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.

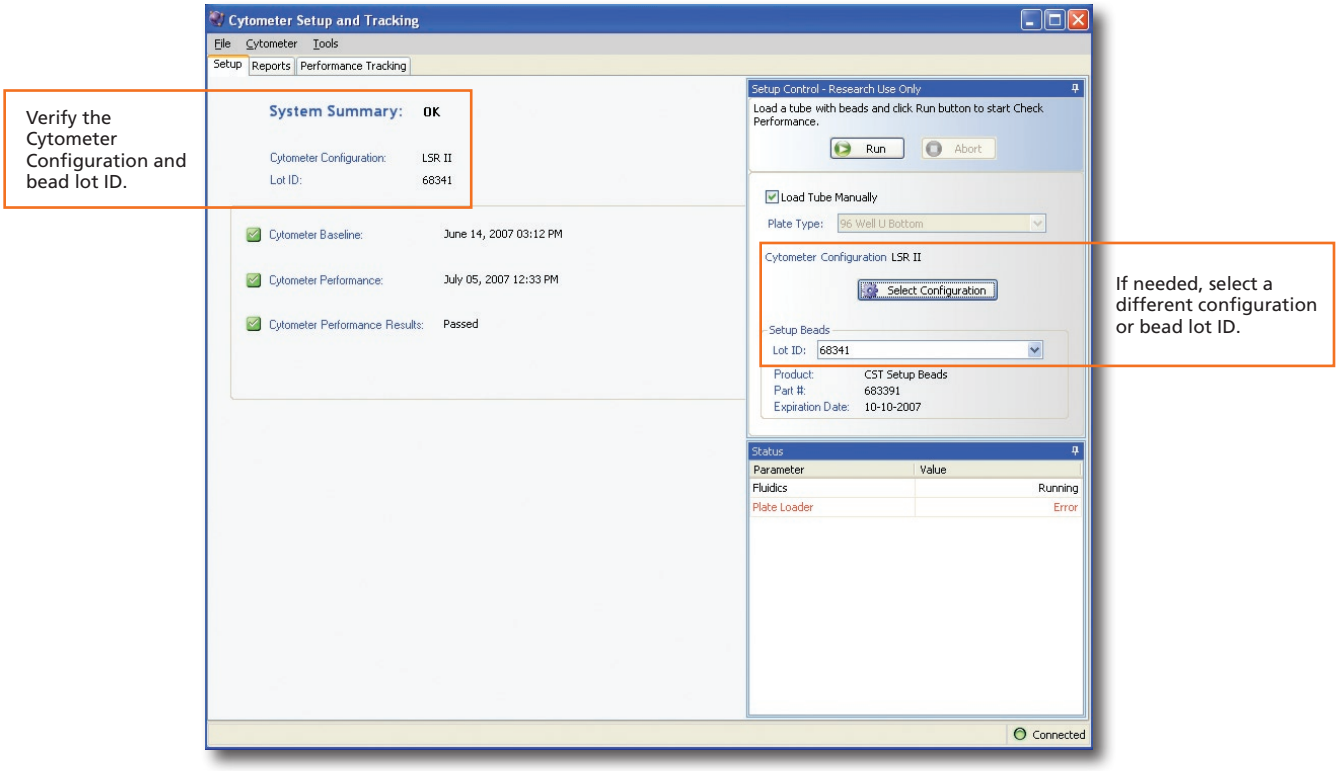
BD Helping all people live healthy lives

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.

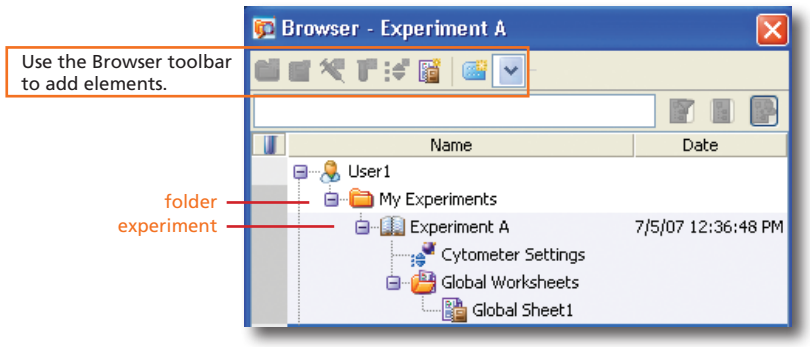


2. Run the BD™ Cytometer Setup and Tracking beads.
3. View the Cytometer Performance Report.
4. Close the Cytometer Setup and Tracking window.

