MULTIPARAMETER STOCHASTIC FLUORESCENCE LABELING OF INDIVIDUAL CELLS AND CELL LINEAGES

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The multicolor cell-labeling method 'Brainbow' is a powerful tool for multiple applications such as mapping dendrites and axons through the nervous system, following individual cells during development, or analyzing cell lineages [1]–[3]. In Brainbow and related methods, unique color tags are generated by the stochastic combinatorial expression of three fluorescent proteins of different colors. However, the number of generated hues is limited, and not enough to reliably distinguish large tissue samples. Moreover, these methods occupy three fluorescence channels; this makes difficult an additional labeling of objects of interest. We present a method of multiparametric labeling of cells, called FLIMbow, that drastically increases the number of generated labels by combining idea of Brainbow and the Fluorescence Lifetime Imaging Microscopy (FLIM) visualizing method. Here we present that imaging in FLIM mode allows to distinguish 2-3 individual FPs with different fluorescence lifetimes and their combinations within one spectral channel (Figure 1). We also demonstrate that the combination of 2 or 3 channels significantly increases the achievable number of unique "color" tags and their reliable discrimination.

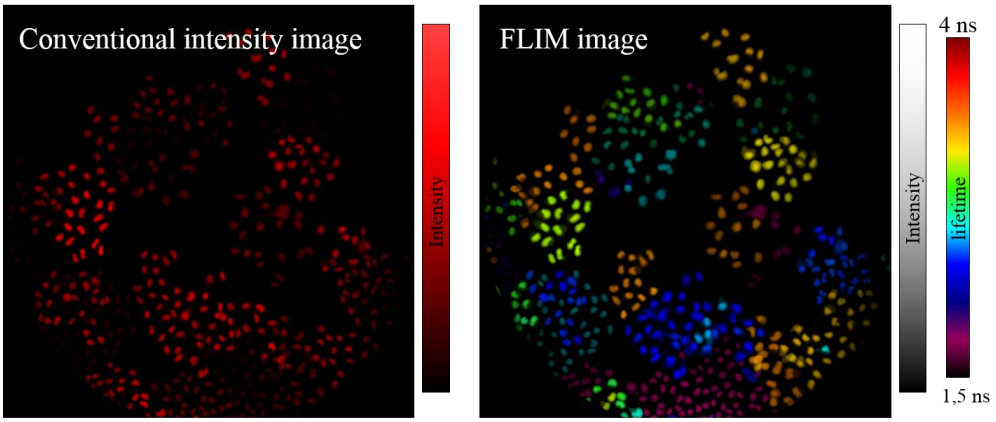

Figure 1. Comparison of a conventional intensity image in red channel and a FLIM image.

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