HITACHI SCIENTIFIC INSTRUMENT
TECHNICAL DATA STEM

SHEET NO. 6

SUBJECT: PREPARATION OF BIOLOGICAL TISSUE SECTIONS USING FIB TECHNIQUE AND MICROSCOPY

INSTRUMENT: THE FB-2000A FOCUSED ION BEAM SYSTEM
THE HD-2000 ULTRA-THIN FILM EVALUATION SYSTEM

CONTENTS

1. INTRODUCTION

Specimen preparation using FIB (focused ion beam) technique has been employed for electronic materials and devices as well as many other fields of science and industry. One of the unique features of this technique is that it allows specimen preparation with the help of SIM or scanning ion microscopy so that it permits site-specific specimen preparation. We have reported on FIB/(S)TEM system integration with which we have achieved a site-specific specimen preparation as thin as 0.1 μm. Coupled with the development of micro-sampling technique, we have allowed direct TEM specimen preparation from bulky samples. Recently the FIB technique has generated interest in polymer and biological material fields. We report here on some applications of the FIB micro-sampling technique for preparation of biological tissue sections and some microscopy results.

2. METHOD

2.1 Specimen and purpose of observation

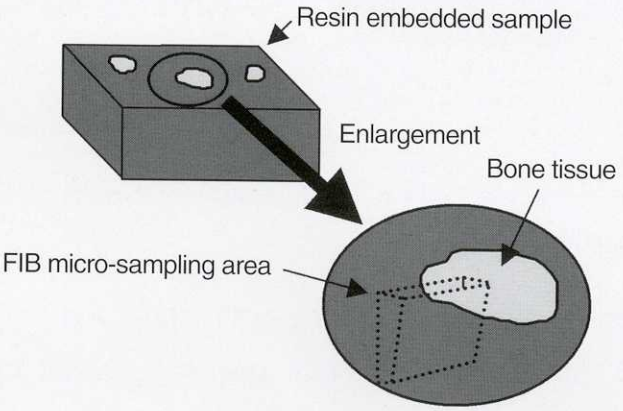
We have observed the knee joint and cells surrounding the joint of a rat.

2.2 Specimen preparation

Fig. 1 shows specimen preparation procedures. Biological materials were embedded in resin and a part of it was mechanically polished. We examined the polished specimen by using backscattered electron imaging and elemental mapping techniques (Fig. 1-1) so that we might locate specific area(s) of interest prior to FIB milling. We then cut out the specific area of interest from a bulky sample using micro-sampling technique (Fig. 1-2). The cutout sample was fixed on a specimen stub using W-deposition function of the FIB (Fig. 1-3). Then, a part of it was FIB milled to a thin section for cross-sectional microscopy (Fig. 1-4).

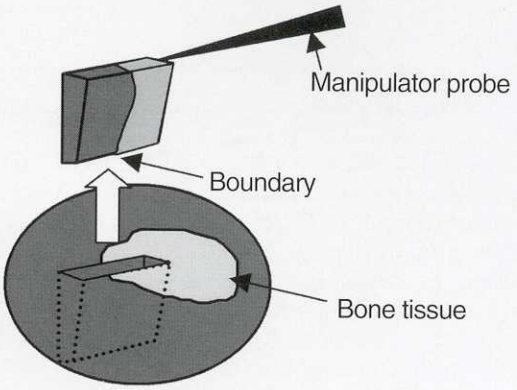
(1) Locating the area of interest

- BSE image observation
- X-ray mapping image observation

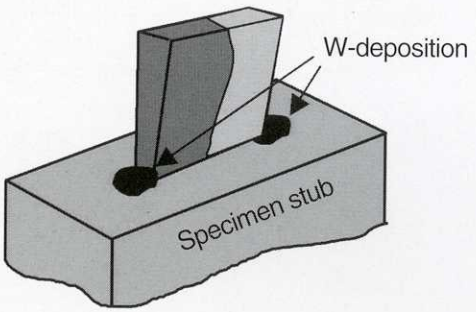


(2) Cutting out a specific area of interest

- FIB micro-sampling



(3) Fixing the cutout sample



(4) FIB milling of the cutout sample to a thin section

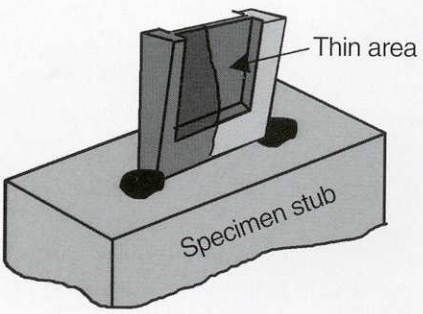


Fig. 1 Specimen preparation procedure

2.3 Instrument

We have used the FB-2000A for FIB milling. We have used the S-4700 FE-SEM with EMAX energy dispersive X-ray spectrometer for BSE imaging and X-ray mapping respectively. We have used the HD-2000 (200 kV STEM) for observation of a prepared specimen.

