Damage Less Cross Sectioning Method for Biominerals and its applications

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Pearls or pearl shells are one of typical jewelry items in the world due to their beautiful color and stability of structure. Nowadays, we already understand that they have a unique color of gloss caused by the difference in visible light interference due to the fine multilayered structures. And it is called ‘Nacre’ consisting of multi layers of aragonite and protein. However, we have been studying their mysterious formation mechanism of structure in mineral crystallography, biology or other fields until now [1][2][3]. Moreover, many researchers have reported in the biomimetic field in order to develop artificially reproduced colors and strength, etc. as new high performance ecological materials for industrial products [4].

In such various research fields, it is important to understand detailed information on nano scale. Scanning Electron Microcopy (SEM) is one of the powerful techniques to characterize the structure of biological materials. On the other hand, it is usually very difficult to observe the cross section of nano structures in Biomineral samples.

Recently we succeeded in developing cross section method using Ar ion beam which can process cross section of microstructures. However, most of biominerals in shells consist of carbonate minerals (e.g. calcite, aragonite) and coexist with proteins, which are very weak for heating and are easily damaged by irradiation with Ar ion or even electron beam. Therefore, it was difficult to understand micro and nano structures of nacre in abalone shells from the cross section observation by SEM after Ar ion milling.

In this study, we challenged to prepare cross section samples of the abalone nacre layer by Ar ion beam milling using sample cooling system called cross section polisher (JEOL IB-19520CCP). And we compared Ar^+ ion milling samples with and without cooling.

Figure 1 shows a comparison between FE-SEM images of samples with and without cooling during Ar^+ ion milling process. For the sample without cooling, a lot of micro pores appear at protein layer. Whereas there are no micro pores in the sample cooled during Ar^+ ion milling process. And it is clearly observed that aragonite and protein are formed layer by layer as shown in Figure 1.

Furthermore, we tried EBSD orientation mapping by a newly developed EBSD detector (Oxford instruments Symmetry) which is adopted in a supersensitive CMOS device for first acquisition and lower voltage.

As a result of the EBSD analysis, we could get high quality maps of the Ar^+ ion milled cross section sample prepared using the cooling system as shown in Figure 2.

Usually, biominerals such as an abalone shell are very sensitive even for electron beam. Nevertheless, it is possible to analyze crystal orientation at 5 kV that is low acceleration voltage condition for EBSD by using the CMOS type EBSD detector.

We believe that the combination of Ar^+ ion milling with sample cooling and low voltage EBSD technique is powerful for structural analysis in biomineral field.

References

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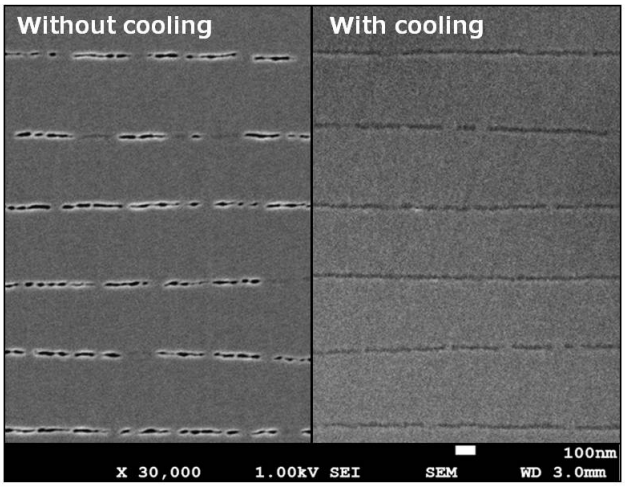

Fig. 1 FE-SEM images of an abalone nacre layers. Samples are processed by Ar ion beam with and without cooling.

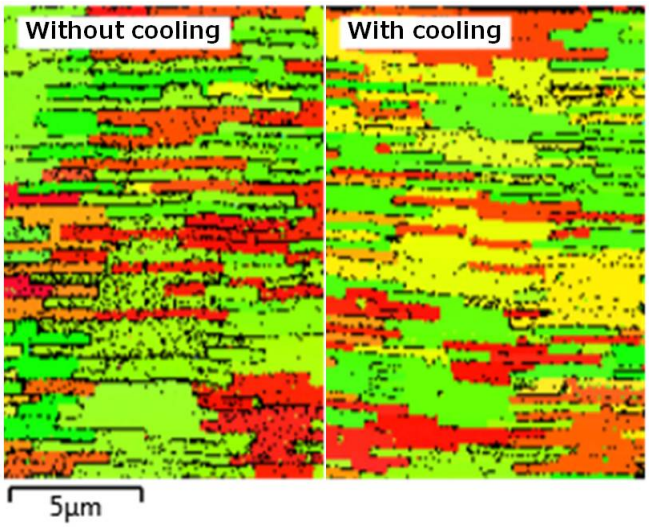

Fig. 2 EBSD maps of abalone nacre layers. (Acc. 5 kV, P.C. 5.7 nA, S.S. 100 nm, Without noise reduction) Black points in maps are index errors.

