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CytoFLEX System Performance Evaluation

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ABSTRACT

Performance testing of cytometry instruments is utilized to characterize the capability of the cytometer to perform high complexity applications. We have tested the new CytoFLEX flow cytometer against the performance measurements for sensitivity, dynamic range, population resolution, linearity, stability, reproducibility, and small particle resolution. Commercially available bead standards were used to evaluate performance expectations across multiple parameters.

The CytoFLEX flow cytometer was evaluated at La Jolla Bioengineering Institute, Purdue University Cytometry Laboratory (PUCL) and the Beckman Coulter, Miami site. Additional data was collected through a collaborative effort with Al Donnenberg from University of Pittsburgh Cancer Center and School of Medicine at Sun Yat-Sen University, Guangzhou, China. Each instrument was equipped with three lasers (405nm, 488nm and 638nm) and 12 parameters with 9 fluorescence detectors. Instruments were equipped with the enhanced Violet laser Side Scatter Channel (VSSC) option for small particle detection..

MATERIAL AND METHODS

CytoFLEX multicolor flow cytometer equipped with enhanced Violet laser Side Scatter Channel (VSSC) option (manufactured by Beckman Coulter)

To calculate the daily Quality Control rCV (robust CV) through the CytExpert Software, CytoFLEX QC Beads were used. The QC beads were collected on three different CytoFLEX instruments. Robust CV is approximately the 75th percentile minus the 25th percentile divided by the median. And is not as skewed by outlying values as the CV.

Spherotech 8 Peak Rainbow Beads were used for resolution, linearity, stability and separation testing.

For Microparticle testing the following were used:

Laboratory 1: Beckman Coulter Miami
Spherotech beads. 0.5 µm and 0.2 µm.

Laboratory 2: La Jolla Bioengineering Institute
Polybead Polystyrene Sampler Kit III PolySciences # 16905-1
Polybead Polystyrene Sampler Kit II PolySciences # 21756-1

Laboratory 3: PUCL.
PolySciences beads 0.5, 0.4, 0.35, 0.3, and 0.2 µm beads

Laboratory 4: School of Medicine, Sun Yat-Sen University, Guangzhou, China
Beckman Coulter Particle Sizing Standards 0.5 µm, 0.3 µm, and 0.2 µm latex beads (non-fluorescent) mixture for testing. Beads were diluted using UPW (UltraPure Water).

Cytometer comparison was conducted against a competitor system configured with equivalent lasers and filter setup.

Separation index is described as the separation of adjacent peaks of Rainbow beads measured by the difference between the peak MFIs, divided by the geometric mean of their standard deviations.

Separation Index is measured using the following formula:

Separation Index = (MFIpeak2-MFIpeak1)/(SQRT(SDpeak1^2+SDpeak2^2)/2)

RESULTS

Figure 1. rCV analysis of QC beads over 18 days

QC beads were run on the instrument for 18 days. Twelve Startup and Shutdown cycles were captured and rCV were tracked via Levey-Jennings. The data below is relative parameters for each Laser.

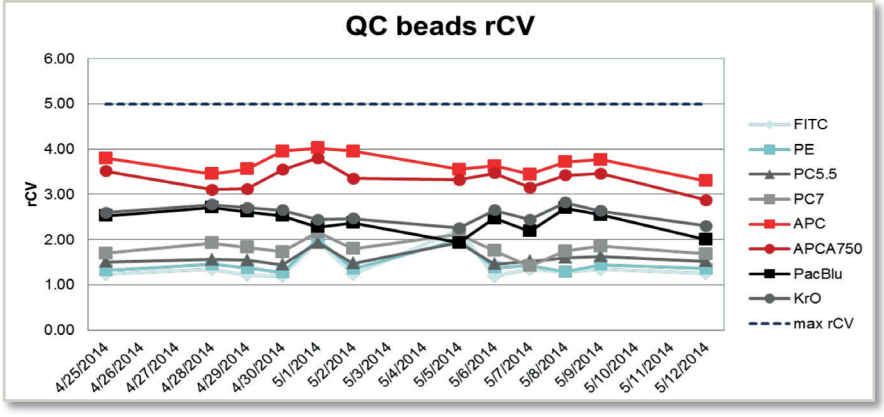


Figure 2. rCVs on 8 peak beads run continuously for one hour period

Spherotech 8 Peak Rainbow beads were run continuously for one hour on the CytoFLEX unit. rCV data was collected on Peak#5 PE: 2.12%, APC: 4.40% and BV421: 2.34% showing the stability of the system.

