



METHODOLOGIES FOR THE VISUALIZATION AND RECONSTRUCTION OF THREE-DIMENSIONAL FOSSILS FROM THE SILURIAN HEREFORDSHIRE LAGERSTÄTTE

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ABSTRACT

The dominantly soft-bodied fauna of the Silurian (Wenlock) Herefordshire Lagerstätte of England is preserved as three-dimensional calcitic fossils within calcareous nodules. These fossils are difficult to interpret from study of sections produced by splitting, and are difficult also to isolate from their matrix. CT scanning of nodules is feasible, but is expensive and unlikely to be able to image the fossils at a sufficiently high resolution. We describe a technique based on serial grinding and digital photography which enables the capture of three-dimensional morphological information from the fossils. Computer video files are used to package the resulting datasets for inspection. Three-dimensional reconstructions can be created by a method involving the manual tracing of outlines on each image, but we consider this approach inappropriate in this instance. Datasets from grinding are treated instead as volumes, which are rendered either directly to produce virtual X-ray images, or by the computation of isosurfaces which are then visualized by ray-tracing. Rendering is performed from sequential angles to produce video files of rotating three-dimensional models. The method is illustrated with reconstructions of a trilobite and graptolite associated with the Herefordshire soft-bodied fauna, and of the worm-like mollusc *Acaenoplax hayae*. Reconstructions produced in this way can be retouched easily and manipulated to remove noise and preservational artifacts, to dissect out structures for detailed study, or to combine part and counterpart into a single reconstruction. The approach will provide the primary means of study of the Herefordshire fauna, and has the potential to provide a method of obtaining and/or communicating morphological information in a variety of palaeontological applications.

KEY WORDS: isosurface, reconstruction, Silurian, soft-bodied, three-dimensional.

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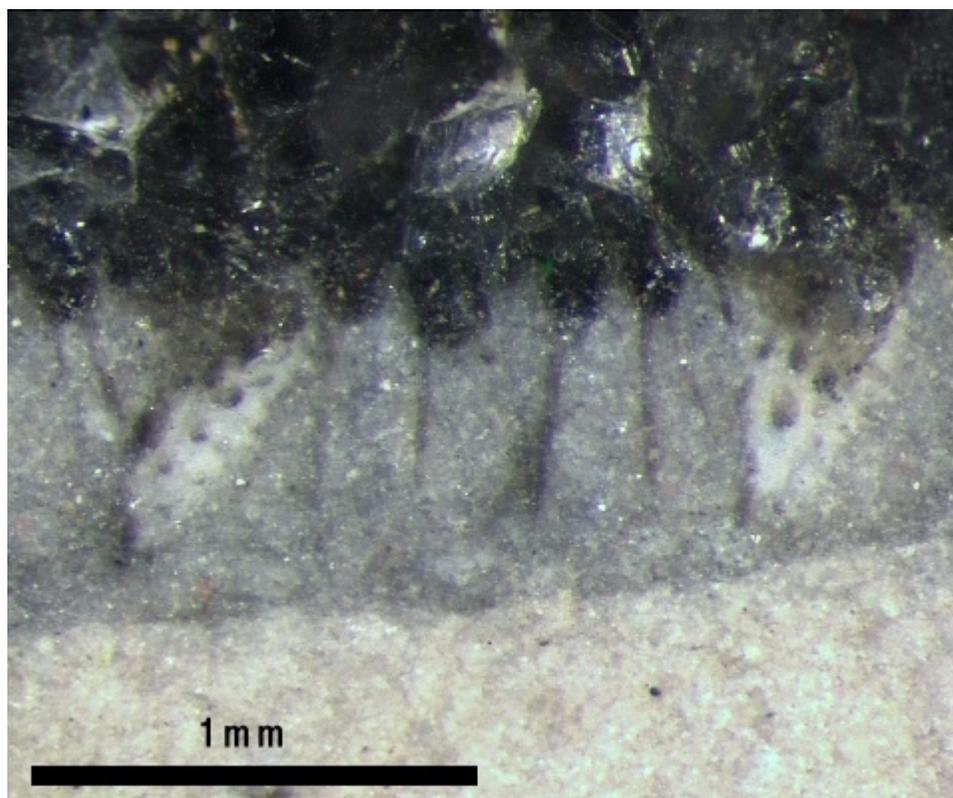


Figure 1. Detail of fine spines preserved on margin of *Acaenoplax hayae* Sutton et al. (2001), from the Herefordshire Lagerstätte; OUM C.29503b.

INTRODUCTION

The non-biomineralized fauna of the Herefordshire Lagerstätte (Briggs et al. 1996) represents a rare opportunity to sample the true taxonomic composition and palaeoecology of a Silurian benthic community. Fossils occur in spherical to subelliptical calcareous nodules found in a locally thick bentonite deposit in the Coalbrookdale Formation, Wenlock Series. They vary in size from under a millimetre to several centimetres, with worms, sponges, and non-biomineralized arthropods dominating the assemblage. All taxa are preserved in three dimensions, with no indication of post-mortem disturbance or compression. While some fossils preserve organic material (e.g., the graptolites) and others siliceous skeletons (e.g., the radiolarians), for the most part specimens consist of dark-coloured sparry calcite

shapes within a lighter-coloured matrix (Briggs et al. 1996; see also Orr et al. 2000). Few unambiguous internal features of the organisms are known, but external details are commonly reproduced with a degree of fidelity sufficient to preserve fine spines 30 μm or less in diameter (Figure 1).

