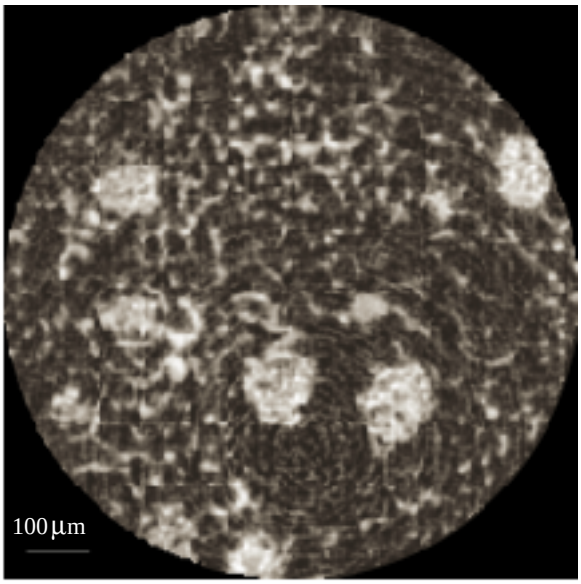
Global Heterogeneity of Glomerular Volume-distribution Evaluated by Three-dimensional Analysis using Synchrotron Radiation X-ray MicroCT

An estimated glomerular volume within the restricted histological sections has been a conventional index for renal dysfunction. Subtle aspects of glomerular remodeling are, however, difficulties with the conventional approach that compresses three dimensions into two. Therefore, we aimed at visualizing thee dimensional (3-D) glomerular micro-structures using an X-ray microCT (μCT) at beamline **BL20B2**, to accurately measure glomerular volumes throughout a cortex and quantitatively evaluate global heterogeneity of the volume-distribution in a rat with spontaneous diabetes mellitus (DM).

Using the Otsuka Long Evans Tokushima Fatty (OLETF) rats as rats of early stage DM (28 weeks) and control rats (Long Evans Tokushima Fatty; LETO, of the same age, the vessels of the left kidney was filled up with contrast media ($BaSO_4$ + India ink + 8% gelatin). A sampled renal column (ϕ 3 - 4 mm) was observed using two μCT systems; BL20B2 μCT for the highest image quality (3 - 6 μm in resolution) and commercially available μCT (10 μm in resolution) (Fig. 1) for data accumulation. The glomerular volumes more than 400 per sample were computed and normalized to body weight. Heterogeneity in glomerular volume-distribution was evaluated by coefficient variation (CV).

By stereomicroscopic observation, we confirmed complete-filling of the vessels with the contrast media. The glomeruli in OLETF were characterized by irregular stereostructures, whereas those in LETO were characterized by regular glomerular

(a) CT-imaging of renal cortex



(b) 3D-volume rendering image of the glomerulus

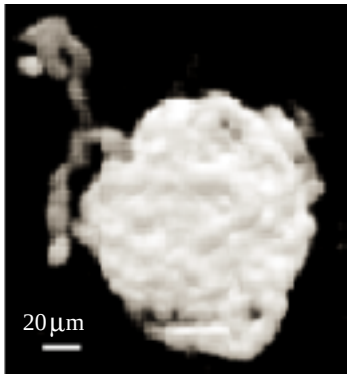


Fig. 1. Visualization of renal cortex by high resolution (3 μm) microCT.

structure. The CV of glomerular volume was significantly larger in OLETF than LETO (2.0 *vs.* 0.15, $p < 0.01$). In addition, absolute glomerular volume was larger in OLETF than LETO ($2.3 \pm 0.2 \times 10^6$ *vs.* $1.6 \pm 0.2 \times 10^6$ μm^3, $p < 0.01$). The normalized glomerular volume was, however, comparable (NS), indicating glomerular enlargement in early DM may associate with increase in body mass. Both μCT provided similar results.

Highly heterogeneous glomerular volume-distribution in DM rat was quantitatively demonstrated using our developed 3-D glomerular micro-structural visualizing method. This technique provides a new aspect for the evaluation of global heterogeneity in glomerular remodeling under the chronic renal dysfunction, which may provide more sensitive insight into early complications of diabetic nephropathy.

On the glomerular volume evaluation, the SPring-8 μCT and the conventional μCT showed the similar results. However, the SPring-8 μCT system with the high resolution is required for evaluation of the more detailed microvasculature.

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