Increasing the Accuracy of 3D Heart Models Based on Micro-Computed Tomography

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Abstract

Micro-CT is an imaging method offering much higher resolution than standard clinical CT, with up to 0.5 µm/pixel for the objects 1-2mm. It captures detailed three-dimensional images, merging micro and macro anatomy of organs. This study aimed to develop an effective tissue processing protocol for micro-CT of cardiovascular systems. Twenty pig hearts were processed through isolation, perfusion, and flushing with isotonic solution to clear clots. Various immersion and injection reagents, like KI2 and liquid BaSO4, were tested under different conditions. Eight hearts were scanned using GE Nanotom S at AGH's Micro and Nano Tomography Lab, AGH University, Krakow, Poland. The best results came from injecting liquid BaSO4 into coronary arteries at cold temperatures, preventing blockage and leaks. Immersion methods proved less effective. Despite minor gamma corrections in post-processing, this approach yielded optimal imaging.

1. Introduction

X-ray microtomography, or micro-computed tomography (microCT), is a non-invasive imaging technique that visualizes the internal structure of objects through two-dimensional projections from various angles. While based on the same principles as conventional computed tomography, microCT achieves higher resolution due to a smaller radiation focal spot (1).

MicroCT can scan objects of varying sizes, typically from a few millimeters to several tens of centimeters, depending on the device specifications. Specialized systems exist for larger specimens (1,2). Its primary applications include investigations in materials engineering, geology, the food industry, and the polymer industry (3).

Recently, there has been increasing interest in using microCT for post-mortem imaging of ex vivo organs, particularly bone structures (4,5) and easily contrasting tissues that enhance attenuation differentiation. This evolving field seeks optimal protocols for visualizing muscular, vascular, and nervous structures of the heart (6). This study aimed to establish an effective ex vivo tissue processing protocol for microCT imaging to improve the accuracy of three-dimensional anatomical models of the cardiovascular system.

1. Materials and methods

2.1. Enchacing accuracy

Resolution, contrast, and contrast enhancement techniques, which will be discussed below, influence the accuracy of the models.

2.2. Sample material and ex-vivo tissue processing

Twenty pig hearts were harvested within 24 hours before the intervention. The entire mediastinum and its associated structures were excised to prevent damage during the collection process. The vessels leading to the heart and lungs were prepared from the collected tissues. An iso-osmotic physiological saline solution (PBS) was employed to cleanse the hearts, thereby minimizing cellular fluid loss. The atria and ventricles were thoroughly rinsed via the primary arterial and venous trunks to eliminate blood clots. The right and left coronary arteries were cannulated to facilitate the removal of residual blood from the vascular bed. To maintain the hearts in a relaxed state, tubes with diameters ranging from 0.8 to 1.5 cm were attached to the aorta, pulmonary trunk, main vein, and inferior vena cava, allowing fluid to be introduced into the

atrium and ventricle, thereby relaxing the heart muscle. To prevent fluid backflow into the heart under high pressure, specially designed stoppers shaped like cones and tailored to the diameter of the tubes were utilized, which were produced using a 3D resin printer. Additionally, the lungs and their connecting structures to the heart were tied off to prevent fluid leakage.

1.3. Contrasting the research material

Effective X-ray attenuation is achieved through the use of contrast agents with high atomic numbers, such as barium sulfate and iodine. A suitable carrier or solvent must deliver the contrast agent without compromising tissue integrity. However, excessive contrast density may obstruct its passage through smaller vascular subdivisions, while insufficient density may lead to inadequate retention during the scanning process. Consequently, this study conducted a comparative analysis of barium- and iodine-based contrast agents, along with their respective carriers. The optimal concentration for the iodine-based contrast agent was determined.

Following tissue preparation, the contrast application commenced. Both immersion and intravascular contrast techniques (for the left and right coronary arteries) were utilized (Figure 1). The immersion contrasts included using 3% and 10% potassium iodide (KI2) solutions, with some specimens undergoing immersion in buffered formalin beforehand. Intravascularly, various mixtures were employed, including a liquid solution of BaSO4, epoxy resins containing BaSO4, epoxy glue mixed with a solvent, and a 10% iodine solution. Out of 20 hearts collected, only 8 were successfully scanned. The exclusion criteria included the identification of damage at the microcirculatory level (where contrast leaked outside the heart) or damage to one of the coronary arteries during the cannulation preparation, which hindered the delivery of contrast to the entire circulatory system.

2.4. Micro-computed tomography imaging

Scans were performed at the AGH University of Krakow, Poland at the Micro and Nano Computer Tomography Laboratory (LMiNT) on a General Electric Nanotom S. Before scanning, the specimens were placed in 3 cm of water, locked in a plastic container to prevent them from changing their position during several hours of scanning. The parameters used during the scanning process were constant, chosen based on previous experience with soft tissue scans: (current (I) = 200A, voltage (U) = 80V, resolution = 60 μm).

