Palaeontologia Electronica
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NEUTRON TOMOGRAPHY OF INTERNAL STRUCTURES OF VERTEBRATE REMAINS: A COMPARISON WITH X-RAY COMPUTED TOMOGRAPHY

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ABSTRACT

Neutron tomography has been applied as a novel, non-invasive technique for 3-D visualization and characterization of internal structures of vertebrate remains. Whereas X-ray computed tomography maps regions of different densities within an object, neutron tomography is sensitive to differences in the concentration of some light materials like hydrogen. Compared to X-ray computed tomography (CT), the image quality of neutron tomography (NT) is strongly influenced by the resin materials used for the reconstruction and conservation of the objects. Stabilizing metal inclusions in bones leads to a strong scattering of X-rays in CT, whereas these inclusions can be better penetrated and therefore lead to less disturbing contrasts in NT. The maximum cross-section of rocks and fossilized bone material that can be penetrated by X-rays in a medical CT scanner is approximately 40-50 cm, whereas neutrons can penetrate rocks or fossilized bone material with a cross-sectional thickness up to about 12 cm. The spatial resolution of NT (0.1-0.27 mm) is better than in a medical CT scanner (0.35-0.5 mm). In the special case of the studied sauropod vertebrae, the density of the fossil bones, the high amount of marly sediment matrix within openings of the bones, and the presence of multiple fractures filled with glue decreased the quality of the neutron tomographic images. On the other hand, neutron tomographic images displayed a detailed account of the distribution of glue within the fossil remains. The combination of computed tomographic and neutron tomographic data therefore helped to increase the information about the internal structure of sauropod vertebrae. The decision of which technique to use will in the end be dictated by the research questions, the preservation and material properties of the object.

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PE Article Number: 8.2.30A

Submission: 17 June 2004. Acceptance: 16 June 2005

Schwarz, Daniela, Vontobel, Peter, Lehmann, Eberhard H., Meyer, Christian A., and Bongartz, Georg, 2005. Neutron Tomography of Internal Structures of Vertebrate Remains: A Comparison with X-ray Computed Tomography. *Palaeontologia Electronica* Vol. 8, Issue 2; 30A:11p, 800KB;
http://palaeo-electronica.org/paleo/2005_2/icht/issue2_05.htm

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KEY WORDS: Neutron tomography; X-ray computed tomography; sauropod vertebra; concentration; density; sediment; glue

INTRODUCTION

The technology of tomography provides information about the material composition in cross-sections through an object. Tomographic procedures therefore provide non–invasive three-dimensional (3-D) visualization and characterization of objects from transmission data. X-ray computed tomography (CT) and neutron tomography (NT) have been used since the 1970s (Lehmann et al. 2000), but hitherto mainly CT has been applied to investigate palaeontological objects (e.g., Britt 1993; Brochu 2003; Conroy and Vannier 1984; Ketcham and Carlson 2001; Rowe et al. 1999; Tykoski et al. 2002). A research project on sauropod biomechanics at the Natural History Museum Basel/Switzerland gave us the possibility to test NT as a tool for the investigation of fossilized sauropod vertebrae at the Paul-Scherrer-Institute in Villigen/Switzerland and compare the results with CT images.

The vertebrae of sauropod dinosaurs are often hollowed out by a complex system of cavities that is interpreted to be pneumatic (Janensch 1947; Britt 1993; Wedel 2003a, b). Knowing the distribution and pattern of formation of hollow spaces within sauropod vertebrae is important for phylogenetic (Wilson and Sereno 1998; Wedel 2003b), ontogenetic (Britt 1993; Wedel 2003a) and biomechanic (Henderson 2003) challenges. The project at the Natural History Museum Basel comprises a constructional morphological analysis of the axial skeleton of sauropod dinosaurs. It is aimed to reconstruct soft part anatomy like muscles, ligaments and airsac systems of different sauropod taxa as well as to work out the mechanical properties of the vertebrae and ribs and overall flexibility of the axial skeleton with the help of a Finite Elements Analysis (FEA). The results of those reconstructions are used to devise a bracing system of the neck, trunk and tail of several sauropods, and in the end will give new insights into sauropod biomechanics and physiology.

NT and CT are used to get multiple cross-sections through sauropod vertebrae and ribs, to define the distribution, size and development of pneumatic structures within the axial skeleton and to extract 3-D polygon-surface models of the scanned specimens. Traditionally, mainly X-ray examination and X-ray tomography have been applied for investigating internal structures of vertebrate remains, which is probably due to the broad availability of this technique in hospitals and scientific institutions. However, limits exist in the technique of X-ray tomography, for example in the size of the specimens investigated or if metal inclusions are present. Trying possible alternative investigation methods may therefore increase the variety of techniques applicable to certain research questions. Comparisons between X-ray tomographic and neutron tomographic scans are made here to identify: i) if NT is an appropriate method for the investigation of diagenetically altered bone fragments; ii) the benefits and limits of NT analysis of vertebrate remains; iii) differences between CT and NT images; and iv) how differential preservation and preparation of the specimens affects both techniques. The results of those comparisons are outlined below.

