MULTIFOCAL FLIM SYSTEM WITH 0.25 BILLION TCSPC EVENTS/SECOND

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For high precision FLIM, time-correlated single photon counting (TCSPC) is unparalleled in its measurement accuracy particularly for multi-exponential decays[1, 2]. Until recently, high speed FLIM could only be performed using modulated or time-gated image intensifier systems[3, 4] as TCSPC was fundamentally limited with respect to photon counting rate in implementations of laser scanning microscopy[1].

We have since demonstrated multifocal fluorescence lifetime imaging microscopy for multiphoton (MM-FLIM) applications utilizing TCSPC[5, 6] which increases the acquisition rate of high resolution fluorescence lifetime imaging by a factor of 64 by parallelizing excitation and detection. The system consists of a two dimensional array of ultrafast beams (generated using a spatial light modulator) which are then optically conjugated with a Megaframe camera [16] consisting of 32×32 individual 10-bit time-to-digital convertor (TDC) array with integrated single-photon avalanche diodes (SPADs), each of which operates in TCSPC mode and provides FLIM capability. Although each individual SPAD in the array is capable of measuring a count rate of up to 30MHz, there are limitations to the number of counts which can be transmitted via USB2 (~20MHz count rate for the whole array assuming each count is 16-bits long). Due to these constraints in data transfer we were only able to use a fraction of the Megaframe camera size (8×8) in TCSPC mode.

We report the development of a massively parallelised MM-FLIM laser scanning system incorporating a USB3 based data transfer mechanism[7] with the ability to acquire ~0.25 million photon arrival events per second. This allows us to operate in full frame mode (32×32 beamlets) unlocking more potential from the Megaframe camera for FLIM imaging. To evaluate the lifetime imaging performance and capabilities of the MM-FLIM system we will demonstrate its use in monitoring dynamic interactions in live-cell imaging with FRET.

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