Vision in the Small: Reconstructing the Structure of Protein Macromolecules from Cryo-Electron Micrographs

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Abstract

Single particle reconstruction using Cryo-Electron Microscopy (cryo-EM) is an emerging technique in structural biology for estimating the 3-D structure (density) of protein macromolecules. Unlike tomography where a large number of images of a specimen can be acquired, the number of images of an individual particle is limited because of radiation damage. Instead, the specimen consists of identical copies of the same protein macro-molecule embedded in vitreous ice at random and unknown 3-D orientations. Because the images are extremely noisy, thousands to hundreds-of-thousands of projections are needed to achieve the desired resolution of 5Å. Along with differences of the imaging modality compared to photographs, single particle reconstruction provides a unique set of challenges to existing computer vision algorithms. Here, we introduce the challenge and opportunity of reconstruction from transmission electron micrographs, and briefly describe our contributions in areas of particle detection, contrast transfer function (CTF) estimation, and initial 3-D model construction.

Reconstructing the Structure of Protein Macromolecules

One of the most exciting challenges for biology today is understanding the molecular machinery of the cell as a working, dynamic system. Critical to this understanding is determining the 3-D structure of protein macromolecules, a task that is often accomplished using x-ray crystallography. The technique of cryo electron microscopy (cryo-EM) has a unique role to play in addressing this challenge as it can provide structural information of large macromolecular complexes in a variety of conformational and compositional states while preserved under close to physiological conditions. Traditionally the methods for cryo-EM have been time consuming and labor intensive, involving data acquisition, analysis and averaging of thousands to hundreds of thousands of images (views) of the individual macro-molecular complexes. Thus, over the last few years there has been considerable interest and substantial effort devoted to developing automated methods to improve the accuracy, robustness, ease of use, and throughput of cryo-EM [1, 2, 3, 12, 15, 17], and this abstract considers three aspects originally presented in [9, 10, 11].

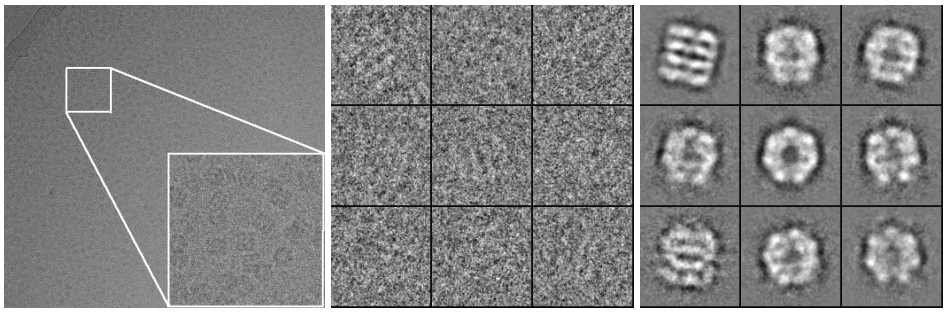


Figure 1: The image on the left shows a typical cryo-EM micrograph containing several images of a macro-molecule called GroEL. The inset shows a zoomed portion of the micrograph. The image in the center shows nine projections selected from a micrograph. Many such projections ($\sim 10,000$) are clustered into 50-100 classes. The image on the right shows the class averages of nine arbitrarily chosen classes. The class averages have significantly better signal to noise ratio at the expense of finer detail (high resolution information) contained in the raw projections.

One of the advantages of using cyro-EM is that 3-D structure can be determined without the need for crystallization. It is often very difficult to crystallize large molecules. Even when crystallization is possible, the structure constrained in crystalline form can differ from the structure of the macromolecule in its native environment. Cryo-EM therefore presents an attractive alternative for structure estimation from a biological point of view. Though freezing the specimen in vitreous ice preserves it in its close to native state, the lack of crystalline lattice means that many projections cannot be trivially averaged to improve the signal to noise ratio.

While the intensity in a photograph is related to the light (radiance) reflected from surfaces in a scene, the intensity at a point in an image produced in transmission electron microscopy, like an x-ray, is related to the integral of the scene density along a 3D line segment between the radiation source and a point on the detector (image plane). Projection can modelled as orthographic. Computed Tomography (CT) is a technique for reconstructing the 3D density from a collection of 2D images (aka projections) taken with a known relation between the radiation source/image plane and the scene. This is akin to 3D reconstruction from multiple photographs when the camera geometry is known (multi-view stereo).

