BD FACSCanto™ II Flow Cytometer Instructions For Use

IVD CE

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Trademarks

FCC Information

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

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

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

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

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

Notice

BD Biosciences delivers software and workstations that are intended for running the cytometers supplied by BD Biosciences. It is the responsibility of the buyer/user to ensure that all added electronic files including software and transport media are virus free. If the workstation is used for Internet access or purposes other than those specified by BD Biosciences, it is the buyer/user's responsibility to install and maintain up-to-date virus protection software. BD Biosciences does not make any warranty with respect to the workstation remaining virus free after installation. BD Biosciences is not liable for any claims related to or resulting from buyer/user's failure to install and maintain virus protection.

Regulatory Information

For In Vitro Diagnostic Use

Laser Safety Information

BD FACSCanto II cytometers are Class 1 laser products.

History

Revision	Date	Change Made
23-12882-00 Rev. 01	10/2010	Updated for BENEX contact information changes.
23-12882-01 Rev. 01	2/2012	Updated for BD FACSDiva 7.0. Updated Regulatory Class I Laser information from Roman numeral to Arabic 1.
23-14527-00	3/2013	Updated for BD FACSDiva 8.0 and BD FACSCanto clinical software 3.0.
23-14527-01	3/2015	Added Flow Cytometer to cover. Updated Intended use statement for 510K submission. Added the RxOnly statement.
23-20269-00	9/2019	Updated new part number, Diva 9.0 and Windows 10 information.

Contents

Preface

This chapter contains the following information:

About Instructions for Use

This document contains the information necessary to operate your BD FACSCanto™ II flow cytometer. Most cytometer functions are controlled by BD FACSCanto™ clinical software and BD FACSDiva™ software. BD FACSCanto clinical software contains modules for dedicated clinical applications with automatic gating algorithms, while BD FACSDiva software is non–application specific. Use BD FACSCanto clinical software for performing cytometer quality control.

BD Biosciences recommends that first-time users of this cytometer take advantage of operator training offered with the sale of every new cytometer.

The *BD FACSCanto II Instructions For Use* assumes you have a working knowledge of basic Microsoft® Windows® operation.

For US:

Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

Conventions

The following tables list conventions used throughout this guide.

Table 1 Hazard symbols

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

a. Although these symbols appear in color on the cytometer, they are in black and white throughout this document; their meaning remains unchanged.

Table 2 Text and keyboard conventions

Convention	Use
NOTE	Describes important features or instructions
Italics	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
Bold	Bold text indicates software elements such as windows, menus, buttons, and tabs that are used to complete tasks.
>	The arrow indicates a menu selection. For example, "select **File > Print**" means to select Print from the File menu.
Ctrl+X	When used with key names, a plus sign means to press two keys simultaneously. For example, Ctrl+P means to hold down the Control key while pressing the letter *p*.

For US:

Symbol	Meaning
Rx Only	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

Technical Assistance

For technical questions or assistance in solving a problem:

- See the Troubleshooting section.
- See the BD Biosciences website: bdbiosciences.com

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, part number, and serial number; software version and computer system specifications
- Error messages, if any
- Details of recent cytometer performance

For support from in the US and Canada, call 877.232.8995.

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

Intended Use for Outside US

The BD FACSCanto II system is intended for use as an In Vitro Diagnostic device for identification and enumeration of lymphocyte subsets in human cells in suspension.

Intended Use for US

The BD FACSCanto II flow cytometers (4-2-2 and 5-3 configurations) function as part of a system with dedicated clinical software intended for use with cleared

or approved in vitro diagnostic (IVD) assays that are indicated for use with the instrument for the identification and enumeration of human cell subsets. Only six detection channels using a blue (488 nm) and a red (633 nm) laser have been cleared for in vitro diagnostic use. For use with or without the BD FACS™ Sample Prep Assistant III.

For in vitro diagnostic use.

Intended Use for US

The BD FACSCanto II flow cytometer (4-2 configuration) is intended for use as an In Vitro Diagnostic device for identification and enumeration of lymphocyte subsets in human cells in suspension.

- Immunophenotyping in clinical laboratories, using previously cleared in vitro diagnostic assays for flow cytometry.
- Identification and enumeration of lymphocyte subsets in human cells in suspension.
- For in vitro diagnostic use.
- For use with or without the BD FACS Sample Prep Assistant III

1

System Overview

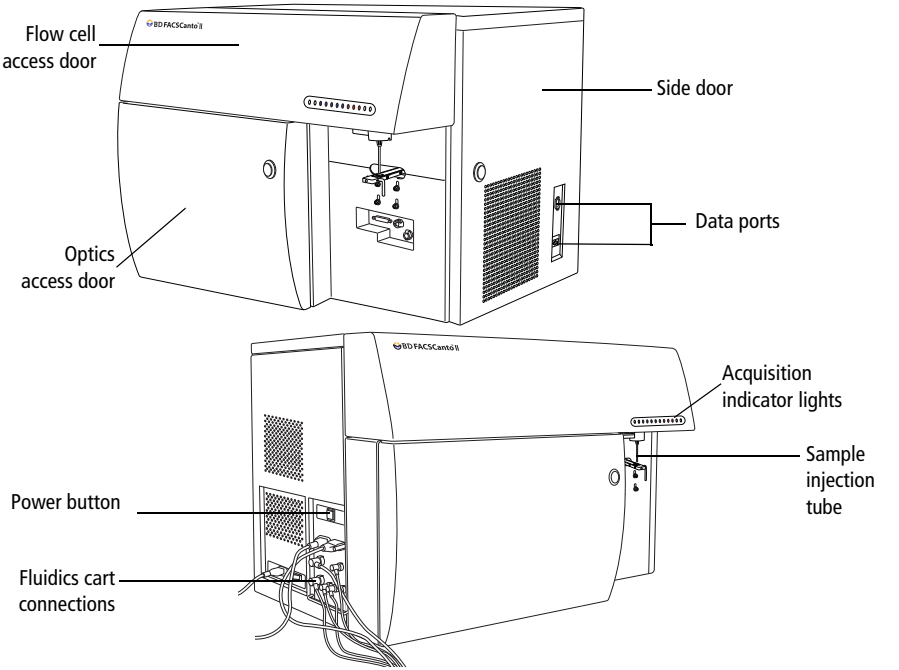
This chapter contains the following information:

System Components

The BD FACSCanto II system consists of a flow cytometer, a self-contained fluidics cart, and the BD FACSCanto II workstation. System options include an automated sample loader and a barcode reader.

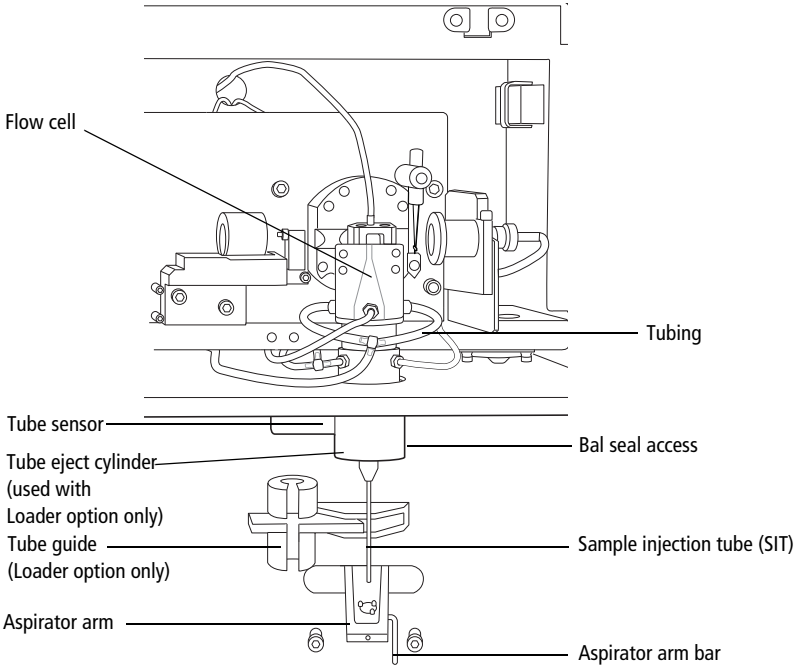
Flow Cytometer

Figure 1-1 Flow cytometer components



⚠ Do not place heavy objects on top of the cytometer at any time. Doing so could cause alteration of data.

Fluidics



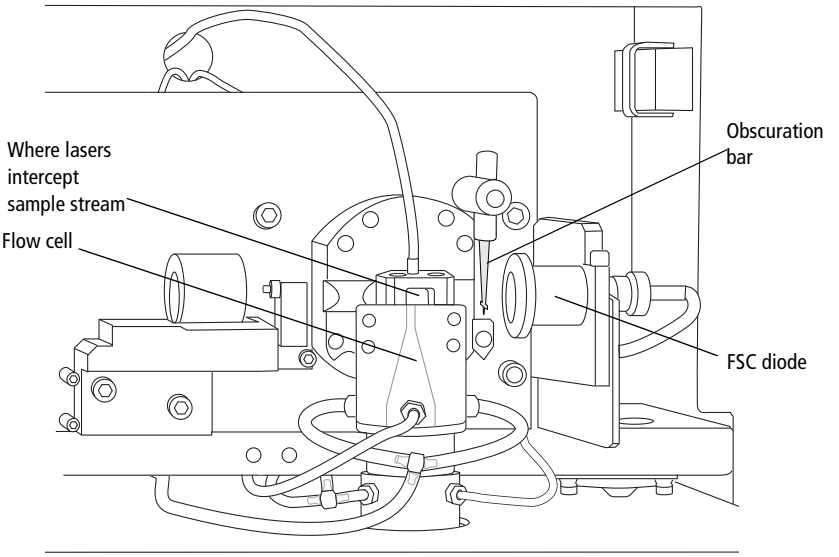
When you install tubes onto the sample injection tube (SIT), a pump within the fluidics cart pressurizes the plenum, which then provides sheath fluid to the flow cell. At the same time, the sample tube is pressurized and sample is pushed up the SIT and into the flow cell.

When you remove tubes from the SIT, the cytometer cleans the SIT by flushing sheath solution down the inside and outside of the tube. The flushed sheath solution is aspirated by the aspirator arm.

SIT cleaning between tubes is automatic when you use BD FACSCanto clinical software. In BD FACSDiva software, SIT cleaning between tubes is automatic unless you disable it by clearing the SIT Flush checkbox on the Acquisition Dashboard.

Optics

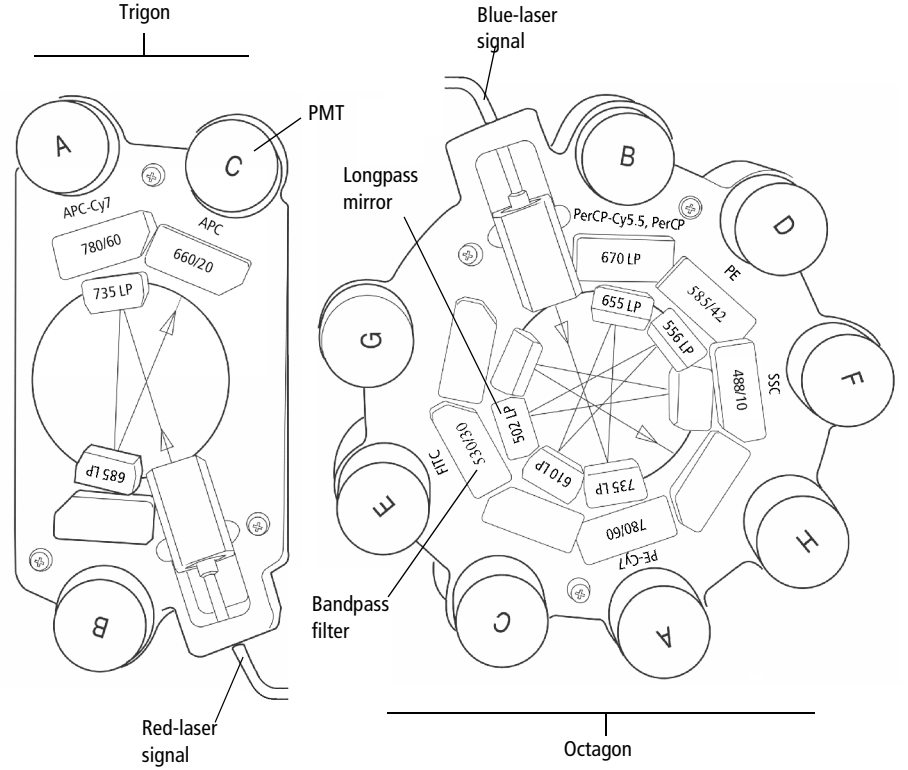
Once the sample moves into the flow cell, particles move in single file through the laser beams. The scattered and emitted light from these particles provides information about their size, shape, granularity, and fluorescence properties.



From the flow cell, laser-excited and scattered light is routed to the detector arrays, which consist of photomultiplier tubes (PMTs) arranged in one octagon and one trigon.

The octagon contains five PMTs and detects light excited and scattered by the 488-nm (blue) laser. One PMT in the octagon collects side scatter (SSC) signals.

Figure 1-2 Trigon and octagon detector arrays (4-2 configuration)



Detector Array (Laser)	PMT Position	LP Mirror	BP Filter or LP Mirror	Intended Dye
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy™7
	B	655	670	PerCP-Cy™5.5 or PerCP
	C	610	Blank optical holder	—
	D	556	585/42	PE
	E	502	530/30	FITC
	F	Blank optical holder	488/10 and pinhole	SSC
	G	Blank optical holder	Blank optical holder	—
	H	—	Blank optical holder	—
Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	685	Blank optical holder	—
	C	—	660/20	APC

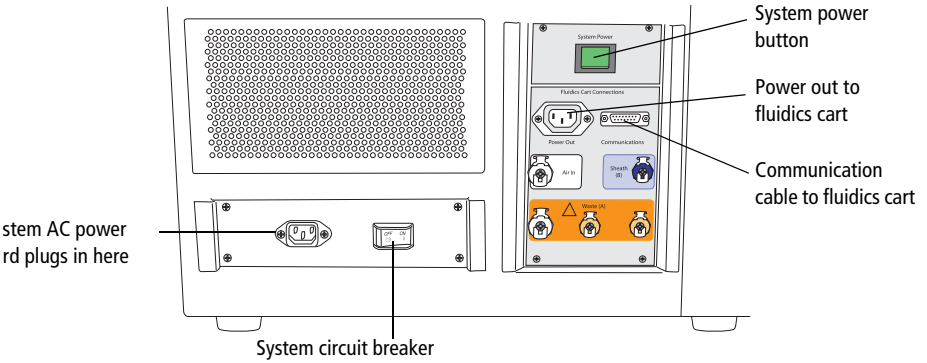
Blank optical holders do not contain optical filters. They are used in the octagon and trigon to prevent unwanted light from interfering with fluorescence signal.

Electronics

Power to the cytometer, lasers, and fluidics cart is supplied by a power cord from the cytometer plugged directly into a standard electrical outlet. We recommend using an uninterruptible power supply (UPS) unit to maintain cytometer power during a power outage. The system power button turns on the cytometer and fluidics cart, and powers the lasers.

NOTE If a power failure occurs during a run, sample can leak from the SIT. To prevent a biohazardous spill, place an empty tube on the SIT. Once power is restored, remove the tube and perform a fluidics startup before resuming the run.

Figure 1-3 Flow cytometer power panel

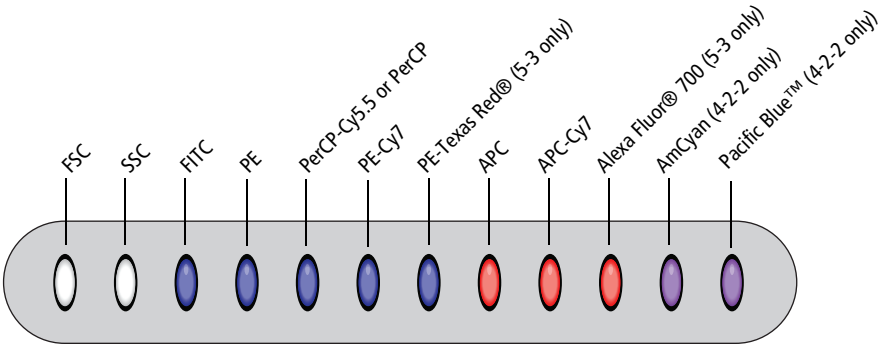


The system circuit breaker is located next to the AC power cord. The breaker will need to be reset if there is a power surge in the laboratory.

Acquisition indicator lights are located on the flow cell access cover on the front of the cytometer (see Figure 1-4 on page 24). Each light corresponds to a detector in the collection optics subsystem, and blinks when the signal at that detector reaches a preset level. Acquisition threshold levels (set using the software) override the presets.

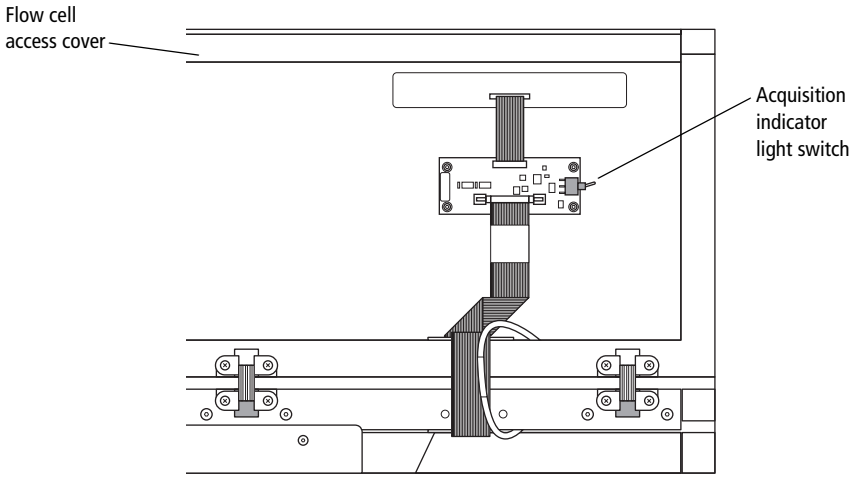
Lights are activated only when the system is acquiring data, and only the indicators corresponding to currently active parameters will blink.

Figure 1-4 Detector order of acquisition indicator lights



The acquisition indicator lights can be switched off. The on/off switch is located inside the flow cell access cover.

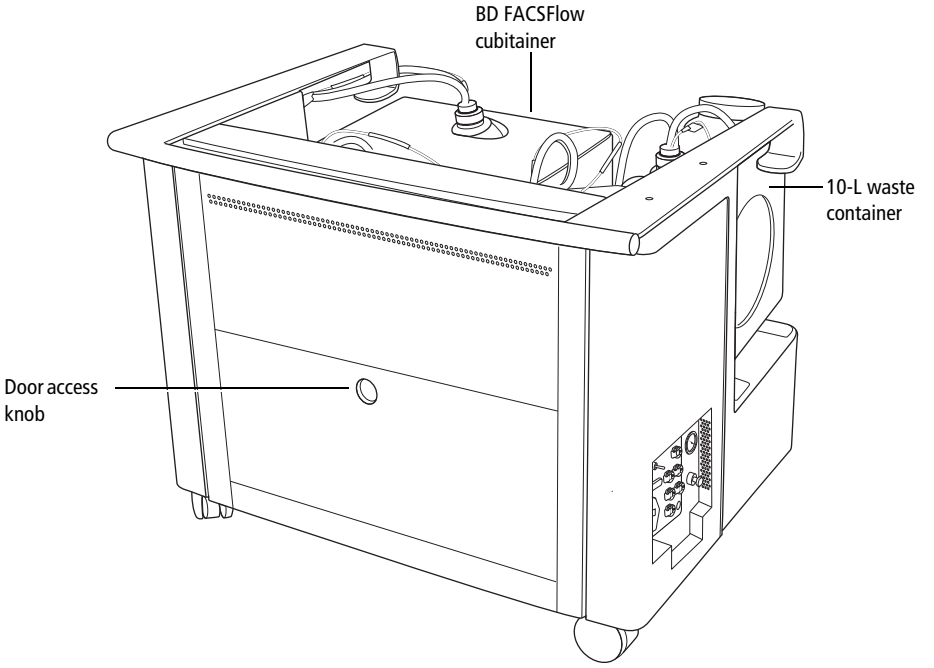
Figure 1-5 Acquisition indicator light switch



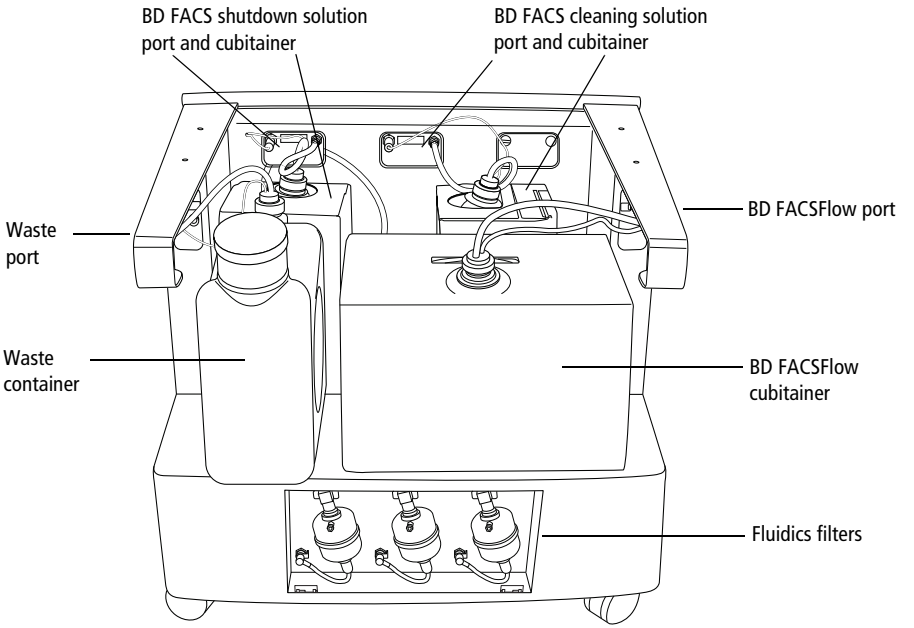
Fluidics Cart

The fluidics cart provides filtered sheath and cleaning fluids to the cytometer, and collects system waste products. The cart supplies the required air pressure and vacuum, which eliminates the need for an external source (although the cart can be hooked up to an in-house air source).

Figure 1-6 Fluidics cart



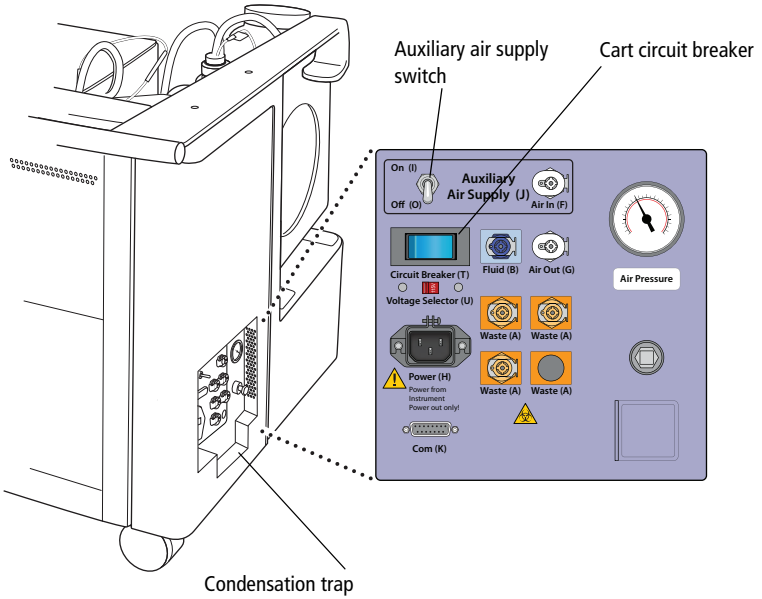
Containers and Ports



Controls

The fluidics cart connects to the flow cytometer unit by way of cables and tubing. When you turn on the power to the cytometer, the fluidics cart powers on also. Under ordinary circumstances, you do not need to adjust any of the switches on the cart's power panel. Leave the auxiliary air supply switch off unless the cart has been attached to an in-house air supply by BD Biosciences service personnel. Leave the cart circuit breaker on at all times.

Figure 1-7 Fluidics cart panel

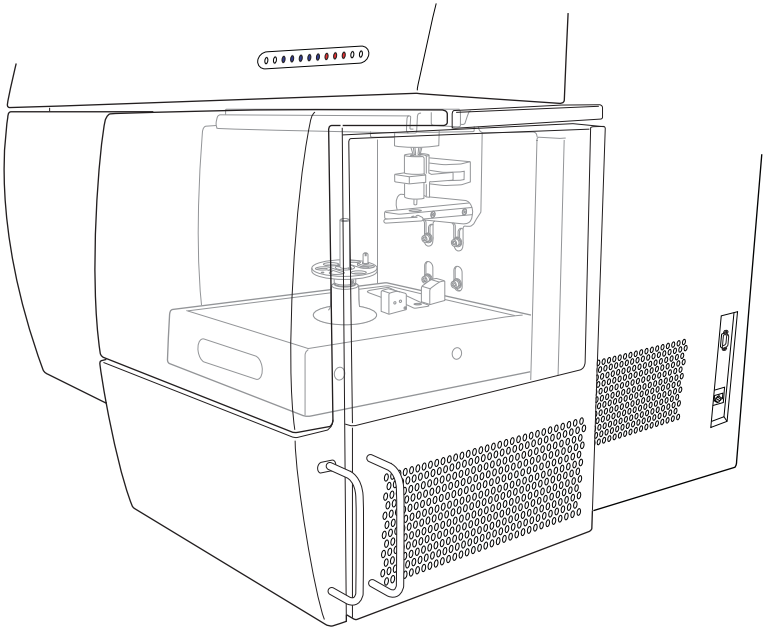


Condensation Trap

The fluidics cart condensation trap is located beneath the controls panel (). Empty the trap during the daily shutdown procedure.

BD FACS Loader (Optional)

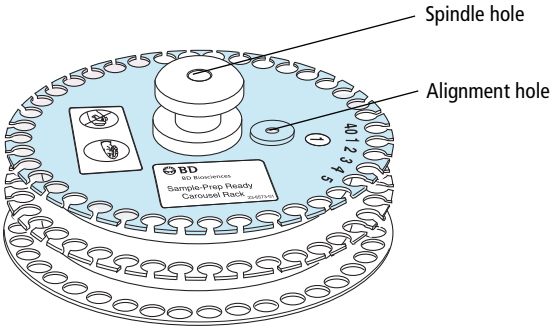
The BD FACS™ Loader (Loader) automatically introduces prepared samples to the cytometer. The Loader is controlled by BD FACSCanto clinical software or BD FACSDiva software.



Carousel

The carousel accommodates up to forty 12 x 75-mm tubes. Each carousel has a unique ID printed on top and on an optically read label inside.

Figure 1-8 Sample-Prep Ready carousel



NOTE The Loader is compatible only with the green-tinted carousels labeled *Sample Prep Ready*.

⚠ Not all manufactured 12 x 75-mm tubes have been checked for proper functionality on the Loader. BD Biosciences has validated only disposable, 12 x 75-mm Falcon® polystyrene test tubes, BD Trucount™ tubes, and BD FACS™ 7-color setup bead tubes.

Barcode Reader (Optional)

The barcode reader is a hand-held device that plugs into the USB port on the BD FACSCanto workstation. The barcode reader reads most barcode standards, including Codabar, Code 128, Code 39 with checksum, and Interleaved 2 and 5. It reads information from the BD FACS 7-color setup beads label into BD FACSCanto clinical software, as well as coded patient information into a worklist.

For detailed information including how to program the barcode reader for other barcode standards, refer to the manufacturer's documentation.

BD Applications Running in Windows 10 OS

BD FACSDiva software version 9.0 and BD FACSCanto clinical software version 4.0 run in the Microsoft Windows 10 64-bit operating system. The applications will not run in older versions of the Windows operating system.

The basic functionality of the applications remains the same as in the previous versions.

System Requirements

Software

Both included software packages must be installed:

- BD FACSCanto clinical software version 4.0 or later

FCS files created with BD FACSCanto clinical software version 2.4 or later cannot be used with an earlier version of BD FACSCanto clinical software. Earlier versions will show incorrect results.

- BD FACSDiva software version 9.0 or later

Workstation

BD FACSCanto II workstation purchased through BD Biosciences

Compatible Tubes

- 12 x 75-mm polystyrene test tubes (Falcon tubes)
- 12 x 75-mm BD Trucount tubes
- BD FACS 7-color setup bead tubes

Bulk Fluids

- BD FACSFlow™ solution
- BD™ FACSClean solution
- BD FACS™ shutdown solution
- Bleach (waste container)

Fluids Required for External Cleaning

- BD FACSClean solution
- Deionized (DI) water

Setup Beads

BD FACS 7-color setup beads for use with BD FACSCanto clinical software

2

Software Windows and Toolbars

This chapter contains information about the following software:

BD FACSCanto Clinical Software Workspace

After you log in, the main window appears. Table 2-1 provides a brief overview of window components.

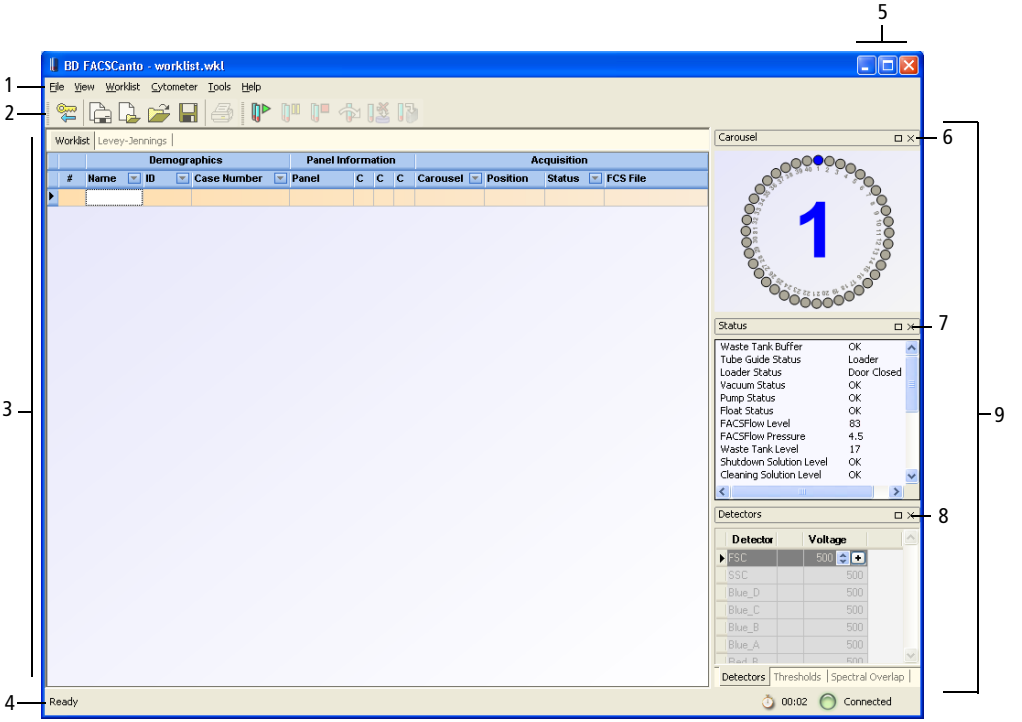


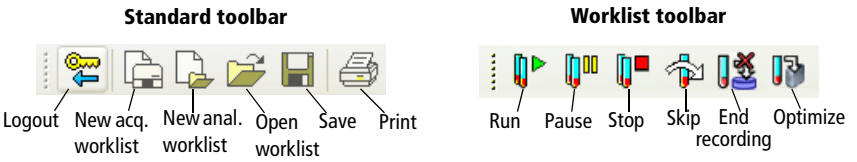
Table 2-1 Main window components

Number	Component	Function
1.	Menu bar	Contains the File, View, Worklist, Cytometer, Tools, and Help menus
2.	Toolbars	Contains buttons that provide quick access to menu commands. See BD FACSCanto Toolbars on page 35.

