Proc. XIth Int. Cong. on Electron Microscopy, Kyoto, 1986

A NEW HIGH RESOLUTION SCANNING ELECTRON MICROSCOPE AND ITS APPLICATION TO BIOLOGICAL MATERIALS

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Since the scanning electron microscope (SEM) was put to practical use, about 20 years has passed. As the SEM provides seemingly three-dimensional images, it becomes to be used in wide fields, such as biology, technology and medicine. In spite of rapid progress in instrument design during the past few years, the SEM could not have the resolving power under 2nm, which was considerably worth than that of transmission electron microscope (TEM). As to the limit of the resolution in SE mode, it was theoretically said that the resolution can not be improved beyond 2nm in a balk specimen as metals, because the lateral distribution of secondary electron is calculated about 1nm for normal incidence[1]. In contrast, we reported that the theoretical model of metals does not exactly apply to biological materials of low density covered with thin metal films of high density, and the resolution of the SEM will be able to attain under 1nm in case of biological specimens[2].

Based on our results, we designed together with Hitachi Co. Ltd. a new SEM (UHS-T1) with ultra-high resolution (Fig. 1). This instrument was equipped with a field emission source and the objective lens with short focal length (f=3.6mm) for reducing the electron probe strongly. On calculation the probe size was estimated to 0.4mm in diameter at 30kV and the probe size of 0.5nm was confirmed by observation in STEM mode. On observation of biological materials (rat motor nerve cells) which was previously coated with platinum by a ion sputter coater, the SEM shows 0.5nm resolution when the width of the dark space criterion is used (Fig.2).

Main production design specifications of this SEM are as follows:

Electron Optics

Electron gun: field emission source / Accelerating voltage:1-30 kV / Field emission voltage: 0-6.3 kV / Magnification: 150x-600,000x / Lens system: 2 stage electromagnetic lens / Objective lens: f 3.6mm, Cs 1.6mm, Cc 2.0mm

Specimen Stage

Side entry specimen stage (eucentric)
Specimen size: 2mm diameter x 2mm high / Specimen exchange: Airlock-prepumping system

CRT

Viewing CRT: 9" 3sets / Recording CRT: ultra-high resolution type 1 set

Photo-apparatus

Film size: 4x5 inches / Lens: Rodagon 135mm (F 5.6)

Vacuum system

Vacuum pumps: Ion pump (60 l/sec)x 1 and (20 l/sec)x 2

Turbo molecular pump (340 1/sec)x 1
Rotary pump (174 1/sec)x 2

Ultimate vacuum

Electron Gun: $1x10^{-8}$ Pa / 1st and 2nd intermediate chambers: $3x10^{-8}$ Pa - $1.8x10^{-7}$ Pa / Specimen chamber: $3x10^{-5}$ Pa or more better

Application to biological materials

The newly designed high resolution SEM is mainly used at extremely high magnifications (300,000-800,000 folds). To make use of the characteristic, very fine objects such as macromolecules or viruses seem to be more suitable than cell organelles, cells and tissues.

For specimen preparation also more precise techniques are requested. As to metal coating, especially, the routine methods with ordinary vacuum evaporators or diode sputter coaters are quite unusable, because the coated metal particles are clearly resolved by the new SEM. To minimize this artificial figure, we constructed a new sputter coater with a ion beam gun (Fig. 4). With this apparatus we could get much finer particles of Pt, W and Ta. In the present study, this ion beam sputter coater was used for metal coating.

1. Ribosomes

Until today the ribosomes did not become a object of SEM studies, because the resolving power of SEM was not enough to clarify the fine structure of ribosomes. For the new SEM, however, this object seems to be a quite suitable one. We chose it as the first sample for our study.

Specimens were prepared as follows: Adults rats were used. After removal of the blood, animals were perfused with the fixative; a mixture of 0.5% glutaraldehyde and 0.5% formaldehyde in phosphate buffer solution (pH7.4). Small blocks of the spinal cord were removed from the animals. After this step, the specimens were treated by the osmium-DMSO-osmium method[3]. The dried specimens were coated very lightly (0.7nm) with

