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Research Article

Micro-Computed Tomography Assessment of Rat Bone Microstructures: Effects of Acquisition Resolution and Rotation Range

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Abstract

Introduction: Micro-computed tomography (micro-CT) has become the gold standard for evaluating bone microarchitectures in small animal models. Various studies have employed micro-CT to investigate rat bones. However, results can be significantly influenced by rotation range (RR) and scan resolution (SR).

Objective: Through our study, we tried to assess effects of RR and SR during Micro-computed tomography scan.

Materials and Methods: In this study, nine femurs were scanned based on four different acquisition scenarios using all possible combinations of two SRs (SR: 9 µm/pixel and 18 µm/pixel) and two RRs (RR: 180° and 360°).

Results: The scan durations and file sizes were statistically significantly different between different groups: A-C ($p = 0.004$; $p = 0.002$), B-C ($p = 0.001$; $p = 0.001$), and B-D ($p = 0.004$; $p= 0.007$). No statistically significant differences between groups were observed for the bone volume ($p = 0.1589$), trabecular number ($p = 0.4160$), trabecular separation ($p = 0.6251$), or volume of closed pores ($p = 0.0538$). In contrast, trabecular thicknesses were statistically significantly different between various groups: A-D ($p = 0.03$) and B-D ($p = 0.043$). Furthermore, the cortical bone morphometry corresponding to the number of closed pores was statistically significantly different between various groups: A-C ($p = 0.004$), A-D ($p= 0.001$), and B-D ($p = 0.011$).

Conclusion: SR and RR affect acquisition time, data storage, also the quantitative results of trabecular bone micro-CT assessments.

Keywords: Micro-Computed Tomography; Rotation Range; Scan Resolution; Pixel Size; Trabecular Bone

Introduction

Micro-computed tomography (micro-CT) is an extensively used, non-destructive, X-ray transmission imaging method [1-5]. X-rays are emitted by a generator, travel through a sample, and are recorded by a detector to produce a radiograph referred to as a projection image. The sample is then rotated by a fraction of a degree and another projection image is acquired at the new orientation. This procedure is repeated until the sample has been rotated by 180° or 360° to produce a series of projection images. The acquired projection images are then processed using dedicated computer software to reveal the internal structures of the object. The processed images are commonly referred to as reconstructed images or cross sections [6]. Reconstructed images can be modeled as 3D volumetric objects to facilitate quantitative analysis or simple visualization [2].

The micro-CT technique has been extensively used to characterize the microstructures of materials in various fields, such as tissue engineering, geosciences, medical devices, pharmaceutical packaging, developmental biology, and dentistry [7]. Micro-CT has become the gold standard for evaluating bone morphologies and microarchitectures in small animal models *ex vivo* [4]. Various studies have employed micro-CT to study rat bones [8-17]. However, results can be significantly affected by sample rotation and acquisition resolution.

Following the acquisition of a projection image, the sample is rotated by a fraction of a degree (typically ≤ 0.5°). Both the X-ray source and detector pair are rotated when scanning *in vivo*, while only the sample is rotated during *ex vivo* imaging. A new projection image is typically acquired at each step through a 360° rotation range (RR). However, a 180° RR can be used to reduce scan time because the projection images captured from 0° to 180° are mirror images of those captured from 180° to 360°. Typically, a smaller step-size results in thinner cross sections.

The smallest possible voxel size (i.e. highest possible scan resolution, SR) should ideally be used for all scans. However, a higher SR requires a longer acquisition time because more projections must be acquired, which also results in larger datasets. Voxel size can significantly affect results when analyzing smaller structures, such as rat trabeculae [1].

Aim of the Study

The goal of this study was to investigate the effects of SR and RR on (i) morphometric results, as well as (ii) acquisition time and data storage.

Materials and Methods

Samples

Nine 10-week-old Wistar rats (250 to 270g) were used as subjects in this study. The principles of laboratory animal care (NIH publication 85-23, 1985) and national laws for animal use were observed in this study, which was authorized by the Ethical Committee for Animal Research of the University (XXX). Euthanasia was performed via decapitation, following anesthesia. The left femurs were dissected from the rats and all soft tissue was removed. The samples were then identified and submerged in ethanol.

