

BD™ High Throughput Sampler User's Guide

For Research Use Only

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APC-Cy7: US 5,714,386

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NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense.

Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

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Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.

Notice

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History

Revision	Date	Change Made
338642	10/04	Initial release
640756	3/06	Updated to include BD FACSDiva software, version 5.0, and compatibility with BD FACSCanto and BD FACSCanto II flow cytometers.
642224	6/07	Updated to include BD FACSDiva software, version 6.0, added configuration and performance tracking module, operational improvements, and increased cytometer-specific improvements.
23-11232-00 Rev. 01	4/2012	Updated for BD FACSDiva 7.0 software and BD LSRFortessa flow cytometer.

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About This Guide

This guide describes how to set up and operate the BD™ High Throughput Sampler (HTS) option with the BD™ LSR II, BD LSRFortessa™, BD FACSCanto™, and BD FACSCanto™ II flow cytometers. You should know how to operate your flow cytometer before using the HTS. For important safety information, see the safety guide provided with the HTS.

Cytometer function is controlled by BD FACSDiva™ software. In this guide, you will find a description of BD FACSDiva software features specific to the HTS.




BD LSR II, BD LSRFortessa, BD FACSCanto, and BD FACSCanto II flow cytometers modified with the HTS can acquire samples from plates or tubes. Even when acquiring samples using the HTS, consult the appropriate cytometer user's guide for information about flow cytometer operation, daily shutdown, maintenance, and troubleshooting.

The *BD High Throughput Sampler User's Guide* assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, see the documentation provided with your computer.

Conventions

The following tables list the conventions used throughout this guide. Table 1 lists the symbols that are used in this guide or on safety labels to alert you to a potential hazard. Text and keyboard conventions are shown in Table 2.

Table 1 Hazard symbols^a

Symbol	Meaning
	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death
	Risk of electric shock
	Biological risk

a. Although these symbols appear in color on the cytometer, they are in black and white throughout this user's guide and their meaning remains unchanged.

Table 2 Text and keyboard conventions

Convention	Use
NOTE	Describes important features or instructions.
<i>Italics</i>	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, “select File > Print ” means to select Print from the File menu.
Ctrl+X	When used with key names, a plus symbol means to press two keys simultaneously. For example, Ctrl+P means to hold down the Control key while pressing the letter <i>p</i> .

Technical Assistance

For technical questions or assistance in solving a problem:

- Read the section of the user's guide specific to the operation you are performing.
- See Chapter 5, Troubleshooting.

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

When contacting BD Biosciences, have the following information available:

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

For cytometer support from within the US and Canada, call 877.232.8995.

Customers outside the US and Canada, contact your local BD representative or distributor.

If you need to send your HTS back to BD Biosciences for repair, see Appendix B on page 165 for instructions. Note that depot repair procedures might be different outside of the United States. Contact your local BD Biosciences service representative for information for your region.

New Functions for the HTS

BD FACSDiva software version 7.0 includes the following new functions that work specifically with the HTS:

- **Automation.** This function allows you to connect to BD FACSDiva software via a server socket to issue commands such as creating experiments and recording plates through the use of a published command Application Programming Interface (API). You can set up a walkaway type of automation process where clients can coordinate interactions between external hardware (such as robotics), third party software, BD FACSDiva software, and the cytometer.
- **Baseline restore.** This function is available for the BD FACSCanto, BD LSR II, and BD LSRFortessa cytometers. At the start of data acquisition and only when data is recorded, BD FACSDiva software ignores the initial data for a period of time defined by the baseline restore period.

System Overview

The HTS is a compact, high-speed sample loading device for use with the BD LSR II, BD LSRFortessa, BD FACSCanto, and BD FACSCanto II flow cytometers. BD FACSDiva software controls the sample loader, providing automated acquisition of samples from a multiwell plate.

The following topics are covered in this chapter:

- Hardware Overview on page 14
- Components on page 17
- Cytometer Connections on page 21
- Setting Up for Plate-Based Acquisition on page 28
- Acquisition Sampler Modes on page 34

Hardware Overview

The HTS provides the following basic functionality:

- Acquires samples from 96- and 384-well plates (standard depth)
- Includes two throughput modes: standard and high
- Minimizes carryover
- Provides user-definable mixing and sample introduction protocols
- Is user-installable (software and HTS only, initial installation excluded)
- Supports immunophenotyping assays

The HTS can process a 96-well plate in approximately 44 minutes in standard mode and approximately 15 minutes in high-throughput mode using the default settings listed in Table 1-1.

Table 1-1 Default throughput mode settings

Setting	Standard Mode	High-Throughput Mode
Sample flow rate (µL/sec)	1	1
Sample volume (µL)	10	2
Mixing volume (µL)	100	50
Mixing speed (µL/sec)	180	200
Number of mixes (cycles)	2	2
Wash volume (µL)	400	200
Approximate acquisition time (min)	44	15



For more information, see Loader Settings on page 51.

The HTS is installed on the cytometer by a BD Biosciences service engineer. Once installed, the HTS enables quick conversion of the flow cytometer from tube to plate-based acquisition.

Figure 1-1 HTS on a BD LSR II flow cytometer



Figure 1-2 HTS on a BD LSRFortessa flow cytometer (front orientation)



Figure 1-3 HTS on a BD LSRFortessa flow cytometer (side orientation)



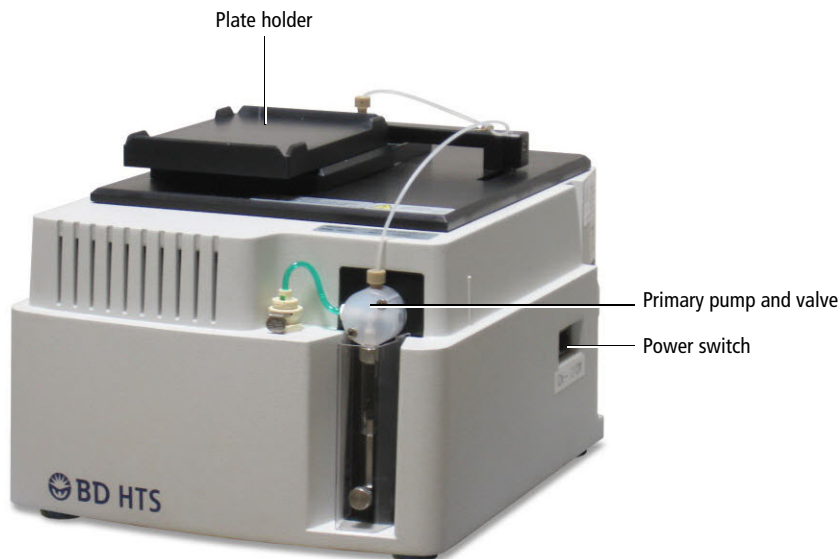
Figure 1-4 HTS on a BD FACSCanto II flow cytometer



Components

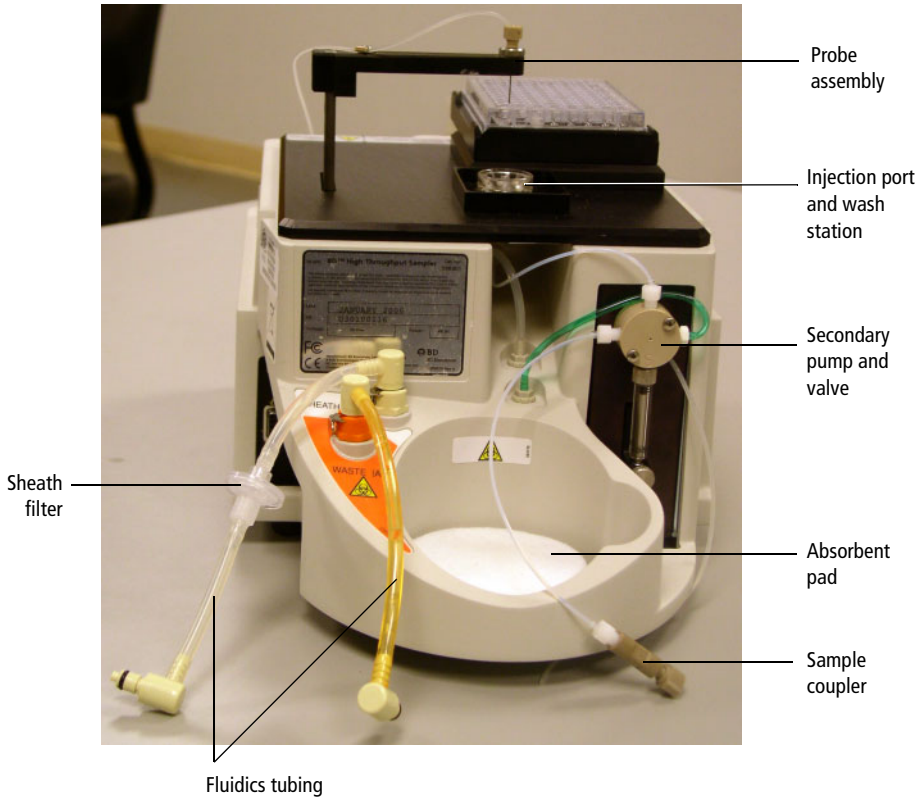
The following figures show the hardware components of the HTS.

Figure 1-5 HTS for BD LSR II, BD LSRFortessa, and BD FACSCanto cytometers (front view)



Component	Description
Plate holder	Moves left to right and front to back to position the plate so the probe can pick up sample.
Primary pump and valve	Enables mixing and aspiration of sample and delivers it to the flow cell in standard mode.
Power switch	Turns the HTS on and off. Has an LED indicator.

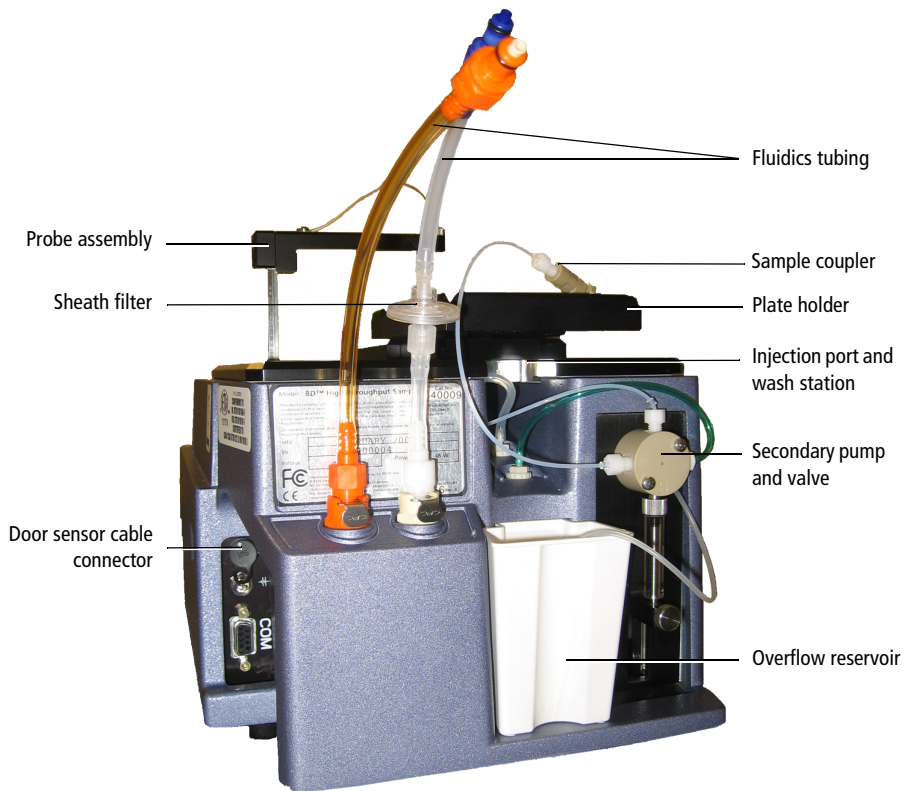
Figure 1-6 HTS for BD LSR II, BD LSRFortessa, and BD FACSCanto cytometers (rear view)



Component	Description
Sheath filter	Filters incoming sheath fluid to the HTS.
Fluidics tubing	Carries sheath (clear) and waste (orange).
Probe assembly	Moves front to back and up and down to transfer sample between the plate holder and the injection port/wash station.
Injection port/wash station	Provides an interface for sample injection and probe washing.

Component	Description
Secondary pump and valve	Delivers sample to the flow cell in high-throughput mode.
Absorbent pad	Collects potential overflow from the injection port/wash station or drips from the cytometer sample injection tube (SIT).
Sample coupler	Connector between the HTS and the cytometer SIT.

Figure 1-7 HTS for BD FACSCanto II (rear view)



Component	Description
Probe assembly	Moves front to back and up and down to transfer sample between the plate holder and the injection port/wash station.
Sheath filter	Filters incoming sheath fluid to the HTS.
Door sensor cable connector	Detects if the safety door is open.
Fluidics tubing	Carries sheath (clear) and waste (orange).
Sample coupler	Connector between the HTS and the cytometer SIT.
Plate holder	Moves left to right and front to back to position plate so the probe can pick up sample.
Injection port/wash station	Provides an interface for sample injection and probe washing.
Secondary pump and valve	Delivers sample to the flow cell in high-throughput mode.
Overflow reservoir	Collects potential overflow from the injection port/wash station or drips from the cytometer SIT.

Cytometer Connections

Cytometer Interface Panel

The HTS is installed on the base plate or in the enclosure (BD FACSCanto II only). Sheath and waste travel between the cytometer and the HTS via connectors in the cytometer interface panel. The power cable, communication cable, and door sensor cable (BD FACSCanto II only) that connect to the HTS can be found at the cytometer interface panel. See Figure 1-8 to Figure 1-11 for the connection points for the HTS and see Installing the HTS on page 179 for detailed instructions.

Figure 1-8 Cytometer interface panel (BD LSR II)

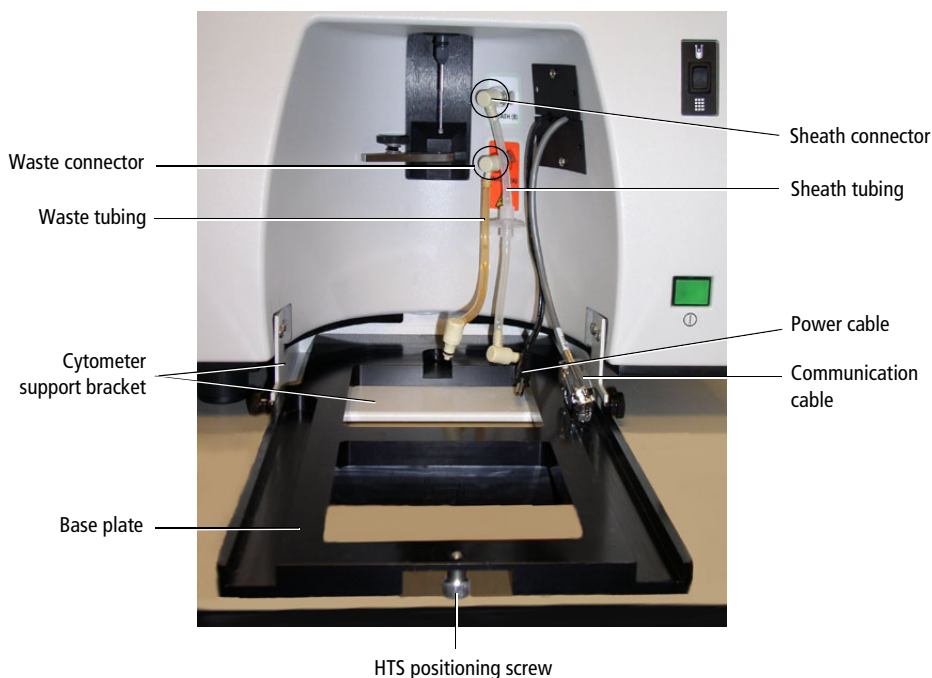


Figure 1-9 Cytometer interface panel (BD LSRFortessa)

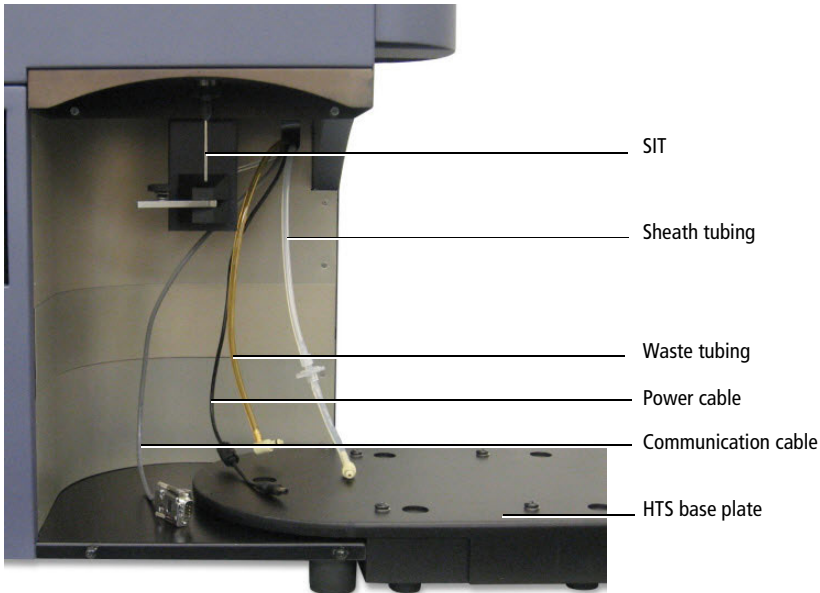


Figure 1-10 Cytometer interface panel (BD FACSCanto)

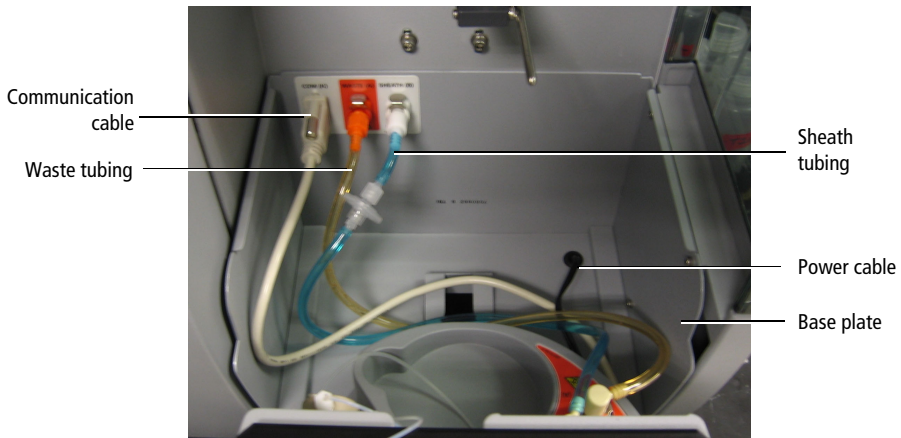
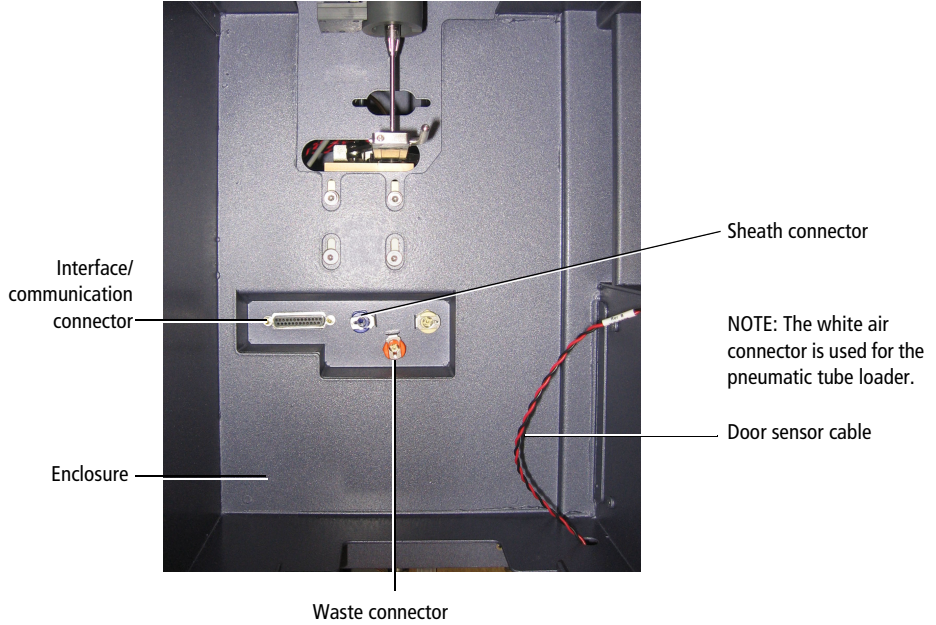


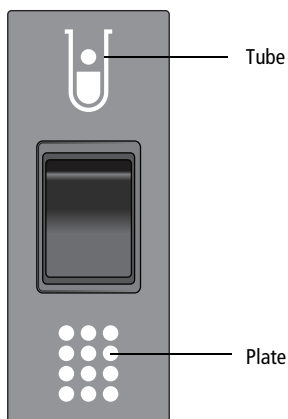
Figure 1-11 Cytometer interface panel (BD FACSCanto II)



Tube/Plate Acquisition Mode Switch

The BD LSR II and BD LSRFortessa interface panels also include an acquisition mode switch.

Figure 1-12 Tube/plate acquisition mode switch for HTS



- In Tube mode (U), the droplet containment module (DCM) vacuum functions normally—the vacuum is on when the arm is positioned to the side and off when the arm is centered. Backdripping from the SIT is contained when the DCM sleeve is installed.
- In Plate mode (3x3 grid), the DCM vacuum on the cytometer is disabled. Drip containment is provided by the absorbent pad in back of the HTS (Figure 1-6 on page 18).



Figure 1-13 Tube/plate acquisition mode switch on the BD LSR II flow cytometer

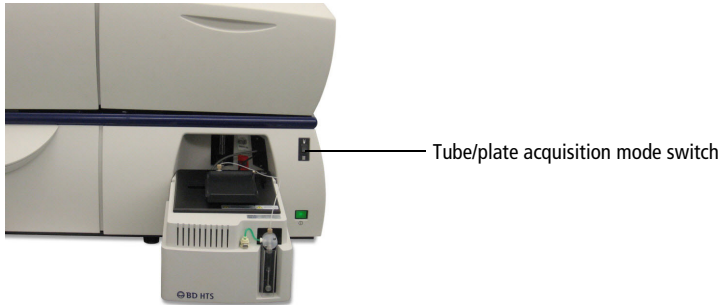


Figure 1-14 Tube/plate acquisition mode switch on the BD LSRFortessa flow cytometer



Sample Coupler

A sample coupler connects the injection port tubing on the HTS to the cytometer SIT (Figure 1-19 on page 33).

For the BD LSR II and BD LSRFortessa, the DCM sleeve is replaced by the SIT protector. The SIT protector is a modified sleeve that prevents the sample injection tube from bending during installation of the HTS sample coupler.

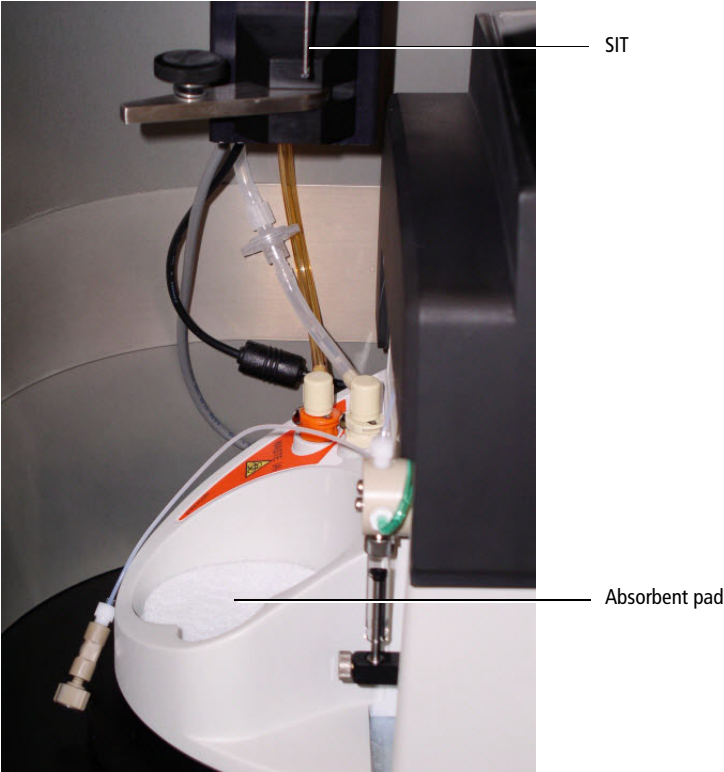
For the BD FACSCanto and BD FACSCanto II, the sample coupler is installed on the SIT, while the aspirator arm rests against the sample coupler. Flip the aspirator arm bar to the back (as shown in Figure 1-19 on page 33) to ensure that it does not come in contact with the HTS probe. This position also ensures that the aspirator arm is able to detect an installed sample coupler.

Biohazardous Waste

When you are not acquiring samples using the HTS, install the DCM sleeve and switch the cytometer to tube mode. For more information, see Setting Up for Tube-Based Acquisition on page 103.



While connecting or disconnecting the HTS, backdripping from the SIT is contained by the absorbent pad in the rear of the HTS.

Figure 1-15 HTS absorbent pad on the BD LSRFortessa flow cytometer




Setting Up for Plate-Based Acquisition

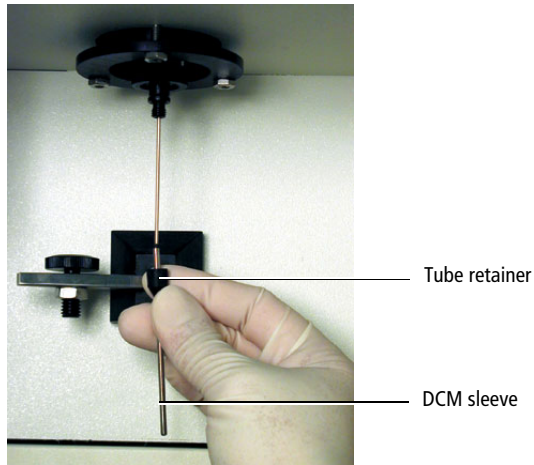
This section describes how to set up the cytometer for plate-based acquisition. When switching from tube mode to plate mode, you need to place the acquisition mode switch in plate mode, replace the DCM sleeve with the SIT protector, and connect the sample coupler.

  Any cytometer surface that comes in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling cytometer hardware. Wear suitable protective clothing and gloves. For instrument precautions, see Biohazardous Waste on page 26.

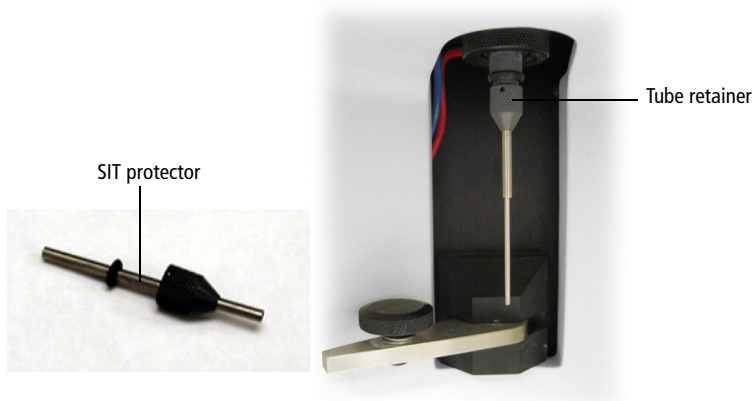
Setting Up for Plate-Based Acquisition (BD LSR II and BD LSRFortessa)

To switch from tube mode to plate mode:

- 1 If necessary, install the HTS.
See Installing the HTS on page 179.
- 2 Set the acquisition control switch to plate mode () .
- 3 Unscrew the tube retainer that holds the DCM sleeve onto the SIT and carefully remove the sleeve.



- 4 Install the SIT protector.



- a Slide the protector over the SIT and push up on the tube retainer until you can screw it onto the SIT.
 - b Tighten the tube retainer.
- 5 Carefully attach the HTS sample coupler to the cytometer SIT.
 - a Move the aspirator arm to the left.

