

Some Techniques and Applications of Scanning Electron Microscopy in the
Fields of Marine Science*

D. A. WALKER and D. E. BUCKLEY
Atlantic Oceanographic Laboratory, Bedford Institute, Dartmouth, N. S.

Introduction

Many of the specialized research disciplines of marine science involve the study of minute particles or organisms. The nature and detailed structure of these microscopic materials are often the key to important oceanic processes or environments. For this reason much effort is expended by the marine scientist in attempting to obtain data on the size, shape, and ultrastructure of such diverse materials as the testate Foraminiferida (Protozoa), clay minerals, organic rich aggregate particles, and crystals of minerals precipitated on the sea floor.

In recent years the development of the Scanning Electron Microscope (S. E. M.) has demonstrated that this instrument can be used by scientists to obtain images of materials having features in the micron and submicron size range. A concise and descriptive review of the applications of this type of microscopy is given in a recent paper by Kimoto and Russ (1969). A simplified explanation of the functions of the S. E. M. may be found in a paper by Gillott (1969). A few references dealing with applications appropriate to the sciences of biology, micro-paleontology, and geology are: Bé (1968), Small and Marsalek (1969), Hay and Sandberg (1967), Bartlett (1968), Honjo and Berggren (1967), Heslop-Harrison (1968), Gillott (1969), and a paper which appeared in Machine Design, July 1969, author anonymous.

A JEOLCO JSM-2 Scanning Electron Microscope was installed at the Atlantic Oceanographic Laboratory, Bedford Institute in the early part of 1969. Since this installation considerable experience has been gained with respect to applications of this instrument to the needs of marine scientists, and several techniques for mounting and viewing specimens have been tested. This paper describes some of these techniques and specific applications, illustrated with a few examples of photomicrographs.

Specimen Preparation

All specimens were mounted on copper or aluminum blocks. The blocks were prepared by gentle polishing of one end with fine emery paper in order to remove any grooves or scratches formed as a result of cutting the blocks. For ease of orientation, once the blocks have been placed in the S. E. M. , quadrants may be scratched on the polished surface and each quadrant numbered at the intersection. The blocks were then finally polished with a fine liquid abrasive (e. g. Brasso) and placed in an organic solvent, such as CCl₄, to remove contamination due to handling.

Sample preparation prior to mounting usually consisted of washing with deionized water in order to remove salts normally present in sea water which would crystallize upon drying. All samples were dried completely, generally in a desiccator. In the case of foraminifera collected from plankton tows, there is usually an abundance of organic material adhering to the tests. Such samples were treated in a low temperature asher (e. g. Tracerlab LTA 600) to remove all organic material by an oxidation process.

The specific technique of mounting the samples on the block depended on the type and size of material examined. In mounting foraminiferal tests the block was first coated with a thin film of collodion (C. A. Godden, A. O. L. , oral communication) to render the surface adhesive. The collodion was allowed to dry for about 5 minutes. The foraminiferal tests were then transferred to the block using a fine camel hair brush moistened with iso-amyl acetate. The small amount of iso-amyl acetate adhering to the test acted as a solvent for the collodion, allowing the tests to be cemented to the block. The tests were spaced over the block in such a way that during the subsequent metallic coating process one test did not shadow another.

Clay minerals were mounted directly onto the polished surface of the block. No adhesive was needed to mount particles smaller than 10 . In the event that extremely small clay

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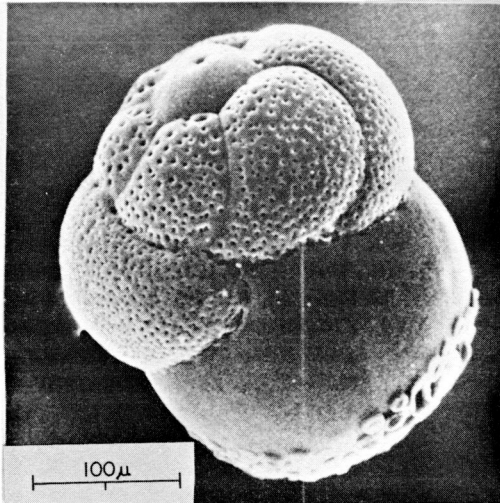


FIGURE 1 - *R. globularis* - view of entire test of pelagic phase, dextrally coiled test proper on upper portion of photograph, float chamber on lower portion, x 260.