In Cryo-EM, a specimen is first frozen in a very thin layer of vitreous (non-crystalline) ice and then imaged with a transmission electron microscope. To reconstruct protein macromolecules (aka particle) from the resulting micrographs, CT cannot be used directly for at least two reasons. First, the energy densities used to acquire a micrograph may damage the macromolecule, and so only a single usable high resolution image is obtained per instance of a protein. However, when the macromolecule can be induced to assume only one (or a small number of) stable confirmations, then multiple copies can be imaged in turn. Generally, individual particles are frozen into a random and unknown orientation in the ice; the distribution of orientations may be nonuniform since some orientations may be more common. Consequently, to reconstruct 3-D density using CT, the orientation of each macromolecule must be determined from the image data.

The second challenge stems from the low signal to noise ratio. See Fig. 1.a,b for an example electron micrograph. It is apparent that even detecting and locating particles is difficult for a human; furthermore it is not reasonable to extract and match local feature points in images (e.g., corners) and perform structure-from-motion (SFM) using conventional computer vision techniques. Yet, to achieve a desired resolution (< 5Å) in spite of the noise, researchers use between 1,000 and 100,000 projections, about two orders of magnitude more images than typically used in conventional SFM problems.

A single micrograph such as the one in Fig. 1.a contains noisy projections of several identical randomly oriented particles. The individual particles are selected and cropped from the micrograph. As can be seen in Fig. 1.b, individual projections are extremely noisy. The signal to noise ratio can be improved by clustering a large number ($\sim 10,000$) of projections into a few classes ($\sim 50-100$) and averaging within each class; see Fig. 1.c. Even though averaging leads to smoothing of high resolution information, the detail in the class averages is sufficient for the purpose of reconstructing an initial model at a resolution of about $30-40$Å.

Within the cryo-EM community, a set of techniques for solving the reconstruction problem have emerged [3], and implementations are available [4, 7, 13]. The process is essentially the following: First, a rough, usually low resolution and possibly distorted initial density (initial model) is constructed by some means (e.g., low resolution, higher dose electron micrographs, x-ray crystallography, single axis or random conical tomography, known structure of related molecules, assumed structure from other means, etc.). This model is used to initiate an iterative process where the image plane orientations relative to the current 3D model are determined (pose estimation), and then the 3D density (a new model) is reconstructed using CT techniques. The process repeats with this new model. It should be noted that each iteration may take 12 hours to run, and a full reconstruction may take a few weeks. In the end, the ability of the iterative process to converge to the correct solution depends critically on the accuracy of the initial model, and when it does converge, the number of required iterations also depends upon the accuracy of the initial model.

The overall processing pipeline can be summarized by the following steps.

1. Specimen preparation.
2. Automated acquisition of electron micrographs.
3. Calibration.
4. Particle detection.
5. Constructing an initial 3-D model.
6. Refinement of the 3-D model.

In our collaborative work between the microscopists and biologists at the Scripps Research Institute and the computer vision researchers at UCSD, we have addressed a number of aspects of this processing pipeline. The Leginon system has been developed to automatically control an electron microscope, select regions of a specimen having suitable ice thickness, and acquire a large number of micrographs to be used as input [2, 12]

A critical step in the processing and analysis of cryo-EM images involves the estimation of the factors that modulate the image and which must be corrected in order to generate an accurate 3-D reconstruction of the specimen. Principal among these is the contrast transfer function (CTF) of the microscope. The effect of the CTF is to introduce

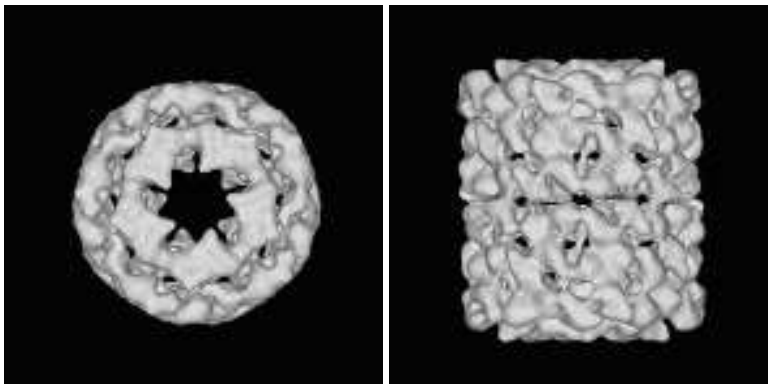


Figure 2: Reconstruction: Top and side views of Keyhole Lympet Hemocyanin.

