BD FACSCanto Clinical Software Reference Manual

For In Vitro Diagnostic Use

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Notice

BD Biosciences delivers software and workstations that are intended for running the instruments 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
337977 Rev. A	4/04	Initial release
339859 Rev. A	9/04	US IVD release, new limitation added
339863 Rev. A	9/04	New limitation added; updated shutdown procedure; new title page
343372 Rev. A	9/05	Updated for 6-color reagent and BD FACSCanto clinical software v2.0; removed Running Samples chapter, added Software Reports chapter
640802 Rev. A	5/06	Updated for BD FACSCanto clinical software v2.1
643086 Rev. A	7/07	Updated for BD FACSCanto clinical software v2.2

Contents

About This Manual

This manual contains reference information about BD FACSCanto™ clinical software. Designed for the BD FACSCanto™ and the BD FACSCanto™ II flow cytometers, the software handles setup, acquisition, analysis, and automated loading of samples.

For information about cytometer components, maintenance, and troubleshooting, and instructions on how to run samples using the software, refer to the printed instructions for use for either the BD FACSCanto or the BD FACSCanto II flow cytometer. Further details can be found in the reference manual, provided on the documentation CD, for either the BD FACSCanto or the BD FACSCanto II flow cytometer. For application-specific instructions, refer to the individual application guides and information supplied with the reagents.

The *BD FACSCanto Clinical Software Reference Manual* assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, refer to the documentation provided with your computer.

Before using BD FACSCanto clinical software, print and review the ReadMe file for BD FACSCanto clinical software that is included on both the software and documentation CD. It contains important information that is not included in the printed or electronic documentation.

Conventions

The following tables list conventions used throughout this manual. Table 1 lists symbols that are used to alert you to a potential hazard. Text and keyboard conventions are shown in Table 2.

Table 1 Hazard symbols

Symbol	Meaning
⚠	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death

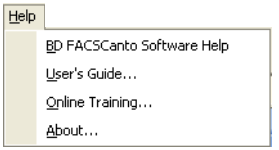
Table 2 Text and keyboard conventions

Convention	Use
☑ **Tip**	Highlights features or hints that can save time and prevent difficulties
NOTICE	Describes important features or instructions
Italics	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	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 *p*.

Technical Assistance

For technical questions or assistance:

- In BD FACSCanto clinical software, select Help > BD FACSCanto Software Help. Use the full-text online search feature to locate topics specific to the operation you are performing.



- In BD FACSCanto clinical software, select Help > Online Training to access online training courses on the BD Biosciences website.
- See Chapter 6, Troubleshooting.

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

When contacting BD Biosciences, have the following information available:

- Product name, catalog number, and serial number
- Error messages
- Details of system performance

For instrument support within the US, call (877) 232-8995, prompt 2, 2.

For support within Canada, call (888) 259-0187.

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

1

Introduction

BD FACSCanto clinical software streamlines your workflow by combining instrument QC and setup, acquisition and analysis, and optional automated sample loading in one easy-to-use package. Compensation settings are automatically recalculated during voltage adjustments. Auto-gating algorithms isolate populations of interest, but the software allows manual gating, if necessary. Internal QC checks the validity of your results. You can print results in a customizable lab report.

This chapter discusses these topics:

Requirements

Hardware

- BD FACSCanto or BD FACSCanto II flow cytometer and fluidics cart
- BD FACSCanto workstation
- BD Biosciences recommended printer
- (Optional) BD FACS™ Loader for automated acquisition

Software

- Microsoft Windows XP Professional OS with Service Pack 2 or later
- Microsoft .NET Framework v1.1*
- BD FACSDiva™ software v5.0 (installs firmware components required for acquisition workstations)
- Adobe® Acrobat® Reader® software v6.0*

Reagents

- BD FACS™ 7-color setup beads
- BD Multitest™ reagents for sample staining
- BD FACSFlow™ sheath fluid, BD™ FACSClean solution, and BD FACS™ shutdown solution for cytometer operation

  For other flow cytometer requirements, refer to the reference manual or instructions for use for either the BD FACSCanto or the BD FACSCanto II flow cytometer.

* Installed by the BD FACSCanto clinical software installer, if not already installed

Compatibility

Data Files

BD FACSCanto clinical software writes flow cytometry standard (FCS) 3.0 data files. The software reads FCS data files produced by BD FACSCanto clinical software only.

Do not read FCS files created with version 2.2 using an earlier version of BD FACSCanto software. Earlier versions will show incorrect results.

Result Files

The software generates result files in comma separated value (CSV) format, readable by a spreadsheet application such as Microsoft Excel®.

Reports

The software automatically creates setup reports and application setup reports in PDF format, and can also be set to automatically generate lab reports in PDF format.

Worklists

BD FACSCanto clinical software can import worklists from BD FACS™ Sample Prep Assistant (SPA) software v2.0, v3.0, and v3.01.

Other Software

FCS and setup files created within BD FACSCanto clinical software can be imported into BD FACSDiva software v5.0 and v6.0.

Interference

There are no known incompatibilities with BD FACSCanto clinical software. Virus scanning programs could slow down the software's processing speed.

Limitations

- For in vitro diagnostic use (IVD) when used with IVD reagents. Refer to the reagent package insert for application-specific limitations.
- For use only with BD FACS™ 7-color setup beads for setup and reagents (eg, BD Multitest™) that have been cleared for use on the BD FACSCanto and BD FACSCanto II flow cytometers.
- This software is for use only on the BD FACSCanto and BD FACSCanto II flow cytometers.

2

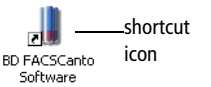
Starting Up

Use this chapter to familiarize yourself with BD FACSCanto software the first time you use it.

Starting the Software

To start BD FACSCanto clinical software, do one of the following:

- Double-click the shortcut icon on the desktop.



- Select Start > Programs > BD FACSCanto Software > BD FACSCanto Software.

The Login dialog appears.

Logging In

The lab administrator should be the first user to log in to the software. A BD Biosciences service representative will give the password to the administrator, who can then access the software and set up users. See Setting Up New Users on page 85.

☑ **Tip** Keep a copy of the administrator password in a secure location in case you forget it.

To log in, use the following procedure.

1. Select your User ID from the menu.
2. Enter your password, then click Login.

   The main application window appears.

Software Overview

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

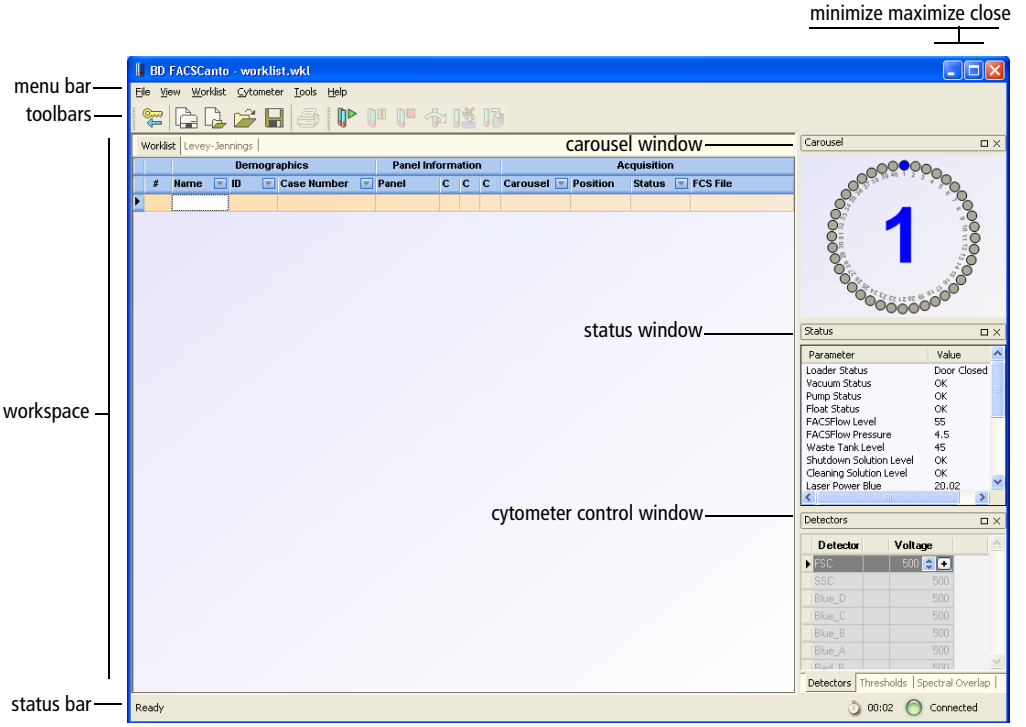


Table 2-1 Main window components

Component	Function
Menu bar	Contains the File, View, Worklist, Cytometer, Tools, and Help menus. See Application Menus on page 148.
Toolbars	Contain buttons that provide quick access to menu commands. See Becoming Familiar with Toolbars on page 19.
Workspace	Displays the Worklist, Acquisition, Lab Report, and Levey-Jennings tabs, depending upon where you are in the workflow.
Status bar	Provides information about the cytometer's current state, the cytometer-software connection, and the amount of time elapsed since login.

Table 2-1 Main window components (continued)

Component	Function
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.
Carousel window	Shows a graphic representation of a carousel rack and the rack ID of the currently selected sample. See Using the Carousel Window on page 20.
Status window	Provides information on the current status of the flow cytometer. See Viewing Status Indicators on page 21.
Cytometer control windows	Includes Detectors, Thresholds, and Spectral Overlap tabs. For information, refer to the instructions for use for your cytometer.

Becoming Familiar with Toolbars

Toolbars can be moved within the main window or hidden.

- To move a toolbar, drag the grab bar to a new position on the screen.

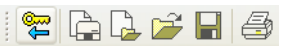
  

- To hide a toolbar, select it from the View menu.

  To show a hidden toolbar, select it again.

Use buttons on the software toolbars for the following.

Standard Toolbar




Log out


Create a new, blank acquisition worklist


Create a new analysis worklist


Open an existing worklist


Save the current worklist


Print the current workspace view

Worklist Toolbar


Run


Pause


Stop


Skip


End Recording


Optimize


Add selected files

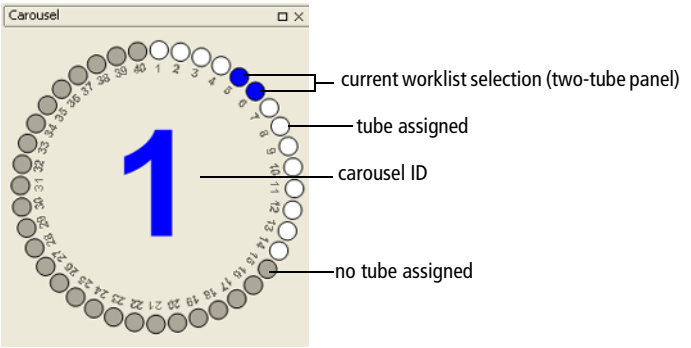

Add files in folder

Using the Carousel Window

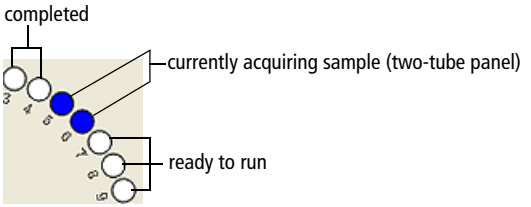
The Carousel window shows a graphic representation of a Loader carousel.

When you are setting up a worklist, the Carousel window shows the carousel ID for the currently selected sample.

As you enter sample information and assign tubes, carousel positions change from gray (unassigned) to white (assigned). The sample currently selected in the worklist is represented by one or two blue circles, depending upon the number of tubes in the panel.



During a run, the window shows the ID of the carousel that is currently running. Blue indicates the position of the current sample.

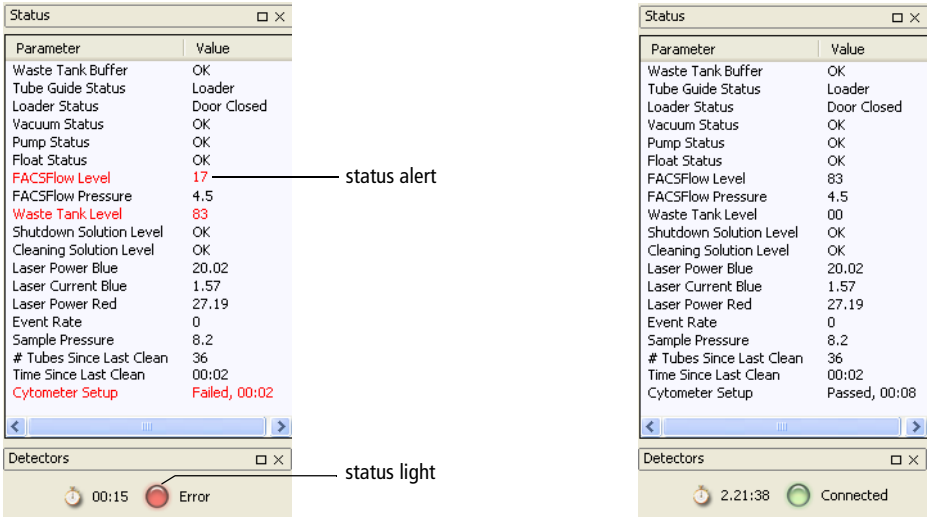


Viewing Status Indicators

Status window parameters and values differ depending on whether the software is connected to the BD FACSCanto or the BD FACSCanto II flow cytometer.

The Status window displays important information about the cytometer. See Figure 2-2. The light on the Status bar turns red if there is something you must fix before you can continue. In the Status window, the text for a parameter turns red to alert you that a problem exists, although you might still be able to run the software. See Table 2-2 on page 22 for information about each parameter.

Figure 2-1 BD FACSCanto II cytometer Status window



error conditions

no error conditions

Table 2-2 Status window

Parameter	Acceptable Range	Details
Waste Tank Buffer	—	OK or Error For errors, see on page 120.
Tube Guide Status[a]	—	Loader or Manual Indicates tube loading method.
Loader Status	—	Door Open, Door Closed, or Error For Loader troubleshooting, refer to the reference manual for either the BD FACSCanto or BD FACSCanto II flow cytometer.
Vacuum, Pump, Float Status	—	OK or Error For errors, see on page 120.

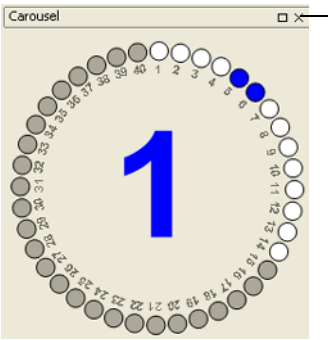
Parameter	Acceptable Range	Details
FACSFlow Level	0–83%	Six levels[b] or three levels[a] shown; refill the tank when the text turns red.
FACSFlow Pressure	Set by Service	Call BD Biosciences when the pressure is out of range.
Waste Tank Level	0–99%	Seven levels[b] or three levels[a] shown; empty the tank when the text turns red.
Shutdown or Cleaning Solution Level	Empty/Full	OK or Empty Refill the indicated tank when empty.
Laser Power Blue, Laser Current Blue, Laser Power Red	Set by Service	Call BD Biosciences when the laser power or current is out of range.
Event Rate	—	For information only; value not adjustable
Sample Pressure	—	For information only; value not adjustable
# Tubes Since Last Clean	—	For information only; shows number of tubes run using BD FACSCanto clinical software since last clean Note that *clean,* in this case, indicates that either Fluidics Shutdown and/or Monthly Clean was run.
Cytometer Setup	Passed/Failed	Text turns red if you accepted a failed setup; the software allows you to proceed with failed setup.
	0–24 hours	Run Setup when more than 24 hours have elapsed since last setup. The text turns red when setup is over 24 hours old; the software allows you to proceed with the old setup. In *total hours*:*minutes* format

a. BD FACSCanto II flow cytometer
b. BD FACSCanto flow cytometer

Rearranging Window Components

You can rearrange the main window as needed. Hide, move, resize, or merge the Carousel, Status, and cytometer control windows.

To close a window, click the Close button in the title bar of the window.



To view the window again, select the name of the window from the View menu.

- To move a window, drag the title bar to a new position on the screen. You can position a window to fill the upper half of the screen by dragging the window to the upper-left corner of the worklist.

  To return the window to the right side of the screen, double-click the window's title bar.

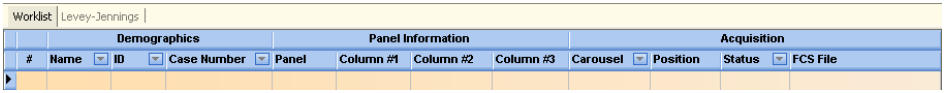
- To resize a window, place the mouse pointer over the window's border. When the pointer changes to a double-headed arrow, drag the border.

- To merge windows, drag one window on top of another. Each window is represented in the shared space by a tab.

  To separate a merged window, drag the tab of the window out of the window's frame.

Becoming Familiar with the Worklist

Enter all sample and panel information into the worklist. You can also import a worklist to automatically fill in the appropriate fields.



Use the keyboard or barcode reader to enter sample information in worklist fields. Use the keyboard or mouse to select the Panel type and Carousel rack ID.

The column headers under Panel Information change, depending on which panel you are running, and which line is currently selected.

You can create, save, and print worklists.

- For information on creating a worklist, refer to the instructions for your cytometer.
- For information on saving and reusing a worklist, see Using an Acquisition Worklist as a Template on page 56.
- For information on printing a worklist, see Printing a Worklist on page 59.

Worklist Symbols

The following symbols might appear as you enter information into the worklist.


Indicates the currently selected field or line of information.

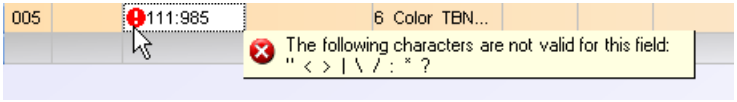

Indicates that the current field is editable.


Indicates that information in the current field has been changed.

Indicates the next line without entries.

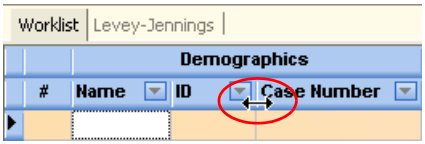
Indicates a problem with a worklist entry.

Place your cursor over the symbol for more information about the problem. For example, in the following figure, a pop-up with the information *The following characters are not valid for this field* indicates that the colon (:) must be removed from the entry.



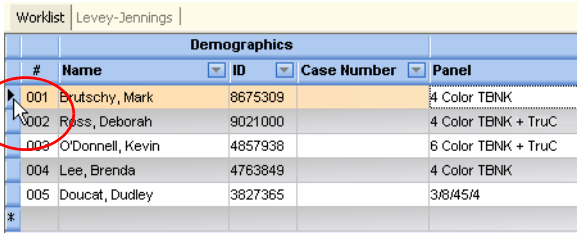
Resizing Worklist Columns

You can expand or shrink the width of worklist columns by dragging column dividers to the right or left.



Deleting Samples from a Worklist

1 Select a row of sample information as shown.



2 Select Worklist > Delete Sample.

Clearing a Field Entry

Press the Esc key to clear an entry from a field. Press the Esc key a second time to clear the entire row.

Filtering Worklist Entries

You can filter a worklist to show only a specified type of entry. To use this feature, click the ▼ next to a column header and select a value.

Figure 2-2 Filtering the sample Name column

For example, in Figure 2-2, selecting *Lee, Brenda* will show all samples or entries in the worklist labeled with that sample name, and hide (filter out) all other entries.

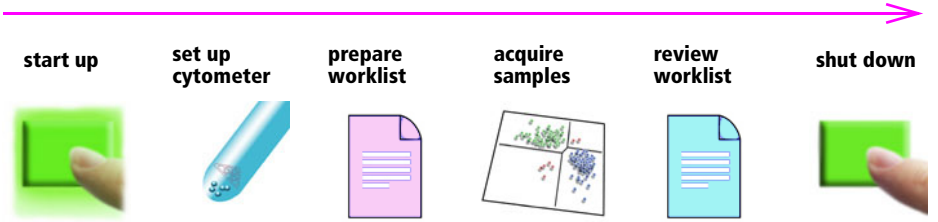
Different columns offer different filtering choices. The following table shows choices that are available for all filterable columns.

Table 2-3 Filter choices

Filter Choices	Meaning
All	No filter applied; worklist shows all entries.
Blanks	Worklist shows only rows that have no entries in this column.
NonBlanks	Worklist shows only rows that have entries in this column.

Understanding the Workflow

The following shows an overview of the workflow when you are using the software for an acquisition worklist.



Turn on system.

Prepare fluidics.

Run setup beads.

Run optimization sample(s).

Enter sample information.

Run worklist.

Optimize.

Change gating.

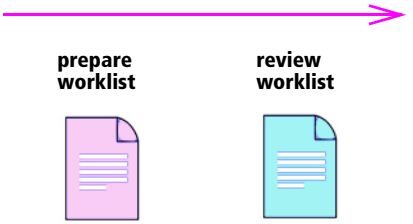
Review sample analyses.

Change gating.

Perform fluidics shutdown.

Turn off system.

The following shows an overview of the workflow during analysis.



Import FCS files or worklist.

Review sample information.

Review sample analyses.

Change gating, if required.

Print lab reports.

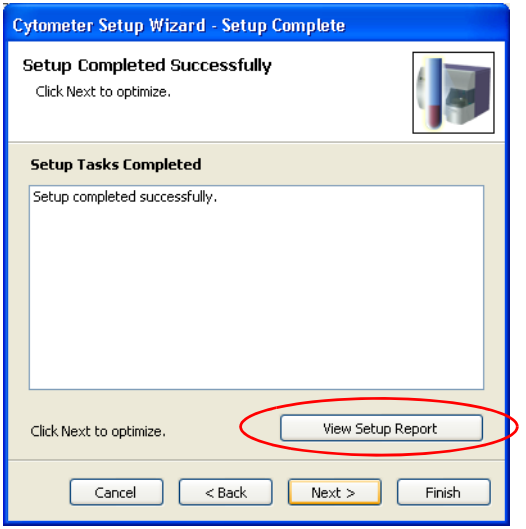
3

Reports

The software generates four types of reports, which are described in this chapter:

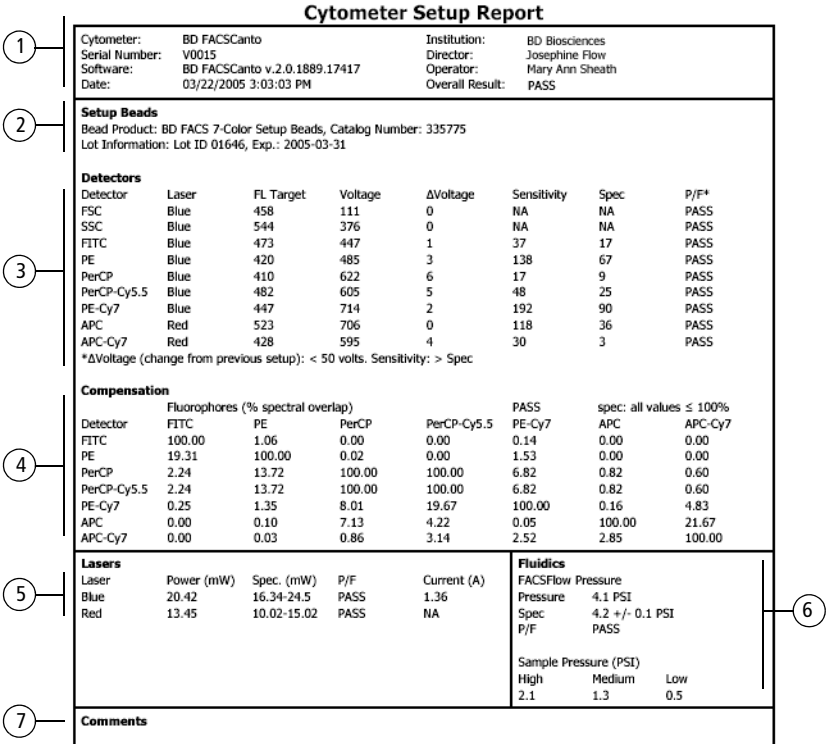
Cytometer Setup Reports

The Cytometer Setup Report becomes available after you run cytometer setup with BD FACS 7-color setup beads. You can access the report by clicking View Setup Report in the Cytometer Setup Wizard prior to optimization.



