BD FACSDiva Software Quick Reference Guide for BD FACSAria Cell Sorters

This guide contains instructions for using BD FACSDiva™ software version 6.0 with BD FACSAria™ cell sorters.

Workflow Overview

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

Start Up System ▶ Check Performance ▶ Set Up Experiment ▶ Record Data ▶ Analyze Data ▶ Shut Down System

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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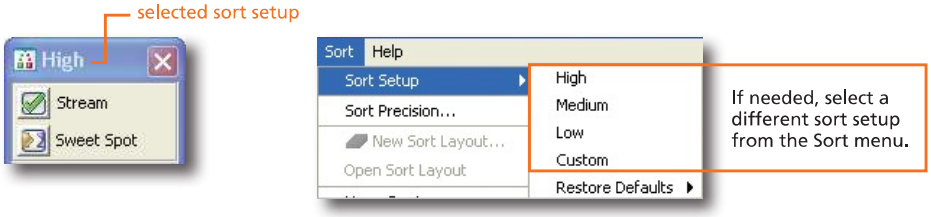
Starting Up the System

1. 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. Verify that the appropriate sort setup is selected and perform fluidics startup.



5. Optimize the breakoff.

Checking Cytometer Performance

1. Turn off the Sweet Spot and 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.
5. Verify that the breakoff is stable and turn on the Sweet Spot.

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Setting Up the Experiment

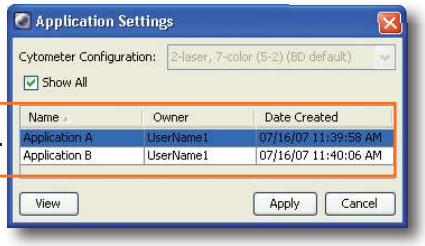
1. Create a new experiment and apply the Doublet Discrimination analysis template.

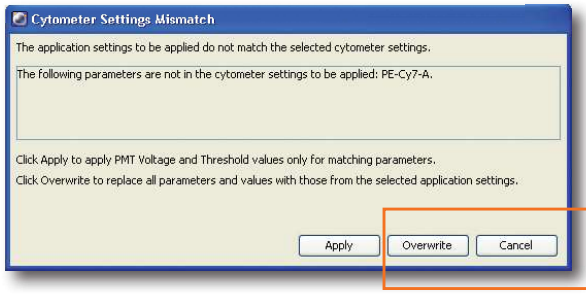
2. Select Cytometer Settings in the Browser and select height and width for the FSC and SSC parameters in the Inspector window.

3. Right-click Cytometer Settings in the Browser and select Application Settings > Apply.

See page 6 for additional information about creating application settings.

Select an application setting.



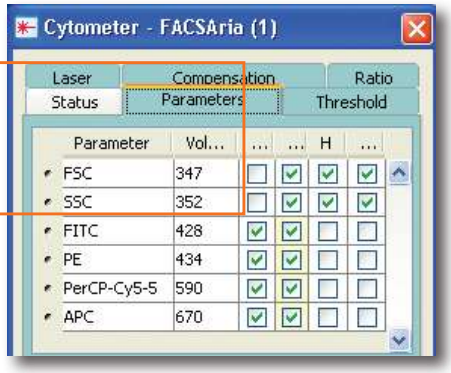


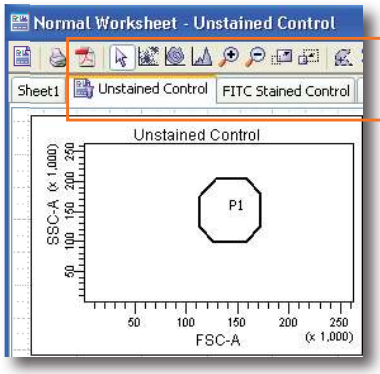
Click Overwrite if necessary.

