SHEET NO. 102

SUBJECT: MICROSCOPY OF BIOLOGICAL SECTIONS USING A LOW VOLTAGE STEM TECHNIQUE

INSTRUMENT: THE S-5200 ULTRA-HIGH RESOLUTION SCANNING ELECTRON MICROSCOPE

CONTENTS

1. INTRODUCTION

A low voltage STEM technique using a scanning electron microscope (SEM) allows high contrast images owing to high electron scattering and making visible subtle changes of densities and compositions in specimen structures. This technique has long been used for microscopy of unstained polymers[1]. Recently, this technique has been used also for semiconductor materials associated with specimen preparation using FIB systems[2]. TEM technique has been playing a leading role for microscopy of biological sections. We have used the S-5200 ultra-high resolution scanning microscope with STEM attachment for microcopy of biological sections. We have examined if it is possible to observe biological sections with good imaging contrast. We have also studied X-ray microanalysis of unstained sections. We report here on some of our initial results.

Fig. 1 A general view of the S-5200 ultra-high resolution scanning electron microscope

2. SPECIFICATIONS OF THE S-5200 WITH STEM ATTACHMENT

Fig. 2 shows a schematic diagram of the S-5200 with STEM and X-ray detectors. The S-5200 has an objective lens of in-lens design[3]. An electron probe is scanned across the specimen and transmitted electrons are collected by STEM detector for image formation. STEM images are displayed on CRT monitor. For X-ray microanalysis, an EDX detector and the specimen is positioned in the gap of the lens. This geometry allows a high X-ray take-off angle as well as a large solid angle of the detector. Table 1 shows specifications of the S-5200 in STEM mode and X-ray micro-analysis mode. We have used Noran's Vantage EDX (Energy Dispersive X-ray spectrometer) system.

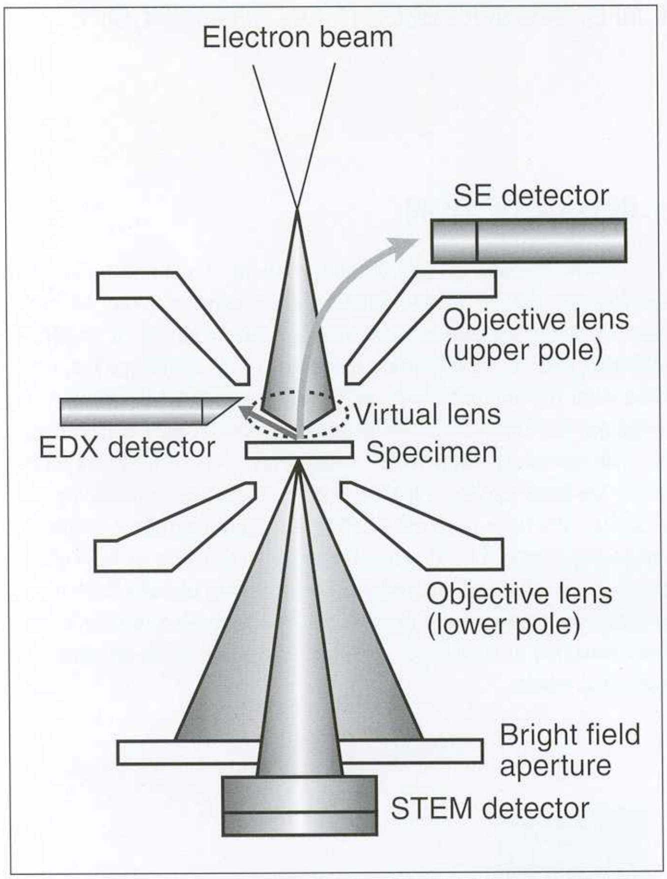


Fig. 2 A schematic diagram of the S-5200 with STEM/X-ray detectors

Table 1 Specifications of the S-5200 with STEM/EDX detector

Item	Specifications
STEM resolution	0.6 nm at 30 kV
Accelerating voltage	0.5 ~ 30 kV
Magnification	~ ×2,000,000
X-ray detector	0.21 sr (Solid angle) 20° (X-ray take-off angle)

