

Fortessa 4-15 Voltages and Brightness Index

- Mouse spleen cells were stained with 0.1 μg anti-CD4 (clone GK1.5) and run on the 4-15 at nine different voltages to compare the staining indexes between fluorophores

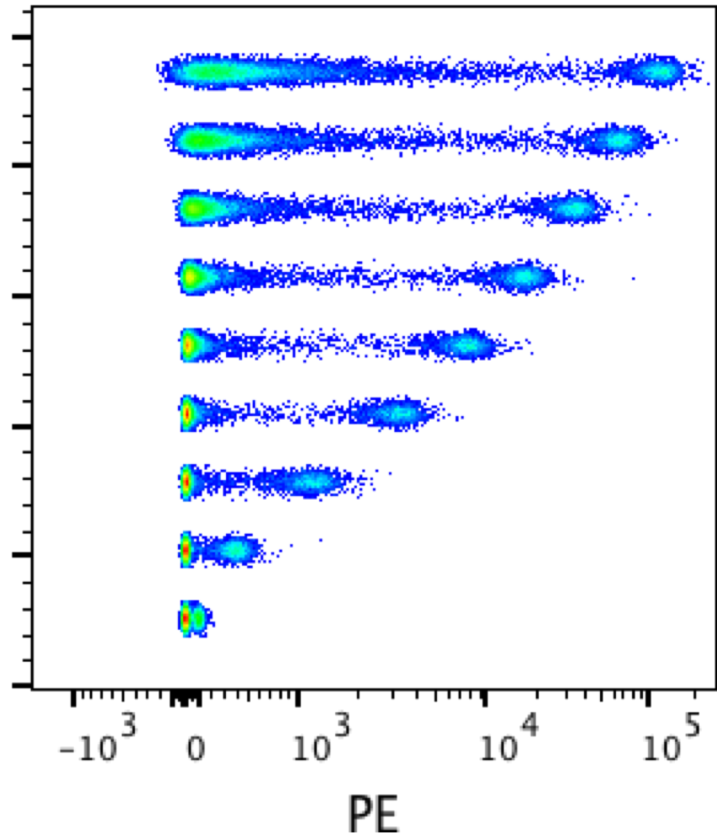
Recommended Voltages and Brightness

- 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.

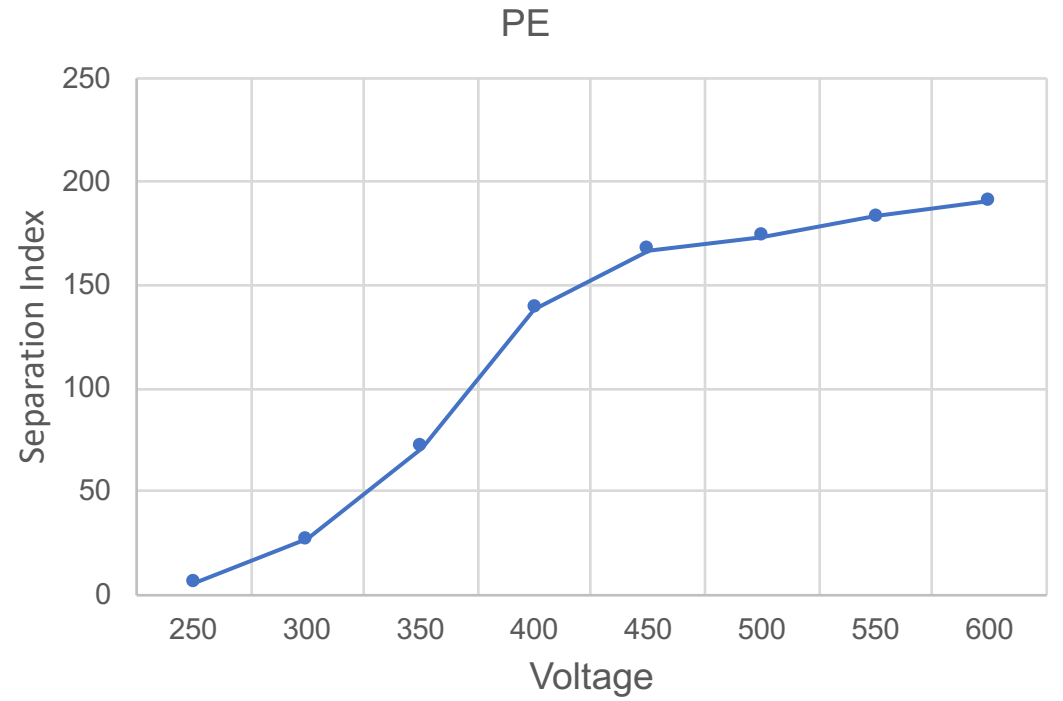
| Laser | Filter | Fluorophore tested | Starting voltage |
|-------|--------|--------------------|------------------|
| 405 | 450/50 | BV421 | 500 |
| 405 | 525/50 | BV510 | 450 |
| 405 | 610/20 | BV605 | 550 |
| 405 | 660/20 | BV650 | 661 |
| 405 | 710/50 | BV711 | 581 |
| 488 | 525/50 | AF488 | 550 |
| 488 | 710/50 | PerCP-Cy5.5 | 643 |
| 561 | 582/15 | PE | 657 |
| 561 | 610/20 | PE-Dazzle595 | 584 |
| 561 | 660/20 | PE-Cy5 | 686 |
| 561 | 730/45 | PE-Cy5.5 | 477 |
| 561 | 780/40 | PE-Cy7 | 502 |
| 640 | 670/30 | APC | 529 |
| 640 | 710/50 | AF700 | 600 |
| 640 | 780/60 | APC-Cy7 | 600 |

| Fluorophore tested | Separation Index | Brightness Estimation |
|--------------------|------------------|-----------------------|
| PE | 187 | Bright |
| PE-Dazzle595 | 175 | Bright |
| APC | 174 | Bright |
| BV421 | 166 | Bright |
| PE-Cy7 | 158 | Bright |
| BV711 | 150 | Bright |
| BV605 | 120 | Moderate |
| PerCP-Cy5.5 | 98 | Moderate |
| AF488 | 79 | Dim |
| BV510 | 70 | Dim |
| Pacific Blue | 69 | Dim |
| APC-Cy7 | 52 | Dim |
| AF700 | 41 | Very Dim |
| BV650 | not tested | |
| PE-Cy5 | not tested | |
| PE-Cy5.5 | not tested | |

PE



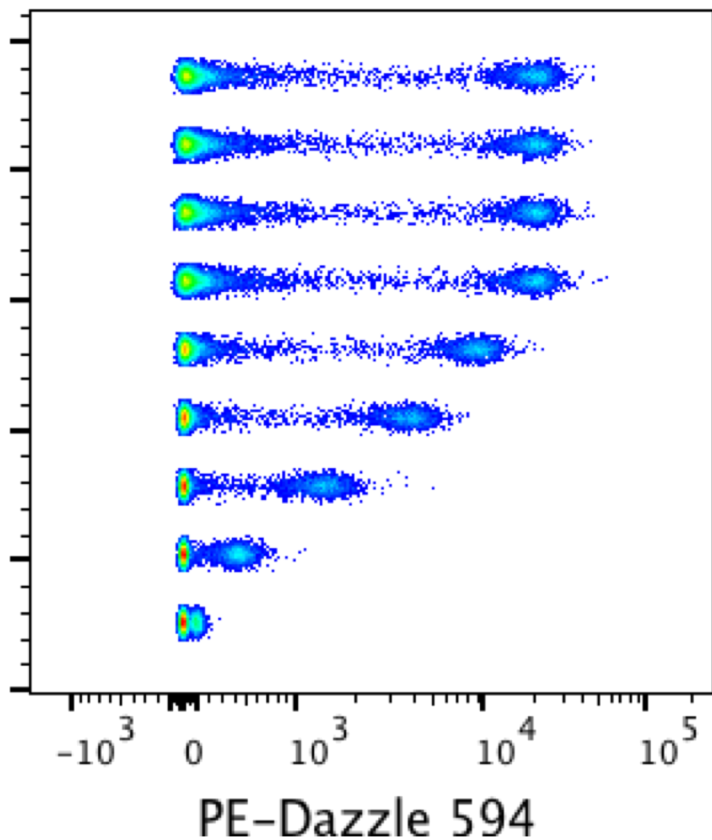
| Voltage | SI |
|---------|------------|
| 650 | 187.932182 |
| 600 | 190.755247 |
| 550 | 182.846504 |
| 500 | 173.225565 |
| 450 | 166.714127 |
| 400 | 138.556032 |
| 350 | 71.8171036 |
| 300 | 27.2511615 |
| 250 | 5.71339474 |