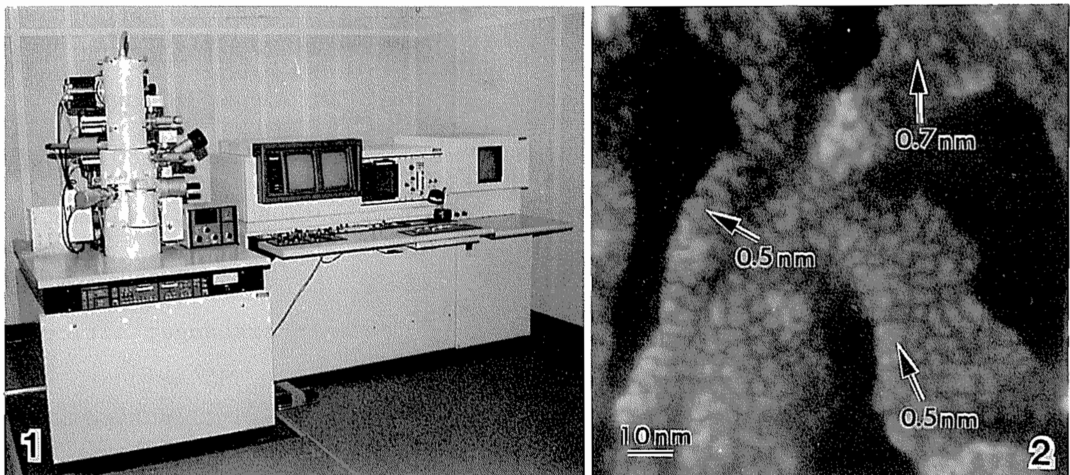

Fig.1 Outside view of an ultra-high resolution SEM UHS-T1.

Fig.2 Platinum particles coated by a diode sputter coater on a membrane of a Golgi complex from a rat motor nerve cell.

platinum in an ion-beam sputter coater which was newly designed by us.

Concerning the shape of ribosomes, some models have been presented by studies with the negative staining method. These models show that the ribosome has a definite form and it consists of large and small subunit. In the present study, strangely, such a definite form was not found in ribosomes. Their shape was quite irregular and different individually, though they consisted of two subunits. The size of ribosomes also varied remarkably. Why ribosomes in our sample did not show a definite form, is still being studied. The ribosomes arranged linearly, hair-pin like or spirally to make polysomes. Between each ribosomes of the polysome thread-like structure could be seen. They have about 3nm in width, sometimes 5nm, and each thread stretched from gaps between two subunits to those of neighbouring ribosomes. Judging from their position these threads may be correspond to messenger RNA (Fig.3, arrows).

2. T_2 bacteriophage

Bacteriophages have been sometimes used as test sample to check the ability of the high resolution SEM[4,5]. In the present study also T_2 phages mounted on carbon discs were observed. The samples were supplied by Dr. Takayama, Department of bacteriology of our Medical school. The suspension of the T_2 phage was treated with 1% glutaraldehyde. After having been rinsed, the samples were post-fixed with 1% osmium tetroxide solution. Then they were stained by a conductive staining method, dehydrated and dried in a critical point dryer.

In the present study, substructures of the bacteriophages such as head, tail and base plate could be clearly distinguished (Fig. 6). Especially, tail fibers extending from base plate were seen (Fig. 5 & 7). In explicably, the cross banding of the tail sheath reported by negative staining method was not observed.

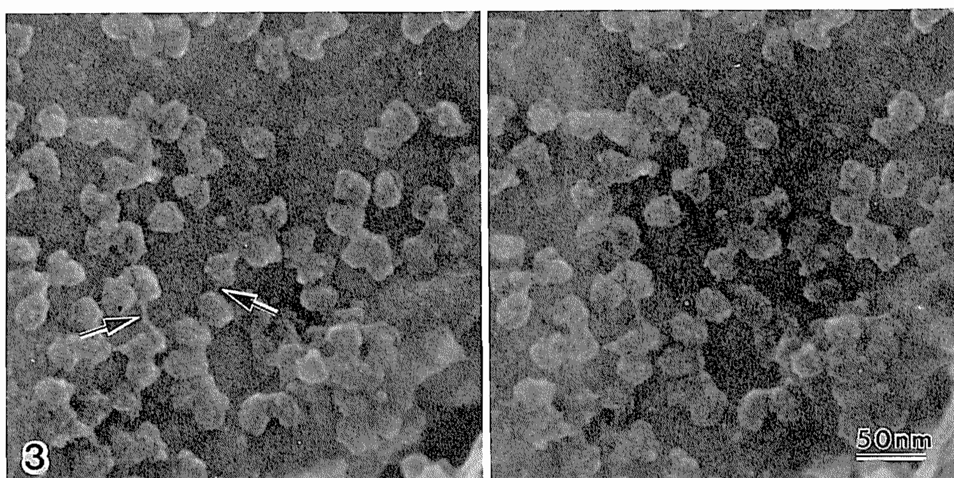

Fig.3 Stereo pair of polysomes. The shape of the ribosomes is irregular and different individually. Between ribosomes fine threads can be seen (arrows).

