

# BD FACSCanto II Instructions For Use

**IVD** For In Vitro Diagnostic Use **CE**

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## **Patents**

PerCP: US 4,876,190

APC-Cy7: US 5,714,386

## 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, can 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 Règlement 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.

## History

Revision	Date	Change Made
640773 Rev. A	5/06	Initial release
642098 Rev. A	9/06	Incorporate changes for filing
642239 Rev. A	6/07	Updated for BD FACSDiva software v6.0



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# About These Instructions

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These instructions for use contain 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.

# Conventions

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The following tables list conventions used throughout this guide.

**Table 1** Hazard symbols<sup>a</sup>

Symbol	Meaning
	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death
	Risk of electric shock
	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
 <b>Tip</b>	Highlights features or hints that can save time and prevent difficulties
<i>Italics</i>	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, “choose File > Print” means to choose Print from the File menu.
Ctrl-X	When used with key names, a dash means to press two keys simultaneously. For example, Ctrl-P means to hold down the Control key while pressing the letter <i>p</i> .

## Introduction

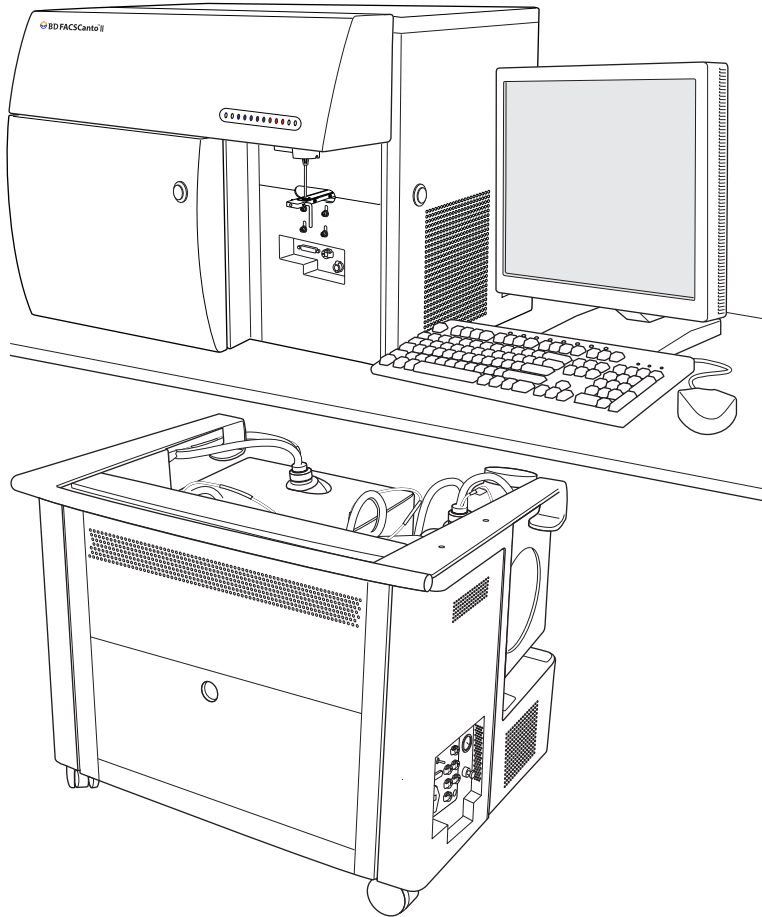
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The BD FACSCanto II system is intended for use as an In Vitro Diagnostic device for identification and enumeration of lymphocyte subsets in human cells in suspension for flow cytometry.

# System Components and Theory of Operation

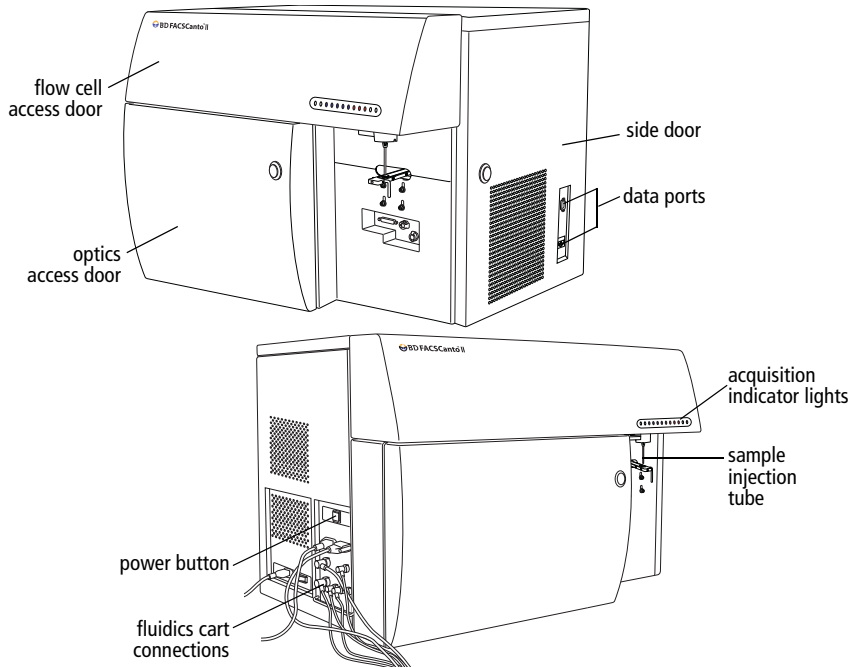
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The BD FACSCanto II system consists of a flow cytometer, a self-contained fluidics cart, and the BD FACSCanto II workstation. System options include an automated sample loader and a barcode reader.



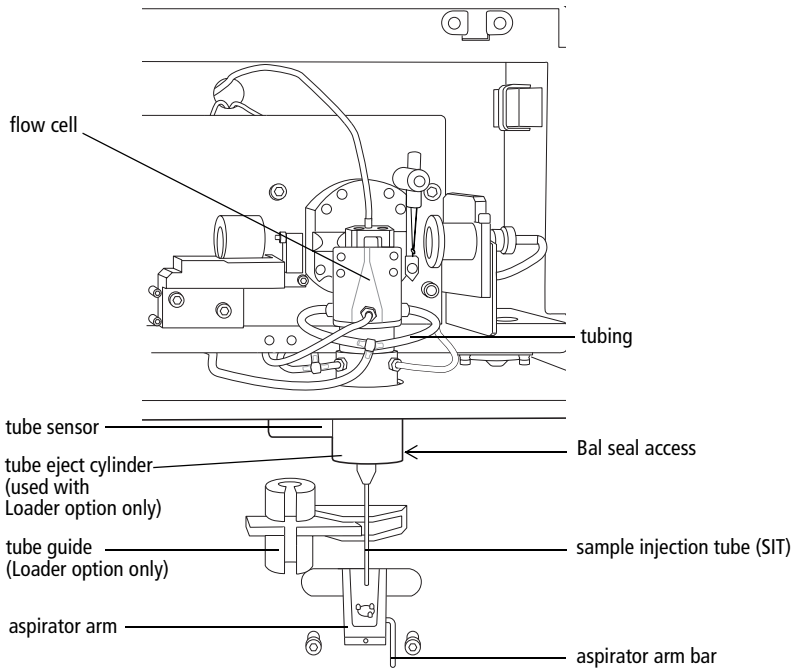
# Flow Cytometer Components

**Figure 1-1** BD FACSCanto II flow cytometer



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

## Fluidics Components



## Fluidics Theory of Operation

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.

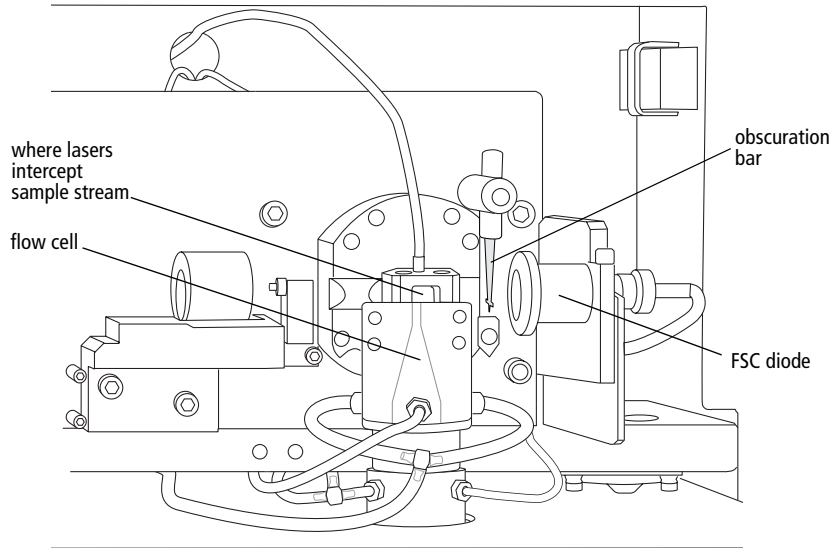
When you remove tubes from the SIT, the cytometer cleans the SIT by flushing sheath solution down the inside and outside of the tube. The flushed sheath solution is aspirated by the aspirator arm.

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 deselecting the SIT Flush checkbox on the Acquisition Dashboard.



## Optics Components and Theory of Operation

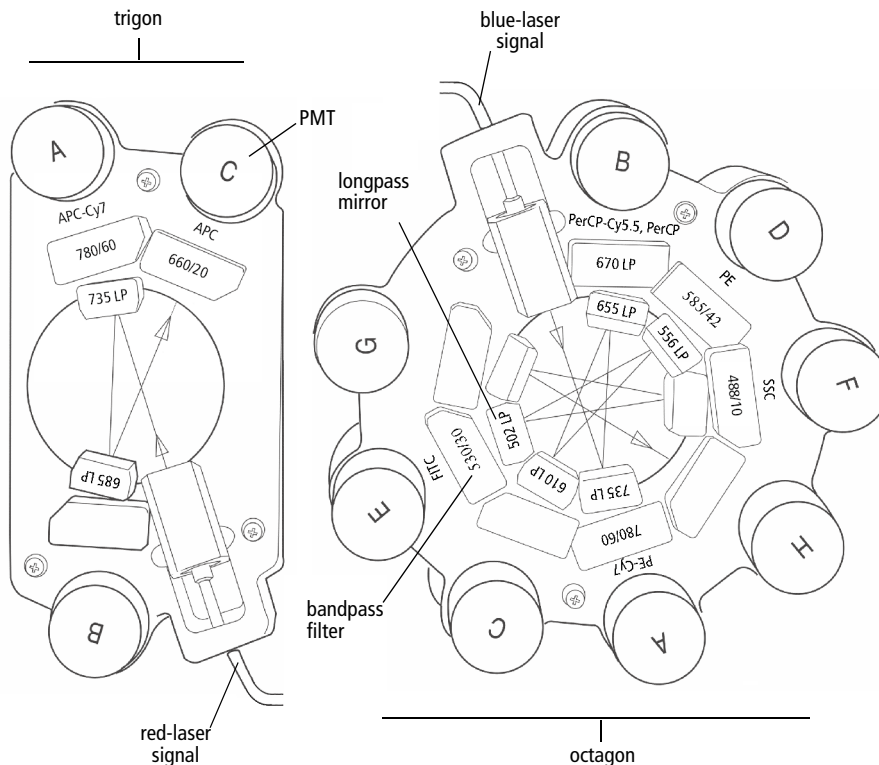
Once the sample moves into the flow cell, particles move in single file through the laser beams. The scattered and emitted light from these particles provides information about their size, shape, granularity, and fluorescence properties.



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 (a 4-2 optical configuration).

In the 4-2 configuration, 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. The trigon contains two PMTs and detects light excited by the 633-nm (red) laser. The 4-2 configuration octagon and trigon arrays have the filter and mirror combinations shown in Figure 1-2, and Table 1-1 on page 19.

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



**Table 1-1** Trigon and octagon optical filters (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 <sup>TM</sup> 7
	B	655	670	PerCP-Cy <sup>TM</sup> 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

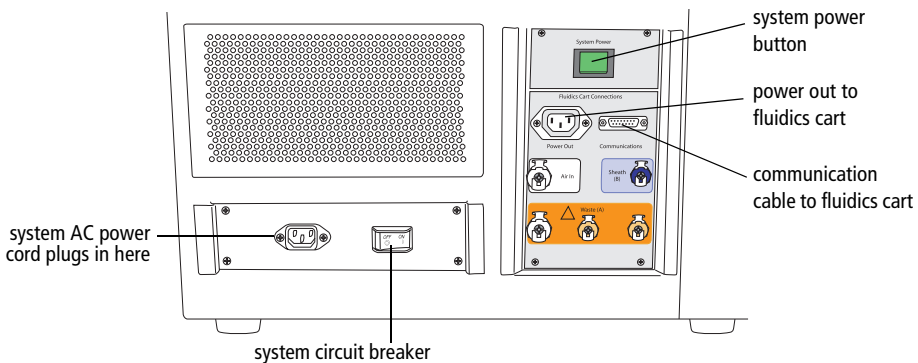
Blank optical holders do not contain optical filters. They are used in the trigon and octagon to prevent unwanted light from interfering with fluorescence signal.

## Electronics Components

Power to the cytometer, lasers, and fluidics cart is supplied by a power cord from the cytometer plugged directly into a standard electrical outlet. BD recommends 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.

**NOTICE** 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

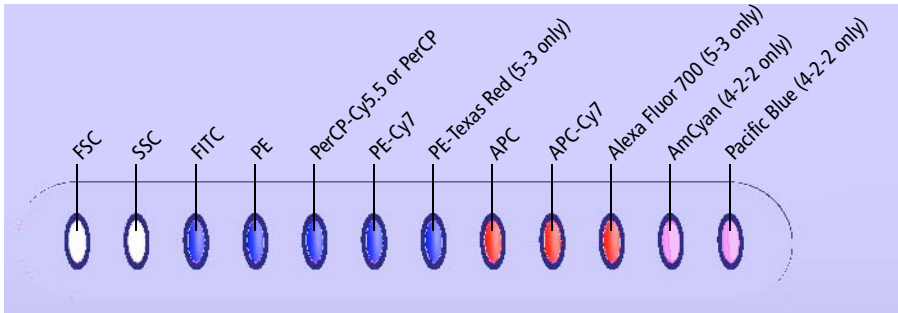


The system circuit breaker is located next to the AC power cord. The breaker will need to be reset if there is a power surge in the laboratory.

Acquisition indicator lights are located on the flow cell access cover on the front of the cytometer (see Figure 1-4 on page 21). 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. See Figure 1-5 on page 21.

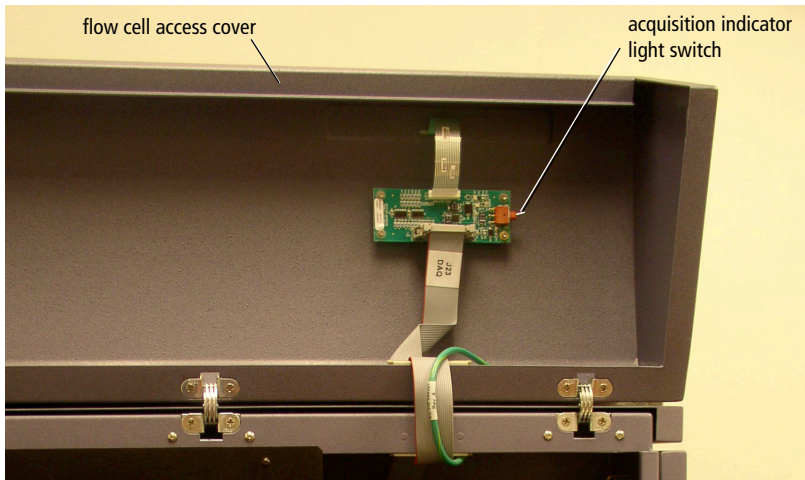
Lights are activated only when the system is acquiring data, and only the indicators corresponding to currently active parameters will blink.

**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.

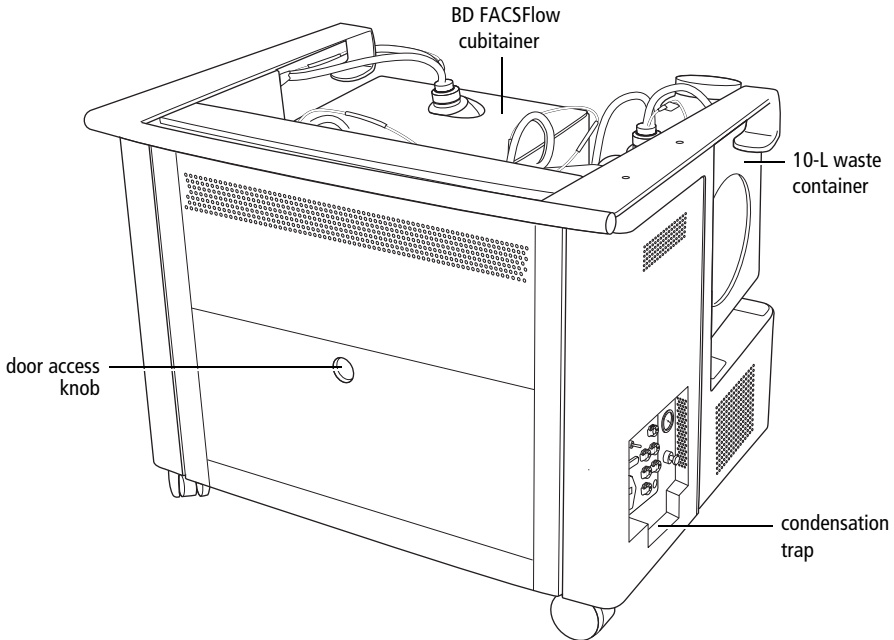
**Figure 1-5** Acquisition indicator light switch



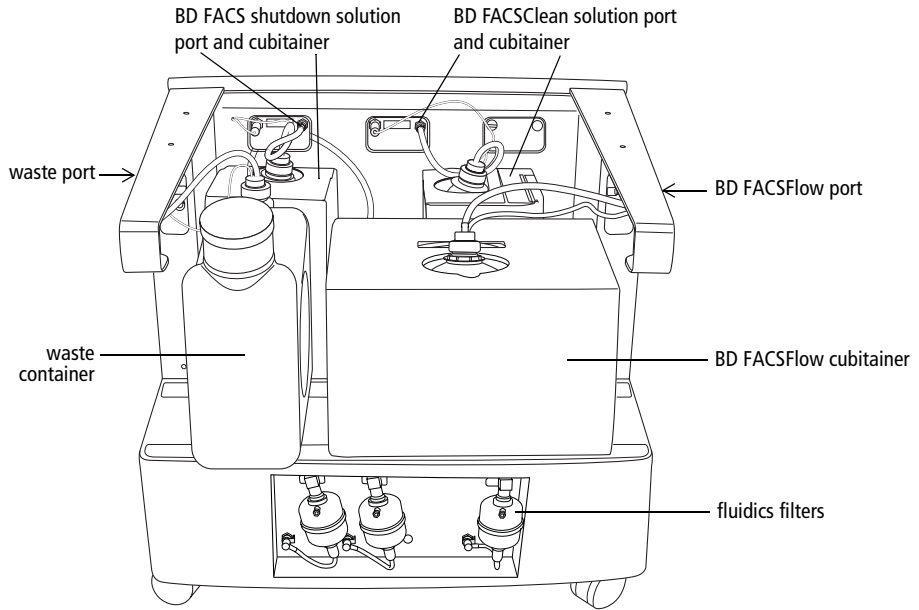
# Fluidics Cart

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



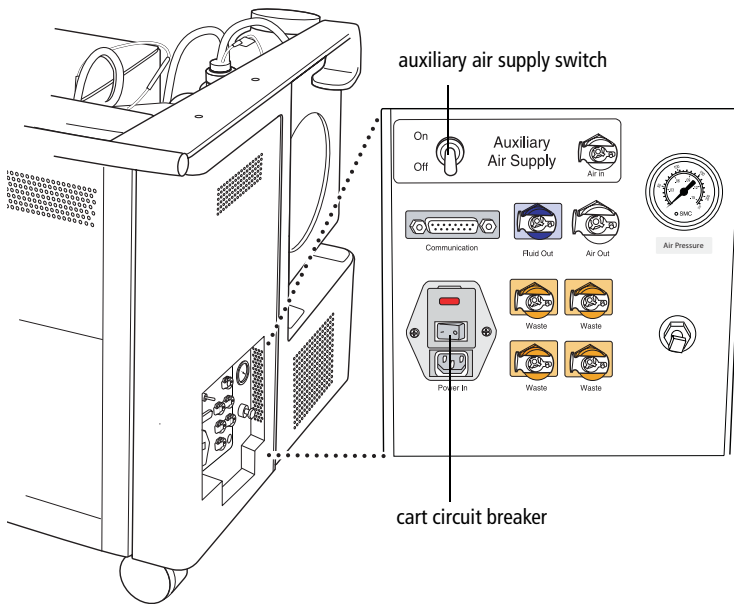
## Containers and Ports



## 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



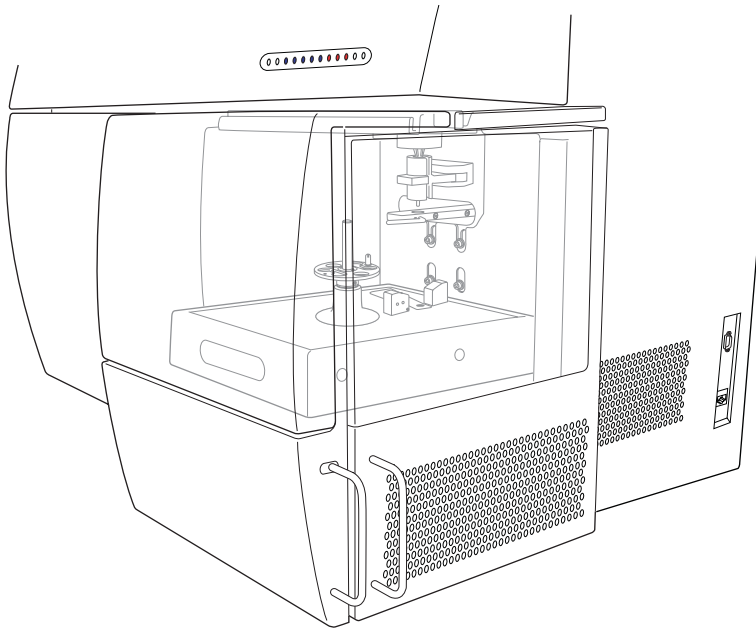
## Condensation Trap

The fluidics cart condensation trap is located beneath the controls panel (see Figure 1-6 on page 22). Empty the trap during the daily shutdown procedure.



## BD FACS Loader (Optional)

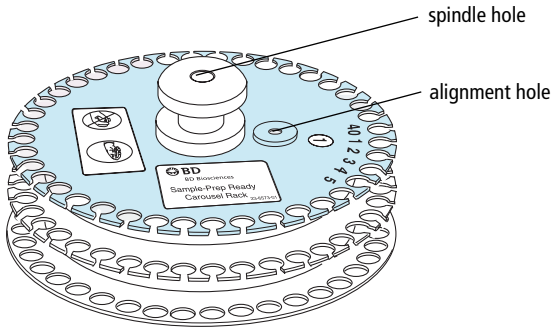
The BD FACS™ Loader automatically introduces prepared samples to the cytometer. Two sliding doors protect you from moving parts during operation. The Loader is controlled by BD FACSCanto clinical software or BD FACSDiva software.



## 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



**NOTICE** The Loader is compatible only with the green-tinted carousels labeled “Sample Prep Ready” (Figure 1-8).



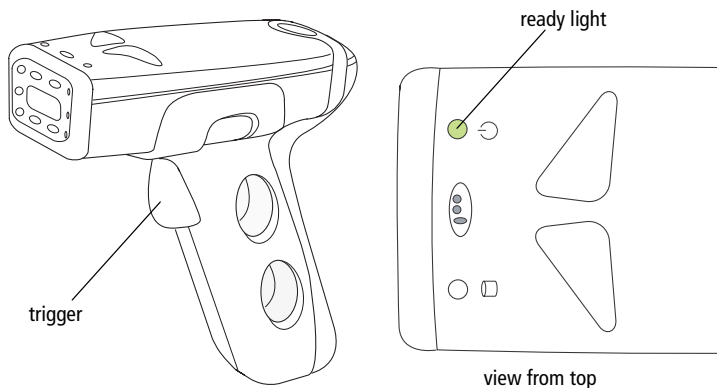
Not all manufactured 12 x 75-mm tubes have been checked for proper functionality on the Loader. BD Biosciences has validated only disposable, 12 x 75-mm BD Falcon polystyrene test tubes, BD Trucount tubes, and BD FACS 7-color setup bead tubes.

## Barcode Reader (Optional)

The Opticon LG2 Imager is a hand-held barcode reader that plugs into the USB port on the BD FACSCanto II computer workstation.

The barcode reader reads most barcode standards, including Codabar, Code 128, Code 39 with checksum, and PDF417. It reads information from the BD FACST<sup>TM</sup> 7-color setup beads label into BD FACSCanto clinical software, and also reads coded patient information into a worklist. For operating instructions, see Chapter 3 on page 39.

**Figure 1-9** Typical barcode reader (example)



# System Requirements

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## Software

Both included software packages must be installed:

- BD FACSCanto clinical software v2.1



Do not read FCS files created with v2.1 into previous versions of BD FACSCanto clinical software. Previous versions will show incorrect results.

- BD FACSDiva software v6.0

**NOTICE** BD FACSDiva software v6.0 must be installed for BD FACSCanto clinical software v2.1 to operate.

## Workstation

BD FACSCanto II workstation purchased through BD Biosciences

## Compatible Tubes

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

## Bulk Fluids

- BD FACSFlow solution
- BD™ FACSClean solution
- BD FACST™ 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



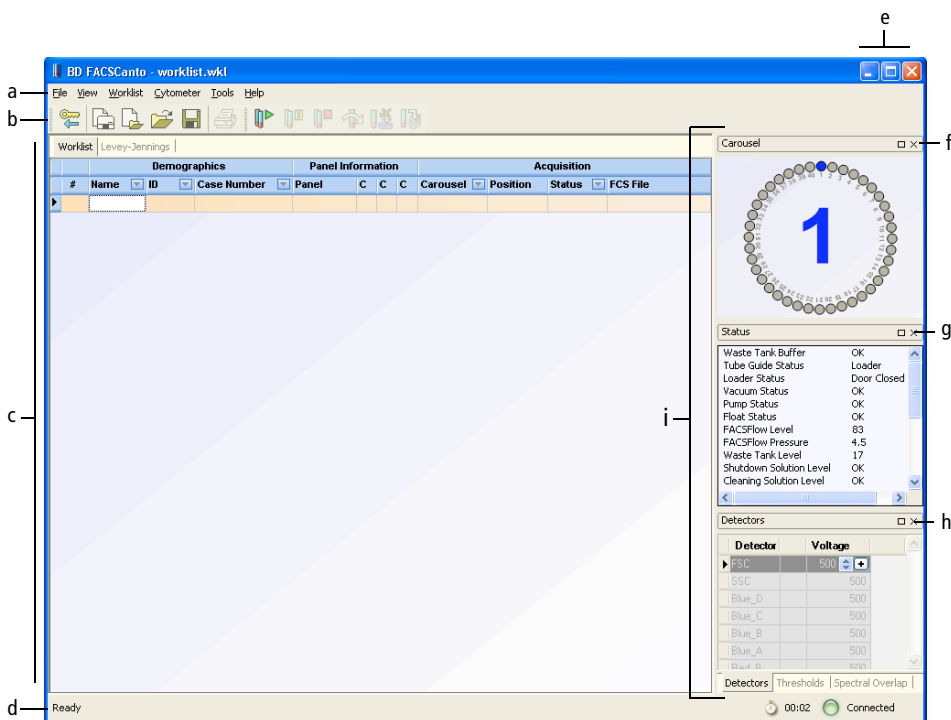
## Software Windows and Toolbars

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- BD FACSCanto Clinical Software Workspace on page 32
- BD FACSCanto Toolbars on page 33
- BD FACSDiva Software Workspace on page 34

# BD FACSCanto Clinical Software Workspace

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



**Table 2-2** Main window components

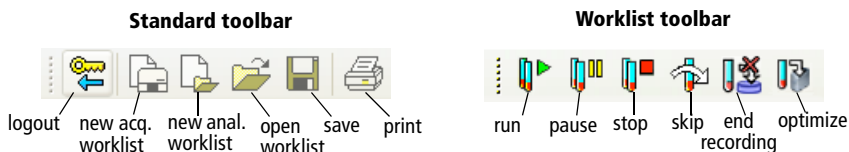
Component	Function
a. menu bar	Contains the File, View, Worklist, Cytometer, Tools, and Help menus
b. toolbars	Contains buttons that provide quick access to menu commands; see BD FACSCanto Toolbars on page 33.



