TECHNIQUES FOR ELECTRON MICROSCOPY

Edited by

DESMOND KAY

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FOREWORD

Electron microscopy is an art as much as it is a science. Its applications extend into almost all branches of pure and applied science – biological as well as inorganic. The preparative methods needed in its practice are correspondingly multifarious. Each type of tissue in biology, each type of metal in metallurgy, requires special treatment if sections (or films) thin enough for electron microscopy are to be obtained in good condition and with minimum risk of artefact.

The armamentarium of methods now available has been built up over the past twenty years or so, at first slowly and with many false starts, and more recently at a rapid rate and with greater finality. To keep abreast of progress in these matters is particularly difficult, in that it is being made in many countries and is published in a great variety of journals. When the Electron Microscopy Group of the Institute of Physics was formed in 1946, one of its first preoccupations was the production of a survey of the current state of preparative techniques. It appeared in 1950 as one of the quarterly parts of the *Journal of the Royal Microscopical Society* under the title of 'The Practice of Electron Microscopy'. The subject was already extensive enough to require a dozen contributors for coverage, with Dr. D. G. Drummond as editor.

It is not too much to say that the rapid growth of electron microscopy in Great Britain during the next decade was based mainly on 'Drummond'. It found much use in other parts of the world also. For several years now it has been out of print and the need for an up-to-date successor has become urgent. The present text, prepared by a new team under the editorship of Dr. Kay, has been written in response to that demand. Nearly three times the length of the earlier handbook, with a greater variety of techniques described and many more references, it reflects the advances made in a decade which has seen first the perfection of ultra-thin sectioning of biological material and more recently the controlled preparation of very thin films of metal from bulk material. May this new text serve as guide and counsellor for the new wave of electron microscopists as fruitfully as 'Drummond' has done for their predecessors.

V. E. COSSLETT

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CHAPTER ONE

THE OPERATION OF THE ELECTRON MICROSCOPE

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INTRODUCTION

This introductory chapter to a volume dealing principally with techniques of specimen preparation for electron microscopy is designed to cover the principles of operation of an electron microscope. No attempt will be made to describe the theory of the design of the instrument, or its various aberrations. For a discussion of these matters, the reader is referred to several excellent books (Zworykin *et al.*, 1945; Cosslett, 1951; Hall, 1953; review by Haine, 1954), which cover the ground in detail.

The central requirement met by the designer is a low spherical aberration of the objective lens. This sets the limit of resolving power which will be achievable with the instrument. Once this has been set, the designer attempts to ensure that other possible limitations to performance are unimportant in comparison. The principal factors are:

1. H.T. stability } in order to reduce the effects of chromatic
2. Lens current stability } aberration.
3. Mechanical stability.
4. Accuracy of mechanical movements.
5. Residual astigmatism of the objective lens.
6. Magnetic screening.

Items 1-4 are within the control of manufacturing tolerances, and provided that the instrument is functioning correctly, will not concern the operator. In most high performance microscopes, these limit the resolution to a value somewhat worse than that defined by spherical aberration of the objective lens. Items 6 and 3 establish limits to the tolerable variable magnetic field and floor vibration in the site in which the microscope is installed. These are usually checked before installation, and should not again require consideration. If a fault has developed, it will readily be detected by the testing described on page 14.

The residual astigmatism of the objective lens cannot in general be reduced to a tolerable limit by care in manufacture, and it must be corrected by the operator, as described later.

The explanation of the operation of the electron microscope has been written so as to be applicable to any instrument. Reference will, however, be made to specific features of some of the high resolution microscopes now available. Those referred to are:

Siemens Elmiskop I
A.E.I. EM. 6

Philips EM. 200
RCA EMU. 3
Japan Electron Optics JEM. 5

In this chapter, the type number only will be used.

ALIGNMENT

The quality of the image from an electron lens falls off very rapidly away from its axis, and the designed performance of the microscope usually assumes that the axes of the various lenses are in line, so that the optimum conditions are achieved at the centre of the viewing screen or photographic plate.

The mechanical tolerances required to achieve such alignment of the electron lenses are not achievable in practice, and the instrument is therefore provided with adjustments which permit the lenses to be moved relative to one another. Not all these alignments are of equal importance, and in the interest of simplicity, some or all of the possible movements are dispensed with in microscopes of different manufacture. However, it is only in the very low performance instruments that alignments are all pre-set, and there is an increasing tendency to offer the operator fuller control of lens alignment, culminating in the recent Philips microscope (van Dorsten & van der Brock, 1958) where all lenses are independently alignable.

Quite apart from the effect on instrumental performance, the convenience of operation is greatly improved if the lenses are correctly aligned. A lens will project an image of a point on its axis to some other point on its axis, whatever its strength. An off-axial point, however, will be imaged at different distances from the axis depending on the strength of the lens. If successive lenses are not in line, changes in operating conditions may lead to undesirable movements of the image.

The important requirements to be met by alignment are:

1. The illumination should be sufficiently bright to enable objects to be viewed and focused at the highest magnification for which the instrument is designed. The illumination should remain centred as its intensity is varied.
2. The object should not sweep out of view as the objective focus is varied.
3. Change of magnification should be possible without loss of the field of view, or of the illumination on it.

If these conditions are met, full flexibility in operation with a minimum of adjustments can be obtained, and they enable the optimum conditions of illumination and magnification to be selected for any particular specimens.

Function of alignments usually provided

The minimum alignments required are as follows:

Traverse of the electron gun with respect to the condenser lens system.

Traverse of the illuminating system (condenser and gun) as a unit with respect to the objective lens.