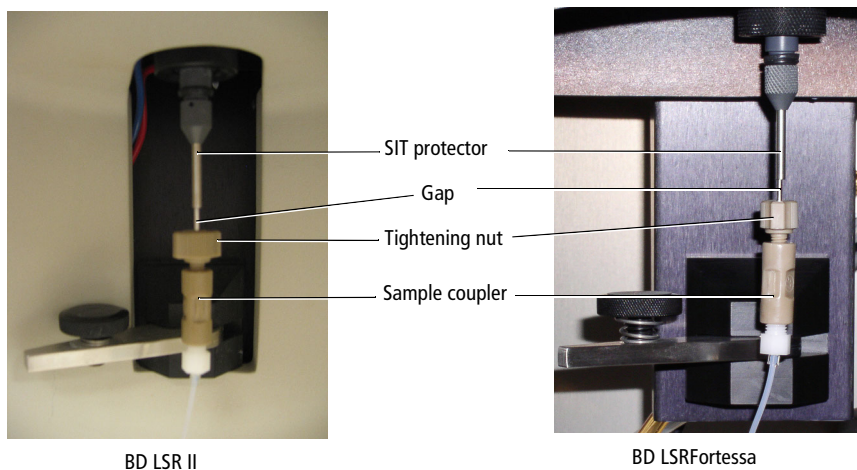
- b** Slide the sample coupler onto the SIT until you reach a hard stop. Make sure the sample coupler tubing is not kinked or twisted.
- c** Hold the coupler with one hand while you tighten the top nut with the other hand.

There should be a gap between the tightening nut and the bottom of the SIT protector (Figure 1-16 on page 30). If you do not see a gap, unscrew the tube retainer, push the SIT protector all the way up, and retighten the tube retainer.

- d** Make sure the sample coupler is securely connected to the SIT.



Figure 1-16 Installing the sample coupler on the BD LSR II or BD LSRFortessa



- 6** Turn on the HTS.

The power switch is on the right side of the HTS. See Figure 1-17.

Figure 1-17 Power switch (BD LSR II flow cytometer)

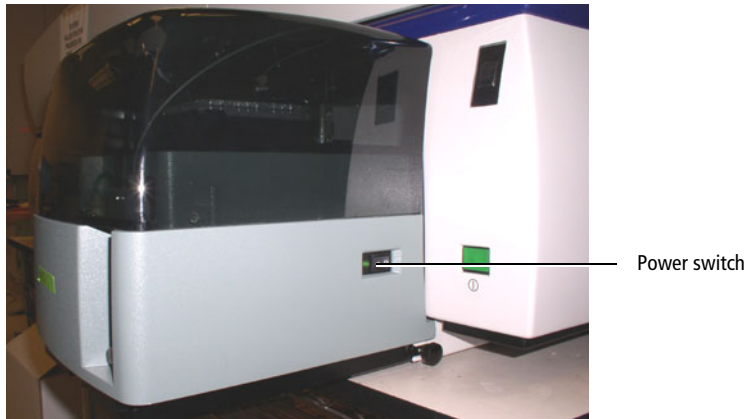


Figure 1-18 Power switch (BD LSRFortessa flow cytometer)



NOTE (BD LSR II and BD LSRFortessa) Soft standby mode is not available when you are acquiring samples in plate mode. See your cytometer user's guide for information about soft standby mode.

Setting Up for Plate-Based Acquisition (BD FACSCanto and BD FACSCanto II)

To switch from tube mode to plate mode:

- 1 Turn on the cytometer and the HTS.

(BD FACSCanto) Turn on the cytometer, followed by the HTS.

(BD FACSCanto II) The HTS turns on automatically when the cytometer is powered on.

- 2 Remove/open the HTS safety cover.

(BD FACSCanto) Tilt the front of the cover up, then slide the cover forward to remove it.

(BD FACSCanto II) Slide open the covers. Slide the front cover to the left and slide the side cover to the back.

- 3 Perform a sheath fluid exchange.

See Exchanging the Sheath Fluid (BD FACSCanto and BD FACSCanto II) on page 107.

- 4 Attach the HTS sample coupler to the cytometer SIT.

- a Move the aspirator arm to the left.

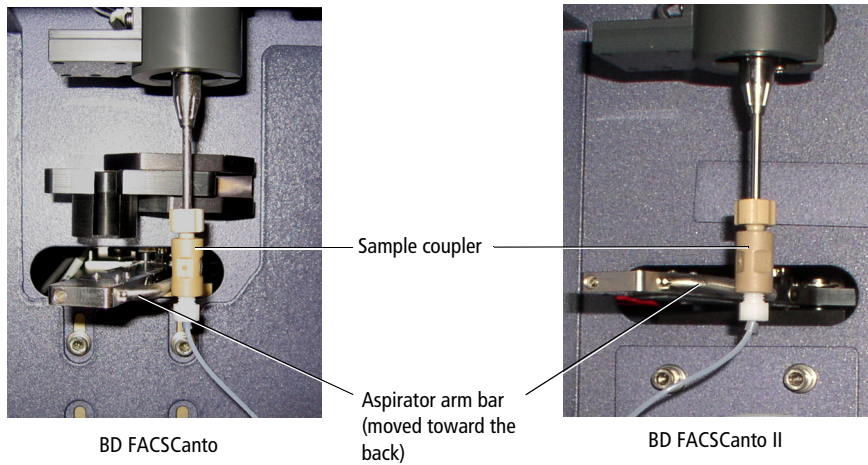
- b Slide the sample coupler onto the SIT until you reach a hard stop. Make sure the sample coupler tubing is not kinked or twisted.

- c Hold the coupler with one hand while you tighten the top nut with the other hand.

- d Allow the aspirator arm to rest against the sample coupler.

NOTE Flip the aspirator arm bar backwards to ensure that it does not come in contact with the HTS probe.

Figure 1-19 Installing the sample coupler on the BD FACSCanto or BD FACSCanto II



- 5 Make sure the sample coupler is securely connected to the SIT and prime the HTS twice by selecting **HTS > Prime**.

Acquisition Sampler Modes

During acquisition, the HTS processes samples differently based on the throughput mode. Table 1-2 compares acquisition in each mode.

Table 1-2 Comparison of acquisition sequence in standard and high-throughput mode

Sequence	Standard mode	High-throughput mode
1	<ul style="list-style-type: none"> • Raises the probe to the cleaning position • Flushes the probe using the primary pump • Turns on the waste pump 	<ul style="list-style-type: none"> • Raises the probe to the cleaning position • Flushes the probe using the primary pump • Turns on the waste pump
2	<ul style="list-style-type: none"> • Raises the probe • Refills the primary pump • Turns off the waste pump • Draws a separator bubble into the tip of the probe 	<ul style="list-style-type: none"> • Raises the probe • Refills the primary pump • Draws a separator bubble into the tip of the probe
3	Moves the probe and the plate holder so the probe is positioned above the required well	Moves the probe and the plate holder so the probe is positioned above the required well
4	Lowers the probe and mixes the sample	Lowers the probe and mixes the sample
5	Aspirates the requested sample volume plus 20 μL of aspirated excess volume (required by the system) along with a separator bubble	<ul style="list-style-type: none"> • Aspirates a fixed volume of 22 μL of mixed sample (independent of the sample volume entered in the software) along with a separator bubble • Turns off the waste pump
6	Moves the probe up, over, and down into the injection port	Moves the probe up, over, and down into the injection port

Sequence	Standard mode	High-throughput mode
7	A non-selectable, fixed boost command transports sample to the flow cell to prime the sample flow path	A non-selectable, fixed boost command transports sample to the flow cell to prime the sample flow path
8	When the boost command is done, BD FACSDiva software begins acquiring the sample	<ul style="list-style-type: none"> • When the boost command is done, delivers the sample to the flow cell using the secondary (high-throughput) pump • BD FACSDiva software begins acquiring the sample • Simultaneously raises the probe to the cleaning position and the primary pump begins processing the next well in parallel to acquisition
9	After the last well in a sequence, raises the probe to the cleaning position and performs a final flush and refill	After the last well in a sequence, raises the probe to the cleaning position and performs a final flush and refill
10	Lowers the probe into the injection port/wash station	Lowers the probe into the injection port/wash station

Loader Settings

Sample Well Volumes

There are different volumes to take into consideration when choosing loader settings. Figure 1-20 shows different well volumes and Table 1-3 defines each volume type.

Figure 1-20 Well volumes

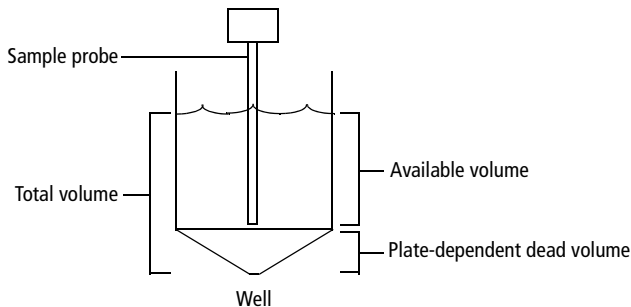


Table 1-3 Volume type

Volume type	Definition
Well volume	Volume that well can hold filled to the brim
Dispensed volume	Volume pipetted into well minus aspirated excess volume
Aspirated excess volume	Standard mode = 20 μ L
Available volume	Volume pipetted into well minus aspirated excess volume minus dead volume
Minimum volume	50 μ L for both standard and high-throughput modes for 96-well plates
Mixing volume	Approximately one-half the available volume NOTE A mixing volume that is larger than the available volume introduces air bubbles into the sample
Dead volume	Volume in the bottom of the well that the probe cannot reach
Sample volume	Amount of sample requested for analysis in BD FACSDiva software

Mixing

HTS mixing efficiency is impacted by the viscosity of the sample in the well. Adjust the mixing volume, speed, and the number of mixes to obtain the most homogeneous population for acquisition.

A greater number of mixes might allow you to decrease the mixing volume and speed, but this could impact sample throughput. Increasing the mixing speed and volume might improve throughput and mixing efficiency, but it could also introduce air bubbles in the sample well. In addition, increased mixing speed could compromise the separator bubble between the sample and sheath, resulting in sporadic event rates and possibly higher carryover.

Use default values as a starting point (see Table 2-4 on page 54) with a mixing volume of one-half the total sample volume. Note that samples are mixed less effectively in flat-bottom wells than U- or V-bottom wells. When using flat-bottom wells, you might want to mix the sample well before pipetting it into the plate, and acquire the sample before it has a chance to settle.

Carryover

You can vary the amount of wash volume to minimize carryover. In general, a greater wash volume results in less carryover, but the greater the volume, the slower the system throughput.

The minimum volume is determined by the sample and mixing volume. To start with, try a 400- μ L wash for 10 μ L of sample volume. As the sample and mixing volumes decrease, the wash volume can also be reduced.



Throughput

The speed at which the HTS completes a plate depends on the acquisition time (sample volume/sample rate), acquisition mode (high throughput or standard), mixing volume and speed, number of mixes, and wash volume. To achieve optimal throughput, we recommend that you concentrate the sample, reduce the sample volume (10 μ L or less), increase the sample rate (1 μ L/sec or greater), minimize the number of mixes (2 or less), and decrease the wash volume (400 μ L or less).

NOTE The BD FACSCanto II maximum event rate is 10,000 events/second.

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BD FACSDiva Software Overview

This chapter describes the BD FACSDiva software features necessary to operate the HTS with the BD LSR II, BD LSRFortessa, BD FACSCanto, and BD FACSCanto II flow cytometers. For an in-depth description of software components not described in this chapter, see the *BD FACSDiva Software Reference Manual*.

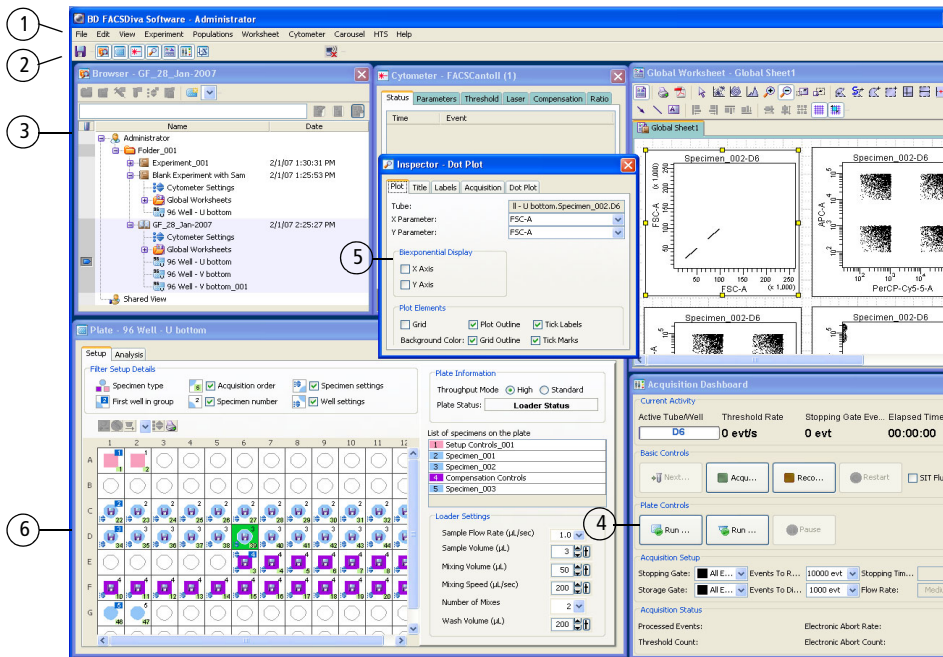
The following topic is covered in this chapter:

- Workspace Components on page 40

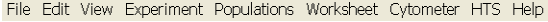


Workspace Components





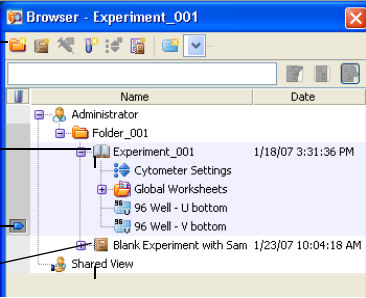
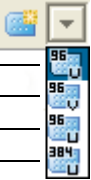
When you start BD FACSDiva software, the workspace opens. Windows containing the main application components are displayed within the workspace. Display additional windows by clicking buttons on the Workspace toolbar. The workspace components (Figure 2-1) are described in the following table.


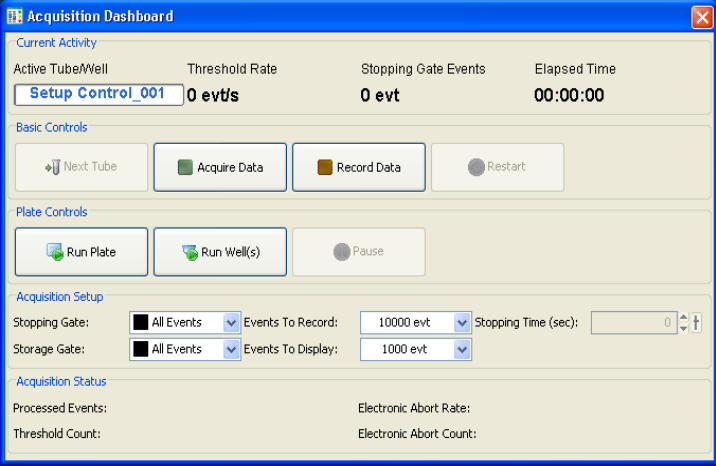
Figure 2-1 BD FACSDiva workspace displaying an open experiment and the Plate window



The following table describes some of the more common workspace components specific to running the HTS.

Number	Common Workspace Components
1	<p>Use the commands in the menu bar to operate the software.</p>  <ul style="list-style-type: none"> • Use the Experiment menu to add a new experiment to the Browser or open a template. A new experiment automatically contains global cytometer settings and a global worksheet. For information, see Creating Experiments on page 72. • Use the commands in the HTS menu to perform maintenance procedures on the HTS. For information, see Maintenance on page 111.
2	<p>Click a button on the Workspace toolbar to hide or show the corresponding window.</p>  <p>Windows can be resized by dragging a border or corner. Click the Plate button () to view the Plate window.</p>

Number	Common Workspace Components
<p data-bbox="266 215 292 240">3</p> 	<ul style="list-style-type: none"> <li data-bbox="350 215 1079 272">• Use the Browser window to create and set up experiments and view experimental data hierarchically. <p data-bbox="377 285 938 313">In the Browser, double-click an experiment to open it.</p> <ul style="list-style-type: none"> <li data-bbox="350 329 1130 386">• Click the New Experiment button () on the Browser toolbar to create a new experiment. <li data-bbox="350 402 1069 459">• Click the New Plate button () on the Browser toolbar to add a default 96-well U-bottom plate to the open experiment. <li data-bbox="350 475 1110 532">• Click the arrow next to the New Plate button () to select a new plate type to add to the experiment. <p data-bbox="377 545 796 573">The type you select becomes the default.</p> <div data-bbox="350 586 850 881">  <p data-bbox="391 594 458 643">Browser toolbar</p> <p data-bbox="364 699 458 748">Open experiment</p> <p data-bbox="357 773 458 821">Current tube pointer</p> <p data-bbox="364 837 458 886">Closed experiment</p> </div> <div data-bbox="532 919 850 1097">  <ul style="list-style-type: none"> <li data-bbox="555 967 743 995">96-well U-bottom plate <li data-bbox="555 1000 743 1027">96-well V-bottom plate <li data-bbox="541 1032 743 1060">96-well flat-bottom plate <li data-bbox="532 1065 743 1092">384-well flat-bottom plate </div>

Number	Common Workspace Components
<p data-bbox="314 215 333 237">4</p> 	<p data-bbox="397 215 1177 329">Use the Acquisition Dashboard to acquire and record well data. Use the Plate controls to acquire and record wells in sequence using the selected throughput mode. Run Plate runs the wells from the current position to the end of the plate. Run Well(s) runs the selected wells only.</p> <p data-bbox="397 345 1120 399">Use the Basic Controls to manually acquire or record selected wells in standard mode using the current loader settings.</p>  <p data-bbox="397 889 1139 915">You can expand (show) and contract (hide) the Acquisition Dashboard.</p> <p data-bbox="397 932 1177 1045">To show or hide the Plate Controls, Acquisition Setup, or Acquisition Status sections of the Acquisition Dashboard, right-click the Acquisition Dashboard in any blank area (except for Basic Controls) and select a section to show or hide from the shortcut menu.</p> <p data-bbox="397 1062 1096 1115">You can resize the Acquisition Dashboard using standard Windows methods.</p>


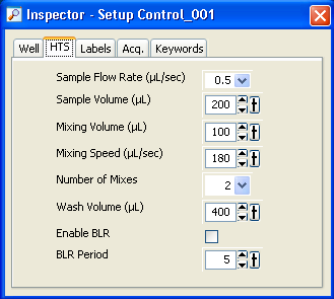

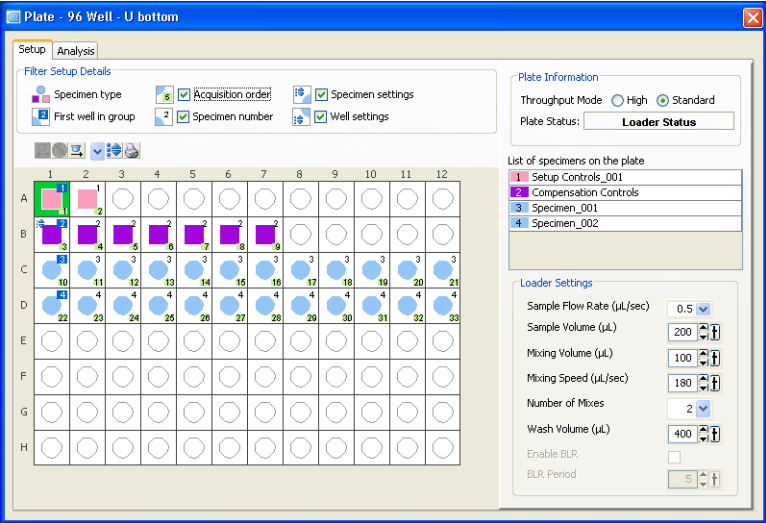
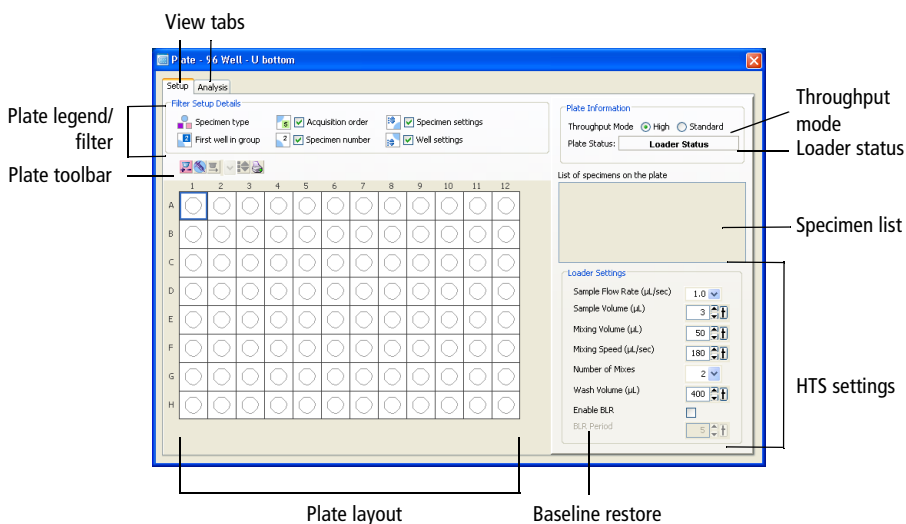
Number	Common Workspace Components
<p>5</p> 	 <p>Use the Inspector to view or modify the attributes of a single object or set of objects on the worksheet or plate, or in the Browser. The contents of the Inspector change depending on what is selected in the workspace.</p> <p>The Inspector can be used to view or change HTS (Loader) settings. See Loader Settings on page 51.</p>
<p>6</p> 	<p>Set up, acquire, and analyze plate-based experiments in the Plate window. Use this window also to select the throughput mode and adjust loader settings.</p> 

Plate Window

Most of the features for running plate-based experiments on the HTS are located in the Plate window (Figure 2-2 on page 45). The tabs at the top of the Plate window represent views that organize the workflow for the plate. The Setup view is for setting up the experiment and the cytometer, adjusting cytometer settings, and acquiring and analyzing samples from a plate. The Analysis view is for assigning keywords to wells and analyzing data after a run. The Plate window is available when an experiment is open and you double-click the plate in the Browser.

Figure 2-2 Setup view of the Plate window



See the following sections for a description of components in, and the functionality of, each view.

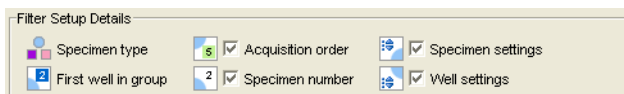
Setup View Components

The Setup view contains controls for designing experiments, running plate-based acquisition, and monitoring acquisition status. For instructions on setting up an experiment, see [Creating Experiments](#) on page 72. All entries made in the Setup view can be saved as a template, either for general projects or as a default experiment for a user-defined project.

The following components are available in the Setup view.

Plate Filter

The Filter Setup Details area provides a legend indicating the type of specimens in the plate and allows you to filter (hide or show) acquisition order, specimen number, specimen settings, and/or well settings.



Clear the checkbox next to acquisition order, specimen number, specimen settings, and/or well settings to hide the symbol in the plate layout.

Icon	Category	Description
	Specimen type	Indicates the type of control or sample assigned to a given well. The pink square represents a setup control, the purple square a compensation control, and the blue circle a specimen.
	First well in group	A dark blue square appears for the specimen number in the upper-right corner of the first well for each specimen.
	Acquisition order	The order (sequence number) in which each well will be acquired appears with a green background in the bottom-right corner of the well.
	Specimen number	The specimen number appears in the upper-right corner. Each well belonging to the same specimen has the same specimen number.






Icon	Category	Description
	Specimen settings	When cytometer settings are added to a specimen, the cytometer settings icon appears in the upper-left corner of the well.
	Well settings	When cytometer settings are added to a well, the cytometer settings icon appears in the lower-left corner of the well.

Plate Toolbar



The Plate toolbar contains the following buttons.

Icon	Command Name	Description
	Add Setup Controls	Select a well or group of wells, then click the Add Setup Controls button to add well(s) to the plate layout for adjusting cytometer settings. The wells are for setup only, and are not recorded.
	Add Specimen Wells	Select a well or group of wells, then click the Add Specimen Wells button to add a specimen to the plate layout. Wells are run in the order they are selected.
	Add Well	Select a well that has already been defined, then click the Add Well button to add another sample or set up a control well. The new well is inserted to the right of or below the selected well. Click the arrow to the right of the Add Well button to select a horizontal or vertical orientation, depending on whether you want to add the new well to the right of or below the selected well.




Icon	Command Name	Description
	Add Cytometer Settings	Select a well, then click the Add Cytometer Settings button to add cytometer settings to the sample. The Cytometer Settings icon () is added to the well in the bottom-left corner. Select a specimen from the specimen list, then click the Add Cytometer Settings button to add cytometer settings to the selected specimen. The Cytometer Settings icon is added to the first well in the top-left corner.
	Print	Click the Print button to print the Setup view. To save a record of acquisition status for each well, print the Setup view after acquisition is complete. The printout contains the plate name, plate type, name of the view, and legend that indicates well status. For more information on well status, see Plate Layout on this page and Table 2-2 on page 50.

Plate Layout

The plate layout is a representation of a multiwell plate. The plate layout in the Setup view is where you add samples, setup controls, and cytometer settings to the plate. You can also copy and paste HTS settings, rearrange samples, add wells, assign keywords, and set recording rules. Once you have set up the plate layout, you can print the view to use as a guide when adding samples to the multiwell plate. For information on the functions you can perform in Setup view, see Setup View Functions on page 56.

The plate layout in the Setup view displays the run sequence and status of each well.

- Wells are numbered according to sample type (setup controls first, compensation controls second, specimens last), and then the order assigned.
- During setup or acquisition, wells are colored according to their status. See Table 2-1.

- After acquisition is complete, wells are colored according to acquisition status. See Table 2-2 on page 50.

The basic layout of each well appears as follows:

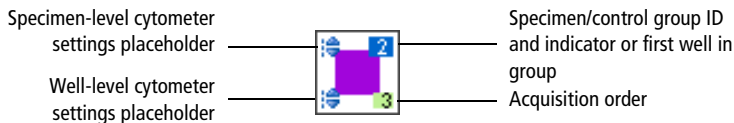












Table 2-1 Assessing well status during setup or acquisition

Well	Status
	Selected for acquisition
	Selected for acquisition and contains previously recorded data
	Acquiring
	Recorded
	Selected for acquisition and contains previously recorded data from aborted run ^a
	Selected for acquisition, aspirated, and aborted ^a

a. The error message is cleared from the Status tab of the Cytometer Settings window when acquisition begins.

Table 2-2 Assessing well status once acquisition is complete

Well	Status
 A blue circle with a white 'H' inside, a '2' in the top right, and a '6' in the bottom right.	Well contains data—acquisition successful
 A blue circle with a '2' in the top right and a '6' in the bottom right.	Well contains no data
 A red square with a white 'H' inside, a '2' in the top right, and a '6' in the bottom right.	Well contains data—recording aborted
 A 4x4 grid with a blue square in the center, a '2' in the top right, and a '6' in the bottom right.	Well contains no data—acquisition aborted



To keep a record of acquisition status, print a copy of the Setup view immediately after acquisition is finished, and refer to the printout if you reanalyze or export the data. If you export a data file or experiment that contains incomplete data, the software cannot recognize that the file is incomplete.

Compensation Controls

You can create label-specific compensation controls. See the *BD FACSDiva Software Reference Manual* for information.

Throughput Mode

The default mode at startup is high throughput.

Select throughput mode by clicking the corresponding mode button in the Setup view. You can also select the throughput mode in the Plate Inspector.

Throughput Mode High Standard

Default acquisition settings and limits change when you switch between standard and high throughput mode, so select the mode first when setting up a plate.

NOTE The throughput mode applies to the entire plate, so all wells on the plate are acquired in the same mode, except for setup and compensation control wells, which are always run in standard mode.

The following acquisition times can be achieved with the settings specified in Table 1-1 on page 14.

- In high-throughput mode, the HTS can process a 96-well plate in approximately 15 minutes.
- In standard mode, the HTS can process a 96-well plate in approximately 44 minutes.

For details on how the HTS acquires samples in each mode, see Acquisition Sampler Modes on page 34.

Loader Status

Monitor status messages for the HTS Loader during acquisition by viewing the Loader status field. For Loader errors, see HTS Troubleshooting on page 154.

Loader Settings

The loader (HTS) settings shown in the Setup view are the current settings for the selected well or plate. You can modify these settings in either the Setup view or the Well Inspector. Default loader settings are provided for each throughput mode. You will need to optimize these settings for the plate type and assay you are running. You cannot change HTS settings during acquisition or when a sequence is in process.

