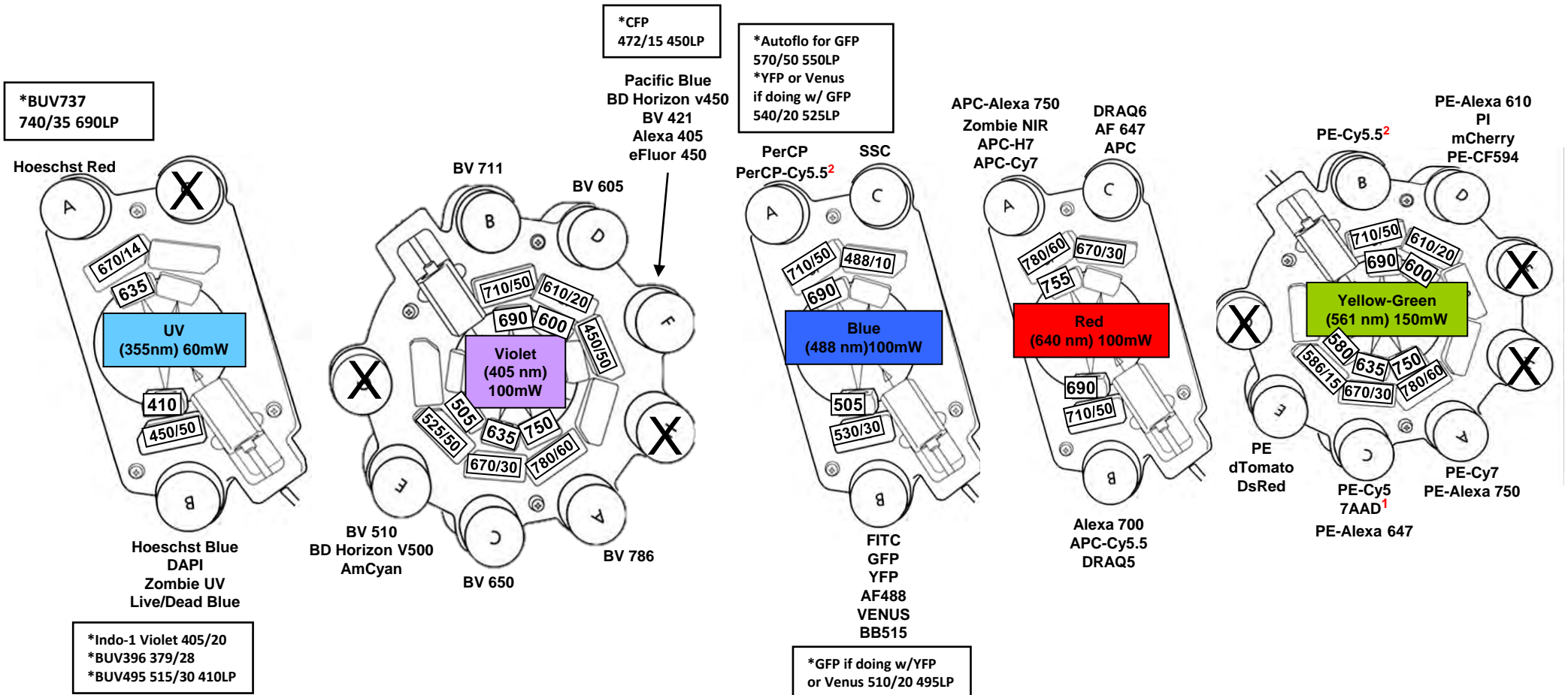


FACS Aria Fusion Configuration (5-Laser Aria)



Note: There may be other compatible fluorophores/dyes that are not listed here. Always check fluorochrome combinations prior to running an experiment!

*Denotes filter change

¹ 7AAD and PI may be problematic depending on your fluorochrome panel.

² PerCP-Cy5.5 and PE-Cy5.5 cannot be combined.

