M2 and/or PhD proposal
Institut Fresnel – Marseille - France

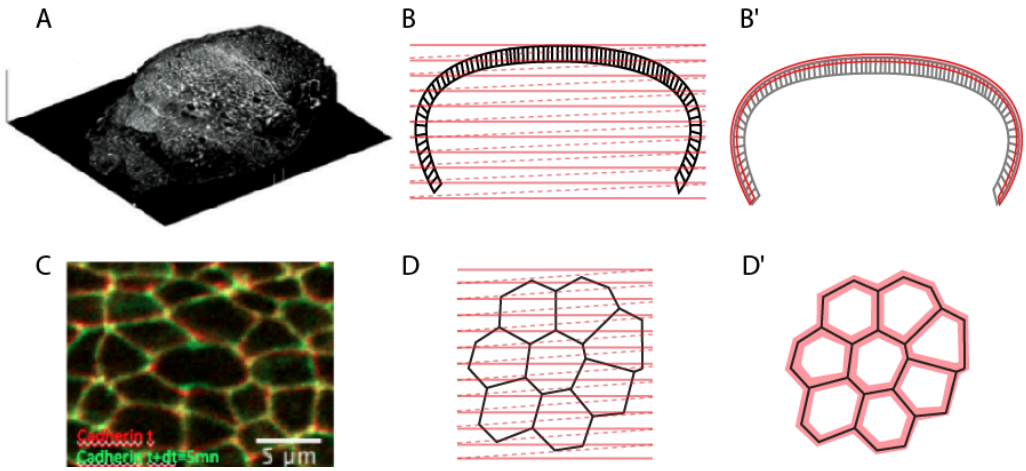
Smart scans: imaging biological tissues faster and with less damage using informed scans.

Name of the mentors: Loïc Le Goff & Hervé Rigneault
Email address: loic.le-goff@fresnel.fr, herve.Rigneault@fresnel.fr
Phone number: 0491282898
Mail address: Institut Fresnel
Aix-Marseille Université
Campus de St Jérome
13013 Marseille, France

The context:
The MOSAIC team at Institut Fresnel gathers physicists and biologists to tackle biological questions from a physics and engineering point of view.
web site: http://www.fresnel.fr

Introducing the subject:
The confocal microscope and its different variations have fostered a revolution in life sciences: one can now image entire tissues at a sub-cellular resolution in living organisms. However, confocal microscopes (linear or non-linear) are slow and quite phototoxic because they scan the excitation laser in the entire field of view in a raster fashion, which takes time and significant amount of light.

Summary of the project:
The present project aims at developing a novel confocal microscope that is faster and uses an order of magnitude less light to perform volumetric imaging. In order to do so, the system will use prior information of the imaged sample to focus exclusively on the structures of interest and thus avoid wasting light and time on regions that bear no information. The prior information will first come from the user informing on the structure of the imaged tissue. For example, the tissue may be a curved cell monolayer in which we want to image the apical shape of cells. The prior will also come from the redundancy from one time point to the next. Indeed, the tissue will not dramatically change in time, and this information should be used in order to "focus" the scanning.
We illustrate the concept with a concrete example (see Figure, below). We study the growth of Drosophila tissues, which are organized in curved monolayers (Fig. A). We are interested in the apical part of the cells where the morphogenetic forces concentrate. A traditional confocal microscope will scan the entire parallelepiped that encloses the tissue (raster scan in red in Fig. B), while the informed scan will exclusively scan a shell that enclose the apical region of the tissue (in red in Fig. B'). Likewise, in the plane of the tissue, cell shapes are redundant from one time point to the next (see how two successive snapshots in green and red overlap in Fig. C). Rather than scanning the entire surface (Fig. D), the informed scanning will explore a sub-surface using the shape of cells at the previous time as a starting point (D').



The informed scan introduces a new paradigm in microscopy, where the imaging process adapts in real time to the information content of the sample. Starting from a custom build confocal microscope, the student will develop the informed scan and use it to image Drosophila embryonic tissues so as to demonstrate the benefit of the approach. In a second step, extension to non-linear contrasts such as two-photon fluorescence and coherent Raman imaging will be treated. Finally, the approach will be combined with adaptive optics.

The Mosaic group is looking for a M2 or PhD candidate interested in microscopy, signal processing and biology.

Selected references:

LeGoff , Lecuit Mechanical forces and growth in animal tissues. Cold Spr Harb Persp Biol pii: a019232 (2015)
Heemskerk, Lecuit, LeGoff, Dynamic clonal analysis based on chronic in vivo imaging allows multiscale quantification of growth in the Drosophila wing disc, Development 141, 2339 (2014)
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Lecuit, Le Goff, Orchestrating size and shape during morphogenesis, Nature 450, 189 (2007)
Berto, Andresen, Rigneault. Background free stimulated Raman spectroscopy and microscopy. Phys. Rev. Lett 112, 053905 (2014)
Berto, Gachet, Bon, Monneret, Rigneault. Wide-Field Vibrational Phase Imaging. Phys. Rev. Lett. 109, 093902 (2012)
Andresen, Berto, Rigneault. Stimulated-Raman scattering microscopy by spectral focussing and fiber-generated soliton as Stokes pulse. Opt Lett 36, 2387-2389 (2011)

Keywords : confocal microscope (linear and non linear contrast); in vivo imaging; real time analysis
Required skills : optics, image analysis, real-time control