High resolution electron tomography

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ABSTRACT

Reaching atomic resolution in 3D has been the ultimate goal in the field of electron tomography for many years. Significant progress, both on the theoretical as well as the experimental side has recently resulted in several exciting examples demonstrating the ability to visualise atoms in 3D. In this paper, we will review the different steps that have pushed the resolution in 3D to the atomic level. A broad range of methodologies and practical examples together with their impact on materials science will be discussed. Finally, we will provide an outlook and will describe future challenges in the field of high resolution electron tomography.

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1. Introduction

Modern transmission electron microscopy (TEM) can be considered as an advanced tool for nanocharacterisation. Aberration correctors nowadays enable us to obtain a spatial resolution below 1 Å. Nevertheless, one should never forget that TEM images are only two-dimensional (2D) projections of three-dimensional (3D) objects. To overcome this problem, 3D electron microscopy or so-called “electron tomography” has been used successfully in the field of biology for several decades [1,2], but the resolution is limited to the nanometre range because of several parameters such as beam damage, the thickness of the sample and the sample preparation [3,4]. For inorganic materials, beam damage might be a bottleneck for specific samples, but there are more important problems to overcome. Conventional bright field TEM images of crystalline materials are often dominated by Bragg scattering and for certain orientations, the interaction is non-linear. This violates the so-called “projection requirement”, which states that each image of a tilt series for electron tomography should be a monotonic projection of a physical property of the sample under investigation [5]. In materials science, electron tomography was therefore initially only used for polymers [6] and porous materials [7]. Once the combination of electron tomography and high angle annular dark field scanning transmission electron microscopy (HAADF STEM) was demonstrated by Midgley and Weyland [8], electron tomography became a standard characterisation method in materials science as well.

Over the years, different electron microscopy techniques such as bright field TEM (BF TEM), HAADF STEM, annular dark field TEM, electron holography and energy filtered TEM (EFTEM) have all been extended to 3D, providing a world of new information on structure function relationships across a broad range of samples and applications [9–17]. For many years, the ultimate goal has been to achieve electron tomography with atomic resolution. Although this is not yet a standard possibility for all structures, significant progress has recently been achieved using different approaches that will be further explained in this paper.

2. What is “resolution” in 3D?

In conventional electron tomography, also referred to as “continuous electron tomography”, a tilt series of TEM images is acquired by tilting the sample over a large tilt range, with an increment of typically 1° or 2°. After alignment of the projection images, using e.g. cross-correlation, the tilt series is combined into a 3D reconstruction of the original object through a mathematical algorithm. Using this approach, the quality of such a reconstruction is very often predominantly determined by the number of projections. Unfortunately, it is often impossible to tilt the sample over the full 360°, since there is only limited spacing for the sample holder in between the objective pole pieces of the microscope or because of shadowing effects that occur at higher tilt angles. When using a single tilt tomography holder, a tilt range of typically ±80° can be covered. The consequent lack of projection images for a certain range of angles leads to a so-called “missing wedge” of information [8]. Artefacts caused by the missing wedge, such as fanning and elongation effects are a major source of loss of resolution in the final 3D reconstruction [18]. This loss of resolution can be particularly observed along the axes perpendicular to the tilt axis. It can therefore be expected that the resolution of a reconstruction obtained by electron tomography is anisotropic.

However, defining a measure for the resolution that can be obtained from an electron tomography experiment is far from
straightforward. The conventional method to evaluate the resolution of such a reconstruction is determined by a measure called the Crowther criterion [19]. Assuming a ±90° single-axis tilt series that is perfectly aligned, the criterion yields a measure for the resolution defined along three orthogonal axes. The effect of the missing wedge was afterwards included in the measure by Radermacher [20].

According to this definition, the resolution along the axis parallel to the tilt axis (Y) is only limited by the resolution of the input images. For the direction (X) which is perpendicular to Y and the optical axis (Z), the resolution is determined by the number of projections and the thickness of the specimen. This is shown in the formula below:

\[ d_x = \frac{\pi D}{N} \]

where \( D \) is the diameter of the reconstructed volume and \( N \) is the number of projections. The resolution along the optical axis (Z) is determined by multiplying the resolution in the X direction by an elongation factor \( (e_x) \), which is related to the maximum achievable tilt angle. For a single tilt geometry this factor is given by:

\[ e_x = \sqrt{\frac{x + \sin x \cos x}{x - \sin x \cos x}} \]

With \( x \) being the maximum tilt angle. Since \( e_x > 1 \), the resolution along the Z axis is expected to be worse in comparison to the X and Y axis. The resolution along the three directions therefore follows:

\[ d_z > d_x > d_y \]