FACS Aria Fusion: Laser and filter configuration

Laser	Detector	Long Pass	Band Pass	Optimal fluors
355 nm 60 mW	B	410	450/50	DAPI, Hoechst blue, Zombie UV, Live/Dead blue
	A	635	670/14	Hoechst red
405 nm 100 mW	F	NA	450/50	BV 421, Pacific blue, eFluor 450, Alexa 405
	E	505	525/50	BV 510, V500, eFluor 506, AmCyan, Live/Dead aqua
	D	600	610/20	BV 605, SB 600
	C	635	670/30	BV 650, SB 645
	B	690	710/50	BV 711, SB 702
	A	750	780/60	BV 786, SB 780
488 nm 100 mW	B	505	530/30	FITC, Alexa 488, BB 515, GFP, YFP, Venus
	A	690	710/50	PerCP, PerCP-Cy5.5**, PerCP-e710, BB 700
561 nm 150 mW	E	580	586/15	PE, tdTomato, DsRed
	D	600	610/20	PE-CF594, PE-Dazzle594, PE-e610, mCherry, PI*
	C	635	670/30	PE-Cy5, 7-AAD*
	B	690	710/50	PE-Cy5.5**, NovaYellow 700
	A	750	780/60	PE-Cy7
637 nm 100 mW	C	665	670/30	APC, Alexa 647, DRAQ7, Live/Dead far-red
	B	690	710/50	Alexa 700, DRAQ5
	A	755	780/60	APC-Cy7, Zombie NIR, Live/Dead near-IR

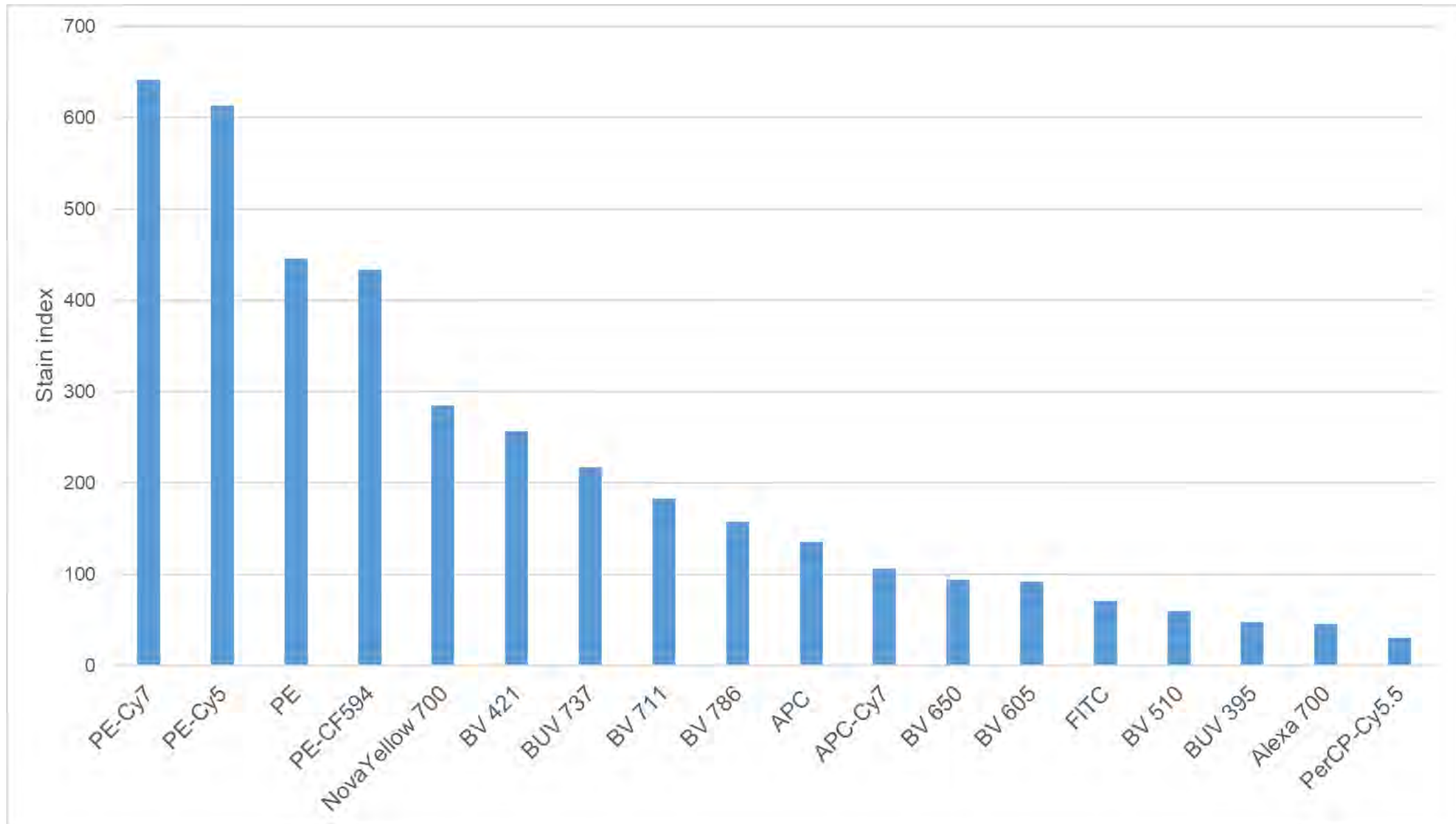
*PI and/or 7-AAD may be problematic depending on the panel

