
SHORTER COMMUNICATIONS

APPLICATION OF THE SCANNING ELECTRON MICROSCOPE IN PALEOBOTANY¹

THOMAS N. TAYLOR

Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago

Detailed investigations of plant microfossils and microdetails of megafossils have always been difficult to study because of our inability to observe and adequately record minute morphological surface features. The imaging potential of the scanning electron microscope (SEM) now provides the opportunity to observe and illustrate these fine details on specimens which have previously been too small for direct observation, or have complex microtopographical surface patterns that have been impossible to accurately interpret. Furthermore, improved resolution and magnification available with the scanning electron microscope now provide the opportunity to directly observe and illustrate morphological features that have not previously been seen.

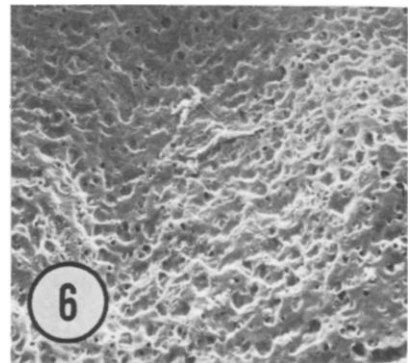
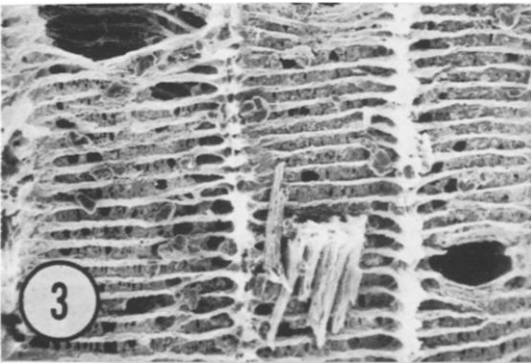
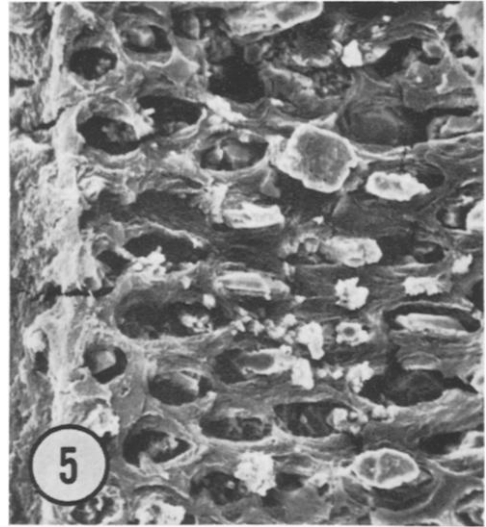
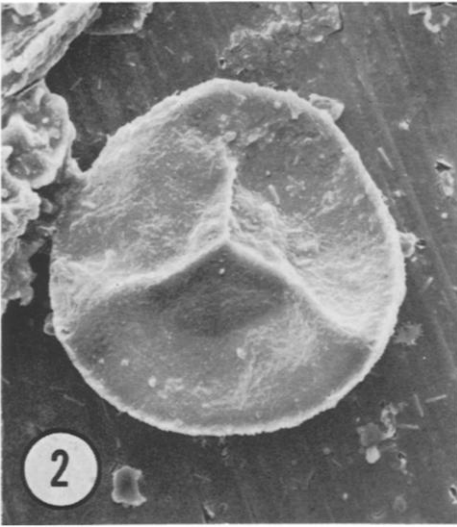
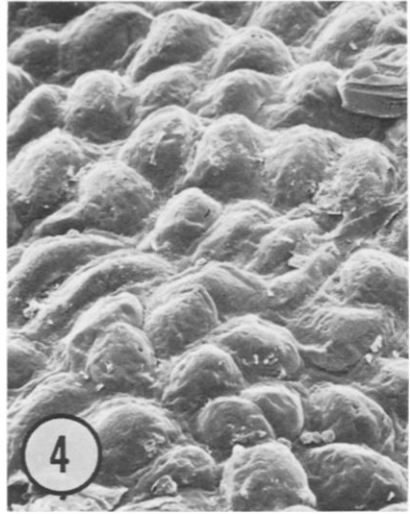
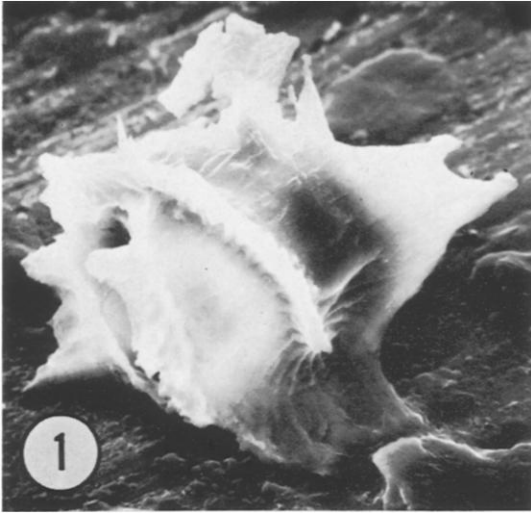
Although the theoretical basis for a scanning electron microscope was suggested in 1935, the first operational instrument was not constructed until 1938.² Commercial availability of the instrument was announced in 1965. Presently there are three commercial instruments available, and at least two additional manufacturers with scopes ready to market.

