Programmable lifetime imaging (PLI) to investigate association of EGFR complexes on the surfaces of cancer cell derived exosomes

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Understanding the mechanisms driving drug resistance in cancer patients is fundamental for continued progression towards effective treatment. Human epidermal growth factor receptor (HER/ErbB) network rewiring, through receptor dimerization, is believed to play such a role. Therefore, it is highly desirable to detect and monitor such phenomena non-invasively, in the clinic. Time-correlated single photon counting fluorescent lifetime imaging (FLIM) is a highly sensitive and non-invasive method to detect protein interactions. Modern FLIM systems typically comprise a laser scanning module and point detector to analyse the fluorescence of an assay on a pixel-by-pixel basis. Confocal sectioning improves the signal-to-noise and resolution of TCSPC data, yet suffers from being particularly slow; typical acquisition times can take up to five minutes per frame. Our lab has recently significantly improved upon this by parallelising up to 1024 confocal points simultaneously, reducing acquisition times to seconds. However, for sparse image fields where data is located in a small percentage of pixels, considerable time is wasted scanning every pixel in the field-of-view. As such, we introduce a new fluorescence lifetime data acquisition technique whereby data is collected sequentially and automatically from features of interest within the field-of-view, greatly improving the efficiency of data acquisition. We can then reconstruct, for the first time, lifetime images based on the localization information of such features. Furthermore, the system is flexible such that if the entire frame is not necessary, the user can select particular areas within the total field-of-view to measure, further improving acquisition times. We demonstrate the advantage of the system by imaging H1975 cell derived exosomes containing the marker GFP-CD63 under evanescent field illumination. Additionally, we show that the system has sufficient resolution to significantly improve the throughput of a FRET assay detecting association of EGFR complexes on the exosome surface.