Near UV Laser System Performance on the CytoFLEX S

APPLICATION NOTE

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Introduction

CytoFLEX S 375 nm laser is a new addition to the CytoFLEX family of benchtop flow cytometers. The CytoFLEX S maintains the same high sensitivity and high performance expectation from all lasers: 375 nm, 405 nm, 488 nm, 561 nm and 638 nm lasers. The addition of the 375 nm laser, in a spatially separated discrete beam spot, enables excellent excitation of Hoescht, DAPI and brilliant UV dyes. This allows for experimentation with these dyes/ fluorochromes without incurring the cost of a true UV laser.

Material and Methods

CytoFLEX S multicolor flow cytometers were equipped with a blue, red, violet and near UV laser or a blue, violet, yellow/green and near UV laser (see Figure 1 for system configurations with the near UV laser). All samples were assessed on the CytoFLEX S cytometer (Beckman Coulter, Life Sciences) using the settings established during QC and the data analyzed using CytExpert software (Beckman Coulter, Life Sciences).

Num	Configuration	Blue 488 nm						Red 638 nm			Violet 405 nm			NUV 375 nm	
		SSC 488	FITC 505-545	PE 565-607	ECD 605-627	PC5/PerCP 670-727	PC7 755-811	APC 656-666	A-A700 702-725	A-A750 755-811	PB 450 425-470	KO 525 505-545	V610 605-627	DAPI 430-470	Hoechst Red 660-690
1	B4-NUV2	✓	✓	✓		✓	✓							✓	✓
2	B5-R3-NUV2	✓	✓	✓	✓	✓	✓	✓	✓	✓				✓	✓
3	B5-R3-V3-NUV2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Num	Configuration	Blue 488 nm			Violet 405 nm				YG 561 nm				NUV 375 nm	
		SSC 488	FITC 505-545	PC5/PerCP 670-727	PB 450 425-470	KO 525 505-545	V610 605-627	V660 656-666	PE 565-607	ECD 605-627	PC5 670-727	PC7 755-811	DAPI 430-470	Hoechst Red 660-690
4	B2-Y4-NUV2	✓	✓	✓					✓	✓	✓	✓	✓	✓
5	B2-Y4-V4-NUV2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Figure 1. CytoFLEX S configurations with a near UV laser

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For staining of blood samples:

- Prewash 1 mL blood 3 times with PBS (1:4). Centrifuge and aspirate supernatant.
- Resuspend cells in 1 mL PBS + 0.2 % FBS.
- Incubate 100 µL blood with appropriate amount of antibody for 10-15 min at room temperature in the dark.
- Lyse with 1 mL VersaLyse Lysing Solution and fix (1 % paraformaldehyde final) for 10-15 min at room temperature in the dark.
- Wash 1 time with 3 mL PBS
- Resuspend in 1 mL PBS + 1 % paraformaldehyde

Fluorochrome	3 Laser	375 Config.1	375 Config.2
FITC	CD8	CD3	CD8
PE	CD16/CD56	CD16/CD56	CD16/CD56
ECD	CD19	CD19	CD19
PC5.5	CD38	CD38	CD4
PC7	CD25	CD25	CD25
APC	CD45RA	CD45RA	-
APC-A700	CD14	CD14	-
APC-A750	CD4	CD4	-
Pacific Blue	HLA-DR	HLA-DR	HLA-DR
Krome Orange	CD45	CD45	CD45
BV605	CD5	CD5	CD5
BV650	CD20	-	CD20
BV780	CD3	-	-
DAPI/BUV395	-	CD8	CD3
Hoescht/BUV661	-	CD11c	CD11c
PerCP-Cy5.5	-	-	CD14

DAPI/Hoescht staining protocol:

- Prewash 1 mL blood 3 times with PBS (1:4). Centrifuge and aspirate supernatant.
- Resuspend cells in1mL PBS + 0.2 % FBS.
- Incubate 100 µL blood with appropriate amount of viability dye for 10-15 min at room temperature in the dark.
- Lyse with 1 mL VersaLyse Lysing Solution for 10-15 min at room temperature in the dark.
- Wash 1 time with 3 mL PBS
- Resuspend in 1 mL PBS

Spherotech 8 Peak Rainbow Beads were used for resolution studies.