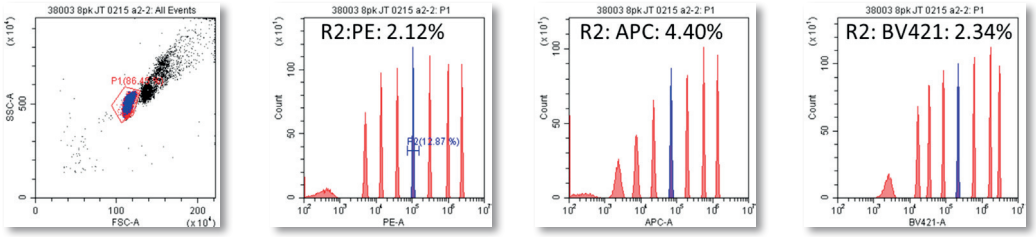
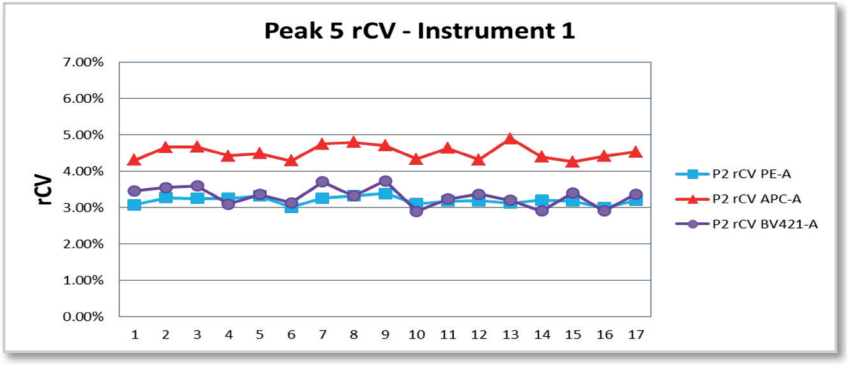


Figure 3.

Spherotech 8 Peak Rainbow beads were run every day for seventeen days on two instruments and the rCV Data was collected and plotted on Peak#5.



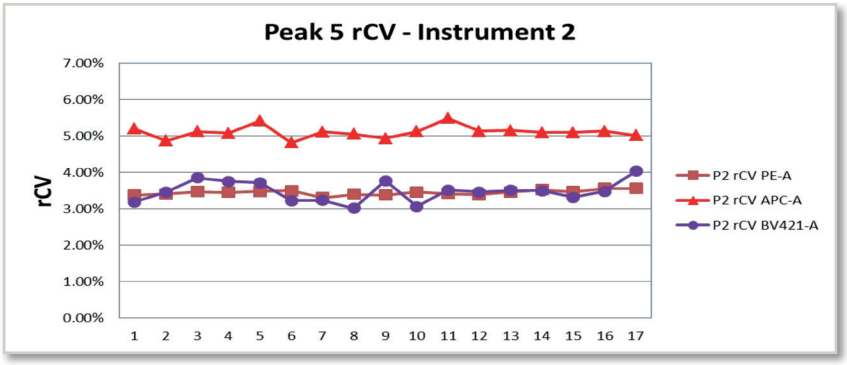


Figure 4.

