BD™ Cytometer Setup and Tracking Application Guide

Software Version 3.0

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Regulatory information

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Notice

History

Revision	Date	Change made
642342 Rev. A	6/07	Initial release
23-12881-00 Rev. 01	10/2010	Updated for BENEX contact information changes.
23-11220-00 Rev. 01	4/2012	Revised for new features in BD FACSDiva v7.0.
23-15134-00	5/2013	Revised for BD FACSDiva v8.0.

Contents

Chapter 9: Software preferences

Chapter 10: Reference information

Chapter 11: Troubleshooting

Glossary

Index

1

Introduction

The following topics are covered in this chapter:

About this guide

Introduction This topic describes the documentation available to support your use of the BD™ Cytometer Setup and Tracking application (CS&T) with BD FACSDiva™ software.

Before you begin This guide assumes that you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, see the documentation provided with your computer.

Help system The following documentation is available on your computer to help you learn how to use the CS&T features:

- *BD Cytometer Setup and Tracking Application Guide*
- *BD FACSDiva Software Reference Manual*
- Your BD cytometer manual

The Help system contains all the information from these manuals and is enhanced with full text search to make it easier to find what you are looking for. You can also use the Index tab to locate topics. The Help system opens in a separate window so you can access the documentation while working in the software. To access the Help system, select Help > FACSDiva Help within BD FACSDiva software.

The manuals also are available as PDFs that can be opened, searched, and printed using Adobe® Acrobat® Reader®. To access the PDFs, select Start > Programs > BD FACSDiva Software.

More information
- Technical assistance (page 9)
- CS&T purpose and functions (page 14)

Technical assistance

Introduction   This topic describes how to obtain technical assistance.

On your computer   In BD FACSDiva software:

- Select **Help > FACSDiva Help** to locate and read topics specific to the operation you are performing.
- Select **Start > Programs > BD FACSDiva Software** to access the online version of the *BD™ Cytometer Setup and Tracking Application Guide* PDF.

Contacting BD Biosciences   When contacting BD Biosciences, have the following information available:

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

Note that BD Biosciences might also request the CS&T log file located at D:\BD\FACSDiva\CST\LogFiles, as well as your exported experiment file.

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

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

More information   • Troubleshooting (page 134)

Limitations

Introduction

This topic describes limitations you need to consider before using CS&T.

CS&T software and beads

The CS&T module and BD FACSDiva™ CS&T research beads (CS&T research beads) are for Research Use Only (RUO). Not for use in diagnostic or therapeutic procedures.

Bead lot file

> ⚠️ **Caution!** We recommend using the Qr (detector efficiency) and Br (optical background) values generated with non-standard configurations for tracking only. Do not use the Qr and Br values for comparison to other parameters.

When CS&T research beads are manufactured, a lot-specific file is created which 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. See the appropriate cytometer reference manual for information on standard configurations.

Upgrading CS&T software

For all instruments, upgrading from BD FACSDiva software version 6.x deletes the CS&T bead lot files and application settings in the catalog. Baseline, performance, and reset target values reports from the previous CS&T beads will still be present after the upgrade. You need to run new baselines and create new application settings for your cytometer configurations with the CS&T research beads.

Neutral density filters

The CS&T module does not support cytometers with neutral density filters installed that are 2.0 or greater. We recommend using a neutral density filter of 1.0.

Cytometer performance check duration

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

Cytometer configurations

Rules for using cytometer configurations are as follows:

- You can import configurations if they are 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.
- Do not start the CS&T module until BD FACSDiva software finishes starting. If you do, a message indicating that the cytometer is not connected is displayed when you click Set Configuration 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, an error message is displayed and the following functions are blocked:
  - Copy/paste tube, specimen, or plate with cytometer settings
  - Copy/paste cytometer settings
  - Apply cytometer settings from the catalog
  - Import cytometer settings

Resetting target values

To normalize a new lot of CS&T research beads to an existing lot, you must reset the target values using the existing lot and the new lot. Verify that you reserve a large enough volume of the existing lot of CS&T research beads to reset the target values. See the *BD FACSDiva CS&T Research Beads* technical data sheet for volume requirements.

Cytometer settings behavior

Laser delay values are always updated to the latest CS&T results.

Area scaling and window extension values belong to the cytometer and are not experiment-specific. Once these settings are modified, either manually or by applying application settings, all existing experiments will display the modified settings.

To reapply the latest CS&T settings to an experiment:

1. Right-click the cytometer settings.

2. Select **Apply Current CST Settings**.

To reapply the application settings to an experiment:

1. Right-click the application settings.

2. Select **Application Settings > Apply**.

For new experiments, a CST Mismatch dialog opens. Click the Details button to view the CS&T settings and verify the correct settings for your experiment.

More information

- About this guide (page 8)
- *BD FACSDiva Software Reference Manual*

2

CS&T overview

The following topics are covered in this chapter:

CS&T purpose and functions

Introduction   This overview describes the purpose and functions of the CS&T application when used with BD FACSDiva software and CS&T research beads.

Before you begin   Before using the CS&T application, make sure you are familiar with general cytometer functions as described in your BD cytometer manual.

Overview   The CS&T workspace in BD FACSDiva software and CS&T research beads can be used on the BD FACSCanto™, BD FACSCanto™ II, BD FACSAria™ II, BD FACSAria™ III, BD FACSAria™ Fusion, BD™ LSR II, and BD LSRFortessa™ flow cytometers. CS&T is for Research Use Only on all flow cytometers.

CS&T baseline and performance checks generate consistent settings for your experiments and provide you with better resolution of dim populations, fewer compensation artifacts, and reproducible data. By tracking your cytometer's performance, you can:

- Monitor performance trends.
- Use any number of lasers and detectors to create custom configurations with predefined or custom parameters, filters, and mirrors.
- Create application-specific settings for all of your experiments.

New functions for CS&T

The CS&T application includes the following new functions:

- New CS&T research beads:
    - Updated laser list with support for corresponding ABD values
    - Updated parameter list to support new fluorochromes
    - Updated filter and mirror lists
- 2D barcode reader support for importing bead lot files for the BD FACSCanto II.
- Automatic update of application settings based on the latest performance check.
- Target values can be reset any number of times.
- Changes to Levey-Jennings (LJ) charts:
    - Select up to 30 LJ charts per page to display or print on LJ performance reports
    - Select up to 30 variables based on parameters to plot on LJ performance charts
    - Option of selecting any two bead lots for use in LJ performance tracking

Baseline checks

Administrators can measure baseline performance for each cytometer configuration by running CS&T research beads. A baseline check does the following:

- Measures median fluorescence intensity (MFI) and percent robust CV (%rCV) for each bead intensity in all fluorescence detectors.
- Uses software algorithms to differentiate the fluorescence signal from each bead type based on size and fluorescence intensity in each detector.
- Evaluates linearity, detector efficiency (Qr), optical background (Br), electronic noise, and laser delays.
- Adjusts photomultiplier (PMT) voltages to maximize population resolution in each detector.

Performance checks

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.

Reports

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.

Application settings

Application settings are associated with a particular cytometer configuration and include the following parameters: PMT voltages (PMTVs), area scaling factors, window extension values, and threshold values. You can reuse cytometer settings for commonly used applications by saving the application settings to the catalog. When application settings are applied to an experiment, the PMTVs in the application settings are automatically updated based upon the most recent performance check.

More information

CS&T workspace overview

Introduction   This topic describes the major elements of the CS&T workspace.

CS&T workspace   The CS&T workspace contains controls that allow you to set up cytometer configurations and gather baseline and performance information.

Setup control

Menu bar

Workspace tabs

System summary

Status window
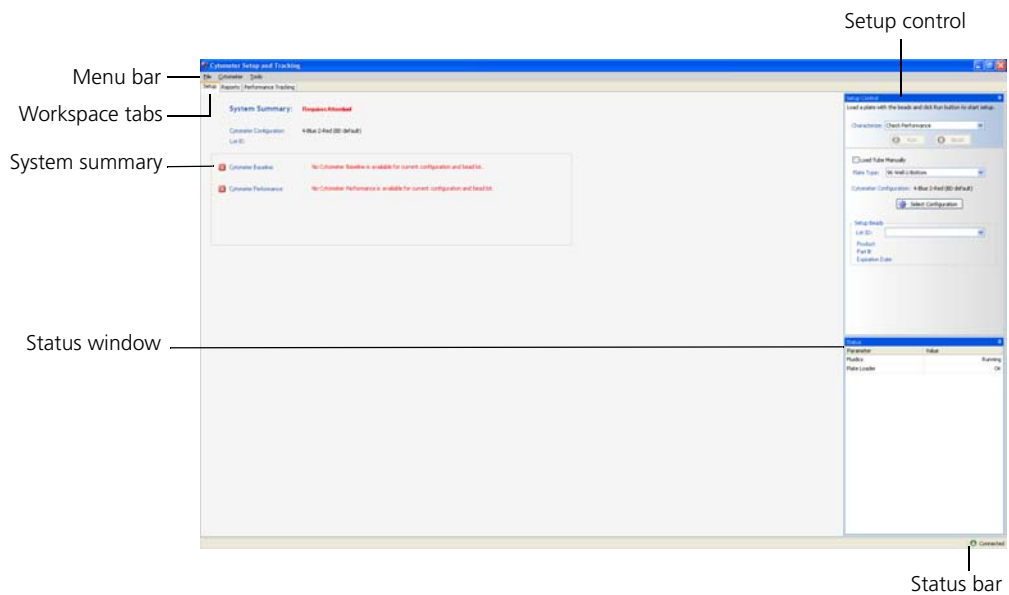
Status bar

Menu bar   The menu bar contains the File, Cytometer, and Tools menus. Select a menu command to perform the corresponding task. When keyboard shortcuts are available, they are listed next to the command.

Status bar   The status bar is located in the lower right corner of the CS&T workspace and indicates the connection status of the cytometer. The cytometer can be in any of the following states.

Cytometer state	Icon
Connecting	○ Connecting...
Connected	○ Connected
Disconnected	● Disconnected

When you select Cytometer > View Configurations or Cytometer > Performance Tracking (LJ) from the BD FACSDiva workspace, the cytometer remains disconnected. When you select Cytometer > CST, the cytometer connects to the CS&T interface.

More information   • CS&T workspace tabs (page 19)

CS&T workspace tabs

Introduction

This topic summarizes the functions of the workspace tabs (Setup, Reports, and Performance Tracking).

Some functions under the workspace tabs are only available for users with administrative privileges. Also, the Load Tube Manually option is only available for systems that have a BD FACS™ Loader (the Loader) or BD™ High Throughput Sampler (HTS) installed on the cytometer.

Setup tab

The Setup tab opens when you open the CS&T workspace. The setup workspace allows you to select bead lots, cytometer configurations, run baseline and performance checks, and reset target values. The options available depend on whether a user has operator or administrator privileges. An operator can only run a performance check.

There are three main windows in the Setup tab:

• In the Setup Control window, select which characterization to run: Define Baseline, Check Performance, or Reset Target Values. Select whether to load tubes manually, view the current cytometer configuration, access the Cytometer Configuration window, or select which lot of setup beads to use.
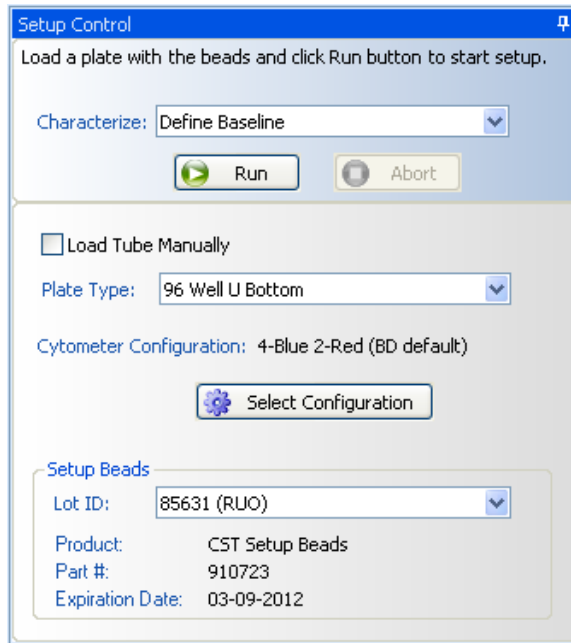
- The System Summary window under the Setup tab displays the cytometer setup status.
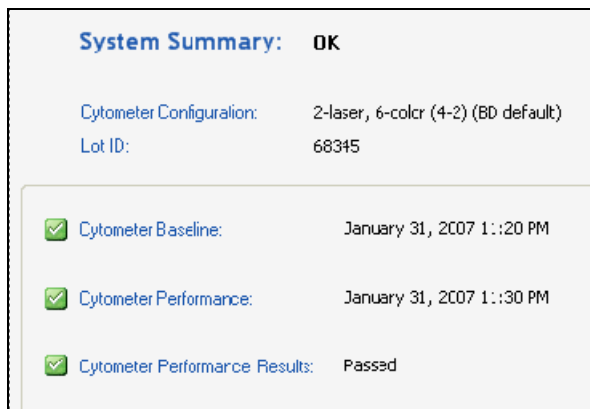
Green checkmark icons indicate that the baseline and performance check have passed. A baseline and performance check displaying yellow warning symbols can still pass cytometer performance. If any of the system summary information is displayed in red, see Troubleshooting (page 133). An expired baseline or performance check displays in red.
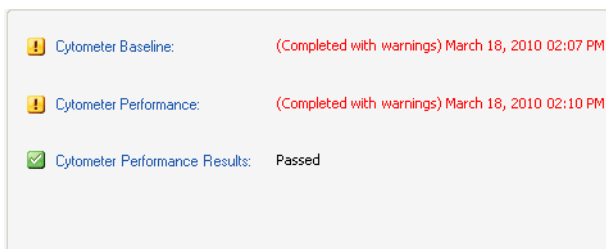
The following table describes the items in the Setup tab of the System Summary window.

Name	Description
System Summary	Status indicators: **OK.** Baseline and performance check passed without warnings. **Requires Attention.** Investigate baseline or performance check.
Cytometer Configuration	Current cytometer configuration.
Lot ID	Bead lot ID number.
Cytometer Baseline	Status indicator icon and date of the last cytometer baseline definition. For status indicator meaning, see the table in Reports tab (page 22).
Cytometer Performance	Status indicator icon and date of the last cytometer performance check.
Cytometer Performance Results	Status indicator icon and results of last cytometer performance check. Indicates either Passed or Failed.

• You can monitor the status of various parameters in the Status window. The parameters shown depend on which cytometer is connected.

Parameter	Value
Blue Laser Current	1.57
Blue Laser Power	20.02
Red Laser Power	27.19
Float Switch	OK
Cleaning Solution	OK
Shutdown Solution	OK
Sample Pressure	8.1
FACSFlow Pressure	4.5
FACSFlow Level	55
Waste Tank	45
Pump	OK
Vacuum	OK

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

Note that the violet laser standby mode is shown in the Status window (if applicable for your system).

Reports tab

Use the Reports tab to quickly check whether a baseline has been defined or if a performance check was completed for a specific configuration.