3. APPLICATIONS

3.1 Microscopy of biological sections

Fig. 3a is a STEM image of a human glomerulus, sectioned at about 0.1 μm, recorded at 15 kV and at ×3,000. We can see glomerular basement membrane and karyoplasma of nucleus (N) clearly. Fig. 3b is an enlargement (×100,000) of foot process of basement membrane. A double membrane structure of foot process shown by an arrow is clearly visible.

Fig. 4 shows another STEM image of foot process, sectioned at about 0.3 μm thick and recorded at 30 kV and at ×100,000. Despite a thickness of 0.3 μm, a double membrane structure of foot process has been made clearly visible.

Next, Fig. 5 shows another STEM image of cancer cells of liver, recorded at 15 kV. It shows a propagating condition of adeno-virus in cancer cells of liver. The adeno-virus is a vector injected for genetic medical care. Fig. 5a shows the virus in nucleus and Fig.5b is an enlargement at ×100,000 showing a propagating condition of adeno-virus of about 80 nm in a lattice format. As shown here, a low voltage STEM technique allows good imaging of biological sections which have usually been observed using TEMs.

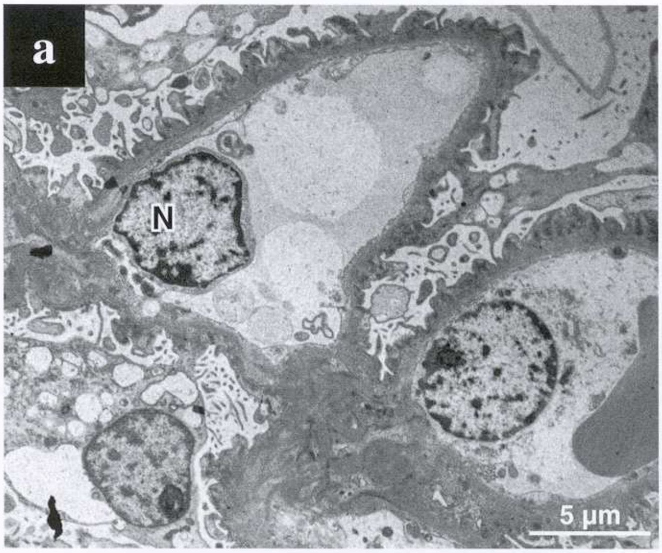


Fig. 3a Human glomerulus, sectioned at about 0.1 µm and recorded at 15 kV and at ×3,000

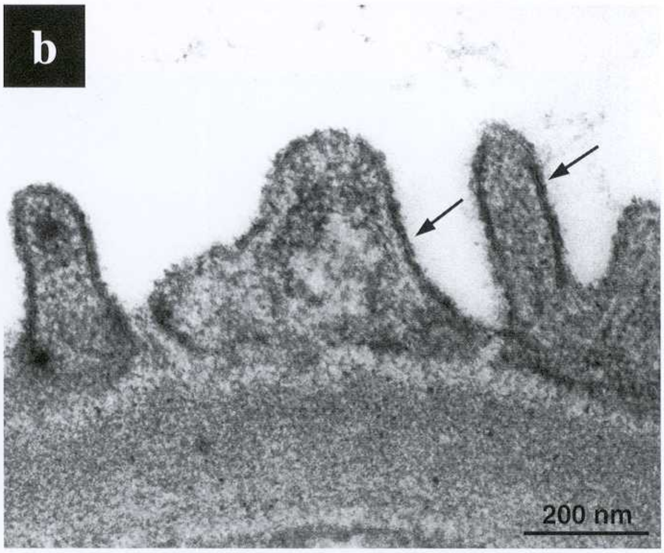


Fig. 3b Foot process of human glomerulus, sectioned at about 0.1 µm and recorded at 15 kV and at ×100,000