Yellow/Green (561nm)

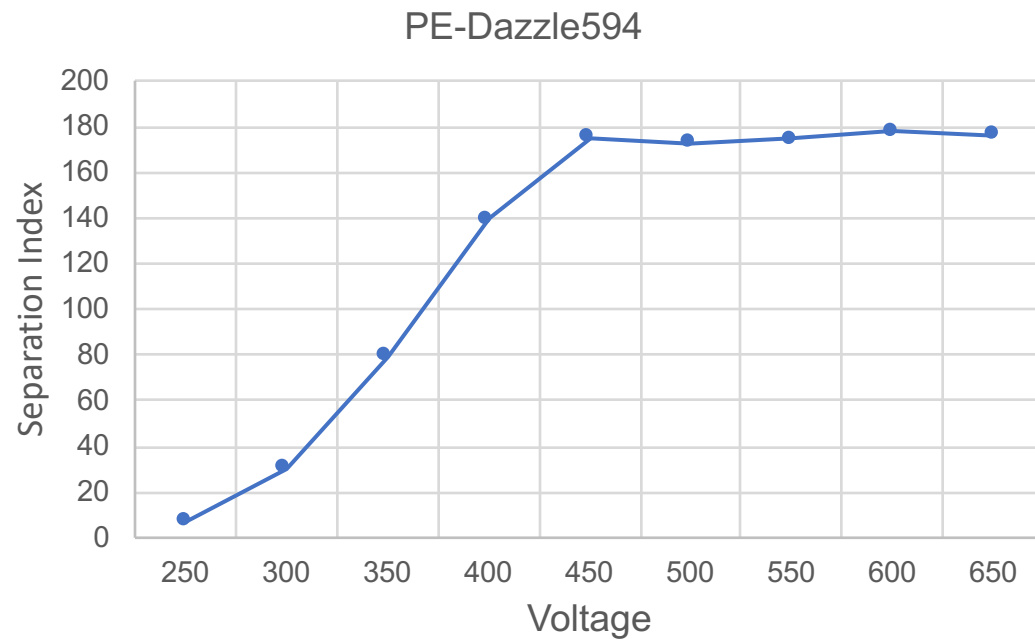
Bright

PE-Dazzle594



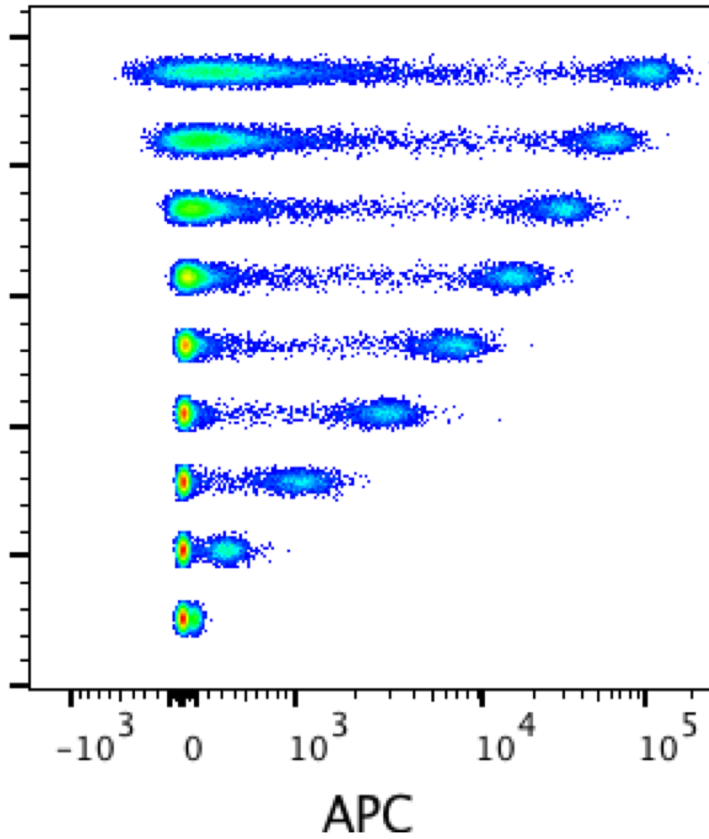
Yellow/Green (561nm)

| Voltage | SI |
|---------|------------|
| 500 | 176.398752 |
| 500 | 178.000753 |
| 500 | 174.227994 |
| 500 | 172.591118 |
| 450 | 174.992489 |
| 400 | 139.268219 |
| 350 | 79.5492042 |
| 300 | 30.2107932 |
| 250 | 6.9028125 |

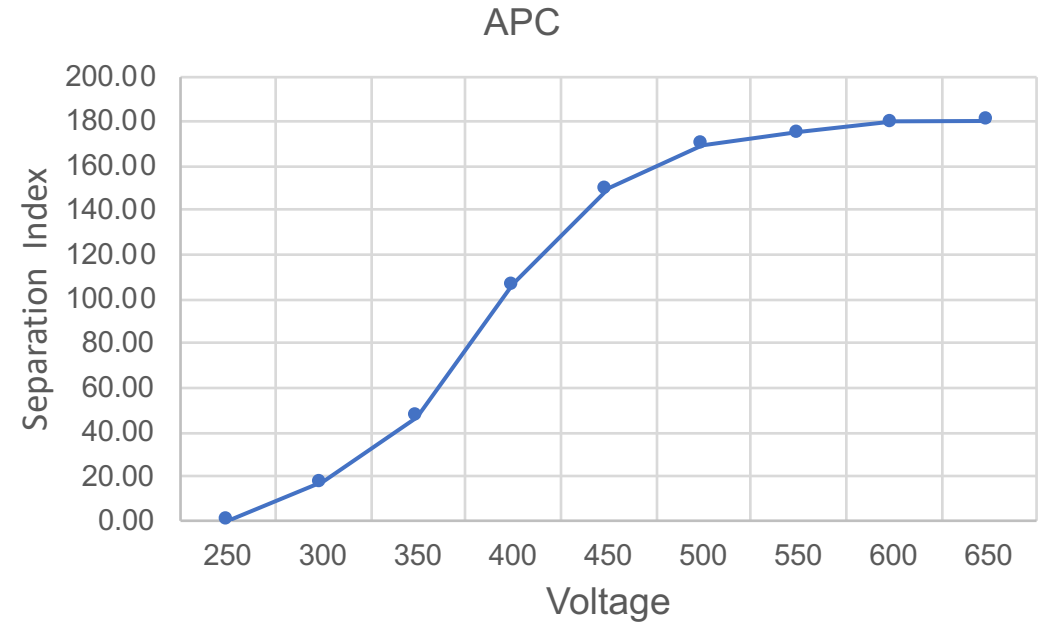


Bright

APC



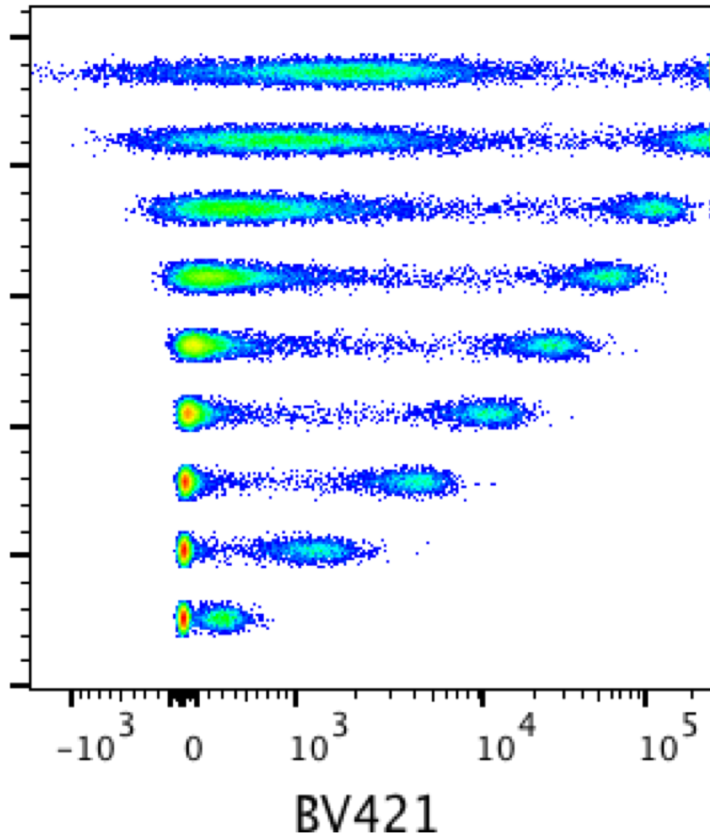
| Voltage | SI |
|---------|------------|
| 650 | 180.249334 |
| 600 | 179.506595 |
| 550 | 174.750569 |
| 500 | 169.527664 |
| 450 | 149.74046 |
| 400 | 106.200703 |
| 350 | 46.9915706 |
| 300 | 17.4834235 |
| 250 | 0 |



