Pushing the resolution limit by interferometric localization microscopy

With the support by the National Natural Science Foundation of China and the Chinese Academy of Sciences, the research team led by Prof. Xu Tao (徐涛) at the National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, developed a new interferometric single molecule localization microscopy with fast modulated structured illumination, which was published in *Nature Methods* (2019, 16(11): 1114—1118).

In the past decade, although various image based central position estimations (termed as "centroid fitting"), such as 2D Gaussian fitting methods, have been commonly used in single molecule localization microscopy (SMLM) to precisely determine the location of each fluorophore, it remains a challenge to improve the single molecule lateral localization precision to the molecular scale (< 2 nm) for high throughput cellular nanostructure imaging.

In this technique named Repetitive Optical Selective Exposure (ROSE), the authors pushed the resolution of SMLM to less than 3 nm (~1 nm localization precision). ROSE utilized six different direction and phase interference fringes to excite the fluorescent molecules, and found that the intensity of the fluorescent molecules is closely related to the phase of the interference fringes. A fluorescence molecule is located by the intensities of multiple excitation patterns of interference fringes, providing around two-fold improvement in the localization precision.

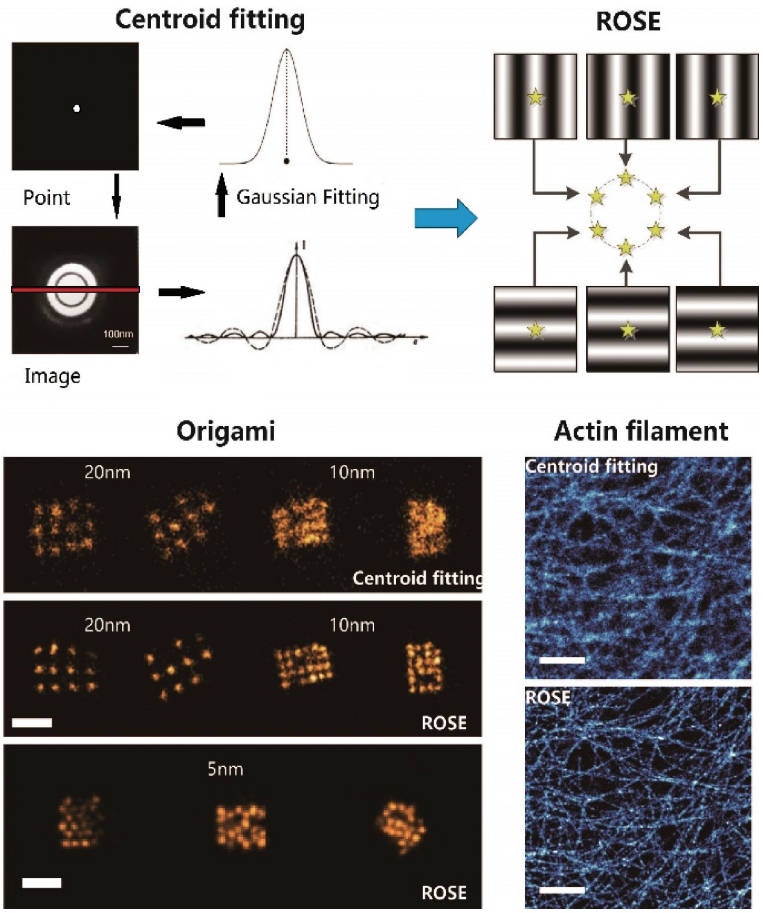

Figure The principle and results of ROSE with DNA origami and cells. (Modified from *Nat Methods*, 2019, 16(11): 1114—1118.)

The authors demonstrated that ROSE could resolve 5 nm separated structure at a resolution of ~3 nm over a large field of view of 25 x 25 μm^2, which means that ROSE has the ability to push the resolving power of SMLM to the molecular scale. They also showed that ROSE had advantages in resolving the hollow structure of single microtubule filaments, small clathrin-coated pits (CCPs) and cellular nanostructures of actin filament. They envisioned that this method could extend the application of SMLM in biomacromolecule dynamic analysis and structural studies at the molecular scale.