THE TECHNIQUES OF CT AND NT SCANNING

X-ray Computed Tomography

The technique of CT works with X-rays interacting mainly with the electron shell of atoms. X-ray attenuation coefficients increase steadily with atomic number Z (Hubbell 1999). Computer tomographic images display differences in density and material Z composition within an object (Van Geet et al. 2000). For a medical CT scan, the object is placed on a table with an X-ray tube (the “gantry“) that rotates around the object’s length axis sending an X-ray fan of 0.5 to 40 mm width. The X-ray beam passes through the object along multiple paths and is recorded on the opposite side by

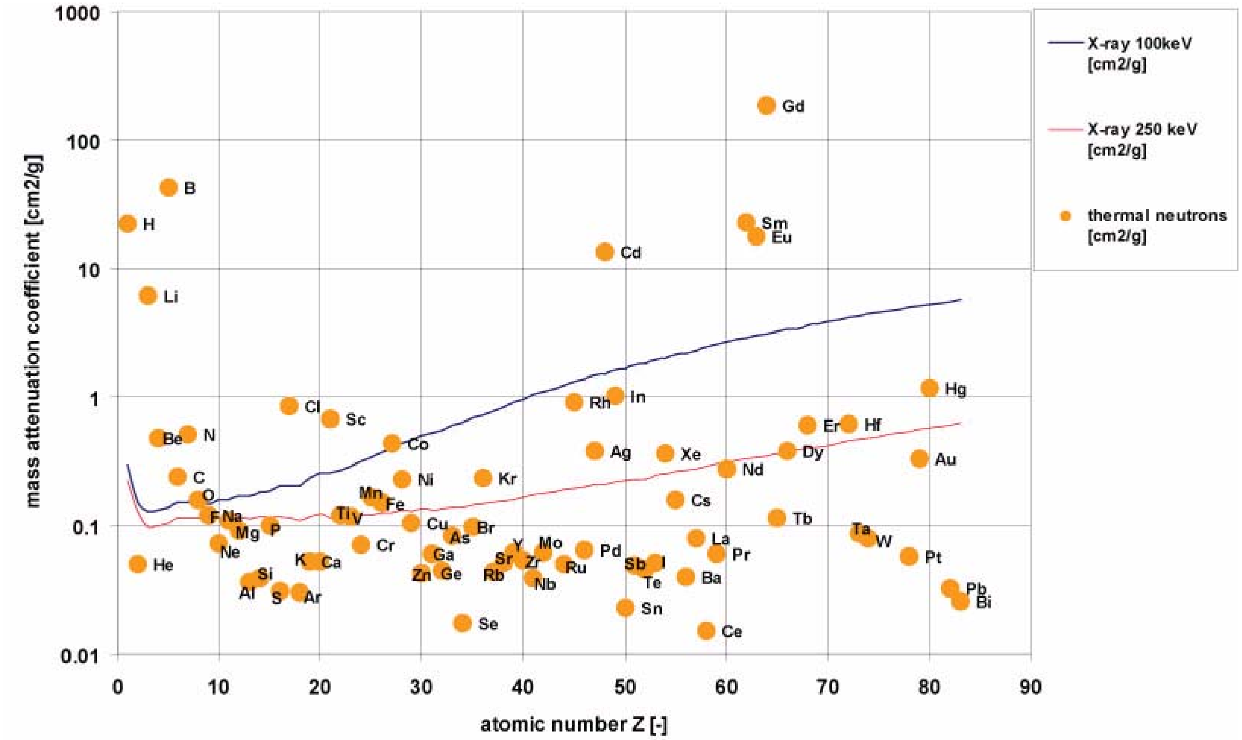

Figure 1. Diagram of X-ray and neutron attenuation coefficient for all elements. X-ray's of 100 or 250 keV penetrate low Z materials easily, whereas high Z materials induce high radiation attenuation (lines). High X-ray energies are needed to penetrate thick geological samples. Thermal neutrons (bullets) are weakly attenuated by silicon or calcium, but show strong attenuation for hydrogen. Even heavy elements are easily transmitted by neutrons. There is no systematic dependence of neutron attenuation with atomic number Z.

detectors, measuring the attenuation of its energy. CT slice images are calculated by using mathematical formulas and algorithms (Kak and Slaney 1988). Recent technology enables decreasing of the slice thickness to less than 1 mm by simultaneous acquisition of multiple slices during one rotation (multi-slice or multi-detector – CT). The option of thin slice imaging is the basis for high resolution imaging with near-to isotropic resolution in all axes. Secondary reconstruction with a 3-D display is helpful for scientific and medical purposes.

The technique of CT was introduced initially as a medical diagnostic tool and found variable applications in vertebrate palaeontology (e.g., Britt 1993; Conroy and Vannier 1984; Rowe et al. 1999). The development of high-resolution industrial-grade CT scanners additionally improved the available image resolution for the objects and provided a technique for the investigation of very small vertebrate remains (Rowe et al. 1999). Exhaustive descriptions of the technique of medical and technical X-ray computed tomography and its application to palaeontological and geological questions are given in Carlson (1993) and Ketcham and Carlson (2001).

Neutron Tomography

Neutron radiography (Domanus 1992) and tomography has been available since 1997 at the Neutron Transmission Radiography Station (NEUTRA http://neutra.web.psi.ch/) of the Paul-Scherrer-Institute (PSI) in Villigen/Switzerland. Like CT the technique is based on the application of the universal law of attenuation of radiation passing through matter. Neutrons carry no electrical charge and interact mainly with atomic nuclei via very short-range forces. Contrary to X-rays, neutrons interact significantly with some light materials (e.g., hydrogenous substances, boron or lithium) and penetrate heavy materials with minimal attenuation. Therefore neutron radiography and tomography is a technique complementary to X-ray radiography and tomography and is particularly sensitive to samples where small amounts of hydrogenous materials occur with weakly interacting materials. The latter yields images with high contrast (Figure 1, Lehmann et al. 1996, 1999).

In the PSI, the neutrons are generated at the "Swiss Spallation Neutron Source" SINQ (http://sinq.web.psi.ch/) by using a particle accelerator to direct a 590 MeV proton beam at a target of heavy metal (lead). The neutrons are then slowed down to thermal energy (~25 meV) in a moderator tank (filled with heavy water D_2O) and extracted by a flight tube forming a collimator, which bundles neutron rays to an almost parallel beam of approximately 35 cm diameter. The beam is transmitted through the object and recorded by a plane position sensitive detector (Lehmann et al. 1996, 1999).

