Journal of Microscopy, Vol. 233, Pt 1 2009, pp. 93–101
Received 7 May 2008; accepted 3 July 2008

Simple 3D images from fossil and Recent micromaterial using light microscopy

J.T. HAUG*, C. HAUG*, A. MAAS*, S.R. FAYERS†,
N.H. TREWIN† & D. WALOSZEK*
**Biosystematic Documentation, University of Ulm, Helmholtzstrasse 20, D-89081 Ulm, Germany*
†Department of Geology and Petroleum Geology, University of Aberdeen, Meston Building, Kings College, Aberdeen, AB24 3UE, U.K.

Key words. Crustaceans, microfossils, 3D reconstruction.

Abstract

We present a technique for extracting 3D information from small-scale fossil and Recent material and give a summary of other contemporary techniques for 3D methods of investigation. The only hardware needed for the here-presented technique is a microscope that can perform dark field and/or differential interference contrast with a mounted digital camera and a computer. Serial images are taken while the focus is successively shifted from the uppermost end of the specimen to the lowermost end, resulting in about 200 photographs. The data are then processed almost completely automatically by successive use of three freely available programs. Firstly, the stack of images is aligned by the use of COMBINEZM, which is used to produce a combined image with a high depth of field. Secondly, the aligned images are cropped and sharp edges extracted with the aid of IMAGEJ. Thirdly, although IMAGEJ is also capable of producing 3D representations, we preferred to process the image stack further using OSIRIX as it has the facility to export various formats. One of the interesting export formats is a virtual Quicktime movie file (QTVR), which can be used for documentation, and stereo images can also be produced from this Quicktime VR. This method is easy to apply and can be used for documenting specimens in 3D (at least some aspects) without having to prepare them. Therefore, it is particularly useful as a safe method for documenting limited material, before using methods that may destroy the specimen of interest, or to investigate type material that cannot be treated with any preparatory technique. As light microscopes are available in most labs and free computer programs are easily accessible, this method can be readily applied.

Correspondence to: Joachim T. Haug. Tel: +49-731-5031008; fax: +49-731-5031009; e-mail: joachim.haug@uni-ulm.de

Introduction

General techniques for acquiring and documenting 3D information

Modern science provides a number of techniques for acquiring 3D information. Classical (medical) computed tomography (CT) or magnetic resonance tomography (MRT) yields a resolution down to 500 μm (Oppitz *et al.*, 2007; Yoon *et al.*, 2008) and is, therefore, not precise enough for most specimens in biology and palaeontology. The same holds true for surface laser scanners or mechanical surface digitizers that can usually likewise be used only for large specimens (Lyons *et al.*, 2000; Wilhite, 2003; Tse *et al.*, 2006). Better resolution is provided by μ-CT (e.g. Polcyn, 2002; Dierick *et al.*, 2007; Penney *et al.*, 2007; Wirkner & Prendini, 2007, and references therein), nano CT or synchrotron CT (Donoghue *et al.*, 2006; Lin *et al.*, 2006; Tafforeau *et al.*, 2006). These techniques may allow a resolution of less than 1 μm. Radiation types other than X-rays can also be applied, for example neutron-tomography (Schwarz *et al.*, 2005) or high-energy magnetic-resonance tomography (Roebroeck *et al.*, 2008). Both methods give a resolution slightly better than medical CTs, but show a different contrast, thus allowing the visualization of structures that are invisible using X-ray.

Confocal laser scanning microscopy (CLSM) also makes it possible to gather information in three dimensions, and as a light microscopic technique it allows resolutions down to less than 1 μm. In a growing number of studies this technique has been mainly used to reconstruct neuro-anatomy or muscle arrangements (e.g. Wanninger & Haszprunar, 2002; Wollesen *et al.*, 2007). It can also be used for other morphological structures (Carotenuto, 1999; Zupo & Buttino, 2001; Buttino *et al.*, 2003; Michels, 2007), and can even be applied to special fluorescent fossil material (Chi *et al.*, 2006; Chen *et al.*, 2007). Apart from tomographic and laser-based methods there are other, more classical 'low-tech' possibilities such as serial sectioning (e.g. Wirkner

& Richter, 2003; Fanenbruck *et al.*, 2004; Fanenbruck & Harzsch, 2005) or serial grinding. The resolutions of these techniques are coupled to the method that is used to inspect the sections/grinds, and are usually the same as for light microscopy (see above). These methods are already quite well known. Størmer (1939), for example used the technique of serial grinding for his reconstructions of the limb morphology of †*Ceraurus pleurexantemus* Green, 1832. Størmer simply copied the outlines of a polished specimen onto a glass plate, ground a certain amount down, copied the new outlines onto glass again and continued to repeat the process. Finally he assembled all the glass plates in a large rack and obtained 3D information on the morphology of the limb of †*C. pleurexantemus*.

Nowadays, as for data inferred from CTs, computer programs are used for the reconstruction of 3D information from serial sections or grindings. The well-known Silurian Herefordshire Lagerstätte, England, provides recent examples of 3D reconstructions based on serial grinding. There the application of serial grinding combined with modern computer-based 3D-reconstruction (Sutton *et al.*, 2001) improved access to the fossils enormously compared with earlier methods (Orr *et al.*, 2000), giving resolutions of 20 μm in the *z*-axis (slightly higher in the *xy*-plane), and in a specimen size range is from 3.5 to about 30 mm. Since the method has been established, an impressive range of taxa has been described from the Herefordshire Lagerstätte using this particular reconstructive method. The taxonomic range spans from different arthropods over trochozoans and tentaculates to echinoderms (Siveter *et al.*, 2007a, b, and references therein). The common principle of most of these methods for reconstructing 3D information from an image stack is usually referred to as a multi-planar reconstruction.

There are many different modern methods and all have certain advantages and disadvantages. The main disadvantage of serial grinding or serial sectioning is that both methods are destructive: at the end the specimen is destroyed and no longer available, an impossible method for type or rare material. In the case of serial sectioning there are at least the sections still present, but in the case of serial grinding nothing is left but the reconstructed virtual specimen. The destruction of specimens is only unproblematic if a large number of specimens of the species of interest is available. For species that are known only from a handful of specimens, or even just the type material, these methods cannot be applied. Also the so-called non-destructive methods have disadvantages. CT-scans, for example, produce images with grey-scaled information, and in many cases the raw data are rather low in contrast. Compared to stained sections, these methods are, therefore, more time consuming in the reconstruction of a virtual specimen. Furthermore, size and method are related in a way that can make it difficult to obtain the information from a specimen; for example it may be too big or too small for the machine used.

