# Comparison of BD FACSLyric™ Instrument Performance in a Global Setting

*Is the Cytometer Setup & QC software module of the BD FACSLyric™ instrument sufficient to monitor instrument performance?*

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## Background

As flow cytometry is a powerful tool to characterize cellular populations, it is critical to have standardized instruments within and across different labs and/or regions for global clinical trials. The Cytometer Setup & QC software in the BD FACSLyric™ instrument should correct for daily fluctuations within one instrument and across instruments using Bright Bead Median Target Values (BBMTV). To assess the capability of the software module to standardize flow cytometry assays, we evaluated the Median Fluorescence Intensity (MFI) between instruments and within instruments over time, using both BD® Cytometer Setup and Tracking (CS&T) beads (BD Biosciences) and SPHERO™ Ultra Rainbow calibration particles (Spherotech).

## Method

To monitor instrument performance and reproducibility of MFI values, experiments were performed across a total of 15 instruments located in four different countries; Belgium (6), USA (4), Taiwan (2) and Australia (3). A specific lot of two types of calibration beads, CS&T beads (LOT 2091889) and Ultra Rainbow calibration particles (LOT AP03), were chosen to monitor all 12 channels of the BD FACSLyric™ instrument.

During the experiments, both types of calibration beads were prepared according to the manufacturer's recommendation and acquired daily for five consecutive days on a total of 15 BD FACSLyric™ instruments. In order to perform the experiments on optimally functioning flow cytometers, acquisition of the beads was always done after a successful performance QC (pQC). To ensure that the resulting MFI values were obtained independently from the built in Cytomer Setup & QC software module, beads were acquired in experiment mode on the Lyse/Wash (LW) setting, without compensation. Next, data were analyzed using FACSuite™ software for all 12 channels, as shown in Figure 1. For CS&T beads, the MFI value of the positive peak was determined, and for the Ultra Rainbow calibration particles, the MFI of the 5th peak was obtained. Statistical analysis was performed on the resulting MFI values for all 12 channels to evaluate stability of MFI values over time and alignment of MFI values across instruments, using the formulas below in MS Excel:

$$\%CV = \frac{SD}{Mean} \times 100$$

$$\%\ difference = \frac{MFI\ reference\ instrument - MFI\ value\ instrument\ of\ choice}{MFI\ reference\ instrument} \times 100$$

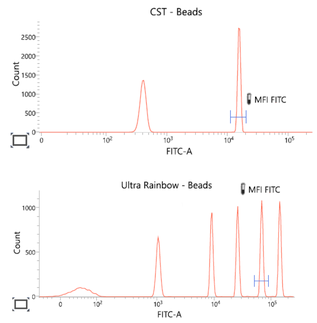
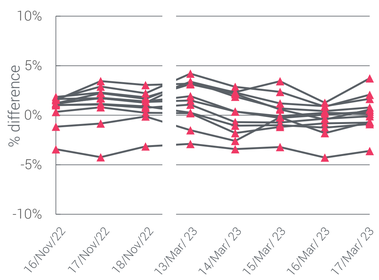



Figure 1: Representative histograms for CS&T beads (top) and Ultra Rainbow beads (bottom). The positive peak for CS&T and 5th peak for Ultra Rainbow beads are chosen for MFI value.

## Results

### MFI values are stable over time




B

| | cQC | | BLT | |
|---|---|---|---|---|
| | #264 | #293 | #246 | #284 |
| FITC | 0.4% | 1.7% | 1.4% | 1.1% |
| PE | 0.2% | 1.9% | 1.6% | 1.2% |
| PerCP-Cy5.5 | 1.3% | 0.9% | 1.3% | 1.8% |
| PE-Cy7 | 1.0% | 2.0% | 0.7% | 1.8% |
| APC | 2.0% | 2.7% | 1.9% | 2.2% |
| APC-R700 | 1.8% | 0.7% | 1.3% | 2.1% |
| APC-Cy7 | 1.4% | 2.0% | 1.5% | 2.6% |
| V450 | 0.4% | 1.2% | 0.9% | 0.5% |
| V500-C | 0.5% | 2.1% | 1.3% | 1.9% |
| BV605 | 0.2% | 2.1% | 0.3% | 2.6% |
| BV711 | 1.5% | 0.2% | 0.6% | 0.3% |
| BV786 | 1.2% | 2.8% | 0.4% | 2.0% |

Figure 2: MFI values from CS&T beads were collected for two non-consecutive weeks, with a time period of five months in between. Data is displayed as %difference between MFI on the first day (15/Nov/2022) of acquisition and the MFI on the days shown on the graph. Representative data from one instrument is shown (**A**). The influence of a cQC and BLT was evaluated in two instruments. The table shows %difference (absolute values) in MFI values from CS&T beads (cQC) or Ultra Rainbow beads (BLT) before and after the execution of a cQC or BLT (**B**).

