# FLISM: FLUORESCENCE LIFTETIME IMAGE SCANNING MICROSCOPY WITH A SPAD ARRAY

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**ABSTRACT:**
Image scanning microscopy (ISM) is a strategy to push the effective resolution in confocal microscopy imaging to its theoretical limit [1]. Until now, available ISM implementations were, for different reasons, not compatible with fluorescence lifetime imaging (FLIM). During the past years, we successfully implemented ISM modifying a standard confocal microscope with a novel single-photon detector array. When used for imaging, each element of the array records a partial view of the sample [2]; then we developed a totally-adaptive method (no calibration required), based on pixel reassignment, to obtain the final super-resolved image.
Here, we present the extension of this technique to fluorescence-lifetime imaging [3]. This is possible because each element of our single-photon avalanche diode (SPAD) array can signal the photon arrival time with a time jitter having FWHM (full-width at half maximum) lower than 200 ps. Photon tags are recorded using a multi-channel time-correlated single-photon counting (TCSPC) system able to work at high photon-rate. A measurement results into a series of three-dimensional fluorescence decay images, where the third dimension is the photon arrival time. In analogy with what is done for imaging, all the decay images (one for each element of the array) are fused together with the same adaptive method to obtain the final super-resolved FLIM image.
Our implementation enables super-resolution FLIM, paving the way for a massive and definitive transition from confocal microscopy to ISM.
Further work will be devoted to increase the maximum photon-rate, with the in-house development of a dedicated electronic hardware and moving to real-time FLIM imaging, developing high-throughput parallel algorithms on massively parallel hardware like GPUs (Graphics Processing Units).

**REFERENCES:**
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