

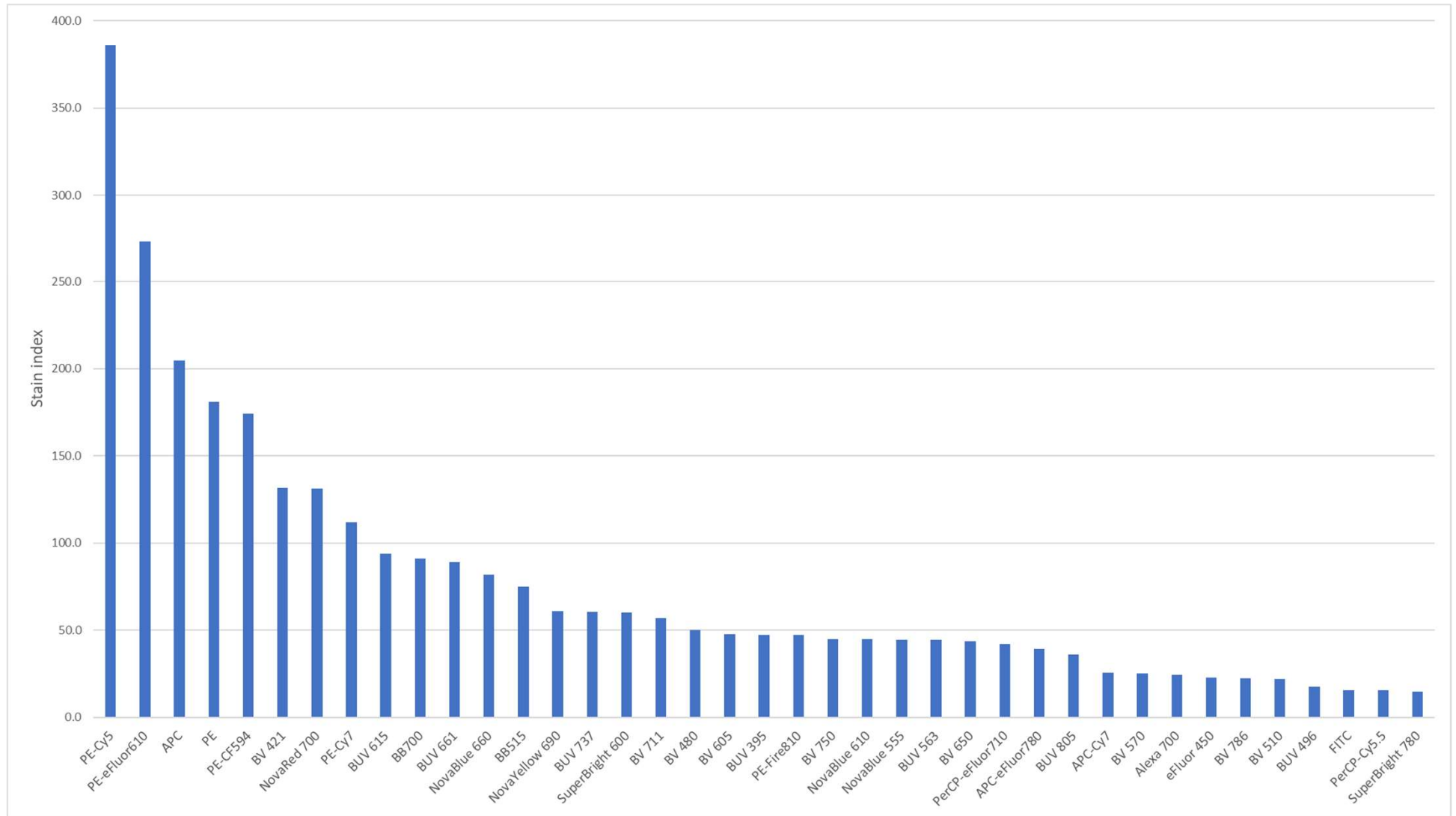
# Bigfoot: Laser and filter configuration

Laser	Band Pass	Primary Detector	Laser	Band Pass	Primary Detector
349 nm 100 mW	387/11	BUV 395	488 nm 125 mW	507/19	FITC, Alexa 488, BB515, GFP
	420/10			549/15	YFP
	434/17	Live/Dead blue		583/30	
	455/14	DAPI, Hoechst blue, Zombie UV		615/24	BB 630
	473/15			670/30	PerCP, BB 660
	507/19	BUV 496, Live/Dead aqua		720/60	PerCP-Cy5.5**, PerCP-e710, BB700
	549/15		750LP	BB 790	
	575/15	BUV 563	561 nm 120 mW	575/15	PE, DsRed
	615/24	BUV 615		589/15	tdTomato
	670/30	BUV 661		605/15	PE-CF594, PE-Dazzle594, PE-e610, mCherry
	728/40	BUV 737		625/15	PE-Texas red, PI*, 7-AAD*
750LP	BUV 805	685/15		PE-Cy5	
420/10	BV 421, Alexa 405	700/13		NovaYellow 700, PE-Cy5.5**	
434/17	SB 436, BFP	720/24			
455/14	eFluor 450, Pacific Blue	760/50		PE-Cy7	
473/15	BV 480	800/12		PE-Fire810	
507/19	BV 510, eFluor 506, V500	832/37			
549/15		860LP			
405 nm 100 mW	575/15	BV 570	640 nm 100 mW	670/30	APC, Alexa 647, Live/Dead far red, DRAQ7
	615/24	BV 605, SB 600		700/13	APC-Cy5.5, DRAQ5
	661/20	BV 650, SB 645		720/24	Alexa 700
	710/20	BV 711, SB 702		760/50	APC-Cy7, Zombie NIR
	747/33	BV 750		770LP	APC-H7, APC-Fire750, Live/Dead near-IR
	770LP	BV 786, SB 780			

