Pre-Reconstruction Filtering Improves Feature Identification in Cryo-Electron Tomography of Cancer-Related Specimen

Lewis WC, Chin JA[1], Chiu W[1], Galaz-Montoya JG[1]

1. National Center for Macromolecular Imaging, and the Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, United States

Introduction

As the function of biological complexes depends on their structure, resolving structures to high resolution can be crucial to understanding and developing drugs to target diseases such as cancer. Earlier research suggests that cryo-electron microscopy (cryoEM) could be used as a non-invasive tool for cancer screening by looking for structural changes in cancer cells or subcellular components. However, this requires that cellular features be unambiguously and faithfully resolved. Cryo-electron tomography (cryoET)[1] is a variant of cryoEM that images subcellular structures via back-projection of a tiltseries, a collection of 2D views of the specimen from different angles (Figure 1).

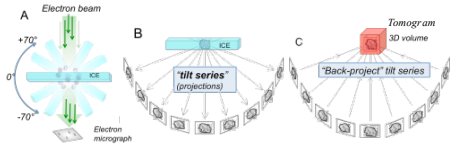

Figure 1. Cryo-Electron Tomography

Schematic of **A)** specimen in vitreous ice being tilted, **B)** images recorded and **C)** back projected into a 3D tomogram, revealing the structure of the specimen.

Once a tomogram has been reconstructed, nanometer and subnanometer resolutions can be achieved by averaging up to several thousand macromolecular complexes or "particles" in near-identical conformations. As cryoET tiltseries typically have low contrast, identifying structures of interest in reconstructed tomograms can be difficult and subject to observer bias. Contrast in tomograms can be improved by applying various filters, such as Gaussian bandpass and Nonlinear Anisotropic Diffusion (NAD) filters. However, the reconstruction process introduces artifacts due to interpolation and missing data in collected tiltseries. These reconstruction artifacts could be accentuated by filters applied to tomograms post-reconstruction, reducing contrast and leading to inaccurate localization of macromolecules.

Objectives

We hypothesize that applying a series of *pre*-reconstruction filters directly to tiltseries can improve contrast and reduce artifacts in the tomograms, facilitating the identification of biological features.

Methods

We developed a pre-filtering protocol that includes the following filters:

1) High-pass filter to get rid of "ice-gradients" and for initial edge enhancement taking feature-size into account.
2) Low-pass filter to smooth noise out, taking the expected resolution into account.
3) A modified preNAD filter for further edge sharpening[4].

We tested our pre-reconstruction filtering protocol on high contrast tiltseries of isolated epsilon 15 virus[2] (Fig. 2. Ai-Ci) and isolated TRiC chaperonin (CCT)[5] (Fig. 2. Aii-Cii) as controls, and low-contrast tiltseries of mutant huntingtin (mHTT) aggregates incubated with TRiC (Figure 2 Aiii-Ciii). We used IMOD software for tomographic reconstruction and EMAN2 for tomogram and tiltseries processing[6]. We compared manual and automated particle picking done using PyTom[3] on raw (Fig. 2. Ai-Aiii), post-filtered (Fig. 2. Bi-Biii) and pre-filtered (Fig. 2. i-Ciii) tomograms. We used an all-vs-all average minimum distance comparison, with manually picked particle coordinates as a gold standard to quantify the effectiveness of automated particle picking with different filtering techniques.

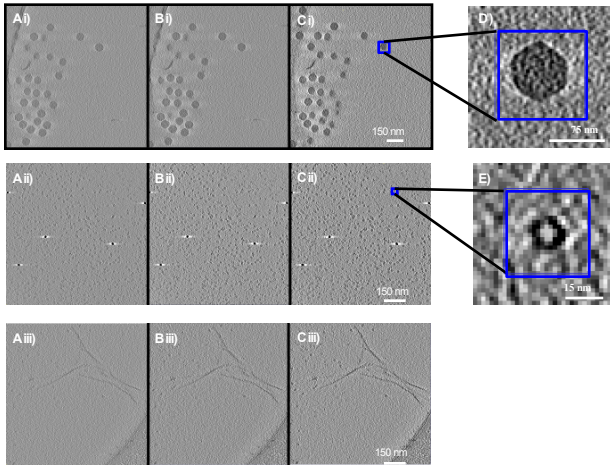

Figure 2. Epsilon 15, TRiC, and TRiC/mHTT Contrast Improvements

Contrast improves from (A) the raw tomogram to (B) the post-NAD filtered tomogram, but a greater improvement is shown by (C) our pre-filtering pipeline. Epsilon 15 (D) and TRiC (E) particles can then be "boxed" from the tomograms.

