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| Author(s) | Honjo, Susumu; Okada, Hisatake |
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# SCANNING ELECTRON MICROSCOPY OF PLANKTONIC FORAMINIFERA: A PREPARATION TECHNIQUE

*by*

Susumu HONJO* and Hisatake OKADA*

(with 2 Text-figures and 3 plates)

(Contributions from the Department of Geology and Mineralogy, Faculty of Science, Hokkaido University. No. 1071)

A scanning (electron) microscope is particularly useful to observe a minute and solid specimen or complex surface topography of a bulk specimen with the dimension far less than the optical limit. A scanning microscope can enlarge a specimen into less than a hundred times to as large as a hundred thousand times with continuous shift of magnification. The brightness of image is almost constant under different magnification. The depth of focus of a scanning microscope is significantly deeper than that of right optical microscope. A scanning microscopic image provides an observer particularly an excellent feeling of three dimensios. An introduction of scanning microscopy is mensioned in KIMOTO and HONJO, 1967.

The scanning microscopy has been already applied to a various field of micropaleontology. HONJO, MINOURA and OKADA (1967) observed coccoliths by a scanning electron microscope. Also, HONJO and BERGGREN (in preparation) studied the test surface structure of planktonic foraminifera. The largest advantage of studing such microfossils under a scanning microscope is, the original specimen is kept undamaged and there would never cause a difficulties in the taxonomic procedure of setting type specimen.

The only requirement for the sample preparation is to cover the surface of a sample with a thin film of electro-conductive material with an uniform thickness. The film should cover everywhere of the view field, and it should be electrically connected with the main body of the microscope throughwhere the charged electrons are ground out. There are a few method to deposit a conductive film on an irregular non-conductive surface. A chemical method, such as silver mirror reaction could be employed, although we have not able to obtain a satisfactory result.

The vacuum deposition technique is a well established method to produce a thin conductive film. A piece of heavy metal is heaten by electric currency or a forcused electron bombardment, then the evaporated metalic particles radiate with

* Department of Geology and Mineralogy, Hokkaido University, Sapporo, Japan.

a straight orbit to adhere on any solid surface forming an uniform, conductive film. The film is usually smooth and strong enough; evaporated metallic particles are bond tightly together with van der Waals' force, sometimes crystalline growth is accompanied. It is possible to deposit extremely thin film such as a few angstromes to as thick as hundreds microns. The electric nature of thin metal film is well studied by engineers for modular electronic application.

Since the evaporated heavy metal particles radiate in a straight orbit, the shadowed side of a specimen is left uncovered by such evaporated particles. This effect is called shadowing effect and has been employed to emphasize relief of a specimen surface in replica technique.

The test of a planktonic foraminifera is usually spherical and the surface is covered by a complex topography, and it is impossible to deposit a film with uniform thickness throughout the surface regardless the height of relief.

A rotary shadowing technique has been successful to avoid the shadowing effect. A sample is rotated and swiveled during evaporation to give even opportunities of being faced against the direction of radiation. Our simple rotating stage does not provide exact geometric evenness, therefore, an extra evaporation is usually required. There are several choices of the materials to be deposited. We found that the pure gold wire, with 0.3 mm in diameter, is most easy to handle and yet the best result being obtained.

Although the thickness of the film should be kept as thin as possible to maintain the original topography, it requires a certain thickness to keep the proper electrical conductivity. We found that a gold film of approximately 100 Å is satisfactory for this purpose.

*Specimen stand*

A small piece of brass or a stainless steel rod is used for a specimen stage. The size of a stand to be prepared is decided on the specification of a particular type microscope to be used. For use of our instrument, (JEOL-JSM, 1967), the diameter of rod is 10 mm thick and 12 mm long. A rod should be carefully cleaned with successive ultrasonic cleaning to clean out machine grease. Any evaporative organic materials should be carefully avoided from a specimen stand at any phase of preparation. This is to maintain a better and cleaner vacuum of column space.

The one end of a rod where a specimen or specimens to be mounted, is better finished to avoid bothering machine marks in the background of obtained picture. Or a thin mica flake is used to obtain an extremely smooth surface. A small mica flake, approximately 7 mm by 7 mm, is mounted on one end of a rod with a small amount of non-evaporative adhesive such as epoxy resine. A fresh mica cleavage surface is obtained by peeling the surface flake off immediately before mounting specimens.

*Mounting of specimen*

A clean specimen (or several specimens) is mounted in a required orientation on an end of a specimen stand or on a mica plate with tragacanth glue for regular micropaleontologic research use. We reported the use of Neoplen adhesive solution for mounting in the last paper (HONJO and BERGGREN, 1967, in press), however, we have found the solution more easily contaminates the specimen surface because of its low viscosity. Outgassing from carefully dried tragacanth glue is almost negligible in case it is a very small amount.

