CytoFLEX S System Performance

TECHNICAL INFORMATION BULLETIN

James Tung, Jeffrey Cobb, Elsa Burgess, Domenic Fenoglio; Life Science, Beckman Coulter, Inc., Miami, FL, United States

Introduction

CytoFLEX S 561nm is a new member to the CytoFLEX family of benchtop flow cytometers. The CytoFLEX S platform design goals are to retain the same high sensitivity and high performance as the original CytoFLEX 3-laser 13-color system. The CytoFLEX S 561 maintains the same high sensitivity and high performance expectation from 405nm, 488nm, and 638nm lasers, and adds additional excitation and high sensitivity detection capability from the 561nm laser. The addition of the 561nm laser, in a spatially separated discrete beam spot, enables better excitation and detection of PE/PE tandems and red fluorescent proteins.

Material and Methods

CytoFLEX S multicolor flow cytometer equipped with blue, red, violet and yellow lasers. All samples were assessed on the CytoFLEX™ cytometer (Beckman Coulter, Life Sciences) using the settings established during QC and the data analyzed using CytExpert software (Beckman Coulter, Life Sciences).

For staining of blood samples: 100 µL of normal donor blood were stained with the appropriate volumes for each reagent. Samples were incubated for 15 minutes at room temperature in the dark. The stained blood samples were then lysed using VersaLyse™ Lysing Solution and incubated for 15 minutes at room temperature in the dark. All lysed samples were handled as follows: 3mL PBS/0.1% NaN3 added, centrifugation for 4 min at 1200 x g, supernatant decanted, re-suspended with 1 mL 1X PBS/0.1% HCHO buffer.

Targets	Fluorophores	Clones
CD3	PE	UCHT1
CD3	ECD	UCHT1
CD3	Pacific Blue	UCHT1
CD4	Pacific Blue	SCFI12T4D11(T4)
CD19	APCA700	J3-119
CD23	ECD	9P25
CD25	PE	B1.49.9
CD38	ECD	LS198
CD56	PE	N901(NKH-1)

Spherotech 8 Peak Rainbow Beads were used for resolution. mCherry and dsTomato bacteria were a kind gift of Teresa Hawley.

Results

The CytoFLEX S system can be configured from a 2 to 4 laser system with blue, red, violet and yellow lasers.

