#13 Evaluation of the Long-Term Stability of the BD™ CS&T Setup Workflow on the BD FACSLyric™ System

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Introduction

For immunofluorescence applications, flow cytometer setup and QC are critical to ensure consistent and reproducible results over time and across cytometer systems. The BD™ Cytometer Setup and Tracking (CS&T) setup workflow on the newly developed multicolor BD FACSLyric™ system incorporates BD™ CS&T IVD beads, BD™ FC beads, and BD FACSuite™ software. CS&T is a user-friendly method that characterizes and maintains the performance of the instrument. The optimal biological application settings can be created in BD FACSuite software and saved using the CS&T bright bead target values for standardization of multiple cytometers with differing optical components. The CS&T setup workflow allows users to define baseline performance and perform daily QC automatically for critical instrument parameters. The workflow also performs daily checks and adjustments for optimal signal resolution, fluorescence target value positions, and laser delays. Levey-Jennings (LJ) plots track daily instrument fluctuations from baseline settings.

The stability of instrument parameters is very important because it affects the fluorescence intensity and stability of biological markers. This study verified the long-term stability of a 10-color BD FACSLyric system that was placed at a clinical study site for biological sample evaluation. The BD CS&T setup workflow with three lots of CS&T IVD beads was used. The BD CS&T setup workflow was run multiple days per week on the cytometer for 11 months to qualify the BD FACSLyric instrument for biological studies. The stability of spillover values (SOVs) and biomarker median fluorescence intensities (MFIs) was observed over a two-month period. The LJ plots were analyzed for instrument QC of the 10-color BD FACSLyric system, as well as fluorescence SOVs and MFIs of six fluorescence channels using T-cell subset biomarkers.

Methods

To evaluate instrument QC for the BD FACSLyric instrument, two drops of CS&T IVD beads were added into 500 µL of BD FACSFlow sheath fluid, then run on the BD FACSLyric instrument to generate a passing instrument QC report.

The BD FC beads were prepared by adding 2 drops of each fluorochrome in the BD FACSuite™ FC bead kit and 1 drop of BD FC beads to each fluorochrome-labeled tube and incubating the tubes for 15:30 min at RT in the dark. After adding 0.5 mL of bead solution to each tube, prepared FC bead tubes were run on the BD FACSLyric system to generate SOVs.

To monitor the stability of SOVs and biomarker MFIs, BD™ Multi-Check Control cells were stained and prepared using the BD Multitest™ 6-color TBNK reagent and run on the BD FACSLyric system.

During the period of the study, BD FACSuite software and three lots of CS&T IVD beads were used to set up and perform QC on the BD FACSLyric instrument. Two lots of BD FC beads were prepared and run on the BD FACSLyric system to establish optimal fluorescence compensation fduring study period. Two lots of BD Multi-Check Control cells were used to test biomarker stability.

Discussion

LJ tracking results of instrument parameters are summarized in Table 1. To analyze LJ-tracking data for a 330-day study, we compared key parameters every 30 days relative to Day-1. A total of 11 percent difference (%diff) values (%diff$_{Day-n}$ = (Day-n – Day-1)/Day-1*100, n = 30, 60, 90, 120 … 330) were generated for each key parameter. The maximum positive or negative values of the 11 difference values are presented in Table 1.

Real-time instrument parameters are plotted in Figure 1 (A-D) for the BD FACSLyric instrument. Due to the large numbers of tracking days, we took the average value of each parameter in a month and plotted our results over the study period, as shown in Figure 1. The SOVs of six fluorescence channels are plotted in Figure 2 using data from representative study days. MFIs of six biomarkers (CD3 FITC, CD16+56 PE, CD45 PerCP-Cy5.5, CD4 PE-Cy7, CD19 APC, and CD8 APC-Cy7) were tracked over time, as presented in Figure 3.

