A New Approach for Electron Tomography: Annular Dark-Field Transmission Electron Microscopy**

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Transmission electron microscopy (TEM) images yield valuable information about the structure and chemistry of (in)organic materials. However, they “only” provide a 2D projection of a 3D object. Therefore, electron tomography was developed to reconstruct objects in three dimensions from a tilt series of TEM images. This technique is well accepted in the life sciences as a method used to study viruses or cells. The resolution in the reconstructions, however, is limited to a few nanometers, mainly because of the low resistance of biological materials to electron-beam damage. Electron-tomography techniques have recently been adopted by researchers in materials science. Here, atomic resolution could be achieved in principle. In practice, however, the resolution is still of the order of one to two nanometers because of the limited stability of the sample holders and the presence of dynamic diffraction in crystalline solids, to name but two reasons. In particular, dynamic diffraction in strongly diffracting materials violates the projection requirement in bright-field (BF) TEM images, since image intensities no longer increase monotonically with sample thickness. In these cases, BF-TEM tomography have been avoided and other techniques such as high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) tomography and energy-filtered transmission electron microscopy (EFTEM) tomography have been successfully applied. Although these methods are very powerful, exposure (scanning) times of 10–30 s per image are required when using HAADF-STEM and, furthermore, scanning noise cannot be avoided. Using EFTEM signal-to-noise ratios are poor and every element map requires the registration of multiple images in each projection, which is disadvantageous if beam-sensitive samples are investigated.

In this work, an alternative approach to electron tomography is proposed: electron tomography is combined with annular dark-field transmission electron microscopy (ADF-TEM). The setup of the technique is illustrated in Figure 1. ADF-TEM uses an annular objective aperture that acts as a central beam stop in the back focal plane of the objective lens. This aperture acts as a central beam stop, in the sense that only electrons scattered between 20–40 mrad are allowed through for further imaging. Secondary electron image of an annular aperture during fabrication using the focused-ion-beam (FIB) system.
Therefore, the projection requirement can be fulfilled and ADF-TEM is a suitable method for electron tomography. Furthermore, the electron-scattering cross sections scale with $Z^n$, where $Z$ equals the atomic number and $n$ approximately equals 3/2. The recorded signal therefore amplifies chemical differences. Since ADF-TEM images require exposure times of only 1–3 s, the acquisition time of a tomographic tilt series can be significantly reduced and scanning noise is absent. In this paper we will demonstrate the applicability of ADF-TEM for tomography and compare the results obtained with other electron-tomography techniques.

BF-TEM and ADF-TEM reconstructions of a sample consisting of CdTe tetrapods are considered first. In Figure 2, individual projections from the BF-TEM and the ADF-TEM series are presented. In this figure it can be seen that the ADF-TEM image yields a higher signal-to-noise ratio in comparison to the BF-TEM image and enables a distinction between CdSe (average $Z = 50$) and CdTe (average $Z = 41$), because of a different mass-thickness contrast. Obviously, a distinction of $\Delta Z = 9$ is possible.

An isosurface visualization of two reconstructed tetrapods from a BF-TEM tilt series is shown in Figure 3a. From this reconstruction it is clear that the morphology of the sample is preserved and it can be concluded that BF-TEM can be used for 3D reconstruction in this case, despite possible contributions from dynamic scattering because the sample is thin in comparison to the extinction oscillations. It can be seen that all branches have a uniform thickness of ca. 5 nm and that three branches of the tetrapods are attached to the C film, with the fourth branch almost perpendicular to this plane. This finding is in contrast to the expected shape of the tetrapod, which is a tetrahedron. It is impossible to extract this information from a single-shot image and the observation reveals that a strong interaction between the C support layer and the tetrapod is present.

An isosurface rendering of another tetrapod from ADF-TEM projections is shown in Figure 3b. The shape of the tetrapod is comparable to those observed in the BF-TEM reconstruction; this result confirms that ADF-TEM imaging is suited for tomographic reconstruction. The chemical sensitivity of the technique makes heavier materials appear brighter in an ADF-TEM image. Therefore, chemically different materials can be distinguished in an ADF-TEM 3D reconstruction even if the difference in average $Z$ is as small as $\Delta Z = 9$. Such chemical sensitivity could not be obtained in the BF-TEM tomogram that shows all tetrapod branches having equal thickness.