One of the biggest disadvantages of most of the modern techniques is their availability. The machines, especially the newer ones, are very expensive and, hence, still not widespread, and the user schedules are often tightly filled. For people at institutions with low research budgets this is a significant disadvantage. Although a method has been published recently to transform a scanning electron microscope (SEM) into a μCT (Tanisako *et al.*, 2005, 2006), it will take some time until CT will be an easily accessible method. The same holds true for most software packages that are needed to reconstruct the 3D information from the data stack.

One of the simpler, but also powerful methods of documenting 3D information is the use of stereo images, no matter whether they are given as anaglyphs, KMQ (method named after the inventors Koschnitzke, Mehnert and Quick) (Kallenborn *et al.*, 1990) or other methods (Purnell, 2003; Gatesy *et al.*, 2005). Stereo images can easily be applied to SEM material or reflective microscopy. Although this method does not give the possibility of viewing the specimen of interest from several angles, it yields additional information compared to usual 2D images (Gatesy *et al.*, 2005) and can be combined with other 3D documenting methods (e.g. Müller, 1983; Walossek, 1993; Knappertsbusch *et al.*, 2006; Siveter *et al.*, 2007a, b, and references therein).

Another possibility for displaying 3D information is modelling. This can, for example, be based on SEM images from several angles (Müller & Walossek, 1988, reconstructing the Cambrian crustacean †*Bredocaris admirabilis* using modelling clay), or in the case of flattened fossils on specimens with several embedding planes (Bruton, 1981). More recent works have used virtual computer-based 3D models based on SEM images for presenting reconstructed morphologies (Haug *et al.*, 2007; Maas *et al.*, 2007). These modelling methods differ from the earlier described methods in not reconstructing a single specimen, but merging the information of several specimens into one 3D representative image.

A frequently underestimated problem of the modern 3D-methods as well as other well-established documenting methods, such as SEM techniques, is the mounting or other processing of the sample, such as coating or embedding (Scott *et al.*, 2000). The mounting that is necessary for some of the methods is not always reversible when it is applied to fragile material. This can be equally problematical as destructive methods for species known from only a few specimens.

Here we present an additional method of documenting 3D information on microfossils that may also be applied to Recent material. This method simply relies on a transmitted light microscope with a mounted camera, and further processing with three programs, which are freely available on the worldwide web. Specimens do not have to be mounted but can simply be put onto a microslide. This method can, therefore, be used to pre-document specimens before applying other (destructive) methods or before irreversible mounting. In both cases this is especially useful when documenting type material.

Material of interest

As our research concentrates mainly on the early development of arthropods, there are two fossil preservation types that are of particular interest. The first one is the 'Orsten' type of preservation. In 'Orsten' fossils, mainly arthropods, the cuticle has been impregnated by calcium phosphate, and the fossil is preserved in a completely uncompressed state in three dimensions, with very fragile structures such as setules down to 0.2 μm diameter preserved. Furthermore, a number of species are preserved with their early developmental stages (e.g. Müller & Walossek, 1986) and up to now no less than five species are also known from at least several, and in some cases even successive instars (Walossek, 1993; Maas *et al.*, 2003; Zhang *et al.*, 2007, and references therein). Because of the small size of the specimens, especially of the early larval stages, the standard method for documenting these is by SEM. As already mentioned, SEM is a semi-destructive method, as the specimens can usually only be re-mounted with difficulties; this is not only true for the highly fragile Swedish 'Orsten' fossil material, but also for critical-point dried extant specimens. SEM itself may also be destructive due to the high energy of the electron beam. A number of specimens of our 'Orsten' collection have already been damaged or even destroyed.

Therefore, an easy to apply non-destructive pre-documenting method such as light microscopy for this type of material will be valuable to a wide range of users. Reflective light microscopy, however, does not yield a magnification high enough to resolve the details and CLSM proved to be unsuccessful, and bright-field microscopy also cannot be applied, because 'Orsten' fossils are rather dark, being brown to black in colour. Therefore, this material has been studied using dark-field microscopy.

As the method, introduced here, is solely based on light microscopy, it can also be applied to fossils that are usually limited to thin sections for bright-field microscopy, for example fossils from the Early Devonian (Pragian) Rhynie and Windyfield cherts of Aberdeenshire, Scotland. Rhynie chert fossils have a long research history (review in Trewin, 2004), and together with new finds from the more recently discovered and closely associated Windyfield chert (Fayers & Trewin, 2004, and references therein), have become well-known for plant fossils (reviews in Cleal & Thomas, 1995; Edwards, 2004), and also for branchiopods (Scourfield, 1940a; Anderson *et al.*, 2004, and references therein), hexapods (Scourfield, 1940b; Fayers & Trewin, 2005, and references therein), chelicerates (Dubinin, 1962; Dunlop *et al.*, 2004; Fayers *et al.*, 2005, and references therein), myriapods (Fayers & Trewin, 2004, and references therein), and the enigmatic euthycarcinoids (Anderson & Trewin, 2003).

All these fossils provide 3D information, and the usual method for documenting these types of fossils is transmitted light microscopy. Therefore, this kind of fossil preservation seemed to be a very good candidate for this new method, applied with bright-field microscopy. A first successful attempt to extract 3D information from Rhynie chert fossils with light microscopy and modern computer software was presented by Kamenz *et al.* (2008). For this study we have used larval branchiopod fossils from the Windyfield Chert (Fayers & Trewin, 2004).

As a third example the method has also been tested on extant crustacean larvae, namely of cirripedes. Recent material was the only material that could be used with differential interference contrast (DIC) methods, as this method of light microscopy should also be tested.

Although the method is applied here solely on the three types of material described above, these are simply tests of three different kinds of transmitted light microscopy. The aim is to develop a fast, easy to apply and cheap method for documenting, visualizing and gaining better understanding of the spatial arrangements of structures in fossil and Recent micromaterial.

Material and methods

General settings

All specimens were investigated at the Biosystematic Documentation, University of Ulm, Germany. They were viewed using a Zeiss Axio Scope (Zeiss, Jena, Germany) and photographed with an Olympus E-20p camera (Olympus, Tokyo, Japan). Further processing was done using a Windows PC (Microsoft, Redmond, WA, U.S.A.) with the freely available software COMBINEZM (http://www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm) and on a MacPro Quad-Core Intel Xeon (Apple, Cupertino, CA, U.S.A.) with the freely available software programs IMAGEJ (NIH, Bethesda, MD, U.S.A.), OSIRIX (http://www.osirix-viewer.com) and GIMP (http://www.gimp.org).