2.4 Specimen preparation procedures

2.4.1 Locating the area of interest by BSE imaging

Fig. 2 shows a secondary electron image (a) and BSE image (b) of the prepared specimen. The secondary electron image (a) shows topographic details of the specimen surface clearly. But it was difficult for us to locate specific areas of interest in the bone tissue. We observed the same specimen using BSE (backscattered electrons). The BSE image (b) shows some white shiny areas which indicate that there are some higher average atomic number materials in these areas than in the rest of the areas.

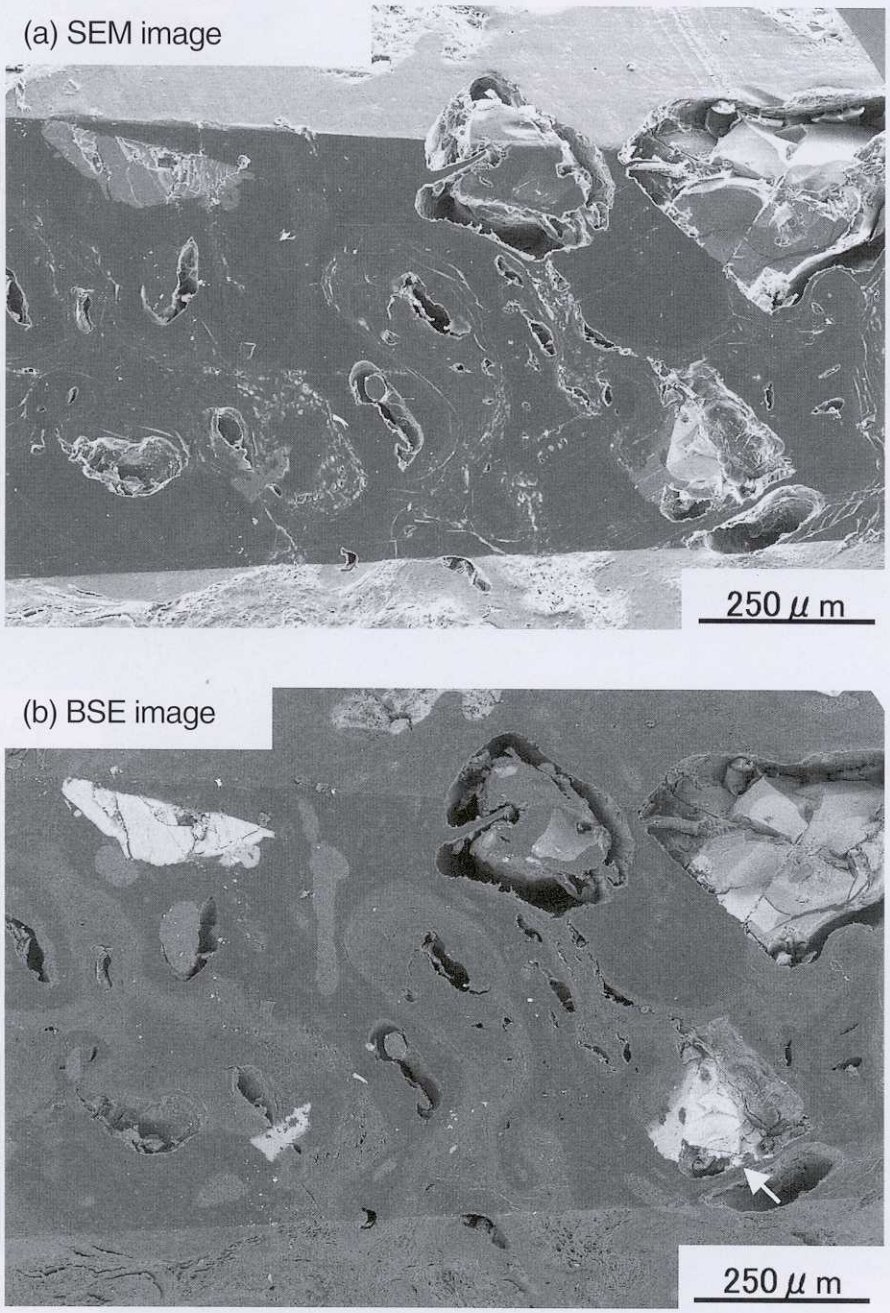


Fig. 2 SEM image (a) and BSE image (b) of bone and peripheral tissues

2.4.2 Composition analysis using X-ray mapping

Fig. 3 shows X-ray mapping of the white shiny area indicated by an arrow in Fig. 2(b). Calcium and phosphor have been detected from these white shiny areas. From this analysis result, we have identified that they are bones.

