Principles And Techniques Of Electron Microscopy

BIOLOGICAL APPLICATIONS

Edited by

M. A. Hayat

VOLUME 9

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PREFACE

This is the ninth volume of a multi-volume series on the principles and techniques employed for studying biological specimens with the aid of an electron microscope. Since its inception in 1970, the series has successfully reflected the growth of electron microscopy in instrumentation as well as in methodology. There was a pressing need to keep readers abreast of the remarkable expansion of the field in recent years and the ever growing importance of its contributions to the understanding of many problems in biological and medical sciences. This treatise serves as an international authoritative source in the field, and is designed to cover important new developments systematically. The treatise departs from the tradition that books on methodology present only the contemporary consensus of knowledge. It is written by scholars, and when they have anticipated the potential usefulness of a new method, they have so stated. The authors have not hesitated to include ideas in progress. The treatise should serve as a guide and survey, which can save a newcomer the tedious search for information scattered in biological journals.

This volume has developed over the years through the joint effort of ten distinguished author-scientists. As a result, a most comprehensive compilation of methods developed and used by a large number of competent scientists has been achieved. The book contains new viewpoints with particular regard to current problems. Areas of disagreement and potential research problems have been pointed out. It is hoped that the readers will become aware that correct interpretation of the information retrieved from electron micrographs is dependent

upon an understanding of the principles underlying the methodology and instrumentation.

The basic approach in this volume is similar to that in the previous eight volumes, in that the methods presented have been tested for their reliability, and are the best of those currently available. The instructions for the preparation and use of various solutions, media, stains, and apparatus are straightforward and complete, and should enable the worker to prepare his or her specimens without outside help. Before undertaking the processing, one should read the entire procedure and prepare necessary solutions and other media. Each chapter is provided with an exhaustive list of references with complete titles. Full author and subject indexes are included at the end of the book.

It is encouraging to know that the previous volumes have been received favorably. It is my impression that this volume will also fulfill its purpose: to provide an understanding of the usefulness, limitations, and potential applications of special methods employed for studying the structure, composition, size, number, and location of cellular components, and to provide details of current improvements in the instrumentation.

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1. ELECTRON MICROSCOPY OF ATOMS

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INTRODUCTION

The electron microscope has the inherent capability of atomic resolution, by virtue of the very short wavelength of medium energy electrons, and the implied capability of somehow "seeing" single atoms. However, since a micrograph is a record of the projection of an object, even a thin specimen such as a single protein molecule would be imaged as a confusing array of overlapping atoms. Fortunately, electrons interact much more strongly with heavy atoms than with light ones, so that single heavy atoms could be localized from a projection by virtue of their high contrast. It is this possibility that has led some biologists to dream of visualizing heavy atoms placed at strategic locations within biological molecules, as bright dots in a sea of faint overlapping images of light atoms. With the recent achievements of electron microscopy in imaging single heavy atoms, the prospects for realizing this dream are becoming increasingly bright.

History

The earliest calculations of single-atom contrast were made by Hillier (1941) and Schiff (1942), followed by much more realistic estimates by Boersch (1947), who showed that a microscope of 3 Å resolution should be capable of visualizing atoms of low atomic number, $Z \gtrsim 3$ by phase contrast bright field and $Z \gtrsim 13$ by dark field microscopy. Soon after, Scherzer (1949) described a practical and elegant method to achieve almost perfect phase contrast by properly balancing

defocus against the inherent spherical aberration of electron lenses. These early theoretical predictions were not immediately followed by experimental verification, simply because electron microscopes were not yet able to achieve atomic resolution ($\lesssim$5 Å). Even when atomic resolution became possible, atoms were not immediately visualized. M. Beer, one of the earliest and most dedicated proponents of the use of specific heavy atom staining of biological molecules, concluded that even two gold atoms were barely detectable by phase-contrast microscopy (Highton and Beer, 1968).

Meanwhile, Müller (1957) had shown conclusively that his field ion microscope (FIM) could clearly image single atoms placed on the end of a very sharp tungsten tip. Although this technique has been of tremendous value to the study of atomic adsorption, desorption, and diffusion, there are few indications that the FIM will ever be useful to biology. The present difficulties in FIM imaging of biological molecules are field denaturation, field desorption, and the fact that imaging seems to involve an uncertain combination of many physical and chemical, bulk and surface properties of the specimen (Machlin *et al.*, 1974).

