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Micro-CT examination of human bone: from biopsies towards the entire organ

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Summary. Micro-CT systems are available that facilitate *ex vivo* examinations of human specimens as big as entire vertebrae, with spatial resolutions in the 10-micrometer range. This opens a new way for looking at entire bones in 3D. Accurate description of the internal microarchitecture of the entire organ can be obtained, at spatial resolutions previously achievable only on excised biopsies. These high resolution scans produce large datasets and come with costs and benefits, which have to be considered in the successful planning of an experiment. The aim of this paper is to present examples of human vertebrae scanned at high resolution (17 μm/pixel), allowing the visualization and quantification of the microarchitecture, and to discuss some aspects of using high resolution scans of such large specimens. The datasets were down-sampled to 34 μm and 68 μm pixel size, and their morphometric parameters compared to those obtained at 17 μm pixel size, in relation to data size and calculation time.

Key words: vertebra, bones, micro-CT, spatial resolution, biopsy.

Riassunto *(Esame dell'osso umano mediante la microtomografia computerizzata: dalle biopsie all'organo intero)*. Oggi esistono sistemi di microtomografia computerizzata in grado di consentire esami *ex vivo* di campioni umani grandi come intere vertebre, con una risoluzione spaziale che rientra nella gamma dei 10 micrometri. Queste tecniche permettono l'apertura verso un nuovo modo di analizzare l'osso in 3D e consentono di ottenere una descrizione accurata della micro architettura interna di un organo intero sfruttando risoluzioni spaziali prima ottenibili solo per mezzo di biopsie escisse. Tali scansioni ad alta risoluzione producono una grande quantità di dati e sono accompagnate da costi e benefici che devono essere considerati nella pianificazione di un esperimento. Lo scopo di questo lavoro è di presentare esempi di vertebre umane scannerizzate ad alta risoluzione (17 μm/pixel), di quantificarne e visualizzarne la micro architettura e di discutere alcuni aspetti legati all'utilizzo di scansioni ad alta risoluzione per campioni così grandi. I *datasets* sono stati ricampionati a 34 μm/pixel e 68 μm/pixel ed i loro parametri morfometrici sono stati confrontati con quelli ricampionati a 17 μm/pixel, e discussi in relazione alla dimensione dei dati ed al tempo di calcolo.

Parole chiave: vertebre, ossa, micro-CT, risoluzione spaziale, biopsia.

INTRODUCTION

Biomedical imaging is seeing a continuous increase in its applications. This is mainly due to the advances in technologies, which facilitate investigations at increasingly higher spatial resolution, thanks to developments in sensors, electronics, micro-mechanics, faster computers, bigger hard drives, more memory, optimized software; and this list is far from being exhaustive. The advent of X-ray microtomography (micro-CT) is a breakthrough in terms of non-destructive imaging, due to its capability of giving a qualitative and quantitative characterisation of the examined sample in two and in three dimensions in the micrometer range. One of the main fields of applications of micro-CT is in the investigation of bone structures. Bone is a hierarchical material, for which depending on the level of investigation, at the organ level (500 μm), structural level (50 μm), tissue level (5 μm) or cellular level (0.5 μm), the corresponding spatial resolution might be used [1]. Micro-CT images contain information about material and structure of the examined sample that can be used in combination with other examination techniques, such as mechanical testing or finite element modelling, for the investigation of the behaviour of the specimen under load, in relation to its composition and structure [2-5].

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Spatial resolution is one of the determinants for accurate quantification of bone microarchitecture [6-9]. For simplicity, in the remainder of this manuscript the terms "spatial resolution" and "pixel size" will be used interchangeably, despite not being synonymous [10]. The pixel size of the projection image used during scanning and the external dimensions of the scanned specimen are linked together, in particular in a cone-beam scanning geometry. The first micro-CT systems using commercially available X-ray sources were *in vitro* systems, in which the specimen was placed on a rotating sample holder, with a fixed X-ray source-detector system [11, 12]. These systems were capable of imaging bone cubes of 8 mm side, at a pixel size 50 μm [11, 13]. In the following years systems with increased spatial resolution became available, and 10-20 μm pixel size became a typical setting for micro-CT characterization of the microarchitecture of human bone samples, validated with 2D histology and 3D physical phantoms [14-16]. However, despite the increase in spatial resolution, for almost 2 decades the typical specimen dimensions were in the range of about 10 mm in cross-sectional length and up to 20 mm in height, in part also due to design limitations of the systems (among these, the detector and CCD camera size), and this is still the typical specimen size for many of the desktop *in vitro* scanners used today. This means that a core (biopsy) has to be physically extracted from the bone to be examined. Developments in desktop micro-CT have made *in vitro* systems available capable of scanning specimens with diameters two or three times larger as in the past, fully contained in the field of view, with resolution in the 5-20 μm range. Similarly, custom made systems at the synchrotron have been shown to be capable of scanning large specimens. An intact human femur (which has typical lengths up to 50 cm) was placed on the sample holder, and the proximal part (13 cm wide) imaged at high resolution (22 μm pixel size) [17].

