Ultra-broadband few-cycle laser pulses for advanced multi-color FLIM-microscopy

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Multi-photon (MP) microscopy is favored by its deep-tissue imaging capabilities, reduced scattering and lowered photo-toxicity. With standard femtosecond (fs) lasers for multi-color imaging either one employs multiple sources for simultaneous excitation or performs multiple scans spectrally tuning a single source. Here, we demonstrate the novel and patent-pending SyncRGB-FLIM method, where a few-cycle ultra-broadband laser source provides an alternative path for multi-color imaging. Within a single scan, the ultra-broadband source allows simultaneous excitation of various chromophores. A single detector channel attached to a time-correlated single photon counting (TCSPC) module enables distinguishing dyes by their characteristic fluorescence lifetimes [1,2]. After compression at the focus of the objective, the few-cycle fs laser delivers sub-10 fs pulses at the focus, with >400 nm bandwidth, covering the two-photon absorption range of RGB emitting chromophores. Advantage compared to a 70 fs laser excitation can be observed in Fig 1, where the MP-FLIM and SyncRGB-FLIM images are compared.

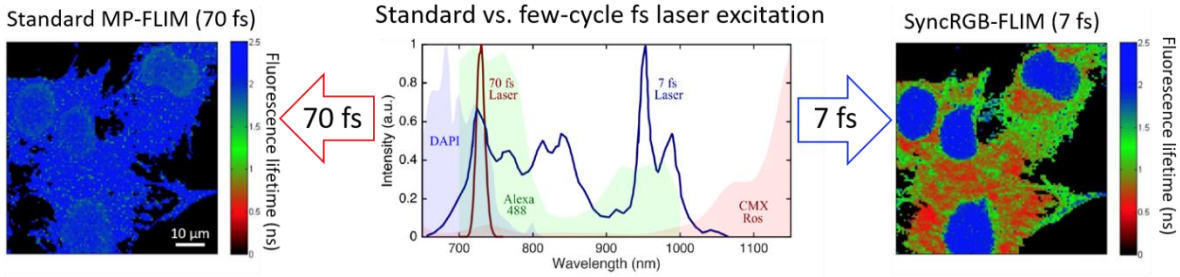

Fig. 1 MP-FLIM image recorded with 70 fs laser source. Laser spectra and two photon absorption profiles of the FlouCells #1 sample (DAPI, Alexaflour488 and CMX Ros). SyncRGB-FLIM image taken with 7fs few cycle laser resolving nuclei, actin filaments and mitochondria simultaneously.

The potential of the SyncRGB-FLIM method to be deployed in nanomedicine research was in a live-cell imaging study. The Ultrabroadband excitation was used to excite the endogenous markers (NADH/NADPH) and simultaneously a fluorescent drug Doxorubicin (DOX). We were able to follow cell uptake and metabolic response simultaneously. Currently, a fast laser-scanning SyncRGB-FLIM demonstrator system is being built with the support of the FCT UTAustin-Portugal ExtreMed project [3].

[1] C. Maibohm, F. Silva, E. Figueiras, P. T. Guerreiro, M. Brito, R. Romero, H. Crespo, and J. B. Nieder, "SyncRGB-FLIM: synchronous fluorescence imaging of red, green and blue dyes enabled by ultra-broadband few-cycle laser excitation and fluorescence lifetime detection," *Biomed. Opt. Express* 10, 1891-1904 (2019)

[2] METHOD AND APPARATUS FOR SIMULTANEOUS NONLINEAR EXCITATION AND DETECTION OF DIFFERENT CHROMOPHORES ACROSS A WIDE SPECTRAL RANGE USING ULTRA-BROADBAND LIGHT PULSES AND TIME-RESOLVED DETECTION (EP 19724610.1-EU, EP 17047249 -US patent applications)

[3] https://utaustinportugal.org/projects/extremed/ , NORTE-01-0247-FEDER-045932.