Light microscopy settings in general and procedure

For all three types of material, we used a 20× objective. The Windyfield chert thin sections were already mounted as polished thin sections fixed onto slides. The 'Orsten' specimens from Swedish material and living cirripedes collected in Helsingør, Denmark, in 2006, were placed on a glass slide with a central dip, the 'Orsten' specimens in a dry state, extant specimens in 96% alcohol and covered with a glass cover slip. The specimens were then photographed, and the focus was altered manually using the scale on the fine adjustment knob, resulting in steps of approximately 0.9 μm. For each specimen the procedure was started at a level above the top of the specimen, where all parts of the specimen were slightly out of focus and continued downwards until all

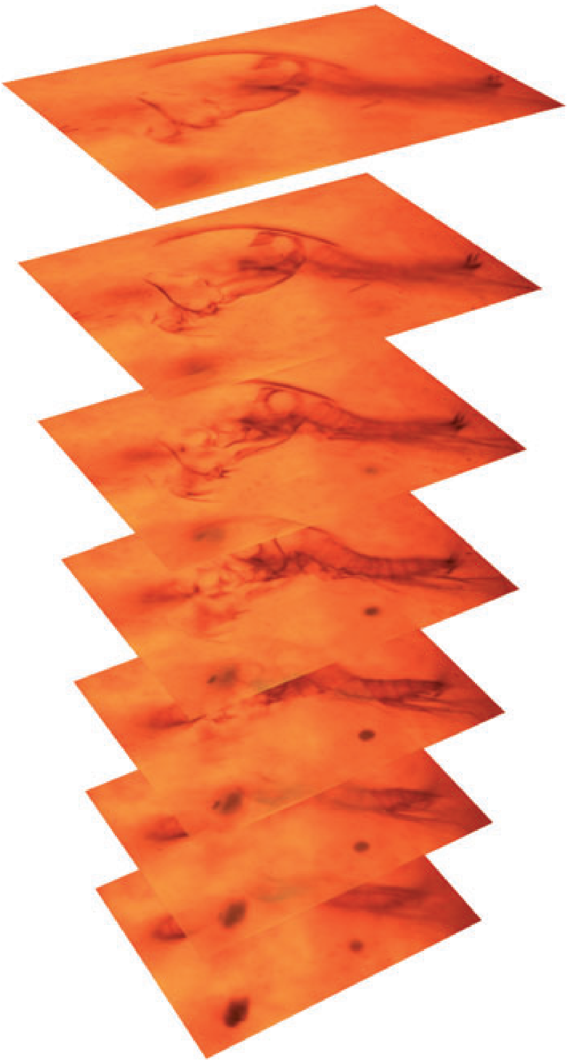

Fig. 1. Seven images of a stack of more than 200 images from a Windyfield chert arthropod specimen (AUGD12449b). The images demonstrate an interesting character of the Windyfield and Rhynie chert material: details out of focus are disguised by the optically dense matrix. Only the sharp areas are later taken into account, the information is then distributed on the *z*-axis, finally resulting in a multi-planar reconstruction.

parts of the specimen became out of focus again (Fig. 1). This procedure usually resulted in about 200 images per specimen.

Dark-field microscopy exemplified on 'Orsten' fossils (Fig. 2)

As noted above the 'Orsten' fossils are very dark. They are brown to black and almost no details can be seen in normal bright-field microscopy. Dark-field microscopy allows highlighting of some details, as the material is very bright in dark field, but proves to have a low depth of field (Fig. 2C). This is in fact an advantage for the applied method, as one only gains information of a very well defined short range in the *z*-axis.

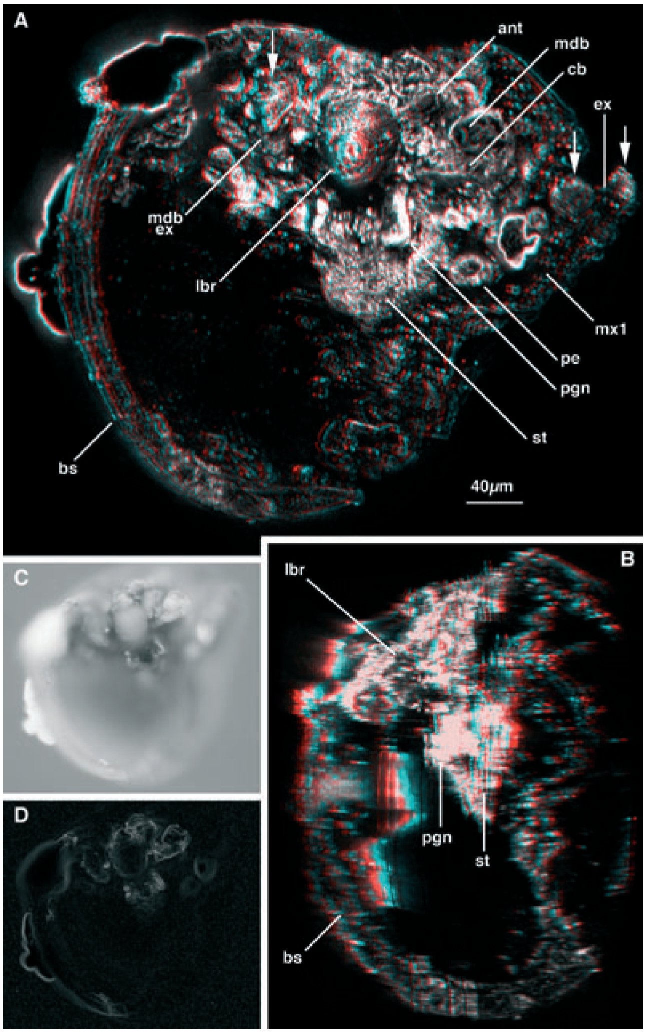

Fig. 2. Single specimen (UB W 358/SPEC 11286, sample 6414, collection of the Institut für Paläontologie in Bonn) of the phosphatocopine species †*Hesslandona unisulcata* Müller, 1982. (A)–(B). Red-cyan stereo images of UB W 358/SPEC 11286. (A) The multi-planar reconstruction of the 'Orsten' fossil is presented in ventral view, displaying a number of details. Arrows indicating the fine preservation of setae. Abbreviations: ant, antenna, second appendage; bs, bivalved shield; cb, coxal blade; ex, exopod, outer ramous; lbr, labrum; mdb, mandible, third appendage; mx1, maxillula, fourth appendage; pe, proximal endite; pgn, paragnath humps and st, sternum. (B) The multi-planar reconstruction of the 'Orsten' fossil is presented in lateral view, perpendicular to the virtual section planes, demonstrating the lower resolution in this direction. (C) Unprocessed image recorded in dark-field microscopy demonstrating the low depth of field. (D) The same image as C, processed with IMAGEJ 'find edges' function.

