TIME-RESOLVED IMAGE SCANNING MICROSCOPY WITH A SPAD ARRAY

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KEY WORDS: image scanning microscopy, fluorescence lifetime imaging, SPAD array.

ABSTRACT:
Image scanning microscopy (ISM) represents one of the strategies to overcome the traditional compromise between signal-to-noise ratio and lateral resolution in confocal microscopy [1].
Recently, we successfully implemented ISM through modifications introduced on the detection plane of a standard confocal laser scanning microscopy (CLSM). We replaced the single detector with a novel SPAD (single-photon avalanche diode) array of 25 detectors (5 by 5 matrix).
Furthermore, we developed strategies based on pixel reassignment and image deconvolution to process the acquired data [2], demonstrating a spatial resolution improvement from ~230nm to ~170nm (far-red region), confirmed by our Fourier ring correlation (FRC) analysis [3]. In particular, we developed a totally blind method that does not require any calibration of the system (i.e. the measurement of the size of the detector on the object plane before imaging) and compensates for a non-perfect alignment of the detector.
In this work, we are presenting the extension of the technique to fluorescence-lifetime imaging microscopy (FLIM). In fact, our SPAD array can be used to perform time-resolved measurements since each of its elements is able to signal the arrival of photons with a time jitter having FWHM < 200ps.
For this purpose, we implemented a multi-channel time-correlated single-photon counting (TCSPC) system able to work at high photon-rate (4 MHz on the entire array). The TCSPC measurement results into a series of three-dimensional fluorescence decay images (the third dimension is photon arrival time), one for each element of the array, that are fused together to obtain the final super-resolved FLIM image. This is possible by extending the blind method described for imaging.
Further work will be devoted to increasing the maximum photon-rate and moving to real-time FLIM imaging (i.e. real-time calculation of the lifetime image).

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