

2.5. Electron diffraction and electron microscopy in structure determination

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2.5.1. Foreword

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Given that electrons have wave properties and the wavelengths lie in a suitable range, the diffraction of electrons by matter is completely analogous to the diffraction of X-rays. While for X-rays the scattering function is the electron-density distribution, for electrons it is the potential distribution which is similarly peaked at the atomic sites. Hence, in principle, electron diffraction may be used as the basis for crystal structure determination. In practice it is used much less widely than X-ray diffraction for the determination of crystal structures but is receiving increasing attention as a means for obtaining structural information not readily accessible with X-ray- or neutron-diffraction techniques.

Electrons having wavelengths comparable with those of the X-rays commonly used in diffraction experiments have energies of the order of 100 eV. For such electrons, the interactions with matter are so strong that they can penetrate only a few layers of atoms on the surfaces of solids. They are used extensively for the study of surface structures by low-energy electron diffraction (LEED) and associated techniques. These techniques are not covered in this series of volumes, which include the principles and practice of only those diffraction and imaging techniques making use of high-energy electrons, having energies in the range of 20 keV to 1 MeV or more, in transmission through thin specimens.

For the most commonly used energy ranges of high-energy electrons, 100 to 400 keV, the wavelengths are about 50 times smaller than for X-rays. Hence the scattering angles are much smaller, of the order of 10^{-2} rad, the recording geometry is relatively simple and the diffraction pattern represents, to a useful first approximation, a planar section of reciprocal space. Extinction distances are hundreds of ångströms, which, when combined with typical lattice spacings, produces rocking-curve widths which are, unlike the X-ray case, a significant fraction of the Bragg angle.

The elastic scattering of electrons by atoms is several orders of magnitude greater than for X-rays. This fact has profound consequences, which in some cases are highly favourable and in other cases are serious hindrances to structure analysis work. On the one hand it implies that electron-diffraction patterns can be obtained from very small single-crystal regions having thicknesses equal to only a few layers of atoms and, with recently developed techniques, having diameters equivalent to only a few interatomic distances. Hence single-crystal patterns can be obtained from microcrystalline phases.

However, the strong scattering of electrons implies that the simple kinematical single-scattering approximation, on which most X-ray diffraction structure analysis is based, fails for electrons except for very thin crystals composed of light-atom materials. Strong dynamical diffraction effects occur for crystals which may be 100 Å thick, or less for heavy-atom materials. As a consequence, the theory of dynamical diffraction for electrons has been well developed, particularly for the particular special diffracting conditions relevant to the transmission of fast electrons (see Chapter 5.2), and observations of dynamical diffraction effects are commonly made and quantitatively interpreted. The possibility has thus arisen of using the observation of

dynamical diffraction effects as the basis for obtaining crystal structure information. The fact that dynamical diffraction is dependent on the relative phases of the diffracted waves then implies that relative phase information can be deduced from the diffraction intensities and the limitations of kinematical diffraction, such as Friedel's law, do not apply. The most immediately practicable method for making use of this possibility is convergent-beam electron diffraction (CBED) as described in Section 2.5.3.

A further important factor, determining the methods for observing electron diffraction, is that, being charged particles, electrons can be focused by electromagnetic lenses. Many of the resolution-limiting aberrations of cylindrical magnetic lenses have now been eliminated through the use of aberration-correction devices, so that for weakly scattering samples the resolution is limited to about 1 Å by electronic and mechanical instabilities. This is more than sufficient to distinguish the individual rows of atoms, parallel to the incident beam, in the principal orientations of most crystalline phases. Thus 'structure images' can be obtained, sometimes showing direct representation of projections of crystal structures [see *IT C* (2004), Section 4.3.8]. However, the complications of dynamical scattering and of the coherent imaging processes are such that the image intensities vary strongly with crystal thickness and tilt, and with the defocus or other parameters of the imaging system, making the interpretation of images difficult except in special circumstances. Fortunately, computer programs are readily available whereby image intensities can be calculated for model structures [see *IT C* (2004), Section 4.3.6]. Hence the means exist for deriving the projection of the structure if only by a process of trial and error and not, as would be desirable, from a direct interpretation of the observations.

