STUDY OF NANNOFOSSILS BY THE SCANNING ELECTRON MICROSCOPE

by

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(with 2 Text-Figures and 2 Plates)

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Coccoliths and allied carbonate nannofossils are opaque to regular electron beams with an accelaration voltage of less than 100 KV. To observe the detailed structure of specimens, a replica of thin carbon film is prepared. The sample is first shadowed in a high vacuum with a heavy metal such as platinum and then evenly coated by a thin film of evaporated carbon. The original sample is desolved completely in hydrochloric acid. The shadowed impression of the surface of the specimen is then viewed by the electron microscope. This technique is called the preshadow preparation method. For the details of the method, refer to HONJO, 1967.

A free coccolith specimen is usually flat, and does not possess extremely high relief. Therefore, shadowing is particularly effective in emphasizing the delicate ornamentation of the surface. Also, carbon particles tend to evenly distributed throughout the sample surface because of its flatness.

Carbonate nannofossils are usually composed of minute crystallites of calcite. They are easily desolved in a weak acid without leaving any opaque material. A preshadow replica represents the surface detail of a specimen in a precise manner. A well prepared carbon replica can reproduce relief separated by only 50Å. This degree of dependability is more than sufficient for nannofossil study.

However, the preshadow technique has several inportant defects. The preparation requires disolving the original specimen. Furthermore, carbon films are easily contaminated or damaged during observation, and it is difficult to preserve them for long periods of time. Also, it is extremely difficult to relocate a particular specimen among others after a microscopic mesh is removed from the microscope. This is mainly due to the narrowness of the field of an electron microscope. The microscopic mesh therefore commonly is discarded after observation and photomicroscopy.

The designation of types thus presents a serious problem, inasmuch as the

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original sample disolved and the replica of the original specimen can not be indefinitely preserved. Many authors therefore have designated photographic negatives as taxonomic types. This obviously is not an adequate nomenclatorial practice. Photographic plates represent only two-dimensional views of a specimen and they also can not be permanently preserved.

The use of a scanning electron microscope provides a solution to many of these problems. The resolution of the scanning electron microscope is far better than that of optical microscope and, somewhat surprisingly, the depth of focus is significantly improved over that of the optical system.

The scanning electron microscope magnifies the surface details of an object in sequential images with a signal spot that is generated by an electron probe and scans the specimen in a regular raster.

The magnification of a scanning microscope ranges from as little as X50 to more than X10000 with smooth continuation. The view field is far greater at low magnifications on the order of 100 times larger than the regular electron microscope. Consequently, it is quite easy to relocate a particular specimen when re-examination is required.

Unlike regular electron microscopic preparation, a specimen is almost untouched during preparation and observation, except for the surface of the sample which is evenly coated by a very thin metal film. This preparation technique is generally easier than the preshadow replica technique, and specimen with a substrate require very little space for storage. A gold-coated specimen can be preserved indefinitely without special care, and are ready for re-examination at any time.

The scanning electron microscope was developed by KNÖLL and ARDENNE as early as 1935. However, mainly because of limited power of resolution, the early attempts did not receive widespread attention. Recent work by EVARHART and others drastically improved the performance of the scanning electron microscope which has now found a great deal of application in solid state physics. CREWES (1966) suggested that the scanning microscope can be used for high resolution studies competitively with a transmission type electron microscope in the near future. There are a few commercialized models available at present. The details of the application of the scanning microscope in biologic and geologic research is reviewed by KIMOTO and HONJO (1967). The coating technique for the scanning electron microscopic study of nannofossils is similar to that for planktonic foraminifera and was described by HONJO and BERRGREN (1967).

A coating of conducting metal such as gold or platinum is obtained by a vacuum evaporation method. There are two points of importance in the coating procedure of nannofossils; first, the thickness of the film should be kept as thin as possible to maintain the original topography of the specimen. However, this film requires a certain minimum thickness to maintain proper electric conductivity. We have found that a gold film of 20 Å is satisfactory for this purpose and also

sufficiently thin. Secondly the thin metal film must cover the surfaces with uniform thickness. Evaporated particles of heavy metals such as gold, travel straight from the radiation source in a high vacuum. Their distribution therefore is strongly influenced by the surface topography of the specimen. A rotary evaporation technique described in this paper is applied to avoid this so-called "shadowing effect".

