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From localizations to structure

Single-particle reconstruction algorithms can be used to obtain 3D structures from 2D localization microscopy images.

Although cryogenic electron microscopy (cryoEM) has emerged as a powerful method for determining the structures of biological molecules, light microscopy methods have lagged behind as tools for structural biology. One reason for this lag is that light microscopy methods are largely diffraction limited and cannot provide details on the Ångstrom scale that are needed for structural biology. Super-resolution microscopy methods, especially those that provide high resolution like single-molecule localization microscopy, are beginning to bridge this gap, paving the way for structural studies.

Marcelo Nollman at the University of Montpellier and colleagues sought to develop a strategy that would allow 3D reconstructions of biological structures to be determined from 2D localization microscopy data sets. To this end, they applied established algorithms developed for single-particle reconstruction of cryoEM structures to localization microscopy images. Although others have used similar approaches, almost all existing methods depend on prior knowledge in the form of a structural template. This can cause problems when a structure is entirely unknown and can lead to a reconstruction that is biased toward the template.

Compared with these existing methods, Nollman's algorithmic approach is model free and does not require a template or knowledge of symmetry, which makes it more useful for generating diverse structures. The researchers demonstrated their approach on experimental data from DNA origami structures and showed that they could model rod and tetrahedron shapes with high accuracy and isotropic resolution. They further showed that the model could accurately reconstruct a complex biomolecule using simulated microscopy data of the T4 bacteriophage. To show that the method works on asymmetric structures, they successfully reconstructed 3D spiral- and 'duckling'-shaped particles from simulated data.

The researchers also showed that their method is robust to problems that are thought to affect localization microscopy—but not cryoEM—like incomplete labeling, labeling density, and background levels. These demonstrations highlight the power of using single-particle reconstruction algorithms with localization microscopy data to generate 3D structures and show that super-resolution microscopy may belong in the structural biology toolbox.

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RESEARCH PAPERS

Salas, D. *et al.* Angular reconstitution-based 3D reconstructions of nanomolecular structures from superresolution light-microscopy images. *Proc. Natl. Acad. Sci. USA* **114**, 9273–9278 (2017).