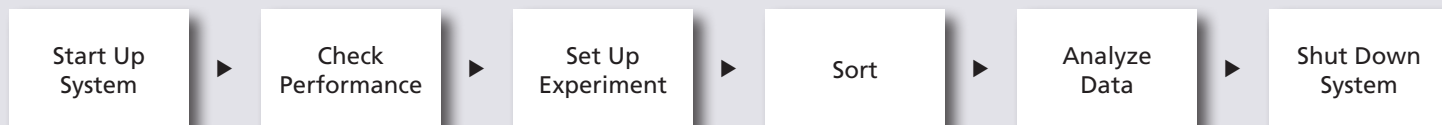


BD FACSDiva Software Quick Reference Guide for BD FACSAria Cell Sorters

This guide contains instructions for using BD FACSDiva™ software version 8.0 with BD FACSAria™ cell sorters.

Workflow Overview

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



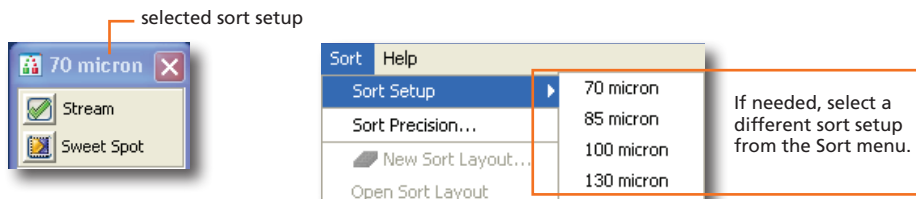
Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings. See the *BD FACSAria User's Guide* for detailed instructions.



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Starting Up the System

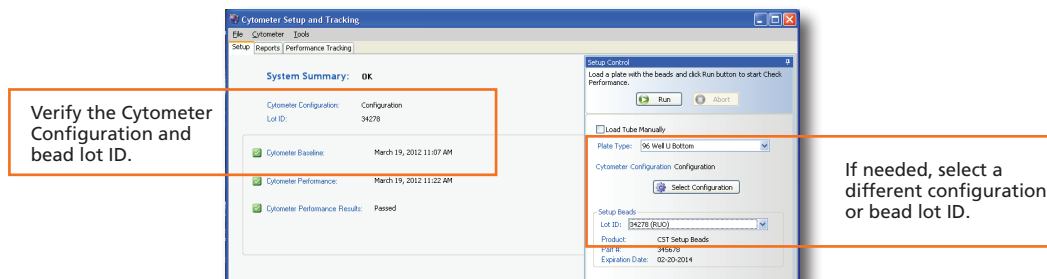
- 1 Turn on the cytometer main power.
- 2 Start up the computer, start BD FACSDiva software, and log in.
- 3 Check fluid levels in the Cytometer window.
- 4 Verify that the appropriate sort setup is selected and perform fluidics startup.



- 5 Optimize the breakoff.

Checking Cytometer Performance

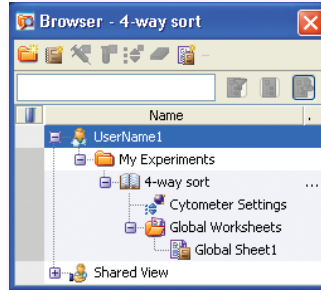
- 1 Turn off the Sweet Spot and select Cytometer > CST.



- 2 Run the BD FACSDiva™ CS&T research 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.