Desktop *in vivo* micro-CT systems are available, for *in vivo* imaging of small animals, such as rats and mice [18-21]. The source-detector system rotates around the animal, similar to a clinical scanner for humans. The pixel size in these scanners can be as small as 9 μm, maintaining a large field of view (*eg.*, 68 mm diameter, scanning length up to 100 mm and more). These systems are also used for *ex vivo* or *in vitro* purposes; due to their gantry design, these are capable of fitting whole excised human bones in their specimen chamber, for example human vertebral bodies, for scans at high resolution [22-24]. For a review of micro-CT scanners available on the market and their capabilities, please see the work by Stock *et al*. [10], or visit the manufacturers' websites.

Thus, nowadays micro-CT systems can facilitate scans of entire excised human bones as large as human vertebrae, totally contained in the field of view, with resolutions in the 10-20 μm pixel range [22, 25, 26], as opposed to previous systems limited at investigating excised bone cores at this resolution. This extraordinary capability now available to researchers, however, comes also at a cost: data acquisition, data processing and data management. Higher resolution and increasing specimen dimensions mean an increasing challenge for operators and the analysis, which cannot be overlooked. For reducing computational costs during the analysis, down-sampling of the datasets might be a viable option, as done in some studies [27, 28].

The aim of this paper is to present examples of human vertebral bodies scanned at high resolution (17 μm pixel size), allowing the visualization and quantification of the microarchitecture, and to discuss aspects of using high resolution scans of such large specimens, in relation to datasize and calculation times. The datasets were downsampled to 34 μm and 68 μm, and their morphometric results compared to those obtained at 17 μm pixel size.

MATERIALS AND METHODS

Specimens

Lumbar vertebral body specimens (L3) from five embalmed cadavers of mean (SD) age at death 83 (7) years were used in this investigation, which are part of an ongoing study, with preparation details explained elsewhere [22]. All samples were free of any serological conditions. Approval to use the specimens for research purposes was granted by the Human Research Ethics Committee at the Royal Adelaide Hospital, South Australia, in accordance with the Declaration of Helsinki, 1975. The micro-CT scanning and analysis procedures have been described elsewhere [22, 26], and are outlined below.

Micro-CT examination

Scanning and reconstruction

Five L3 vertebrae were used for micro-CT scanning. These were dissected from the spine by means of a bandsaw, and the posterior elements were removed from each isolated vertebral body. The micro-CT system used was an *in vivo* animal scanner (Skyscan 1076, *in vivo* micro-CT scanner, Skyscan NV, Kontich, Belgium), as described previously [22, 26]. During scanning, the vertebral body was fixed on a carbon bed, having the specimen axis in common with the rotation axis of the system. Scan settings: source voltage 80 kVp, current 120 μA, rotation step 0.5º, full rotation over 180º, 0.5 mm-thick aluminium filter for beam hardening reduction. The isotropic pixel size was 17.4 μm, exposure time 0.59 seconds, 4 frames averaging. Each vertebra was scanned in three consecutive, automated steps, imaging a third of the specimen's height at each scan step, producing a total of 1266 projection images (422 projections at each scan step). Each projection was 1048×3936 pixels in size (length × side), saved as a 16 bit tiff file (7.9 MB each). The cross-section images were then reconstructed by means of a filtered back-projection algorithm (NRecon software, V 1.4.4, Skyscan, Kontich, Belgium). For each vertebra a stack of up to 2000 cross-sections was reconstructed (1800 on average), corresponding to a maximum reconstructed height of 35 mm (32 mm on average), with an inter-slice distance of 1 pixel (17.4

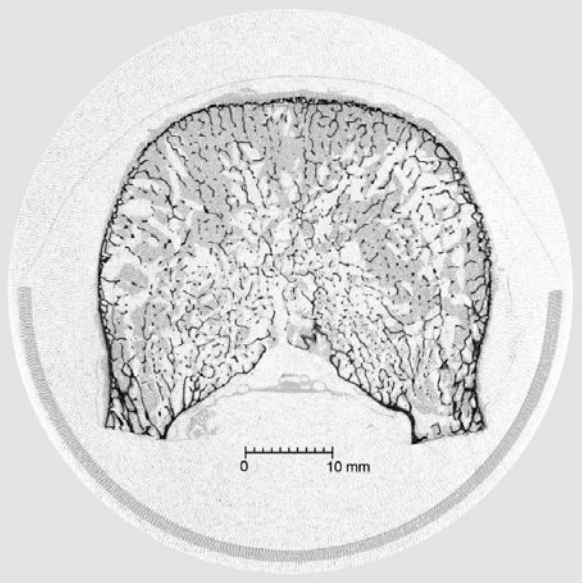

Fig. 1 | *Micro-CT cross-section image of a human vertebral body, at 17.4 µm pixel size (3936 × 3936 pixel, 68.5 × 68.5 mm).*

µm), recreating the full height of the vertebrae. The reconstructed axial cross-section images were of 3936 × 3936 pixels each, with 17.4 µm pixel size (68.5 × 68.5 mm), saved as 8 bit (256 grey levels) BMP files (14.7 MB each file, *Figure 1*). Details regarding dimensions of the cross-sections and size of the reconstructed datasets are summarized in *Table 1*. For each specimen, the total scanning time was approximately 3 hrs, and reconstruction time was on average 30 hrs, respectively. The reconstructions were done on a computer equipped with two dual core Intel Xeon 5160 CPUs, each running at 3.00 GHz, 4 GB memory, OS Windows XP 64 bit. For each vertebra, the generated data size was 9.7 GB for the projection images, and up to 29 GB for the reconstructed cross-section images (26 GB on average).