Usual bright-field microscopy exemplified on Windyfield chert fossils (Fig. 3)

On the Windyfield chert fossils only bright-field microscopy can be applied. Neither dark field, DIC nor CLSM could be applied successfully, or at least did not supply results as detailed as usual bright-field microscopy. Because of the dense chert

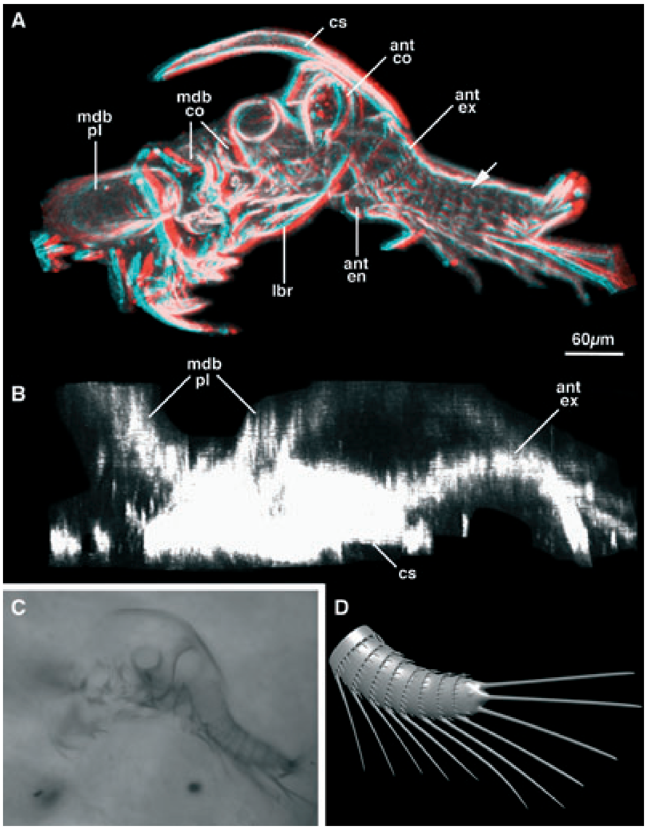

Fig. 3. Single specimen (AUGD12449b, collection of the University of Aberdeen, Department of Geology & Petroleum Geology) of a yet undescribed branchiopod species from the Devonian Windyfield chert. (A) Red-cyan stereo image of specimen AUGD12449b. The multi-planar reconstruction of the fossil is displayed in anterior view. Note that the anterior portion of the head is not present because of the cutting plane of the thin section, and the further posterior morphological structures are hidden because of the dimming effect of the matrix. Arrow indicates the finely preserved small hairs on the rim of the exopodal articles. Abbreviations: ant, antenna, second appendage; cs, cephalic shield; co, coxa; ex, exopod, outer ramous; lbr, labrum; mdb, mandible, third appendage; pl, palp. (B) Dorsal view onto the multi-planar reconstruction of the specimen. It demonstrates the low resolution when looking at the specimen perpendicularly to the virtual section plane. The main information is the position of the limbs, e.g. showing how strongly the exopod of the antenna is bent. The mandibular palps are more weakly contrasted, but recognisable. The cephalic shield hides more details. (C) One image from the stack of more than 200, demonstrating the low depth of field, but showing the fine details such as the fine setules on the exopod of the second antenna. (D) 3D model of the exopod of the antenna of the nauplius of the undescribed branchiopod from the Windyfield chert. The model was produced in the free available software BLENDER. Information obtainable from the single specimen has been taken into account. More details of the exopod and other structures will be added based on comparison with additional specimens.

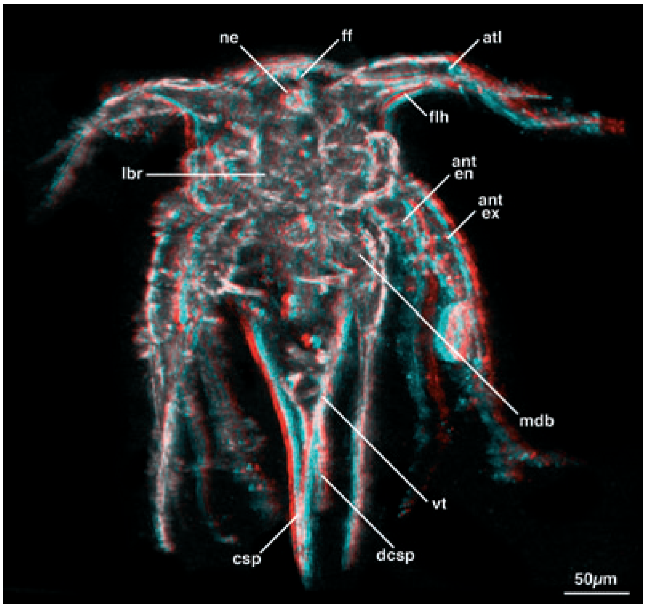

Fig. 4. Red-cyan image of a specimen of an undetermined extant cirripede species. The multi-planar reconstruction of the animal is displayed in ventral view. Abbreviations: ant, antenna, second appendage; atl, antennula, first, uniramous appendage; csp, spine of cephalic shield; dcsp, dorso-caudal spine; ex, exopod, outer ramous; ff, frontal filaments; flh, fronto-lateral horns; lbr, labrum; mdb, mandible, third appendage; ne, naupliar eye and vt, vestigial trunk.

matrix only a limited depth in the *z*-axis is visible at one time, namely the parts in focus (Fig. 3C). Therefore, one image equals a virtual section through the specimen and allows a later multi-planar reconstruction.

DIC exemplified on living crustacean larva (Fig. 4)

DIC also gives a very sharp image that is limited in depth of field. As no fossil material available proved to give useful data with this method, we tested it with Recent material, namely cirripede larvae.