MFI values of 12 channels were evaluated daily for two weeks with a gap of five months in between both weeks. Analysis was performed on all instruments using the same CS&T bead lot. Figure 2A shows representative data for one instrument. All 15 flow cytometers demonstrated an identical trend, in which % difference is <5% when compared to the MFI on the first day of acquisition. These data show that the CS&T software module of the BD FACSLyric™ corrects for daily fluctuations.

As the execution of a characterization QC (cQC) or Bead Lot Transfer (BLT) to a new CS&T lot changes the BBMTV of LW settings, MFI values were assessed before and after cQC and BLT on two instruments (Figure 2B). %difference is <5% (maximum is 2.8%), showing that MFI stability is not influenced by cQC or BLT, which is crucial for testing samples in long term clinical trials.

### Comparison of MFI values between instruments

*Note that our extensive investigation across 15 instruments indicates that instruments #114 and #052 located in EU (grey background in Table 1) are outliers with possible inherent differences in lasers and detectors, or in setup during the initial installation. Further investigation continues to identify the issue in these instruments. Therefore, data from these two instruments are NOT included in the analysis in Figure 3 and Table 1.*

The MFI values were also compared between instruments. To cover different ranges across the MFI spectrum of the cytometers, data from both types of calibration beads were evaluated. Whisker plot analysis reveals a higher variation in MFI values for APC-Cy7 and BV786 when using CS&T beads, and for APC, APC-Cy7 and V450 when using Ultra Rainbow beads (Figure 3). As shown in Table 1 for Ultra Rainbow beads, the variation is further confirmed when reviewing APC and V450, which have a %CV of 17.77% and 11.43%, respectively.

To further investigate which of the instruments are deviating, the %difference was calculated for all instruments using instrument #292 as reference. #292 was chosen as reference because its MFI values were closest to the average of all instruments. Only three instruments show %difference greater then 20% in certain channels with Ultra Rainbow beads. Multiple instruments show %differences between 10% and 20% in several channels with all the beads tested (Table 1).

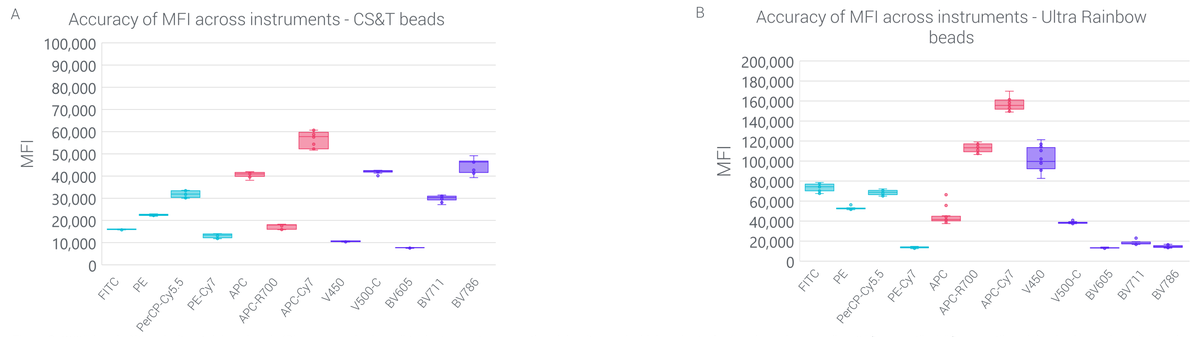



Figure 3: CS&T beads (**A**) and Ultra Rainbow beads (**B**) were acquired on all 15 instruments across the globe. Box and whisker chart of MFI values show median, 25th percentile, 75th percentile, minimal value and maximal value. Data from all instruments, except instruments #114 and #052 located in EU which are considered as outliers and currently under investigation, are shown for each of the 12 channels, with channels of the 488 nm laser shown in blue, channels of the 640 nm laser shown in pink, and channels of the 405 nm laser shown in purple.