Our long-term LJ tracking data showed that for the bright bead MFI, maximum %diff within 330 days relative to Day-1 was within ±2%. For the robust coefficient of variation (rCV%) of the bright bead, the FSC channel showed a significant increase over time, while the rCV% of fluorescence channels remained stable over 11 months. For the fluorescence linearity maximum value, the maximum %diff within 330 days relative to Day-1 was within ±3%. Instrument sensitivity remained stable and within the manufacturer's specification. Decrease of fluorescence resolution as measured by the Qr value was less than 25% over 330 days. For the Br value of the V500-C channel, the maximum %diff relative to Day-1 showed a 105.7% increase within the 330 study days.

Fluorescence spillover values remained stable over two months in the six fluorescence channels. The MFI of lymphocyte subset markers demonstrated stability that was acceptable. Over a two-month period, the CV% values of biomarker MFIs in six fluorescence channels were within 10%.

	Bright bead median (Day-1)	Max % diff within 330 days	Bright bead rCV% (Day-1)	Max % diff within 330 days	Linearity Max channel (Day-1)	Max % diff within 330 days
FSC	120,400	1.5	0.6	133.3	n/a	n/a
SSC	124,219	1.9	1.2	16.7	n/a	n/a
FITC	100,824	0.9	1.3	23.1	222,494	0.8
PE	100,815	0.7	0.9	55.6	225,019	1.0
PerCP-Cy™5.5	99,007	1.6	2	15.0	237,609	-2.0
PE-Cy7	99,069	1.3	3.8	15.8	226,913	-2.1
APC	100,806	1.1	1.8	-11.1	227,424	0.9
APC-R700	100,024	1.2	2.2	-22.7	222,718	0.8
APC-Cy™7	100,667	1.2	2.8	-25.0	225,486	1.0
V450	102,311	-0.6	1.8	11.1	224,451	0.6
V500-C	102,485	1.0	1.6	18.8	225,941	0.9
BV605	101,589	0.8	2.3	4.4	220,207	0.6

Table 1 Maximum %diff relative to Day-1 observed within 330 days

	Sensitivity (Day-1)	Max % diff within 330 days	Resolution Qr (x10³) (Day-1)	Max % diff within 330 days	Br (Day-1)	Max % diff within 330 days
FSC	358	19.8	n/a	n/a	n/a	n/a
SSC	1,373	-3.6	n/a	n/a	n/a	n/a
FITC	620	-16.5	108.1	14.7	89	14.7
PE	1,569	-4.6	964.9	-24.7	134	-24.7
PerCP-Cy5.5	505	19.8	37.6	11.7	35	11.7
PE-Cy7	2,225	27.0	67.5	20.6	3	20.6
APC	601	13.6	55	-13.6	28	-13.6
APC-R700	194	9.8	19.8	8.1	30	8.1
APC-Cy7	182	7.7	31.1	43.1	165	43.1
V450	194	-8.3	495.3	89.4	5,238	89.4
V500-C	178	31.5	125.4	105.7	1,182	105.7
BV605	2,755	37.7	495.7	-9.9	26	-42.3

