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MicroCT Morphometry Analysis of Mouse Cancellous Bone: Intra- and Inter-system Reproducibility

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Abstract

The agreement between measurements and the relative performance reproducibility among different microcomputed tomography (microCT) systems, especially at voxel sizes close to the limit of the instruments, is not known. To compare this reproducibility 3D morphometric analyses of mouse cancellous bone from distal femoral epiphyses were performed using three different ex vivo microCT systems: GE eXplore Locus SP, Scanco μ CT35 and Skyscan 1172. Scans were completed in triplicate at 12 μ m and 8 μ m voxel sizes and morphometry measurements, from which relative values and dependence on voxel size were examined. Global and individual visually assessed thresholds were compared. Variability from repeated scans at 12 μ m voxel size was also examined. Bone volume fraction and trabecular separation values were similar, while values for relative bone surface, trabecular thickness and number varied significantly across the three systems. The greatest differences were measured in trabecular thickness (up to 236%) and number (up to 218%). The relative dependence of measurements on voxel size was highly variable for the trabecular number (from 0% to 20% relative difference between measurements from 12 μ m and 8 μ m voxel size scans, depending on the system). The intra-system reproducibility of all trabecular measurements was also highly variable across the systems and improved for BV/TV in all the systems when a smaller voxel size was used. It improved using a smaller voxel size in all the other parameters examined for the Scanco system, but not consistently so for the GE or the Skyscan system. Our results indicate trabecular morphometry measurements should not be directly compared across microCT systems. In addition, the conditions, including voxel size, for trabecular morphometry studies in mouse bone should be chosen based on the specific microCT system and the measurements of main interest.

Keywords

mouse cancellous bone; bone morphometry; microcomputed tomography; reproducibility

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Introduction

Since its initial development microcomputed tomography (microCT) has become an important tool for the analysis of bone morphology, particularly for cancellous bone. In recent years microCT has been used extensively to characterize alterations of bone density and microarchitecture in human and animal bones due to disease [1-5], pharmacologic intervention or hormonal effects [6-10] and mechanical loading [10-17]. Although synchrotron radiation-based x-ray imaging provides higher resolution, conventional desktop microfocus microCT systems are used in the majority of studies because of greater availability and lower cost. The last few years have witnessed a surge in microCT-based skeletal phenotype reports in mouse models, commercially available instruments have multiplied and researchers have realized the need of consistency to compare findings across studies [18]. This increased use warrants a better understanding of microCT performance in mouse cancellous bone analysis, where the structures analyzed require resolutions close to the limit of the instruments.

The effect of voxel size and other parameters on the precision of microCT measurements have been the subject of numerous studies. In these studies, measurements from human or rat bone were commonly obtained at different voxel sizes and/or compared to similar independent measurements from standard methods, such as histomorphometry, synchrotron radiation microCT or higher resolution scans [2,5,9,19-27]. Comparisons have generally been made on a single system, thus the conclusions about voxel size and repeatability of microfocus microCT as a method were limited to the system used. Yet, comparisons in the literature may be based on different systems, and ideally comparisons should be possible on similar models irrespective of the system.

In this study ex vivo microCT analyses of adult mouse femurs were compared with repeated 8 μ m and 12 μ m voxel size scans to examine the (1) agreement of trabecular morphometry measurements between three of the most commonly used microCT systems, (2) relative dependence of measurements on voxel size of scans, and (3) relative reproducibility of measurements within each system. The results revealed wide discrepancies between the systems in terms of both the measurements' absolute values and the dependence on voxel size. Based on replicate analyses using two different sets of bones scanned on two comparable models of three of the most common systems located at different sites, the different systems' performance, in terms of reproducibility of results for each system and voxel size, was highly variable under the scan conditions recommended by the manufacturers.

Materials and methods

Specimen preparation and scanning

Femurs from a total of nine 12 week-old C57/B6 male mice were studied. A set of three femurs (dataset #1) was analyzed at 12-13 μ m and 7-8 μ m isotropic voxel size to compare relative values of trabecular measurements and their dependence on voxel size. A second larger set of six femurs (dataset #2) was analyzed at 12-13 μ m to study inter-system measurement reproducibility. Scans for the second set were performed on the same systems/models but on different machines and at different facilities than the first set. All femurs were cleaned of soft tissue and stored in 70% alcohol at -4°C until scanning. Before the scans, the bones were positioned with gauze in the sample holder and allowed to reach room temperature. The distal half of femurs from both datasets was scanned three times at both voxel sizes without sample repositioning on 3 scanners: eXplore Locus SP (GE Healthcare, London, Ontario, Canada), Scanco μ CT35 (Scanco Medical, Bassersdorf, Switzerland) and Skyscan 1172 (Skyscan, Aartselaar, Belgium). The scans for each bone were performed

