SCANNING ELECTRON MICROSCOPE AS A TOOL IN GEOLOGY AND BIOLOGY

by

Sizuo KIMOTO* and Susumu HONJO**

(with 5 Text-figures and 6 plates)

(Contributions from the Department of Geology and Mineralogy, Faculty of Science, Hokkaido University. No. 1070)

Preparation techniques in the electron microscopy on biologic or geologic materials have been significantly developed in the recent years. Especially, in field of biology, ultramicrotomy as well as negative stain technique have enabled us to look into the microstructure of organic tissue down to a molecular level. On the other hand, the introduction of super high voltage electron microscope has extended the possibility of transmitted electronmicroscopic observation, penetrating through highly opaque material such as a thicker metalic foil. Geologists have been successful to observe a rock specimen applying elaborated two stage replica preparation technique.

Since reflective electron microscopy is matter fact useless became of its poor resolution and operational difficulties, microscopists depend on the replica when they observe the surface of an opaque or bulky specimen. However, some are impossible to be replicated became they are so fragile, and are not able to stand for the replication treatment. Also, a specimen with a complex surface are often impossible to be replicated.

The single stage replication technique is supposed to be the best method to reproduce the surface topography so far as the obtainable resolution conserned. However, this preparation requires the destruction of the original specimen and it can be a serious impedent in some types of investigation such as taxonony which requires the permanent preservation of an original specimen for the later references (Honjo and Berggren, 1967).

Any kinds of replication technique require certain skillness and the procedures are time consuming as well. The surface observation of a small and fragile biologic specimens such as a tentacles of moth, mold calix are almost impossible to apply the two stage replica method. Neither the single stage method is applicable on such organic tissue because the tissue is hard to be removed by melting in a solvent.

* Research Laboratory, Japan Electron Optics Laboratory Co., Ltd.

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

Microscopists have been confronted to the difficulty of relating an optical image to the electron optical one of the same specimen. There is a certain range of break of magnification between an optical and electron microscope. Especially in the reflective observation of a solid specimen, the magnification jumps into few thousand times directly from a binocular microscope which enlarges the object into merely less than a hundred times. This practice leads one being lost himself in a very narrow view field of strongly magnified image.

A low magnification such as times 200 or even little less is usually available in a regular electron microscope. However, the image of a carbon replica is both ered by the shadow of gridbars which support specimen, carbon replica is usually broken, fractured or winkled. Therefore, only a small part is available for the observation especially when an original surface has a complex topography or the replica is produced from a solid specimen such as a small spheric body.

The scanning electron microscope may be one of the answers to those problems. The principal of a scanning microscope is greatly different from the transmission electron microscope or reflective electron microscope. In the scanning electron microscope, a finely focused electron probe scans across a specimen surface in a manner similar to that of a television raster, and a bombardment of the probe emits secondary or backscattered electrons from the specimen. Those electrons are collected and modulated on a cathode ray tube as a light spot in different brightness. The image is desplayed by scanning the spot synchronously with the probe throughout the screen.

A regular transmission electron microscopic image is formed on a fluorescent screen or on a photographic plate by the electrons which pass through a specimen. The transmitted electrons are scattered elastically or inelastically in the specimen and some of these electrons are obstructed by a small aperture in the focal plane of the objective lens. The resultant absence of such electrons in the image is a principal source of image contrast (Fig. 1).

Scanning electron microscope has been developed chiefly to observe the electrical properties of the semi-conductor divice. However, this instrument has been found to be a powerful method to observe any solid specimen surface under far greater magnification than optical methods. Microscopists have been enjoyed its great depth of focus and the resolution as fine as less than a few hundred angstromes. The scanning electron microscope can magnify the object in less than a hundred times up to more than a ten thousand times without a leap of magnification.

The history of the scanning electron microscope can be traced back to 1935, to an instrument built by Knöll. ARDENNÉ (1937) advocated that scanning microscopy is a promising method to avoid the chromatic error in electron microscope. ARDENNÉ (1940), MCMULLAN (1953) and SMITH (1955) constructed practical models; however, the earlier attempts did not receive particular attention, because the limit of resolution was not drastically improved over the optical micro-

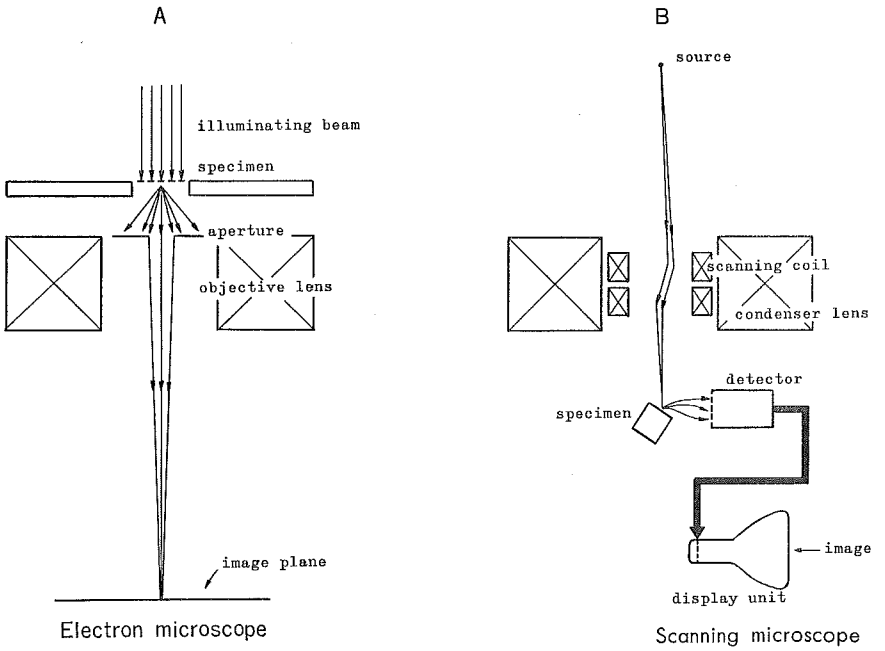

Fig. 1
Principals of image forming in electron micoscope (A) and scanning microscope (B).

scope and the over-all ability of this type of microscope was thought to be quite inferior to a regular transmission electron microscope.

The behavior of the emitted electrons from the electron bombarded surface has been precisely studied by EVERHEART, and the Cambridge group since 1957. Thsoe studies have led to the remarkable improvement of resolution and image contrast of the scanning electron microscope and have found a wide field of application, especially in solid state physics.

Two kinds of electrons are concerned with the image forming in scanning electron microscope. When an electron beam is directed on a small area, backscattered electrons and secondary electrons are emitted from the bombarded spot. The *backscattered electrons* are the original electrons which are emitted from the beam source, then reflected on the surface of a sample. The electron bombardment discharges the sample surface, and the electrons fred by the discharge are called *secondary electrons*. The intensity of backscattered and secondary electrons is influenced by the surface topography and physico-chemical properties of the sample surface.