\*PI and/or 7-AAD may be problematic depending on the panel

\*\*Do not combine PerCP-Cy5.5 and PE-Cy5.5

# Bigfoot: Ranking of fluorochrome brightness



Stain indices were calculated by staining human PBMCs with anti-human CD4 (SK3 clone) conjugated to each of the fluors indicated. Voltage settings were optimized the manufacturer. Data recorded in spectral mode.

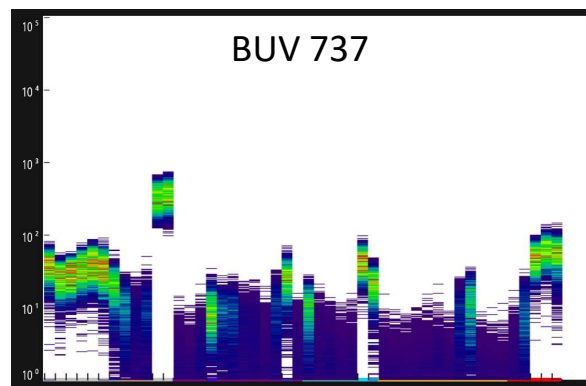
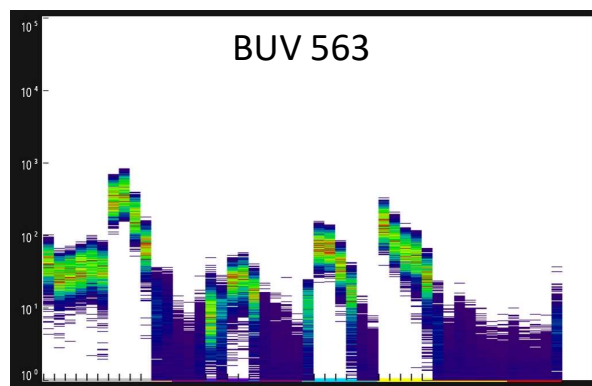
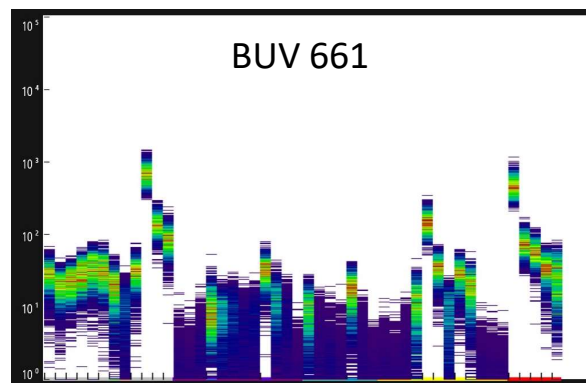
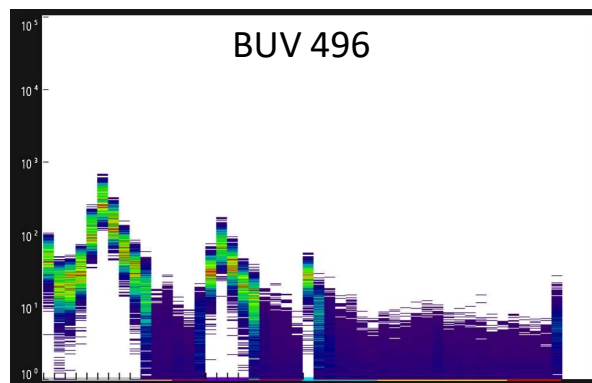
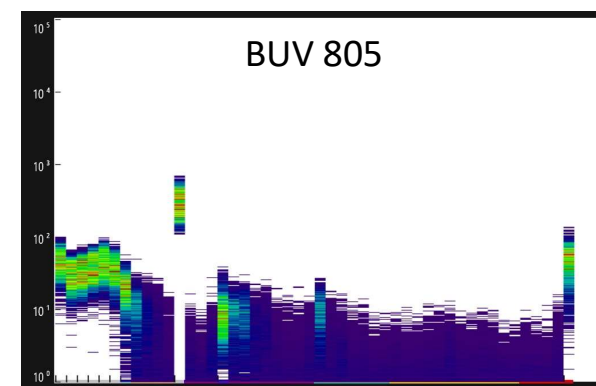
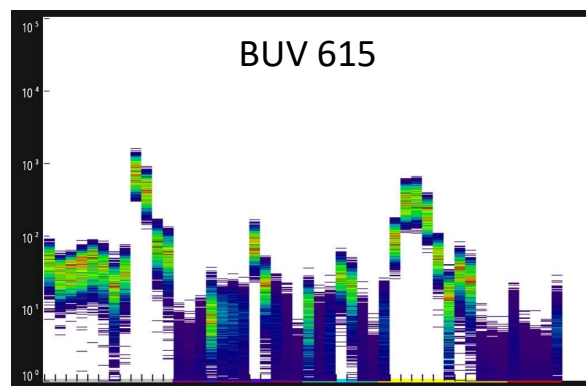
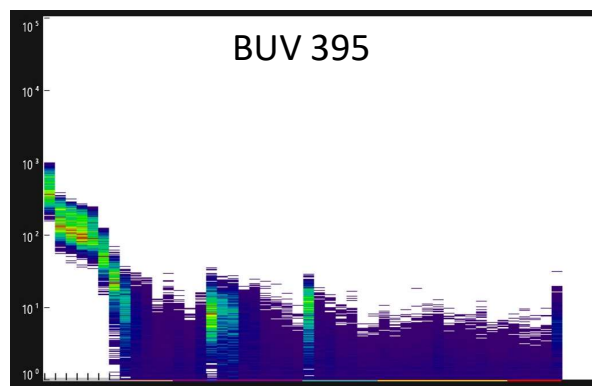
# Bigfoot: Spillover spread matrix (SSM)

