

Mar 26, 2025

Version 1

Cryo ET data collection and processing with Scipion V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlkd55dg5r/v1>

kevin rose¹

¹UC Berkeley



kevin rose

UC Berkeley

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.j8nlkd55dg5r/v1>

Protocol Citation: kevin rose 2025. Cryo ET data collection and processing with Scipion. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.j8nlkd55dg5r/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 25, 2025



Last Modified: March 26, 2025

Protocol Integer ID: 125025

Keywords: ASAPCRN, cryo et data collection, processing with scipion protocol, scipion protocol, subtomogram averaging, data, cryo

Abstract

Protocol for cryo et data collection and subtomogram averaging using Scipion

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-/ $\text{\AA}^2$ 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 Run "imod - Dose filter" on motion corrected tilt series using the Scipion import dose source

- 11 After dose filtering, run the "imod - X-rays eraser" Run
- 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 angstroms/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

Particle picking

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

Data Processing

- 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 appropriate 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