**Table 2-2** Main window components (continued)

Component	Function
c. workspace	Displays the Worklist, Acquisition, Lab Report, and Levey-Jennings tabs, depending on where you are in the workflow
d. status bar	Provides information about the flow cytometer's current state, the cytometer-software connection, and the amount of time elapsed since logon
e. 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
f. Carousel window	Shows a graphic representation of a carousel rack and the rack ID of the currently selected sample
g. Status window	Provides information on the current status of the flow cytometer
h. cytometer control windows	Includes three tabs: <ul style="list-style-type: none"> <li>• Detectors tab</li> <li>• Thresholds tab</li> <li>• Spectral Overlap tab</li> </ul>
i. docking area	Provides a default home for the Carousel, Status, and cytometer control windows

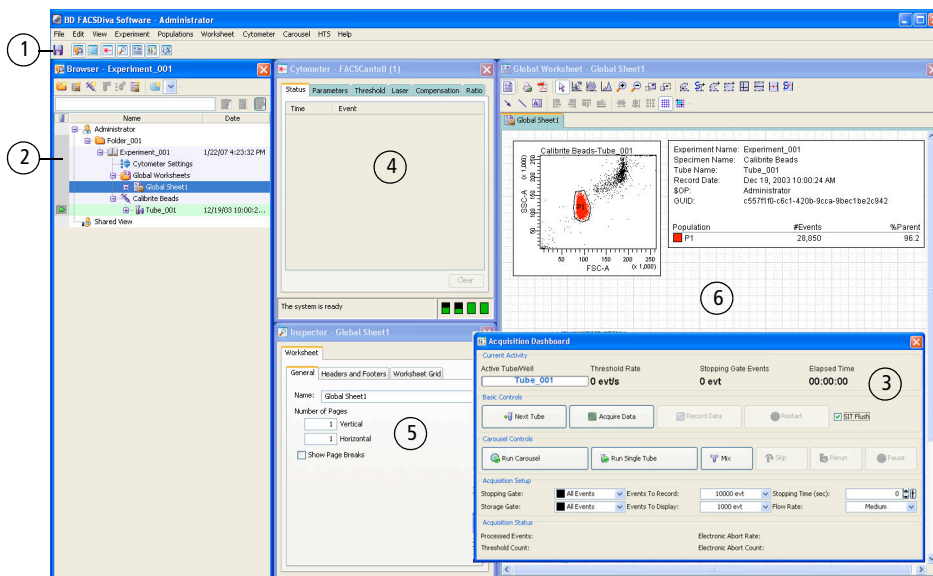
## BD FACSCanto Toolbars



# BD FACSDiva Software Workspace

After you log in, the workspace appears, showing the main application windows (Figure 2-10). Hide or show windows by clicking buttons in the Workspace toolbar (① in the figure).

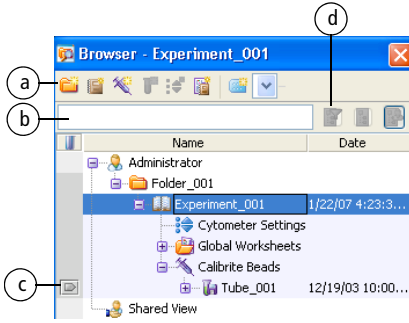
**Figure 2-10** BD FACSDiva workspace



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



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.

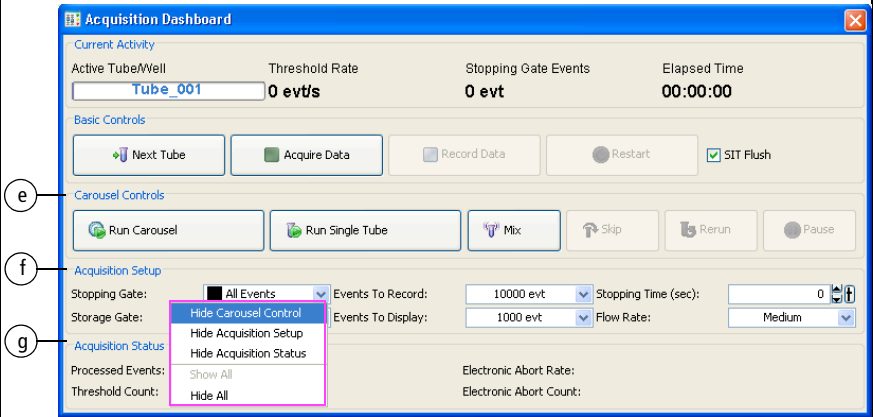
Only one experiment can be open at a time. In an open experiment, you can add, rename, or copy elements, and record or display data.

Click a button in 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




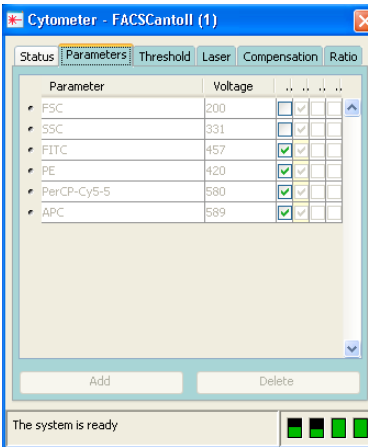

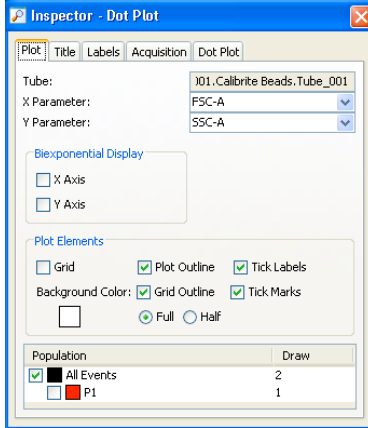
The **Acquisition Dashboard** contains controls for setting up, starting, monitoring data acquisition and recording, and running the Loader. Basic controls are always shown. Carousel Controls (e), Acquisition Setup (f), and Acquisition Status (g) can be hidden or shown by right-clicking anywhere on the 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.



e

f

g

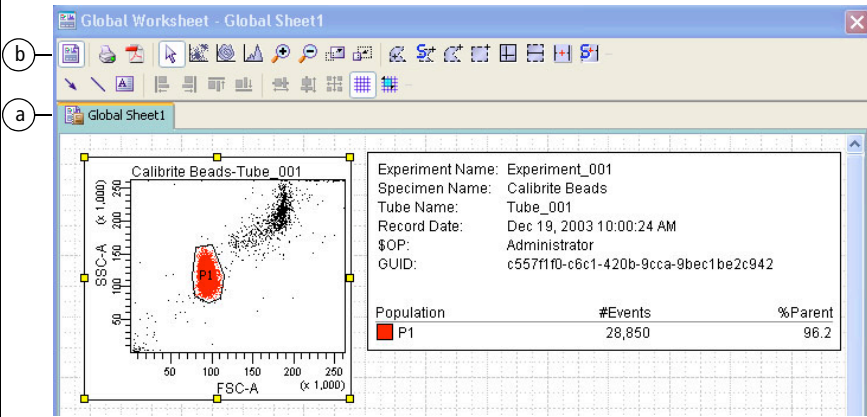
<p>4</p> 	 <table border="1" data-bbox="306 235 655 560"> <thead> <tr> <th>Parameter</th> <th>Voltage</th> <th></th> <th></th> <th></th> <th></th> </tr> </thead> <tbody> <tr> <td>FSC</td> <td>200</td> <td><input type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>SSC</td> <td>331</td> <td><input type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>FITC</td> <td>457</td> <td><input checked="" type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>PE</td> <td>420</td> <td><input checked="" type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>PerCP-Cy5-5</td> <td>580</td> <td><input checked="" type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>APC</td> <td>589</td> <td><input checked="" type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </tbody> </table>	Parameter	Voltage					FSC	200	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	SSC	331	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	FITC	457	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	PE	420	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	PerCP-Cy5-5	580	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	APC	589	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<p>The <b>Cytometer</b> 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.</p> <p>Additional controls might be displayed in this window depending on the cytometer you are running. Refer to your cytometer manual for a description.</p>
Parameter	Voltage																																											
FSC	200	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																																							
SSC	331	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																																							
FITC	457	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																																							
PE	420	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																																							
PerCP-Cy5-5	580	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																																							
APC	589	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																																							
<p>5</p> 	 <p>Tube: J01, Calibrite Beads, Tube_001</p> <p>X Parameter: FSC-A</p> <p>Y Parameter: SSC-A</p> <p><b>Biexponential Display</b></p> <p><input type="checkbox"/> X Axis</p> <p><input type="checkbox"/> Y Axis</p> <p><b>Plot Elements</b></p> <p><input type="checkbox"/> Grid <input checked="" type="checkbox"/> Plot Outline <input checked="" type="checkbox"/> Tick Labels</p> <p>Background Color: <input checked="" type="checkbox"/> Grid Outline <input checked="" type="checkbox"/> Tick Marks</p> <p><input type="checkbox"/> <input checked="" type="radio"/> Full <input type="radio"/> Half</p> <table border="1" data-bbox="315 982 651 1055"> <thead> <tr> <th>Population</th> <th>Draw</th> </tr> </thead> <tbody> <tr> <td><input checked="" type="checkbox"/> All Events</td> <td>2</td> </tr> <tr> <td><input type="checkbox"/> P1</td> <td>1</td> </tr> </tbody> </table>	Population	Draw	<input checked="" type="checkbox"/> All Events	2	<input type="checkbox"/> P1	1	<p>Use the <b>Inspector</b> 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.</p>																																				
Population	Draw																																											
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<input type="checkbox"/> P1	1																																											

6

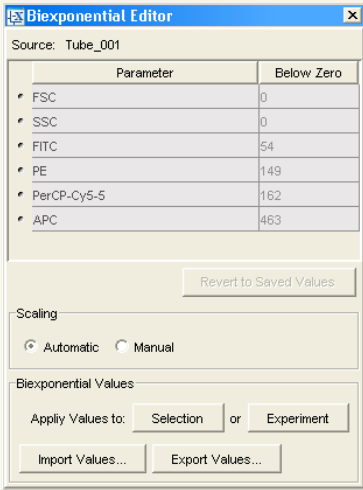
The **Worksheet** window is where you create global or normal worksheets containing plots, gates, statistics, and custom text.

Plots on a *global worksheet* show data for the tube selected with the current tube pointer. Global worksheets are indicated by green tabs (a). 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 in the Worksheet toolbar (b) to switch between the global or normal worksheets view. Then you can create analysis objects or text, or align objects on a worksheet.



EX



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.



# 3

## Barcode Reader Option

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This chapter contains the following information:

- Installing and Using the Barcode Reader on page 40
- Cleaning the Barcode Reader on page 42
- Barcode Symbologies on page 43

# Installing and Using the Barcode Reader

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

**NOTICE** The Opticon LG2 Imager 2D barcode reader available for the BD FACSCanto II system is capable of reading both 1D and 2D barcode symbologies. See Barcode Symbologies on page 43.

The BD FACSCanto II system supports only two uses of the 2D barcode reader:

- reading information from the BD FACS 7-color setup beads label into BD FACSCanto clinical software (2D barcode symbology)
- reading coded patient information from sample tube labels into a worklist (1D barcode symbology)

**NOTICE** The barcode reader supplied with the BD FACSTM Sample Prep Assistant II (SPA II) is a 1D reader that cannot read information from BD FACS 7-color setup beads. Use only the Opticon LG2 Imager 2D barcode reader for reading information from the BD FACS 7-color setup beads label into BD FACSCanto clinical software.

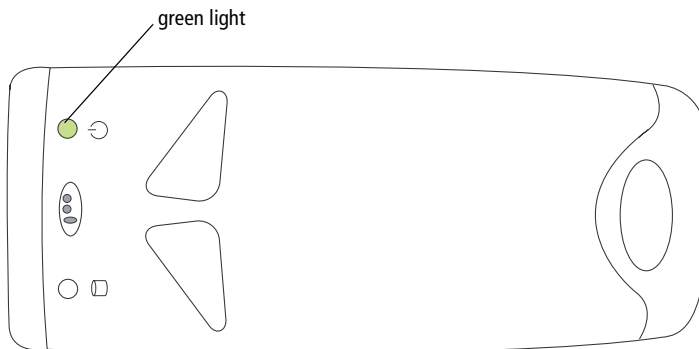
**NOTICE** Contact BD Biosciences before changing default barcode settings.



- 1 Plug the barcode reader into a USB port on your computer.

The reader turns on automatically.

- 2 Make sure the green light on top of the reader is illuminated:



- 3 In BD FACSCanto clinical software, navigate to the fields for which information can be scanned.

- Lot information for BD FACS 7-color setup beads is scanned into the Setup Lot Information dialog from the setup values label supplied with the setup bead kit (NOT from the lot number on the setup bead tubes). See Entering Lot Information with the Barcode Reader on page 53.
- Patient information is scanned from sample labels into the worklist ID field.

- 4 Locate the barcode on the setup values label of BD FACS 7-color setup beads kit, or on the patient sample.



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

- Tip** To make it easier to use, an optional stand is available for the barcode reader. See Barcode Reader Parts on page 229.

- 5 Aim the barcode reader at the center of the barcode. The barcode reader has two focal lengths, 10 cm and 23 cm (4 in. and 9 in.):
  - For reading BD FACS 7-color setup bead labels, hold the barcode reader 23 cm (9 in.) from the labels.
  - For reading patient information from sample tube labels, hold the barcode reader 10 cm (4 in.) from the labels.
- 6 Press and hold the trigger on the barcode reader until you hear a beep.

If the reader does not beep, adjust your distance from the barcode while continuing to hold the trigger.
- Tip** To obtain a reading, keep the bar code reader aimed at the center of the label. Do NOT sweep across the label.
- 7 Compare software field values with the setup beads or sample label.

## Cleaning the Barcode Reader

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

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 FACSClean solution or bleach to clean or disinfect the barcode reader.

# Barcode Symbologies

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Although data entry using barcodes is generally more reliable than manual data entry, it is not guaranteed to be 100% accurate. To increase accuracy, use of barcode symbology checksums is recommended.



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.

By default, the barcode reader has checksums enabled. We recommend you do not disable checksums, or use barcode symbologies without checksums.

## 1D Barcode Symbologies

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

## 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 is required to read all target values with one scan.

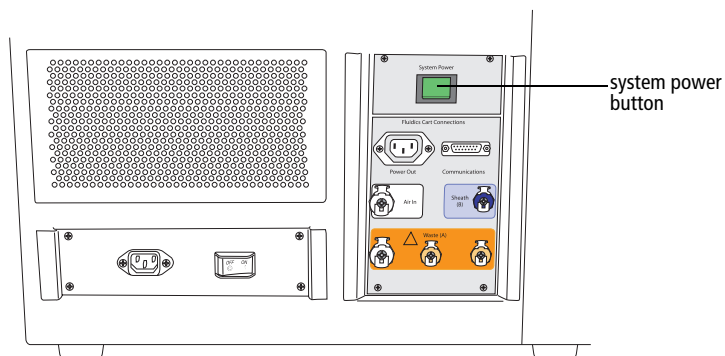
# 4

## Starting Up

This chapter describes cytometer startup for both BD FACSCanto clinical software and BD FACSDiva software.

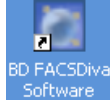
- 1 Turn on the power to the cytometer.

**Figure 4-1** Flow cytometer power panel

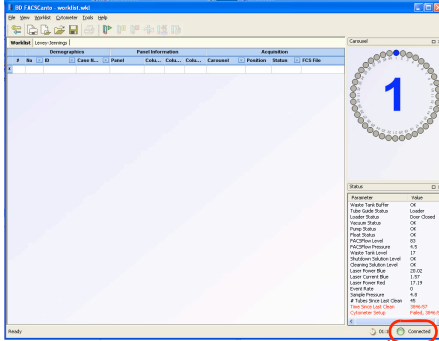


The system power button turns on power to the cytometer, fluidics cart, and lasers.

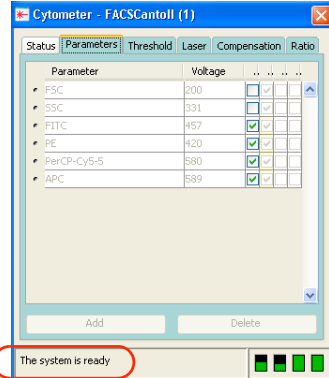
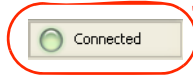
2 Start up the computer, launch the software, and log in.



3 Make sure the software is connected to the cytometer.



BD FACSCanto



BD FACSDiva

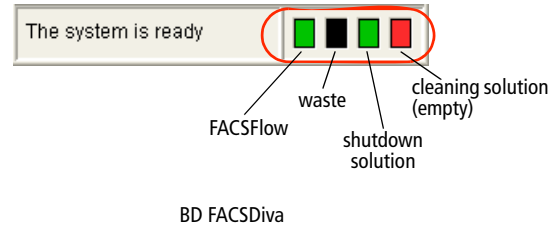
If needed, choose Cytometer > Connect.

#### 4 Check fluid levels.

Low fluid levels or a full waste container are indicated in red.

Parameter	Value
Waste Tank Buffer	OK
Tube Guide Status	Loader
Loader Status	Door Closed
Vacuum Status	OK
Pump Status	OK
Flood Status	OK
FACSFlow Level	17
FACSFlow Pressure	4.5
Waste Tank Level	83
Shutdown Solution Level	OK
Cleaning Solution Level	OK
Laser Power Blue	20.02
Laser Current Blue	1.57
Laser Power Red	17.19
Event Rate	0
Sample Pressure	4.8
# Tubes Since Last Clean	36
Time Since Last Clean	00:02
Cytometer Setup	Failed, 00:02

BD FACSCanto



#### 5 If fluidics startup does not start automatically, choose Cytometer > Fluidics Startup.



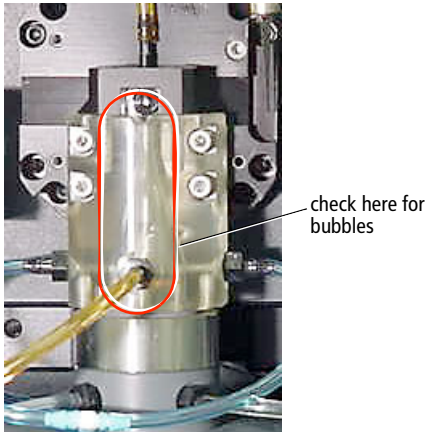
To prevent fluid overflow, make sure there is no tube on the SIT at startup.

#### 6 Click OK at the confirmation dialog.

#### 7 When Fluidics Startup finishes, click OK to close the dialog.

- Lift the flow cell access door to check the flow cell for air bubbles.

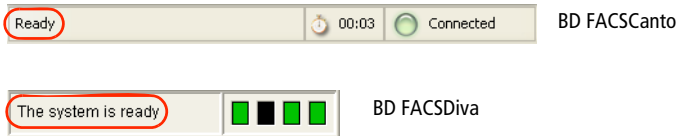
**Figure 4-2** Flow cell



- If you see bubbles choose Cytometer > Cleaning Modes > De-gas Flow Cell.
- Click OK when the completion message appears.
- If you still see bubbles, repeat.

An error message might appear when you open the flow cell access door. To dismiss the message, close the door and wait 30 seconds.

- Check that laser warmup has finished.





# 5

## Cytometer QC and Setup

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This chapter describes how to perform cytometer QC and setup.

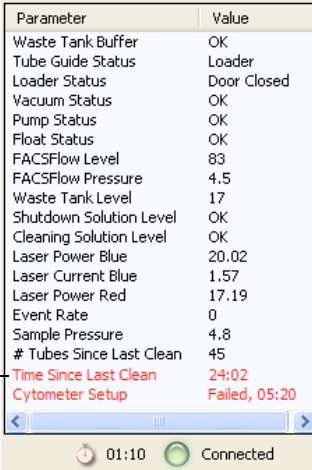
- Performing Automated Setup on page 50
- Optimizing with BD FACSCanto Clinical Software on page 64
- Optimizing with BD FACSDiva Software on page 76

# 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.



Parameter	Value
Waste Tank Buffer	OK
Tube Guide Status	Loader
Loader Status	Door Closed
Vacuum Status	OK
Pump Status	OK
Float Status	OK
FACSFlow Level	83
FACSFlow Pressure	4.5
Waste Tank Level	17
Shutdown Solution Level	OK
Cleaning Solution Level	OK
Laser Power Blue	20.02
Laser Current Blue	1.57
Laser Power Red	17.19
Event Rate	0
Sample Pressure	4.8
# Tubes Since Last Clean	45
Time Since Last Clean	24:02
Cytometer Setup	Failed, 05:20

time since last setup

01:10 Connected

**NOTICE** If the software fails to respond during acquisition and you need to restart the software, perform a fluidics startup prior to resuming acquisition. See BD FACSCanto Software General Issues on page 199 for more information.

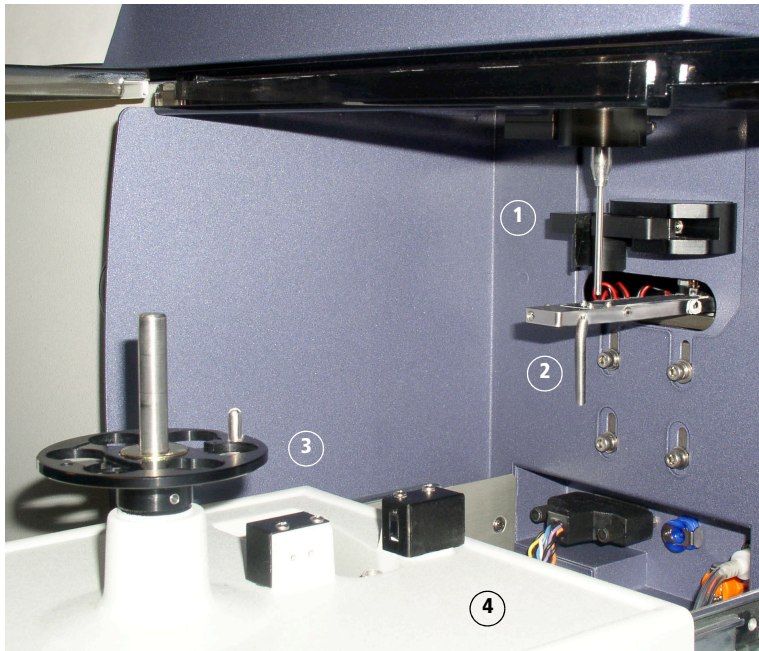
## Running Setup Using Manual Loading

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

 Do not use expired beads. 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 Choose Cytometer > Setup > Standard Setup.
- 4 Select the current bead lot from the Lot ID menu.

**Figure 5-1** Setup Lot Information window

**Cytometer Setup Wizard - Setup Lot Information**

**Bead Lot Information**  
Select the lot information for your bead product.

**Lot ID:**  
D1656

**Bead Product:**  
BD FACS 7-Color  
Setup Beads  
(335775)

**Exp. Date:**  
2005-03-31

New Lot ID  
Scan Barcodes

Detector	Target Value
FSC	544
SSC	544
FITC	473
PE	420
PerCP	410
PerCP-Cy5-5	482
PE-Cy7	447
APC	523
APC-Cy7	426

Cancel < Back Next > Finish

- 5 Check lot ID, targets, and spectral overlap factors in the Cytometer Setup Wizard against the BD FACS 7-color setup beads label. If necessary, enter new values into the Wizard.

See Figure 5-2 on page 53 for an example BD FACS 7-color setup beads label.

**Figure 5-2** Setup beads label (example)

**BD FACSTM 7-Color Setup Beads**

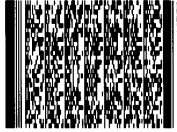
(Cat. No.)  
REF 335775

(Exp.) 2006-12-31

LOT 16472

**Targets**

Scatter/ Fluorophore	Target Value
FSC	468
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	88	100	100
PerCP	97	91	100	100	93	98	90
PerCP - Cy5.5	97	91	100	100	93	98	90
PE - Cy7	100	85	94	82	100	100	86
APC	100	100	88	80	100	100	113
APC - Cy7	100	100	78	72	95	83	100

23-7383-01

- To enter values with the barcode reader, go to the next section.
- To enter values with the keyboard and mouse, go to page 55.

If you do not need to enter new values, go to Loading Tubes on page 56.

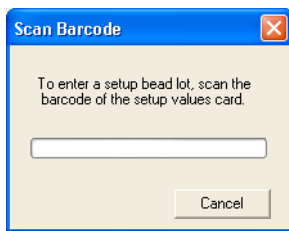
## Entering Lot Information with the Barcode Reader

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  in the Setup Lot Information window of the Wizard (Figure 5-1).

The Scan Barcode dialog appears (Figure 5-3).



**Figure 5-3** Scan Barcode dialog





- 2 Locate the barcode on the BD FACS 7-color setup beads label.

See Figure 5-2 for an example label.

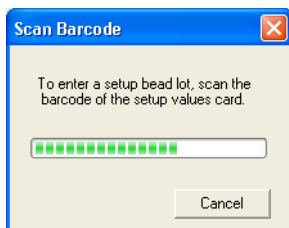
- 3 Hold the barcode reader 23 cm (9 in.) from the BD FACS 7-color setup beads label and aim the barcode reader at the center of the barcode.

  If the barcode reader is used in a manner not specified by BD Biosciences, the inherent safeguards provided may be impaired.

- 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 Tubes on page 56.

## Entering New Lot Information with the Keyboard

- 1 Click  in the Setup Lot Information window of the Wizard (Figure 5-1 on page 52).
- 2 Choose the bead product, enter the lot ID and the expiration date, and click OK.

The lot ID and expiration date are on the setup beads label.



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.

Targets		Spectral Overlap Factors	
Detector		Target Value	
FSC		458	
SSC		544	
FITC		473	
PE		428	
PerCP		410	
PerCP-Cy5.5		482	
PE-Cy7		447	
APC		523	
APC-Cy7		428	

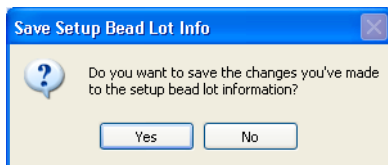
- 4 Click the Spectral Overlap Factors tab, and enter the spectral overlap factors for the bead lot.

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

Targets		Spectral Overlap Factors						
Detector	FITC	PE	PerCP	PerC...	PE-Cy7	APC	APC-...	
FITC	100	95	100	100	100	100	100	
PE	101	100	100	100	62	100	100	
PerCP	97	91	100	100	69	98	100	
PerCP-Cy5.5	97	91	100	100	69	98	100	
PE-Cy7	100	65	94	82	100	100	89	
APC	100	100	68	80	100	100	144	
APC-Cy7	100	100	76	32	91	83	100	

## Loading Tubes

- 1 Click .
- 2 If the Save Setup Bead Lot Info dialog appears, click .



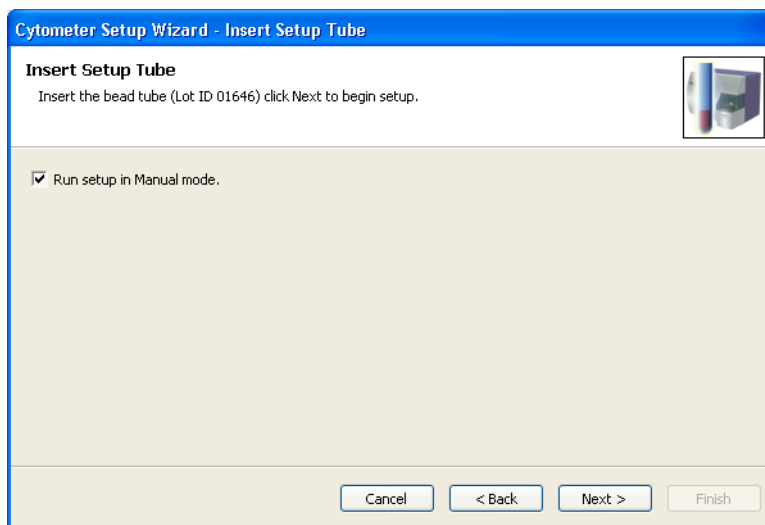
This dialog appears only if you changed lot ID values.



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

See Figure 5-4 on page 57.

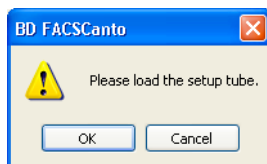


**Figure 5-4** Insert Setup Tube dialog



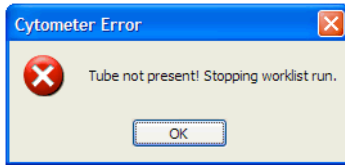
  Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

- 4** When prompted, load the beads tube onto the SIT, using these steps:
  - Push the aspirator arm to the left.
  - 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.
  - 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.

