MEDICAL COMPUTED TOMOGRAPHY (CT) ABLE TO IMAGE THE PAST: AN INVESTIGATION OF DIAGNOSTIC ACCURACY AND IMAGE QUALITY

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DECLARATION

I declare that this Thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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ABSTRACT

The use of X-ray imaging in palaeoanthropology has a long history. Both plain X-rays as well as computed tomography have successfully imaged fossils that have been cleaned and prepared from any encasing materials. There has, however, been very little work done using radiological techniques to image fossils still enclosed in surrounding matrix. Most imaging modalities have been applied post preparation of the fossils. This body of work explores the use of medical computed tomography (XCT) in the pre-preparatory phase of fossil discovery in the South African context. Scanning of breccia blocks from the site of Malapa on XCT concluded that the resultant images were of sufficient quality to enable accurate fossil identification and characterization when measured against the standard of manual preparation. Breccia blocks from Malapa were scanned at high and lower energies using micro CT (µXCT) and XCT respectively. Images were analysed for image quality, artifact and certainty of diagnosis. Results show that lower energy images are deemed superior to higher energy images for this particular application. This finding, taken together with the limitations associated with the use of µXCT for the imaging of the large breccia from Malapa, shows that XCT is the better modality for this specific application. Pre-preparatory XCT scanning can focus both preparation and interpretation of findings. The importance of pre-preparatory XCT imaging is demonstrated by the fact that preparatory techniques and protocols need to be modified from traditional methods in order to minimize the risk of contamination of possible biomolecules. Revision is needed of the peri and post excavation treatment of fossil bones to better preserve the potential of genetic heritage of the past and this research demonstrates the role that XCT can play.

None of the research covered by this body of work has been done before on fossil-bearing matrices. This research should significantly change the way fossil discovery, recovery and preparation is done in the South African context and has potential for application in other palaeontological situations.

Dedicated to:

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1. INTRODUCTION

Fossils offer tangible evidence of the past and are important for the study of the prehistory of life on earth. Through fossils, information can be obtained about the anatomy and behaviour of organisms, evolution of life and habitat development. In the South African context, fossils are formed through diagenesis and object replacement by a wide variety of minerals and typically recovered in blocks of concrete-like rock, referred to as breccia (Berger, 2005, Dirks *et al*., 2010). Fossils encased in breccia are usually only known if they are exposed on the surface, leaving their discovery and recovery otherwise largely to chance. The research presented here focused on radiological assessment of fossils encased in breccia from the South African Plio-Pleistocene site of Malapa (Berger *et al*., 2010) in order to ascertain the viability of using readily available medical computed tomography (XCT) to rapidly and efficiently scan and search potential fossil-bearing blocks for material of interest.

While the use of CT to examine individual fossils, which have been prepared from encasing matrix, to elucidate their anatomy and to reconstruct damaged or distorted specimens has become commonplace (Brunet *et al*., 2002, Zollikofer *et al.*, 2002, Zollikofer *et al.*, 2005), the use of CT in the discovery and recovery phase of fossils has remained limited. Widespread use is made of CT imaging, in particular micro-CT (µXCT) machines, for fossil analysis once these fossils have been prepared and cleaned from surrounding matrix (Conroy *et al*., 1998, Falk *et al*., 2005, Wu and Schepartz, 2009, Balzeau *et al*, 2010 Carlson *et al*., 2011, Berger *et al*., 2015), but little use is made of CT to image matrix conglomerates prior to preparation.

Traditionally fossils have been manually prepared from their encasing matrix. This involves either mechanical or chemical extraction, or both, which is often time consuming and potentially damaging to the fossils themselves (Dirks *et al*., 2010, Pickering *et al*., 2018). Furthermore, the search for fossils immediately below the surface (beyond those made visible by the extraction process, by human induced fracturing or by natural erosion), is typically a haphazard affair, reliant upon the skills of the preparator and random chance. It is often very difficult to completely clean or reconstruct fossils without in some way damaging them. Fossils are also often incomplete or filled with calcified matrix compounding the difficulty of preparation (Wu and Schepartz, 2009). These manual methods of preparation are, in addition, very often destructive to the surrounding matrix, and information not recognized during the process is consequently permanently lost. Traditional methods of fossil preparation often severely limit research and due to the fact that many fossils of interest to palaeontologists are exceedingly rare, other methods have been examined to allow better visualization and interpretation, whilst at the same time potentially preserving the fossil material.

2. BACKGROUND

X- rays have been widely used in the human and veterinary medical fields for diagnostic purposes for many decades. Yet over the years, their role in other disciplines has not been as extensively investigated. Palaeoanthropology is one such discipline. Historical attempts at X-ray imaging of fossil-bearing matrix have been reported as producing poor results, thought to be due to the density of the material, inclusions in the matrix and lack of resolution of images produced by the equipment used (Wu and Schepartz, 2009). There has been limited use of X-rays for exploratory investigations of fossiliferous and potential fossil-bearing rocks in the exploratory phase of palaeontology.

Despite film X- rays not being able to produce desired imagery of fossils encased in rock, the attempted use of X-ray technology in palaeontology has a long history.

"…by far the greatest technical advance was made when radiology began to be used in the examination of anthropological and paleontological materials." … "The Roentgenological examination, moreover, has the great advantage in that it permits the investigator to examine bones without destroying them." (Sigerist, 1951).

X-rays have been used in palaeontology since 1896, when X-ray pictures of fossils were first undertaken by Brühl (Bruhl, 1896) in Berlin and Lemoine (Lemoine, 1896) in Paris, just after Röntgen's initial discovery of X-rays (Hohenstein, 2004). The first definitive work on fossil images was published in 1906 by Branco (Hohenstein, 2004). Others such as Jaekel in 1921 and Mauz in 1929 used X- rays intermittently to image fossils, but it was not until 1934 that Lehmann used X- rays extensively to investigate the marine fossils of the Hunsrück slate (Hohenstein, 2004). Relatively good detail of bony and shell material was obtained, but little definition of the visible soft tissue in this well-preserved assemblage was achieved. Wilhelm Stürmer, a chemical physicist and radiologist at Siemens Corporation, combined Lehmann's experience with his own interest in palaeontology and developed new methods of examining the Hunsrück fossils using X-rays (Selden and Nudds, 2012). Consequently, he produced beautiful radiographs of unprepared slates, using soft X-rays (25–40 kilovolts (KV)) and stereoscopic exposures, combined with high-resolution films and image processing - showing some detail of soft tissue not revealed by conventional techniques that Lehmann had used.

However as demonstrated by these studies, conventional X-rays (and conventional X-ray machines) had limitations. A major limitation of conventional X-rays was that the output was a 2-dimensional (2D) image of 3-dimensional (3D) structures, resulting in superimposition of all structures in the path of the X-ray beam (Wu and Schepartz, 2009). Conventional X-rays did not have good soft tissue resolution and thus lacked the ability to provide detailed information about internal structures. Additionally, many different body tissues (and in the case of fossils, mineralized tissues) have similar abilities to absorb X-rays and thus X-ray images were not able to detect difference between these preserved tissues. Conventional X-ray was, in these early days, not an exact science and a large portion of the X-rays emitted were scattered from the subject, producing a less than complete image.

Godfrey Hounsfield invented computed tomography (CT) in 1972 (Hounsfield, 1973). In a medical CT machine, multiple X-rays and an array of detectors rotate around the object being scanned. A series of cross sectional images are created and post processing allows the production of a virtual 3D image of the object. Compared to conventional X-rays, CT provides higher resolution, cross sectional as well as 3D images. Over time, CT capabilities have improved allowing reduction in scan times, better image resolution, simplification and better efficiency in the process (Robb, 1982). CT also allows potential preparation (sometimes referred to as "segmentation") to occur in a virtual environment, thus removing the possibility of physical damage to the fossils, with the exception of exposing them to X-rays. CT additionally has a number of advantages over traditional X-rays. Its greatest benefit perhaps, is that it can distinguish between substances of differing densities better than conventional radiographs. In brief, each fossil varies in composition due to variances in uptake of minerals in organic materials of differing densities and absorptive abilities, thus parameter adjustment on the CT is necessary for each specimen. The parameters are chosen to highlight the density differences between substances of differing densities. Virtual reconstruction then allows missing pieces to be filled in, to complete the whole. All of this in a non- invasive fashion.

In 1984 Glen Conroy applied XCT scanning for the first time to a matrix filled fossil mammalian cranium (Conroy and Vannier, 1984). Later he used CT to scan hominin fossils (Vannier *et al.*, 1985). More detailed studies would follow, with Conroy and others using CT in the analyses of fossil hominin skulls and fossil teeth (Conroy *et al*., 1990, Grine, 1991, Macho and Thackeray, 1992, Schwartz *et* *al.,* 1998). Morphometric studies focusing on such structures as mandibular cross sections (Daegling and Grine, 1991, Schwartz and Conroy, 1996); femora (Ohman *et al*., 1997) and labyrinthine structures (Spoor, 1993, Spoor *et al.,* 1994, Spoor and Zonneveld, 1995) obtained from CT scans were well received, while those on enamel thickness (Grine, 1991) were questioned (Conroy, 1991, Spoor *et al.,* 1993). One of the main reasons for the questioning was the apparent inability of CT to clearly depict tissue interfaces – at the time, the contact region between adjacent tissues tended to present itself as a blurred boundary on CT images, causing difficulty in the positioning of measuring points. On these CT images, a tissue interface was represented by a gradual, rather than a discontinuous, change in CT numbers. This gradual transition of attenuation values across an interface was attributable to the limitations of the CT scanner's spatial resolution, as determined by the X-ray beam. Moreover, experimental studies showed that objects larger than 1 mm could be measured with an error range of +/- 0.1 mm; objects smaller than 1 mm (such as teeth) were however overestimated owing to the limited spatial resolution of the scanner (Spoor, 1993, Spoor *et al.,* 1993, Spoor and Zonneveld, 1995).

Most of the time, in palaeoanthropological applications, CT has been used with considerable success in assessing skulls. Several early hominin cranial capacities have been re-examined using CT technology. In 1990, Conroy et al. (Conroy *et al*., 1990) used XCT scanning to study the *Australopithecus* MLD 37/38 specimen from Makapansgat, and in 1998, they virtually reconstructed the *Australopithecus* Stw 505 cranium using CT data (Conroy *et al*., 1998). CT scanning was also employed in the assessment of cranial capacity in the Taung child, the type specimen of *Australopithecus africanus* from South Africa (Falk and Clarke, 2007). In 2005 the endocranial features of a *Homo erectus* fossil from Indonesia (Mojokerto child) were described with the aid of CT reconstruction (Balzeau *et al.,* 2005) and in 2005, Falk (Falk *et al*., 2005) produced a virtual endocast of a *Homo floresiensis* skull, from which deductions about language processing were made. The endocranial capacities of both *Australopithecus sediba* and *Homo* *naledi* have been successfully calculated from CT data (Carlson *et al*., 2011, Berger *et al*., 2015).

In 2004, a Chinese – French team (Li *et al*, 2004) used XCT to scan and reconstruct the Yunxian 2 *Homo erectus* cranium, but were limited by the resolution of the CT, so the brain endocast of the fossil was not clearly imaged. In 2007, the Liujiang *Homo* fossil cranium was studied with high-resolution industrial CT and clear images of the exterior and interior structures were obtained (Wu *et al*., 2008).

The rapid increase in the quality of technology has thus been consequently followed by its increasing use on important fossils. Other scientists took advantage of developing technologies and used CT scans to examine various fossil bones. The temporal bones of fossils were examined by Wind (Fairdough, 2010), while fossil hominin postcranial elements were CT scanned by Jungers and Minns (Junger and Minns, 1979). Comparatively good imaging of these individually prepared fossils was obtained, but due to the low energies of XCT and the relatively primitive state of early CT technologies at the time, successes were limited to imaging the interiors of fossil specimens that had been prepared partially or completely out of the surrounding matrix (Stewart, 2009, Daniels, 2011). Reduction of image quality by artifact was commonly encountered, especially when high attenuation materials were found in association with low attenuation materials or when the object being scanned had an irregular shape (McLean *et al*., 2001).

Daegling and Grine used CT to examine fossil and modern mandibles (Daegling and Grine, 1991) and Ruff (Ruff, 1994) analyzed successfully, using CT, the cross-sectional properties of a fossil hominin femur, in order to estimate the femoral strength of an early *Homo* juvenile male skeleton from West Turkana, Kenya (the “Nariokotome boy” KNM-WT 15000). Other functional studies of postcranial bones were also conducted and the suspicion of bipedalism in the “Millennium Man” fossils from Kenya was suggested from CT scans of the proximal femur (Galik *et al*., 2004).

As technologies improved, applications of CT data have moved beyond simply anatomical comparisons of variation and morphology and even analysis of ancient health of early hominins has become possible from the use of CT scans. One study, using high resolution CT of the 130 000-year-old Singa fossil calvaria from Sudan showed inner ear abnormalities suggestive of various pathologies (Spoor *et al.*, 1998). Dental pathologies were elucidated from CTs of the maxilla and mandible of a German woman from ca. 6000 BC (Alt and Buitrago-Téllez, 2004). The cranium of the St Césaire 1 Neanderthal was demonstrated to have a healed fracture on 3D reconstructions of the CT images (Zollikofer *et al*., 2002) and in 2005 Ryan and Milner (Ryan and Milner, 2006) reconstructed a chert arrowhead lodged inside a fossil tibia by using high-resolution CT. Post-mortem taphonomic alterations of bone and not pathological processes were proven by CT of the *Homo erectus* cranium from Lantian (Shang *et al*., 2008). Bony pathology has been eloquently elucidated with µXCT, with the demonstration of the earliest hominin evidence for neoplastic disease (Odes *et al*, 2016, Randolph-Quinney *et al.*, 2016, Odes *et al.*, 2017).

As CT imaging has improved due to software improvements, engineering and improvements to equipment, high resolution, modern CT has also been found to be very useful for studying the delicate internal structure of smaller anatomical structures such as the para nasal sinuses, the inner ear and the microanatomy of the teeth. CT analysis of sinuses was done by Manzi *et al*. (Manzi *et al.*, 2001) on the frontal sinus of the Saccopastore 1 Neanderthal skull and Prossinger *et al*. (Prossinger *et al*., 2003) virtually reconstructed the frontal and sphenoid sinus of three Middle Pleistocene crania from Steinheim, Petralona and Kabwe (Broken Hill). Inner ear structures were eloquently demonstrated on 3D reconstructions of CT images of the inner ear of *Australopithecus* and *Homo erectus*, by Spoor *et al*.

(Spoor *et al*., 1994). High-resolution CT techniques can clearly display the thickness of enamel and dentin and allowed Plotino *et al*. (Plotino *et al*., 2006) to reconstruct molar root canals. Other studies successfully used µXCT scanning to compare the enamel thicknesses of hominoid fossils (Grine, 2004, Olejniczak and Grine, 2005).

In the first application of reparative reconstructive CT to an early hominin fossil, Zollikofer *et al*. (Zollikofer *et al.*, 2002) used CT to virtually reconstruct the left side of the braincase of a Neanderthal fossil skull. The success of this initial work resulted in the application of reconstructive CT technology to other fossils, some more difficult in that the quality of preservation was poorer. In 2005, Zollikofer and Ponce de Leon (Zollikofer and Ponce de Leon, 2005) created a digital representation of the highly crushed Toumai cranium using high-resolution computed tomography. Distortion created by severe crushing of the skull was corrected with these virtual images and allowed important deductions about the mode of locomotion to be postulated. Such reconstructive work has become relatively commonplace, although due to the nature of interpretive work, is still very time consuming.

Consequent with the advances in the machinery associated with CT, great advances in the software that is used to post-process data, have been made. While much post processing had to be done by hand, or skilled programmers just a few years ago, today there are a large number of such post - processing programmes available on the commercial market. All of these various computerized 3D visualization software programmes are available for post processing of the data, with differences in their ability to manipulate the data. Most of these programmes allow sophisticated internal as well as external analysis of fossils, so that inferences can be made about functional anatomy, physiology and phylogenetic relationships (Daniels, 2011), the composition, relative density or form of a fossil being scanned.

Recently virtual extraction and reconstruction of very small specimens was demonstrated, when CT imaging and reconstruction of a partially prepared specimen allowed positive identification of an elephant shrew (*Elephantulus sp*.) from the site of Malapa, South Africa (Val *et al*., 2011). This progress indicates that many of the problems associated with scanners only a few years ago, are being overcome, and the technological advances in the software are at least equal in their importance to the many advances being made in the CT devices themselves.

Other than fossils, other types of archaeological artifacts have also been subjected to CT. Egyptian mummies were first scanned as early as 1977. Using 3D techniques, the mummies were virtually "unwrapped" and dissected (Gardner *et al*., 2004), a practice that has now become commonplace. The Dead Sea Scrolls have been examined with CT and dinosaur eggs have been scanned in China by Zhou *et al.* (Zhou *et al.*, 2006), with the intention of examining developing dinosaurs within the egg, thus removing the need to attempt to prepare very fragile and tiny embryonic bones.

Rapid advances in CT in the 21st century, that ran parallel with significant advances in computing technology, as well as software improvements, have thus made high resolution fossil imaging and reconstruction viable due to expanded CT number scales and the use of special image reformatting software that has provided qualitative and quantitative 3D imaging. Additionally, helical CT – introduced in 1989 - is now significantly better than conventional CT, with higher energy capabilities (Badawi-Fayad *et al*., 2005). Thus, the combination of software and hardware advances has offered considerably greater potential for the application of CT in palaeontology.