The Setup Report does not reflect assay-specific instrument settings. Figure 3-1 on page 33 shows an example of a Cytometer Setup Report.

Figure 3-1 Cytometer Setup Report (example)



Cytometer Setup Report

Cytometer:	BD FACSCanto	Institution:	BD Biosciences
Serial Number:	V0015	Director:	Josephine Flow
Software:	BD FACSCanto v.2.0.1889.17417	Operator:	Mary Ann Sheath
Date:	03/22/2005 3:03:03 PM	Overall Result:	PASS

Setup Beads
Bead Product: BD FACS 7-Color Setup Beads, Catalog Number: 335775
Lot Information: Lot ID 01646, Exp.: 2005-03-31

Detectors

Detector	Laser	FL Target	Voltage	ΔVoltage	Sensitivity	Spec	P/F*
FSC	Blue	458	111	0	NA	NA	PASS
SSC	Blue	544	376	0	NA	NA	PASS
FITC	Blue	473	447	1	37	17	PASS
PE	Blue	420	485	3	138	67	PASS
PerCP	Blue	410	622	6	17	9	PASS
PerCP-Cy5.5	Blue	482	605	5	48	25	PASS
PE-Cy7	Blue	447	714	2	192	90	PASS
APC	Red	523	706	0	118	36	PASS
APC-Cy7	Red	428	595	4	30	3	PASS

*ΔVoltage (change from previous setup): < 50 volts. Sensitivity: > Spec

Compensation

	Fluorophores (% spectral overlap)				PASS	spec: all values ≤ 100%	
Detector	FITC	PE	PerCP	PerCP-Cy5.5	PE-Cy7	APC	APC-Cy7
FITC	100.00	1.06	0.00	0.00	0.14	0.00	0.00
PE	19.31	100.00	0.02	0.00	1.53	0.00	0.00
PerCP	2.24	13.72	100.00	100.00	6.82	0.82	0.60
PerCP-Cy5.5	2.24	13.72	100.00	100.00	6.82	0.82	0.60
PE-Cy7	0.25	1.35	8.01	19.67	100.00	0.16	4.83
APC	0.00	0.10	7.13	4.22	0.05	100.00	21.67
APC-Cy7	0.00	0.03	0.86	3.14	2.52	2.85	100.00

Lasers

Laser	Power (mW)	Spec. (mW)	P/F	Current (A)
Blue	20.42	16.34-24.5	PASS	1.36
Red	13.45	10.02-15.02	PASS	NA

Fluidics

FACSFlow Pressure	
Pressure	4.1 PSI
Spec	4.2 +/- 0.1 PSI
P/F	PASS

Sample Pressure (PSI)

High	Medium	Low
2.1	1.3	0.5

Comments

1	**Report Header**—Contains basic information about the cytometer, software version, institution, operator, and overall result. • PASS indicates that all pass/fail specifications were met. • FAIL indicates that at least one pass/fail specification was out of range.
2	**Setup Beads**—Details the bead product used, its catalog number, lot ID, and expiration date.

③	**Detectors**—Provides the following information (Figure 3-2): • Laser (a)—Indicates which laser excited the stained particle to emit light collected for that detector. • FL Target (b)—Fluorescence target value in log form. The software adjusts the voltage so that the setup bead is at the target value. For more information about the FL Target, refer to the bead package insert. • Voltage (c)—Voltage required to place the beads at the fluorescence target values (b). ΔVoltage (d) indicates the change in volts from the last setup. The difference between the two values should be less than 50 volts. A difference of less than 50 will pass (g). A difference of 50 or greater will fail. • Sensitivity (e)—Measure of the cytometer's ability to resolve dimly stained cells. The measurement includes contributions from efficiency of photon collection, background signal, and intrinsic brightness of each fluorophore. A sensitivity value greater than the Spec value (f) means that the detector passes the sensitivity specification (g). • P/F (g)—Indicates whether the Sensitivity (e) or ΔVoltage (d) passed or failed. A fail in either category will cause an overall Fail (g) for that detector. **Figure 3-2** Columns in the Detectors section of Setup Report **Detectors** *ΔVoltage (change from previous setup): < 50 volts. Sensitivity: > Spec
④	**Compensation**—Displays spectral overlap values calculated during setup for the current voltages. Values ≤100% will pass; values >100% will fail.
⑤	**Lasers**—Provides information about each laser and whether or not it passes the power specifications (in milliwatts) determined by BD Biosciences. The laser current (measured in ampere) is also provided.
⑥	**Fluidics**—Shows whether or not the sheath pressure meets BD Biosciences determined specifications. It also shows sample pressure voltage for low, medium, and high flow rates, a useful troubleshooting measurement.

	a	b	c	d	e	f	g
Detector	Laser	FL Target	Voltage	ΔVoltage	Sensitivity	Spec	P/F*
FSC	Blue	458	111	0	NA	NA	PASS
SSC	Blue	544	376	0	NA	NA	PASS
FITC	Blue	473	447	1	37	17	PASS
PE	Blue	420	485	3	138	67	PASS
PerCP	Blue	410	622	6	17	9	PASS
PerCP-Cy5.5	Blue	482	605	5	48	25	PASS
PE-Cy7	Blue	447	714	2	192	90	PASS
APC	Red	523	706	0	118	36	PASS
APC-Cy7	Red	428	595	4	30	3	PASS

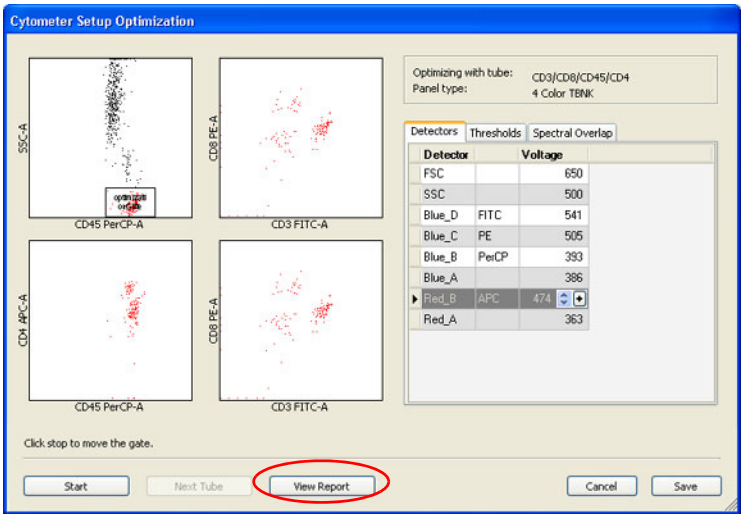
7	**Comments**—Provides an area to write additional information on a Cytometer Setup Report after you print the report.

For help with out of range values on Setup Reports, see Setup Troubleshooting on page 125 or refer to the *BD FACS 7-color setup beads* package insert.

Application Setup Reports

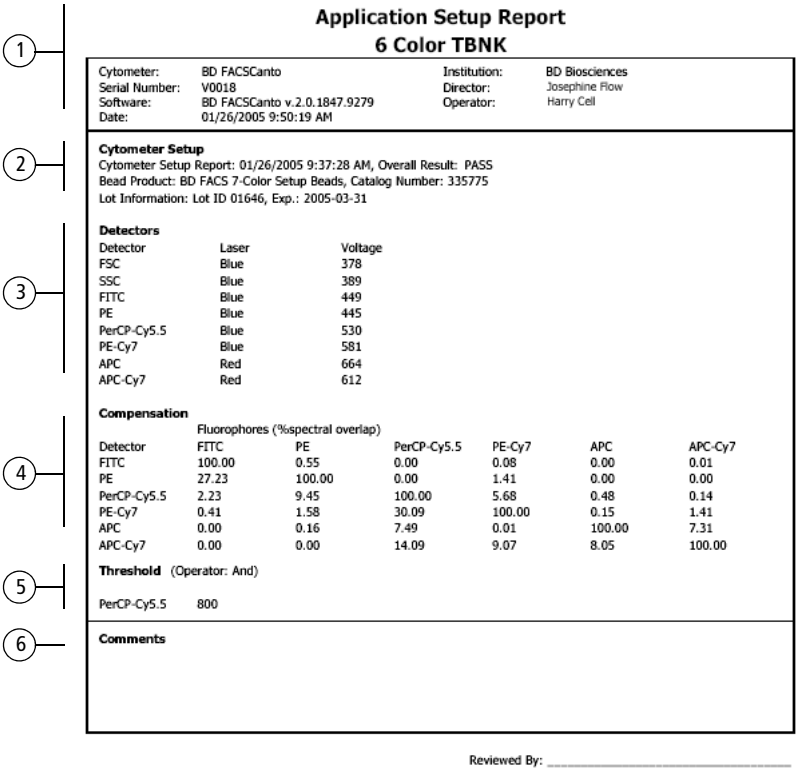
An Application Setup Report shows assay-specific instrument settings. The software calculates and uses these settings if you choose not to optimize with biological samples. If you optimize, your adjustments will be used. Display this report by clicking View Report in the Cytometer Setup Optimization dialog after optimization.

Figure 3-3 Cytometer Setup Optimization dialog



Application Setup Reports do not display any pass or fail information. Pass or fail information is found on the Cytometer Setup Report. Figure 3-4 on page 36 shows an example of an Application Setup Report.

Figure 3-4 Example Application Setup Report



Application Setup Report
6 Color TBNK

Cytometer:	BD FACSCanto	Institution:	BD Biosciences
Serial Number:	V0018	Director:	Josephine Flow
Software:	BD FACSCanto v.2.0.1847.9279	Operator:	Harry Cell
Date:	01/26/2005 9:50:19 AM		

Cytometer Setup
Cytometer Setup Report: 01/26/2005 9:37:28 AM, Overall Result: PASS
Bead Product: BD FACS 7-Color Setup Beads, Catalog Number: 335775
Lot Information: Lot ID 01646, Exp.: 2005-03-31

Detectors

Detector	Laser	Voltage
FSC	Blue	378
SSC	Blue	389
FITC	Blue	449
PE	Blue	445
PerCP-Cy5.5	Blue	530
PE-Cy7	Blue	581
APC	Red	664
APC-Cy7	Red	612

Compensation

	Fluorophores (%spectral overlap)					
Detector	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-Cy7
FITC	100.00	0.55	0.00	0.08	0.00	0.01
PE	27.23	100.00	0.00	1.41	0.00	0.00
PerCP-Cy5.5	2.23	9.45	100.00	5.68	0.48	0.14
PE-Cy7	0.41	1.58	30.09	100.00	0.15	1.41
APC	0.00	0.16	7.49	0.01	100.00	7.31
APC-Cy7	0.00	0.00	14.09	9.07	8.05	100.00

Threshold (Operator: And)

PerCP-Cy5.5	800

Comments

Reviewed By: _____

1	**Report Header—**Contains basic information about the cytometer and operator. The report title specifies the clinical application the values apply to.
2	**Cytometer Setup—**Details the bead product used, its catalog number, lot ID, and expiration date. Information from the cytometer setup (page 32) is used when calculating assay-specific setup.

③	**Detectors—**Shows either BD Biosciences–generated voltages or user-optimized voltages. • **BD Biosciences–generated voltages** means that default values were used to generate the application-specific setup. • After you manually optimize, the software stores **user-optimized voltages**. The next time you run setup, the voltages will update based on both the default values and the last optimization values.
④	**Compensation—**Displays spectral overlap values automatically calculated for the voltages listed in the Detectors section.
⑤	**Threshold—**Indicates the parameter(s) used as the threshold for a clinical application during optimization. It also shows the logical operator in effect for the threshold(s). You can alter the default threshold parameter(s) and the logical operator, if needed. Logical operator choices are as follows: • OR (any one threshold) • AND (all selected thresholds)
⑥	**Comments—**Provides an area to write additional information on an Application Setup Report after you print the report**.**

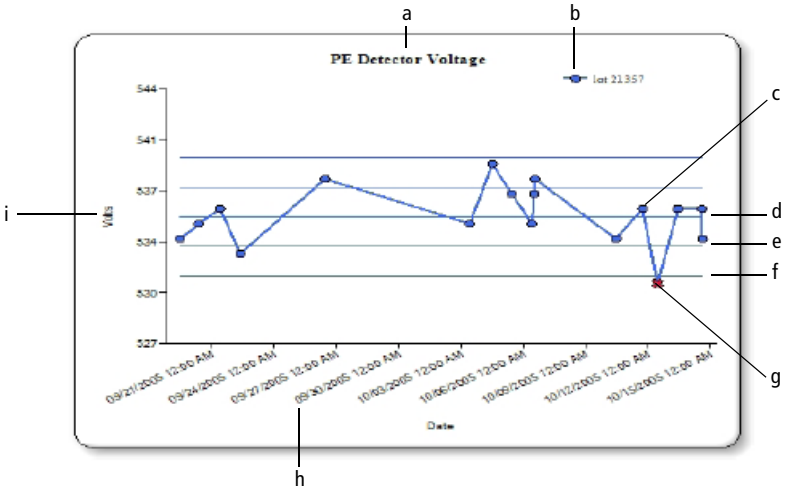
Levey-Jennings Reports

Levey-Jennings Reports contain Levey-Jennings plots, which track the cytometer setup data over time.

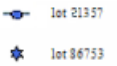
Figure 3-5 Example Levey-Jennings Report

1	**Report Header**—Contains basic information about the cytometer, software version, institution, and operator. The header is always included. Lab managers cannot alter the header.
2	**Levey-Jennings plots**—Included for the detectors, lasers, and other parameters specified by the lab manager in the Levey-Jennings Preferences dialog (see Specifying Levey-Jennings View Preferences on page 93). You can have up to 20 plots in a report.

③ All Levey-Jennings plots contain the same basic elements.



Legend

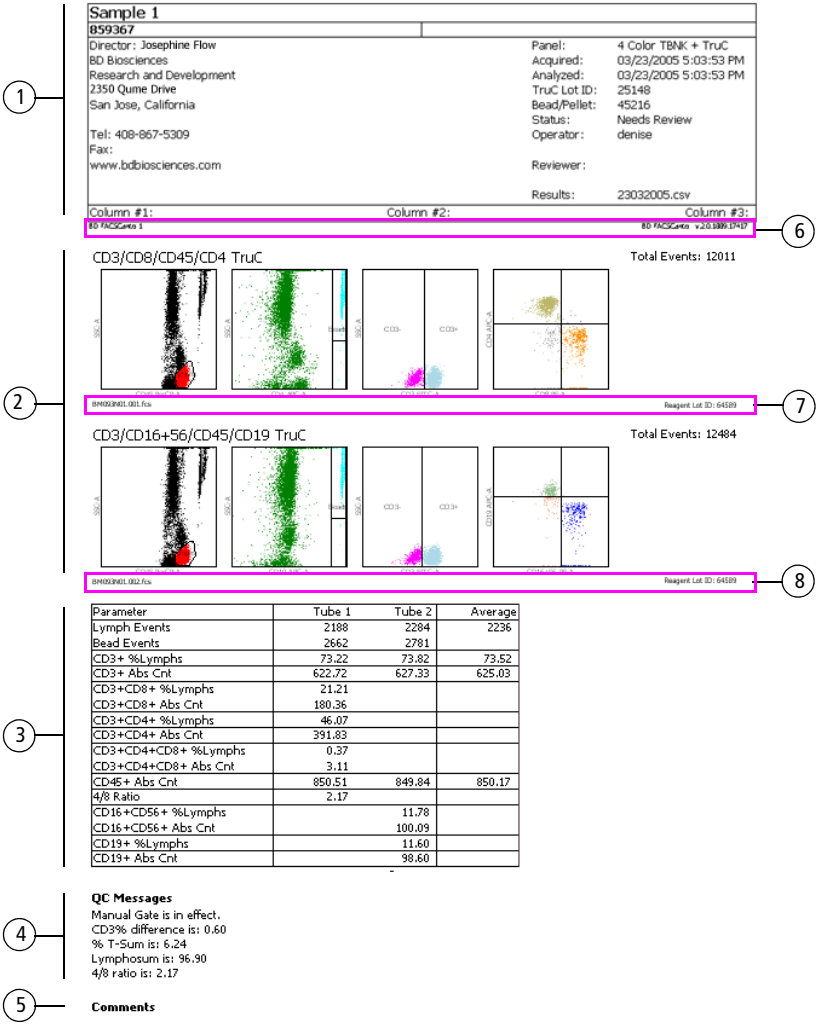
	Element	Description
a	Title	The attribute under observation
b	Lot ID line	If more than one lot ID is used, two lot ID symbols will appear in the plot and legend; only two lot IDs can be visualized by the software in the plots 
c	Data point	A cytometer setup
d	Thick blue line	Mean
e	Thin blue line	+/- 1 standard deviation (SD) from the mean
f	Thin blue line	+/- 2 SD from the mean; an additional line can show +/- 3 SD from the mean, if selected as an alarm boundary; minimum and maximum lab manager–entered values can appear instead of SD
g	Red X	Data point outside alarm boundary
h	x axis	Time, with dates for data points
i	y axis	Value depends upon which attribute is being plotted; can be volts (detectors), sensitivity, mWatts (laser power), amps (laser current), spillover, channel

④ **Comments—**Can be entered on a Levey-Jennings Report by any operator. See Entering Comments into a Lab or LJ Report on page 68 for instructions.

Lab Reports

You can view or print a Lab Report for every analyzed sample in a worklist.

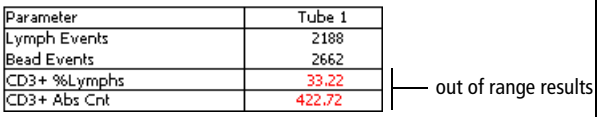
Figure 3-6 Lab Report example



Parameter	Tube 1	Tube 2	Average
Lymph Events	2188	2284	2236
Bead Events	2662	2781	
CD3+ %Lymphs	73.22	73.82	73.52
CD3+ Abs Cnt	622.72	627.33	625.03
CD3+CD8+ %Lymphs	21.21		
CD3+CD8+ Abs Cnt	180.36		
CD3+CD4+ %Lymphs	46.07		
CD3+CD4+ Abs Cnt	391.83		
CD3+CD4+CD8+ %Lymphs	0.37		
CD3+CD4+CD8+ Abs Cnt	3.11		
CD45+ Abs Cnt	850.51	849.84	850.17
4/8 Ratio	2.17		
CD16+CD56+ %Lymphs		11.78	
CD16+CD56+ Abs Cnt		100.09	
CD19+ %Lymphs		11.60	
CD19+ Abs Cnt		98.60	

QC Messages
Manual Gate is in effect.
CD3% difference is: 0.60
% T-Sum is: 6.24
Lymphosum is: 96.90
4/8 ratio is: 2.17

Comments

1	A lab manager chooses which **Report Header** information to include on Lab Reports (see Choosing Header Information on page 110). If a lab manager selects Sample Name and Sample ID, these worklist entries will become the first and second lines of the header (see the sample Lab Report in Figure 3-6 on page 40). Values entered in the panel-specific columns of the worklist will appear here as well. (In Figure 3-6 on page 40, Column #1, Column#2, and Column #3 are the columns specific to the 4 Color TBNK + TruC panel.)
2	This section shows the plots and the analyzed data, as well as the total number of events collected, the reagent lot ID used, and the name of the FCS file generated for each tube.
3	This section reports the results of the analysis for each tube. A lab manager can choose which results to display and alter alarm ranges (see Changing Subset Results for a Reagent on page 104 and Changing Alarm Ranges for Subset Results on page 105). If a result falls outside the alarm range, the text is highlighted in red, and the message *One or more results are outside the alarm range* appears in the QC Messages section of the Lab Report. 
4	**QC Messages** shows the following: • Lab manager–selected quality control values (see Choosing QC Values on page 106) • Message indicating a failure occurred (see QC Messages on page 138) • Message when one or more results are outside the alarm range
5	Any operator can electronically enter or edit **Comments** on a Lab Report**.** See Entering Comments into a Lab or LJ Report on page 68.
6	The small print on the left shows the **serial number** of the cytometer. The small print on the right shows the **software version** used to do the assay.
7	The small print on the left shows the **name of the FCS file.** The small print on the right shows the **reagent lot ID**.
8	The small print on the left shows the **name of the FCS file.** The small print on the right shows the **reagent lot ID**.

4

User Options

Certain software options are available only if you have lab manager privileges. See Lab Manager Options on page 73. The following options are available to all users of BD FACSCanto clinical software.

- Options while Running the Cytometer on page 44
- Understanding Worklist Options on page 54
- Printing on page 58
- Customizing Software Defaults on page 64
- Entering Comments into a Lab or LJ Report on page 68
- Viewing Previous Levey-Jennings Plots on page 70
- Changing Your Password on page 72

Options while Running the Cytometer

Entering New Lot IDs

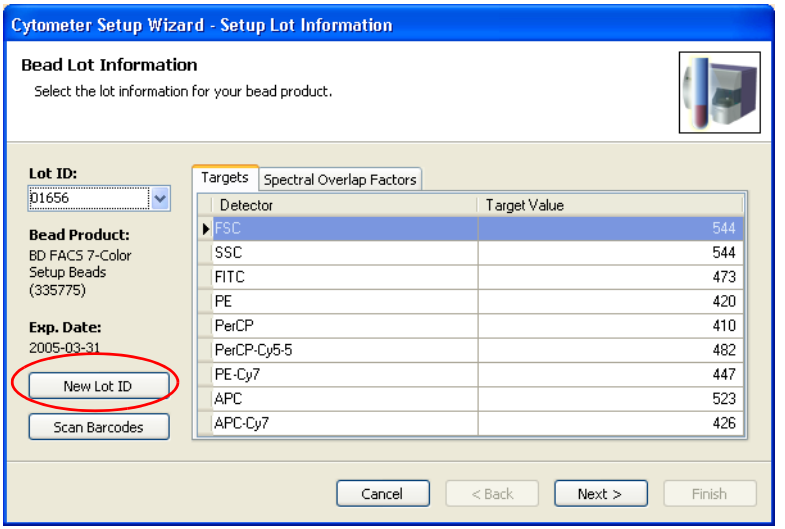
BD FACSCanto clinical software stores lot information for setup beads, reagents, and BD Trucount™ beads. To enter information for a new lot, see the following sections:

Entering Lot Information for Setup Beads Manually

1 Select Cytometer > Setup > Standard Setup.

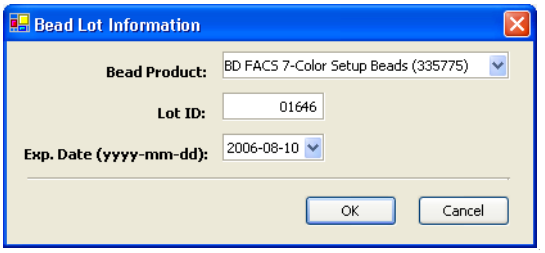
2 Click New Lot ID in the Setup Lot Information dialog.