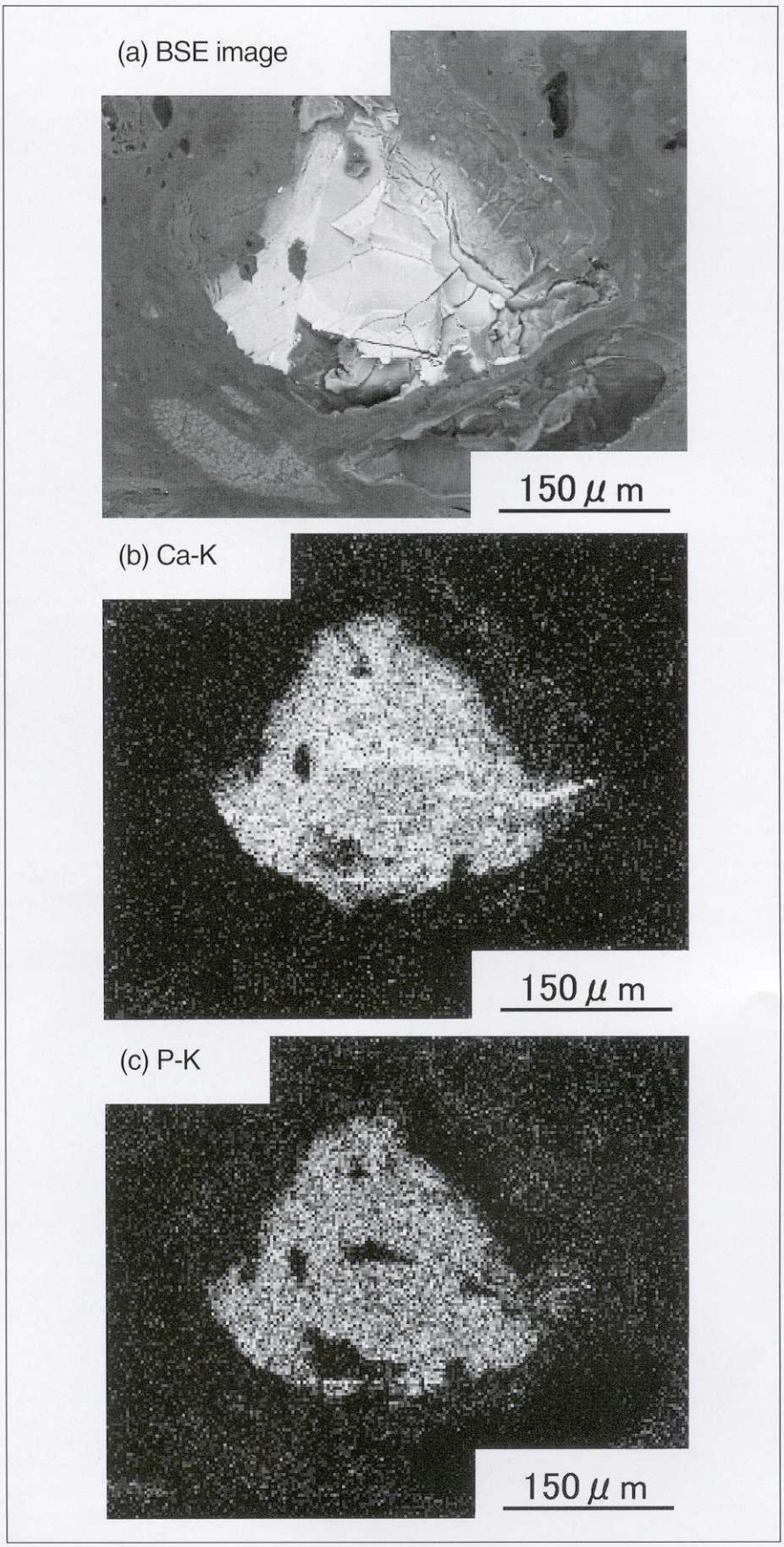


Fig. 3 BSE image (a) and X-ray mapping (b) and (c)

2.4.3 Locating the areas of interest using FIB

Fig. 4 shows BSE image (a) and SIM (scanning ion microscope) image (b) of the same area. The boundary area (shown by an arrow) of bone and peripheral tissues is not visible on SIM image. For locating this area, we have paid attention to the specific structure (shown by △). From this structure, we have set the milling area.

