TIME-RESOLVED MULTIPHOTON MULTIFOCAL FLUORESCENCE MICROSCOPY APPLIED TO EARLY CANCER DIAGNOSIS

Ariane Deniset¹, S. Lévêque-Fort¹, M.P. Fontaine-Aupart¹, G. Roger² and P. Georges²
¹Laboratoire de Photophysique Moléculaire, CNRS UPR 3361, Fédération LUMAT, Université Paris-Sud, Bat.210, 91405 Orsay cedex, France
Phone : 33(0)1 69 15 76 36, E.mail: ariane.deniset@ppm.u-psud.fr
²Laboratoire Charles Fabry, Institut d’optique, CNRS et université Paris-Sud UMR 8501, Université Paris-Sud, Bat. 503, 91405 Orsay cedex, France

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Two-photon microscopy is a key method for biomedical research and cells or tissue imaging, regarding photobleaching and photodamages. Furthermore, the near infrared lasers employed in this case, have higher penetration in tissue, limiting scattering and absorption.

However, one of the main drawbacks of the conventional two-photon microscope is the imaging speed. Indeed, as it is a laser scanning technique where images are acquired point-by-point, it becomes rapidly time-consuming to image 3D volume and could be injurious for biological samples.

To speed up acquisition and preserve samples from too long experiments, the most relevant method consists in illuminating simultaneously the sample with several points, reducing the acquisition time proportionally to the number of excitation points used. The principle of ‘multifocal multiphoton microscopy’ has been demonstrated by several groups [1-3]. In our case, an original arrangement of mirrors and a beamsplitter [3] has been developed, creating an 8×8 beam array, presented on the figure. This approach presents several advantages like a good uniformity of the beams, a high transmission coefficient (90%) and prevents from any crosstalks between the excitation beams.

Compared to other methods, the original microscope we thus developed, allows us to access to a dynamic measurement of the fluorescence and to have a first insight of the fluorescence image of our sample, even before scanning the excitation beams on this sample. We will demonstrate all the advantages of our home-made time-resolved multifocal multiphoton microscope which considerably reduces time acquisition of both fluorescence intensity image and lifetime image. All biomedical samples used will clearly prove the real relevance of this method: more particularly on urothelial and cervical cells for early cancer diagnosis purpose.