapter is available as an option to enable the use of 60-well and 72-well Terasaki plates with the ACDU. A Terasaki plate is installed into the adapter, and then the plate/adapter assembly is loaded onto the ACDU.

Terasaki plate adapter showing location of set screws



Terasaki plate in adapter installed on ACDU

Using the adapter

The adapter has three set screws that enable the fit to be adjusted for the plates.

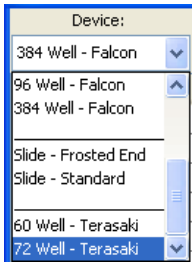
1. Install a Terasaki plate into the adapter with the A10 or A12 well at the bottom left corner of the adapter.
2. Tighten the set screws so the plate is held firmly and does not move within the adapter, but do not over-tighten because you need to be able to remove plates easily.

   The set screws should not have to be adjusted after the initial fitting process.

Using the correct device in the sort layout

During the sort layout setup process, you have to select the correct device in the Sort Layout window so the ACDU knows the layout and location of the wells on the Terasaki plate.

1. In the **Sort Layout** window, select the **Device** menu.
2. Navigate to the bottom of the list, then select the plate you are using (60-well or 72-well).



   The sort layout displays with the correct number of rows and columns.

7

Shutdown and maintenance

This chapter explains the routine maintenance procedures you should follow to keep your BD FACSymphony™ S6 cell sorter in good condition.

Shutdown and maintenance procedures are presented as follows:

Daily shutdown

In the BD FACSymphony™ S6 cell sorter system, the recommended daily shutdown procedure is to run the Clean Flow Cell command with the closed-loop nozzle installed (see the next section). This procedure fills the flow cell with cleaning solution. This is normally sufficient to keep the flow cell clean and operating properly.

If the system is used to process many different sample types, or the system has problems with contamination, you can perform a more extensive cleaning by running the Fluidics Shutdown command. See Fluidics Shutdown (Weekly or As Needed) (page 142).

In addition to one of these procedures, you should also perform an external cleaning. See External cleaning (page 144).

Cleaning the flow cell (daily)

Use the Clean Flow Cell command to run a tube of 1.5% solution of BD® Detergent Solution Concentrate through the sample line and flow cell.

Note: After the procedure is complete, the detergent solution remains in the flow cell until the stream is restarted. The solution should not be left in the flow cell for long periods. For shutdowns that will last more than a day, perform Fluidics Shutdown as described in Fluidics Shutdown (Weekly or As Needed) (page 142).

To clean the flow cell:

1. Turn off the stream.
2. Mix a 1.5% dilution of BD® Detergent Solution Concentrate (catalog number 660585) as described in the instructions provided with the detergent.

   Do not mix BD® Detergent Solution Concentrate with bleach because they produce chlorine gas.

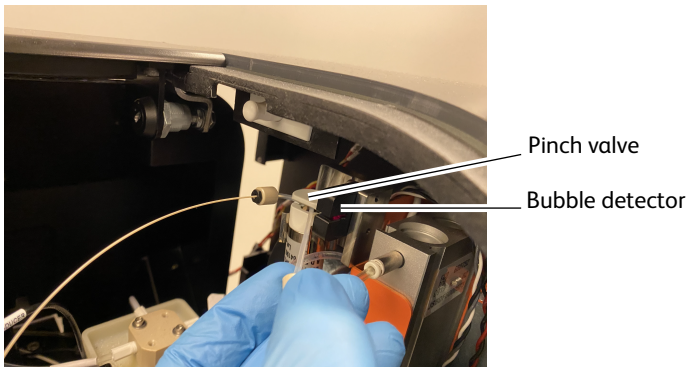
3. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
4. Remove the nozzle and install the integrated closed-loop nozzle.

   If you are using a standard closed-loop nozzle, verify that there is an O-ring in the nozzle before installing it.
5. Select **Cytometer > Cleaning Modes > Clean Flow Cell**.
6. When prompted, install a tube containing the 1.5% BD® Detergent Solution Concentrate, then click **OK**.

   The cytometer loads the tube and fills the flow cell with the detergent solution.
7. Click **OK** when the completion dialog opens.
8. Turn off the lasers using the laser buttons on the Laser Power Panel.
9. Turn off the cytometer main power.
10. Turn off the Temperature Control Option if it was used.
11. Exit BD FACSDiva™ software and shut down the computer.
12. Turn off the in-house air or the compressor.
13. Vent the air pressure from the sheath tank by pulling up on the vent ring.
14. If the cell sorter is installed in a BSC, leave the BSC blower on and close the view screen by lowering the sash to the lowest (ready safe mode) position.

Deep cleaning the flow cell (weekly or as needed)

1. Stop the stream.
2. Install the integrated closed loop nozzle.
3. Carefully pull the tubing out of the bubble detector, taking care not to pull it out of the sample pinch valve.



4. Select **Cytometer > Cleaning Modes > Clean Flow Cell**.
5. Mix a 1.5% dilution of BD® Detergent Solution Concentrate (catalog number 660585) as described in the instructions provided with the detergent.

   Do not mix BD® Detergent Solution Concentrate with bleach because they produce chlorine gas.

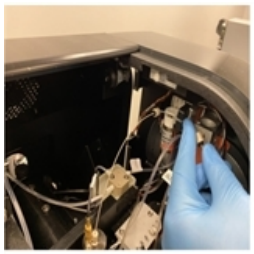
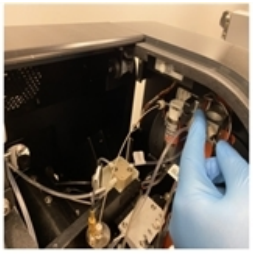
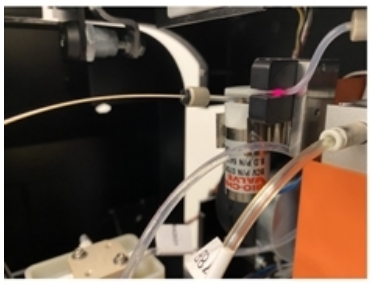
6. When prompted, install a tube with at least 4ml of 1.5% solution of BD® Detergent Solution Concentrate then click **OK**.

   Do not mix BD® Detergent Solution Concentrate with bleach because they produce chlorine gas.

   The cytometer loads the tube and fills the flow cell with the detergent solution.
7. When the completion dialog displays, click **OK**.
8. Select **Cytometer > Cleaning Modes > Clean Flow Cell**.
9. When prompted, install an empty tube, then click **OK**.

   The cytometer loads the tube, which will create bubbles that scrub the inside of the sample path and flow cell.
10. Repeat steps 4 to 9 two more times.

11. Carefully push the tubing back into the bubble detector. Make sure that the tubing is fully inserted. A cotton swab may be used to push it in.

12. Select **Cytometer > Cleaning Modes > Clean Flow Cell**.
13. When prompted, install an empty tube, then click **OK**.

    When the tube loads, the bubble detector should trip and LED should flash. If it does not, check tubing to be sure it is fully inserted and then repeat this procedure from step 12.
14. If the system will be shut down for more than 1 day, perform Fluidics Shutdown as described in Fluidics Shutdown (Weekly or As Needed) (page 142).

Fluidics Shutdown (Weekly or As Needed)

The Fluidics Shutdown command can be used to perform an extensive cleaning if the system is used to process many different sample types, or the system has problems with contamination. This procedure removes sheath fluid from the lines and fills them with 70% ethanol, and cleans the flow cell.

Preparing for shutdown

1. Unload the sample tube, if one is loaded.
2. Turn off the stream.
3. Check the waste container and empty it if needed.

   See Emptying the waste tank (page 97).
4. Check the ethanol shutdown tank and refill it if needed.

   See Refilling the ethanol shutdown tank (page 95).

Running fluidics shutdown

1. Select **Cytometer > Fluidics Shutdown**.

   The **Fluidics Shutdown** dialog opens.
2. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
3. Remove the nozzle from the flow cell assembly and click **Done**.

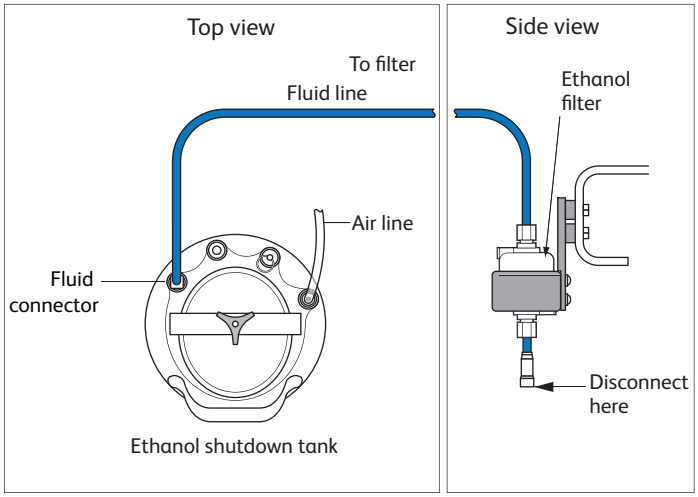
4. Insert the integrated closed-loop nozzle into the flow cell assembly and click **Done**.

   If you are using a standard closed-loop nozzle, verify that there is an O-ring in the nozzle before installing it.

5. Connect the air and fluid lines to the stainless steel ethanol shutdown tank.
   a. Disconnect the air line from the sheath tank and connect it to the air port on the ethanol shutdown tank.
   b. Disconnect the fluid line from the bottom side of the sheath filter and connect it to the ethanol filter on the ethanol shutdown tank.

      Remove the sheath filter and replace with the ethanol filter. Do not run ethanol through the sheath filter.
   c. Click **Done**.

   The system starts the cleaning process, and then displays a message at the bottom of the dialog.



6. When prompted, install a tube containing 3 mL of sterile, filtered DI water on the loading port, then click **Done**.

   The cytometer loads the tube and continues the cleaning process. A progress message is displayed, and then Done is displayed when the process is complete.
7. Click **OK** when you see a message informing you that the system can be turned off.
8. Turn off the in-house air or the compressor.
9. Vent the air pressure from the sheath tank by pulling up on the ring on the pressure relief valve.
10. Turn off the lasers by pressing the lighted buttons on the Laser Power Panel.
11. Turn off the cytometer main power.
12. Turn off the Temperature Control Option if it was used.
13. Exit BD FACSDiva™ software and shut down the computer.

14. If the cell sorter is installed in a BSC, leave the BSC blower on and close the view screen by lowering the sash to the lowest (ready safe mode) position.

External cleaning

To keep the system free from salt buildup, wipe down all cytometer surfaces that have been exposed to sheath fluid. Clean surfaces with a cloth dampened with a 10% bleach solution, followed by DI water.

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.

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

The following surfaces should be inspected and cleaned when necessary:

- Inside the sort chamber
- Deflection plates
- Sample loading port
- Collection devices

Scheduled maintenance

For optimal cytometer functioning, perform the following procedures according to the recommended maintenance schedule.

Procedure	Recommended Frequency
Internal cleaning (page 144)	See the table in the following section.
Purging the fluid filters (page 148)	Weekly
Purging the sheath filter (page 148)	Weekly
Changing the disposable waste cap (see Emptying the waste tank (page 97))	Monthly
Changing the fluid filters (page 148)	Every 6 months
Changing the sheath/ethanol filter (page 149)	Every 6 months
Changing the sample lines (page 150)	Every 4–6 months, or as needed
Changing the air filter (page 154)	Every 6–12 months, depending on cytometer use and the quality of the air
Changing the Sheath Tank Air Filter (page 154)	Every 6 months

Internal cleaning

BD FACSDiva™ software includes four pre-programmed cleaning modes that can be used alone or in combination to provide the required level of cleaning. The following sections describe the different cleaning

modes.

Cleaning Mode	Summary	Frequency
Sample line backflush (page 145)	Flushes the sample line with sheath fluid.	After running samples with adherent cells or dye.
Cleaning the flow cell (daily) (page 140)	Cleans the sample path and the flow cell with DI water.	When indicated by distorted scatter or high CVs, or as a daily shutdown procedure.
Prime after tank refill (page 145)	Primes the fluid lines for the designated fluid(s).	When a fluidics line is unplugged to refill a tank.
Prepare for aseptic sort (page 146)	Decontaminates the complete sheath path and sample path with bleach, DI water, and ethanol.	When needed before aseptic sorting.

Sample line backflush

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. Use the Sample Line Backflush command to perform additional backflushing of the inside of the sample line after a tube is unloaded. Perform the sample line backflush when you observe sample carryover or after running samples with adherent cells or dye.

Note: Keep the stream running while performing the backflush.

1. Select **Cytometer > Cleaning Modes > Sample Line Backflush**.
2. Click **Start** to start the backflush.
3. Click **Stop** to stop the backflush, or click **Cancel** to stop the backflush and close the dialog.

   The backflush does not stop automatically.

Prime after tank refill

Use the Prime After Tank Refill command to prime the fluid lines if a 5-L plastic fluidics container was disconnected for refilling.

1. Turn off the stream.
2. Select **Cytometer > Cleaning Modes > Prime After Tank Refill**.
3. Select the checkboxes for the tanks that were refilled, then click **OK**.

   The cytometer proceeds with priming the specified tanks. A progress dialog opens while the tanks are being primed.
4. Click **OK** when the tank prime is complete.

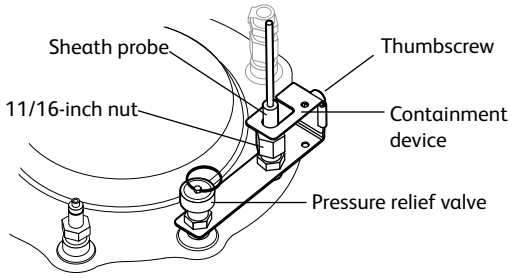
Removing the sheath probe

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

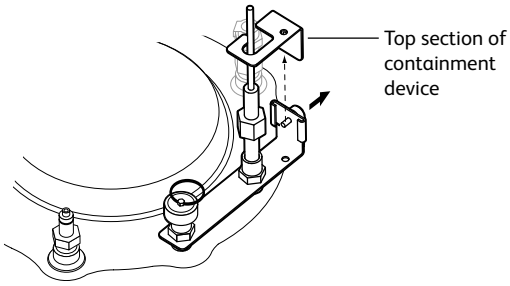
Note: Do not autoclave the sheath probe. It is not designed to withstand the conditions of autoclaving.

Make sure to perform the following steps in sequence, so the containment device works properly.

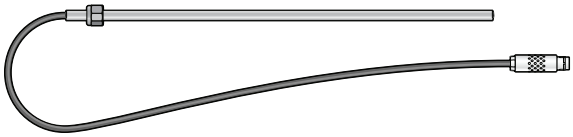
1. Disconnect the air line from the sheath tank.
2. Vent the air pressure from the sheath tank by pulling up on the pressure relief valve. Verify that all of the pressure is released by pulling up a second time.
3. Loosen the nut at the top of the probe with a wrench.



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



6. 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.
7. Decontaminate the sheath probe using 70% ethanol.



Prepare for aseptic sort

Use the Prepare for Aseptic Sort command when you want to decontaminate the entire sheath path. This procedure cleans the system with bleach, DI water, and ethanol.

Perform the following steps before starting the Prepare for Aseptic Sort command:

1. Verify that the pressure has been vented from the sheath tank and the sheath probe has been removed. See the preceding section.
2. Disconnect the fluid and air lines from the sheath tank.
3. Empty the sheath tank and rinse it with DI water.
4. Autoclave the sheath tank at 125 °C and 15 psig for 30 minutes with a 7.5-minute warmup and shutdown cycle.
5. Fill the sterilized sheath tank with sterile sheath fluid.
6. Obtain a new sheath filter to replace the old filter when instructed to do so in the wizard. See Changing the sheath/ethanol filter (page 149).
7. Install the decontaminated sheath probe into the sheath tank and tighten the nut securely with a wrench.

8. Install the top section of the containment device into the bottom section, and then tighten the thumbscrew.
9. Sterilize the DI water sensor by soaking in a 10% bleach solution for 10 minutes.
10. Fill the DI water container with sterile DI water and 3 mL of bleach per liter of DI water, and then reinstall the sterilized DI water sensor.

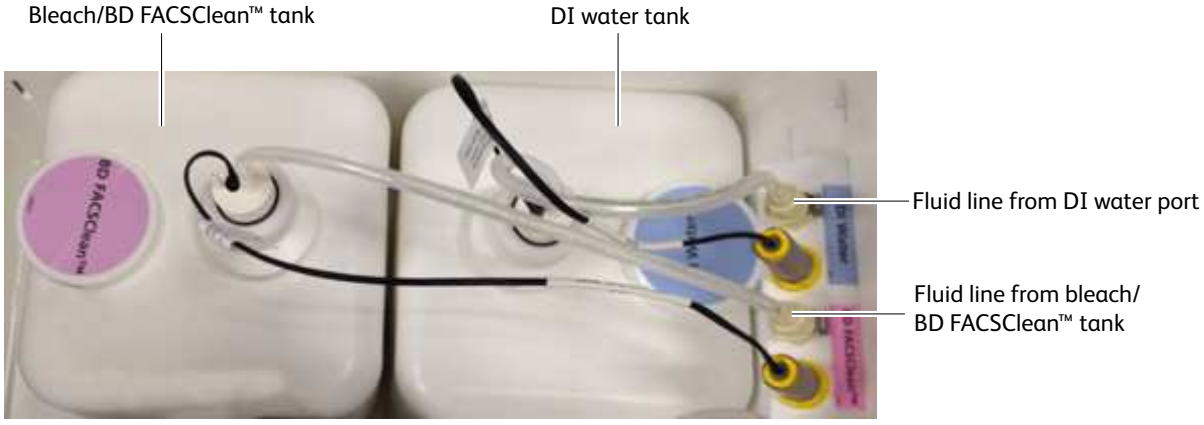
To run the Prepare for Aseptic Sort command:

1. Select **Cytometer > Cleaning Modes > Prepare for Aseptic Sort**.

   Follow the instructions on screen as you perform the procedure.
2. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
3. Install the integrated closed-loop nozzle in the flow cell and click **Done**.