The promise of atomic electron microscopy was finally realized when Crewe *et al.* (1970) reported the first clear images of uranium and thorium atoms, obtained using a scanning transmission electron microscope (STEM) developed by Crewe and Wall (Crewe *et al.*, 1968; Crewe, 1970; Crewe and Wall, 1970). Operated in the dark field mode proposed by its inventor, von Ardenne (1938), the STEM was able to produce high contrast, low noise images by use of very thin, clean carbon support films and by use of the inelastically scattered electrons to reduce the carbon film noise. Soon after, single atom imaging in the conventional transmission electron microscope (CTEM) was also reported (Formanek *et al.*, 1971; Henkelman and Ottensmeyer, 1971; Hashimoto *et al.*, 1971).

This chapter reviews the present state-of-the-art of single atom microscopy and outlines those aspects the author believes will be of importance in the future. It is hoped that it will lead to a better understanding of the theoretical and experimental basis of atomic imaging and will direct interest toward the most important problems in the application of atomic microscopy to biological research.

Single-Atom Microscopes

A brief review of the geometries of several types of microscopes useful for imaging single atoms will make the calculations and experiments presented later more understandable. Schematic illustrations of these microscopes (Figs. 1.1 and 1.2) show the essential similarities and differences among the instruments.

The mainstay of biological microscopy is the bright field conventional transmission electron microscope (CTEM) (Fig. 1.1a). The specimen is illuminated by a nearly parallel beam of coherent electrons, with a small convergence semiangle β_c.

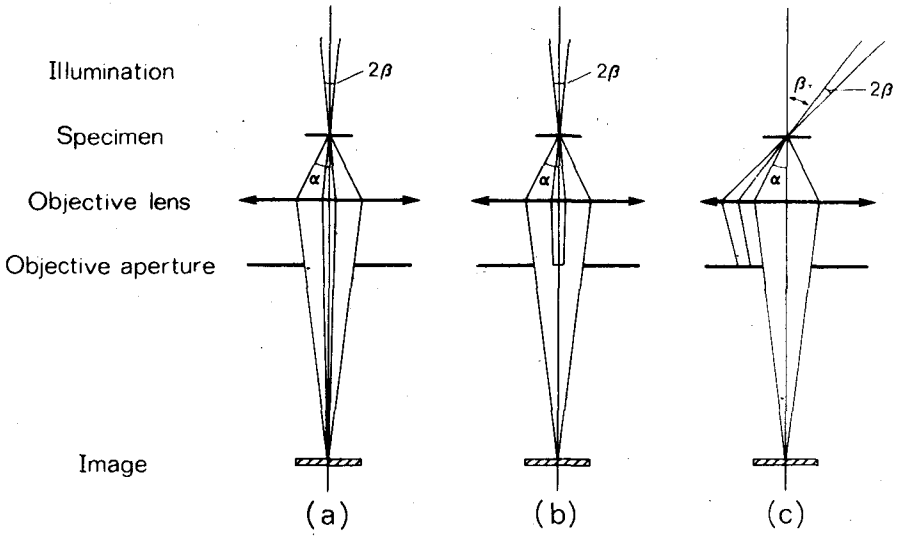

Fig. 1.1 Schematic diagrams of the conventional transmission electron microscopes: (a) bright field, (b) beam stop dark field, and (c) tilted illumination dark field. Angle α is the objective aperture angle and β_C is the convergence angle of illumination.

A magnetic lens is used to image the transmitted electrons that pass through the objective aperture of semiangle α, consisting of the unscattered electrons, most of the inelastically scattered electrons, and ~20% of the elastically scattered electrons. Phase contrast is produced by interference of the unscattered with the elastically scattered electrons. Scattering contrast is formed simultaneously, by stopping most of the elastically scattered electrons at the objective aperture; but is generally much weaker than phase contrast and is therefore ignored. The inelastically scattered electrons are not useful for forming single-atom images because (a) few electrons are inelastically scattered by heavy atoms, (b) inelastic events are not highly localized (Isaacson *et al.*, 1974b), and (c) chromatic aberration prevents the inelastically scattered electrons from being correctly focused (e.g. Crick and Misell, 1971).

To reduce the background caused by the noninformative, unscattered and inelastically scattered electrons, a physical beam stop can be placed at the objective aperture plane, as shown in Fig. 1.1b. This configuration is called a beam stop dark field CTEM. The contrast in this geometry is very high, since the background due to the unscattered electrons is not present.

Dark field can also be achieved by tilting the axis of illumination, by the angle β_T, so that the unscattered beam is stopped by the objective aperture (Fig. 1.1c). This seems to be the most practical dark field geometry for the CTEM, since the unscattered beam can be placed far from the edge of the aper-