A specimen stand is prepared as follows. A small piece of brass or stainless steel rod is prepared. The dimension of this rod for use with our instrument is 10mm in thickness and 12mm in length. A block should be carefully prepared by successive ultrasonic cleaning to remove materials which may evaporate in a high vacuum.

Many specimens are mounted on a small fragment of mica flake by an air blow technique (HONJO, 1967) or simply dripped on this surface with water. The mica flake is mounted carefully with a small amount of epoxy resin on the flat end of the block.

A proper length of thin pure gold wire to be used for shadowing is tightly

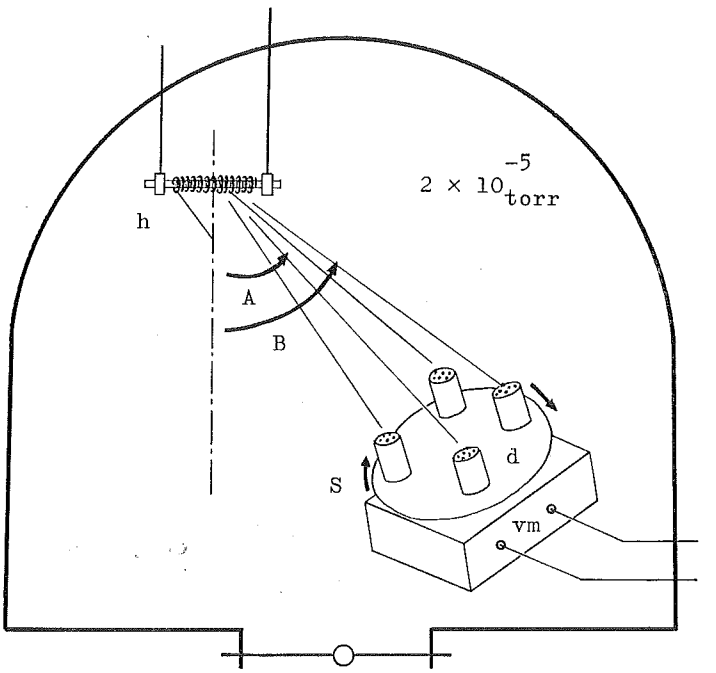

Fig. 1
The specimen stand (S) is mounted on a rotary table (d) of a vacuum motor (vm). The thin gold wire, coiled on a heater (h) is evaporated for deposition. As a rotary table turns, the shadowing angles (A, B, etc.) changes by every moments. This encourages the even deposition of the gold film on the specimen surface.

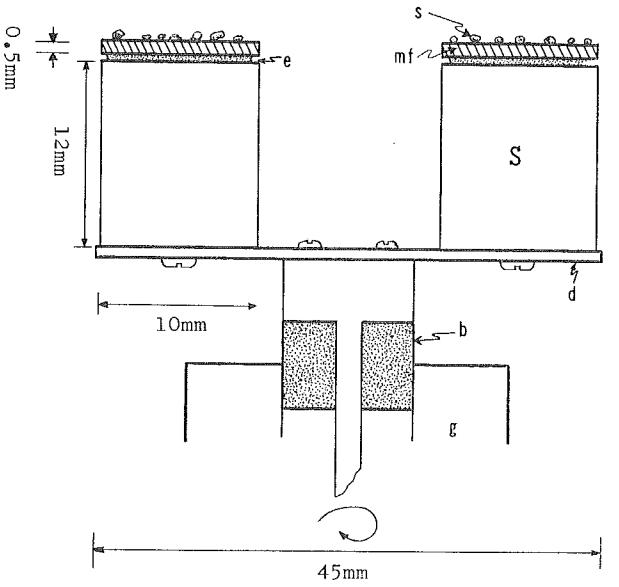

Fig. 2
The samples (s) are mounted on a piece of mica flake (mf). A specimen stand (S) and the mica flake is bonded with epoxy resin (e). The rotary disk (d) is connected with the gear-head (g) of a vacuum motor through a Teflon bearing (b).

coiled around a tungsten radiation source. The mass of the metal to be evaporated is calculated by a fomula provided by BRADLEY (1952).

