Spécialité de Master « Optique, Matière, Paris »
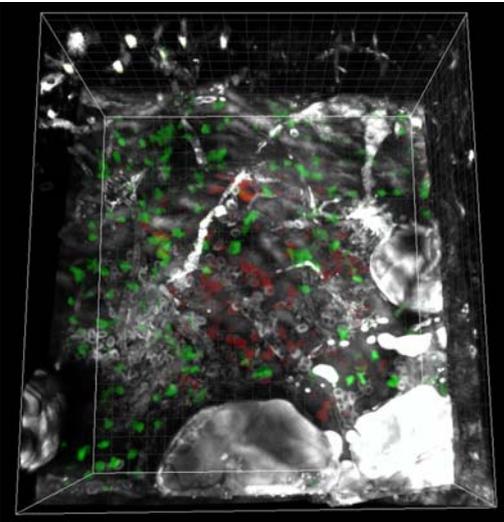
Stage de recherche (4 mois minimum, à partir de début mars)

Proposition de stage

Date de la proposition : 10/2020

Responsable du stage */ internship supervisor*:	
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Nom du Laboratoire / *laboratory name*:	
Code d'identification :LOB	Organisme : Polytechnique - CNRS - Inserm
Site Internet / *web site*: www.lob.polytechnique.edu	
Adresse / *address:* Ecole Polytechnique, 91128 Palaiseau	
Lieu du stage / *internship place*: Ecole Polytechnique	

Titre du stage / *internship title*: **Deep three-photon microscopy of brain and developing tissue**

Keywords: nonlinear optics, microscopy, scattering media, neuroimaging

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 (Horton et al, Nature Photonics 2013). 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 recently built the first dual-color 3P microscope prototype (Guesmi, Light:Sci App 2018).

The objective of the M2 internship will be to characterize and improve the performances of 3P imaging (resolution, depth, contrast, signal level) and its potential for applications in fish and mouse tissues.

This will involve the following implementations: (i) A pulse-splitting system will be validated to **adjust the repetition rate** depending on the application and photodamage thresholds; (ii) A laser source adapted for 2P excitation will be coaligned with the 3P excitation source for comparing 2P and 3P **performance as a function of depth**. This work can be continued with a PhD thesis focusing on more advanced developments (**adaptive optics** for aberration correction; **multiplane** imaging; **multicontrast** imaging) and their application to in-depth live 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 well-known expertise in the field of multiphoton microscopies and their application to tissue studies. The work will involve daily interactions with a group of ~4-5 people, within a local microscopy team of ~25 persons and an active collaborative network (IOGS, Inst Vision, Inst Pasteur, CEA).

Some related references *from our group*: Guesmi, Light:Sci App (2018); Maioli, Biomed Opt Express (2020). https://portail.polytechnique.edu/lob/en/research/advanced-microscopies-tissue-physiology

Ce stage pourra-t-il se prolonger en thèse ? *Possibility of a PhD* **? : Yes**			
Si oui, financement de thèse envisagé/ *financial support for the PhD***:**			
Lumière, Matière, Interactions	**[X]**	Lasers, Optique, Matière	**[X]**