Fluorescence Imaging on Small Space and Time Domains

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Background:

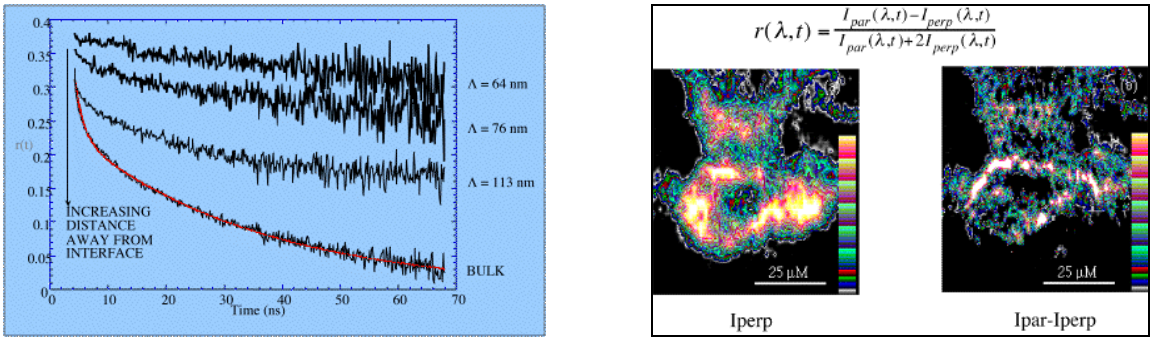
The identification of the degree of localisation and the speciation of fluorescent species is required at increasingly small levels of spatial resolution, particularly in biological samples. Simultaneously, time-resolved fluorescence measurements with high temporal resolution provide additional information to that achievable with conventional intensity-based emission microscopy techniques.

Outcomes:

- Methods for high spatial (nanometre) and temporal (nanosecond, picosecond and femtosecond) imaging of systems such as pharmaceuticals in tissue.
- Methods for such studies at the single molecule level.
- Methods and devices for the quantitative detection of fluorescent biological species

Progress to date

We have developed a range of time-resolved fluorescence imaging techniques, expanding "conventional" techniques. One specific example of this is the extension of total internal reflection fluorescence techniques (e.g. TIRF microscopy) to the picosecond regime. This potentially will provide the generation of more accurate three-dimensional imaging of adsorbed species such as proteins to surfaces. We have also developed time-resolved fluorescence polarisation techniques following TIRF excitation, which provides detailed information regarding the motion of macromolecules near an interface.



Left: *Time-resolved fluorescence anisotropy measurements of fluorescently-labelled (ANS) bovine serum albumin (BSA) following TIR excitation. TIR excitation at various angles of incidence provides depth profiling of protein motion within tens of nanometres of the interface.* Right: *polarised emission images of a porphyrin dye in tissue*

1) **Time-resolved polarised emission TIRF measurements** have been developed and illustrate the ability to probe macromolecular motion near interfaces.
2) **Time-resolved TIRF microscopy has been developed** and applied to preliminary measurements of fluorescence at a cellular level.
3) **Time-resolved Fluorescence Resonance Energy Transfer** (FRET) experiments have been preformed probing changes of protein conformation upon adsorption
4) **A range of advanced optical microscopy techniques** have been developed. These include scanning near field, time-resolved confocal and multiphoton microscopy, single molecule detection/spectroscopy etc. which can be applied to a range of problems and samples.

We wish to develop these techniques further towards the development of methods and devices for the quantitative detection of fluorescent biological species at high spatial and temporal resolution.

We have expertise in and operate a comprehensive range of advanced ultrafast laser and advanced optical microscopy instrumentation, which is available for collaborative projects in a range of areas**.**

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