The accuracy with which the projection of a structure can be deduced from an image, or series of images, improves as the resolution of the microscope improves but is not at all comparable with the accuracy attainable with X-ray diffraction methods. A particular virtue of high-resolution electron microscopy as a structural tool is that it may give information on individual small regions of the sample. Structures can be determined of 'phases' existing over distances of only a few unit cells and the defects and local disorders can be examined, one by one.

The observation of electron-diffraction patterns forms an essential part of the technique of structure imaging in high-resolution electron microscopy, because the diffraction patterns are used to align the crystals to appropriate axial orientations. More generally, for all electron microscopy of crystalline materials the image interpretation depends on knowledge of the diffraction conditions. Fortunately, the diffraction pattern and image of any specimen region can be obtained in rapid succession by a simple switching of lens currents. The ready comparison of the image and diffraction data has become an essential component of the electron microscopy of crystalline materials but has also been of fundamental importance for the development of electron-diffraction theory and techniques. The development of the nanodiffraction method in the field-emission scanning transmission electron microscope (STEM) has allowed micro-diffraction patterns to be obtained from subnanometre-sized regions, and so has become the ideal tool for the structural analysis of the new microcrystalline phases important to nanoscience. The direct phasing of these coherent nanodiffraction patterns is an active field of research.

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The individual specimen regions giving single-crystal electron-diffraction patterns are, with few exceptions, so small that they can be seen only by use of an electron microscope. Hence, historically, it was only after electron microscopes were commonly available that the direct correlations of diffraction intensities with crystal size and shape could be made, and a proper basis was available for the development of the adequate dynamical diffraction theory.

For the complete description of a diffraction pattern or image intensities obtained with electrons, it is necessary to include the effects of inelastic scattering as well as elastic scattering. In contrast to the X-ray diffraction case, the inelastic scattering does not produce just a broad and generally negligible background. The average energy loss for an inelastic scattering event is about 20 eV, which is small compared with the energy of about 100 keV for the incident electrons. The inelastically scattered electrons have a narrow angular distribution and are diffracted in much the same way as the incident or elastically scattered electrons in a crystal. They therefore produce a highly modulated contribution to the diffraction pattern, strongly peaked about the Bragg spot positions (see Chapter 4.3). Also, as a result of the inelastic scattering processes, including thermal diffuse scattering, an effective absorption function must be added in the calculation of intensities for elastically scattered electrons.

The inelastic scattering processes in themselves give information about the specimen in that they provide a measure of the excitations of both the valence-shell and the inner-shell electrons of the solid. The inner-shell electron excitations are characteristic of the type of atom, so that microanalysis of small volumes of specimen material (a few hundreds or thousands of atoms) may be achieved by detecting either the energy losses of the transmitted electrons or the emission of the characteristic X-ray [see *IT C* (2004), Section 4.3.4]. The development of the annular dark field (ADF) mode in STEM provides a favourable detector geometry for microanalysis, in which the forward scattered beam may be passed to an electron energy-loss spectrometer (EELS) for spectral analysis, while scattering at larger angles is collected to form a simultaneous scanning image. The arrangement is particularly efficient because, using a magnetic sector dispersive spectrometer, electrons of all energy losses may be detected simultaneously (parallel detection). Fine structure on the EELS absorption edges is analysed in a manner analogous to soft X-ray absorption spectroscopy, but with a spatial resolution of a few nanometres. The spectra are obtained from points in the corresponding ADF image which can be identified with subnanometre accuracy.

