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Technical Note

The precision of micro-tomography in bone taphonomic experiments and the importance of registration

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ABSTRACT

Micro-computed tomography (μCT) provides micrometric 3D images and has been used in forensic studies for anthropology pubis measurement or insect description for *post mortem* interval estimation. Studies have suggested using registration, a superimposing images method between a reference and a target. This technique avoids positioning bias and increase the precision of μCT. However, no clear study has reported the precision with μCT analysis before or after registration in a forensic field.

One fresh *post mortem* sample of a human cranial vault was collected. Two successive μCT acquisitions (resolution 10 μm) of it were performed without repositioning. The data from the second acquisition were copied and registered by two trained operators (operators 1 and 2). Operator 1 performed a second registration process after 1 week (operator 1 bis). The images were analysed. The bone volume (BV), bone surface (BS), number of trabeculae (TbN), trabecular thickness (TbTh) and mean trabecular distance (TbSp) were compared before and after registration. The mean (±SD), the coefficient of variation (%CV), and the precision error of the standard deviation absolute value and of the coefficient of variation between operators 1 and 2 (inter-subject variability) and between operator 1 and 1 bis (intra-subject variability) were calculated. We also collected the second phalanx of the 2nd, 3rd and 4th fingers on the hands of a second individual. Two successive scans (resolution 27 μm) were performed without repositioning. A comparison (mean ± SD of BV, BS, TbN, TbTh, TbSp) was made between the first and second scans with and without registration, and an ANOVA repeated measures procedure was performed.

For the vault, we show that after 30 registrations for each operator (1, 2 and 1 bis), the mean and %CV were very close for each variable and between operators. For BV and BS, the difference in the mean value was approximately 0.01 (mm^3 and mm^2, respectively). The precision error was higher in the inter-subject registrations for each variable. The precision error magnitude for all variables was very low (<0.01) in absolute value and of %CV. For the fingers, the difference between the first and second scans may be approximately 50% without registration. We found that the second scan without registration is significantly different for BV ($p = 0.006$), BS ($p = 0.007$), TbN ($p = 0.019$) and TbSp ($p = 0.002$).

Knowing the precision of the device (with and without registration) is important to ensure that the accuracy of the μCT results.

1. Introduction

Micro-computed tomography (μCT) is an imaging tool developed in approximately 1980 [1] that provides micrometric 3D images of small pieces for, e.g., engineering, science, and medical studies. Over time, it has grown into a promising tool. The μCT imaging principles have been summarized by Rutty et al. [2], and good practices have been reported notably by Bouxsein et al. [3]. Resolution is one of the critical points with μCT, but several parameters must be correctly chosen to reduce the artefacts during the scan (frame averaging) and reconstruction (beam hardening and ring artefact correction). Currently, it is a useful device for studies on bone architecture and modifications in medical fields such as osteoporosis and in forensic fields such as fracture patterns

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[2] or skull bevelling in gunshot wounds [4]. This technique shows a high correlation between its results and those collected in histological analyses and morphometric measurements on human and animal bones [5–9]. μCT enables one to study a greater volume of bone faster and more easily than other techniques such as histology and morphometry [3,5–8]. After the acquisition of images with μCT, 2D images can be reconstructed in 3D. Morphometric and architectural analyses can be performed on those 3D reconstructed images [3]. μCT is non-destructive and allows samples to be studied over time, as in taphonomic fields. μCT is reported to be a precise tool with good reproducibility [3,5–8]. However, no clear precision has been reported for each μCT device, especially in research over time or when the μCT is used to compare a before-and-after effect on an object. For this type of research field, μCT may have precision errors due to human manipulations of the sample between μCT acquisitions. A μCT scan may last many hours, and errors can simply occur due to micro-movement in the μCT. It is probable that each device shows at least small differences in precision. It is also probable that over time, each device may show precision errors, as other technical devices do. To highlight and increase the precision of measurement with μCT, previous studies [10–12] and methodological guides [3] suggest using registration. Registration is a method for superimposing images between a reference and a target. The basic principle of registration is to place the target in the same 3D position as the reference image. Without registration, multiple μCT morphometric analyses of a specific area of a single object may provide several differences uniquely due to the position of the object during μCT acquisitions. Previous studies [11,13] have showed that μCT measurement are improved with registration. Registration provides the theoretical capacity to compare the architecture of an object over time with μCT without positioning bias. Theoretically again, it allows the good reproducibility of μCT acquisitions over time [3,11].

