SCANNING ELECTRON MICROSCOPY OF UNCOATED FOSSILS

by P. D. TAYLOR

ABSTRACT. The necessity of coating fossils with a conductor prior to scanning electron microscopy is avoided using a system in which backscattered electron images are formed of specimens maintained under a relatively low vacuum in an 'environmental chamber'. Resolution and other image characteristics at low magnifications ($< 500\times$) generally compare favourably with conventional secondary electron images of coated specimens. Charging artefacts are reduced, edge effect is eliminated, and the backscattered electron image appears flatter than a conventional secondary electron image. As well as minimizing sample preparation time, the system is valuable in allowing scanning of fossils for which coating is either undesirable (e.g. type specimens) or difficult (e.g. large specimens).

COMMERCIAL availability of the scanning electron microscope (SEM) during the past twenty years has revolutionized studies of fine-scale morphology. Applications of the SEM in palaeontology have been widespread. The SEM is a routine tool in micropalaeontological studies and during studies of skeletal ultrastructures in macrofossils. Conventional SEM techniques necessitate mounting fossils onto stubs and coating the surface with a conducting material, usually gold or gold-palladium. Although techniques are available for removal of these metallic coatings (Hansen 1968), they can be time-consuming and hazardous to the specimen. Therefore, once applied, coatings are usually looked upon as permanent. Restudy of coated specimens using an optical microscope is difficult because of the high reflectance from the specimen surface. It is common curatorial practice to discourage or even prohibit coating of important specimens such as types, thereby excluding their study with a conventional SEM.

Five years experience has now been gained at the British Museum (Natural History) with a system for scanning uncoated specimens which uses an environmental chamber in conjunction with a backscattered electron detector (Buchanan 1983). The availability of this system is not widely known despite its major advantage in permitting scanning of types, etc. without alteration. The objectives of this paper are to describe the principles of operation of this system, compare images obtained with those of conventional SEM images, and discuss some applications.

PRINCIPLES

To understand the operation of the system for uncoated specimens it is necessary to give a brief account of some of the principles of scanning electron microscopy. A modern text such as Goldstein *et al.* (1981) should be consulted for details.

SEM images are formed by scanning a narrow beam of electrons across the surface of a specimen, collecting and processing the emitted electrons, and displaying them on a cathode ray tube using a visual raster which is synchronized with the beam scan. When an electron beam strikes a specimen, a complex interaction takes place and several kinds of emission occur. For the purpose of scanning electron microscopy the most important emissions are secondary electrons (SE) and backscattered electrons (BSE). SEs are shallow, low-energy emissions resulting from inelastic events which transfer energy from the electron beam to the specimen. BSEs are deeper, high-energy emissions resulting from elastic events during which there is no energy transfer between beam and specimen. Usually two to five times more BSEs are emitted than SEs (Buchanan 1983). Conventional SEM images