NOTE If multiple wells with different loader settings are selected, a red highlight appears around the loader settings that are different.

Loader Settings

Sample Flow Rate (µL/sec) 1.0

Sample Volume (µL) 3

Mixing Volume (µL) 50

Mixing Speed (µL/sec) 200



Number of Mixes 2

Wash Volume (µL) 200

Enable BLR

BLR Period 5

Table 2-3 HTS loader settings

Loader Setting	Description
Sample flow rate	<p>Speed that the syringe aspirates sample from the well in µL/second.</p> <p>To determine the acquisition time, divide the sample volume by the sample rate:</p> $\text{Acquisition time (sec)} = \frac{\text{Sample volume (}\mu\text{L)}}{\text{Sample rate (}\mu\text{L/sec)}}$
Sample volume	<p>Volume of sample aspirated from each well for acquisition.</p> <p> During acquisition in standard mode, the HTS aspirates the selected sample volume (2–200 µL) plus an additional 20 µL from the well. During acquisition in high throughput mode, the HTS aspirates a fixed 22 µL per well even though you can select a sample volume between 2–10 µL.</p> <p> Make sure each well contains sufficient sample for the entered volume plus the dead volume. Insufficient volume can introduce air bubbles into the system.</p> <p>To make sure you do not run out of sample, we recommend that you prepare your plate with a minimum of 250 µL of sample/well for a 96-well plate in standard mode, 100 µL/well for a 96-well plate in high-throughput mode, and 50 µL/well for a 384-well plate (either mode).</p>



Loader Setting	Description
Mixing volume	<p data-bbox="489 212 1089 240">Volume of sample aspirated and dispensed during mixing.</p> <p data-bbox="498 272 548 321"> Make sure each well on your plate contains sufficient sample for mixing. Insufficient volume can introduce air bubbles into the system. We recommend a mixing volume that is one-half the available volume. See Loader Settings on page 35, Sample Well Volumes on page 35, and Mixing on page 36.</p> <p data-bbox="498 467 548 516"> Adequately mix the sample before pipetting it into the plate. Use the mixing cycle only to maintain a homogeneous particle suspension.</p>
Mixing speed	Speed that the syringe aspirates sample from and dispenses sample to the well during mixing.
Number of mixes	Number of mixing cycles that are performed before a sample is aspirated.
Wash volume	Volume of sheath fluid dispensed for rinsing between wells.
Baseline restore	<p data-bbox="489 781 1180 894">At the start of data acquisition, only when data is recorded, BD FACSDiva software version 7.0 or later ignores the initial data for a period of time. That period of time is defined by the baseline restore (BLR) period.</p> <p data-bbox="489 911 1180 1024">BLR functionality is only available on BD FACSCanto, BD LSR II, and BD LSRFortessa cytometers with the HTS. The default state for BLR is off. BLR works in both standard and high throughput modes.</p> <p data-bbox="489 1040 727 1068">To set the BLR period:</p> <ol data-bbox="489 1084 1180 1219" style="list-style-type: none"> <li data-bbox="489 1084 776 1112">1 Select one or more wells. <li data-bbox="489 1128 861 1156">2 Select the Enable BLR checkbox. <li data-bbox="489 1172 1180 1219">3 Set the BLR time within the minimum and maximum period of 5–150. <p data-bbox="516 1235 1180 1325">The software multiplies the BLR period by 10 to set the time in milliseconds. For example, setting the BLR period to 5 equates to a delay of 50 milliseconds before recording data.</p> <p data-bbox="516 1341 1180 1390">Note that when you type in a value, you need to press the Enter key for the value to be saved.</p>

Table 2-4 HTS settings for standard and high-throughput modes

Setting	Standard Mode		High-Throughput Mode	
	Default	Range	Default	Range
Sample flow rate ($\mu\text{L}/\text{sec}$)	1	0.5–3.0	1	0.5–3.0
Sample volume (μL)	10	2–200	2	2–10
Mixing volume (μL) ^a	100	5–100	50	5–100
Mixing speed ($\mu\text{L}/\text{sec}$)	180	25–250	200	25–250
Number of mixes (cycles)	2	0–5	2	0–5
Wash volume (μL)	400	200–800	200	200–800

a. We recommend a mixing volume that is one-half the available volume. See Loader Settings on page 35, Sample Well Volumes on page 35, and Mixing on page 36.

NOTE A mixing volume greater than the available volume introduces air bubbles into the sample.

Shortcut Menu

Right-click a well in the plate layout to open the shortcut menu. From the shortcut menu, you can:

- Cut, copy, and paste wells, cytometer settings, loader settings, and spectral overlap
- Delete wells and settings
- Export settings, analysis templates, and FCS files
- Import cytometer settings
- Apply analysis templates
- Apply and save cytometer settings

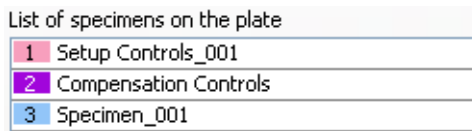
- Link to a setup
- Apply compensation controls
- Apply and save application settings
- Create an experiment layout

For additional information about these commands, see the *BD FACSDiva Software Reference Manual*.

Renaming Specimens in a Plate

To rename specimens in plate view:

- 1 In the **Plate View** window, click once to select the specimen name in the list of specimens on the plate.
- 2 Click the name once more to enter the new name for the specimen.
- 3 Press **Enter**.




Cut, Copy, and Paste Features

You can copy and paste well setup and elements to a blank well. The pasted wells inherit the loader and cytometer settings from their respective sources. This does not apply to the compensation controls specimen because it is unique to the experiment.

To copy and paste wells:

- 1 In **Plate View**, select the wells to be copied.
- 2 From the shortcut menu, select **Copy > Well(s)**.

- 3 Click the down arrow () to set the paste direction to horizontal or vertical.

This automatically adds a new well to the existing specimen in the direction selected.

Or, to paste to a different area of the plate and make a new specimen, select an empty well. From the shortcut menu, select **Paste > Well(s)**.

To copy and paste a specimen:

- 1 Click the specimen in the list of specimens on the plate.
- 2 Right-click a well in that specimen, then select **Copy**.
- 3 Select an empty well, then right-click it and select **Paste**.

If there is room on the plate, the specimen will be pasted.

Setup View Functions

Use the Setup view to set up new plate-based experiments. Once you have added setup controls, samples, and cytometer settings to the plate layout, you can assign keywords, set recording rules, apply a selected assay (analysis template), and copy and paste HTS (loader) settings to selected wells or to the entire plate.

Use the Setup view to perform the following functions:

- Define loader settings
- Optimize cytometer settings while running setup controls
- Monitor well status during acquisition
- Acquire and record wells manually or automatically
- Analyze plate data

Rearranging Samples on the Plate

Rearrange samples and wells in the plate layout by using the Cut, Copy, and Paste functions. Note that specimens and wells cannot be overwritten.

- You can move specimens to different locations in the plate layout as long as there is room to accommodate the entire specimen.
- You can move wells within a specimen to change the acquisition order or move wells from one specimen to another. Wells are pasted either below or to the right of the selected well, depending on the orientation of the Add Well button.

Assigning Keywords

You can create and assign keywords at the experiment or well level. For information on creating experiment keywords, see the *BD FACSDiva Software Reference Manual*. For instructions on creating and assigning keywords at the well level, see Assigning Keywords on page 86.

Setting Recording Rules



You can alter the acquisition stopping criteria for a selected well by:

- Changing the sample flow rate and sample volume in the HTS tab of the Well Inspector.
- Changing the events to record in the Experiment Layout or Acq tab of the Well Inspector.

For information on acquisition stopping criteria, see Running Samples Automatically in Sequence on page 59 and Running Wells Manually on page 61.

Applying an Analysis Template

You can apply an analysis template to selected samples or wells. First select the well, then either:

- Select **Edit > Apply Analysis Template** from the menu bar.
- Right-click and select **Apply > Analysis Template** from the shortcut menu.

For instructions on how to save an Analysis template, see *Exporting an Experiment as a Template* on page 93.

Applying HTS Settings

Apply specific HTS settings to a well or group of wells by using the *Copy/Paste Loader Settings* commands in the shortcut menu.

Copying and Pasting Cytometer Settings

You can copy and paste cytometer settings from one specimen or well to another as long as the specimen or well that you are copying from has cytometer settings. You can copy and paste cytometer settings to and from unrecorded wells only.



To copy settings from one specimen to another:

- 1 Click to select the specimen in the specimen list (see Figure 2-2 on page 45).
- 2 Right-click the selected specimen on the plate and select **Copy Cytometer Settings**.
- 3 Select the specimen you want to copy to in the specimen list.
- 4 Right-click the selected specimen on the plate and select **Paste Cytometer Settings**.

To copy settings from one well to another:

- 1 Click a well to select it.

- 2 Right-click the well and select **Copy Cytometer Settings**.
- 3 Select the well you want to copy to.
- 4 Right-click and select **Paste Cytometer Settings**.

Acquisition Mode

There are two ways to run plate-based acquisition on the HTS: automatically in sequence or manually one well at a time. See *Running Samples Automatically in Sequence* in the following section and *Running Wells Manually* on page 61 for a description of each mode.

Running Samples Automatically in Sequence

When running samples in sequence, use the HTS controls in the Acquisition Dashboard. Data collection proceeds automatically according to sequence number.

Select noncontiguous samples by holding down the Ctrl key while clicking individual specimens, or select a range of samples using the mouse. Wells are run in the following order:

- Setup controls
- Compensation controls
- Specimen wells

NOTE When a sequence is in progress, the ability to select a well in the plate layout is disabled.

During a sequence, the acquisition buttons have the following function:

- **Run Plate/Stop Plate toggle button.** Starts recording data for all wells in the plate or terminates the sequence after recording data for the current well. The system flushes any remaining sample that was aspirated.

- **Run Well(s)/Stop Well(s) toggle button.** Starts recording for all wells selected in the sequence or terminates the sequence after recording data for the current well. The system flushes any remaining sample that was aspirated.
- **Pause/Resume toggle button.** Pauses the sequence after finishing collection of the current well or resumes a paused sequence with the next well in the sequence.

During an uninterrupted sequence, BD FACSDiva software calculates the acquisition time of each well based on sample volume. This acquisition time is referred to as the stopping time, and is calculated as follows:

$$\text{Stop time (msec)} = \frac{\text{Sample volume } (\mu\text{L})}{\text{Flow rate } (\mu\text{L}/\text{sec}) \times 1 \text{ sec}/1,000 \text{ msec}}$$

The software stops acquisition or recording of a well and proceeds to the next well when any of the following stopping rules have been met:

- The specified number of events have been collected
- The stopping time has been reached
- The file has exceeded memory specifications. See the *BD FACSDiva Software Reference Manual* for more information.

NOTE If an HTS error occurs during a run, the run aborts and an error message displays. Once the sequence run is complete, a dialog is displayed with the status of the run and whether any errors occurred. An alert sounds until the dialog is closed.

Running Wells Manually

When running samples in manual mode, use the Basic Controls in the Acquisition Dashboard. You can acquire, record, and restart acquisition on a single selected well as long as a sequence is not currently running. For detailed information on the basic acquisition controls, see the *BD FACSDiva Software Reference Manual*.

To start manual mode, do one of the following:

- Click a well, then click **Acquire Data** under **Basic Controls** to start acquisition.
- Click a well, then click **Record Data** under **Basic Controls** to start recording data.

NOTE While running plate-based acquisition, the Start Acquisition on pointer change preference in the User Preferences window is ignored and the software proceeds as if the preference were set to Off.

NOTE During manual acquisition, high-throughput mode is not applicable.

Stopping time in manual acquisition mode is calculated the same as in automatic sequence mode. The software stops acquisition or recording of a well when any of the following stopping rules have been met:

- The specified number of events have been collected
- The stopping time has been reached
- The file has exceeded memory specifications. See the *BD FACSDiva Software Reference Manual* for more information.
- Acquisition was stopped by clicking the Stop Acquiring button under Basic Controls

Once acquisition of the well is complete, any remaining sample is flushed from the system. The controls in the Acquisition Dashboard are briefly disabled while the loader is reset.

Analysis View Components

The Analysis view provides an interface for:

- Displaying keyword results in the plate layout
- Reviewing and analyzing data collected from a plate
- Starting a batch analysis

For information on performing these functions, see Analysis View Functions on page 65.

The following components are available in the Analysis view.

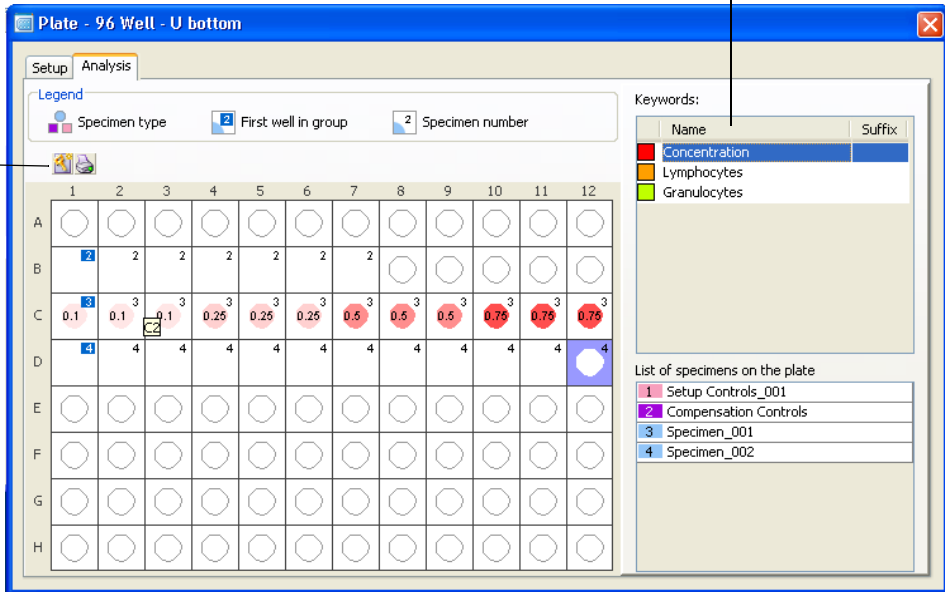
Plate Layout

The plate layout in the default Analysis view displays the acquisition status of each well. See Table 2-2 on page 50 for status indicators.

Figure 2-3 Plate layout showing Keyword Analysis view

Add Keyword button

Keyword list



Add Keyword Button


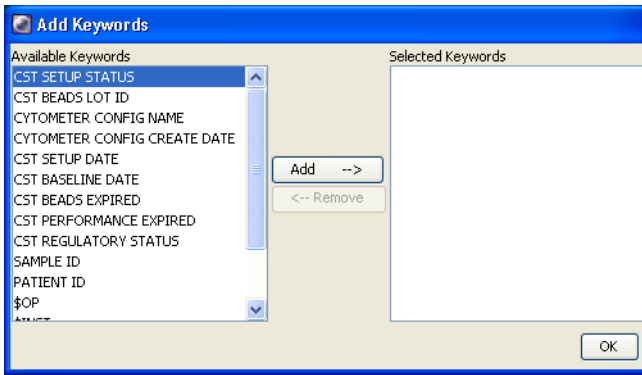

Click the **Add Keyword** button () to bring up the **Add Keywords** dialog (Figure 2-4). The Available Keywords list displays all keywords that were used in the experiment. To assign new keyword, see *Assigning Keywords* on page 86. To view keywords in the **Analysis** view, you must add the Keywords to the Selected Keywords list. For complete instructions, see *Displaying Keywords* on page 95.

Figure 2-4 Add Keywords dialog



Print Button

Click the **Print** button () to print the Analysis view. To save a record of analysis, print this view after analysis is complete. The printout shows the plate layout, legend with keywords, and plate name and type.

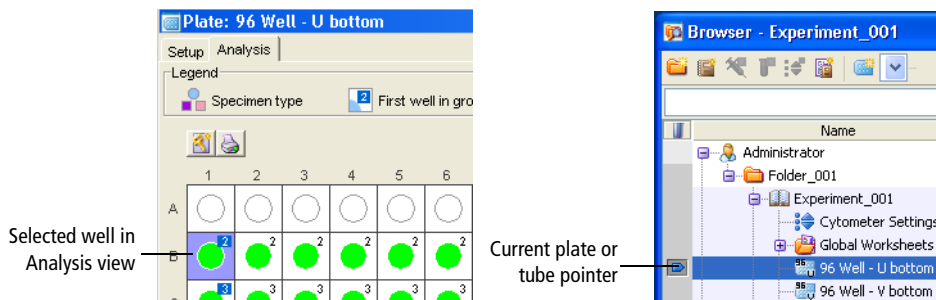
Keywords List

The Keywords list contains the selected keywords for all samples and wells on the plate (Figure 2-3). A maximum of 15 keywords can be added to the list. Add keywords to the list by clicking the **Add** button. See Add Keyword Button on page 63 for more information.

Current Tube Pointer

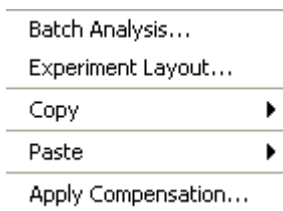
To analyze a well in the Analysis view, click the well to select it. The well is outlined in blue, indicating that the well is selected for analysis. In the Browser, the current tube pointer appears to the left of the plate name. See Figure 2-5 for an example.

Figure 2-5 Analysis indicators



Shortcut Menu

Right-click a selected well at the Analysis view to open the shortcut menu. The following commands are available in the shortcut menu.



Analysis View Functions

Perform the following functions in the Analysis view:

- Display keywords in plate layout
- Analyze plate data
- Perform batch analysis

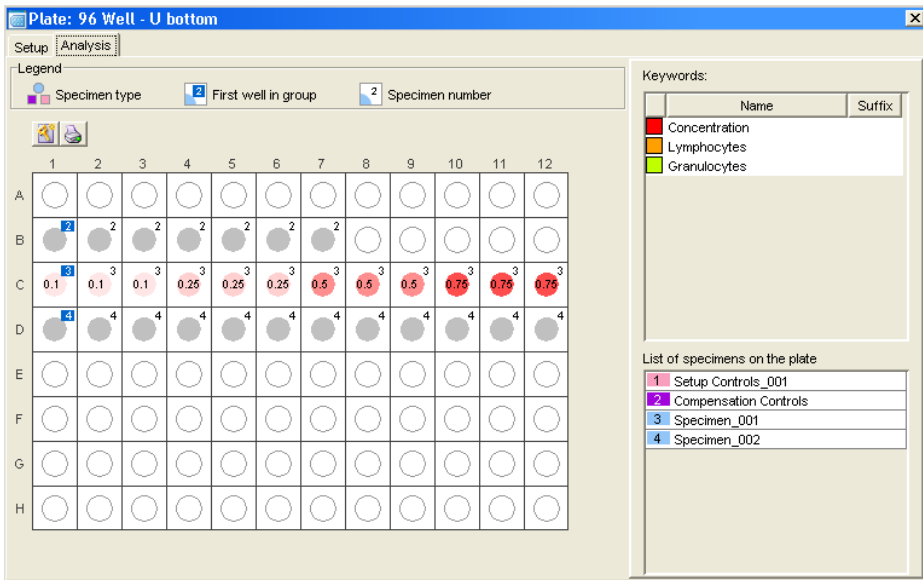
Displaying Keyword Results in the Plate Layout

If keywords were assigned to the plate, clicking on a keyword in the Keywords list toggles the plate layout to show the Keywords Analysis view. Each keyword is shown as a different color. If a keyword was assigned a range of values, a value is displayed in each well. Different values of the same keyword are shown in various shades of the keyword color. If a well has no value assigned, the well is white. To revert back to the default Analysis view, click the keyword in the Keywords list.

Use the Keyword Analysis view to display keyword values for all wells that were assigned the selected keyword. The Keyword list displays all keywords that were used in the current experiment. When you select a keyword, the plate layout shows keyword values and corresponding background colors. Wells are colored according to keyword assignments.

For example, Figure 2-6 displays values for the keyword *Concentration*. In this example, four different concentrations were assigned to wells on the plate. The Keyword Analysis view shows the concentration value assigned to each well.

Figure 2-6 Keyword Analysis view



Analyzing Plate Data

Analyze plate data the same way you would analyze tube data. For detailed information, see the *BD FACSDiva Software Reference Manual*.

Performing Batch Analysis

Use the batch analysis feature to automatically advance a selected set of data for multiple wells through an analysis template on a global worksheet. You can also assign a preferred global worksheet to a specimen or individual wells using the Inspector. You can set up batch analysis to pause between each data file and to print a copy of the analysis before proceeding with the next well.

NOTE To perform batch analysis, you must view data using a global worksheet.

For an example of setting up and performing batch analysis, see *Performing Batch Analysis* on page 99.

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Running Samples

This chapter describes how to set up for plate-based acquisition and acquire samples using the HTS. For general information about the software, see Chapter 2.

NOTE You can automate your plate run in BD FACSDiva 7.0 software. For details, see Automation on page 188.

NOTE If you are running the system for the first time, we recommend that you practice running a sample plate using BD Calibrite™ beads or a similar control sample.

The following topics are covered in this chapter:

- Starting Up on page 70
- Creating Experiments on page 72
- Preparing for Acquisition on page 87
- Performing Compensation on page 89
- Acquiring Data on page 91
- Analyzing Data on page 95

- Setting Up for Tube-Based Acquisition on page 103
- Shutting Down on page 109

Starting Up

This section describes how to start up the cytometer, software, and HTS. Notice that some steps apply only to specific cytometers.

NOTE If you need to install the HTS, follow the procedures in *Installing the HTS* on page 179.

- 1 Start up the flow cytometer as described in the appropriate cytometer manual.

Make sure to refill the sheath container and empty the waste container.

For BD FACSCanto and BD FACSCanto II systems, if a full waste container is detected at the start of or during a run, the run is stopped. If necessary, perform a sheath fluid exchange to use the appropriate sheath solution. See *Exchanging the Sheath Fluid* (BD FACSCanto and BD FACSCanto II) on page 107.



To prevent bubble formation in the flow cell, use only BD FACSTTM sheath solution with surfactant (BD Catalog No. 336524 (US) or 336911 (Europe)) when acquiring samples with the HTS. This sheath solution is intended for research use only, and should be used only with the HTS. It should not be used for sorting or for in vitro diagnostic (IVD) applications. See the product insert for more information.



Add 500 μ L of Sigma[®] Antifoam A Concentrate to the waste tank to prevent foaming of potentially biohazardous waste up around the cap. Mix the Antifoam Concentrate in distilled water to force it into the solution before adding it to the waste tank. Add the Antifoam Concentrate in addition to bleach.



Do not allow the system to run dry, as this could damage the HTS pumps.

- 2 Turn on the HTS.

NOTE The HTS turns on automatically for the BD FACSCanto II.

- 3 Ensure that the sample coupler is installed.

See details under Setting Up for Plate-Based Acquisition on page 28.

- 4 (BD LSR II and BD LSRFortessa) Place the cytometer in Run mode.

- 5 Start up the computer and start BD FACSDiva software.



Verify that no other software applications are running before you start acquisition. BD FACSDiva software performance time can be severely affected if multiple applications are running at the same time.

- 6 At the **Log In** dialog, select your user name, enter your password, and click **OK**.

For instructions on creating a user name and password, see the *BD FACSDiva Software Reference Manual*.

- 7 Verify that the software connects to the cytometer. If the software does not connect, select **Cytometer > Connect**.

View the connectivity status at the bottom of the Cytometer window. The software detects the HTS and automatically runs an initialization sequence.

- 8 After the initialization sequence has finished, select **HTS > Prime**.

- 9 Run the HTS prime sequence 2 to 3 times.

- 10 (BD LSR II and BD LSRFortessa) When the prime sequence is complete, return the cytometer to Standby mode.

11 (BD FACSCanto and BD FACSCanto II) Perform a fluidics startup.

a Select **Cytometer > Fluidics Startup**.

A confirmation dialog opens.

b Click **OK** to begin fluidics startup.


When fluidics startup is done, a completion dialog opens.

c Click **OK**.

Creating Experiments

An experiment is a group of elements used to acquire and analyze data from the HTS. The Browser is where you create experiments and access stored data.






Here are two ways to add experiments to the Browser:

- Click the **New Experiment** button () on the Browser toolbar to create a new, empty experiment with default experiment elements and default cytometer settings.
- Select **Experiment > New Experiment** to create a new experiment based on a saved template with customized elements such as plots, gates, statistics views, and cytometer settings.

The following tutorials describe how to set up an experiment specifically for plate-based acquisition and how to create additional experiments.

Creating a Folder and an Experiment


This section describes how to create a folder and an experiment containing basic analysis options.

- 1 Click the corresponding buttons on the **Workspace** toolbar to display the Browser () , Cytometer () , Inspector () , Worksheet () , and Acquisition Dashboard () windows, as needed.


NOTE As you work in the software, windows can become hidden. Bring a window to the forefront by clicking the corresponding button on the Workspace toolbar.

- 2 Select **Edit > User Preferences** and select the options in the **General** tab that are appropriate for your experiment.

NOTE The options Tube-specific worksheet and Save analysis after recording through global worksheet do not apply to plate runs on the HTS.

- 3 Click the **New Folder** button () on the **Browser** toolbar.
- 4 Rename the folder *Practice*.

To rename any Browser object, select it in the **Browser** and start typing. Press Enter to apply the new name.

- 5 With the **Practice** folder selected, click the **New Experiment** button () on the **Browser** toolbar.

A new, open experiment is added below the Practice folder in the Browser. The experiment contains default cytometer settings and a global worksheet in a Global Worksheets folder.

- 6 Rename the experiment.

For example, use today's date.


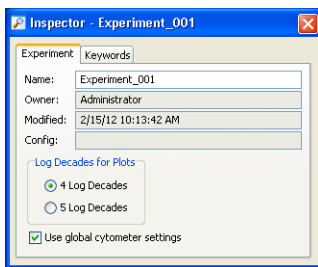

- 7 If necessary, click the **Inspector** button () on the **Workspace** toolbar to show the **Experiment Inspector**. Verify that the **Use global cytometer settings** checkbox is selected (see Figure 3-1).

Figure 3-1 Experiment Inspector



- 8 Click the arrow next to the **New Plate** button () on the **Browser** toolbar and select a plate from the list.

The list contains plates that have been validated with the HTS. For details on each plate type, see Table 3-1 on page 74.

NOTE The HTS is compatible only with standard-depth 96- or 384-well plates.


Table 3-1 Plates compatible with the HTS

Plate Type	Well Capacity (µL)	BD Catalog No.
384 flat-bottom	120	353233
96 flat-bottom	300	353915
96 U-bottom	300	353910
96 V-bottom	340	353263

The selected plate type is added to the experiment.



Make sure you select the plate type that corresponds to the plate you are using. BD FACSDiva software cannot verify that the chosen plate matches the plate on the HTS. If you select the wrong plate, the probe could hit the plate between wells or strike the bottom of a well, resulting in damage to the cytometer.

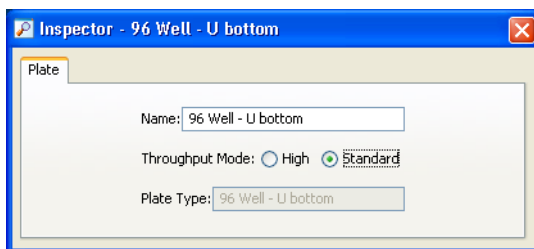
- 9 If necessary, click the **Plate** button () on the **Workspace** toolbar to view the **Plate** window.



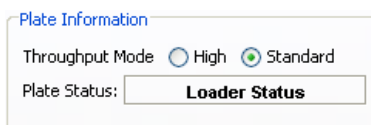
Setting Up the Plate

In this section, you will add setup controls, compensation controls, and samples to the plate to run a 6-color experiment. As an example, you will be recording and analyzing human peripheral blood stained with the following reagents: CD14 FITC/CD16+CD56 PE/CD 8 PerCP-CyTM5.5/CD19 PE-CyTM7/CD3 APC/CD4 APC-Cy7. You will need seven compensation control wells to accommodate the unstained control and the six fluorochromes you are using in your experiment. For information about setting up the plate, see *Setting Up for Plate-Based Acquisition* on page 28.

- 1 If necessary, click the **Inspector** button on the **Workspace** toolbar to view the **Plate Inspector**. Select a **Throughput Mode** of **Standard**.

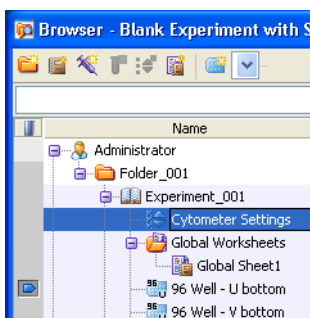


Alternatively, select the throughput mode in the upper-right corner of the **Plate** window's **Setup** view.