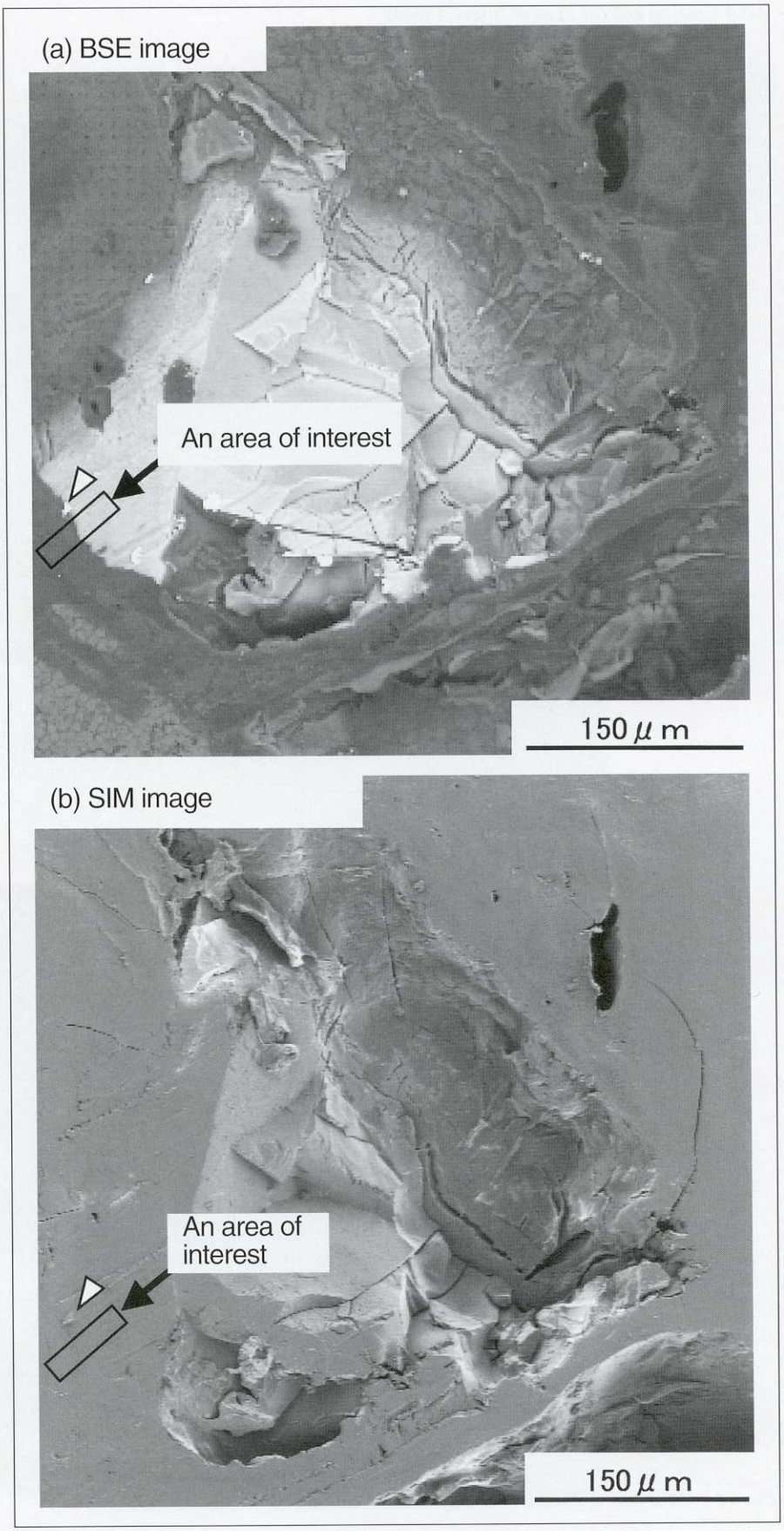


Fig. 4 Locating the specific area of knee joint and cells

2.4.4 Cutting out the area of interest using micro-sampling technique

Fig. 5 shows an SIM image of the specified area on Fig. 4 after W-deposition. The specified area is cut out from the rest of the sample by FIB milling. It is picked up using micro-sampling technique. Fig. 6 shows a general view of the cutout specimen, a part of which has been milled to a thin section by FIB.

