HIGH-DIMENSIONAL SUPER-RESOLUTION IMAGING OF SUBCELLULAR LIPID MEMBRANES WITH STRUCTURED ILLUMINATION

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Structured illumination microscopy (SIM) is widely used in biological research because of its doubled spatial resolution, fast imaging speed, and low photon-toxicity. Recently we demonstrated polarized SIM (pSIM) exploiting the polarization modulation in SIM systems and achieved super-resolution of fluorescent dipoles [1]. Here we further develop a six-dimensional super-resolution imaging technique based on structured illumination for the studying of subcellular lipidomics in vivo. We use one florigenic lipid dye, Nile Red, to universally stain all the lipid membranes and obtain super-resolution mapping of the morphology, polarity, and phase of different lipid membranes from fluorescence intensity, emission spectrum, and polarization modulation. We image and quantitatively mapped the lipid polarity and phase on up-to-eight cellular compartments, including the plasma membrane, nuclear membrane, ER, mitochondria, lipid droplet, lysosome, endosome, and Golgi apparatus.

As the missing cone in the optical transfer function of wide field microscopy brings a high background signal that deteriorates the accuracy of functional measurements, we develop POLarized Optical Sectioning (POLOS) method to remove the bias induced by the out-of-focus background [2]. POLOS applies two structured patterns with π phase shift for each of three illumination patterns, which takes six raw acquisition for a 2D imaging. Afterward, POLOS uses a Fourier filtering technique based on HiLo [3] to attenuate the low-frequency signal of the background signal. Experimental results show that POLOS significantly increases the mapping accuracy of the spectrum and polarization measurement.

Our experiments uncover the heterogeneity of various lipid membranes in live cells. With super-resolution, we discover the different lipid polarity and order even within a single organelle, such as the core and shell of single lipid droplet, or the outer membrane and the cristae of single mitochondria. We also distinguish the lipid heterogeneity within the same type of organelle on different developmental stages, such as the early endosome and late endosome. Based on the heterogeneity of lipid membranes in morphology, emission spectrum, and polarization modulation, we further segment different organelles with artificial intelligence. Therefore, we super-resolve eight cellular compartments simultaneously with only one dye, which potentially relates cellular lipidomics with organelle interactome. Our technique can be performed on off-the-shelf SIM systems, which will immediately advance the research in lipidomics and organelle interactome.

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