sequentially, and then the scanned bone was returned to -4°C . The scans on each system were completed within 2-3 days, and each set of scans was completed within 2-3 months. The scanning conditions were those recommended by the manufacturers for adult mouse bone at each voxel size (Table 1). In the GE and Scanco systems a precisely 12 or $8\mu\text{m}$ voxel size, respectively, is not commonly used due to limitations in size of sample holders, and the closest voxel size possible in the system protocols was used instead. The 12 to $13\mu\text{m}$ scans will be referred to as “ $12\mu\text{m}$ scans” and 7 to $8\mu\text{m}$ scans will be referred to as “ $8\mu\text{m}$ scans” from this point onwards. Scanning at $8\mu\text{m}$ or even $6\mu\text{m}$ voxel size is recommended by all manufacturers for mouse trabecular 3D morphometry analysis using these particular systems, if scanning time is not a concern. Given that we had to choose a voxel size that was attainable by and accommodated the same area for analysis for all the systems, we used $8\mu\text{m}$ scans as our baseline. We compared the $8\mu\text{m}$ data to $12\mu\text{m}$ scans, a voxel size recommended in some earlier systems.

Reconstruction- Segmentation of volumes

Reconstructed volumes were processed using the respective manufacturer’s software for the three systems (Table 2). Calibration for mineral density in the scans was performed with a use of a phantom or automatically, as recommended by the manufacturers (Table 1). The regions of interest (ROIs) for analysis were first defined on the $8\mu\text{m}$ scans. To standardize the ROIs examined among systems, cancellous bone ROIs were created in the reconstructed-calibrated volumes using the largest circles that could be enclosed within the endosteal envelope on the 2D slices (Fig. 1). These ROIs extended from $100\mu\text{m}$ proximal to the growth plate of each femur to the most proximal level on the diaphysis where trabeculae were still discernible, resulting in 4.4 - 4.8mm areas along the bones long axis analyzed. The diameters of the ROI circles for a specific slice from one femur matched at a location were within $\pm 1.5\%$ in all three systems. Identical to the $8\mu\text{m}$ volumes, circles were drawn on the respective slices from $12\mu\text{m}$ scan volumes and equivalent ROIs were created. The identical trabecular ROI for each voxel size was automatically applied to all 3 scans for a given femur/voxel size/system data set.

Bone was segmented from the background and bone marrow in all volumes using two different thresholds, a global and an individual one. Thresholds were visually defined for each femur by two operators (KV, LL) based on minimizing noise while maintaining trabecular interconnectivity for the first $8\mu\text{m}$ scan of each femur. In all cases, the maximum difference of threshold chosen by the two operators was smaller than 5% of the whole mineral density range. Threshold values were defined in g/cm^3 or Hounsfield units, depending on the system. The lowest of the three thresholds determined for the three femurs for each voxel size was assigned as the global threshold for all volumes from a particular system. Two additional threshold values were used to obtain a range of measurements by possible thresholds. These “low” and “high” threshold values corresponded to density values that were 5% lower or higher, respectively, than the global “normal” (Fig. 2). Results from global thresholding refer to the normal threshold. For the individual thresholds, the threshold values as defined on each individual femur were used for thresholding of the particular femur volumes. The Scanco system showed the most distinct separation between cancellous bone and background peaks.

3D morphometry measurements-statistical analysis

Trabecular morphometry was measured using the instrument’s individual software package [19, 21-22, 27] (Table 2). The morphometric parameters examined were bone volume fraction (BV/TV), relative bone surface (BS/BV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp). Reproducibility of measurements was assessed in both datasets through coefficients of variation (CVs) in the three measurements

of every femur/system/voxel size set, calculated as $CV = [\text{standard deviation of values (scans 1-3)} / \text{mean value (scans 1-3)}] * 100$. The larger sample size of dataset #2 allowed us to perform statistical comparisons at the $12\mu\text{m}$ voxel size. All calculations were performed on data from both global and individual thresholding methods.

