MEASURING SINGLE-MOLECULE ORIENTATIONS USING A RADially AND AZIMUTHALLY POLARIZED EPIfluorescence Microscope

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Single-molecule orientation localization microscopy (SMOLM) has been recently developed to measure the orientations of single molecules (SMs) in addition to their positions. In typical SM experiments, a camera detects multiple photons during each acquisition frame. Therefore, the rotational dynamics of SMs are inherently encoded in the detected images, i.e., a rotating molecule can be parameterized by its average orientation $\vec{\mu} = [\mu_x, \mu_y, \mu_z]$, where $\mu_z$ represents the direction parallel to the optical axis, and wobble solid angle $\Omega$ over a unit sphere.

Since the polarization of light emitted by SMs depends on their orientations, a widely used method to measure molecular orientation is to add a polarizing beam splitter to a standard microscope to create $x$- and $y$-polarized ($xy$Pol) imaging channels, which can sensitively differentiate $x$- and $y$-oriented molecules. However, it is difficult to measure the orientation of molecules with non-zero $\mu_z$ components since the photons emitted by $z$-oriented molecules are evenly distributed between the $x$- and $y$-polarization channels. Here, we propose using a vortex half-wave plate placed at the back focal plane of the imaging system to convert radially and azimuthally polarized light to $x$- and $y$-polarized light, creating a radially and azimuthally polarized (raPol) standard point spread function (PSF). This method measures the orientations of $z$-oriented molecules more precisely than $xy$Pol since light emitted by $z$-oriented molecules is radially polarized.

To demonstrate raPol imaging, we imaged Nile Red (NR) molecules in DPPC lipid bilayers with 40% cholesterol; molecular crowding causes the NR to be mostly parallel to the optical axis ($z$-oriented) [1]. The raw raPol fluorescence images [Fig. 1(a)] exhibit higher contrast than those of $xy$Pol [Fig. 1(b)]. Further Cramér-Rao bound analyses show that the precision for measuring both the average orientation $\vec{\mu}$ [Fig. 1(c)] and wobble angle $\Omega$ using raPol [Fig. 1(d)] is superior to those using $xy$Pol. Surprisingly, our analysis shows that raPol also outperforms many engineered PSFs.