

# A Study on the Usefulness of 3D Imaging in Micro-CT for Observing the Microstructure of Mice

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## 흰쥐 미세구조 관찰을 위한 Micro-CT 3D 영상의 유용성에 관한 연구

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요 약 본 연구는 실시간 동적영상(Real-time dynamic image)을 획득할 수 있는 고해상도 엑스선 영상장치인 마이크로 CT 을 이용하여 흰쥐의 미세 혈관구조를 관찰함은 물론 국내 원광 방사선영상 과학연구 센터에서 개발한 마이크로 CT 에 대한 유용성을 알아보고자 한다. 흰쥐 몸 전체의 2D 영상을 얻은 후 MIP(maximum intensity projection), VRT(volume rendering technique)기법을 이용하여 혈관구조의 조영된 3D 영상을 얻을 수 있었고 이 3D 혈관 영상을 머리, 복부, 심장과 몸 전체의 혈관시스템으로 각각 분류하였다.

주제어 : 마이크로 CT | 미세구조 | 3D영상

**Abstract** In this thesis we observe microvascular structure in mice by using micro-computed tomography (CT), which is high-resolution X-ray imaging equipment that can acquire Real-time dynamic image, and it aims to investigate the usefulness of micro-CT developed by Institute for Radiological Imaging Science Wonkwang University School of Medicine.

After acquiring the systemic images of rats, contrast-enhanced 3D images of vascular structures could be acquired by using Maximum Intensity Projection (MIP) and Volume Rending Technique (VRT), This was divided into each vascular system of head, abdomen and heart and systemic vascular system.

**Key Words** : Micro CT | microvasculature | 3D image

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## 1. Introduction

Optical techniques such as those for imaging and computer graphics by X-ray imaging equipment have been developing[1], as has the equipment used with these imaging techniques, including MRI, CT, US, PET, and optical imaging. Multi-Detector row CT (MDCT) has developed quickly since spiral CT was developed in the 1990s, and currently, there are 256-channel MDCTs on the market. These MDCTs have a spatial resolution of 1-0.23mm. The resolution and scan time of the MDCT have been improved, and there is not much difference in the imaging systems used for small animals. However, its resolution is much less than that of micro-CT, which can be an issue since small animals are much smaller than the human body[2].

High-resolution micro-CT can be used to observe the progress and elapsed time of a disease without sacrificing small animals using non-invasive methods by imaging. This technology has played a very important role in biotech research since Alexander Sasov published articles on X-ray micro-CT in Russia in 1982, and since the first SKYSCAN1072 device was introduced in 1997[1].

Micro X-ray CT equipment can produce images with much higher resolution than other medical imaging systems. Micro X-ray CT systems can use X-ray-generating equipment using a light source with size of several  $\mu\text{m}$  or less. This technology is used to explore the structure and changes of the structure of biological cells in a non-invasive way, using a hypersensitive detector due to the relatively low strength of the X-ray light source. Specific proteins can also be imaged using an immuno-chemical technique, and real-time dynamic images (R) can be obtained for use in the development of in-vivo diagnosis and treatment techniques.[2] Until now, methods of scanning small animals with high resolution have not been common, so methods have been used

that involve sacrificing and dissecting a large number of animals, or taking out and analyzing their organs and tissues and then processing the results statistically[3]. In contrast, micro-CT provides a method of examining the biological changes in small animals over their lifetime without sacrificing them. Recently, with the developments of computer hardware and software, images with high spatial resolution can be obtained even in small specimens using micro-CT. This technique has gradually been regarded as an important imaging test method for animal experiments.[4-5] Compared to the images obtained in commonly used thin-slice MDCT, the images obtained from micro-CT have very high resolution, so they are assumed to be more accurate for discovering microscopic structures and lesions. However, the imaging of micro-vascular morphological structures of small animals is still lacking. In particular, due to low resolution, it is very difficult to view biological changes over time, and to visualize the structure of the blood vessels in the head, the shape of the heart, and the entire anatomical micro-vascular systems of a white mouse in vivo.

This study attempts to provide basic figures that can be applied to clinical images by observing the micro-vascular structures of the whole body of a mouse. Images of the micro-components of mice were obtained through various imaging acquisition techniques with a micro-CT imaging system developed at the Wonkwang University Institute for Radiological Imaging Science.

