and so addresses a crucial problem in structural biology. This is done by the remarkably successful method of single-particle image reconstruction, in which images of the same protein, lying in random orientations within a thin film of vitreous ice, are combined in the correct orientation to form a three-dimensional reconstructed charge-density map at nanometre or better resolution. The summation over many particles achieves the same radiation-damage-reduction effect as does crystallographic redundancy in protein crystallography. Finally, Section 2.5.8 describes experience with the application of numerical direct methods to the phase problem in electron diffraction. Although direct imaging 'solves' the phase problem, there are many practical problems in combining electron-microdiffraction intensities with corresponding high-resolution images of a structure over a large tilt range. In cases where multiple scattering can be minimized, some success has therefore been obtained using direct phasing methods, as reviewed in this section.

For most inorganic materials the complications of many-beam dynamical diffraction processes prevent the direct application of these techniques of image analysis, which depend on having a linear relationship between the image intensity and the value of the projected potential distribution of the sample. The smaller sensitivities to radiation damage can, to some extent, remove the need for the application of such methods by allowing direct visualization of structure with ultra-high-resolution images and the use of microdiffraction techniques.

2.5.2. Electron diffraction and electron microscopy[1]

By J. M. Cowley

2.5.2.1. Introduction

The contributions of electron scattering to the study of the structures of crystalline solids are many and diverse. This section will deal only with the scattering of high-energy electrons (in the energy range of 10^4 to 10^6 eV) in transmission through thin samples of crystalline solids and the derivation of information on crystal structures from diffraction patterns and high-resolution images. The range of wavelengths considered is from about 0.122 Å (12.2 pm) for 10 kV electrons to 0.0087 Å (0.87 pm) for 1 MeV electrons. Given that the scattering amplitudes of atoms for electrons have much the same form and variation with $(\sin\theta)/\lambda$ as for X-rays, it is apparent that the angular range for strong scattering of electrons will be of the order of 10^{-2} rad. Only under special circumstances, usually involving multiple elastic and inelastic scattering from very thick specimens, are scattering angles of more than 10^{-1} rad of importance.

The strength of the interaction of electrons with matter is greater than that of X-rays by two or three orders of magnitude. The single-scattering, first Born approximation fails significantly for scattering from single heavy atoms. Diffracted beams from single crystals may attain intensities comparable with that of the incident beam for crystal thicknesses of 10^2 Å, rather than 10^4 Å or more. It follows that electrons may be used for the study of very thin samples, and that dynamical scattering effects, or the coherent interaction of multiply scattered electron waves, will modify the diffracted amplitudes in a significant way for all but very thin specimens containing only light atoms.

The experimental techniques for electron scattering are largely determined by the possibility of focusing electron beams by use of strong axial magnetic fields, which act as electron lenses having focal lengths as short as 1 mm or less. Electron microscopes employing such lenses have been produced with resolutions approaching 1 Å. With such instruments, images showing individual isolated atoms of moderately high atomic number may be obtained. The resolution available is sufficient to distinguish neighbouring rows of adjacent atoms in the projected structures of thin crystals viewed in favourable orientations. It is therefore possible in many cases to obtain information on the structure of crystals and of crystal defects by direct inspection of electron micrographs.

The electromagnetic electron lenses may also be used to form electron beams of very small diameter and very high intensity. In particular, by the use of cold field-emission electron guns, it is possible to obtain a current of 10^{-10} A in an electron beam of diameter 10 Å or less with a beam divergence of less than 10^{-2} rad, *i.e.* a current density of 10^4 A cm^{-2} or more. The magnitudes of the electron scattering amplitudes then imply that detectable signals may be obtained in diffraction from assemblies of fewer than 10^2 atoms. On the other hand, electron beams may readily be collimated to better than 10^{-6} rad.

The cross sections for inelastic scattering processes are, in general, less than for the elastic scattering of electrons, but signals may be obtained by the observation of electron energy losses, or the production of secondary radiations, which allow the analysis of chemical compositions or electronic excited states for regions of the crystal 100 Å or less in diameter.

On the other hand, the transfer to the sample of large amounts of energy through inelastic scattering processes produces radiation damage which may severely limit the applicability of the imaging and diffraction techniques, especially for biological and organic materials, unless the information is gathered from large specimen volumes with low incident electron beam densities.

Structure analysis of crystals can be performed using electron diffraction in the same way as with X-ray or neutron diffraction. The mathematical expressions and the procedures are much the same. However, there are peculiarities of the electron-diffraction case which should be noted.

(1) Structure analysis based on electron diffraction is possible for thin specimens for which the conditions for kinematical scattering are approached, *e.g.* for thin mosaic single-crystal specimens, for thin polycrystalline films having a preferred orientation of very small crystallites or for very extensive, very thin single crystals of biological molecules such as membranes one or a few molecules thick.

(2) Dynamical diffraction effects are used explicitly in the determination of crystal symmetry (with no Friedel's law limitations) and for the measurement of structure amplitudes with high accuracy.

(3) For many radiation-resistant materials, the structures of crystals and of some molecules may be determined directly by imaging atom positions in projections of the crystal with a resolution of 2 Å or better. The information on atom positions is not dependent on the periodicity of the crystal and so it is equally possible to determine the structures of individual crystal defects in favourable cases.

(4) Techniques of microanalysis may be applied to the determination of the chemical composition of regions of diameter 100 Å or less using the same instrument as for diffraction, so that the chemical information may be correlated directly with morphological and structural information.

(5) Crystal-structure information may be derived from regions containing as few as 10^2 or 10^3 atoms, including very small crystals and single or multiple layers of atoms on surfaces.

The material of this section is also reviewed in the text by Spence (2003).

2.5.2.2. The interactions of electrons with matter

(1) The *elastic* scattering of electrons results from the interaction of the charged electrons with the electrostatic potential distribution, $\varphi(\mathbf{r})$, of the atoms or crystals. An incident electron of kinetic energy eW gains energy $e\varphi(\mathbf{r})$ in the potential field. Alternatively it may be stated that an incident electron wave of

[1] Questions related to this section may be addressed to Professor J. C. H. Spence (see list of contributing authors).