23-9550-00

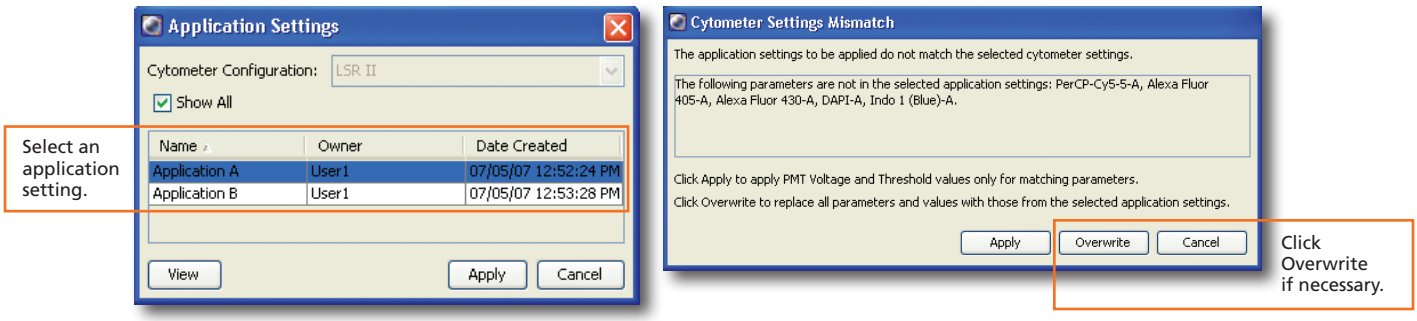
Setting Up the Experiment

1. Create Browser elements.

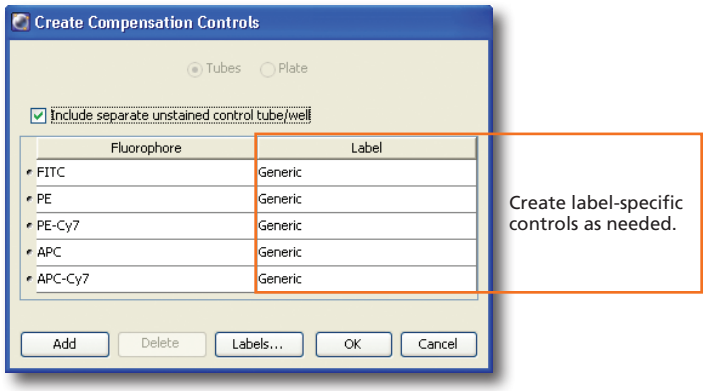


2. Right-click Cytometer Settings in the Browser. Select Application Settings > Apply.

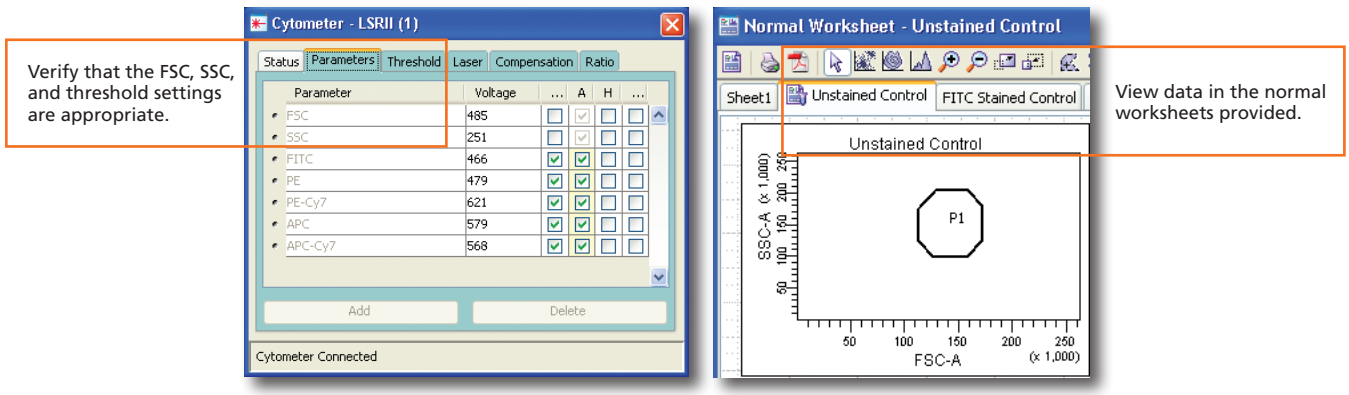
See page 5 for additional information about creating application settings.



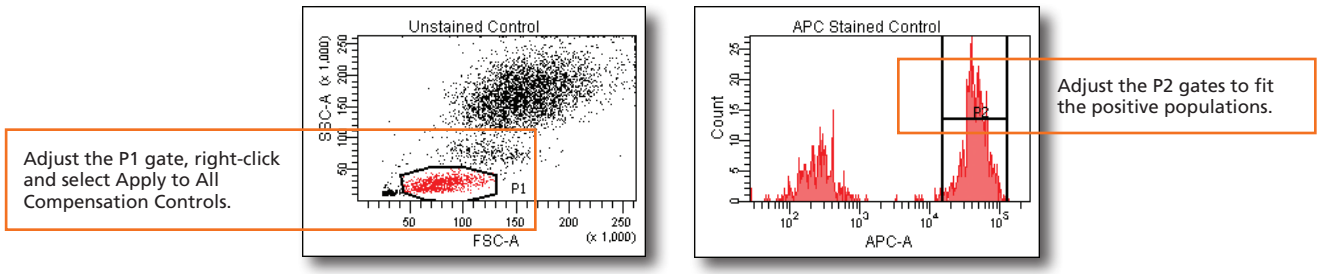