Table 2-1 Main window components (continued)

Number	Component	Function
3.	Workspace	Displays the Worklist, Acquisition, Lab Report, and Levey-Jennings tabs, depending on where you are in the workflow
4.	Status bar	Provides information about the flow cytometer's current state, the cytometer-software connection, and the amount of time elapsed since login
5.	Minimize, Maximize, and Close buttons (in title bar)	**Minimize button**. Reduces the application to a button on the Windows taskbar **Maximize button**. Fills the screen with the main window **Close button**. Exits the application and prompts the Fluidics Shutdown procedure
6.	Carousel window	Shows a graphic representation of a carousel rack and the rack ID of the currently selected sample
7.	Status window	Provides information on the current status of the flow cytometer
8.	Cytometer control windows	Include three tabs: • Detectors tab • Thresholds tab • Spectral Overlap tab
9.	Docking area	Provides a default home for the Carousel, Status, and Cytometer Control windows

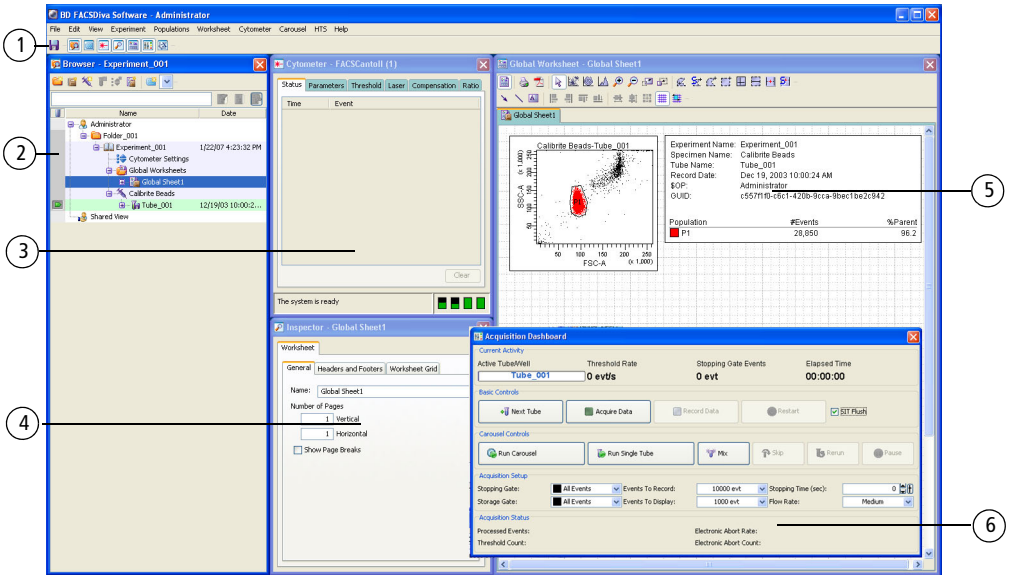
BD FACSCanto Toolbars



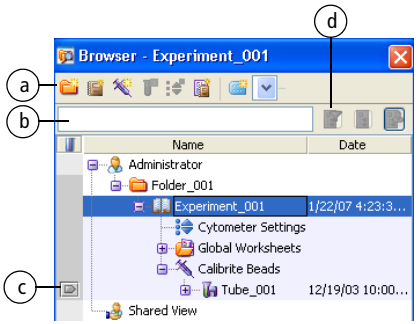
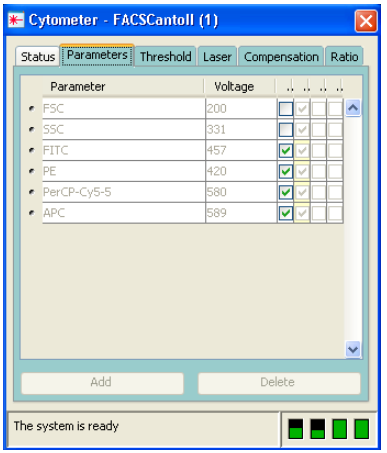
BD FACSDiva Software Workspace

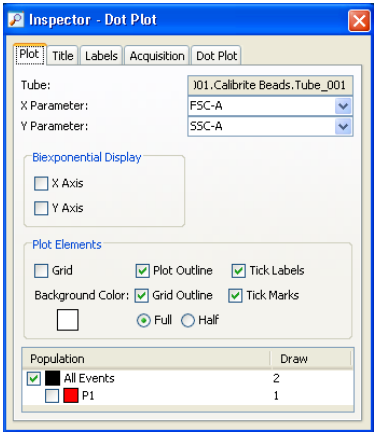
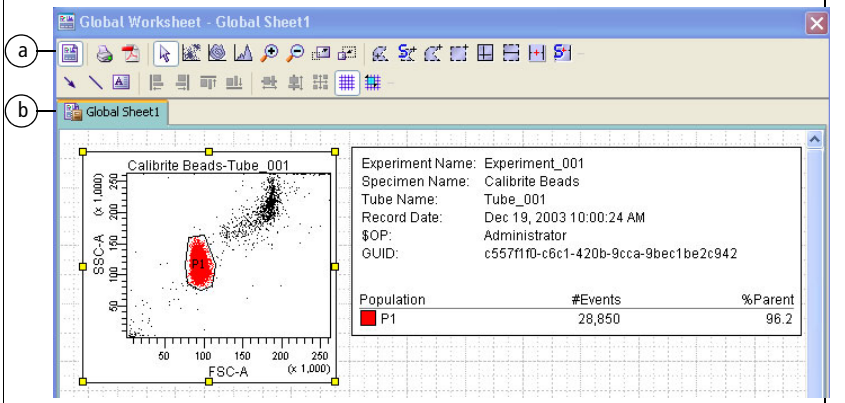
After you log in, the workspace appears, showing the main application windows (Figure 2-1).

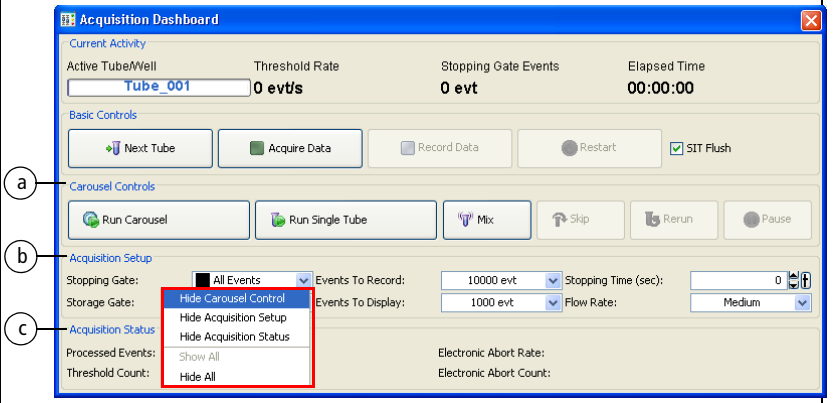
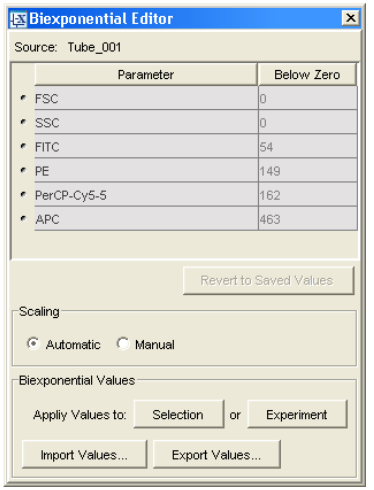
Figure 2-1 BD FACSDiva workspace



Number	Description
1	Click a button on the Workspace toolbar to hide or show the corresponding window. Most windows can be resized by dragging a border or corner. 

Number	Description
2 	 The Browser lists folders, experiments, and experimental elements in a hierarchical view, provides an interface for setting up experiments, and contains a current tube pointer (c) indicating the tube for which acquisition or analysis data will be shown. Only one experiment can be open at a time. In an open experiment, you can add, rename, or copy elements, and record or display data. Click a button on the Browser toolbar (a) to add elements to the Browser. To display a particular experiment, enter a term in the search field (b) and click the Find button (d).
3 	 The Cytometer window shows the cytometer connectivity status. When the cytometer is connected, the window also shows status messages. If an experiment is open and the current tube pointer is set, the window displays cytometer settings for the current acquisition tube and laser controls.

Number	Description
4	 Use the Inspector to view or modify the attributes of one or more objects in the worksheet or Browser. The contents of the Inspector change depending on what is selected. For example, this Inspector shows plot options.
5	The Worksheet window is where you create global or normal worksheets containing plots, gates, statistics, and custom text. Plots on a global worksheet show data for the tube selected with the current tube pointer. Global worksheets are indicated by green tabs (b). Plots on a normal worksheet are tube specific. Normal worksheets are indicated by white tabs for active worksheets and gray tabs for inactive worksheets. Click the button on the Worksheet toolbar (a) to switch between the global or normal worksheets view. Then you can create analysis objects or text, or align objects on a worksheet. 

Number	Description
6 	The Acquisition Dashboard contains controls for setting up, starting, monitoring data acquisition and recording, and running the Loader. Basic controls are always shown. Carousel Controls (a), Acquisition Setup (b), and Acquisition Status (c) can be hidden or shown by right-clicking anywhere on the Dashboard (except buttons and fields or in Basic Controls) to display the Show/Hide menu and making your selection. You can resize the Dashboard by dragging an edge or corner of the window. 
	 Unlike other workspace windows, the Biexponential Editor is hidden by default. When you are using automatic scaling, there is no need to show the Biexponential Editor. Use the Biexponential Editor to manually adjust biexponential scales, export and import scale values, and apply values to other elements in an experiment.

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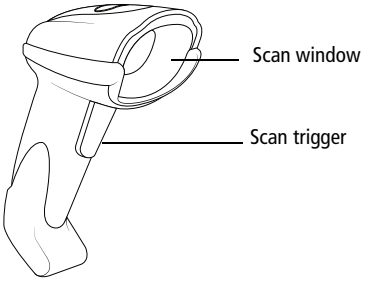
3

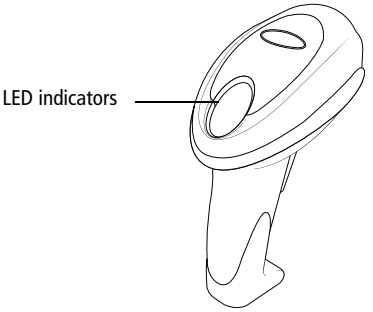
Barcode Reader Option

This chapter provides the following barcode information:

Installing and Using the Barcode Reader

The handheld barcode reader plugs into the USB port on the system computer workstation. The barcode reader reads most current barcode standards. See the manufacturer's documentation for details on safety, how to use the reader, maintenance, and for the complete list of standards.





When to Use the Barcode Reader

Use the barcode reader to scan the following labels when using the BD FACSCanto system:

- BD FACS 7-color setup beads label
- Sample ID label on sample tube

NOTE Only use the barcode reader that is supplied with your instrument to perform these tasks.

Important Barcode Reader Information

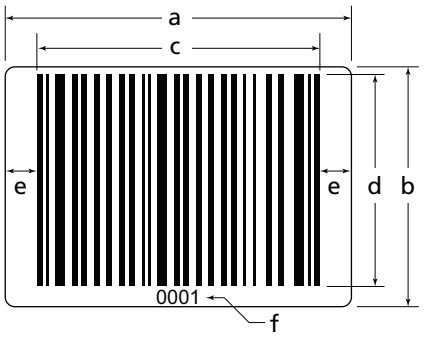
To prevent laser injury, do not stare into the beams or aim the scanner at another person's eyes while the trigger is depressed.

For accurate results, do not photocopy or enlarge the barcodes that are included with the reagent. Scan the barcodes exactly as they are provided.

- Contact BD Biosciences before changing default barcode settings.

Barcode Label Specifications

The following table lists the details for barcode labels that are used on tubes.

Specification	Description
Supported symbologies (types)	Code 128, Code 39, Codabar, Interleaved 2 of 5 You do not need to specify the type of symbols for labels on tubes. The barcode reader can sense differences automatically.
Narrow element (width of the narrowest bar in a label)	10 mil (0.25 mm, 0.01 in.) or greater
Dimensions  	a - Max label length: 44.45 mm (1.75 in.) This length should include the barcode symbol and quiet zone. b - Max label height: Cannot exceed 20 mm more than the circumference of the tube. c - Max symbol length: 37.45 mm (1.47 in.) d - Minimum symbol height: 19.05 mm (0.75 in.). We recommend the maximum symbol height possible because this makes it easier to orient tubes within a rack. e - Minimum quiet zone: 3.5 mm (0.14 in.) at each end of the symbol.
Human readable	f - Size and placement of the human readable to be determined by the user.
Label thickness	Thickness of label and adhesive not to exceed 3.9 mil. (0.099 mm, 0.0039 in.)
Finish	Print labels on material with a matte finish.

Specification	Description
Placement of label on tube	The label must be placed a minimum of 12 mm (0.47 in.) from the bottom of the tube. The label must be placed so the bars are perpendicular to the length of the body of the tube.

Cleaning the Barcode Reader

For best performance, keep the front window of the barcode reader clean. Do not touch the window directly, and wipe it only with a soft, non-abrasive cloth moistened with one of the following:

- Isopropyl alcohol
- Ethyl alcohol (denatured grade)

⚠ Do not use BD FACS™ cleaning solution or bleach to clean or disinfect the barcode reader.

Barcode Symbologies

Checksums

Although data entry using barcodes is generally more reliable than manual data entry, it is not guaranteed to be 100% accurate. By default, the barcode reader has checksums enabled. To increase accuracy, we recommend you do not disable checksums, or use barcode symbologies without checksums.

⚠ Using barcode symbologies with checksums disabled increases the likelihood of incorrect information transfer, including sample ID assignments. This can result in a mismatch of sample IDs and sample results.

1D Barcode Symbologies

BD Biosciences has evaluated the following 1D barcode symbologies for use with the BD FACSCanto II flow cytometer, and has the following recommendations.

Barcode Symbology	Recommendation
Code 128	Preferred.
Code 39	Acceptable if barcode labels are printed with the checksum digit. By default, the barcode reader recognizes the checksum digit when reading the Code 39 symbology. However, if labels are printed without a checksum digit, contact your BD service representative for instructions on disabling the checksum feature.
Codabar	The barcode reader does not support the checksum feature when reading the Codabar symbology.
Interleaved 2 of 5	Acceptable

2D Barcode Symbologies

BD Biosciences has evaluated 2D barcode symbology to read the target values of BD FACS 7-color setup beads when using BD FACSCanto clinical software. 2D barcode symbology must be installed in order to read all target values with one scan.

Label Recommendations

Observe these recommendations for optimal performance.

- Labels must be clean and not yellowed.
- Use labels prior to expiration date.
- Label must not have defects such as spots, lines, missing sections, cuts, folds, or density problems.

- Bars must be well defined and bar edges must not be irregular.

Barcode Error Rate Guidelines

- Code 128 and 39 are more accurate and have lower error rates than Codabar and Interleaved 2 of 5.
- CLSI recommends Code 128 because of its accuracy, compact form, and self-checking capabilities.
- A checksum greatly increases accuracy. If possible, use a checksum with Codabar and Interleaved 2 of 5 because they are less accurate symbologies.
- If available, select the fixed length option since this is more accurate than variable length.

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4

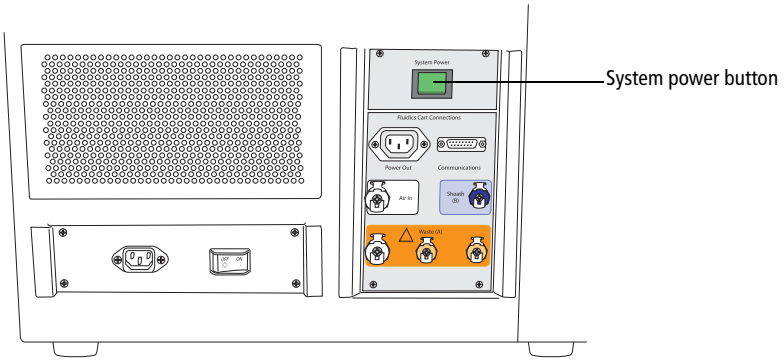
Starting Up

This chapter provides the following information on how to start up the cytometer, BD FACSCanto clinical software, and BD FACSDiva software:

Starting the System

1 Turn on the power to the cytometer.

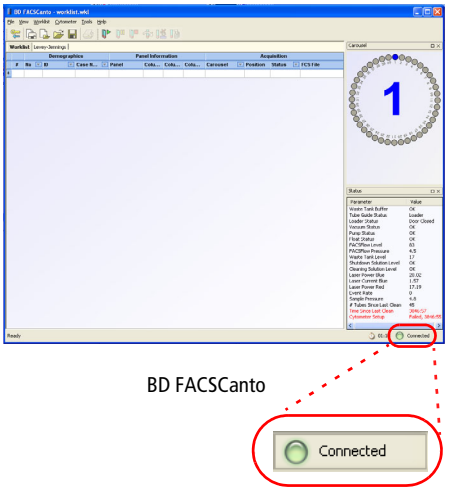
Figure 4-1 Flow cytometer power panel



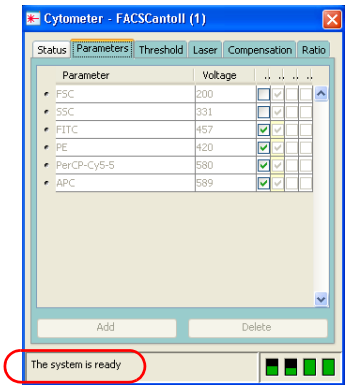
The system power button turns on power to the cytometer, fluidics cart, and lasers.

2 Start up the computer, start the software, and log in.

3 Make sure the software is connected to the cytometer.



BD FACSCanto



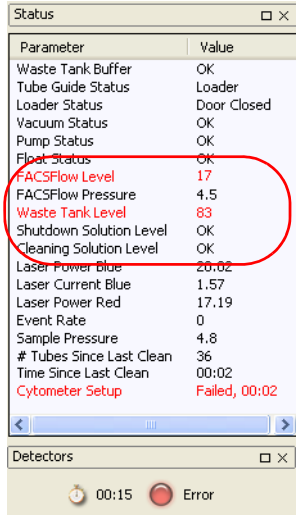
BD FACSDiva

If needed, click **Cytometer > Connect**.

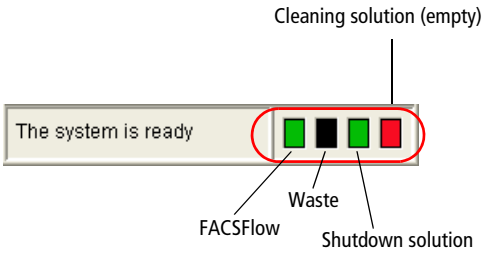
Checking Fluid Levels

1 After startup, check fluid levels.

A low fluid level or a full waste container is indicated in red.



BD FACSCanto



BD FACSDiva

2 If fluidics startup does not start automatically, click **Cytometer > Fluidics Startup**.

⚠ To prevent fluid overflow, make sure there is no tube on the SIT at startup.

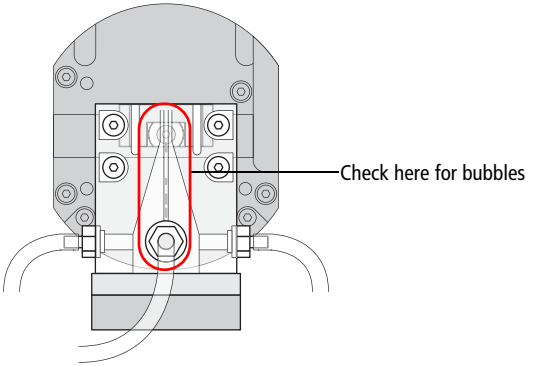
3 Click **OK** at the confirmation dialog.

4 When Fluidics Startup finishes, click **OK** to close the dialog.

Checking for Air Bubbles

1 After checking fluid levels, lift the flow cell access door to check the flow cell for air bubbles.

Figure 4-2 Flow cell



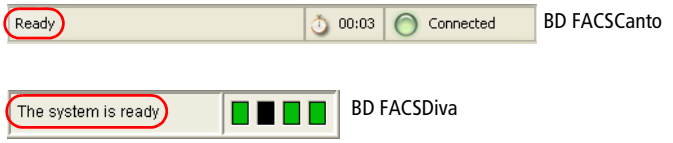
2 If you don't see air bubbles, go to step 5. If you see bubbles, click **Cytometer > Cleaning Modes > De-gas Flow Cell**.

3 Click **OK** when the completion message appears.

4 If you still see bubbles, repeat.

NOTE If an error message appears when you open the flow cell access door, close the message by closing the door and waiting 30 seconds.

5 When you are done, confirm that laser warmup has finished before proceeding.



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5

Cytometer QC and Setup

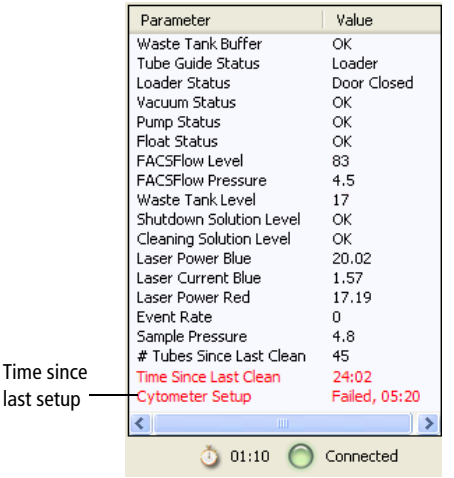
This chapter provides the following information on how to perform cytometer QC and setup:

Performing Automated Setup

Whether you run samples in BD FACSCanto clinical software or BD FACSDiva software, use BD FACSCanto clinical software to run automated setup and QC.

During setup, detector voltages are adjusted to place setup beads at defined target values, sensitivity values are measured, and spectral overlap values are calculated and applied to compensate data for fluorescence spillover. Use the Levey-Jennings feature in BD FACSCanto clinical software to automatically track cytometer setup values over time, and to monitor cytometer performance and see shifts or trends in parameters as they occur.

Run setup once every 24 hours, using BD FACS 7-color setup beads. The software tracks the time between setups and displays it in the Status window. A setup age of more than 24 hours appears in red. Running a successful setup resets the timer.



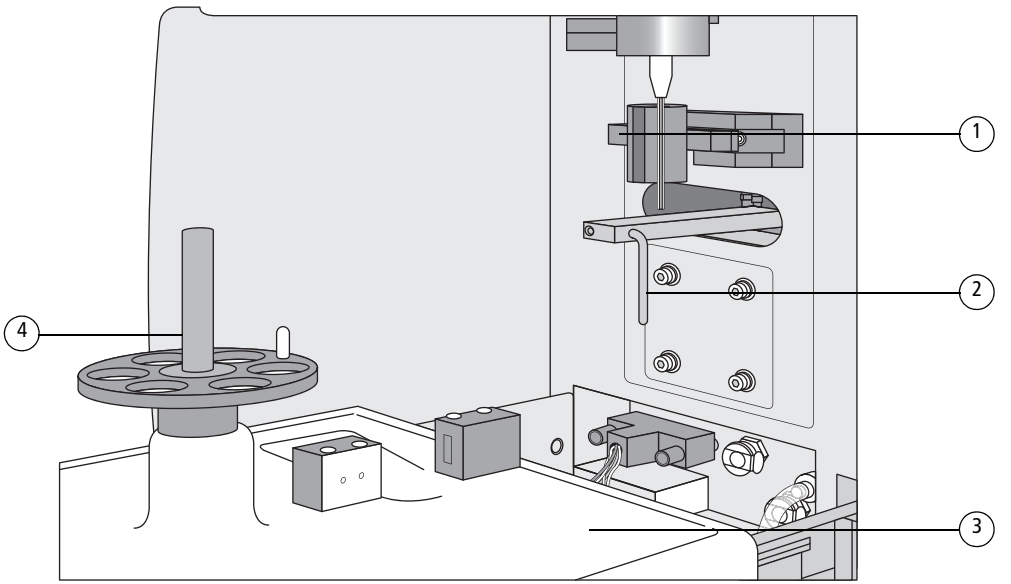
Running Setup Using Manual Loading

1 Prepare BD FACS 7-color setup beads (see the instructions supplied with the beads).

⚠ Do not use beads that have passed their expiration date. Doing so can cause incorrect setup results.

2 Prepare the cytometer for manual loading.

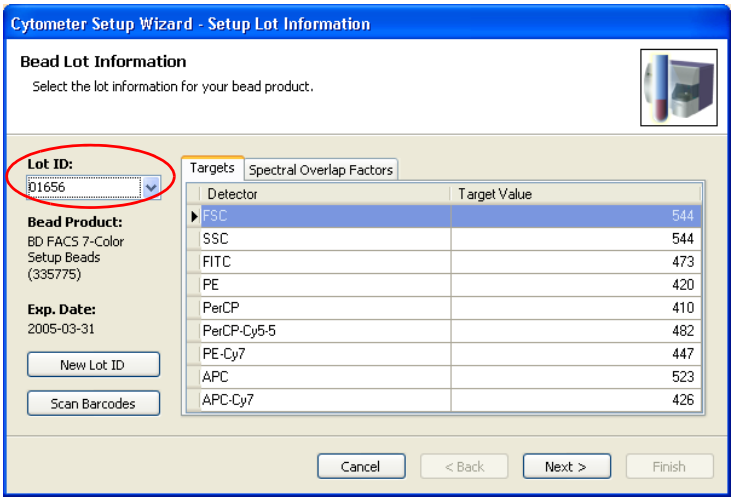
1. Tube guide pushed back
2. Aspirator arm bar vertical
3. Drawer pulled out
4. Carousel removed



3 Select **Cytometer > Setup > Standard Setup** to open the Cytometer Setup Wizard.

4 Select the current bead lot from the **Lot ID** menu.

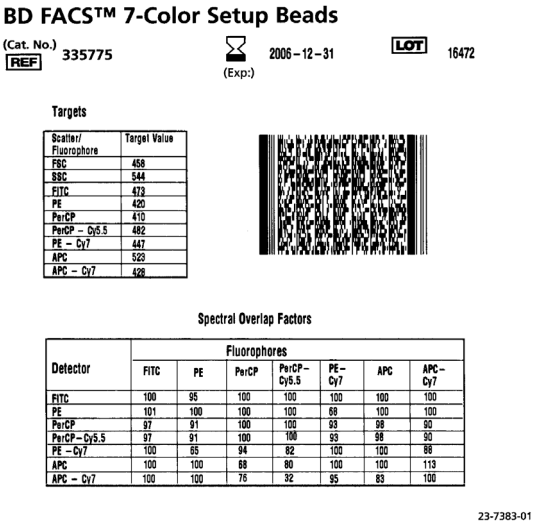
Figure 5-1 Setup Lot Information window



5 Check lot ID, targets, and spectral overlap factors in the **Setup Lot Information** window against the BD FACS 7-color setup beads label.

See Figure 5-2 on page 59 for an example BD FACS 7-color setup beads label.

Figure 5-2 Setup beads label (example)



BD FACS™ 7-Color Setup Beads

(Cat. No.) REF 335775

(Exp:) 2006-12-31

LOT 16472

Targets

Scatter/ Fluorophore	Target Value
FSC	458
SSC	544
FITC	473
PE	420
PerCP	410
PerCP – Cy5.5	482
PE – Cy7	447
APC	523
APC – Cy7	428

Spectral Overlap Factors

Detector	Fluorophores						
	FITC	PE	PerCP	PerCP–Cy5.5	PE–Cy7	APC	APC–Cy7
FITC	100	95	100	100	100	100	100
PE	101	100	100	100	68	100	100
PerCP	97	91	100	100	93	98	90
PerCP–Cy5.5	97	91	100	100	93	98	90
PE–Cy7	100	65	94	82	100	100	88
APC	100	100	68	80	100	100	113
APC – Cy7	100	100	76	32	95	83	100

23-7383-01

6 If necessary, enter new values.

- To enter values using the barcode reader, go to the next section.
- To enter values using the keyboard and mouse, go to page 61.

7 If you do not need to enter new values, click **Next** and go to Loading and Running Beads Tubes (Manual) on page 62.

Entering Lot Information with the Barcode Reader

Although data entry using barcodes is generally more reliable than manual data entry, it is not guaranteed to be 100% accurate. To increase accuracy when using the barcode reader, enabling the checksum feature is recommended. For more information on using the barcode reader, see Chapter 3.

1 Click **Scan Barcodes** in the **Setup Lot Information** window of the Wizard (Figure 5-1 on page 58).

The Scan Barcode dialog opens.

2 Locate the barcode on the BD FACS 7-color setup beads label. See Figure 5-2 on page 59.

3 Hold the barcode reader 23 cm (9 in.) from the BD FACS 7-color setup beads label and aim the barcode reader at the center of the barcode.

4 Press and hold the trigger on the barcode reader until you hear a beep.

To prevent laser injury, do not stare into the beams or aim the scanner at another person's eyes while the trigger is depressed.

The progress bar fills and the dialog closes when you successfully scan the barcode.

If the reader does not beep, adjust your distance from the barcode while continuing to hold the trigger.

5 Check software entries for accuracy of reading the setup beads label.

6 Go to Loading and Running Beads Tubes (Manual) on page 62.

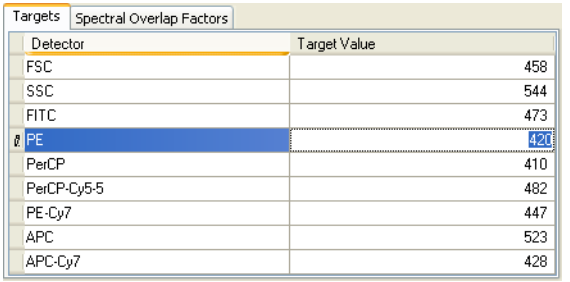
Entering New Lot Information with the Keyboard

1 Click **New Lot ID** in the **Setup Lot Information** window of the Wizard (Figure 5-1 on page 58).

2 Select the bead product, enter the lot ID and the expiration date from the setup beads label, and click **OK**.

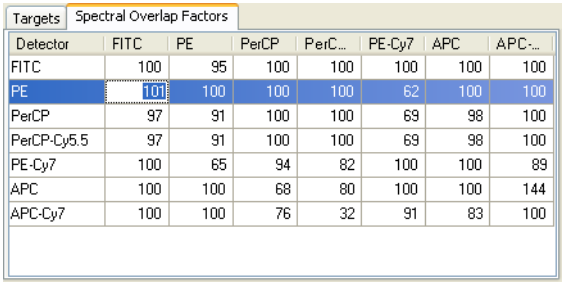
⚠ Do not use the barcode on the tube.

3 Enter the target values for the bead lot. Select the current value in the **Target Value** field and enter the new value. Repeat until you have edited all target values.



4 Click the **Spectral Overlap Factors** tab, and enter the spectral overlap factors for the bead lot.

Select a current value and enter the new value. Repeat until you have edited all spectral overlap factors.



