

Fortessa X20 5-18 Voltages and Brightness Index

- Mouse spleen cells were stained with 0.1 μg anti-CD4 (clone GK1.5) and run on the X20 at eight different voltages to compare the staining indexes between fluorophores

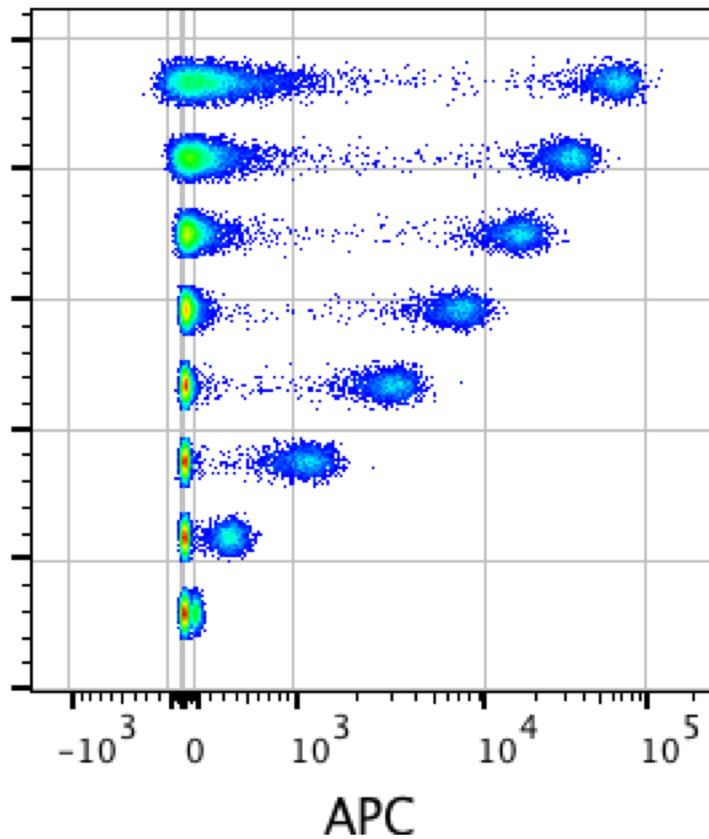
Recommended Voltages and Brightness

- These recommended voltages are a good starting point. If your single stain control is too bright, the voltage should be lowered. In general, increasing the voltage above the recommended settings has no benefit.

Laser	Laser	Filter	Fluorophore tested	Starting voltage
UV	355	379/28	BUV396	450
UV	355	450/50	DAPI	458
UV	355	515/30	BUV496	600
UV	355	740/35	BUV737	600
UV	355	820/60	BUV805	600
Violet	405	450/50	BV421	450
Violet	405	525/50	BV510	500
Violet	405	610/20	BV605	600
Violet	405	710/50	BV711	600
Violet	405	780/60	BV786	514
Blue	488	530/30	AF488	500
Blue	488	710/50	PerCP-Cy5.5	600
Yellow/Green	561	586/15	PE	500
Yellow/Green	561	610/20	PE-Dazzle595	450
Yellow/Green	561	780/60	PE-Cy7	450
Red	640	670/30	APC	500
Red	640	710/50	AF700	600
Red	640	780/60	APC-Cy7	600

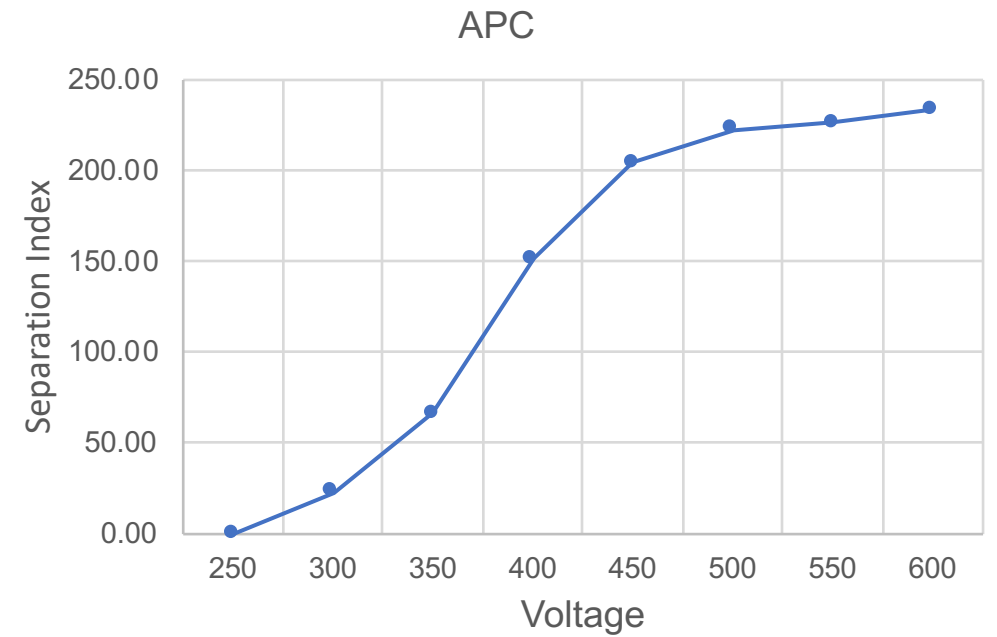
Fluorophore tested	Separation Index	Brightness Estimation
APC	223	Very Bright
PE	222	Very Bright
PE-Dazzle595	214	Very Bright
BV421	198	Very Bright
PE-Cy7	181	Bright
BV786	178	Bright
BV711	172	Bright
BV605	135	Moderate
PerCP-Cy5.5	113	Moderate
BUV737	104	Moderate
AF700	86	Dim
BV510	85	Dim
AF488	85	Dim
BUV396	71	Dim
APC-Cy7	71	Dim
Pacific Blue	61	Dim
BUV496	33	Very Dim
BUV805	30	Very Dim
DAPI	not tested	

APC



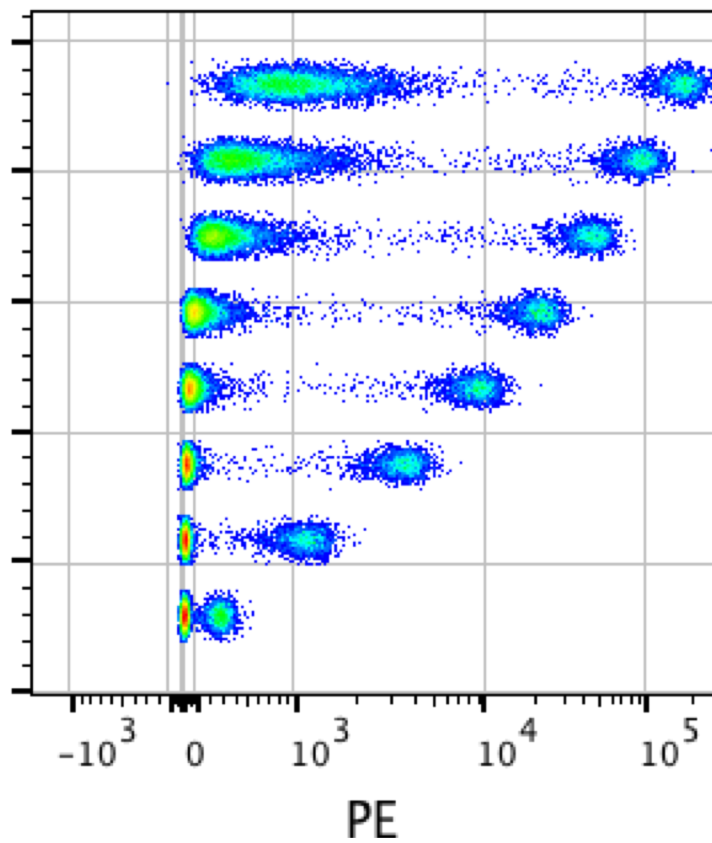
Red (640nm)

Voltage	SI
600	233.76
550	226.35
500	222.94
450	204.57
400	151.09
350	66.10
300	22.68
250	0.00



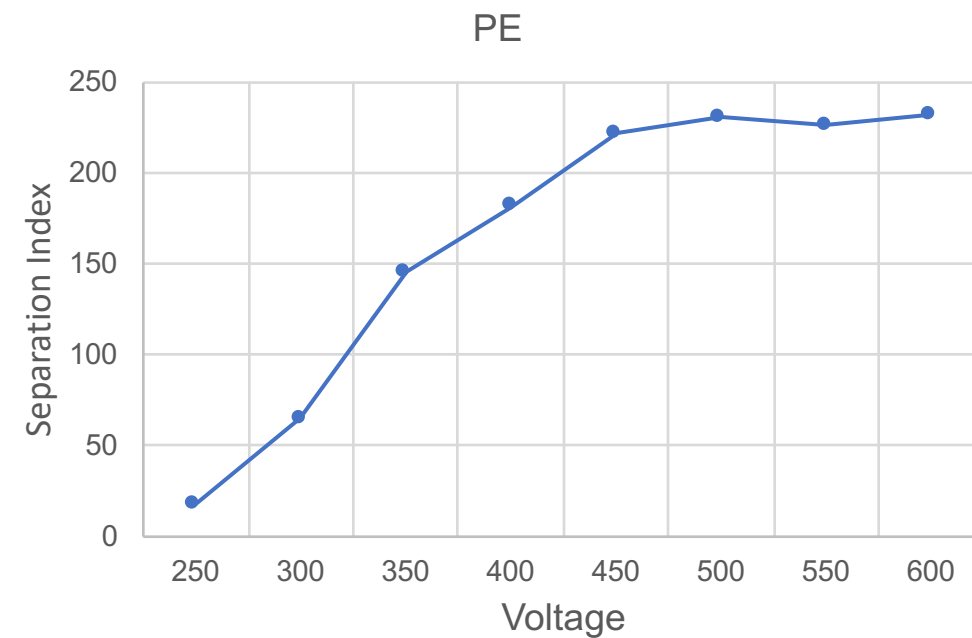
Very Bright

PE



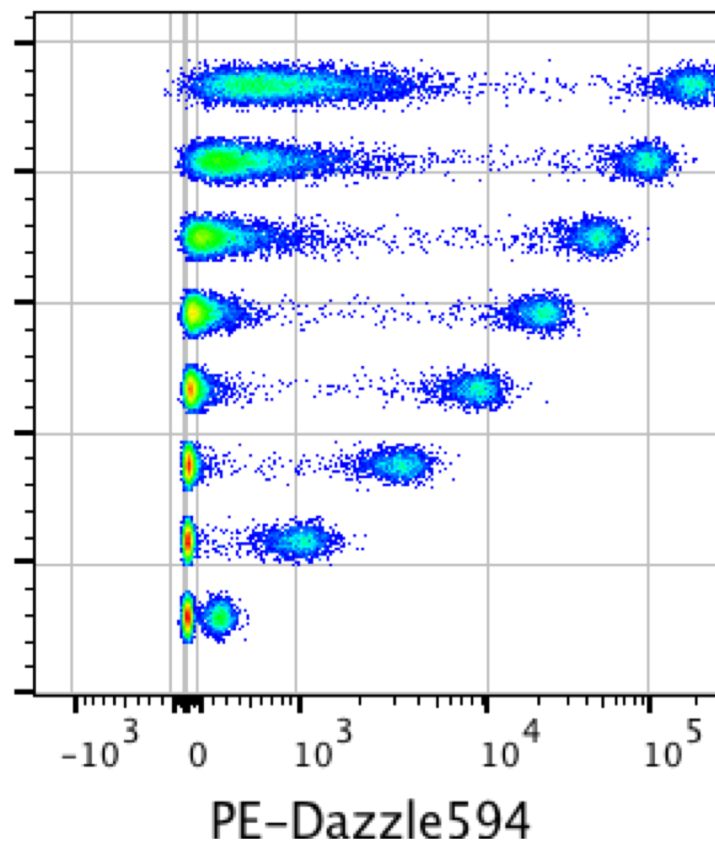
Yellow/Green (561nm)