Although the Crowther criterion is mostly used as the standard definition in studies concerning electron tomography, it does not account for effects such as misalignment of the tilt series, the information limit of the microscope, the signal-to-noise ratio of the individual images but also not for the reconstruction algorithm that is used. Obviously, these factors will have a tremendous influence on the quality of a 3D reconstruction. Therefore, a novel, quantitative resolution measure was recently proposed that also determines the resolution in three orthogonal directions [21]. Using this approach, edge profiles of reconstructed particles or features in a 3D reconstruction are fitted to a step edge function. In this manner, all possible factors that might influence the final resolution are taken into account. It must be noted that independent of the method that is used to define resolution, it is clear that the missing wedge will have a major influence.

It should be pointed out that the use of the expression “atomic resolution 3D reconstruction” is in fact more often related to the visualisation of individual atoms in 3D rather than being based on a definition such as the Crowther criterion. Once the atoms can be visualised in 3D, reconstructions can be interpreted quantitatively in terms of numbers for the atom positions. In that case, classical resolution criteria are no longer appropriate. Then, the use of an alternative criterion relating resolution to statistical measurement precision will be very promising [22].

3. Improving the resolution: optimisation of acquisition

For reasons explained above, the missing wedge has been considered as one of the most important challenges in the field of electron tomography for many years. It is clear that because of the missing wedge, the resolution is anisotropic and inferior along the direction where the information is missing. In order to improve the quality and the resolution of a 3D reconstruction, based on continuous electron tomography, it is therefore necessary to overcome missing wedge artefacts as much as possible.

In order to minimise the missing wedge, new types of tomography sample holders were developed and subsequent acquisition schemes were proposed. For example, “dual-axis tilt electron tomography” is an approach in which a second tilt series is acquired along a tilt axis that is perpendicular to the tilt axis of the first series. Hereby, the missing wedge can be reduced to a missing pyramid leading to an improvement of the quality of the 3D reconstruction [23,24]. An alternative is the so-called “on-axis tilt tomography” technique, which is based on the use of a needle-shaped sample that is prepared by focussed ion beam (FIB) milling. The needle, having a diameter of approximately 100–300 nm, is attached to a dedicated tomography holder and in this manner, one is able to rotate the needle over a tilt range of 360° eliminating missing wedge artefacts [25–27]. An example of a study in which on-axis tilt tomography is used, is presented in Fig. 1. In this study, a porous La₂Zr₂O₇ layer was investigated [26,28]. Orthoslices through a 3D reconstruction based on a needle-like sample show that missing wedge effects are absent using on-axis tilt tomography. The effect of the missing wedge is also illustrated in Fig. 1 where 3D reconstructions are presented that are obtained by eliminating a range of projections from the original tilt series.

Although the missing wedge is completely eliminated when using on-axis tilt tomography, it is unlikely that the approach might lead to atomic resolution in 3D in a straightforward manner. One important bottleneck is the thickness of the needle: when decreasing the diameter, required to obtain high resolution (S)TEM projection images, the stability of the sample during the tilt series will be a limiting factor. Furthermore, the approach is only applicable in studies for which a needle shaped sample can be prepared (e.g. by FIB).

4. Improving the resolution: advanced reconstruction algorithms

In addition to optimising the acquisition of a tilt series, an alternative approach to improve the resolution of a 3D reconstruction is the use of advanced reconstruction algorithms. An important reconstruction algorithm is the Discrete Algebraic Reconstruction Technique (DART) [29,30]. DART is an iterative algorithm for tomographic reconstruction, which alternates between steps of the SIRT algorithm and certain discretisation steps. The discretization is done by exploiting prior knowledge based on the fact that the studied structure consistently is composed of regions with homogeneous density, resulting in well-defined local contrast [29]. It has been shown recently that this technique leads to 3D reconstructions with better quality for bright field TEM [31] as well as HAADF STEM [26,30]. Missing wedge artefacts are greatly reduced and in addition, the algorithm has the advantage that segmentation is carried out during the reconstruction, leading to a more straightforward quantification of the results [26]. This is also illustrated in Fig. 1, where orthoslices through 3D reconstructions obtained using SIRT were compared to reconstructions carried out using DART. It can be seen that the quality of the DART reconstruction is almost not influenced by the missing wedge, even when the tilt range is only ±60°.

