BD Cytometer Setup and Tracking Application Guide

for BD FACS Digital Flow Cytometers

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Patents

PerCP: US 4,876,190

BD Trucount tubes: US 5,723,218 and 5,187,288

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
642342 Rev. A	6/07	Initial release

Contents

About This Guide

This manual describes how to use the Cytometer Setup and Tracking features in BD FACSDiva™ software. Before using this manual, make sure you are familiar with general cytometer functions, as described in the cytometer manual.

The *BD Cytometer Setup and Tracking Application Guide* assumes that 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.

Documentation

The following online documentation is available to help you learn how to use the cytometer setup and tracking features.

- The *BD Cytometer Setup and Tracking Application Guide* is available as a PDF that can be opened, searched, and printed using Adobe® Acrobat® Reader. To access the PDF, choose Start > Programs > BD FACSDiva software.

- The online help system contains the BD FACSDiva Software Reference Manual, the Cytometer Setup and Tracking Application Guide, and your cytometer manual. The online help contains all information from the manuals, enhanced with full text search to make it easier to find what you are looking for. The online help opens in a separate window so you can access the documentation while working in the software. To access the online help system, choose Help > Online Help within BD FACSDiva software.

- For online customer training on the Cytometer Setup and Tracking features, choose Help > Online Training.

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[a]

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

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

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 solving a problem:

- In BD FACSDiva software, choose Help > Online Help. Locate and read topics specific to the operation you are performing.
- In BD FACSDiva software, choose Help > Online Training.
- Choose Start > Programs > BD FACSDiva software to access the Cytometer Setup and Tracking PDF and look in the Troubleshooting section.

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

When contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number; software version and computer system specifications
- Any error messages
- Details of recent cytometer performance
- Log file for Cytometer Setup and Tracking (do not alter anything in the Log file)

BD Biosciences might also request the console.log and LogFile.xml files located in C:\Program Files\BD FACSDiva Software\CST\log, as well as your exported experiment file.

For cytometer support from within the US, call (877) 232-8995.

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

Customers outside the US and Canada should contact their local BD representative or distributor.

Limitations

- The Cytometer Setup and Tracking module and BD Cytometer Setup and Tracking beads are for Research Use Only (RUO). Not for use in diagnostic or therapeutic procedures.

- Cytometer Setup and Tracking does not support cytometers with neutral density filters installed that are 2.0 or greater. BD recommends using a neutral density filter of 1.0.

- The amount of time required to complete a baseline definition or cytometer performance check is dependent on the cytometer configuration, fluidics, and performance.

- To normalize a new lot of Cytometer Setup and Tracking beads to an existing lot, you must run Reset Target Values using the existing lot and the new lot. Verify that you reserve a large enough volume of the existing lot of Cytometer Setup and Tracking beads to run Reset Target Values.

- When Cytometer Setup and Tracking beads are manufactured, a lot-specific file is created that contains bead lot information. This bead lot information is used to characterize your cytometer and to normalize one bead lot to another when switching bead lots. Qr and Br values generated while defining baseline are calculated using the bead lot information in the lot-specific file. When generated on BD cytometers with standard configurations (lasers, mirrors, and filters), the Qr and Br values can be used for comparison to other parameters and for tracking. Refer to the appropriate cytometer reference manual for information on standard configurations.

BD recommends using the Qr and Br values generated with non-standard configurations for tracking only. Do not use the Qr and Br values for comparison to other parameters.

- You can import configurations only from the same cytometer model.

- You cannot import a configuration into the Cytometer Configuration window that has the same name as an existing configuration. To import the configuration, you must first rename it.

- Before you export a configuration, copy and rename it.

- Do not start the Cytometer Setup and Tracking module until BD FACSDiva software finishes starting. If you do, a message indicating the cytometer is not connected appears when you select the Set Configuration button in the Cytometer Configuration window.

- Cytometer settings from the catalog or an experiment cannot be shared if they are associated with different configurations. If you use cytometer settings in an experiment that are associated with a different configuration, the following functions are blocked and an error message appears: 1) Copy/paste tube, specimen, or plate with cytometer settings, 2) Copy/paste cytometer settings, 3) Apply cytometer settings from the catalog, and 4) Import cytometer settings.

- Experiment functionality varies depending on the experiment template it was created from.

    - New experiments created from the Blank Experiment or Blank Experiment with Tube template have the following functionality:

        - Current CST settings are automatically applied

        - Application Settings menu is accessible

    - New experiments without specimen- or tube-level cytometer settings that are created from any other experiment template have the following functionality:

        - Current CST settings are not automatically applied

        - The Application Settings menu is not accessible until you right-click the experiment level cytometer settings and choose Apply Current CST Settings.

1

Introduction

The following topics are covered in this chapter:

Overview

The Cytometer Setup and Tracking features in BD FACSDiva v6.0 software, when used with BD Cytometer Setup and Tracking beads, allow you to reproducibly set up the BD FACSCanto™, BD FACSAria™, and BD™ LSR II flow cytometer platforms. With Cytometer Setup and Tracking you can:

- Define cytometer baseline performance
- Track cytometer performance
- Establish and automatically update application-specific settings

How it Works

The Cytometer Setup and Tracking features in BD FACSDiva software are used to define the baseline performance of your cytometer. You perform setup for each cytometer configuration by running BD Cytometer Setup and Tracking beads. Median fluorescence intensity (MFI) and percent robust CV (%rCV) are measured for each bead intensity in all fluorescence detectors. Software algorithms differentiate the fluorescence signal from each bead type based on size and fluorescence intensity in each detector. Linearity, detector efficiency (Qr), optical background (Br), electronic noise, and laser delays are all evaluated. PMT voltages are then adjusted to maximize population resolution in each detector.

Once baseline measurements are defined, the beads are used to run performance checks to measure variation from those baseline measurements. Laser delays, area scaling factors, and PMT voltages are adjusted. User-defined application settings are updated to the new performance check values.

Baseline and Performance Check reports are automatically created and contain all performance measurements determined by the Cytometer Setup and Tracking process. Performance check values are plotted on Levey-Jennings charts, allowing you to track cytometer performance and spot trends.

Benefits

The Cytometer Setup and Tracking features generate consistent settings for your experiments. Using Cytometer Setup and Tracking provides you with better resolution of dim populations, fewer compensation artifacts, and reproducible data. By tracking your cytometer's performance, you can monitor performance trends. The flexible features allow you to use any number of lasers and detectors, create custom configurations using pre-defined or custom parameters, filters, and mirrors, and create application-specific settings for all of your experiments.

Before Getting Started

Before you can use the Cytometer Setup and Tracking features, a user with administrator privileges (usually the lab manager or supervisor) must perform some initial setup tasks. See Administrative Tasks on page 29 for instructions on performing initial setup of the software. It is best if your lab has only one administrator who sets up and maintains all user accounts. Refer to the *BD FACSDiva Software Reference Manual* for information on administering accounts. See the next section for an overview of the Cytometer Setup and Tracking workflow.

Cytometer Setup and Tracking Workflow Overview

The following table shows tasks that can be performed with the Cytometer Setup and Tracking features and page references to task instructions. Tasks are separated into those performed by the administrator and those performed by all users.

All Users	
1	Start BD FACSDiva software v6.0.
2	Choose Cytometer > CST.
Administrator (As Needed)	
1	Import bead lot information. See page 30.
2	Create custom configurations. See page 40.
3	Define baseline values for configurations. See page 48.
4	Reset target values for new bead lots. See page 65.
All Users (Daily or per Experiment)	
1	Check configuration. If appropriate, go to step 3; if not, go to step 2.
2	Select a configuration. See page 45.
3	Check baseline status. If defined, go to step 5; if not, go to step 4.
4	Ask administrator to define baseline. See page 49.
5	Check performance status. If not expired, go to step 7; if expired, go to step 6.
6	Run a performance check. See page 91.
7	Exit Cytometer Setup and Tracking.
8	In the BD FACSDiva workspace, do one of the following to an existing experiment: • Create or apply application settings (page 98). • Apply current CST settings (page 104).

2

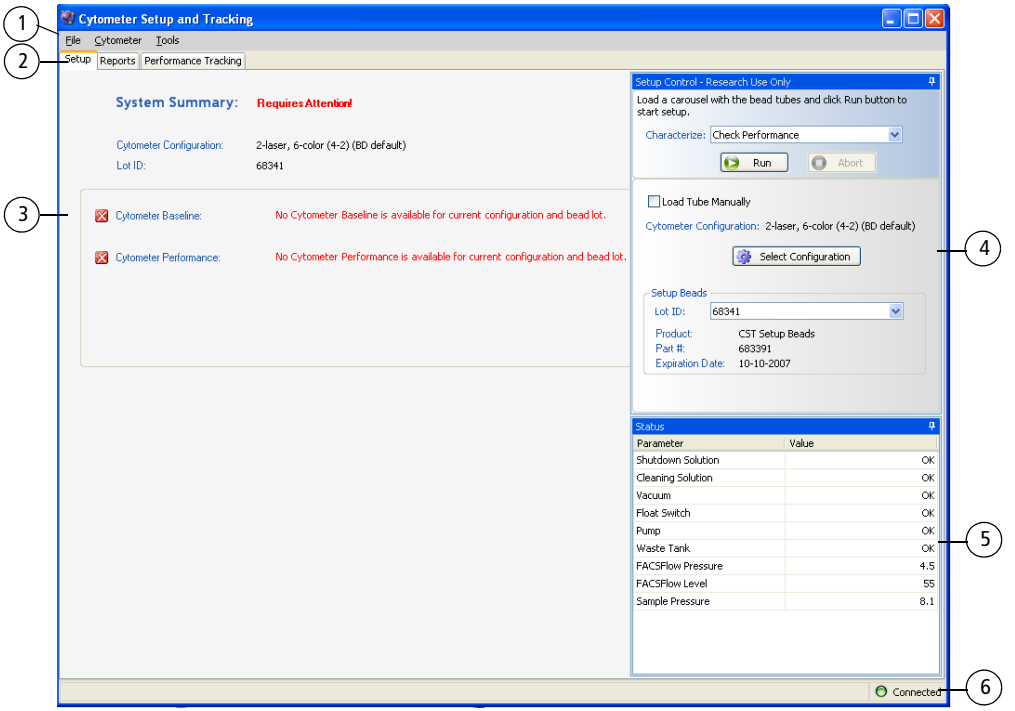
Software Windows and Toolbars

The following topics are covered in this chapter:

Cytometer Setup and Tracking Workspace

From BD FACSDiva software, access the cytometer setup and tracking features by choosing Cytometer > CST. The Cytometer Setup and Tracking workspace appears. Access most Cytometer Setup and Tracking functions from either the menu bar or the workspace tabs.

Figure 2-1 Cytometer Setup and Tracking workspace (when first opened)



1	Menu Bar—See page 19.	4	Setup Control—See page 25.
2	Workspace Tabs—See page 19.	5	Status—See page 26.
3	System Summary—See page 24.	6	Status Bar—See the following Notice and page 27.

NOTICE When you choose Cytometer > CST, the cytometer disconnects from the BD FACSDiva interface and connects to the Cytometer Setup and Tracking interface. The following message appears on the BD FACSDiva status bar at the bottom left corner of the screen 

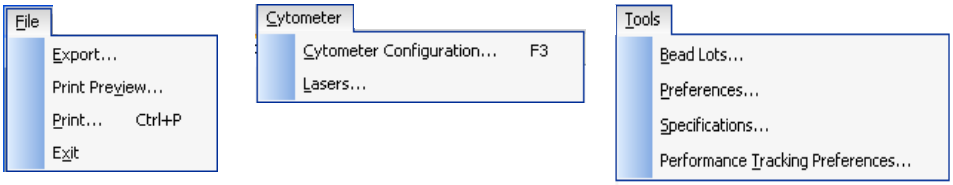
.

If you click in the BD FACSDiva workspace while the Cytometer Setup and Tracking interface is loading, the Cytometer Setup and Tracking workspace will appear behind the BD FACSDiva workspace. If the Cytometer Setup and Tracking workspace does not appear within a few seconds, check the Windows task bar and open the workspace from there.

Menu Bar

The menu bar contains the menus shown in Figure 2-2. Choose a menu command to perform the corresponding task. When keyboard shortcuts are available, they are listed next to the command.

Figure 2-2 Cytometer Setup and Tracking menus



Workspace Tabs

The workspace tabs are separate work areas in the Cytometer Setup and Tracking workspace. Click a tab to view its contents. See the following sections for a brief overview of each tab.

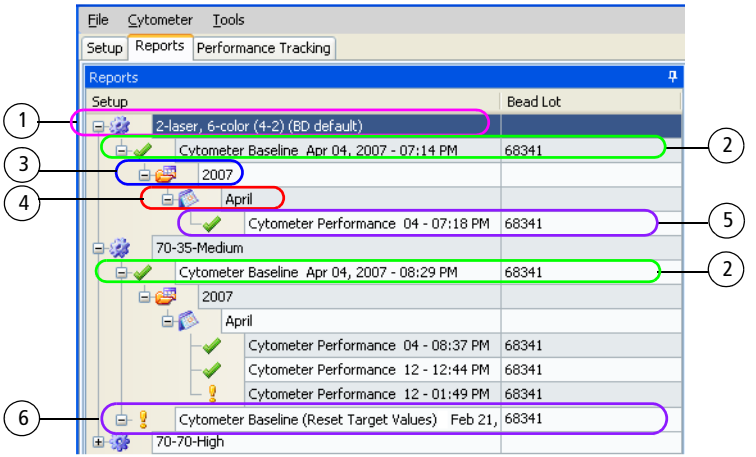
Setup

The Setup tab appears when you open the Cytometer Setup and Tracking workspace.

Reports

View Setup reports in the Reports tab browser (see Figure 2-3). Reports are organized by configuration (1). The Cytometer Baseline report is listed first (2). To view, click the report in the browser. The report is displayed in the window next to the browser. Cytometer Performance reports (5) are listed under the Cytometer Baseline report and are stored in folders organized by year (3) and month (4). Click the (+/-) icons to open and close each folder. If data for more than one bead lot exists, the Baseline Definition (Reset Target Values) report (6) for each new lot is listed as well.

Figure 2-3 Reports tab browser



☑ **Tip** Use the Reports view to quickly check whether a baseline has been defined, or a performance check was completed, for a specific configuration.

1	Configuration name
2	Cytometer Baseline report
3	Folder named with the year the performance check was run

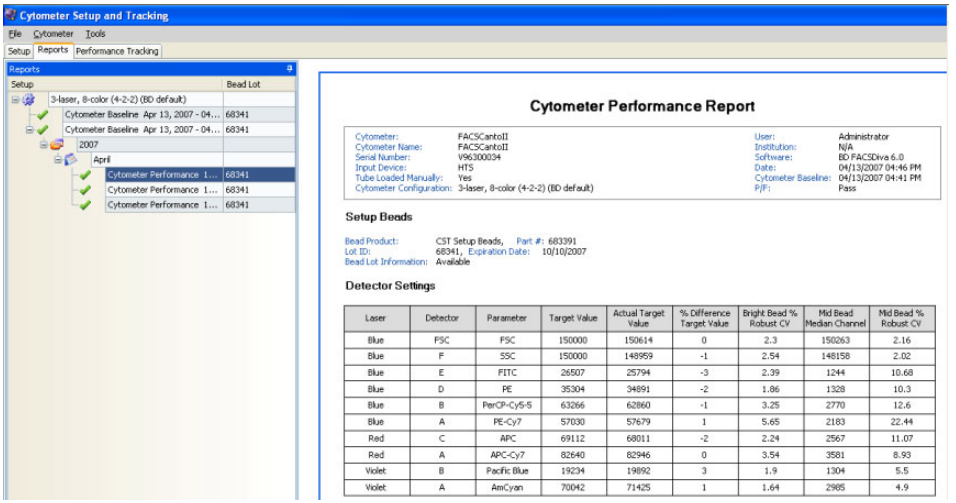
4	Folder named with the month the performance check was run
5	Cytometer Performance report
6	Cytometer Baseline with target values reset

Icons under the Reports tab indicate the status of each setup. See Table 2-1 to view status icons and their meaning.

Table 2-1 Reports view icons

Status Icons	Meaning	Organizational Icons	Meaning
	Passed		Monthly folder
	Failed		Yearly folder
	Some values out of recommended ranges	**Other Icons**	**Meaning**
			Configuration

Figure 2-4 Example Reports tab browser and Cytometer Performance report

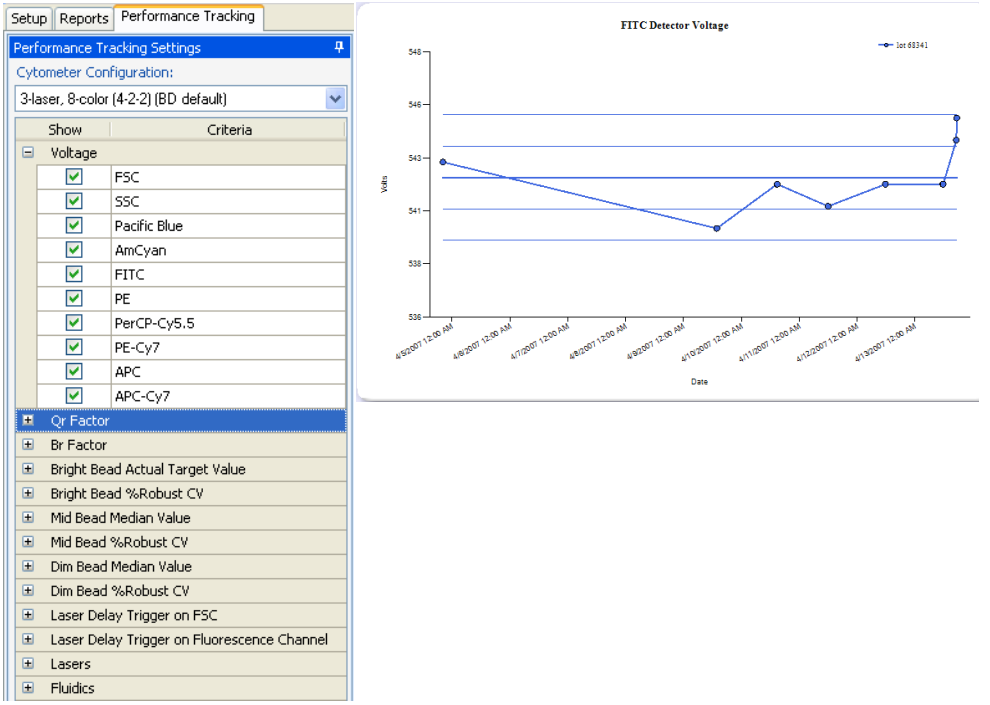


Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead % Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
Blue	FSC	FSC	150000	150614	0	2.3	150263	2.16
Blue	F	SSC	150000	148959	-1	2.54	148158	2.02
Blue	E	FITC	26507	25794	-3	2.39	1244	10.68
Blue	D	PE	35304	34891	-2	1.86	1328	10.3
Blue	B	PerCP-Cy5-5	63266	62860	-1	3.25	2770	12.6
Blue	A	PE-Cy7	57030	57679	1	5.65	2183	22.44
Red	C	APC	69112	68011	-2	2.24	2567	11.07
Red	A	APC-Cy7	82640	82946	0	3.54	3581	8.93
Violet	B	Pacific Blue	19234	19892	3	1.9	1304	5.5
Violet	A	AmCyan	70042	71425	1	1.64	2985	4.9

Performance Tracking

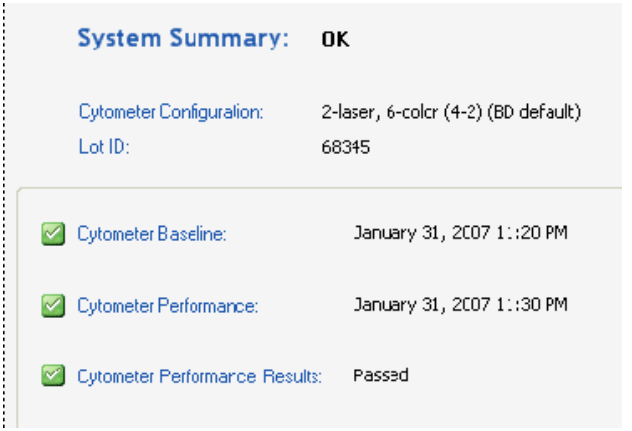
The Performance Tracking tab is where you choose which performance values you want to display and view Levey-Jennings charts of the data (Figure 2-5). You can choose to view up to 20 criteria at one time. Performance values can include PMT voltages, optical background (Br), fluorescence detection efficiency (Qr), and %robust CVs for beads.

