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🌐 In situ Cryo ET data collection and processing with Scipion

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We use this protocol and it's working

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Abstract

cryo et data collection and subtomogram averaging using Scipion

Troubleshooting

Cryo ET data collection

1 Grids containing lamellae were retrieved from the Aquilos and immediately stored in nitrogen or loaded into a 300 kV Titan G2, G3, or G4 Krios. The autogrids containing lamellae were loaded such that the pre-tilt axis induced by FIB milling was perpendicular to the tilt axis of the microscope. Serial EM was used for all downstream steps.

2 Montage maps were generated for the entire autogrid using a low grid atlas magnification in Serial EM to identify lamellae positions.

3 Using a higher resolution medium mag, generate a square map at each lamellae site.

4 Switch to collection mag and perform the registration. Using this mag, draw Polygons at each lamellae to generate polygon montages. These polygons will be used to guide the data collection process

5 To measure cross sectional diameters of organelles, these polygon montages can be opened in IMOD. Diameters can be measured using the measurement tool.

6 Collect tilt series using a dose-symmetric scheme starting from 10-15 degree lamellae pre-tilt with increments of 3 degrees in groups of 2 tilts. Use a defocus that varies between tilt-series from -2 to -6 μm with a step size of 0.25 μm. We typically use a total dose of 120 e-/ Å2 for each tilt series. For collection on the G4, frames were saved in Electron Event Representation (EER) format.

Install the Scipion environment

7 Visit https://github.com/scipion-em and follow instructions to install Scipion as well as the plugins necessary for data processing in the Scipion environment

Tomogram generation, denoising, and membrane segmentation

8 Import tilt series using the "tomo - import tilt-series movies" Run and input all necessary microscope parameters into the required fields corresponding to the mdoc values from each tilt series and apply the gain reference appropriately

9 Motion correct imported tilt series using the "motioncorr - align tilt-series movies" Run and bin the data to the real pixel value

10 After dose filtering, run the "imod - X-rays eraser" Run

11   Run "imod - Dose filter" on motion corrected tilt series using the Scipion import dose source

12   In parallel with dose filtering, also run "cistem - tilt-series ctffind" on motion corrected tilt series and adjust input parameters as needed

13   Once all Runs are finished, use the "aretomo - tilt-series align and reconstruct" Run using the X-ray erased tilt series as an input to align the tilt series stack as an interpolated tilt series with no tomogram reconstruction

14   After alignment, apply "imod - CTF correction" using the aligned tilt series stack and the CTF estimation using a defocus tolerance of 200 nm and interpolation width of 15 px

15   Once tilt series have been CTF corrected, Run "aretomo - tilt-series align and reconstruct" and reconstruct WBP tomograms. We typically bin our data 4 to 5 times such that the final binned pixel sizes is approximately 10 angs/pixel. Adjust additional input parameters as necessary

16   Denoise tomograms using the "tomo3d - denoise tomogram" Run with the EED method and 10 iterations using a 0.5 sigma gaussian filter

17   Segment membranes in tomograms using the "membrain - tomogram membrane segmentation" Run and adjust input parameters as necessary

18   Begin manually picking particles using the "sphire - cryolo tomo picking (manual)" Run

19   Extract resultant subtomos from manual picking using the "reliontomo - Extract subtomos" and include the CTF and tilt series from above. Modify reconstruct parameters as necessary

20   Extract resultant subtomos from manual picking using the "reliontomo - Extract subtomos" and include the CTF and tilt series from above. Modify reconstruct parameters as necessary

21   Generate an initial model from subtomograms using the "reliontomo - 3D initial model" Run with approrpriate symmetry and mask size

22   Refine the initial model using "reliontomo - 3D auto-refine" using the initial model as a reference with appropriate filtering and mask diameter with a 7.5 degree initial angular sampling interval and 1.8 local searches from auto-sampling

23 Perform 3D classification to identify bad picks as well as alternate classes from manual picks using "reliontomo - 3D classification" and the auto-refined average as a reference. We usually use at least 3 classes.

24 Use "reliontomo - Reconstruct particle" to generate a subtomogram average structure from each resulting class with appropriate box size and binning

25 Generate a mask in ChimeraX and import the volume into Scipion using "pwem - import volumes" and resize the volume to match the pixel size of your subtomogram average using "xmipp3 - crop/resize volumes." Convert this to a mask using "relion - create 3d mask" with a low pass filter of 30 angstroms, and a soft endge of 8 with an initial threshold appropriate for your volume

26 Run "reliontomo - Post-processing" using the average output by the reconstruct particle job and the solvent mask generated in the previous step. Run with -1.0 calibrated pixel size and default settings to determine the resolution of your subtomogram average

27 To build models that fit the EM density maps for each class, use the AlphaFold server (**https://alphafoldserver.com/**) to generate an initial protein model of your protein or complex. Relax this model into the EM density map using ISOLDE and the AlphaFold pAE matrix as restraints.