METHODS IN MICROBIOLOGY

VOLUME 8

METHODS in MICROBIOLOGY

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PREFACE

With the publication of Volume 7 the initial Series of "Methods in Microbiology" was completed. As work on the Series progressed, we were encouraged by receiving several suggestions for further topics and offers of contributions over and above the areas covered in the planned Volumes, and we decided to put some of these together to form a single continuation Volume.

Inevitably the topics are diverse and there is no connecting theme for Volume 8. Nevertheless, we have continued with our policy of treating subjects which are not adequately detailed elsewhere in the literature, or which represent technical developments at the advancing forefront of microbiology—attractive by virtue of their evident potential rather than by their established value in the armoury of the research worker.

As with earlier Volumes, we have allowed individual contributors largely to determine the nature of their presentations. In some areas it is clearly appropriate and valuable to present detailed operating instructions; in others a more general orientation with adequate references to technical methods is more useful. As with earlier Volumes we are grateful for the friendly co-operation we have received from authors during the preparation of Volume 8. We have no firm plans for continuing the Series but will re-assess the situation periodically to see whether advances in technique and methodology suggest that the production of a further Volume would be useful.

J. R. NORRIS

D. W. RIBBONS

March, 1973

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CHAPTER I

Scanning Electron Microscopy

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I. INTRODUCTION

The concept of scanning electron microscopy dates back to that of conventional transmission electron microscopy. The initial patent application for a scanning electron microscope was made in 1927 (Stintzing, 1929),

but the first instrument was not built until 1938 (Von Ardenne, 1938). Shortly thereafter though, several instruments were constructed for experimental use. Only a few scanning electron micrographs appeared in the literature (Zworykin *et al.*, 1942). During the middle to late forties, emphasis was placed on instrumental development; and as a result of the concerted efforts of Oatley *et al.* (1965) in England, the first commercial instrument was produced. Now there is available a variety of commercial designs.

The scanning electron microscope has several features that render it advantageous for various kinds of microscopic observation. Information can be obtained from different electron beam-induced signals that include (1) secondary electrons, (2) back-scattered electrons, (3) X-rays, (4) visible light or infrared energy, and (5) currents from semiconductors. Thus, it is possible to gather information on the chemical composition and electrical properties of biological materials as well as interaction of such materials with specific stains. The scanning electron microscope affords image production comparable to light, ultraviolet, fluorescence, and X-ray microscopes. Furthermore, it has great depth of focus and produces images with three-dimensional quality. Specimen preparation usually is reduced to a minimum and, sometimes, requires no chemical fixation or physical pretreatment. Consequently, biological material can be viewed directly as it actually is, only magnified.

This Chapter is intended to provide a better insight into the usefulness of the scanning electron microscope, to summarize the principles of scanning electron microscopy; to describe briefly the methods for specimen preparation; and to outline some applications in microbiology. For a comprehensive treatise on the theory and principles of scanning electron microscopy, the reader is referred to the text by Thornton (1968). Other theoretical considerations of microscopy were presented earlier (see Quesnel, this Series, Volume 5A) as were methods of specimen preparation for electron microscopy (see Greenhalgh and Evans, this Series, Volume 4).

II. THE SCANNING ELECTRON MICROSCOPE

A. Imaging system

A fundamental and unique characteristic of the scanning electron microscope is its imaging system. Fig. 1 compares the imaging system of a scanning microscope to those of optical and transmission electron microscopes. In a light microscope (Fig. 1a), the light beam passes through a transparent specimen, and the resulting image is magnified by glass lenses. An analogous situation exists in a transmission electron microscope