Unlike the conventional transmission and reflection electron microscope, the scanning electron microscope produces a magnified image without the necessity of lenses between the specimen and viewing screen. The SEM does not have as great a resolving power as the transmission electron microscope; however, sample preparation is far easier in scanning electron microscopy and can be performed by persons who are relatively unskilled and who may have difficulty in transmission electron microscopy replication techniques. The SEM differs from the light microscope system in having greater flexibility of magnifications (20×–50,000×, corresponding to scanned areas of 5.0 mm to 2 μm

¹ The illustrations for this paper were taken on the Cambridge STEREOSCAN MARK IIA (Cambridge Instrument Co. Ltd.) at the Engis Equipment Company, Morton Grove, Illinois. I wish to acknowledge the assistance of Mr. Arnold Young for making the instrument available, and to express my gratitude to Mr. Franco Rossi and his staff for providing technical assistance and for taking many of the photographs. This investigation was supported by National Science Foundation Grant GB-6834.

² The reader is referred to Moellenstedt & Lenz (1963), Oatley, Nixon & Pease (1965), and Hay & Sandberg (1968) for a historical review of the development of the scanning electron microscope.

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- FIG. 1. *Bowmanites dawsoni* spore. Pennsylvanian. 510×.
FIG. 2. Proximal view of *Endosporites* spore. Pennsylvanian. 525×.
FIG. 3. Longitudinal section of *Lepidodendron* secondary xylem showing three tracheids with horizontal scalariform secondary thickenings and smaller vertical fimbrials. Specimen prepared for viewing by dissolving away matrix (calcium carbonate) with dilute hydrochloric acid. Pennsylvanian. 220×.
FIG. 4. Surface view of nucellar cuticle of Paleozoic seed. Pennsylvanian. 200×.
FIG. 5. Longitudinal section of Paleozoic seed fern (*Medullosa*) tracheid showing pits in wall. Pennsylvanian. 500×.
FIG. 6. Proximal surface of *Endosporites* spore showing detailed ornamentation of wall. Pennsylvanian. 2,000×.