As has been noted, these advances in technology have made the use of CT technology in the analysis of fossils which have been prepared out from encasing matrix, common place (Balzeau, 2010). As can be seen from the described work, most of the CT work to date has been performed on specimens which have been partially or completely removed from surrounding matrix. However, the application of CT technology to large matrix conglomerates that potentially contains fossils, has lagged behind these many advances in the visualization and study of fossils that have been prepared out from their encasing matrices. Very little work has been done to image, via CT, large fossil- bearing matrix conglomerates fresh out of the field. This has, in part, been due to the demand to apply these new imaging technologies to fossils that have already been prepared, or are currently under study, and also to the fact that many matrices that potentially contain fossils have not been considered suitable for such imaging, based upon earlier non-rigorous and occasional tests. Furthermore, as palaeontologists and palaeontological technicians have not typically been trained in the interpretation of CT images, the perception seems to have existed that it would be difficult or near impossible to identify fossils still encased in anything but minute amounts of rock. Specifically, previous attempts to use CT to image rocks with potential palaeoanthropological interest had resulted in generally poor results and little effort had been made to apply these methods in the early part of the 21st century (Wu and Schepartz, 2009).

2.1 Available scanning technologies

The scanning technologies available for imaging include medical CT (XCT), micro CT (µXCT), synchrotron, neutron microtomography (NXCT) and magnetic resonance imaging (MRI).

XCT machines utilize multiple X-rays and an array of detectors which rotate around the object being scanned. A series of cross sectional images are created and post processing allows the production of a virtual 3D image of the object. X-rays in conventional CT are polychromatic with a wide range of energies – this can lead to artifact production due to the stronger attenuation of X- rays with low energy. Monochromatisation eliminates this artifact problem but is only feasible in systems such as synchrotrons or linear electron accelerators (Vogel, 2005).

XCT is used primarily for diagnostic human imaging and can reach a spatial resolution of 0.5 mm. When used for fossils, XCT has however been perceived to be limited in its capability of providing data on internal structure when fossils are heavily mineralized (Conroy *et al*., 1990, Conroy *et al.*, 1998).

µXCT has penetration capabilities much better than medical scanners due to its wider energy ranges and ability to achieve higher energy beams, which are potentially more useful in the more heavily mineralized specimens. µXCT reaches a resolution of 5-20 µm, but has the disadvantage of the scanning time, at present, being long and the machines are not freely available for use by the scientific community (Weber and Bookstein, 2011). µXCT systems employ the following optimizations (Ketcham and Carlson, 2001):

- Use of higher energy X-rays, which may be more effective at penetrating dense materials.

- Use of smaller X-ray focal spots, providing increased resolution at a cost in X-ray output.

- Use of finer, more densely packed X-ray detectors, which also increases resolution at a cost in detection efficiency.

- Use of longer exposure times, increasing the signal-to-noise ratio to compensate for the loss in signal from the diminished output and efficiency of the source and detectors.

- Rotation of the specimen rather than the source and detector.

A synchrotron uses powerful magnets and radio frequency waves to accelerate negatively charged electrons along a stainless-steel tube, where they reach high speed. As the magnets are turned on and off, electrons get pulled along the ring of tubes. Since the fast-moving electrons emit a continuous spectrum of light, with various wavelengths and strengths, scientists can pick whatever wavelength they need for their experiments e.g. visible light, ultraviolet light or X-rays (Manke, 2009).

Synchrotron-generated X-ray beams provide the following advantages (Tafforeau *et al.*, 2006):

- The option to vary the energy of the radiation enables the investigation of objects with very different absorption coefficients within the same structure.
- The high parallelism of the beam limits imaging artifacts.
- The high beam coherence provides very high image contrast.
- Monochromatisation avoids beam hardening thus reducing this artifact.
- Slice thickness down to 0.3 µm.

However, the main disadvantages of synchrotron scanning are:

- The huge cost for use of the facility.
- The scarcity of venues.
- Excessively long scanning times.

NXCT differs from XCT and µXCT, in that it utilizes neutrons and these can penetrate materials opaque to X-rays. Organic material strongly attenuates these neutron beams. NXCT may thus be appropriate for imaging organically preserved fossils as a complement to XCT or µXCT (Winkler, 2006). NXCT has shown promising results in being able to differentiate otherwise similar dense materials - recent studies documented the use of NXCT to view fossils encased in breccia (Le Roux *et al*., 1997a, Le Roux *et al*., 1997b, Beaudet *et al.*, 2016). However, NXCT may induce hazardous levels of radioactivity in some geological materials, which leaves these imaged samples radioactive and necessitate the samples to be isolated for a long time after the imaging. Additionally, there are currently no functioning NXCT machines in South Africa, where this breccia triage is needed. NXCT necessitates much longer scanning times when compared to XCT and is also limited to smaller fields of view.

MRI maps properties related to the chemical environment of certain elements, rather than mapping radiation attenuation. MRI has been considered to be poorly suited to geological material (Sutton, 2008) and at present does not compete with µXCT or NXCT. MRI machines are not easily accessible to palaeontological researchers, scans are very expensive and scan times long compared to XCT.

For these above reasons and disadvantages of the varying scanning modalities, synchrotron, NXCT and MRI were not considered suitable for the mass screening of breccia from Malapa.

2.2 Effect of radiation on fossils

Fossils may have the potential to contain ancient DNA and whilst the effect of radiation on living tissue is well investigated, little has been done to research the impact radiation may have on ancient DNA (Grieshaber *et al*., 2008). Recent work has shown that irradiation of fossils may have a detrimental effect on ancient DNA when the total surface dose exceeds 200 Gray, so the study recommended using as low a dose as possible when scanning fossils as well as using resolution no higher than necessary to achieve the desired outcome (Immel *et al.*, 2016). The value of 200 Gray is far higher than any dose from a XCT or µXCT scan (8000 times higher than the highest dose for a medical CT scan) (Immel *et al*., 2016).

2.3 Factors that affect quality of XCT image

The quality of a XCT image is affected by several factors viz:

a) Spatial resolution

b) Contrast resolution

c) Artifacts

Enhancing or suppressing any of these characteristics depends upon the imaging interests and it is important to realize that changing XCT parameters such as section thickness, algorithms and field of view (FOV) have a profound effect on the overall quality of the XCT image. XCT image quality is dependent upon balancing these parameters to produce the best possible image for the object being scanned (Reddinger, 1998). XCT parameters can be manipulated to either decrease or eliminate the adverse effects of these characteristics. Generally, there is a trade-off when XCT parameters are manipulated. For example, if a bone reconstruction algorithm is utilized to increase spatial resolution, image noise increases which degrades contrast or soft tissue resolution. Increasing technical factors such as milliamp seconds (mAs) or peak kilovoltage (kVp) decreases image noise but increases dose.

a) Spatial resolution is the ability to resolve, as separate forms, small objects that are very close together. There are a variety of interrelated factors affecting the degree of spatial resolution in a XCT image. These factors are i) matrix size, ii) pixel size, iii) voxel size, iv) field of view (FOV), v) slice thickness, vi) focal spot size, vii) blur and viii) pitch (Seeram, 1994, Reddinger, 1998, Romans, 2012).

i) & ii) & iii) To create an image, the system must segment raw data into tiny sections. A matrix is a grid that is used to break the data into columns and rows of tiny squares. Each square is a pixel – a 2-dimensional unit. When CT slice thickness is factored in, the unit is called a “voxel”- a 3-dimensional unit. The matrix size refers to how many pixels are present in the grid. A 512 matrix will have 512 pixels across the rows and 512 pixels down the columns. The most common matrix sizes used in CT are 256, 512, and 1024 (Reddinger, 1998). The information contained in each pixel is averaged so that one density number (Hounsfield unit (HU)) is assigned to each pixel, therefore small pixels/large voxels produce images with less blurring, better detail and improved spatial resolution. Because an object may not lie entirely within a pixel, the pixel should be half the size of the object, to increase the likelihood of the object being resolved (Reddinger, 1998).

iv) The FOV determines how much of the total raw data available will be used to create an image. Decreasing the FOV will decrease the pixel size and hence improve spatial resolution (Seeram, 1994, Romans, 2012).

v) Spatial resolution is also affected by slice thickness. Slice thickness is the primary factor affecting the degree of volume averaging in the image (Seeram, 1994). A thicker slice will contain more volume averaging. Decreasing the slice thickness affects the resolution in two ways. First, it reduces the amount of tissue averaged together, increasing resolution but second, it will increase the image noise, which if not compensated for, will decrease the resolution (Seeram, 1994).

vi) A small focal spot size will improve resolution in the image but cannot withstand heat as well as a larger focal spot. Therefore, an increase in mAs often necessitates a larger focal spot (Reddinger, 1998, Romans, 2012).

vii) Sharpness is the ability of a system to define an edge. It is measured by the amount of blur in a system (Reddinger, 1998, Romans, 2012). Sources of blur in CT include geometric blur from the focal spot size, detector blur, absorption blur and motion blur - the last not an issue in this study.

viii) Spiral scanning moves the object through the X-ray beam in a continuous manner. This creates a continuous data set that can later be sliced in many ways during the reconstruction phase. The selected pitch value determines how fast the object is moved and how much the X-ray beam is "spread" over the object. The pitch in multi slice spiral CT is defined as the ratio of the table movement over the detector collimation (slice thickness) in the study (Reddinger, 1998, Romans, 2012). The detail that is required, in the slice thickness direction, limits how much the pitch can be increased. Increasing the pitch value can limit the detail that can be achieved in the direction of movement, by increasing the effective width of the beam and the associated blurring.

There are parameters that a CT technologist can manipulate to increase the spatial resolution when scanning. The use a bone, sharp, high frequency algorithm during reconstruction mathematically enhances the edges of structures by diminishing structural blurring. However, the added algorithm produces statistical interference, which results in an increase in image noise. The increase in image noise decreases contrast resolution.

b) Contrast resolution is the ability to differentiate objects with slightly different densities. Factors that affect contrast resolution (Seeram, 1994, Reddinger, 1998, Romans, 2012), and thus the size of the object that is visible, are i) contrast scale, ii) contrast-detail response, iii) receiver operator characteristics (ROC), iv) quantum noise and v) dose.

i) Contrast scale is affected by the window width and the window level. Narrow window widths will improve low-contrast discrimination in the image.

ii) Contrast-detail response informs that for a given technique, the level of contrast that is visible will decrease as the object size decreases. Thus, smaller objects are harder to see than bigger ones.

iii) ROC describe the fact that different observers will look at the same image and evaluate it differently, making assessment subjective.

iv) Quantum noise is the result of too few X-ray photons reaching the detectors. Noise is the portion of a signal that contains no information. Noise is characterized by a grainy appearance of the image. Any factor that limits the number of attenuated photons at the detector will increase image noise. Anatomical structure size, reduction of slice thickness without increasing technical factors, decreasing pixel size and scatter radiation are all factors that contribute to image noise. Smoothing algorithms can help to reduce the visibility of noise by averaging each pixel with its neighbour. Similarly, wide window widths also help disguise noise.

v) Noise and radiation dose are linked; as radiation dose (mAs) increases, image noise is suppressed. As the noise decreases, small low-contrast objects are more visible.

c) XCT artifacts can affect the quality of the image, sometimes to the point of making them diagnostically unusable. An artifact is any distortion or error in the image that is unrelated to the subject being studied (Reddinger, 1998) and falls into 4 categories (Barret and Keat, 2004):

i) Physics-based artifacts result from the physical processes involved in the acquisition of CT data.

ii) Subject-based artifacts are caused by factors such as subject movement or the presence of metallic materials in or on the subject.

iii) Scanner-based artifacts result from imperfections in scanner function.

iv) Helical and multi section technique artifacts are produced by the image reconstruction process.

Design features incorporated into modern XCT scanners minimize some types of artifacts, and some can be partially corrected by the scanner software. However, in many instances, careful positioning and optimum selection of scanning parameters are the most important factors in avoiding XCT artifacts (Barret and Keat, 2004).

The types of artifact encountered are (Barret and Keat, 2004):

- Star artifacts are caused by stationary high attenuating objects such as metal.

- Streak artifacts may be caused by motion.

- Partial voluming is caused by two differently attenuating structures occupying one voxel. This results in the measured attenuation being an average of the two. This appears as "blurring" over sharp edges and can be partially overcome by scanning using thinner slices.

- Ring artifacts are caused by faulty detectors. The malfunctioning detector will falsely produce a ring in an image.

- Beam hardening occurs when there is more attenuation in the centre of the object than around the edge. An X-ray beam is composed of individual photons with a range of energies. As the beam passes through an object, it becomes “harder”, that is to say its mean energy increases, because the lower-energy photons are absorbed more rapidly than the higher-energy photons. Correction is attempted by filtration, calibration correction and software manipulation.

- Geometrical artifacts are caused by the diverging CT slices being wider at the edges or narrower at the centre. This means slices may either overlap at the edge or have a gap between them at the centre. This results in either overlapping or absent information.

- Aliasing artifacts are due to very sharp and high contrast structure boundaries being displayed as a lower contrast series of lines or streaks (Reddinger 1998).

2.4 Visualization of fossils on XCT

The grey levels depicted in a XCT image correspond to the attenuation of the X-ray beam as it passes through each voxel. The attenuation of the X-ray relates to the energy of the X-ray as well as to the density and atomic number of the material being imaged. The XCT machine uses specialized algorithms to reconstruct the distribution of X-ray attenuation in the slice plane and by summating this information from a contiguous series of images, data for a volume can be acquired (Ketcham and Carlson, 2001). Grey scale is the way in which the grey shades in a black and white image are spread. The grey shades are correlated with the digital pixel values of the image, with ranges of pixel values assigned to a certain grey shade. A grey scale will typically contain 256 grey shades (G.E. Healthcare, 2012). One can change the pixel values that are assigned to a grey shade as one looks at the image. In this process, a window width and a window level are defined and by doing this, the grey scale is assigned to a certain range of Hounsfield numbers.

Physiologically, humans only perceive about sixteen levels of grey (Adcock, 2002). The XCT machine will allow one to determine which matrix number should be printed as white and which should be printed as black. The numerical range between the white and black levels establishes the XCT window "width". “Window width” is defined as the range of XCT numbers (in Hounsfield units) included in the grey-scale display of the XCT image, with varying ranges, depending on the type of machine. The centre of that numerical range becomes the window "level". The window width is divided by sixteen to determine the numbers which are included in each individual grey tone.

XCT machines use a 12–bit scale of CT numbers in which 4096 values are possible, whilst newer systems and post processing systems use 16-bit or 64-bit scales which allows values to range from 0 - 65 535 (Ketcham and Carlson, 2001).

Unlike fresh living bone, fossilized bone from Malapa has a different XCT appearance. Fresh bone appears generally white on XCT images with very high, positive Hounsfield unit readings (+700 to +3000 HU), reflecting the calcium content. A Hounsfield unit is the value scale comparing density of varying materials relative to the density of water. Air is given a CT number of -1000 and water is given a value of 0, causing most soft tissues to have values ranging from -100 to +100 and bone to range from +600 to over +2000 (Zatz, 1981).

The process of fossilization involves the dissolving and replacement of the original minerals in the bone with other minerals, as well as often crystal formation within spaces and other alterations to the material. This process results in a heavy, rock-like copy of the original object. The fossil often has the same shape as the original object but is chemically more like a rock. Some of the original hydroxy-apatite (a major bone constituent) remains, although it is saturated with silica (rock), calcium carbonate (lime) or other minerals (Carpenter, 2001). This results in a change in density and thus appearance and Hounsfield unit reading. Fossilized bone examined in this study, typically appears grey-black on XCT with Hounsfield units ranging from +300 to +1500 HU.

Features that were used to help identify possible hominin bones from other animal bones were gross skeletal anatomy (if fossils were complete enough), and cortical thickness – hominins typically having thicker cortical bone that other non–humans (the thickness being relative when compared to the size of the marrow cavity (Croker *et al.,* 2009)).

2.5 The Malapa site

The site of Malapa (site U.W. 88) (Zipfel and Berger, 2009), represents a rich early hominin locality in Africa. Dating to 1.977 +/- 0.002 million years ago (Pickering *et al.*, 2011) it contains remains of several individuals, all attributed to the species *Australopithecus sediba*. These remains are found alongside an abundant, well preserved fauna (Dirks *et al*., 2010, Kuhn *et al.,* 2011). The hominin skeletons of Malapa display critical areas of anatomy that have, in many cases, not been seen in such completeness or lacking distortion, in the entirety of the early hominin fossil record (Berger *et al*., 2010, Carlson *et al*., 2011, Kibii *et al*., 2011, Kivell *et al*., 2011). It is postulated that all of this well-preserved material was accumulated during a seemingly rapid depositional event that occurred over a few days, weeks or months (Dirks *et al*., 2010, Pickering *et al*., 2011).

The site of Malapa was first discovered in 2008, in the dolomitic region of the Cradle of Humankind World Heritage Site, northwest of Johannesburg, South Africa (Berger *et al*., 2010). The locality is recognized as a de-roofed cave of at least 25 x 20 metres, in an area where limited limestone mining had taken place, probably during the late 19th or early 20th century (Dirks *et al*., 2010).

3. PLoS ARTICLE (Smilg and Berger, 2015)

3.1 Discovering hominins – application of medical computed tomography (CT) to fossil-bearing rocks from the site of Malapa, South Africa

Contribution: Smilg: 85% (conducted research and wrote manuscript)

Berger: 15% (original idea, reviewed manuscript, funded research)

In the South African context, computed tomography (CT) has been applied to individually prepared fossils and small rocks containing fossils but has not been utilized on large breccia blocks as a means of discovering fossils, and particularly fossil hominins.