Figure 2-5 Performance tracking selection and Levey-Jennings charts



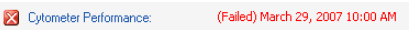
System Summary

The System Summary under the Setup tab displays the cytometer setup status.



If any of the System Summary information is displayed in red, see the recommendations in the following table.

Name	Description
System Summary	Overall status of the system indicates: **OK**—Baseline and performance check passed without warnings. **Requires Attention—investigate baseline or performance check:** • Were baseline and performance check run? • Have baseline or performance check expired? • Does baseline display warnings? • Has performance check failed or does it display warnings?
Cytometer Configuration	Current cytometer configuration
Lot ID	Bead lot ID number

Cytometer Baseline	Status indicator[a] icon and date of last cytometer baseline definition. If this message is displayed, review the report to troubleshoot issues, then continue. 
Cytometer Performance	Status indicator icon and date of last Cytometer Performance check. If this message is displayed, review the report to troubleshoot issues, then continue.  If this message is displayed, troubleshoot and resolve possible issues. If you cannot resolve the problem, assess whether your experiment will be impacted if you continue. 
Cytometer Performance Results	Status indicator icon and results of last cytometer performance check. Indicates either Passed or Failed. If Failed, refer to Performance Check Troubleshooting on page 115.

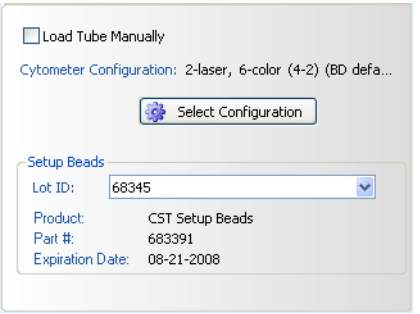
a. For status indicator meaning, see Table 2-1 on page 21.

Setup Control

In the Setup Control window under the Setup tab, you choose which characterization to run. Choose from Define Baseline, Check Performance, or Reset Target Values.

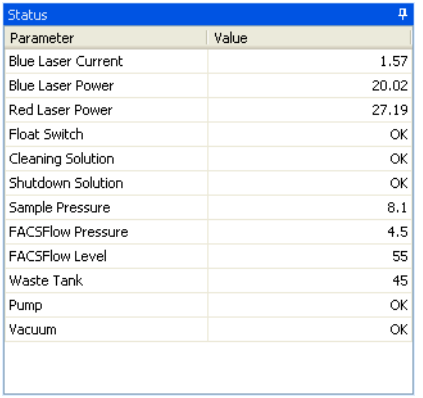
Use the Setup Beads section of the Setup Control window to choose whether to load tubes manually, view the current cytometer configuration, access the Cytometer Configuration window, or choose which lot of setup beads to use.

Figure 2-6 Setup Control



Status

Monitor status of various parameters in the read-only Status window. The parameters shown depend on which cytometer is connected.



NOTICE Values displayed in red indicate a problem. For more details, refer to your cytometer user's guide.

Status Bar

The Status Bar in the lower right corner of the Cytometer Setup and Tracking workspace indicates the connection status of the cytometer. The cytometer can be in any of the following states.

Table 2-2 Status bar icons

Cytometer State	Icon
Connecting	
Connected	
Disconnected	

NOTICE When you choose Cytometer > View Configurations or Performance Tracking (LJ) from the BD FACSDiva workspace, the cytometer remains disconnected. Only when you choose Cytometer > CST does the cytometer connect to the Cytometer Setup and Tracking interface.

3

Administrative Tasks

Before you can use the Cytometer Setup and Tracking features, a user with administrator privileges (usually the lab manager or supervisor) must perform some initial setup tasks. It is best if your lab has only one administrator who performs these tasks.

The following topics are covered in this chapter:

Bead Lot Information

When Cytometer Setup and Tracking beads are manufactured, a lot-specific file is created that contains bead lot information. This bead lot information is used to characterize your cytometer and to normalize one bead lot to another when switching bead lots. Qr and Br values generated while defining a baseline are calculated using the bead lot information in the lot-specific file. When generated on BD cytometers with standard configurations (lasers, mirrors, and filters), the Qr and Br values can be used for comparison to other parameters and for tracking. Refer to the appropriate cytometer reference manual for information on standard configurations.

⚠ BD recommends using the Qr and Br values generated with non-standard configurations for tracking only. Do not use the Qr and Br values for comparison to other parameters.

When BD FACSDiva software was loaded on your workstation, bead lot information for the current lot of Cytometer Setup and Tracking beads was automatically copied to the software folder. Before running setup, check whether this bead lot matches the lot of Cytometer Setup and Tracking beads you are using. For how to view bead lot information, see Figure 3-1 on page 32.

Verifying and Importing Bead Lot Information

1. Log in to BD FACSDiva software as an administrator.
2. Choose Cytometer > CST.
3. In the Cytometer Setup and Tracking workspace, choose Tools > Bead Lots and do one of the following:
   - Verify that the Setup Beads tab is displayed, the bead lot you are using is selected, and bead part number, lot ID, and expiration date appear in the appropriate fields. Go to step 8 on page 33.

- If your bead lot does not appear in the Lot IDs list, go to step 4.

NOTICE The lot ID number is located on the Cytometer Setup and Tracking beads vial.

4 In the Bead Lots dialog, click Import.

An Open dialog to the Bead Lots folder appears.

NOTICE If the bead lot already exists in the Setup Beads section of the Setup Control window, a warning message appears. If you import the same bead lot information over the information for a currently existing bead lot, BD recommends rerunning the baseline definition.

5 If you cannot locate a lot-specific file for your bead lot, go to Downloading a Lot-Specific File on page 33.

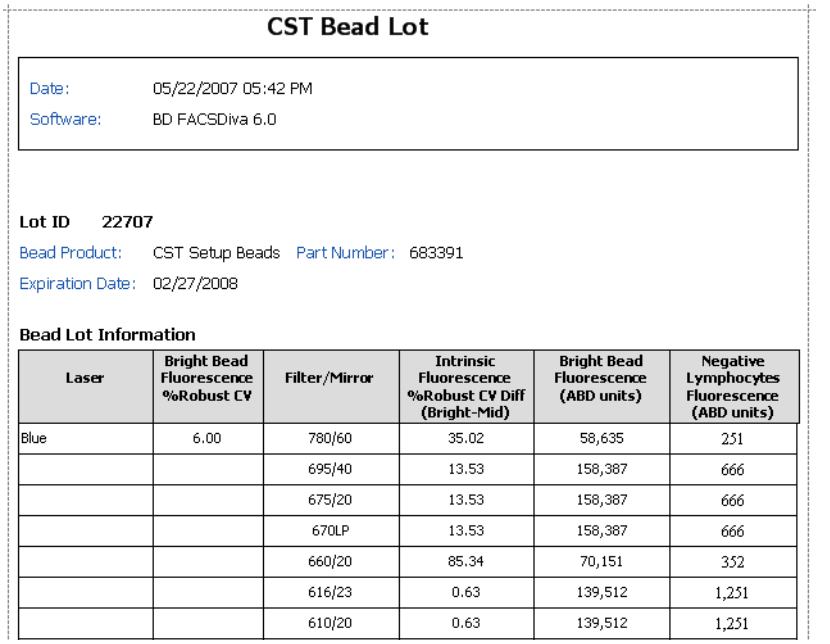
6 Select the bead lot file (ending in .bls). Click Open.

The bead lot information is automatically entered.

7 (Optional) To view the bead lot information, click the Beads Info tab in the Bead Lots dialog. Select the appropriate bead lot from the Lot ID field.

Figure 3-1 Bead Lots dialog

8 (Optional) To print the setup beads information, click Print.



CST Bead Lot

Date: 05/22/2007 05:42 PM
Software: BD FACSDiva 6.0

Lot ID **22707**
Bead Product: CST Setup Beads Part Number: 683391
Expiration Date: 02/27/2008

Bead Lot Information

Laser	Bright Bead Fluorescence %Robust CV	Filter/Mirror	Intrinsic Fluorescence %Robust CV Diff (Bright-Mid)	Bright Bead Fluorescence (ABD units)	Negative Lymphocytes Fluorescence (ABD units)
Blue	6.00	780/60	35.02	58,635	251
		695/40	13.53	158,387	666
		675/20	13.53	158,387	666
		670LP	13.53	158,387	666
		660/20	85.34	70,151	352
		616/23	0.63	139,512	1,251
		610/20	0.63	139,512	1,251

Downloading a Lot-Specific File

To obtain a lot-specific file for your current lot of Cytometer Setup and Tracking beads, do the following:

1 Go to bdbiosciences.com/CSandT.

2 Download the file to your workstation or appropriate transport medium, and then save the file to C:\Program Files\BD FACSDiva Software\CST\Bead Lot.

3 Follow steps 4 through 8 in Verifying and Importing Bead Lot Information, starting on page 30.

Entering a Bead Lot ID Manually

To fully utilize the Cytometer Setup and Tracking features, it is necessary to import the lot-specific file with bead lot information for your beads. If you are using a non-standard configuration on your cytometer or will not be using the tracking features, you can enter the bead lot ID manually.

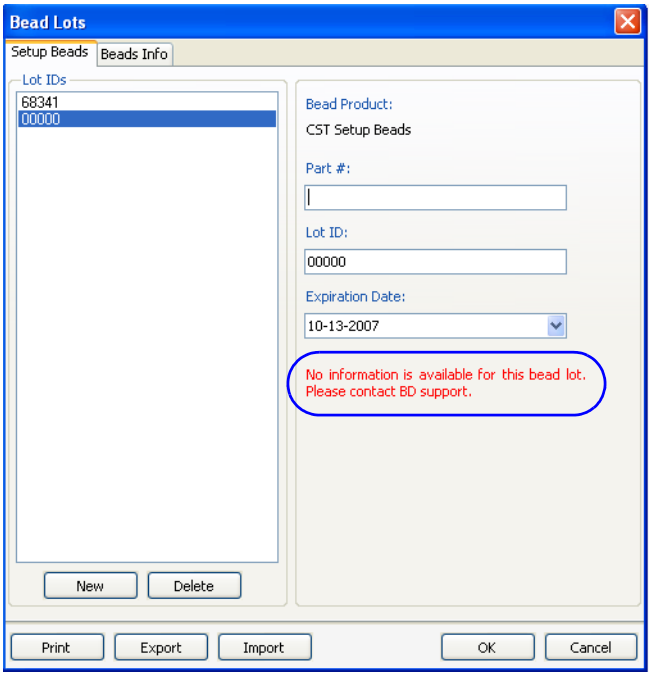
The lot ID number is located on the Cytometer Setup and Tracking beads vial.

1 Choose Cytometer > CST.

2 In the Cytometer Setup and Tracking workspace, choose Tools > Bead Lots.

3 Click New.

The software adds a line of zeros to the Lot IDs list.



Notice the message (circled on the right) indicating that bead lot information is not available for the new lot.

4. In the corresponding fields, enter the 6-digit part number and the 5-digit lot ID number for the beads.

5. Click the check mark at the right of the Expiration Date field and choose the date from the calendar that is shown.

6. (Optional) Click Print to print the setup beads information.

7. Click OK to save the lot information.

Deleting Bead Lots and Lot Information

BD does not recommend deleting bead lots and lot information. If you delete a bead lot and its lot information, you can no longer run a baseline or update performance check trending data for that lot.

1. To delete bead lot information, click the bead lot in the Bead ID list, and click Delete.

2. Click Yes in the confirmation dialog.

Exporting Bead Lot Information

To export bead lot information (eg, from one networked workstation to another) do the following:

1. In the Cytometer Setup and Tracking workspace, choose Tools > Bead Lots.

2. Under the Setup Beads tab, click Export.

   The Export Bead Lots dialog appears.

3. Navigate to and click the folder of choice. Click Save.

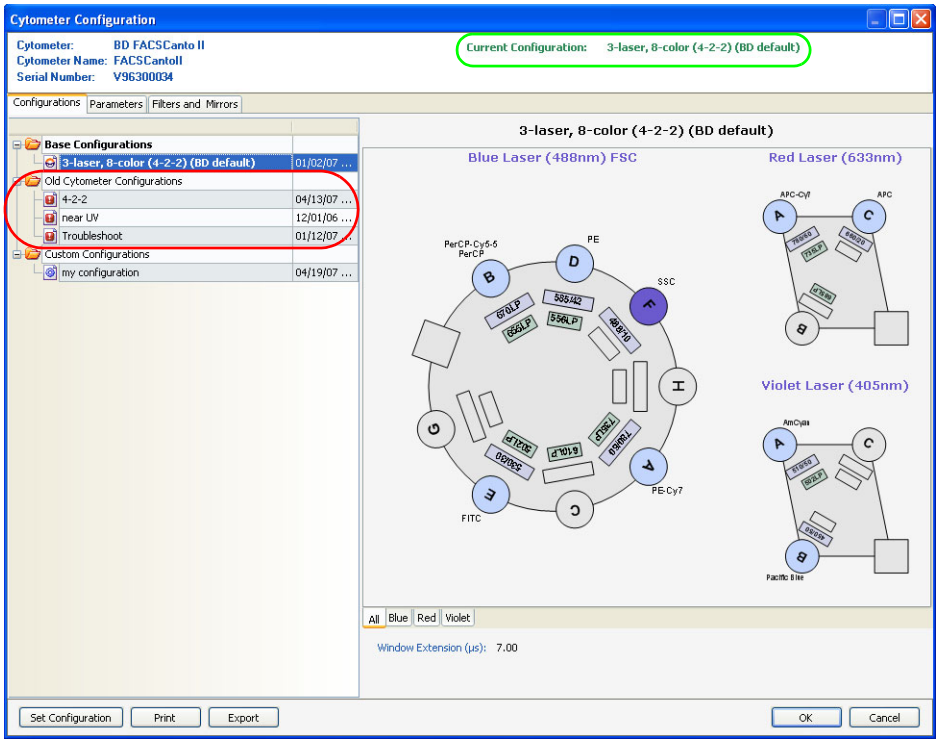
Cytometer Configurations

The physical configuration of a cytometer is the combination of lasers, detector arrays, filters, and dichroic mirrors inside the cytometer. BD FACSDiva software refers to this as the *base configuration*. Before you can begin using the Cytometer Setup and Tracking features, a configuration matching your cytometer must be created within the software. This is typically done by the BD Biosciences field service engineer during installation. This base configuration serves as the template from which custom configurations can be created.

Only users with administrative access can create, modify, or delete *custom configurations*. Custom configurations can be created for the different filter, mirror, and fluorophore combinations used in your lab. Custom configurations can also include other information (eg, cytometer-specific information, comments, etc). Any user can then set the appropriate configuration for a particular experiment. Once a configuration is set, it is listed as the *current configuration* (circled at the top right in Figure 3-2 on page 37) in the Cytometer Configuration window.

NOTICE When upgrading from a previous version of BD FACSDiva software (v5.0.x or earlier), the base configuration is automatically set to what was used previously minus filter and mirror information, which can then be added. (See Appendix A for upgrade information and page 44 for assigning filters and mirrors.) View additional configurations created in the previous version by opening the Old Cytometer Configurations folder in the Configurations list. See Figure 3-2 on page 37 for the location of the Old Cytometer Configurations folder (circled at the left).

Figure 3-2 BD default cytometer configuration for BD FACSCanto II flow cytometer



Creating New Parameters, Filters, and Mirrors

Before creating a new configuration or editing an existing configuration, verify that the necessary fluorophores, filters, and mirrors are defined under the Parameters and Filters and Mirrors tabs. If they are not defined, you can create custom parameters, filters, and mirrors.

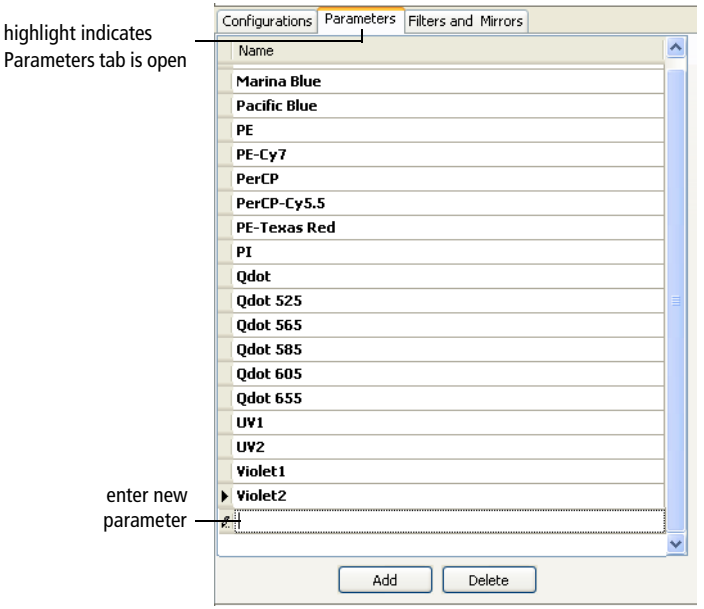
Adding to the Parameters List

The parameter names assigned to each detector are the names that will appear under the Parameters tab in the Cytometer Inspector or Cytometer window. All available parameter names are listed under the Parameters tab of the Cytometer Configuration window.

