TAGGING PHOTONS IN TIME AND SPACE: THE BRIGHT FUTURE OF FLUORESCENCE LASER SCANNING MICROSCOPY

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Laser scanning microscopy (LSM) is one of the most common microscopy architectures for fluorescence imaging and spectroscopy. In a typical fluorescence LSM: (i) the objective lens focuses a laser beam to generate an effective excitation region on a well-defined position of the sample; (ii) the same objective lens images such excitation region on the sensitive area of a single-element detector, which generates an electronic signal "proportional" to the collected fluorescence photons; (iii) the microscope data acquisition card registers the signal. Finally, in the context of imaging, the beam scanning system sweeps the excitation region across the sample, and the signals recorded at each pixel allow building a digital image.

Since, the image of the excitation region is integrated spatially – across the sensitive area of the detector – and temporally – along the pixel-dwell time for imaging or the microsecond bins for spectroscopy – any additional information potentially encoded in the fluorescence dynamics and the image of excitation region is lost.

To solve this limitation, we have upgraded a typical LSM by replacing its single-element detector with a novel asynchronous-readout single-photon-avalanche-diode (SPAD) array detector [1] and its data acquisition card with a multi-channels time-tagging architecture. In a nutshell, each collected photons is tagged with a nanometre scale spatial signature – its position within the image of the excitation region, and a picosecond scale temporal signature – its arrival-time with respect to a reference clock, e.g., the sync of a picosecond pulsed laser.

Here, we show selected examples in which this novel photon spatiotemporal information (together with the classical spatiotemporal information provided by a LSM, i.e., the pixel position within the LSM field-of-view and the microsecond sampling) is explored to improve most of the characteristics of LSM, such as the effective spatial resolution [1] and the penetrations depth [2]. Furthermore, we show how the same spatiotemporal photons' tags can be used to augment the information content of LSM as molecular quantitative assay and as tool to investigate molecular dynamics, structures and interactions.

[1] M. Castello, et al. "A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM," *Nat. Methods,* **16**(2), 175-178 (2019)

[2] S. Koho, et al., "Easy two-photon image-scanning microscopy with SPAD array and blind image reconstruction," *bioRxiv* (2019) doi.org/10.1101/563288