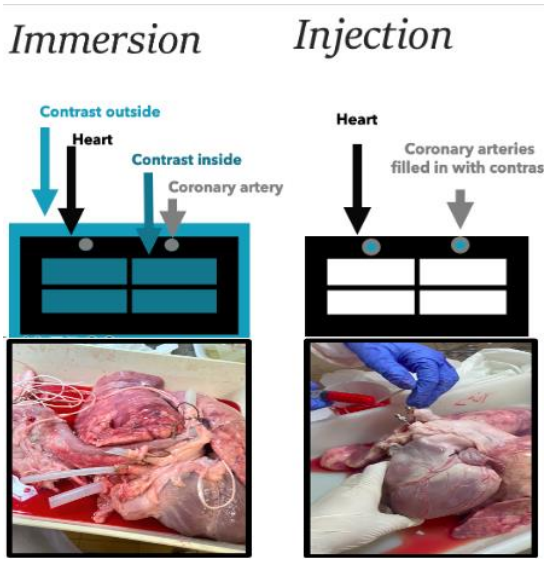

Figure 1. Schematic representation of contrast enhancement techniques: injection and immersion. Images of animal specimens during the application of each technique are presented below each diagram.

2.5. Data Processing

The provided microCT data exhibited the following characteristics: high resolution, cyclically recurring gamma disturbances, spherical artifacts, inability to import data into multiple medical imaging segmentation programs. After a successful import attempt, a stack of DICOM format data was created. The next step was trying to import the data into various medical programs to export them to a series of nii/nifti or DICOM formats. This operation was successfully performed, and the data were loaded into software allowing for analysis and 3D reconstruction as 3D Slicer and Dragonfly. Gamma disturbances did not affect the pixel value difference. To correct the cyclically recurring disturbances (Figure 2), Adobe software (Photoshop) was used with an automatic white balance function.

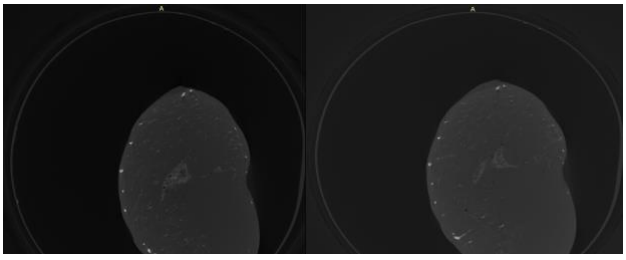

Figure 2. Cyclically recurring gamma disturbances (grey value).

3. Results and discussion

Below each paragraph, tables are presented comparing the contrast agent, contrast technique, and density with the DICOM images. The first row of the table indicates the contrast technique (injection (IJ) or immersion (IM)). The second row specifies the substance and carrier. The third row denotes the density of the substance. The fourth row displays the DICOM images.

Injection with low-density iodine did not allow for contrasting structures (Heart 1). Injection with 10% iodine allowed for visualization of major vessel walls, contrast did not last at the lumen of the arteries (Heart 2).

Heart 1	Heart 2
IJ	IJ
KI2 3%	KI2 10%
Low	

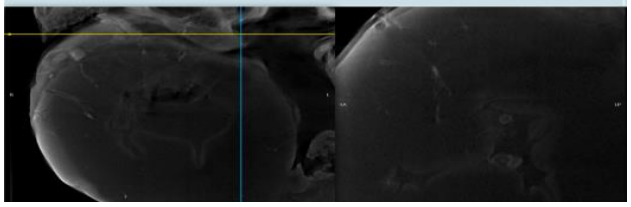

Figure 3. In the comparison of the low-concentration and high-concentration iodine contrast without a carrier, the injection technique was used.

Immersion contrasts did not allow vessel visualization. Immersion in a 3% KI2 solution did not allow for contrasting any structures (Heart 6). Visible differences in tissue attenuation level were achieved after immersion in a 10% KI2 solution. After 14 days, the contrast entered the muscle at a distance from the walls of 0.4 (Heart 7) to 0.8 mm (Heart 8). Formalin increased absorption of the contrast.

Heart 6	Heart 7	Heart 8
IM	IM	
KI2 3%	KI2 10%	Formalin+ KI2 10%
Low	Low	

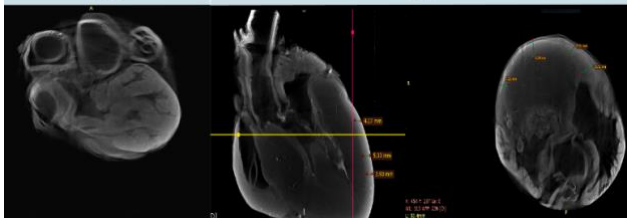

Figure 4. Comparison of immersion technique with different concentrations of iodine.

