Scanning Electron Microscopy and X-Ray Microanalysis

A Text for Biologists, Materials Scientists, and Geologists

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Preface

This book has evolved by processes of selection and expansion from its predecessor, *Practical Scanning Electron Microscopy* (PSEM), published by Plenum Press in 1975. The interaction of the authors with students at the Short Course on Scanning Electron Microscopy and X-Ray Microanalysis held annually at Lehigh University has helped greatly in developing this textbook. The material has been chosen to provide a student with a general introduction to the techniques of scanning electron microscopy and x-ray microanalysis suitable for application in such fields as biology, geology, solid state physics, and materials science. Following the format of PSEM, this book gives the student a basic knowledge of (1) the user-controlled functions of the electron optics of the scanning electron microscope and electron microprobe, (2) the characteristics of electron-beam–sample interactions, (3) image formation and interpretation, (4) x-ray spectrometry, and (5) quantitative x-ray microanalysis. Each of these topics has been updated and in most cases expanded over the material presented in PSEM in order to give the reader sufficient coverage to understand these topics and apply the information in the laboratory. Throughout the text, we have attempted to emphasize practical aspects of the techniques, describing those instrument parameters which the microscopist can and must manipulate to obtain optimum information from the specimen. Certain areas in particular have been expanded in response to their increasing importance in the SEM field. Thus energy-dispersive x-ray spectrometry, which has undergone a tremendous surge in growth, is treated in substantial detail. Moreover, we have come to realize the importance of developing careful procedures for qualitative x-ray microanalysis, that is, the identification of the elemental constituents present in a sample; suitable procedures for both energy-dispersive and wavelength-dispersive x-ray spectrometry are described.

The most conspicuous addition to the book is the material on biological specimen preparation and coating. Because of the great difficulties in properly preparing a biological sample for SEM examination and analysis, this topic has been considered in detail. It should be recognized that this material is of value not only to biologists, but also to many nonbiological disciplines in which fragile samples, often containing water or other fluids, must be prepared for the SEM. These include polymers, pigments, corrosion products, textiles, and many others.

For the convenience of readers who are confronted with a need for numerical information on important parameters for SEM and x-ray microanalysis calculations, we have included a data base of frequently used information, including x-ray energies of principal lines, mass absorption coefficients, backscattering factors, and others.

Some material from PSEM has been deleted in preparing this book. Some chapters, including "Contrast Mechanisms of Special Interest in Materials Science" and "Ion Microprobe Mass Analysis," have been removed. These topics and several others will be presented in a companion volume tentatively titled *Advanced Topics in Scanning Electron Microscopy and Microanalysis*, a specialist volume which is specifically intended for advanced workers who have completed an introductory course.

The authors wish to thank their many colleagues who have contributed to this volume by their kindness in allowing us to use material from their publications, by their criticism of PSEM and the present manuscript, and by their general support. One of the authors (J. I. Goldstein) wishes to acknowledge the research support and encouragement from the Planetary Materials Program of the National Aeronautics and Space Administration and from the Geochemistry Program of the Earth Sciences Division of the National Science Foundation. Special thanks go to Betty Fekete Zdinak and Louise Valkenburg of Lehigh for their extra efforts in the preparation of the original manuscript, to Carol Swyt of the National Institutes of Health for her constructive criticisms of many of the original chapters, to Roger Bolon and Mike Ciccarelli of General Electric, and Bob Myklebust and Harvey Yakowitz of the National Bureau of Standards, for specific contributions.

The Authors

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Introduction

In our rapidly expanding technology, the scientist is required to observe, analyze, and correctly explain phenomena occurring on a micrometer (μm) or submicrometer scale. The scanning electron microscope and electron microprobe are two powerful instruments which permit the observation and characterization of heterogeneous organic and inorganic materials and surfaces on such a local scale. In both instruments, the area to be examined, or the microvolume to be analyzed, is irradiated with a finely focused electron beam, which may be static or swept in a raster across the surface of the specimen. The types of signals produced when the electron beam impinges on a specimen surface include secondary electrons, backscattered electrons, Auger electrons, characteristic x-rays, and photons of various energies. These signals are obtained from specific emission volumes within the sample and can be used to examine many characteristics of the sample (composition, surface topography, crystallography, etc.).

In the scanning electron microscope (SEM), the signals of greatest interest are the secondary and backscattered electrons, since these vary as a result of differences in surface topography as the electron beam is swept across the specimen. The secondary electron emission is confined to a volume near the beam impact area, permitting images to be obtained at relatively high resolution. The three dimensional appearance of the images is due to the large depth of field of the scanning electron microscope as well as to the shadow relief effect of the secondary electron contrast. Other signals are available which prove similarly useful in many cases.