In the Reports window, you can view Reports Setup (see the following figure). Reports are organized by configuration. The Cytometer Baseline report for each configuration is listed first and is displayed in the window next to the browser. Cytometer Performance reports are listed under the Cytometer Baseline report and are stored in folders organized by year and month. If data for more than one bead lot exists, the Baseline Definition (Reset Target Values) report for each new lot is also listed.
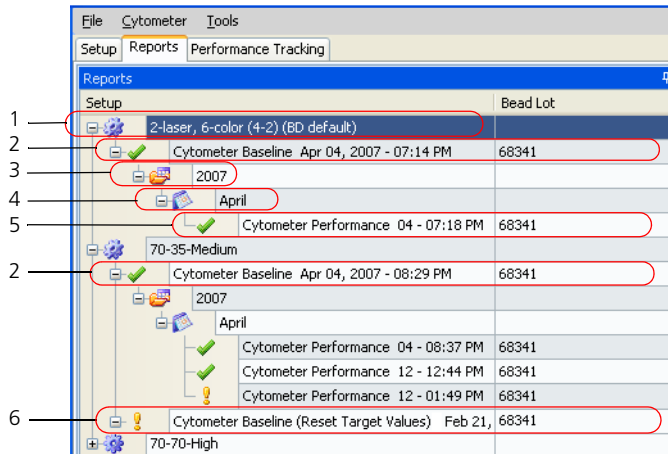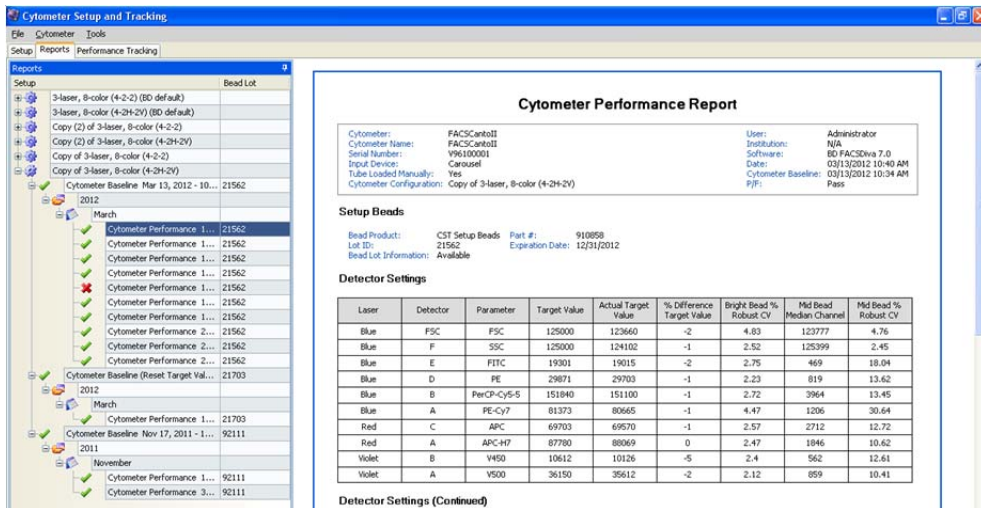
No.	Description
1	Cytometer configuration
2	Cytometer Baseline report
3	Folder named with the year the performance check was run
4	Folder named with the month the performance check was run
5	Cytometer Performance report
6	Cytometer Baseline with target values reset

The following table describes the icons under the Reports tab which indicate the status of each setup.

Icon	Description
✔	Passed
✖	Failed
❗	Some values out of recommended ranges

Icon	Description
	Monthly folder
	Yearly folder
	Configuration

The following figure shows an example Reports tab window with the Cytometer Performance report open.

Performance Tracking tab

The Performance Tracking tab is used to select which performance values you want to display and to view Levey-Jennings data charts. You can select to view up to 30 criteria at one time. Performance values can include PMTVs, optical background (Br), fluorescence detection efficiency (Qr), and %robust CVs for beads.
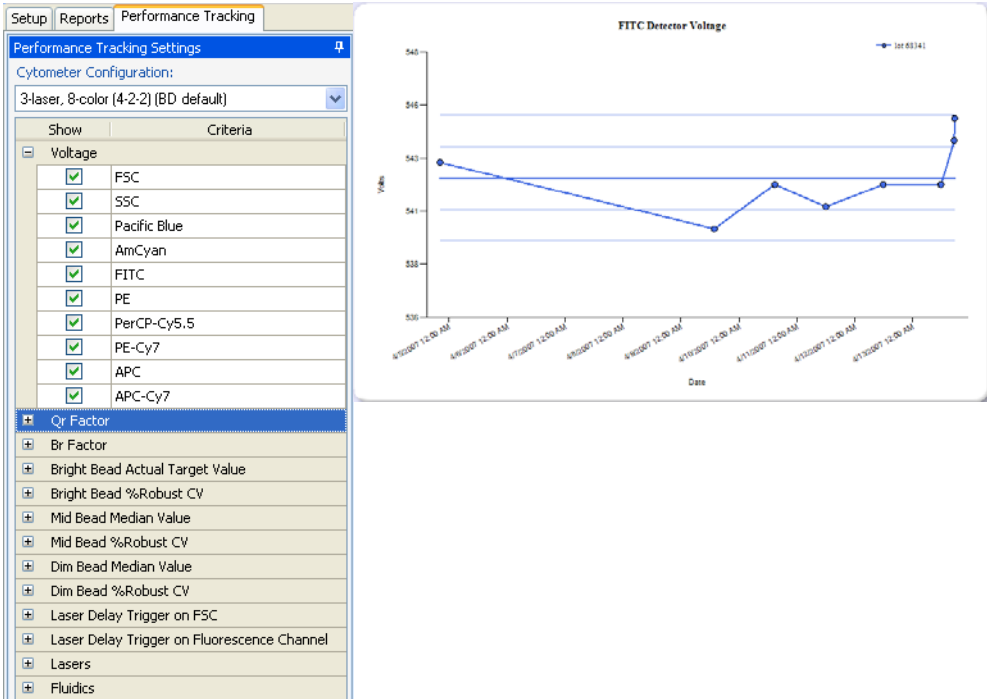
More information
- CS&T workspace overview (page 17)

CS&T workflow

Introduction This topic shows the tasks that can be performed with the CS&T functions and shows task workflows. Tasks are separated into those performed by the administrator and those performed by all users.

Before you begin Before you can use the CS&T functions, 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 sets up and maintains all user accounts. See the *BD FACSDiva Software Reference Manual* for information on managing accounts.

Routine tasks workflow This is the daily workflow for all users. See Routine tasks workflow (page 30).

Initial setup tasks workflow Before all users can perform the routine workflow, a user with administrator privileges needs to create the cytometer configurations, import the bead lot information, and run a baseline definition. These tasks are usually done in the order listed for initial setup and then on an as-needed basis.

Stage	Description
1	Cytometer configuration workflow (page 68)
2	Bead lot information workflow (page 102)
3	Baseline definition workflow (page 86)

Reset target values workflow When you need to switch to a new lot of CS&T research beads, a user with administrator privileges needs to reset the target values. See Reset target values workflow (page 102).

More information   •   CS&T purpose and functions (page 14)

•   *BD FACSDiva Software Reference Manual*

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3

Routine tasks

The following topics are covered in this chapter:

Routine tasks workflow

Introduction

This topic describes the routine tasks workflow. These tasks are typically performed daily. Use the CS&T features to set up, optimize, and track your cytometer settings to save time and ensure reproducible setup every day.

Before you begin

Before you can use the CS&T functions, a user with administrator privileges (usually the lab manager or supervisor) must perform some initial setup tasks. See Initial setup tasks workflow (page 26).

Routine tasks workflow

The workflow for routine tasks includes these stages.

Stage	Description
1	Starting the CS&T application (page 31)
2	Verifying the cytometer configuration (page 32)
3	Setting up for a performance check (page 33)
4	Running a performance check (page 35)
5	Applying CS&T settings (page 38)
6	Applying application settings to an experiment (page 40)

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

More information

- CS&T purpose and functions (page 14)
- CS&T workflow (page 26)

Starting the CS&T application

Introduction   This topic describes the startup procedure for the CS&T application.

Before you begin   Before you begin:

- Start your flow cytometer as instructed in the cytometer reference manual.
- Make sure you allow the cytometer lasers sufficient time to warm up.
- If an HTS is connected to your system, decide if you will run beads in a single tube or a multiwell plate.
- If a Loader is connected to your system, decide whether you will run the tube of beads manually or automatically.

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

Procedure   **To start the CS&T application:**

1. Log in to BD FACSDiva software.

2. From the **BD FACSDiva** workspace, select **Cytometer > CST**.

    The CS&T workspace opens.

    When you select **Cytometer > CST,** the cytometer disconnects from the BD FACSDiva interface and connects to the CS&T interface. The following message is displayed in the BD FACSDiva status bar at the bottom of the screen.

    BD FACSDiva Software is in CST mode, not accepting user input.

If you click in the BD FACSDiva interface while the CS&T interface is loading, the CS&T workspace is hidden behind the BD FACSDiva workspace. If the CS&T workspace does not open within a few seconds, check the Windows task bar and open the workspace from there.

Next step   Proceed to Verifying the cytometer configuration (page 32).

More information   •   Routine tasks workflow (page 30)

Verifying the cytometer configuration

Introduction   This topic describes how to verify the configuration for the cytometer you are using.

Procedure   **To verify the cytometer configuration:**

1.   In the **System Summary** window, verify the following.
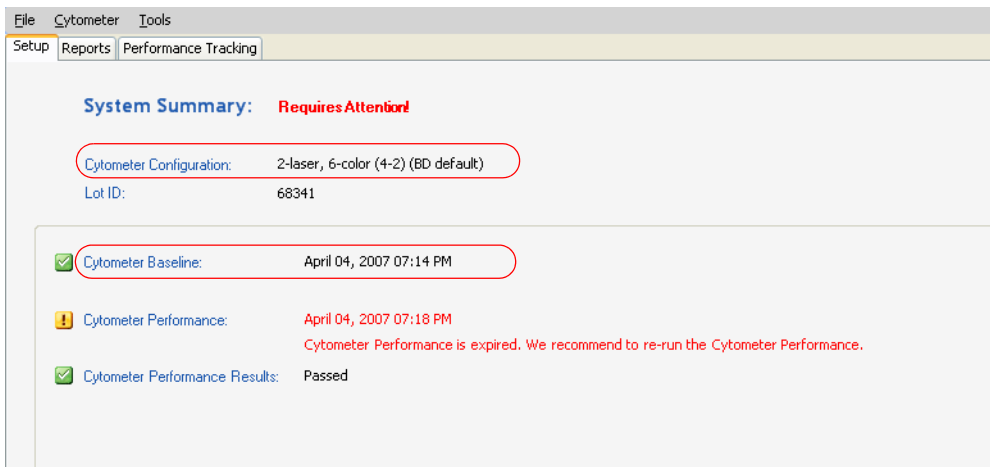
- Check that the cytometer configuration is appropriate for your experiment. If not, select another configuration. See Setting a configuration as the current configuration (page 80).

- Check that the cytometer baseline has been defined and is valid. If not, ask your administrator to define a new baseline. See Baseline definition workflow (page 86).

Next step Proceed to Setting up for a performance check (page 33).

More information
- Routine tasks workflow (page 30)
- CS&T workspace overview (page 17)

Setting up for a performance check

Introduction This topic describes how to set up the workspace, and how to prepare and load the CS&T research beads for a performance check.

Procedure **To set up for a performance check:**

1. Prepare the setup beads for tubes or a plate as instructed in the *BD FACSDiva CS&T Research Beads* technical data sheet.

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

2. Verify that the setup beads lot ID selected matches your current lot of CS&T research beads.

The bead lot ID can be found on the CS&T research beads vial. If not, have the administrator import the bead lot for you or use the menu to select the appropriate lot.

Bead lot ID
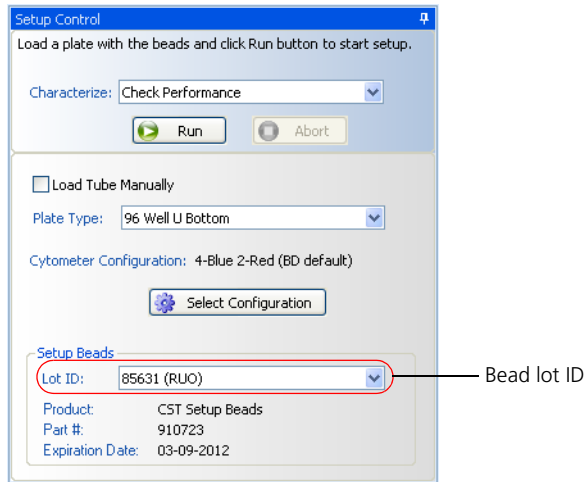
3. Load the CS&T research beads, then do one of the following:

   - If you are loading tubes manually, place the beads tube on the cytometer and select the **Load Tube Manually** checkbox.

   - If you are using the Loader, place the carousel on the Loader and clear the **Load Tube Manually** checkbox.

   - If you are using the HTS, place the plate on the HTS, clear the **Load Tube Manually** checkbox, and select the appropriate plate type from the **Plate Type** menu.

4. If you are manually loading tubes on a BD LSR II or BD LSRFortessa, set the flow rate to low.

   For all other platforms, the software sets the flow rate. If you are using an HTS, the HTS software sets the pressure.

Next step   Proceed to .

More information	• Routine tasks workflow (page 30)
	• Bead lot information workflow (page 102)
	• *BD FACSDiva CS&T Research Beads* technical data sheet.

Running a performance check

Introduction   This topic describes how to run a performance check.

Cautions   Running a performance check takes approximately 5 minutes, depending on your system. We recommend that you run a performance check every 24 hours.

⚠️   **Caution!**   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.

If you run an experiment with a failed performance check or a performance check with warnings, the CS&T keyword in the FCS file displays a failed or warning condition.

A failed performance check will impact application settings. For more information, see Application settings workflow (page 44).

Procedure   **To run a performance check:**

1. In the **Setup Control** window, select **Check Performance** and click **Run**.

   Note that if you are logged in as an operator, **Check Performance** is the only option shown in the **Characterize** menu.

After a brief pause, the Checking Cytometer Performance window opens.
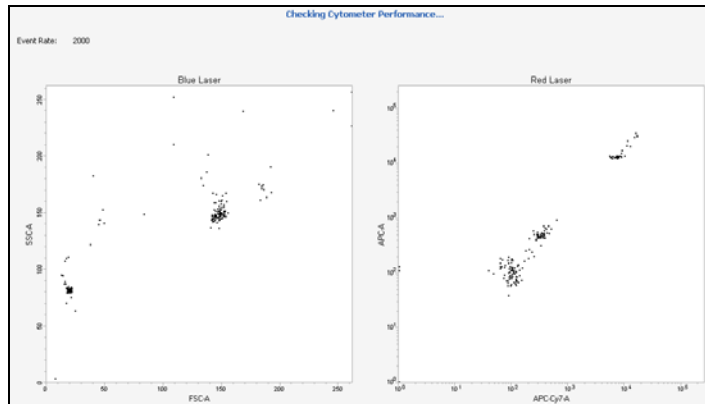
During the performance check, the following occur:

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

2. Once the performance check is complete, a dialog opens. Do one of the following:

- To view the Cytometer Performance report, click **View Report**. See Cytometer Performance report (page 62).

- To complete the performance check and return to the **Setup** view of the workspace, click **Finish**.

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

The cytometer performance results are displayed in the System Summary window.

— Date and time of run

— Pass/Fail results
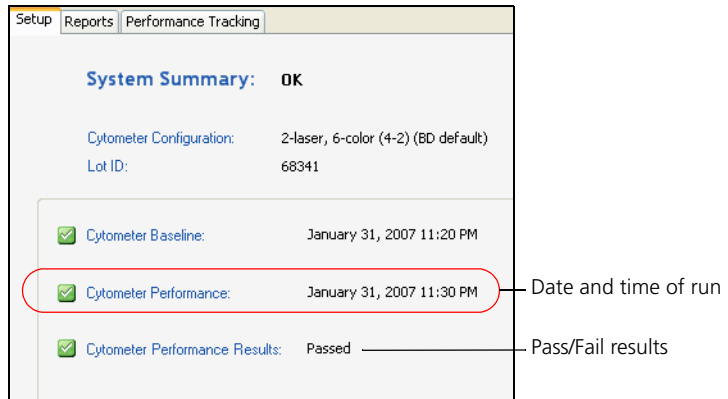
- If the performance check was successful, results are shown as passed along with the date and time of the run.
- If the performance check passed with warnings, a message is displayed in the System Summary window.
- If the performance check failed, results are shown as failed.

  For information on troubleshooting a failed performance check, see Troubleshooting (page 133).

Next step   Proceed to Applying CS&T settings (page 38).

More information • Routine tasks workflow (page 30)
                      • Cytometer Performance report (page 62)

Applying CS&T settings

Introduction

This topic describes how to apply CS&T settings.

About applying CS&T settings

When CS&T settings are applied to new experiments, the settings applied include PMT voltages, threshold, laser delays, and area scaling factors. (The window extension values are generated from the cytometer configuration.)

When CS&T settings are applied to existing experiments, the settings applied include PMT voltages, laser delays, area scaling factors, and window extensions. Threshold values remain the same.

You can apply new CS&T 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

Ways to apply CS&T settings

CS&T settings can be applied through the following:

- Applying CS&T settings through the CST Mismatch dialog (page 39)
- Applying current CS&T settings manually (page 39)
- Enabling the Use current CS&T settings preference (page 117)

About the CST Mismatch dialog

If there is a mismatch between the CS&T settings and those in BD FACSDiva software, the CS&T dialog opens to indicate what the differences are, and gives you the following options:

- **Use CST Settings.** Select this option if you want to use the settings generated from the most recent performance check.

- **Keep BD FACSDiva Settings.** Select this option if you want to keep the settings that are associated with your cytometer.