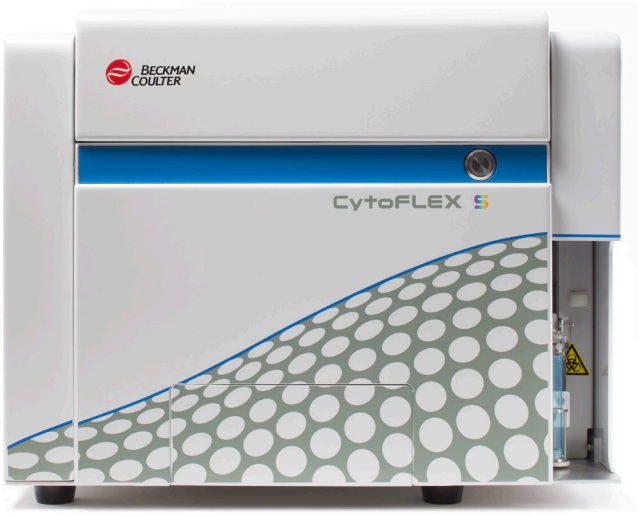

Figure 1A. The CytoFLEX S sytem

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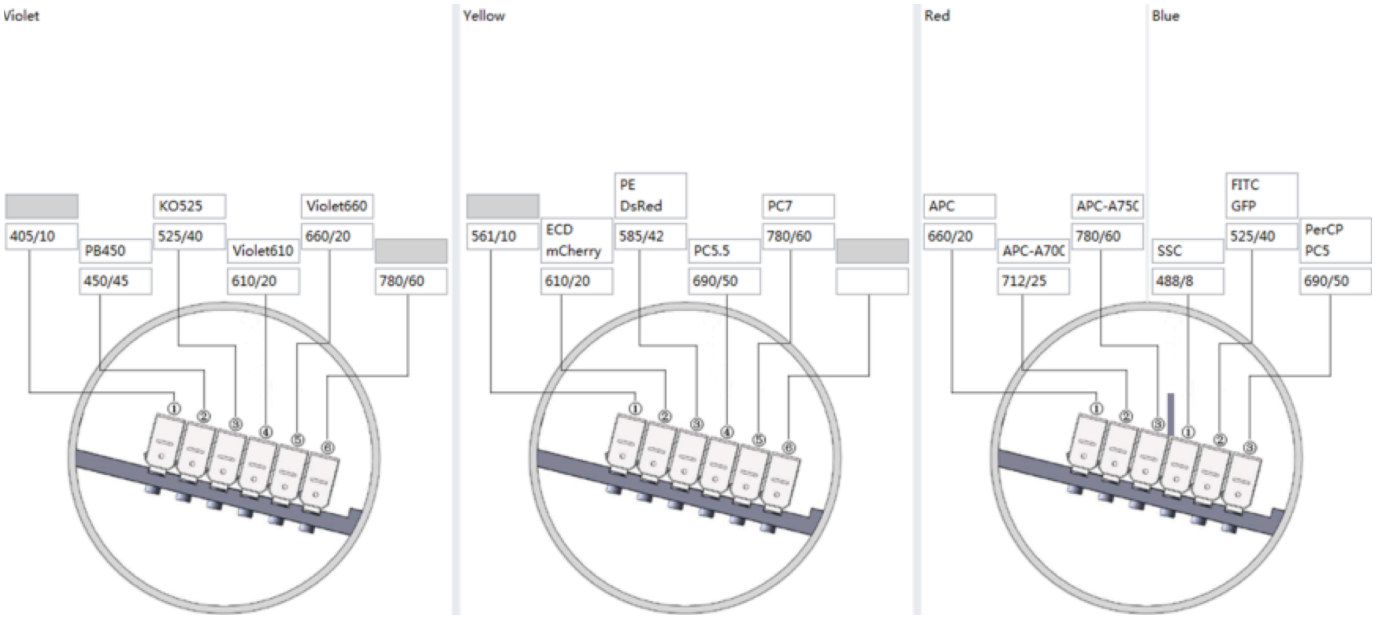

Figure 1B. The CytoFLEX S configuration

Figure 1C. The CytoFLEX S detector configuration

Common fluorochromes and fluorescent proteins can be detected on the CytoFLEX S.

WDM	Laser	Fluorescent Channel	CytoFLEX S Channel Names	Commonly used Fluorescent Dyes
WDM 1	405-nm	450/45 BP	PB450	Pacific Blue™ dye, V450, eFluor™ 450, BV421
		525/40 BP	KO525	Krome Orange, AmCyan, V500, BV510
		610/20 BP	Violet610	BV605, Qdot® 605, mCherry
		660/20 BP	Violet660	BV650, Qdot® 655
WDM 2	561-nm	610/20 BP	ECD mCherry	ECD, PE-Texas Red®, PE-CF594, PI, mCherry
		585/42 BP	PE DsRed	PE, PI, DsRed, TdTomato
		690/50 BP	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI
		780/60 BP	PC7	PC7
WDM 3	488-nm	525/40 BP	FITC GFP	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
		690/50 BP	PC5 PerCP	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI
	638-nm	780/60 BP	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor™ 780
		712/25 BP	APC-A700	APC-A700, Alexa Fluor™ 700
		660/20 BP	APC	APC, Alexa Fluor™ 647, eFluor™ 660

Figure 2: Common fluorochromes for the CytoFLEX S

To demonstrate the functionality and sensitivity, Spherotech 8 peak rainbow bead data was collected on the system.