5 Click **Next** and go to Loading and Running Beads Tubes (Manual).

Loading and Running Beads Tubes (Manual)

Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

1 Click **Next**.

2 If you changed Lot ID values and the **Save Setup Bead Lot Info** dialog appears, click **Yes**.

3 Select **Run setup in Manual mode**, and click **Next**.

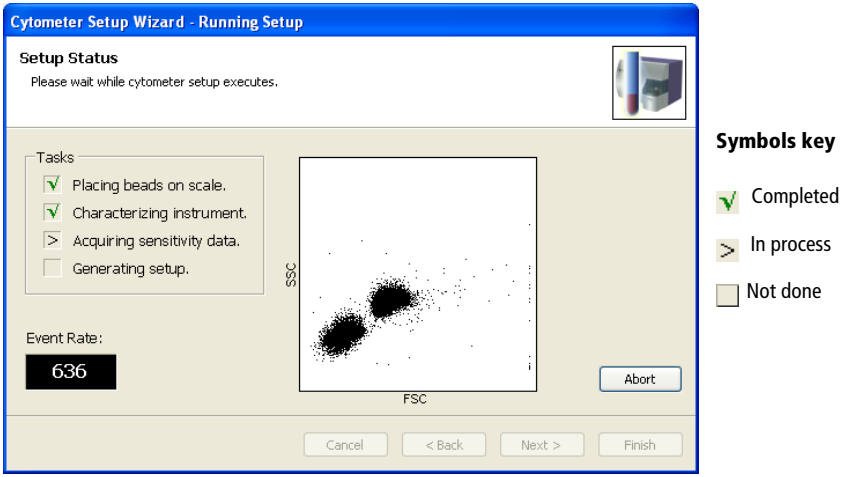
4 When prompted, load the beads tube onto the SIT, using these steps:

a Push the aspirator arm to the left.

b Place the beads tube on the SIT, ensure the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.

c Center the aspirator arm under the beads tube. There are three sensor pins on the aspirator arm. The bottom of the tube should sit within the center of the pins.

5 Click **OK** and wait for setup to finish.



The software adjusts cytometer settings to place the beads on scale. Note that it is normal for the beads to move to the baseline and back on scale during this process.

6 Unload the beads tube when prompted, using these steps:

a Hold the sample tube while pushing the aspirator arm to the left.

b Remove the tube from the SIT.

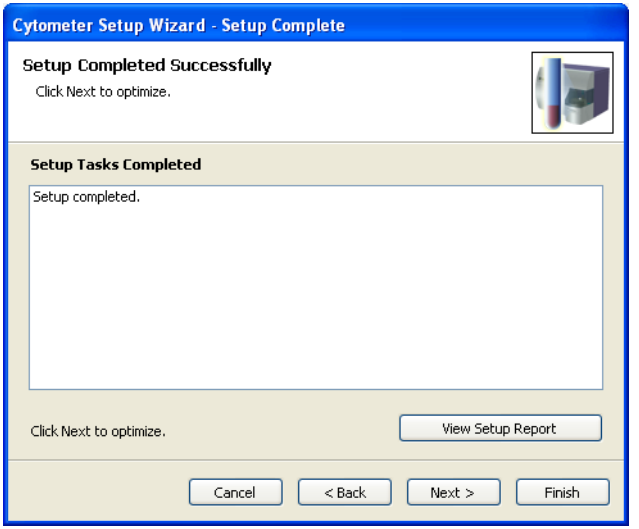
c Release the aspirator arm.

A SIT Flush occurs when the aspirator arm comes to center.

Accepting and Managing Setup Information (Manual)

Incorrect values are saved if you accept a setup that is unsuccessful or setup results that are out of range. If setup is unsuccessful or if setup results are out of range, *do not* click Finish. Note the message provided by the software (for example, Figure 5-3) and consult BD FACSCanto Clinical Software Troubleshooting on page 196.

Figure 5-3 Successful Setup Complete dialog



If setup is successful, see Table 5-1 on page 65.

Table 5-1 Options after setup

Task	Button	Additional Information
View setup results	View Setup Report	The report contains cytometer QC and pass/fail information. You can print the report from this view.
Discard current results	Cancel	You will be given the option to use the last setup results.
Run setup again	Back	
Optimize setup values using BD FACSCanto clinical software	Next When prompted, click **Yes** to save your results and continue.	Proceed to Optimizing with BD FACSCanto Clinical Software on page 70.
Exit setup and save new setup results	Finish	The software calculates and saves lyse/wash and lyse/no-wash setups that you can use with BD FACSCanto clinical software or BD FACSDiva software. PDF files of Setup Reports are automatically saved in the D:\BD\FACSCanto\SetupReports folder.
(Optional) Save setup results and optimize setup values using BD FACSDiva software	Finish	Proceed to Optimizing with BD FACSDiva Software on page 102.

NOTE It is important to monitor setup data for trends. See Reviewing Levey-Jennings Reports on page 79.

Running Setup Using the Loader

1 Prepare BD FACS 7-color setup beads (see the instructions supplied with the beads).

NOTE Do not use beads that have passed their expiration date. Doing so can cause incorrect setup results.

2 Prepare the cytometer for automatic loading (Figure 8-3 on page 134).

3 Select **Cytometer > Setup > Standard Setup** to open the Cytometer Setup Wizard.

4 Select the current bead lot from the **Lot ID** menu.

5 Check the Lot ID, Targets, and Spectral Overlap Factors in the **Setup Lot Information** window against the BD FACS 7-color setup beads label.

6 If needed, enter new values and click **Next**.

See page 60 for how to enter information using the barcode reader.

7 If you changed Lot ID values and the **Save Setup Bead Lot Info** dialog appears, click **Yes**.

Loading and Running Beads Tubes (Loader)

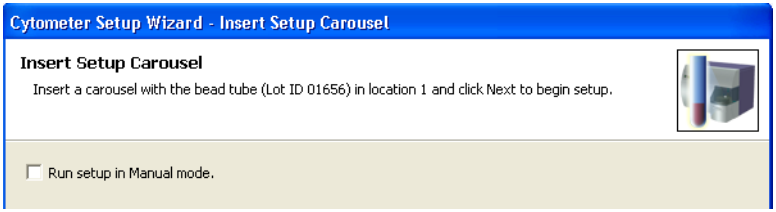
1 Place the beads tube in position 1 of a carousel and the optimization tubes in the positions following.

For instructions, see step 3 on page 135.

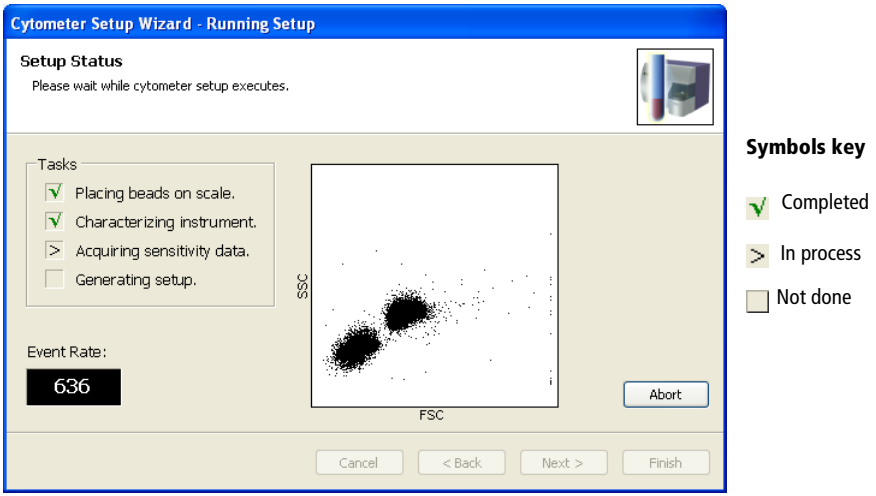
2 Install the carousel onto the Loader.

For instructions, see step 4 on page 135 and step 5 on page 135.

3 Clear the **Run setup in Manual mode** checkbox, and click **Next**.



4 Wait for setup to finish.



The software adjusts cytometer settings to place the beads on scale. Note that it is normal for the beads to move to the baseline and back on scale during this process.

Accepting and Managing Setup Information (Loader)

⚠ Incorrect values are saved if you accept a setup that is unsuccessful or setup results that are out of range. If setup is unsuccessful or if setup results are out of range, *do not* click Finish. Note the message provided by the software (see Figure 5-3 on page 64) and consult BD FACSCanto Clinical Software Troubleshooting on page 196.

If setup is successful, see Table 5-1 on page 65.

NOTE It is important to monitor setup data for trends. See Reviewing Levey-Jennings Reports on page 79.

6

Running Samples with BD FACSCanto Clinical Software

This chapter provides the following information on how to use BD FACSCanto clinical software features to run and review worklist data:

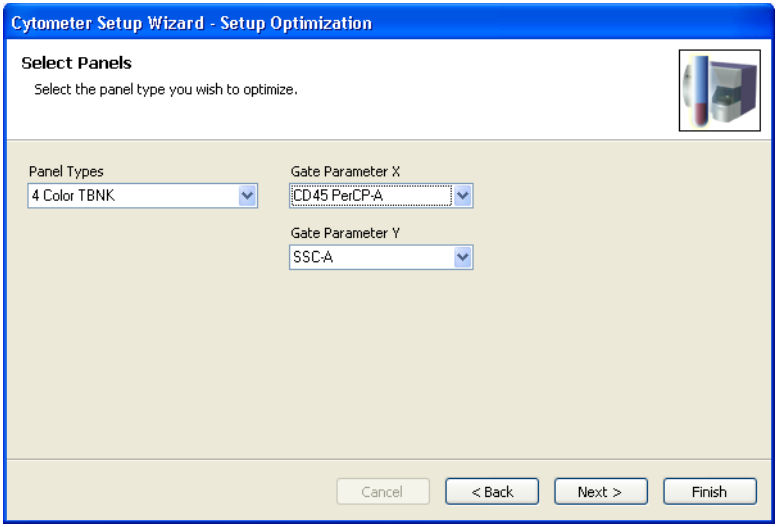
Optimizing with BD FACSCanto Clinical Software

During optimization, you can adjust thresholds, detector voltages, and spectral overlap values for a panel type. The software uses BD Biosciences default settings the first time you optimize. When you make changes, the new settings apply to all tubes and samples of the selected panel type.

Acquiring Data and Optimizing Settings

1 Select a panel type and parameters from the menus and click **Next**.

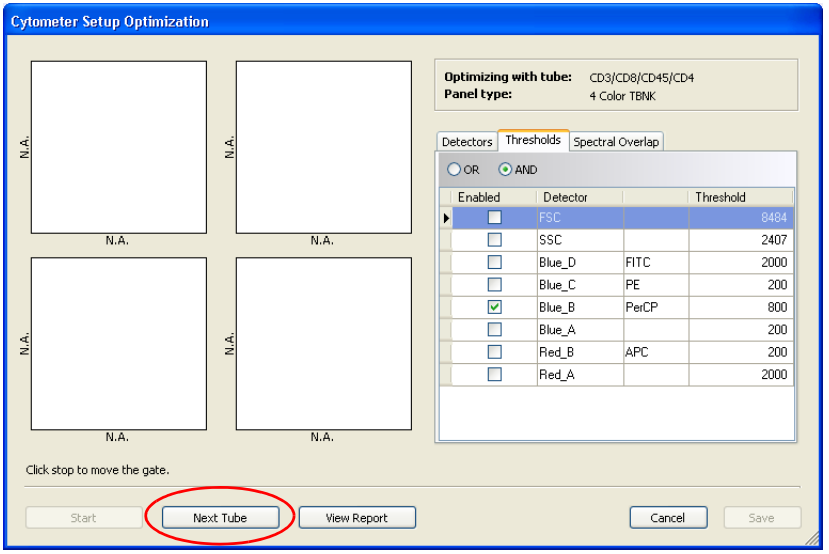
NOTE The 4-color and 6-color TBNK panels are the same, with or without BD Trucount tubes.



Gate Parameter X and Gate Parameter Y refer to the plot parameters for the first optimization plot, the plot that contains a gate around the cells of interest.

2 (Loader only) Make sure the optimization tubes are installed in the correct carousel positions, and click **Next**.

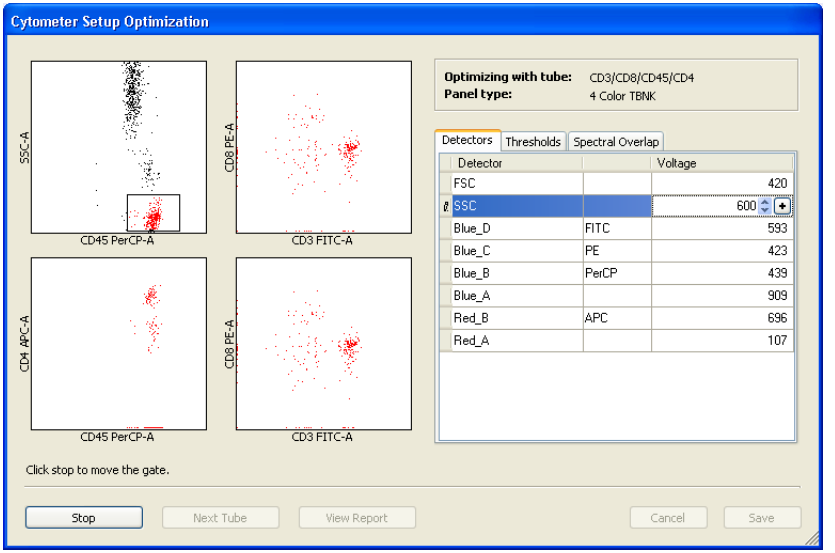
3 Click **Next Tube**.



4 (Manual only) Install the first optimization tube when prompted, and click **OK**.

5 In the **Cytometer Setup Optimization** window, click **Start**.

- Acquisition begins, and events appear in the plots.
- Right-click the axis labels on a plot to select other parameters to be displayed.



6 Optimize settings, as needed.

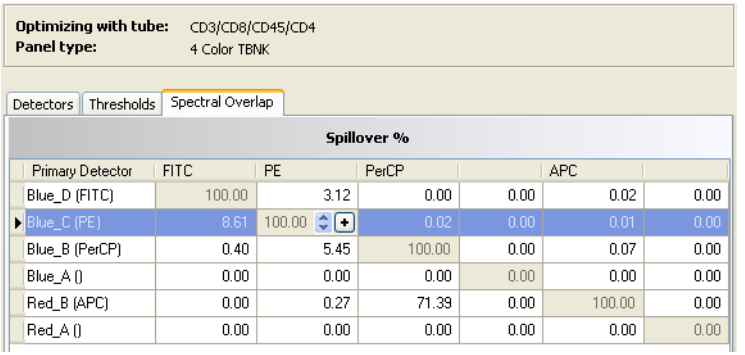
- To adjust the optimization gate, click **Stop**. The acquisition display stops updating. Move the gate by dragging the gate border, or resize the gate by selecting a corner and dragging. When you are ready to proceed, click **Start**.

- To adjust detectors, thresholds, or spectral overlap settings, click the corresponding tab and use controls in the tab to adjust the settings. For instructions, see Using Cytometer Controls on page 76.

  Spectral overlap values are automatically recalculated when you adjust voltages.

NOTE Threshold and side scatter are the most frequently optimized parameters for TBNK assays.

For clinical applications that use tandem conjugates such as APC-Cy7 or PE-Cy7, spectral overlap varies from lot to lot. Because BD FACSCanto clinical software setup targets the average lot, you might need to adjust spectral overlap settings for these reagents.



7 If this is the last tube, proceed to Saving the Optimized Setup Results on page 74. Otherwise, continue to the following section.

Running Additional Optimization Tubes

1 Click **Stop**, and then **Next Tube**.

2 (Manual only) Remove the current tube when prompted, using the following method:

a When the aspirator arm lowers, hold your sample tube while you push the aspirator arm to the left.

b Remove the tube from the SIT.

c Release the aspirator arm.

SIT cleaning occurs when the aspirator arm comes to center.

⚠ If you move the arm without holding the tube, the tube could fall off the SIT and expose you to potentially biohazardous sample.

NOTE To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 183.

3 (Manual only) When prompted, place the next tube on the SIT, and click **OK**.

4 Repeat steps 3 through 6 in the preceding section starting on page 71.

Saving the Optimized Setup Results

1 When there are no more tubes to optimize, click **Stop**, and then **Save**.

Optimized setup results are saved to the *panel name.opt* file in the C:\ProgramData\BD\Shared\Setup Results folder. (For example, 4-color TBNK optimized setups are saved to the *4 color TBNK.opt* file and 6-color TBNK optimized setups are saved to the *6 color TBNK.opt* file.)

The software also saves PDF files of the Application Setup Report.

2 Unload the optimization tube or remove the carousel.

3 Click **Finish**.

Viewing and Printing the Application Setup Report

The Application Setup Report reflects the optimized settings. You can view and print it before optimization is complete.

- From the Cytometer Setup Optimization window, click **View Report**.
- Click the **Print** button on the toolbar to print the report.

When setup results are saved, the software also saves PDF files of the reports. Navigate to D:\BD\FACSCanto\SetupReports to view and print these reports.

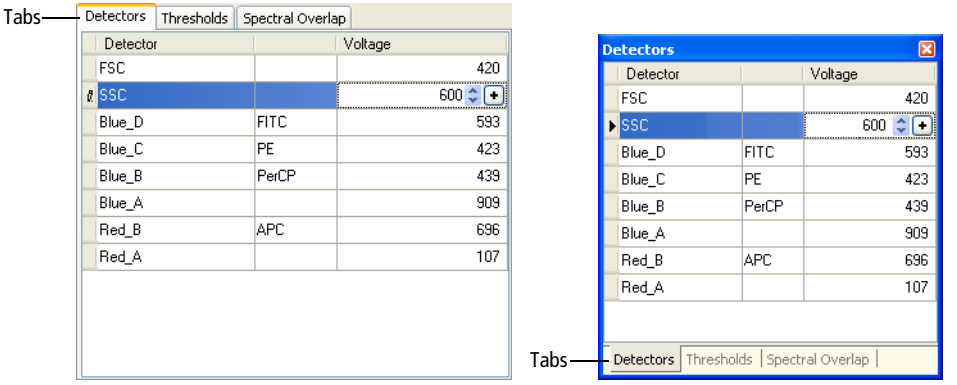
Maintaining User-Specific Optimization Settings

Task	Procedure	Additional Information
Maintain settings for a single user	**1** Run setup. **2** Select an application and optimize.	User-optimized setup is calculated for the selected application and panel type.
Maintain settings for multiple users	**1** Copy the *panel type*.opt file. **2** Rename it with a unique name (for example, *4 color TBNK_username.opt*), and save it in your choice of location. **3** To use your optimized results, exit the software, rename the file with its original file name (for example, 4 color TBNK.opt or 6 color TBNK.opt), and move it back into the Setup Results folder. **4** When prompted, overwrite the current file. **5** Restart the software, and run setup.	The file is in C:\ProgramData\BD\Shared\Setup Results.
Restore BD Biosciences default settings	**1** While the software is not running, delete the *panel type*.opt file. **2** Restart the software, and run setup.	The software automatically rebuilds the *panel type*.opt file with BD-defined settings.

Using Cytometer Controls

You can adjust cytometer controls only during optimization (immediately after setup), or after pausing sample acquisition during a worklist run. Cytometer controls are disabled when a worklist is running.

There are three types of cytometer controls: Detectors, Thresholds, and Spectral Overlap. Click a tab to access the corresponding controls, or select an option from the **View** menu.



After setup, in Wizard

During acquisition

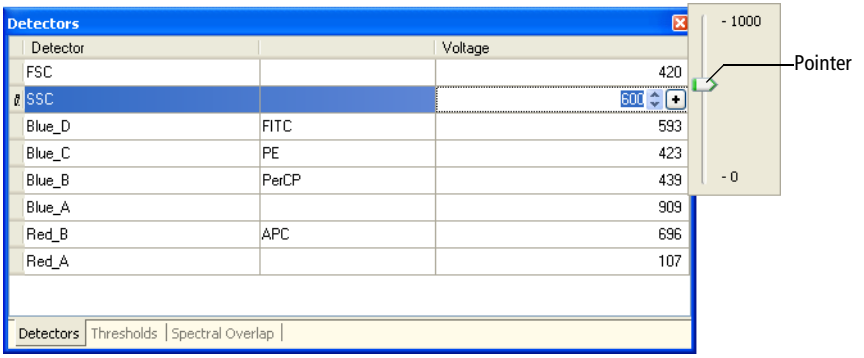
Adjusting Detectors

Adjust the signal for events displayed in plots by changing detector voltages. Higher voltages amplify the signal. Lower voltages decrease the signal. BD FACSCanto clinical software automatically recalculates spectral overlap when you change detector voltages.

To change a setting, click in the field containing the value you want to change. Up and down arrows and a plus sign (+) appear. Change the value using one of the following methods:

- Select the value and type in a new one.
- Click the up and down arrows.

- Click the plus sign (+), and drag the pointer to a new setting.
- Press Ctrl+↑/↓ to adjust settings in minor increments, or Ctrl+Page Up/Page Down to adjust settings in larger increments.



Adjusting Thresholds

A threshold sets a channel number below which events will not be processed. Use thresholds to filter out unwanted events. You can set one or more thresholds at a time, and select whether any one (OR) or all (AND) need to be met.

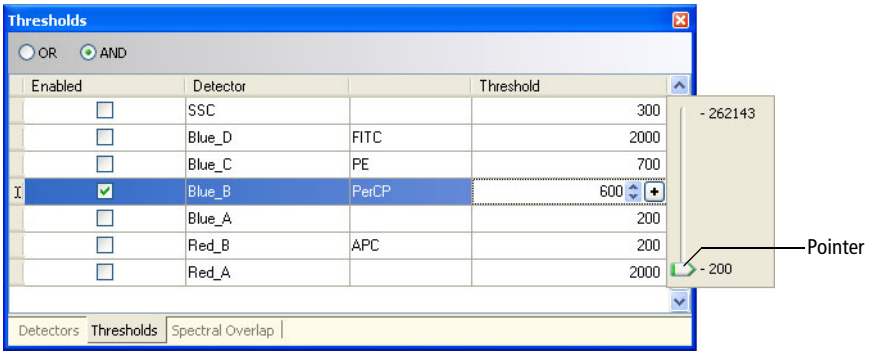
To set a threshold:

1 Enable a parameter as a threshold by selecting the checkbox beside it.

2 Click in the associated **Value** field to set the threshold value.

Use one of the editing methods specified in Adjusting Detectors on page 76.

Figure 6-1 Adjusting Threshold



3 (Optional) Enable and set another threshold parameter.

a Select the associated checkbox.

b Edit the threshold value.

c Select **OR** (any of the parameter thresholds can be met) or **AND** (all of the parameter thresholds must be met).

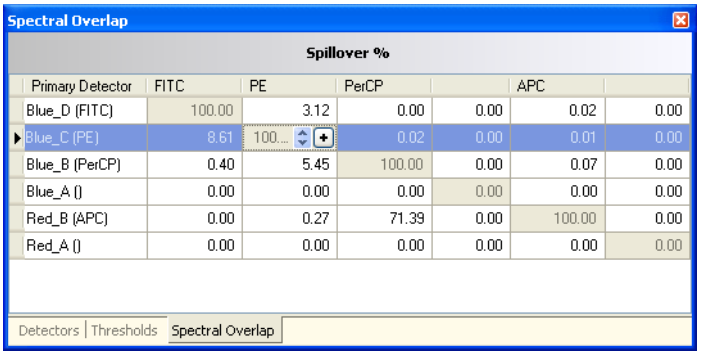
Adjusting Spectral Overlap

Fluorochromes emit light over a range of wavelengths. During cytometer setup, fluorescence spillover is automatically determined and corrected. If necessary, you can use the spectral overlap controls to make manual adjustments.

1 Click in the **Spillover %** value field that needs correction (Figure 6-2 on page 79).

2 Use one of the editing methods specified in Adjusting Detectors on page 76.

Figure 6-2 Adjusting Spectral Overlap

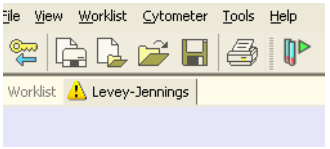


Reviewing Levey-Jennings Reports

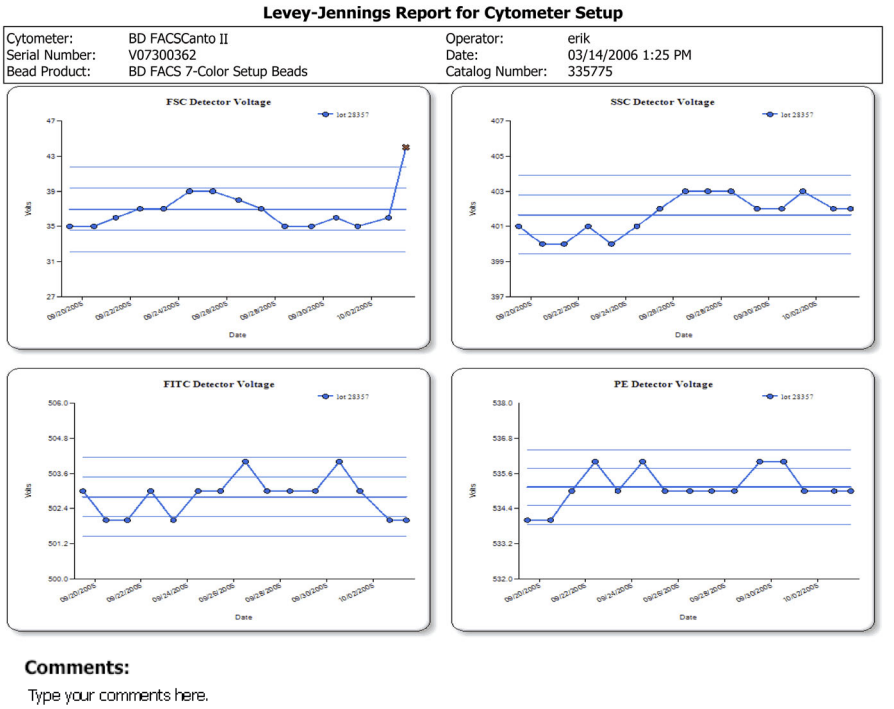
The software automatically creates a Levey-Jennings Report from the cytometer setup data. To view the report:

1 From the main window, click the **Levey-Jennings** tab.

An exclamation mark on the tab indicates an out-of-range value on the report.



2 Check the plots in the report.



Parameters outside the limits set by the lab manager are shown with a red *x* in the affected plot.

3 To add comments to the report, click **Comments**.

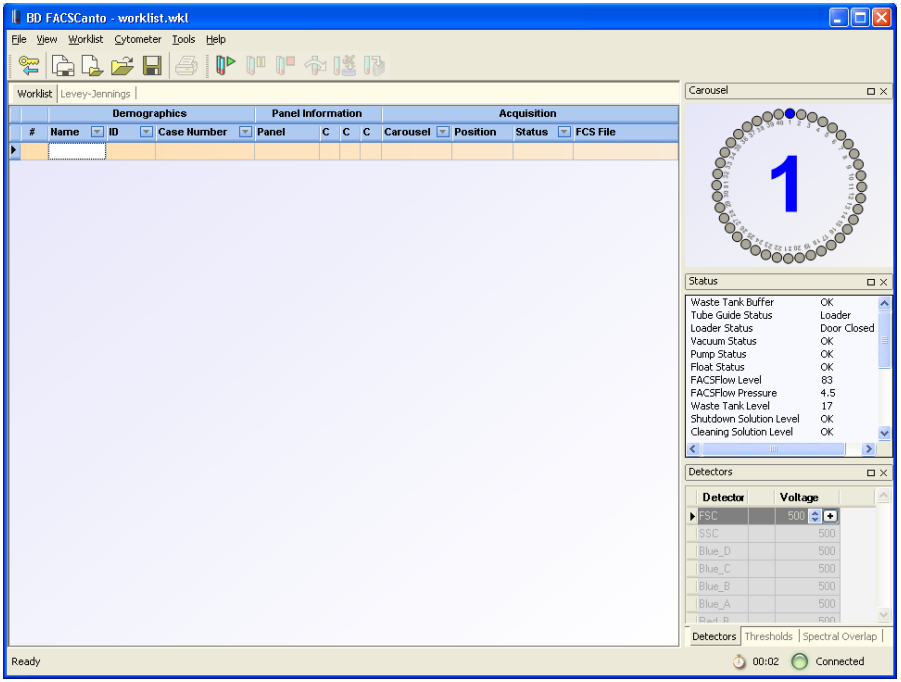
Enter text into the **Comments** field (up to 2,500 characters), and click **OK**.

To print the report, click the **Print** button.

Running an Acquisition Worklist

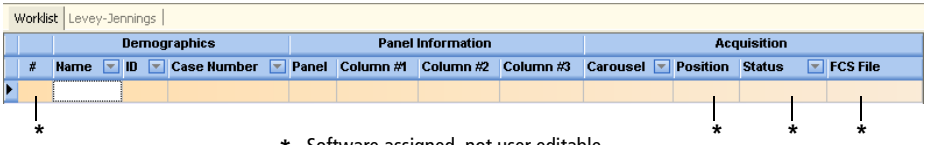
An acquisition worklist allows you to run a group of samples, optimize cytometer settings and save the data, and obtain automated analysis.

When you first open BD FACSCanto clinical software, a blank worklist appears.



Entering Information into a Worklist

A worklist can contain information for up to 200 samples.



1 (Optional) Enter a name.

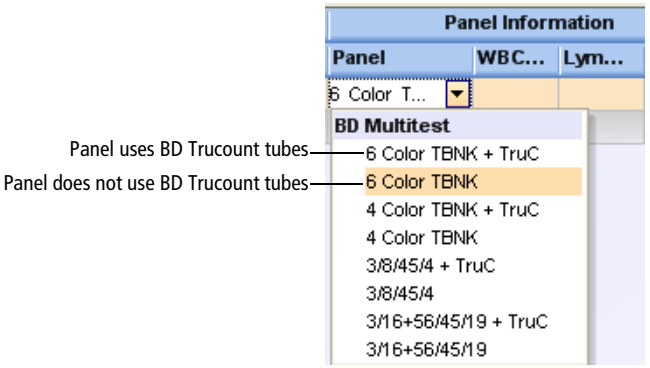
2 Enter an ID.

To automatically enter sample IDs, use the optional barcode reader.

3 (Optional) Enter a case number.

4 Select a panel.

- Either click in the **Panel** field, click the down arrow, and select a panel, or
- Press Alt+ the down arrow to access the **Panel** menu, and use the arrow keys to select a panel.



If you select a 4-color or a 6-color TBNK panel that does not use BD Trucount tubes, to calculate absolute counts you need to either

- Enter the WBC count (x 1,000) and the lymphs (%), or
- Enter the absolute lymphocyte count (x 1,000)

where $\text{counts} = \frac{\text{cells}/\mu\text{L}}{1000}$.

If you try to enter all three values, the software will alert you with a dialog.

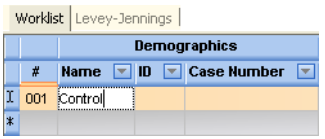
5 (Loader only) Select a carousel ID in the **Carousel** field.

Click the down arrow in the field, and select a number. The number you select must match the number on top of the carousel.

Running a Process Control

We recommend that you always run a process control using your optimized settings before running test samples (unknowns). Process control results are automatically exported to a spreadsheet file in C:\ProgramData\BD\FACSCanto \DataFiles. If the file already exists, results are appended. Use the file to track results over time.

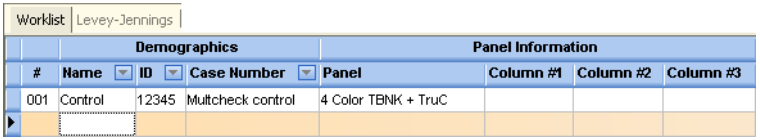
1 In the **Name** field, enter *Control*.



Names other than *Control* (not case sensitive) will not be exported into the control-specific file.