*Deposition of metalic film*

A proper amount of thin pure gold wire for use in shadowing preparation in electron microscope, is tightly but broadly coiled around a tungsten radiation source. Mount a specimen stand on a rotary disk of an in-vacuum mother: set the evaporation source at proper position relative to the sample. The radiation source is placed at a position approximately 35 to 40 degrees from the normal to the disk. The disk itself swivels for 15 degrees.

The position relative to the radiation source changes accordingly as the sample swivels around a tilted axis of rotation and encourages the even deposition of metalic film throughout the irregular surface of a sample. The velocity of rotation of the rotary disk has to be decided from the rate of deposition of metalic film. Since the evaporation temperature of gold wire is relatively low and so the evaporation is rapid. When it is started, fast rotation, such as four times every second is prefered.

Introduction of such rotation into a bell-jar without breaking the high vacuum is not an easy task. We have constructed an epoxy-sealed small induction motor with reducing gearhead for this purpose. The parts which require grease lubrication are all replaced by Teflon bearings to avoid outgassing during operation in high vacuum (Text Figure 1).

The apparatus is covered by a bell-jar and evacuated. A vacuum at the lower range of $10^{-5}$ torr is required for better result. The proper rate of deposition for scanning microscopic observation is not well studied but usually takes several second to finish when tungsten heater method is applied for the evaporation of gold wire. A common vacuum unit with a combination of a rotary mechanical pump and an oil diffusion pump, for the use of shadowing practice in electron microscopic sample preparation, can be employed.

Every viewing field should now be covered by a continuous thin gold film. To make sure that the mica plate and specimen stand are electrically connected, a very small amount of electroconductive paint is droped to cover the sidewall of a specimen stand as well as a mica surface. The drop of paint should be dried carefully without remaining the residue of evaporative materials. The electrical con-

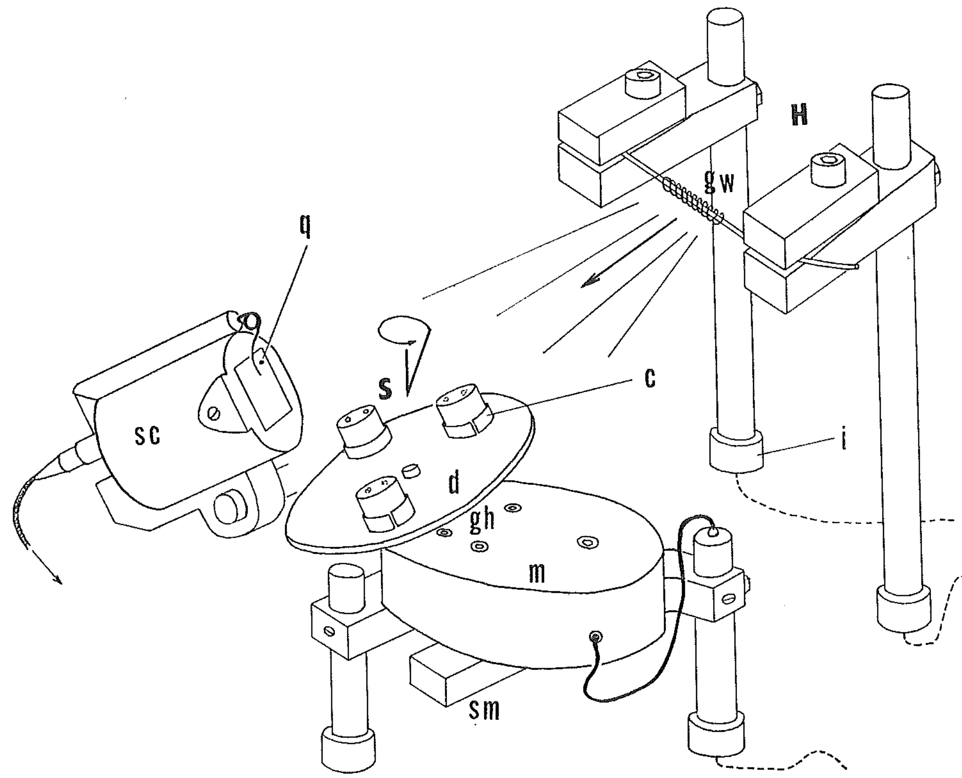


**Fig. 1**
The outfit inside of vacuum belljar.
Specimen stands (S) are placed on a rotary disk (d) with clumps (c), of in-vacuum motor(m). A reducing gearhead and swivel mechanism rotate samples during the gold wire (gw) is evaporated from the heater (H). The deposition of film is monitored by a sensor-head (sc) which indicates the thickness of metal layer deposited on a quartz transducer (q).

ductivity between the vicinity of a specimen and a specimen holder of a microscope should be checked by a proper resistance meter.