| | | | | % difference | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | #292 (MFI) | %CV of MFI | US #058 | US #061 | US #158 | US #161 | EU #114* | EU #052* | EU #265 | EU #264 | EU #292 | EU #293 | TW #246 | TW #248 | AUS #020 | AUS #099 | AUS #353 |
| CS&T beads | FITC | 16.197 | 0,95% | 1,0% | 1,9% | 0,8% | -0,1% | 1,7% | 0,3% | 1,6% | 1,1% | 0,0% | 0,3% | 0,6% | 1,1% | 2,9% | 2,8% | 1,6% |
| | PE | 22.561 | 1,29% | -1,3% | 0,2% | -0,9% | -1,9% | 1,2% | 0,0% | 1,6% | 0,9% | 0,0% | -0,2% | -0,2% | 1,0% | 1,5% | 2,9% | 0,3% |
| | PerCP-Cy5.5 | 31.852 | 3,97% | -3,1% | 0,3% | -4,9% | -4,9% | -1,7% | -5,7% | 4,8% | 5,3% | 0,0% | -2,0% | -1,5% | -0,4% | 5,8% | 3,5% | -5,4% |
| | PE-Cy7 | 13.120 | 6,64% | -4,8% | -2,2% | -6,4% | -6,2% | -15,0% | -16,8% | 7,1% | 10,1% | 0,0% | -7,4% | -4,2% | -3,3% | 9,8% | 7,1% | -7,3% |
| | APC | 41.232 | 2,65% | -1,0% | -0,5% | -1,2% | -1,8% | 4,2% | 4,4% | 7,6% | 3,4% | 0,0% | 2,0% | 0,1% | 1,4% | 4,1% | 2,7% | 0,6% |
| | APC-R700 | 17.534 | 5,36% | -4,4% | -2,6% | -2,3% | -3,3% | -9,0% | -9,5% | 9,4% | 8,7% | 0,0% | -3,0% | -0,7% | 0,9% | 9,6% | 6,4% | -2,5% |
| | APC-Cy7 | 57.765 | 5,71% | -5,0% | -2,9% | -1,7% | -3,4% | -16,8% | -14,0% | 9,6% | 10,5% | 0,0% | -5,0% | -1,0% | 0,1% | 9,6% | 5,9% | 0,3% |
| | V450 | 10.850 | 1,87% | 2,0% | 5,1% | 1,2% | 0,5% | 1,6% | 4,7% | 1,0% | -0,1% | 0,0% | 1,7% | -0,8% | 1,5% | 4,0% | 4,5% | 2,9% |
| | V500-C | 42.233 | 1,62% | -0,6% | 4,9% | -0,9% | -0,7% | -0,3% | 4,4% | 1,1% | 0,1% | 0,0% | 0,0% | -1,3% | 0,3% | 2,2% | 1,1% | 0,3% |
| | BV605 | 7.775 | 1,41% | 0,5% | 4,2% | -0,1% | -0,7% | 0,5% | 5,8% | 1,1% | -0,3% | 0,0% | 1,5% | -0,7% | 1,3% | 2,6% | 1,7% | 0,9% |
| | BV711 | 30.323 | 4,31% | 2,0% | -2,1% | -1,7% | -2,4% | -5,7% | -7,5% | 3,4% | 7,2% | 0,0% | -1,5% | -2,7% | -0,9% | 10,6% | 3,5% | -3,7% |
| | BV786 | 46.307 | 6,51% | -0,3% | -0,4% | -0,9% | -1,8% | -20,4% | -10,7% | 7,8% | 10,9% | 0,0% | -6,2% | -2,1% | -1,0% | 15,1% | 10,2% | 0,1% |
| Ultra Rainbow beads | FITC | 78,659 | 5.25% | 14.0% | 9.2% | 4.0% | 1.7% | 12.1% | 9.7% | 10.7% | 1.1% | 0.0% | 2.7% | 3.3% | 8.8% | 10.7% | 14.1% | 5.5% |
| | PE | 53,350 | 2.24% | 1.2% | 2.3% | 0.5% | 0.5% | 0.6% | -0.9% | 1.4% | 1.9% | 0.0% | 3.6% | 1.3% | 1.6% | -5.5% | 1.9% | 3.2% |
| | PerCP-Cy5.5 | 68,861 | 3.46% | 0.9% | 3.1% | -4.8% | -3.1% | 2.3% | -4.0% | 5.5% | 5.6% | 0.0% | 1.3% | -4.0% | 0.1% | -2.4% | 3.8% | -0.9% |
| | PE-Cy7 | 13,838 | 5.21% | 2.2% | 1.8% | 0.8% | 0.5% | -10.1% | -13.2% | 5.9% | 10.5% | 0.0% | -6.7% | -6.4% | -1.8% | -4.7% | 7.8% | -1.4% |
| | APC | 45,340 | 17.77% | 8.9% | 5.0% | 13.3% | 8.6% | -47.3% | -38.0% | 8.3% | 8.8% | 0.0% | 2.3% | 17.3% | 14.7% | -22.8% | -46.4% | 3.3% |
| | APC-R700 | 116,892 | 3.74% | 6.8% | 6.2% | 5.1% | 3.0% | -10.4% | -10.1% | 8.0% | 8.8% | 0.0% | -1.1% | -0.5% | 2.5% | -1.9% | 4.3% | 0.6% |