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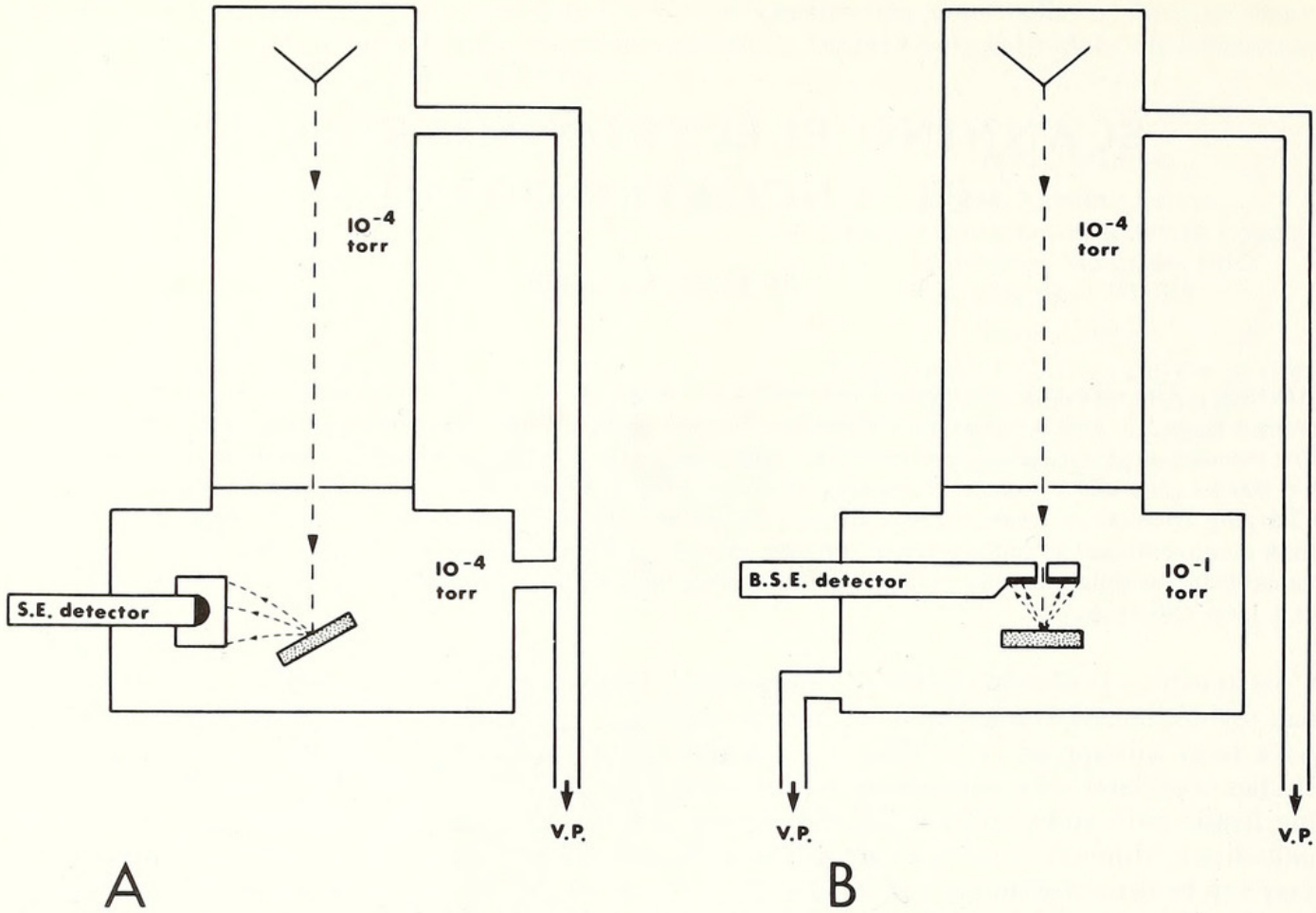

TEXT-FIG. 1. Diagrams showing the essential features of a SEM: A, operating conventially using secondary electrons for scanning coated specimens: B, adapted for scanning uncoated specimens using backscattered electrons. VP = vacuum pump; SE = secondary electron; BSE = backscattered electron.

comprise mostly SEs which are attracted to a detector, generally an Everhart–Thornley scintillator-photomultiplier, positively charged and located to the side of the specimen (text-fig. 1A). Although some BSEs are also detected, high resolution BSE imaging requires a special detector. The system described here for uncoated specimens uses a scintillator BSE detector. Unlike the SE detector, this detector is uncharged and is positioned directly above the specimen (text-fig. 1B).

Conventional SEM requires that the specimen chamber as well as the microscope column be maintained at a high vacuum (about 10^{-4} torr) which prevents electrical discharge and interference with the electron beam by air molecules. The system for scanning uncoated specimens also operates with a high vacuum in the microscope column but has a separate vacuum pump for the specimen chamber ('environmental chamber') which is held at a relatively low vacuum (about 10^{-1}–10^{-2} torr). The electron beam passes through a 200 micron aperture (text-fig. 1B) which is sufficiently small to allow the differential vacuum between column and chamber to be maintained. The strong positive charge of SE detectors prohibits their use in conjunction with an environmental chamber in which the low vacuum would cause electrical discharge.