Previous attempts at XCT imaging of rocks from other South African sites for this purpose yielded disappointing results. For this study, 109 fossil-bearing rocks from the site of Malapa were scanned with XCT prior to manual preparation. The resultant images were assessed for accuracy of fossil identification and characterization against the standard of manual preparation. The accurate identification of fossils, including those of early hominins, that were not visible on the surface of individual blocks, is shown to be possible. Knowledge of bony as well as radiological anatomy is deemed essential for accurate interpretation of findings, as is familiarity and experience with digital imaging techniques, their production limitations and pitfalls of post processing manipulation.

By using XCT for breccia triage, the discovery of unexpected fossils is reduced, thus lowering the potential that fossils could be damaged through accidental encounter during routine preparation, or even entirely missed. XCT imaging is shown to be reliable, readily available, cost effective and accurate in finding fossils within matrix conglomerates. Improvements in XCT equipment and in XCT image quality are such that XCT is now a viable imaging modality for this palaeontological application. XCT scanners are shown by this study to be capable of producing images that allow for the accurate identification and often characterization of fossils. The correlation of these predicted findings with the actual findings post manual preparation is sufficiently concordant to change the traditional course of the handling of fossil-bearing blocks.

In order to maximize the use of limited resources and manual preparatory skills as well as to curtail costs, this research suggests that prior to manual preparation, blocks should undergo scanning with XCT scanners and “virtual” assessment of contents should be undertaken by suitably qualified individuals to allow for prioritization of rocks for manual preparation. Information can be extracted without damage to the matrix and hence allows the potential for preservation of these remains for future generations of scientists, ensuring that as technology advances, enough direct physical evidence has been left behind on which to apply new methods of analysis in the future.

RESEARCH ARTICLE

Discovering Hominins - Application of Medical Computed Tomography (CT) to Fossil-Bearing Rocks from the Site of Malapa, South Africa

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Abstract

In the South African context, computed tomography (CT) has been used applied to individually prepared fossils and small rocks containing fossils, but has not been utilized on large breccia blocks as a means of discovering fossils, and particularly fossil hominins. Previous attempts at CT imaging of rocks from other South African sites for this purpose yielded disappointing results. For this study, 109 fossil- bearing rocks from the site of Malapa, South Africa were scanned with medical CT prior to manual preparation. The resultant images were assessed for accuracy of fossil identification and characterization against the standard of manual preparation. The accurate identification of fossils, including those of early hominins, that were not visible on the surface of individual blocks, is shown to be possible. The discovery of unexpected fossils is reduced, thus lowering the potential that fossils could be damaged through accidental encounter during routine preparation, or even entirely missed. This study should significantly change the way fossil discovery, recovery and preparation is done in the South African context and has potential for application in other palaeontological situations. Medical CT imaging is shown to be reliable, readily available, cost effective and accurate in finding fossils within matrix conglomerates. Improvements in CT equipment and in CT image quality are such that medical CT is now a viable imaging modality for this palaeontological application.

Introduction

Fossils offer tangible evidence of the past and are important for the study of the prehistory of life on Earth. They are typically formed through diagenesis and object replacement by a wide variety of minerals and elements [1]. In the Cradle of Humankind (COH) World Heritage site [2], fossils from the Plio-Pleistocene era are usually found in dolomitic limestone caves, encased in hard calcified sediments. These are often referred to as breccias or calcified clastic matrix [1,3]. This breccia encases bones and varies in its hardness and density. Whilst the strength of the rock has protected the fossils, the density of the rock also makes extraction of the fossils from their surrounding matrix difficult.

Traditionally fossils have been manually prepared from their encasing matrix, or prepared using methods involving acetic or other acids. This involves both mechanical or chemical extraction which is often time consuming and potentially damaging to the fossils themselves [3]. Furthermore, the search for fossils immediately below the surface of the rock being prepared (beyond those made visible by the extraction process or by natural erosion), is typically a haphazard affair, traditionally completely reliant upon the skills of the preparator and random chance. It is therefore often very difficult to completely clean or reconstruct fossils without in some way damaging them. Compounding the difficulty of preparation, fossils are also often incomplete or filled with calcified matrix [4]. These manual methods of preparation are, in addition, destructive to the surrounding matrix and information not recognized during the process may be permanently lost. Even advances in preparation using automated techniques suffer these same problems [5]. Traditional methods of fossil preparation often severely limit research and due to the fact that many fossils of interest to palaeontologists are exceedingly rare, other methods have been examined to allow better visualization and interpretation, whilst at the same time potentially preserving the fossil material and associated matrix. Advances in computer technology, software and the quality of X-rays machines have seen an increase in the use of these X-ray based modalities to “virtually” prepare fossils [6]

X-rays have been commonly used for medical diagnosis since their discovery in 1895 by Wilhelm Röntgen. The use of X-rays in palaeontology dates back to 1896, when Brühl [7] in Berlin and Lemoine [8] in Paris first used X- rays to image fossils. Branco [9] produced the first published work on the use of X-rays for fossil imaging in 1906, followed by Jaekel in 1921, Mautz in 1929 and Lehmann in 1934, who investigated the marine fossils of the Hünsruck slate with X-ray images [9]. Wilhelm Stürmer, a chemical physicist and radiologist at Siemens Corporation, combined Lehmann’s experience with his own interest in palaeontology and developed new methods of examining the Hunsrück fossils using X-rays [10]. Consequently he produced detailed radiographs of unprepared slates, using soft X-rays (25–40 KV) and stereoscopic exposures, combined with high-resolution films and image processing. These showed some detail of soft tissue not revealed by the conventional techniques that Lehmann had used.

Historical attempts at X-ray imaging of fossil bearing matrix has typically been reported as producing poor results, thought to be due to the density of the material, inclusions in the matrix and lack of resolution of images produced by the equipment used [4]. A major limitation of conventional X-rays was a 2 dimensional image of 3 dimensional structures, resulting in superimposition of all structures in the path of the X- ray beam [4]. Conventional X- rays did not have good differential tissue resolution and thus lacked the ability to provide detailed information about internal structures. Additionally, in the case of fossils, mineralized tissues have similar abilities to absorb X-rays and thus X-ray images were not able to detect difference between these preserved tissues and between them and the surrounding matrix.

Computed Tomography (CT) was invented in 1972 by Godfrey Hounsfield [11]. Compared to conventional X-rays, CT provides higher resolution and cross sectional as well as 3D images. CT additionally has a number of advantages over traditional X- rays. Its greatest benefit perhaps is that it can distinguish between substances of differing densities better than conventional radiographs.

CT was introduced 43 years ago, but its use for palaeoanthropological applications has still to be fully exploited. In 1991, Grine stated that “*the employment of CT in palaeontology is* *potentially even more problematic because diagenetic factors that may affect the mineralization of fossil teeth can only but add to the factors that can confound the use of CT*" [12].

It however has been recognized that CT was able to acquire interior information non-destructively from irreplaceable fossil specimens [13,14]. In 1984 Conroy applied CT scanning to a mammalian cranium and after that success he used CT to scan hominin fossils [15]. More detailed studies would follow, with Conroy and others using CT in the analysis of fossil hominin skulls and fossil dental enamel thickness amongst others [12,16,17]. Morphometric studies focusing on such structures as mandibular cross sections [18,19]; femora [20] and labyrinthine structures [21–23] obtained from CT scans were well received, while those on enamel thickness [12] were questioned [24,25].

In palaeoanthropological applications, CT has been used mostly to assess skulls.[16,26–30] But other bones have been examined by CT, including temporal bones, mandibles, femurs and other post cranial elements [18,31–37]. As CT imaging has improved due to software improvements and engineering improvements to equipment, high resolution, modern CT has also been found to be very useful for studying the delicate internal structure of smaller anatomical structures such as the para nasal sinuses, the inner ear and the microanatomy of teeth [21,38–42].

Rapid advances in CT in the 21st century, that run parallel with significant advances in computing technology, as well as software improvements, have made high resolution fossil imaging and reconstruction viable due to expanded CT number scales and the use of special image reformatting software that has provided qualitative and quantitative 3D imaging [6]. Additionally, helical CT—introduced in 1989 [27]—is now significantly better than conventional CT, with higher energy (mAs) capabilities [43]. Thus the combination of software and hardware advances has offered considerably greater potential for the application of CT in palaeontology.

These advances in technology have made the use of CT in the analysis of prepared fossils common place [44]. Most of the CT work to date has been performed on prepared or partially prepared specimens. However, the application of CT to matrix that potentially contains fossils has lagged behind these many advances in the visualization and study of prepared fossils. Very little work has been done to image, via CT, large fossil- bearing matrix conglomerates fresh out of the field. This has, in part, been due to the demand to apply these new technologies to fossils that have already been prepared, or are currently under study, and also to the fact that many matrices that potentially contain fossils have not been previously considered suitable for such imaging, based upon earlier non-rigorous and occasional tests. Furthermore, as palaeontologists and palaeontological technicians have not typically been trained in the interpretation of CT images, the perception seems to have existed that it would be difficult or near impossible to identify fossils still encased in anything but minute amounts of rock. Specifically, previous attempts to use CT to image rocks with potential palaeoanthropological interest has resulted in generally poor results and little effort has been made to apply these methods in the 21st century [4].

Advances in CT technologies, combined with the discovery of sites and localities with denser matrix, containing fewer inclusions have, however, shown promising results for the application of CT technologies to unprepared sediments [45]. A study by Bollinger and colleagues [46] describes the use of multi detector CT in locating, identifying and examining fossil remains of 3 crocodilians embedded in hard shale whilst Rahman and colleagues [47] saw the combination of computer science and the study of past life as creating "*an incredibly exciting field*".

In February 2009, a breccia block discovered at Malapa, was found to contain the diaphysis of a humerus (later to be assigned to *A. sediba* MH1). In April 2009, this block was undergoing manual preparation when a portion of a maxilla was uncovered. This maxilla appeared to belong to an early hominid. Due to its potential importance and prior to further preparation, better visualization was sought of what might be hidden from the preparator's view. On 21 April 2009, the first CT scans of the Malapa material were performed. The visualized maxillary bone was in fact part of an entire juvenile cranium (MH1). The quality of visualization obtained from the CT images gave the first hint that the Malapa calcified clastic sediments were particularly suitable to X-ray penetration.

This discovery also laid the groundwork for the present research and a process of scanning of unprepared blocks was begun.

The aim of this research was to determine the viability of medical CT scanning for use in the identification and characterization of fossils within unprepared matrix blocks from the fossil hominin bearing site of Malapa in the Cradle of Humankind World Heritage site, against the gold standard of traditional block preparation using manual techniques to expose fossils. If successful, such methods could prove cost effective and preserve and protect material, while allowing greater success in discovering and recognizing important fossils.

Materials

The site of Malapa lies to the north of Johannesburg, South Africa in an area known as the Cradle of Humankind (COH)—a UNESCO World Heritage Site declared due to its important hominin fossil—bearing localities [2]. In the late 19^{th} and early 20^{th} century, lime miners traversed this area in search of mineable lime. The lime miners test blasted many sites in their search for economically mineable lime, leaving behind many localities that are only slightly damaged by such activities. The site known as "Malapa" is one such area. It represents a deroofed cave that has been exposed by years of erosion [3]. After some initial limited blasting, the miners appear to have abandoned further mining activity. Even this limited mining activity, however, left large rocks strewn across the surface of the site. It is some of these rocks that have been collected from the site and taken to what was then the Institute of Human Evolution (IHE) and is now the Evolutionary Studies Institute (ESI) at the University of the Witwatersrand for analysis and examination in this study. The blocks are variable in size. For many of the blocks, their exact context within the fossil deposit on the site is known and recorded, for others, the exact location of recovery is not known, only their presence within the miners' dumps at the site are known as well as their association with the site. The blocks for scanning were chosen due to the presence of visible bone on the exterior of the blocks or due to their potential to yield fossils as determined by their position on the site. 109 blocks were scanned and analyzed. Each block was assigned a "B" number as well as a "UW88" site number for identification purposes.

A medical CT scanner at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) —Somatom Definition AS 40 from Siemens (Erlangen, Germany)—was used for the scanning of the 109 blocks. For post processing and interrogation of the medical CT scan data, the images were stored on compact disc (CD) and Digital Imaging and Communication in Medicine (DICOM) images were assessed on an Apple MacBook (Mac OS X version 10.5.8) with OsiriX software (version 3.5.1–64 bit). The CT reader is a diagnostic Radiologist, trained in radiological human anatomy and cross sectional imaging.

Methods

Excavations at the site of Malapa governed by an excavation permit as follows:

Issuing Body: South African Heritage Resources Agency (SAHRA)

Permit Holder: Lee R. Berger

Permit ID: 1946

Case ID: 6407

Validity: 15 January 2015–31 January 2018

The fossil blocks are under the custodial care of the Evolutionary Studies Institute (ESI) at the University of the Witwatersrand, Johannesburg, South Africa.

109 blocks from the Malapa site were scanned. Site/specimen numbers (designated by UW 88) and Block numbers (designated by "B" numbers) were assigned to each block. Preliminary visual identification was made on each block, prior to scanning, of any bone visible on the surface. This identification was done by technical staff of the IHE. Radiographers assisted with the production of the CT images.

CT scanning parameters were chosen–Table 1.

During reconstruction of the raw CT data, kernels are used to enhance spatial and contrast resolution. The kernel is a reconstruction parameter affecting image sharpness and noise by applying a specific mathematical algorithm that digitally filters the raw data during reconstruction. The authors experimented with different kernels, visually assessing the image for suitability of fossil identification. It was found that the H70h was overall best for the specimens scanned. This used a high resolution reconstruction kernel producing a sharper image, although greater noise. This kernel was found to improve bone/fossil visualization with edge enhancement and better spatial resolution. Interpretation of the CT images of the 109 blocks was done prior to block preparation and a colour code was assigned to each block to denote the findings—Table 2

Following completed analysis of all 109 CT scans, representative blocks were prepared manually by preparators in the IHE. Due to the costly, time consuming nature of manual preparation, 44 blocks were chosen for this manual preparation, after communication between the scientists and the radiologist. Blocks were chosen using a combination of the colour coding assessment assigned from the CT analysis as well as the deemed importance of each block. The latter used visualized surface findings in combination with the deemed importance of the location at which the block was found on the site. The actual specimen findings following manual preparation of these 44 blocks were documented and correlation between the CT findings and the actual findings was made (Table 3).

Results and Discussion

The surface findings, CT findings and manual preparation findings are tabulated in Table 3 for each block by site/specimen number and block number. The colour code assigned to each block following CT analysis is tabulated. 109 blocks were scanned and 44 manually prepared. The findings are recorded in Table 3.

Unlike fresh living bone, fossilized bone from Malapa has a different CT appearance. Fresh bone appears generally white on CT images with very high, positive Hounsfield unit readings (+700 to +3000 HU), reflecting the calcium content (Fig 1).

Table 1. CT Scanning parameters used for the scanning of fossil breccia.

Matrix size	512 x 512
Field of View (FOV)	Individualized according to size of rock
Slice thickness	1 mm
Pitch	0.45
mAs	360
kVp	140

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Table 2. Colour assignment depicting CT findings.

Colour assigned to block	CT Findings
Red	Identifiable bone—probable hominin/primate
White	Identifiable bone—not hominin/primate
Yellow	Non identifiable bone or absence of bone

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The process of fossilization involves the dissolving and replacement of the original minerals in the bone with other minerals, as well as often crystal formation within spaces and other alterations to the material [48]. This process typically results in a mineralized copy of the original object. The fossil has the same shape as the original object, but is chemically more like a rock. Some of the original bio apatite (a major bone constituent) remains, although it is saturated with silica (rock), calcium carbonate (lime) or other minerals [48]. This results in a change in density and thus appearance and Hounsfield unit reading. Fossilized bone examined in this study, typically appears grey—black on CT with Hounsfield units ranging from +300 to +1500 HU (Fig 2).

Features that were used to help differentiate possible hominin bones from other animal bones were gross skeletal anatomy (if fossils were complete enough), and cortical thickness—hominin limb bones typically having thicker cortical bone that other non—hominin bones (the thickness being relative when compared to the whole bone thickness) [49].

On analysis of the correlation of the CT findings and the manual preparation findings the following were found:

31 of the 44 blocks prepared (70.5%) had concordant findings on CT and actual preparation.

9 of the 44 cases (20.4%) showed minor variances.

4 of the 44 cases (9.1%) were discordant.

Results were deemed to be concordant when the CT predictions of the identification of the bone as well as the taxonomic designation were the same as findings on manual preparation—(Figs 3 and 4). Discordant results arose when the prediction of findings from the CT had mis—identified the bones, when correlated with the actual findings post manual preparation. Minor variances were assigned when the CT had predicted the type of bone but was unable to identify the specific bone or the bone was mis-assigned taxonomically. Manual preparation then confirmed the identification of the bone. This occurred when the bone was small and fragmentary, making CT identification difficult.

Examination of the 9 cases of minor variance where CT had predicted the type of bone but could not identify further showed that this was due to several factors:

1. Where the bone was found to be crushed and fragmented, accuracy of prediction diminished with partial voluming causing erroneous appearances on CT—which were interpreted as bony anatomy.
2. CT over predicted possible hominin bones. Hominin prediction was done predominantly by assessing relative cortical thickness, and over estimation could have been due to similar density matrix making accurate cortical thickness measurement difficult.
3. The size of the fossil bones correlated with the accuracy of prediction. Bigger bones were more easily correctly identified from CT images than were small bones.

Table 3. CT findings vs preparation findings.