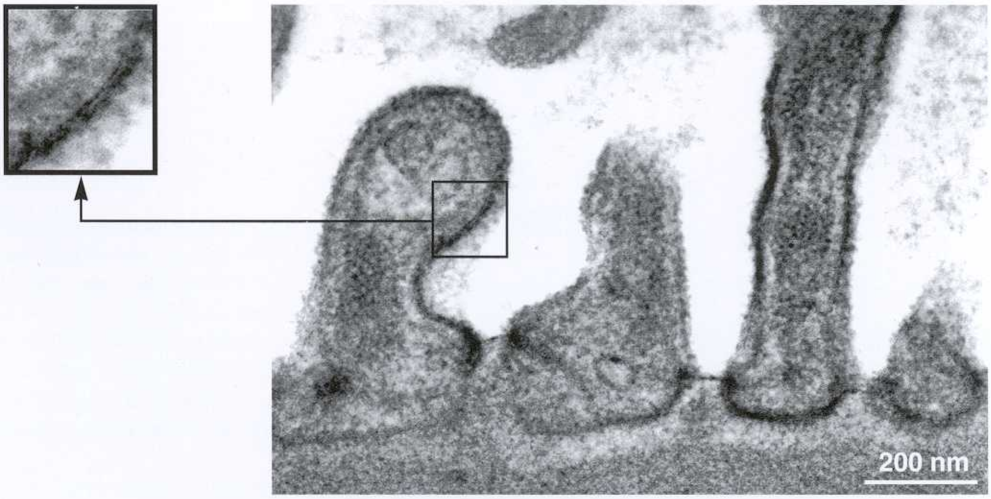
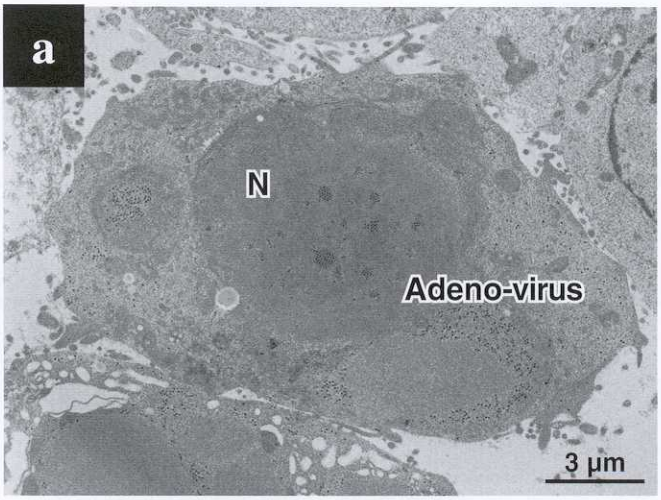
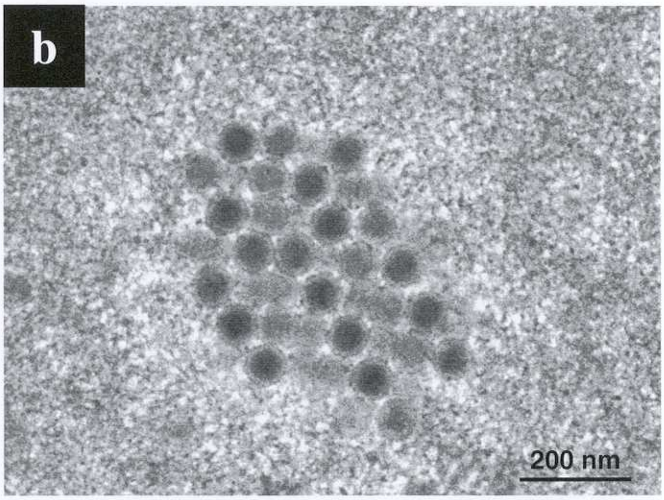