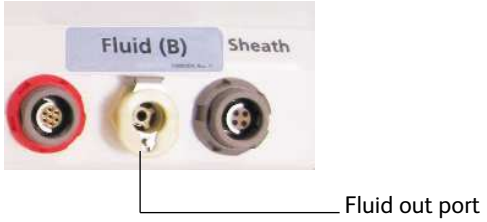
   If you are using a standard closed-loop nozzle, verify that there is an O-ring in the nozzle before installing it.
4. Remove the fluid line from the DI water port, connect the fluid line from the bleach/BD FACSClean™ container to the DI water port, then click **Done**.

   Do not disconnect the sensors from either container.



5. Disconnect the bleach/BD FACSClean™ fluid line from the DI water port, and connect it back to the bleach container port, then reconnect the DI fluid line to the DI water port, and click **Done**.
6. Disconnect the sheath fluid line from the sheath filter (at the output side) and connect it to the fluid out port on the connectors panel on the side of the fluidics drawer. Click **Done**.

   The system cleaning takes approximately 20 minutes.



7. Disconnect the fluid line from the fluid out port on the side of the fluidics drawer and connect it to a new 0.2-µm sheath filter. See Changing the sheath/ethanol filter (page 149).
8. Remove the old sheath filter and connect the new 0.2-µm sheath filter to the liquid port of the sterilized sheath tank.
9. Reconnect the fluid and air lines to the sheath tank.
10. To complete the process, select one of the two options.

- To continue running samples, perform fluidics startup.
- To turn off the system, perform the flow cell cleaning procedure.

Purging the fluid filters

Once a week, purge air from the fluid filters for the 5-L plastic containers by opening the bleeder valve on the top of each filter. This ensures that the filters will not dry out.

1. Open the bleeder valve a small amount and leave it open until fluid seeps out through the valve.

   Note: If the fluid does not seep, close the valve, prime the fluid lines, and try again. It might take a few minutes for the air to purge.
2. Close the valve.
3. Wipe up any excess fluid that might have dripped into the fluidics drawer.

Purging the sheath filter

Once a week, purge air from the sheath filter by opening the bleeder valve on the top of the filter. The sheath tank is pressurized, so do this task carefully to avoid spraying sheath fluid on any equipment.

1. Place a small container under the bleeder valve to catch any fluid.
2. Slowly open the bleeder valve a small amount and leave it open until fluid seeps out through the valve.
3. Close the valve.
4. Wipe up any excess fluid that might have dripped into the fluidics drawer.

Changing the fluid filters

We recommend changing the fluid filters every six months. Spare filters are included with the accessory kit.

1. Remove the filter by pressing the tabs on each quick-disconnect coupling.
2. Install the new filter with the flow arrow point down and connect the quick-disconnect couplings.
3. Write the current date on the new filter so you will know when to replace it.
4. Open the bleeder valve on top of the filter a small amount and leave it open until fluid seeps out through the valve.
5. Close the valve.
6. Wipe up any excess fluid that might have dripped into the fluidics drawer.

Changing the sheath/ethanol filter

Introduction

The sheath/ethanol filter can be used to filter either sheath fluid or ethanol fluid. You need to change the 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, or an ethanol filter. After the filter is installed for one of these purposes, however, you cannot use it for a different purpose. Because of this, you should label each filter so that you know which one to use for each purpose.

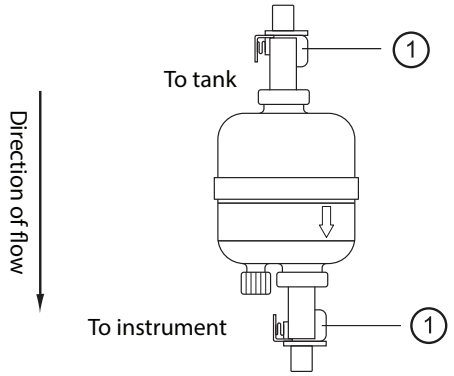
When you use the filter as a sheath filter, you should change it every six months, or when increased debris in an FSC vs SSC plot indicates that the sheath filter needs to be replaced.

Spare filters are included with the accessory kit.

Procedure

To change the sheath/ethanol filter:

1. Turn off the stream.
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 the pressing the metal tabs on each end.



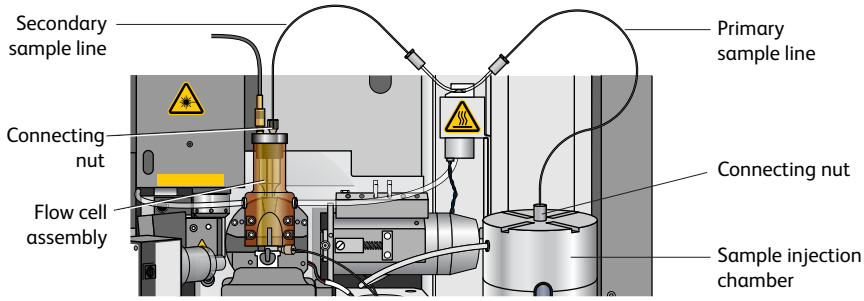
Item	Description
1	Tabs

5. Write the current date on the filter so that you will know when to replace it.
6. 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.
7. Reconnect the air line and check for leaks when the pressure is turned on.

Changing the sample lines

The primary sample line between the sample injection chamber and the pinch valve should be changed every 4–6 months or when decreased event rates indicate that the sample line might be clogged. The secondary sample line between the pinch valve and the cuvette flow cell needs changing only when it is kinked or clogged.

As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.



To withstand the high pressures generated by the BD FACSymphony™ S6 cell sorter flow cytometer, the sample lines are attached using a two-piece compression fitting, in which a cone-shaped ferrule is compressed onto the tubing as the connecting nut is tightened.

To replace the tubing, you will need a 12-inch length of replacement tubing for the primary sample line, or a 7-inch length for the secondary line. Replacement tubing is supplied in the accessory kit. The ferrules and connecting nuts can be reused when the tubing is replaced.

All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle used tubing and fittings as if capable of transmitting infection. Wear suitable protective clothing, eyewear, and gloves.

Changing the primary sample line

To replace the primary sample line, you will need a 12-inch length of replacement tubing from the accessory kit. There is a different fitting at each end of the tubing, so the procedure is divided into two sections.

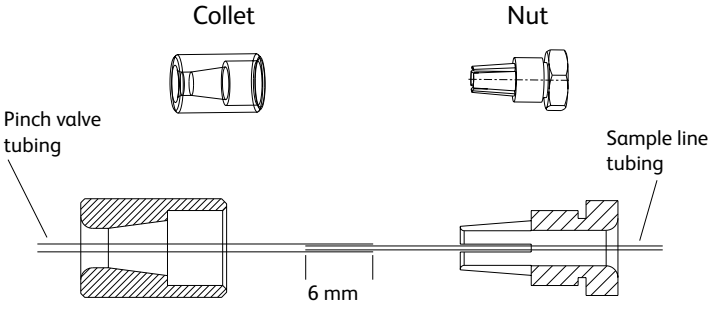
Assembling the collet fitting at the pinch valve

At the pinch valve end of the primary sample line, a collet fitting joins the sample line to the pinch valve tubing.

To replace the tubing:

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Turn the stream off (if needed). Make sure the loading port is in the unload position.
3. Unscrew the nut from the collet fitting and pull the nut and collet apart.
4. Pull the pinch valve tubing out of the nut, and then pull the sample line out of the pinch valve tubing.
5. Locate a new 12-inch piece of sample line tubing.

a. Slide the collet over the pinch valve tubing, then slide the nut over the new sample line tubing.



b. Slide the pinch valve tubing over one end of the sample line tubing until approximately 6 mm of the sample line is inside the pinch valve tubing.

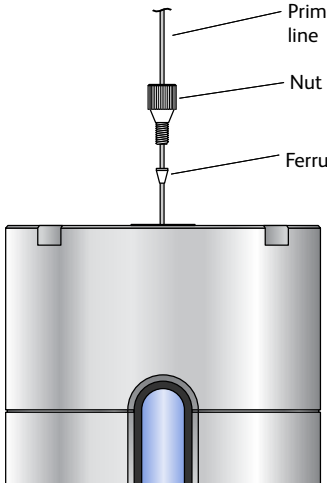
c. Slide the pinch valve tubing on the inside of the teeth of the nut until it stops.

Ensure that the pinch valve tubing is inserted inside the teeth of the nut. Failure to do so can cause the bubble detector to malfunction.

d. Couple both pieces of the fitting together and then tighten until finger-tight.

Primary sample line – sample injection end

At the sample injection end of the primary sample line, a compression fitting secures the sample line at the top of the sample injection chamber.



1. Turn the stream off (if needed). Make sure the loading port is in the unload position.
2. If there is a sample line filter installed, remove it by pulling it off the sample line.

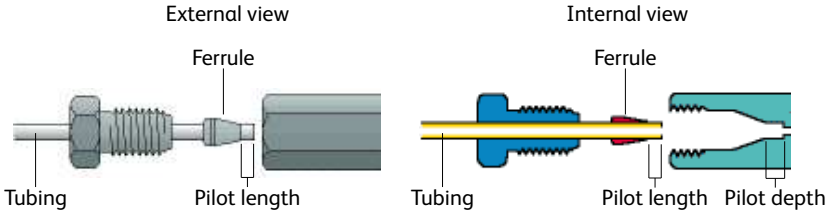
   To gain access to the filter, see Installing or removing a sample line filter (page 159).
3. Unscrew the connecting nut at the top of the sample injection chamber and slowly pull out the sample line.
4. Ensure that a cone-shaped ferrule is attached to the sample line.

   If the ferrule was left behind in the injection chamber fitting, gently push the tip of the ferrule-removal tool (included in a small vial inside the accessory kit) into the top of the ferrule and pull the ferrule straight out.

   After using the tool, you might need to replace the ferrule. If the ferrule is damaged, replace it with a spare (included in the accessory kit).

5. Slide the ferrule and nut off the end of the sample line.
6. Slide the nut and then the ferrule onto the end of the new sample tubing.

   Leave approximately 5 inches (12.7 cm) of tubing extending out of the sample injection chamber end. (This length can be adjusted depending on the depth of your sample tube.) This length is referred to as the pilot.



7. Insert the pilot tubing into its fitting, ensuring that the tubing reaches the intended pilot depth.

   Do not to bend the primary sample line during insertion.

   Insert the sample line into the sample injection chamber fitting. Push the tubing from the top until it is slightly above the bottom of the chamber viewing window. Finger-tighten the nut on top of the chamber so the sample line is secure.

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

8. Check the fitting connections at both ends to make sure they are not leaking.

   Turn on the stream, load a tube of DI water, and make sure none of the fittings are leaking. If needed, unload the tube, turn off the stream, and tighten the fittings. After tightening, if leaking still occurs, replace the ferrule.

   Make sure all fittings are securely tightened. If any fitting is loose, the tubing could detach during high-pressure operation, exposing the operator to potentially biohazardous sample spray.

9. Verify the length of the sample line.

   The sample line should 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.

   See Installing or removing a sample line filter (page 159) for detailed instructions on verifying sample line length. Disregard any steps that do not apply.

Changing the secondary sample line

This section describes changing the secondary sample line. There is a different fitting at each end of the tubing, so the procedure is divided into two sections.

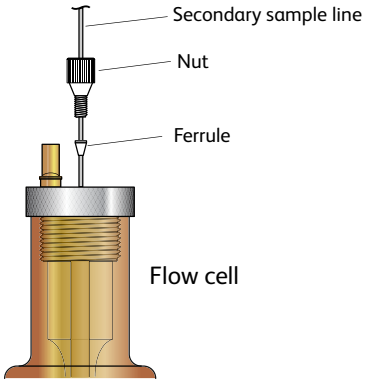
As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.

Pinch valve end

The procedure to replace the secondary sample line at the pinch valve end is the same as for the primary sample line, except the replacement line is a 7-inch length. See Assembling the collet fitting at the pinch valve (page 150).

Flow cell end

1. Turn the stream off.
2. Unscrew the connecting nut at the top of the flow cell and slowly pull out the sample line.



3. Ensure that a cone-shaped ferrule is attached to the sample line.

   If the ferrule was left behind in the flow cell fitting, gently push the tip of the ferrule-removal tool (included in the accessory kit) into the top of the ferrule and pull the ferrule straight out.

   After using the tool, you might need to replace the ferrule. If the ferrule is damaged, replace it with a spare (included in the accessory kit).
4. Slide the ferrule and nut off the end of the sample line.
5. Slide the nut and then the ferrule onto the end of the new sample tubing.

   Leave approximately 0.1 inch (0.25 cm) of tubing extending out of the ferrule. This length is referred to as the pilot.
6. Insert the pilot tubing into its fitting at the top of the flow cell, ensuring that the tubing reaches the intended pilot depth.

   Within the cuvette flow cell fitting, make sure 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.
7. Finger-tighten the nut at the top of the flow cell to secure the sample line.

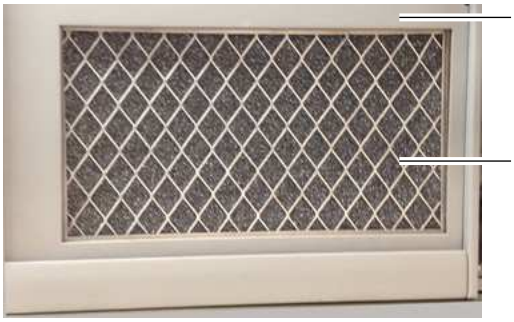
   Do not overtighten the nut and do not use tools. Over-tightening the nut can kink or damage the tubing.
8. Check the fitting connections at both ends to make sure they are not leaking.

   Turn on the stream, load a tube of DI water, and make sure none of the fittings are leaking. If needed, unload the tube, turn off the stream, and tighten the fittings. After tightening, if leaking still occurs, replace the ferrule.

   Make sure all fittings are securely tightened. If any fitting is loose, the tubing could detach during high-pressure operation, exposing the operator to potentially biohazardous sample spray.

Changing the air filter

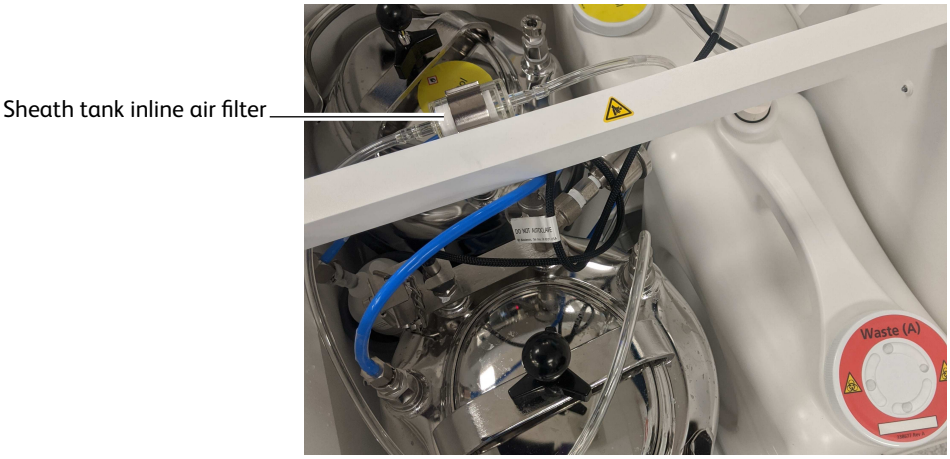
The BD FACSymphony™ S6 cell sorter has an air filter in the sort collection chamber door.



To change the filter in the sort collection chamber door, slide out the old filter and slide in the new one. To slide out the old filter, release the locking screw behind the door. See Replacing the air filter (page 199) for more information.

Changing the Sheath Tank Air Filter

Check the inline air filter on the sheath tank air line periodically for any signs of debris or discoloration. Replace with a new air filter from the accessory kit every six months, or sooner if needed.



1. Turn off the cytometer.
2. Pull the tubing off each end of the air filter.
3. Install a new filter with the directional arrow pointing toward the sheath tank.

Unscheduled maintenance

There are several cytometer components that should be cleaned periodically or checked for wear and replaced if necessary. See the indicated sections for the following maintenance procedures.

Procedure	Recommended Frequency
Changing the integrated nozzle (page 155)	As needed for different sized particles
Cleaning the integrated nozzle (page 156)	When stream irregularities indicate that the nozzle is clogged
Temporary replacement of a seal (page 158)	As needed when seal is lost or damaged in an integrated nozzle
Closed-Loop Nozzle Maintenance (page 158)	As needed
Installing or removing a sample line filter (page 159)	When the sample line filter needs to be installed or changed
Changing the pinch valve tubing (page 161)	As needed
Cleaning the camera windows (page 163)	When smudges appear in the Breakoff or Side Stream windows
Removing the deflection plates (page 165)	As needed to clean the deflection plates
Lubricating the sample injection chamber O-ring (page 166)	As needed when the O-ring is dry
Using custom optical filters (page 167)	As needed
Cleaning the optical filters (page 168)	As needed when changing a filter
Removing or installing the FSC ND filter (page 168)	As needed

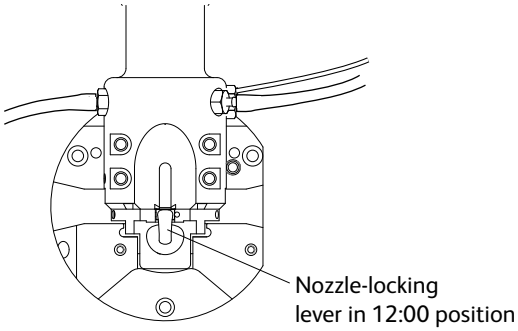
Changing the integrated nozzle

Three sizes of nozzles are provided with your cytometer: 70, 85, and 100 µm. A 130-µm nozzle can be ordered as an option. The size is marked on the nozzle. The closed-loop nozzle used for cleaning and shutdown procedures is also changed with this procedure.

Any cytometer 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.

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Turn off the stream and open the flow cell access door.

3. Turn the nozzle-locking lever counterclockwise to the 9:00 position, and pull the nozzle out of the cuvette flow cell.



4. Insert the new nozzle into the flow cell (with the top side facing up) and push it gently all the way forward until it stops.
5. Turn the nozzle-locking lever clockwise to the 12:00 position.
6. Turn on the stream and make sure it flows smoothly from the nozzle into the center of the waste aspirator.