An adverse effect of the inelastic scattering processes, however, is that the transfer of energy to the specimen material results in radiation damage; this is a serious limitation of the application of electron-scattering methods to radiation-sensitive materials such as organic, biological and many inorganic compounds. The amount of radiation damage increases rapidly as the amount of information per unit volume, derived from the elastic scattering, is increased, *i.e.* as the microscope resolution is improved or as the specimen volume irradiated during a diffraction experiment is decreased. At the current limits of microscopic resolution, radiation damage is a significant factor even for the radiation-resistant materials such as semiconductors and alloys.

In the historical development of electron-diffraction techniques the progress has depended to an important extent on the level of understanding of the dynamical diffraction processes and this understanding has followed, to a considerable degree, from the availability of electron microscopes. For the first 20 years of the development, with few exceptions, the lack of a precise knowledge of the specimen morphology meant that diffraction intensities were influenced to an unpredictable degree by dynamical scattering and the impression grew that electron-diffraction intensities could not meaningfully be interpreted.

It was the group in the Soviet Union, led initially by Dr Z. G. Pinsker and later by Dr B. K. Vainshtein and others, which showed that patterns from thin layers of a powder of microcrystals could be interpreted reliably by use of the kinematical approximation. The averaging over crystal orientation reduced the dynamical diffraction effects to the extent that practical structure analysis was feasible. The development of the techniques of using films of crystallites having strongly preferred orientations, to give patterns somewhat analogous to the X-ray rotation patterns, provided the basis for the collection of three-dimensional diffraction data on which many structure analyses have been based [see Section 2.5.4 and *IT C* (2004), Section 4.3.5].

In recent years improvements in the techniques of specimen preparation and in the knowledge of the conditions under which dynamical diffraction effects become significant have allowed progress to be made with the use of high-energy electron-diffraction patterns from thin single crystals for crystal structure analysis. Particularly for crystals of light-atom materials, including biological and organic compounds, the methods of structure analysis developed for X-ray diffraction, including the direct methods (see Section 2.5.8), have been successfully applied in an increasing number of cases. Often it is possible to deduce some structural information from high-resolution electron-microscope images and this information may be combined with that from the diffraction intensities to assist the structure analysis process [see *IT C* (2004), Section 4.3.8.8].

The determination of crystal symmetry by use of CBED (Section 2.5.3) and the accurate determination of structure amplitudes by use of methods depending on the observation of dynamical diffraction effects [*IT C* (2004), Section 4.3.7] came later, after the information on morphologies of crystals, and the precision electron optics associated with electron microscopes, became available. This powerful convergent-beam microdiffraction method has now been widely adopted as the preferred method for space-group determination of microphases, quasicrystals, incommensurate, twinned and other imperfectly crystalline structures. Advantage is taken of the fact that multiple scattering preserves information on the absence of inversion symmetry, while the use of an electron probe which is smaller than a mosaic block allows extinction-free structure-factor measurements to be made. Finally, an enhanced sensitivity to ionicity is obtained from electron-diffraction measurements of structure factors by the very large difference between electron scattering factors for atoms and those for ions at small angles. This section by M. Tanaka replaces the corresponding section by the late P. Goodman in previous editions, which researchers may also find useful.

In spite of the problem of radiation damage, a great deal of progress has been made in the study of organic and biological materials by electron-scattering methods. In some respects these materials are very favourable because, with only light atoms present, the scattering from thin films can be treated using the kinematical approximation without serious error. Because of the problem of radiation damage, however, special techniques have been evolved to maximize the information on the required structural aspects with minimum irradiation of the specimen. Image-processing techniques have been evolved to take advantage of the redundancy of information from a periodic structure and the means have been devised for combining information from multiple images and diffraction data to reconstruct specimen structure in three dimensions. These techniques are outlined in Sections 2.5.5, 2.5.6 and 2.5.7. Section 2.5.6, written for the first and second editions by Boris Vainshtein, has been revised and extended for this third edition by Pawel Penczek. It deals with the general theory of three-dimensional reconstruction from projections and compares several popular methods. Section 2.5.7 describes the application of electron-microscope imaging to the structure analysis of proteins which cannot be crystallized,

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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 microscopy¹

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).