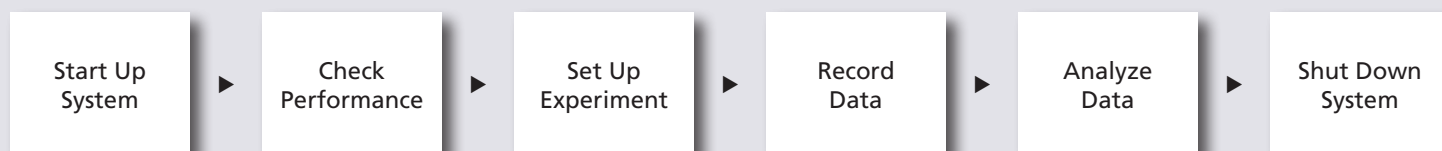


BD FACSDiva Software Quick Reference Guide for the BD LSR II

This guide contains instructions for using BD FACSDiva™ software version 6.0 and later with BD™ LSR II flow cytometers.

Workflow Overview

The following figure shows the steps for daily workflow using BD FACSDiva software.



Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.



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Starting Up the System

- 1 Start up the cytometer and computer.
- 2 Start BD FACSDiva software and log in.
- 3 Prepare the fluidics tanks and remove bubbles from the fluidics system.
- 4 Verify that the optical filters are appropriate for your experiment.

Checking Cytometer Performance

- 1 Select Cytometer > CST.

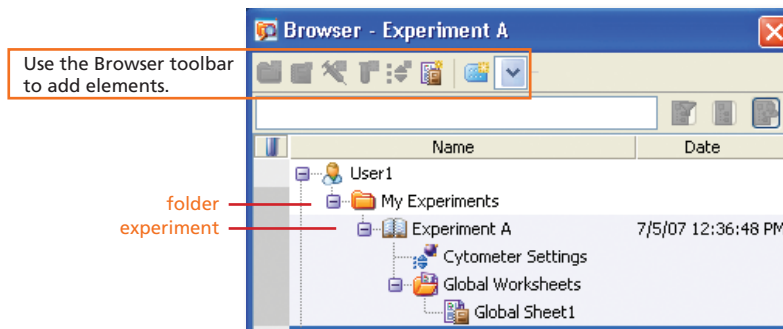
Verify the Cytometer Configuration and bead lot ID.

If needed, select a different configuration or bead lot ID.

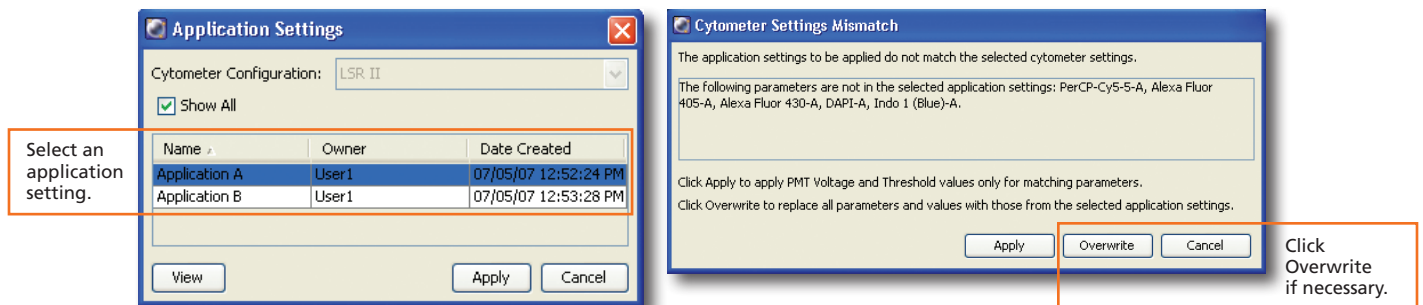
- 2 Run the BD™ Cytometer Setup and Tracking beads.
- 3 View the Cytometer Performance Report.
- 4 Close the Cytometer Setup and Tracking window.

