SCANNING ELECTRON MICROSCOPY: A REVIEW AND REPORT OF RESEARCH IN WOOD SCIENCE[1]

Bernard M. Collett
Forest Products Laboratory, University of California, Richmond 94804

ABSTRACT

Scanning electron microscopy is discussed in light of its principles, advantages, and applications. Comparisons of this system are made with the light microscopic and transmission electron systems. A cross section of pertinent literature on the scanning electron microscope, its development and use, has been integrated into the initial sections to provide a reference base for this general field. A detailed literature view on the use of this system in the field of wood science has also been included.

The result of the author's research on wood through use of the scanning electron microscope is reported. Effect of techniques used to prepare specimens for viewing by this method and the effect of the environment inside the microscope itself were determined. A means for preserving original green structure of wood was determined by studying the bordered pit structure in redwood. Finally, ⅜-inch plywood was used in exploring means for improving image contrast at the wood-adhesive interface. Use of much reduced incident electron-beam voltage on uncoated specimens showed promise as a means of studying distribution patterns in wood containing materials of different conductivity.

INTRODUCTION

Although first developed in the early 1930's and perfected to a high degree in the late 1950's, the scanning electron microscope and scanning beam equipment based on its principle have been slow to find their proper fields of application. Perhaps the great impact of transmission electron microscopy in almost every field of research was a main factor in this. The void between the transmission electron microscope and the light microscope, plus the limitations and disadvantages of each, apparently had to be more fully appreciated before scanning electron microscopy could find its proper place.

In 1965 the scanning electron microscope became commercially available, and since then there has been a great spurt in use of this equipment as a research tool. The utility of the scanning electron beam principle is rapidly increasing in microscopy, and many other applications of its versatility are being exploited.

This paper discusses fundamentals and principles of the scanning electron microscope and reviews the literature concerning use of electron microscopy in wood science. Research carried out by the author through use of the scanning electron microscope is also discussed.

FUNDAMENTALS AND PRINCIPLES OF OPERATION

Development of scanning electron microscopy

Scanning electron microscopy (SEM) had its beginning in the development of the conventional transmission electron microscope (TEM) by German physicists of the early 1900's (Mulvey 1967). Although it was not first applied to microscopes (Knoll 1935), the soundness of the scanning electron beam principle was established and its separate development as a microscopic system continued in the 1930's (Von Ardenne 1938). The war interrupted German development of SEM, and research shifted to the United States in the early war years (Zworykin, Hillier, and Snyder 1942). Nixon (1969) recently reviewed in detail this early period of development in SEM.

[1] Acknowledgment is made to Dr. T. E. Everhart, College of Engineering, Electronics Research Laboratory, University of California, Berkeley, under whose direction the Cambridge Stereoscan Mark II Scanning Electron Microscope is operated. This equipment was purchased under Grant No. GB-6428 from the National Science Foundation, and is operated under Grant No. GM15536 from the National Institute of Health.

The most significant period in SEM developmental research began at the University of Cambridge in 1948, and the first really efficient and reliable microscope was produced in 1952 as a result of these efforts (McMullan 1952, 1953). Refinement and further development of the system took place almost exclusively at Cambridge over the ensuing few years (Smith 1956; Wells 1957; Everhart 1958). The contributions of this research and development effort have also been reviewed by Nixon (1968).

As a result of the Cambridge work, the Cambridge Instrument Company began producing commercial scanning election microscope systems in 1965, and more recently two Japanese firms have marketed SEM systems (Kimoto 1967; Fujiyasu, Hara, and Tamura 1968).

Some fundamental considerations

Resolution is a term basic to all microscopy. It is the point at which two objects lose their separate identities and at which it is impossible to be confident that one is observing two adjacent objects in the microscope (Jensen and Park 1967). Resolution has a "theoretical" and a "practical" limit, depending on the particular imaging system.

The theoretical limit is defined by wave theory and depends on the wavelength of the electromagnetic radiation used to make the observation. This theory predicts that diffraction (bending of waves) occurs when the size of the object viewed is about the same as the wavelength of the radiation used, and in the limit of resolution, this is about one-half wavelength (Hay and Sandberg 1967). In a light imaging system, the predominant radiation is blue light at about 4500 Å (1Å = 10^{-8} cm). Wave theory thus predicts resolution limit of a light microscope to be around 2000 to 2500 Å. Electrons are the form of radiation used in electron imaging systems. Considering electrons as wave phenomena, the wavelength depends on their energy and this in turn depends on the accelerating voltage driving the particle. The higher this voltage, the shorter the wavelength. For example, a 100 keV (kiloelectron volt) beam-energy generates a wavelength of about 0.050 Å, and so the limit of resolution would be in the range of .025 Å (Hay and Sandberg 1967; Pease 1968).

The practical resolution of a system is determined by four factors that reduce the efficiency of any imaging system. These are diffraction, chromatic aberration, spherical aberration, and astigmation (Wischnitzer 1962). Diffraction is the principal offender in light imaging systems, and since it is bending of waves that determines the theoretical limit, the light microscope actually resolves at a level near its theoretical limit. Practically, resolution in the range of 2500 to 3000 Å is possible, ultraviolet light being used to advantage for the greater resolutions (Hay and Sandberg 1967; Pease 1968). Spherical aberration causes the greatest problem in electron beams, and it occurs when the electromagnetic lenses in the imaging column pull with a greater force on electrons passing near the periphery of the beam than electrons in the center of the beam. Energy changes that cause wavelength variations result. As a result, the practical level of resolution in TEM is about 5 to 10 Å in transmission, and about 40 to 50 Å with replicated specimens (Hay and Sandberg 1967; Pease 1968; Ilvessalo-Pfaffli and Laamanen 1969). Although magnification capability of a system may be theoretically large, practical magnification ranges are limited by resolution capabilities of the system used.

Principle of the scanning electron microscope

In understanding the SEM microscopic system, it is advantageous to compare it with light and TEM microscopic systems. Figure 1 is a schematic illustration of the light and TEM systems, while Fig. 2 depicts the scanning microscope.

Figure 1 compares TEM and compound light microscopic systems. They are analogous systems if the light microscope is thought of as being rotated 180° as shown in the figure. A cathode, which is the source of electrons, corresponds to the lamp of the light system and is usually a tungsten wire

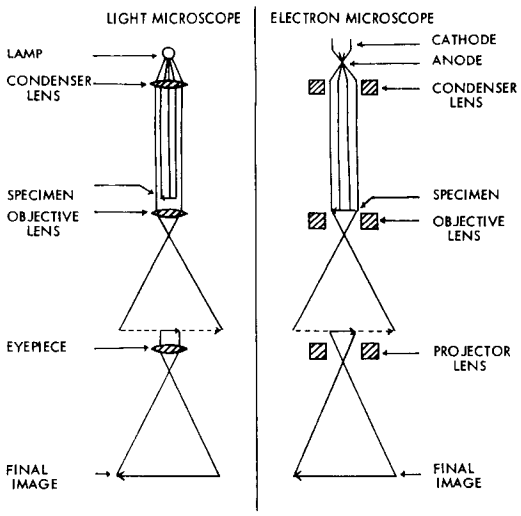


FIG. 1. Comparison between components of the light microscope and the transmission electron microscope (from Jensen and Park 1967).

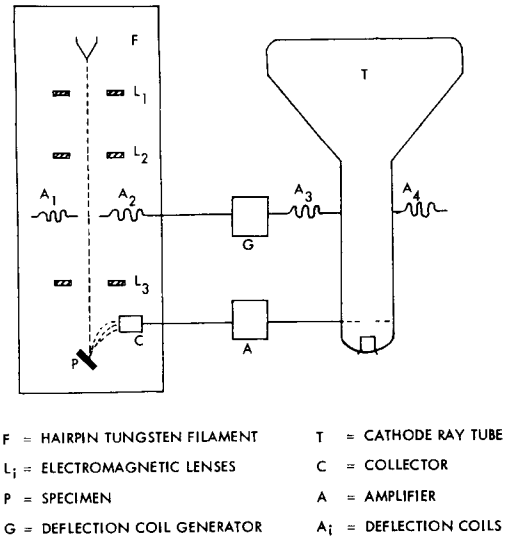


FIG. 2. Components of the scanning electron microscope (from Oatley 1966).

filament. The potential in TEM at which the cathode is held with respect to anode ranges from 50,000 to 100,000 keV. Voltages much below 50,000 keV are not suitable for TEM, because their penetration powers are insufficient. Accelerated electrons enter the electron optical system beyond the anode and are focused by the electromagnetic lenses. The image results from electron penetration of the specimen, and contrast is a function of the absorption and scattering of these transmitted electrons. The transmitted electrons impinge upon and excite to various degrees a phosphorescent screen, thus producing image buildup (Hall 1966; Kay 1965).

TEM requires extremely thin specimens for penetration and transmission of electrons (less than 500 Å for good imaging). This in effect renders such specimens two-dimensional, and the advantage of the large depth of field capabilities of TEM is lost in direct observation. Only through replica techniques can depth of field in TEM be used fully. Replication allows surface study, but all of the several methods available are tedious, time-consuming, and require special training (Liese and Côté 1960; Côté, Koran, and Day 1964; Fengel 1967).

The scanning beam system depicted in Fig. 2 differs considerably from both light and TEM. The first difference is that accelerating voltages are lower, varying from 1000 to 50,000 keV (generally, operation is around 20 keV). The second is that the specimen is located beyond the electromagnetic lenses. These lenses focus the electron beam to a minute spot on the surface of a solid specimen (the term "scanning" derives from the fact that this electron spot, or point source of radiation is made to sweep over the specimen surface by the deflections coils). The spot movement is at a well-defined velocity and in a well-defined pattern of lines (termed the raster). Line by line this rectangular raster is swept out, each line being built up of a large number of picture elements, each one of which is the size of the electron-spot diameter (Thornton 1968; Oatley, Nixon and Pease 1965).

