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Wide-field Time Resolved Microscopy for in-situ Lipid Phase Dynamics[1] NEDA DADASHVAND, FELIX SCHUPP, CHRISTINA OTHON, Wesleyan University — We have developed a new time-resolved fluorescence platform which enables us to follow the molecular orientation and dynamics of a lipid monolayer at the air – water interface. Confocal microscopy is limited in its ability to characterize dynamic orientation changes within cellular membranes. By implementing an all reflective Cassegrain objective we minimize dispersion while eliminating the restriction of collinear excitation. We investigate the miscibility transition of a ternary lipid mixture, DPPC / DOPC/ Cholesterol, using a combination of fluorescence imaging and spectroscopy. The technique affords unprecedented dynamic characterization for lipid orientation as the monolayer is forced from the liquid to the gel phase. We demonstrate the applicability of this device by contrasting the time-resolved fluorescence signal of three different lipid probes (NBD-PC), (BIODIPY), (Dil) which show different orientation and dynamic freedom when bound to the lipid layer for a range of lipid phases. Using this technique we can resolve highly dynamic processes such as the insertion of peptide and proteins into the lipid membrane.

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Neda Dadashvand
Wesleyan University

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