3. Select Experiment > Compensation Setup > Create Compensation Controls.



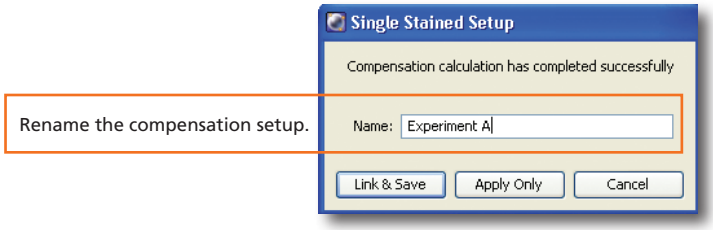
4. Install the unstained control onto the cytometer. Click Acquire Data.



5. Record data for the compensation control tubes.



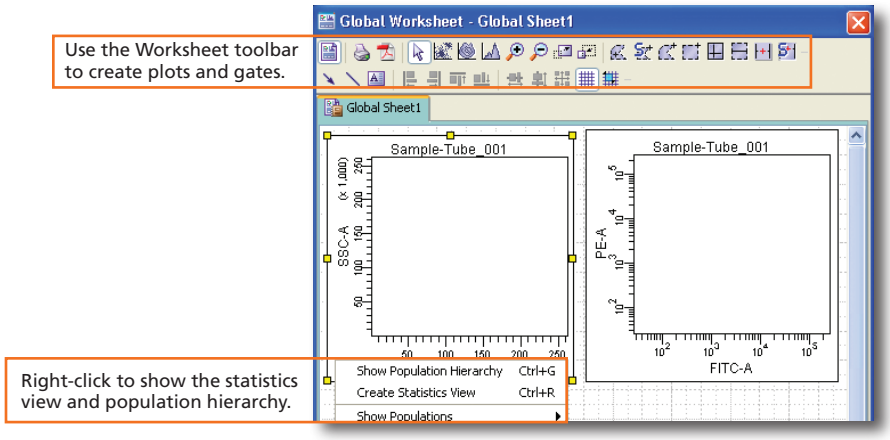
6. Select Experiment > Compensation Setup > Calculate Compensation.



