

3D Elemental Mapping by Array Tomography Particle Analysis

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Array Tomography (AT) is a method for three-dimensional (3D) volume imaging of soft materials such as biological or polymer materials by using Scanning Electron Microscopy (SEM). Serial ultrathin sections prepared with ultramicrotome are placed on substrates and then observed with SEM [1,2,3]. And 3D volume images are reconstructed by stacking from two-dimensional (2D) images of each section. Therefore, it is a very powerful technique to understand complex structures in soft materials. However, very few studies of 3D elemental mapping have been reported since there is less characteristic X-ray emission from ultrathin sections as compared to bulk. On the other hand, artifacts that involve both electron beam damage and specimen charging are substantially reduced, even if elemental mapping requires electron beam at high incident voltage with large probe current, due to the fact that the sections are placed on a conductive substrate directly. Our previous work [4] focused this advantage of AT on 3D elemental mapping of silver nanoparticles in mouse liver tissue by using high solid angle multi Energy Dispersive X-ray Spectrometry (EDS). As the result, 767 particles were detected in a tissue volume of 12 μm x 9.0 μm x 6.1 μm , but particles with diameter less than 40 nm were difficult to detect due to insufficient X-ray counts from these particles. Thus, we have tried 3D elemental mapping by AT particle analysis, in order to detect smaller particles than 40 nm with same acquisition time as the previous report in 2017.

Particle analysis was carried out by using JSM-7900F (JEOL Ltd.) with high solid angle multi EDS detectors: two X-max^N150 mm² and one X-max Extreme 100 mm² windowless detector (Oxford instruments). Mouse liver tissues were fixed by formaldehyde and immunolabeled with gold nanoparticles. Then the tissues were stained by uranyl acetate after silver enhancement. The resin-embedded tissues were cut at 100 nm and 61 serial sections were placed on Ultra Flat Carbon substrate (JEOL Ltd.). The 61 sections were analyzed under the following conditions in order to apply particle analysis: incident voltage 7.0 kV, probe current 1.6 nA, magnification x10,000 and working distance 10 mm. A backscattered electron image of 8192 x 6144 pixels was taken for 100 sec/frame with a dwell time of 2 μs /pixel. Around one hundred bright particles were detected in each frame, and every particle was analyzed by using high solid angle multi EDS for 1 sec. The particles containing more than 1 wt% of silver were labeled, and other particles were unlabeled.

As the result, we obtained 61 images of silver nanoparticles labeled using particle analysis. Each image was acquired for about 6 min in Figure 1. 3D volume image was reconstructed by stacking software (alignment: Image J, segmentation: Colorist from System In Frontier Inc., Tokyo Japan, reconstruction: Visualizer-kai from System In Frontier Inc.) with 61 images as shown in Figure 2. 2370 silver nanoparticles were detected in a tissue volume of 12 μm x 9.0 μm x 6.1 μm , and the average diameter was 61 nm and the minimum diameter was 8 nm in Figure 3. Here, we have been able to detect particles with diameter less than 40 nm using SEM-AT particle analysis method.

References:

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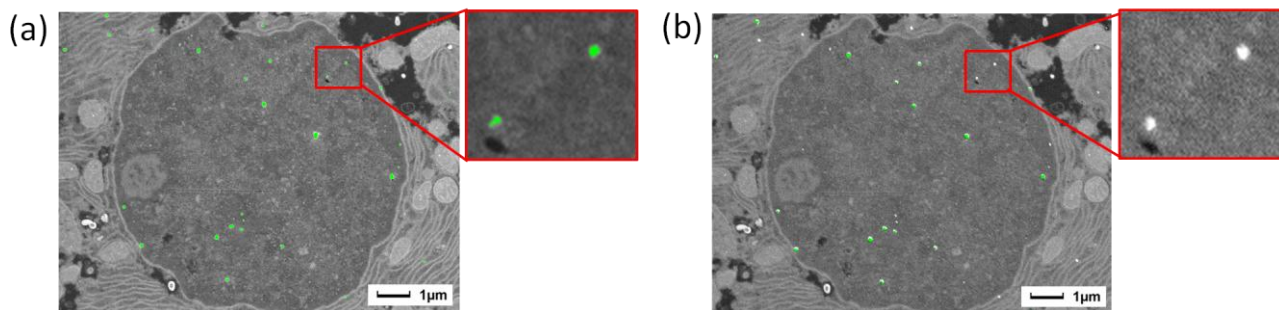