For tomographic images, the object is placed on a rotating table and turned in small angular steps for 180° while a Peltier-cooled CCD-camera system takes several projections. From the single projections, slices perpendicular to the rotation axis are reconstructed by a tomographic reconstruction algorithm using "filtered backprojection." The slices are then collected in an image stack that can be visualized, edited and exported into different file formats using a 3-D rendering software.

MATERIAL AND METHODS

Remains of diplodocid saupropds m the Late Jurassic (Kimmeridgian) Morrison Formation of Wyoming were provided by the Saurier-Museum Aathal/Switzerland for the NT and CT analyses. The sample consisted of five cervical vertebrae and one cervical rib of approximately 10 cm in length, with each belonging to juvenile individuals. A 40 cm long midcervical vertebra of an adult specimen did not fit the rotating table for the NT and therefore was only analyzed using CT. One caudal vertebra of 12 cm length was scanned both with CT and NT to compare with a vertebra without internal cavities.

The fossil bones of all scanned sauropod remains were diagenetically altered (Zocco and Schwartz 1994). With the exception of the caudal vertebra and the cervical rib, all specimens are lateromedially slightly distorted and compressed. The internal cavities, openings and foramina are filled with siliciclastic mud characterized by a calcareous cement (Ayer 2000). The bones are partially badly fractured and most fractures and cracks were filled with quick drying cyano-acrylate resin. The bone surfaces were partially slightly painted and missing parts of the vertebrae have been remodelled with polyester cast resin.

The CT scans were performed with a Multidetector CT-scanner (Sensation 16, Siemens, Erlangen; Germany) at the Department of Medical Radiology of the University Hospital Basel. All vertebrae were scanned with their anteroposterior long axis perpendicular to the scanner rotation axis, so that the resulting image stack runs through the vertebra either from anteriorly to posteriorly or vice versa. Therefore, the maximum path length of X-rays through the largest specimen was 17.9 cm. The parameter setting was 140 kV and 350 MA and a primary collimation of 16 x 0.75 mm. The raw data were reconstructed applying a standard algorithm for human osseous structures using the standard CT imaging processor with the imaging software version VA 70C. The pixel dimensions of the camera were 512 x 512 [pixels], and the resulting field of view was 598 x 323 mm. All CT data were reconstructed in all orthogonal planes at 3 mm thickness and additionally along dedicated planes along anatomical structures. The basic overlapping axial datasets were saved as DICOM files with a defined dimension of 1 x 1 x 1 mm/voxel, and to allow for further 3-D reconstructions. Scanning and data processing took approximately 10 minutes per specimen.

The NT scans were performed in the Neutron Transmissions Radiography Station NEUTRA at the PSI in Villigen/Switzerland (Vontobel et al. 2003). Samples were positioned on a rotary table at position 3 of NEUTRA, i.e., providing a neutron flux of about 3.6 10^6 [n/cm^2/s] and a collimation ratio L/D = 550. While the samples were rotated over 180^o, 240-300 transmission projections were taken. The maximum path length of neutrons through the largest specimen was 12 cm. Neutrons were converted into light by a ^{6}Li based neutron scintillator screen with thickness of 0.25 mm, which was imaged with a 1024 X 1024 pixel CCD camera (DV434) from Andor technology (http://www.andor-tech.com). The resulting field of view was 279 x 279 mm. After exposure normalization and flatfield correction the projection data were reconstructed into slices by filtered backprojection. The voxel data were saved into DICOM-format with a defined dimension of 0.272 x 0.272 x 0.272 mm/voxel. The time needed for the NT scans was 1.5 to 3 hours per specimen, and together with the data processing was up to 5 hours per specimen.

From both CT and NT scans, resulting tomographic images were edited with ImageJ and OsiriX software for MacIntosh. The quality of the images was improved by individual changes of brightness and contrast, gray levels and sharpening with image processing tools. The image stacks were also exported into movie files (Figures 2, 3). Three-dimensional polygon-surface models (stl-format) were extracted from the NT data with the help of VG Studio software and from the CT data with the help of Mimics 8.0 software.

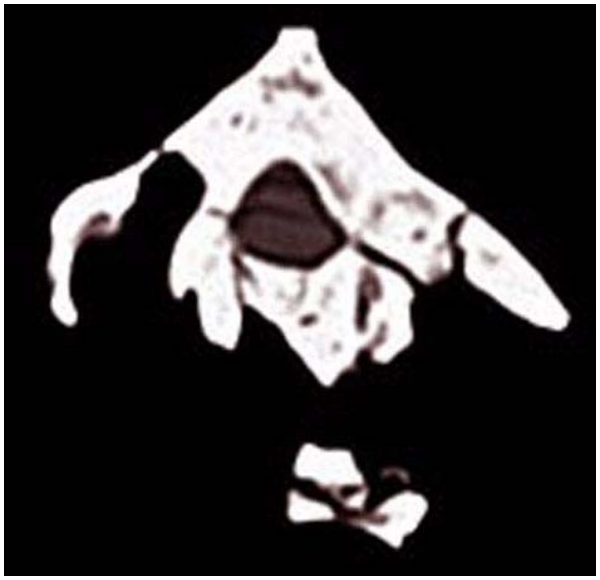

Figure 2. Animation of X-ray attenuation through an axis (No. H25-1) of an undetermined diplodocid. The movie is composed of axial slices from the cranial to caudal regions, taken through the vertebra. For the left lateral view of No. H25-1 see Figure 4a. Green scale bar below the object is 10 cm.

