Practical Methods in ELECTRON MICROSCOPY

Volume 1

AUDREY M. GLAUERT

Part I

SPECIMEN PREPARATION IN MATERIALS SCIENCE

P. J. Goodhew

Part II

ELECTRON DIFFRACTION AND OPTICAL DIFFRACTION TECHNIQUES

B. E. P. Beeston

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Editor's preface

Electron microscopy is now a standard technique with wide applications in all branches of Science and Technology, and every year a large number of students and research workers start to use the electron microscope and require to be introduced to the instrument and to the techniques for the preparation of specimens. Many books are available describing the techniques of electron microscopy in general terms, but the authors of Practical Methods in Electron Microscopy consider that there is an urgent need for a comprehensive series of laboratory handbooks in which all the techniques of electron microscopy are described in sufficient detail to enable the isolated worker to carry them out successfully. The series of books will eventually cover the whole range of techniques for electron microscopy, including the instrument itself, methods of specimen preparation in biology and the materials sciences, and the analysis of electron micrographs. Only well-established techniques which have been used successfully outside their laboratory of origin will be included.

Great care has been taken in the selection of the authors since it is well known that it is not possible to describe a technique with sufficient practical detail for it to be followed accurately unless one is familiar with the technique oneself. This fact is only too obvious in certain 'one author' texts in which the information provided quickly ceases to be of any practical value once the author moves outside the field of his own experience.

Each book of the series will start from first principles, assuming no specialist knowledge, and will be complete in itself. Following the successful innovation, made by the same publishers in the parallel series Laboratory

Techniques in Biochemistry and Molecular Biology (edited by T. S. Work and E. Work), each book will be included, together with one or two others of the series, in a hardback edition suitable for libraries and will also be available in a cheap edition for individual use in the laboratory. Each book will be revised, independently of the others, at such times as the authors and editor consider necessary, thus keeping the series of books continuously up-to-date.

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Cambridge, England

AUDREY M. GLAUERT, Sc. D.
General editor

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SPECIMEN PREPARATION IN MATERIALS SCIENCE

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P. J. GOODHEW

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Chapter 1

Introduction

It can fairly be said that specimen preparation is the *sine qua non* of electron microscopy. However there are almost as many techniques or variations of techniques published as there are electron microscopes to utilize them. Hence it is the intention of this book to gather together those techniques which have gained wider currency than their laboratory of origin and therefore may be considered not to depend solely on the art of their inventor. Electron microscopists should take to heart the comment of a colleague who said: 'In Birmingham phosphoric acid worked every time, nitric never; in Bristol phosphoric acid never works, but nitric always.' For this reason wherever possible at least two approaches to every problem have been included.

In attempting to obtain a more reliable guide to preparation techniques than is suggested by the above comment it is sensible first to analyse the requirements of a specimen suitable for electron microscopy.

1.1 Requirements from specimen preparation techniques

There are many conditions which the ideal specimen should fulfil and most preparation methods involve a compromise with respect to some of them. The most obvious requirement, that the specimen be transparent to electrons, is clearly of great importance, but it should not be thought that it is the only prerequisite for an electron microscope investigation. Of equal importance is that the structure visible in the prepared specimen is representative of that found in the bulk material; in other words the microstructure should be unaltered by the preparation procedure. It is because of this requirement that the preponderance of methods described in this book are chemical or electro-chemical in nature. It should be borne in mind, however, that for

many materials it has never been demonstrated that the structure observed in the electron microscope is typical of the bulk structure. This is a problem which is starting to be solved by the use of million volt or greater electron microscopes (see § 1.3).

Another limitation to the type of specimen that is suitable is that the interpretation of microstructure may become difficult if too many structural features are present in the field of view. This is a disadvantage of the thicker specimens used in high voltage microscopy and means that stereo photographic techniques must often be used to elucidate the structure. A further factor affecting the choice of specimen thickness is the resolution required from the specimen. Many structural features, although themselves small (e.g. dislocations), give rise to images of much larger dimensions such that resolution is not a limiting factor. Other studies, however, may require the observation of smaller features (e.g. lattice resolution) and here the thickness of the specimen may limit the microscope resolution and hence the detail which may be observed. This arises because of the increase in inelastic scattering of electrons from a thicker specimen leading to loss of contrast, loss of intensity, increase in chromatic aberration and hence a poorer resolution. It is important therefore to consider specimen thickness requirements in the light of the resolution required.