Fig. 4 Foot process of human glomerulus, sectioned at about 0.3 µm and recorded at 30 kV and at ×100,000



(a) Magnification: ×6,000

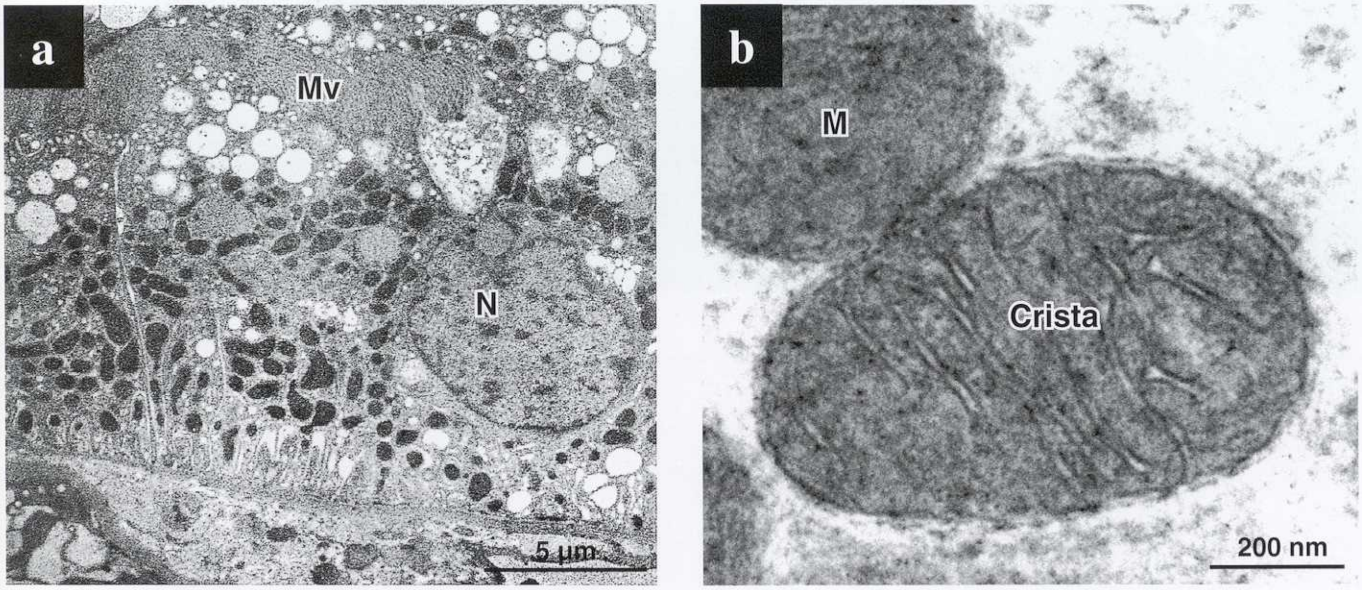


(b) Magnification: ×100,000

Fig. 5 A propagating condition of cancer cells in liver, sectioned at about 0.1 µm and recorded at 30 kV

3.2 Microscopy of unstained sections

We have studied uriniferous tubule of unstained human nephritis kidney, sectioned at about 0.1 µm. Fig. 6a was recorded at 15 kV. It shows nucleus (N), mitochondria (M), and microvillus (Mv) clearly. Fig. 6b is an enlargement of mitochondria showing crista in it at good contrast.



(a) Magnification: ×5,000 (b) Magnification: ×80,000

Fig. 6 Uriniferous tubule of unstained human nephritis kidney, sectioned at about 0.1 µm and recorded at 15 kV

3.3 X-ray microanalysis

Fig. 7 shows a STEM image of unstained human skin of argyria, sectioned at about 0.1 µm. We can see a high electron density particle sedimentation (shown by an arrow) on collagen at high contrast. Fig. 8 shows a result of X-ray microanalysis of this particle analyzed at 15 kV and a probe current of 100 pA. It was identified as silver sulfide. Fig. 9 shows X-ray mapping images of the same silver particle taken at 7, 15 and 30 kV. The lower the voltage, the closer the silver particle to a STEM image and the better the S/N ratio of the mapping image. At 7 kV, the small particle of about 30 nm shown by an arrow is seen in the mapping image. This result shows that a low voltage X-ray microanalysis of thin sections improves X-ray generation and that mapping images of a good S/N ratio are available.