The specimen stand is then mounted on a rotary table; the evaporation source is placed at a proper position relative to the sample; This position is approximately 35 to 40 degrees from a normal to the rotation disk (Text-Figure 1).

This apparatus is then covered by a bell jar and air is evacuated to 2×10^{-5} torr. The gold wire is evaporated by applying a strong current while the sample disk is rotated. Our experience indicates that slow rate of deposition from a rather remote evaporation source is preferable and we therefore use a vacuum motor with a rate of four revolutions per second.

As is shown in figure 1 of plate 1, the view-field of the scanning microscope covers an area of approximately 100 microns at a magnification of X1000. Therefore, a particular specimen, such as that illustrated in figure 3, can easily be located by reference to the adjacent specimens.

The transmission electron photomicrographs of preshadow replicas (right row) and the scanning micrographs of gold-coated specimens of the same species (left row) are compared in the plate 2. The over-all resolution clearly is better in the transmission photographs; however, the three dimensional arrangement of the constituent crystallites of the coccoliths are better represented by the scanning electron

phtomicrographs.

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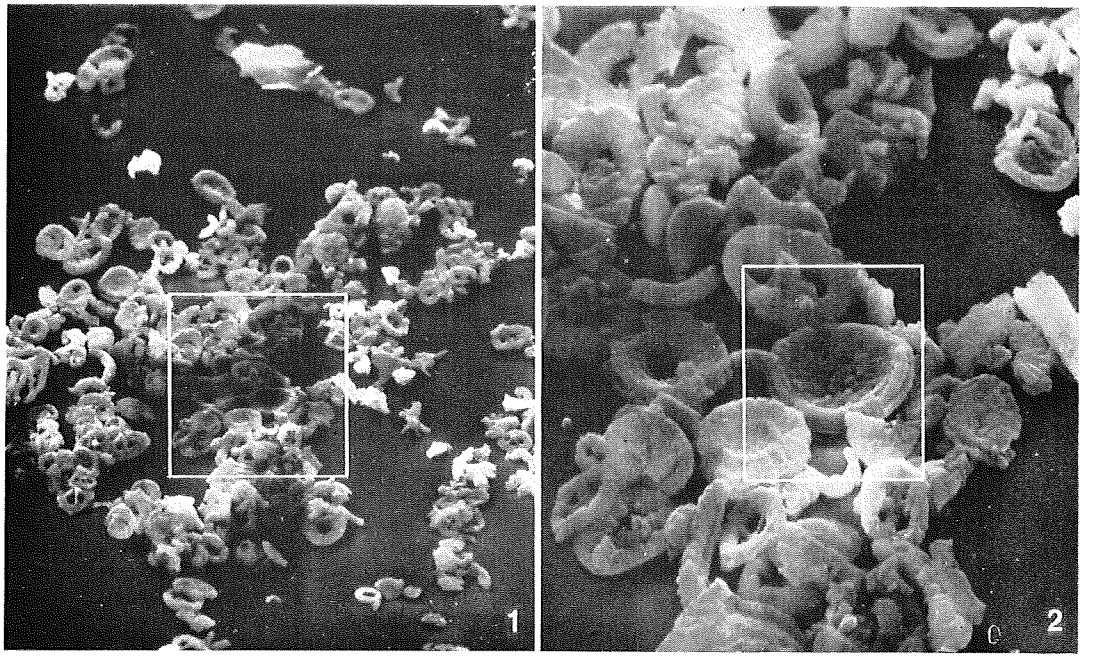
PLATE 51 AND EXPLANATION

Explanation of Plate 51

The view-field of the scanning microscope covers an area of approximately 100 microns on one edge of a display-tube, as is shown in figure 1. Therefore, a particular specimen such as that illustrated in figure 3 (*Cribrosphaerella* cf. *ehrenbergi* (Arkhangelsky)), can easily be located by reference to the adjacent specimens, through an intermediate magnification such as figure 2. A complex arrangement of calcite crystallite are well observed in figure 4 (An undescribed species of *Chiastozygus*).