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

Common fluors requiring filter changes:

- BUV395: use 355 nm detector B with 379/28 BP
- BUV 737: use 355 nm detector A with 740/35 BP
- GFP + YFP: for GFP use 488 nm detector B with 510/20 BP, for YFP use 488 nm detector A with 540/20 BP

Aria 1 (Fusion): 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 by voltration of CD4 single-stained PBMCs.

Aria 1 (Fusion): Spillover spread matrix (SSM) and total spread matrix (TSM)

SSM

	BUV396	BUV737	BV421	BV510	BV605	BV650	BV711	BV786	FITC	PCP-Cy5.5	PE	PE-CF594	PE-Cy5	PE-Cy5.5	PE-Cy7	APC	Alexa 700	APC-Cy7	sum	
BUV 395		0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
BUV 737	0.00		0.09	0.00	0.00	0.22	0.62	0.80	0.00	2.20	0.00	0.00	0.15	0.61	0.60	0.28	3.11	0.86		9.53
BV 421	0.00	0.00		0.13	0.09	0.06	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.39
BV 510	0.00	0.26	0.04		0.99	0.82	0.64	0.60	0.00	0.19	0.32	0.38	0.00	0.00	0.00	0.28	0.83	0.00		5.36
BV 605	0.00	0.47	0.18	0.00		1.44	1.29	1.02	0.00	1.21	1.25	1.44	1.60	0.93	0.52	0.88	1.82	0.00		14.03
BV 650	0.00	0.56	0.22	0.00	0.88		1.78	1.27	0.00	1.10	0.33	0.52	1.47	0.84	0.44	1.96	3.30	0.38		15.04
BV 711	0.00	0.77	0.17	0.00	0.07	0.37		1.69	0.00	1.75	0.00	0.00	0.47	0.71	0.58	0.74	4.47	0.77		12.56
BV 786	0.00	0.34	0.26	0.00	0.08	0.13	0.32		0.00	0.20	0.00	0.00	0.01	0.10	0.39	0.00	0.52	0.47		2.81
FITC	0.00	0.00	0.00	0.00	0.12	0.01	0.00	0.03		0.18	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.50
PerCP-Cy5.5	0.00	0.43	0.04	0.00	0.00	0.52	0.90	0.86	0.00		0.00	0.00	0.86	0.94	0.60	1.05	2.34	0.44		8.97
PE	0.00	0.05	0.00	0.00	0.32	0.23	0.23	0.11	0.06	0.94		1.00	0.89	0.48	0.22	0.20	0.19	0.00		4.92
PE-CF594	0.00	0.13	0.00	0.00	0.41	0.34	0.37	0.19	0.00	1.90	0.73		1.65	0.91	0.54	0.38	0.51	0.00		8.07
PE-Cy5	0.00	0.21	0.05	0.00	0.11	0.40	0.68	0.26	0.00	3.92	0.17	0.13		1.28	0.75	1.42	1.71	0.27		11.33
NovaYellow 700	0.00	0.14	0.02	0.00	0.09	0.25	0.47	0.11	0.00	2.40	0.43	0.32	1.70		0.76	2.10	8.42	0.55		17.75
PE-Cy7	0.00	0.14	0.05	0.00	0.00	0.04	0.07	0.57	0.00	0.36	0.20	0.11	0.13	0.20		0.00	0.02	0.25		2.13
APC	0.00	0.26	0.00	0.00	0.07	0.35	0.35	0.23	0.00	0.98	0.00	0.10	1.76	0.70	0.46		2.90	0.49		8.64
Alexa 700	0.00	0.28	0.03	0.00	0.00	0.00	0.31	0.32	0.00	0.41	0.00	0.00	0.26	0.54	0.48	0.30		0.62		3.55
APC-Cy7	0.00	0.24	0.03	0.00	0.00	0.10	0.12	0.74	0.00	0.16	0.00	0.00	0.56	0.27	1.53	0.88	1.12			5.75
																				65.35