Figure 3. Animation of neutron attenuation through an axis (No. H25-1) of an undetermined diplodocid sauropod. Like Figure 2, the movie is composed of axial slices from cranial to caudal regions, taken through the vertebra. For the left lateral view of No. H25-1 see Figure 4a. Green scale bar below the object is 10 cm.

RESULTS AND DISCUSSION

The CT and NT images differ from each other strongly in quality and displayed contrasts. In the CT images boundaries between cavities filled with sediment and surrounding fossil bone are sharply marked (Figures 2, 4, 5). A significant contrast appears between darkly depicted sediment matrix and brightly depicted fossil bone. Comparatively in the NT images the contrast between sediment matrix and fossil bone is low and boundaries between different materials are blurred (Figures 3, 4, 5). Sediment-filled cavities (e.g., canals and foramina within the vertebrae) are easy to identify in the CT images and difficult to trace in the NT images (Figures 4c-d, 5c). The high contrast between sediment matrix and fossil bone in the CT images mirrors the differences in density and material Z between the two materials (Figure 1, Table 1). In the NT images, the low contrast between fossil bone and sediment matrix is a response to the small difference in the linear attenuation coefficient for thermal neutrons of calcium (i.e., the main constituent of apatite) and silica (i.e., the main constituent of sediment around and within the vertebrae) (Table 1). The additional blur in the NT slices is due to a higher scattering background induced by the hydrogen content of resin material and the use of a neutron area detector. Because both neutron and X-ray attenuation are dependent on the type of sediment, the contrast between fossil bone and sediment would probably be higher with a different sediment composition. For example, in the neutron radiography of an ichthyosaur head embedded in a shale matrix, the bone structures are clearly visible and distinguishable from the sediment matrix (Figure 6).

Table 1. Linear attenuation coefficients of X-ray and neutrons for elements of the bone and marly sediment matrix investigated here.

Element	X-ray mu [1/cm]	neutron sigma [1/cm]
Ca	0.26	0.08
O	0.16	0.17
Si	0.33	0.11
P	0.25	0.12
F	0.14	0.20
H	0.02	3.44
Cl	0.23	1.33

The gray level contrast between fossil bone and larger amounts of polyester cast resin is high in both CT and NT images (Figures 2, 3, 5). In the CT images, glued fractures and smaller cracks in the vertebrae appear dark like the background and are not distinguished from the latter by the X-rays, and very thin fractures are not displayed. However, this effect can be overcome by a different calibration of the CT scanner to bone density and/or using

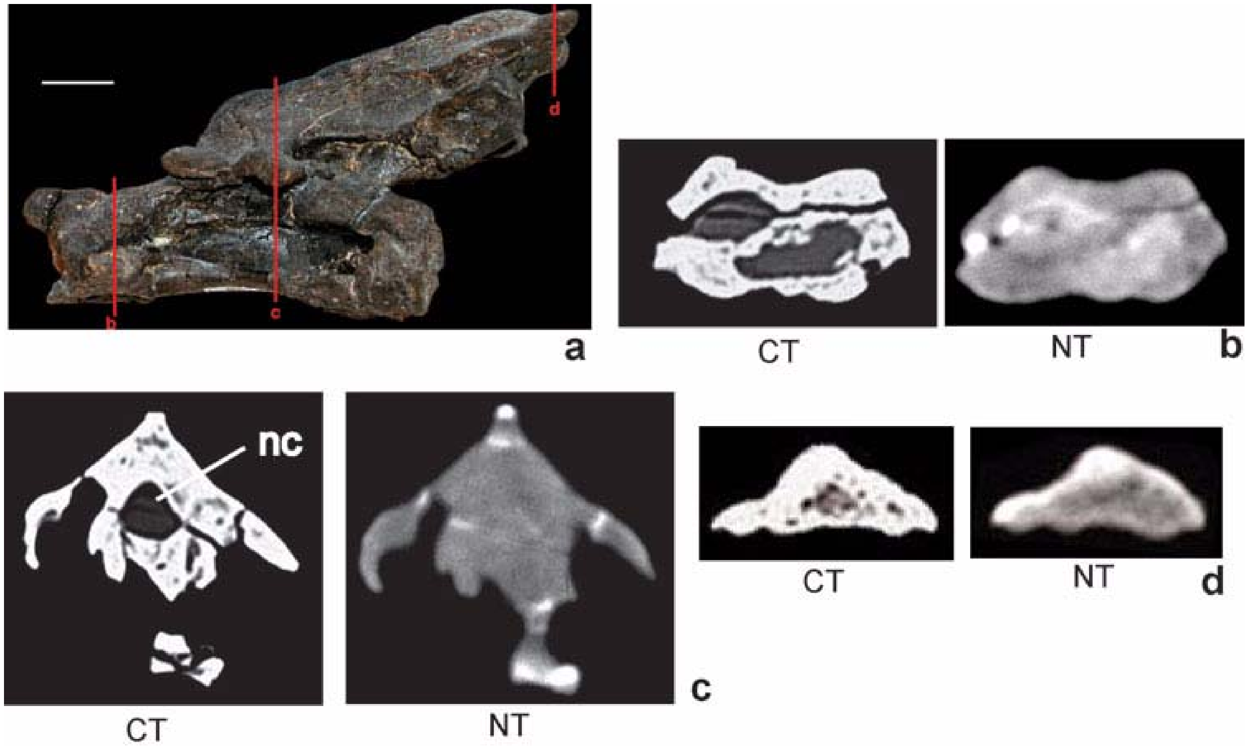

Figure 4. Axis (No. H25-1) of an undetermined diplodocid sauropod a) in left lateral view, red bars indicate axial cross-sections displayed in Figure 4b-d, scale bar (white) is 20 mm; b) axial section through the condyle: in the CT image (left) two large cavities filled with sediment and several smaller foramina are visible whereas in the NT image (right) even the large cavities are difficult to trace; c) axial section through the region of the diapophysis: in the CT image (left) several cavities and foramina are visible whereas the glue (quick drying adhesive) within the fractures is displayed as black as the background, in the NT image (right) the glue is detectable by its bright colour, nc = neural canal; d) axial section through the caudal part of the spinal process, only in the CT image (left) details of internal hollow spaces in the bone are visible.