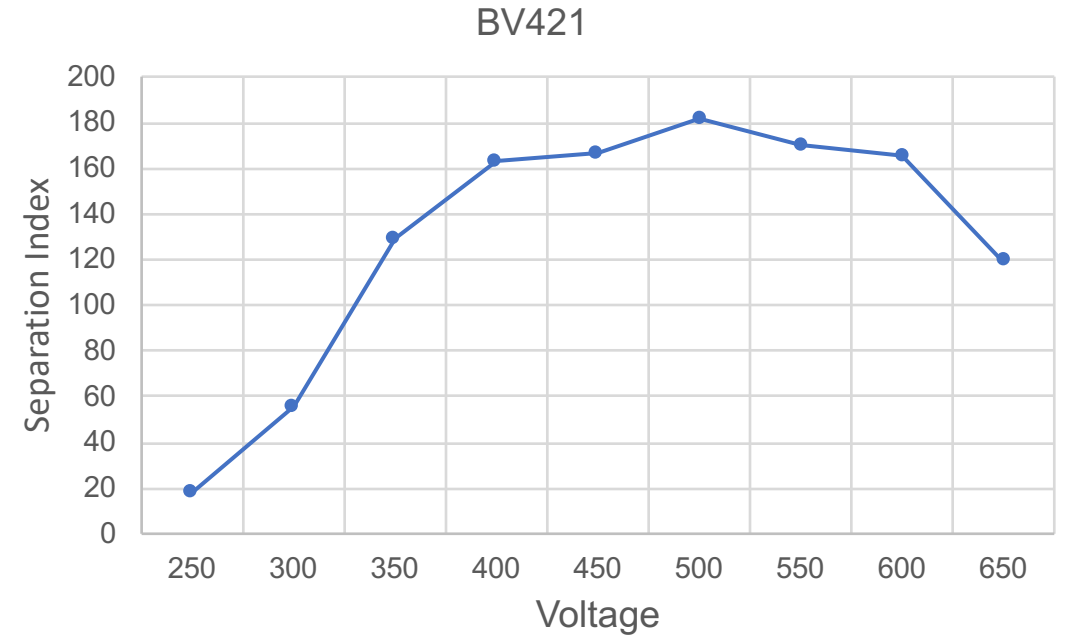
Red (640nm)

Bright

BV421



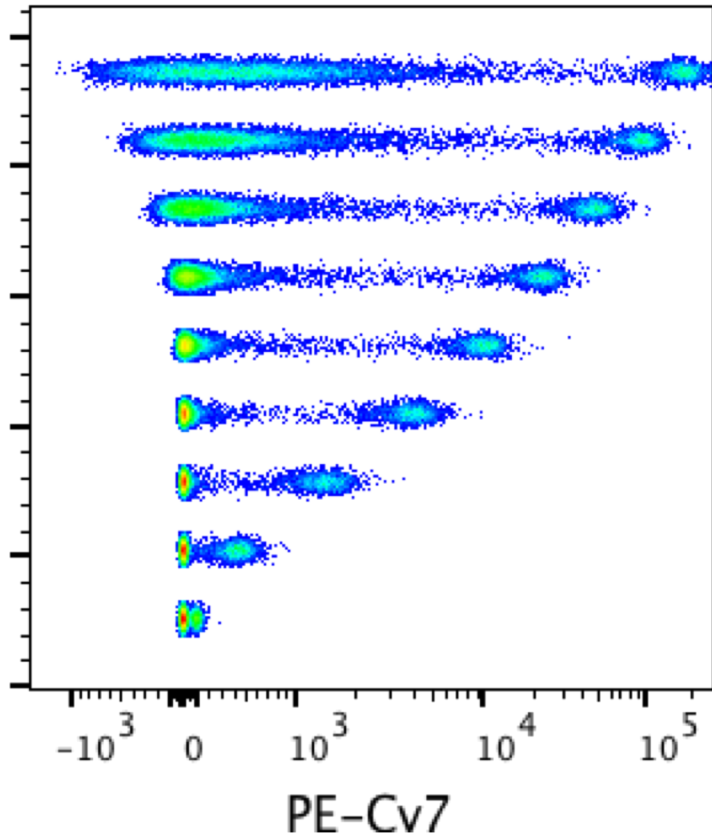
| Voltage | SI |
|---------|------------|
| 650 | 118.778411 |
| 600 | 165.34717 |
| 550 | 169.553677 |
| 500 | 181.628672 |
| 450 | 166.150986 |
| 400 | 162.829786 |
| 350 | 129.147627 |
| 300 | 55.5903361 |
| 250 | 18.0742464 |



Violet (405 nm)