All of the specimens in plate 1 and 2 are prepared from a Niabrara chalk sample, collected from western Kansas, U. S. A. The thickness of the gold film which covers entire view field is approximately 20 Å, deposited by a rotation evaporation technique.

Plate 51



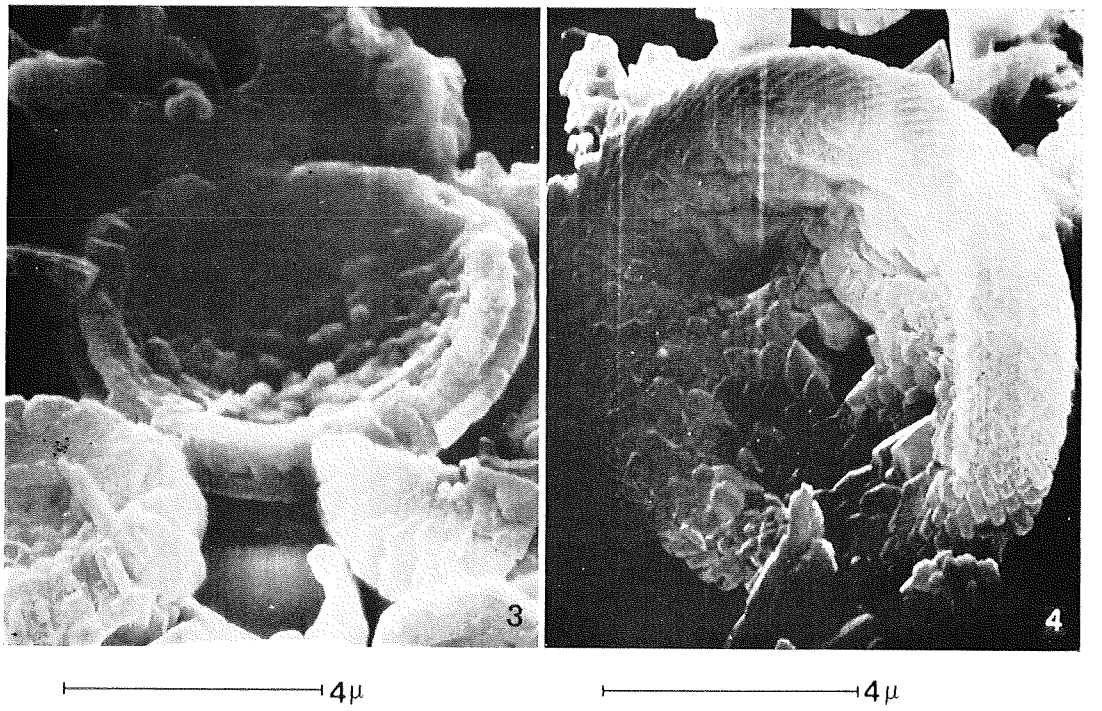


PLATE 52 AND EXPLANATION

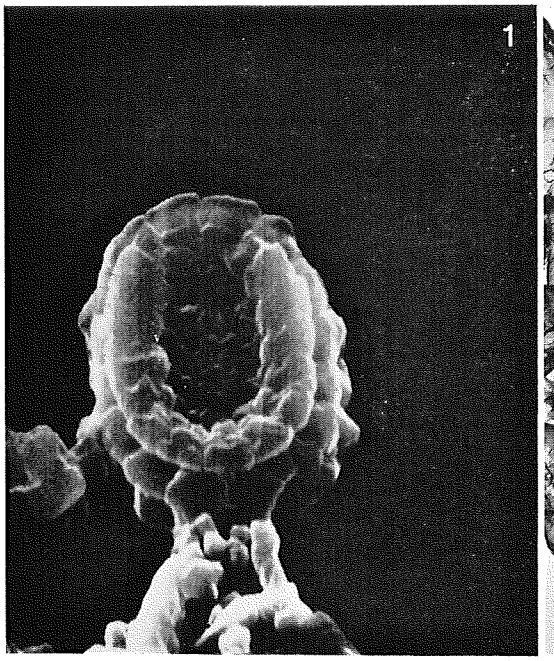
Explanation of Plate 52

The transmission electron photomicrographs of preshadow replicas (figure 2 and 4) and the scanning micrographs of gold-coated specimens of the same species (figure 1, for figure 2 and figure 3, for figure 4). The over-all resolution clearly is better in the transmission photographs; however, the three dimensional arrangement of the constituent crystallites of the coccoliths are better represented by scanning electron micrographs.