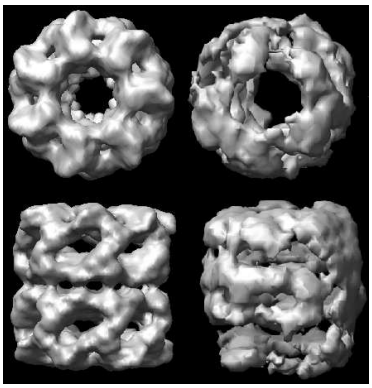
spatial frequency dependent oscillations into the Fourier space representation of the image. The theory of contrast transfer in the electron microscope [5, 6] provides a parametric form for the CTF, the envelope function and the background noise. In our own work [10], we have a completely automated algorithm for estimating the parameters of the contrast transfer function (CTF) of a transmission electron microscope including estimation of the astigmatism. Once estimated, the micrographs can be corrected in order to generate an accurate 3-D reconstruction of the specimen.

In [11] we demonstrated how to detect particles in noisy micrographs like the one in Fig. 1 using a cascaded detector based on the face detector of Viola and Jones [14]. The detector is trained on manually selected examples of the particle of interest and randomly selected regions of a micrograph (non-particles). In [16], it was shown to be one of the most effective methods for particle detection in a benchmark dataset of Keyhole Lympet Hemocyanin (KLH). An example of a reconstruction of KLH using the automatically detected particles is shown in Fig. 2.

Finally, in [11], we introduced an automated technique for reconstructing a 3-D initial model from multiple electron micrographs without information about orientation of the particle in each image. The method is based on three ideas: First, between any two projections (i and j) of a 3-D volume (e.g. a particle), we can compute a constraint on the relative 3-D orientation between the two views. For two images with orthographic viewing directions $\mathbf{v}_i$ and $\mathbf{v}_j$, there is a 3-D direction $\mathbf{v}_i \times \mathbf{v}_j$ which projects onto each image as directions ϕ_{ij} and ϕ_{ji}. These directions can be determined from a projection image (e.g., a transmission electron micrograph, an x-ray, etc.) using a Radon transform. Identifying ϕ_{ij} and ϕ_{ji} provides two constraints, called the *common lines constraint*, on the relative orientation of two views i and j. Second, given three images, there is a common line constraint between each pair of images; for such a triplet, the relative orientations are fully determined in principle up to a reflection ambiguity. Yet because the estimates of ϕ_{ij} can be noisy and include outliers (mismatches), [9] introduces a robust technique for estimating the 3-D projection orientation by denoising a common lines matrix $[\phi_{ij}]$. Finally, once the orientations are determined, the 3-D density is readily estimated using the Fourier Slice Theorem, which states that the 2-D Fourier transform of a projection of a 3-D density is a central slice through the 3-D Fourier transform of the density.

Figure 3.a shows two views of a macro-molecule called GroEL produced from a published 11.5 Å reconstruction [8] as well as the initial model produced with our tech-

a.



b.

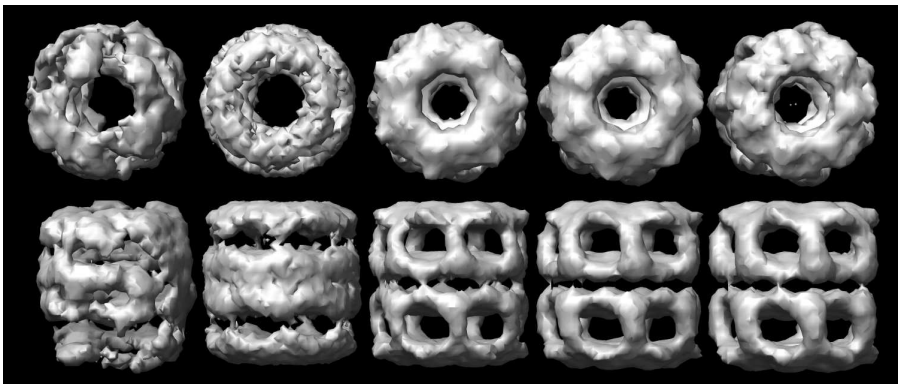


Figure 3: a. The left column shows the top and side views of a macro-molecule called GroEL produced from a 11.5 Å reconstruction [8] in a publicly available Molecular Structure Database. The right column shows the initial model estimated using our method. b. Starting with the initial model on the left, each column shows two views after each iteration of refinement using routines available in EMAN [7].

nique. 15,839 projections of GroEL were clustered into 40 classes, and the corresponding 40 class averages were generated. A few examples of the class averages are shown in Fig. 1.c. The initial model was refined using four iterations of standard refinement routines in EMAN using all 15,839 projections [7]. Fig. 3 shows the progress of refinement where each iteration takes 9 hours.