*Control of the thickness of metallic film*

The thickness of the deposited metal film is a function of (1) distance between evaporation source and sample, and (2) the mass of evaporating material. The approximate thickness can be known by calculation of the formula provided by BRADLEY (1961).

We have been successfully using a transducer type film thickness monitor.

The resonance frequency of a quartz transducer is reduced with mass increase when evaporated gold is made to adhere to the surface of the transducer. A transducer is placed in the same vacuum during the operation, at the position where it is showered with the evaporated gold particles which are uniformly coating the sample. The change of frequency is detected and transformed into direct current which operates a cut-off relay circuit at a present current which corresponds to a required thickness of film (Text-Figure 2).

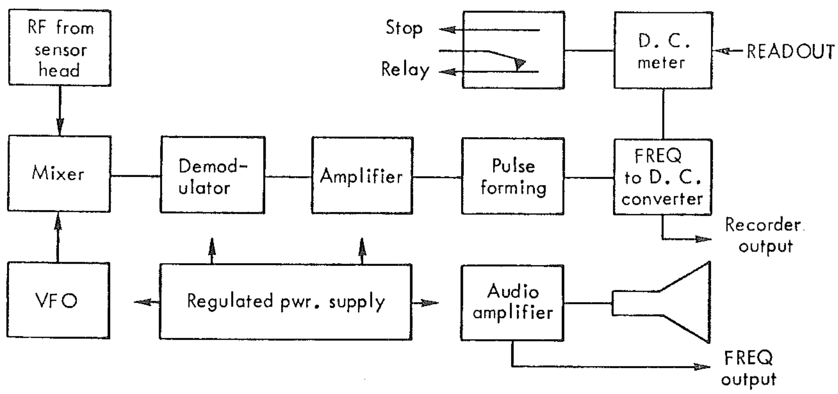


**Fig. 2**
A block diagram of an electronic film thickness monitor.

## *Storage of samples*

The samples are now ready for scanning microscopic observation. Metal coated specimens could be preserved for very long period of time under a normal condition. However, in case future re-examination by a scanning microscope being expected, it is preferred to keep coated specimen stands in a desiccator to prevent a possible chance of growing micro-organisms on the glue material. They should be contained in totally dust free condition. A grease free Fleon gas blower is available through commercial route for cleaning dust on samples.

Gold coating on microfossils encourages the better shadow contrast of optical microscopic image under high magnification. A reflactive optical photomicrograph of gold coated foraminifera test is demonstrated in Fig. 2 and 4, Plate 3, in comparison with the scanning microscopic image of the identical spot.