Figure 1, 2. Undescribed species of *Zygodiscus*. The middle of the bridge in figure 3, is covered by an unidentified object.

Figure 1, 2. Undescribed species of *Cretadiscus*. The specimen illustrated in figure 1 is titled forward approximately by 35 degree.

Plate 52



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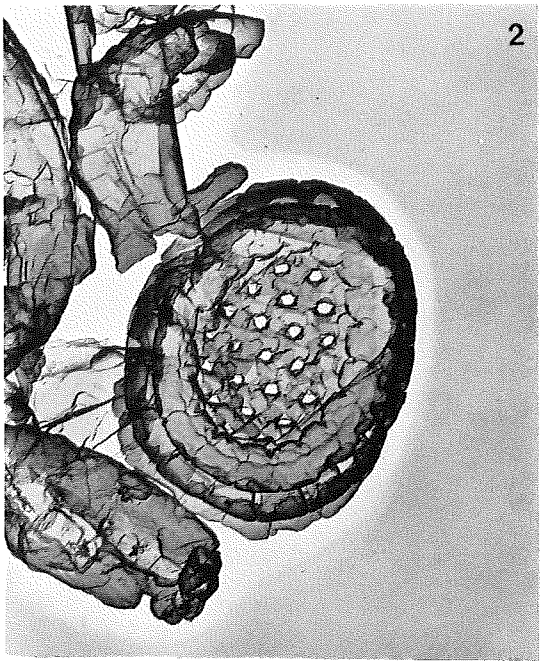

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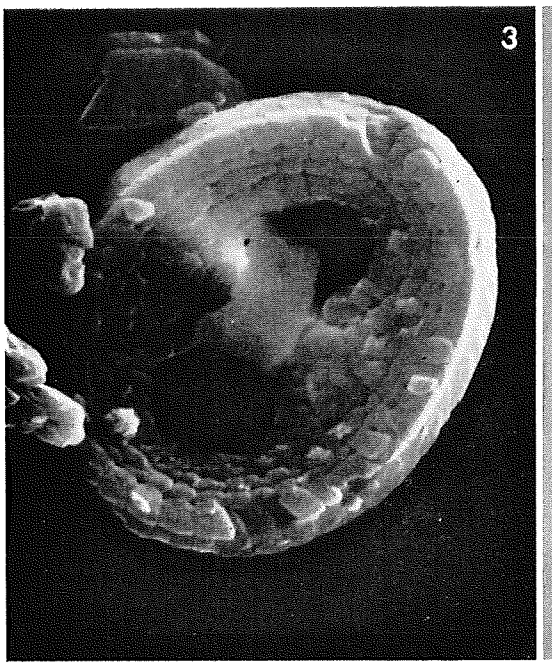

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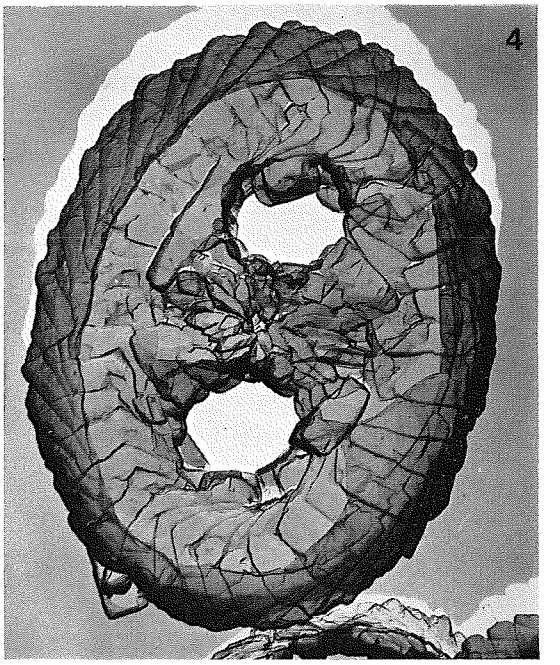

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