2 In the **ID** field, enter the control's lot ID.

3 (Optional) Enter a case number and a short comment in this field.



4 Select a panel.

5 Run the control as you would a typical sample.

Acquiring Samples

1 Enter all sample information into a worklist.

2 (Loader) Prepare to acquire with the Loader.

⚠ For accurate results when loading, match tube positions to those listed on the printout. Make sure to use the carousel rack with the appropriate Carousel number.

a Print the worklist. Load tubes into the carousel(s) according to the printout

b Set up the cytometer for automatic loading (page 134).

c Insert the carousel into the Loader, and close the Loader doors.

NOTE Ensure that the carousel is inserted all the way in. If it is not, the tube can become jammed in place. Open and close the door twice to release the jam.

3 Verify that the cytometer is ready.

Check the status bar at the bottom of the main window, and the **Status** window. If an error condition exists, resolve it before starting the acquisition.

4 From the **View** menu, select to display Detectors, Spectral Overlap, and Threshold.

5 Click the **Run** button.

A dialog appears asking if you want to save the worklist.

6 Click **Yes**. Specify a file name and storage location in the next dialog.

(Loader) The carousel briefly mixes the samples, and acquisition begins.

7 (Manual) Click **Ignore** to load tubes manually.

Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

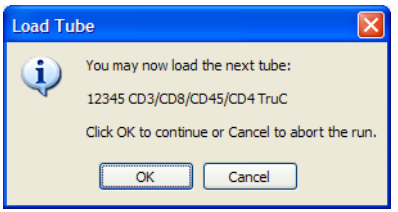
8 (Manual) When prompted, install the tube containing the first worklist sample.

a Push the aspirator arm to the left.

b Place the tube on the SIT, ensure the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.

c Center the aspirator arm under the tube. There are three sensor pins on the aspirator arm. The bottom of the tube should sit within the center of the pins.

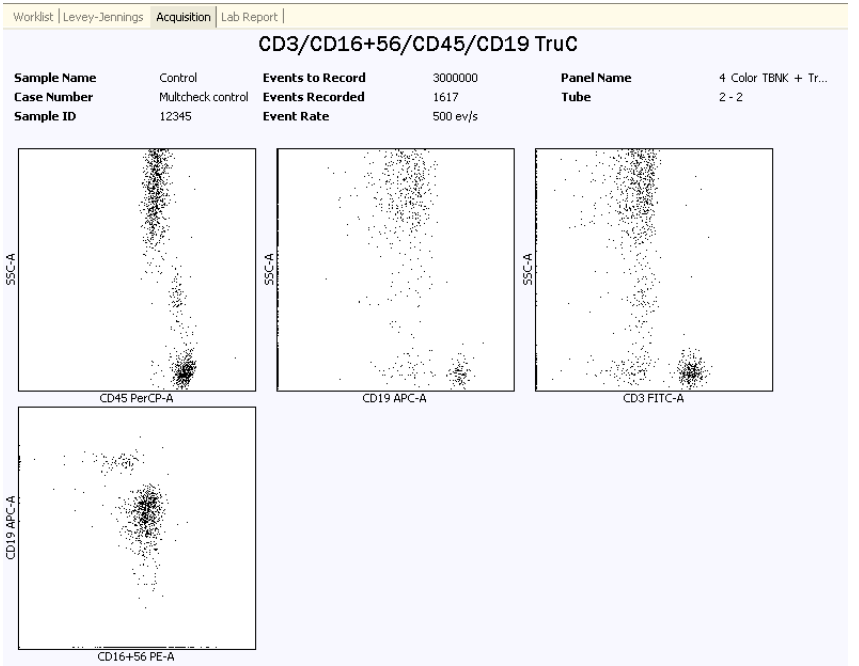
d Click **OK**.



Observing and Recording Event Data

The Acquisition tab comes into view and events appear once data has been acquired for the first tube. See Figure 6-3.

Figure 6-3 Acquisition tab



1 Observe event data.

Data recording begins as soon as the flow stabilizes. Recording stops automatically after the designated acquisition targets have been met.

After you click the **Run** button and before data recording begins, you can select from the following options.

Table 6-1 Options before recording

Option	Procedure	Additional Information
Optimize detectors, thresholds, or spectral overlap	**1** Click the **Pause** button. **2** Click the **Optimize** button.	See page 89.
Change plot parameters	**1** Right-click an axis label. **2** Select a different parameter.	
Proceed with recording	Do nothing.	
Stop recording data for the worklist	**1** Click the **Pause** button. **2** Click the **Stop** button.	If recording was in progress, data for the current tube is discarded and no FCS file is saved.

2 View the Lab Report for recorded data.

If the Lab Report countdown is set to On, the Lab Report is shown for the designated time. You can pause and edit the report during the countdown. See Inspecting Lab Reports on page 90 for instructions.

Acquiring and Recording the Remaining Samples

Loader acquisition of the next tube starts automatically.

The **Status** and **FCS File** fields update as each sample is recorded.

1 (Manual) After data has been recorded for the first tube, remove the tube at the prompt and install the next tube.

NOTE To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 183.

a Hold your sample tube while you push the aspirator arm to the left.

b Remove the tube from the SIT.

c Release the aspirator arm.

If you move the arm without holding the tube, the tube could fall off the SIT and expose you to potentially biohazardous sample.

NOTE If you do not move the aspirator arm to the left and/or unload a tube when prompted, an error message appears. If this happens, click **OK**. Push the arm to the left and/or unload the tube. Wait for the Load Tube message before you install the next tube.

SIT flush occurs when the aspirator arm comes to center. When cleaning is finished, you are prompted to load the next tube.

2 (Manual) Repeat the sequence starting with step 7 on page 85 until there are no more samples.

3 (Loader) Load the next carousel when prompted, and click **OK**.

4 After all samples have been run, review the worklist.

5 After the last run of the day, shut down the system.

See Chapter 9 for instructions.

Options During Acquisition

Do any of the following during acquisition:

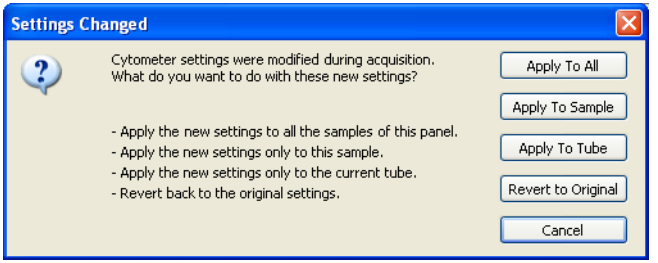
- Adjust voltages, thresholds, and spectral overlap values (page 89)
- Inspect recorded data on the Lab Report (page 90)
- Skip tubes or samples (page 94)
- Stop recording and acquisition (page 94)
- Add samples to a worklist (page 95)

Optimizing During a Run

For instructions on using optimization controls, see Using Cytometer Controls on page 76.

1. At the Acquisition view, click the **Pause** button on the toolbar to pause.
2. Click the **Optimize** button.

   Live events are displayed without being recorded.
3. Use Detectors controls to adjust detector voltages.
4. Use Thresholds controls to adjust the threshold.
5. Use Spectral Overlap controls to adjust compensation settings.
6. Click the **Run** button to return to data recording.
7. Decide how to apply the optimized changes and click the corresponding button.



The software applies the updated settings and finishes running the tube (unless you click **Cancel** or **Revert to Original**).

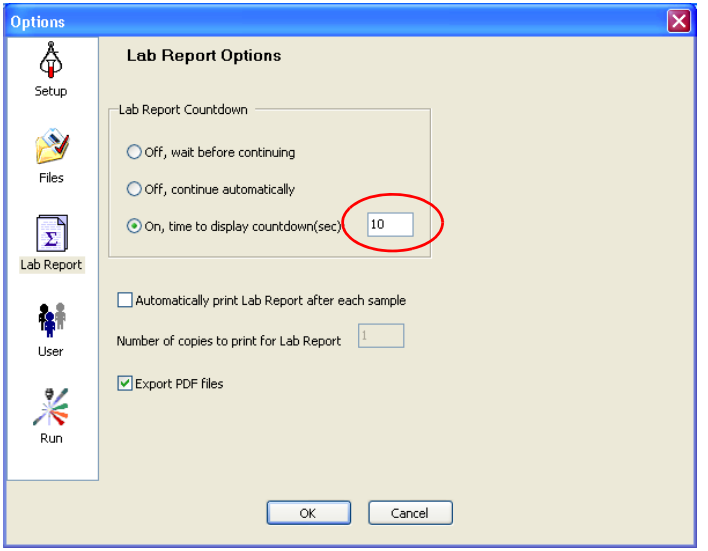
⚠ The software overwrites all events recorded prior to optimizing for the current tube.

Inspecting Lab Reports

To inspect a Lab Report during acquisition, set the Lab Report display countdown.

Specifying a Display Time for the Lab Report Countdown

1. Select **Tools > Options**.
2. Click the **Lab Report** button.
3. Select **On, time to display countdown (sec)**.
4. Enter a number of seconds, from 1 to 10, and click **OK**.



If the Lab Report countdown is set, recorded data displays in the Lab Report view. A message shows the time remaining to view the report.

During the countdown, you can pause the run and do any of the following:

- Re-Gating a Plot Manually
- Reapplying the Auto-Gate on page 92
- Re-Running a Tube on page 93
- Verifying Report Review on page 93

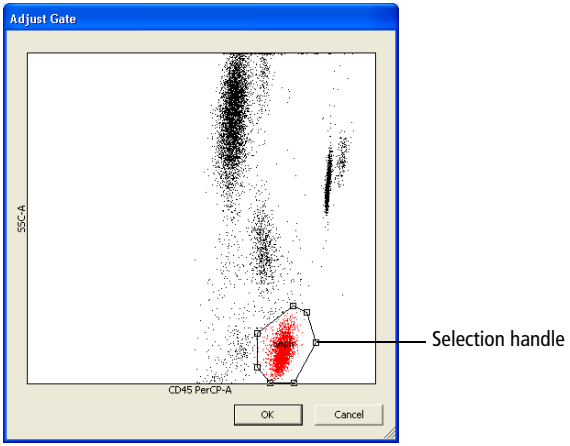
Re-Gating a Plot Manually

You can wait until the entire worklist has completed to review plots on the Lab Report. However, if you prefer to re-gate plots during a worklist run, follow these steps.

1 In the **Lab Report Countdown** dialog, click **Pause**.

2 Click any plot to adjust its gate.

The selected plot appears in an enlarged view.



3 Adjust the gate.

a Select the gate by clicking its boundary.

b Drag the selection handle to adjust the shape or size of the gate.

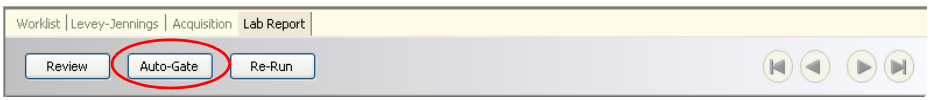
c Drag the gate boundary between selection handles to move the gate.

Gate changes apply only to the current tube.

4 Click **OK**.

Reapplying the Auto-Gate

To override gate changes and return to the automatic gate, click **Auto-Gate**.



When the confirmation message appears, click **Yes**.

Re-Running a Tube

After you inspect the data on the Lab Report, you can elect to re-run the tube. Re-running a tube overwrites the previous data for it.

1 Click **Re-Run** at the top of the Lab Report view.

2 Select the tubes you want to re-run, and click **OK**.

You can re-run all tubes or a single tube of the current sample.

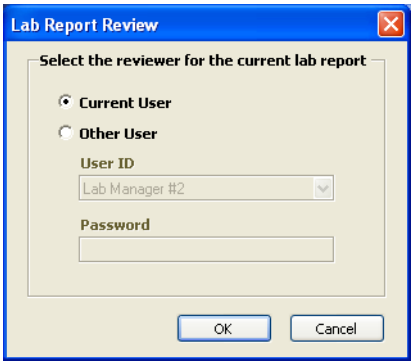
Verifying Report Review

You can append a virtual signature to reviewed reports.

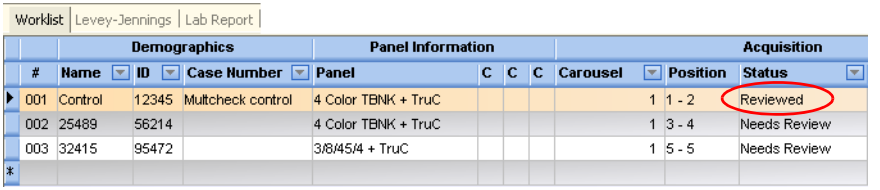
1 Click **Review** at the top of the Lab Report view.

2 Select a reviewer, and click **OK**.

- Select **Current User** for the current, logged-in user.
- Select **Other User** for a different user. Select the User ID from the menu, and enter a password.



The corresponding worklist Status field changes to *Reviewed*.



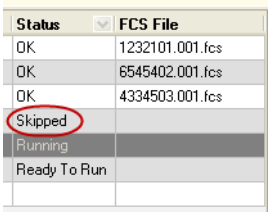
Skipping a Tube

1 Under the **Acquisition** tab, click the **Pause** button.

2 Click the **Skip** button.

3 Select whether you want to skip to the **Next Tube** or **Next Sample**.

Skipped tubes are indicated on the worklist.



Stopping Recording

To stop recording data for the current sample, click the **End Recording** button under the **Acquisition** tab.

The software saves the data already recorded for that tube and proceeds to the Lab Report view or starts acquisition of the next sample.

Adding Samples to the Worklist

You can stop worklist acquisition and add samples to the worklist.

1 Click the **Pause** button, followed by the **Stop** button to stop the acquisition.

2 If a sample tube was loaded manually, remove the tube from the SIT.

3 Enter new sample information into the worklist.

4 Click the **Run** button to resume.

Reviewing an Analysis Worklist

An analysis worklist allows you to reanalyze FCS files previously created by BD FACSCanto clinical software. Only FCS files created in BD FACSCanto clinical software can be processed in an analysis worklist. Do not modify BD FACSCanto FCS files with other software applications.

Every time you reanalyze an FCS file, only the latest analysis is saved.

⚠ FCS files created with BD FACSCanto clinical software version 2.4 or later cannot be used with an earlier version of BD FACSCanto clinical software software. Earlier versions will show incorrect results.

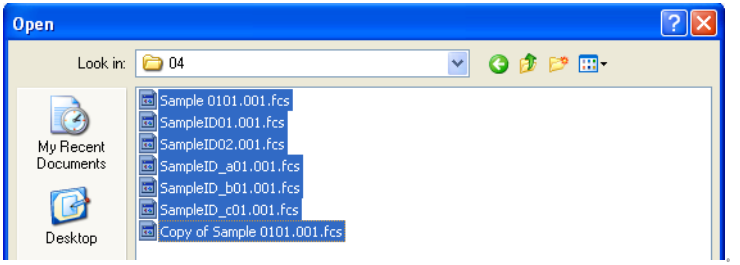
Creating a New Analysis Worklist

1 Select **File > New Analysis Worklist**.

2 Add FCS files.

By default, the software stores FCS files in a dated folder in D:\BDFACSCantoFCSFiles.

- To add selected files, click the **Add Files** button, navigate to the folder containing the files, and select them. Click **Open**.



Only the FCS file for the first tube in a panel is displayed. All tubes in the panel are automatically imported when you select this file.

- To add all files in a folder (including files in subfolders), click the **Add Folder** button, locate and select the folder, and click **OK**.

3 Verify that the worklist contains all required files.

4 Continue to add FCS files (up to 200).

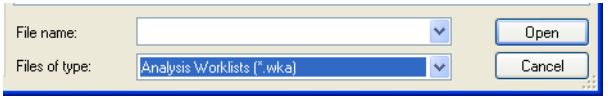
Opening an Existing Analysis Worklist

1 Select **File > Open Worklist**.

2 Navigate to the folder containing your saved worklists.

By default, worklists are stored in D:\BD\FACSCanto\Worklists.

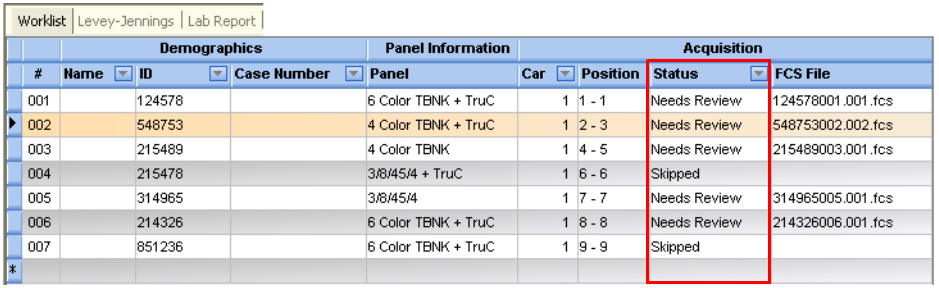
3 From the **Files of Type** menu at the bottom of the window, select **Analysis Worklists** (*.wka).



4 Select an analysis worklist, and click **Open**.

Reviewing a Lab Report

After all samples in a worklist have been processed, inspect the Lab Report for each sample. You can review Lab Reports after each sample is run, or after an acquisition worklist is complete. The following table describes Status entries.



	Demographics			Panel Information		Acquisition		
#	Name	ID	Case Number	Panel	Car	Position	Status	FCS File
001		124578		6 Color TBNK + TruC	1	1 - 1	Needs Review	124578001.001.fcs
002		548753		4 Color TBNK + TruC	1	2 - 3	Needs Review	548753002.002.fcs
003		215489		4 Color TBNK	1	4 - 5	Needs Review	215489003.001.fcs
004		215478		3/8/45/4 + TruC	1	6 - 6	Skipped	
005		314965		3/8/45/4	1	7 - 7	Needs Review	314965005.001.fcs
006		214326		6 Color TBNK + TruC	1	8 - 8	Needs Review	214326006.001.fcs
007		851236		6 Color TBNK + TruC	1	9 - 9	Skipped	

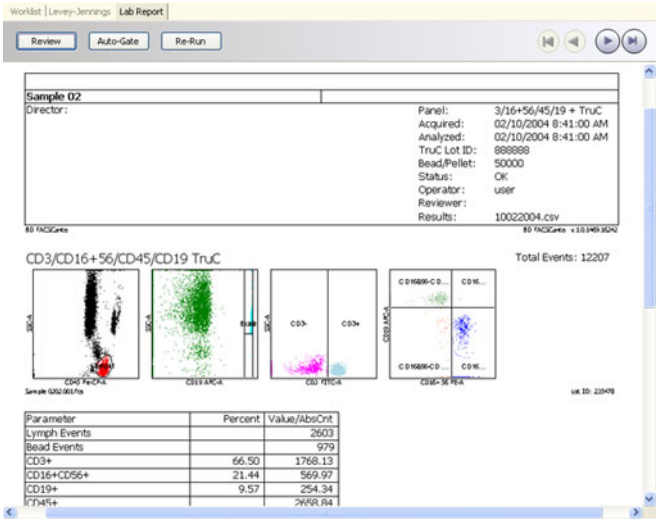
Status	Meaning
Ready To Run	All columns filled in and sample ready for acquisition
Not Prepped	Sample skipped—prep on SPA II or SPA III unsuccessful
Running	Sample currently being acquired
OK	Sample successfully acquired and analyzed with no QC messages
Partial	A tube belonging to the sample was successfully acquired or analyzed, while another tube was skipped
Incomplete	Not all tubes in a multitube panel were recorded
Skipped	All tubes belonging to the sample were skipped or aborted by the user
Needs Review	Results out of alarm range, or a QC message appears on the Lab Report
Reviewed	Sample marked as reviewed

To review a Lab Report, do the following.

1 Double-click the **Status** field for the sample that needs review.

The Lab Report for that sample appears.

Figure 6-4 Lab Report



- You can adjust gates, revert to auto-gates, or append a virtual signature, ie Review, to the report.

- For suggestions on addressing QC messages, see page 210.

2 Continue reviewing reports as needed.

Use  to navigate to other Lab Reports in the worklist.

3 Save the worklist.

Saving a worklist allows you to reanalyze data and review Lab Reports at a later time and use the worklist as a template.

Running Cleaning Tubes

1. Create a worklist.

   a. Enter *Clean* as the Sample ID.

   b. Select a two-tube panel (such as 4 color TBNK) from the **panel** field menu.

⚠ Each panel has default acquisition targets. A lab manager should set the time target (Max time for acquisition) for the two-tube panel to be used for the Clean to no less than 300 seconds (5 minutes).

2. Click the **Run** button.

3. When prompted to save the worklist, click **No**.

4. (Loader Only) Click **Ignore** to run tubes manually.

5. When prompted, install a tube with ≤3 mL of BD FACS cleaning solution or 10% bleach on the SIT. Firmly push up on the tube until it comes to a complete stop and is fully seated.

6. Click **OK**.

7. After 5 minutes, click the **End Recording** button.

   Do not click **Pause**.

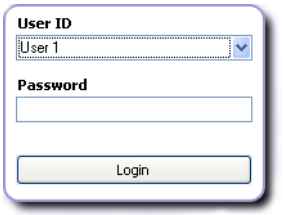
8. When prompted, unload the tube.

9. Install a tube with ≤3 mL of DI water. Firmly push up on the tube until it comes to a complete stop and is fully seated.

10. Repeat steps 6 through 8.

Logging Out

To stop using the software without shutting down the flow cytometer, click the **Logout** button to log out.



The next user can now log in.

7

Running Samples with BD FACSDiva Software

This chapter provides the following information on how to use BD FACSDiva software features to record and analyze sample data:

Optimizing with BD FACSDiva Software

To optimize cytometer settings in BD FACSDiva software:

1 Exit BD FACSCanto clinical software, if necessary.

   a Select **File > Exit**.

   b Select **Exit only** in the dialog, and click **OK**.

2 Start BD FACSDiva software, enter your user name and password, and click **OK**.

Verifying Cytometer Configuration and User Preferences

Do not change the cytometer configuration without consulting BD Biosciences. Doing so can invalidate results.

For accurate data results, the octagon and trigon arrays must match the current Cytometer Configuration.

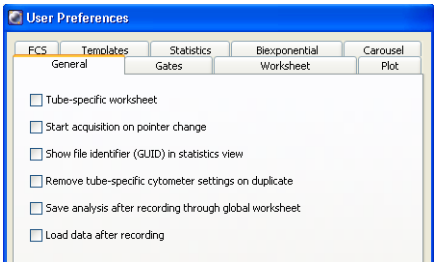
1 Select **Cytometer > View Configurations** and verify the current parameters.

   If changes are necessary, contact your Lab Manager.

2 Verify that the filters are appropriate to run FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, and APC-Cy7 fluorochromes.

3 Exit CS&T, then select **Edit > User Preferences** and clear all options in the **General** tab.

Figure 7-1 User Preferences General tab



4 Click **OK** to save the changes.

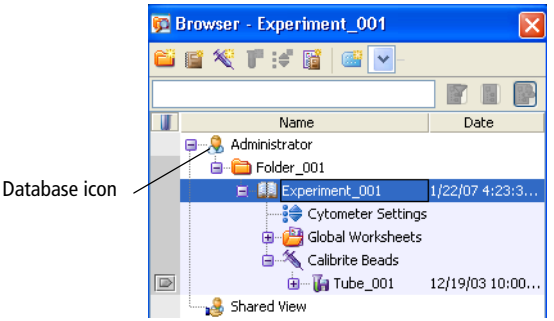
Creating the Experiment

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

2 (Optional) Create a folder:

a Select your database icon in the **Browser**, and click the **New Folder** button on the **Browser** toolbar.

b Rename the folder.

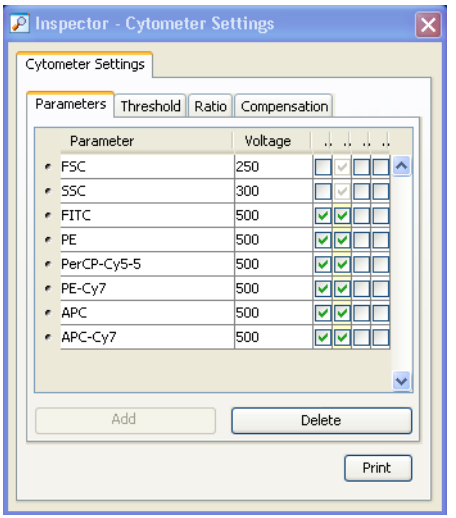


3 Select the folder and click the **New Experiment** button to create a new experiment.

An open experiment appears.

4 Rename the experiment.

5 Select the experiment-level cytometer settings, and click the **Parameters** tab in the **Inspector**.



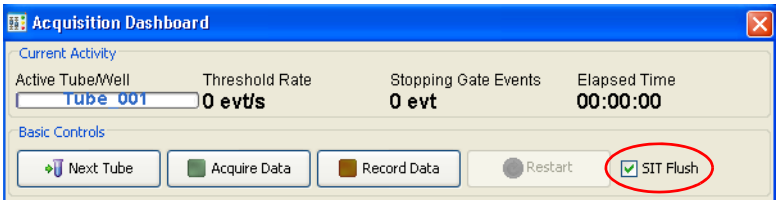
6 Change, add, or delete parameters as needed.

- To change, select a parameter, and select a new one from the menu.
- To add, click **Add**. A new line appears. Select it, and select a parameter from the menu.

  The Add button is enabled only when fewer than the maximum number of parameters are shown in the Inspector.
- To delete, click the selection button next to the parameter and click **Delete**.

7 In the **Acquisition Dashboard**, verify that the **SIT Flush** checkbox is selected.

We recommend leaving the SIT Flush option enabled to minimize carryover between tubes.



Applying the Setup Results

1 Right-click the experiment-level cytometer settings, and select **Link Setup**.

2 Select the setup that you want to link from the **Compensation Setup** dialog.

BD FACSCanto clinical software generated a Lyse/No-Wash and a Lyse/Wash setup.

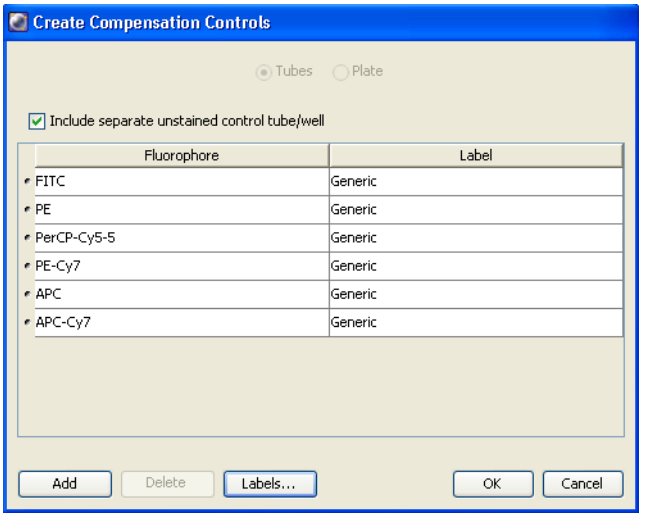
NOTE Entries in bolded text are setups created in BD FACSDiva software.

3 Click **Link**.

Creating Compensation Controls

1 Unlink the **Compensation Setup** if it has been previously linked.

2 Select **Experiment > Compensation Setup > Create Compensation Controls**.

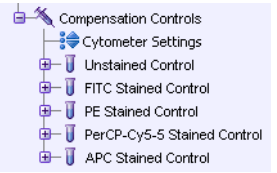


3 (Optional) Edit the labels associated with parameters.

Edit labels when your experiment contains samples stained with the same fluorophore conjugated to different antibodies (labels) that require different compensation values. Compensation differences are especially noticeable in tandem conjugates, due to lot-to-lot variation.

4 Click **OK**.

The software adds a compensation control, containing stained control tubes and one unstained control tube, to your experiment. Normal worksheets containing the appropriate plots are added for each compensation tube.



Optimizing Cytometer Settings

When you performed cytometer QC, voltage settings were adjusted to set each parameter at a target value. These settings might not be appropriate for the stained sample(s) you plan to analyze. Before recording data, adjust FSC, SSC, and threshold settings; gate on the population of interest (such as lymphocytes); and adjust voltages to optimize fluorescence signal.

For these adjustments, you will need an unstained control sample. It is important to perform these steps in order, since some adjustments influence others.


Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

Running the Unstained Control

1 Install the unstained control tube on the cytometer.

a Push the aspirator arm to the left.

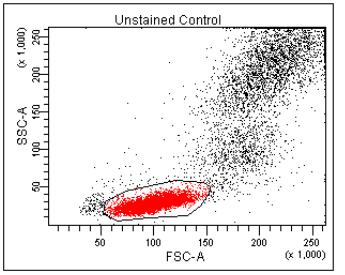
b Place the tube onto the SIT, ensure the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.

c Center the aspirator arm under the tube. There are three sensor pins on the aspirator arm. The bottom of the tube should sit within the center of the pins.

2 Verify that the green current tube pointer is set to the unstained control tube in the **Browser**. Click **Acquire Data**.

3 Adjust the FSC and SSC voltages to appropriately display the scatter properties of the unstained control.

Figure 7-2 Voltages adjusted



4 Click the **Threshold** tab and adjust the FSC Threshold, if needed.

Set the threshold to remove most of the debris without cutting off the lymphocyte population (Figure 7-2).

5 Adjust the P1 gate on the Unstained Control worksheet to surround only the lymphocyte population (Figure 7-2).

Select the gate by clicking on the boundary. Once you select the gate, you can drag to move it, or drag any of the selection handles to change the gate shape and size.

6 Once the gate is adjusted, right-click its boundary and select **Apply to All Compensation Controls**.

This applies your gate changes to the P1 gates on the remaining compensation worksheets.

7 Optimize the voltages by making sure that the negative populations for each fluorescence parameter are on scale, using the fluorescence histograms on the Unstained Control worksheet.

8 Click **Stop Acquiring** to stop acquisition.

9 When acquisition stops, remove the unstained control tube.

a Hold the sample tube while pushing the aspirator arm to the left.

b Remove the tube from the SIT.

c Release the aspirator arm.

SIT cleaning occurs when the aspirator arm comes to center.

d Wait for the **Progress** dialog to close before loading the next tube onto the SIT.


If you move the arm without holding the tube, the tube could fall off the SIT and expose you to potentially biohazardous sample.

NOTE To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 183.

Running the Stained Sample and Recording Data

1 Install the stained sample tube on the cytometer and click **Acquire Data**.

a Push the aspirator arm to the left.

b Place the tube onto the SIT, ensure the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.

c Center the aspirator arm under the tube. There are three sensor pins on the aspirator arm. The bottom of the tube should sit within the center of the pins, but not touch them. A correctly seated tube will be above the aspirator.

2 If necessary, decrease the voltages to place the positive population for each fluorescent parameter on scale.

3 Click **Stop Acquiring** to stop acquisition.

4 Remove the stained sample tube.

5 Re-install the unstained sample tube and click **Acquire Data**.

6 Wait 3 to 5 seconds, then click **Record Data** to record data for the unstained control tube.

7 When acquisition stops, remove the tube.

⚠ Do not change the PMT voltages after the first compensation tube has been recorded. To calculate compensation, all tubes must be recorded with the same PMT voltage settings. If you need to adjust the PMT voltage for a subsequent compensation tube, delete the current compensation specimen, recreate the specimen, and run all the compensation tubes again.

Calculating Compensation

For these adjustments, you will need a single-stained control sample for each parameter to be measured.

1 Install the first stained control tube onto the cytometer.

2 In the **Acquisition Dashboard**, click **Next Tube**.

3 Click **Acquire Data**.

4 Verify that the P1 gate encircles the population of interest, and click **Record**.

5 When recording is finished, remove the tube.

NOTE To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 183.

a Hold your sample tube while you push the aspirator arm to the left.

b Remove the tube from the SIT.

c Release the aspirator arm.

If you move the arm without holding the tube, the tube could fall off the SIT and expose you to potentially biohazardous sample.

