A UNIVERSAL AND EASY-TO-USE FD-FLIM SYSTEM ALLOWING SENSITIVE AND RAPID FLUORESCENCE LIFETIME IMAGING

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Fluorescence lifetime imaging (FLIM) is rapidly taking up a prominent position within the field of microscopy. By providing an additional dimension to the commonly used spectral imaging approaches, it allows multi-probe detection by lifetime discrimination, and sensitive and quantitative intensity-independent FRET measurements. Lifetime imaging in the time domain by time-correlated (TCSPC) and gating techniques has now become state of the art. While providing accurate measurements, they are, however, slow and limited in their resolution. Imaging in the frequency domain (FD) allows for faster imaging, but takes up a privileged position in the researcher's repertoire of analytical techniques and remains confined to few specialized laboratories, often with home-built systems.

We present a new, inexpensive, simple and user friendly FD-FLIM system for rapid and sensitive lifetime imaging. It consists of a newly developed high-resolution solid-state FD-FLIM CMOS camera (PCO), and a dedicated laser coupler (Rapp OptoElectronic) for excitation with stable high-frequency modulated lasers (Omicron Laserage) that can be fitted to existing wide-field microscopes without adaptations. The camera is based on the on-chip lock-in detection technology that we introduced in an early prototype of an all-solid-state FLIM camera for nanosecond fluorescence lifetimes [1,2]. Technological developments have now resulted in a scientific camera for fast lifetime imaging at high (1024 square) spatial resolution that combines the advantages of frequency- and time-domain instruments. Together with the development of a dedicated homogeneous wide-field laser illumination solution, we now provide a comprehensive and easy-to-use package for a broad range of biological applications.

We will exemplify the use of this FD-FLIM system for multiple applications; with sensitive high-resolution imaging of protein dimerisation, rapid Ca^{2+}-dependent ion channel regulation as well as tissue imaging and probe multiplexing. We foresee the contribution of this system to the increasing demand for quantitative investigations in a wide range of biological and biomedical applications.

References:

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