To add new parameter names to the list, perform the following steps:

1. Log in to BD FACSDiva software as an administrator.
2. Choose Cytometer > View Configurations.
3. Click the Parameters tab to open the Parameters list.
4. Click Add, enter the new parameter name, and click OK to close the Cytometer Configuration window and save the changes.

   Parameter names must be unique within the list and cannot include commas or periods. Spaces at the beginning or end of the name are automatically removed.



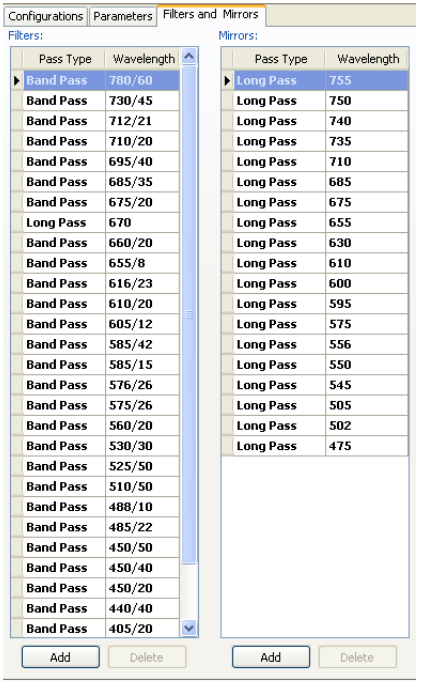
NOTICE You cannot add SSC to the Parameters list. See Setting SSC on page 43.

Adding to the Filters and Mirrors Lists

All available filters and mirrors are listed under the Filters and Mirrors tab of the Cytometer Configuration window. To add new filters or mirrors to the lists, perform the following steps:

1. Choose Cytometer > View Configurations, if necessary.

2. In the Cytometer Configuration window, click the Filters and Mirrors tab to open both the Filters list and the Mirrors list.



3. To add to either list, click Add, choose a pass type, enter the new wavelength, and click OK to close the Cytometer Configuration window and save the changes.

Creating Custom Configurations

You can create a custom configuration by adding a new blank configuration to the configuration list, or preferably by copying and editing an existing configuration. To use a custom configuration in an experiment, a baseline must be defined. See page 49 for instructions on defining a baseline.

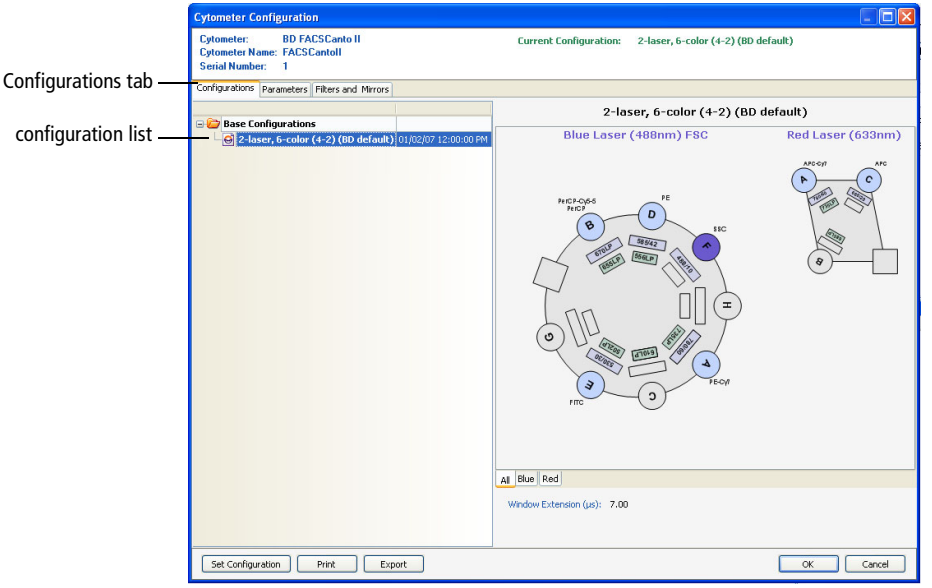
Follow these steps to create a custom configuration.

1 Verify that you are logged in to the software as an administrator.

2 Choose Cytometer > View Configurations.

The Cytometer Configuration window appears (Figure 3-3).

Figure 3-3 Cytometer Configuration window



NOTICE By default, FSC is detected from the blue laser. If you need to change this default, call your BD Biosciences service representative.

3 Create a folder for your custom configurations.

Right-click the Base Configurations folder, choose New Folder, rename the folder, and press Enter.

4 Add a custom configuration to the new folder.

- To add a new blank configuration, right-click the new folder, choose New Configuration, rename the configuration, and then press Enter. The following characters cannot be entered: \ / : * ? " < > |

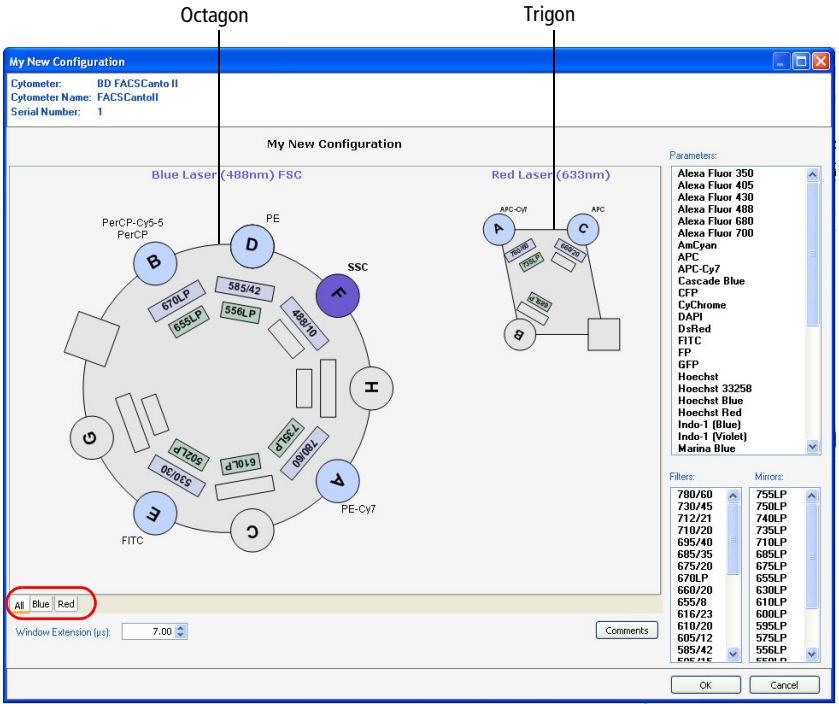
  A blank configuration appears in the Cytometer Configuration window that includes the base configuration lasers, detector arrays, FSC position, and active detectors.

- To edit an existing configuration, right-click a configuration in the configuration list and choose Copy. Then right-click your custom configurations folder and choose Paste. Rename the configuration and press Enter.

5 In the configuration list, double-click the new configuration.

The My New Configuration window appears showing a graphical representation of the detector arrays in your cytometer. The detector arrays are arranged as either octagons or trigons. See Figure 3-4 on page 42.

Figure 3-4 My New Configuration window



The circles represent the different detectors. A gray circle indicates an unavailable detector. You cannot add parameters to an unavailable detector. A light blue circle indicates an available detector. You can assign parameters from the Parameters list to any available detector. A dark blue circle indicates SSC.

The outer set of rectangles (next to the circles) represents the filters. The inner set of rectangles represents the mirrors. You can assign filters and mirrors only if the detector for that filter or mirror is available (light blue).

NOTICE A configuration cannot be used in Cytometer Setup and Tracking until each detector is fully configured with filters and mirrors. Also, there must be at least one fluorescence parameter defined on each laser.

To see the detector array in an enlarged view, click a laser tab (circled at bottom left in Figure 3-4). Click the All tab to return to the default view.

- If you created a new blank configuration, the parameters, filters, and mirrors will be blank. Assign the appropriate parameters, filters, and mirrors to the configuration.

- If you copied an existing configuration, parameters, filters, and mirrors are already assigned. Edit the assigned parameters, filters, and mirrors as necessary.

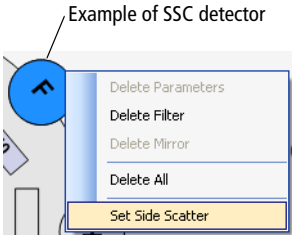
NOTICE Some detectors might be unavailable, based on your cytometer's optics.

Setting SSC

An SSC parameter must be defined to run the Cytometer Setup and Tracking features. When your cytometer was installed, a default configuration was created. When creating new configurations, refer to this configuration for the correct SSC detector assignment.

1 To set SSC, right-click the appropriate detector.

The position of SSC is based on your detector array. Refer to your cytometer manual for more information.



2 Choose Set Side Scatter from the menu.

☑ **Tip** Once SSC is set, the detector is locked. To unlock the detector, delete the parameter.

Assigning Parameters, Filters, and Mirrors

1. To apply a parameter, filter, or mirror to the configuration, drag the one you want from its respective list onto the appropriate graphical representation of the detector, filter, or mirror. If a parameter, filter, or mirror is already assigned, the existing one is overwritten by the added one.

   - Assign the parameters—To add multiple contiguous parameters, Shift-click the parameter names and drag them onto the appropriate detector. To add multiple noncontiguous parameters, Ctrl-click the parameter names and drag them onto the appropriate detector. Parameter names are listed in alphabetical order. To change a parameter, drag the appropriate parameter from the Parameters list to the available detector.

   - Assign the filters—Drag the filter name from the Filters list onto the appropriate outer rectangle for that detector.

   - Assign the mirrors—Drag the mirror name from the Mirrors list onto the appropriate inner rectangle for that detector.

   NOTICE The software will not allow you to place a mirror in a filter location, or vice versa.

2. To delete an assigned parameter, filter, or mirror, right-click the appropriate detector (colored circle) and choose to delete the parameter, filter, mirror, or all.

Adding Comments

1. Click Comments.

2. Enter text in the Cytometer Configuration Comments dialog box. Click OK to save comments.

3. When everything is labeled, click OK to save the changes.

Deleting Configurations

You cannot delete BD-default configurations or the current configuration.

To delete a custom configuration, right-click the configuration in the list, and choose Delete.

Printing Configurations

To print the current cytometer configuration, click Print.

The printout includes the name of the user currently logged in, date and time printed, information about the cytometer, configuration name and details, and a graphic representation of the configuration.

Setting the Current Configuration

1. To make the new configuration the current configuration, select the configuration in the list and click Set Configuration.

   The Current Configuration name changes at the top of the Cytometer Configuration window.

⚠ You must click Set Configuration for the new configuration to apply. For accurate data results, always verify that the cytometer optics match the current cytometer configuration.

2. Click OK to finish.

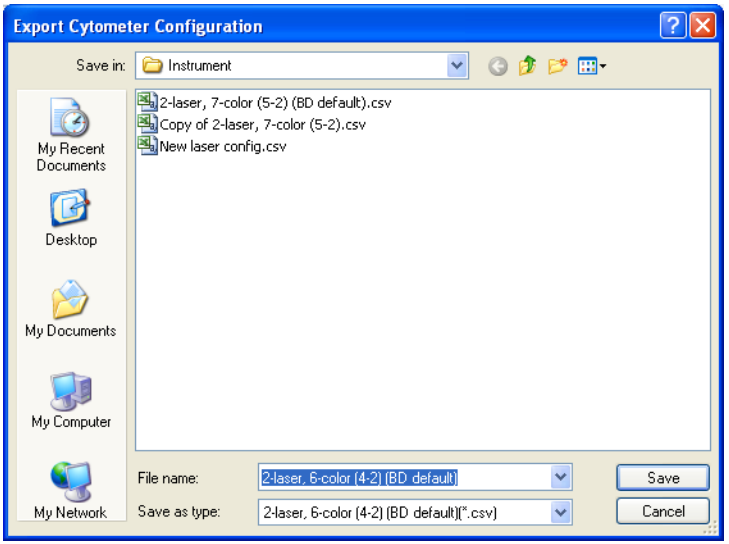
☑ **Tip** To ensure that the correct parameters appear on your Parameters tab, set the configuration you want to use *before* you create a new experiment.

Exporting Configurations

You can export custom configurations for use on a different workstation.

1. Select Cytometer > View Configurations.
2. In the Cytometer Configuration window, right-click a custom configuration in the list and choose Copy.
3. Right click a folder containing custom configurations, and choose Paste.
4. Rename the configuration.
5. Right click the new configuration and select Export Configuration.
6. Click Save in the Export dialog that appears.

   By default, exported configurations are saved in D:\BDExport\Instrument.



Importing Configurations

1. Transfer the exported cytometer configuration file to the secondary workstation. Rename the file if you have not already done so.
2. Log into BD FACSDiva software as administrator, and choose Cytometer > View Configurations.
3. In the Cytometer Configuration window, right-click the folder you want to import the configuration into, and choose Import Configuration.
4. Navigate to and select your saved or exported file and click Open.
5. Select the configuration from the list.
6. Click Set Configuration to make the imported configuration the Current Configuration.

Verify that the imported configuration has the same number of lasers and detectors as the base configuration and is from the same cytometer type.

Baseline Definition

You can define baseline performance of your cytometer by running BD Cytometer Setup and Tracking beads with BD FACSDiva software. While defining baseline, the software executes the following steps.

1	Performs laser setup • Sets laser delay • Sets area scaling factor
2	Identifies the beads • Sets gates on the bright, mid, and dim beads • Adjusts PMT voltages to set bright beads to approximately 100,000 MFI
3	Collects data samples Collects data at different PMT voltages to set median fluorescence values of bright beads to fixed target values
4	Determines baseline PMT voltages based on performance • Calculates %rCV of mid beads and median fluorescence for bright, mid, and dim beads • Fits %rCV vs median curve for each fluorescence detector • Calculates baseline voltage based on optimal fitted curve, and linearity and amplification factor for each fluorescence detector • Generates and displays PMT voltages results
5	Determines target values • Sets each detector to baseline PMT voltages and collects data • Gates bright beads • Calculates baseline target values for each detector
6	Generates results • Calculates Qr, Br, %rCVs, rSDs for each fluorescence detector • Generates baseline target values • Displays results

Defining a Baseline

BD recommends that you redefine a baseline if you change the physical configuration of your cytometer, or perform periodic maintenance or other procedures that can alter the performance or electronic noise level of your system.

NOTICE It takes approximately 30 minutes to define a baseline. The time required to define a baseline is dependent on cytometer configuration, fluidics, and cytometer performance.

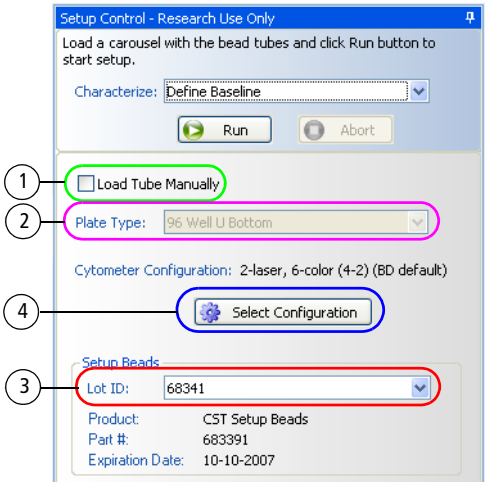
Allow cytometer lasers sufficient warm-up time. Refer to the cytometer manual for requirements.

1. Start the computer, cytometer, and software. Log in to BD FACSDiva software as an administrator.

   Start your system as instructed in the cytometer reference manual.

2. Choose Cytometer > CST.

3. In the Setup Control window, do the following:



- If a BD FACS™ Loader or BD™ High Throughput Sampler (HTS) is connected to your system, decide whether to run beads in a tube or a multiwell plate.
  - Select the Load Tube Manually checkbox (1) to load tubes manually.
  - Clear the Load Tube Manually checkbox to use the Loader or HTS.
  - Select the plate type (2) to use the HTS.
- Verify that the Setup Beads lot ID selected (3) matches your current lot of Cytometer Setup and Tracking beads. If not, see Verifying and Importing Bead Lot Information on page 30.
- Click Select Configuration (4) to open the Cytometer Configuration window.

4 Select a configuration that matches the physical configuration of your cytometer, click Set Configuration, and click OK.

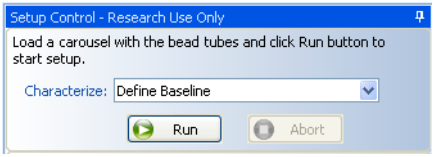
5 Prepare the setup beads for tubes or a plate as instructed in the *BD Cytometer Setup and Tracking Beads* data sheet.

6 Load the beads.

- If you are loading tubes manually, place the beads tube on the cytometer.
- If you are using the Loader, place the carousel on the Loader.
- If you are using the HTS, place the plate on the HTS.

7 If your cytometer is an LSR II, set the flow rate to low. For all other platforms, the software sets the flow rate.

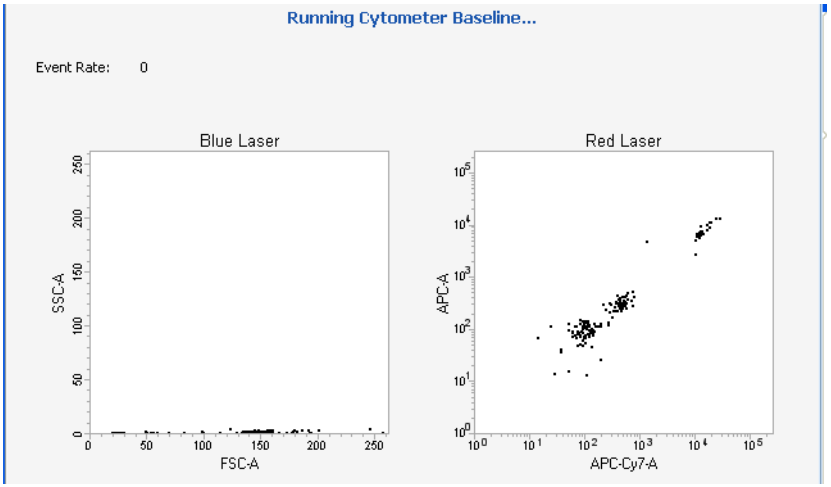
8 In the Setup Control window, choose Define Baseline, and click Run.



After a brief pause, the Running Cytometer Baseline window appears.

☑ **Tip** To view different plot parameters during acquisition, right-click the parameter and choose another from the menu.

NOTICE Multiply the x- and y-axis values by 1,000 to obtain the proper scale on the FSC and SSC acquisition plots.