## 2. Subjects and methods of experiment

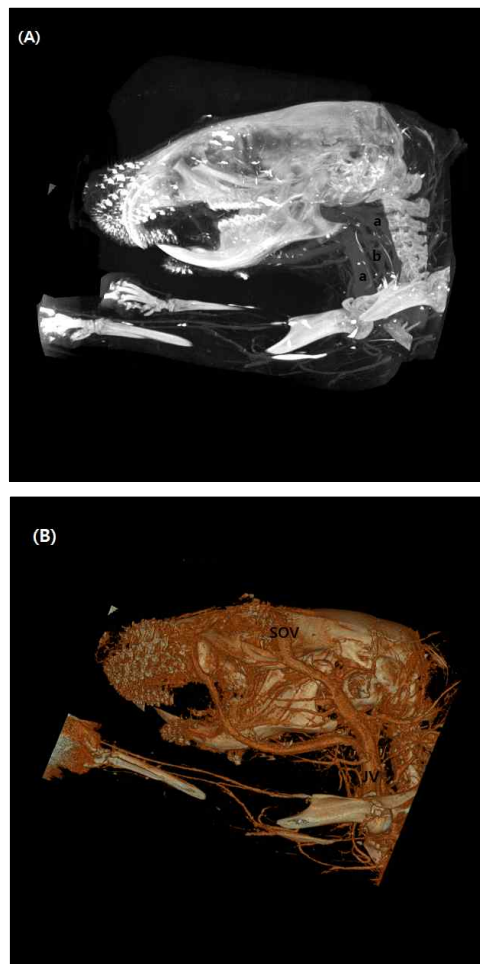
The subjects were two 7 to 12-week-old BALB/C mice with a weight of 20-25 g. To anesthetize these mice, 4% isoflurane was infused with oxygen in a chamber. According to another research report, there were some differences in the viscosity of gelatin, so a

mixture of 40% BaSO<sub>4</sub> by volume and 5% gelatin by weight was produced using 4 g of barium sulfate (BaSO<sub>4</sub>), 0.5 g of gelatin, and 10ml of distilled water. However, this study used Solotop Suspension 140 contrast medium mixed with 140 g of barium sulfate (BaSO<sub>4</sub>) per 100ml with distilled water at a mixture ratio of 50:50. After infusing the mixed Solotop contrast medium into the white mice's tail veins at 0.01 ml per 1 g of body weight, CT scans were performed. Images were obtained 6 minutes after the infusion of the contrast medium, and each of the experiments was repeated five times

### 3. Result and Discussion

2D cross-section data were obtained five times with approximately 6 minutes of scanning to obtain images of organs and the micro-vascular structures of the head, heart, and whole body using micro-CT. Since it is difficult to evaluate 2D sectional data due to visual obstructions, all 2D data were transmitted to a workstation. As a result of applying Maximum Intensity Projection (MIP) and Volume Rendering Technique (VRT), contrasted 3D images could be obtained in vivo. The best of 5 images are shown in Figures 1 through 4, which showed reproducibility.

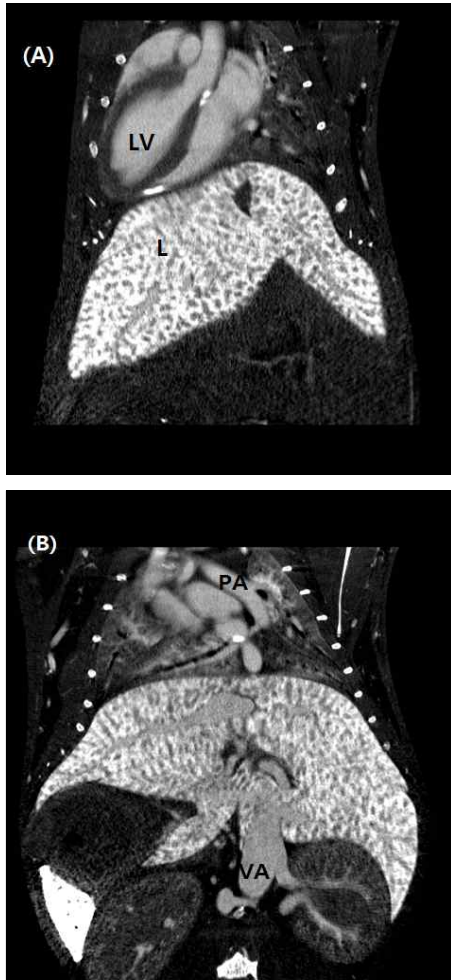
Figure 1(A) shows images of large blood vessels from the heart toward the cerebrum observed on the sagittal plane after virtually cutting the head to observe the shape of the head and the blood vessel structure. The major blood vessel structure, external jugular veins (a), and extra-cranial arteries (b) in the blood system of the cerebrum could be observed by the MIP technique. As shown in Figure 1B, clear images of the external jugular vein (JV) and the SupraOrbital Vein (SOV) could be obtained.