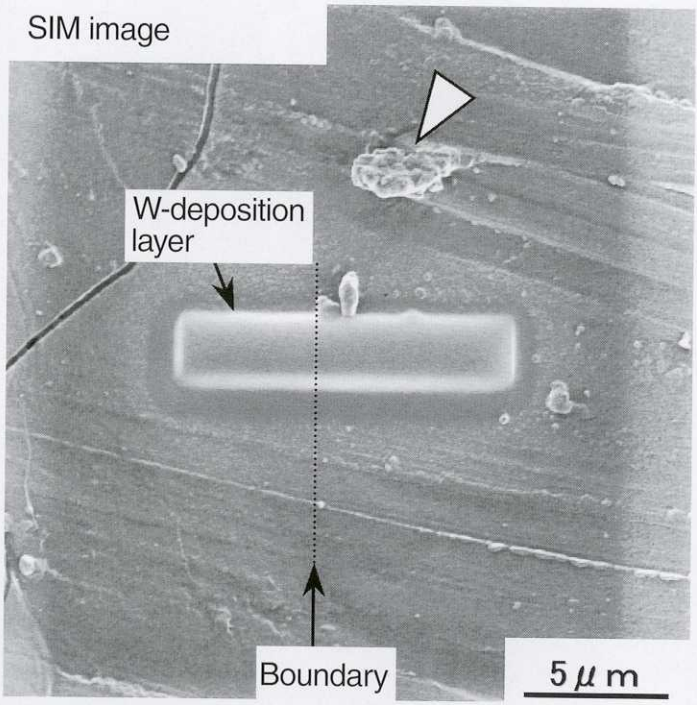


Fig. 5 SIM image of W-deposition area

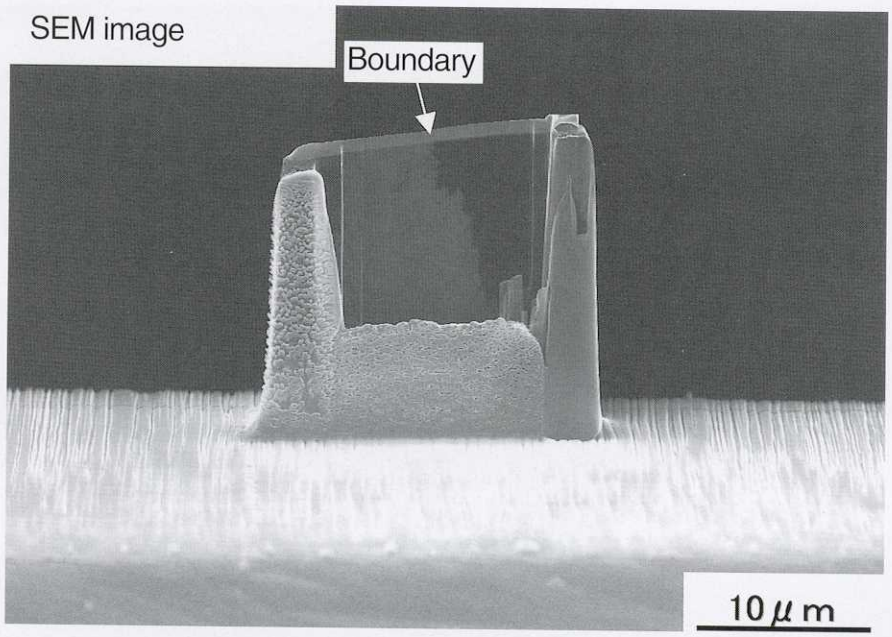


Fig. 6 A general view of thin specimen prepared by FIB miiling

3. RESULTS OF OBSERVATION

Figs. 7(a) and (b) show cross-sectional images of knee joint and cells surrounding the joint. The boundary area shows complex conditions of bone and peripheral tissues. Fig. 7(b) shows collagen structures in the bone tissue. The observation shows that FIB technique is quite useful for specimen preparation of biological hard tissues.

