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Exploring hominin and non-hominin primate dental fossil remains with neutron microtomography

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Abstract

Fossil dental remains are an archive of unique information for paleobiological studies. Computed microtomography based on X-ray microfocus sources (X-μCT) and Synchrotron Radiation (SR-μCT) allow subtle quantification at the micron and sub-micron scale of the meso- and microstructural signature imprinted in the mineralized tissues, such as enamel and dentine, through high-resolution "virtual histology". Nonetheless, depending on the degree of alterations undergone during fossilization, X-ray analyses of tooth tissues do not always provide distinct imaging contrasts, thus preventing the extraction of essential morphological and anatomical details. We illustrate here by three examples the successful application of neutron microtomography (n-μCT) in cases where X-rays have previously failed to deliver contrasts between dental tissues of fossilized specimen.

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Keywords: Neutron Radiography; Neutron Imaging; Neutron Computed Tomography; CCD Camera; Paleontology

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1. Introduction

Fossil dental remains represent the most common evidence testifying the life of extinct taxa in a given region at a given time. In parallel with results from research on developmental biology and genetics, advances in comparative structural morphology of fossil and living primate dental remains show that a significant amount of valuable biological information is preserved in the internal structure of tooth crowns and roots (rev. in Macchiarelli et al., 2013). Since the first discovery of X-rays by Röntgen in 1895, paleontology was among the first scientific fields to use radiography to study human fossil remains (Walkhoff, 1903; Gorjanović-Kramberger, 1906). These early applications already discovered substantial differences between the Neanderthal and modern human dental structure. In response to the potentially conflicting requirements of preservation vs. scientific fruition/exploitation of the fossil record, the available computed microtomography technologies based on X-ray microfocus sources (X-μCT) and Synchrotron Radiation (SR-μCT) allow nowadays the quantification at the micron and sub-micron scale of the meso-/microstructure of the mineralized biological tissues through high-contrast and high-resolution "virtual histology" (Macchiarelli et al., 2008; Smith and Tafforeau, 2008). Nonetheless, depending on the degree of alterations undergone during fossilization, X-ray analyses of dental tissues do not always provide a distinct structural signal (Fig. 1), thus preventing the extraction of essential paleobiological evidence (e.g., Schwarz et al., 2005; Smith et al., 2009; Zanolli et al., 2015).

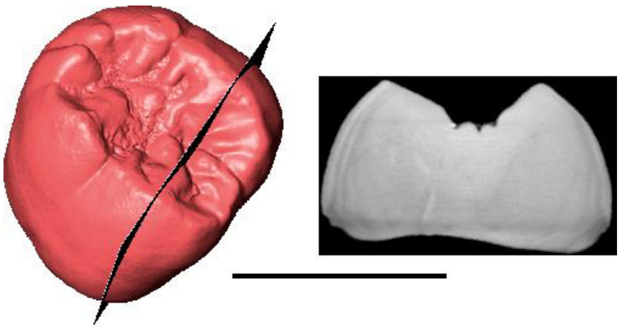

Fig. 1. X-ray analyses of many fossil hominid teeth from Indonesia exhibit no contrast between the enamel and the dentine (Zanolli, 2011).

Besides phase-contrast SR-μCT, a new complementary tool was recently developed to investigate the internal structure of dense materials: neutron microtomography (n-μCT). Even if neutron were discovered in 1932, their use in 3D imaging was developed only recently, when high quality neutron sources and detector systems became available (Tremsin et al., 2011). Neutrons have an absorption profile (interaction mechanism of neutrons with matter) that differs from X-rays (Kardjilov et al., 2003; Winkler, 2006; Sutton, 2008; Tremsin et al., 2015). Because of their unique ability to penetrate materials opaque to X-rays, neutron-based analytical techniques such as neutron radiography and n-μCT represent an effective investigative tool for imaging the fossil material with a better contrast resolution despite the variably hazy appearance of the inner structural signal (Zanolli et al., 2013). We illustrate here the formidable potential of applying n-μCT to the fossil record, and in particular to heavily mineralised remains such as teeth.