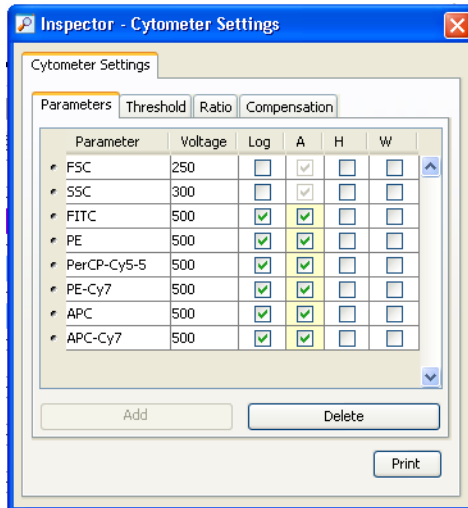
NOTE Setup and compensation control wells are always acquired in standard mode, even if high-throughput mode is selected in the Plate Inspector. Sample wells are acquired using the throughput mode selected in the Plate Inspector unless you acquire individual wells manually (in which case high-throughput mode is not an option). See *Running Wells Manually* on page 61 for more information.


- 2 In the **Browser**, click the **Experiment (global) Cytometer Settings**.



- 3 Delete any unnecessary parameters in the **Parameters** tab of the **Cytometer Inspector**.

The parameters should match those shown in the following figure.



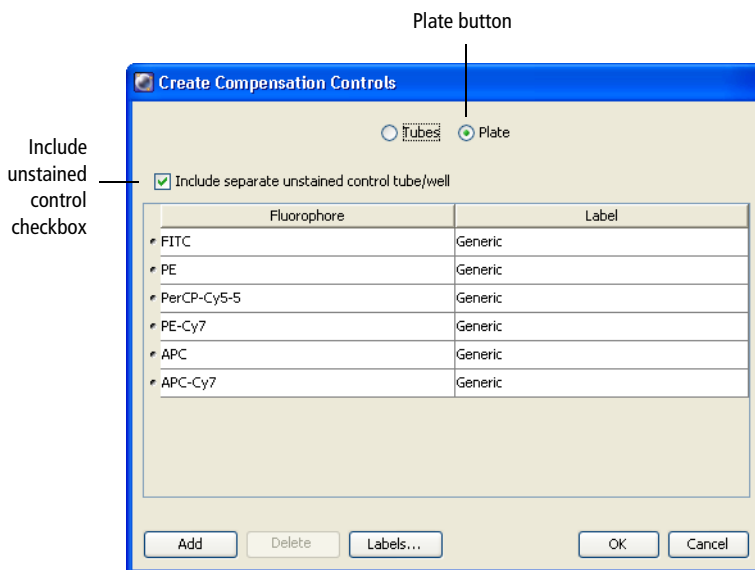
- 4 Click and drag to select wells A1 and A2 in the plate layout.
- 5 Click the **Add Setup Controls** button () on the **Plate** toolbar to add setup control wells to the experiment.

These two wells will be used for unstained controls to set the threshold and PMTs.

NOTE Designate at least two wells as setup controls to make sure that you do not run out of sample while optimizing cytometer settings.


- 6 Select well B1, then select **Experiment > Compensation Setup > Create Compensation Controls**.
- 7 Verify that the options in Figure 3-2 on page 78 are selected.

Figure 3-2 Create Compensation Controls dialog



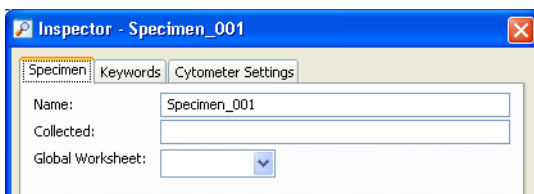
- 8 Click **OK**.

The specified compensation controls are added to wells B1–B7.

- 9 Click and drag to select the following wells, then click the **Add Specimen Wells** button () to add two specimens to the plate layout.

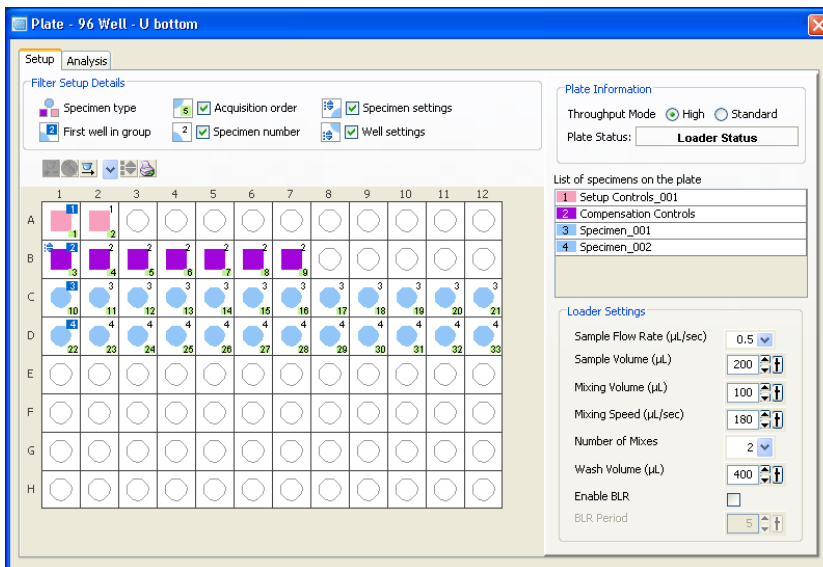
Wells	Name
C1–C12	Specimen_001
D1–D12	Specimen_002

NOTE You can change the specimen name using the Specimen Inspector.



The plate layout should look similar to Figure 3-3.

Figure 3-3 Setup view



10 Select **Experiment > Experiment Layout**, then click the **Labels** tab (Figure 3-4 on page 81).

11 Define the fluorophore labels for both specimens.

Fluorophore	Label
APC	CD3
APC-Cy7	CD4

Fluorophore	Label
PerCP-Cy5.5	CD8
FITC	CD14
PE	CD16+CD56
PE-Cy7	CD19

- a Select the FITC column of Specimen_001 and Specimen_002.
- b Click in the **Label** field and enter *CD14*.

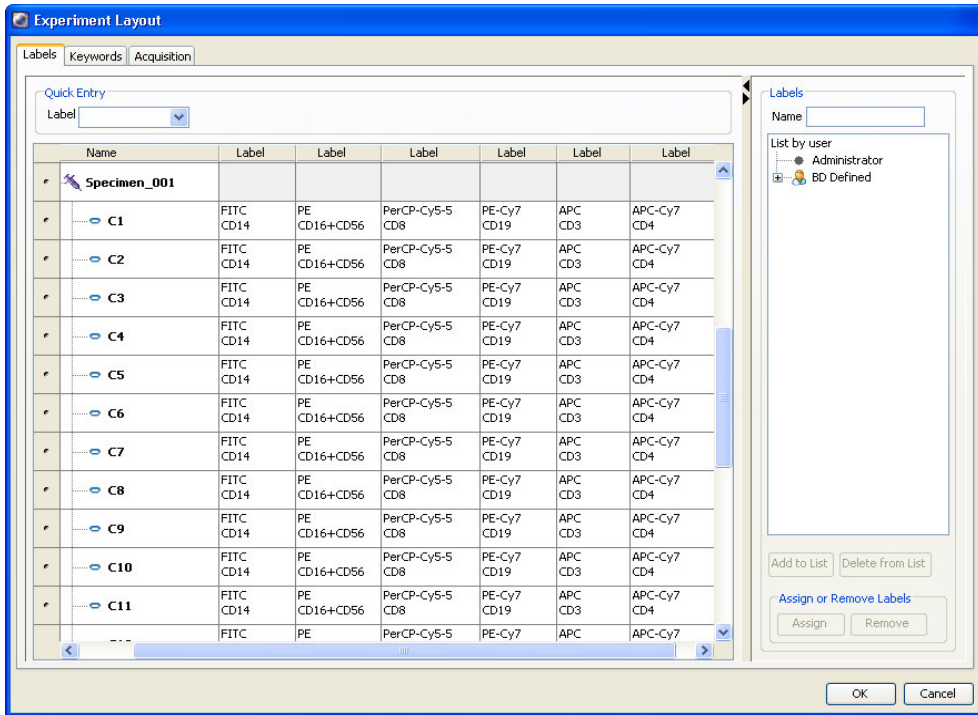
The selected fields are labeled *CD14*.

- c Label the remaining fluorophores.

NOTE There are other fast ways to create labels. See the *BD FACSDiva Software Reference Manual*.

The Experiment Layout should look similar to Figure 3-4.

Figure 3-4 Labeled fluorophores in the Experiment Layout



- Click the **Acquisition** tab in the **Experiment Layout** (Figure 3-5), and set the number of events to record.

Name	Events to Record
Compensation controls	10,000
Specimens	10,000

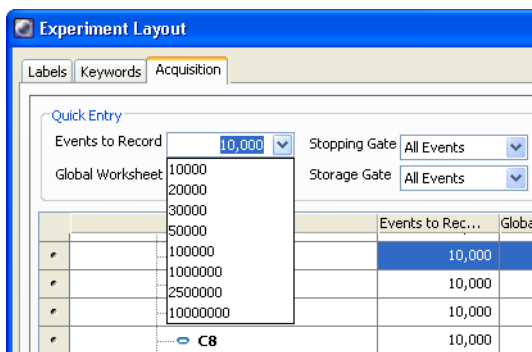
NOTE Setup controls are acquired, but not recorded. We recommend that you set the aspirated volume for the setup controls high enough so you can optimize settings before you reach the BD FACSDiva stopping time.

NOTE Press Ctrl+Shift+End to set multiple fields at one time, and then type in the number of events in one of the fields. All selected fields will reflect the new number of events.

NOTE Another way to add a value to the **Events to Record** field using the list is to select the field you want to change, then double-click the item in the list to apply it to the field.

See the *BD FACSDiva Software Reference Manual* for more ways to add events.

Figure 3-5 Acquisition tab of the Experiment Layout



- 13 Click **OK** to save the changes.

Setting Up the Worksheet

This section describes how to set up plots in a global worksheet.

- 1** Create an FSC vs SSC plot on the global worksheet.

The plot parameters should be FSC-A and SSC-A.

- 2** Create a PE histogram next to the FSC vs SSC dot plot and change the plot parameter to PE-A.

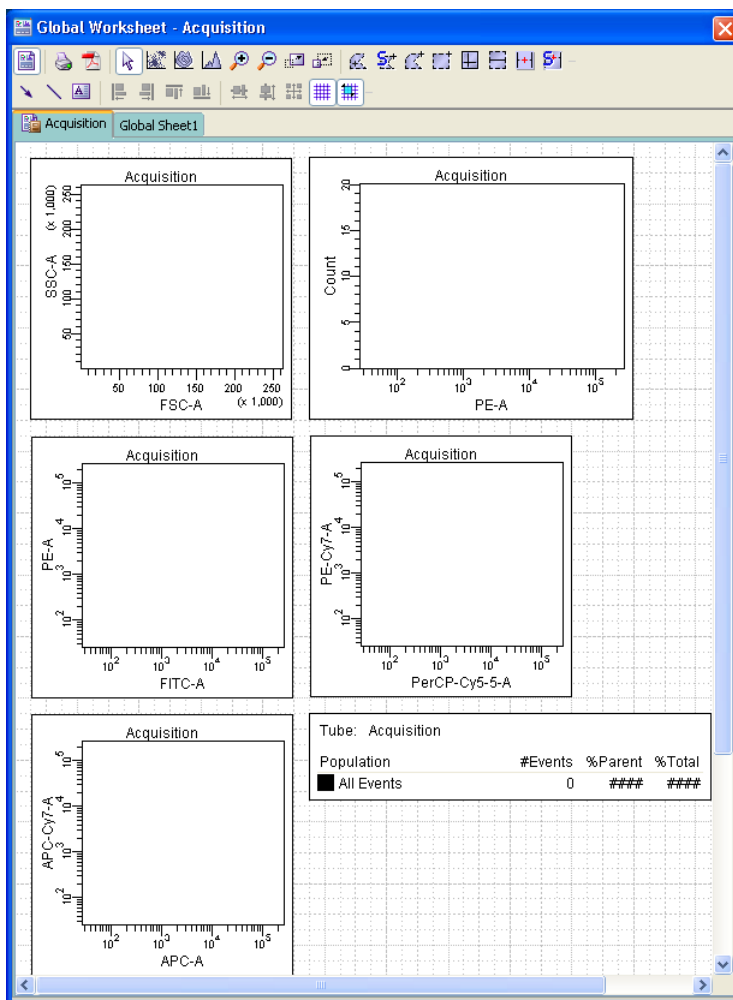
- 3** Create the following dot plots, choosing the appropriate parameters:

- FITC vs PE
- PerCP-Cy5.5 vs PE-Cy7
- APC vs APC-Cy7

- 4** Show a population hierarchy.

The worksheet should look similar to Figure 3-6 on page 84.

Figure 3-6 Plots on a global worksheet



NOTE Alternatively, you can view the unstained normal worksheet, and select **Edit > Select All > Copy**. Then view the global worksheet, and right-click and select **Paste**. Draw the gates (FSC vs SSC plot and all histograms) or you can copy the gates from an unstained normal worksheet. During acquisition, drag the gates to the populations of interest.

- 5 Assign a name to the global worksheet.

In the **Browser**, select the worksheet, then select **Rename** from the shortcut menu.

You are now ready to set up the cytometer for acquisition.

Exporting a Plate as a Template

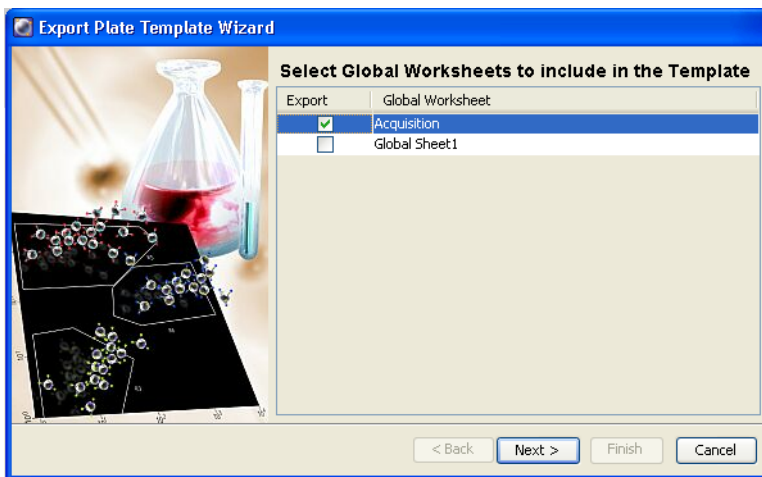
After you have set up a plate, you can export it as a template. Creating a plate template can save you setup time if you routinely use the same sample setup. Plate templates include keywords, labels, cytometer settings, and HTS settings, but do not include recorded data.

- 1 Select 96 well - U bottom in the **Browser**, then select **File > Export > Plate Template**.

Alternatively, in the **Browser**, select the plate to be exported. Then select **Export > Plate Template** in the shortcut menu.

The Export Plate Template Wizard opens. The bold text at the top of the dialog tells you what to do on each screen. See Figure 3-7 on page 86.

Figure 3-7 Export Plate Template Wizard



- 2 Select the global worksheet to include in the template.
- 3 Click **Next**.
- 4 Select the template type and enter a name.

The template type is used to group similar templates so they are easy to find. At a minimum, you need to enter only the type and name when you are exporting a plate template. The remaining screens are optional.

NOTE Select the **Lock Template** checkbox when you want to prevent other users from overwriting your template.

- 5 Click **Next**, then click **Finish**.

Assigning Keywords

Keywords are used to enter information about the wells, such as sample type and preparation details. For more information, see the *BD FACSDiva Software Reference Manual*.

Preparing for Acquisition

This section describes how to optimize cytometer settings and to perform compensation.

Optimizing Cytometer and HTS Settings

The default settings provide a good starting point for achieving optimal throughput in either mode, but they should be optimized for your plate type and sample volume.

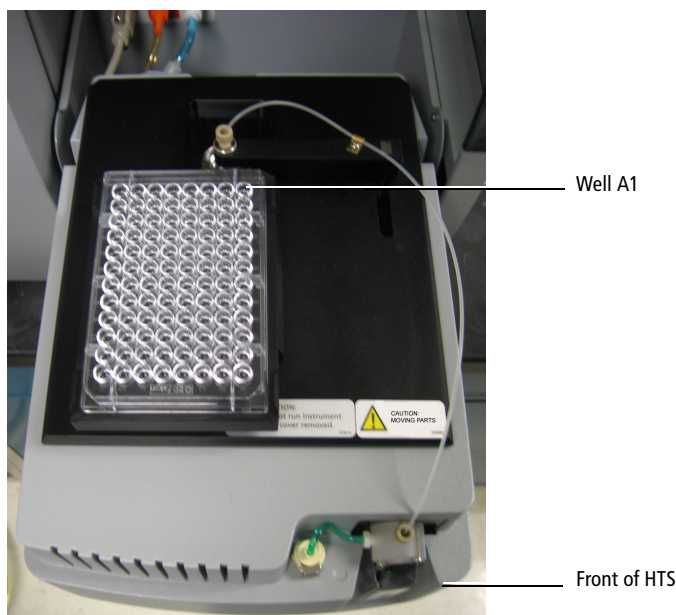
- 1 Click the **Setup** tab of the **Plate** window.
- 2 Click each setup control well, view the **Cytometer Settings**, and verify that they are the same as defined in **Setting Up the Plate** on page 75.
- 3 Remove the safety cover on the HTS and place the prepared plate on the plate holder.



To prevent damage to the HTS probe, always remove the multiwell plate cover before you put the plate on the holder.

Make sure the plate corresponds to the type selected in the software. Orient the plate with well A1 at the back-right corner of the stage. See Figure 3-8 on page 88.

Figure 3-8 Orienting the plate (BD FACSCanto example)




- 4 Replace the HTS safety cover.
- 5 (BD LSR II and BD LSRFortessa) Press the RUN button on the cytometer.



Do not put the BD LSR II in Standby during HTS acquisition. BD FACSDiva software cannot continue the run when the cytometer is in Standby, and it can cause damage to the flow cell.

- 6 Select the two setup control wells in the plate layout of the **Setup** view.

The well is outlined in green .

- 7 Click **Run Well(s)** in the **Acquisition Dashboard**.

If necessary, select **View > Acquisition Dashboard**.

The HTS homes and primes the probe, and then aspirates the unstained control from the first setup control well.

NOTE Setup and compensation wells are always acquired in standard mode, even if high-throughput mode is selected in the Plate window.



Verify that no other software applications are running before you start acquisition. BD FACSDiva software performance time can be severely affected if multiple applications are running at the same time.

- 8** If needed, adjust the FSC, SSC, threshold, and PMTs using the unstained control.

For more information on optimizing cytometer settings, see the appropriate cytometer user's guide.

NOTE Acquisition stopping time is determined by BD FACSDiva software, according to sample volume/sample rate. If you run out of sample before you finish optimizing settings, allow the HTS to move to the next setup control well and continue optimization from there.

- 9** Click **Stop Well(s)** once you have optimized settings.

The HTS goes through a purge cycle.

NOTE No data file is saved for setup control wells.

Performing Compensation

Once you have optimized the cytometer settings, you are ready to run the compensation controls and calculate compensation.

NOTE You can run compensation controls only once during an experiment. If you need to re-run compensation controls, you must create a new experiment.



- 1** Select wells B1 through B7 in the plate layout.
- 2** Click **Run Well(s)** in the **Acquisition Dashboard**.

The compensation wells are run in the order they were created.

NOTE You must select a well(s) in the Setup view, and a global worksheet must be in view, to enable the Run Well(s) button.



After the last compensation control has been recorded, the Sequence Done dialog opens.

- 3 Click **OK**.
- 4 Toggle to the normal worksheet for the compensation control wells.
- 5 Select the FITC Stained Control worksheet in the **Worksheet** window.
- 6 Verify that the P1 gate encompasses the singlet bead population.
- 7 Verify that the autointerval gate encompasses the positive peak in each of the plots on all worksheets.
- 8 Select **Cytometer > Cytometer Setup > Calculate Compensation**.

If the calculation is successful, a dialog opens.

- 9 Name the compensation setup after the experiment, then click **OK**.

For information on performing compensation, see the *BD FACSDiva Software Reference Manual*.

NOTE Compensation controls can be run individually through their normal worksheets using the Basic Controls in the Acquisition Dashboard.

Acquiring Data

Once you have calculated compensation, you are ready to record your samples.

- 1 Click the **Worksheets View** button to return to the global worksheet.
- 2 Select well C1 in the plate layout.
- 3 Click **Run Plate** in the **Acquisition Dashboard**.

Wells are acquired in the order they were created.

As each well is injected into the cytometer SIT, events appear in plots on the global worksheet. If an error occurs, an error message appears, and the well in the plate layout is colored according to its status. Well status is indicated by the color outlining each well, as described in Plate Layout on page 48.



The HTS is equipped with a safety interlock that prevents the cytometer from running when the safety cover is removed or opened. Do not remove or open the safety cover while samples are being processed. To pause or stop acquisition, click the corresponding buttons on the **Acquisition Dashboard** before you remove or open the safety cover.

At the end of the run, a dialog opens indicating that the run is complete.

- 4 Click **OK** to close the completion dialog.
- 5 Open the safety cover and remove the plate.

NOTE Access the plate only after sample processing is complete and the probe is no longer moving.

- 6 Click the **Print** button on the **Plate** toolbar to print a record of the **Setup** view and legend.



Keep a record of the acquisition status by printing a copy of the Setup view immediately after acquisition is complete. Refer to the printout if you reanalyze or export the data. If you export a data file or an experiment that contains incomplete data, the software cannot recognize that the file is incomplete.

- 7 Select the appropriate settings and printer, then click **OK**.

Use the printout to:

- View the acquisition run order according to well numbering.
- Determine the well status after acquisition. (See Plate Layout on page 48.)

- 8 At the end of each day or shift, clean and service the cytometer as described in Daily Maintenance on page 112.

Pausing the HTS

To pause a run, click **Pause** in the **Acquisition Dashboard**. The HTS finishes processing the current well (or wells in high-throughput mode), and then remains in a suspended state until you choose to continue.

- To continue the run, click **Resume**.
- To stop the run, click **Stop Well(s)** or **Stop Plate**.

This sequence allows you to stop the run early to minimize sample loss.

Stopping the HTS

The HTS stops automatically when plate acquisition is finished. Do the following if you need to stop the HTS in the middle of a run.



If you stop the HTS during a run in standard mode, the current well will be lost. If you stop the HTS in high throughput mode, the current and next well will be lost. Do not stop acquisition if your sample volume is limited.



- 1 Click **Stop Plate** or **Stop Well(s)** in the **Acquisition Dashboard**.

The HTS finishes the sequence in progress, stops, and then the Sequence Done dialog opens.

NOTE If you are using the Basic Controls to acquire, simply click **Stop Acquiring**.

- 2 Click **OK**.

Exporting an Experiment as a Template

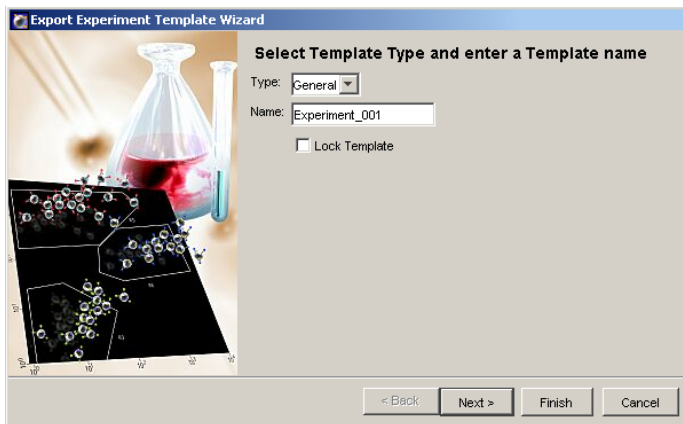
Now that you have completed an experiment with cytometer and HTS settings, you can export it as a template. Creating analysis templates for assays that you typically run saves setup time. Experiment templates include plates, keywords, labels, worksheet elements and worksheets (including all settings such as page breaks), cytometer settings, and HTS settings, but do not include recorded data.

- 1 Select Experiment_001 (or whatever you named it) in the **Browser**.
- 2 Select **File > Export > Experiment Template**.

Alternatively, from the shortcut menu, select **Export > Experiment Template**.

NOTE Make sure you select the **Experiment Template** command, and not the **Experiments** command.

The Export Experiment Template Wizard opens. The bold text at the top of the dialog tells you what to do at each screen.



- 3 Enter *Practice* for the template type, and *6-Color Analysis* for the template name.

The template type is used to group similar templates so they are easy to find. At a minimum, you need to enter only the type and name when you are creating a template. The remaining screens are optional.

NOTE Select the **Lock Template** checkbox when you want to prevent other users from overwriting your template with the same name.

- 4 Click **Next**, **Next**, and then **Finish**.

NOTE To export a **Panel** template, select a specimen in the **Plate** window and follow steps 1 to 4.

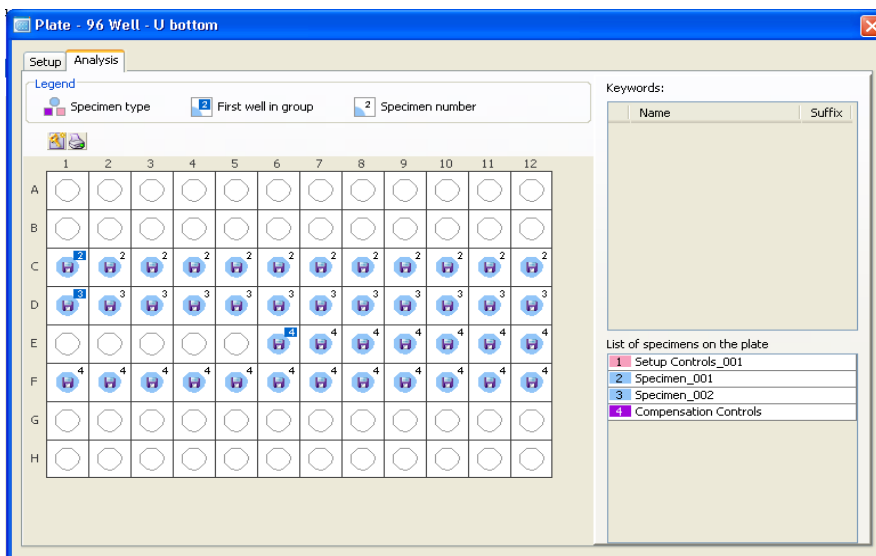
Analyzing Data


This section describes how to use the analysis view and the batch analysis feature in BD FACSDiva software to analyze data from plate-based acquisition.

Displaying Keywords

- 1 Click the **Analysis** tab to display the Analysis view.

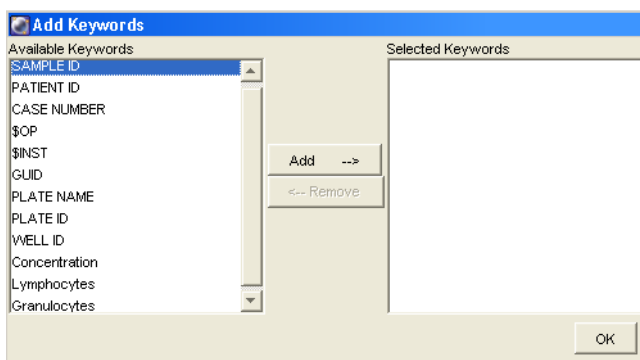
The Analysis view shows the acquisition status of each well.



- 2 Click the **Add Keywords** button () on the **Plate** toolbar.

The Add Keywords dialog opens. See Figure 3-9 on page 96.

Figure 3-9 Add Keywords dialog



- 3** Alt+Shift+Click the keywords *Concentration*, *Lymphocytes*, and *Granulocytes* from the **Available Keywords** list (on the left side of the dialog). The *Concentration*, *Lymphocytes*, and *Granulocytes* keywords were created for this example.

- 4** Click **Add** to move the keywords to the **Selected Keywords** column, then click **OK**.