The mechanism of resolution and imaging in SEM involves those items depicted on the right side of Fig. 2. In a cathode-ray tube (CRT), a second electron spot is generated and caused to scan the fluorescent screen of the tube with a synchronized pattern of lines. This synchronization in

TABLE 1. *Performance capabilities of three imaging systems*

Performance factor	Imaging system		
	Light	SEM	TEM
Useful magnification	10 × – 2500 ×	20 × – 50,000 ×	500 × – 500,000 ×
Practical resolution	2000–2500 Å	100–300 Å	5 – 10 Å[1]
Depth-of-field:			
50 ×	20 μ	≃ 1 cm	——
5000 ×	——	100 μ	80 μ

[1] With replicated specimens = 40 Å.

scanning is achieved through the twin-deflection coil arrangement between the CRT and the electron gun column. The synchronous twin-beam system localizes at any given moment a known spot on the specimen surface with a known spot on the screen of the CRT imaging tube. Thus there is a one-to-one correspondence due to this time-sequencing of object-image points. This is termed "localization" (Hayes and Pease 1968) in SEM and it is equivalent to resolution. Once a known spot of the specimen has been localized on the image screen, the physical size of that spot determines the resolution of the system (Hayes and Pease 1968; Oatley 1966), and therefore resolution in SEM depends on how small a spot the electron beam can be focused to, on the surface of the specimen. Detail less than this is not possible to resolve. The problem of making a small spot has occasioned much investigation and resultant achievement (Zworykin, Hillier, and Snyder 1942; McMullan 1953; Smith 1960). Pease and Nixon (1965) achieved a 50 Å diameter spot on their scope and state that this agreed experimentally with the theoretical resolution of SEM using conventional CRT's with tungsten hairpin filaments. Future equipment may have a 5 to 10 Å limit, however, if recent work using field-emission cathodes proves practical (Crew, Wall, and Welter 1968; Crew 1969). As with TEM, however, SEM also has a practical level of resolution, which is between 100 Å and 300 Å (Oatley, Nixon and Pease 1965; Hayes and Pease 1968). The greatest loss in resolution is due to secondary electrons arising beneath the specimen surface, which cause noise interference and distortion (Everhart, Wells and Oatley 1959).

Magnification in SEM is simply the ratio of the linear dimension of the field scanned on the specimen surface to that of the CRT imaging screen (the ratio of the size of the two synchronous rasters) (Smith and Oatley 1955; Everhart et al. 1960). As in the other imaging systems, useful magnification is determined by resolution. In SEM, theoretical magnifications of 10^5 are possible, but in practice about 50,000 is the maximum (Oatley 1966).

Table 1 compares the three imaging systems discussed in terms of resolution, magnification, and depth of field capabilities (more will be said on depth of field below).

In light and TEM systems, information transfer involves focusing of energy (light, electrons) that has been transmitted through the specimen onto the imaging screen (the eye or a phosphorescent screen). That is to say, the same energy used to illuminate the specimen is transmitted and collected. In SEM, resolution and information transfer are achieved separately. A spot on the image screen is correlated to a known spot on the specimen surface (resolution). The impingement of this primary electron beam excites various kinds of radiation at the solid surface of the specimen. Any one of these kinds of radiation is then amplified and impressed upon the synchronous beam of the CRT, the spot of which is scanning the screen in correspondence with the movement of the primary electron beam over the specimen surface. This is information transfer. Figure 3 illustrates the various types of information that arise when a solid speci-

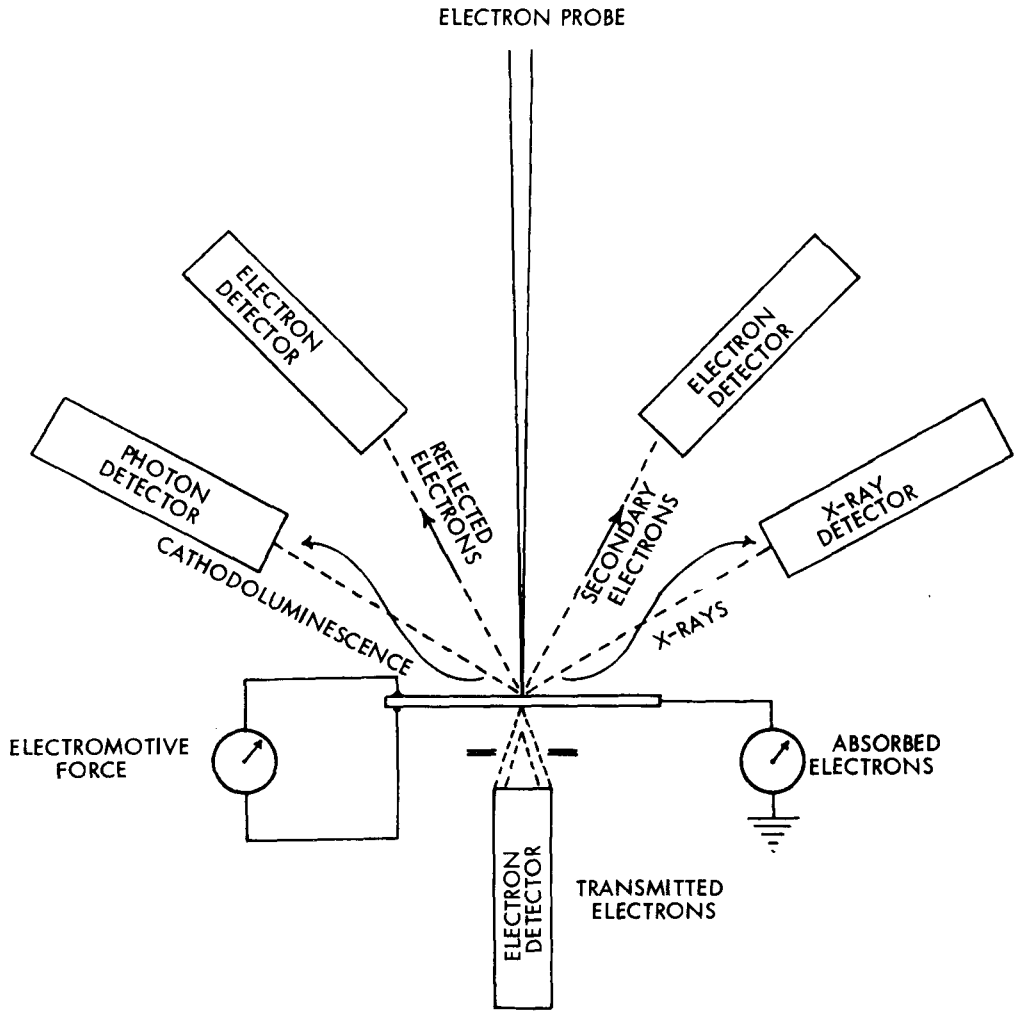


FIG. 3. Information retrievable at specimen surface because of bombardment by incident electron probe (from Kimoto 1967).

men surface is bombarded with electrons. Information can be impressed on the CRT beam by collecting and modulating absorbed electrons, backscattered electrons, secondary electrons, photons (cathodoluminescence) and electromotive force patterns. These are called "detection modes."

The above indicates the great versatility of the electron beam principle, its use as a microscope being only one application. (Other applications alluded to in Fig. 3 are discussed below.) However, it is the secondary electron detection mode that is of most importance for its greatest potential as a microscope. When secondary electrons are collected, amplified, and used to modulate the brightness of the CRT spot, a three-dimensional image of the object surface is built up.

The secondary-electron detection mode

SEM finds its greatest use as a microscope in the study of solid specimen surfaces. Because of its great depth of field capabilities, materials with rough topography are particularly well suited for SEM investigation. This capability is primarily due to the secondary electron detection

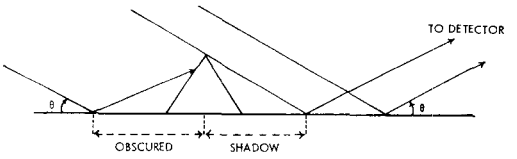


FIG. 4. Path of incident and reflected electrons in region of surface irregularity (from Atack and Smith 1956).

mode. The electrons leaving a surface because of impingement of the primary beam on that surface are one of two types: slow-moving, low-energy (less than 50 Ev) electrons called secondaries, or electrons with energies ranging from 50 Ev up to the energy of the primary beam (usually about 20 keV (McMullan 1953; Everhart, Wells and Oatley 1959; Moellenstedt and Lenz 1963). These latter are called reflected electrons. Because of their high energy, they travel straight paths from surface to detector, but because surface irregularities may block the path, a loss of detail may result in the image. Figure 4 shows how this effect leads to shadows and obscurities in the reflected electron image (Atack and Smith 1956). Thus, even though the reflected electron image may have high clarity, depth of field is lacking (Kimoto 1967).

Secondary electrons travel curved paths from surface to detector primarily because of attraction caused by the positive potential of the accelerating electrode, which attracts the slow-moving, low-energy particles. Placement of the detector to take advantage of this results in an illuminating effect in which secondaries are gathered from areas obscured by surface irregularities (Oatley, Nixon and Pease 1965; Oatley 1966; Everhart, Wells and Oatley 1959). From this phenomenon arises the great depth of field capabilities of SEM with secondary electron imaging. Secondaries arising from obscured areas are collected, and the information they carry is reproduced in the image buildup.

Ever since the scanning beam principle found wide use in microscopy, the secondary electron detection mode has been the object of much research. Everhart (Everhart 1958; Everhart, Wells and Oatley 1959; Everhart et al. 1960) has done much to improve the contrast mechanism in SEM and has shown upon what factors it depends. He found, for example, that secondary electron yield is most greatly influenced by variations between the angle of incidence of the primary beam and the local normal to the surface of the specimen, a factor highly dependent on surface topography (Everhart, Wells, and Oatley 1959). Because of this, most objects are tilted at an angle of 15 to 45° from the horizontal, but the resultant "foreshortening" in the image or photomicrograph is not serious from the standpoint of interpretation. The characteristics of this phenomenon and methods of dealing with it have been discussed by Eichen, Fitchmun, and Sefton (1969).