	BUV 395	BUV 496	BUV 563	BUV 615	BUV 661	BUV 737	BUV 805	BV 421	eFluor 450	BV 480	BV 510	BV 570	SB 600	BV 650	BV 711	BV 750	SB 780	FITC	NB 555	NB 610	PerCP-e710	PE	PE-e610	PE-Cy5	NV 690	PE-Cy7	APC	NR 700	APC-e780	sum
BUV 395		0.76	0.31	0.11	0.02	0.00	0.00	0.09	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.06	0.01	0.11	1.68
BUV 496	0.49		0.86	0.67	0.66	0.20	0.15	0.17	0.28	0.85	1.00	0.77	0.47	0.19	0.06	0.00	0.06	0.17	0.38	0.18	0.00	0.45	0.53	0.00	0.00	0.00	0.35	0.05	0.00	8.99
BUV 563	0.37	0.38		1.23	1.18	0.49	0.33	0.10	0.08	0.50	0.61	0.66	0.46	0.25	0.09	0.05	0.03	0.13	0.49	0.53	0.18	0.93	1.02	0.48	0.23	0.07	0.63	0.10	0.06	11.67
BUV 615	0.29	0.22	0.23		1.99	0.84	0.75	0.07	0.00	0.00	0.03	0.24	0.70	0.47	0.28	0.18	0.04	0.10	0.09	0.63	0.25	0.44	1.56	0.71	0.44	0.26	1.11	0.15	0.05	12.11
BUV 661	0.28	0.19	0.11	0.39		1.19	1.06	0.06	0.00	0.00	0.00	0.00	0.18	0.48	0.38	0.29	0.07	0.00	0.00	0.31	0.29	0.07	0.41	0.75	0.41	0.27	1.85	0.55	0.50	10.08
BUV 737	0.48	0.33	0.14	0.08	0.33		2.45	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.62	0.25	0.00	0.00	0.00	0.56	0.00	0.00	0.10	0.25	0.35	0.28	0.43	0.87	8.10
BUV 805	0.62	0.36	0.15	0.17	0.00	0.14		0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.15	0.05	0.98	3.12	
BV 421	0.07	0.61	0.20	0.07	0.00	0.03	0.04		0.96	1.13	0.98	0.38	0.23	0.07	0.00	0.05	0.07	0.00	0.00	0.07	0.00	0.21	0.13	0.00	0.00	0.00	0.00	0.02	0.04	5.38
eFluor 450	0.00	0.33	0.21	0.00	0.00	0.00	0.00	0.54		1.65	1.40	0.72	0.35	0.00	0.00	0.09	0.09	0.00	0.00	0.12	0.00	0.35	0.25	0.00	0.00	0.00	0.00	0.10	0.10	6.12
BV 480	0.11	0.76	0.41	0.31	0.31	0.08	0.08	0.29	0.48		1.96	1.01	0.72	0.43	0.20	0.14	0.10	0.21	0.30	0.22	0.03	0.54	0.43	0.07	0.04	0.00	0.17	0.05	0.00	9.45
BV 510	0.10	1.08	0.70	0.73	0.89	0.39	0.27	0.29	0.46	1.55		1.42	1.31	0.79	0.50	0.37	0.11	0.00	0.18	0.34	0.05	0.70	0.77	0.09	0.06	0.00	0.43	0.12	0.07	13.77
BV 570	0.00	0.26	0.49	0.74	0.84	0.26	0.21	0.59	0.56	0.94	1.03		1.82	1.02	0.61	0.39	0.09	0.00	0.41	0.67	0.19	1.21	1.39	0.66	0.40	0.15	0.43	0.16	0.00	15.51
SuperBright 600	0.00	0.00	0.27	0.87	1.19	0.64	0.49	0.34	0.39	0.51	0.48	0.68		1.47	0.81	0.71	0.31	0.00	0.12	0.70	0.27	0.52	1.51	0.79	0.47	0.31	0.67	0.24	0.08	14.84
BV 650	0.07	0.00	0.00	0.56	1.80	0.94	0.86	0.49	0.47	0.65	0.41	0.25	1.32		1.34	1.11	0.51	0.00	0.00	0.44	0.29	0.20	0.88	0.84	0.52	0.32	1.43	0.69	0.27	16.67