Only secondary electrons are detected for image production in regular scanning microscopy. The resulted secondary electrons are so weak and they are amplified

after collected by an energy analyzer. The beam spot of electrons which are emitted from a heated filament, is focused on a sample and is swept in a regular raster with a deflection coil which is synchronized with a modulated light spot on the tube, and the integration of such light spots forms the enlarged image of the object.

Experimental Apparatus:

Our experiments are carried out with a JSM scanning electron microscope, manufactured by Japan Electron Optics Laboratory Co., Ltd., Tokyo.

The whole outfit of instrument consists of three blocks. They are the main cabinet which includes an electron optical system, specimen chamber, detector system, etc. Electronic circuits and display unit are facilitated in a control console. A power supply box is independent.

An electron beam emitted from a heated tungsten filament is finely focused on a specimen surface by an electron optical system which is provided in the main column. The electron optical system is composed of a triode electron gun, a single condenser lens, an objective lens and a astigmator. This system produces finely focused electron probe with variable energy between 5 to 50 KV. Double scanning coils are mounted in the objective lens and make the probe sweeping on a specimen. The magnetic deflection system controls the scanning of the probe in a manner similar to that of a television raster across the specimen.

In this experiment, a 45 degree inclined specimen stage is used. A horizontal specimen stage is available to examine a larger specimen as 25 mm in diameter. A specimen up to 12 mm in diameter, 10 mm in thickness can be fixed onto a specimen holder for a inclined specimen stage. In either case, a specimen can be shifted in two orthogonal directions to examine any desired specific area. Specimens can be exchanged within few minutes through an simple airlock system without breaking the vacuum of the main part of microscopic column.

The secondary electrons are emitted as a result of excitation by the incident electron probe and the most of them has low energies which are less than a few hundredse V. While the backscattered electrons are a portion of the incident electrons which are backscattered from the specimen and their energies are nearly as large as those of the incident ones. The secondary and the backscattered electrons are detected by the separate detector systems respectively.

The collecting system for secondary electron consists of electrostatic focusing electrodes and a positively biased scintillator which is optically coupled to a photomultiplier tube through a translucent light pipe. The collected secondary electrons are accelerated to 10 KV before exciting the scintillator in order to produce a sufficient light intensity. The light energy is amplified through a photomultiplier tube. The collecting efficiency can be controlled by adjusting the potentials which are supplied onto a focusing electrodes.

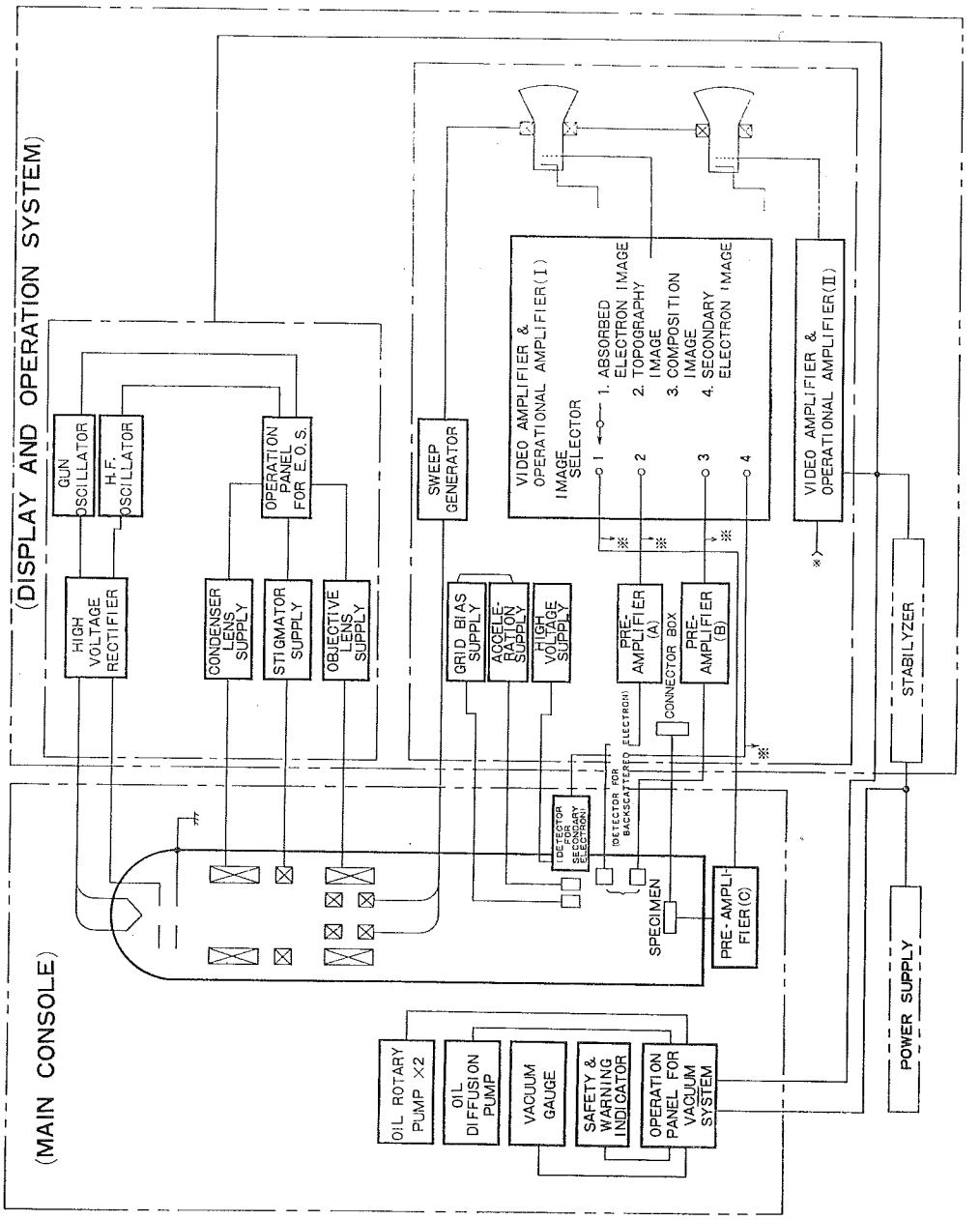

Fig. 2
Block-diagram of JEOL-JSM scanning microscope.

The detector for backscattered electrons consists of pair of silicon p-n junctions. This simple detector facilitates an efficient amplification of the higher energy electrons (Fig. 2).

