

#2	Development of a Fluorescence Lifetime Imaging Module for Multimodal Microscopy	
Fluorescence Lifetime Imaging Microscopy (FLIM) is one of the most powerful quantitative imaging techniques in modern biophotonics. Unlike conventional fluorescence microscopy, which relies on emission intensity, FLIM measures the fluorescence decay time of molecules, providing quantitative information about their biochemical environment that is largely independent of fluorophore concentration, excitation intensity, and optical losses. As a result, FLIM enables label-free assessment of cellular metabolism, protein interactions, tissue composition, and molecular microenvironments. In ophthalmology, endogenous fluorophores constitute valuable biomarkers of retinal metabolism, oxidative stress and extracellular matrix remodeling. Combining FLIM with nonlinear imaging modalities such as Two-Photon Excited Fluorescence (TPEF) and Second Harmonic Generation (SHG) allows the simultaneous acquisition of structural, functional and molecular information from the same biological sample, providing a comprehensive characterization of ocular tissues. At BIOSIMLab, we are developing a next-generation multimodal microscopy platform that integrates fluorescence, FLIM, SHG, hyperspectral imaging and advanced computational analysis for the study of retinal physiology, myopia and ocular biomechanics. A key challenge is the development of a robust FLIM subsystem capable of synchronizing ultrafast laser excitation, photon detection and lifetime reconstruction with high temporal precision. This project is ideal for students interested in optical instrumentation, photonics, electronics and scientific software development, offering hands-on experience in the implementation of cutting-edge FLIM technology.		
Project	The student will participate in the design, integration and experimental validation of a Fluorescence Lifetime Imaging (FLIM) module for BIOSIMLab's multimodal microscopy platform. The work will include hardware integration, synchronization electronics, photon-counting instrumentation, software implementation and lifetime reconstruction algorithms, culminating in biological validation experiments.	
Objectives	• Design the FLIM acquisition architecture. • Integrate synchronization & photon-counting electronics • Develop FLIM reconstruction algorithms.	• Characterize the system's temporal/optical performance • Validate the FLIM module using biological samples and endogenous fluorophores.
Skills acquired	• Fluorescence Lifetime Imaging Microscopy (FLIM) • Time-Correlated Single Photon Counting (TCSPC) • Optical instrumentation & synchronization electronics	• Signal processing and lifetime analysis • Biomedical photonics and experimental validation
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Selected References	Becker W. Advanced Time-Correlated Single Photon Counting Techniques. Springer (2005). Lakowicz J.R. Principles of Fluorescence Spectroscopy. Springer. Dysli C. et al. Fluorescence Lifetime Imaging Ophthalmoscopy. Progress in Retinal & Eye Research (2017). Tang J.A.H. et al. AO Fluorescence Lifetime Imaging Ophthalmoscopy. Biomedical Optics Express (2022). Viñas-Peña M. et al. Fluorescence lifetimes of scleral tissues after riboflavin-mediated crosslinking. IOVS (2023)	