Micro-CT

In this study, only the left femurs of the rats were considered. Each femur was scanned four times using a SkyScan 1176 (Bruker Micro-CT, Belgium) micro-CT system. All nine samples were scanned according to four different acquisition scenarios using all possible combinations of two SRs (SR: 9 µm/pixel and 18 µm/pixel) and two RRs (RR: 180° and 360°) with 0.2° increments (Table 1). The X-ray source voltage and current for acquisition were set to 50 kV and 500 µA, respectively. An aluminum filter with a thickness of 0.5 mm was adopted. The first steps of image acquisition included sample preparation and positioning. In this study, samples were aligned with the horizontal axis of the scanner and placed on a stand. All the samples were placed in the same original position to avoid any bias introduced by initial positioning. No additional scan medium was used for imaging specimens in this study because different media can affect X-ray attenuation [3].

Reconstruction and 3D analysis were performed using the NRecon (v1.6.10.2) and CT Analyzer (v1.15.4.0) standardized software without any post processing, such as beam hardening, alignment correction, or smoothing. According to the manufacturer's designated procedures, an automatic global threshold was used with the software to ensure objective and reproducible results. A specific portion with a thickness of 8 mm was reconstructed for each of the nine femurs. All 3D analyses were performed over the same volume of interest. Additionally, we investigated the characterization time and disc space required to store the data corresponding to each acquisition scenario to evaluate the efficiency in terms of time and resources.

		Scan Resolution	
		9 (µm/ pixel)	**18 (µm/ pixel)**
Rotation range	180°	A (9 and 180)	C (18 and 180)
	360°	B (9 and 360)	D (18 and 360)

Table 1: Different acquisition scenarios using all possible combinations of two scan resolution (9 µm/pixel and 18 µm/pixel) and two rotation range.

*The letters represent groups.

In this study, the most commonly cited morphometric indices were evaluated based on the captured 3D images, namely bone volume (BV), trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular thickness (Tb.Th). Cortical bone morphometry was evaluated based on the number of closed pores (Po.N(cl)) and volume of closed pores (Po.V(cl)).

Statistical analysis

Statistical analysis was conducted using the SPSS Statistical Package (IBM® SPSS® Statistics version 24.0, IBM, USA). Descriptive statistics were calculated for each group. The normality of the data distribution was analyzed using the Shapiro Wilk test. The Kruskal Wallis and Dunn post hoc tests were used to compare scan times, file sizes, Tb.N, Tb.Sp, and Po.N(cl) between different groups. The ANOVA and Tukey post hoc tests were used to compare BVs, Tb.Th, and Po.V(cl) between various groups. The differences between groups were considered to be statistically significant when $p < 0.05$.

Results

The longest scan durations can be observed for groups A and B (9 µm/pixel) (3481.5 and 6663.9s, respectively). The scan durations are statistically significantly different between different groups: A-C ($p = 0.004$), B-C ($p = 0.001$), and B-D ($p = 0.004$), as shown in table 2. The smallest file sizes can be observed for groups C and D (18 µm/pixel) (26.350 GB and 44.150 GB, respectively). The file sizes exhibit statistically significant differences between different groups: A-C ($p = 0.002$), B-C ($p = 0.001$), and B-D ($p= 0.007$), as shown in table 3.

No statistically significant differences between groups can be observed for BV ($p = 0.1589$) (Table 4), Tb.N ($p = 0.4160$) (Table

Groups	N	Mean	SD	Min	Max	p-value
A	9	3481.5	330.0	2877.0	3732.0	a
B	9	6663.9	17.1	6657.0	6706.0	b, c
C	9	854.4	7.2	840.0	859.0	a, b
D	9	1567.0	1.1	1566.0	1569.0	c

Table 2: The effect of scan resolution and rotation range on the scan time.

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$; a = .004; b = .001; c = .004.