Setting Up the Experiment

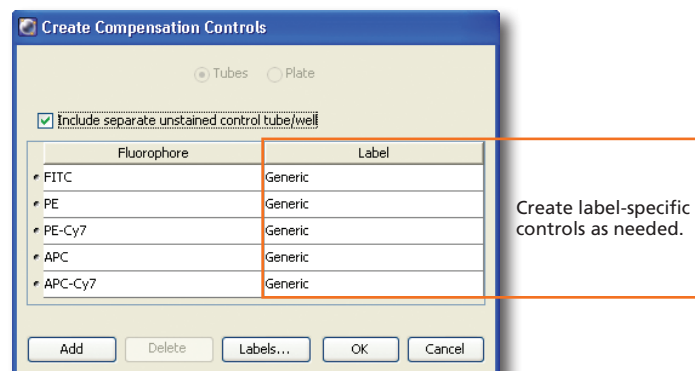
- 1 Create Browser elements.



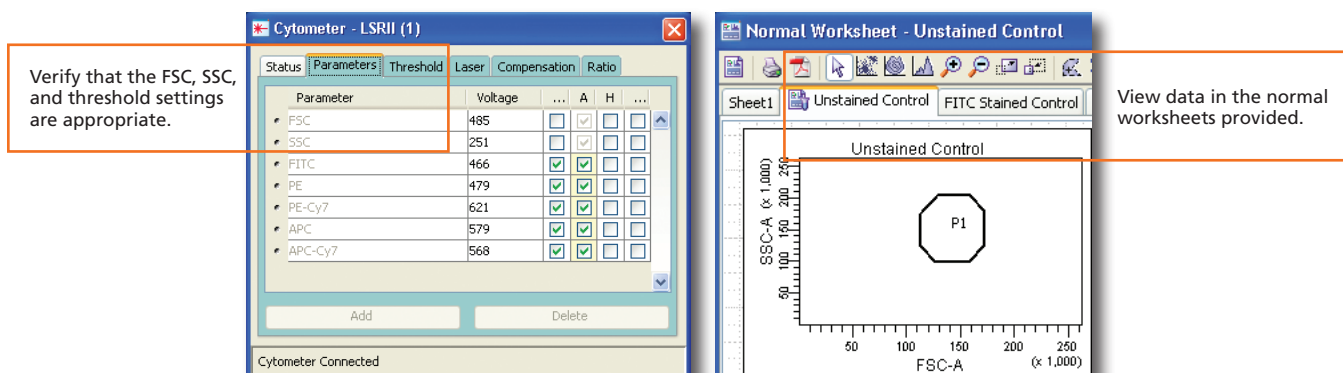
- 2 Right-click Cytometer Settings in the Browser. Select Application Settings > Apply. See page 5 for additional information about creating application settings.



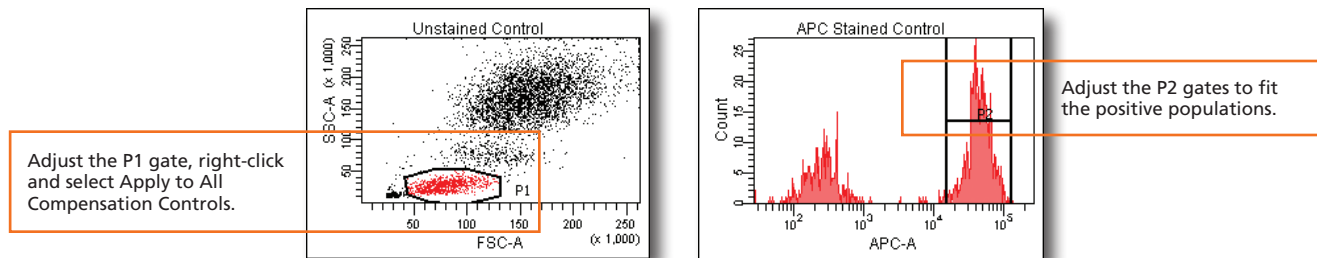
- 3 Select Experiment > Compensation Setup > Create Compensation Controls.



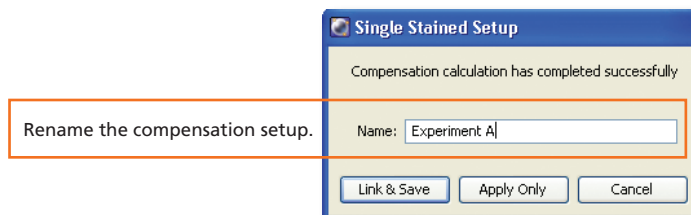
- 4 Install the unstained control onto the cytometer. Click Acquire Data.



