

#1 Mini2P. Design and Development of a Compact 3D-Printed Two-Photon Microscope

Two-photon fluorescence microscopy (2PM) is one of the most powerful techniques for biological imaging, enabling high-resolution visualization of living tissues hundreds of micrometres below the tissue surface while minimizing photobleaching and phototoxicity. By restricting fluorescence excitation to the focal volume, two-photon microscopy provides intrinsic optical sectioning and excellent imaging contrast, making it particularly valuable for ophthalmology, neuroscience, developmental biology and tissue engineering. Despite its outstanding capabilities, commercial multiphoton microscopes remain expensive, bulky and difficult to customize for emerging research applications. Recent advances in ultrafast fiber lasers, compact scanning systems, additive manufacturing and open-source electronics now enable the development of flexible, modular and affordable imaging platforms that can be tailored to specific scientific questions. **At BIOSIMLab**, we are developing a new generation of compact multiphoton microscopes specifically designed for ocular imaging and multimodal microscopy. This platform will provide the foundation for future systems integrating Two-Photon Excited Fluorescence (TPEF), Second Harmonic Generation (SHG), Fluorescence Lifetime Imaging Microscopy (FLIM), hyperspectral imaging and adaptive optics. These technologies will enable the investigation of retinal metabolism, collagen organization & biomechanics with unprecedented resolution.

Project	This project offers an excellent opportunity for students interested in optical engineering, biomedical instrumentation and laser technologies to participate in the complete development cycle of an advanced photonic imaging system, from optical design and mechanical integration to experimental validation.	
Objectives	• Design a modular TP microscope architecture. • Develop custom 3D-printed opto-mechanics • Integrate laser scanning and detection modules.	• Characterize the system's optical performance • Validation using biological samples.
Skills acquired	• Optical system design & Biomedical instrumentation • Laser microscopy	• Opto-mechanical engineering • Experimental system characterization
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Selected References	<i>Denk W. et al. Two-photon laser scanning fluorescence microscopy. Science (1990).</i> <i>Helmchen F. & Denk W. Deep tissue two-photon microscopy. Nature Methods (2005).</i> <i>Horton N.G. et al. In vivo three-photon microscopy of subcortical structures. Nature Photonics (2013).</i> <i>Tang J.A.H. et al. AO fluorescence lifetime imaging ophthalmoscopy. Biomedical Optics Express (2022).</i>	