Analysis of internal structure for Radiolarian microfossil by SEM via Ar^+ ion beam cutting method

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Radiolarians are marine unicellular planktonic protozoa with a skeleton consisting mainly of opaline silica or strontium sulfate. Among these groups, Nassellaria, Collodaria and Entactinaria have initial spicule within or without the shell. The configuration of the initial spicule is the most important trait of the higher classification for these groups. Because of excellent fossil record for 500 million years, Radiolaria are invaluable for determining geological age and palaeoenvironment using marker species. Therefore, analyzing initial spicular system of Radiolaria is useful for enhancing reliable classification and reconstructing evolutionary lineage. In general, SEM is a useful tool to observe surface morphology of Radiolaria, but it is not easy to directly observe internal structure of the shell. In recent years, it has been reported that internal structures of radiolarian genus *Glomeropyle* were analyzed by X-ray micro CT using SEM [1]. However, since the resolution of X-ray micro CT using SEM is around 380 nm/pixel, another method is needed to observe much finer internal structures such as a resolution less than 100 nm. Presently we are applying a new method. The new method is possible to cut fragile shells of Radiolaria without any mechanical stress due to broad Ar^+ ion beam cutting. Therefore, the internal structure in the shell can be clearly observed using SEM.

The feature of the Ar^+ ion beam cutting method is to prepare a 1 mm wide cross section of the sample in a short time and not apply mechanical stress to the sample [2]. Moreover, the accuracy of the processing position can be achieved to around 5 μm in combination with optical microscope. In this study, we have analyzed initial spicular system of *G. grantmackiei*, which was reported by Aita et al.(2017), [3].

Figures 1 (a) and (b) show SEM images of the specimen before and after Ar^+ ion beam cutting, respectively. Processing conditions for Ar^+ ion beam cutting were set to an accelerating voltage of 5.0 kV and a process time of 30 min using the Cross section polisher, IB-19530CP (JEOL Ltd.). JSM-7200F (JEOL Ltd.), which has high spatial resolution and large depth of focus, was used for observation of processed specimen. Figure 1 (b) shows the result that the outer shell of *G. grantmackiei* was processed without damaging any internal structure by Ar^+ ion beam cutting. Three dimensional internal structures are clearly observed from the window fabricated by Ar^+ ion beam cutting (Fig. 2 (a)). This internal structure well agreed with the initial spicule system (Fig. 2 (b)) analyzed using X-ray micro CT [1]. In addition, quartz crystals due to diagenetic process exist on the surface of the spicule in the magnified SEM image (Fig. 2 (c)).

As the result, the newly developed cutting method using Ar^+ ion beam can be applied for observing much finer internal structure of Radiolaria *G. grantmackiei*. And this method is a powerful new tool to analyze internal structures of fragile and fine specimens of microfossils like Radiolaria.

Reference

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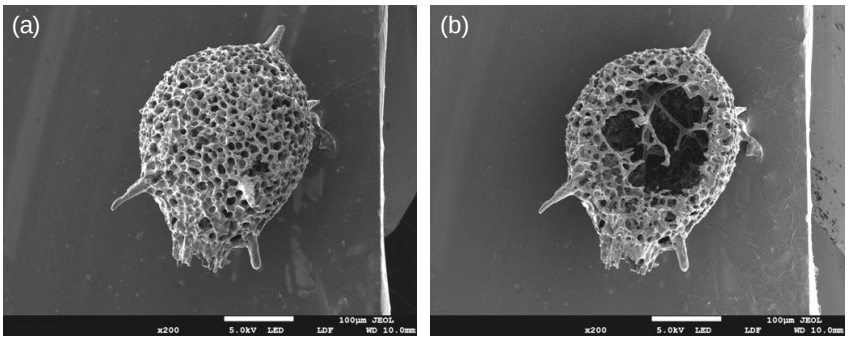

Fig. 1. Images of *G. grantmackiei* (a) before (b) after Ar^+ ion beam cutting.

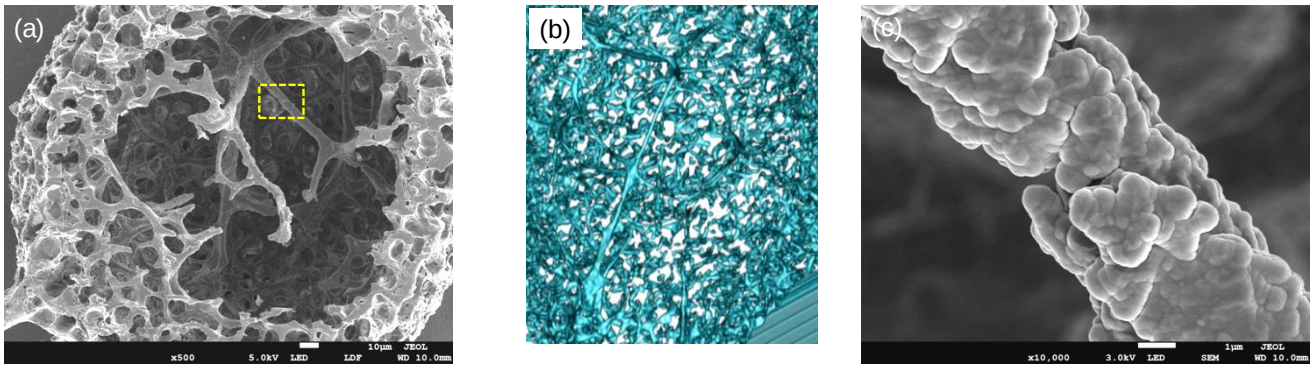

Fig. 2. (a) SEM image of the internal structure observed from the window, (b) initial spicular system analyzed by using X-ray micro CT , (c) SEM image of the magnified spicule in the square area of (a).