- Record data for the compensation control tubes.

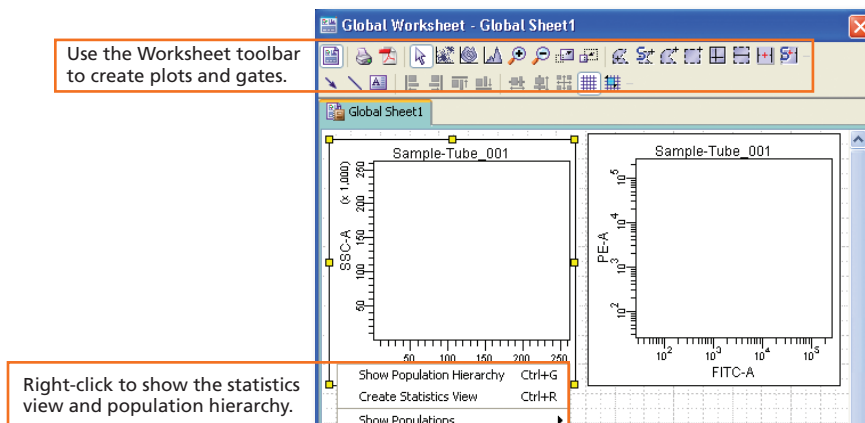


- Select Experiment > Compensation Setup > Calculate Compensation.

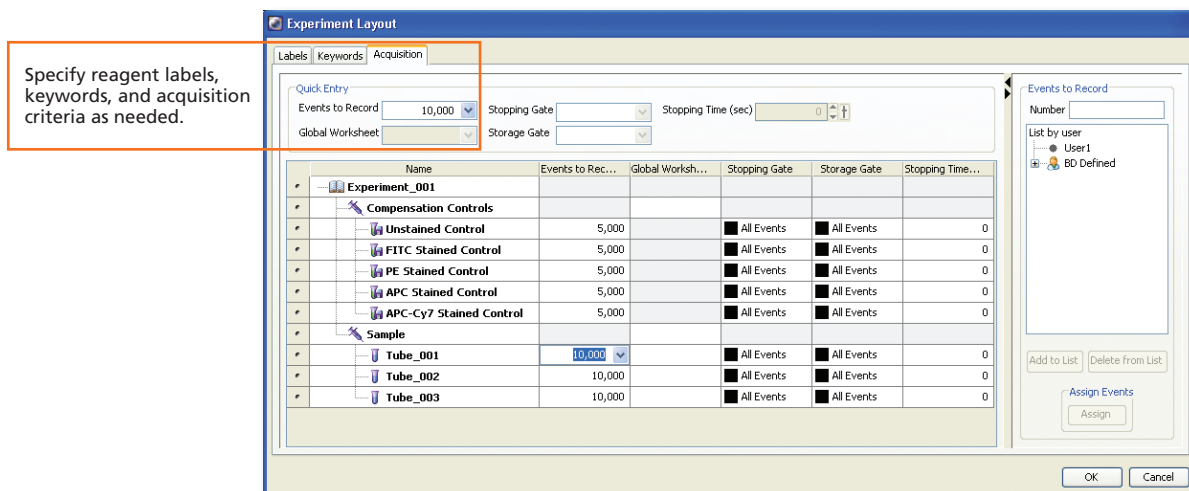


Recording Specimen Data

- Create Browser elements.
- Create the plots, gates, and statistics needed for recording.



- Make entries in the Experiment Layout.



- Record data.

Analyzing Data

- 1 Create plots, gates, and statistics needed for analysis.

The screenshot shows the BD FACS software interface. The **Browser** window on the left displays a tree view of experiments and global worksheets. The **Global Worksheet - Global Sheet2** window on the right shows a scatter plot of SSC-A vs FSC-A for Sample-Tube_001, with a red gate labeled 'Parent'. The **Inspector - Dot Plot** window at the bottom shows the plot settings, including the X Parameter (FSC-A) and Y Parameter (SSC-A).

Annotations:

- Create new global worksheets.** Points to the 'Global Worksheets' folder in the Browser.
- Create custom text and graphics.** Points to the Global Worksheet window.
- Customize plots using the Plot Inspector.** Points to the Inspector - Dot Plot window.