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PLATE 17 AND EXPLANATION

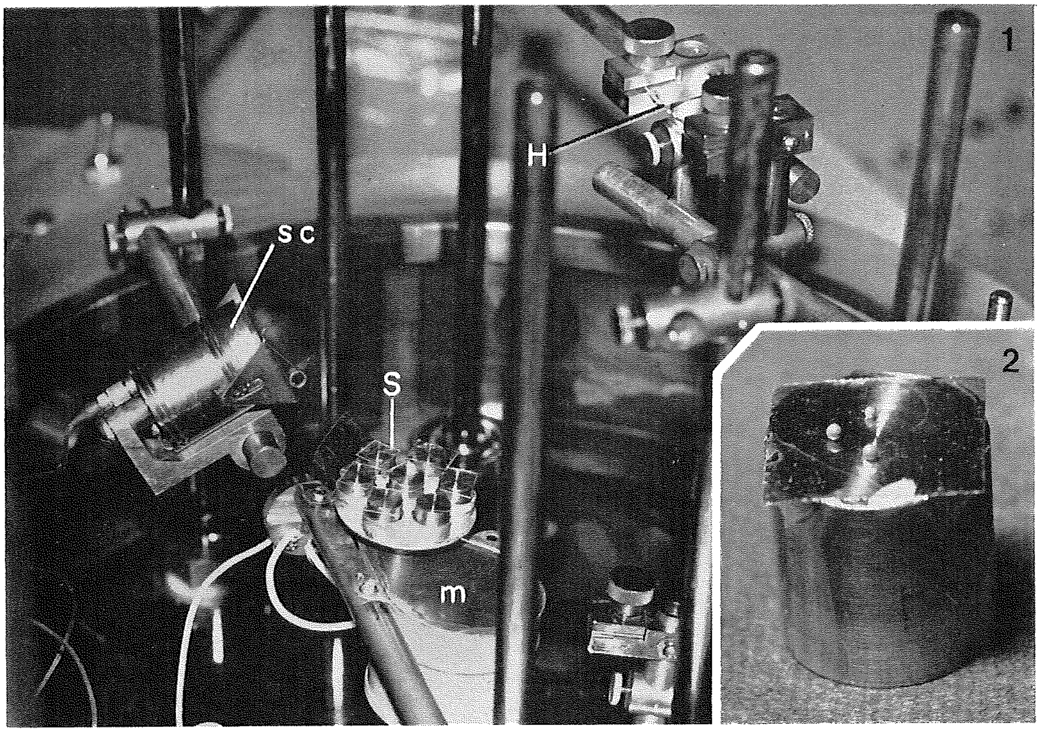
## Explanation of Plate 17

**Fig. 1** Arrangement of sample stands (S), heater unit (H) and sensor module of film thickness monitor. Specimen stands are mounted on a in-vacuum motor with reducing gear head and swivel mechanism.

**Fig. 2** Close up of a specimen stand with mica flake. After a proper thickness of gold film is deposited touch up by conductive paint to secure the connection between a brass stand and evaporated film.

**Fig. 3** An automatic vacuum plant for sample preparation. After setting required vacuum level at preset dial, it is automatically obtained by the electronic valve operation, control unit (cp) and neumatic valve stystem (mv). A relay system of a film thickness monitor (dtm) automatically shut off the heater current when preset film thickness is deposited on a sample.

**Plate 17**



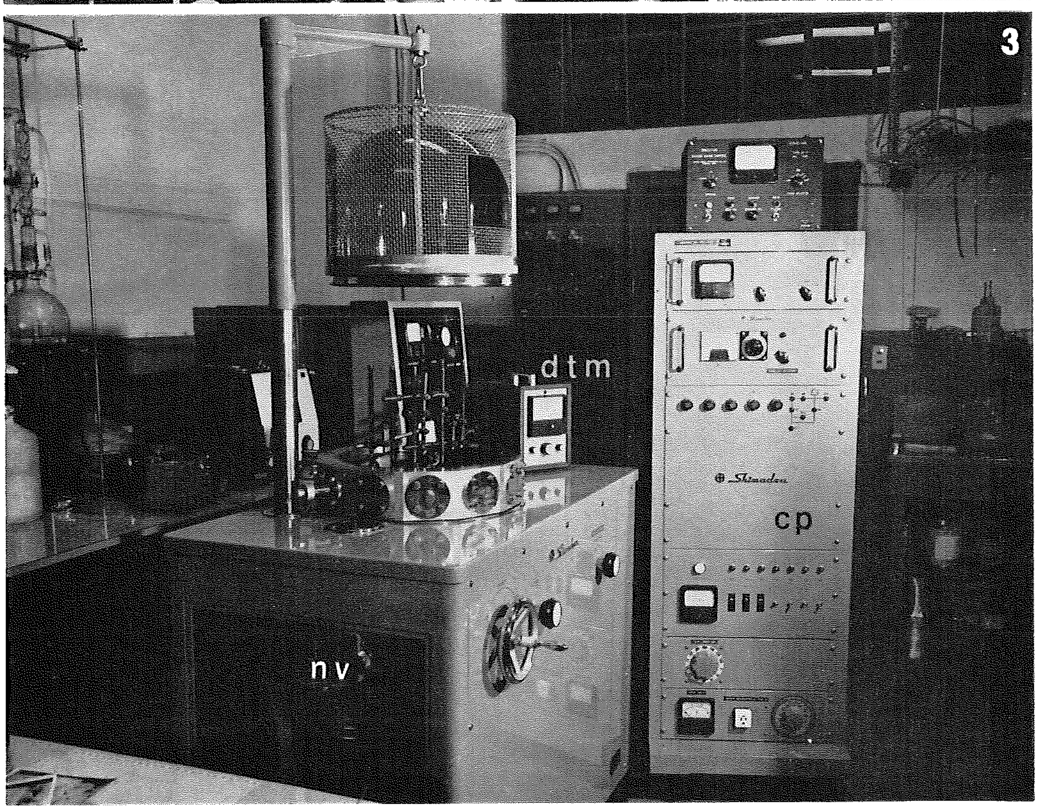



PLATE 18 AND EXPLANATION

**Explanation of plate 18**

Scanning microscopic photomicrographs of *Globigerina chapinani* in a series of different magnification.
The conductive film should cover every surface hopefully in uniform thickness, especially complex topography such as deep holes shown in Fig. 4

