SINGLE WAVELENGTH EXCITATION DUAL COLOR FLIM FOR MULTIPLEX KINASE ACTIVITY MEASUREMENTS IN LIVING CELLS

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Genetically encoded Förster Resonance Energy Transfert (FRET) biosensors are powerful tools for monitoring spatiotemporal biochemical activities in living samples. By labelling a probe protein with a pair of fluorescent proteins, FRET measurement allows to follow a conformational change of the probe sensor to a specific activity. A very exciting challenge is to follow two FRET biosensors at the same time in the same sample. But the multiplex approach suffers from two limitations: (i) a spectral bleed through of the first acceptor in the second donor emission band that depends on the concentration of the two biosensors and (ii) the multiple excitation wavelengths which necessitates sequential acquisition that is not adequate to follow fast signal changes in highly dynamic biochemical activities.

Here, we report a method alleviating from both limitations. Taking advantage of the long stoke shift of LSSmOrange[1], we have used 440 nm single excitation wavelength of the two donor mTFP1 and LSSmOrange and a dual color FLIM to simultaneously measure two genetically encoded FRET biosensors. Moreover, thanks to the non-fluorescent acceptor sREAcCh [2] for mTFP1 and of red-shifted mKate2 for LSSmOrange, we were able to neglect any spectral bleed trough. These acquisitions were carried out on our fastFLIM system [3]. This prototype combines a supercontinuum laser, a spinning disk system and a fast-gated intensifier coupled to a CCD camera which permits optical sectioning, spatial and temporal resolution. With a dual spectral system we were able to detect fluorescence lifetime images of mTFP1 and LSSmOrange simultaneously. The methodology allows measuring sequences of biosensor measurements at a frequency up to 1Hz. We validated our approach by applying this methodology to simultaneously ERK and PKA activation using EKAR2G [4] and AKAR4 [5] biosensors respectively modified with mTFP1/sREAcCh and LSSmOrange/mKate2 fluorescent protein pairs.