More practical, but no less important, requirements of the specimen are that it should if possible be visible with the naked eye and susceptible to handling without damage. This is a major advantage of the disc techniques described in Chapter 3. Other desirable features are that it should be sufficiently rigid not to suffer deformation during examination in the microscope and that the area of suitable thickness shall be extensive to give the operator a chance to assess what features are truly 'typical'. Much time (and temper) can be saved if the specimen can easily be removed from the microscope and stored for some time in the laboratory without oxidising, breaking or otherwise changing its structure. On specimens such as these, results can be checked or more extensive studies performed some time after the original investigation.

The preparation methods which produce specimens with all or some of these desirable characteristics need above all to be reproducible. Small bulk specimens are often the result of long series of experiments and may even be unique. Obviously a technique with a low success rate is expensive in experimental time and material as well as valuable microscope time. At a conservative estimate as much time is spent in the preparation of specimens as in their study in the microscope and microscope investigation can therefore be

speeded up tremendously by the use of quick, reproducible (and preferably simple and inexpensive) specimen preparation techniques. To the attainment of this end the remainder of this book is dedicated.

1.2 Specimen thickness for 100 kV microscopy

It is not easy to define the maximum useful specimen thickness of any particular material since it will depend on the level of electron intensity which the operator can tolerate. Most modern microscopes can be fitted with image intensifying devices which allow operation at what would be very low screen intensities and on such machines the tolerable specimen thickness may be greater. However, although such devices may increase the usable thickness by up to a factor of two, the maximum thickness for some materials is still very low. Also there is some evidence that the apparent width of the images of crystal defects may increase with specimen thickness, and this could lead to misleading quantitative results (Sahashi 1969).

Electron intensity is reduced as the beam passes through a specimen by any process, elastic or inelastic, which scatters electrons off the electron-optical axis and thus prevents them contributing to the image. The transmitted intensity depends very strongly on the electron scattering factor of the material, which increases with atomic number. Thus for amorphous specimens of the same thickness, light elements will transmit a far larger proportion of the incident beam than heavier elements. While it is possible to see through several hundred nanometres of amorphous Al at 100 kV, the equivalent brightness is obtained only with a few tens of nanometres or less of uranium.

For crystalline materials Bragg reflection may occur (coherent, elastic scattering) and by a process of repeated diffraction an apparently anomalous thickness of material can be penetrated. This process is very dependent on the orientation of the specimen with respect to the electron beam and hence in a typical slightly buckled specimen only certain areas which happen to be in the correct orientation will show this enhanced transparency. However, now tilting stages are available on virtually all microscopes the specimen can be manipulated so that almost any area of it can be put in the condition of 'anomalous absorption' as it is called. Using this effect much greater thicknesses of crystalline specimens can be examined than is possible with amorphous samples. Several micrometres of Al have been penetrated at 100 kV and over a hundred nanometres of uranium.

1.3 Penetration of electrons and accelerating voltage

Specimens only a hundred nm thick or less are rarely typical of bulk material (as well as being difficult to produce) and hence efforts have been made to increase the permissible thickness by increasing the accelerating voltage in the electron microscope. At the time of writing (1971) microscopes of 1.2 MeV are available commercially and 3 MeV machines are under evaluation.

Appreciably thicker specimens can be penetrated by making a relatively small increase in accelerating voltage. Thus much work on glass and ceramic systems and fairly heavy metals has been simplified by the use of the 200 kV microscope, and significantly increased penetration has resulted as the accelerating voltage has been raised all the way up to 1000 kV. Present estimates of the thickness of crystalline specimens which it is possible to penetrate at 1000 kV are 4–8 μm for aluminium (Dupouy and Perrier 1964), 1–2 μm for copper (Sharp and Poole 1968), and 1–2 μm for iron (Hale 1966 and Keown 1969). Recent calculations which take into account many-beam diffraction effects suggest that these estimates may be optimistic (McElroy and Gale 1971).

In general it has been found that the use of higher accelerating voltages does not simplify specimen preparation techniques; it merely makes study of heavy atom systems possible and gives larger transparent areas in other specimens. Thus specimen preparation methods have not been evolved or modified for such work. Perhaps a little less care is needed in the final thinning and also the thicker parts of the specimens, being more rigid, are less susceptible to damage during handling. Otherwise the techniques described in the following chapters are equally applicable to specimens intended for any transmission microscope.

1.4 Direct formation of specimens

Many electron microscope investigations have been carried out on specimens directly produced as thin films* from a few nm to a few μm thick. In the early days of electron microscopy such specimens gave a very useful insight into the types of structure which might be viewed by this technique and indeed thin films showed many of the features we now recognize in specimens

* As far as possible the term 'film' has been used for a thin specimen built up by deposition and 'foil' for a specimen, quite possibly of similar dimensions, produced by thinning from the bulk.