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Exploring the potential of neutron imaging for life sciences on IMAT

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ABSTRACT

Neutron imaging has been employed in life sciences in recent years and has proven to be a viable technique for studying internal features without compromising integrity and internal structure of samples in addition to being complementary to other methods such as X-ray or magnetic resonance imaging. Within the last decade, a neutron imaging beamline, IMAT, was designed and built at the ISIS Neutron and Muon Source, UK, to meet the increasing demand for neutron imaging applications in various fields spanning from materials engineering to biology. In this paper, we present the first neutron imaging experiments on different biological samples during the scientific commissioning of the IMAT beamline mainly intended to explore the beamline's capabilities and its potential as a non-invasive investigation tool in fields such as agriculture (soil-plants systems), palaeontology and dentistry.

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1. INTRODUCTION

Neutrons form a highly penetrating radiation that passes through matter without damaging or structurally modifying it. This property makes neutrons an ideal tool for many kinds of complementary material investigations. Moreover, the strong interaction of neutrons with hydrogen, and their ability to distinguish between hydrogen and deuterium with no radiation damage, makes neutrons a good probe for imaging biological specimens. Among the experimental neutron techniques being developed in biology, one could mention: neutron activation analysis related to the capture of neutrons, which is applied for isotope and element analysis (Yunus *et al.*, 2010); small-angle neutron scattering (Teixeira *et al.*, 2008) used to understand the interaction between the complex molecular systems that make up living cells; and neutron imaging (Anderson, McGreevy & Bilheux, 2009) based on the capture and scattering of neutrons which provides information about the inner structure and, indirectly, about the composition of a sample due to the fact that different materials attenuate neutrons to a greater or lesser extent. While small-angle neutron scattering is well exploited in life sciences research, neutron imaging has become more appealing in the recent years through development and improvement of both the sources and the detector systems.

2. NEUTRON IMAGING FACILITY IN UK, IMAT BEAMLINE

The neutron imaging facilities, e.g. CONRAD at BER-II (Kardjilov *et al.*, 2016), ANTARES at FRM-II (Heinz Maier-Leibnitz Zentrum *et al.*, 2015), NEUTRA (Lehmann *et al.*, 2001) and ICON (Kaestner *et al.*, 2011) at PSI, RADEN at J-PARC (Kiyanagi *et al.*, 2013), CG-1D at Oak Ridge Laboratory (Santodonato *et al.*, 2015), have to serve the demands of an increasing number of users worldwide whether they are under operation at research reactors or spallation sources.

IMAT (Imaging and Materials Science & Engineering) is the first neutron imaging and diffraction beamline at the ISIS Pulsed Neutron and Muon Source, based at Rutherford Appleton Laboratory (RAL) in the UK. Design of the instrument (Burca *et al.*, 2013; Kockelmann *et al.*, 2015) started in 2008, and construction was finished in 2016. Since then, engineering and scientific commissioning has mainly focused on the neutron imaging capability, although neutron diffraction will eventually be routinely available on the same beamline for samples that can benefit from characterisation using both techniques.

The IMAT instrument utilises neutrons received from the newer Target Station 2 at the ISIS neutron source, which produces short-duration (<1 μs) neutron pulses at 10 Hz. These neutrons first pass through a coupled moderator filled with liquid hydrogen cooled to 18 K, which slows them to velocities on the order of 10^3 m·s^{-1} without unduly lengthening the duration of the pulse. As the wavelength of a neutron is inversely proportional to its velocity,

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these slowed neutrons have a spread of wavelengths comparable to the atomic spacing in ordinary solid matter. Neutrons are transported from the moderator to the IMAT instrument using a supermirror straight neutron guide of 42.8 m length. The required band of wavelengths is selected using choppers: spinning assemblies that block the neutron path at specific times during their rotation such that only neutrons within a specific range of velocities (and hence wavelengths) are capable of reaching the instrument from the target where the neutrons are produced. IMAT uses three sets of choppers, one for filtering out unwanted high-energy neutrons and gamma radiation, and two for defining the required wavelength band while preventing faster neutrons overtaking slower neutrons from the previous pulse.

