Miltenyi Biotec

MACSQuant® Analyzer X

Simultaneous detection of multiple fluorescent proteins using a high-throughput flow cytometer

Introduction

Fluorescence-based techniques are crucial for studying cellular mechanisms as well as cellular structures and functions, including interactions of molecules in biological systems for drug discovery.[1,2] Fluorescent gene products, such as green fluorescent protein (GFP) and its variants, are accepted by molecular biologists as tools that can be linked to cellular proteins so that scientists can visualize components of living systems and understand specific protein-protein interactions as well as transfection efficiency.

Although many researchers use microscopy both to measure transfection efficiency and to identify protein-protein interactions, it can be difficult to distinguish cellular autofluorescence from true fluorescent signals of both the transfected proteins and protein-protein interactions. Therefore unlike flow cytometry technology, microscopy results provide more qualitative than quantitative information. In addition to providing quantitative accuracy of fluorescent protein expression, multiparametric flow cytometry can simultaneously use additional markers, such as DNA and specific cell surface proteins, to identify different cell subsets of interest, for example, proliferating cells, apoptotic cells, etc.

The MACSQuant Analyzer X (MACSQuant X) is a fully-automated flow cytometer consisting of fluidics designed to provide rapid sampling that increase sample throughput. A 384-well plate can be processed in less than one hour, with a reliable absolute cell count still being obtained in the absence of counting beads. The orbital shaker on the MACSQuant X ensures that the automated mixing of samples across the entire plate is standardized and reliable. This application note describes simultaneous measurement of four different fluorescent proteins using the MACSQuant X in a single assay.

Materials and methods

To assess whether four optically distinct fluorescent proteins could be reliably detected by the MACSQuant X in a single cell, four different human cell lines transfected with BFP, GFP, iRFP, and TdTomato were analyzed, both individually and in combination. Cells were plated at equal concentrations in a 96-well plate. Control wells containing cells expressing a single fluorescent protein were used for compensation, while combinations of the proteins were analyzed. Detection filters for all channels in the MACSQuant X were left open to monitor any spillover effects (table 1). Data were acquired and analyzed using the MACSQuantify™ Software.

Excitation laser	Emission filter (nm)	Channel in MACSQuant X	Detection of Fluorescent protein
405 nm (violet)	450/50 nm	V1	BFP
	525/50 nm	V2	
488 nm (blue)	525/50 nm	B1	GFP
	585/40 nm	B2	TdTomato
	655-730 nm	B3	
	750 nm LP	B4	
635 nm (red)	655-730 nm	R1	iRFP
	750 nm LP	R2	

Table 1: Fluorescent protein detection in MACSQuant X.

Results

Acquired cells from each sample were gated based on their forward and side scatter parameters. Doublets were excluded by gating within the FSC-A vs. FSC-H parameter (example shown in fig. 1). All single fluorescent protein-expressing cells showed specific fluorescent signal in their respective detection channel, as shown in table 1 (fig. 1, 2, 3, and 4 respectively). No significant signal could be observed in the adjacent detection channels (fig. 1, 2, 3, and 4). Analysis of the double and triple fluorescent signals also showed specific fluorescent signals in the intended channel with minimal spillover detected by adjacent channels (data not shown). Specific fluorescent signals with high resolution and low spillover remained equivalent in the samples containing all four fluorescent proteins (fig. 5).