[Fig. 1] Sagittal Maximum intensity projections (MIP) A and 3D reconstruction B showing murine (BALBc) cerebral vessels clearly stand out from the skeleton (white) in this enhanced micro-CT image of a normal living mouse. (A) The anatomy of the external jugular veins (a) and extracranial arteries (b). (B) External jugular vein (JV) and supra-orbital vein (SOV) as determined by in vivo CTA in a C57BL/6 mouse.

Figure 2(A) shows a 3D image expressing the liver (L) and the left ventricle (LV) in the four-way sagittal plane of the heart and the abdomen. In the contrast image, up to 4 normally contrasted chambers could be observed. Figure 2(B) shows the coronal plane of the heart and the abdomen, in which the heart structure and pulmonary arteries (PA) could be observed. A

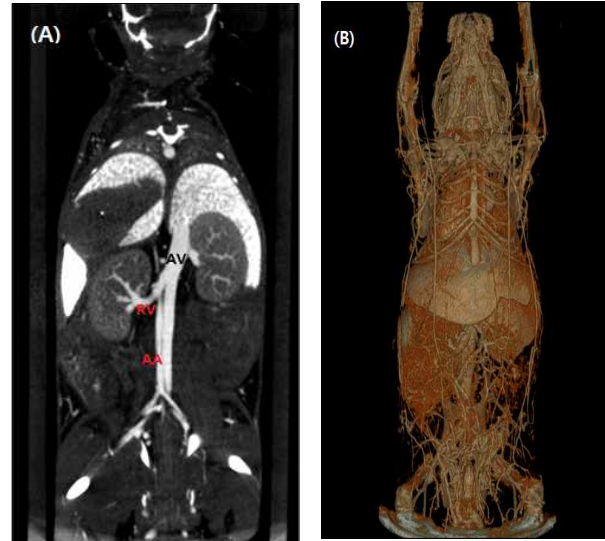
notable overview of the major blood vessels of each of the organs could be obtained, and the smallest blood vessels such as those of the kidney and the glomerulus could be observed.



[Fig. 2] The isotropic resolution in micro-CT allows for re-slicing along any arbitrary plane without loss of resolution. (A) The oblique sagittal plane demonstrates the left ventricle (LV) and liver (L). (B) A number of anatomical features are indicated, including pulmonary arteries (PA) and the abdominal vein (AV).

Figure 3(A) shows the coronal plane of the abdomen, clearly expressing the abdominal vein (VC), renal vein (RV), and abdominal artery (AA). The micro-vascular scan and kidney shape are also observed. The 3D VRT

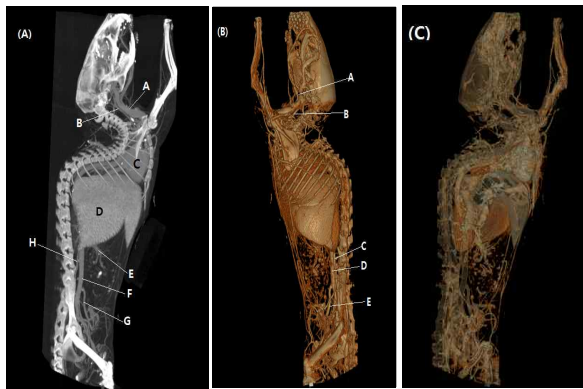
image in Figure 3(B) visualizes the small blood vessels of the 100µm epidermis, and expresses more three-dimensional and quantitative imaging.



[Fig. 3] Reconstructed coronal cross-sectional images (A) and 3D reconstruction (B) showing that the murine (BALBc) vascular system clearly stands out from the skeleton (white) in this enhanced micro-CT image of a normal living mouse. (A) The coronal plane demonstrates the abdominal vein (AV), renal vein (RV), and abdominal artery (AA). (B) Surface blood vessel as determined by in vivovCTA in a C57BL/6 mouse.

