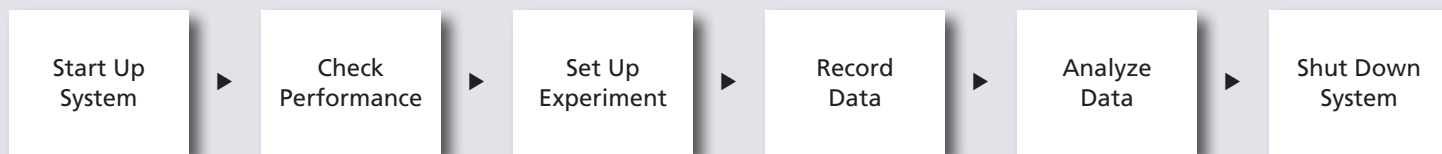


BD FACSDiva Software Quick Reference Guide for the BD LSR II or BD LSRFortessa

This guide contains instructions for using BD FACSDiva™ software version 8.0 and later with BD™ LSR II or BD LSRFortessa™ flow cytometers.

Workflow Overview

The following figure shows the daily flow cytometry workflow when 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.



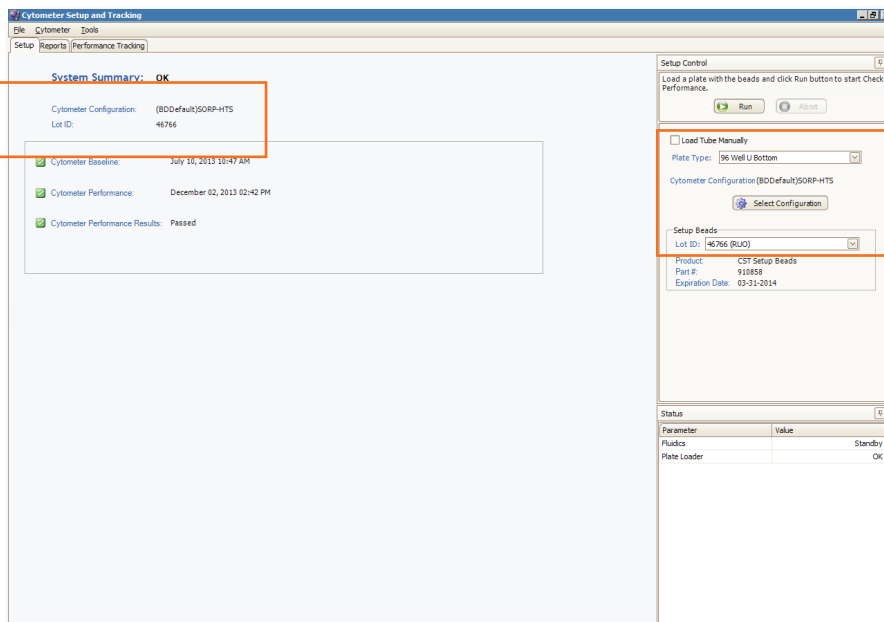
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Starting Up the System

- 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

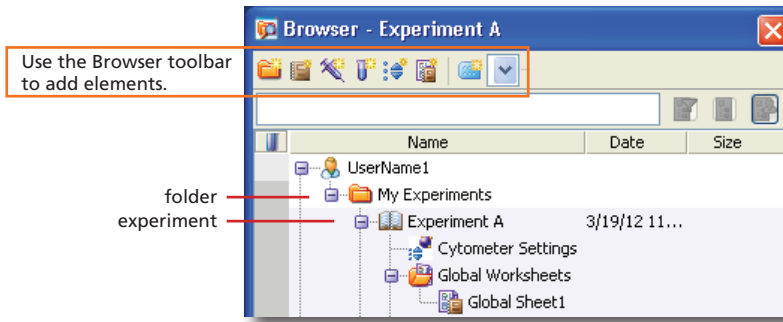
- 1 Select Cytometer > CST.



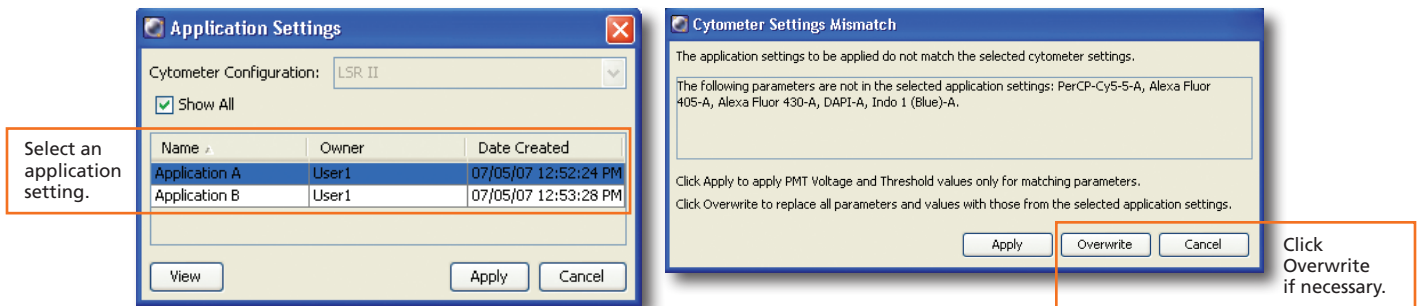
- 2 Run the BD FACSDiva™ CS&T research beads.
- 3 View the Cytometer Performance Report.
- 4 Close the Cytometer Setup and Tracking window.