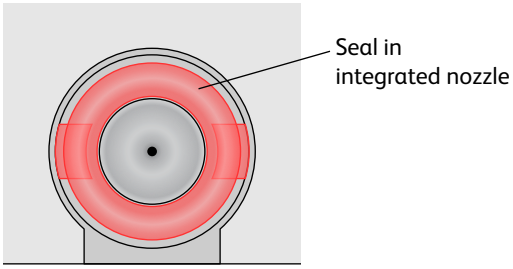
   If the stream is flowing but the breakoff is too long or the gap is unsteady, this could indicate that there are bubbles in the flow cell. If these conditions occur, turn off the stream, wait for 10 seconds, and turn on the stream again.

   If you see any dripping or spraying, or the stream image appears abnormal, turn off the stream and see Troubleshooting the stream (page 170).

Note: After changing the nozzle, you might need to adjust the angle of the sort block to re-center the stream in the aspirator. To do so, loosen the adjustment screws on both sides of the deflection plates and rotate the sort block. Tighten the screws when the stream is centered in the aspirator. For further assistance, see Troubleshooting the stream (page 170).

Cleaning the integrated nozzle

Use the following procedure to clean the nozzle when the stream appears blocked or distorted. To verify that the nozzle is clogged, examine the opening at the center of the seal area under a microscope.



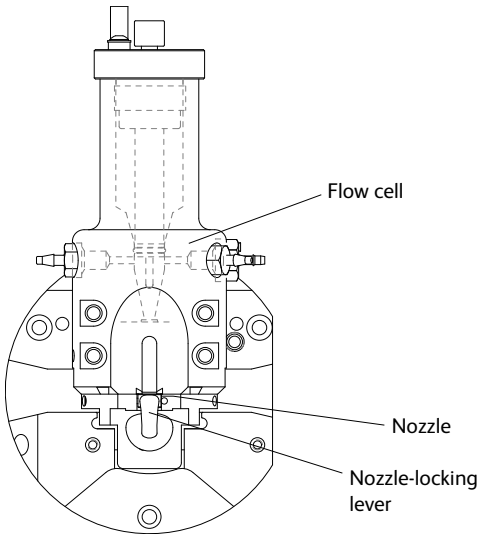
All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle nozzles as if capable of transmitting infection. Wear suitable protective clothing, eyewear, and gloves.

To clean the integrated nozzle:

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Remove the nozzle from the flow cell by turning the nozzle-locking lever counterclockwise to the 6:00 position, then pull the nozzle straight out.
3. Sonicate the nozzle for approximately 1 minute in a test tube containing DI water. Repeat the sonication until the nozzle is clean.

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

4. Allow the nozzle to air dry for a few minutes. Do not wipe the nozzle with anything, because it could leave fibers or other contamination.
5. Insert the nozzle into the flow cell with top side facing up and push it gently forward until it stops.
6. Turn the nozzle-locking lever clockwise to the 12:00 position.
7. Turn on the stream and make sure it flows through the nozzle properly.



Note: After re-installing the nozzle, you might need to change the angle of the sort block to re-center the stream in the aspirator. To do so, loosen the adjustment screws on both sides of the deflection plates and rotate the sort block. Tighten the screws when the stream is centered in the aspirator. For further assistance, see Troubleshooting the stream (page 170).

Handling the integrated nozzle

In addition to following proper cleaning instructions, follow these precautions when handling the integrated nozzles.

Precaution	Result
Always use the integrated closed-loop nozzle for cleaning and shutdown procedures.	Keeps the flow cell clean and reduces the chances for clogs. A clean flow cell provides improved sensitivity and higher performance.
Do not expose integrated nozzles for long periods of time to bleach or detergents. However, you can prepare for the aseptic sort procedure without causing any problems to the O-ring in the integrated nozzle.	Prevents the seal from coming loose and falling out.
Do not expose integrated nozzles to strong base solutions such as Contrad 70.	Prevents the seal from coming loose and falling out. Any contact with such solutions might damage the seal.
Do not wipe the surface of the seal with anything.	Prevents damage to the seal that could result in leaking.

Temporary replacement of a seal

The standard O-ring can be used as a short-term replacement in the integrated nozzle, if the original seal has been lost or damaged, but a new integrated nozzle will provide better long-term usability. See Accessory kit (page 184) for part numbers for ordering integrated nozzles.

Standard O-rings (part number 333084) are supplied in the accessory kit. The following procedure describes how to install a standard O-ring in an integrated nozzle.

1. Make sure the groove in the nozzle is clean.

   If the any part of the seal is still in the nozzle groove, sonicate the nozzle in a bleach solution until the seal comes out. Rinse the nozzle in DI water after sonicating.
2. Use the wooden end of a cotton swab, or similar tool, to install the O-ring in the nozzle groove, then allow the nozzle to air dry for a few minutes.

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

Closed-Loop Nozzle Maintenance

The closed-loop nozzle and related tubing should be cleaned if there are any indications of clogging or kinked tubing.

Cleaning the integrated closed-loop nozzle

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Turn off the stream and open the flow cell access door.

3. Remove the closed-loop nozzle from the cuvette flow cell.

   Turn the nozzle-locking lever counterclockwise to the 6:00 position. Remove the nozzle by pulling it straight out.

4. Unscrew the nut on the side of the nozzle to remove the tubing.

   Make sure that the ferrule stays on the tubing as you remove it.

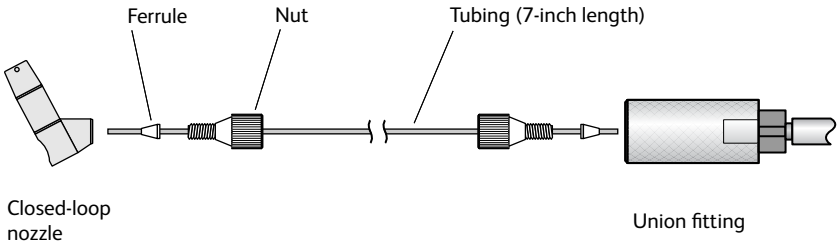
5. Sonicate the nozzle for approximately 1 minute.

   Sonicate the nozzle in a test tube containing DI water or a mild detergent. Repeat the sonication as needed until the nozzle is clean.

6. Make sure that the ferrule is on the tubing, then screw the nut back into the hole in the side of the closed-loop nozzle.

Replacing the tubing on the closed-loop nozzle

To replace the tubing on the closed-loop nozzle, use this procedure. Use a 7-inch length of sample tubing from the accessory kit as the replacement.



To replace the tubing:

1. Unscrew the nut from the closed-loop nozzle and from the union fitting and pull out the tubing from both places.

   Make sure that the ferrule comes out on the tubing on both ends. If not, use the ferrule tool to remove it.

2. Slide the nut and ferrule off each end of the tubing.
3. Slide the nut and ferrule onto the new tubing.

   Insert the tubing into the closed-loop nozzle and slowly tighten the nut until secure. Do not over-tighten. Make sure that the tubing is pushed all the way in while tightening the nut.

4. Insert the tubing into the union fitting and slowly tighten the nut until secure. Do not over-tighten. Pull gently on the tubing to ensure that it is secure.

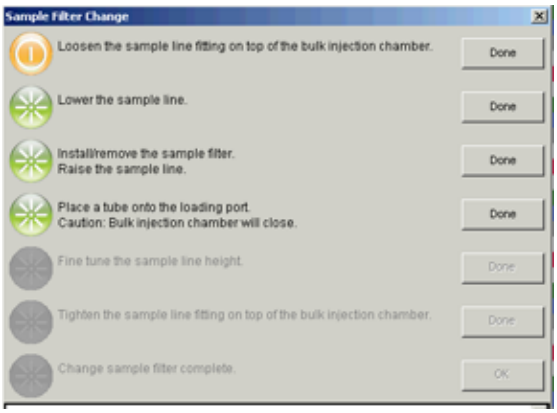
Installing or removing a sample line filter

Sample filters can be installed on the end of the sample line to filter out large particles from a sample. Pre-filtering the sample before beginning any sorting is recommended.

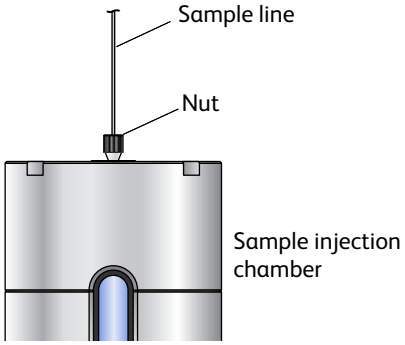
Two sizes of sample line filters are included in the accessories kit: 35 micron (green) and 50 micron (blue). The filters can be changed as often as required. The sample filters are not intended for use with the 1-mL microtubes.

Note: When a sample filter is installed, the sample flow rate can be slowed down due to the effect of particles in the sample fluid clogging the filter.

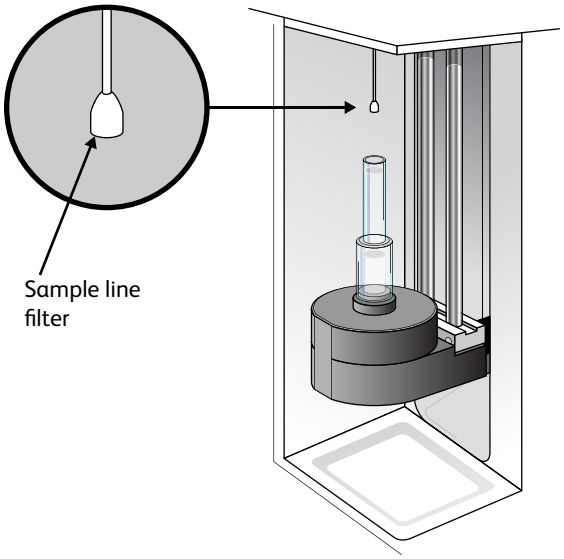
1. With the stream turned on, select **Change Sample Filter** from the **Cytometer** menu.
2. A wizard opens with the instructions.



3. Loosen the sample line fitting nut at the top of the injection chamber to allow the sample line to slide freely through the fitting.



4. Push the sample line down so the end is below the bottom of the sample injection chamber.



5. Install the sample line filter by sliding it onto the end of the line, then click **Done**.

   Do not bend the sample line while installing the filter.
6. Pull the sample line up to operation height, slightly above the chamber viewing window.
7. Place a tube onto the loading port.

   Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. Keep your hands and clothing away from the loading port when a tube is loading or unloading. Do not place objects underneath the loading port.
8. Click **Done** to raise the sample injection chamber.

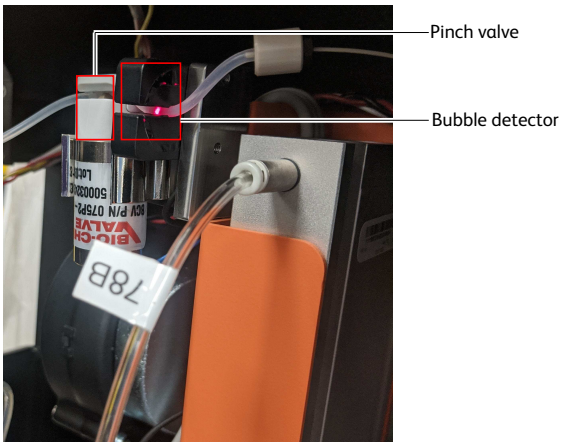
   Be careful of a pinching hazard as the sample injection chamber is raised.

   The sample line should not bow or bend when a tube is loaded.
9. Adjust the sample line height if needed.
10. Tighten the sample line fitting and click **Done**.

    The bulk injection chamber is lowered and the sample purge mode is turned on for about 5 seconds until several drips exit the filter. A message is displayed at the bottom of the wizard.
11. Click **Done** to complete the process.

Changing the pinch valve tubing

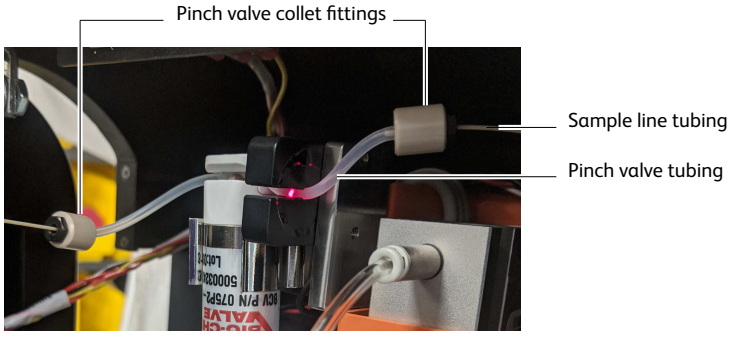
The tubing that runs through the bubble detector and the pinch valve should be changed as needed. The system ships with replacement tubing cut into 3-inch lengths, found in the accessory kit.



To replace the tubing:

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Turn off the stream. Make sure the loading port is in the unload position.
3. Pull the existing pinch valve tubing out of the slot in the pinch valve and the bubble detector.

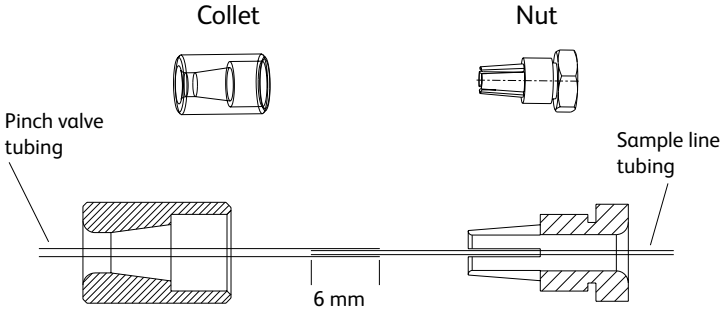
   Grasp the tubing with two fingers next to the pinch valve and two fingers next to the bubble detector. Pull the tubing straight out from the slots. The tubing pops out with a small amount of pulling action.



4. Unscrew the nut on the black collet fitting at each end of the tubing, and pull the pinch valve tubing out of both fittings.

   Leave both pieces of the fitting on the sample line tubing.
5. Install a new 3-inch piece of pinch valve tubing.
   a. Slip the collet over one end of the new pinch valve tubing.
   b. Slide the pinch valve tubing over the sample line tubing until approximately 6 mm of the sample line is inside the pinch valve tubing.
   c. Slide the pinch valve tubing on the inside of the teeth of the nut until it stops.
   d. Couple both pieces of the fitting together and then tighten until finger-tight.
   e. Repeat steps a through d at the other end of the tubing.

f. Check to see that both ends of the new pinch valve tubing are held securely in the compression fittings.



6. Install the new pinch valve tubing into the slots in the pinch valve and bubble detector.

   Make sure that the tubing goes all the way into the back of each slot. The sample line can drip if the pinch valve tubing is not all the way into the slot in the pinch valve and the bubble detector.

Cleaning the camera windows

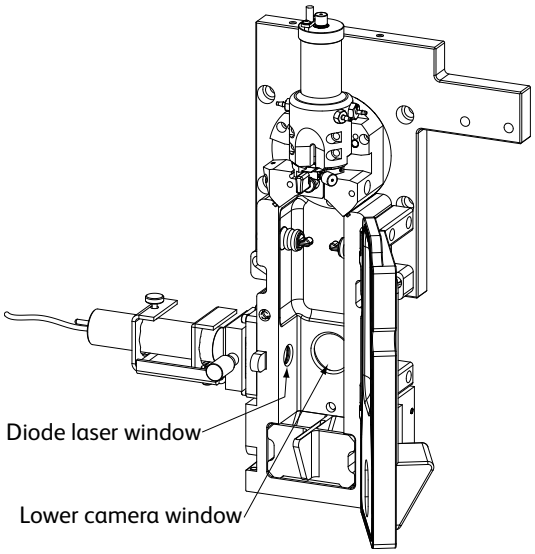
Lower camera window

Clean the lower camera window and the diode laser window when you have trouble viewing the side streams or you cannot set the drop delay using Accudrop.

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

To clean the lower camera window and the diode laser window:

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Ensure that the deflection plates are turned off (warning light is not illuminated).
3. Turn off the stream.
4. Open the sort block door.
5. Wipe the windows with a soft, lint-free cloth soaked with DI water, and then dry the windows.



Upper camera window

Clean the strobe lens and upper camera window when smudges appear in the processed (digitized) image in the Breakoff window, or when dark spots appear to interfere with Sweet Spot monitoring. You might need to clean these components after a clog, or after sheath fluid has leaked or sprayed. Follow the steps in this section to clean the strobe lens and upper camera window.

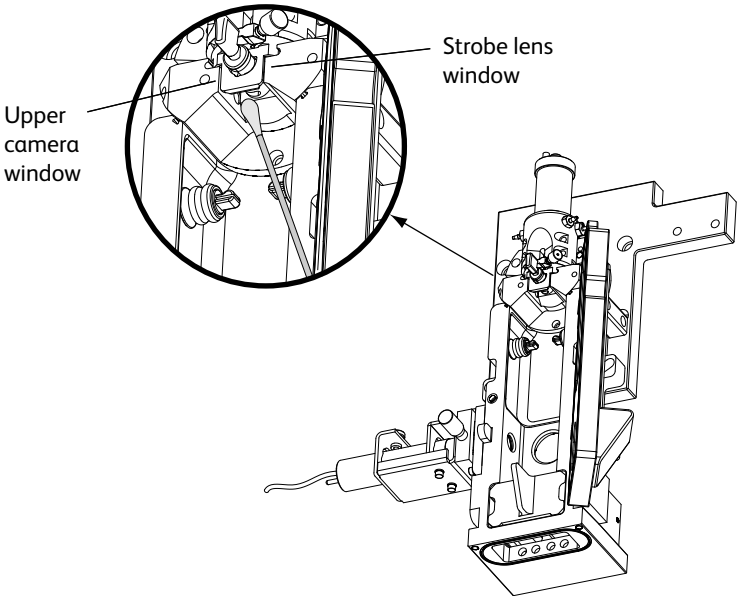
A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the plate voltage is on. The plates remain energized even when the sort block door is open.

