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X-RAY TOMOGRAPHY ANALYSIS AND APPLICATIONS OF μ-CT TECHNIQUES FOR 3D VISUALISATION IN PALAEOBIOLOGY

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1. INTRODUCTION

X-ray computed tomography techniques have been prevalent within areas of palaeobiological research for more than 15 years [1]. These techniques have enabled researchers to examine the morphology of fossil and modern organisms both non-destructively and in three-dimensions. Software tools for working with these datasets provides the ability to perform analyses ranging from simple linear measurements or volume estimations, to full three-dimensional digital reconstruction and testing of morphological features against functional hypotheses. The range and diversity of applications of such tomographic analyses, both in Palaeobiology and across the Earth Sciences, continues to expand [2].

Micro-computed tomography (μ-CT), using scanners such as the Nikon XTH 225 ST, which are quite widely available at various UK institutions, sit comfortably in the middle of a range of closely associated techniques allowing 3D visualisation of palaeontological specimens (e.g., synchrotron tomography, μ-CT, medical-CT, external surface scanning and photogrammetry) [3]. The μ-CT technique has applicability across a wide spectrum, with utility both for samples that may be up to 1 m in length, where achievable voxel (the 3D pixel) sizes in resultant datasets may be in the 100 – 200 μm range, and down to samples that may themselves be only 100 - 200 μm in size, where voxel data can be achieved down to 1 - 3 μm. Some scanners (e.g., Zeiss Versa) are optimised to this smaller end of the spectrum, allowing even sub-micron resolution.

X-ray tomography as a technique has three fundamental characteristics that determine its value to a range of research objectives. Firstly, X-ray penetration of samples provides a non-destructive means of revealing hidden features. Whereas in past decades fossils embedded within matrix may have had to be physically extracted (with associated dangers to the specimen) or destructively sampled (e.g., serial sectioning), X-ray tomography provides an alternative route to revealing internal structures. The pattern of X-ray attenuation through a sample reveals differences in the underlying density and composition of a sample, made visible as a set of tomographic (cross-sectional) reconstructed slices. There is also value in using CT to collect preliminary data to inform further sample preparation (e.g., for curatorial purposes) or as a precursor to other analytical techniques. Secondly, it allows capture of those morphological features in three-dimensions. Reconstructed slices are loaded into visualisation software from which either automated or careful, painstaking, manual segmentation, labels features within a dataset that can then be extracted and translated into 3D. Distinct layers or features can be viewed independently or used as input in downstream analyses. Thirdly, the resolutions achievable with modern μ-CT techniques expose details and features only possible through microscopic techniques, but in three-dimensions. For example, detailing individual chambers in foraminifera or trabecular bone structures within skeletal elements.

A recent study on the inner ear morphology in fossil marine reptiles [4] neatly illustrates these advantages of a μ-CT-based approach. The authors used μ-CT scans of fossil marine reptiles to generate 3D models and perform a comparative analysis of the complex 3D labyrinth (inner ear) morphology. The labyrinth would otherwise often have been invisible on the surface of fossils, and equally impossible to assess meaningfully without the 3D preservation of information. In this case, the CT reconstructions enabled detailed insights into relationships between changes in the morphology and varying aquatic lifestyles in these extinct marine reptile groups.

Computed tomography scanners pass current through a filament to generate a beam of electrons that is focussed onto a 'target', typically a cylinder of tungsten, leading to the emission of a cone-shaped beam of X-rays. This travels towards a scintillator detector that records varying grayscale values dependent on the number of X-rays detected at each pixel on the detector. Thus, with a sample positioned in the path of the X-rays, a simple X-ray radiograph is produced. To retrieve tomographic information, the sample is rotated through 360° on an axis perpendicular to the beam, with radiography images (termed 'projections') recorded at set intervals. For optimal results, approximately 2,000 - 3,000 individual projection images are captured during the 360° rotation of the sample (i.e., at every 0.1 - 0.2 degrees).

The completed set of projections acts as input data for reconstruction software that uses algorithms to compute the tomographic data. The reconstructed data thus looks like a stack of cross-sectional images through a sample, hopefully displaying varying density properties as differences in grayscale values, and which forms the basis for any subsequent analysis.

Visualisation of the results three-dimensionally can be achieved in a number of ways (Fig. 1). Volume rendering directly displays each voxel of a dataset according to its grayscale value, with images manipulated by adjusting the range of visible values or changing the level of transparency. Alternatively, 3D surfaces (meshes) can be computed once areas of an image have been 'labelled' (using grayscale thresholds, or photoshop-style tools, to label pixels as belonging to different materials, e.g., magic wand, lasso, or manual paintbrush selection tools). The material properties of the sample and the quality of the scan will determine whether 3D visualisation of regions of interest can be achieved almost instantly, or whether manual slice-by-slice user input is required, sometimes over weeks or even months as can be the case with difficult fossil datasets. Various software packages facilitate this production of 3D visualisations (e.g., Avizo/Amira, SPIERS, Drishti, Dragonfly, and ImageJ).