All taxonomic and palaeoecological study of the Herefordshire biota must be underpinned by careful resolution of the morphologies of the fossils preserved. However, while three-dimensional preservation is clearly richer in morphological information than the two-dimensional or near two-dimensional modes of most other Konservat-Lagerstätten (e.g., the Burgess Shale, see Allison and Briggs 1991), it introduces special problems. Surfaces exposed by splitting nodules are essentially randomly oriented with respect to the organism and show only those structures

that happen to be intersected by the split. Structures of fossils are thus easily overlooked or misinterpreted. Furthermore, it is frequently difficult to distinguish the genuine termination of a structure, such as the body of a worm, from the situation where it passes out of the plane of section. These factors make attempts to reconstruct morphology from such 'natural' splits difficult and feasible only when a large number of specimens are known or when the morphology is relatively simple. If essential morphological data are to be obtained for the majority of taxa, to which neither of these conditions apply, then as much of the three-dimensional form as possible must be extracted from a single specimen.

The simplest and most common approach to determining the three-dimensional form of a fossil is to separate it from the matrix, either chemically or mechanically. Chemical dissolution techniques, though widely used in many branches of palaeontology (including the recovery of Konservat-Lagerstätte fossils such as the Cambrian 'Orsten' faunas; see Müller et al. 1995) require fossils and matrix to be chemically dissimilar. Although dissolution has been used to extract microfossils such as radiolarians from the Herefordshire nodules (Briggs et al. 1996), the predominantly calcitic nature of both matrix and macrofossils precludes its use in the study of most of the faunal elements. Mechanical excavation of the Herefordshire fossils is possible and has some utility for the study of large and robust structures (e.g., Briggs et al. 1996, fig. 1c, e). However, it is both extremely time consuming and inappropriate for resolving small or fragile structures, and thus is not a satisfactory approach for the study of the fauna as a whole. In view of these difficulties, our study has concentrated not on the physical isolation of the fossils but on techniques based on serial slices, in which

many cross-sections are obtained from parallel planes intersecting the fossil. Data of this form can be collected in several different ways, but all approaches produce datasets that can be reconstructed into three dimensions with the aid of a computer.

APPROACHES TO SLICE DATA ACQUISITION

Non-destructive approaches

Two commonly used medical scanning technologies, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), use non-invasive techniques to produce 'slice-based' datasets representing respectively, the X-ray absorption, and degree of magnetically induced nuclear resonance of materials in the sample. The latter technique is not well suited to solid geological materials, but CT has been used successfully to provide three-dimensional data on fossils (Hamada et al. 1991; Torres 1999). Although our preliminary experiments have shown that the Herefordshire fossils within nodules can be detected by CT scanners, generally available equipment is capable at best of slice spacings and pixel sizes of around 0.3 mm to 0.5 mm. Specialised CT equipment (e.g., *the University of Texas High Resolution X-ray Computed Tomography Facility*) can, under ideal conditions, achieve resolutions over an order of magnitude finer than this, and might thus in principle be a viable tool for studying the fauna. However, time on such equipment is expensive and its ability to consistently distinguish fossil from matrix in the Herefordshire material at high resolutions is as yet unproven. While CT may provide a valuable tool for investigating large or extremely rare taxa, we do not consider it to be a viable approach to the routine study of this fauna.

Serial slicing

Serial sectioning of fossils is the normal approach to the collection of 'slice-based' datasets. However, the term 'serial sectioning' is commonly used to encompass both serial slicing by saw or blade and serial grinding (Ager 1965, p. 213). These are substantially different techniques, and we prefer to use the separate terms to distinguish between them.

Serial slicing is less commonly used on fossils than serial grinding, although it does allow much of the original material to be retained whilst providing the required multiplanar views. The method is best suited to larger specimens than the Herefordshire fossils because intact slices substantially thinner than 1 mm are extremely difficult to produce by sawing. In contrast, biological microtome equipment can generate exceptionally fine slices (as small as 1 μm). However, microtomy requires a non-brittle sample capable of forming a thin cohesive slice—properties not associated with crystalline materials. Almost certainly for these reasons, experiments on the use of microtomy on Herefordshire fossils were unsuccessful, despite their vacuum impregnation with resin.

Serial grinding

Serial grinding involves the sequential removal by abrasion of small thicknesses of material from a single planar surface which is photographed at each stage. Although this approach is destructive, it suffers from none of the problems of a lack of cohesiveness associated with slicing, and inherently produces very flat polished surfaces which are ideal for photography. For these reasons we consider it the only viable approach to obtaining high-quality serial images from the Herefordshire fossils. Serial grinding is not a new technique and has been used extensively on fossils of widely differing sizes (e.g., Sollas 1913; Stensiø 1927; Ager 1956-67;

Baker 1978; Hammer 1999). Grinding has been carried out by other researchers with a variety of pieces of dedicated equipment, the most popular of which have been variants of the 'Croft Grinder' (Croft 1950; see also Ager 1965; Sandy 1989). The principle differences between our data acquisition technique and those used by most previous authors are the relative simplicity of the grinding device, and the method of data capture—photography at regular intervals rather than manually prepared drawings often at irregular intervals.

Considerations for Data Acquisition by Serial Grinding Photography.

The destructive nature of serial grinding places special demands on the photography of each freshly exposed surface. The photographic technique used must not only capture as much information as possible, but it must have a very low failure rate, because images cannot be re-taken after grinding has progressed. Digital rather than conventional photography is therefore a key element of our method since digital images can be downloaded instantly to a computer where they can be checked for quality before grinding continues. The use of a digital camera also offers significant economic savings as well as removing the need for images to be scanned into a computer for reconstruction.

