

Visualize Identified Molecules Of A Cryo-Tomogram

Robert Brandt

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Cryogen electron tomography (Cryo-ET) has the ability to image macromolecules of cells in situ. To identify the molecules in a tomogram and determine their positions, template matching can be performed. The result of template matching is a set of points together with the estimated orientations of the matched molecule. Template Matching packages have their own way to store this information. This Xtra provides a module that allows to import the STAR files generated by STOPGAP sub-tomogram averaging package (Wan et al., 2020) and visualize the template molecule at its locations within the tomogram with a specialized rendering module. The module renders models of the template molecule at atomic resolution in the pose they have been detected by template matching.

This Xtra also provides a module to crop pointclouds. It will be shown how to create a molecule (point-)cloud corresponding to a sub-volume of the tomogram as specified by a ROI Box.

The data we are using in this Xtra is a cryo tomogram showing only two types of macromolecules. They have been created from purified solutions of 70S ribosomes (E.coli) and 20S proteasomes (T. acidophilum). The molecules have been located and identified using PDB 4V4R and PDB 3J9I as template models and STOPGAP template matching software. The tomogram as provided in the Xtra is downsampled by a factor of 4 and low-pass filtered with a Gaussian of 1 sigma. Details on acquisition and postprocessing are in Khavnekar et al 2023.

Wan, W., Khavnekar, S., Wagner, J., Erdmann, P., Baumeister, W. „STOPGAP: A Software Package for Subtomogram Averaging and Refinement” Microscopy and Microanalysis 26 S2, 2020

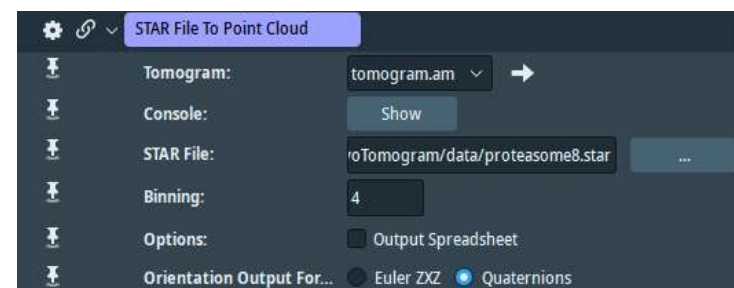
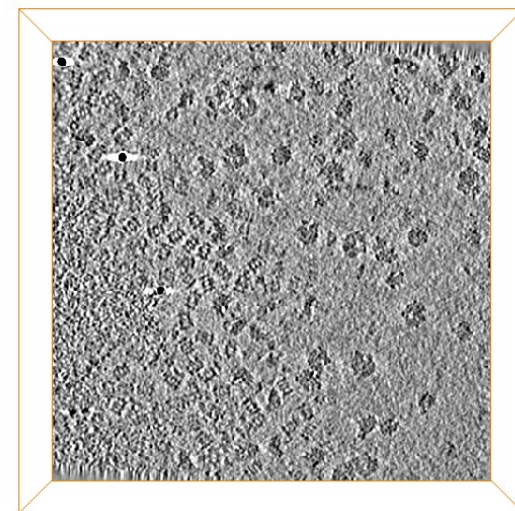
Khavnekar S, Wan W, Majumder P, Wietrzynski W, Erdmann PS and Plitzko JM. Multishot tomography for high-resolution subtomogram averaging. Journal of Structural Biology 2023, 215(1); 107911.

Create Pointcloud from STOPGAP Motiflist

- Open set-up.hx

This opens the tomogram and visualizes it with an Ortho Slice. We see various molecule densities that correspond to proteasomes and ribosomes respectively.

- First we want to see the TM results for the proteasomes. For that we open the pdb model for 20S proteasomes from data/3j9i-pdb.amf.
- Right-click tomogram.am and select *Xtra > Cryo EM > Star File to Point Cloud*
- In the Properties of *STAR File to Point Cloud* set port *STAR File* to data/proteasome8.star
- Set port *Binning* to 4.
- Clicking *Apply* creates a new object *proteasome8.pointcloud*
- Right-click *proteasome8.pointcloud* and select *Display > Molecule Cloud View*
- Connect port *Molecule* in *Molecule Cloud View* with *3j9i-pdb.amf*.

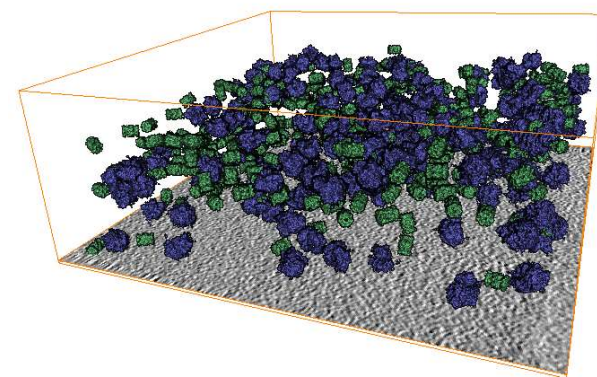
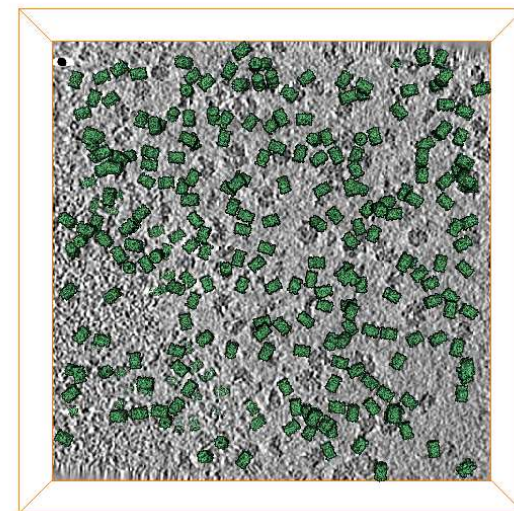


Create Pointcloud from STOPGAP Motiflist

- In *Molecule Cloud View* select *chains* in port *Color*
- Double-click the yellow field in port *Color* and choose a color from the palette.

Now we repeat this procedure for the ribosomes.

- The ribosome molecular model can be loaded from `data/4v4r-pdb.amf`.
- Configure port *STAR File* in *STAR File To Point Cloud* with `data/ribosome8.star` and click *Apply*:
- Connect port *Molecule Cloud* in *Molecule Cloud View* with `ribosome8.pointcloud`.
- Select an appropriate color in port *Color*.



Cropping a Molecule Cloud according to a ROI Box

- Open `setup_cropping.hx`

This opens a sub-volume of the tomogram and visualizes it with an Ortho Slice. We also see the proteasomes of the entire tomogram.

- Right-click `tomogram_subvol.am` and select *ROI Box* from the center column of the Module Finder.
- Connect the *ROI* connection port of *Molecule Cloud View* with the *ROI Box*.

This creates the visualization that only displays the molecules within the region of interest. Now we want to crop the molecule cloud in the same way to have a data object with these molecules only.

- Right click the molecule cloud and select from *Xtra > Cryo EM > Crop Point Cloud*.
- Connect its *ROI Box* connection port with the *ROI Box* and click *Apply*.
- A new data object, *proteasome8-cropped*, is created which can be saved in Amira format.

If for cropping the tomogram Amira has been used, the bounding box of the sub-volume describes its position correctly in real-world coordinates. Therefore, the *ROI Box* connected to the sub-volume will correctly crop the molecule cloud since it inherited its bounding box from the tomogram.

