Mapping time-resolved and emission depolarization behaviour of evanescent wave-induced emission

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Total internal reflection (TIR) (or evanescent wave (EW)) induced fluorescence techniques can be used to study the photophysical properties of dyes and polymers in close (~100 nm) proximity to interfaces. Varying the angle of incidence of the excitation light affords a level of control over the penetration depth of the evanescent field into the medium of lower refractive index, and thereby provides a means to probe properties that vary from the interfacial region to the bulk solution. By recording fluorescence decay information following EW-excitation provides significant advantages and additional information to that accessible through steady-state measurements.

The unique capabilities of TIR fluorescence methods can also be exploited by using polarised excitation and emission detection.[1] We have performed time-resolved EW-induced fluorescence anisotropy measurements to probe molecular photophysics, motion and conformational change in the interfacial region. This approach allows some discrimination of phenomena to be probed between processes occurring in- and out-of the plane of the interface.

We have also implemented polarised, steady-state fluorescence anisotropy mapping on the µm scale using both conventional and TIR fluorescence wide-field microscopy. Through this approach spatial variations in emission depolarising phenomena, such as molecular rotational motion or energy migration, can be visualised, and changes in this behaviour can be monitored.

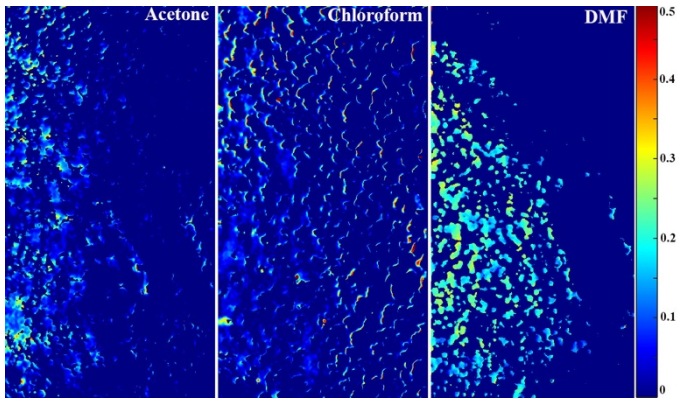

Figure 1. Fluorescence anisotropy histogram of air-dried Coumarin 6 ~100 nm thin films cast from a range of solvents

Coupled with time-resolved fluorescence imaging, fluorescence anisotropy imaging can provide a great deal of detail on the microenvironment within a thin film. The results of fluorescence anisotropy imaging measurements on a range of systems, included dyes in polymer films (Fig. 1), aligned molecular and polymeric systems and probing molecular crowding effects within cells (Fig. 2),[2] will be discussed.

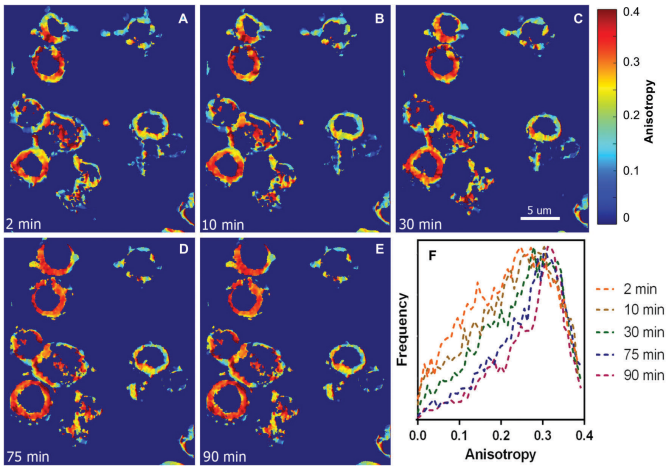

Figure 2. Real time monitoring of cytoplasmic crowding effect in live cells. (A–E) Fluorescence anisotropy images of TPE-Py-NCS stained Neuro 2A cells taken at different time 55 points after being exposed to 450 mM sorbitol. (F) Fluorescence anisotropy distribution histograms from panels A–E. [TPE-Py-NCS] = 10 mM. Scale bar: 5 μm.[2]

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References:

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