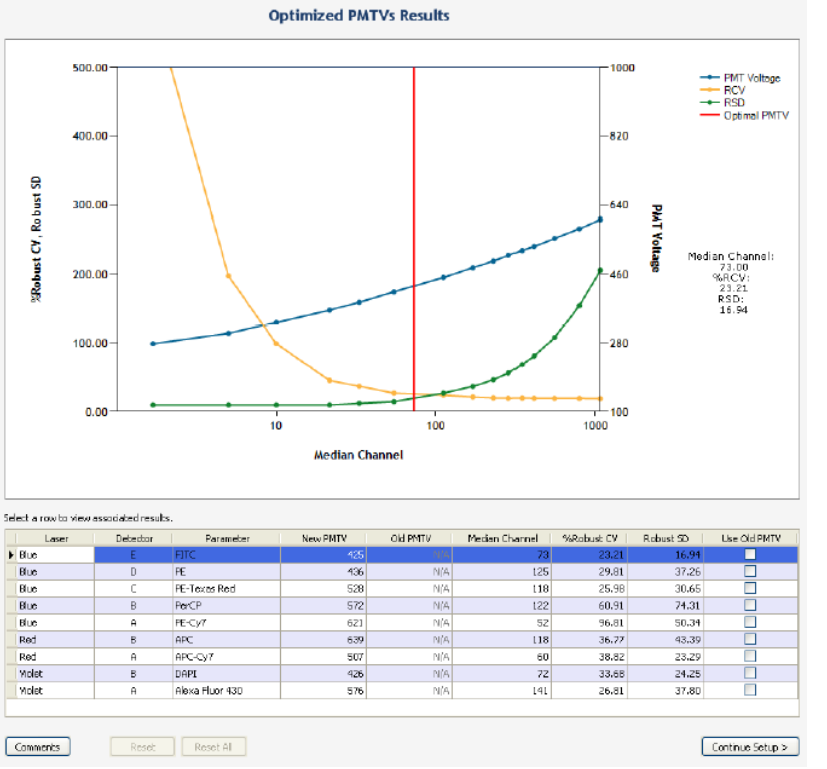


NOTICE During flow stabilization, events might appear in plots. Counters do not update until data is acquired.

- Laser setup is performed.
- Beads are identified.

- Data samples are collected.
- Once the data samples are collected, the PMTVs (PMT voltages) Results window appears. See Figure 3-5.

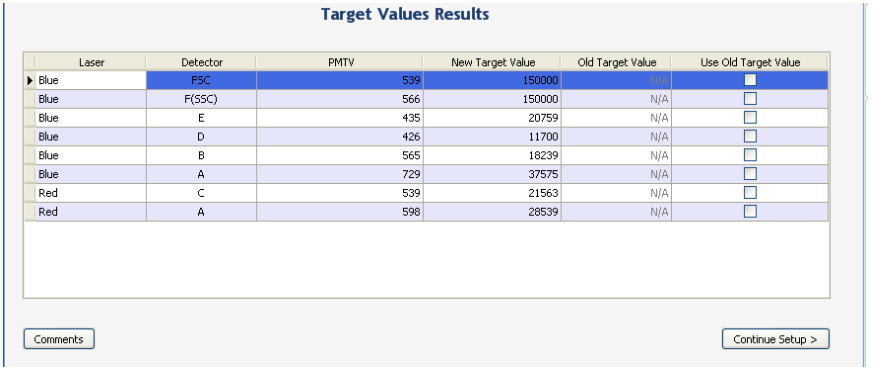
Figure 3-5 PMTVs Results window



See Reviewing Baseline PMTV Selection Plots on page 54 for instructions on how to use the features in this window.

9 After viewing the results, click Continue Setup.

The Running Cytometer Baseline window appears briefly, followed by the Target Values Results window.



See Reviewing Target Value Results on page 57 for instructions on how to use the features in this window.

10 Click Continue Setup.

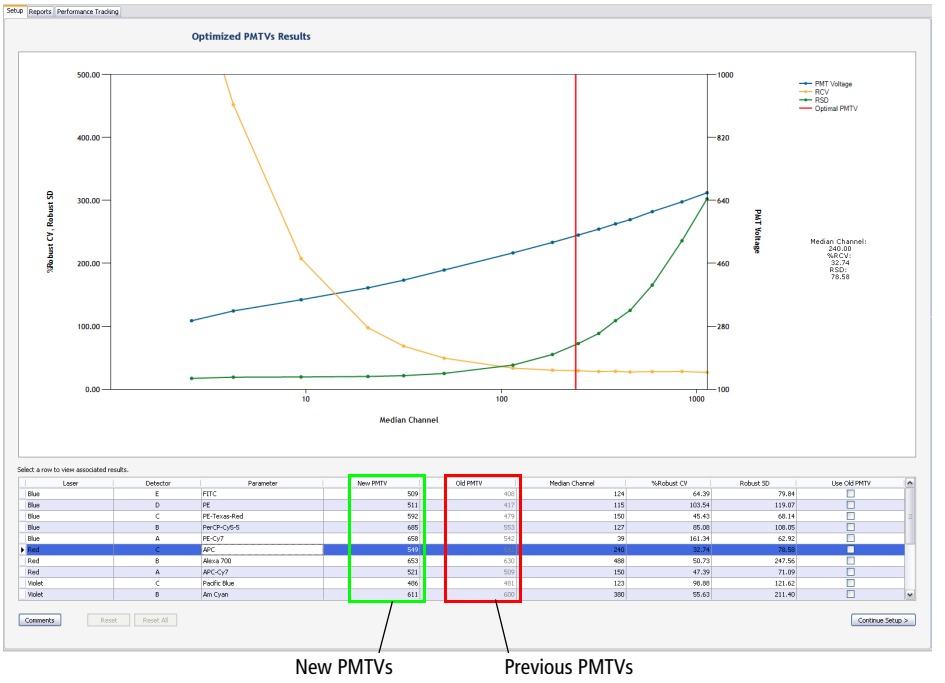
Once a baseline has been defined, a message appears.

- To view the Baseline Report, click View Report. See Reviewing the Baseline Report on page 57.
- To complete baseline definition and return to the Setup View of the workspace, click Finish.

11 Remove the tube from the cytometer, carousel from the Loader, or plate from the HTS when prompted.

Reviewing Baseline PMTV Selection Plots

The baseline PMTV (PMT voltages) selection plot displays rSD (robust standard deviation) vs median channel value, %rCV (percent robust coefficient of variation) vs median channel value, and PMT Voltage (PMTV) vs median channel value. The plot shows baseline performance characteristics for your system and baseline PMTVs (indicated by the vertical red line) for each detector. This line indicates the voltage at which the values for the beads are 10 times the standard deviation of electronic noise. By default, the plot shows the first detector in the table. To view results for a specific detector, click the corresponding row in the table.

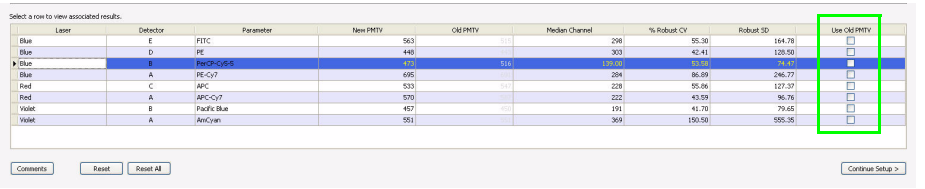


New PMTVs for the current baseline appear in the results table. If a baseline was previously defined for this configuration, PMTVs for the previous baseline also appear in the results table.

You can choose to use the new PMTVs, previous PMTVs, or adjust PMTVs manually.

- To use the new PMTVs, click Continue Setup.
- To use the previous PMTVs, see Choosing PMTVs from the Previous Baseline Definition.
- To adjust PMTVs manually, see Adjusting PMTVs Manually.

Choosing PMTVs from the Previous Baseline Definition



Choose from the following options:

- Select Use Old PMTV for a specific detector by selecting that checkbox.
- Select Use Old PMTV for all detectors by selecting all checkboxes.

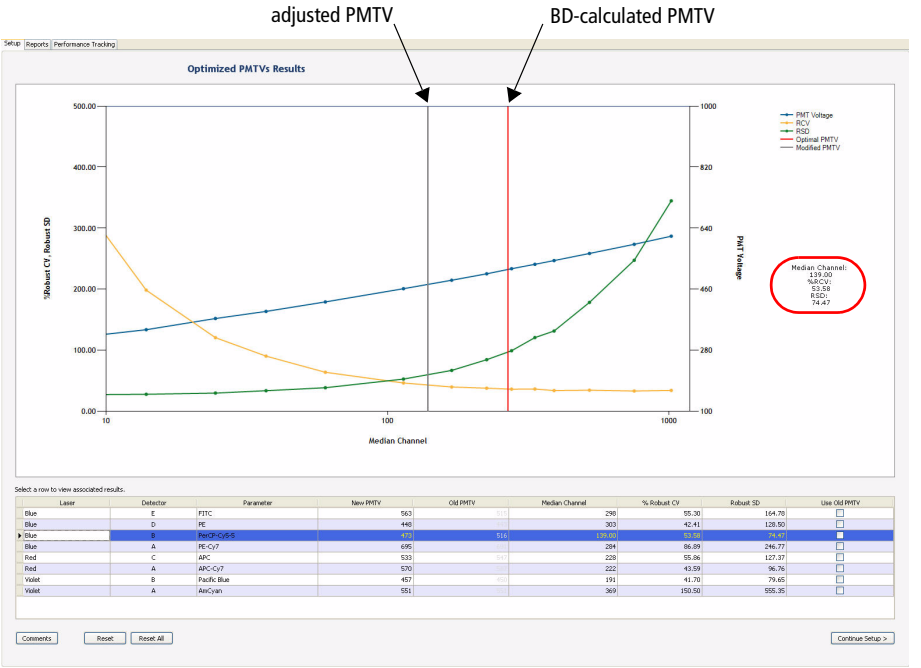
☑ **Tip** If you decide to use the previous values rather than the values obtained when defining this baseline, click Comments to note this.

Adjusting PMTVs Manually

⚠ BD recommends that you use the PMTVs derived by the software. Only advanced users should attempt to adjust settings manually.

1. In the table, click the corresponding row to display data for a specific detector.
2. In the PMTV Results plot, click and move the red line to adjust the PMTVs, or enter a new median channel value in the table.

- The plot changes to display the adjusted PMTV (black line) in relation to the BD-calculated PMTV setting (red line).
- The values for Median Channel, rCV, and rSD (circled) automatically update.



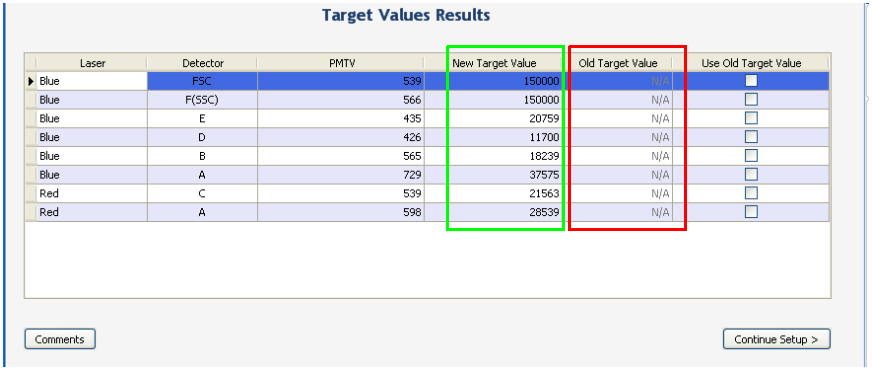
3 Adjust PMTVs for the remaining detectors, if necessary.

4 Choose from the following options:

- To use the new settings, click Continue Setup.
- To move a PMTV back to the original setting, select the detector row, and click Reset.
- To reset all detectors back to the original settings, click Reset All.

☑ **Tip** If you decide to use the adjusted values rather than the BD-calculated values obtained when defining this baseline, click Comments to note this.

Reviewing Target Value Results



New target values for the new baseline definition appear in the results table (rectangle on the left). If a baseline was previously defined for this configuration, old target values for the previous baseline also appear in the results table (rectangle on the right).

- To use new target values from this baseline, click Continue Setup.
- To use target values from the previous baseline, select Use Old Target Value for one or more detectors. Click Continue Setup.

☑ **Tip** If you decide to use the previous target values rather than the new target values obtained when defining this baseline, click Comments to note this.

Reviewing the Baseline Report

The Baseline Report contains information about the cytometer, setup bead lot information, detector settings, cytometer settings, and plots of PMTV-optimized results and linearity results. Information in the report varies depending on the cytometer used. Figure 3-6 on page 58 shows the first page of an example Baseline Report. Descriptions of measurements are shown, beginning with Detector Settings on page 59. For information on troubleshooting cytometer baseline reports, see page 112.

Figure 3-6 Example Cytometer Baseline Report

Cytometer Baseline Report

Cytometer:	FACSCantoII	User:	Administrator
Cytometer Name:	FACSCantoII	Institution:	N/A
Serial Number:	V96300005	Software:	BD FACSDiva 6.0
Input Device:	Carousel	Date:	05/02/2007 03:50 PM
Tube Loaded Manually:	Yes		
Cytometer Configuration:	4-2-2 with APC - H7 WE 2.0		

Setup Beads

Bead Product: CST Setup Beads, Part #: 683391
Lot ID: 22707, Expiration Date: 02/27/2008
Bead Lot Information: Available

Detector Settings

Laser	Detector	Parameter	PMTV	New Target Value	Old Target Value	Bright Bead %Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV	Dim Bead Median Channel	Dim Bead % Robust CV
Blue	FSC	FSC	500	150000	N/A	1.11	134228	0.92	22399	1.39
Blue	F	SSC	458	150000	N/A	3.04	142193	2.61	69186	1.79
Blue	E	FITC	477	16324	N/A	2.02	713	9.65	88	37.62
Blue	D	PE	421	21115	N/A	1.59	755	8.58	163	27.72
Blue	B	PerCP-Cy5-5	496	38804	N/A	3.01	1145	13.73	168	36.19
Blue	A	PE-Cy7	609	43385	N/A	4.85	807	24.59	79	81.54
Red	C	APC	518	36406	N/A	2.53	1776	11.4	155	40.13
Red	A	APC-Cy7	546	46681	N/A	3.43	1264	11.64	134	34.81
Violet	B	Pacific Blue	425	12081	N/A	1.42	708	5.36	81	26.55
Violet	A	AmCyan	537	149770	N/A	1.35	6908	5.08	469	29.27

Detector Settings (Continued)

Laser	Detector	Parameter	Linearity Min Channel	Linearity Max Channel	Slope	Intercept	Electronic Noise Robust SD	Qr	Br
Blue	FSC	FSC	N/A	N/A	0.0039	3.1	N/A	N/A	N/A
Blue	F	SSC	N/A	N/A	7.3808	-14.5	N/A	N/A	N/A
Blue	E	FITC	99	238108	7.5067	-15.9	17.7	0.0275	360
Blue	D	PE	107	240721	7.4566	-15.2	17.7	0.2598	388
Blue	B	PerCP-Cy5-5	100	241173	7.4524	-15.5	16.7	0.0128	42
Blue	A	PE-Cy7	26	237118	7.4390	-16.1	18.7	0.0168	10
Red	C	APC	35	239395	7.3780	-15.5	13	0.0164	117
Red	A	APC-Cy7	51	234108	7.3075	-15.3	13.9	0.0037	1184
Violet	B	Pacific Blue	36	230473	7.2374	-14.9	12.6	0.1024	1395
Violet	A	AmCyan	691	247284	7.7543	-16.0	39.2	0.0176	2704

Specifications

Violet Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)
Blue Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)
Red Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)

Research Use Only Page 1 of 9

Detector Settings

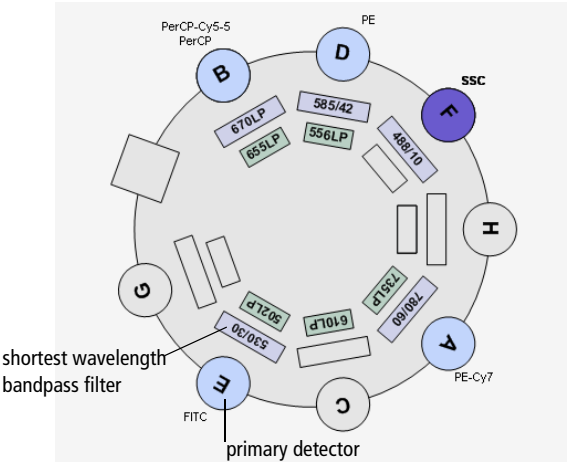
Measurement	Description/Function
Laser	Laser name
Detector	Scatter or fluorescence detector
Parameter	Fluorochrome name (assigned)
PMTV	Photomultiplier tube voltage (PMTV) required to place the dim beads population at 10 times the robust standard deviation of electronic noise
New Target Value	Measured fluorescence intensity of the bright beads at the baseline PMTV—determined during a new baseline definition
Old Target Value	Old (previous) fluorescence intensity of the bright beads at the baseline PMTV—determined by old (previous) baseline
Bright Bead % Robust CV	Percent robust coefficient of variation of bright beads
Mid Bead Median Channel	Median fluorescence intensity (MFI) value of mid beads—term used in the calculation of photon detection efficiency (Qr) and linearity
Mid Bead % Robust CV	Percent robust coefficient of variation of the mid beads—term used in the calculation of photon detection efficiency (Qr)
Dim Bead Median Channel	MFI value of dim beads—term used in the calculation of relative optical background fluorescence (Br) in the detector. (Dim bead brightness approximates that of negatively stained lymphocytes.)
Dim Bead % Robust CV	Percent robust coefficient of variation of dim beads—term used in the determination of relative optical background (Br) in the detector
Linearity Min Channel	Minimum value for the acceptable linear range of the detector
Linearity Max Channel	Maximum value for the acceptable linear range of the detector

Slope	Slope of the detector gain for bright beads (log MFI vs log PMT voltages)
Intercept	Intercept of the detector response for bright beads (log MFI vs log PMT voltages)
Electronic Noise Robust SD	Robust standard deviation (rSD) of the electronic noise in the particular detector—term used to predict the minimum acceptable signal levels required for the best attainable resolution and sensitivity for this system
Qr	Relative fluorescence detection efficiency—used for tracking light collection efficiency of a detector
Br	Relative optical background signal—used for tracking optical background levels in a detector

Specifications

These are BD recommended values for the bright bead % robust CV for the primary detector in BD cytometers with standard configurations (lasers, mirrors, and filters). Refer to the appropriate cytometer reference manual for information on standard configurations.

The primary detector on the array is the detector with the shortest wavelength (lowest number) bandpass filter. However, SSC cannot be a primary detector. In the following figure, E is the primary detector. Since F is designated as SSC, the detector with the next lowest bandpass filter is E.



Laser Settings

Measurements shown in this section of the report are cytometer dependent.