Registration. Although the processing of images from serial grinding is discussed below, one aspect must be considered here as it impacts on the early stages of the technique. Before any reconstruction can be attempted images must be 'registered' (or aligned) so that the arrays of pixels line up correctly. This can be accomplished in one of two ways: 1) the sample can be placed in exactly the same position relative to the field of view of the camera for each image capture; or 2) the sample can be processed so that it

contains physical ‘fiduciary’ (or alignment) markings of some sort, that can be used subsequently to align the images. The second approach is simpler in practice and is preferred in our work. Fiduciary markings used in similar work by previous authors are, typically, straight holes drilled perpendicular to the grinding surface so that they appear in the corners of the image (e.g., Herbert 1999, figure 3.2; Baker 1978). However, holes less than 0.5 mm in diameter cannot be produced reliably with current drilling technology and larger holes would be unacceptable in the context of this study. Consequently, we use two perpendicular edges (see below) as fiduciary structures, which are roughly aligned in the field of view when images are first captured. These edges are used to guide precise digital alignment during the registration phase of post-processing (see Appendix 1, section 1).

METHODOLOGY FOR GRINDING AND DATA CAPTURE

Specimens selected for serial grinding (Figure 2.1) are first sawed to produce a small block with two faces perpendicular to each other and to the direction of grinding, and as close to the specimen as possible. These side faces are then polished to obtain the sharp edges at their junction with the ground face (Figure 2.2). The block is set within a resin cuboid or cylinder (Figure 2.3), that is then mounted with wax onto a Wirtz Buehler Slide Holder (Figure 2.4). Note that while Figure 2 illustrates a grinding run performed on a single fossil, multiple specimens are normally mounted within one resin block for greater efficiency. The slide-holder consists of an inner metal cylinder that, by means of a screw thread, can be positioned accurately to protrude any required distance beyond the plane of an outer hard ceramic disc. This enables a fixed thickness to be

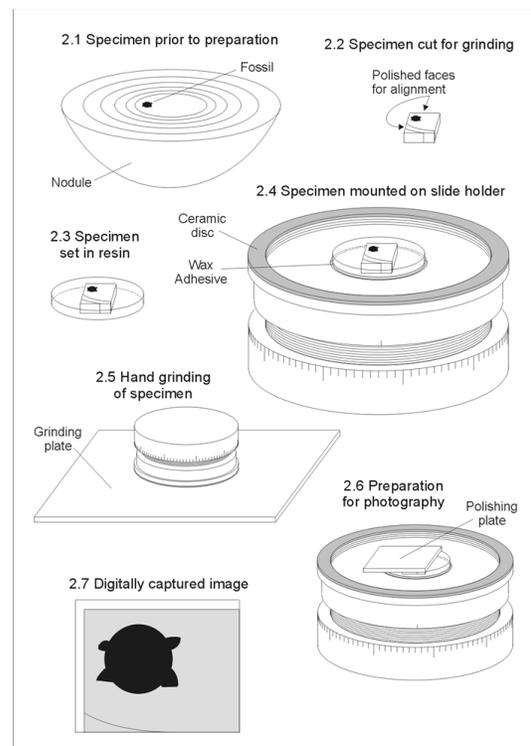


Figure 2. Grinding process.

ground away before the ceramic comes into contact with the grinding plate and prevents any further removal of material.

Specimens are ground manually on a glass plate (Figure 2.5) with 600 grade carborundum powder and water to remove thicknesses of approximately 30-50 μm at a time. After each thickness is removed, the slide holder is inverted and placed on its base. The surface is then washed and polished for a few seconds with a small flat plate coated in fine-grade aluminium oxide powder and water (Figure 2.6). This final polishing improves image quality without removing any appreciable thickness of sample. The surface is then washed again, and the slide-holder placed under a Leica MZ8 binocular microscope. An image of each specimen and alignment edges (Figure 2.7) is then captured by means of a digital camera attached to the microscope operating at 1000 by 800 pixel resolution in 24-bit colour. The image is checked for quality prior to filing as part of

a sequential set. All images for a grinding run are taken at the same magnification, with the same camera settings, and under consistent lighting conditions; only the focus is re-adjusted for each image. The grinding process is then repeated for as many iterations as required. The time taken per image depends on how many specimens are mounted at once for a grinding run, but on average it takes six minutes to grind a 30 μm interval and one minute to photograph each specimen. After completion of a grinding run each dataset is prepared for use by registration of the images (see Appendix 1, section 1).

VISUALIZATION AND RECONSTRUCTION

Slice videos

Computer video files can be assembled directly from the datasets resulting from grinding (see Appendix 1, section 2), providing a simple way of leafing backwards and forwards through the slice images (Figure 3). These files form an invaluable tool for the exploration of datasets and, although less striking than full three-dimensional reconstructions, are often more useful for tracing the origins and termination of small or obscure structures. Nonetheless, they do not constitute

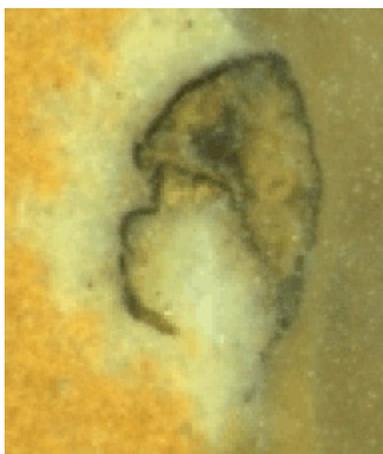


Figure 3. Slice video showing raw dataset (distal portion of monograptid graptolite; OUM C.29526a).

a three-dimensional reconstruction in the accepted sense and are best used to complement rather than substitute for such reconstructions.