Results

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

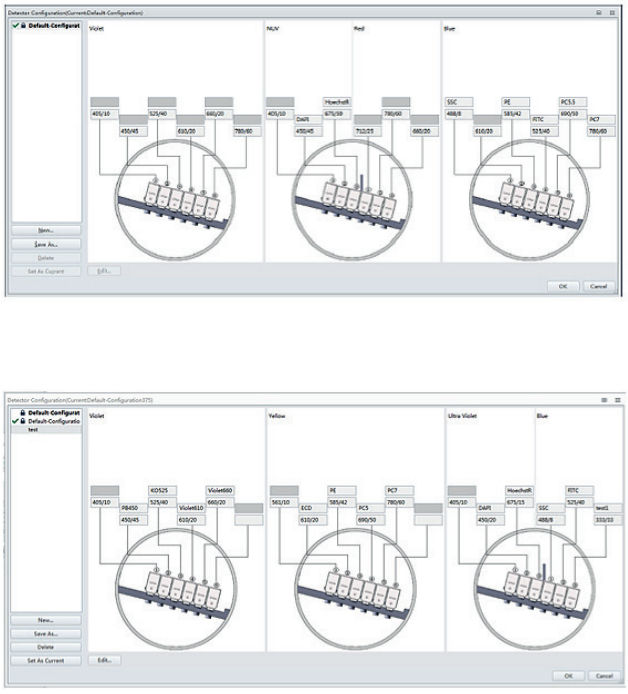

Figure 2. The detector configurations for CytoFLEX S with near UV laser

Common fluorochromes and fluorescent proteins can be detected on the CytoFLEX S. Importantly the near UV laser allows for the detection of BUV dyes, DAPI and Hoescht.

Commonly used Fluorescent Dyes	Laser	Fluorescent Channel	B78558	B78561	B78559	B78560	B78557
			6	8	10	12	13
DAPI, Hoechst Blue	375 nm	450/45 BP	✓	✓	✓	✓	✓
Hoechst Red		675/30 BP	✓	✓	✓	✓	✓
Pacific Blue dye, V450, eFluor 450, BV421	405 nm	450/45 BP				✓	✓
Krome Orange, AmCyan, V500, BV510		525/40 BP				✓	✓
BV605, Qdot 605		610/20 BP				✓	✓
BV650, Qdot 655		660/20 BP				✓	✓
FITC, Alexa Fluor 488, CFSE, Fluo-3	488 nm	525/40 BP	✓	✓	✓	✓	✓
PE, PI		585/42 BP	✓	✓	✓	✓	✓
ECD, PE-Texas Red, PE-CF594, PI		610/20 BP	✓		✓		✓
PC5.5, PC5, PerCP, PerCP-Cy5.5, PI		690/50 BP	✓		✓		✓
PC7		780/60 BP			✓		✓
PE, PI	561 nm	585/42 BP		✓		✓	
ECD, PE-Texas Red, PE-CF594, PI		610/20 BP		✓		✓	
PC5.5, PC5, PerCP, PerCP-Cy5.5, PI		690/50 BP		✓		✓	
PC7		780/60 BP		✓		✓	
APC, Alexa Fluor 647, eFluor 660	638 nm	660/20 BP			✓		✓
APC-A700, Alexa Fluor 700		712/25 BP			✓		✓
APC-A750, APC-Cy7, APC-H7, APC-eFluor 780		780/60 BP			✓		✓

Figure 3: Common fluorochromes for the CytoFLEX S with near UV laser

To demonstrate the functionality and sensitivity of the CytoFLEX S system, Spherotech 8 peak rainbow bead data was collected on two system configurations containing the near UV laser.

