2pm 11 Mar 2015 Christopher Dunsby (Imperial)

Ingram Lecture Theatre

Tittle: 'Multi-dimensional fluorescence imaging: for cell biology, high content analysis and label-free tissue imaging'

Abstract:

This talk will review our work on quantitative fluorescence microscopy techniques, with an emphasis on fluorescence lifetime imaging (FLIM). FLIM can provide readouts of variations in the local molecular environment of fluorophores, which may be exploited using small molecule fluorescent probes or used to sense the proximity of other fluorophores via Forster resonant energy transfer (FRET). Because FLIM is an inherently ratiometric technique that is relatively insensitive to probe concentration or signal attenuation, it is increasingly applied to read out FRET of appropriately labelled protein-protein interactions to study cell signalling networks.

For cell biology we have developed FLIM microscopes to study fixed and live cells, including high-speed optically sectioned FLIM microscopes for rapid 3-D imaging of cell biology. In order to allow drug discovery to take more advantage of FLIM and FRET, e.g. to screen for protein interactions, we have developed automated optically sectioned multiwell plate readers that can image a 96 well plate in less than ~15 minutes, including all sample translation, focussing and FLIM. Together with our associated analysis software, FLIMfit, we believe that this technology makes FLIM a practical tool for high content analysis (HCA) including for live cell assays. We have recently used this technology to measure dose response curves in an exemplar assay reading out aggregation of the Gag protein in the presence of an inhibitor.

We are also developing a high-speed microscopy technique called oblique plane microscopy (OPM) capable of performing real time 3-D imaging of live cells. This method allows us to study events such as calcium sparks and waves in cardiac myocytes in 2-D at 660 frames per second and in 3-D at 25 volumes per second.

For clinical applications, spectrally resolved FLIM can be applied to study autofluorescence to provide label-free molecular contrast in biological tissue and we are applying this to study skin cancer. We are also developing a novel approach for multiphoton microscopy enabling 3-D scanning with no distal moving parts that has the potential to provide endoscopic imaging with sub-cellular resolution via a sub-millimetre diameter endoscope.