TIME-RESOLVED STED MICROSCOPY WITH SINGLE-PHOTON DETECTOR ARRAY: A PERFECT SYNERGY

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Image scanning microscopy successfully overcomes the trade-off between resolution and signal-to-noise ratio of traditional confocal microscopes by considering the spatial distribution of the fluorescence emission light and by reassigning the detected photons accordingly (i.e., pixel-reassignment). A recent implementation [1] upgrades a confocal to an image scanning microscope by substituting the traditional single-element detector with a SPAD array detector. Notably, the SPAD array samples the fluorescence signal from the detection/probing volume both in space (such as cameras) and in time (such as single element detectors, e.g., SPADs), providing potentially significant extra information for a variety of experimental contexts. To fully exploit this advantage, we present a versatile FPGA-based time-resolved microscopy platform that parallelly acquires all the SPAD array signals with a sub-nanosecond temporal resolution, thanks to a digital frequency domain (DFD) architecture. In the context of stimulated emission depletion (STED) microscopy, we leverage the platform to decrease the STED power needed to achieve a target spatial resolution [2]. In particular, we show how to synergically exploit both the spatial and temporal extra information to implement a new and dedicated photon-reassignment method for STED microscopy. This method not only takes advantage of the ISM principle but also compensates for all the different sources of background which typically reduce resolution and imaging quality in STED microscopy, i.e., incomplete depletion [3,4], direct-excitation from the STED beam, and out-of-focus signal. Additionally, the platform allows for fluorescence lifetime imaging and a straightforward pulsed interleaved excitation (PIE) implementation, enabling dual-colour STED microscopy.
These results significantly encourage the transition from single element detectors to SPAD arrays for laser scanning microscopy techniques.

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