2. μ-CT OPERATION & SETTINGS

The operator of the μ-CT scanner has control over a number of variables that can be manipulated according to the requirements of the sample in question. There is often no single optimum, but rather a range within which results are achievable, and normally there is a need to accept some trade-offs – most commonly in assessing the quality of a scan required against the time (and associated costs) to produce such a result.

Figure 1. Three-dimensional visualisations of the skull and neck of a modern kestrel specimen. Left a volume rendering of the skull beneath the external soft tissues, and right, the same but generated as a 3D surface mesh.

The beam emitted in μ-CT scanners is polychromatic, composed of a spectrum of X-rays of varying energy up to a user-defined maximum kV. At higher energies the beam will achieve better penetration through a specimen, without which scans will fail to reveal internal features. In samples where the composition may not yield high contrast (i.e., density or composition differences are small), it is optimal to select the lowest kV that provides sufficient penetration (determined via the grayscale histogram of the detector image). The contrast is achieved primarily from the lower end of the energy spectrum (the energies that are more strongly attenuated), and so if the beam energy is too high most of the X-rays will travel relatively unhindered through the sample and thus would not contribute to producing contrast in the final images (the contrast arising from differences in the linear attenuation coefficient within the sample). The thickness of the sample and the density of its composition will both contribute to determining the optimal parameters. For scans of fossil/rock specimens, beam energies up to or above 200 kV may be required. Whereas scans of biological material, or samples of much smaller size (e.g., 1 - 2 mm particles) beam energies of below 120 kV would be more common (e.g., [5]).

The current (μA) used affects the overall brightness of the images (by increasing the number of X-rays generated) allowing you to maximise the signal generated up to the point where the detector starts to become saturated. Increasing the exposure time for the projections will have the same effect (the number of X-rays detected increases because the length of detection is increased,) and the net result from either an increase in current or exposure (all other variables being equal) should be a cleaner, less noisy image in the resulting reconstructed slices.

Other variables influencing scan quality includes the use of filtering (metal plates located between source and object to remove lower-energy X-rays from the beam), which can reduce artefacts or be used to reduce brightness in background areas and thus allow higher power (> kV +/or μA) in order to improve X-ray penetration. The use of analogue or digital gain on the detector panel can help amplify any signal produced, and finally the application of 'frame averaging', where multiple images are taken and the results averaged into a single image, can also have a dramatic effect in improving signal-to-noise ratio (but will proportionately increase the length of the scan).

Ultimately in the appropriate selection of scan parameters a wide range of factors should be considered. However, the primary concern should always be to ensure the necessary minimum of X-ray penetration through the specimen is achieved to clearly reveal internal features, and then secondly to focus on achieving the best contrast between structures of interest and any background material.

3. APPLICATIONS ACROSS PALAEOBIOLOGY AND THE EARTH SCIENCES

Below a number of applications of µ-CT scanning are briefly discussed that both reveal the variety of research areas that can benefit from µ-CT approaches and that also highlight the value attained by matching the scanner operation and parameter settings to the needs of the studies and their respective research questions.

Foraminifera (single-celled marine organisms with an external shell composed of multiple chambers reflecting the organism's development), imaged under synchrotron conditions, have been studied for number of years to detail aspects of their biology and inform on evolutionary processes (e.g., [6]). However, tomography at the larger µ-CT scale can also reveal insights, and while the level of detail recovered cannot compete with synchrotron approaches, there is a significant advantage in the speed of data collection and ability to study 3D data in large sample sizes. Imaging forams sits at the smallest end of the spectrum of specimen sizes that are possible to examine on systems such as the Nikon XTH225 ST, where it is possible to attain voxel sizes down to ~3 µm. However, this is sufficient for a number of research questions, including quantification of the number and sizes of individual chambers (which can be as small as 15 - 20 µm in diameter), and which is informative to the study of potential stresses within the ecological system or the plastic adaptation of organisms to external stressors (e.g., as with climate change). For a single scan, the field of view at these resolutions is in the region of 5 - 6 mm in diameter, which makes it is possible to image multiple (upwards of 30 individuals) in a single scan, while keeping the individual specimens far enough apart from one another to avoid introducing any significant artefacts (Fig. 2). As scan times can also be as little as 25 minutes, this allows the rapid generation of large sample sizes suitable for broad comparative analysis [7].