Morphometric analysis, full resolution (17 µm pixel size)

The volume scanned by micro-CT comprised the entire vertebral body. From the stack of contiguous cross-section images, a volume of interest (VOI) containing only trabecular bone was extracted (software CT Analyser V 1.8.0.5, 64 bit Skyscan, Kontich, Belgium). The VOI was constructed from a stack of regions of interest (ROI) encompassing the whole trabecular bone compartment in each slice, excluding the cortex. It extended through the whole vertebra, starting at a distance of 1 mm from both endplates, and corresponded to an average height of 20 ± 2 mm (1127 ± 104 sections, mean ± SD). For calculation of the morphometric parameters, the cross-section images were segmented (thresholded) into bone and non-bone using a uniform threshold algorithm, with a single threshold value applied to all specimens [22, 26]. The morphometric parameters were calculated both using 2D methods based on slice by slice calculations (model-dependent), as well in 3D using direct model-independent methods as follows (software CT Analyser).

In 2D, for each slice, the trabecular bone volume fraction (BV/TV, %) was calculated as the sum of the pixels marked as bone within the ROI, divided by the total area of the ROI [29]. The trabecular thickness (Tb.Th, µm), trabecular separation (Tb.Sp, µm) and trabecular number (Tb.N, 1/mm), were calculated using bone area and bone perimeter calculations, based on the plate model, as described in detail by the guidelines of the American Society of Bone and Mineral Research [29].

In 3D, the BV/TV was calculated as the voxels segmented as bone divided by the voxels constituting the examined VOI, using the marching cubes method [30]. The Tb.Th and Tb.Sp were calculated using direct model-independent methods using the sphere-fitting algorithm, whereas Tb.N was calculated as Tb.N = (BV/TV)/Tb.Th [31-33]. Also the Structure Model Index (SMI) was calculated, which provides information describing the topology of the examined structure in 3D, with values ranging from 0 (plate like) to 3 (rod-like), and values in between indicating an intermediate structure composed of plates and rods [34].

For each vertebra, the extracted VOI for the calculation of the morphometric parameters was on average 6 GB in file size *(Table 1)*. The morphometric calculations of the parameters in 2D (model-dependent)

Table 1 | *Details of reconstructed micro-CT cross-section images and datasets, at 17 µm/pix and at downsampled pixel sizes*

	17 µm/pix	34 µm/pix	68 µm/pix
Single cross-section image:			
image dimension (mm)	68 x 68	68 x 68	68 x 68
image dimension (pix)	3936 x 3936	1968 x 1968	984 x 984
file size (8-bit bmp)	14.7 MB	3.7 MB	0.9 MB
Dataset of cross-section images, file size:			
Entire vertebra (H= 32 ± 2 mm)	26.4 GB	3.3 GB	423 MB
trabecular VOI* (H= 20 ± 2mm)	6.3 GB	591 MB	102 MB

**the trabecular VOI was constructed from a stack of cross-section images, which were resized to the trabecular ROI boundaries; H = height.*

were straightforward (CT Analyser) (average calculation time per specimen: 29 minutes). The 3D parameters were more calculation intensive, in particular for the model-independent Tb.Th and Tb.Sp (on average, 90 hrs per specimen for all the 3D parameters).

The cortical thickness (Ct.Th, μm) was calculated in 3D, using the model-independent thickness method based on the sphere-fitting algorithm [32]. Similar to the trabecular bone, the full resolution dataset (17 μm pixel size) was a computationally intensive task (calculation time 30 hrs per specimen).

Morphometric analysis on downsampled datasets (34 and 68 μm pixel size)

Next, the reconstructed vertebral datasets were down-sampled by a factor of two (34 μm pixel size), obtaining trabecular VOI datasets of 591 MB in size on average (full cross-sectional image datasets 3.3 GB on average, *Table 1*) (software TConv v 2.1, Skyscan, Kontich, Belgium). Over these trabecular VOI datasets, the morphometric parameters were recalculated, with lower calculation times (on average, 3 min for 2D parameters, 76 min for 3D parameters, per specimen). Also the calculation time for the Ct.Th was decreased (on average, 38 min per specimen).