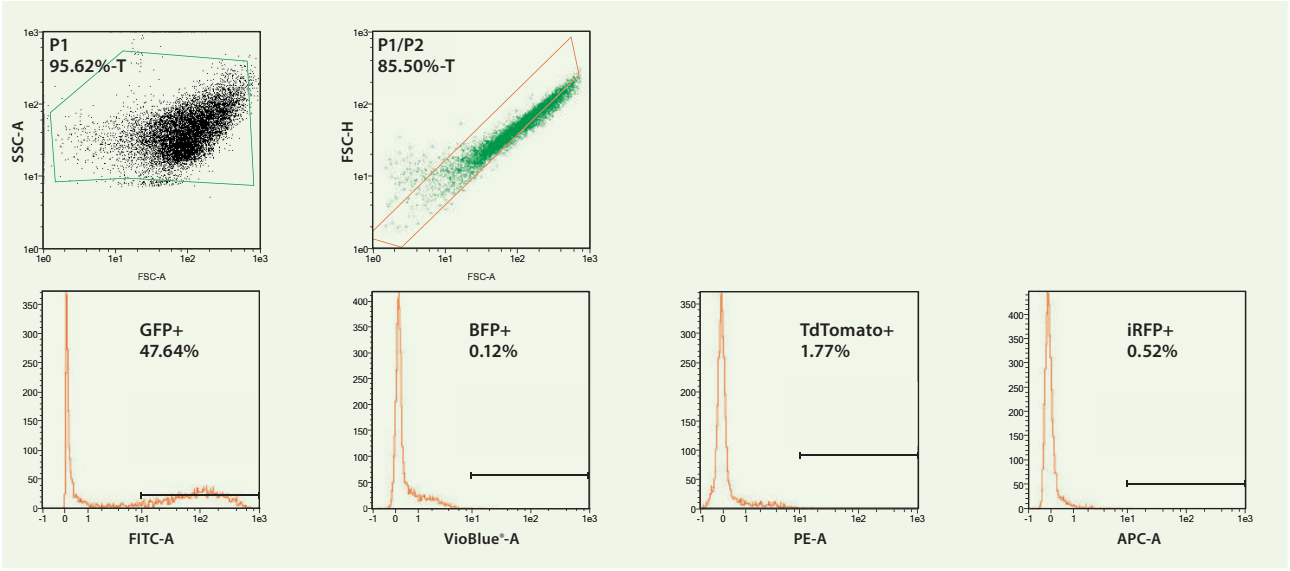

Figure 1: Detection of fluorescent protein expression in GFP-transfected cells.

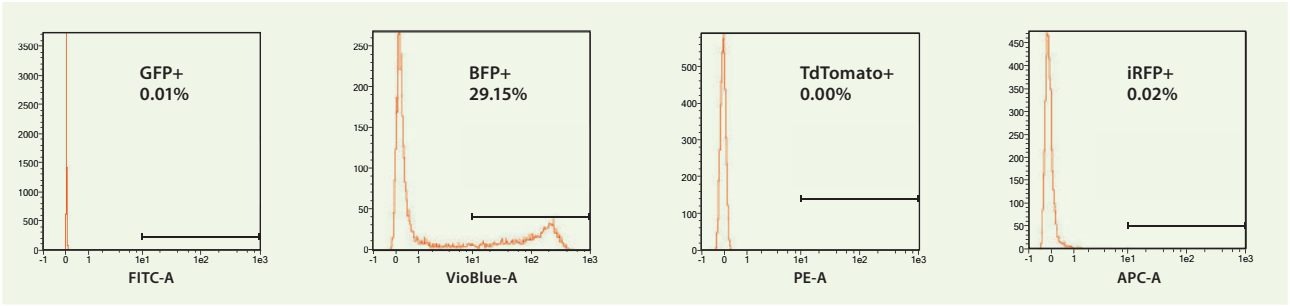

Figure 2: Detection of fluorescent protein expression in BFP-transfected cells.

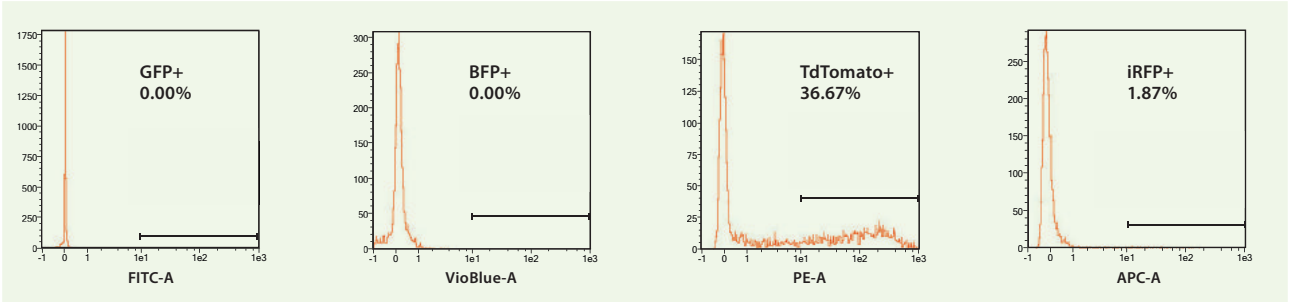

Figure 3: Detection of fluorescent protein expression in TdTomato-transfected cells.

