Chapter 5

General discussion

Electron tomography is a powerful method with great potential for the three-dimensional (3D) characterization of samples in the nm-resolution range in the biological and materials sciences (Baumeister et al., 1999; Koster et al., 2000; McEwen and Marko 2001). It is unique for its ability to investigate individual structures, which sets it apart from 3D methods like angular reconstitution (electron microscopy), nuclear magnetic resonance spectroscopy (NMR), electron and X-ray crystallography that rely on averaging over unit cells or particles or the assumption of certain symmetries.

The general applicability pairs with a high demand on the versatility of the instrumentation, which typically includes a slow-scan CCD camera and a high-tilt sample stage and holders—apart from the need for a remote controllable electron microscope and, in addition, for the expertise necessary to operate this instrumental set-up. This led to a situation where electron tomography is mainly applied by, or in collaboration with, laboratories that are also involved in the software development for the automation/reconstruction of tomographic tilt series.

Recently this picture has begun to change. International congresses (Ringberg Castle, Germany, 1997; Amsterdam, The Netherlands, 2001), special issues of the Journal of Structural Biology dedicated to electron tomography, and various excellent applications that emerged in the last few years (see e.g. special issue of JSB, 138, 2002) illustrated the power of electron tomography and a wider audience has become interested in the routine inclusion of the method in their investigations.

Manufacturers of electron microscopes (FEI Co., Eindhoven, The Netherlands; JEOL, Ltd., Akishima, Japan) as well as independent suppliers of hard-/software for electron microscopy (Emispec, Inc., Tempe, AZ; TVIPS, GmbH, Gauting, Germany), have seen the signs on the wall and provide systems for the automated collection of tilt series that include not only the latest developments that evolved in the specialized laboratories, but can also be used by non-experts. Members of our
Chapter 5

group have been involved in this process from the early beginnings, like autotuning for electron microscopy (Koster et al. 1987), to an extension of the same approach for tilted samples (presented in chapter 3 of this publication; Ziese et al, 2002c) and the work about pre-calibration tomography that considerably speeds up data acquisition (chapter 2; Ziese et al, 2002).

Presently, the developments have evolved to a point were the main interest in tomography with transmission electron microscopy shifts from methodic or technical investigations, like e.g. the value of high voltage or energy filtering (Grimm et al., 1997) for thick samples, to the realization that the next big step towards the widespread application will be made in the field of image processing. Software for 3D reconstructions from tomographic data series, developed in specialized laboratories like e.g. IMOD, Boulder Laboratory for 3D Fine Structure or IVE, Macromolecular Structure Group, UCSF, certainly take account of necessary aspects of the reconstruction process, but could be characterized as still being in a ‘homegrown’ state, that certainly does not appeal to non-experts. A recent congress on electron microscopy (ICEM 15) displayed first attempts of software suppliers (JEOL Ltd., Tietz GmbH) to provide programs that guide inexperienced users through the reconstruction progress—but the commercial availability of robust programs for the 3D reconstruction of any kind of sample, including marker-less alignment, still seems to lie in the near future.

Another challenge in the widespread application of electron tomography that has to be dealt with is the difficulty provided by the interpretation of 3D reconstructions (tomograms). While the results of single particle analysis can readily be displayed as surface rendered maps, the depiction of the volumetric information contained in tomograms remains problematic. Showing single slices of the reconstruction is of course a lossless method, but one ‘cannot see the wood for the trees’, especially when there are only modest contrast differences in the sample that prohibit thresholding. Volume rendering emphasizes a bit more the 3D structure, but otherwise suffers the same problem. As a result most investigators have mainly used software for hand-drawing outlines of interesting structures inside tomograms. Examples for single-slice (Figs. 7A and 7C), surface-rendered (Figs. 7B and 7D) and modelled (Fig. 8B) representations of tomograms can be found in chapter 2. Recently, there are trends in
the development of methods for automated segmentation (see special issue of *JSB*, 138, 2002).

Examples for other aspects of computer science that will influence and facilitate electron microscopy and thus electron tomography in the near future are remote (tele) microscopy and distributed computing/grid services (see e.g. Voelkl et al., 1998; Hadida-Hassan et al., 1999). Both approaches revolve around the idea that specialized resources (like high-voltage electron microscopes) might not be available locally to all investigators and should thus be shared over fast (inter)national computer networks. The investigation of dangerous material under security containment (like e.g. infectious particles) also benefits from the advances in the area of remote microscopy.

While electron tomography has matured and become an established method and a commercially available product in the biological sciences, the field of materials sciences has only discovered it during the last few years. While we have shown that transmission electron tomography can be useful in the investigation of zeolite crystals (see addendum to chapter 2), others provided evidence that HAADF-STEM tomography can be an outcome for high-resolution work on crystalline samples (Midgley et al. 2001) and a combination of electron energy loss spectroscopy with transmission electron tomography can yield insight into element distributions in samples (Weyland and Midgley 2001). Vice versa, investigations in the materials sciences have impact on biological applications, as we have re-adopted the technique to show that HAADF-STEM tomography can be useful for the 3D localization of ultrasmall gold labels in cell sections (chapter 4; Ziese et al., 2002b). This can become valuable in the determination of functions and interactions of proteins, as addressed in the field of cell dynamics and proteomics.