TIME-RESOLVED (STED) IMAGE SCANNING MICROSCOPY WITH A SPAD ARRAY DETECTOR

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KEY WORDS: Time-resolved spectroscopy, ISM, FLISM, STED.

Image scanning microscopy (ISM) [1,2] stands as a powerful and promising fluorescence investigation technique for the Life sciences: it successfully overcomes the long-standing trade-off between the spatial resolution and the signal-to-noise ratio (SNR) of confocal laser scanning microscopy (CLSM), while preserving all its key features, the optical sectioning capability *in-primis*. We recently proposed and validated a straightforward implementation of ISM [3], obtained by equipping a typical CLSM system with a novel asynchronous-readout single photon avalanche diode (SPAD) array detector, and we demonstrated the superior performances of such a microscopy platform with respect to traditional CLSM. Here, we exploit the single-photon timing capabilities of our SPAD array detector to combine our ISM implementation with time-resolved spectroscopic essays and greatly enhance the information content of the experiment. In particular, we leverage both the photons' time-of-arrival and photons' spatial distribution information – simultaneously and exclusively provided by our SPAD array detector – to introduce fluorescence lifetime image scanning microscopy (FLISM). We demonstrate that FLISM provides higher spatial resolution and higher precision compared to conventional lifetime imaging systems. We then investigate the photons' spatiotemporal information provided by our SPAD array module in the context of time-resolved stimulated emission depletion (STED) microscopy [4]. This new platform allows to reduce the STED beam intensity needed to achieve a target spatial resolution, hence mitigating the chances of photo-damaging the sample [5].

In conclusion, we believe that the straightforward compatibility with a variety of temporal spectroscopy essays is a pivotal feature of the microscopy platform based on the SPAD array detector, and it may greatly contribute to its potential diffusion.

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