Repeated measures analysis of variance was performed on the morphologic parameters from dataset #1 to determine the effect of voxel size and thresholding mode (Systat v10.2 and v12.2, Systat Software Inc., Chicago, IL). Reproducibility was also examined using a repeated measures of variance of the CVs from dataset #2 and the combined data. Post-hoc comparisons were made by Neuman-Keuls test. Alpha was set at 0.05 for all comparisons.

Results

The 3D morphometry measurements varied widely across the three systems studied (Fig. 3). This variability was highest for Tb.Th and Tb.N with over two-fold differences between systems, followed by BS/BV with average 33% and 16% differences at $8\mu\text{m}$ and $12\mu\text{m}$ voxel size, respectively, as measured using a global threshold. The range of measurements between the systems was larger than the range of values produced by high and low thresholds within a single system (Table 3). Tb.Th, Tb.N and BS/BV were significantly different across systems at both voxel sizes for both thresholds. BV/TV was similar among the three systems in the $12\mu\text{m}$ scans but showed a substantial difference (27% by global thresholding) between GE and the other two systems in the $8\mu\text{m}$ scans, while Tb.Sp was the most consistent parameter among systems at both voxel sizes.

The relative changes from $8\mu\text{m}$ to $12\mu\text{m}$ ($8\mu\text{m}/12\mu\text{m}$ changes) in trabecular morphometry also varied (Fig. 4, shown only for global thresholding). The relative changes in BS/BV and Tb.Th were unidirectional with increasing voxel size across systems, while the direction of these changes varied in the remaining measurements for the particular sample and the system. Relative ratios of $8\mu\text{m}/12\mu\text{m}$ BV/TV, BS/BV and Tb.N changes varied among systems, e.g. a 20% decrease in Tb.N by the Skyscan system compared to less than 1% for the other two systems. Tb.Sp measurements were minimally affected by the voxel size of the scans in all 3 systems. Finally, relative trends from $8\mu\text{m}$ to $12\mu\text{m}$ were independent of thresholding method for each particular system.

When reproducibility was examined, the CVs for the morphometric measurements varied across the three systems (Fig. 5). Although most of the coefficients of variation were lower in dataset#2 compared to dataset#1, as expected from the higher number of samples, their relative between the systems values were similar in the two datasets. CVs of all measurements differed significantly between at least two of the systems, and was higher for the GE eXplorer system than for the other two in most cases. Reproducibility, obtained from the $12\mu\text{m}$ voxel size scans of dataset #2, was as follows (coefficients of variation for GE, Skyscan, Scanco systems, respectively): BV/TV: $2.5 \pm 0.9, 0.7 \pm 0.5, 0.4 \pm 0.3$, BS/BV: $3.2 \pm 0.6, 0.5 \pm 0.4, 1.0 \pm 0.2$, Tb.Th: $4.3 \pm 1.6, 0.7 \pm 0.3, 1.0 \pm 0.4$, Tb.N: $1.9 \pm 1.1, 0.3 \pm 0.1, 1.1 \pm 0.6$, Tb.Sp: $2.0 \pm 1.1, 0.2 \pm 0.1, 0.5 \pm 0.4$. One system (Scanco uCT35) showed a consistently higher reproducibility in the lower voxel size scans, otherwise reproducibility depended on the particular parameter examined. Average CVs for BV/TV were lower in $8\mu\text{m}$ scans compared to $12\mu\text{m}$ scans in all systems. For Tb.N and Tb.Sp two of three systems showed lower CVs at $8\mu\text{m}$ than $12\mu\text{m}$, while for BS/BV and Tb.Th the reverse pattern was observed. On one system (Scanco) all morphometry measurements were more reproducible at the $8\mu\text{m}$ than at the $12\mu\text{m}$ voxel size.

Discussion

In the present study three widely used state-of-the-art microCT systems were used *ex vivo* in repeated analyses of mouse femurs at two commonly used voxel sizes. In addition to comparing values of measurements from different systems and their dependence on voxel size, the study also examined the relative reproducibility of these measurements using a single system. The voxel sizes examined were close to the limit that commercially available benchtop systems can achieve. Two different thresholding methods were compared, since the optimal thresholding approach is still under debate [28-29]. With the exception of Tb.Sp and possibly BV/TV, the inter-system reproducibility of 3D morphometry measurements was poor at the different voxel sizes tested. Voxel size dependence of all trabecular bone measurements between systems and intra-system reproducibility of these measurements were highly variable. The voxel size dependence of measurements was greater than previously described for thicker structures and lower voxel sizes [9,30].