- **Details.** Click this button to view the differences between the two settings to help with your selection.
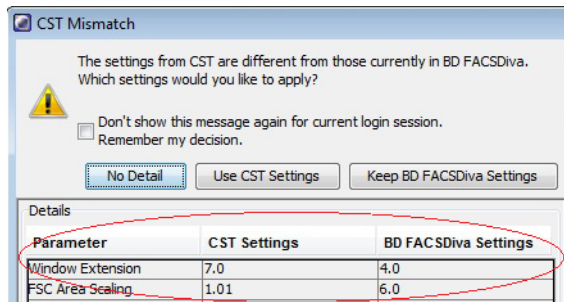
Applying CS&T settings through the CST Mismatch dialog

To apply CS&T settings:

1. Select **File > Exit** to close the CS&T window and connect back to the BD FACSDiva interface.

2. Click **Use CST Settings** in the **CST Mismatch** dialog that opens.

Applying current CS&T settings manually

Users who change area scaling or window extension values from the default should manually apply CS&T settings each time they reuse an existing experiment to ensure that the proper settings are applied to the experiment.

Note: You cannot apply CS&T settings to an experiment that is linked to compensation settings.

To manually apply the current CS&T settings to an experiment:

1. In the **BD FACSDiva** workspace, right-click the experiment-level cytometer settings.

2. Select **Apply Current CST Settings**.

   If the CS&T settings have expired or have warnings associated with them, a warning dialog opens.

3. Click **OK** to use the current CS&T settings or **Cancel** to use the default cytometer settings.

Next step	Proceed to Applying application settings to an experiment (page 40).

More information	• Routine tasks workflow (page 30)
	• Use current CS&T settings preference (page 117)

Applying application settings to an experiment

Introduction

This topic describes how to apply application settings to an experiment.

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

- The cytometer settings are renamed Application Settings and the cytometer settings icon in the Browser changes.
- The cytometer settings are updated with the selected parameters, threshold settings, area scaling factors, and window extension values included in the application settings.
- PMTVs and laser delays in the application settings are automatically updated based on the performance check.

Before you begin

- Before you can apply application settings to your experiment, you must have run a performance check for the configuration you are using. Use the Reports tab to quickly check whether a performance check exists for a specific configuration.
- Verify that the correct user preferences are set for your experiment. See the *BD FACSDiva Software Reference Manual* for more information.

Applying application settings to a new experiment

To apply application settings to a new experiment:

1. Click the **New Experiment** icon on the **Browser** toolbar.

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

2. In the new experiment, right-click the experiment-level cytometer settings and select **Application Settings > Apply**.

3. In the **Application Settings** catalog, select the application settings file you want, then click **Apply**.

4. If the parameters are not the same, a mismatch dialog opens. Do one of the following:

   • Click **Overwrite** to delete the parameters not in the application settings.

   • Click **Apply** to keep all the parameters currently in the experiment.

   Voltages are set for the parameters in the application settings only.

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

Updating application settings for templates or existing experiments

Application settings in templates or existing experiments do not automatically update when a new performance check is run. Application settings must be applied to an existing experiment to be updated.

To update application settings for an existing experiment:

1. In the experiment, right-click the application settings and select **Application Settings > Apply**.

2. In the **Application Settings** catalog, select the application settings file you want, then click **Apply**.

Note: If cytometer settings are linked with a compensation setup, application settings cannot be applied. Unlink from the compensation setup first before applying the application settings.

Next step

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

More information

- Routine tasks workflow (page 30)
- Application settings workflow (page 44)
- Creating application settings (page 46)

4

Application settings

The following topics are covered in this chapter:

Application settings workflow

Introduction

This topic describes application settings and the steps for creating them.

About application settings

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 the following parameters needed for the application:

- PMTVs
- Area scaling factors
- Window extension values
- Threshold values

Application settings do not include compensation values. See the *BD FACSDiva Software Reference Manual* for instructions on applying compensation, recording data, analyzing data, and shutting down the system.

Once applied, application settings do not update with future performance checks. See Updating application settings for templates or existing experiments (page 41) for instructions.

Each time an administrator defines a new baseline, verify that application settings are still appropriate. Adjustment might be necessary. See Baseline definition workflow (page 86).

Application settings workflow

The workflow for creating application settings includes these stages.

Stage	Description
1	Creating application settings (page 46)
2	(If needed) Adjusting the size of application settings target boxes (page 49)
3	Optimizing application settings (page 50)

About application settings target boxes

Introduction   This topic describes application target boxes and how to use them.

Application target box components

An application target box is displayed on plots created using the Applications Worksheet function. The target box represents a range that the software determines is optimal for resolving dimly fluorescing populations.
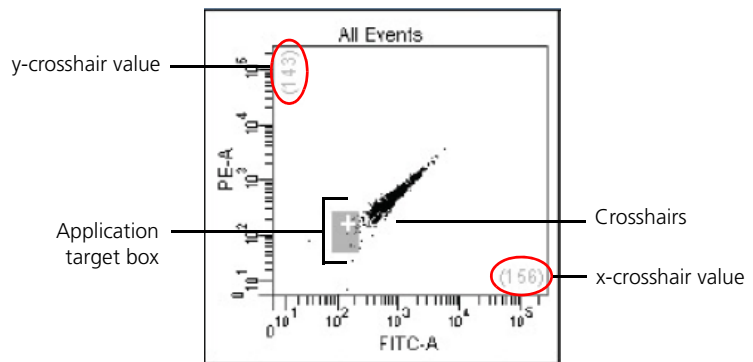
- The size of the application target box represents the standard deviation (SD) of electronic noise of the dim beads in each detector. The default value is 2 SD, but can be set from 1 to 4 SD depending on the application. See Adjusting the size of application settings target boxes (page 49).

- 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 red in the preceding figure).

How application target boxes are used

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 adjust the 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, we recommend maintaining the negative populations within or above the shaded areas.

The starting PMTVs 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. Note that this behavior, in general, applies more to lymphocytes than other cell types.

More information

- Application settings workflow (page 44)
- Adjusting the size of application settings target boxes (page 49)

Creating application settings

Introduction

This topic describes how to create application settings.

Before you begin

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

By default, the Application Target box is set to display a range of 2 SD for electronic noise of the dim beads. See Adjusting the size of application settings target boxes (page 49).

Procedure

To create application settings:

1. Exit CS&T by selecting **File > Exit**.

2. If the **CST Mismatch** dialog opens, click **Use CST Settings** to use the latest CS&T cytometer settings from the currently set configuration.
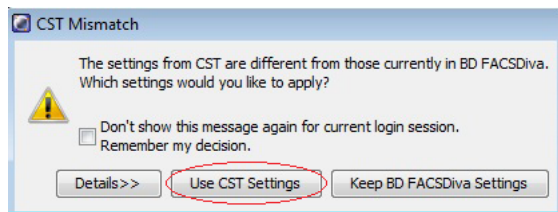
See Use current CS&T settings preference (page 117) for more information.

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

   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.

4. In the new experiment, click the experiment-level cytometer settings.

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

6. In the **Browser**, right-click the experiment-level cytometer settings.

7. Select **Application Settings > Create Worksheet**.

   A new global worksheet with plots specifically configured for each parameter is created. Each plot contains an application target box.

   Note: If needed, click Acquire to display the application target boxes.

Application targets
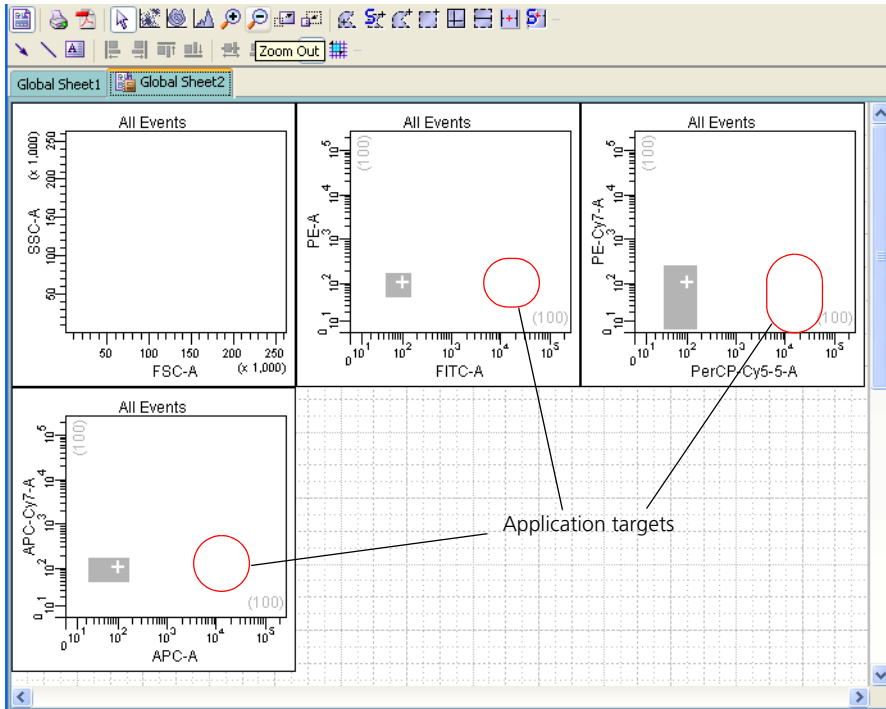
Next step	If needed, go to Adjusting the size of application settings target boxes (page 49) or proceed to Optimizing application settings (page 50).
More information	• Routine tasks workflow (page 30)
	• Optimizing application settings (page 50)

Adjusting the size of application settings target boxes

Introduction
This topic describes how to use adjust the size of the application settings target boxes.

Procedure
To change the size of the application settings target box:

1. In the worksheet, select the plot you want to change.

2. Open the **Plot** tab of the **Dot Plot Inspector.**

3. Under **Application Target Areas,** click the arrow to display either the **X** or **Y Axis SD** menus, and select a number.
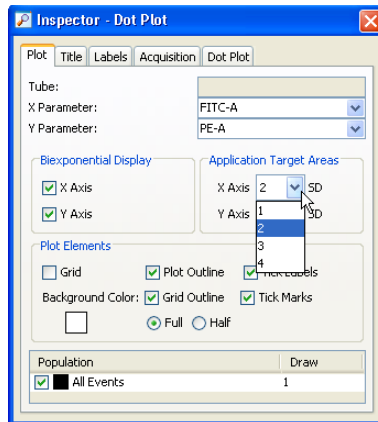
4. Repeat for the other axis.

Next step
Proceed to Optimizing application settings (page 50).

More information
*   About application settings target boxes (page 45)
*   Application settings workflow (page 44)

Optimizing application settings

Introduction   This topic describes how to optimize application settings.

When to optimize   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.

Note that optimizing application settings must be done with the cells of interest for the application, not beads or other standard particles.

Procedure   **To optimize settings for the application:**

1. Run a positively stained control with fluorochromes for each parameter or individual single-stained samples (one for each fluorochrome used in the application).

2. In the **Cytometer** panel, optimize the following settings for your application, if necessary:

   - FSC and SSC voltages
   - Threshold value
   - Area scaling factors. Note that adjusting area scaling results in a global effect for all users without notifying the other users of your adjustments.
   - Fluorescence PMT voltages (to ensure that positive populations are on scale and in the linear range of the detector)

3. Right-click the experiment-level cytometer settings, and select **Application Settings > Save**.

   The Save dialog opens.

4. Name the application settings, then click **OK**.

More information
- Application settings workflow (page 44)
- Creating application settings (page 46)

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5

Reports

The following topics are covered in this chapter:

About CS&T reports

Introduction

This topic describes the CS&T report. The three different report types are Performance, Baseline, and Reset Target Values. The different CS&T reports contain common information such as detector settings, specifications, laser settings, and Levey-Jennings charts.

Detector settings descriptions

The following table describes settings on CS&T reports.

Setting	Description
Laser	Laser name
Detector	Scatter or fluorescence detector
Parameter	Fluorochrome name (assigned)
PMTV	PMTV required to place the dim beads population at 10 times the robust standard deviation of electronic noise
New Target Value	Measured median fluorescence intensity of the bright beads at the baseline PMTV determined during a new baseline definition
Old Target Value	Old (previous) median 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 the bright beads.
Mid Bead Median Channel	MFI value of mid beads. A 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. A term used in the calculation of photon detection efficiency (Qr).

Setting	Description
Dim Bead Median Channel	MFI value of dim beads. A term used in the calculation of relative optical background fluorescence (Br).
Dim Bead % Robust CV	Percent robust coefficient of variation of dim beads. A term used in the determination of relative optical background (Br).
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 PMTVs)
Intercept	Intercept of the detector response for bright beads (log MFI vs log PMTVs)
Electronic Noise Robust SD	Robust standard deviation (rSD) of the electronic noise in the particular detector. A 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   For the primary detector of each laser in BD cytometers with standard configurations (lasers, mirrors, and filters), the BD-recommended maximum value for the bright bead % robust CV is 6.00.

See the appropriate cytometer reference manual for information on standard configurations.

Primary detector   The primary detector on the array is the detector with the shortest wavelength (lowest number) bandpass filter. However, side scatter (SSC) cannot be a primary detector. Each detector array has 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.

Shortest wavelength bandpass filter

Primary detector
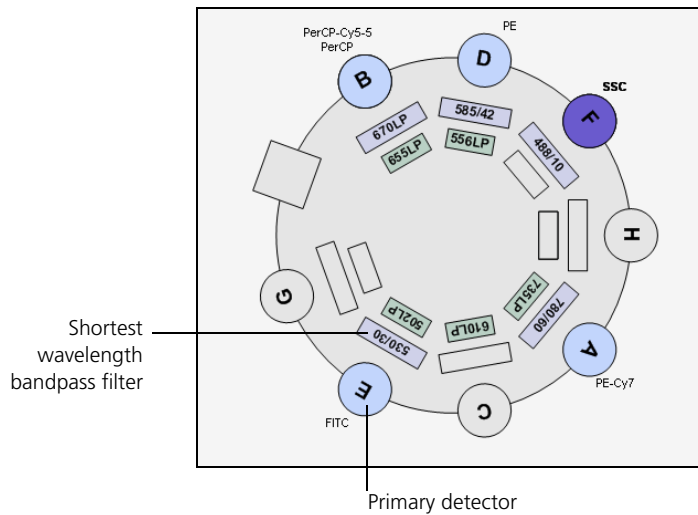
Laser settings   This section contains cytometer-dependent measurements.

Laser Settings

Laser	Power (mW)	Power Spec. (mW)	Current (mA)	Current Guidelines (mA)	Delay (Trigger on FSC)	Delay (Trigger on Fluorescence)	Area Scaling Factor
Violet	59.82	47.84 - 71.76	N/A	N/A	-32.25	-31.93	1.14
Blue	20.44	16.35 - 24.53	1.39	1.14 - 1.70	0.00	0.00	1.39
Red	15.00	12.24 - 18.36	N/A	N/A	31.43	31.75	1.18

Window Extension: 7.00
FSC Area Scaling Factor: 0.90

The following table describes the laser settings measurements on CS&T reports.

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 Guidelines (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 has PMTV results plots for each detector (as seen on the Baseline report).
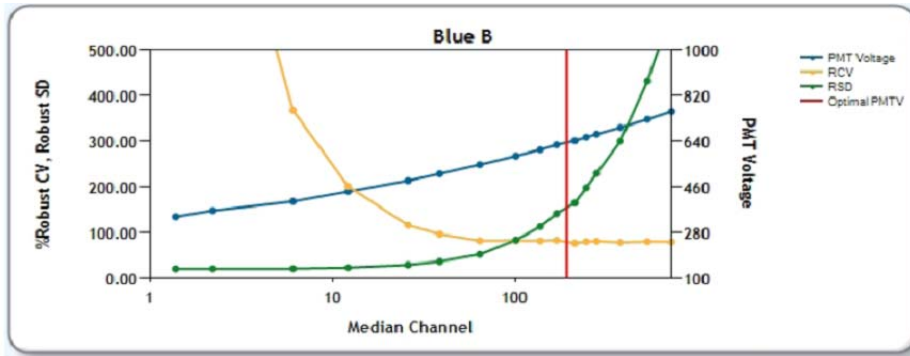
The following table describes the PMTV plot measurements found on CS&T reports.

Measurement	Description
PMT Voltage	Voltage applied to the photomultiplier tube
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 has linearity plots for each detector (as seen on the Baseline report).

- The vertical yellow lines at each end of the plot indicate the minimum and maximum channels, which the CS&T application considers linear.
- Minimum and maximum channel values are circled in red (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 mid beads across the detector response. If the mean of the ratio is greater than 2%, the results are not considered linear.