Figure 4(A) shows an image of the sagittal plane including the head, throat, lungs, and abdomen, which expressed a better image than the blood vessel structure in Figure 3 with the MIP technique. The external jugular veins (A), extra-cranial arteries (B), heart (C), liver (D), renal artery (E), abdominal artery (F) and iliac artery (G) are well expressed, and other micro-vessels could be visualized. Figure 4(B) is a VRT image, which could visualize more three-dimensional, factual, and quantitative information by giving the different areas of the parts different transmittance and color. Figure 4(C) shows the blood vessels of the whole body in more detail with imaging

that removed bones or organs, but not blood vessels.



[Fig. 4] Reconstructed sagittal cross-section images A, B and 3D reconstruction C showing of murine (BALBc) vascular system clearly stand out from the skeleton (white) in this enhanced micro-CT image of a normal living mouse. (A); the sagittal plane demonstrates the external Jugular Veins (A), common carotid artery (B), heart (C), liver (D), renal artery (E), Abdominal Artery (F), iliac artery (G). (B); number of anatomical features are indicated including Jugular Veins (A), common carotid artery (B), Abdominal Artery (D), iliac artery (E).

Rodents like mice are used in various ways in basic experiments in many academic fields, such as disease, pathologic study, and micro-structural morphology, but there is almost no anatomical imaging information about them.[6] The blood vessel structure of the whole body of a white mouse, including the head, is composed of complex and subtle forms similar to human blood vessel structure, and its size is 300  $\mu\text{m}$  or less. As such, it is very difficult to visualize the structure. New attempts at imaging techniques to obtain higher resolution will historically be evaluated as a significant achievement in many scientific areas, including life science.

Previously, to visualize the micro-vascular structure of a small animal, each part was removed and a biopsy specimen without pre-treatment was used, or the parts

were observed through an optical microscope after infusing a contrast medium into a blood vessel. However, optical images do not have good transmission power, so it is difficult to visualize deep parts in humans, and there is much signal scattering, so it is inadequate to make and quantify tomography.[7] In addition, a system that can obtain high resolution such as electron microscopy and atomic force microscopy needs a physical or chemical pre-treatment process for a biopsy specimen to be observed, so the specimen cannot be observed in a living state, and it is difficult to observe thick samples. In order to compensate for these shortcomings, using micro osteotomy, a cross section of each part is produced for observation. Yet, a large number of single slices should be produced, and the complete organ tissue is damaged in the process of producing a slice, making some research impossible. Thus, this technique is insufficient for understanding the micro-vascular structure of an entire area. [8]

There has been much research to analyze the structure and shape of blood vessels, but focus has mostly been centered on applications to human bodies, with limitations in analyzing micro-vessels. There is still no system to visualize the micro-vessels of the whole body. Using mice models, which are morphologically and functionally most similar to humans among experimental animals, Kwon-ha Yoon et al.[9] studied the changes and features of the micro-structure of a variety of animal models. It was reported that in a cirrhosis model, 3D observation of the micro-structure of the portal vein through micro-CT regarding the impacts of angiopoietin on the liver blood flow in the hepatic portal vein provided information beyond the pathological structure. Recently, in osteoporosis research and the study of arthritis, quantitative information about bone and cartilage were provided, and functional information about the volume, surface area, thickness, continuity, and intensity of trabecular bone could not be determined by other imaging equipment. Erik L. Ritman et al[10]. reported

that the 3D quantitative anatomical characteristics imaging in the blood vessel of the lungs and bronchi could be visualized, so new information about the temporal transit of the pulmonary blood flow along the branch of dynamic movements and anatomical structure of the Pulmonary Arteries (PA) could be provided. Seung-hyun You et al. observed the micro-vascular structure of a complete kidney by micro-CT, and obtained three-dimensional imaging information. Garcia-Sanz et al.[11] reported the results of division of the kidney according to the micro-vascular distribution using micro-CT in white mice into four regions.

This study divided the head, heart, and abdomen through the visualization of the blood vessel structure of the whole body to visualize the micro-vascular structure of each part. In addition, the imaging directions of each part on the sagittal plane and the coronal plane are shown, so that quantified information like a more accurate size of the blood vessels could be visualized. Until now, most researchers have obtained images using damaged organ tissue, while this study could obtain images by the contrast enhancement of living white mice. Because it was difficult to evaluate 2D sectional data of each part since they were not visible, all 2D data were transmitted to a workstation. As a result of the use of MIP and VRP, contrasted 3D images of the organs and micro-vascular structure of the head, heart, and whole body could be obtained.

