Brilliant Violet™ Considerations for Multicolor Flow Cytometry

BV421™

- BV421™ has an emission spectrum that is more narrow than that of Pacific Blue™, 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 most cell surface staining, BV421™ is significantly brighter than most 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%.
- It may be possible to use BV421™ together with Pacific Blue™, but very specific and narrow bandpass filters would be needed. BioLegend does not recommend attempting this for the average flow user as compensation requirements will likely be very high. In addition, stray light from the violet laser can also increase the background signal for BV421™.

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™.
- Use the 575LP filter when using BV570™ with Horizon™ V500 or Fixable Live/Dead Aqua in order to prevent unnecessary spillover and with instruments have equipped with 561 nm 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.*

Using a 575LP for the PE detector also helps to reduce the spillover of BV570™ into PE.

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 provide excellent signal-to-noise ratio with brightness on par with that of BV421™ or PE. On a brightness scale of 1-5 with 5 being the brightest, we would give this a 5.
- BV605™ has very little compensation requirements with all other lasers. When used in a panel with BV570™ and BV650™ on the violet laser, PMT voltage balancing will be required, which means that the default CS&T settings will likely not be optimal when using these together. Additionally, there may be spillover into the PE-TR detector, which is a common detector for 561/532nm laser-equipped instruments.
- The standard Qdot® 605 filter for this PMT, 610/20 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 ratio with brightness rated at 4 on a scale of 1-5 with 5 being the brightest.
- BV650™ has some slight compensation requirements with APC, due to its partial excitation by the 633 nm laser. The compensation requirements will be minimal and would 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 the 660/20 or 670/20 BP filter to minimize spillover.