Laser Settings

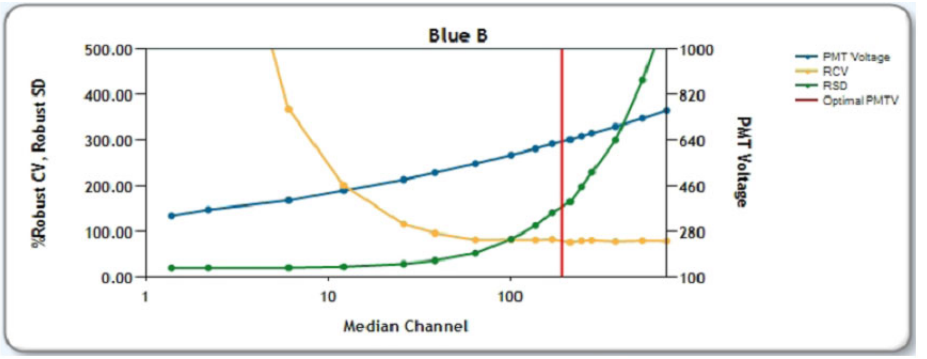
Laser	Power (mW)	Power Spec. (mW)	Current (mA)	Current Spec. (mA)	Delay (Trigger on FSC)	Delay (Trigger on Fluorescence)	Area Scaling Factor
Violet	57.62	46.08 - 69.12	N/A	N/A	-34.58	-33.84	1.10
Blue	20.44	16.35 - 24.53	1.61	1.34 - 2.00	0.00	0.00	1.35
Red	18.03	14.54 - 21.80	N/A	N/A	29.97	30.81	1.14

Window Extension: 7.00
FSC Area Scaling Factor: 0.91

Measurement	Description
Laser	Laser name
Power (mW)	Laser power—measured in milliwatts (BD FACSCanto platform only)
Power Spec (mW)	Laser power specification in milliwatts (BD FACSCanto platform only)
Current (mA)	Laser current—measured in milliamperes (BD FACSCanto platform only)
Current Spec (mA)	Laser current specification (BD FACSCanto platform only)
Delay (Trigger on FSC)	Laser delay values when thresholding on FSC
Delay (Trigger on Fluorescence)	Laser delay values when thresholding on fluorescence
Area Scaling Factor	Area scaling factors that are determined by setting area and height values on the bright 3-µm beads.

PMTV Plots

This section of the report shows PMTV results plots for each detector.



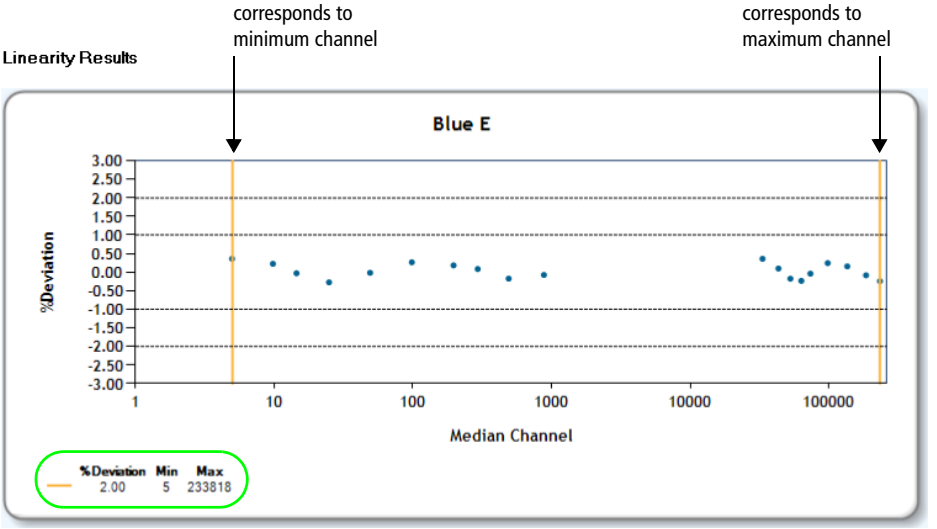
Measurement	Description
PMT Voltage	Voltage applied to photomultiplier tube (PMT)
rCV	Percent robust coefficient of variation of the dim bead
rSD	Robust standard deviation of the dim bead
Optimal PMTV	PMT setting required to place dim beads at their target: 10x the rSD of electronic noise

Linearity Results

This section of the report shows linearity plots for each detector.

- The vertical yellow lines at each end of the plot indicate the minimum and maximum channels, which the CST application considers linear.
- Minimum and maximum channel values are circled in green (lower left).
- The software assesses linearity at the upper and lower ends of the scale, so the concentration of data is in that area.

The acceptable linear range is determined by measuring the ratio of bright beads to dim beads across the detector response. If the mean of the ratio is greater than 2%, the results are not considered linear.



Reset Target Values

Before switching to a new bead lot, run Reset Target Values with the existing bead lot and the new bead lot. The software uses this information to normalize cytometer tracking (resets the target values of the new lot to the same PMTVs as the existing lot so the plots are comparable). Resetting target values also provides for reproducible setup when using application settings.

⚠ Verify that you reserve a large enough volume of the existing lot of Cytometer Setup and Tracking beads to run Reset Target Values.

NOTICE If it is necessary to define a new baseline, the software will prompt you to do so.

Before Running Reset Target Values

Before running Reset Target Values, download the lot-specific file for the new bead lot. See Downloading a Lot-Specific File on page 33 for instructions. For information about the lot-specific file, see Bead Lot Information on page 30.

Running Reset Target Values

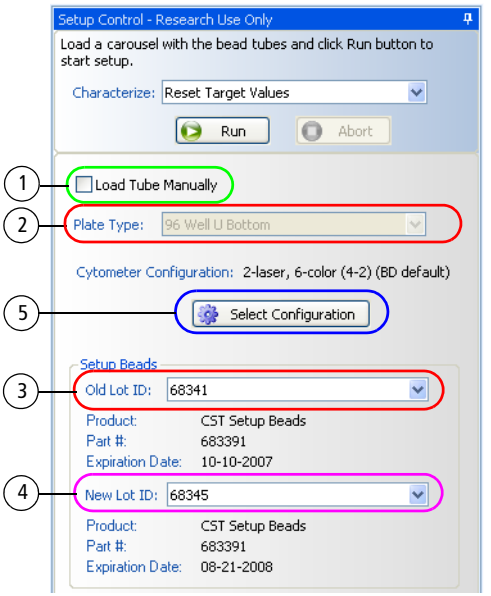
Allow cytometer lasers sufficient warm-up time. Refer to the cytometer manual for requirements.

1. Start the computer, cytometer, and software. Log in to BD FACSDiva software as an administrator.

   Start your system as instructed in the cytometer reference manual.

2. Choose Cytometer > CST.

3 In the Setup Control window, do the following:



- If a BD FACS Loader or BD High Throughput Sampler is connected to your system, decide whether to run beads in a tube or a multiwell plate.
  - Select the Load Tube Manually checkbox ((1)) to load tubes manually.
  - Clear the Load Tube Manually checkbox to use the Loader or HTS.
  - Select the plate type ((2)) to use the HTS.
- Verify that the Setup Beads lot IDs selected for the old (existing) beads lot ((3)) and the new beads lot ((4)) are correct. If not, see Verifying and Importing Bead Lot Information on page 30.
- Click Select Configuration ((5)) to open the Cytometer Configuration window.

4 Select a configuration that matches the physical configuration of your cytometer, click Set Configuration, and click OK.

NOTICE Run Reset Target Values on every cytometer configuration for which you are tracking performance values and that has a baseline defined.

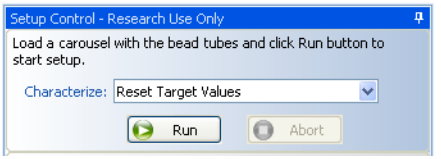
5 Prepare the setup beads for tubes or a plate as instructed in the *BD Cytometer Setup and Tracking Beads* data sheet.

6 Load the beads.

- If you are loading tubes manually, place the old beads lot in the first tube, and put the tube on the cytometer. (The software will prompt you when it is time to load the tube with the new beads lot.)
- If you are using the Loader, place the tube with the old beads lot in the first position, the tube with the new beads lot in the second position, and place the carousel on the Loader.
- If you are using the HTS, place the old beads lot in the first well (A1), the new beads lot in the second well (A2), and place the plate on the HTS.

7 If your cytometer is an LSR II, set the flow rate to low. For all other platforms, the software sets the flow rate.

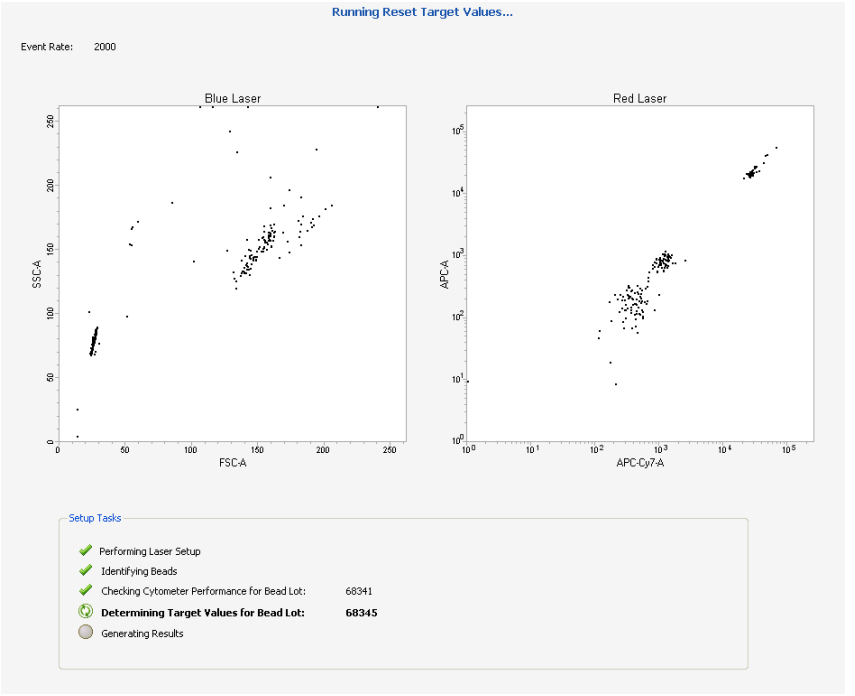
8 In the Setup Control window, choose Reset Target Values, and click Run.



After a brief pause, the Resetting Target Values window appears.

☑ **Tip** To view different plot parameters during acquisition, right-click the parameter and choose another from the menu.

NOTICE Multiply the x- and y-axis values by 1,000 to obtain the proper scale on the FSC and SSC acquisition plots.



NOTICE During flow stabilization, events might appear in plots. Counters do not update until data is acquired.

- Laser setup is performed.
- Beads are identified.
- Cytometer performance for the current bead lot is checked.
- Target values for the new bead lot are determined.
- Results are generated, and a completion message appears.

NOTICE A Cytometer Performance report for the old beads lot is generated as well as the Reset Target Values report.

9 Choose from the following options:

- To view the Reset Target Values Report, click View Report. See Reviewing the Reset Target Values Report.
- To complete resetting target values and return to the Setup View of the workspace, click Finish.

10 Remove the tube from the cytometer, carousel from the Loader, or plate from the HTS when prompted.

NOTICE If it is necessary to define a new baseline, the software will prompt you to do so.

Reviewing the Reset Target Values Report

The Reset Target Values report contains information about the cytometer, old and new setup beads lot information, detector settings for the old and new beads lots, laser settings and specifications, and cytometer settings. Figure 3-7 on page 70 shows the first page of an example Reset Target Values Report. See Detector Settings on page 71 for a description and explanation of measurements.

Figure 3-7 Example Reset Target Values Report

Cytometer Baseline Report (Reset Target Values)

Cytometer:	FACSCantoII	User:	Administrator
Cytometer Name:	FACSCantoII	Institution:	N/A
Serial Number:	V96300005	Software:	BD FACSDiva 6.0
Input Device:	HTS	Date:	05/17/2007 04:12 PM
Setup Bead (old lot) Position:	A1	Cytometer Baseline:	05/02/2007 03:50 PM
Setup Bead (new lot) Position:	A2		
Cytometer Configuration:	4-2-2 with APC - H7 WE 2.0		

Old Setup Beads

Bead Product: CST Setup Beads, Part #: 683391
Lot ID: 22707, Expiration Date: 02/27/2008
Bead Lot Information: Available

New Setup Beads

Bead Product: CST Setup Beads, Part #: 910723
Lot ID: 42307, Expiration Date: 04/23/2008
Bead Lot Information: Available

Detector Settings

Laser	Detector	Parameter	PMTV	Target Value Old Lot	Target Value New Lot	Bright Bead % Robust CV
Blue	FSC	FSC	512	150000	150000	1.37
Blue	F	SSC	464	150000	150000	3.67
Blue	E	FITC	475	16324	18126	2.08
Blue	D	PE	420	21115	21667	1.73
Blue	B	PerCP-Cy5-5	492	38804	32926	3.13
Blue	A	PE-Cy7	603	43385	33233	4.80
Red	C	APC	515	36406	33707	2.94
Red	A	APC-Cy7	542	46681	35285	3.23
Violet	B	Pacific Blue	427	12081	13666	2.23
Violet	A	AmCyan	541	149770	157094	2.16

Detector Settings (Continued)

Laser	Detector	Parameter	Mid Bead Median Channel	Mid Bead % Robust CV	Dim Bead Median Channel	Dim Bead % Robust CV	Qr	Br
Blue	FSC	FSC	152637	1.18	25586	1.57	N/A	N/A
Blue	F	SSC	151930	3.40	73094	2.49	N/A	N/A
Blue	E	FITC	806	8.89	100	34.66	0.0288	496
Blue	D	PE	772	8.78	172	26.96	0.2526	408
Blue	B	PerCP-Cy5-5	1192	12.68	154	37.69	0.0137	85
Blue	A	PE-Cy7	878	22.28	64	93.80	0.0188	23
Red	C	APC	1838	11.11	135	41.46	0.0148	26
Red	A	APC-Cy7	1342	10.40	75	49.42	0.0037	774
Violet	B	Pacific Blue	732	6.15	87	32.95	0.0925	3484
Violet	A	AmCyan	7036	5.73	519	34.99	0.0151	4993

Specifications

Violet Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)
Blue Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)
Red Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)

Research Use Only

Page 1 of 2

Detector Settings

Measurement	Description/Function
Laser	Laser name
Detector	Scatter or fluorescence detector
Parameter	Fluorochrome name (assigned)
PMTV	Photomultiplier tube voltage (PMTV) required to place the dim beads population at 10 times the robust standard deviation of electronic noise
Target Value Old Lot	Old (previous) fluorescence intensity of the bright beads at the baseline PMTV—determined by old (previous) baseline
Target Value New Lot	Measured fluorescence intensity of the bright beads at the baseline PMTV—determined during a new baseline definition
Bright Bead % Robust CV	Percent robust coefficient of variation of bright beads
Mid Bead Median Channel	Median fluorescence intensity (MFI) value of mid beads—term used in the calculation of photon detection efficiency (Qr) and linearity
Mid Bead % Robust CV	Percent robust coefficient of variation of the mid beads—term used in the calculation of photon detection efficiency (Qr)
Dim Bead Median Channel	MFI value of dim beads—term used in the calculation of relative optical background fluorescence (Br) in the detector. (Dim bead brightness approximates that of negatively stained lymphocytes.)
Dim Bead % Robust CV	Percent robust coefficient of variation of dim beads—term used in the determination of relative optical background (Br) in the detector
Qr	Relative fluorescence detection efficiency—used for tracking light collection efficiency of a detector
Br	Relative optical background signal—used for tracking optical background levels in a detector

Specifications

These are BD recommended values for the bright bead % robust CV for the primary detector in BD cytometers with standard configurations (lasers, mirrors, and filters). Refer to the appropriate cytometer reference manual for information on standard configurations.

Laser Settings

Measurements shown in this table are cytometer dependent.

Laser Settings

Laser	Power (mW)	Power Spec. (mW)	Current (mA)	Current Spec. (mA)	Delay (Trigger on FSC)	Delay (Trigger on Fluorescence)	Area Scaling Factor
Violet	57.62	46.08 - 69.12	N/A	N/A	-34.58	-33.84	1.10
Blue	20.44	16.35 - 24.53	1.61	1.34 - 2.00	0.00	0.00	1.35
Red	18.03	14.54 - 21.80	N/A	N/A	29.97	30.81	1.14

Window Extension: 7.00
FSC Area Scaling Factor: 0.91

Measurement	Description
Laser	Laser name
Power (mW)	Laser power—measured in milliwatts (BD FACSCanto platform only)
Power Spec (mW)	Laser power specification in milliwatts (BD FACSCanto flow cytometer only)
Current (mA)	Laser current—measured in milliamperes (BD FACSCanto platform only)
Current Spec (mA)	Laser current specification (BD FACSCanto flow cytometer only)
Delay (Trigger on FSC)	Laser delay values when thresholding on FSC
Delay (Trigger on Fluorescence)	Laser delay values when thresholding on fluorescence
Area Scaling Factor	Area scaling factors that are determined by setting area and height values on the bright 3-µm beads

Preferences

Administrators can make the following modifications to software defaults:

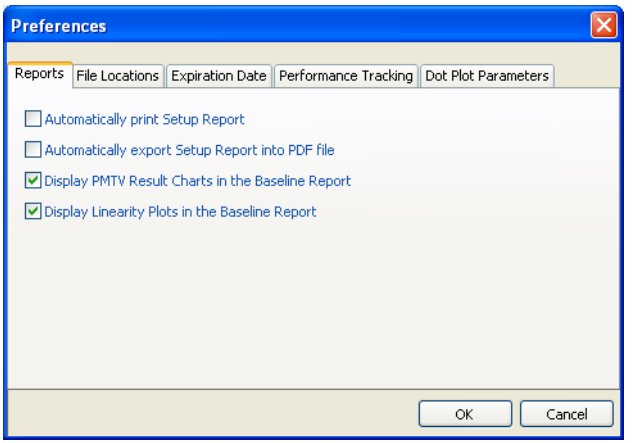
- Changing Report Preferences on page 74
- Switching the Automatic Save File Destination on page 75
- Resetting Expiration Dating on page 76
- Choosing the Number of Entries in the Performance Tracking File on page 77
- Choosing Dot Plot Parameters on page 78

To begin, Choose Tools > Preferences on the Cytometer Setup and Tracking menu bar to open the Preferences window.

NOTICE If you have made changes under more than one tab, clicking Cancel under one tab cancels changes made in all tabs.

Changing Report Preferences

Under the Reports tab, choose an option by selecting the checkbox.