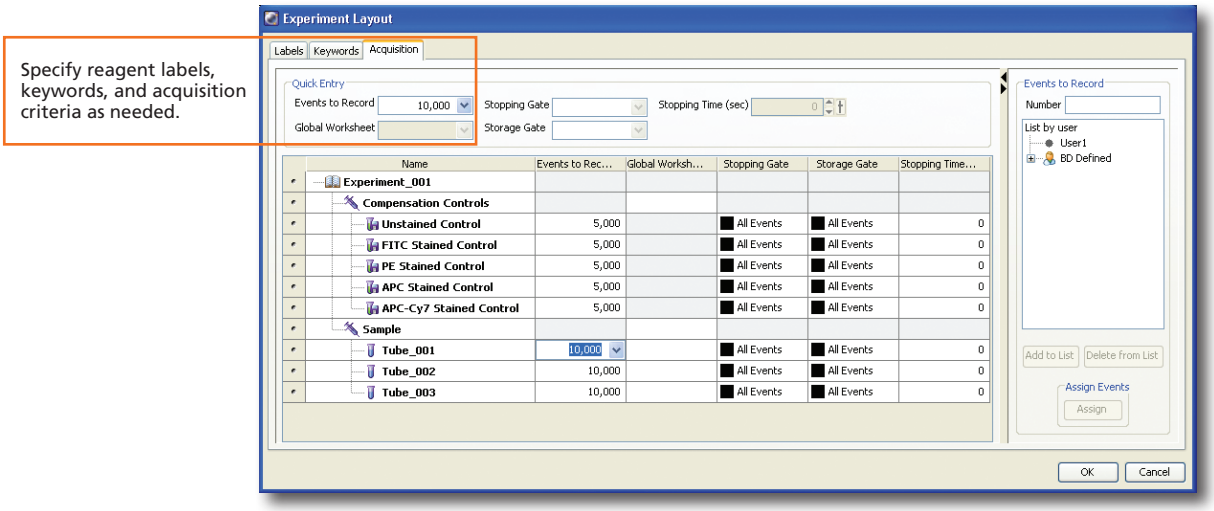
Recording Specimen Data

1. Create Browser elements.

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



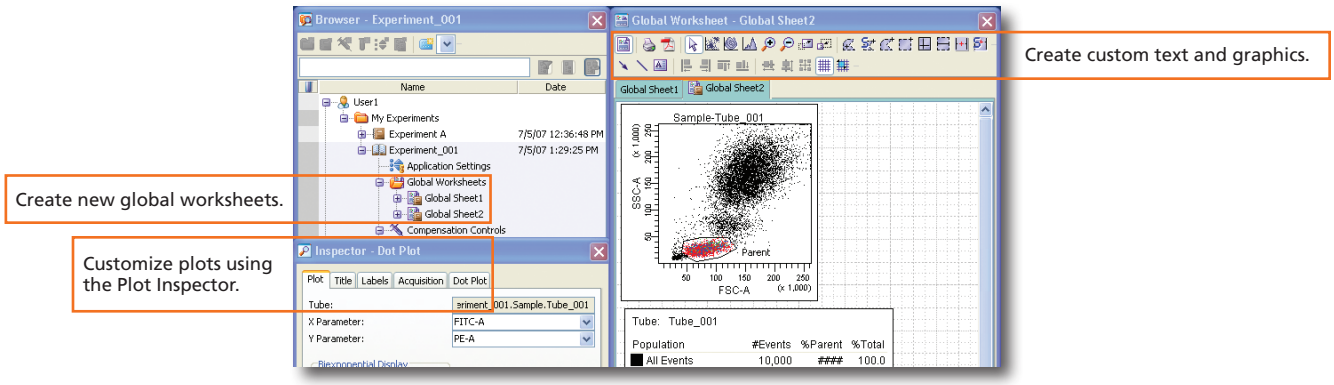
3. Make entries in the Experiment Layout.



