Dual-Laser Instrument Setup

Dual-Laser Cytometer Setup, Data Acquisition, and Analysis

The Cytometer setup information is for dual-laser FACSCalibur™ flow cytometers only. The BD FACSComp software is useful for setting up the flow cytometer. CellQuest™ software is required for analyzing samples and formatting data for subsequent analysis using the BD CBA software.

1. Preparation of Cytometer Setup Beads:

1. Add 50 µl of Cytometer Setup Beads to a cytometer setup tube labeled A.
2. Add 450 µl of Wash Buffer to Tube A.
3. Proceed to Section 2.

2. Instrument Setup with FACSComp software and CaliBRITE beads:

1. Perform instrument start-up.
2. Perform flow check.
3. Prepare tubes of CaliBRITE beads for 4-color, dual-laser instrument setup and open FACSComp software.
4. Launch FACSComp software.
5. Run FACSComp software in Lyse/No Wash mode. Time-delay calibration must be performed.
6. Proceed to Section 3.

NOTE: For detailed information on using FACSComp with CaliBRITE beads to set up the flow cytometer, refer to the *FACSComp Software User's Guide* and the *CaliBRITE Beads* Package insert. FACSComp Version 4.2 contains a BD CBA preference setting to automatically save a BD CBA calibration file at the successful completion of any Lyse/No Wash assay. The BD CBA calibration file provides the optimization for FSC, SSC, and threshold settings as described in Section 3, Steps 3 - 5. Optimization of the fluorescence parameter settings is still required (*i.e.,* PMT settings, see Section 3, Step 7).

3. Instrument Setup with the Cytometer Setup Beads:

1. Launch CellQuest software and open the CBA Dual-Laser Instrument Setup template.

NOTE: The CBA Dual-Laser Instrument Setup template may be downloaded via the Internet from: http://www.bdbiosciences.com/pharmingen/cba

2. Connect to the cytometer. Make sure that the second laser is turned on.
3. Set SSC (side light scatter) and FSC (forward light scatter) to Log mode.
4. Decrease the SSC PMT voltage by 100 from what FACSComp set.
5. Set the Threshold to FSC at 650.
6. Set all compensation values to 0.0%. Any use of compensation above 0 may adversely affect performance of the CBA Kit when using the dual-laser acquisition template.
7. In Setup mode, run Cytometer Setup Beads Tube A. Follow the setup instructions alongside Figures 1 - 3.

NOTE: Pause and restart acquisition frequently during the instrument setup procedure in order to reset detected values after settings adjustments.

Adjust gate R1 so that the singlet bead population is located in gate R1 (Figure 1).

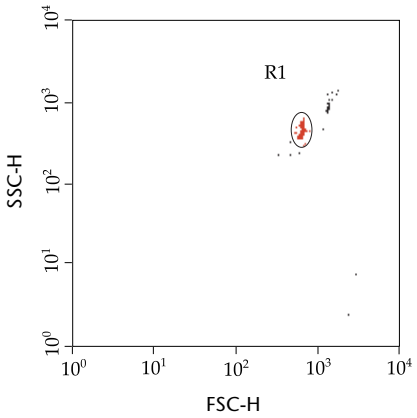
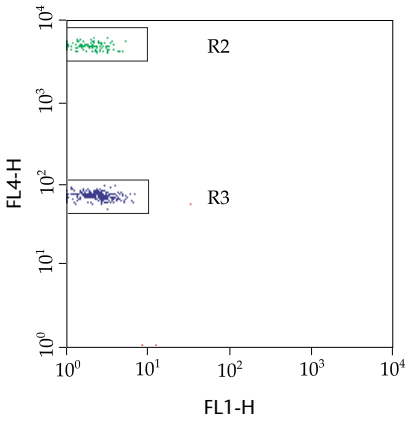


Figure 1

Adjust the FL4 PMT so that the median of the top FL4 bead population's intensity is around 5000 (Figure 2). Adjust gate R3 as necessary so that the dim FL4 bead population is located in gate R3 (Figure 2). Do not adjust the R2 gate.

Adjust the FL1 PMT value so that the median of FL1 is approximately 2.0 - 2.5 (Figure 2).

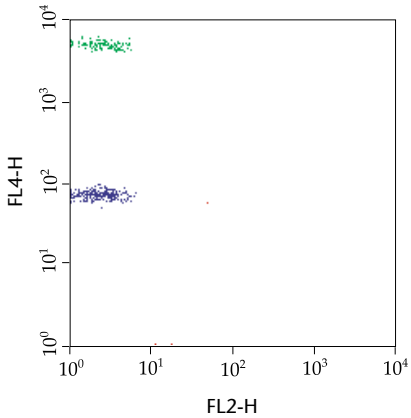


Bright Beads (R2)
FL4 Median: 5139.70

FL1 Median: 2.19

Figure 2

Adjust the FL2 PMT value so that the median of FL2 is approximately 2.0 - 2.5 (Figure 3). Save and print the optimized instrument settings.