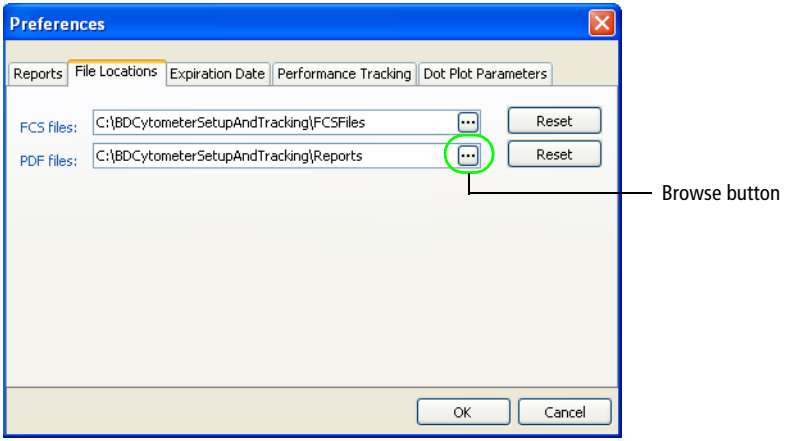


Options include the following:

- Automatically print the Setup Report
- Automatically export the Setup Report into a PDF file
- Display PMTV Results Charts in the Baseline Report
- Display linearity plots in the Baseline Report

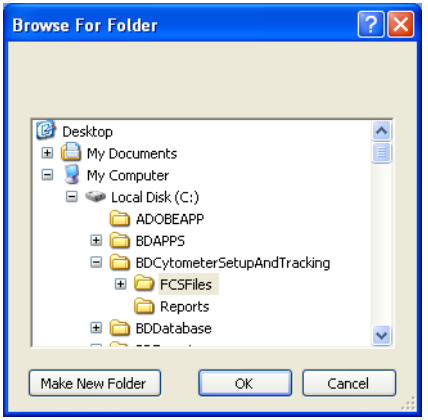
Switching the Automatic Save File Destination

1 In the Preferences window, click the File Locations tab.



2 To save files to a different location, click the Browse button next to the corresponding file path you want to change.

The Browse for Folder dialog appears.



3 Do one of the following:

- Navigate to and select a new folder. Click OK.
- Navigate to the destination, click Make New Folder, name the folder, and click OK.

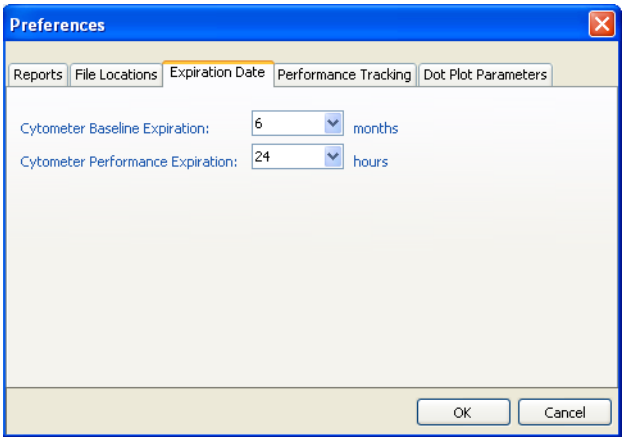
In each case, the file pathway is updated.

4 To go back to the default pathway, click the Reset button next to the file pathway you changed.

Resetting Expiration Dating

The expiration date affects the status of the run date shown in the System Summary under the Setup tab. If the expiration date for cytometer baseline or cytometer performance has expired, the run date appears in red.

1 In the Preferences window, click the Expiration Date tab.

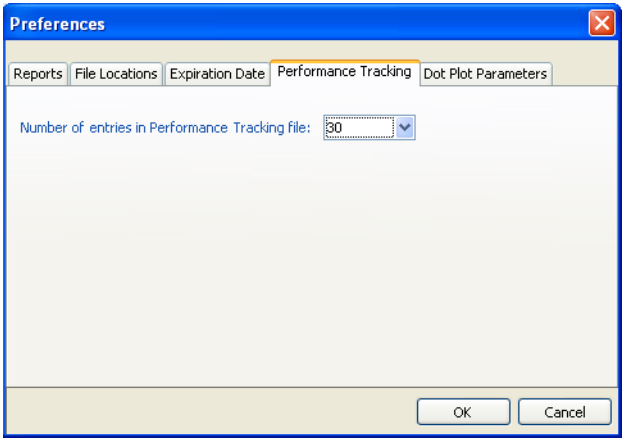


2 Choose a new expiration date from one of the menus.

- Expiration dating for the Baseline definition ranges from 1–12 months.
- Expiration dating for the Performance Check ranges from 8–48 hours.

Choosing the Number of Entries in the Performance Tracking File

1 In the Preferences window, click the Performance Tracking tab.



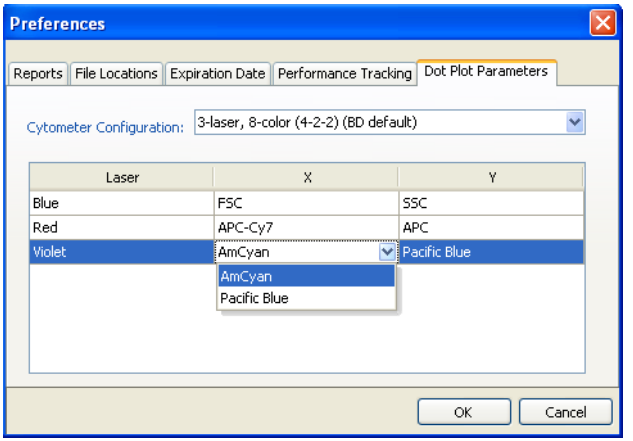
2 Choose from 30, 60, or an unlimited number of data points to display in the Performance Tracking plots.

- If you choose a fixed number of data points, data is overwritten on a first in, first out basis.
- If you choose the unlimited option, the time it takes to load the plots increases as the number of data points increases.

Choosing Dot Plot Parameters

The selections you make in this tab affect the plot parameters displayed when running cytometer performance and defining a baseline.

1. In the Preferences window, click the Dot Plot Parameters tab.
2. Choose the Cytometer Configuration you want to change.
3. Choose the x- and y-parameters for each plot by clicking in the parameter field and choosing from the menu.

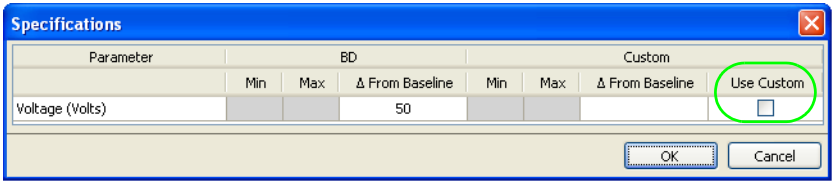


4. Click OK to save all preferences and close the Preferences dialog.

Specifications

By default, the software fails the performance check and flags a parameter voltage value in the Cytometer Performance report if the value varies by more than 50 volts from the baseline. Use the Specifications dialog to set your own specification range.

1 Choose Tools > Specifications on the Cytometer Setup and Tracking menu bar to open the Specifications window.

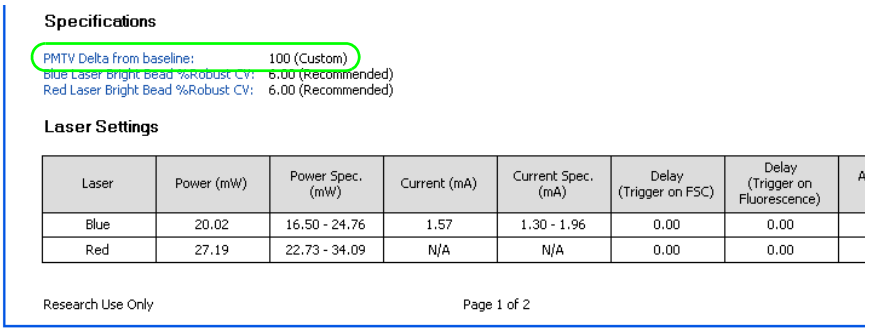


2 Select the Use Custom checkbox (circled), enter a number between 0 and 200 in the Custom Δ (Change) From Baseline field, and click OK.

To close the dialog without making changes, click Cancel.

NOTICE You cannot change the BD-specified value.

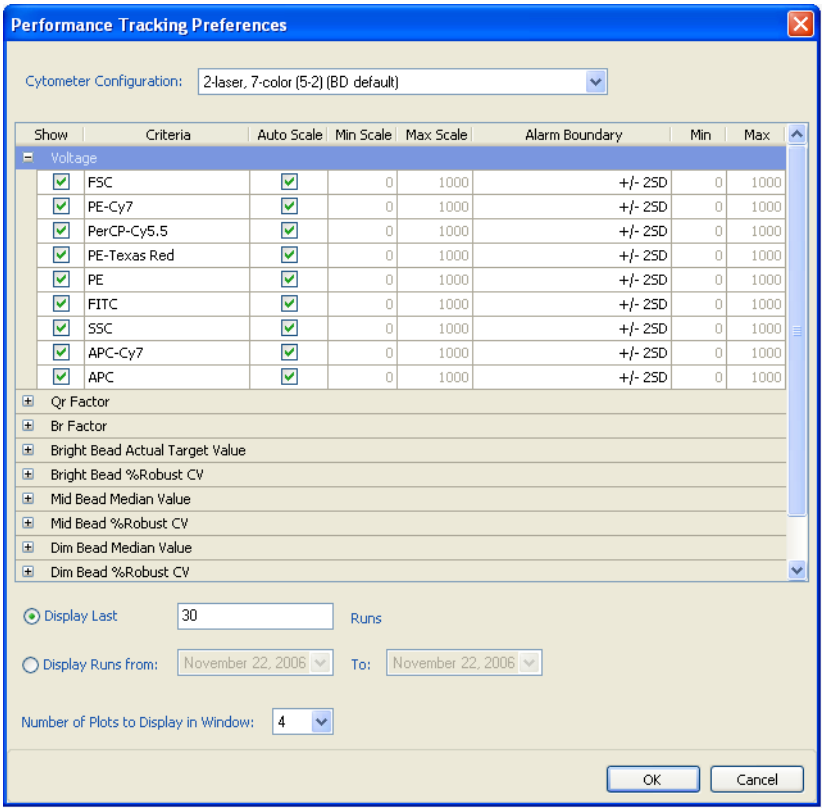
The next time you run a cytometer performance check, the *PMTV Delta from baseline* field (circled) in the Specifications section of the Cytometer Performance report shows the custom-defined value.



Performance Tracking Preferences

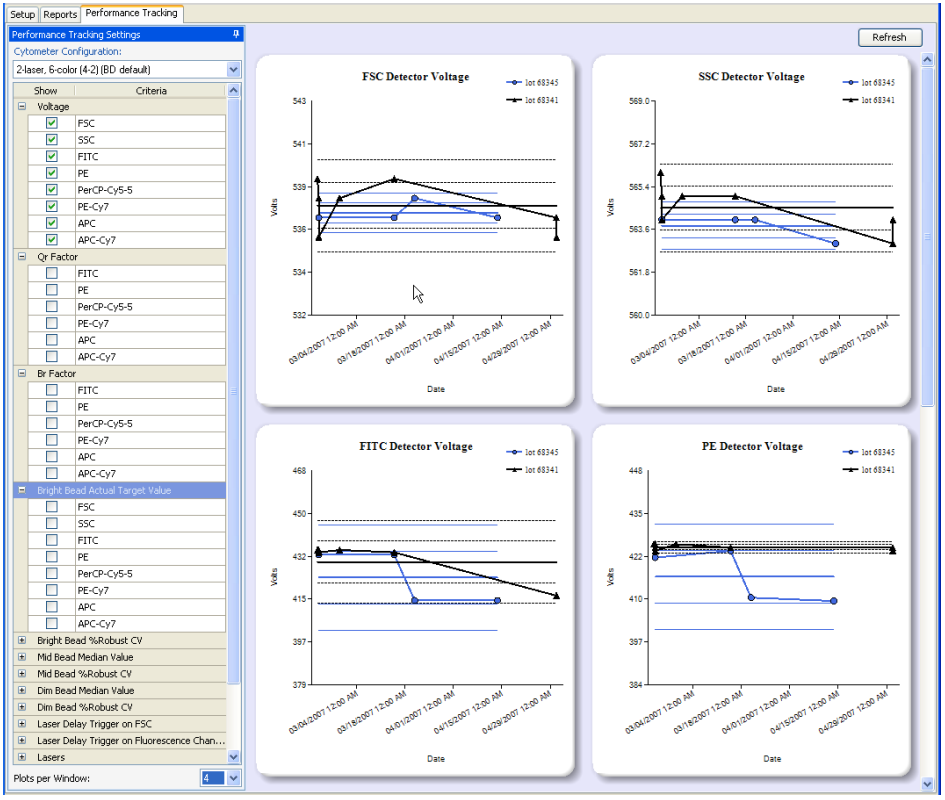
In the Performance Tracking Preferences window, choose the performance criteria from the Cytometer Performance report that you want to display. Your choices appear under the Performance Tracking tab of the Cytometer Setup and Tracking workspace. You can display different criteria for different cytometer configurations. See Reviewing the Baseline Report on page 57 and Reviewing the Cytometer Performance Report on page 93 for an explanation of criteria.

Choose Tools > Performance Tracking Preferences to open the Performance Tracking Preferences window.

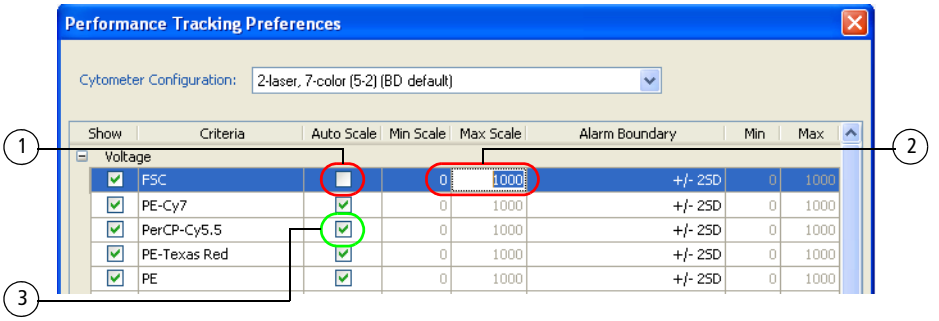


You can choose up to 20 different criteria to display under the Performance Tracking tab. Each plot displays data points for one criterion. You can display data for more than one bead lot on each plot, as shown in Figure 3-8.

Figure 3-8 Performance Tracking tab



Selecting Scale

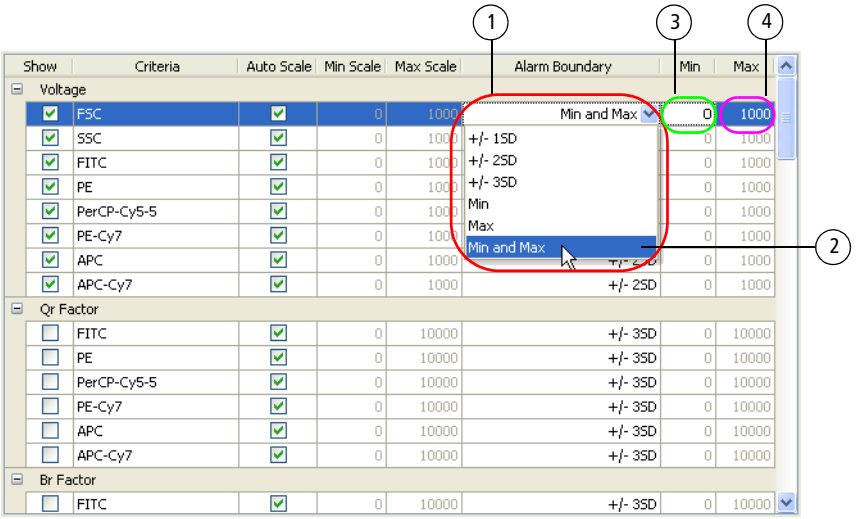


- If you clear Auto Scale ((1)), enter Min Scale and Max Scale ((2)) values. Choose values between –1,299,999 and 300,000. The Min Scale value must be lower than the Max Scale value.

- If you select Auto Scale ((3)), the software automatically chooses an appropriate display range for the data.

Modifying Alarm Boundaries

You can modify alarm boundaries to meet the specific needs of your lab. Choose different boundaries for specific criteria, or use the same boundaries for all.



NOTICE Any changes made to the alarm boundaries after you have been tracking performance will not be reflected on the Levey-Jennings charts until you next run Cytometer Setup and Tracking beads.

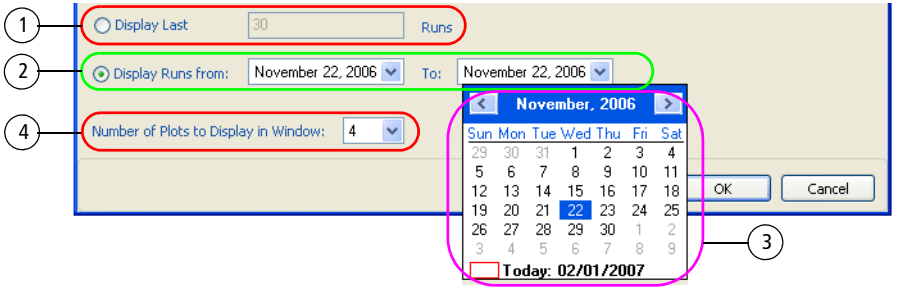
1 For each criterion, choose an appropriate alarm boundary (1) from the menu.

Alarm boundary choices include the following: ±1 to 3 SD, minimum and maximum boundary, minimum boundary only, maximum boundary only.

2 If you choose Min and Max (2) boundary, enter a minimum value in the Min field (3) and a maximum value in the Max field (4), as appropriate.

Selecting Display Criteria

Use the lower half of the Performance Tracking Preferences window to select display criteria.



1 Choose how to display performance data.

- To display data from the last number of runs, click *Display Last* (1) and choose between 30, 60, or unlimited number of runs.
- To display data by date range, click *Display Runs From* (2), choose From and To dates by clicking the menu and selecting dates from the calendar view (3).

2 Choose to display 1, 2, or 4 Levey-Jennings charts per page (4) under the Performance Tracking tab. See Figure 3-8 on page 81.

Results Files

The software generates results files in CSV format, readable by a spreadsheet application such as Microsoft® Excel. You can export custom cytometer configuration information, Cytometer Setup and Tracking bead information, baseline results, and performance results.

Exporting Results Files

1. Select the results file you want to export.
2. Choose File > Export.
3. In the Export dialog that appears, enter a file name, choose a destination folder, and click Save.

   The file is saved in CSV format to the specified folder.

4

Workflow and Application Tasks

The following topics are covered in this chapter:

Routine Workflow Tasks

Using the Cytometer Setup and Tracking features to set up, optimize, and track your cytometer settings saves time and ensures reproducible setup every day. This section guides you through a typical workflow for all users.

