Tomography using a scanning transmission electron microscope (STEM) offers intriguing possibilities for the three-dimensional imaging of micron-thick, biological specimens and assemblies of nanostructures, where the image resolution is potentially limited only by plural elastic scattering in the sample. A good understanding of the relationship between material thickness and spatial resolution is required, with particular emphasis on the competition between beam divergence (a geometrical effect from the converged STEM probe) and beam spreading (an unavoidable broadening due to plural elastic scattering). We show that beam divergence dominates beam spreading for typical embedding polymers beyond the 100-nm thickness range and that minimization of this effect leads to enhanced spatial resolution. The problems are more pronounced in spherical-aberration-corrected instruments where the depth of field is shorter.

1. Introduction

Advances in instrumentation and technique have increased the interest in electron tomography for biology [1,2] and materials science [3]. For problems involving polymers and composites [4], organic low-K dielectrics, photonic bandgap materials [5] and integrated circuits [6], tomography continues to show promise in providing quantitative information previously unattainable by two-dimensional (2D) electron microscopy.

The optimum mode for electron tomography, TEM or scanning transmission electron microscope (STEM), for investigating thin, biological weak-phase objects, continues to be debated [7]. However, for sample thicknesses on the order of a few hundred nanometers, STEM might offer some advantages [8] since there need not be a post-specimen imaging lens and hence no chromatic blurring from energy losses in the sample. For the case of TEM, an increase in energy spread in thick samples results in a decrease in resolution due to chromatic aberrations. Inclusion of an energy filter can improve the resolution, but this also reduces the recorded intensity significantly [9,10].

Electron tomography requires the acquisition of a series of 2D projection images tilted in small increments, from which a three-dimensional (3D) object can be digitally reconstructed. In TEM mode, diffraction artifacts at different tilts may not be straightforward to interpret and phase contrast effects may require mathematical post-processing [11]. STEM using a high-angle annular dark field detector (HAADF) [12] minimizes this problem since the intensity in each image pixel is the sum of elastically scattered electrons over an annular range that excludes the Bragg-scattered electrons that are responsible for generating diffraction contrast.

Ideally the spatial resolution should remain constant, independent of depth into the sample. However, beam spreading from plural elastic scattering of the electrons within the sample [10] and beam divergence due to the geometry of a convergent beam both challenge this condition. In STEM mode, beam spreading detrimentally affects image resolution for features located at the exit (bottom) surface, while features located at the entrance (top) surface are imaged with the resolution determined by the diameter of the point spread function. Consequently, top features are more reliably reproduced in a STEM image compared to bottom features, a result also known as the top–bottom effect [13]. In TEM mode, the reverse is true [14], where resolution of bottom features are influenced largely by just the chromatic errors and top features are influenced by both plural scattering and chromatic aberration [15].

Since beam spreading depends on material properties and the kinetic energy of the electrons, it cannot be modified without changing the beam voltage. Beam divergence, on the other hand, can be minimized by decreasing the convergence angle of the
microscope. Often, the effect of beam divergence is underestimated and beam spreading is thought to set the resolution limit.

Earlier measurements by Beorchia et al. [16] illustrate the relation between spatial resolution and thickness for polymer substrates. With the advances in brightness that commercial FEG electron sources offer, an analysis of the problem with particular emphasis on the effects of beam divergence and beam spreading is essential. We demonstrate that for typical convergence angles for high-resolution atomic imaging, the dominant effect is the beam divergence in polymer films thinner than 1.1 μm. Tilting the sample increases the projected thickness, thereby enhancing the effects of beam spreading and beam divergence. In such cases, a tilt series of a thick specimen could fail to produce a reliable tomographic reconstruction. By simply selecting the proper convergence angle, we can optimize the spatial resolution and improve the accuracy of the reconstruction.

2. Experimental

2.1. Instrumentation

A 200-kV FEI Tecnai F20 STEM system was used for all measurements. The optimal convergence angle for high-resolution STEM imaging set by the 1.2-mm spherical aberration coefficient is 9.6 mrad [17], where the resulting resolution is 1.6 Å and the depth of field is 22 nm. The actual convergence angle during normal operation was measured to be 10 ± 0.1 mrad.