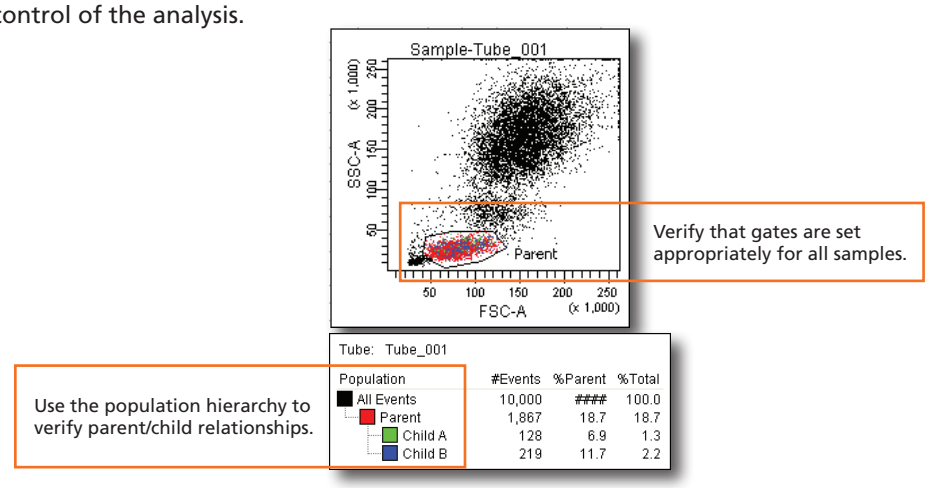
4. Record data.

Analyzing Data

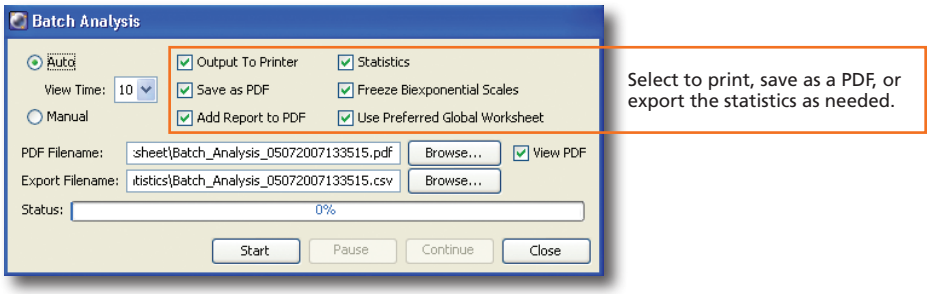
1. Create plots, gates, and statistics needed for analysis.



2. Perform quality control of the analysis.



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).



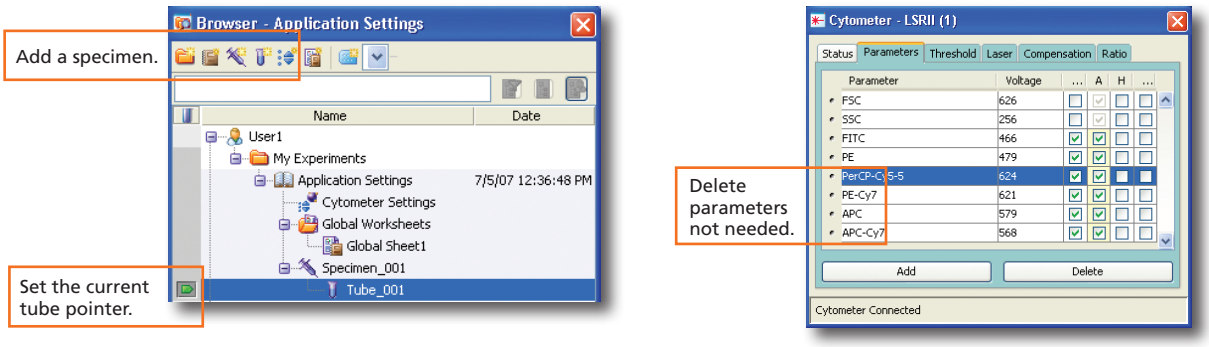
Shutting Down the System

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.

