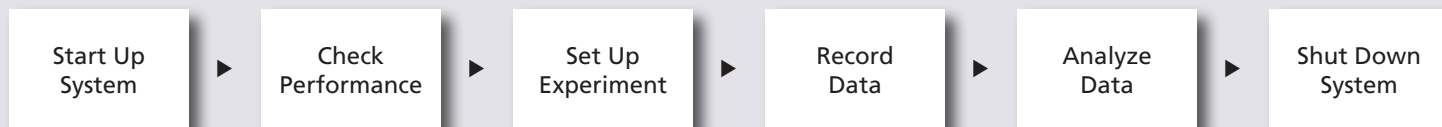


BD FACSDiva Software Quick Reference Guide for BD FACSCanto Systems

This guide contains instructions for using BD FACSDiva™ software version 8.0 and later with the BD FACSCanto™ product family.

Workflow Overview

The following figure shows the daily flow cytometry workflow when using BD FACSDiva software.



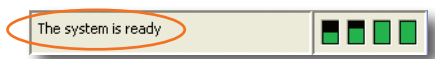
Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.



Helping all people
live healthy lives

Starting Up the System

- 1 Turn on the cytometer main power.
- 2 Start up the computer, start BD FACSDiva software, and log in.
- 3 Check fluid levels in the Cytometer window.
- 4 Select Cytometer > Fluidics Startup if automatic cleaning is disabled.
- 5 Check the flow cell for air bubbles.
- 6 Check that laser warmup has finished, indicated by a ready status.



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.

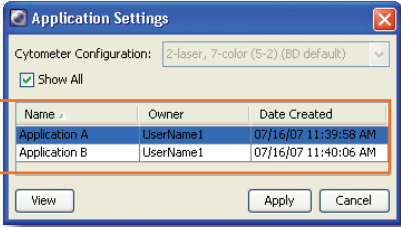
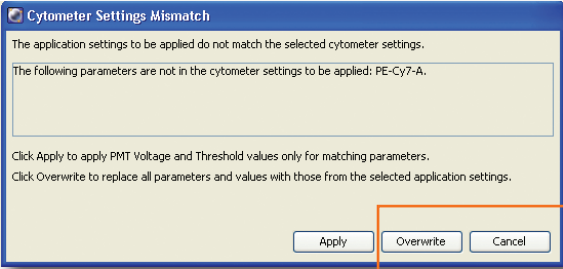
- 2 Run the BD FACSDiva™ CS&T research beads.
- 3 View the Cytometer Performance Report.
- 4 Close the Cytometer Setup and Tracking window.

Setting Up the Experiment

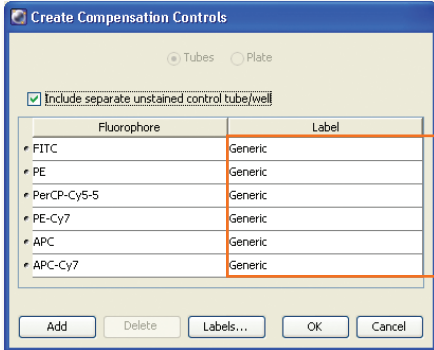
- 1 Select Edit > User Preferences and verify that selected preferences are appropriate.
- 2 Create an experiment in the Browser.
- 3 Right-click  Cytometer Settings in the Browser. Select Application Settings > Apply.

Select an application setting.

Click Overwrite if necessary.





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



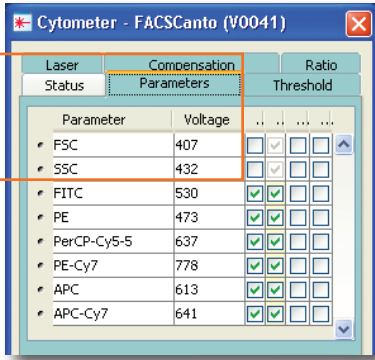
Fluorophore	Label
FITC	Generic
PE	Generic
PerCP-Cy5-5	Generic
PE-Cy7	Generic
APC	Generic
APC-Cy7	Generic

Create label-specific controls as needed.

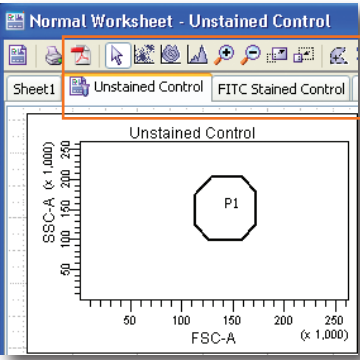
- 5 Install the unstained control tube onto the cytometer. Click .

Verify that the FSC, SSC, and threshold settings are appropriate.

View data in the normal worksheets provided.