Figure 4-1 Setup Lot Information dialog



3 Select the bead product, enter the lot ID and the expiration date, and click OK.

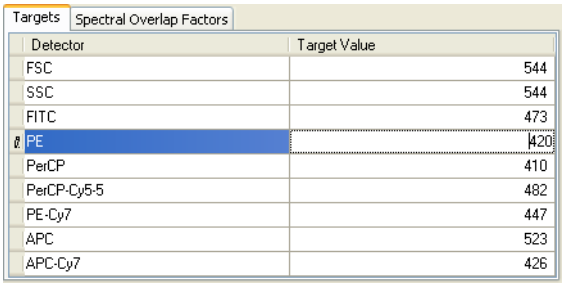
Figure 4-2



For BD FACS 7-color setup beads, locate this information on the foil reagent packet, the target values sticker, or the side of the reagent box. **Do not** use the numbers on the setup beads tube.

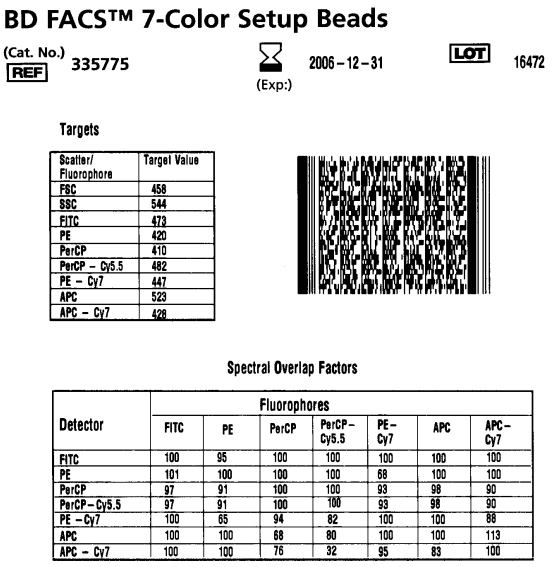
4 In the Setup Lot Information dialog, enter the target values for the bead lot.

To change a target value, select the current value in the Target Value field and enter the new value. Repeat until you have edited all target values.



Target values determine where the software places the setup beads during detector and spectral overlap adjustments. They must be edited for every new bead lot. Target values are provided with each box of beads. See Figure 4-3 on page 47 for an example setup beads label.

Figure 4-3 Example setup beads label



BD FACS™ 7-Color Setup Beads

(Cat. No.) REF 335775

(Exp:) 2006-12-31

LOT 16472

Targets

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

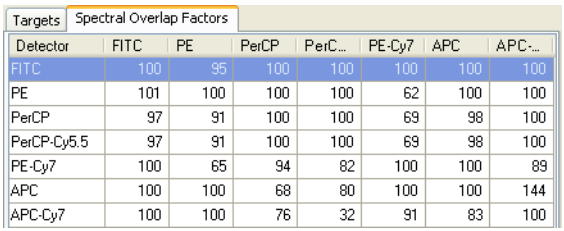
Spectral Overlap Factors

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

5 Click the Spectral Overlap Factors tab, then enter the spectral overlap factors for the bead lot.

Spectral overlap factors correct for mismatches in spectral overlap between the setup beads and cells so that the sample cells will be properly compensated. They must be edited for every new bead lot. Spectral overlap factors are provided with each box of beads.

To change a value, select the field containing the value you want to change and enter the new value. Repeat until all required values are entered.



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

6 Click Finish.

Using the Barcode Reader for the BD FACSCanto System

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

1D Barcode Symbologies

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, enable the checksum feature.

⚠ Using barcode symbologies without checksums enabled increases the likelihood of incorrect information transfer, including sample ID assignments. This can result in a mismatch of sample IDs and sample results.

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

Using the Optional Stand

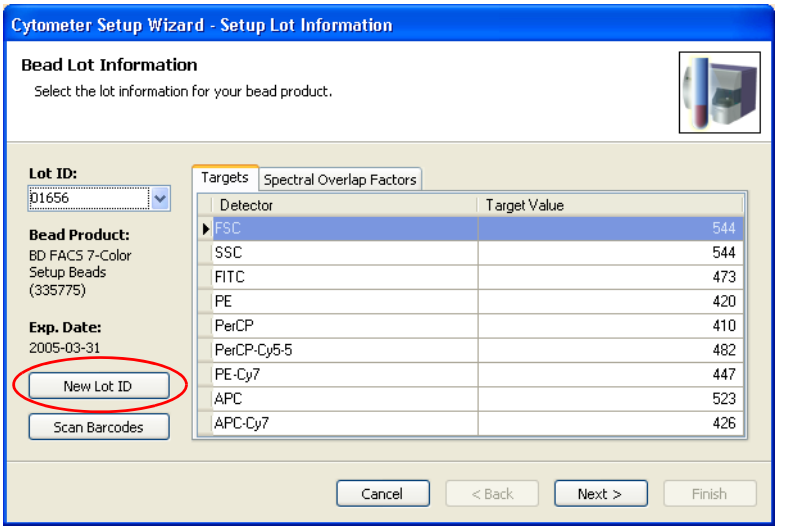
If you use the barcode reader with the stand, place the tube over the hole on the platform.

Entering Lot Information for Setup Beads with the Barcode Reader

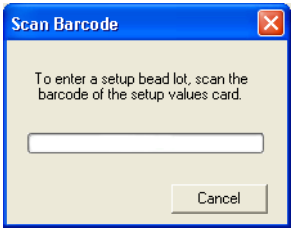
⚠ If the barcode reader is used in a manner not specified by BD Biosciences, the inherent safeguards provided may be impaired.

1 Select Cytometer > Setup > Standard Setup.

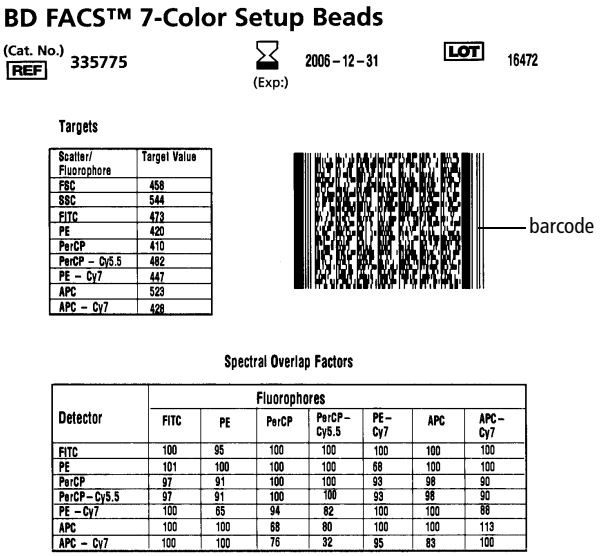
2 Click Scan Barcodes in the Setup Lot Information dialog.



The following dialog appears:



3 Scan the barcode, located on the BD FACS 7-color setup beads label.



BD FACS™ 7-Color Setup Beads

(Cat. No.) REF 335775 (Exp:) 2006-12-31 LOT 16472

Targets

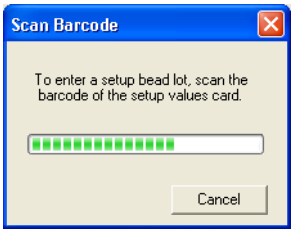
Scatter/ Fluorophore	Target Value
FSC	458
SSC	544
FITC	473
PE	420
PerCP	410
PerCP – Cy5.5	482
PE – Cy7	447
APC	523
APC – Cy7	428

barcode

Spectral Overlap Factors

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

The progress bar fills and the dialog closes when the barcode scan is successful.



The lot ID, expiration date, target values, and spectral overlap factors for the bead product appear on the appropriate tab of the Setup Lot Information dialog.

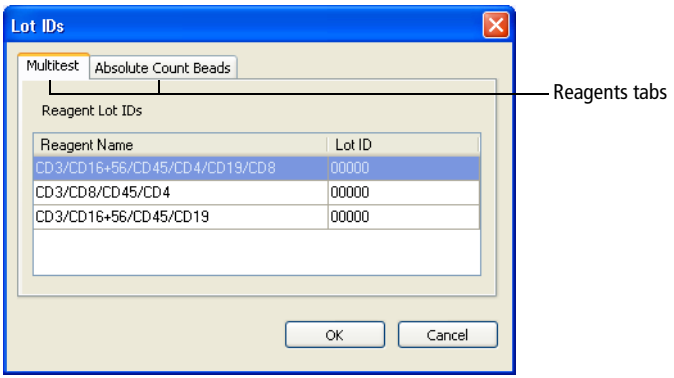
See Entering Lot Information for Setup Beads Manually on page 45 for more information.

4 Check all affected software fields for accuracy against the setup beads label.

Entering a Reagent Lot ID Manually

1 Select Tools > Lot IDs.

2 On the appropriate Reagents tab, select a Reagent Name from the list and enter the new Lot ID. Click OK.

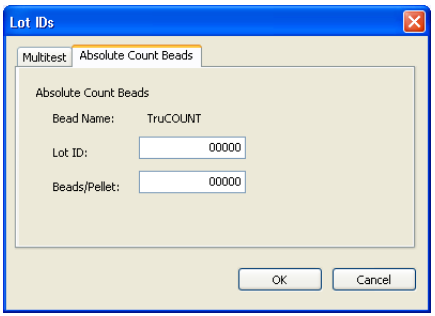


Entering an Absolute Count Bead Lot ID

1 Select Tools > Lot IDs.

2 In the Lot IDs dialog, click the Absolute Count Beads tab.

3 Enter the Lot ID and Beads/Pellet, which can be found on the pouch.



4 Click OK.

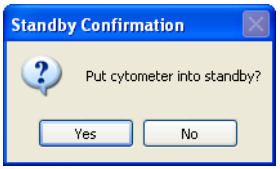
To obtain accurate results, it is critical that you enter this information accurately. Double-check your entry.

Placing the Cytometer in Standby

Placing the cytometer in standby disconnects the software from the cytometer, which allows you to run BD FACSDiva software without quitting BD FACSCanto clinical software.

1 Select Cytometer > Standby.

2 Click Yes in the confirmation dialog.



The fluidics shut down and the software disconnects from the cytometer.

Note the messages in the status bar:

Connecting to the Cytometer

After standby, and whenever you start the software before turning on the cytometer, you will need to connect the software to the cytometer.

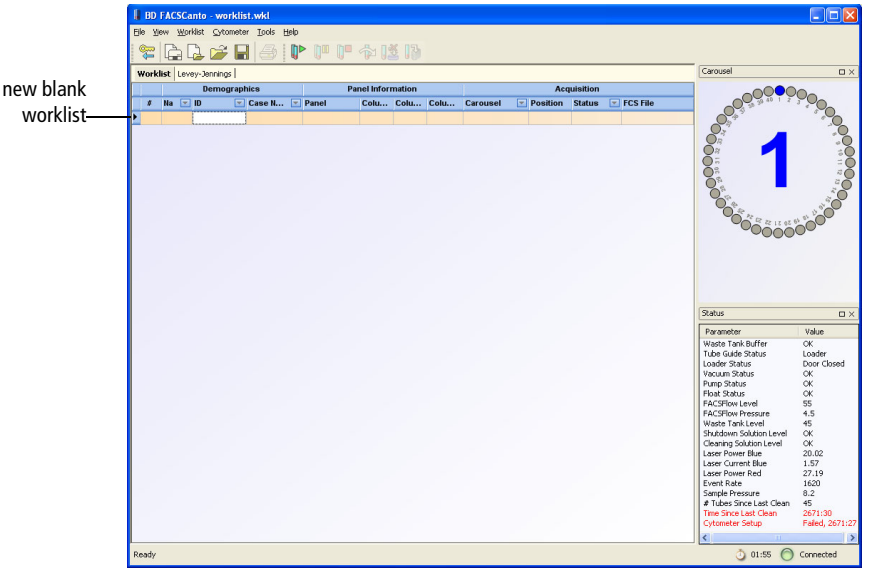
To connect to the cytometer, select Cytometer > Connect.

A message indicating that the cytometer is connecting appears in the status bar:

Fluidics startup runs automatically, only if enabled by the lab manager, and fluidics shutdown was done previously.

Understanding Worklist Options

When you first open BD FACSCanto software, a new blank worklist appears by default.



You can enter information into this worklist, or you can:

- Open an existing acquisition worklist.
- Start a new acquisition worklist using a saved worklist as a template.
- Import a Sample Prep Assistant worklist.

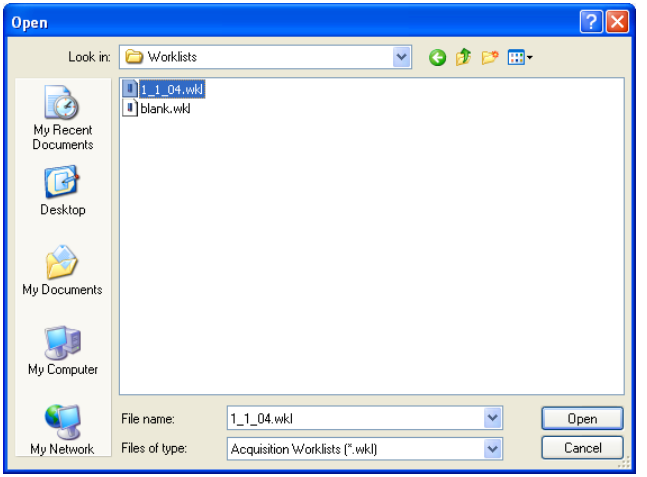
For information on entering information into a worklist, refer to the instructions for use for either the BD FACSCanto or the BD FACSCanto II flow cytometer.

Opening an Existing Worklist

1 Select File > Open Worklist.

2 Navigate to and select an acquisition worklist (WKL), and click Open.

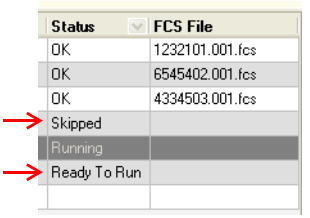
By default, worklists are saved in the C:\Program Files\BD FACSCanto Software\Worklists folder.



3 View the status of worklist samples in the Status fields.

For a description of Status entries, refer to Reviewing a Worklist in the instructions for use for your cytometer.

If an existing acquisition worklist contains samples that were not yet run, you can run the worklist again. You can also add samples to the worklist. Refer to the instructions for use for your cytometer.



Once an acquisition worklist contains FCS files for each sample in the worklist, you cannot run it again. To run a skipped tube, double-click the tube's Status field, and from the Lab Report view, click the Re-Run button.

Using an Acquisition Worklist as a Template

Any saved acquisition worklist can be reused as a template. When you open a saved worklist as described in this section, sample information and FCS file name fields are cleared. Enter new sample information to use the worklist again.

1 Select File > New Acquisition Worklist.

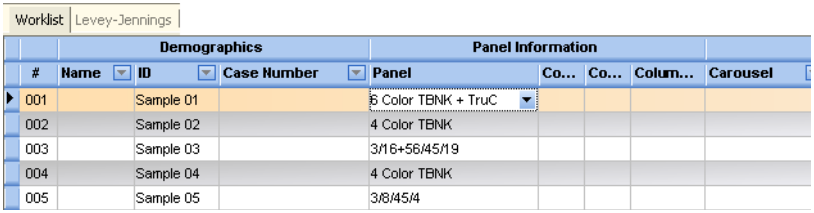
2 Navigate to the folder containing your saved worklists and select a file. Click Open.

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

☑ **Tip** Start a new, empty worklist by selecting *blank.wkl* from the worklists folder (this file comes with the software). Or, you can click on the toolbar.

3 (Optional) Enter new sample IDs for each sample.

The software retains the panel type and carousel information from your saved worklist, and assigns default sample IDs. (Sample IDs partially determine the FCS file name for each sample.) Keep or change the information, as needed. You can also enter sample names and case numbers, if you like.



Importing a Worklist from BD FACS SPA

You can import sample information from a worklist created in BD FACS Sample Prep Assistant (SPA) software version 2.0 or later.

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

1 Select 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, then click Open.

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.

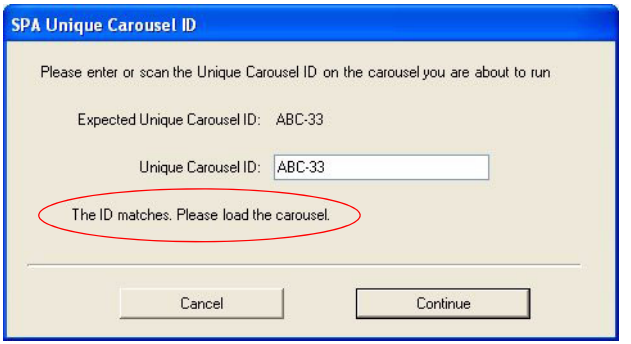
3 Review the imported information and edit missing or incorrect entries, if needed.

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

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

5 Select Run. If the SPA Worklist you run contains a Unique Carousel ID, a dialog is displayed telling you to enter or scan the ID for the carousel you are about to run.

6 If you enter the correct ID, the following dialog is displayed. Click Continue to continue with the run.



If the Unique Carousel ID does not match, it is indicated in the dialog.

Printing

All of the lists and reports in BD FACSCanto software offer the option to print, some automatically, some manually. For information about printing options, see the following sections.

- Printing a Worklist on this page
- Printing a Lab Report on page 61
- Printing a Setup Report on page 62
- Printing a Levey-Jennings Report on page 63

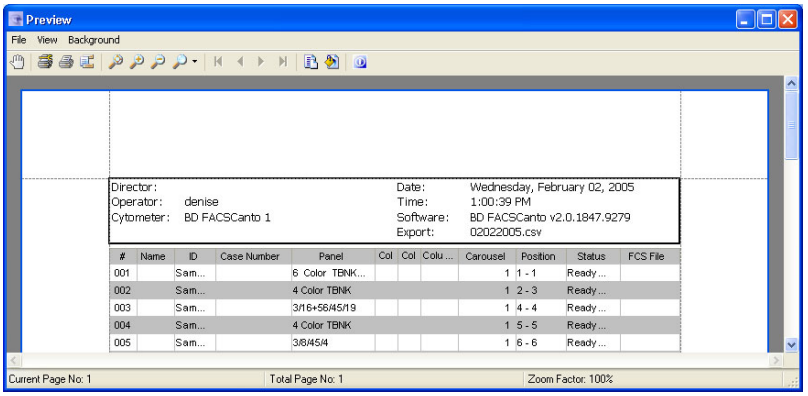
Printing a Worklist

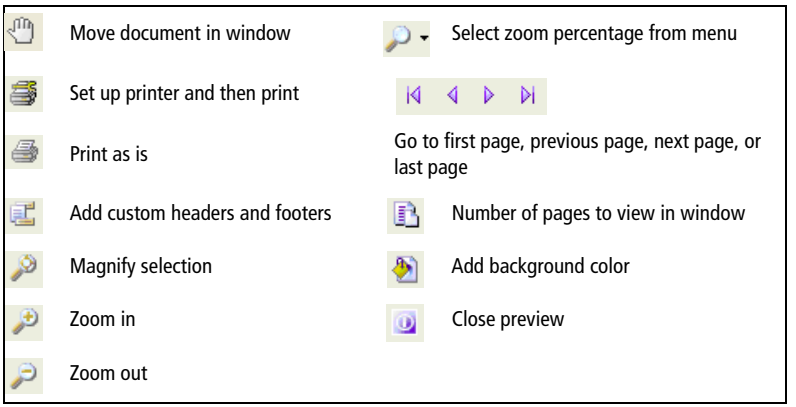
You can print a worklist before running it to assist you when loading a carousel. You can also print after a worklist finishes to serve as a summary report of the samples run.

Always preview a worklist before printing it to ensure that all required information is visible before you print it.

1. Select File > Print Preview.
2. Use buttons in the Preview window to set up for printing (Figure 4-4 on page 60).

Figure 4-4 Using Print Preview buttons





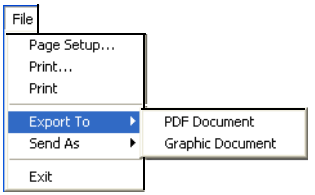
- To print the worklist, select File > Print.

3 To exit, select File > Exit.

Exporting the Worklist to Another Format

Alternatively, you can make the worklist into a PDF document or a graphic document, using these steps.

1. Select File > Print Preview.
2. From the Preview window's main menu, select File > Export To > PDF Document or Graphic Document.



Printing a Lab Report

To print a Lab Report, select File > Print while viewing it.

To print all Lab Reports in a worklist at once, select File > Print All Lab Reports.

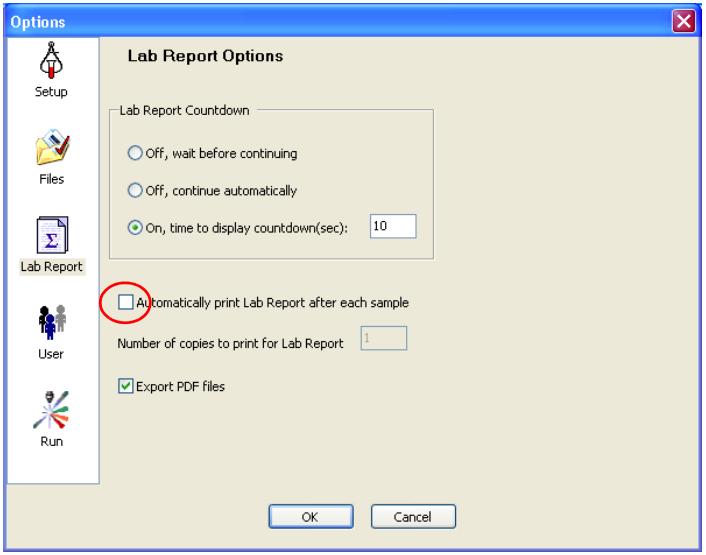
Printing a Lab Report Automatically

You can set Lab Report Options to print Lab Reports automatically after the cytometer acquires each sample. This option applies only to the current user. Preferences are saved from one login session to the next.

1. Select Tools > Options.
2. Click 

.

   The Lab Report Options dialog appears.



3 Select the checkbox to *Automatically print Lab Report after each sample*.

4 Enter the number of report copies to print per sample, and click OK.

You can print up to 10 copies.

Printing a Setup Report

To print a Setup Report, select File > Print while viewing it.

Setup Reports are automatically saved as PDF files in C:\Program Files\BD FACSCanto Software\SetupReports.

If you do not print a report immediately after setup, you can open and print the PDF file. For information on how files are named, see Table 5-1 on page 80.

Printing Setup Reports Automatically

You can print Setup Reports automatically after both setup and optimized setup. This option applies only to the current user. Preferences are saved from one login session to the next.

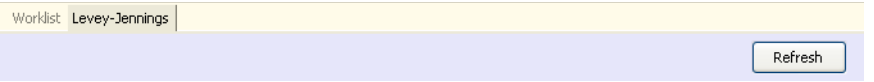
☑ **Tip** Enable this option to make sure formatted reports are printed after setup and optimization.

1. Select Tools > Options.
2. Click 

.
3. Select the checkbox to *Automatically print Setup Report* and click OK.

Printing a Levey-Jennings Report

1. In the main window, select the Levey-Jennings tab.



2. Select File > Print.

Previewing a Levey-Jennings Report Before Printing

1. Select File > Print Preview.
2. Use buttons in the Preview window to set up for printing (Figure 4-4 on page 60).
3. To print the report, select File > Print.
4. To exit, select File > Exit.

