Supporting Information

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Staining Protocol According to Histological Eosin-Based Procedure

The mouse organ was surgically removed and immediately placed in a 50-mL Falcon Centrifuge Tube (neoLab), which was filled with a fixative solution containing 10 mL of 4% (vol/vol) formaldehyde solution (FA, derived from a 37% acid-free FA solution stabilized with ~10% methanol from Carl Roth; further dilution with DPBS without calcium and magnesium). The sample was refrigerated for 48 h and then washed with phosphate saline buffer solution (DPBS without calcium and magnesium, pH 7.0–7.3; Thermo Fisher Scientific) for 1 h. The mouse organ was placed in the staining solution of eosin y 0.1% (wt/vol) in distilled water (product number E4382, stain certified by the Biological Stain Commission; Sigma-Aldrich). The soft-tissue sample was stained with 2 mL of staining solution for 24 h (the soft-tissue sample was moving freely within the sample container). During the incubation time the soft-tissue sample was kept on a horizontal shaking plate allowing for a smooth rocking of 60 rpm. After staining, the soft-tissue sample was carefully removed from the sample container and access of staining agent was softly patted with a cellulose tissue paper. The soft-tissue sample was stored in an Eppendorf tube above an ethanol vapor phase [the Eppendorf tube contained a few drops of 70% (vol/vol) ethanol at the bottom of the tube].

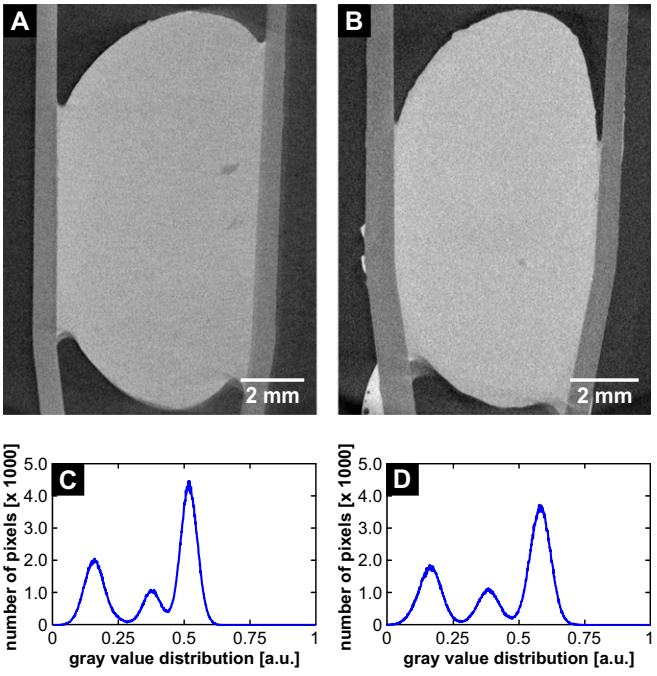

Fig. S1. CT slices of the same whole mouse kidney before and after staining following the standard histological staining protocol. The Xradia Versa 500 microCT was used to acquire both data sets under identical acquisition parameters. The voxel size in both data sets is around 18 μm. (*A*) Overview image of the unstained mouse kidney. (*B*) Overview image of the same mouse kidney sample shown in *A* after staining. (*C*) Histogram of CT slice shown in *A*, and (*D*) Histogram of CT slice shown in *B*.

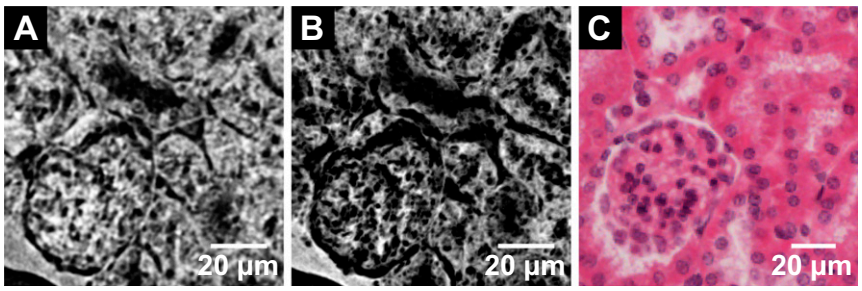

Fig. S2. CT slices of nanoCT (*A* and *B*) data of the cortex region in comparison with the histological microscopic slide (*C*) derived from the same mouse kidney after application of the developed eosin-based staining protocol. Furthermore, compatibility with conventional histological methods is shown, i.e., the counterstaining with hematoxylin was applied to the histological microscopic slide. (*A*) NanoCT image of the same mouse kidney sample after staining and CPD showing detailed structures of the cortex region: Renal corpuscle with glomerulus, Bowman's capsule, and renal cortex with convoluted tubules. (*B*) Minimum intensity projection slice of the same nanoCT data set shown in *A* corresponding to a virtual slice thickness of ~7 µm. (*C*) Representative histological microscopic slide with an approximate thickness of 7 µm obtained from the same mouse kidney sample after the applied eosin-based staining, embedding in a paraffin block, sectioning, and counterstaining with hematoxylin. Displayed are renal corpuscle with glomerulus, Bowman's capsule, and renal cortex with convoluted tubules showing highlighted cell nuclei in purple due to counterstain.