Corresponds to minimum channel

Corresponds to maximum channel

Linearity Results
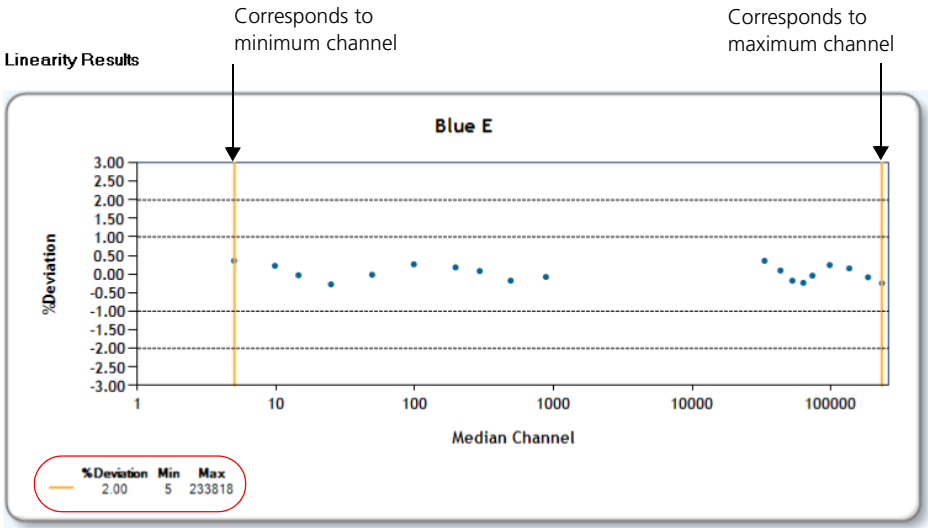
More information

- Cytometer Baseline report (page 60)
- Cytometer Performance report (page 62)
- Reset Target Values report (page 64)

Cytometer Baseline report

Introduction　　This topic describes the Cytometer Baseline report.

Description　　The Cytometer Baseline report contains information about the cytometer, setup bead lot, detector settings, cytometer settings, and plots of PMTV-optimized results and linearity results. The information in the report depends on the cytometer used. The following figure shows the first page of an example Baseline report. See the table under About CS&T reports (page 54) for descriptions of each setting.

For information on troubleshooting Cytometer Baseline reports, see Troubleshooting (page 133).

Cytometer Baseline Report

Cytometer:	FACSCantoII		User:	Administrator
Cytometer Name:	FACSCantoII		Institution:	N/A
Serial Number:	V96100001		Software:	BD FACSDiva 7.0
Input Device:	Carousel		Date:	03/13/2012 10:34 AM
Tube Loaded Manually:	Yes			
Cytometer Configuration:	Copy of 3-laser, 8-color (4-2H-2V)			

Setup Beads

Bead Product:	CST Setup Beads	Part #:	910858
Lot ID:	21562	Expiration Date:	12/31/2012
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	574	125000	N/A	4.22	134946	4.23	19451	21.68
Blue	F	SSC	443	125000	N/A	3.21	117244	3.14	59287	2.76
Blue	E	FITC	566	19301	N/A	3.1	477	17.52	93	52.33
Blue	D	PE	449	29871	N/A	2.67	823	13.88	163	47.34
Blue	B	PerCP-Cy5-5	741	151840	N/A	3.05	3973	13.56	413	43.49
Blue	A	PE-Cy7	645	81373	N/A	4.67	1217	30.86	90	121.3
Red	C	APC	525	69703	N/A	2.46	2712	12.33	417	31.74
Red	A	APC-H7	554	87780	N/A	2.04	1836	10.34	160	41.12
Violet	B	V450	459	10612	N/A	2.51	588	12.67	102	59.3
Violet	A	V500	467	36150	N/A	2.25	873	10.48	147	37.93

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	0.0040	2.8	N/A	N/A	N/A
Blue	F	SSC	N/A	N/A	7.3208	-14.3	N/A	N/A	N/A
Blue	E	FITC	252	231343	7.4231	-16.2	28.5	0.0220	167
Blue	D	PE	44	235066	7.3735	-15.1	26.6	0.1352	266
Blue	B	PerCP-Cy5-5	138	233619	7.4285	-16.1	29.5	0.0064	68
Blue	A	PE-Cy7	205	238583	7.5499	-16.3	27.5	0.0101	8
Red	C	APC	182	240503	7.4547	-15.4	25.2	0.0160	30
Red	A	APC-H7	23	235727	7.3838	-15.3	22.6	0.0036	823
Violet	B	V450	130	236185	7.4143	-15.7	23.7	0.0748	3895
Violet	A	V500	21	168340	7.4822	-15.4	22.1	0.0224	1847

More information
- About CS&T reports (page 54)
- Baseline definition workflow (page 86)

Cytometer Performance report

Introduction   This topic describes the Cytometer Performance report.

Description   The Cytometer Performance report contains cytometer information, setup bead lot information, detector and laser settings, specifications, cytometer settings, and pass/fail results. The following figure shows the first page of an example Cytometer Performance report. See the table under About CS&T reports (page 54) for descriptions of each setting.

Specifications   We recommend the following values:

- PMTV delta from baseline: The default is 50 V. Users with administrative privileges can modify the specification. See Setting the specification range for PMTVs (page 122).

- Maximum 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 Troubleshooting (page 133).

Cytometer Performance Report

Cytometer:	FACSCantoII	User:	Administrator
Cytometer Name:	FACSCantoII	Institution:	N/A
Serial Number:	V96100001	Software:	BD FACSDiva 7.0
Input Device:	Carousel	Date:	03/13/2012 10:40 AM
Tube Loaded Manually:	Yes	Cytometer Baseline:	03/13/2012 10:34 AM
Cytometer Configuration:	Copy of 3-laser, 8-color (4-2H-2V)	P/F:	Pass

Setup Beads

Bead Product:	CST Setup Beads	Part #:	910858
Lot ID:	21562	Expiration Date:	12/31/2012
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	125000	123660	-2	4.83	123777	4.76
Blue	F	SSC	125000	124102	-1	2.52	125399	2.45
Blue	E	FITC	19301	19015	-2	2.75	469	18.04
Blue	D	PE	29871	29703	-1	2.23	819	13.62
Blue	B	PerCP-Cy5-5	151840	151100	-1	2.72	3964	13.45
Blue	A	PE-Cy7	81373	80665	-1	4.47	1206	30.64
Red	C	APC	69703	69570	-1	2.57	2712	12.72
Red	A	APC-H7	87780	88069	0	2.47	1846	10.62
Violet	B	V450	10612	10126	-5	2.4	562	12.61
Violet	A	V500	36150	35612	-2	2.12	859	10.41

Detector Settings (Continued)

Laser	Detector	Parameter	Dim Bead Median Channel	Dim Bead % Robust CV	PMTV	Δ PMTV	Qr	Br	P/F
Blue	FSC	FSC	17908	25.01	565	-9	N/A	N/A	Pass
Blue	F	SSC	63439	2.11	446	3	N/A	N/A	Pass
Blue	E	FITC	93	53.11	565	-1	0.0204	160	Pass
Blue	D	PE	161	48.57	448	-1	0.1484	323	Pass
Blue	B	PerCP-Cy5-5	413	42.93	740	-1	0.0064	53	Pass
Blue	A	PE-Cy7	89	122.78	644	-1	0.0103	10	Pass
Red	C	APC	413	33.04	526	1	0.0150	37	Pass
Red	A	APC-H7	161	42.21	556	2	0.0035	896	Pass
Violet	B	V450	97	60.34	457	-2	0.0830	4444	Pass
Violet	A	V500	145	39.38	467	0	0.0242	2377	Pass

More information

- About CS&T reports (page 54)
- Routine tasks workflow (page 30)

Reset Target Values report

Introduction   This topic describes the Reset Target Values report.

Description   The Reset Target Values report contains information about the cytometer, old and new bead lot information, detector settings for the old and new bead lots, laser settings and specifications, and cytometer settings. The following image shows the first page of an example Reset Target Values report. See the table in the About CS&T reports (page 54) for a description and explanation of measurements.

For information on troubleshooting cytometer Reset Target Values reports, see Troubleshooting (page 133).

Cytometer Baseline Report (Reset Target Values)

Cytometer:	FACSCantoII
Cytometer Name:	FACSCantoII
Serial Number:	V96100001
Input Device:	Carousel
Tube Loaded Manually:	Yes
Cytometer Configuration:	Copy of 3-laser, 8-color (4-2H-2V)

User:	Administrator
Institution:	N/A
Software:	BD FACSDiva 7.0
Date:	03/16/2012 02:12 PM
Cytometer Baseline:	03/13/2012 10:34 AM

Old Setup Beads

Bead Product: CST Setup Beads, Part #: 910858
Lot ID: 21562, Expiration Date: 12/31/2012
Bead Lot Information: Available

New Setup Beads

Bead Product: CST Setup Beads, Part #: 910858
Lot ID: 21703, Expiration Date: 12/31/2012
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	568	125000	125000	5.23
Blue	F	SSC	445	125000	125000	4.43
Blue	E	FITC	568	19301	19918	4.09
Blue	D	PE	451	29871	30682	3.85
Blue	B	PerCP-Cy5-5	743	151840	112447	4.24
Blue	A	PE-Cy7	647	81373	47096	6.19
Red	C	APC	526	69703	66263	2.67
Red	A	APC-H7	555	87780	53112	2.28
Violet	B	V450	460	10612	10900	2.91
Violet	A	V500	469	36150	37139	2.73

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	122728	5.21	17791	25.54	N/A	N/A
Blue	F	SSC	121227	4.35	61193	3.90	N/A	N/A
Blue	E	FITC	472	18.21	91	51.67	0.0190	70
Blue	D	PE	796	14.90	162	49.00	0.1199	267
Blue	B	PerCP-Cy5-5	3155	15.64	440	40.43	0.0057	0
Blue	A	PE-Cy7	912	36.78	103	116.63	0.0094	11
Red	C	APC	2451	13.45	421	32.12	0.0145	3
Red	A	APC-H7	1392	12.58	201	35.34	0.0033	389
Violet	B	V450	683	10.91	107	52.05	0.0665	2902
Violet	A	V500	966	9.11	146	30.12	0.0233	700

More information

- About CS&T reports (page 54)
- Reset target values workflow (page 102)

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6

Cytometer configurations

The following topics are covered in this chapter:

Cytometer configuration workflow

Introduction   This topic describes the cytometer configuration workflow.

Custom cytometer configuration workflow   The workflow for creating a custom cytometer configuration includes these stages.

Stage	Description
1	(If needed) Adding parameters, filters, and mirrors (page 72)
2	Creating custom configurations (page 75)
3	Editing custom configurations (page 77)
4	Setting a configuration as the current configuration (page 80)
5	Printing the current configuration (page 81)

**More information**   • CS&T workflow (page 26)

About cytometer configurations

Introduction This topic describes the types of configurations used in BD FACSDiva software and their relationship to the physical configuration of the cytometer and the combination of lasers, detector arrays, filters, and dichroic mirrors inside the cytometer.

Cytometer Configuration window The Cytometer Configuration window is shown in the following figure.
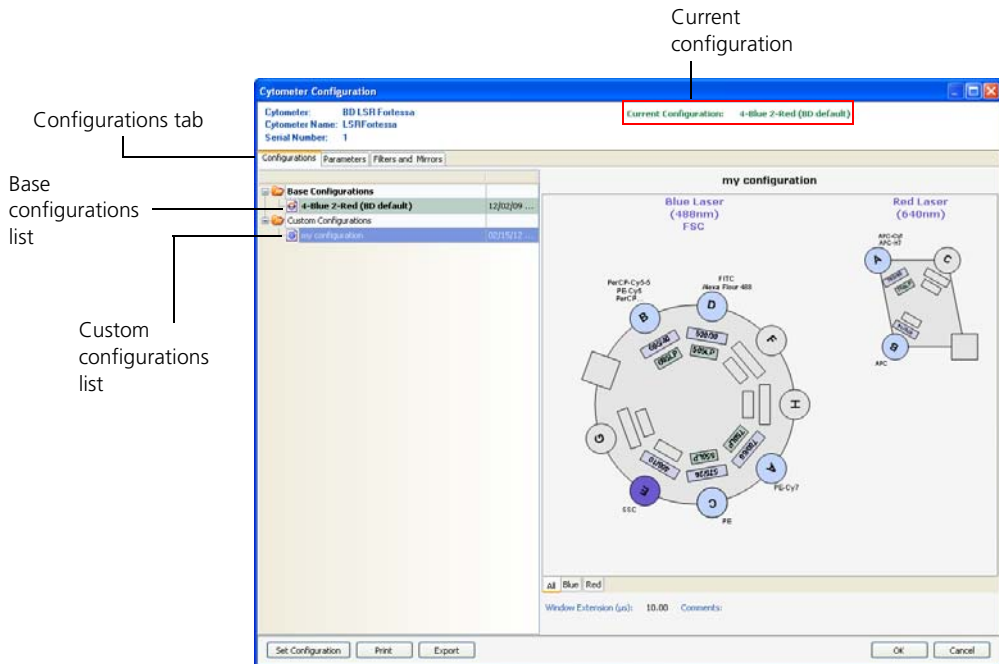
Base configuration Before you use the CS&T functions, a configuration matching your cytometer's physical configuration must be created within the software. This is typically done by the BD Biosciences field service engineer during installation. This base configuration serves as the template from which custom configurations can be created.

Custom and current configurations

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 (for example, 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 in the Cytometer Configuration window.

When upgrading from a previous version of BD FACSDiva software (v5.x or later), the base configuration is automatically set to what was used previously minus filter and mirror information, which can then be added.

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 and add them to the lists.

More information

About detector arrays

Introduction

This topic illustrates the detector arrays, which are graphical representations of the physical setup of the cytometer. These representations are used to create and edit custom configurations.

Configuration editing window

The configuration editing window has a graphical representation of the detector arrays in your cytometer. Detector arrays are arranged as either octagons or trigons. You can edit custom configurations in this window. See the following figure.
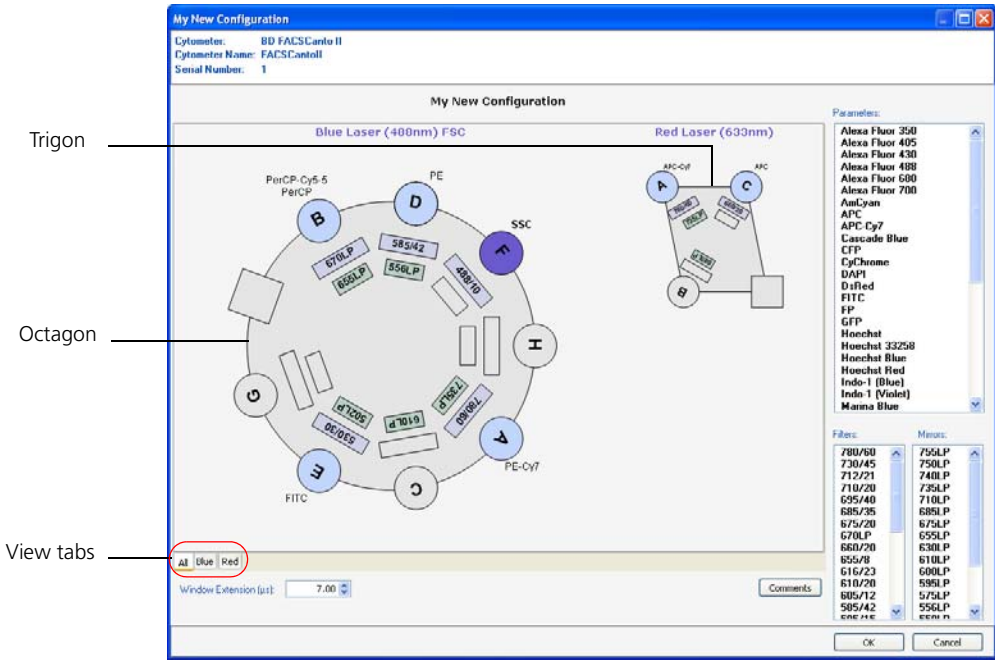
The following table describes the components that represent the detectors, filters, and mirrors in the configuration editing window.