6 Install the next tube onto the SIT.

7 Repeat steps 2 through 6 until data for all stained control tubes has been recorded.

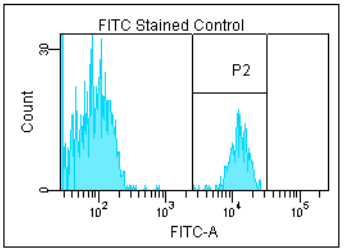
Adjusting Gates

1 Make sure the current tube pointer is set to the first stained control (FITC Stained Control) tube.

2 Double-click the FITC Stained Control tube in the **Browser** to locate the corresponding plots on the normal worksheet.

3 If needed, move the P2 gate to encompass the fluorescence-positive population.

Figure 7-3 Gating the positive population



4 Make sure the current tube pointer is set to the next stained control tube; double-click the tube in the **Browser** to locate the corresponding plots on the normal worksheet.

5 Repeat steps 3 and 4 for the remaining compensation tubes.

Creating a Compensation Matrix

1 Select **Experiment > Compensation Setup > Calculate Compensation**.

If the calculation is successful, a dialog appears prompting you for the name of the compensation setup.

2 Enter a name for the compensation setup, and select either **Link and Save** to link the setup to the experiment's cytometer settings, or **Apply Only**. Click **OK**.

NOTE We recommend that you confirm the compensation setup by running a process control before you run samples.

Sample Reagents

For the following experiment, data will be recorded and analyzed for two tubes of human peripheral blood stained with the following reagents:

- CD3 FITC
- CD16+CD56 PE
- CD45 PerCP-Cy5.5
- CD4 PE-Cy7
- CD19 APC
- CD8 APC-Cy7

For other analysis methods, see the information supplied with the reagent.

Setting Up the Global Worksheet

This section shows you how to use a global worksheet to preview and record data for multiple samples.

1. Open the experiment in which you optimized cytometer settings.

2. Create a new specimen by clicking the **New Specimen** button. Rename it *LWB*.

3. Create two tubes under the LWB specimen, and rename them, for example, *TBNK_001* and *TBNK_002*.

   To create a second tube, select the specimen, and click the **New Tube** button.

4. Create a global worksheet.

   - If the **Default global worksheet** preference is enabled in User Preferences (default option), the global worksheet is already present. Expand the Global Worksheets folder to locate and rename the worksheet.

   - If the **Default global worksheet** preference is disabled, create a global worksheet by clicking the **New Global Worksheet** button in the **Browser** toolbar.

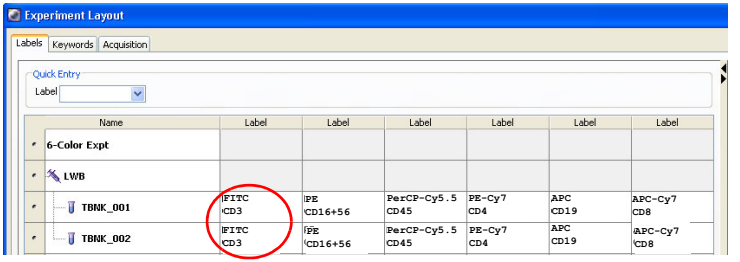
5. Rename the global worksheet *Record Data*.

6. Use the Experiment Layout to define parameter labels and to specify the number of events to record for each tube. Labels will appear on the plot axes and in all statistics views.

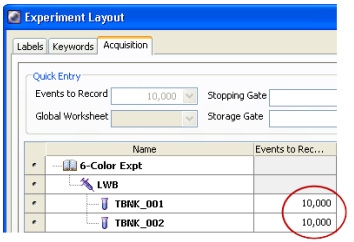
   a. Select **Experiment > Experiment Layout**.

   b. Under the **Labels** tab, enter appropriate labels for the tube. For example, enter CD3 in the **FITC** field. Use the Tab key to move to the next field (Figure 7-4).

Figure 7-4 Entering parameter labels



c Under the **Acquisition** tab, verify 10,000 events to record for tubes TBNK_001 and TBNK_002. Click **OK**.



7 On the global worksheet, create plots for previewing the data.

For example, create PerCP-Cy5.5 vs SSC, FITC vs APC, FITC vs PE, FITC vs PE-Cy7, and FITC vs APC-Cy7 dot plots.

Setting SPA User Preferences

Importing a Worklist from the SPA Software

You can import sample information from a worklist created in BD FACS™ Sample Prep Assistant (SPA) software. When you import a SPA worklist, the XML file contains defined keywords and elements used to populate an experiment. All of the keywords and elements are associated with the samples in

the imported SPA worklist (XML) file. In addition, the order of the keywords and elements within the experiment layout matches the order found in the worklist.

To import the worklist, all reagent and panel names must exactly match those used in BD FACSDiva software.

To import a SPA worklist:

1. (Optional) In Sample Prep Assistant software, print a report of the worklist you want to import into BD FACSDiva software.
2. Verify that tube and panel names from the SPA worklist exactly match those in the BD FACSDiva panel template.
3. If there is a discrepancy, create a new panel template in BD FACSDiva software to match the reagent panel in the SPA software.
4. Select **File > Import > Worklist**.
5. Navigate to and select a worklist, and click **Import**.

   NOTE If you need to edit and delete keywords and elements belonging to the imported worklist, open the worklist before importing and review the current information. Edit missing or incorrect entries, as needed, and save.

   BD FACSDiva software creates a locked experiment that includes all specimens prepared in the SPA worklist.
6. Following the import, select a specimen and open the **Inspector** window to review the imported information.

Editing an Imported SPA Worklist

The Edit > User Preferences - General tab option, Allow editing of imported SPA worklists, is enabled by default.

You can edit and delete some of the keywords and elements belonging to the imported worklist.

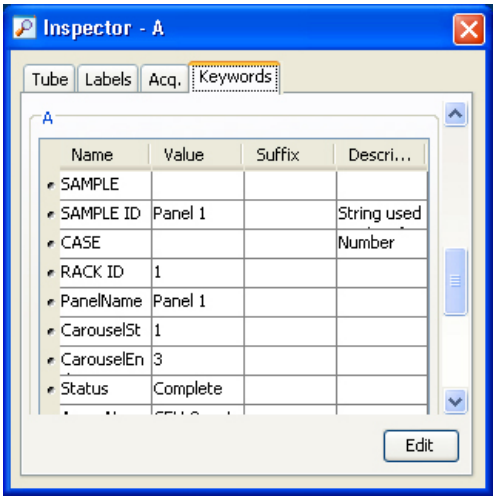
1 Select a specimen.

2 Open the **Inspector** window.

3 Click the **Keywords** tab.

Keywords that are associated with the SPA worklist are shown, based on the imported XML file.

NOTE The following keywords cannot be edited or deleted: SAMPLE NAME, SAMPLE ID, CASE NUMBER, PANEL NAME, PATIENT ID.



4 Select an item, change the information as necessary, and click **Edit**.

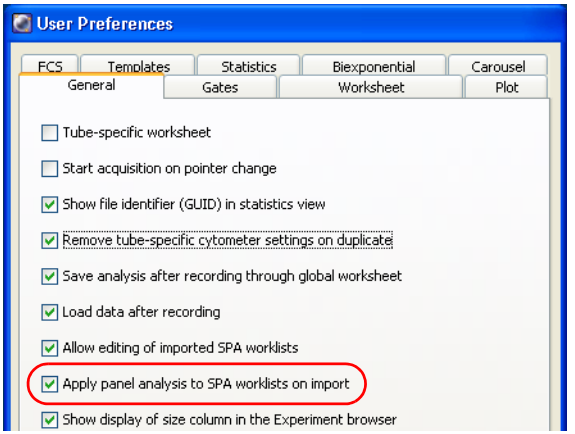
Applying Panel Analysis to SPA Worklists on Import

A panel is a collection of tests, reagents, or markers commonly used together in the same experiment. A panel template is used to analyze the experiment data. The panel template applies changes to the normal worksheet elements of a selected specimen. When you import a SPA worklist, you have the option to manually or automatically apply a panel template to specimens in an experiment.

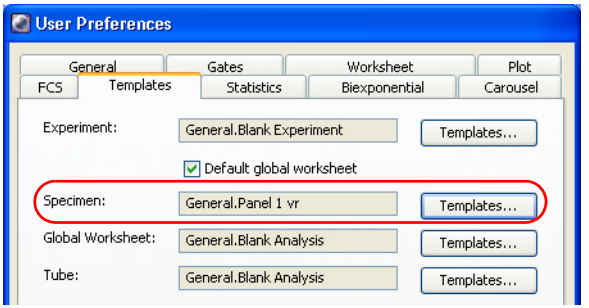
The Apply panel analysis to SPA worklists on import option is enabled by default. A panel template is automatically applied to the experiment. When this option is cleared and you import a SPA worklist, the Panel Templates dialog appears for you to select a panel to import.

1 Select **Edit > User Preferences - General** tab.

2 Confirm that **Apply panel analysis to SPA worklists on import** is selected.



3 Click the **Templates** tab.



4 Click the **Templates** button for the specimen.

The Panel Templates dialog opens.

5 Select a panel to import and use as the default template, and click **OK**.

The changes are saved with your login name and are retained from one session to the next.

Recording Data

If you want to save a copy of the data displayed in the global worksheet with each recorded tube, enable the **Save analysis after recording through global worksheet** preference in the **User Preferences** window (Figure 7-1 on page 103). If you do not want to save a copy of the data, leave the preference disabled.


Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

1 Install the first sample tube onto the cytometer.

   a Push the aspirator arm to the left.

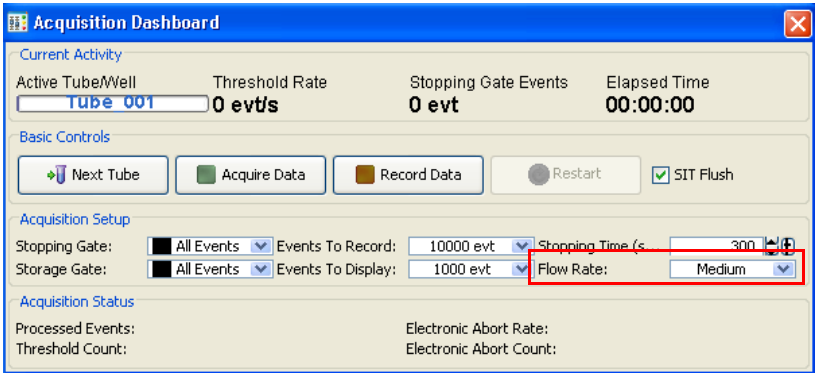
   b Place the tube on the SIT, ensure the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.

   c Center the aspirator arm under the tube. There are three sensor pins on the aspirator arm. The bottom of the tube should sit within the center of the pins, but not touch them. A correctly seated tube will be above the aspirator.

2 In **Acquisition Setup**, verify that you are using an appropriate flow rate and that the **SIT Flush** checkbox is selected.

- Hide or show acquisition setup information by right-clicking the Dashboard anywhere outside the **Basic Controls** area and selecting the option from the menu.

We recommend leaving the SIT Flush option enabled to minimize carryover between tubes.



- Low = approximately 10 µL/min of sample
- Medium = approximately 60 µL/min of sample
- High = approximately 120 µL/min of sample

3 Move the current tube pointer to the first tube. Click **Acquire Data**.

4 While data is being acquired, draw a gate around the lymphocytes. Set the other plots to show data from the lymphocyte population.

5 Click **Record Data**.

6 When all events have been recorded, unload the sample tube when the aspirator arm lowers.

a When the aspirator arm lowers, hold your sample tube while you push the aspirator arm to the left.

b Remove the tube from the SIT.

c Release the aspirator arm.

If you move the arm without holding the tube, the tube could fall off the SIT and expose you to potentially biohazardous sample.

NOTE If the tube is not removed within approximately 30 seconds, the aspirator arm will lock against the tube. You will be prompted to either continue acquiring or remove the tube.

7 When the SIT flush is complete, load the next tube.

8 Click **Next Tube**, and then **Acquire Data**.

9 Preview the data in the global worksheet. Click **Record Data**.

10 Repeat steps 6 through 9 until data has been recorded for all tubes.

(Optional) To print the experiment-level cytometer settings or the Cytometer Status report, right-click the cytometer settings icon and select **Print**.

Analyzing Data

1 Create a new global worksheet. Rename it *TBNK Analysis*.

2 Select the first tube under the LWB specimen and create the following dot plots: PerCP-Cy5.5 vs SSC, FITC vs APC, FITC vs PE, FITC vs PE-Cy7, and FITC vs APC-Cy7.

3 Resize the plots so they fit on one page.

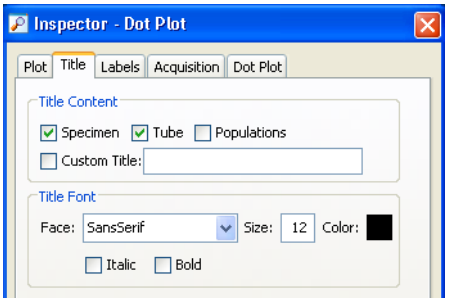
By default, the plots will fit on one page if you select three across.

4 On the PerCP-Cy5.5 vs SSC plot, draw a gate around the lymphocytes. Use the population hierarchy to rename the population *Lymphocytes*.

5 Select all plots except the PerCP-Cy5.5 vs SSC plot and specify to show only the Lymphocyte population.

Hold down the Control key while you select successive plots. Once all plots are selected, select the checkbox next to Lymphocytes in the **Plot Inspector**.

6 Select all plots and click the **Title** tab in the **Plot Inspector**. Select the checkboxes to display the tube and specimen names in the plot titles.



7 Create a statistics view. Edit the view to show the Lymphocyte population and subpopulations, and to display the mean for all fluorochromes.

To create a statistics view, right-click any plot and select **Create Statistics View**. The resulting statistics view lists the number of events, %Parent, and means of the plot parameters for all populations displayed in the plot.

8 Draw a gate around the CD19 positive population on the CD3 FITC vs CD19 APC plot. Name the population *B Cells*.

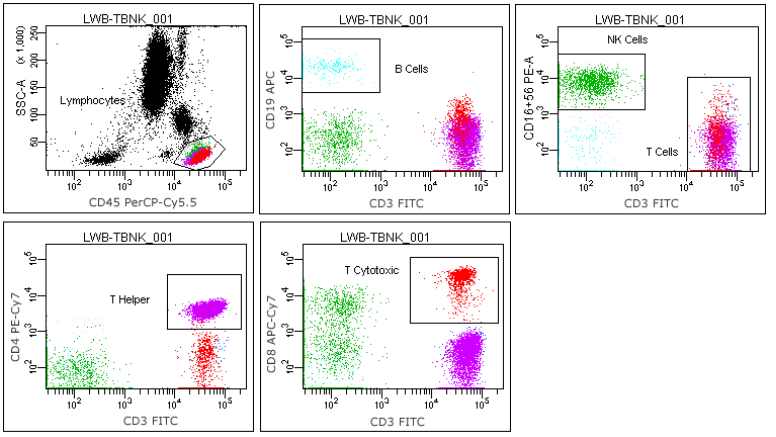
9 Draw a gate around the CD16+56 positive population on the CD3 FITC vs CD16+56 PE plot. Name the population *NK Cells*.

10 Draw a gate around the double-positive population on the CD3 FITC vs CD4 PE-Cy7 plot. Name the population *T Helper*.

11 Draw a gate around the double-positive population on the CD3 FITC vs CD8 APC-Cy7 plot. Name the population *T Cytotoxic*.

12 Print the analysis.

Figure 7-5 Lymphocyte analysis (example)



Tube: TBNK_001

Population	#Events	%Parent	%Total
All Events	30,000		100.0
Lymphocytes	8,535	28.4	28.4
B Cells	374	4.4	1.2
NK Cells	2,025	23.7	6.8
T Cells	6,039	70.8	20.1
T Helper	4,639	54.4	15.5
T Cytotoxic	1,312	15.4	4.4

Experiment Name: Immunophenotype
Specimen Name: LWB
Tube Name: TBNK_001
Record Date: Aug 9, 2004 5:39:18 PM
$OP: Administrator

			FSC-H	SSC-A	CD3 FITC	CD16+56 PE	CD45 PerCP-Cy5.5	CD4 PE-Cy7	CD19 APC	CD8 APC-C
Population	#Events	%Parent	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Lymphocytes	8,535	28.4	87,756	22,800	29,539	2,036	5,938	1,096	35,638	2,415
B Cells	374	4.4	85,999	21,558	27,181	17	5	22,434	73	27
NK Cells	2,025	23.7	100,881	29,823	29,666	8,399	3,014	59	93	14
T Cells	6,039	70.8	82,899	20,344	29,733	54	7,361	139	50,320	3,392
T Helper	4,639	54.4	81,702	19,264	28,556	-3	363	160	52,514	4,414
T Cytotoxic	1,312	15.4	86,586	23,853	33,525	216	33,069	79	41,671	75

Reusing the Analysis

Now that the analysis strategy has been defined, use it to analyze the remaining tubes in the experiment. Global worksheets allow you to apply an analysis strategy to a series of data files without saving the analysis each time. After previewing the data, you can print the analysis or save it to a tube-specific (normal) worksheet.

1 Move the current tube pointer to the next tube under the LWB specimen.

2 View the data on the global worksheet. Make adjustments to gates, as needed.

Adjustments will also apply to the next tube viewed on the global worksheet. If you don't want to alter the global worksheet, save the analysis as described in the next section and make adjustments on the tube's normal worksheet.

Saving the Analysis

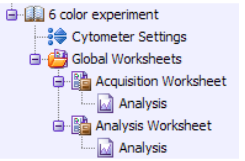
Since the analysis objects were created on a global worksheet, the analysis will not be saved with each tube. If you want to save a copy of the analysis with any tube, do the following.

1 Expand the TBNK Analysis global worksheet in the **Browser**.

2 On the **Worksheet** toolbar, click the **Global Worksheets** button to switch to the normal worksheet view.

3 Create a new normal worksheet for the destination tube. Rename the worksheet.

4 In the **Browser**, right-click the analysis object below the global worksheet icon and select **Copy**.



5 Select the tube in the **Browser**. Right-click the tube icon and select **Paste**.

The elements on the global worksheet are copied to the new normal worksheet. View the analysis by double-clicking the tube in the **Browser**.

NOTE Automatically save a copy of the analysis with each tube by enabling the **Save Analysis After Recording** preference before you record data. In this case, the analysis plots are placed on the normal worksheet that is open at the time of recording. To control where the plots are placed, create a new normal (tube-specific) worksheet before data is recorded.

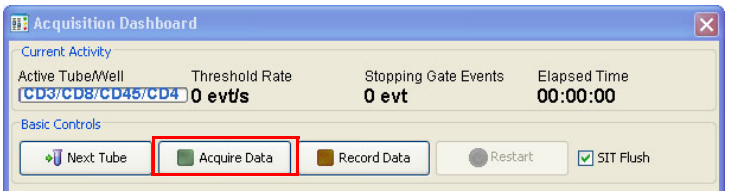
Running Cleaning Tubes

Use this procedure when cleaning the cytometer manually (without the Loader).

NOTE When the Loader is installed, use the alternate procedure for Running Cleaning Tubes on the Loader on page 143.

Install a tube with ≤3 mL of BD FACS cleaning solution or 10% bleach on the SIT. Firmly push up on the tube until it comes to a complete stop.

1 Under **Basic Controls**, click **Acquire Data**.



2 Watch the time in the **Elapsed Time** field.

3 After 300 seconds (5 minutes), click **Stop Acquiring** and remove the tube.

4 Install a tube with ≤3 mL of DI water on the SIT. Firmly push up on the tube until it comes to a complete stop and is fully seated.

5 Repeat steps 1 and 3.

Logging Out

Log out when you are finished using BD FACSDiva software, but are not ready to shut down the system.

1 Select **File > Log Out**.

2 If the system will be used to run samples after you log out, select **Log out only**.

NOTE If you select Log out after a fluidics shutdown, keep in mind that fluidics shutdown can take up to 5 minutes to complete.

The BD FACSDiva workspace is hidden and the Log In dialog appears. The system is available for the next operator to log in.

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8

Using the Loader with BD FACSDiva Software

This chapter provides the following information about using the BD FACS Loader with BD FACSDiva software:

Getting Ready

1. Start up the cytometer, workstation, and software.

2. Perform cytometer quality control and sample optimization.

3. (Optional) Open a new experiment, and copy your optimized cytometer settings from step 2 to the new experiment.

   Alternatively, you can work with the experiment used during sample optimization and add new specimens to that.

   NOTE You must use a global worksheet for data display when acquiring samples with the Loader.

4. Add specimens and tubes to your experiment (see Figure 8-1 on page 129).

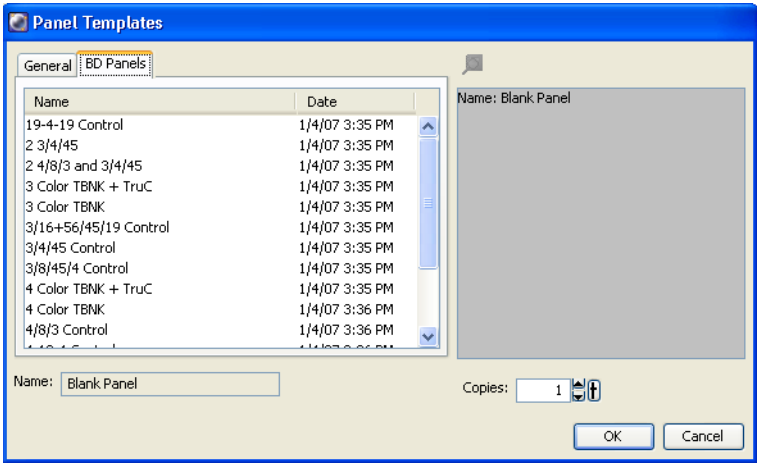
   To add a predefined panel:

   a. Select any **Browser** item within the open experiment, and select **Experiment > New Specimen**.

   b. Click the **BD Panels** tab and select a panel.

   c. Specify the number of copies (each copy creates a new specimen in the **Browser**), and click **OK**.

Figure 8-1 Panel Templates dialog



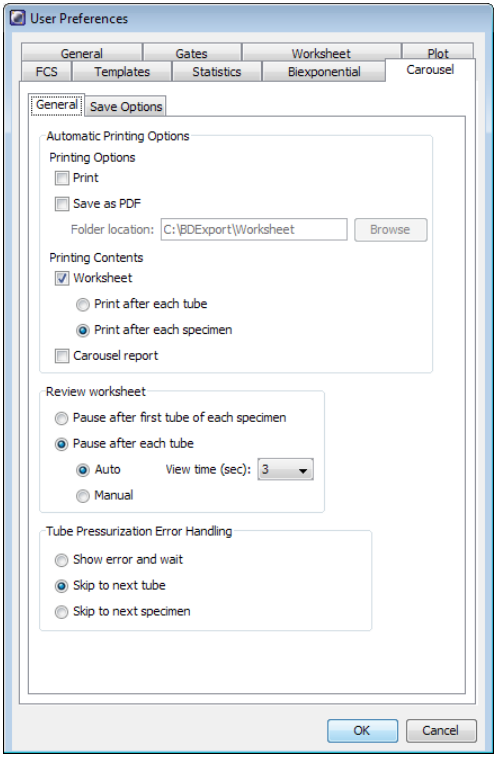
5 If you imported panels, inspect the information and the cytometer settings for each panel type to make sure they are complete and appropriate.

Assigning Carousels and Verifying Run Settings

1 Select **Edit > User Preferences.**

2 Click the **Carousel tab,** then click the **General** tab.

3 Make selections for printing and pausing.

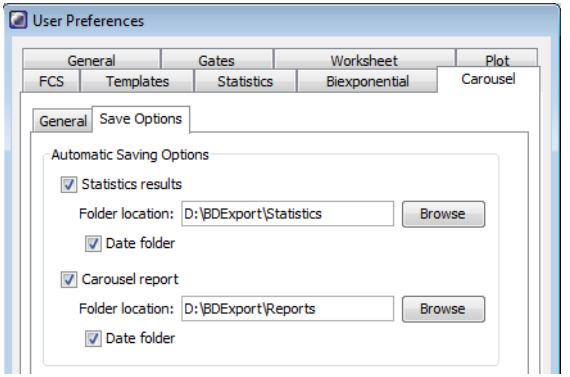


4 Select how you want the Loader to handle any tube pressurization errors that occur during the run.

Tube Pressurization Error Handling Options	Function
Show error and wait	Stops the run and waits for you to select whether to abort the run, skip the tube, or skip to the next specimen
Skip to next tube	Automatically moves to the next tube if an error is encountered
Skip to next specimen	Automatically moves to the next specimen if an error is encountered

NOTE To make changes to the Tube Pressurization Error Handling options for the current run only, use the Carousel Setup window. See Figure 8-2 on page 132.

5 Click the **Carousel** tab and then click the **Save Options** tab, and specify whether and where to automatically save the statistics results and Carousel Report.



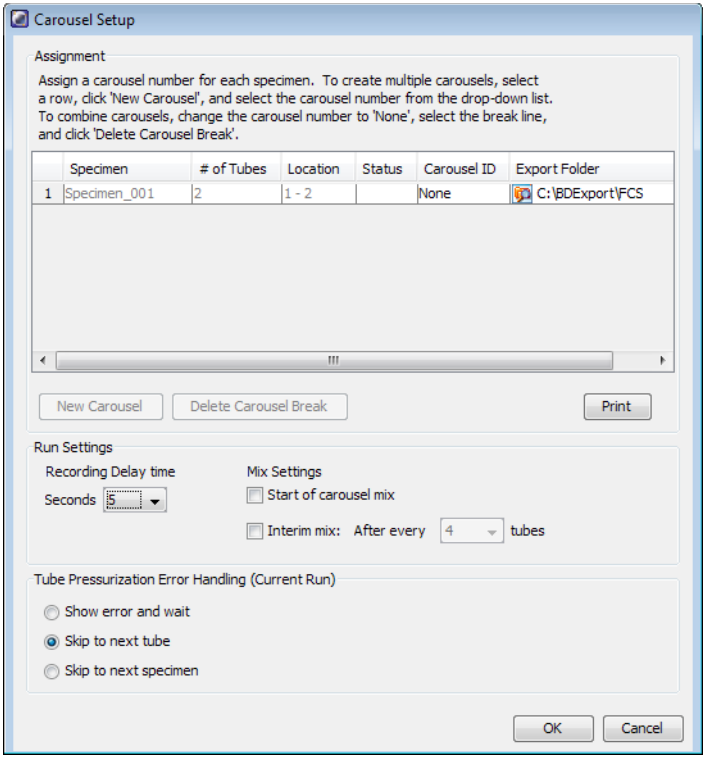
6 Click **OK** to save changes.

7 Select **Carousel > Carousel Setup**.

All specimens or panels in the experiment are listed at the top of the Carousel Setup window, in a worklist format. Carousel locations are automatically assigned.

NOTE Specimens to be run using the carousel must have 40 tubes or fewer.

Figure 8-2 Carousel Setup window



8 (Optional) Specify a folder to export the FCS data from a carousel run.

Click the folder in the **Export Folder** column and select a directory. D:\BDExport\FCS is the default folder.

9 (Optional) Insert or delete carousel breaks.

Carousel breaks, represented by a thick horizontal line, indicate the start of a new carousel. The software automatically inserts them whenever a carousel cannot contain the next specimen's tubes. To change carousel assignments for tubes, insert and delete breaks.

You can insert or delete breaks only on an unassigned carousel (the carousel ID is None).

- To insert, select a row, click **New Carousel**, and select an ID number from the list.
- To delete, select **None** from the **Carousel ID** list, select the break line, and click **Delete Carousel Break**.

10 Click in the **Carousel ID** field for each specimen and assign a carousel ID from the list to each specimen.

11 Click **Print** to print a record of tube and carousel assignments, as well as the FCS directory selected for each specimen.

Use the printout as a guide when filling the carousels.

12 Verify the Run Settings in the **Carousel Setup** window.

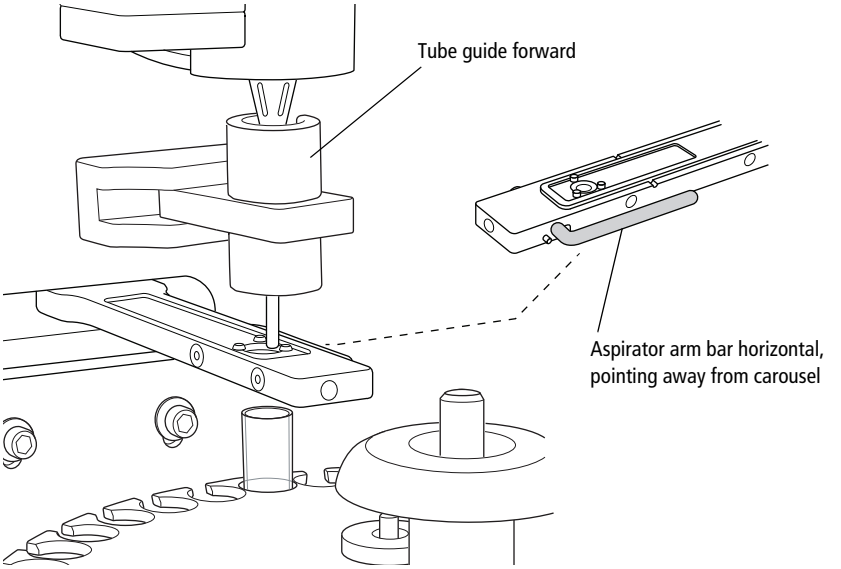
Run Settings Options	Range	Function
Recording Delay time	3–15 seconds	Allows acquisition to stabilize before recording begins
Start of carousel mix		Performs a 10-second mix when the run begins
Interim mix	After every 1–20 tubes	Performs a mix after a specified number of tubes
Mix duration	3–25 seconds	Allows you to set the duration of the interim mix

13 Click **OK** to save changes.

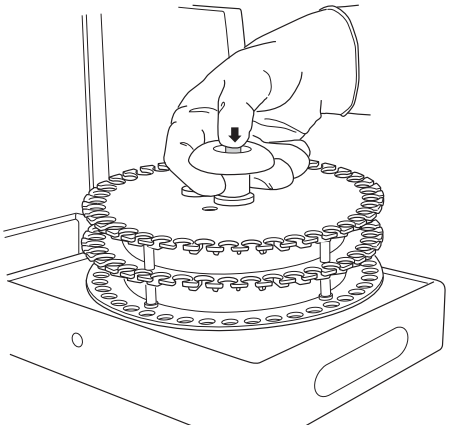
Preparing the Loader

1 Set up the cytometer for automatic loading.

Figure 8-3 Automatic loading



2 Gently remove the carousel from the cytometer as shown.



3 Vortex the sample tubes and place them uncapped in the carousel(s) according to the worklist.

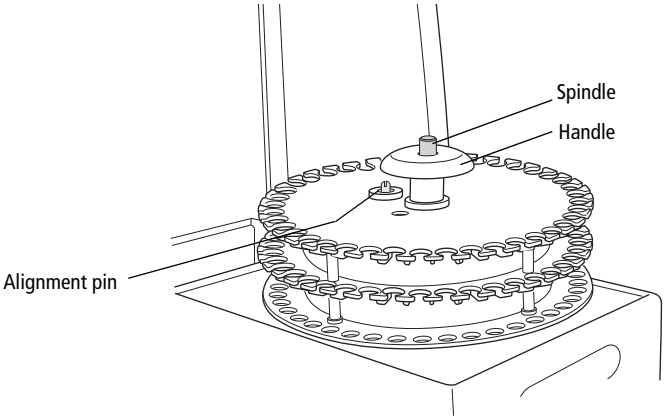
For accurate results, match the tubes to those listed on the printed worklist or Carousel Setup (BD FACSDiva software).

