Applications for the MACSQuant® VYB

Analysis of fluorescent proteins

Background

The use of flow cytometric techniques for the analysis of cell populations enables scientists to evaluate cells based on both intrinsic and extrinsic properties. Using fluorescent markers such as dyes, antibodies, or fluorescent reporters multiple characteristics of thousands of cells can be investigated within a matter of minutes. Fluorescent protein reporters are used for many applications, including analysis of transcription factors, cell differentiation, cell expression patterns, protein localization, and transfection efficiency. However, the wide array of available fluorescent reporters calls for high-capacity flow cytometers offering the simultaneous detection of even closely related fluorescent spectra (table 1). The MACSQuant® VYB is equipped with three excitation lasers with wavelengths of 405 nm, 488 nm, and 561 nm and can detect up to eight different fluorescent colors. This configuration allows for simultaneous detection of multiple fluorescent proteins such as GFP, YFP, mCherry, and CFP.

Detection of multiple fluorescent cells using reporter cell lines

The MACSQuant VYB is configured to detect multiple fluorescent cells in any sample. HEK293 cell lines provided by Clontech expressing AmCyan (emission max: 489 nm), ZsGreen (emission max: 505 nm), and tdTomato (emission max: 581 nm) were analyzed on the MACSQuant VYB (figure 1). The filter configuration of the MACSQuant VYB facilitates a clear distinction of these fluorochromes using the channels V1, B1, and Y2. Each of these colors is excited by a separate laser, therefore minimizing the spectral overlap and the need for compensation.

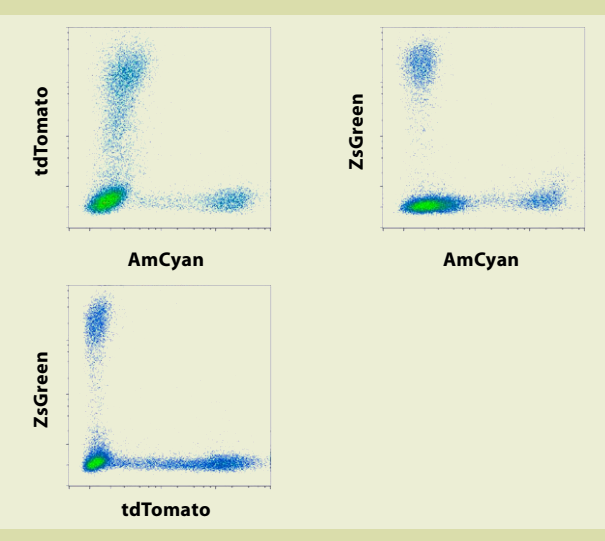

Figure 1: HEK cells expressing AmCyan, ZsGreen, or tdTomato and untransfected cells were analyzed using the MACSQuant VYB.

Laser	Band Pass Filter	Channel	Miltenyi Biotec dyes	Other dyes
Violet 405 nm	450/50 nm	V1	VioBlue	CFP, BFP, DAPI, Pacific Blue, BD Horizon V450, Cascade Blue, AlexaFluor405, eFluor450, DyeCycle Violet, Hoechst Dyes, AmCyan
Violet 405 nm	525/50 nm	V2	VioGreen	vGFP, Pacific Orange, Krome Orange, BD Horizon V500, AlexaFluor430, AmCyan
Blue 488 nm	525/50 nm	B1	FITC	GFP, YFP, DyLight488, CFSE, AlexaFluor488, ZsGreen
Blue 488 nm	614/50 nm	B2	PI	LSS-mKate, YFP, 7-AAD
Yellow 561 nm	586/15 nm	Y1	PE	
Yellow 561 nm	615/20 nm	Y2		mCherry, dsRed, tdTomato, PE-Texas Red, Texas Red
Yellow 561 nm	661/20 nm	Y3	APC	mKate, AlexaFluor647, PE-Cy5, PE-Cy5.5
Yellow 561 nm	750 nm LP	Y4	PE-Vio770, APC-Vio770	mPlum, PE-Cy7, PE-AlexaFluor750, APC-Cy7, APC-H7, APC-AlexaFluor750, APC-eFluor780

Table 1: Table of the MACSQuant VYB channels with the fluorescent proteins and dyes for each channel.

Simultaneous detection of GFP and YFP

The simultaneous detection of GFP and YFP can be challenging with many flow cytometers due to similar emission wavelengths of the two proteins. However, only GFP will be excited at 405 nm. Combining this signal and the YFP signal obtained with a 488 nm laser, discrimination of both signals is possible (figure 2).

