Brighter is Better:
The Introduction of Brilliant Violet™ Conjugated Antibodies for Flow Cytometry

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Abstract

There is always a need for improved and more sensitive fluorophores in research applications. Brilliant Violet 421™ is a novel polymeric, highly sensitive fluorophore excited by the violet laser, for use in flow cytometry and microscopy. It is ideal for producing antibody conjugates that provide significantly improved signal-to-noise ratios, as much as 12-fold, compared to comparable fluorochromes excited by the violet laser, such as Pacific Blue™. These antibodies are fully compatible for use in multi-color flow cytometry applications, maintaining expected phenotypic frequencies, and are also useful for intracellular staining of cytokines. Furthermore, they work well with common commercial cell staining, fixation, and permeabilization solutions, proving their versatility and ease of use. Thus, antibodies conjugated to these novel Brilliant Violet™ fluorophores could prove to be an enabling technology allowing researchers to resolve lowly expressed antigens with greater resolution and accuracy, providing new and expanded capabilities in flow cytometry using the violet laser.

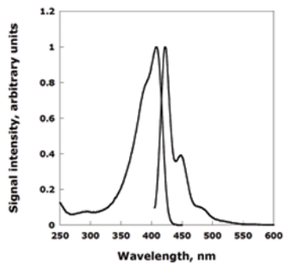
Introduction

Researchers are often limited by the properties of the fluorochrome used in flow cytometry experiments, such as lack of brightness to resolve dim populations, loss of signal after fixation, and excessive compensation requirements. These common problems can be traced back to limitations in dye chemistry. Synthetic organic dyes like Alexa and Cy dyes are too small to absorb enough energy to be bright, while proteins like PE and APC are bright but lack stability post-fixation and lack environmental stability. Here we introduce the first of an entirely novel family of fluorophores called Brilliant Violet™. Brilliant Violet 421™, the first in this family, is as stable as organic dyes but has a signal:noise brightness on par or better than PE. Our data demonstrates the exceptional brightness of BV421™ and its usefulness in cell sorting. We demonstrate how BV421™ antibodies dramatically improve resolution of dim populations and rare populations, and increase the dynamic range of intermediate, transitional populations.

Table 1. Comparison of BV421™ with other fluorochrome families

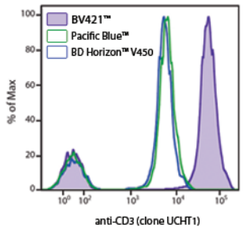
	Brilliant Violet 421™	Violet laser organic dyes (PB, V450)	Phycobiliproteins (PE, APC)	Quantum dots
Laser excitation (nm)	405	405	488, 561, 633	350, 405
Discrete excitation	+++	+++	+++	-
Discrete emission	+++	++	++	+++
Brightness	+++	+	+++	+++
Stability with fixation	+++	+++	+	++
Photostability for microscopy	+++	-	-	+++
Suitability for intracellular staining	+++	+++	+++	+
Compatibility with standard buffers	+++	+++	+++	-
No disposal requirements	+++	+++	+++	-
Antibody selection	++	++	+++	+

Figure 1. Excitation and Emission Spectra of BV421™



BV421™ can be detected using the commonly used bandpass filter: 450/50 nm. With a narrow emission spectrum, BV421™ requires less compensation into the AmCyan detector than Pacific Blue™.

Figure 2. BV421™ brightness is superior to Pacific Blue™ and BD Horizon™ V450 .

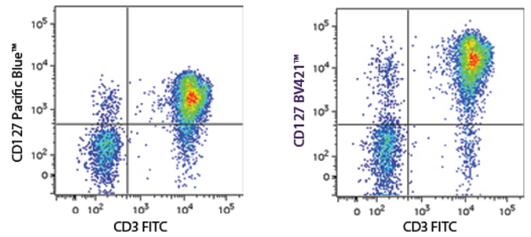


Stain Index

Specificity /Clone	Fluorochrome	Laser Excitation	Stain Index
CD3/UCHT1	Brilliant Violet 421™	405 nm	262
CD3/UCHT1	PE	561 nm	170
CD3/UCHT1	Pacific Blue™	405 nm	59
CD3/UCHT1	BD Horizon™ V450	405 nm	56

Whole blood cells were stained with anti-CD3 conjugated to the above fluorochromes, lysed, washed, fixed and analyzed on the BD LSR II flow cytometer. The data represents the lymphocyte gated population. The stain index values indicated are derived at the optimal concentration for each conjugate.

Figure 3. BV421™ resolves CD127-dim and negative populations.