To prevent binding during loading, make sure that each tube has a maximum of two labels. Flatten labels completely before placing a tube in the carousel.

4 Install the first carousel to be acquired on the Loader.

Position the spindle hole in the handle over the center spindle of the Loader drawer. Rotate the carousel until the alignment guide pin fits into the small alignment hole at the top of the carousel. Press down firmly to seat the carousel.

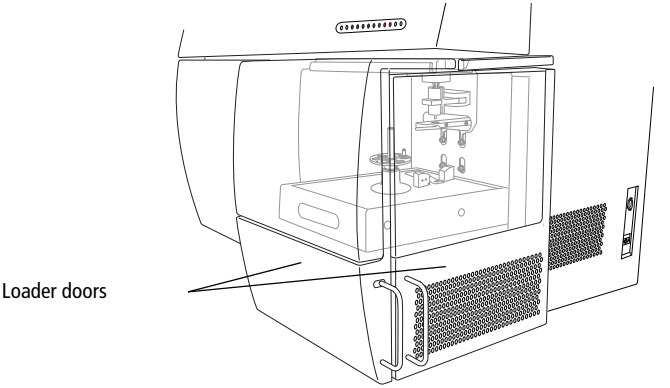
Figure 8-4 Carousel components



5 Close the Loader drawer completely, and close the Loader doors.

The Loader scans and positions the carousel at tube 1.

⚠ To run the Loader, the doors must be closed. Tubes will not load with the doors open. The currently running tube will be unloaded if you open the doors during a run.



Running Samples

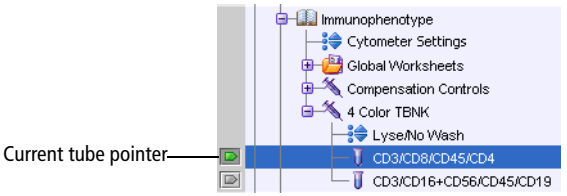
Before the Run

If the stopping time is 0, the software uses a default stopping gate based on number of events. When you enter a time, the software stops either when it reaches the total number of events, or when it reaches the time limit, whichever comes first.

NOTE We recommend that you always enter a stopping time. Setting a time limit ensures that the carousel doesn't stall on one tube if that tube should contain a dilute sample or rare events.

To run samples:

1 To activate the acquisition controls, move the current tube pointer to the first tube in your experiment.

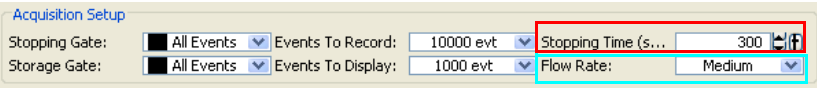


2 In **Acquisition Setup** (Figure 8-5):

a Verify that you are using an appropriate flow rate.

b Enter a stopping time (seconds).

Figure 8-5 Acquisition Setup in Acquisition Dashboard



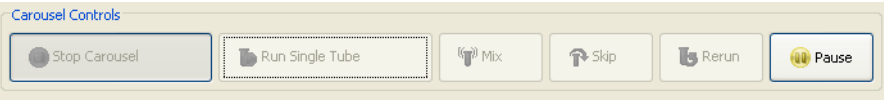
3 In **Carousel Controls**, click **Run Carousel**.

NOTE If the Carousel Controls are not displayed on the dashboard, right-click on the dashboard and select **Carousel Controls**.

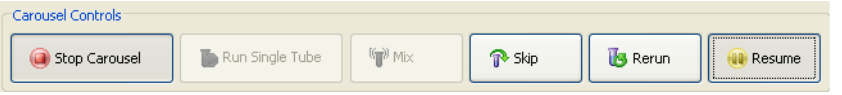
4 View data for the first tube on the global worksheet.

The ID of the currently running carousel, along with the tube's position, is displayed in Carousel Controls.

5 If necessary, click **Pause** and make adjustments to cytometer settings or the analysis.



6 Click **Resume** to proceed with acquisition of the next tube.



During the Run

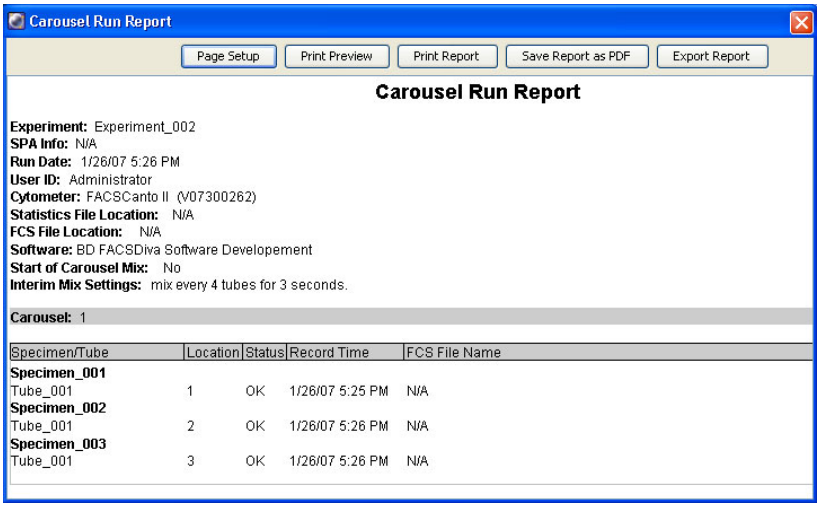
Data for the current tube is displayed in the global worksheet view for the amount of time specified in General Carousel preferences. Any changes to the global worksheet remain in effect for the rest of the tubes that use the same worksheet.

Tube processing continues until the carousel run is complete. Repeat step 4 through step 6 in the preceding section for the remaining tubes on the carousel.

To run more than one carousel:

1. Remove the carousel.
2. Load the next carousel when prompted. Click **Continue**.
3. When done, review the Carousel Run Report. Any tube errors that occurred during the run are highlighted.

Figure 8-6 Example Carousel Run Report



After the Run

The report prints automatically if specified under the General tab of the Carousel tab under User Preferences. If you selected automatic saving under the Save Options tab of the Carousel tab, the report is saved to the designated directory. You can also save the report as a PDF or export it.

Skipping or Re-Running Samples

When you re-run a sample, existing data is not overwritten if you have enabled one of the following:

- If the Export FCS after recording preference is enabled, a new FCS file is saved as: <*Specimen Name*>_<*TubeName*>_<*DDMMYYYYHHMMSS*>.fcs, where *DDMMYYYY* represents the date and *HHMMSS* represents the time the new file was saved. If the exported file is from an experiment created from an imported SPA worklist, the keyword PANEL NAME and its value are written to the FCS file header.

- If the *Automatic Saving* preference is enabled for statistics results, a new row is written to the statistics results file.

Skipping or Re-running Tubes or Specimens During a Run

To skip a tube or specimen:

1 Click **Skip** in **Carousel Controls**.

2 In the dialog that appears, select **tube** or **specimen**.

The carousel moves to the next tube or specimen.

To re-run a tube or specimen:

1 Click **Rerun** in **Carousel Controls**.

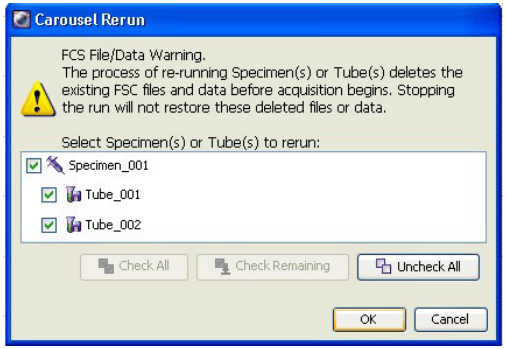
2 In the dialog that appears, select either **tube** or **specimen**.

The carousel moves to re-run the selected tube or specimen.

Re-Running Tubes or Specimens After Acquisition

1 Click **Run Carousel** in **Carousel Controls**.

2 In the re-run dialog that appears, select the tubes or specimens you want to re-run. Click **OK** to close the dialog.



NOTE When you re-run a sample, existing data is stored only if the Export FCS after recording preference is enabled.

Adding Tubes to an Existing Carousel

1 In the current experiment, add a specimen.

2 Select **Carousel > Carousel Setup**.

3 Delete the Carousel break.

The Carousel ID is automatically assigned to the new specimen in the worklist.

4 Click **Run Carousel** in **Carousel Controls**.

5 In the re-run dialog that opens, select the new tubes or specimen. Click **OK** to close the dialog.

Re-Running a Carousel

1 After all tubes in the carousel are recorded, click **Run Carousel** in **Carousel Controls**.

2 In the dialog that appears, select all tubes in the carousel. Click **OK** to close the dialog.

The run begins.

Stopping a Run

To stop a carousel, click **Stop Carousel** in **Carousel Controls**. The run stops and the Carousel Report appears.

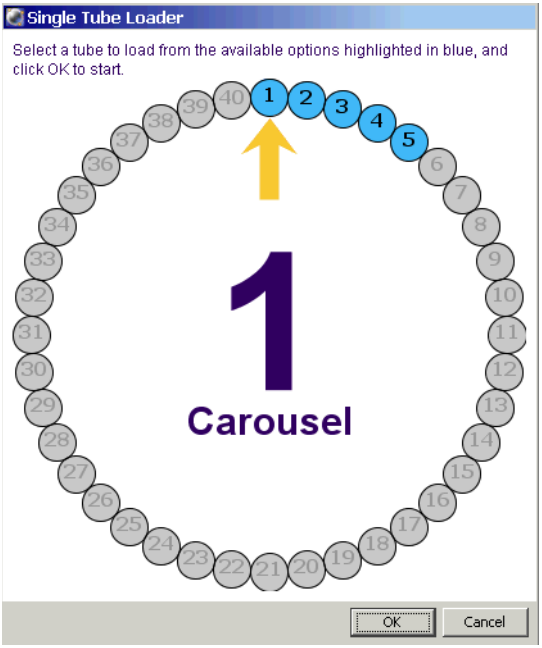
Running a Single Tube

1 To run a single tube using the carousel, click **Run Single Tube** in **Carousel Controls**.

2 In the dialog that appears, select the tube you want to run. Click **OK** to close the dialog and begin the run.

See Figure 8-7 on page 143.

Figure 8-7 Single Tube Loader dialog

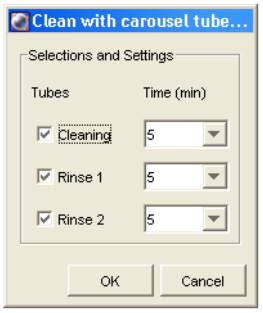


3 When the tube is finished, click **Unload** in the **Acquisition Dashboard**.

Running Cleaning Tubes on the Loader

1 In **Acquisition Setup**, check that flow rate is set to **Medium**.

2 Select **Carousel > Clean**. Select the tube type and number of minutes to run each tube.



3 Click **OK**.

4 Move the current tube pointer to the first cleaning tube.

5 Place tubes filled with the appropriate solution in the designated positions in the cleaning rack.

We recommend using at least BD FACS cleaning solution and DI water.

6 Click **OK** to begin cleaning.

9

Shutting Down

This chapter provides the following information for shutting down your application software and instrument:

About Shutdown

To prevent fluid overflow, do not leave a tube on the SIT during fluidics shutdown.

- Turn off the cytometer when not in use to preserve laser life.
- At the end of the day, shut down the cytometer after running a fluidics shutdown.
- After every 4 hours of operation, and during cytometer shutdown, you will hear a hiss as condensed water discharges from the fluidics cart pumps into the condensation trap.

Shutting Down BD FACSCanto Clinical Software

1. Select **File > Exit**, and select **Run fluidics shutdown and exit.**
2. When fluidics shutdown completes, the software closes.
3. Turn off the cytometer power.
4. Shut down the computer.

Shutting Down BD FACSDiva Software

1. Select **Cytometer > Fluidics Shutdown**, and then follow all prompts.
2. Turn off the cytometer power.
3. Exit BD FACSDiva software.
4. In the dialog that opens, verify that **Exit application only** is selected and click **OK**.

5 Shut down the computer.

Cleaning the Surfaces

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

To prevent damage, do not use isopropyl alcohol or ethanol on any cytometer or fluidics cart surface.

Following shutdown:

1 Wet a soft, lint-free cloth with DI water and gently wipe down the following surfaces daily to remove salt buildup:

- SIT

  Be careful not to bend the SIT.

- Aspirator arm

  Wipe all exposed surfaces of the aspirator arm that are outside the cytometer chassis.

2 Use a dry lint-free cloth to remove residual moisture.

3 Place an empty tube on the SIT to catch any BD shutdown solution that might drip from the SIT.

4 Empty the condensation trap located underneath the fluidics cart power panel. See Figure 1-7 on page 27.

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10

Maintenance

This chapter describes the following maintenance procedures:

Scheduled Maintenance

For optimal cytometer functioning, perform the following maintenance according to the recommended schedule. Not following the scheduled maintenance procedures as listed can result in incorrect results or data not being detected.

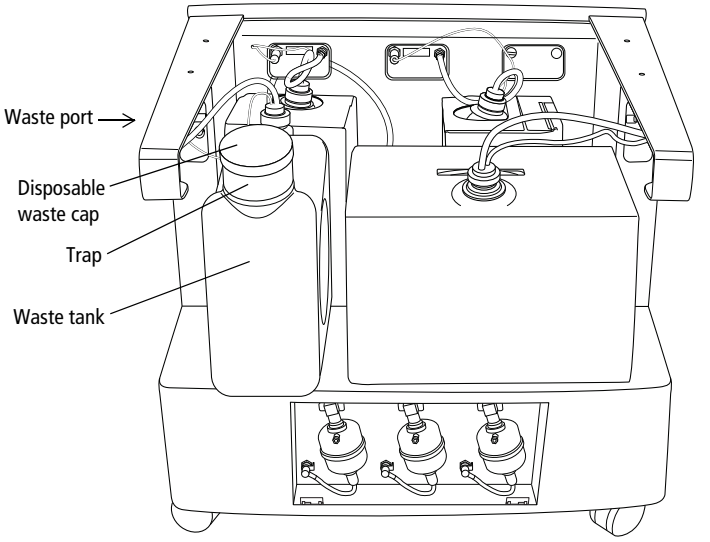
Procedure	Description	Frequency
Fluidics startup	Replaces BD FACS shutdown solution with BD FACSFlow solution See Chapter 4	Daily
Fluidics shutdown	Replaces BD FACSFlow solution with BD FACS shutdown solution See Chapter 9	Daily
Empty the waste	—	Daily and as needed
Wipe down the SIT and aspirator arm	Prevents saline deposit buildup See Chapter 9	Daily
Empty the condensation trap on the fluidics cart	Prevents overflow of the condensation trap See Chapter 9	Daily
Purge fluid filters	Removes air from fluid filters, ensuring that they will not dry out	Weekly
Change the waste container cap	—	Monthly
Clean fluidics (Long Clean)	Cleans the internal sheath path with BD FACS cleaning solution, then rinses with BD FACS shutdown solution	Monthly and before service calls

Procedure	Description	Frequency
Replace the air filter	Ensures proper cytometer performance	Every 6 months
Replace fluid filters	Keeps fluids free of particulates	Every 6 months or when increased debris is observed in FSC vs SSC plots
Schedule preventative maintenance by BD service	—	Every 6 months

Emptying the Waste Container

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

Figure 10-1 Location of waste port on fluidics cart



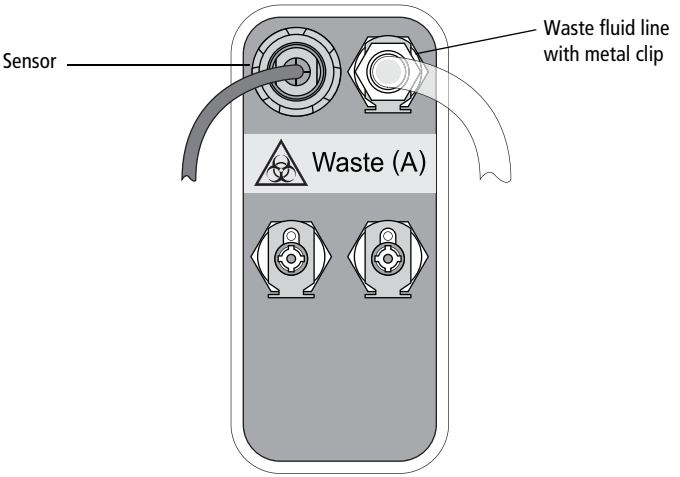
1 Ensure that the cytometer is not acquiring events.

2 Detach the waste container's sensor line and fluid line from the fluidics cart waste port (Figure 10-2 on page 153).

a Pull the sensor straight out.

b Press the metal clip on the fluid line.

Figure 10-2 Closeup of waste port



The waste container can become pressurized when the cytometer is running. Always disconnect the container from the fluidics cart before you empty it. Wait at least 30 seconds for pressure to dissipate before you remove the waste cap or level sensor cap.

3 Remove the disposable waste cap and attached trap from the container. Place the assembly on the bench label-side up.

Do not wet the cap. If you see liquid inside the trap, treat it as biohazardous waste. Remove the drain plug and fully drain the liquid. Rinse the trap and drain plug with 10% bleach, followed by DI water. Replace the drain plug.

4 Empty the bleach-exposed waste.

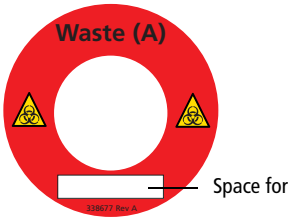
5 Add approximately 1 L of bleach to the empty waste container (10-L container).

6 If one month has passed since the last cap change:

a Detach the cap from the trap.

b Replace it with a new cap.

c Write the date on the cap label.



7 Screw the cap assembly onto the waste container and hand-tighten until it is fully closed.

To prevent waste container overpressurization, do not overtighten the trap or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants such as Teflon® tape or other adhesives.

8 Re-attach the sensor line and fluid line.

Purging the Fluidics Filters

NOTE The cytometer must be on (system pressurized) during this procedure.

1 Prime the fluidics lines.

a Select **Cytometer > Cleaning Modes > Prime After Tank Refill**.

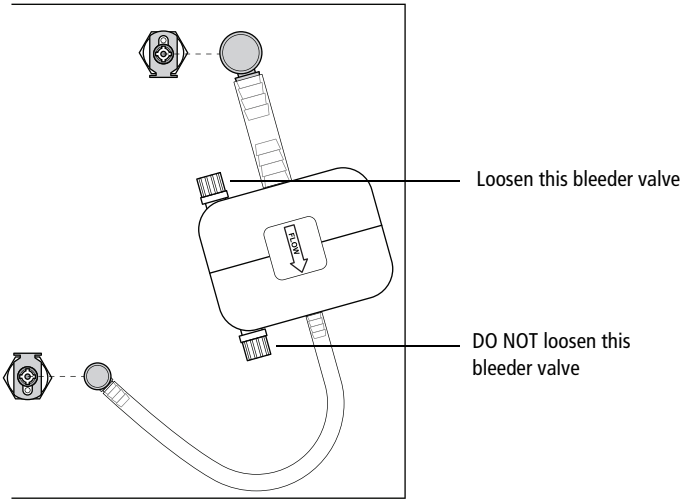
b Select all the checkboxes for the cubitainers.

c When the completion message appears, click **OK**.

2 Place a few paper towels beneath the filter to be purged.

3 Loosen the bleeder valve near the top of the filter by turning it counterclockwise. Do not completely unscrew the valve. If you do, it will come off.

NOTE Do not loosen the bottom bleeder valve. Ensure that it is tightened.



4 Wait until fluid seeps out.

Fluid should seep from the open valve within 30 seconds. If no fluid appears, make sure that the corresponding cubitainer is not empty or detached from the cart. If the cubitainer contains fluid and the fluid lines are attached and primed, the filter might be airlocked. See Removing an Air Lock on page 164.

5 Close the bleeder valve by turning it clockwise.

6 Repeat steps 2 through 5 with the remaining filters.

Cleaning the Fluidics System (Long Clean)

This procedure takes about 75 minutes to complete.

1 Check all fluid levels to make sure they are at least half full in order to complete the long clean. Fluid levels include FACSClean and Shutdown. Empty the waste if needed.

2 Select **Cytometer > Cleaning Modes > Long Clean**.

A confirmation dialog appears.

3 Click **OK** to continue. Once you have begun the Long Clean, you cannot cancel the process.

4 Click **OK** when the completion message appears.

⚠ If the completion message has not appeared after 90 minutes, verify that there are no error messages in the Status tab of the Cytometer window. If the cleaning mode fails, see Fluidics Cart Troubleshooting on page 195.

5 Select to shut down or continue.

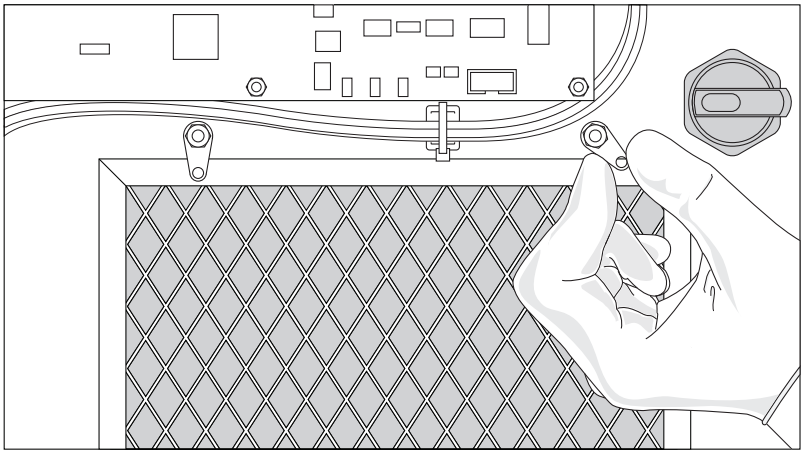
- To shut down, exit the software and turn off the power to the cytometer.
- To continue, select **Cytometer > Fluidics Startup**.

If setup fails after a long clean, repeat Fluidics Startup until setup passes.

Replacing the Air Filter

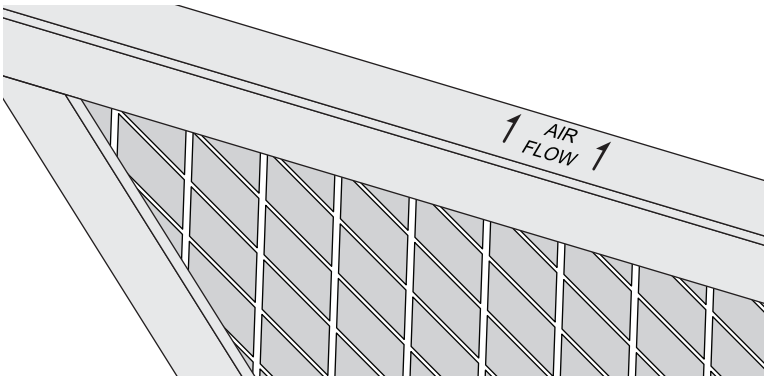
1 Turn off the cytometer power.

2 On the door's interior, turn the tabs located along the upper edge of the filter to release the old filter.



Dispose of the old filter. It cannot be reused.

3 Install a new filter. Ensure that the arrows etched on edges of the new filter point in toward the cytometer.



4 Turn the tabs to hold the filter.

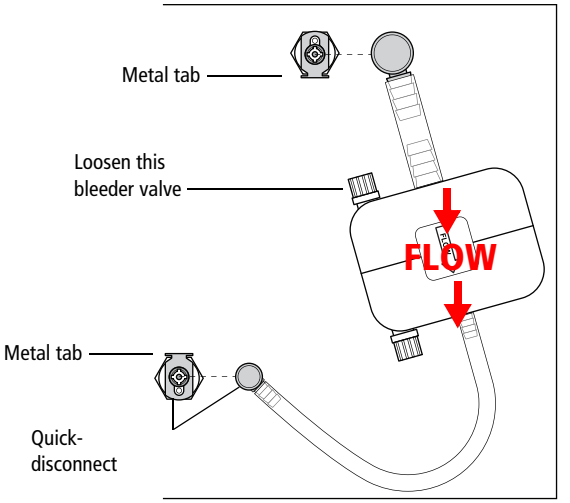
Replacing Fluidics Filters

The fluidics cart should be on during this procedure.

1 Place a few paper towels beneath the filter.

2 Remove the filter by pressing the metal tabs on each quick-disconnect coupling (Figure 10-3).

Figure 10-3 Removing the filter



3 Position the new filter and connect the couplings.

Write the replacement date on the filter so you will know when to replace it.

4 Loosen the top bleeder valve by turning it counterclockwise.

NOTE Do not loosen the bottom bleeder valve. Ensure that it is tightened.

5 Wait until fluid seeps out.

Fluid should seep from the open valve within 30 seconds. If no fluid appears, ensure that the corresponding cubitainer is not empty or detached from the cart. If the cubitainer contains fluid and the fluid lines are attached and primed, the filter might be airlocked. See Removing an Air Lock on page 164.

6 Close the bleeder valve by turning it clockwise.

Repeat the procedure for other fluid filters to be replaced.

Unscheduled Maintenance

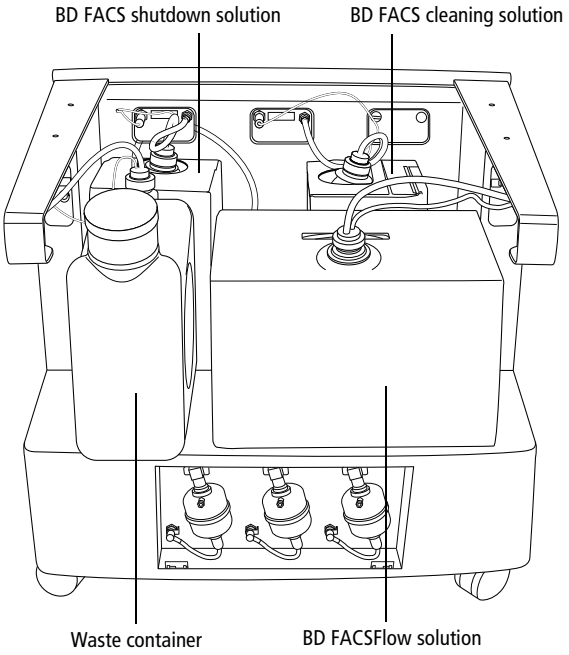
Perform these maintenance procedures as indicated in the table.

Procedure	Description	When to Perform
Replace cubitainers	—	As needed
Prime fluid lines	Removes air from fluid lines	Whenever a fluidics line is disconnected to change a cubitainer or perform other maintenance
Remove an air lock	Removes air from the filter and restores the flow of fluid	When fluidics are not functioning properly (the flow cell will not fill, or there is backflush into the sample tube)
Clean external surfaces	Keeps surfaces free from salt buildup after exposure to sheath fluid	As needed
De-gas Flow Cell	Removes bubbles from the flow cell	As needed during daily startup
Clean Flow Cell	Runs BD FACS cleaning solution through the SIT and flow cell	When poor optical performance indicates additional cleaning is needed

Procedure	Description	When to Perform
Bubble Filter Purge	Removes air from the bubble filter	If fluidics run dry, or when CVs are poor
Clean the fluidics system for storage	Cleans out the fluidics lines with BD FACS cleaning solution, then fills them with BD FACS shutdown solution	Before long-term storage
Replace the Bal seal	Replaces a worn Bal seal	When the seal becomes worn or cracked (sample tubes will not pressurize)
Reconnect fluidics cart tubing	—	As needed
Replace fluidics level sensors	—	When instructed to by a BD Biosciences service representative

Changing a Cubitainer

Three fluidics cubitainers (disposable boxes of approved fluids) and a waste container fit onto the cart in the following configuration.

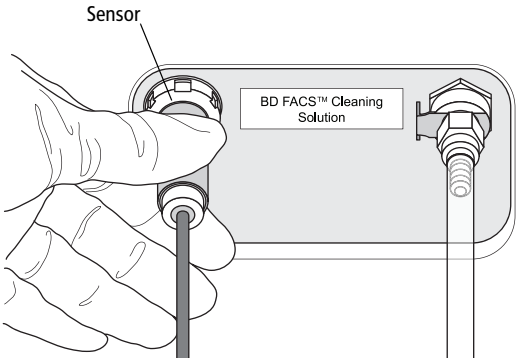


Each cubitainer and the waste container have their own color-coded ports. To change a cubitainer:

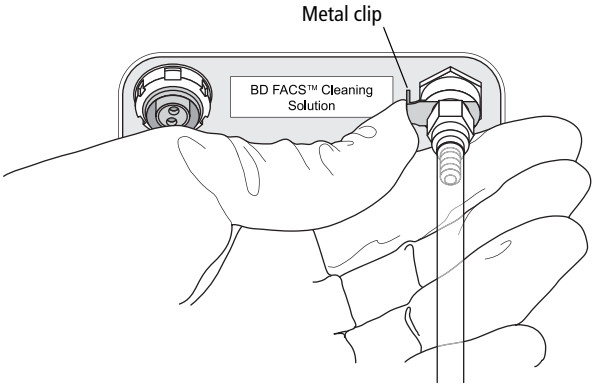
1 Ensure that the cytometer is not acquiring events.

2 Detach the sensor and fluid line from the cart.

a Pull the sensor straight out.



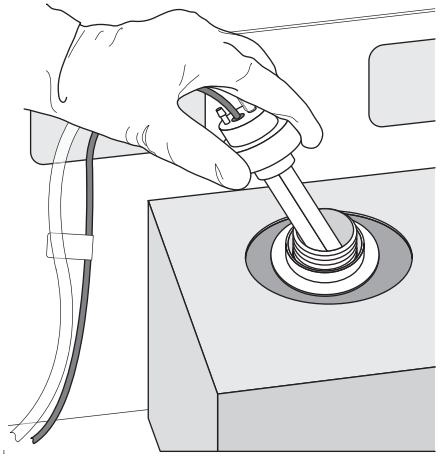
b Press the metal clip on the fluid line.



⚠ You could damage the sensor line if you leave it connected while changing a cubitainer.

3 Unscrew the cap on the cubitainer.

4 Remove the cap and sensor assembly and set it aside.



5 Put a new cubitainer onto the fluidics cart.

⚠ To ensure that the appropriate solutions are dispensed, match the label on the container to the port on the fluidics cart.

6 Replace the cap assembly and hand-tighten it until it is fully closed.

7 Reattach the sensor line and fluid line to the cart.

- To attach the sensor line, gently rotate it until the connection aligns, and then push.
- To attach the fluid line, push the coupling into the port until it clicks.

8 Prime the fluidics.

Important: Continue with the procedure in Priming Fluidics Lines on page 164.

Priming Fluidics Lines

1 Select **Cytometer > Cleaning Modes > Prime After Tank Refill**.

2 Select the checkboxes for the cubitainers you changed. Click **OK**.

3 Click **OK** when the completion message is displayed.

NOTE If the fluidics are not functioning properly, perform Removing an Air Lock.

