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STOPGAP_refine: Tilt series refinement for high-resolution subtomogramaveraging

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Cryo-electron tomography (Cryo-ET) is currently the only technique available for investigating molecular detail of complex biological specimens such as membrane-associated assembles, pleomorphic viruses, or cellular/organelle sections. In electron tomography, the sample is physically rotated in the microscope, and images are acquired at discreet tilt angles resulting in a tilt series. Tilt series are then aligned using either fiducial markers or cross-correlation based approaches. The aligned series of projections are then used to reconstruct the three-dimensional (3D) tomographic volume. A suboptimal tilt series alignment deteriorates the quality of the tomographic reconstruction and attenuates the high-resolution information contained. Poor Initial alignment, in turn, affects the attainable resolution for subtomogram averaging. However, it has recently been shown that the information gathered from subtomogram averaging can be exploited to refine the poor initial alignment(Himes & Zhang, 2018; Tegunov et al., 2021).

STOPGAP_refine is an extension to STOPGAP, an open-source, MATLAB-based software package for subtomogram averaging. Its focus is on enhancing the tilt series alignment using subtomograms as fiducial markers. The subtomogram average and the refined positions of the individual subtomograms are then used to create a simulated tilt series. A fiducial model is generated by tracking the projection of individual subtomograms on each tilt image using a patch-based cross-correlation approach. Tracked fiducials are then used to solve for local deformations in projection images to enable accurate alignment of the original tilt series. The resulting tomographic volume preserves the high-resolution information and consequently improves the resolution of subtomogram averages. STOPGAP_refine is also compatible with other processing pipelines.

Here we show that STOPGAP_refine facilitates subnanometer resolution subtomogram average of 70S ribosome from the Multishot (beam image shift) tilt series data acquired on automated FIB milled lamellae. Furthermore, we also show that the refinement approach implemented in STOPGAP_refine improves the quality of the entire tomographic volume, enabling the use of one macromolecule species as fiducials to improve the subtomogram averaging resolution of another species.

References

Himes, B. A., & Zhang, P. (2018). emClarity: software for high-resolution cryo-electron tomography and subtomogram averaging. *Nature Methods*, *15*(11), 955–961. https://doi.org/10.1038/s41592-018-0167-z

Tegunov, D., Xue, L., Dienemann, C., Cramer, P., &Mahamid, J. (2021). Multi-particle cryo-EM refinement with M visualizes ribosome-antibiotic complex at 3.5 Å in cells. *Nature Methods*, *18*(2), 186–193. https://doi.org/10.1038/s41592-020-01054-7