Practical Electron Tomography

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Despite the potential of the technique, electron tomography has yet to be widely used by biologists. This is in part related to the rather daunting list of equipment and expertise that are required. Thanks to continuing advances in theory and instrumentation, tomography is now more feasible for the non-specialist. One barrier that has essentially disappeared is the expense of computational resources. In view of this progress, it is time to give more attention to practical issues that need to be considered when embarking on a tomographic project. The following recommendations and comments are derived from experience gained during two long-term collaborative projects[1,2].

The specimen

Tomographic reconstruction results in a three dimensional description of an individual EM specimen, most commonly a section, and is therefore applicable to problems in which ultrastructural details within the thickness of the specimen are obscured in single micrographs. Information that can be recovered using tomography includes the 3D shape of particles, and the arrangement and disposition of overlapping fibrous and membranous structures. It is a mistake to assume that tomography will compensate for poor contrast in the different structures that must be discriminated. Although contrast enhancement can be used on the final reconstruction, it is advisable to invest time in maximizing the contrast and definition of the original specimen, rather than in trying later to find an image processing solution. For sections, this may involve exploring alternatives to standard uranyl-lead staining. The early literature on staining includes a number of formulations with great promise[3]. Exploratory tests must also be carried out to determine the most reliable method of stabilizing the specimen and adding gold particles as fiduciary markers[2]. The final specimen grids should be unbent, and contain flat, untorn sections. The selection of nominal section thickness depends on the nature of the question, and must be matched to the KV available, bearing in mind that at high tilt, the electron beam traverses about 3 times the specimen thickness. Although in principle, HVEM is ideal for handling thicker specimens with minimal beam damage, in practice, the IVEM range from 150KV to 300KV is often more suitable given the inherent low contrast of HVEM instruments. Also HVEMs tend to be of older design, making it difficult to collect adequate tilt series.

Data collection

Before starting to collect data, it is important to consider the resolution required by the project, since this is directly related to the angular separation between the tilt images. Resolution is limited by the angular separation of the tilt data, the maximum tilt angle, and the voxel size. Of these, the latter is trivial, and can be selected at the time of image acquisition to be non-limiting. The angular separation of the tilt data sets a formal limit to resolution (d) in the plan (xy)of the specimen:

$$d = \pi D/N \quad (1)^4$$

where D is specimen 'diameter', and N the number of equally spaced tilt images between -90° and +90°. As discussed elsewhere[5], the appropriate value of D depends on the nature of the specimen. In the 'worst case' situation of a section completely filled with specimen detail, D is the maximum distance traversed by the beam at maximum tilt, while for a spherical particle that is not overlapped with other specimen elements throughout the tilt range, D is effectively the particle diameter. The lack of a complete angular range further limits the resolution in the 'z' direction, approximately twofold for a ±60° data set[6].

Simple modeling experiments in which projections are made from computer generated images, and then 'reconstructed' by weighted back projection suggest that there are conditions where valid information is present beyond the limit set by equation (1). As pointed out[4], this is to be expected for images that are not densely packed with information. It is also possible to use modeling to examine the effects of different high tilt restrictions. As expected, reducing the angular range leads to circular elements becoming elongated in the 'z' direction as the edges become ill-defined at the top and bottom. Another effect of tilt restriction is the severe degradation of linear features in a wedge-shaped region defined by the missing angular range. To what extent the results from reconstructions of rather simple images should be heeded when selecting resolution levels for real reconstructions is not clear. The modeling data do underscore the need for tomographic data sets of specimens with known structure. Sections containing a mixture of spherical and rod-like viruses could produce very informative results.

Most specimens lose mass during electron beam exposure, the damage leading to substantial shrinkage of sections in the 'z' direction. Data sets collected while the sample is shrinking yield invalid reconstructions, a conclusion verified by modeling experiments. At present, two strategies can be used to minimize the effect: pre-irradiate the specimen until most of the shrinkage has taken place, or reduce the shrinkage using low-dose microscopy of cooled specimens. The latter approach must be regarded as still in the development stage, and the stability of cryo-holders at high tilt remains a problem. If the pre-shrinking method is used, what is the effect on the specimen. Experiments using Lowicryl K11M sections containing a dispersion of colloidal gold beads or osmium ammine stained nuclei suggest that beam damage does not result in a simple uniform contraction in 'z'. Rather, stable components such as stain aggregates tend to stay in place, as the embedding medium collapses around them.

