Towards Single-Photon Microscopy: Exploiting Extra Spatio-Temporal Information Provided by SPAD Array Detector in Laser Scanning Microscopy

Giorgio Tortarolo[1,2], Marco Castello[1], Mauro Buttafava[3], Sami Koho[1], Eli Slenders[1], Alessandro Rossetta[1,2,4], Paolo Bianchini[4], Federica Villa[3], Alberto Diaspro[4,5], Alberto Tosi[3], Giuseppe Vicidomini[1]

[1]Molecular Microscopy and Spectroscopy, Istituto Italiano di Tecnologia, Genoa, Italy, [2]DIBRIS, University of Genoa, Genoa, Italy, [3]DEIB, Politecnico di Milano, Milan, Italy, [4]Optical Nanoscopy & NIC@IIT, Istituto Italiano di Tecnologia, Genoa, Italy, [5]DIFI, University of Genoa, Genoa, Italy

Laser scanning microscopy is one of the most common architecture in fluorescence microscopy, e.g., confocal, two-photon excitation (TPE), and stimulated emission depletion (STED) microscopy. In a nutshell: (i) the objective lens focuses the laser beam(s) to the sample and generates an effective excitation spot which is raster scanned across the sample; (ii) for each position/pixel the fluorescent image of the spot is projected into a single-element detector, which – typically – spatially and temporally integrates the fluorescent light over the detector sensitive area and during the pixel dwell-time, thus providing a single-intensity value per pixel; (iii) all pixel intensity values are registered to build up a digital image. It is clear that the spatio-temporal integration performed by the single-element detector hinders any additional information potentially encoded in the dynamic and image of the fluorescent spot.

To address this limitation, we recently upgraded [1] the detection unit of a laser scanning microscope, replacing the traditional single-element detector with a novel SPAD (single photon avalanche diode) array detector (Fig. 1 A).

First, we have shown that the additional spatial information provided by such a detector allows to overcome the tradeoff between resolution and signal-to-noise ratio (SNR) proper of confocal microscopy (Fig 1 B): indeed, this architecture represents the natural implementation of image scanning microscopy (ISM). The same spatial information is explored in a STED microscope [2] to mitigate the usually non-negligible chance of photo-damaging the sample with the high-intensity STED beam, and in a TPE microscope to compensate distortions/aberrations occurring for deep-imaging.

We then exploited the temporal information, in particular the single-photon timing ability of the SPAD array – all detector elements are fully parallel with < 200 ps timing jitter and 40 MHz maximum count-rate – to combine intensity and fluorescence lifetime (FL) imaging: the results show higher spatial resolution and better accuracy of the lifetime estimate with respect to the confocal counterpart (Fig 1 B).

Lastly, we discuss how the proposed SPAD-based laser scanning microscope can be used in the context of single-molecule experiments, such as imaging, tracking, and spectroscopy. We envisage that this implementation will trigger a transition from single-molecule microscopy to single-photon microscopy.

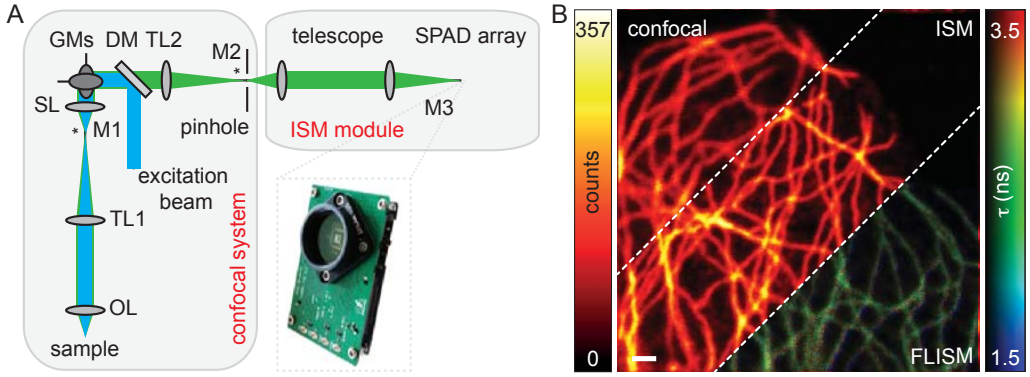

Fig. 1. (A) Scheme of the SPAD-based laser scanning microscope. (B) Example of imaging with the SPAD-based system. The image compares confocal microscopy, image scanning microscopy (ISM), and fluorescence lifetime ISM.

References

[1] Castello, M. et al. A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM. Nat. Methods 16, 175–178 (2019)

[2] Vicidomini, G., Bianchini, P. & Diaspro, A. STED super-resolved microscopy. Nat. Methods 15, 173–182 (2018)