doi:10.1017/S1431927622006067
Microsc. Microanal. 28 (Suppl 1), 2022

3D Imaging and Elemental Analysis of Biological Samples

Yuuki Yamaguchi[1], Yukari Moriya[1], Tomohiro Haruta[1] and Shunsuke Asahina[1]

[1.] JEOL Ltd. Akishima, Tokyo, Japan.

Array Tomography (AT) is a powerful technique which enables us to observe three-dimensional (3D) structures of biological specimens using Scanning Electron Microscopy (SEM) [1-4]. Furthermore, it is possible to acquire 3D information on elemental distribution in the specimens by the combination of AT and Energy Dispersive X-ray Spectroscopy (EDS). 3D information of elemental distribution by AT-EDS can be expected to apply for understanding drug delivery system. For example, AT-EDS would be possible to reveal distribution of drugs in cells by labeling the drugs with nanoparticles before the drugs are ingested in the cell. We used *Paramecium* as a model sample for experiment with AT-EDS because *Paramecium* is easy to ingest and preserve nanoparticles. Recently, we have reported the result of 3D imaging and elemental analysis of *Paramecium* which ingests gold, silver and silica nanoparticles by using AT-EDS [5]. In that study, *Paramecium* was cultured firstly in water with gold nanoparticles, secondly in water with silver nanoparticles, and finally in water with silica nanoparticles. As the result, gold was most detected in a phagosome of *Paramecium*. And a small amount of silica was detected, but silver was not detected in the phagosome. It leads the suggestion that *Paramecium* selectively ingested gold, silver or silica nanoparticles. However, the order of ingestion for the nanoparticles may cause the selectivity. In this study, we attempted to further verify the selective ingestion habits of *Paramecium* by culturing *Paramecium* in an environment where nanoparticles of gold, silver and silica can be simultaneously ingested.

Paramecium was cultured in water with homogeneously mixed gold nanoparticles, silver nanoparticles, and silica nanoparticles for 1 hour. The *Paramecium* was fixed with glutaraldehyde and OsO_4 and then block stained with uranium. Ultrathin sections totaling 370 slices with a thickness of 200 nm were prepared by an ultramicrotome after being embedded with resin. Finally, they were mounted on a carbon substrate (Ultra Flat Carbon: UFC, JEOL Ltd.). Conventionally, ultrathin sections are stained by heavy metal such as lead for enhancing the contrast of backscattered electron (BSE) images. However, lead is easy to deposit on the surface of sections. The deposition may be recognized as nanoparticles on BSE images of sections. Therefore, we did not stain the sections by lead. The 3D volume imaging was carried out by FE-SEM (JSM-IT800SHL, JEOL Ltd.). Scintillator BSE Detector (SBED) with high responsibility and high sensitivity is mounted on JSM-IT800SHL. Serial two dimensional (2D) images of each section were acquired with high throughput in a short time using SBED. Furthermore, the 2D images were acquired automatically by using dedicated software (Array Tomography Supporter, JEOL Ltd.). A 3D image of the whole structure of *Paramecium* was reconstructed by stacking the 2D images. Elemental analysis for nanoparticles in phagosomes was carried out by EDS (JED, JEOL Ltd.) mounted on the JSM-IT800SHL.

2D images of 370 slices were obtained for 8 hours by using SBED. In this case, it took 78 seconds to obtain one image (10240 x 3840 pixels) of each slice including stage moving. In case of using conventional BSE detector which is semiconductor type, it took 180 seconds to obtain one image (10240 x 3840 pixels) of each slice. This means that SBED enables us to take the images in over two times shorter than using conventional BSE detector. A 3D image of *Paramecium* was reconstructed with a volume of 250 μm x 90 μm x 70 μm by stacking the 2D images (Figure 1a). Several phagosomes were

founded in this 3D structure. A slice image of a phagosome in the XY plane is shown in Figure 1b. As a result of elemental analysis, all particles of gold, silver and silica were detected in a phagosome (Figure 2). In this study, all of gold, silver and silica nanoparticles were detected in a phagosome of *Paramecium* by culturing *Paramecium* in water with homogeneously mixed gold, silver and silica nanoparticles. It is suggested that the selectivity of nanoparticles in *Paramecium* is caused by the order of ingestion for the nanoparticles.