| | APC-Cy7 | 160,621 | 3.85% | 3.1% | 4.9% | 1.4% | 3.1% | -13.5% | -13.0% | 7.2% | 6.0% | 0.0% | -5.8% | -1.9% | 3.6% | -0.5% | 6.7% | 4.7% |
| | V450 | 97,941 | 11.43% | -12.8% | 5.7% | -16.6% | -4.2% | -13.9% | 4.3% | -15.1% | 7.3% | 0.0% | -23.9% | -1.7% | 15.5% | -1.3% | -19.4% | 5.8% |
| | V500-C | 37,857 | 2.41% | -1.5% | -1.5% | -4.2% | -1.1% | -5.0% | 3.0% | 1.3% | -1.0% | 0.0% | -1.2% | 1.1% | 0.4% | -1.6% | -3.6% | -7.8% |
| | BV605 | 13,016 | 2.66% | -0.9% | 1.9% | -5.7% | -2.1% | -3.9% | 2.8% | -3.4% | -1.9% | 0.0% | -2.2% | 1.5% | -0.5% | -1.1% | -3.8% | -7.8% |
| | BV711 | 17,780 | 9.40% | 1.7% | 6.2% | -7.2% | 1.6% | -13.8% | -6.7% | 3.1% | 3.3% | 0.0% | -7.2% | 0.0% | 3.1% | -29.5% | 5.9% | -9.3% |
| | BV786 | 15,133 | 7.74% | 6.3% | 9.1% | -1.9% | 4.1% | -19.8% | -6.6% | 8.6% | 11.5% | 0.0% | -8.5% | 2.6% | 4.6% | -11.2% | 13.9% | -3.4% |
| Fc beads | PE-Cy7 | 143,129 | 4.78% | ND | ND | ND | ND | -17.0% | -19.4% | 5.1% | 5.4% | 0.0% | -4.5% | ND | ND | ND | ND | ND |
| | APC | 76,612 | 0.72% | ND | ND | ND | ND | -13.0% | -15.4% | 1.4% | -0.1% | 0.0% | 0.8% | ND | ND | ND | ND | ND |
| | APC-Cy7 | 34,862 | 8.59% | ND | ND | ND | ND | -10.1% | -5.2% | 12.9% | 16.2% | 0.0% | 2.3% | ND | ND | ND | ND | ND |
| | BV786 | 96,577 | 4.66% | ND | ND | ND | ND | -8.6% | -7.3% | 4.9% | 10.8% | 0.0% | 5.0% | ND | ND | ND | ND | ND |

Table 1: %difference of all instruments is calculated to reference instrument #292. US = United States of America, EU = Europe (Belgium), TW = Taiwan, AUS = Australia. %CV (Coefficient of Variation) of MFI over all instruments, except instruments #114 and #052, is shown. %difference/%CV <10%: black; 10%-20%: purple; >20%: pink. Data are from acquisition of CS&T beads, Ultra Rainbow beads and Fc beads. * Instruments #114 and #052, located in EU are considered as outlier instruments and are currently under investigation. ND: Note Done.

## Conclusion

Evaluation of MFI values across all 12 channels for an extended period shows that the BD FACSLyric™ instrument is capable of generating reproducible results over time. However, the data from calibration beads show that the Cytometer Setup & QC software module is not able to ensure optimal alignment of MFI values across multiple instruments for all channels. The most significant differences were observed on the APC, APC-Cy7, V450 and BV786 channels in some of the instruments.

Variation of MFI values across different BD FACSLyric™ instruments were more significant than anticipated. This highlights the importance of selecting instruments with similar MFI values during assay validation and, when possible, incorporating quantification beads for normalization of MFI values for global clinical trials.

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