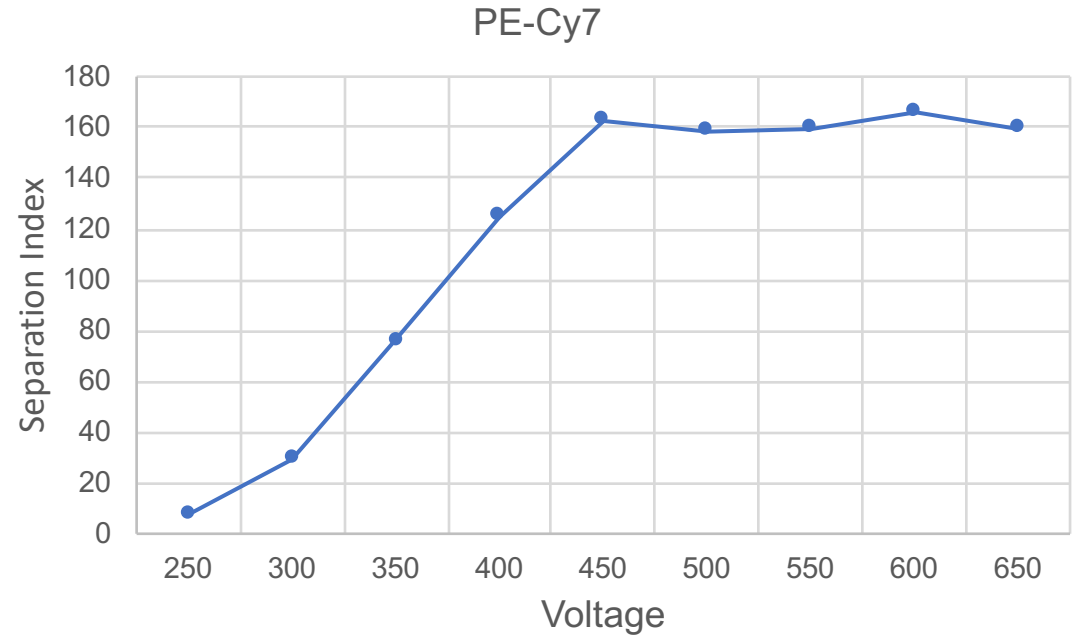
Bright

PE-Cy7



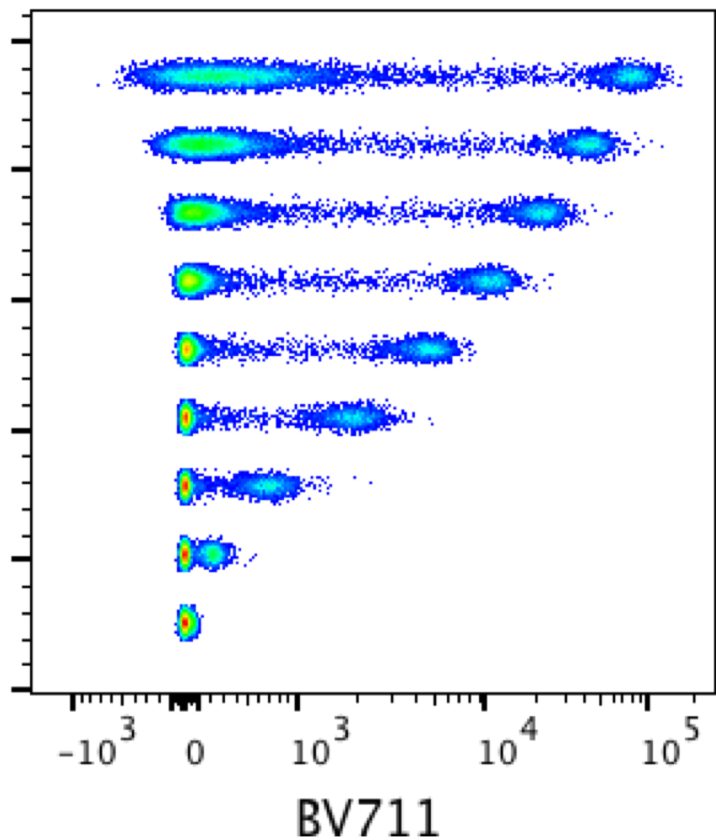
Yellow/Green (561nm)

| Voltage | SI |
|---------|------------|
| 650 | 159.812233 |
| 600 | 165.969073 |
| 550 | 159.810073 |
| 500 | 158.644151 |
| 450 | 162.897514 |
| 400 | 124.948675 |
| 350 | 76.0606825 |
| 300 | 29.5765864 |
| 250 | 7.59464844 |

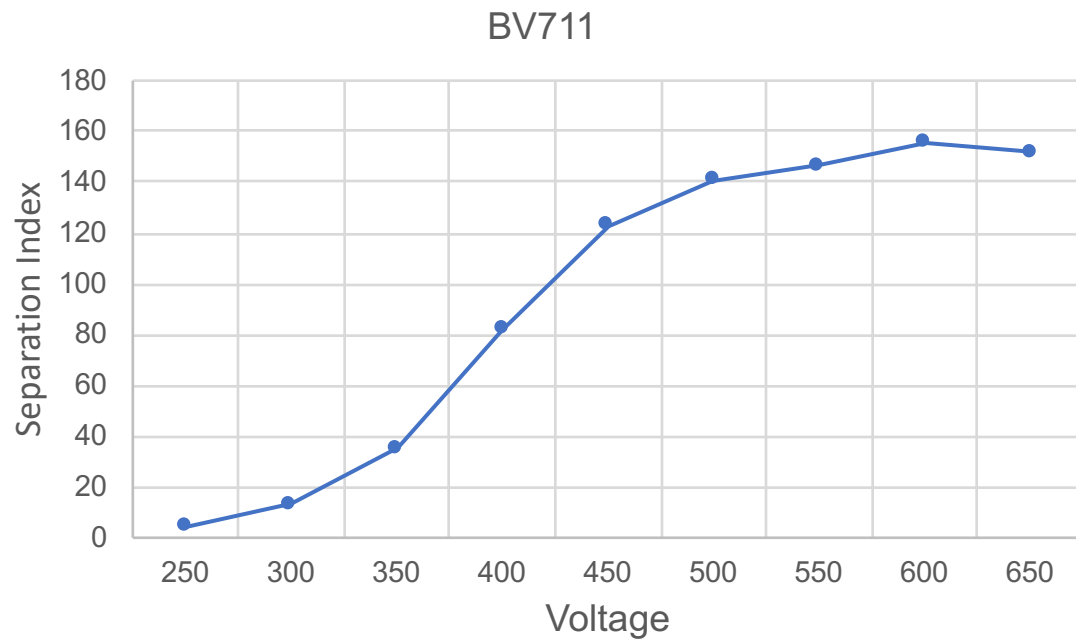


Bright

BV711



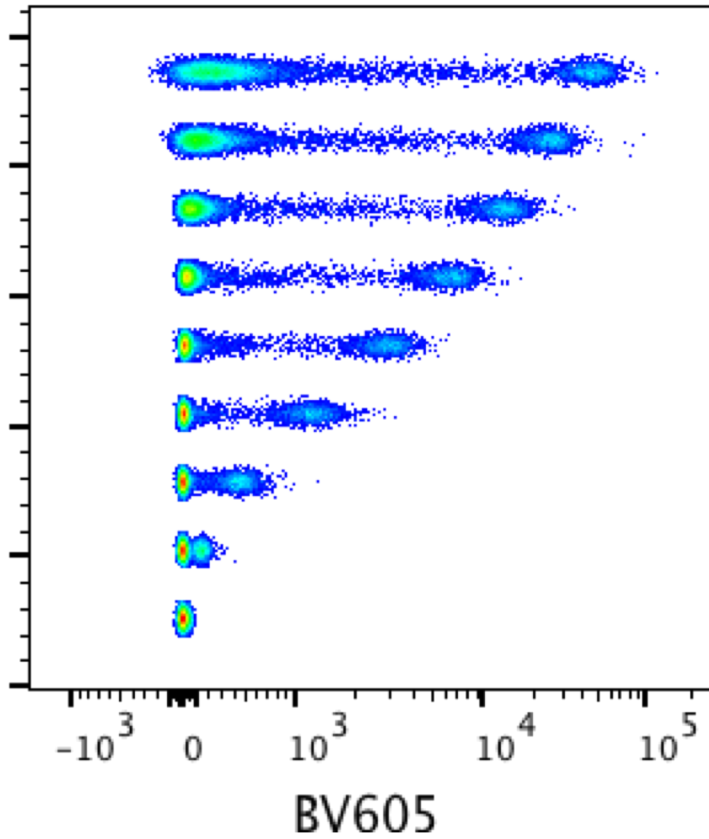
| Voltage | SI |
|---------|------------|
| 650 | 151.555641 |
| 600 | 155.452702 |
| 550 | 146.391489 |
| 500 | 140.498061 |
| 450 | 123.109682 |
| 400 | 82.1727727 |
| 350 | 35.3103406 |
| 300 | 12.9564234 |
| 250 | 4.21444341 |



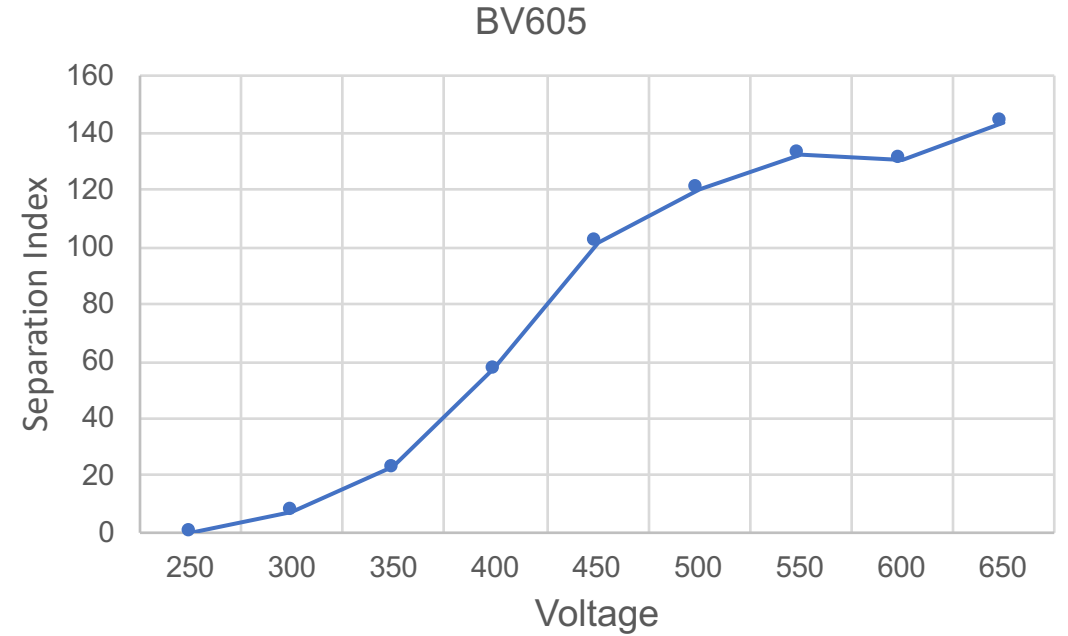
Violet (405 nm)