2. Material and methods

2.1. Fossil material

The extent of such problems and the investigative limitations in signal retrieving are illustrated here by a few examples of paleontological dental remains from distinct chrono-geographic contexts: a highly mineralized jaw fragment attributed to *Homo erectus* (Sangiran 1b) from Java, Indonesia, a fossil orangutan molar (SMF-8889) from Indonesia and a cercopithecoid upper jaw (STS 1039) from the Sterkfontein Cave, South Africa, still embedded in a consolidated breccia rock matrix. These specimens were first unsuccessfully examined by X-µCT (for STS 1039, see Beaudet et al., 2016). The Indonesian *Homo erectus* mandible fragment Sangiran 1b (Fig. 2) is one of the most emblematic fossil human remains from the Sangiran Dome, on Java Island. The isolated fossil orangutan tooth SMF-8889 represents one of the abundant hominid dental elements also recovered at the Sangiran Dome (Fig. 2). The latter two specimens are permanently stored at the Senckenberg Research Institute and Natural History Museum of Frankfurt, Germany. The block of sedimentary breccia bearing the partial cranium STS 1039 (Fig. 2) is permanently stored at the Ditsong National Museum of Natural History, Pretoria, South Africa.

Fig. 2. The fossil hominid specimens Sangiran 1b and SMF-8889 from Indonesia (above) and the South African cercopithecoid maxilla STS 1039 embedded in hard breccia rock (below). Scale bar: 1cm.

2.2. Applying n-µCT to fossil material

When using X-µCT and SR-µCT, the imaging contrasts between materials result from X-ray interactions with atomic electrons (i.e., photoelectric and Compton effects), which depend on the atomic number of the elements of interest. Indeed, contrasts obtained with X-ray imaging are due to the different natural absorption and scattering of X-rays and to the different electron cloud densities surrounding the atoms of the analyzed materials. Since the neutrons are only being absorbed or scattered by the nucleus of the atom, as being neutral particles, this principle does not apply to neutron-based analyses. Instead n-µCT's imaging contrast depends only on the internal composition of the nuclei in the sample, often showing high attenuation (absorption and scattering) differences between neighbouring elements, and even between isotopes of the same element (Kardjilov et al., 2003; Winkler, 2006; Tremsin et al., 2015). Hydrogen is a special case, which attenuates neutron beams by equal-mass scattering, while minerals made of heavier elements, usually constituting geological and paleontological material, are easily penetrated and additionally show high contrast levels between bone and matrix (breccias) (Schwarz et al., 2005; Sutton, 2008). In summary, neutron radiography and n-µCT are bound to deliver high imaging contrasts that are very different from X-rays, sometimes even complementary (Sutton, 2008). In this study, neutrons help to distinguish between the dense materials representing the matrix, bone, tooth enamel and dentine. Previous examinations with X-rays and synchrotron radiation demonstrated that some teeth show sharp contrast between enamel and dentine, and others show no contrast at all, while neutron microtomography still shows significant contrast. This is a hint that samples differ in terms of the fossilization process and embedding in soil, where some exchange of minerals must have happened. There are currently no data available about which elements are actually present in the samples, and cause the contrast for X-rays and neutrons. Possible examination methods are X-ray fluorescence, and Prompt Gamma Neutron Activation Analysis. As soon as our current and future tomography experiments have identified a significant representative set of samples, a detailed examination for elemental composition is envisaged.

Between 2013 and 2015, the specimens were detailed by n-μCT at the ANTARES Imaging facility (SR4a beamline). The measurements were carried out according to the following parameters: The neutron beam originated from the cold source of the FRM II reactor, with an energy range mostly from 3 to 20 meV, a collimation ratio of L/D=500 (ratio between sample-detector distance and collimator aperture) and an intensity of 6.4×10^7 n/cm^{2}s. A 20 μm Gadox screen was used to detect neutrons. Both a cooled scientific CCD camera (Andor ikon-L) and cooled scientific CMOS camera (Andor NEO) were used as detectors. The final virtual volume was reconstructed with an isotropic voxel size of 20.5 μm for Sangiran 1b and of 75.0 μm for SMF-8889 and STS 1039.

