SPPIN's seminar winter-spring 2023

Mardi, 21 Mars, 10h00, R229 (in presence and online)

SPPIN SAINTS-PERES Paris Institute for the Neurosciences SEMINARS

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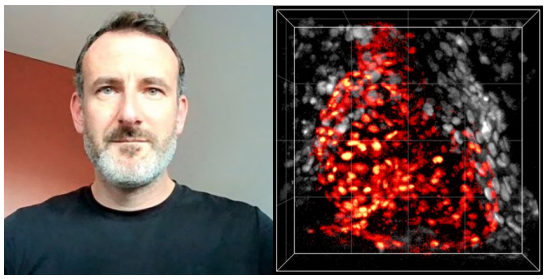
Speeding up multiphoton microscopy with light-sheet illumination



Multiphoton microscopy has unique advantages for in vivo imaging, including a large imaging depth and the ability to perform multimodal imaging by combining nonlinear fluorescence excitation with other contrast mechanisms, such as second harmonic generation (SHG). However, its relatively slow acquisition speed in the MHz pixel rate range, has long limited its ability to monitor fast biological dynamics or to image large samples at multiple scales. In the last decade, several strategies have emerged to achieve fast multiphoton imaging beyond 10 MHz pixel rate, including fast point scanning or multifocal illumination. Here, we first present the advantages of light-sheet illumination among these strategies. In multiphoton light-sheet microscopy, the illumination parallelization is achieved with a single weakly focused beam without loss of signal or axial resolution. By using an illumination axis orthogonal to the detection axis, this parallelization is generated in the same direction as the beam propagation. This results in a very efficient use of the photon budget, which is critical in the case of nonlinear excitation. Fast imaging with high pixel rate is then achieved with lower peak intensity, mean power and longer pixel dwell time compared to other approaches. To demonstrate this property we investigated the experimental parameters governing the linear and nonlinear photodamage induced by the specific illumination conditions used in multiphoton light-sheet illumination. By devising a systematic experimental workflow using live embryo imaging, we show that the limiting parameters differ from those previously reported using point-scanning multiphoton microscopy. As a result, further optimization of the illumination parameters leads to a 10-fold increase in the signal without inducing additional photodamage. Such a significant improvement opens new opportunities for fast in vivo imaging with multiphoton light-sheet microscopy. Finally, we will present our work aimed at combining two-photon excited fluorescence with second harmonic generation in a light-sheet microscope. This allowed us to perform fast in vivo SHG imaging of nanoprobes in live embryos.

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