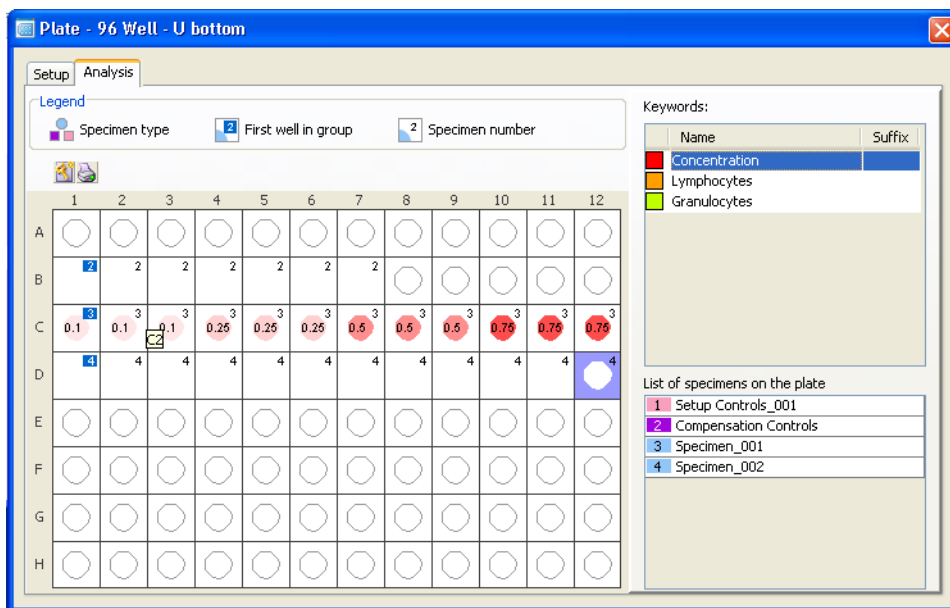
The three keywords now appear in the Keywords list in the Analysis view.


- 5** Click the keyword *Concentration* in the **Keywords** list.

The Analysis view changes to the Keyword view (Figure 3-10 on page 97). If values were assigned to the selected keyword, the values are displayed in this view.

In this example, the selected keyword is *Concentration* and the values are *0.10*, *0.25*, *0.50*, and *0.75*.

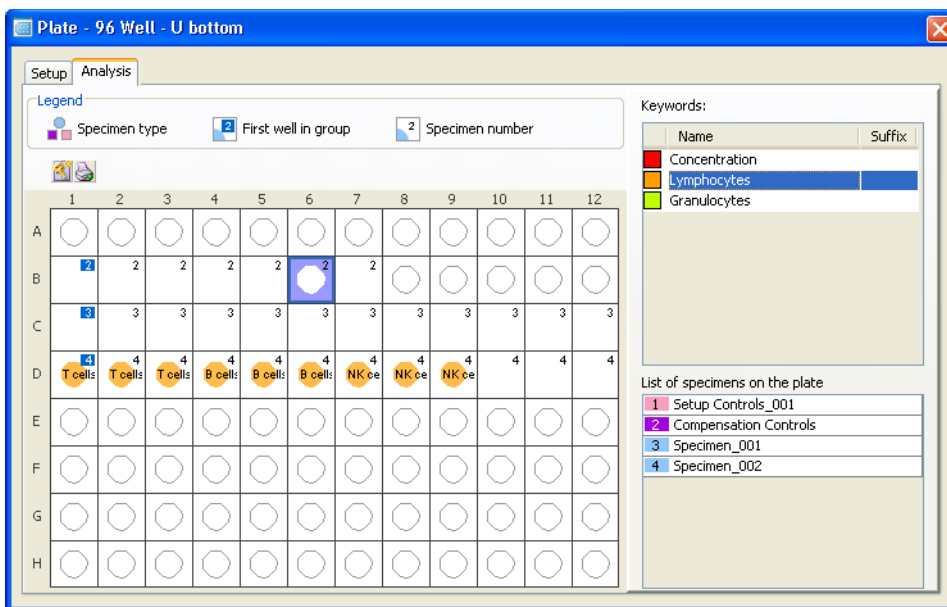
Figure 3-10 Keyword Analysis view showing concentration values



- 6 Click the **Print** button () on the **Plate** toolbar to print the concentration keyword view and legend.
- 7 Select the appropriate print options, then click **OK**.
Save the printout for your records.
- 8 Click the keyword *Lymphocytes* in the **Keywords** list.

The Keyword view displays the values for the keyword *Lymphocytes*. See Figure 3-11 on page 98.

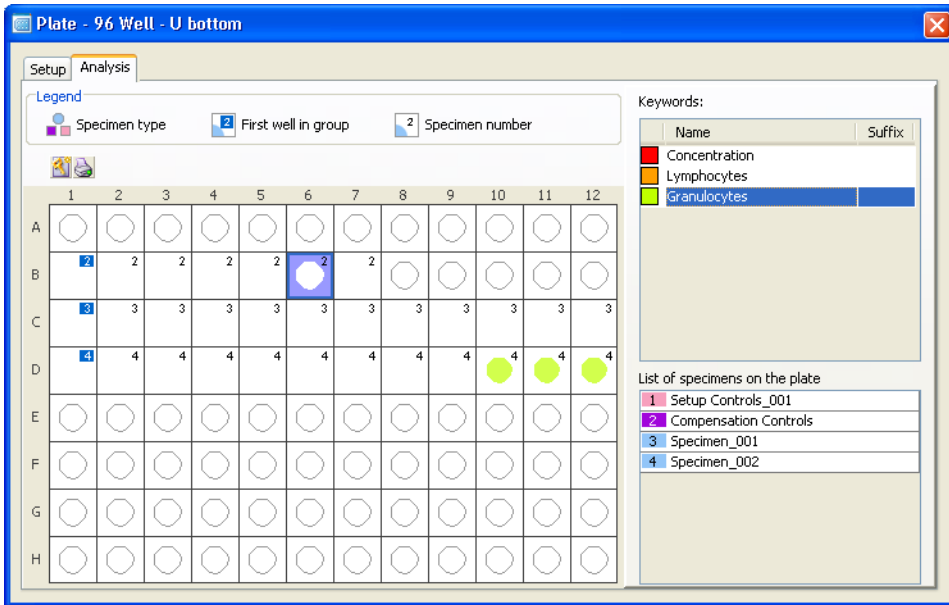
Figure 3-11 Keyword Analysis view showing lymphocytes values



- 9 Print the *Lymphocytes* Keyword view and legend.
- 10 Click the keyword *Granulocytes* in the **Keywords** list.

The wells that were designated with the keyword *Granulocytes* are colored yellow-green, but do not list a value since none was assigned.

Figure 3-12 Keyword Analysis view showing granulocytes wells



11 Print the *Granulocytes* Keyword view and legend.

Performing Data Analysis

Analyze plate data in the same manner you would analyze tube data using BD FACSDiva software. For detailed instructions, see the *BD FACSDiva Software Reference Manual*.

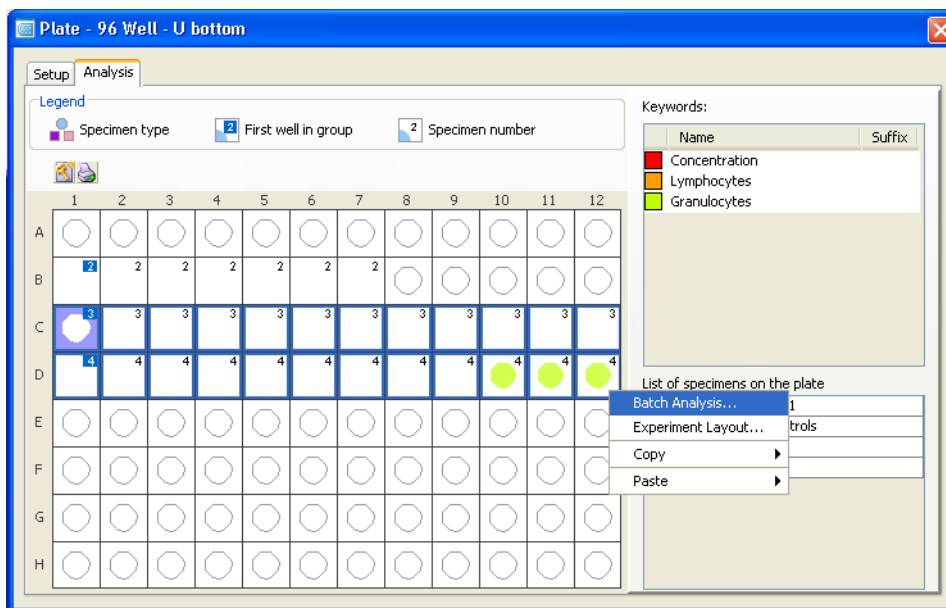
Performing Batch Analysis

Do the following to set up batch analysis:

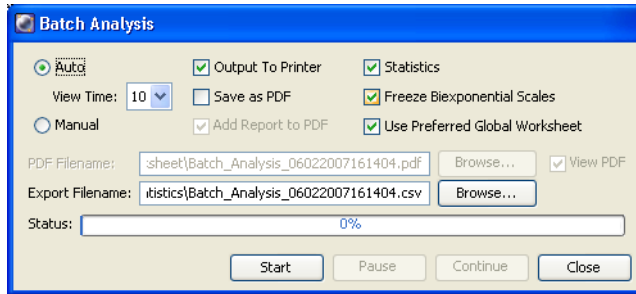
- 1 Open an experiment containing the plate data that you want to analyze.
- 2 Double-click the plate in the **Browser** to open the **Plate** window.

- 3 Click the **Analysis** tab to display the **Analysis** view.
- 4 In the global worksheet, verify that the gates enclose the appropriate populations.
- 5 In the plate layout, select the wells for batch analysis, right-click to open the shortcut menu, and then select **Batch Analysis**.

NOTE If you wish to print, you must assign a printer before starting batch analysis.



The **Batch Analysis** dialog opens.



6 Select the type of analysis to be done.

- Select **Auto** to analyze all files with no user intervention. Data is displayed in the global worksheet for the amount of time specified in the **View Time** field (in seconds) before analysis of the next well begins. Make adjustments to your analysis during this pause or let analysis proceed automatically. Select zero only if you want to process the batch without reviewing the data between wells.
- Select **Manual** to pause the batch after data is loaded for each well. Click the **Continue** button to proceed with analysis of the next well. The **View Time** field is disabled when you select **Manual** analysis.
- You can print worksheets or export statistics before data for the next well is loaded.

Output to Printer. Print a copy of the analysis for each well.

Statistics. Export statistics to a single comma separated value (CSV) file for the batch. The resulting file can be opened with a spreadsheet application such as Microsoft Excel®. Depending on the auto-export format selected in User Preferences, each well adds a new row or column of results to the file. For each population in the statistics view, the software adds parameter statistics in the order in which they appear in the view. A new header row is added if you add or delete statistics or parameters during batch analysis. You cannot add, remove, or edit statistics views while the batch is running.

- **Save as PDF.** The Add Report to PDF and View PDF checkboxes become active. If Add Report to PDF is selected, the Batch Analysis Report is added to the top of the worksheet's PDF file. If View PDF is selected, the PDF is automatically displayed at the completion of the batch analysis.
- Specify whether to let biexponential scales fluctuate, if applicable. When the Freeze Biexponential Scales checkbox is selected, biexponential scaling does not change during batch analysis. All data is processed using scales from the tube where the current tube pointer is set. For information on biexponential scales, see the *BD FACSDiva Software Reference Manual*.

Preferred global worksheets can be used with wells and specimens. For information on using preferred global worksheets, see the *BD FACSDiva Software Reference Manual*.

- 7 Click **Start** to begin batch analysis.

A dialog informs you when batch analysis is complete.

NOTE A plate GUID (globally unique identifier) appears in the statistics view. This number is unique for each plate.



Maintaining Data

Maintain the database by keeping the size below recommended limits, backing up data on a regular basis, and deleting experiments and plates that you no longer need. Follow the precautions and instructions for database management given in the *BD FACSDiva Software Reference Manual*.

Setting Up for Tube-Based Acquisition


This section describes how to change from plate-based acquisition to tube-based acquisition.

Setting Up for Tube-Based Acquisition (BD LSR II and BD LSRFortessa)

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


To switch from plate mode to tube mode:

- 1 Perform the daily clean procedure. See Daily Cleaning on page 112.
- 2 Place the cytometer in Standby mode.
- 3 Switch off the HTS power.
- 4 Detach the sample coupler from the cytometer SIT by unscrewing the top thumbscrew. Once the coupler feels free, gently pull it straight down from the SIT.

 To avoid bending the SIT, pull straight down on the coupler. Do not pull the coupler at an angle.

- 5 Remove the SIT protector.

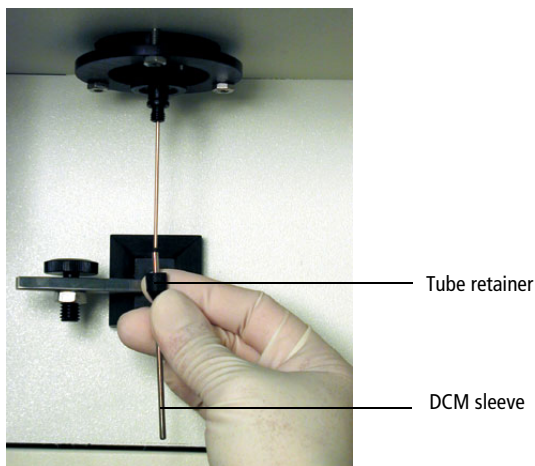
Unscrew the tube retainer and slide the SIT protector straight down.

 To avoid bending the SIT, do not slide the SIT protector at an angle.



- 6 Reinstall the standard droplet containment module (DCM) sleeve (Figure 3-13 on page 104).
 - a Slide the sleeve straight up over the SIT.
 - b Screw the tube retainer on to secure it.

Figure 3-13 Installing the DCM sleeve



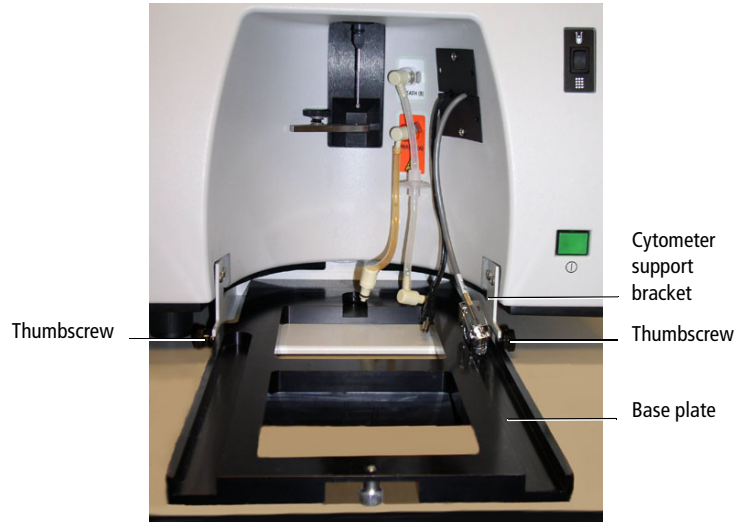
- 7 Install a tube of deionized (DI) water on the SIT and place the tube support arm under the tube.
- 8 Switch the acquisition control switch to tube mode (U).
- 9 Uninstall the HTS.
 - a Detach the HTS from the cytometer (see Removing the HTS on page 166 and Figure 3-14 on page 105).
 - b Remove the base plate by loosening the thumbscrews at the bottom of the cytometer support bracket (see step 13 on page 142).

NOTE If you are not going to use the HTS for a week or longer, follow the instructions under Placing the HTS in Long-Term Storage on page 140.



To prevent personal injury or damage to the HTS, disconnect the HTS from the cytometer and remove the HTS before running tube-based acquisition.

Figure 3-14 BD High Throughput Sampler detached from cytometer



- 10** If applicable, perform the monthly cleaning as outlined in Monthly Cleaning on page 119, install a new sheath filter, and fill the sheath tank with BD FACSTFlow™ sheath fluid.

Exchanging the Sheath Fluid (BD LSR II and BD LSRFortessa)

Follow the instructions under Monthly Cleaning (BD LSR II and BD LSRFortessa) on page 119. In step 16, replace the sheath filter with a new filter instead of reconnecting the old filter.

NOTE If you exchanged BD FACSToGo with BD FACS sheath solution with surfactant, install the HTS sample coupler, then prime the system twice by selecting **HTS > Prime**. This exchanges the fluid in the syringes with BD FACS sheath solution with surfactant.

Setting Up for Tube-Based Acquisition (BD FACSCanto and BD FACSCanto II)

Before running BD FACSCanto clinical software, you must exchange BD FACS sheath solution with surfactant with BD FACSToGo sheath fluid. Version 2.4 of the clinical software cannot connect in acquisition mode if the sheath type is set to BD FACS sheath solution with surfactant.

- 1 Perform a sheath exchange to replace BD FACS sheath solution with surfactant with BD FACSToGo sheath fluid.

See Exchanging the Sheath Fluid (BD FACSCanto and BD FACSCanto II) on page 107.

- 2 Detach the sample coupler from the cytometer SIT by unscrewing the top thumbscrew. Once the coupler feels free, gently pull it straight down from the SIT.



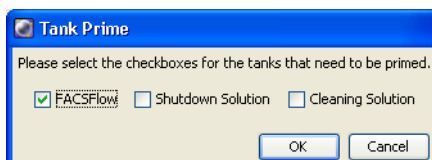
To avoid bending the SIT, pull straight down on the coupler. Do not pull the coupler at an angle.

NOTE For the BD FACSCanto, place the sample coupler in the clamp on the catch tray when the coupler is not in use (see Figure B-12 on page 183).

Exchanging the Sheath Fluid (BD FACSCanto and BD FACSCanto II)

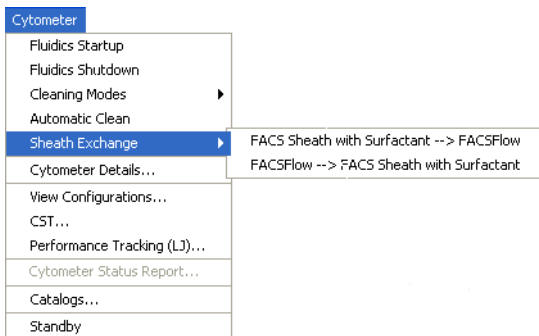
Perform this procedure to exchange one sheath fluid with another. For example, when switching from plate-based to tube-based acquisition, follow these steps to exchange BD FACS sheath solution with surfactant with BD FACSFlow.

- 1 Remove the sample coupler from the SIT.
- 2 Install a cubitainer containing the sheath fluid you want to use.
- 3 Empty the waste tank, if necessary.
- 4 Select **Cytometer > Cleaning Modes > Prime after Tank Refill** to display the **Tank Prime** window.
- 5 Select **FACSFlow**, then click **OK**.



NOTE It may be necessary to purge air from the sheath filter on the wet cart, then prime two or three times. See your cytometer user's guide for more information on troubleshooting air in the fluid lines.

- 6 Select **Cytometer > Sheath Exchange**, and select the desired sheath exchange.



A confirmation dialog opens.

- 7 Click **OK**.
- 8 Click **OK** in the next dialog to confirm the duration.

A dialog opens informing you that the sheath exchange is complete.

- 9 Click **OK**.

NOTE When the sheath type is BD FACSFlow, the sheath fluidics level indicator (■) is green. When the sheath type is BD FACS sheath solution with surfactant, the level indicator is white.

NOTE If you exchanged BD FACSFlow with BD FACS sheath solution with surfactant, install the HTS sample coupler, then prime the HTS twice by selecting **HTS > Prime**. This exchanges the fluid in the syringes with BD FACS sheath solution with surfactant.

Shutting Down

Perform the daily cleaning procedure before shutting down the system.

- 1** Run the daily cleaning procedure (see Daily Cleaning on page 112).
 - 2** (BD FACSCanto and BD FACSCanto II) Perform a fluidics shutdown. See Fluidics Shutdown in the following section.
 - 3** (BD LSR II and BD LSRFortessa) Prime the HTS.
 - a** Place the cytometer in Run mode.
 - b** Select **HTS > Prime**.
 - c** When priming is complete, click **OK** in the dialog, then place the cytometer in Standby mode.
- NOTE** To prime the cytometer, press the Prime button on the fluid control panel on the cytometer itself.
- 4** (BD FACSCanto, BD LSR II, and BD LSRFortessa) Turn off the power to the HTS.
 - 5** Turn off the power to the cytometer.
 - 6** Quit the software (see Quitting the Software on page 110).

Fluidics Shutdown

(BD FACSCanto and BD FACSCanto II) Perform a fluidics shutdown before quitting the software.

- 1 Ensure that the sample coupler is attached.

NOTE Always run the fluidics shutdown with the sample coupler attached to rinse the syringes and fill them with BD FACS shutdown solution.

- 2 Select **Cytometer > Fluidics Shutdown**.

A confirmation dialog opens.

- 3 Click **OK**.

A dialog opens when fluidics shutdown is complete.

- 4 Click **OK**.

Quitting the Software

If you have a BD FACSCanto or BD FACSCanto II, you must perform a fluidics shutdown by selecting **File > Quit** before quitting the software (see Fluidics Shutdown on page 110). All Browser and worksheet elements are automatically saved when you quit the application.

4

Maintenance

This chapter provides maintenance procedures to keep your HTS in good working order.

NOTE You can also use the BD Biosciences HTS Customer Care Kit to replace clogged connectors, tubing, and syringes on your HTS. Using the HTS Customer Care Kit reduces downtime and the need for depot repair (see Depot Repair Procedures on page 165). For detailed information about using the HTS Customer Care Kit to prevent clogs and optimize system performance, see the *HTS Customer Care Kits User's Guide*.

The following maintenance procedures are covered in this chapter.

- Daily Maintenance on page 112
- Monthly Maintenance on page 119
- Periodic Maintenance on page 122
- Unscheduled Maintenance on page 144

Daily Maintenance

When running the HTS daily, perform the following at the end of every day (or shift):

- Daily Cleaning on this page
- Cytometer Inspection and Servicing on page 116

NOTE (BD LSR II and BD LSRFortessa) When running samples containing acridine orange or propidium iodide (or any nucleic dyes), run the daily clean twice. First, run 70% isopropyl alcohol in wells A1–A4 and BD™ FACSClean or a 10% bleach solution in wells B1–B4. Second, run BD™ FACSRinse solution in wells A1–A4, followed by DI water in wells B1–B4.

Daily Cleaning

During the daily clean procedure, the cytometer samples cleaning solution and then DI water from predefined wells and performs a sequence of mixing, aspiration, and rinsing. Software prompts guide you through the cleaning sequence. Perform the cleaning procedure at the end of every day or shift while the cytometer is in plate-based mode. Allow 15 minutes to complete this procedure.

Materials Needed

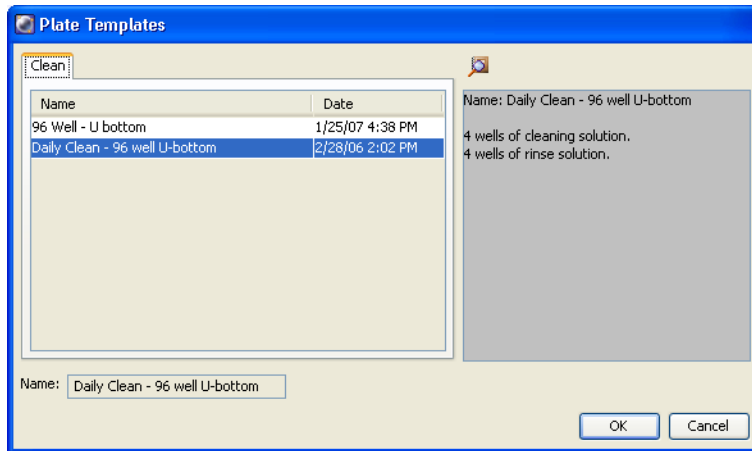
- BD FACSClean or a fresh 10% bleach solution (1 part bleach in 9 parts DI water)
- BD FACSRinse solution
- DI water

Running the Daily Clean

- 1 Select **HTS > Clean**.

The Plate Templates dialog opens(Figure 4-1 on page 113).

Figure 4-1 Plate Templates dialog



- 2 Select the **Daily Clean - 96 well U-bottom** template, if not already selected.

If you do not have a U-bottom plate for cleaning, you can set up your own cleaning template.

- 3 Click **OK**.
 - The plate interface changes to show the Daily Clean Protocol view (Figure 4-2 on page 114) and the confirmation dialog opens.

Figure 4-2 Daily Clean Protocol view

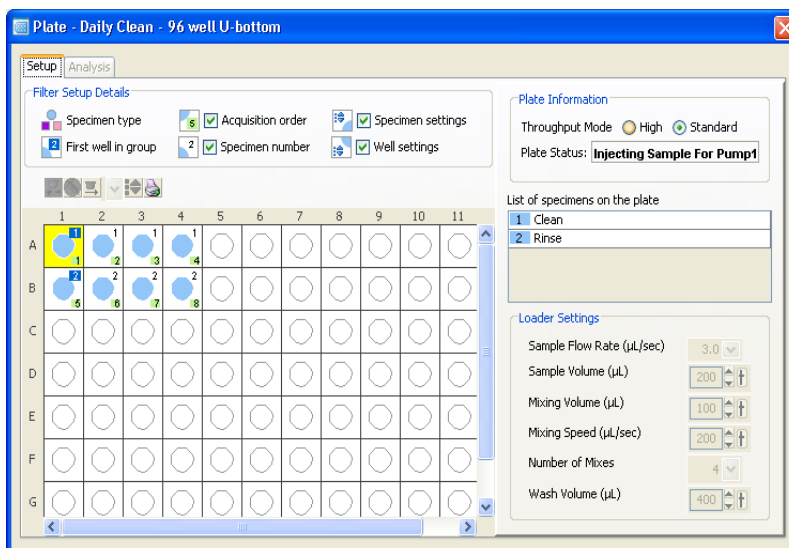
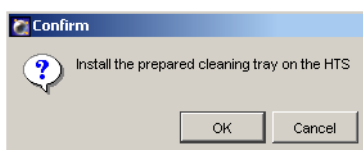




Figure 4-3 Cleaning confirmation dialog



NOTE Do not click OK at this time.

- 4 Fill the wells of a 96-well plate according to the following table.

Wells	Solution	Volume (μL)
A1–A4	BD FACSClean (or a 10% bleach solution)	200
B1–B4	DI water	200

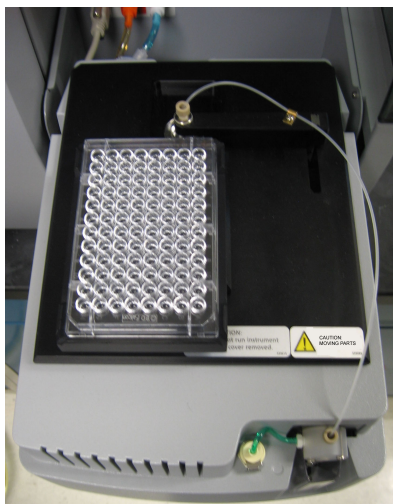
  To ensure that the 10% bleach solution retains its full germicidal effect, prepare a fresh solution daily.

NOTE If you wish to run BD FACSRinse solution as part of the daily cleaning procedure, repeat the daily clean using BD FACSRinse solution in wells A1–A4 and DI water in wells B1–B4.

- 5 Remove or open the safety cover and place the plate on the plate holder.

Make sure the plate corresponds to the type selected in the software. Orient the plate with well A1 on the back-right corner of the stage. See Figure 4-4 on page 115. Verify that the sample coupler is properly installed and is not leaking.

Figure 4-4 Orienting the plate



- 6 Replace the safety cover.
- 7 (BD LSR II and BD LSRFortessa) Place the cytometer in Run mode.
- 8 Verify that the Cytometer window displays *Cytometer Connected*.
- 9 Select well A1 (the first well for the cleaning protocol).

- 10 Click **OK** to close the **Cleaning Confirmation** dialog (Figure 4-3 on page 114).

The cytometer goes through a homing sequence, and cleaning begins. Note that the cleaning procedure can take up to 15 minutes.

- 11 Click **OK** when the completion dialog opens.
- 12 Remove and discard the multiwell plate, or rinse it for use on another day.