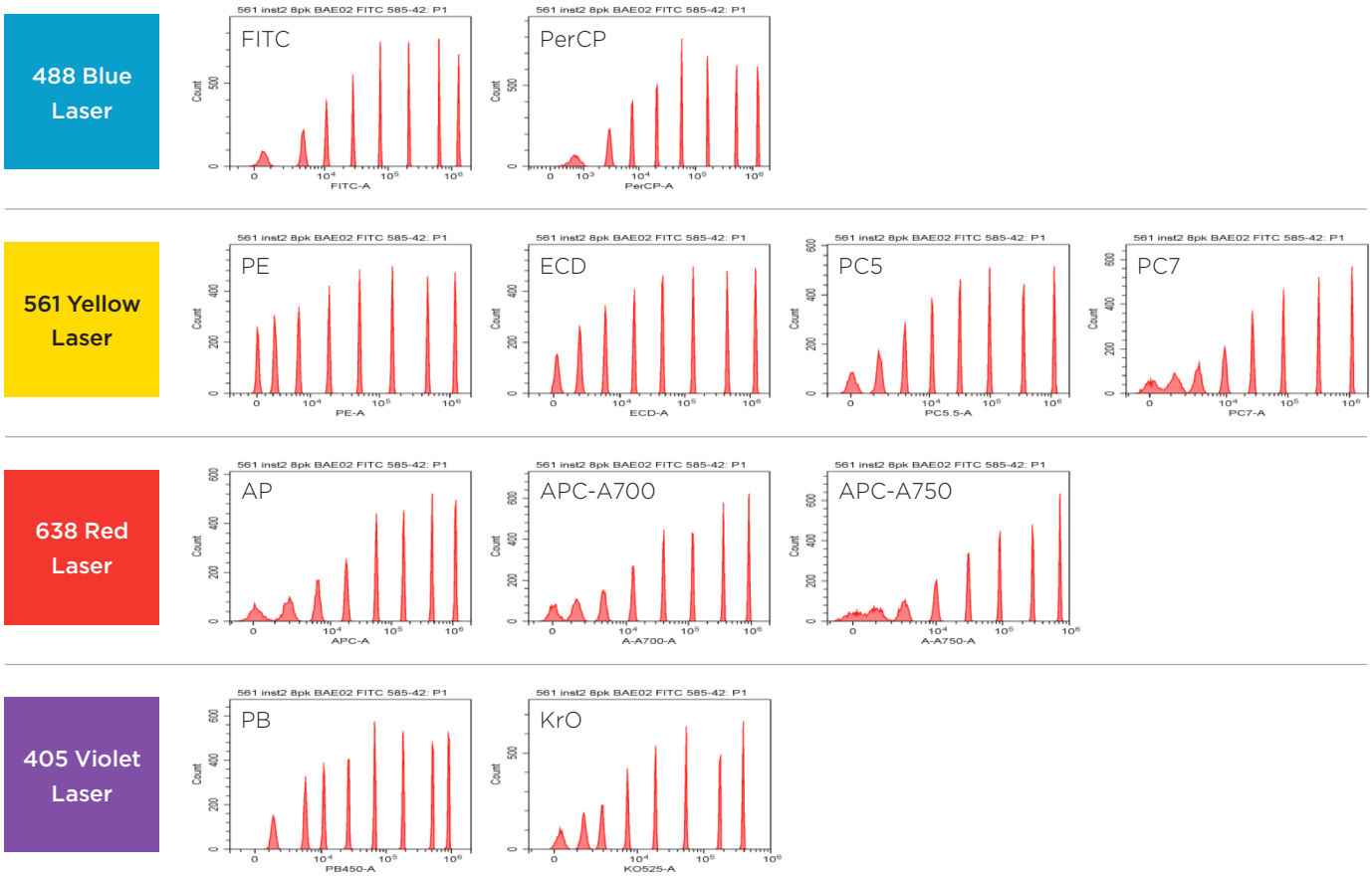

Figure 3. Resolution of Spherotech Rainbow Calibration Particles. Note that the 8 peak beads are well resolved in the PC7 and APC-A750 channels.

Importantly, the performance of the CytoFLEX and CytoFLEX S is comparable.

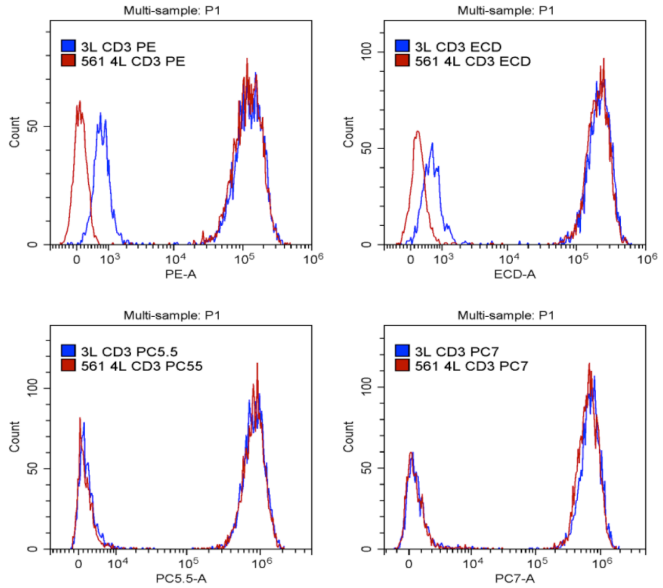

Figure 4. The CytoFLEX S instrument (4L) offers the same high performance compared to the CytoFLEX (3L).

