Ultramicroscopy 115 (2012) 41–49

Contents lists available at SciVerse ScienceDirect

Ultramicroscopy

journal homepage: www.elsevier.com/locate/ultramic

Discrete tomography of demanding samples based on a modified SIRT algorithm

Andreas Zürner [a], Markus Döblinger [a], Valentina Cauda [a,b], Ruoshan Wei [c], Thomas Bein [a,*]

[a] Department of Chemistry and Center for NanoScience (CeNS), University of Munich (LMU), Butenandtstr. 5-13 (E), 81377 Munich, Germany
[b] CSHR, Italian Institute of Technology (IIT), C. so Trento 21, 10129 Turin, Italy
[c] Walter Schottky Institute, Technische Universität München, Am Coulombwall 3, 85748 Garching, Germany

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form
21 January 2012
Accepted 26 January 2012
Available online 3 February 2012

Keywords:
Electron tomography
Simultaneous Iterative Reconstruction Technique (SIRT)
Discrete Algebraic Reconstruction Technique (DART)
Scanning Transmission Electron Microscopy (STEM)

ABSTRACT

The 3D structure of three particularly challenging samples was reconstructed by electron tomography. Due to sample limitations resulting in a large missing wedge and large tilt increments respectively the 3D structure could not be reconstructed by standard iterative algorithms; even a recently developed discrete algorithm failed until the input parameters for discrete reconstruction were improved. These challenges were addressed by adding a mask in each step of the preceding standard iterative reconstruction, setting all voxels known to be vacuum as zero, thus improving the segmentation and the 3D starting model. The position of these vacuum voxels is obtained from TEM images or other measurement data.

1. Introduction

In the field of materials science, electron tomography has developed rapidly in recent years with the emergence of various new techniques [1–6]. The increase of available methods has brought new potential insights in 3D structures through electron tomography, for example via added chemical or atomic number information. Most notably, Scanning Transmission Electron Microscopy (STEM) coupled with High-Angle Annular Dark Field (HAADF) or Annular Dark Field (ADF) imaging emerged to be a very useful method [7,8], as most of the drawbacks of the 3D—TEM technique, like Fresnel diffraction and diffraction contrast, are overcome. In addition, STEM–HAADF is also chemically sensitive; it is able to resolve very small structures and does not suffer from phase contrast. Thus it enables the 3D structure and composition to be mapped simultaneously at high spatial resolution. However, as for many other non-conventional techniques in electron microscopy, issues of sample preparation and beam sensitivity have frequently become the limiting factor for obtaining high resolution data. In electron tomography, a further complication is related to the limited range of accessible sample tilt angles—the so-called missing wedge [9,10]. It arises from the limited space between the objective lens pole pieces, the finite thickness of the sample holder as well as the geometry of the sample support and the sample itself. It causes a blurring of the reconstructed objects along the beam direction at zero tilt. For example, for a tilt range of $\pm 70^\circ$ the elongation for reconstructions with standard reconstruction techniques can be estimated to 30% [11], therefore the angular range usually is $\pm 70^\circ$ or higher.

The choice of the angular sampling rate can be another crucial factor limiting the resolution of the reconstruction [9]. Hence, small tilt increments of around 2° or less are commonly used for series acquisitions. As a consequence, the required number of images per series by far exceeds the maximum dose for many beam sensitive specimen. This problem can be overcome to a certain extent using the imaging technique least prone to beam damage [7,12,13] or by adjusting the acceleration voltage [14] and the specimen temperature [6]. On the other hand, these measures may be unsuitable because of limited penetration depth in the case of lower acceleration voltages or sample drift under cryo-conditions, especially in STEM mode because of its long exposure times.

FBP (Filtered Back Projection) and SIRT (Simultaneous Iterative Reconstruction Technique) are the most popular reconstruction methods in electron tomography. In both cases every TEM image is smeared back into object space along the original pathway (so-called backprojection), but only SIRT additionally reconciles the original projections with those calculated from the actual reconstruction and minimizes the differences in an iterative process.

* Corresponding author. Tel.: +49 89 2180 77623; fax: +49 89 2180 77622.
E-mail address: bein@lmu.de (T. Bein).

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doi:10.1016/j.ultramic.2012.01.015

The fidelity of the reconstruction can thereby be improved but the problems due to the missing wedge remain [15]. Moreover, both methods produce a blurring of the reconstructed objects, which affects the segmentation step after reconstruction. If a sample consists of only a few different objects having different densities, discrete tomography can be used for the 3D reconstruction taking advantage of the reduced number of possible values in the reconstructed volume. As a consequence of the drastic reduction of the number of potential solutions, high quality tomographic reconstructions can be obtained with significantly smaller data sets. For example, the sophisticated reconstruction algorithm proposed by Batenburg and Sijbers (Discrete Algebraic Reconstruction Technique, DART) [16] was successfully applied to a reduced angular range, between -48° and $+74^{\circ}$ and to a reduced number of exposures of only 15 images [2]. It was noted that the assignment of gray scales is essential for the quality of the reconstruction and that sometimes the gray values had to be refined by a trial-and-error process.