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

5. Load the unstained control tube.

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



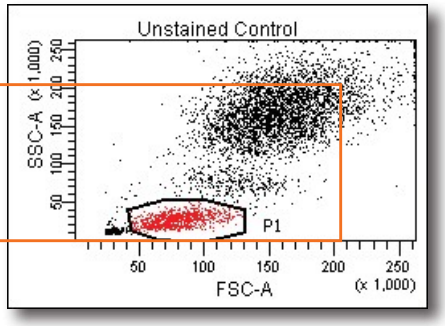


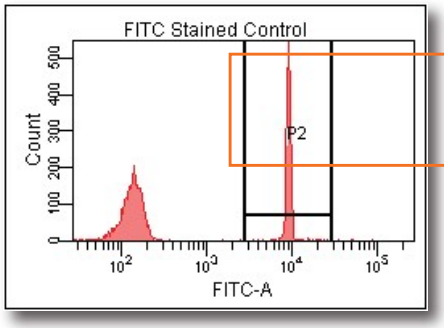
View data in the normal worksheets provided.

6. Record data for the compensation control tubes.

7. View the recorded data and gate the positive populations.

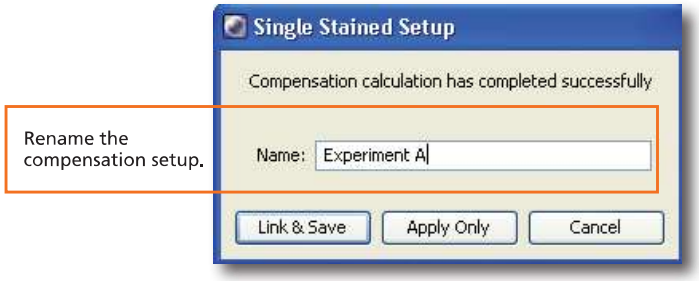
Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.



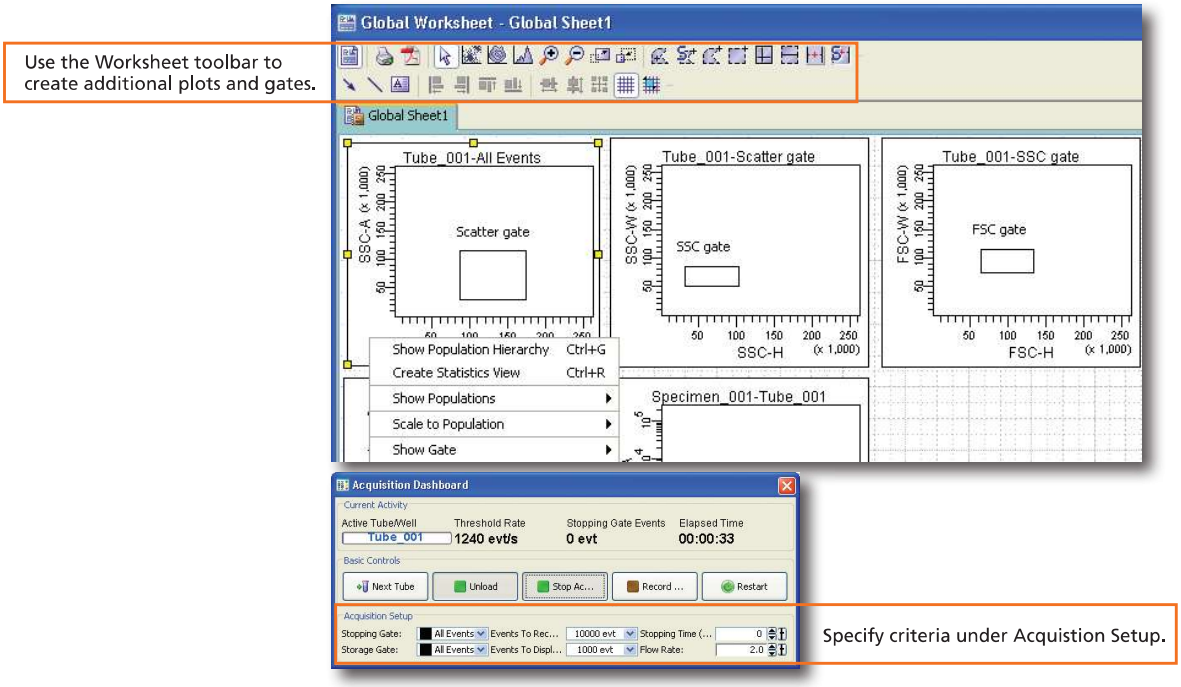


Adjust the P2 gates to fit the positive populations.

