

# **In-Situ Visualization of 2D Phase Separation of Oxidized Phospholipid and Cholesterol**

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While self-diffusion of surfactant molecules has long been investigated with tools such as photobleaching, studying the inter-mixing of two or more surfactants remains a challenge primarily due to hurdle in preparing well-defined initial condition. In this poster session, we present a technique that enables the formation of clear boundary between two lipid domains, namely ‘droplet merging technique’. This was achieved by initially preparing two monolayers: (1) fluorescence-tagged lipid monolayer on a planar water surface and (2) Chol monolayer adsorbed on a water droplet. Droplet monolayer was then incorporated into planar monolayer by coalescing the water droplet. By using this technique, we observed the phase separation of oxidized dioleoylphosphatidylcholine (oxDOPC) and cholesterol (Chol). Additional analyses including line tension measurement and numerical analysis reveals oxidation-dose dependent manner of their immiscibility. Our findings suggest that phospholipid oxidation can induce aberrant phase separation of lipid membrane, leading to disruption of membrane organization. The technique presented in our study can be potentially utilized for assessing the interfacial mixing properties of various surface-active molecules.