The yellow 561 laser is known to have a performance advantage when looking at dim populations with specific dyes. The CytoFLEX S is better able to resolve dim populations on the PE and ECD channels.

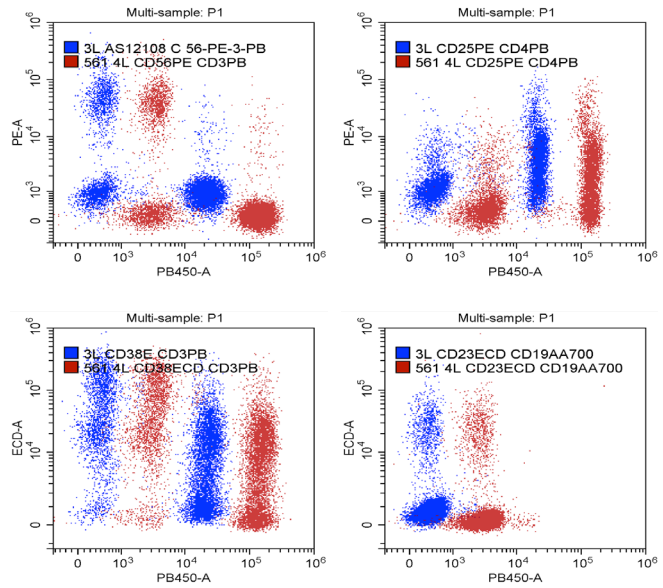

Figure 5. The 561 4L instrument resolves the dim markers better on PE and ECD channels.

Lastly, as fruit fluorescent dyes are more prevalent, the mCherry expressing bacteria and beads were subject to analysis to show the functionality of the system for these dyes.

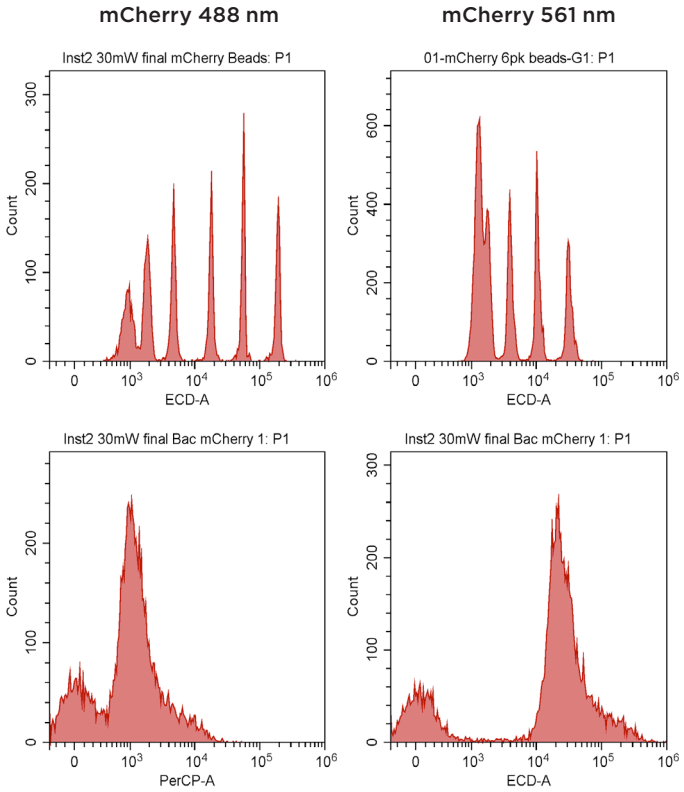

Figure 6. The 561 4L instrument can detect and resolve mCherry 6 peak beads (Clonetech) and mCherry bacteria

Conclusion

CytoFLEX S 561nm clearly demonstrates the capability to excite and detect PE, PE tandems, and red fluorescent proteins. High sensitivity and high performance are maintained in the new CytoFLEX S design. The dim-populations in the PE and ECD channels are better resolved in the CyotFLEX S 561nm instrument due to the maximal PE excitation efficiency. Red fluorescent proteins such as mCherry, are well excited and resolved with the 561nm laser. The CytoFLEX S platform has been designed for the addition of a 4th laser, with the 561nm laser as first product offering in the CytoFLEX S product line.

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