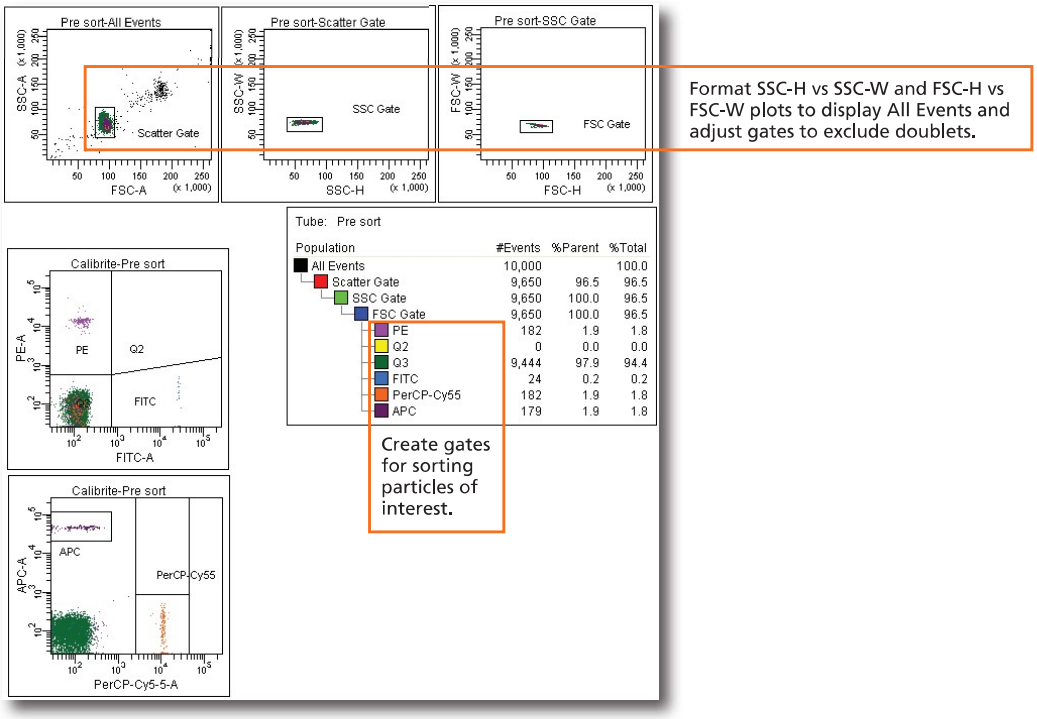
8. Select Experiment > Compensation Setup > Calculate Compensation.



9. Record pre-sort data.

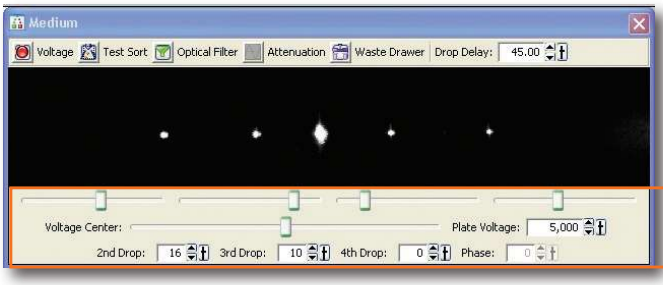


10. Create sorting gates.



Sorting

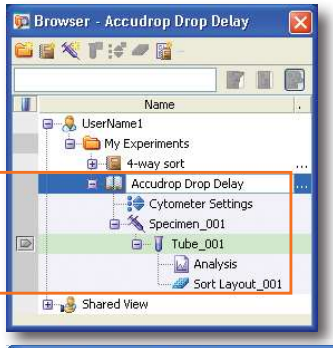
1. Install the collection tubes and optimize the side steams.