- 2 Perform quality control of the analysis.

The screenshot shows a scatter plot of SSC-A vs FSC-A for Sample-Tube_001, with a red gate labeled 'Parent'. Below the plot is a table showing the population hierarchy for Tube_001.

Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
Parent	1,867	18.7	18.7
Child A	128	6.9	1.3
Child B	219	11.7	2.2

Annotations:

- Verify that gates are set appropriately for all samples.** Points to the scatter plot.
- Use the population hierarchy to verify parent/child relationships.** Points to the population hierarchy table.

- 3 Do one of the following to print or export the results.

- Select File > Print to print the active worksheet.
- Select File > Export to export selected elements.
- Right-click a specimen or experiment and select Batch Analysis (using a global worksheet).

The screenshot shows the **Batch Analysis** dialog box. It has two tabs: **Auto** and **Manual**. The **Auto** tab is selected, and the following options are checked:

- ☒ Output To Printer
- ☒ Save as PDF
- ☒ Add Report to PDF
- ☒ Statistics
- ☒ Freeze Biexponential Scales
- ☒ Use Preferred Global Worksheet

The PDF Filename is set to 'sheet\Batch_Analysis_05072007133515.pdf' and the Export Filename is set to 'statistics\Batch_Analysis_05072007133515.csv'. The Status bar shows 0% completion. Buttons for Start, Pause, Continue, and Close are at the bottom.

Annotation:

- Select to print, save as a PDF, or export the statistics as needed.** Points to the checked options in the Batch Analysis dialog.

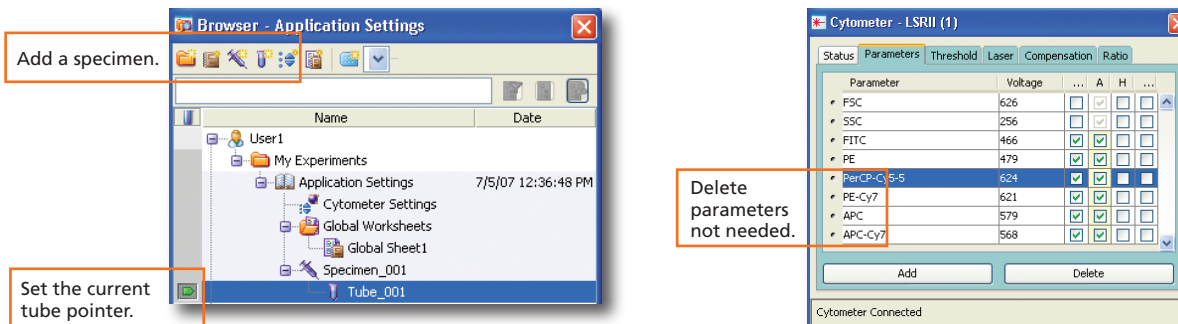
Shutting Down the System

- 1 Clean the fluidics.
- 2 Select File > Quit.
- 3 Turn off the cytometer and computer.

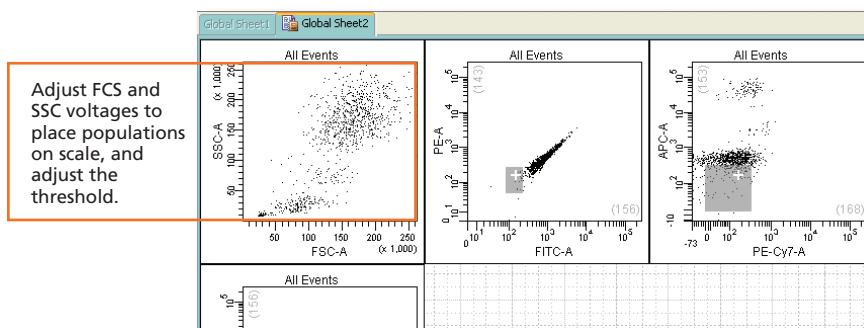
Creating Application Settings

Before creating application settings, perform the cytometer startup procedure according to your cytometer user's guide and run a performance check.