The column vacuum is an important factor for the signal gain. The better the vacuum, the image characteristics are improved. This relation is more clitical in scanning microscope than regular transmission electron microscope. At least 10^{-5} torr or better vacuum is required in between the specimen and the secondary electron collecting electrode. In our experiment, rough vacuum is obtained by mechanical rotally pumps and final vacuum, at the better range of 10^{-5} torr, is furnished by oil diffusion pumps.

Characteristics of Image:

The scanning electron microscopy provides various information from a same specimen simultaneously, such as the intensity of secondary electrons, backscattered electrons, absorbed electrons, electromotive force, cathodoluminescence and X-rays. However, we would like to limit our discussion on the secondary and backscattered electron images, because these two images are the most important for our purpose.

As it was explained before, the secondary electrons provide better topographic image of surface. Secondary electrons are emitted and collected by a collector even from a deep hole on the specimen surface. This is due to the characteristics of the secondary electrons. Because of their low energy, secondary electrons travel in a curved path to the collecting system by biasing the focusing electrodes to the suitable potentials. Therefore, examination with secondary electrons is especially useful for rough surfaces such as fractures of rocks or metalic fractures, fibers, biological specimens, or etched surfaces of carbonate rocks (Fig. 3).

The backscattered electrons travels in an almost straight line with higher energy. The intensity of backscattered electrons depends on the average atomic number of specimen at the incident electron probe. The higher the atomic number, the stronger the intensity of backscattered electrons. Therefore, the backscattered electron image can be used for descriminating the elemental composition of a surface (Fig. 4).

Resolution. The minimum distance between two points being separated as two is defined as the resolution. The resolution (δ) of an optical microscope is the function of the numerical aperture of the objective lens (a) and the wave length of light (λ). Their relation is; $\delta = \lambda/a$.

The numerical aperture of an optical microscope could not be more than 1.4. When (λ) is 550 mμ, (δ) is approximately 444 mμ, when (a) is 1.25. In case the monochromatic ultra-violet ray with the wave length of 275 mμ, is utilized, (λ) is a quarter

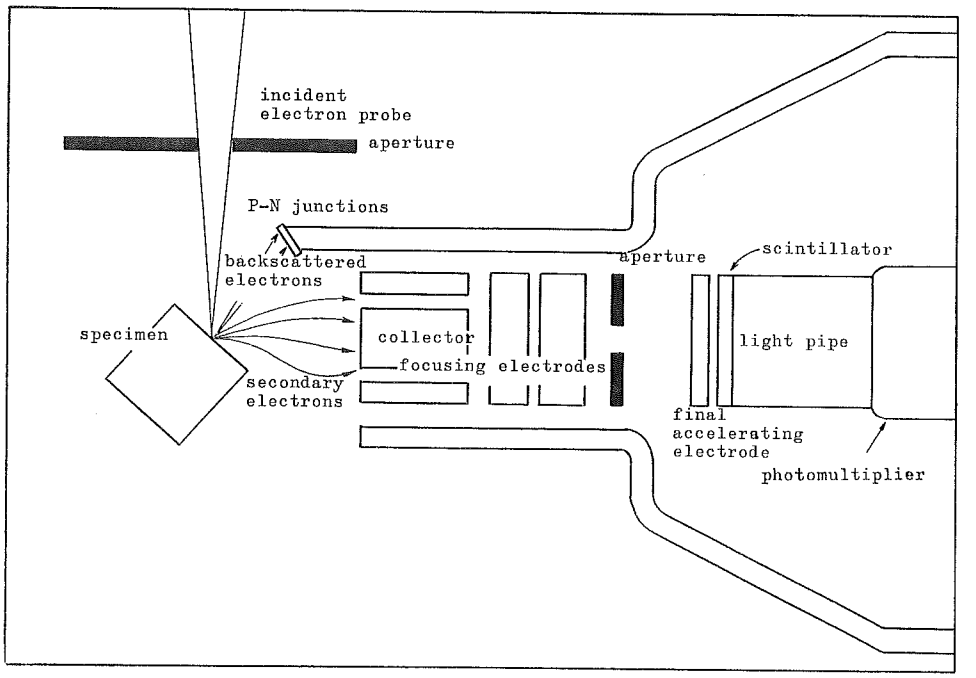

Fig. 3
A detail of emitted electron collection system.

of a micron, and it is the practical limit of the resolution an optical microscope.

On the other hand, the numerical aperture of electron microscope is extremely small. However, as the wave length (λ) of an electron beam is short, the practical resolution is as low as few angstromes at the optimum use of microscope.

The secondary electrons are emitted only from the bombarded area because of their low energy. As the energy level of the backscattered electrons are much higher, the backscattered electrons can be emitted from the interior parts of specimens. In other word, the resolution of the secondary electron image is essentially depend upon the diameter of the probe, while that of the backscattered electron image depends on the composition of the surface and energy of the primary electron probe.

The minimum probe diameter in our experiment is approximately 150 Å, therefore the final resolution should be equal to this. However, the currency of the emitted secondary electrons is so small, and the resolution is influenced by the signal noise ratio of the electronic system. The instrument used in this experiment has the resolution better than 500 Å. The resolution can be enhanced close to 150 Å under the optimum condition.

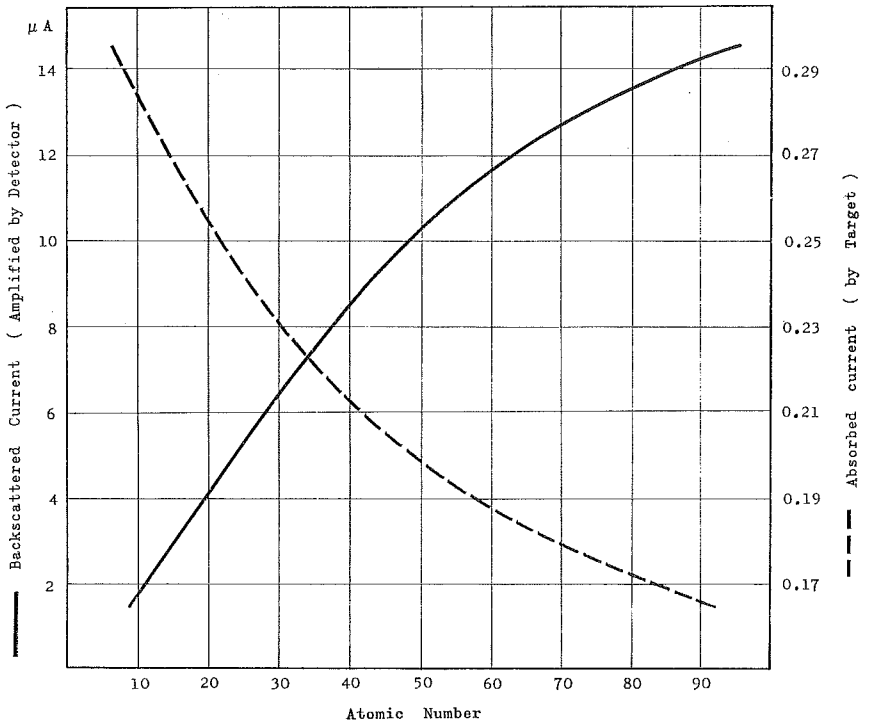

Fig. 4
The relation between intensity of backscattered current and the atomic number of the bombarded specimen.