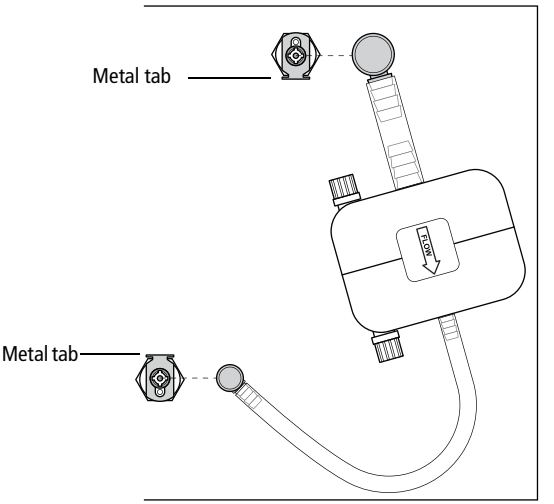
Removing an Air Lock

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

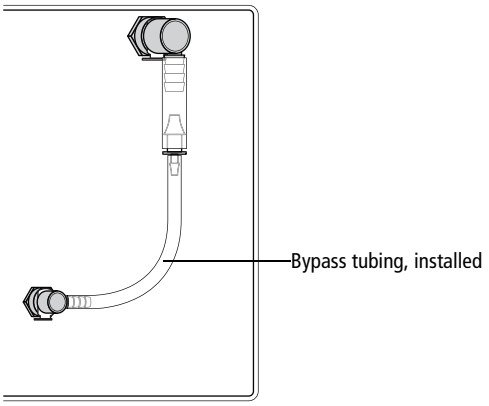
1 Place a few paper towels beneath the air-locked filter.

2 Remove the filter by pressing the metal tabs.

Figure 10-4 Fluidics filter



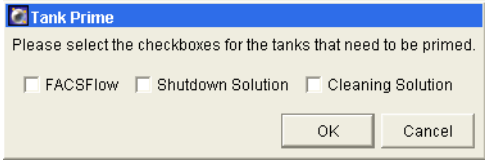
3 Install bypass tubing in place of the filter.



4 Select **Cytometer > Cleaning Modes > Prime After Tank Refill.**

5 Select the checkbox that corresponds to the filter you have bypassed.

BD FACSCanto clinical software



BD FACSDiva software

6 Click **OK**.

7 Ensure that fluid is moving through the bypass tubing.

8 When the prime finishes, remove the bypass tubing.

9 Reattach the filter to the fluidics cart.

10 Repeat the Priming Fluidics Lines procedure.

a Select **Cytometer > Cleaning Modes > Prime After Tank Refill**.

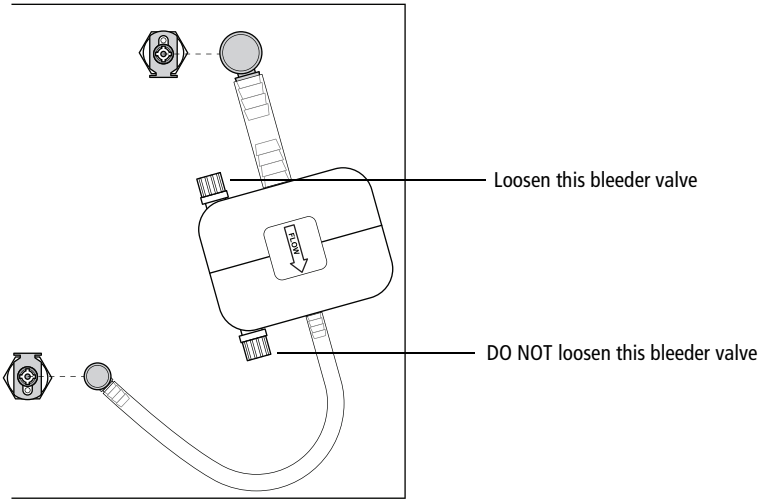
b Select the checkbox for the filter you bypassed.

c Click **OK** when the completion message is displayed.

11 Open the bleeder valve and wait for fluid to seep out. Close the valve.

If no fluid seeps out, repeat steps 10 and 11.

Figure 10-5 Bleeder valves on fluidics filter



Cleaning External Surfaces

To prevent saline deposit buildup, wipe down all external cytometer surfaces that have been exposed to sheath fluid.

Cleaning Guidelines

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

To prevent damage, do not use isopropyl alcohol or ethanol on any cytometer or fluidics cart surface.

NOTE Do not use BD FACS cleaning solution or bleach to clean or disinfect the barcode reader. See Cleaning the Barcode Reader on page 45.

Over time, saline deposits may develop on the interior surface of the SIT Bal seal retainer, causing an incomplete SIT flush between samples. If this occurs, you might need to remove and clean the retainer with DI water. See Figure 10-6 on page 170.

Cleaning Procedure

To avoid potential shock, always switch off the power and unplug the AC power cord before you begin cleaning.

1. Switch off the cytometer power and unplug the AC power cord.
2. Wipe all accessible surfaces with BD FACS cleaning solution.
3. Wet a fresh cloth with DI water and wipe all solution or bleach-exposed surfaces to prevent corrosion.
4. Wipe all exposed surfaces with a clean, dry cloth.

Removing Bubbles from the Flow Cell

1 Select **Cytometer > Cleaning Modes > De-gas Flow Cell**.

2 Click **OK** when the completion message is displayed.

3 Check the flow cell for bubbles.

If you still see bubbles, repeat the process. You might need to de-gas the flow cell several times.

Cleaning the Flow Cell

1 Select **Cytometer > Cleaning Modes > Clean Flow Cell**.

2 If you have a Loader installed, remove the carousel.

3 When prompted, manually install a tube containing approximately 2 mL of BD FACS cleaning solution onto the SIT, and click **OK**.

A progress message is displayed during the cleaning.

4 After the completion message is displayed, wait 5 minutes.

Waiting allows BD FACS cleaning solution to dissolve deposits in the flow cell cuvette.

5 Click **OK**.

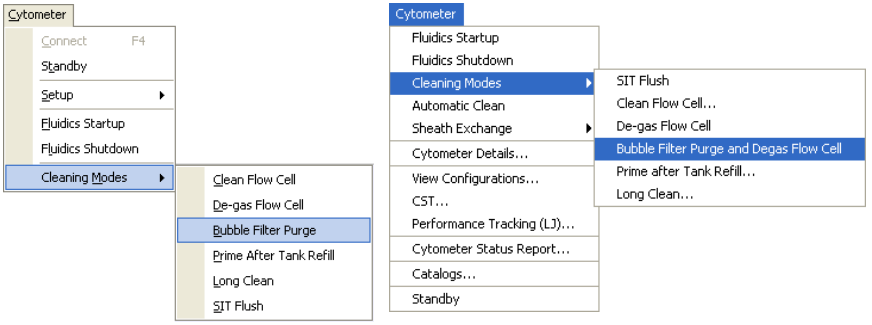
6 Remove the tube from the SIT.

7 Clear BD FACS cleaning solution from the flow cell and fluidics lines:

- Before running setup or samples, run Fluidics Startup.
- To shut down without running more samples, run Fluidics Shutdown.

Purging the Bubble Filter

1 Select **Cytometer > Cleaning Modes > Bubble Filter Purge** (or **Bubble Filter Purge and Degas Flow Cell**).



BD FACSCanto clinical software

BD FACSDiva software

2 Wait while the purge finishes.

3 Click **OK** when the completion message displays.

4 Repeat steps 1 through 3 until bubble-free liquid enters the flow cell from the bubble filter.

5 If you are using BD FACSCanto clinical software, after performing Bubble Filter Purge, select **Cytometer > Cleaning Modes > De-gas Flow Cell**.

Cleaning the Fluidics System for Storage

1 Perform steps 1 through 4 in Cleaning the Fluidics System (Long Clean) on page 156.

2 Exit the software and turn off the power to the cytometer.

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

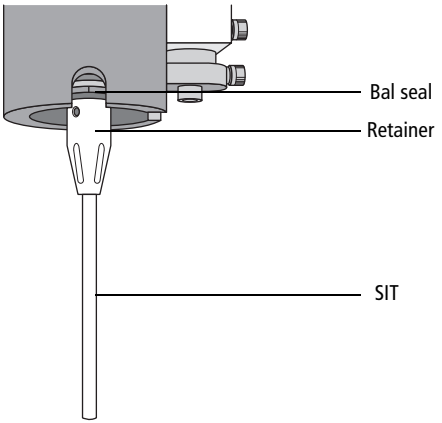
3 Wipe down the SIT and the aspirator arm with DI water. See Shutting Down on page 145 for more information.

Replacing the Bal Seal

Over time, saline deposits may develop on the interior surface of the SIT Bal seal retainer, causing an incomplete SIT flush between samples. Therefore, you should always rinse the retainer in DI water when you replace a Bal seal.

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

Figure 10-6 Bal seal and retainer



1 Turn off the cytometer.

2 If you have a Loader installed:

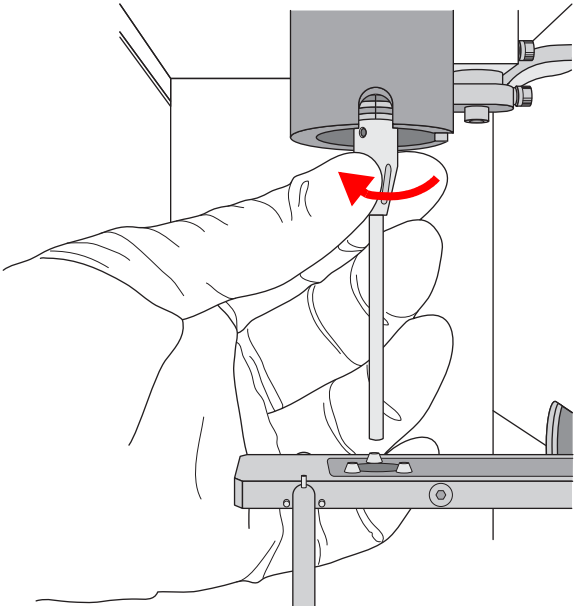
   a Open the Loader doors.

   b Pull out the drawer.

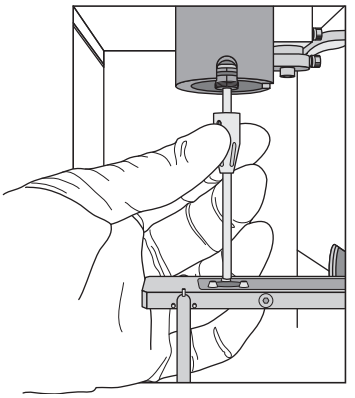
   c If needed, remove the carousel.

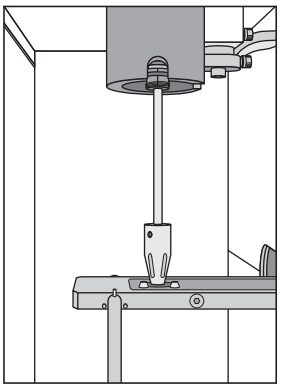
d Push the tube guide back to the manual position. Ensure that the tube guide is pushed back and the aspirator arm bar is in the vertical position.

3 Unscrew the retainer by turning it in the direction shown.

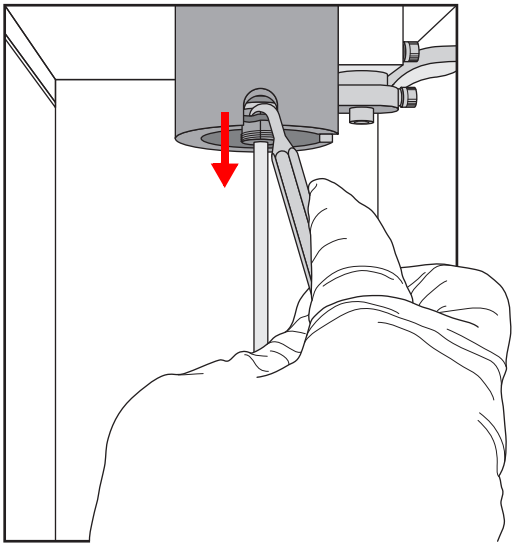


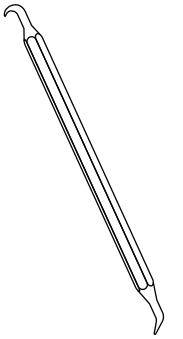
4 Lower the retainer down the SIT and let it rest on the aspirator arm.





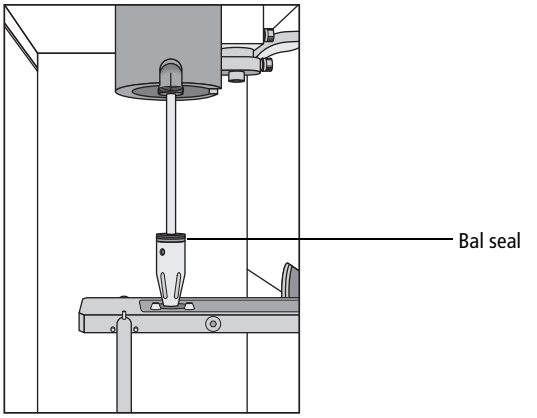
5 Access the Bal seal at the notch on the right side of the SIT assembly. Unseat the Bal seal by using the Bal seal removal tool to gently pull it downward.





Bal seal removal tool

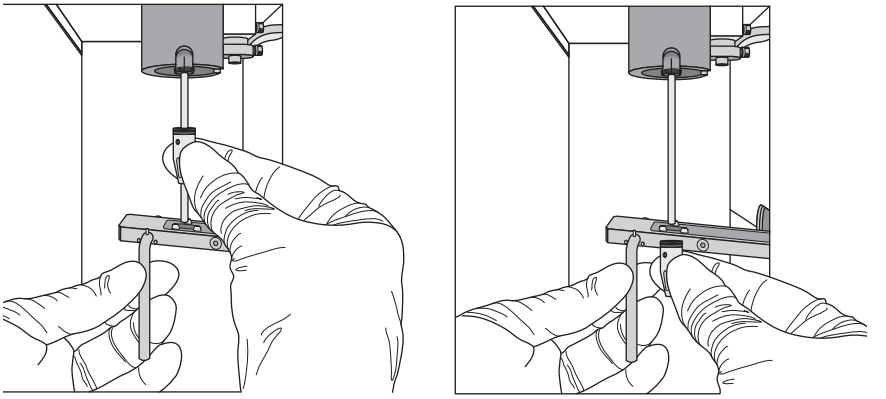
6 Allow the Bal seal to slide down the SIT and rest on top of the retainer.



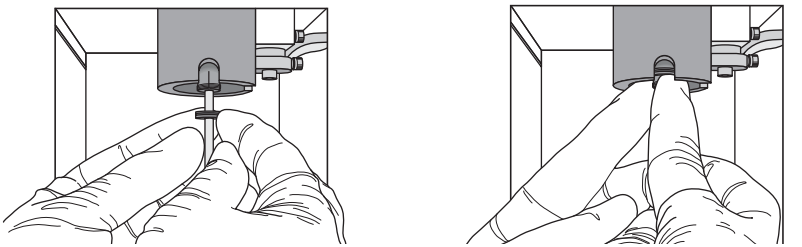
7 Center the Bal seal on the retainer. With one hand, support the retainer and Bal seal and with the other hand, move the aspirator arm to the left. Lower the retainer and Bal seal from the SIT.

See Figure 10-7.

Figure 10-7 Removing the Bal seal and retainer from the SIT

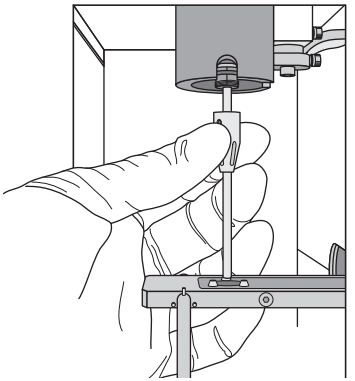


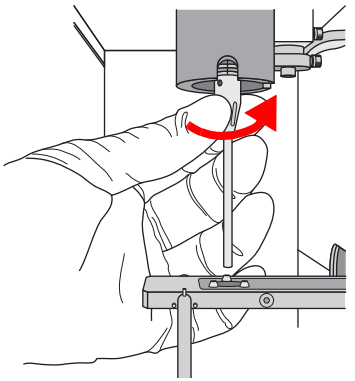
8 Hold the new Bal seal spring-side up (see figure), and slide it up the SIT. Use both hands to gently push it into its seated position.



9 Rinse the retainer in DI water before replacing it on the SIT.

10 Reinstall the retainer over the SIT and tighten in the direction shown.





If the Bal seal is not seated firmly in position, replacing the retainer will seat it correctly.

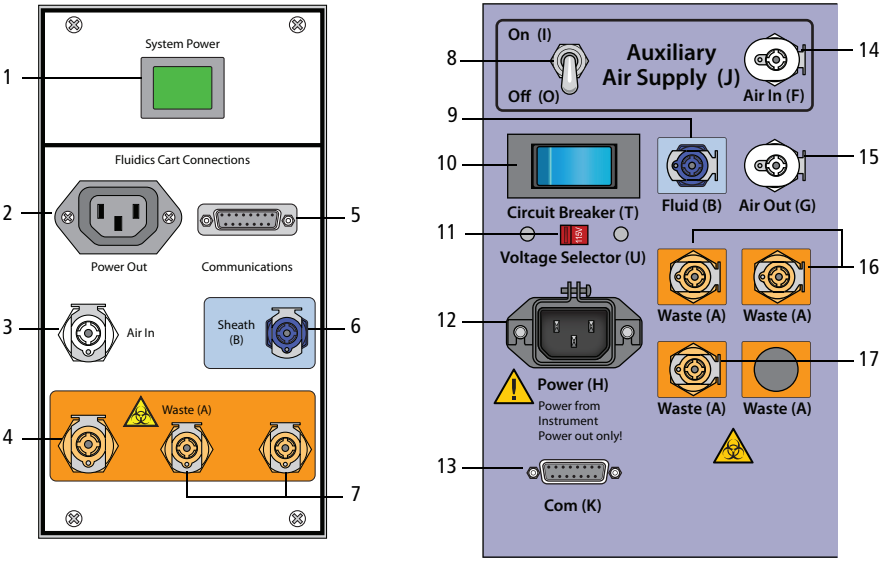
11 Test the installation by manually loading a tube onto the SIT and running fluid.

12 If you are using a Loader, ensure that the system is ready for automatic loading. Move the tube guide into place on the SIT and move the aspirator arm bar to the back.

Reconnecting the Fluidics Cart Tubing

The ports and connectors are color coded.

Figure 10-8 Cytometer and fluidics cart ports



Cytometer ports and their corresponding fluidics cart ports are listed in Table 10-1 on page 176. See Table 10-2 for port functions.

Table 10-1 Correspondence of cytometer ports to fluidics cart ports

Port or Button on Cytometer	Port on Fluidics Cart
1. System Power	—
2. Power Out	12. Power In
3. Air In	15. Air Out (G)
4. Waste (A)	17. Waste (A)
5. Communications	13. Com (K)
6. Sheath (B)	9. Fluid (B)
7. Waste (A)	16. Waste (A)
—	8. On/Off
—	14. Air In (F)

Do not plug the fluidics cart power cord into a wall outlet. Plug the cord into the cytometer only. This ensures proper electrical grounding and protects against electrical shock or damage to the cytometer.

Table 10-2 Function of ports, buttons, and switches

Port or Switch	Additional Information
1. System Power	Powers both the cytometer and fluidics cart
2. Power Out	Connects to the fluidics cart
3. Air In	70 ±5 psi
4. Waste (A)	Waste out (aspirated)
5. Communications	Data port
6. Sheath (B)	BD FACSFlow solution port
7. Waste (A)	Waste out (non-aspirated)
8. On/Off	Auxiliary air supply switch. Keep in the off position unless connected to house air.

Table 10-2 Function of ports, buttons, and switches (continued)

Port or Switch	Additional Information
9. Fluid Out (B)	BD FACSFlow solution port
10. Circuit breaker (T)	Fluidics cart circuit breaker
11. Voltage selector (U)	Fluidics cart voltage selector
12. Power In	Connects to the cytometer. Do not connect to a wall outlet.
13. Com (K)	Data port
14. Auxiliary Air In (F)	No tubing on this port unless connected to house air
15. Air Out (G)	Sends compressed air to the cytometer
16. Waste (A)	Waste in (non-aspirated)
17. Waste (A)	Waste in (aspirated)

Replacing the Fluidics Level Sensors

Before you replace a suspected faulty sensor, try rinsing it with DI water to restore operation.

If you are changing the sensor on the waste container, use proper precaution and wear suitable protective clothing, eyewear, and gloves. All biological specimens, and materials coming into contact with them, can transmit potentially fatal disease.

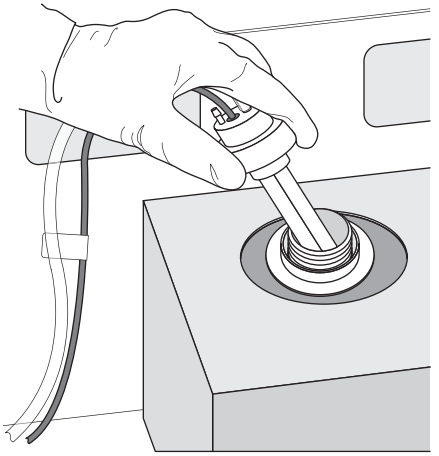
The waste container can become pressurized when the cytometer is running. Always disconnect the waste container from the fluidics cart and wait at least 30 seconds for pressure to dissipate before you remove the level sensor cap.

1 Ensure that the cytometer is not acquiring events.

2 Detach the sensor and fluid line from the cart.

3 Unscrew the cap on the cubitainer.

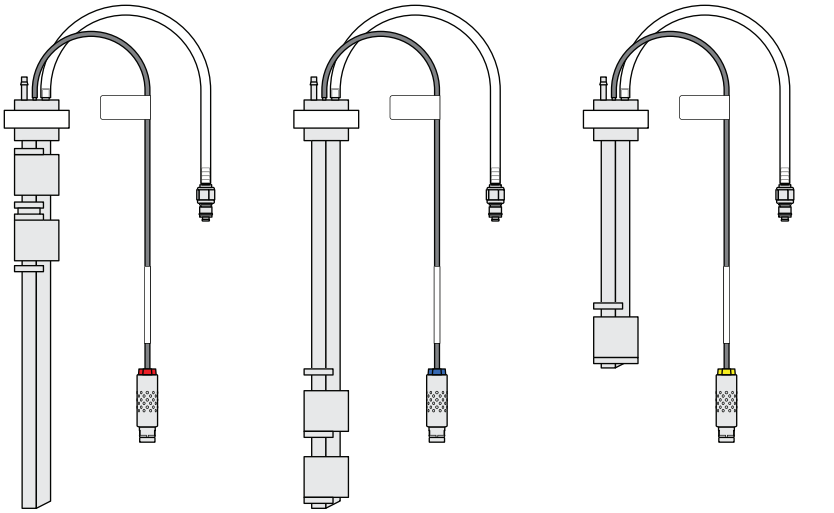
4 Remove the level sensor assembly and discard into a suitable receptacle.



5 Put a new level sensor assembly on the cubitainer or waste container. Hand-tighten the cap until it is fully closed.

⚠ To ensure that the appropriate solutions are dispensed, make sure the label on the container matches the labeled port on the fluidics cart.

Figure 10-9 Level sensor assemblies



Waste level sensor (red connector)

BD FACSFlow level sensor (blue connector)

BD FACS cleaning solution and BD FACS shutdown solution level sensor (yellow connector)

6 Reattach the sensor line and fluidics lines.

7 If you replaced any level sensors (other than the waste sensor), prime the affected fluidics lines. Continue with the Priming Fluidics Lines procedure on page 164.

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11

Troubleshooting

This chapter contains the following information, which will help you to troubleshoot issues that might arise when using this cytometer:

Instrument Troubleshooting

The instrument consists of the cytometer, the optional Loader, and the fluidics cart. The following sections provide tips for troubleshooting the instrument.

If you need additional assistance, contact BD Biosciences. Have the following information available: product name, catalog number, and serial number; any error messages; and 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. See our website, bdbiosciences.com, for up-to-date contact information.

Cytometer Troubleshooting

Observation	Possible Causes	Recommended Solutions
Flow cell will not fill	Air in bubble filter	For BD FACSCanto clinical software, run the Bubble Filter Purge command at least once (you might need to run it several times). When finished, run De-Gas Flow Cell. For BD FACSDiva software, run the Bubble Filter Purge and De-Gas Flow Cell command at least once (you might need to run it several times).
	Fluidics cart power off	Turn on the power to the fluidics cart by resetting the fluidics cart circuit breaker switch (Figure 1-7 on page 27). Always use the cytometer power button, located on the left side of the cytometer, to turn the system off and on.
	No pressure in the plenum	Check the air supply components (see Figure 10-8 on page 175). • Check the air supply connections. • Check the air supply tubing for kinks.
	Sheath line disconnected	**1** Check the cubitainer-to-fluidics cart and fluidics cart–to-cytometer connections. See Reconnecting the Fluidics Cart Tubing on page 175. **2** Check the tubing for kinks.
	BD FACSFlow cubitainer empty	Replace the BD FACSFlow cubitainer. See Changing a Cubitainer on page 161.

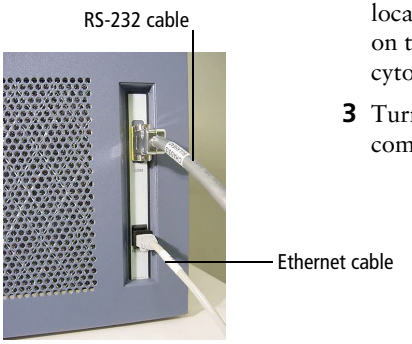
Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Flow cell will not fill (continued)	Air in the BD FACSFlow filter (fluidics cart)	Purge air from the filter. See Priming Fluidics Lines on page 164.
	Air lock in the BD FACSFlow filter (on fluidics cart)	See Removing an Air Lock on page 164.
	No air pressure in the fluidics cart	• If the cart is attached to an auxiliary air supply, switch on the auxiliary air. • If the cart is not attached to an auxiliary air supply, switch off the auxiliary air.

Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Fluid backfill into sample tube	Cracked or defective tube	⚠ Data could be lost when transferring to a new tube. • If using a BD Trucount tube, do not transfer sample to a new BD Trucount tube because that would result in incorrect absolute counts. Re-stain the sample and use a new BD Trucount tube. • If using a non-BD Trucount tube, transfer sample to a new, non-BD Trucount tube. • Make sure you are using appropriate tubes. See System Requirements on page 31.
	Bal seal improperly installed or worn	Reinstall or replace the Bal seal. See Replacing the Bal Seal on page 170.
	Air lock in BD FACSFlow filter	See Removing an Air Lock on page 164.
	Tube partially removed from SIT after acquisition	Once you start to remove a tube from the SIT, complete the action.
Low laser power indication	Flow cell access door open	Close the door completely.
	Laser power output is below requirement	Call BD Biosciences.
Backflushing of sheath fluid into sample tube	Aspirator arm bumped or moved to side, but tube was not removed	**1** Repeat the tube removal sequence. **2** Perform a manual SIT flush.

Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Fluidics pressure errors	Air lock in filter	Check the filter in the fluidics cart. Verify that the bottom bleeder valve on the filter is fully tightened. Open the top bleeder valve. If no fluid leaks out, remove the air lock as described in Removing an Air Lock on page 164.
Cytometer on, no response to software commands	Bad keyboard or mouse connection	Check the keyboard or mouse connections to computer. See the documentation that came with your workstation.
	Communication failure between computer and cytometer 	**1** Turn off the computer and the cytometer. **2** Reseat the Ethernet cable, located next to the power cord on the right side of the flow cytometer. **3** Turn on the cytometer, then the computer.
	User-installed firewall	• Contact your system administrator to open a port in the firewall for the cytometer. • Call BD Biosciences.
QC fails after Long Clean	Residual BD FACS cleaning solution in lines	Run Fluidics Startup to flush the system with sheath fluid. Repeat until QC passes.

Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Tube does not load, or sample tube does not fit snugly on SIT	Improper tubes used	Use only uncapped 12 x 75-mm Falcon polystyrene test tubes, BD Trucount tubes, and BD FACS 7-color setup beads tubes. See System Requirements on page 31.
	Defective or cracked tube	⚠ Data could be lost when transferring to a new tube. • If using a BD Trucount tube, do not transfer sample to a new BD Trucount tube because that would result in incorrect absolute counts. Re-stain the sample and use a new BD Trucount tube. • If using a non-BD Trucount tube, transfer sample to a new, non-BD Trucount tube. • Make sure you are using appropriate tubes. See System Requirements on page 31.
	Bal seal improperly installed or worn	Reinstall or replace the Bal seal. See Replacing the Bal Seal on page 170.
Cytometer and fluidics cart will not turn on	Power cord disconnected from wall socket or cytometer	Reconnect the power cord to the wall socket or the cytometer.
	Cytometer circuit breaker tripped	Switch on the cytometer circuit breaker, followed by the cytometer power (if off).
	Blown fuse in cytometer controller	Turn off the cytometer main power and call BD Biosciences.

Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Fluid leaking from SIT or aspirator arm	Interior valve failure or leak	**1** Turn off the cytometer power. **2** Clean up the liquid, using proper precautions. **3** Call BD Biosciences.
	Waste line to fluidics cart disconnected	**1** Turn off the cytometer power. **2** Clean up the liquid, using proper precautions. **3** Check that waste lines are securely plugged in. **4** Turn on the cytometer power.
Liquid leakage around cytometer base	Interior valve failure	**1** Turn off the cytometer power. **2** Clean up the liquid, using proper precautions. **3** Call BD Biosciences.
Aspirator arm locked, cannot unload tube	Software not responding	⚠ Do not use force to remove tube from the SIT. **1** Press Ctrl+Alt+Delete to open Task Manager. **2** Select **End Task** to close the BD FACSCanto clinical software or BD FACSDiva software application. **3** Restart the software and unload the tube.
	Pressure loss	Verify that the fluidics cart is powered on and that air pressure hoses are properly connected.

Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Fluidics error after replacing sheath fluid or emptying waste	Status change not yet detected by software	After replacing or emptying fluids, wait until the software detects a status change before restarting acquisition. It can take up to 30 seconds for the software to detect a fluid level change.

Loader Troubleshooting

Observation	Possible Causes	Recommended Solutions
Tube does not load	Tube not completely raised	Ensure that the Loader drawer is completely shut.
		Ensure that the tube is free of bulky labels or tape.
	Tube lifter hitting carousel during ascent	Ensure that the carousel is properly engaged with the alignment guide pin. If the problem persists, contact BD Biosciences for assistance.
	Tube lifter failure	Contact BD Biosciences.

Loader Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Tube does not unload	Tube lifter failure	**1** Log out and close BD FACSDiva software. **2** If the lifter remains in the up position, turn off the cytometer power for approximately 5 minutes. **3** If the lifter still remains in the up position, contact BD Biosciences. **4** If the lifter returns to the down position, remove the Loader cover and the carousel. **5** Clean salt buildup from the base of the tube lifter. **6** Restart the cytometer and log in to BD FACSDiva software. **7** Replace the carousel and the Loader cover. If the problem persists, contact BD Biosciences.
	Status change not yet detected by software	After replacing or emptying fluids, wait until the software detects a status change before restarting acquisition. It can take up to 30 seconds for the software to detect a fluid level change.

Loader Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Tube missed, or missing tube messages in software	Salt crystal buildup on optical sensors	Clean the tube optical sensor (outer sensor), as described in Cleaning External Surfaces on page 167.
	Condensation on tube	Wipe the tube.
	Tube label reflection	Turn the tube.
Tube not detected	Optical sensor is wet or dirty	**1** Clean the sensor. **2** Allow time for the sensor to dry.
Loader stalls or emits a grinding noise	Door opened during Loader initialization	When the Loader stops moving, open and close the Loader doors. Do not open the Loader doors while the Loader is in motion.
Tube runs dry, Loader not advancing to next sample	Dilute sample or rare events	Set a stopping time in BD FACSDiva software. See Chapter 7, Running Samples with BD FACSDiva Software.
	Bubble in flow cell diverts stream	De-gas the flow cell. See Removing Bubbles from the Flow Cell on page 168.
	Communication error	**1** Restart the system. **2** Perform Fluidics Startup. See Chapter 4, Starting Up.