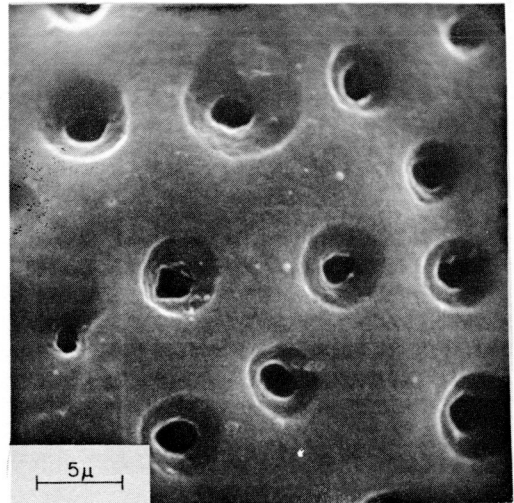


FIGURE 2 - *R. globularis*, pore structure on outer surface of test proper, x 3,000.

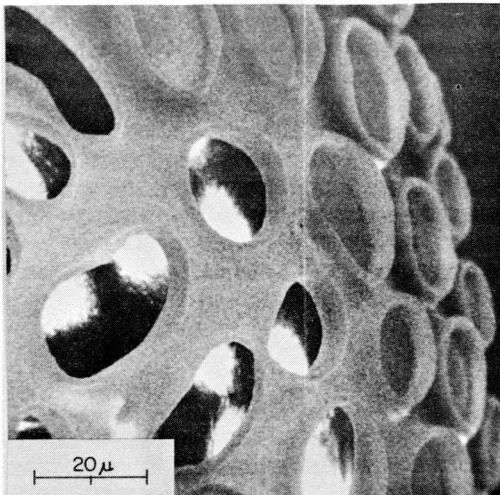


FIGURE 3 - *R. globularis*, embossed float chamber pores, showing protective membrane intact in pores on right side of photograph; inner gas filled chamber is visible through pores lacking the organic membrane, x 1,000.

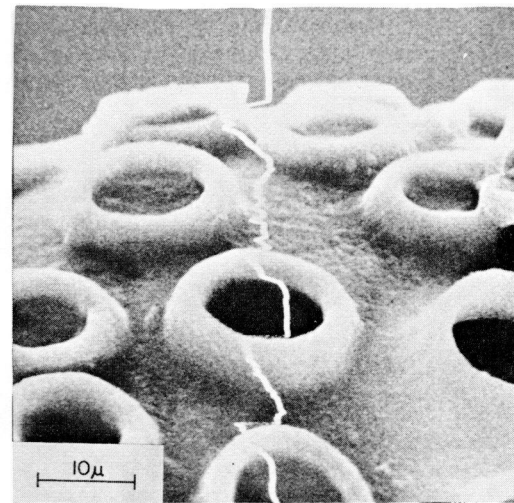


FIGURE 4 - *R. globularis*, line profile mode is illustrated, enabling measurements to be made of height of embossed portion of pores, x 1,700.

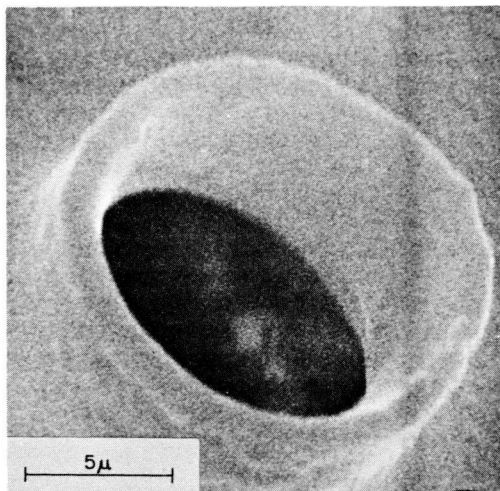


FIGURE 5 - *R. globularis*, single embossed float chamber pore, x 5,250.

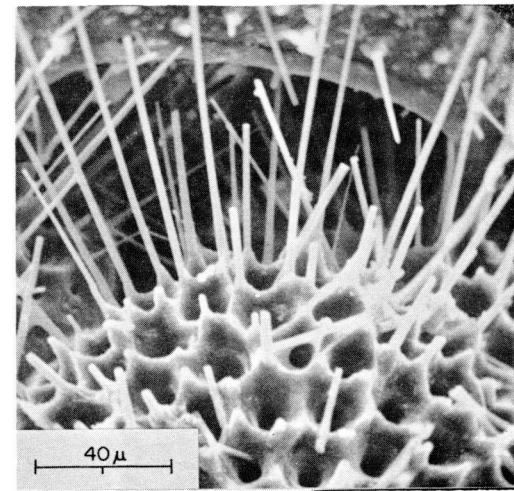


FIGURE 6 - *Glo sacculifer*, great depth of field is illustrated here in view of apertural region, X600.