Conclusion

The BD FACSLyric demonstrated stable performance in all critical instrument parameters, such as bright bead median fluorescence, robust coefficients of variation (rCV%) of the bright bead, instrument sensitivity, fluorescence resolution, and linearity range. System stability of SOVs and biomarker MFIs was satisfactory. The BD CS&T setup workflow is a comprehensive, easy-to-use method for cytometer standardization in long-term studies.
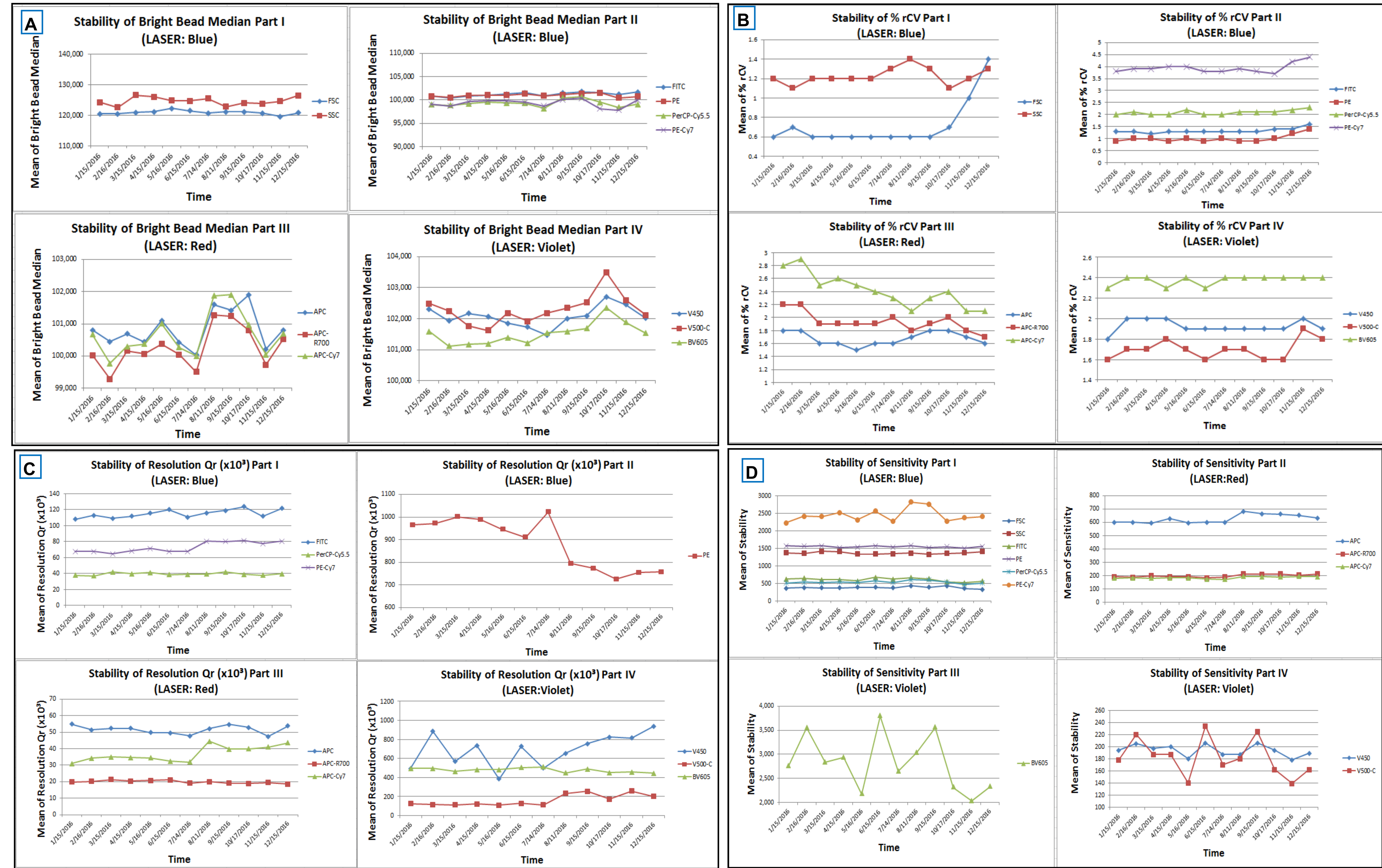

