# VISUALIZING MEMBRANE COMPOSITION AND AMYLOID AGGREGATE ORGANIZATION USING SINGLE-MOLECULE 3D ORIENTATION IMAGING

**Tianben Ding, Jin Lu, Tingting Wu, Matthew D. Lew**

**Department of Electrical and Systems Engineering**
**Washington University in St. Louis**
**One Brookings Drive, St. Louis, Missouri, 63130 United States**
**E-mail: mdlew@wustl.edu**

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Conventional single-molecule localization microscopy (SMLM) uses repeated blinking of fluorescent molecules to reconstruct images of biological structures with resolution beyond the Abbé diffraction limit, and recent developments like MINFLUX have pushed resolution limits to the single nanometer regime. Complementing these techniques, we have developed single-molecule orientation localization microscopy (SMOLM) to measure the orientations of single molecules (SMs) in addition to their positions. In SMOLM, images of blinking single molecules are used to jointly estimate their positions $(x, y, z)$, orientations $(\bar{\mu}_x, \bar{\mu}_y, \bar{\mu}_z)$, and rotational "wobble" (parameterized by solid angle $\Omega \in [0,\ 2\pi]$) during a camera frame. SMOLM leverages both engineered point-spread functions (PSFs) and regularized maximum likelihood estimators to enable both the translational and rotational dynamics of ~$10^5$ single molecules to be measured with nanoscale precision.

Our measurements show that the 3D orientation and wobble of Nile red (NR) molecules are extremely sensitive to the chemical composition of lipid bilayers [1]. For example, in a DPPC bilayer without cholesterol, NR exhibits a tilted out-of-plane orientation (polar angle θ=26.0°), and as the cholesterol concentration increases to 40%, the tilt angle decreases dramatically (θ=8.7°). In addition, NR within liquid-ordered vs. liquid-disordered domains shows a ~4° difference in polar (tilt) angle and a ~0.3π sr difference in wobble (rotational diffusion) angle, making it possible to detect these domains via SMOLM directly.

NR also transiently binds to aggregates of amyloid beta peptide (Aβ42). We have used SMOLM to probe the binding orientations of NR relative to the fiber superstructure. Interestingly, our analysis shows that fiber bundles are not always resolvable via SMLM due to limited localization precision, but SMOLM is able to detect disordered fiber organization through broader distributions of both SM orientations and wobble dynamics. SMOLM opens the door to visualizing dynamic reorganization of amyloid aggregates in real time.

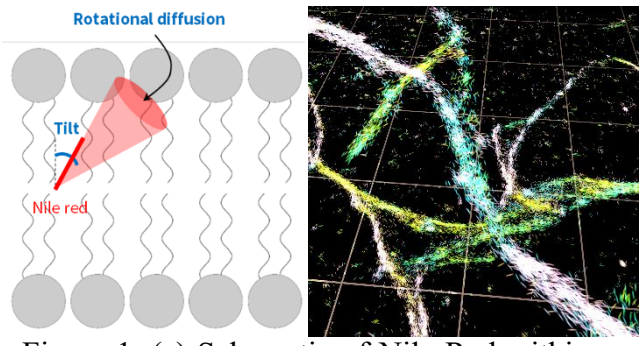


Figure 1. (a) Schematic of Nile Red within a lipid bilayer. (b) SMOLM rendering of Nile red orientations on amyloid fibers, color-coded by in-plane orientation. Grid = 1 μm.

[1] J. Lu, H. Mazidi, T. Ding, O. Zhang, and M. D. Lew, "Single-molecule 3D orientation imaging reveals nanoscale compositional heterogeneity in lipid membranes," *Angew. Chem. Int. Ed.* **59**, 17572–17579 (2020).

[2] T. Ding, T. Wu, H. Mazidi, O. Zhang, and M. D. Lew. "Single-molecule orientation localization microscopy for resolving structural heterogeneities between amyloid fibrils," *Optica* **7**, 602 (2020).