Development of a multibeam scanning high frame-rate confocal FLIM microscope and its use for studies of protein-protein interactions in living cells

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Fluorescence lifetime imaging (FLIM) is a powerful technique for the measurement of protein-protein interactions via Förster resonance energy transfer (FRET). We have developed a time-correlated single photon counting (TCSPC)-based confocal FLIM microscope capable of imaging at frame rates > 1 Hz by scanning an array of visible beamlets generated using a spatial light modulator[1]. Our compact design uses a single tip-tilt piezo mirror to scan the beamlets across the sample and de-scan the resultant fluorescence which is imaged onto a an array detector comprising 32x32 individual single photon avalanche diodes (SPADs) with circular active areas of 6 µm diameter and temporal resolution of ~60 ps.[2] Each SPAD serves as both a confocal pinhole and FLIM detector. In addition to TCSPC detection a centre of mass method (CMM)[3, 4] for rapid estimation and inspection of fluorescence lifetimes is implemented in FPGA embedded FLIM processors. This microscope maintains the spatio-temporal accuracy of conventional TCSPC-based single beam scanning confocal FLIM whilst increasing the frame rate by more than two orders of magnitude.

We present dynamic FRET-FLIM measurements of interactions between fascin-GFP and actin-RFP in peripheral protrusions known as filopodia in live HeLa cells. Fascin, a protein that is upregulated in human carcinomas, bundles parallel actin fibres in filopodia[5, 6]. Using our novel confocal FLIM system we have studied the spatio-temporal dynamics of the fascin-actin interactions in stable, growing and retracting filopodia on a timescale of ~ 1 s. Our data highlight the necessity for high frame-rate FLIM for measuring protein-protein interactions in dynamic filopodia, and demonstrate the capabilities of our system for measuring these interactions via time-lapse FRET-FLIM.

References

1. Poland, S.P., Krstajic, N., Knight, R.D., Henderson, R.K. & Ameer-Beg, S.M. *Optics Letters* **39**, 2431-2434 (2014).
2. Richardson, J. et al. *Proceedings of the IEEE 2009 Custom Integrated Circuits Conference* (2009).
3. Li, D.U., Rae, B., Andrews, R., Arlt, J. & Henderson, R. *Journal of Biomedical Optics* **15** (2010).
4. Poland, S.P. et al. *Optics Express* **24**, 6899-6915 (2016).
5. Jayo, A. & Parsons, M. *Int. J. Biochem. Cell Biol.* **42**, 1614-1617 (2010).
6. Vignjevic, D., Kojima, S., Svitkina, T. & Borisy, G.G. *Journal of Cell Biology* **174**, 863-875 (2006).