Voltage	SI
600	232.35
550	225.99
500	230.91
450	221.56
400	181.26
350	145.37
300	65.05
250	18.03



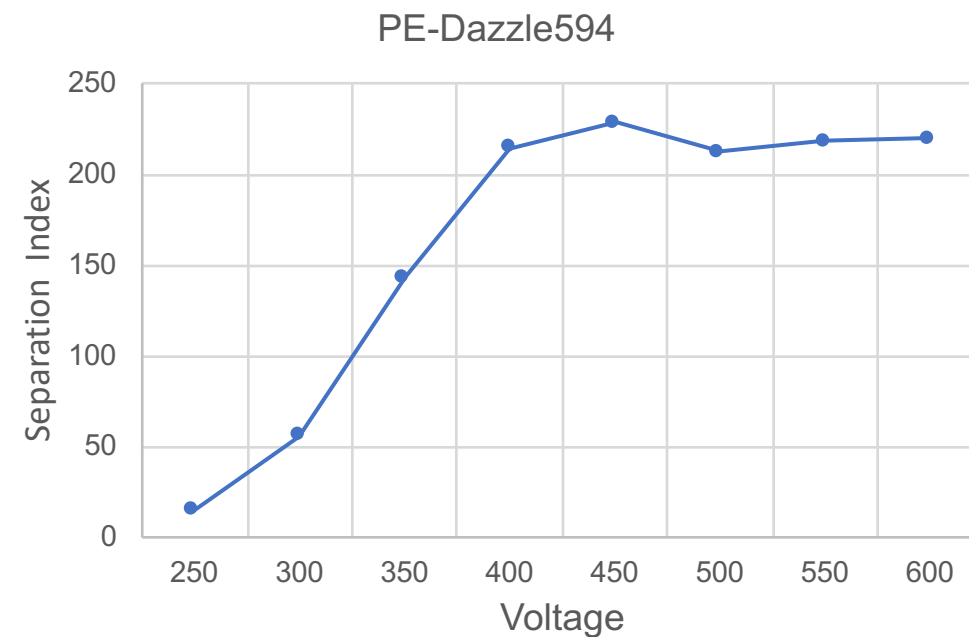
Very Bright

PE-Dazzle594



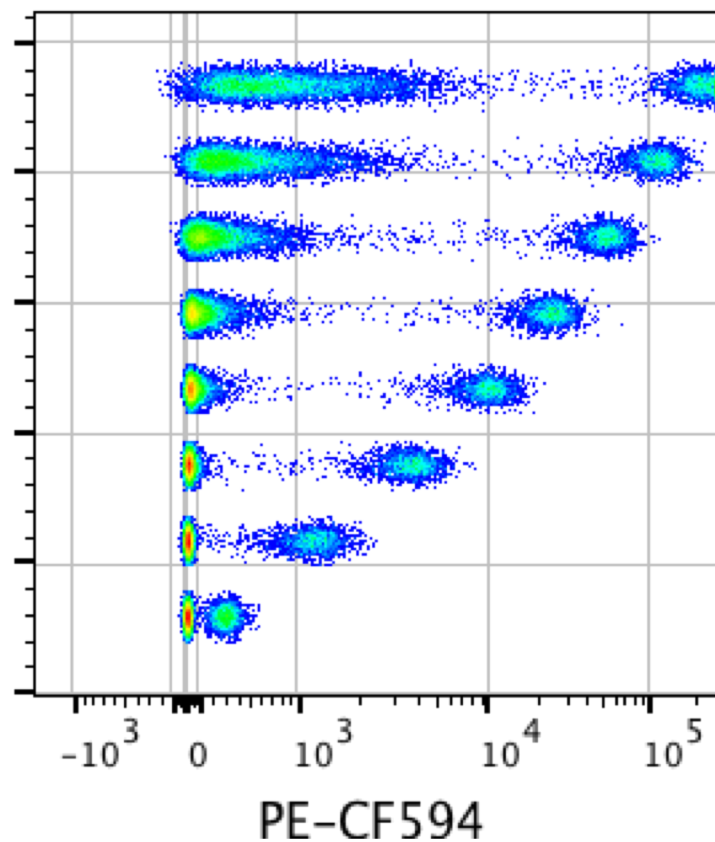
Yellow/Green (561nm)

Voltage	SI
600	219.11
550	217.90
500	212.39
450	228.57
400	214.12
350	143.07
300	55.57
250	15.07



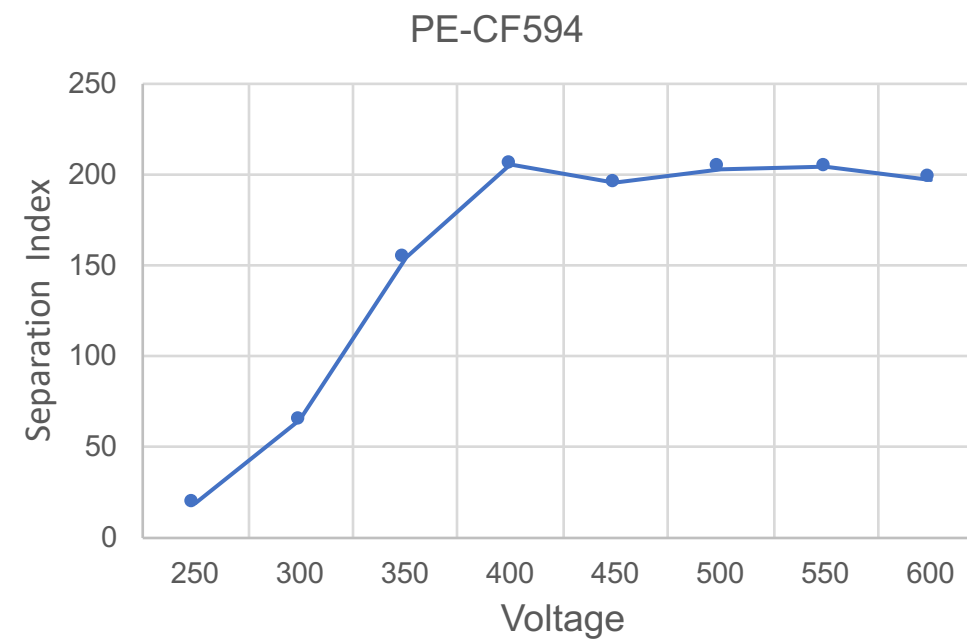
Very Bright

PE-CF594



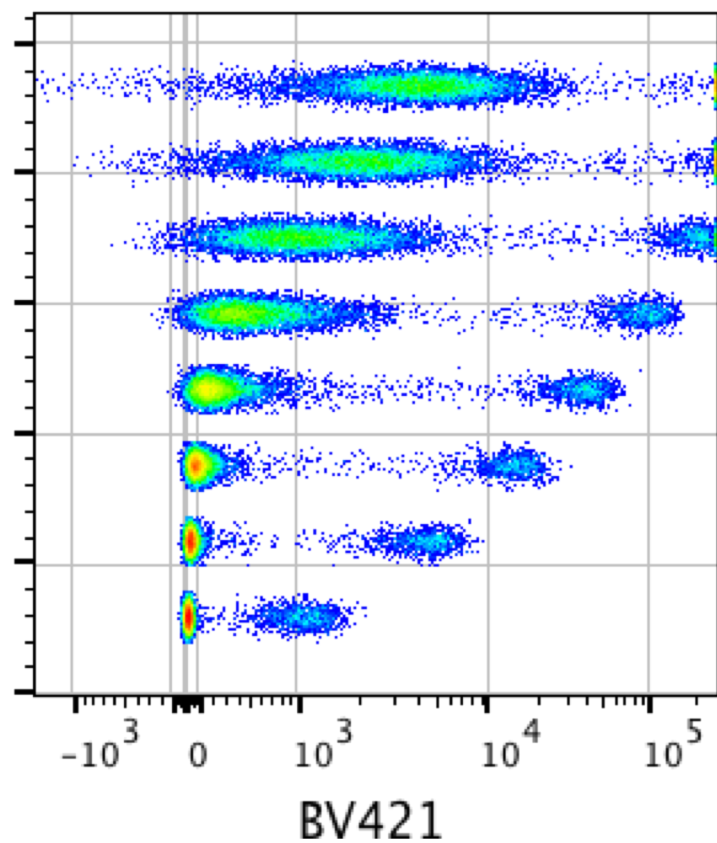
Yellow/Green (561nm)

Voltage	SI
600	197.67
550	204.27
500	203.47
450	195.17
400	205.62
350	153.83
300	64.36
250	19.08



