

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

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Two-photon microscopy is a key method for biomedical research and cells or tissue imaging, regarding photobleaching and photodammages. 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.

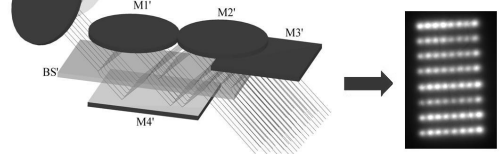


Figure: Beamsplitter principle and image of the 64 excitation beams in the focal plane

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.

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