PRINCIPLES AND PRACTICE OF ELECTRON MICROSCOPE OPERATION

ALAN W. AGAR

RONALD H. ALDERSON and DAWN CHESCOE

PRINCIPLES AND PRACTICE OF ELECTRON MICROSCOPE OPERATION

ALAN W. AGAR
Agar Aids
Bishop's Stortford, Hertfordshire

RONALD H. ALDERSON
Ickleton
Saffron Walden, Essex

and DAWN CHESCOE
AEI Scientific Apparatus Ltd
Manchester

NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM · LONDON
AMERICAN ELSEVIER PUBLISHING CO., INC. – NEW YORK

Library of Congress Catalog Card Number 73-94298
North-Holland ISBN series 0 7204 4250 8
volume 0 7204 4254 0
American Elsevier ISBN 0 444 10644 8

Publishers:

NORTH-HOLLAND PUBLISHING COMPANY–AMSTERDAM
NORTH-HOLLAND PUBLISHING COMPANY, LTD.–LONDON
Sole Distributors for the U.S.A. and Canada:
AMERICAN ELSEVIER PUBLISHING COMPANY, INC.
52 VANDERBILT AVENUE
NEW YORK, N.Y. 10017

Printed in The Netherlands

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.

Strangeways Research Laboratory
Cambridge, England

AUDREY M. GLAUERT, Sc.D.
General editor

Acknowledgements

Much of the presentation of this book was developed during a long series of training courses in the use of the transmission electron microscope. We have been greatly helped over the years by the many 'students' who have asked awkward questions and stimulated new lines of thought. We must acknowledge the debt we owe to Dr. M. E. Haine, Prof. T. Mulvey and Mr. R. S. Page who unstintingly shared their experience and knowledge.

We particularly wish to thank the Editor for her many suggestions for the improvement of the presentation.

We are indebted to the following for permission to reproduce micrographs and figures: Dr. D. L. Allinson (9.8), Dr. V. E. Cosslett (9.3, 9.4), Prof. J. M. Cowley and Dr. S. Iijima (8.4), Dr. S. L. Cundy (9.13), Prof. G. Dupouy (2.27, 3.21. 9.11), Dr. A. M. Glauert (8.1), Dr. K. H. Hale and Miss M. Henderson-Brown (9.5), Dr. B. Hudson and Dr. M. J. Makin (6.3, 6.4), Prof. M. Iwanaga (7.8), Prof. J. B. LePoole (9.7), Dr. P. R. Swann (2.24, 9.9, 9.10), Prof. R. C. Williams (6.6), Dr. D. B. Wittry (9.13), AEI Scientific Apparatus Ltd. (2.4, 2.8, 2.10, 2.11, 2.18, 2.19, 2.20, 2.23, 2.32, 3.3, 3.5, 3.6, 3.7, 3.9, 3.19, 5.1, 5.6, 5.7, 5.11, 6.1, 6.2, 8.7, 9.2, 9.6, 9.12, 9.15), Ilford Ltd. (7.6), JEOL Ltd. (2.1, 2.31), Ebtec Inc. (2.6, 2.7), N.V. Philips (2.2, 2.9, 2.13, 2.17, 2.22, 2.25, 2.29, 5.3), Siemens Ltd. (2.3, 2.21, 2.26, 2.28), Smethurst High Light Ltd. (2.12), Zeiss Ltd. (9.1).

One of us (AWA) is also grateful for the use of electron microscope facilities at the Strangeways Research Laboratory, Cambridge, and at Pye-Unicam Ltd., for obtaining a number of micrographs used for illustration.

Contents

Chapter 1

The basic principles of the electron microscope

The aim of this book is to provide the background needed for an operator to obtain the best results from an electron microscope. To this end, the basic principles of the instrument are first described, followed by the physical construction of the microscope. The details of alignment and operation of the instrument follow, so that an operator can gain a clear idea of the functions of the controls available to him. The foregoing chapters should also have explained some of the potentialities as well as the difficulties of the design of an electron microscope, so that new possibilities and basic limitation become apparent.

Apart from understanding the operation of the instrument the operator must record the information which the instrument renders visible and important photographic techniques which are not always properly observed are detailed in one chapter. The basic mechanism of image formation (quite different from that in the light microscope) is described so that there is a background for intelligent interpretation of the image structure that is obtained. Chapters are also devoted to routine maintenance and fault-finding.

Optical microscopy has suffered greatly because it always appeared to be easy to achieve results without understanding how the instrument should be used. Electron microscopes are perhaps fortunate in appearing to be sufficiently complex so that they induce prospective operators to attempt to understand them before proceeding with their research programmes. It is the hope of the authors that this book will aid this process.