Component	Description	Meaning
Circles	Detectors	Photomultiplier tube
Gray circle	Unavailable detector	You cannot add parameters to an unavailable detector.
Light blue circle	Available detector	You can assign parameters from the Parameters list to any available detector.
Purple circle	SSC	Side scatter
Outer set of rectangles	Filters	You can assign filters and mirrors only if the associated detector is available
Inner set of rectangles	Mirrors	

Changing the detector array view	**To change the detector array view, do one of the following:**
	• Click the **All** tab to view all detector arrays.
	• Click the tab for a particular laser (eg, Blue or Red) to view only the detectors for that laser.
More information	• About cytometer configurations (page 69)
	• Adding parameters, filters, and mirrors (page 72)

Adding parameters, filters, and mirrors

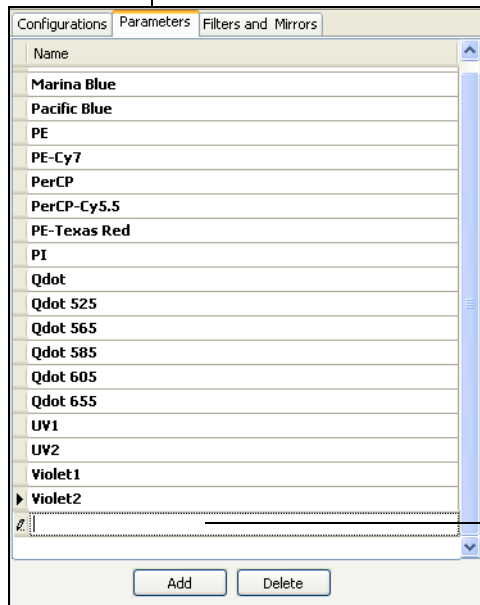|	
Introduction	This topic describes how to add parameters, filters, and mirrors to the selection lists for use in your cytometer configuration.
	A configuration cannot be used in CS&T until each detector is fully configured with filters and mirrors. Also, there must be at least one fluorescence parameter defined on each laser.
	You need to be an administrator to perform these procedures.
Adding new parameter names	The parameter names assigned to each detector are the names that are displayed 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 a new parameter name to the list:
	1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**. The Cytometer Configuration window opens.
	2. Click the **Parameters** tab to open the **Parameters** list.
	3. Click **Add**, then enter the new parameter name.

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.

Highlight indicates that the
Parameters tab is open

Configurations	Parameters	Filters and Mirrors

Name
Marina Blue
Pacific Blue
PE
PE-Cy7
PerCP
PerCP-Cy5.5
PE-Texas Red
PI
Qdot
Qdot 525
Qdot 565
Qdot 585
Qdot 605
Qdot 655
UV1
UV2
Violet1
▶ **Violet2**

Enter new parameter

Add   Delete

Note that you cannot add SSC to the Parameters list. See Setting SSC (page 78).

4. Click **OK** to close the **Cytometer Configuration** window and save the changes.

|	
Adding new filters and mirrors	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:

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

   The Cytometer Configuration window opens.

2. In the **Cytometer Configuration** window, click the **Filters and Mirrors** tab to open both the **Filters** list and the **Mirrors** list.
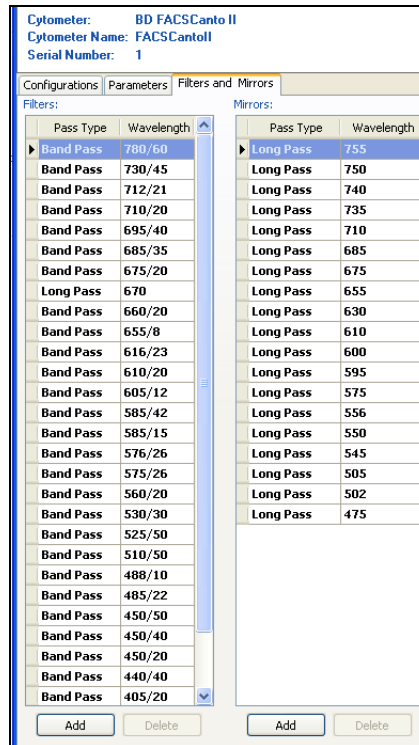
3. To add to either list, click **Add**, select a pass type, and enter the new wavelength.

4. Click **OK** to close the **Cytometer Configuration** window and save the changes.

Next step Proceed to Creating custom configurations (page 75).

More information
- About detector arrays (page 70)
- Cytometer configuration workflow (page 68)

Creating custom configurations

Introduction This topic describes how to create a custom configuration by copying and editing an existing configuration, which is the preferred method, or by creating a new configuration.

You need to be an administrator to perform these procedures.

- If you want to copy and edit an existing configuration, then the parameters, filters, and mirrors are assigned.
- If you want to create a new configuration, the parameters, filters, and mirrors are blank.

Copying an existing configuration **To copy an existing configuration:**

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

    The Cytometer Configuration window opens.

2. Create a custom configurations folder by right-clicking in the configuration list and selecting **New Folder.**

3. Name the folder *Custom Configurations*.

4. Right-click the configuration you want in the configuration list and select **Copy.**

5. Right-click your custom configurations folder and select **Paste.**

6. Rename the configuration and press **Enter**.

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

   The configuration editing window opens showing a graphical representation of the detector arrays in your cytometer.

Creating a new configuration

To create a new configuration:

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

   The Cytometer Configuration window opens.

2. Create a folder for your custom configurations.

   a. Right-click the **Base Configurations** folder.

   b. Select **New Folder**, rename the folder, and press **Enter**.

3. Add a new configuration to the new folder.

   a. Right-click the new folder and select **New Configuration.**

   b. Rename the configuration and press **Enter**.

      The following characters cannot be used in the name: \ / : * ? " < > |

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

      Depending on the optics of your cytometer, some detectors may not be available.

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

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

   The configuration editing window opens showing a graphical representation of the detector arrays in your cytometer.

Next step	Proceed to Editing custom configurations (page 77).

More information	• Cytometer configuration workflow (page 68)
	• About cytometer configurations (page 69)

Editing custom configurations

Introduction

This topic describes how to set SSC; assign or delete a parameter, filter, or mirror; and add a comment to your new configuration.

Configurations cannot be edited once a baseline has been run.

You need to be an administrator to perform these procedures.

Before you begin

Before you can edit a custom configuration, you need to add parameter names, filter names, and mirror names to their respective lists. If needed, see Adding parameters, filters, and mirrors (page 72).

To edit configurations you have to right-click a configuration and select **Edit Configuration**, or double-click a configuration. This opens the configuration editing window.

Setting SSC

An SSC parameter must be defined to run the CS&T application.

To set SSC:

1. In the configuration editing window, right-click the appropriate detector (dark blue circle).

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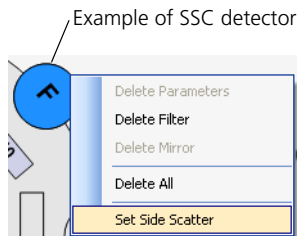
The position of SSC is based on your detector array. See your cytometer manual for more information.

2. Select **Set Side Scatter** from the menu.

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

Assigning parameters

Parameters, filters, or mirrors you apply overwrite the existing ones. Parameter names are listed in alphabetical order.

You must place the parameter in the correct detector (based on filter/mirror combination and fluorochromes emission spectrum) for data to be seen in the software. Note that CS&T will not display an error message if a parameter is placed on an inappropriate detector.

To assign a parameter, do one of the following:

- To add a single parameter, drag the appropriate parameter from the **Parameters** list to the available detector.
- 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.

Assigning filters

To assign a filter:

1. Drag the filter name from the **Filters** list onto the appropriate outer rectangle for that detector.

Assigning mirrors

To assign a mirror:

1. Drag the mirror name from the **Mirrors** list onto the appropriate inner rectangle for that detector.

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

Deleting assigned parameters, filters or mirrors

To delete an assigned parameter, filter, or mirror:

1. Right-click the appropriate detector (colored circle).

2. Select **Delete Parameter, Delete Filter, Delete Mirror,** or **Delete All**.

Adding comments

To add a comment:

1. Click **Comments**.

2. Enter text in the **Cytometer Configuration Comments** dialog, then click **OK**.

3. Click **OK** to close the configuration editing window.

Next step

Proceed to .

More information	• Cytometer configuration workflow (page 68)
	• Adding parameters, filters, and mirrors (page 72)

Setting a configuration as the current configuration

Introduction	This topic describes how to set a configuration as the current configuration.
Procedure	To ensure that the correct parameters are displayed on the Parameters tab in the BD FACSDiva workspace, set the configuration you want to use before you create a new experiment.

To set the current configuration:

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

2. In the **Cytometer Configuration** window, select the configuration in the list and click **Set Configuration**.

   A confirmation dialog opens.

3. Click **OK**.

   The current configuration name changes at the top of the Cytometer Configuration window.

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

4. Click **OK** to finish.

Next step	Proceed to Printing the current configuration (page 81).

More information	• CS&T workflow (page 26)
	• Cytometer configuration workflow (page 68)
	• About cytometer configurations (page 69)

Printing the current configuration

Introduction   This topic describes how to print your current configuration.

Procedure   **To print the current cytometer configuration:**

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

   The Cytometer Configuration window opens.

2. 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.

More information	• CS&T workflow (page 26)
	• Cytometer configuration workflow (page 68)
	• About cytometer configurations (page 69)

Deleting configurations

Introduction   This topic describes how to delete custom configurations.

BD-default configurations and the current configuration cannot be deleted.

Procedure   You need to be an administrator to perform this procedure.

To delete a custom configuration:

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

   The Cytometer Configuration window opens.

2. Right-click the configuration in the list.

3. Select **Delete**.

4. Click **OK** in the confirmation dialog.

More information   •

Transferring a configuration file

Introduction   This topic describes how to export a custom configuration and then import it into another workstation.

Make sure that the exported cytometer configuration name is a unique name that does not already exist on the secondary workstation. Modify the name before importing the configuration if needed.

**Exporting a
configuration**

To export a custom configuration:

1. In the CS&T workspace, select **Cytometer > Cytometer Configuration**.

2. In the **Cytometer Configuration** window, right-click a custom configuration in the list and select **Export Configuration**.

3. Click **Save** in the **Export** dialog that opens.

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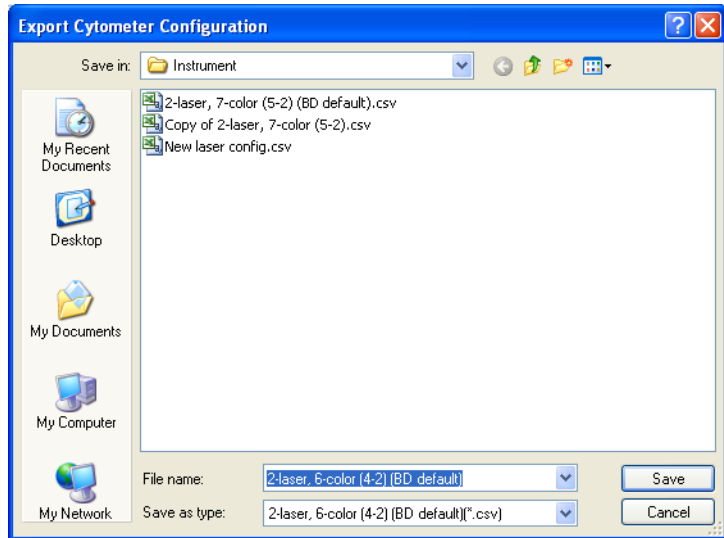
Importing configurations

You need to be an administrator to perform this procedure.

To import a configuration:

1. Transfer the exported cytometer configuration file to the secondary workstation.

2. Log in to BD FACSDiva software.

3. Select **Cytometer > View Configurations**.

   The Cytometer Configuration window opens.

4. Right-click the folder you want to import the configuration into, then select **Import Configuration**.

5. Navigate to and select your saved or exported file and click **Open**.

6. Select the configuration from the list.

7. Click **Set Configuration** to make the imported configuration the current configuration.

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

More information

- About cytometer configurations (page 69)

7

Baseline definition

The following topics are covered in this chapter:

Baseline definition workflow

Introduction

This topic describes the baseline definition process, which uses the CS&T application with CS&T research beads to set the basic performance criteria for your cytometer.

Baseline definition process

When you run a baseline definition, CS&T performs the following steps.

Step	Task	Description
1	Performs laser setup.	• Sets the laser delay. • Sets the area scaling factor.
2	Identifies the beads.	• Sets gates on the bright, mid, and dim beads. • Adjusts the PMTVs to set the bright beads to approximately 100,000 MFI.
3	Collects data samples.	Collects data at different PMTVs to set MFI values of bright beads to fixed target values.

Step	Task	Description
4	Determines baseline PMTVs.	• Calculates %rCV of dim beads and MFI for bright, mid, and dim beads. • Fits %rCV vs median curve for each fluorescence detector. • Calculates the baseline voltage based on optimal fitted curve, and linearity and amplification factor for each fluorescence detector. • Generates and displays PMTVs results.
5	Determines target values.	• Sets each detector to the baseline PMTVs and collects data. • Gates the bright beads. • Calculates the baseline target values for each detector.
6	Generates results.	• Calculates Qr, Br, %rCVs, and rSDs for each fluorescence detector. • Generates baseline target values. • Displays results.

Baseline definition workflow

The workflow for running the baseline definition includes these stages.

Stage	Description
1	Setting up for a baseline definition (page 88)
2	Running a baseline definition (page 90)
3	(If needed) Adjusting baseline definition PMTVs (page 94)
4	(If needed) Adjusting baseline definition target values (page 98)

Setting up for a baseline definition

Introduction This topic describes how to set up the workspace, and prepare and load the CS&T research beads for a baseline definition.

You need to be an administrator to perform this procedure.

When to perform When you change the physical configuration of your cytometer and perform periodic maintenance or other procedures that may alter performance or increase electronic noise, we recommend that you run a new baseline definition.

Limitations It takes approximately 30 minutes to define a baseline. The time required is dependent on cytometer configuration, fluidics, and performance. Allow cytometer lasers sufficient time to warm up. (See the laser requirements in your cytometer manual.)

Procedure **To set up the baseline definition:**

1. Select **Cytometer > CST**.

   The CS&T workspace opens.

2. From the **Setup Control** window, click **Select Configuration** to open the **Cytometer Configuration** window.

3. Select a configuration that matches the physical configuration of your cytometer.

4. Click **Set Configuration**, then click **OK**.

5. Verify that the selected setup bead lot ID matches your new lot of CS&T research beads.

Bead lot ID

6. Prepare the setup beads for tubes or a plate as instructed in the *BD FACSDiva CS&T Research Beads* technical data sheet.

7. Load the CS&T research beads, then do one of the following:

   - If you are loading tubes manually, place the beads tube on the cytometer and select the **Load Tube Manually** checkbox.

   - If you are using the Loader, place the carousel on the Loader and clear the **Load Tube Manually** checkbox.

   - If you are using the HTS, place the plate on the HTS, clear the **Load Tube Manually** checkbox, and select the appropriate plate type from the **Plate Type** menu.

8. If you are manually loading tubes on a BD LSR II or BD LSRFortessa, set the flow rate to low.

   For all other platforms, the software sets the flow rate. If you are using an HTS, the HTS software sets the pressure.

Next step   Proceed to .

•   *BD FACSDiva CS&T Research Beads* technical data sheet.

Running a baseline definition

Introduction   This topic describes how to run a baseline definition.

You need to be an Administrator to perform this procedure.

Procedure   **To run the baseline definition:**

1.   In the **Setup Control** window, select **Define Baseline** from the **Characterize** menu, then click **Run**.
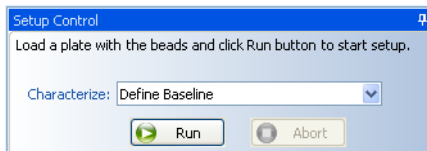
A confirmation dialog opens.