Because the detection of the tissue interfaces is based on attenuation at the boundary of a structure in both X-ray and neutron-based microtomography, we performed a threshold-based segmentation with manual corrections, using the software Avizo v.7.0 (Visualization Sciences Group Inc.).

3. Preliminary results

Our results show that, even when X-μCT fails to produce any contrast between the dental tissue components, the inner morphology of even highly mineralized remains can still be recognized in the n-μCT images (Fig. 3). In fact, n-μCT imaging allowed us to segment enamel from the underlying dentine. Neutron analyses enable retrieving information concerning the subtle structural morphology of the bone and teeth, even if these are enclosed in a consolidated sedimentary block that has a similar density (Fig. 3C-D).

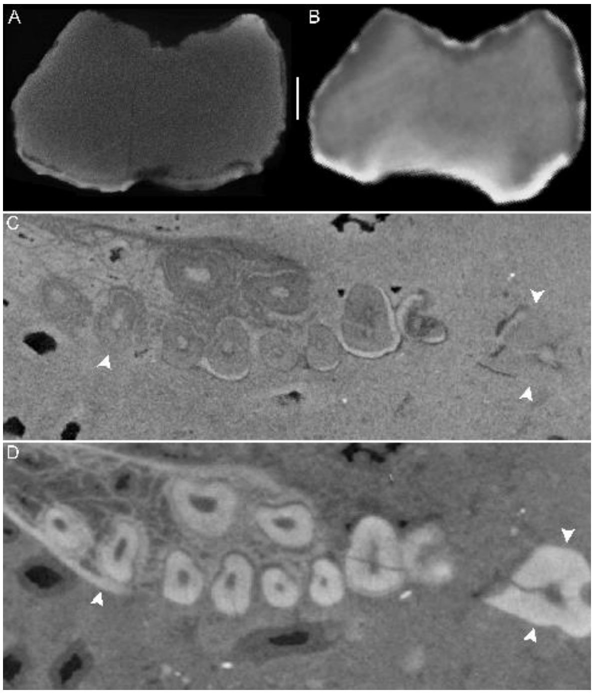

Fig. 3. X-μCT slice of SMF-8889 (A) showing no contrast compared with the n-μCT record (B) that displays well-visible boundaries between enamel (dark grey) and dentine (light grey). A similar comparison between the X-μCT record of STS 1039 (C) and the n-μCT imaging (D), with arrows pointing at some dental and bony features that are not visible in the former but clearly identified in the latter.

In addition, whereas it was not possible with X-rays, the tooth internal structure of the three specimens scanned by n-μCT was virtually extracted and imaged in 3D (Fig. 4). This method allowed us for the first time to satisfactorily quantify the internal signature of precious fossil specimens and thus to (re)assess their taxonomic identity, which is crucial for paleobiological research. Our analytical experiment demonstrates that, besides phase-contrast SR-μCT, neutron microtomography, whose absorption profile differs from X-rays by being more strongly attenuated by organic than mineral materials, represents an effective investigative tool for imaging fossil remains at a high contrast resolution despite the usual hazy appearance of their structural signal.