- Starting the System on this page
- Verifying the Cytometer Configuration on page 90
- Running a Performance Check on page 91

Refer to the *BD FACSDiva Software Reference Manual* for instructions on applying compensation, recording data, analyzing data, and shutting down the system.

Before you begin, ensure that the software's administrator has performed all necessary tasks to set up the software for your use.

Starting the System

Start your system as instructed in the cytometer reference manual. Be sure to allow cytometer lasers sufficient warm-up time.

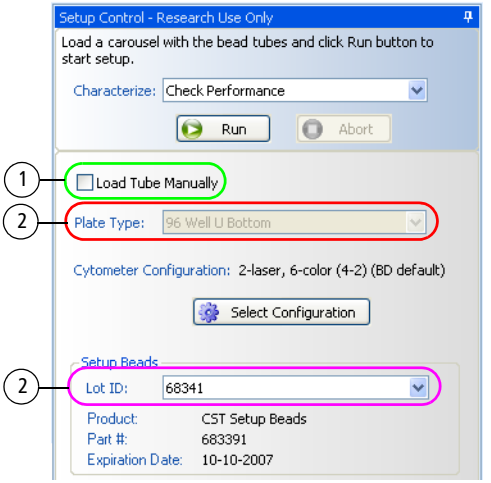
1. Start the computer, cytometer, and software. Log in to BD FACSDiva software.
2. Prepare the setup beads for tubes or a plate as instructed in the *BD Cytometer Setup and Tracking beads* data sheet.
3. Choose Cytometer > CST.

NOTICE When you choose Cytometer > CST, the cytometer disconnects from the BD FACSDiva interface and connects to the Cytometer Setup and Tracking interface. The following message appears in the BD FACSDiva status bar at the bottom of the screen: 

.

If you click in the BD FACSDiva interface while the Cytometer Setup and Tracking interface is loading, the Cytometer Setup and Tracking workspace will appear behind the BD FACSDiva workspace. If the Cytometer Setup and Tracking workspace does not appear within a few seconds, check the Windows task bar and open the workspace from there.

4 In the Setup Control window, do the following:



- If a BD FACS Loader or BD High Throughput Sampler is connected to your system, decide whether to run beads in a tube or a multiwell plate.
  - Select the Load Tube Manually checkbox (1) to load tubes manually.
  - Clear the Load Tube Manually checkbox to use the Loader or HTS.
  - Select a plate type (2) to use the HTS.
- Verify that the Setup Beads lot ID selected (3) matches your current lot of Cytometer Setup and Tracking beads. If not, have the administrator import the bead lot for you or use the menu to choose the appropriate lot.

NOTICE Always use the same loading method (tubes, Loader, or plates) when running the performance check. Using different methods might cause CV variations.

☑ **Tip** Place the configuration in a specific folder. When you run the performance check, choose the cytometer configuration from that folder.

Verifying the Cytometer Configuration

1 In the System Summary, verify the following:

- Check that the Cytometer Configuration (1) is appropriate for your experiment. If not, select another configuration. See Setting the Current Configuration on page 45.

☑ **Tip** Print the configuration for a map of filters and mirrors. For printing instructions, see Printing Configurations on page 45.

- Check that the cytometer baseline has been defined and is valid (2). If not, see Defining a Baseline on page 49.

2 Load the beads.

- If you are loading tubes manually, place the beads tube on the cytometer.

- If you are using the Loader, place the carousel on the Loader.
- If you are using the HTS, place the plate on the HTS.

3 If your cytometer is an LSR II, set the flow rate to low. For all other platforms, the software sets the flow rate.

Running a Performance Check

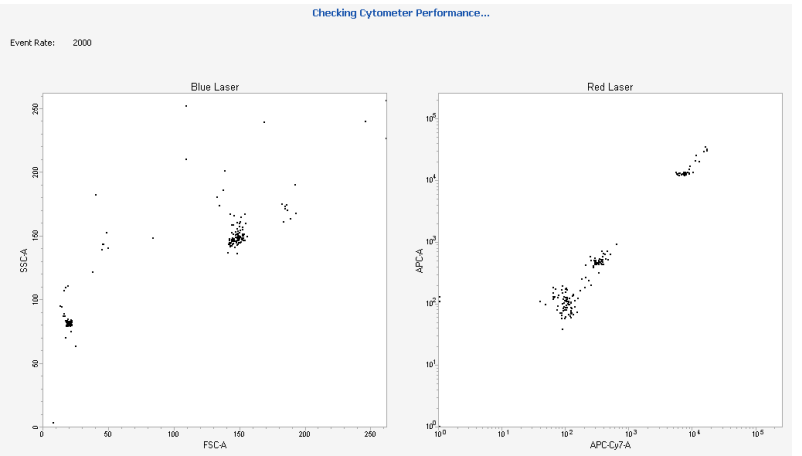
Running a performance check takes approximately 5 minutes, depending on your system.

BD Biosciences recommends that you run a performance check every 24 hours.

1 In the Setup Control window, choose to run a performance check and click Run.

After a brief pause, the Checking Cytometer Performance window appears.

NOTICE Multiply the x- and y-axis values by 1,000 to obtain the proper scale on the FSC and SSC acquisition plots.



☑ **Tip** To view different plot parameters during acquisition, right-click the parameter and choose another from the menu.

NOTICE During flow stabilization, events might appear in plots. Counters do not update until data is acquired.

- Laser setup is performed.
- Beads are identified.
- Cytometer setup is performed.
- Results are generated.

2 Once the performance check is complete, a message appears.

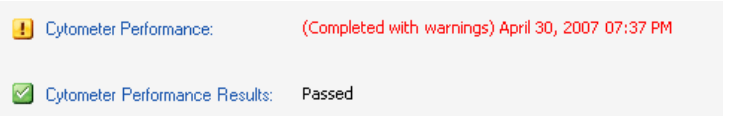
- To view the Cytometer Performance Report, click View Report. See Reviewing the Cytometer Performance Report on page 93.
- To complete the performance check and return to the Setup View of the workspace, click Finish.

3 Remove the tube from the cytometer, carousel from the Loader, or plate from the HTS when prompted.

4 The cytometer performance results appear in the System Summary.

- If Cytometer Performance was successful, cytometer performance results are shown as passed along with the date and time of the run (circled in green in the figure preceding).

- If Cytometer Performance passed with warnings, a message similar to the following appears in the System Summary. For information on troubleshooting a performance check, see page 115.



- If Cytometer Performance failed, the following message appears. For information on troubleshooting a failed performance check, see page 115.

BD FACSDiva software does not prevent you from running your experiment with a failed performance check. However, failed settings might impact your experiment and Levey-Jennings charts. Be sure to assess the impact on your experiment before you proceed.

NOTICE If you run an experiment with a failed performance check or a performance check with warnings, the CST keyword in the FSC file displays a Failed or Warning condition.

NOTICE A failed performance check will impact application settings. Therefore, save a new application settings file. For more information, see Application Settings Tasks on page 96.

Reviewing the Cytometer Performance Report

The Cytometer Performance report contains cytometer information, setup beads lot information, detector and laser settings, specifications, cytometer settings, and pass/fail results. Figure 4-1 on page 94 shows the first page of an example Cytometer Performance report. Descriptions of measurements are listed, beginning with Detector Settings on page 95.

Figure 4-1 Example Cytometer Performance report

Cytometer Performance Report

Cytometer:	FACSCantoII	User:	Administrator
Cytometer Name:	FACSCantoII	Institution:	N/A
Serial Number:	V96300005	Software:	BD FACSDiva 6.0
Input Device:	HTS	Date:	05/18/2007 07:50 AM
Setup Bead Position:	A1	Cytometer Baseline:	05/17/2007 04:12 PM
Cytometer Configuration:	4-2-2 with APC - H7 WE 2.0	P/F:	Pass

Setup Beads

Bead Product: CST Setup Beads, Part #: 910723
Lot ID: 42307, Expiration Date: 04/23/2008
Bead Lot Information: Available

Detector Settings

Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead % Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
Blue	FSC	FSC	150000	150118	0	1.34	149749	1.15
Blue	F	SSC	150000	150017	0	3.25	150380	2.9
Blue	E	FITC	18126	17621	-3	1.98	784	9.19
Blue	D	PE	21667	21373	-2	1.68	760	8.96
Blue	B	PerCP-Cy5-5	32926	31861	-4	3.07	1161	12.75
Blue	A	PE-Cy7	33233	31977	-4	4.88	849	22.46
Red	C	APC	33707	33040	-2	2.82	1804	10.56
Red	A	APC-Cy7	35285	34670	-2	3.17	1329	9.69
Violet	B	Pacific Blue	13666	13439	-2	1.76	724	6.08
Violet	A	AmCyan	157094	156844	-1	1.68	7057	5.7

Detector Settings (Continued)

Laser	Detector	Parameter	Dim Bead Median Channel	Dim Bead % Robust CV	PMTV	Δ PMTV	Qr	Br	P/F
Blue	FSC	FSC	24743	1.43	511	11	N/A	N/A	Pass
Blue	F	SSC	72208	2.03	463	5	N/A	N/A	Pass
Blue	E	FITC	97	36.06	474	-3	0.0266	488	Pass
Blue	D	PE	172	27.16	419	-2	0.2388	396	Pass
Blue	B	PerCP-Cy5-5	148	36.74	489	-7	0.0132	14	Pass
Blue	A	PE-Cy7	61	94.63	599	-10	0.0184	22	Pass
Red	C	APC	143	38.26	506	-12	0.0166	42	Pass
Red	A	APC-Cy7	81	44.48	533	-13	0.0044	973	Pass
Violet	B	Pacific Blue	87	31.32	426	1	0.0805	2648	Pass
Violet	A	AmCyan	523	34.7	541	4	0.0141	4516	Pass

Specifications

PMTV Delta from baseline: 50 (BD)
Violet Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)
Blue Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)
Red Laser Primary Channel Bright Bead %Robust CV: 6.00 (Recommended)

Research Use Only

Page 1 of 2

Detector Settings

Measurement	Description
Laser	Laser name
Detector	Scatter or fluorescence detector
Parameter	Fluorochrome name (assigned)
Target Value	Target value defined by baseline
Actual Target Value	Target value measured during performance check
% Difference Target Value	Percent difference between baseline target value and target value determined during the performance check
Bright Bead % Robust CV	Percent robust coefficient of variation of bright beads
Mid Bead Median Channel	Median fluorescence intensity (MFI) value of mid beads—term used in the calculation of photon detection efficiency (Qr) and linearity
Mid Bead % Robust CV	Percent robust coefficient of variation of mid beads—term used in the calculation of photon detection efficiency (Qr)
Dim Bead Median Channel	MFI value of dim beads—term used in the calculation of relative optical background fluorescence (Br) in the detector. (Dim bead brightness approximates that of negatively stained lymphocytes.)
Dim Bead % Robust CV	Percent robust coefficient of variation of dim beads—term used in the determination of relative optical background (Br) in the detector
PMTV	PMT voltage value from current performance check
ΔPMTV	Difference between PMT voltage value for baseline check and current performance check
Qr	Relative fluorescence detection efficiency—used for tracking light collection efficiency of a detector

Br	Relative optical background signal—used for tracking optical background levels in a detector
P/F	Pass or Fail—cytometer performance passes if difference between baseline PMT setting and current PMT setting is within 50 V (BD recommended) or a custom-defined setting (see Specifications on page 79)

Specifications

These values are the BD recommended values for the following:

- PMTV delta from baseline—the default is 50 V. Users with administrative privileges can modify the specification. See Specifications on page 79.
- Bright bead %robust CV for the primary detector of each laser—this value is 6.00 for BD cytometers with standard configurations (lasers, filters, and mirrors).

For information on troubleshooting Cytometer Performance reports, see page 115.

Application Settings Tasks

This section guides you through application settings tasks.

- Working With Application Target Boxes on page 97
- Creating Application Settings on page 98
- Using Application Settings in an Experiment on page 102

Refer to the *BD FACSDiva Software Reference Manual* for instructions on applying compensation, recording data, analyzing data, and shutting down the system.

Application settings provide an easy and reproducible way to reuse cytometer settings for commonly used applications. Application settings are associated with a cytometer configuration and include parameters needed for the application: PMT voltages, area scaling factors, window extension values, and threshold values. Note that application settings do not include compensation values.

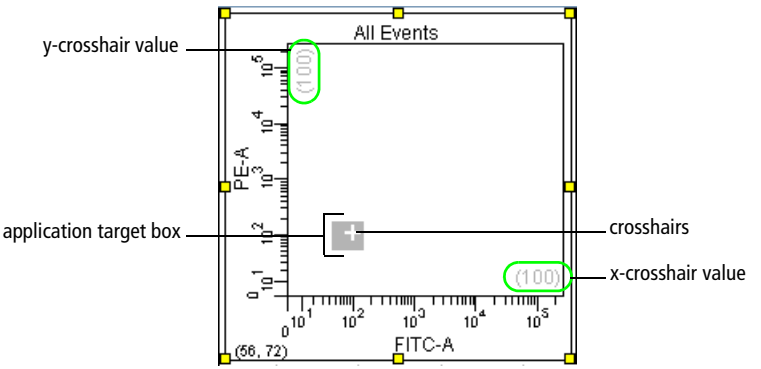
Each time you run a performance check for a configuration, application settings associated with that configuration are updated.

Before you can create application settings, you must run a performance check with the cytometer configuration that will be used for the application.

NOTICE Each time you define a new baseline, verify that application settings are still appropriate. Adjustment might be necessary.

Working With Application Target Boxes

An application target box appears on plots created with the Applications Worksheet feature. The target box represents a range that the software determines is optimal for resolving dimly fluorescing populations.



- The size of the application target box represents the SD of electronic noise of the dim beads in each detector. The default value is 2 SD, but can be set from 1 to 4 SD depending on the application. See Creating Application Settings on page 98.

- The crosshairs in each application target box represent 10 times the standard deviation of electronic noise for that detector.

- The x-crosshair and the y-crosshair values are displayed next to each axis (circled in green in the preceding figure).

Use the Application Target boxes as guides for adjusting the PMTVs for samples. The software sets the PMTVs to provide appropriate resolution with dimly fluorescing populations. These settings might not be optimal for all sample types and might require adjustment.

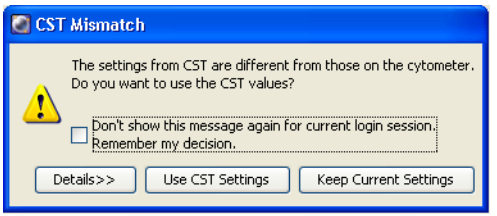
Use the Application Target boxes to decide whether to increase or decrease PMTVs. Placing the negative population below these Application Target boxes might compromise low-end resolution. However, this might be required to bring the bright staining population on scale. In general, BD recommends maintaining the negative populations within the shaded areas.

NOTICE The starting PMT voltages generated by the software might place some of the negative populations below the application target boxes. This is normal for some parameters and will not affect resolution for these parameters.

Creating Application Settings

1 Exit Cytometer Setup and Tracking by choosing File > Exit.

The following dialog appears.



- If you choose Use CST Settings, the latest CST cytometer settings from the currently set configuration will be applied.

- If you choose Keep Current Settings, the cytometer settings last used for the logged in user will be applied.

2 In the BD FACSDiva workspace, click the New Experiment button on the Browser toolbar.

NOTICE If you changed the default experiment template for the Browser toolbar, create the experiment from the Blank Experiment or Blank Experiment with Sample Tube default experiment template.

3 In the new experiment, click the experiment-level Cytometer Settings.

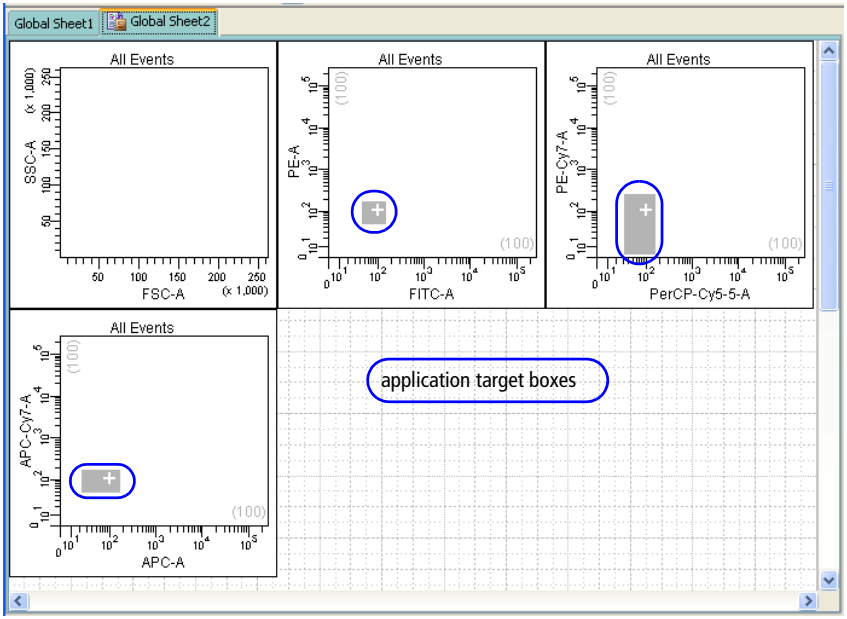
4 In the Cytometer Settings Inspector, delete unused parameters for the application.

5 In the Browser, right-click the experiment-level Cytometer Settings, and choose Application Settings > Create Worksheet.

A new global worksheet with plots specifically configured for each parameter is created. Each plot contains an application target box (circled in blue in Figure 4-2 on page 100).

By default, the application target box is set to display a range of 2 SD for electronic noise of the dim beads.

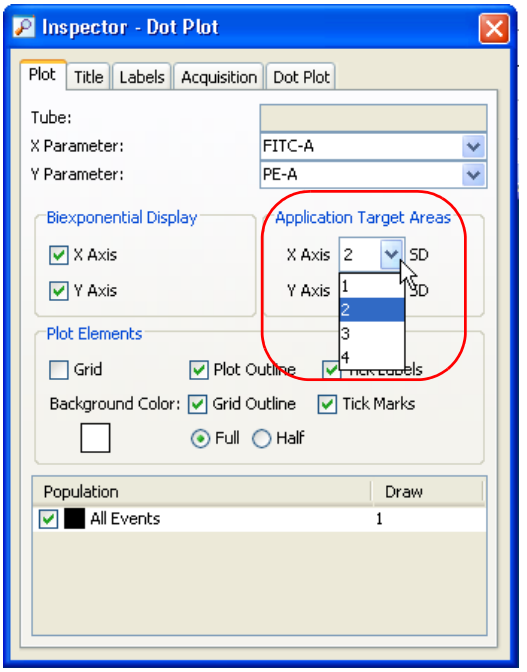
Figure 4-2 Global worksheet showing application target boxes



6 (Optional) To change the size of the application target box, do the following:

- In the Worksheet, select the plot you want to change.
- Open the Plot tab of the Dot Plot Inspector. See Figure 4-3 on page 101.
- In the Application Target Areas, click the arrow to display either the X or Y Axis SD menus, and choose a number.
- Repeat for the other axis.

