# Simultaneous Two-Colour TIRF using Solvatochromic Probes Enables Study of Membrane Biophysics at the Immune synapse

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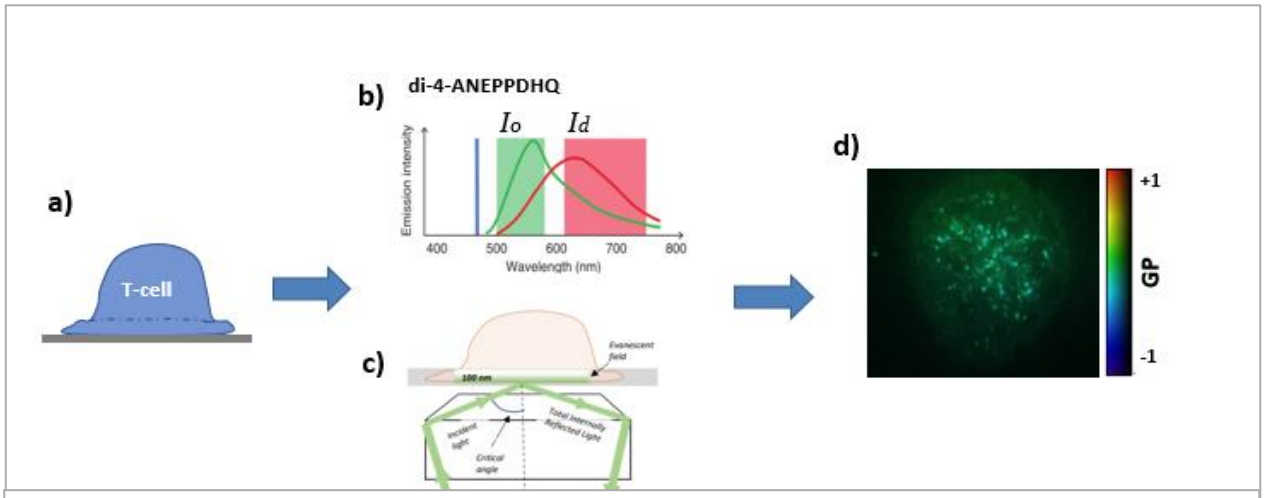
Total internal reflection microscopy (TIRF) allows real time imaging of events occurring at the cell-glass interface, such as trafficking of vesicles and proteins. We use a 2-channel TIRF set-up in combination with a novel class of fluorescent probes, called environmentally-sensitive dyes, to directly visualise the level of lipid packing within both live and fixed cells. A generalised polarisation (GP) parameter provides ratiometric quantification of the lipid order, which has been shown to have a crucial role in regulating various cell functions. Current analysis software can then correlate the GP with live-cell dynamic data, or perform cross-correlation with fixed cell data. This allows us to simultaneously image intracellular events while recording the membrane order, or other biophysical parameters, within the biological system.




***Methods: a)*** *Model immune synapse system* ***b)*** *Solvatochromic probes can distinguish between lipid ordered (Io) and disordered (Id) phases* ***c)*** *TIRF microscopy for visualisation of plasma membrane associated events* ***d)*** *Pseudo-coloured GP image of the immune synapse (colour represents level of lipid order, measured by generalised polarisation (GP) parameter)*

Our system of interest is the T-cell immunological synapse, which is a highly coordinated junction formed between a lymphocyte and its antigen-presenting cell. It forms as a result of successful recognition of a T-cell with its pathogen and is a crucial part of the adaptive immune response. Any dysregulation in the intricate trafficking pathways that regulate this tightly controlled system could result in immune and autoimmune diseases. Using advanced fluorescence imaging, labelling and analysis methods described above, we study vesicle trafficking at the immune synapse, with particular interest in discovering the potential role of membrane order in regulating this complex network.