Customizing Software Defaults

Customizing the Lab Report Countdown

Lab managers set the default for the Lab Report countdown, but each user can set a user-specific preference that will be saved from one login session to the next.

The Lab Report countdown controls the amount of time the Lab Report displays at the end of sample acquisition. During the countdown, you can pause and re-gate the current sample (showing in the Lab Report view).

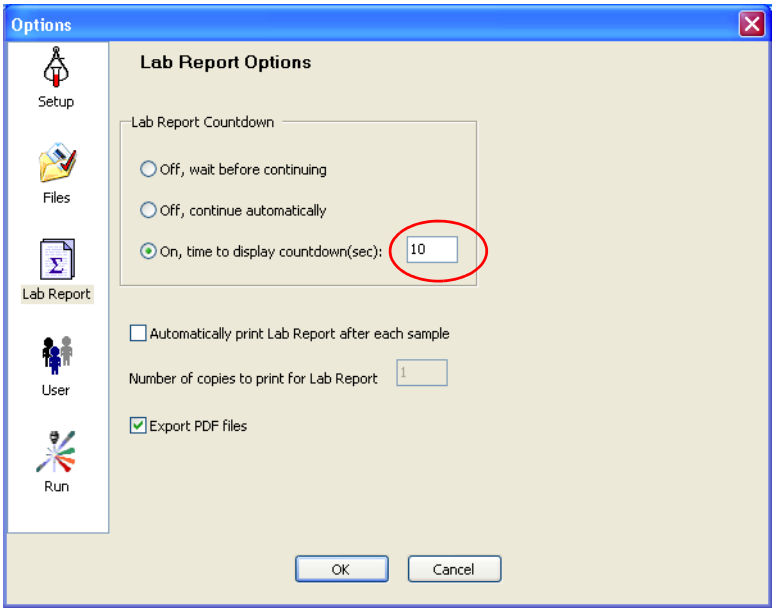
Options for customizing the Lab Report countdown are as follows:

Option	Explanation
Off, wait before continuing	Countdown dialog does not appear. The Lab Report appears prior to the Acquisition view for the next tube. You must click Start to continue to the next tube.
Off, continue automatically	Countdown dialog does not appear. Acquisition of next tube starts automatically.
On, time to display countdown (sec)	Countdown dialog appears, allowing you to pause and re-gate, if necessary.

Specifying a Display Time for the Lab Report Countdown

1. Select Tools > Options.
2. Click 

.
3. Select *On, time to display countdown (sec)*.
4. Enter a number of seconds, from 1 to 10, and click OK.

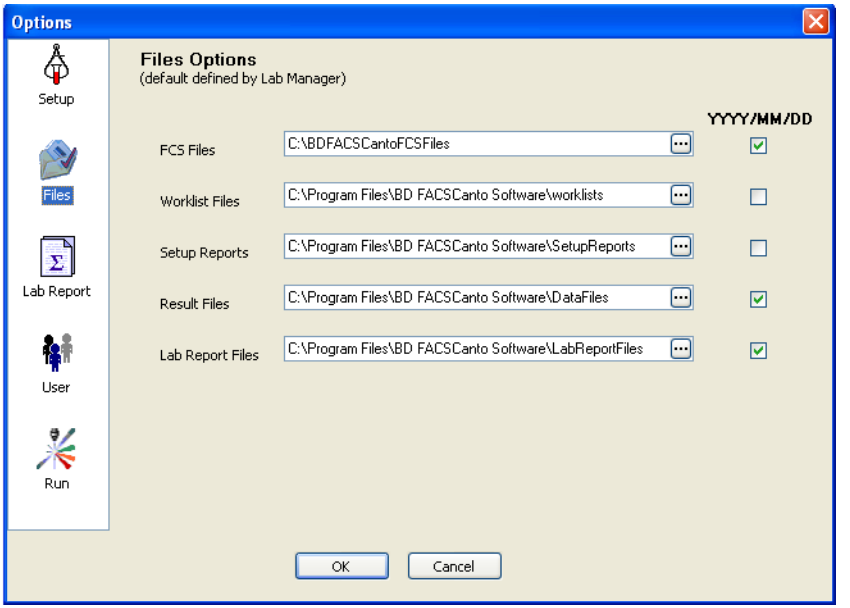


Customizing File Locations

Lab managers set default file storage locations, but each user can make user-specific selections that will be saved from one login session to the next.

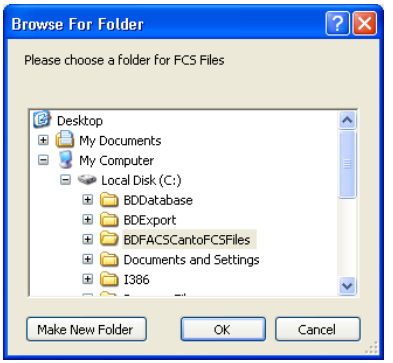
1. Select Tools > Options.
2. Click  .

3. Enter a new storage location for a file type.



Enter a new location or find a location by browsing.

- To browse, click ....
- Select or create a folder.



- Click OK.

4 To designate that a type of file is stored in a location consisting of year, month, and date folders, ensure that the directory checkbox (YYYY/MM/DD) is selected for that type of file.

For FCS Files, Result Files, and Lab Report Files, the YYYY/MM/DD checkboxes are selected by default. For Worklist Files or Setup Reports, the YYYY/MM/DD checkboxes are not selected by default. You can select or clear these checkboxes for any of the file types.

5 Click OK to save changes.

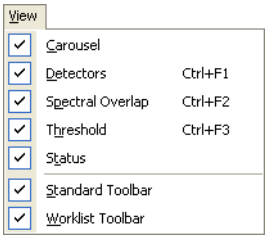
Customizing Windows and Toolbars

You can show, hide, change the appearance of, or relocate many of the windows and toolbars in the main window. The change lasts until you log off or quit the software.

Hiding Windows and Toolbars

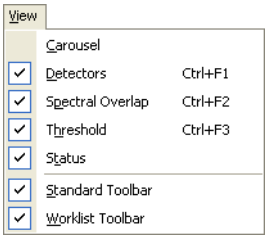
1 From the main menu, select View.

The ✓ indicates visible windows or toolbars.



2 Select a window or toolbar to hide and then select it.

The ✓ disappears, and the window becomes hidden.



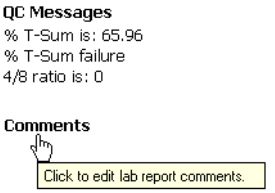
To show the window or toolbar again, reselect it in the menu.

Entering Comments into a Lab or LJ Report

Lab Reports and Levey-Jennings (LJ) Reports allow any reviewer to enter text into the Comments field. To enter or edit comments, do the following:

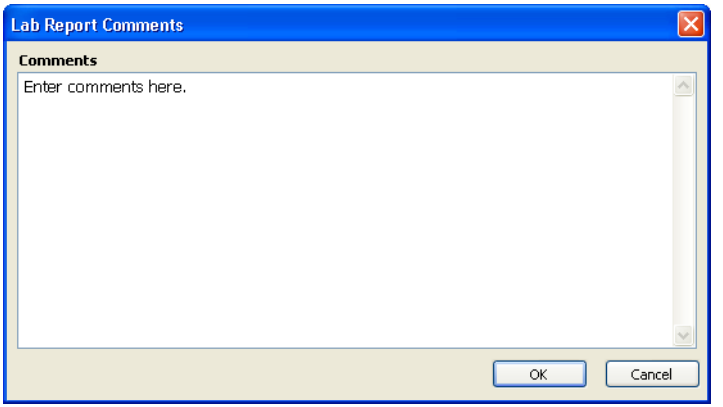
1 At the Lab Report or Levey-Jennings Report view, click Comments.

Figure 4-5 Example Lab Report



A dialog appears.

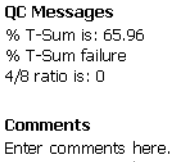
2 Enter text into the Comments field or edit the existing text.



☑ **Tip** Copy and paste unformatted text into the Lab Report Comments dialog.

3 Click OK.

Your comment appears on the report view and the printed report.



Viewing Previous Levey-Jennings Plots

While LJ data from an unlimited number of runs can be stored in the *BD FACS Setup Beads-7 colors LJ.csv* file, the LJ plots in the software can display data from only the last 100 runs at one time. The lab manager might therefore save previously created LJ data in renamed CSV files.

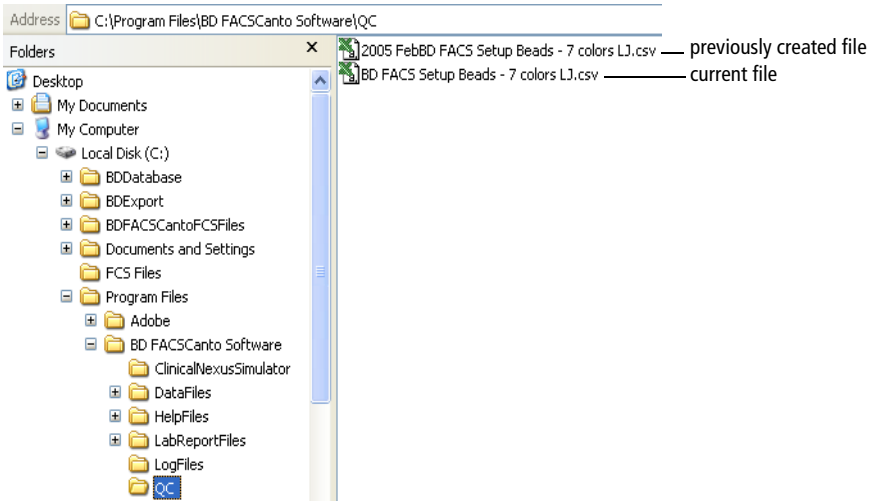
To look at a set of previously created and saved LJ plots, use the following procedure:

1 Navigate to the C:\Program Files\BD FACSCanto Software\QC folder (Figure 4-6 on page 71).

2 Rename the current *BD FACS Setup Beads-7 colors LJ.csv* file with a name of your choice.

The software reads and creates LJ plots from the data in this file.

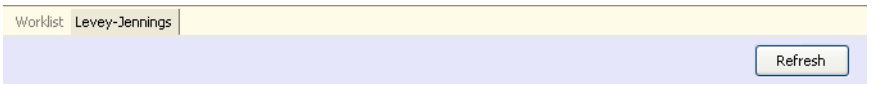
Figure 4-6 Location of current file



3 Rename the previously created file to *BD FACS Setup Beads-7 colors LJ.csv*.

In Figure 4-6, the previously created file is *2005 FebBD FACS Setup Beads-7 colors LJ.csv*.

4 In the main window, select the Levey-Jennings tab.



5 Click Refresh.

The software reads the previous files, which display in the Levey-Jennings view.

Viewing Current Levey-Jennings Plots

The software automatically updates the LJ plots after you accept a cytometer setup. To view the current LJ plots, simply select the Levey-Jennings tab. There is no need to click the Refresh button.

Changing Your Password

To change your password, follow these steps.

1. Select Tools > Options.
2. Click 

.
3. Click Change Password.
4. Enter your old password, a new password of up to 30 characters, and then confirm the new password. Click OK.

You can use any alphanumeric character. Passwords are case sensitive.

5. Click OK to close the Options dialog.

5

Lab Manager Options

In BD FACSCanto clinical software, lab managers administer user accounts and set defaults for functions such as printing, fluidics startup, and what appears on a Lab Report or Levey-Jennings Report. Whoever installs the software and creates the first account during installation assumes lab manager status. That individual can then create other user accounts, including more lab managers. There can be one or many lab managers.

You must have Windows XP administrator privileges to install the software (page 74). You must have lab manager privileges to do the following:

Most examples within this chapter show 4- and 6-color TBNK panel details. For clinical application defaults and examples, refer to the individual clinical application guides.

Installing the Software

BD FACSCanto software is already installed on your computer. Follow these steps if you need to re-install the software.

Before you begin, uninstall the current software. (See Uninstalling the Software on page 79.) Make sure you have no programs running that might conflict with the installer software.

1. Insert the BD FACSCanto software installation CD into the CD-ROM drive.

   The installer should start up automatically. If it doesn't, use Windows Explorer to view the CD contents, and double-click the Setup.exe icon.

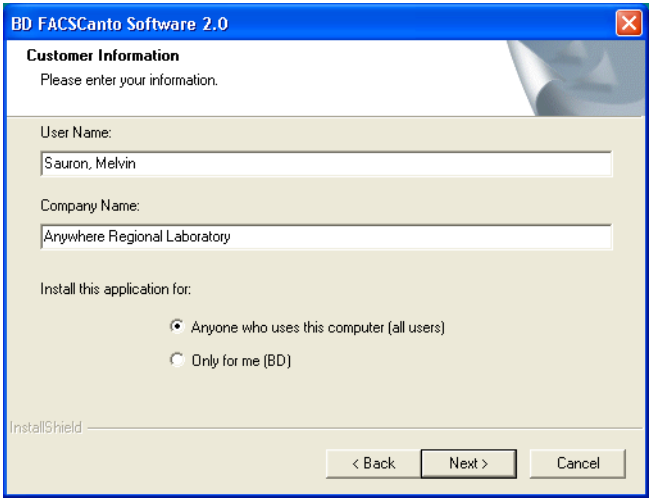
2. Click Next to begin the installation.

3. If you are prompted to do so, install Microsoft .NET Framework.

   Installation will not continue if you do not have Microsoft .NET Framework software installed.

4. Review and accept the license agreement.

5. Enter your user name and company name. Click Next.

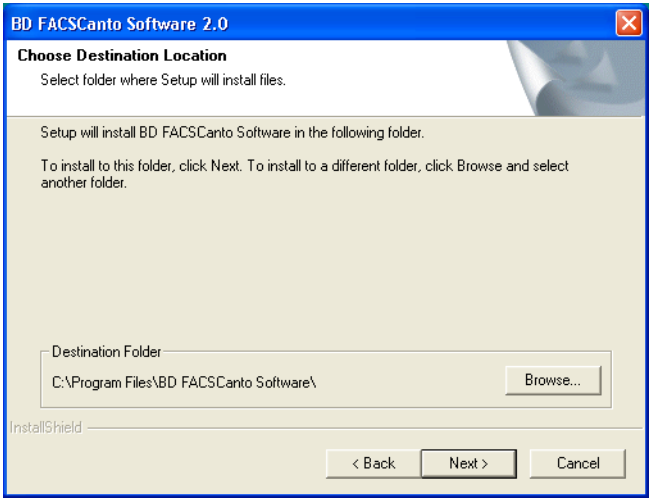


⚠ Keep the default of *Anyone who uses this computer (all users)*. Otherwise, only the person who installed the software will have access to it.

6 If you are prompted to do so, click OK to install Adobe Acrobat Reader.

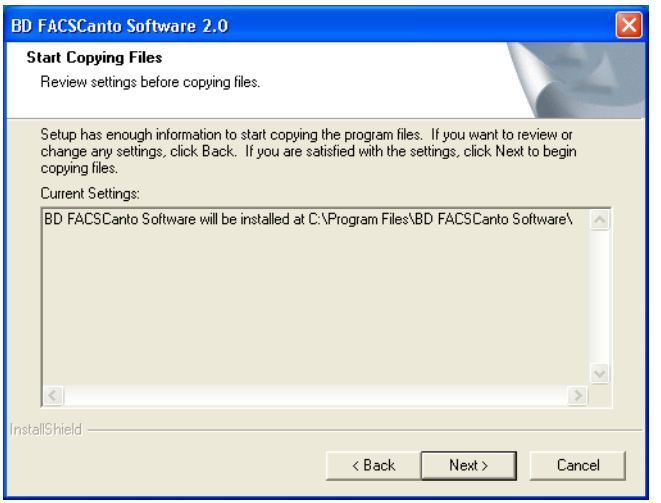
You need Acrobat Reader to view PDF documents. Follow the instructions in the dialogs and accept all default options to install Acrobat Reader.

7 Review the setup information and click Next.



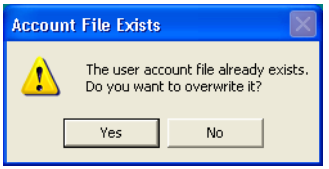
By default, the installer places all components in C:\Program Files\BD FACSCanto Software. To install components in a different location, click the Browse button and navigate to a different folder.

8 Review the installation settings and click Next.



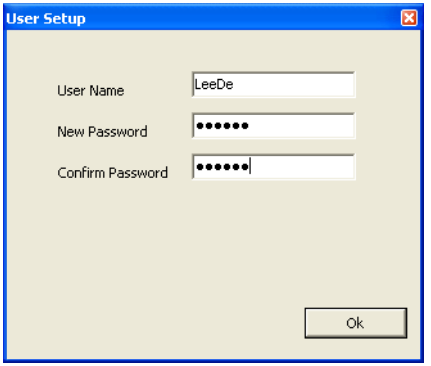
The installer loads the software and its support files on the selected hard disk.

9 When prompted, choose to save or overwrite your existing user account file.

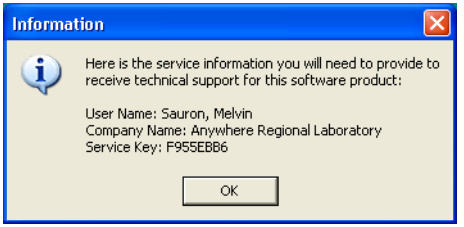


- To reuse your existing user information, click No and go to step 10.
- To start a new list of users, click Yes.

  Create the first user account when prompted. This user will have lab manager privileges. Enter the user name and password, then click OK.



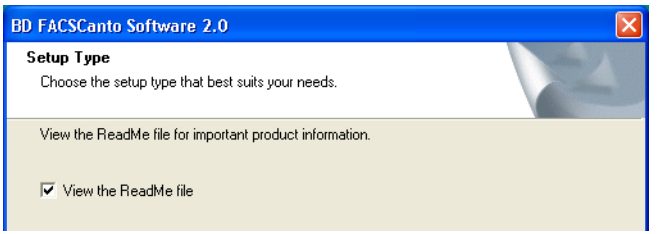
10 Write down the number provided for your service key and click OK.



⚠ Write the service key here: _____. You will not be able to obtain technical support for your software without this key.

11 When prompted, click Next to view the ReadMe file in English.

Translations are provided on the documentation CD.



Print and review the ReadMe file, and then click the Close box to return to installation.

A message appears when installation is complete.

12 Click Finish to complete the installation.

The installer places a shortcut to BD FACSCanto software and the PDF version of this manual in the Start menu, and a shortcut to the software on the desktop.

The installer places the software and all supporting files in one of three folders on the specified drive.

- D:\BDFACSCantoFCSFiles
- C:\Program Files\BD FACSCanto Software
- C:\Program Files\Common Files\BD

Translated copies of the PDF version of this manual can be found on the BD FACSCanto documentation CD.

Uninstalling the Software

Follow the steps in this section if you need to uninstall the software. Uninstalling will not remove your data files.

1. From the Windows Start menu, select Settings > Control Panel > Add or Remove Programs.
2. Select BD FACSCanto Software, and click Change/Remove.
3. Click OK to confirm.
4. Follow the prompts on the screen to remove all installed components, then click Finish.
5. Close the Add or Remove Programs window.

Managing Files

The software creates the following file types (Table 5-1).

⚠ **Do not** change the properties of these files, or BD FACSCanto clinical software might not run. For example, do not make them Read-only.

Do not open support files in another software application while BD FACSCanto clinical software is running.

☑ **Tip** To open a file while running BD FACSCanto clinical software, make a copy of the file and open the copy.

Table 5-1 Files

File Type	Default Name	Default Location	Description
FCS (.fcs)	*SampleID[3-digit worklist entry number].[3-digit tube number]*.fcs	D:\BDFACSCanto FCSFiles*yyyy**Month**dd*	Saves event data, cytometer settings, analysis (including manual gates), and any user comments for a tube; FCS 3.0 format used
Levey-Jennings (.csv)	BD FACS Setup Beads - 7 colors LJ.csv	C:\Program Files\BD FACSCanto Software\QC	Contains the following QC data for 30, 60, or an unlimited number of samples (if designated by the lab manager): • Run date and time • Cytometer setup bead lot ID • Detector voltages • Sample and sheath pressure • Spectral overlap values • Power and spec for each laser, current for blue laser
Acquisition Worklist (.wkl)	Worklist.wkl	C:\Program Files\ BD FACSCanto Software\Worklists	List of sample information and associated FCS files

Table 5-1 Files (continued)

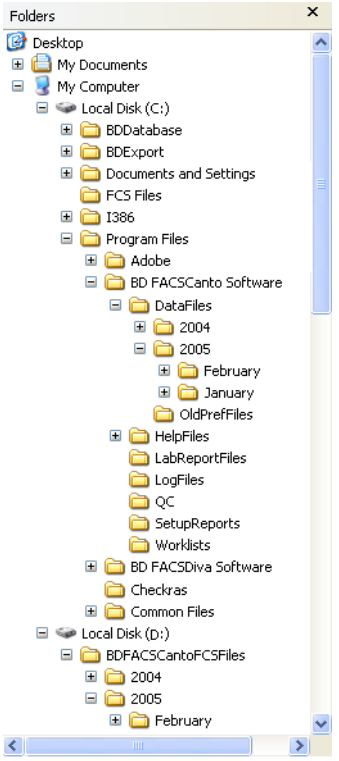
File Type	Default Name	Default Location	Description
Analysis Worklist (.wka)	Worklist.wka	C:\Program Files\ BD FACSCanto Software\Worklists	List of sample information and associated FCS files
Setup Report (.pdf)	*setup type*_yyyyMM dd_hhmm.pdf	C:\Program Files\BD FACSCanto Software\SetupReports	Consists of a PDF file of a Setup Report from a specific time and date, saved automatically
Lab Report (.pdf)	*SampleID[3-digit worklist entry number]* .pdf	C:\Program Files\BD FACSCanto Software\LabReport Files*yyyy**Month**dd*	Consists of a PDF file of a Lab Report from a specific time and date, saved if specified by lab manager
Usage Trace (.csv)	*yyyy Month.csv;* for example, 2003 March.csv	C:\Program Files\Common Files\BD	Tracks the following information for all users: • User name • Full name • Application name • Role • Department • Institution • Login time, login date • Logout time, logout date
Setup Results (.dat)	SetupResult. dat	C:\Program Files\Common Files\BD\Setup Results	Contains data from the latest saved setup
Optimized Setup Result (.opt)	*panel type*.opt; for example, 4 Color TBNK.opt	C:\Program Files\Common Files\BD\Setup Results	Contains data from the latest optimized setup
Result (.csv)	*ddMMyyyy*.csv	C:\Program Files\BD FACSCanto Software\DataFiles\ *yyyy**Month**dd*	Tracks sample and panel information and all subset results on a daily basis

Table 5-1 Files (continued)

File Type	Default Name	Default Location	Description
Process Control Results (.csv)	*LotID*.csv	C:\Program Files\BD FACSCanto Software\DataFiles	Tracks results of process control samples to an export-friendly data file (CSV format)

The following figure shows how the folders are organized.