To clean the strobe lens and upper camera window:

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Ensure that the deflection plates are turned off (warning light is not illuminated).
3. Turn off the stream.
4. Open the sort block door.
5. Place 1–2 drops of DI water or ethanol on a cotton swab.
6. Click the **Breakoff** window and select **Raw Image**.

   Visible smudges are more apparent in the raw image view.
7. While viewing the image in the **Breakoff** window, insert the swab just below the bottom of the flow cell.

   The strobe lens and upper camera windows are located behind two circular openings on either side of the top of the sort chamber. You will see the end of the swab in the Breakoff window when you intercept either opening.



8. Gently wipe the upper camera window, and then the strobe lens (opposite the window) to remove any saline.
9. Repeat with isopropyl alcohol or 70% ethanol until clean.

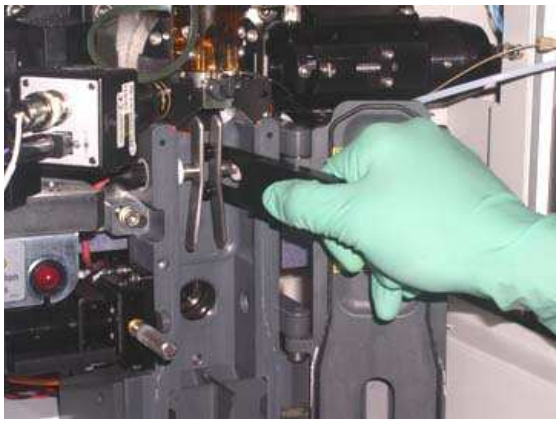
Removing the deflection plates

You can remove the deflection plates for cleaning by pulling the plates out using the deflection plate removal tool, supplied in the accessory kit.

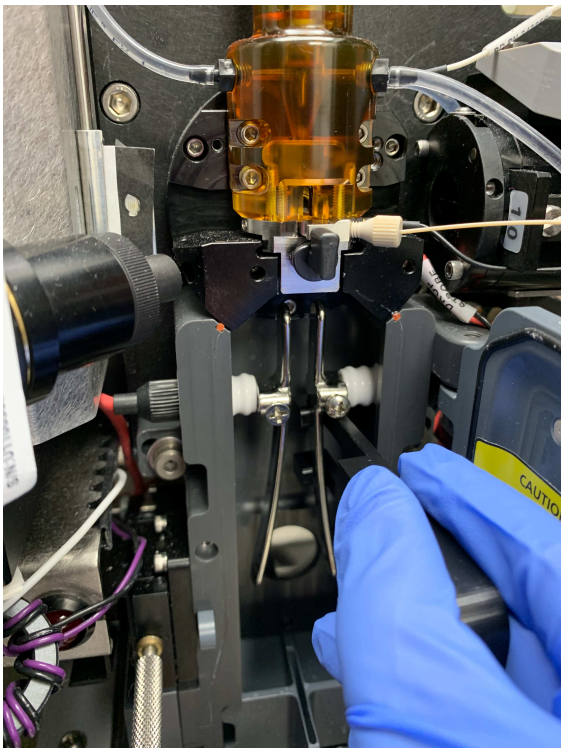
1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Make sure that the deflection plates are turned off.
3. Open the sort block door.
4. Slide the deflection plate removal tool behind one of the plates and pull straight out from the sort block.

   Hold your thumb on the plate (or use your other hand) as you pull it out so it does not fall as you remove it.

2.5-inch deflection plates



3.5-inch deflection plates

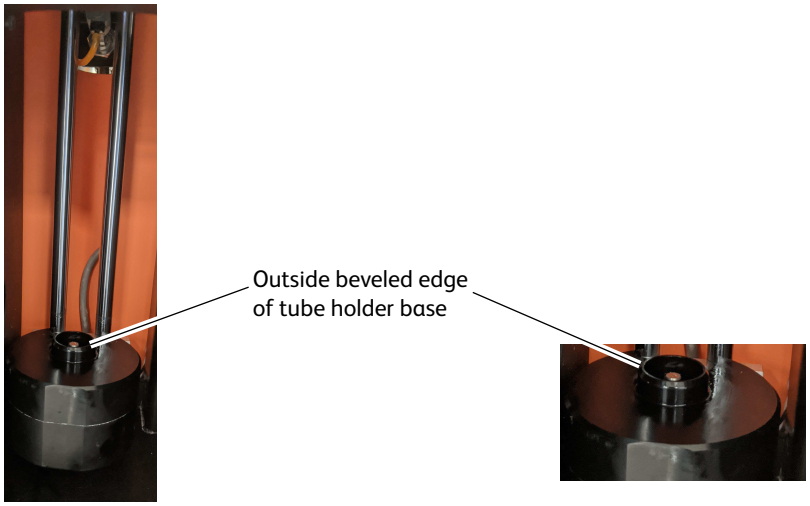


Lubricating the sample injection chamber O-ring

The O-ring at the bottom of the sample injection chamber should be lubricated as needed to maintain proper operation.

1. Verify that the loading port is in the down position, and open the hinged cover.
2. Check the O-ring (located at the opening in the bottom of the chamber) to see if it is dry.
3. If the O-ring is dry, it must be lubricated with O-ring lubricant from the accessory kit.
   a. Remove the clear plastic tube holder from the tube holder base.
   b. Apply a small amount of O-ring lubricant to the outside beveled edge of the tube holder base. This is where the base contacts the O-ring inside the sample injection chamber.
   c. Wipe off any excess lubricant.

d. Replace the tube holder on the base.



Using custom optical filters

If you want to install a custom filter or dichroic, the filter should comply with the following specifications.

Filter Characteristic	Dichroic LP Filters	BP Filters	Glass Filters
Diameter	0.492 in. (12.5 mm)	0.492 in. (12.5 mm)	0.492 in. (12.5 mm)
Thickness	0.16 ±0.005 in. (0.408 ± 0.127 mm)	0.079 in. (2.00 mm)	0.098 in. (2.5 mm)
Minimum clear aperture	0.562 in. (14.3 mm)	0.85 in. (21.6 mm)	
Incident angle	11 ±1°	0°	

For the longpass filters, the surface that faces the center of the HPC should be coated directly on its surface, not between two or more pieces of glass. The coating should transmit >70% of the wavelength range you want the filter to transmit, with a minimum transmission of >50%, and it should reflect >90% of the wavelength range you want the filter to reflect.

The opposite surface (facing away from the center) should be coated with an anti-reflective coating with a minimum reflection of £1% of the wavelength range you want the filter to reflect.

Note: Filters must be installed in front of each PMT to block unwanted laser light. For the dichroic, carefully assemble the glass filter in the holder using the retaining spring. Assemble the filter as per the supplier's specification. The angle of the dichroic is critical to achieving optimal results.

Note: Whenever you modify the filter set on your cytometer, you will need to create a new cytometer configuration to identify which PMT (identified by laser color and letter) will detect the emitted light. See Custom configurations (page 61) for instructions.

Cleaning the optical filters

Optical filters should be inspected occasionally and cleaned as necessary. The frequency will depend on how often the filters are handled.

When cleaning or replacing a filter, handle with care to avoid scratching the surface and to prevent the filter from falling out of the holder. Use cotton swabs, optical lens paper, and spectral-grade methanol or absolute ethanol in a dropper bottle (do not use acetone) to clean the optical filters.

1. Wrap a triangular section of the lens paper around the cotton end of a cotton swab. Moisten and seal the end with a few drops of alcohol.
2. Holding the cotton swab in a horizontal position, gently rub any spots on the filter surface and wipe clean.
3. Allow the solvent to evaporate and check the filter surface for streaks.
4. Inspect a ¼-inch–diameter section in the center of the filter for scratches.

   Filters are coated with different dielectrics that can get scratched. If you see scratches, replace the filter.
5. Insert the cleaned filter into the HPC array.

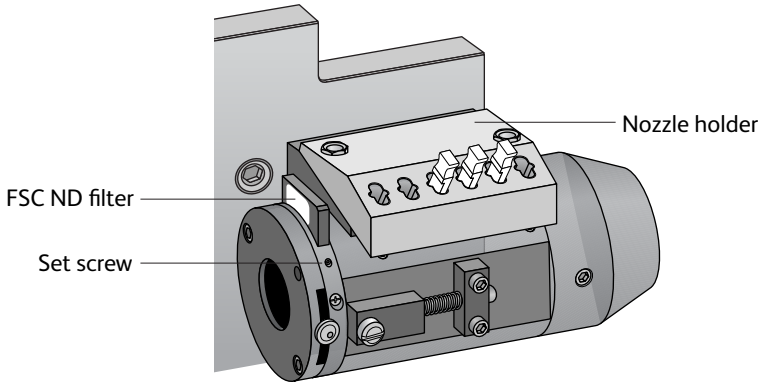
   Make sure the filters are pushed all the way in.

Removing or installing the FSC ND filter

For applications involving large particles in which events appear off scale on the FSC axis with a voltage of zero, keep the FSC ND filter in place to decrease the FSC signal and keep the events on scale. For applications involving small particles (for example, bacteria or platelets), you might need to remove the FSC ND filter as follows.

1. As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.
2. Open the flow cell access door.
3. Locate and pull out the FSC ND filter.

   The filter is installed at the left end of the FSC detector block, just to the right of the flow cell. To remove the filter, loosen the set screw and pull the filter out of the slot.



4. To reinstall the filter, slide it into the slot with the filter side down and the label facing the flow cell.

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

8

Troubleshooting

The tips in this chapter are designed to help you troubleshoot your experiments. Additional troubleshooting information can be found in the *BD FACSDiva™ Software Reference Manual*.

Note: As a precautionary measure, prior to opening the Flow Cell access door, use the **Stop** button in the laser control software to put the lasers in standby and then close the laser control software. This will turn off the laser diode, but will keep the diode temperature constant.

If additional assistance is required, contact your local BD Biosciences technical support representative. See Technical assistance (page 9).

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

Troubleshooting the stream

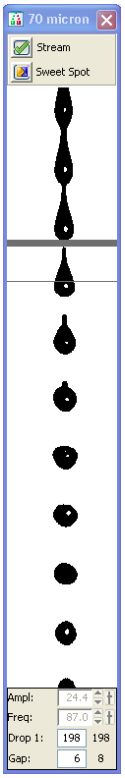
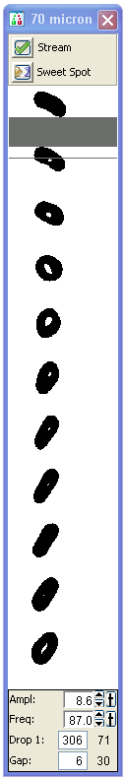
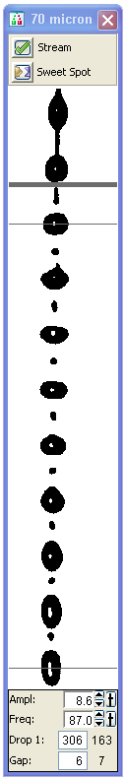
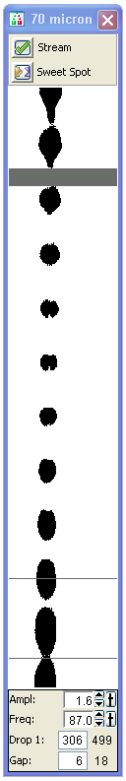
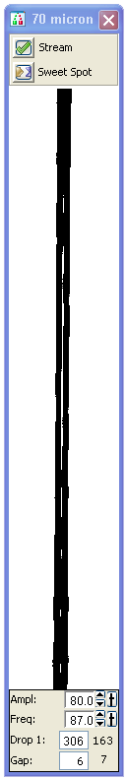
Observation	Possible Causes	Recommended Solutions
Stream not in center of aspirator	Difference in keyed stream position between nozzles	If you have just changed the nozzle, use an Allen wrench to loosen the screws on either side of the sort block. Adjust the angle of the sort block until the stream flows into the center of the waste aspirator, and then tighten the screws.
	Nozzle inserted improperly	Turn off the stream. Remove the nozzle and ensure that the seal or O-ring is in place. Re-insert the nozzle and slide the nozzle in until it stops, then close the locking lever.
	Clogged or damaged 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 integrated nozzle (page 156). • If the nozzle appears damaged, replace it. See Changing the integrated nozzle (page 155).
Plate arcing with no events thereafter	Arcing error comes from the droplet board (not from the fluidics board). Board will turn everything off to prevent destroying plates and for circuit protection. If no notification is sent to the board, the software will not reset the board.	Dry the plates and sort block. Reset the instrument and software.
Performance check failed	Incorrect filters	Confirm that the filters used during the performance check match the filters used to define the baseline.
No stream or dripping stream	Nozzle inserted improperly	Turn off the stream. Remove the nozzle. See Changing the integrated nozzle (page 155) for instructions.
	Clogged or damaged 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 integrated nozzle (page 156). • If the nozzle appears damaged, replace it. See Changing the integrated nozzle (page 155).
Stream control disabled or no stream when stream control clicked	Air lock in fluidics filter	Prime the system with the corresponding fluid. If the control is still disabled, remove the filter, install the bypass tubing, and repeat the priming procedure until you see fluid in the line. When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.
	Communication failure between workstation and cytometer	Exit the software and restart it.

Observation	Possible Causes	Recommended Solutions
No stream when Stream control clicked	Sheath tank low or empty	Refill the sheath tank. See Refilling the sheath tank (page 94). **NOTE** When the empty tank warning message is not closed after 15 minutes, the stream shuts off automatically.
	Air in sheath line	Prime the sheath tank. See Prime after tank refill (page 145).
	Air in sheath filter	Purge the sheath filter. See Purging the sheath filter (page 148).
	Dry filter	Open the bleeder valve to purge the filter. See Purging the fluid filters (page 148).
Fanning around center stream	Nozzle inserted improperly	Re-insert the nozzle. Push it gently all the way forward without rocking it from side to side.
	House air or compressor is off (gauge reads zero)	Turn on house air or compressor.
	Sheath tank lid not properly seated (gauge reads <80 psi)	Remove sheath tank lid, reseal properly.
Unstable stream	Debris in flow cell or nozzle	Remove the nozzle and run the stream with no nozzle in place for approximately 10 seconds. (Click the Stream control on, then off.) Sonicate the nozzle and re-install it.
	Fluid line connected to ethanol shutdown tank	Move fluid and air lines to the sheath tank, then perform a fluidics startup. See Performing fluidics startup (page 90).
Stream stops shortly after starting stream	Interlock cable is disconnected	Check cable. Call BD Service.
Stream stops when loading tube	Sample Injection Chamber obstructed Sample Injection Chamber misaligned	Check tube loading area is clear. Check chamber base O-ring is properly seated. Call BD Service.
Stream stops after tube unloads	Sample Injection Chamber obstructed Sample Injection Chamber misaligned	Check that nothing is below the Sample Injection Chamber base. Ensure Sample Injection Chamber skin is positioned all the way back. Call BD Service.
Stream stops randomly	Emergency Off switch pressed	Reset switch. Call BD Service.
Leaking or spraying around nozzle	Defective or damaged integrated nozzle seal	Replace the defective seal with a standard O-ring. See Temporary replacement of a seal (page 158).
	Nozzle inserted improperly	Turn off the stream. Remove the nozzle. See Changing the integrated nozzle (page 155) for instructions.
	Extra O-ring is blocking the nozzle	Remove the nozzle and use a cotton swab to clear out the cuvette.
Drop breakoff is too long	Bubbles in flow cell	Open the flow cell access door and check for bubbles in the flow cell. If they are visible, turn off the stream, wait a few seconds, and turn on the

Observation	Possible Causes	Recommended Solutions
		stream again.
	Attenuation is on	Turn off attenuation.
	Amplitude is too low	Increase the amplitude until you can see drops. If you need a very high amplitude (>70 volts) to see drops, there might be air bubbles in the flow cell.
	Nozzle inserted improperly	Turn off the stream. Remove the nozzle and ensure that the seal or O-ring is in place. Re-insert the nozzle and slide the nozzle in until it stops, then close the locking lever.
Problems using Auto Delay feature	Stream is not stable	Make sure that the stream is stable before starting to run auto delay.
	Diode laser does not fully intercept the sorting streams	Adjust the diode laser to intercept streams in the middle, producing the biggest and brightest spots in the left and center images.
	Event rate is too low or too high	Adjust the flow rate to increase or decrease the event rate.

Troubleshooting the breakoff

Use the following examples to help troubleshoot problems with the breakoff image.

Normal stream image		Abnormal Stream Images				
						
	Possible Causes	Nozzle inserted improperly	Nozzle inserted improperly or orifice is off center	Partial clog	Wet or dirty strobe lens	Attenuation is on at wrong pressure
	Recommended Solutions	Remove the nozzle and re-insert it.	Remove the nozzle and re-insert it.	Remove the nozzle, clean it, and then re-insert it.	Clean the lens as described in Cleaning the camera windows (page 163).	Turn off attenuation in the Side Stream window.

Sorting troubleshooting

Observation	Possible Causes	Recommended Solutions
Unstable breakoff while Sweet Spot is engaged	Residual ethanol in system	Allow the system to run until the breakoff stabilizes.
	Target Drop 1 value is out of range for drop spacing	Use an actual Drop 1 value for the target. Remember to repeat the drop delay setup each time the target value is changed.
	Nozzle is clogged or inserted improperly	See Responding to a nozzle clog during a sort with the AMO (page 195).
	Dirty strobe lens or upper camera window	Clean the lens and the window as described in Cleaning the camera windows (page 163).
	Air in sheath filter	Purge the sheath filter. See Purging the sheath filter (page 148).
	Debris in flow cell or nozzle	Remove the nozzle and run the stream with no nozzle in place for approximately 10 seconds. (Click the Stream control on, and then off.) Sonicate the nozzle and re-install it.
Center stream image is dim or not visible in the Side Stream window	Camera window is dirty	Clean the lower camera window. See Cleaning the camera windows (page 163).
	Stream is not intercepting the diode laser	Adjust the micrometer dial on the laser to make the stream intercept as bright as possible.
Center stream is off center when the plate voltage is turned on	Voltage center too low or too high	Adjust the Voltage Center slider to put the center stream back to center.
	Saline spray on deflection plates or in sort block	Clean the deflection plates and the area around them.
Arcing between deflection plates	Salt bridge	Clean and dry the deflection plates and the area around and behind the plates.

