Guidelines for Journal Authors Using the BD Accuri™ C6

Technical Bulletin

Contents

Introduction

Journal editors, reviewers, and readers who are unfamiliar with the BD Accuri™ C6 flow cytometer might be surprised by its unusual signal detection system, fixed voltages, and daily instrument QC procedures. Authors may find it useful to provide a brief description of these features so that audiences understand the methodology.

The first section of this technical bulletin suggests language to describe the BD Accuri C6 flow cytometer in a typical Materials and Methods section. Authors might want to highlight this information for editors and reviewers when submitting papers for publication. The second section includes advice on labeling and scaling plots in a typical Results section, as well as fluorophore/detector/filter combinations.

Instrument description for Methods and Materials section

This section contains a suggested description of the BD Accuri C6 and its features for a typical Methods and Materials section of a journal article. Suggested language appears in blue type, while commentary appears in black type.

You might want to call editors' and reviewers' attention to this section, since the voltage setting process differs from other cytometers with which they may be familiar.

Equipment: BD Accuri™ C6 flow cytometer

- *Lasers: 488 nm and 640 nm*
- *Optical filters (standard): FL1 533/30 [or 530/30*] nm, FL2 585/40 nm, FL3 >670 nm, FL4 675/25 nm*
- *Additional instrument and operational specifications can be found at:* ***bdbiosciences.com/instruments/accuri***

Voltage setting, fluorescence detection optimization, and instrument QC

The optical alignment and voltage settings for the BD Accuri C6 flow cytometer are optimized and locked down during the manufacturing process. Two industry-standard fluorescent beads are used: Spherotech 8-Peak Validation Beads (BD Biosciences Cat. No. 653144, for voltage settings on FL1, FL2, FL3, FSC, and SSC) and Spherotech 6-Peak Validation Beads (Cat. No. 653145, for voltage setting on FL4). The bright peak channel position, and the separation between negative and dim peaks on all four fluorescence detectors, are determined by instrument manufacturing specifications, and the optics and electronics of each instrument are tuned to meet this standard.[1,2]

Daily instrument QC was performed using Spherotech 8- and 6-Peak Validation Beads to ensure that the BD Accuri C6 was performing within specification immediately prior to data collection.

The BD Accuri C6 is a digital cytometer equipped with linear amplifiers and 24-bit digitization on all detectors. Data is stored and displayed in 16.7 million channels (approximately 7.2 log display). The range of channels displayed for each detector (light scatter and fluorescence) was determined using appropriate experimental and staining controls. [Identify controls used, as described in the next section of this technical bulletin.]

Fluorescence compensation was set using single-stained controls, and matching-median [or automated†] compensation algorithms were applied.[1,3]

References

1. Maecker HT, Trotter J. Flow cytometry controls, instrument setup, and the determination of positivity. *Cytometry.* 2006;69A:1037-1042.
2. Wood JCS, Hoffman RA. Evaluating fluorescence sensitivity on flow cytometers: an overview. *Cytometry.* 1998;33:256-259.
3. Roederer M. Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. *Cytometry.* 2001;45:194-205.

* Check the filter holder of the individual BD Accuri C6 instrument for the FL1 filter specification.

† Matching-median compensation is used in BD Accuri™ C6 software, while automated compensation algorithms are available in some third-party flow cytometric analysis programs.

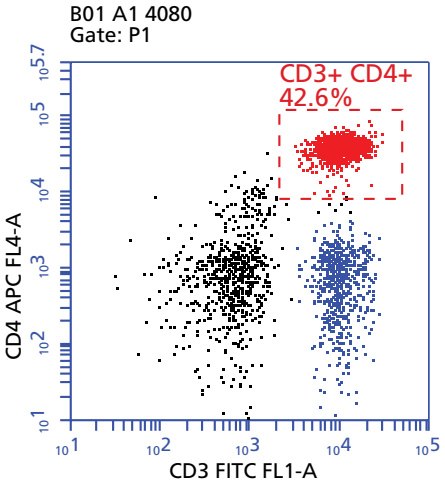

Figure 1. Sample axis labels.

Axis labels should include the fluorophore (in this case, APC or FITC) and CD designation (CD3 or CD4) or antibody name, if applicable.

