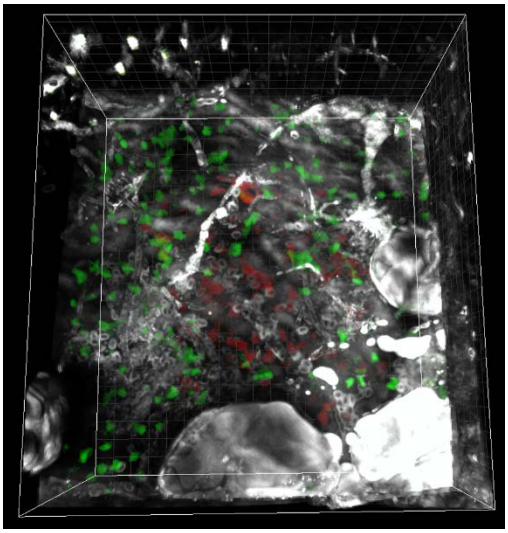
Master/PhD thesis proposal

Laboratoire d'Optique et Biosciences
Ecole Polytechnique, Palaiseau

Multicontrast 3-photon microscopy for deep imaging of brain and developing tissue

Keywords: *nonlinear optics;* scattering media; neuroimaging



Scientific description: Two-photon (2P) microscopy uses a focused infrared femtosecond laser (900 nm) to record fluorescence images with 3D cellular resolution at depths reaching 300-500 µm inside scattering biological tissues. Thanks to these properties, it is a widely used technique in neuroscience and embryology. However, deeper fluorescence imaging is very difficult because scattering degrades the image contrast. A very promising technique to **push this depth limit** has been introduced recently, based on **three-photon (3P) excitation**, which provides better excitation confinement in the presence of scattering. This approach is made possible thanks to a new generation of infrared sources (1300-1700 nm, 50 fs, 1 MHz) and makes it possible to reach depths exceeding 1 mm in tissues or to image through the skull of small animals. This new imaging method opens **novel application possibilities**, and at the same time motivates **technological developments** and optimizations. The microscopy group at LOB is pioneering 3P imaging, and has demonstrated the first dual-color 3P microscope using innovative multibeam laser sources.

The general objective of the project is to improve the performances of 3P imaging by combining complementary contrast modalities, and to explore its potential for in-depth microscopy of fish and mouse tissues.

The internship will first focus on a novel label-free contrast modality called **third-order sum-frequency generation (TSFG) microscopy**. TSFG signals can be obtained by mixing two femtosecond infrared beams. Our group has recently found that this signal is sensitive to hemoglobin and can be used to analyze flowing red blood cells in vivo (Ferrer Ortas 2023). The intern will continue the characterization of this contrast, and optimize the simultaneous detection of TSFG and 3P fluorescence signals on independent detectors.

This work can be continued with a PhD thesis focusing on more advanced developments (such as **adaptive optics** for aberration correction at large depths; **multiplane** excitation for faster imaging) and their application to in-depth and **long-term imaging of developing tissues**.

***Profile*:** The candidate should have a background in physics / experimental optics, and a motivation to work in an interdisciplinary environment. Knowledge in programming and image/signal processing will be an asset, as the goal of the developments is to extract quantitative parameters from the images. The project will involve experimental nonlinear microscopy, data analysis, numerical simulations, and biological samples manipulation.

Environment: The project will take place in the 'Advanced microscopies' group of the Lab for Optics and Biosciences at Ecole Polytechnique (LOB). Our team has a strong expertise in the field of multiphoton microscopies and their application to tissue studies. The work will involve daily interactions with a group of ~4 people, within a local microscopy team of ~25 persons and an active collaborative network (IOGS, Amplitude, Inst Vision, Pasteur, INMED).

Some related references: Ferrer-Ortas, Light:Sci Appl (2023); Guesmi, Light:Sci App (2018). https://lob.ip-paris.fr/recherche/microscopies-avancees-physiologie-des-tissus

Techniques/methods: Nonlinear optical microscopy. Femtosecond lasers. Image analysis. Numerical simulations. Biological samples handling.
Applicant skills: Experimental optics. Programming. Taste for interface work (physics/ biology/ informatics). Good level of English.
Supervisor:
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Location: LOB, Ecole Polytechnique, Palaiseau
Possibility for a PhD thesis: Yes