The instrument has only two condenser lenses so source size and convergence angle cannot be varied independently as in a three-condenser arrangement. Either an additional lens is necessary, or a physical aperture must also be changed. Once the smallest probe-forming aperture is inserted, the convergence angle can be further reduced by decreasing the objective lens strength and re-focusing using the final condenser lens (C2). A quicker way is to take advantage of the minicondenser lens, which controls the switching between TEM and STEM mode in combined TEM/STEM systems. By reversing the minicondenser lens current while operating in STEM mode, the beam becomes more parallel, producing a substantially narrower convergence angle. We were able to reduce the convergence angle to 0.2 mrad using this simple technique. The appropriate adjustments in the optical alignment were saved, thereby allowing fast switching between the 10 and 2 mrad convergence angle settings. This also allows the objective lens strength optimally set for eucentric focus to be preserved, and minimizes drift in the optical alignments. An alternative technique for significantly reducing the convergence angle is through the Microprobe STEM mode [18].

The convergence angle α can be determined on a crystalline sample by

\[ \frac{a}{d} = \frac{\alpha}{\theta_b} \]  

(1)

where a is the aperture diameter, b is the spacing between Bragg discs, and θb is the Bragg angle for a particular reflection [17]. All convergence angles were measured by recording the CBED patterns of Si oriented onto the (110) axis and using the (111) reflection for θb.

2.2. Methodology

Various polymer film thicknesses up to 1 μm were prepared by ultramicrotoming Quetol 651, a widely used embedding medium for biological samples [19]. In order to characterize the spreading and divergence of the beam, gold nanoparticles were deposited on top and bottom of the films, similar to a method implemented by Beorchia et al. [16]. On the bottom side of the film, an additional 30 Å of amorphous carbon was sputtered to avoid charging and to increase thermal stability. The areas of interest were additionally flooded with electrons in TEM mode for roughly 20 min to allow for the film to undergo any shrinkage and reach a stable thickness.

The original size of the nanoparticles, determined by measuring the full width at half maximum (FWHM) of top particles in focus, was 6.4 ± 0.4 nm. The average particle diameter was consistent for all regions used for the analysis and variations stayed within the error.

The physical thicknesses of the films were measured by t/λ, EELS measurements [20] for thicknesses less than 600 nm. Beyond this sample thickness, insufficient signal enters the detector to make reliable measurements. Instead parallax measurements were used to obtain the thickness for films thicker than 600 nm.

The inelastic mean free path, λe, was calibrated to a sample of known thickness by acquiring a full tilt series of elastic images and using electron tomography to reconstruct a full 3D image from which the sample thickness was determined. From EELS measurements on the same sample, the inelastic mean free path for Quetol 651 was found to be 110 ± 10 nm.

Beam spreading can be defined in several ways. The 90% beam radius is a popular choice as it can be described by a simple analytical model, and scales as the 3/2 power of the thickness [21]. It is a useful measure for trace-element microanalysis but less predictive for imaging because it is dominated by the far tails from infrequent large-angle scattering events, which are often not detectable at the lower signal to noise ratios used in imaging. A more appropriate measure for evaluating image resolution is the FWHM of the beam spread. The FWHM has a very different thickness dependence to the 90% radius and shows a much less pronounced spreading with sample thickness than does 90% radius [6].

The effects of beam spreading and beam divergence can be separated by imaging features at the entrance and exit of the sample. Figs. 1a and b illustrate the method of isolating and characterizing beam spreading and divergence, respectively. The FWHM of the bottom particle in focus is affected predominantly by plural scattering, and hence measures beam spreading. Likewise, keeping the bottom particle still in focus, the FWHM of a top particle is affected only by the defocused beam as there is no material above for plural scattering to occur, and hence measures beam divergence.

Assuming that the geometrical divergence, the diffraction limit and the original particle size are Gaussians, we can approximate the FWHM of the top particle, λtop, by adding these three different contributions in quadrature:

\[ \lambda_{top} = \sqrt{\left(2 \tan \theta_i \right)^2 + \left(\frac{0.61 \lambda}{2} \right)^2 + \lambda_0^2} \]  

(2)

where r is the thickness of the sample, α is the convergence angle, λ is the electron wavelength and λ0 is the FWHM of the original particle size.