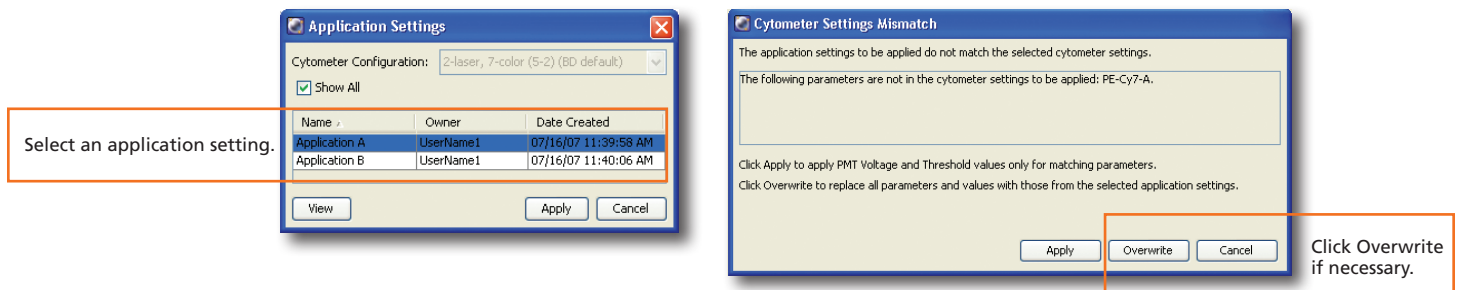
Setting Up the Experiment

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



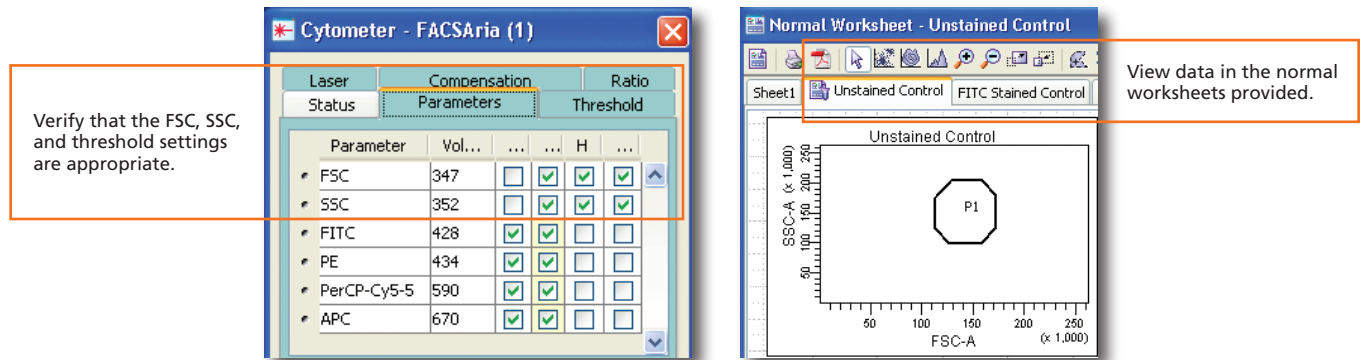
- 2 Select **Cytometer Settings** in the Browser and select the height and width for the FSC and SSC parameters in the Inspector window.

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



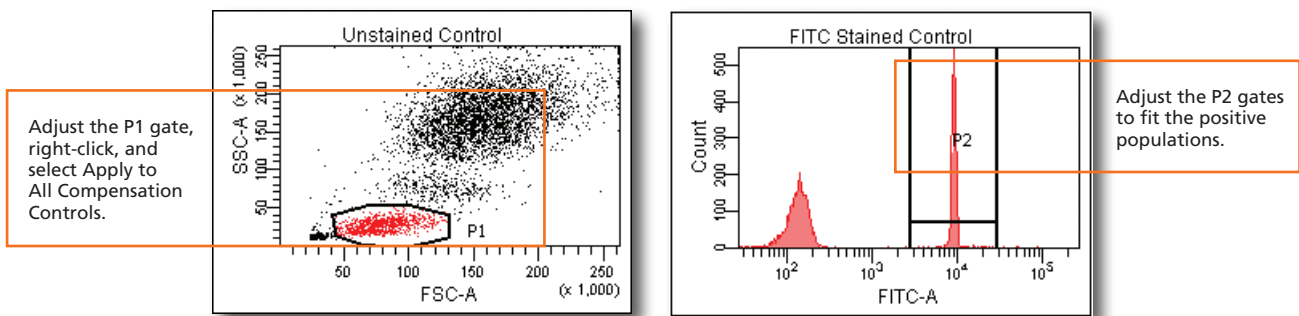
- 4 Select Experiment > Compensation Setup > Create Compensation Controls.