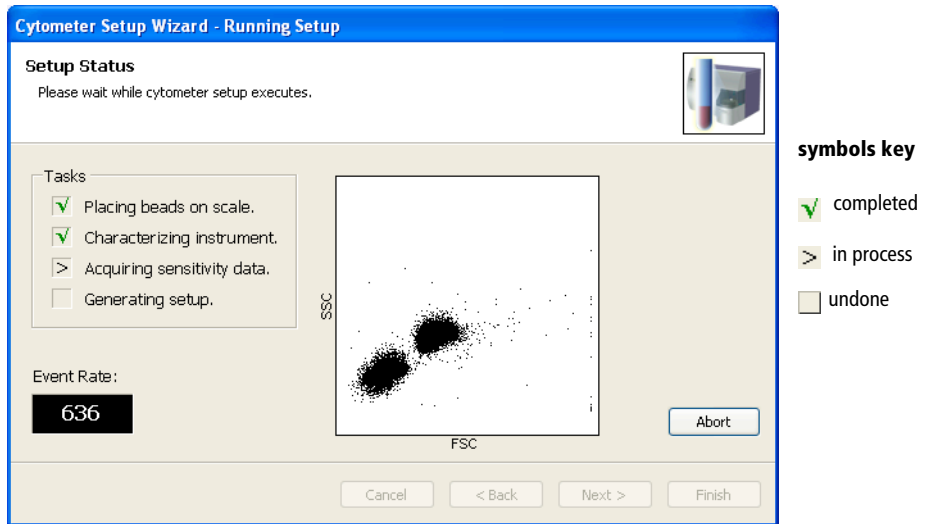


- 5 Click .

If the tube is not fully seated, the following error message appears. Click OK. Remove the tube and repeat step 4 to properly reinstall it.

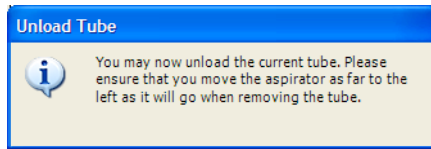


- 6 Wait for setup to finish.



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.

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



- Hold the sample tube while pushing the aspirator arm to the left.
- Remove the tube from the SIT.
- Release the aspirator arm.

SIT cleaning occurs when the aspirator arm comes to center.

8 Decide what to do next:

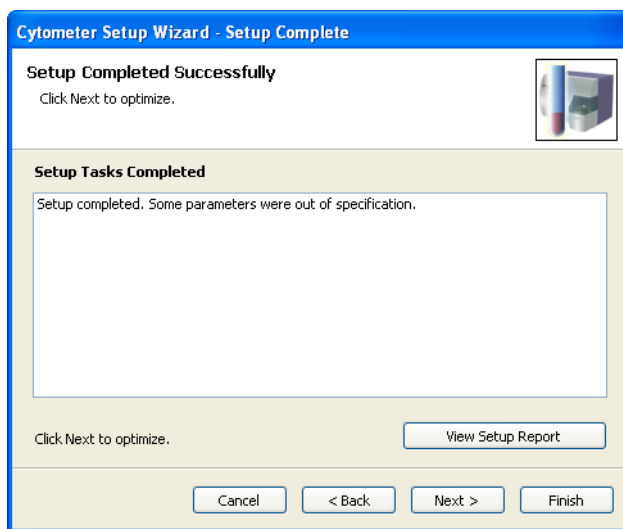


Incorrect values are saved if you accept a setup that is unsuccessful or setup results that are out of range.






- If setup is unsuccessful or if setup results are out of range, **do not** click Finish. Note the message provided by the software (eg, Figure 5-5 on page 60) and consult BD FACSCanto Clinical Software Troubleshooting on page 199.
- If setup is successful, see Table 5-1 on page 60.

**NOTICE** It is important to monitor setup data for trends. See Reviewing Levey-Jennings Reports on page 74.



**Figure 5-5** Successful Setup Complete dialog



**Table 5-1** Options after Setup

To...	Click...	Additional Information
View setup results		The report contains cytometer QC and pass/fail information. You can print the report from this view.
Discard current results		You will be given the option to use the last setup results.
Run setup again		
Optimize setup values using BD FACSCanto clinical software	 When prompted, click  to save your results and continue.	Proceed to Optimizing with BD FACSCanto Clinical Software on page 64.

**Table 5-1** Options after Setup

To...	Click...	Additional Information
Exit setup and save new setup results		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 C:\Program Files\BD FACSCanto Software\SetupReports folder.
(Optional) Save setup results and optimize setup values using BD FACSDiva software		Proceed to Optimizing with BD FACSDiva Software on page 76.

## Running Setup Using the Loader


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

**NOTICE** Do not use setup beads after their expiration date. Doing so can cause incorrect setup results.


- 2 Prepare the cytometer for automatic loading (Figure 8-3 on page 136).
- 3 Choose Cytometer > Setup > Standard Setup.

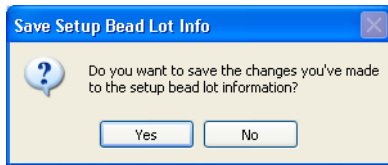
The Cytometer Setup Wizard appears.

- 4 Select the current bead lot from the Lot ID drop-down menu.
- 5 Check the Lot ID, Targets, and Spectral Overlap Factors in the Cytometer Setup Wizard against the BD FACS 7-color setup beads label.

- 6 Enter new values into the Wizard; Click  .

See page 53 for how to enter information with the barcode reader.

- 7 If the Save Setup Bead Lot Info dialog appears, click  .




This dialog appears only if you changed lot ID values.

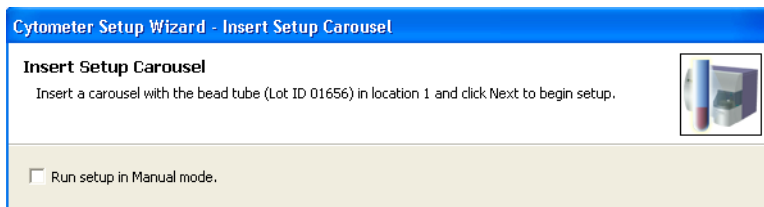
- 8 Place the bead tube in position 1 of a carousel and the optimization tubes in the positions following.

For instructions, see step 3 on page 137.

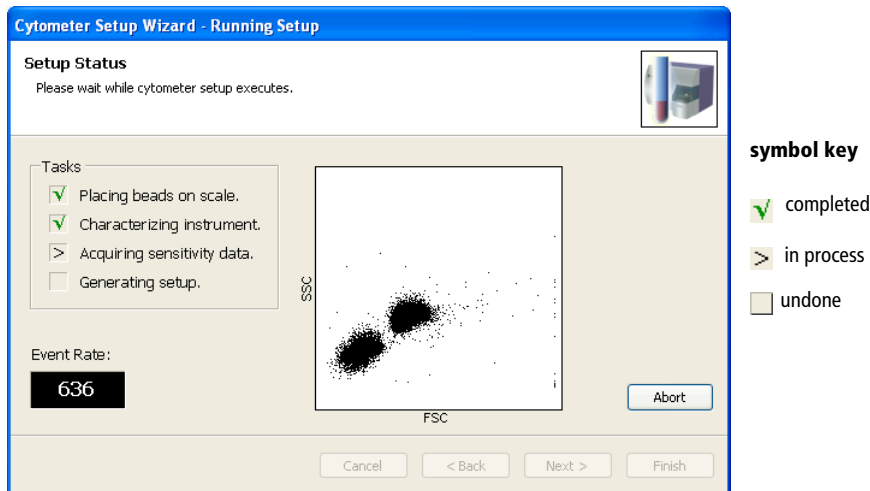
- 9 Install the carousel onto the Loader.

For instructions, see step 4 on page 137 and step 5 on page 138.

- 10 Deselect *Run setup in Manual mode*, and click  .



**11** 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.

**12** Decide what to do next:



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 5-5 on page 60) and consult BD FACSCanto Clinical Software Troubleshooting on page 199.
- If setup is successful, see Table 5-1 on page 60.

**13** It is important to monitor setup data for trends. See Reviewing Levey-Jennings Reports on page 74.

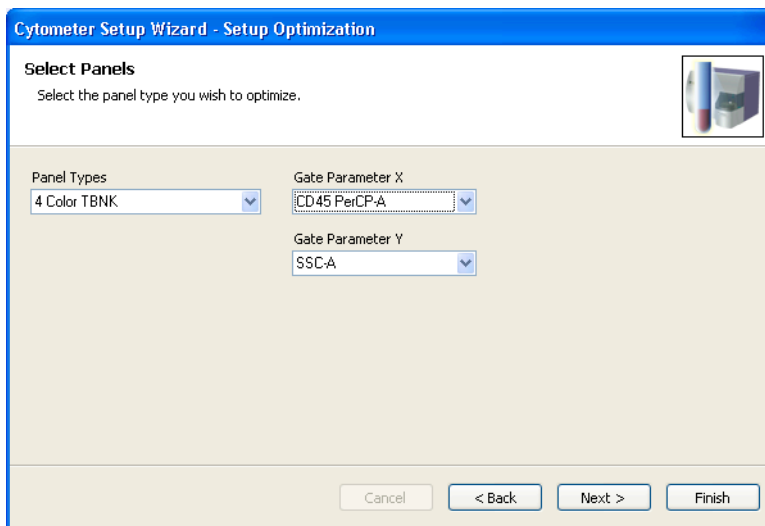
**14** If you are using BD FACSDiva software to acquire samples, close BD FACSCanto clinical software.

# 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 this panel type.


**NOTICE** If the software fails to respond during acquisition and you need to restart the software, perform a fluidics startup prior to resuming acquisition. See BD FACSCanto Software General Issues on page 199 for more information.

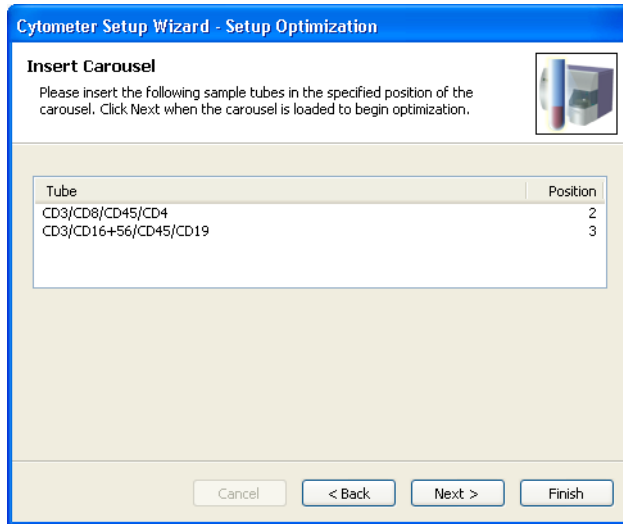
- 1 Select a panel type and parameters from the menus and click .



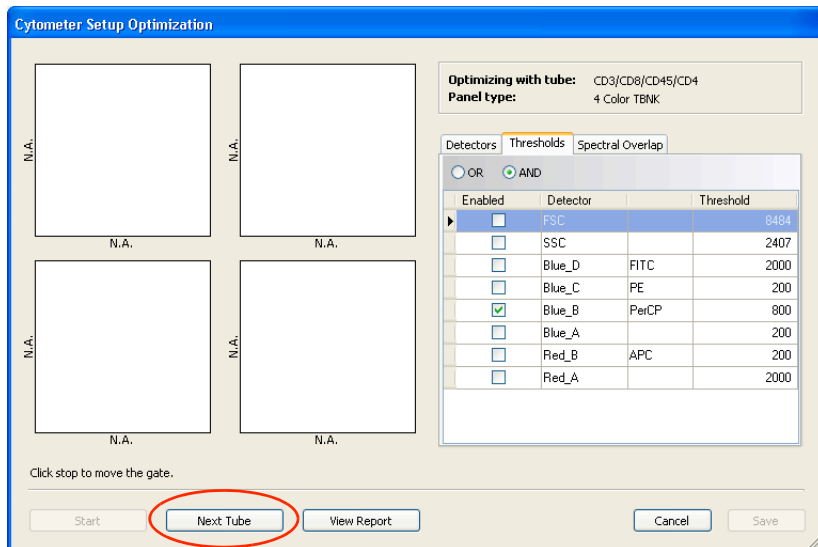
Gate Parameter X and Gate Parameter Y refer to the plot parameters for the first optimization plot, the plot that contains a gate around the cells of interest.



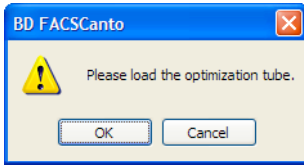
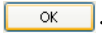
- 2 (Loader only) Make sure the optimization tubes are installed in the correct carousel positions, and click .



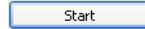
- 3 Click .



- 4 (Manual only) Install the first optimization tube when prompted, and click



- 5 At the Cytometer Setup Optimization screen, click



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

**Cytometer Setup Optimization**

Optimizing with tube: CD3/CD8/CD45/CD4  
Panel type: 4 Color TBNK



Detectors		Thresholds	Spectral Overlap
Detector			Voltage
FSC			420
SSC			600
Blue_D	FITC		593
Blue_C	PE		423
Blue_B	PerCP		439
Blue_A			909
Red_B	APC		696
Red_A			107

Click stop to move the gate.

Stop Next Tube View Report Cancel Save

## 6 Optimize settings, as needed.

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

- To adjust the optimization gate, click —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 .
- To adjust detectors, thresholds, or spectral overlap settings, click on the corresponding tab. Use controls in the tab to adjust the settings. For instructions, see Using Cytometer Controls on page 71.

**Tip** Spectral overlap values are automatically recalculated when you adjust voltages.



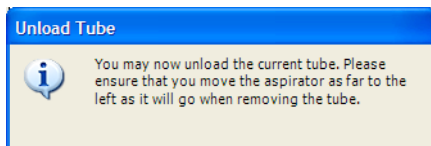
For clinical applications that use tandem conjugates such as APC-Cy7 or PE-Cy7, spectral overlap varies from lot to lot. Because BD FACSCanto clinical software setup targets the average lot, you might need to adjust spectral overlap settings for these reagents.

Optimizing with tube: CD3/CD8/CD45/CD4							
Panel type: 4 Color TBNK							
Detectors   Thresholds   Spectral Overlap							
Spillover %							
Primary Detector	FITC	PE	PerCP	APC			
Blue_D (FITC)	100.00	3.12	0.00	0.00	0.02	0.00	
▶ Blue_C (PE)	8.61	100.00	0.02	0.00	0.01	0.00	
Blue_B (PerCP)	0.40	5.45	100.00	0.00	0.07	0.00	
Blue_A ()	0.00	0.00	0.00	0.00	0.00	0.00	
Red_B (APC)	0.00	0.27	71.39	0.00	100.00	0.00	
Red_A ()	0.00	0.00	0.00	0.00	0.00	0.00	

- 7 If there are more tubes, click , and then  .

If this is the last tube, proceed to step 11 on page 69.

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



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






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

- Remove the tube from the SIT.
- Release the aspirator arm.


SIT cleaning occurs when the aspirator arm comes to center.

**NOTICE** To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 190.



- 8 (Manual only) When prompted, place the next tube on the SIT, and click  .
- 9 Repeat steps 3 through 6.

- 10** (Optional) To view the Application Setup Report, click , and then  .

The Application Setup Report reflects the optimized settings.

Click  in the toolbar to print the report.

- Tip** You can also save an electronic copy of the report and print it later. When you click Save, the software automatically saves PDF files of Application Setup Reports in C:\Program Files\BD FACSCanto Software\SetupReports.

- 11** When there are no more tubes to optimize, click , and then  .

Optimized setup results are saved to the *panel name.opt* file in the C:\Program Files\Common Files\BD\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.)

- 12** Unload the setup tube or remove the carousel.

- 13** Click  .

Changes will be saved for future setups. See the table on page 70 for instructions on saving user-specific optimization settings or reverting to default settings.

## Maintaining User-Specific Optimization Settings

To....	Do This....	Additional Information
Maintain settings for a single user	<ol style="list-style-type: none"> <li>1 Run setup.</li> <li>2 Select an application and optimize.</li> </ol>	User-optimized setup is calculated for the selected application and panel type.
Maintain settings for multiple users	<ol style="list-style-type: none"> <li>1 Copy the <i>panel type.opt</i> file.</li> <li>2 Rename it with a unique name (eg, 4 color <i>TBNK_username.opt</i>), and save it in your choice of location.</li> <li>3 To use your optimized results, quit the software, rename the file with its original file name (eg, 4 color <i>TBNK.opt</i> or 6 color <i>TBNK.opt</i>), and move it back into the Setup Results folder.</li> <li>4 When prompted, overwrite the current file.</li> <li>5 Restart the software, and run setup.</li> </ol>	The file is in C:\Program Files\Common Files\BD\Setup Results.
Restore BD Biosciences default settings	<ol style="list-style-type: none"> <li>1 While the software is not running, delete the <i>panel type.opt</i> file.</li> <li>2 Restart the software, and run setup.</li> </ol>	The software automatically rebuilds the <i>panel type.opt</i> file with BD-defined settings.

# Using Cytometer Controls

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

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

tabs —

Detectors		
Detector		Voltage
FSC		420
SSC		600
Blue_D	FITC	593
Blue_C	PE	423
Blue_B	PerCP	439
Blue_A		909
Red_B	APC	696
Red_A		107

after Setup, in Wizard

Detectors		
Detector		Voltage
FSC		420
SSC		600
Blue_D	FITC	593
Blue_C	PE	423
Blue_B	PerCP	439
Blue_A		909
Red_B	APC	696
Red_A		107

tabs —


during acquisition

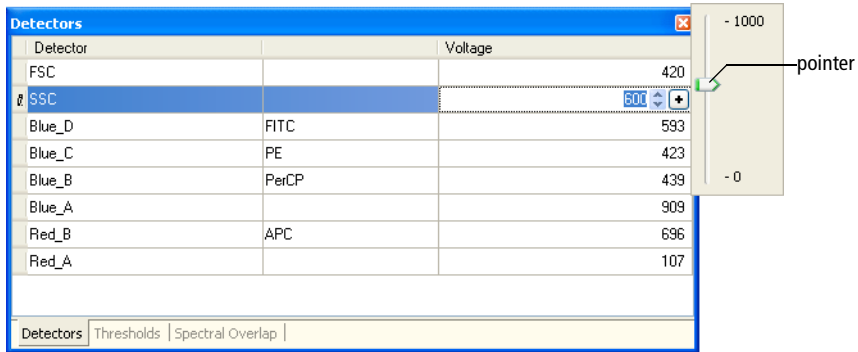
## Adjusting Detectors

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 **+** appear. Change the value as follows:

- highlight the value and type in a new one
- click the up and down arrows

- click the , 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

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

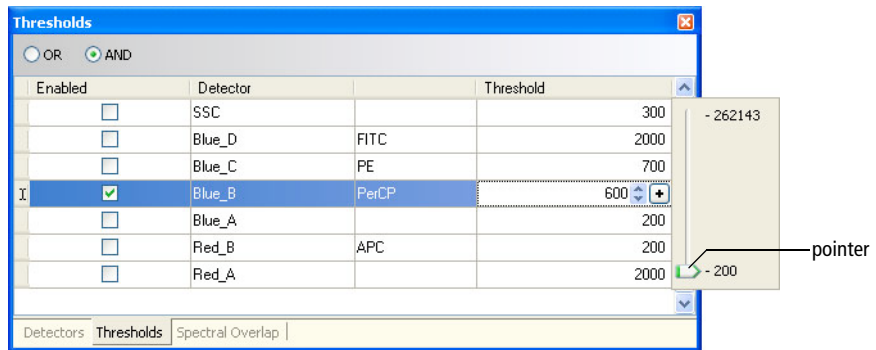
To set a threshold:

- 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 (Figure 5-6 on page 73).

Use one of the editing methods specified in Adjusting Detectors on page 71.



**Figure 5-6** Adjusting Threshold



**3** (Optional) Enable and set another threshold parameter.

- Select the associated checkbox.
- Edit the threshold value.
- 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 5-7 on page 74).
- 2** Use one of the editing methods specified in Adjusting Detectors on page 71.

**Figure 5-7** Adjusting Spectral Overlap


Spectral Overlap							
Spillover %							
Primary Detector	FITC	PE	PerCP		APC		
Blue_D (FITC)	100.00	3.12	0.00	0.00	0.02	0.00	
Blue_C (PE)	8.61	100.00	0.02	0.00	0.01	0.00	
Blue_B (PerCP)	0.40	5.45	100.00	0.00	0.07	0.00	
Blue_A ()	0.00	0.00	0.00	0.00	0.00	0.00	
Red_B (APC)	0.00	0.27	71.39	0.00	100.00	0.00	
Red_A ()	0.00	0.00	0.00	0.00	0.00	0.00	

## 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, select the Levey-Jennings tab.



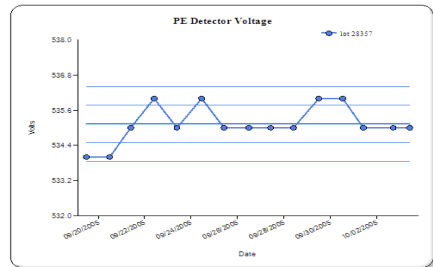
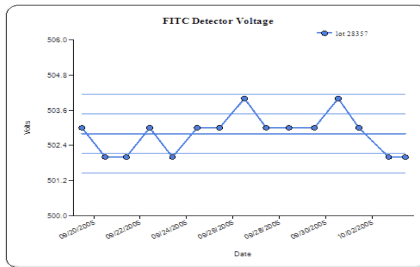
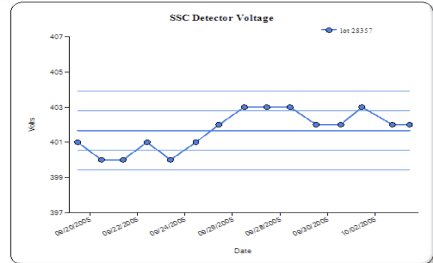
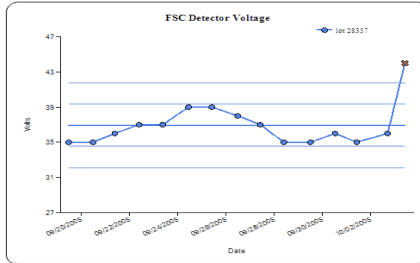
An  on the tab indicates an out-of-range value on the report:



2 Check the plots in the report.

**Levey-Jennings Report for Cytometer Setup**

Cytometer:	BD FACSCanto II	Operator:	erik
Serial Number:	V07300362	Date:	03/14/2006 1:25 PM
Bead Product:	BD FACS 7-Color Setup Beads	Catalog Number:	335775



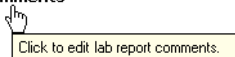
**Comments:**

Type your comments here.

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

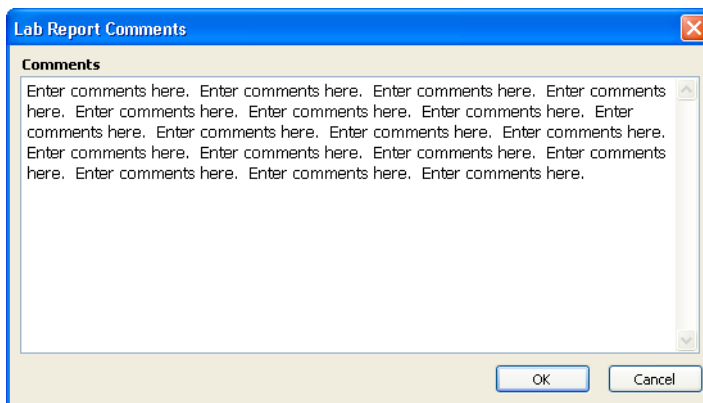
3 To add comments to the report, click Comments:


**Comments**



Enter text into the Comments field (up to 2500 characters), and click OK. See Figure 5-8 on page 76.

**Figure 5-8** Enter Lab Report Comments




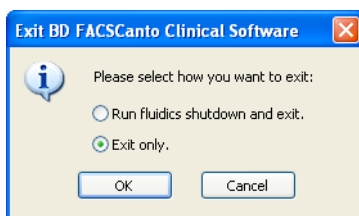
- 4 To print the report, Click .

## Optimizing with BD FACSDiva Software

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To optimize cytometer settings in BD FACSDiva software:

- 1 Quit BD FACSCanto clinical software, if necessary.
- 2 Select *Exit only*, and click .



- 3 Launch 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.

- 1** Choose 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.

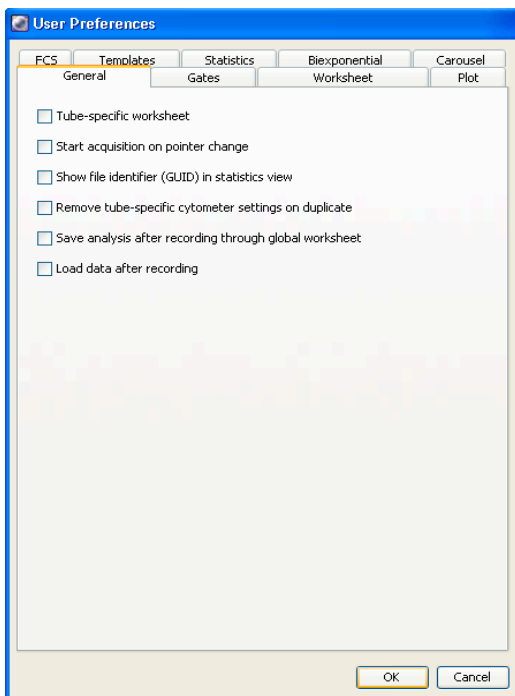


For accurate data results, the octagon and trigon arrays must match the current Cytometer Configuration.

- 3** Choose Edit > User Preferences and deselect all options in the General tab.







See Figure 5-9 on page 78.

**Figure 5-9** User Preferences General tab




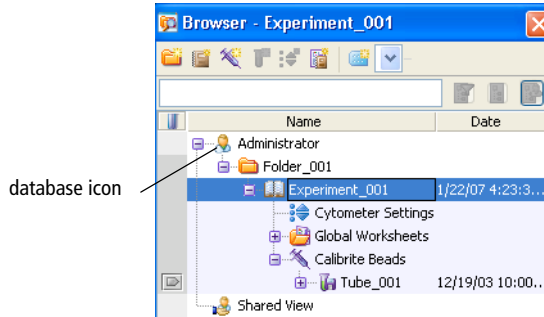
- 4 Click OK to save the changes.

## Creating the Experiment

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

**2** (Optional) Create a folder:

Select your database icon in the Browser, and click  in the Browser toolbar. Rename the folder.



**3** Select the folder, click  to create a new experiment.

An open experiment appears.

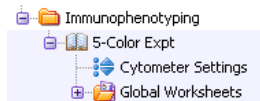
open experiment



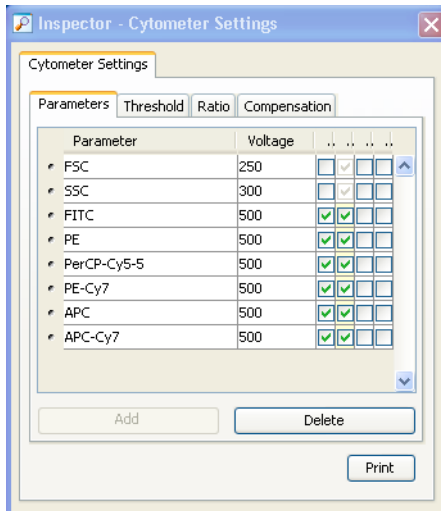
closed experiment



**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 choose a new one from the drop-down menu.
  - To add, click Add—a new line appears. Select it, and choose a parameter from the drop-down menu.

**NOTICE** The Add button is enabled only when less than the maximum number of parameters are shown in the Inspector.

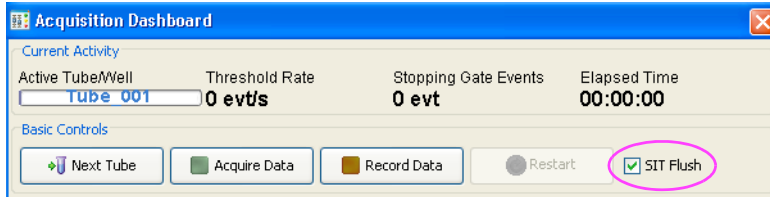
- To delete, click the selection button next to the parameter and click Delete.



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



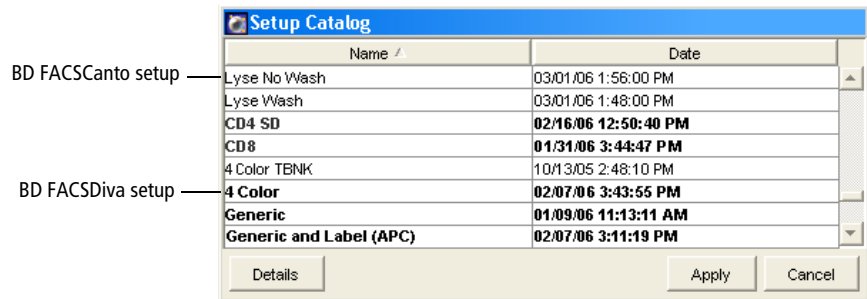
BD recommends leaving the SIT Flush option enabled to minimize carryover between tubes.