Construction of three-dimensional models

The ‘surfaces’ method. Two methodologies exist for the construction of three-dimensional models from data based on slices (Brown and Herbert 1996; Herbert 1999). The first, the ‘surfaces’ approach (Herbert 1999; see also Chapman 1989), begins with a manual interpretation step, tracing all structures of interest from each slice image (Figure 4.1-4.3). Traced outlines are digitized into line segments forming closed loops (Figure 4.4). The reconstruction algorithm assembles these stacked two-dimensional loops into a three-dimensional geometry, typically a mesh of triangular facets (Figure 4.5), which can be rendered into a two-dimensional view by a number of standard computer graphics techniques. The two key advantages of this method are that it, (1) does not require photographic images of

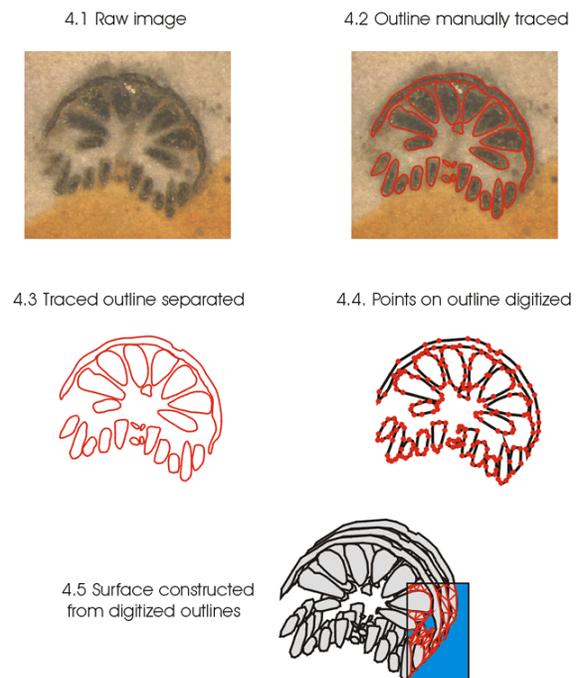


Figure 4. Vector-based reconstruction process of Herbert (1999).

the slices and, (2) is capable of working with low-quality, sparsely-spaced data. Neither of these is relevant to the present study. This method also has several disadvantages, especially in the context of the Herefordshire fauna. The initial interpretation stage is extremely time-consuming when a large number of slice images are involved, and also threatens the objectivity of the reconstruction as structures must be delineated before the three-dimensional form becomes apparent. When the margins of the fossil structures are unclear (as is often the case with the Herefordshire fossils), there is a danger that subjective interpretations will influence the reconstructed morphology. In addition, the algorithmic reconstruction of stacked two-dimensional loops into three-dimensional surfaces is subject to the ‘correspondence problem’—that is, the difficulty of determining the manner in which loops in subsequent slices connect to each other. Herbert (1999) approached this problem with algorithms that made *a priori* assumptions about the morphology of the object to be reconstructed, an approach that is clearly not viable for the reconstruction of poorly understood morphologies such as those of many Herefordshire fossils. Other available approaches require the user to determine correspondence, a procedure that is both time consuming and represents another potential source of subjectivity.

Volume-based methods. The second reconstruction methodology, that of volume-based reconstruction, is preferred in the present study. Volume representation is a three-dimensional extension of ‘raster’ or ‘bitmap’ representations of two-dimensional images, in which an image is modelled as a rectangular array of regularly spaced pixels. A volume dataset thus consists of a three-dimensional array of regularly spaced volume elements or ‘voxels’, each stored as a number representing the

value of some property of the object at that point. The sequential slice images generated by the grinding technique (after minor post-processing — see Appendix 1, section 3), represent a volume of this sort in which the values of the voxels represent the reflectivity of the surface as captured by the digital camera. Volume-based three-dimensional reconstructions, largely utilizing datasets from MR or CT scanners, are routinely used in medical applications and have been used to produce volume-based reconstructions of fossils (e.g., Hamada et al. 1991; Torres 1999). These methods have also been applied to optically captured volumes. Brown and Herbert (1996) attempted to produce volume-based models from serial section data, although the low resolution of the graptolite sections on which that work was based precluded a successful reconstruction. More successfully, Hammer (1999) reconstructed a halysitid coral colony from volume data obtained by serial grinding and captured with a flatbed scanner.

Volume rendering. There are various techniques, collectively known as volume rendering, for the direct visualization of volume data. Although they vary in detail, all involve forms of ‘ray casting’, where the computer calculates an array of virtual light rays ‘shone’ into the volume from a specified angle, determines their fate according to mathematical rules based on the values of voxels in the volume, and builds a two-dimensional image from the results of these calculations. We have used mean-intensity volume rendering, where the final intensity of the virtual ray is determined by the mean values of the voxels it encounters. This algorithm produces a virtual ‘X-ray’ image of the volume from any angle the user chooses (Figure 5). Like true X-radiographs, the images are a relatively poor aid to visualizing the outer surface of objects, but are effective for imaging of large or strongly absorbent

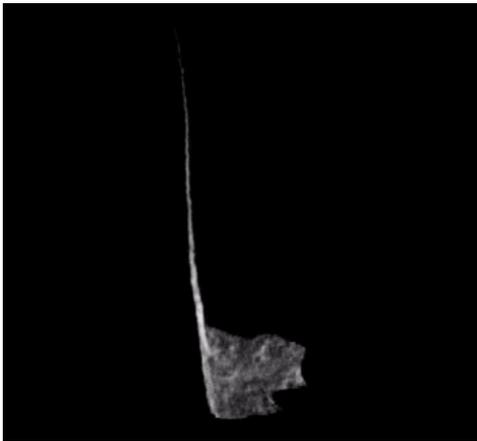


Figure 5. Volume-rendered 'X-ray' visualization of distal portion of monograptid graptolite; OUM C.29526a. 165 slices at 30 μm interval. Specimen is approximately 5 mm long.

