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Tomographic techniques for the study of exceptionally preserved fossils

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Three-dimensional fossils, especially those preserving soft-part anatomy, are a rich source of palaeontological information; they can, however, be difficult to work with. Imaging of serial planes through an object (tomography) allows study of both the inside and outside of three-dimensional fossils. Tomography may be performed using physical grinding or sawing coupled with photography, through optical techniques of serial focusing, or using a variety of scanning technologies such as neutron tomography, magnetic resonance imaging and most usefully X-ray computed tomography. This latter technique is applicable at a variety of scales, and when combined with a synchrotron X-ray source can produce very high-quality data that may be augmented by phase-contrast information to enhance contrast. Tomographic data can be visualized in several ways, the most effective of which is the production of isosurface-based ‘virtual fossils’ that can be manipulated and dissected interactively.

Keywords: tomography; fossil; visualization

1. INTRODUCTION

Organisms are three-dimensional structures, and to understand their biology we are required to consider them as such. For palaeobiologists, this requirement can be problematic, as fossilization processes may reduce three-dimensional morphology to less informative two-dimensional fossils. While three-dimensional preservation of mineralized fossils is common, the processes that produce soft tissue preservation are typically less helpful; most Konservats-Lagerstätten (deposits with exceptionally preserved fossils) preserve flattened soft tissues. Three-dimensional preservation of unmineralized tissue does occur and is less rare than generally supposed; examples include the Herefordshire (Briggs et al. 1996), La Voulte Sûr Rhone (Wilby et al. 1996), Gogo (Ahlberg 1989) and Orsten (Waloszek 2003) Lagerstätten. However, this type of material can present a more subtle problem; how to extract three-dimensional morphological information from the host rock. This issue is not restricted to exceptionally preserved material, but pervades all work with three-dimensional fossils. Isolation methods, of which several exist, are one solution. Fossils may simply ‘drop out’ or be naturally washed out of rocks; wet sieving of poorly consolidated sediments mimics this process. Specimens may also be extracted chemically, for example, by dissolving the matrix (e.g. Aldridge 1990). These approaches are effective where applicable, but are prone to losing associations between disarticulated or weakly connected parts of fossils and to damaging delicate structures. Specimens can also be physically ‘prepared’ out using needles, drills or gas-jet powder abrasive tools (e.g. Whybrow & Lindsay 1990); while usually preserving associations, this approach may also damage delicate structures, scales poorly to small specimens and cannot always expose all of a specimen. Finally, isolation of a fossil only provides access only to its surface; study of morphological data from the interior of a three-dimensionally preserved fossil requires the preparation of a tomographic dataset.

Tomography is the representation of three-dimensional structure as a series of two-dimensional images formed from parallel sections (figure 1); a tomoscan is a single sectional image and a tomoscan is a device used in the preparation of a tomographic dataset (a series of tomoscans). Tomographic datasets can be studied directly or used as a basis for three-dimensional reconstructions of the original structure (§3). Tomographic datasets where image scale, resolution and slice spacing are constant can be treated as volumes, in which tomogram pixels are voxels (volume elements), measurements of some property at points on a three-dimensional grid.

Tomography (§2) is typically time consuming, may require expensive tomographs and may be destructive. Tomographic datasets can also be problematic to interpret, even when aided by digital visualization techniques (§3). Nonetheless, tomography provides the only effective means of imaging the internal structure of three-dimensional fossils and does not suffer from data loss problems associated with isolation techniques. While applicable to any three-dimensional fossil, tomography is particularly important for exceptionally preserved material, where the extraction of maximal morphological data from rare specimens is paramount.