2.   Click **OK**.

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

View plot parameters as they are acquired by right-clicking the parameter, and selecting another from the menu.
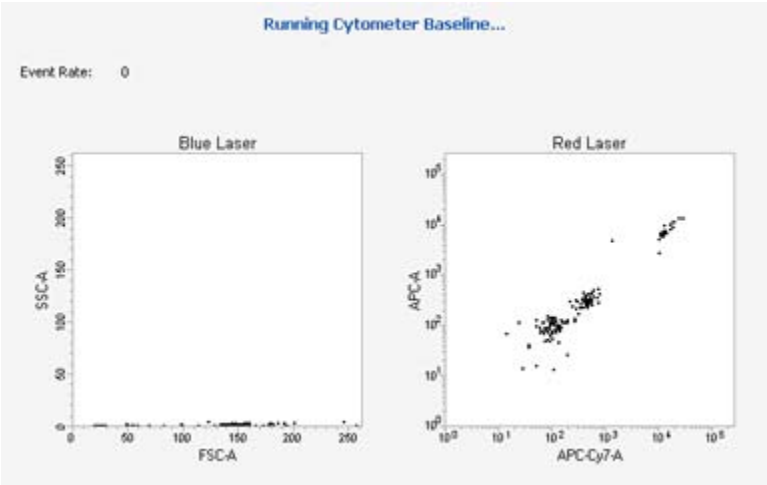
Once the data samples are collected, the Optimized PMTVs Results window opens.
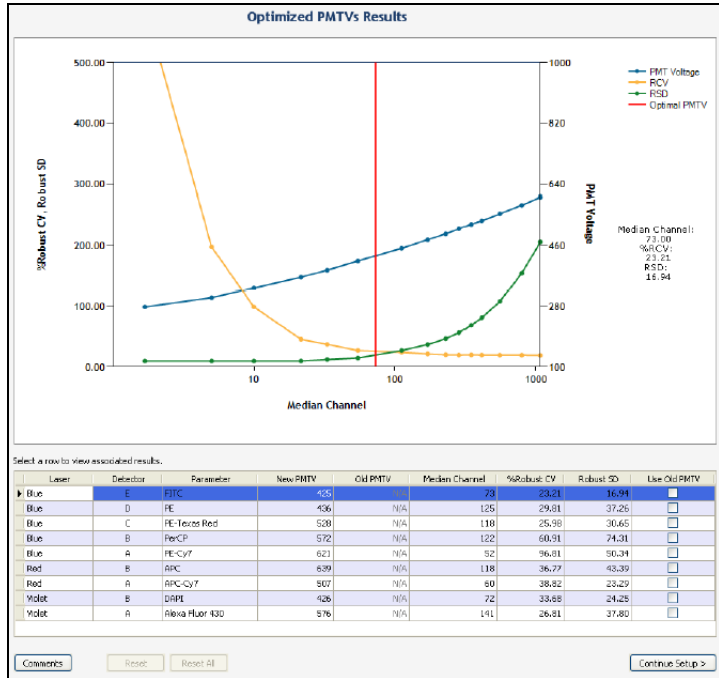
Advanced users can see About baseline PMTV selection plots (page 94) for instructions on making adjustments, if necessary.

3. After viewing the results, click **Continue Setup**.

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

Target Values Results

Laser	Detector	PMTV	New Target Value	Old Target Value	Use Old Target Value
▶ Blue	FSC	539	150000		☐
Blue	F(SSC)	566	150000	N/A	☐
Blue	E	435	20759	N/A	☐
Blue	D	426	11700	N/A	☐
Blue	B	565	18239	N/A	☐
Blue	A	729	37575	N/A	☐
Red	C	539	21563	N/A	☐
Red	A	598	28539	N/A	☐

[Comments]   [Continue Setup >]

> See Adjusting baseline definition target values (page 98) for instructions on how to use the functions in this window.

4. Click **Continue Setup**.

   Once a baseline has been defined, a dialog opens.

   - To view the Baseline Report, click **View Report**. See Cytometer Baseline report (page 60).
   - To complete baseline definition and return to the **Setup** view of the workspace, click **Finish**.

5. Remove the tube from the cytometer, the carousel from the Loader, or the plate from the HTS.

Next step   If needed, proceed to Adjusting baseline definition PMTVs (page 94).

More information
- CS&T workflow (page 26)
- Baseline definition workflow (page 86)
- Cytometer Baseline report (page 60)

Adjusting baseline definition PMTVs

Introduction

This topic describes how to review, adjust, and manage baseline PMTV selection plots.

You need to be an administrator to perform these procedures.

About baseline PMTV selection plots

The baseline PMTV selection plot displays:

- rSD (robust standard deviation) vs median channel value
- %rCV (percent robust coefficient of variation) vs median channel value
- 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.

Viewing detector results

To view results for a specific detector:

1. Click the corresponding row in the table.

   New PMTVs for the current baseline are displayed in the results table.

   If a baseline was previously defined for this configuration, PMTVs for the previous baseline also are displayed in the results table.

2. Select the new PMTVs, previous PMTVs, or adjust the PMTVs manually.

   - To use the new PMTVs, click **Continue Setup**.
   - To use the previous PMTVs, see Selecting PMTVs from the previous baseline definition (page 96).
   - To adjust PMTVs manually, see Adjusting PMTVs manually (page 96).
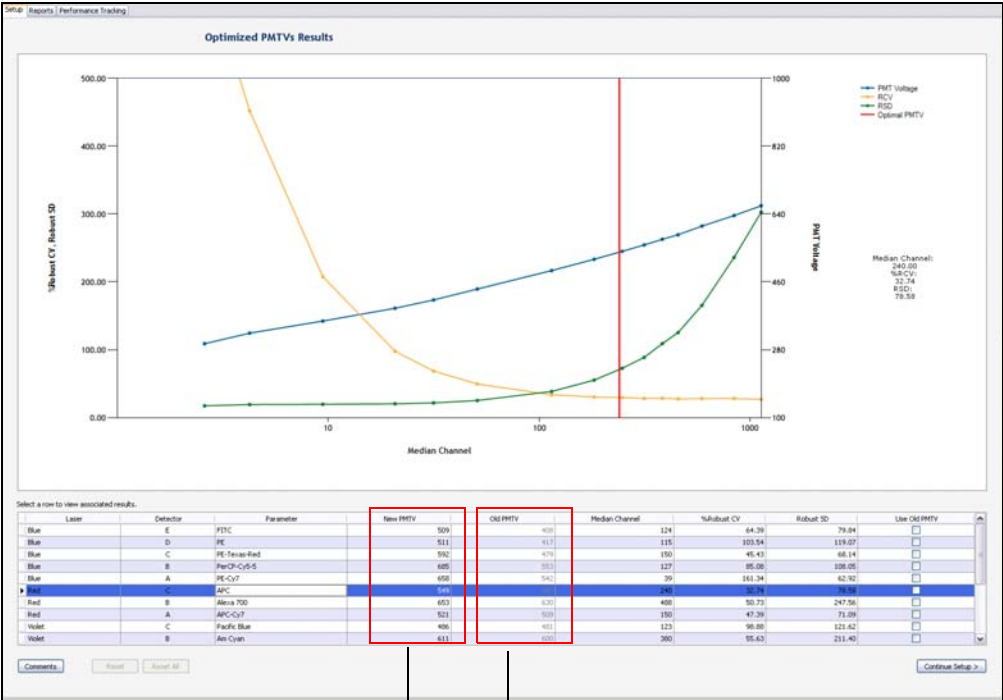
New PMTVs   Previous PMTVs

Selecting PMTVs from the previous baseline definition

Standard Deviations

To select PMTV settings from the previous baseline definition:

1. Do one of the following:

    • Select **Use Old PMTV** for a specific detector by selecting that checkbox.

    • Select **Use Old PMTV** for all detectors by selecting all checkboxes.

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

2. Click **Continue Setup**.

Adjusting PMTVs manually

Caution! We recommend that you use the PMTVs derived by the software. Only advanced users should attempt to adjust settings manually.

To adjust PMTVs 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.

Adjusted PMTV   BD-calculated PMTV

Median Channel
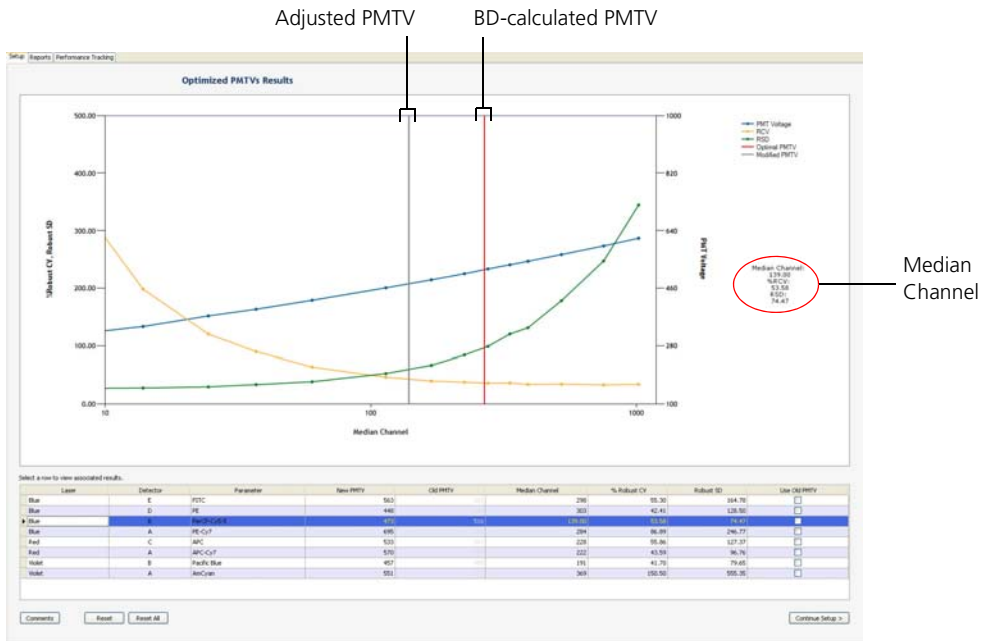
3. Adjust PMTVs for the remaining detectors, if necessary.

4. Select 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**.

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

Next step   Proceed to Adjusting baseline definition target values (page 98).

More information	• CS&T workflow (page 26)
	• Baseline definition workflow (page 86)
	• Cytometer Baseline report (page 60)

Adjusting baseline definition target values

Introduction

This topic describes how to adjust baseline definition target values.

You need to be an Administrator to perform this procedure.

Reviewing target value results

When you review target value results, notice that the new target values for the newly run baseline definition are displayed in the results table (the rectangle on the left). If a baseline was previously defined for this configuration, old target values for the previous baseline also are displayed in the results table (the rectangle on the right).

Target Values Results

Laser	Detector	PMTV	New Target Value	Old Target Value	Use Old Target Value
▶ Blue	FSC	539	150000	N/A	☐
Blue	F(SSC)	566	150000	N/A	☐
Blue	E	435	20759	N/A	☐
Blue	D	426	11700	N/A	☐
Blue	B	565	18239	N/A	☐
Blue	A	729	37575	N/A	☐
Red	C	539	21563	N/A	☐
Red	A	598	28539	N/A	☐

[Comments]   [Continue Setup >]

To specify the target values to use, do one of the following:

- 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, then click **Continue Setup**.

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. Selecting previous target values is recommended when the previous and current baselines have been run with the same lot of CS&T research beads.

More information
- CS&T workflow (page 26)
- Baseline definition workflow (page 86)
- Cytometer Baseline report (page 60)

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8

Bead lot management

The following topics are covered in this chapter:

Bead lot information workflow

Introduction   This topic describes bead lot information and how it affects your cytometer settings. The bead lot workflow involves downloading bead lot file information from the BD Biosciences website and importing the file into your workstation.

Current bead lot information   When BD FACSDiva software was loaded on your workstation, bead lot information for the current lot of CS&T research beads was automatically copied to the software folder. During CS&T setup, before you run a baseline and/or performance check, verify that this bead lot matches the lot of CS&T research beads you are using.

New bead lot workflow   The workflow for adding a new bead lot includes these stages.

Stage	Description
1	Downloading a bead lot file (page 103)
2	Importing a bead lot file (page 104)

More information   • CS&T workflow (page 26)

Reset target values workflow

Introduction   This topic describes the reset target values workflow. You need to reset the target values when you switch to a new lot of CS&T research beads. You can also choose to run a new baseline when you switch to a new lot of beads, depending on how soon it will be before the current baseline expires.

Resetting target values workflow

The workflow for resetting target values includes these stages.

Stage	Description
1	Downloading a bead lot file (page 103)
2	Importing a bead lot file (page 104)
3	Setting up for a reset target values run (page 107)
4	Resetting target values (page 109)

More information

- CS&T workflow (page 26)

Downloading a bead lot file

Introduction

This topic describes how to download a lot-specific file from the BD Biosciences website.

Before you begin

Have the CS&T research beads vial available to verify the bead lot ID number, which is on the CS&T research beads vial.

Procedure

To download a lot-specific file for your current lot of CS&T research beads:

1. Go to bdbiosciences.com/csandt and follow the download instructions.

Next step

Proceed to Importing a bead lot file (page 104).

More information

- CS&T workflow (page 26)
- Importing a bead lot file (page 104)

Importing a bead lot file

Introduction

This topic describes how to import a bead lot file.

You need to be an administrator to perform this procedure.

Procedure

To import a bead lot file:

1. Select **Cytometer > CST**.

2. In the **CS&T** workspace, select **Tools > Bead Lots**.

    The Bead Lots dialog opens.

3. Click **Import**.

4. In the **Open** dialog, select the bead lot file (.bls) and click **Open**.

    The bead lot information is automatically entered into the CS&T workspace.

    Note that you should import a bead lot file for CS&T research beads.
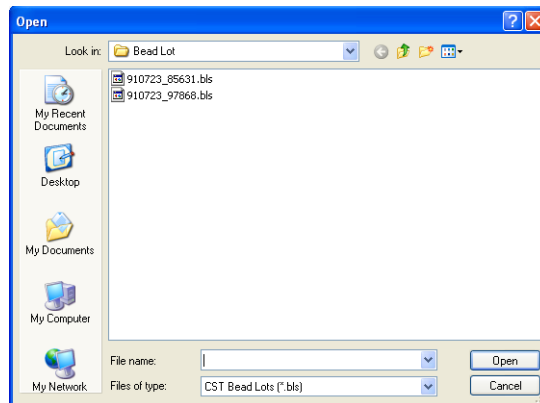
If the bead lot already exists in the Setup Beads section of the Setup Control window, a warning dialog opens.
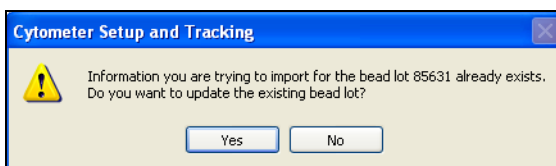
More information
- CS&T workflow (page 26)
- Downloading a bead lot file (page 103)

About resetting target values

Introduction   This topic describes how to reset target values when you switch to a new bead lot.

You need to be an administrator to perform this procedure.

Purpose   Before switching to a new bead lot, you need to run Reset Target Values with the existing bead lot and the new bead lot. (Alternatively, you can run a new baseline with 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.

Note that you can reset target values any number of times.

Before you begin   Before resetting target values:

- Download and import the lot-specific file for the new bead lot. See Downloading a bead lot file (page 103) and Importing a bead lot file (page 104) for instructions.

- Reserve a large enough volume of the existing lot of CS&T research beads to reset the target values.

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

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

- Set up the bead lot ID and select a configuration in the Setup Control window of the CS&T workspace.

Reset the target values on every cytometer configuration that has a baseline defined and for which you are tracking performance values.

Process   During this task, the following occur:

- 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.
- A completion dialog opens.

During flow stabilization, events might appear in plots, and counters do not update until all data is acquired.

Once the target values have been reset, a Cytometer Performance report for the old bead lot is generated, as well as the Reset Target Values report.

Setting up for a reset target values run

Introduction This topic describes how to set up the workspace, and prepare and load the CS&T research beads for a reset target values run.

Before you begin Before you begin, do the following:

• Download the lot-specific file for the new bead lot. See Downloading a bead lot file (page 103).

• Import the lot-specific file for the new bead lot. See Importing a bead lot file (page 104).

Procedure **To set up for a reset target values run:**

1. Select **Cytometer > CST**.

   The cytometer disconnects from the BD FACSDiva workspace and connects to the CS&T workspace.

2. If a Loader or HTS is connected to your system, decide whether to run beads in a tube or a multiwell plate, then do one of the following:

   • Select the **Load Tube Manually** checkbox to load tubes manually.

   • Clear the **Load Tube Manually** checkbox to use the Loader or HTS and select the appropriate plate type from the **Plate Type** menu.

3. Verify that the setup bead lot IDs selected for the old (existing) bead lot and the new bead lot are correct.
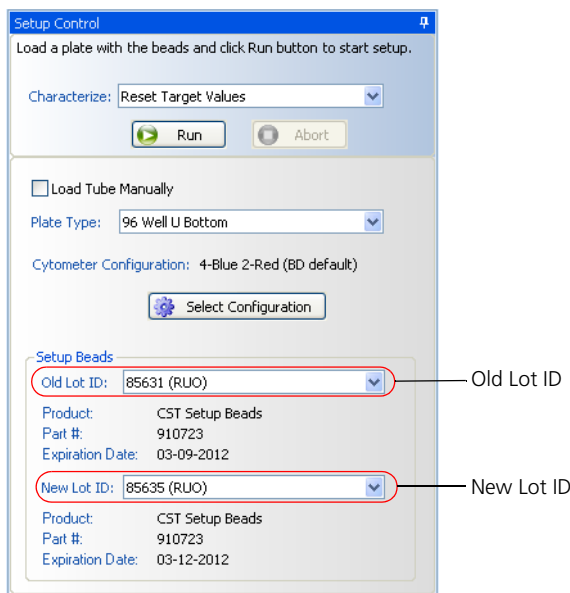
4. Click **Select Configuration** to open the **Cytometer Configuration** window.