- 5 Load the unstained control tube.

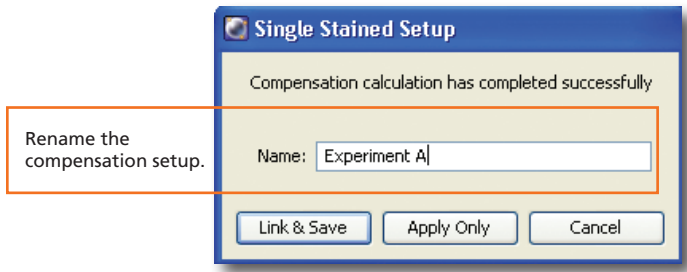


- 6 Record data for the compensation control tubes.

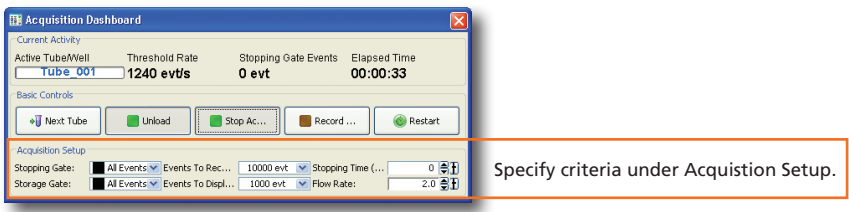
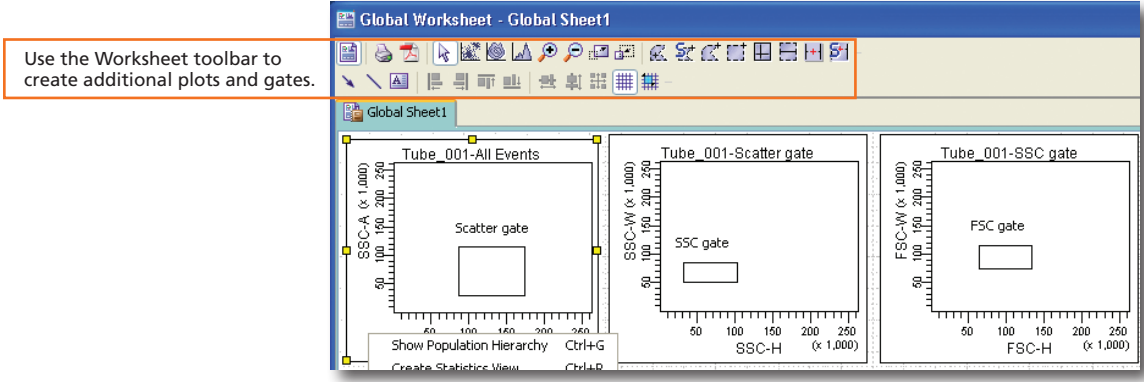
- 7 View the recorded data and gate the positive populations.