Setting Up the Experiment

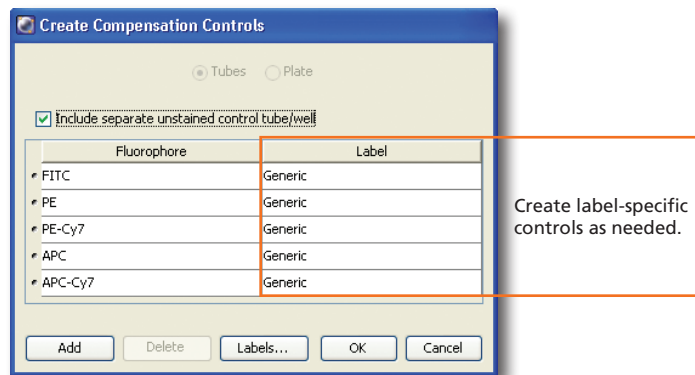
- 1 Create Browser elements.



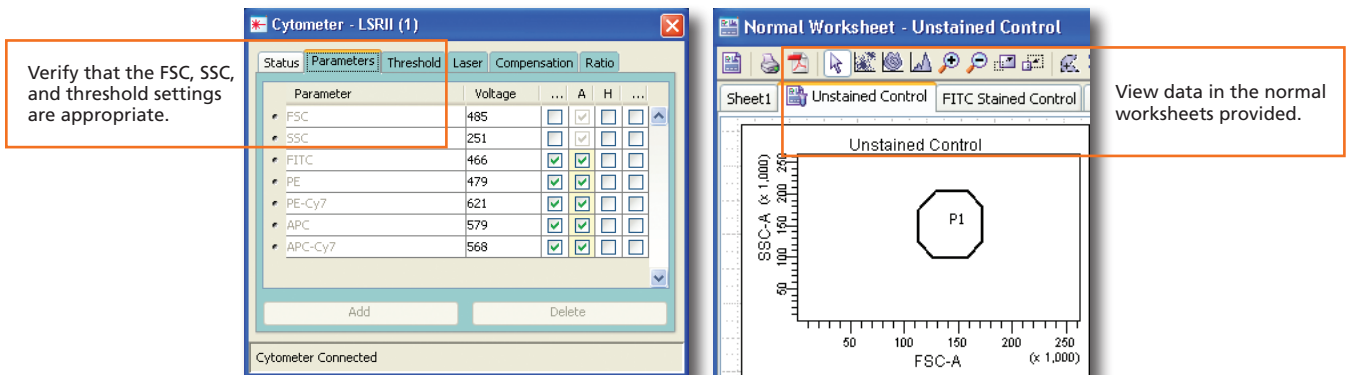
- 2 Right-click **Cytometer Settings** in the Browser. Select Application Settings > Apply.



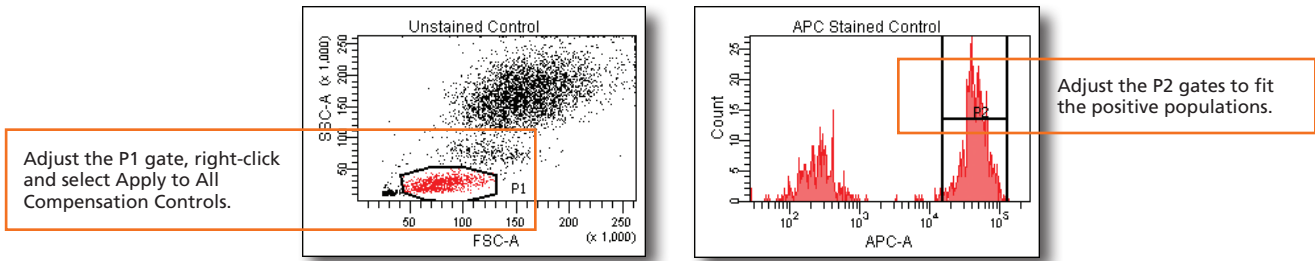
- 3 Select Experiment > Compensation Setup > Create Compensation Controls.



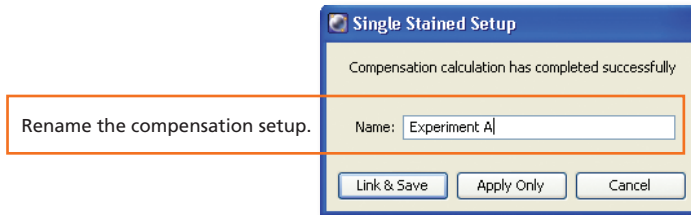
- 4 Install the unstained control tube onto the cytometer. Click **Acquire Data**.