5. Select a configuration that matches the physical configuration of your cytometer.

6. Click **Set Configuration**, then click **OK**.

7. Prepare the setup beads for tubes or a plate as instructed in the *BD FACSDiva CS&T Research Beads* technical data sheet.

8. Load the beads on the cytometer, then do one of the following:

   • If you are loading tubes manually, add the old bead lot into the first tube, and place the tube on the cytometer. (The software will prompt you when to load the tube with the new bead lot.)

   • If you are using the Loader, place the tube with the old bead lot in the first position, the tube with the new bead lot

in the second position, and place the carousel on the Loader.

- If you are using the HTS, add the old bead lot into the first well (A1), the new bead lot in the second well (A2), and place the plate on the HTS.

9. If you are manually loading tubes on a BD LSR II or BD LSRFortessa, set the flow rate to low.

   For all other platforms, the software sets the flow rate. If you are using an HTS, the HTS software sets the pressure.

Next step   Proceed to Resetting target values (page 109).

More information
- Reset target values workflow (page 102)
- *BD FACSDiva CS&T Research Beads* technical data sheet

Resetting target values

Introduction   This topic describes how to reset target values.

You need to be an administrator to perform this procedure.

Procedure   **To reset target values:**

1. In the **Characterize** menu, select **Reset Target Values.**

2. Click **Run**.
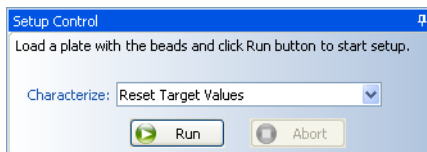
After a brief pause, the Resetting Target Values window opens.

- (Optional) To view different plot parameters during acquisition, right-click the parameter and select another from the menu.

- (Optional) Multiply the x- and y-axis values by 1,000 to obtain the proper scale on the FSC and SSC acquisition plots
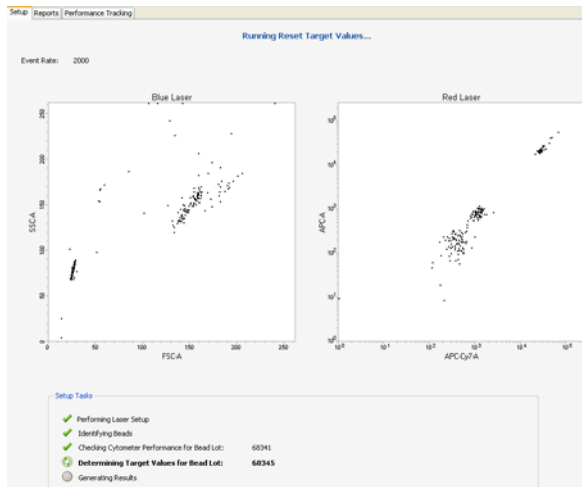
3. Select one of the following options:

   - To view the Reset Target Values report, click **View Report**. See Reset Target Values report (page 64).

   - To complete resetting target values and return to the **Setup View** of the workspace, click **Finish**.

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

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

Entering bead lot information manually

Introduction   This topic describes how to manually enter a bead lot ID. This is done if you are using a non-standard configuration on your cytometer or will not be using the tracking features.

You need to be an administrator to perform this procedure.

Before you begin   Have the CS&T research beads vial available to verify the bead lot ID number, which is on the CS&T research beads vial.

Procedure   **To enter the bead lot ID manually:**

1. Select **Cytometer > CST**.

2. In the **CS&T** workspace, select **Tools > Bead Lots**.

   The Bead Lots dialog opens.

3. Click **New**.

The software adds a line of zeros to the Lot IDs list. Notice the message indicating that bead lot information is not available for the new lot.
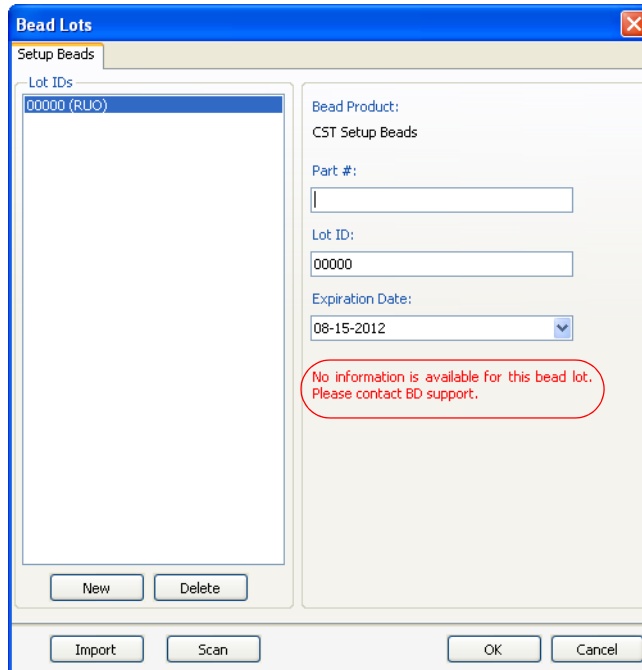
4. In the corresponding fields, enter the 6-digit part number and the 5-digit lot ID number for the beads.

5. From the **Expiration Date** menu, select the date from the calendar.

6. Click **OK** to save the lot information.

More information

- Bead lot information workflow (page 102)
- Importing a bead lot file (page 104)

Scanning bead lot information

Introduction

This topic describes how to scan bead lot information using the barcode scanner.

You need to be an administrator to perform this procedure.

Procedure

To scan bead lot information using the barcode scanner:

1. Select **Cytometer > CST.**

2. In the **CS&T** workspace, select **Tools > Bead Lots**.

   The Bead Lots dialog opens.

3. Click **Scan,** then scan the new bead lot barcode card inside the BD FACSDiva CS&T Research Beads technical data sheet.

   The bead lot information is automatically entered into the CS&T workspace.

More information

- Bead lot information workflow (page 102)
- Importing a bead lot file (page 104)

Deleting bead lots

Introduction

This topic describes how to delete bead lots.

You need to be an Administrator to perform this procedure.

We do not recommend deleting bead lots or 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.

Procedure

To delete bead lot information:

1. Select **Cytometer > CST**.

2. In the **CS&T** workspace, select **Tools > Bead Lots**.

   The Bead Lots dialog opens.

3. Select the bead lot in the **Lot IDs** list, then click **Delete**.

4. Click **Yes** in the confirmation dialog.

More information

- Bead lot information workflow (page 102)

9

Software preferences

The following topics are covered in this chapter:

About software preferences

Introduction

This topic describes the CS&T software preferences.

Use of current CS&T settings

You can apply the current CS&T settings from your last performance check on an experiment-by-experiment basis or have the current CS&T settings applied automatically after a performance check. See Use current CS&T settings preference (page 117).

Report preferences

You can change how the CS&T reports are displayed, saved, exported, and printed. Also, you can set the expiration dates for the baseline definition and performance check. See Changing report and file preferences (page 118).

Specification range

You can set the range for the delta PMTV specification. Values measured outside this range fail the performance check and are flagged on the performance report. See Setting the specification range for PMTVs (page 122).

Performance tracking preferences

You can select which performance criteria are displayed on the performance report. You can also set the autoscaling parameters for the plots and alarm boundaries for the performance criteria. See Setting performance tracking preferences (page 123).

More information

- CS&T purpose and functions (page 14)

Use current CS&T settings preference

Introduction

This topic describes the Use current CS&T settings preference.

About CS&T settings

When CS&T settings are applied to new experiments, the settings applied include PMT voltages, threshold, laser delays, and area scaling factors. (The window extension values are generated from the cytometer configuration.)

When CS&T settings are applied to existing experiments, the settings applied include PMT voltages, laser delays, area scaling factors, and window extensions. Threshold values remain the same.

About the Use current CS&T settings preference

The Use current CS&T settings preference is not enabled by default. If the preference is not enabled, the software displays a dialog giving you a choice to use BD FACSDiva settings or the new CS&T settings. BD FACSDiva settings are the settings last used in the software. If the preference is enabled, the software will automatically use CS&T settings for new experiments. This is equivalent to always selecting the Use CST Settings button in the CST Mismatch dialog. See About the CST Mismatch dialog (page 38) for more information.

Administrative privileges are needed to enable this preference.

Note: If you change area scaling or window extension values from the default, then you should leave this preference cleared to ensure that the proper settings are applied to the experiment.

Enabling the Use current CS&T settings preference

To enable the Use current CS&T settings preference:

1. In the **BD FACSDiva** workspace, select **File > Administration.**

2. Select a user.

3. Under **Access Privileges**, select the **Use current CS&T settings** checkbox.

4. Click **Save.**

Changing report and file preferences

Introduction   This topic describes the modifications that an Administrator can make to the default settings for reports, file saving, expiration dating, performance tracking, and dot plot parameters.

Note that if you have made changes under more than one tab, clicking Cancel cancels the changes made in all tabs.

**Changing report preferences**   **To change report preferences:**

1.   In the CS&T workspace, select **Tools > Preferences**.

     The Preferences dialog opens.

2.   Click the **Reports** tab.

3.   Select options by selecting the corresponding checkboxes.

4.   Click **OK**.

Changing the automatic Save File destination   **To change the automatic save file destination:**

1.   In the CS&T workspace, select **Tools > Preferences**.

     The Preferences dialog opens.

2.   Click the **File Locations** tab.

3. Click the **Browse** button next to the file path you want to change.
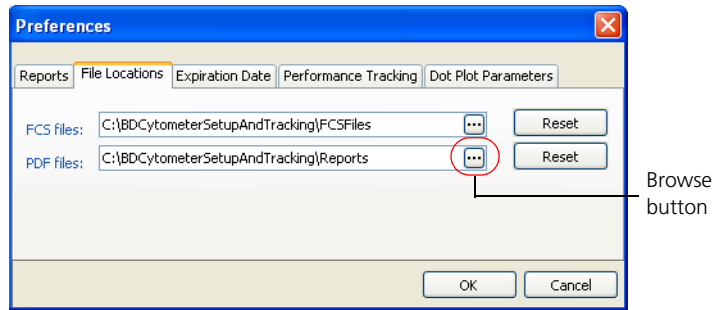
The Browse For Folder dialog opens.
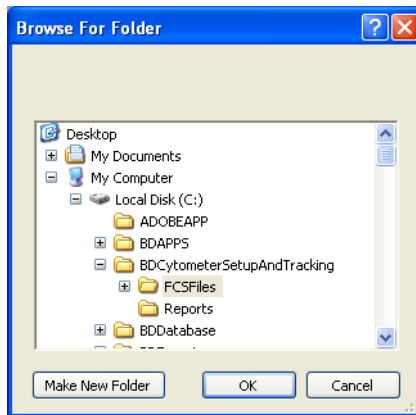
4. Do one of the following:

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

In each case, the file pathway is updated.

5. (If needed) To go back to the default pathway, click the **Reset** button next to the file pathway you changed.

FCS files:	C:\BDCytometerSetupAndTracking\FCSFiles [...] Reset
PDF files:	C:\BDCytometerSetupAndTracking\Reports [...] Reset
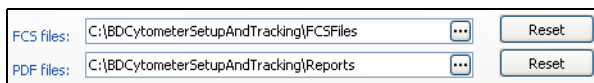
6. Click **OK**.

Resetting expiration dating

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

To reset the expiration dating:

1. In the CS&T workspace, select **Tools > Preferences**.

   The Preferences dialog opens.

2. Click the **Expiration Date** tab.

3. Select 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.

4. Click **OK.**

Selecting entries in the performance tracking file

You can select the total number of entries to display in your cytometer's performance tracking file.

To select the number of entries in the performance tracking file:

1. In the CS&T workspace, select **Tools > Preferences.**

   The Preferences dialog opens.

2. Click the **Performance Tracking** tab.

3. Select 30, 60, or an unlimited number of data points to display in the performance tracking plots.

   If you select a fixed number of data points, data is overwritten on a first in, first out basis.

   If you select the unlimited option, the time it takes to load the plots increases as the number of data points increases.

4. Click **OK.**

Selecting dot plot parameters

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

To select dot plot parameters:

1. In the CS&T workspace, select **Tools > Preferences.**

   The Preferences dialog opens.

2. Click the **Dot Plot Parameters** tab.

3. Select the configuration you want to change from the **Cytometer Configuration** menu.

4. Select the x- and y-parameters for each plot by clicking in the parameter field and selecting from the menu.
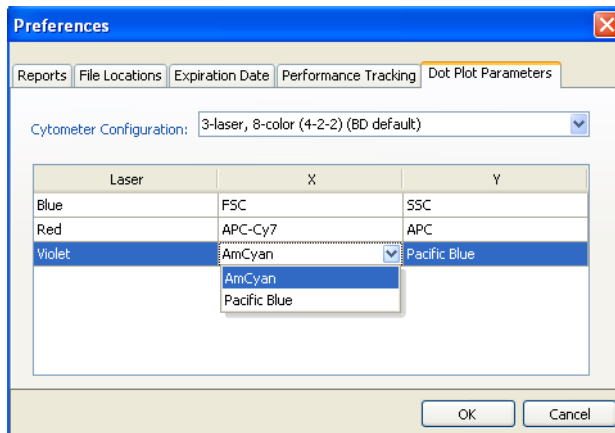
5. Click **OK.**

Setting the specification range for PMTVs

Introduction   This topic describes how to set your own specification range.

If a value varies by more than 50 volts from the baseline (the default setting), the performance check fails and the software flags a parameter voltage value in the Cytometer Performance report. When you want to expand or narrow this range, use the following procedure.

You need to be an administrator to perform this procedure.

Procedure   **To set your own specification range:**

1. In the CS&T workspace, select **Tools > Specifications**.

2. Select the **Use Custom** checkbox.
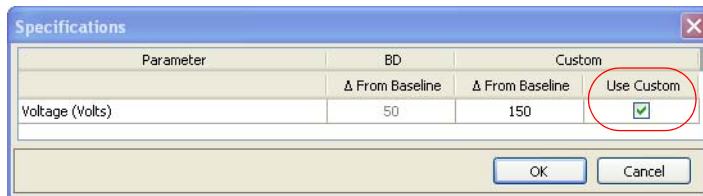
3. Enter a number between 0 and 200 in the **Custom Delta (Change) From Baseline** field.

4. Click **OK**.

   Note that you cannot change the BD-specified value.

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

   Specifications

   PMTV Delta from baseline: 100 (Custom)
   Blue Laser Bright Bead %Robust CV: 6.00 (Recommended)
   Red Laser Bright Bead %Robust CV: 6.00 (Recommended)

More information   •   Cytometer Performance report (page 62)

Setting performance tracking preferences

Introduction   This topic describes how to set the autoscale range, modify alarm boundaries, and select performance criteria to display in the Cytometer Performance report.

Setting performance tracking criteria   **To set performance tracking criteria:**

1. In the CS&T workspace, select **Tools > Performance Tracking Preferences**.

The Performance Tracking Preferences window opens.
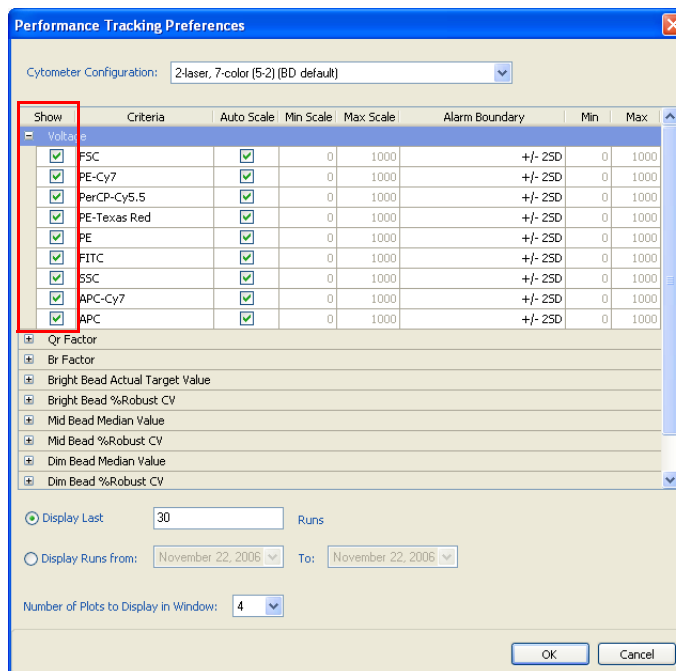
2. Select the performance criteria from the Cytometer Performance report that you want to display.

Your options are displayed under the Performance Tracking tab of the CS&T workspace. You can display different criteria for different cytometer configurations. See About CS&T reports (page 54) for an explanation of criteria.

You can select up to 30 different criteria to display under the Performance Tracking tab. Each plot displays data points for one criterion.

