Book Review

Practical Electron Microscopy for Biologists.

Second edition, Geoffrey A. Meek. John Wiley and Sons, Inc., London, 1976, 528 p., \$34.

In the preface to the first edition of this book, the author stated, "There are several good textbooks dealing with biological techniques in electron microscopy, and several dealing with electron optics and the design of electron microscopes. This book is intended to fill the gap between instrument design on the one hand and specimen preparation on the other." The author succeeds very well in attaining the general objective. In some of his specific aims, e.g., to enable the reader "to make the best use of an electron microscope," the author does superbly; in other aims, e.g., to enable the reader "to interpret electron micrographs," he falls short of his goals.

The book has 19 chapters organized into three parts: the electron microscope, using the electron microscope, and specimen preparation. There is also an "epilogue—the future of electron microscopy," an appendix devoted to the specifications of commercial electron microscopes, an appendix containing a list of general suppliers of materials and accessories for biological electron microscopy, a very respectable bibliography, and a useful index.

The quality of the writing is excellent. The author succeeds in making both interesting and clear even the most complex concepts of electron optics and the intricacies of microscope operation. He presents a very thorough compilation and analysis of the structure and function of each significant component of an electron microscope. In addition, he enumerates the essential features, mission, advantages, and disadvantages of the wide variety of instruments composing the burgeoning class of microscopes collectively called electron microscopes, but now consisting principally of the flood-beam transmission electron microscope (TEM), the flood-beam reflection electron microscope (TEM/R), the scanning transmission electron microscope (STEM), the scanning electron microscope (SEM), and the electron microprobe X-ray microanalyser (EXMA), but also including

electron emission microscopes. Although this is rather formidable fare for the typical biologist, the author's skill in presentation makes it surprisingly palatable. All of this is done with an astonishingly small number of typographical and minor errors, considering the technical complexity of the material.

One such error which really should be corrected in future printings or editions occurs on page 22 where the author states that "the maximum overall magnification which can usefully be employed is simply the ratio of the resolution of the microscope to the resolution of the eye." This is obviously inverted. (The author has stated the relationship properly on page 6.) Some improvement could be made in presenting this concept if the author told the reader that the maximum useful magnification which a microscope can provide is determined by a number of factors which ultimately define the resolving power available and that dividing that resolving power into the resolving power of the eye gives the maximum useful magnification which one should seek from his microscope image. It would be well to state at this time that the *necessary* magnification is determined by dividing the resolving power of the eye by the resolution required by the specimen and that there is no point in seeking a final magnification of 1,000,000 if one needs only to resolve 20 Å, for a magnification of 100,000 would suffice. This assumes, of course, that the microscope lenses are capable of resolving the 20 Å. In spite of this relatively minor aberration, chapter 1 presents the basic principles of optics in as lucid a manner as this reviewer has ever encountered.

Chapter 2 provides an excellent review of the early developmental history of the electron microscope. Chapter 3 classifies and clarifies the instrumentation and gives necessary attention to the alternate relationships between a specimen and impinging electrons; i.e., the specimen may transmit, reflect, absorb, or emit electrons or it may emit positive ions or electromagnetic radiation. Chapter 4 is a superb discussion of properties of magnetic electron lenses. In chapter 5, the author discusses the physical processes involved in image formation, but he

causes a bit of confusion by stating that a 500–600 Å thick section is capable of providing a resolution of about 15–25 Å, a statement in conflict with Cosslett's Rule (chapter 4), viz., that resolution is approximately equal to 0.1 times the section thickness. Biologists should not be confounded this way! Chapter 6 is a very fine presentation of the anatomy of the TEM. Chapter 7 is an excellent coverage of the vacuum system and its reasons for being. Chapter 8 covers electronic systems well, but probably in more detail than is necessary for biologists.

Chapter 9 merits special attention for its coverage of "commercial transmission electron microscopes." It presents some facts that should be known to all who aspire to control an electron microscope facility and especially to those who approve of the expenditure of the funds for these facilities. If the information provided in this chapter had been known and heeded by granting institutions, millions of dollars in support of unneeded instrumentation could have been saved. With inflating costs, the information is still most pertinent and justifies making this chapter required reading for all personnel involved in electron microscopy. Beyond the financial consideration, however, is the subtle message that almost unbelievable amounts of time can be expended in maintaining an instrument for which the research has no need. One of the most priceless quotes in the book occurs on page 200: "Once it has been accepted that extreme resolving power is seldom either possible or necessary, and that instruments capable of extreme resolution are sought more as a matter of laboratory prestige than as a necessity, the choice of an instrument becomes at once more logical."

Chapter 10 contains basic information concerning microscope installation which should be routinely handled by the installation engineer. It certainly is useful for the microscopist to be aware of the facts, however.