In cases where isolation is not viable (e.g. the Herefordshire Lagerstätte; Briggs et al. 1996), tomography may represent the only method capable of resolving any three-dimensional morphology. Even where specimens have already been isolated, tomographic methods remain of use for investigating internal structures (e.g. Donoghue *m.sutton@imperial.ac.uk

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2. TOMOGRAPHIC DATA CAPTURE

(a) Physical–optical tomography

Prior to the advent of ‘scanning’ techniques (§2b), tomograms were of necessity prepared through the physical exposure of sections and optical imaging of surfaces; these approaches retain utility. Palaeontological tomography was pioneered by Sollas (1903), whose approach involved serial grinding using a custom-made apparatus, and photography followed by manual tracing of structure. Sollas primarily applied his technique to vertebrate fossils (e.g. Sollas & Sollas 1913), as did Stensiö (1927) in his classic studies of the cranial anatomy of Devonian fish. The approach is, however, adaptable to a variety of scales and taxonomic groups, and various grinding tomograms (e.g. Simpson 1933; Croft 1950; Ager 1965; Sutton et al. 2001) have been used on a wide range of fossils; studies of the internal structures of fossil brachiopods, in particular, have become heavily reliant on Sollas-style tomography since the pioneering work of Muir-Wood (1934).

Variants on the Sollas method include serial sawing, in which fine parallel saw-cuts are used to expose tomographic planes; sawing is less destructive, as material between saw-cuts is preserved, fine annular or diamond wire saws removing as little as 200–300 μm material. However, saw-cut spacings below 1–2 mm are impractical, and this variant is hence limited to larger fossils (e.g. vertebrates, Kermack 1970), or to obtaining rough reconstructions of smaller fossils (e.g. Sutton et al. 2005b, p. 1002). Saw-exposed surfaces are also less well polished for photography than ground surfaces, and datasets comprise tomograms facing in alternating directions and with alternating spacings, complicating visualization. Serial slicing using a microtome blade is also possible for the study of ‘soft fossils’ (e.g. the decalcified mammal skulls of Kielen-Jaworowska et al. 1986), but is not practical for mineralized fossils.

Means of data capture other than direct photography also exist, the most important of which is the use of acetate peels (Katz & Friedman 1965) to provide a physical record of exposed surfaces that transcend the limitations of any image capture device. Peels can be combined with chemical staining to enhance visual contrast between different material types in the surface. Acetate peel tomography has been used at least as broadly as ‘classical’ Sollas tomography in palaeontology; important examples include the study of exceptionally preserved plant fossils (e.g. Long 1960), brachiopods (a recent example is Baker 2005), carpoids (e.g. Jeffries & Lewis 1978) and vertebrates (e.g. Romer 1941). Typically, workers have relied on peels to record data, not supplementing them with direct photography of surfaces; for the purposes of digital reconstruction this is unfortunate, as even the best peel datasets are prone to data loss from tears and bubbles, to inconsistent contrast between slices and to wrinkling effects. These problems are difficult to correct and degrade three-dimensional models; where a tomographic dataset is being captured with visualization in mind, the use of peels alone should be avoided.

Recent physical–optical tomographic work has returned to direct photography of surfaces, but replaced film with digital photography (or scanning; see Hammer 1999). Digital images are convenient for digital visualization and allow image quality to be checked before the next section is generated, reducing the potential for information loss. The investigation of the Silurian Herefordshire Lagerstätte (e.g. Sutton et al. 2001, 2004a, b; Siveter et al. 2003, 2004, 2007) provides an extensive example of the application of a modern physical–optical tomography technique to exceptionally preserved fossils. These studies, covering a range of small invertebrate taxa, use high-resolution serial grinding (at 20–30 μm intervals) coupled with photography via a microscope-mounted digital camera (figure 3a). The same technique was used by Thomson et al. (2003) to analyse the enigmatic Devonian vertebrate Palaeospondylus; tomographic studies of Late Proterozoic calcified metazoans have also been undertaken by Grotzinger et al. (2000; see also Watters & Grotzinger 2001), based on direct photography rather than microscope-mounted digital photography.