In order to create a well-collimated neutron beam suitable for radiography, the neutron beam passes through a pinhole aperture with one of six different selectable diameters (5, 10, 20, 40, 80 and 100 mm). As the distance from this aperture to the sample position and detector is ~10 m (the sample typically being close to the imaging plane of the detector), the beam can be assumed to be near-parallel, and the resulting projected image of the sample is not magnified. Accordingly, samples that are smaller than the active area of the detector can be analysed in a single acquisition, while images of larger samples can be assembled piecewise by translating the sample on a motorised stage between successive acquisitions. Rotation of the sample also enables tomographic reconstruction from individual radiographs collected at a series of angles (Anderson *et al.*, 2009). A photograph of the IMAT facility is shown in Figure 1.

For the experiments presented in this paper, neutron radiographs were imaged with an optical camera box (Finocchiaro *et al.*, 2013), with a field-of-view varying from 50×50 mm^2 to 200×200 mm^2, using a defined continuous wavelength range. This setup detects and records the image by means of a scintillator screen, mirror, lens and a Zyla sCMOS 4.2 Plus

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camera (www.andor.com). It should be noted that, IMAT also supports the use of a second detector capable of recording a full neutron spectrum for each of its 55 µm pixels, albeit over a smaller 28×28 mm^2 field of view (Tremsin *et al.*, 2013).

3. DATA COLLECTION, PROCESSING AND ANALYSIS

The basic principle of neutron radiography (similar to X-ray radiography) is that a neutron beam passing through the sample is attenuated. The detector registers the fraction of the initial beam intensity that remains after it has been transmitted by each point in the object. The relationship between the incident intensity I_0 and the transmitted intensity I is given by the Beer-Lambert law defined as,

$$I = I_0 e^{-\mu t}$$

where μ is the attenuation coefficient along the path of the neutron through the sample and depends on the selected material and its density and t is the thickness of the sample along this path. For all test measurements presented in this paper, samples were mounted on the tomography stage positioned on the translation/rotation stage system (Figure 1) between the neutron beam flight tube and the detector. The main consideration in selecting the detector for an experiment was its capacity to resolve details of interest with good spatial and temporal resolution. Moreover, for each radiographic setup, the L/D ratio (beam collimation, L being pinhole to sample distance, and D pinhole diameter) was modified based on conditions including the neutron flux, spatial resolution of the detector, and size of the sample. A normalisation procedure was used to eliminate/minimise inhomogeneities of the images obtained that result from the spatial variations in beam intensity, the response of the camera to the beam, or from the electronic noise generated by the camera system. To achieve this, radiographies of the same exposure time were collected without the sample present in the beam (open beam) and with the beam shutter closed (dark field). The image taken with

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the sample in the beam (at the same energy as the open beam) was normalised to the open beam after subtracting the dark field from both images. This was done automatically in the software used for reconstruction, or using ImageJ (Rasband *et al.*, 1997).

The reconstruction of the sample projections into a 2D slice of the object was done using commercial software, e.g. Octopus (Octopus 8.8, inCT, Ghent, Belgium) (https://octopusimaging.eu) or the open-source Python package TomoPy (Argonne National Laboratory; https://tomopy.readthedocs.io/en/latest/). When the reconstruction was completed the volume data obtained was imported into Thermo Scientific™ Avizo 3D volume rendering software (FEI Visualization Sciences Group; www.fei.com/software/amira-avizo) where the volumetric object was generated and rendered from the stack of image slices.

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4. EXEMPLAR MEASUREMENTS

We have decided to choose and measure samples of high complexity (in terms of shape, composition, inner structure, dimensions) during the scientific commissioning of IMAT to test the feasibility and the instrument's capabilities for neutron imaging applications in future interdisciplinary life sciences projects.

4.1. Palaeontology

Palaeontology is a research field where neutron tomography has significant potential as a non-destructive technique complementing X-ray computed tomography (CT). Over the last decade, it has been used occasionally to study fossil specimens (de Beer *et al.*, 2008; Laaß *et al.*, 2011; Mays *et al.*, 2017). Fossil preservation mechanisms are variable and some specimens have poor X-ray attenuation contrast. This issue is particularly acute, for example, when a fossil and its host rock have similar composition and density: for example the Herefordshire Lagerstätte has calcite ($CaCO_3$) fossils within calcium carbonate nodules

(Sutton *et al.*, 2001; Briggs *et al.*, 2012; Briggs *et al.*, 2017). In these cases, X-ray CT provides insufficient detail to fully resolve the preserved fossilized structures (Sutton *et al.*, 2014). To test the suitability of IMAT for studying fossils with poor X-ray attenuation contrast, we applied neutron tomography to fossil crabs from the Eocene of Spain (Figure 2a) that were previously imaged using X-ray CT with limited success (Figure 2b). Imaging at IMAT was carried out using the optical camera box with sCMOS camera (58 µm pixel size; http://doi.org/10.5281/zenodo.825926). The results show that neutron tomography can be successful in probing samples with low X-ray attenuation, capturing previously unseen features from the interior of fossils and making it possible to distinguish between fossil and matrix, even in material with limited internal density contrast (Figure 2c).

