rapidFLIM - Quantitiative Lifetime Imaging at High Frame Rates and Lifetime based Multi-Species Analysis

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In life sciences, increasing the speed of Fluorescence Lifetime Imaging (FLIM) is essential for imaging dynamic processes such as protein interactions, chemical reactions or highly mobile species in live cells. 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. This hardware combination achieves excellent photon statistics in significantly shorter time spans, allowing fast processes to be measured with the high spatial resolution offered by confocal microscopy. The separation of overlapping fluorescence emission bands in biological samples has been improved over the last years by using spectral confocal microscopy in combination with linear unmixing. However, the separation of multiple labels remains challenging, especially when strong tissue autofluorescence (AF) outshines specifically labeled structures. Combining this spectral approach with FLIM based on simultaneous acquisition of both spectral and lifetime parameters can significantly improve the separation quality between multiple labels and tissue AF. We show application results using both spectrally resolved (sFLIM) and high speed confocal fluorescence lifetime imaging (rapidFLIM). We particularly focus on life science applications to pave the way to monitoring sub-second dynamics in live cell imaging, including lifetime based Förster Resonant Energy Transfer (FLIM-FRET) imaging. Furthermore we show how the inevitable pulse-pile-up occurring in detector signals at high photon flux can be corrected for and how this data acquisition scheme excels in terms of photon collection efficiency in comparison to other approaches.