(=dark) structures that otherwise may be obscured by less substantial features.

Isosurfaces. While volume data can thus be rendered directly, the preferred approach for medical visualization from CT or MR data is usually to generate polygon mesh surfaces known as isosurfaces. These are generated by algorithms that connect points of a constant (user-determined) intensity within the volume. If the threshold level is chosen correctly, an isosurface generated from a dataset such as shown in Figure 3 should correspond to the original external surface of the organism. Isosurfaces can be rendered with any of several computer graphics techniques available for the visualization of geometrically defined surfaces. Ray-tracing, though the most computationally expensive of these techniques, provides the clearest and most natural visualizations (Figure 6). Although volume-rendered 'X-ray' reconstructions have some application to the Herefordshire fossils, ray-traced isosurfaces are our preferred means of three-dimensional visualization. Reconstructions produced in this way retain the objectivity of volume data without compromising the clarity and sharpness of the rendered images.



Figure 6. Ray-traced isosurface visualization of anterior portion of a cranium of the trilobite *Tapinocalymene vulpecula* Siveter 1980; OUM C.29527a. 172 slices at 50 μm interval. Specimen is approximately 16 mm wide. Top is anterior.

Animation. Isosurfaces have been used previously to produce static images of fossils (e.g., Torres 1999; Hammer 1999; Brown and Herbert 1996). While such images have some illustrative value, a true appreciation of three-dimensional morphology is best achieved with an interactive model. We produce these (for both isosurface and volume rendering techniques) from a batch of images rendered from sequential angles and assembled into a computer video file that can be presented as a reconstruction of the specimen (Figures 7-11). As with video files assembled directly from slice images, these files can be played backwards or forwards and provide a simple but effective means of viewing the reconstruction from a variety of angles. They also enable the reconstruction to be distributed easily for viewing on any modern computer without the need for dedicated software.

Pitfalls for three-dimensional reconstructions

Transparency of calcite. The nature of the Herefordshire material necessitates guarding against two potential pitfalls. The first concerns the assumption that each image represents the intersection of the fossil with a single plane. The calcite that comprises the fossils is normally dark and

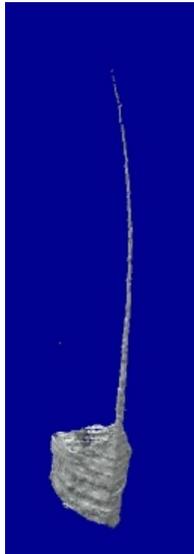


Figure 7. AVI format video file of ray-traced isosurface reconstruction of distal portion of monograptid graptolite; OUM C.29526a. 165 slices at 30 μm interval. Specimen is approximately 5 mm long.

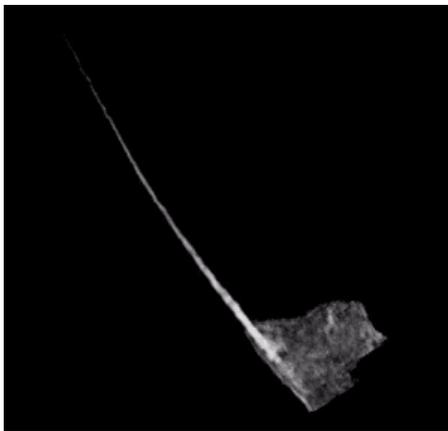


Figure 8. AVI format video file of Volume-rendered 'X-ray' reconstruction of distal portion of monograptid graptolite; OUM C.29526a. 165 slices at 30 μm interval. Specimen is approximately 5 mm long.

opaque, but translucent regions can reveal features beneath the surface plane or be relatively light in shade when matrix lies only a short distance below the plane. Such occurrences, which have the potential to invalidate reconstructions partially, are detected by inspection of the slice images and corrected by manual retouching (see Appendix 1, Section 3).

'Halo' effects. Light-coloured regions, presumed to be reduction haloes, often

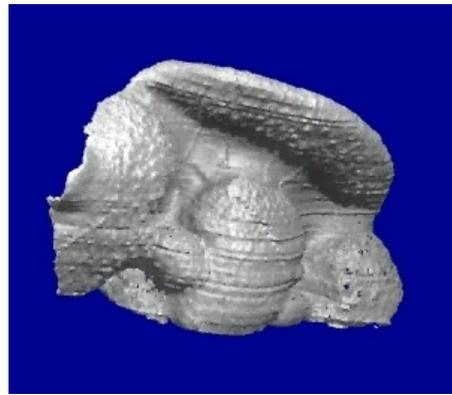


Figure 9. AVI format video file of ray-traced isosurface reconstruction of anterior portion of a cranium of the trilobite *Tapinocalymene vulpecula* Siveter 1980; OUM C.29527a. 172 slices at 50 μm interval. Specimen is approximately 16 mm wide.