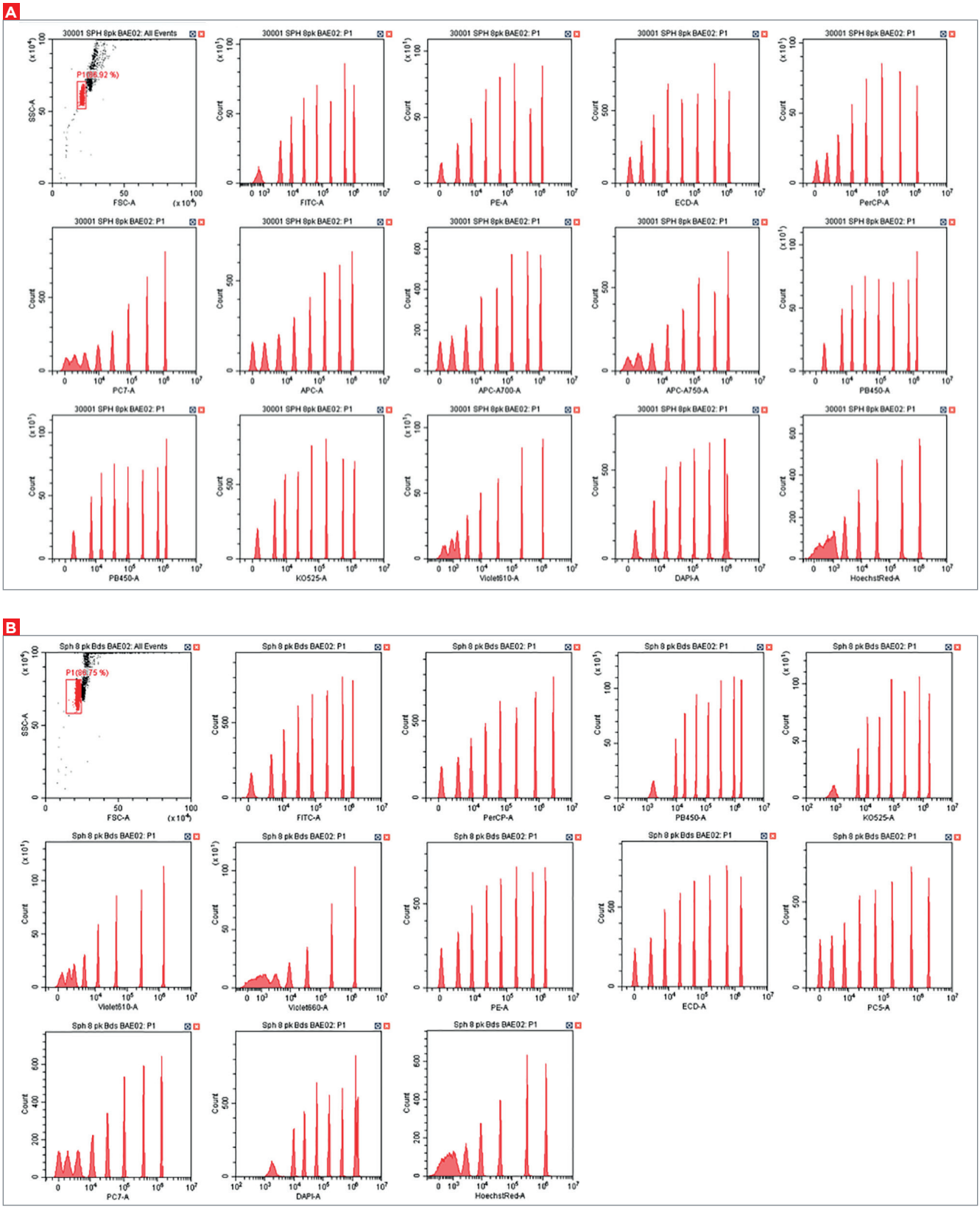

Figure 4. Resolution of Spherotech Rainbow Calibration Particles.

A) Data collected on configuration 1 which contains blue, red, violet and near UV lasers (B5-R3-V3-NUV2).
B) Data collected on configuration 2 which contains blue, yellow, violet and near UV lasers (B2-Y4-V4-NUV2).

To demonstrate the usability of the near UV laser for Hoescht and DAPI staining, blood samples were stained with both dyes. As shown in Figure 4, dead cells were easily identifiable with both DAPI and Hoescht. Importantly, dead cells were separated from live cells by at least 1.5 decades.