Two factors peculiar to secondary electron imaging are related to their action at the specimen surface. First, only secondaries arising at the specimen surface contribute to the image buildup; those arising below the surface (the maximum depth of penetration being only about 100 Å) impair resolution and alter contrast (Everhart, Wells, and Oatley 1959). Second, secondaries show little response to variation in electron density of the surface material (atomic weight differences) with respect to contrast formation (Oatley, Nixon, and Pease 1965). This is in contrast to higher energy reflected and transmitted electrons (Sternglass 1954).

Other detection modes

The versatility of the scanning beam principle can be underscored by noting some of its applications in other detection modes depicted in Fig. 3.

One of the first and most practical uses resulted from image buildup using the X-rays emitted from the specimen surface. This is termed "electron-probe microanalysis," and is used in studying elemental composition distributions (Crosslett and Duncumb 1957; Crosslett 1966; Norville 1962; Macres et al. 1968).

Surface potential differences at low primary beam voltages, first studied as a

source of contrast formation (Oatley and Everhart 1957), have become a valuable feature of SEM in studying electromotive force distributions and patterns in semiconductors and integrated circuits (Oatley, Nixon, and Pease 1965; Oatley 1966; Kimoto 1967; Kimoto, Hashimoto, and Mase 1968; Everhart, Wells, and Oatley 1959; Everhart et al. 1960).

In the cathodoluminescent mode, light quanta (photons) excited at the specimen surface by the primary beam are collected and used to modulate the brightness of the CRT image (Smith 1956; Thornton 1968). The pattern of luminescence of a material, sometimes enhanced by selective luminescent dyes, is used to study surface composition and as a contrast mechanism (Pease and Hayes 1966).

Finally, one of the most interesting aspects of developmental work is the attempt to incorporate the capabilities of both transmission and scanning electron microscopes in one instrument (Cowley and Strojnik 1969).

Advantages and disadvantages of SEM

One of the great advantages of SEM lies in its extremely simplified specimen preparation techniques. The detailed methods of TEM in ultrathin specimen preparation and surface replication are eliminated because of direct observation of the solid surface of the object. If the material is inorganic and not subject to shrinkage due to moisture loss, all that is required is preparation of the surface and affixing to a mounting stub. The surface to be viewed may be microtomed, sliced, split, or fractured. If the material is hygroscopic and subject to structural change with moisture variation, special drying techniques of varying degrees of complexity may be needed, depending on the material's moisture sensitivity. This is due to one of the major disadvantages in all electron microscopy: electrons are highly absorbed by matter, including air, and therefore the specimen chamber of the microscope must have a high vacuum during viewing. The vacuum is usually in the range of 10^{-4} torr (one torr = 1 mm hg). The effects of such an environment on the structure and characteristics of the material must be understood, and so work has been done in various fields to determine proper handling methods of various materials. For example, Echlin (1968), in a study of a wide variety of materials, classified them on the basis of their sensitivity to moisture removal. Probably the most novel studies involving sensitive materials have been those done on the various life development stages of living insects (Pease and Hayes 1966; Pease et al. 1966; Sokoloff et al. 1967). There have also been some results published regarding solvent drying techniques (Merchant 1957; Oatley, Nixon, and Pease 1965; Echlin 1968), and freeze-drying methods (Oatley, Nixon, and Pease 1965; Thornley 1960).

Another fact that may or may not be a disadvantage in SEM is the requirement that nonconducting surfaces must be metallized. Primary beam electrons will, upon surface bombardment, build up a static charge if not conducted to ground. For nonconducting materials, the metal coating serves this function. Static change buildup on nonconducting surfaces causes brightness variation in the image, which impairs resolution, alters contrast, and masks areas of the surface taking on the charge. The coating process requires evaporation of the metal onto the specimen surface at a vacuum about the same as is required in the electron gun column (10^{-4} torr). Because this also exposes the material to a severe environment that may contribute to undesirable surface modifications, some work has been done to circumvent the process. Thornley (1960) tried reducing the primary beam voltage to below 6 keV, with the idea that the charge buildup rate would be reduced. The method proved to be satisfactory in avoiding charging artifacts, but a degree of resolution and clarity was lost. Sikorski et al. (1967) used a commercial airosol antistatic coating, which provided satisfactory conductance except at high resolutions.

A great advantage of SEM is the tremendous specimen size range that can be

selected. The maximum is around one cm^3. Such a surface can be rapidly scanned at low magnification and allows "zooming" in on areas of particular interest with no adjustment for magnification change. Such capabilities have allowed the recent tracing of nerve fibers from one cell to another for the first time (Lewis, Everhart, and Zeevi 1969). On the other end of the size spectrum, since secondary electrons arise within about the first 100 Å, high-resolution, three-dimensional images can be obtained from quite thin sections (McDonald and Hayes 1968, Echlin 1968).

In summary, the most important advantages of this microscopic system are: (1) rapid and simple specimen preparation; (2) access to study of large surface areas; (3) intermediate levels of resolution with respect to light and TEM systems; (4) great depth of field; (5) alternatives as the choice of information retrieval arising at the surface under the action of electron bombardment; (6) capability for specimen orientation changes during observation (i.e. rotation and tilt of specimen holder); and (7) availability of a large range of magnifications requiring little or no refocusing for large alterations.

SCANNING ELECTRON MICROSCOPY IN WOOD SCIENCE

The use of SEM in the study of wood and wood products began almost immediately after it became a practical tool and long before the system was marketed commercially. This was due partially to the ideal surface and structural makeup of wood that lends itself well to this type of investigation. Perhaps in a greater part, however, it was due to the interest in wood of K. C. A. Smith, one of the pioneer developers of the microscope (Smith 1956). With Atack (1956), he published results of the first application of SEM in this field in a study of groundwood pulp fiber. This was followed by a series of research efforts that investigated several aspects of pulp and paper (Smith 1959; Buchanan and Smith 1960).

The early research efforts initiated by Smith were carried further by several additional investigations over the ensuing four years (Buchanan and Washburn 1962; Buchanan and Lindsay 1962; Forgacs 1963; Buchanan and Washburn 1964). The main goal in these works was to exploit the capabilities of SEM in studying pulp fiber produced by different methods, with the intent of characterizing fiber morphology, modes of structural damage and other aspects of pulp technology. However, comparatively little pertaining to techniques in preparation and handling of wood for viewing in the SEM was included in these studies. The first work that took into account the hygroscopic nature of wood was done by Washburn and Buchanan (1964). By comparison of air-dried pulp fiber webs with specimens freeze-dried from a range of moisture levels, the degree of surface modifications was evaluated.

The above series of research investigations ended what might be termed the early era of SEM application in wood science. In 1965, commercial models of the microscope were marketed. Perhaps time was needed for researchers to find the proper areas for application of SEM, as it wasn't until 1968 that published work regarding use of this tool began to appear. Its most extensive use appears to have been in Germany, where the principle was developed 40 years earlier. Resch and Blaschke (1968) published the first example of the use of SEM as a tool in the study of wood anatomy, and this was followed by a brief note by Wagenfuhr and Zimmer (1968). Both of these articles appear to have been written primarily to demonstrate the capability of SEM in this area of research, as the micrographs gave no indication of special drying or preparation precautions.

After publication of the earlier studies on pulp and paper, it was not until 1969 that the first articles on use of SEM in research appeared. Wagenfuhr (1969) studied adhesive-wood interfaces of foil-overlaid particleboard and microroughness in decorative papers receiving various surface treatments. Both TEM and SEM photomicrographs were used in illustrating surface

TABLE 2. *Results of vacuum-evaporation treatment on wood moisture content*

Test set No.[1]	Moisture content		Time, min.		Temperature rise, °F	
	Initial	Final	To 10^{-4} torr	In coating	To 10^{-4} torr	In coating
1	26.3%	0.6%	60[2]	3	2.2°	12.2°[2]
2	13.6%	0.5%	80	3	4.5°	21.0°
3	9.8%	0.9%	40	—	—	—

[1] Average of 6 samples for each set.
[2] Differences in time and temperature between sets 1 and 2 are primarily due to running set 2 immediately following set 1, and reflect equipment inefficiencies.

characteristics of various materials. Findlay and Levy (1969) used SEM in cursory investigation of wood decay and further demonstrated its capability in wood anatomy with illustrations of small cubes of wood cut true to the three planes of orientation. Finally, in what is probably the most wide-ranging demonstration of surface topography capabilities of SEM, a Finnish publication (Ilvessalo-Pfaffli and Laamanen 1969) showed photomicrographs of various types of paper, crystals, metal and synthetic wire, and fabrics, as well as some excellent pictures of wood.

At the University of California Forest Products Laboratory, SEM has been used with striking results in the study of the bordered pit structure in white fir (Schlink 1969). This work attempted to explain the high permeability of white fir wetwood, and SEM was used to study the split radial surface of solvent-exchange dried specimens. In research still in progress at this laboratory, SEM has proved a valuable tool in characterizing and determining preservative distribution in wood (Resch and Arganbright 1968).

SOME INVESTIGATIONS ON WOOD USING SCANNING ELECTRON MICROSCOPY

SEM was used by the author to investigate: (a) the effect of specimen preparation on wood moisture content, (b) the means of preserving original wood structure for study by SEM, and (c) the adhesive distribution at the glue-wood interface of plywood, with emphasis on contrast improvement.

As pointed out earlier, the vacuum required in the vacuum-evaporator used in coating and in the electron gun column is about 10^{-4} torr. Wood, being a nonconductor at low moisture levels, must be coated for best results. Therefore, the vacuum-evaporator was used to determine what happens to wood moisture content during preparation. Because conditions in the evaporator are the same as those in the gun column, the effect in it should be the same as those in the column.