FL2 Median: 2.31

Figure 3

4. Data Acquisition:

1. Open the Dual-laser acquisition template.

   NOTE: The Dual-laser acquisition template may be downloaded via the Internet from: http://www.bdbiosciences.com/pharmingen/cba

2. Set acquisition mode and retrieve the optimized instrument settings from Section 1.3.
3. In the Acquisition and Storage window, set the resolution to 1024.
4. Set number of events to be counted at 1800 of R1 gated events. (This will ensure that the sample file contains approximately 300 events per Capture Bead).
5. Set number of events to be collected to "all events". Saving all events collected will ensure that no true bead events are lost due to incorrect gating.
6. Click the Parameters Saved button. In the new window, ensure that only FSC-H, SSC-H, FL1-H, FL2-H, and FL4-H are checked. Click Okay.

   NOTE: The BD CBA software will evaluate data in five parameters (FSC, SSC, FL1, FL2, and FL4). It is essential that additional detectors be turned off.

7. In Setup mode, run Tube #1, and using the FSC vs. SSC dot plot, place the R1 region gate around the singlet bead population (see Figure 1).
8. Samples are now ready to be acquired.
9. Begin sample acquisition with the flow rate set at HIGH.

   NOTE: Run the negative control tube (0 pg/ml standards) before any of the recombinant standard tubes. Run the control assay tubes before any unknown test assay tubes. Run the tubes in the order listed in the manual of the CBA Kit being used.

   To facilitate analysis of data files using the BD CBA software and to avoid confusion, add a numeric suffix to each file that corresponds to the assay tube number (*i.e.,* Tube #1 containing 0 pg/ml could be saved as KT032598.001). The file name must be alphanumeric (*i.e.,* contain at least one letter).

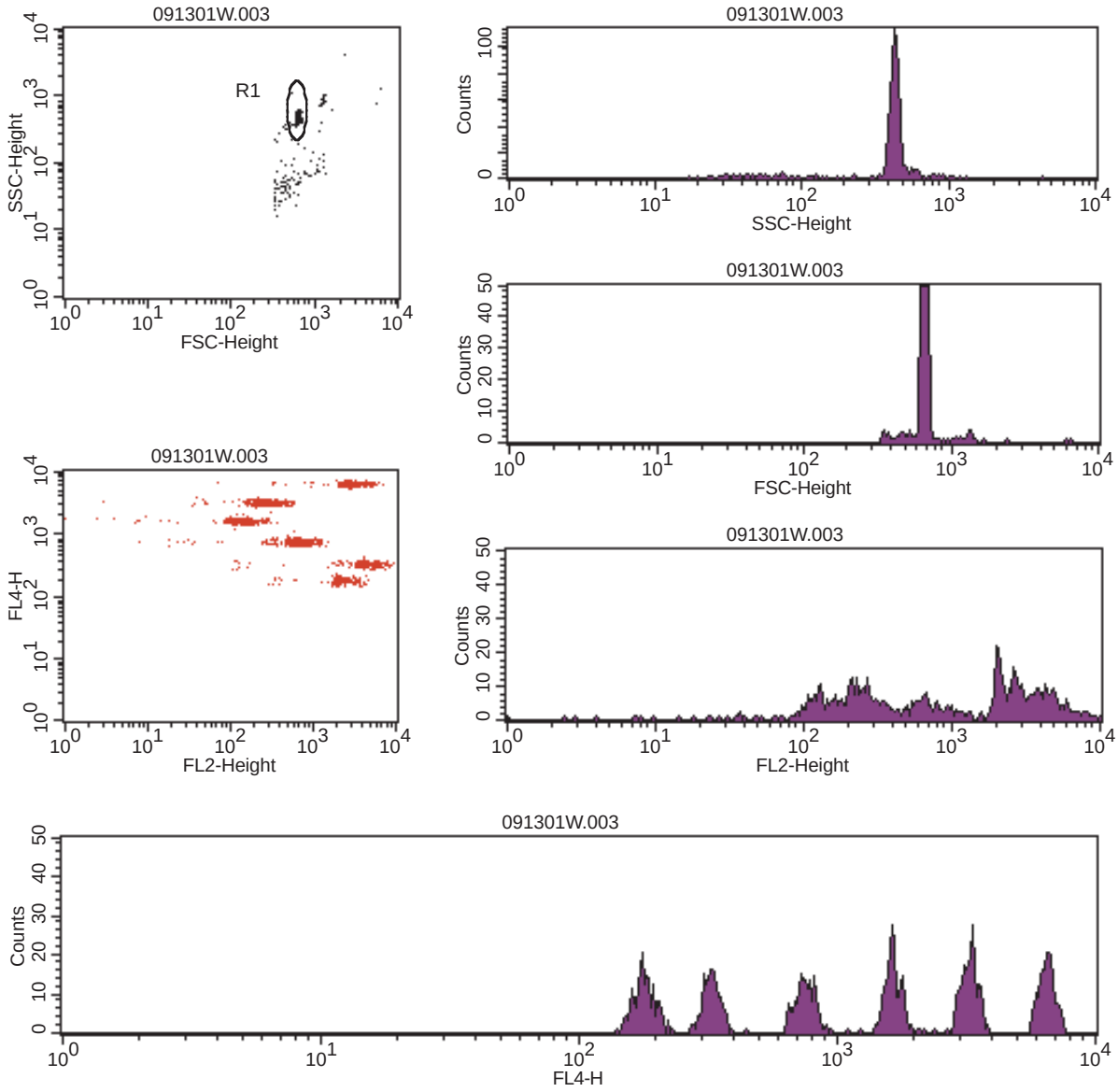


Figure 4: Dual-laser acquisition template example