Master 2 internship for physicists
Année 2017-2018

Title: **Chicken embryo studies with inverted SPIM**

Laboratory: INSTITUT LUMIERE MATIERE UMR CNRS 5306
Laboratory director: Philippe DUGOURD
Team: “Interfaces and Non Linear Optics" = ONLI group
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Job: Master 2 internship (physics)
PhD possibilities: yes (“doctorale school” grant)
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Partner 1: ILM, UMR CNRS 5306
ONLI (Non-Linear Optics and Interfaces) team
The ONLI group, led by Pierre-François BREVET, has a recorded history of publications in the field of nonlinear optics of nanoparticles. It currently develops light sheet microscopy imaging.

Partner 2 : INMG - UMR 5310
Morphogenesis and tissue growth in embryonic development
This group led by Christophe MARCELLE has been created recently the arrival of professor Marcelle from his group in Australia.

Scientific problem:
Light Sheet Microscopy, like Selective Plane Illumination Microscope (SPIM) is becoming more and more popular in developmental biology and for various biomedical applications because it allows long term 3D imaging (optical sections during several days), because of a very low phototoxicity or bleaching. This can moreover be achieved with a high temporal resolution compared to traditional confocal acquisition for 3D imaging. Indeed, in this technique, the excitation light is shaped into a small light sheet and the detection is set on a distinct optical path (usually at 90°): the sample is only illuminated in the plane of focus of the observation, this is radically different from the usual excitation scheme used in conventional fluorescent microscopy, where the whole sample is illuminated, and this is why phototoxicity is so low (see Figure 1). This technique is commonly used for *in vivo* biological experiments but might be useful as well to investigate on a model tissue long term effects of a given stress parameter (chemicals, drugs, electromagnetic field, ultrasound, mechanical constraints…) without adding another source of stress due to phototoxity.

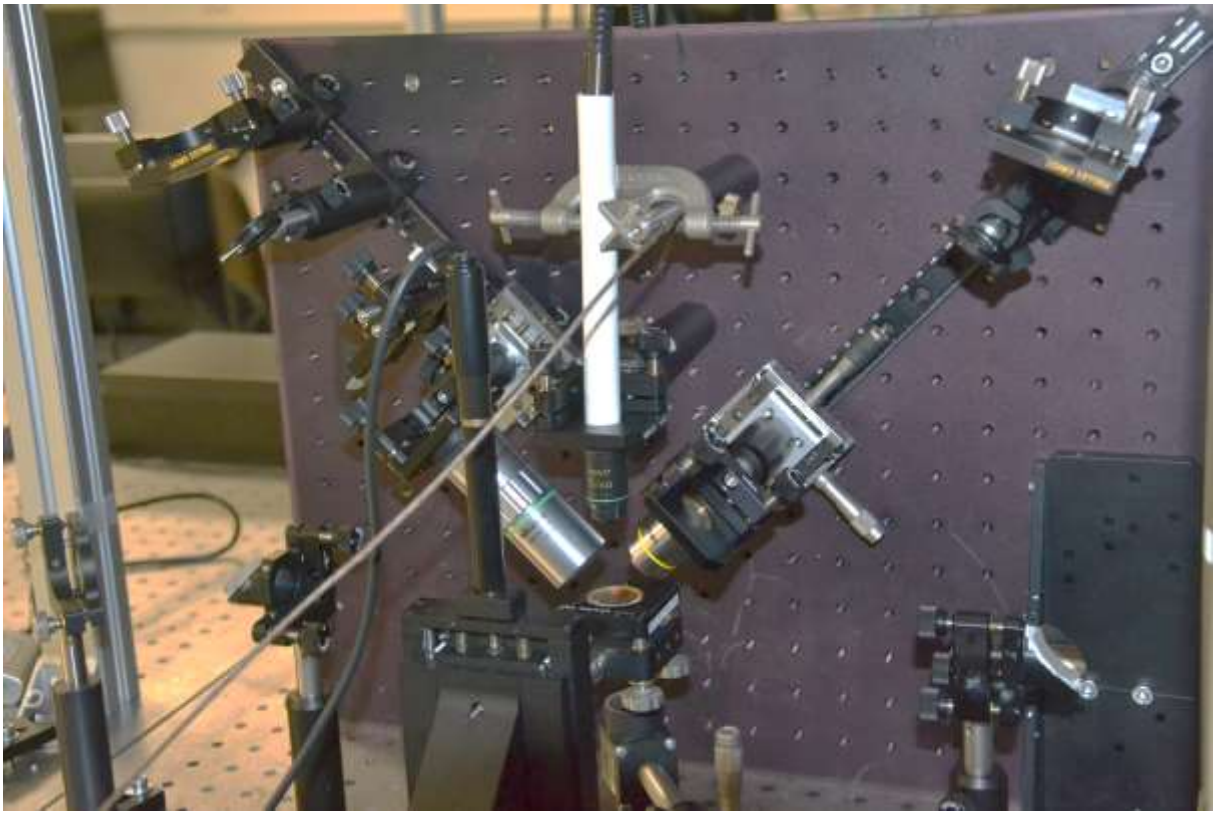

Figure 1 : (a) iSPIM set-up (Inverted Selective Plane Illumination Microscopy).

Partner 2 are using skeletal muscle formation in the chick embryo as a powerful model to understand how cells within tissues display complex behaviors while being exposed to an ever-changing cellular environment. The model requires observing the embryo from above, *i.e.* with an iSPIM (inverted SPIM see Figure 1), equipped with a powerful (20-40X) objective and placed in a humid, heated chamber. Observing live tissues for extended periods of time requires a laser system that generates no photo-toxicity. Moreover, the (~150Hz) heart-beat is (albeit slightly) propagating throughout the entire embryo; this requires a fast scanning system that will freeze this slight movement. The light sheet microscope is particularly adapted to such purposes.

Tasks:

During the internship, integrated in the partner 1 team, the first part of the job will be to design, test and characterize a thermal and humidity regulation system to keep the chick embryo alive during the overall imaging process. The second part is to implement some image processing with open source program such as ImageJ to enhance the quality of the image through the embryo development and the skeletal muscle formation. These developments will be done in tight collaboration with partner 2 to obtain the chick embryo and analyze the images.