Spherotech 8 peak rainbow bead data for 3 Laser 10 Color detectors.
Data provided by University of Pittsburgh Cancer Center

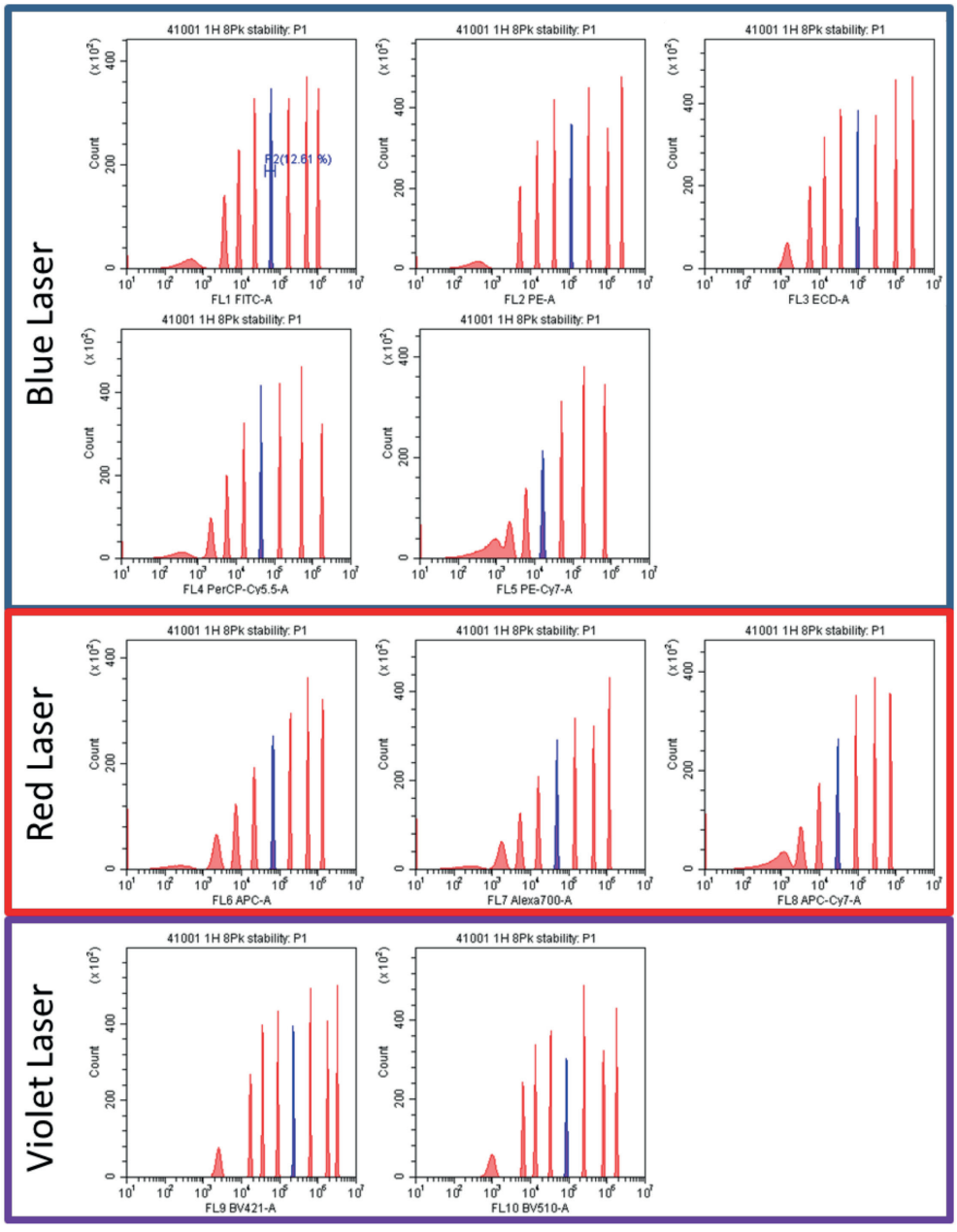


Figure 5.

Spherotech 8 peak rainbow bead data calculated for a Separation Index between bead peaks as compared to a standard High Complexity research Cytometer.