The results should be interpreted in the context of limitations related to the analysis of a limited number of scans from multiple systems operated at different centers and with different processing software. The particular mouse strain examined has been widely used in microCT studies, but differences in cancellous bone volume across strains [31,32] could produce different results. The number of samples and voxel sizes tested was relatively low for practical considerations. The comparative observations made here could not distinguish between the scanning/reconstruction component of the systems examined versus the processing algorithms. A separate study of data obtained from a single system and processed using each manufacturer's software would be more appropriate for this purpose. In addition, whereas the effect of threshold was examined, thresholding methodology cannot be definitively excluded as a contributing factor in the observed differences between the systems. We were unable to use a single threshold value for all three systems because of inter-system differences in both the calibration of mineral density and the distribution of mineral densities within an object. The three systems use different methods for calibration, e.g. pre-reconstruction vs. post-reconstruction calibration based on a phantom scan vs. algorithm-based calibration (Table 1). In addition, the separation between cancellous bone and background peaks differed substantially, e.g. there was much less superimposition of density values from the cancellous bone and the background in the 12 μ m Scanco scans than in the other two systems (Figure 2). Finally, we did not attempt to assess the true resolution at the 8 μ m and 12 μ m voxel sizes in the different systems.

None the less, morphometry measurements from the three systems differed significantly. Similar observations, albeit at a lower magnitude, for two of the systems examined here (Skyscan and Scanco) were previously reported for rat trabecular microarchitecture [27]. In this earlier rat study, Tb.Th and Tb.N showed the greatest differences, which were explained as an effect of thresholding. Differences in Tb.Th and Tb.N values reported here were 50-80% and 100-150%, respectively, depending on the voxel size (Table 3). An even larger difference, up to 140% depending on the system and the voxel size, was found between Tb.Th values across the three instruments. At either voxel size these variations were higher than the range of values generated by varying the threshold $\pm 5\%$. Therefore, based on our data, the previously reported differences in 3D measurements [27] most likely also reflected differences in scan and reconstruction protocols or integration of measurements between systems, not only thresholding. The more pronounced differences in Tb.Th and Tb.N reported here likely reflect the smaller feature size of mouse compared to rat cancellous bone. For most parameters analyzed, discrepancies between systems were reduced when the higher voxel size was used for the same measurement.

The source of the observed poor inter-system reproducibility may differ between morphometric parameters. The discrepancies in the 3D measurements may relate to scanning or reconstruction and/or processing software-related factors. Scanning or reconstruction variability could determine whether voxels are assigned to bone or background during reconstruction. Relevant processing software-related factors include the methods for integration of measurements used by each system [2,22,27,33] and the “tolerance” of a particular processing software to the presence of noise. For Tb.Th and Tb.Sp, the maximum fitted spheres model [34] is used by all systems (Table 2) but implementation of the algorithm may differ. Examining the source of inter-system discrepancies requires systematic variation of scanning- and processing-related variables and was beyond the scope of the current study. In addition, comparative studies generally use a single instrument and processing software, and therefore absolute values of measurements are not critical for determining differences expressed as a percentage of a reference value.

Comparing voxel size dependence of measurements across systems was the second objective of this study. In general, sensitivity to voxel size was greatest for BS/BV and Tb.Th and least for Tb.Sp. In human bone samples, variability of the 3D morphometry parameters has been described as a result of increasing scanning voxel size [9,30], degradation of similar images through reconstruction at larger sizes [9,23] or decreasing angular rotation steps [5]. In the previous human bone studies, multiples of a reference voxel size were examined. The reasons postulated for these relative changes included the partial volume effect, reduced surface detail, artifactual merging of adjacent trabeculae or loss of thin trabeculae at the higher voxel size [5,9,30]. In our study the range of voxel size examined was relatively small, as multiples of the lowest one attainable would not be sufficient for mouse cancellous bone analysis. Substantial changes were observed here with an only 50% such change.