Digital visualizations can, in theory, be constructed from any physical–optical dataset (§3), but high-fidelity visualizations require high-resolution datasets (low slice spacings, high resolution within each tomogram) with a minimum of missing data, and consistent spacing, brightness/contrast and image scale. Tomographic planes should be parallel, and the appearance in a tomogram of structures outside the plane, due to crystal transparency for instance, should be avoided. Prior to reconstruction individual tomograms must be ‘registered’ (digitally aligned); formally, they must be transformed so that the vector offset in real space between point (x, y) in tomograms n and n+1 is perpendicular to the tomographic planes. Automated registration is possible, but has not been satisfactorily demonstrated for palaeontological material. Instead, interactive software is used to manually shift and/or rotate each tomogram. The accuracy of this process, and hence the fidelity of visualizations, is greatly improved if fiduciary structures are emplaced before data capture commences; these are typically drilled holes or polished surfaces perpendicular to the tomographic plane (Sutton et al. 2001).

(b) Optical tomography

Tomography of translucent materials can be achieved through ‘optical sectioning’, where tomograms are obtained by imaging within a single focal plane. Confocal laser scanning microscopy (CLSM) is a specialized technique for single-plane focus, widely used in biology (e.g. Amos & White 2003). While geological materials are not all suited to CLSM, most variants of which require
fluorescent organic samples, it has been applied to Precambrian microfossils preserved in chert (Schopf et al. 2006, see also figure 3d) and to isolated siliceous microfossils (O’Connor 1996). Conventional microscopy can also provide sufficient separation of focal planes for tomography, as demonstrated by the reconstruction of arachnid respiratory structures from the Devonian Rhynie chert Lagerstätte (Kamenz et al. 2008). Optical tomography is non-destructive and can resolve sub-micron scale structure. However, it requires a relatively unimpeded light path to and from the specimen, and is hence only applicable to ‘clean’ translucent materials, typically hosted within thin sections. CLSM is sensitive to the absorbance of light by both fossil and host rock and imaging is typically limited to top of samples; Schopf et al. (2006) reported difficulties in obtaining clean data at depths below 150 \( \mu m \).

(c) Scanning technologies

Several technologies can generate tomograms without visible light penetration or physical exposure of surfaces. The most widely used of these is X-ray computed tomography (CT) which produces tomograms representing X-ray attenuation maps. In its simplest form, a CT scanner consists of an X-ray source, a detector and a means of rotating these with respect to the sample; medical scanners rotate the source/detector pair, while scanners for inanimate specimens rotate the sample on a stage. Volume CT is the scanning mode normally used in a palaeontological context; here the detector is a two-dimensional charge-coupled device (CCD) array used to capture digital radiographs of the sample from many rotational positions, typically every degree of a 180° rotation (figure 2). Tomograms perpendicular to the axis of rotation are derived from this data computationally, using a filtered back projection algorithm to implement an inverse Radon transform (e.g. Kak & Slaney 2001). This scanning methodology, formally ‘X-ray computed axial tomography’, produces isotropic data (voxels are cubic), which is ideal for visualization. Many other CT variants exist and many details are here glossed over; see Ketcham & Carlson (2001) and Kalendar (2006) for exhaustive treatments.

The resolution (voxel count) of a tomographic dataset from an axial scan is directly proportional to detector resolution, but the range of absolute voxel sizes a scanner can achieve depends on the physical configuration and precision of the device, and varies from millimetres to less than 1 \( \mu m \). Within these limits, absolute voxel size is proportional to the maximum sample dimension perpendicular to the rotational axis, as tomogram computation requires each radiographic image to contain all of the sample that lies in the tomographic plane. This requirement restricts resolution from fossils within flat slabs of rock; axial scanning is best suited to near equidimensional samples, or elongate samples that can be rotated around their long axis and scanned in sections down their length. Dierick et al. (2007) have, however, demonstrated a ‘region of interest’ scanning technique that avoids this stricture, at least for fossils in the relatively homogenous medium of amber.

