Supercontinuum Radiation for the Development of Automated Microscopy Platforms

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Project Objectives

The main objective of this project is to develop super-continuum-enabled, multi-modal fluorescence microscopy platforms for bioimaging, drug screening applications and research projects related to neurode-generative diseases.

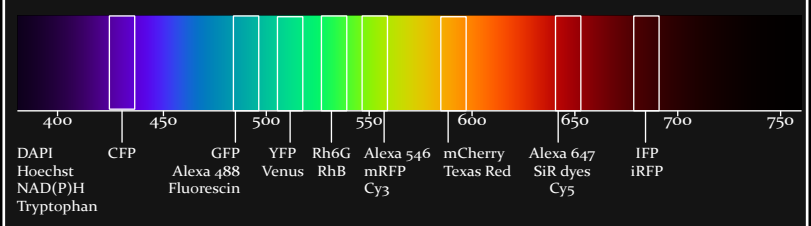
Supercontinuum sources make excellent illumination sources for our fluorescence lifetime imaging (FLIM) platforms because of their:

1. Broad bandwidth (full visible spectrum+IR)
2. Pulsed output (MHz repetition rates)
3. Spatial coherence and high power (>1 mw/nm)

FLIM measures the rate of decay of fluorescence emission, which contains valuable information about the micro-environment of the fluorescent species. FLIM can be used to quantitatively measure pH, tempe-rature, viscosity, ion concentration, FRET, biosensor activity, protein aggregation.

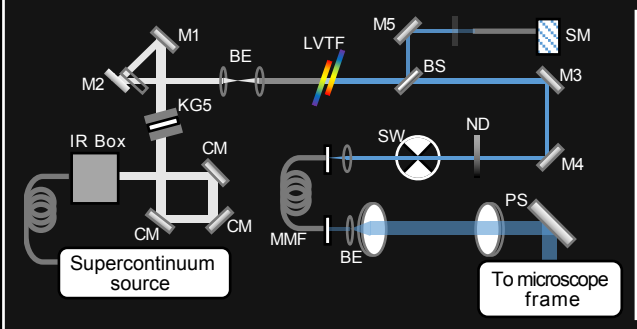
We have built two FLIM platforms incorporating super-continuum sources. They are already in use in research projects but need upgrades for fully automated, mul-ti-modal and UV-compatible functionalities. (project deliverable D5.7)

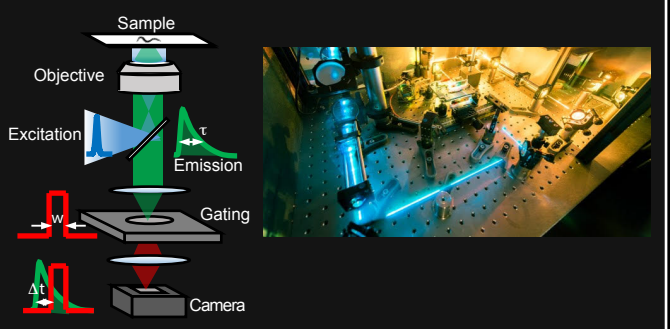
A single broadband SC source can excite many fluorophores