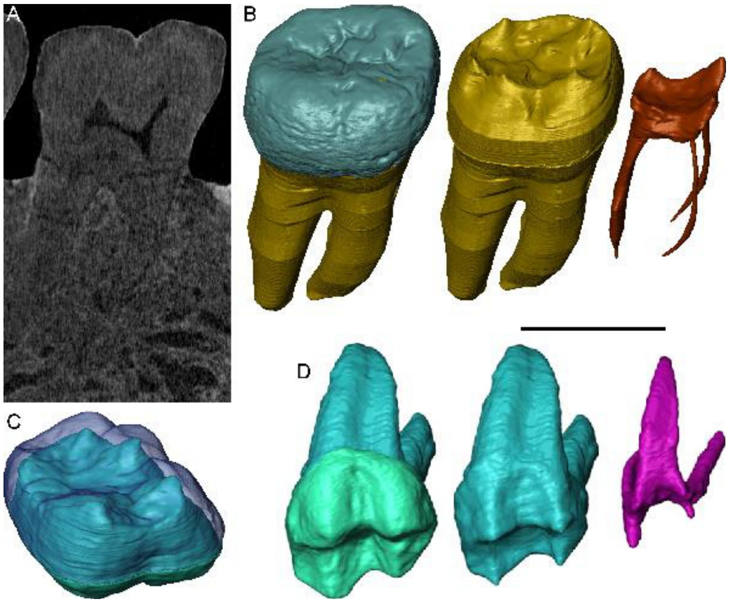

Fig. 4. The n-μCT record (A) of the Indonesian specimen Sangiran 1b enabled extracting and reconstructing the 3D internal structure of the molars (B). Similarly, the 3D internal signature of the fossil orangutan molar SMF-8889 from Java (C; Zanolli et al., 2013) and of the South African fossil cercopithecoid teeth of STS 1039 (D; Beaudet et al., 2016) which were virtually rendered with accuracy.

4. Discussion and concluding remarks

When X-μCT does not enable extracting the internal structural signal, n-μCT represents a suitable alternative, as it is also non-destructive. But a strict protocol should be followed in order to ensure the safety of the fossil material analysed and maximize the chances to get appropriate contrast levels with neutron imaging. In particular, high-intensity neutron bombardment can induce the radioactivity of some heavy elements, like cobalt or europium, that exhibit a long-period half-life, thus requiring the storage of the analysed material in a radioactive-proof safe (Sutton, 2008). For this reason, we preliminary tested to irradiate non-valuable samples (like sediment and fossil fragments) that were found close to the specimens to be analysed in order to check for the presence of such heavy elements (fortunately these elements are generally rare in a sedimentary context). Each of the n-μCT experiments conducted for this study lasted between 20 and 22 hours. During the acquisition, some short-lived radioactive isotopes were activated and the specimens showed radiation levels up to 300 μSv/h for a few hours, but decreased to natural level of radioactivity (below 0.5 μSv/h) in less than 10 days. In addition, n-μCT analyses are strongly influenced by the resins used for preservation and preparation of fossil materials, as high amounts of glue and polyester resin can significantly decrease the quality of the images (Schwarz et al., 2005). For this reason, it is recommended to scan specimens with minimum or without resin coating. Following these precautions, we successfully used n-μCT for the inspection of three dentognathic specimens (i.e., the Indonesian *Homo erectus* mandible Sangiran 1b, the fossil orangutan molar SMF-8889 and the breccia block embedding the partial monkey cranium STS 1039 from Sterkfontein). These three specimens come from different geological contexts (from open air sites, for the Indonesian material, and from a cave system, for the South African jaw), exhibit variable size (from c. 16*14*10 mm for the isolated tooth SMF-8889 to a block measuring 135*70*30 mm), and include several dense mineralized materials (with fossilized dental tissues enamel, dentine and cement, as well as bone and sedimentary matrix). Despite these variable conditions, all the specimens displayed sufficient contrasts to study their internal structure (Fig. 4).

So far, neutron tomography has been used to detail some vertebrate fossil specimens (Schwarz et al., 2005; Grellet-Tinner et al., 2011) and we present here the first application of high-resolution n-µCT (with a reconstructed voxel size of 20.5 micrometers) on fossil hominin specimens. This is a critical step forward as many tooth specimens from Indonesia do not exhibit discernible internal contrasts when using X-µCT and SR-µCT (Smith et al., 2009; Zanolli et al., 2015). Thus, this new approach opens stimulating ways to investigate in 3D the fossil record and unlock hidden information that was previously not accessible with X-rays. Furthermore, this is the first time such a highly promising analytical approach is applied to virtually disclose and 3D render the content of matrix embedded primate remains from famous South African hominin-bearing cave sites (Beaudet et al., 2016). Our experience with these three fossil specimens, coming from various chronogeographical and geological contexts show how promising n-µCT can be to help solving commonly encountered issues in paleontology.

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