Definition. The definition of microscopic image is depend on the astigmatism of the probe spot and chromatic error of the probe beam.

The astigmatism can be minimized with the electronically controlled stigmator. Since the supply of high voltage to the electron gun is highly stablized by the stablizing circuit, chromatic error is almost negligible.

The number of scanning lines on the final dispay is also an important factor for the image demarcation. In our experimental instrument, 1,000 scanning lines are available for the maximum performance.

Depth of focus. In an optical microscope, the depth of focus is in reverse relation to the numerical aperture of the objective lens (a) and the total magnification. Therefore, the depth of focus (T) is shallower at a higher magnification. When a total magnification is (M), and the index of the material surrounding the object is n; $T(\mu) \fallingdotseq 0.24\ n/aM$.

For instance, a Cargille's oil (n=1.515) immersed specimen is observed with

a high magnification optical system with an object lens of N.A. 1.37, ×90, with ×10 ocular, the depth of focus (T) is less than 0.3 μ. Such a shallow focul depth is especially of trouble in the reflective observation of surface.

However, in a scanning electron microscope, the depth of focus of probe beam is very deep because the objective lens has an extremely small numerical aperture like an ordinary electron microscope. The relation between the total magnification and the depth of focus is shown in Fig. 5.

Because of their low energy, the emitted secondary electrons travel in a curved path to the collecting system by biasing the focusing electrodes to the suitable potentials. Therefore, secondary electrons which are emitted from complex topography such as ones from the bottom of a deep holes can be collected.

Brightness of image. The brightness of the image is not influenced with a shift of magnification except within a extremely high magnification range. The intensity of electron probe is not affected with the change of magnification. This is a noted advantage to regular transmission electron microscope where an image becomes significantly darker at higher magnification.

The deviation of brightness in the view field is especially of trouble in trans-

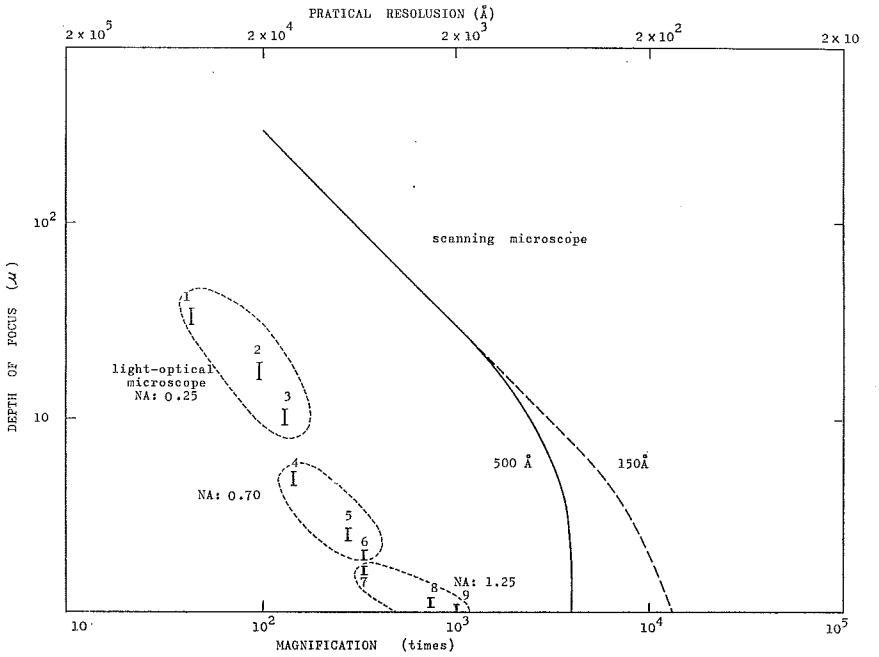

Fig. 5
The relation between practical resolution (A), magnification (times) and depth of focus (μ).

mission electron microscope at a low magnification. In the scanning electron microscope, view field as wide as 2 mm by 2 mm, can be photographed with a complete homogenity of brightness. The resulted picture is similar to a reflective image which is illuminated by a flood lump from 45 degree direction.

The total brightness of displayed image is the function of the brightness of the modulated spot and the scanning speed. The dark and bright contrast of a displayed image can be adjusted electronically at an observer's convenience.

Magnification. A magnification is determined by a ratio of the length of a scanning line on the cathode-ray tube *v. s.* the length of scanning line on the specimen. As a practice, the magnifications can be changed over a very wide range by controlling the deflections of the electron probe in the main column.

A contenuous shift of magnification from X 100 to X 100,000 can be obtained at the accelerating voltage of 25 KV. The minimum magnification is less than 30 X with a lower accelerating voltage. A special specimen stage with a longer working distance (the distance between the lower part of the objective lens and specimen) is usually required for under X 100 observation.

The best image quality is obtained between X 1,000 and X 5,000. This is exactly the area where the both light optical and electron microscope fail to cover (HONJO and BERGGREN, 1967).

The focus readjustment is not necessary when the magnification is shifted from one to the other observing the same spot. While in the transmission electron microscope, refocusing is usually required especially when magnification is shifted within a higher magnification range.

The final image on the display tube is photographed on 35 mm high-speed film. When the scanning line is 1,000, the space between two adjacent sweep lines are 0.1 mm. Therefore, the photographic enlargement is limited within a few times of the size of displayed image. In our experiment, the display screen is 10 cm by 10 cm.

The resolution of the backscattered electron image is between 5,000 Å and 1,000 Å. It is particularly inferior for a surface with higher relief.

Specimen Preparation:

Specimen preparation is less complex than a replica method or regular disectioning technique for tissue study. All what should be prepared is to affix a specimen onto a specimen holder for a conductive specimen. The specimen surface is coated with an evaporated conductive thin film in case the specimen is an insulator.

The purpose of this evaporation is to give conductivity to an insulator specimen in order to prevent electric charge on the surface. This practice is fundamentally different from so called "shadowing" procedure in replica preparation. Shadowing

technique is to emphasize the surface topography throwing a radiation of evaporated heavy metal from an angle.

The sample is coated with evaporated metal, hopefully in an even thickness throughout the specimen surface to be observed. The thickness of coating can be thin as 30 Å or less. This much of coated layer is usually negligible under strong magnification.