Specimens of ponderosa pine were prepared at near maximum size (about 1 cm^3). These were conditioned from the green to three levels of moisture (26.3%, 13.6%, and 9.8%), and were then weighed. The two higher moisture-level sample sets were evacuated to 10^{-4} torr, after which the evaporator was turned on to simulate the coating process. Samples were not actually coated, but time and temperature conditions were noted. The lowest moisture-level test set was evacuated to 10^{-4} torr, but no coating process was simulated. This would

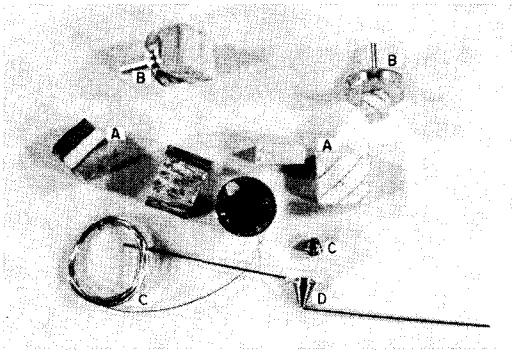


FIG. 5. Specimens (A), mounting stubs (B), coating wire (C) and tungsten filament (D), used in preparing samples for viewing in the scanning electron microscope.

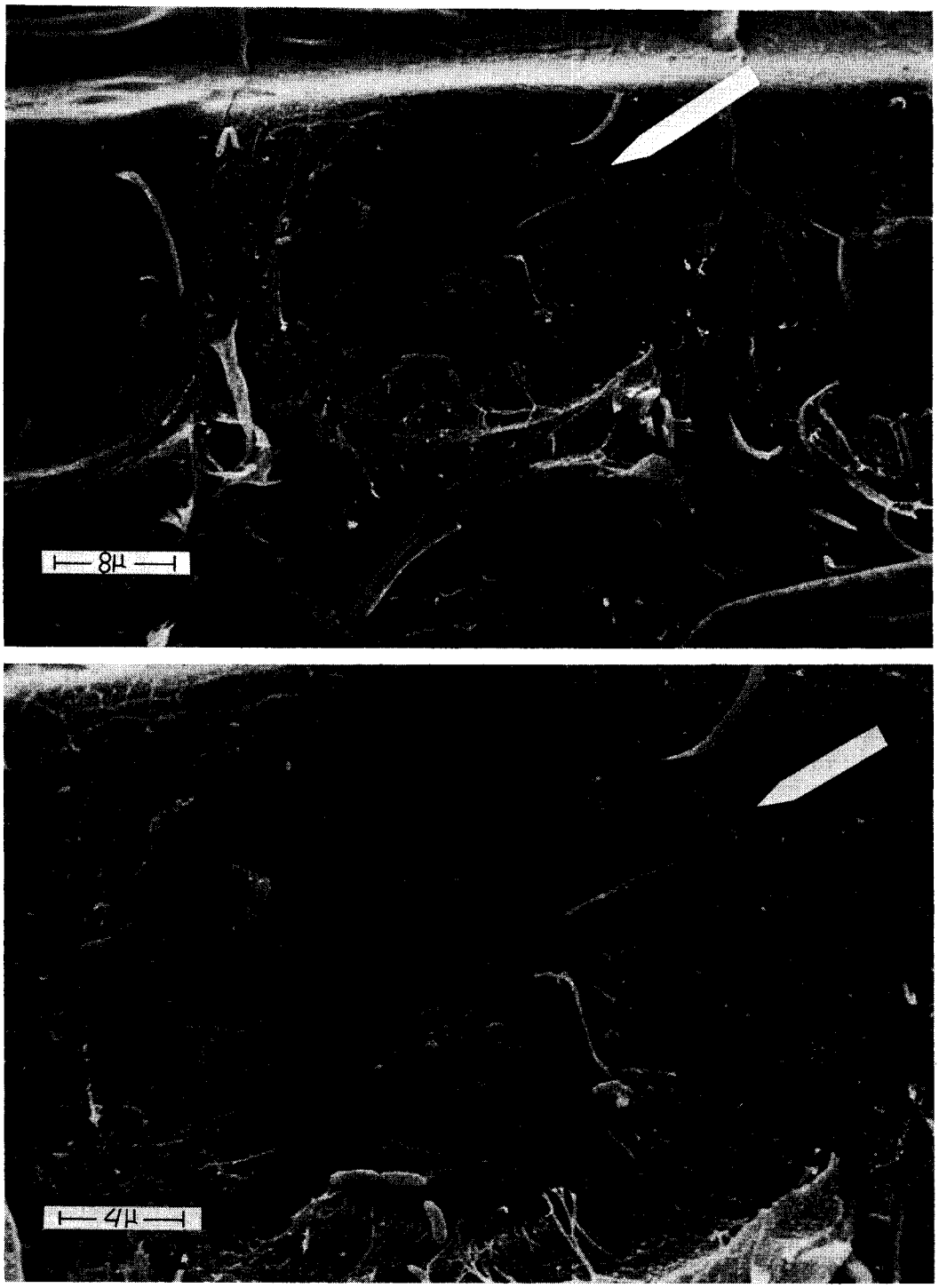


FIG. 6. Bordered pit structure of the split radial surface of redwood sapwood. Pit membranes are either missing or aspirated because no precautions were taken in preparing the specimens for viewing. (2250 ×, 4500 ×)

therefore simulate what would happen to a hygroscopic material in the electron gun column. Immediately following evacuation, samples were removed and weighed, and their moisture contents were calculated. Table 2 summarizes this work. The first two sets had final moisture levels of around 0.5%; this condition results from coating. The third test set had a final moisture level of 0.9%. As the evaporator was not used after 10^{-4} torr was attained, this is the condition samples would approximate if exposed directly to the specimen chamber of the electron gun column.

Thus, regardless of the initial moisture level, wood exposed to the environment of the SEM specimen chamber will end up having a moisture content of about 1%. If the wood is metallized prior to viewing, as is usually the case, moisture content will be even lower because of heat generated in evaporation of the coating metal.

Later, it was found that the coating process could be speeded up markedly by evacuating to around 10^{-3} torr. This is because the metal coating serves solely to make the surface conducting in SEM. In TEM, the coating procedure is termed "shadowcasting" because the metal source must be located at a definite angle to the specimen surface (the pattern of buildup ultimately giving the contrast observed from the replica). For best results, 10^{-4} torr or more is needed. Because contrast in SEM results primarily from variation in surface topography, and not by variation in the metal coating thickness, a lesser degree of evacuation is satisfactory and saves time. Gold or gold-palladium (60%–40%) coating wire gave the best results from the standpoint of ease and efficiency in evaporating. Figure 5 illustrates typical specimen size and mounting-stub design, and gives examples of the tungsten wire evaporator basket and coating wire used in SEM.

Table 2 suggests that any attempt to use SEM to study original structure of wood will require special drying precautions. A simple yet satisfactory method of accomplishing this was sought. It was decided that if the bordered pit structure could be maintained in its unaspirated, original condition, the technique used in achieving this would also insure against modification of wood structure and anatomy. Figure 6 shows the results of not taking precautions in drying. Pit structure is either completely lacking, or the torus structure is tightly aspirated. This is the type of result illustrated by photomicrographs in those publications discussed in the review section.

FIG. 7. Bordered pit structure of the split radial surface of redwood sapwood. Solvent-exchange technique used achieved poor results. Note bacteria on the pit torus. (2100 ×)

To determine a simple method that would do the job, several cursory solvent-exchange methods were tried. In general, the results were poor, the best example found being that shown in Fig. 7. Finally, the solvent-exchange method based on that used by Thomas (Thomas and Nicholas 1966; Thomas 1969) in TEM investigations was used. This involved four steps:

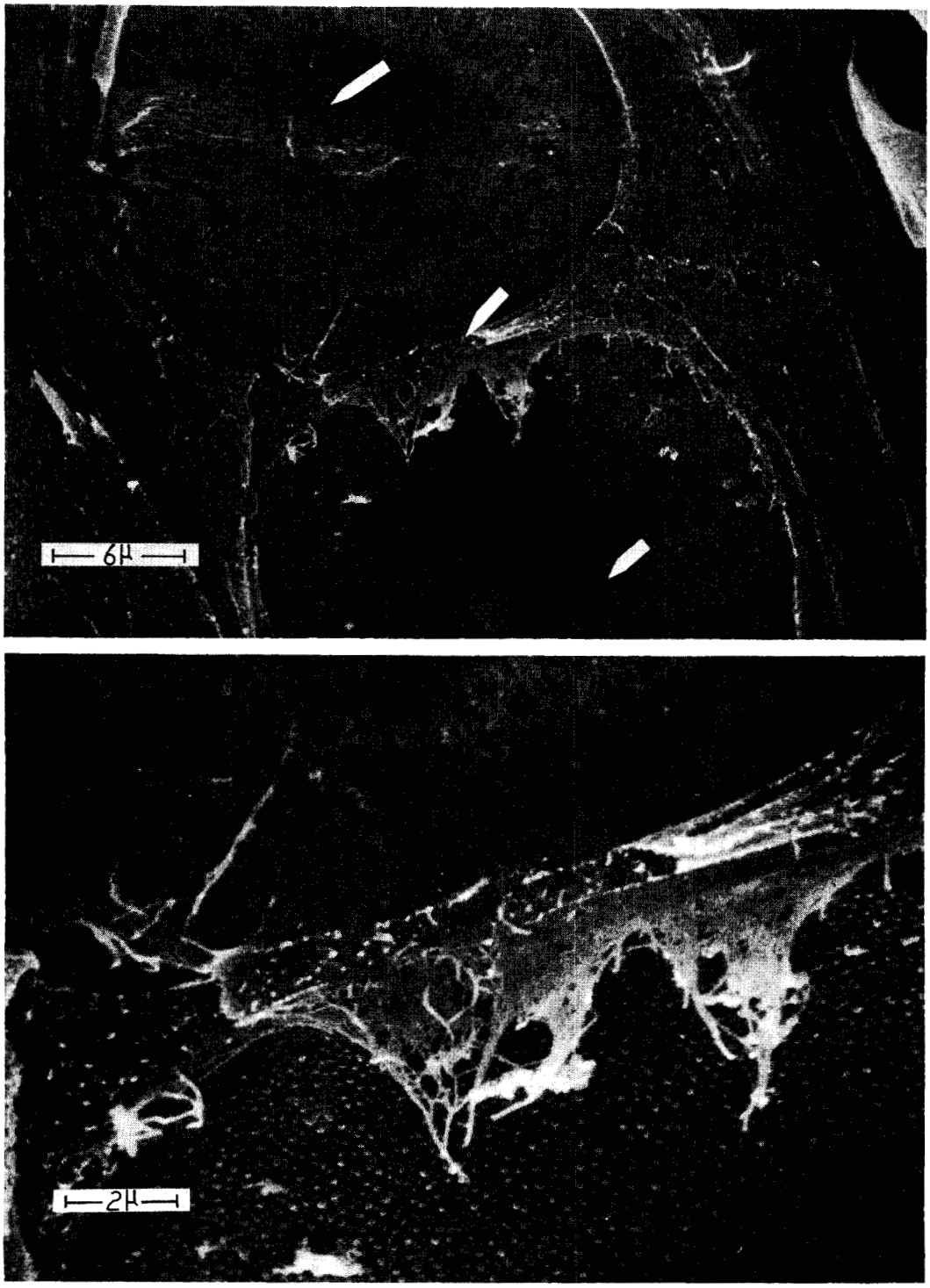
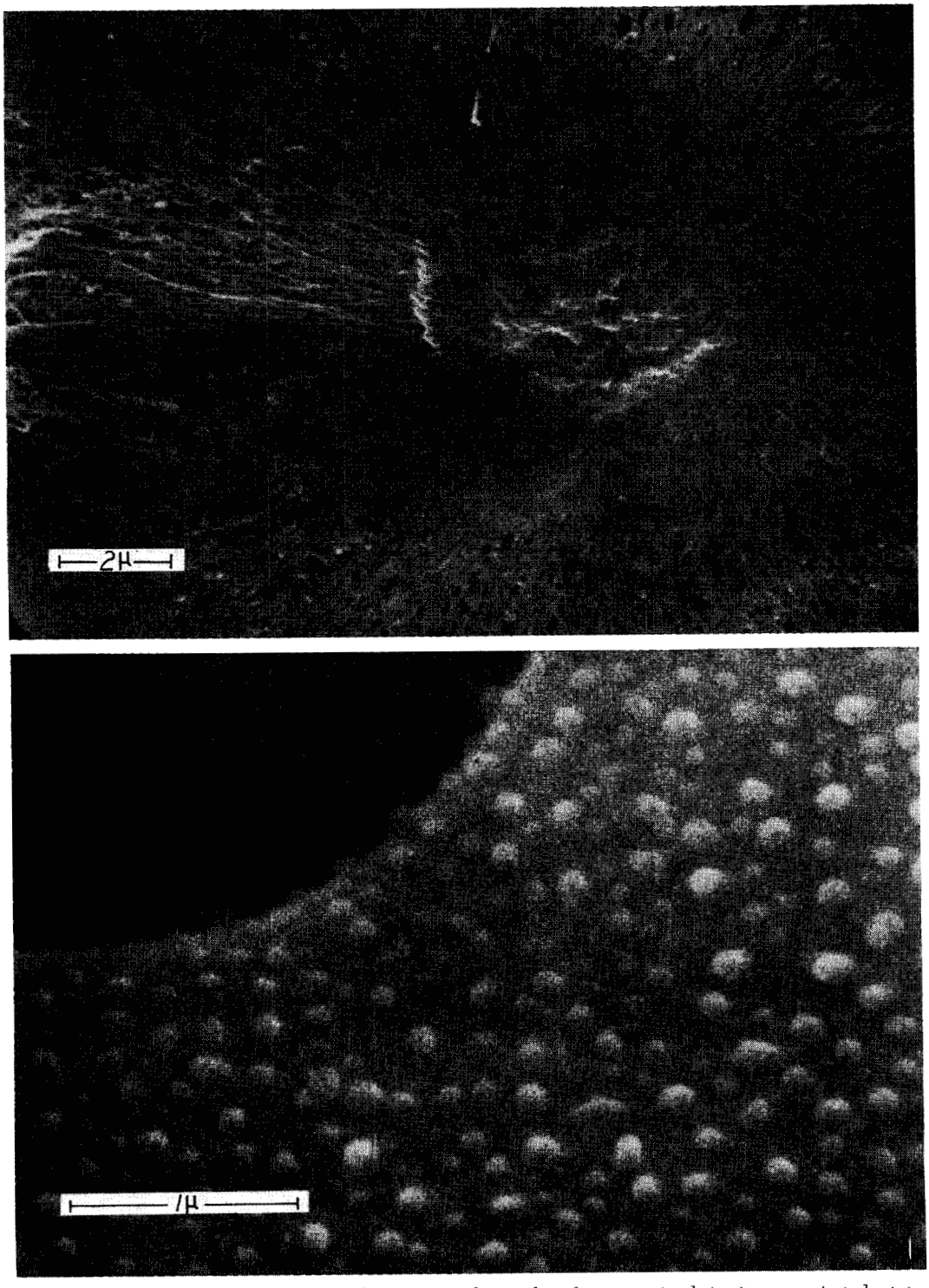


FIG. 8. Bordered pit structure of redwood prepared by the solvent-exchange method of Thomas (Thomas 1969; Thomas and Nicholas 1966). One of the two adjacent pits has had its torus torn away,



exposing the warty layer, while the other pit membrane has been retained in its unaspirated state. (3250 ×, 8300 ×, 8400 ×, 35000 ×)

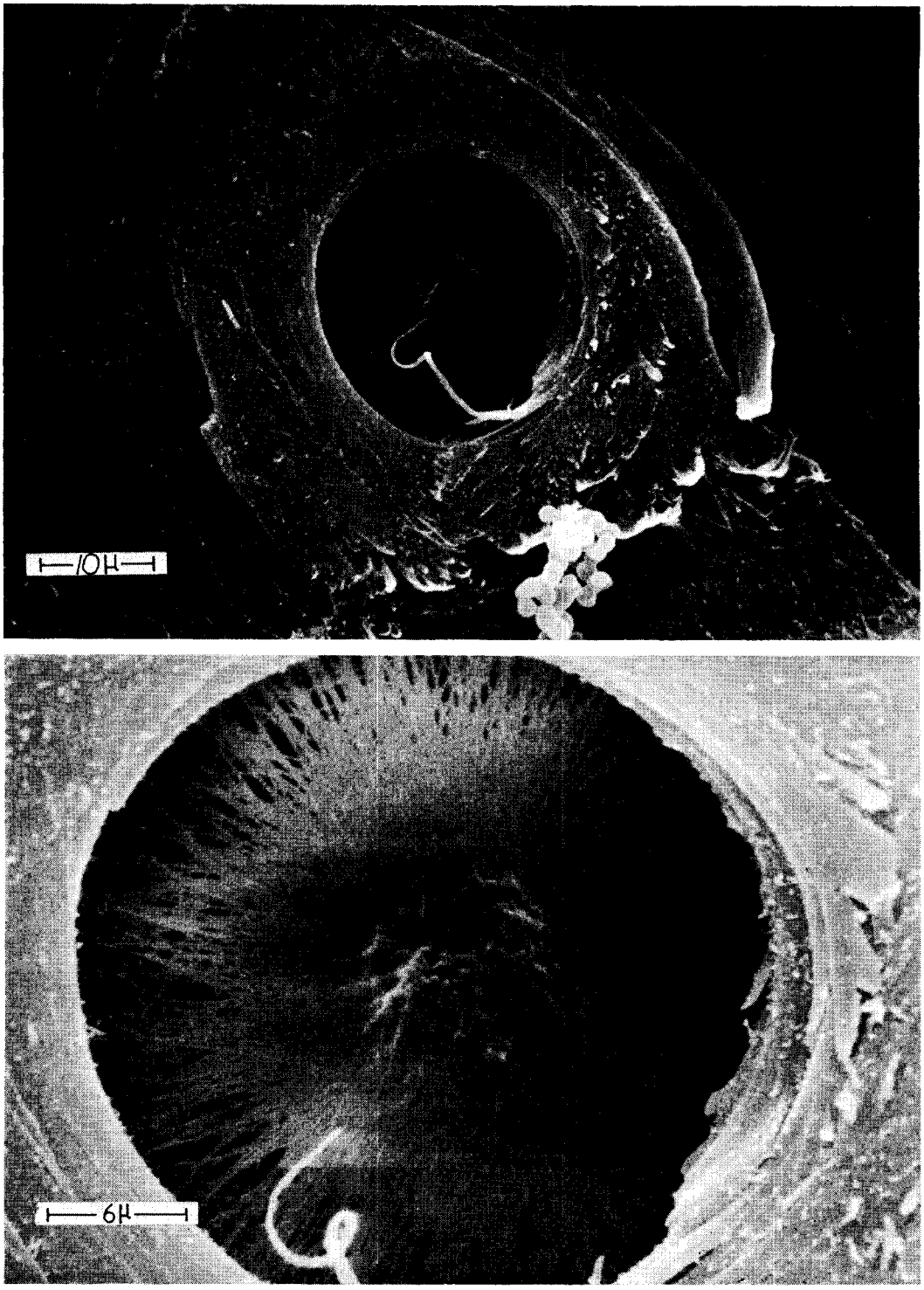


FIG. 9. More pit structure from the material shown in Fig. 8. Note the overhanging border, illustrating depth of field capability of SEM. (1650 ×, 3400 ×)

