

## Preparation of a High Quality Cross Section of a Bone Tissue for SEM --Application of the Cross-section Polisher to a Biological Specimen--

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### 1. Introduction

A bone tissue of a mouse tail, composed of hard and soft materials, was polished with the Cross-section Polisher (CP) for obtaining wide and smooth cross-section. The prepared specimen was observed with a SEM and analyzed with an EDS.

### 2. Materials and Methods

#### (1) Specimen: mouse tail

##### Cross-section Polisher

It is a specimen preparation tool for cross-sectioning which uses an Ar ion beam. Usually a specimen is covered with a shielding plate and a broad Ar ion beam is irradiated on it. A cross section of the specimen is produced along to the edge of the shielding plate.

##### Specimen preparation procedure

- (1) Perfusion fixation: 2.5% glutaraldehyde / 0.1M PBS (pH 7.4)
- (2) Washing: 0.1M PBS
- (3) Post fixation: 2% osmium tetroxide / 0.1M PBS (4°C / 1.5h)
- (4) Dehydration: ethanol
- (5) Decantation: propyleneoxide
- (6) Embedding: EPON812 (TAAB)
- (7) Polishing: diamond lapping sheet (30  $\mu$  m, 10  $\mu$  m, 3  $\mu$  m) → diamond paste (1  $\mu$  m)
- (8) CP polishing

##### CP polishing

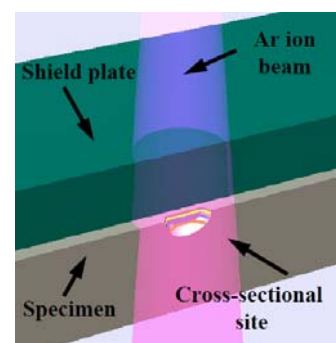
Mechanically polished specimen was mounted on a rotation holder of CP.

An Ar ion beam was irradiated on the rotating specimen with grazing incidence of 2 to 4 degrees.

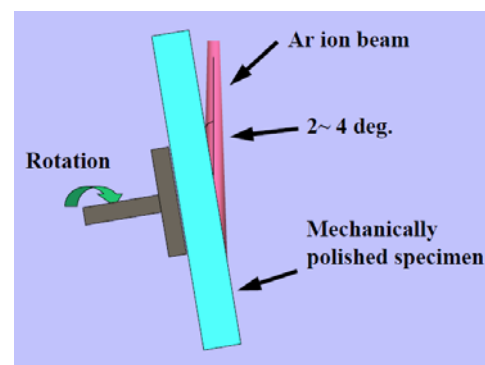
Accelerating voltage: 4 kV  
Ion beam current: 150  $\mu$  A  
Processing time: 4h



**Cross-section Polisher  
(IB-09010CP)**



**Principle of CP**



**Rotation holder for CP**

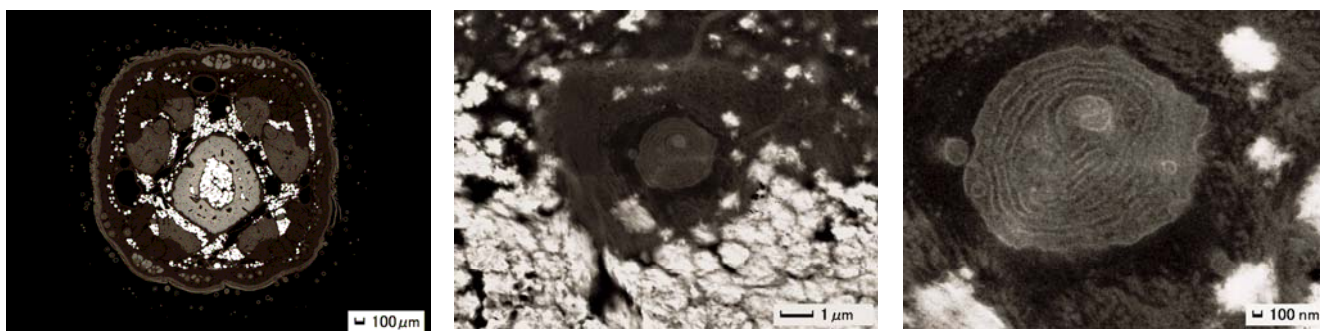
### 3. Result and Discussion

#### (1) SEM Observation

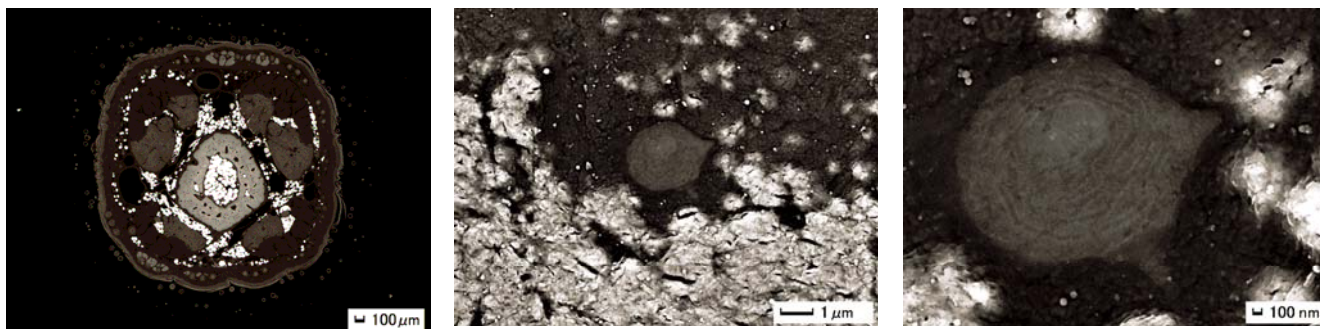
A CP-polished specimen and a mechanically polished specimen were observed at 10 kV in the backscattered electron mode with JSM-7001F Field Emission Scanning Electron Microscope.

