

The BrightEyes_TTM: an Open-Source Multi-Channel Time-Tagging Module for Single-Photon Laser-Scanning Microscopy

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Laser-scanning microscopy (LSM) is experiencing a high-tech revolution due to the introduction of high-throughput single-photon array detectors. These detectors gave access to an entirely new set of spatiotemporal information normally lost in conventional LSM, thus triggering a new imaging paradigm, the so-called single-photon LSM (SP-LSM).

Nowadays, the specifications of single-photon array detectors are constantly improving, also thanks to the well-established single-photon-avalanche diode (SPAD) array technology [1]. Within this context, there is an increasing need for data acquisition platforms able to harvest the information provided by this new category of detectors. We refer in particular to multi-channel time-tagging modules capable of connecting to a single-photon LSM and cope with the mega-sized temporal information delivered in parallel by each element of the detector array.

Therefore, in order to fill the gap between detector array performances and the lack of a benchmarking data-acquisition architecture for single-photon LSM applications, we developed an open-source FPGA-based multi-channel time-tagging module (TTM) that can be upgraded, modified and customized to satisfy the always-growing needs of the microscopy-makers.

The TTM is a time-to-digital converter (TDC)-based real-time acquisition apparatus that works as a passive plug-and-play device and can be operated, with minimal modifications, in pre-existing LSM setups. To demonstrate its functioning, we connected the module to a SP-LSM equipped with a SPAD array detector, and we demonstrated that current specifications allow for fluorescence lifetime image scanning microscopy (FLISM)[2] and fluorescence lifetime fluctuation spectroscopy (FLFS)[3] experiments.

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

[2] M. Buttafava *et al.*, “SPAD-based asynchronous-readout array detectors for image-scanning microscopy”, *Optica*, **7**(7), 755-765 (2019).

[3] E. Slenders *et al.*, “Confocal-based fluorescence fluctuation spectroscopy with a SPAD array detector”, *Light: Science and Appl.*, in-press (2021).