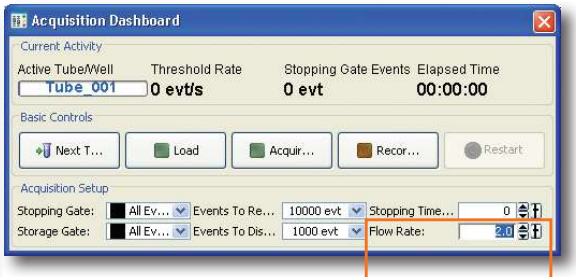
Adjust values and sliders as needed.

2. Remove the collection tubes.

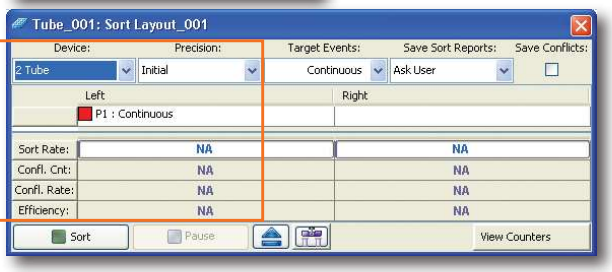
3. Set the drop delay using the Accudrop Drop Delay experiment template and Accudrop beads.



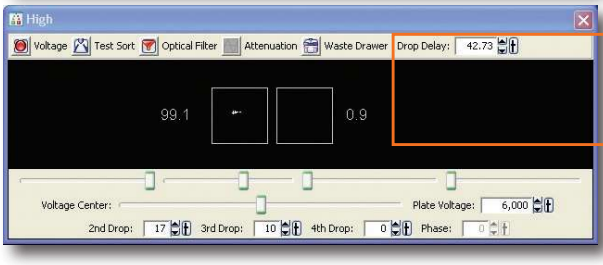
Bring in the provided experiment template.



Adjust the flow rate until the threshold rate is ~3000 to 4,000 events/second.

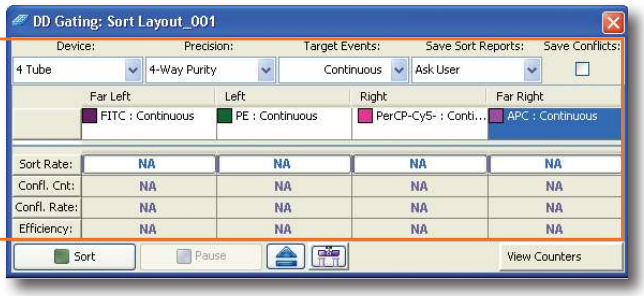


Start with the Precision mode set to Initial and then switch to Fine Tine.



Sort beads and adjust the Drop Delay until ~ 100% are sorted to the left.

4. Open your sorting experiment and set up the Sort Layout.



Make appropriate selections and entries.

5. Install the collection tubes, start the sort, and monitor the stream during sorting.

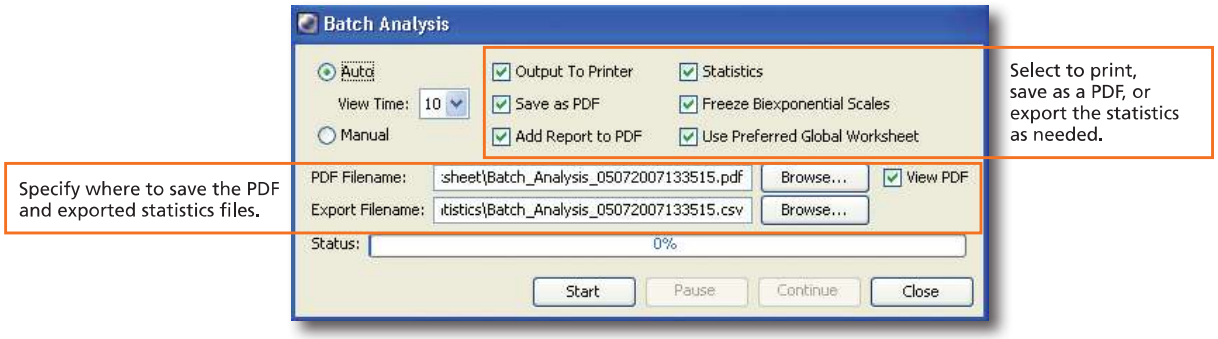
   Troubleshoot as needed using the following table as a guide.