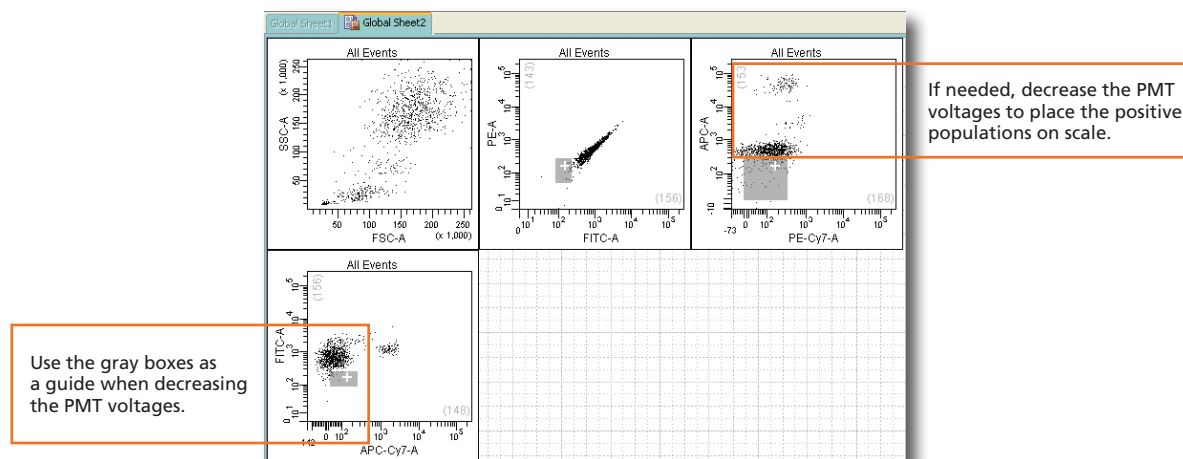
- 1 Create a new experiment.



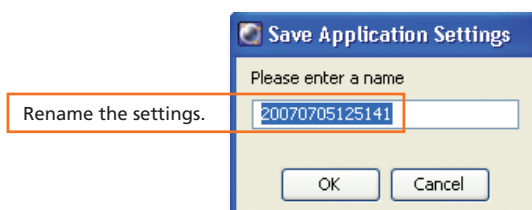
- 2 Right-click Cytometer Settings in the Browser. Select Application Settings > Create Worksheet.
- 3 Install the unstained control onto the cytometer. Click Acquire Data .
- 4 Adjust the cytometer settings.



- 5 Acquire the single-stained controls.



- 6 Right-click Cytometer Settings in the Browser. Select Application Settings > Save.



Cytometer Settings Icons in the Browser



Default or user-defined settings have been applied.



CS&T defined settings are applied.



User-defined application settings have been applied.



A compensation setup has been linked to the application settings.



A compensation setup has been linked to the cytometer settings.

Cytometer Setups in the Catalog

Setting type	Description	When to save	How to save	How to apply	What is saved Parameters, PMTV, and threshold Area scaling and laser delay** Compensation		
Application Settings	User-optimized settings for sample type and reagent combination. CS&T updates these settings to account for daily variations.	Create once for each application you are running. Verify the settings after defining the baseline and when you change reagent lots.	Right-click the Cytometer Settings in the Browser and select Application Settings > Save.	Right-click the Cytometer Settings in the Browser and select Application Settings > Apply. Select an application setting from the catalog.	✓	✓*	—
Compensation Setup	Settings that include automatically calculated compensation values created by BD FACSDiva software using single-stained controls. Not updated by CS&T.	Create daily for each experiment.	Select Experiment > Compensation Setup > Calculate Compensation. In the Single Stained dialog, select Link & Save.	Right-click the Cytometer Settings in the Browser and select Link Setup. Select a compensation setup from the catalog.	✓	✓	✓
Cytometer Settings	User-optimized settings, not updated by CS&T or linked to a compensation setup.	As needed.	Right-click the Cytometer Settings in the Browser and select Save to Catalog.	Right-click the Cytometer Settings in the Browser and select Apply from Catalog. Select a cytometer settings from the catalog.	✓	✓	✓

* The area scaling is adjusted for the CS&T beads only. You may need to readjust this setting for your cell type.

** The latest optimized laser delay setting is always used on the cytometer. When the saved cytometer settings are re-applied, the laser delay does not change to the value saved with the settings. The laser delay setting is saved for reference only.