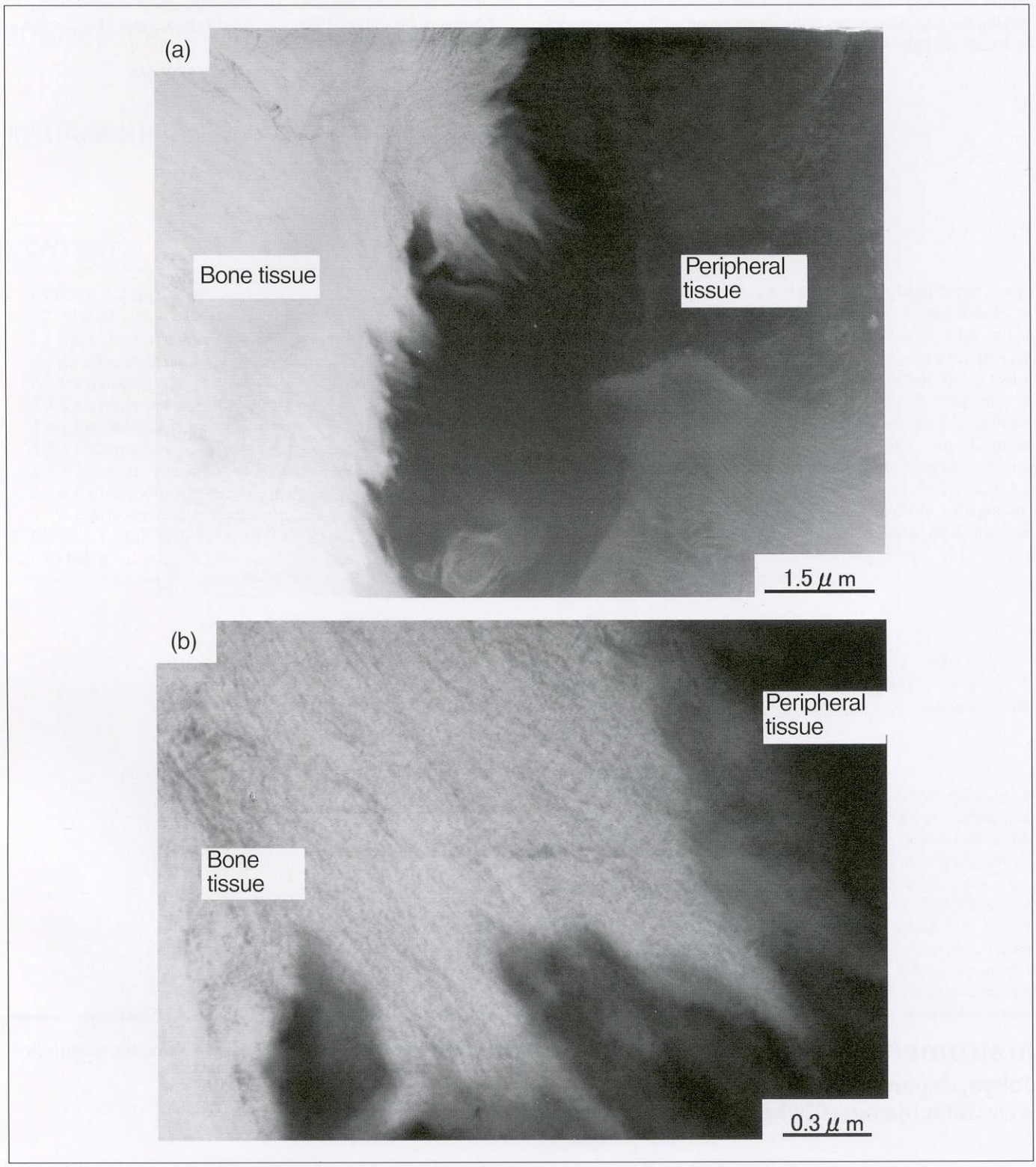


Fig. 7 Cross-sectional image of knee joint and cells

4. CLOSING

We have introduced thin specimen preparation of boundary areas of bone/peripheral tissues using FIB technique. Since it was difficult to locate specific areas of interest by using SIM image alone, we used BSE and X-ray mapping images, both of which are easily available using SEMs with EDX spectrometer. Taking the best advantages of available instruments, it is possible to locate specific areas of interest without difficulty. We have confirmed that FIB technique is useful for thin specimen preparation of biological hard tissues without any serious damage. We trust that this technique will be useful for specimen preparation of specific areas of interest with polymers and other biological tissues. We wish to thank Dr. Antonio Nanci (University of Montreal, Faculty of Dentistry Stronatorogy Dept.) for providing us with precious samples.

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