Chapter 1

General introduction

Electron tomography is a method for obtaining three-dimensional (3D) structural information from electron micrographs. It can be applied to a wide range of samples that can be prepared for transmission electron microscopy (TEM)—may they be of biological origin like e.g. cryo or thin plastic sections of cells and tissue or of material science origin like solid catalysts and electronic devices.

A strong advantage of electron tomography is that it does not depend on averaging over unit cells or particles or on the assumption and exploitation of symmetry in samples, as is the case for methods like angular reconstitution (electron microscopy), nuclear magnetic resonance spectroscopy (NMR), electron and X-ray crystallography. Electron tomography can thus be applied to truly unique structures—the only restriction being that the registered image contrast in the electron micrograph must be a projection of some physical characteristic of the sample, e.g. its mass/electron density. This projection requirement implies that, besides conventional bright-field transmission electron microscopy, also energy filtering (zero-loss or element specific) or high angular annular dark-field (HAADF) scanning transmission electron microscopy (STEM) can be utilized in electron tomography.

Electron tomography data acquisition can be regarded as a kind of super-stereology. Instead of images taken from only two different vantage points, a tomographic data set might consist of more than 151 images taken over an angular range of 150°. The more images, and the larger the angular range, the higher the resolution will be within a 3D reconstruction. A rule-of-thumb for the achievable resolution equals three times the thickness of the sample divided by the number of images (for detailed calculations see Radermacher, 1992). Thus for a 300 nm thick sample the resolution would correspond to 6 nm for the 151 images. Though the theory for the 3D reconstruction from projections (weighted back-projection, inverse radon transform) is available for almost a century (Radon, 1917), and the first applications to electron micrographs were published some 30 years ago (DeRosier and Klug, 1968),
the technical burden connected with the acquisition of so many images, each preceded by the rotation of the sample holder and the re-centering and re-focusing of the feature of interest, prevented the application of the technique as a routine tool for 3D structural investigations in the nm-resolution range. One of the reasons electron tomography gained pace during the last decade (Dierksen et al., 1992; Koster et al., 1992) was based on the availability of slow-scan CCD cameras for image acquisition, fast computer systems for on-line image processing and computer controllable electron microscopes. With these instrumental elements available, automated systems for electron tomography data acquisition could be developed—making the method even suitable for the investigation of extremely beam sensitive samples like preparations of frozen-hydrated material (Dierksen et al., 1993 and 1995).

In spite of the fact that the automation efforts in the nineties enabled the application of electron tomography to a variety of samples suitable for transmission electron microscopy, the amount of time and expertise needed for the acquisition of a tilt series discouraged the majority of potential users from routine usage. In chapter 2 we describe a method for automated electron tomography data collection that reduces acquisition time by a factor of five, enabling data collection in less than an hour (Ziese et al, 2002). The method includes a pre-calibration step—measurement and correction of image shift and defocus change at low magnification to detect displacements that might be as large as several µm—before image acquisition. This step avoids switching back and forth between high and low magnification during image acquisition, which is a very time consuming step regarding the electron magnetic lens stabilizations needed. The method is based on the fact that these dislocations occur due to a displacement of the feature of interest from the eucentric height, a displacement of the optical axis from the tilt axis and some movements intrinsic to the sample holder/stage combination and are thus almost predictable.

Pre-calibration electron tomography has been used as an approved method for routine data acquisition in our laboratory for the last two years and has been adapted by several commercial suppliers of software for automated tomography (Emispec, Inc., Tempe, AZ; FEI Co., Eindhoven, The Netherlands; TVIPS, GmbH, Gauting, Germany) in the meantime. The addendum to chapter 2 exemplifies the application of the method with a material science and a biological sample. The first example
shows that the 3D structural investigation of zeolites, key catalytic materials in applications such as hydro-isomerization of alkanes and hydrocracking of heavy petroleum fractions, can lead to a better understanding of the catalytic activity and selectivity of these materials. The second example illustrates by the 3D study of the Golgi complex that electron tomography of high-pressure frozen and freeze-substituted sections of cell organelles can aid in investigations in cell dynamics and proteomics.

In chapter 3, we discuss a method for the correction of autofocusing errors due to specimen tilt (Ziese et al., 2002c), as this would disable accurate defocus prediction in e.g. pre-calibration tomography. Defocus determination using the beam-tilt method can be hampered for low magnifications and high-tilt angles due to a defocus ramp in the images. The method for the correct cross-correlation (XCF) of two images of a tilted sample acquired under tilted-beam conditions is a modification of the cosine stretch used in the alignment of images acquired under different tilt angles.

As mentioned before, electron tomography is not restricted to the use of transmission electron microscopy. Image acquisition in HAADF-STEM mode makes the method readily available for e.g. crystalline samples, which cannot be acquired in TEM mode as they show angle dependent diffraction contrast. As it was shown that nm-sized inclusions in crystalline material can be detected by this method (Midgely et al., 2001) we have applied the approach to the detection of ultrasmall immuno-gold labels absorbed to heavy-metal stained plastic sections of biological material. Our initial experiments discussed in chapter 4 (Ziese et al., 2002b) provide good evidence that HAADF-STEM electron tomography could be a useful tool for the accurate 3D immuno-localization of proteins. So far, the method is time consuming as all images were acquired by manual operation of the STEM, but we assume that pre-calibration will be applicable in automation and thus provide routine application of STEM tomography.

Finally, chapter 5 discusses our work in the context of recent trends in automation and application of electron tomography.