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Micro-X-ray imaging of ethanol fixed biological soft tissue

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1 Introduction

Recently, a phase-contrast X-ray imaging technique for detecting contrast from differences in refractive index, was developed. This technique has approximately 1000 times greater sensitivity than that of the conventional absorption technique [1-4]. However, spatial resolution of phase-contrast X-ray imaging technique is limited by the thickness of half mirror (analyzer). Absorption-contrast X-ray imaging allows to obtain higher spatial resolution less than 1 micrometer, however the image-contrast reduces significantly.

To enhance the image-contrast that enables to obtain the higher spatial resolution, we changed the fixation technique of the biological sample from commonly used formalin to ethanol. Ethanol fixation is popularly used to prepare specimens for biopsy processing and rapid intra-operative pathological diagnosis. It exhibits comparable histology results and superior immune-staining results to formalin. We surmise that the physical property increases density differences against back ground and the dehydration by ethanol increases the soft tissue density. Here, preliminary phase-contrast X-ray imaging with ethanol fixation were performed, and the image-contrast was significantly improved in brain [5, 6], heart [7] and kidney [8, 9] of rat.

2 Experiment

This study used six male rats (age, 10 weeks). All rats were anesthetized, and cannulation to the apex of left ventricle was carried out surgically for perfusion. First, physiological saline solution was injected from the apex, and the whole blood in the vessel was replaced to eliminate blood coagulation artifacts. Then, 10% formalin or 100% ethanol was perfused for fixation, and the samples were steeped in each fixing solution. The experimental protocol was approved by the President of Kitasato University through the judgment of the Animal Care and Use Committee of Kitasato University.

Phase-contrast X-ray CT system with crystal interferometer was set at the vertical wiggler beam-line 14C of the Photon Factory, Tsukuba, Japan. X-ray CCD camera with a pixel size of 13 x 13 μm^2 was used. The X-ray energy was set at 35 keV, and projection number was 250 per 180 degree. CT image was reconstructed FBP.

After phase-contrast X-ray CT imaging, all specimens fixed by each solution (ethanol and formalin) were sliced into 3-μm-thick sections and stained with hematoxylin and eosin (HE).

The density was measurement in phase-contrast X-ray CT images. And histological image analysis was also performed by using image-manipulating software (NIH image 1.63)

3 Results and Discussion

Here, we report the experiment of kidney in detail. Phase-contrast X-ray imaging using a two-crystal X-ray interferometer was able to clearly visualize the fine renal structures such as cortex, medulla and the vessels in both formalin-fixed and ethanol-fixed kidneys. Especially, the image-contrast of ethanol-fixed kidneys was much better than that of formalin-fixed kidneys due to physically enhance the density difference between the soft tissue and surrounded ethanol solution with lower density ($0.79 g/cm^3$) than that of formalin solution ($1.01 g/cm^3$). Actually in histological image, the shrinkage of soft tissue was observed in ethanol-fixed kidney.

In quantitative analysis, the ethanol fixation technique enhances approximately 3.1 times higher image contrast; the effect of shrinkage is estimated approximately 2-times and the physical effect of ethanol is approximately 28%. Thus, the ethanol-fixation technique enables to enhance the image contrast in phase-contrast X-ray imaging. Histological conditions of various organs were examined as preliminary studies by using phase-contrast X-ray CT system.

The ethanol is easily volatilized, and fixed soft tissue having exact histological structure remains. Here, we are planning to obtain high spatial resolution image with X-ray CT system at room condition.

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