a high-resolution industrial X-ray scanner (Ketcham and Carlson 2001). In contrast to adhesives or resin, the presence of metal inclusions like iron (e.g., for stabilisation of the bones), would lead to a strong attenuation of the X-rays. Therefore, metal within objects causes a significant noise and decrease of CT image quality (Figure 7). High atomic number elements like metals can be better penetrated by neutrons (Figure 1), resulting in a much smaller decrease of image quality than in a CT image.

In the NT images, filling of cracks and fractures with glue is detectable by its high neutron attenuation shown as very bright regions (Figure 5b). The most blurred boundaries between areas filled with glue and fossil bone are due to diffusion of the glue in the bone matrix surrounding the fractures (Figures 4c, 5b-d) or due to the strongly scattered neutrons in hydrogen. Generally, the more light material like glue or polyester cast resin is present in the remains, the stronger the neutrons are deflected, causing significant noise and a decrease of image quality. This effect could be minimized by the use of a collimating linear neutron detector instead of the neutron area detector. The strong display of glue was helpful in the case of the sauropod vertebrae to distinguish between glue-filled cracks and pneumatic structures, and to show additional small, glue-filled pneumatic canals that were not displayed with CT.

The density of the fossil bone and a maximal thickness of the scanned vertebrae (17.9 cm) influenced image quality in the CT scans. Larger objects (up to 20 cm) generally were depicted with less resolution and a stronger blur than the smaller objects (about 10 cm). The imaging of dense material can be optimized by using an industrial X-ray CT with high voltage instead of a medical X-ray CT (Ketcham and Carlson 2001; Rowe et al. 1999, 2001; Tykoski 2002). However, as it was mentioned above, X-ray attenuation is additionally influenced by the type of sediment around or within the fossil bone. In NT, the maximal penetration thickness of non-organic material (i.e., rocks, sediment