## Applying the Setup Results

- 1 Right-click the experiment-level cytometer settings, and choose Apply Setup.
- 2 Select a setup from the Setup Catalog.

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

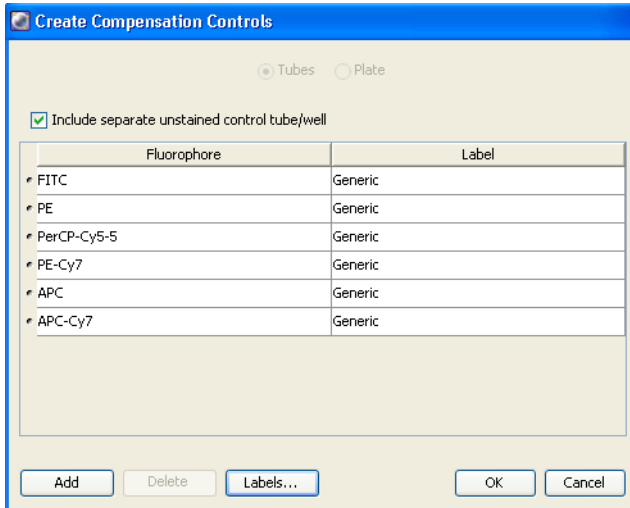


**NOTICE** Entries in bolded text are setups created in BD FACSDiva software.

- 3 Click Apply.

# Creating Compensation Controls

- 1 Choose Experiment > Compensation Setup > Create Compensation Controls.

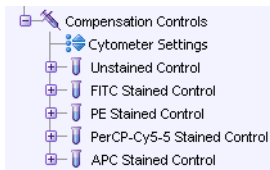


- 2 (Optional) Edit the labels associated with parameters.

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.

- 3 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

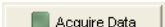
When you performed cytometer QC, voltage settings were adjusted to set each parameter at a target value. These settings might not be appropriate for the stained sample(s) you plan to analyze. Before recording data, adjust FSC, SSC, and threshold settings; gate on the population of interest (such as lymphocytes);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

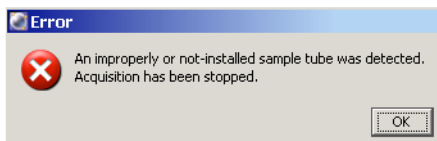
**NOTICE** If the software fails to respond during acquisition and you need to restart the software, perform a fluidics startup prior to resuming acquisition. See BD FACSDiva Software General Issues on page 215 for more information.



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.

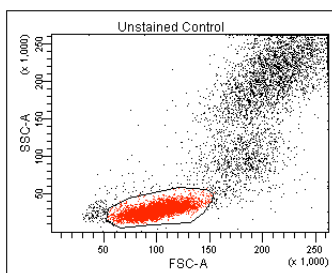
- 1 Install the unstained control tube on the cytometer.
  - Push the aspirator arm to the left.
  - Place the tube onto the SIT, ensure the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.
  - Center the aspirator arm under the tube. There are three sensor 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  .

If the tube is not fully seated, the following error message appears. Click OK. Remove the tube and repeat step 1 to properly reinstall it.



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

**Figure 5-10** 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 5-10).

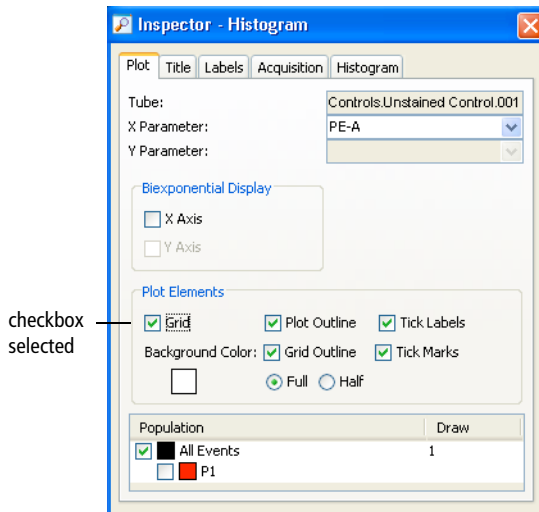
- 5 Adjust the P1 gate on the Unstained Control worksheet to surround only the lymphocyte population (Figure 5-10).

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

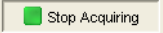
- 6 Once the gate is adjusted, right-click its boundary and choose Apply to All Compensation Controls.

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

- 7 Select all fluorescence histograms on the Unstained Control worksheet.
- 8 In the Plot Inspector, select the Grid checkbox:



Gridlines are used to delineate log decades on plots. In a four-log display, values are displayed from 26–262,143. Thus, the first log decade ranges from 26–262. The lines are shown only in plots that display log parameters.

- 9 Optimize the voltages to place the negative population for each fluorescent parameter on scale.
- 10 Click  to stop acquisition.
- 11 When acquisition stops, remove the unstained control tube.

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



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

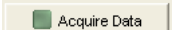
- Remove the tube from the SIT.

- Release the aspirator arm.

SIT cleaning occurs when the aspirator arm comes to center.

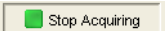
**NOTICE** To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 190.

- When the Progress dialog finishes, you can load the next tube onto the SIT.


**12** Install the stained sample tube on the cytometer and click  .

- Push the aspirator arm to the left.
- 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.
- 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.

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

**14** Click  to stop acquisition.

**15** Remove the stained sample tube.

**16** Re-install the unstained sample tube and click  .

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

Data acquisition stops when acquisition criteria have been met.

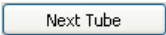

- 18 When acquisition stops, remove the tube.



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.

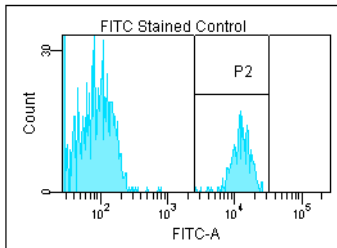
- 1 Install the first stained control tube onto the cytometer.
- 2 In the Acquisition Dashboard, click  .  
This moves the current tube pointer to the next tube in the Browser.
- 3 Click  .
- 4 Verify the P1 gate encircles the population of interest, and click Record.
- 5 When recording is finished, click Remove Tube.
- 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 next 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 5-11** 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 Choose 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.

**NOTICE** BD Biosciences recommends that you confirm the compensation setup by running a process control before you run samples.



# 6

## Running Samples with BD FACSCanto Clinical Software

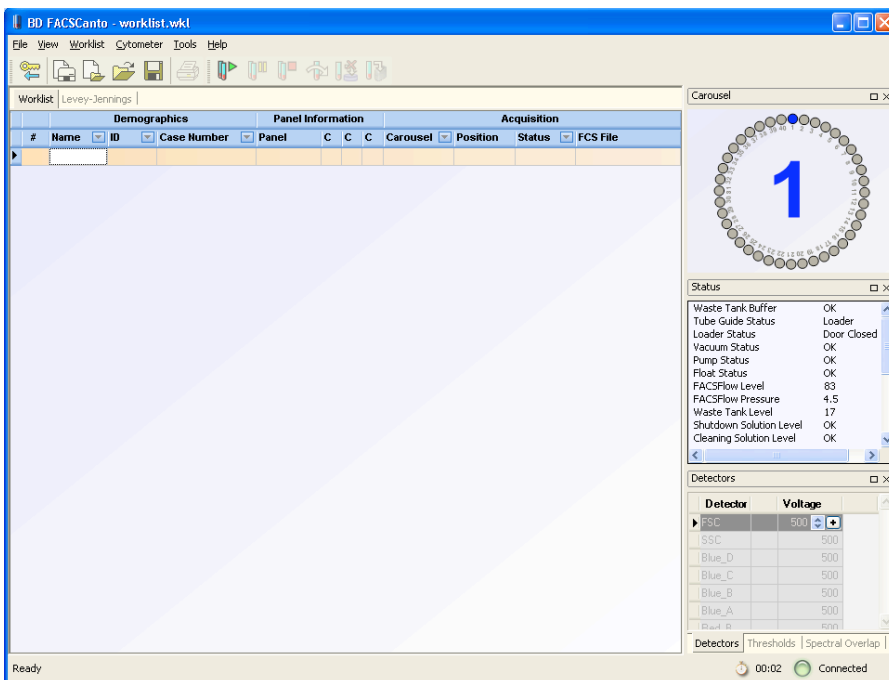
---

- Running an Acquisition Worklist on page 90
- Reviewing an Analysis Worklist on page 108
- Reviewing a Worklist on page 111
- Logging Out on page 113

# 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.

Demographics		Panel Information			Acquisition						
#	Name	ID	Case Number	Panel	Column #1	Column #2	Column #3	Carousel	Position	Status	FCS File
*									*	*	*

\* software assigned, not user editable

1 (Optional) Enter a name.

2 Enter an ID.


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

3 (Optional) Enter a case number.

4 Choose a panel.



For accurate results, make sure you choose the correct panel for your sample type.

- Either click in the Panel field, click the , and choose a panel, or
- Press Alt-↓ to access the Panel menu, and use the arrow keys to choose a panel.

Panel Information			
Panel	Col...	Col...	Col...

panel uses Trucount tubes

panel does not use Trucount tubes

**BD Multitest**

- 6 Color TBNK + TruC
- 4 Color TBNK + TruC
- 4 Color TBNK
- 3/8/45/4 + TruC
- 3/8/45/4
- 3/16+56/45/19 + TruC
- 3/16+56/45/19


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

- enter the WBC count (x 1000) and the lymphs (%), or
- enter the absolute lymphocyte count (x 1000)

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.

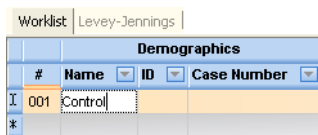
**5** (Loader only) Choose a carousel ID in the Carousel field.

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

## Running a Process Control

BD Biosciences recommends 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. Use the file to track results over time.

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



Demographics			
#	Name	ID	Case Number
I. 001	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.

Results are exported as *[lotID].csv* to C:\Program Files\BD FACSCanto Software\DataFiles. If the file already exists, results are appended.

- 3 (Optional) Enter a case number.

You can also enter a short comment in this field.

Worklist		Demographics			Panel Information		
#	Name	ID	Case Number	Panel	Column #1	Column #2	Column #3
001	Control	12345	Multichk control	4 Color TBNK + TruC			

- 4 Select a panel.
- 5 Run the control as you would a typical sample.

## Acquiring Samples

**NOTICE** If the software fails to respond during acquisition and you need to restart the software, perform a fluidics startup prior to resuming acquisition. See BD FACSCanto Software General Issues on page 199 for more information.

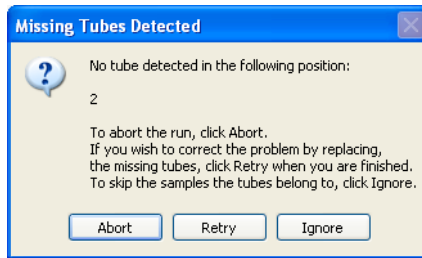
- 1 Enter all sample information into a worklist.
- 2 (Loader) Prepare to acquire with the Loader.
  - Print the worklist; load tubes into the carousel(s) according to the printout.



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

If the software cannot find a tube when starting a run, a message appears requiring operator response. See Figure 6-1 on page 94.

**Figure 6-1** Missing Tubes Detected dialog



- Set up the cytometer for automatic loading (page 136).
- Insert the carousel into the Loader, and close the Loader doors.

The worklist will not run with the Loader doors open.

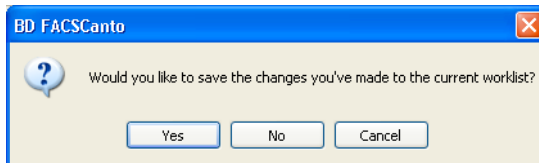
**3** Verify that the cytometer is ready.

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 worklist.

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

**5** Click .

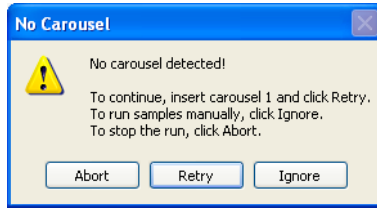
A dialog appears asking if you want to save the worklist:





**6** Click ; specify a file name and storage location in the next dialog box.

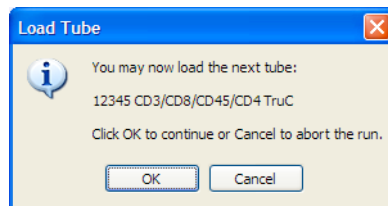
(Loader) The carousel briefly mixes the samples, and acquisition begins.

- 7 If no carousel is found, click  to load tubes manually.

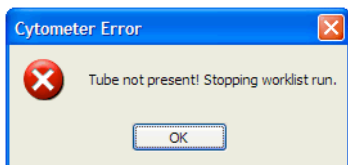


  Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

- 8 (Manual) When prompted, install the tube containing the first worklist sample.
- Push the aspirator arm to the left.
  - 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.
  - 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.
- 9 Click .

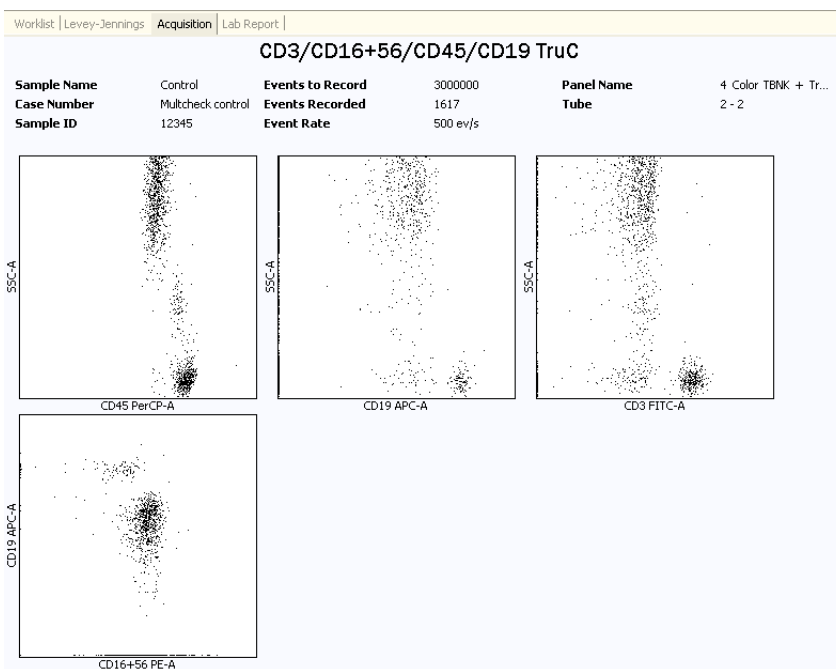


If the tube is not fully seated, the following error message appears. Click OK. Remove the tube and repeat step 8 to properly reinstall it.



The Acquisition tab comes into view and events appear. See Figure 6-2 on page 96.


**Figure 6-2** Acquisition tab







**10** 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  and before data recording begins, choose from the following options.

**Table 6-1** Options before recording

To...	Do This	Additional Information
Optimize detectors, thresholds, or spectral overlap	<ol style="list-style-type: none"> <li>1 Click .</li> <li>2 Click .</li> </ol>	See page 100.
Change plot parameters	<ol style="list-style-type: none"> <li>1 Right-click on an axis label.</li> <li>2 Choose a different parameter.</li> </ol>	
Proceed with recording	Do nothing.	
Stop recording data for the worklist	<ol style="list-style-type: none"> <li>1 Click .</li> <li>2 Click .</li> </ol>	If recording was in progress, data for the current tube is discarded and no FCS file is saved.

**11** View the Lab Report for recorded data.

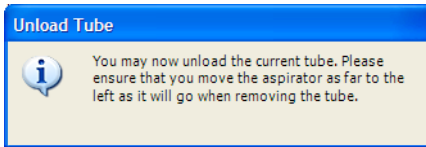
If the Lab Report countdown is set to On, the Lab Report is shown for the designated time. You can pause and edit the report during the countdown. See Inspecting Lab Reports on page 101 for instructions.

**12** (Loader) Acquisition of the next tube starts automatically.

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

Status	FCS File
OK	45433401.001.fcs
Running	
Ready To Run	
Ready To Run	
Ready To Run	
Ready To Run	

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



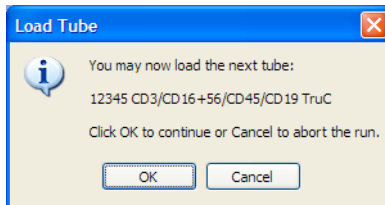
- Hold your sample tube while you push the aspirator arm to the left.



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


- Remove the tube from the SIT.
- Release the aspirator arm.

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




**NOTICE** If you do not unload a tube when prompted, the following message appears.



If this happens, click , and unload the tube. Wait for the Load Tube message before you install the next tube.

**NOTICE** To prevent backflow into the tube, follow the tube removal sequence exactly. For more information, see Cytometer Troubleshooting on page 190.

- 14** (Manual) Repeat steps 7 through 11 until there are no more samples.
- 15** (Loader) Load the next carousel when prompted, and click .
- 16** After all samples have been run, review the worklist.
- 17** After the last run of the day, shut down the system.

See Chapter 9 on page 147, for instructions.




## Options During Acquisition

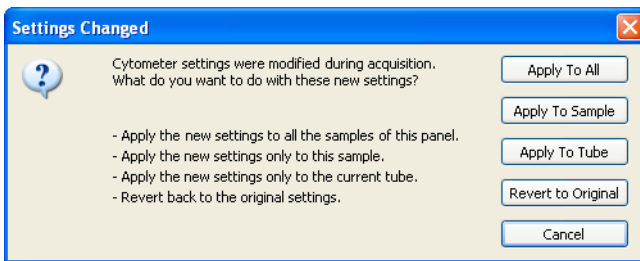
Do any of the following during acquisition:

- Adjust voltages, thresholds, and spectral overlap values. See *Optimizing During a Run* (page 100).
- Inspect recorded data on the Lab Report (page 101).
- Skip tubes or samples (page 106).
- Stop recording and acquisition (page 106).
- Add samples to a worklist (page 106).

## Optimizing During a Run

For instructions on using optimization controls, see Using Cytometer Controls on page 71.

- 1 At the Acquisition view, click  on the toolbar to pause.
- 2 Click .  
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  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).





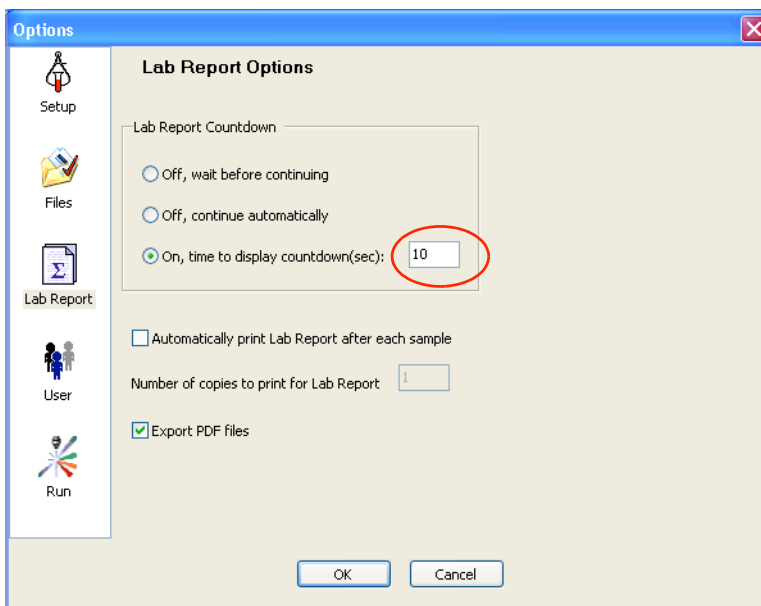
The software overwrites all events recorded prior to optimizing for the current tube.

## Inspecting Lab Reports

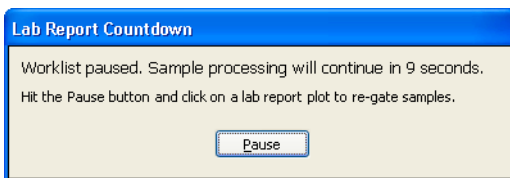
To inspect a Lab Report during acquisition, set the Lab Report countdown.

### Specifying a Display Time for the Lab Report Countdown

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



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.



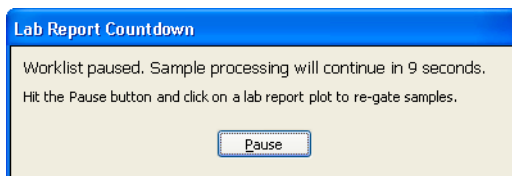
During the countdown, you can pause the run and do any of the following:

- Re-Gating a Plot Manually (below)
- Reapplying the Auto-Gate on page 104
- Re-Running a Tube on page 104
- Verifying Report Review on page 105

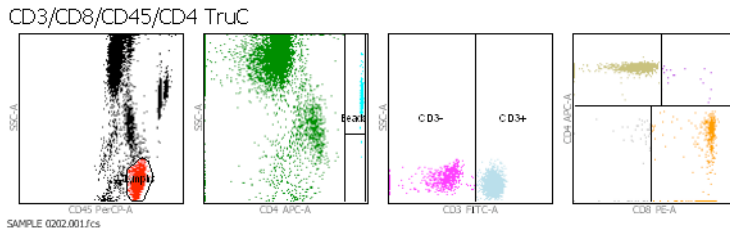
### Re-Gating a Plot Manually

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.

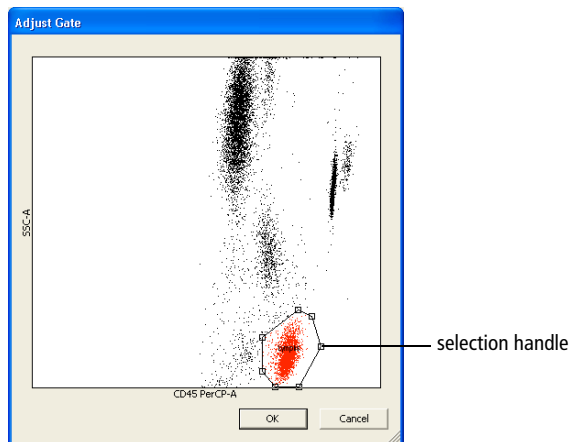
- 1 At the Lab Report Countdown dialog, click Pause.



2 Click any plot to adjust its gate.



The selected plot appears in an enlarged view:



3 Adjust the gate.

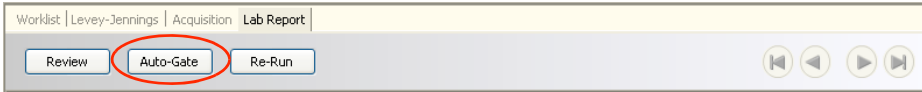
- Select the gate by clicking its boundary.
- Drag the selection handle to adjust the shape or size of the gate.
- Drag the gate boundary between selection handles to move the gate.


Gate changes apply only to the current tube.

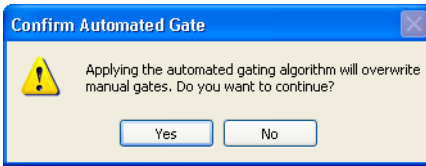
4 Click  .

## Reapplying the Auto-Gate

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





When the confirmation message appears, click .

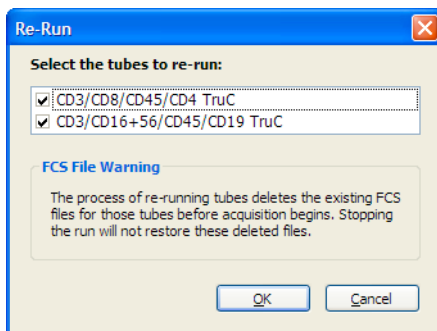


## Re-Running a Tube

After you inspect the data on the Lab Report, you can elect to re-run the tube.

- 1 Click  at the top of the Lab Report view.
- 2 Select the tubes you want to re-run, and click .

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

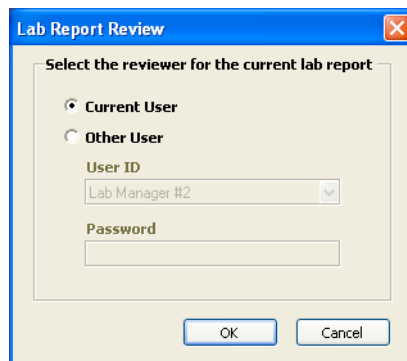




## Verifying Report Review

BD FACSCanto clinical software provides an interface where you can append a virtual signature to reviewed reports.

- 1 Click  at the top of the Lab Report view.
- 2 Select a reviewer, and click  .
  - Select Current User for the current, logged-in user.
  - Select Other User for a different user. Choose the User ID from the menu, and enter a password.





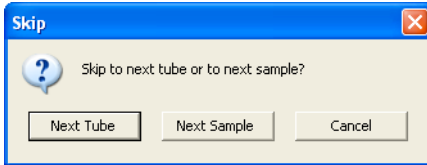
The image shows a dialog box titled "Lab Report Review" with a close button (X) in the top right corner. The main text inside the dialog is "Select the reviewer for the current lab report". There are two radio button options: "Current User" (which is selected) and "Other User". Below the "Other User" option, there is a "User ID" label and a dropdown menu showing "Lab Manager #2". Below that is a "Password" label and an empty text input field. At the bottom of the dialog are two buttons: "OK" and "Cancel".

The corresponding worklist Status field changes to *Reviewed*.

Worklist		Levey-Jennings		Lab Report						
Demographics				Panel Information			Acquisition			
#	Name	ID	Case Number	Panel	C	C	C	Carousel	Position	Status
▶ 001	Control	12345	Multichannel control	4 Color TBNK + TruC				1	1 - 2	Reviewed
002	25489	56214		4 Color TBNK + TruC				1	3 - 4	Needs Review
003	32415	95472		3/8/45/4 + TruC				1	5 - 5	Needs Review
*										

## Skipping a Tube


- 1 In the Acquisition tab, click .
- 2 Click .
- 3 Select whether you want to skip to the Next Tube or Next Sample:



Skipped tubes are indicated on the worklist:

Status	FCS File
DK	1232101.001.fcs
DK	6545402.001.fcs
DK	4334503.001.fcs
Skipped	
Running	
Ready To Run	



## Stopping Recording


To stop recording data for the current sample, click  at 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 add samples to a worklist when it is stopped.

- 1 Click  and then  to stop the worklist.
- 2 If a sample tube was loaded manually, remove the tube from the SIT.

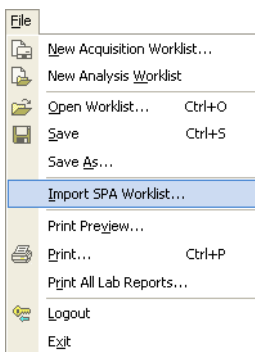
- 3 Enter new sample information into the worklist.
- 4 Click  to resume.

## Importing a Worklist from SPA Software


You can import sample information from a worklist created in BD FACS Sample Prep Assistant (SPA) software v2.0 or v3.0.

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

- 1 Choose File > Import SPA Worklist.



Sample information can be imported only into a new, blank worklist; you cannot add imported information to a worklist that is already started.

- 2 Navigate to and select a worklist, and click .

By default, SPA worklists are stored in Program Files/BDApps/SPA/DataFiles on the system where Sample Prep Assistant software is installed.

**Tip** Create a SPA Worklist folder within the BD FACSCanto Worklists folder to help locate files.

- 3 Review the imported information; edit missing or incorrect entries, if needed.

- 4 If you are running samples with the Loader, verify carousel IDs for each sample, and print the worklist.

If carousel IDs are missing or incorrect, choose the correct carousel ID from the drop-down menu. Print the worklist, and use it as a guide when you are filling the carousels.

## Reviewing an Analysis Worklist

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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.

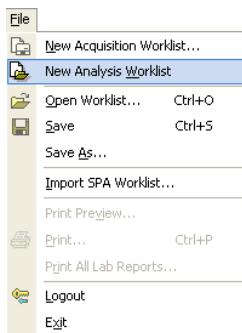
Every time you reanalyze an FCS file, only the latest analysis is saved.



Do not read FCS files created with v2.1 into previous versions of BD FACSCanto clinical software. Previous versions will show incorrect results.



## Creating a New Analysis Worklist

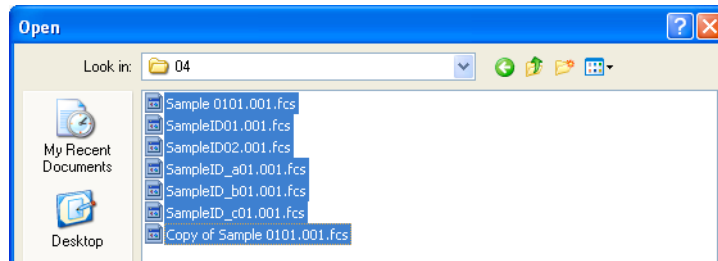
- 1 Choose File > New Analysis Worklist.