The intra-system reproducibility was highly variable and depended on the measurement. Scans on two units of the same type were performed for datasets #1 and #2 to ensure that the reproducibility of measurements for a particular system would be independent of the particular instrument used. Precise normalization of different scanners for scanner age, condition or calibration was not possible. The larger sample (dataset #2) was used for statistical comparison but was limited to triplicate scans, while more repeats are theoretically needed due to technical considerations. The relative coefficient of variation agreement between datasets #1 for a 12 μ m voxel size and dataset #2 implied that these findings were not a result of random variation in the condition of the instruments. In general, BV/TV and Tb.Sp appeared to be the most robust measurements, showing highest intra-system reproducibility (CVs were 0.5 or lower) in at least two of the systems, Scanco and Skyscan. Tb.N and Tb.Sp presented the biggest discrepancies in intra-system reproducibility among systems, where coefficients of variation varied up to 8-fold (Tb.N) or 12-fold (Tb.Sp). Indirect vs. direct measurement of Tb.N may be a factor for the observed difference in CVs between the Scanco and the GE or the Skyscan system (7-fold lower and 4-fold lower, respectively). The overall reproducibility from both datasets examined was higher than previously reported for analyses of rodent cancellous bone [27,35], with measured CVs being lower than the values reported using similar systems. For example, CVs for Tb.Sp measurements varied by a factor of 6 or more between the earlier results and our results. The lower CVs in our study most probably reflect the analysis of identical volumes of interest across the triplicate scans. Our focus was the system-related reproducibility, while earlier studies examined the overall reproducibility of microCT as a method. CVs were previously shown equivalent across systems [27], while we found significant inter-system differences. Lack of equivalent scan settings (Table 1) may contribute to this effect, although the effective radiation dose in the GE eXplore system, which showed the highest CVs, was actually higher at 6 μ m and 12 μ m scans than this in the other two systems. We chose to use typical conditions suggested by the respective manufacturer, rather than constant settings

across systems to optimize performance for each system's hardware and geometry. Our results might be different if the scan settings were varied, but this broader analysis was beyond the scope of our study.

An important finding for two of three systems was the absence of a clear correlation between higher reproducibility and smaller voxel size, which may be specific to the size of the structures examined. Although a wide range of voxel sizes has been used for microCT scanning, the prevailing view is that lower sizes provide higher image quality and more reliable measurements [9,23-24]. At the same time, measurement reproducibility is critical to the statistical robustness of the analysis. Given the artifacts described at smaller voxel sizes [9,30,36], measurements from 12 μ m scans would be expected to have lower reproducibility compared to data from 8 μ m scans. However, for two of three systems BS/BV and Tb.Th measurements were less variable at 12 μ m than at 8 μ m. Explanations include possible increased noise, and more pronounced penumbral blurring with decreased voxel size [37-38]. For voxel sizes close to the limit of the systems, a de facto compromise exists between accuracy (defined as the true measurements' value) and reproducibility (consistency during repeated analyses). Finally, regarding reproducibility, performance of scanners is less critical when group or treatment differences in measurements are large relative to the variability obtained from repeated scans of the same object.

In conclusion, we suggest the following need to be recognized when analyzing mouse trabecular bone by microCT. First, absolute values cannot be compared across microCT systems for all measurements, for at least the range of structure sizes examined here. Therefore, comparisons to the literature should be made with care. Second, a range of voxel sizes and associated conditions must be tested before selecting the settings to image structures close to the system resolution, such as mouse cancellous bone. Changes in voxel size near the limit of the system potentially create large differences in measured values depending on manufacturer's hardware and reconstruction method, although the effective resolution may not be changing as much as the voxel size. As a result, a smaller voxel size does not always improve the reproducibility of the results. Finally, in all cases the choice of scan parameters must be based on the particular outcome measurements relevant to the research question of interest.

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References

- [1]. Ciarelli TE, Fyhrie DP, Schaffler MB, Goldstein SA. Variations in three-dimensional cancellous bone architecture of the proximal femur in female hip fractures and in controls. *J. Bone Miner. Res.* 2000; 15:32–40. [PubMed: 10646112]
- [2]. Hildebrand T, Laib A, Müller R, Dequeker J, Rüegsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: microstructural data from spine, femur, iliac crest, and calcaneus. *J. Bone Miner. Res.* 1999; 14:1167–1177. [PubMed: 10404017]
- [3]. Kinney JH, Ladd AJ. The relationship between three-dimensional connectivity and the elastic properties of trabecular bone. *J. Bone Miner. Res.* 1998; 13:839–845. [PubMed: 9610748]
- [4]. Kinney JH, Lane NE, Haupt DL. In vivo, three-dimensional microscopy of trabecular bone. *J. Bone Miner. Res.* 1995; 10:264–270. [PubMed: 7754806]