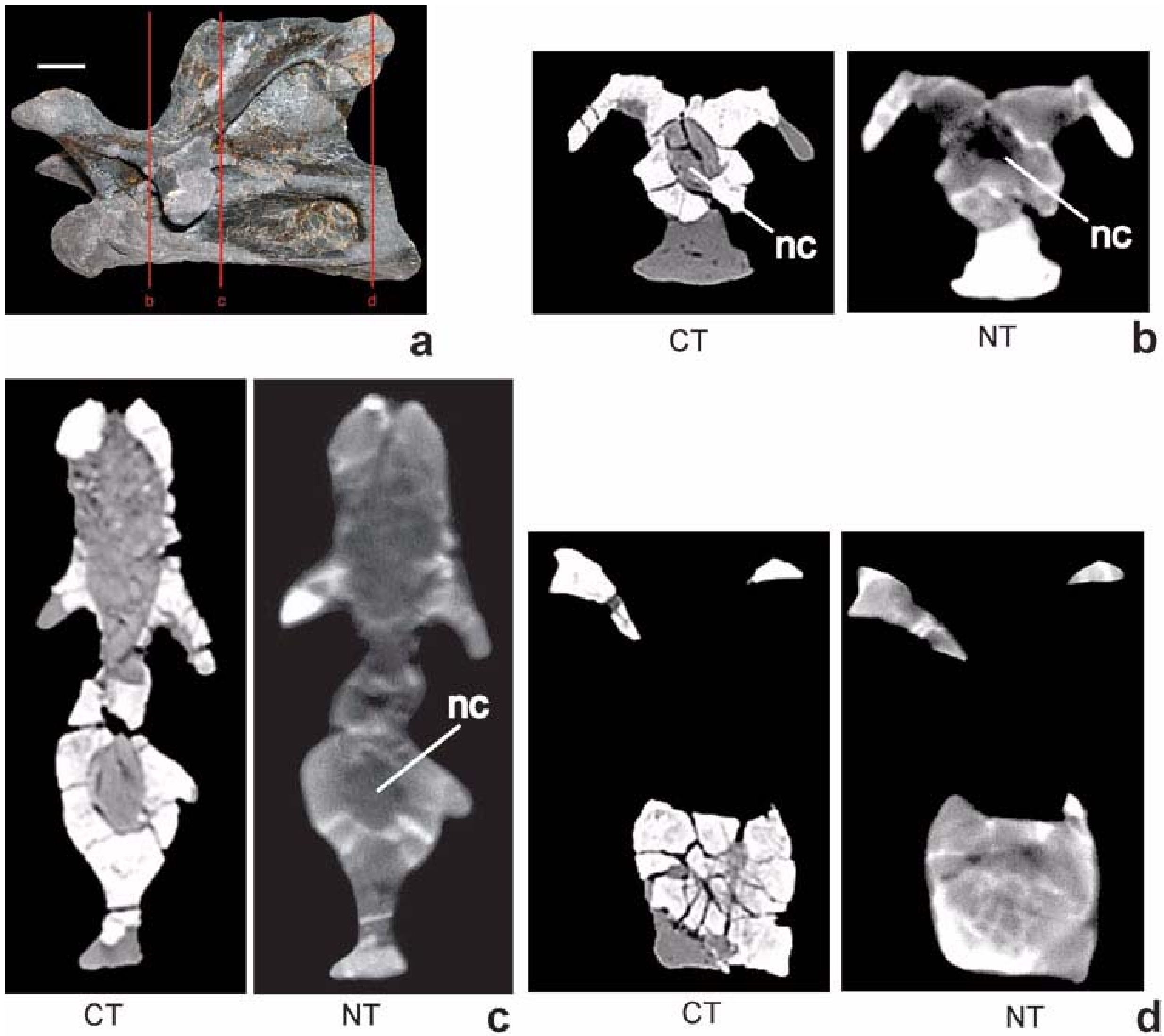

Figure 5. Fourth cervical vertebra (No. H25-2) of an undetermined diplodocid sauropod with broken parts of vertebral body and diapophysis modelled in polyester cast resin a) in left lateral view, red bars indicate axial cross-sections displayed in Figure 5b-d, scale bar (white) is 20 mm; b) axial section in the cranial third of the vertebra: both in the CT image (left) and the NT image (right), the polyester cast resin can be distinguished from bone, but only in the NT image the resin has a different colour than the matrix; nc = neural canal; c) axial section through the region of the diapophysis: in the CT image (left) margins between cavities filled with sediment are sharp but glue within the fractures is not displayed, whereas in the NT image (right) margins between sediment and bone are blurred and amounts of glue within the fractures is visible; d) axial section through the caudal part of the vertebral body and the postzygapophyses: the differences between X-ray CT and NT result in differences in the kind of displaying fractures filled with glue or polyester cast resin.

and fossilized bone) is about 10 cm, but we found no problem with penetration of a vertebra of 12 cm thickness. Image quality in NT depends strongly on the material composition of an object, and in the case of fossil bone material (apatite) and siliciclastic sediment (mainly silica and calcareous cement), the minimal difference in the linear neutron attenuation coefficients leads to low contrast.

There is no difference between the 3-D polygon-surface models extracted from the volume data of the CT and NT scans. The resulting surface models are of high quality and allow 3-D modelling of flexibility between single vertebrae as well as building models of the objects with stereolithography (Figure 8).

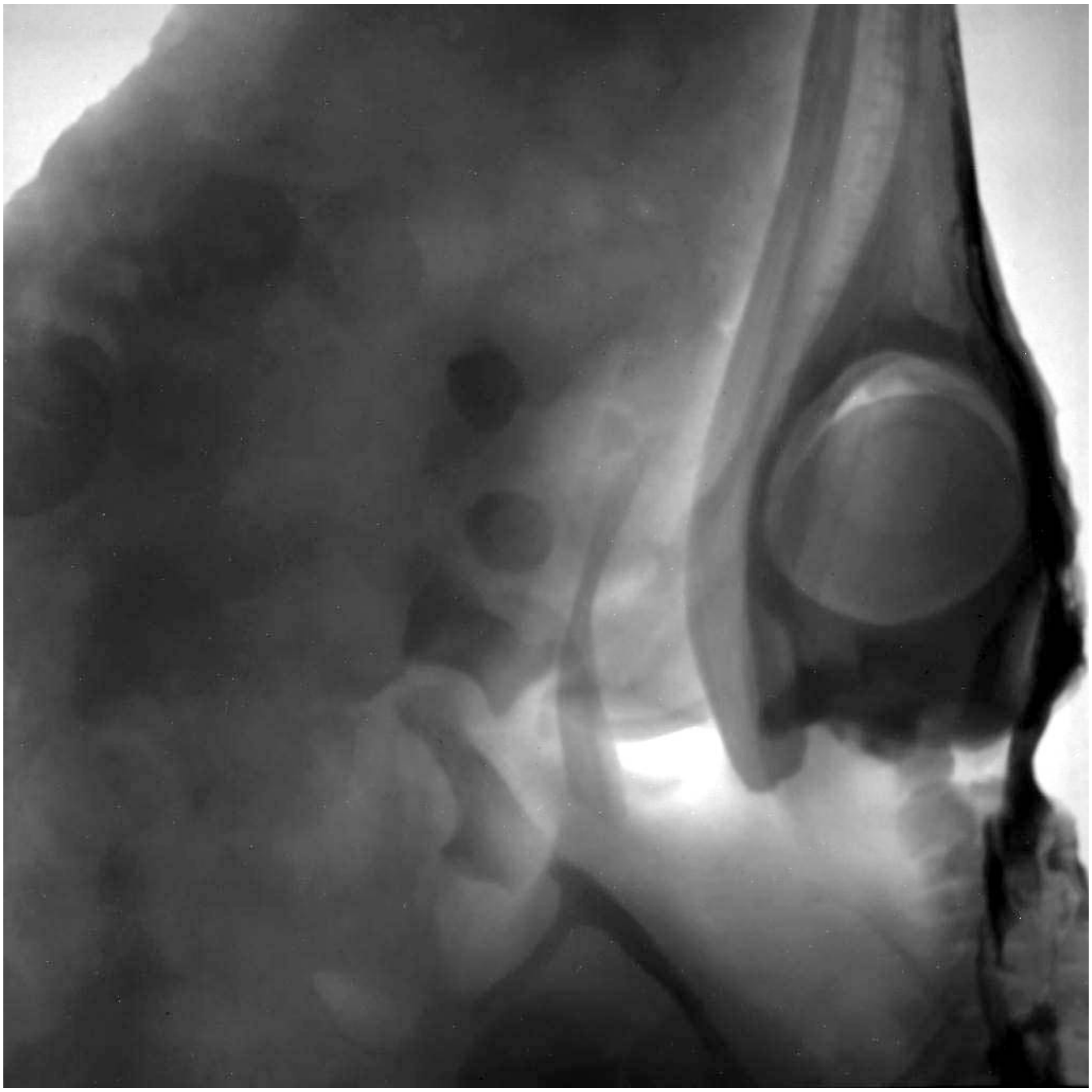

Figure 6. Neutron radiographic image of the head of an ichthyosaur imbedded in a shale. The image displays the orbital region of the skull with caudal part of the rostrum, orbit, braincase and first neck vertebrae (right side, from top to bottom) and some isolated bones of the forelimb (center of the image). The bone structure is clearly visible and can be distinguished from the pelitic matrix. This investigation was done on behalf of Urs Oberli (St. Gallen, Switzerland). Scale bar is 20 mm.