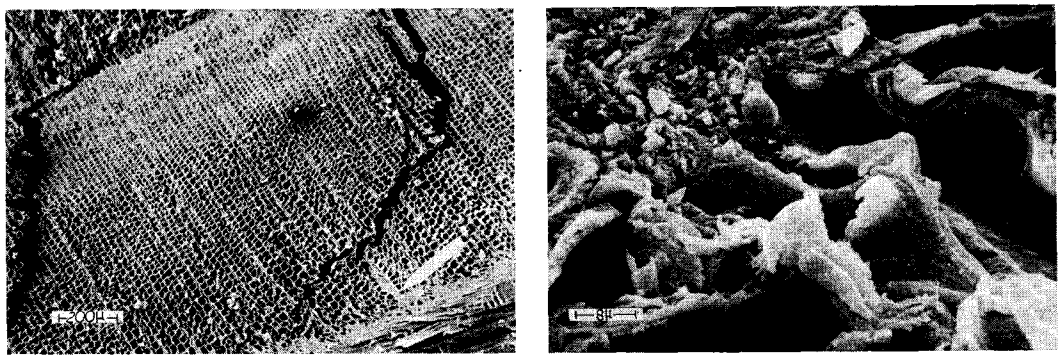


FIG. 10. The plywood glueline at 20 keV. Note the glue radiating up the ray at point of arrow. Contrast between glue and wood is poor, even in the higher magnification photo. (40 ×, 1050 ×)

1) Methanol—12 hr (overnight), followed by three exchanges at 3-hr intervals.
2) Acetone—same schedule.
3) N-pentane—same schedule.
4) Dry from n-pentane in preheated oven for 15 min at 65 C. Store over desiccant until ready for use.

Figures 8 and 9 show typical results. All observations were made on the split-radial surface of redwood sapwood. The surfaces were prepared by splitting after removal from the desiccator and just prior to coating. Primary magnifications (before enlargement) were made up to 22,000× with good clarity and resolution. The results give clear indication of the capability of SEM for anatomic study on the "semi-ultramicroscopic" level.

During these initial studies on wood structure, various adhesive-bonded wood products were prepared and observed with the microscope. The lack of contrast noted between the wood substrate and the adhesive in the SEM image proved to be a limiting factor in its use for study of the character and distribution of glue in wood structure. Thus, attempts were made to explore techniques of improving this contrast.

The glueline of ⅜-inch redwood plywood prepared in the laboratory was chosen for study. Two methods seemed to hold promise of improving contrast between surface materials of widely different nature—these were cathodoluminescence, and low primary beam voltage. Cathodoluminescence involves the collection and modulation of light quanta (photons) excited by the impinging electron beam. The idea here was that if the luminescent properties of the adhesive and the wood were different enough, or if they could be made so with dyes, then useful distribution patterns might result. The literature (Thornley 1960) cites use of low primary beam voltage to eliminate the need for coating in nonconducting materials. The thought behind its use as a contrast mechanism is that if a surface contained materials of widely different conducting properties, and if the incident beam voltage were low enough to prevent or subdue the masking effect of brightness buildup from static charge, then such conductance patterns would be apparent in the image.

TABLE 3. *Plywood specimens*[1] *prepared for glueline examination with scanning electron microscope*

Sample number	Mode of operation	
	Low primary-beam voltage	Cathodoluminescence
1	Control	—
2	—	Control
3	—	0.5% Rhodamine B*
4	—	1.0% Rhodamine B*
5	10% Lead, in solution*	—
6	10% Lead Powder*	—

[1] ⅜" plywood from ⅛" redwood veneer, using standard hot-press phenol formaldehyde glueline.
* All percentage additions to the glue mix were based on the resin solids of the glue.

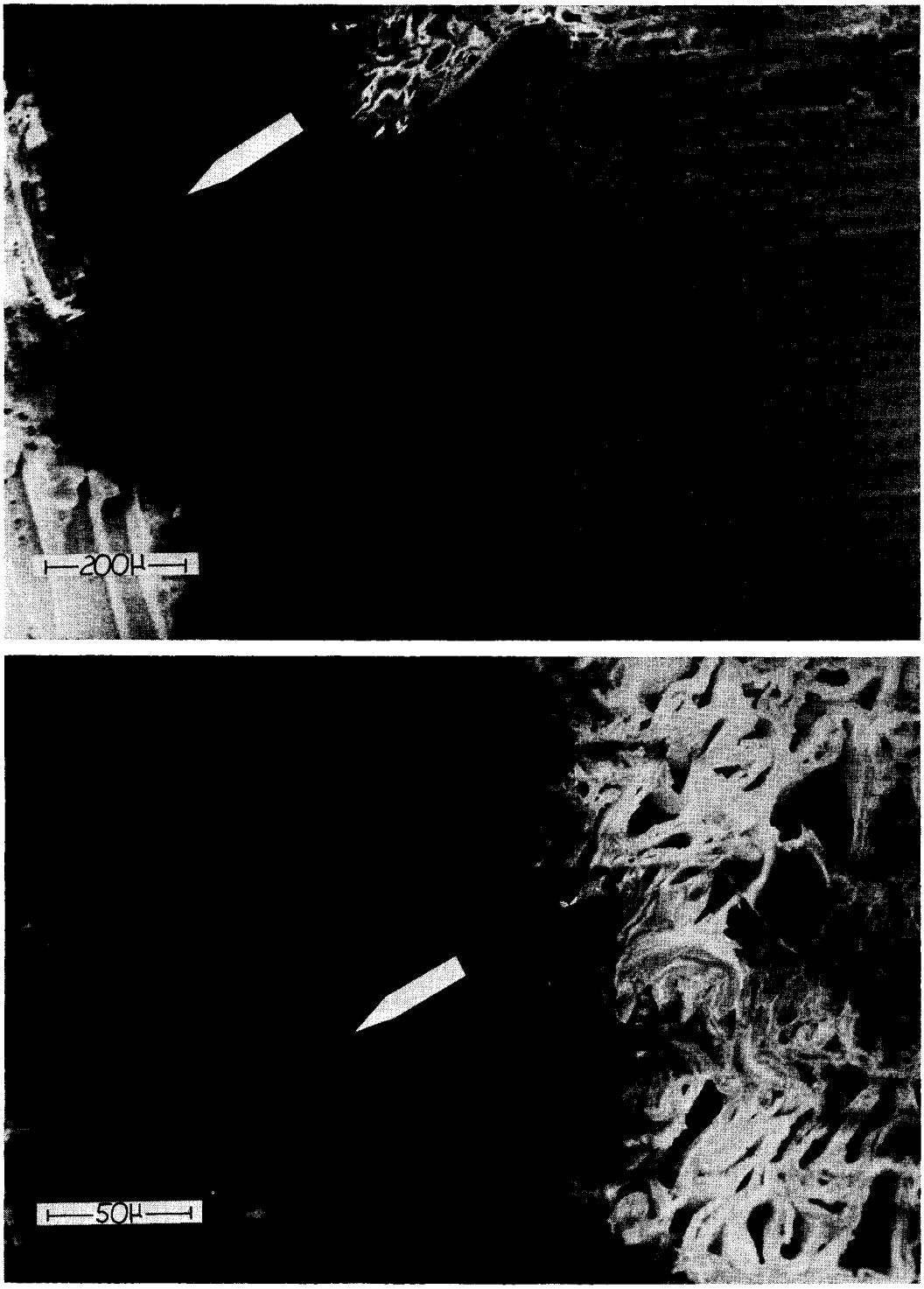


FIG. 11. Lead-containing plywood glueline at 3 keV primary beam voltage onto uncoated specimens. Arrows indicate the glueline. Note penetration of crushed cells adjacent to glue. (120 ×, 450 ×)

Table 3 summarizes the study conducted on these specimens prepared for viewing under the above-described conditions. The plywood was made with a standard, hot-press phenol formaldehyde glue. In addition to control samples, two treatments were used in the glueline of the other samples prepared. For the low-voltage study, lead (PbO) was dissolved in the caustic addition of the glue mix used for one sample, and merely mixed in powder form in the other. The idea was possibly to increase conductance by the presence of a metal atom in the glueline, as well as to test the theory that secondary electrons do not respond to changes in electron density of the surface materials (Oatley, Nixon, and Pease 1965). The dye dissolved in the glue mix of the cathodoluminescent samples was an attempt to improve the luminescent properties of the glueline over that of the wood. Other dyes that have better luminescent potential (e.g. anthracene) were tried but were found to be incompatible with the glue.

Figure 10 is typical of samples coated and viewed at normal operating voltages, regardless of the type of glueline treatment. The glueline of this sample contained lead, which did not help in contrast formation at normal beam voltages for secondary electron detection.

Figures 11 and 12 are examples of uncoated samples with and without lead-treated gluelines, respectively. Both have been observed at a primary beam voltage of 3 keV. Some loss of clarity results at this voltage level. The general contrast between the glueline and the wood substrate is very distinct in both specimens, but the lead-containing sample is the better of the two. The indistinct interface suggests that the cell walls of at least the first two adjacent elements are penetrated with adhesive. Also, the rays appear to contain adhesive that has radiated out considerably farther. There appears to be no filling of a cell lumen with glue unless that lumen is exposed to entry of the glue. The higher magnifications of areas bordering those where the apparent glue penetration begins to fade indicate streaks of penetration out into the cell structure. It is realized that some of the patterns of contrast resulting could be due to cutting artifacts, such as the redistribution of the glue as the knife slices through. Interpretation is made still more difficult by the natural darkness of depressions beyond the normal depth of field capabilities of the equipment. However, the technique of using low SEM voltages for viewing uncoated specimens has potential as a means of studying distribution patterns of materials in wood. It perhaps may have application not only for adhesives, but also for preservatives, paint and film interfaces, and other such areas.

