Zoological Studies 34, Supplement I (1995)

Zoological Studies

Energy Filtering and Exit Surface Wavefront Reconstruction of Thick Biological Specimens - Technical Development for Three Dimensional Electron Microscope Tomography

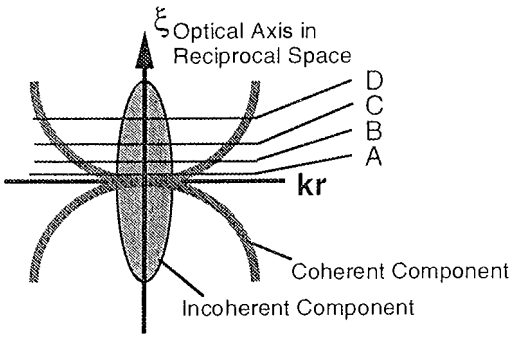
Karen F. Han, John W. Sedat and David A. Agard

Graduate Group in Biophysics, Howard Hughes Medical Institute, Department of Biochmistry and Biophysics, University of California San Francisco, CA 94143, USA

High resolution studies of cellular organellse and nuclear structures are often investigated by using transmission electron microscopy (TEM). Three dimensional (3D) reconstruction is essential to elucidate high resolution substructures of these macromolecular assemblies. There are two main approaches to obtain 3D reconstructions in TEM: serial thin sections and tomography. Our laboratory uses the latter technique which has the advantage of nearly isotropic resolution in 3D with the reconstruction resolution to be much higher than the former. 3D tomography is the reconstruction of an object by combining multiple projection views of the object at different tilt angles. In order to accurately reconstruct these structures, it is essential to understand the relationship between the TEM image intensity and specimen mass density. Due to the natural dimensions of these structures, they are considered thick (0.3-1.0μm at high specimen tilt angles) for TEM studies. Compared with thin specimens (0.1 μm), the imaging of thick specimens are additionally complicated by multiple scattering which gives rise to the incoherent and partially coherent components that degrade the images. Two techniques were used to analyze the mechanism of image formation for thick specimens: electron energy-loss spectroscopic imaging (ESI), and the exit wavefront reconstrction using a through focus series. We showed that: 1) there is a significant amount of coherent transfer in thick specimen imaging, 2) the coherent component in the images are contributed almost exclusively by elastically scattered electrons, 3) the incoherent component are mostly contributed by multiple elastic and inelastic scattering, and 4) exit wavefront reconstruction can act in part as an energy filter.

Electron-specimen interactions include single and multiple, elastic and inelastic scattering. Multiple and inelastic scattering events give rise to nonlinear imaging effects which complicates the interpretation of the images collected. In addition, due to the chromatic aberration of the TEM's objective lens, multiply scattered indlastic electrons cause a blurring of the image due to an effective broadening of the focus spread. We have shown previously that when imaging our typical thick specimens at 200 keV, only the elastic (zero-loss) and plasmon (single inelastically scattered) electrons contribute to high resolution images. Most inelastically scattered electrons are multiply scattered and contribute to low resolution images.

The coherent component of the image can be extracted in a three-dimensional power spectrum of a through focus series. The coherent information in the opwer spectrum lies on a parabola which is the Ewald sphere4-6. In such restroations, the components which are not mutually coherent through focus are naturally excluded, including multiple and inelastic scattering. By analyzing the 3D power spectrum of a through focus series, one can quantify the relative amount of coherent and imcoherent components. Figrue 1 shows a few representative cross-sections of the Ewald sphere from the unfiltered esit wavefront of a 0.5μm specimen (epon embedded, stained with uranyl acetate and lead citrate) at 200 keV. As the schematic diagram shows, the coherent component is on the outer circle, and the noise or



incoherent component is at the center. Contrary to common assumption, coherent transfer is significant in these thick specimens. Ehen the same analysis was done on the thuough focus series of only slastically scattered electrons (or zero-loss filtering), we found that the coherent component is greatly enhanced (Fig.2). The central component is still quite prominent perhaps due to multiple elastic scattering. When normalized by total counts, the central component is reduced in the filtered series. Images from the plasmon electrons (25 eV-loss) contain very weak parabola component and a large central component, while all images from other energy ranges contain only the central (incoherent) component. An analogous study was done on a 0.7 μm specimen where we showed that only the zero-loss filtered series contain the parabola (coherent) component (data not shown). We conclude that the coherent component of thick specimen TEM images are contributed by elastically scattered electrons, and the incoherent component contains mostly multiple inelastic and some multiple elastic electrons.