particles were to be studied (e. g. $< 0.5\mu$), the surface of the block was coated with gold in order to fill any minute scratches left from polishing. It proved desirable to avoid the use of an adhesive coating, such as collodion, as it tended to warp and crack when the small clay particles were viewed under high magnification ($> \times 10^4$). A very dilute suspension of clays (< 10 mg/litre) was prepared in deionized water, and one or two drops of this suspension transferred to the specimen block using a Pasteur pipette. The sample was then desiccated at room temperature, and finally heated to 65°C to complete the drying process.

Small chips of whole rock samples were prepared by drying to remove water in pores and void spaces. The chips then were cemented to the block surface with a small amount of electronic silver paint (e. g. Dotite). The surface of a whole rock sample should be a fresh fracture surface that has not been abraded or smoothed by any means.

Some experience was gained in mounting other types of material which include diatoms, marine organic particulate matter, polyhedral protein inclusion bodies in insect cells, human teeth, and metal fractures. In all of these cases the method of preparation was one of those described above, except for certain organisms, such as diatoms, which were freeze-dried prior to mounting (Small and Marsalek, 1969).

Once mounted on the block specimens were coated with a thin film (e. g. $200-500 \text{ \AA}$) of metal, either gold or aluminum, to render the sample electrically conductive. We have found that most geologic materials can be viewed at magnifications less than $\times 3,000$ without metallic coating. This may be an important consideration in cases where it is desirable to obtain electron or X-ray spectrographic data simultaneously with the imaging capabilities of the S. E. M.

Metallic coating was accomplished by evaporating a small amount of metal in a high vacuum evaporator in which the mounted specimens were placed. For our purposes an Edwards Model 3 High Vacuum Evaporator was used in which a vacuum greater than 10^{-4} Torr was necessary for satisfactory distribution of evaporated metal. We have found that aluminum coating was satisfactory with respect to image quality, but was subject to deterioration due to oxidation. Sylvester-Bradley (1969) has found that aluminum coating is, in some respects, better than gold in that specimens may be retrieved more easily for other types of studies.

Applications

1. Biological and Micropalaeontological:

Studies of the ultrastructure of the testate foraminifera can be applied to problems in the fields of micropalaeontology and marine ecology. Perhaps one of the greatest uses of the S. E. M. in the micropalaeontology of foraminifera will be to completely revise the taxonomy of this order, as suggested by Bartlett (1968). In the field of marine ecology Bé (1968) has suggested that shell porosity of planktonic foraminifera varies according to the latitudinal location of the various species, in that there is an increasing gradient of shell porosity from the polar to the subpolar, temperate, subtropical, and tropical planktonics. Bé anticipates being able to detect climatic changes through the geologic ages based on such information.

A description of ultrastructural features which have been studied using the S. E. M. follows. Figures 1-5 are scanning electron micrographs of the calcareous tests of the foraminifer *Rosalina globularis* (*Tretomphalus bulloides*). Figure 1 illustrates the adult gamont (pelagic), the dextrally coiled portion being the test proper; the large rounded chamber on the lower portion of the photograph is the float chamber, possessing embossed pores through which the gametes are released for gametogamic reproduction. Figure 2 shows, at a higher magnification, representative pores on the outer surface of the test proper; these pores are, on the average, $3-5\mu$ in diameter, depending on the particular chamber on which the pores are located. This photograph illustrates the calcite layering of the test, showing three well-defined layers; similar evidence is given in transmission electron micrographs of *Rosalina floridana* (Angell, 1967). Figure 3 illustrates the embossed float chamber pores. The organic membrane, which seals these pores from the external environment until such time as the gametes are released, is still present in some of the pores (shown on the right side of the photograph). Looking through the pores lacking the protective membrane, one is able to view the outer wall of the inner gas-filled chamber. The float chamber pores are considerably

larger than those of the test proper, about 10-12 μ inside diameter and 15-18 μ outside diameter. Figure 4 illustrates the line profile lode of the S. E. M., which is achieved by stopping the horizontal scan of the electron beam. With this capability one is able to study surface topography. For example, a measurement of about 6 μ is obtained for the height of the embossed pores on the float chamber. Figure 5 is a further enlargement of a single float chamber pore, illustrating the embossed lip. One of the advantages of the S. E. M. is the depth of field available, as illustrated in figure 6 which shows the apertural region of a spinose planktonic foraminifer Globigerinoides sacculifer.

