Qr and Br in BD FACSDiva v6 Software: Parameters for Characterizing Detector Performance

Alan Stall, PhD, Director, Advanced Cytometry Technologies
BD Biosciences, San Diego, CA

Application Note

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Introduction

In BD FACSDiva™ version 6 software, Cytometer Setup and Tracking (CS&T) is used to characterize and monitor performance of BD flow cytometers, and can also be used for comparing different detectors and instruments or developing multicolor panels in certain situations. This application note explains the technical background for the Qr and Br parameters used by CS&T, and describes the benefits and limitations for using these parameters on different cytometer configurations.

In flow cytometry, no single term adequately characterizes a cytometer's fluorescence detector performance. Since fluorescence signals are always measured in the presence of some (optical) background, it takes minimally two terms, Q and B, to characterize detector performance. The detector efficiency, Q, for measuring a particular fluorochrome, is defined as *the number of photoelectrons measured per molecule of equivalent soluble fluorochrome* (MESF). The optical background, B, is also reported in MESF units. Together, they combine to characterize the performance of a detector[1-4] and are powerful tools for measuring and tracking cytometer resolution sensitivity.

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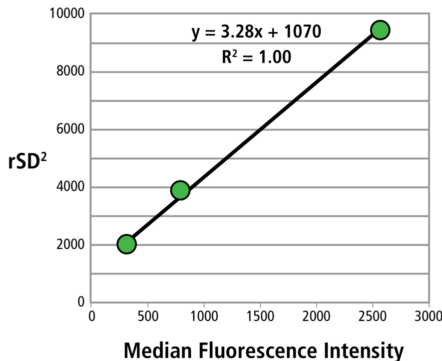

Figure 1.

How are Qr and Br determined in BD FACSDiva v6 software?

Mathematically, ***Q = 1/ (Mean [MESF] x CV²)***.

Q and B can be calculated from the slope and intercept of a plot of SD^2 vs the median of the three beads (Figure 1). Q is equal to the inverse of the slope of this plot and B is equal to Q x Intercept. In Figure 1, Q = 1/3.28 = 0.306. B = 0.306 x 1070 = 326.

In BD FACSDiva v6 software, Q and B are similarly estimated statistically from the medians, rSDs, and rCVs[5] of the Bright, Mid, and Dim CS&T beads for each fluorescence detector. These measured values are corrected for the intrinsic CV and autofluorescence of these beads, using values from the bead lot file as described later.

The ABD unit

Q and B as originally defined are reported in units of MESF.[2-4] However, since MESF standards do not exist for most fluorochromes used in flow cytometry, BD FACSDiva v6 software calculates Q and B using a relative fluorescence unit, the Arbitrary BD (ABD) unit. When measured in ABD units, Q and B are designated Qr and Br respectively.

The ABD unit, although not a calibration standard per se, has a practical association to antibodies bound per cell. Careful measurements with BD Quantibrite™ standards and PE anti-CD4 antibodies (very bright fluorochromes) have shown that, on fixed human blood lymphocytes, there are approximately 40,000 antibodies bound per CD4-positive cell.[6] By carefully normalizing the fluorescence intensity of the bright CS&T bead to that of CD4 conjugates in every available color, ABD units can be assigned to the CS&T bright bead for each fluorochrome conjugate. One ABD unit is approximately equivalent to the fluorescence of one fluorochrome-conjugated anti-CD4 antibody. Therefore, BD FACSDiva v6 software characterizes performance relative to factory measured CD4 brightness for each color, and Qr and Br are reported as *photoelectrons per ABD unit*.

CS&T bead lot information

Every lot of Cytometer Setup and Tracking beads has an associated file (bead lot file) available online (**bdbiosciences.com/csandt**). Data from the appropriate bead lot file is used by BD FACSDiva v6 software to adjust and normalize the values of Qr and Br. This file contains lot-specific values that are used to:

1. Correct the bead data for the intrinsic CVs of that set of beads.
2. Correct for differences between CS&T bead lots to ensure consistency of values.
3. Normalize the Qr and Br values based upon the ABD units and fluorescent value for each bead.

To normalize calculations of Qr and Br in ABD units, the appropriate bead lot files must be downloaded and entered into the Cytometer Setup and Tracking features of BD FACSDiva software (see the *BD Cytometer Setup and Tracking Application Guide*).

These lot-specific values depend upon the laser excitation and the emission spectra of the fluorescence being measured and are specific for each BD qualified detector configuration as defined by the laser and filter set for the BD FACSAria,™ BD FACSCanto™, and BD™ LSR II platforms.

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BD Qualified Detector Configurations Specified in Bead Lot Files			
Blue (488 nm) ≤30mW	Red (635 nm) ≤25mW	Violet (405 nm) ≤60mW	UV (355 nm) ≤50mW
780/60	780/60	655/8	530/30
710/50	730/45	605/40	450/50
695/40	712/21	605/12	450/40
675/20	710/20	585/15	450/20
670/14	710/50	560/20	440/40
670LP	685/35	530/30	
660/20	660/20	525/50	
616/23		510/50	
610/20		450/50	
585/42		450/20	
575/26		440/40	
575/25		450/40	
576/26			
530/30			

Table 1.

BD detector configurations

BD qualified fluorescence detector configurations are defined by the combination of lasers and filters (see Table 1). Each of these configurations has respective ABD and intrinsic CV correction factors specified in the bead lot information file. Special Order Research Product (SORP) instruments might contain user-specified detector configurations that are not among the qualified configurations described in the table. Similarly, users might use their own filter combinations for other fluorochrome reagents such as propidium iodide, green fluorescent protein, or mCherry.

In non-qualified configurations, the Qr and Br values are not normalized to ABD units, nor are intrinsic CV differences between lots corrected. However, these non-normalized Qr values are still valid and serve as a powerful metric to track changes in fluorescence detector performance over time. This performance tracking is valid because a 50% change in a non-normalized Qr value is functionally equivalent to a 50% change in a normalized Qr value in terms of relative instrument performance. Since the bead lots are not corrected for differences in intrinsic CV, different bead lots can give slightly different Qr values for non-qualified detectors. Therefore a small change in the Levey-Jennings Qr tracking chart may be seen when a new bead lot is used. In addition, while non-normalized Qr values are useful for tracking, they should not be used for direct comparison with normalized Qr values of BD qualified detectors on the same instrument or between instruments.

Qr limitations

While Qr is a very powerful tool for characterizing an individual detector and tracking its performance over time, it is limited when comparing detector configurations from different instruments. In BD FACSDiva software, the fluorescence intensity of the CS&T beads is normalized through the ABD units for BD standard fluorochromes such as FITC or PE. However, the dyes used in the CS&T beads have fluorescence spectra different from the actual fluorochrome conjugates used on the flow cytometer. Thus, differences in the optical filters used in detector configurations of various instrument platforms might result in Qr values that do not fully represent the true relative difference in Q. In addition, note that even with "identical" instruments using defined MESF standards, because of normal manufacturing variations in the PMTs, lenses, and optical filters for a given fluorescence detector, the value of Q or Qr for equivalent detectors can vary as much as two to three-fold between two optimized flow cytometers.

Conclusion

In BD FACSDiva v6 software, Qr and Br are powerful new metrics to automatically and reproducibly determine initial performance and monitor it on a daily basis for each cytometer configuration used in the lab.

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BD Biosciences
2350 Qume Drive
San Jose, CA 95131
bdbiosciences.com

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