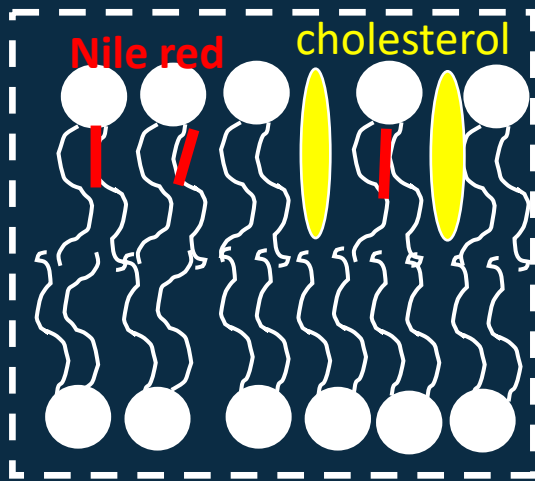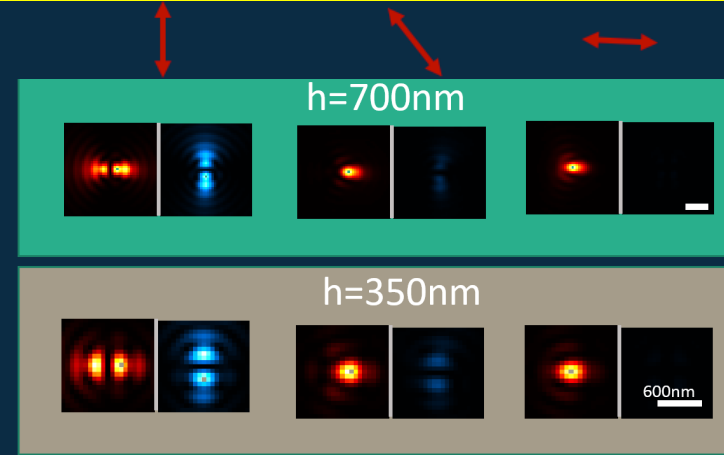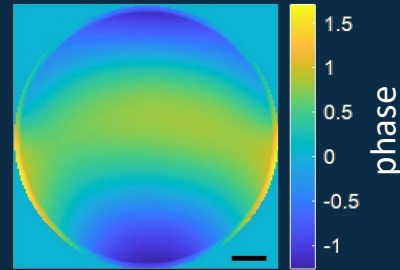**Accurate super-resolution microscopy of the 3D orientations and 3D locations of single molecules within lipid membranes**

3D spherical lipid bilayer

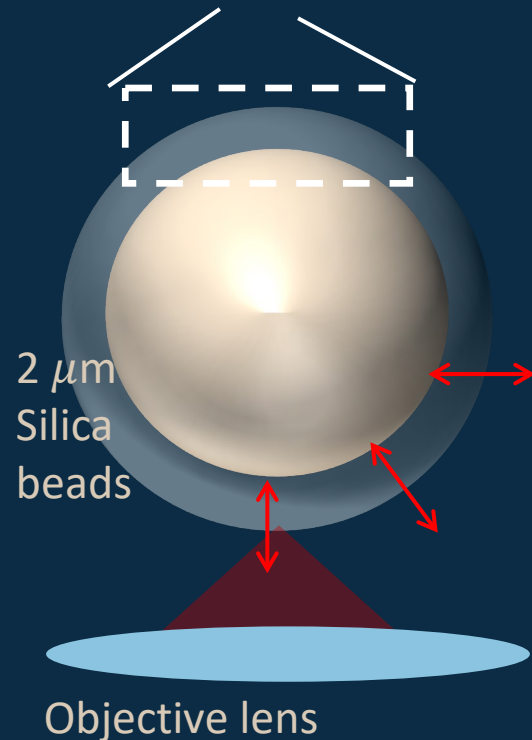Nile red   cholesterol

3D orientation+3D location measurement

pixOL phase mask

phase

h=700nm

h=350nm

600nm

pixOL phase mask encodes the 3D orientation and 3D location information into the shape of PSF

2 μm Silica beads

Objective lens

Reveal the distribution of cholesterol within lipid membrane

300 nm <h< 400 nm   500 nm < h < 600 nm   700 nm < h < 800 nm   900 nm < h < 1000 nm   1100 nm < h < 1200 nm

DPPC+ cholesterol

Perpendicular orientation

90°
180°   0°
-90°

400 nm

DPPC-only

Random orientation