3D PARTICLE AVERAGING AND DETECTION OF MACROMOLECULAR SYMMETRY IN LOCALIZATION MICROSCOPY

Hamidreza Heydarian[1], Maarten Joosten[1], Florian Schueder[2], Ralf Jungmann[2], Jonas Ries[3], Sjoerd Stallinga[1], and Bernd Rieger[1]
[1] Department of Imaging Physics, TU Delft, Delft, The Netherlands
[2] Max-Planck-Institute of Biochemistry, Martinsried, Germany
[3] Cell Biology and Biophysics Unit, EMBL, Heidelberg, Germany
Email: h.heydarian@tudelft.nl

KEY WORDS: localization microscopy, 3D particle fusion, super-resolution reconstruction

Single molecule localization microscopy (SMLM) shows promise for quantitative structural analysis of subcellular complexes with a resolution well below the diffraction limit. Photon scarcity and labeling deficiency, however, limit the achievable resolution by means of SMLM. Moreover, due to experimental limitations the axial resolution is typically ~2-3 times worse in lateral direction. Proper alignment of repeated structures ("particle fusion") in a 2D/3D SMLM measurement can overcome these limiting factors and to push for isotropic resolution.

The existing approaches for SMLM particle fusion can be classified into routines that are borrowed from SPA in EM[1] or the methods that use a model for the structure to be reconstructed[2]. While the first approaches are completely ignoring the differences in image formation model between EM and SMLM, the second ones are susceptible to the template-bias problem.

We have extended our 2D template-free particle fusion approach[3] to 3D which enables the alignment of 3D particles with very low degree of labelling (DOL) and anisotropic localization uncertainties. Besides, the developed approach can efficiently detect structural symmetry from the image data giving insight into the morphology and functional properties of subcellular structures.

We have evaluated our developed method on various experimental datasets containing NUP107 and NUP96 subcomplexes of the nuclear pore complex (NPC) and tetrahedron-shaped DNA-origami nanostructures imaged with two different imaging setups. **Figure 1a-b** show the results of fusing 306 and 356 NPCs imaged using STORM and PAINT. Here, we achieved a two orders of magnitude SNR amplification, and FSC-resolution values as low as 14-16 nm. We further retrieved the 8-fold rotational symmetry of the NUP96 assembly and the full tetragonal symmetry of a 3D tetrahedron DNA-origami, without any prior knowledge imposed on the data (**Figure 1c-d**).

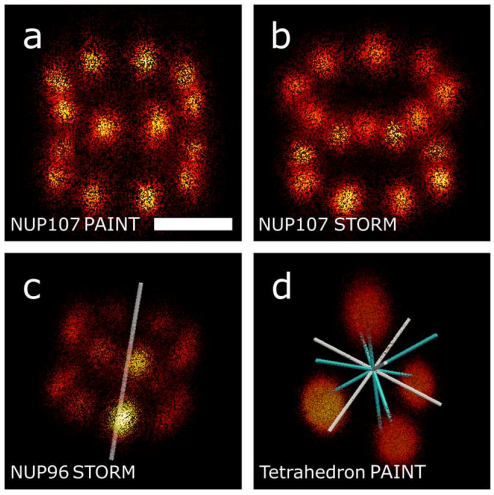

Figure 1 | 3D particle fusion and symmetry detection in SMLM. The result of fusing (**a**) 306 PAINT and (**b**) 356 STORM NUP107 particles. (**c-d**) Symmetry group detection from fusion of (**c**) 300 STORM NUP96 particles and (**d**) 400 3D PAINT DNA-origami tetrahedrons. The bars depict the estimated rotational symmetry axes for these two structures (scale bar = 50 nm).

1. Salas, D. et al. Proc. Nat. Ac. Sci. 114**,** 9273–9278, 2017.
2. Broeken, J. et al. Meth. App. Fluores. 3**,** 014003, 2015.
3. Heydarian, H. et al., Nature Methods. Vol. 15, 781, 2018.