The chemical sensitivity of ADF-TEM imaging is similar to HAADF-STEM imaging where the image follows a dependence close to $Z^2$. It is therefore instructive to compare a 3D reconstruction obtained using ADF-TEM to one using HAADF-STEM. This comparison is carried out for a sample that consists of C nanotubes filled with Cu nanoparticles. In Figure 4 an ADF-TEM image of the above sample is compared to a HAADF-STEM image of the same specimen. It can be seen that the HAADF-STEM image yields larger intensity differences because of the larger exponent of $Z$. Nevertheless, the intensity difference between the C nanotube and the Cu nanoparticle is similarly distinguishable in the ADF-TEM image. It should be noted that the ADF-TEM image is recorded in a single acquisition with an exposure time of 1 s, whereas the HAADF-STEM image requires a scan of
Figure 4. a) HAADF-STEM image of a C nanotube filled with a Cu nanoparticle, the scanning time is approximately 10 s. b) ADF-TEM image recorded for the same sample with an exposure time of 1 s.

approximately 10 s. More details on the acquisition of the ADF-TEM and HAADF-STEM images of the tilt series for tomography can be found in the Experimental section.

The isosurface rendering of the HAADF-STEM reconstruction is shown in Figure 5a. In this image, the C nanotube can be clearly distinguished from the Cu nanoparticle and it is also clear that a tail of Cu is present in the nanotube, which is not obvious from conventional 2D TEM images. The corresponding ADF-TEM reconstruction, shown in Figure 5b and c, is in good agreement with the previous reconstruction shown in Figure 5a. Again, the C nanotube, Cu nanoparticle, and the Cu tail are easily detected. This second example again proves that ADF-TEM is an alternative approach for electron tomography with the advantages provided by imaging using a parallel beam and single shots.

In summary, we have demonstrated the applicability of ADF-TEM for tomography. When comparing this technique with BF-TEM tomography it is obvious that both techniques can be complementary: BF-TEM tomography yields information about the morphology, whereas ADF-TEM tomography provides chemical information in the TEM mode, similar to HAADF-STEM tomography, although HAADF-STEM images yield a higher contrast difference for different Z values. Indeed, when ADF-TEM tomography is compared to HAADF-STEM tomography, it is clear that both techniques yield comparable results. It has been noted that the individual projections using ADF-TEM are acquired in TEM mode with exposure times of typically one to three seconds, without the presence of scanning noise. This makes the technique extremely useful in cases where a reduction of the acquisition time is desired; thus reducing beam damage also. Although ADF-TEM was originally developed to avoid the presence of Bragg scattering, the high image contrast obtained suggests that ADF-TEM tomography is not only interesting for materials science but also for biological applications.

Experimental

Aperture Preparation: The annular objective apertures are fabricated by a focused-ion-beam (FIB) system (FEI FIB DB Strata 235). In the back focal plane of the objective lens of the microscope, the annular aperture acts as a beam stop and the diameter of the inner and outer rings are chosen in such a way that only the electrons scattered between 20 and 40 mrad are allowed to pass the aperture. More experimental details on the fabrication of the apertures can be found in our previous work [9].

CdTe Tetrapods: CdTe tetrapods were dispersed on a holey carbon film together with fiducial Au markers [12]. Both the BF-TEM and ADF-TEM tilt-series images were recorded using a Philips CM300 microscope with a dedicated single-tilt holder with a tilt angle of ±70°. This holder was developed at the National Center for Electron Microscopy (NCEM) at the Lawrence Berkeley National Laboratory (LBNL), Berkeley, CA, USA. The sample was attached to the holder by Ag paint to reduce shadowing that could be caused by a clamping system. The BF-TEM series was recorded at 2° tilt-angle intervals over the tilt-angle range –64 to +70°, and the ADF-TEM series of a similar sample was recorded using a 1° tilt-angle interval over the range –67 to +62°. The alignment and reconstruction (weighted back projection) were computed with routines from the TOM toolbox [14]. Surface rendering of the final reconstruction was carried out using Modeller Light software [15].

C Nanotubes Filled with Cu Nanoparticles: The preparation and 2D characterization of the sample was previously described by Tao et al. [16]. The nanotubes were dispersed on a holey C layer. The HAADF-STEM reconstruction of the sample was based on a tilt series acquired on a Tecnai G² 20 X-TWIN microscope, equipped with Xplore3D software and using a Fischione ultra-high-tilt tomography holder [17]. For the acquisition, a nonlinear Saxton tilt scheme was used and the angular range covered –70 to +70°. Inspc3D was used for the alignment, reconstruction (which consisted of an iterative simultaneous iterative reconstruction tomography routine of 20 cycles), and visualization. The corresponding ADF-TEM reconstruction was obtained from a tilt series recorded on a Philips CM30 microscope (without compu-stage); tilting, repositioning, and refocusing was done manually with intervals of 2°. The angular range covered by the single-tilt tomography holder, which was developed at the University of Delft, The Netherlands [18], was in this case limited to –60 to +60°. The reconstruction was based on the TOM toolbox, and the final surface rendering is carried out using the Modeller Light software.

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