SINGLE MOLECULE IMAGING OF FLUORESCENCE ANISOTROPY

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Given the inherent complexity of biological systems, it is necessary to go beyond ensemble measurements and attain information at the single molecule level to accurately probe molecular properties. Single molecule imaging can examine real-time conformational dynamics [1], which is often the underlying cause of heterogeneity in molecular distributions in terms of dipole orientations, spectra, or intramolecular distances, in both stable and unstable systems.

A combination of polarisation-resolved detection and 2-colour alternating laser excitation (ALEX) allows quantification of the anisotropy and stoichiometry of the fluorophores present [2]. As a result, it is possible to accurately quantify Förster resonance energy transfer (FRET). This technique provides a rapid approach for probing the fluorophore's environment in terms of viscosity, interactions between molecules, and ligand-substrate binding.

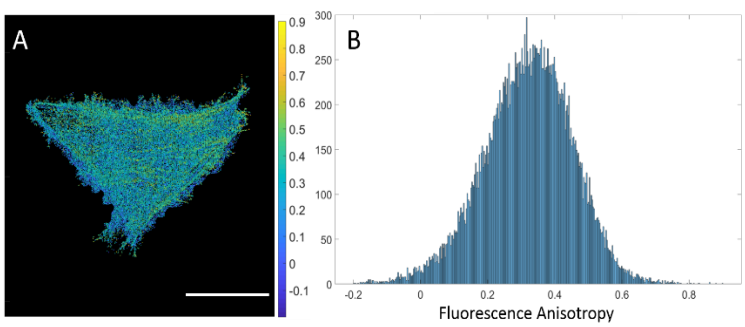

Figure 1: Single molecule anisotropy imaging. (A) An image of a fixed Hela cells transfected with Life Act-mEos2 shown with the associated (B) fluorescence anisotropy histogram. Scale bar: 20 μm.

Here we present an optimised TIRF microscope in conjunction with ALEX and steady state fluorescence anisotropy detection [3] for single molecule imaging. Validation and determination of the limits of the technique were accomplished via measurements of isolated fluorescent proteins. This technique was subsequently applied to elucidating the complex and poorly understood dimerization behaviour of transmembrane proteins CXCR4 and EGFR upon ligand activation.

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