Results

Contrast and feature identification in tomograms improved with our proposed pre-filtering protocol when compared to unfiltered and post-filtered tomograms. We found that automated particle picking in pre-filtered tomograms was ~78% (ε15), ~22% (isolated TRiC), and ~20% (TRiC/mHTT) closer to manual particle picking than in post-filtered tomograms (Figure 3). Clearly, in our results post-filtering provides little benefit over not filtering at all.

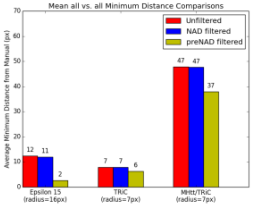

Figure 3. Pre-Filtering and Particle Autoboxing

In each of the control tomograms, our all-vs-all average minimum distance metric shows an improvement in automated particle picking by PyTom[3] from unfiltered to post-filtered and from post-filtered to pre-filtered tomograms.

Conclusions

Pre-filtering increases the contrast in tomograms. This facilitates the identification of features in tomograms, yields better resolved structures, and aids in the interpretation of their biological function. Our pre-filtering protocol still needs to be tested on a wider array of specimens, but these preliminary results suggest that researchers using cryoET should use pre-filtering rather than post-filtering to enhance contrast and identify biological features. We plan to apply pre-filtering protocols to TRiC and platelets to investigate the structural basis of their established roles in cancer pathogenesis. Improvements in the direct visualization of biological structures in cancer-related systems can unveil new cellular and molecular mechanisms that will further our understanding of this disease and aid the discovery of novel drug targets.

Acknowledgements

This research was funded by the CPRIT Summer Undergraduate Program in Computational Cancer Biology, training grant award RP 140113 from the Cancer Prevention & Research Institute of Texas (CPRIT), by Ovarian Cancer Research Foundation Grant #5-258813, and by NIH Grant P41GM103832.

References

1. Auer, M. (2000). Three-dimensional electron cryo-microscopy as a powerful structural tool in molecular medicine. Journal of Molecular Medicine (Berlin, Germany), 78(4), 191–202. http://doi.org/10.1007/s001090000101
2. Chang, J. T., Schmid, M. F., Haase-Pettingell, C., Weigele, P. R., King, J. a., & Chiu, W. (2010). Visualizing the Structural Changes of Bacteriophage Epsilon15 and Its Salmonella Host during Infection. Journal of Molecular Biology, 402(4), 731–740. http://doi.org/10.1016/j.jmb.2010.07.058
3. Hrabe, T., Chen, Y., Pfeffer, S., Kuhn Cuellar, L., Mangold, A. V., & Förster, F. (2012). PyTom: A python-based toolbox for localization of macromolecules in cryo-electron tomograms and subtomogram analysis. Journal of Structural Biology, 178(2), 177–188. http://doi.org/10.1016/j.jsb.2011.12.003
4. Maiorca, M., Hanssen, E., Kazmierczak, E., Maco, B., Kudryashev, M., Hall, R., … Tilley, L. (2012). Improving the quality of electron tomography image volumes using pre-reconstruction filtering. Journal of Structural Biology, 180(1), 132–142. http://doi.org/10.1016/j.jsb.2012.05.019
5. Shahmoradian, S. H., Galaz-Montoya, J. G., Schmid, M. F., Cong, Y., Ma, B., Spiess, C., … Chiu, W. (2013). TRiC's tricks inhibit huntingtin aggregation. eLife, 2013(2), 1–17. http://doi.org/10.7554/eLife.00710
6. Tang, G., Peng, L., Baldwin, P. R., Mann, D. S., Jiang, W., Rees, I., & Ludtke, S. J. (2007). EMAN2: An extensible image processing suite for electron microscopy. Journal of Structural Biology, 157(1), 38–46. http://doi.org/10.1016/j.jsb.2006.05.009