From the results above, we showed that restored specimen esit wavefront from and unfiltered through focus series effectively 'filters' out the inelastically scattered electrons. This applies for thick specimens because most inelastically scattered electrons are multiply scattered and therefore contribute very little to the coherent component of the images. By virtue of excluding the multiple scattering component, the restored images displayed enhanced contrast with higher resolution. Thus in thick specimen imaging. the combined use of energy filtering and through focus reconstruction is optimal to recover the exit wave (Fig.3). The interpretation of the aberration corrected images are proposed as follows: the amplitude component coming from the absroptive effect

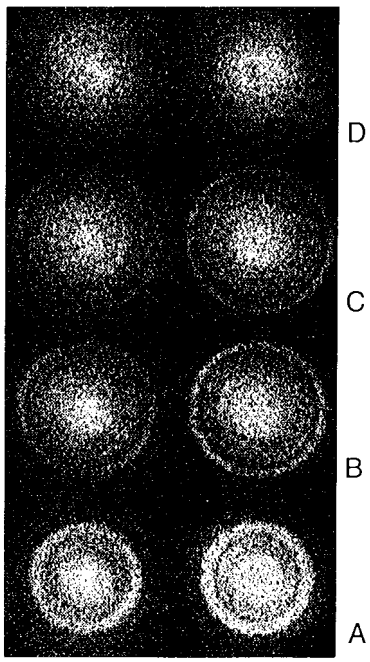


Fig. 1. Fig. 2.

Fig. 1. Selected cross sections through the Eeald Sphere of 0.5mm thick specimen, unfiltered, at 200keV, displayed in order of increasing reciprocal z. (Eesolution limit is 2.4nm^{-1})

Fig. 2. Selected cross sections as in Figure 1, but from the zero-loss filtered series, demonstrating an enhanced coherent component. (Resolution limit is 2.4nm^{-1})

has a logarithmic relation to the mass density of the specimen, and the high resolution phase component is linearly related. This has direct implications to the three dimensional reconstructions of these specimens where aberrations were not corrected and a simple direct relationship was assumed.

ACKNOWLEDGMENTS

The authors thank A. J. Gubbens for the use of the Gatan Imaging Fliter and M. Braun feld for the specimens used in this study. K.F.H. is supported by the Howard Hughes Medical Imstitute Predoctoral Fellowship in the Biological Scienced. This work is supported by grants from the National Institutes of Health (GM 31627 for D.A.A.; GM25101 for J.W.S.) and by Howard Hughes Medical Institute.

REFERENCES

Reimer L, R Rennekamp, I Fromm, M Langenfeld. 1991. Contrast in the electron spectroscopic imaging mode of a TEM. IV.

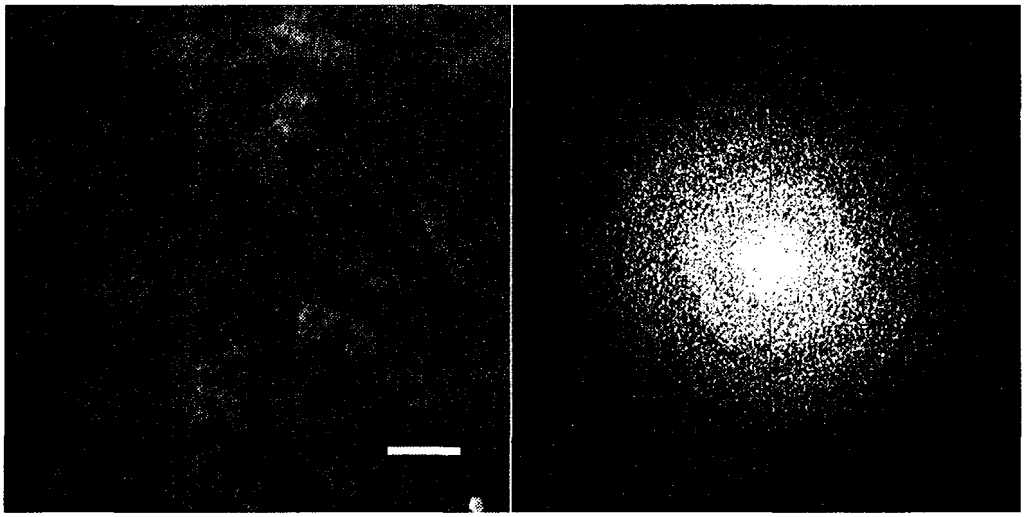


Fig. 3. Reconstruction of the projected specimen mass-density combined from the amplitude and phase components of the restored exit wavefront. Diffractogram resolution limit is 2.4nm $^{-1}$. Scale bar: 50nm. The specimen is eponembed-ded microtubules from an in vitro centrosome preparation.

Thick specimens imaged by the most-probable energy loss. Journal of Microscopy, **162:** 3-14.

Han K, A Gubbens, A Koster, M Braunfeld, J Sedat, D Agard, 1993. Analysis of electron-specimen interactions of thick biological specimens in transmission electron microscopy at 200 keV. Proc. 52nd Ann Microscopy Society of America, 204-205.

Han K, J Sedat, D Agard. 1995. Mechanism of Image Forma- tion for Thick Biological Specimens: Exit wavefront reconstruction and electron energy-lwss spectroscopic imaging. Journal of Microscopy, April (in Press).

Van Fyck, D. Op de Beeck, M. 1990. New direct methods for phase and structure retrieval in HREM. Proc. of 12th Int'l Congress for Electron Microscopy, 26-17.

Taniguchi Y, T Ikuta, R Shimizu. 1991. Assessment of image formation by three-dimensional power spectrum in transmis- sion electron microscopy. Journal of Electron Microscopy, **40:** 5-10.

Coene W, G Janssen, Op de Beeck, M. Van Dyck, D. 1992. Phase retrieval through focus variation for ultra-resolution in fiele - emission transmission electron microscopy. Physical Re- view Letters, **69:** 3743-3746.