RapidFLIM a technology for fast, quantitative lifetime imaging and fluorescence lifetime-activated droplet sorting in microfluidic systems

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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. We particularly focus on life science applications where being able to monitor sub-second dynamics is of greatest interest in live cell imaging, including lifetime based Förster Resonant Energy Transfer (FLIM-FRET) imaging. We also 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.

In recent years, chip-based droplet microfluidics has established itself as a standard technique in a diverse filed of research and can be combined with the rapidFLIM approach. One of the reasons behind its rise to prominence is the technique's efficiency and elegance in actively steering processes in complex microfluidic networks. We present here a novel and highly efficient approach for droplet manipulation in a lab-on-a-chip device, called Fluorescence Lifetime-Activated Droplet Sorting (FLADS) [1].

In contrast to the widely used Fluorescence Activated Droplet Sorting (FADS), FLADS uses fluorescence lifetime instead of intensity to discriminate between droplets. Using this lifetime information provides multiple advantages: the detected values are mostly independent of sample concentration, it allows differentiating between fluorophores emitting in the same spectral range as fluorescence lifetimes are compound specific. Furthermore, as lifetime can be influenced by environmental conditions, it could even be used for sensing applications (e.g., pH value or temperature).

The proof-of-concept setup described here is a lab-on-a-chip microfluidic device that uses on-the-fly fluorescence lifetime determination of passing droplets, using commercial Time-Correlated Single Photon Counting (TCSPC) technology in conjunction with a newly developed open source LabVIEW program. The droplets are sorted downstream from the detection point by means of dielectrophoretic force based on the individual droplet's average fluorescence lifetimes. The approach worked reliably at individual substrate concentrations from 1 nM to 1 mM. This not only allowed reliable sorting of droplets containing species with different fluorescence lifetimes but also enabled differentiation of mixtures in individual droplets.