2. Mineralogical:

Data obtained for identifying discrete minerals or for quantitative analyses of mineral assemblages have been obtained most commonly by means of the optical petrographic microscope or X-ray diffraction equipment. Both methods require considerable time for preparation of samples and also require skill and experience on the part of the analyst to make the correct identifications. There are some disadvantages in the use of these techniques which can be overcome to some extent by use of a combination of X-ray analysis, petrographic microscope observation, chemical tests, and examination in the scanning electron microscope.

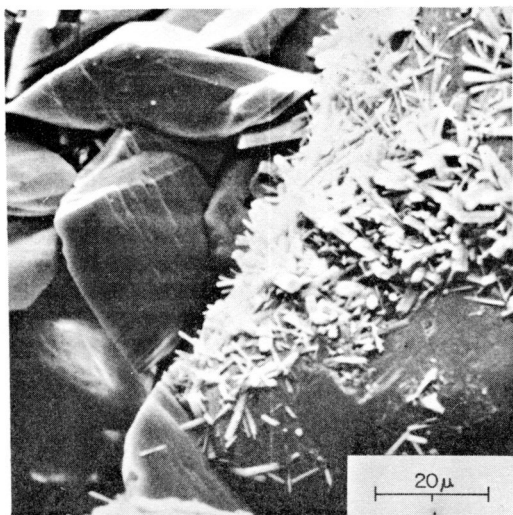


FIGURE 7 - Fracture surface of carbonate cemented sandstone with small crystals of apatite minerals on surface of quartz grain (right half of photograph). Carbonate crystals are siderite. x 1,000.

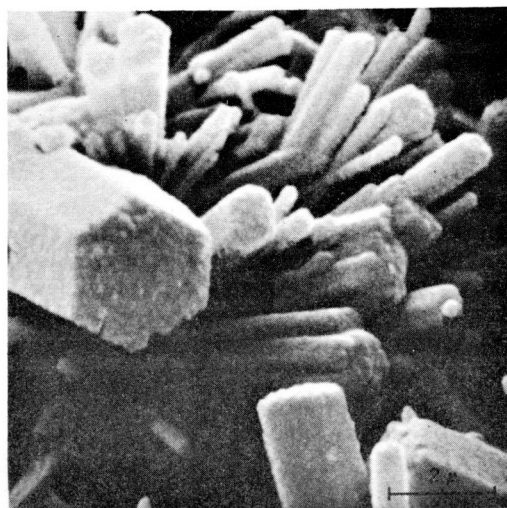


FIGURE 8 - Apatite crystals on fracture surface of carbonate cemented sandstone. Crystals are typically well formed with clusters oriented in subparallel directions. Granular appearance may indicate some solution. x 10,000.

An example of the use of the S. E. M. to resolve the mineralogy of a minor constituent in a marine carbonate rock is illustrated by figures 7 and 8. This particular specimen had been examined as a thin section on a polarizing petrographic microscope and was observed to be a carbonate cemented sandstone (King et al. 1970). A powdered sample of the same material when analysed by X-ray diffraction was found to contain about 15% apatite $[\text{Ca}_5(\text{F}, \text{Cl})(\text{PO}_4)_3]$ minerals, although no apatite minerals could be seen in thin sections of the sample. The sample was then chemically tested for phosphate content which confirmed the presence of phosphate. When a small chip of the sample was examined in the S. E. M. the X-ray and chemical data were confirmed, and details of the relationships between the minerals of the rock were determined.

Figure 7 is a view of a quartz grain on the right half of the photograph which is encrusted with delicate long prismatic crystals of apatite. The crystals do not appear on the surfaces of the carbonate mineral (siderite), indicating two distinct phases of precipitation. Figure 8 is a more highly magnified view of the apatite crystals and shows the typical unstrained hexagonal crystals. Examination of these photographs yields an explanation as to why these crystals could not be seen in thin section. The average size is considerably smaller than can be seen through a petrographic microscope. In addition the cutting and grinding action would have abraded the fragile crystals from the surface of the larger detrital grains. The time required to make the S. E. M. observations was less than either that by the petrographic microscope or the X-ray diffractometer.

3. Size Analysis:

This type of analysis is applied through practically every branch of science. The methods have become extremely varied and complex. Size analyses have been conducted by means of sieves, settling tubes, X-ray, conductivity cells, and microscopes. Each method measures a slightly different aspect of the particle size, and each method has certain limitations as to the range of sizes that can be analysed. Several of these methods determine size based on the mean diameter of equivalent spheres (e. g. settling tube, conductivity counter, sieves), however these methods are unable to account for variable shape factors. Only a method capable of measuring along three axes will yield true size and shape data. Normally three-dimensional measurements can only be made megascopically, however this capability does exist with the use of the S. E. M.