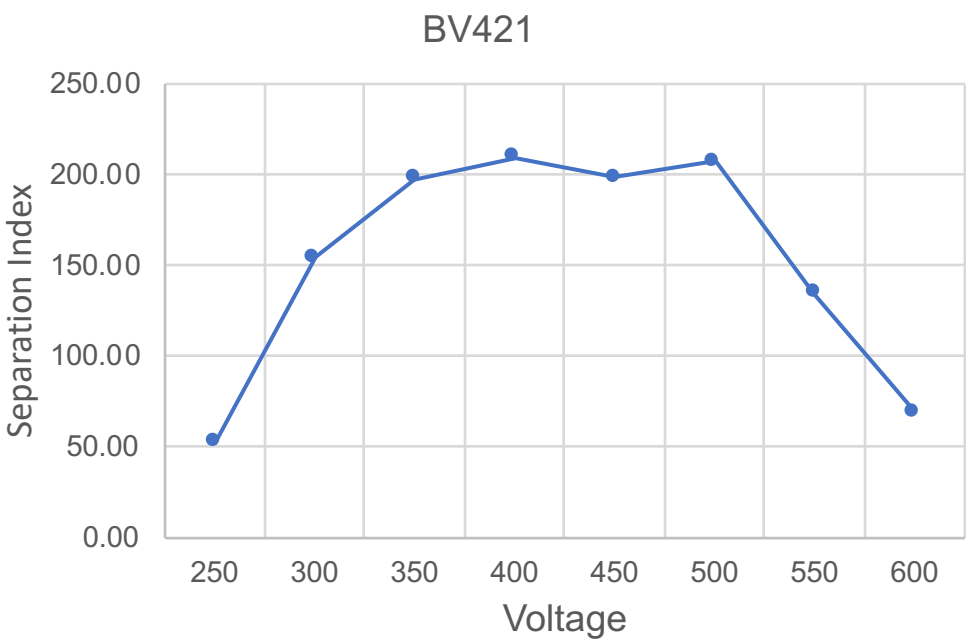
Very Bright

BV421



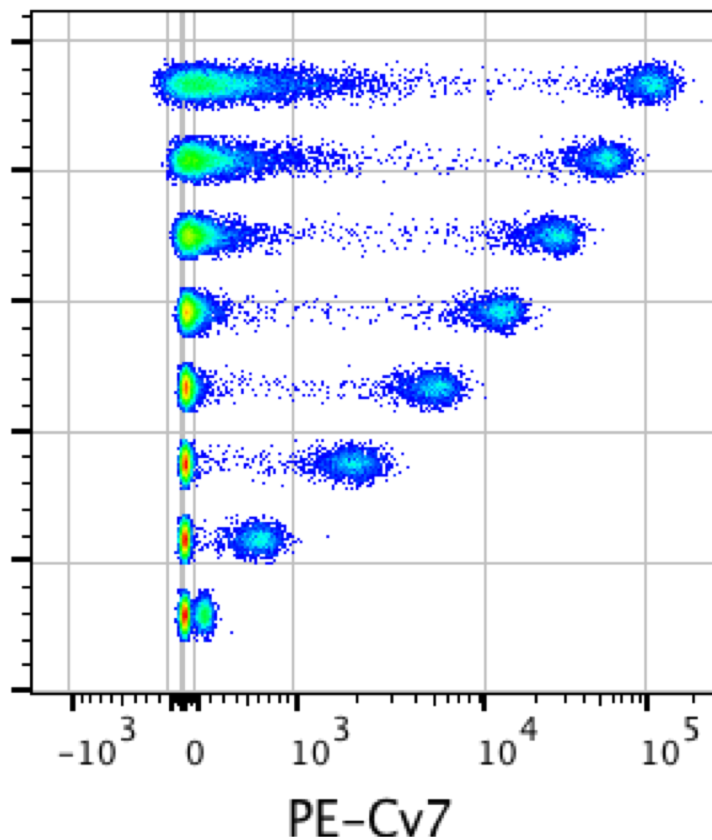
Violet (405 nm)

Voltage	SI
600	69.21
550	134.72
500	207.43
450	198.24
400	209.15
350	197.97
300	154.33
250	52.30



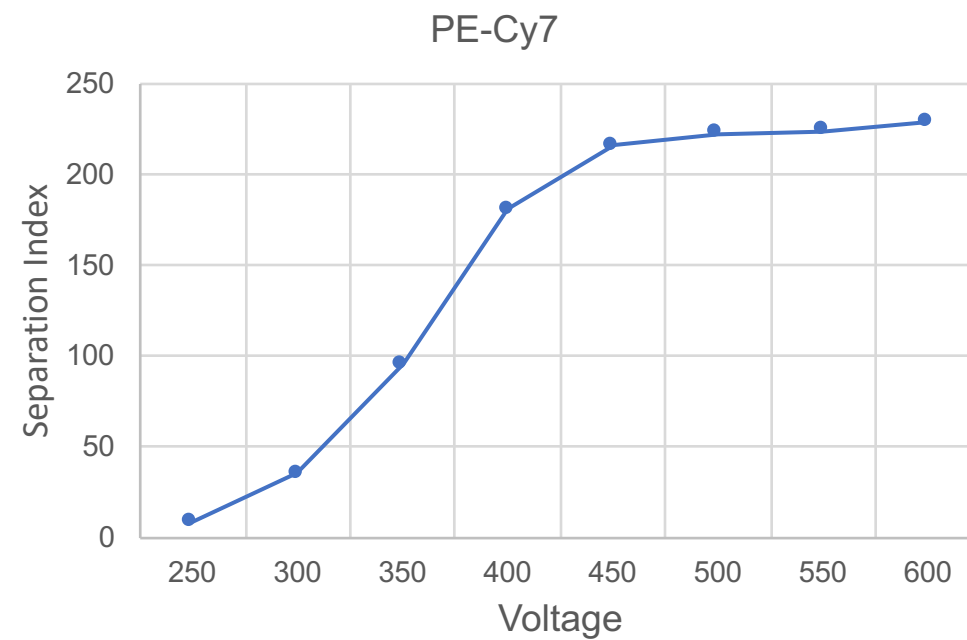
Very Bright

PE-Cy7



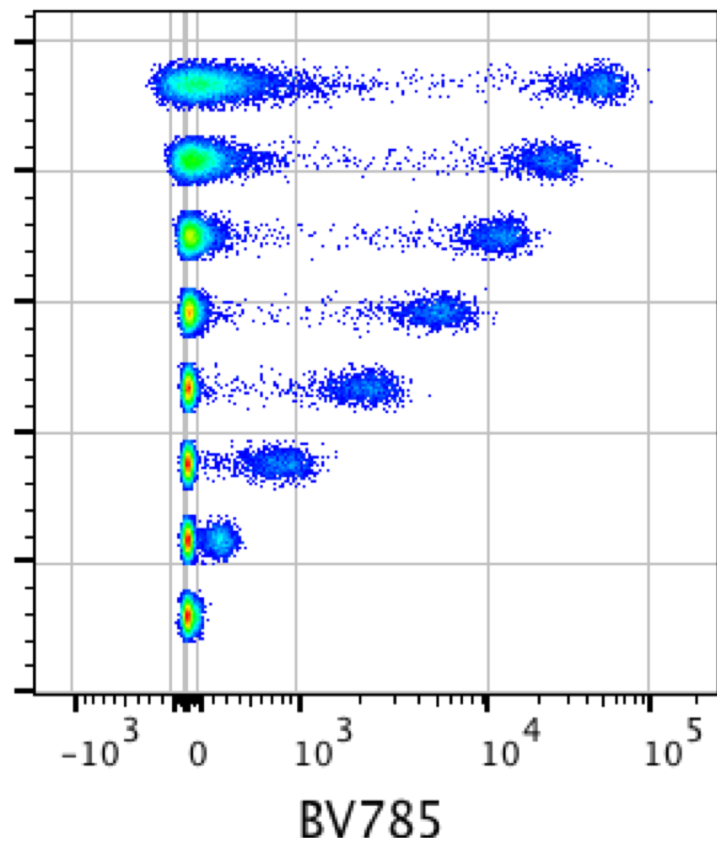
Yellow/Green (561nm)

Voltage	SI
600	229.07
550	223.78
500	222.64
450	216.36
400	180.93
350	95.78
300	35.57
250	9.00



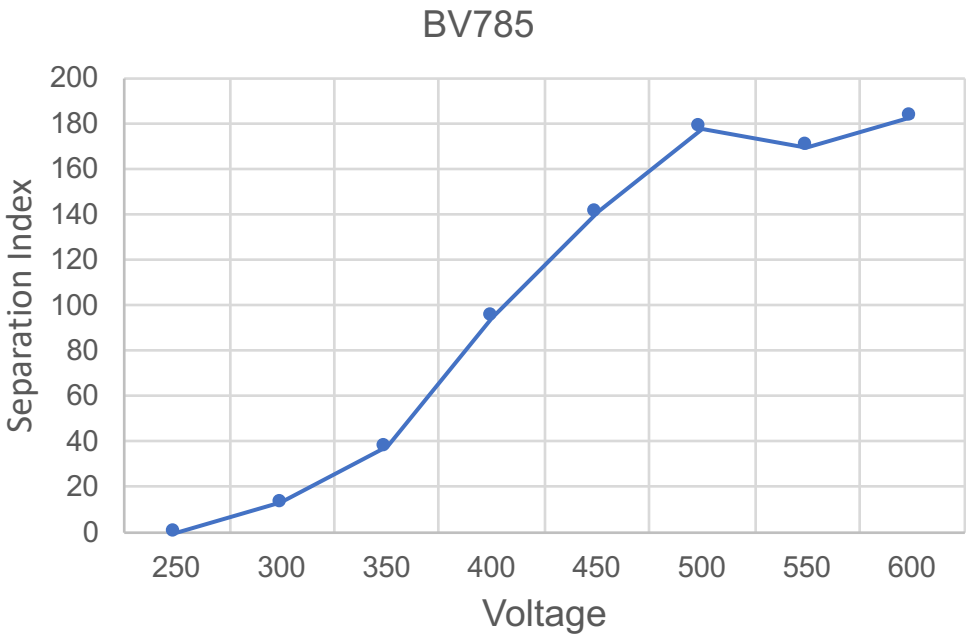
Bright

BV785



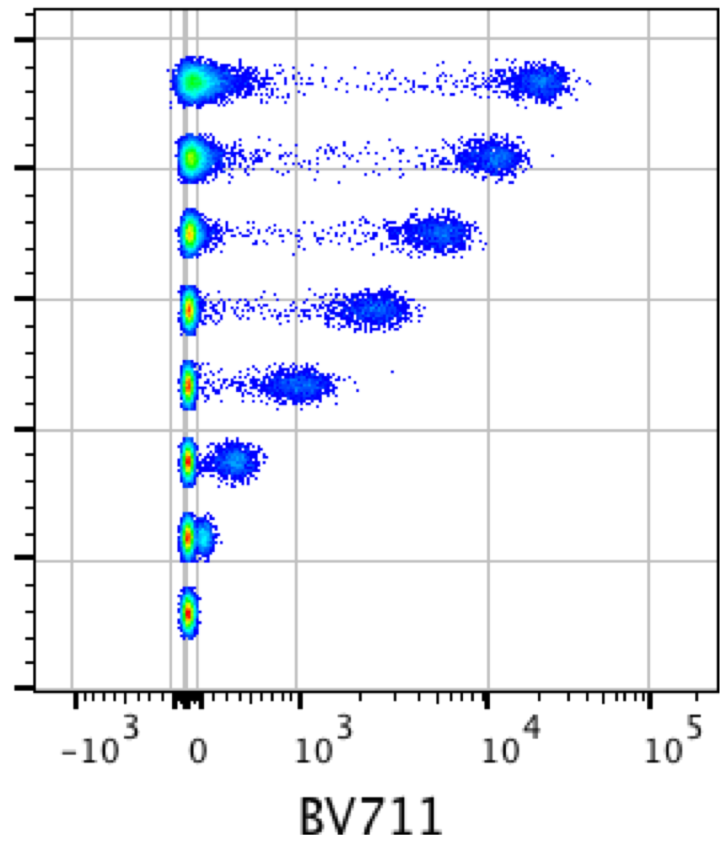
Violet (405 nm)