In our experiment, a piece of gold wire is evaporated in a high vacuum. The sample is swiveled in an angle to the horizon as well as rotated several times per second in a vacuum, to expose the surface evenly to the radiation source. The detail of the technique is described in HONJO and OKADA, 1967.

The number of secondary electrons emitted from a specimen surface increases when the energy of an incident probe is low; an insulator sample can be observed without conductive coating. However, the resolution is sacrified in an operation with low energy probe.

The energy dissipation of probe beam in the specimen is much lower than that of transmitted electron beam of regular electron microscope. Therefore, fragile specimens can be examined without being damaged by electron beam bombardment.

Conclusion:

A scanning microscope is particularly useful to observe a minute and solid specimen, or complex surface topography of a bulk specimen with the dimension of beyond the optical limit. It provides an observer a familiar three dimensional image which is somewhat similar to a binocular microscopic image with sufficient illumination. The resolution of the microscope is as little as 150 Å, while an optical resolution is usually more than 5,000 Å.

A scanning microscope can enlarge a specimen into less than a hundred times to as large as a hundred thousand times without a bold leap of magnification. The brightness of image is almost constant under different magnification. Therefore, a particular spot can be enlarged through contenuous sift of magnification starting form a weak enlargement where the view field is wide.

An observation with a few thousand times magnification has been of problem with both optical and electron microscope. Scanning microscope is found to bridge the break which the conventional microscopies have not been able to fill. An object with the dimension of around an optical limit is particularly good for the scanning electron microscopy.

A scanning electron microscope has significantly deep focus distance. As the emitted secondary electrons can travel in a curved orbit, clear image can be produced from a complex topography such as a deep hole on a specimen surface. Those characteristics of image provides an observer particularly good feeling of

three dimensions.

The specimen preparation is less tedium than surface replication or disectioning procedure. The preparation does not require the destruction of the original specimen. Only thin metal film coating, which is thin enough and does not affect the original topography, is required. A specimen to be observed is usually keep unaltred during observation; a particular spot on the surface is exposed to a finely focused sweeping electron probe within a very short time. A coated specimen can be preserved permanently for later reference.

A specimen to be observed is subjected under high vacuum during observation. In case the specimen is an insulator, it is also placed in high vacuum for conductive coating before observation. A specimen with considerable amount of free water or evaporative material is obviously deformed by the rapid evaporation. Therefore, scanning electron microscopy is not applicable for the most of biological tissue specimens.

Our sincere thanks due to the Japan Electron Optical Company, Tokyo, for the generous permission of using their research equipments. We are grateful to Kiyoshi MASE, Yuji NAKAJIMA, Hisatake OKADA and Nachio MINOURA for their valuable technical assistance. We are grateful to Sumio KUMANO for his editorial help. A JEOL-JSM scanning electron microscope has been used throughout this research. A part of this project is supported by Grant-in-Aid A-091008 from the Japanese Ministry of Education.

References

ARDENNÉ, M. (1938): Das Elektronen Rastermikroskop, Praktische Aufühung, Zeitschrift für Technische Physik, vol. 19, pp. 407–416.

BRADLEY, D. E. (1965): Replica and Shadowing Techniques. In Techniques for Electron Microscopy, ed. Kay, D. H., Philadelphia, particularly p. 137.

CREWS, C. A. (1966): Scanning Electron Microscope: Is High Resolution Possible?, *Science*, vol. 154, pp. 729–738.

HONJO, S. (1967): Electron Microscopic Techniques in Carbonate Geology and Nannofossil Study, Rep. Res. Kansas Geol. Sur. (to be published).

HONJO, S. and BERGGREN, W. A. (1967): A Study of Planktonic Forminifera by Scanning Electron Microscope, Micropaleonology, vol. 13 (to be published).

HONJO, S., MINOURA, N. and OKADA, H. (1967): Study of Nannofossils by the Scanning Electron Microscope, Jour. Fac. Sci., Hokkaido Univ. Ser. 4, vol. 13, pp. 427–431.

HONJO, S. and OKADA, H. (1967): Scanning Electron Microscopy of Planktonic Forminifera; A Preparation Technique, Jour. Fac. Sci., Hokkaido Univ., Ser. 4, vol. 15, no. 1 (to be published).

KIMOTO, S. and HASHIMOTO, H. (1964): Stereoscopic Observation in Scanning Micro-

scopy Using Multiple Detectors, *Read to* Symp. Electrothemics and Metall., Electrochem. Soc., Washington, D. C., John Wiley and Sons, N. Y.

KIMOTO S. HASHIMOTO, H. and SATO, M. (1966): On a Scanning Type Electron Microscope, Sixth International Congress for Electron Microscopy, Kyoto, Japan, vol. 1, pp. 197–198, Maruzen Co., Tokyo.

KNÖLL, M. (1935): Aufladepotential und Schundäremission electronenbestrahlter Körper, Zeitschrift fur Technische Physik, vol. 16, pp. 467–475.

MCMULLAN, D. (1953): An Improved Scanning Electron Microscope for Opaque Specimens, Proc. Inst. Elct. Engneer. vol. 100, pp. 245–259.

OATLEY, C. W. (1958): The Scanning Electron Microscope, *New Scientist*, vol. 5, p. 155.

(Manuscript recieved July 16, 1967)

PLATE 11 AND EXPLANATION

Explanation of Plate 11

Fig. 1 High resolution scanning photomicrograph of selenium polycrystal surface, Demonstrating the resolution nearly clears 150 Å at the maximum performnce of the instrument.

Figs. 2, 3 Backscattered electron (2) and secondary electron photomicrograph (3) of same polished surface of nickel alloy. Different composition of element can be descriminated in a black-and-white contrast like it is shown in Fig. 2 A vague image which is similar to Fig. 2 in Fig. 3 may not be due to the elemental difference but slight relief on a polished surface. It is detected by secondary electron emission.

Fig. 4 Outline of JEOL-JSM scanning electron microscope.
Left : electron optical system with main column and specimen chamber.
Right : control console and two display screens. One of the screens is affixed 35 mm camera for recording of image. Power unit does not come into sight.