Computer processing

An alignment of images may be necessary, because most microscopes have a slight *x*-axis and/or *y*-axis displacement when focusing through. The easiest way to get rid of this is using the program CombineZM. This software was originally designed as an image fusion program, i.e. to produce images with a high depth of field from a stack of images differing in the focal plane. This method leads to very sharp images, but, of course, 'flattens' the 3D information. The program is also capable of automatic rescaling, displacing or rotating images with different automatic algorithms, in order to place the outlines of objects in a stack correctly above each other, when the difference of two successive images is not too large. The stack of images obtained with the light microscope has simply to be loaded in, becomes aligned automatically and in the end is exported as TIF files.

Further image processing was done by using the software IMAGEJ. The most powerful tool of this program is the 'find edges' function. This function displays all sharp edges; all other areas become black (Fig. 2D). Further manipulation can easily be applied, such as cropping the image to a smaller size, or structures that are recognized as not belonging to the animal can be cut out. This is especially necessary for the Windyfield chert material as the matrix contains a lot of particles that should be cut away to give a clearer view on the fossil. The chert used in this study is unusually clear; increasing difficulty would be experienced with inclusion-rich, or more opaque varieties of the chert.

Based on the information from the Windyfield chert specimen a partial reconstruction was modelled using the software BLENDER. Therefore images from anterior, dorsal and lateral view were taken from the Quicktime VR and 'redrawn' in three dimensions (Fig. 3D).

Although IMAGEJ is capable of producing 3D reconstructions, we prefer to export the images as a tiff-stack and process it further in the DICOM-viewer OSIRIX. This software is in our experience faster in displaying the 3D-reconstruction than IMAGEJ, and it has some very interesting export formats. The stacks are simply loaded in OSIRIX and reconstructed using the Maximum Intensity Projection (MIP) function. From these reconstructions very useful Quicktime VR files (Lyons & Head, 1998) can be exported. The details of the models in the *z*-axis are not as precise as in the *xy*-plane. The Quicktime VR has been used to produce stereo images that give a good 3D-impression (Supporting Video Clip 1).

Stereo images can be processed further to red-cyan stereos, e.g. with the help of the software GIMP. This cannot usually be done directly from the specimens with transmitted light microscopy and is, therefore, seen as one of the advantages of the new method. Another possibility to display the results and to give a good impression of the 3D-reconstruction is given by producing a 'fly-through' (Supporting Video Clip 2). This should be calculated from three scene images. The first should be some degrees rotated around the *x*-axis of the direct view on the *xy*-plane, the second shows directly the view on the *xy*-plane and the third one should again be a rotation of the direct view on the *xy*-plane but from the other side. This video should be viewed as an endless loop that goes back and forth. This display gives a very easy depth impression without using additional tools, for example spectacles for red-cyan-stereos. A complete turn-around view is problematical for these kinds of models as it produces an optical depth inversion illusion (Supporting Video Clip 3).

Discussion

All methods yielded 3D images of example specimens each for 'Orsten' material (Fig. 2), Windyfield chert material (Fig. 3) and Recent material (Fig. 4). For each specimen a 3D representative was produced as a multi-planar reconstruction, exported as a Quicktime VR and from this red-cyan stereo images were produced. The advantages and disadvantages are discussed as follows.

Necessary effort in application of the new method

As already stated the specimens did not require any complex treatments but were simply placed on slides and put under the microscope. Taking the images took up to 30 min per specimen, because the camera was rather slow. Processing of the stack went in most steps automatically. Calculating time for alignment was up to 30 min in COMBINEZM, processing in IMAGEJ and OSIRIX took some minutes for each specimen, up to 1 h for the Windyfield chert specimen where a lot of alien particles were cut away manually. Calculating times for QTVR or flythrough Quicktime file were around 20 min. Thus, the maximum active time effort is not longer than 2 h in the worst case, and all in all it may take up to two and a half hours to have the final 3D representative.

Advantages and disadvantages compared to other methods

In general, if one has to work up a large number of specimens the procedure may be quite time consuming as an additional method. The largest disadvantage may, of course, be that you lose the original surface information of the specimen such as colour or texture during the software processing. Thus, this information has to be documented with other methods that are specially adapted for such kinds of documentation (e.g. Scott *et al.*, 2000). The greatest advantage of this method is, of course, that it can be easily applied and simply relies on widespread and easily accessible hardware and cost-free software.

The dark-field method applied to 'Orsten' material produced a quite precise 3D representative (Fig. 2A), even though the resolution is lower in the *z*-axis (Fig. 2B). Even the setae on the endites and exopods can be recognized. However, the method is less precise than SEM images and produced artefacts where alien particles are attached to the specimen (Fig. 2A). The method could not be tested for higher magnifications, because our microscope was not able to perform dark field on higher magnifications. It is still very useful as a backup documentation before applying other methods such as SEM.

For the Windyfield chert material this documenting method is in our view a great step forward (Fig. 3A). The information in the *z*-axis is rather limited (Fig. 3B), but still holds information about the orientation of the various parts of the specimen. Common transmitted light microscopy does not give the opportunity to produce stereo images. The stereo image of the Windyfield chert specimens shown here is at first rather unusual as they appear transparent. Therefore, it may take a while until the depth impression becomes visible to the observer. (Fig. 3A). The complete display of the animal is greatly improved, as the depth of field is rather limited when viewing the Windyfield chert fossils under the microscope, and it is difficult to get a correct impression of the whole animal (Fig. 3C). The 3D representation gives a good impression of the 3D arrangement and still shows details, such as the small setae on the rim of each exopodal article. The method is, of course, still limited by the transparency of the chert, but provides a new opportunity for documenting and presenting the 3D information found in Windyfield chert fossils. Compared to the method applied by Kamenz *et al.* (2008) the method presented here is inferior in *z*-axis resolution, but is much faster to apply and is based exclusively on freely available software. Additionally, it does not suffer from the blur effects mentioned in Kamenz *et al.* (2008).

The information obtained through the new method can be used as a basis to model 3D reconstructions, and take data from more than one specimen into account. Three-dimensional models based on SEM data have proven to be a powerful tool in understanding and presenting the morphology of an animal. A first attempt was produced in reconstructing the exopod of the antenna (Fig. 3D), but this is only a first step to reconstruct the whole animal. These modelled reconstructions are facilitated by the new method, since it is able to provide more spatial information on the structures.

