Modern µCT: analysis of whole mouse kidney down to capillary level

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Background and current situation: Nephron number and glomerular volume are the key features in renal morphometry. The accurate estimation of these parameters has become increasingly important because their alterations may play a significant pathophysiological role in the development and/or progression of a range of nephropathies and various kidney-related pathologies.

Nowadays the gold-standard method of the kidney morphometry is the exhaustive physical fractionator/dissector method (often combined with Cavalieri for kidney volume estimation). Although accepted as standard in the lab animal research, it is *extremely time-consuming and laborious* and therefore rather rarely performed.

Aim: to develop a technique that would allow ***fast and reliable*** estimation of such parameters as nephron number, glomerular volume, glomerular size distribution and kidney volume.

Equipment and material:

- SkyScan 1172 - ex-vivo µCT with resolution down to 0.6µm (isotropic voxel size)
- novel Angiofil contrast agent (Fumedica AG, Switzerland)

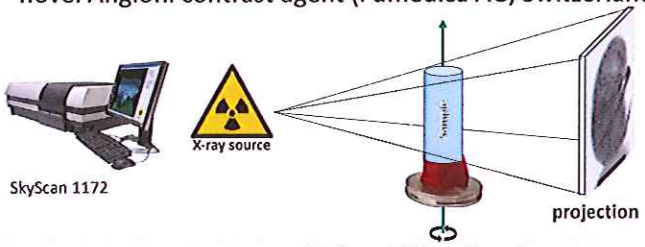

Fig.1. Our ex-vivo µCT-scanner - SkyScan 1172 and how it works.

Technique/Experimental design: adult mice were anaesthetised and their thoracic aorta catheterised. After washing blood out the mice were perfused with the novel Angiofil contrast agent. Later, the kidneys were scanned at approx. 2µm spatial resolution. Thereafter, they underwent one of the following procedures:

1. More detailed vascular analysis: digestion of the tissue in order to obtain the corrosion vascular cast (fig. 2).
2. Histological analysis (the site of interest may be determined using µCT-data): with the help of the special treatment, the casting agent was eliminated from the vessels in order to not interfere with the cutting and other steps of histological preparation. See fig.3.

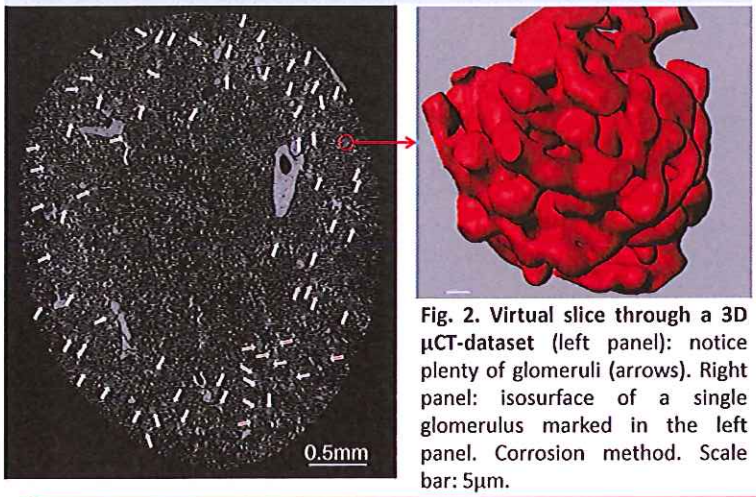

Fig. 2. Virtual slice through a 3D µCT-dataset (left panel): notice plenty of glomeruli (arrows). Right panel: isosurface of a single glomerulus marked in the left panel. Corrosion method. Scale bar: 5µm.

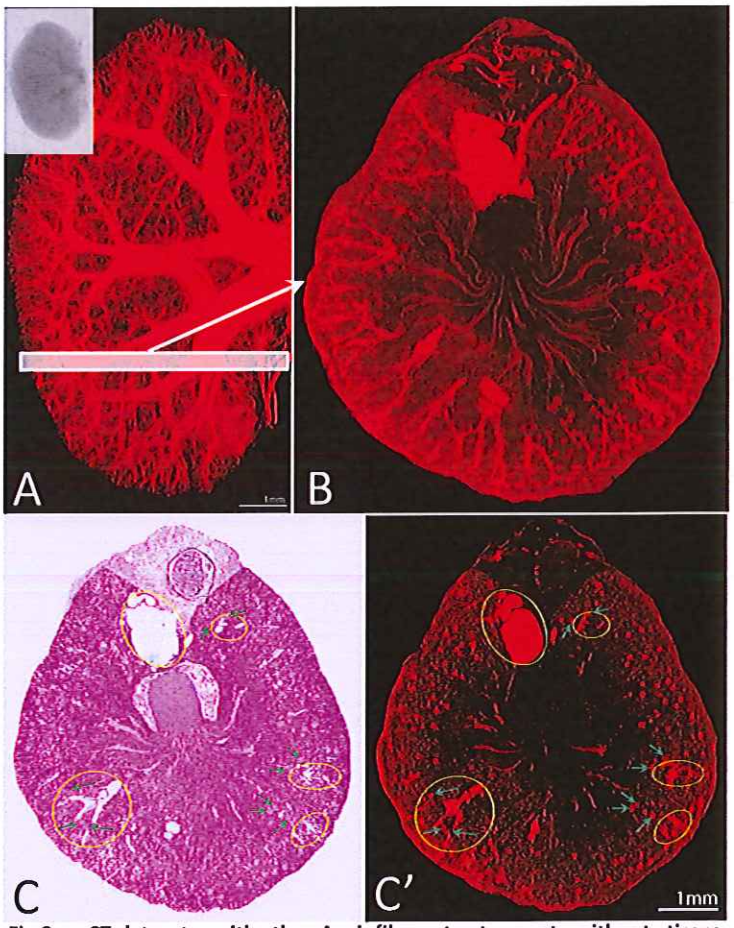

Fig.3. µCT-datasets with the Angiofil contrast agent without tissue digestion are of superior quality. A: µCT-volume-rendered overview of the whole kidney after reconstruction from single projections (insert). Panel **B** displays the volume of interest as indicated in **A**: all glomeruli are easily recognizable. Panel C and C' show corresponding histological section (C) and a volume-rendered section through a µCT-3D-dataset (C'): note the encircled groups of vessels on both sections as well as glomeruli in their vicinity.

Results: using Angiofil contrast agent we obtained 3D-µCT-datasets of superior quality, which are sufficient for obtaining all kidney morphometry parameters. Moreover, the same kidney may be used for the histological analysis of the site of interest.

Conclusions:

- The developed technique allows ***fast (<24hours) and reliable kidney morphometry*** based on high-resolution µCT-scans of the kidney vasculature in ***3D***. We can assess the parameters: number of glomeruli, total glomerular volume, volume (size) of single glomeruli, glomerular volume distribution etc.
- Besides classical kidney morphometry, it can provide data on the ***vasculature down to the capillary level***, what makes the technique even more beneficial for studying pathological processes involving the vasculature.
- Possibility of ***further histological analysis of the same kidney*** is another major advantage of the presented method.