Few-cycle ultra-broadband beam scanning microscope prototype

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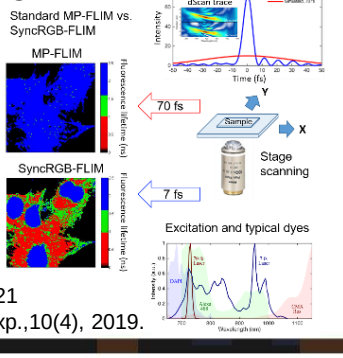
Introduction and background

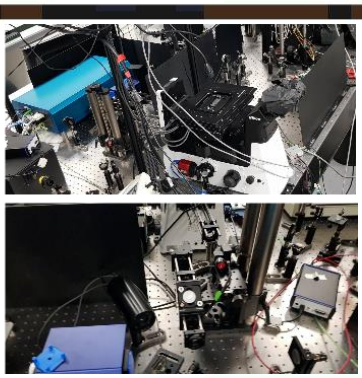
The patented[1] SyncRGB-FLIM method has been successful showcased with all its merits in a *stage scanning* microscope configuration[2], but not in a fast *beam scanning* configuration due to the difficulty of pulse control through a scanning system.



1. US Patent App. 17/047,249, 2021
2. Maibohm et.al., Biomed. Opt. Exp.,10(4), 2019.

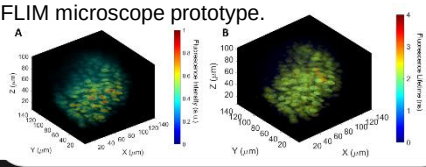
Current status

A beam scanning SyncRGB-FLIM microscope prototype has successfully been developed where the sub 10 fs laser pulse is maintained through a scanning system (A) reaching the microscope platform with a high NA microscope objective (B). The prototype is equipped with custom written scanning software providing device control, as well as live multi exponential fluorescence lifetime fitting and representation.



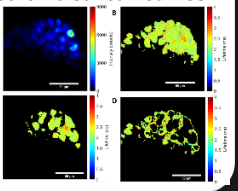
3D Spheroid imaging

3D fluorescence intensity (A) and fluorescence lifetime (B) stack of a A549 cell based spheroid with H33342-labelled nuclei recorded with the beam scanning SyncRGB-FLIM microscope prototype.



Separation of components based on fl. lifetime

For a slice at z=48µm signals were separated based on distinct lifetimes. Intensity (A), FLIM (B), separated , H33342 labelled nuclei (C), label-free cytoplasm (D).



Summery, next steps and partners

- With the SyncRGB-FLIM prototype we are able to perform fast beam scanning imaging with sub 10 fs laser pulses on relevant biological samples.
- Next is the inclusion of active pulse shaping for further control and optimization as well as functional imaging in context of nanomedicine.

1 INL International Iberian Nanotechnology Laboratory
2 sphere ULTRAFAST PHOTONICS
3 IFIMUP–IN Material Physics Institute of the University of Porto
3 U.PORTO Faculdade de Ciências Universidade do Porto

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