Plate 11

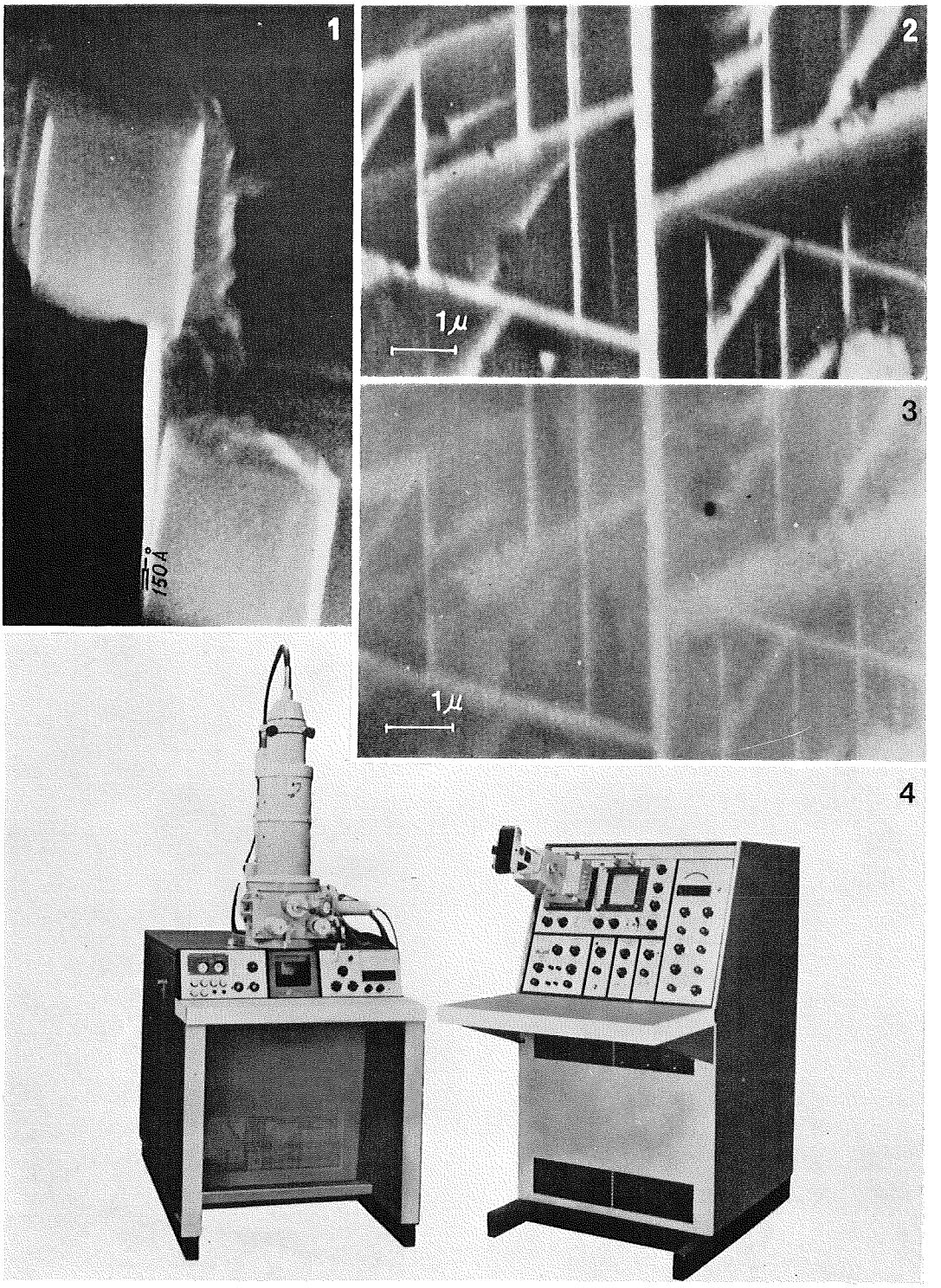

PLATE 12 AND EXPLANATION

Explanation of Plate 12

Scanning photomicrograph of *Hantkenina alabamaensis* CUSHMAN

Fig. 1 A time consuming sketch of a microfossils can be replaced by the low magnification scanning micrograph.

Fig. 2, 3 The magnification between X 1,000 to 5,000 times is particularly usuful. The depth of focus is still deep enough at X 5,000 for this much of relief.

Fig. 4 Close up photo of a perforation in Fig. 3 Secondary electron image provides better image of complex topography even from the bottom of a hole.