BV 711	0.17	0.00	0.09	0.00	0.52	1.38	1.42	0.61	0.54	0.73	0.48	0.23	0.00	0.42		1.89	0.86	0.00	0.00	0.06	0.45	0.11	0.14	0.26	0.36	0.34	0.73	0.92	0.86	13.57
BV 750	0.21	0.00	0.00	0.00	0.00	1.05	1.60	0.55	0.55	0.78	0.66	0.28	0.12	0.00	0.90		1.17	0.00	0.00	0.00	0.31	0.16	0.10	0.00	0.10	0.29	0.21	0.24	0.86	10.13
SuperBright 780	0.26	0.00	0.00	0.00	0.00	0.48	2.11	0.70	0.83	1.27	0.56	0.21	0.00	0.03	0.23	1.92		0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.28	0.15	0.09	1.33	10.60
FITC	0.00	0.27	0.25	0.18	0.00	0.00	0.00	0.01	0.00	0.13	0.65	0.51	0.21	0.09	0.00	0.00	0.00	0.63	0.91	0.53	0.11	0.51	0.36	0.38	0.13	0.00	0.09	0.08	0.00	5.39
NovaBlue 555	0.00	0.00	0.26	0.20	0.00	0.02	0.00	0.02	0.00	0.00	0.51	0.58	0.23	0.00	0.10	0.00	0.00	0.00	0.63	0.83	0.20	0.89	0.71	0.58	0.26	0.00	0.21	0.13	0.00	6.36
NovaBlue 610	0.00	0.00	0.00	0.46	0.30	0.11	0.00	0.06	0.00	0.00	0.26	0.58	0.39	0.39	0.06	0.00	0.37	0.44	0.57	0.46	0.57	0.46	1.30	0.99	0.65	0.28	0.46	0.21	0.00	8.34
PerCP-eFluor710	0.00	0.00	0.00	0.00	0.60	0.99	0.91	0.25	0.00	0.00	0.00	0.00	0.55	1.89	1.45	0.70	0.00	0.00	0.24	0.09	0.26	0.62	0.86	0.62	0.86	0.62	0.83	0.90	0.95	12.71
PE	0.00	0.11	0.35	0.37	0.37	0.12	0.08	0.15	0.05	0.25	0.46	0.89	0.77	0.39	0.26	0.11	0.02	0.15	0.43	0.69	0.29	1.14	0.62	0.36	0.19	0.25	0.11	0.00	8.97	
PE-eFluor610	0.00	0.00	0.14	0.39	0.44	0.16	0.09	0.08	0.01	0.05	0.12	0.37	0.74	0.44	0.30	0.13	0.03	0.14	0.22	1.08	0.34	0.63	0.93	0.48	0.22	0.32	0.15	0.00	7.99	
PE-Cy5	0.03	0.00	0.03	0.07	0.59	0.33	0.28	0.09	0.00	0.00	0.13	0.18	0.44	0.56	0.30	0.10	0.02	0.14	0.69	0.73	0.29	0.62	0.93	0.51	1.03	0.41	0.29	0.00	8.80	
NovaYellow 690	0.00	0.00	0.00	0.08	0.29	0.23	0.09	0.08	0.00	0.00	0.00	0.00	0.14	0.55	0.15	0.03	0.00	0.23	0.48	0.80	0.61	0.77	1.06	0.67	0.96	0.76	0.38	0.00	8.37	
PE-Cy7	0.07	0.00	0.06	0.13	0.00	0.19	0.69	0.06	0.00	0.00	0.31	0.15	0.11	0.29	0.50	0.41	0.00	0.26	0.32	0.39	0.51	0.49	0.36	0.31	0.15	0.08	0.62	0.00	6.46	
APC	0.00	0.00	0.00	0.07	0.69	0.40	0.28	0.06	0.00	0.00	0.00	0.15	0.47	0.34	0.27	0.07	0.00	0.00	0.37	0.26	0.09	0.35	1.01	0.46	0.29	0.50	0.50	0.00	6.63	
NovaRed 700	0.00	0.00	0.00	0.00	0.27	0.36	0.25	0.04	0.00	0.00	0.00	0.00	0.00	0.25	0.54	0.36	0.09	0.00	0.00	0.21	0.23	0.08	0.20	0.57	0.59	0.32	1.54	0.69	6.57	
APC-eFluor780	0.10	0.00	0.00	0.00	0.35	0.25	0.75	0.01	0.00	0.00	0.00	0.00	0.00	0.23	0.13	0.35	0.45	0.12	0.00	0.18	0.14	0.00	0.17	0.48	0.23	0.67	0.81	0.29	5.70	