Plate 18

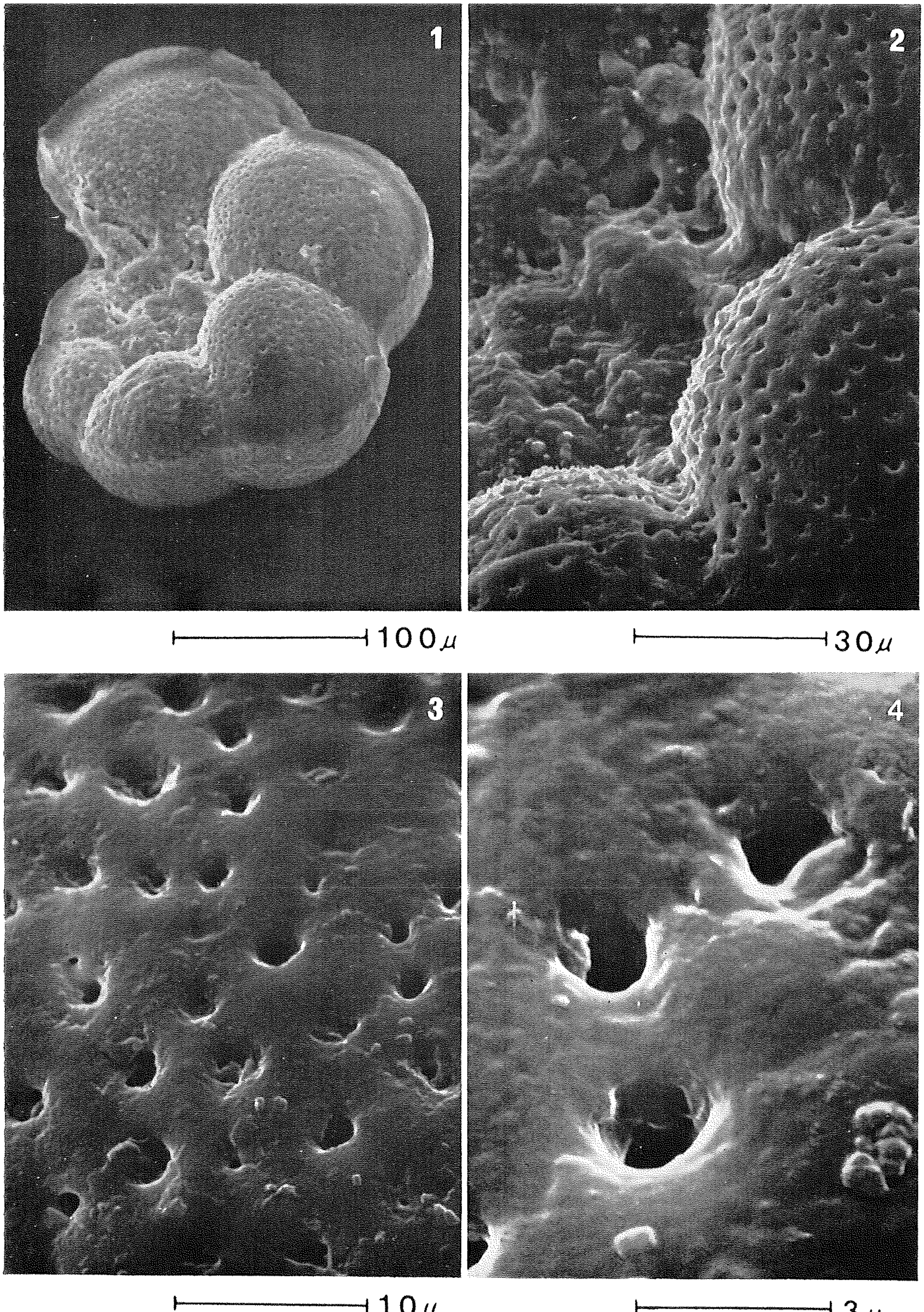


PLATE 19 AND EXPLANATION

## Explanation of Plate 19

Scanning microscopic and reflective optical images are compared. Specimens shown here are all coated by gold film. The depth of focus is overwhelming in scanning photomicrograph either low (Fig. land 2) or high (Figure 3, 4) magnification. The reflective photomicrographs are taken by an Ultrapark type microscope.

Fig. 1 and 2 *Globigerina tripartita* KOCH.

Fig. 3 and 4 A part of *Globorotalia mayer* CUSHMAN and ELLISON.

Plate 19