A further down-sampling by a factor 4 (68 μm pixel size) was performed on the original cross-section images, which per specimen gave VOI datasets of 102 MB on average (entire down-sampled dataset 423 MB on average, *Table 1*). The calculation times over these VOI datasets were much lower (on average, 30 s for 2D parameters, and 4 min 30 s for 3D parameters, per specimen). The calculation times for the Ct.Th were also decreased (31 s per specimen).

Statistics

Descriptive statistics were collated, with average values and standard deviations for each morphometric parameter calculated on the original dataset and on the two down-sampled datasets.

The values of the morphometric parameters were tested for normality by means of a Shapiro-Wilks test. To test for differences in morphometric parameters at varying pixel size, on the normally distributed data a one-way ANOVA for repeated measures was performed, whereas on the not normally distributed data a non-parametric Friedman test was performed. For those morphometric parameters showing statistical significance in these tests, further analysis was done, to test for differences between the data at lower resolutions (38 μm and 68 μm pixel size) and data at highest resolution (17 μm pixel size); a paired t-test was performed for the normally distributed data, whereas a Wilcoxon signed rank test was performed for the not normally distributed data. The significance level was set to $p = 0.05$.

RESULTS

A three-dimensional micro-CT representation of an entire vertebral body, the trabecular bone compartment and the cortical compartment, over which the morphometric parameters were calculated, is shown in *Figure 2A-C*. In *Figure 2 D* a cylindrical sub-volume (10 mm diameter, 20 mm height) of trabecular bone extracted from the vertebra micro-CT dataset is shown, analogous to a virtual bone biopsy.

The values of the morphometric parameters for both the trabecular and cortical bone compartment showed significant changes with varying pixel size, both in 2D and in 3D ($p < 0.05$). *Table 2* reports a summary of the analysis at each pixel size, with average values, standard deviations, and p values for the comparisons with the 17 μm/pixel dataset.

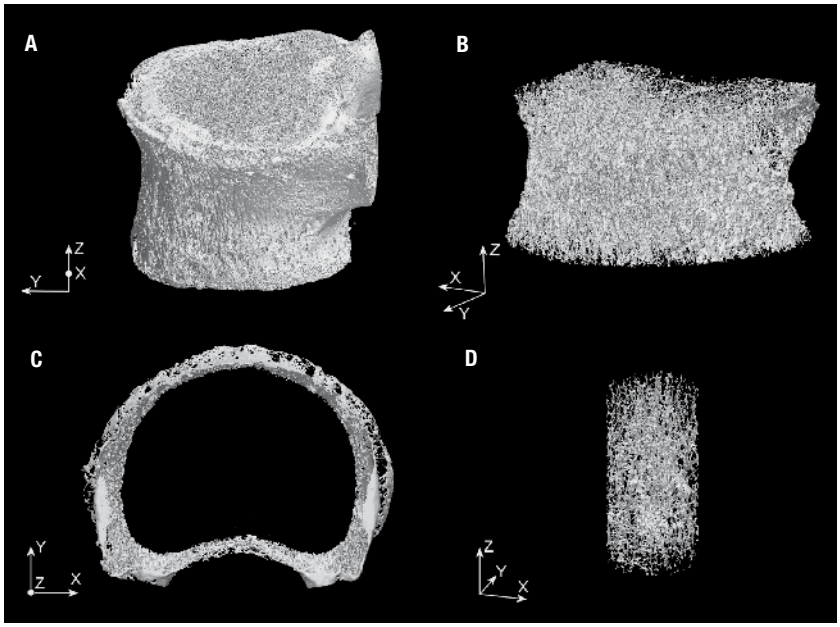

Fig. 2 | *Three-dimensional micro-CT images of a vertebral body scanned at 17.4 μm pixel size. (A) entire vertebral body (B) trabecular bone compartment (VOI) within the endplates, over which the trabecular bone morphometric parameters were calculated (C) cortical bone compartment, superior-inferior view (D) cylindrical subvolume of trabecular bone, 10 mm in diameter, 20 mm in height.*

Table 2 | *Morphometric parameters (average ± SD) calculated at 17 µm/pix and at downsampled pixel sizes*

	17 µm/pix	34 µm/pix	68 µm/pix
2D			
BV/TV (%):	5.2 ± 1.6	5.2 ± 1.6	4.9 ± 1.7 *
Tb.Th (µm):	57 ± 5	61 ± 5#	69 ± 4#
Tb.Sp (µm):	1135 ± 391	1215 ± 410**	1503 ± 535*
Tb.N (1/mm):	0.90 ± 0.24	0.84 ± 0.23**	0.69 ± 0.21**
3D			
BV/TV (%):	5.1 ± 1.6	4.9 ± 1.6**	4.2 ± 1.5 ***
Tb.Th (µm):	133± 2	165 ± 6#	203 ± 11#
Tb.Sp (µm):	1342 ±362	1375 ± 367**	1477 ± 382 *
Tb.N (1/mm):	0.38 ± 0.12	0.30 ± 0.10**	0.21 ± 0.08**
SMI:	1.78 ± 0.32	1.83 ± 0.29*	2.01 ± 0.25**
Ct.Th (µm):	231 ± 26	265 ± 28***	360 ± 35***

Comparison with data at 17 µm/pix
**: p < 0.05 paired t-test*
***: p < 0.01 paired t-test*
****: p < 0.001 paired t-test*
#: p < 0.05 Wilcoxon signed rank test

In *Figure 3* graphs are shown, with the percentage changes calculated for each parameter relative to the 17 µm/pixel dataset.