Calculation of the FWHM of the bottom particle was performed by a Monte Carlo algorithm [22]. The algorithm simulates a scanning electron beam that enters perpendicular to the film. For each scan position the elastically scattered trajectories of the electron at the exit surface are used to determine their paths to the post-specimen detectors. Electrons scattered into an annular range corresponding to the physical dimensions of the HAADF are then collected to generate an intensity profile of a gold particle sitting on the bottom of the film. The FWHM of the profile is then extracted and compared to that of the experiment. An elastic mean free path, λe, of 360 nm was used, approximately
determined from the inelastic mean free path using
\[ \lambda_e = \frac{2 \lambda_i \ln(2/\theta_e)}{Z} \] where \( Z \) is the atomic number and \( \theta_e \) describes the characteristic angle corresponding to the mean energy loss [20]. The expression has an accuracy of \( \sim 20\% \).

3. Results and discussion

Measured and calculated particle size due to beam spreading and divergence are summarized in Figs. 1c and d. For all measurements the bottom particles were kept in focus. The beam spreading effect determines the FWHM of the bottom particles and the beam divergence effect determines the FWHM of the top particles.

For a 10-mrad convergence angle (Fig. 1c) the beam divergence effect dominates the beam spreading effect and sets the resolution limit for measurements up to a micron in thickness. Decreasing the convergence angle to 2 mrad reduces beam divergence; so the effect becomes comparable to the beam spreading. The depth of field, \( T \), is defined by

\[ T = \frac{d}{2} = 0.61 \lambda_e \] where \( d \) is the probe size or the diffraction limit, which increases from 15 to 383 nm by the change in convergence angle from 10 to 2 mrad. As a trade off, the STEM resolution effectively defined by the probe size degrades from 1.5 to 7.6 Å from the increased diffraction blur. A 1-mrad convergence angle would offer a depth of field of 1.5 μm at a cost of a 1.5-nm probe size.

For the 2-mrad setting, the difference between top and bottom particles is less pronounced than for the 10-mrad setting. The discrepancy between experiment and theory in Fig. 1d could be accounted for by the 20\% systematic error of Eq. (3), resulting in a possible overestimate of the elastic mean free path used in the Monte Carlo calculation.

The divergence can also be shown in terms of a defocus series of the point spread function. Fig. 2 shows the quantum mechanically calculated defocus series of a 20-mrad convergence angle for an aberration corrected system using \( C_3 = -8 \) μm and \( C_5 = 20 \) mm [23], and that of an uncorrected 10- and 2-mrad convergence angle with \( C_3 = 1.2 \) mm. With a 20- or 10-mrad convergence angle and defocus values on the order of a 100 nm, the intensity profile of the probe is no longer concentrated in the center, but toward the outer tails, leading to a donut or ring shape. This defocused point spread function creates artifacts such as the deformation of solid particles into rings as shown in Fig. 3. By focusing on features buried a few hundred nanometers into the sample, features at the sample entrance surface are no longer accurate representations, but artifacts of the ring-shaped probe convolved with the original features. At 2 mrad, the point spread

Fig. 1. (a) and (b) Method for characterizing beam spreading and beam divergence. (a) Focusing on the bottom particle and measuring its FWHM provide a measure of the beam spreading. (b) With the bottom particle still in focus, measuring the FWHM of a top particle provides a measure of the beam divergence. (c) and (d) With bottom particle in focus, measured FWHM of top (solid circles) and bottom particles (open squares), and the calculated FWHM of top particle using Eq. (2) (solid curve) and bottom particle using Monte Carlo simulation (dotted curve). (c) A 10-mrad convergence angle setting shows beam divergence dominating beam spreading. (d) A 2-mrad convergence angle setting shows beam divergence significantly reduced. Original particle sizes are 6.4 ± 0.4 nm.
function stays consistent through its depth of field. Original features are safely preserved in this range, and are only subject to beam spreading.

When features near the top of the sample are focused, features near the bottom of the sample are imaged with the convolved contributions of beam spreading and divergence.

**Fig. 4** displays a comparison between images recorded with a 10 mrad and a 2 mrad probe-forming aperture with the beam focused on top particles sitting on a 600-nm thick film. The bottom particles appear as a faint haze using a 10-mrad setting, but by decreasing the angle to 2 mrad they come into focus. The depth of field at 2 mrad is less than the thickness of the film, which accounts for a slight geometrical contribution to the resolution near the exit side of the film in addition to the beam spreading manifested in the small increase of the FWHM of the bottom particle. The same camera length of 100 mm was used for both convergence angles. The lower intensity in the 2 mrad setting is due to the different electron optics of the illumination system.