Data provided by University of Pittsburgh Cancer Center

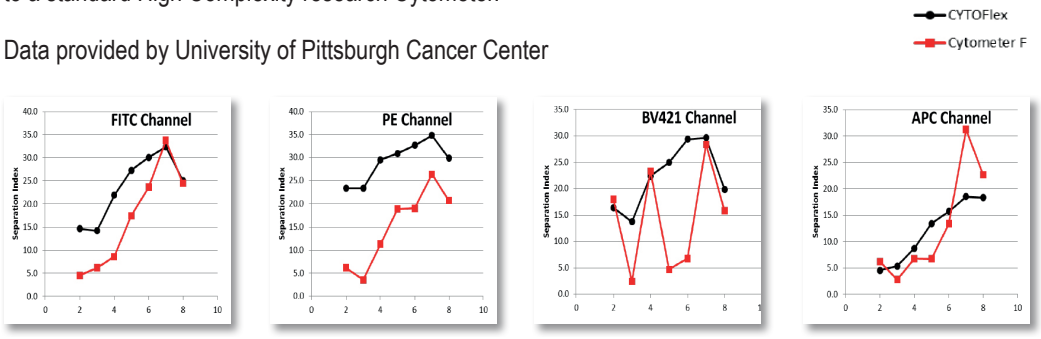


Figure 6.

Spherotech 8 peak rainbow bead data calculated for a Separation Index between bead peaks as compared to a standard High Complexity research Cytometer. Data is Specific for Red Channel Sensitivity.