In this paper examples are presented showing tomographic reconstructions from series with even fewer exposures and smaller tilt ranges. These examples show how rather extreme acquisition parameters can still yield high quality results if additional information gained from experimental measurement data is included in the reconstruction process. Furthermore, an unconventional image alignment procedure is presented since the common cross correlation and particle tracking failed due to the large angular increments of 15°. By combining these new techniques it is possible to visualize nano objects from projection series which would not result in suitable 3D images with conventional reconstruction techniques.

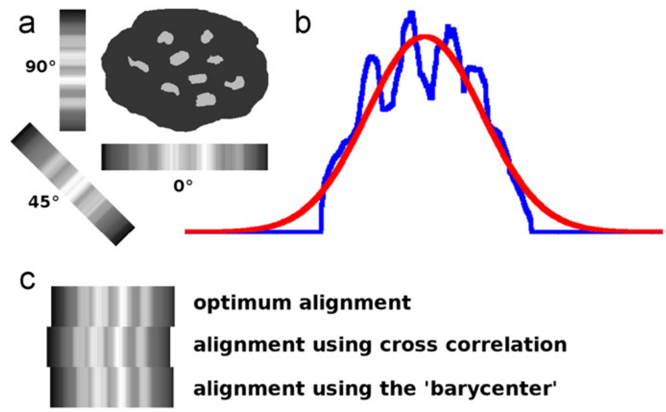

Fig. 1. "Barycenter" alignment procedure. (a) Two dimensional object with its corresponding projections at 0°, 45° and 90°. The intensity distribution of the projections differs strongly because of the large angular increments. (b) Gaussian fitted to the intensity distribution of the projection at 0°. (c) Alignment of the 90° projection with respect to the other projections. The "barycenter" alignment results in a smaller deviation from the optimum as compared to the alignment based on cross correlation.

2. Methods

2.1. Alignment using the "barycenter"

Large angular increments often prevent standard image alignment by cross correlation or by tracking of heavy metal nanoclusters because of the large positional changes. In the case of nearly spherical particles the alignment shifts can be calculated by integration in both dimensions of each background corrected projection and fitting a Gauss function to these data. The "barycenter" alignment shifts all projections such that the maxima of their Gaussians are aligned. An illustration of the alignment is shown in Fig. 1.

2.2. Masked SIRT algorithm

In order to perform discrete tomography the DART algorithm [16] was integrated into the free tomography reconstruction package TOM toolbox [17]. DART uses as input an estimate of different grayscales corresponding to different compositions of the specimen. These values are determined from an initial SIRT reconstruction [2] and the segmentation procedure is performed automatically during reconstruction. Due to the fact that SIRT tends to distribute intensities to a bigger volume, these gray values are always underestimated. Often, the underestimated gray values are attributed to poor-quality SIRT reconstructions, obtained from only a few projections or from a small tilt range. Hence, calculating a SIRT starting volume as realistic as possible is essential for a successful DART reconstruction. For this reason, the initial SIRT reconstruction was modified by an additional "masking" step (see Fig. 2a). The mask is an approximation of the real object volume obtained from real measurement data. In each SIRT iteration step all voxels outside the mask will be set to zero. As a consequence, the intensity a standard SIRT reconstruction would have scattered over "vacuum voxels" is now added to the object's voxels and increases their gray value. The reconstruction procedure is illustrated by a test object consisting of an oval particle lying on a thin membrane as shown in Fig. 2b. Particle and membrane were assigned the same gray value of 3.0. Standard and masked SIRT reconstructions were calculated from nine projections of the test object ($\pm 60^{\circ}$, 15° steps). The standard SIRT reconstruction resulted in a histogram showing a broad peak lying considerably below the assigned voxel value for object and membrane. In contrast, the histogram from the masked SIRT reconstruction shows a pronounced peak at the correct gray value of the particle (Fig. 2c).

2.3. Setting up the mask

According to different types of samples two procedures for creating masks were developed.

2.3.1. Individual particles

For the reconstruction of individual particles a mask is calculated by subtracting the mean value from all projections, setting positive values to one and negative values to zero (Fig. 3a and b). Afterwards, a volume is reconstructed with these "black and white" projections using one simple backprojection step. Every voxel encountering a zero value at least once during the backprojections is defined as vacuum. The remaining voxels are a rough approximation of the nanoparticle's shape (see Fig. 3c). A similar procedure was used by Saghi et al. [18] to reconstruct the shape of convex particles.

2.3.2. Continuous membranes and metal layers

The shape, position and thickness of continuous membranes or metal layers perpendicular to the 0° viewing direction cannot be determined from projections. However, small particles and defects on their upper and lower surface show up in reconstructions as brighter and darker spots, respectively. The localization of these spots is simplified if a thin volume of the standard SIRT reconstruction perpendicular to the tilt axis is integrated in the direction of the tilt axis (Fig. 4). Applying this technique to different volumes gives two sets of data points—indicating the position of the upper and the lower surface. The mask is generated by fitting planes to these points. It is emphasized that this procedure does not reconstruct the membrane but helps to improve the reconstruction of the object