UW Number	Block number	Scan venue	Priority	Surface ID	CT Findings	Volume cm^3	Preparation findings	CT vs preparation findings
UW88-1316	B001	CMJAH	yellow	nil	nil	12304	Unidentified fragment	concordant
UW88-1342	B027	CMJAH	yellow	nil	fragments	862	Pelvic fragment	concordant
UW88-1365	B050	CMJAH	red	nil	Phalanx, long bone (tibia/ulna)	1451	Distal bovid metapodial, bovid metacarpal	minor variance
UW88-1368	B053	CMJAH	yellow	Bone fragments	fragments	285	Bone fragments	concordant
UW88-1376	B061	CMJAH	white	nil	Long bone (tibia)	2672		
UW88-1388	B073	CMJAH	white	nil	Femoral head	60	Bovid vertebra	discordant
UW88-1393	B078	CMJAH	yellow	nil	nil	238		
UW88-1396	B081	CMJAH	yellow	nil	Crystal, crushed bone	1030		
UW88-1421	B106	CMJAH	yellow	nil	Fragments	496	Fragments	concordant
UW88-1428	B113	CMJAH	yellow	nil	Long bone fragments	919	Carnivore metacarpal and long bone. Small mammal humerus and tibia	minor variance
UW88-1440	B125	CMJAH	red	nil	Fragments, possible scapula	418	Fragments. Parts of flat bone and long bone with cortical manganese	minor variance
UW88-1443	B128	CMJAH	yellow	Bovid antler fragment	Long bone fragment	41		
UW88-1456	B141	CMJAH	yellow	nil	fragments	782	1st proximal phalanx, lateral end clavicle	minor variance
UW88-1462	B147	CMJAH	yellow	nil	nil	1297		
UW88-1472	B157	CMJAH	yellow	nil	fragments	1932	fragments	concordant
UW88-1476	B161	CMJAH	yellow	Long bone	Surface bone	897		
UW88-1479	B164	CMJAH	yellow	nil	Long bone	1700	Cervical vertebra bovid	discordant
UW88-1483	B168	CMJAH	yellow	nil	nil	2843	Tiny caudal vertebra bovid	discordant
UW88-1487	B172	CMJAH	yellow	Microfauna (tooth)	nil	682		
UW88-1491	B176	CMJAH	yellow	nil	nil	190		
UW88-1505	B190	CMJAH	yellow	fragment	fragment	2522	fragment	concordant
UW88-1506	B191	CMJAH	white	Rib fragment, micro fauna	Ulna bovid	473		
UW88-1523	B208	CMJAH	red	micro fauna	Malleolus/tibia ball joint	111		
UW88-1557	B242	CMJAH	yellow	nil	Long bone bovid	293		

(*Continued*)

Table 3. (*Continued*)

UW Number	Block number	Scan venue	Priority	Surface ID	CT Findings	Volume cm^3	Preparation findings	CT vs preparation findings
UW88-1560	B245	CMJAH	red	nil	Hominin vertebra and rib	5387	Lumbar vertebra and rib from *A. sediba*. Body rib bovid II	concordant
UW88-1564	B249	CMJAH	yellow	Flow stone	Tubular bone	2720		
UW88-1566	B251	CMJAH	yellow	nil	Long bone fragment	1113		
UW88-1578	B263	CMJAH	yellow	Bovid rib fragment	Fragments	3797		
UW88-1586	B271	CMJAH	white	nil	Primate ribs, long bone fragments, artefact ++	4802		
UW88-1594	B279	CMJAH	yellow	nil	Fragments	5364		
UW88-1600	B285	CMJAH	yellow	Long bone fragment	fragments	3635		
UW88-1601	B286	CMJAH	yellow	nil	Long bone fragments	195	calcaneus	discordant
UW88-1613	B298	CMJAH	yellow	nil	Fragments	2269		
UW88-1629	B314	CMJAH	yellow	nil	Fragments	2224	Fragments	concordant
UW88-1631	B315b	CMJAH	yellow	nil	Fragments	7984		
UW88-1638	B322	CMJAH	yellow	Small mammal	nil	5491		
UW88-1650	B334	CMJAH	yellow	Snails, fly pupae, manganese, flowstone	fragments	7182		
UW88-1654	B338	CMJAH	white	nil	2 long bones (tibia/ fibula)	792	Juvenile bovid tibia + fibula shaft fragments	concordant
UW88-1656	B340	CMJAH	white	fragments	Ribs/long bones	2987	Ribs + long bones	concordant
UW88-1658	B342	CMJAH	yellow	nil	Fragments	1362		
UW88-1670	B354	CMJAH	white	nil	Fragments, mandible piece	1782	Bovid III mandible ramus + fragments	concordant
UW88-1687	B371	CMJAH	white	nil	Long bone	1833		
UW88-1691	B375	CMJAH	white	nil	Ribs articulating with vertebrae, long bone, artefact ++	4315	5 x Articulated sub adult bovid vertebrae, with 2 ribs. Possible primate ulna	concordant
UW88-1695	B379	CMJAH	white	Pupae	Complex bone shape, rib	4316	Canid mandible, ribs	minor variance
UW88-1704	B388	CMJAH	yellow	nil	Thin curved bone, cranial fragments, artefact ++	2356	Bovid phalanx and skull fragments	concordant
UW88-1705	B389	CMJAH	yellow	Rat mandible	Fragments	1237		
UW88-1718	B402	CMJAH	yellow	Fragments	nil	8733		
UW88-1728	B412	CMJAH	yellow	Fly pupae, rock fragments	Bone fragments	1274		

(*Continued*)

Table 3. (*Continued*)

UW Number	Block number	Scan venue	Priority	Surface ID	CT Findings	Volume cm^3	Preparation findings	CT vs preparation findings
UW88-1729	B413	CMJAH	yellow	nil	nil	10087	nil	concordant
UW88-1733	B417	CMJAH	yellow	? Bird bone, insect damage	nil	3349		
UW88-1753	B437	CMJAH	yellow	? Rabbit tooth	Fragments	477		
UW88-1754	B438	CMJAH	yellow	Dolomite inclusion	Fragments bone	1092		
UW88-1758	B442	CMJAH	white	nil	Vertebral elements, ribs	3403	Bovid vertebrae and ribs	concordant
UW88-1762	B446	CMJAH	white	nil	Long bone -crushed	1286		
UW88-1769	B453	CMJAH	red	nil	Fragments	420	Crushed bone fragments	concordant
UW88-1781	B465	CMJAH	white	nil	Bovid vertebra	3656	Thoracic bovid III vertebra	concordant
UW88-1785	B469	CMJAH	white	Clay nodules	Flat bone, fragments	1395	Flat bone fragment	concordant
UW88-1789	B473	CMJAH	yellow	Fragments, micro fauna,? burrows	Fragments bone	3063		
UW88-1791	B475	CMJAH	yellow	Fly pupae	Fragments bone	2615		
UW88-1792	B476	CMJAH	yellow	? Burrows	Small cube-like objects	2537		
UW88-1793	B477	CMJAH	yellow	nil	nil	1370		
UW88-1799	B483	CMJAH	yellow	Fragments bone, quartz	Fragments bone	3547		
UW88-1806	B490	CMJAH	white	nil	Bone fragments, distal femur, ribs/flat bone	2778	Bovid II distal femur, rib fragments bovid III	concordant
UW88-1807	B491	CMJAH	white	Bovid mandible	Mandible with teeth	9625	Bovid mandible and teeth	concordant
UW88-1812	B496	CMJAH	yellow	nil	Fragments bone	1587	Bone fragments	concordant
UW88-1813	B497	CMJAH	yellow	Long bone shaft fragment	Unidentifiable bone	5739		
UW88-1816	B500	CMJAH	yellow	nil	Bone fragments	3419	Bone fragments	concordant
UW88-1822	B506	CMJAH	yellow	$CaCO_3$ stalactite, snail shells	Fragments bone	1804	Fragment mammalian rib	minor variance
UW88-1833	B517	CMJAH	white	nil	Vertebral, rib and long bone fragments	4378		
UW88-1834	B518	CMJAH	yellow	Fragments	Fragments bone	3806		
UW88-1836	B520	CMJAH	yellow	nil	Fragments bone	5699		
UW88-1840	B524	CMJAH	yellow	? stone tools	nil	4384		
UW88-1845	B529	CMJAH	yellow	inclusion	Fragments bone	2155		

(*Continued*)

Table 3. (*Continued*)

UW Number	Block number	Scan venue	Priority	Surface ID	CT Findings	Volume cm^3	Preparation findings	CT vs preparation findings
UW88-1853	B537	CMJAH	yellow	Rock inclusion, fly pupae	Fragments	1013		
UW88-1857	B541	CMJAH	yellow	Dolomite inclusion	Fragments bone	2786		
UW88-1860	B544	CMJAH	yellow	nil	Fragments bone	3564		
UW88-1862	B546	CMJAH	yellow	nil	Fragments bone	1891	Bone fragments	concordant
UW88-1863	B547	CMJAH	yellow	Rock flakes	Fragments bone	2180		
UW88-1870	B554	CMJAH	white	nil	Long bones, vertebra, rib	1712	Small cat pelvis in articulation with vertebra and femur	minor variance
UW88-1871	B555	CMJAH	yellow	Clay nodule	Fragments bone	1234		
UW88-1876	B560	CMJAH	red	nil	Long bone fragment	5817	Long bone fragments	concordant
UW88-1877	B561	CMJAH	yellow	nil	nil	2422		
UW88-1879	B563	CMJAH	yellow	Dolomitic inclusion, rib fragment	Fragments bone, artefact ++	2535		
UW88-1887	B571	CMJAH	yellow	nil	nil	1390		
UW88-1888	B572	CMJAH	yellow	Fly pupae	nil	1853		
UW88-1904	B588	CMJAH	white	nil	Rib fragment, metapodial	1164		
UW88-1905	B589	CMJAH	white	nil	Flat bone	1717	Flat bone	concordant
UW88-1906	B590	CMJAH	yellow	Rock flake, fly pupae, snail shell	Fragments	1005	Bovid 2nd phalanx fragment	minor variance
UW88-1910	B594	CMJAH	white	Air pockets	Long bone fragment	1727		
UW88-1912	B596	CMJAH	yellow	nil	Long bone fragment, artefact++	4985	Bird tibia—proximal fragment	minor variance
UW88-1918	B602	CMJAH	yellow	? Burrows	Bone fragment	2444		
UW88-1925	B609	CMJAH	yellow	airspaces	Fragments	1305		
UW88-1932	B616	CMJAH	yellow	nil	Bone fragments	1064		
UW88-1943	B627	CMJAH	yellow	nil	nil	1310		
UW88-1951	B635	CMJAH	yellow	nil	Fragments	1010		
UW88-1954	B638	CMJAH	yellow	Fragments bone	Fragments bone	939		
UW88-1955	B639	CMJAH	red	? Burrows,? organics	Distal femur/proximal tibia	1365	Proximal tibia	concordant
UW88-1962	B646	CMJAH	yellow	nil	nil	1113		

(*Continued*)

Table 3. (*Continued*)

UW Number	Block number	Scan venue	Priority	Surface ID	CT Findings	Volume cm^3	Preparation findings	CT vs preparation findings
UW88-1971	B655	CMJAH	yellow	nil	Bone fragments	457	Bone fragments	concordant
UW88-1972	B656	CMJAH	yellow	Microfauna, dolomite	Fragments	924		
UW88-1988	B672	CMJAH	yellow	Fly pupae	Bone fragments	2613		
UW88-1999	B683	CMJAH	white	Burrows	Pelvic fragment	827	Pelvic fragment	concordant
UW88-2008	B692	CMJAH	white	nil	Femur large bovid	944	Distal bovid femur	concordant
UW88-2014	B698	CMJAH	white	nil	Bone fragment	2094	Bovid bone fragments	concordant
UW88-2040	B724	CMJAH	yellow	Dolomite/quartz	Long bone fragments	2481		
UW88-2044	B728	CMJAH	yellow	nil	fragments	1329		
UW88-2015	B735	CMJAH	yellow	nil	Fragments	2258		
UW88-2053	B737	CMJAH	yellow	nil	Fragments	738	Fragments	concordant
UW88-2386	B1070	CMJAH	yellow	nil	nil	680		

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Analysis of the 4 cases where there was CT/preparation discordance showed:

1. Size of specimen again played a role in accuracy of assessment. Smaller bones were more easily missed or mis-identified.
2. Artifact contributed to poor CT- preparation correlation.
3. Complex bony architecture, such as facial bones and pelvises, were more difficult that tubular, long bones, to correctly identify.
4. Vertebrae were somewhat problematic, especially when small.
5. In 2 cases, (B164 and B286), the discordance was thought to be due to confusion in block numbering with the blocks scanned not in fact being the blocks prepared.

The CT images of the discordant cases were re-evaluated following preparation findings. Re-evaluation could not convincingly find the bones that had originally been missed, nor improve identification of visualized bones. As round shapes may be poorly seen in a single plane, careful multi planar evaluation was done, but this did not improve their identification. Visualization of each scan in multiple planes was shown to be essential for complete evaluation, as objects could sometimes be well seen in one projection, but poorly visualized in an orthogonal view [50]—tubular structure (long bones *etc*.) are well seen when viewed along their long axis but less well seen if only a transverse, short axis is viewed. These findings might suggest that the round shape is more easily missed on CT viewing, as several of the dissimilar cases, involved vertebrae or a calcaneus—the common factor here seemingly possibly the shape, although cataloguing error (where block numbers was changed between CT scanning and manual preparation) was strongly suspected in a couple of the cases.

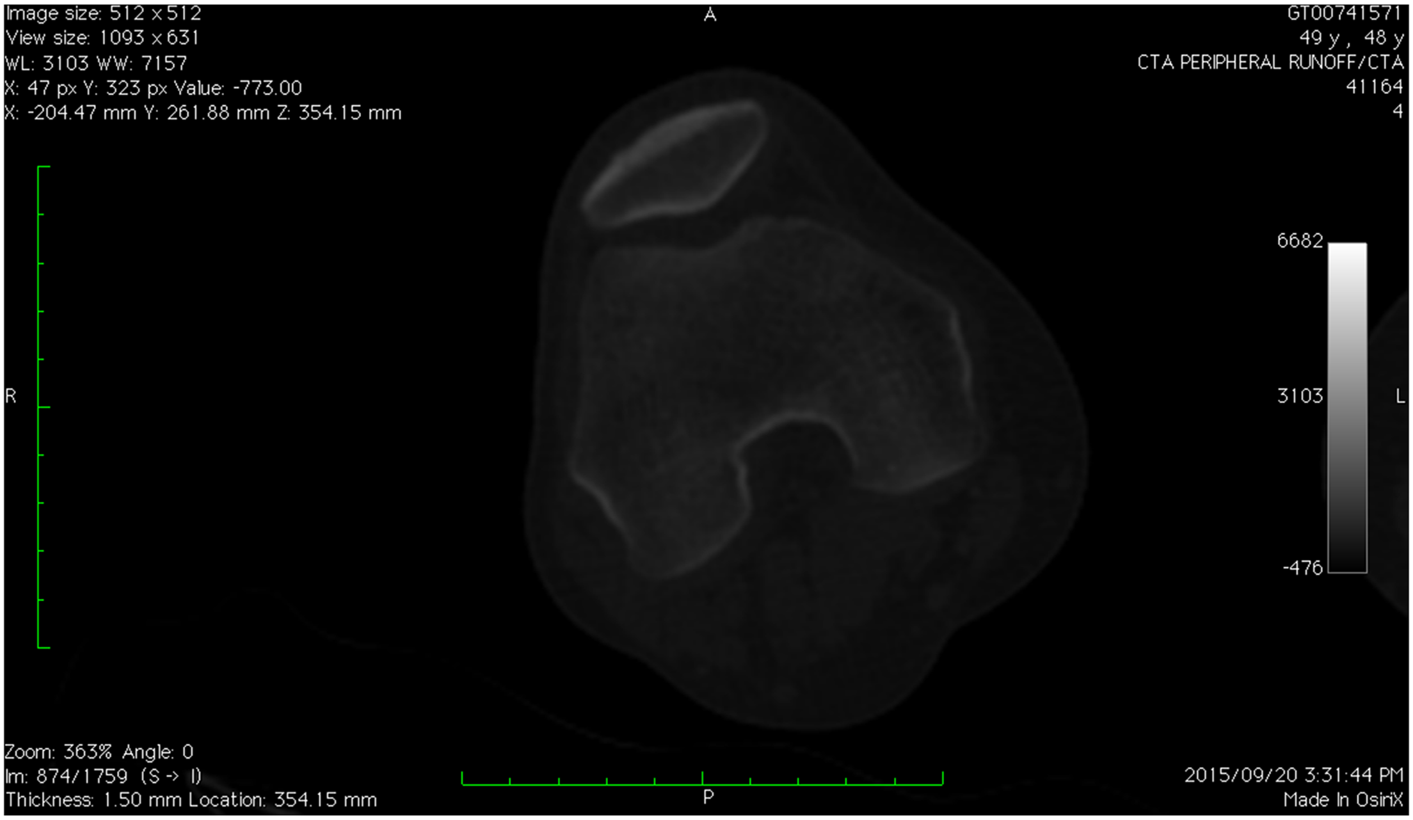

Fig 1. CT image of a distal femur of a living human—fresh bone. The cortex of the bone appears white with the mineralized matrix also appearing as shades of white to grey. Basic iterative reconstruction technique. Scale = 10 cm total—each bar = 1 cm.

