X-Ray Phase-contrast Microtomography for Visualizing of Renal Microstructures in Albino-Panda-Albino Hamsters

Small laboratory animal model of human diseases such as the albino-panda-albino (APA) hamster, which is known to develop spontaneous renal focal segmental glomerulosclerosis (FSGS) with age, has been used in basic and premedical research of the kidney. Since the pathological diagnosis of FSGS with a scattered small pathological lesion is often missed when only a few sliced sections are examined, a sufficiently large sample, the sample site, and serial sliced sections are often required for exact diagnosis.

X-ray crystal interferometer-based (Bonse-Hart type [1]) X-ray phase-contrast microtomography (phase-contrast microtomography) [2] enables us to reveal the microstructures of soft tissue, such as cancerous mass, necrosis, fibrous capsule, fat tissue and surrounding normal tissue without the use of a contrast agent [3]. An experimental imaging by phase-contrast microtomography was performed with fixed renal specimens of hamster to confirm the feasibility of this technique as a nondestructive method. This system consists of a triple Laue-case X-ray interferometer with a 40-μm-thick analyzer [4], a sample cell and an X-ray CCD camera with the pixel size of 4.34 μm (Fig. 1). Experiments were carried out at the undulator beamline **BL20XU**.

In our study [5], albino-panda-albino (APA) hamsters and age-matched Syrian hamsters were used. After whole-body perfusion with physiological saline solution, the kidneys were quickly extracted and fixed with formalin. The images of glomeruli and tubular structures were similar to those observed using 40-100 folds magnification on an optical microscope. In four specimens from two female APA hamsters, 7 scattered lesions were detected. Wedge-shaped pathological lesions including mild atrophic tubular walls, markedly dilated tubular lumen, high-density glomeruli and widening of Bowman's space were observed quantitatively. Wall thickness and lumen diameter of tubules were calculated in normal and abnormal areas of the cortex. The thinnest tubular wall was about 12 μm. The glomerular volume was calculated and compared between the normal (n = 25) and abnormal glomeruli (n = 17) (Fig. 2(a)). The ratio of Bowman's space against glomerular volume in abnormal glomerulus was higher than those in normal glomerulus (Fig. 2(b)). In addition, the mean density of glomerulus in pathological regions was higher than that in normal regions (Fig. 2(c)). On measuring the density of normal glomerulus, the percentile of abnormal glomerulus [normal mean density + 2SD (standard deviation)] was approximately 57.7% in pathological regions in APA hamster.

The renal microvasculature was extracted from 3D phase-contrast microtomography images using the density threshold-based rendering technique. The distributions of the glomeruli, renal arteries and veins were successfully visualized. Glomerular capillary tufts formed by capillary loop and surrounding tubules and vessels were clearly observed (Fig. 3).

In this experiment, blood was washed out from renal vessels to eliminate artifacts. After the injection of physiological saline, the hepatic vessel of the rat could be visualized clearly by X-ray phase-contrast radiography, whereas the X-ray absorption-contrast image could not reveal the vessel at the same X-ray dose and energy [6]. This procedure was much easier and is considered to preserve more the physiological condition than using conventional contrast agents with high viscosity. The fine density difference among physiological saline within tubules, vessels, and surrounding soft tissue of kidney was discriminated sufficiently on the images. In addition, by volume

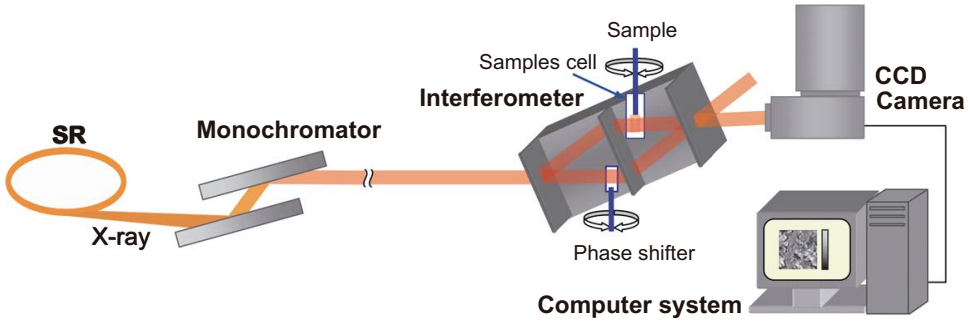

Fig. 1. System of X-ray crystal interferometer-based X-ray phase-contrast microtomography. [5]