Medical CT scanners are optimized for human-scale objects and have been used since the early 1980s (e.g. Tate & Cann 1982; Conroy & Vannier 1984) to study large vertebrate fossils. Their use is now routine in this field, particularly to visualize material enclosed in matrix or structures internal to a specimen (e.g. Brochu 2003), or where three-dimensional models are required for functional analysis (e.g. Rayfield et al. 2001). While occasionally used to image invertebrates (Hamada et al. (1991) is an early example), medical CT scanners have minimum voxel sizes of several hundred microns, and are unsuitable for analysing small objects such as most exceptionally preserved invertebrate fossils.

In the last 10 years, X-ray micro-tomography (XMT) scanners have become available, which apply the axial CT method on smaller scales. Initially designed for engineering and materials applications, they are well suited to scanning small fossils. The University of Texas High-Resolution X-ray Computed Tomography Facility has been responsible for the majority of palaeontological XMT studies; it is capable of resolving voxels of less than 5 \( \mu m \) in size. Laboratory-scale XMT systems with comparable specifications now exist in several institutions worldwide, and lower specification desktop scanners are also available; these provide an available and effective means of recovering tomographic data from most millimetre- to centimetre-scale material and have become important tools for the study of smaller vertebrate (e.g. Rowe et al. 2001), plant and invertebrate fossils. Examples include the visualization of gill-slit like structures in the carpoid Jaekelocarpus (Dominguez et al. 2002), investigation of the chiton-like multiplacophoran mollusc Polysacos (Vendrasco et al. 2004) and taxonomy of rudist corals (Molineux et al. 2007); imaging of Eocene fruits and seeds from the London Clay (Devore et al. 2006), spiders in amber (Penny et al. 2007) and Bembridge Marl insects (figure 3c) demonstrates the applicability of XMT to exceptionally preserved material.

Data quality from laboratory-based XMT is limited by the X-ray sources available; these are polychromatic, reducing precision and causing ‘beam-hardening’ artefacts (brightening towards edges), and may be too weak to penetrate some X-ray dense specimens or provide good discrimination between materials of similar X-ray density. A variant XMT technique uses a synchrotron, a form of particle accelerator, as a bright monochromatic X-ray source. Tomography systems on synchrotron beam lines are provided with very high-resolution detectors to complement the source; synchrotron radiation X-ray tomographic microscopy (SRXTM) can thus produce tomographic data of exceptional resolution and clarity. The study of sub-millimetre scale phosphatized Cambrian embryos by Donoghue et al. (2006; figure 3e,f) performed...
sparry calcite fossils from largely micritic matrix. An approach that may assist with such difficult specimens exploits the coherence of synchrotron X-ray sources to implement a ‘propagation phase-contrast’ technique (Tafforeau et al. 2006), where X-ray interference information provides augmented boundary detection and is overlayed on absorbance data. Related to this method is synchrotron holotomography (Claetens et al. 2006), which produces tomograms solely containing phase-contrast information. The applicability to fossils of both approaches was demonstrated by Tafforeau et al. (2006) and Friis et al. (2007) have subsequently used propagation phase-contrast XMT to study charcoalized Cretaceous seeds.

Laminography is a technique for generating X-ray attenuation tomograms, which does not require full sample rotation, substituting differential movement of sample and detector to simulate ‘focus’ on a plane. The approach is well suited to flat objects problematic for conventional CT, such as fossils in slabs of rock. Analogue laminography is in many ways unsatisfactory, but ‘computed laminography’ (or ‘digital X-ray tomosynthesis’), which uses tomogram reconstruction algorithms analogous to those of CT, represents an important development (Dobbins & Godfrey 2003). The collimated monochrome X-rays from synchrotron sources are particularly well suited to this procedure (Helfen et al. 2005), and while conventional computed laminography has yet to be applied to fossils, Tafforeau et al. (2006) have demonstrated the viability of synchrotron laminography for palaeontological material.