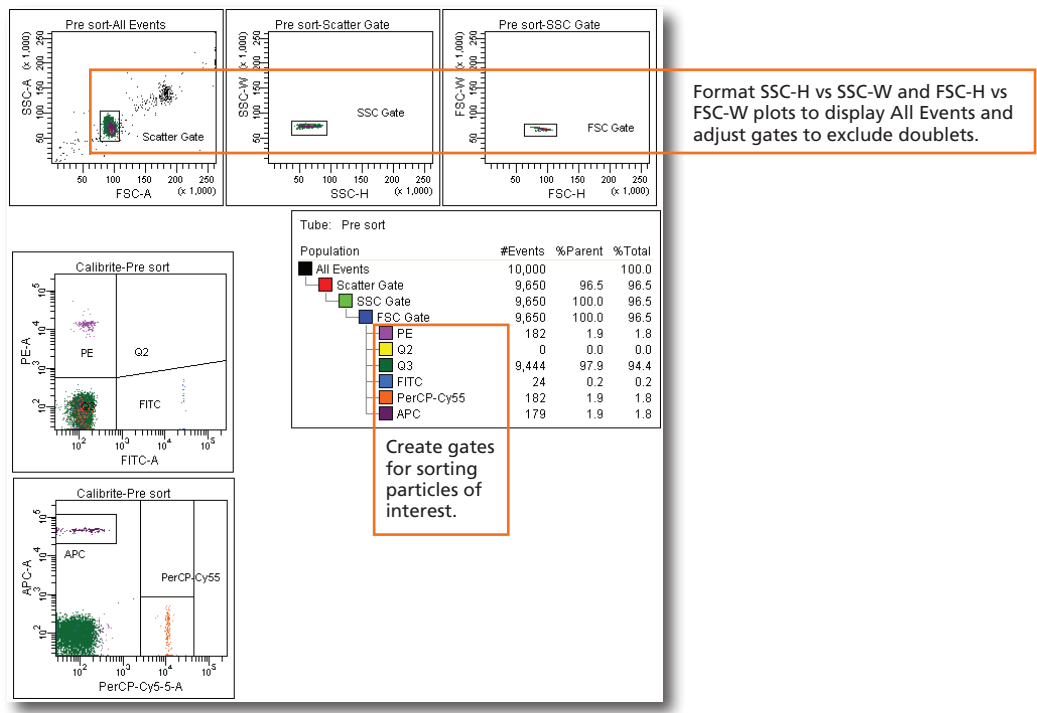
- 8 Select Experiment > Compensation Setup > Calculate Compensation.



- 9 Record pre-sort data.



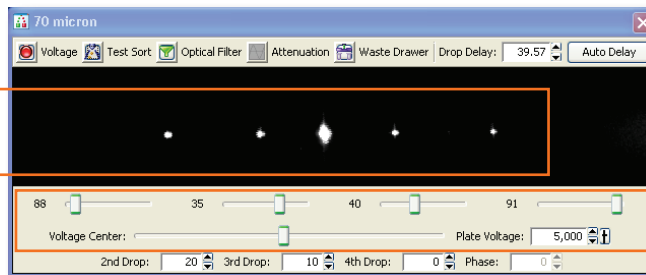
- 10 Create sorting gates.



Sorting

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

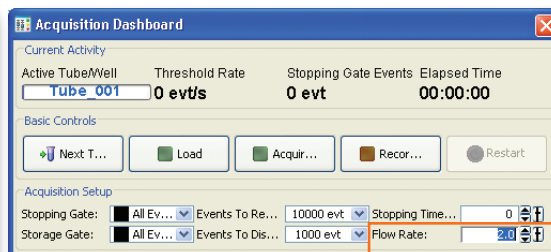
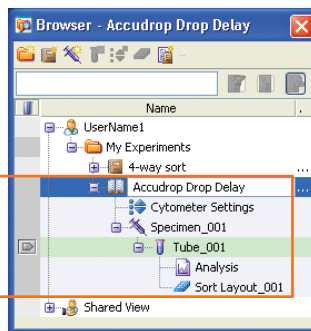
Adjust the micrometer dial on the diode laser until the center stream is bright.



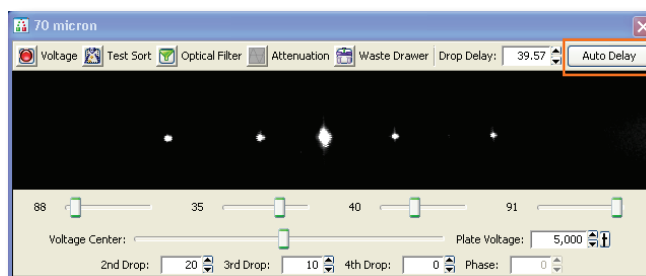
Adjust sliders as needed.