## 2 Add FCS files.

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

- To add selected files, click , navigate to the folder containing the files, and select them. Click .



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 , locate and select the folder, and click .

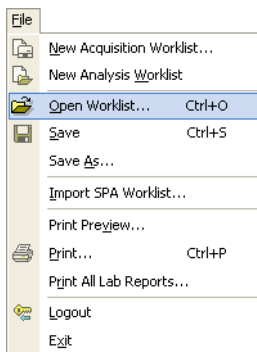
## 3 Verify that the worklist contains all required files.

You cannot edit an analysis worklist.

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

## Opening an Existing Analysis Worklist

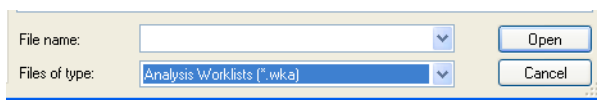
- 1 Choose File > Open Worklist.




- 2 Navigate to the folder containing your saved worklists.

By default, worklists are stored in C:\Program Files\BD FACSCanto Software\Worklists.

- 3 From the Files of Type drop-down menu at the bottom of the window, choose Analysis Worklists (\*.wka).



- 4 Select an analysis worklist, and click .

# Reviewing a Worklist

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.

Worklist		Levey-Jennings		Lab Report				
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	
*								

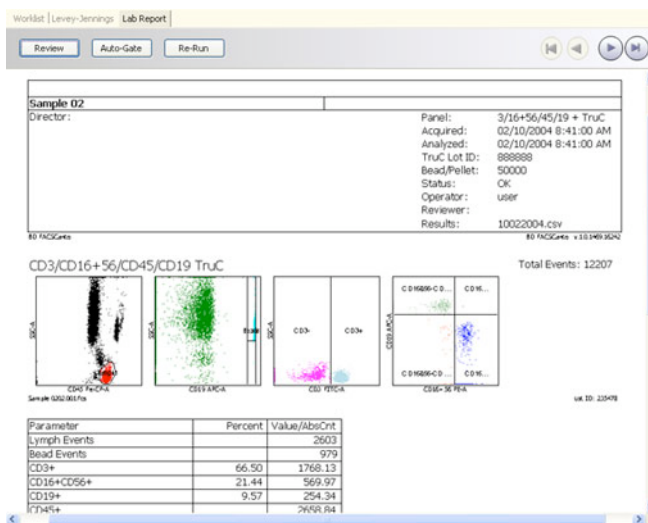
Status	Meaning
Ready To Run	All columns filled in and sample ready for acquisition
Not Prepped	Sample skipped—prep on SPA II unsuccessful
Running	Sample currently being acquired
OK	Sample successfully acquired and analyzed with no QC messages
Partial	A tube belonging to the sample was successfully acquired or analyzed, while another tube was skipped
Incomplete	Not all tubes in a multi-tube panel were recorded
Skipped	All tubes belonging to the sample were skipped or aborted by 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 6-3 on page 112).

**Figure 6-3** Lab Report




- To adjust gates, see page 102; to revert to auto-gates, see page 104.
- For suggestions on addressing QC messages, see page 210.
- To append a virtual signature to reviewed reports, see page 105.

**2** (Acquisition worklists only) To re-run a tube or all tubes for the sample, click  (see Re-Running a Tube on page 104).

Re-running a tube overwrites all data for that tube. You must put the sample tube on the SIT to re-run it; you cannot re-run a data file or a sample in an analysis worklist.

**3** Continue reviewing reports as needed.

Use  to navigate to other Lab Reports in the worklist.


**4** Save the worklist.

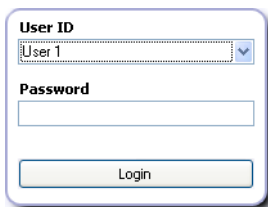
Saving a worklist allows you to reanalyze data and review Lab Reports at a later time and use the worklist as a template.



# Logging Out

---

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



A login dialog box with a blue border and a drop shadow. It contains a 'User ID' dropdown menu with 'User 1' selected, a 'Password' text input field, and a 'Login' button at the bottom.

The next user can now log in.



# 7

## Running Samples with BD FACSDiva Software

---


This section describes how to use BD FACSDiva software features to record and analyze sample data. As an example, data will be recorded and analyzed for two tubes of human peripheral blood stained with the following reagents:

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

For other analysis methods, refer to 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. To switch between the normal and global worksheet view, click the Global Worksheets button on the Worksheet toolbar ()




BD FACSDiva software v5.0 is required to run the BD FACSCanto II flow cytometer. Older versions of BD FACSDiva software will not work with a BD FACSCanto II flow cytometer.

- 1 Open the experiment in which you optimized cytometer settings.
- 2 Create a new specimen (); 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 ()

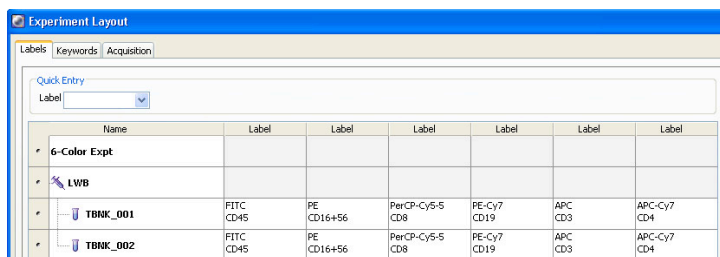
- 4 Create a global worksheet; rename it *Record Data*.
  - If the *Default global worksheet* preference is enabled in User Preferences (default option), the global worksheet is already present. Expand the Global Worksheets folder to locate and rename the worksheet.
  - If the *Default global worksheet* preference is disabled, create a global worksheet by clicking the New Global Worksheet button in the Browser toolbar ()
- 5 Use the Experiment Layout dialog 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.

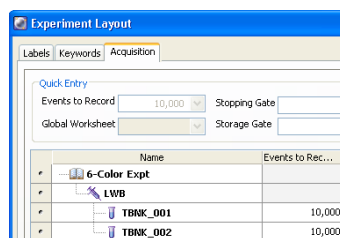
- Choose Experiment > Experiment Layout.

- In the Labels tab, enter appropriate labels for the tube. For example, enter CD45 in the FITC field. Use the Tab key to move to the next field (Figure 7-1).

**Figure 7-1** Entering parameter labels



- In the Acquisition tab, verify 10,000 events to record for tubes TBNK\_001 and TBNK\_002; click OK.



- 6 On the global worksheet, create plots for previewing the data.



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

# 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 5-9 on page 78). If you do not want to save a copy of the data, leave the preference disabled.

**NOTICE** If the software fails to respond during acquisition and you need to restart the software, perform a fluidics startup prior to resuming acquisition. See BD FACSDiva Software General Issues on page 215 for more information.

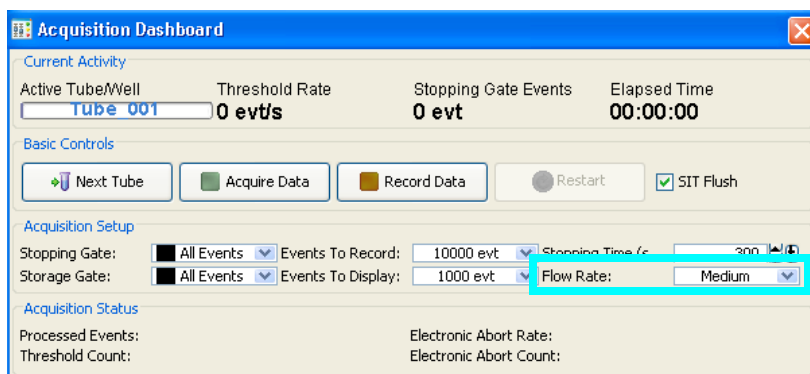
  Always wear gloves when manually loading samples. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples that might contain biohazardous waste.

- 1 Install the first sample tube onto the cytometer.
  - Push the aspirator arm to the left.
  - 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.
  - 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 In Acquisition Setup (Figure 7-2 on page 119), verify that you are using an appropriate flow rate and that the SIT Flush checkbox is selected.

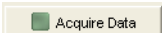
 BD recommends leaving the SIT Flush option enabled to minimize carryover between tubes.

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

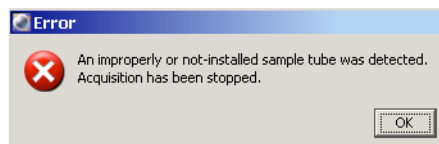
**Figure 7-2** Acquisition Dashboard showing Acquisition Setup

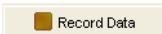



- Low = approximately 10  $\mu\text{L}/\text{min}$  of sample
- Medium = approximately 60  $\mu\text{L}/\text{min}$  of sample
- High = approximately 120  $\mu\text{L}/\text{min}$  of sample

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

If the tube is not fully seated, the following error message appears. Click OK. Remove the tube and repeat step 1 to properly reinstall it.



- 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 .

- 6 When all events have been recorded, click  and unload the sample tube when the aspirator arm lowers.
- When the aspirator arm lowers, hold your sample tube while you push the aspirator arm to the left.

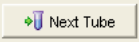




If you move the arm without holding the tube, the tube could fall off the SIT and expose you to potentially biohazardous sample.

- Remove the tube from the SIT.
- Release the aspirator arm.

SIT cleaning occurs when the aspirator arm comes to center.

**NOTICE** 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 , and then .
- 9 Preview the data in the global worksheet. Click .
- 10 Repeat steps 6 through 9 until data has been recorded for all tubes.
- 11 (Optional) To print the experiment-level cytometer settings or the Cytometer Status report, right-click the cytometer settings icon and choose Print.

## Importing a Worklist from SPA Software

You can import sample information from a worklist created in BD FACS Sample Prep Assistant (SPA) software v2.0 or v3.0.

- 1 (Optional) In Sample Prep Assistant software, print a report of the worklist you want to import into BD FACSDiva software.

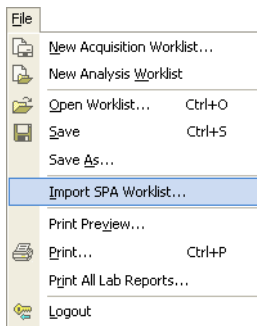



- 2 Verify tube and panel names from the SPA worklist exactly match those in the BD FACSDiva panel template.



To import the worklist, all tube names in the panel template and the panel template name in BD FACSDiva software must exactly match the tube names and reagent panel name used to prepare samples in the SPA software.

- 3 If there is a discrepancy, create a new panel template in BD FACSDiva software to match the reagent panel in the SPA software; otherwise, go to step 4.
- 4 Choose File > Import > Worklist.



- 5 Navigate to and select a worklist, and click .

By default, SPA worklists are stored in Program Files/BDApps/SPA/DataFiles on the system where Sample Prep Assistant software is installed.

BD FACSDiva software creates a locked experiment that includes all specimens prepared in the SPA worklist.

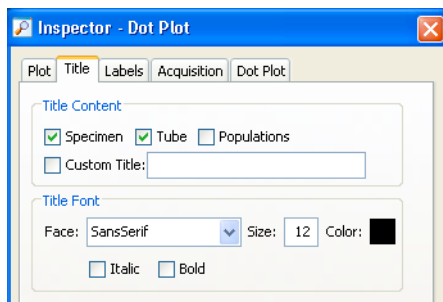
# Analyzing Data

---

- 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: FITC vs SSC, APC vs PE-Cy7, APC vs PE, APC vs APC-Cy7, and APC vs PerCP-Cy5.5.
- 3 Resize the plots so they fit on one page.
- Tip** By default, the plots will fit on one page if you select three across.
- 4 On the FITC vs SSC plot, draw a gate around the lymphocytes; use the Population Hierarchy to rename the population *Lymphocytes*.
- 5 Select all plots except the FITC 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, click 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.



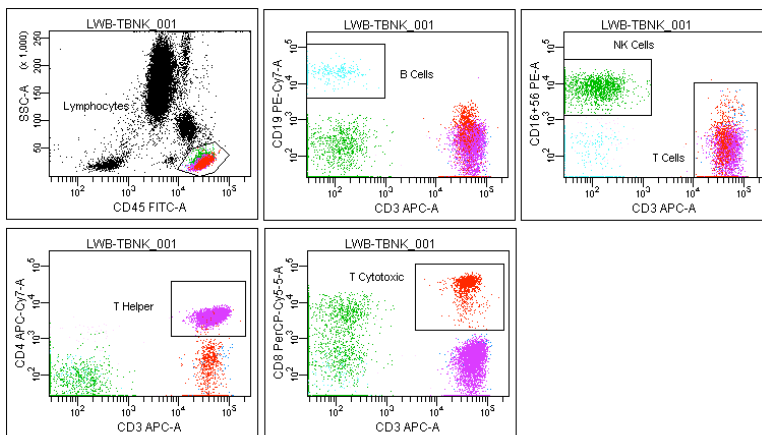
- 7** Create a statistics view; edit the view to show the Lymphocyte population and subpopulations, and to display the mean for all fluorochromes.

To create a statistics view, right-click any plot and choose 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 APC vs CD19 PE-Cy7 plot; name the population *B Cells*.
- 9** Draw a gate around the CD16+56–positive population on the CD3 APC vs CD16+56 PE plot; name the population *NK Cells*.
- 10** Draw a gate around the double-positive population on the CD3 APC vs CD4 APC-Cy7 plot, and name the population *T Helper*.
- 11** Draw a gate around the double-positive population on the CD3 APC vs CD8 PerCP-Cy5-5 plot; name the population *T Cytotoxic*.

## 12 Print the analysis.

**Figure 7-3** 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

Population	#Events	%Parent	FSC-H Mean	SSC-A Mean	CD45 FITC-A Mean	CD16+56 P... Mean	CD8 PerCP... Mean	CD19 PE-C... Mean	CD3 APC-A Mean	CD4 APC... 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 (see the following section, Saving the Analysis).

- 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.

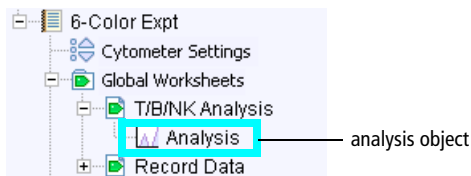
Adjustments will also apply to the next tube viewed on the global worksheet. If you don't want to alter the global worksheet, save the analysis as described in the next section and make adjustments on the tube's normal worksheet.

## 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 choose Copy.



- 5 Select the tube in the Browser; right-click the tube icon and choose 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.

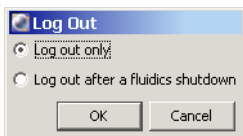
- Tip** Automatically save a copy of the analysis with each tube by enabling the Save Analysis After Recording preference before you record data. In this case, the analysis plots are placed on the normal worksheet that is open at the time of recording. To control where the plots are placed, create a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

# Logging Out

---

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

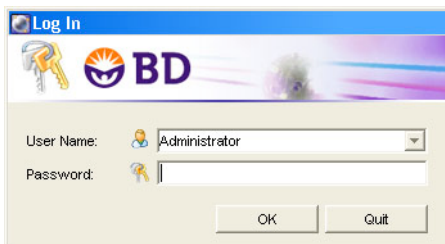
- 1 Choose File > Log Out.



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

**NOTICE** If you select *Log out after a fluidics shutdown*, keep in mind 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.







# 8

## Using the Loader with BD FACSDiva Software

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- Getting Ready on page 130
- Assigning Carousels and Verifying Run Settings on page 132
- Preparing the Loader on page 136
- Running Samples on page 139
- Running Cleaning Tubes on the Loader on page 146

# 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 also add specimens to the experiment used during sample optimization (step 4).

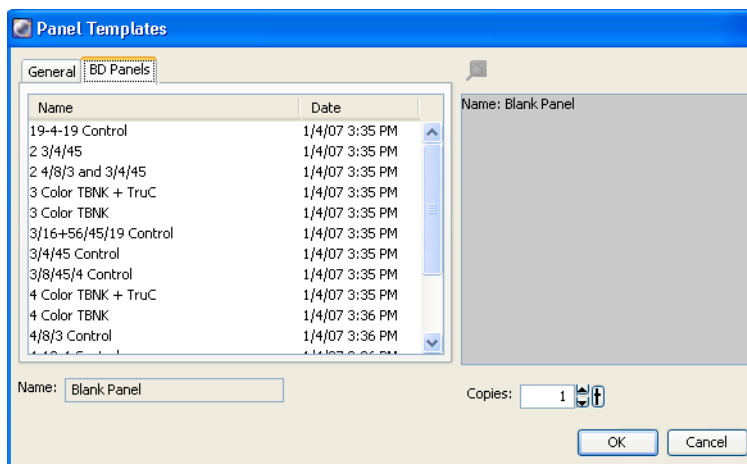
**NOTICE** You must use a global worksheet for data display when acquiring samples with the Loader.

- 4 Add specimens and tubes to your experiment (see Figure 8-1 on page 131).

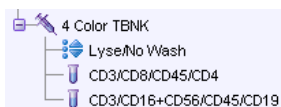
To add a predefined panel:

- Select any Browser item within the open experiment, and choose Experiment > New Specimen.
- Click the BD Panels tab and select a panel.
- Specify the number of copies (each copy creates a new specimen in the Browser), and click OK.

**Figure 8-1** Panel Templates dialog

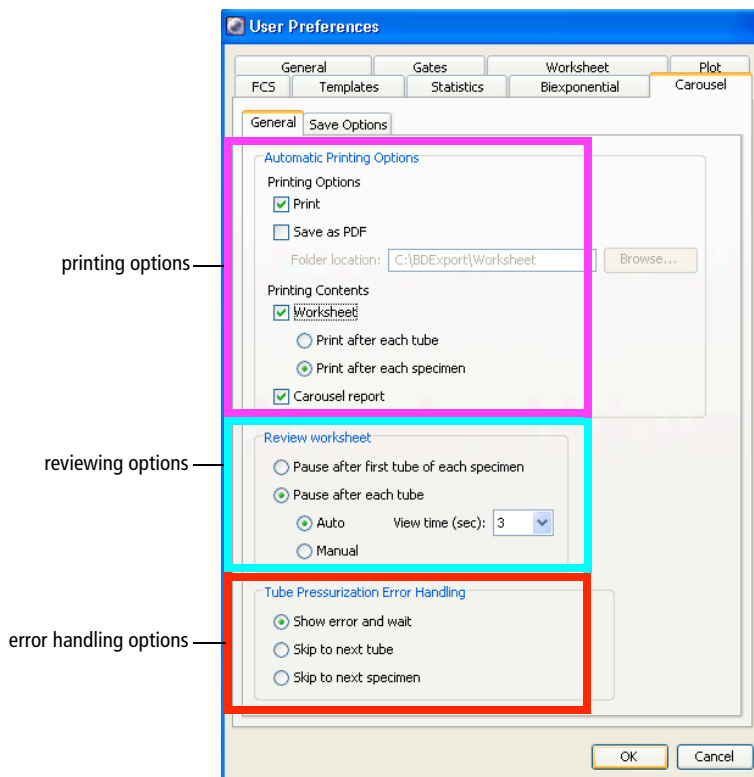


- 5 If you imported panels, inspect the information and the cytometer settings for each panel type to make sure they are complete and appropriate.



# Assigning Carousels and Verifying Run Settings

- 1 Choose Edit > User Preferences > Carousel tab > General tab, and make selections for printing and pausing.



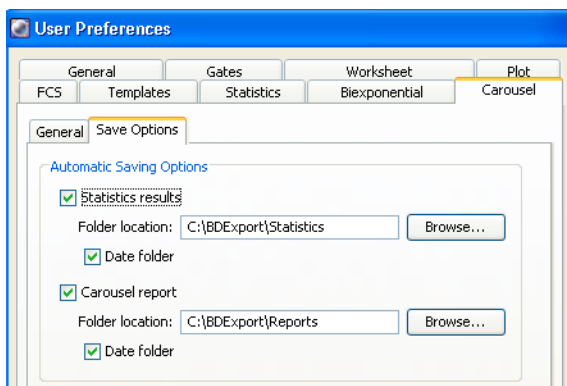
- 2 Select how you want the Loader to handle any tube pressurization errors that occur during the run.

Tube Pressurization Error Handling Options	Function
Show error and wait	Stops the run and waits for you to choose 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

If a tube or specimen is skipped during the run, this will be highlighted in the Carousel Report.

**NOTICE** 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 134.

- 3 Click the Carousel tab > Save Options tab, and choose whether and where to automatically save the statistics results and Carousel Report.



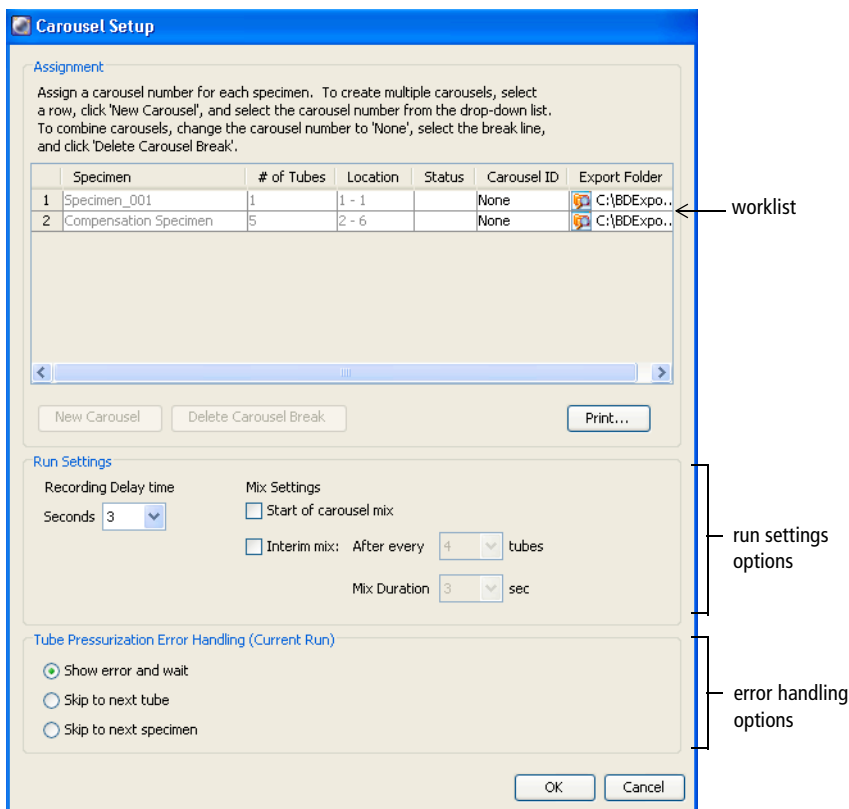
- 4 Click OK to save changes.

**NOTICE** Options chosen in the User Preferences dialog are applied globally.

**5** Choose Carousel > Carousel Setup.

All specimens or panels in the experiment are listed at the top of the Carousel Setup window. This is your worklist. Carousel locations are automatically assigned. You will need to assign a carousel ID to each specimen (see step 7).

**Figure 8-2** Carousel Setup window



You can choose to export the FCS data from a carousel run. Click the folder in the Export Folder column and choose a directory. C:\BDEExport\FCS is the default folder.

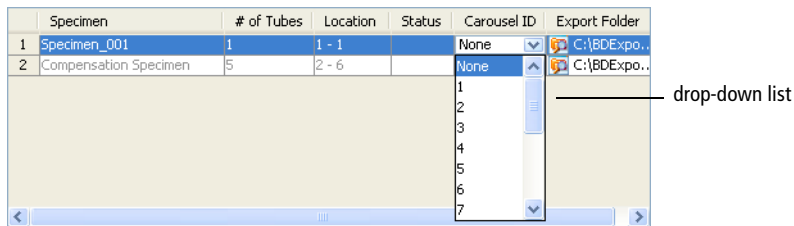
**6** (Optional) Insert or delete carousel breaks.

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 (carousel ID is None).

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

**7** From the menu, assign a carousel ID to each specimen.



**8** 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.

**9** Verify the Run Settings.

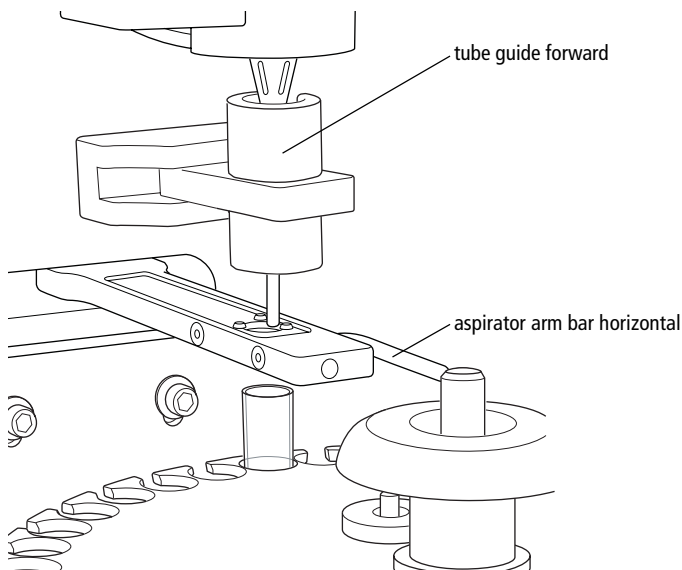
See Figure 8-2 on page 134.

Run Settings Options	Range	Function
Acquisition time	3–15 seconds	Allows acquisition to stabilize before recording begins
Start of carousel mix		Performs 10-second mix when 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

## Preparing the Loader

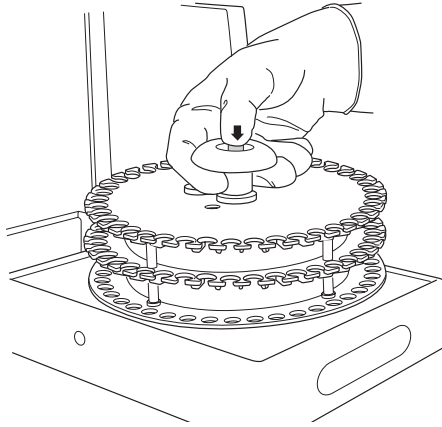
- 1 Set up the cytometer for automatic loading.

**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 (BD FACSCanto clinical software) or Carousel Setup (BD FACSDiva software). Print out a copy of tube assignments and use the printout as a guide when filling each carousel.

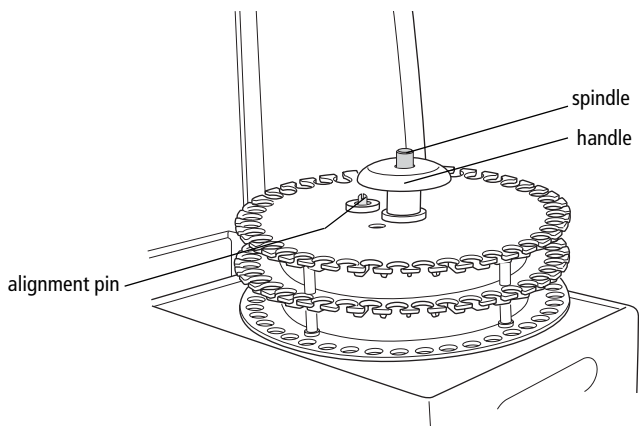


To prevent binding during loading, make sure label thickness per tube is less than three labels. Flatten labels completely before placing a tube in the carousel.

- 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** Installing the carousel

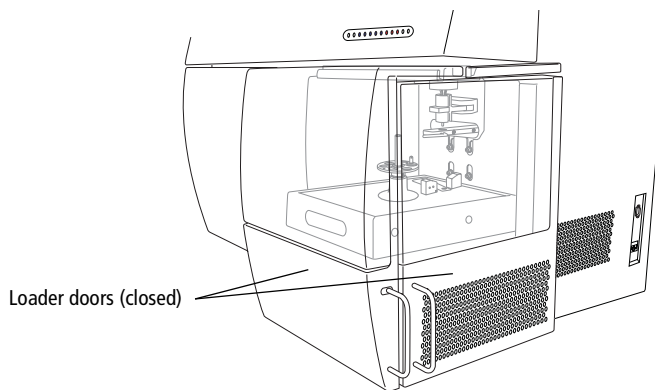


- 5 Close the Loader drawer completely, and close the Loader doors.

The Loader scans and positions the carousel at tube 1.



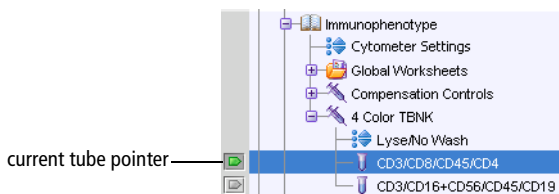
To run the Loader, the doors must be closed. Tubes will not load with the doors open. The currently running tube will be unloaded if you open the doors during a run.