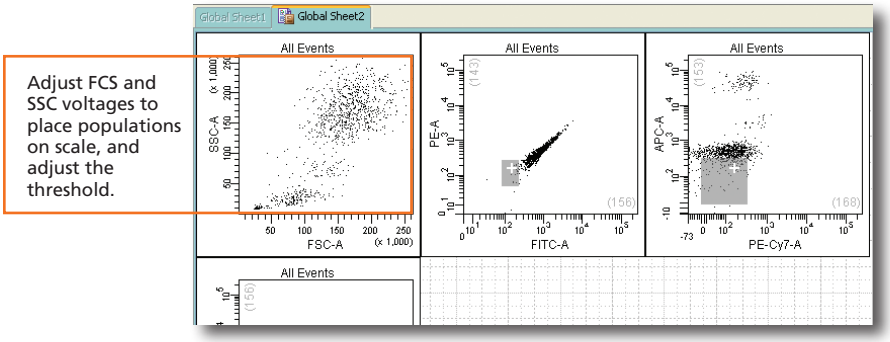
1. Create a new experiment.



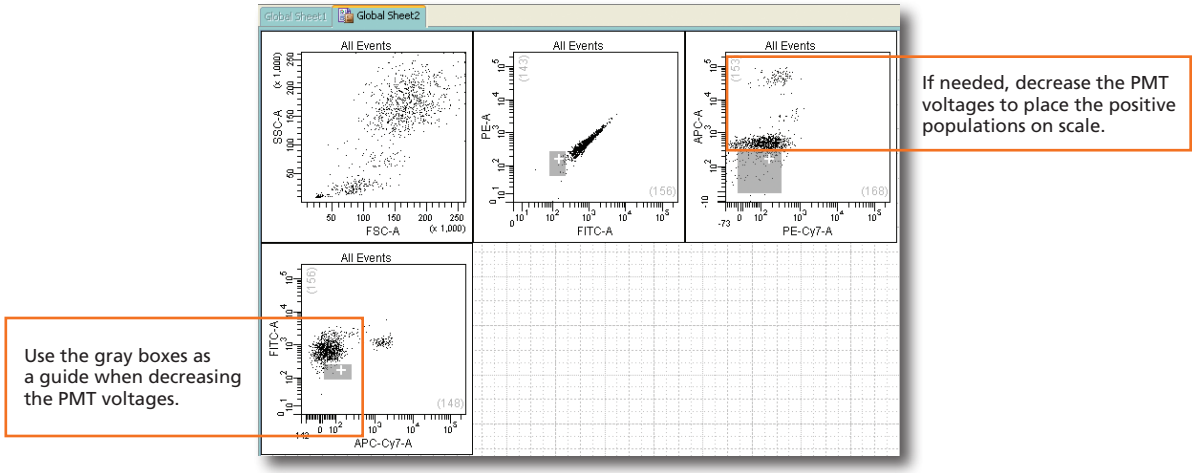
2. Right-click Cytometer Settings in the Browser. Select Application Settings > Create Worksheet.

3. Install the unstained control onto the cytometer. Click Acquire Data .

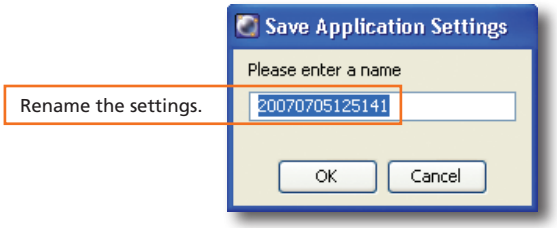
4. Adjust the cytometer settings.



5. Acquire the single-stained controls.

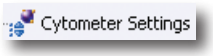


6. Right-click Cytometer Settings in the Browser. Select Application Settings > Save.



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	What is saved: Area scaling and laser delay**	What is saved: 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.