The seven chapters, comprising part II of the book, contain a wealth of information pertinent to the efficient operation of an electron microscope. They are well worth the reading time for any beginner, or old timer, in the field. Occasionally, one might object to a minor point in these chapters. For example, in reference to chapter 11, the reviewer, having operated an electron microscope for 25 years without ever having tilted an objective lens, cannot really

regard such an action as a "basic minimum adjustment."

Chapter 12 could well terrify and totally discourage any biologist contemplating venturing into the field of electron microscopy. For example, under "stable electronics," the author sets operating conditions for high-resolution operation which can only leave the biologist operator with the impression that aspirations for such operation are essentially impractical and impossible of attainment. Few biologists using the TEM could take the time or have the skill and experience to determine that (1) "the stabilities of the high tension voltage and lens currents are within the limits specified by the manufacturer, generally a few parts in a million," (2) "all reference batteries are fresh and in good condition," (3) "all valves are correctly aged and stable (not brand new and not more than 10% down on emission)," (4) "there are no dirty or intermittent contacts in any of the switches and potentiometers used to control lens currents and high tension." When one adds to these, and other, instrumental and specimen requirements the necessity that the operator be "in the right mood of almost limitless patience," be "undisturbed," and be "prepared to work far into the night," the situation becomes nearly hopeless, or one arrives at the conclusion that biological electron microscopists are truly a breed apart. (Such may be the case!) Not one electron microscopist in a thousand could hope to satisfy all these conditions. The reader might well conclude, at this point, that mere mortals should not attempt high-resolution operation and that instruments capable of such resolution belong only in the hands of a highly select and protected few. The situation is very nearly this rigorous! Little that is pertinent has been omitted from this chapter. However, in discussing the taking of a through-focus series, the author mentions the necessity for taking the series rapidly "to avoid circuit drift and contamination," but he omits pointing out that the effect of contamination is to move the image above focus.

Chapter 13 contains valuable practical information about the preparation and use of test specimens. Chapter 14 restates many basic points related to photographic techniques. Chapter 15 presents a number of generally known facts related to maintenance and fault-finding. This chapter is most valuable for its

advisory, "LEAVE WELL ALONE!" Chapter 16 is of relatively little value to the biologist for the cost and complexity of the high-voltage instrument can be justified for only a very few research workers. Chapter 17 also has little value for the biologist, but ultra-high vacuum physicists will undoubtedly find it useful. Were it not for the fact that there are so few errors in the book, the misuse of depth of focus for depth of field on page 403 would be really disturbing. The disturbance is ameliorated, however, by the recollection that the author clearly defined these terms in chapter 4.

Part III, comprised of chapters 18 and 19, was somewhat disappointing. This was partly due to the fact that almost no mention was made of the various buffers which serve as vehicles for the fixatives, that few biologists could accept the concept of nonbuffered fixatives, that acrolein and paraformaldehyde fixatives were omitted, that most biologists insist upon a buffer wash following postfixation in osmium tetroxide, that Spurr's epoxy embedding method was omitted, and that one is led to believe that a glass knife will give better sections, even initially, than the best diamond knife. (The reviewer would really like to have a few plates of that glass!) In spite of these deficiencies, the information contained in these two chapters is worth reviewing, especially that in chapter 19 on particulate material.

The epilogue is a source of some delight to the reviewer, who has devoted a significant proportion of the last quarter century to practicing and introducing other biologists to the practice of the art of electron microscopy, for it states that the "place of the TEM in future research involving the use of microscopes is as assured as that of the light microscope."

Appendix I provides specifications of several instruments in each of the three classes, i.e., basic, medium performance, and high performance.

Appendix II provides a list of the principal general suppliers of materials and accessories for biological electron microscopy. This is particularly valuable for the beginner.

The bibliography is generally excellent. One notable omission is the 1971 paperback by Dawes which might well have been included under "Elementary Books" for chapters 18 and 19. The index is quite useful.

Being somewhat of a sentimentalist, the reviewer would have liked to see the names of James Hillier, Cecil E. Hall, Robley W. Williams, Humberto Fernández-Morán, and Daniel C. Pease included with those of Palade, Porter, and Sjöstrand in the epilogue. Hillier, in particular, was nearly unique among these true pioneers and greats in this fascinating field, for he made significant early contributions in *both* electron optics *and* biological techniques.

As a final comment, it is as true in 1977 as it has been in the past 25 years that the very best way to learn to use the electron microscope as a research tool in biology is to affix oneself firmly to a practicing artist and to pay strict attention to everything he says and does, for, as the author states in his first-edition preface, "... there is no substitute for an apprenticeship spent in a top-class electron microscope laboratory." It can be a lot of fun, too!

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