Figure 10. AVI format video file of ray-traced isosurface reconstruction of anterior fragment of the mollusc *Acaenoplax hayae* Sutton et al. 2001; OUM C.29532. 190 slices at 30 μm interval. Specimen is approximately 5 mm long.

occur around specimens of certain taxa (Figure 12). Fossil material within these haloes is often no darker than the matrix outside them, making the selection of a single isosurface threshold level for the whole fossil impossible. Reconstructions may therefore fail to include extremities within these regions that are nonetheless clearly visible on the slice images. While this problem can be overcome by careful manual darkening of these regions, a simpler alternative is often to prepare a dissection (see below) of the inside of a light

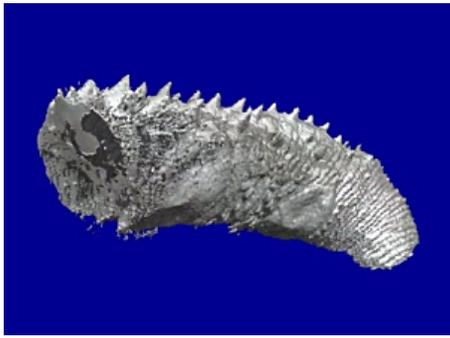


Figure 11. AVI format video file of ray-traced isosurface reconstruction of antero-median fragment of the mollusc *Acaenoplax hayae* Sutton et al. 2001; OUM C.29529. 243 slices at 30 μ m interval. Specimen is approximately 7 mm long.

region, and to reconstruct this section separately.

EXTENSIONS OF RECONSTRUCTION TECHNIQUES

Whole fossil reconstructions

Reconstructions from a single grinding run typically comprise just part of a fossil, as grinding normally commences from the plane along which the nodule was originally fractured. Reconstructions of entire

fossils may be obtained by gluing part and counterpart together before sawing to size so that the entire fossil can then be ground through as one unit. However, this approach can produce artifacts from imperfect alignment of the glued surfaces or from the glue itself. Alternatively and more satisfactorily, part and counterpart can be reconstructed separately and reassembled digitally before rendering. Figure 13 is an example of a whole fossil reconstructed in this way.

Dissection of models

Reconstructions of portions of a fossil, or of a subset of the structures present, are often of great utility. A strength of the volume-based approach described here is the ease with which such 'dissected' reconstructions can be prepared. Copies of the appropriate slice images are simply manipulated using image editing software to remove structures or portions of the fossil that are not required (see Appendix 1, section 3), and are then rendered in the normal way. Slice videos and pre-dissection three-dimensional reconstructions



Figure 12. Reduction haloes surrounding an unnamed polychaete annelid; OUM C.29525a.

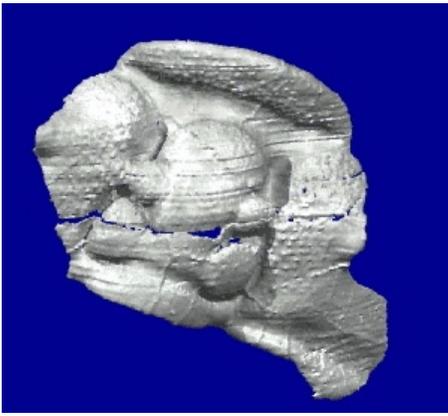


Figure 13. AVI format video file of ray-traced isosurface reconstruction integrating part and counterpart of a cranidium of the trilobite *Tapinocalymene vulpecula* Siveter 1980; OUM C.29527a-b. 29527a (anterior): 172 slices at 50 μm interval. 29527b (posterior): 197 slices at 50 μm interval. Specimen is approximately 13.5 mm long medially.

provide the ideal tools to guide such dissection work, and ensure that unwanted structures are removed accurately.

APPLICATIONS OF RECONSTRUCTION TECHNIQUES

The techniques described herein, although largely illustrated with well-understood biomineralized taxa, have been developed to elucidate the morphology of the essentially soft-bodied organisms that dominate the Herefordshire fauna (such as *Acaenoplax hayae*, Figures 10-11, see also Sutton et al. 2001), and provide the primary means for palaeontological studies. However, they are easily adapted to other situations and essentially require only that the fossil preserves three-dimensional form and is visually distinguishable from matrix. Despite the destructive nature of grinding we thus expect that similar applications, whose main goal is simply to determine morphology, will arise in other situations where isolation of the fossils in question is impractical.

These techniques also have broader potential applications within palaeontol-

ogy. Virtual models of fossils are useful tools for the dissemination of morphological information, as they contain more information than static images, yet can be copied, distributed, and viewed quickly and at minimal cost. Applications in education can be envisaged as a means of familiarizing students with morphology of fossils, particularly where material is too large, small, or rare to be physically manipulated. Computer-based models may also prove to be an efficient way of conveying morphology to professional palaeontologists, particularly those less familiar with the group in question. These techniques might also provide a methodology for capturing morphological information as a prelude to further computer-based modelling work. Such studies might include the reconstruction of skeletal structures (e.g., conodont feeding apparatus) from isolated material, the illustration of theoretical models of unpreserved soft parts (e.g., brachiopod musculature), and studies of functional morphology that require digital models (e.g., finite element stress analysis).

