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Surface reconstruction in scanning electron microscopy and its application to biological fields

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Scanning electron microscopy (SEM) has been widely used for three-dimensional (3D) surface imaging of biological samples. However, it is often very difficult to measure quantitatively the 3D relation of surface structures (e.g., height, length and/or diameter measurement). To overcome this difficulty, several techniques for obtaining height information in SEM have been proposed to date. Among them, the multiple-detectors method, focusing method and stereometric technique are considered to have potentials for the general applications of sample measurements. Under these circumstances, we have introduced the fundamental innovation of the multiple-detectors method for SEM, because this has some advantages in measuring samples from the view-point of resolution, processing speed, repeat accuracy, and ease of use. In our system, backscattered electron (BSE) signals- produced from a sample - are detected by multiple detectors. Because the detected signals are related to the surface inclination of the area probed by the scanning beam, the surface topography can be obtained with quantitative information at nanometer-scale resolution.

This type of SEM, or 3D-SEM, has a long history of development (Lebiedzik 1979) and has been produced by some SEM manufacturers according to the requirements of the markets. However, the resolution and accuracy in measurement have been insufficient for general use of this instrument, in comparison with other microscopes such as the scanning probe microscope and confocal microscope. Thus, the purpose of this study is to introduce the intrinsic ability of 3D-SEM in 3D observation

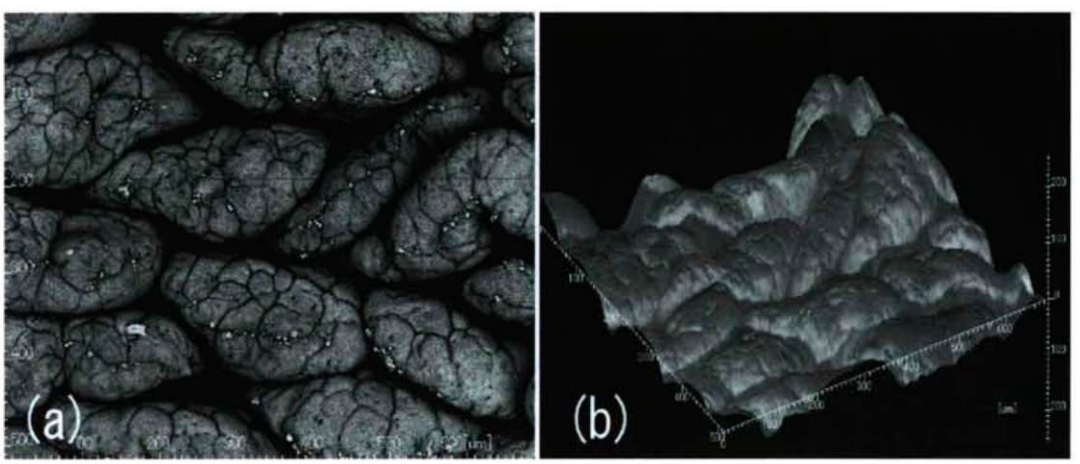

Figure 1A, 1B. A result of 3D observation by our 3D-SEM is shown in the figure below ((a) secondary electron (SE) image, (b) 3D-image obtained through four BSE detectors. Sample: rat intestinal villi). Measurement accuracy increases successfully, because some important improvements have been performed.

and measurement of samples. We also show the usefulness of 3D-SEM for the surface reconstruction of biological samples. Because 3D-SEM is comparatively unconstrained by the sample condition (e.g., size, roughness), focal length, magnification, scanning speed, it has great mobility in 3D-measurement of various samples. The development of reconstruction principle of the surface topography from BSE signals, as well as the quality of acquisition data, is important to improve performance.

Lebiedzik J (1979) An automatic topographical surface reconstruction in the SEM. Scanning 2:230-237.

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