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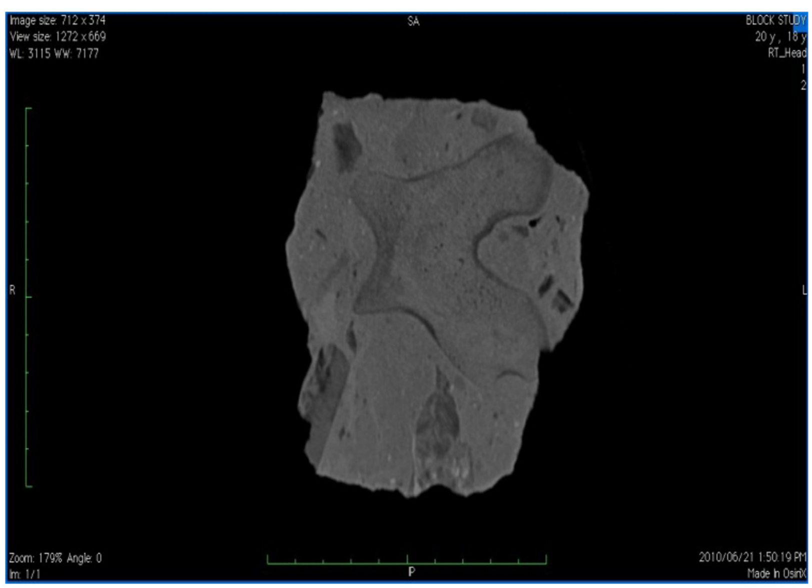

Fig 2. CT image of fossil of a distal bovid femur embedded entirely in matrix. The edges of the fossil appear dark grey. The central body of the fossil is of a very similar grey colour to the surrounding matrix. Small air pockets and inclusions appear black or nearly black. H70h kernel used. Scale = 10 cm total—each bar = 1 cm.

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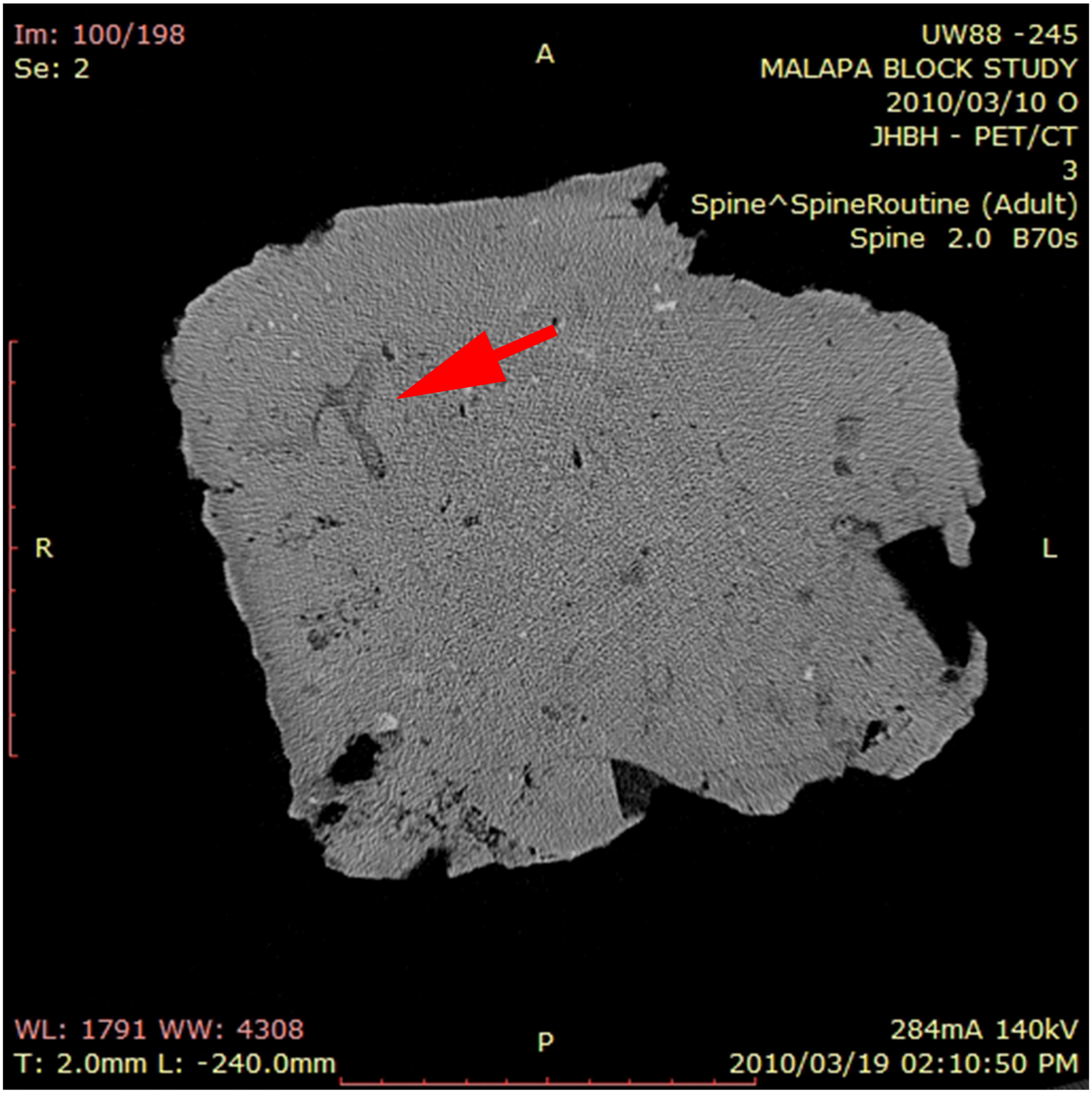

Fig 3. B 245 (UW 88–152)—Hominin vertebra identified on CT (arrow). Seen in sagittal projection.

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Importantly it was noted that when viewing, the CT image should be magnified according to the object being sought. As the FOV varies according to block size, all images are initially processed to occupy the same viewer space, regardless of the blocks' true size. Thus small objects may be very difficult to find unless the image is magnified so that the visualized size of an object mimics more closely its true size. Magnification during viewing causes enlargement of a small area by selecting that small area within the total digital field and making it cover the

Fig 4. B 245 (UW88 152)–*Australopithecus sediba's* vertebra after preparation.

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full display. This differs from the process of changing the FOV during image reconstruction. Reducing the FOV has the effect of increasing image detail, magnification does not do this. Without adjustment of the magnification when viewing, perspective is forgotten and smaller fossils e.g. teeth may be overlooked.

Medical CT has, over time, become standardized due to the fact that there are a limited number of different scanning subjects [50]. However, in contrast, the use of CT for palaeontological investigations requires case by case selection of scanning parameters to optimize the contrast between objects of interest whilst minimizing artifact.

Grey scale is the way in which the grey shades in a black and white image are spread. The grey shades are correlated with the digital pixel values of the image, with ranges of pixel values assigned to a certain grey shade. The CT machine will allow one to determine which matrix number should be printed as white and which should be printed as black. The numerical range between the white and black levels establishes the CT window "width". "Window width" is defined as the range of CT numbers (in Hounsfield units) included in the grey—scale display of the CT image, ranging from 1 to 2000 or 3000, depending on the type of machine. The centre of that numerical range becomes the window "level". The window width is divided by sixteen to determine the numbers which are included in each individual grey tone.

A grey scale on the display will typically contain 256 grey shades [51], whereas the Hounsfield scale has 4096 values. The range of grey scale units used during post processing varies from the Hounsfield unit scale generated by the CT machines as most medical CT systems use a 12-bit scale where 4096 values are possible, but the post processing software makes use of 16 or 64- bit scales where the range is 0–65 535 [50]. This scale does not purport to equate to the actual density of the geological materials, but allows relative comparison of densities. Ketcham et al [50] when commenting on CT use for geosciences states that *"For geological purposes, it is commonly more desirable to select the reconstruction parameters to maximize the CT-value contrast for each scanned object. This makes viewer experience with image processing essential for accurate interpretation."*

The human observer can perceive no more than around 900 shades of gray. Therefore, there is an upper limit to the amount of grayscales in a medical viewing application. Display systems that are able to show 1,024 simultaneous shades of gray (10 bits) are sufficient for medical imaging. Display systems exceeding this specification will present to the human observer shades of gray that cannot be discriminated from each other anymore.[52]

The grey scale of the individual images was adjusted to reach a "best view" depiction of block contents. It has been noted by CT researchers that it is possible to set the window inappropriately and completely miss the important diagnostic information from a particular study [53]. If the window is set too wide, each grey tone will include such a wide range of tissue density that a potential object is likely to be indistinguishable from the surrounding material.

The CT reader is trained in anatomical bony recognition and has training and significant experience in digital imaging. As noted by several authors [50,54], this is essential for accurate CT prediction of fossil findings. Readers need to be familiar with the complexities of CT post processing and image manipulation as findings can be "lost" in the CT image, should the incorrect manipulations be performed or poor settings used for viewing. [54,55]

Artifact production can degrade the CT image and hinder interpretation. Modern CT machines are developed with built-in artifact reduction features, including filters, calibration correction, automatic tube current modulation and scanner software [56]. Artifact noted on the CT scans in this study was more marked in the breccia of larger volume, but interpretation was still possible. Beam hardening artifact was identified to varying degrees on the CT scans as streaking emanating from the rock's surface and through the rock. Willis *et al* [57] showed that the higher photon attenuation and irregular shape of fossilized material lead to severe streak artifact resulting from abrupt changes in X-ray transmission intensity across an object and was often associated with long straight edges of high attenuation material. Scanning at a higher kV results in a harder X-ray beam, and thus less beam hardening artifacts, hence the choice of the maximum KVp of 140 on the medical scanner.

Of note, was that no hominin fossils were missed on CT predictions, in blocks prepared. This may be due to the general relative thicker cortices [49] of hominin bones over other animals, thus facilitating their identification even when round in shape. Not only was CT convincingly able to identify the presence of fossil bone, but good visualization allowed good predictive identification and characterization. CT scans offer a quick and non-destructive method of imaging. Data is obtained in a digital format that allows 3 dimensional representations of an object to be created. Post processing of this data allows reconstructions, measurements and a variety of analyses to be performed.

There are however limitations to the use of CT in palaeontological research, many of these are being overcome as hardware and software improvements occur and advancements in technology are made. The CT image can be manipulated and visualized depending on the required application. Often 2 D analyses along orthogonal planes are sufficient for skeletal structures, but additional information can be gained from 3 D image reconstructions. Traditionally these 3D reconstructions are calculated from CT values based on CT grey scale numbers. If quantitative measurements are needed from the CT data, segmentation techniques are often necessary to separate features of interest based on criteria other than CT values, as use of CT numbers may be complicated by partial volume averaging effects [58]. Manipulation of CT parameters at the time of scanning, to lessen imaging artifact, can hinder precise image acquisition [59]. Partial voluming and limits in spatial resolution are important constraints of CT [23]. Scientists have tested and validated the accuracy of these latter two constraints as regards the accuracy and reproducibility of measurements and the definition of landmark coordinates [60–62].

The use of medical CT was specifically investigated, as oppose to industrial CT with micro CT capabilities or other scanning technologies (e.g. synchrotron) for the following reasons:

- Industrial micro CT is presently not readily available in South Africa [55], scanning times are longer than medical CT (hours versus seconds on a medical scanner) [63] and size and weight restrictions on these micro CT machines are significantly more limiting than on medical CT scanners. (The micro CT at the University of Witwatersrand takes a maximum diameter of +/- 20 cm and a weight of 50–60 kg, whereas medical CT allows diameter of 80–90 cm and weights of between 200–300 kg).
- Synchrotron scanners are very scarce, immensely costly and very time consuming.
- Both micro CT and synchrotron imaging generate very large data sets, necessitating dedicated computers and software with large data handling capabilities. These are expensive and not as freely available as ordinary laptop or desktop computers that can handle the DICOM data generated from the medical scanner. In addition, medical CT data can be analyzed with software that is free.

Conclusions

In 2009, Wu [4] stated "*The suitability of medical CT for the study of hominin fossils is limited by its low X-ray dosage that is unable to penetrate highly mineralized and matrix-filled specimens*." Scanning of 109 matrix fossil- bearing rocks from the site of Malapa yielded CT images of a quality that, coupled with modern software post processing programmes and a suitably trained and experienced reader, allowed for the accurate prediction of the fossil contents of the blocks.

Medical CT scanners are shown by this study to be capable of producing images that allow for the accurate identification and often characterization of fossils. The correlation of these predicted findings with the actual findings post manual preparation is sufficiently concordant to change the traditional course of the handling of fossil bearing blocks. In order to maximize the use of limited resources and manual preparatory skills as well as to curtail costs, this research suggests that prior to manual preparation, blocks should undergo scanning with medical CT scanners and "virtual" assessment of contents should be undertaken by suitably qualified individuals to allow for prioritization of rocks for manual preparation. Knowledge of bony as well as radiological anatomy is deemed essential for accurate interpretation of findings, as is familiarity and experience with digital imaging techniques, their production limitations and pit falls of post processing manipulation.

For the first time in South African palaeoanthropological work hominin fossils have been imaged within their matrix before any preparation had been performed. The relationships of all the bones could be eloquently demonstrated on post- processing of the CT images. This afforded scientists the unique opportunity to, up front, assess and plan the further investigation and preparation of this block—something never achieved in South Africa before.

The results of this study have shown conclusively the viability and value of the use of medical CT imaging to assess possible fossil-containing rocks for fossil remains. It has also demonstrated that there is considerable advantage to being able to know the contents of a rock ahead of costly, time-consuming "blind" manual preparation. This allows decisions to be made as regards the most efficient use of resources, manpower and allocation of funds, in addition to allowing planning of the course of action for each fossil. Information can be extracted without damage to the matrix and hence allows the potential for preservation of these remains for future generations of scientists, ensuring that as technology advances, enough direct physical evidence has been left behind on which to apply new methods of analysis in the future.

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4. SAJS ARTICLE (Smilg, 2017)

4.1 Finding fossils in Malapa breccia – medical CT scanning or micro-CT scanning?

New ways have been sought to better deal with the many Malapa breccia requiring assessment. The idea of triage (prioritizing for preparation) of breccia by imaging prior to manual preparation has been explored in the PLoS article. The work presented in the PLoS article demonstrated good correlation and suggested that XCT is a valuable imaging modality in the triage process.

In 2009, Wu (Wu and Schepartz, 2009) stated *"The suitability of medical CT for the study of hominin fossils is limited by its low X-ray dosage that is unable to penetrate highly mineralized and matrix-filled specimens."* As high energy X-rays can potentially penetrate breccia more effectively than lower energy beams, the effectiveness of lower energies with regards to image quality and object penetration was explored in SAJS article. This study demonstrates that lower energy beams produce superior images for prioritizing breccia for preparation. Additionally, this article concluded that in the application of breccia triage, the penetrating ability of a lower energy beam is not detrimental to the outcome. Additionally, contrasting the use of XCT and µXCT for the application of breccia triage, it was found that overall the use of XCT was deemed preferable. XCT scanners are numerous, accessible, fast and relatively cost effective when compared to µXCT scanners – the latter are not freely available, scanning times are much longer and there are significant limitations on size and weight of scannable objects.

Finding fossils in Malapa breccia – medical CT scanning or micro-CT scanning?

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Computed tomography (CT) imaging of fossils has revolutionised the field of palaeontology, allowing researchers to gain a better understanding of fossil anatomy, preservation and conservation. Micro focus X-ray computed tomography (µXCT) has been far more extensively used for these purposes than medical CT (XCT) – mostly because of the exquisite detail that the µXCT scanning modality, using slices of micron thicknesses, can produce. High energy X-rays can potentially penetrate breccia more effectively than lower energy beams. This study demonstrates that lower energy beams produce superior images for prioritising breccia for preparation. Additionally, XCT scanners are numerous, accessible, fast and relatively cost-effective when compared to µXCT scanners – the latter are not freely available, scanning times are much longer and there are significant limitations on the size and weight of scannable objects. Breccia blocks from Malapa were scanned at high and lower energy and images were analysed for image quality, artifact and certainty of diagnosis. Results show that lower energy images are deemed superior to higher energy images for this particular application. This finding, taken together with the limitations associated with the use of µXCT for the imaging of the large breccia from Malapa, shows that XCT is the better modality for this specific application. The ability to choose fossil-bearing breccia, ahead of manual mechanical preparation by laboratory technicians, would allow for the optimal use of limited resources, manual preparatory skills as well as the curtailment of costs.

Significance:

- ‘Blind’ manual preparation of fossil-bearing breccia is a costly and time-consuming exercise – and often results in a low yield.
- The ability to triage fossil-bearing breccia ahead of manual preparation would allow for the optimal use of limited resources.
- Medical CT is better than micro-CT to triage breccia to allow for prioritisation of rocks for manual preparation.

Background

The use of computed tomography (CT) in the analysis of fossils has become common place[1-4], although most of the CT work to date has been performed on prepared or partially prepared specimens[3,5,6]. The application of CT for matrix that potentially contains fossils has lagged behind these many advances in the visualisation and study of prepared fossils.

Micro CT (µXCT) scanners have become increasingly popular in the imaging of prepared fossils as a result of the combination of their ability to produce high-resolution images because of much smaller slice thicknesses (in the micron range), increased spatial resolution and more variable energy capabilities (especially in the higher energy ranges) than medical CT (XCT) scanners; in fact, µXCT dominates the current fossil literature as the X-ray modality of choice for virtual analysis of prepared fossils.[7-10]

The site of Malapa has yielded hundreds of breccia blocks, which have the potential to contain fossils of the hominin *Australopithecus sediba*. Traditionally, breccia has undergone manual preparation – with a single block taking months to adequately prepare, with no guarantee of obtaining any fossils. This process is costly – both in time and money – and is not a prudent use of scarce preparatory skills.

New ways have been sought to better deal with the many Malapa breccia requiring assessment. The idea of triage (prioritising for preparation) of breccia by imaging prior to manual preparation has been explored. Mass use of XCT to image Malapa breccia has been undertaken and the predicted findings from imaging have been compared to the actual post-preparation findings.[11] Smilg and Berger[11] demonstrated good correlation and suggested that XCT is a valuable imaging modality in the triage process. There is considerable advantage to being able to know the contents of a rock ahead of costly, time-consuming ‘blind’ manual preparation. Knowledge of breccia contents before manual preparation allows decisions to be made as to the most efficient use of resources, personnel and funds, in addition to allowing planning of the course of action desired for the preparation of fossil material.