In the electron probe microanalyzer (EPMA), frequently referred to as the electron microprobe, the primary radiation of interest is the characteristic x-rays which are emitted as a result of the electron bombardment. The analysis of the characteristic x-radiation can yield both qualitative and quantitative compositional information from regions of a specimen as small as a few micrometers in diameter.

Historically, the scanning electron microscope and electron microprobe evolved as separate instruments. It is obvious on inspection, however, that these two instruments are quite similar but differ mainly in the way in which they are utilized. The development of each of these instruments (SEM and EPMA) and the differences and similarities of modern commercial instruments are discussed in this chapter.

1.1. Evolution of the Scanning Electron Microscope

The scanning electron microscope (SEM) is one of the most versatile instruments available for the examination and analysis of the microstructural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5 nm (50 Å) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5 nm (25 Å) (Broers, 1974b). The high-resolution micrograph, shown in Figure 1.1, was taken with an advanced commercial SEM under typical operating conditions.

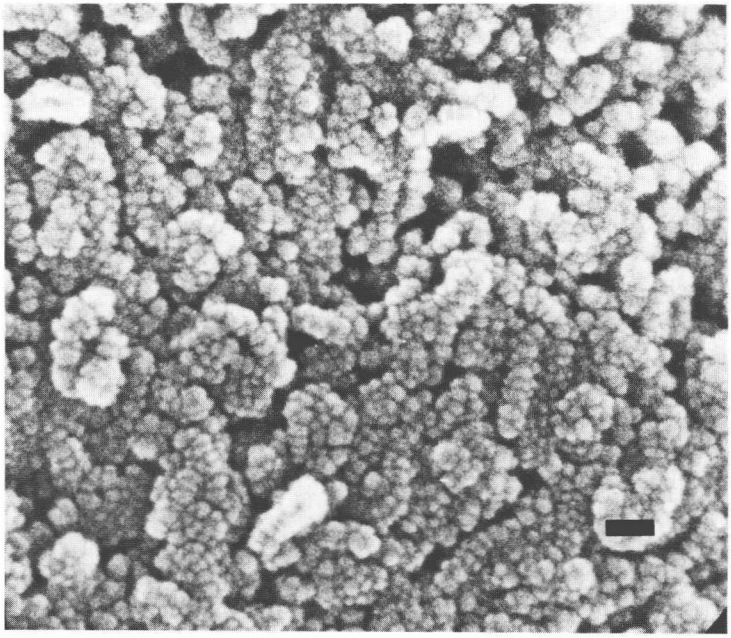


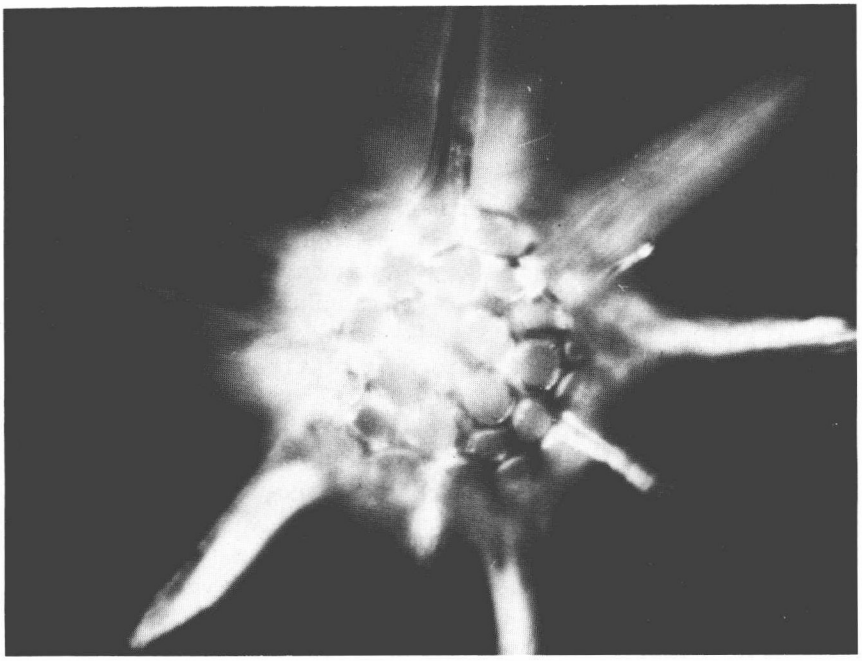
Figure 1.1. High-resolution micrograph of Au:Pd coating on magnetic tape. The image was taken with a JEOL 100CX equipped with a standard tungsten filament. Marker = 30 nm. (Micrograph courtesy of J. Geller, JEOL.)

Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field. Figure 1.2a shows the skeleton of a small marine organism (the radiolarian *Trochodiscus longispinus*) viewed optically, and Figure 1.2b as viewed with the SEM. The greater depth of field of the SEM provides much more information about the specimen. In fact, the SEM literature indicates that it is this feature which is of the most value to the SEM user. Most SEM micrographs have been produced with magnifications below 8000 diameters. At these magnifications the SEM is operating well within its resolution capabilities. Figure 1.3 is a micrograph of a pollen grain of *Ipomoea purpurea L.* (morning glory) and shows in one picture the complex surface topography of the wall of this single plant cell. The only other way to obtain this type of detailed information would be to painstakingly reconstruct the three-dimensional structure from planar serial sections observed in the transmission electron microscope. It would be difficult to make a faithful replica of such a detailed and irregular surface.

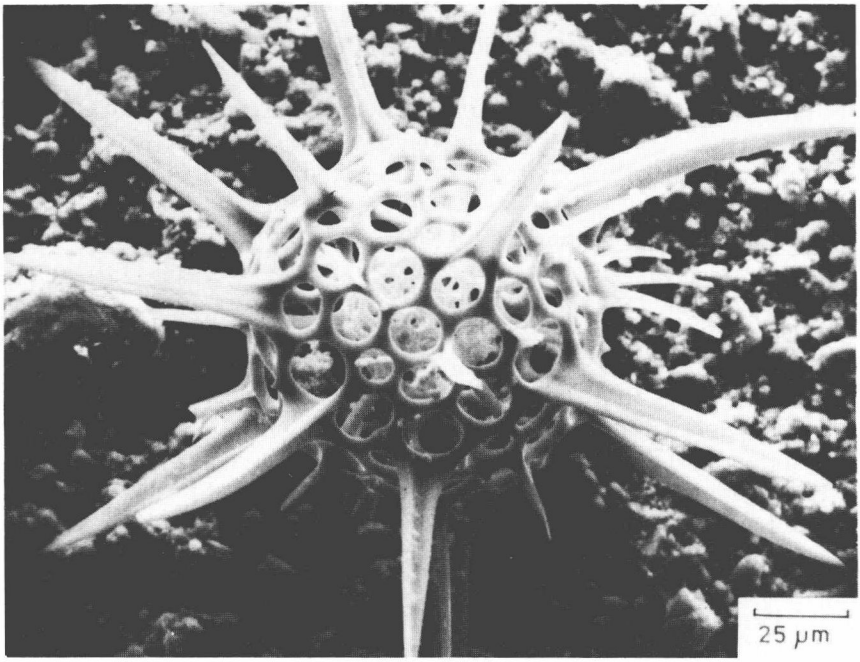
The SEM is also capable of examining objects at very low magnification. This feature is useful in forensic studies as well as other fields. An example of a low-magnification micrograph of an archeological subject is shown in Figure 1.4.

The basic components of the SEM are the lens system, electron gun, electron collector, visual and recording cathode ray tubes (CRTs), and the electronics associated with them. The first successful commercial packaging of these components (the Cambridge Scientific Instruments Mark I instrument) was offered in 1965. Considering the present popularity of the SEM, the fact that 23 years passed between the time Zworykin, Hillier, and Snyder (1942) published the basis for a modern SEM and this development seems incredible. The purpose of this brief historical introduction is to point out the pioneers of scanning electron microscopy and in the process trace the evolution of the instrument.

The earliest recognized work describing the construction of a scanning electron microscope is that of von Ardenne in 1938. In fact, von Ardenne added scan coils to a transmission electron microscope (TEM) and in so doing produced what amounts to the first scanning transmission electron microscope (STEM). Both the theoretical base and practical aspects of STEM were discussed in fairly complete detail. The first STEM micrograph was of a ZnO crystal imaged at an operating voltage of 23 kV, at a magnification of 8000 × and with a spatial resolution between 50 and 100 nm. The photograph contained 400 × 400 scan lines and took 20 min to record (von Ardenne, 1938a, b) because the film was mechanically scanned in synchronism with the beam. The instrument had two electrostatic condenser lenses, with the scan coils being placed between the lenses. The instrument possessed a CRT but it was not used to photograph the image (von Ardenne, 1938a, b).



(a)



(b)

Figure 1.2. (a) Optical micrograph of the radiolarian *Trochodiscus longispinus*. (b) SEM micrograph of same radiolarian shown in (a). The depth of focus and superior resolving capability in this micrograph are apparent.