DISCUSSION

Examination of whole human bones in the 10-micrometer range in three dimensions opens a new method of looking at bones. High resolution imaging of large samples produces a large amount of data, which has to be stored on hard drives, loaded into memory, and for which data managing becomes an important point in a study, in particular if a high number of specimens have to be investigated.

As shown in this study, the micro-CT scan of a whole human vertebra at high resolution (17 µm pixel size) produces an amount of data for which the calculation of morphometric parameters can be challenging, depending also on the available software and hardware capabilities. Depending on the morphometric parameter and on the software implementation, calculation of parameters in 3D (model-independent) can be a major computational task, compared to parameters in 2D (model-dependent).

To speed up the calculations, a down-sampling of the datasets was performed. By considering the higher resolution scan at 17 µm as the most accurate, down-sampling the reconstructed dataset to 34 µm and 68 µm pixel size introduced some expected loss in accuracy in the morphometric parameters (*Table 2, Figure 3*), as previously described in the literature [6, 7]. With increasing pixel size, independently of the methods used for the calculation (either 2D or 3D), the values of BV/TV and Tb.N showed a statistically significant decrease, whereas Tb.Th and Tb.Sp showed significant increase. Similarly, Ct.Th and SMI showed significant increase with increasing pixel size. In 3D, Ct.Th and Tb.Th were the parameters most sensitive to pixel size change (+ 56% and + 53%, respectively, at 68 µm with respect to 17 µm pixel size), whereas SMI and Tb.Sp were the least sensitive (+ 13% and + 10, respectively, at 68 µm with respect to 17 µm pixel size). Regarding the actual values of the morphometric parameters calculated in 2D and 3D, as shown in *Table 2*, differences are noticeable in values between 2D methods and 3D (direct) methods, in particular for Tb.Th. This is likely due to the plate model assumptions in 2D, as discussed extensively in the literature [15, 32]. Indeed, the SMI, with a value of 1.8, indicates that the examined trabecular structure is more rod-like than plate-like.

Nonetheless, down-sampling of the dataset was very effective for speeding up calculations. A change in isotropic voxel size by a factor of 2 changes the whole volume by a factor of 8, with direct effect on hard drive space and on calculation times. Indeed, down-sampling the dataset to 34 µm/pixel (factor 2) reduced the calculation times of the 3D morphometric parameters from several hours (days) to a few hours per vertebra; down-sampling to 68 µm/pixel (factor 4) gave results within a few minutes. Similarly, if the dataset is to be used for finite element analysis, where often a voxel-conversion is done, a pixel size of 17 µm for specimens of this size is beyond the capabilities of common FEA application softwares

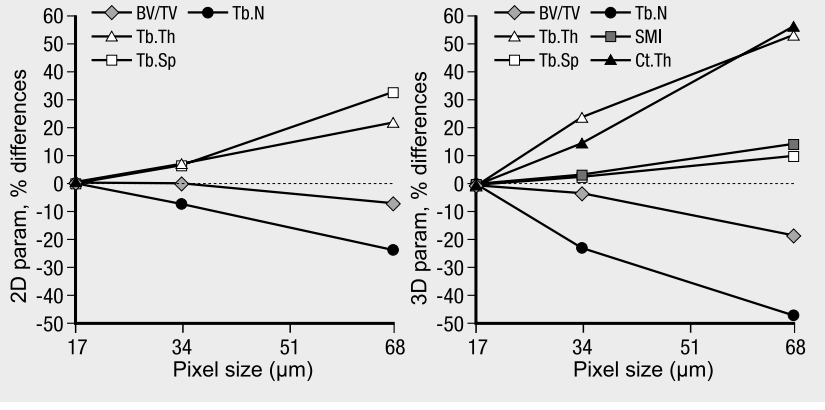

Fig. 3 | *Percentage changes of the morphometric parameters at varying pixel size, with reference to the 17 µm/pixel dataset. Left: 2D parameters. Right: 3D parameters.*

and computers, for which the datasets are typically down-sampled to 60 μm/pixel, and run on supercomputers (*eg.*, 880 processors in parallel, 1800 GB memory [24, 28]). It is clear that with increasing resolution, specimen size and amount of data to be analysed, the capabilities in terms of hardware and software have to be scaled accordingly as well. As a test, the morphometric calculations were repeated on a desktop computer with superior specifications compared to the first one used, equipped with two quad-core Intel Xeon E5640 CPUs, each running at 2.66 GHz, 48 GB memory, OS Windows 7, 64-bit, available at the School of Computer Science, Engineering and Mathematics, Flinders University. The calculation times were further reduced, in particular for the 3D calculations at full resolution (on average, 12 hrs for trabecular bone parameters, 3 hrs for Ct.Th). Down-sampling the datasets did further reduce calculation times (at 34 μm pixel size: 40 min for the trabecular bone parameters and 11 min for Ct.Th. At 68 μm pixel size: 3 min for the trabecular bone parameters and 48 s for Ct.Th). The calculation times for 2D parameters were comparable to those obtained with the first computer used.