- [5]. Chappard D, Retaillieu-Gaborit N, Legrand E, Basle MF, Audran M. Comparison insight bone measurements by histomorphometry and microCT. *J. Bone Miner. Res.* 2005; 20:1177–1184. [PubMed: 15940370]
- [6]. Chen P, Miller PD, Recker R, Resch H, Rana A, Pavo I, Sipos AA. Increases in BMD correlate with improvements in bone microarchitecture with teriparatide treatment in postmenopausal women with osteoporosis. *J. Bone Miner. Res.* 2007; 22:1173–1180. [PubMed: 17451369]
- [7]. Dai R, Ma Y, Sheng Z, Jin Y, Zhang Y, Fang L, Fan H, Liao E. Effects of genistein on vertebral trabecular bone microstructure, bone mineral density, microcracks, osteocyte density, and bone strength in ovariectomized rats. *J. Bone Miner. Metab.* 2008; 26:342–349. [PubMed: 18600400]
- [8]. Liu J, Xu K, Wen G, Guo H, Li S, Wu X, Dai R, Sheng Z, Liao E. Comparison of the effects of genistein and zoledronic acid on the bone loss in OPG-deficient mice. *Bone.* 2008; 42:950–959. [PubMed: 18337202]
- [9]. Kim DG, Christopherson GT, Dong XN, Fyhrie DP, Yeni YN. The effect of microcomputed tomography scanning and reconstruction voxel size on the accuracy of stereological measurements in human cancellous bone. *Bone.* 2004; 35:1375–1382. [PubMed: 15589219]
- [10]. Pajamäki I, Sievänen H, Kannus P, Jokihaara J, Vuohelainen T, Järvinen TL. Skeletal effects of estrogen and mechanical loading are structurally distinct. *Bone.* 2008; 43:748–757. [PubMed: 18620089]
- [11]. Guldberg RE, Caldwell NJ, Guo XE, Goulet RW, Hollister SJ, Goldstein SA. Mechanical stimulation of tissue repair in the hydraulic bone chamber. *J. Bone Miner. Res.* 1997; 12:1295–302. [PubMed: 9258761]
- [12]. Robling AG, Hinant FM, Burr DB, Turner CH. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *J. Bone Miner. Res.* 2002; 17:1545–1554. [PubMed: 12162508]
- [13]. van der Meulen MCH, Morgan TG, Yang X, Baldini TH, Myers ER, Wright TM, Bostrom MPG. Cancellous bone adaptation to in vivo loading in a rabbit model. *Bone.* 2006; 38:871–877. [PubMed: 16431171]
- [14]. Gardner MJ, van der Meulen MCH, Demetrakopoulos D, Wright TM, Myers ER, Bostrom MPG. In vivo cyclic axial compression affects bone healing in the mouse tibia. *J. Orthop. Res.* 2006; 24:1679–1686. [PubMed: 16788988]
- [15]. Follet H, Peyrin F, Vidal-Salle E, Bonnassie A, Rumelhart C, Meunier PJ. Intrinsic mechanical properties of trabecular calcaneus determined by finite-element models using 3D synchrotron microtomography. *J. Biomech.* 2007; 40:2174–2183. [PubMed: 17196599]
- [16]. Gardner MJ, Ricciardi BF, Wright TM, Bostrom MPG, van der Meulen MCH. Pause insertions during cyclic in vivo loading affect bone healing. *Clin. Orthop. Relat. Res.* 2008; 466:1232–1238. [PubMed: 18273676]
- [17]. Lam H, Qin YX. The effects of frequency-dependent dynamic muscle stimulation on inhibition of trabecular bone loss in a disuse model. *Bone.* 2008; 43:1093–1100. [PubMed: 18757047]
- [18]. Buxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents Using Micro-Computed Tomography. *J. Bone Miner. Res.* 2010; 25:1468–1486. [PubMed: 20533309]
- [19]. Bagi CM, Hanson N, Andresen C, Pero R, Lariviere R, Turner CH, Laib A. The use of micro-CT to evaluate cortical bone geometry and strength in nude rats: correlation with mechanical testing, pQCT and DXA. *Bone.* 2006; 38:136–144. [PubMed: 16301011]
- [20]. Kothari M, Keaveny TM, Lin JC, Newitt DC, Genant HK, Majumdar S. Impact of spatial resolution on the prediction of trabecular architecture parameters. *Bone.* 1998; 22:437–443. [PubMed: 9600776]
- [21]. Chappard C, Marchadier A, Benhamou CL. Side-to-side and within-side variability of 3D bone microarchitecture by conventional micro-computed tomography of paired iliac crest biopsies. *Bone.* 2008; 43:203–208. [PubMed: 18439891]
- [22]. Pothuau L, Carceller P, Hans D. Correlations between grey-level variations in 2D projection images (TBS) and 3D microarchitecture: applications in the study of human trabecular bone microarchitecture. *Bone.* 2008; 42:775–787. [PubMed: 18234577]

