Brilliant Violet™ Considerations for Multicolor Flow Cytometry

BV421™

- BV421™ has an emission spectrum that is more narrow than that of Pacific Blue™, BD Horizon™ V450, eFluor® 450, thus, there is less spillover into neighboring channels, such as AmCyan or Horizon™ V500.
- BV421™ is excitable to some degree by UV laser (350-355 nm) (~25% of maximal excitation), so some compensation would be required when used in combination with UV-excited fluorophores. However, BV421™ is consistently demonstrated to be compatible with viability probes Fixable Live/Dead Blue or DAPI excited off the UV laser.
- For cell surface staining, BV421™ is significantly brighter than other spectrally equivalent fluorophores including Pacific Blue™, BD Horizon™ V450, eFluor® 450, and Alexa Fluor® 405. It has an extinction coefficient of 2,500,000 $M^{-1}cm^{-1}$ at 405 nm, and an aqueous solution quantum yield of 65 ± 5%.
- The BV421 channel (450/50 off the violet laser) can appear to have higher background than is a result of much higher autofluorescent emission in that range.

BV510™

- BV510™ has a similar emission spectrum to that of Horizon™ V500 and so, we recommend using the bandpass filter setting being used for Horizon™ V500 or Live/Dead Aqua (510/50 or 525/50).
- BV510™ is excitable by the UV laser (350-355 nm), so some compensation would be required when used in combination with UV-excited fluorophores, but is not excited by other lasers, such as the 488, 532 or 561 nm lasers.
- For cell surface staining, BV510™ is significantly brighter than equivalent fluorophores including Pacific Orange™ and BD Horizon™ V500.
- When using BV510™ with other Brilliant Violet™ fluorophores, compensation and PMT voltage balancing with BV570™ and some BV605™ will be required. A good strategy when choosing markers for BV510 and BV570 is to choose markers that will not be co-expressed on the same cell.

BV570™

- BV570™ is excited by the violet laser at 405 nm and emits optimally at 570 nm, and can be used in place of Pacific Orange™, Qdot® 565, Qdot® 585, eFluor® 565NC, and eFluor® 585NC.
- BV570™ antibody conjugates provide a good signal-to-noise ratio, although not as bright as BV421™. On a brightness scale of 1-5 with 5 being the brightest, we would give this a 2-3.
- The optimal bandpass filter (585/42) is typically not the default filter on most instruments. Be sure that this bandpass filter is correctly configured on the instrument before using BV570™.
- Using a longer wavelength dichroic mirror, such as 570LP, will help manage the spillover of Horizon™ V500 or Fixable Live/Dead Aqua into the BV570 channel when they are being analyzed simultaneously and will reduce laser signal on instruments equipped with a 561nm laser line. In most other cases, using a LP filter between 545 and 556 nm is acceptable.

- BV570™ has an emission spectrum very similar to that of PE and it can be partially excited by the green laser (532 nm) and the Yellow-Green laser (561 nm) and to lower extend by the blue laser (488nm). This raises potential compensation issues when using the two fluorophores together in a multicolor panel. In order to minimize spill-over/compensation requirements for the PE channel, we advise that users adjust the PMT-V for BV570™ to be higher than PMT-V for PE. The data below is an example of % compensation requirements for two PMT-voltage scenarios, one in which the BV570™ PMT-V is higher and one where the PE PMT-V is higher. Note the % compensation into BV421™ and PE is significantly less when the BV570™ PMT-V is higher.

PMT	BV570	Voltage
PE	5.70%	520
BV421	6.00%	536
BV570		550
PE	20.42%	600
BV421	12.57%	600
BV570		550

**This is only an example for one specific instrument and configuration. Optimization will be required for your specific instrument and configuration. This rule of thumb is true for all aspects of voltage optimization between fluors that experience cross-beam excitation and thus cross-beam spillover.*

BV605™

- BV605™ is excited by the violet laser at 405 nm and emits optimally at 603 nm, and can be used in place of Qdot® 605 and eFluor® 605NC.
- BV605™ antibody conjugates have excellent signal-to-noise as it is very bright and its emission range contains very little background due to autofluorescence. On a brightness scale of 1-5 with 5 being the brightest, we would give this a 5, although not as bright as BV421™.
- BV605™ has very little cross-beam compensation requirements with the 488nm laser. However, there may be some spillover into the PE-TR detector on 561/532nm laser-equipped instruments.
- When used in a panel with BV570™ and BV650™ on the violet laser, PMT voltage balancing will be required between those neighboring channels to manage spillover values, by adjusting the detector voltage until the primary detector MFI is larger than that of any of the secondary detectors.
- The standard Qdot® 605 filter for this PMT, 610/20 nm with a 595LP dichroic, works well for BV605™ detection.

BV650™

- BV650™ is excited by the violet laser at 405 nm and emits optimally at 645 nm, and can be used in place of Qdot® 655 and eFluor® 650NC.
- BV650™ antibody conjugates provide excellent signal-to-noise with brightness rated at 4 on a scale of 1-5 with 5 being the brightest and very little autofluorescent background in its emission range.
- BV650™ has some slight compensation requirements with APC, due to its partial excitation by the 633 or 640 nm laser. The compensation requirements will be minimal and do not normally require any special adjustments.

- The standard Qdot® 655 filter for this PMT, 660/20 with a 630nm LP dichroic, works well for BV650™ detection. When being used together with BV605™, we would recommend a 20nm BP filter window to minimize spillover.

BV711™

- BV711™ is excited by the violet laser at 405 nm and emits optimally at 711 nm, and can be used in place of Qdot® 705 and eFluor® 700NC.
- BV711™ antibody conjugates provide good brightness rated at 4 on a scale of 1-5 with 5 being the brightest.
- The standard Qdot® 700 filter for this PMT, 710/50 with a 685nm LP dichroic, works well for BV711™ detection.
- BV711™ has some spillover into 633 laser-excited fluorophores, due to its partial excitation by the 633 nm laser, as well as moderate spillover into PerCP/Cy5.5. The compensation requirements are manageable and would not normally require any special adjustments.
- BV711™ will have moderate spillover into BV785™. Compensation requirements may be high, but manageable. When used together, we recommend BV785™ for more highly expressed antigens and BV711™ for lower expressing antigens.

BV785™

- BV785™ is excited by the violet laser at 405 nm and emits optimally at 785 nm, and can be used in place of Qdot® 800.
- BV785™ antibody conjugates provide good brightness rated at 3 on a scale of 1-5 with 5 being the brightest. BV785™ has some cross-beam excitation by the red laser and thus a small amount of spillover into APC/Cy7 channel.
- The standard Qdot® 800 filter for this PMT, 780/60 with a 750 nm LP dichroic, works well for BV785™ detection.