On the other hand, if it is likely a major effort, and if then for practical reasons the dataset might possibly be down-sampled for the analysis [27, 28], one might ask whether it is really necessary to use a high resolution scan. The answer depends on the research question, on the minimum acceptable error for the study purpose, and on the resources available in terms of time, operators, and computer capabilities.

Human trabecular bone can have structures as thin as 80-100 μm, especially in anatomical sites such as the vertebrae, and using a resolution capable of resolving these structures is important for accurate description of the specimen [32]. It has been shown that for quantifying trabecular bone microarchitecture, scanning at high resolutions (20 μm pixel size) and following down-sampling to lower resolutions, (*eg*. to 50 μm and 110 μm [8]), gives better outcomes (minor effect of artificial thickening and of loosening of trabecular struts) than direct scanning of the specimens at lower resolution [8]. On down-sampled datasets, it was shown that for a pixel size ranging from 14 μm up to 175 μm, depending on the morphometric parameter, there is a monotonic increase or decrease in values, suggesting that with proper calibration, values of higher resolution parameters can be restored [6]. Similarly, investigations on cortical porosity have shown resolution-dependent outcomes, with down-sampled datasets giving more accurate results compared to datasets obtained from lower resolution scans (investigated pixel size between 5 and 40 μm) [35]. Hence, studies in the literature suggest that high resolution scans are more likely to ensure a higher accuracy in the description of the specimens even after down-sampling, compared to low resolution scans with similar large pixel size.

The data produced by a micro-CT scan of a whole bone gives extraordinary freedom of choice: regions of interest can be extracted *ad hoc*, ranging from the largest possible volume of interest including the whole organ to sub-volumes of various shapes [22, 36], parallelepipeds [24], or cylinders [37, 38]. The latter are equivalent to virtual biopsies, for which if a high resolution scan is done, a subsequent extraction of a smaller VOI produces a data size analogous to a high resolution scan of a cored sample *(Figure 2 D)*. An advantage over real biopsies is the possibility of simply placing the region of interest via software within the specimen, with the choice of later changing its position, size and shape, and as such not having the need of coring the sample. This gives also the possibility of focusing on diverse subregions within the same bone [22, 36, 37]. For example, the cortical and trabecular bone can be analysed separately *(Figure 2B-C)*, and their relative contribution to the overall mechanical behaviour of the bone in combination with mechanical testing or FEA analysed [28, 39], which would be difficult to achieve otherwise.

In the present case, the scan and reconstruction at 17 μm pixel size for entire human vertebral bodies occupied 36 GB on average (9.7 GB scanning, 26.4 GB reconstruction). If a number of 15 or 20 specimens have to be investigated, as is often done in studies, the data size easily enters the TB range for this resolution. Apart from the computational component related to generating experimental results, also the pure data managing itself, that is transferring data and creating backups, can be quite time and resource consuming for datasets as large as these. However, due to continuous developments in technologies, datasets of this size might become normality, depending also on the experiments and research question.

As a further trial, a full scan at 9 μm isotropic pixel size was done on one vertebra (full vertebral height of 32 mm). The data acquisition time was 9 hrs, generating 39 GB in projection images (rotation step 0.5°, 1266 files, each tiff file 7872 x 2096 pixels, 32 MB). Each reconstructed cross-section image was big 60 MB (7872 x 7872 pixels, equal to 68 mm x 68 mm, 9 μm pixel size), saved as 8 bit bmp each, interslice distance 1 pixel. The total cross-section reconstruction time was 7 days (NRecon, Skyscan), generating 213 GB of data (3550 bmp cross-sections, reconstruction of full vertebral height, 32 mm). It can be expected that by using high speed solutions provided by the manufacturer including cluster-reconstruction, the time needed for cross-section reconstruction might substantially be reduced for these very large datasets. Nonetheless, data analysis in 3D for one entire vertebra of this size at this pixel size (9 μm, 213 GB) is a major effort, and performing a study on 15 (or more) entire human bones of this size at this high resolution, might be at the limit of practicality (if not beyond), of most laboratory capabilities today. Again, apart from down-sampling the dataset, this is likely a technological hurdle that might be overcome, depending also on the study purpose.

A limitation of this study is the low sample size. The specimens were obtained from cadavers with

a relatively advanced age at death, and as such are more representative of an aged population subgroup [32]. The analysed vertebrae were harvested from five different cadavers, thus providing some inter-individual diversity in the study. As the aim was to present examples of micro-CT scans of large human specimens as big as entire vertebral bodies, at high resolution, and to discuss aspects related to data size and calculation times, this sample size can be considered adequate for the purpose.

