Staining-based X-ray tomography for visualizing the three dimensional micro-structure of soft-tissue specimens

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Summary: To investigate the micro-morphology of biological specimens in 3D, we developed a method, which is based on X-ray active staining and a laboratory-based nanoscopic X-ray tomography setup. We show that this method reproduces the tissue architecture with an at least similar level of detail as conventional light microscopy. Beyond that, the volume data allows for tracking crucial structures in 3D.

1. INTRODUCTION

In most biological studies, accessing the 3D micro-structure of a specimen is essential. For imaging biological soft tissue in 3D, conventionally light or electron microscopy techniques are used [1, 2]. These techniques, however, have a number of drawbacks. Confocal light microscopy and transmission electron microscopy tomography are limited regarding the sample thicknesses. Serial-sectioning or block-face imaging approaches overcome these constraints, but are destructive and prone to sectioning and image registration artifacts [1]. Reaching resolutions well below one micrometer, the field of nanoscopic X-ray computed tomography (NanoCT) is becoming a valuable tool for non-destructively revealing the inner 3D morphology on a (sub)cellular scale [3]. The inherently weak X-ray attenuation contrast of soft tissue requires the use of X-ray active contrast agents [4]. Here, we present a method, which combines a cytoplasm-specific X-ray active stain based on eosin and a NanoCT setup, providing resolutions down to 100 nm [2, 5]. We acquired NanoCT data of mouse kidney soft tissue from the cortex region and evaluated the resulting NanoCT slices in comparison to conventional light microscopy. Furthermore, we used the volume data to model a glomerulus, a central filter unit in the kidney cortex, and track a proximal tubule in 3D. Thereby, we demonstrate the immense potential of this method to visualize and analyze soft-tissue specimens [5].

2. EXPERIMENTAL METHOD

The sample preparation protocol, including the X-ray active eosin staining, was carried out for an entire mouse kidney, as described in [5]. The organ was fixated under acidic conditions. Briefly, after washing the sample with phosphate-buffered saline solution, it was placed in a staining solution of eosin y and was kept on a horizontal shaking plate over 24 h. After staining, the kidney was cut into smaller volumes and critical-point dried.
The 3D data was acquired with the NanoCT setup [2], which is based on geometric magnification. It consists of a nanofocus X-ray source (Excillum AB) and a single-photon counting detector (Pilatus 300K-W, Dectris). An effective voxel size of 400 nm was chosen. 1599 projections were acquired over 360 °. The exposure time per projection was 3 s, resulting in a total acquisition time of 2 h 40 min. The projections were processed with a Richardson-Lucy deconvolution algorithm. For better contrast and to correct for edge enhancement effects, Paganin's phase-retrieval was applied [6]. The data was reconstructed with a filtered backprojection algorithm and visualized with Avizo Fire 8.1 (ThermoFisher Scientific).

3. RESULTS

The resulting NanoCT data, displayed in Fig. 1, perfectly reproduce the kidney cortex tissue of a mouse at the same level of detail as conventional light microscopy. The NanoCT slices in Fig. 1(A) and (B) show tubular

