

## Comparing vascular casts of murine kidneys with and without tissue corrosion

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**INTRODUCTION:** Vascular corrosion casting is a well-established technique for obtaining the vasculature by perfusing the blood vessels with a hardening material and then corroding the surrounding tissues. The resulting cast of the vascular lumen can be imaged using micro computed tomography ( $\mu$ CT) to obtain the entire 3D structure. We compared the structure of a vascular corrosion cast of a mouse kidney to that of a kidney cast with added contrast agent, but no corrosion.

**METHODS:** Two mice were anaesthetized with pentobarbital and perfused through the left heart ventricle with heparin 25000 u/l in PBS, 4 % formaldehyde in PBS, and the casting resin PU4ii (vasQtec, Switzerland).<sup>1</sup> For the corrosion, the tissue was macerated in 5-10 % KOH. The plastic casts were washed, freeze-dried, and coated with OsO<sub>4</sub> for enhanced contrast. For the contrast agent casting, 1,3-diiodobenzene was admixed to the PU4ii mixture to an equivalent of 90 mg iodine per ml before perfusion, and the tissue was not corroded afterwards.

Both samples were imaged on a phoenix nanotom m  $\mu$ CT scanner (Sensing & Inspection Technologies GmbH, Wunstorf, Germany) with a voxel size of 1.5  $\mu$ m. The corroded sample was imaged in air with an accelerating voltage of 100 kV, while the non-corroded sample was measured in PBS using an accelerating voltage of 40 kV.

**RESULTS:** Both protocols allowed for the acquisition of the vascular (micro-)structure of the kidney. While the corroded cast had the better signal-to-noise ratio and fewer bubbles within the cast, there were some significant structural deviations. In Figure 1, roughly a quarter of a representative section of each kidney is shown. The corroded cast has a rougher surface and some large empty areas without blood vessels are visible at the image center. These features were not found in the non-corroded cast. Additionally, the vasa recta are much more tightly bundled in the latter.

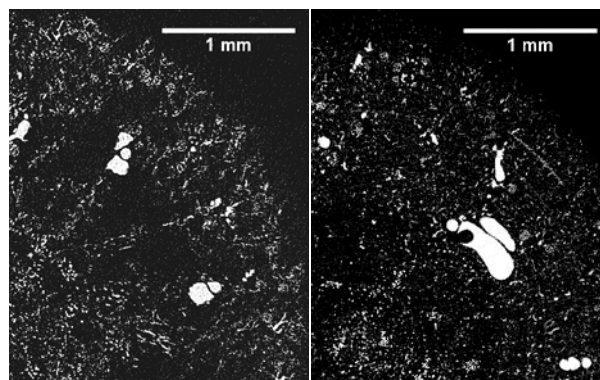


Fig. 1:  $\mu$ CT image slices of vascular corrosion casts after the corrosion of the surrounding tissue (left) and without corrosion (right).

**DISCUSSION & CONCLUSIONS:** The overall vascular structure is comparable in both cases. This observation is expected, since parameters such as blood vessel surface, volume, and connectivity depend on the casted volume and will remain unaffected by the corrosion procedure. Other parameters including the distances between vessels can change, affecting structural relationships. Depending on the research question, it may thus be necessary to image the vasculature in the perfused tissue before corrosion. Since the X-ray absorption of tissue is higher than that of air, achieving the necessary signal-to-noise ratio can prove challenging, especially when trying to resolve the microstructures close to the limit of spatial resolution. This obstacle is balanced by the possibility of performing multi-modal imaging on tissue and vascular cast when the tissue is intact and not corroded or cut into slices. Therefore, we view casting without corrosion followed by multi-modal imaging as a promising tool for 3D functional analysis of organs including the kidney.

**REFERENCES:** <sup>1</sup> T. Krucker, A. Lang, E.P. Meyer (2006) *Microscopy Research and Techniques* **69**:138-147.

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