Groups	N	Mean	SD	Min	Max	p-value
A	9	26.350	8.966	13.5	37.2	a
B	9	44.150	9.633	32.8	58.0	b, c
C	9	3.958	0.866	1.9	4.5	a, b
D	9	6.002	0.875	5.1	7.8	c

Table 3: The effect of scan resolution and rotation range on the size of file (GB).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$; a = .002; b = .001; c = .007.

Groups	N	Mean	SD	Min	Max	p-value
A	9	76.538	6.888	69.292	89.099	0.1589
B	9	79.448	8.022	70.788	91.311	
C	9	83.309	9.216	74.027	98.579	
D	9	85.442	8.644	73.634	99.873	

Table 4: The effect of scan resolution and rotation range on the bone volume (BV).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$.

Groups	N	Mean	SD	Min	Max	p-value
A	9	0.0684	0.0439	0.0270	0.1510	0.4160
B	9	0.0451	0.0216	0.0260	0.0880	
C	9	0.0375	0.0076	0.0250	0.0470	
D	9	0.0785	0.1227	0.0200	0.3810	

Table 5: The effect of scan resolution and rotation range on the trabecular number (Tb.N).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.005$.

Groups	N	Mean	SD	Min	Max	p-value
A	9	6.793	0.8284	5.437	7.444	0.6251
B	9	7.186	0.3061	6.532	7.481	
C	9	7.282	0.1583	7.028	7.468	
D	9	7.243	0.1650	7.028	7.539	

Table 6: The effect of scan resolution and rotation range on the trabecular separation (Tb.Sp).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$.

Groups	N	Mean	SD	Min	Max	p-value
A	9	0.4711	0.2576	0.1850	0.9760	0.0538
B	9	0.5156	0.3451	0.1890	1.2020	
C	9	0.8961	0.5903	0.3820	1.9200	
D	9	0.8994	0.5051	0.4260	1.9910	

Table 7: The effect of scan resolution and rotation range on the volume of closed pores (Po.V(cl)).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$.

Groups	N	Mean	SD	Min	Max	p-value
A	9	0.3550	0.0434	0.2980	0.4210	a
B	9	0.3850	0.0507	0.3190	0.4860	b, d
C	9	0.4248	0.0523	0.3450	0.5050	c
D	9	0.4640	0.0743	0.3810	0.6070	a, d

Table 8: The effect of scan resolution and rotation range on the trabecular thickness (Tb.Th).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$; a = .03; b = .043.

Groups	N	Mean	SD	Min	Max	p-value
A	9	1971.6	561.1	1358	3157	a, c
B	9	1370.5	483.6	846	2217	b, d
C	9	764.3	194.7	574	1050	a
D	9	610.8	161.4	395	810	c, d

Table 9: The effect of scan resolution and rotation range on the number of closed pores (Po.N(cl)).

N: Sample Size; SD: Standard Deviation; Min: Minimum; Max: Maximum.

$p < 0.05$; a = .004; c = .001; d = .011.

5), Tb.Sp (p = 0.6251) (Table 6), or PoV(cl) (p = 0.0538) (Table 7). In contrast, the Tb.Th values are statistically significantly different between various groups: A-D (p = 0.03) and B-D (p = 0.043) (Table 8). Furthermore, the cortical bone morphometries corresponding to the Po.N(cl) values are statistically significantly different between various groups: A-C (p = 0.004), A-D (p = 0.001), and B-D (p = 0.011) (Table 9).

Discussion

In this study, we assessed morphometric parameters in the 3D volumes of femurs for all test conditions. However, changes in SR and RR led to differences in terms of acquisition time, data storage, Tb.Th and Po.N(cl).

It is important to investigate the effects of SR and RR on morphometric bone assessments because micro-CT has been widely used in numerous preclinical studies. The SR and RR of micro-CT systems can be modified to reduce radiation doses. Thus far, no study has evaluated the effects of SR and RR on the morphometric results of micro-CT.

The results of this study suggest that higher resolution and complete rotation (9 μm/pixel and 360°, respectively) of the X-ray source increase the scan time and file size compared to lower resolution and incomplete rotation (18 μm/pixel and 180°, respectively).