- Record data for the compensation control tubes.

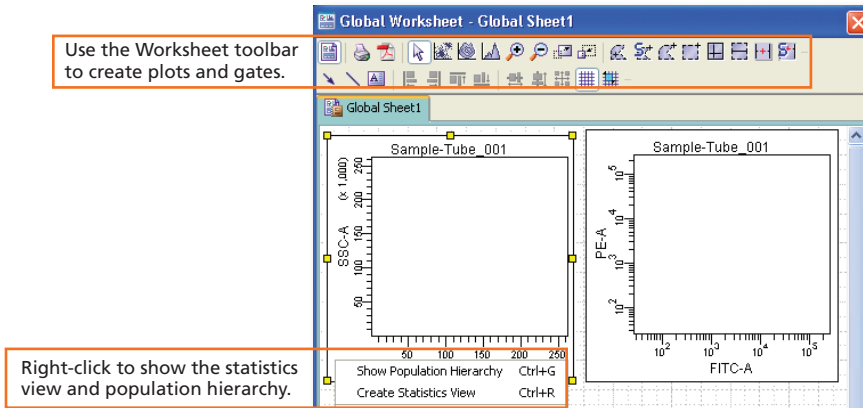


- Select Experiment > Compensation Setup > Calculate Compensation.

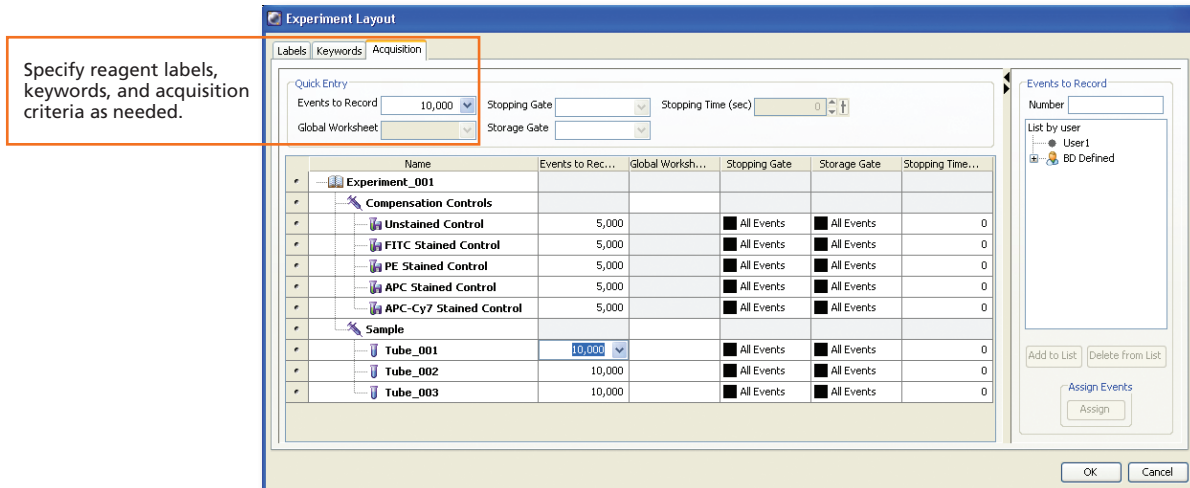


Recording Specimen Data

- 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 software interface with three windows: 'Browser - Experiment_001', 'Global Worksheet - Global Sheet2', and 'Inspector - Dot Plot'. The Browser window shows a tree view of experiments and global worksheets. The Global Worksheet window shows a scatter plot of SSC-A vs FSC-A for 'Sample-Tube_001' with a red gate labeled 'Parent'. The Plot Inspector window shows settings for the plot, including 'Tube: Sample_Tube_001', 'X Parameter: FITC-A', and 'Y Parameter: PE-A'.

Annotations:

- Global Worksheet - Global Sheet2: Create custom text and graphics.
- Browser - Experiment_001: Create new global worksheets.
- Inspector - Dot Plot: Customize plots using the Plot Inspector.

- 2 Verify 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: Tube_001'.

Population	#Events	%Parent	%Total
All Events	30,000	###	100.0
Parent	7,966	26.6	26.6
Child A	437	5.5	1.5
Child B	1,645	20.7	5.5

Annotations:

- Scatter plot: Verify that gates are set appropriately for all samples.
- Population hierarchy table: Use the population hierarchy to verify parent/child relationships.

- 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 with the following options:

- Auto (selected) / Manual
- View Time: 10
- Output To Printer (checked)
- Save as PDF (checked)
- Save as XML (checked)
- Add Report (checked)
- Statistics (checked)
- Freeze Biexponential Scales (checked)
- Use Preferred Global Worksheet (checked)
- Save Specimen Reports (unchecked)
- View PDF (checked)

Annotations:

- Batch Analysis dialog: Select the options needed.
- Batch Analysis dialog: Specify where to save the PDF, XML, and exported statistics files.

Shutting Down the System

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