Bright

BV605



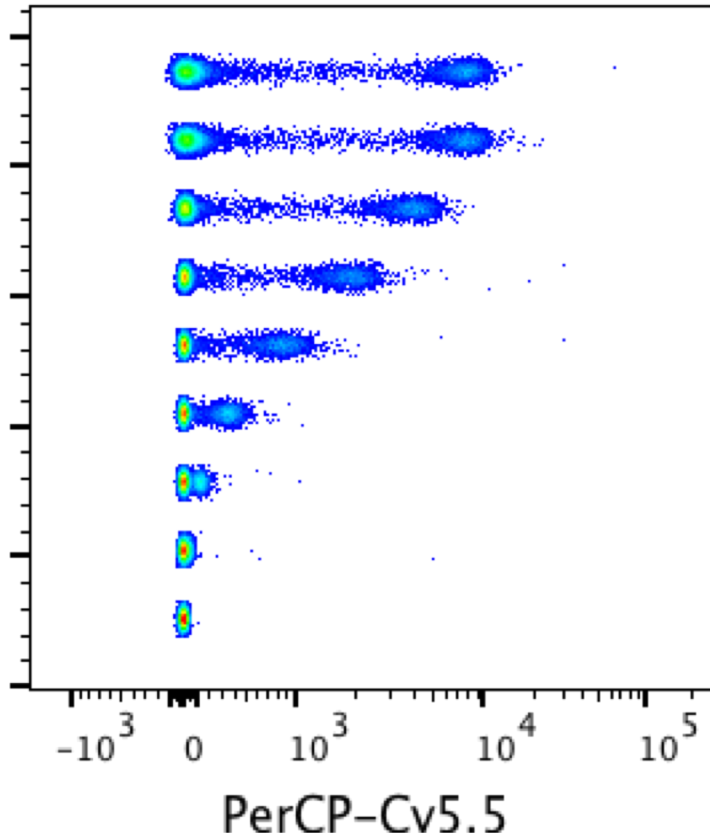
| Voltage | SI |
|---------|------------|
| 650 | 143.546268 |
| 600 | 130.816914 |
| 550 | 132.60784 |
| 500 | 120.30547 |
| 450 | 101.645353 |
| 400 | 57.5092544 |
| 350 | 22.7887598 |
| 300 | 7.52694301 |
| 250 | 0 |



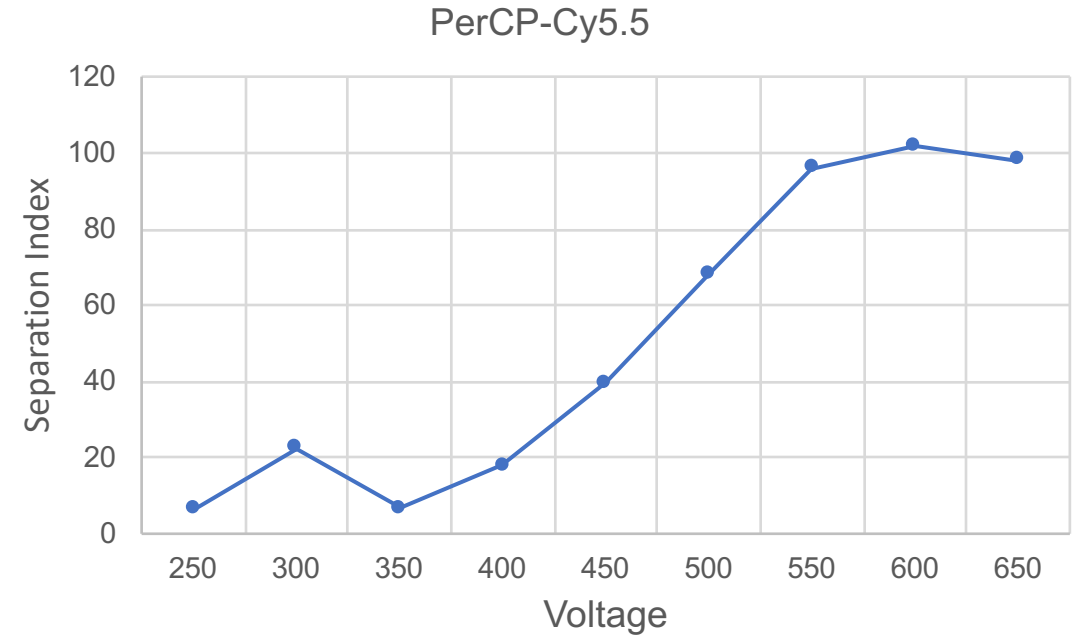
Violet (405 nm)

Moderate

PerCP-Cy5.5



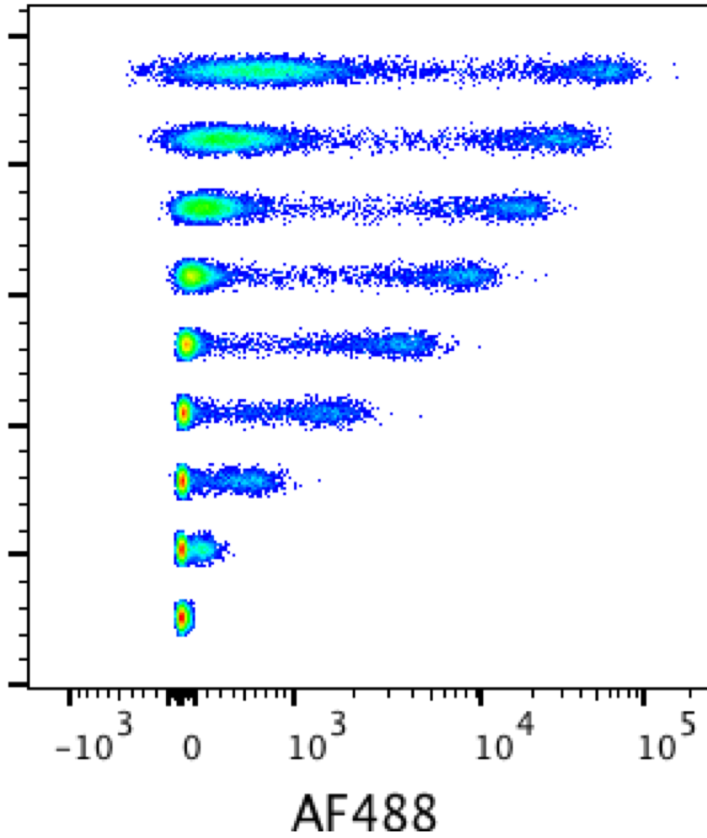
| Voltage | SI |
|---------|------------|
| 650 | 98.1701043 |
| 600 | 101.803454 |
| 550 | 95.9506317 |
| 500 | 68.3973523 |
| 450 | 39.5282847 |
| 400 | 17.8732631 |
| 350 | 6.81901145 |
| 300 | 22.3170123 |
| 250 | 6.22399556 |



