Protocol: Experiment Setup (BD Digital Instruments)

Steps:

1. Perform once per instrument configuration, or after major service:
    a. Open CST and run “define baseline” routine.

2. Perform at the start of each day the instrument is used:
    1. Open CST and run “daily performance check”.

3. Perform for experiments involving NEW panels of antibodies (after Ab titration and optimization of Ab combinations):
    1. Open a new experiment using CST baseline settings.
    2. Run single-stained CompBeads for each antibody in the panel, and check that they are at least 2x brighter in their primary detector vs other detectors; if not, increase the voltage of the primary detector.
    3. Run fully stained cells and decrease the voltage for any detector in which events are in the highest channel. Increase the voltage for any detector in which negative populations are below zero (this is only for asthetic purposes, and should theoretically not be necessary).
    4. Re-run single-stained CompBeads and calculate compensation.
    5. Repeat steps 2-4 until no further voltage adjustments are needed.
    6. Save application-specific settings in Diva.

4. Perform for experiments in which application-specific settings have already been defined as in step 3:
    1. Open a new experiment using CST baseline settings.
    2. Apply the saved application-specific settings for the panel being run.
    3. Run single-stained CompBeads and calculate compensation.

5. Run experiment samples, followed by appropriate shutdown procedure for the instrument.

Notes:

1. CST baseline settings correspond to the minimum voltages required to maximize resolution sensitivity in each detector. Increasing voltages above the baseline doesn’t improve resolution sensitivity. Decreasing voltages should only be done if events are in the highest channel, since their fluorescence otherwise will not be measured accurately.

2. The use of stained CompBeads to optimize settings is an attempt to “balance” detector voltages so that compensation is minimized. However, if such adjustments are needed, it indicates a potentially poor combination of reagents with regard to spillover.

3. Application-specific settings are stored as an offset from the baseline CST settings. As such, they require that a valid CST performance check has been run. Note that this is different from storing and recalling fixed voltages.

4. Because voltages will vary slightly from experiment to experiment, it is essential to run compensation controls for each experiment. Even if voltages are not changed, the use of tandem dyes requires experiment-specific compensation due to lot-to-lot variability and reagent degradation.

5. CompBeads are generally preferable to single-stained cells as compensation controls, as long as the antibody efficiently binds the appropriate bead.

Protocol: Experiment Setup (BD Digital Instruments)

Reagent Recommendations:

Reagent	**Catalog Number**	**Instructions for Use**
BD CST beads	641319 (50 tests) or 642412 (150 tests)	Use 1 drop + 350 μL FACS Flow buffer
BD CompBeads	552843 (anti-mouse κ) 552844 (anti-rat κ) 552845 (anti-rat/hamster κ)	Use 1 drop per sample. Negative CompBeads can be included in each tube and/or run as a separate negative control (the latter is preferable).