TSM

	BUV396	BUV737	BV421	BV510	BV605	BV650	BV711	BV786	FITC	PCP-Cy5.5	PE	PE-CF594	PE-Cy5	PE-Cy5.5	PE-Cy7	APC	Alexa 700	APC-Cy7	sum	
BUV 395	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUV 737	0	0	0	0	0	19	56	68	0	192	0	0	15	50	52	28	260	78		818
BV 421	0	0	0	10	14	0	7	0	0	0	6	0	0	0	0	0	0	0	0	37
BV 510	0	15	0	0	54	41	34	32	7	14	18	20	0	8	0	21	49	0		312
BV 605	0	27	0	0	0	83	83	59	0	75	69	90	96	53	32	52	114	0		835
BV 650	0	36	10	0	53	0	109	79	0	62	19	32	92	50	28	122	196	25		913
BV 711	0	71	17	0	12	35	0	157	0	160	0	0	41	71	53	70	461	68		1216
BV 786	0	30	21	0	9	9	31	0	0	20	0	0	0	0	35	0	44	44		243
FITC	0	0	0	0	9	0	0	7	0	14	0	0	0	0	0	0	0	0	0	30
PerCP-Cy5.5	0	23	0	0	6	28	49	46	0	0	0	0	43	50	35	55	118	24		479
PE	0	9	0	0	37	28	25	13	10	119	0	120	104	62	28	28	29	0		612
PE-CF594	0	18	0	0	52	42	51	22	0	237	90	0	206	117	66	44	65	0		1011
PE-Cy5	0	44	10	0	25	86	139	57	0	938	35	27	0	274	155	323	363	62		2539
NovaYellow 700	0	18	0	0	9	35	71	17	0	339	62	44	236	0	104	264	1092	69		2362
PE-Cy7	0	24	0	0	9	0	10	97	0	60	31	18	18	32	0	0	0	42		342
APC	0	27	0	0	9	31	34	24	0	93	0	11	170	66	41	0	276	41		824
Alexa 700	0	20	0	0	0	0	25	21	0	34	0	0	24	38	35	25	0	45		268
APC-Cy7	0	17	0	0	0	0	7	46	0	10	0	0	38	19	98	62	71	0		368

8437

Spread matrices were 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 voltration of CD4 single-stained PBMCs.