Creating and Saving Optimal Settings for BD FACSDiva-Based Platforms

Run Baseline Definition and Performance Checks

↓

Determine 2.5 x rSD_{EN} with unstained cells

↓

Verify stained cells within the linearity of each parameter

↓

Standardize remaining cytometers

↓

Save application settings

Reusing optimal settings in BD FACSDiva software over time

To reuse your saved optimal settings over time, follow these steps to update your instrument settings for that day.

Run Daily Performance Check

↓

Open Experiment, Apply Application Settings

↓

Generate Compensation Settings and Save

↓

Acquire Data

For additional information, see the Technical Bulletin: Standardizing Application Setup Across Multiple Flow Cytometers Using BD FACSDiva Version 6 Software.

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BD FACSDiva™ Software

Standardizing Application Settings Across Multiple Cytometers

Multicolor flow cytometry is increasingly used in research laboratories to generate more data from a given experiment.

A key aspect of effectively using multicolor flow is ensuring that the experiment can be performed consistently over time and across instruments. Standardizing experiments using application settings can ensure consistency of results over time across multiple systems. To achieve this consistency, we recommend the use of the Application Settings workflow in BD FACSDiva software.

To create and save the optimal settings for specific experiments, follow these basic steps:

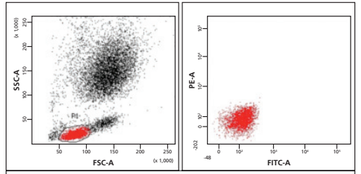
Step 1: Run baseline definitions and performance checks
Step 2: Determine 2.5 x rSD_{EN} with unstained cells
Step 3: Verify stained cells within linearity of each parameter
Step 4: Standardize remaining cytometers
Step 5: Save optimal application settings

Step 1: Run baseline definition and daily performance check

a. Verify that CS&T Baseline has been run for the selected configuration.
b. In the CS&T application, run a Daily Performance Check on each cytometer.
c. Record the rSD_{EN} and linearity max for each fluorescence channel for each cytometer.

Step 2: Determine 2.5 x rSD_{EN} with unstained cells

a. Multiply the rSD_{EN} by 2.5 for all fluorescence channels.
b. Compare these values across all cytometers and record the highest value for each channel.
c. Adjust the PMT voltage (PMTV) for each channel until the rSD for unstained cells reaches this value.

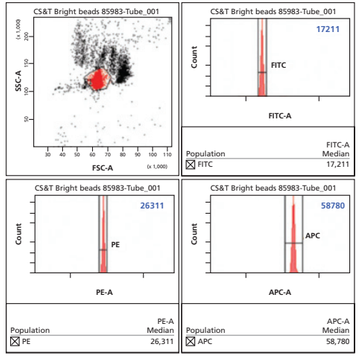


Population	FITC-A rSD	PE-A rSD	FITC-A rSD	PerCP-... rSD	APC-A rSD	APC-H7-A rSD	Pacific B... rSD	AmCyan... rSD
P1	70	77	70	66	68	77	65	66

An example of a worksheet used to acquire unstained cells and adjust PMTVs until the target rSD values (2.5 x rSD_{EN}) are reached.

Step 3: Verify stained cells within the linearity of each parameter

a. Divide the linearity max value by 2 for all fluorescence channels.
b. Compare these values across all cytometers and record the lowest value for each channel.
c. Using highly positive cell populations, verify that the median fluorescence intensity (MFI) of the positive population falls at or below this number. *Note: If the MFI exceeds this value for any channel, lower the PMTV.*



An example of a worksheet used to verify that MFI values for stained cells fall within the linearity of each parameter.

Step 4: Standardize the remaining cytometers

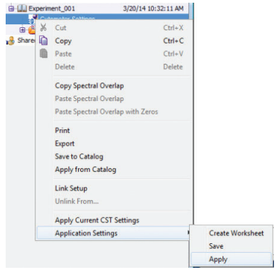
a. Using BD™ CompBead particles (BD CompBeads) stained with antibodies from your experiment, record 20,000 events.

Note: CS&T beads (bright peak) can be used for instruments with identical optical configurations.

b. Determine and record the MFI of the positive population for every channel.
c. On the remaining cytometers, using the same lot of BD Comp Beads and each reagent, adjust the PMTV for each channel until the target MFI is reached.

Step 5: Save application settings

a. Create application settings by right-clicking the settings under the experiment, then selecting **Application Settings** > **Save**.



b. A template may also be created and shared across instruments with identical configurations. Right-click the experiment, and select **Export** > **Experiment Template**.