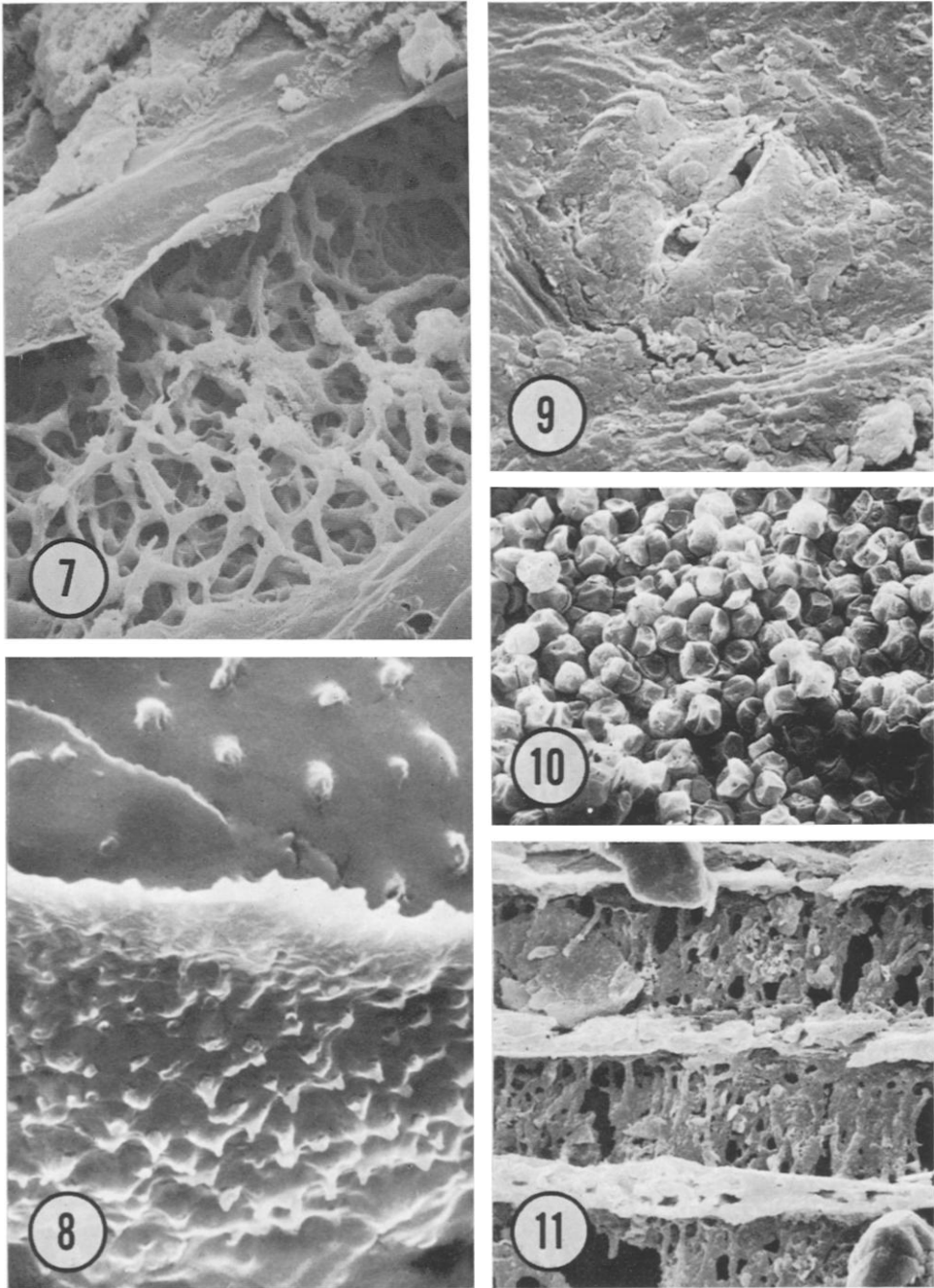


FIG. 7. Tear in surface of Paleozoic seed megaspore membrane showing ultra-architecture inside. Pennsylvanian. 2,000 \times .

FIG. 8. Side view of *Lycospora* spore showing differences in proximal and distal surface ornamentation patterns. Pennsylvanian. 9,000 \times .

FIG. 9. Surface view of angiosperm leaf stomate. Eocene. 2,100 \times .

square on the specimen), improved resolution of 20–50 times, and increased depth of focus of about 300–500 times. The capacity to focus through certain substances, to observe internal features, and to make direct measurements, as well as being able to resolve color, remain useful aspects of light optical systems that cannot be duplicated at present in scanning electron microscopy.

In both the light microscope and transmission electron microscope the incident radiation is focused on the object and results in a magnified image. In light optical systems the image is related to wavelength and number of photons reflected, transmitted, or diffracted from the object. The image produced in the transmission electron microscope is related to electron scattering and diffraction by the extremely thin layer of the specimen. In scanning electron microscopy the illuminating radiation is not imaged after impingement with the object, and therefore any signal produced by the interaction of the primary electron beam and the specimen may be used as a source of information. Such signals as secondary electrons, backscattered electrons, X-rays, Auger electrons, visible light and infrared photons, and a variety of differential electrical currents are all potential sources of information in scanning electron microscopy.

In scanning electron microscopy an electron beam is demagnified to a spot approximately 100 Å in diameter by passing it through a series of three electromagnetic condenser lenses. This beam is scanned over the surface of the specimen in a square raster pattern by deflection coils which carry current from a saw-tooth generator. Low energy secondary electrons emitted from the specimen are picked up, amplified, and displayed on a cathode ray tube. Emission differential of electrons from various areas of the specimen surface produce contrast that is viewed as the image of the specimen. The magnification obtained is the ratio of the linear dimensions of the raster on the specimen and that of the cathode ray tube.

Sample preparation of fossil plant material is generally easy and requires little time. The microfossils illustrated in Figures 1 and 2 were macerated from the surrounding matrix, deposited on a standard aluminum specimen disc in a drop of water, and allowed to dry at room temperature. Larger specimens (Figs. 3–5, 9) were attached to specimen discs with a small drop of commercial household cement.

To insure that specimens are electrically conductive the samples are rotated in a high vacuum while a carbon or metal film is vapor deposited onto the surface. The conductive coating must be deposited evenly over the specimen surface; thick enough to prevent electrical charging, and thin enough so that microdetails of the surface are not masked. Because of the high vacuum requirement for vapor coating, extant plant material that might be compared with fossil specimens must now undergo freeze drying and/or freeze-etching sublimation preparation prior to coating. The use of electrostatic aerosol sprays may make high vacuum vapor coating of some extant plant materials unnecessary.