Figure 5-1 Folder locations



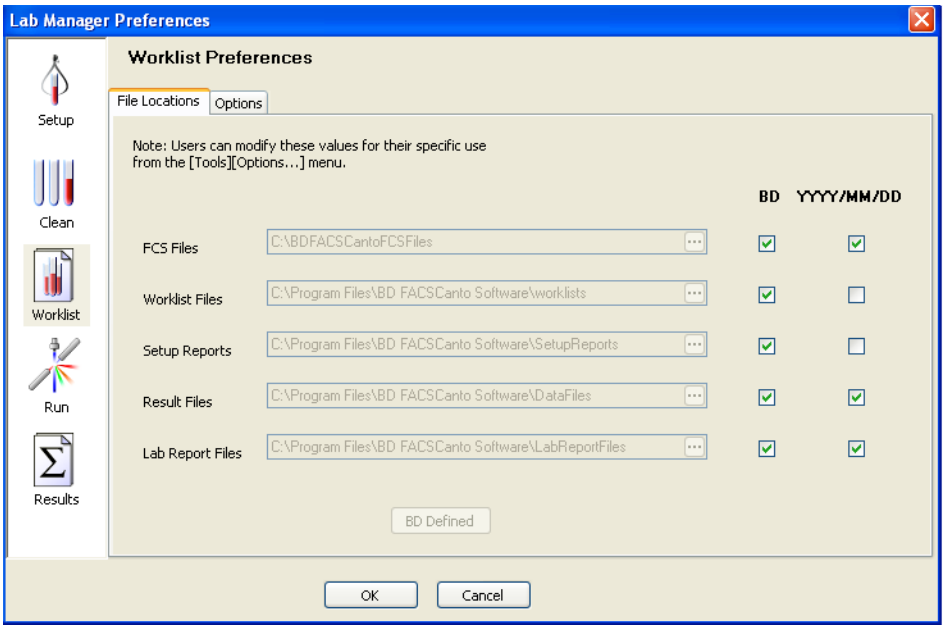
Changing Default File Locations

The lab manager can change default file locations for FCS files, worklist files, Setup Reports, and result files. These changes apply to all users, but do not overwrite individual user preferences.

1. Select Tools > Lab Manager Preferences.
2. Click 

.
3. Click the File Locations tab.
4. Clear the checkbox beside a file type.

   Doing so allows you to change from the BD default.



5 Enter a new location, or find a location by browsing.

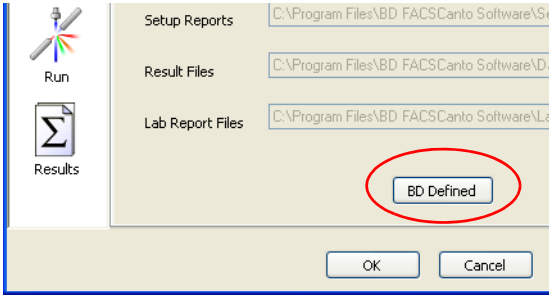
- To browse, click ....
- Select or create a folder.
- Click OK.

6 To designate that a type of file is stored in a location consisting of year, month, and date folders, ensure that the directory checkbox (YYYY/MM/DD) is selected for that type of file.

For FCS Files, Result Files, and Lab Report Files, the YYYY/MM/DD checkboxes are selected by default. For Worklist Files or Setup Reports, the YYYY/MM/DD checkboxes are not selected by default. You can select or clear these checkboxes for any of the file types.

7 To revert to the BD-defined storage location, select the checkbox next to the file type.

To revert all file locations back to BD defaults, click BD Defined.



8 Click OK to save changes.

Managing User Accounts

NOTICE User account information is stored in a file with the administrator password. If you lose the password, user information could also be lost. Keep a copy of the administrator password in a secure location in case you forget it.

Setting Up New Users

Before other users can run samples, the lab manager needs to set up user accounts.

1. From the main menu, select Tools > Users and Passwords.
2. (Optional) Create a list of departments.
   - Click Departments.
   - Click New Department.

     A new department name appears in the Department list.
   - Enter the following information in each field.

Field or Menu	Explanation
Department Name	Up to 30 characters
Director Name	(Optional) Up to 30 characters

Field or Menu	Explanation
Institution Name	(Optional) Up to 30 characters
Address	(Optional) Up to 3 lines, with 40 characters per line
Tel. No.	(Optional) Up to 30 characters
Fax No.	(Optional) Up to 30 characters
URL	(Optional) Up to 200 characters

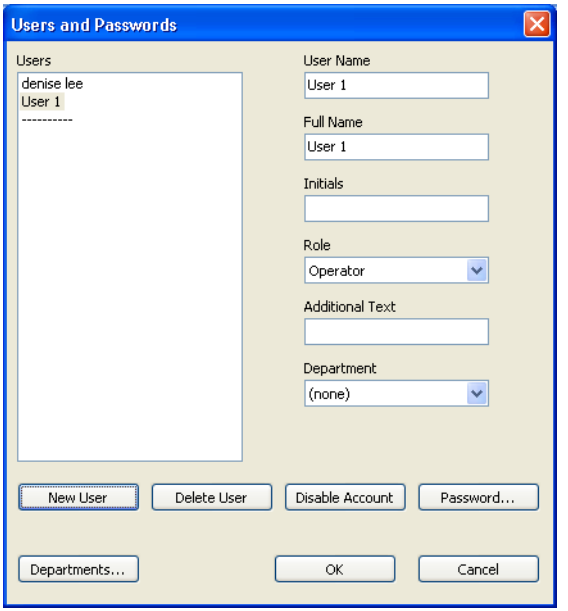
- Continue to add departments as needed.

  You can create up to 50 departments.

- Click OK when you are done.

3 Create a list of users.

- Click New User.



The default name (*User 1*) appears on the list.

- Enter the following information for each user.

Field or Menu	Explanation
User Name	Up to 25 characters—name the user selects from the menu during login and enters with the password
Full Name	Up to 30 characters—name that appears on report headers
Initials	Up to 10 characters
Role	
• Operator	Non-administrative privileges only, cannot add new users
• Lab Manager	Administrative privileges, includes approved user-list creation
Additional Text	Up to 30 characters
Department	User's department affiliation Select from the list of predefined departments.

- Continue to add users.

  You can have up to 50 enabled users (including lab managers).

4 Create a password for each new user.

- Select a user.
- Click Password.
- Enter a password and confirm it.

- Click OK.
- Continue until all users have passwords.

5 Click OK to close the Users and Passwords dialog.

Editing User Information

To edit user information:

1 Select Tools > Users and Passwords.

2 Select a user name from the list.

3 Edit information as needed.

4 To change the password, click Password; enter the new password and confirm it, and then click OK.

5 Click OK.

Deleting Users

To delete a user, follow these steps. Deleted user names are removed from the login menu, but their FCS files are retained.

1 Select Tools > Users and Passwords.

2 Select a user from the list.

3 Click Delete User.

4 Click Yes to confirm.

5 Click OK.

You cannot delete the currently logged-in user (yourself).

Disabling User Accounts

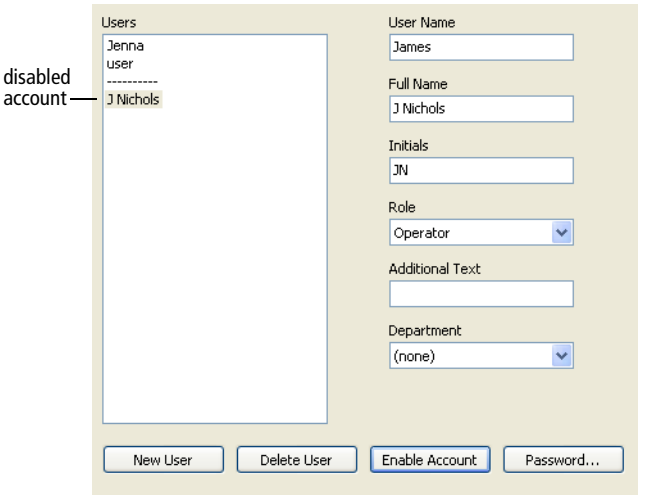
Disabling a user account prevents a user from logging in to the software.

1 Select Tools > Users and Passwords.

2 Select a user from the list.

3 Click Disable Account.

The disabled account appears beneath the dashed line.



4 Click OK.

The account holder can no longer log in to the software.

Enabling User Accounts

To enable a user account that was previously disabled:

1. Select Tools > Users and Passwords.
2. Select a disabled account from the list.
3. Click Enable Account.
4. Click OK.

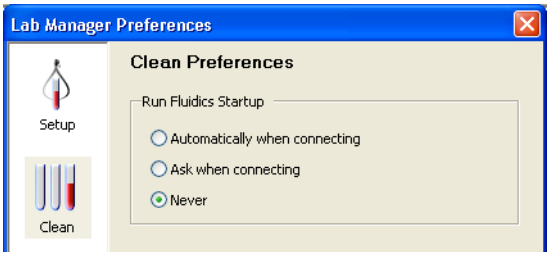
The user will regain login privileges.

Changing Fluidics Startup Preferences

By default, the cytometer does not run fluidics startup automatically. To change to an automatic fluidics startup (occurs when you start up, if it is needed, or the first time you connect to the cytometer), follow these steps.

1. Select Tools > Lab Manager Preferences.
2. Click 

.
3. Select when to run fluidics startup.



Option	Explanation
Automatically when connecting	Fluidics startup runs automatically at startup or when you connect after standby, if needed. (It is needed if a fluidics shutdown was run previously.)
Ask when connecting	Whenever the software connects to the cytometer, a dialog asks whether you want to run fluidics startup. This will happen at startup or when you connect after standby, if needed.
Never	Automatic fluidics startup never occurs automatically.

The default is *Never*.

4 Click OK.

Changing Setup Preferences

You can make the following changes to cytometer setup preferences:

- Changing the Levey-Jennings File Preferences on page 92
- Specifying Levey-Jennings View Preferences on page 93
- Hiding the “Reviewed By” Field on page 96
- Printing the Setup Report Automatically on page 96

Changing the Levey-Jennings File Preferences

You can change the default number of entries (30) stored in the LJ file.

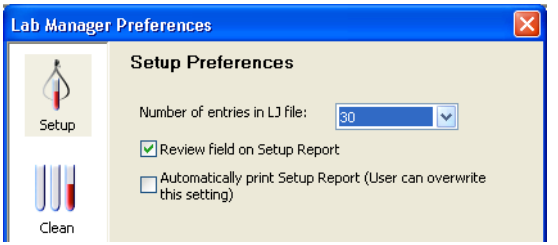
1 Select Tools > Lab Manager Preferences.

2 Click 

.

3 Select a total number of entries to store.

Select 30, 60, or unlimited entries.



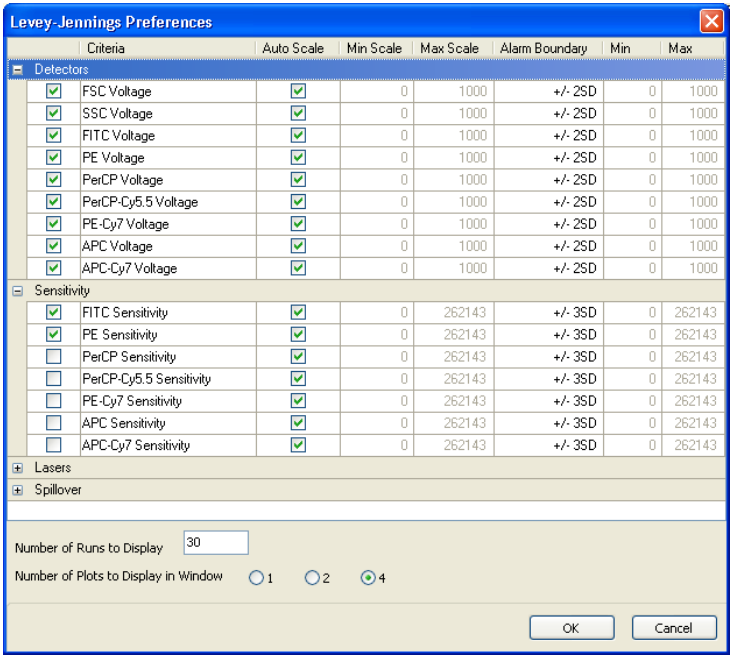
4 Click OK.

Specifying Levey-Jennings View Preferences

You can specify how much of the LJ data to display in the Levey-Jennings view and in the Levey-Jennings Report.

1 Select Tools > Levey-Jennings.

The Levey-Jennings Preferences dialog appears.



2 To show a parameter, select the checkbox beside it.

If you don't want a parameter to show in the Levey-Jennings view or report, clear its checkbox.

Select up to 20 parameters to display in LJ plots.

3 Change the scale, if needed, for a parameter's Levey-Jennings plot.

By default, Auto Scale is selected, and the software fits the scale to the data points to be displayed. To change to a custom scale with fixed top and bottom values, do the following:

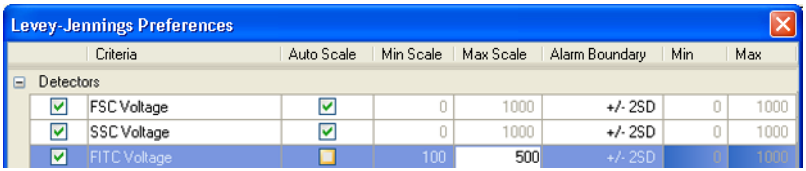
- Select a parameter.

  In Figure 5-2, FITC Voltage is selected.

- Clear the Auto Scale checkbox.

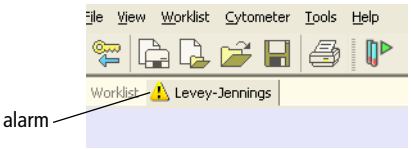
- Enter the minimum and maximum values for the scale into the appropriate fields.

Figure 5-2 Changing the scale for Levey-Jennings FITC data



4 Change the alarm boundaries, if needed, for a parameter's Levey-Jennings plot.

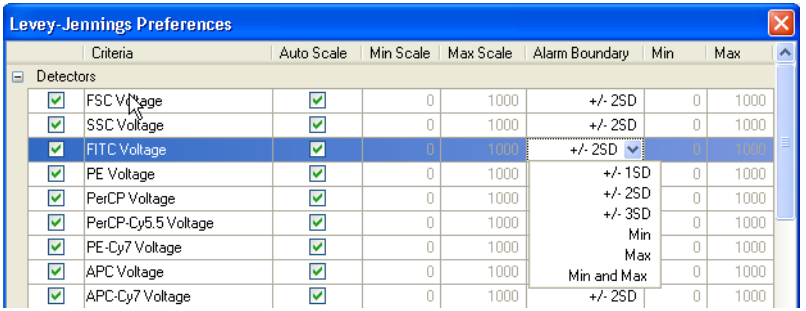
An alarm symbol will appear on the Levey-Jennings view tab when a data point falls outside the specified boundaries.



To change the default boundaries, do the following:

- Select a parameter.

- From the Alarm Boundary menu, make a selection.



For example, a choice of +/- 3SD means that an alarm will appear if the data is outside the boundaries of -3 to +3 standard deviations of the data mean.

- To enter a custom minimum or maximum, select Min, Max, or Min and Max. Then, enter the required Min or Max into the appropriate field.

⚠ No alarm will appear for a parameter when data falls outside the boundaries unless you choose to display a LJ plot for that parameter.

5 Change the number of runs to display in each LJ plot.

You can display the data from up to 100 cytometer setups in each LJ plot.

6 Select the number of plots to display together in each LJ view window.

You can view 1, 2, or 4 plots at a time in the Levey-Jennings view window. How you group plots determines how they print in the Levey-Jennings Report.

7 Click OK when done changing Levey-Jennings preferences.

Hiding the "Reviewed By" Field

You can hide the *Reviewed by* field that by default appears on Setup Reports.

To hide this field, follow these steps.

1. Select Tools > Lab Manager Preferences.
2. Click 

.
3. Clear the checkbox beside *Review field on Setup Report*.
4. Click OK.

Printing the Setup Report Automatically

By default, the software does not print Setup Reports automatically after cytometer setup. To change the default to automatic printing after setup (pre-optimized and optimized), follow these steps.

1. Select Tools > Lab Manager Preferences.
2. Click 

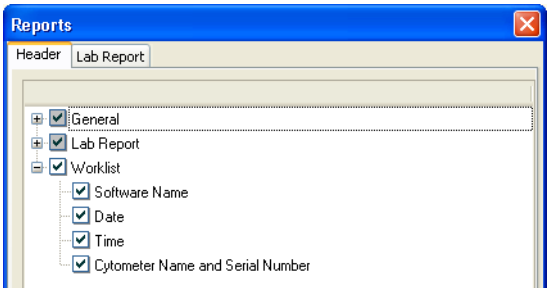
.
3. Select the *Automatically print Setup Report* checkbox.
4. Click OK.

Changing Worklist Report Header Preferences

To select header information specific to the Worklist Report, do the following.

1. Select Tools > Reports.
2. On the Header tab, click the ⊞ beside Worklist.

   A list of choices expands.



3. Select the header information you want to show on the Worklist Report.

   Clear the items you do not want on the Worklist Report.
4. Click OK when you have finished.

Changing Acquisition Preferences

Acquisition preferences vary according to the application you are running. You can make the following changes in cytometer acquisition:

- Changing Plots in the Acquisition View on page 98
- Changing Acquisition Targets on page 99
- Changing the Lag Time Before Recording on page 100

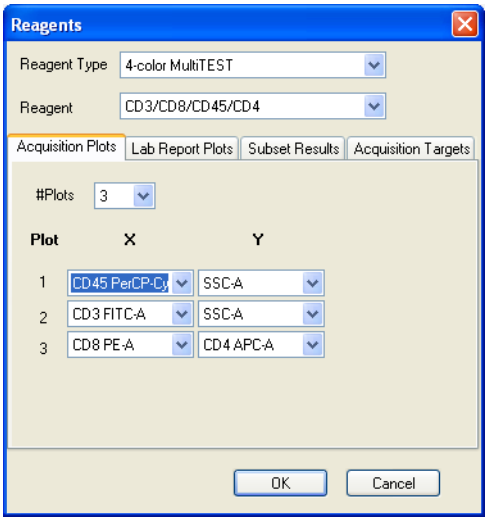
- Changing the Lab Report Countdown on page 101

Changing Plots in the Acquisition View

You can change default plots in the acquisition view for a reagent as follows.

1 Select Tools > Reagents.

2 Select a reagent type and reagent from the respective menus.



3 On the Acquisition Plots tab, select the number of plots to show during acquisition.

4 Select parameters for the x- and y-axes for each plot.

Parameter choices will vary depending on the reagent.

5 Continue to select reagents and modify acquisition plots, if appropriate.

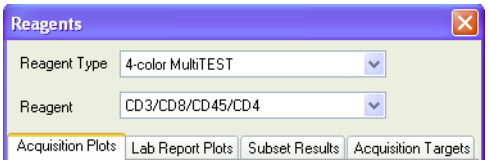
6 Click OK when you are done.

The software will save the new acquisition plot templates.

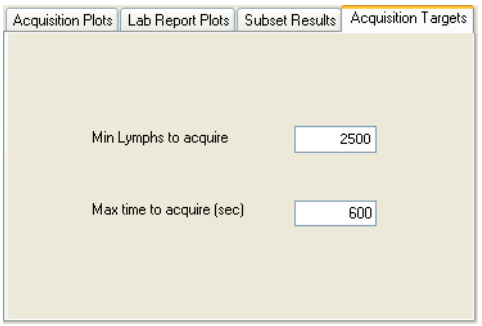
Changing Acquisition Targets

To change the acquisition targets for a reagent, follow these steps.

1. Select Tools > Reagents.
2. Select a reagent type and a reagent from the respective menus.



3. Click the Acquisition Targets tab.
4. Enter a minimum number of lymphs.



   You can enter 0–3,000,000 lymphs. If you enter 0, the software will ignore lymphocytes as a target and use time as the acquisition target instead.

5. Enter a maximum time.

   You can enter 0–900 seconds. If you enter 0, the software will ignore time as a target and use lymphs as the acquisition target instead.

   When you specify both lymphs and time, acquisition stops when one of the criteria is met.

Time and lymphs cannot both be 0.

6 Continue to select reagents and modify the acquisition targets, if appropriate.

7 Click OK when you are done.

The software will save the new acquisition targets.

☑ **Tip** You don't need to click OK after each target. You can define all targets and then click OK once at the end.

Changing the Lag Time Before Recording

The lag time occurs between the start of acquisition (when you click Run) and the start of data recording. This lag prevents data from being recorded while the sample flow stabilizes. Follow these steps to change the default lag time of 10 seconds.

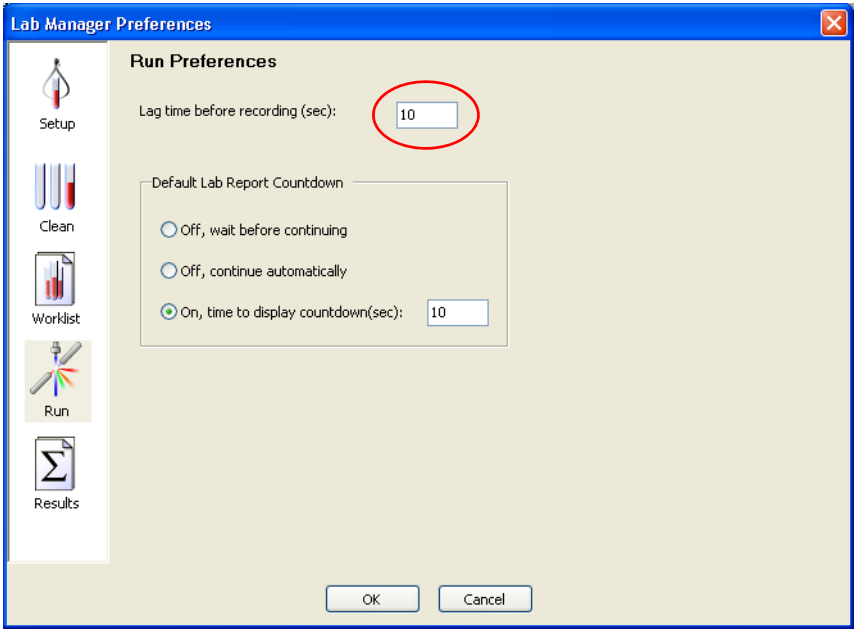
1 Select Tools > Lab Manager Preferences.

2 Click 

.

3 Enter a lag time.

The acceptable range is 3–15 seconds.



4 Click OK.

Changing the Lab Report Countdown

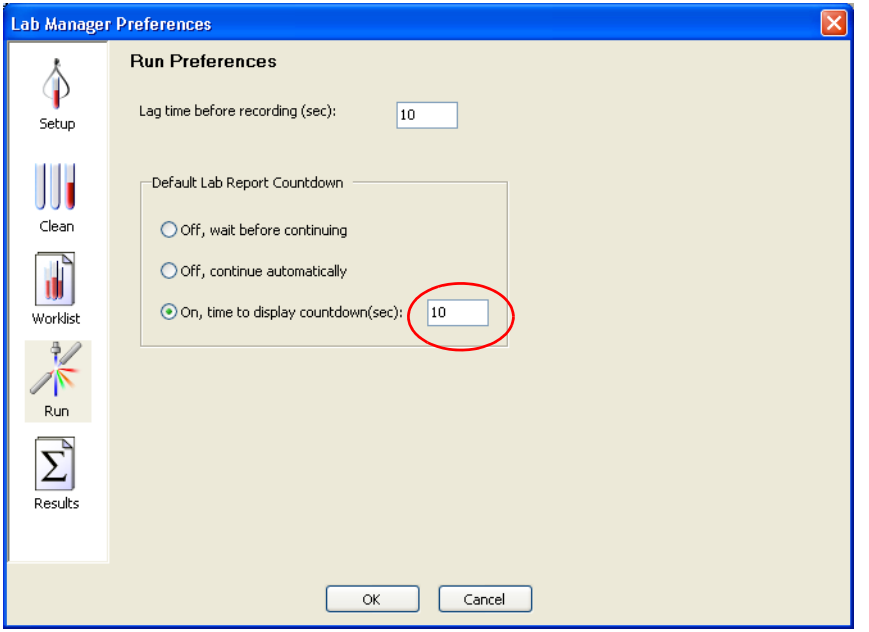
The Lab Report countdown controls the amount of time the Lab Report displays at the end of sample acquisition. Lab managers set the default time, but each user can set a user-specific preference that is saved from one login session to the next.