Observation	Possible Causes	Recommended Solutions
ACDU sorting failure	Insufficient stream voltage	Increase the voltage for the far left stream.
	Splash shield not installed	Install the splash shield. See Installing the sorting hardware (page 132).
Side stream position reversal, where the streams appear to be associated with the wrong voltage slider.	Voltage sliders are set too far in or too far out.	Move sliders in or out so they control the correct side streams.
No deflection or insufficient deflection	Insufficient voltage	• Increase the side-stream voltages using the slider controls. • Increase the plate voltage.
	Stream-charging wire is loose or missing	Verify that the stream-charging wire is inserted all the way into the barb.
	Salt bridge	Turn off the stream. Remove the nozzle. See Changing the integrated nozzle (page 155) for instructions.
	Sorting paused because actual Drop 1 value is out of range	Wait until the Sweet Spot adjusts the amplitude to achieve the Drop 1 target. If this happens repeatedly during sorting, there might be debris in the nozzle or flow cell. See Troubleshooting the stream (page 170) for suggestions.
Fanning around center or side streams	Nozzle inserted improperly	Turn off the stream. Remove the nozzle and ensure that the seal or O-ring is in place. Re-insert the nozzle and slide the nozzle in until it stops, then close the locking lever.
	Incorrect Gap	Turn off the Sweet Spot and adjust the Gap amplitude to a value that is midway between the outer limit tolerances. Turn on the Sweet Spot after the adjustment.
	Sweet Spot is off	Turn on the Sweet Spot.
	Incorrect sort precision mode	Verify that the sort precision mode is appropriate for your sorting requirements. See Sort Precision Modes (page 52).
	2nd, 3rd, or 4th Drop values not optimized	Adjust the 2nd, 3rd, and 4th Drop settings to tighten the center stream and fine-tune the side streams.
	Particles too big for nozzle	Change the nozzle. See Changing the integrated nozzle (page 155).
Sort button disabled	Current tube pointer not set to current tube	Click to move the current tube pointer to the appropriate tube.
Population not listed in Add menu on sort layout	Population defined using snap-to gate	Redefine the population using another gate type.
	Viewing sort layout for another tube	Open or create a sort layout for the current acquisition tube.

Observation	Possible Causes	Recommended Solutions
Sort layout counters not updating	Viewing sort layout for another tube	Open or create a sort layout for the current acquisition tube.
High sort conflict rate	Event rate is too high for drop drive frequency	Decrease the event rate.
	Gating conflict	Verify the gating hierarchy.
	Purity mask is too high	Decrease the purity mask.
Erratic sort rate	Flow rate is too high	Decrease the flow rate.
Unexpected sort results	Incorrect drop delay	Reset the drop delay. See Determining the drop delay – automatic method (page 126).
	Incorrect sort precision mode	Verify that the sort precision mode is appropriate for your sorting requirements. See Sort Precision Modes (page 52).
	Sweet Spot is off	Keep the Sweet Spot on during sorting.
	Drop 1 changed after setting drop delay	Reset the drop delay each time you change the Drop 1 value. See Determining the drop delay – automatic method (page 126).
	Laser delay changed after setting drop delay	Reset the drop delay each time you change the laser delay. See Determining the drop delay – automatic method (page 126).
	Incorrect logic in population hierarchy	Verify the gating strategy.
	Sorting parent and child populations into two different tubes	If you try to sort a parent and its child population into two tubes, BD FACSDiva™ software ignores the child events in both tubes. Create a new subset under the parent population consisting of NOT (child). Sort the child population into one tube and the NOT (child) population into another tube.

Acquisition troubleshooting

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Load or Acquire Data	Current tube pointer is not set to current tube	Click to move the current tube pointer to the appropriate tube.
	Laser shutter is engaged	Make sure the flow cell access door is completely closed.
	Laser power is off	Turn on the laser power.
	Laser delay set incorrectly	Adjust the laser-delay settings. See Manual adjustment of laser delay (page 116).
	Viewing plots for a different tube	Double-click the current tube in the Browser to display the plots for that tube.
	Incorrect population(s) in plot	Right-click the plot and select Show Populations. Verify that the appropriate populations are displayed.
	Uncolored events in plot	• Format the plot to display all events. • Assign a color to the population displayed in the plot. • Verify the population drawing order.
	Current cytometer configuration different from optical setup	Verify that the cytometer optics match the current cytometer configuration.
	No sample in the tube	Add sample to the tube or install a new sample tube.
	Sample is not mixed properly	Increase the sample agitation rate. See Sample agitation (page 57).
	Sample line is clogged	Perform a sample line backflush. See Sample line backflush (page 145). If necessary, change the sample line.

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Acquire Data (continued)	Sample filter is clogged	Replace the sample filter.
	Threshold not set to the correct parameter (usually FSC)	Set the threshold to the correct parameter for your application.
	Multiple threshold parameters not set correctly	Verify that the correct Boolean logic (And/Or) was used for the threshold parameters.
	Threshold channel too low or too high	Adjust the threshold channel. See Calculating compensation (page 110).
	Optical filter(s) not completely seated	Make sure that the filters are pushed all the way in.
	FSC area scaling is incorrect	Ensure that the FSC-A matches the FSC-H value.
No fluorescence signal	Current cytometer configuration different from optical setup	Verify that the cytometer optics match the current cytometer configuration.
	Wrong filter installed or filter not completely seated	Make sure that the appropriate filter is installed for each fluorochrome. Make sure that the filters are pushed all the way in.
	Laser delay is set incorrectly	Adjust the laser-delay settings. See Manual adjustment of laser delay (page 116).
Low area signal	Area scaling is too low	Adjust area scaling for the corresponding laser. See Adjusting area scaling (page 104).
Unexpected events in plot	Incorrect logic in population hierarchy	Verify the gating strategy.
	Incorrect population (s) in plot	Right-click the plot and select Show Populations. Verify that the appropriate populations are displayed.
	Incorrect drawing order	Verify that the required population is not hidden by another population. Right-click the plot and select Order Populations by Count.
Erratic event rate	Sample aggregates	Filter the sample.
	Bulk injection O-ring is worn	Contact your BD Biosciences service engineer.
	Sample is contaminated	Re-stain the sample, making sure the tube is clean.
	Sheath tank low	Fill the sheath container.
Unexpectedly high event rate	Threshold channel is too low	Adjust the threshold channel. See Calculating compensation (page 110).
	Sample is too	Dilute the sample.

Observation	Possible Causes	Recommended Solutions
	concentrated	
	Flow rate is too high	Decrease the flow rate in the Acquisition Dashboard.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and turn on the stream again.
Unexpectedly low event rate	Sample not adequately mixed	Increase the sample agitation rate. See Sample agitation (page 57).
	Threshold channel is too high	Adjust the threshold channel. See Calculating compensation (page 110).
	Sample is too dilute	Concentrate the sample.
	Sample line is clogged or kinked	Backflush the sample line. See Sample line backflush (page 145). If necessary, change the sample line.
		Look for visible kinks in the line. If kinks are found, change the sample line. See Changing the sample lines (page 150).
Unexpectedly low event rate (continued)	Sample line installed incorrectly	Verify the sample line installation. See Changing the sample lines (page 150).
	Sample aggregates	Filter the sample.
	Memory is full	Compare the processed event rate in BD FACSDiva™ software with the threshold counter. If the event rate is much lower, exit and then restart the application.
Distorted populations or high CVs	Cytometer settings adjusted incorrectly	Optimize the scatter parameters. See Calculating compensation (page 110).
	Flow rate is too high	Decrease the flow rate in the Acquisition Dashboard.
	Window extension is too low	Increase the window extension.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and turn on the stream again.
	Nozzle is clogged or dirty	Clean the nozzle as described in Changing the integrated nozzle (page 155).
	Flow cell is dirty	Clean the flow cell. See Cleaning the flow cell (daily) (page 140). Let DI water sit for 15 minutes before turning on the stream. Repeat as needed.
	Poor sample preparation	Repeat sample preparation.
	Area scaling is too low	Verify area scaling. See Adjusting area scaling (page 104).
Excessive amount of debris in plots	Threshold channel is too low	Increase the threshold channel. See Calculating compensation (page 110).
	Dead cells or debris in sample	Examine the sample under a microscope.

Observation	Possible Causes	Recommended Solutions
	Sample is contaminated	Re-stain the sample, making sure the tube is clean.
High electronic abort rate (>10% of system event rate)	Window extension is too high	Decrease the window extension.
	Threshold channel is too low	Increase the threshold channel.
	Event rate is too high	Decrease the flow rate in the Acquisition Dashboard.
	Sample is aggregated	Filter the sample.
	Sample is too concentrated	Dilute the sample.
Fewer events than expected in gated population	Window extension set incorrectly	Adjust the window extension, if needed. See the *BD FACSDiva™ Software Reference Manual* for information.
	Laser delay set incorrectly	Adjust the laser-delay settings. See Manual adjustment of laser delay (page 116).
	Plot is zoomed	Unzoom the plot or make the gate bigger.
	Events left out of the gate	When drawing a gate, make sure that events on the axis are included.
Increasing threshold results in decreased area signal	Window extension is too low	Slightly increase the window extension to maximize the area signal. Increasing the window extension too much results in more electronic aborts or high CVs.
Area measurement off scale while the height measurement is on scale	Area scaling is too high	Decrease area scaling to move the area measurement back on scale. If necessary, adjust area scaling to make the area measurement match the height measurement.
Cannot delete from Parameters, Threshold, Compensation, or Ratio tab views	Row not selected	Select the row using the selection button.
	Data already recorded	Create a new tube.
Cannot connect to CS&T	External air supply is <80 PSI	Check the air pressure gauge on the air supply panel or sheath tank. The air pressure should be 80–100 PSI. • If the air pressure is within range, contact your BD Biosciences service engineer. • If the air pressure is <80 PSI, close the release valve and reseal the lid.
Negative Q values	Higher power lasers than in previous cell sorters	1. Rerun the CST baseline. 2. When the software pauses at the **Optimized PMT Results** window, set median channel values that are <200 to 200. 3. Click **OK** to finish the run.

Fluidics troubleshooting

Observation	Possible Causes	Recommended Solutions
No fluid in line during system prime	Air lock in sheath or fluidics filter	Remove the filter for the corresponding fluid, install the bypass tubing, and run Prime After Tank Refill. Repeat the priming procedure until you see fluid in the line. When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.
Prepare for aseptic sort fails	Air lock in filter	Remove the filter for the corresponding fluid, install the bypass tubing, and run Prime After Tank Refill. Repeat the priming procedure until you see fluid in the line. When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.
	Fluid line is detached	Verify the fluid line connections on the fluidics drawer and on the cytometer. Push firmly on each line to ensure that it is connected.
Fluidics air flow <80 PSI	Air leak	Contact your BD Biosciences service engineer.
Fluidics air flow >100 PSI	Regulator not adjusted properly	Contact your BD Biosciences service engineer.
Fluid leak under fluidics drawer or below side door	Condensation from pressure relief valve	This is a normal phenomenon that occurs when water is condensed from room air. Condensation is greater in humid environments. To avoid slipping, wipe up any water daily.
	Bleeder valve is open	Check and close all bleeder valves for fluid and sheath filters.
	Broken fluid line	Contact BD Biosciences.
Sample injection chamber does not close and causes a BISH or BISO error message	O-ring at bottom of sample injection chamber is dry and causing chamber to stick	Lubricate the O-ring and tube holder. See Lubricating the sample injection chamber O-ring (page 166).

Electronics troubleshooting

Observation	Possible Causes	Recommended Solutions
"Cytometer Disconnected" in Cytometer window	Cytometer power is off	Turn on the cytometer main power.
	Communication failure between workstation and cytometer	• Exit the software and then restart it. • If restarting does not work, reset the cytometer electronics: switch off the main power, wait 10 seconds until the system is fully depressurized, and then switch the power back on. • Restart the computer and the cytometer.
	Ethernet cable between workstation and cytometer is disconnected	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address changed	Enter the correct IP address. Call BD Biosciences for assistance.
Lasers are not powered on	Main laser power button is not switched on.	Switch the main laser power button on.
	USB data loss.	Unplug and replug the USB cable at the side of the sorter.
"Master DAQ Overflow" in Cytometer window	Event rate is too high	Decrease the event rate or verify the threshold.
	Dirty flow cell	Clean the flow cell. See Cleaning the flow cell (daily) (page 140).
"Cytometer not responding" in Status tab	Unknown	Perform the suggestions for a communication failure, above.

9

Supplies and consumables

This chapter provides a list of supplies and options that are available for the BD FACSymphony™ S6 cell sorter.

- To order spare parts and consumables from BD Biosciences from within the US, call 877.232.8995 or go to bdbiosciences.com.

  Outside the US, contact your local BD Biosciences representative.
- To order cytometer options, contact your sales representative.

This information is correct at the time of publication. For up-to-date information, see our website (bdbiosciences.com).

- Cytometer supplies (page 184)
- Consumables (page 188)

Cytometer supplies

Accessory kit

The cytometer is shipped with an accessory kit containing the following items. Use these part numbers if you need to order any replacements.

Tank-related parts

Item	Part Number
Waste sensor probe (6-level)	334915
Auxiliary 5-L container	333504
Baffle for 10-L waste tank (2 pack)	338505
Cap for 5-L container (3)	335916
Sheath tank assembly	641915
Ethanol tank assembly	641916
Auxiliary sensor probe (ethanol)	642874
O-ring, tank, ethyl and sheath	642877
Wet cart liquid filter	644320
Disposable waste tank caps (pack of 12)	338854
Waste tank with label	643909
Ethanol tank with label	643910
DI water tank with label	643911
BD FACSClean™ tank with label	643912
Sheath sensor probe	644109
Pressure gauge, 0-100 PSI	646024
Pipe fitting, 0.25 in.	645400
Vacuum lid utility knob	648233
Ball knob, 1 in. diameter	642833
Auxiliary sensor probe (non-ethanol)	343835

Optical Filters

Item	Part Number
FSC 0.5 neutral density filter assembly	337104
FSC 1.0 neutral density filter assembly	337108
FSC 1.5 neutral density filter assembly	338651
FSC 2.0 neutral density filter assembly	338652
Filter (Chroma) HQ710/50	640471
Mirror (Omega) 675/20	19-62774-15
Filter holders (3)	336102
Filter clip (5)	640563

Sort collection devices

Item	Part Number
15-mL centrifuge tube holder	333430
12 × 75-mm test tube holder	333456
Six-way 5-mL amber tube holder	500050957
Six-way 5-mL tube holder top	500050958
Six-way 1.5-mL tube holder	665647
1-mL microtube holder	333457
Four-way 12 × 75-mm collection tube holder	641454
Two-way 12 × 75-mm collection tube holder	500032421
Two-way 15-mL collection tube holder	641612
Four-way 1-mL collection tube holder	641614
Splash guard	641615
Temp control 15-mL tube holder	647168
Temp control 12 × 75-mm tube holder	647206
Temp control Eppendorf tube holder	647207
Four-way 1.5-mL Eppendorf collection tube holder	644349
Terasaki plate adaptor	652905
Adapter tray for microscope slides	335630
Six-way 5-mL temp control tube holder	665346

Maintenance-replacable items

Item	Part Number
Sample injection tubing (12-in. lengths)	641059
Sample injection tubing (7-in. lengths)	641475
Standard nozzle O-rings	333084
Ferrule	335108
Capsule filter 0.2 micron	336945
O-ring for collection-tube holder, 1.75 in. internal diameter, 1.87 in. external diameter (9)	337897
O-rings 0.176 in. internal diameter, 0.318 in. external diameter (10)	340086
Drop charge cable assembly	343358
Barbed Fitting, 1/4-28 in. × 1/16 in. internal diameter tubing (3)	343508
Lubricant for O-rings	347306
Peek nut fitting	348578
O-rings 0.859 in. internal diameter (3)	641860
Pinch valve tubing	641900
Reducing union fitting 1/16 in. – 1/32 in.	643571
Rotary shaft seal	644054
Universal top section for collection tube holders (2)	651439
Coupling insert valved 1/4 in.	641543
Tube compression fitting 3/8 in. through	641920
Coupling body valved 1/4 in.	641542
Window BI sort head	657582
Fitting street tee 1/4M × 1/4F × 1/4hex	657798
Collet	19-66455-00
Collet nut large diameter	663282
Connector female luer to 10/32 in.	59-10102-00
Collet nut	19-66456-00
Sort block deflection plate, left 2.5 in.	642579
Sort block plate deflection plate, right 2.5 in.	640042
Sort block deflection plate, left 3.5 in.	5000061176
Sort block deflection plate, right 3.5 in.	5000061177

Cables

Item	Part Number
Power cords: • Main power cord • Cordset for continental Europe • Cordset for UK • Cordset for Australia/Asia	 • 333694 • 334140 • 334141 • 334175
R1 CAT-5 cable	334965
Coaxial cable BNC-M to BNC-F 2 ft.	342824
Cable, extension, DB9FEM-DB 8 ft.	332907

Nozzles

Item	Part Number
Closed loop nozzle assembly	644395
Integrated nozzles: • 70 micron (2) • 85 micron (2) • 100 micron	 • 647339 • 647340 • 647341
Nozzle holster	642884
Nozzle locking lever (spring and plunger included)	7001349

Tools

Item	Part Number
Plastic O-ring pick tool	331430
Ferrule-removal tool	335690
Magnifying glass	337599
Tool alignment 1.5-mL side stream	658214
Deflection plate removal tool	643197
Hex wrench set	98-10004-00

Other replacement parts

The following items may not be included in the accessory kit, but you can use the indicated part numbers to order spare or replacement parts.