CONCLUSIONS

The comparison of certain aspects of both techniques can be summarized as follows. The differences in the information and contrast at the CT and NT images result from the differences in the attenuation properties and detection techniques of X-rays and neutrons. Additionally, image quality and contrast in both techniques depends strongly on calibration of the instruments and their resolution. For optimal comparison, the resulting 3-D data volumes have to be co-registered. In the example of the sauropod vertebrae, NT analyses are strongly influenced by the resins used for preservation and preparation of the scanned objects. The special combination of materials like fossil bone (apatite) and sediment matrix (silica) and a high amount of glue and polyester resin significantly decrease the quality of the images. In contrast, information about the distribution and content of glue within fractures and cavities of the bone are quickly available without recalibration of the instruments. Furthermore, glue-filled pneumatic canals that were not visible in the CT images were visible in the NT images. The medical CT scans of the sauropod vertebrae are only minimally affected by preservation and preparation of the scanned objects, but information like glue distribution was diminished. Comparing images of both techniques

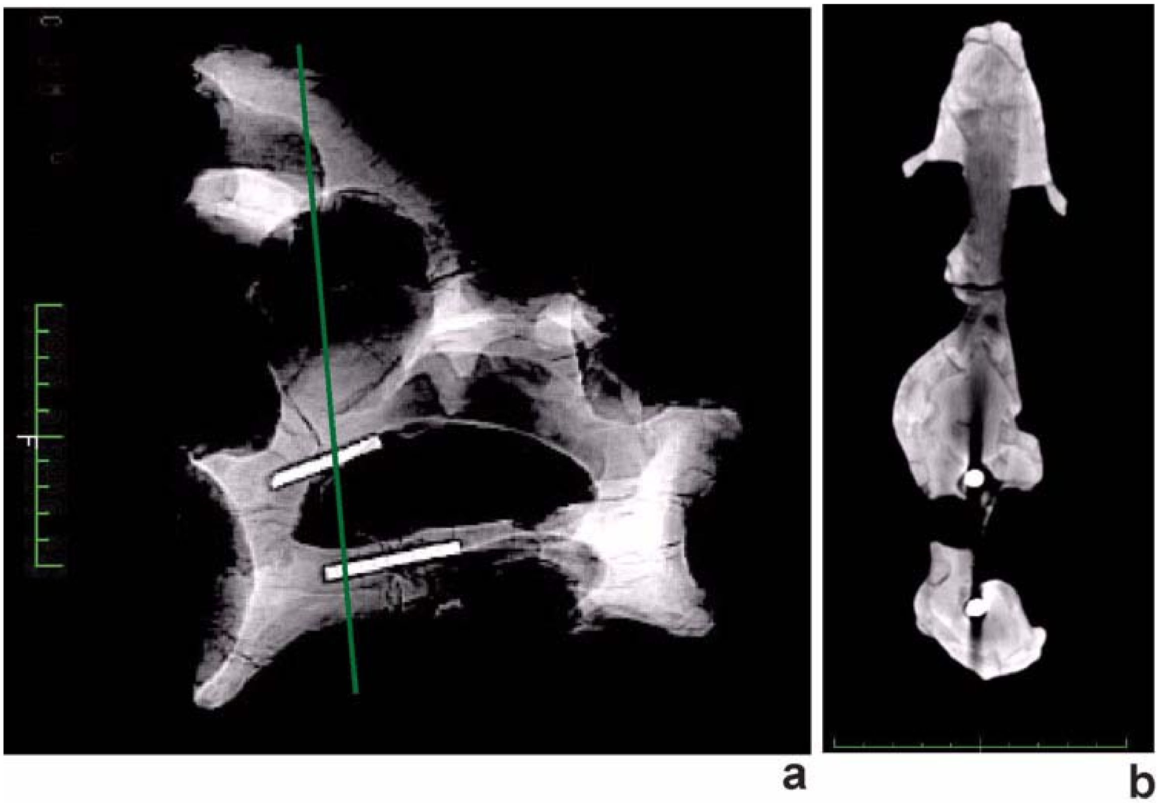

Figure 7. CT scan of a cross-section through the axis of *Brachiosaurus brancai* (SI 71) with two metal rods inside, analyzed by the Institute for Small Pets of the Free University in Berlin a) lateral overview about the vertebral with the location of the metal rods, b) transverse cross-section (position see green line in a), through axis showing the metal rods. The X-rays are strongly scattered, which considerably decreases the image quality. Green scale bar is 10 cm.

therefore helps to collect information about the scanned objects.

The time effort necessary for the CT scans depends strongly on the cross-section of the objects, the type of CT scanner and the available instruments and software. A CT scan together with reconstruction from the raw data can take between 5 and 60 minutes. In contrast, NT scans require a larger amount of time, because many radiographs are taken with the camera and the object has to be rotated on a table. Together with the reconstruction from the raw data, between 30 minutes and 5 hours might be required for one complete NT scan. The costs of a CT and an NT scan both depend on the object size and the scanner, and for this study, ranged from 500 and 1000 Euro. It should also be mentioned that medical CT is broadly available in many hospitals, whereas NT is bound to the presence of a neutron source, and therefore available only in few places.