# Running Samples

**NOTICE** If the software fails to respond during acquisition and you need to restart the software, perform a fluidics startup prior to resuming acquisition. See BD FACSDiva Software General Issues on page 215 for more information.

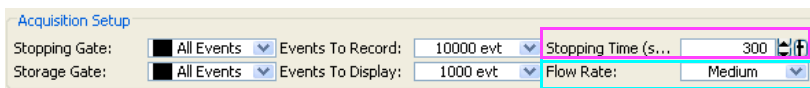
- 1 To activate the acquisition controls, select the first tube in your experiment with the current tube pointer.



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

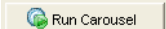
- Verify that you are using an appropriate flow rate.
- Enter a stopping time (seconds).

**Figure 8-5** Acquisition Setup in Acquisition Dashboard



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.

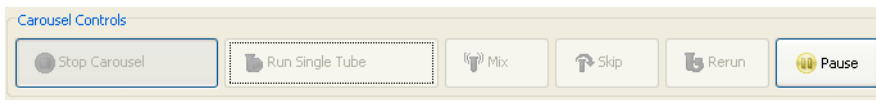
- Tip** BD Biosciences recommends that you always enter a stopping time. Setting a time limit ensures that the carousel doesn't stall on one tube if that tube should contain a dilute sample or rare events.

- 3 In Carousel Controls, click .

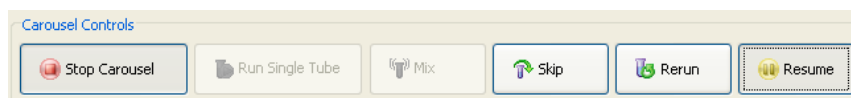
- 4 View data for the first tube on the global worksheet.

The ID of the currently running carousel along with the tube's position appear 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.



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 is complete. Repeat step 4 through step 6 for the remaining tubes on the carousel.

- 7 Load the next carousel when prompted; click Continue.
- 8 When done, review the Carousel Run Report (Figure 8-6 on page 141).

Any tube errors that occurred during the run are highlighted.

**Figure 8-6** Example Carousel Run Report

The screenshot shows a window titled "Carousel Run Report" with a blue title bar and a close button. Below the title bar is a menu bar with buttons for "Page Setup", "Print Preview", "Print Report", "Save Report as PDF", and "Export Report". The main content area is titled "Carousel Run Report" and contains the following text:

**Experiment:** Experiment\_002  
**SPA Info:** N/A  
**Run Date:** 1/26/07 5:26 PM  
**User ID:** Administrator  
**Cytometer:** FACSCanto II (V07300262)  
**Statistics File Location:** N/A  
**FCS File Location:** N/A  
**Software:** BD FACSDiva Software Development  
**Start of Carousel Mix:** No  
**Interim Mix Settings:** mix every 4 tubes for 3 seconds.

Below the text is a section labeled "Carousel: 1" followed by a table with the following data:

Specimen/Tube	Location	Status	Record Time	FCS File Name
<b>Specimen_001</b> Tube_001	1	OK	1/26/07 5:25 PM	N/A
<b>Specimen_002</b> Tube_001	2	OK	1/26/07 5:26 PM	N/A
<b>Specimen_003</b> Tube_001	3	OK	1/26/07 5:26 PM	N/A

The report prints automatically if specified in the General tab of the Carousel tab under the User Preferences. If you selected the automatic saving preference in 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 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 *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, choose tube or specimen.

The carousel moves to the next tube or specimen.

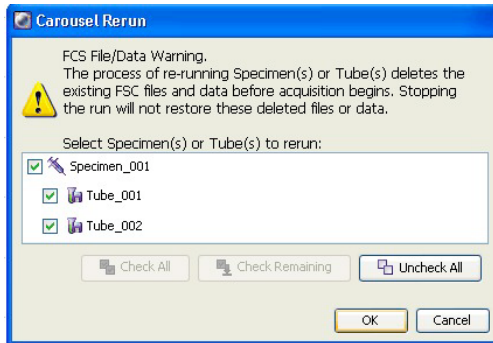
To re-run a tube or specimen:

- 1 Click  Rerun in Carousel Controls.
- 2 In the dialog that appears, choose either tube or specimen.

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

## Re-running Tubes or Specimens After Acquisition

- 1 Choose Carousel > Carousel Setup.
- 2 In the worklist, change the Carousel ID to None for all specimens that you want to re-run.
- 3 Click New Carousel and reassign the carousel rack; click OK to close the window.
- 4 Click Run Carousel in Carousel Controls.
- 5 In the re-run dialog that appears, select the tubes or specimens you want to re-run; click OK to close the dialog.



## Adding Tubes to an Existing Carousel

- 1 In the current experiment, add a specimen.
- 2 Choose 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 appears, 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  in Carousel Controls. The run stops and the Carousel Report appears.

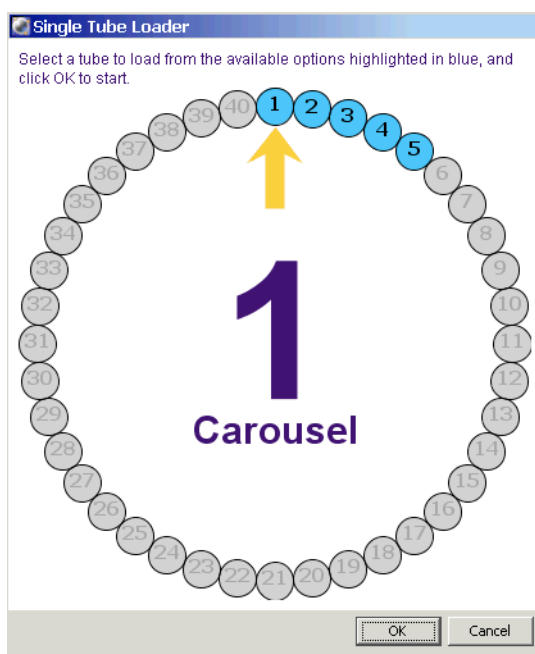


## Running a Single Tube

- 1 To run a single tube using the carousel, click  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 145.

**Figure 8-7** Single Tube Loader dialog

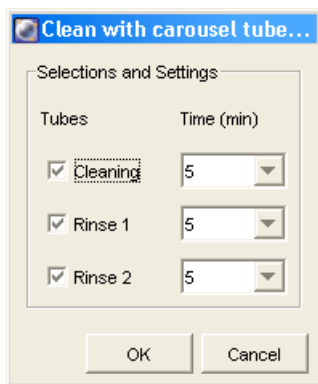


- 3 When the tube is finished, click  in the Acquisition Dashboard.

# Running Cleaning Tubes on the Loader

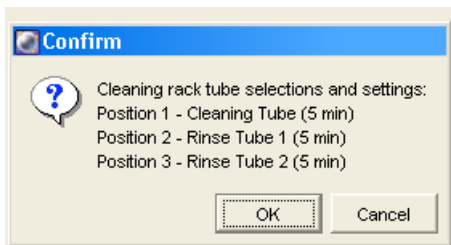
---

- 1 In Acquisition Setup, check flow rate; set to Medium.
- 2 Choose 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.

BD recommends using at least BD FACSClean solution and DI water.



- 6 Click OK to begin cleaning.

# Shutting Down

---



At the end of the day, shut down the cytometer.

## BD FACSCanto Clinical Software

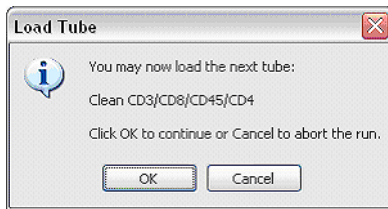
- 1 Create a worklist.
  - Enter *Clean* as the Sample ID.
  - Choose a two-tube panel (such as 4 color TBNK) from the panel field menu.







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 .
- 3 When prompted to save the worklist, click No.
- 4 (Loader Only) Click  to run tubes manually.

- 5 When prompted, install a tube with  $\leq 3$  mL of BD FACSClean 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 .
- 7 After 5 minutes, click .
- Do not click .
- 8 When prompted, unload the tube.
- 9 Install a tube with  $\leq 3$  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.

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

- 11 Choose File > Exit, and select *Run fluidics shutdown and exit*.

BD Biosciences recommends turning off the cytometer when not in use to preserve laser life.

**NOTICE** After every 4 hours of operation, and during cytometer shutdown, you will hear a hiss when condensed water discharges from the fluidics cart pumps into the condensation trap.

- 12 When fluidics shutdown completes, the software closes.
- 13 Turn off cytometer power.

- 14 Shut down the computer.
- 15 Wet a soft, lint-free cloth with DI water and wipe down the following surfaces daily to prevent salt buildup:
  - SIT  

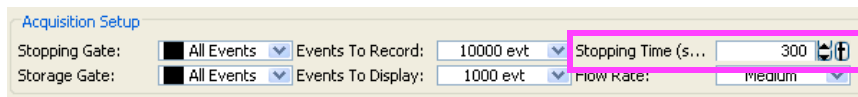
Be careful not to bend the SIT.
  - aspirator arm  

Wipe all exposed surfaces of the aspirator arm that are outside the cytometer chassis.
- 16 Use a dry lint-free cloth to remove residual moisture.
  - Tip** Place an empty tube on the SIT to catch any BD shutdown solution that might drip from the SIT.
- 17 Empty the condensation trap located underneath the fluidics cart power panel. See Figure 1-6 on page 22.

## BD FACSDiva Software

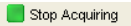
Use this procedure when cleaning the cytometer manually (without the Loader). Use the procedure, Running Cleaning Tubes on the Loader on page 146, when the Loader is installed.

- 1 Install a tube with  $\leq 3$  mL of BD FACSClean solution or 10% bleach on the SIT. Firmly push up on the tube until it comes to a complete stop.
- 2 In Acquisition Setup, set stopping time to 300 sec (5 min).



- 3 In Basic Controls, click Acquire Data.



- 4 After 300 sec (5 min), click  and remove the tube.
- 5 Install a tube with  $\leq 3$  mL of DI water on the SIT. Firmly push up on the tube until it comes to a complete stop and is fully seated.
- 6 Repeat steps 3 and 4.



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

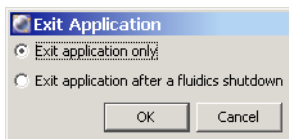
- 7 Choose Cytometer > Fluidics Shutdown, and then follow all prompts.
- 8 Turn off cytometer power.

BD Biosciences recommends turning off the cytometer when not in use to preserve laser life.

**NOTICE** 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.

- 9 Quit BD FACSDiva software.

The following dialog appears. Verify *Exit application only* is selected and click OK.



- 10** Shut down the computer.
- 11** 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.
- 12** Use a dry lint-free cloth to remove residual moisture.
  - Tip** Place an empty tube on the SIT to catch any BD shutdown solution that might drip from the SIT.
- 13** Empty the condensation trap located underneath the fluidics cart power panel. See Figure 1-6 on page 22.





# 10

## Maintenance

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- Scheduled Maintenance on page 154
- Unscheduled Maintenance on page 164

# Scheduled Maintenance

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

<b>Procedure</b>	<b>Description and Location</b>	<b>Frequency</b>
Fluidics startup	Replaces BD FACS shutdown solution with BD FACSFlow solution See step 5 on page 47.	Daily
Fluidics shutdown	Replaces BD FACSFlow solution with BD FACS shutdown solution See Shutting Down on page 147.	Daily
Empty the waste	See Emptying the Waste Container on page 156.	Daily and as needed
Wipe down SIT and aspirator arm	Prevents saline deposit buildup See step 15 on page 149 and step 11 on page 151.	Daily
Empty condensation trap on the fluidics cart	Prevents overflow of condensation trap See step 17 on page 149 and step 13 on page 151.	Daily
Purge fluid filters	Removes air from fluid filters, ensuring they will not dry out See Purging the Fluidics Filters on page 158.	Weekly
Change waste container cap	See step 6 on page 158.	Monthly

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<b>Procedure</b>	<b>Description and Location</b>	<b>Frequency</b>
Decontaminate fluidics (Long Clean)	Decontaminates the internal sheath path with BD FACSClean solution, then rinses with BD FACS shutdown solution See page 160.	Monthly and before service calls
Replace air filter	Ensures proper cytometer performance See Replacing the Air Filter on page 161.	Every 6 months
Replace fluid filters	Keeps fluids free of particulates See Replacing Fluidics Filters on page 162.	Every 6 months or when increased debris observed in FSC vs SSC plots
Schedule preventative maintenance by BD service	—	Every 6 months

# Emptying the Waste Container

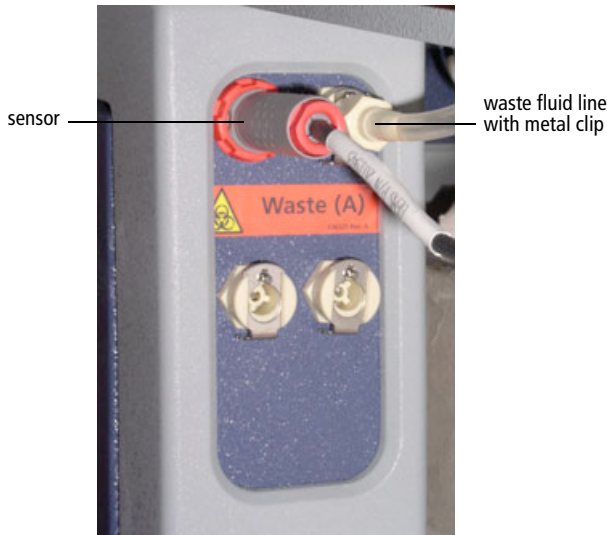
  All biological specimens and materials coming into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, expose waste container contents to bleach (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions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

**Figure 10-1** Location of waste port on fluidics cart



- 1 Ensure 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 157).
  - Pull the sensor straight out.
  - Press the metal clip on the fluid line.

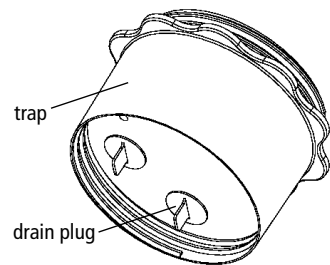
**Figure 10-2** Closeup of waste port



**⚠️ ⚠️** 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.

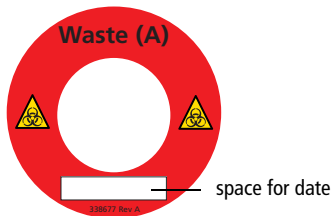
**3** Remove the disposable waste cap and attached trap from the container; place the assembly on the bench label-side up.



**⚠️ ⚠️** 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:
  - Detach the cap from the trap.
  - Replace it with a new cap.
  - Write the date on the cap label.



  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.

- 7 Screw the cap assembly onto the waste container and hand-tighten until it is fully closed.
- 8 Re-attach the sensor line and fluid line.

## Purging the Fluidics Filters

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

- 1 Run the Prime After Tank Refill for all fluids to ensure all fluid lines are primed.

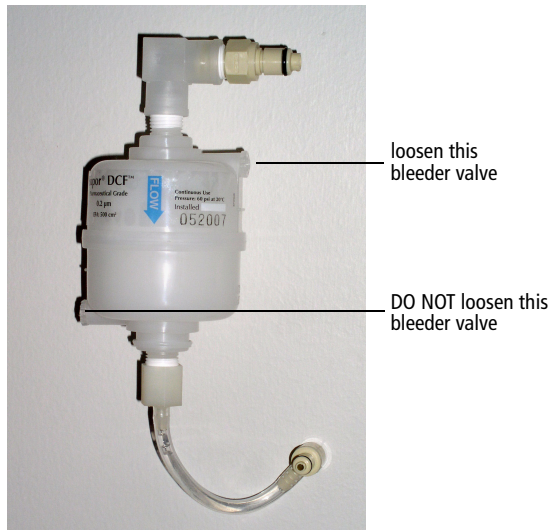
See Priming Fluidics Lines on page 169.

- 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.

**NOTICE** Do not loosen the bottom bleeder valve. Ensure it is tightened.

- Tip** Do not completely unscrew the valve. If you do, it will come off.



- 4 Wait until fluid seeps out.

Fluid should seep from the open valve within 30 seconds. If no fluid appears, make sure 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 169.

- 5 Close the valve by turning it clockwise.
- 6 Repeat steps 2 through 5 with the remaining filters.

# Decontaminating the Fluidics System (Long Clean)

**NOTICE** This procedure takes about 75 minutes to complete.

- 1 Check all fluid levels. Empty the waste if needed.



The Cytometer menu in BD FACSCanto clinical software differs slightly from the BD FACSDiva Cytometer menu. Instructions in this and the following sections list the BD FACSCanto menu first, followed by the BD FACSDiva menu, in parentheses.

- 2 Select Cytometer > Cleaning Modes > Long Clean.

A confirmation dialog is displayed.

- 3 Click OK to continue. Once you have begun the Long Clean, you cannot abort 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 198.

- 5 Choose to shut down or continue.

- To shut down, quit the software and turn off the power to the cytometer.
- To continue, choose Cytometer > Fluidics Startup.

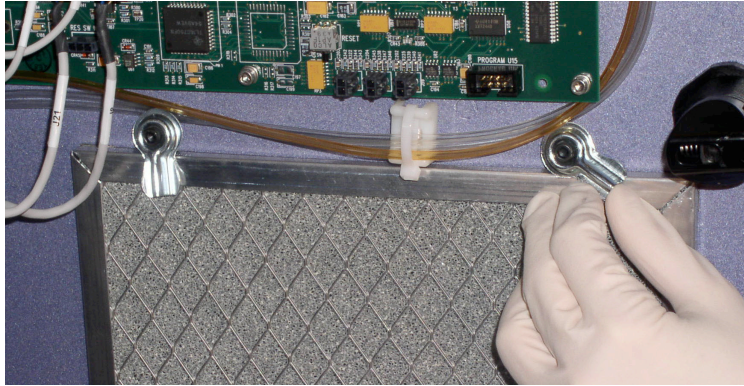


**Tip** If setup fails after a long clean, repeat Fluidics Startup until setup passes.



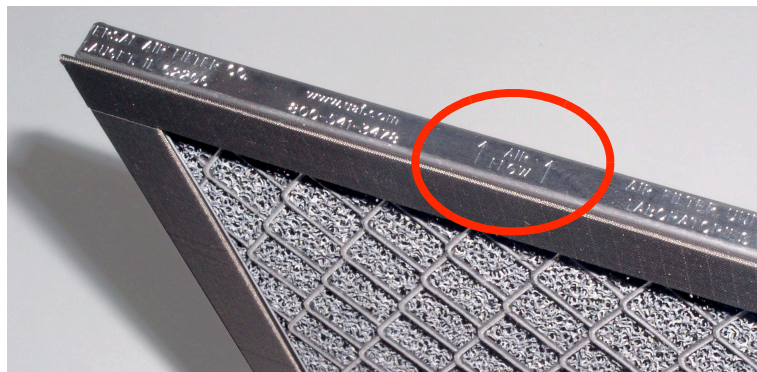
## Replacing the Air Filter

- 1 Turn off the cytometer power.
- 2 On the cytometer, open the right side door by pressing the black button, and then twisting and pulling the handle that pops out.
- 3 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.

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



**5** Turn the tabs to hold the filter.

**6** Close the side door.

 To avoid cytometer damage, do not close the door on any tubing or wires.

## Replacing Fluidics Filters

The fluidics cart should be on during this procedure.

Change the fluid filters when you see increased debris in FSC vs SSC plots, or every 6 months.

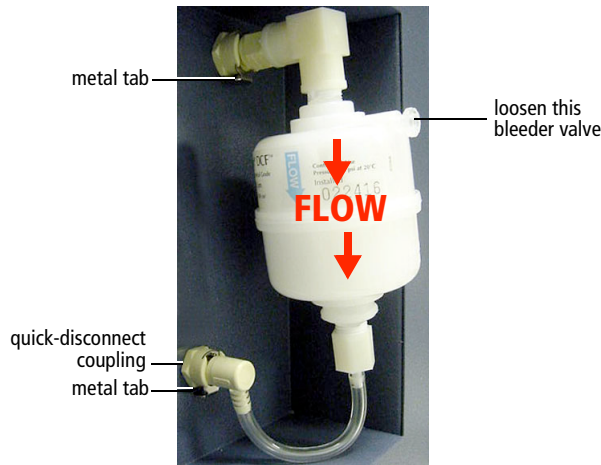


**1** Place a few paper towels beneath the filter.

**2** Remove the filter by pressing the metal tabs on each quick-disconnect coupling.

See Figure 10-3 on page 163.

**Figure 10-3** Removing the filter



**3** Position the new filter and connect the couplings.

**Tip** Write today's date on the filter so you will know when to replace it.

**4** Loosen the top bleeder valve by turning it counterclockwise.

**NOTICE** Do not loosen the bottom bleeder valve. Ensure it is tightened.

**5** Wait until fluid seeps out.

Fluid should seep from the open valve within 30 seconds. If no fluid appears, ensure 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 169.

**6** Close the valve by turning it clockwise.

Repeat the procedure for other fluid filters to be replaced.

# Unscheduled Maintenance

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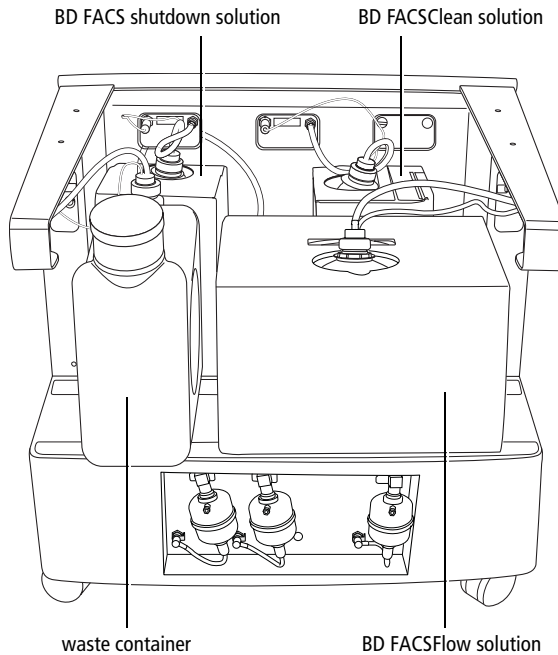
Perform these maintenance procedures as indicated in the table.

<b>Procedure</b>	<b>Description and Location</b>	<b>When to Perform</b>
Replace cubitainers	See Changing a Cubitainer on page 166.	As needed
Prime fluid lines	Removes air from fluid lines See Priming Fluidics Lines on page 169.	Whenever a fluidics line is disconnected to change a cubitainer or perform other maintenance
Remove an air lock	Removes air from filter and restores flow of fluid See Removing an Air Lock on page 169.	When fluidics are not functioning properly (flow cell will not fill, or there is backflush into sample tube)
Clean external surfaces	Keeps surfaces free from salt buildup after exposure to sheath fluid See Cleaning External Surfaces on page 171.	As needed
De-gas Flow Cell	Removes bubbles from the flow cell See See Removing Bubbles from the Flow Cell on page 172.	As needed during daily startup
Clean Flow Cell	Runs BD FACSClean solution through the SIT and flow cell See Cleaning the Flow Cell on page 173.	When poor optical performance indicates additional cleaning is needed
Bubble Filter Purge	Removes air from the bubble filter See Purging the Bubble Filter on page 174.	If fluidics run dry, or when CVs are poor

<b>Procedure</b>	<b>Description and Location</b>	<b>When to Perform</b>
Decontaminate the fluidics system for storage	Cleans out the fluidics lines with BD FACSClean solution, then fills them with BD FACS shutdown solution  See Decontaminating the Fluidics System for Storage on page 175.	Before long-term storage
Replace the Bal seal	Replaces worn Bal seal  See Replacing the Bal Seal on page 175.	When the seal becomes worn or cracked (sample tubes will not pressurize)
Reconnect fluidics cart tubing	See Reconnecting the Fluidics Cart Tubing on page 181.	As needed
Replace fluidics level sensors	See Replacing the Fluidics Level Sensors on page 183.	When instructed to by a BD Biosciences service representative
Replace fluidics cart fuses	See Replacing the Fluidics Cart Fuses on page 186.	When cart will not function because of power surge or other electrical event

# Changing a Cubitainer

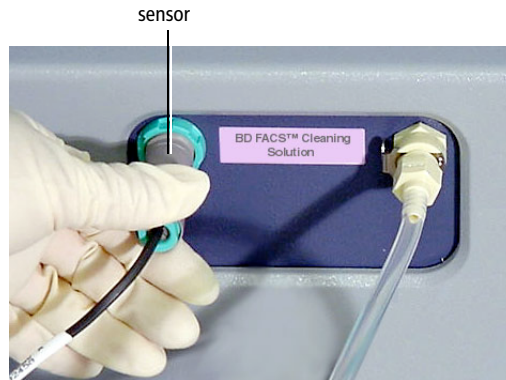
Three fluidics cubitainers (disposable boxes of approved fluids) and a waste container fit onto the cart in the following configuration:



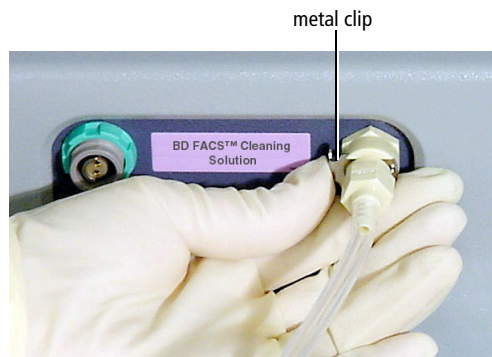
Each cubitainer and the waste container has its own color-coded port.


- 1** Ensure the cytometer is not acquiring events.
- 2** Detach the sensor and fluid line from the cart.

- Pull the sensor straight out.



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



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

- 8 Prime the fluidics.

Important: Continue with the procedure in Priming Fluidics Lines on page 169.





## Priming Fluidics Lines

- 1 Choose 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 displays.

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

## Removing an Air Lock

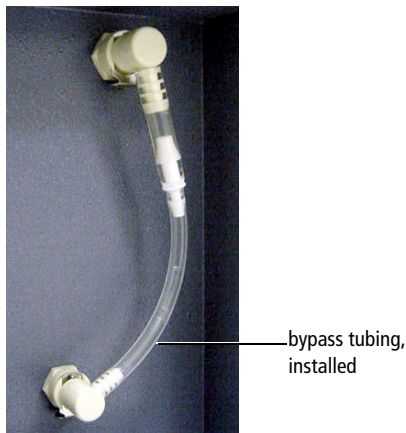
  All cytometer surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning cytometer surfaces. Wear suitable protective clothing and gloves.

- 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



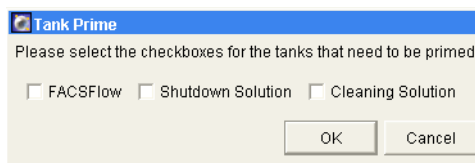
- 3 Install bypass tubing in place of the filter.



- 4 Choose Cytometer > Cleaning Modes > Prime After Tank Refill.
- 5 Select the checkbox that corresponds to the filter you have bypassed.
- 6 Click OK.



BD FACSCanto clinical software



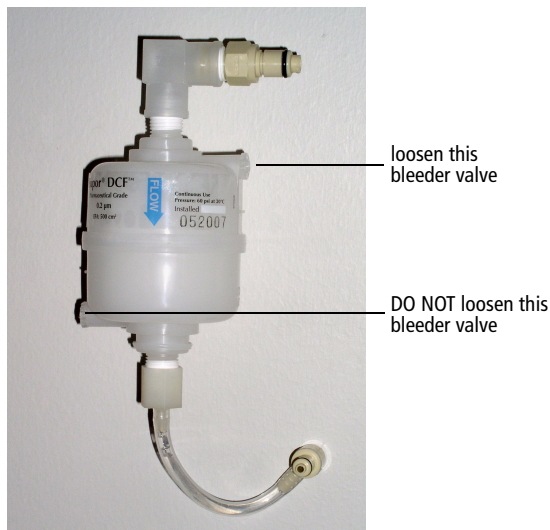
BD FACSDiva software

- 7 Ensure 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 Prime After Tank Refill command.
- 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.



**NOTICE** 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 175 and steps 3, 4, 9, and 10 of the Replacing the Bal Seal procedure that starts on page 175.

**NOTICE** Do not use BD FACSClean solution or bleach to clean or disinfect the barcode reader. See Cleaning the Barcode Reader on page 42.

  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.

**1** Switch off the cytometer power and unplug the AC power cord.

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

**2** Wipe all accessible surfaces with BD FACSClean solution.

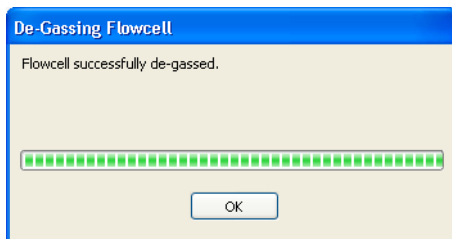
**3** Wet a fresh cloth with DI water and wipe all bleach-exposed surfaces to prevent corrosion.