Item	Part No.
Autoclavable 10-L container	340261
Warning labels	335600

Consumables

Cytometer setup particles

Particle	Supplier	Catalog No.
BD Calibrite™ Beads	BD Biosciences	
• Two-color kit (unlabeled, FITC, PE)		• 349502
• Three-color kit (unlabeled, FITC, PE, PerCP)		• 340486
• PerCP beads		• 340497
• PerCP-Cy5.5 beads		• 345036
• APC beads		• 340487
Fluoresbrite® Yellow-Green 2-µm beads (for the 405-nm laser)	Polysciences Inc. (800) 523-2575	18604
Spherotech Rainbow Calibration Particles, 3.0–3.4 µm	BD Biosciences	• 559123 (8 peaks) • 556286 • 556291 (brightest peak in 556286)
BD FACS™ Accudrop Beads	BD Biosciences	345249
BD FACSDiva™ CS&T Research Beads	BD Biosciences	641319 (1 vial) 642412 (3 vials)

Reagents

Reagent	Supplier	Catalog No.
BD FACSFlow™ sheath fluid	BD Biosciences	342003
BD® Detergent Solution Concentrate	BD Biosciences	660585
Detergent liquid cond	BD Biosciences	99-30109-00
BD FACSClean™ solution	BD Biosciences	340345
Ethanol	Various	–
Chlorine bleach (5% sodium hypochlorite)	Clorox® or other major supplier (to ensure that the bleach is at the correct concentration and free of particulate matter)	–
Monoclonal antibodies	BD Biosciences[a]	–
Dyes and fluorochromes	Life Technologies (800) 438-2209 Sigma (800) 325-3010	–
BD FACS™ Lysing Solution	BD Biosciences	349202

[a]See the BD Biosciences Immunocytometry Products Catalog or the BD Biosciences website, bdbiosciences.com.

Labware

Item	Supplier	Catalog No.
1-mL microtubes	Bio-Rad Laboratories (800) 424-6723	223-9391 (1,000 per box)
1.5-mL Eppendorf tubes	Various	–
5-mL polystyrene test tubes, 12 × 75-mm • Uncapped, 125 per bag • Capped, 125 per bag • Capped, 25 per bag • With cell-strainer cap, 25 per bag	BD Biosciences	 • 343675 • 352054 • 352058 • 352235
15-mL conical centrifuge tubes • Polypropylene, 50/bag • Polypropylene, 125/bag • Polypropylene, 50/rack • Polystyrene, 125/bag • Polystyrene, 50/rack	BD Biosciences	 • 352196 • 352096 • 352097 • 352095 • 352099

AMS/AMO consumables

Item	Part Number
Air filter for ACDU cabinet (set of 3)	334821
ULPA filter and tubing replacement kit (set of 3)	334822
Pre-filter	333595
Hydrophobic filter for sort block door, 0.2 micron, 25 mm	651177

BD FACSymphony™ S6 Cell Sorter consumables

Item	Part Number
Swab, micro head 3.2 × 1.5 × 10, 70-mm LG (100/pkg)	643290
Cotton-tipped applicators (100/pkg)	99-30122-00
Air filter, inline, 0.3 micron (2)	641913
Sample inline filters (35 micron) Qty 50	649048
Sample inline filters (50 micron) Qty 50	649049
Hydrophobic filter for sort block door, 0.2 micron, 25 mm	651177
Sheath fluid filter (also used for ethanol shutdown tank filter)	661744

10

BD® Aerosol Management Option

The BD® Aerosol Management Option (AMO) is a device that uses an attached vacuum source to rapidly evacuate aerosolized particles through an ultra-low penetrating air (ULPA) filter during routine sorting or analysis.

The following topics are described in this chapter:

Option components

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

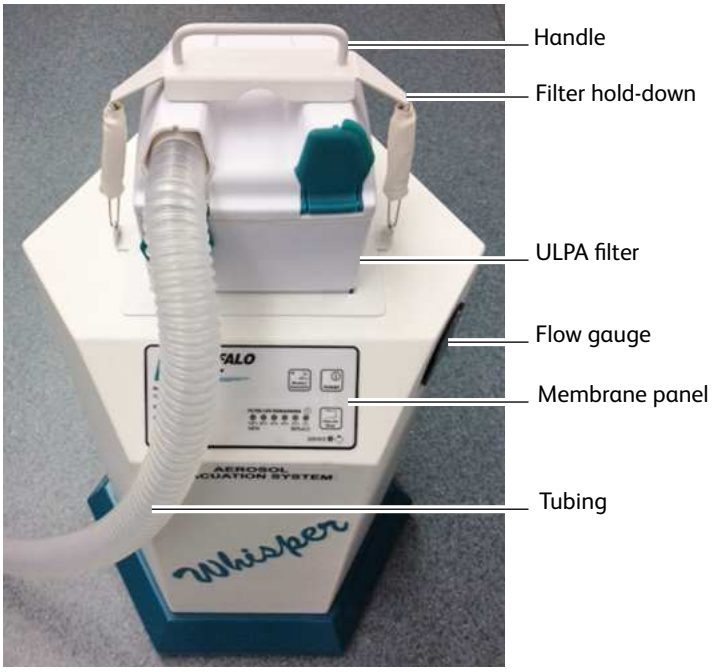
- An evacuator to generate negative pressure
- An ULPA filter to trap particles, with attached tubing that connects the evacuator to the instrument
- An air filter for the sort collection chamber door
- A hydrophobic filter on the sort block door
- A hinged cover on the sample injection chamber

The BD® Aerosol Management Option does not eliminate the health risks of working with biohazardous material and must be used in conjunction with good laboratory practice.

The BD® Aerosol Management Option is for research use only. It is not for use in diagnostic or therapeutic procedures.

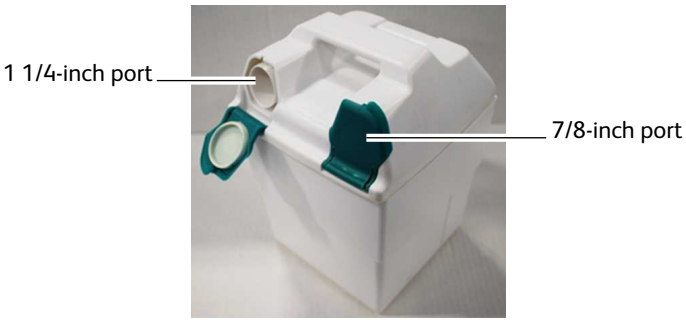
Evacuator

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



ULPA filter

The ULPA filter used in the BD AMO captures and retains 99.9995% of all particles down to and including particles 0.12 microns in size, according to the manufacturer's specifications.



Operating the BD® Aerosol Management Option

Starting up the evacuator

Before starting up the evacuator, make sure that:

- The ULPA filter is completely seated against the bottom of the evacuator filter well
- One end of the tubing is securely attached to the 1 1/4-inch (left) port on the ULPA filter

  Note: Ensure that the 7/8-inch (right) port is closed.
- The other end of the tubing is attached to the manifold located in the connection panel on the back of the cytometer

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

To start up the evacuator:

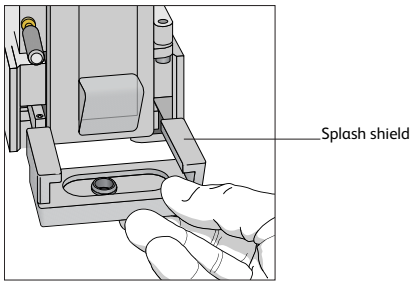
1. Install the splash shield or the tube holder below the aspirator drawer.

   The splash shield is required for sorting into a multiwell plate or onto a slide.

   To install the splash shield:

   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.



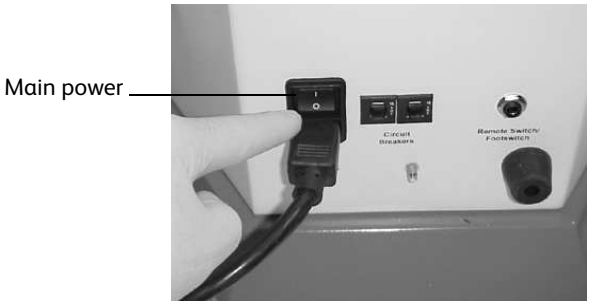
2. Ensure that an air filter is installed in the sort collection chamber door.

   The filter traps airborne dust that could clog the ULPA filter. The filter should be changed on a monthly basis. See Replacing the air filter (page 199).

3. Close the sort collection chamber door.

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

4. Switch on the main power on the back of the evacuator.



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

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

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

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

7. Verify that the filter flow gauge reads less than 2.4 inches of H2O.

   For a new filter, the gauge should read 1.1–1.4 inches of H2O. As the filter is used, the reading will increase. If the gauge reads 2.4 inches of H2O or greater, replace the filter. See Replacing the ULPA filter (page 197).

New filter

Used filter

Setting up for sorting

To set up for sorting:

1. Start up the flow cytometer system. See Cytometer startup (page 88).
2. Start up the evacuator as described in Starting up the evacuator (page 193).
3. Follow the standard sort setup procedure. See Setting up for sorting (page 120).

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

Responding to a nozzle clog during a sort with the AMO

If the stream is disturbed during the sort (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes (if Sweet Spot is on). The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog, do not open the sort collection chamber door or access the sort tubes before following this procedure.

Cell sorters that use droplet generation methods, such as the BD FACSymphony™ S6 cell sorter, can produce aerosols around the sample stream. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door and the sort collection chamber door closed during sorting. Follow these steps to stop sample flow and evacuate potential aerosols before opening the sort collection chamber door.

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

1. If the stream has not already shut down automatically, turn off the stream by clicking the **Stream** button (with a checkmark) at the top of the **Breakoff** window.

   This will shut off the stream, unload the sample, and close the aspirator drawer.
2. Increase the air evacuation rate on the AMO unit to 100%.
3. Open the aspirator drawer using software controls.
4. Wait at least 60 seconds.

   This procedure will clear aerosols from the sort chamber.
5. Close the aspirator drawer.
6. Turn on the stream and view the breakoff.

   If the clog is removed, the breakoff will be similar to the breakoff before the clog.
7. If the clog is not cleared, turn the stream on and off several times to see if the clog will clear itself.

8. If the clog is not removed, turn the stream off and perform the Clean Flow Cell procedure with DI water (see Cleaning the flow cell (daily) (page 140)), followed by turning the stream on to see if the clog has cleared.
9. Open the aspirator drawer and evacuate for at least 60 seconds before closing the aspirator drawer again.
10. You can now open the sort collection chamber and remove the sort collection device.
11. If it is necessary to change nozzles or remove a clog from a nozzle, see Cleaning the integrated nozzle (page 156).
12. With stream turned off, open the sort block door and dry the plates and surfaces as needed.
13. When removing collection tubes, be aware that the outside of the tube is potentially contaminated. Use alcohol swabs or bleach to wipe the outsides of tubes.
14. Set the AMO unit back to 20% vacuum.
15. Make sure that all chamber doors are closed and restart the stream.
16. Perform these tasks if needed:
    - Turn on the Sweet Spot
    - Check the drop delay
    - Check the side stream deflection

Turning off the evacuator

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

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

Maintenance

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

- Change the ULPA filter and attached tubing when the flow gauge indicator is >2.4 at a 20% flow setting or when the red filter-life indicator LED is blinking. See Replacing the ULPA filter (page 197).

  Two spare filters and replacement tubing are included with the AMO. To order additional replacement kits, contact your local BD Biosciences representative.

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

- Change the air filter in the sort collection chamber door on a monthly basis. The filter traps airborne dust that could clog the ULPA filter. Regular replacement of the air filter will extend the life of your ULPA filter. See Replacing the air filter (page 199).
- Do not touch the Filter Life Reset button during normal operation. Doing so could shut down the evacuator and prevent the collection of aerosols.
- Do not disconnect the tubing from the instrument manifold outlet or the ULPA filter unless you are changing the filter. Repeated removal and reattachment of the tubing could loosen the connection and disrupt airflow.
- To ensure optimal airflow, keep the tubing free of kinks and away from sharp or heavy objects. Do not crush or puncture the tubing. Ensure that the tubing is securely attached at both ends before turning on the evacuator power.

- Keep the sort collection chamber free of potentially obstructive debris, such as Kimwipes® or disposable pipets.
- Replace the hydrophobic filter on the sort block door every six months.

Replacing the ULPA filter

Replace the filter when either of the following conditions occur:

- The filter-flow gauge reads 2.4 inches of H2O or greater at 20% suction.
- The red filter-life indicator LED is blinking.

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

To replace the ULPA filter:

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

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

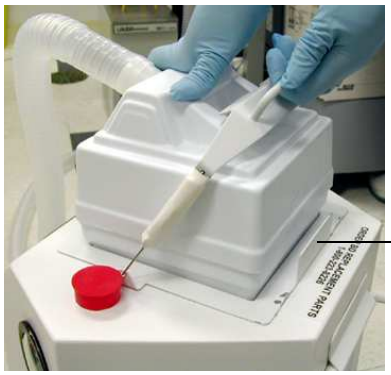
2. Disconnect the tubing from the manifold.

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

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

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

   Note: The photos in this section show the discontinued ULPA filter. The new ULPA filter has two ports (1 1/4-inch and 7/8-inch).

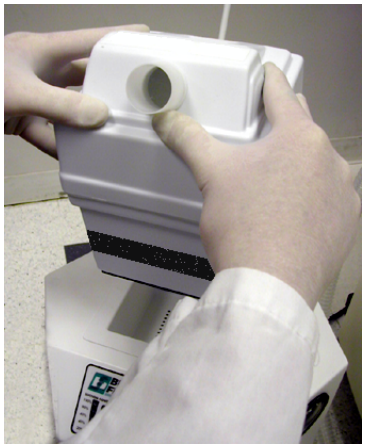


4. Lift off the ULPA filter and attached tubing from the evacuator and dispose of both the filter and the tubing.

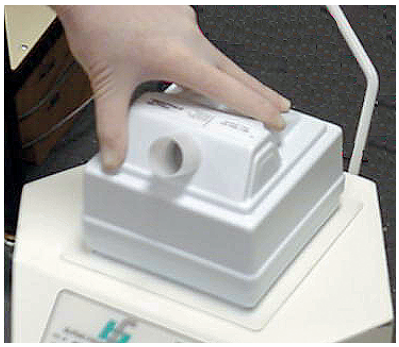
   

   All biological specimens and materials coming into contact with them can transmit potentially fatal disease. Handle the ULPA filter, attached tubing, and all instrument hardware as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations.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

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



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



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

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

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

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

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

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

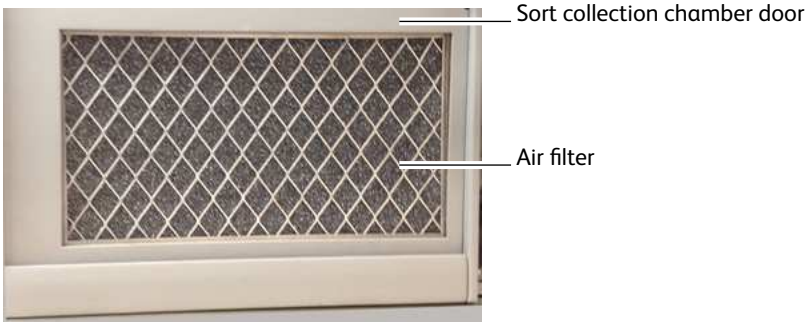
Note: For optimal evacuation of aerosols, ensure that the tubing is securely connected at both ends.

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

Replacing the air filter

To extend the life of your ULPA filter, we recommend that you replace the air filter on a monthly basis. The filter traps airborne dust that could clog the ULPA filter. Regular replacement of the air filter will extend the life of your ULPA filter.

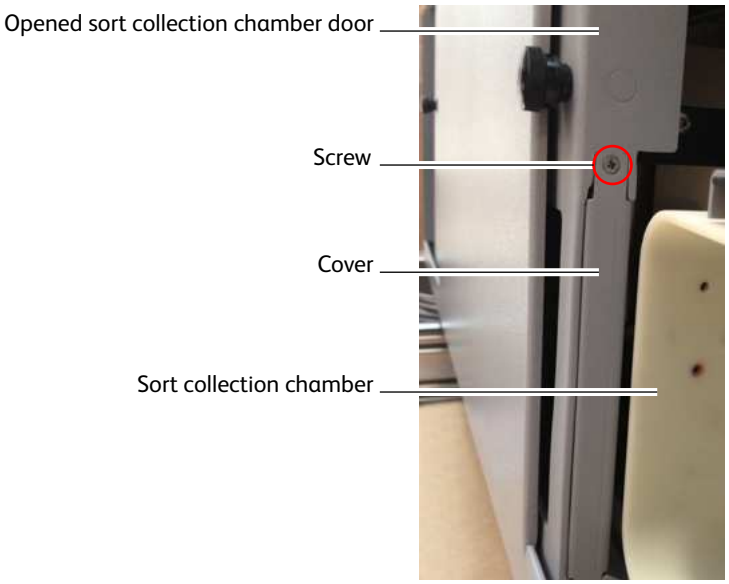
The air filter is located inside the sort collection chamber door.



To replace the air filter:

1. Slide open the sort collection chamber door.

   The air filter is held in place by a cover.



2. Unscrew the screw at the top of the cover.
3. Pull off the cover and slide out the air filter.

   Grasp the filter and then carefully slide it out of the door. While it is still inside the BSC, bag the air filter to avoid exposure to biohazardous agents.

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

4. Install a new air filter in the door.

   Slide the new filter in from the right.

   Note: Make sure to install the filter with the grid side facing out so that it is visible through the closed door.

Grid side faces out

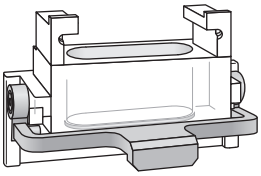
Non-grid side faces in

5. Reinstall the cover and tighten the screw.

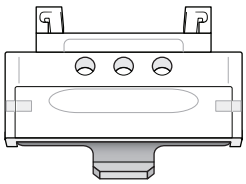
Upgrades for the AMO

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

Contact BD Customer Support for information on the upgrade kits.



Universal top (front view)



Universal top (back view)

Troubleshooting the AMO

The tips in this section are provided to help you troubleshoot issues that arise when using the BD® Aerosol Management Option. For cytometer-specific troubleshooting, see Troubleshooting (page 169).

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.

