Project title: Use of AI for comparative phasor plot analyses in the evaluation of Fluorescence Lifetime Imaging (FLIM) analyses of biological samples

Supervisors: Simone Baltrusch (German PI), Barry Sanders (Canadian PI)

Current state of the art

Fluorescence lifetime imaging (FLIM) generates an image based on differences in the decay rate of the excited state of a fluorescent sample. FLIM is therefore a fluorescence imaging technique in which the contrast is based on the lifetime of individual fluorophores rather than their emission spectra. Fluorescence lifetime is defined as the average time a molecule remains in an excited state before returning to its ground state by emitting a photon. FLIM can be used to detect both, dyes and endogenous fluorophores in biological samples. New developments in FLIM setup are focused on higher speed and better spatial resolution, achieved using advanced detectors and faster readout methods making the technique unique to investigate tissue and organoids under native conditions [1-4].

Since fluorescence lifetime does not depend on concentration, absorption by the sample, sample thickness, photo-bleaching and/or excitation intensity, it is more robust than intensity-based methods. However, fluorescence lifetime depends on a variety of environmental parameters such as pH, ion or oxygen concentration, molecular binding, or proximity to energy acceptors, occurring in biological samples. This can be exploited in biological samples to separate different structures from one another [2].

Time-Correlated Single Photon Counting (TCSPC) is used to determine the fluorescence lifetime. In TCSPC, one measures the time between sample excitation by a pulsed laser and the arrival of the emitted photon at the detector. TCSPC requires a defined “start”, provided by the electronics steering the laser pulse or a photodiode, and a defined “stop” signal, realized by detection with single-photon sensitive detectors (e.g. Single Photon Avalanche Diodes, SPADs). The measurement of this time delay is repeated many times to account for the statistical nature of the fluorophore’s emission. The delay times are sorted into a histogram that plots the occurrence of emission over time after the excitation pulse. To acquire a fluorescence lifetime image, the photons must be attributed to the different pixels, which is done by storing the absolute arrival times of the photons additionally to the relative arrival time in respect to the laser pulse. Line and frame marker signals from the scanner of the confocal microscope are additionally recorded to sort the time stream of photons into the different pixels [4].

However, compared to the usual exponential fit used in the analysis of lifetime decay, phasor plot analysis creates the possibility for the detailed analysis of complex biological samples [5-7]. It generates a two-dimensional (2D) diagram of the lifetime characteristics per pixel of an image by Fourier transformation of the decay data, without the need to define the number of different lifetimes in advance. In the phasor plot, pixels from structures composed of identical biomolecules form distinct clusters representing their unique fluorescence lifetimes. When clusters overlap or appear intermediate, it indicates the presence of structures containing similar but not identical biomolecules or mixtures of multiple species, as their phasor positions represent weighted combinations of individual molecular lifetimes. By creating a phasor plot database of biological baseline conditions (e.g. physiological untreated tissue or intact organoids), it should therefore be possible to detect changes in similar or similarly prepared samples using an AI-based software routine. Such an approach is interesting for different biological questions but has not yet been addressed.

Research goals and working program

The aim of this project is

- to establish FLIM Imaging of *Caenorhabditis elegans* using the MicroTime 200 (PicoQuant) system. *Caenorhabditis elegans* is a tiny, transparent nematode worm that serves as an important model organism in biomedical research. It is the ideal target structure for this approach because it has a well-organized anatomy allowing detection of the autofluorescence lifetime. As it is already used for studies on ageing, diseases and genetics, since many of its genes and signalling pathways also occur in humans, the expected results are highly relevant. [Month 1-9]
- to use established FLIM imaging to investigate large number of worms in the same stage, which can be produced using age-matched cultivation and to analyse the results by matched phasor-blot analysis (Fig. 1B physiological) [Month 6-18].
- to write an AI based software algorithm based on the library generated in [2] [Month 18-30]
- to test the generated algorithm by investigating and analysing manipulated *Caenorhabditis elegans* (Fig. 1B condition A/ B).

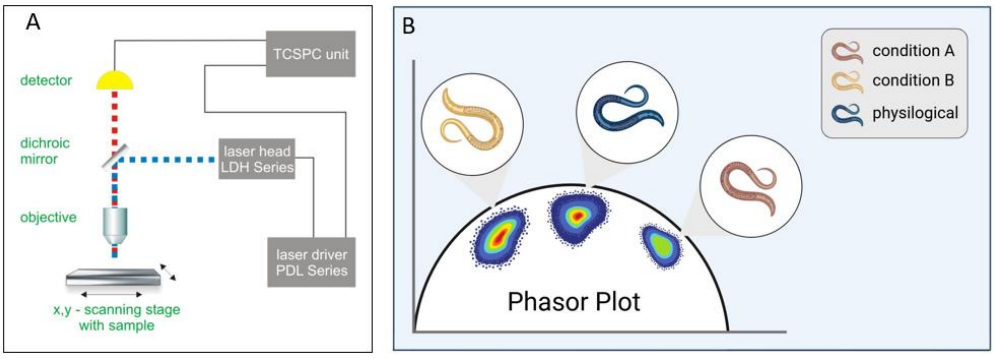

Fig. 1 A: Principle of FLIM [4] B: Workflow Scheme AI-based detection of changes in FLIM phasor plot profile in *Caenorhabditis elegans*

[1] J. R. Lakowicz, Ed., "Instrumentation for Fluorescence Spectroscopy," in Principles of Fluorescence Spectroscopy, Boston, MA: Springer US, 2006, pp. 27–61. doi: 10.1007/978-0-387-46312-4_2.

[2] M. Y. Berezin and S. Achilefu, "Fluorescence Lifetime Measurements and Biological Imaging," Chem. Rev., vol. 110, no. 5, pp. 2641–2684, May 2010, doi: 10.1021/cr900343z.

[3] M. Köllner and J. Wolfrum, "How many photons are necessary for fluorescence-lifetime measurements?," Chemical Physics Letters, vol. 200, no. 1, pp. 199–204, Nov. 1992, doi: 10.1016/0009-2614(92)87068-Z.

[4] https://www.picoquant.com/applications/category/life-science/fluorescence-lifetime-imaging-flim

[5] J. Enderlein and R. Erdmann, "Fast fitting of multi-exponential decay curves," Optics Communications, vol. 134, no. 1, pp. 371–378, Jan. 1997, doi: 10.1016/S0030-4018(96)00384-7.

[6] V. Mannam, Y. Zhang, X. Yuan, C. Ravasio, and S. S. Howard, "Machine learning for faster and smarter fluorescence lifetime imaging microscopy," J. Phys. Photonics, vol. 2, no. 4, p. 042005, Sep. 2020, doi: 10.1088/2515-7647/abac1a.

[7] M. K. Rahim et al., "Phasor Analysis of Fluorescence Lifetime Enables Quantitative Multiplexed Molecular Imaging of Three Probes," Anal Chem, vol. 94, no. 41, pp. 14185–14194, Oct. 2022, doi: 10.1021/acs.analchem.2c02149.