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APPENDIX 1: DETAILS OF COMPUTER-BASED PROCESSING

This represents a step-by-step documentation of the computer-based elements of the reconstruction process used to produce slice videos and isosurface models. For each step we provide both a ‘package-independent’ overview (in bold), followed by ‘package-specific’ details of our method. The latter are intended only as a description of our procedures, not as a set of instructions for those unfamiliar with the software involved, nor indeed as an exclusive recommendation of any specific software package—equivalents of all major packages used are available for most platforms. Details of software packages used in the study are given in Appendix 2, and terminology specific to these packages is placed within inverted commas on first usage.

1) Registration and cropping

Images are first registered (aligned), using the alignment edges in the images as a guide. An image-editing software package is used to shift and rotate the images manually, so that the edges are in exactly the same position. Once registered, the images are cropped to remove extraneous background, including registration edges. All images must be cropped using exactly the same parameters so that alignment is preserved.

One image, normally the first, is chosen as the datum for alignment. This image is loaded into *PhotoPaint*, and a new ‘object’ is created to lie above the background. Straight lines of a single pixel in width are drawn on this object to correspond to the alignment edges of the image beneath. The ‘Eraser’ tool is then used to remove portions of these lines, so they appear as coarse dashes. Oblique lines are also drawn on the object from edge to edge near each of the four corners of the

image; these ensure that the object is exactly the same size as the image, which assists with placement in the next step. Once the alignment object is complete it is placed into the clipboard with a copy command and the file closed.

The registration process now commences, and the following procedure is performed for each image. The image to be aligned is loaded, and the alignment object is inserted with the ‘Paste As New Object’ command—this alignment object is not moved subsequently, but used as a guide for aligning the image. The image itself is converted to an object with the ‘Create From Background’ command. This image object is then shifted manually with the ‘Object Picker’ tool selected, by use of the cursor keys, so that the point where the two alignment edges meet is correctly positioned. The rotational centre of the object is then dragged to that point, and the correct rotation of the image applied by means of trial and error entries of angles in the ‘Tool Settings Roll-up’. When and only when the correct rotation is determined, it is applied with the ‘Transform’ button on the roll-up, and any final single-pixel shifts required for perfect alignment are performed with the cursor keys. Finally, the alignment object is removed from the image which is saved in its original format. This process must be carried out for each image in the dataset and is thus fairly time-consuming. Much of the work can be automated, however, by recording actions as ‘scripts’. We normally make use of one script to create the necessary objects, and another to remove the alignment object after alignment. In practice, we find that it is possible to register up to 60 images per hour.

After registration, the images are cropped to size with the ‘Deskew Crop’ tool. Care must be taken that the crop

area set includes all of the fossil on every image. Once the correct crop size is determined, the action is recorded as a script, and then applied to all images with the 'Batch Playback' command.

2) Post-processing for preparation of slice videos

Slice videos, if required, can be prepared directly from the registered and cropped image set using a suitable software package. Typically such videos are prepared from a second copy of this dataset, the images of which are colour balanced and/or reduced in size prior to video production. This processing is carried out with image-editing software. Size reduction is necessary only if the resulting video would otherwise be too large to store or play on any given computer.

To prepare for video production, a copy of the registered and cropped image set is made, and the following *PhotoPaint* operations are applied first to one image, recorded as a script, and then applied to each image by means of the Batch Playback command.

- a. Colour is balanced to taste with the 'Level Equalization' command.
- b. Image size is reduced, if necessary, with the 'Resample' command.

Processed images are opened as a file sequence video in *Ray Dream*, and then saved as an AVI video file in Cinepak format.

3) Post-processing for isosurface reconstruction

To produce a dataset suitable for the isosurface algorithm, the registered and cropped dataset must be converted from colour to monochrome, inverted (so that fossil is light and matrix dark), and reduced in size if the restrictions of the isosurface package so demand. We find that the Hereford-

shire fossils are usually more clearly distinguished from the matrix if the conversion to monochrome is performed using the red channel only. These operations, which must be identically performed on all images in the dataset, are carried out with an image editing software package.

Any required retouching of images should be carried out at this step, although in most cases we prefer to reconstruct an un-retouched or minimally retouched set of images first, then return to this point in the process to perform any required 'cleaning' on a second copy of the dataset. Retouching is carried out manually using an image-editing software package. In most cases it comprises the deletion of objects in the matrix that appear in the reconstruction but are clearly not part of the main fossil, or of structures visible through translucent regions of calcite. Retouching can also include the brightening of structures that are clearly part of the main fossil, but are too dark to appear in the reconstruction (i.e., are falling below the chosen isosurface threshold, usually because of transparency problems). We find it helpful to choose an isosurface threshold during the retouching step, so that these problems can be assessed within the image editing software package.

Post-processing operations are performed on each image using *PhotoPaint*. As in Step 2 above, they are first performed on one image, the actions recorded as a script, and then applied to each image by means of the Batch Playback command.

- a. The image is converted to monochrome. This can be accomplished with the 'Convert to Greyscale (8-bit)' command. If only the red channel is required (see above), the Level Equalization command is used to remove the green and

blue signals, the Convert to Greyscale (8-bit) command is then applied, and finally the 'Brightness-Contrast-Intensity' command is used to enhance the visual clarity of the image (which will be too dark).

b. The image is inverted using the 'Invert' command.

c. If necessary, the image is resampled to a smaller size. AVS (see below) can only accept volumes up to 255 x 255 x 255 voxels in size, so the image size must be reduced to 255 x 255 pixels or smaller before a reconstruction can be attempted. Resampling ideally is carried out with an integral divisor (e.g., at 50% or 33.33%); arbitrary values can introduce an element of blurring, although this is rarely of critical importance. The final image width must also be a multiple of 4 to satisfy AVSCONV. This can be accomplished either by padding with black space using the 'Paper Size' command, or by further careful cropping.