**4** Wipe all exposed surfaces with a clean, dry cloth.

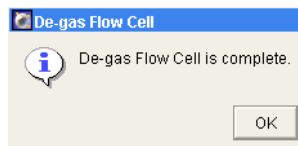
## Removing Bubbles from the Flow Cell

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

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



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BD FACSDiva software

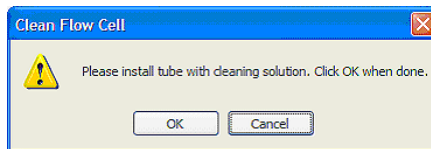
- 3 Check the flow cell for bubbles.

If you still see bubbles, repeat the process.

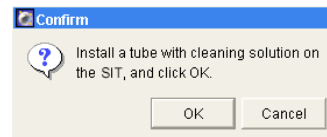
**Tip** You might need to de-gas a new flow cell several times to clear bubbles.

## Cleaning the Flow Cell

- 1 Choose 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 cleaning solution onto the SIT, and click OK.



BD FACSCanto clinical software



BD FACSDiva software

A progress message is displayed during the cleaning.

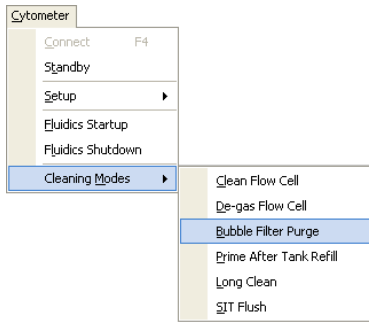
- 4 After the completion message displays, wait 5 minutes.

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

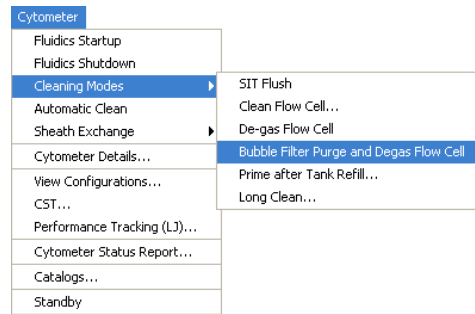
- 5 Click OK.
- 6 Remove the tube from the SIT.
- 7 Clear BD FACSClean solution from the flow cell and fluidics lines:
  - Before running setup or samples, run Fluidics Startup.
  - To shut down without running more samples, run Fluidics Shutdown.

# Purging the Bubble Filter

- 1 Choose Cytometer > Cleaning Modes > Bubble Filter Purge (Bubble Filter Purge and Degas Flow Cell).

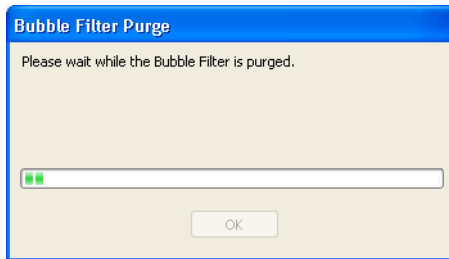


BD FACSCanto clinical software

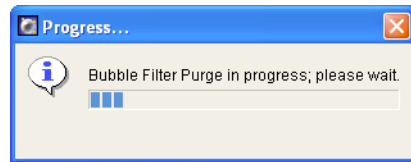


BD FACSDiva software

- 2 Wait while the purge finishes.



BD FACSCanto clinical software



BD FACSDiva software

- 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, choose Cytometer > Cleaning Modes > De-gas Flow Cell.

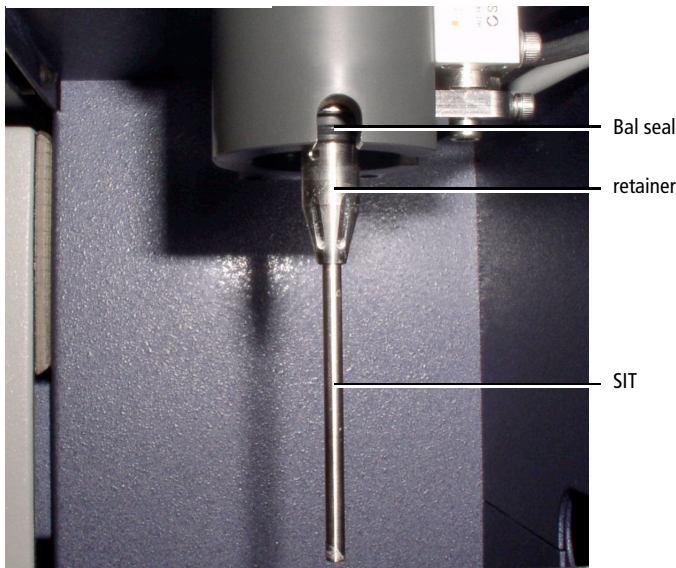
## Decontaminating the Fluidics System for Storage

- 1 Perform steps 1 through 4 in Decontaminating the Fluidics System (Long Clean) on page 160.
- 2 Shut down the software and turn off the power to the cytometer.
- 3 Wipe down the SIT and the aspirator arm with DI water. See Shutting Down on page 147 for more information.

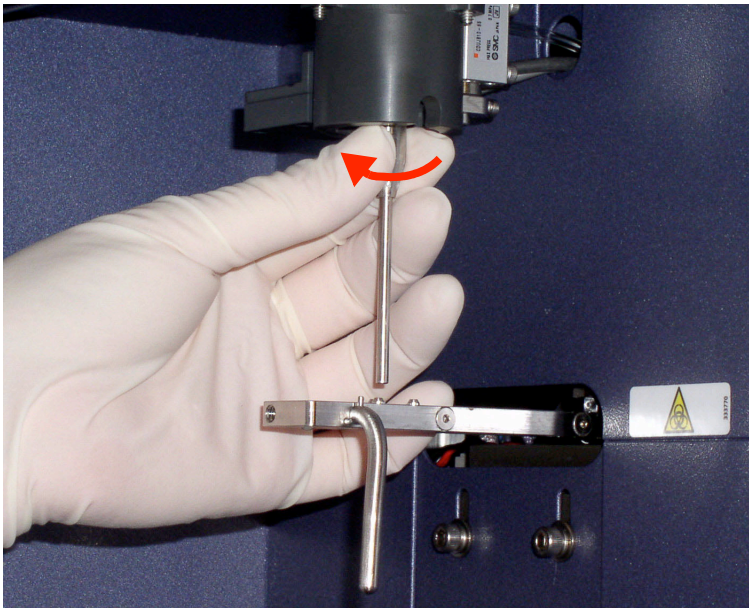
## Replacing the Bal Seal

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.

**Figure 10-6** Bal seal and retainer

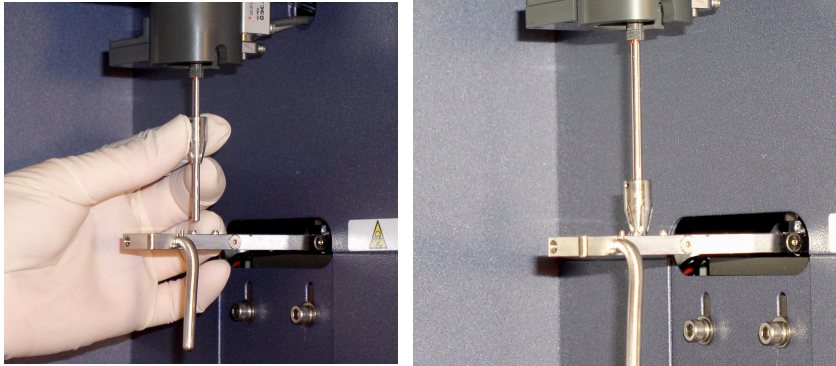


- 1** Turn off the cytometer.
- 2** If you have a Loader installed:
  - Open the Loader doors.
  - Pull out the drawer.
  - If needed, remove the carousel.
  - Move the tube guide to manual position. See step 2 on page 51.
- 3** Unscrew the retainer by turning it in the direction shown.

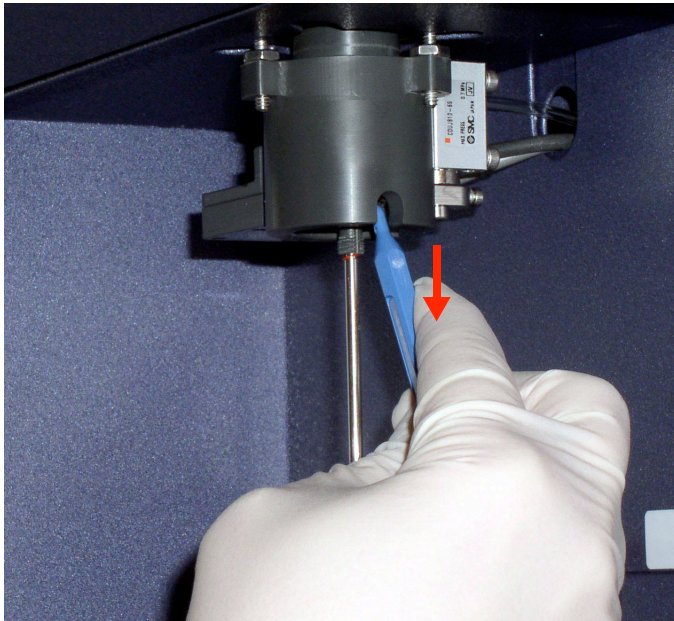




- 4 Lower the retainer down the SIT and let it rest on the aspirator arm.



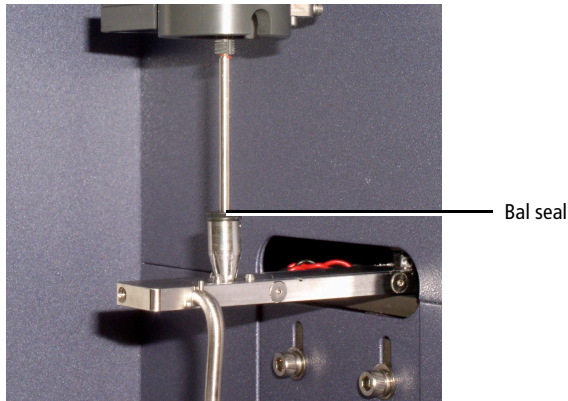
- 5 Access the Bal seal at the notch on the right side of the SIT assembly. Unseat the Bal seal by using the Bal seal removal tool (Figure 10-7 on page 178) to gently pull it downward.



**Figure 10-7** Bal seal removal tool



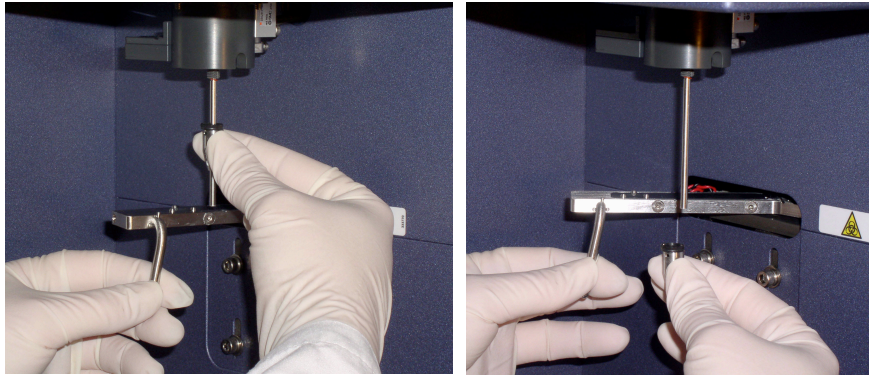
- 6** Allow the Bal seal to slide down the SIT and rest on top of the retainer.



- 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 10-8 on page 179.

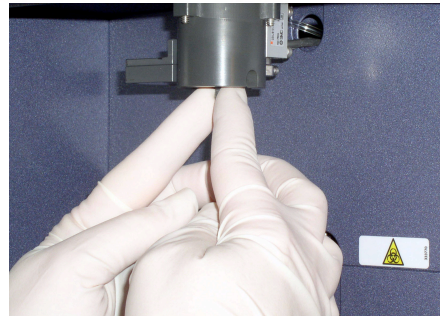
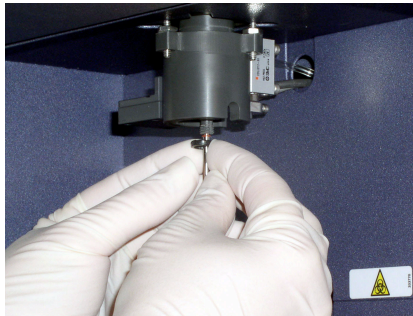
**Figure 10-8** Removing the Bal seal and retainer from the SIT



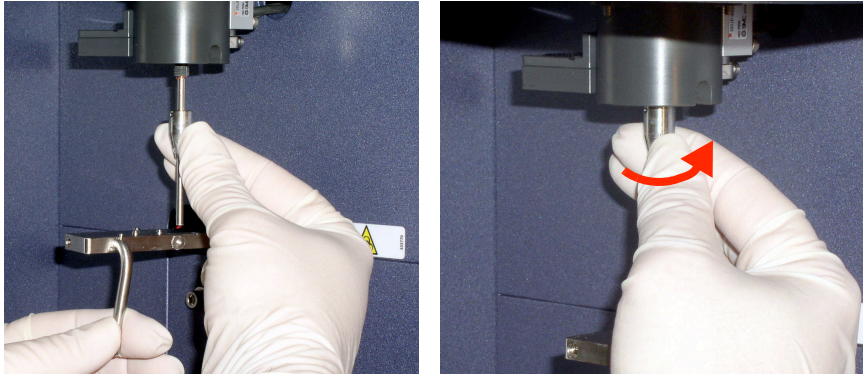
- 8** Hold the new Bal seal spring-side up (see figure), and slide it up the SIT. Use both hands to gently push it into its seated position.

**Tip** If the Bal seal is not quite in its seated position, replacing the retainer will seat it correctly.

Bal seal,  
spring side up



- 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.

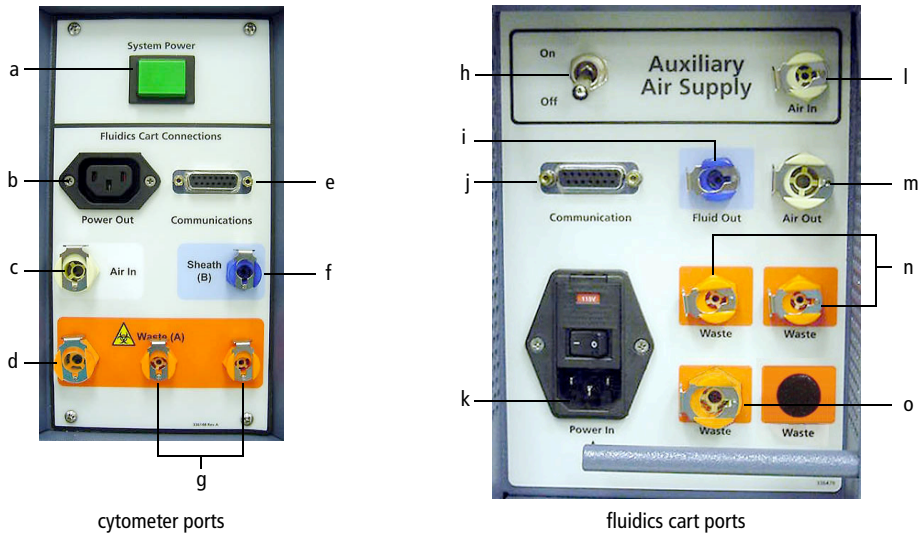


- 11 Test the installation by manually loading a tube onto the SIT and running fluid.
- 12 If you are using a Loader, ensure that the system is ready for automatic loading. See Figure 8-3 on page 136.

# Reconnecting the Fluidics Cart Tubing

☑ **Tip** The ports and connectors are color coded.

**Figure 10-9** Cytometer and fluidics cart ports



Cytometer ports and their corresponding fluidics cart ports are listed in Table 10-1 on page 182. 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
a. System Power	—
b. Power Out	k. Power In
c. Air In	m. Air Out
d. Waste (A)	o. Waste
e. Communications	j. Communication
f. Sheath (B)	i. Fluid Out
g. Waste (A)	n. Waste
—	h. On/Off
—	l. Air In



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
a. System Power	Powers both cytometer and fluidics cart
b. Power Out	Connects to fluidics cart
c. Air In	70±5 psi
d. Waste (A)	Waste out (aspirated)
e. Communications	Data port
f. Sheath (B)	BD FACSCanto solution port
g. Waste (A)	Waste out (non-aspirated)
h. On/Off	Auxiliary air supply switch. Keep in off position unless connected to house air.



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

Port or Switch	Additional Information
i. Fluid Out	BD FACSDiagnostic solution port
j. Communication	Data port
k. Power In	Connects to cytometer. Do not connect to wall outlet
l. Auxiliary Air In	There will be no tubing on this port unless connected to house air.
m. Air Out	Sends compressed air to cytometer
n. Waste	Waste in (non-aspirated)
o. Waste	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.

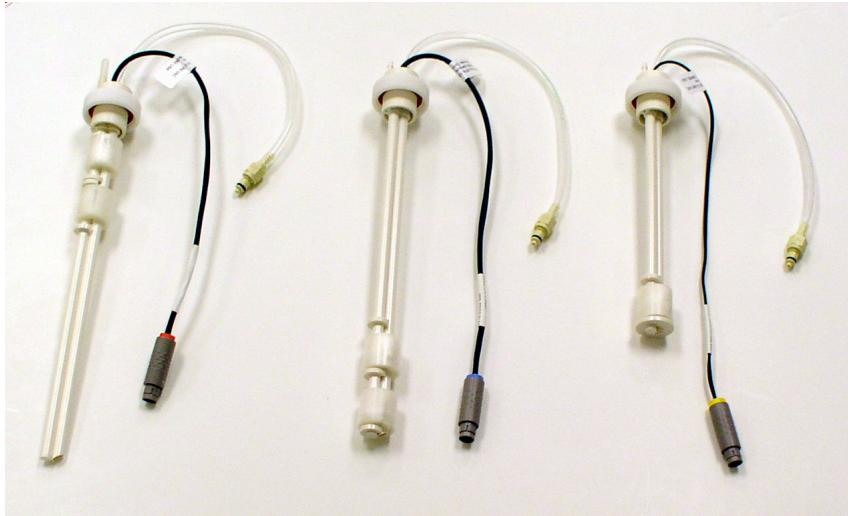
- 1 Ensure that the cytometer is not acquiring events.
- 2 Detach the sensor and fluid line from the cart.
- 3 Unscrew the cap on the cubitainer.
- 4 Remove the level sensor assembly and discard into a suitable receptacle.





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

**Figure 10-10** Level sensor assemblies



waste level sensor  
(red connector)

BD FACSTow level sensor  
(blue connector)

BD FACSClean solution and  
BD FACSClean shutdown solution  
level sensor (yellow connector)

- 6 Reattach the sensor line and fluidics lines.





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

- 7 If you replaced any level sensors (other than the waste sensor), prime the affected fluidics lines.

**Important:** Continue with the procedure in Priming Fluidics Lines on page 169.

## Replacing the Fluidics Cart Fuses

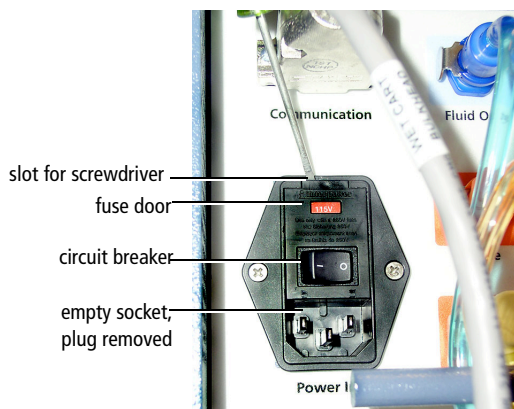
- 1 Shut down the cytometer and turn off the cytometer power.

  To protect against shock, always turn off the cytometer and unplug the power cord before performing this procedure.

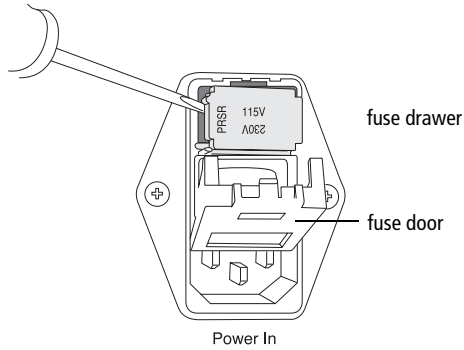
- 2 Unplug the power cord from the wall outlet and the fluidics cart.

Removing the plug allows easier access to the fuse door.

- 3 Insert a small screwdriver into the slot and gently pry the fuse door open.

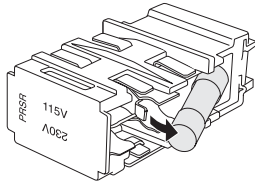


- 4 Gently pry out the fuse drawer until you can grip it.



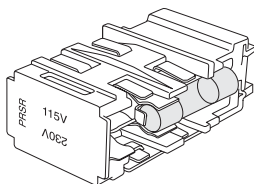
- 5 Remove the fuse drawer, and remove and dispose of the old fuses.

- Tip** Note the positions of the old fuses before you remove them. Duplicate these positions with the new fuses.

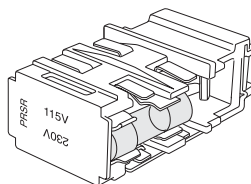


**6** Replace both fuses.

Ensure that the fuses are positioned as shown.



right

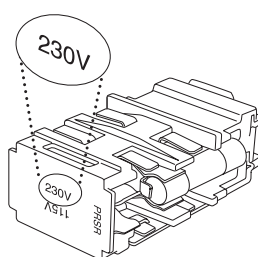
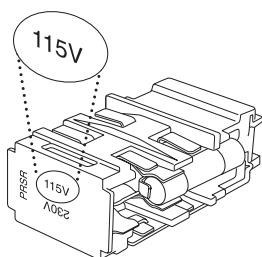


wrong



For protection against risk of fire, replace fuses only with those provided by BD Biosciences.

**7** Make sure the text for your country's voltage is right-side up.



**8** Slide the drawer back into the cytometer until it snaps into place.

**9** Close the fuse access door.

**10** Reconnect the power cord to the fluidics cart.

**11** Plug the cytometer power cord into the wall outlet and switch on the power.

## Troubleshooting

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The tips in this section can help you troubleshoot issues that might arise when using this cytometer. 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, call (877) 232-8995. For support from within Canada, call (888) 259-0187. Customers outside the US and Canada, contact your local BD representative or distributor. Refer to our website, [bdbiosciences.com](http://bdbiosciences.com), for up-to-date contact information.

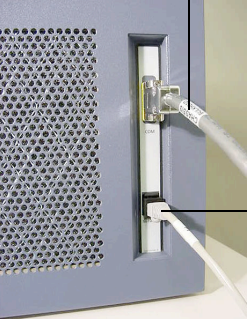
# Cytometer Troubleshooting

Observation	Possible Causes	Recommended Solutions
Flow cell will not fill	Air in bubble filter	<p>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.</p> <p>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).</p>
	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 24). Always use the cytometer power button, located on the left side of the cytometer, to turn the system off and on.
	No pressure in plenum	<p>Check air supply components (see Figure 10-9 on page 181).</p> <ul style="list-style-type: none"> <li>• Check air supply connections.</li> <li>• Check air supply tubing for kinks.</li> </ul>
	Sheath line disconnected	<p><b>1</b> Check cubitainer-to-fluidics cart, and fluidics cart-to-cytometer connections. See Reconnecting the Fluidics Cart Tubing on page 181.</p> <p><b>2</b> Check tubing for kinks.</p>
	BD FACSFlow cubitainer empty	Replace the BD FACSFlow cubitainer. See Changing a Cubitainer on page 166.

# Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Flow cell will not fill (continued)	Air in BD FACSTFlow filter (fluidics cart)	Purge air from the filter. See Priming Fluidics Lines on page 169.
	Air lock in BD FACSTFlow filter (on fluidics cart)	See Removing an Air Lock on page 169.
	No air pressure in cart	<ul style="list-style-type: none"> <li>• If the cart is attached to an auxiliary air supply, switch on auxiliary air.</li> <li>• If the cart is not attached to an auxiliary air supply, switch off auxiliary air.</li> </ul>
Fluid backfill into sample tube	Cracked tube	<ul style="list-style-type: none"> <li>• Transfer sample to new tube.</li> <li>• Make sure you are using appropriate tubes. See System Requirements on page 28.</li> </ul>
	Bal seal improperly installed or worn	Reinstall or replace the Bal seal. See Replacing the Bal Seal on page 175.
	Air lock in BD FACSTFlow filter	See Removing an Air Lock on page 169.
	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	<ol style="list-style-type: none"> <li><b>1</b> Repeat tube removal sequence.</li> <li><b>2</b> Perform a manual SIT flush.</li> </ol>

# Cytometer Troubleshooting (continued)


Observation	Possible Causes	Recommended Solutions
Fluidics pressure errors	Air lock in filter	Check the filter in the fluidics cart. Verify 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 169.
Cytometer on, no response to software commands	Bad keyboard or mouse connection	Check keyboard or mouse connections to computer. Refer to the documentation that came with your workstation.
	Communication failure between computer and cytometer	<ol style="list-style-type: none"> <li>1 Turn off the computer and the cytometer.</li> <li>2 Reseat the Ethernet cable, located next to the power cord on the right side of the flow cytometer.</li> <li>3 Turn on the cytometer, then the computer.</li> </ol>
		
	User-installed firewall	<ul style="list-style-type: none"> <li>• Contact your system administrator to open a port in the firewall for cytometer.</li> <li>• Call BD Biosciences.</li> </ul>
QC fails after Long Clean	Residual BD FACSClean 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 BD Falcon polystyrene test tubes, BD Trucount tubes, and BD FACS 7-color setup beads tubes. See System Requirements on page 28.
	Defective or cracked tube	<ul style="list-style-type: none"> <li>• Transfer sample to new tube.</li> <li>• Make sure you are using appropriate tubes. See System Requirements on page 28.</li> </ul>
	Bal seal improperly installed or worn	Reinstall or replace the Bal seal. See Replacing the Bal Seal on page 175.
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.
Fluid leaking from SIT or aspirator arm	Interior valve failure or leak	<ol style="list-style-type: none"> <li>1 Turn off the cytometer power.</li> <li>2 Clean up the liquid, using proper precautions.</li> <li>3 Call BD Biosciences.</li> </ol>
	Waste line to fluidics cart disconnected	<ol style="list-style-type: none"> <li>1 Turn off the cytometer power.</li> <li>2 Clean up the liquid, using proper precautions.</li> <li>3 Check that waste lines are securely plugged in.</li> <li>4 Turn on the cytometer power.</li> </ol>

## Cytometer Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Liquid leakage around cytometer base	Interior valve failure	<ol style="list-style-type: none"> <li>1 Turn off the cytometer power.</li> <li>2 Clean up the liquid, using proper precautions.</li> <li>3 Call BD Biosciences.</li> </ol>
Aspirator arm locked, cannot unload tube	Software not responding	<p> Do not use force to remove tube from SIT.</p> <ol style="list-style-type: none"> <li>1 Press Ctrl-Alt-Delete to open Task Manager.</li> <li>2 Choose End Task to close BD FACSCanto clinical software or BD FACSDiva software application.</li> <li>3 Restart software and unload tube.</li> </ol>
	Pressure loss	Verify fluidics cart is powered on and that air pressure hoses are properly connected.
Fluidics error after replacing sheath fluid or emptying waste	Status change not yet detected by software	<p>After replacing or emptying fluids, wait until software detects status change before restarting acquisition.</p> <p>It can take up to 30 seconds for the software to detect a fluid level change.</p>

# Loader Troubleshooting

Observation	Possible Causes	Recommended Solutions
Tube does not load	Tube not completely raised	<p>Ensure the Loader drawer is completely shut.</p> <p>Ensure the tube is free of bulky labels or tape.</p>
	Tube lifter hitting carousel during ascent	Ensure 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.
Tube does not unload	Tube lifter failure	<p><b>1</b> If the tube lifter is in the up position, turn off the cytometer or Loader power for approximately 5 minutes.</p> <p>The air in the tube lifter will decompress, lowering the tube lifter.</p> <p><b>2</b> Remove the tube.</p> <p>If the problem persists, contact BD Biosciences.</p>
	Status change not yet detected by software	<p>After replacing or emptying fluids, wait until software detects a status change before restarting acquisition.</p> <p>It can take up to 30 seconds for the software to detect a fluid level change.</p>

## 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 <i>Cleaning External Surfaces</i> on page 171.
	Condensation on tube	Wipe tube.
	Tube label reflection	Turn tube.
Tube not detected	Optical sensor is wet or dirty	<ul style="list-style-type: none"> <li>• Clean sensor</li> <li>• Allow time for sensor to dry</li> </ul>
Loader stalls or emits a grinding noise	Door opened during Loader initialization	<p>When the Loader stops moving, open and close the Loader doors.</p> <p>Do not open the Loader doors while the Loader is in motion.</p>
Tube runs dry, Loader not advancing to next sample	Dilute sample or rare events	Set a stopping time in BD FACSDiva software. See step 2 on page 139.
	Bubble in flow cell diverts stream	Degas flow cell. See <i>Removing Bubbles from the Flow Cell</i> on page 172.
	Communication error	<ol style="list-style-type: none"> <li><b>1</b> Restart the system.</li> <li><b>2</b> Perform fluidics startup. See step 5 on page 47.</li> </ol>

# Loader Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Test tube stuck on SIT, software not responding	<ul style="list-style-type: none"> <li>• Cytometer not set up for automatic loading</li> <li>• Software problem</li> </ul>	<p>Remove the tube from the SIT manually.</p> <ol style="list-style-type: none"> <li><b>1</b> Hold the tube and move the aspirator arm aside.</li> <li><b>2</b> Gently pull the tube off the SIT.</li> <li><b>3</b> Release the aspirator arm.</li> <li><b>4</b> Restart the software.</li> <li><b>5</b> If needed, modify the cytometer for automatic loading.</li> </ol>
Carousel not rotating correctly	<p>Carousel not engaged with alignment pin on drawer</p> <hr/> <p>Aspirator arm bar vertical</p>	<p>Rotate the carousel on the spindle until the alignment guide pin engages with the alignment hole, and press down. See step 4 on page 137. If the problem persists, contact BD Biosciences for assistance.</p> <hr/> <p>Rotate the bar to the forward horizontal position.</p>

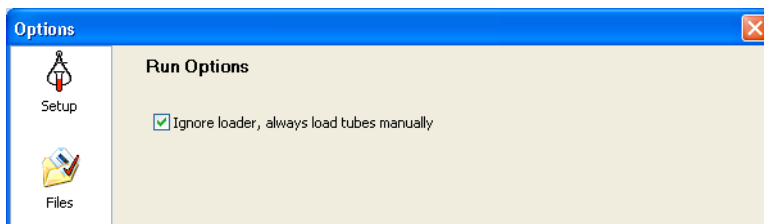
## Disabling the Loader in BD FACSCanto Clinical Software

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 appear.

