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Quick 3D Elemental Mapping of Biological Tissues using Super High Solid Angle EDS

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Three-dimensional (3D) volume imaging of biological tissues by Array Tomography (AT) is a powerful technique which enables us to understand fine complex structures in large volumes [1,2,3]. Especially, many studies of brain networks such as Connectomics have been reported with AT [4]. However, very few studies of 3D elemental mapping with AT have been challenged since high quality elemental mapping takes a long acquisition time for each thin section. On the other hand, artifacts that involve both electron beam damage and specimen charging are substantially reduced with thin sections. Therefore AT has a chance to acquire elemental mapping. Actually, we reported 3D elemental mapping of silver nanoparticles in mouse liver tissue by using high solid angle multi Energy Dispersive X-ray Spectroscopy (EDS) in Microscopy and Microanalysis 2017[5]. As a result, it was possible to detect silver nanoparticles. However, other elements such as heavy metal stain were not able to be detected because the concentration of heavy metals is extremely low, making detection of the X-rays almost impossible. Thus, we have challenged 3D elemental mapping of heavy metal stain as well as silver nanoparticles by using super high solid angle EDS.

We prepared two different types of specimens, mouse liver tissue and mouse cerebellum tissue.
1) Mouse liver tissue was fixed by formaldehyde. Then the tissue was stained by osmium tetraoxide and uranyl acetate after immunolabeling with gold nanoparticles and silver enhancement. The resin-embedded tissue was cut at a thickness of 100 nm and 61 serial sections were placed on Ultra Flat Carbon substrate (JEOL Ltd.). 2) Mouse cerebellum tissue was fixed with formaldehyde. Then the tissue was stained with osmium tetraoxide and uranyl acetate after immunolabeling by synaptobrevine with gold nanoparticles and silver enhancement. Synaptic vesicles were labeled by the synaptobrevine. The resin-embedded tissue was cut at 100 nm and 64 serial sections were placed on Ultra Flat Carbon substrate (JEOL Ltd.). The 64 sections were stained with uranium acetate and lead citrate.
Elemental mapping was carried out by using JSM-7900F (JEOL Ltd.) with super high solid angle EDS detector: XFlash®5060FlatQUAD (Bruker). EDS analysis conditions were as follows: incident voltage 7.0 kV, probe current 16 nA, magnification x5,000 or 10,000 and working distance 10 mm. Acquisition time was 2 min by live time. 3D volume images were then reconstructed by the software (alignment: Image J, segmentation: Colorist from System In Frontier Inc., Tokyo Japan, reconstruction: Visualizer-kai from System In Frontier Inc.).

As a result, we obtained 3D elemental maps of liver tissue in 12 μm x 9.0 μm x 6.1 μm volume. Both silver nanoparticles and osmium were clearly obtained as 3D elemental mapping by this method in Figure 1. And Figure 2 shows that we obtained 3D elemental maps of cerebellum tissue in 24 μm x 18 μm x 6.4 μm volume. Uranium map and silver nanoparticles map were also obtained. Here, we have been able to obtain 3D elemental mapping of even a few amount of uranium or osmium as well as silver nanoparticles by using super high solid angle EDS.

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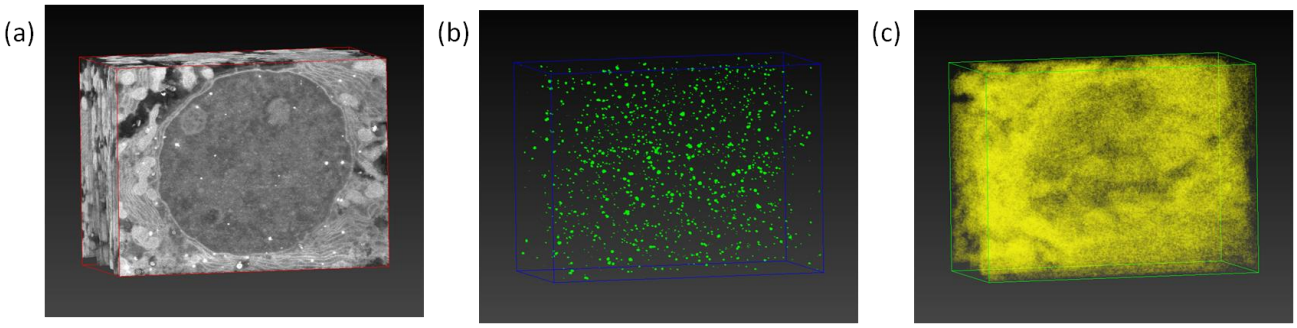

Figure 1. 3D elemental maps of liver tissue. 3D volume is 12 μm x 9.0 μm x 6.1 μm. (a) Backscattered electron image. (b) Silver map. (c) Osmium map.

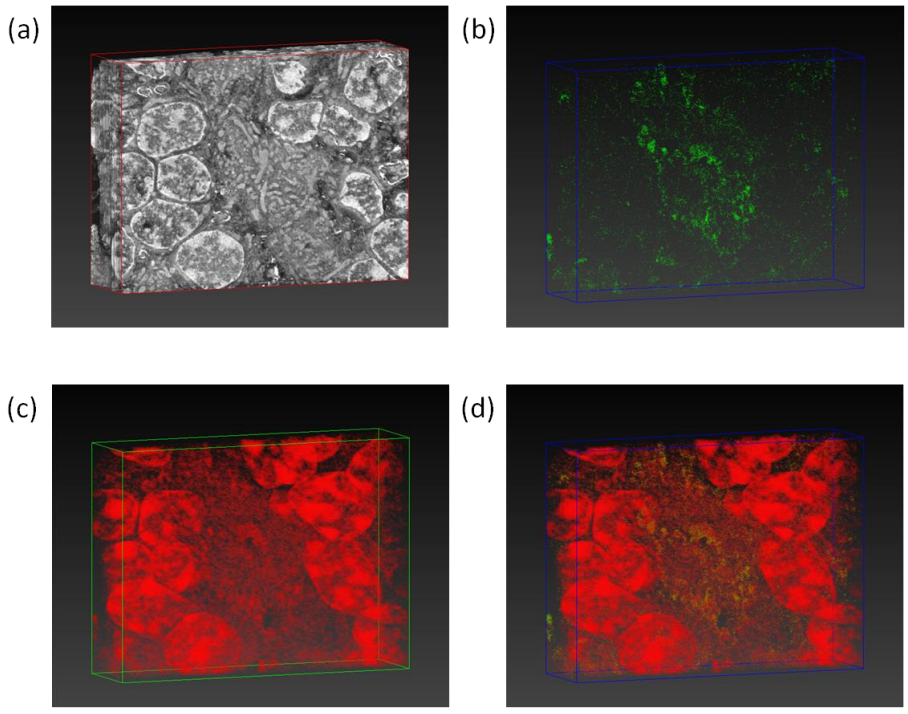

Figure 2. 3D elemental maps of cerebellum tissue. 3D volume is 24 μm x 18 μm x 6.4 μm. (a) Backscattered electron image. (b) Silver map. (c) Uranium map. (d)Merged map of silver and uranium.

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