This pilot study provides an excellent opportunity to introduce scientists from different branches of the palaeontological community to the capabilities of IMAT. Furthermore, they will facilitate a deeper understanding of the strengths and weaknesses of neutron tomography in comparison to the traditional X-ray CT scanning, thus helping the geoscience community target future neutron tomography applications.

4.2. Soil/plants science

One of the essential functions of plant roots is the uptake and transport of water. Due to increasing water restraints on agriculture, there is strong motivation to truly understand plant water uptake. This requires new methods that allow the measurement of water fluxes in roots and the soils that surround them. However, X-rays are not well-suited to characterisation of water distribution, since the contrast ratios between water, plant roots, and other soil constituents are poor. The high neutron attenuation coefficient of hydrogen makes neutron radiography and tomography very attractive techniques for measuring water dynamics in plant-soil systems (Matsushima *et al.*, 2008; Menon *et al.*, 2007; Totzke *et al.*, 2017; Warren

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et al., 2013; Zarebanadkouki *et al.*, 2013) and map the water content distribution in soils (Carminati *et al.*, 2007; Cheng *et al.*, 2012).

On IMAT we propose to develop similar methods to understand water flux in soils, driven by root activity in order to inform models of water dynamics in agricultural systems. The first step of our soil-plant system research project was to investigate the water distribution into the soil using neutron tomography. A compost specimen was measured because it contained a wide range of elements (organic and inorganic) and could absorb many times its own weight in water, resulting in improved soil water and nutrient availability (Figure 3).

Further tomographic acquisitions will be carried out using different neutron beam and detector parameters, allowing benchmarking of the instrument's performance in elucidating soil water distributions. Moreover, reconstructed neutron data will complement the X-ray CT reconstructions (Keyes *et al.*, 2017; Keyes *et al.*, 2016) in order to validate models describing the dynamics of water transport in the soil region immediately surrounding plant roots.

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4.3. Dentistry application

The first neutron tomography of a human tooth (second molar) on IMAT was acquired using optical camera box equipped with Andor Zyla 4.2 PLUS sCMOS with 2048×2048 pixels, each pixel of 30×30 µm. A neutron flux density of 10^7 neutrons/cm^2/sec at a beam collimation of 250 reached the sample. The neutron tomography scan of the tooth lasted almost 9 hours, and had 1049 projections over 360° rotation, with 6 flat field and 6 dark field images and 30 second exposure time per image. The Fourier grid reconstruction algorithm from the module tomopy.recon.algorithm (Dowd *et al.*, 1999; Rivers, 2012) was applied for reconstruction, revealing the internal structure of the tooth (pulp chamber, dentin and enamel), which was then virtually extracted and imaged in 3D with Avizo software (Figure 4). These initial measurements allow the quantification of the internal signature of the tooth with potential applications including the investigation of endodontic processes and forensic dentistry, or even analysis of bone implant biomaterials if one considers the recent improvements in high-resolution neutron imaging (spatial resolution of 5 μm) (Jakubek *et al.*, 2006; Tremsin *et al.*, 2008).

5. CONCLUSION

We have shown throughout this paper that IMAT is definitely a reliable neutron imaging tool to be successfully employed in cutting-edge interdisciplinary research of high complexity which bring together experts from different research fields. Because IMAT is a newly built instrument exemplar measurements are of paramount importance since they are an effective means of identifying the best possible matches between the instrument capabilities and applications. The results acquired prove that as well as being complementary to X-ray computed tomography, neutron tomography is also an efficient non-destructive investigation method that can contribute significantly to the progress of life sciences research.