Fig. 7 Human unstained skin of argyria, sectioned at about 0.1 µm and recorded at 15 kV

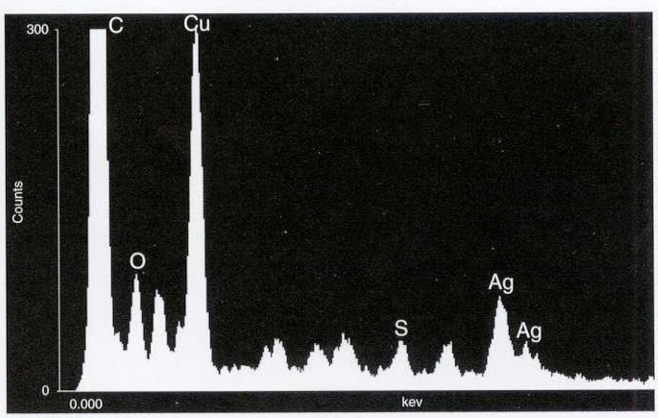


Fig. 8 X-ray microanalysis taken at 15 kV

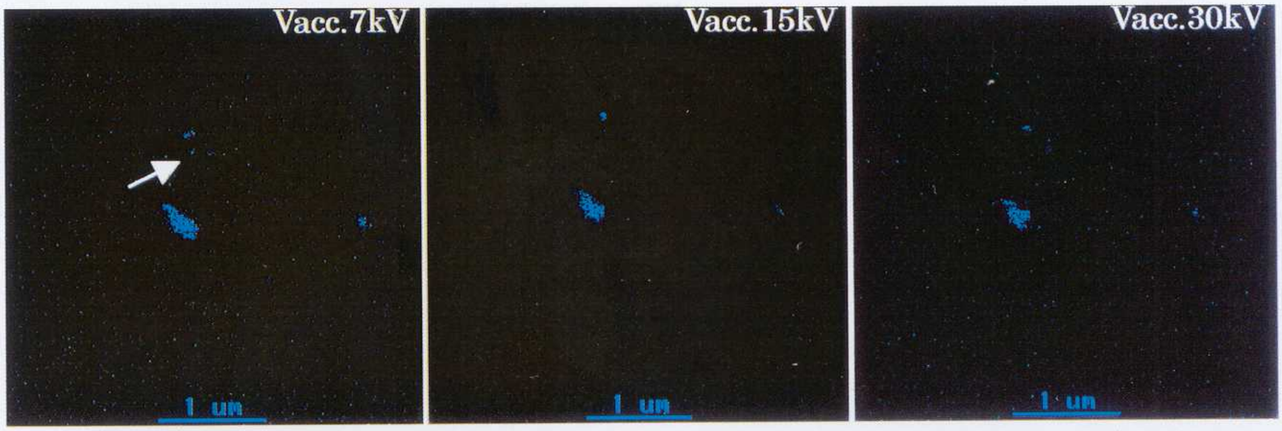


Fig. 9 X-ray mapping images (Ag-L) of human skin of argyria

4. CLOSING REMARKS

We have introduced a low voltage STEM technique using the S-5200 for microscopy of biological sections. It was confirmed that image contrast and resolution were satisfactory for observation of biological sections. It was also confirmed that a low voltage of 15 kV or lower results in large electron scattering within the sample which contributes to better image contrast and X-ray emission yield. This is a favorable sign for microscopy of cryosections, and microanalysis of small concentrations in cells in the future.

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