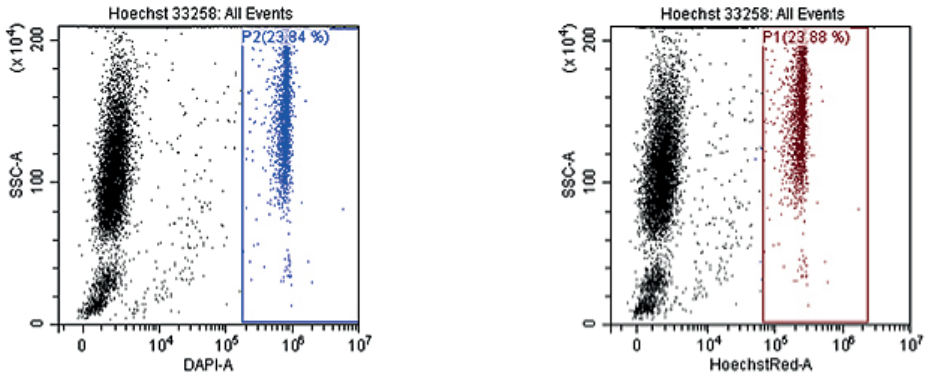

Figure 5. Viability as determined by DAPI and Hoescht. Blood samples were stained with DAPI and Hoescht and run on configuration 2 of CytoFLEX S as described above.

Brilliant UV fluorochromes (BUV) can be utilized when designing high complexity cocktails and give additional flexibility in panel design. To this end, a multicolor panel was designed using two BUV dyes and run on the CytoFLEX S.