Observation	Cause	Recommended Solution
Sorting pauses automatically.	If the Drop 1 or Gap values are out of range, sorting pauses while the amplitude is being adjusted.	Wait for sorting to continue. If this happens often, stop the sort, turn off the Sweet Spot, and refer to the *BD FACSAria User's Guide* for instructions on troubleshooting an unstable breakoff.
Sorting stops automatically. • The sample tube is unloaded • The stream is turned off • The waste drawer closes • The deflection plates are turned off • A message appears informing you that a clog has been detected	The Drop 1 or Gap values could not be matched automatically.	Turn on the stream and see if Drop 1 returns to the original value. • If Drop 1 returns to the original value, then set the Sweet Spot and continue sorting. • If Drop 1 returns to a different value but the breakoff still looks good, set the Sweet Spot, optimize the drop delay, and continue sorting. • If the stream is unstable or leaking, follow the directions in the *BD FACSAria User's Guide* to clean a clogged nozzle. When finished, set the Sweet Spot, optimize the drop delay, and continue sorting.

6. Stop the sort and save a copy of the sort report.

Analyzing Data

1. Record post-sort data.
2. Check the sort purity.
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).



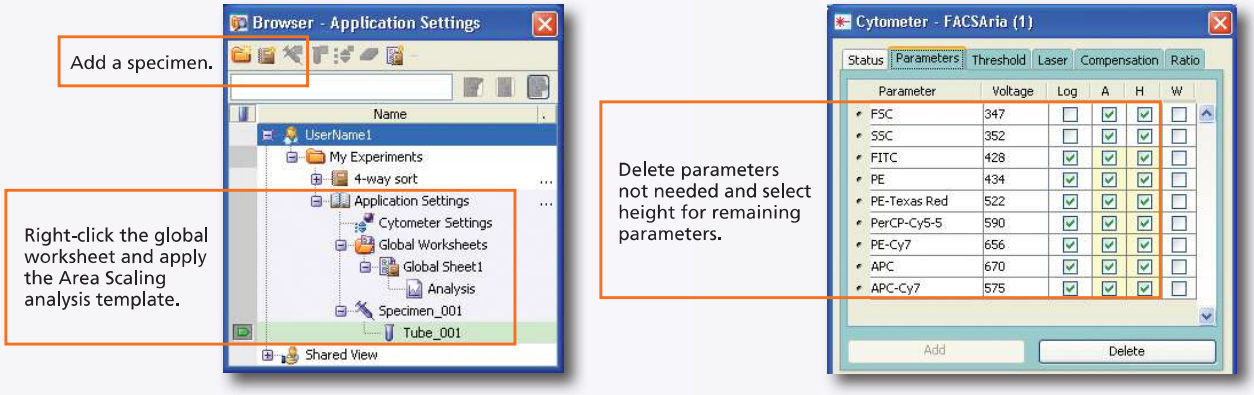
Shutting Down the System

1. Open the flow cell access door.
2. Turn off the stream.
3. Perform a fluidics shutdown.
4. Empty the waste and refill the DI water if prompted.
5. Run the cleaning and rinsing solutions when prompted.
6. Turn off the cytometer main power and shut down the computer.

Creating Application Settings

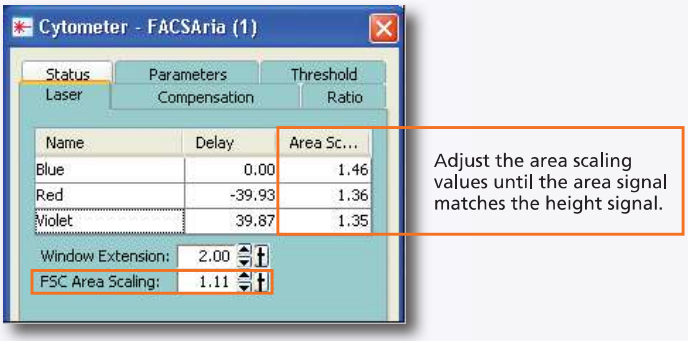
Before creating application settings, perform the cytometer startup procedure according to your cytometer user's guide and run a performance check.