1 Select Tools > Lab Manager Preferences.

2 Click 

.

3 Enter a number of seconds.



The acceptable range is 0–10 seconds.

4 Click OK.

Changing Lab Report Preferences

You can make the following changes to Lab Report defaults:

- Changing Plots in the Lab Report View on page 103
- Changing Subset Results for a Reagent on page 104
- Changing Alarm Ranges for Subset Results on page 105
- Choosing QC Values on page 106
- Hiding Error Messages on page 109

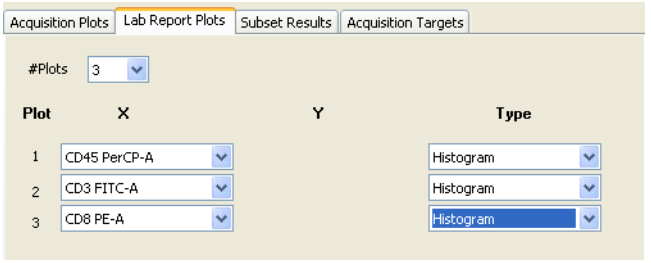
- Disabling Comments on page 110
- Choosing the Lab Report Language on page 110
- Choosing Header Information on page 110
- Automatically Printing the Lab Report on page 111

Changing Plots in the Lab Report View

Follow these steps to change the default plots at the Lab Report view.

1. Select Tools > Reagents.
2. Select a reagent type and reagent from the respective menus.

3. Click the Lab Report Plots tab.
4. Select the number of plots to show.
5. Select a type for each plot (dot plot or histogram).



A histogram presents single-parameter data. The horizontal x-axis shows signal intensity and the vertical y-axis shows the number of events.

A dot plot presents two-parameter data. Each axis displays the values of one parameter. A dot represents an event.

6 Select parameters for the x- and y-axes for each plot.

For histograms, you can only select the x-axis parameter.

Parameter choices will vary depending on the reagent.

7 Continue to select reagents and modify Lab Report plots, if appropriate.

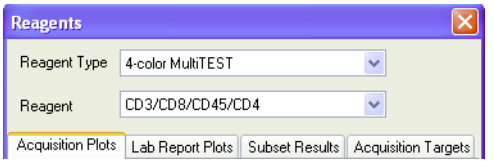
8 Click OK when you are done.

Changing Subset Results for a Reagent

To change the default options for subset results, which show on the Lab Report, follow these steps.

1 Select Tools > Reagents.

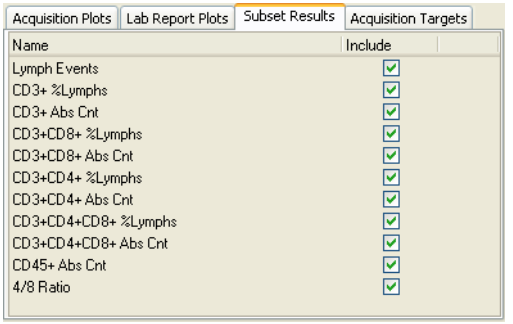
2 Select a reagent type and reagent from the menus.



3 Click the Subset Results tab.

4 Select checkboxes for the subset results to include.

Clear checkboxes for the subset results to exclude.



Subset results choices will vary, depending on the reagent.

5 Click OK when you are done.

Changing Alarm Ranges for Subset Results

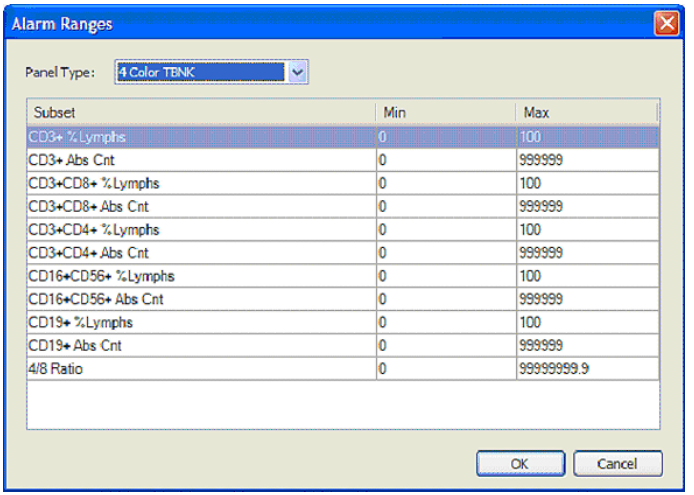
When a result falls outside of the specified range, the software prints the result in red text on the Lab Report to bring attention to it. You can change the default alarm ranges for subset results.

BD Biosciences recommends that you establish appropriate ranges for your laboratory.

1 Select Tools > Alarm Ranges.

2 Select a panel type.

Figure 5-3 Default alarm ranges



3 Enter a minimum and maximum alarm value for each subset.

The values shown in Figure 5-3 are the defaults and define the lower and upper range limits for the subsets.

4 Click OK when you are done.

Choosing QC Values

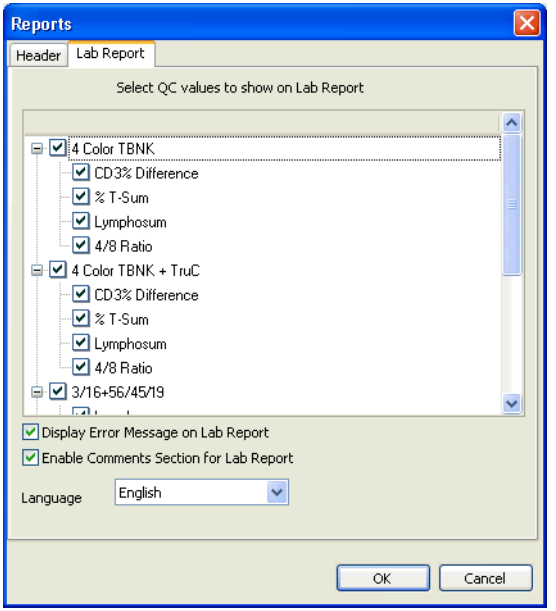
Follow these steps to select which QC values to display on your Lab Reports.

1 Select Tools > Reports.

2 Click the Lab Report tab.

3 Click the ⊞ beside a panel.

A list expands beneath the panel.



4 Select the QC results you want to show on the Lab Report.

Clear those that you do not want on the Lab Report. You can also choose to hide all QC results for the panel by clearing the checkbox beside the panel.

5 Click OK when you have finished.

Deciding Which QC Results to Include

QC results provide information about the analytic reliability of the cytometer and your methods. Which results you include will depend upon the panels you run and your laboratory's needs.

If you receive QC error messages on your Lab Report, follow these general suggestions.

1 Check the gating for the tube.

2 See QC Messages on page 138.

CD3% Difference

This QC result looks at result consistency within a panel.

$CD3^+$, as a percentage of lymphocytes, is enumerated for each tube in the panel. The difference between the percentages is reported.

$$\text{CD3 \% consistency} = \text{maximum } CD3^+ \%\text{lymphs} - \text{minimum } CD3^+ \%\text{lymphs}$$

% T-Sum

$CD4^+$ and $CD8^+$ cells are enumerated as a percentage of lymphocytes. $CD3^+$, as a percentage of lymphocytes, is enumerated for each of the two tubes, and the two values are averaged.

The following result is reported:

$$\text{T-sum} = CD3^+ - (CD4^+ + CD8^+)$$

$CD4^+CD8^+$ events are included in both $CD4^+$ and $CD8^+$ counts. Thus, the double-positive events are counted twice.

If the absolute value of the T-Sum is greater than 10, % *T-Sum failure* appears on the Lab Report.

Lymphosum

The lymphosum is an internal QC check.

$CD19^+$ and $CD16+56^+$ cells are enumerated as a percentage of lymphocytes. $CD3^+$, as a percentage of lymphocytes, is enumerated for each tube in the panel. For two tube panels, the values are averaged.

The following result is reported:

$$\text{Lymphosum} = \langle \text{Average } CD3^+ \rangle + CD19^+ + CD16+56^+$$

If the lymphosum falls beyond the default 95–105% range, *Lymphosum failure* appears on the Lab Report.

4/8 Ratio

The software will determine a CD4/CD8 ratio if a panel contains one or more reagents with a $CD3^+CD4^+$ population, and one or more reagents with a $CD3^+CD8^+$ population. $CD4^+CD8^+$ events are included in both $CD4^+$ and $CD8^+$ counts. Thus, the double-positive events are counted twice.

For a panel containing one $CD3^+CD4^+$ and one $CD3^+CD8^+$ population, the software uses this formula:

$$4/8 \text{ ratio} = \frac{CD3^+CD4^+ \text{events}}{CD3^+CD8^+ \text{events}}$$

Hiding Error Messages

By default, the Lab Report displays error messages. You can set the software so no error messages display on the report. However, results outside the alarm range will still appear in red text, and a Needs Review status will still appear in the Status column for samples that require it.

To hide error messages, follow these steps.

1. Select Tools > Reports.
2. Click the Lab Report tab.
3. Clear the checkbox beside *Display Error Message on Lab Report*.
4. Click OK.

To show error messages again, select the checkbox beside *Display Error Message on Lab Report*.

Disabling Comments

By default, the Lab Report allows you to enter comments. To disable comments, follow these steps.

1. Select Tools > Reports.
2. Select the Lab Report tab.
3. Clear the checkbox beside *Enable Comments Section for Lab Report*.
4. Click OK.

To enable comments again, select the checkbox beside *Enable Comments Section for Lab Report*.

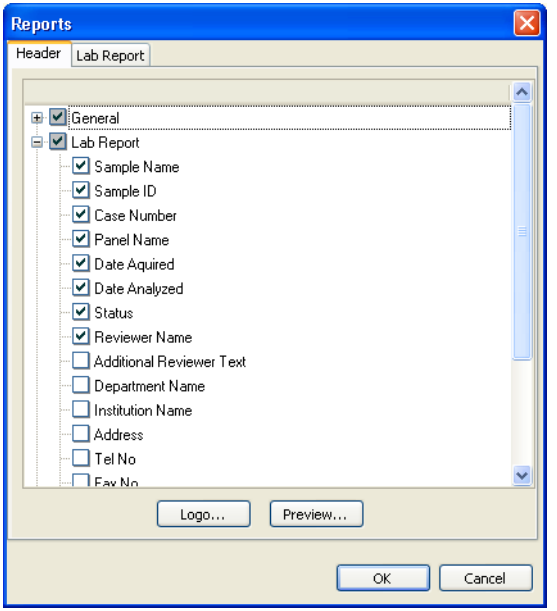
Choosing the Lab Report Language

1. Select Tools > Reports.
2. Click the Lab Report tab.
3. Select a language from the menu.
4. Click OK.

Choosing Header Information

You can specify which header information to include on Lab Reports.

1. Select Tools > Reports.
2. On the Header tab, click the beside Lab Report.



3 Select the header items you want to show on the Lab Report.

Clear the items that you do not want on the Lab Report.

4 Click OK when you have finished.

Automatically Printing the Lab Report

By default, the software does not automatically print Lab Reports. To enable automatic printing, do the following.

1 Select Tools > Lab Manager Preferences.

2 Click 

.

3 Click the Options tab.

4 Select the *Automatically print lab reports* checkbox.

5 Enter the number of copies to print.

You can enter up to 10.

6 Click OK.

Disabling Automatic PDF Creation of Lab Reports

By default, the software automatically creates PDF files of the Lab Reports. To disable automatic PDF creation, do the following:

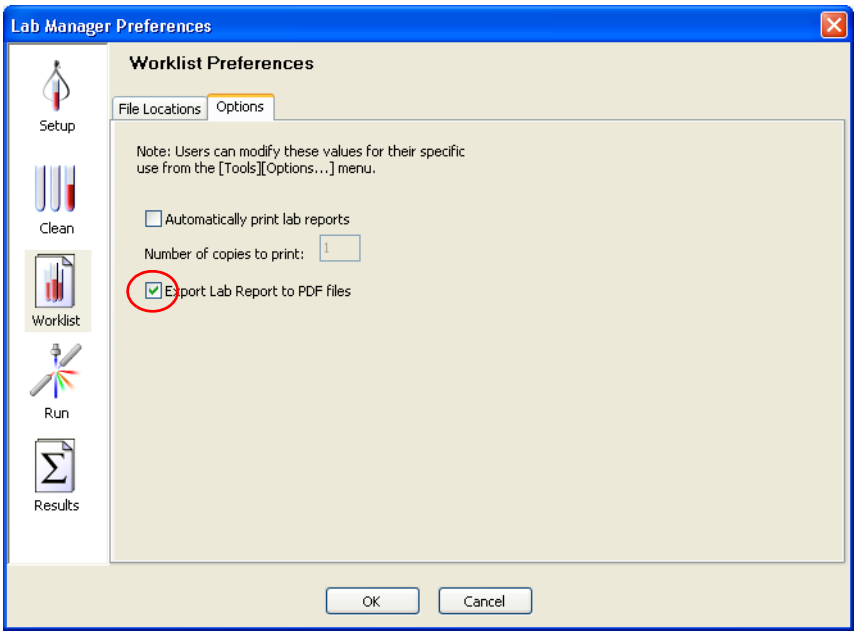
1 Select Tools > Lab Manager Preferences.

2 Click 

.

3 Click the Options tab.

4 Clear the *Export Lab Report to PDF files* checkbox.



5 Click OK.

Other Options

You can make the following changes to these defaults:

- Setting Results Preferences on page 113
- Customizing Header Information for Both Lab and Setup Reports on page 115
- Adding a Logo to Reports on page 116

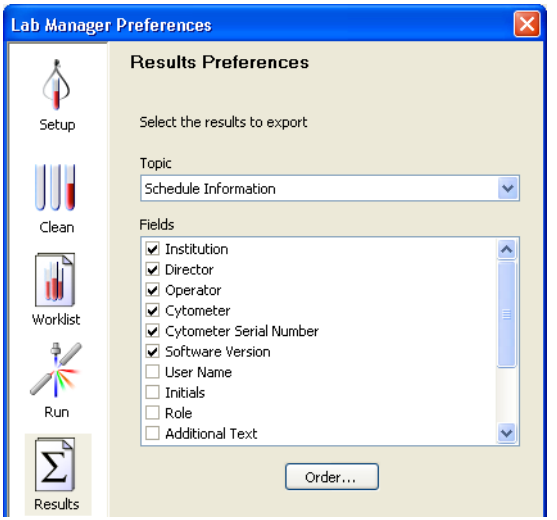
Setting Results Preferences

You can choose which results are exported to the results file that is created daily.

1 Select Tools > Lab Manager Preferences.

2 Click 

.



3 Select a topic from the menu.

4 Select or clear fields associated with the topic.

The information for selected fields will be exported.

The information for cleared fields will not be exported.

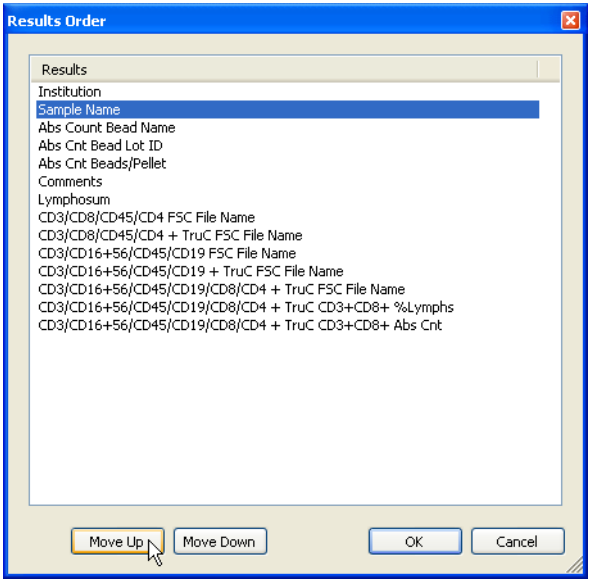
5 Select another topic.

6 Select or clear fields associated with the topic.

7 Continue until all needed topics and fields have been included for export.

8 Select the order for exported information.

- Click Order.
- Select a line of information.



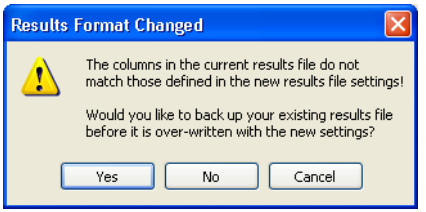
- Click Move Up or Move Down until the information is in the preferred order.
- Repeat as needed.
- Click OK when you are done.

9 Click OK to save the new results for export.

When you change the type of information included in the results file, headers in the new file are different from the previous file.

Because the new format is incompatible with the old one, you need to either back up your previous file and start a new file, or overwrite the previous file with the new one.

10 If prompted, decide whether to back up your existing file.



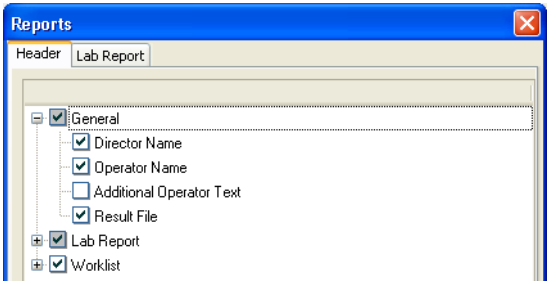
- Click Yes to back up your existing file.
- Click No to overwrite your previous file with the new file.
- Click Cancel to return to the Results Preferences window.

Customizing Header Information for Both Lab and Setup Reports

You can select header information that will apply to both Lab Reports and Setup Reports. You cannot change Levey-Jennings Report headers.

1 Select Tools > Reports.

2 On the Header tab, click the ⊞ beside General.



3 Select the header information you want to show on reports.

Clear the items you do not want on reports.

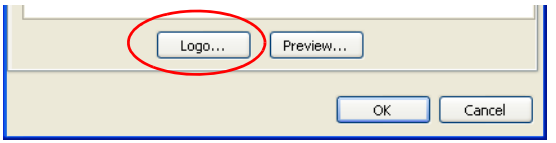
4 Click OK when you have finished.

Adding a Logo to Reports

You can import JPG, BMP, and PNG files to use as logos on reports.

1 Select Tools > Reports.

2 On the Header tab, click Logo.

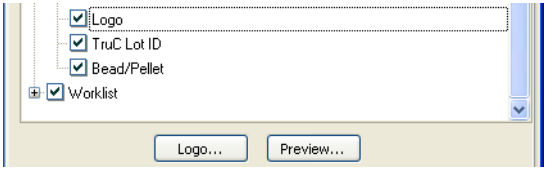


3 Locate a file to import.

4 Click Open.

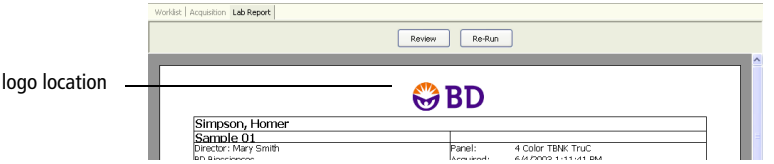
The software scales the image file to fit in the report header.

5 On the Reports Header tab, click the ⊞ next to Lab Report; scroll down and select the checkbox next to Logo.



6 (Optional) Click Preview to view an example of a report header.

7 Click OK to save the new logo to all headers.



6

Troubleshooting

The tips in this section can help you troubleshoot issues that might arise when using this software. For instrument troubleshooting, refer to the reference manual or instructions for your cytometer. You can also find application-specific troubleshooting in the information supplied with reagents and reagent kits.

If you need additional assistance, contact BD Biosciences. Refer to our website, bdbiosciences.com, for up-to-date contact information.

Troubleshooting suggestions can be found under the following topics:

General Software Troubleshooting

Observation or Error Message	Possible Causes	Recommended Solutions
Software not responding	Software frozen	Press Ctrl+Shift-Esc. Locate BD FACSCanto software in the Windows Task Manager; click End Task. ⚠ If acquisition is in progress, data will be lost.
Software not connecting to cytometer, or connection error messages	Multiple	**1** If there are any error messages, follow the directions on screen. **2** Make sure the cytometer power is on. **3** Check the Ethernet cable connection to the cytometer and computer. **4** Verify the fluidics cart is plugged in and the power on. **5** Shut down the software, computer, and cytometer, and then restart them.
Barcode reader errors	Dirty barcode reader window	Clean the barcode reader window with isopropyl or ethyl alcohol and try again.
	Blurred or damaged barcode label	Try scanning with a duplicate label (if available), or enter data manually.

General Software Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Fluidics pressure errors	Kinked tubing	Remove any kinks in tubing to the fluidics cart.
	Detached tubing	Check fluidics connections between the fluidics cart and cytometer. Make sure the waste tank is properly connected to the cart. If this is unsuccessful, call BD Biosciences.
	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 the reference manual for your cytometer.
	Fluidics cart power is switched off or Auxiliary air switch is in the wrong position	Verify the settings of the power switch and auxiliary air switch on the fluidics cart. Refer to the reference manual for your cytometer, if needed.
	Multiple	Follow the directions on the screen.

General Software Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Tube pressurization errors	Cracked tube	• Transfer the sample to new tube. • Make sure you are using appropriate tubes.
	Drop at the top of the tube	Dry the inside of the tube with a cotton swab and re-run.
	Bal seal is improperly installed or worn	Reinstall or replace the Bal seal. Refer to the reference manual for your cytometer for instructions.
	Loader misaligned	**1** Try running in manual mode instead of automatic. Refer to the reference manual for your cytometer for assistance. **2** If unsuccessful, call BD Biosciences.
	Wrong tubes used	Make sure you are using recommended tubes. Refer to the reference manual for your cytometer for a list.
	Tubing to fluidics cart is kinked or disconnected	Reconnect or straighten the tubing. Refer to the reference manual or instructions for use for your cytometer.

General Software Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Tube pressurization errors (continued)	Fluidics cart power is switched off or Auxiliary air switch is in the wrong position	Verify the settings of the power switch and auxiliary air switch on the fluidics cart. Refer to the reference manual for your cytometer, if needed.
	BD FACSCanto flow cytometer only: Adapter lever not in manual mode position	BD FACSCanto flow cytometer only: Change the adapter lever position.
Vacuum error	Kinked tubing	Remove kinks in fluidics cart tubing.
	Vacuum tubing to waste cart is disconnected	Reconnect the waste tank or tubing, and remove kinks. Refer to the reference manual or instructions for use for your cytometer.
	Waste tank is disconnected, waste tubing is disconnected or pinched	Reconnect waste tank or tubing, and remove kinks. Refer to the reference manual or instructions for use for your cytometer.
	Fluidics cart power is switched off	Turn the fluidics cart power on. Refer to the reference manual for your cytometer, if needed.
	Clogged aspirator arm	Call BD Biosciences.
Float or pump error	Air lock in filter	Check the filter in the fluidics cart. Verify that the bottom bleeder valve on the filter is fully tightened. Open the top bleeder valve. If no fluid leaks out, remove the air lock as described in the reference manual for your cytometer.