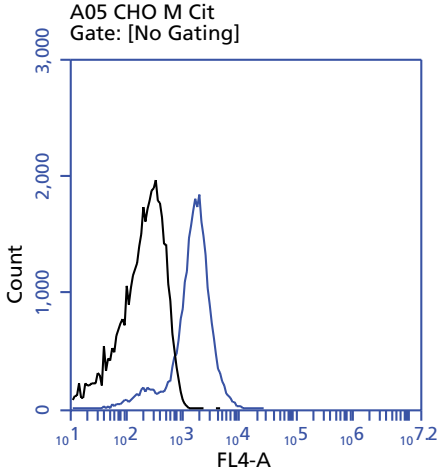

Figure 2. Fluorescence of negative controls can vary widely.

The background or autofluorescence of different particles can vary widely. Negative fluorescence for 1.0-µm beads (black) falls between channels 10 and 1,000, while most negative fluorescence for CHO cells (blue) falls between 1,000 and 10,000.

Detectors, filters, axis labels, and scaling for Results section

When describing the results of particular experiments, specify the detector position and optical filter used to detect each fluorophore, for example, FITC (FL1 533/30 nm), PE (FL2 585/40 nm), APC (FL4 675/25 nm).

Axis labels of displayed plots should include the dye or fluorophore, and antibody names, if applicable (see Figure 1). A detector designation can also be useful.

We recommend scaling plots of analyzed data based on the position of an unstained or "negative" control sample. The position of this population determines the lowest channel displayed in the plot, and will vary depending on the particle type and negative control used. Figure 2 shows negative control histograms for a small particle (in this case, a 1-µm bead) and a large, highly autofluorescent, Chinese Hamster Ovary (CHO) cell line, which fall in very different channel ranges.

Choose the lowest displayed channel so that at least 95% of all negative control events are on scale. In Figure 2, for example, start the plot at 10 for 1-µm beads and 1,000 for the CHO cell line. This is analogous to adjusting the voltage setting to place the negative control in the first decade of display. Once the lowest channel is chosen, do not change it for subsequent, related samples. You can set the highest displayed channel to be 4 logs greater than the lowest, or leave it unset to display the entire data range above the control.

Figure 3 shows two equivalent histograms of overlaid Yellow Fluorescent Protein (YFP) transfected and control transfected (black) CHO cells, differing only in the scaling. Starting the graph at 1,000, the bottom of the negative control distribution (Figure 3B), shows more detail and uses space more efficiently than starting the graph at 10 (Figure 3A).

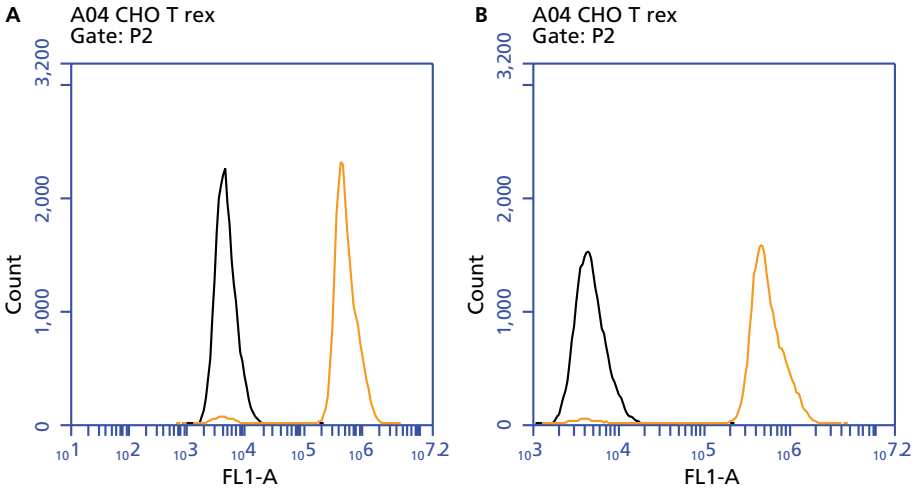

Figure 3. Scale axes so that negative controls appear in the first decade.

Overlays of CHO cells, either untransfected (black) or transfected with YFP (orange).
A. *Scaling the graph to display the full channel range, from 10 to 16.7 million, wastes space and sacrifices detail.* ***B.*** *Starting the graph at 1,000 shows more detail.*

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