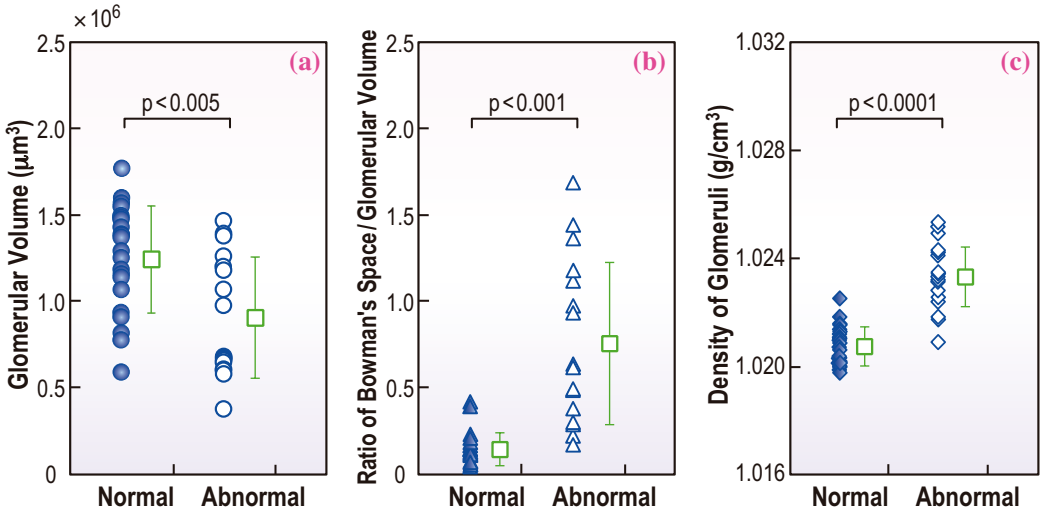

Fig. 2. Quantitative analysis of glomerulus images obtained by X-ray phase-contrast microtomography. **(a)** The mean volumes are $(1.24 \pm 0.31) \times 10^6$ μm^3 and $(0.91 \pm 0.35) \times 10^6$ μm^3 in normal and abnormal glomeruli, respectively ($p < 0.005$). **(b)** The ratio of Bowman's space against glomerular volume in abnormal glomeruli was higher than those in normal glomeruli (0.74 ± 0.47 *vs.* 0.13 ± 0.10, $p < 0.001$). **(c)** The density of abnormal glomerulus is about 2.6 mg/cm^3 higher than that of normal glomerulus (1023.3 ± 1.1 mg/cm^3 *vs.* 1020.7 ± 0.7 mg/cm^3, $p < 0.0001$). [5]

rendering technique, the renal microstructures and microvasculature could be depicted from the same specimen at one scan without any contrast agents.

We considered that phase-contrast microtomography might be used as a powerful auxiliary tool for pre-histological evaluation to detect and analyze the position of a small scattering lesion and its spread in the renal disease models.

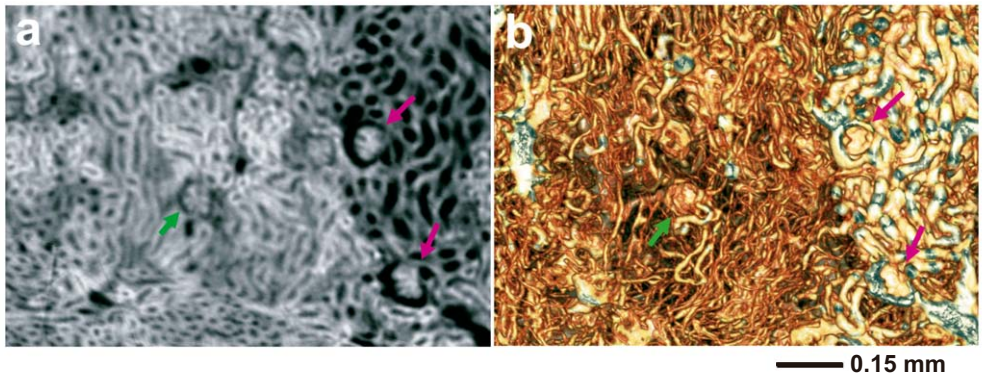

Fig. 3. X-ray phase-contrast microtomogram of renal cortex **(a)** and three-dimensional extracted renal microvasculature and tubule **(b)**. The distributions of renal arteries and veins were successfully visualized. The capillary tufts are clearly observed in normal glomerulus (green arrow), whereas the widening of Bowman's space and the indistinct pattern of the capillary loop are observed in abnormal glomerulus (pink arrow). In the three-dimensional image, non-dilated tubules and non-atrophic glomeruli are shown in normal cortical areas, whereas dilated tubules and atrophic glomeruli are clearly revealed in FSGS lesions. [5]

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