Non-conducting specimens, for example, the great majority of fossils, must normally be coated with a conducting material prior to scanning in order to prevent charging and enhance electron emission. Uncoated specimens irradiated by an electron beam accumulate a negative charge which can cause image distortion or at best bright spots of enhanced emission on the image. Coating allows this charge to run to earth via the specimen stub. Satisfactory images of uncoated specimens

(Howden and Ling 1974) may sometimes be obtained by using a low beam voltage (*c.*5KV) but resolution is usually poor. In the system for scanning uncoated specimens residual air molecules present in the specimen chamber dissipate charge on the specimen by ionization. Argon gas can be introduced into the chamber to assist this process. The specimen need not be grounded via the stub and coating is therefore unnecessary.

IMAGE CHARACTERISTICS

There are considerable differences between conventional SE images of coated specimens and BSE images of uncoated specimens. These differences must be appreciated when interpreting morphology from the microscope screen or from micrographs. Therefore a specimen of the cheilostome bryozoan *Metrarabdotos moniliferum* (Milne Edwards) from the Pliocene Coralline Crag of Suffolk was selected for a comparative study. The specimen was first examined uncoated using BSEs, and then coated with gold-palladium and re-examined using SEs. Micrographs obtained by the two methods at three magnifications (× 30, × 150, × 550) are shown in Plate 52 (see Cheetham (1968) for optical micrographs of *M. moniliferum*). The beam voltage used for both series was 15 KV, specimen working distance was the same, and brightness and contrast level equivalent.

At low magnification resolution appears to be about the same for the uncoated as the conventional coated image. However, resolution tends to become noticeably inferior to that of conventional images at magnifications above about 500 ×. Uncoated BSE images in excess of 1K × have rarely proven satisfactory with the system in operation at the BM(NH).

SE images are considerably more three-dimensional than BSE images. This is well-illustrated by comparing figs. 1 and 2 of Plate 52. Zooecial frontal walls appear relatively flat in the BSE image but ridged and elevated in the SE image. This important difference in image characteristics must be taken into account when interpreting morphology from the SEM. BSE images of coated specimens are similarly flat. The cause of the difference may be the relative locations of BSE and SE detectors (text-fig. 1); BSE detectors are positioned directly above the specimen whereas SE detectors are positioned laterally to the specimen and receive more electrons from the side of the specimen that is closer.

Specimen edges and protuberances (e.g. spines) produce high levels of emission in SE images. This 'edge effect' is absent from BSE images. For example, compare the fractured left-hand edge of the specimen in Plate 52, figs. 1 and 2. Edge effect is particularly pronounced in SE images of specimens on a black background. The bright circumference of specimens in SE images contributes greatly to the aesthetic appeal of scanning electron micrographs. However, lack of edge effect in BSE images means that they are closer to the appearance of specimens viewed with an optical microscope.

Electron emission from cavities or depressions in specimen surfaces is greater in SE images than BSE images. For example, the areolar pores are brighter in the SE image shown in Plate 52, fig. 4 than the corresponding BSE image of Plate 52, fig. 3. The uncoated BSE image may be regarded as superior in lacking artificially high levels of pore brightness that would not be seen with an optical microscope. Additionally, charge accumulation within pores where coating may be inadequate is a problem of many coated SE images (e.g. Pl. 52, fig. 6). This is invariably absent from BSE images (e.g. pl. 52, fig. 5).

Whereas the relative brightness of SE images depends mostly on specimen relief, that of BSE images is determined also by the elemental composition of the specimen. This high atomic number contrast has been utilized extensively in BSE studies of clay mineralogy (e.g. Pye and Krinsley 1983) and may also be valuable in some palaeontological contexts, for example, to enhance the distinction between fossils embedded in a matrix of a different composition. However, high atomic number contrast can be disadvantageous in emphasizing the presence of adherent particles of dirt, sediment, or glue (e.g. Taylor 1984, fig. 1B).

Most BSEs are emitted at high angles to the specimen surface; the number of BSEs emitted at low take-off angles diminishes rapidly. Therefore with the BSE detector positioned directly above

the specimen, most BSEs are detected from subhorizontal surfaces of the specimen (i.e. surfaces perpendicular to the electron beam) and few from subvertical surfaces (i.e. surfaces parallel to the beam). Relatively flat, untilted specimens produce the most satisfactory images whereas images of specimens of high relief or tilted specimens can be unsatisfactory. Perspective views and stereo pairs can be less successful of uncoated specimens using BSE imaging.