264.07

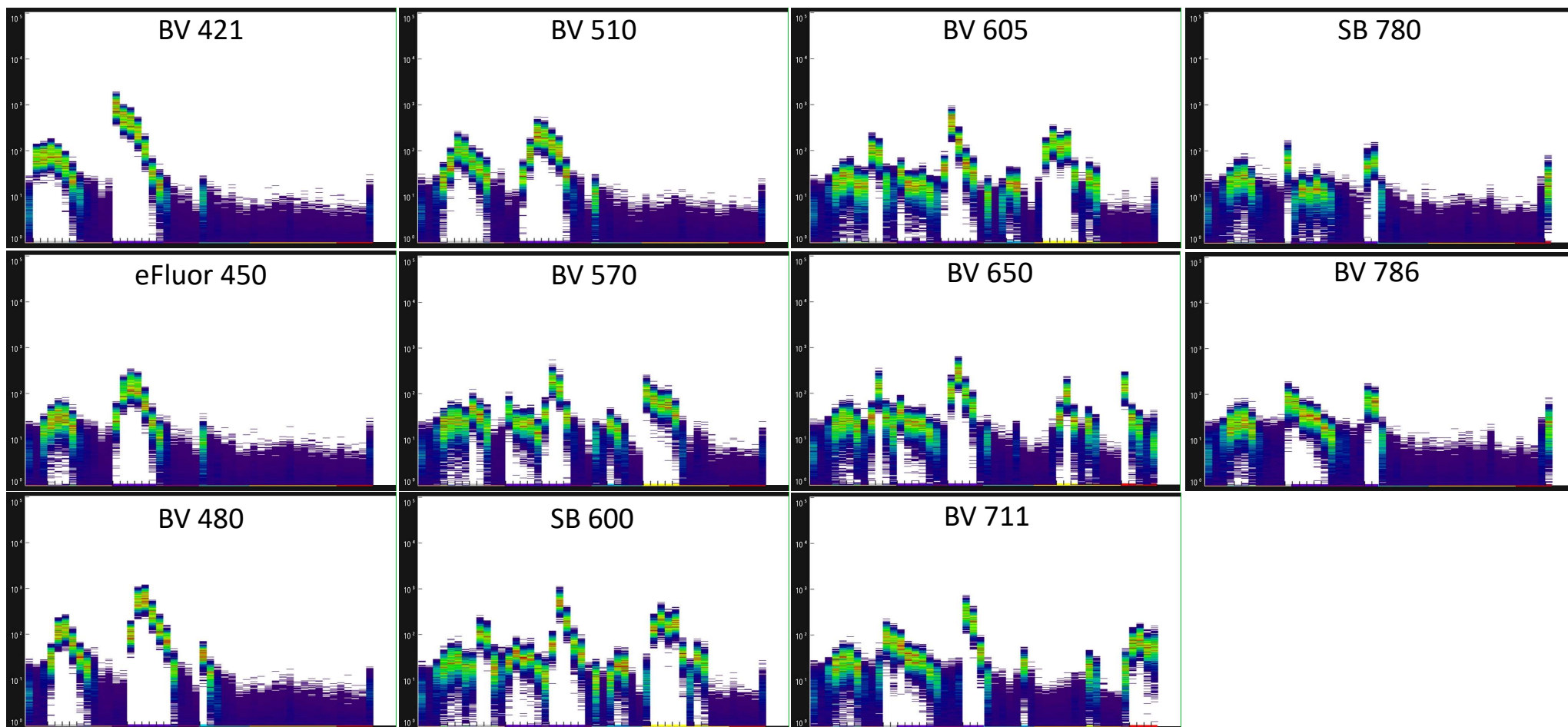
Spread matrix was calculated from CD4 single-stained human PBMCs using the method described in Nguyen, et al, *Cytometry A*. 2013 March; 83(3): 306-315. Voltage settings were optimized by the manufacturer. Data recorded in spectral mode.