The effectiveness of XCT with regard to image quality and object penetration was explored. The quality of an X-ray beam is the measurement of the penetrating power of the photons, which depends on the energy of the photons, the atomic number (Z), the density and the thickness of the object being scanned.[12] Peak kilovoltage (kVp) governs the penetrating power of photons – the higher the kVp, the more the beam penetrates the object. Hence the question was posed as to whether higher kilovoltage beams would produce images from the breccia that would be better quality than those produced from lower kilovoltage beams. XCT is limited in its kilovoltage, with the maximum being 140 kV, whereas µXCT is capable of higher kilovoltage. As a consequence of the better penetrative ability of high energy X-rays,[13] it may be expected that high energy scanning would be superior to lower energy scanning when it is applied to large, dense breccia blocks.

High and lower energy images of breccia were obtained to address this question of image quality from the Malapa breccia for triage purposes. μXCT was used to obtain the higher kilovoltage values that XCT was unable to generate. The spectrum of potential imaging characteristics available from μXCT was not researched for this particular application.

The image quality of lower energy and high energy images when applied to objects within breccia blocks was compared. Material for this experiment was selected from the fossil hominin bearing site of Malapa in the Cradle of Humankind World Heritage site because of the importance of fossils from this site, the many blocks collected from which there are no fossils visible on the surface, and the sheer number of blocks retrieved that require assessment.

The possibility of the use of neutron microtomography (NCT or n-μCT) or magnetic resonance imaging (MRI) was considered. NCT differs from X-rays in that neutrons can penetrate materials that are opaque to X-rays and organic material strongly attenuates these neutron beams. NCT may thus be appropriate for imaging organically preserved fossils as a complement to XCT or μXCT.[14] NCT has shown promising results in being able to differentiate otherwise similar dense materials – a recent study has documented the use of NCT to view a fossil encased in breccia.[15]

However, NCT may induce hazardous levels of radioactivity in some geological materials which leaves these imaged samples radioactive and necessitates that the samples be isolated for a long time after the imaging. Additionally, there are currently no functioning NCT machines in South Africa, where this breccia triage is needed. NCT necessitates much longer scanning times when compared to XCT and is also limited to smaller fields of view.

MRI maps properties related to the chemical environment of certain elements, rather than mapping radiation attenuation. MRI has been considered to be poorly suited to geological material[16] and at present does not compete with μXCT or NCT. MRI machines are not easily accessible to palaeontological researchers, scans are very expensive and scan times are long compared with those of XCT. For these reasons, both NCT and MRI were not considered suitable for the mass screening of breccia from Malapa.

Fossils may have the potential to contain ancient DNA and whilst the effect of radiation on living tissue has been well investigated, little has been done to research the impact that radiation may have on ancient DNA.[17] Recent work has shown that radiation of fossils may have a detrimental effect on ancient DNA when the total surface dose exceeds 200 Gray, so these researchers recommended using as low a dose as possible when scanning fossils as well as using resolution no higher than necessary to achieve the desired outcome.[18] The value of 200 Gray is far higher than any dose from a XCT or μXCT scan (8000 times higher than the highest dose for a medical CT scan).[18]

The Malapa site

The site of Malapa (site UW88)[19] represents an unusually rich early hominin locality in Africa[20-24], dating to 1.977 ± 0.002 million years ago (Ma)[25]. The site contains remains of several individuals, all ascribed to *A. sediba.*[20] These remains are found alongside an abundant, well-preserved fauna.[24] It is postulated that this well-preserved material was accumulated during a seemingly rapid depositional event that occurred over a few days, weeks or months.[26] The site of Malapa is located in the region of the Cradle of Humankind World Heritage Site, northwest of Johannesburg, South Africa. The locality is recognised as a de-roofed cave of at least 25 x 20 m, in an area where limited limestone mining had taken place, probably during the late 19th or early 20th century, almost certainly before Robert Broom began exploring the area in the mid-1930s.[26]

Materials and methods

A total of 15 breccia blocks from the site of Malapa were chosen to be scanned at differing kVp using both XCT and μXCT. Blocks were selected taking cognisance of the limitations on weight and volume presented by the University of the Witwatersrand's micro-CT scanner (a maximum permissible weight of 50 kg).

Lower energy scanning was performed on a Philips Brilliance 128 slice CT at Charlotte Maxeke Johannesburg Academic Hospital (South Africa), whilst high energy scanning was performed on a Nikon Metrology XTH 225/320 LC dual source industrial CT system at the University of the Witwatersrand. Both data sets were analysed on AMIRA 5.4.5 Ink.

This experiment was intended to simulate a real-world situation. After discussions with the principal scientists in charge of the Malapa project, it was decided that if mass screening of breccia was to be implemented, it would be impractical to adjust energy levels and scanning parameters for every block in order to optimise image quality, because of the desire to scan large numbers of rocks in a single session and the possibility that the scanning would be overseen by technicians not familiar with CT images. Hence the imaging parameters would have to be constant and pre-decided. The parameters selected are given in Table 1. A single energy parameter was selected for each system based upon initial tests that resulted in a good quality image from both machines. Reference was also made to prior work done with low energy scanning.[11] Micro-CT images were reconstructed with micro-CT Pro v2.2 associated with the Nikon Metrology XTH 225/320 LC dual source industrial CT system.

Table 1: Parameters used for scanning on medical CT (XCT) and micro-CT (μXCT)

XCT	μXCT
140 kVp	300 kVp
360 mAs	340 μA
1-mm slice thickness	4 frames per second
Automatic artifact correction	Ring artifact correction
Pitch 0.45	Frame averaging of 2
512 x 512 matrix	3000 projections
	Copper filtration of 3.4 mm

kVp, peak kilovoltage; mAs, milliamp seconds; μA, micro amps

Three objects, thought to represent potential fossils, were chosen from within each block for evaluation. The same object was identified on images from each modality and displayed on a two-dimensional image with the same orientation and alignment specific to the object under assessment. The viewing parameters (including greyscale setting and magnification) were fixed for all readers, with no manipulation allowed. Readers were supplied with identical static two-dimensional images. Qualitative visual assessment was done by each reader independently.

Each object was evaluated for overall image quality, certainty of diagnostic accuracy and imaging artifact. There is no objective definition of image quality – it is a matter of the observer's subjective judgement. CT artifacts can affect the quality of the images, sometimes to the point of making them diagnostically unusable. Artifact is any distortion or error in the image that is unrelated to the subject being studied.[12] Artifact production can degrade the CT image and hinder interpretation, but modern CT machines are developed with built-in artifact reduction features, including filters, calibration correction, automatic tube current modulation and scanner software.[27]

The images were rated according to a qualitative, visual five-point scale for each parameter given in Table 2.

The objective was to compare these parameters between lower kilovoltage and higher kilovoltage images. The scores from an individual reader for each object, per criterion, for each energy were compared, rather than analysing inter-reader variability. Results were evaluated by two diagnostic radiologists and three palaeontological scientists. These results are summarised in Appendices 1 and 2 in the supplementary material.

Table 2: Scale used to determine rating of image for each parameter assessed

Rating	Overall image quality	Diagnostic certainty	Artifact
5	Excellent delineation: 100%	Full and confident certainty	Absence of artifact
4	Clear/good delineation: >75–<100%	Good certainty	Mild artifact, not interfering with diagnosis
3	Adequate delineation: >50–<75%	Adequate certainty	Moderate artifact, slightly interfering with diagnosis
2	Marginally acceptable delineation: >25–<50%	Marginal certainty	Pronounced artifact, interfering with diagnosis but still possible to arrive at diagnosis
1	Unacceptable delineation: <25%	Uncertain	Artifact completely hinders diagnosis

Table 3: Summary of reader analysis showing number of reads for which readers scored the 140 kVp or 300 kVp image higher and the number of reads for which the images were scored equally. Overall, lower energy was deemed to produce better images when images were assessed for image quality, certainty of diagnosis and presence of artifact.

kVp scores	Artifact assessment reads	Certainty assessment reads	Quality assessment reads	Fraction of total reads	Percentage for kVp setting
140 kVp scored higher	114	136	151	401/630	63.65
300 kVp scored higher	27	14	25	66/630	10.48
140 kVp score = 300 kVp score	84	30	49	163/630	25.87

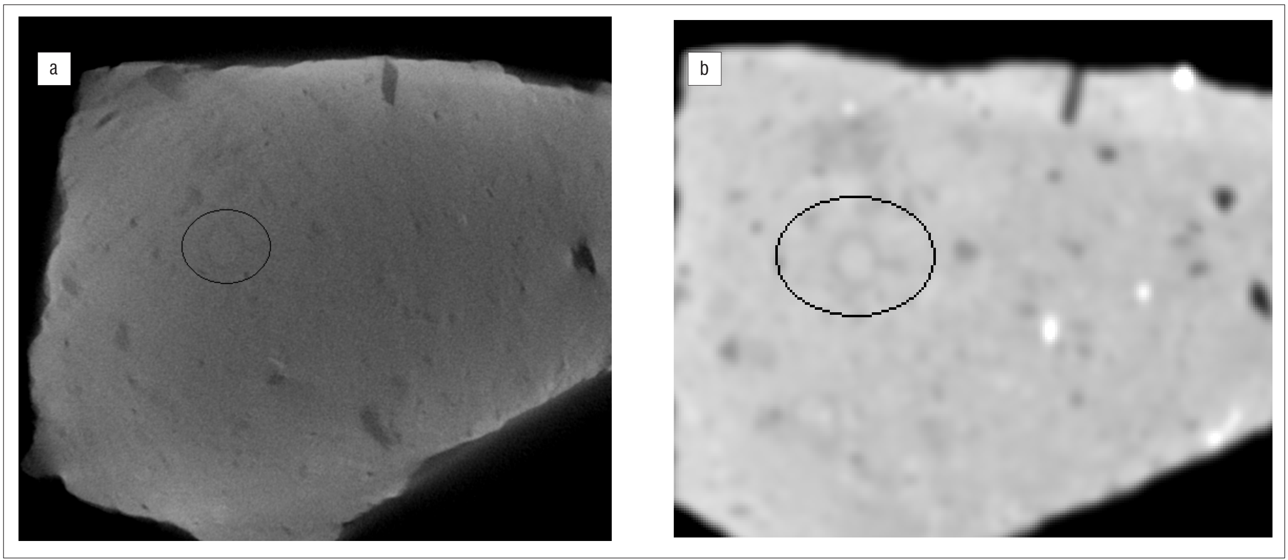

Figure 1: Object 2 within block 3195 (ringed). The potential fossil is visualised in the same plane and alignment on both modalities: (a) 300 kVp and (b) 140 kVp. Overall the readers deemed the image from lower energy to be better with fewer artifacts than that from higher energy.

Results

Results were assessed to determine which kVp choice scored higher (indicating better overall image) per reader, per object and per criterion, and whether the reader assigned the same score per criterion per object – the latter indicating an indeterminate result of neither kilovoltage setting producing a superior image (Table 3).

When considering the presence or absence of artifacts, the readers found that the lower energy scans produced better interpretive images in 114 of 225 reads (Figure 1). In 27 of 225 reads, high energy scanning was found to produce better interpretive images and 84 of 225 reads were indeterminate as to one scanning energy being better than the other.

Overall quality was deemed to be better in 151 of 225 reads for lower energy scans, whilst higher energy scans were deemed better in 25 of 225 reads (Figure 2). An indeterminate result, with both energy levels scoring equally, was obtained in 49 of 225 reads.

One reader did not rate for certainty of diagnosis for either modality because of a lack of confidence in cross-sectional identification. Results here identified lower energy scans as being superior in 136 of 180 reads (Figure 3). Higher energy scans were deemed better in 14 of 180 reads and results were indeterminate in 30 of 180 reads.

Overall percentages indicate that for all three criteria, the images obtained from lower energy scanning produced better, more diagnostic and more useful images with fewer artifacts in 63.65% of reads. Higher energy was deemed to deliver better images in 10.48% of reads and both modalities yielded equitable images in 25.87% of reads.

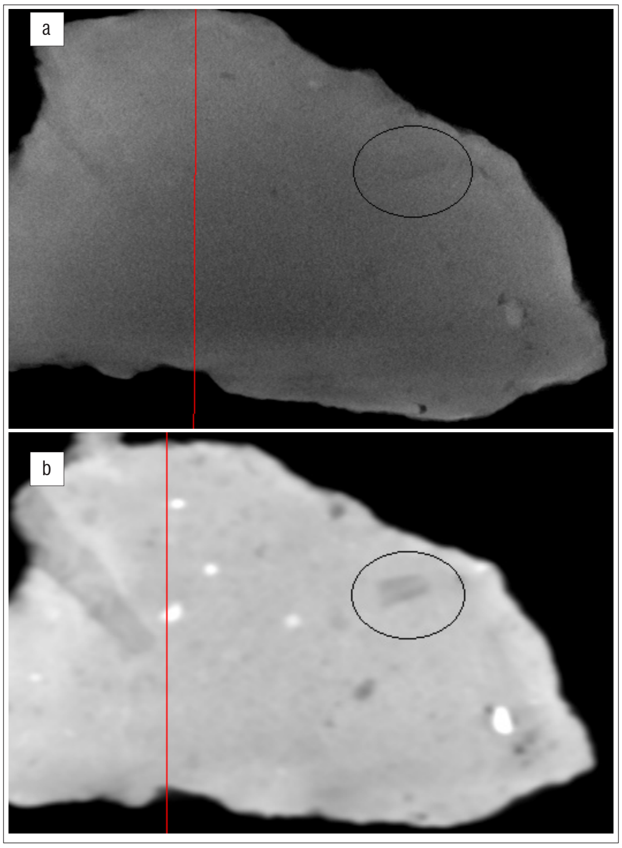

Figure 2: Object 3 within block 1343 (ringed). The potential fossil is visualised in the same plane and alignment on both modalities: (a) 300 kVp and (b) 140 kVp. Overall the readers deemed the image from lower energy to have better image quality than that from higher energy.

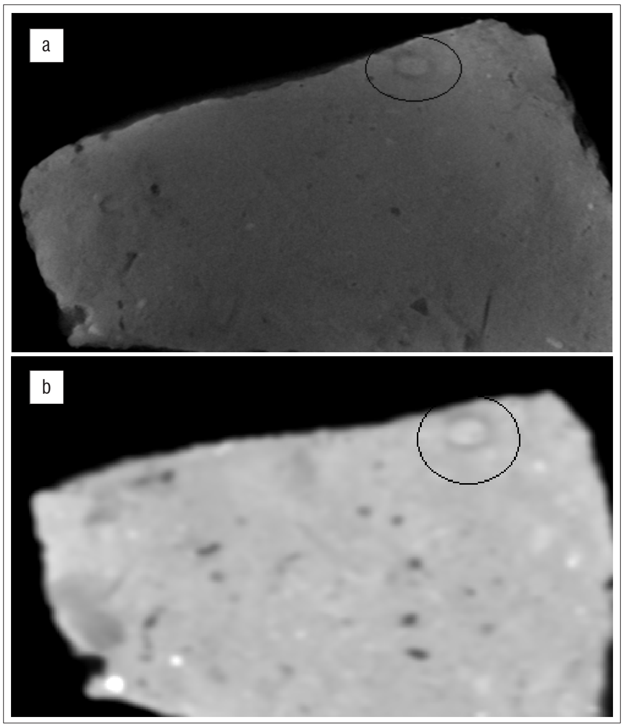

Figure 3: Object 2 within block 3728 (ringed). The potential fossil is visualised in the same plane and alignment on both modalities: (a) 300 kVp and (b) 140 kVp. Overall the readers deemed the image from lower energy to offer more certainty of diagnosis than that from higher energy.

Discussion

In producing a CT image, both milliamp seconds (mAs) and kVp must be chosen to ensure sufficient delivery of X-rays to achieve acceptable image quality.[28] The selection of kVp sets the energy of the X-rays reaching the object. The energy of an X-ray defines its penetrative ability[13], as well as the expected attenuation of the ray as it passes through materials of different densities. High energy X-rays, when compared to lower energy rays, may penetrate certain objects more effectively but are less sensitive to changes in material density and composition.

Each object varies in density and atomic number – both of which impact the X-ray beams' attenuation. Lower energy settings decrease the overall signal and result in increased noise, which can degrade the image. Increasing technical factors, such as mAs or kVp, decrease image noise but also increase dose. Because of the inanimate and inorganic nature of the material being studied, dose was not as important as it would be with living material. As X-rays traverse an object they are attenuated by scattering and absorption. This attenuation is a result of three processes[13]: photoelectric absorption, Compton scattering and pair production. Medical scanners (which use lower X-ray energy up to 140 kV) have more photoelectric effect and lower Compton scatter, whereas in higher energy μXCTs, attenuation is greater from Compton scatter.[13] This difference is important as it explains why low energy X-rays are more sensitive to differences in composition than higher energy ones. The photoelectric absorption is proportional to $Z^{4\text{-}5}$ (atomic number of atom in the material to the power 4-5) whereas Compton absorption is proportional only to Z (atomic number).[29] Thus two materials could be differentiated in lower energy CT, but at higher energies may be indistinguishable. Despite a better penetration ability, high energy scanning may not differentiate objects as well as lower energy scanning.

The quality of a CT image is affected by several factors; enhancing or suppressing any of these factors depends upon the imaging interests.[12] CT image quality is dependent upon balancing these parameters to produce the best possible image for the object being scanned. CT parameters can be manipulated to either decrease or eliminate the adverse effects of these characteristics. Generally, there is a trade-off when CT parameters are manipulated.