There are parallels from this to applications in volcanology, where projects have in recent years utilised CT based approaches to capture 3D information on individual ash particles. For example, µ-CT approaches enable calculation of properties such as surface area to volume ratio, centre of gravity location, porosity, or sphericity, with bearing on the understanding of volcanic processes and comparative analysis of particles from specific eruptions [5]. Recent ongoing work by Claudio

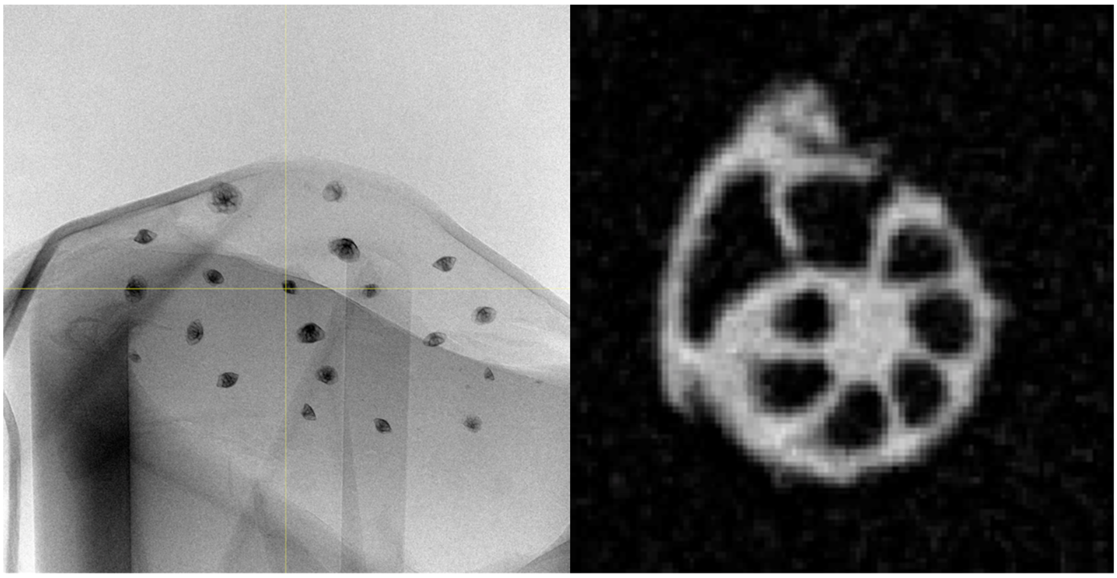

Figure 2. Imaging of foraminifera using Nikon XTH 225 ST. Left: X-ray projection image of scan setup, with 20 forams dispersed across ~4 mm diameter region of double-sided tape fixed to a diagonally-cut section of pipette tip acting as holder. Right: Indication of quality of data in the resulting reconstructed slices, with low resolution relative to the individual foram size, but sufficient detail attained to assess key features of the individual chambers.

Contreras & colleagues in Bristol has evaluated the results of varying scan parameters in the study of silicic pumice samples and is evaluating the impact of these on downstream quantification (Fig. 3). A typical advantage in this area compared to working with fossils, is that it is often possible to use destructive techniques in the preparation of a sample (e.g., producing thin cylinders which enable positioning of the sample to attain the best resolution, and which minimises the risk of artefacts appearing in the image (constant thickness of the sample from all angles in its 360° rotation). However, even in these cases, assessing (perhaps by pilot scans) the levels of noise (graininess) in a dataset, and its potential to affect subsequent analysis, is critical to adjusting the scan protocols (e.g. choosing to apply greater levels of frame averaging or longer exposure times to improve the signal-to-noise ratio).

A long-standing pathway in application of μ-CT approaches to palaeobiological questions, is in the generation of 3D models of fossils as a precursor to finite element analysis and assessment of the biomechanical performance of morphologies under simulated loading patterns [8]. These techniques can inform key questions around life in the past but depend on capturing preserved 3D morphology and in some cases digitally reconstructing a specimen [9]. Generating scans of such rock/fossil material of enough quality to produce accurate 3D representations can be difficult to achieve depending on the type of preservation.

Furthermore, to inform appropriate loading parameters for the biomechanical modelling, 3D reconstruction of muscle anatomy in fossil species can be developed, perhaps informed by morphological features in the geometry of the fossil or informed by the study of modern analogues

