Informative three-dimensional survey of cell/tissue architectures in thick paraffin sections by simple low-vacuum scanning microscopy

Sawaguchi, A.[1], Kaminura, T.[2], Yamashita, A.[3], Takahashi, N.[1], Ichikawa, K.[2], Aoyama, F.[1] and Asada, Y.[3]

[1] Department of Anatomy, Faculty of Medicine, University of Miyazaki, Japan, [2] Hitachi High-Technologies Corporation, Japan, [3] Division of Pathophysiology, Department of Pathology, Faculty of Medicine, University of Miyazaki, Japan

One of the major goals of biological microscopy is to elucidate the structural evidence with which we can correlate functional activity. Recent advances in bio-medical researches, such as the production of regenerative organ from induced pluripotent stem (iPS) cells and the morphological changes induced by CRISPR/Cas9 mediated genome editing, require three-dimensional analysis in those cell/tissue architectures. High-resolution imaging by electron microscopy remains superior to elucidate complex cell/tissue architectures. Scanning electron microscopy (SEM) provides three-dimensional information of specimen surfaces by collecting electrons reflected from the surface (backscattered electrons: BSE) and that forced out of the surface (secondary electrons: SE). The low-vacuum SEM allows the BSE imaging of non-conductive biological samples because the negative charge accumulations on the non-conductive materials are subject to be eliminated with the positive ions in residual gas molecules.

For light microscopic examinations, paraffin wax continues to be the universal embedding medium for histological analysis, immunohistochemistry, and diagnostic histopathology mainly because it is inexpensive and easily handled for sectioning. However, the images obtained from such thin slices (5 - 10 m in thickness) are chiefly two-dimensional: length and width, and there remains a challenge for us to reconstruct the missing third dimension by examining many sections of the three-dimensional cell/tissue architectures. To address the problems, the present study developed a three-dimensional survey of the cell/tissue architectures in 30 m-thick paraffin sections, by taking the advantages of backscattered electron imaging in low-vacuum scanning electron microscope. The application provided a simple three-dimensional survey of the cell/tissue architectures embedded in the thick paraffin section by which the missing third dimension rescued in our vision.

As results, in the kidney, the podocytes and their processes were clearly observed covering the glomerulus. The 30 m-thickness facilitated investigation on a face-side, instead of sectioned, image of the epithelium and endothelium that is rarely seen within conventional thin section. In the testis, differentiated sperms were three-dimensionally assembled in the middle of seminiferous tubule. Further application to vascular injury thrombus formation revealed the distinctive networks of fibrin fibers and platelets, capturing the erythrocytes into the thrombus. The four segmented BSE detector provided topographic bird's-eye images for three-dimensional understanding of the cell/tissue architectures at electron microscopic level. We describe the precise procedures accompanied by representative electron micrographs of normal rat organs, experimental thrombus formation, and three-dimensionally cultured tumor cells.