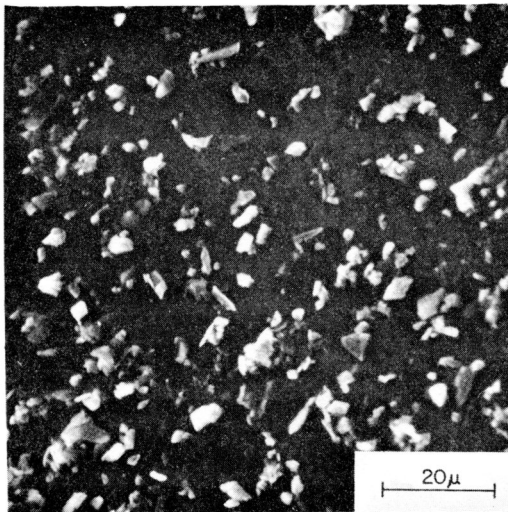


FIGURE 9 - Dispersed clay sized minerals (2-4 μ). Mineral assemblage consists of biotite, chlorite, hornblend, feldspars, and quartz. Sample was dispersed on an aluminum block. x 1,000.

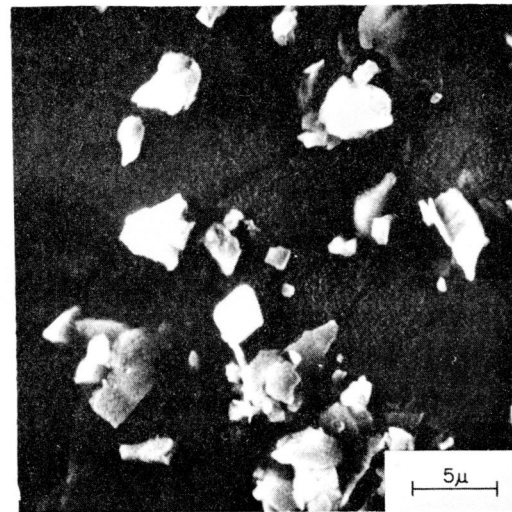


FIGURE 10 - Detrital grains of biotite (thin plates lower centre and upper right), chlorite? (partially curled plate at right), feldspar rhomb at centre, x 3,000.

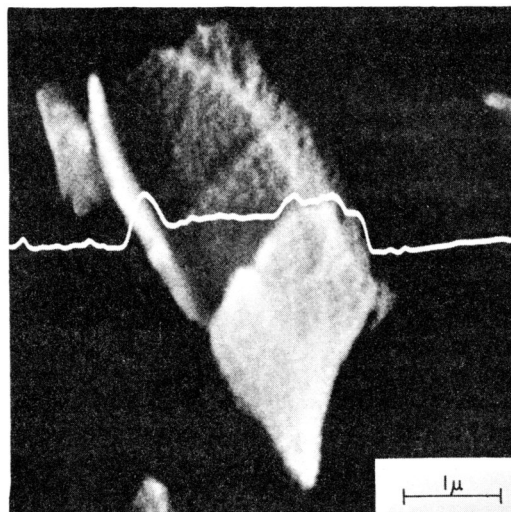


FIGURE 11 - Layered silicate partially curled as result of dehydration (chlorite?). Line profile through centre of photograph gives graphic display of thickness of particle. x 17,000.

The most difficult analysis for all these methods is one involving nonspherical particles that are in the colloidal size range. Natural clay minerals ($< 4\mu$) are extremely difficult to analyse because they are tabular plates, and because they behave as a colloid in electrolytes. Use of the S. E. M. for particle size analysis of clay minerals is demonstrated by figures 9, 10, and 11. These photographs are views of clay minerals dispersed on aluminum blocks. The suspensions from which the clay minerals were taken were dilute (ca. 10 mg. /litre) and were salt free. It can be seen that there was practically no flocculation of the clay minerals as the aqueous suspension was dried on the sample block. Figure 9 shows an assemblage of clay minerals (chlorite, mica), and some amphiboles and quartz having a diameter of 2-4. Figure 10 is a more highly magnified view of the same sample. It can be seen that some details of the crystallinity can be resolved at this magnification. Figure 11 illustrates the use of the line profile technique referred to earlier. Here the line profile can be employed to obtain an estimate of the topography and thickness of the particle.

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