General Software Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
	Cytometer malfunction	Call BD Biosciences.
BD FACSCanto II flow cytometer only: Tube not present error	Tube is not fully seated	Remove and then reinstall the tube; verify that the tube is fully seated against the top plate.
	Tube is not aligned on the SIT	Remove and then reinstall the tube; verify that the tube is straight and fully seated.
	Cracked tube	Transfer the sample to a new tube.
BD FACSCanto II flow cytometer only: Tube guide not present error	Tube guide is not in place	Verify that the tube guide is in place.
	Tube guide sensor malfunction	Call BD Biosciences.

Setup Troubleshooting

- For help with messages that appear in the setup wizard, see the next section.
- For help with messages that appear on a Setup Report, see Setup Report Failure Messages on page 130.
- For help with preparing the setup beads, refer to the *BD FACS 7-color setup beads* package insert.

Setup Wizard Messages

Messages	Possible Causes	Recommended Solutions
No acquisition events were received from cytometer	Bubbles are in the flow cell	Check the flow cell for bubbles. If found, run the De-gas Flow Cell command. Refer to the reference manual for your cytometer for assistance.
	No setup beads are in the sample tube	Prepare another tube of beads and run setup again.
	Laser shutter is engaged	Make sure the flow cell access door is completely closed.
	Detector failure	**1** Verify that all optical filters and holders are in place within the octagon and trigon. **2** Call BD Biosciences.
	Laser failure	Check the laser power in the Status window. If it is outside the acceptable range, call BD Biosciences.
	Clogged SIT	Clean the flow cell as described in the reference manual for your cytometer. If this doesn't help, call BD Biosciences.
	Internal firmware error	Restart the instrument.

Setup Wizard Messages (continued)

Messages	Possible Causes	Recommended Solutions
Failed to place [*name of beads*] on scale. or Failed to find [*name of beads*]	Bubbles in the flow cell	Check the flow cell for bubbles. If found, run the De-gas Flow Cell command. Refer to the reference manual for your cytometer for assistance.
	Software is using saved settings from a failed setup	Delete the SetupResults.dat file from C:\Program Files\ Common Files\BD\Setup Results, and run the setup again.
	Detector failure	**1** Ensure that all optical filters and holders are in place within the octagon and trigon. **2** Call BD Biosciences.
	Laser failure	Check the laser power status in the Status window. If it is outside the acceptable range, call BD Biosciences.
	Wrong beads used for setup	Use only BD FACS 7-color setup beads.
	Incorrect laser delay	Call BD Biosciences.
No valid data points	Internal setup error	Delete the SetupResults.dat file from C:\Program Files\ Common Files\BD\Setup Results, and run the setup again.

Setup Wizard Messages (continued)

Messages	Possible Causes	Recommended Solutions
A communication error was encountered	Communication problem between hardware and software	**1** Turn on the power. **2** Connect the Ethernet cable to the cytometer and computer. **3** Shut down the software, computer, and cytometer, and restart them.
	Fluidics cart is 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 was aborted	Perform the setup again.
Cytometer setup was aborted because the loader door was opened	Loader cover was removed	Perform setup again, keeping the loader cover closed.
	Communication error	Exit setup and start again. Make sure the Loader door status is *Open* (even if you are not using the Loader).
There is a vacuum error	See Vacuum error on page 123.	See Vacuum error on page 123.
There is a pump error	Disrupted communication between fluidics cart and cytometer	Make sure the communications cable from cytometer to fluidics cart is attached at both ends. Refer to the reference manual or instructions for use for your cytometer.

Setup Wizard Messages (continued)

Messages	Possible Causes	Recommended Solutions
There is a float switch error	Plenum fails to fill with fluid	
	• Sheath tank is not attached to the fluidics cart	Reconnect the sheath cubitainer. Refer to the instructions for use for your cytometer.
	• Sheath tubing from the fluidics cart to the cytometer is not attached or is pinched	Reconnect the sheath tubing. Refer to the reference manual or instructions for use for your cytometer.
	• Sheath filter is not primed	Always select Cytometer > Cleaning Modes > Prime After Tank Refill after changing the sheath cubitainer.
	• Air lock is in the sheath filter	Remove the air lock as described in the reference manual or instructions for use for your cytometer.
	• Air lock is in the bubble filter	Perform a bubble filter purge. Refer to the reference manual or instructions for use for your cytometer.

Setup Report Failure Messages

Failed	Possible Causes	Recommended Solutions
Detector voltage	Wrong target value was used	Make sure the target values in the software match those printed on the setup bead card. If needed, enter new targets. See Entering Lot Information for Setup Beads Manually on page 45.
	Bubbles in the flow cell	Check the flow cell for bubbles. If found, run the De-gas Flow Cell command. Refer to the reference manual for your cytometer for assistance.
	Weak laser	Check the laser power status in the Status window. If it is outside the acceptable range, call BD Biosciences.
	Change in ambient temperature since last setup	Accept the failed setup, then run setup again.
	Change in bead lot	Changing the bead lot might generate what appears to be a detector voltage failure. This could be due to bead lot variation; no failure has occurred. Note for your records that the bead lot was changed. If you accept the failed setup, the next time you run setup, the software will account for the bead lot difference, and no failure should be generated.

Setup Report Failure Messages (continued)

Failed	Possible Causes	Recommended Solutions
Sensitivity	Flow cell is contaminated	Clean the flow cell as described in the instructions for use for your cytometer.
	Optical problem	Make sure all filters are firmly seated in the octagon and trigon.
	Wrong target values	Match the target values in the software to those printed on the reagent box. Re-enter them, if needed.
	Bead tube suspension is too old	Run setup again with a fresh tube of setup beads.
Spectral overlap	Expired bead lot	Run setup again with a fresh tube of setup beads.
	Software is 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.
	Wrong values	Verify the spectral overlap values match those provided with the BD FACS 7-color setup beads being used. Re-enter them, if needed.
	Optical problem	Make sure all filters are firmly seated in the octagon and trigon.
Laser power	Laser failure	Check the laser power status in the Status window. If it is outside the acceptable range, call BD Biosciences.

Setup Report Failure Messages (continued)

Failed	Possible Causes	Recommended Solutions
Sheath pressure	Kinked or clogged sheath line	**1** Remove any kinks in tubing to the fluidics cart. **2** Call BD Biosciences.
	Clogged or airlocked sheath filter	Check the sheath filter. Open the bleeder valves on the filter. If no fluid leaks out, remove the air lock as described in the reference manual for your cytometer.
	Fluidics cart is powered off	**1** Check the power cord to the fluidics cart. Make sure it is plugged in to the cart and to the cytometer. **2** Check the circuit breaker on the fluidics cart. Make sure it is in the on position. **3** Check the fuses. Replace if necessary. Refer to the reference manual for your cytometer.
	Pump failure	Call BD Biosciences.

Levey-Jennings Errors and Messages

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

Acquisition Troubleshooting

Observation	Possible Causes	Recommended Solutions
No events in plots after clicking Run	Laser shutter is engaged	Make sure the flow cell access door is completely closed.
	No sample is in the tube	Add sample to the tube or install a new sample tube.
	Cracked tube	• Transfer the sample to a new tube. • Make sure you are using appropriate tubes.
	Sample was not mixed properly	Make sure you vortex the sample before loading it onto the SIT.
	Threshold is not set to the correct parameter	Set the threshold to the correct parameter for your application.
	Threshold is too low or too high	Adjust the threshold. Refer to the instructions for use for your cytometer.
	Detector voltage is too low	Increase the voltage. Refer to the instructions for use for your cytometer.
	SIT is clogged	**1** Clean the flow cell. Refer to the reference manual for your cytometer. **2** If unsuccessful, call BD Biosciences.
Fewer events than expected in gated population	Events left out of the gate	When changing or moving a gate, make sure events on the axis are included.
	Incorrect plot parameters	Right-click the plot axes and verify that appropriate parameters are displayed.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unexpected events in plot	Incorrect gating	Verify the gating.
	Wrong target value was used	Make sure the target values in the software match those printed on the reagent box. If needed, enter new targets. See Entering Lot Information for Setup Beads Manually on page 45.
Unexpectedly high event rate	Threshold is too low	Increase the threshold. Refer to the instructions for use for your cytometer.
	Sample is too concentrated	Dilute the sample.
	Bubbles are in the flow cell	Check the flow cell for bubbles. If found, run the De-gas Flow Cell command. Refer to the reference manual for your cytometer for assistance.
Unexpectedly low event rate	Sample was not adequately mixed	Vortex the sample before running it on cytometer.
	Threshold is too high	Decrease the threshold. Refer to the instructions for use for your cytometer.
	SIT is clogged	**1** Clean the flow cell. Refer to the reference manual for your cytometer. **2** If unsuccessful, call BD Biosciences.
	Sample aggregates	Prepare a new sample. Filter before staining.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Distorted populations or unexpected pattern in plot	Cytometer settings are adjusted incorrectly	Optimize the scatter parameters.
	Bubbles are in the flow cell	Check the flow cell for bubbles. If found, run the De-gas Flow Cell command. Refer to the reference manual for your cytometer for assistance.
	Flow cell is dirty	Clean the flow cell.
	Poor sample preparation	Do the sample preparation again.
	Wrong panel was selected	Check the panel selection; make sure the plot parameters are appropriate for your assay.
	Incorrect gating	Verify the gating.
	Tubes were run in the wrong order	Re-run the tubes in the correct order.
	SIT was not cleaned between tubes	• BD FACSCanto flow cytometer: Make sure you push the aspirator arm all the way to the left when loading a tube manually. This activates the flush between sample tubes. • BD FACSCanto II flow cytometer: Select Cytometer > Cleaning Modes > SIT Flush to perform an extra SIT Flush. If an extra SIT flush is regularly required, call BD Biosciences.

Acquisition Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Excessive amount of debris in plots	Threshold is too low	Increase the threshold. Refer to the instructions for use for your cytometer.
	Dead cells or debris are in the sample	Examine the sample under a microscope.
	Sample is contaminated	Re-stain the sample, making sure the tube is clean.
	Stained sample is too old	Check the reagent package insert for directions.
	Internal filter failure	Call BD Biosciences.
FCS file not created	PC hard disk is full	Check the available disk space. If the disk is full, do the following: **1** Delete unnecessary files to make room for new FCS files. **2** Run disk utilities on a regular basis to prevent accumulation of unnecessary files or disk corruption.

Analysis Troubleshooting for 4- and 6-Color TBNK

- For help with messages that appear on a Lab Report, see QC Messages.
- For help with visual identification of dot plot problems, see 4- and 6-Color TBNK Troubleshooting on page 143.

You can also refer to the individual application guides for non–4- and 6-color TBNK troubleshooting.

QC Messages

Message	Possible Causes	Recommended Solutions
Lymph gate failure: Gate manually	No usable lymph gate	• Adjust the lymph gate manually. • If necessary, run the sample again.
No beads detected	BD Trucount tubes were not used	Re-run the sample using a BD Trucount tube.
	Wrong panel was chosen	Select a panel that does not rely on BD Trucount tubes.
	Missing bead pellet	Handle BD Trucount tubes according to the package insert. Re-run the sample with a new tube.

QC Messages (continued)

Message	Possible Causes	Recommended Solutions
Sample quality questionable	Donor-specific anomaly	Adjust gates manually to include the required subsets.
	Insufficient mixing during sample preparation: cell populations elongated in the CD3 vs SSC and FITC vs PE plots	Re-stain the sample. Vortex after blood and reagent are added to the tube. Make sure no blood remains on the side of the tube.
	Sample not lysed adequately: cell populations in the CD45 vs SSC plot extend upward	Prepare the sample again, ensuring complete lysis.
	Aged blood and/or stained sample: granulocytes have low side scatter in the CD45 vs SSC plot; no distinct monocyte population is present	Refer to your reagent insert for stability limitations.
	Excessive mixing: debris is encroaching on populations in the CD45 vs SSC plot	Re-stain the sample and run it again.
	Not enough reagent was added during sample preparation: fluorescence parameters are dimmer than expected	Re-stain the sample, following the instructions in the reagent insert.
	Low voltage for SSC detector	Increase the SSC voltage and run the sample(s) again.
	Multiple	Look at the dot plots in 4- and 6-Color TBNK Troubleshooting on page 143. Re-run the sample.
	Sample was not mixed properly	Vortex the sample before loading it onto the SIT.

QC Messages (continued)

Message	Possible Causes	Recommended Solutions
Default [*gate name*] gating used: Visually inspect	Very few events in the indicated gate	Inspect the plot(s) containing the indicated gate and make sure the required events are included. Adjust the gate, if needed.
	Inappropriate instrument settings	Check the instrument settings values. Run setup again, if needed.
Insufficient beads acquired (<500)	Not using BD Trucount tubes	Re-run using BD Trucount tubes.
	Missing bead pellet	Handle BD Trucount tubes according to the package insert. Re-run using a new tube.
	Sample was not mixed properly	Make sure you vortex the sample before loading it onto the SIT.
	Cell concentration is too high	Dilute the sample, re-stain, and run it again.
		Change the acquisition targets in Reagents Tools (lab manager only). See Changing Acquisition Targets on page 99.
	Sample aggregates	Filter the sample.
Less than 2,500 Lymphocytes collected *or* Could not acquire the user-requested number of lymphocytes	Lymphopenic sample	Determine if the number of lymphocytes collected meets your laboratory's criteria. Adjust the acquisition time, if needed (lab managers only). See Changing Acquisition Targets on page 99.
	Excessive debris	Increase the threshold. Refer to the instructions for use for your cytometer.

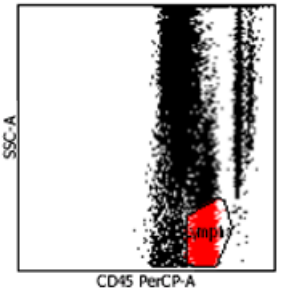
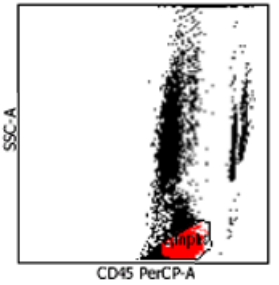
QC Messages (continued)

Message	Possible Causes	Recommended Solutions
Manual gate is in effect	Gates were adjusted manually	Verify that all gates are placed correctly.
% T-sum failure	Large number of double-positive or double-negative T cells	Inspect the gates and include all required events. Adjust the gates manually, if needed.
Lymphosum failure	Staining error	**1** Check the dot plot. **2** Check the reagent package insert and follow the instructions. Review the pipetting technique.
	Events incorrectly classified as T, B, or NK	Inspect the gates and make sure required events are included. Adjust the gates manually, if needed.
CD3% difference failure	Inter-tube value is outside CDC guidelines (see page 157)	Follow your laboratory QC guidelines on how to use this data.
Run as Field Service Engineer	Data was collected under BD Service login name	Log in to the software with the correct login name and run the sample(s) again.
Cytometer settings were generated from a failed setup result	Data was collected with instrument settings from a failed setup	Run the setup, make sure that it passes, and run the sample(s) again.
Sheath pressure low during recording: Visually inspect	Data was collected with low sheath pressure	Inspect the data. If needed, resolve the sheath pressure error and run the sample(s) again. See Fluidics pressure errors on page 121.

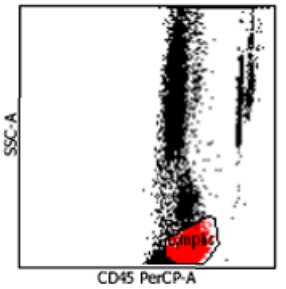
QC Messages (continued)

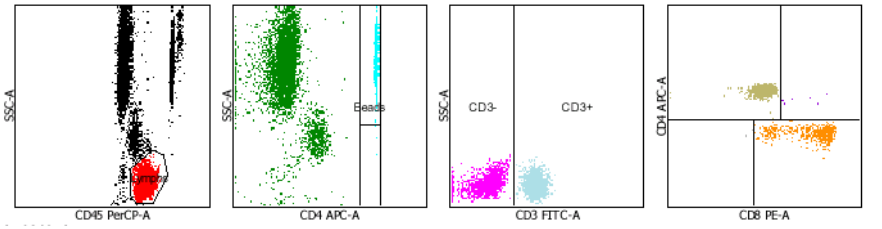
Message	Possible Causes	Recommended Solutions
Laser power low during recording: Visually inspect	Data was collected with low laser power	Inspect the data to see if it meets your laboratory QC guidelines. If the laser power is outside the acceptable range, call BD Biosciences.
One or more results are outside the alarm range	Data is outside the alarm ranges defined by the lab manager	Inspect the data. Adjust the gates manually, if needed. If necessary, adjust the alarm ranges (lab managers only). See Changing Alarm Ranges for Subset Results on page 105.

4- and 6-Color TBNK Troubleshooting

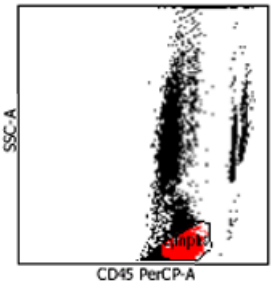
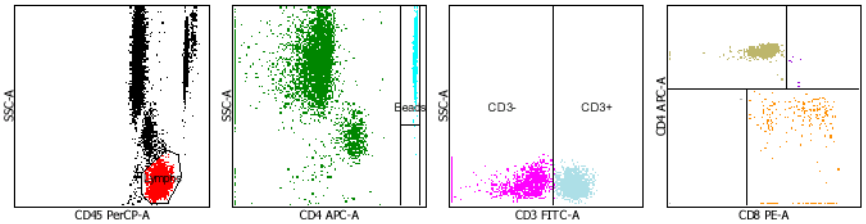
Observation	Possible Causes	Recommended Solutions
Cell populations in CD45 vs SSC extend upward 	Inadequate lysing of sample	Prepare the sample again, and ensure complete lysis.
Granulocytes with low side scatter in CD45 vs SSC plot, no distinct monocyte population 	Aged blood or stained cells	Refer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

4- and 6-Color TBNK Troubleshooting (continued)

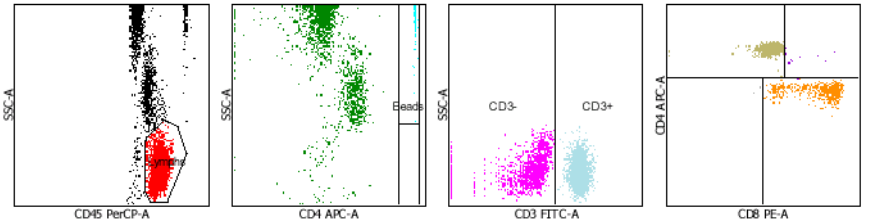
Observation	Possible Causes	Recommended Solutions
Debris encroaching on populations in CD45 vs SSC plot 	• Excessive mixing • Aged blood or stained cells	Prepare the sample again.
Cell populations not captured in gates	Donor-specific anomaly	Use manual gating to include the subsets.
Fluorescence parameters dimmer than expected	Not enough reagent was added to the tube during sample preparation	Refer to the reagent package insert for the staining procedure.
	Incorrect setup	Run cytometer setup again; run a process control.
	Laser failure	Check the laser power status in the Status window. If it is outside the acceptable range, call BD Biosciences.



4- and 6-Color TBNK Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Vertically compressed populations	Side scatter is too low	Reacquire the sample. Set SSC so that granulocytes reach to top of the CD45 vs SSC plot.
	Lipemic sample	Check the reagent package insert for instructions.
		
Indistinct populations; events sparse or missing from one population; lack of separation between $CD3^-$ and $CD3^+$ cluster	Incorrect spectral overlap	Re-run setup, optimizing for the application. Re-run the sample.
		
Granulocytes cut off at top of plot; stretched monocyte population	High SSC	Re-run setup, optimizing for the application. Re-run the sample, lowering the SSC.

4- and 6-Color TBNK Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
		

Disabling the Loader

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

1. Select Tools > Options.
2. Click 

.
3. Select the Ignore Loader checkbox in the Run Options dialog.
4. Click OK.

Appendix A

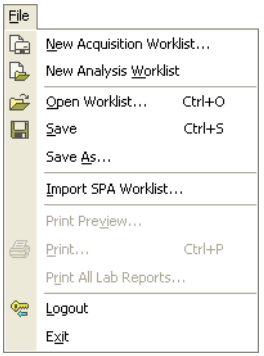
Menus and Keyboard Shortcuts

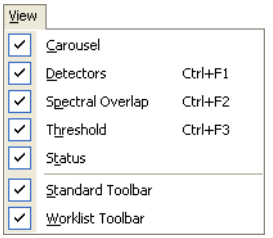
This appendix provides a visual map of all BD FACSCanto software menus and a list of the available keyboard shortcuts.

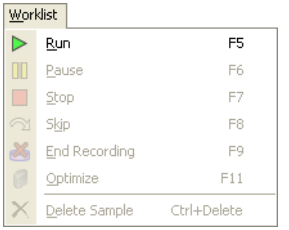
Menus

Application Menus

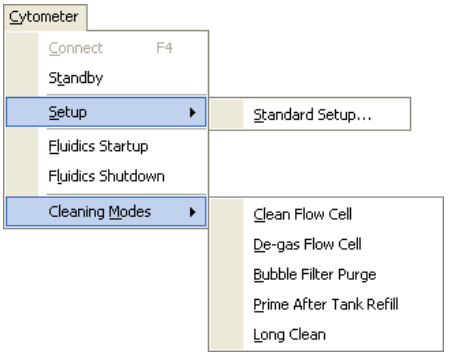
The Cytometer > Cleaning Modes menu differs depending on whether the software is connected to the BD FACSCanto or the BD FACSCanto II flow cytometer.



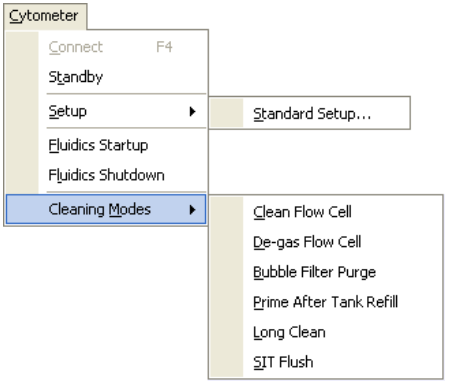




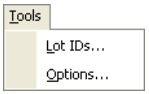
Cytometer menu, BD FACSCanto flow cytometer



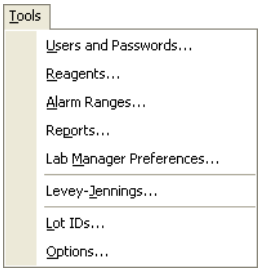
Cytometer menu, BD FACSCanto II flow cytometer



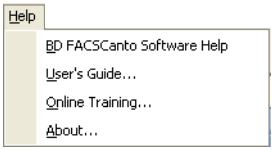
Tools menu, all users



Tools menu, lab managers



Help menu

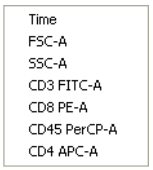


Contextual Menus

Activate contextual menus by right-clicking on the indicated software components.

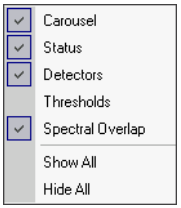
Plot Parameters

Right-click on x or y parameters of dot plots.



Hide/Show Windows

Right-click on title bars of Carousel, Status, Detectors, Thresholds, and Spectral Overlap windows.



Sort Worklist

Right-click on worklist field headers.

Keyboard Shortcuts

Keyboard shortcuts are provided for the following functions.