Retouching, if required, is also carried out in *PhotoPaint*, mainly by use of the 'Eraser' tool to delete objects, and the 'Effect' tool to brighten others. The 'Threshold' command can be used to apply the chosen isosurface threshold level and determine what will and what will not appear in the reconstruction. The threshold effect can then simply be removed with the 'Undo' command.

4) Isosurface production

The post-processed image set must first be converted into whatever volume format the isosurface software requires, using appropriate software. Once informed of the appropriate threshold level the isosurface software can then generate the surface. The surface may then need to be converted to a format that can be read by the ray-tracing software employed.

We convert the dataset to AVS volume format using the custom AVSCONV pro-

gram. This requires the images be in Windows BMP format (uncompressed), be consecutively numbered with three digits (i.e., file names of the form Name001.bmp, Name002.bmp), and placed in a single directory. The user selects the first file, clicks the process button, and when requested enters the number of files in the dataset. On completion, the program places the AVS format data file in the same directory, with the name 'output.dat'.

An AVS 'network' is created, as follows. The data file produced in Step 3 is read with the 'read volume' module, which feeds the 'average down' module. This should be set with relatively high x, y, and z coefficients (2 or more) at this step. The output from the 'average down' module is fed to the right hand input port of the 'isosurface' module, the output of which is sent to the right hand port of the 'geometry viewer' module. The isosurface level should now be set to produce the best possible reconstruction; as noted above we normally find it helpful to choose this level during retouching. When the isosurface appears correct, the coefficients of the 'average down' module should be reduced to 1, and the output of the 'isosurface' module connected to the 'write geom' module, which should be set to produce a 'DXF' file. As the processing involved in the network can be time consuming, we find it helpful to disable the flow executive whilst making changes to parameters of these modules.

DXF files produced by the 'write geom' module can be very large. We find that files much over 100MB in size are impractical to render in Step 5 below. In some cases therefore, particularly with datasets containing a large number of images, the final isosurface is produced with x and y settings of 2 rather than 1 in the 'average down' module. Although we normally prefer not to average down in the z axis, if

alignment errors are producing strong artifacts (lineation parallel with the plane of grinding), a z setting of 2 can help to smooth these out, as well as to reduce output file size.

5) Rendering

Geometry files describing isosurfaces are read into a ray-tracing software package, or any other three-dimensional visualization package. Isosurfaces are stretched to correct for differences in voxel spacing within slices and between slices (this may be done during Step 4 instead, if the software allows). If multiple isosurfaces are to be joined (e.g., Figure 11), they are both imported into one scene, and then manually moved into a contiguous position. The visualization package is then used to generate a video file of the specimen rotating in the desired fashion.

DXF files produced by AVS are read into *Ray Dream* with the 'Import' command. All the default import options are left unaltered, except 'Scaling Conversion Factor' which is set to '0.5 cm' rather than

'1 in.'. Once imported, the isosurface is stretched in the z axis to correct for slice spacing—the z scale property of the isosurface object is set to $100\% \times (\text{Pixels/mm in resampled images}) / (\text{slices/mm})$. Any other objects are imported and treated in the same way, and carefully moved into position relative to each other. When bringing two objects together, we prefer first to bring a single corresponding point into contact, move the 'hotpoint of rotation' to that point, and then rotate the object into position using the 'virtual trackball'. A 'shader' is then applied to the object(s), lights placed in the scene, and an animation set up to rotate the object in the desired fashion over a time period of several seconds. The 'production frame' is carefully positioned and sized to the minimum size necessary to contain the object at all steps of the animation. The animation is then rendered within *Ray Dream*, normally with an image width of 300 to 400 pixels and a frame rate of 15 or 18 frames per second. It is saved as an AVI video file in Cinepak format, which forms the final reconstruction.

**APPENDIX 2:
SOFTWARE AND EQUIPMENT**

1) Commercial Software

Image editing:

PhotoPaint version 8 (for Microsoft Windows)

Corel Corporation
Corporate Headquarters
1600 Carling Avenue
Ottawa, Ontario
K1Z 8R7, Canada
www.corel.com

Rendering and production of slice videos:

Ray Dream 3D version 1.02 (for Microsoft Windows)

MetaCreations Corporation
6303 Carpinteria Ave.
Carpinteria, CA 93013, USA
www.metacreations.com

Isosurface production:

AVS version 5 (for Solaris)
Advanced Visual Systems Inc.
300 Fifth Avenue Waltham,
MA 0245, USA
www.avs.com

2) Free Software

Conversion of image files to AVS volume format:

AVSCONV version 1.1 (for

Microsoft Windows)

Author: Mark Sutton

URL for download:

palaeo-eletronica/2001_1/s2/program
Export from AVS geometry format to DXF for import into RayDream:

'write geom' AVS module [Must be installed into AVS system]

Author: Georg Thallinger

URL for download:

[http://www.iavsc.org/repository/avs5/
source/data_output/write_geom/](http://www.iavsc.org/repository/avs5/source/data_output/write_geom/)

3) Equipment used

SV Micro Digital Microscope Camera
Sound Vision, Inc.

492 Old Connecticut Path
Framingham, MA 01701

<http://www.soundvisioninc.com/>

Wirtz-Buehler 'Slide Holder' (hand-held grinder)

Buehler Krautkramer UK
Milburn Hill Road

University of Warwick Science Park
Coventry CV4 7HS United Kingdom

<http://www.buehlerltd.com/>