The DART algorithm uses the prior knowledge that a reconstruction only contains a limited number of grey values. A different kind of prior knowledge is exploited when using compressive sensing based reconstruction algorithms [32,33]. A specific variant of this approach is “Total Variation Minimization” (TVM), where it is assumed that the object that needs to be reconstructed has a sparse gradient at the nanometre scale. For objects at the nanoscale, it is indeed often valid to assume that boundaries between different compounds are sharp, leading to a sparse gradient of the object. TVM has the advantage that the resulting 3D reconstructions again suffer less from the missing wedge. Also a lim-
ited number of projections is sufficient to obtain a high quality reconstruction. In a recent study, a combination of TVM and discrete tomography was proposed, in which the threshold intensities from a TVM reconstruction serve as the grey values that are requested for a discrete reconstruction [34]. In the majority of the studies where advanced reconstruction algorithms have been applied so far, electron tomography with a resolution in the nanometre range was aimed at. Nevertheless, it is clear that these algorithms will play a key role when atomic resolution in 3D is the final goal.

5. Seeing atoms in 3 dimensions

Despite all of the progress that was made towards improving the resolution in 3D as well as the ability to obtain quantitative results, atomic resolution in 3D remained the ultimate goal in the field of electron tomography for many years [12,35]. Even though the underlying theory for atomic resolution tomography has been well-understood [35–37], experimental results were lacking until recently. The development of aberration corrected TEM opened up a new level of characterisation in 2D, but it was still far from straightforward to extend the results to 3D. Significant progress, pushing the resolution in 3D to the atomic level, has recently been achieved using different methodologies [38–41].

5.1. 3D atomic resolution reconstructions of occupancies using a fixed grid

A first approach is based on the acquisition of a limited number of HAADF STEM images that are acquired along different zone axes. As shown in Fig. 2, a 3D reconstruction at the atomic scale could be obtained for a Ag nanoparticle with a diameter of approximately 3 nm, which was embedded in an Al matrix [38]. The reconstruction was based on only 2 HAADF STEM images obtained when viewing the same particle along the [100] and the [100] zone-axis (see Fig 2a and d, respectively). Using advanced statistical parameter estimation, the parameters of an empirical physics-based model have been estimated in the least squares sense. The refined models are shown in Fig. 2b and e for the particles shown in the white boxed regions of Fig. 2a and d. Based on the estimated parameters, scattered intensities have been computed for each atomic column. The high sensitivity of these scattered intensities for the number of atoms could be exploited to count the number of atoms in a column with single atom precision [38]. The resulting counting results are shown in Fig. 2c and f and have been used as an input for discrete tomography. During the reconstruction, the following prior knowledge is incorporated: (i) all of the atoms lie on a face-centred-cubic (fcc) grid; (ii) the particle is connected and contains no holes; (iii) the number of edges should be minimised. Using a custom implementation of the simulated annealing algorithm [42], a reconstruction was computed that satisfies these three properties. The computed 3D reconstruction of the Ag nanocluster viewed along three different directions is shown in Fig. 2g. An excellent match was found when comparing the 3D reconstruction with additional projection images that were acquired along additional zone axes. The feasibility of this approach was already discussed earlier in a theoretical paper where it was explained that the discreteness that is exploited is the fact that crystals can be thought of as discrete assemblies of atoms [36]. In this manner, a

![Image](image-url)
very limited number of 2D images is sufficient to obtain a 3D reconstruction with atomic resolution.

Although the technique was experimentally first applied to nanocrystals present in a stabilizing matrix, the approach is certainly more generally applicable and can also be used to study, free-standing nanocrystals. As an example, Fig. 3 illustrates the result of a study that was performed for colloidal core–shell semiconductor nanocrystals that form an important class of opto-electronic materials [39]. Rod-shaped PbSe(core)-CdSe(shell) hetero-nanocrystals grown by Cd-for-Pb cation-exchange have been imaged along the [110] direction using aberration corrected HAADF STEM (Fig. 3a). Because of the $Z^2$-dependence of the contrast, the core and shell can clearly be distinguished. Nevertheless, the image corresponds to a 2D projection of a 3D object. In order to gain further understanding of the mechanism of cation exchange at the nanoscale and the chemistry of the PbSe-CdSe interfaces, it is of great importance to determine the 3D morphology of the core as well as the 3D atomic structure of the interface between the core and the shell of the nanorod.

Using the atom counting procedure, the number of Pb atoms in the core could be quantified for [110], [100], and [010] zone axes projections. Next, discrete tomography is used to reconstruct the occupancies of the Pb positions in 3D. The resulting reconstruction is presented along different viewing directions in Fig. 3b. The symmetry of the core is clearly different from what could be expected based on a single 2D image. In addition, incomplete Pb-planes are
observed at the interface. These findings suggest that cation exchange during growth proceeds in a layer-by-layer fashion along certain crystallographic planes. The speed of the exchange depends on the directions in the crystal. From the results presented here, it is clear that the combination of aberration corrected TEM and advanced computational methods have opened the road to atomic resolution electron tomography.