Whole blood was stained with anti-CD3 FITC and anti-CD127 conjugated to Pacific Blue™ or BV421™. The data shown is at the optimal concentration for each conjugate. BV421™ significantly improves resolution of dim versus negative cell populations.

Figure 4. BV421™ is suitable for intracellular staining using standard fixation/permeabilization buffers.

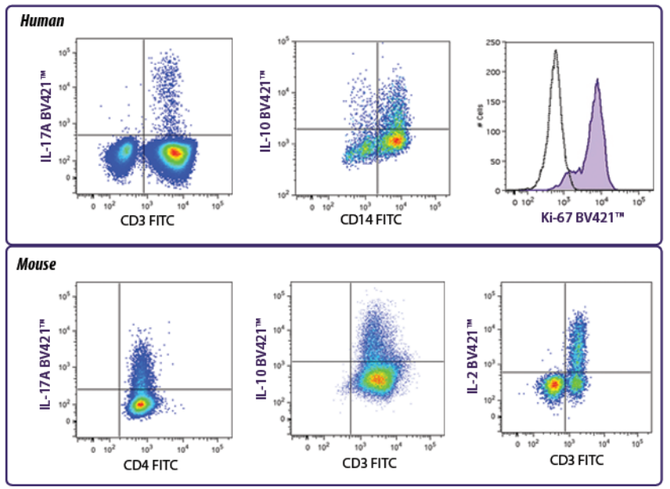
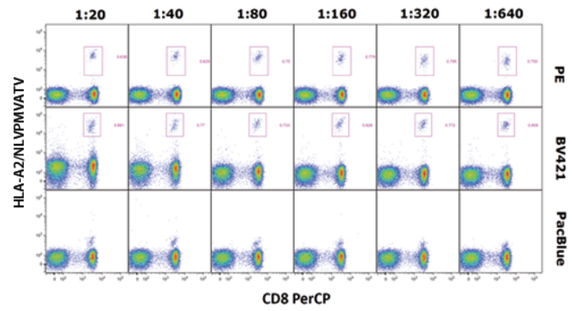
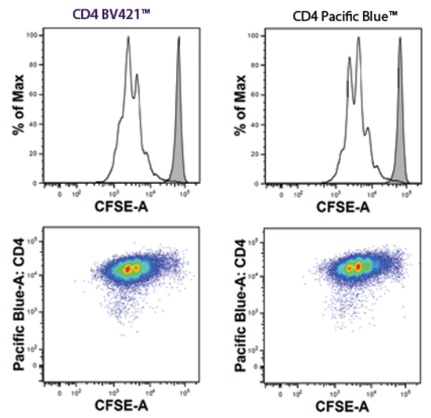


Figure 5. BV421™-labeled tetramer displays expected phenotypic frequencies.



Human blood cells from a CMV+ donor were labeled with titrations of CMV peptide (NLVPMVATV)-loaded human HLA-A2 tetramer bound to Streptavidin-BV421™, -PE, or -Pacific Blue™. The data shows optimal staining with BV421™ when comparing equivalent tetramer concentration and equivalent phenotypic frequencies compared to PE. Data provided by Dr. Rick Willis and John Altman, Emory/Yerkes.

Figure 6. BV421™ is nontoxic and suitable for cell sorting applications.



Pooled Balb/c spleen and lymph node cells were stained with CD4-Pacific Blue™ or CD4-BV421™ and sorted for CD4 positive cells. The cells were labeled with CFSE and stimulated in 96-well flat-bottom plates coated with anti-CD3/anti-CD28 (1 µg/ml each) at 1 x 10^5 cells/well. On day 4, the cells were stained with CD4-Pacific Blue™ and DAPI, and CFSE dilution was analysed in DAPI-CD4+ cells (triplicates). The data demonstrates equal responsiveness of CD4-Pacific Blue™ and CD4-BV421™-sorted cells to stimulation , suggesting that BV421™ is non-toxic and does not interfere with cell stimulation or viability. Data provided by Aras Toker and Jochen Hühn, Helmholtz Centre for Infection Research.

Conclusions

- Brilliant Violet 421™ is the brightest fluorochrome available for the Pacific Blue™ detector excited by the 405 nm violet laser. For surface antigens, it can provide signal-to-noise equivalent to or better than PE.
- BV421™ antibodies are suitable for intracellular staining.
- BV421™ antibodies detect subpopulations at expected phenotypic frequencies.
- BV421™ is non-toxic and suitable for sorting applications.

Learn more about Brilliant Violet™ antibodies at
www.biolegend.com/brilliantviolet.