Since there are now many different models of microscope in service throughout the world, it is impracticable to describe constructions and

procedures which are completely applicable to each one – and indeed the instruction manual will describe the best procedure for operating a particular instrument. This book aims to create an informed background for the operation of electron microscopes so that they may be used more intelligently and at a higher level of performance. Since the majority of those using the electron microscope are in the biological sciences, an attempt has been made to present the information in a descriptive rather than a mathematical form.

It will be apparent from the aims of this book that it has no pretensions to be a complete survey of the electron optics of the instrument. Those wishing to look deeper into this subject can consult a number of useful books (Zworykin et al. 1945; Cosslett 1951; Hall 1953; Haine 1954, 1961; Siegel 1964; Grivet 1972; Hawkes 1972).

1.1 The use of electrons for microscopy

A microscope is an instrument designed to render visible objects which are too small to be seen by the unaided eye. For particles greater than a few tenths of a micrometre in diameter, the light microscope is adequate, but for very small objects the light microscope fails because the wavelength of visible light is large compared with the objects to be examined.

When light rays emanating from a point, pass through a lens of semi-angular aperture α (Fig. 1.1), they form an image which is no longer a point but with the intensity spread out in what is known as an Airy disc (Fig. 1.2). The distance between the two minima on either side of the main intensity peak is given by $D = 1.22\lambda/n \sin\alpha$, where λ is the wavelength of the light, and n the refractive index of the material in which the object lies. When two

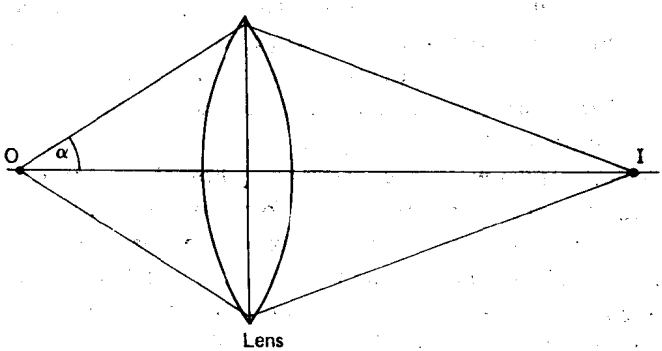

Fig. 1.1. Lens aperture defining Airy disc. The semi-angle α of the lens subtended from the object point O defines the image disc diameter at I (see Fig. 1.2 for intensity profile).

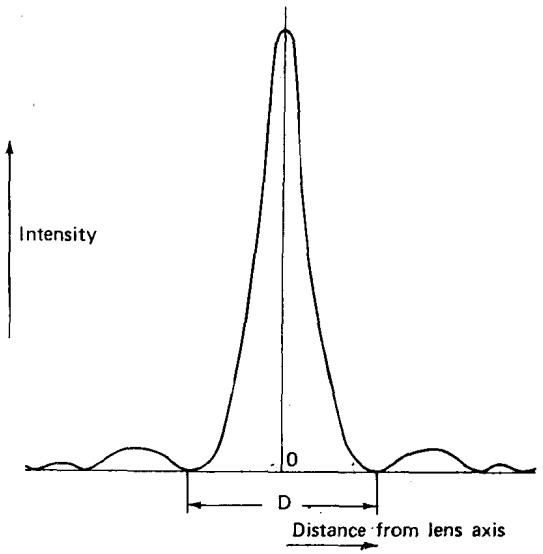

Fig. 1.2. Intensity profile of the Airy disc at the image point I. The ordinate represents image intensity. O represents the lens axis. The abscissae are distances from the lens axis. D is the diameter of the central intensity disc, given by $D = 1.22\lambda/n \sin \alpha$.

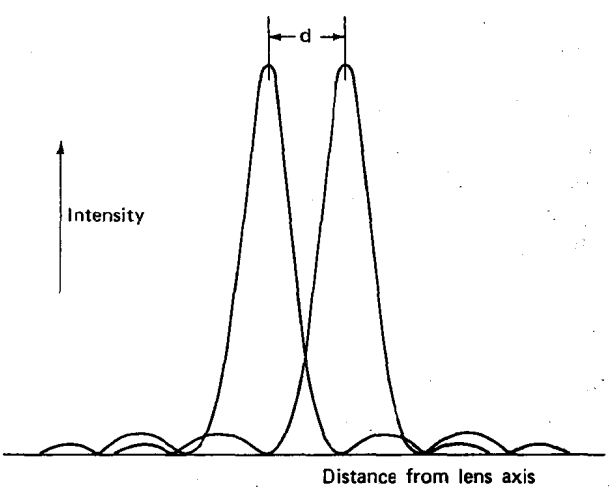

Fig. 1.3. The definition of resolution in terms of Airy disc separation. The central maximum of one image point coincides with the first minimum of intensity of the adjacent (just resolved) image point, distance $d = 0.61\lambda/n \sin \alpha$.

emitting points of the object lie very close together (Fig. 1.3) the intensity patterns in the image will overlap. The resolution of the system is defined as the distance between the maxima when the maximum intensity from one point is coincident with the first minimum from the other point. The separation of the two points is then

$$d = \frac{0.61\lambda}{n \sin \alpha} \tag{1}$$

Note that this separation does not depend on any property of the lens except its semi-angular aperture.