Voltage	SI
600	183.08
550	170.13
500	178.06
450	141.07
400	94.87
350	37.52
300	12.99
250	0.00

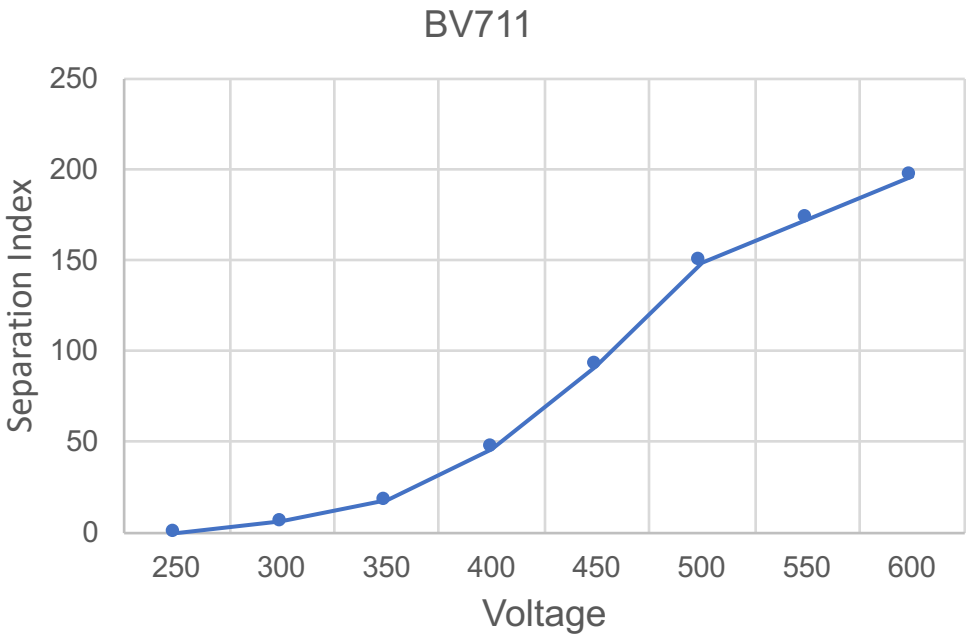


Bright

BV711



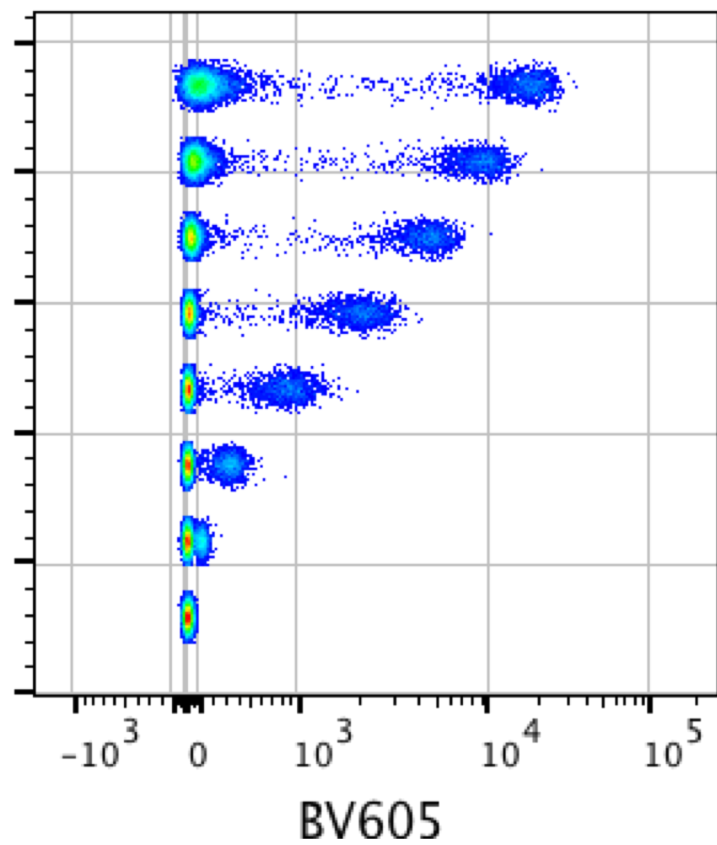
Voltage	SI
600	196.35
550	172.38
500	149.58
450	92.25
400	46.79
350	18.17
300	5.75
250	0.00



Violet (405 nm)

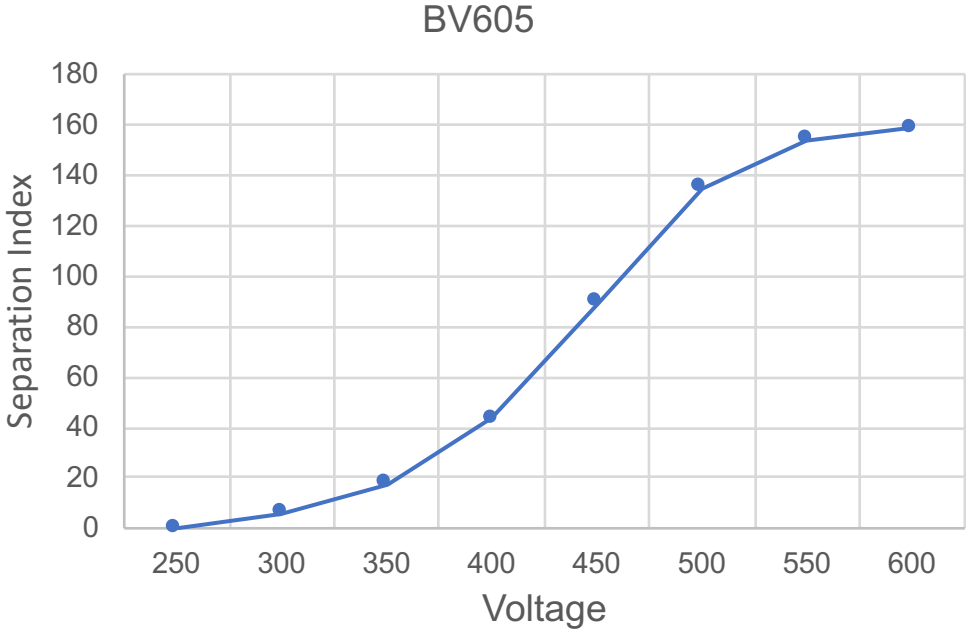
Bright

BV605



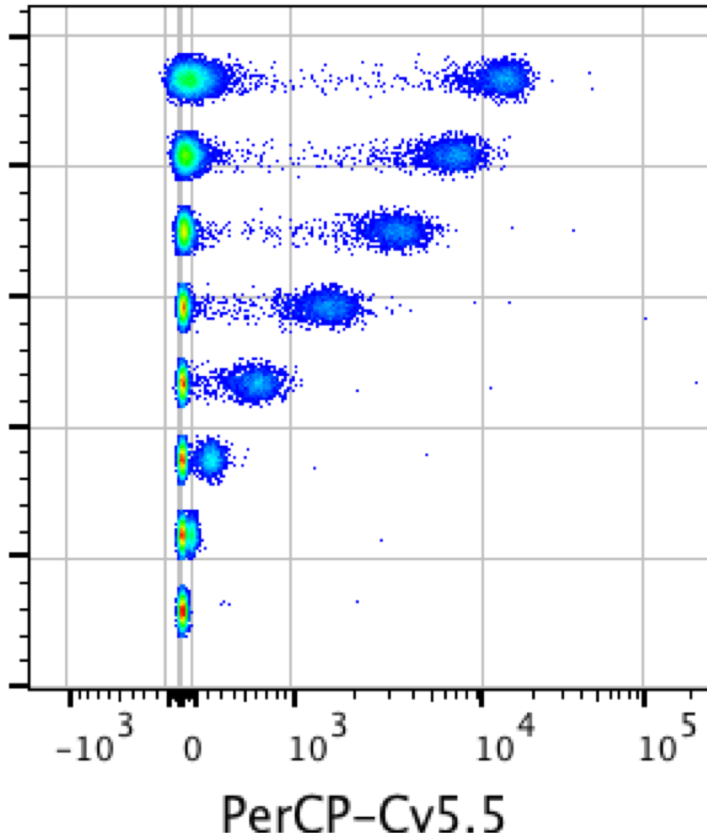
Violet (405 nm)

Voltage	SI
600	158.81
550	153.98
500	134.93
450	89.33
400	43.57
350	17.82
300	5.98
250	0.00