It has also to be acknowledged that clinical CT systems, which compared to micro-CT systems have larger fields of view and pixel sizes in the order of 0.5-1 mm, are increasing in spatial resolution. Although their increase in resolution for *in vivo* scans on humans is limited by the related increase in radiation dose [40], clinical CT systems with a pixel size of 82 μm are available, for scanning peripheral parts of the human body, such as the wrist and ankle. This enables *in vivo* quantification of the bone microarchitecture in patients, in terms of trabecular bone and cortical bone, with a spatial resolution that approximates that of low-resolution micro-CT [41]; these systems are also increasingly used for research purposes *ex vivo* [36, 37].

In conclusion, in having access to micro-CT systems capable of making scans of large volumes such as entire bones at resolutions in the 10-micrometer range, researchers might also consider aspects in relation to the research question, available resources, hard drive space, efforts, and also to financial costs linked to this analysis, in particular if the examination is done by a third party service. For years many micro-CT users wished (and many users still wish) they could scan entire bones, at the highest resolution. Newly developed micro-CT devices indeed open the doors to scans of this type, for which now researchers are facing the challenge of handling and analysing the data, in order to take full advantage of the wealth of high resolution information. Downsampling of the datasets is a viable option; nonetheless, managing and analysing large datasets plays an increasingly important part in a study, in particular if a high number of specimens have to be investigated. The capability of providing microarchitectural data at high resolution, on specimens from the size of biopsies up to entire human bones, confirms micro-CT as an invaluable tool for non-destructive investigations in 3D.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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References

1. Müller R. Hierarchical microimaging of bone structure and function. *Nat Rev Rheumatol* 2009;5(7):373-81.
2. van Rietbergen B. Micro-FE analyses of bone: state of the art. *Adv Exp Med Biol* 2001;496:21-30.
3. Homminga J, McCreadie BR, Weinans H, Huiskes R. The dependence of the elastic properties of osteoporotic cancellous bone on volume fraction and fabric. *J Biomech* 2003;36(10):1461-7.
4. Perilli E, Baleani M, Ohman C, Fognani R, Baruffaldi F, Viceconti M. Dependence of mechanical compressive strength on local variations in microarchitecture in cancellous bone of proximal human femur. *J Biomech* 2008;41(2):438-46.
5. Nazarian A, Müller R. Time-lapsed microstructural imaging of bone failure behavior. *J Biomech* 2004;37(1):55-65.
6. Müller R, Koller B, Hildebrand T, Laib A, Gianolini S, Rüegsegger P. Resolution dependency of microstructural properties of cancellous bone based on three-dimensional mu-tomography. *Technol Health Care* 1996;4(1):113-9.
7. Peyrin R, Salome M, Cloettens P, Laval-Jeantet A, Ritman E, Rüegsegger P. Micro-CT examination of trabecular bone samples at different resolutions: 14, 7, and 2 micron level. *Technol Health Care* 1998;6:391-401.
8. 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(6):1375-82.
9. 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(5): 437-43.
10. Stock SR. *Microcomputed tomography: methodology and applications.* Boca Raton FL, US: CRC Press, Taylor and Francis Group; 2009.
11. Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M. The direct examination of three-dimensional bone architecture *in vitro* by computed tomography. *J Bone Miner Res* 1989;4(1):3-11.
12. Sasov AY. Microtomography. Part 1: Methods and equipment. *J Microsc* 1987;147(2):169-78.
13. Kuhn JL, Goldstein SA, Feldkamp LA, Goulet RW, Jesion G. Evaluation of a microcomputed tomography system to study trabecular bone structure. *J Orthop Res* 1990;8(6):833-42.
14. Müller R, Van Campenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T, Rüegsegger P. Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. *Bone* 1998;23(1):59-66.
15. Chappard D, Retailleau-Gaborit N, Legrand E, Basle MF, Audran M. Comparison insight bone measurements by histomorphometry and microCT. *J Bone Miner Res* 2005;20(7):1177-84.
16. Perilli E, Baruffaldi F, Bisi MC, Cristofolini L, Cappello A. A physical phantom for the calibration of three-dimensional X-ray microtomography examination. *J Microsc* 2006;222(Pt 2):124-34.

