Quantitative Ultra-fast FLIM and Lifetime based Multi-Species Analysis

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Increasing the speed of Fluorescence Lifetime Imaging (FLIM) is essential for imaging dynamic processes in life science. The rapidFLIM approach dramatically reduces acquisition times through a combination of fast beam scanning, hybrid photomultiplier detectors which are capable of handling very high count rates, and TCSPC modules with ultra short dead times. With the new FLIMbee fast scanning add-on for the MicroTime 200, this technique can be used with our microscopy platform as well as being offered as an upgrade kit for conventional Laser Scanning Microscopes (LSMs). With this hardware combination, excellent photon statistics can be achieved in significantly shorter time spans, allowing fast processes to be measured with the high resolution offered in confocal microscopy. Depending on image size, rapidFLIM enables following dynamic processes like protein interactions, chemical reactions or highly mobile species in live cell imaging with a rate of several frames per second.

The separation of overlapping fluorescence emissions in biological samples has been improved in the last years by using spectral confocal microscopy in combination with linear unmixing. However, the separation of multiple labels in biological samples remains challenging, especially when strong tissue autofluorescence (AF) overshadows specific labeled structures. Combining the spectral approach with fluorescence lifetime measurements based on a simultaneous acquisition of both spectral and lifetime parameters could significantly improve the separation quality between multiple labels and tissue AF. We demonstrate this approach in highly autofluorescent human lung tissue, where the fluorescence signals from specific stainings are weaker than tissue AF. We use dual colour Pulsed Interlevaed Excitation (PIE) in conjunction with a spectral FLIM (sFLIM) detection system featuring eight separate TCSPC timing channels and analyze the data by applying a unique pattern matching technique (1).

(1) Multi-target spectrally resolved fluorescence lifetime imaging microscopy, T. Niehörster et al., Nature Methods, 257-262, **13(3)**, 2016