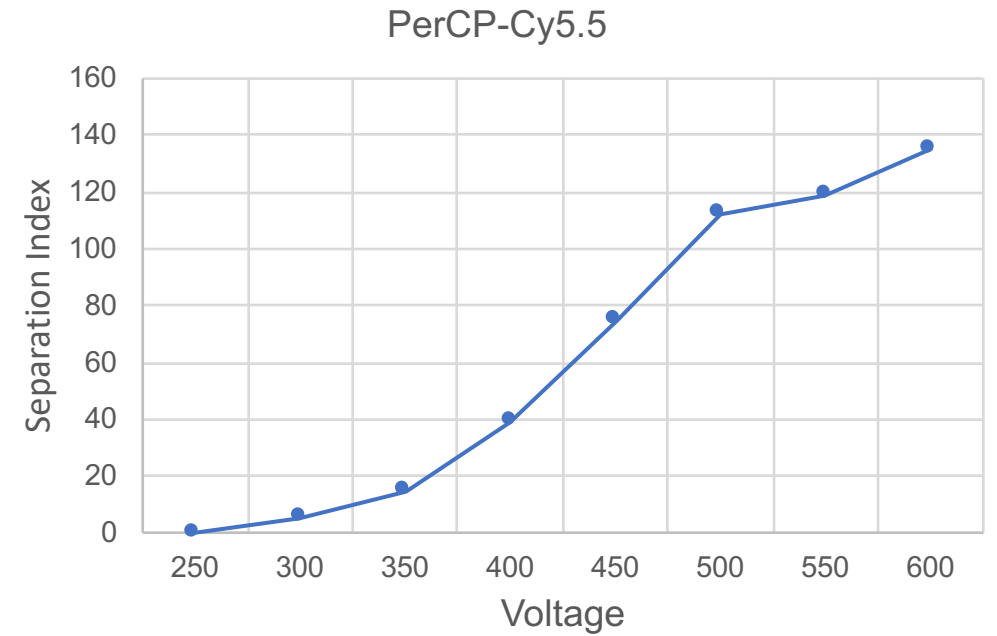
Moderate

PerCP-Cy5.5



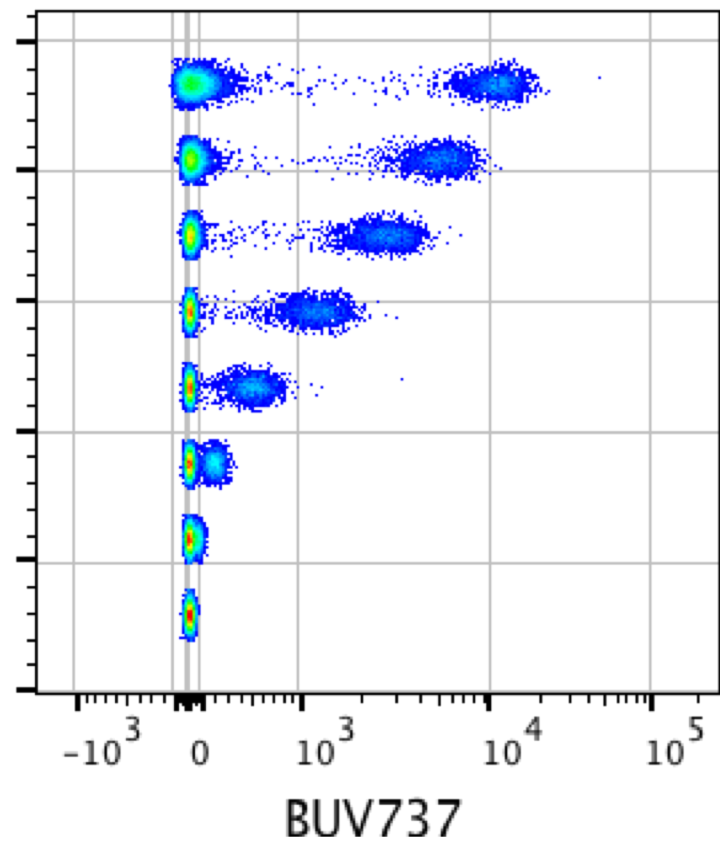
Blue (488 nm)

Voltage	SI
600	135.17
550	118.78
500	112.51
450	74.81
400	38.91
350	14.47
300	64.64
250	18.73



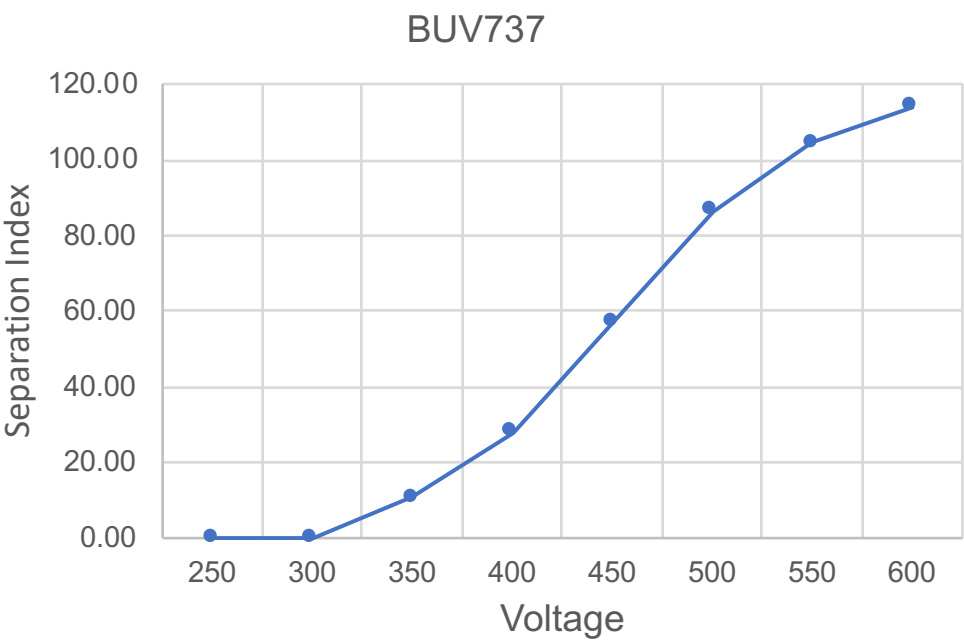
Moderate

BUV737



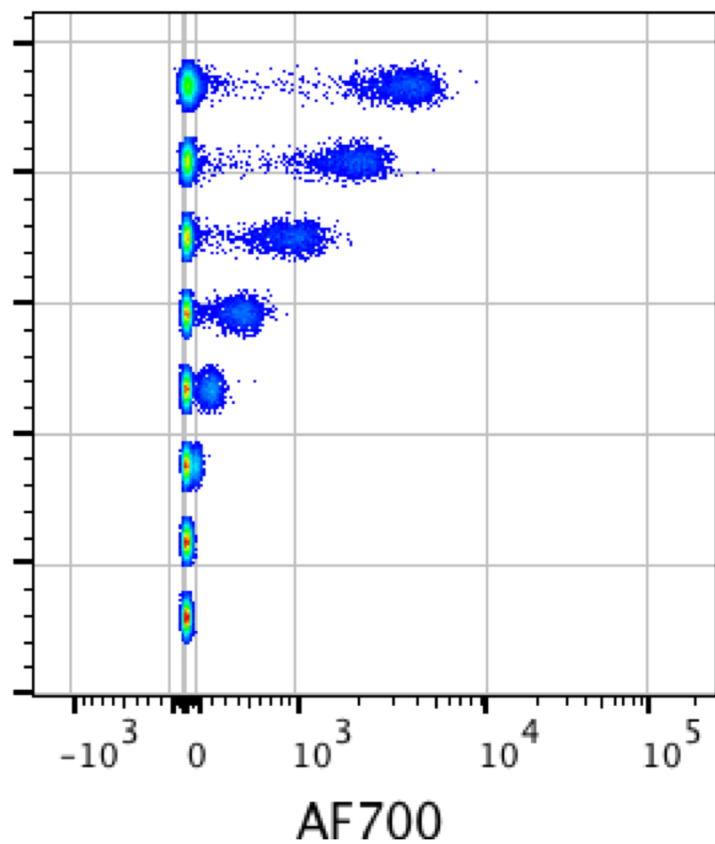
UV (355 nm)

Voltage	SI
600	113.82
550	104.38
500	86.46
450	56.90
400	28.03
350	10.61
300	0.00
250	0.00



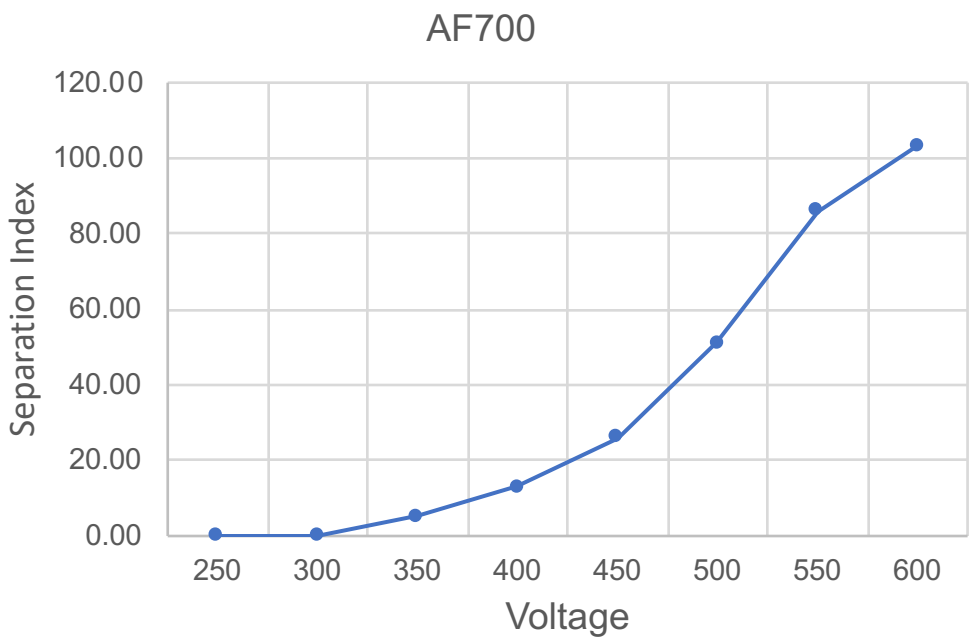
Moderate

AF700



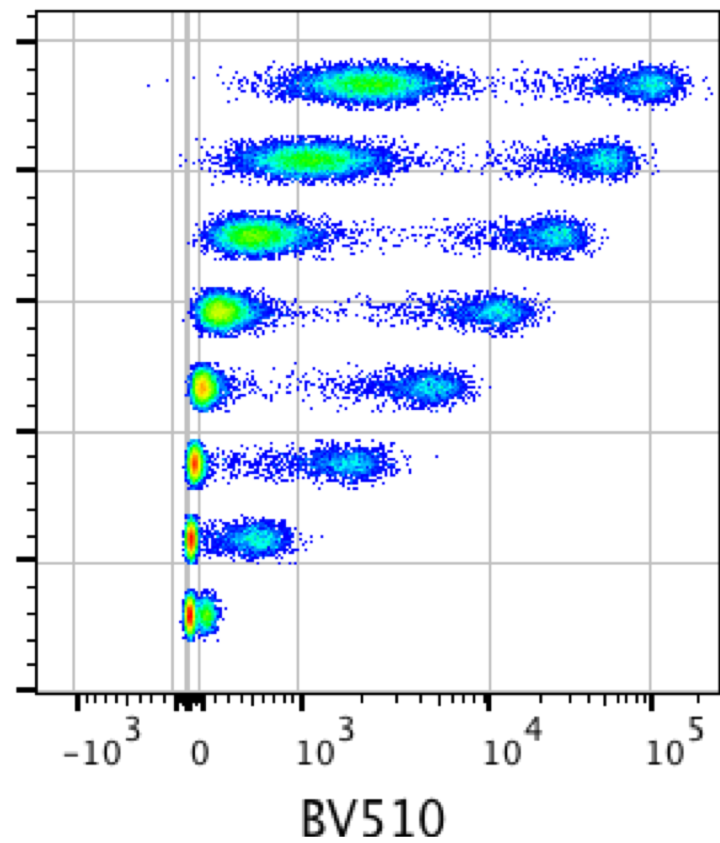
Red (640nm)