Computed axial tomography can use any penetrative radiation differentially absorbed by a sample; neutron tomography (NT) represents one such alternative. The absorption profile of neutrons is very different from that of X-rays; they are, for instance, more strongly attenuated by organic material than by most rock. NT is hence appropriate for the imaging of organically preserved fossils (e.g. plants, see Winkler 2006); it may also be useful for larger vertebrate material (Schwarz et al. 2005) as a complement to X-ray CT. NT resolution is, however, lower than that of XMT and SRXTM in terms of minimum voxel size (approx. 100 µm; Winkler 2006), and high-intensity neutron bombardment can induce hazardous levels of radioactivity in some geological materials, for example, those containing cobalt or europium (M. Dawson 2008, personal communication); samples may thus need to be interred for months or years after NT study.

Magnetic resonance imaging (MRI) is a tomographic technique widely used in medicine; it maps not radiation attenuation but properties relating to the chemical environment of certain elements, especially hydrogen. Historically, MRI has been considered poorly suited to geological materials, but has been used, for example, to image a fluid-filled mouldic vertebrate fossil (Clark et al. 2004) and to investigate pore space disturbance by trace fossils (Gingras et al. 2002). Mietchen et al. (2008) have recently demonstrated MRI micro-tomograms of invertebrate fossils (figure 3c) with approximately 50 µm voxels, though with relatively low overall resolution. At present, MRI does not compete with XMT or NT for the imaging of morphology, but the multidimensional nature of MRI signals has potential to provide rich three-dimensional data on the chemical composition of palaeontological specimens.
3. VISUALIZING TOMOGRAPHIC DATA

Tomographic datasets can be studied directly, but are more informative when used for three-dimensional visualization. The most primitive visualizations are physical models, introduced to palaeontology by Sollas (1903) who built models from layered wax cut-outs based on tomographic tracings, stacked and melted together. There are many drawbacks to this laborious approach. Virtual fossils—digital reconstructions from tomographic datasets—provide a more practical means of studying morphology, particularly when manipulated interactively in stereo-capable viewing software. They can be viewed from any position, dissected or made locally translucent, be coloured to pick out structures, are easily copied, and retain associations between non-contiguous structures. The production of physical models from virtual fossils is possible using rapid-prototyping technologies (e.g. Zhang et al. 2000); virtual models are normally preferable for research purposes, but physical models retain some utility, for example, in museum displays.

The production of a digital visualization follows similar principles whatever the source of the tomographic dataset, although most physical–optical data first require registration (§2a). Two visualization approaches exist. First, structures can be modelled using two-dimensional vector graphic objects in each tomogram; typically these are spline curves, either closed loops or open paths. Identification of outlines can theoretically be automated, but high noise levels in most palaeontological data make this impractical; manual placement of curves, using a raster tomogram as a guide, is the norm (e.g. Kamenz et al. 2008). To produce a model, corresponding curves are surfaced with a triangle mesh. This approach is relatively inexpensive computationally and is well suited to pre-existing data in the form of traced outlines; it was thus the first approach used for palaeontological physical–optical datasets (e.g. Chapman 1989; Herbert 1999). Enforced human interpretation is involved, as tracings must be made on every slice; this may sometimes be desirable (problematic regions cannot be ‘glossed over’, for instance), but equally ‘graded’ regions are difficult to represent, and the process is always laborious. Reconstruction requires the identification of correspondences between objects in adjacent tomograms; this can be problematic for complex structures (e.g. branching networks) which are difficult to model.

