Parallelized Fluorescence lifetime imaging microscopy (FLIM) based on photon reassignment

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KEY WORDS: Fluorescence lifetime imaging microscopy, super resolution, photon reassignment, parallel detection.

1. INTRODUCTION

FLIM is a key fluorescence microscopy technique to map the environment and interaction of fluorescent probes. The combination of TCSPC and confocal laser scanning meets almost all the imaging requirements of FLIM ideally, however, the imaging resolution is constrained by the diffraction limit, and the imaging speed is also limited due to pile-up effects caused by dead time and signal processing time of the time-measurement circuity [1].

2. METHODS

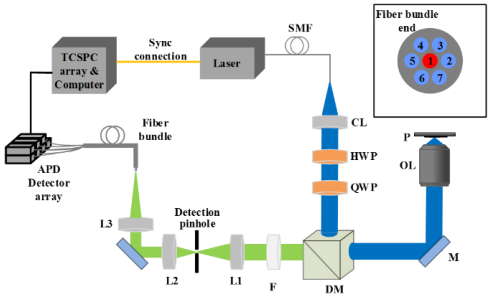

Figure 1: Schematic of parallel-detection based FLIM system.

To deal with the referred problems, we propose a novel parallelized FLIM method based on photon reassignment with a detector (APD) array and TCSPC array. The introduction of parallel detection can solve the pile-up effect caused by the dead-time in TCSPC measurement, which can enhance the effective photon detection efficiency and notably increase the fluorescence lifetime imaging speed. More importantly, the resolution and signal-noise ratio (SNR) can be enhanced based on photon reassignment.

3. RESULTS

As seen in Fig. 2, the experiment results of fluorescent beads with the size of 100nm show that the sub-diffraction resolution and faster scanning speed is realized by our method. The experiment results show that the resolution of our method is enhanced by a factor of 1.5 and the imaging speed can be at least increased by a factor of 2.5 compared with conventional confocal TCSPC FLIM system. We envision that the proposed FLIM method based on parallel detection will allow for super-resolution fluorescence lifetime real-time imaging of live cell.

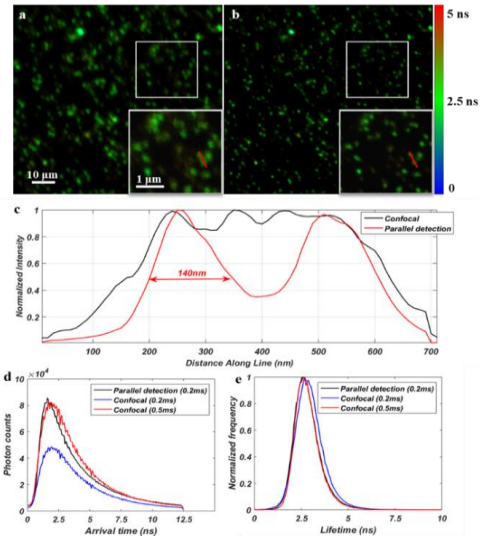

Figure 2: The experiments results of fluorescent beads

[1] M. Patting, M. Wahl, P. Kapusta, and R. Erdmann, "Dead-time effects in TCSPC data analysis," in *Proc. SPIE*, 2007, 658307.