Voltage	SI
600	103.34
550	86.14
500	51.06
450	25.99
400	13.05
350	5.04
300	0.00
250	0.00



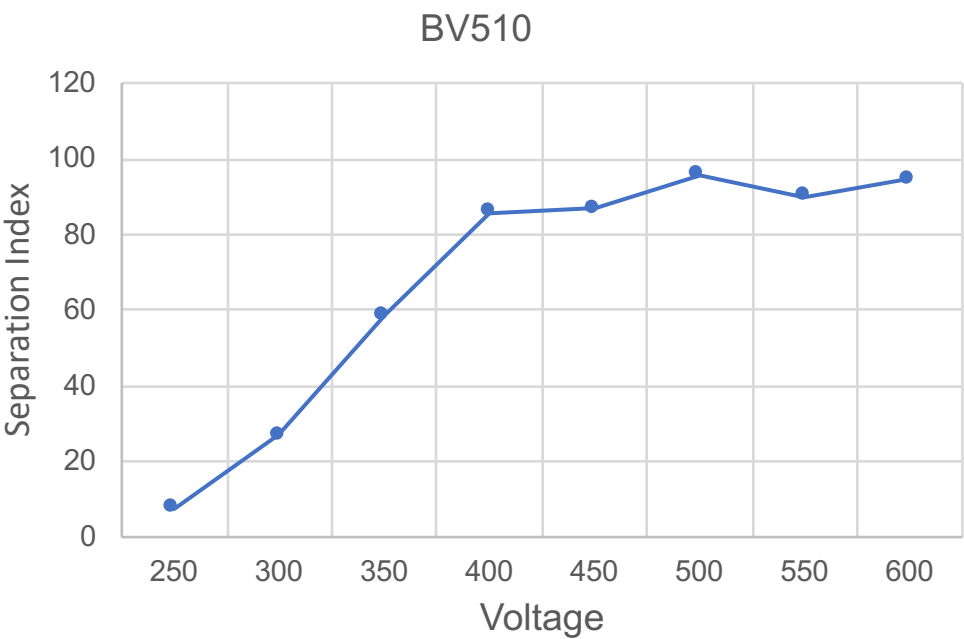
Dim

BV510



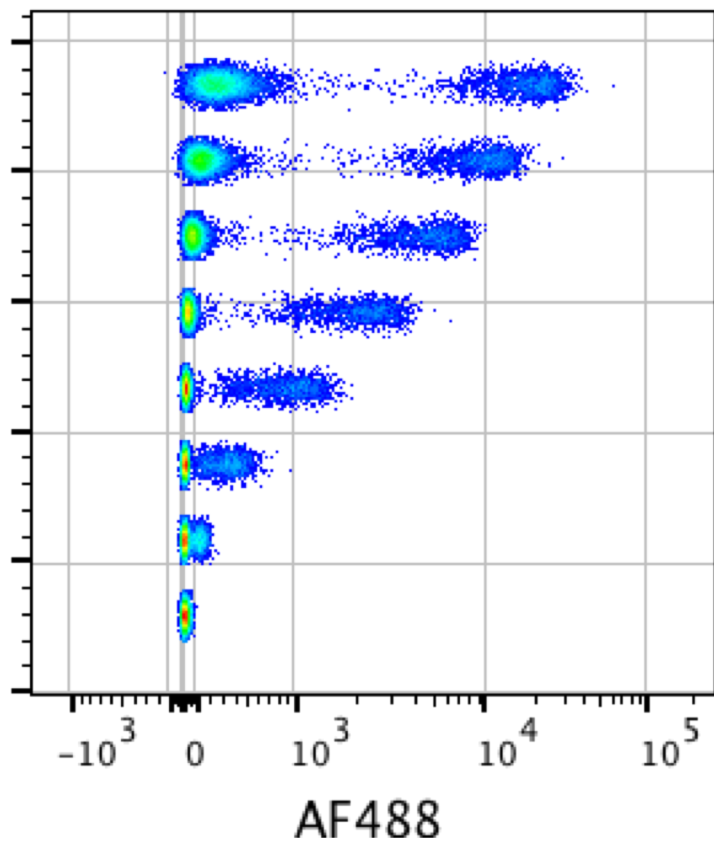
Violet (405 nm)

Voltage	SI
600	94.59
550	89.89
500	95.58
450	86.70
400	85.86
350	58.49
300	26.76
250	7.47



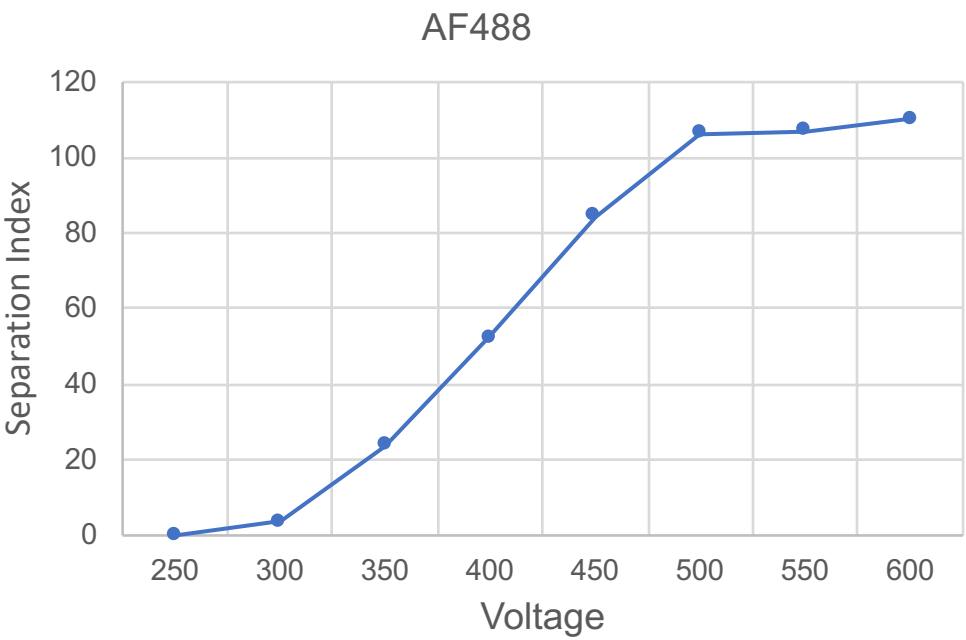
Dim

AF488



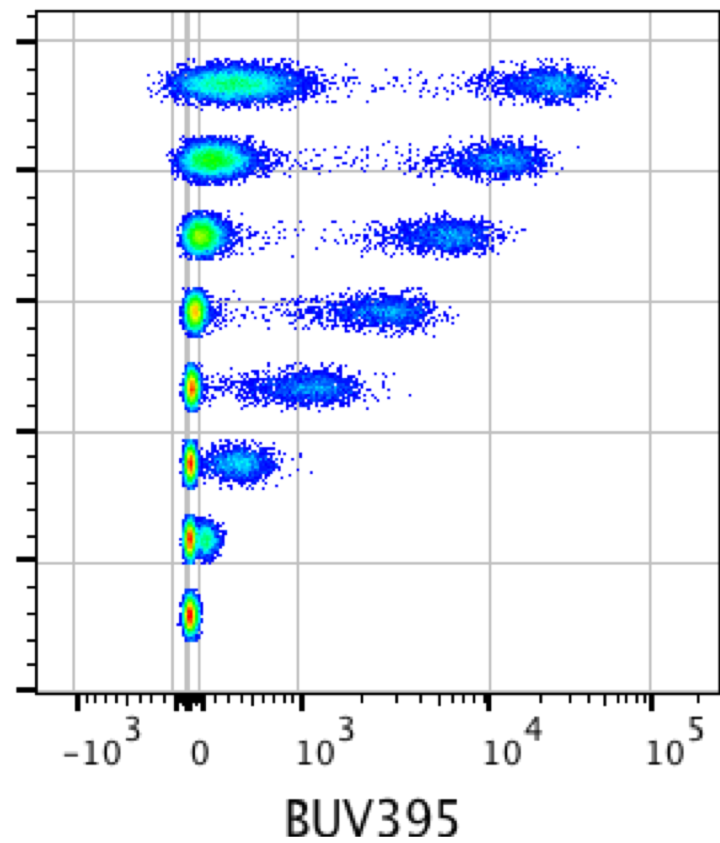
Blue (488 nm)

Voltage	SI
600	110.319051
550	107.059713
500	106.427185
450	84.5464549
400	52.2941018
350	23.6905768
300	3.5511971
250	0