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

Control Panel Troubleshooting

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

Filter Flow Gauge Troubleshooting

Observation	Possible Cause	Recommended Solution
Zero reading on filter flow gauge	Power off	Press the power button on the membrane panel of the evacuator.
	Filter defective	Replace the filter.
	Filter improperly seated in evacuator	Re-seat the filter with the evacuator power off.
	Tubing loose or not connected	Ensure that the tubing is securely connected below the sort chamber and to the filter module.
	Tubing kinked or damaged	Inspect the tubing for kinks or punctures. Replace the tubing, if needed.
	Wrong tubing type or part	Ensure that the correct type of tubing is in use.
Erratic reading on filter flow gauge	Defective filter	Replace the filter.
	Filter improperly seated in evacuator	Re-seat the filter with the evacuator power off.
Off-scale reading on filter flow gauge	Tubing or sort collection chamber obstructed	• Inspect the tubing for kinks or punctures. Replace the tubing, if needed. • Check for obstructions in the sort collection chamber. Remove any obstruction.
	Filter clogged or saturated	Replace the filter as described in Replacing the ULPA filter (page 197).

Specifications

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

Evacuator

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

ULPA filter module

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

Air filter

- Filter medium is an open-cell polyurethane foam
- Medium specially coated for improved fire retardation and fungi resistance
- High dust-trapping capacity, low air resistance

- Can be used in a wide variety of climatic conditions
- Rated UL 94 HF-1

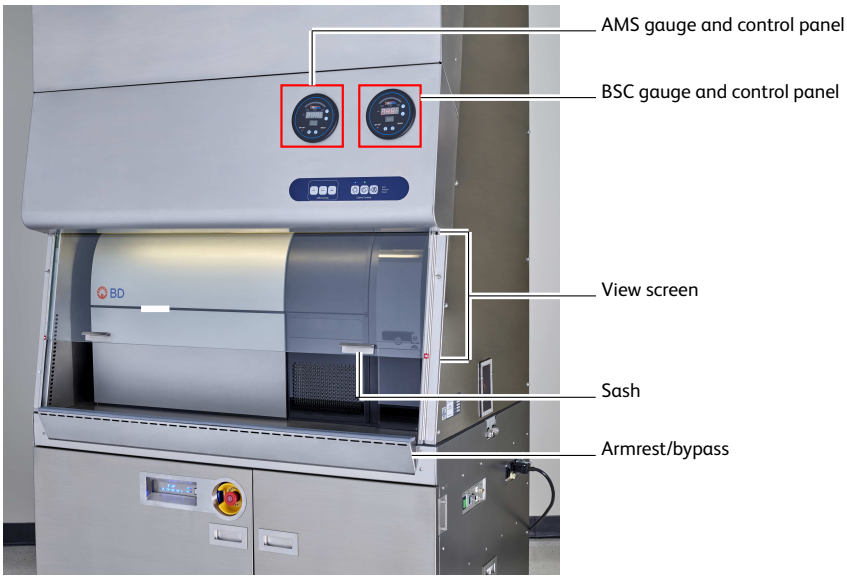
11

Biosafety Cabinet Option

The following topics are covered in this chapter:

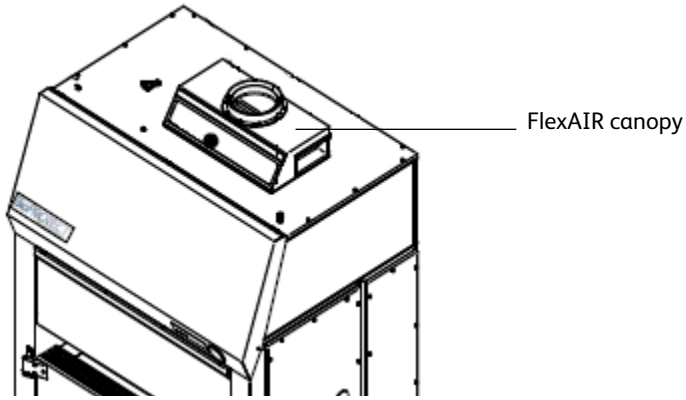
Biosafety cabinet overview

The optional biosafety cabinet (BSC) is designed to be used with the BD FACSymphony™ S6 cell sorter, with the sorting hardware located inside the BSC. Biosafety protection is provided by negative pressure and controlled air flow within the cabinet. Before entering the work area or leaving the top of the cabinet, air passes through high-efficiency particulate air (HEPA) filters. An integrated Aerosol Management System (AMS) operates independently from the BSC blower and provides additional biosafety protection. The AMS evacuates the sort chamber during sorting and exhausts that air through a separate HEPA filter. Air flow probes in the BSC and AMS monitor exhaust air flow. An audible alarm is triggered if the air pressure differential (BSC) or air flow (AMS) drops to and unsafe level.



Operating the BD FACSymphony™ S6 cell sorter inside the BSC can generate temperatures above the acceptable range of 17.5–22.5 °C (63.5–72.5 °F). Monitor the temperature inside the BSC to ensure that it does not exceed this range.

An optional FlexAIR® canopy can be purchased to connect the BSC to an in-house exhaust system. The FlexAIR canopy can help alleviate excess heat inside the BSC. Lowering the temperature inside the lab will also help keep the temperature down.



For safety information about the BSC, see the *BD FACSymphony™ S6 cell sorter Safety and Limitations Guide*. Additional BSC information not contained in this guide can be found in The Baker Company *Biosafety Cabinet for BD FACSymphony™ S6 cell sorter Operator's Manual*.

View screen

The view screen is made from safety plate glass and is the barrier between the BSC work area and the user.

- Maximum opening: The view screen can be opened to a maximum of 18 inches to allow temporary full access to the work area.
- Minimum opening: The view screen can be fully closed.

Sash

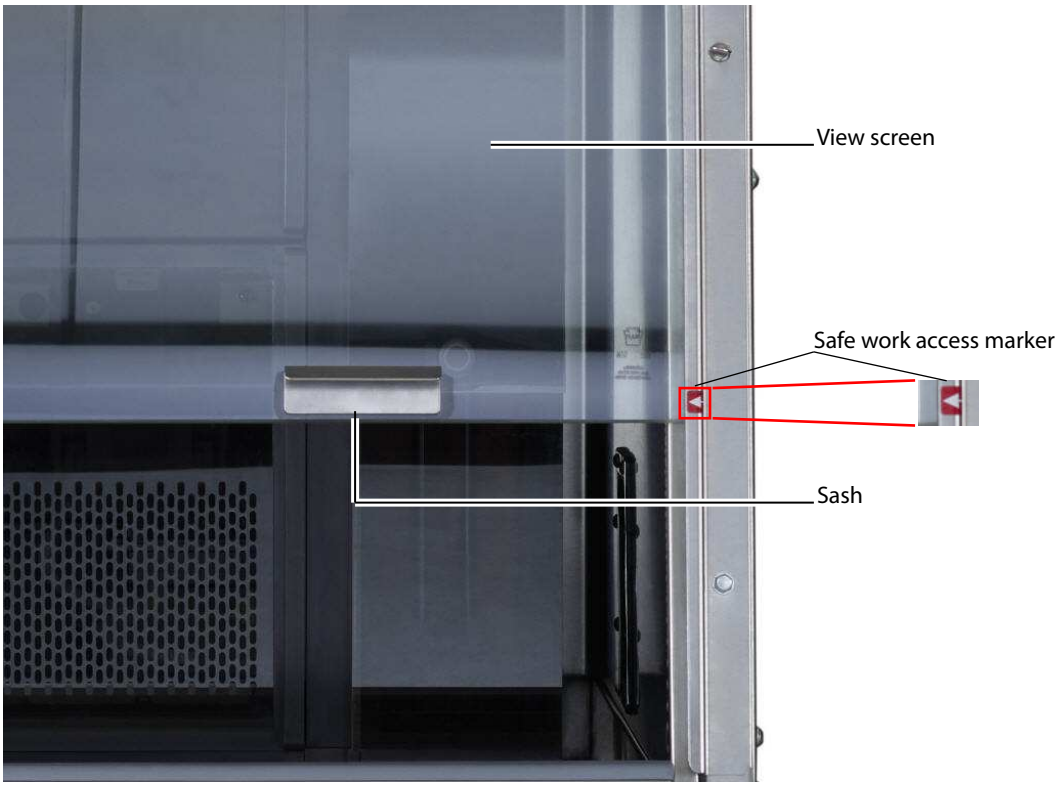
The sash is the bottom edge of the view screen. Grip the sash and slowly:

- Pull down to close the view screen
- Push up to open the view screen

Safe Work access opening

The safe work access opening is 8 inches, which provides biosafety protection. If the sash is raised above 8 inches for more than 3 seconds, an alarm sounds.

The safe work access opening is marked by a red arrow located on both sides of the BSC frame. Ensure that the sash is at this level each time you work in the BSC.



Ready safe mode

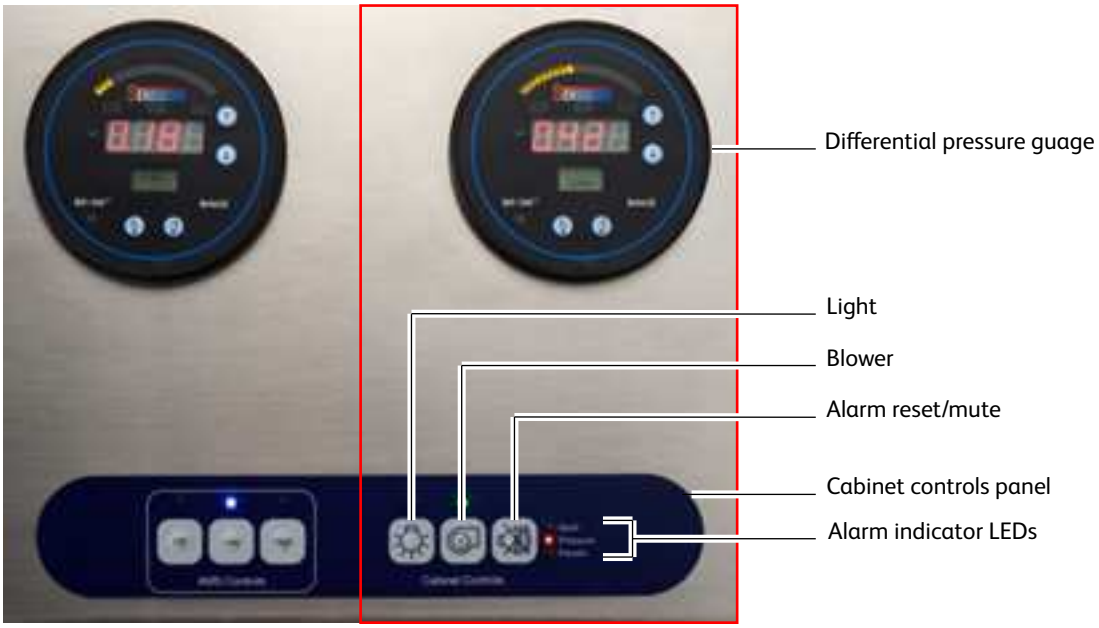
The ready safe mode is an energy saving mode for when the BSC is not in use. To put the BSC in ready safe mode:

- Keep the blower on.
- Lower the sash to fully close the view screen.
- Check the differential pressure gauge to verify that the pressure decreases. (See View screen (page 207) for more information.)

We recommend that you leave the BSC in ready safe mode when it is not being used.

Cabinet gauge and controls

The BSC differential pressure gauge and cabinet controls are located on the front of the BSC in the lower right corner. (The BSC and AMS controls are located on the same panel.) The BSC controls are on the right side of the panel.



Differential pressure gauge

The differential pressure gauge displays the difference in air pressure inside the BSC vs outside the BSC, measured in inch water column (inch WC). The reading should be the value obtained during certification ±20%. (See your BSC certification paperwork.) If the value drops below this level, the pressure alarm will sound and an orange indicator LED will flash. See Buttons and LED indicators (page 208).

Buttons and LED indicators

The following table describes the function of the BSC control buttons and the meaning of the LED indicators located above each button.

Button	Function	LED indicates	LED color	Comments
Light	Turns the light on and off	Power is on	Blue	Two fluorescent lamps light the work area.
Blower	Turns the blower on and off	Power is on	Green	The blower creates the air flow patterns inside the BSC.
		Power is off	Flashing green	–
Alarm	Mutes the alarm for 5 minutes or resets it	See LED alarm indicators (page 209).		

LED alarm indicators

LED indicators to the right of the Alarm button are illuminated when there is an alarm condition.The following table describes the LEDs and conditions that trigger them.

Alarm	LED Color	Condition
Sash	Flashing red	• The sash is raised above the 8-inch safe opening height. • The sash is lowered to fully close the view screen (until pressure decreases).
Pressure	Flashing orange	• The air pressure differential drops to an unsafe exhaust airflow level. • The air pressure differential has not reached the proper level. (This occurs when the blower is first turned on.)
	Solid orange	The air pressure problem was corrected. Press the alarm button to reset the alarm.
FlexAIR[a] (if installed)	Flashing yellow	The air flow through the canopy drops to an unsafe level.

Audible alarms

An LED indicator to the right of the alarm button is illuminated when there is an alarm condition. Audible alarms differ for each alarm type. The following table describes the alarms and conditions that trigger them.

LED Indicator	Audible Alarm	Condition
Sash	Sounds once per second.	The sash is positioned at an unsafe level for more than 3 seconds.
Pressure	Sounds four times per second followed by a two-second delay.	• The air pressure differential drops to an unsafe exhaust air flow level. • The air pressure differential has not reached the proper level. (This occurs when the blower is first turned on.)
FlexAIR[b] (if installed)	Sounds two times per second.	The air flow through the canopy drops to an unsafe level.

[a]The optional FlexAIR canopy can be purchased to connect the BSC to an in-house exhaust system.

[b]The optional FlexAIR canopy can be purchased to connect the BSC to an in-house exhaust system.

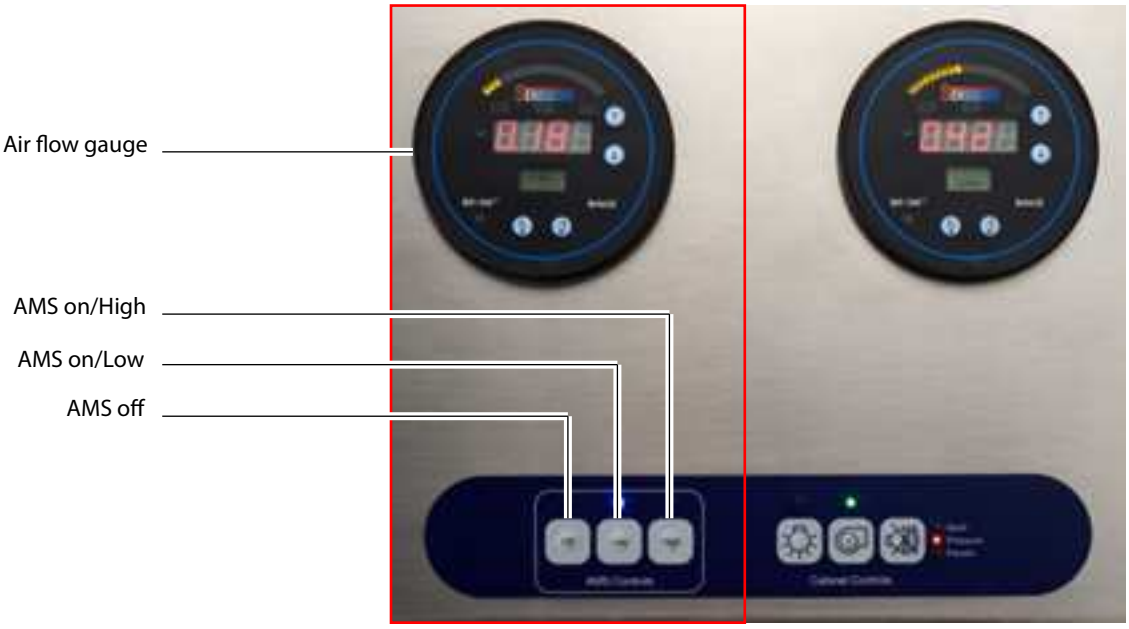
For detailed instructions on how to use the BSC, what the alarm conditions indicate, and how to reset them, see The Baker Company *Biosafety Cabinet for BD FACSymphony™ S6 cell sorter Operator's Manual*.

Aerosol Management System

An AMS is incorporated into the BSC to rapidly evacuate aerosolized particles from the sort collection chamber during sorting or analysis. Equipped with a separate HEPA filter, the AMS operates independently of the BSC. The AMS will continue to operate if the BSC blower fails, providing an additional level of biosafety protection.

AMS gauge and controls

The AMS air flow gauge and controls are located on the front of the BSC in the lower right corner, to the left of the BSC controls.



Air flow gauge

The air flow gauge indicates the amount of suction being applied to evacuate the sort collection chamber and the aspirator drawer. The air flow is measured in CFM.

The air flow value should be:

- Low: 14 CFM ±1 CFM
- High: >26 CFM

See Buttons and LED indicators (page 210) and Audible alarms (page 211).

Buttons and LED indicators

The following table describes the functions of the AMS control buttons and the meaning of the LED indicators located above each button.

Button	Function	LED Color	Comments
AMS off	Turns the AMS off	Blue	Turn the AMS off when it is not in use.
AMS on/Low	Turns the AMS and low suction on. Turns low suction off.	Blue	Use low suction during normal sorting.
AMS on/High	Turns the AMS and high suction on. Turns high suction off.	Blue	Use high suction while removing a clog.

Audible alarms

An LED indicator will flash above the corresponding button when there is an alarm condition. The following table describes the meaning of the LED indicators and the alarms and conditions that trigger them.

LED Indicator	LED Color	Audible Alarm?	Condition
AMS on/Low	Flashing orange	Yes	The air flow is outside the range of 14 ±1 CFM. (This also occurs when the AMS is first turned on.)
AMS on/High	Flashing orange	Yes	The air flow is <26 CFM. (This also occurs when the AMS is first turned on.)

Operating the AMS

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

1. To turn on the AMS, press either the Low or the High button.
   - A blue indicator LED is illuminated over the button.
   - The air flow gauge goes through a startup sequence and then equilibrates.
2. Operate the AMS in:
   - Low during normal sorting.
   - High while removing a clog. See Responding to a nozzle clog during a sort (page 211) to troubleshoot a nozzle clog.
3. Press the AMS Off button to turn off the AMS.
   - A blue indicator LED is illuminated over the AMS off button.
   - The flow air gauge shuts off.

