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

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.

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.

Results
We demonstrate for the first time that an ultrabroadband 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].

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 ultrabroadband few-cycle laser excitation and fluorescence lifetime detection, Biomedical Optics Express 10 (4), 1891-1904

Problems overcome by SyncRGB-FLIM Method, compared to traditional multicolor bioimaging
• Multiple scans which may lead to phototoxicity
• Wavelength tuning
• Multi-detector setup

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 ultrabroadband 7 fs laser, with adapted single photon absorption spectra of DAPI (cell nuclei), Alexafluor 488 (actin filaments) and Mitotracker Red (CMX) (mitochondria).