5.2. 3D atomic resolution reconstructions of atom positions

The discrete approach that was used in the studies explained in Section 5.1 assumes that the atoms are positioned on a (fixed) face-centred-cubic lattice and that the particle contains no holes. These assumptions provide an excellent start for the investigations described above, but deviations from a fixed grid, caused by defects, strain or lattice relaxation might be important parameters that determine the physical properties of nanomaterials. Studying these effects is therefore considered as the next challenge in the field of electron tomography at the atomic scale and again different approaches have been reported.

5.3. Continuous electron tomography at the atomic scale

As explained above, when using continuous tomography, a tilt series consisting of a large number of projection images is acquired. One may wonder if this approach can lead to visualisation of individual atoms in case the projection images yield atomic resolution.

Fullerene-like nanostructures were investigated with a sub-nanometre spatial resolution (0.3 ± 0.6 ± 0.6) nm³ by Bar Sadan and co-workers [12]. In this work, a tilt series was recorded with a tilt range of ±60° and a fixed 3° increment. The series was acquired in BF TEM mode using a microscope aligned at negative Cs imaging conditions. The tomographic reconstruction, which was obtained for MoS2 octahedral nanoparticles could be interpreted in terms of the atomic structure.

More recently, Scott et al. reported a 3D reconstruction at a resolution of 0.24 nm. A icosahedral multiply twinned Au nanoparticle with a diameter of ~10 nm was hereby investigated [40]. Although not all atoms could be located in this reconstruction, individual atoms could be observed in some parts of the nanoparticle and valuable information concerning lattice parameters and grains inside the particle could be obtained. The reconstruction was obtained without using any prior information and was based on the so-called equally-sloped tomography approach [43]. Rather than using a tilt series based on an equal tilt increment, increments in the acquired series are based on angles having equal slopes. In total 69 images were required to obtain this 3D reconstruction.

All routes towards atomic resolutions that are described in the manuscript so far each have their own advantages: using the approach based on discrete tomography, only a limited number of images is required, but prior knowledge on the lattice is required. Using the methodology reported by Scott et al. [40], no prior knowledge is assumed, but a relatively large number of images is necessary and not all atoms in the nanoparticle could be visualised. This might be related to the presence of the missing wedge, which will also play an important role for reconstructions at the atomic scale. An alternative approach, based on compressive sensing was recently reported [44].

5.3.1. Electron tomography at the atomic scale based on compressive sensing

The advantages of using compressive sensing for electron tomography with nanoscale resolution were recently reported and are summarised elsewhere in this paper [32,33]. It was shown that the approach is able to compensate for missing wedge artefacts, the technique therefore holds great promise for 3D reconstructions at the atomic scale. For such reconstructions, one possibility is to exploit the sparsity of the object since only a limited number of voxels in the reconstruction are expected to contain an atom and most voxels will correspond to vacuum [44]. An important advantage of this approach is that the actual positions of the atoms can be revealed without using any assumptions concerning the crystal lattice. Another advantage is that because of the incorporation of sparsity during the reconstruction, a limited number of projections is sufficient to create a faithful reconstruction of the atomic lattice.

Mathematically, a tomographic reconstruction corresponds to reconstructing an object x starting from its projections b, which are acquired by a projection operator A. The tomographic reconstruction then often corresponds to iteratively solving the following minimisation problem:

\[
\hat{x} = \arg\min_x \|Ax - b\|_2^2
\]

When implementing compressive sensing, an additional penalty parameter \(\lambda\) is introduced leading to a simultaneous minimisation of the projection error and the L1-norm of the object (i.e. the sum of the absolute values of all the voxels in the reconstructed object) [45]:

\[
\hat{x} = \arg\min_x \left[\|Ax - b\|_2^2 + \lambda \|x\|_1 \right]
\]

The methodology was applied for Au nanorods and the results are presented in Fig. 3. In order to obtain the 3D reconstruction, 4 different high resolution HAADF STEM images were acquired along different zone axes. Alignment of the images was based on a centre of mass calculation for each projection [40]. The reconstructions were calculated using the iterative algorithm based on compressive sensing as explained above. In Fig. 4a, orthoslices through the reconstruction of a Au nanorod are presented and it is clear that the fcc crystal lattice of the rod has been reproduced without using prior knowledge on the atomic structure. From these reconstructions, the boundary as well as the tip facets of the rod have been precisely identified and also atomic steps at the surface can be detected. Our results indicate that surface facets are different when using slightly different experimental parameters during