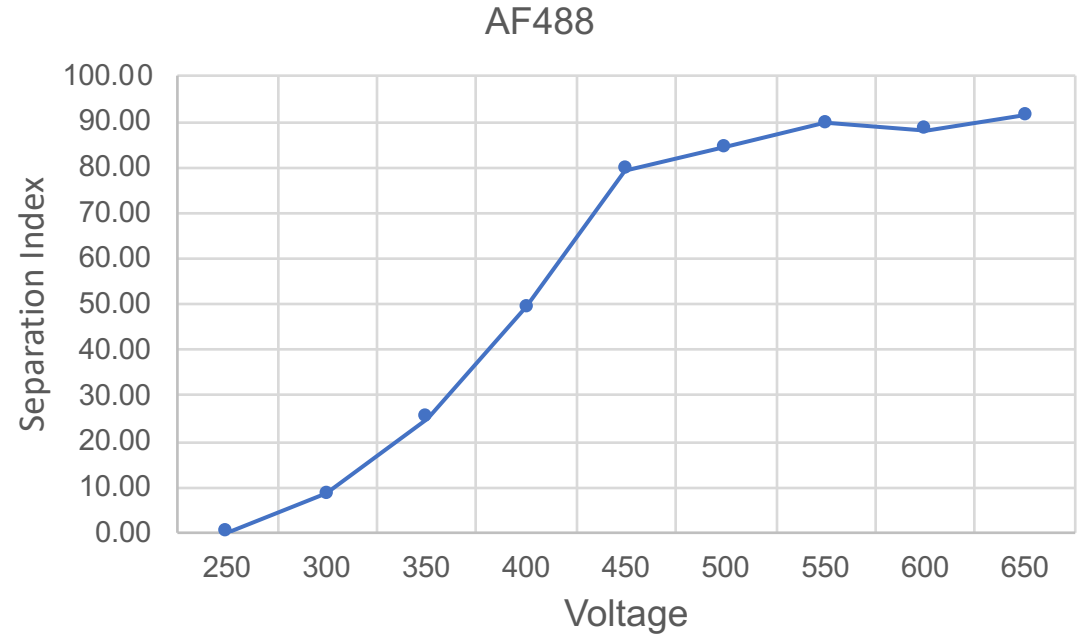
Blue (488 nm)

Moderate

AF488



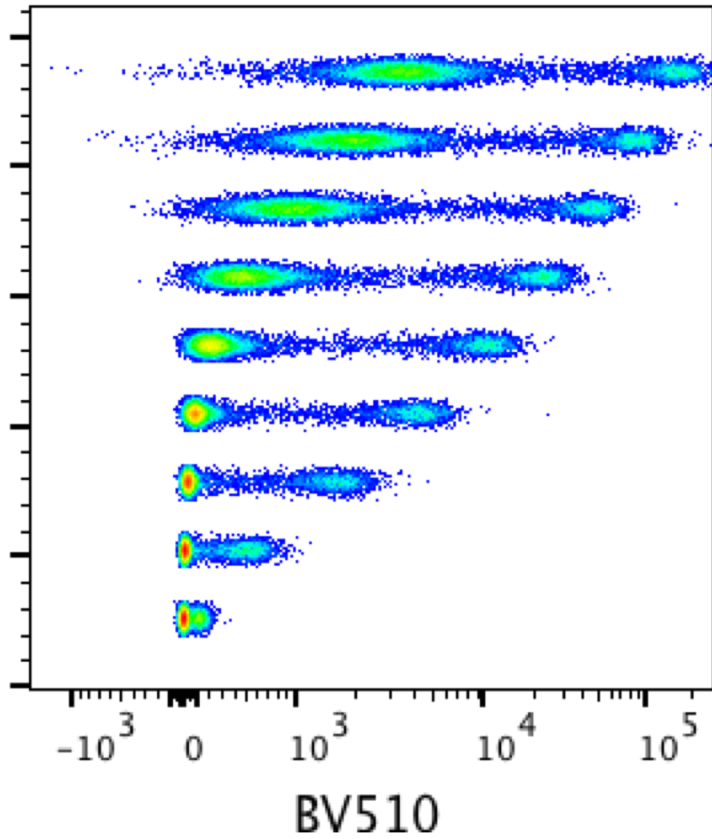
| Voltage | SI |
|---------|-------|
| 650 | 91.39 |
| 600 | 87.97 |
| 550 | 89.58 |
| 500 | 84.25 |
| 450 | 79.36 |
| 400 | 49.30 |
| 350 | 25.11 |
| 300 | 8.38 |
| 250 | 0.00 |



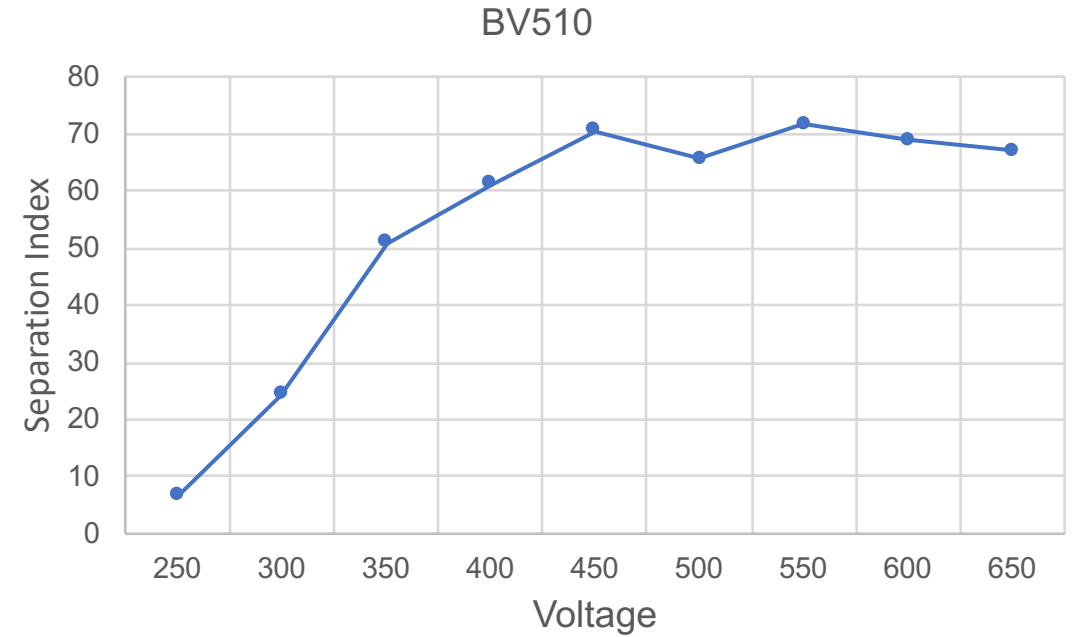
Blue (488 nm)

Dim

BV510



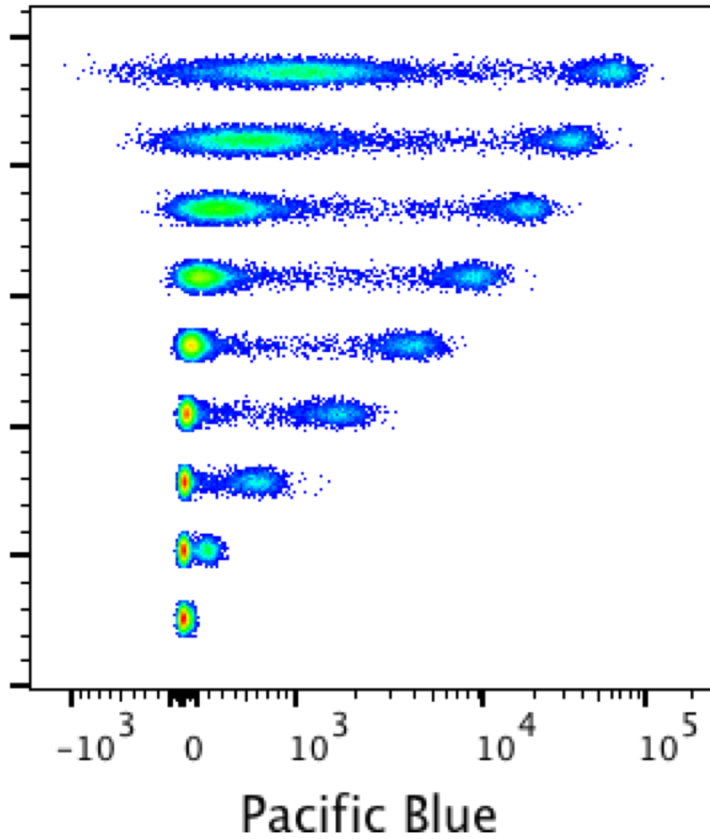
| Voltage | SI |
|---------|------------|
| 650 | 66.8954312 |
| 600 | 68.9270412 |
| 550 | 71.7244134 |
| 500 | 65.5952901 |
| 450 | 70.5326613 |
| 400 | 61.1933037 |
| 350 | 50.9278269 |
| 300 | 24.2408808 |
| 250 | 6.51661182 |