Figure 1. Backscattered electron images with silver nanoparticles labeled green.
 (a) Particle analysis. (b) Previous report [4].

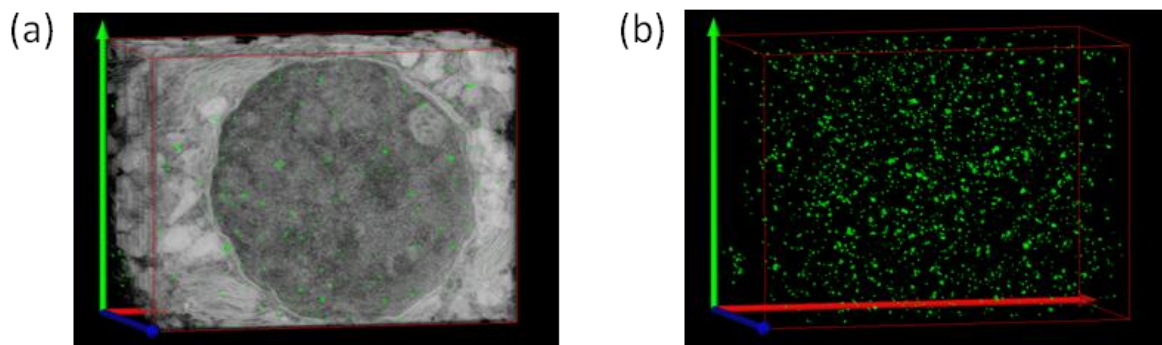


Figure 2. (a) 3D volume images of mouse liver tissue with green labeled silver nanoparticles.
 (b) 3D distribution of silver nanoparticles.

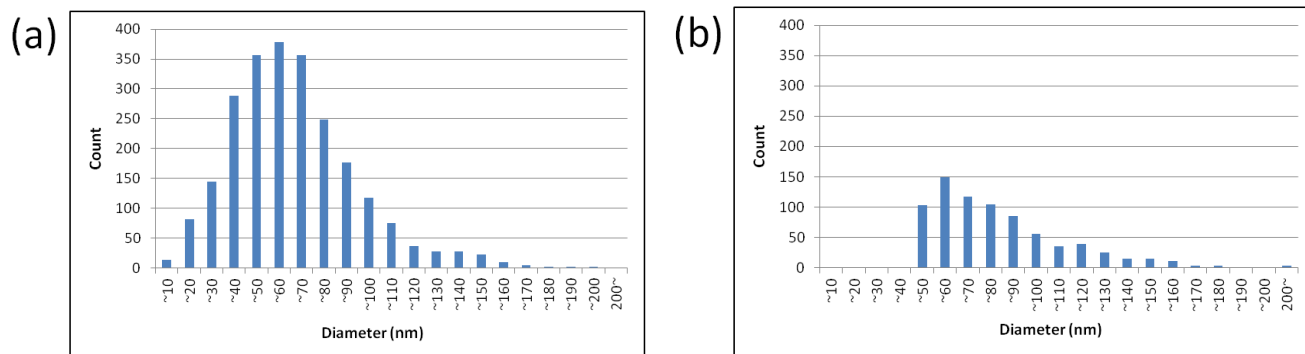


Figure 3. Size distribution of silver nanoparticles in 3D volume images. (a) AT particle analysis.
 (b) Previous report [4].