The specimen chamber of the instrument will accommodate samples as large as 2.3 cm in diameter and approximately 3.0 mm high. Larger specimens can be examined by using modified stages, however, rotational and linear movements are restricted. Under normal operation the specimen stage has linear

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FIG. 10. Surface view of *Endosporites* spore tetrads within sporangium. Pennsylvanian. 50×.

FIG. 11. Portion of an arborescent lycopod tracheid showing delicate fimbrials between horizontal scalariform bars. Pennsylvanian. 2,200×.

movement capability along x, y, and z axes, as well as rotation and tilt around two axes.

The most obvious use of the scanning electron microscope as a research tool in paleobotany is in the area of palynology and in the study of other plant microfossils. Plant microfossils may broadly be defined as any microscopic particle indicating evidence of having originated from plants. The most commonly investigated plant microfossils are pollen grains and spores. The durable nature of these fossils through geologic time, and the fact that pollen grains of each genus (and in some instances each species) often have distinctive morphologic forms and ornamentation patterns, has made the study of pollen and spores a significant area of investigation in the development of stratigraphic correlation and in the reconstruction of ancient floras and environments.

The identity of these microfossils is based almost entirely on size, shape, and sculpturing or ornamentation of the outer, and in some instances, inner surfaces of the wall. The minuteness and often complex ornamentation patterns of the wall plus resolution limitations of the light microscope make critical examination of such surface features in some instances impossible. In many cases the actual configuration must be inferred from optical effects. The higher magnifications and the improved resolution capabilities available with the scanning electron microscope provide a superior method of observing these details of ornamentation that are the basis of identification.

The effectiveness of plant microfossil studies regarding identification, description, and classification depend not only upon direct examination by the researcher but, of equal importance, upon illustrations for comparison and reference purposes and for reporting and publication. Prior to the introduction of the SEM, illustration of microfossils relied almost exclusively on line drawings that represent a composite of the specimen. Line drawings of this kind, however, often omit details that were not considered significant by the investigator or were simply not observed. Light microscope photographs are more objective, however, they inherently suffer from limitations of resolution, magnification, and depth of field. The scanning electron microscope provides an objective and accurate means of illustrating the three-dimensional surface architecture of microfossils (Figs. 6, 8).

The ability to make stereopairs by tilting the specimen about one of the rotational axes increases the three-dimensionality of the specimen and also provides an accurate means of measuring microtopographic features. Comprehensive specimen stage movements of the SEM that allow the same specimen to be viewed on different sides and at different angles should be useful in determining ornamentation variability of single specimens and within taxa. The potential of the scanning electron microscope in palynological research is demonstrated by the illustrations of several Paleozoic small spores (Figs. 1, 2, 6, 8, 10). Recent studies by Echlin (1968) and Heslop-Harrison (1968) have utilized the scanning electron microscope to investigate extant pollen wall ontogeny. Similar "ontogenetic" studies of fossil pollen and spores should be rewarding.

Additional plant microfossils that should prove excellent subjects for the SEM include: diatom frustules, charophyte oogonia, dinoflagellate cysts and armor plates, spores and reproductive structures of fungi, single cells (e.g., fibers and tracheids) and cutinous residues of megafossils, and numerous algal resting spores.

Several studies in recent years have demonstrated that some ultrastructural details of fossil plant material are preserved and can be investigated with the transmission electron microscope (Ehrlich & Hall, 1959; Pettitt, 1966). Ultrastructural studies of the megaspore membrane of Paleozoic seeds in our laboratory indicate the consistent occurrence of a thick spongy layer forming the bulk

of the membrane. Examination of this layer by the SEM reveals the nature of this ultra-architecture and provides a graphic demonstration of the great depth of field available with this instrument (Fig. 7).

The examination of petrified Paleozoic wood (Figs. 3, 5, 11) reveals new and interesting information concerning anatomical features of secondary wall development. The opportunity to observe secondary wall deposition patterns and ultra-architectural features in fossil woods will contribute significantly to the solution of problems relating to identification, ontogeny, and phylogeny.

Investigation of fine-grained impression and compression megafossils promises to be a rewarding area of study through the availability of scanning electron optics (Fig. 9). Studies of epidermal cell types and patterns have proved useful in the identification, classification, and interpretation of phylogenies within groups of both living and fossil plants. The ability to directly observe the surface (Fig. 4) of different preservation modes for epidermal cell outlines greatly increases the opportunity to determine such relationships for plant parts now classified solely on the basis of morphologic form.

The increased resolving power and magnification available with the SEM offer a potential for revealing heretofore unnoticed microdetails or surface markings of a large number of problematic fossils whose affinities are presently obscure and which may be more adequately understood after this information is available.

The applications mentioned above for the scanning electron microscope in the study of fossil plants only scratch the surface of potential uses for this remarkable instrument. Greater availability of the instrument and continued technological developments in such areas as increased resolution should provide interesting and significant advances in many aspects of paleobotany.

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