The alternative (and more popular) approach uses volume rendering; here tomogram pixels are treated as voxels in a three-dimensional array (§1). Scalar values for voxels are required; where data are multidimensional, as for colour photography, they are first reduced to scalar (monochrome) form. Volumes can be rendered directly by projecting virtual beams through them, and attenuating these computationally in any desired way; this approach requires no user intervention prior to reconstruction, but produces difficult to interpret radiograph-like images (e.g. Sutton et al. 2001). Direct volume rendering is also not supported by most graphics hardware, and is hence relatively slow. Much more commonly, one or more isosurfaces are computed from the volume; the data are first thresholded to identify voxels brighter than an arbitrary user-defined threshold, and a triangle mesh representing the surface defined by these is generated, normally using the marching cubes algorithm (Lorensen & Cline 1987). This approach provides visually clean surface-based models without requiring tracing of structures, and does not suffer from the connectivity problems of spline surfacing. Interpretation is not enforced but is possible through local raising or lowering of voxel values, pushing individual voxels above or below the threshold and hence incorporating or removing them from the isosurface. Careful ‘editing’ in this manner allows the removal of noise (figure 3e), and accurate modelling of any visually identifiable structure (Sutton et al. 2001). Interpretation can thus be employed where necessary or skipped where time constraints or data quality do not require or permit it.

While many studies have worked with single isosurface reconstructions (such as figure 3e), most visualization software allows specification of regions-of-interest and multiple threshold levels. Multiple isosurfaces representing different anatomical structures and/or preservational materials (e.g. Sutton et al. 2005b) can thus be assembled into a composite model, using pseudo colour to differentiate these elements (e.g. Sutton et al. 2005a; Donoghue et al. 2006; figure 3f,g); elements can also be toggled on/off during visualization to allow ‘virtual dissection’.

Isosurface reconstruction is ideal for datasets where the fossil is a solid region, distinct in shade from the matrix. It is less effective for gradational structures, fossils distinguished by textural differences or preserved as outlines such as the organic film fossils of the Rhyne Chert (Fayers & Trewin 2004). The approach works best for isotropic or sub-isotropic data such as that from axial CT; where tomogram spacing is large with respect to pixel spacing, reconstructions can be ‘blocky’. Finally, isosurfaces have a higher triangle count than spline-based surfaces, which can make them cumbersome to handle, although secondary simplification can mitigate this problem.

Where interactivity is required, triangle meshes from isosurfaces or spline surfaces can be rendered in real-time using graphics hardware (figure 3e,f); high-quality preprepared images or animations can alternately be prepared using the slower technique of ray tracing, which provides more realistic shadow and lighting effects (figure 3g).

4. SUMMARY

Tomography is the optimal approach to the study of internal structures in three-dimensional fossils, but visualization difficulties, coupled with the laborious and destructive nature of physical–optical techniques, long limited its use. Tomography is now enjoying a renaissance, driven by digital visualization and new scanning technologies. Physical–optical methods remain attractive for some material; they can work at high resolutions, record optical data in colour (which in some cases provides optimal fossil/matrix contrast), and require no dedicated equipment. Optical tomography provides a straightforward method for gathering very high-resolution data; though restricted to small scales and translucent materials, it may be the best approach when applicable. Variants of X-ray CT (XMT and SRXTM) are currently the methods of choice for most fossils; these cover a range of different scales and capture high-quality datasets rapidly. Conventional laboratory-based CT/XMT systems are capable of
resolving fine detail in most specimens (figure 3e) and should normally be used as a first resort, but very small or otherwise ‘difficult’ specimens (e.g. those too dense to be penetrated by conventional X-ray sources, or with very low X-ray attenuation contrast) may require a synchrotron source. Fundamental limitations of all variants to material with X-ray attenuation contrast and to sub-equadimensional samples can be problematic, though new synchrotron techniques (phase-contrast tomography, hologtotomography and laminography) may partially overcome these problems. Other scanning technologies (NT and MRI) are currently less broadly applicable, though NT may be preferable to CT for relatively large organically preserved fossils.

High-quality tomographic datasets can now be produced from almost any three-dimensional material and very effectively studied through interactive digital visualization. This ‘virtual palaeontology’ approach represents a powerful new means of working with previously difficult material, greatly augmenting the ability of palaeontologists to extract maximal data from our most information-rich resource; exceptionally preserved three-dimensional fossils.

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