The best results were obtained after injection contrasts. High-density contrast carriers, such as epoxy glue mixed with a solvent (Heart 5), hindered injection and passage through smaller capillaries. They solidified during injection, ending the vascular bed filling process. Low-density contrasts (epoxy resin, liquid BaSO4 solution) allowed passage through arterial vessels and arterial capillaries to venous and venous vessels without blocking in transit. However, epoxy resin (Heart 4) left BaSO4 residue in transit, unlike the liquid BaSO4 solution (Heart 3). Due to the contrast tightening properties under cold temperatures or activators, the liquid solution and epoxy resin could be applied without time limitation.

Heart 3	Heart 4	Heart 5
IJ		
Liquid BaSO4	Epoxy BaSO4	Epoxy glue + solvent + KI2 10%
Low/ high after freezing	Low/ high after 5 minutes of reaction with activator	High

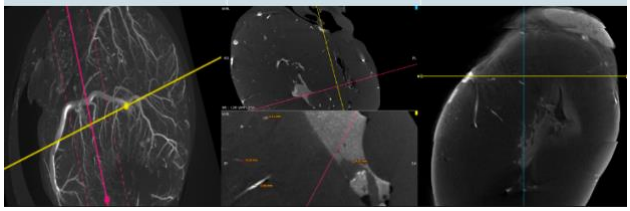

Figure 5. Comparison of the barium sulfate contrast with high concentration iodine contrast. The density during the scanning time of each contrast was high.

The obtained high-resolution microCT data set contained approximately 1800-2200 DICOM format files. To perform the analysis and 3D reconstruction, such a large amount of data had to be set in a stack of images.

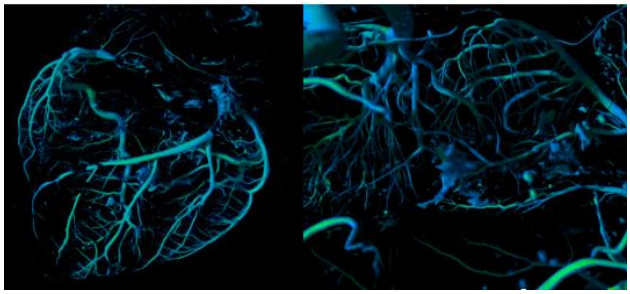

Figure 6. 3D reconstruction of the heart with Liquid Barium Sulfate Solution at Dragonfly Software.

Additionally, importing a stack of images into 3D visualization software was not possible without reducing the cyclically recurring gamma disturbances. Reducing these disturbances in Adobe Photoshop software, allowing DICOM file import, did not affect the pixel value of HA (Hounsfield Units). The developed and tested protocol allowed obtained a view of the vessels at the level of detail

0, 11mm (Figure 7).

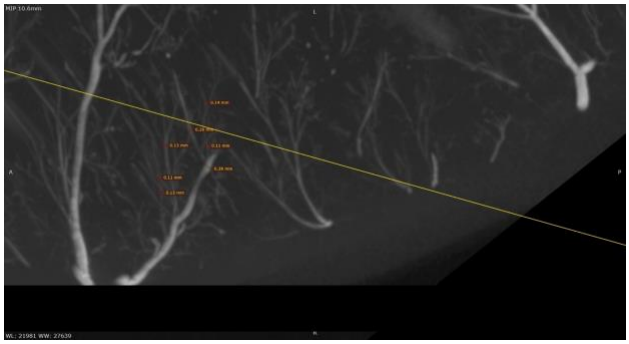

Figure 7. View of obtained microCT data from Dicom Viewer, WL: 21981, WW:27639, measurements of the level of detail, e.g. one of the smallest vessel diameters: 0,11 mm.

4. Conclusion

This study highlights the efficacy of barium sulfate ($BaSO_4$) contrast media over iodine-based contrast agents, particularly in the context of injection techniques. The optimal use of contrast relies heavily on the manipulation of its density; a low-density during placement is crucial, while a high density at the time of scanning is necessary to ensure adequate retention within the vascular system. Employing an activator or cooling mechanism effectively maintains this balance. Furthermore, the analysis of high-resolution micro-CT data necessitated advanced processing tools, which successfully minimized artifacts without compromising pixel integrity. The findings of this research pave the way for non-destructive studies of vascularization, offering significant implications for understanding and addressing ischemic heart disease. Overall, the results underscore the importance of selecting appropriate contrast media and refining imaging techniques to enhance post-mortem diagnostic capabilities in cardiovascular health.

5. Acknowledgment

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5. References

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