Compact-sized Cutting System for a Serial-block-face Scanning Electron Microscopy

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3D morphologies of living matters have close relations with the functionalities. To recognize the relationships, 3D observation of the biological tissues has been crucial in biology and medical science [1]. Among some 3D observation methods such as serial-block-face scanning electron microscopy (SBF-SEM) [2], focused ion beam SEM [1,3,4], array tomography [5], and an automated tape-collecting ultra-microtome [6], SBF-SEM is relatively fast and has large field of view with a high resolution of tens of nanometers. It obtains a 3D structure of a sample as a series of cross-sectional images by repeating slicing and imaging of the top surface of the specimen's block. However, a commercial system for SBF-SEM can be used only in some specific SEM systems. Therefore, we have studied a compact cutting device, which can incorporate into a general-purpose SEM to attain SBF-SEM. cutting resin samples with a thickness of a few tens of nanometer has been demonstrated [7]. In this study, we installed the cutting device into a general-purpose SEM and demonstrated fully automated 3D observation of kidney tissue of a mouse. Figure 1 shows the compact-sized cutting device. The total size was 75×75×45 mm. A diamond knife with a tip angle of 35 degrees (Syntek, Co. LTD, Japan) is attached to the x-directional stage driven by the piezoelectric actuator (MTDD04S60F12, Mechano transformer corp., Japan) with a stroke of a few tens of micrometers. A sample placed on the z-directional actuator (MTKK15S170f1320PS, Mechano transformer corp., Japan) is driven with a stroke of 150 μm and precision of a few nanometers. Both of the actuators were equipped with a mechanical transformer mechanism to amplify the stroke [8]. The cutting device was attached to the stage of a Schottky field emission scanning electron microscope (JSM-7900F, JEOL, Japan). The control system was constructed using PXI system and LabVIEW 2018 (National Instruments Corp. USA). It controls the motion of the two actuators and capturing images by interacting with SEM software through Ethernet. Voltages for x- and z-actuators in the SEM were magnified by respective voltage amplifiers (MTAD-3001, MTAD1110, Mechano transformer corp., Japan) and fed into SEM using a vacuum electric feedthrough attached to the SEM extension port. Samples were prepared based on the NICMIR protocol [9] modifying the staining and embedding process. A mouse's kidney was stained by TI blue (Nisshin EM Co., Ltd, Japan) and embedded in EPON 812 (TAAB, UK) containing Ketjenblack (Ketjenblack EC600JD, Lion Specialty Chemicals Co., Japan) to reduce charging [10]. Finally, it was coated by Osmium tetroxide. It was trimmed into the trapezoidal-shaped pillar with a top-width of 20 μm. Figure 2(a) shows a diamond knife and the sample pillar in the SEM. SEM images were obtained using a back-scattered electron detector with an acceleration voltage of 3 kV. The voltage step of the z-actuator between cycles was 2.5 mV, which corresponds to a cutting depth of approximately 50 nm. Figure 2(b) shows the SEM images of every ten cross-sections with a magnification of three thousand. Cutting in each cycle was succeeded, and the kidney tissue's fine textures were obtained with enough contrast. Total 42 images were obtained, and the required time for each cycle was approximately one minute. The limitation was the relatively short stroke of the x-directional actuator, which narrows the cutting area into a few tens of micrometers. It also caused the accumulation of sliced chips near the sample, which sometimes disturbed

capturing surface images. Although some limitations still need to be cleared, these results demonstrated the feasibility of our device for SBF-SEM.
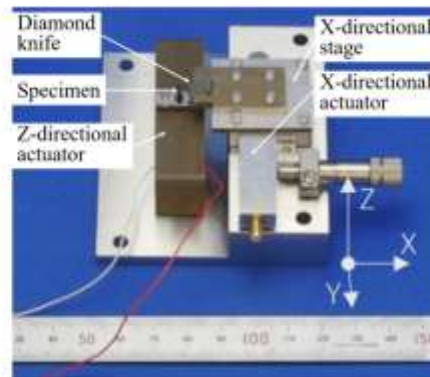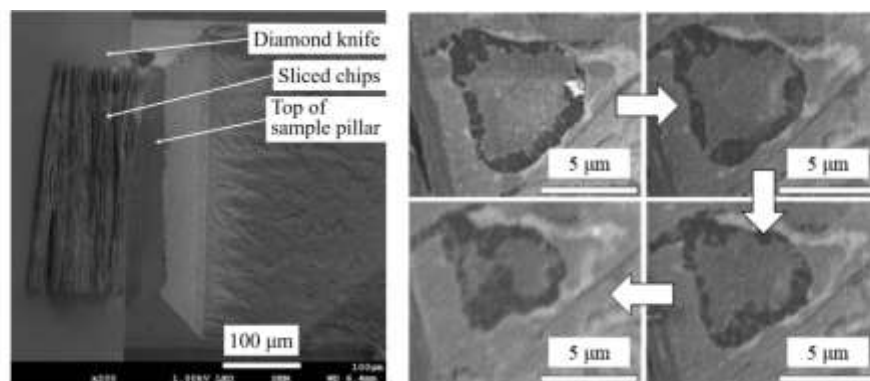

Figure 1. Compact-sized cutting device

Figure 2. (a: left) Sample and knife image. (b: right) Series of cross-sectional images

References
1. D. Wei, et al., BioTechniques. 53 (2012) 41–48.
2. W. Denk and H. Horstmann, PLoS Biology. 2 (2004) e329.
3. J. A. W. Heymann, et al., Journal of Structural Biology. 155 (2006) 63–73.
4. G. Knott, et al., J. Neurosci. 28 (2008) 2959.
5. K. D. Micheva and S. J. Smith, Neuron. 55 (2007) 25–36.
6. R. Schalek, et al., Microscopy and Microanalysis. 17 (2011) 966–967
7. Y. Nakade, et al., Proc. 8th Int. Conf. of Asian Society for Precision Engineering and Nanotechnology(ASPEN2019), (2019) A26.
8. S. K. Chee, et al., International Journal of Automation Technology. 7 (2013) 71–82.
9. Deerinck TJ, et al., https://ncmir.ucsd.edu/sbem-protocol (2020.2.10 accessed)
10. J. A. Courson, et al., PLOS ONE. 14 (2019) e0224434.