Loader Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Test tube stuck on SIT, software not responding	• Cytometer not set up for automatic loading • Software problem	Remove the tube from the SIT manually. **1** Hold the tube and move the aspirator arm aside. **2** Gently pull the tube off the SIT. **3** Release the aspirator arm. **4** Restart the software. **5** If needed, modify the cytometer for automatic loading.
Carousel not rotating correctly	Carousel not engaged with alignment pin on drawer	Rotate the carousel on the spindle until the alignment guide pin engages with the alignment hole, and press down. See Figure 8-5 on page 137. If the problem persists, contact BD Biosciences for assistance.
	Aspirator arm bar vertical	Rotate the bar to the forward horizontal position.

Disabling the Loader in BD FACSCanto Clinical Software

If a problem occurs that requires you to temporarily disable the Loader and run tubes manually, you can make the software behave as though a Loader is not part of your system. Dialogs reminding you to insert the Loader, and other software notifications, no longer are displayed.

NOTE You can only make this change if you are logged in as the Lab Manager.

1. In BD FACSCanto clinical software, select **Tools > Options**.
2. Click the **Run** button.
3. Select the **Ignore loader, always load tubes manually** checkbox in the **Run Options** dialog.
4. Click **OK**.

Fluidics Cart Troubleshooting

Observation	Possible Causes	Recommended Solutions
Water leakage around fluidics cart base	• Normal condensation overflow from pressure relief valve • Extremely humid climate	**1** Turn off the cytometer power. **2** Clean up the water. **3** Empty the condensation trap in the fluidics cart daily. See Figure 1-6 on page 25.
	Bleeder valve on fluidics cart filter open	**1** Turn off the cytometer power. **2** Clean up the liquid. **3** Check and close all bleeder valves. See Purging the Fluidics Filters on page 154.
	Broken fluid line	Contact BD Biosciences.
Fluidics cart will not power on, cytometer on	Cart circuit breaker off	Switch on the fluidics cart circuit breaker.
	Auxiliary air supply switch is on, cart not normally connected to auxiliary air	Toggle the auxiliary air supply off. When auxiliary air is on, the cart's own air pumps turn off.
	Power cord from fluidics cart to cytometer disconnected	Connect both ends of the cord.

BD FACSCanto Clinical Software Troubleshooting

Acquisition Indicator Lights

The acquisition indicator lights may provide important information when troubleshooting software related issues. Ensure that these lights are on (see Electronics on page 23).

BD FACSCanto Clinical Software General Issues

Observation or Error Message	Possible Causes	Recommended Solutions
Software not responding	Software frozen	⚠ If acquisition is in progress, data will be lost when you click **End Task**. **1** Press Ctrl+Shift+Esc. Locate BD FACSCanto clinical software in the Windows Task Manager. Click **End Task**. **2** Restart the software. To prevent carryover, perform Fluidics Startup before acquiring data by selecting **Cytometer > Fluidics Startup**.
Software does not connect to cytometer	BD FACSDiva software running	• Exit BD FACSDiva software. OR • In BD FACSDiva software, put the cytometer in standby: select **Cytometer > Standby**.
	Cytometer power is off	Turn the power on.
	Internal firmware error	Cycle the power on the cytometer.
	Ethernet disconnected	See page 186.

BD FACSCanto Clinical Software General Issues

Observation or Error Message	Possible Causes	Recommended Solutions
Connection error messages	Various	• Follow any message directions. • Ensure that the cytometer power is on. • Check the Ethernet cable connection to the cytometer and computer. • Verify that the fluidics cart is plugged in and the power is on. • Exit the software, shut down the computer and cytometer, and then restart them.
Barcode reader error	Dirty barcode reader window	Clean the barcode reader window with isopropyl or ethyl alcohol and try again.
	Blurred or damaged barcode label	Try scanning with a duplicate label (if available), or enter the data manually.

BD FACSCanto Clinical Software General Issues

Observation or Error Message	Possible Causes	Recommended Solutions
Tube not present error	Tube not seated on SIT	Push the tube all the way up onto the SIT.
	Cracked or defective tube	⚠ Data could be lost when transferring to a new tube. • If using a BD Trucount tube, do not transfer sample to a new BD Trucount tube because that would result in incorrect absolute counts. Re-stain the sample and use a new BD Trucount tube. • If using a non-BD Trucount tube, transfer sample to a new, non-BD Trucount tube. • Make sure you are using appropriate tubes. See System Requirements on page 31.
	Fluid at top of tube prevents detection	Wipe the inside top of the tube with a clean cotton swab. Dispose of the swab with biohazardous materials.
	Tube sensor wet or damaged	• Allow time for the sensor to dry. • Reduce solution volumes to ≤3 mL. • Call BD Biosciences.

BD FACSCanto Clinical Software General Issues

Observation or Error Message	Possible Causes	Recommended Solutions
Tube pressurization errors	Cracked or defective tube	⚠ Data could be lost when transferring to a new tube. • If using a BD Trucount tube, do not transfer sample to a new BD Trucount tube because that would result in incorrect absolute counts. Re-stain the sample and use a new BD Trucount tube. • If using a non-BD Trucount tube, transfer sample to a new, non-BD Trucount tube. • Make sure you are using appropriate tubes. See System Requirements on page 31.
	Drop at top of tube	Dry the inside of the tube with a cotton swab and re-run.
	Bal seal improperly installed or worn	Reinstall or replace the Bal seal. See page 170 for instructions.
	Loader misaligned	**1** Try running the cytometer in manual mode instead of automatic. See Running Setup Using Manual Loading on page 57 for converting to manual mode. **2** If unsuccessful, call BD Biosciences.
	Wrong tubes used	Make sure that you are using the recommended tubes. See System Requirements on page 31.

BD FACSCanto Clinical Software General Issues

Observation or Error Message	Possible Causes	Recommended Solutions
Tube pressurization errors (continued)	Tubing to fluidics cart kinked or disconnected	Reconnect or straighten the tubing. See page 175.
Tube not detected	Tube not seated on Bal seal properly	• Ensure that the tube is all the way up onto the Bal seal. • Ensure that you are using appropriate tubes. See System Requirements on page 31. • Replace or reinstall the Bal seal.
Vacuum error	Tubing to fluidics cart kinked or disconnected	Reconnect or straighten the tubing. See page 175.

BD FACSCanto Clinical Software Setup Wizard Messages

Messages	Possible Causes	Recommended Solutions
No acquisition events were received from cytometer	Communication problem between hardware and software	**1** Turn on the power. **2** Connect the Ethernet cable to the cytometer and computer. **3** Exit the software, shut down the computer and cytometer, and restart them. **4** To prevent carryover, perform a Fluidics Startup before acquiring data. Select **Cytometer > Fluidics Startup**.
	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.
	Laser failure	Check the laser power in the Status window. If it is outside the acceptable range, call BD Biosciences.
	Clogged SIT	Clean the flow cell.
Failed to place [*name of beads*] on scale. or Failed to find [*name of beads*]	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.
	Software using saved settings from a failed setup	Delete the SetupResult.dat file from C:\ProgramData\BD\Shared\Setup Results, and run the setup again.
No valid data points	Internal setup error	Delete the SetupResult.dat file from C:\ProgramData\BD\Shared\Setup Results, and run setup again.

BD FACSCanto Clinical Software Setup Wizard Messages (continued)

Messages	Possible Causes	Recommended Solutions
A communication error was encountered	Communication problem between hardware and software	**1** Turn on the power. **2** Connect the Ethernet cable to the cytometer and computer. **3** Exit the software, shut down the computer and cytometer, and restart them.
	Fluidics cart off or disconnected	Switch on the fluidics cart circuit breaker. Make sure that the power cable is connected at both ends.
Cytometer setup was aborted by user	Setup aborted	Perform setup again.
Cytometer setup was aborted because the loader doors were opened	Loader doors opened	Perform setup again, keeping the Loader doors closed.
There is a vacuum error	Vacuum tubing to waste cart disconnected	Reconnect the waste tank or tubing, and remove kinks.
	Waste tank disconnected, waste tubing disconnected or pinched	Reconnect the waste tank or tubing, and remove kinks.
	Clogged aspirator arm	Call BD Biosciences.

BD FACSCanto Clinical Software Setup Wizard Messages (continued)

Messages	Possible Causes	Recommended Solutions
There is a float switch error	Sheath tank not attached to fluidics cart	Reconnect the sheath cubitainer or tubing, and remove kinks.
	Sheath tubing from fluidics cart to cytometer not attached or pinched	Reconnect the waste tank or tubing, and remove kinks.
	Sheath filter not primed	Always prime after changing a cubitainer.
	Fluidics air lock	See Removing an Air Lock on page 164.
	Fluidics cart off or disconnected	Switch on the fluidics cart circuit breaker; make sure the power cable is connected at both ends.
	Low or no pressure	• Check the air supply connections. • Check the air pressure gauge.

BD FACSCanto Clinical Software Setup Report Failure Messages

Failed	Possible Causes	Recommended Solutions
Detector voltage	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.
	Wrong target value used	Match the target values in the software to those printed on the BD FACS 7-color setup beads label. Re-enter them, if needed.
	SetupResults.dat file deleted	Accept the failed setup, and then re-run setup.
Sensitivity	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.
	Dirty flow cell	Clean the flow cell.
Spectral overlap	Expired bead lot	Run setup again with a fresh tube of setup beads.
	Software using saved setting from a failed setup	Delete the SetupResult.dat file from C:\ProgramData\BD\Shared\ Setup Results, and run setup again.
	Incorrect spectral overlap factors used	Match the spectral overlap factors in the software to those printed on the BD FACS 7-color setup beads label.
Laser power	Laser failure	Check the laser power in the Status window. If outside the acceptable range, call BD Biosciences.
Sheath pressure	Kinked or clogged sheath line	Remove any kinks in the tubing to the fluidics cart.
	Clogged or airlocked sheath filter	Check the sheath filter. Open the bleeder valves. If no fluid leaks out, remove the air lock.

BD FACSCanto Clinical Software Levey-Jennings Errors and Messages

Observation	Possible Causes	Recommended Solutions
LJ plots empty, no data, no error message is displayed	*BD FACS Setup Beads-7 colors LJ.csv* file missing	• If the file was renamed, rename it with its original name, and click **Refresh**. • If the file was moved from the default directory, move it back, and then click **Refresh**.
LJ plots empty, no data, error message is displayed	*BD FACS Setup Beads-7 colors LJ.csv* file corrupted or contains invalid data	Delete the *BD FACS Setup Beads-7 colors LJ.csv* file, and run setup again. The software will create a new set of plots.
	BD FACS Setup Beads-7 colors LJ.csv file open or in use by another application	**1** Close the file or application. **2** Click **Refresh**.

BD FACSCanto Clinical Software Acquisition

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Run, acquisition indicator lights are blinking as expected	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
	Detector voltage too low	Increase the voltage.
No events in plots after clicking Run; acquisition indicator lights, except for those corresponding to threshold parameters, are blinking as expected	Threshold too low or too high	Adjust the threshold.
	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
No events in plots after clicking Run, NO acquisition indicator lights are blinking (no fluorescence signal)	Cracked or defective tube	⚠ Data could be lost when transferring to a new tube. • If using a BD Trucount tube, do not transfer sample to a new BD Trucount tube because that would result in incorrect absolute counts. Re-stain the sample and use a new BD Trucount tube. • If using a non-BD Trucount tube, transfer sample to a new, non-BD Trucount tube. • Make sure you are using appropriate tubes. See System Requirements on page 31.
	SIT clogged	Clean the flow cell.
	Current cytometer configuration different from optical setup	Verify that the cytometer configuration corresponds to the cytometer's optical setup.
	Laser delay set incorrectly	Contact BD Service.

BD FACSCanto Clinical Software Acquisition

Observation	Possible Causes	Recommended Solutions
Incorrect results/data not detected	Wrong panel was selected	Check the panel selection; make sure the plot parameters are appropriate for your assay.
	A BD Trucount panel was selected when there was no BD Trucount tube	Re-run the sample and select a panel without BD Trucount tubes listed.

BD FACSCanto Clinical Software Acquisition

Observation	Possible Causes	Recommended Solutions
	A panel with no BD Trucount tube was selected when there was a BD Trucount tube	Re-run the sample and select a panel with BD Trucount tubes listed.
	Expired BD FACS 7-color setup beads used	Redo with current BD FACS 7-color setup beads.
Incorrect absolute count results	Incorrect hematology results were entered in the worklist	Enter correct hematology results in the worklist and rerun the sample.
Unexpectedly high event rate	Threshold too low	Increase the threshold.
	Sample too concentrated	Dilute the sample.
	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.
Unexpectedly low event rate	Threshold too high	Decrease the threshold.
	SIT clogged	Clean the flow cell.
Excessive amount of debris in plots	Threshold too low	Increase the threshold.
	Dead cells or debris in sample	Examine the sample under a microscope.
	Stained sample too old	Check the reagent package insert.
FCS file not created	PC hard disk full	**1** Delete unnecessary files. **2** Run disk utilities regularly.

BD FACSCanto Clinical Software Acquisition

Observation	Possible Causes	Recommended Solutions
Acquisition stops	High event rate (>10,000 events/sec) with more than 8 compensated parameters	• Increase the threshold. • Decrease the voltage on the threshold parameter. • Dilute the sample. • Define only parameters of interest in the parameters list.
Distorted populations or unexpected pattern in plot	Cytometer settings adjusted incorrectly	Optimize the scatter parameters.
	Bubbles in flow cell	Check for bubbles. If found, run De-gas Flowcell.
	Flow cell dirty	Clean the flow cell.
	SIT not cleaned between tubes	Select **Cytometer > Cleaning Modes > SIT Flush**.
	Incorrect gating	Verify the gating.

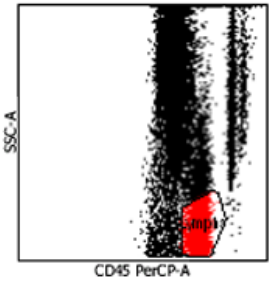
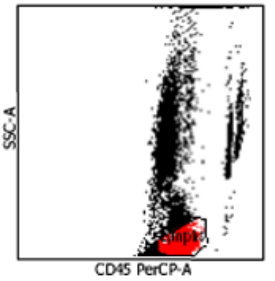
BD FACSCanto Clinical Software TBNK Analysis QC Messages

Message	Possible Causes	Recommended Solutions
Sample quality questionable	Donor-specific anomaly	Adjust gates manually to include required subsets.
	Insufficient mixing during sample preparation: cell populations elongated in CD3 vs SSC and FITC vs PE plots	Re-stain the sample. Vortex after the blood and reagent are added to the tube. Make sure that no blood remains on the side of the tube.
	Sample not lysed adequately: cell populations in CD45 vs SSC extend upward	Prepare the sample again, ensuring complete lysis.
	Aged blood and/or stained sample: granulocytes have low side scatter in CD45 vs SSC plot. No distinct monocyte population	See the information supplied with the reagent for stability limitations.
	Excessive mixing: debris encroaching on populations in CD45 vs SSC plot	Re-stain the sample and run it again.
	Low voltage for SSC detector	Increase the SSC voltage and run the sample(s) again.
	Various	Look at the dot plots in BD FACSCanto Clinical Software Four- and Six-Color TBNK on page 212. Re-run the sample.
	Sample not mixed properly	Vortex prior to loading the sample onto the SIT.

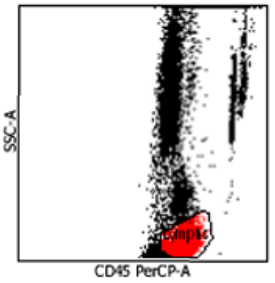
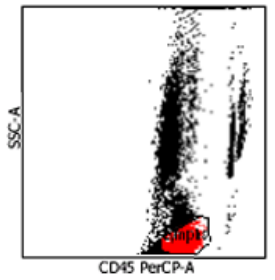
BD FACSCanto Clinical Software TBNK Analysis QC Messages (continued)

Message	Possible Causes	Recommended Solutions
Insufficient beads acquired (<500)	Cell concentration too high	Dilute the sample, re-stain, and run it again.
% T-sum failure	Large number of double-positive or double-negative T cells	Inspect the gates and include all required events. Adjust gates manually, if needed.
Lymphosum failure	Events incorrectly classified as T, B, or NK	Inspect the gates and make sure that required events are included. Adjust gates manually, if needed.
Laser power low during recording: Visually inspect.	Data collected with low laser power	Inspect the data. If the power is outside the acceptable range, call BD Biosciences.
Lymph gate failure: gate manually.	No usable lymph gate	• Adjust the lymph gate manually. • If necessary, run the sample again.

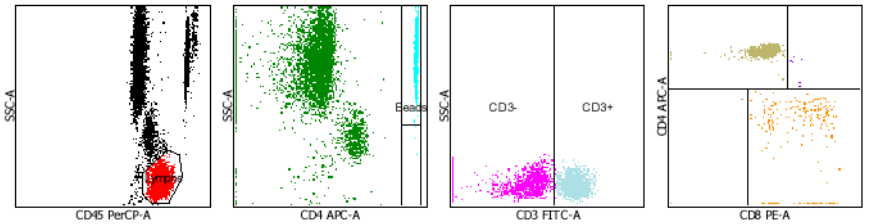
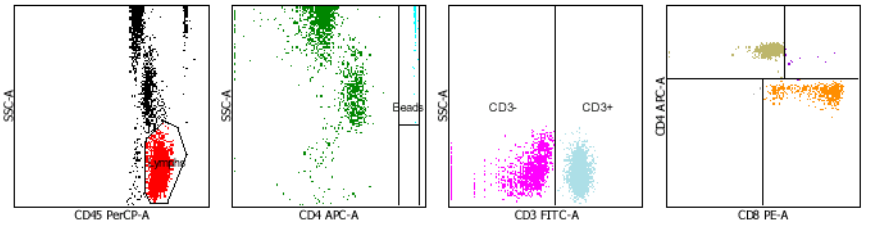
BD FACSCanto Clinical Software Four- and Six-Color TBNK

Observation	Possible Causes	Recommended Solutions
Cell populations in CD45 vs SSC extend upward 	Inadequate lysing of sample	Prepare the sample again, and ensure complete lysis.
Granulocytes with low side scatter in CD45 vs SSC plot, no distinct monocyte population 	Aged blood or stained cells	See the reagent package insert for stability limitations.

BD FACSCanto Clinical Software Four- and Six-Color TBNK (continued)

Observation	Possible Causes	Recommended Solutions
Debris encroaching on populations in CD45 vs SSC plot 	Excessive mixing	Prepare the sample again.
	Aged blood or stained cells	Prepare the sample again.
Vertically compressed populations 	Side scatter too low	Reacquire the sample. Set SSC so granulocytes reach to top of the CD45 vs SSC plot.
	Lipemic sample	Check the reagent package insert for instructions.

BD FACSCanto Clinical Software Four- and Six-Color TBNK (continued)

Observation	Possible Causes	Recommended Solutions
Indistinct populations. Events sparse or missing from one population. Lack of separation between $CD3^-$ and $CD3^+$ cluster	Incorrect spectral overlap	Re-run setup, optimizing for the application. Re-run the sample.
		
Granulocytes cut off at top of plot; stretched monocyte population	High SSC	Re-run setup, optimizing for the application. Re-run the sample, lowering the SSC.
		

BD FACSDiva Software Troubleshooting

Acquisition Indicator Lights

The acquisition indicator lights may provide important information when troubleshooting software related issues. Ensure that these lights are on (see Electronics on page 23).

BD FACSDiva Software General Issues

Observation	Possible Causes	Recommended Solutions
Cytometer Disconnected in Cytometer window (software does not connect to cytometer)	BD FACSCanto clinical software running	• Exit BD FACSCanto clinical software. • In BD FACSCanto clinical software, put the cytometer in standby: select **Cytometer > Standby**.
	Cytometer power is off	Turn the power on.
	Internal firmware error	Cycle the power on the cytometer.
	Communication failure between software and cytometer	• Exit the software and restart it. • If unsuccessful, cycle the cytometer power. Restart the computer.
	Ethernet cable disconnected	Unplug and then plug in the cable connectors.
Cytometer not responding status message	Various	Switch the cytometer power off, and then on. Restart the computer.

BD FACSDiva Software General Issues (continued)

Observation	Possible Causes	Recommended Solutions
Unable to access database	Adaptive Server Anywhere™ not running	Verify that the database server has been started. **1** Select **Start > Settings > Control Panel** and double-click the **Administrative Tools** icon. **2** Double-click the **Services** icon. **3** Select **Adaptive Server Anywhere**. If the **Start** button is available, click the button to start the database server. If the buttons are dimmed, the database could be loading.
Software not responding	Saving or loading large data file	Look for screen activity. If there is no activity, wait 1–2 minutes, and then restart the software.
	Calculating large number of statistics	Calculating statistics is memory intensive. When calculating many statistics on a large number of displayed events, wait 1–2 minutes before using the software.
	Waiting for response from cytometer	Wait until the cytometer action is complete. If after 2 minutes no timeout is received, restart the cytometer and software. After restarting the software, to prevent carryover, perform a Fluidics Startup before acquiring data: Select **Cytometer > Fluidics Startup**.
Software message *Hardware key not accessible...*	Security module disconnected	Reconnect the security module to the USB port, and restart the software.

BD FACSDiva Software General Issues (continued)

Observation	Possible Causes	Recommended Solutions
Error message in Status tab	(See text in error message)	Follow instructions in the error message.
	Communication or fluidics error	Shut down the computer and cytometer, and restart them. If the message is displayed again, contact technical support. Provide the exact wording of the status message.
Faulty screen display, display objects distorted or unreadable	Non-standard DPI setting	Revert to the default DPI: Right-click the desktop and select **Properties**. Click the **Settings** tab. Click the **Advanced** button. With the **General** tab selected, use the menu to set the DPI to *96* (normal size).

BD FACSDiva Software Cytometer Setup

Observation	Possible Causes	Recommended Solutions
Error creating compensation tubes	Naming conflict with existing tube or worksheet	**1** Locate the tube or worksheet named *(ParameterName)* Stained Control, and change the name. **2** Create the compensation tubes again.
Error calculating compensation	PMT voltages inconsistent between compensation tubes	Re-record all compensation tubes with the same PMT settings.
	Wrong tube run for stained control tube	Re-record all compensation tubes and recalculate.
	Insufficient separation between positive and negative populations	Adjust compensation to see if it improves. If it does not, re-record the compensation tubes, draw new gates, and calculate compensation again. If automatic compensation fails, perform manually.

BD FACSDiva Software Acquisition

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Acquire, acquisition indicator lights are blinking as expected (viewing normal worksheet)	Current tube pointer not on current tube	Move the current tube pointer in front of the appropriate tube.
	Viewing plots for a different tube	Double-click the current tube in the **Browser** to display the plots for that tube.
	Incorrect population(s) in plot	Right-click the plot and select **Show Populations**. Inspect the populations.

BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
(Viewing normal or global worksheet)	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
	Detector voltage too low	Increase the voltage.
No events in plots after clicking Acquire. Acquisition indicator lights, except for those corresponding to threshold parameters, are blinking as expected.	Threshold too low or too high	Adjust the threshold.
	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.

BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Acquire, NO acquisition indicator lights are blinking	Cracked or defective tube	⚠ Data could be lost when transferring to a new tube. • If using a BD Trucount tube, do not transfer sample to a new BD Trucount tube because that would result in incorrect absolute counts. Re-stain the sample and use a new BD Trucount tube. • If using a non-BD Trucount tube, transfer sample to a new, non-BD Trucount tube. • Make sure you are using appropriate tubes. See System Requirements on page 31.
	SIT clogged	Clean the flow cell.
	Current cytometer configuration different from optical setup	Verify that the cytometer configuration corresponds to the cytometer's optical setup. If it does not, contact your System Administrator.
	Incorrect sample preparation	Re-stain and re-run samples.
	Indicator light switch off	Turn the indicator light switch on.
	Laser delay set incorrectly	Contact your lab manager to correct the laser delay settings.
	Area scaling factor set incorrectly	Contact your lab manager to correct the area scaling factor settings.

BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
Fluorescence signal missing	Current cytometer configuration different from optical setup	Verify that the cytometer configuration corresponds to the cytometer's optical setup, and the parameter list includes appropriate parameters.
	Laser delay set incorrectly	Contact your lab manager to correct the laser delay settings.
	Area scaling set incorrectly	Contact your lab manager to correct the area scaling settings.
	Fluorescence threshold set too high	Adjust the threshold.
	Incorrect sample preparation	Re-stain and re-run samples.
Unexpected results after clicking Next	Current tube pointer on wrong tube	Put the current tube pointer in front of the appropriate tube before clicking **Next**.
Unexpectedly high event rate	Threshold too low	Increase the threshold.
	Sample flow rate too high	Decrease the flow rate.
	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.
Unexpectedly low event rate	Threshold too high	Decrease the threshold.
Erratic event rate	Sample aggregates	Filter the sample.
Distorted scatter parameters	Cytometer settings adjusted incorrectly	Optimize the scatter parameters.
	Bubbles in flow cell	Check for bubbles. If found, run De-Gas Flowcell.

BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
Excessive amount of debris in plots	Threshold set too low	Increase the threshold.
	Dirty sheath filter	Replace the sheath filter.
	Dirty flow cell	Clean the flow cell. See page 168.
High CVs	Sample flow rate too high	Decrease the flow rate.
	Window extension set incorrectly	Contact the lab manager to set the window extension to 7.0.
Acquisition stops	High event rate (>10,000 events/sec) with more than 8 compensated parameters	• Increase the threshold. • Decrease the voltage on the threshold parameter. • Dilute the sample. • Define only channels of interest in the parameter list.
Fewer events than expected in gated population	Window extension set incorrectly	Contact the lab manager to set the window extension to 7.0.
	On-axis events left out of gate	Include events on the axis.
	Gates improperly set	Check the gating strategy.
	Dirty sheath filter	Replace the sheath filter.
	Dirty flow cell	Clean the flow cell. See page 168.
High electronic abort rate (>10% of system event rate)	Event rate too high	Decrease the flow rate.
	Sample too concentrated	Dilute the sample.
	Threshold set too low	Increase the threshold.
	Window extension set incorrectly	Contact the lab manager to set the window extension to 7.0.

BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
Increasing threshold results in decreased area signal	Window extension set too low	Contact the lab manager to set the window extension to 7.0.
Area measurement off-scale while height measurement is on scale	Area scaling set too high	Contact your lab manager to correct area scaling settings.

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Appendix A

Technical Specifications

This appendix contains information for the following specifications:

For barcode reader specifications, see the information supplied by the manufacturer.

Cytometer Specifications

Description	Specification
Dimensions	Height: 64 cm (25.2 in.) Width: 91 cm (35.7 in.) Depth: 61 cm (24 in.)
Workspace dimensions	Height (with flow cell access door open): 85 cm (33.5 in.)
	Unit designed to fit a lab bench 55.9 cm (22 in.) deep.
Operational clearances, cytometer	Left side: 30 cm (11.8 in.) between the unit and other objects or a wall to permit proper air flow and access to the main power button and circuit breaker
	Right side: 30 cm (11.8 in.) between the unit and other objects or a wall to permit proper air flow
	Top: 22.5 cm (8.9 in.) between the unit and other objects or a wall to permit opening of the flow cell access door
Weight	Less than or equal to 146 kg (320 lb), cytometer only, excluding the Loader and computer
	Maximum 168 kg (370 lb), including the Loader
Power requirements	100/115/230 VAC (50–60 Hz)
	Current: 5 A at 115 VAC 2.5 A at 230 VAC
Power consumption	500 W

Environment

Description	Specification
Storage temperature	5–40°C (41–104°F)
Operating temperature	16–31°C (59–86°F)
Operating relative humidity	20–80% (noncondensing)
Noise level	Less than or equal to 62 dBA
Facilities	No special room requirements

Performance

Description	Specification
Fluorescence threshold sensitivities	FITC <100 MESF PE <50 MESF
Forward and side scatter sensitivity	Platelets can be resolved from noise
Forward scatter sensitivity	1 micron
Side scatter sensitivity	0.5 micron

Optics

Laser Specifications

The following Class 3B lasers are mounted on the BD FACSCanto II cytometer.

Laser	Wavelength (nm)	Power (mW)
Blue	488	20
Red	633	17
Violet	405	30

These lasers are contained within the cytometer. Therefore the BD FACSCanto II flow cytometer is a Class 1 laser product.

Excitation Optics

Description	Specification
Optical platform	Fixed optical assembly
Beam geometry (all lasers)	9 x 65-µm elliptical beam

Emission Optics

Description	Specification
Collection lens	Optical gel-coupled to flow cell Numerical aperture (NA) = 1.2
Fluorescence detection	6 to 8 photomultiplier tube detectors: Wavelength ranges detected from the 488-nm laser: • 750–810 nm (PE-Cy7) • 670–735 nm (PerCP-Cy5.5) • 610–637 nm (PE-Texas Red®, optional) • 564–606 nm (PE) • 515–545 nm (FITC) Wavelength ranges detected from the 633-nm laser: • 750–810 nm (APC-Cy7) • 701–723 nm (Alexa Fluor® 700, optional) • 650–670 nm (APC) Wavelength ranges detected from the 405-nm laser: • 502–535 nm (AmCyan) • 425–475 nm (Pacific Blue™)
Forward scatter detection	Photodiode with 488/10 bandpass filter
Side scatter detection	PMT with 488/10 bandpass filter

Fluidics

Description	Specification
General operation	Integrated fluidics cart with automated startup, shutdown, and cleaning cycles
Sheath consumption	1.1 L/h, normal operation Less than1.0 mL/h, standby
Sample flow rates	Assay dependent, controlled automatically by BD FACSCanto clinical software. Nominal rates: Low = 10 µL/min Medium = 60 µL/min High = 120 µL/min
Sample acquisition rate	10,000 events/sec with <10% abort rate (8 parameters)
Recommended maximum particle size	50 µm

Signal Processing

Description	Specification
Workstation resolution	262,144-channel resolution
Data acquisition channels	8 to 10 parameters: 6 to 8 fluorescence and 2 scatter parameters
Fluorescence compensation	No limit to inter- and intra-beam compensation
Pulse processing	Height, area, and width measurements available for any parameter (BD FACSDiva software)
Time	Can be correlated to any parameter
Channel threshold	Available for any parameter from all lasers

Fluidics Cart Specifications

Description	Specification
Dimensions	Height: 64 cm (25.2 in.) Width: 79 cm (31.1 in.) Depth: 61 cm (24 in.)
Operational clearances	Fluidics cart, side air vent: 20 cm (7.9 in.) between the air vent and other objects or a wall to permit proper air flow
	Fluidics cart, door air vent: 20 cm (7.9 in.) between the door and other objects or a wall to permit proper air flow
Weight	Less than or equal to 51 kg (112 lb), fluidics cart only, excluding tanks
	Less than or equal to 82 kg (180 lb), with tanks full
Facilities	No air supply or vacuum required

Capacity

Description	Specification
BD FACSFlow cubitainer	20 L
BD FACS cleaning solution cubitainer	5 L
BD FACS shutdown solution cubitainer	5 L
Waste tank	10 L

BD FACS Loader Specifications

Description	Specification
Carousel compatibility	Loader carousels, numbers 1–16
Tube compatibility	
• Carousel	Accommodates up to 40 uncapped 12 x 75-mm tubes • Falcon polystyrene test tubes • BD Trucount tubes • BD FACS 7-color setup bead tubes
• Thickness of accumulated labels	Less than or equal to 0.125 mm (5 mils) no more than 3 labels thick

Appendix B

Assays

In the US, the following assays have been cleared for the BD FACSCanto II cytometer:

- BD Multitest™ 6-color TBNK with BD Trucount™ tubes
- BD Multitest™ 6-color TBNK without BD Trucount™ tubes
- BD Multitest™ IMK kit with BD Trucount™ tubes
- BD Multitest™ IMK kit without BD Trucount™ tubes
- BD™ HLA-B27
- BD™ Stem Cell Enumeration Assay

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