FLIM platforms

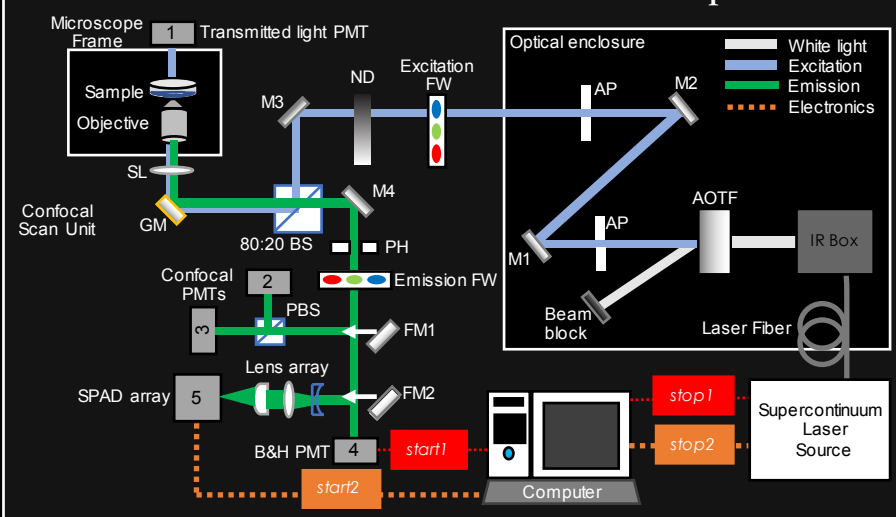
A. Automated time-gated FLIM platform

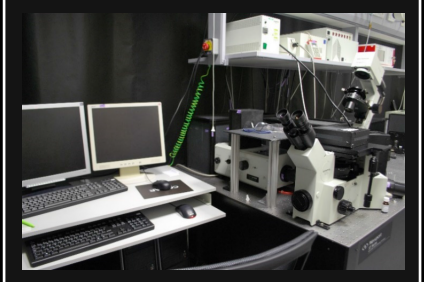




- Collects photons for every image pixel simultaneously using fast gating.
- Reduces FLIM acquisition time from a few minutes to few seconds.
- Future work will enable unsupervised imaging for hours.

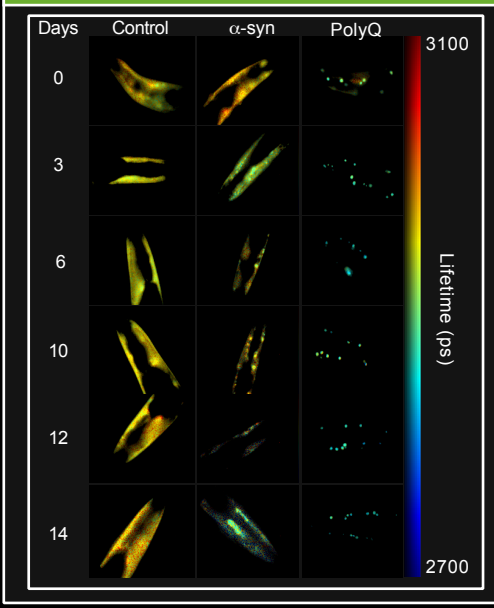
B. Motorised multi-modal TCSPC FLIM platform





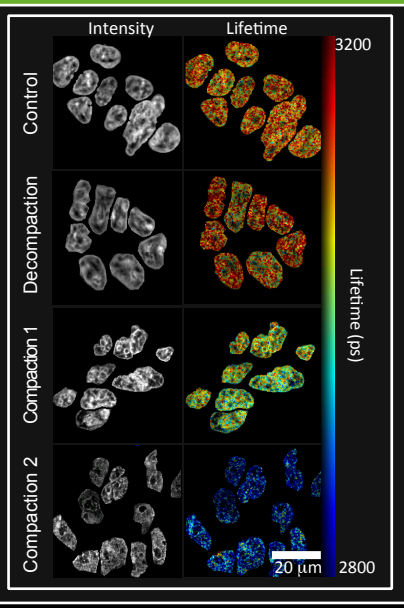
- Based on a confocal microscope. Measures arrival times of single photons pixel-by-pixel.
- SPAD array detector being tested (Micro Photon Devices, Italy) to reduce acqusition times.
- 5 detectors can be used for multi-modal imaging (intensity, lifetime, polarisation).

Biological and biomaterials applications



Application of TG-FLIM platform, adapted from [4]:

- Fast detection of amyloids in *C. elegans*
- Tracking the onset of neuro-degenerative diseases throughout the animal life cycle.
- Screening of phenotypes and drugs.



Application of TCSPC-FLIM platform:

- Developing assays to monitor nuclear compaction states in living cells, especial-ly in stem cells, adapted from [2]
- Monitoring efficacy of gene therapies delivered to cells through nanoparticles and metal organic frameworks [5]

Secondments and Publications

Secondments planned:

a. CNRS (Besancon, France)
Month 29, 1 week, Feb 2019

Publications planned:

[1] Poudel et al. 'Supercontinuum radiation in microscopy and bioimaging applications' (in preparation for JOSA B)
[2] Poudel et al. 'Fluorescence lifetime of SiR-DNA reports on nuclear compaction state of live stem cells' (in preparation for Nature Communications)
[3] Huang et al. 'Intrinsically aggregation-prone proteins form amyloid-like aggregates and contribute to tissue aging in C. elegans' (Submitted to PNAS)
[4] Laine et al. 'Fast fluorescence lifetime imaging reveals the maturation process of α-synuclein aggregates in ageing C. elegans' (in preparation for PNAS)
[5] Teplensky et al. 'Gene knockdown using siRNA loaded highly porous metal-organic frameworks' (in preparation for Nature Materials)

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