Figure 1. Tracking results of instrument parameters for the BD FACSLyric established by the CS&T setup workflow
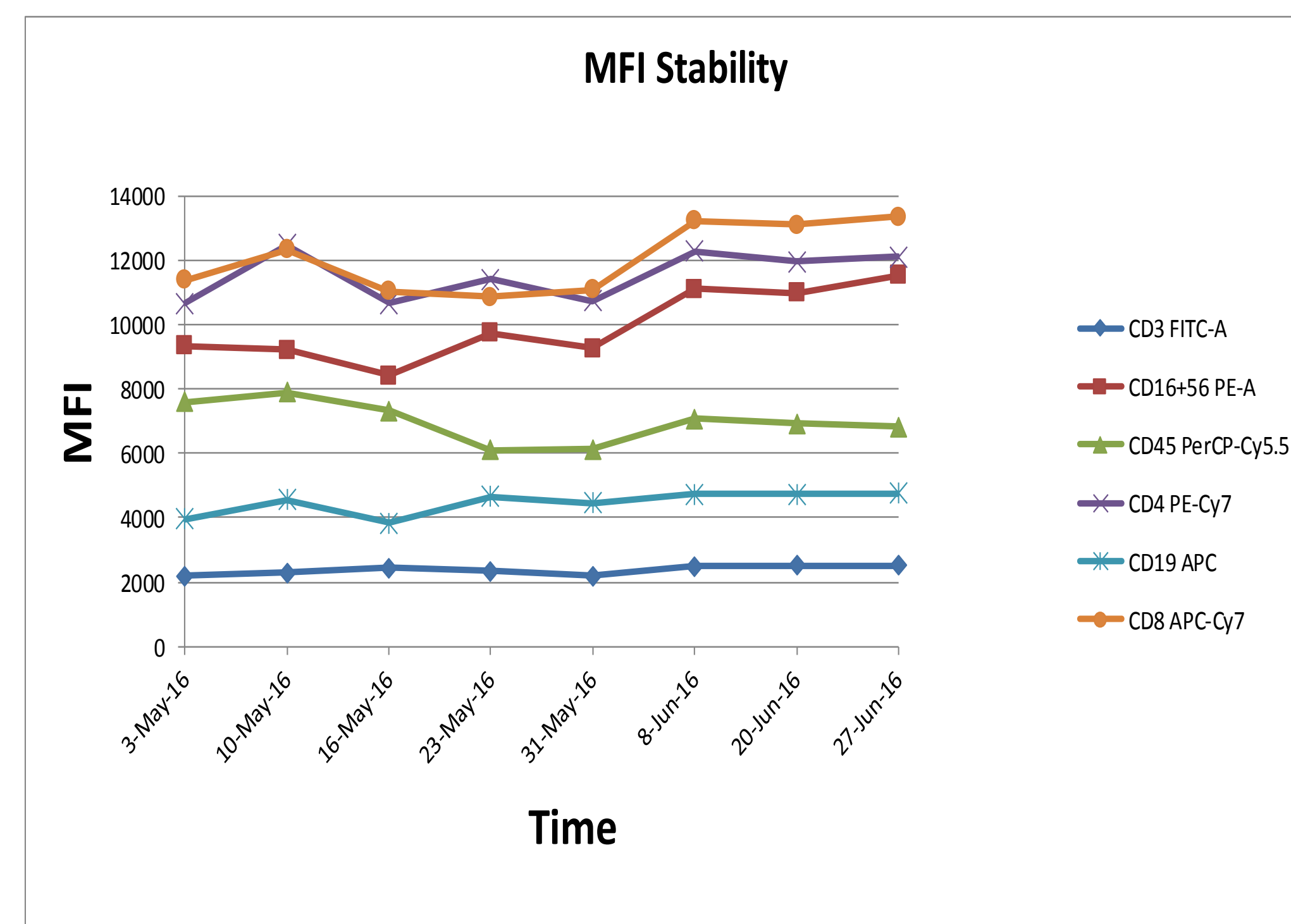

