Vio® Dyes in Spectral Flow Cytometry

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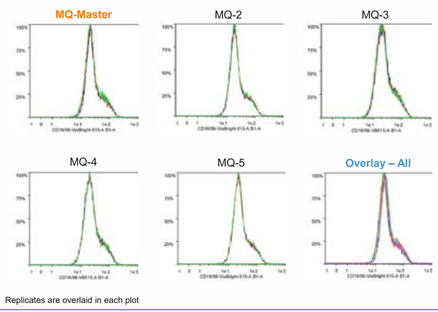
Introduction

MACSQuant® Analyzer Flow Cytometers

High levels of irreproducibility in pre-clinical research cause high economic damage and lead to longer drug development times and in turn, to higher costs for approved drugs and treatments. The major part of irreproducibility is influenced directly by the experimental setup, reagents, data analysis and instrument variation.
The MACSQuant Analyzer flow cytometers address the issue of instrument variation by putting in place a workflow that enables the harmonization of data quality across instruments, sites, users and time by automated recompensation and calibration to minimize detector variabilities and deliver equivalent MFI by smart gain technology. Additionally, algorithm-based data analysis modes reduce operational variabilities.

Figure 1



Replicates are overlaid in each plot

Vio® Dyes and REAfinity™ Antibodies

Flow cytometry has an inherent need to continuously increase the number of fluorochromes to meet the growing demand of sophisticated multicolor analysis. However, fluorochromes vary greatly with regard to their fluorescence intensity. In particular for antigens with low expression levels, it is crucial to select an appropriate fluorochrome-conjugate to obtain sufficiently bright signals for reliable analysis.
Vio Dyes represent a family of fluorochromes for flow cytometry and fluorescence microscopy developed by Miltenyi Biotec. to be an ideal choice for multicolor applications with several advantages:

- High fluorescence intensity with optimal laser and filter compatibility for minimal spillover and compensation
- High stain index for excellent signal to noise ratio and clear population discrimination

Vio Dye bioconjugates are combined with REAfinity antibodies. Recombinant engineering of the antibodies was performed with the flow cytometrical application in mind. A specifically mutated human IgG1 sequence in the constant region of all REAfinity antibodies combines two important benefits when it comes to experiment design, workflow and hands-on time for the user. Unspecific binding to Fc receptors is eliminated, making the Fc blocking step unnecessary. Additionally the universal IgG1 sequence requires only a single isotype control, reducing sample volume drastically.
A biologically and chemically defined production process results in a high lot-to-lot consistency and purity, compared to traditional mouse or rat hybridoma-derived monoclonal antibodies, which is tested thoroughly with biochemical and cell-based methods.

Spectral Flow Cytometry

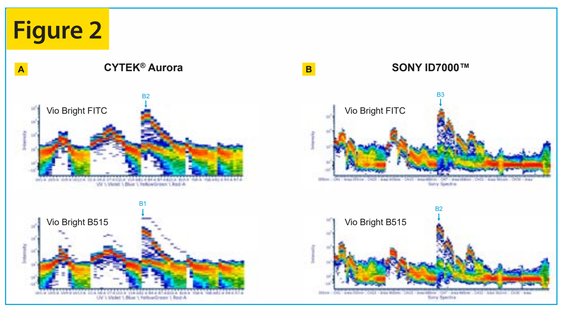
Even though classical flow cytometry is a well-established method with high performance devices available and in use for groundbreaking research, it has reached its limits in the past few years. The demand for multicolor assays seems to have outgrown the limitations of bandpass and dichroic filters where large portions of the available fluorescence signal remain undetected.
In spectral flow cytometry, ideally the full emission spectrum of the fluorophores is captured by a large number of detectors (e.g. 32 or 64) upon excitation by multiple lasers. This approach results in a more detailed signature for each fluorophore. In contrast to classical flow, particularly narrow emission peaks are not really necessary. Instead, it is important that the dyes exhibit unique signatures. The expansion of acquired data is accompanied by an automated and reliable spectral unmixing algorithm that identifies and subtracts cell autofluorescence and separates fluorochrome signals that have very similar emission signatures.

Methods

MACS® Comp Beads (10 min at RT) and Veri-Cells™ PBMC (by BioLegend, 20 min at 4°C) were stained according to protocol with CD4 (REA623) conjugates of various dyes. An unstained control of compensation beads and cells is essential for the automated spectral unmixing of signals. However, the following data are not unmixed to emphasize differences of fluorochrome signal patterns on beads and cells. Before measurement on the Cytek® Aurora, a target channel was defined for each fluorochrome. For the Sony ID7000™ a range of wavelength is chosen to detect fluorescence intensity.

Results

Figure 2



A comparison between bead- and cell stainings with Vio Bright V423 shows a good match of detection patterns between both carrier materials. Cell staining on the Cytek® Aurora shows a higher spreading in intensity with almost no outliers, while the bead staining is more compact with several data points outside of the majority. On the Sony ID7000™ cell- and bead staining result in a much more similar detection pattern, still with a greater spread but less outliers on the cells (Figure 2).
Comparing different Vio Dyes alongside classical fluorochromes, the data revealed unique emission patterns that would be detected in the same channel by classical flow cytometers. For example, on the Aurora Vio Bright FITC gives the strongest signal in the B2 channel, while Vio Bright B515 give the strongest signal in the B1 channel. On the ID7000, Vio Bright FITC gives the strongest signal in the B3 channel, while Vio Bright B515 give the strongest signal in the B2 channel. (Figure 4)