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Figure 1. *The IMAT beamline: neutron imaging setup with the optical camera box mounted on the robotic arm*

Figure 2. *(a) Fossil crabs from the Eocene (40 million years old) of Spain; (b) slice through reconstructed X-ray CT (scanned on a Nikon Metrology HMX ST 225 system at the Natural History Museum, London, using a 1.0 mm thick Copper filter, 225 kV voltage and 180 µA current, giving a tomographic dataset with a voxel size of 46 µm) of fossil crabs; (c) slice through reconstructed neutron tomography of fossil crabs on IMAT.*

Figure 3. *(a) Photograph of the NT experimental setup of the quartz glass tube (boron free) filled with compost, 10 mm in diameter. Radiographies were recorded with Zyla sCMOS 4.2 Plus camera (30 µm pixel size) and computed to a 3D volume stack (b) The attenuation profile of the neutron data shows that the water is predominantly held in the clay aggregates, rather than in the hard mineral grains. The soil texture (poorly described by NT) will be complemented by the future synchrotron X-rays CT.*

Figure 4. *(a) Photograph of human tooth; (b) Neutron radiograph of tooth; (c-e) Tomographic 3D reconstruction of tooth with 3 segmented areas showing (pulp chamber, dentin and enamel)*

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Figure 1

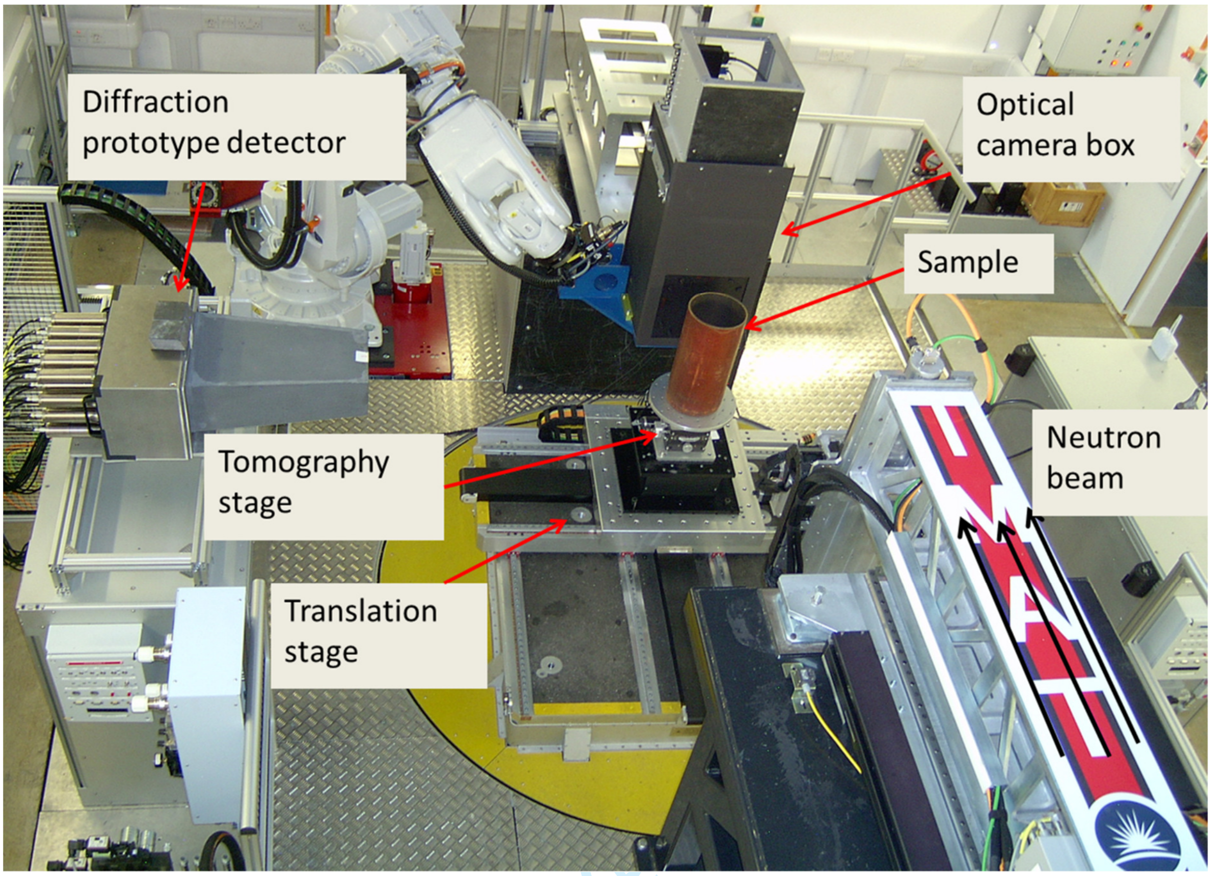


Figure 2

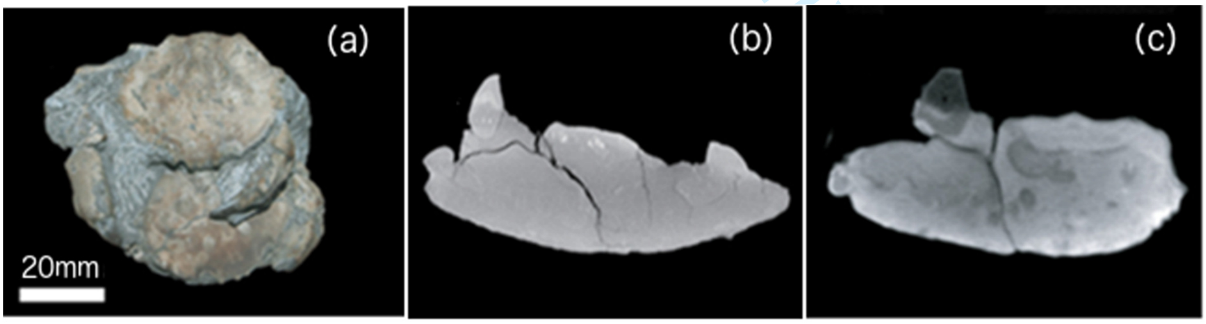


Figure 3

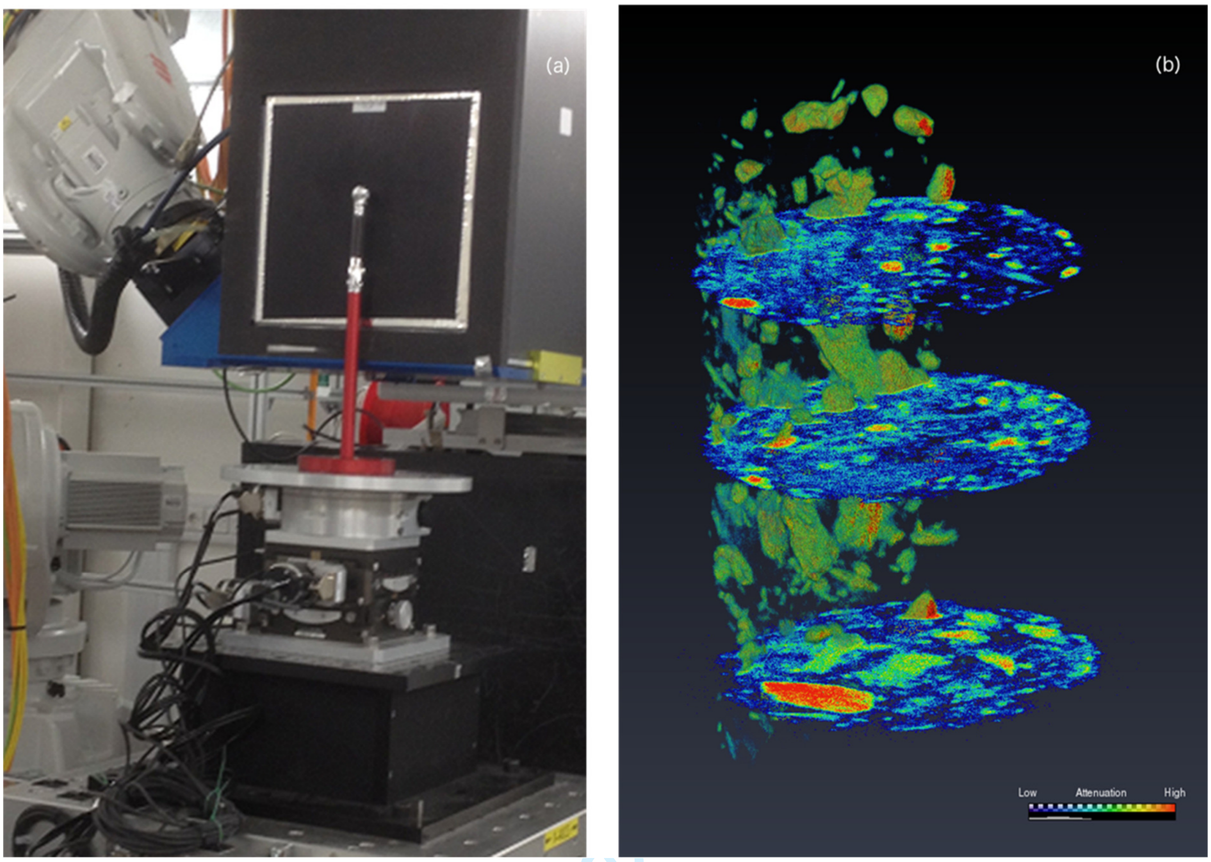
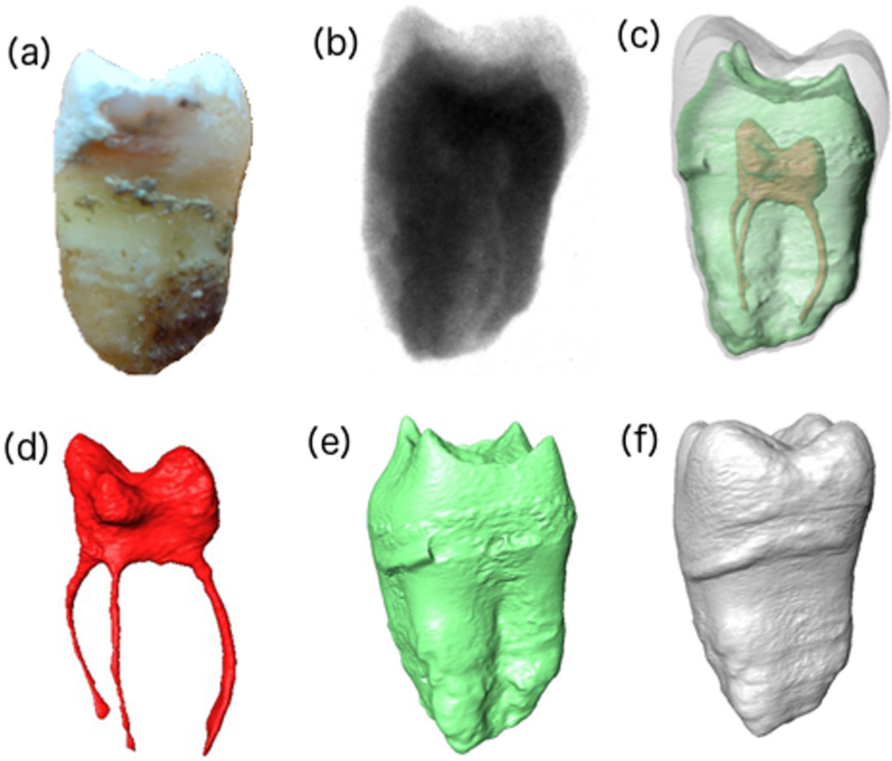


Figure 4



Lay description

Neutrons form a highly penetrating radiation passing through matter without damaging or structurally modifying it, a property that makes them the ideal tool for many kinds of complementary material investigations. Moreover, the strong interaction of neutrons with hydrogen and their ability to distinguish between hydrogen and deuterium with no radiation damage make neutrons a good probe for imaging biological specimens. The recent technological developments of sources and detectors improved the capabilities of neutron imaging instruments and also have facilitated the use of neutron imaging on a much wider scale than before. Neutron imaging is proving its advantages as being complementary to other known methods of investigation such as X-ray imaging or magnetic resonance imaging and it is no surprise that it is not only employed in engineering or archeaology, but also in life sciences. This definitely opens new perspectives for a more interdisciplinary approach in contemporary science. Within the last decade a neutron imaging beamline, IMAT, was designed and built at the ISIS Neutron and Muon Source, UK, to meet the increasing demands of researchers from different fields, spanning from materials engineering to biology. The results presented here, acquired from first measurements on different biological samples during the scientific commissioning of IMAT beamline show the instrument capability and its suitability to palaeontology, agriculture (soil-plants systems) or dentistry applications.

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