At low magnification, there are no differences in both preparation methods. At high magnification, fine structures of a soft bone cell which seemed to be mitochondria, endoplasmic reticulum and collagen fibers are clearly observed in the CP-polished specimen. On the contrary, many small cracks are observed and fine structures are not observed clearly in the mechanically polished specimen.

#### CP polishing

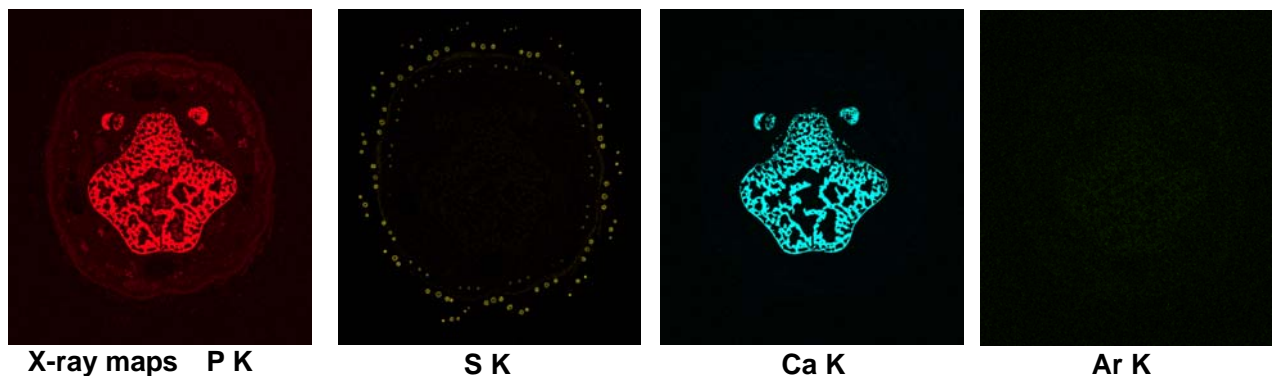
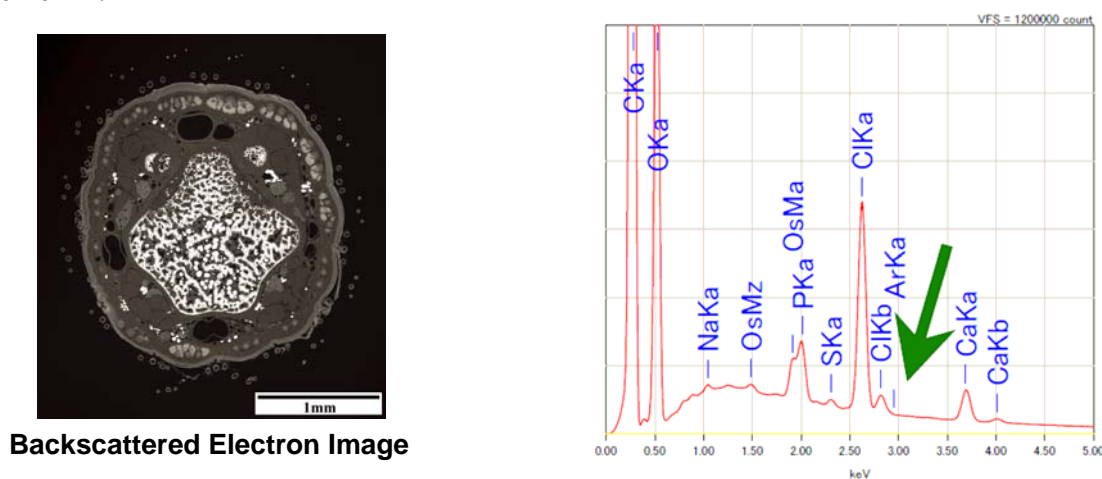


#### Mechanically polishing



## (2) EDS Mapping

A CP-polished specimen was analyzed at 15kV with JSM-6390LA Scanning Electron Microscope. The EDS spectrum was obtained from a whole field of view. Ca, P and S are detected but Ar is not detected (Arrow). It shows CP-polishing is excellent specimen preparation technique also for elemental analysis. X-ray maps show localization of Ca and P in a bone tissue and S in a skin and tail-hair. Ar map is formed with the background intensity at an energy of Ar and does not mean localization of Ar.



## 4. Conclusion

- (1) Cross section of very wide area over 3 mm in diameter was polished.
- (2) Very smooth cross-sectional surface was obtained in spite of composed material of hard and soft tissues.
- (3) SEM observation ranging several ten to several ten thousand times was possible for one specimen.  
Fine structures of a cell were observed at high magnification.
- (4) With EDS analysis, main elements from the tissue were detected but Ar from the CP polishing process was not detected.

## 5. References

- [1] M. Shibata: JEOL News, [39] (2004) p.28
- [2] M. Shibata. S. Asahina. T. Negishi. Proc. 8 APEM, (Kanazawa 2004) p.258