# Bigfoot: Spectral profile of fluorochromes with 349 nm excitation



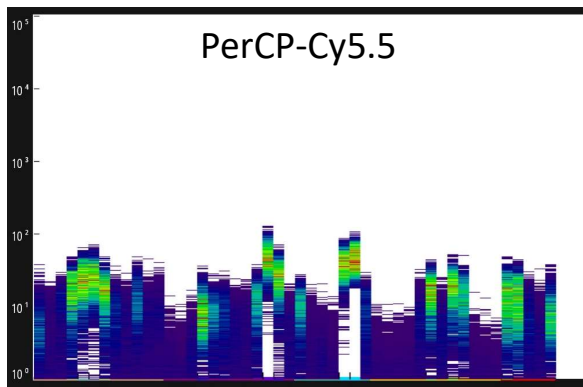
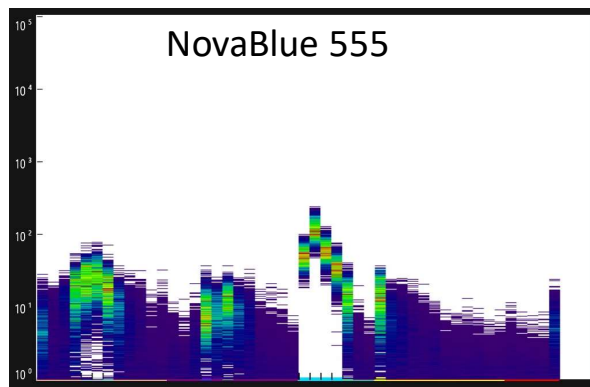
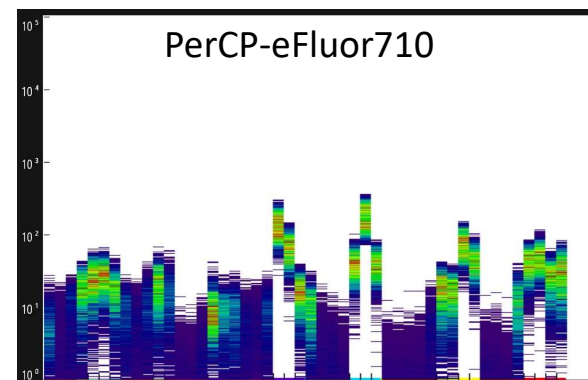
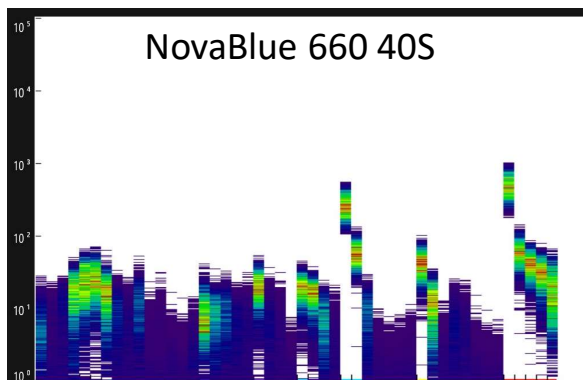
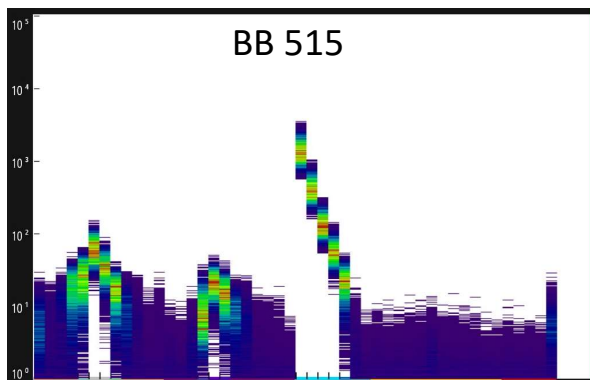
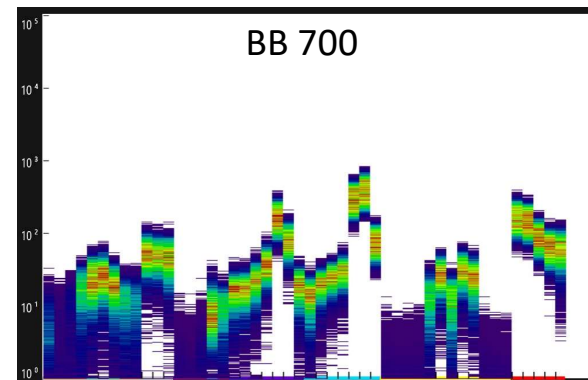
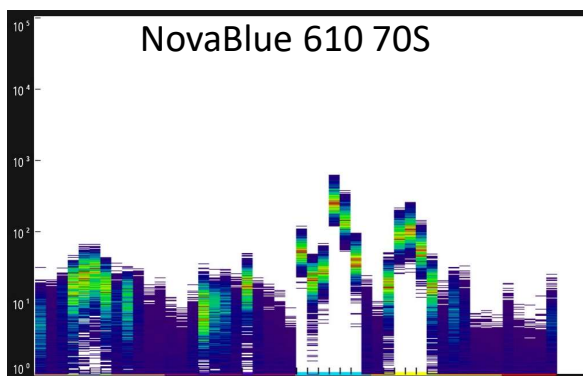
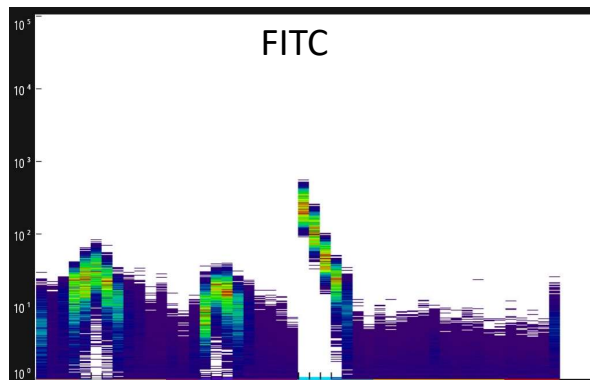
Stain indices were calculated by staining human PBMCs with anti-human CD4 (SK3 clone) conjugated to each of the fluors indicated. Voltage settings were optimized the manufacturer. Data recorded in spectral mode.