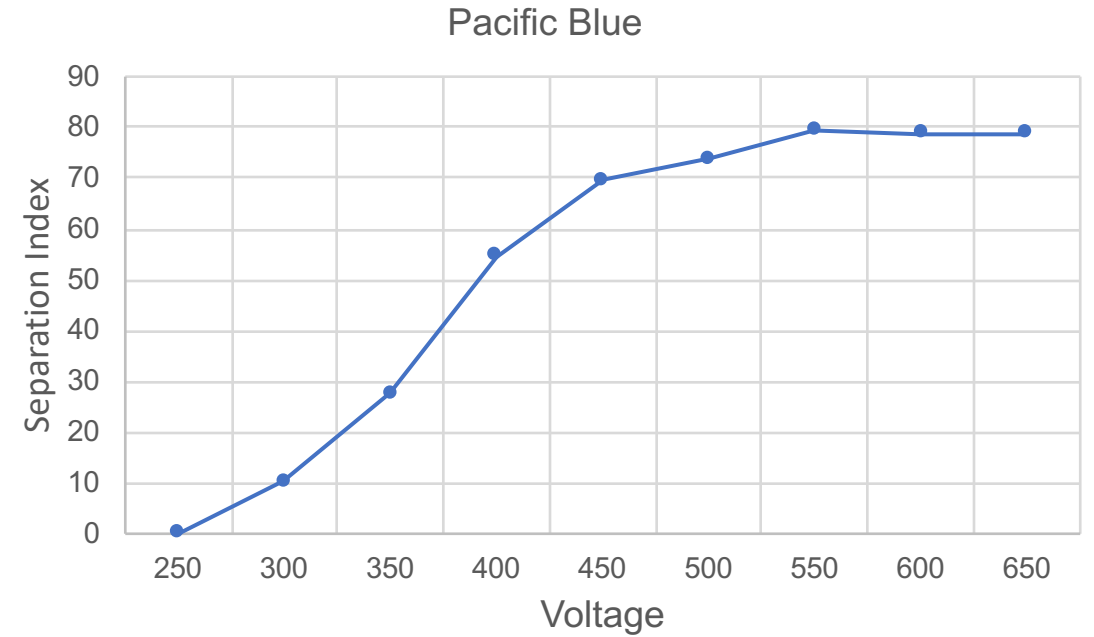
Violet (405 nm)

Dim

Pacific Blue



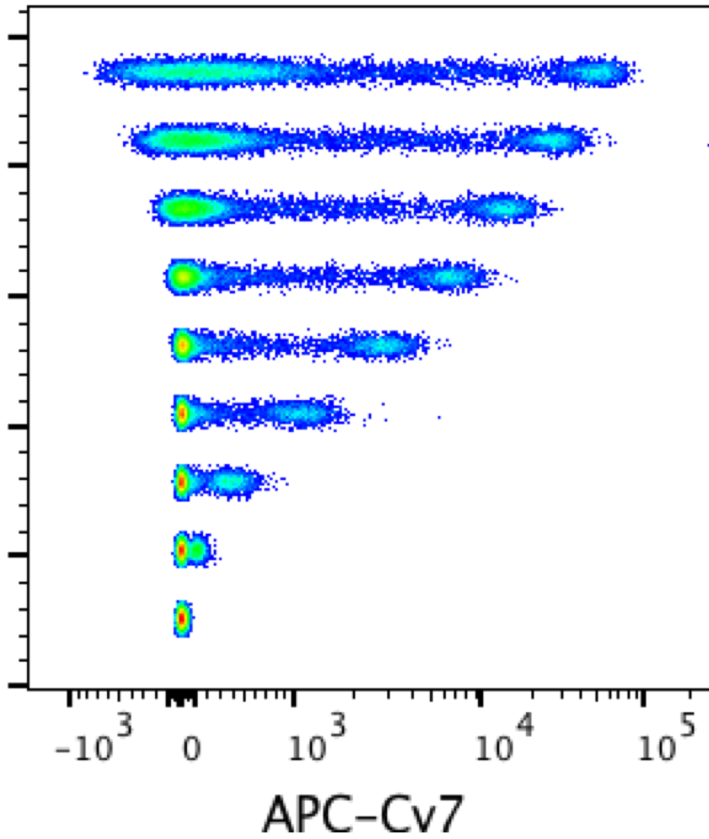
| Voltage | SI |
|---------|------------|
| 650 | 78.6864216 |
| 600 | 78.7039923 |
| 550 | 79.4097794 |
| 500 | 73.8234722 |
| 450 | 69.7037838 |
| 400 | 54.6544326 |
| 350 | 27.5428801 |
| 300 | 10.2346821 |
| 250 | 0 |



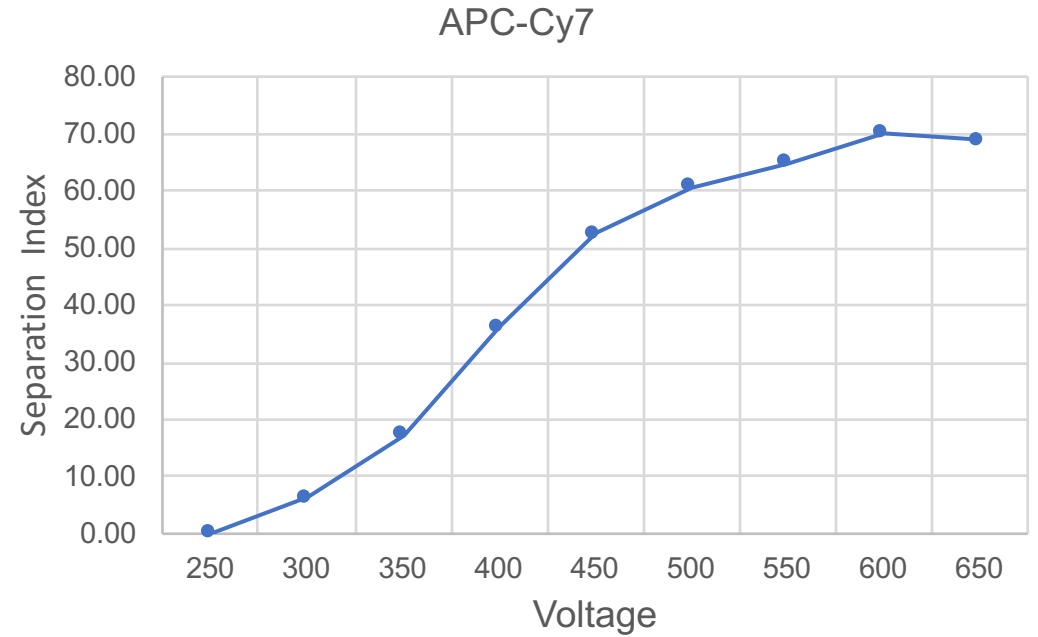
Violet (405 nm)

Dim

APC-Cy7



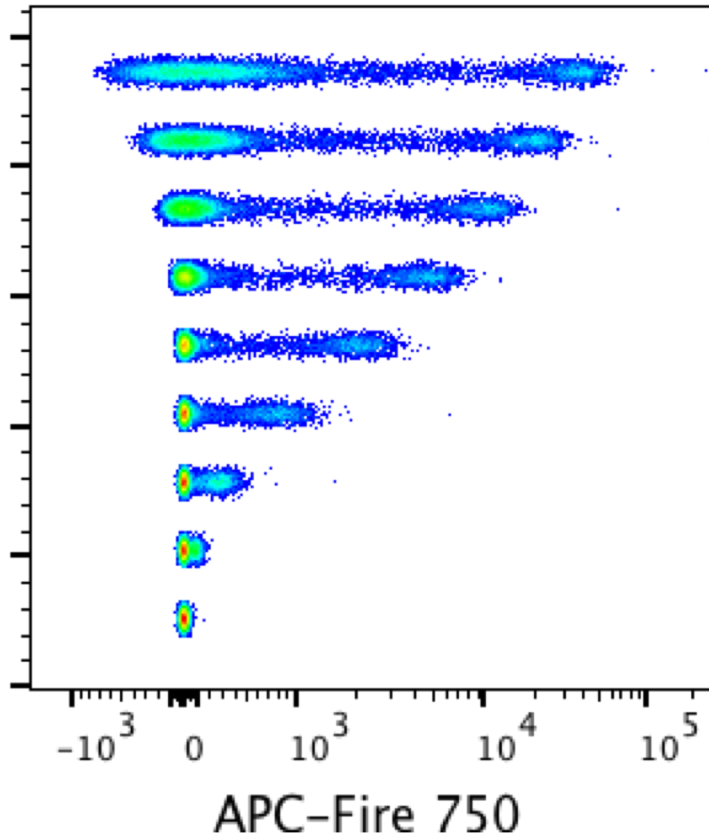
| Voltage | SI |
|---------|------------|
| 650 | 68.8828501 |
| 600 | 70.0759844 |
| 550 | 64.8724383 |
| 500 | 60.6633121 |
| 450 | 52.6217964 |
| 400 | 36.1542468 |
| 350 | 17.3398007 |
| 300 | 6.30132154 |
| 250 | 0 |