Figure 3

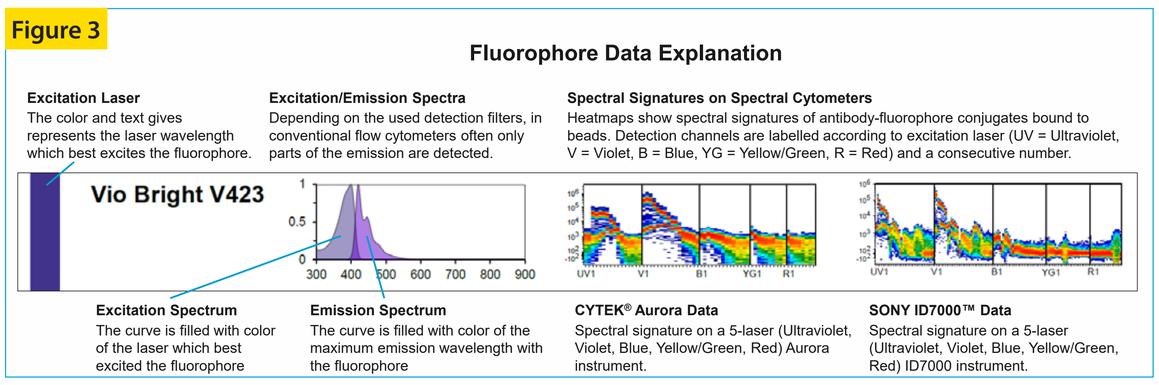
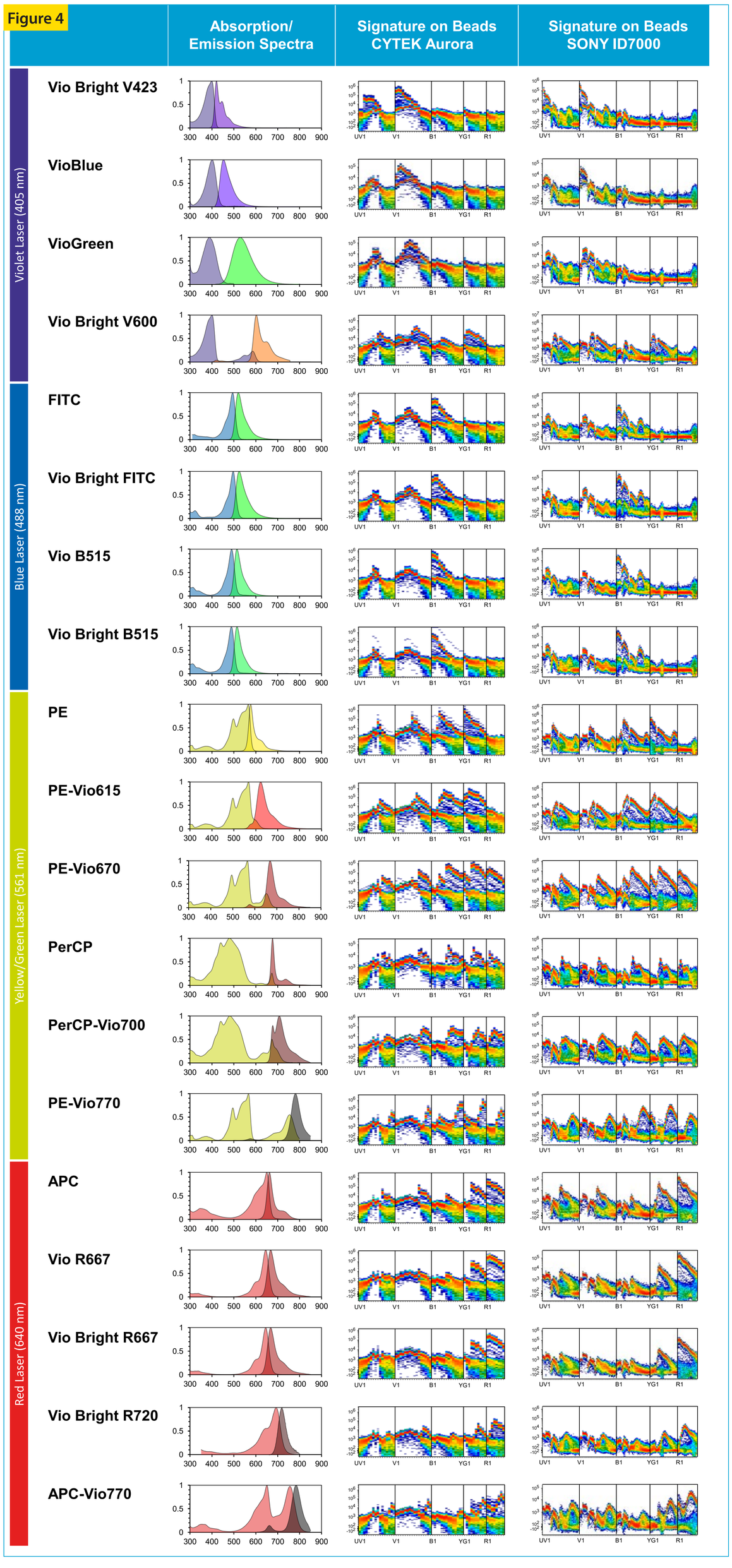


Figure 4



Conclusion

The unique signal patterns on beads and cells make the Vio and Vio Bright conjugates interesting candidates for the application in spectral flow cytometry.

The added value of the Vio Dyes was especially demonstrated on the spectral signature difference of Vio Bright V515 and Vio Bright FITC. This should allow a simultaneous application of dyes that have been mutually exclusive in panel design before.

Acknowledgements

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