DISCUSSION

There are many applications of BSE imaging of uncoated specimens. Most importantly it allows scanning of specimens for which coating is not permitted or is deemed undesirable. For example, type specimens can be examined with the SEM without alteration or damage. Increasing use of fine scale morphological features in taxonomic discrimination means that SEM study of types is becoming crucial to species characterization in some groups. For example, SEM study of the uncoated holotype of the Cretaceous cheilostome bryozoan *Charixa vennensis* Lang revealed that the spine bases supposedly diagnostic of the species were not present (Taylor, in press). Even when optical microscopy is capable of resolving the detailed morphology of type specimens it may be impossible to record these details adequately by photo-micrography in which depth of field is a problem. SEM micrographs provide an obvious solution to difficulties of illustration.

Although several SEMs are equipped with large specimen chambers, specimens much larger than stub diameter (12 mm) can be impossible to scan because of difficulties in applying an adequate coating to large specimens. Inadequate coating commonly leads to charging artefacts. Uncoated specimens over 10 cm in diameter have been scanned successfully using the system in operation at the BM(NH).

Removing the need to coat specimens not only reduces sample preparation time but also eliminates curatorial problems associated with coated specimens glued permanently to a stub. In the uncoated system, cleaned and dried fossils are simply fixed temporarily onto a stub using plasticine or a similar mounting medium. They can be removed from the stub immediately after scanning; no special provision need be made for storing stub-mounted fossils. Fragile specimens on their original mounts (e.g. card-mounted specimens) can be scanned *in-situ* without risking the damage that removal may entail. Savings in time become especially important when specimens need to be repeatedly coated and scanned after periods of treatment (e.g. etching).

A wide taxonomic range of specimens of differing chemical composition have been scanned successfully using the system in operation at the BM(NH). These include fossils of calcitic bryozoans, goethite-encrusted bryozoans and silicified bryozoans (Taylor and Curry 1985), latex casts and shale impressions of fossil plants (Hill *et al.* 1985), phosphatic problematica (Taylor 1984), fossil ostracodes (Neale 1985), and Recent spicular forminifers (Brönnimann and Whittaker 1983, pl. 4), fish teeth (Greenwood 1983) and insects (Day 1984).

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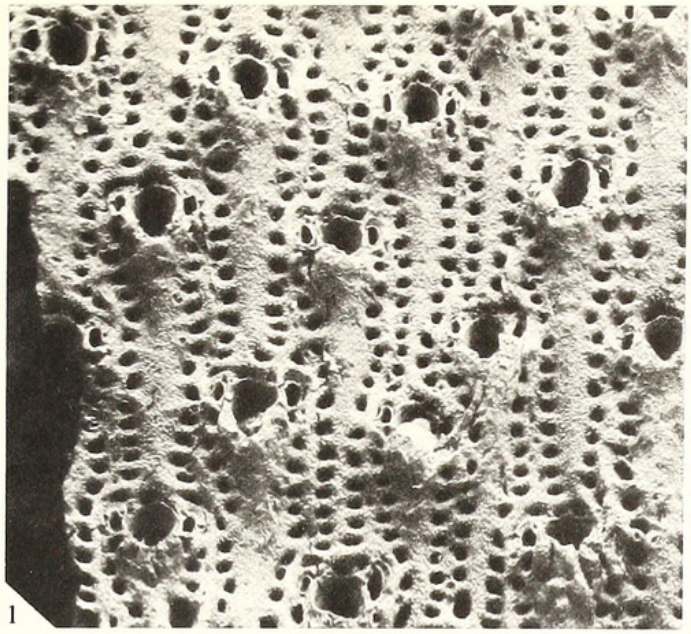
EXPLANATION OF PLATE 52