Although CT scanners using high kVp (μXCT) could potentially be more effective at penetrating large breccia and can produce images with increased resolution as a result of much thinner slices, their disadvantages are their limitations in the size/weight of scannable objects, the long scan times needed as well as their limited availability to many scientists. In addition, the huge databases generated from the scan data necessitate specialised computer hardware and software for analysis, which are costly and not readily available to many researchers.

In contrast, medical CT machines are accessible as a consequence of their widespread use in clinical medical situations and are found in most hospitals and radiology practices, have a weight restriction of up to 200 kg and offer fast, reliable imaging and greater throughput of scanned objects. The databases are manageable on basic modern computers and data can be assessed with easily available (often free) software packages. Finally, at present, medical CT scanning, on a case by case basis, is typically significantly cheaper than micro-CT scanning, largely because of differences in initial machine cost as well as the presence of a greater number of medical CT machines in the community.

To be effective for the screening of Malapa breccia, the imaging modality used should:

- be cost effective, both in time and money
- be quick
- be repeatable
- use predetermined parameters that would negate the need for specialist attendance at scanning sessions
- be regularly readily accessible
- be able to accommodate large pieces of breccia.

The μXCT machines in the Gauteng area that are available to the Malapa project are limited in that:

- they have prolonged application and waiting times
- they are costly
- scanning is time consuming (±6 h/block)
- there are significant limitations in the weight and dimensions of objects to be scanned
- they generate large databases which make assessment time consuming and post processing limited to specialised programmes.

Conclusions

Fossils, particularly of hominins, are highly sought-after objects in the search for human origins, but 'blind' manual mechanical preparation of fossil-bearing breccia is a costly and time-consuming exercise – often with a low yield. The ability to triage breccia ahead of manual preparation would allow for the optimal use of limited resources, manual preparatory skills as well as the curtailment of costs.

Microcomputed tomography is well established as an imaging modality for the imaging of prepared fossils. Because of its potential to penetrate breccia more effectively by using high energy X-rays, it might be considered the modality of choice for breccia triage.

This study shows that in the application of breccia triage, the penetrating ability of a lower energy beam is not detrimental to the outcome.

Given the many other limitations of μXCT faced by the Malapa team, it is of significant advantage to researchers interested in a high throughput of potentially low value material, in search of high value material (as is the case with using CT scanners to triage potentially fossil-bearing blocks), that the differential penetration of lower and higher energy beams does not have a significant impact on image quality and that XCT is overall the better choice over μXCT for this application. The study does not seek to generalise the contribution of XCT and acknowledges that for other applications, μXCT may be the modality of choice. But for the purpose of fossil identification within large rocks and for breccia triage for the breccia originating from the Malapa site, it has been shown that XCT is superior to μXCT for this particular palaeontological application. Application of these findings can now be expanded to breccia from other fossil sites.

Additionally, as the effects of radiation on ancient DNA are still not clear, consideration should be given to the accumulative dose of radiation to which an individual fossil is exposed. It is recommended that the lowest possible dose necessary to achieve the desired outcome is used, as well as the lowest resolution possible to achieve the desired result. The dose increases at about the square power of the increase of resolution.[18] Thus, another reason to advocate the application of lower energy scanning over higher energy scanning for breccia triage is that the potential and ability to access ancient DNA from fossil specimens still needs research and elucidation.

Use of medical CT scanning of fossil-bearing breccia is thus an alternative to random block preparation. In order to maximise the use of limited resources and manual preparatory skills, as well as to curtail costs, this research shows that, prior to manual preparation, blocks should undergo scanning with medical CT scanners and virtual assessment of contents should be undertaken to allow for prioritisation of rocks for manual preparation.

Triage of fossil-bearing breccia using medical CT scanners will shorten the time from breccia removal from the field to the retrieval of relevant fossil specimens for interrogation, publication and dissemination of their information.

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Appendix 1: Scoring of high energy images per breccia block, per object, per reader, per criterion

Blocks	Readers														
	1			2			3			4			5		
UW88 numbers	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact
1343-1	2	2	3	3	2	5	2	2	2	1		1	2	2	2
1343-2	1	1	2	5	5	5	2	2	4	1		5	2	2	3
1343-3	2	2	4	2	2	5	3	3	2	1		5	2	2	3
1456-1	2	1	4	1	1	3	2	3	3	1		5	2	2	2
1456-2	3	3	3	4	3	4	2	2	2	1		1	2	3	2
1456-3	3	3	4	5	5	4	2	2	2	2		5	2	1	2
1461-1	1	1	4	5	5	5	1	1	2	1		5	2	2	2
1461-2	1	1	4	1	1	5	1	1	2	1		5	2	1	2
1461-3	2	2	4	1	1	2	1	1	1	1		3	3	3	3
1485-1	1	1	4	1	1	5	2	2	2	1		4	2	2	2
1485-2	3	3	4	3	3	5	2	2	1	2		5	3	2	3
1485-3	3	3	4	3	3	5	2	2	2	2		5	3	3	3

Blocks	Readers														
	1			2			3			4			5		
UW88 numbers	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact
1506-1	2	1	4	3	3	5	2	2	2	2		5	3	3	3
1506-2	2	1	3	3	3	4	2	2	2	2		4	2	3	2
1506-3	2	2	4	2	2	4	2	2	2	2		4	2	3	2
1519-1	1	1	4	1	1	5	4	2	2	1		3	2	2	2
1519-2	3	3	4	4	4	4	4	2	2	3		1	3	3	3
1519-3	1	1	5	1	1	5	3	2	3	1		1	2	2	2
1655-1	4	3	5	3	3	5	4	3	3	2		5	2	3	3
1655-2	4	4	5	3	3	5	4	3	4	3		5	2	3	3
1655-3	3	3	4	3	3	4	3	2	3	3		4	3	2	2
1781-1	3	2	5	3	3	4	2	1	2	3		5	2	3	3
1781-2	1	1	4	1	1	5	2	1	2	1		5	2	2	2
1781-3	2	2	5	2	2	3	1	1	2	1		1	2	2	2
2206-1	2	2	4	4	4	4	4	2	2	4		1	2	1	2
2206-2	3	3	4	3	3	5	3	2	3	4		5	2	2	2
2206-3	2	1	3	3	3	4	4	2	3	4		5	2	1	2
2255-1	2	2	4	3	3	4	3	2	3	2		5	2	2	2
2255-2	1	1	4	1	1	4	2	1	2	1		5	2	2	2
2255-3	2	2	4	3	2	5	2	1	1	2		5	2	2	2

Blocks	Readers														
	1			2			3			4			5		
UW88 numbers	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact
2314-1	1	1	5	2	2	5	2	2	2	1		1	2	2	2
2314-2	1	1	4	1	1	5	2	2	3	1		1	2	1	2
2314-3	3	3	4	4	4	5	2	2	2	2		1	2	1	2
2318-1	3	2	3	3	2	3	2	2	2	2		5	2	2	2
2318-2	1	1	4	1	1	5	2	2	3	1		5	2	1	1
2318-3	3	3	4	4	4	4	2	1	2	1		1	2	3	3
2370-1	3	3	4	2	2	5	2	2	3	2		5	2	3	3
2370-2	1	1	4	1	1	5	2	1	2	2		5	2	2	2
2370-3	3	2	4	3	3	5	2	2	2	3		5	2	2	2
3195-1	1	1	3	1	1	5	2	4	4	1		5	2	1	2
3195-2	1	1	4	1	1	5	2	3	2	1		1	2	2	2
3195-3	1	1	4	1	1	5	2	3	2	1		5	2	1	2
3728-1	1	1	4	4	4	4	2	2	3	1		3	2	1	2
3728-2	3	2	4	3	3	3	2	1	2	1		3	2	2	2
3728-3	1	1	4	1	1	5	2	1	2	3		3	2	2	2

Appendix 2: Scoring of lower energy images per breccia block, per object, per reader, per criterion

Blocks	Readers														
	1			2			3			4			5		
UW88 numbers	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact
1343-1	4	4	5	5	5	4	4	3	4	2		4	3	2	3
1343-2	4	4	5	4	4	4	2	2	4	3		4	2	3	3
1343-3	4	4	5	3	3	3	4	4	5	2		4	2	3	3
1456-1	4	3	5	4	4	4	4	3	4	2		4	3	3	3
1456-2	4	4	4	5	5	4	3	3	5	3		4	3	2	3
1456-3	4	4	4	4	3	3	4	3	4	2		4	3	3	3
1461-1	3	3	5	2	1	5	4	4	4	1		5	2	2	3
1461-2	3	3	5	4	3	4	4	4	4	2		4	2	2	3
1461-3	4	4	4	5	4	4	4	4	4	2		4	2	1	2
1485-1	4	4	3	5	3	4	5	4	5	3		1	2	2	2
1485-2	4	3	4	4	5	5	4	3	4	2		5	2	3	3
1485-3	4	3	4	4	3	5	4	2	3	1		5	2	3	3
1506-1	3	3	5	3	4	4	3	3	4	1		5	2	3	3
1506-2	3	3	5	3	4	5	4	3	5	2		5	2	3	3
1506-3	3	3	5	3	3	4	3	4	4	1		5	2	3	2
1519-1	2	2	4	2	2	5	5	2	4	1		5	4	3	3
1519-2	5	4	4	5	5	4	5	2	4	3		1	4	2	2
1519-3	3	3	5	5	4	5	5	3	4	1		3	4	3	3

Blocks	Readers														
	1			2			3			4			5		
UW88 numbers	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact
1655-1	4	3	5	3	4	5	3	2	4	1		5	2	2	2
1655-2	4	4	5	5	5	5	3	2	5	2		5	2	3	3
1655-3	3	3	4	4	3	4	4	3	4	2		3	2	2	2
1781-1	4	3	5	5	5	5	3	3	2	3		5	4	4	3
1781-2	4	3	5	5	5	5	4	3	3	2		5	4	4	3
1781-3	5	4	4	5	5	4	4	3	3	3		1	4	2	2
2206-1	5	4	4	5	5	5	3	4	4	2		5	2	2	2
2206-2	4	3	5	4	4	3	3	3	3	2		5	3	3	3
2206-3	3	3	5	3	3	5	3	3	4	2		5	3	3	3
2255-1	4	3	5	5	5	5	5	2	4	2		5	3	3	3
2255-2	2	2	5	4	4	5	5	2	5	2		5	4	3	3
2255-3	3	3	5	4	4	5	5	2	4	2		5	4	3	3
2314-1	2	2	4	5	5	5	3	3	4	2		4	2	3	3
2314-2	2	3	5	3	2	4	4	4	4	1		4	2	3	3
2314-3	5	4	4	5	5	4	3	3	4	3		1	2	2	2
2318-1	4	3	5	5	4	5	3	3	4	2		5	2	3	3
2318-2	3	3	5	3	2	5	3	2	4	2		5	2	2	2
2318-3	5	4	4	5	5	4	3	2	4	3		1	2	2	2

Blocks	Readers														
	1			2			3			4			5		
UW88 numbers	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact	Image quality	Certainty	Artifact
2370-1	4	3	5	5	4	5	4	2	4	2		5	2	2	2
2370-2	2	2	4	2	2	4	4	3	4	1		5	2	2	2
2370-3	4	3	5	5	5	5	3	3	4	2		5	3	3	3
3195-1	2	2	5	2	2	5	4	3	4	1		5	2	2	2
3195-2	2	2	5	4	3	5	4	2	3	1		3	2	2	3
3195-3	2	2	4	2	2	5	4	3	3	1		5	2	3	3
3728-1	3	3	5	3	3	5	3	4	4	1		3	3	2	2
3728-2	3	3	5	5	4	5	3	4	4	1		3	3	3	3
3728-3	2	2	4	3	3	4	3	3	4	1		3	3	3	3

5. SUBMITTED FOR PUBLICATION - UNDER REVIEW

5.1 How air changed fossil preparation – a case report from Malapa, South Africa.

This case study illustrates how pre-preparatory XCT imaging of fossil-containing breccia from the site of Malapa can focus both preparation and interpretation of findings. The importance of pre-preparatory XCT imaging is demonstrated by the fact that preparatory techniques and protocols were modified from traditional methods in order to minimize the risk of contamination of possible biomolecules. Analysis of the images resulted in the discovery of critical information that allowed for at least two further major discoveries to be made. Critical contributions to dietary habits of this hominin (Henry *et al*., 2012) and the presence of possible organics (Keeling, 2017) may have been hampered by contamination or damage if the presence of an air layer, seen on XCT, had not focused attention on the areas/ideas prior to preparation. Preparatory techniques were modified to address questions raised by the presence of air noted on the XCT images. This maximised the information extracted from the contents in order to address the current research questions. Until recently, recovery and analysis of genetic information encoded in ancient DNA sequences from hominin fossils were impossible. Recent advances in molecular biology offers technical tools to obtain ancient DNA sequences from well-preserved fossils and has opened the possibilities to directly study genetic changes in fossil species to address various biological and paleontological questions.

Without the *in-situ* visualization of the skull within the encasing breccia, the presence of the air spaces would have gone undetected. Manual preparation would

not have identified them as present or significant and traditional preparation methods may have hindered the interpretation of macro-information (dermal tissue, dental calculus, potential hair) or even possible biomolecular analysis, that remains untested to date.

Revision is needed of the peri- and post-excavation treatment of fossil bones to better preserve the potential of genetic heritage of the past. The procedures for handling and storage of the fossil material used here, should be applied routinely to the fossils from Malapa and could be extended across other palaeoanthropological and palaeontological studies, particularly in future investigations of assemblages that may contain residues or remnants of soft tissues.

HOW AIR CHANGED FOSSIL PREPARATION - A CASE REPORT FROM MALAPA, SOUTH AFRICA

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ABSTRACT

Preparators involved in the manual preparation of fossil material require dexterity skills and patience as well as a good knowledge of anatomy, geology and sedimentology in order to maximize the precision of the fossil extraction process. Information not recognized during this preparation process may be lost. This case study illustrates how pre- preparatory computed tomography (CT) imaging of fossil- containing breccia from the site of Malapa focussed both preparation and interpretation of findings and allowed for retrieval of biomolecules that might have been contaminated by or overlooked with traditional methods.

The identification, on CT images, of air spaces adjacent to the cranial bones and teeth of the skull of *Au. sediba* MH1 facilitated the search for possible organics adjacent to the former and the finding of calculus adjacent to the latter. Critical contributions to dietary habits of this hominin and the presence of possible organics may have been hampered by contamination or damaged if the presence of an air layer, seen on CT, had not focused attention on the areas/ideas prior to preparation.

This maximised the information extracted from the contents in order to address the current research questions.

Key words

Medical CT, hominin, *Au sediba*, non-invasive, breccia, preparation protocols

HOW AIR CHANGED FOSSIL PREPARATION - A CASE REPORT FROM MALAPA, SOUTH AFRICA

Medical computed tomography (XCT) scanning, prior to preparation of breccia blocks from the site of Malapa, has been shown to be valuable and accurate in predicting fossil contents of breccia (Smilg and Berger, 2015). This case study demonstrates how knowledge, ahead of preparation, can focus researchers' attention to discoveries that may be overlooked or destroyed during traditional mechanical preparation methods. The implementation of regular XCT scanning on fossils from brecciated contexts prior to preparation, allows for specific protocols to be employed during the course of preparation in order to preserve suspected areas of importance and potentially minimize possible contamination of specific areas that may be useful for studies that have not been traditionally thought important to ancient fossils.

During the search for Plio-Pleistocene-aged fossils in southern Africa, particularly in karstic situations, field expeditions often yield rocks that could potentially contain fossils. In laboratories that deal with these, the traditional practices of preparation of these rocks have hinged on manual or chemical extraction of the fossils they very often contain. Often, fossilized bone and potential areas of preserved anatomy around these bones, are hidden within the block, invisible to the naked eye. Proper recovery procedures, however, have the potential to maximize the amount and quality of biological and cultural information a block, or a fossil within a block, might yield.

Early publications dealing with fossil preparation, recognized that for a preparator to apply the best methods of discovering and removing fossils from rocks, the preparator should have a thorough knowledge of anatomy, geology and sedimentology as well as manual dexterity skills and patience (Whybrow, 1985).

Mechanical preparation utilizes tools that apply external physical force to the matrix in order to expose possible fossil bones to both identify them, and eventually release them from the encasing matrix. This can be a complex task requiring an understanding of forces, materials and their interactions and may requires the preparator to "guess" at anatomy yet to be revealed, based on the preparator's personal level of experience. Fossil bone, not having the internal strength of fresh bone, can be easily damaged during this effectively "blind" process of preparation. The difficulty of preparation can further be complicated by the difficulty of visually distinguishing encasing matrix of no biological or anatomical importance from fossil bone (Wylie, 2009).

Chemical extractions utilize various compounds to dissolve the surrounding matrix, but these can slowly dissolve bone as well as invisible, but important, biological information such as DNA or non-bony organic remains, so not only the fossil bone itself needs to be protected before being subjected to the chemicals, but sometimes the surrounding matrix should be conserved.

'You only see what you look for, you only look for what you know' is a saying used by radiologists trained to look at complex images. Hence knowledge is the key to successful recognition. This can, and should, be applied in the field of fossil preparation. Ravetz (Ravetz, 1996) cites technicians as a potential source of error in science because they often only recognize anticipated data results, so when the unexpected and contrary results appear, the technician may make a judgement that could affect the outcome for that fossil, and potentially for unrecognized information it may hold.

Many of the tools and techniques used by today's preparators have changed little since the origins of the field. However, in recent years new technologies have been applied pre- and during preparation of these fossils.

When applied to fossil hominins particularly, computed tomography (CT), particularly micro CT (µXCT), has been extensively used in assessing fossils which have been prepared, or partially prepared, from the matrix in which they were found. The use of CT in the analysis of fossils that have undergone preparation from their encasing matrix, has become common place (Balzeau, 2010). The widespread application of CT to fossil-bearing breccia that have not yet having undergone preparation, however, has lagged behind that for the study of fossils that have been prepared from the surrounding matrix.

