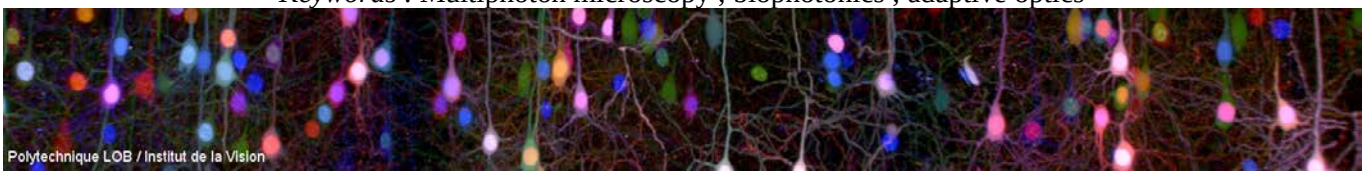
Laboratory for optics and biosciences
Ecole Polytechnique – CNRS – INSERM – IP Paris

M2+PhD project

Brain-wide structural imaging with multiphoton microscopy

Keywords : Multiphoton microscopy ; biophotonics ; adaptive optics



***Scientific context*:** In vertebrates, the anatomy and structure of the brain are established during early development, and any abnormalities during these crucial stages can lead to serious pathologies. However, the mechanisms shaping brain regions are still poorly understood, due to the technical difficulty of mapping opaque tissue at different development stages with sub-cellular precision. To address these questions, the Laboratory of Optics and Biosciences (LOB, Ecole Polytechnique, Palaiseau) has developed in collaboration with the Institute of Vision (IdV, Paris) an original platform for large-volume 3D microscopy of ex vivo mouse tissue, called chromatic multiphoton serial microscopy (ChroMS). This method is based on the frequency-mixed color two-photon microscopy developed at the LOB (Mahou, Nat Methods 2012), combined with serial sectioning, and with the 'brainbow' approach developed at the IdV and allowing the color labeling of neurons in a mouse brain (Livet, Nature 2007). A first demonstration of the performance of the ChroMS approach has been published recently (Abdeladim 2019, https://doi.org/10.1038/s41467-019-09552-9) and the team is currently pursuing the improvement of the technology.

***Objectives of the internship*:**
A **first objective** of the internship will be to explore the use of the platform for brain-wide **mapping of label-free nonlinear signals** such as third-harmonic generation (THG). The exploration of this new application of the existing ChroMS platform will be the occasion to learn its operation, from sample mounting to data reconstruction.
One **second objective** of the internship will be to explore **simple adaptive optics approaches** to minimize the progressive in-depth degradation of the resolution and signal to noise ratio. Indeed, deep-tissue imaging is difficult due to aberrations caused by refractive index heterogeneity. This effect complicates image quantification, even with a serial sectioning approach. One strategy will be to **homogenize the refractive index** of the tissue with a water-soluble index matching medium, and to measure the benefit of this approach for 3D imaging. A complementary development will be to implement a motorized correction collar for the microscope objective, in order to **minimize spherical aberration**. This implementation will be based on a design available in open access, involving the 3D printing and the software control of the system. The validation of the system will then be realized on the ChroMS microscope with the help of host team members. The aim will be to establish optimal corrections according to tissue type and imaging depth.
The perspectives will be, with our collaborators, to record reference large-scale 3D datasets of brain tissue, and to benchmark automated image analysis strategies for extracting quantitative data on cell and tissue morphology. The internship can be followed by a PhD thesis on the development of volumic color microscopy and large-image analysis workflows, and their application to study the development of brain circuits, in the framework of a collaborative project funded by the European Research Council (ERC).
***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. The project will involve experimental nonlinear microscopy (femtosecond laser alignment, data acquisition), data analysis, programming, 3D printing, basic tissue preparations.
Environment: The project will take place in the 'Advanced microscopies' group of the LOB at Ecole Polytechnique (Palaiseau), in collaboration with IdV (Paris). Our team has a known expertise in the field of multiphoton microscopies and their application to tissue studies. The work will be cosupervised by Pierre Mahou (IR Polytechnique), Hugo Blanc (PhD student) and Emmanuel Beaurepaire (CNRS).
Website of the LOB advanced microscopies team:
https://portail.polytechnique.edu/lob/en/recherche/advanced-microscopies-tissue-physiology

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Possibility of a PhD. Funding available: ERC.