- 2 Remove the collection tubes.
- 3 Set the drop delay using the Accudrop Drop Delay experiment template and BD FACST[™] Accudrop beads.

Bring in the provided experiment template.

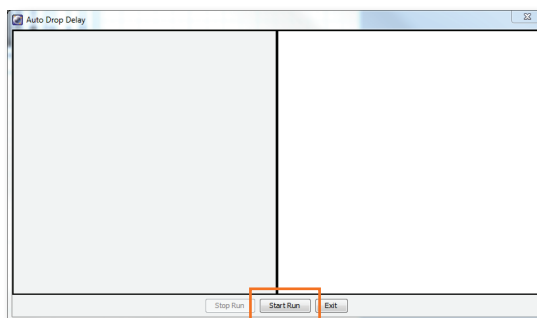


Adjust the flow rate until the threshold rate is appropriate for your nozzle size.



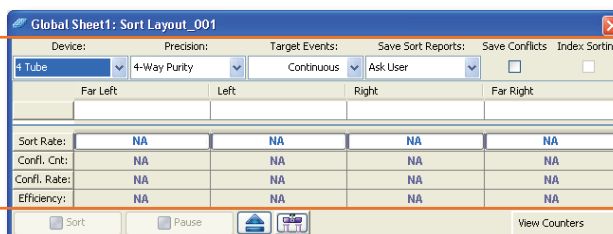
Sort beads, then click Auto Delay.

- 4 Click Start Run in the dialog and monitor the progress until complete.



- 5 Open your sorting experiment and set up the Sort Layout.

Make appropriate selections and entries.



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

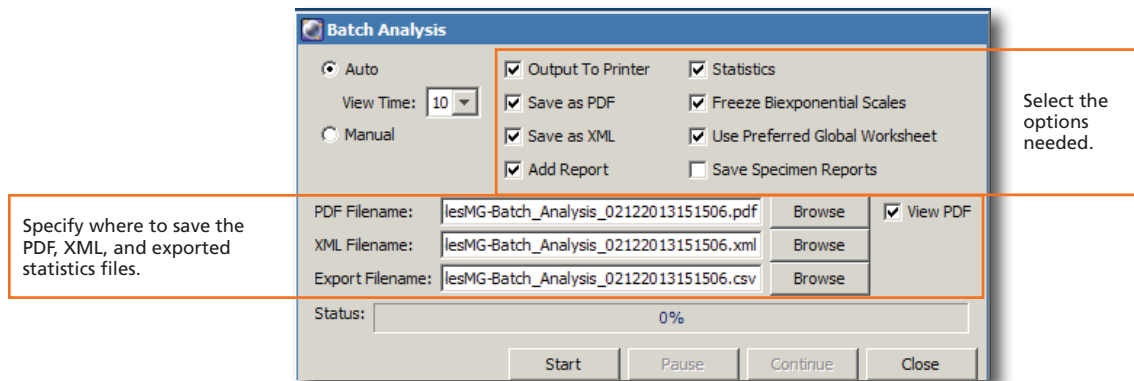
Troubleshoot as needed using the following table as a guide.

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 see the <i>BD FACSAria User's Guide</i> for instructions on troubleshooting an unstable breakoff.
Sorting stops automatically. <ul style="list-style-type: none"> 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 could not be matched automatically.	Turn on the stream and see if Drop 1 returns to the original value. <ul style="list-style-type: none"> 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 <i>BD FACSAria User's Guide</i> to clean a clogged nozzle. When finished, set the Sweet Spot, optimize the drop delay, and continue sorting.

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

Analyzing Data

- Record post-sort data.
- Check the sort purity.
- 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

- Perform a fluidics shutdown or clean the flow cell.
- Perform external cleaning.
- Turn off the cytometer main power and shut down the computer.