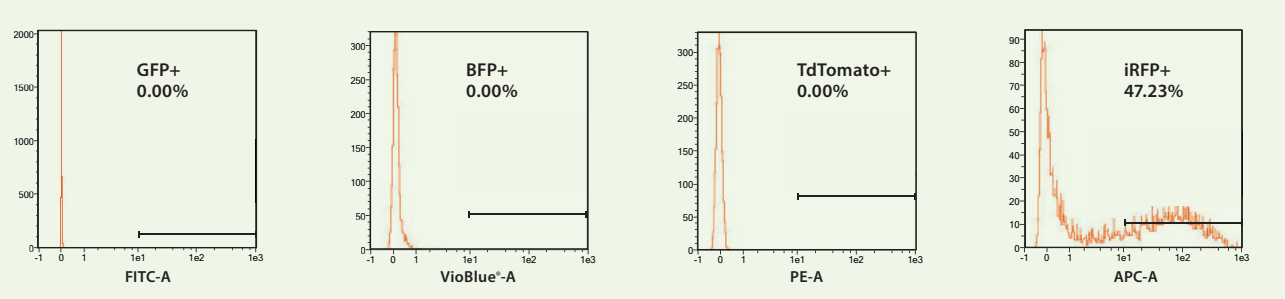

Figure 4: Detection of fluorescent protein expression in iRFP-transfected cells.

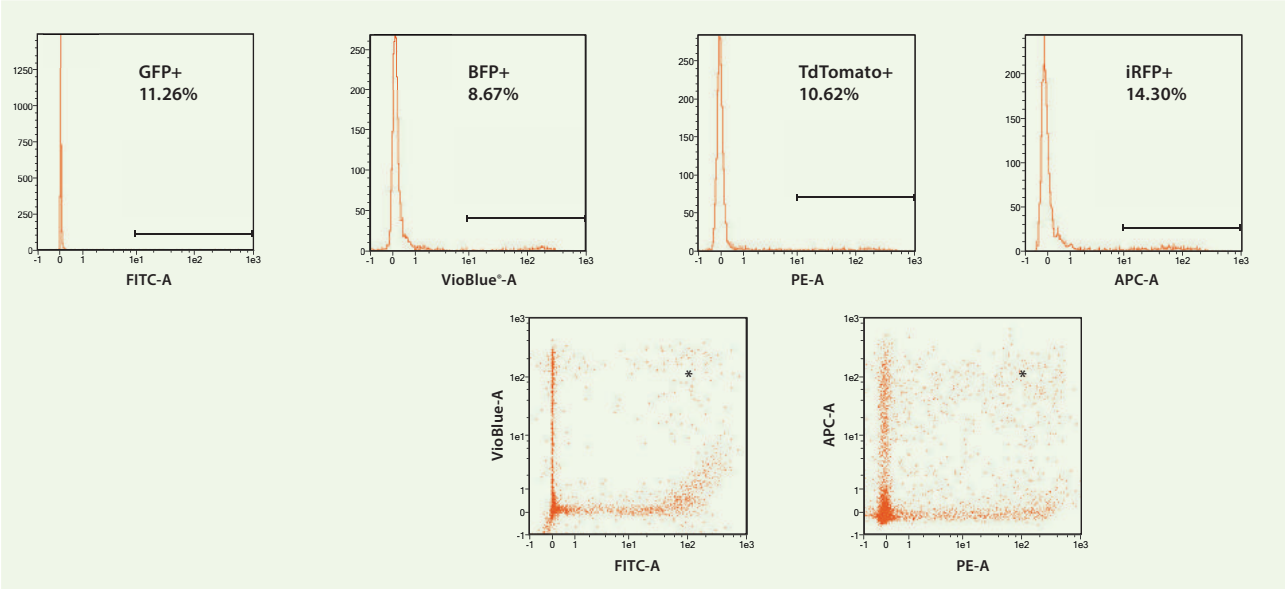

Figure 5: Detection of fluorescent protein expression in cells transfected with all four fluorescent protein. Cells marked with asterisk shows co-transfected cells.

Conclusion

The high throughput MACSQuant® X was capable of successfully detecting four different fluorescent proteins in different combinations in a single assay, with minimal spillover effect and at high resolution. Furthermore, enhanced speed for sample acquisition helps to accelerate the throughput of the assay, making it the perfect fit for large-scale screening assays.

References

1. Chakraborty, C. *et al.* (2009)
Current Pharmaceutical Design 15: 3552–3570

2. Neefjes, J. and Dantuma, N. P. (2004)
Nature Reviews Drug Discovery 3: 58–69

Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

V.01