Cytometer Inspection and Servicing

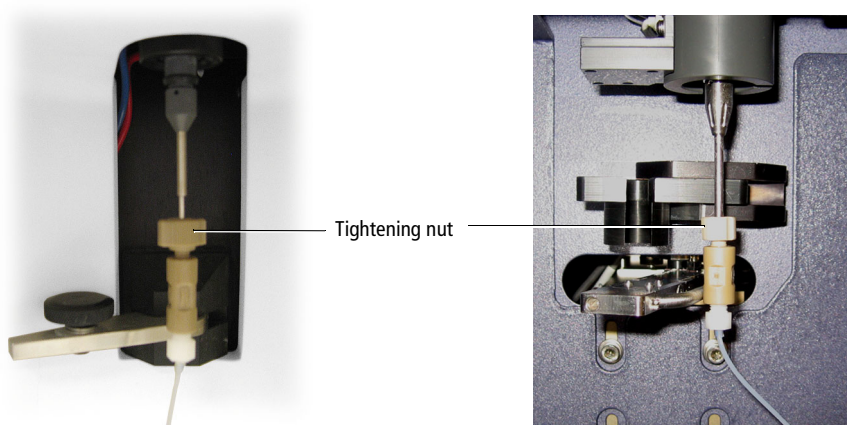
Inspect the following cytometer components at the end of each day and service them, as needed.



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

- Sample coupler

Check for leaks around the sample coupler on the cytometer SIT. If necessary, tighten the top nut to secure the sample coupler to the SIT.



If the coupler continues to leak after you tighten the fitting, remove and then reinstall the coupler as follows.

- a** Hold the coupler with one hand while you unscrew the tightening nut with the other hand.
- b** When the coupler is loose, pull it straight down.
- c** Slide the coupler back up until you reach a hard stop. Make sure the sample coupler tubing is not kinked or twisted.
- d** Hold the coupler with one hand while you tighten the tightening nut with the other hand.

If the coupler is still leaking, replace it. See Replacing the Sample Coupler and Tubing on page 130.

- Injection port/wash station

Check this area for residual liquid. Liquid appearing around the injection port/wash station may indicate a clog. If you suspect a clog, see HTS Troubleshooting on page 154.

- Base plate, probe arm assembly, and plate holder

Dampen a clean, lint-free cloth with distilled or DI water and wipe down only the black surfaces of these components, as needed.

- Sample probe

Make sure the probe is straight. If it is bent or shows signs of wear, replace the probe. See Replacing the Probe on page 136.

- Pumps

- Wipe up any spills on or around the pumps.
- Inspect the fittings to make sure they are tight. See Inspecting the Thumbscrew Fittings on page 149 and Inspecting the Hexagonal Fittings on page 150.
- Inspect the pump syringes for leakage. If your system has been used extensively and the syringes appear worn, replace them. See Replacing a Pump Syringe on page 132.

- (BD LSR II, BD LSRFortessa, and BD FACSCanto) Absorbent pad

Inspect the absorbent pad at the back of the HTS to make sure it is not saturated. Replace the pad, if needed.

Monthly Maintenance

When running the HTS daily, perform the following cleaning procedures at the end of every month. If you are using the HTS less frequently, adjust the monthly maintenance schedule accordingly.

- Monthly Cleaning on page 119
- Surface Inspection and Cleaning on page 121

Monthly Cleaning

Monthly Cleaning (BD LSR II and BD LSRFortessa)

To perform the monthly clean you will need DI water and BD FACSClean or a fresh 10% bleach solution (1 part bleach in 9 parts DI water).

- 1 Bypass the cytometer sheath filter.
 - a Press the quick-disconnectors on both sides of the filter assembly.
 - b Remove the filter assembly.
 - c Reconnect the two fluidic lines.
- 2 Remove the sheath line filter on the supply line from the cytometer to the HTS. See Sheath line and filter (example shown for BD LSR II) on page 127.
- 3 Remove the sheath tank and replace the sheath fluid with BD FACSClean solution or a freshly prepared 10% bleach solution. Replace the tank.



To ensure that the 10% bleach solution retains its full germicidal effect, prepare a fresh solution daily.

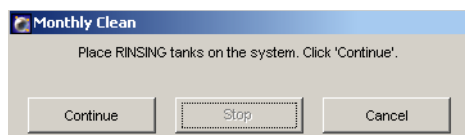
- 4 Open the roller clamp on the sheath line that enters the side of the cytometer, for approximately 10 seconds, to remove excess air from the sheath line.
- 5 Close the roller clamp.
- 6 Prime the cytometer two to three times to bring the cleaning fluid to the flow cell.
- 7 Place the cytometer in Run mode.
- 8 Select **HTS > Prime** and repeat the prime.
- 9 Select **HTS > Monthly Cleaning**.

The Monthly Clean dialog opens.

- 10 Click **Continue**.

A dialog appears while cleaning is in progress. This can take up to 30 minutes.

- 11 When prompted, remove the tank and replace the cleaning solution with DI water.



- 12 Reinstall the tank.

A progress dialog appears while rinsing is in progress. This can take up to 30 minutes.

- 13 Click **Continue**.
- 14 Click **OK** when monthly cleaning is complete.

NOTE If you have been running BD FACSRinse solution as part of a biweekly cleaning procedure, you can repeat the monthly cleaning using BD FACSRinse solution followed by DI water.

- 15 Disconnect the tank, fill it with sheath solution, and reconnect it.
- 16 Reconnect the cytometer sheath filter from step 1.

NOTE If you are performing a sheath fluid exchange, install a new filter.

- 17 Reconnect the HTS sheath line filter from step 2.
- 18 Check for bubbles and prime, if necessary.

Monthly Cleaning (BD FACSCanto and BD FACSCanto II)

- 1 Ensure that the sample coupler is properly installed and the HTS is powered on.
- 2 Empty the waste tank, if necessary.
- 3 Ensure that the BD FACSClean and BD FACS shutdown solution cubitainers are full and connected.
- 4 Select **Cytometer > Long Clean**.
- 5 Click **OK** when the long clean is complete.

Surface Inspection and Cleaning



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

- Use BD FACSClean solution (or a 10% bleach solution) followed by DI water to wipe down the HTS enclosure and cover on a monthly basis, or when needed.

- Inspect the pump syringes for leaks and tighten fittings, if required. See Inspecting the Thumbscrew Fittings on page 149.

Periodic Maintenance

To ensure optimal performance of your HTS system, replace the following cytometer components according to the recommended replacement schedule in Table 4-1. Note that the extent of maintenance will vary depending on how much you use your system. Use the schedule in Table 4-1 as a guideline.

A maintenance log is provided in Appendix A to keep track of when each procedure is performed.

Table 4-1 Recommended replacement schedule

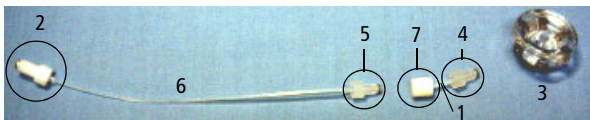
Component	Part No.	Replacement Schedule	Procedure
Sample injection tubing ^a	335526 (A) 335451 (F)	Every 6 months	page 123
Sheath filter	335710	Every 6 months	page 126
Air filter	336218	Yearly or as needed	page 128
Sampler coupler and tubing (BD LSR II and BD LSRFortessa)	335452	Yearly or as needed	page 130
Sampler coupler and tubing (BD FACSCanto and BD FACSCanto II)	339340	Yearly or as needed	page 130
Syringes	339047	Yearly or as needed	page 132
Probe	34389017	Yearly or as needed	page 136
Quick-connector O-ring	343618	Yearly or as needed	page 137
SIT protector and O-ring (BD LSR II and BD LSRFortessa)	335345	Yearly or as needed	page 138

a. See Figure 4-5 on page 123.



Replacing the Sample Injection Tubing

The sample injection tubing connects the injection port/wash station to the secondary pump valve tubing. Replace the tubing every 6 months.

Figure 4-5 Disconnecting sample injection tubing from injection port/wash station



Number	Description
1	Sample injection tubing
2	Hex screw
3	Sample injection port/wash station
4	Thumbscrew
5	Thumbscrew
6	Secondary pump valve tubing
7	Connector

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

- 1 Perform the monthly clean procedure to decontaminate the sample injection port/wash station.

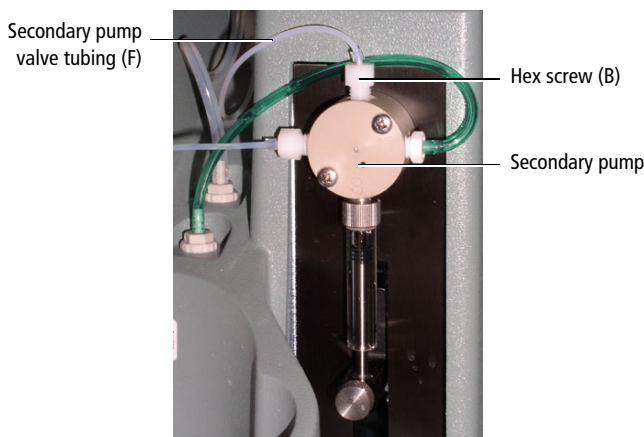
See Monthly Cleaning on page 119.

- 2 Turn off the HTS power switch (BD FACSCanto, BD LSR II, and BD LSRFortessa), or turn off the cytometer (BD FACSCanto II).
- 3 For easy access, remove the HTS.

See Removing the HTS on page 166 for instructions.

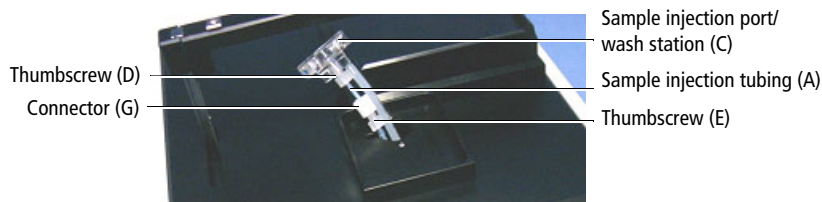
- 4 Move the probe arm up, then push it toward the front of the HTS.
- 5 Remove the sample injection tubing (A) from the HTS. See Figure 4-7 on page 124.
 - a If necessary, disconnect the top hex screw (B) from the secondary pump using the 5/16-in wrench in the accessory kit (Figure 4-5 on page 123 and Figure 4-6).

Figure 4-6 Hex screw on secondary pump



- b Turn the sample injection port/wash station (C) slightly to disengage the magnetic force, then lift it up to expose the thumbscrew (D) on the sample injection tubing (A) and the thumbscrew (E) on the secondary pump valve tubing (F) (Figure 4-7).

Figure 4-7 Accessing the tubing



- c** Unscrew the thumbscrew (E) from connector (G) and remove the secondary pump valve tubing (F) (Figure 4-5 on page 123 and Figure 4-7).
 - d** Disconnect the sample injection tubing (A) from the sample injection port/wash station (C) by unscrewing the thumbscrew (D) (Figure 4-7) and remove the sample injection tubing (A).
- 6** Discard the sample injection tubing (A) and the secondary pump valve tubing (F) as biohazardous waste.
- 7** Install new sample injection tubing.

Replacement tubing is included in the accessory kit.

- a** Screw the thumbscrew (D) onto the sample injection port/wash station (C).
- b** Screw the thumbscrew (E) from the secondary pump valve tubing into connector (G).

NOTE Take care to screw the thumbscrews on straight. If you attach them at an angle, you could strip the threads.

- 8** Insert the secondary pump valve tubing (F) into the opening of the HTS base and route the tubing to the secondary pump. Then, lower the sample injection port/wash station (C) onto the HTS base plate.



The magnetic force will secure the sample injection port/wash station in place.

- 9** Reconnect the secondary pump valve tubing (F) to the secondary pump by tightening the hex screw (B).
- 10** Reinstall the HTS.

Follow the instructions for Installing the HTS on page 179.

Replacing the HTS Sheath Filter

The HTS sheath filter is integrated in the fluid line that supplies sheath fluid to the HTS (Figure 4-9 on page 127). Replace the filter every 6 months.

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

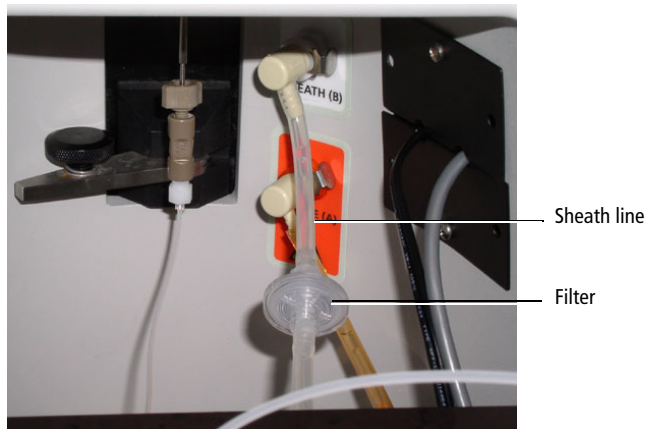
- 1 Loosen the positioning screw holding the HTS to the cytometer support bracket (Figure 4-8).

Figure 4-8 Loosening the positioning screw (example shown for BD LSR II)



- 2 Slide the HTS towards you by doing the following:
 - a Slightly lift the HTS to clear the screw.
 - b Being careful not to strain the sheath tubing, slide the HTS towards you until you can access the filter.

Figure 4-9 Sheath line and filter (example shown for BD LSR II)



- 3** (BD FACSCanto) Remove the catch tray.

Pull the HTS towards you slightly to allow room for removing the catch tray.

- 4** Label the sheath line connectors.

To keep track of the flow direction, label each quick-connector so you know which end attaches to the cytometer (C), and which end attaches to the HTS.

- 5** Remove the sheath line.

Press the metal tabs to release the two off-white quick-connectors, one on the cytometer interface panel and the other on the back of the HTS.

- 6** Remove the filter.

- a** Detach the filter from one tubing segment by holding the filter while you twist the Luer connection.

- b** Repeat this operation for the other tubing segment.

- 7** Install the new filter.

- a** Attach the tubing segment with the male connector to the female filter connection. Hold the filter while you twist the Luer lock ring.
 - b** Attach the tubing segment with the female connector to the male filter connection. Hold the filter while you twist the Luer female connector.
- 8** Reinstall the sheath line between the cytometer interface panel and the HTS.

Attach the quick-connectors at each end.

NOTE Although the sheath filter is a screen filter that provides identical performance regardless of the flow direction, we recommend that you always install the sheath line with the lock-ring end toward the cytometer panel (connector labeled C). This prevents any foreign material accumulated on one side of the filter from flushing into the HTS if the filter is reversed.

- 9** Reinstall the HTS.
 - a** (BD FACSCanto) Install the rear catch tray by routing the sample coupler underneath the catch tray tongue and sample coupler slot. Push the tray in, making sure the coupler line is not crimped or kinked.
 - b** Push in the HTS and tighten the positioning screw holding the HTS to the cytometer support bracket.

Cleaning the Air Filter

The air filter element is located within a filter retainer and fan guard attached to the base plate of the HTS. To clean or replace the air filter, follow these steps.

- 1** Remove the HTS from the cytometer.

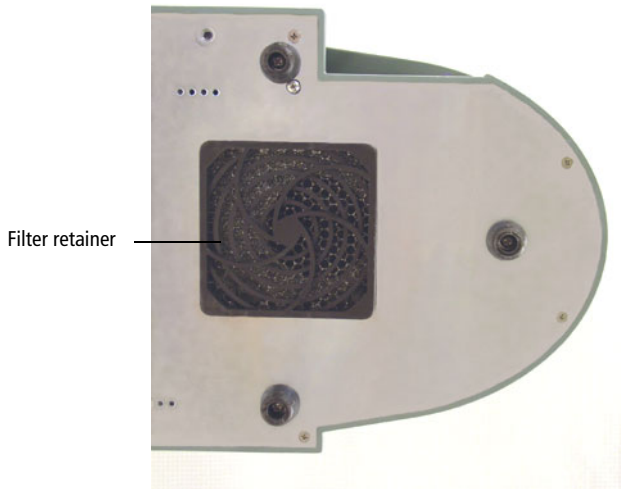
For detailed instructions, see Removing the HTS on page 166.

- 2** Place the HTS on its side.

NOTE To avoid scratching the finish, handle the HTS with care. Do not drag the HTS on the laboratory bench.

3 Remove the filter retainer.

The filter retainer is located within the air filter element in the base plate of the HTS.



- a** Place your middle and index fingers of each hand on the opposite edges of the filter retainer. Place your thumbs on the fan guard through the retainer apertures.
- b** Support the fan guard with your thumbs as you pull out the retainer with your fingers.

NOTE To prevent the HTS from tipping, use your thumbs to hold the HTS in place while pulling.

4 Remove the filter and clean or replace it as needed.



Filter

Filter retainer

To clean the filter, rinse it well with water. Dry it completely before you reinstall it.

5 Place the clean filter inside the filter retainer.

6 Reinstall the filter retainer.

Center it over the fan guard and push evenly on the opposite edges to snap it into place.



7 Place the HTS right-side up.

8 Reinstall the HTS on the cytometer.

For detailed instructions, see the Installing the HTS on page 179.

Replacing the Sample Coupler and Tubing

If you frequently remove the sample coupler to run the cytometer in tube mode, replace the coupler yearly or if the coupler continues to leak after you have tightened the fitting.

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

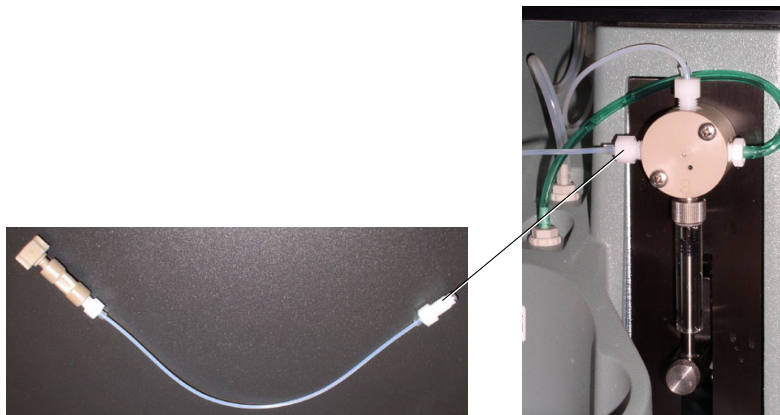
1 Remove the HTS from the cytometer.

For detailed instructions, see Removing the HTS on page 166.

2 Detach the sample coupler tubing.

Unscrew the tubing fitting from the secondary pump valve (Figure 4-10) and discard the sample coupler and tubing as biohazardous waste.

Figure 4-10 Detaching the sample coupler and tubing



3 Install a new sample coupler and tubing.

Replacement tubing is included in the accessory kit. Connect the new tubing fitting to the port in the secondary pump valve. Tighten the tubing fitting until it is finger-tight.



Valves contain sealing washers in each port. If you over-tighten the fitting, the sealing washers can be compressed, resulting in a blocked port. Tighten the fitting until it contacts the sealing washer, then turn the fitting an additional 1/6 to 1/4 turn. If leaking is observed, tighten the fitting no more than an additional 1/8 turn.

4 Reinstall the HTS on the cytometer.

For detailed instructions, see Installing the HTS on page 179.

Replacing a Pump Syringe

Glass syringes are used in the primary and secondary pumps in the HTS. Replace the syringes once a year or whenever leaks occur or pumping accuracy is suspect. To begin this procedure, the cytometer and software must be running.



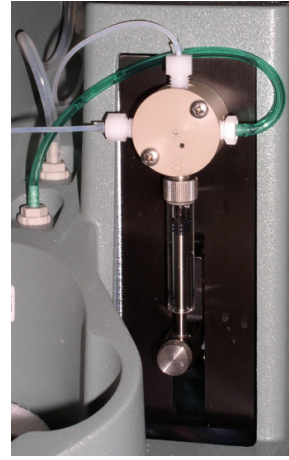
All cytometer surfaces and hardware that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when changing the syringe. Wear suitable protective clothing, eyewear, and gloves.

- 1 Before replacing the pump syringe, perform the monthly clean procedure.
See Monthly Cleaning on page 119.
- 2 (BD LSR II and BD LSRFortessa) Place the cytometer in Run mode.
- 3 Remove the liquid from the syringe by performing steps 1 through 11 in Placing the HTS in Long-Term Storage on page 140.
- 4 Turn off the HTS power switch (BD FACSCanto, BD LSR II, and BD LSRFortessa), or turn off the cytometer (BD FACSCanto II). Keep the software running on the workstation.
- 5 If you are replacing:
 - The primary pump syringe located at the front of the HTS, skip to step 6.
 - The secondary pump syringe located at the back of the HTS, remove the HTS. See Removing the HTS on page 166.

Figure 4-11 Primary and secondary pumps



Primary syringe pump

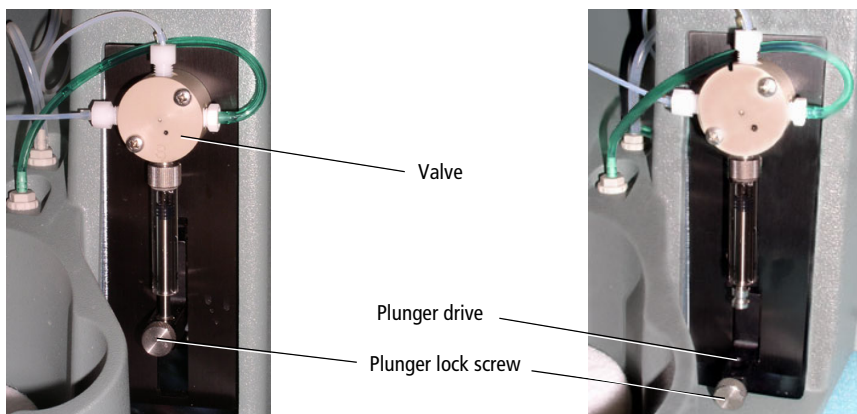


Secondary syringe pump

- 6** Push the syringe plunger up to the top of the syringe barrel.
- 7** Loosen the plunger lock screw approximately three full turns counterclockwise (Figure 4-12 on page 134).
- 8** Lower the plunger drive by pushing down on the plunger lock screw.

See Figure 4-12 on page 134.

Figure 4-12 Lowering the plunger



- 9** Unscrew the syringe from the valve.

Grasp the metal ring at the top of the syringe and turn it counterclockwise.

- 10** Discard the syringe into a biohazardous sharps container.

- 11** Install the new syringe (Figure 4-13 on page 135).

- a** Screw the syringe into the valve (see *a* in Figure 4-13).

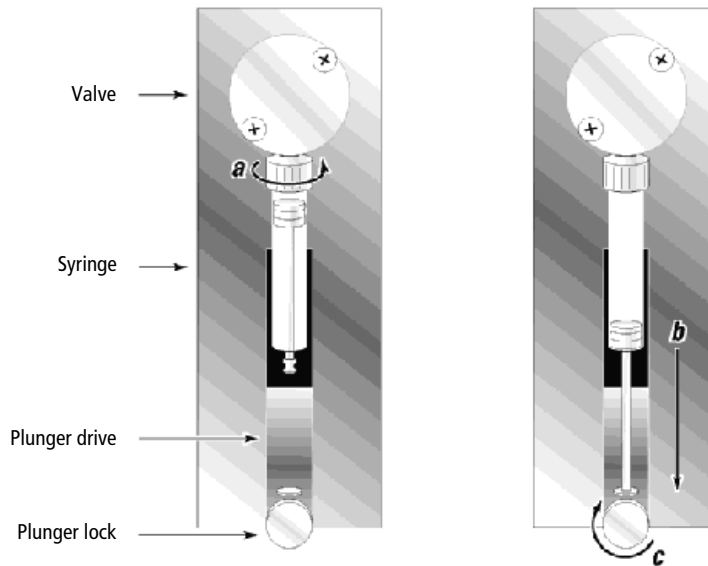
Screw the syringe into the valve port until it contacts the seal washer, then turn the syringe an additional 1/6 to 1/4 turn. If leakage is observed, tighten a maximum of an additional 1/8 turn.

- b** Pull down the syringe plunger until it meets the plunger drive (see *b* in Figure 4-13).

- c** Tighten the plunger lock screw (see *c* in Figure 4-13 on page 135).

Make sure the plunger lock screw is secured tightly.

Figure 4-13 Installing the syringe



12 If you:

- Replaced the primary pump syringe, skip to step 13.
- Replaced the secondary pump syringe, reconnect the HTS to the cytometer by following the procedure in *Installing the HTS* on page 179.

13 Refill the pump.

- (BD LSR II and BD LSRFortessa) Verify that the cytometer is still in Run mode.
- (BD FACSCanto II) Turn on the cytometer.
- Reattach the sheath line and filter in back of the HTS.
- (BD FACSCanto, BD LSR II, and BD LSRFortessa) Turn on the HTS.

- (BD LSR II and BD LSRFortessa) Select **HTS > Prime** to prime the fluidics until the plunger barrel is full of sheath fluid. If you see any bubbles, continue priming until the bubbles are gone.
- (BD FACSCanto and BD FACSCanto II) Select **Cytometer > Fluidics Startup** to perform a fluidics startup. Verify that the plunger barrel is full of sheath fluid. If you see any bubbles, select **HTS > Prime** and repeat until all bubbles are gone.

Replacing the Probe

The probe is located at the end of the probe assembly arm. Replace the probe whenever it is bent or permanently clogged. A spare probe is included in the accessory kit.



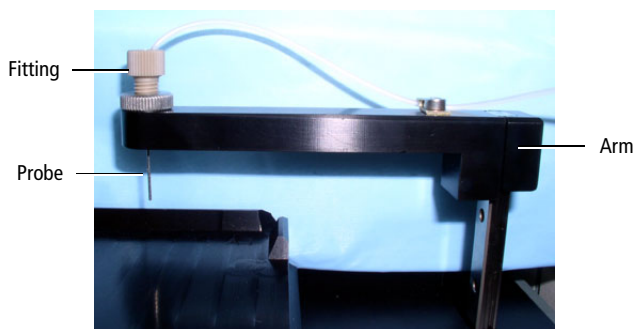
  All cytometer surfaces and hardware that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when replacing the probe. Wear suitable protective clothing, eyewear, and gloves.

Figure 4-14 Probe assembly components





- 1 Unscrew the fitting at the top of the probe (Figure 4-14).
- 2 Remove the probe by unscrewing it from the arm, and discard the probe into a biohazard sharps container.

- 3 Install a new probe.
- 4 Reattach the fitting at the top of the probe.

Replacing the Quick-Connector O-Rings

The sheath and waste lines are attached to the cytometer interface panel and the HTS using quick-connectors. There is an additional waste line attached to the cytometer waste tank using a quick-connector. Replace the quick-connector O-rings once a year or whenever a connector is leaking.

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



- 1 Disconnect the quick-connector.
- 2 Remove the O-ring using a flat, small screwdriver. Discard the O-ring as biohazardous waste.



- 3 Install a new O-ring, pushing it into place with your thumbs.
- 4 Reconnect the quick-connector.


Replacing the SIT Protector and O-Ring (BD LSR II and BD LSRFortessa)

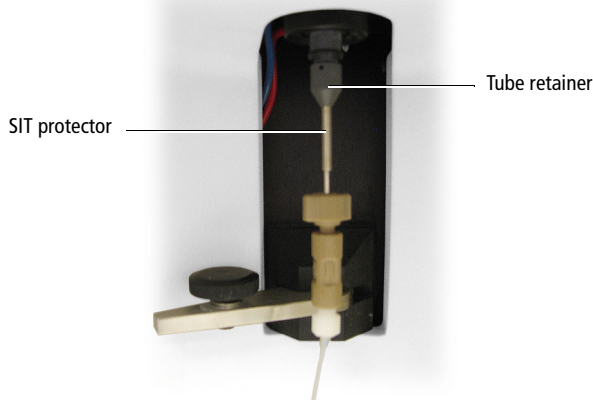
The SIT protector is a modified sleeve that prevents the sample injection tube from bending during installation of the HTS sample coupler. An O-ring between the SIT protector and the tube retainer keeps the protector in place. Replace the O-ring when the SIT protector slips down by itself.

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

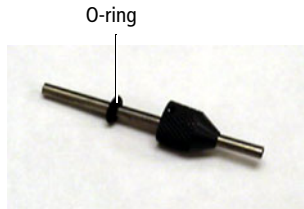
- 1 Remove the safety cover from the HTS.
- 2 Detach the sample coupler from the cytometer SIT.
- 3 Remove the SIT protector.

Unscrew the tube retainer and slide the SIT protector straight down.

 To avoid bending the SIT, do not slide the SIT protector at an angle.



- 4 Slide the tube retainer down the metal tubing to find the O-ring.



If the O-ring is stuck inside the retainer, push the retainer to the top of the tubing and work it down at an angle until the O-ring is free.

- 5 Remove the O-ring and discard it as biohazardous waste.
- 6 Install a new O-ring and slide it to about the middle of the tube.

O-rings are included in the accessory kit.

- 7 Slide the SIT protector straight onto the SIT.



To avoid bending the SIT, do not slide the SIT protector at an angle.

- 8 While holding the tube, gently push up on the retainer screw and screw it on to secure it.
- 9 Reattach the sample coupler to the cytometer SIT.