Function	Key Combination	When to Use Shortcut
New Acquisition Worklist	Ctrl+N	When no worklist is running
Open Worklist	Ctrl+O	When no worklist is running
Save Worklist	Ctrl+S	When worklist is stopped or completed
Print	Ctrl+P	Anytime except when Lab Report is in view
Hide/Show Detectors	Ctrl+1	Anytime
Hide/Show Spectral Overlap	Ctrl+F2	Anytime
Hide/Show Thresholds	Ctrl+F3	Anytime
Delete Sample	Ctrl+Delete	When sample is selected in worklist
Cut	Ctrl+X	When item is selected; works in most text-edit fields
Copy	Ctrl+C	When item is selected; works in most text-edit fields
Paste	Ctrl+V	When item is selected; works in most text-edit fields

Menu Command Shortcuts

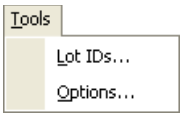
To select menu commands using the keyboard, you must first open a menu using these key combinations:

Open File Menu	Alt+F
Open View Menu	Alt+V
Open Worklist Menu	Alt+W
Open Cytometer menu	Alt+C
Open Tools menu	Alt+T
Open Help menu	Alt+H

Then, you can select a command using its designated keyboard shortcut (indicated by an underline).

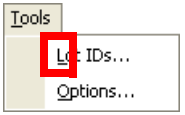
For example, to select the Lot IDs command from the Tools menu, follow these steps.

1. Press Alt+T to open the Tools menu.



2. Press L to open Lot IDs.

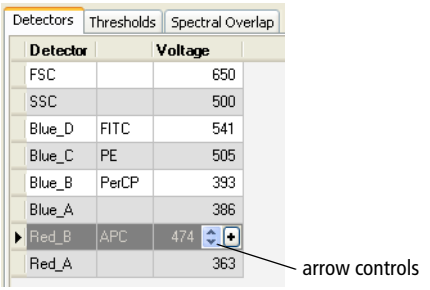
   The underline beneath the L indicates the keyboard shortcut.



   (To access the Options dialog, you would press O.)

Instrument Control Window Shortcuts

Use the following keys to increase or decrease values for the Detectors, Thresholds, and Spectral Overlap windows.



Function	Key Combination	When to Use Shortcut
Large increase or decrease in value	Ctrl+Page Up Ctrl+Page Down	When row is selected
Small increase or decrease in value	Ctrl+up arrow Ctrl+down arrow	When row is selected

Function Keys

Use the function keys on the keyboard to operate the flow cytometer.

Function	Function Key	When to Use Shortcut
Connect to cytometer	F4	Software is disconnected from the cytometer or the cytometer is in Standby
 Run	F5	For an acquisition worklist, the laser must be warmed-up; a prepared worklist has entries in all required fields
 Pause	F6	Acquisition or Analysis worklist is in progress
 Stop	F7	Acquisition or Analysis worklist is in progress and paused
 Skip	F8	Acquisition worklist is in progress and paused
 End Recording	F9	Acquisition worklist is in progress
 Optimize	F11	Acquisition worklist is in progress and paused
 Add Data Files	F12	Analysis worklist is open

Appendix B

Technical Overview for BD Multitest 4- and 6-Color Reagents

This appendix covers these topics:

Panels and Reagents

BD Biosciences provides seven panels. You cannot edit or delete these panels. The number of sample tubes and the reagents associated with the tubes for each panel appear in the columns to the right.

Panel	Tubes	Reagents
4 Color TBNK	1	CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC
	2	CD3 FITC/CD16+56 PE/CD45 PerCP/CD19 APC
4 Color TBNK + TruC	1	CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC + BD Trucount tube
	2	CD3 FITC/CD16+56 PE/CD45 PerCP/CD19 APC + BD Trucount tube
3/16+56/45/19	1	CD3 FITC/CD16+56 PE/CD45 PerCP/CD19 APC
3/16+56/45/19 + TruC	1	CD3 FITC/CD16+56 PE/CD45 PerCP/CD19 APC + BD Trucount tube
3/8/45/4	1	CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC
3/8/45/4 + TruC	1	CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC + BD Trucount tube
6 Color TBNK + TruC	1	CD3 FITC/CD16+56 PE/CD45 PerCP-Cy5.5/CD4 PE-Cy7/ CD19 APC/CD8 APC-Cy7 + BD Trucount tube

Acquisition Stopping Criteria

The following acquisition stopping criteria are used by BD FACSCanto clinical software for reagents for which the expert lymph gate is applied in CD45 vs SSC.

The software collects 10,000 total events, applies the expert lymph gate, and calculates the number of lymphocytes in the gate. The software continues acquiring until the user-specified number of lymphocytes is reached. If fewer than the CDC-recommended number of lymphocytes are obtained (at least 2,500*), a QC Message appears on the Lab Report.

BD FACSCanto clinical software always reports a subset value regardless of whether the acquisition criteria were met.

NOTICE Once the software finishes acquiring a sample, it recalculates the expert lymph gate and reapplies it. The gate boundaries might change slightly, sometimes resulting in fewer than 2,500 lymphocytes being reported on the Lab Report.

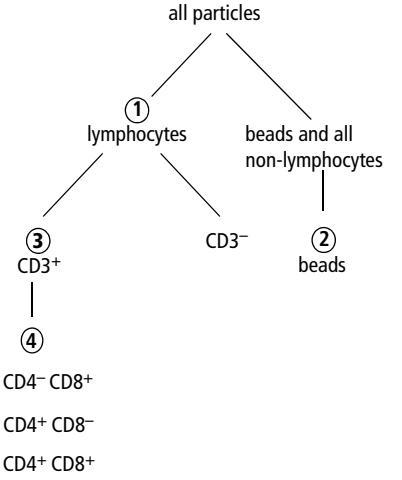
* Mandy FF, Nicholson JK, McDougal JS. Guidelines for performing single-platform absolute $CD4^+$ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 2003;52(RR-2):1-13.

Gate Hierarchy

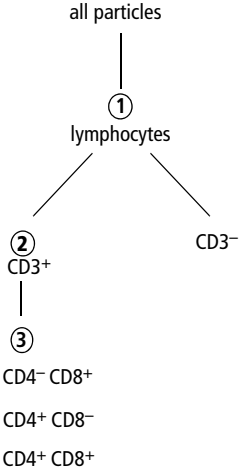
The following information describes the subset order and plots BD FACSCanto clinical software uses when analyzing samples stained with BD Multitest 4- and 6-color reagents.

BD Multitest 4-Color Reagents

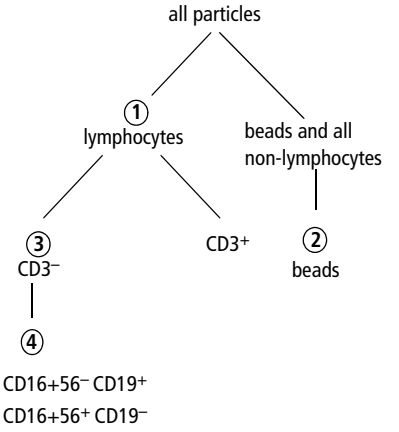
3/8/45/4 + TruC

Plot	Population(s) of Interest	Visual Representation of Hierarchy
1 CD45 vs SSC	Lymphocytes	
2 CD4 vs SSC	BD Trucount beads	
3 CD3 vs SSC	$CD3^+$	
4 CD8 vs CD4	$CD4^-CD8^+$	
	$CD4^+CD8^-$	
	$CD4^+CD8^+$	

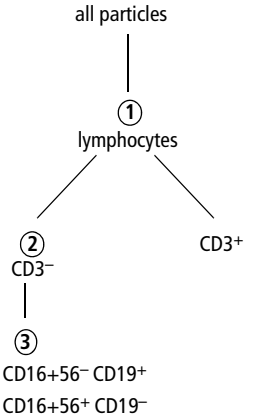
3/8/45/4

Plot	Population(s) of Interest	Visual Representation of Hierarchy
1 CD45 vs SSC	Lymphocytes	
2 CD3 vs SSC	$CD3^+$	
3 CD8 vs CD4	$CD4^-CD8^+$	
	$CD4^+CD8^-$	
	$CD4^+CD8^+$	

3/16+56/45/19 + TruC

Plot	Population(s) of Interest	Visual Representation of Hierarchy
1 CD45 vs SSC	Lymphocytes	
2 CD19 vs SSC	BD Trucount beads	
3 CD3 vs SSC	$CD3^-$ cells	
4 CD16+56 vs CD19	$CD16+56^- CD19^+$ cells	
	$CD16+56^+ CD19^-$ cells	

3/16+56/45/19

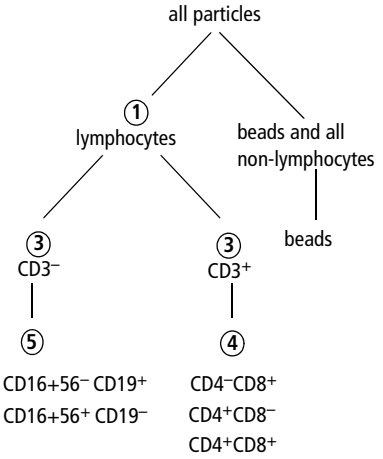
Plot	Population(s) of Interest	Visual Representation of Hierarchy
1 CD45 vs SSC	Lymphocytes	
2 CD3 vs SSC	$CD3^-$ cells	
3 CD16+56 vs CD19	$CD16+56^- CD19^+$ cells	
	$CD16+56^+ CD19^-$ cells	

BD Multitest 6-Color Reagent

3/16+56/45/4/19/8 + TruC

Plot	Population(s) of Interest
1 CD45 vs SSC	Lymphocytes
2 CD19 vs SSC	BD Trucount beads
3 CD3 vs SSC	$CD3^-$ cells $CD3^+$ cells
4 CD4 vs CD8	$CD3^+$ cell subsets: $CD4^-CD8^+$ cells $CD4^+CD8^-$ cells $CD4^+CD8^+$ cells
5 CD16+56 vs CD19	$CD3^-$ cell subsets $(CD16+CD56)^-CD19^+$ cells $(CD16+CD56)^+CD19^-$ cells

Visual Representation of Hierarchy



Visual Check for BD Multitest Reagents

The following is a step-by-step guide for visually inspecting plots to determine the integrity of BD Multitest 4-color stained samples. Not all plots will be present for every reagent.

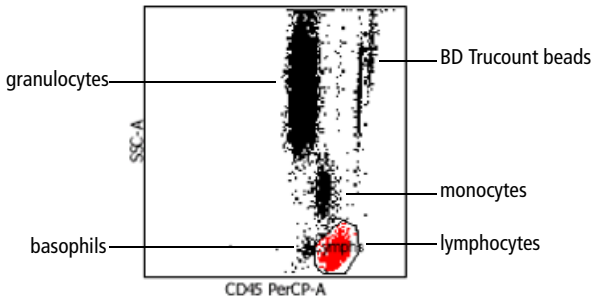
⚠ Lipemic samples are problematic. You must visually inspect the data.

1 Inspect the CD45 vs SSC plot (Figure B-1).

For all reagents, an acceptable CD45 vs SSC plot displays:

- Distinct lymphocyte, monocyte, granulocyte clusters
- BD Trucount beads (if a BD Trucount tube was used)
- A compact lymphocyte cluster with low SSC
- A possible basophil cluster
- Threshold set to ensure a visible valley between debris and the $CD45^+$ cluster

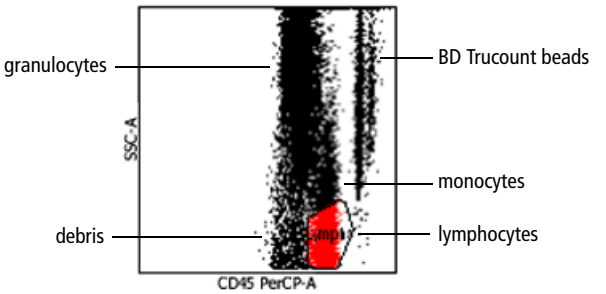
Figure B-1 Acceptable CD45 vs SSC plot



A questionable CD45 vs SSC plot *might* display the following (see Figure B-2):

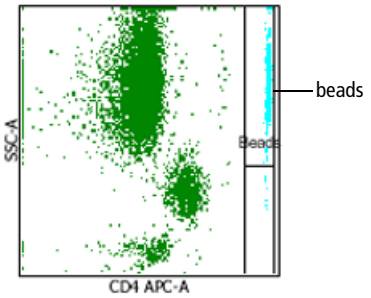
- Dispersed lymphocyte, granulocyte, or monocyte cluster
- Lymphocyte cluster merged with debris or monocytes
- Granulocyte cluster losing SSC
- No visible monocyte cluster
- No discernible clusters other than the lymphocyte cluster
- Change in the relative position of the monocyte cluster to the lymphocyte cluster

Figure B-2 Questionable CD45 vs SSC plot



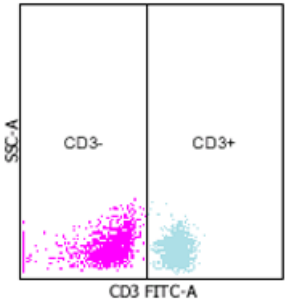
2 Inspect the CD4 vs SSC plot.

For reagents using BD Trucount tubes, the CD4 vs SSC plot displays a distinct bead cluster.



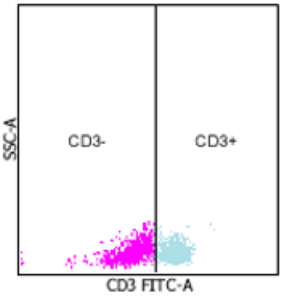
3 Inspect the CD3 vs SSC plot.

An acceptable CD3 vs SSC plot displays distinct cell clusters.



A questionable CD3 vs SSC plot *might* display any of the following:

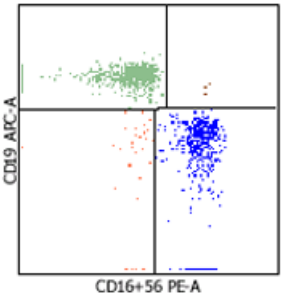
- Dispersed cell clusters
- Merged clusters
- Lack of distinction between $CD3^-$ and $CD3^+$ cluster



If you are still uncertain about the sample integrity, take corrective action such as restaining or redrawing the sample.

4 Inspect the CD16+56 vs CD19 plot, if present.

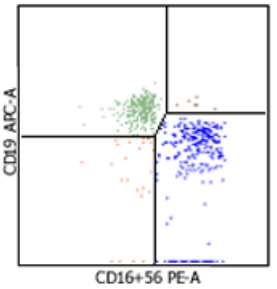
An acceptable CD16+56 vs CD19 plot displays distinct cell clusters.



A questionable CD16+56 vs CD19 plot *might* display any of the following:

- Dispersed cell clusters

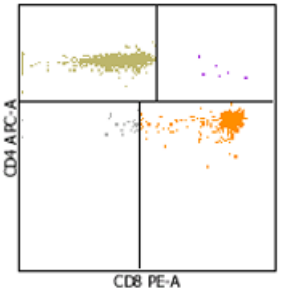
- Merged clusters

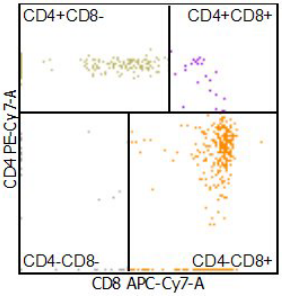


If you are still uncertain about the sample integrity, take corrective action such as restaining or redrawing the sample.

5 Inspect the CD8 vs CD4 plot(s), if present.

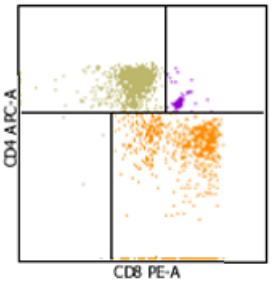
An acceptable CD8 vs CD4 plot displays distinct cell clusters.





A questionable CD8 vs CD4 plot *might* display any of the following:

- Dispersed cell clusters
- Merged clusters



If you are still uncertain about the sample integrity, take corrective action such as restaining or redrawing the sample.

Default Settings

The software contains the following default values for BD Multitest 4-color and 6-color reagents. Refer to application-specific guides for software defaults specific to those assays.

Options Defaults

Preference	Menu Item	Value
Setup	Automatically print Setup Report	Not selected

Preference	Menu Item	Value
Files	File Locations	
	• FCS Files	• C:\BDFACSCantoFCSFiles*yyyy**Month**dd*[a]
	• Worklist Files	• C:\Program Files\BD FACSCanto Software\worklists
	• Setup Report Files	• C:\Program Files\BD FACSCanto Software\Setup Reports
	• Result Files	• C:\Program Files\BD FACSCanto Software\DataFiles*yyyy**Month**dd*
Lab Report	Lab Report Countdown	10 seconds
	Automatically print Lab Report after each sample	Not selected
	Number of copies to print for Lab Report	—

a. Where *yyyy* represents a four-digit year (eg, 2007) and *dd* represents a two-digit day (eg, 02).

Reagent Defaults

Only lab managers can change the following preferences.

4-Color Multitest

Reagent				
CD3/CD8/ CD45/CD4	Acquisition Plots (left to right on Lab Report)	# Plots	3	
		Plot 1	X axis	CD45 PerCP-A
			Y axis	SSC-A
		Plot 2	X axis	CD3 FITC-A
			Y axis	SSC-A
		Plot 3	X axis	CD8 PE-A
			Y axis	CD4 APC-A
	Subset Results	Include	All selected	
	Acquisition Targets	Min Lymphs to acquire	2,500	
		Max time to acquire (sec)	300	

4-Color Multitest (continued)

Reagent				
CD3/CD16+56/ CD45/CD19	Acquisition Plots (left to right on Lab Report)	# Plots	3	
		Plot 1	X axis	CD45 PerCP-A
			Y axis	SSC-A
		Plot 2	X axis	CD3 FITC-A
			Y axis	SSC-A
		Plot 3	X axis	CD16+56 PE-A
			Y axis	CD19 APC-A
	Subset Results	Include	All selected	
	Acquisition Targets	Min Lymphs to acquire	2,500	
		Max time to acquire (sec)	300	

4-Color Multitest TruC

Reagent				
CD3/CD8/ CD45/CD4 TruC	Acquisition Plots (left to right on Lab Report)	# Plots	4	
		Plot 1	X axis	CD45 PerCP-A
			Y axis	SSC-A
		Plot 2	X axis	CD4 APC-A
			Y axis	SSC-A
		Plot 3	X axis	CD3 FITC-A
			Y axis	SSC-A
		Plot 4	X axis	CD8 PE-A
			Y axis	CD4 APC-A
	Subset Results	Include	All selected	
	Acquisition Targets	Min Lymphs to acquire	2,500	
		Max time to acquire (sec)	300	

4-Color Multitest TruC (continued)

Reagent				
CD3/CD16+56/ CD45/CD19 TruC	Acquisition Plots (left to right on Lab Report)	# Plots	4	
		Plot 1	X axis	CD45 PerCP-A
			Y axis	SSC-A
		Plot 2	X axis	CD19 APC-A
			Y axis	SSC-A
		Plot 3	X axis	CD3 FITC-A
			Y axis	SSC-A
		Plot 4	X axis	CD16+56 PE-A
			Y axis	CD19 APC-A
	Subset Results	Include	All selected	
	Acquisition Targets	Min Lymphs to acquire	2,500	
		Max time to acquire (sec)	300	

6-Color Multitest TruC

Reagent				
CD3/CD16+56/ CD45/CD4/ CD19/CD8/ TruC	Acquisition Plots (left to right on Lab Report)	# Plots	5	
		Plot 1	X axis	CD45 PerCP-Cy5.5-A
			Y axis	SSC-A
		Plot 2	X axis	CD19 APC-A
			Y axis	SSC-A
		Plot 3	X axis	CD3 FITC-A
			Y axis	SSC-A
		Plot 4	X axis	CD8 APC-Cy7-A
			Y axis	CD4 PE-Cy7-A
		Plot 5	X axis	CD16+56 PE-A
			Y axis	CD19 APC-A
	Subset Results	Include	All selected	
	Acquisition Targets	Min Lymphs to acquire	2,500	
		Max time to acquire (sec)	300	

Alarm Ranges Defaults

Only lab managers can change the following preferences.

Subset	Min	Max
$CD3^+$ %Lymphs	0	100
$CD3^+$ Abs Cnt	0	999999
$CD3^+CD8^+$ %Lymphs	0	100
$CD3^+CD8^+$ Abs Cnt	0	999999
$CD3^+CD4^+$ %Lymphs	0	100
$CD3^+CD4^+$ Abs Cnt	0	999999
$CD16+56^+$ %Lymphs	0	100
$CD16+56^+$ Abs Cnt	0	999999
$CD19^+$ %Lymphs	0	100
$CD19^+$ Abs Cnt	0	999999
4/8 Ratio	0.0	99999999.9

Report Defaults

By default, reports include the following. Only lab managers can make changes to these options.

Report Option	Default Content
General header information	Director Name Operator Name Name of Result File
Lab Report header information	Sample Name Sample ID Case Number Panel Name Date Acquired Date Analyzed Status Reviewer Name
Worklist header information	Software Name Date Time Cytometer Name and Serial Number
QC values to show on Lab Report:	
• 4 Color TBNK (+ TruC)	• CD3% difference, % T-Sum, Lymphosum, 4/8 ratio
• 3/16+56/45/19 (+ TruC)	• Lymphosum
• 3/8/45/4 (+ TruC)	• CD3% difference, 4/8 ratio
• 6 Color TBNK (+ TruC)	• % T-Sum, Lymphosum, 4/8 ratio
Display Error Message on Lab Report	Selected

Report Option	Default Content
Enable Comments Section for Lab Report	Selected
Language	English

Lab Manager Preferences Defaults

Only lab managers can change the following preferences.

Preference	Menu Item	Value
Setup	Number of entries in LJ file	30
	Review field on Setup Report	Selected
	Automatically print Setup Report	Not selected
Clean	Fluidics Startup	Never
Worklist	File Locations tab	
	• FCS Files	• D:\BDFACSCantoFCSFiles*yyyy**Month**dd*[a]
	• Worklist Files	• C:\Program Files\BD FACSCanto Software\Worklists
	• Setup Report Files	• C:\Program Files\BD FACSCanto Software\SetupReports
	• Result Files	• C:\Program Files\BD FACSCanto Software\DataFiles*yyyy**Month**dd*
	• Lab Report files	• C:\Program Files\BD FACSCanto Software\LabReportFiles
	Options tab	
	• Automatically print Lab Reports	• Not selected
	• Number of copies to print	• —
	• Export Lab Reports to PDF files	• Selected
Run	Lag time before recording (sec)	10 seconds
	Default Lab Report countdown	On, time to display countdown (sec): 10

Preference	Menu Item	Value
Results	Schedule Information	The following values are selected: • Institution • Director • Operator • Cytometer • Cytometer Serial Number • Software Version
	Sample Information	The following values are selected: • Sample Name • Sample ID • Case Number • Panel Name • Collection Date • Date Analyzed • Abs Cnt Bead Name • Abs Cnt Bead Lot ID • Abs Cnt Beads/Pellet • Comments
	Cross Tube Results	All values are selected.
	CD3/CD8/CD45/CD4	All values are selected.
	CD3/CD8/CD45/CD4 TruC	All values are selected.
	CD3/CD16+56/CD45/CD19	All values are selected.
	CD3/CD16+56/CD45/CD19 TruC	All values are selected.
	CD3/CD16+56/CD45/CD4/ CD19/CD8 TruC	All values are selected.

a. Where *yyyy* represents a four-digit year (eg, 2007) and *dd* represents a two-digit day (eg, 02).

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