Acknowledgments

Part of this work was conducted at the National Resource for Automated Molecular Microscopy which is supported by NIH through the NCRR P41 program (RR17573). D. Kriegman and S. Mallick were supported under grant NSF EIA-03-03622, S. Agarwal was supported under NSF CCF-04-26858, and S. Belongie was supported under NSF CAREER 0448615, the Alfred P. Sloan Research Fellowship, and DOE under contract No. W-7405-ENG-48.

References

[1] C. R. Booth, W. Jiang, W. Baker, Z. Hong Zhou, S. J. Ludtke, and W. Chiu. A 9Å single particle reconstruction from ccd captured images on a 200kv electron cryomicroscope. *Journal of Structural Biology*, 147:116–127, 2004.

[2] B. Carragher, N. Kisserberth, D. Kriegman, R. A. Milligan, C. S. Potter, J. Pulokas, and A. Reilein. LEGINON: An automated system for acquisition of images from vitreous ice specimens. *Journal of Structural Biology*, 132:33–45, 2000.

[3] J. Frank. *Three-Dimensional Electron Microscopy of Macromolecular Assemblies*. Oxford University Press, 2006.

[4] J. Frank, M. Radermacher, P. Penczek, J. Zhu, et al. SPIDER and WEB: processing and visualization of images in 3D electron microscopy and related fields . *J. of Struct. Bio.*, 116:190–199, 1996.

[5] P. W. Hawkes. The electron microscope as a structure projector. *Electron tomography*, pages 17–38, 1992.

[6] P. W. Hawkes and E. Kasper. *Principles of electron optics: Wave Optics*, volume 3. Academic Press, London, 1994.

[7] S. Ludtke, P. Baldwin, and W. Chiu. EMAN: Semiautomated software for high-resolution single-particle reconstructions. *J. Struc. Bio.*, 122:82–97, 1999.

[8] S. Ludtke, J. Jakana, J. Song, D. Chuang, and W. Chiu. An 11.5 A Single Particle Reconstruction of GroEL Using EMAN. *J. of Mol. Bio.*, 314:241–250, 2001.

[9] S. Mallick, S. Agarwal, D. Kriegman, S. Belongie, B. Carragher, and C. Potter. Structure and View Estimation for Tomographic Reconstruction: A Bayesian Approach. *Computer Vision and Pattern Recognition*, 2:2253–2260, 2006.

[10] S. Mallick, D. Kriegman, B. Carragher, and C. Potter. ACE:Automated CTF Estimation. *Ultramicroscopy*, 104:8–29, 2005.

[11] S. Mallick, D. Kriegman, and Y. Zhu. Detecting Particles in cryo-EM micrographs using learned features. *Journal of Structural Biology*, pages 52–62, January 2004.

[12] C. S. Potter, H. Chu, B. Frey, C. Green, N. Kisserberth, T. J. Madden, K. L. Miller, K. Nahrstedt, J. Pulokas, A. Reilein, and et al. Leginon: A system for fully automated acquisition of 1000 micrographs a day. *Ultramicroscopy*, 77:153–161, 1999.

[13] M. Van Heel. Single-particle electron microscopy: towards atomic resolution. *Quart. Rev. of Bio. phy.*, 33:307–369, 2000.

[14] Paul Viola and Machael Jones. Robust real-time object detection. *Technical Report CRL*, 2002.

[15] P. Zhang, A Beatty, J. L. S. Milne, and S. Subramaniam. Automated data collection with tecnai 12 electron microscope: applications for molecular imaging by cryomicroscopy. *Journal of Structural Biology*, 135:251–261, 2001.

[16] Y. Zhu, B. Carragher, R. Glaeser, D. Fellmann, C. Bajaj, M Bern, F Mouche, F Haas, R Hall, D Kriegman, S Ludtke, S Mallick, P Penczek, A Roseman, F Sigworth, N Volkmann, and C. Potter. Automatic particle selection: results of a comparative study. *Journal of Structural Biology*, pages 3–14, 2004.

[17] Y. Zhu, B. Carragher, D. Kriegman, and C. S. Potter. Automated identification of filaments in cryo-electron microscopy images. *Journal of Structural Biology*, 135:302–312, 2001.