- 1** In BD FACSCanto clinical software, choose Tools > Options.

- 2** Click  .

- 3** Select the checkbox in the Run Options dialog:



4 Click .

## Fluidics Cart Troubleshooting


Observation	Possible Causes	Recommended Solutions
Water leakage around fluidics cart base	<ul style="list-style-type: none"> <li>• Normal condensation overflow from pressure relief valve</li> <li>• Extremely humid climate</li> </ul>	<ol style="list-style-type: none"> <li>1 Turn off the cytometer power.</li> <li>2 Clean up the water.</li> <li>3 Empty the condensation trap in the fluidics cart daily. See Figure 1-6 on page 22.</li> </ol>
	Bleeder valve on fluidics cart filter open	<ol style="list-style-type: none"> <li>1 Turn off the cytometer power.</li> <li>2 Clean up the liquid.</li> <li>3 Check and close all bleeder valves. See Purging the Fluidics Filters on page 158.</li> </ol>
	Broken fluid line	Contact BD Biosciences.
Fluidics cart will not power on, cytometer on	Cart circuit breaker off	Switch on the 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.
	Fuse blown	Replace the fluidics cart fuses. See Replacing the Fluidics Cart Fuses on page 186.

# 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 Components on page 20).

## BD FACSCanto Software General Issues

Observation or Error Message	Possible Causes	Recommended Solutions
Software not responding	Software frozen	<ol style="list-style-type: none"><li>1 Press Ctrl-Shift-Esc. Locate BD FACSCanto clinical software in the Windows Task Manager; click End Task.</li></ol> <p> If acquisition is in progress, data will be lost.</p> <ol style="list-style-type: none"><li>2 Restart software. To prevent carryover, perform fluidics startup before acquiring data by choosing Cytometer &gt; Fluidics Startup.</li></ol>
Software does not connect to cytometer	BD FACSDiva software running	<ul style="list-style-type: none"><li>• Quit BD FACSDiva software.</li></ul> OR <ul style="list-style-type: none"><li>• In BD FACSDiva software, put the cytometer in standby: choose Cytometer &gt; Standby.</li></ul>
	Cytometer power is off	Turn power on.
	Internal firmware error	Cycle power on cytometer.
	Ethernet disconnected	See page 192.

## BD FACSCanto Software General Issues (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Connection error messages	Various	<ul style="list-style-type: none"><li>• Follow any message directions.</li><li>• Ensure the cytometer power is on.</li><li>• Check the Ethernet cable connection to the cytometer and computer.</li><li>• Verify the fluidics cart is plugged in and the power on.</li><li>• Shut down the software, computer, and cytometer, and then restart them.</li></ul>
Barcode reader error	Dirty barcode reader window	Clean 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 data manually.



## BD FACSCanto Software General Issues (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Tube not present error	Tube not seated on SIT	Push tube all the way up onto the SIT.
	Cracked tube	<ul style="list-style-type: none"><li>• Transfer sample to new tube.</li><li>• Make sure you are using appropriate tubes. See System Requirements on page 28.</li></ul>
	Fluid at top of tube prevents detection	Wipe inside top of tube with clean cotton swab. Dispose of swab with biohazardous materials.
	Tube sensor wet or damaged	<ul style="list-style-type: none"><li>• Allow time for sensor to dry.</li><li>• Reduce solution volumes to <math>\leq 3</math> mL.</li><li>• Call BD Biosciences.</li></ul>

## BD FACSCanto Software General Issues (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Tube pressurization errors	Cracked tube	<ul style="list-style-type: none"> <li>• Transfer sample to new tube.</li> <li>• Make sure you are using appropriate tubes.</li> </ul>
	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 175 for instructions.
	Loader misaligned	<ol style="list-style-type: none"> <li>1 Try running in manual mode instead of automatic. See step 2 on page 51 for converting to manual mode.</li> <li>2 If unsuccessful, call BD Biosciences.</li> </ol>
	Wrong tubes used	Make sure you are using recommended tubes. See System Requirements on page 28.
	Tubing to fluidics cart kinked or disconnected	Reconnect or straighten the tubing. See page 181.
Tube not detected	Tube not seated on Bal seal properly	<ul style="list-style-type: none"> <li>• Ensure that the tube is all the way up onto the Bal seal.</li> <li>• Ensure you are using appropriate tubes. See System Requirements on page 28.</li> <li>• Replace or reinstall Bal seal.</li> </ul>
Vacuum error	Tubing to fluidics cart kinked or disconnected	Reconnect or straighten the tubing. See page 181.

## BD FACSCanto Software Setup Wizard Messages

Messages	Possible Causes	Recommended Solutions
No acquisition events were received from cytometer	Communication problem between hardware and software	<b>1</b> Turn on the power.
		<b>2</b> Connect the Ethernet cable to the cytometer and computer.
		<b>3</b> Shut down the software, computer, and cytometer, and restart them.
		<b>4</b> To prevent carryover, perform a fluidics startup before acquiring data. Choose 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 outside the acceptable range, call BD Biosciences.
	Clogged SIT	Clean the flow cell.
Failed to place [ <i>name of beads</i> ] on scale.	Bubbles in flow cell	Check for bubbles. If found, run De-gas Flowcell.
or Failed to find [ <i>name of beads</i> ]	Software using saved settings from a failed setup	Delete the SetupResults.dat file from C:\Program Files\Common Files\BD\Setup Results, and run setup again.
No valid data points	Internal setup error	Delete the SetupResults.dat file from C:\Program Files\Common Files\BD\Setup Results, and run setup again.

# BD FACSCanto Software Setup Wizard Messages

Messages	Possible Causes	Recommended Solutions
A communication error was encountered	Communication problem between hardware and software	<ol style="list-style-type: none"> <li><b>1</b> Turn on the power.</li> <li><b>2</b> Connect the Ethernet cable to the cytometer and computer.</li> <li><b>3</b> Shut down the software, computer, and cytometer, and restart them.</li> </ol>
	Fluidics cart off or disconnected	Switch on the fluidics cart circuit breaker; make sure 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 loader doors closed.
There is a vacuum error	Vacuum tubing to waste cart disconnected	Reconnect waste tank or tubing, and remove kinks.
	Waste tank disconnected, waste tubing disconnected or pinched	
	Clogged aspirator arm	Call BD Biosciences.

## BD FACSCanto Software Setup Wizard Messages

Messages	Possible Causes	Recommended Solutions
There is a float switch error	Sheath tank not attached to fluidics cart	Reconnect sheath cubitainer or tubing, and remove kinks.
	Sheath tubing from fluidics cart to cytometer not attached or pinched	
	Sheath filter not primed	Always prime after changing a cubitainer.
	Fluidics airlock	See Removing an Air Lock on page 169.
	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	<ul style="list-style-type: none"> <li>• Check air supply connections.</li> <li>• Check air pressure gauge.</li> </ul>

## BD FACSCanto 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.
	Setup.dat file deleted	Accept the failed setup, and then re-run Setup.

## BD FACSCanto Software Setup Report Failure Messages (continued)

Failed	Possible Causes	Recommended Solutions
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 fresh tube of setup beads.
	Software using saved setting from a failed setup	Delete the SetupResults.dat file from C:\Program Files\Common Files\BD\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 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 Software Levey-Jennings Errors and Messages

Observation	Possible Causes	Recommended Solutions
LJ plots empty, no data, no error message appears	<i>BD FACS Setup Beads-7 colors LJ.csv</i> file missing	<ul style="list-style-type: none"> <li>If the file was renamed, rename it with its original name, and click Refresh.</li> <li>If the file was moved from the default directory, move it back, and then click Refresh.</li> </ul>
LJ plots empty, no data, error message appears	<i>BD FACS Setup Beads-7 colors LJ.csv</i> file corrupted or contains invalid data	Delete the <i>BD FACS Setup Beads-7 colors LJ.csv</i> file, and run setup again. The software will create a new set of plots.
	<i>BD FACS Setup Beads-7 colors LJ.csv</i> file open or in use by another application	<ol style="list-style-type: none"> <li>Close the file or application.</li> <li>Click Refresh.</li> </ol>

## BD FACSCanto 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.

## BD FACSCanto Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Run, NO acquisition indicator lights are blinking (no fluorescent signal)	Cracked tube	<ul style="list-style-type: none"> <li>• Transfer sample to new tube.</li> <li>• Make sure you are using appropriate tubes.</li> </ul>
	SIT clogged	Clean the flow cell.
	Current cytometer configuration different from optical setup	Verify the cytometer configuration corresponds to the cytometer's optical setup.
	Laser delay set incorrectly	Contact BD Service.
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	<ol style="list-style-type: none"> <li><b>1</b> Delete unnecessary files.</li> <li><b>2</b> Run disk utilities regularly.</li> </ol>



## BD FACSCanto Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition stops	High event rate (>10,000 events/sec) with more than 8 compensated parameters	<ul style="list-style-type: none"> <li>• Increase threshold.</li> <li>• Decrease threshold channel gain.</li> <li>• Dilute sample.</li> <li>• Define only channels of interest in parameter list.</li> </ul>
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	<ul style="list-style-type: none"> <li>• Choose Cytometer &gt; Cleaning Modes &gt; SIT Flush.</li> </ul>
	Incorrect gating	Verify the gating.

# BD FACSCanto Software TBNK Analysis

## QC Messages

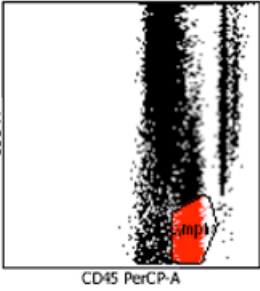
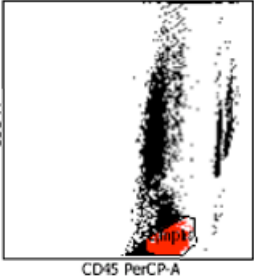
Message	Possible Causes	Recommended Solutions
Sample quality questionable	Donor-specific anomaly	Adjust gates manually to include required subsets.
	Insufficient mixing during sample preparation: cell populations elongated in CD3 vs SSC and FITC vs PE plots	Re-stain the sample. Vortex after blood and reagent are added to tube. Make sure no blood remains on the side of the tube.
	Sample not lysed adequately: cell populations in CD45 vs SSC extend upward	Prepare 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	Refer to 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 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 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 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	<ul style="list-style-type: none"> <li>• Adjust the lymph gate manually.</li> <li>• If necessary, run the sample again.</li> </ul>

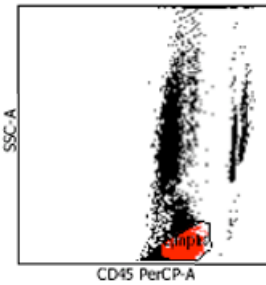
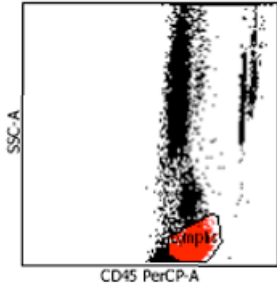
# BD FACSCanto Software Four- and Six-Color TBNK

The following examples provide some assistance in troubleshooting BD Multitest reagent plots.

Observation	Possible Causes	Recommended Solutions
Cell populations in CD45 vs SSC extend upward 	Inadequate lysing of sample	Prepare 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	Refer to the reagent package insert for stability limitations.

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

Observation	Possible Causes	Recommended Solutions
Debris encroaching on populations in CD45 vs SSC plot	<ul style="list-style-type: none"> <li>Excessive mixing</li> <li>Aged blood or stained cells</li> </ul>	Prepare sample again.
Vertically compressed populations	Side scatter too low	Reacquire sample. Set SSC so granulocytes reach to top of CD45 vs SSC plot.
	Lipemic sample	Check reagent package insert for instructions.



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

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

# BD FACSDiva Software Troubleshooting

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## Acquisition Indicator Lights

The acquisition indicator lights may provide important information when troubleshooting software related issues. Ensure that these lights are on (see Electronics Components on page 20).

## BD FACSDiva Software General Issues


Observation	Possible Causes	Recommended Solutions
<i>Cytometer Disconnected</i> in Cytometer window (software does not connect to cytometer)	BD FACSCanto clinical software running	<ul style="list-style-type: none"><li>Quit BD FACSCanto clinical software.</li><li>In BD FACSCanto clinical software, put the cytometer in standby: choose Cytometer &gt; Standby.</li></ul>
	Cytometer power is off	Turn power on.
	Internal firmware error	Cycle power on cytometer.
	Communication failure between software and cytometer	<ul style="list-style-type: none"><li>Quit the software and restart it.</li><li>If unsuccessful, cycle the cytometer power. Restart the computer.</li></ul>
	Ethernet cable disconnected	Unplug and then plug in the cable connectors.
<i>Cytometer not responding</i> status message	Various	Switch the cytometer power off, and then on. Restart the computer.

## BD FACSDiva Software General Issues (continued)

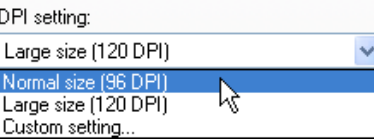
Observation	Possible Causes	Recommended Solutions
Unable to access database	Adaptive Server Anywhere™ not running	<p>Verify that the database server has been started.</p> <ol style="list-style-type: none"> <li>1 Choose Start &gt; Settings &gt; Control Panel and double-click the Administrative Tools icon.</li> <li>2 Double-click the Services icon.</li> <li>3 Select Adaptive Server Anywhere. If the Start button is enabled, click the button to start up the database server. If the buttons are greyed out, the database could be loading.</li> </ol>
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	<p>Wait until cytometer action is complete. If after 2 minutes no timeout is received, restart cytometer and software.</p> <p>After restarting software, to prevent carryover, perform a fluidics startup before acquiring data:</p> <p>Choose Cytometer &gt; Fluidics Startup.</p>

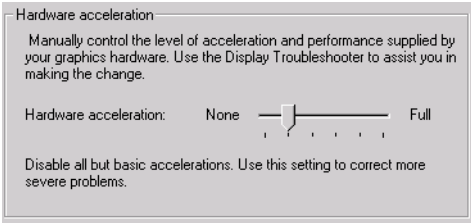


## BD FACSDiva Software General Issues (continued)

Observation	Possible Causes	Recommended Solutions
Software not responding (continued)		<p><b>1</b> Press Ctrl-Shift-Esc. Locate BD FACSCanto clinical software in the Windows Task Manager; click End Task.</p> <p> If acquisition is in progress, data will be lost.</p> <p><b>2</b> Restart software. To prevent carryover, perform fluidics startup before acquiring data by choosing Cytometer &gt; Fluidics Startup.</p>
Software message “Hardware key not accessible...”	Security module disconnected	Reconnect the security module to the USB port, and restart the software.
Error message in Status tab	(Refer to text in error message)	Follow instructions in error message.
	Communication or fluidics error	Shut down the computer and cytometer, and restart them. If the message reappears, contact technical support. Provide exact wording of the status message.

## BD FACSDiva Software General Issues (continued)

Observation	Possible Causes	Recommended Solutions
Faulty screen display, display objects distorted or unreadable	Pointer shadow enabled	<p>Disable the pointer shadow:</p> <p>Select Start &gt; Settings &gt; Control Panel. Double-click the Mouse icon and select the Pointers tab. Deselect the Enable pointer shadow checkbox. Click OK.</p>
	Graphics hardware acceleration set too high	<p>Decrease the acceleration:</p> <p>Right-click on the desktop and select Properties. Click the Settings tab. Click the Advanced button. Click the Troubleshooting tab and drag the hardware acceleration pointer to the left.</p>
Non-standard DPI setting		<p>Revert to the default DPI:</p> <p>Right-click on the desktop and select Properties. Click the Settings tab. Click the Advanced button. With the General tab selected, use the drop-down menu to set DPI to 96 (normal size).</p>
		



## BD FACSDiva Software Cytometer Setup

Observation	Possible Causes	Recommended Solutions
Error creating compensation tubes	Naming conflict with existing tube or worksheet	<ol style="list-style-type: none"><li>1 Locate the tube or worksheet named (<i>ParameterName</i>) Stained Control, and change the name.</li><li>2 Create the compensation tubes again.</li></ol>
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)  (viewing normal or global 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 choose Show Populations; inspect the populations.
	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
No events in plots after clicking Acquire; acquisition indicator lights, except for those corresponding to threshold parameters, are blinking as expected	Detector voltage too low	Increase the voltage.
	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 tube	<ul style="list-style-type: none"> <li>• Transfer sample to new tube.</li> <li>• Make sure you are using appropriate tubes.</li> </ul>
	SIT clogged	Clean the flow cell.
	Current cytometer configuration different from optical setup	Verify 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 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 area scaling factor settings.
Fluorescent signal missing	Current cytometer configuration different from optical setup	Verify the cytometer configuration corresponds to the cytometer's optical setup, and 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 laser delay settings.
	Fluorescence threshold set too high	Adjust threshold.
	Incorrect sample preparation	Re-stain and re-run samples.

## BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
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.
Excessive amount of debris in plots	Threshold set too low	Increase the threshold.
	Dirty sheath filter	Replace sheath filter.
	Dirty flow cell	Clean flow cell. See page 173.
High CVs	Sample flow rate too high	Decrease the flow rate.
	Window extension set incorrectly	Contact lab manager to set the window extension to 7.0.

## BD FACSDiva Software Acquisition (continued)

Observation	Possible Causes	Recommended Solutions
Acquisition stops	High event rate (>10,000 events/sec) with more than 8 compensated parameters	<ul style="list-style-type: none"> <li>• Increase threshold.</li> <li>• Decrease threshold channel gain.</li> <li>• Dilute sample.</li> <li>• Define only channels of interest in parameter list.</li> </ul>
Fewer events than expected in gated population	Window extension set incorrectly	Contact 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 gating strategy.
	Dirty sheath filter	Replace sheath filter.
	Dirty flow cell	Clean flow cell. See page 173.
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 lab manager to set the window extension to 7.0.
Increasing threshold results in decreased area signal	Window extension set too low	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.

## BD FACSDiva Software Analysis

Observation	Possible Causes	Recommended Solutions
Fewer events than expected in gated population	Current tube pointer on wrong tube	Move the current tube pointer to the correct tube.
	On-axis events left out of gate	Include events on the axis.
	Gates improperly set	Check gating strategy.
Missing analysis objects on worksheet	Analysis objects obscured by other objects	Double-click the tube containing the analysis objects. Select the objects on the worksheet and move them.
Differing statistics between exported and imported file	Compensated data with negative values set to zero during FCS 2.0 export	<ul style="list-style-type: none"> <li>• Export the original data as FCS 3.0.</li> <li>• Export FCS 2.0 data with compensation disabled.</li> <li>• If statistical differences are significant, re-record the file.</li> </ul>



# Display Troubleshooting

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Observation	Possible Causes	Recommended Solutions
Software screen appears distorted (height is compressed; New Lot ID button is missing from Cytometer Setup Wizard)	<ul style="list-style-type: none"><li>• Display setting was changed from 96 dpi to 120 dpi</li></ul>	<ol style="list-style-type: none"><li><b>1</b> Right-click the desktop and select Properties from the menu.</li><li><b>2</b> Click the Settings tab.</li><li><b>3</b> Click Advanced.</li><li><b>4</b> Click the General tab.</li><li><b>5</b> Select Normal Size (96 DPI) from the DPI Setting menu.</li><li><b>6</b> Click OK to restart the computer.</li></ol>

---



# Appendix A

## Supplies and Replacement Parts

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To order supplies and options, contact your local BD Biosciences representative.

For updated information and part numbers, refer to our website, [bdbiosciences.com](http://bdbiosciences.com).

- Cytometer Supplies on page 228
- Consumables on page 230

# Cytometer Supplies

---

## Installation Kit

The cytometer ships with an installation kit including the following items, many of which are used during installation. Use these part numbers to order replacements.

<b>Item</b>	<b>Part No.</b>
Wet cart liquid filter assembly	33566307
1/4-in. elbow couplings	333072
6-ft cordset for US power (15A, 5–15P/320-C13)	337219
2.5-m cordset for Australian power (10A C13)	335696
2.5-m cordset for European power (10A C13)	335697
2.5-m cordset for UK power (10A C13 R/A)	335698
Filter bypass assembly	33576007
Bal seal for SIT	343509
Bal seal removal tool	331430
12 x 75-mm tubes	343675
10-L waste container	340261
Vented cap for waste container (pack of 12)	33885407
Waste cap baffle	33850507
Fluidics cart fuses: 2.5-A 250V Slo-blo Type T	343565
Loader front door assembly (Loader option only)	34401007
Loader side door assembly (Loader option only)	34349207
Preventive maintenance kit	64106807
Plastic O-ring pick tool	331430

## Other Replacement Parts

<b>Item</b>	<b>Part No.</b>
Sheath sensor probe (2-level)	338979
Waste sensor probe (2-level)	338978
Auxiliary sensor probe (1-level)	343835
Air filter (side door)	336303
Bal seal retainer	640116
Carousel kit, carousels 1–4	332727
Carousel kit, carousels 5–8	332728
Carousel kit, carousels 9–12	332729
Carousel kit, carousels 13–16	332730

## Barcode Reader Parts

<b>Item</b>	<b>Part No.</b>
2D barcode reader	344025
Stand (bar code reader)	344026

# Consumables

---

## Cytometer Setup

Particle	Supplier	Catalog No.
BD FACS 7-color setup beads	BD Biosciences (877) 232-8995	335775

## Reagents

Reagent	Supplier	Catalog No.
BD FACSCFlow sheath fluid	BD Biosciences (877) 232-8995	340398 (US and Latin America) 342003 (other countries)
BD FACSClean solution	BD Biosciences	340345
BD FACS shutdown solution	BD Biosciences	334224
Monoclonal antibodies	BD Biosciences	a
BD FACSTM lysing solution <sup>b</sup>	BD Biosciences	349202

a. Refer to the BD Biosciences Immunocytometry Products Catalog or the BD Biosciences website (bdbiosciences.com).

b. US Patent Nos. 4,654,312; 4,902,613; 5,098,849

## Labware

Item	Supplier	Catalog No.
5-mL polystyrene test tubes, 12 x 75-mm (BD Falcon tubes) <ul style="list-style-type: none"><li>• uncapped, 125 per bag</li><li>• capped, 125 per bag</li><li>• capped, 25 per bag</li><li>• with cell-strainer cap, 25 per bag</li></ul>	BD Biosciences (877) 232-8995	<ul style="list-style-type: none"><li>• 352052</li><li>• 352054</li><li>• 352058</li><li>• 352235</li></ul>





# Appendix B

## Technical Specifications

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- Cytometer Specifications on page 234
- Fluidics Cart Specifications on page 238
- BD FACS Loader Specifications on page 239

For barcode reader specifications, refer to the information supplied by the manufacturer.

# Cytometer Specifications

---

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 lab bench 55.9 cm (22 in.) depth.
Operational clearances, cytometer	Left side: 30 cm (11.8 in.) between unit and other objects or wall to permit proper air flow and access to the main power button and circuit breaker  Right side: 30 cm (11.8 in.) between unit and other objects or wall to permit proper air flow  Top: 22.5 cm (8.9 in.) between unit and other objects or wall to permit opening of flow cell access door
Weight	≤146 kg (320 lb)—cytometer only, excluding Loader and computer  Maximum 168 kg (370 lb)—including Loader
Power requirements	100/115/230 VAC (50–60 Hz)  Current: 5A at 115 VAC 2.5A at 230 VAC
Power consumption	500 W

## Environment

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

## Performance

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 IIIb (3B) lasers are mounted on the BD FACSCanto II cytometer.

Manufacturer	Model	Wavelength (nm)	Power (mW)
Coherent	Sapphire 488-20	488	20
JDS Uniphase	1144-P	633	17
Point Source (optional)	iFLEX2000-P-1-405-0.65-30-NP	405	30

These lasers are contained within the cytometer, therefore the BD FACSCanto II flow cytometer is a Class I (1) laser product.

## Excitation Optics

Optical platform	Fixed optical assembly
Beam geometry (all lasers)	9 $\mu\text{m}$ x 65 $\mu\text{m}$ elliptical beam

## Emission Optics

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 488-nm laser: <ul style="list-style-type: none"><li>• 750–810 nm (PE-Cy7)</li><li>• 670–735 nm (PerCP-Cy5.5)</li><li>• 610–637 nm (PE-Texas Red®, optional)</li><li>• 564–606 nm (PE)</li><li>• 515–545 nm (FITC)</li></ul> Wavelength ranges detected from 633-nm laser: <ul style="list-style-type: none"><li>• 750–810 nm (APC-Cy7)</li><li>• 701–723 nm (Alexa Fluor® 700, optional)</li><li>• 650–670 nm (APC)</li></ul> Wavelength ranges detected from 405-nm laser: <ul style="list-style-type: none"><li>• 502–535 nm (AmCyan)</li><li>• 425–475 nm (Pacific Blue™)</li></ul>
Forward scatter detection	Photodiode with 488/10 bandpass filter
Side scatter detection	PMT with 488/10 bandpass filter

## Fluidics

General operation	Integrated fluidics cart with automated startup, shutdown, and cleaning cycles
Sheath consumption	1.1 L/hr, normal operation <1.0 mL/hr, standby
Sample flow rates	Assay dependent, controlled automatically by BD FACSCanto clinical software. Nominal rates: Low = 10 $\mu$ L/min Medium = 60 $\mu$ L/min High = 120 $\mu$ L/min
Sample acquisition rate	10,000 events/sec with <10% abort rate (8 parameters)
Recommended maximum particle size	50 $\mu$ m

## Signal Processing

Workstation resolution	262,144-channel resolution
Data acquisition channels	8 to 10 parameters: 6 to 8 fluorescent 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

---

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 air vent and other objects or wall to permit proper air flow  Fluidics cart, door air vent: 20 cm (7.9 in.) between door and other objects or wall to permit proper air flow
Weight	≤51 kg (112 lb)—fluidics cart only, excluding tanks  ≤82 kg (180 lb)—with tanks full
Facilities	No air supply or vacuum required

## Capacity

BD FACSTFlow cubitainer	20 L
BD FACSClean solution cubitainer	5 L
BD FACS shutdown solution cubitainer	5 L
Waste tank	10 L

# BD FACS Loader Specifications

---

Carousel compatibility	Loader carousels, numbers 1–16
Tube compatibility	
• Carousel	Accommodates up to 40 uncapped 12 x 75-mm tubes <ul style="list-style-type: none"><li>• BD Falcon polystyrene test tubes</li><li>• BD Trucount tubes</li><li>• BD FACS 7-color setup bead tubes</li></ul>
• Thickness of accumulated labels	≤0.125 mm (5 mils) no more than 3 labels thick
Tube sample volume (maximum)	≤1.07 mL





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