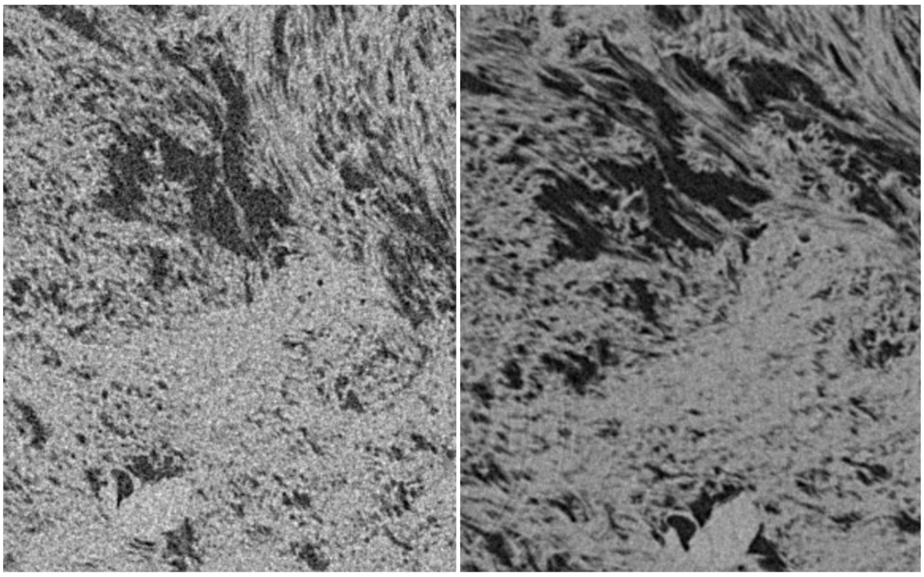

Figure 3. Left, a close-up on a region of pumice from a relatively fast 25 minute scan; and right, the same slice from a second scan utilising double the exposure time and 4x frame averaging on each projection image (orientation is slightly rotated relative to left image). The second image is visibly less 'grainy' leading to better distinctions between the pumice (grey) and the air spaces within (black), but at a cost (of time and therefore money).

(e.g., [10]). To reveal soft tissue morphology directly using μ-CT a contrast-enhanced technique is applied. The most common approach is iodine staining, which takes advantage of differential uptake of the iodine in the various tissue structures, and the results of which can be quite dramatic (Fig. 4).

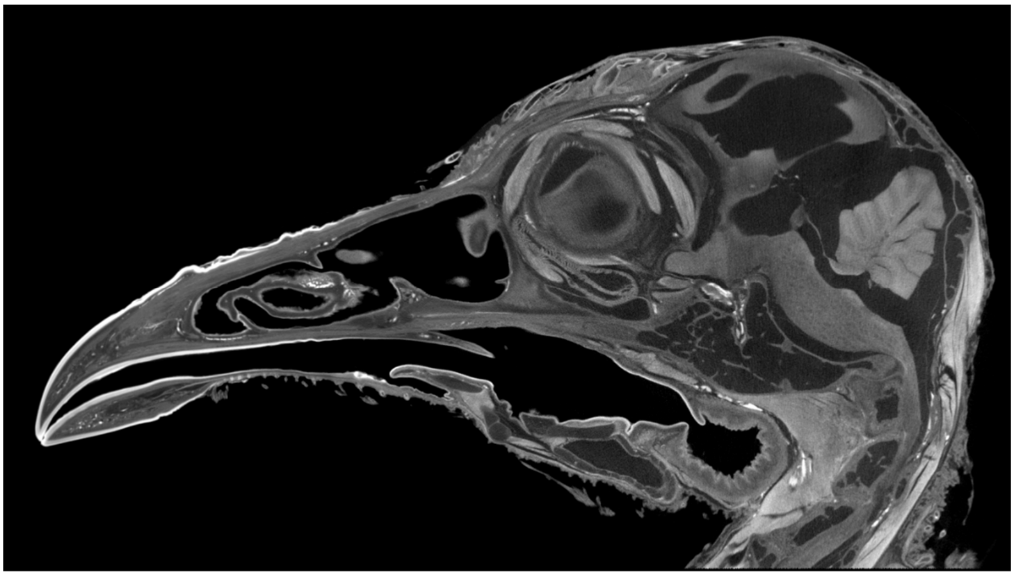

Figure 4. Example of slice information attained from iodine staining to reveal soft tissue structures in biological specimens, here on the head of a Eudromia specimen revealing soft tissue structures within the skull.

A further application, also at the interface of palaeontology and engineering approaches is in examining fluid flow around particular objects/specimens via CFD (computational fluid dynamics). Rahman *et al.* [11] utilised X-ray tomography to generate a 3D model of their fossil specimen, applied some digital reconstruction (to overcome incomplete preservation), and examined simulations of the patterns of water flow around the model to inform on various hypotheses related to feeding ecology in fossil stem-group echinoderms.

4. *CONCLUSIONS*

In conclusion, the use of μ-CT approaches to produce 3D visualisations and digital datasets from downstream analysis is a well-established technique, but one in which its application is continuing to expand across areas both within Palaeobiology and across the Earth Sciences. I have highlighted some of the variables that influence the production of reconstructed scan datasets and the need to select optimal settings and evaluate trade-offs between different parameters to produce the most appropriate study design or achieve the best quality results.

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