As a practical demonstration, HeLa cells stained with osmium and uranyl acetate were imaged using 10- and 2-mrad convergence angles as shown in **Fig. 5**. The cells were embedded in 400-nm thick polymer film. A camera length of 100 and 490 mm was used for the 10- and 2-mrad settings, respectively, resulting in roughly twice more signal in the 2 mrad image. However, the noise levels were 6.1% of the signal for the 10-mrad angle and 5.6% for the 2-mrad angle, permitting a fair comparison. Quick inspection of the area in **Fig. 5a** shows that the film thickness is non-uniform over the field of view, with a depression in the top-right region. **Fig. 5b** shows that both convergence angle settings identically reproduce a common feature near the bottom of the image. In the depressed area near the top-right region, **Fig. 5c** shows the wall of an organelle imaged with better clarity and sharpness for the 2-mrad setting than for the 10-mrad setting. Such a demonstration elucidates the consequences of a tomographic tilt series where beam spreading and beam divergence increases with the projected thickness.

The eucentric focus is commonly located slightly below the midpoint of the film’s depth, effectively halving the impact of beam divergence. However, the typical depth of field is ~20 nm in an uncorrected instrument and ~5–10 nm in an aberration corrected system.
corrected system, so both distances (even after focusing on the midpoint) are much less than a typical 70 to 100 nm-thick microtomed section. Furthermore, at high tilts, where the effective thickness can more than triple, features at the top are subject to beam divergence and features on the bottom are subject to both beam divergence and beam spreading. When reconstructing a tilt series, blurring of features at large tilt angles corresponds to a damping of high spatial frequencies in the corresponding Fourier slice. This results in a loss of reconstructed resolution, most noticeably in the depth direction [24]. In samples thinner than the depth of field, minimizing the beam divergence can improve the reconstructed resolution.

Although beam spreading cannot be removed, limiting the 3D size of a sample by means of advanced sample preparation techniques such as the focused ion beam can restrict the projected thickness at large tilts. Since the projected thickness at high tilts grows indefinitely only when the length of the sample in the direction perpendicular to the tilt axis and electron beam is infinite, controlling this length can bound the projected thickness of a tilted sample. As an example, a typical 0.5-μm thick mesoporous support whose width in the direction perpendicular to the tilt axis and electron beam is 1 μm will have a maximum projected thickness of around 1.12 μm, limiting the effect of beam spreading to this thickness. Likewise, the effect of beam divergence is also bounded, but can be further reduced by choosing the appropriate convergence angle, permitting a significant decrease in the blurring of features throughout the depth of the sample.

It is worth noting that the improved spatial resolution in a spherical-aberration-corrected microscope is obtained by increasing the convergence angle by a factor of 2–4 over an uncorrected instrument, resulting in a decreased depth of field Eq. (4). In tomography, the resolution function $d$ is more likely to be set by
the accuracy of the alignment in the reconstruction, rather than the diffraction limit itself. As a consequence, the reduction in depth of field and corresponding degradation in resolution for a corrected system compared to its uncorrected counterpart is likely to be by a factor of 2–4 rather than 4–16. In either limit, the corrector will degrade the resolution for conventional tomography if the sample thickness exceeds the new depth of field. Instead, to take full advantage of the corrector, it may become necessary to record a through-focal series at each tilt in order to extend the depth of field in software. (This should not be confused with dynamic focusing, which records different \((x,y)\) points at different \(z\) settings. Here we require multiple \(z\) data for the same \((x,y)\) settings.) The simplest method to process the through-focal series is to sum all images in the through-focal series, which can be shown to be mathematically equivalent to recording a projection image with an infinite depth of field [23].

4. Conclusion

In very thick (i.e. several microns thick) organic or low-Z materials, beam spreading determines the resolution limit. In moderately thick samples (0.1–1 \(\mu\)m for uncorrected instruments), beam divergence or equivalently the depth of field limits the resolution. In the 0.1–1-\(\mu\)m sample thickness range, the resolution can be optimized by balancing the geometrical divergence against the diffraction limit. For samples thinner than the depth of field, the resolution is dominated by the beam probe size and changing the convergence angle is unnecessary. With the proper adjustment of the optical geometry we can improve the spatial resolution in thick samples, a method especially important for electron tomography. The increased convergence angle in aberration-corrected systems will extend depth-of-field blurring to thinner samples, down to \(\sim 30–50\)-nm thick, requiring additional collection and processing steps.
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