The decision of which technique to use will therefore be dictated by the research questions, the preservation and material properties of the object. From the comparisons undertaken in this work, it seems that in most cases, X-ray CT will yield the best image results. Special cases, in which NT would be an adequate or even better technique, could be:

- to investigate objects containing metal inclusions;
- to investigate unprepared fossil bones to analyze the distribution of sediment in their internal cavities, including specimens containing lithologies other than the marls described here; and
- if there are fossils that have to be investigated in a forensic or historical context, for example to determine the nature of historical preparation of a museum specimen (e.g., identifying the parts that have been glued or modelled with resin materials).

At the moment, NT has not been extensively utilized in vertebrate palaeontology. To fully leverage this technique, future research might focus on identifying what types of sediment-bone tapho-

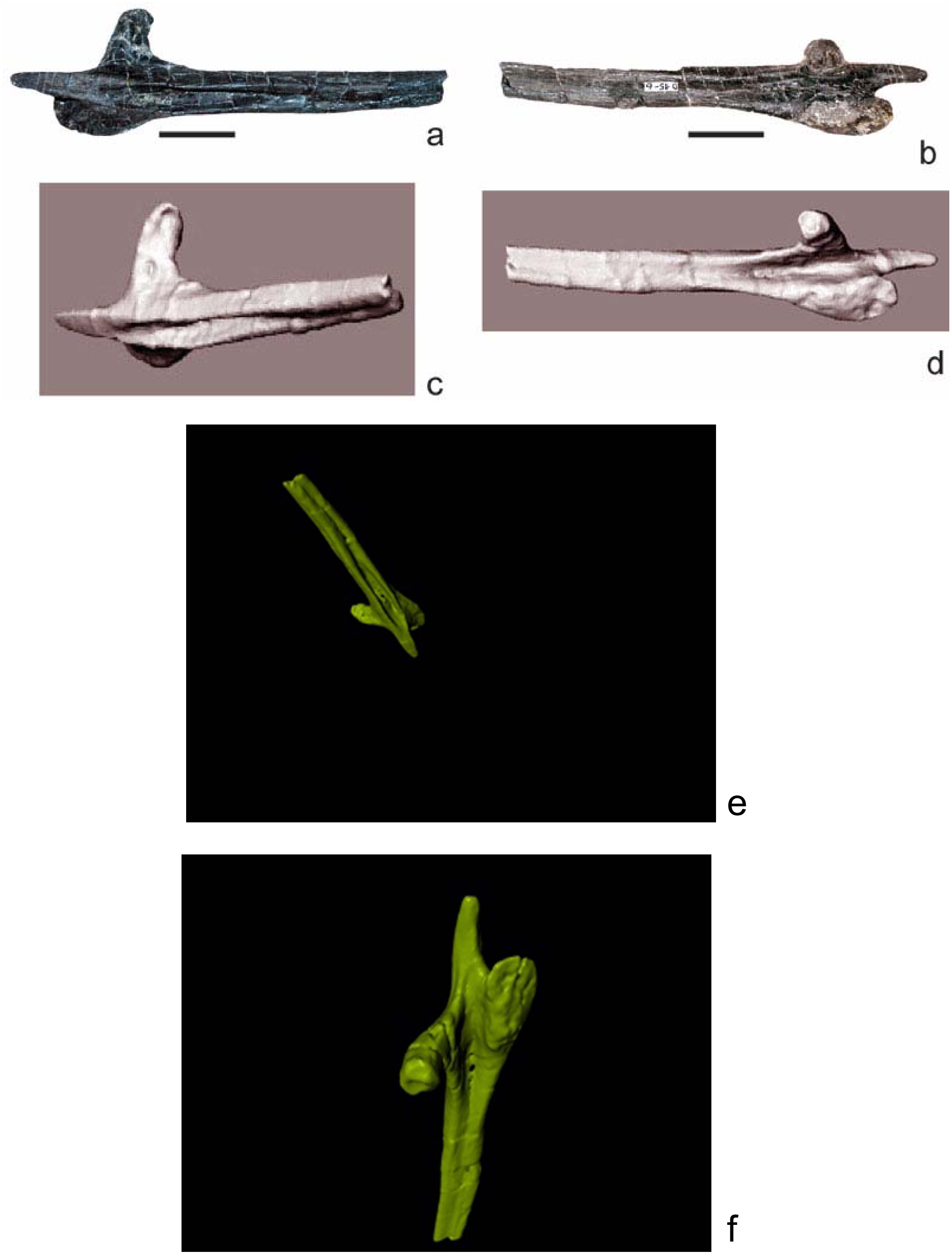

Figure 8. Cervical rib (No. D15-6) of an undetermined diplodocid sauropod a) in external and b) in internal view. Scale bar is 10 mm. 3-D polygon surface model of the same cervical rib, based on neutron tomography volume data. External view illustrated in c) and internal view in d). Animation of external view in e) and animation of internal view in f). Surface models were constructed using VG Studio and Cinema 4D 8R.

nomic modes yield optimal contrasts for determining fossil vertebrate anatomy.

ACKNOWLEDGEMENTS

We thank H.-J. Siber and Y. Siber from the Saurier-Museum Aathal for giving us access to their collections and especially to allow us to use some of the material for the tomographies. We are furthermore indebted to G. Ralli (DMR) for processing the X-ray computed tomographies and R. Schumacher (FHBB) for helping us with data processing. Thanks also to J. Habersetzer for helpful discussions of our results and interpretations and hints. We are also grateful to the two anonymous reviewers, who gave valuable hints and suggestions for the improvement of the manuscript and the application of neutron tomography to vertebrate palaeontology. We are especially grateful to the executive editors of PE, P.D. Polly and J.W. Hagadorn, for their support and help with this manuscript. Financial support for D. Schwarz is provided by the Swiss National Science Foundation (SNF No. 200021-101494/1).

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