In a light microscope, using an oil-immersion objective lens, the value of $n \sin \alpha$ may be as large as 1.4, and taking a wavelength of 500 nm (1 nm = 10^{-9} metres) the resolution limit is seen to be about 200 nm (2000 AU in the more familiar Ångstrom Units). Because the designers of glass lenses have over the years learnt how to correct most of the lens defects, it has proved possible to build instruments which approach close to this limiting performance. This limitation, called the diffraction limit, is due to the size of the wavelength of light and no further improvement can be expected without using a different illumination of shorter wavelength.

It was shown by De Broglie (1924) that an accelerated electron beam has an effective wavelength λ (in nm) given by

$$\lambda = 0.1 \sqrt{\frac{150}{V}} \tag{2}$$

where V is the accelerating voltage. For $V = 60{,}000$ V, $\lambda = 0.005$ nm, a wavelength shorter by a factor of 10^5 than visible light. In principle, therefore, an electron microscope should be capable of imaging atomic structures, if one considers only the wavelength (diffraction) limitation. As will be seen later other factors limit the achievable resolution to a considerably poorer figure.

1.2 Electron lenses

Although one can in principle use either electrostatic or magnetic lenses to focus a beam of electrons, practical instruments exclusively employ magnetic lenses as they can be made with smaller defects than electrostatic lenses, so only magnetic lenses will be considered here. The magnetic field required to form an electron lens is a locally strong axial field in the direction of the electron beam. The envelope of the electron beam is exactly analogous to that of a light beam passing through a converging lens, except that the electrons

travel in a helical path through the lens; this additional rotary motion is a characteristic of magnetic lenses (Fig. 1.4). Since the rotary motion does not affect the focusing behaviour of the lens, the ray diagrams for a beam through an electron lens are identical with those for a light optical lens, and the lens formulae for light optics apply.

In a magnetic electron lens the focal length is determined by the field strength in the lens gap and by the speed of the electrons (determined by the accelerating voltage). With a given lens geometry, the strength of the field in the pole piece gap is proportional to the excitation of the lens, usually

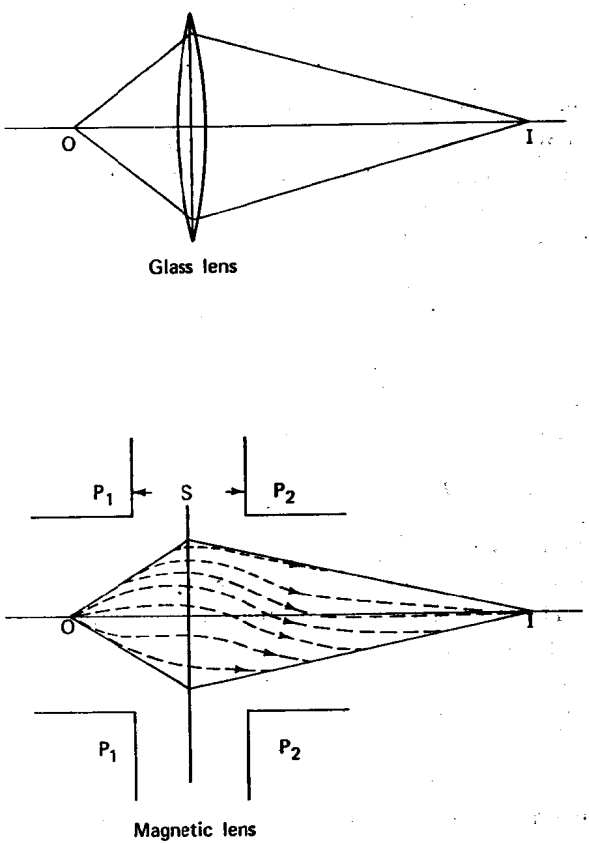

Fig. 1.4. Comparison of light and electron lenses. Each lens forms an image I from the object point O, so that the geometrical image formation is identical, but the electrons rotate in a spiral trajectory about the lens axis as they pass through the magnetic field formed between the pole pieces P_1 and P_2. The dotted lines indicate electron paths over the cone surface.