MEDICAL PHYSICS

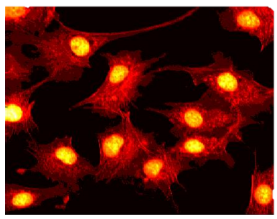
UTAustin Portugal

SyncRGB-FLIM: Synchronous fluorescence imaging of Red, Green and Blue dyes enabled by ultra-broadband few-cycle laser excitation and fluorescence lifetime detection

Christian Maibohm[1]
Francisco Silva[2]
Edite Figueiras[1]
Paulo T. Guerreiro[2]
Marina Brito[1]
Rosa Romero[2,3]
Helder Crespo[2,3]
Jana B. Nieder[1]

Background

Bioimaging is one of the most used characterization technique for medical diagnosis. Often only a small time window is available to analyze complex samples containing multiple chromophores, e.g. live cell or tissue samples.



Problems overcome by SyncRGB-FLIM Method, compared to traditional multicolor bioimaging

- *Multiple scans which may lead to phototoxicity*
- *Wavelength tuning*
- *Multi-detector setup*

Methodology

Here we aim to shorten the required bioimaging time by a simultaneous excitation/ detection scheme that provides multi emitter information from a single scan.

This is realized by using a few-cycle laser source able to simultaneously excite chromophores of different colors. Its ultrabroadband spectrum for excitation is combined with a time-correlated single-photon counting (TCSPC) detection scheme. The localization of different chromophores in the cell can be identified based on their fluorescence decay properties.

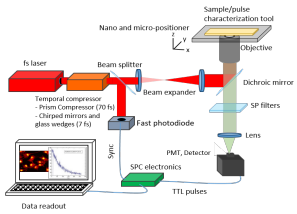


Fig 2: SyncRGB-FLIM setup. *Few-cycle broadband laser source, temporal compressor, and a sample scanning inverted microscope equipped with a single PMT detector synchronized with the pulsed laser via fast single-photon counting electronics.*

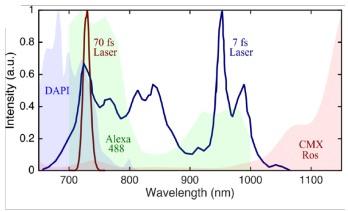


Fig 3: *Normalized spectra of a 70 fs laser and the ultra broadband 7 fs laser, with adapted single photon absorption spectra of, DAPI (cell nuclei), Alexfluor 488 (actin filaments) and Mito Tracker Red (CMX) (mitochondria).*

Results

We demonstrate for the first time that an ultra-broadband 7 femtosecond (fs) few-cycle laser can be used for multicolor nonlinear imaging in a single channel detection geometry, when employing a time-resolved fluorescence detection scheme.

On a multi-chromophore-labelled cell sample we show that the few-cycle laser can efficiently excite the multiple chromophores over a >400 nm two-photon absorption range and distinguish the three different labels via their fluorescence lifetime differences [1, 2].

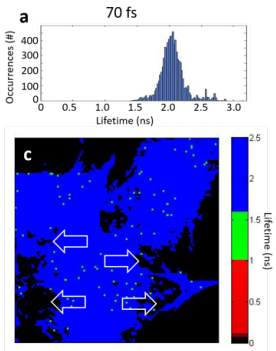


Fig 4: *SyncRGB FLIM images of 2D FluoCells® #1 sample. Average fluorescence lifetime histograms for the a) 70 fs and b) 7 fs laser systems taken from the image data shown in c) and d) for the 70 fs and 7 fs , respectively. Arrows highlight areas to which 70fs is insensitive, while with the 7fs excitation and FLIM detection signals from all 3 dyes can be distinguished.*

Impact/Conclusions

The novel SyncRGB-FLIM multi-color bioimaging technique opens the door to real-time multi-color studies, where its single-scan operation translates into reduced laser exposure of the sample and more photoprotective conditions for biological specimens, such as *in vitro* cells and tissues, as well as for *in vivo* applications. On the way to establishing the SyncRGB-FLIM technique for diagnostics in clinics, the next step is to demonstrate advantages on pre-clinical diagnostics of patient samples.

References

[1] Maibohm, Silva, Figueiras, Guerreiro, Brito, Romero, Crespo, Nieder **SyncRGB-FLIM: synchronous fluorescence imaging of red, green and blue dyes enabled by ultra-broadband few-cycle laser excitation and fluorescence lifetime detection,** Biomedical Optics Express 10 (4), 1891-1904

[2] **PCT/IB 2019/14435 'Method and apparatus for simultaneous nonlinear excitation and detection across a wide spectral range using ultra-broadband light pulses and time-resolved detection', PCT – International,** Applicants: **INL, Sphere Ultrafast Photonics, S.A.,** Inventors: **Nieder, Maibohm, Silva, Crespo, Romero**

ANI Agência Nacional de Inovação · NORTE2020 · PORTUGAL 2020 · União Europeia Fundo Europeu de Desenvolvimento Regional

UTAustin Portugal · FCT Fundação para a Ciência e a Tecnologia · TEXAS The University of Texas at Austin

1 INL International Iberian Nanotechnology Laboratory

2 sphere ULTRAFAST PHOTONICS

3 IFIMUP–IN Material Physics Institute of the University of Porto

U.PORTO FC Faculdade de Ciências Universidade do Porto