By using micro-CT, much imaging information about small animals like rodents can be obtained. but problems that have not yet been solved remain in various research processes and experiments:

1. Despite micro-CT having many advantages, its scanning parts are narrow, so the scanning is very limited. The equipment is also expensive, which limits its use.
2. The current technique of micro-CT imaging provides data for many useful experiments. However, in in vivoscanning, there challenges in

terms of scan speed, irradiation research, and soft tissue contrasting difference, so the technique is still lacking for expressing the micro-vascular imaging of the whole body. Nevertheless, this did not greatly affect the present study.

3. SSD imaging loses information about the actual density of the tissue by the configuration method of the imaging. If partial volume averaging effects are overlapped, the quality of the imaging is noticeably reduced. According to Rubin et al., in neo-artery stenosis patients, MIP imaging showed a highly sensitive and unique level compared to SSD imaging. Also, in stenosis degree evaluation, MIP imaging was more accurate.[12-13] Nevertheless, the analysis of small blood vessels may still make mistakes of overrating or underrating the degree of stenosis.
4. To contrast the micro-vessels of white mouse organs with micro-CT, the particles of the contrast medium should overall be evenly distributed to the entire blood vessel system, and the contrasting should be done while maintaining proper temperature so that the particles of the contrast medium are not weighted by gravity. A heat pad was used to maintain the temperature at 34°C but there was a limitation in maintaining the particles of the contrast medium appropriately. In the micro-CT image, unlike bones, the blood vessels have very low contrast. The X-ray contrast medium used in most clinics is based on iodine atoms, which is soluble in water, so it is appropriate for clinical radiology, but it cannot be used as a contrast medium for small animals. Thus, the selection of the contrast medium of barium sulfate (BaSO<sub>4</sub>) used in this study depended on the researcher, so it was difficult to prove its objectivity. However, images that might not be problematic in the results of this research were obtained.

## 5. Conclusion

This study obtained blood vessel contrast images of the whole body of white mice using the micro-CT developed by the Wonkwang University Institute for Radiological Imaging Science. In addition, the blood vessel systems of the head, abdomen, heart, and whole body were classified. Maximum Intensity Projection (MIP) and Volume Rendering Technique (VRT) were used for the 2D sectional data of each part to obtain contrasted 3D images of the blood vessel structures. The conclusions are as follows:

1. To observe the shape of the head and the blood vessel structure of a mouse, the large blood vessels from the heart toward the cerebrum were visualized on the sagittal plane. The major blood vessel structure, external jugular veins, and extra-cranial arteries in the blood system of the cerebrum could be observed by the MIP technique, and clear images from the external jugular vein and the supraorbital vein could be obtained.
2. The heart and the abdomen were visualized on the four-way sagittal plane. 3D images expressing the liver and the left ventricle were obtained, and in particular, a normally contrasted heart imaging structure could be observed. Regarding the observation level, contrast images in which up to 4 chambers could be observed were obtained.
3. In The coronal plane of the heart and the abdomen, the abdominal vein, renal vein, and abdominal artery (AA) could be expressed well, through which micro-vessels and the liver, heart, and the kidney shape could be observed. 3D VRT images express three-dimensional and quantitative images, so that micro-vessels with 100  $\mu\text{m}$  epidermis could be observed.
4. In the sagittal plane including the head, throat, lungs, and abdomen, external jugular veins,

extra-cranial arteries, the heart, liver, and renal artery, and the abdominal artery and iliac artery could be observed. Other micro-vessels were expressed in 3D imaging and VRT imaging, so that the realistic and quantitative information could be observed visually.

In a half-way vital histological analysis on small animals of which the internal organs and micro-vessels are too small, once they are sliced, their tissue is damaged, so other examinations cannot be carried out. Accordingly, as the methods of obtaining internal structures or tissue status with a non-invasive method at higher resolution have been developed, research on the observation of the micro-vascular structures of small animals have been carried out, but there has been almost no studies on visualizing the blood vessel structures of the whole body of white mice in vivo.

Through the present study, in vivo 3D images and MIP images of the blood vessel systems of the whole body of white mice could be obtained. However, imaging techniques using micro-CT are still at a very rudimentary stage with critical points. And yet, if much research is continuously carried out, not only the organs and blood vessel structures of small animals but also imaging information with nano-level high resolution can be provided. Through the observations of the forms and functions of the micro-structures of vital phenomena, it is expected that models can be derived for evaluating new drugs and medicine currently used in clinics. The results of these efforts can contribute to improving human welfare.

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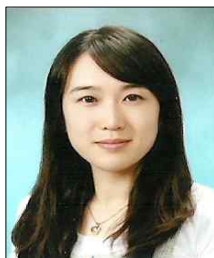
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