- a Slide the sample coupler onto the SIT until you reach a hard stop.



Make sure the sample coupler tubing is not kinked or twisted.

- b Hold the coupler with one hand while you tighten the top nut with the other hand.

Note that there should be a gap between the tightening nut and the bottom of the SIT protector. If you don't see a gap, unscrew the tube retainer, push the SIT protector all the way up, and retighten the tube retainer.

Placing the HTS in Long-Term Storage

To prevent salt deposits from forming in the fluidics system, perform the following procedure if you will not be using your HTS for a week or more. See *Cleaning the HTS* on page 169.

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

Placing the HTS in Long-Term Storage (BD LSR II and BD LSRFortessa)

- 1 Remove the HTS safety cover.
- 2 Locate and loosen the positioning screw that secures the HTS to the cytometer support bracket (Figure 4-15 on page 140).

Figure 4-15 Loosening the positioning screw



- 3 Grasp the sides of the HTS and slide it forward until it meets the stop in the cytometer support bracket.

- Lift the HTS off of the cytometer support bracket by doing the following:



To prevent personal injury or damage to the HTS, do not slide the HTS out so far that it becomes unbalanced or the strain of the tubing bends the SIT.

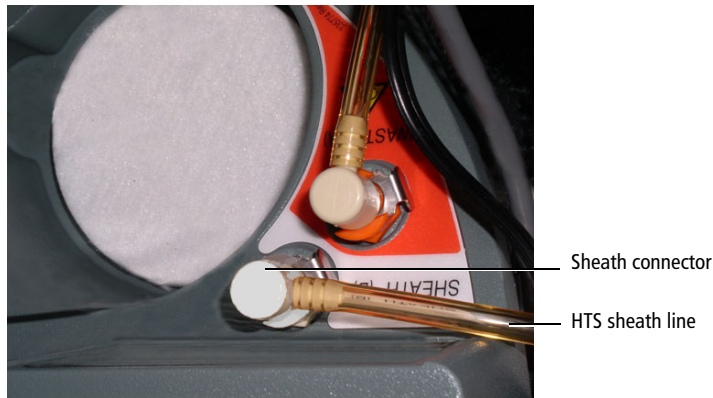
- Slightly lift the HTS to clear the positioning screw.
- Being careful not to strain the sheath tubing, slide the HTS towards you until you can access the filter.

This allows you better access to the connections on the back of the HTS.

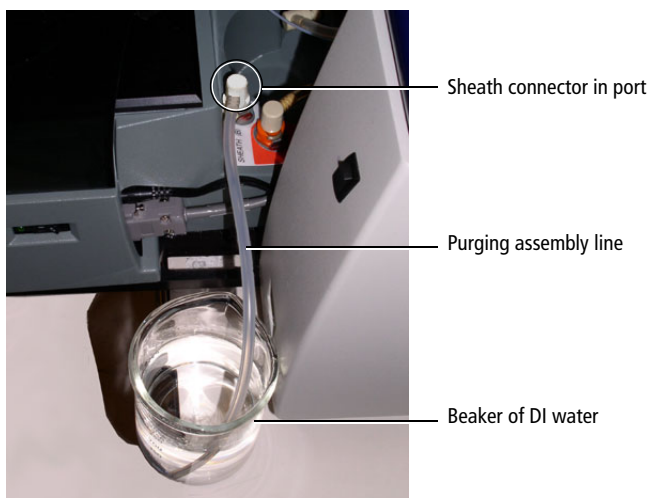
- Detach the sheath line from the sheath port on the back of the HTS (Figure 4-16 on page 141).

Press the metal button to release the connector. Leave the line attached to the cytometer interface panel.

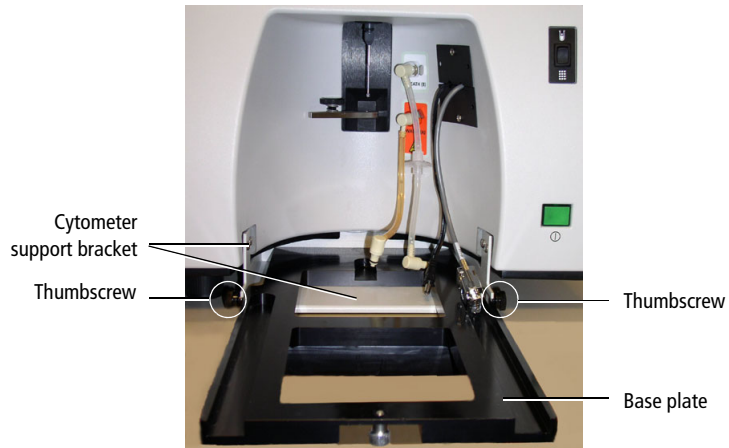
Figure 4-16 HTS sheath and waste lines



- Connect the purging assembly line to the sheath port.
The purging assembly line is located in the spares kit.
- Put the end of the purging assembly line into a 500-mL beaker containing DI water.



- 8** Put the safety cover on the HTS.
- 9** Select **HTS > Prime** and repeat nine times.
Priming will replace the sheath fluid with DI water.
- 10** Remove the end of the purging assembly line from the DI water and lay it on the benchtop.
- 11** Remove the purging assembly line.
- 12** Detach the HTS from the cytometer by following the steps in *Removing the HTS* on page 166.
- 13** Remove the HTS base plate.
 - a** Unscrew the two thumbscrews on each side of the base plate.



- b** Pull the base plate toward you until it disengages from the cytometer support bracket.
- c** Store the base plate with the HTS.

Placing the HTS in Long-Term Storage (BD FACSCanto and BD FACSCanto II)

- 1** Ensure that the sample coupler is installed and the HTS is turned on.
- 2** Perform a fluidic shutdown.
See Fluidics Shutdown on page 110 for details.
- 3** If required, remove the HTS from the cytometer.
See Removing the HTS on page 166.

Unscheduled Maintenance

Perform the following procedures when necessary or when you are directed to do so by a BD Biosciences service representative.

- Homing the Sample Probe on page 144
- Priming the HTS on page 145
- Performing a Motion Test on page 146
- Verifying the Sample Probe Position on page 147
- Inspecting the Thumbscrew Fittings on page 149
- Inspecting the Hexagonal Fittings on page 150
- Declogging the SIT on page 150

Homing the Sample Probe

During normal operation, the sample probe goes through a homing sequence during initialization. Perform the following procedure to manually send the probe to the home position, or when directed by a BD Biosciences service representative during troubleshooting.

- 1** (BD LSR II and BD LSRFortessa) Place the cytometer in Run mode, and ensure that the sample coupler is installed.

The software automatically primes the HTS pumps during a homing operation. To prevent pressure from building in the flow cell, the cytometer must be in Run mode before the homing sequence begins. If you try to run the Home command when the cytometer is not in Run mode, the software indicates that the fluidic system is not ready.

(BD FACSCanto and BD FACSCanto II) Ensure that the sample coupler is installed and the HTS is turned on.

- 2 Select **HTS > Home**.
- 3 Verify that the HTS properly performs the following homing sequence:
 - a The probe moves to the home position, if it is not already there.
 - b The probe moves up.
 - c The plate holder moves left, and then slightly forward.
 - d The probe moves down.
 - e The plate holder moves right to the home position.

After this sequence, the valves initialize (priming occurs) and a completion dialog opens.

- 4 Click **OK**.

Priming the HTS

Prime the HTS when you set it up for the first time (for example, after a depot repair procedure), when you notice bubbles in the pump syringe barrel, or to clear a clog in the HTS fluidics. The Prime command automatically primes both pumps.

- 1 (BD LSR II and BD LSRFortessa) Ensure that the sample coupler is installed, and place the cytometer in Run mode.

To prevent pressure from building in the flow cell, the cytometer must be in Run mode before priming begins. If you try to run the Prime command when the cytometer is not in Run mode, the software indicates that the fluidic system is not ready.

(BD FACSCanto and BD FACSCanto II) Ensure that the sample coupler is installed and the HTS is turned on.

- 2 Select **HTS > Prime**.

- 3 Verify that the pumps are primed and the HTS tubing fills with sheath fluid.

Once priming is complete, a completion dialog opens.

- 4 Click OK.

Performing a Motion Test

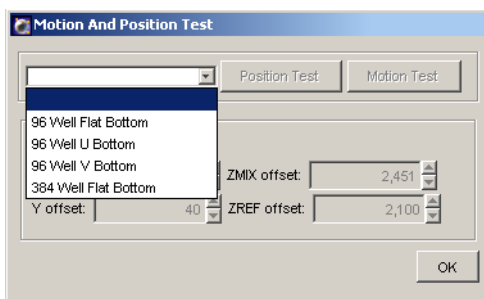
You might need to perform a motion test when you set up the HTS for the first time (for example, after a depot repair procedure), when axis movement is suspect, or when you are directed to do so by a BD Biosciences service representative during cytometer troubleshooting.

Note that this test executes the same sequence whether a 96- or 384-well plate is selected. For the most accurate troubleshooting, use a 384-well plate to verify that the mechanism reaches the extreme positions.

- 1 Select **HTS > Motion and Position Test**.

The Motion and Position Test dialog opens.

- 2 Select the plate type from the menu.



After you select the plate type, the Motion Test button becomes enabled.

- 3 Install the corresponding plate onto the plate holder and replace the safety cover.

- 4** Click **Motion Test**.
- 5** Verify that the sample probe properly performs the following homing sequence:
 - a** The probe travels to the home position.
 - b** The probe moves up, and then moves to the extreme positions for a selected plate type.
 - c** The probe returns to the injection port and a completion dialog opens.
- 6** Click **OK**.

If the motion test fails, contact your local BD Biosciences service representative.

Verifying the Sample Probe Position

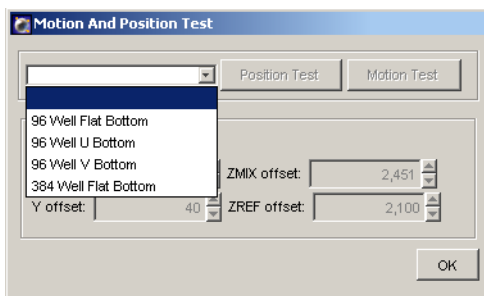
This test positions the probe at the center of the nearest and farthest wells on a plate so you can visually verify the mechanical positioning of the probe.

To run this test, you must select a plate type in BD FACSDiva software and install the corresponding plate on the plate holder. The test can be run with a 96- or 384-well plate.

- 1** Select **HTS > Motion and Position Test**.

The Motion and Position Test dialog opens.

- 2** Select the plate type from the menu.



After you select the plate type, the Position Test button becomes enabled.

- 3 Install the corresponding plate onto the plate holder and replace the safety cover.
- 4 Click **Position Test**.
- 5 Verify that the sample probe properly performs the following sequence:
 - a The probe travels to the home position.
 - b The probe moves up, and then moves to well A1.

The Position Test dialog opens.

- 6 Remove or open the safety cover and verify that the probe is in the center of the nearest well (position A1).
- 7 Replace or close the safety cover, then click **OK**.
- 8 Remove or open the safety cover and verify that the probe is in the center of the farthest well (position H12).
- 9 Replace or close the safety cover and click **OK**.

The probe moves to well H12 and the Position Test dialog opens.

The probe returns to the injection port and a completion dialog opens.

- 10 Click **OK**.

Reinitializing the HTS

If you switch off the HTS, and then switch it back on again while the software is running, you must reinitialize the HTS.

- 1 Select **HTS > Reinitialize**.

A completion dialog opens.

- 2 Click **OK**.

Inspecting the Thumbscrew Fittings

Thumbscrew fittings are installed in the following tubing locations. Check and tighten these thumbscrews periodically, and replace them when needed.





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

- **Primary pump valve to probe.** There are male thumbscrew fittings at both ends of the tubing.
- **Sample injection tubing.** There is a male thumbscrew fitting at the sample injection port/wash station and a female thumbscrew fitting at the extension tubing to the secondary pump valve.
- **Extension tubing to the secondary pump valve.** There is a male thumbscrew fitting at the sample injection tubing end.

Inspecting the Hexagonal Fittings

Hexagonal male fittings are located at the following locations. Check and tighten these fittings periodically, and replace them when needed.



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


- Left port of the primary pump
- Top, left, and right ports of the secondary pump

NOTE Valves contain sealing washers in each port. Over-tightening the fittings can compress the seal washers resulting in a blocked port. Insert a fitting or syringe into a port until it contacts the seal washer, then turn the fitting or syringe an additional 1/6 to 1/4 turn. If leakage is observed, tighten a maximum of an additional 1/8 turn.

Declogging the SIT

Perform this procedure to dislodge a clog in the SIT only when instructed to do so by BD Biosciences. You need a cleaning stylus to complete this procedure.

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

 To avoid a service call, do not insert the cleaning stylus all the way into the SIT. Allow at least an inch of the stylus to protrude from the end of the SIT while declogging.

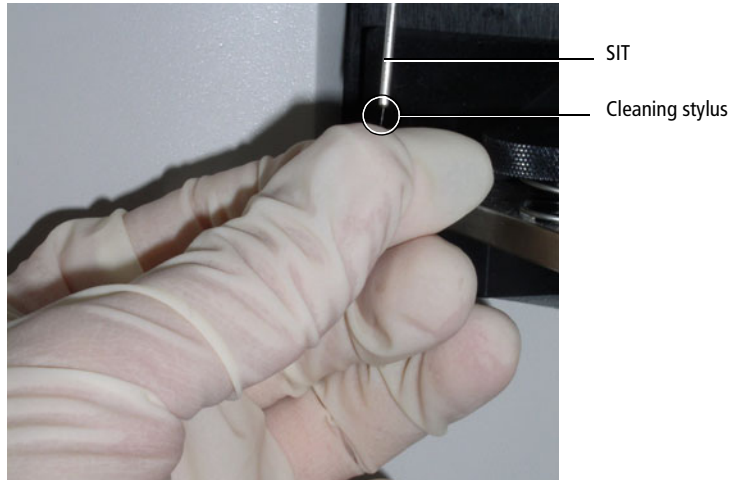
- 1 Remove the sample coupler.

- 2 About one inch from one end of the cleaning stylus, bend it at a 90° angle.

Bending the end of the cleaning stylus prevents you from inserting it too far into the SIT.

- 3 Hold the bent end of the cleaning stylus, and thread about three-quarters of the stylus up into the SIT (see Figure 4-17).

Figure 4-17 Declogging the SIT



- 4 Gently pull the cleaning stylus most of the way out of the SIT and then push it back in. Repeat several times.
- 5 Remove the cleaning stylus from the SIT, rinse the stylus with BD FACSClean solution followed by DI water, and dry the stylus.
- 6 Replace the sample coupler.
- 7 Select **HTS > Prime**.

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Troubleshooting

The tips in this section are provided to help you troubleshoot issues that might arise when using the HTS. For additional troubleshooting assistance, see the appropriate cytometer user's guide.

If additional assistance is required, contact your local BD Biosciences service representative. See Technical Assistance on page xi.

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

- HTS Troubleshooting on page 154
- Acquisition Troubleshooting on page 155
- BD FACSDiva Troubleshooting on page 158

HTS Troubleshooting

Observation	Possible Causes	Recommended Solutions
Bubbles in sample wells	Mixing volume is too great	Reduce the mixing volume. See Loader Settings on page 35, Sample Well Volumes on page 35, and Mixing on page 36 for recommendations.
	Too many mixes	Reduce the number of mixes.
	Insufficient sample volume	Increase the sample volume.
Leaking around sample coupler or cytometer SIT	Coupler or tube retainer is loose or worn	Check the fittings and tighten them as needed.
Leaking around pump syringes	Fitting is loose	Tighten the fitting, and replace it if necessary. See Inspecting the Thumbscrew Fittings on page 149.
(BD LSR II and BD LSRFortessa) DCM system running when tube arm is to the side	Acquisition switch is in tube mode	Switch to plate mode.
Bubbles in waste tank	No antifoam concentrate is being used	Add 500 µL Sigma Antifoam A Concentrate to the waste tank.
Fluid accumulating around the injection port/wash station	Sample probe assembly is bent	Replace the sample probe assembly.
	Clogged wash station	<ul style="list-style-type: none"> • Add water to the injection port/wash station. Insert the cleaning stylus into the drain on the side of the wash station. • Replace the wash station assembly.
Sheath float switch time-out error during sheath exchange	Air in the fluid lines (BD FACSCanto and BD FACSCanto II)	<ul style="list-style-type: none"> • Select Cytometer > Cleaning Modes > Prime after Tank Refill. • Bleed the air out of sheath filter.

Acquisition Troubleshooting

Observation	Possible Causes	Recommended Solutions
Unexpectedly low event rate, or no events in plots	Sheath is not pressurized	(BD LSR II and BD LSRFortessa) Ensure that the pressure relief valve on the cytometer sheath tank is in the closed position.
	Sample injection tubing is kinked or damaged	Replace the tubing. See Replacing the Sample Injection Tubing on page 123.
	Sampler coupler tubing is kinked or damaged	Replace the tubing. See Replacing the Sample Coupler and Tubing on page 130.
	Clogged probe	Run the daily cleaning procedure to clean the probe. See Daily Cleaning on page 112. If the probe remains clogged, replace it. See Replacing the Probe on page 136.
	Clogged SIT	Check for clogs in the SIT using the cleaning stylus. See Declogging the SIT on page 150 for procedure. See Table A-2 on page 161 for cleaning stylus ordering information. If the SIT remains clogged, replace it. See Replacing the Sample Injection Tubing on page 123.
	Incorrect cytometer settings	<ul style="list-style-type: none"> • Ensure that the cytometer settings are correct. • Run setup controls to optimize cytometer settings for the sample type.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unexpectedly low event rate, or no events in plots (continued)	Waste tank back pressure	<ul style="list-style-type: none"> • Ensure that the waste fittings are connected and not damaged. • (BD LSR II and BD LSRFortessa) Ensure that the waste lines are not kinked. • Ensure that antifoam concentrate is added to the waste tank.
	Cytometer malfunction	<ul style="list-style-type: none"> • Manually run a tube of beads and verify that events appear in plots.
	Bubble in the sample well(s)	Gently tap the plate to ensure that all wells are bubble-free, then reacquire the sample.
	Bubbles in the flow cell	Ensure that BD FACS sheath solution with surfactant is used during plate-based acquisition.
	Insufficient sample or no sample in the well	Ensure that there is sufficient sample in the well.
	Bubbles in the syringe	Prime the HTS. Select HTS > Prime .
	Filter cap is wet	(BD FACSCanto and BD FACSCanto II) Replace the filter cap.
Unexpectedly low number of events recorded	Insufficient stopping criteria	Make sure to set the target number of events high enough that you do not run out of events before you reach the software-based acquisition time (sample volume/sample rate).

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
High carryover	Mixing volume is too great	Decrease the mixing volume.
	Too many mixes	Reduce the number of mixes.
	Sample volume is too large	Decrease the sample volume.
	Wash volume is too low	Increase the wash volume.
	Sample coupler is not completely installed	Slide the sample coupler on the SIT until you reach a hard stop.
	Damaged injection tubing	Replace the sample injection tubing. See Replacing the Sample Injection Tubing on page 123.

BD FACSDiva Troubleshooting

Observation	Possible Causes	Recommended Solutions
System error	Cover error	Replace the cover and resume the run.
	Back pressure error	<ul style="list-style-type: none"> • Ensure that the waste line to the cytometer is properly installed and is not kinked. • Ensure that the filter on the waste tank is not clogged.
	Motion error	Select HTS > Home and resume the run.
	Pump error	Select HTS > Home and resume the run.
Plots not refreshing	Incorrect worksheet displayed	Ensure that the global worksheet is displayed during a sequenced, plate-based acquisition.
Run buttons not enabled	No global worksheet is selected	Switch to the global worksheet view by clicking the Worksheet View button.
	Well(s) not selected	Select well(s).
(BD FACSCanto and BD FACSCanto II) HTS not included in cleaning modes	Sample coupler not installed	Ensure that the sample coupler is installed before cleaning modes are selected.
	No power	Ensure that the HTS is powered on.
	Cover is open	Close the cover.

Appendix A

Consumables and Replacement Parts

This appendix provides a list of supplies and replacement parts for your HTS. To order spare parts and consumables from within the US, call 877.232.8995 or go to bdbiosciences.com. In other countries, contact your local BD Biosciences representative.

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

You will find the following in this appendix:

- Cytometer Supplies on page 160
- Maintenance Log on page 162

Cytometer Supplies

The HTS is shipped with a preventive maintenance kit including replacement parts for one year of maintenance, and an accessory kit containing other replacement parts. Use the following part numbers if you need to order part replacements. Note that parts in the preventive maintenance kit can also be ordered individually.

Table A-1 Preventive maintenance kit: part no. 337042

Item	Part No.	Kit Quantity
Glass syringe, 500 μ L	344912	2
Tubing assembly, secondary pump to cytometer (BD LSR II and BD LSRFortessa)	335452	1
Tubing assembly, secondary pump to cytometer (BD FACSCanto and BD FACSCanto II)	339340	1
Tubing assembly, probe to primary pump	335454	1
Tubing assembly, injection port	335526	2
Probe and tubing assembly	34389017	1
HTS sheath filter (2)	335710	2
Quick-disconnect O-ring	343618	7
O-ring sleeve retainer (BD LSR II and BD LSRFortessa)	343620	1
Air filter	336218	1
Absorbent pad (BD LSR II, BD LSRFortessa, and BD FACSCanto)	343502	1
Injection tubing to secondary pump	335451	2

Table A-2 Other replacement parts and reagents

Item	Part No.
SIT protector assembly (BD LSR II and BD LSRFortessa)	335345
BD LSR II and BD LSRFortessa sheath line assembly	336076
BD LSR II and BD LSRFortessa waste line assembly	336077
BD FACSCanto sheath line assembly	7000949
BD FACSCanto waste line assembly	7000950
BD FACSCanto II sheath line assembly	700097807
BD FACSCanto II waste line assembly	700097707
Line declogger tool	343583
Air filter retainer	336474
5/16-inch open-ended wrench	337016
BD FACS sheath solution with surfactant	336524
BD FACSClean solution	340345
BD FACSRinse solution	340346
Anti-foam concentrate, 25-g bottle	334887
Cleaning stylus	343648
Purging assembly line (BD LSR II and BD LSRFortessa)	340088
HTS safety cover (BD LSR II, BD LSRFortessa, and BD FACSCanto)	640404
2-ft serial cable (BD FACSCanto)	640737
HTS catch tray (BD FACSCanto)	640576
HTS power/communication cable (BD FACSCanto II)	344368

Maintenance Log

Use the following log to keep track of maintenance procedures on your HTS. You can photocopy the log and keep it next to the cytometer, or use it as a guide to design your own.

Daily Maintenance	Procedure
Daily Cleaning	page 112
Cytometer Inspection and Servicing <ul style="list-style-type: none"> • Sample coupler • Base plate, probe assembly, plate holder • Sample probe • Pumps • Absorbent pad 	page 116

Monthly Maintenance	Procedure	Month Completed (Initials/Date)					
Monthly Cleaning	page 119						
Surface Inspection and Cleaning	page 121						

Periodic Maintenance	Procedure	Completed (Initials/Date)					
Replacing the Sample Injection Tubing	page 123						
Replacing the HTS Sheath Filter	page 126						
Cleaning the Air Filter	page 128						
Replacing the Sample Coupler and Tubing	page 130						

Periodic Maintenance	Procedure	Completed (Initials/Date)					
Replacing a Pump Syringe	page 132						
Replacing the Probe	page 136						
Replacing the Quick-Connector O-Rings	page 137						
Replacing the SIT Protector and O-Ring (BD LSR II and BD LSRFortessa)	page 138						
Placing the HTS in Long-Term Storage	page 140						

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Appendix B

Depot Repair Procedures

This appendix contains procedures to perform if your HTS needs repair and depot repair service is available in your region.



NOTE Depot repair procedures might be different outside the United States. Contact your local BD Biosciences service representative for information for your region.

The following procedures are covered in this appendix:

- Removing the HTS on page 166
- Cleaning the HTS on page 169
- Unpacking the Replacement HTS on page 177
- Installing the HTS on page 179
- Packing the HTS for Shipping on page 184

Removing the HTS

Follow this procedure to detach your HTS from the cytometer to perform maintenance specified in this user's guide, or before sending it in to BD Biosciences for depot repair.

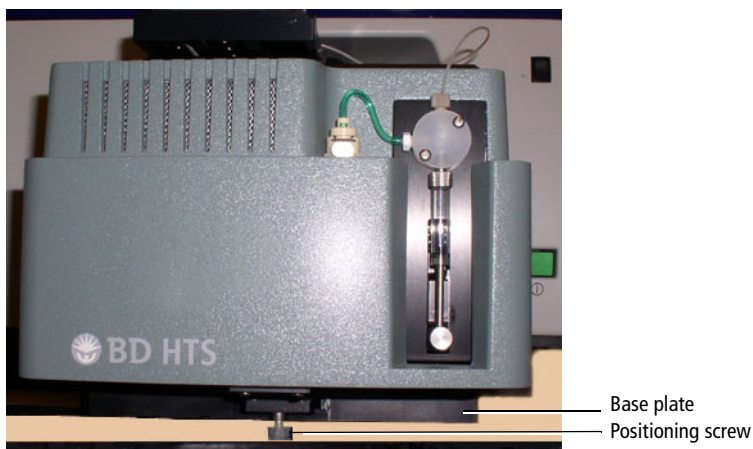
  All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when handling cytometer parts. Wear suitable protective clothing, eyewear, and gloves.

NOTE If you are able to run the system, decontaminate the HTS before you remove it by performing the monthly clean procedure. See Monthly Maintenance on page 119. If you cannot run the system, decontaminate it after removal as described in Cleaning the HTS on page 169.

- 1** (BD LSR II, BD LSRFortessa, and BD FACSCanto) Switch off the HTS power.
- 2** Shut down the cytometer.
- 3** Remove or open the HTS safety cover.
- 4** Detach the sample coupler from the cytometer SIT.
 - a** Unscrew the top nut.
 - b** Pull the sample coupler down and away from the SIT, leaving the nut attached to the sample coupler.
- 5** Locate and loosen the positioning screw that secures the HTS to the base plate.

See Figure B-1 on page 167.

Figure B-1 Location of positioning screw (example of BD LSR II)



- 6** Grasp the sides (or back) of the HTS and slide it forward until it meets the stop in the base plate.
- 7** (BD FACSCanto II) Remove the probe (see Replacing the Probe on page 136) and the overflow reservoir.
- 8** (BD FACSCanto) Remove the catch tray.
- 9** (BD FACSCanto, BD LSR II, and BD LSRFortessa) Lift the HTS off of the base plate by doing the following:



To prevent personal injury or damage to the HTS, do not slide the HTS out so far that it becomes unbalanced.

- a** Slightly lift the HTS to clear the positioning screw.
- b** Be careful not to strain the sheath tubing and slide the HTS towards you until you can access the filter.

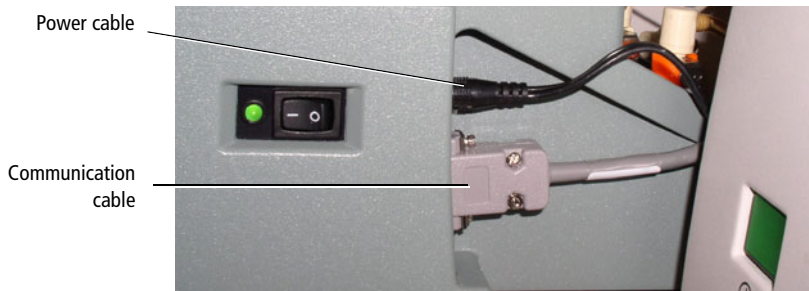
This allows you better access to the connections on the back of the HTS.

- 10** Disconnect the sheath and waste lines from the cytometer.

- 11** (BD LSR II, BD LSRFortessa, and BD FACSCanto) Disconnect the power and communication cables from the rear right side of the HTS.

