NEW FLUORESCENCE MICROSCOPY APPROACHES
FOR DETECTING NANO SCALE LIPID PHASE SEPARATION

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The lipid raft hypothesis postulated the existence of highly dynamic, nano-scaled lipid domains in cellular membranes that are regulated by the dynamic lipid phase behaviour. Phase segregation into liquid ordered phase (Lo) and liquid disordered phases (Ld) yields biophysically and biochemically discrete platforms. However, the existence and the role of the nanoscale lipid phases in cell membranes remain controversial, mainly because of the technical difficulties in directly detecting small and transient lipid domains.

Here, I will present the evidence of nanoscale phase separation in the plasma membrane of live cells. We combined the phasor analysis of spectrally resolved fluorescence lifetime (FLIM) data with the membrane order-sensitive dye, Laurdan, to acquire information about the local polarity and dipole relaxation rate, which indicates the amount of water molecules and their mobility in the membrane, respectively. Data obtained in live cells indicated a coexistence of two lipid phases with different polarity and hydration. In addition, we have recently developed fluorescence spectral correlation spectroscopy (FSCS) to obtain spectral cross-talk free auto- and cross-correlation functions for probes with highly overlapping emission spectra, using a photon weighting approach. When applied to plasma membrane stained with a new bright and photo-stable lipid phase sensitive dye NR12S, it was possible to simultaneously obtain correlation curves corresponding to the dynamics of the NR12S single molecules located in Lo and Ld phases. Surprisingly, we found that in the outer leaflet of plasma membrane of COS7 cells, the correlation time for NR12S in Lo phase was shorter than for the dye in Ld phase. The cross-correlation function indicated that the membrane was phase separated with dye molecules switching between the phases on a millisecond time scale. Next we plan to test how various cell treatments change the lipid phase coverage and dynamics and to link the observed NR12S diffusion behaviour with either lipid composition or cytoskeleton structure.