Plate 12

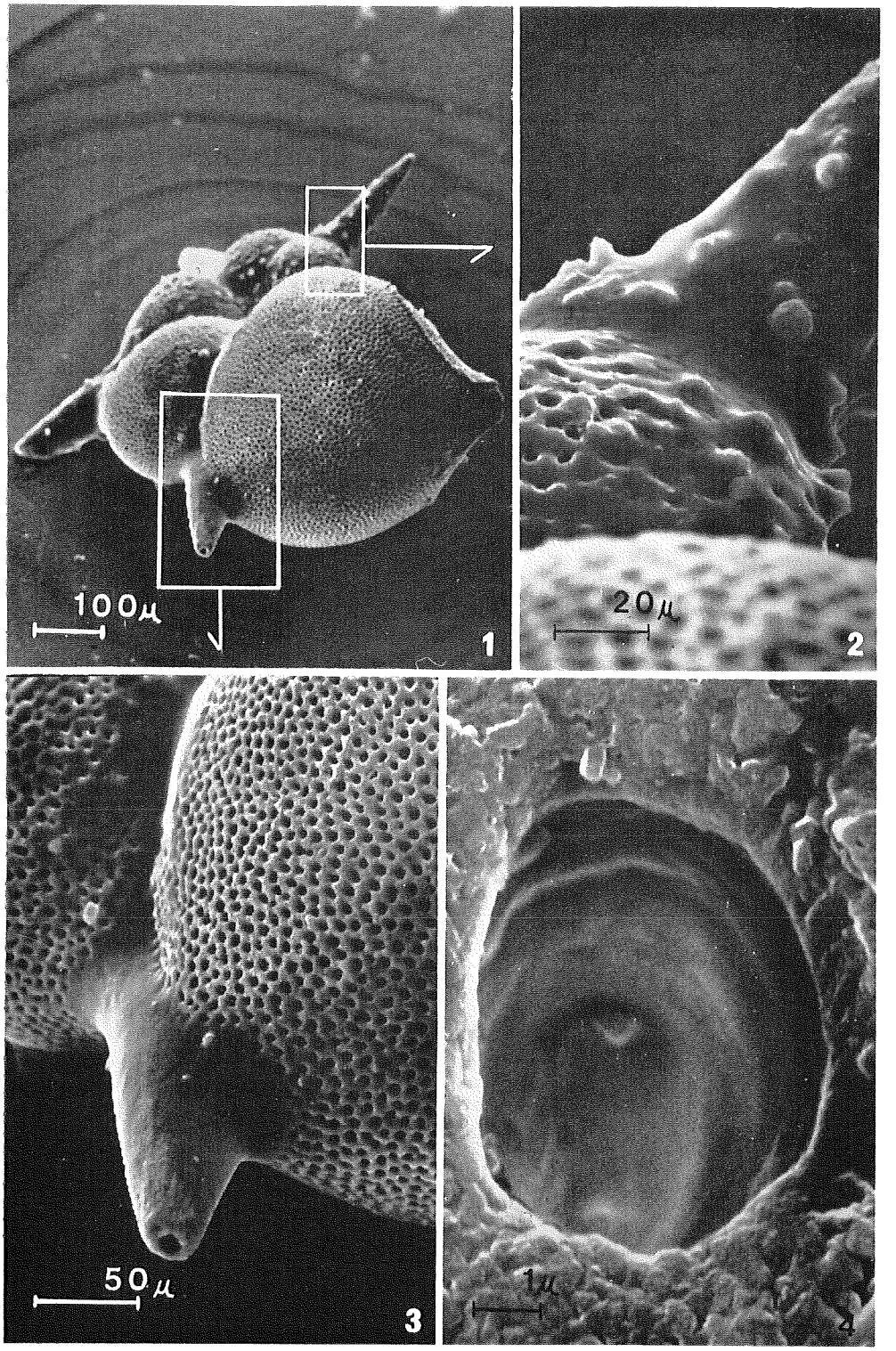

PLATE 13 AND EXPLANATION

EXplanation of Plate 13

Recent pollen grains and the close up. Species unknown.

Plate 13

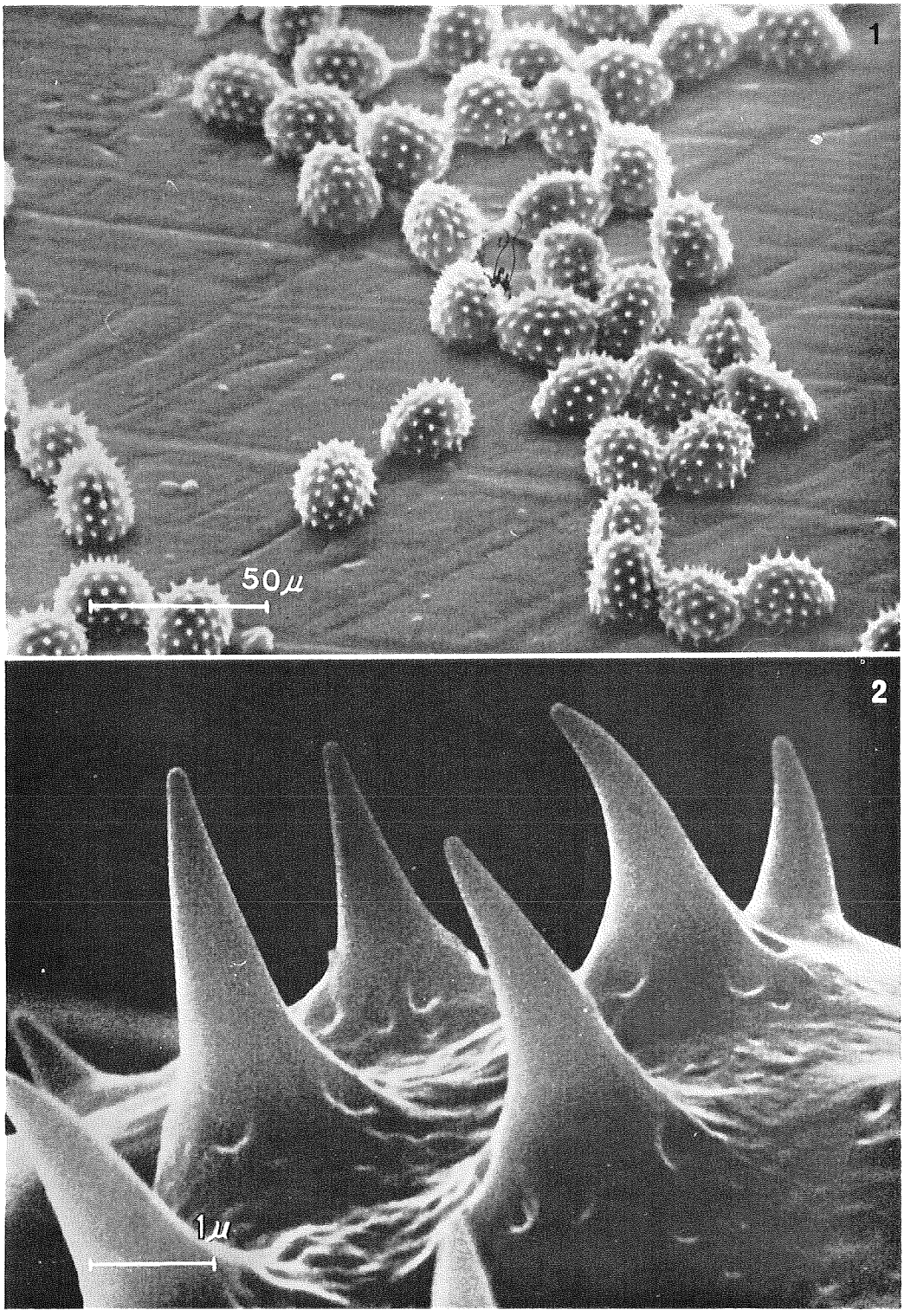

PLATE 14 AND EXPLANATION

Explanation of Plate 14

A cluster of moth. As the specimen was subjected in a high vacuum, it was dehydrated and significantly deformed, *v. i.* a "calix shin" is turned inside out. Stems are constricted by dehydration. Notice the magnificent depth of focus.