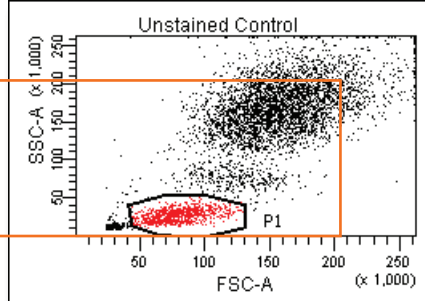
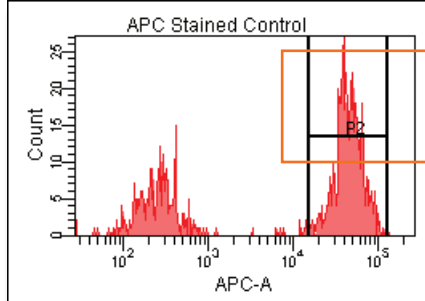
Parameter	Voltage	Ratio	Threshold
FSC	407		
SSC	432		
FITC	530		
PE	473		
PerCP-Cy5-5	637		
PE-Cy7	778		
APC	613		
APC-Cy7	641		



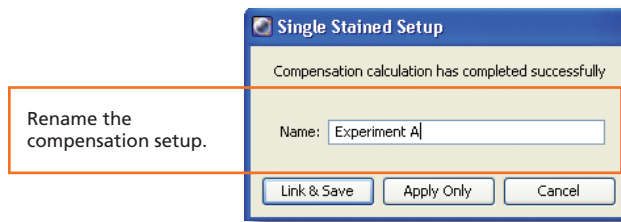
- 6 Record data for the compensation control tubes.
- 7 View the recorded data and gate the positive populations.

Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.

Adjust the P2 gates to fit the positive populations.

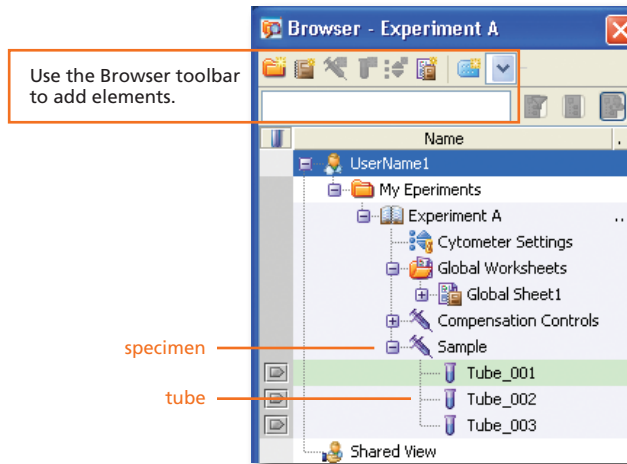



- 8 Select Experiment > Compensation Setup > Calculate Compensation.

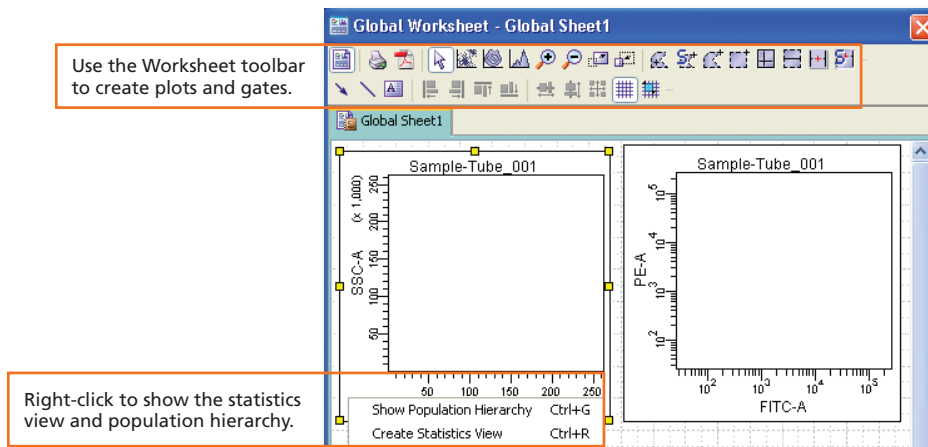


Recording Specimen Data

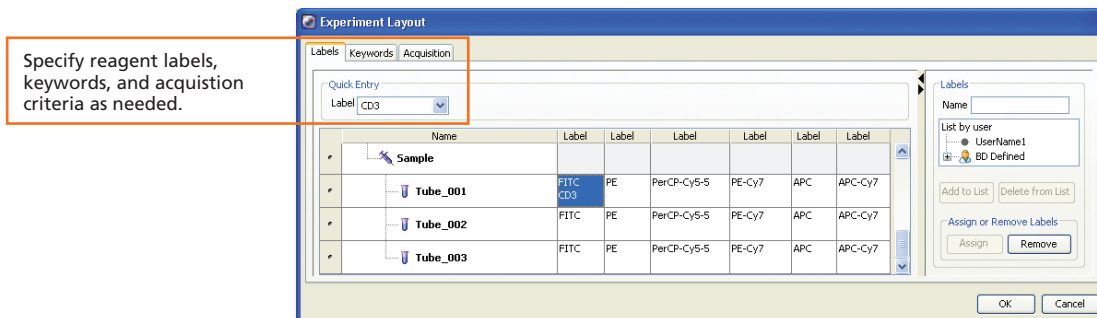
- 1 Create Browser elements.



- 2 Create plots, gates, and statistics needed for recording.



- 3 Make entries in the Experiment Layout.



- 4 Record data.

Analyzing Data

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

Create new global worksheets.

Customize plots using the Plot Inspector.

Create custom text and graphics.

- 2 Verify the analysis.

Verify that gates are set appropriately for all samples.

Use the population hierarchy to verify parent/child relationships.

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

- 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).

Specify where to save the PDF, XML, and exported statistics files.

Select the options needed.

Shutting Down the System

- 1 Perform a fluidics shutdown.
- 2 Empty the waste and refill fluids if prompted to do so.
- 3 Turn off the cytometer main power and shut down the computer.