Responding to a nozzle clog during a sort

If the stream is disturbed during the sort (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes (if the Sweet Spot is on). The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog, do not open the sort collection chamber door or access the sort tubes before following this procedure.

Cell sorters that use droplet generation methods, such as the BD FACSymphony™ S6 cell sorter, can produce aerosols around the sample stream. When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door and the sort collection chamber door closed during sorting. Follow these steps to stop sample flow and evacuate potential aerosols before opening the sort collection chamber door.

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

1. Keep the sort chamber door closed.
2. If the stream has not already shut down automatically, turn off the stream by clicking the **Stream** button (with a checkmark) at the top of the **Breakoff** window.

   This will shut off the stream, unload the sample, and close the aspirator drawer.
3. Increase the air evacuation rate to high.
4. Open the aspirator drawer using the software controls.
5. Wait at least 60 seconds.

   This procedure will clear aerosols from the sort chamber.
6. Close the aspirator drawer.
7. Turn on the stream and view the breakoff.

   If the clog is removed, the breakoff will be similar to the breakoff before the clog.
8. If the clog is not cleared, turn the stream on and off several times to see if the clog has cleared.
9. If the clog is not removed, turn the stream off and perform the Clean Flow Cell procedure with DI water (see Cleaning the flow cell (daily) (page 140)), followed by turning the stream on to see if the clog has cleared.
10. Open the aspirator drawer and evacuate for at least 60 seconds before closing the aspirator drawer again.
11. You can now open the sort collection chamber and remove the sort collection device. Be aware that the surfaces of the device are potentially contaminated.

    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.
12. If it is necessary to change nozzles or remove a clog from a nozzle, see Cleaning the integrated nozzle (page 156).
13. With stream turned off, open the sort block door and dry the plates and surfaces as needed.
14. When removing collection tubes, be aware that the outside of the tube is potentially contaminated. Use alcohol swabs or bleach to wipe the outsides of tubes.
15. Press the AMS Low button.
16. Make sure that all chamber doors are closed and restart the stream.
17. Perform these tasks if needed:
    - Turn on the Sweet Spot
    - Check the drop delay
    - Check the side stream deflection

Working in the BSC

When working inside the BSC, we recommend that you:

- Keep the blower on at all times.
- Keep the AMS on while sorting.
- Keep the view screen closed to the safe-access opening of 8 inches each time you work in the BSC.
- Do not work in the BSC if an alarm sounds and the corresponding indicator is illuminated. See the The Baker Company *Biosafety Cabinet for BD FACSymphony™ S6 cell sorter Operator's Manual* for troubleshooting information. If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.
- Do not block the intake grilles or the diffusers because it obstructs air flow.

  Note: Consult a qualified safety professional for decontamination procedures appropriate for you laboratory.
- Decontaminate the BSC and cytometer before:
  - Turning off the blower.
  - Having maintenance or service performed.
- Keep the blower on at all times and keep the view screen closed when the BSC is not being used, especially if you want the inside of the BSC to remain contamination-free.
- Use an adjustable chair for comfort.

Cleaning the BSC

Use this procedure to clean the BSC after sorting potentially infectious agents.

Materials

- 10% chlorine bleach (0.5% sodium hypochlorite) solution

  Bleach can cause stainless steel to pit and crack. Ensure that you wipe down the BSC with 70% isopropyl alcohol to remove the bleach solution.
- 70% isopropyl alcohol
- Paper towels

Procedure

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

To clean the BSC:

1. Wipe down the following areas using paper towels and 10% bleach (0.5% sodium hypochlorite) solution:
   - Floor of the BSC in front of the cytometer, and on each side
   - Inside and outside surfaces of the view screen
   - Armrest/bypass
   - Cabinet Controls panel
   - Any other areas that might be contaminated
2. Repeat step 1 using 70% isopropyl alcohol.

Cleaning the base pan

Note: Before cleaning the base pan, clean the inside of the BSC. See Cleaning the BSC (page 213).

Use the following procedure to clean the base pan.

Materials

- 10% chlorine bleach (0.5% sodium hypochlorite)

  Bleach can cause stainless steel to pit and crack. Ensure that you wipe down the BSC with 70% isopropyl alcohol to remove the bleach solution.

- 70% isopropyl alcohol
- Paper towels
- A cleaning tool to reach into the base pan

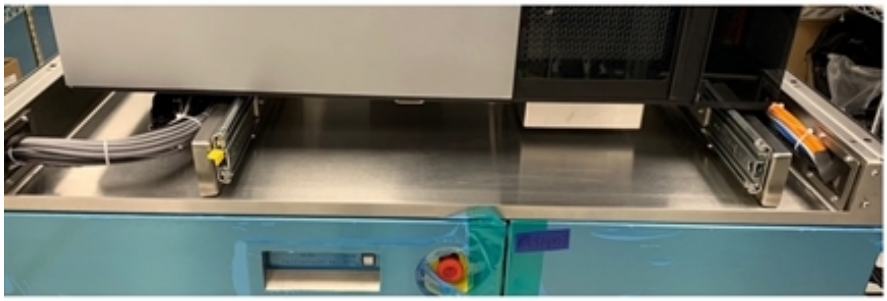
Procedure

Any surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning the base pan. Wear suitable protective clothing, eyewear, and gloves.

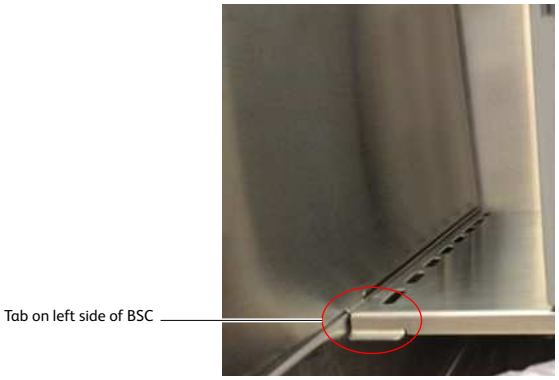
To clean the BSC:

1. Press in on the armrest/bypass and lift it up.

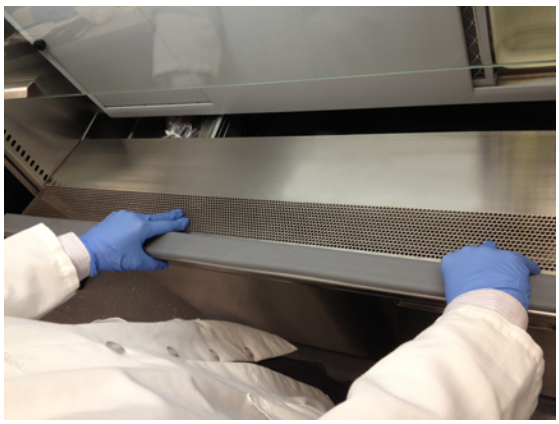
2. Remove the armrest/bypass.
3. Wipe down the armrest/bypass using paper towels and 10% bleach (0.5% sodium hypochlorite) solution.
4. Wipe down the armrest/bypass using paper towels and 70% isopropyl alcohol.
5. Set the armrest/bypass aside.
6. Wipe down the base pan using a cleaning tool and a 10% bleach (0.5% sodium hypochlorite) solution. Ensure that you also wipe down the areas underneath the electronic cables.



7. Wipe down the base pan using a cleaning tool and 70% isopropyl alcohol.
8. Check the position of the tabs above the base pan on either side of the BSC.



9. Reinstall the armrest/bypass by sliding it into position over the tabs.



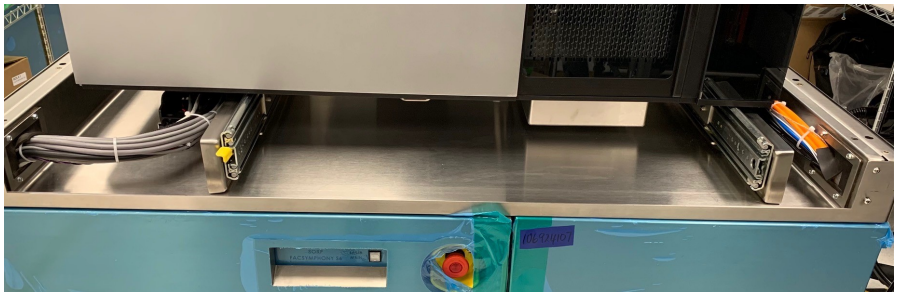
10. Lock the armrest/bypass into place by pushing it down and back so that the bottom snaps into place behind the lip of the base pan.

Replacing the electronics cable plastic sleeve

Some of the electronics cables for the BD FACSymphony™ S6 cell sorter are located under the cytometer in the base pan. The cables are wrapped in plastic sleeves to protect the cables from exposure to liquids. When cleaning the base pan, check the plastic sleeves and replace them as necessary. Replacements are located in the spares kit.

To replace the plastic sleeves:

1. Remove the armrest/bypass by following step 1 through step 5 in Cleaning the base pan (page 214).
2. Check the plastic sleeves covering the electronic cables in the left and right sides of the base pan.



3. If necessary, remove the plastic sleeves by carefully removing the ties at each end of the sleeve and peeling off the plastic sleeve.
4. Wrap the cable bundles with new plastic sleeves and secure with ties at each end.

12

BD FACSymphony™ S6 Temperature Control Option

The BD FACSymphony™ S6 Temperature Control Option can be used to control the temperature of sorted samples in the BD FACSymphony™ S6 cell sorter flow cytometer.

The following topics are described:

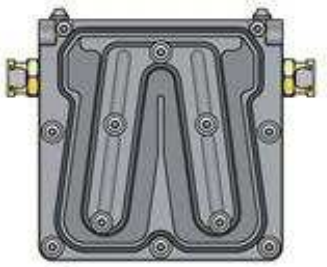
Option components

The BD FACSymphony™ S6 Temperature Control Option includes the following:

- A recirculating water bath
- Specially designed collection tube holders with ports for recirculating water

Tube holders are available in these styles: two-way 15-mL, four-way 12 × 75-mm, four-way 1.5-mL Eppendorf, and six-way 12 × 75-mm tubes.

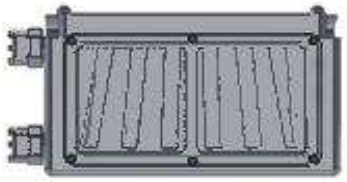
Water bath



Two-way 15 mL

Four-way 12 × 75 mm

Four-way 1.5 mL Eppendorf



Six-way 12 × 75 mm

When acquiring biohazardous samples, follow universal precautions at all times. Keep the sort block door and the sort collection chamber door closed during sorting. When sorting biohazardous samples into the temperature control tube holders, we recommend that you use the BD® Aerosol Management Option (for systems without a BSC) or the BD® Aerosol Management System (for systems with a BSC). If the nozzle develops a clog, see Responding to a nozzle clog during a sort with the AMO (page 195) or Responding to a nozzle clog during a sort (page 211) (for systems with the AMS).

The BD FACSymphony™ S6 Temperature Control Option is for research use only. It is not for use in diagnostic or therapeutic procedures.

Using the BD FACSymphony™ S6 Temperature Control Option

Note: Before you start the recirculating water bath, you must attach the tubing to the appropriate collection device.

Setting 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 counter-clockwise.

   Remove the top cover to access the knob, which is located inside the water bath toward the back. See the operating instructions supplied with the water bath for additional details on this process. This is referred to as position 1.
4. Connect the clear tubing end of the insulated hoses to the input and output ports on the water bath.

   Slide the tubing over the hose barbs and twist gently while installing to get the tubing completely over the barbs.

5. Connect the insulated hoses from the recirculating water bath to the ports on the right side of the cytometer base.

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

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

Setting up the tube holder

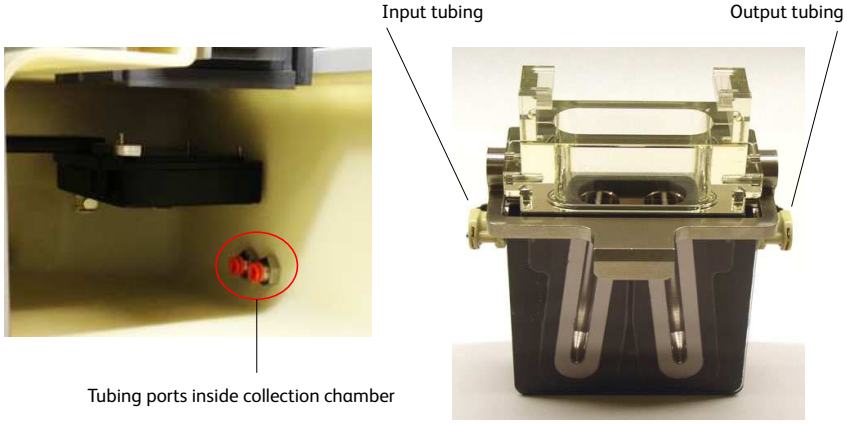
This section describes how to install the temperature control tube holder on the instrument and how to attach the recirculating water tubing to the tube holder.

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 and gloves.

1. Place collection tubes in the temperature control tube holder.
2. Attach the recirculating water tubing to the tube holder.
   a. Open the sort collection chamber door.

      NOTE The tubing ports are labeled *In* and *Out*. Attach the input tubing to the port on the left side of the collection tube holder, and the output tubing to the port on the right side.

   b. To attach the tubing, push it into the port until the tubing snaps into place.



   If you need to remove the tubing, push in the orange collar as you pull the tubing out of the port.
3. Install the tube holder on the instrument.
4. Install the temperature control tube holder.
5. Close the sort collection chamber door and start up the water bath.

Setting up the ACDU stage

This section describes how to attach the recirculating water tubing to the stage used with the automated cell deposition unit (ACDU).

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.

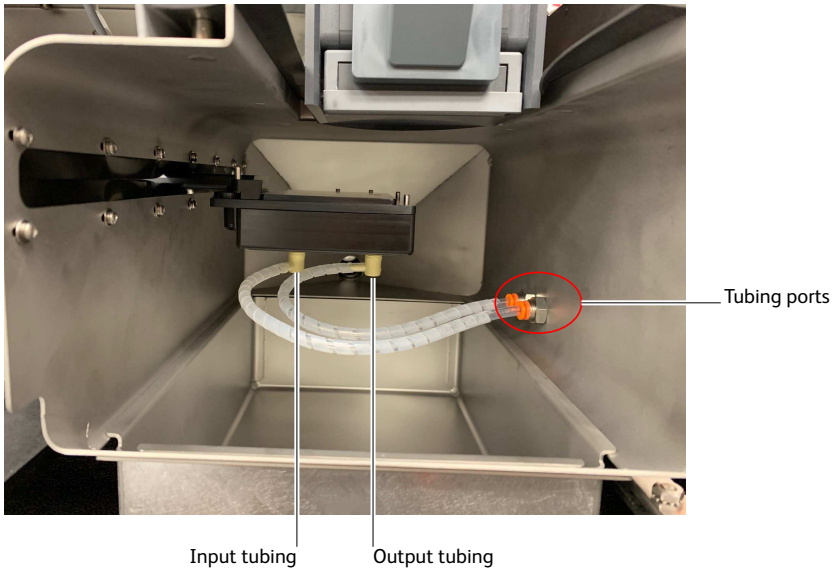
1. Install the splash shield below the aspirator drawer.
   a. Close the sort block door and open the sort collection chamber door, if needed. (The sort block door must be closed to open the collection chamber door.)
   b. Remove the tube holder, if one is installed.

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

2. Click the **Access Stage** button to bring the ACDU stage to the front.
   a. Open an experiment, if one is not already open, and create a sort layout for any of the tubes.
   b. In the **Sort Layout** view, click the **Access Stage** button to move the stage to the front of the sort collection chamber.
3. Attach the recirculating water tubing to the ACDU stage.

   Note: The tubing ports are labeled *In* and *Out*. Attach the input tubing to the port on the left side of the stage, and the output tubing to the port on the right side.

   To attach the tubing, push it into the port until the tubing snaps into place.



   If you need to remove the tubing, push in the orange collar 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 up the water bath.

Starting up the water bath

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

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

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

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

Required Sample Temp (˚C)	Water Bath Setting (˚C) at Room Temperature of 15 ˚C	Water Bath Setting (˚C) at Room Temperature of 30 ˚C
4	2	2
37	43	41
42	52	45

3. Wait at least 90 minutes to allow the recirculating water to reach the required temperature. The duration may be longer if switching over a large temperature range or when attempting to cool the sample.

Maintenance

To maintain the recirculating water bath, see the documentation provided by the manufacturer.

Tube holders

Remove the tube holders when you are finished using them, and clean them periodically before storage.

When you detach the recirculating water tubing from the tube holder, any fluid remaining in the tubing can leak into the sort collection chamber. To ensure that fluid is aspirated from the sort collection chamber, make sure that the sort chamber aspirator pump is on (do not turn off the instrument main power) before you detach the tubing. Use caution when handling tubing containing hot water.

To detach the recirculating water tubing, push in the orange collar as you pull the tubing out of the port.

Clean the temperature control tube holders by wiping them down with an appropriate cleaning fluid (for example, 70% ethanol, 5% bleach, or DI water). Dry them with a lint-free cloth before storage.

Recirculating water tubing

Inspect the tubing periodically for leaks, plugs, or contaminants. If needed, remove the tubing and clean it with an appropriate cleaning solution, or replace the tubing. Contact your BD Biosciences service representative for replacement tubing.

Specifications

Specifications for the recirculating water bath are as follows.

Note: The following specifications are for the US version only.

The BD FACSymphony™ S6 Temperature Control Option includes the Lauda® Ecoline cooling/heating bath, model RE 106.

- Operating temperature range: –20–+20 °C
- Ambient temperature range: 5–40 °C
- Heater power for 115 V/60 Hz: 1.3 kW
- Maximum flow rate at pump output of 5: 17 L/min
- Maximum bath volume: 4–6 L
- Power consumption for 115 V/60 Hz: 1.4 kW

For more information, see the manufacturer's documentation.

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Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, California 95131 USA

BD Biosciences
European Customer Support
Tel +32.53.720.600
help.biosciences@bd.com

bdbiosciences.com
ResearchApplications@bd.com