

Imaging Lipid Organization with Fluorescence-detected 2-photon Linear Dichroism

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In studying cellular membranes, there are limited methods available for non-invasive, quantitative studies of assembly and dynamics of lipids. Here we present a method for measuring the orientation of fluorescent probes in cell membranes using fluorescence-detected linear dichroism.

Linear dichroism (excitation anisotropy) is a function only of chromophore orientation and so does not require assumptions concerning any depolarizing process that occurs following excitation. We have imaged linear dichroism using a wide-field multi-photon microscope that utilises multiple foci and an imaging detector, such that the sample can be scanned and imaged rapidly. This multi-beam, multi-photon approach provides rapid fluorescence imaging with intrinsic sectioning and minimal photo-damage. For this application it also provides increased photo-selection, resulting in higher sensitivity for samples with a low degree of orientation, and additional parameters to resolve an orientation distribution.

We have developed a model to extract the fluorophore orientation from the linear dichroism data. This orientation is studied for several different lipid probes bound to the plasma membrane of live cells and including recently reported membrane nanotubes. To study dynamic changes in lipid order, time lapse imaging following cholesterol extraction was studied (see Figure 1). The probe with a chromophore located on the surface undergoes negligible orientation change; whereas the probe with a chromophore buried between the acyl-chains of neighbouring lipids loses orientation rapidly. This is consistent with the role cholesterol plays in providing order and rigidity to the lipid tails in the membrane. We will also present initial data on imaging the molecular orientation at the immune synapse- the intercellular contact between immune cells.

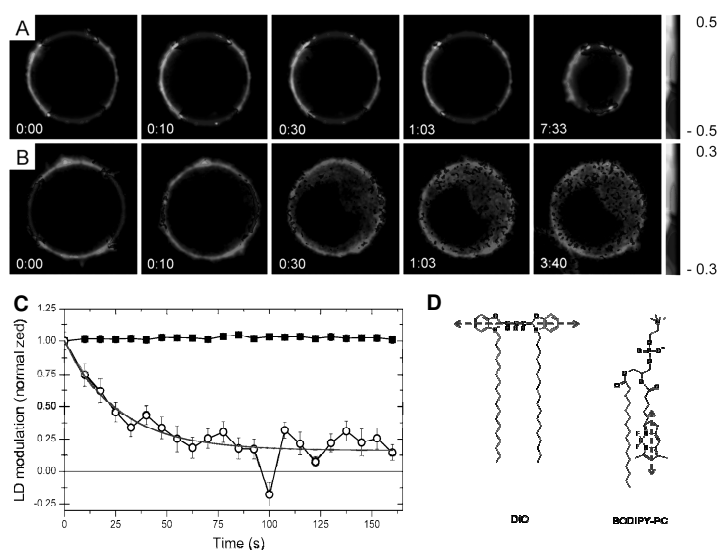


Figure 1. A, B: Images of plasma membrane stained with DiO and BODIPY-PC respectively, at time points following cholesterol extraction. C: graph demonstrating how orientation of DiO (surface probe) remains constant whereas as for BODIPY (buried probe) it decays rapidly. D: Structure of DiO and BODIPY-PC lipid probes, with their respective dipole moments shown as a dashed line.