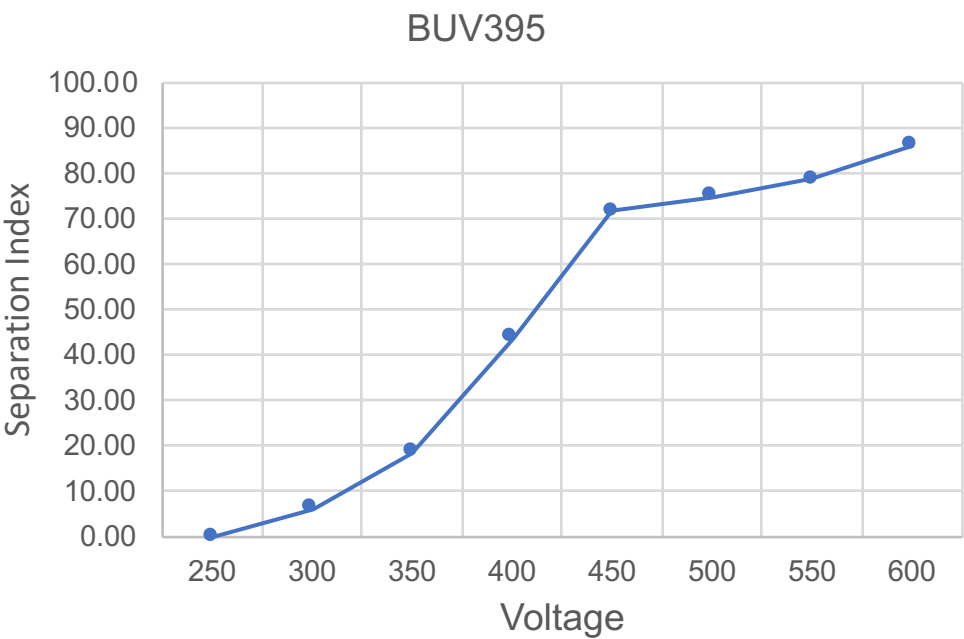
Dim

BUV395



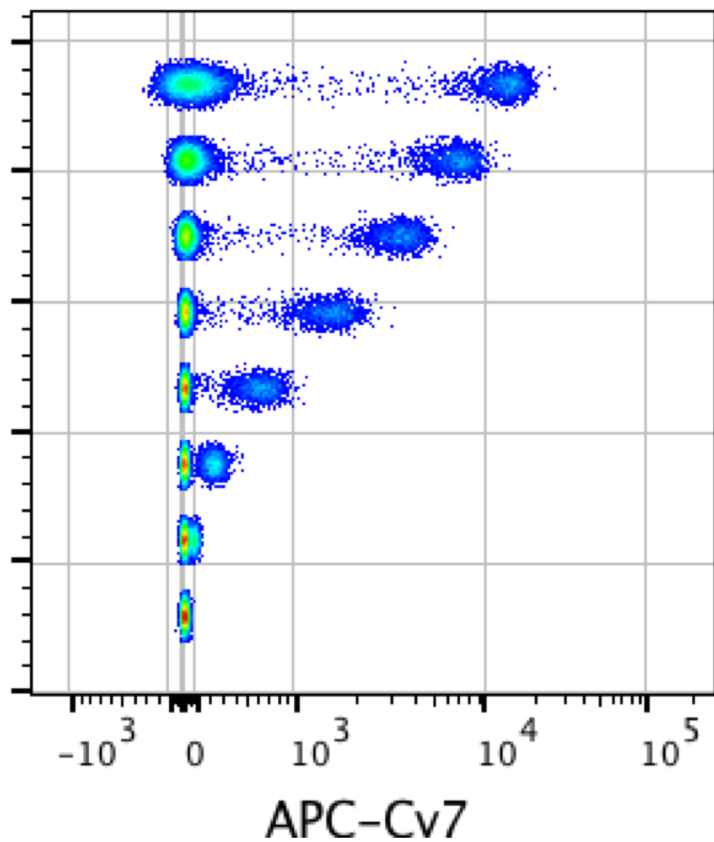
UV (355 nm)

Voltage	SI
600	86.18
550	78.90
500	74.95
450	71.73
400	43.89
350	18.59
300	6.22
250	0.00



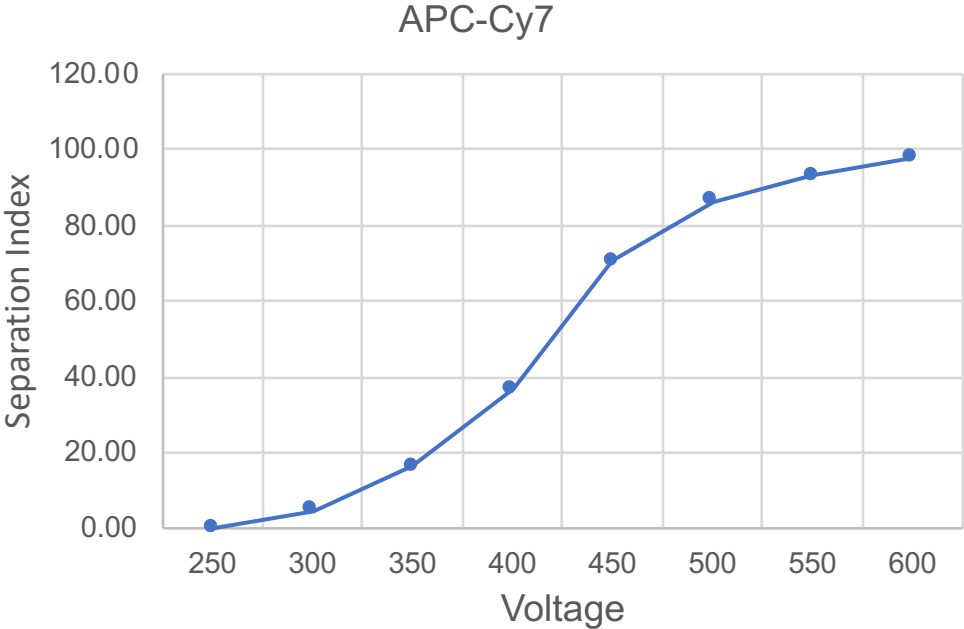
Dim

APC-Cy7



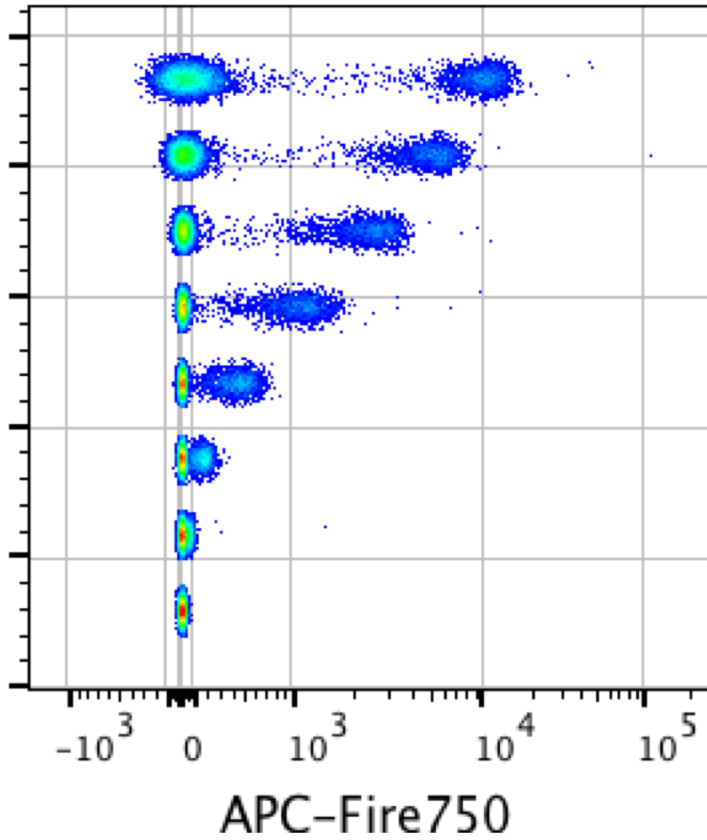
Red (640nm)

Voltage	SI
600	97.86
550	92.98
500	86.51
450	70.57
400	36.58
350	16.16
300	4.50
250	0.00



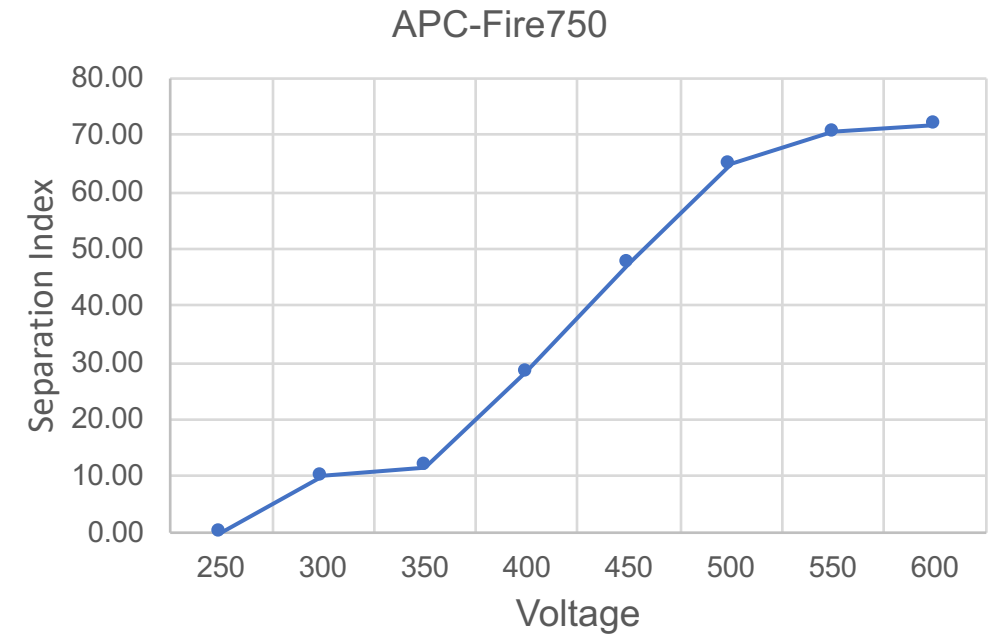
Dim

APC-Fire750



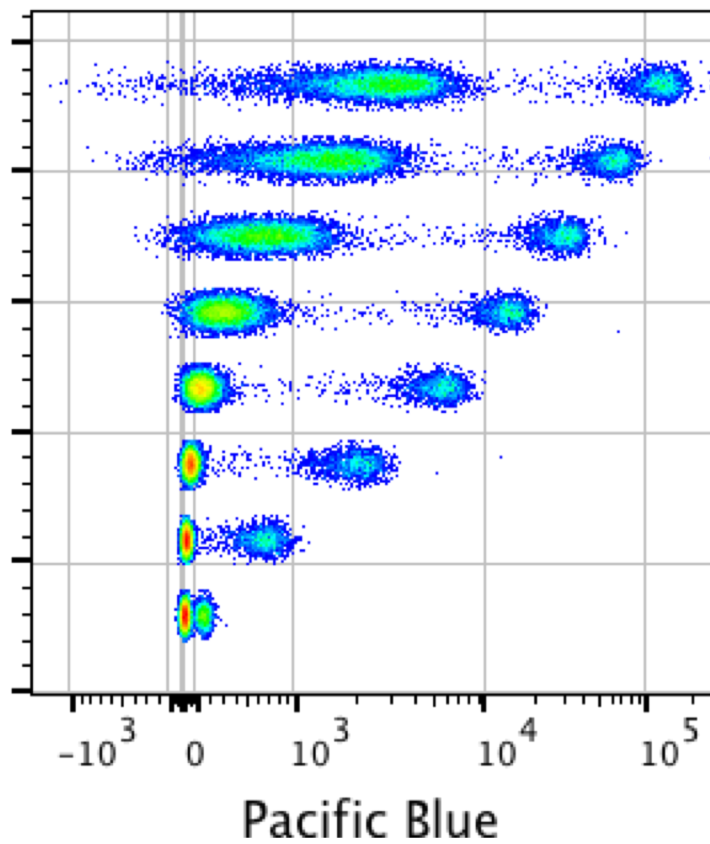
Red (640nm)