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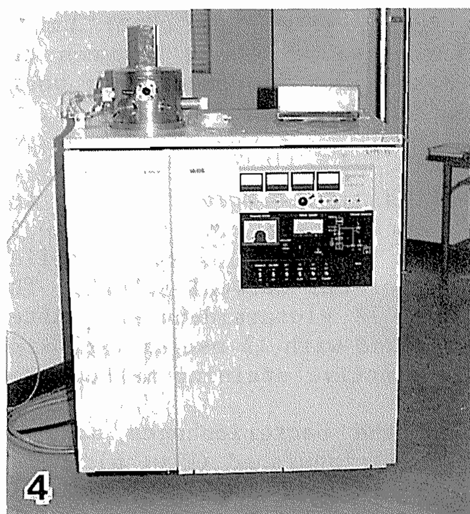


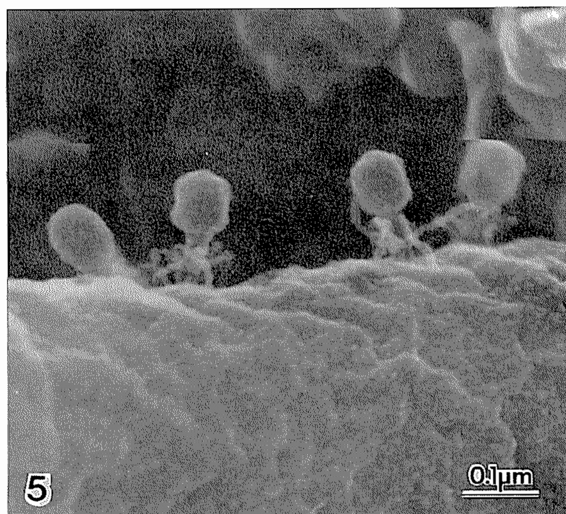
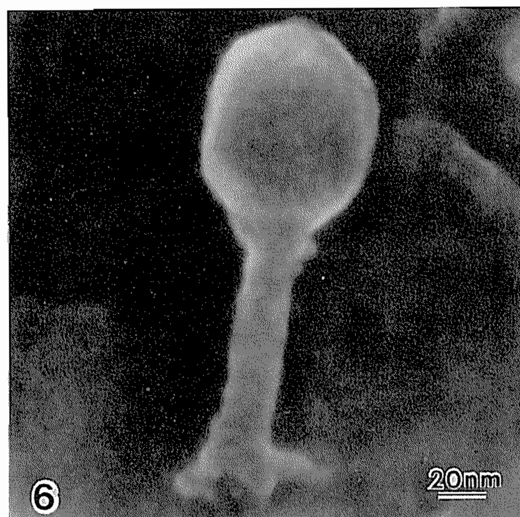


Fig.4 Outside view of the newly designed ion-beam sputter coater.
Fig.5 T_2 phages on a E. coli.



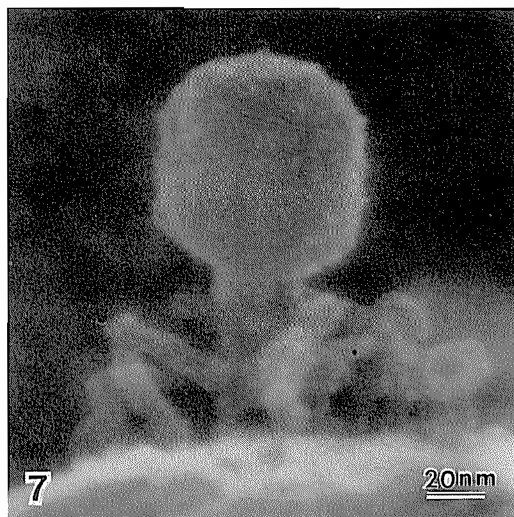


Fig.6 High magnified T_2 phage. The head, tail, base-plate can be seen.
Fig.7 High magnified T_2 phage. Tail fibers extending from base-plate are clearly seen.