For DIC-microscopy the method was less successful (Fig. 4). The model still shows a number of details, for example the subdivision of the exopod is visible, but compared to the dark field and bright field it looks more blurred. By contrast to the first two methods where the final models show the same precision of information compared to the original images of the stack, the 3D image from the DIC images seems to be blurred compared to the single images. This may be caused by the fact that DIC does produce a sharp image of a limited area in *z*-axis, but still has a smooth halo around the sharp areas. The method applied on DIC image stacks is, therefore, seen as inferior to SEM or CLSM, but still gives an opportunity to infer 3D information from a specimen when other methods are not available. As extant material can usually also be documented with dark-field microscopy we prefer this method, as it produced much clearer data.

The method in general enables the production of stereo images from a transmitted light microscope (Figs 2A, 3A and 4). Stereo images have already proven to be a powerful documenting tool with other methods (Kallenborn *et al.*, 1990; Purnell 2003; Gatesy *et al.*, 2005; Siveter *et al.*, 2007a, b, and references therein). The method is completely non-destructive and does not need preparations of the specimens and can thus be applied to type material (Scott *et al.*, 2000).

Future improvements

Further improvements may promote the presented '3Ds from light microscopy' method. An automatic microscope that is capable of producing a stack of images automatically might, of course, shorten the time effort, but like other newer machines it is not as widespread and easily available as a normal light microscope. For Recent material the possibility of staining should be tested in the future. Although the models do not have a high solution in the *z*-axis, they still hold spatial information (Figs 2B and 3B). Thus, based on these 3D representations it is possible to produce 3D models (Fig. 3D) comparable to those based on SEM images of 'Orsten' fossils (Haug *et al.*, 2007; Maas *et al.*, 2007), but simply based on different views on a QTVR 3D representation shown here. A first attempt is given in Fig. 3D. Other fossil and Recent micromaterial needs to be tested using the method described here. Fossiliferous cherts that are usually prepared as thin sections seem to have a high potential for study using the presented method, as demonstrated here by the results on Windyfield chert material (Fig. 2A).

Conclusions

The new '3Ds from light microscopy' method can be applied to many types of microfossils, which typically are studied under bright field and especially dark-field light microscopy. The '3D light microscopy' produces detailed 3D images that yield useful information for better understanding the spatial arrangements of structures. The method requires only a simple light microscope with a mounted digital camera, a PC and the processing with three freely available types of computer software. This is seen as one of the main advantages of the method as it allows the production of 3D information with rather low-tech equipment, that is widespread and, therefore, easily accessible. Thus, it holds the potential to become a widespread study method for both fossil and Recent material that cannot be sectioned or ground down, such as type material. Secondly, the method provides new opportunities for research workers and museum curators to produce 'back-up' documentation, because it requires no special treatment of the specimens and can be applied before applying other techniques. Thirdly, it provides a powerful documentation tool for fossils that yield 3D information, but where study is limited to light microscopy on thin sections as is the case with Rhynie and Windyfield chert fossils.

Acknowledgements

We thank two anonymous referees for improvement of language and scientific content of this publication. Thanks to the people that spent their time programming free available software, in our case COMBINEZM, IMAGEJ, OSIRIX, GIMP and BLENDER. Thanks also to the Department of Cell Biology and Anatomy of the University of Ulm, especially to Dr. Stefan Liebau, for testing the CLSM on Rhynie material. The EU SyntheSys program is thanked for funding the trip to Copenhagen for C.H. (DK-TAF-2171), where the cirripedes were caught. Special thanks to Dr. Jørgen Olesen and Dr. Jens T. Høeg, Copenhagen, for providing scientific and technical support, such as providing a plankton net and giving hints for catching. We are also grateful to Dipl.-Biol. Gerd Mayer, Ulm, for discovering COMBINEZM and to Dipl.-Geol. Martin Stein, Uppsala, for discovering BLENDER. Thanks to the DFG for funding the research of J.T.H. (WA-754/15–1).

References

Anderson, L.I. & Trewin, N.H. (2003) An early Devonian arthropod fauna from the Windyfield cherts, Aberdeenshire, Scotland. *Palaeontology* **46**, 467–509.

Anderson, L.I., Crighton, W.R.B. & Hass, H. (2004) A new univalve crustacean from the Early Devonian Rhynie chert hot-spring complex. *Trans. Roy. Soc. Edinb.* **94**, 355–369.

Bruton, D.L. (1981) The arthropod *Sidneyia inexpectans*, Middle Cambrian, Burgess Shale, British Columbia. *Phil. Trans. R. Soc. Lond.* **295**, 619–653.

Buttino, I., Ianora, A., Carotenuto, Y., Zupo, V. & Milarto, A. (2003) Use of the confocal laser scanning microscope in studies on the developmental biology of marine crustaceans. *Microsc. Res. Tech.* **60**, 458–464.

Carotenuto, Y. (1999) Morphological analysis of larval stages of *Temora stylifera* (Copepoda, Calanoida) from the Mediterranean Sea. *J. Plankton Res.* **21**, 1613–1632.

Chen, J.-Y., Schopf, J.W., Bottjer, D.J. *et al.* (2007) Raman spectra of a Lower Cambrian ctenophore embryo from southwestern Shaanxi, China. *PNAS* **104**, 6289–6292.

Chi, H., Xio, Z., Fu, D. & Lu, Z. (2006) Analysis of fluorescence from algae fossils of the Neoproterozoic Doushantuo formation of China by confocal laser scanning microscope. *Microsc. Res. Tech.* **69**, 253–259.

Cleal, C.J. & Thomas, B.A. (1995) *Palaeozoic Palaeobotany of Great Britain*. Chapman & Hall, London.

Dierick, M., Cnudde, V., Masschaele, B., Vlassenbroeck, J., van Hoorebeke, L., Jacobs, P. (2007) Micro-CT of fossils preserved in amber. *Nucl. Instrum. Meth. A* **580**, 641–643.

Donoghue, P.C.J., Bengtson, S., Dong, X.-P. *et al.* (2006) Synchrotron X-ray tomographic microscopy of fossil embryos. *Nature* **442**, 680–683.

Dubinin, V.B. (1962) Class Acaromorpha: mites or gnathosomic chelicerate arthropods. *Fundamentals of Palaeontology*. (ed. by B.B. Rodendorf), pp. 447–473. Acad. Sci USSR, Moscow.

Dunlop, J.A., Anderson, L.I., Kerp, H. & Hass, H. (2004) A harvestman (Arachnida: Opiliones) from the early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Trans. Roy. Soc. Edinb.* **94**, 341–354.