Automated collection of tilt series images is becoming a reality in several laboratories[7,8], and promises to make tomography simpler, more accurate, and less damaging to the specimen. Without this advance, data collection is tedious and time consuming, and one must expect to reject a high proportion of data sets that cannot be aligned, or show obvious specimen changes between the start and finish. Nevertheless, even at worst, the time spent on data collection will be small compared to the time spent on interpretation! If possible, data should be collected beyond the ±60° limits of standard goniometers through the use of a high tilt holder.

Alignment and reconstruction

A number of algorithms for alignment and reconstruction are in active use, and have been demonstrated to yield faithful 3D information[9]. It would be a valuable service to the EM community if the different reconstruction strategies could be compared using identical input data sets.

Interpretation

Interpreting the resulting cube of numbers (voxels) representing the 3D density distribution of the specimen involves consideration of the resolution in the x, y, and z directions, and ways to turn the numerical data into images that best represent the salient features of the volume. Strategies have to be developed on a case by case basis. Often, it is not possible to obtain useful information from shaded surface representations, where a voxel threshold that distinguishes specimen from background, and more sophisticated tools such as volume rendering where levels of opacity and contrast can be used to view the entire volume. The final challenge for the tomographer is to find a way to impart the interpretation in the printed page medium that is convincing to reviewer and reader. Perhaps this problem will be alleviated in the future by the further development and use of depositories for volume data that can be accessed over the network (e.g. http//indy.cnb.uam.es), and form an essential part of a publication. ■

1. C.L. Woodcock, B.F. McEwen, and J. Frank, *J. Cell Sci.* 99(1991)107.
2. R.A. Horowitz et al., *J. Cell Biol.* 125(1994)1.
3. R.A. Horowitz and C.L. Woodcock, *J. Histochem. Cytochem.* 40(1992)123.
4. R.A. Crowther, D.J. DeRosier, and A. Klug. *Proc. Roy. Soc. London A 317(1970)319.*
5. C.L. Woodcock, in J Frank, Ed, *Electron Tomography* New York: Plenum Press (1992)313.
6. M. Radermacher, in J Frank, Ed, *Electron Tomography* New York: Plenum Press (1992)91.
7. A.J. Koster et al., *MSA Bulletin* 23(1993)176.
8. D. Typke et al., in B. Jouffrey and C. Colliex, Eds, *Proc. 13th Intl. Congress EM* Vol 1 Les Ulis: Les Editions de Physique (1993)523.
9. J. Frank, Ed. *Electron Tomography* New York: Plenum Press (1992).

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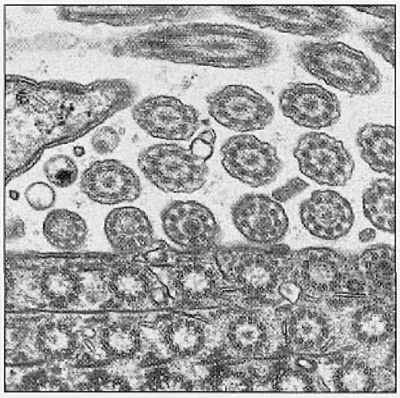

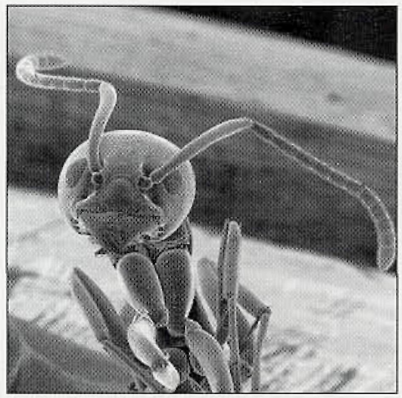

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