Towards multi-photon quantum Image Scanning Microscopy for biological systems

Riccardo Bolzoni[1], **Margaux Bouzin**[1], **Giuseppe Chirico**[1] **and Maddalena Collini**[1]

1. *University of Milan-Bicocca, Department of Physics, Piazza della Scienza 3, 20126, Milano, Italy*

Abstract: The quantum nature of light can be exploited in microscopy to achieve super-resolution optical imaging. We present the groundwork and preliminary results on the development of such a quantum-based acquisition system.

In Confocal Laser Scanning Microscopy (CLSM) one can theoretically achieve an improved lateral spatial resolution by reducing the size of the pinhole filtering the light reaching the detector, but the resulting drop in signal practically limits the applicability of this approach. Image Scanning Microscopy (ISM) circumvents this problem by substituting the pinhole-detector pair with an array of small detectors, simultaneously achieving high resolution without loss of signal [1,2]. Additionally, the parallel acquisition of the single detectors allows to implement correlation-based methods to investigate the statistics of the photons arrival, potentially leading to an even greater resolution improvement and reduced background noise [3,4].

We present here the groundwork for the development of an imaging system combining multi-photon excitation (MPE) with an array of 49 SPAD detectors, aiming at improving the intrinsically lower lateral and axial resolutions of MPE, while maintaining its advantage of depth penetration in thick samples such as biological tissues. The project initially focused on coupling the detector to the imaging system and developing an image analysis and reconstruction routine for the classical implementation of ISM, combining the 49 images acquired by the SPADs into a single super-resolved frame.

Notably, the Point Spread Functions (PSFs) of the individual detector elements are shifted and deformed with respected to the central one. These effects will be further accentuated in the quantum correlation-based approach, and it is therefore essential to identify an effective reconstruction method. For both the classical and quantum cases, the image series can be combined by properly shifting and aligning the images before summing them (an approach termed Pixel Reassignment) or by employing a form of image deconvolution based on the measurement of the PSFs themselves. Experimental tests show the expected resolution improvement (Fig. 1a,b). At the same time, deconvolution highlights the ability to overcome limitations in the Signal-to-Noise ratio, effectively reaching a greater improvement that would be relevant in real-case applications on biological samples (Fig. 1c). This analysis, together with preliminary simulations on the behaviour of photon correlations, will be the basis for near-future work on the quantum reconstruction.

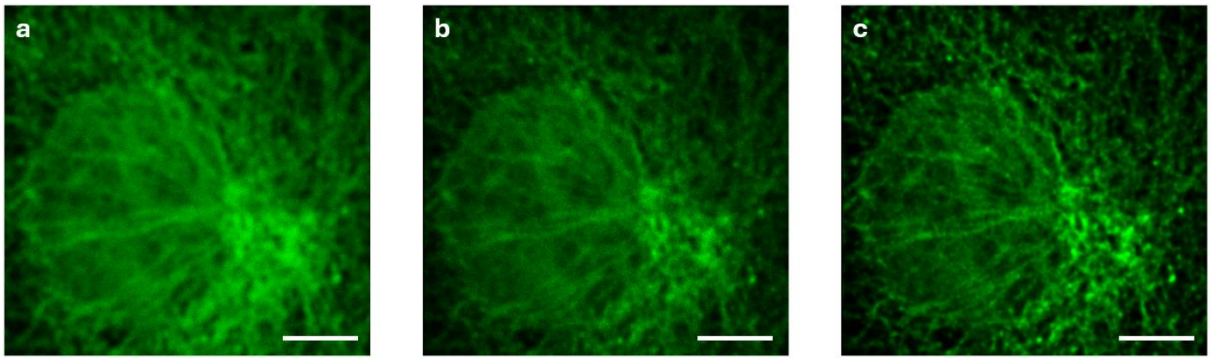

Fig. 1 Exemplary images of microtubules in bovine artery cells stained with AlexaFluor 488. (a) multi-photon image acquired with a photomultiplier tube. (b) ISM image after pixel reassignment. (c) deconvoluted ISM image. Scalebar is 5 µm for all images.

References

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