Plate 14

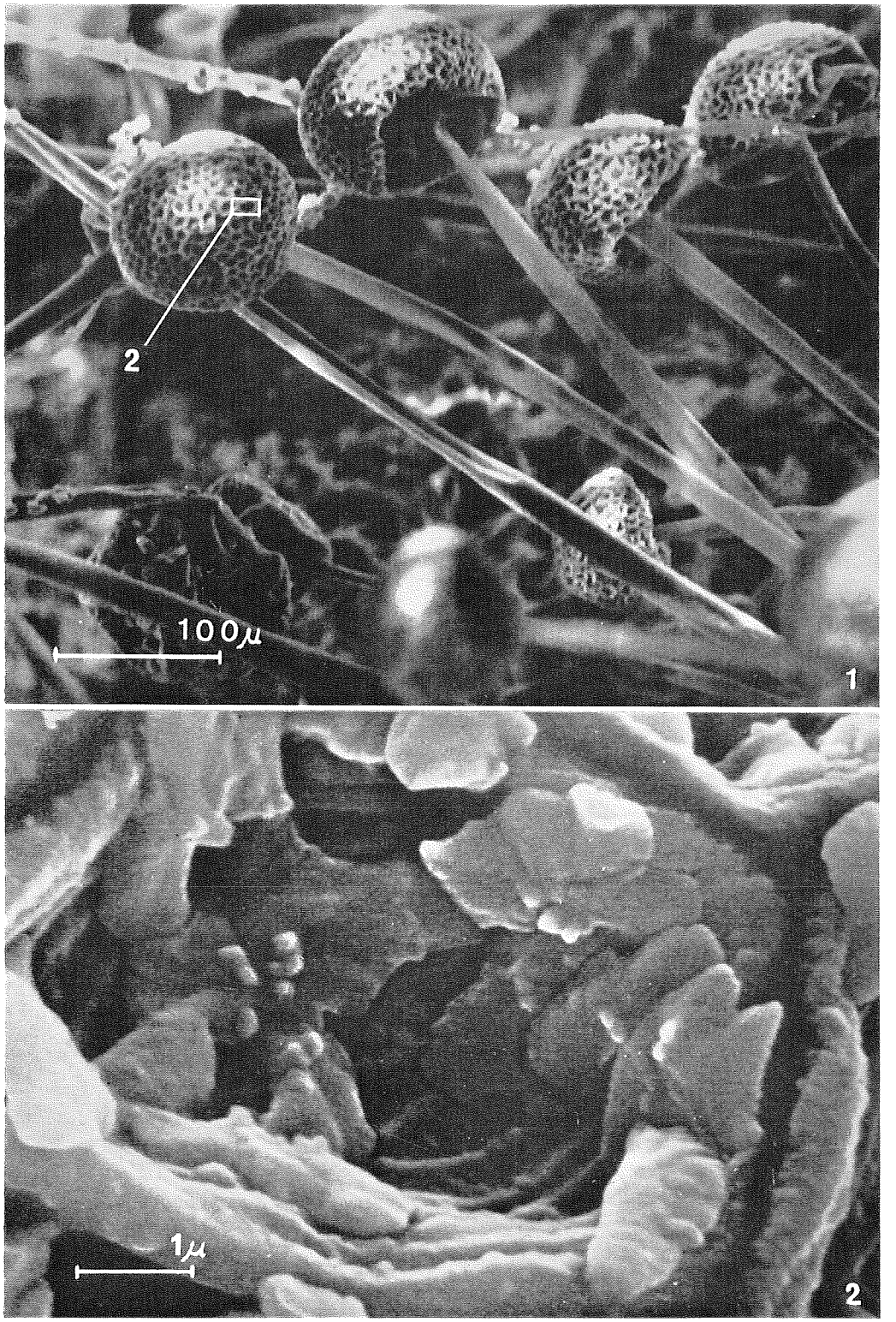

PLATE 15 AND EXPLANATION

Explanation of Plate 15

Proboscis of a moth of unidentified species. Of note is the presence of two types of sensilla, cones and pegs sunk in shallow depressions on the surface (Upper left, right). They are likely to be chemoreceptors. Peculiar patters on a segment surface (lower left, right).

Plate 15

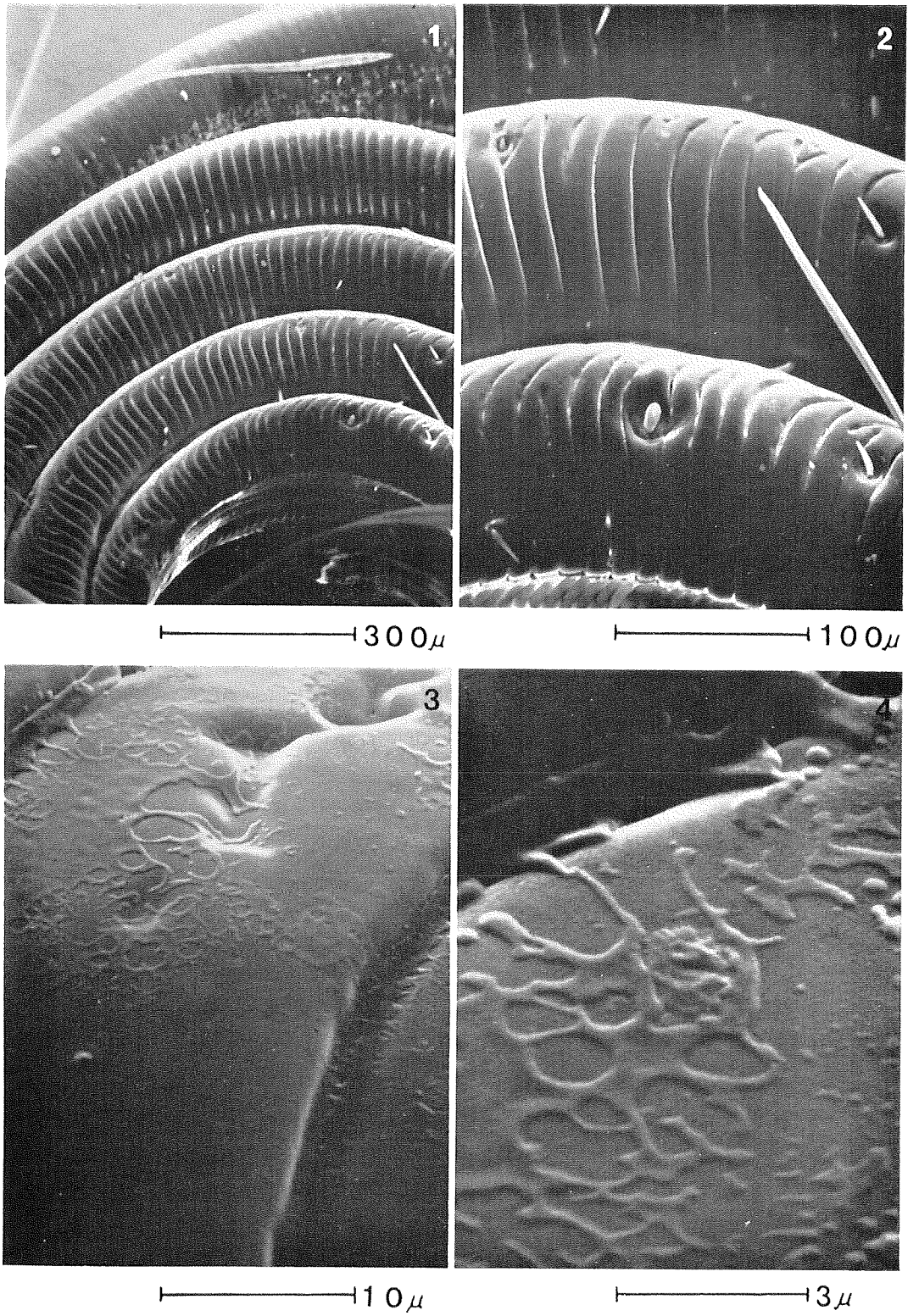

PLATE 16 AND EXPLANATION

Explanation of Plate 16

Butterfly scales (**Fig**. 1, 2) and their texture (**Fig** 3, 4)

Plate 16