3D in SEM, (S)TEM, Ion Imaging, incl. FIB-SEM and SBF-SEM

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Focused Ion Beam Tomography as a versatile tool in basic biomedical research

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In mammals urine is filtered from the blood circulation in the kidney. The primary filtrate is formed in the glomerulus that is surrounded by Bowmans space and enclosed by Bowmans capsule. In the glomerulus the primary filtrate is formed by ultrafiltration across the glomerular filtration barrier, which consists of a fenestrated endothelium, the basement membrane and the slit diaphragm between the podocyte feet processes [1]. Alterations in the structure and morphology of this delicate structures as well as changes in their number and size have been associated with renal and systemic diseases [2].

Up to now different imaging approaches were used to analzye and reconstruct renal tissue like magnetic resonance imaging (MRI) or light and electron microscopy (EM). The combination of different imaging techniques is necessary to cover the range of scales from whole organ to macromolecular ultrastructure. However, each technique suffers from limitations e.g. MRI from the low resolution and e.g. electron microscopy from the susceptibility to artifacts and from the low volume imaging capability.

The method of sequentially milling a resin embedded biological structure with a focused ion beam (FIB) and imaging the emerging surface with a scanning electron microscope (SEM) has several advantages in biomedical research compared to the methods mentioned before. Larger volumes (> 10 μm^3) of interesting structures than in serial sectioning EM can be visualized with large fields of view (100 x 100 µm) obtaining SEM resolution.

In this study we applied focused ion beam (FIB) tomography to visualize the 3D cellular architecture of stained and resin embedded wild type mouse glomerulus. We reconstructed a large volume (40% of a complete glomerulus) from renal tissue with 100 nm resolution and a smaller part of the filtration barrier with a 6.4 fold higher magnification and 20 nm resolution. We visualized the structure and the interface between podocytes and capillaries inside the wild type mouse glomerulus. Podocytes that were in direct contact with capillaries formed a cavity between the cell body and the vessel as well as cellular extensions between distant podocytes. Furthermore, we performed direct measurements of surface area, volume and number of podocytes inside the glomerulus.

The unique volume and resolution range of FIB/SEM tomography offers the possibility to visualize and further analyze multicellular systems of high medical relevance (kidney), which are inacessible by other imaging techniques like micro comuputer tomography (CT) due to lower resolution and transmission electron microscopy (TEM) due to volume constraints.

1. Neal, C.R. et al. Journal of the American Society of Nephrology:JASN, 16(5) (2005)
2. Beeman, S.C. et al. American Journal of Physiology. Renal Physiology, 300(6) (2011)