# Bigfoot: Spectral profile of fluorochromes with 405 nm excitation



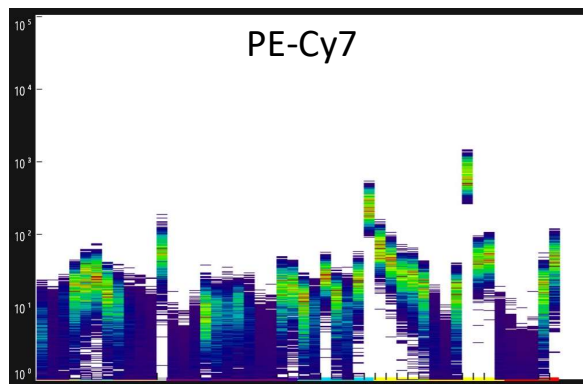
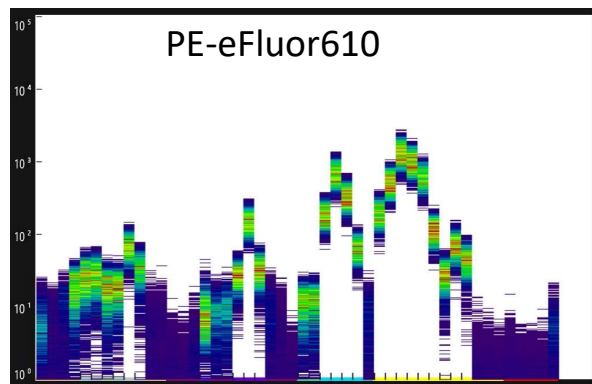
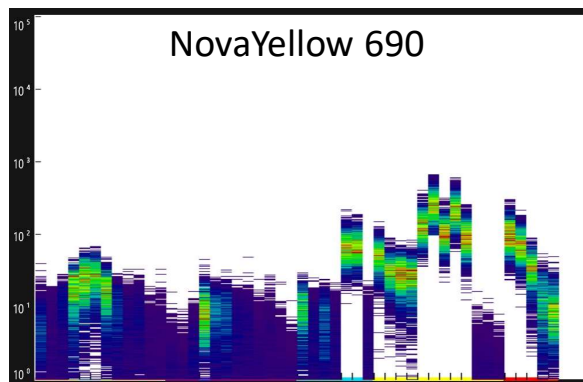
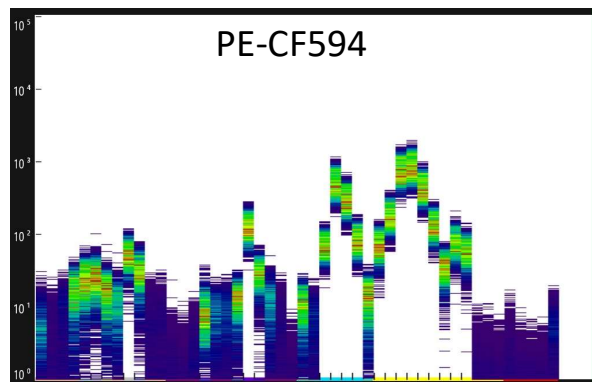
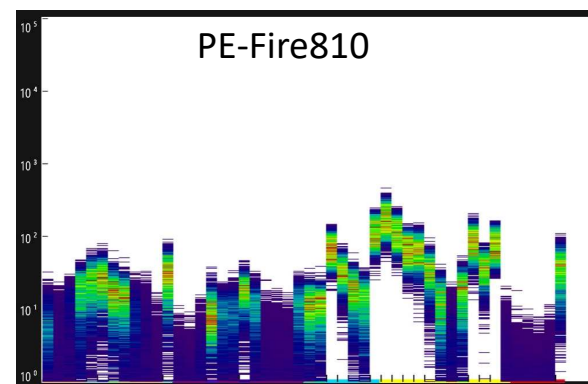
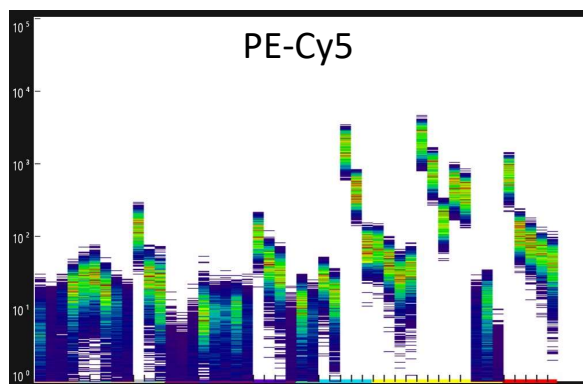
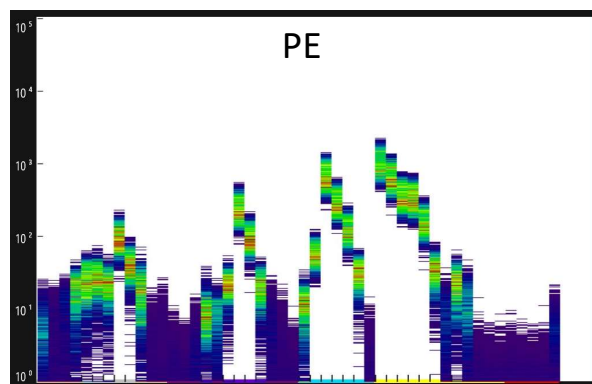
Stain indices were calculated by staining human PBMCs with anti-human CD4 (SK3 clone) conjugated to each of the fluors indicated. Voltage settings were optimized the manufacturer. Data recorded in spectral mode.