Edwards, D. (2004) Embryophytic sporophytes in the Rhynie and Windyfield cherts. *Trans. Roy. Soc. Edinburgh* **94**, 397–410.

Fanenbruck, M. & Harzsch, S. (2005) A brain atlas of *Godzilliognomus frondosus* Yager, 1989 (Remipedia, Godzilliidae) and comparison with the brain of *Speleonectes tulumensis* Yager, 1987 (Remipedia, Speleonectidae): implications for arthropod relationships. *Arthropod Struct. Dev.* **34**, 343–378.

Fanenbruck, M., Harzsch, S. & Wägele, J.W. (2004) The brain of the Remipedia (Crustacea) and an alternative hypothesis on their phylogenetic relationships. *PNAS* **101**, 3868–3873.

Fayers, S.R. & Trewin, N.H. (2004) A review of the palaeoenvironments and biota of the Windyfield chert. *Trans. Roy. Soc. Edinburgh* **94**, 325–339.

Fayers, S.R. & Trewin, N.H. (2005) A hexapod from the early Devonian Windyfield chert, Rhynie, Scotland. *Palaeontology* **48**, 1117–1130.

Fayers, S.R., Dunlop, J.A. & Trewin, N.H. (2005) A new early Devonian trigonotarbid arachnid from the Windyfield chert, Rhynie, Scotland. *J. Syst. Palaeontol.* **2**, 269–284.

Gatesy, S.M., Shubin, N.H. & Jenkins, F.A. Jr. (2005) Anaglyph stereo imaging of dinosaur track morphology and microtopography. *Palaeontol. Electron.* **8**, art.10, http://www.palaeo-electronica.org/2005_1/gatesy10/issue1_05.htm (last accessed 16 December 2008).

Haug, J.T., Maas, A., & Waloszek, D. (2007) Ontogeny and paleoecology of two Cambrian cyclopes. *Wiss. Mitt. Inst. Geol. TU Bergakad. Freiberg* **36**, 50–51.

Kallenborn, H.G., Wisser, A. & Nachtigall, W. (1990) 3-D SEM atlas of insect morphology. *Vol. 1. Heteroptera. BIONA report 7. Schriftenr. Akad. Wiss. Lit., Mainz, Komm. Biol. Kybern.* (ed. by W. Nachtigall), pp. 1–164. Gustav Fischer, Stuttgart.

Kamenz, C., Dunlop, J.A., Scholtz, G., Kerp, H. & Hass, H. (2008) Microanatomy of early Devonian book lungs. *Biol. Lett.* **4**, 212–215.

Knappertsbusch, M.W., Brown, K.R. & Rüegg, H.R. (2006) Positioning and enhanced stereographic imaging of microfossils in reflected light. *Palaeontol. Electron.* **9**, art.8, http://www.palaeo-electronica.org/2006_2/reflect/index.html (last accessed 16 December 2008).

Lin, J.-P., Scott, A.C., Li, C.-W., Wu, H.-J., Ausich, W.I., Zhao, Y.-L. & Hwu, Y.-K. (2006) Silicified egg clusters from a middle Cambrian burgess shale-type deposit, Guizhou, south China. *Geology* **34**, 1037–1040.

Lyons, P.D. & Head, L. (1998) Quicktime VR. A powerful new illustrative tool for micropaleontological research. *Palaeontol. Electron.* **1**, art.9, http://palaeo-electronica.org/1998_2/lyons/issue2.htm (last accessed 16 December 2008).

Lyons, P.D., Rioux, M. & Patterson, R.T. (2000) Application of a three-dimensional color laser scanner to paleontology: an interactive model of a juvenile *Tylosaurus* sp. Basisphenoid-Basioccipital. *Palaeontol. Electron.* **3**, art.4, http://www.palaeo-electronica.org/2000_2/mosasaur/issue2_00.htm (last accessed 16 December 2008).

Maas, A., Waloszek, D. & Müller, K.J. (2003) Morphology, ontogeny and phylogeny of the Phosphatocopina (Crustacea) from the upper Cambrian 'Orsten' of Sweden. *Fossils Strata* **49**, 1–238.

Maas, A., Waloszek, D., Haug, J.T. & Müller, K.J. (2007) A possible larval roundworm from the Cambrian 'Orsten' and its bearing on the phylogeny of Cycloneuralia. *Mem. Ass. Australas. Palaeontol.* **34**, 499–519.

Michels, J. (2007) Confocal laser scanning microscopy: using cuticular autofluorescence for high resolution morphological imaging in small crustaceans. *J. Microsc.* **227**, 1–7.

Müller, K.J. (1983) Crustacea with preserved soft parts from the Upper Cambrian of Sweden. *Lethaia* **16**, 93–109.

Müller, K.J. & Walossek D. (1986). Arthropod larvae from the upper Cambrian of Sweden. *Trans. Roy. Soc. Edinb.* **77**, 157–179.

Müller, K.J. & Walossek, D. (1988) External morphology and larval development of the Upper cambrian maxillopod *Bredocaris admirabilis*. *Fossils Strata* **23**, 1–70.

Oppitz, M., Pintaske, J., Kehlbach, R., Schick, F., Schriek, G. & Busch, C. (2007) Magnetic resonance imaging of iron-oxide labeled SK-Mel 28 human melanoma cells in the chick embryo using a clinical whole body MRI scanner. *Magn. Reson. Mater. Phy.* **20**, 1–9.

Orr, P.J., Siveter, De.J., Briggs, D.E.G., Siveter, Da.J. & Sutton, M.D. (2000) A new arthropod from the Silurian Konservat-Lagerstätte of Herefordshire, UK. *Proc. Roy. Soc. Lond. B* **267**, 1497–1504.

Penney, D., Dierick, M., Cnudde, V., Masschaele, B., Vlassenbroeck, J., van Hoorebeke, L. & Jacobs, P. (2007) First fossil Micropholcommatidae (Araneae), imaged in Eocene Paris amber using X-ray computed tomography. *Zootaxa* **1623**, 47–53.

Polcyn, M.J., Rogers, J.V. II, Kobayashi, Y. & Jacobs, L.L. (2002) Computed tomography of an *Anolis* lizard in Dominican amber: systematic, taphonomic biogeographic, and evolutionary implications. *Palaeontol. Electron.* **5**, art.1, http://www.palaeo-electronica.org/2002_1/amber/issue1_02.htm (last accessed 16 December 2008).