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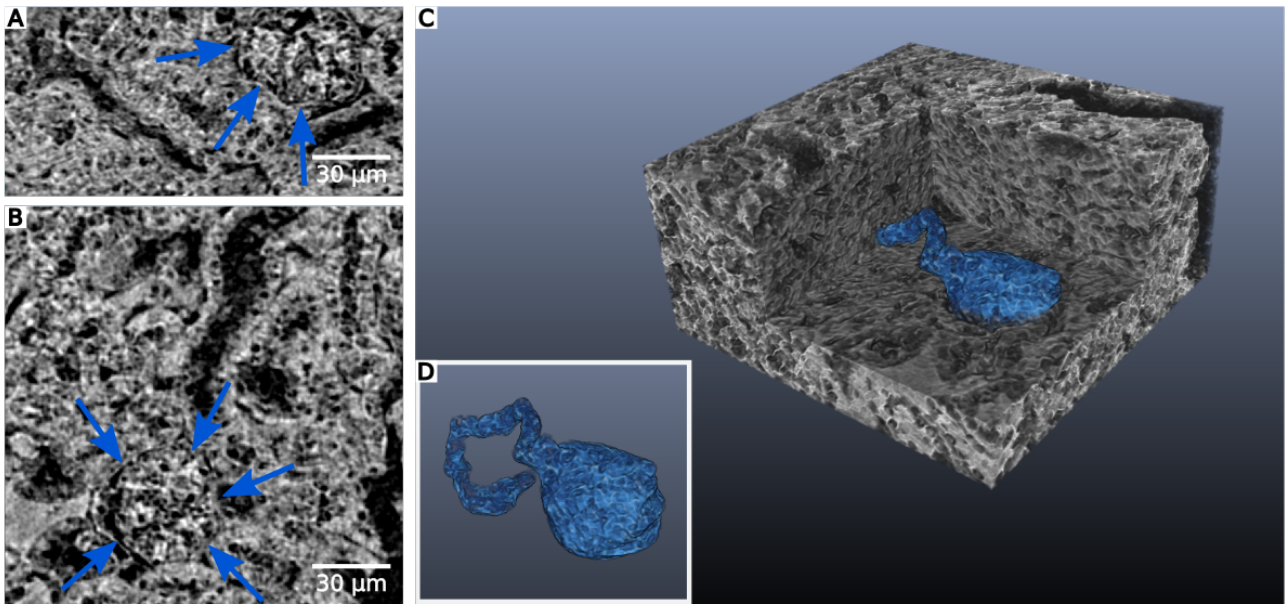

Figure 1: NanoCT slices and 3D rendering of a mouse kidney cortex (effective voxel size ≈ 400 nm). (A) and (B) showing two orthogonal NanoCT slices through a cuboid volume with a glomerulus (blue arrows) embedded into the tubular network. (C) Perspective view of a volume rendering of the same cuboid volume with the glomerulus and the proximal tubule modeled in 3D (blue). (D) Perspective view of the 3D modeled glomerulus and the tubule without the surrounding tissue.

tissue featuring a glomerulus, which is indicated by blue arrows. The X-ray active eosin stain binds to protein structures and consequently stains mostly the cytoplasm. Thereby, it provides adequate contrast throughout the whole specimen and allows for identifying the characteristic structures. Since the stain does not target the cell nuclei, they are visualized as black dots, which are clearly visible in Fig. 1(A) and (B). Beyond depicting the relevant tissue structures in 2D, this method allows for tracking the structures, displayed in Fig. 1(A) and (B), in 3D. Fig. 1(C) and (D) show the glomerulus and the first part of the proximal tubule modeled in 3D. This provides deeper insights into the structural context and configuration of the micro-architecture of the specimen, which is not possible with 2D imaging methods. In contrast to other high-resolution X-ray CT devices, our NanoCT setup is based on geometric magnification. As a result, the magnification can be continuously adjusted by modifying the distances of the sample to the X-ray source and the detector. Thus, the NanoCT is rather versatile. Due to this versatility of both the NanoCT setup and the X-ray eosin stain, the presented method is suitable for various biological applications. These results point out the tremendous potential of this method for biological research.

References

1. Denk, W. & Horstmann, H. Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biology* **2,** 1901–1909 (2004).
2. Müller, M. *et al.* Myoanatomy of the velvet worm leg revealed by laboratory-based nanofocus X-ray source tomography. *Proceedings of the National Academy of Sciences* **114,** 12378–12383 (2017).
3. Withers, P. J. X-ray nanotomography. *Materials Today* **10,** 26–34 (2007).
4. Metscher, B. D. Micro CT for comparative morphology: Simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC Physiology* **9** (2009).
5. Busse, M. *et al.* Three-dimensional virtual histology enabled through cytoplasm-specific X-ray stain for microscopic and nanoscopic computed tomography. *Proceedings of the National Academy of Sciences* **115,** 2293–2298 (2018).
6. Paganin, D., Mayo, S. C., Gureyev, T. E., Miller, P. R. & Wilkins, S. W. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *Journal of Microscopy* **206,** 33–40 (2002).