# Bigfoot: Spectral profile of fluorochromes with 488 nm excitation



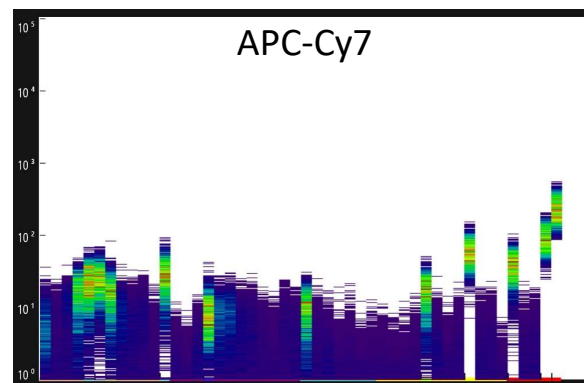
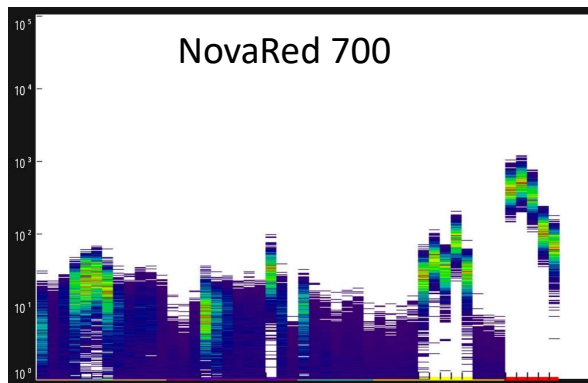
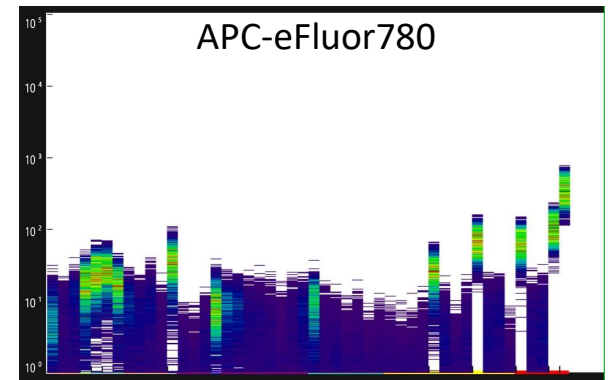
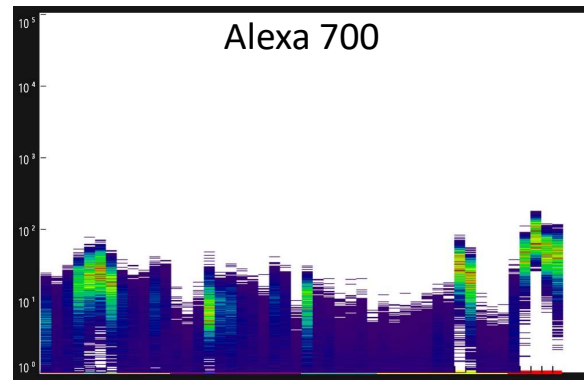
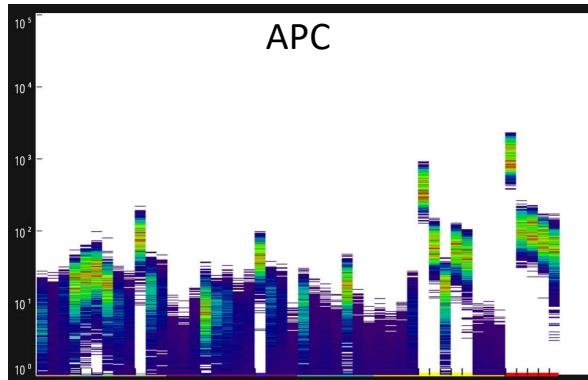
Stain indices were calculated by staining human PBMCs with anti-human CD4 (SK3 clone) conjugated to each of the fluors indicated. Voltage settings were optimized the manufacturer. Data recorded in spectral mode.

# Bigfoot: Spectral profile of fluorochromes with 561 nm excitation



Stain indices were calculated by staining human PBMCs with anti-human CD4 (SK3 clone) conjugated to each of the fluors indicated. Voltage settings were optimized the manufacturer. Data recorded in spectral mode.

# Bigfoot: Spectral profile of fluorochromes with 640 nm excitation



Stain indices were calculated by staining human PBMCs with anti-human CD4 (SK3 clone) conjugated to each of the fluors indicated. Voltage settings were optimized the manufacturer. Data recorded in spectral mode.