Data provided by University of Pittsburgh Cancer Center

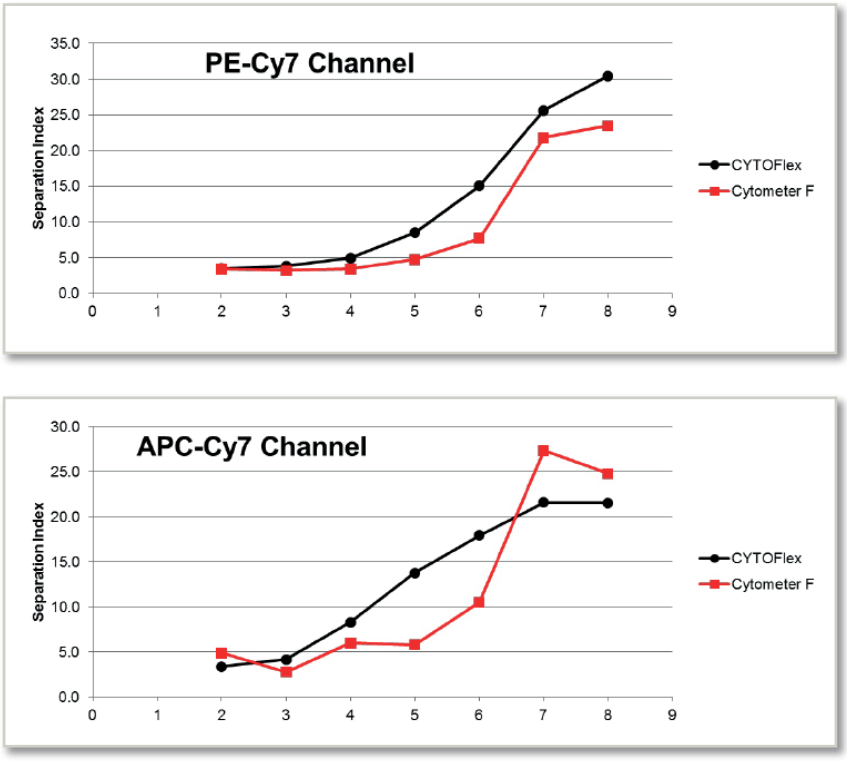
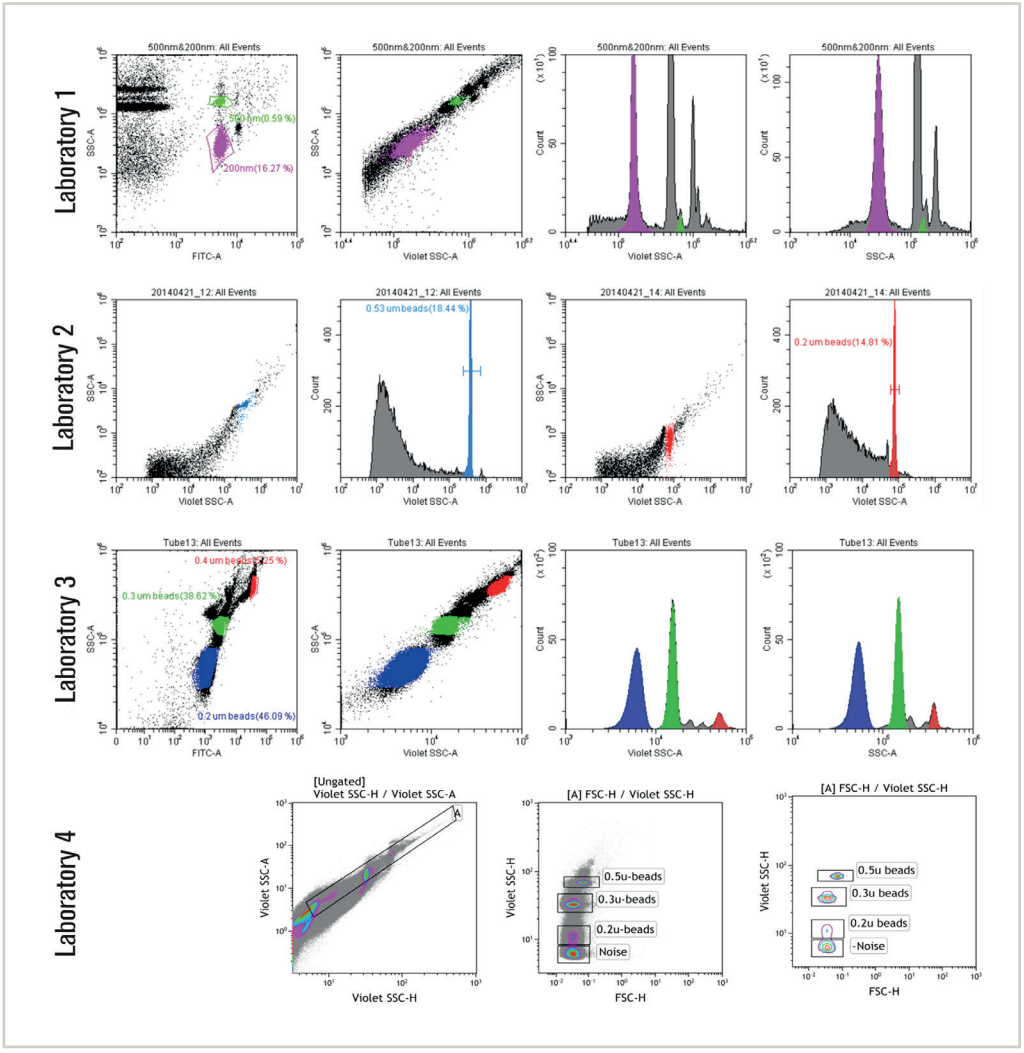


Figure 7.

Microparticle Detection. Four separate labs ran bead based small particles detected using side scatter and VSSC.

Data provided by University of Pittsburgh Cancer Center, PUCL, La Jolla Bioengineering Institute, Sun Yat-Sen University Medical College.



SUMMARY

Performance evaluation results show the CytoFLEX cytometer is stable, reproducible and sensitive especially in violet and far red channels. The system's ability to separate populations as measured by the separation index is overall superior to comparable instrumentation.

Small particle evaluation showed clear resolution of 0.2 μm particles from noise using SSC from the Violet laser. Moreover, the CytoFLEX flow cytometer can clearly distinguish between different sizes of submicron beads.