Figs. 1–6. Scanning electron micrographs of the cheilostome bryozoan *Metrarabdotos moniliferum* (Milne Edwards) from the Pliocene Coralline Crag of Gedgrove, Suffolk; British Museum (Natural History) D54322. 1, 3, and 5 are backscattered electron images of the specimen uncoated: 2, 4, and 6 are conventional secondary electron images of the specimen coated with Au-Pd. 1, 2, colony surface; the frontal walls of the autozooecia appear more convex in the SE image, and enhanced emission (edge effect) causes the fractured edge of the specimen (lower left) to appear bright, × 30. 3, 4, autozooecial orifice flanked by adventitious avicularia; note the increased brightness of the areolar pores in the SE image relative to the BSE image, × 150. 5, 6, adventitious avicularium (left) and areolar pores; the BSE image has poorer resolution but lacks the charging artefact present in the lowermost areolar pore of the SE image, × 550.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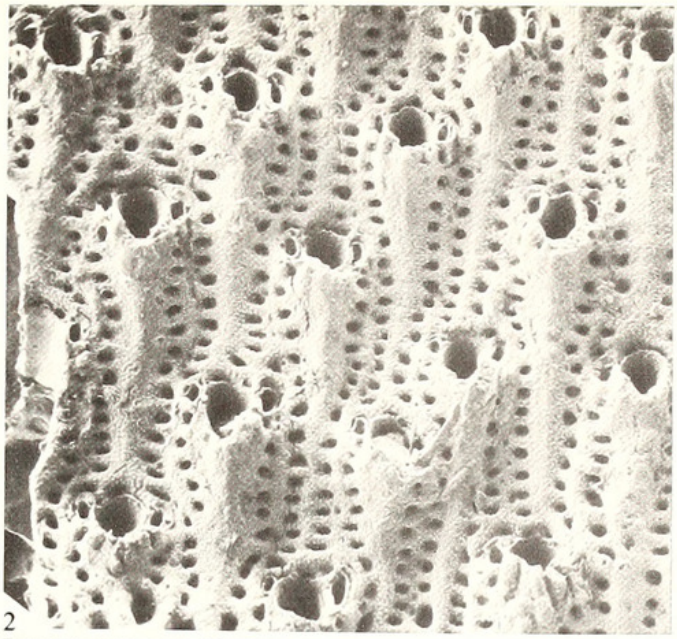