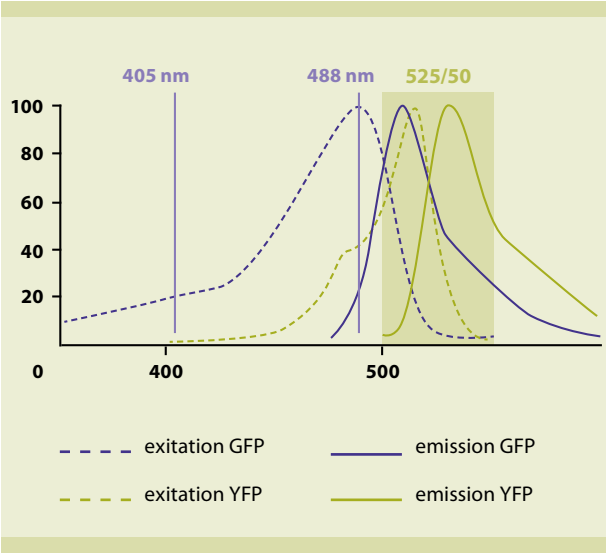

Figure 2: The excitation and emission spectra of GFP (blue) and YFP (green). Shaded area represents the bandpass filter used for collection of the emission spectra of GFP and YFP. The V2 and B1 channels of the MACSQuant VYB both use the 525/50 filter which enables detection of GFP and YFP separately.

Using the MACSQuant Analyzer 10 or the MACSQuant VYB, GFP and YFP can be distinguished by utilizing the 405 nm laser and the channel V2 to detect GFP fluorescence. At the 405 nm excitation, only GFP is detectable within this channel and can therefore be distinguished from the YFP signal by applying compensation to the B1 channel of the 488 nm laser. When proper compensation has been applied, the two cell populations expressing GFP or YFP can be clearly distinguished (figure 3A).

Further, along with GFP and YFP, many other fluorescent proteins can be used simultaneously. In the experiment shown here, GFP, YFP, mCherry, and CFP reporter cell lines were analyzed in one experiment (figure 3).

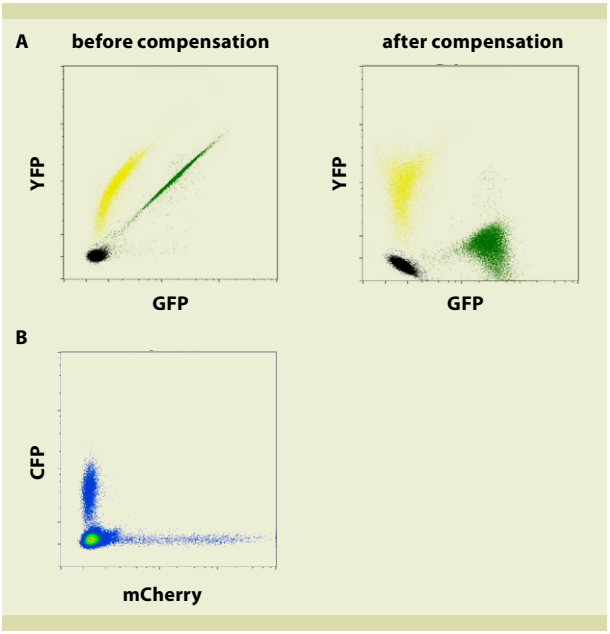

Figure 3: CHO cells transfected with GFP, YFP, mCherry, or CFP were analyzed with the MACSQuant VYB. GFP and YFP were detected using the V2 and B1 channels. (A) Dot plot data of GPF and YFP before and after compensation. (B) Dot plot data of mCherry and CFP.

Conclusions

The MACSQuant VYB is a versatile 3 laser, 10-parameter flow cytometer that has enhanced capabilities for detection of fluorescent protein reporters. As shown here using reporter cell lines, there are a number of fluorescent markers that can be distinguished using this instrument. Virtually any cell can be analyzed based on a number of fluorescent parameters as well as by size and granularity. The power of flow cytometry to analyze thousands of cells per second combined with the ability of the MACSQuant VYB to detect a large range of fluorescent proteins makes this a powerful tool for a wide range of research fields, such as stem cell detection and differentiation, neuroscience, development, cell biology, cancer research, plant, and marine research.

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