Selecting the scale   **To change the autoscale parameters:**

1. Do one of the following:

    •   Clear the **Auto Scale** checkbox and enter the **Min Scale** and **Max Scale** values.

        The Min Scale value must be lower than the Max Scale value. Select values between –1,299,999 and 300,000.

    •   Select the **Auto Scale** checkbox**.**

        The software automatically selects an appropriate display range.

Modifying alarm boundaries   You can modify alarm boundaries to meet the needs of your laboratory. Select different boundaries for specific criteria, or use the same boundaries for all criteria.
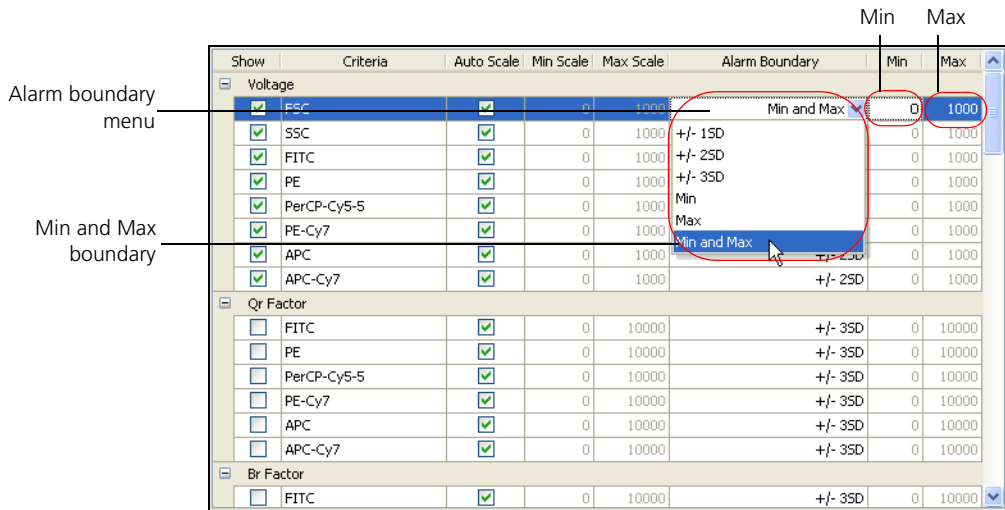
Any changes made to the alarm boundaries after you have been tracking performance will not be reflected on the Levey-Jennings charts until after the next time you run CS&T research beads.

To modify an alarm boundary:

1. For each criterion, select an appropriate alarm boundary 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 select Min and Max boundary, enter a minimum value in the **Min** field and a maximum value in the **Max** field.

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

Display Last

Display Runs

Number of LJ charts

Calendar view
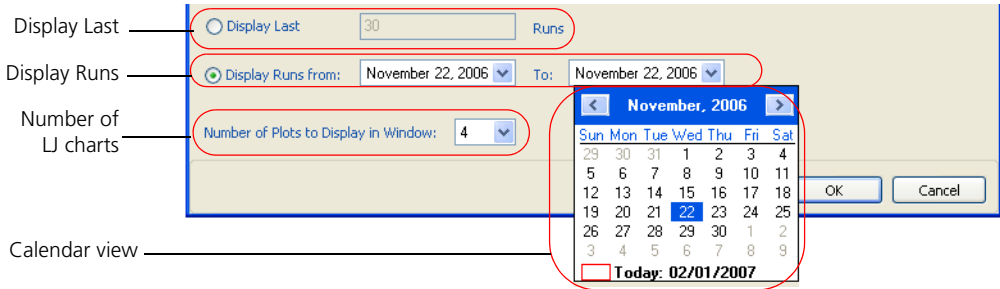
To select display criteria:

1. Select how to display performance data. Do one of the following:

    • To display data from the last number of runs, click **Display Last** and select 30, 60, or an unlimited number of runs.

    • To display data by date range, click **Display Runs From**, select **From** and **To** dates by clicking the menu and selecting dates from the calendar view.

2. Select to display 1, 2, or 4 Levey-Jennings charts per page under the **Performance Tracking** tab.

    You can select from 1 to 30 charts per page for display on the Levey-Jennings Performance report.

More information   • Cytometer Performance report (page 62)

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10

Reference information

The following topics are covered in this chapter:

Exporting results files

Introduction

This topic describes how to export results data files.

Description of results files

The software generates results files in comma-separated value (CSV) format, which is readable by a spreadsheet application such as Microsoft Excel®. You can export baseline results and performance results.

Procedure

To export results files:

1. In the **Reports** tab, select the results file you want to export.

2. Select **File > Export**.

3. In the **Export** dialog, enter a file name.

4. Select a destination folder, then click **Save**.

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

More information

- About cytometer configurations (page 69)
- About CS&T reports (page 54)

Setting up an experiment for offline analysis

Introduction

This topic describes how to set up experiments on a workstation that is not connected to a cytometer. The setup process is first done on an acquisition workstation and then completed on an analysis workstation.

Make sure that the exported cytometer configuration name is a unique name that does not already exist on the analysis workstation. Modify the name before importing the configuration if needed.

Exporting a configuration from an acquisition workstation

To export a configuration from an acquisition workstation:

1. Start BD FACSDiva software.

2. Select **Cytometer > View Configurations.**

   The Cytometer Configuration window opens.

3. Select the configuration and click **Export**.

4. In the **Export Cytometer Configuration** dialog, select where to save the configuration, then click **Save**.

5. Click **Cancel** to close the Cytometer Configuration window.

6. Exit CS&T.

Importing a configuration on an analysis workstation

You must be an administrator to perform this task.

To import a configuration on an analysis workstation:

1. Start BD FACSDiva software.

2. Select **Cytometer > View Configuration.**

   The Cytometer Configuration window opens.

3. Right-click in the **Configurations** list and select **New Folder**.

4. Rename the new folder.

5. Right-click the new folder and select **Import Configuration**.

   The Import Cytometer Configuration dialog opens.

6. Browse to the location of your configuration file and click **Open**.

7. Click **Set Configuration**.

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

More information
- About cytometer configurations (page 69)

11

Troubleshooting

The following topics are covered in this chapter:

-

Troubleshooting

Introduction   This section provides information to help you troubleshoot problems with CS&T.

For additional information, see the *BD FACSDiva Software Reference Manual.* For cytometer troubleshooting, see 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.

No beads detected

Possible causes	Recommended solutions
Beads were not mixed prior to dispensing	Gently vortex the bead vial, prepare a fresh bead suspension, and re-run the tube or well.
Beads are too dilute	
No beads were added to the tube or wells	
Debris in the bead suspension	
Incorrect beads were added	
Low flow rate was improperly calibrated	Contact BD Biosciences.
Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See your flow cytometer reference manual for more information.
Clogs within sample tubes and lines	Check the fluidics for clogs and debris. See your flow cytometer reference manual for more information.

Possible causes	Recommended solutions
Back pressure in the waste lines	Check the waste tank vent for obstructions.
Neutral density filter 2.0 or greater is installed in the FSC detector	Replace the filter with a lower rated neutral density filter.
High scatter noise (FSC or SSC)	• Perform monthly maintenance. • Clean the flow cell. • Contact BD Biosciences.

CS&T interface does not start

Possible causes	Recommended solutions
Error message is hidden behind the BD FACSDiva workspace	Minimize the BD FACSDiva workspace to see the error message.

A custom configuration has a red exclamation mark

Possible causes	Recommended solutions
The configuration was created from a different base configuration in the previous version of software.	Recreate the custom configuration using the base configuration on your system.

Unable to connect to the cytometer after exiting CS&T

Possible causes	Recommended solutions
Configuration file was not successfully sent to the cytometer	1. Select **Cytometer > View Configuration**. 2. Set the appropriate configuration. 3. Close CS&T.

Sample rate is too low to complete analysis

Possible causes	Recommended solutions
Beads were not mixed prior to dispensing	Gently vortex the bead vial, prepare a fresh bead suspension, and re-run the tube or well.
Beads are too dilute	
Debris in the bead suspension	
Incorrect beads added	
Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See your flow cytometer reference manual for more information.
Back pressure in the waste lines	Check the waste tank vent for obstructions.
Low flow rate	● Prepare a fresh bead suspension, increasing the number of drops of CS&T research beads. Re-run the tube or well. ● Contact BD Biosciences.

Apply Current CS&T Settings is not available in the shortcut menu

Possible causes	Recommended solutions
Experiment has specimen- or tube-level cytometer settings	Delete the specimen- or tube-level cytometer settings.

Apply Application Settings is not available in the shortcut menu

Possible causes	Recommended solutions
● Experiment has specimen- or tube-level cytometer settings ● Experiment does not have an associated configuration	Apply the current CS&T settings to the experiment-level cytometer settings.

CS&T keywords are not displayed in the statistics FCS file header

Possible causes	Recommended solutions
Cytometer settings are no longer the current CS&T settings values	Right-click the experiment-level cytometer settings and select **Apply Current CST Settings**.

Create Worksheet is not available in the shortcut menu

Possible causes	Recommended solutions
● Experiment has specimen- or tube-level cytometer settings ● Experiment does not have an associated configuration	Apply the current CS&T settings to the experiment-level cytometer settings.
● Performance check was not performed	Run a performance check.
● CS&T settings were not applied	Apply the current CS&T settings to the experiment-level cytometer settings.

Unable to set laser delays

Possible causes	Recommended solutions
Beads were not mixed prior to dispensing	Gently vortex the bead vial, prepare a fresh bead suspension, and re-run the tube or well.
Beads are too dilute	
Debris in the bead suspension	
Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See your flow cytometer reference manual for more information.
• Unstable sheath pressure • Backpressure in the waste line	Check the fluidics for leaks, bubbles, and clogs. See your flow cytometer reference manual for more information.
High scatter noise (FSC or SSC)	• Perform monthly maintenance. • Clean the flow cell. • Contact BD Biosciences.

Unable to use a panel template to create a new specimen

Possible causes	Recommended solutions
Panel template is associated with a configuration that differs from the experiment configuration	Use a panel template that does not contain cytometer settings.

Performance (Qr, Br, SD$_{EN}$, PMTV, etc) differs after changing the bead lot

Possible causes	Recommended solutions
Lot-specific information for the new bead lot is unavailable	Download the current lot-specific information for the CS&T research beads. See Downloading a bead lot file (page 103).

Response curves displayed in baseline results plots are uncharacteristic

Possible causes	Recommended solutions
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%

Possible causes	Recommended solutions
Beads have expired	Prepare a fresh bead suspension using the new bead lot and re-run the tube or well.
Beads were exposed to direct light	Prepare a fresh bead suspension and re-run the tube or well.
Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See your flow cytometer manual for more information.
Insufficient warm-up time for the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. See your cytometer manual for requirements.
Dirty flow cell	• For the BD LSR platform, perform the monthly cleaning procedure. See your cytometer manual for instructions. • For the BD FACSCanto or BD FACSAria platforms, perform the clean flow cell procedure. See your cytometer manual for instructions.
Suboptimal filter and mirror configuration	See your cytometer manual for standard configurations.
Cytometer alignment has changed	Contact BD Biosciences.

Unable to calculate Qr

Possible causes	Recommended solutions
Insufficient bead intensity in the selected parameter	See Limitations (page 10).
Beads have expired	Prepare a fresh bead suspension using the new bead lot and re-run the tube or well.
Beads were exposed to direct light	Prepare a fresh bead suspension and re-run the tube or well.
Low dim bead %rCV caused by high laser power or low sheath pressure	Contact BD Biosciences.
Debris in the bead suspension	Gently vortex the bead vial, prepare a fresh bead suspension, and re-run the tube or well.

All values for a particular fluorochrome show as NA

Possible causes	Recommended solutions
PMT failure	• Prepare a new bead solution and re-run the baseline. • Perform the long clean procedure on the cytometer. See your cytometer reference manual for instructions. • Contact BD Biosciences.
Incorrect filters used	See Limitations (page 10).

%rCV and rSD show as NA

Possible causes	Recommended solutions
Insufficient bead intensity in the selected parameter	See Limitations (page 10).

Linearity warning: Unable to reach maximum channel with maximum PMTV

Possible causes	Recommended solutions
Insufficient bead intensity in the selected parameter	See Limitations (page 10).
Beads have expired	Prepare a fresh bead suspension using the new bead lot and re-run the tube or well.
Beads were exposed to direct light	Prepare a fresh bead suspension and re-run the tube or well.
Debris in the bead suspension	Gently vortex the bead vial, prepare a fresh bead suspension, and re-run the tube or well.
Insufficient warm-up time for the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. See your cytometer manual for requirements.
Dirty flow cell	- For the BD LSR platform, perform the monthly cleaning procedure. See your cytometer manual for instructions. - For the BD FACSCanto or BD FACSAria platforms, perform the clean flow cell procedure. See your cytometer manual for instructions.
Cytometer alignment has changed	Contact BD Biosciences.

Area scaling factor (ASF) value flagged

Possible causes	Recommended solutions
• Unstable fluidics • Air bubbles in the flow cell or sheath filter	• Purge the flow cell. • Perform the monthly cleaning procedure. See your cytometer manual for instructions. • Debubble the sheath filter. • Contact BD Biosciences.
• Ratio of Minimum ASF to Maximum ASF >3 • Laser is out of alignment	Contact BD Biosciences.

PMT settings change >50 volts (or user-specified value) between performance checks

Possible causes	Recommended solutions
Beads have expired	Prepare a fresh bead suspension using the new bead lot and re-run the tube or well.
Beads were exposed to direct light	Prepare a fresh bead suspension and re-run the tube or well.
Baseline was not reset after cytometer maintenance	Redefine the baseline. See Running a baseline definition (page 90).
Incorrect filters or mirrors are installed	Make sure that the selected configuration matches the physical configuration of the cytometer.
Dirty flow cell	• For the BD LSR platform, perform the monthly cleaning procedure. See your cytometer manual for instructions. • For the BD FACSCanto or BD FACSAria platforms, perform the clean flow cell procedure. See your cytometer manual for instructions.

Possible causes	Recommended solutions
Air bubbles in the flow cell	Check the fluidics for bubbles and debris. See your cytometer manual for more information.
Insufficient warm-up of the cytometer lasers	Allow the cytometer lasers sufficient warm-up time. See your cytometer manual for requirements.
Cytometer alignment has changed	Contact BD Biosciences.

FSC settings change >50 volts (or user specified value) between performance checks

Possible causes	Recommended solutions
Incorrect neutral density filter is installed (BD FACSAria platform only)	Install the correct neutral density filter.

CS&T System Summary - Requires attention

Possible causes	Recommended solutions
Investigate baseline or performance check	• Run new baseline and performance checks. • Do not use expired baseline or performance checks. • Check for baseline warnings. • Check for performance check failure and/or warnings.

Performance check aborted

Possible causes	Recommended solutions
The rCV ratio of dim to mid beads is less than 1.5	● Prepare a fresh bead suspension and re-run the performance check. ● Perform the monthly cleaning procedure. See your cytometer manual for instructions. ● Contact BD Biosciences.
Insufficient warm-up time of the cytometer lasers	Allow the cytometer lasers sufficient time to warm up. See your cytometer manual for laser warm-up requirements.

Glossary

A

application settings
Cytometer settings that are linked with a specific cytometer configuration and automatically adjusted according to the results obtained during the performance check.

area scaling
A correction factor that places area measurements on the same scale as height measurements.

B

background (Br)
Relative optical background fluorescence.

C

CS&T research beads
Particles used to automatically perform basic cytometer setup and performance tracking.

CS&T workspace
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 from secondary parameters is removed so that fluorescence values for a parameter reflect only the fluorescence of the primary fluorophore.

cytometer settings   Collection of values for parameters measured, photomultiplier tube (PMT) voltages, threshold, compensation, and any ratio measurements.

Cytometer Setup and Tracking   A group of setup and cytometer management functions in BD FACSDiva software.

G

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

L

laser delay   Amount of time between signals from different laser intercepts.

M

median fluorescence intensity (MFI)   The median channel of the cytometer response for a population.

P

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.

Q

Qr   Relative fluorescence detection efficiency for each fluorescence detector.

R

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:

$$\theta_{median} = \mathbf{med}_i\{\mathbf{x}_i\}$$

From that, the median absolute deviation is computed:

$$\sigma_{median} = \mathbf{med}_i\{(|\mathbf{x}_i - \theta_{median}|)\}$$

Then the robust standard deviation is computed.

The constant is the following:

$$\sigma_{median} = \sigma_{median} / \phi^{-1}(0.75)$$

S

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 the following:

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

T

threshold A trigger signal and level of discrimination to eliminate unwanted events. Only events with parameter values above the threshold will be analyzed.

W

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.

Index