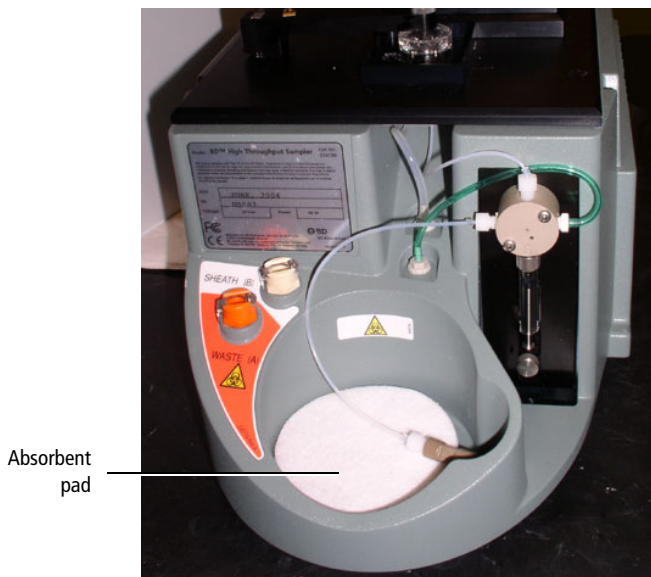
(BD FACSCanto II) Disconnect the interface/communication cable from the cytometer. If necessary, pull the HTS out slightly to access the cable, which is attached to the cytometer at the back-left corner of the HTS.

To detach the communication cable, unscrew the two connector screws and pull out the cable.



- 12** (BD FACSCanto II) Lift the front feet of the HTS just over the front edge of the enclosure and tilt the HTS at a 45° angle towards you to allow you access to the door sensor cable on the right side of the HTS. Unplug the door sensor cable by pulling back on the ferrule. Place the cable over the right side of the enclosure/carrier.
- 13** Carefully lift the HTS and place it on the benchtop next to the cytometer.
- 14** (BD LSR II, BD LSRFortessa, and BD FACSCanto) Remove the absorbent pad from the drip reservoir and discard it as biohazardous waste (Figure B-2).



Figure B-2 Drip reservoir containing absorbent pad (BD LSR II, BD LSRFortessa, and BD FACSCanto only)





- 15 Place the sample tubing and attached sample coupler inside the drip reservoir.



Cleaning the HTS

You must decontaminate both the internal fluidics and external surfaces of your HTS before it is repaired. If you were able to run a monthly clean before you removed the HTS, the internal fluidics are decontaminated. Skip to Decontaminating External Surfaces on page 175. If you were not able to run a monthly clean, proceed with the following section.

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

  To prevent shock, verify that the cytometer is turned off and the power cable is detached before you start cleaning.

Use a 10% bleach solution to decontaminate the internal fluidics and external surfaces of the HTS.

  To ensure that the 10% bleach solution retains its full germicidal effect, prepare a fresh solution daily.

To prepare the bleach solution:

- 1 Pour 45 mL of DI water into a 100-mL beaker.
- 2 Carefully add 5 mL of undiluted bleach to the same beaker.
- 3 Gently swirl the solution to mix.

Decontaminating the Fluidics Manually

Materials Needed

- Household bleach
- DI water
- Disposable plastic pipettes
- Line declogger, part number 343583 from the accessories kit (for manual fluidics decontamination only)

Cleaning the Fluidics

Perform this procedure to decontaminate the internal fluidics of a non-functional HTS.

- 1 Remove the sample probe and tubing that connects the probe to the primary pump. Discard the probe and tubing (Figure B-3 on page 171).

For details on removing the probe, see Replacing the Probe on page 136.



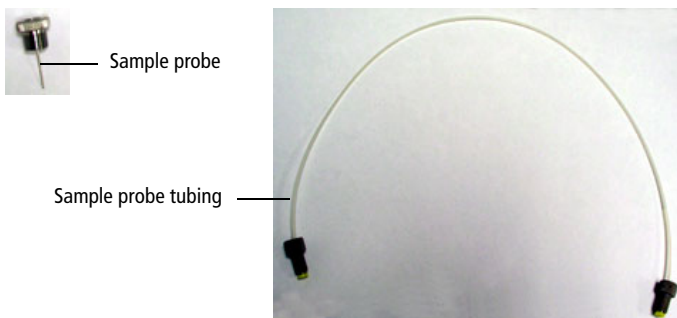
  All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Treat the sample probe and tubing as biohazardous waste and dispose of them according to local regulations.

Figure B-3 Removing the sample probe and tubing



- 2 Pipette a 10% bleach solution into the injection port/wash station.

Using a disposable pipette, fill the injection port/wash station completely, being careful not to overfill it.

- 3 Connect the line declogger tool to the waste connector on the back of the HTS.

Figure B-4 Attaching the declogger tool



- 4** Slowly pull back the plunger of the declogger tool until all of the bleach solution is drawn into the barrel.
- 5** Disconnect the declogger tool and discard the bleach solution according to local regulations.
- 6** Repeat step 2.
- 7** Reattach the declogger tool, and slowly remove about half of the bleach solution. Allow the remaining bleach to sit for 30 minutes.

Keep the declogger tool attached to the waste port during the 30 minutes.
- 8** Use the declogger tool to remove the rest of the bleach solution.
- 9** Disconnect the declogger tool and discard the bleach solution according to local regulations.

Rinsing the Fluidics

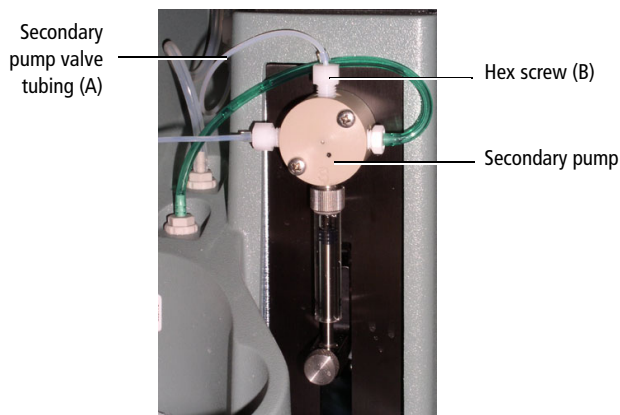


All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Treat the secondary pump valve tubing as biohazardous waste and dispose of it according to local regulations.

- 1** Pipette DI water into the injection port/wash station.

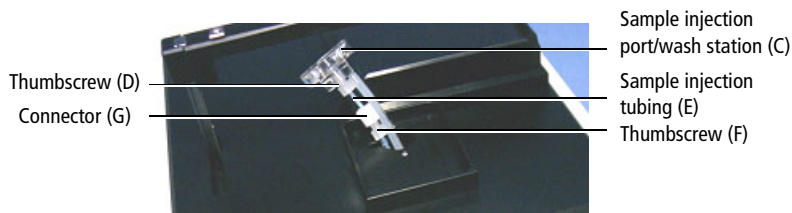
Using a disposable pipette, fill the injection port/wash station completely, being careful not to overfill it.
- 2** Connect the line declogger tool to the waste connector on the back of the HTS.
- 3** Slowly pull back the plunger of the declogger tool until all of the DI water is drawn into the barrel.
- 4** Repeat step 1 and step 3.
- 5** Disconnect the declogger tool and discard the DI water.
- 6** Carefully dry the declogger tool and return it to the HTS accessory kit.
- 7** Remove and discard the secondary pump valve tubing (A).
 - a** Disconnect the top hex screw (B) from the secondary pump using the 5/16-in. wrench in the accessory kit (Figure B-5).

Figure B-5 Hex screw on secondary pump



- b** Turn the sample injection port/wash station (C) slightly to disengage the magnetic force, then lift it up to expose the thumbscrew (D) on the sample injection tubing (E) and the thumbscrew (F) on the secondary pump valve tubing (A) (Figure B-6).

Figure B-6 Accessing the tubing



- c** Unscrew the thumbscrew (F) from the connector (G) and remove the secondary pump valve tubing (A) (Figure B-6).
- 8** Detach the sample coupler tubing from the secondary pump valve and discard the tubing as biohazardous waste.
- 9** Cover the open ports with parafilm or tape.

Decontaminating External Surfaces

Materials Needed

- 10% bleach solution
- DI water
- Paper towels

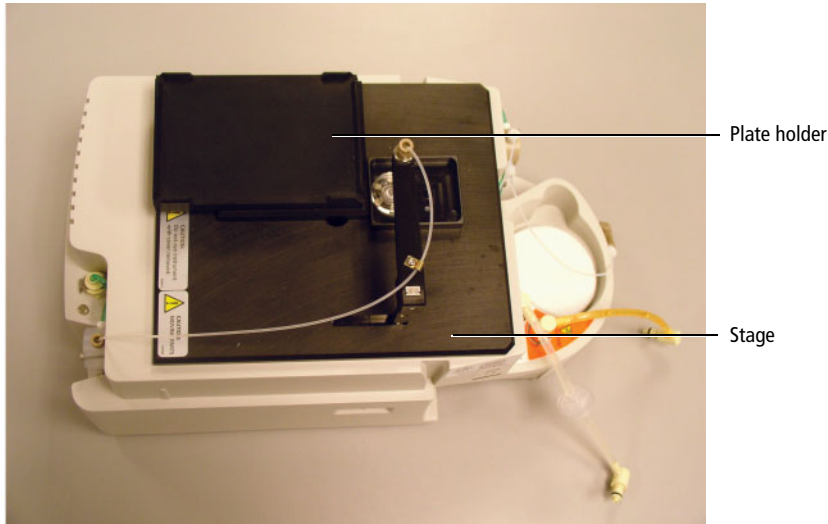
Cleaning the Surface of the HTS



To prevent HTS damage, do not pour or squirt bleach solution directly onto the HTS.

- 1 Saturate some paper towels with a 10% bleach solution.
- 2 Move the plate holder to the far left and wipe down the surface of the stage that was under the plate holder using the saturated paper towels (see Figure B-7 on page 176).

Figure B-7 HTS top view



3 Repeat step 2 moving the plate holder to the far right, front position, and then back position.

4 Clean the remaining external surfaces of the HTS using paper towels saturated with a 10% bleach solution.

Make sure all surfaces are clean.

5 Dispose of the paper towels used for cleaning as biohazardous waste.

6 Rinse all surfaces by repeating steps 1 through 5, substituting DI water for the bleach solution.

7 Allow the HTS to dry thoroughly.

Unpacking the Replacement HTS



An HTS without a cover weighs approximately 22 lb. To prevent personal injury or damage to the HTS, follow the guidelines on Lifting Heavy Objects on page 177 to remove the replacement HTS from its shipping container.

NOTE If you are sending back an existing HTS for depot repair, save all shipping materials and ship the existing HTS in this new container. It contains paperwork for shipping and two labels verifying that the HTS you are returning was decontaminated.

Before You Proceed With Unpacking

- Make sure you have adequate space:
 - On the floor near the cytometer to place the shipping container.
 - On the table or bench near the cytometer to place the new HTS.
- Make sure you:
 - Read through these instructions before attempting to unpack the new HTS.
 - Are ready to install the new HTS. See Installing the HTS on page 179.

Lifting Heavy Objects



To avoid personal injury or damage to the HTS, follow these guidelines for lifting heavy objects from the floor.

- 1 Stand in front of the object, and then sit on your heels (squat).
- 2 Place your hands under the base of the object, ensure the load is balanced, and bring the object as close to your lap as possible.

- 3 While keeping your spine in a natural position and your head up, lift the object using the force of your legs.

Do not twist your back when lifting. Pivot on your heels.

Unpacking the HTS

- 1 With the arrows on the box pointing up, open the shipping container.

Remember to save all paperwork and shipping materials to repack the HTS to be returned.



- 2 Remove the top piece of foam and set it aside.
- 3 Using proper lifting techniques, carefully lift the HTS out of the container and place it on a table near the cytometer.



The HTS is encased in plastic to protect it during shipment. The plastic can make the HTS slippery and difficult to grasp. To prevent personal injury or damage to the HTS, use caution when lifting the HTS out of the container.

- 4 Remove the HTS from the plastic bag.
- 5 Remove the small I-shaped piece of foam that secures the HTS probe assembly and plate holder, and set it aside.

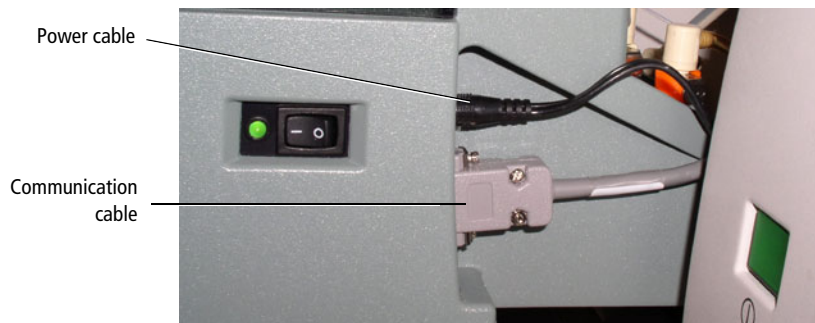
Installing the HTS

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

Installing the HTS (BD LSR II and BD LSRFortessa)

- 1 To avoid damaging the HTS probe, lower it into the injection port/wash station.
- 2 Place the HTS on the base plate.
- 3 Make sure the HTS support legs fit into the bracket grooves. With the legs in the grooves, slide the HTS as far forward as possible.
- 4 Connect the power and communication cables to their respective ports on the rear right side of the HTS (see Figure B-8 on page 179).

Figure B-8 Connecting the power and communication cables (BD LSR II)



- 5 Connect the sheath line (with filter) and waste line to their respective ports on the back of the HTS.

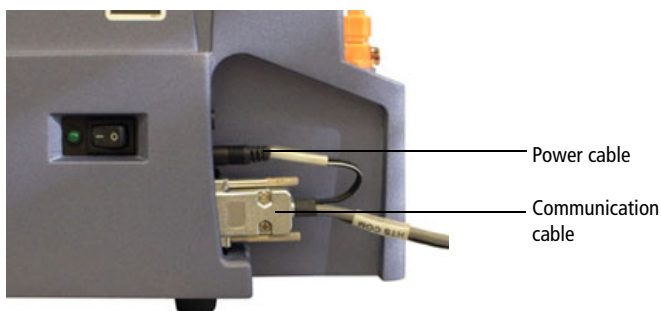
- 6 Slide the HTS all the way back toward the cytometer being careful not to crimp the fluidics tubing or communication cable.
- 7 (BD LSR II) Tighten the positioning screw under the front of the base plate.
- 8 See Setting Up for Plate-Based Acquisition (BD LSR II and BD LSRFortessa) on page 28 to complete the installation of the HTS.

Installing the HTS (BD FACSCanto and BD FACSCanto II)

- 1 To avoid damaging the HTS probe:
 - (BD FACSCanto) Lower it into the injection port/wash station.
 - (BD FACSCanto II) Remove the probe and the overflow reservoir. See Replacing the Probe on page 136.
- 2 Install the HTS.
 - (BD FACSCanto) Place the HTS on the base plate.
 - (BD FACSCanto II) Open the doors to the enclosure and place the door sensor cable that is attached to the cytometer over the right side of the enclosure.

Bring the HTS close to the cytometer while you sit in a chair with the HTS in your lap. Make sure the HTS support legs fit into the bracket grooves. With the legs in the grooves, slide the HTS as far forward as possible.
- 3 Connect the cables.
 - (BD FACSCanto) Connect the power and communication cables to their respective ports on the rear right side of the HTS (see Figure B-9 on page 181).

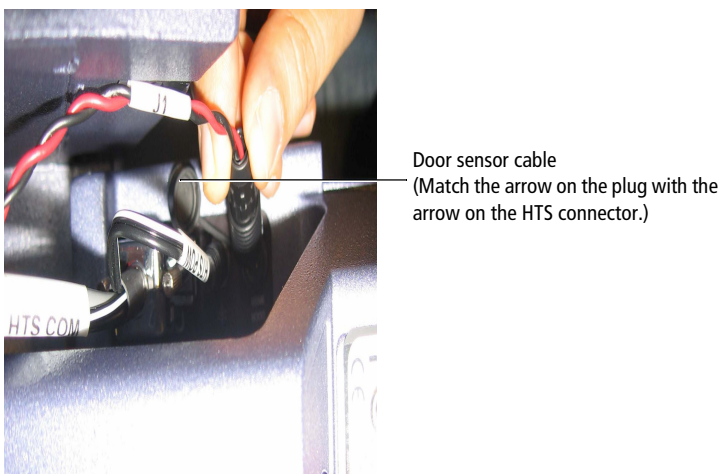
Figure B-9 Connecting the power and communication cables (BD FACSCanto)



- (BD FACSCanto II) Tilt the HTS at a 45° angle towards you to allow you access to the door sensor connector on the right side of the HTS. Plug the door sensor connector into the HTS, matching the white arrow on the plug with the arrow on the connector (see Figure B-10).

If necessary, slide the HTS in until you can connect the interface/communication cable to the cytometer (see Figure B-11 on page 182). Tighten the two connector screws after the communication cable is attached.

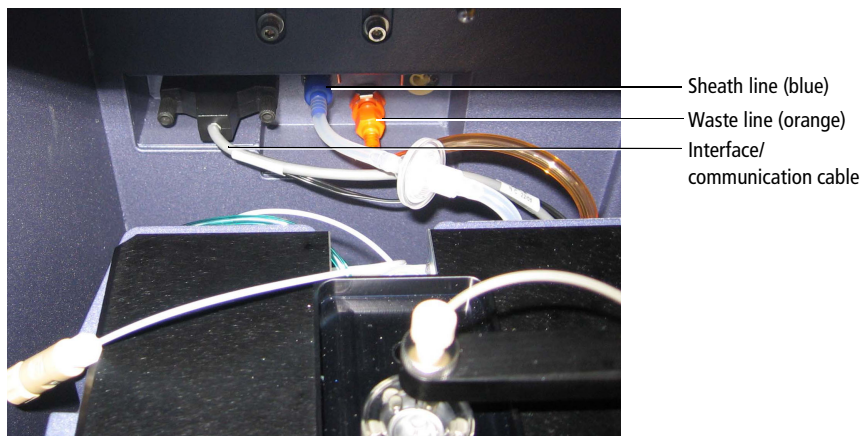
Figure B-10 Plugging the door sensor cable into the HTS (BD FACSCanto II only)



- 4 (BD FACSCanto II) Install the overflow reservoir with the lip facing towards you, and place the overpressure tubing into the reservoir (see Figure 1-7 on page 19).
- 5 Connect the sheath line (with filter) and waste line to their respective ports on the back of the HTS (or back of the cytometer for BD FACSCanto II) (see Figure B-11).

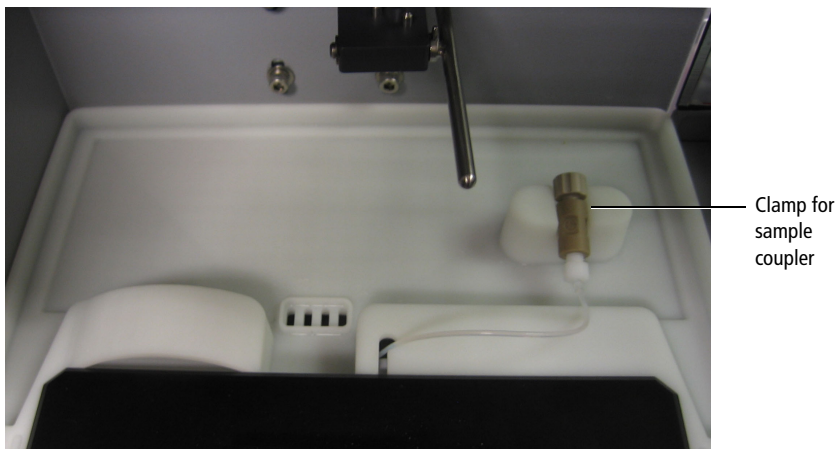
NOTE The white connector on the BD FACSCanto II is not used for the HTS.

Figure B-11 Connecting BD FACSCanto II interface cable to HTS



- 6 (BD FACSCanto) Install the rear catch tray.
 - a Route the sample coupler underneath the catch tray tongue and sample coupler slot.
 - b Push the tray in, making sure the coupler line is not crimped or kinked. The clamp on the tray is used for the sample coupler when it is not in use.

Figure B-12 Catch tray installed at the back of the BD FACSCanto



- 7 Slide the HTS all the way back toward the cytometer being careful not to crimp the fluidics tubing or communication cable.
- 8 (BD FACSCanto II) Install the probe (see Replacing the Probe on page 136).
- 9 Tighten the positioning screw under the front of the base plate/enclosure.
- 10 See Setting Up for Plate-Based Acquisition (BD FACSCanto and BD FACSCanto II) on page 32 to complete the installation of the HTS.

Packing the HTS for Shipping

Use the following procedure to pack the inoperable HTS prior to shipping.

NOTE Pack the HTS in the shipping container that contained the replacement HTS. It should contain the following:

- Shipping paperwork
- Decontamination label
- Small I-shaped piece of foam
- Plastic bag
- Top piece of foam
- Bottom piece of foam

Placing the HTS into its Shipping Container

- 1 Make sure the HTS was decontaminated according to the procedures in Cleaning the HTS on page 169.
- 2 Disconnect the sheath and waste lines from the HTS.
- 3 Disconnect the interface/communication cable from the HTS.
- 4 (BD FACSCanto II) Disconnect the door sensor cable from the HTS.
- 5 Initial and date the decontamination labels. Stick one on the plate holder and the other on the outside of the shipping carton.

Enter the initials of the person who decontaminated the HTS.
- 6 Fill out the Cytometer Return form.

7 Place the small I-shaped piece of foam between the probe assembly arm and plate holder to secure them.

8 Slide the HTS into the plastic bag.



The plastic might make the HTS slippery and hard to grasp. To prevent personal injury or damage to the HTS, use caution when placing the HTS in the shipping container.

9 Using proper lifting techniques, place the bagged HTS in the shipping container, making sure the arrows on the box are pointing up.

For proper lifting techniques, see *Lifting Heavy Objects* on page 177. Place the HTS on top of the bottom piece of foam.

10 Place the top piece of foam over the HTS.

11 Lay the Cytometer Return form on top of the foam, and close and seal the flaps of the shipping container.

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Appendix C

Automation

This appendix describes the remote automation server application programming interface (API).

The following topics are covered in this appendix:

- About Automation on page 188
- Establishing a Connection to the Automation Server on page 188
- Command Syntax on page 189
- API Command List on page 191
- Error Handling and Return Codes on page 195

Automation

About Automation

Automation for the HTS is a function that allows you to connect to BD FACSDiva software (via a server socket) and issue commands, such as creating experiments and recording plates through the use of a published command API. It is designed to help you set up a walkaway type of automation process in which clients can coordinate interactions between external hardware (such as robotics), third-party software, the BD FACSDiva software application, and the cytometer.

Establishing a Connection to the Automation Server

To run plate automation you can use your own proprietary software application to connect to the BD FACSDiva API. The application can be run from the same computer that is connected to the instrument or from a different computer that is located on the network. Users who want to use this new process would typically write an application that acts as a driver, in terms of switching between their robotics application (which adds and removes plates) and the BD FACSDiva software application (which configures and runs plates once they are loaded).

The BD FACSDiva software remote automation server allows clients to connect to the automation server Port 247. Once BD FACSDiva software has been installed, client applications use the IP address of this computer and the Port address 247 to establish a connection with the application.

To establish a connection:

- 1 Start BD FACSDiva software and log in to the application.
- 2 Locate the server icon on the workspace toolbar and click it to turn the server on.



Server icon off



Server icon on

- 3 The server starts up and is ready to accept client connections. The client application can now connect to the server, using the IP address and the port address, and can now send published commands to the automation server.
 - Use a `java.net.Socket` object for a Java client.
 - Use a `System.Net.Sockets.TcpClient` object for a C# or C++ client.

NOTE The communications channel established between the server and the client is a synchronous communication channel. The reason for implementing synchronous communications is so that the client is blocked by the server until the process initiated by the passed command has either finished its process or has stopped due to an error. This allows the client to know exactly when a process is complete.

Command Syntax

All of the commands that are part of the remote automation API will follow the same syntactical design in terms of issuing commands to the automation server. The following is an example which shows the structural pattern to be used when assembling these types of commands:

- **CmdName.** The published name of the command as outlined in this document (case sensitive).
- **ParamName.** Any published parameters for the specified command (not case sensitive).
- **ParamValue.** Any published parameter values that coincide with the specified parameter name (not case sensitive).
- **Brackets [].** The beginning and ending delimiters for a list of parameter name/value pairs.
- **Commas.** The delimiter used to separate multiple parameters.
- **Equals Sign.** Used to associate a parameter value with a parameter name.

Syntax Example

`CmdName[ParamName1=ParamValue 1, ParamValue2=ParamValue 2]`

Additional Information

- Spaces are allowed within the command parameter section. For example, they are allowed between the brackets [], but are not required. Only the spaces within the parameter value area (between the quotes “”) will be preserved.
- Only one command can be sent to the server at a time. The client must wait until it receives the return code before sending any subsequent commands.
- Each command is sent to the server as a byte array and the data returned to the client is also sent as a byte array. Both arrays have a size limit set to 256 bytes.

API Command List

Table C-1 New Experiment Command

Command Name	Command Description	Parameter Names	Parameter Values	Parameter Description
CmdCommNewExp	Creates a new experiment within the currently selected Diva folder.	Name (optional)	<user-defined name>	The display name for the experiment in the experiment Browser.

Table C-2 New Plate Command

Command Name	Command Description	Parameter Names	Parameter Values	Parameter Description
CmdCommNewPlate	Creates a new plate within the currently selected Diva experiment.	Name (optional)	<user-defined name>	The display name for the plate in the experiment Browser.
		Type (mandatory)	96FlatWell 96UWell 96VWell 384FlatWell	The type of plate to be created within the open experiment.

Table C-3 Setup Plate Command

Command Name	Command Description	Parameter Names	Parameter Values	Parameter Description
CmdCommSetupPlate	Configures the currently selected Diva plate in terms of creating the specimen and setting the Loader settings.	Throughput (optional)	High (Default) Standard	High represents high-throughput mode and standard represent standard throughput mode.
		SampleFlowRate (optional)	Min Value: 0.5 Max Value: 3.0 Increment Value: 0.5 Default Value: 1.0	The flow rate to use for the sample measured as $\mu\text{L}/\text{sec}$.
		SampleVolume (optional)	Min Value: 2 STD Mode Max: 200 HTS Mode Max: 10 Increment Value: 1 Default Value: 2	The volume used for the sample measured as μL .

Command Name	Command Description	Parameter Names	Parameter Values	Parameter Description
CmdCommSetupPlate (continued)	Configures the currently selected Diva plate in terms of creating the specimen and setting the Loader settings. (continued)	Mixing Volume (optional)	Min Value: 5 (BD LSR platform) Max Value: 80 (BD FACSCanto platform) Max Value: 100 Increment Value: 1 Default Value: 50	The mixing volume to use measured as μL .
		Mixing Speed (optional)	Min Value: 25 Max Value: 250 Increment Value: 1 Default Value: 200	The mixing speed to use measured as $\mu\text{L}/\text{sec}$.
		NumOfMixes (optional)	Min Value: 0 Max Value: 5 Increment Value: 1 Default Value: 2	The number of times to perform the mixing operation.
		WashVolume (optional)	Min Value: 200 Max Value: 800 Increment Value: 1 Default Value: 200	The washing volume to use measured as μL .

Table C-4 Setup Acquisition Command

Command Name	Command Description	Parameter Names	Parameter Values	Parameter Description
CmdCommSetupAcquisition	Sets up the acquisition rules used for acquiring data.	NumOfEvents (optional)	Min Value: 1 Max Value: N/A Increment Value: 1 Default Value: 10,000	The stopping rule related to the number of events to record.

Table C-5 Run Plate Command

Command Name	Command Description	Parameter Names	Parameter Values	Parameter Description
CmdCommRunPlate	Runs the currently selected Diva plate.	N/A	N/A	N/A

Error Handling and Return Codes

Table C-6 New Experiment Command

Status Type	Return Code
Command Completed Successfully	0
Unknown Error	-1
Invalid Command Name	1000
Invalid Experiment Name	1001

Table C-7 New Plate Command

Status Type	Return Code
Command Completed Successfully	0
Unknown Error	-1
Invalid Command Name	1000
Invalid Plate Name	1001
Experiment Not Open	1002
Unable To Create Plate	1003
Invalid Plate Type Specified	2000

Table C-8 Setup Plate Command

Status Type	Return Code
Command Completed Successfully	0
Unknown Error	-1
Invalid Command Name	1000

Status Type	Return Code
Experiment Not Open	1002
Unable To Create Specimen	1004
Target Plate Not Found	1005

Table C-9 Setup Acquisition Command

Status Type	Return Code
Command Completed Successfully	0
Unknown Error	-1
Invalid Command Name	1000
Experiment Not Open	1002
Unable To Create Specimen	1004
Target Plate Not Found	1005

Table C-10 Run Plate Command

Status Type	Return Code
Command Completed Successfully	0
Unknown Error	-1
Invalid Command Name	1000
Experiment Not Open	1002
Unable To Create Specimen	1004
Target Plate Not Found	1005
Instrument Not Connected	1006
Unable To Run Plate	2100

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