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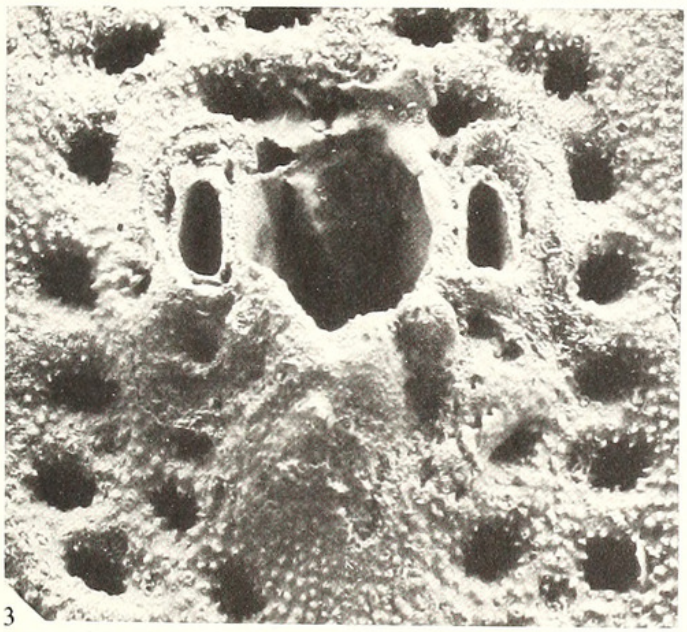
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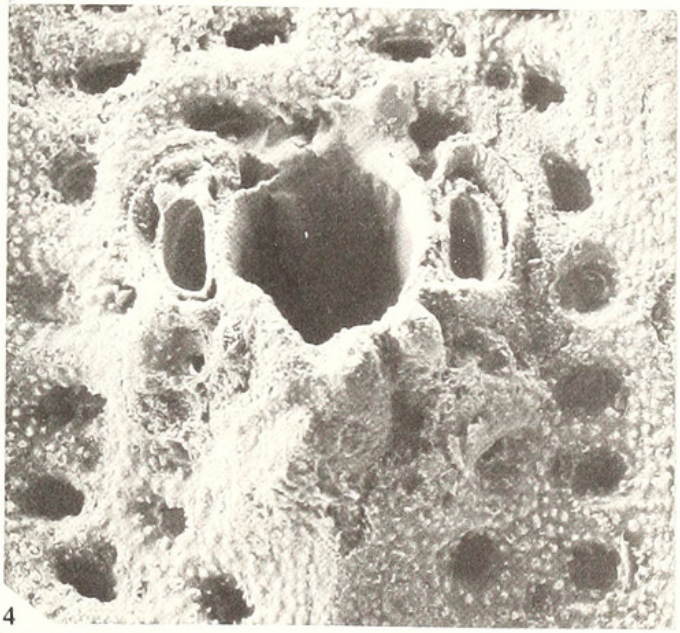
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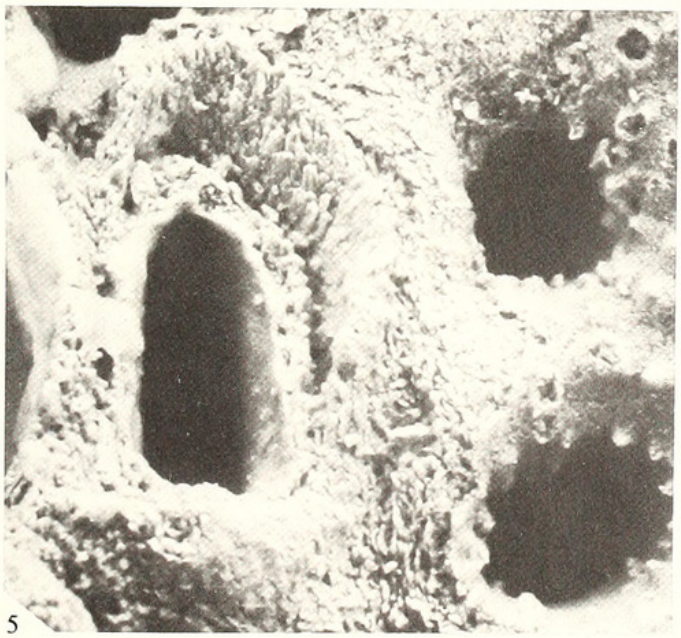
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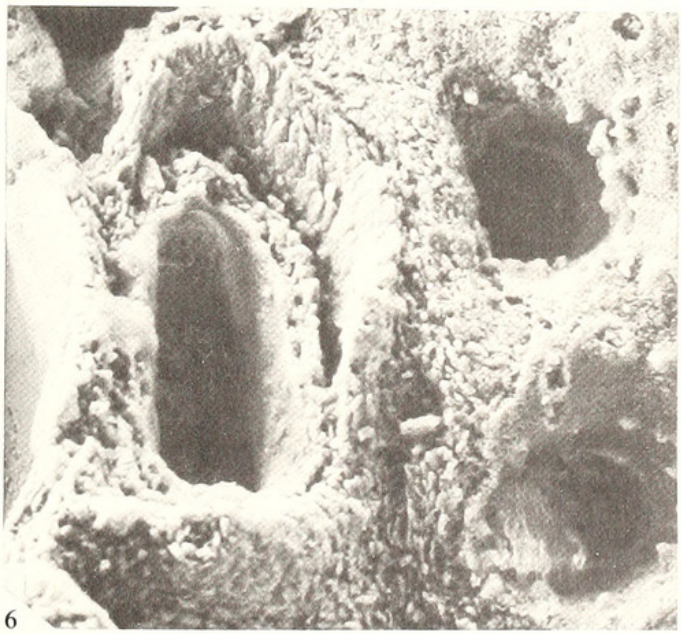
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APPENDIX

Instrumentation. The equipment used at the BM(NH) for scanning uncoated specimens consists of an ISI 60A SEM fitted with an ETPSEMRA Robinson Detector (for BSE) and a CFAS unit (charge free anticontamination system which provides the low vacuum environmental chamber for the specimen). These are marketed by Expo-SEM, Moat Farm, Church Road, Milden, Ipswich, Suffolk IP7 7AF. The Robinson Detector is available for most makes of SEM, and the possibility of developing CFAS units for SEMs other than the ISI are being explored (A. J. Ditheridge, pers. comm. May 1985).