- [23]. Müller R, Rüeegsegger P. Analysis of mechanical properties of cancellous bone under conditions of simulated bone atrophy. *J. Biomech.* 1996; 29:1053–1060. [PubMed: 8817372]
- [24]. Müller R, Van Campenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T, Rüeegsegger P. Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. *Bone.* 1998; 23:59–66. [PubMed: 9662131]
- [25]. Nazarian A, Snyder BD, Zurakowski D, Müller R. Quantitative micro-computed tomography: a non-invasive method to assess equivalent bone mineral density. *Bone.* 2008; 43:302–11. [PubMed: 18539557]
- [26]. Jiang Y, Zhao J, White DL, Genant HK. Micro CT and Micro MR imaging of 3D architecture of animal skeleton. *J. Musculoskelet. Neuronal Interact.* 2000; 1:45–51. [PubMed: 15758525]
- [27]. Bonnet N, Laroche N, Vico L, Dolleens E, Courteix D, Benhamou CL. Assessment of trabecular bone microarchitecture by two different x-ray microcomputed tomographs: A comparative study using Skyscan and Scanco devices. *Med. Phys.* 2009; 36:1286–1297. [PubMed: 19472637]
- [28]. Hara T, Tanck E, Homminga J, Huiskes R. The influence of microcomputed tomography threshold variations on the assessment of structural and mechanical trabecular bone properties. *Bone.* 2002; 31:107–109. [PubMed: 12110421]
- [29]. Kuhn J, Goldstein SA, Feldkamp LA, Goulet RW, Jesion G. Evaluation of a microcomputed tomography system to study trabecular bone structure. *J. Orthop. Res.* 1990; 8:833–842. [PubMed: 2213340]
- [30]. Peyrin F, Salome M, Cloetens P, Laval-Jeantet AM, Ritman E, Rüeegsegger P. Micro-CT examinations of trabecular bone samples at different resolutions: 14, 7 and 2 micron level. *Technol. Health Care.* 1998; 6:391–301. [PubMed: 10100941]
- [31]. Beamer WG, Donahue LR, Rosen CJ, Baylink DJ. Genetic variability in adult bone density among inbred strains of mice. *Bone.* 1996; 18:397–403. [PubMed: 8739896]
- [32]. Schneider P, Stauber M, Voide R, Stampanoni M, Donahue LR, Muller R. Ultrastructural properties in cortical bone vary greatly in two inbred strains of mice as assessed by synchrotron light based micro-CT and nano-CT. *J. Bone Miner. Res.* 2007; 22:1557–1570. [PubMed: 17605631]
- [33]. Remy E, Thiel E. Medial axis for chamfer distances: computing look-up tables and neighbourhoods in 2D or 3D. *Pattern Recognition Letters.* 2002; 23:649–661.
- [34]. Rüeegsegger P, Hildebrand T. A new method for the model-independent assessment of thickness in three-dimensional images. *J. Microsc.* 1997; 185:67–75.
- [35]. Kohler T, Beyeler M, Webster D, Müller R. Compartmental bone morphometry in the mouse femur: reproducibility and resolution dependence of microtomographic measurements. *Calcif Tiss. Int.* 2005; 77:281–290.
- [36]. Chappard C, Basillais A, Benhamou L, Bonassie A, Brunet-Imbault B, Bonnet N, Peyrin F. Comparison of synchrotron radiation and conventional x-ray microcomputed tomography for assessing trabecular bone microarchitecture of human femoral heads. *Med. Phys.* 2006; 33:3568–3577. [PubMed: 17022253]
- [37]. Feldkamp LA, Davis LC, Kress JW. Practical cone-beam algorithm. *J. Opt. Soc. Am.* 1984; 1:612–619.
- [38]. Flannery BP, Deckman HW, Roberge WG, D'Amico KL. Three-dimensional X-ray microtomography. *Science.* 1987; 237:1439–1444. [PubMed: 17816787]

Research Highlights

- > Agreement of cancellous bone measurements by microComputed Tomography systems.
- > 3D measurements significantly different between systems, depending on the measurement.
- > Dependence of the measurements on voxel spacing and reproducibility also different.
- > Concluded that 3D measurements cannot be directly compared across systems.
- > Choice of voxel spacing should be made also based on system.