Figure 3. MFIs of CD3 FITC, CD16+CD56 PE, CD45 PerCP-Cy5.5, CD4 PE-Cy7, CD19 APC, and CD8 APC-Cy7 were plotted with time on dates which BD Multi-Check Controls were stained with BD Multitest 6-Color TBNK reagent and acquired on the BD FACSLyric instrument. Over our study period, the mean MFI with CV% of each fluorescence channel was: FITC (2,708, 6.4%), PE (11,387, 10%), PerCP-Cy5.5 (7,988, 8.5%), PE-Cy7 (13,203, 6.9%), APC (5,099, 7.8%), APC-Cy7 (13,782, 8.4%).
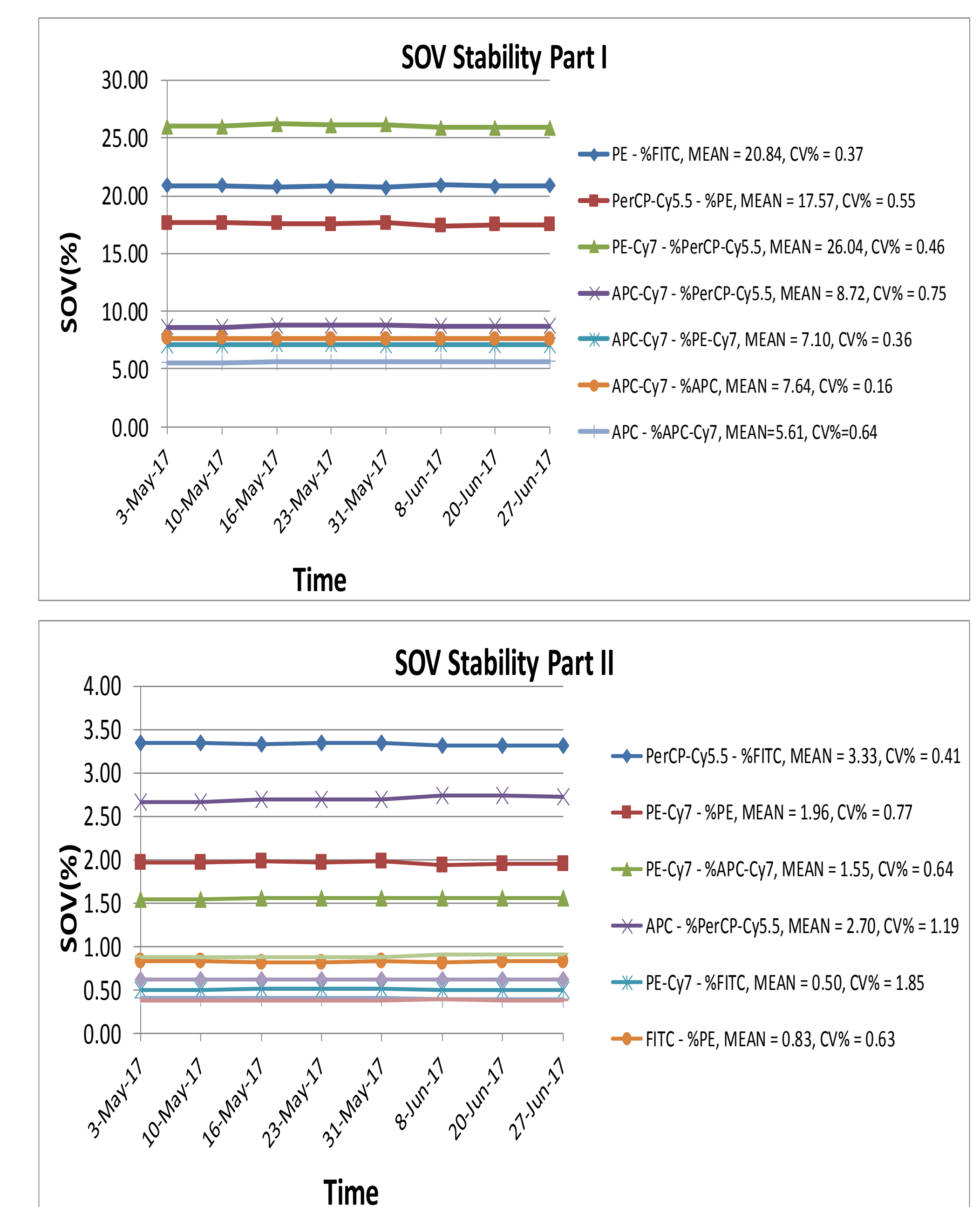

Figure 2. Spillover values (SOV%) of FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, and APC-Cy7 channels from each of the six fluorescence channels. SOV data was extracted after CS&T QC was performed on the study day (SOVs were stored in the FCS files on the study day). Over the study period, the mean values of SOVs with CV% values are listed in the figure legend. Other SOV% values were close to zero and are not listed here.

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