17. Baruffaldi F, Bettuzzi M, Bianconi D, Brancaccio R, Cornacchia S, Lanconelli N, Mancini L, Morigi MP, Pasini A, Perilli E, Romani D, Rossi A, Casali F. An Innovative CCD-Based High-Resolution CT System for Analysis of Trabecular Bone Tissue. *IEEE Trans Nucl Sci* 2006;53(5 (Pt. 1)):2584-90.
18. Waarsing JH, Day JS, Verhaar JA, Ederveen AG, Weinans H. Bone loss dynamics result in trabecular alignment in aging and ovariectomized rats. *J Orthop Res*, 2006;24(5):926-35.
19. Boyd SK, Davison P, Müller R, Gasser JA. Monitoring individual morphological changes over time in ovariectomized rats by *in vivo* micro-computed tomography. *Bone* 2006;39(4):854-62. Epub 2006 Jun 6.
20. Brouwers JE, Lambers FM, Gasser JA, van Rietbergen B, Huiskes R. Bone degeneration and recovery after early and late bisphosphonate treatment of ovariectomized wistar rats assessed by *in vivo* micro-computed tomography. *Calcif Tissue Int* 2008;82(3):202-11.
21. Perilli E, Le V, Ma B, Salmon P, Reynolds K, Fazzalari NL. Detecting early bone changes using *in vivo* micro-CT in ovariectomized, zoledronic acid-treated, and sham-operated rats. *Osteoporos Int* 2010;21(8):1371-82.
22. Briggs AM, Perilli E, Parkinson IH, Wrigley TV, Fazzalari NL, Kantor S, Wark JD. Novel assessment of subregional bone mineral density using DXA and pQCT and subregional microarchitecture using micro-CT in whole human vertebrae: applications, methods, and correspondence between technologies. *J Clin Densitom* 2010;13(2):161-74.
23. Wegrzyn J, Roux JP, Arlot ME, Boutroy S, Vilayphiou N, Guyen O, Delmas PD, Chapurlat R, Bouxsein ML. Determinants of the mechanical behavior of human lumbar vertebrae after simulated mild fracture. *J Bone Miner Res* 2011; 26(4):739-46.
24. Fields AJ, Eswaran SK, Jekir MG, Keaveny TM. Role of trabecular microarchitecture in whole-vertebral body biomechanical behavior. *J Bone Miner Res* 2009;24(9):1523-30.
25. Perilli E, Briggs AM, Kantor S, Codrington J, Wark JD, Parkinson IH, Fazzalari NL. Whole vertebral body strength predicted by bone mineral density from DXA and by bone microarchitecture from micro-CT. *Osteoporos Int* 2011;22(Suppl 4):S542.
26. Perilli E, Briggs AM, Kantor S, Codrington J, Wark JD, Parkinson IH, Fazzalari NL. Failure strength of human vertebrae: prediction using bone mineral density measured by DXA and bone volume by micro-CT. *Bone* 2012(in press).
27. Fields AJ, Lee GL, Liu XS, Jekir MG, Guo XE, Keaveny TM. Influence of vertical trabeculae on the compressive strength of the human vertebra. J Bone Miner Res 2011;26(2):263-9.
28. Fields AJ, Lee GL, Keaveny TM. Mechanisms of initial endplate failure in the human vertebral body. *J Biomech* 2010; 43(16):3126-31.
29. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res* 1987;2(6):595-610.
30. Lorensen WE, Cline HE. Marching Cubes: A high resolution 3D surface construction algorithm. *Computer Graphics* 1987:21(4):163-9.
31. Parkinson IH, Fazzalari NL. Interrelationships between structural parameters of cancellous bone reveal accelerated structural change at low bone volume. *J Bone Miner Res* 2003; 18(12):2200-5.
32. 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(7):1167-74.
33. Hildebrand T, Rüegsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. *J Microsc* 1997;185:67-75.
34. Hildebrand T, Rüegsegger P. Quantification of Bone Microarchitecture with the Structure Model Index. *Comput Methods Biomech Biomed Engin* 1997; 1(1):15-23.
35. Cooper D, Turinsky A, Sensen C, Hallgrimsson B. Effect of voxel size on 3D micro-CT analysis of cortical bone porosity. *Calcif Tissue Int* 2007;80(3):211-9.
36. Hulme PA, Boyd SK, Ferguson SJ. Regional variation in vertebral bone morphology and its contribution to vertebral fracture strength. *Bone* 2007; 41(6):946-57.
37. Wegrzyn J, Roux JP, Arlot ME, Boutroy S, Vilayphiou N, Guyen O, Delmas PD, Chapurlat R, Bouxsein ML. Role of trabecular microarchitecture and its heterogeneity parameters in the mechanical behavior of *ex vivo* human L3 vertebrae. *J Bone Miner Res* 2010;25(11):2324-31.
38. Kim DG, Hunt CA, Zauel R, Fyhrie DP, Yeni YN. The effect of regional variations of the trabecular bone properties on the compressive strength of human vertebral bodies. *Ann Biomed Eng* 2007;35(11):1907-13.
39. Roux JP, Wegrzyn J, Arlot ME, Guyen O, Delmas PD, Chapurlat R, Bouxsein ML. Contribution of trabecular and cortical components to biomechanical behavior of human vertebrae: an *ex vivo* study. *J Bone Miner Res* 2010;25(2):356-61.
40. Müller R, Hahn M, Vogel M, Delling G, Rüegsegger P. Morphometric analysis of noninvasively assessed bone biopsies: comparison of high-resolution computed tomography and histologic sections. *Bone* 1996;18(3):215-20.
41. Boutroy S, Bouxsein ML, Munoz F, Delmas PD. *In vivo* assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab* 2005;90(12):6508-15.