In forensic fields, μCT has been used in studies for injury analysis. Thali et al. [14] used it to analyse external and internal stabbing in bone. Fais et al. [15] recently showed the use of μCT to reveal laryngeal fractures in a manual strangulation. Dicken et al. [16] reported the use of μCT to study bloodstain sizes and shapes throughout fabrics. Rutty et al. [2], in 2013, summarized the multiple uses of μCT in forensic in the study of gunshot wounds and the study of tool marks on bone. μCT can also be used to study the pubis bone for age determination in forensic anthropology [2,17]. In forensic odontology, μCT has been used [18,19] to examine the enamel, dentine, and pulp cavity for age at death estimation. Someda et al. [20] used μCT to examined how age estimation could be affected by gender and region with a focus on the mandibular central incisors and explored the relationship between age and age-related changes in the pulp/tooth volume ratio. In paleopathology and anthropology, μCT has been cited with comparable results to histology for the examination of chronic osteomyelitis, syphilis, hyperostosis frontalis interna, hyperparathyroidism, osteomyelosclerosis and healing following trauma [21]. Rutty et al. [2] demonstrated the use of μCT to perform "virtual dissection" of bone matrix and especially the healing stage of rib fracture in child abuse.

In taphonomy, μCT has been used to estimate the *post mortem* interval (PMI). Richards et al. [26] used it for the study of pupae development of blowflies and insect anatomy [22]. Le Garff et al. [23] reported the use of μCT and registration for PMI estimation with the observation the early trabecular human bone modifications after 4 weeks of controlled conservation.

If the precision of μCT measurements has not been significantly explored in other science fields, it has not been explored at all in forensics. However, precision is important when standard observations must be performed. This type of protocol would probably use registration to improve the precision of the measurements as has been recommended [3,10–12]. It would be used in such studies as fracture pattern descriptions or standard bone characterizations for age estimation in anthropology. Estimating the precision of μCT and registration also seems critical when repeated measures must be taken, such as for insect anatomy description for PMI estimation or for bone changes over time.

The aim of the present study is to determine the precision of a μCT device with and without registration on human *post mortem* bone. The second aim is to obtain data about inter- and intra-operator differences when the registration process is performed.

2. Material and methods

2.1. Bone sample

Fresh human bone without conservation treatment was used in this study. We used a cranial vault and fingers from two bodies. To comply with ethical standards and French laws, the bones were obtained from individuals who had made a specific science body donation.

The individuals were selected to be man (to limit the risk of osteoporosis), dead from a natural cause and without any bone, hormonal or cancer pathology (to limit bone modification due to those pathologies).

The vault was cut transversally above the eyebrow arches (1 cm above the temporal suture) from a man dead from a natural cause at 82 years old. The bone was manually defleshed. One sample of the cranial bone was extracted from the vault with a 9-mm-diameter titan trepan Stoma ® in the right parietal area.

The fingers were also taken from another man, also dead from a natural cause at 81 years old. The second phalanxes of the 2nd, 3rd and 4th fingers were taken on the right and the left hands of the body. The bones were manually defleshed.

2.2. μCT parameters

After extraction, the skull sample was scanned two times successively without repositioning with our μCT (Bruker © SkyScan 1172 high resolution μCT, hardware version 6, software version 1.5). Reconstruction and analysis of the scanning were performed after acquisition. The μCT parameters were identical for the two analyses.

The 6 finger samples were also scanned two times successively without repositioning with the same μCT but with different parameters.

After acquisition, a reconstruction was performed using the NRecon© software (version 1.6.9.8). The μCT parameters (acquisition and reconstruction) are summarized in Table 1.

2.3. Methodology of registration

After reconstruction, the first acquisition was named "the reference", and the second was termed "the target". The registration was performed with a standardization protocol. The methodology was as follows: prior to registration, a difference in 3D conformation between the reference and the target (Fig. 1) was observed, then in the registration process, the operator began with a manual registration, which consisted of moving the target into the same 3D location as the reference (Fig. 2). In this process, the reference is white, and the target is black. If a good registration is performed, the result is close to fully grey. Then, an automatic 3D displacement was performed using the Dataviewer® software (version 1.5.1.2) to obtain a precise and standardized superimposition of the two datasets (Fig. 3). The parameters of the automatic