The smallest possible pixel size must be used for all micro-CT scans, although this results in longer acquisition times and larger datasets [18]. This finding is supported by the fact that voxel sizes can significantly affect results [1] when analyzing small structures (20 to 70 μm), which are in the order of the smallest voxel size of most available micro-CT systems (1 to 10 μm). Therefore, the relationship between SR and scan time must be considered [4]. In this study, SR and RR significantly affected the scan time based on the required number of scan steps.

It was determined that varying SR and RR affects the number of projections, file size, and scan time. Lower resolution and complete rotation are recommended for evaluating bone microstructures. However, it is also important to consider the type of acquisition (*ex vivo* or *in vivo*) and to evaluate the effects of anesthesia time and radiation doses on animals [19].

The standard method used for quantitatively describing bone architectures involves calculating morphometric indices. This method is often referred to as quantitative morphometry [4]. Quantitative morphometry using micro-CT is a precise and verifiable imaging technique that has been used extensively to assess microarchitectural bones to investigate different diseases and their treatments, including osteoporosis, gene therapy, tissue engineering, and biomaterials. This method has also been useful for the validation of additional techniques aimed at investigating bone microstructures in clinical settings [20].

Currently available micro-CT units provide an isotropic voxel size on the order of a few micrometers, which is sufficient for investigating structures such as rodent trabeculae.

Several studies have assessed microstructural tissue properties in femurs, particularly trabecular bones [8-17]. However, different resolutions and rotation ranges have been used, but not always communicated, yielding varied results. Trabecular microarchitectures can be examined on different scales and different studies have focused on different types of information that can be extracted at each scale. Furthermore, comparing the morphometric parameters obtained from images acquired with different resolutions or rotations can produce varied results.

Previous studies on micro-CT resolution have also observed the dependence of trabecular bone parameters on voxel size [21-23]. Isaksson., *et al.* [23] used micro-CT to investigate the effects of image resolution on bone microstructure parameters in healthy and osteoporotic trabecular bones. It was determined that the initially detected differences between normal and osteoporotic groups diminished with increasing image voxel size. Sode., *et al.* [22] demonstrated that three non-metric indices (SMI, Conn.D and DA) of trabecular bone structures are affected by the spatial resolution of micro-CT images. The murine tibiae in the above study were resampled to isotropic voxel sizes of 18, 27, 36, 54, and 72 μm. Peyrin., *et al.* [21] evaluated ten vertebrae samples from healthy females of varying ages (33 to 90), which were imaged at various resolutions (cubic voxel sizes of 14, 6.7, and 1.4 μm). The morphometric parameters extracted from the different images showed good agreement with the results of simulations evaluating the effects of spatial resolution on structure parameters. The findings of this study are in agreement with the results discussed above, suggesting that large micro-CT voxel sizes may not provide an accurate description of trabecular bones.

Micro-CT images may be corrupted by artifacts, which are defined as visual structures in reconstructed data that were not present in a scanned object [24]. In particular, the presence of dense materials, such as metals, tends to generate "metal artifacts" in micro-CT images. The presence of such metal artifacts can affect im-

age quality in several ways, ranging from bright streaks radiating from metallic objects and dark areas in their vicinities to complete loss of information between adjacent dense objects [25,26].

Image data size affects aliasing and a few types of artifacts. Larger image datasets offer more information for volumetric reconstruction, which minimizes artifacts. However, this also increases image acquisition time and storage requirements. Image requirements for a given application should be carefully evaluated to ensure acceptable image quality while reducing time and hardware storage demands.

The results of this study demonstrated that SR and RR can affect the quantitative (i.e. morphometric) results of bone micro-CT assessments. A consistent SR with a varying RR produced no significant differences in trabecular parameters. However, varying SR values can lead to differences in trabecular parameters. This indicates that a complete rotation range (360°) is not required because results mainly depend on SR, facilitating shorter image acquisition times. This is particularly beneficial when micro-CT is used for imaging live small animals.

Conclusion

SR and RR should be carefully considered during micro-CT acquisition because they can affect the quantitative results of bone micro-CT assessments. Additionally, SR and RR also affect acquisition time and data storage.

Conflict of Interest

Declare if any financial interest or any conflict of interest exists.

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