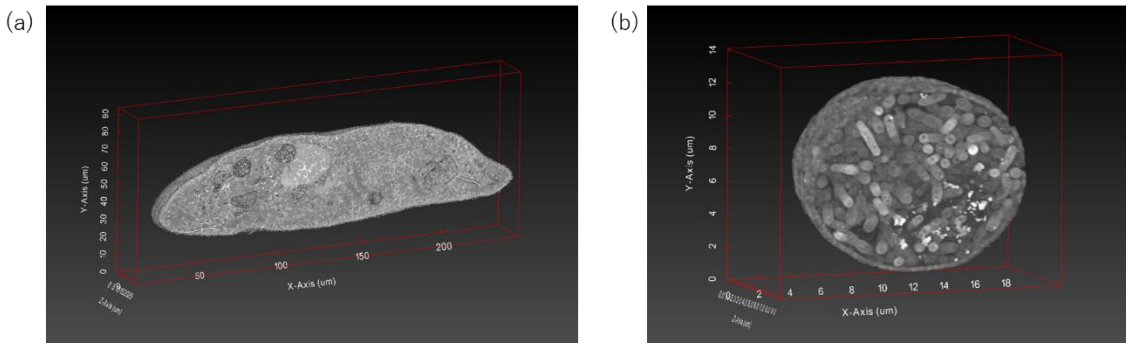

Figure 1. 3D image for whole structure of *Paramecium* (a) and a phagosome (b).

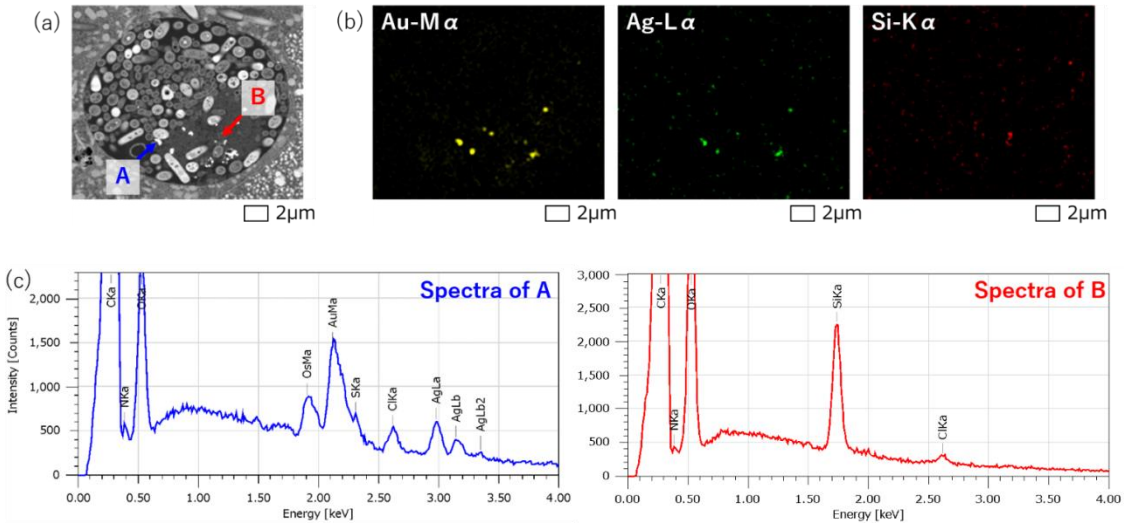

Figure 2. Elemental analysis of nanoparticles in a phagosome. (a) BSE image. (b) Elemental maps of Au-Mα line, Ag-Lα line and Si-Kα line. (c) X-ray spectra of points A and B on the BSE image.

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https://doi.org/10.1017/S1431927622006067 Published online by Cambridge University Press