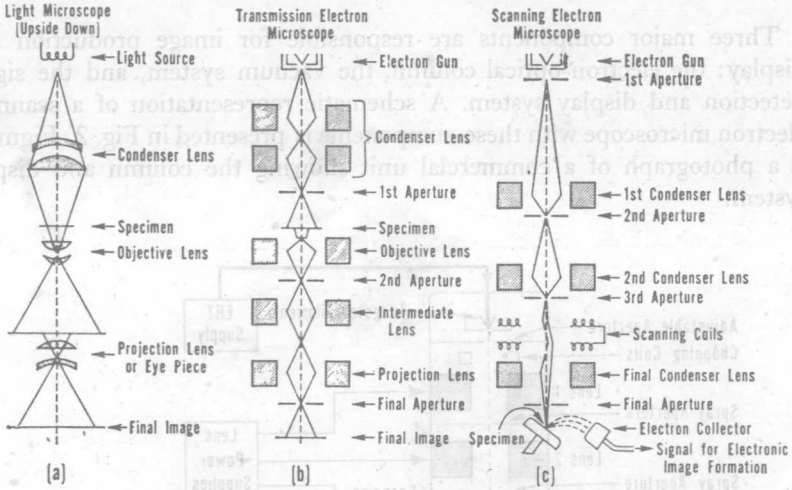

Fig. 1. Diagrammatic comparison of imaging systems in (a) optical microscope, (b) transmission electron microscope, and (c) scanning electron microscope.

(Fig. 1b) where the electrons likewise pass through the specimen and are focused by electron lenses to form an enlarged image on a fluorescent screen or a photographic plate. The image produced by light optics contains an abundance of information. Such aspects as chemical composition, localized interaction of biochemical stains, and general specimen shape can be determined. Of course, the major limitation of a light microscope is its low resolving power. In contrast, electron optics of a conventional transmission electron microscope provide high-resolution images. An inherent limitation of this system, however, is a restricted amount of image information content.

The scanning electron microscope has a quite different imaging system (Fig. 1c) that depends upon electron beam radiation for localization and upon visible light energy for information gathering. Electrons are formed into a fine probe as a result of demagnification by condenser lenses. The probe is moved over the surface of a specimen in a rectangular or zigzag pattern by two pairs of scanning coils powered by a generator. At the same time, the generator current passes through scanning coils of a cathode ray tube to produce a magnified raster identical to that on the specimen. Electrons emitted from the specimen are collected and the resulting current is amplified for regulating brightness of the cathode ray tube. In this way, an enlarged picture of the specimen is viewed on the face of the cathode ray tube with point-by-point correspondence. Such a system provides both high-resolution images and a great deal of information about the image.

Three major components are responsible for image production and display: the electron-optical column, the vacuum system, and the signal detection and display system. A schematic representation of a scanning electron microscope with these components is presented in Fig. 2. Figure 3 is a photograph of a commercial unit showing the column and display system.

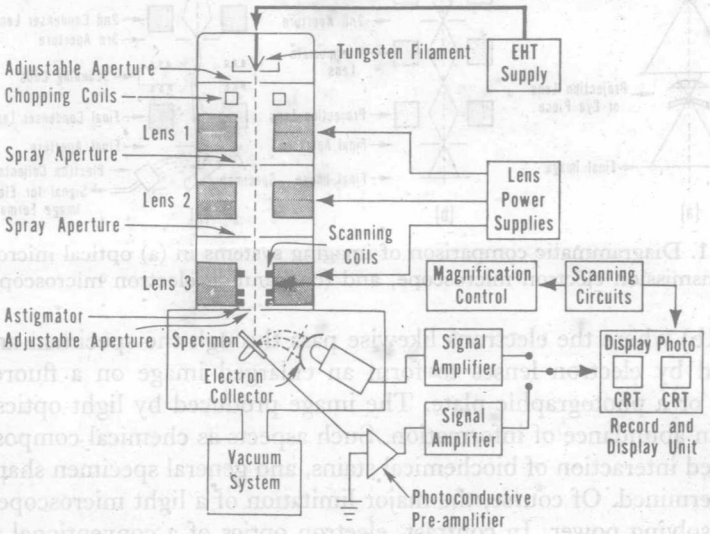

Fig. 2. Schematic diagram of a scanning electron microscope. CRT, cathode ray tube; EHT, electron gun-accelerating voltage.