The U.W. 88-1355 block from Malapa serves as an important case study in the history of the science of palaeoanthropology, as it demonstrates the importance of pre-preparation visualization of fossils within blocks. During routine manual preparation of breccia block U.W.88-1355 from Malapa, a very small portion of a probable maxilla was revealed by manual preparation. This maxilla appeared to be hominin. Under normal practices, this block would have continued with manual preparation without any imaging. However, the block was subjected to XCT imaging in an attempt to better visualize the probable maxilla that was identified at the start of manual preparation. Due to its potential importance and prior to further preparation, better visualization was sought of what might be hidden from the preparator's view. The resultant images demonstrated that the visualized maxillary bone was part of a juvenile cranium (later to be described as the type specimen of the hominin species, *Au. sediba* Malapa hominin 1 *(*MH1) (Berger et al., 2010).

During analysis of the XCT scans of the cranium, unusually good visualization was noted and analysis attributed this to the presence of air between much of the fossil skull and the encasing breccia. This air space allowed for the interface between fossil and matrix to be clearly differentiated radiologically. Air spaces were particularly noted to be preserved associated with the cranial vault (Fig. 1) and with the dentition (Fig. 2). These air spaces directed researchers' attention to these areas, raising questions about the possible reasons for their existence. It is important to note that these air spaces would have gone undetected during normal manual preparation as they would have been destroyed during the course of "blind" preparation.

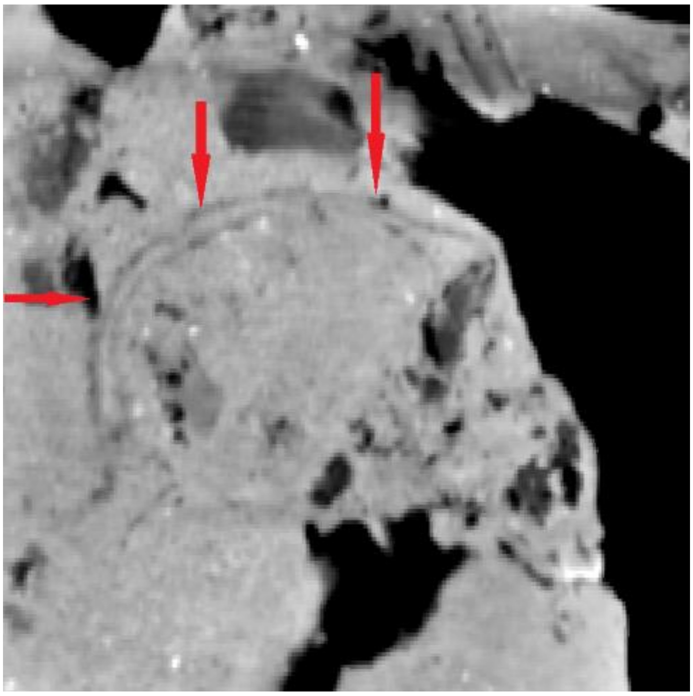

Figure 1: U.W.88-1355 - XCT slice showing sagittal projection of skull encased within the breccia. The air space (arrows) around the cranial vault enhances the visualization.

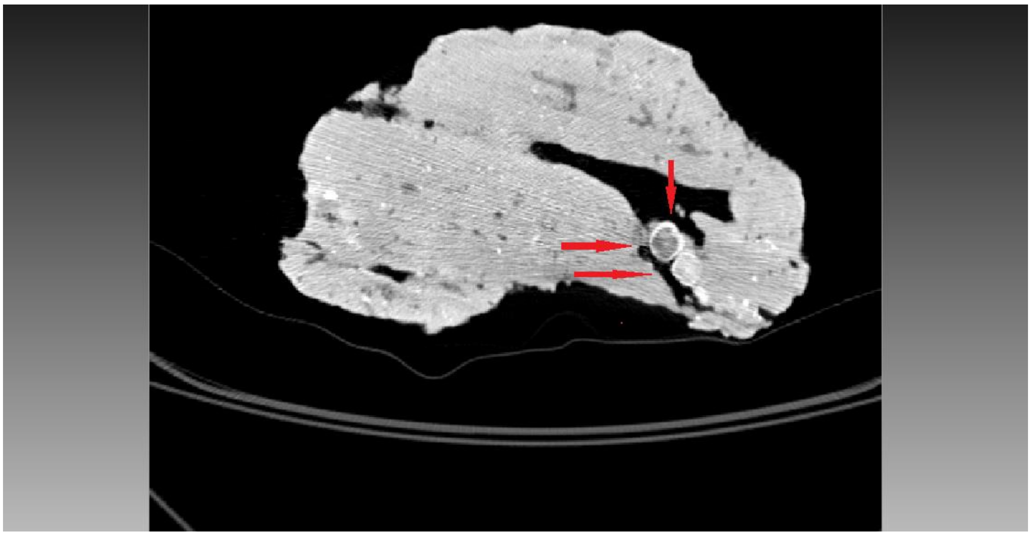

Figure 2: U.W. 88-1355 - XCT slice showing two teeth in axial projection with adjacent air space (arrows) focusing researchers' attention to this uncontaminated area.

In order to address the question of why there were air spaces in the immediate presence of specific areas of anatomy, deep within solid rock, it was important to approach the preparation of the highlighted areas differently to normal practice. Normal mechanical preparatory methods would not have identified this air layer and preparation would have continued down to the fossil surface immediately, with possible destruction and/or contamination of the area resulting in the loss of potential information associated with the area immediately above the surface of the fossil.

In order to access these areas of potential biological interest, near to or adjacent to the fossil bones that were associated with air spaces, without damaging or contaminating them, preparation was carefully done down to, and around the air spaces. The calcified clastic matrix surrounding the air spaces was removed intact. To ensure that the fossil material was kept in an environment that minimized contamination or damage, additional handling and storage protocols, beyond those normally used, were set by the investigating scientists. All scientists handling the material were required to wear latex gloves and surgical masks. The specimens were enclosed in aluminium foil and stored in vacuum-sealed plastic bags. Each specimen was then placed in its own plastic container and these locked in a temperature -controlled safe within the laboratory, for additional protection. This ensured that only the primary scientific team members had access to the specimens, thus ensuring the handling protocols set were strictly followed when the material was examined. These protocols would not have been followed if the areas of interest had not been identified using XCT prior to preparation. In fact, despite the experience and skills of the preparator and the supervising scientists, it is highly likely these areas of biological interest would have been destroyed.

The origin of the air pockets observed with MH1, directly associated with the regions of interest, may be explained by potential similarities to forensic studies, where in a calcite-rich environment a concrete crust possibly formed over the remains, protecting them – and as the remains decomposed, or after the bloat stage, an air-pocket remained (Schotsmans, 2011).

In the case of the areas of interest around the dentition, focussed preparation, limiting external contamination of the dental surfaces, showed staining of the associated teeth. This staining was identified to be dental calculus (Henry et al., 2012), and resulted in the first recognition of plant food material held within calculus for an ancient South African hominin.

Preserved plaque build-up around the edges of the teeth is a pathology not studied prior to this instance in early hominins. This study yielded the first direct evidence of hominin dietary behaviour (Henry et al., 2012), as researchers determined the partial diet of these hominins by analysing tiny plant phytoliths found in this calculus. Never before had the feeding ecology of South African early hominins been investigated in this manner. Of course, early hominin diet is central to the study of human origins in general, and the interpretation of the palaeoenvironment in which these hominins existed. This dental calculus likely could have been destroyed, or at least contaminated during preparation, if not for first recognizing the air spaces on XCT.

Examination of the cranial vault sample of the same specimen highlighted by the air pocket, showed a reddish-brown discoloration on the surface of the calcified clastic matrix as well as what appeared to be anatomical features. The residue had a thickness to it, which together with its location, suggested the possibility that soft parts in the form of dermal tissue might have been preserved, compressed against the rock during fossilization (Keeling, 2017).

Once again, prior knowledge of this space allowed researchers to investigate whether indeed this was fossilized dermal tissue and whether original organics could be recovered. Examination of the adjacent preserved rock and the bone at this area revealed the possibility of mineralized skin tissue and the body of evidence suggests that the specimens are indeed organic in origin (Keeling, 2017). This examination is the first investigation into potential soft tissue preservation in the early hominin record and is of significant importance.

These two cases indicate that both non-traditional biological remains (in the first instance dental calculus, and the second dermal tissue) can offer the possibility that potential biomolecules, preserved with the remains, can be recovered. These previously unrecognized areas of importance are now of potentially great value to science. They may prove to be sources of ancient proteins or even be the possible source of ancient DNA. The analysis of ancient DNA has the potential to provide answers to archaeological, paleontological, and anthropological questions. But DNA preservation is, by its very nature, rare and the analyses are often limited, due to inefficient recovery of ancient genetic material or even the destruction of the areas that potentially hold proteins or DNA prior to their recognition. The case study of the Malapa hominin cranium, and the discovery of critical areas of interest demonstrates that not only should care be taken to avoid direct handling of the material that will be used for analysis (Pruvost et al., 2007), but the XCT offers a further tool in the hands of the investigating scientist to preserve areas of potential interest and importance.

Conclusion

This case study highlights the value of XCT scanning and image analysis prior to manual preparation of breccia from the site of Malapa and thus demonstrates its value in general in this particular record in southern Africa, and perhaps in other regions where similar fossilization events occur. Preparatory techniques were modified to address questions raised by the presence of air spaces noted on the XCT images and these resulted in two significant discoveries related to palaeoanthropology. The XCT scans focused attention on aspects of the rocks and fossils that could have been contaminated, damaged or even destroyed during unfocussed manual preparation. The identification of these airspaces highlighted the regions of the cranial vault surface and the teeth, focussing thought as to their origin and allowing potential destruction, or further contamination of these structures to be limited prior to exposure and analysis.

Without the *in-situ* visualization of the skull within the encasing breccia, the presence of the air spaces would have gone undetected. Manual preparation would not have identified them as present or significant and traditional preparation methods may have hindered the interpretation of macro-information (dermal tissue, dental calculus, potential hair) or even possible biomolecular analysis, that remains untested to date.

The importance of pre-preparatory imaging of fossiliferous breccia with XCT is demonstrated by this study which resulted in the discovery of crucial information that allowed for at least two further major discoveries to be made. Critical contributions to dietary habits of this hominin and the presence of possible organics may have been hampered by contamination or damage if the presence of an air layer seen on XCT had not focussed attention on the areas/ideas prior to manual preparation.

Until recently, recovery and analysis of genetic information encoded in ancient DNA sequences from hominin fossils were impossible. Recent advances in molecular biology offers technical tools to obtain ancient DNA sequences from well-preserved fossils and has opened the possibilities to directly study genetic changes in fossil species to address various biological and paleontological questions.

Revision is needed of the peri- and post-preparation treatment of fossil bones to better preserve the potential of genetic heritage of the past and this should include routine XCT of blocks that may contain fossils. The procedure for handling and storage of the fossil material used here, could be applied routinely to palaeoanthropological and palaeontological studies in the region, particularly in future investigations of assemblages that may contain residues or remnants of soft tissues.

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6. CONCLUSION

In 1974 CT was used for the first time in palaeontological research (Fourie, 1974). Over time technical improvements were largely responsible for its successful use in palaeontology. It has been recognized by scientists that CT was able to reliably acquire interior information non-destructively from irreplaceable fossil specimens (Conroy and Vannier, 1984, Haubitz *et al*., 1988).

The term "computational palaeontology" was introduced in 1996 by Oyvind Hammer (Elewa, 2011), a Norwegian palaeontologist and mathematician. The term referred to the use of mathematical models, simulation and computers in palaeontology. Palaeontological science and radiological techniques have a long history of collaboration starting from the discovery of X-ray (Fourie, 1974). The inclusion of these biomedical diagnostic tools into anatomical and palaeontological works is rapidly expanding. Palaeoradiology has been used to describe the study of palaeontological material using modern imaging methods (Tafforeau *et al*., 2006). CT scans allow researchers to move beyond the external observation, physical handling and mechanical treatment of fossil remains.

There are however limitations to the use of CT in palaeontological research, many of these are being overcome as hardware and software improvements occur and advances in technology are made. The CT image can be manipulated and visualized depending on the required application. Often 2D analyses along orthogonal planes are sufficient for skeletal structures, but additional information can be gained from 3D image reconstructions. Traditionally these 3D reconstructions are calculated from CT values based on CT grey scale numbers. If quantitative measurements are needed from the CT data, segmentation techniques are often necessary to separate features of interest based on criteria other than CT values, as use of CT numbers may be complicated by partial volume averaging effects (Peyton *et al*., 1992). Manipulation of CT parameters at the time of scanning, to lessen imaging artifact, can hinder precise image acquisition (Spoor and Zonneveld, 1994). Partial voluming and limits in spatial resolution are important constraints of CT (Spoor and Zonneveld, 1995). Scientists have tested and validated the accuracy of these latter 2 constraints as regards the accuracy and reproducibility of measurements and the definition of landmark coordinates (Richtsmeier *et al*., 1995, Zollikofer *et al*., 2005, Coleman and Colbert, 2007).

With specific focus on hominin fossils, CT has been applied to this area of palaeontological research in the following ways:

1) **Virtual fossil reconstruction** (Brunet *et al*., 2002, Zollikofer *et al*., 2002, Zollikofer *et al*., 2005). Hominin fossils are often fragmented and distorted following manual preparation techniques. Physical reconstruction may be invasive, destructive and irreversible. It is highly subjective and reliant on the skills of individual preparators. CT imaging of these prepared fossils allows this reconstruction to take place in a virtual environment, eliminating many of the former problems (Zollikofer and Ponce de Leon, 2005).

2) **Virtual endocast studies and brain morphology** (Conroy *et al*., 1998, Falk *et al*., 2005, Carlson *et al*., 2011, Berger *et al*., 2015). Naturally occurring fossilized endocasts are rare, but these endocasts supply the most direct evidence for studies of hominin brain evolution which allow scientists to make inferences about functional anatomy, physiology, and phylogenetic relationships (Wu *et al*., 2007). CT production of brain endocasts facilitates palaeoneurology studies.

3) **Biomechanical analysis of the hominin skeleton** (Daegling and Grine, 1991, Ruff, 1994, Galik *et al*., 2004). CT has allowed analysis of fossil mandibles and skeletons that in turn allows comment on masticatory and locomotive functions respectively.

4) **Early hominin health studies** (Zollikofer *et al*., 2002, Gardner *et al*., 2004, Ryan and Milner, 2006, Odes *et al*., 2016, Randolph-Quinney *et al*., 2016, Odes *et*

al., 2017). CT is an excellent tool to investigate hominin health issues as it clearly images the anatomy of internal and external structures.

5) **Skeletal and dental microanatomy** (Spoor *et al.,* 1993, Spoor *et al*., 1994, Manzi *et al*., 2001, Prossinger et al., 2003, Olejniczak and Grine, 2005). CT is particularly suited to imaging micro anatomical structures e.g. Sinuses, inner ear and microanatomy of teeth.

6) **Skeletal morphology**

There is a move towards the combined use of various CT machines – scanning material at different resolutions. A rock can be scanned at full size with moderate resolution on a medical scanner and then micro focus and synchrotron CT analysis done of sub samples of different sizes.

The non-destructive nature of CT has huge advantages to the research potential of specimens in museum collections and can contribute to global scientific collaboration and communication (Matthews, 2016). CT scanning can facilitate data sharing amongst scientists. Diverse CT systems with differing performance characteristics are designed for different functions and it can be hard for researchers to choose the most appropriate CT machine (Scherf, 2013). The CT method of choice for a project depends on the research question and the structure's dimensions that are going to be investigated.

This research involving the Malapa breccias that underwent XCT imaging prior to preparation, convincingly supports the implementation of routine XCT imaging in the early stages of fossil discovery while fossils are still embedded in surrounding matrix. It supports the use of XCT in the identification and analysis process for the Malapa breccia and should be considered in the investigation of fossil-bearing conglomerates from other sites.

The publications presented for this doctorate represent a unique body of research that makes a significant and original contribution to the field of radiopalaeoanthropology.

Never before has the use of XCT been investigated for the application of breccia triage on this scale. For the first time in South African palaeoanthropological work, hominin fossils have been imaged within their matrix before any preparation had been performed. The results of this study have shown conclusively the viability and value of the use of XCT imaging to assess possible fossil-containing rocks for fossil remains.

The second issue addressed was the comparison of µXCT to XCT for pre-preparatory assessment of fossiliferous breccia from Malapa. µXCT is a modality that is routinely applied to image fossils that have been prepared out of and cleaned of the surrounding matrix. For the virtual assessment of prepared fossils, µXCT is undoubtedly superior, in most instances, to XCT. However, for the assessment of large, unprepared breccia blocks, XCT was shown to be as good as, if not superior to µXCT. Results showed that lower energy images are deemed superior to higher energy images for this particular application. This finding, taken together with the limitations associated with the use of µXCT for the imaging of the large breccia from Malapa, shows that XCT is the better modality for this specific application.

The use of XCT imaging prior to fossil preparation focused attention on and directed preparation to areas that would have gone unnoticed if traditional preparatory methods had been routinely applied. Protocols were modified to address the preservation of material to allow the search for possible biomolecules.

Revision is needed of the peri and post excavation treatment of fossil bones to better preserve the potential of genetic heritage of the past and this research demonstrates the role that XCT can play.

None of the research covered by this body of work has been done before on fossil bearing matrices. This research should significantly change the way fossil discovery, recovery and preparation is done in the South African context and has potential for application in other palaeontological situations.

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