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**Fig. 3.** (a) 2D HAADF STEM image of a PbSe–CdSe core–shell structure. (b) Different viewing directions of the 3D reconstruction of the Pb lattice forming the core of the nanorod.
parameter based reconstruction algorithm has been proposed [50] in aberration corrected STEM setup [49]. To overcome this problem, a strong elongation effect, sometimes larger than a factor of 30, that is present along the electron beam direction for a typical microscope settings. Theoretically, it is shown that the resolution can be pushed to the atomic level in all three dimensions and that the positions of all atoms can be retrieved. By using scanning convergent beam electron microscopy (SCEM), a technique in which the collection optics (of a double aberration corrected TEM) is arranged symmetrically to the illumination optics, the elongation might be significantly reduced [51]. The potential of different S/TEM based techniques was investigated theoretically and it was hereby concluded that energy filtered SCEM (EFSCEM) is the most promising approach, especially in combination with correction of the chromatic aberration [49,52,53].

5.4. Alternative approaches towards atomic resolution tomography

A completely different approach that has gained interest with respect to reaching atomic resolution in 3D is by exploiting the reduced depth of focus for aberration corrected HAADF STEM. When the convergence angle is increased to 30 mrad or more, only very thin slices of a sample can be brought into focus at the same time. In this manner, the technique which is referred to as “optical depth sectioning” can be used to obtain 3D information on a sample. Because of the high signal-to-noise ratio of an aberration corrected microscope it is even possible to detect individual impurity atoms inside the volume of a TEM sample [48]. A drawback of the technique is a strong elongation effect, sometimes larger than a factor of 30, that is present along the electron beam direction for a typical aberration corrected STEM setup [49]. To overcome this problem, a parameter based reconstruction algorithm has been proposed [50] using prior knowledge about the constituent atom types and the microscope settings. Theoretically, it is shown that the resolution can be pushed to the atomic level in all three dimensions and that

6. Further advances and future challenges

All of the abilities and 3D reconstruction tools described above will certainly play an important role in the further characterisation of nanostructures. However, we are always confronted with even more challenging samples that force us to push the limits even further. An example of such a material consists of ultra-small nanoparticles or clusters, having sizes below 1 nm. There is a clear need for a complete characterisation in 3D since these materials can no longer be considered as periodic objects. The main bottleneck hampering a 3D investigation at the atomic scale is that these clusters may rotate or show structural changes during investigation by TEM [54]. As a consequence of this dynamical behaviour none of the conventional electron tomography methods described in this paper, even those that are based on a limited number of projections, can be applied. At the other hand, the intrinsic energy transfer from the electron beam to the cluster can be considered as a unique possibility to investigate the transformation between energetically excited configurations of a specific cluster. This was exactly the idea behind a recent study that focused on the dynamical behaviour of an ultra-small Ge clusters, consisting of less than 25 atoms. Aberration corrected HAADF STEM was used to acquire series of 2D images. From these series, specific frames were selected and analysed using statistical parameter estimation theory. In this manner, the number of atoms at each position could be determined. In order to extend the 2D structural information that is available to 3D, ab initio calculations were carried out. As an input, different starting configurations, all in agreement with the experimental 2D HAADF STEM images, were used. All of the relaxed cluster configurations, illustrated in Fig. 5, stay relatively close to the input structures. However, only those configurations in which a planar base structure was assumed, were found to be

**Fig. 4.** (a) Three orthogonal slices through the reconstruction of a Au nanorod, showing individual atom positions. The facets composing the morphology can be determined. (b) Slices through the 3D $\varepsilon_{zz}$ strain measurement indicate an outward relaxation of the atoms at the tip of the nanorod.
still compatible with the 2D experimental images. In this manner, reliable 3D structural models are obtained for the ultra-small clusters. This approach can be considered as an important step on the route towards atomic resolution characterisation of the dynamical behaviour of materials using electron tomography.

It is clear that major advances towards 3D reconstructions at the atomic scale have been made recently by different groups using different techniques. Although several (nano)materials have been visualised atom by atom, new challenges are currently emerging. One of these goals is to visualise defects or interfaces with the same resolution as demonstrated so far. Obviously, the achievement of atomic resolution in 3D should not be considered as the end of a quest, but as the start of a new journey in the field of 3D electron microscopy and materials science in general.

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Fig. 5. The left column presents the counting results obtained for three different configurations of a single ultra-small Ge cluster. Green, red and blue dots correspond to 1, 2 and 3 atoms respectively. The 3D results of the ab initio calculations are shown at right along different viewing directions.

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