Results at low voltage on uncoated specimens showed contrast regardless of lead content of the glueline. However, it was generally superior for the dissolved lead specimen (No. 5 in Table 3). The point to be stressed is that materials of differing conductance, or in which conductance can be differentially enhanced, yield contrast patterns in the secondary electron image. The areas of greater conductance appear darker. The underlying principle hinges on the relative variation in the numbers of secondary electrons escaping across the surface. Where surface conductance differs, the potential across the surface varies. This is believed to cause microelectric fields at the surface that influence the escape of the low-energy secondary electrons.

The cathodoluminescent study did not give any usable contrast buildup. The pattern of luminescence was nearly uniform, regardless of presence of dye. Perhaps the method may still prove useful if much more selective and stronger luminescent additives could be found.

CONCLUSIONS

This article has sought to discuss scanning electron microscopy in terms of its principles, applications, and advantages with respect to other imaging systems. In the section on fundamentals and principles, a selection of pertinent literature was integrated into the discussion to provide a good introduction into this general field.

The literature pertaining to the applica-

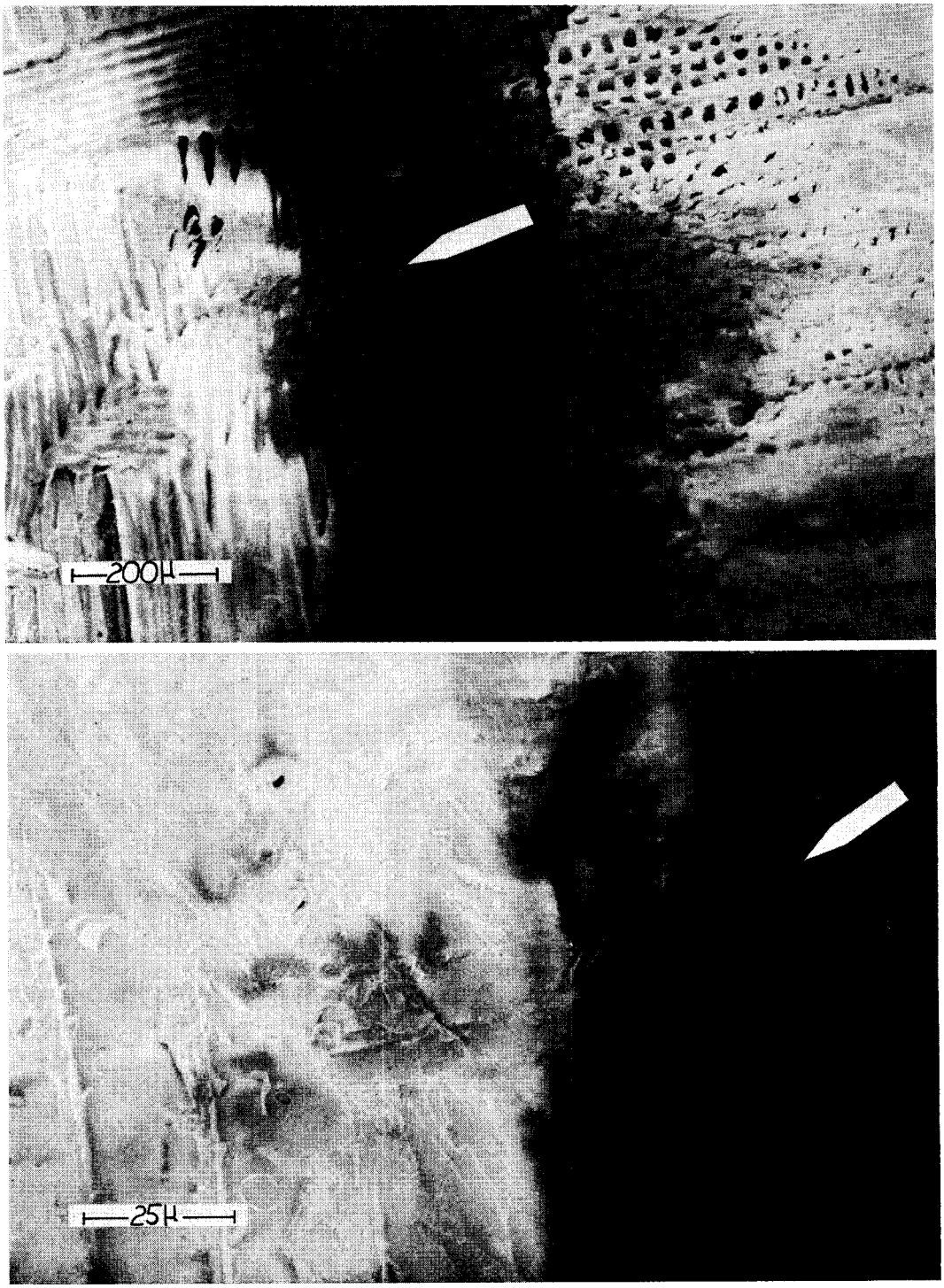


FIG. 12. Lead-free plywood glueline at 3 keV primary beam voltage onto uncoated specimens. Arrows indicate the glueline. Contrast between glue and wood is marked even without special treatment of the glue. (100 ×, 875 ×)

tion of scanning electron microscopy in the field of wood science was reviewed in depth. Finally, the investigations of the author using this equipment were discussed. This work centered around determining suitable specimen-preparation techniques and effects on wood of preparation for, and viewing in, the scanning electron microscope. The results illustrate well the capabilities of SEM as a tool for study of wood structure and anatomy on a semiultramicroscopic level. The final phase of work sought to improve contrast in the SEM image of wood-adhesive interfaces. Use of low primary beam voltage (3 keV) on uncoated specimens showed promise as a means of studying distribution patterns in wood containing materials of different conductivity.

REFERENCES

ARDENNE, M. VON. 1938. Das electronen rastermikroskop: Theoretische grundlagen. Zeitschrift fur Physik, **109**(9 & 10): 553–572.

ATACK, D., AND K. C. A. SMITH. 1956. The scanning electron microscope, a new tool in fibre technology. Pulp and Paper Magazine of Canada, **57**(3): T245–T251.

BUCHANAN, J. G., AND K. C. A. SMITH. 1960. Preliminary studies of damage in papermaking wood using the scanning microscope. Proc. European Regional Conference on Electron Microscopy. (A. L. Houwink and B. J. Spit, eds.) v. 1, p. 547–550. Delft.

———, AND O. V. WASHBURN. 1962. The surface and tensile fractures of chemical fibre handsheets as observed with the scanning electron microscope. Pulp and Paper Magazine of Canada, **63**(10): T485–T493.

———, AND ———. 1964. The surface and tensile fractures of groundwood handsheets as observed with the scanning electron microscope. Pulp and Paper Magazine of Canada, **65**(2): T52–T60.

———, AND R. A. LINDSAY. 1962. A note on structure of paper as revealed by the scanning electron microscope. The formation and structure of paper. Transactions of the Symposium, Technical Section of the British Paper and Board Makers' Association. Oxford. (Francis Bolam, ed.) v. 1, p. 101–108.

CÔTÉ, W. A., JR., Z. KORAN, AND A. C. DAY. 1964. Replica techniques for electron microscopy of wood and paper. TAPPI, **47**(8): 477–484.

COWLEY, J. M., AND A. STROJNIK. 1969. Design and application of a high voltage transmission scanning electron microscope. Proc., 27th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Claitors' Publishing Division, Baton Rouge, p. 106–107.

CREW, A. V. 1969. Closing the gap—A 5 Å scanning microscope. Proc. 27th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Claitors' Publishing Division, Baton Rouge, p. 6–7.

———, J. WALL, AND L. M. WELTER. 1968. A high resolution scanning microscope. Proc. 26th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux ed.). Claitors' Publishing Division, Baton Rouge, p. 356–357.

CROSSLETT, V. E. 1966. Scanning microscopy with electrons and X-rays, p. 11–12. *In* R. Uyeda [ed.], Electron microscopy, v. 2. Maruzen Co., Ltd., Tokyo.

———, AND P. DUNCUMB. 1957. A scanning microscope with either electron or X-ray recording. Electron microscopy. Proc. of the Stockholm Conf. (F. S. Sjostrand and J. Rhodin, eds.). Academic Press, New York, p. 12–14.

ECHLIN, P. 1968. The use of the scanning reflection microscope in the study of plant and microbial material. J. Roy. Microscopical Soc., **88**(3): 407–418.

EICHEN, E., D. R. FITCHMUN, AND L. R. SEFTON. 1969. Interpretation of micrographs from a scanning electron microscope. Proc., 27th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux ed.). Claitors' Publishing Division, Baton Rouge, p. 22–23.

EVERHART, T. E. 1958. Contrast formation in the scanning electron microscope. Ph.D. Dissertation. University of Cambridge (England).

———, K. C. A. SMITH, O. C. WELLS, AND C. W. OATLEY. 1960. Recent developments in scanning electron microscopy. Vierter Internationater Kongress Fur Elektronen-mikroskopie (Herausgegeben von G. Mollenstedt, H. Niehrs, and E. Ruska). Band I, p. 269–273. Springer-Verlag, Berlin (in English).

———, O. C. WELLS, AND C. W. OATLEY 1959. Factors affecting contrast and resolution in the scanning electron microscope. J. Electronics and Control, **7**(2): 97–111.

FENGEL, D. 1967. Ultramicrotomy, its applications in wood research. Wood Science and Technology, **1**(3): 191–204.

FINDLAY, G. W. D., AND J. F. LEVY. 1969. Scanning electron microscopy as an aid to the study of wood anatomy and decay. J. Inst. Wood Science, **4**(5): 57–63.

FORGACS, O. L. 1963. The characterization of mechanical pulps. Pulp and Paper Magazine of Canada (Convention Issue), **64**(C): T89–T118.

FUJIYASU, T., K. HARA, AND H. TAMURA. 1968. Hitachi scanning electron microscope. Proc., 26th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Clai-

tors' Publishing Division, Baton Rouge, p. 374–375.