Figure 4-3 Dot Plot Inspector



7 Run a positively stained control with fluorochromes for each parameter.

8 In the Cytometer window, optimize the settings for your application, if necessary.

- FSC and SSC voltages
- Threshold value
- Area scaling factors

9 Use the application target boxes as guides for adjusting fluorescence voltages for your specific application, as needed.

- If the positively stained populations are on scale, no adjustment is necessary.
- If the positively stained populations are off scale, lower the PMTVs until the populations are on scale and in the linear range for the detector.

10 Right click the experiment level cytometer settings and choose Application Settings > Save.

A save dialog appears.

11 Name the application settings, and click OK.

Using Application Settings in an Experiment

Before you can apply application settings to your experiment, you must have a performance check for the configuration you are using.

☑ **Tip** Use the Reports view to quickly check whether a performance check exists for a specific configuration.

1 In the BD FACSDiva workspace, choose Edit > User Preferences to verify that the correct user preferences are set for your experiment.

2 Click the New Experiment button on the Browser toolbar.

NOTICE If you changed the default experiment template for the Browser toolbar, create the experiment from the Blank Experiment with Sample Tube default experiment template.

Applying Application Settings

1. In the new experiment, right-click the experiment-level Cytometer Settings and choose Application Settings > Apply.

2. In the Application Settings catalog, select the application settings file you saved in Creating Application Settings on page 98. Click Apply.

   If the parameters are not the same, a mismatch dialog appears.

   - Click Overwrite to change all.
   - Click Apply to change common parameter.

   For more information, refer to the *BD FACSDiva Software Reference Manual*.

   When applications settings are applied to an experiment, the following occurs:

   - The cytometer settings are renamed application settings and the cytometer settings icon in the Browser changes.
   - The cytometer settings are updated with the parameters included in the application settings, optimized PMT voltages, threshold settings, area scaling factors, and window extension values.

Performing Compensation

Once application settings have been applied, perform compensation according to your laboratory's standard procedure. Refer to the *BD FACSDiva Software Reference Manual* for instructions.

Current Cytometer Setup and Tracking Settings

To apply the current Cytometer Setup and Tracking settings to an existing experiment, do the following:

In the experiment, right-click the experiment-level Cytometer Settings and choose Apply Current CST Settings.

If the Cytometer Setup and Tracking settings have expired or have warnings associated with them, a warning message appears. Click OK to use the Cytometer Setup and Tracking settings or Cancel to use the default cytometer settings.

NOTICE You can apply current Cytometer Setup and Tracking settings to an experiment only if the experiment meets the following criteria:

- Does not contain specimen- or tube-level cytometer settings
- Does not contain recorded data

Experiment Setup for Offline Analysis

Follow this procedure to set up experiments on an Analysis workstation that is not connected to a cytometer.

1. Start the Cytometer Setup and Tracking module.
2. On the acquisition workstation, select the configuration you want copy.
3. Copy the configuration, rename it, and save it.
4. Right-click the configuration and choose Export Configuration.
5. In the Export dialog, choose where to save the configuration and click Save.

6 On the analysis work station, import the exported configuration from the acquisition workstation.

7 In the Cytometer Configuration window, select the imported configuration and click Set Configuration.

8 Click Yes in the message dialog that appears asking whether you want to change the configuration.

9 Quit Cytometer Setup and Tracking.

The analysis workstation is ready to create experiments for use on the acquisition workstation.

5

Troubleshooting

This section provides assistance for specific Cytometer Setup and Tracking problems. For general software troubleshooting, refer to the *BD FACSDiva Software Reference Manual*. For cytometer troubleshooting, refer to your cytometer reference manual. If additional assistance is required, contact your local BD Biosciences technical support representative. Go to our website, bdbiosciences.com, for up-to-date contact information.

General Troubleshooting

Observation or Error Message	Possible Causes	Recommended Solutions
No beads detected	Beads not mixed prior to dispensing	Vortex the beads vial, prepare a fresh beads suspension, and rerun the tube or well.
	Beads too dilute	
	No beads added to the tube or wells	
	Debris in the beads suspension	
	Incorrect beads added	
	Low flow rate improperly calibrated	Contact BD Biosciences.
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. Refer to the flow cytometer reference manual for more information.
	Clogs within sample tubes and lines	Check the fluidics for clogs and debris. Refer to the flow cytometer reference manual for more information.
	Backpressure in waste lines	Check the waste tank vent for obstruction.
	Neutral density filter 2.0 installed in the FSC detector	Replace the filter with a lower rated neutral density filter.
	High scatter noise (FSC or SSC)	• Perform monthly maintenance. • Call BD Biosciences.
Cytometer Setup and Tracking interface does not start	Error message hidden behind the BD FACSDiva workspace	Minimize the BD FACSDiva workspace to see the error message.

General Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Unable to connect to the cytometer after exiting Cytometer Setup and Tracking	Configuration file was not successfully sent to cytometer	**1** Choose Cytometer > View Configuration. **2** Set the appropriate configuration. **3** Close Cytometer Setup and Tracking.
Sample rate too low to complete analysis	Beads not mixed prior to dispensing	Vortex the beads vial, prepare a fresh beads suspension, and rerun the tube or well.
	Beads too dilute	
	Debris in the beads suspension	
	Incorrect beads added	
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. Refer to the flow cytometer reference manual for more information.
	Backpressure in waste lines	Check the waste tank vent for obstruction.
	Low flow rate	• Prepare a fresh beads suspension, increasing the number of drops of Cytometer Setup and Tracking beads. Rerun the tube or well. • Contact BD Biosciences.
Apply Current CST Settings not available in the shortcut menu	Experiment has specimen- or tube-level cytometer settings	Delete the specimen- or tube-level cytometer settings.

General Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Apply Application Settings not available in shortcut menu	• Experiment has specimen- or tube-level cytometer settings • Experiment does not have an associated configuration	Apply the current CST settings to the experiment-level cytometer settings.
CST keywords not displayed in the Statistics FCS file header	Cytometer settings are no longer the current CST settings values	Right-click the experiment level cytometer settings and choose Apply Current CST Settings.
Create Worksheet not available in shortcut menu	• Experiment has specimen- or tube-level cytometer settings • Experiment does not have an associated configuration	Apply the current CST settings to the experiment-level cytometer settings.
	• Performance check not performed	Run a performance check.
	• CST settings were not applied	Apply the current CST settings to the experiment-level cytometer settings.

General Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Unable to set laser delays	Beads not mixed prior to dispensing	Vortex the beads vial, prepare a fresh beads suspension, and rerun the tube or well.
	Beads too dilute	
	Debris in the beads suspension	
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. Refer to the flow cytometer reference manual for more information.
	Unstable sheath pressure	Check the fluidics for leaks, bubbles and clogs. Refer to the flow cytometer reference manual for more information.
	High scatter noise (FSC or SSC)	• Perform monthly maintenance. • Call BD Biosciences.
Unable to use panel template to create new specimen	Panel template associated with a configuration that differs from experiment configuration	Use a panel template that does not contain cytometer settings.

Baseline Troubleshooting

Observation or Error Message	Possible Causes	Recommended Solutions
Performance (Qr, Br, SD_{EN}, PMTV, etc) differs after changing beads lot	Lot-specific information for new beads lot unavailable	Download the current lot-specific information for the Cytometer Setup and Tracking beads. See Before Running Reset Target Values on page 65.
Response curves displayed in baseline results plots are uncharacteristic	Dim beads are not well resolved in channel.	Try using different filters to better resolve the dim beads from the mid beads.
Alignment warning: Bright bead % rCV for primary channel is greater than 6%	Beads expired	Prepare a fresh beads suspension using the new beads lot and rerun the tube or well.
	Beads exposed to direct light	Prepare a fresh beads suspension and rerun the tube or well.
	Air bubbles in flow cell or sheath filter	Check the fluidics for bubbles and debris. Refer to the flow cytometer manual for more information.
	Insufficient warm-up time of the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. Refer to the cytometer manual for requirements.
	Dirty flow cell	Perform the monthly cleaning procedure. Refer to the cytometer manual for instructions.
	Suboptimal filter and mirror configuration	Refer to the cytometer manual for standard configurations.
	Cytometer alignment changed	Contact BD Biosciences.

Baseline Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Unable to calculate Qr	Insufficient bead intensity in the selected parameter	See page x for filter and mirror limitations.
	Beads expired	Prepare a fresh beads suspension using the new beads lot and rerun the tube or well.
	Beads exposed to direct light	Prepare a fresh beads suspension and rerun the tube or well.
	Low dim bead %rCV caused by high laser power or low sheath pressure	• Refer to the cytometer manual for standard cytometer configurations. • Contact BD Biosciences.
	Debris in the beads suspension	Vortex the beads vial, prepare a fresh beads suspension, and rerun the tube or well.
All values for a particular fluorochrome appear as NA	PMT failure	• Prepare a new bead solution and rerun the baseline. • Perform the long clean procedure on the cytometer. Refer to the cytometer reference manual for instructions. • Contact BD Biosciences.
	Incorrect filters used	See page x for filter and mirror limitations.
%rCV and rSD NA	Insufficient bead intensity in the selected parameter	See page x for filter and mirror limitations.

Baseline Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Linearity warning: Unable to reach maximum channel with maximum PMTV	Insufficient bead intensity in the selected parameter	See page x for filter and mirror limitations.
	Beads expired	Prepare a fresh beads suspension using the new beads lot and rerun the tube or well.
	Beads exposed to direct light	Prepare a fresh beads suspension and rerun tube or well.
	Debris in the beads suspension	Vortex the beads vial, prepare a fresh beads suspension, and rerun the tube or well.
	Insufficient warm-up time of the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. Refer to the cytometer manual for requirements.
	Dirty flow cell	Perform the monthly cleaning procedure. Refer to the cytometer manual for instructions.
	Cytometer alignment changed	Contact BD Biosciences.
Area Scaling Factor (ASF) value flagged	• Unstable fluidics • Air bubbles in the flow cell or sheath filter	• Purge the flow cell. • Perform monthly cleaning procedure. Refer to the cytometer manual for instructions. • Contact BD Biosciences.
	• Ratio of Minimum ASF to Maximum ASF >3 • Laser is out of alignment	Contact BD Biosciences.

Performance Check Troubleshooting

Observation or Error Message	Possible Causes	Recommended Solutions
PMT settings change >50 volts (or user specified value) between performance checks	Beads expired	Prepare a fresh beads suspension using the new beads lot and rerun the tube or well.
	Beads exposed to direct light	Prepare a fresh beads suspension and rerun the tube or well.
	Baseline not reset after cytometer maintenance	Redefine the baseline. See Baseline Definition on page 48.
	Incorrect filters or mirrors installed	Make sure that the selected configuration matches the physical configuration of the cytometer.
	Dirty flow cell	Perform the monthly cleaning procedure. Refer to the cytometer manual for instructions.
	Air bubbles in the flow cell	Check the fluidics for bubbles and debris. Refer to the cytometer manual for more information.
	Insufficient warm-up of the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. Refer to the cytometer manual for requirements.
	Cytometer alignment changed	Contact BD Biosciences.
FSC settings change >50 volts (or user specified value) between performance checks	Incorrect neutral density filter installed (BD FACSAria platform only)	Install the correct neutral density filter. (BD FACSAria platform only)

Performance Check Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Performance check failure	The rCV ratio of dim to mid beads is less than 1.5	• Prepare a fresh beads suspension and rerun the performance check. • Perform the monthly cleaning procedure. Refer to the cytometer manual for instructions. • Troubleshoot the detector in BD FACSDiva software.
	Insufficient warm-up time of the cytometer lasers	Allow the cytometer lasers sufficient time to warm up. Refer to the cytometer manual for laser warm-up requirements.
Alignment warning: Bright bead %rCV for primary channel is greater than 6%	Beads expired	Prepare a fresh beads suspension using the new beads lot and rerun the tube or well.
	Beads exposed to direct light	Prepare a fresh beads suspension and rerun the tube or well.
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. Refer to the cytometer manual for more information.
	Insufficient warm-up time of the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. Refer to the cytometer manual for requirements.
	Dirty flow cell	Perform the monthly cleaning procedure. Refer to the cytometer manual for instructions.
	Suboptimal filter and mirror configuration	Refer to the cytometer manual for standard configurations.
	Cytometer alignment changed	Contact BD Biosciences.

Performance Check Troubleshooting (continued)

Observation or Error Message	Possible Causes	Recommended Solutions
Unable to calculate Qr	Insufficient bead intensity in the selected parameter	See page x for filter and mirror limitations.
	Beads expired	Prepare a fresh beads suspension using the new beads lot and rerun the tube or well.
	Beads exposed to direct light	Prepare a fresh beads suspension and rerun the tube or well.
	Low dim bead %rCV caused by high laser power or low sheath pressure	• Refer to the cytometer manual for standard cytometer configurations. • Contact BD Biosciences.
	Debris in the beads suspension	Vortex the beads vial, prepare a fresh beads suspension, and rerun the tube or well.
Area Scaling Factor (ASF) value flagged	• Unstable fluidics • Air bubbles in the flow cell or sheath filter	• Purge the flow cell. • Perform the monthly cleaning procedure. Refer to the cytometer manual for instructions. • Contact BD Biosciences.
	• Ratio of Minimum ASF to Maximum ASF >3 • Laser is out of alignment	Contact BD Biosciences.

Reset Target Values Troubleshooting

Observation or Error Message	Possible Causes	Recommended Solutions
Performance (Qr, Br, SD_{EN}, PMTV, etc) differs after changing beads lot	Lot-specific information for the new beads lot unavailable	Download the current lot-specific information for the Cytometer Setup and Tracking beads. See Before Running Reset Target Values on page 65.

Glossary

application settings	Cytometer settings that are linked with a specific cytometer configuration and automatically adjusted according to results obtained during the Cytometer Setup and Tracking daily setup
area scaling	A correction factor that places area measurements on the same scale as height measurements
Background (B)	Optical background fluorescence
Br factor	Relative optical background fluorescence
Cytometer Setup and Tracking beads	Particles used to automatically perform basic cytometer setup and performance tracking
Cytometer Setup and Tracking workspace window	Computer work area in which cytometer setup and tracking tasks are performed
coefficient of variation (CV)	The standard deviation of the data divided by the mean of the data; typically expressed as a percentage (also known as Relative Standard Deviation) When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.
compensation	The process by which spillover fluorescence is removed from secondary parameters so that fluorescence values for a parameter reflect only the fluorescence of the primary fluorophore
cytometer settings	Collection of values for parameters measured, photomultiplier (PMT) voltages, threshold, compensation, and any ratio measurements

cytometer setup and tracking
A group of setup and cytometer management features in BD FACSDiva software

global worksheet
Worksheet for which elements can be used to display multiple data sets by moving the current tube pointer

See also Worksheet window.

laser delay
Amount of time between signals from different laser intercepts

Median Fluorescence Intensity (MFI)
The median channel of the cytometer response for a population

parameter
Measurement of a cell property that is ascertained as the cell passes through the laser beam

Each parameter is the output of a single photomultiplier tube or photodiode, measuring fluorescent or scattered light.

population
Data subset defined by a gate or interval

relative fluorescence detection efficiency (Qr)
Relative fluorescence detection efficiency for each fluorescent detector.

robust coefficient of variation (rCV)
Robust coefficient of variation is calculated as follows:

%rCV = ((rSD)/median) x 100)

robust standard deviation (rSD)
The rSD is calculated as follows.

The median of the data sample is computed:

$$\theta_{median} = med_i\{x_i\}$$

From that, the median absolute deviation is computed:

$$\sigma_{median} = med_i\{(|x_i - \theta_{median}|)\}$$

Then the robust standard deviation is computed:

The constant is: $\sigma_{median} = \sigma_{median} / \phi^{-1}(0.75)$

specimen
A Browser object representing the type of material to be analyzed, the collection date, and user-defined keywords

spectral overlap Fluorescence detected in a channel other than the one for which it is intended

standard deviation (SD) A measure of the spread around the mean for events within a defined population, defined as

$$SD = \sqrt{\sum_{i=1}^{n} (X_i - \overline{X})^2 / (n-1)}$$

threshold A trigger signal and level of discrimination to eliminate unwanted events

Only events with parameter values above the threshold will be analyzed.

window extension Extension of the time during which a pulse is sampled

Worksheet window Window for viewing analysis objects on normal worksheets or global worksheets

workspace window A component in the BD FACSDiva software workspace

Workspace windows can be hidden or shown, resized, and closed. The visibility, size, and position are saved when you exit the software and are restored when you start the software the next time.

Appendix A

Upgrading BD FACSDiva Software

This appendix describes how to upgrade BD FACSDiva software v4.1.2 or v5.0 to v6.0 and how to set your cytometer configuration after the upgrade.

Upgrading the Software

1. Start the current version of BD FACSDiva software (either v4.1.2 or v5.0).
2. Select the instrument configuration that corresponds to your cytometer's physical configuration.
3. Exit the current version of BD FACSDiva software.
4. Load BD FACSDiva software v6.0 according to the installation instructions.
5. Start BD FACSDiva software v6.0.

Setting a Configuration

You need to set a configuration using the Cytometer Setup and Tracking interface even if you will not use its features. After upgrading the software, you can set a configuration defined in a previous version or set a configuration defined in BD FACSDiva software v6.0.

- During the upgrade process, configurations defined in the previous software version are copied to an Old Cytometer Configurations folder.
- During the upgrade process, the current configuration in the previous software version becomes the base configuration in the current version. The base configuration will not have filter and mirror information defined.

  NOTICE Filter and mirror information is required only when using the Cytometer Setup and Tracking features.

Setting a Configuration and Exiting Cytometer Setup and Tracking

1. From BD FACSDiva software v6.0, choose Cytometer > View Configurations to open the Cytometer Configurations window.
2. In the Configurations list, do one of the following:
   - Open the Base Configuration folder and select the base configuration.
   - Open the Old Cytometer Configurations folder and select a configuration that corresponds to your cytometer's physical configuration.
3. Click Set Configuration.
4. Click OK to close the Cytometer Configurations window.
5. Choose File > Exit to close the Cytometer Setup and Tracking workspace.

Setting a Configuration and Using Cytometer Setup and Tracking

1. From BD FACSDiva software v6.0, choose Cytometer > CST.
2. In the Setup Control window, click Select Configuration.
3. In the Configurations list, open the Base Configuration folder and select the base configuration.
4. Double-click to edit the configuration.
5. Assign filter and mirror information. See Assigning Parameters, Filters, and Mirrors on page 44.
6. Click Set Configuration.
7. Define a baseline. See page 49.
8. Run a performance check. See page 91.
9. Choose Exit > CST.
10. Set up and run your experiment in the BD FACSDiva workspace.

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