Purnell, M.A. (2003) Casting, replication, and anaglyph stereo imaging of microscopic detail in fossils, with examples from conodonts and other jawless vertebrates. *Palaeontol. Electron.* **6**, art.8, http://www.palaeo-electronica.org/2003_2/rubber/issue2_03.htm (last accessed 16 December 2008).

Roebroeck, A., Galuske, R., Formisano, E. *et al.* (2008) High-resolution diffusion tensor imaging and tractography of the human optic chiasm at 9.4 T. *NeuroImage* **39**, 157–168.

Schwarz, D., Vontobel, P., Lehmann, E.H., Meyer, C.A. & Bongartz, G. (2005) Neutron tomography of internal structures of vertebrate remains: a comparison with X-ray computed tomography. *Palaeontol. Electron.* **8**, art.30, http://palaeo-electronica.org/2005_2/neutron/issue2_05.htm (last accessed 16 December 2008).

Scott, D.B., Takayanagi, Y., Hasegawa, S. & Saito, T. (2000) Illustration and reevaluation of affinities of Neogene Foraminifera described From Japan. *Palaeontol. Electron.* **3**, art.5, http://palaeo-electronica.org/2000_2/foram/issue2_00.htm (last accessed 16 December 2008).

Scourfield, D.J. (1940a) Two new and nearly complete specimens of young stages of the Devonian fossil Crustacean *Lepidocaris rhyniensis*. *Proc. Linn. Soc.* **152**, 290–298.

Scourfield, D.J. (1940b) The oldest known fossil insect *Rhyniella praecursor* (Hirst & Maulik) – further details from additional specimens. *Proc. Linn. Soc.* **152**, 113–131.

Siveter, De.J., Fortey, R.A., Sutton, M.D., Briggs, D.E.G. & Siveter, Da.J. (2007a) A Silurian 'marrelomorph' arthropod. *Proc. Roy. Soc. Lond. B* **274**, 2223–2229.

Siveter, De.J., Sutton, M.D., Briggs, D.E.G. & Siveter, Da.J. (2007b) A new probable stem lineage crustacean with three-dimensionally preserved soft parts from the Herefordshire (Silurian) Lagerstätte, UK. *Proc. Roy. Soc. Lond. B* **274**, 2099–2107.

Størmer, L. (1939) Studies on trilobite morphology. Part I. The thoracic appendages and their phylogenetic significance. *Norsk Geol. Tidsskr.* **19**, 143–273.

Sutton, M.D., Briggs, D.E.G., Siveter, Da.J. & Siveter, De.J. (2001) Methodologies for the Visualization and Reconstruction of Three-dimensional Fossils from the Silurian Herefordshire Lagerstätte. *Palaeontol. Electron.* **4**, art.2, http://palaeo-electronica.org/2001_1/s2/issue1_01.htm (last accessed 16 December 2008).

Tafforeau, P., Boistel, R., Boller, E. *et al.* (2006) Applications of X-ray synchrotron microtomography for non-destructive 3D studies of paleontological specimens. *Appl. Phys. A* **83**, 195–202.

Tanisako, A., Hori, A., Okumura, A., Miyata, C., Kuzuryu, C., Obi, T. & Yoshimura, H. (2005) Micro-CT of *Pseudocneorhinus bifasciatus* by projection X-ray microscopy. *J. Electron Microsc.* **54**, 379–383.

Tanisako, A., Tsuruta, S., Hori, A. *et al.* (2006) Micro-CT of small insects by projection X-ray microscopy. *Proc. SPIE* **6318**, art.63180B.

Trewin, N.H. (2004) History of research on the geology and palaeontology of the Rhynie area, Aberdeenshire, Scotland. *Trans. Roy. Soc. Edinb.* **94**, 285–297.

Tse, K., Van Der Wall, H. & Vu, D.H. (2006) Use of laser 3D surface digitizer in data collection and 3D modelling of anatomical structures. *Proc. SPIE* **6056**, art.60560T.

Walossek, D. (1993) The upper Cambrian *Rehbachiella* and the phylogeny of Branchiopoda and Crustacea. *Fossils Strata* **32**, 1–202.

Wanninger, A. & Haszprunar, G. (2002) Muscle development in *Antalis entalis* (Mollusca, Scaphopoda) and its significance for Scaphopod relationships. *J. Morphol.* **254**, 53–64.

Wilhite, R. (2003) Digitizing large fossil skeletal elements for three-dimensional applications. *Palaeontol. Electron.* **5**, art.8, http://www.palaeo-electronica.org/2002_2/scan/issue2_02.htm (last accessed 16 December 2008).

Wirkner, C.S. & Prendini, L. (2007) Comparative morphology of the hemolymph vascular system in scorpions–a survey using corrosion casting, MicroCT, and 3D-reconstruction. *J. Morphol.* **268**, 401–413.

Wirkner, C.S. & Richter, S. (2003) The circulatory system in Phreatoicidea: implications for the isopod ground pattern and peracarid phylogeny. *Arthropod Struct. Dev.* **32**, 337–347.

Wollesen, T., Wanninger, A. & Klussmann-Kolb, A. (2007) Neurogenesis of cephalic sensory organs of *Aplysia californica*. *Cell Tissue Res.* **330**, 361–379.

Yoon, D.Y., Moon, J.H., Kim, H.K., Choi, C.S., Chang, S.K., Yun, E.J. & Seo, Y.L. (2008) Comparison of low-dose CT and MR for measurement of intra-abdominal adipose tissue: a phantom and human study. *Acad. Radiol.* **15**, 62–70.

Zhang, X.-G., Siveter, Da.J., Waloszek, D. & Maas, A. (2007) An epipodite-bearing crown-group crustacean from the lower Cambrian. *Nature* **449**, 595–598.

Zupo, V. & Buttino, I. (2001) Larval development of decapod crustaceans investigated by confocal microscopy: an application to *Hippolyte inermis* (Natantia). *Mar. Biol.* **138**, 965–973.

Supporting Information

Additional Supporting information may be found in the online version of this article.

Video Clip S1. QTVR of the specimen of †*Hesslandona unisulcata* Müller, 1982. The virtual specimen can be rotated by clicking on it and moving the mouse.

Video Clip S2. Fly-through movie of the specimen of †*Hesslandona unisulcata* Müller, 1982. The movement of the specimen mediates a depth impression without using spectacles or other equipment.

Video Clip S3. 180° degree rotation of the specimen of †*Hesslandona unisulcata* Müller, 1982. Note the depth inversion illusion at the end of the rotation.