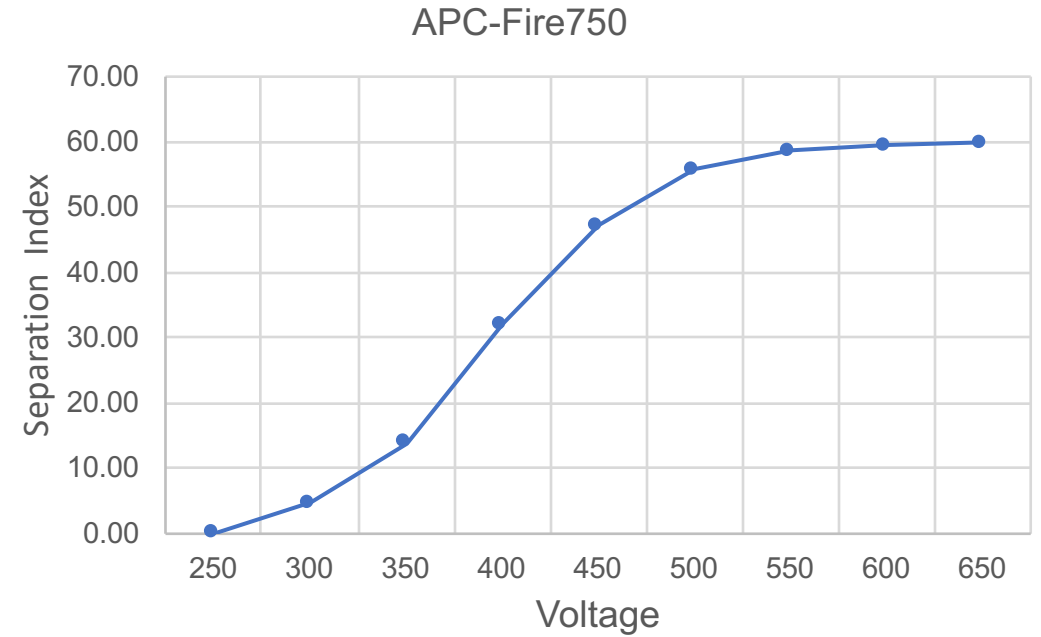
Red (640nm)

Dim

APC-Fire750



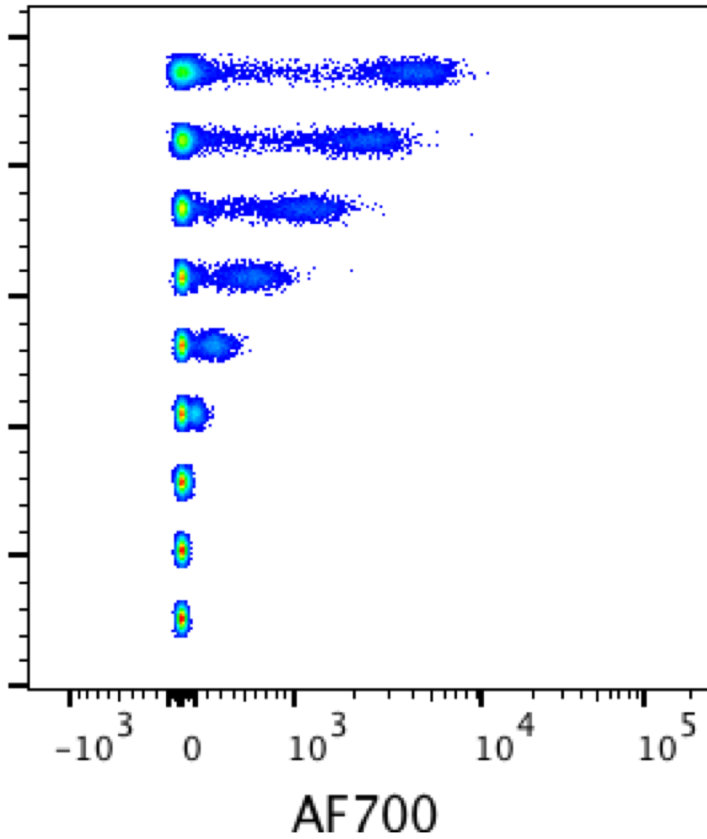
| Voltage | SI |
|---------|------------|
| 650 | 59.936998 |
| 600 | 59.4498603 |
| 550 | 58.7077989 |
| 500 | 55.6729485 |
| 450 | 47.2707658 |
| 400 | 31.9965605 |
| 350 | 13.8595942 |
| 300 | 4.73837232 |
| 250 | 0 |



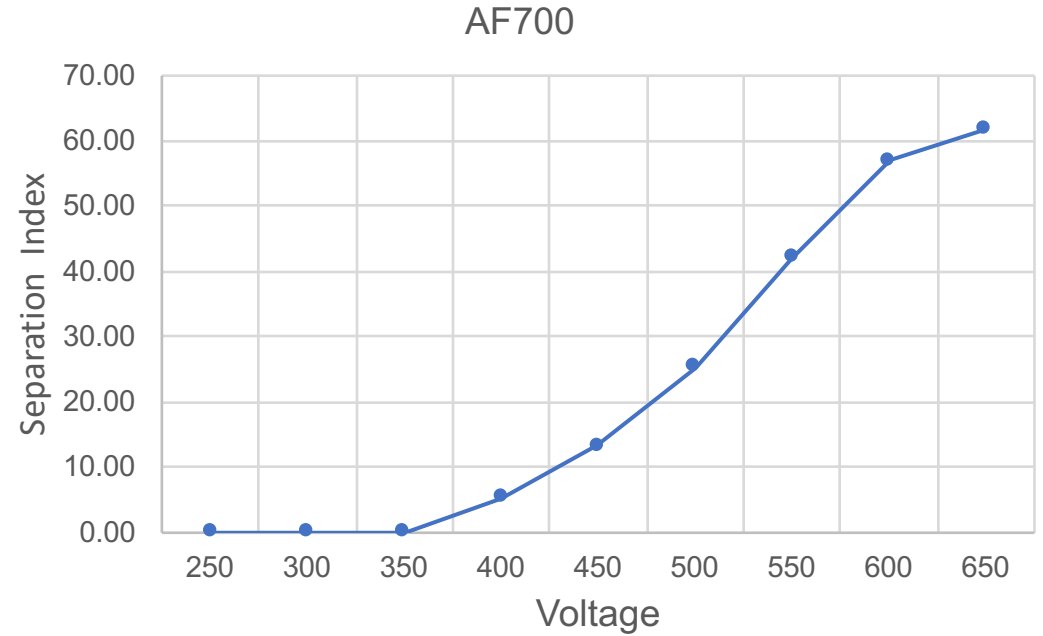
Red (640nm)

Dim

AF700



| Voltage | SI |
|---------|------------|
| 650 | 61.7831825 |
| 600 | 56.9684283 |
| 550 | 41.9792603 |
| 500 | 25.3162093 |
| 450 | 13.2164769 |
| 400 | 5.35296152 |
| 350 | 0 |
| 300 | 0 |
| 250 | 0 |



Red (640nm)

Very Dim