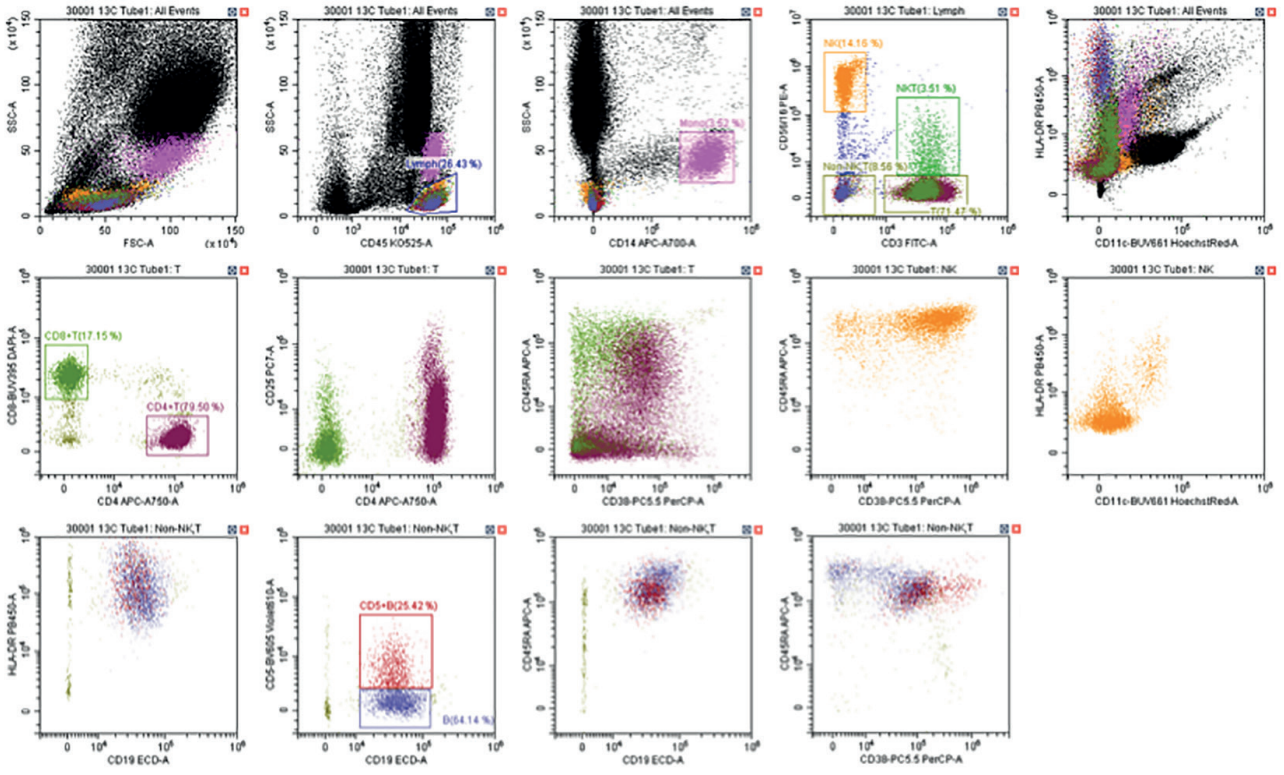

Figure 6. The CytoFLEX S 375 can detect simultaneously 13 colors, including two dyes in the near UV channels (DAPI and Hoechst Red). CD8-BUV395 positive cells were easily identified from negative cells. Similarly, NK cell activation was evaluated by CD11c BUV-661 staining.

Lastly to further evaluate the BUV portfolio of dyes on the CytoFLEX S, single color experiments were performed on whole blood samples using the appropriate bp filter on the HoeschtRed Channel. The results are shown in Figure 7.

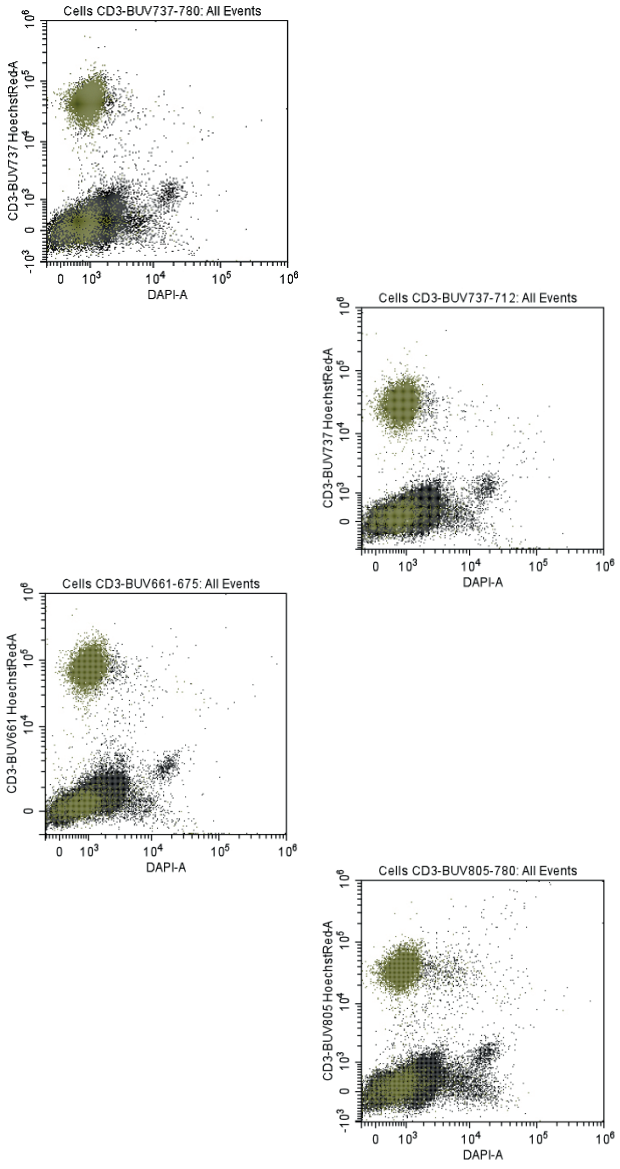

Figure 7. Whole blood stained with antibodies with BUV661, BUV737, and BUV805. Excellent separation between positive and negative samples is observed with all the BUV dyes.

Conclusion

CytoFLEX S with the near UV laser clearly demonstrates the capability to resolve samples when using Hoescht and DAPI stains. Similarly, the excellent resolution of samples stained with brilliant UV dyes demonstrates the functionality of the CytoFLEX S with this family of dyes. High sensitivity and high performance are maintained in the CytoFLEX S design.

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