FAST FLUORESCENCE LIFETIME IMAGING FOR THE ANALYSIS OF SIGNIFICANT BIOLOGICAL FUNCTIONS WITH FLUORESCENCE BIOSENSORS

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Fluorescence biosensors have allowed the measurement of many metabolites, signaling cues and other signal transduction events [1] in live cells to enable a better understanding of cellular dynamics. Biosensors have been the tool of choice for dynamic changes over time. The use of such biosensors required fine tuning of experimental conditions and calibrations. Additional because of the often short temporal window for many changes within cells, the tool of choice for such biosensors has been the use of calibrated fluorescence intensities, often used in a ratiometric mode. In deed many biosensors exhibit a change either in their excitation or emission spectra that can then be followed by ratiometric imaging. Another imaging modality that could be well suited for the use of biosensors is fluorescence lifetime imaging (FLIM). Nevertheless historically the need for high photon budgets and statistics while acquiring at low photon flux to avoid artefacts made FLIM difficult to use in the fast paced environment of biosensors. The fast acquisition of FLIM images (down to 30 ms for a FLIM image with sufficient statistics) on the Leica SP8 FALCON and the tight integration with all other confocal modalities (tile scans, time lapse, 3D stacks, lambda scan and multiphoton) have opened the door to use a wide range of biosensors with FLIM. The intensity and concentration independence of FLIM simplifies the process of implementation for biosensors and the speed allows to tackle high number of events in live cell experiments.

[1] Rodriguez EA, Campbell RE, Lin JY, Lin MZ, Miyawaki A, Palmer AE, Shu X, Zhang J, Tsien RY. The Growing and Glowing Toolbox of Fluorescent and Photoactive Proteins. *Trends Biochem Sci.* 2017 Feb;42(2):111-129. doi: 10.1016/j.tibs.2016.09.010. Epub 2016 Nov 1. Review.