B. Electron-optical column

The electron-optical system (see Fig. 2) is composed of an electron gun, two to four electron lenses, a set of apertures, an astigmator, and a set of beam modulating coils (chopping coils). Usually the electron gun is of triode design (Fig. 4) and produces a crossover of high-current density (Thornton, 1968). It contains a cathode consisting of a tungsten hairpin filament housed in a cylindrical shield with a circular aperture of about 2-mm diameter, and an annular cylindrical anode with a coaxial aperture. Electrons are boiled off the tungsten filament, heated by a high-voltage (EHT or electron gun-accelerating voltage) source, and passed through an aperture in the cylindrical shield that is negatively biased. The shield aids in controlling the electron source by forming a crossover point, or disc of least confusion, through which all electrons pass. By adjusting the bias voltage, the position of the crossover point can be regulated. The anode,

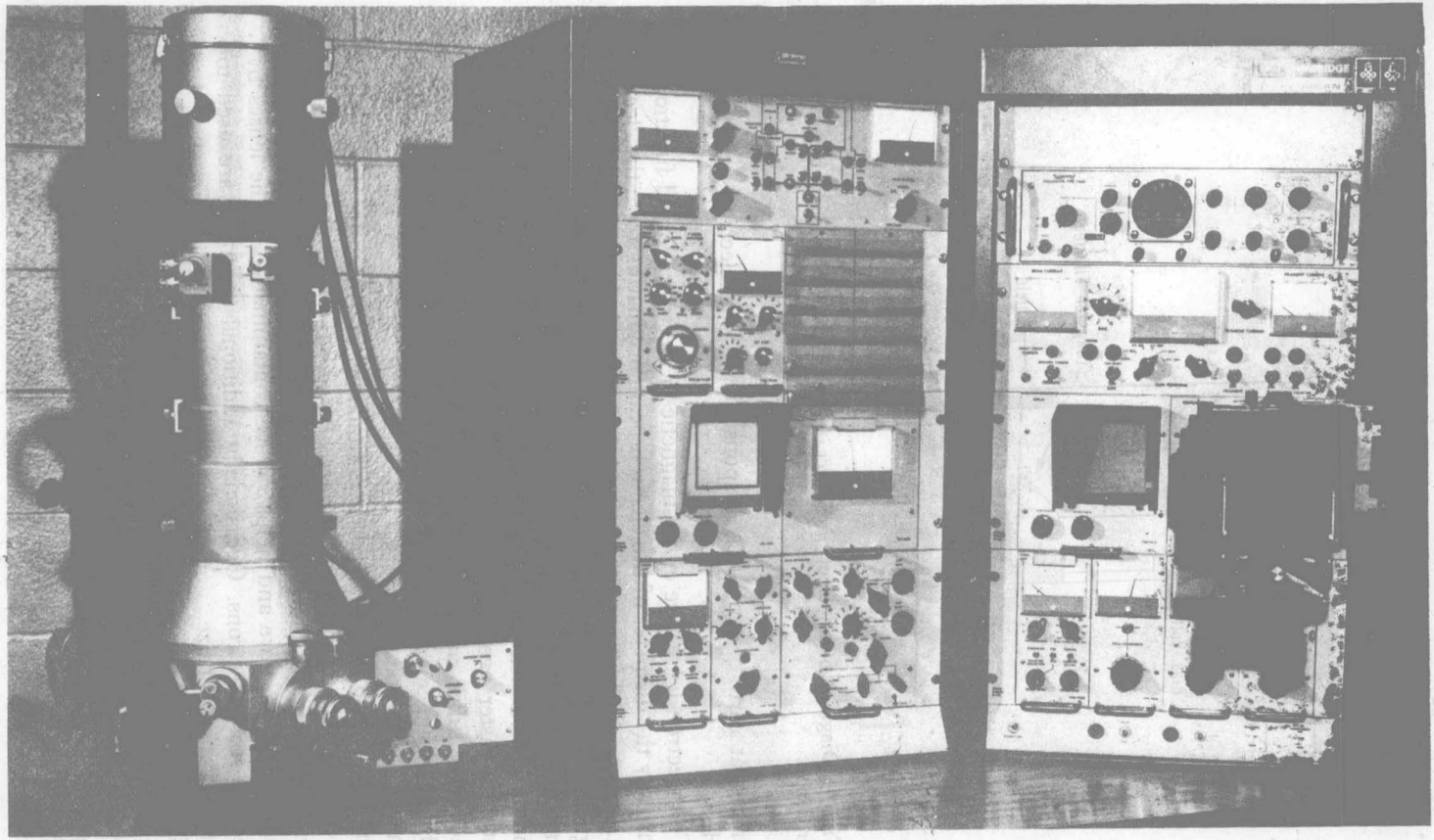

Fig. 3. Photograph of a "Stereoscan" scanning electron microscope (Mark 2A, Cambridge Scientific Instruments Ltd., Cambridge, England).