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Optics and Photonics Group Lunchtime Seminar

"Protein-protein interactions at the cellular interface: Biophotonics approaches to quantitative FRET measurements"

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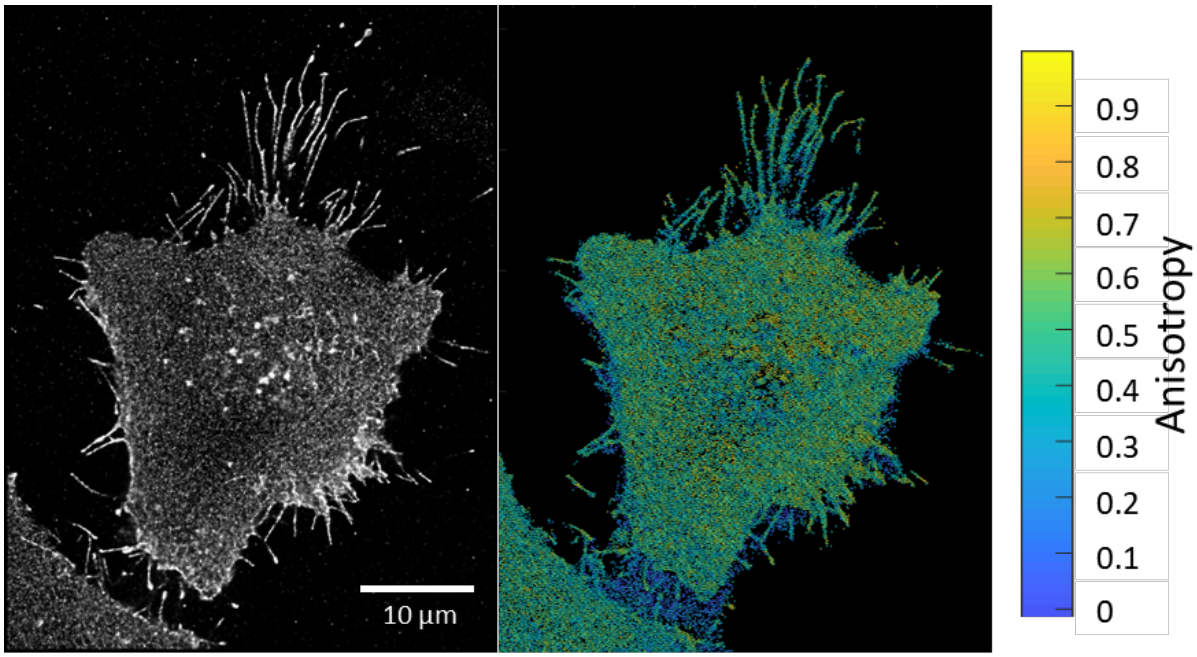

1:00pm Thursday 9th May 2019
203 Tower building
All Welcome

Abstract

http://optics.nottingham.ac.uk/wiki/Talks_2019

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The control of cellular function and intracellular signalling is clearly complex and highly regulated. Many signalling cascades have been extensively investigated and the relationships between proteins have been partially delineated using biochemical techniques. Microscopical techniques coupled with immunocytochemical methods have allowed researchers to image the relative localization of multiple signalling molecules. However, both types of measurement assume (different degrees of) cell homogeneity, which means that important variations in behaviour across the cell, and localisation of signalling events to specific parts of the cell, will be missed. Many intracellular structures are heterogeneous below the 200 nm length scale resolvable by wide-field microscopy. Measurement of the near-field localization of protein complexes may be achieved by the detection of FÃűrster resonant energy transfer (FRET) between protein-conjugated fluorophores. FRET is a non-radiative, dipole-dipole coupling process whereby energy from an excited donor fluorophore is transferred to an acceptor fluorophore in close proximity. The dependence of the coupling efficiency varies with the inverse sixth power of the distance between acceptor and donor and is typically described in terms of the FÃűrster radius (distance at which the efficiency of energy transfer is 50%), typically of the order 1-10 nm. Since the process depletes the excited state population of the donor, FRET will both reduce the fluorescence intensity and fluorescence lifetime of the donor. The advantage of using donor fluorescence lifetime to detect FRET is that the method is independent of fluorophore concentration, donor-acceptor stoichiometry and optical path length and is therefore well suited to studies in intact cells. Combined with confocal or multiphoton techniques to examine the localization of effects in cellular compartments, FLIM/FRET allows us to determine populations of interacting protein species on a point-by-point basis at each resolved voxel in the cell. The use of ‘ensemble’ FRET/FLIM techniques to probe protein-protein interactions in intact cells is now an established technique. We and others have adapted the FLIM-based protein-protein interaction assays to directly monitor post-translational modifications (PTMs) of proteins (such as phosphorylation by PKC, ubiquitination and sumoylation) within live and fixed cells.

In this seminar, I will discuss recent developments in high-speed, multiphoton FLIM which allow us to image fluorescence lifetime changes due to FRET in a fraction of a second using multifocal multiphoton microscopy. Examples of protein-protein interactions in the ErbB network will be presented. I will show our latest implementation of multifocal multiphoton microscopy offering imaging at up to 0.5 billion photons per second with imaging acquisition of up to 50 frames per second.

Finally, I will present a recently developed FRET technique, which allows quantification of protein interaction by acceptor fluorescence anisotropy down to the single molecule level and shows significant promise in simplicity and speed. The technique provides a direct read-out of interaction with a good dynamic range and also permits elimination of false positives linked to direct excitation of the acceptor. I will show that this analysis method is comparable to FLIM for quantification of protein-protein interactions by FRET and offers significant advantages in terms of speed and dynamic range at typically observed FRET efficiencies.