Spécialité de Master « Optique, Matière, Plasmas »

Stage de recherche (4 mois minimum, à partir de début mars 2010)

Proposition de stage pour l'année 2009-2010 (ne pas dépasser 1 page)

Date de la proposition : 22nd october 009

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Lieu du stage / *internship place*: Institut Fresnel, équipe MOSAIC

Titre du stage / *internship title*: Polarimetric Microscopy Imaging in Biological Samples

Résumé / *summary*

Two-photon fluorescence microscopy have been considerably developed in the last decade, and is now widely used to solve fundamental biological issues and bio-medical diagnostics applications.[1] This optical regime generates fluorescence from a near Infra-Red pulsed laser excitation, which penetrates deeper into biological tissues than visible light, and provides an intrinsic optical resolution of about 300nm for imaging.

We have developed in the MOSAIC team an experiment which consists in varying the polarization of the excitation light to measure the orientation of fluorescent molecules in biological samples, such as lipid membranes, plasma cell membranes or cells in tissues.[2] The polarization state of the incident beam in a two-photon microscope is rotated and two-photon fluorescence images are successively recorded for the different states of polarization : from the recorded stack of images, it is possible to retrieve in a specific location the information on how the molecules are oriented, and if they are highly ordered (such as in a cystal) or highly disordered (such as in a liquid).

This information is crucial for biologists who have pointed out the important relation between bio-molecules orientational order and biological functions. In our experiment, the fluorescence signal is obtained from molecules that are previously added into the membrane : they are therefore reporters of how the local environment is ordered;

The actual limit of this experiment is its long acquisition time. There is indeed a need for faster measurements, especially in cell membranes that undergo time-dependent transformations due to an external stimulation for instance. In addition, the amount of data recorded (typically 90 images, for 90 incident polarization states) requires an adequate image processing and analysis. The goal of this project is to develop a protocol for a polarization-resolved two-photon microscopy technique which will be able to provide a fast information on the local orientation of bio-molecules in a biological sample. It will for instance rely on a faster polarization-tuning device (using an electro-optic modulator), or on the use of a lower number of input polarization states. A second part of this project is the development of an image processing tool that will allow a direct 2D view of the molecular order after treatment of the data. This will require analysing locally the polarization dependence of the signal and reconstructing the relevant information in each pixel of the image.

Such a work will be an important step towards the realization of a new microscopy tool, which will be essential for biologists since polarization provides rich information. A tight collaboration is in place with the Centre d'Immunology, Marseille Luminy for biological samples development. The following skills will be developed in the project :

Laboratory optical bench optics, microscopy, optoelectronics, lasers / Bio-imaging, fluorescence spectroscopy / Image analysis, image processing

References :

1. W. Denk, J.H. Strickler, W.W Webb, Science 248, 73-76 (1990).
2. A. Gasecka, T.-J. Han, C. Favard, B.R. Cho, S. Brasselet, accepted in Biophys. J. (2009).
3. L. Gao, L. Jin, P. Xue, J. Xu, Y. Wang, H. Ma, D. Chen, Opt. Expr. 14 (11), 4727 (2006).

Toutes les rubriques ci-dessous doivent obligatoirement être remplies

Ce stage pourra-t-il se prolonger en thèse ? *Possibility of a PhD* **? : OUI**

Si oui, financement de thèse envisagé/ *financial support for the PhD***: ERASMUS MUNDUS- DOC**

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