1. Create a new experiment.



2. Format plots and statistics on the global worksheet as needed.

3. Load appropriate tubes and optimize area scaling values.

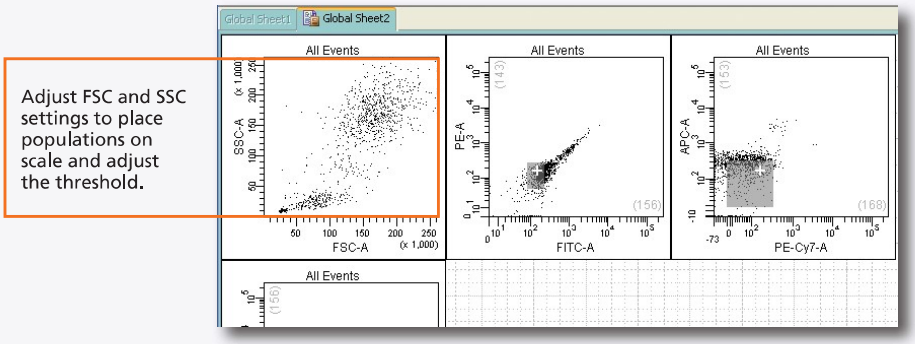


4. Clear height checkboxes for all parameters.

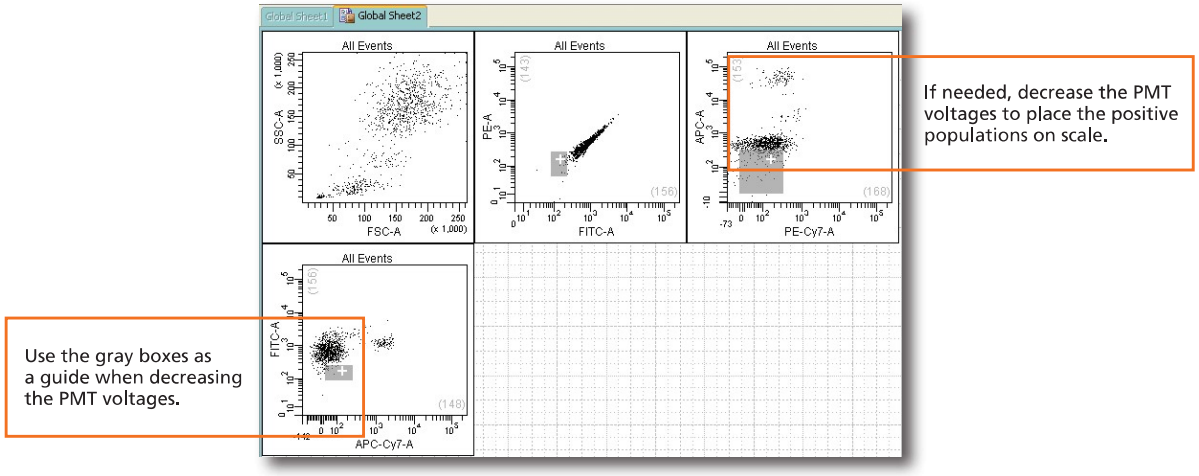
5. Right-click Cytometer Settings in the Browser. Select Application Settings > Create Worksheet.

6. Load the unstained control tube onto the cytometer.

7. Adjust the cytometer settings.



8 Acquire single-stained controls.

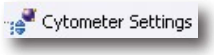


9 Right-click Cytometer Settings in the Browser. Select Application Settings > Save.

Cytometer Settings Overview

Cytometer Settings Icons in the Browser

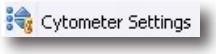
Default or user-defined settings have been applied.



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.