Voltage	SI
600	71.80
550	70.48
500	64.99
450	47.56
400	28.31
350	11.64
300	9.88
250	0.00



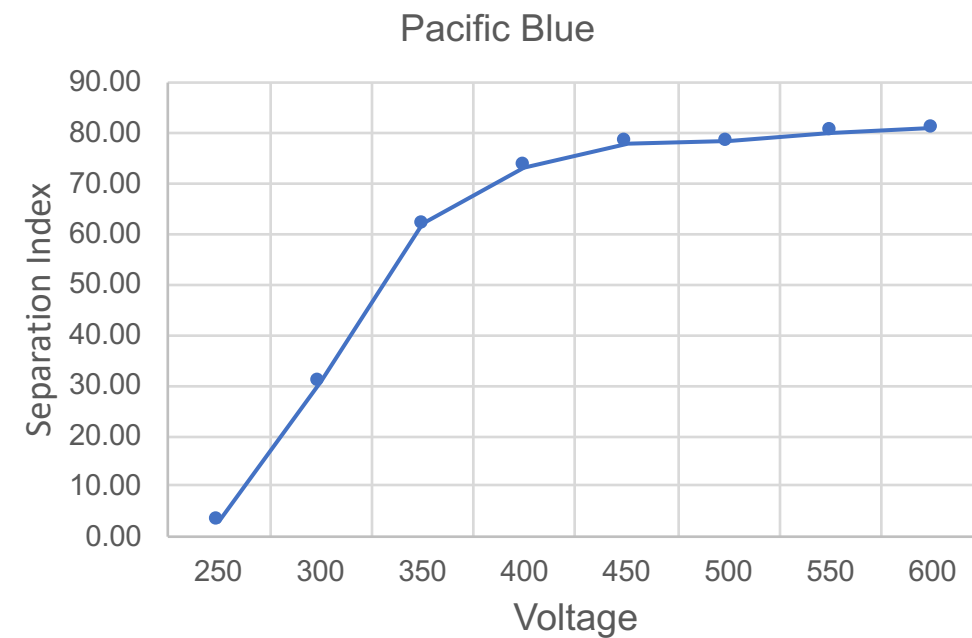
Dim

Pacific Blue



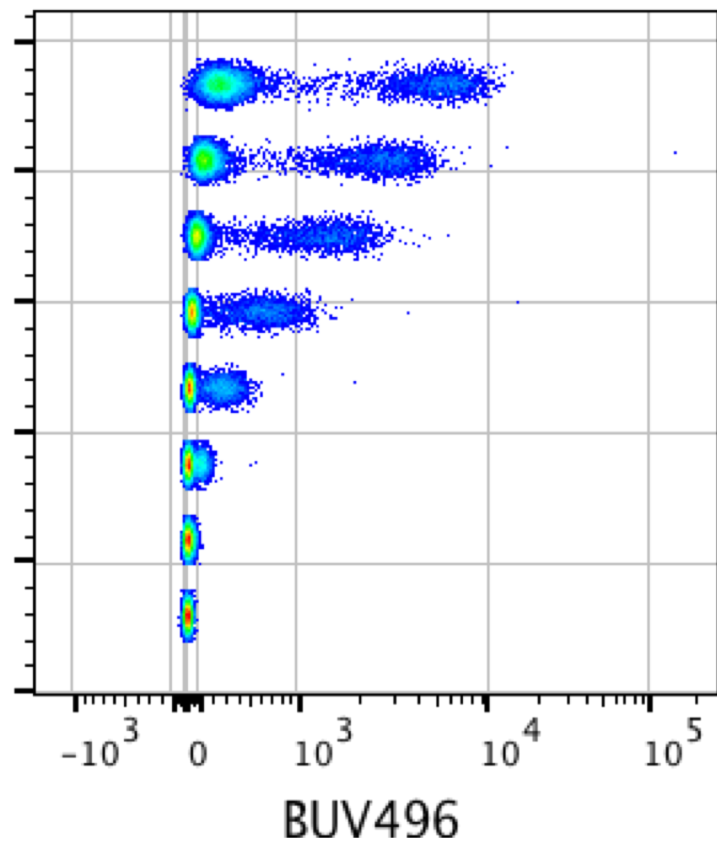
Violet (405 nm)

Voltage	SI
600	81.01
550	80.21
500	78.24
450	78.00
400	73.26
350	61.80
300	30.67
250	2.98



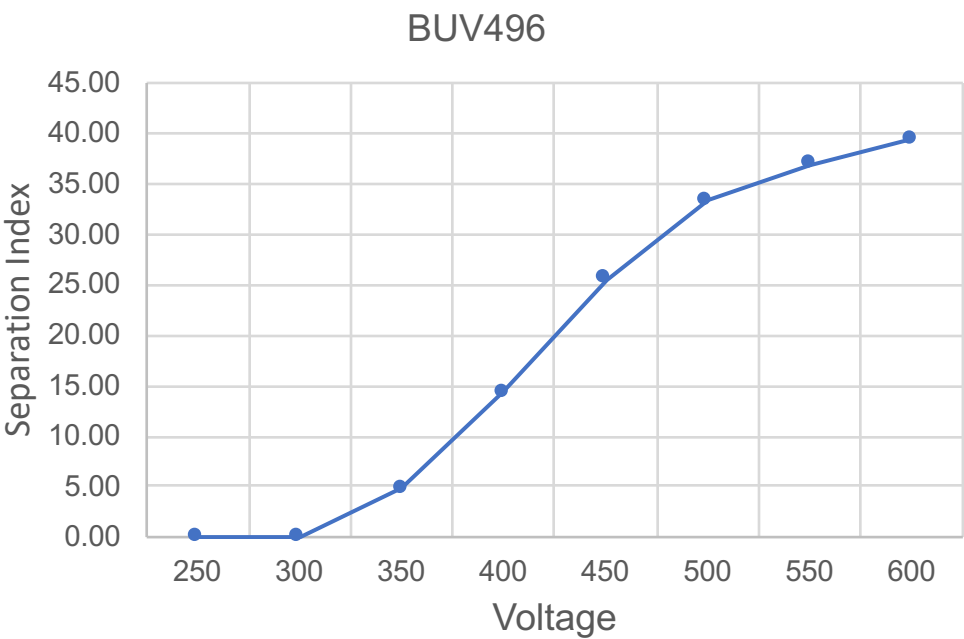
Dim

BUV496



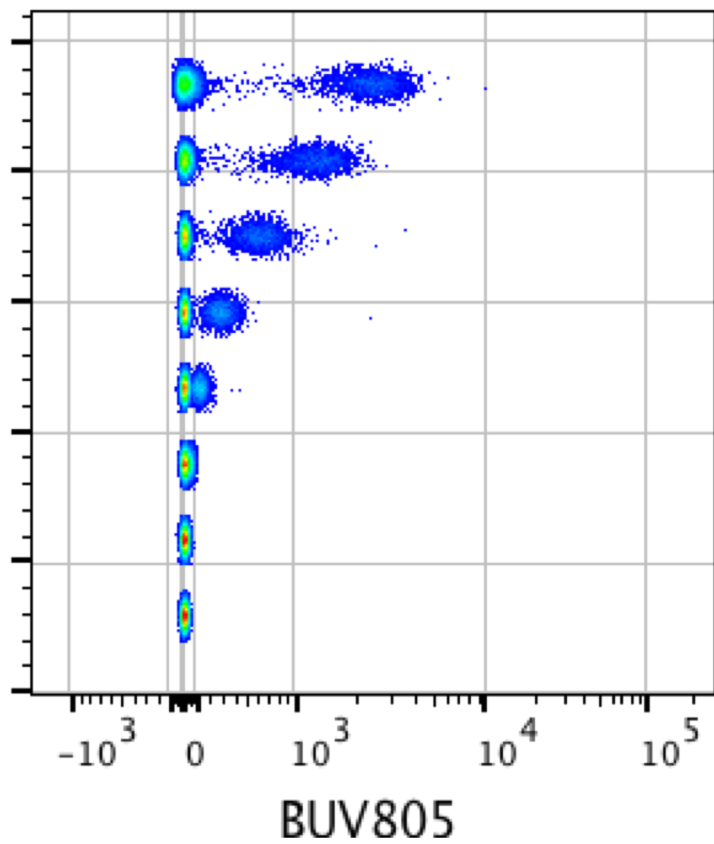
UV (355 nm)

Voltage	SI
600	39.45
550	36.85
500	33.35
450	25.51
400	14.31
350	4.86
300	0.00
250	0.00



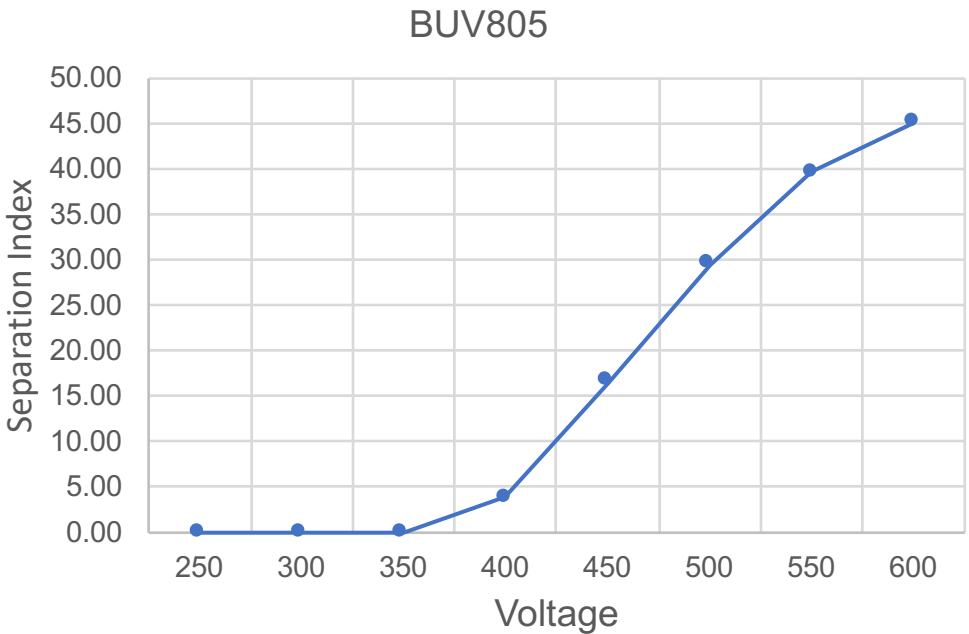
Very Dim

BUV805



UV (355 nm)

Voltage	SI
600	45.11
550	39.73
500	29.51
450	16.56
400	3.86
350	0.00
300	0.00
250	0.00



Very Dim