Fig. 1. Schematic of region of interest (ROI) location on one analyzed femur. Most distal vertical projection (slice) in 8µm and at 12µm scans examined with all three systems is shown. ROI contour indicated on the slices. Note that the image gray scales cannot be directly compared between the systems, due to different grayscale values and range settings.

Fig. 2. Distribution of mineral densities in the same femur in 8 μ m scans. Upper: distribution of mineral densities from the whole bone and trabecular ROIs from a GE eXplore Locus scan (trabecular ROI distribution shown also on the lower graph). Lower: distribution of mineral densities in trabecular ROIs from scans in the three systems. Positions of normal, low and high global thresholds and of background and bone peaks are noted.

Fig. 3.
Mean and standard deviation of measurements as obtained by the three systems. Results from global and individual thresholding modes.

Fig. 4.
Relative changes in values between 8μm and 12μm measurements $[(\text{value at } 8\mu\text{m}) - (\text{value at } 12\mu\text{m})] / (\text{value at } 8\mu\text{m})$. Results from global thresholding.

Fig. 5. Coefficients of variation (CV) of measurements from triplicate 8μm and 12μm scans of the 3femur sample (dataset#1-left panel) and 12μm scans of the 6 femur sample (dataset#2-right panel). Results from global thresholding.

Table 1

Systems and scan conditions used.

GE explore Locus SP		Scanco μ CT35		Skyscan 1172	
13um	8um	12um	7um	12um	8um
2 x 2	1 x 1	2 x 2	1 x 1	2 x 2	2 x 2
80	80	55	55	59	59
80	80	145	145	127	127
200°	200°	200°	200°	195°	195°
0.4°	0.4°	0.36°	0.36°	0.4°	0.4°
2,000	4,000	400	400	590	590
2	5	2	2	5	5
1h	4h	2.1 hr	3.8hr	33 min	33 min
1.0 ml polypropylene tube	manufacturer's acrylic holder	manufacturer's acrylic holder	manufacturer's acrylic holder	15ml polypropylene tube	15ml polypropylene tube
0.5 mm Al	0.5 mm Al	0.5 mm Al	0.5 mm Al	0.5 mm Al	0.5 mm Al
Pre-3D reconstruction/ based on phantom air-water-bone absorption coefficients	Automatic/ conditions-specific built-in algorithms	Post-3D reconstruction/ based on phantom water-bone absorption coefficients	filter	Mineral density calibration	

Table 2

3D morphometry measurements. Principles of integration by system.

Processing software	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	REFERENCE
GE Micro-View ABA 2.2	directly measured / marching cubes method/	directly measured / marching cubes method/	directly measured / as mean chord length using chord length distribution method	indirectly measured: $= (BV/TV)/Tb.Sp \times Tb.Th$	directly measured/ as mean pore thickness using chord length distribution method	[22]
Scanco uCT processing software/ HP DECWindows Motif 1.6.	directly measured/ tetrahedrons in volume of BS triangulated surface	directly measured/ marching cubes method (BS)	directly measured/ distance transformation- maximum fitted spheres method	directly measured/ distance transformation- distance between midaxes from maximum fitted spheres method	directly measured/ distance transformation- maximum fitted spheres method	[2-34]
Skyscan CT Analyzer 1.7.5.1	directly measured / marching cubes method/	directly measured / marching cubes method	directly measured/ maximum fitted spheres method	indirectly measured: $= (BV/TV)/Tb.Th$	directly measured/ maximum fitted spheres method	[33]

Table 3

Averages of measurements from triplicate scans using low and high global thresholds.

GE explore Locus SP		Scanco μ CT35		Skyscan1172		
8 μ m	12 μ m	8 μ m	12 μ m	8 μ m	12 μ m	
0.079-0.088	0.101-0.116	0.092-0.107	0.087-0.112	0.099-0.112	0.088-0.114	BV/TV (fraction)
91.96-95.20	69.39-73.59	69.04-74.94	59.75-66.62	78.23-83.46	71.02-78.78	BS/BV (1/mm)
0.0212-0.0219	0.0273-0.0289	0.0365-0.394	0.0406-0.0448	0.0496-0.0518	0.0593-0.0626	Tb.Th (mm)
3.71-3.98	3.67-3.95	3.77-3.83	3.64-3.82	2.00-2.16	1.48-1.81	Tb.N (1/mm)
0.236-0.256	0.230-0.252	0.258-0.264	0.266-0.282	0.259-0.262	0.273-0.288	Tb.Sp (mm)