HALL, C. E. 1966. Introduction to electron microscopy. 2nd ed. McGraw-Hill Book Co., New York. 397 p.

HAY, W. W., AND P. A. SANDBERG. 1967. The scanning electron microscope, a major breakthrough for micropaleontology. Micropaleontology, **13**(4): 407–418.

HAYES, T. L., AND R. F. W. PEASE. 1968. The scanning electron microscope: principles and applications in biology and medicine. Advances in biological and medical physics. v. 12, p. 85–137. Academic Press, New York and London.

ILVESSALO-PFAFFLI, M., AND J. LAAMANEN. 1969. Stereoscan-elektronimikroskooppiuusi laite pintatopografian tutkimiseen. Paperi Ja Puu-Papper O. Tra, **51**(9): 649–661.

JENSEN, W. A., AND R. B. PARK. 1967. Cell ultrastructure. Wadsworth Publishing Company, Inc., Belmont, California. 60 p.

KAY, D. H. 1965. Techniques for electron microscopy. 2nd ed. F. A. Davis Co., Philadelphia. 560 p.

KIMOTO, S. 1967. On a scanning electron microscope. Bulletin No. SM-67013. Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan. 15 p.

———, H. HASHIMOTO, AND K. MASE. 1968. Voltage contrast in scanning electron microscopy. Proc., 26th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Claitors' Publishing Division, Baton Rouge, p. 366–367.

KNOLL, M. 1935. Aufladepotential und sekund a remission elektronen bestrahlter korper. Zeitschrift fur Technische Physik, **16**(11): 467–475.

LEWIS, E. R., T. E. EVERHART, AND Y. Y. ZEEVI. 1969. Studying neural organization in aplysia with the scanning electron microscope. Science, **165**(3898): 1140–1143.

LIESE, W., AND W. A. CÔTÉ, JR. 1960. Electron microscopy of wood: results of the first ten years of research. Proc., 5th World Forestry Congress. v. 2, p. 1288–1298.

MACRES, V. G., O. PRESTON, N. C. YEW, AND R. BUCHANAN. 1968. A combined scanning electron microscope/electron microprobe analyzer. Proc., 26th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Claitors' Publishing Division, Baton Rouge, p. 368–369.

MCDONALD, L. W., AND T. L. HAYES. 1968. Scanning microscopy of human blood and marrow smears. Proc., 26th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Claitors' Publishing Division, Baton Rouge, p. 166–167.

MCMULLAN, D. 1952. Investigations relating to the design of electron microscopes. Ph.D. Dissertation, University of Cambridge (England).

———, 1953. An improved scanning electron microscope for opaque specimens. Proc. Institution of Electrical Engineers. v. 100, Pt. 2, p. 245–256.

MERCHANT, M. V. 1957. A study of water-swollen cellulose fibers which have been liquid-exhanged and dried from hydrocarbons. TAPPI, **40**(9): 771–781.

MOELLENSTEDT, G., AND F. LENZ. 1963. Electron emission microscopy. Advances in electronics and electron physics. v. 18, p. 251–329. Academic Press, New York, London.

MULVEY, T. 1967. The history of the electron microscope. Proc. Roy. Microscopical Soc. v. 2, Pt. 1, p. 207–227.

NIXON, W. C. 1968. Proc., 1st IITRI Symposium on Scanning Electron Microscopy. Illinois Institute of Technology Research Institute, Chicago, p. 55.

———. 1969. Early scanning electron microscopy. Proc., 27th Ann. Meeting, Electron Microscopy Society of America (C. J. Arceneaux, ed.). Claitors' Publishing Division, Baton Rouge, p. 2–3.

NORVILLE, L. R. 1962. Electron microprobe analysis utilizing the electron beam scanning system. Electron microscopy. 5th Int. Congress for Electron Microscopy. v. 1 (S. S. Breese, Jr., ed.). Academic Press, New York, London, p. EE 14–EE 15.

OATLEY, C. W. 1966. The scanning electron microscope. Science Progress, **54**(21): 483–495.

———, AND T. E. EVERHART. 1957. The examination of p-n junctions with the scanning electron microscope. J. Electronics, **2**(6): 568–570.

———, W. C. NIXON, AND R. F. W. PEASE. 1965. Scanning electron microscopy. Advances in Electrons and Electron Physics. v. 21, p. 181–247. Academic Press, New York, London.

PEASE, R. F. W. 1968. Scanning electron microscopy. Laboratory Management, **6**(9): 13–14, 32–38.

———, AND T. L. HAYES. 1966. Scanning electron microscopy of biological material. Nature, **210**(5040): 1049.

———, ———, A. S. CAMP, AND N. M. AMER. 1966. Electron microscopy of living insects. Science, **154**(3753): 1185–1186.

———, AND W. C. NIXON. 1965. High resolution scanning electron microscopy. J. Scientific Instruments, **42**(2): 81–85.

RESCH, A., AND R. BLASCHKE. 1968. Uber die Anwendung des Rasterelektronenmikroscopes in der Holzanatomie. Planta, **78**(1): 85–88.

RESCH, H., AND D. G. ARGANBRIGHT. 1968. Some basic aspects of the cellon process. Service Report No. 35.01.47, Progress Report No. 7. University of California, Forest Products Laboratory. 7 p.

SCHLINK, C. G. R. 1969. Pit structure in sapwood and heartwood of white fir, *Abies concolor*, (Gord. & Glend.) Lindl. Masters'

Thesis. University of California, Berkeley. 54 p.

SIKORSKI, J., J. A. NOTT, J. S. MOSS, AND T. BUCKLEY. 1967. A new preparation technique for the examination of polymers in the scanning electron microscope. Proc. Roy. Microscopical Soc. v. 2, Pt. 4, p. 431.

SMITH, K. C. A. 1956. The scanning electron microscope and its field of application. Ph.D. Dissertation, University of Cambridge (England).

———. 1959. Scanning electron microscopy in pulp and paper research. Pulp and Paper Magazine of Canada, **60**(12): T366–T371.

———. 1960. A versatile scanning electron microscope. Proc. European Regional Conference on Electron Microscopy (A. L. Houwink and B. J. Spit, eds.). v. 1, p. 177–180. Delft.

———, AND C. W. OATLEY. 1955. The scanning electron microscope and its field of application. Brit. J. Appl. Physics, **6**(11): 391–399.

SOKOLOFF, A., T. L. HAYES, R. F. W. PEASE, AND M. ACKERMANN. 1967. Tribolium castaneum: morphology of "aureate" revealed by the scanning electron microscope. Science, **157**(3787): 443–445.

STERNGLASS, E. J. 1954. Backscattering of kilovolt electrons from solids. The Physical Review, **95**(2): 345–358.

THOMAS, R. J. 1969. The ultrastructure of southern pine bordered pit membranes as revealed by specialized drying techniques. Wood and Fiber, **1**(2): 110–123.

———, AND S. D. NICHOLAS. 1966. Pit membrane structure in loblolly pine as influenced by solvent-exchange drying. Forest Prod. J., **16**(3): 53–56.

THORNLEY, R. F. M. 1960. Recent developments in scanning electron microscopy. Proc. European Regional Conference on Electron Microscopy. (A. L. Houwink and B. J. Spit, eds.), v. 1, p. 173–176. Delft.

THORNTON, P. R. 1968. Scanning electron microscopy. Chapman and Hall Ltd., London. 368 p.

WAGENFUHR, R. 1969. Elektronenmikroskopische Untersuchungen der Struktur von Grenz-und Oberflachen in der Holztechnik. Holztechnologie, **10**(1): 37–40.

———, AND F. ZIMMER. 1968. Die Holzoberflache unter dem Elektronenmikroskop. Holztechnologie, **9**(3): 151–152.

WASHBURN, O. V., AND J. G. BUCHANAN. 1964. Changes in web structure on pressing and drying. Pulp and Paper Magazine of Canada, **65**(9): T400–T408.

WELLS, O. C. 1957. The construction of a scanning electron microscope and its application to the study of fibers. Ph.D. Dissertation. University of Cambridge (England).

WISCHNITZER, S. 1962. Introduction to electron microscopy. Pergamon Press, Inc., New York. 132 p.

ZWORYKIN, V. K., J. HILLIER, AND R. L. SNYDER. 1942. A scanning electron microscope. American Society for Testing Materials, Bulletin No. 117, p. 15–23.

BAMBER, R. K., and G. W. DAVIES. 1969. Lignification of ray parenchyma cell walls in the wood of *Pinus radiata* D. Don. *Holzforschung* 23(3): 83–84 (E. eg.). Observations of UV absorption confirm that the genus *Pinus* are not lignified and support the view that they become lignified in the heartwood. (A)

SHARP, R. F., and H. O. W. EGGINS. 1969. A perfusion technique for culturing fungi on wood. *J. Inst. Wood Sci.* 4(4): 24–31 (E.e). A new culturing system for growing and isolating wood-decaying microfungi is described. A nutrient solution is perfused through fiber glass sleeving to maintain the desired moisture content of beechwood veneer. Ground particles of the decaying wood were used to inoculate selective cellulose agars for colonization studies. (J.D.W.)

LIESE, W., and C. MENDE. 1969. Histometric investigations on the culms of two Indian bamboo species with reference to the portion of the various cell elements. *Holzforsch. und Holzverwert.* 21(5): 113–117 (G. g e). Culms of *Dendrocalamus structus Nees* and *Bambusa tulda Roxb.* were investigated as to proportion of parenchyma and sclerenchyma cells, vessels, and sieve tubes. The difference between the two species is not statistically significant. Parenchyma cells, vessels, and sieve tubes predominate in the inner half of the culm wall; fibers predominate in the outer half. Amount of parenchyma cells decreased with increasing height up to the crown region where it increased. Percentage of vessels and sieve tubes increased with increasing height. (A)