# Stacked digital imaging of foraminifera

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## ABSTRACT

*This paper describes a technique of imaging foraminifera by combining a series of digital photographs in automated software to produce a single optical image with high resolution and superior depth of focus. Results can be obtained that are nearly as good as low-power SEM images, but have the great advantage of showing the hyaline tests as they appear to the working eye under a binocular microscope, as the foraminifera do not need to be coated in gold.*

*Digital imaging should be utilised more in documentation of foraminifera. The benefits of a low-cost and easy method of imaging include faster learning of new faunas, leading to more consistent species concepts. Workers can now publish fully illustrated works on complex morphometric series. Commercial service companies can now illustrate important taxa / faunal components and emphasise good micropalaeontological data in a way that the written word cannot.*

## THE IMPORTANCE OF IMAGING FORAMINIFERA

Illustrations of foraminifera are important for a variety of reasons. They have complex morphologies that change through ontogeny, evolutionary phylogeny and under eco-phenotypic influences. Some species have a distinctly different gerontic stage (*Globigerinoides sacculifer/ fistulosus, Sphaeroidinellopsis / Sphaeroidina* - see Figure 4, and even *Orbulina*), upon which generic or specific identification is based. Strongly expressed in the larger foraminifera is the acceleration of juvenile and adult to reach the specialised gerontic stage, as traced through evolutionary succession. There is gradual evolution, which affects where different workers place interspecific boundaries, and of course bias between lumpers and splitters in taxonomy. There is the problem of iterative [repeated] evolution, and for specimens extracted from indurated sediment the problem of poor preservation. All these mean that foraminifera, particularly the fossils, do not have simple, static shapes. These changeable, gradational, fossils are especially unsuited for species definition based on "ideal" types - a method inherited from ICZN rules based on more stable metazoan organisms[1]

In spite of these well-known aspects and pitfalls, the most common summary of foraminiferal observation is the data table, data chart, or text listing the key marker species. Data tables or charts inherently assume that the taxonomic units *are* discrete. A symbol or a number is placed in a box for each taxonomic entity present or, inversely, it is considered not to occur.

There is therefore an important gap between this data record and the actual observations, which is the quality or reliability of identification. A name implies 100% certainty, and the modifier aff. (*affinis* in Latin means "related to") or cf. (*confer* in Latin means "bring together" or "compare") just implies some uncertainty, not how much, or for what reason.

While it is easy to criticise data tables or charts, it is much harder to suggest a practical alternative. The easiest way to add a taxonomic qualifier is to take the most important age and environmental markers in a sample and photograph them. This way the reader is taken out of the ambiguity of concepts such as gradualism or of words (possibly in a second language) and can see if their experience agrees with the observation. Such images are also the best way to learn micropalaeontology, as again text can be ambiguous, and may not fairly reflect important factors such as texture (hispid as opposed to spinose), or degree of roundness (tumescence as in *tumida*; a subjective amount of fattening), and so on.

## HOW?

The "why" above is well known to foraminiferologists and is stated as background for non-specialists. The "how" has always been a problem. Light microscopy is barely adequate for the largest foraminifera and, for the majority of smaller benthic and planktonic species, the optical limitations are severe. Publications usually have images taken under scanning electron microscopes [SEM], which are expensive, time consuming and require specimens to be coated in gold, thereby making the hyaline tests

[1] *Metazoans have problematic gradations that challenge species concepts, such as in "Ring Species" as in seagulls (Liebers et al., 2004) or* Ensatina *salamanders in California (Highton 1998, Dawkins 2004), but while it is the exception in metazoans it is a common pattern in foraminifera*

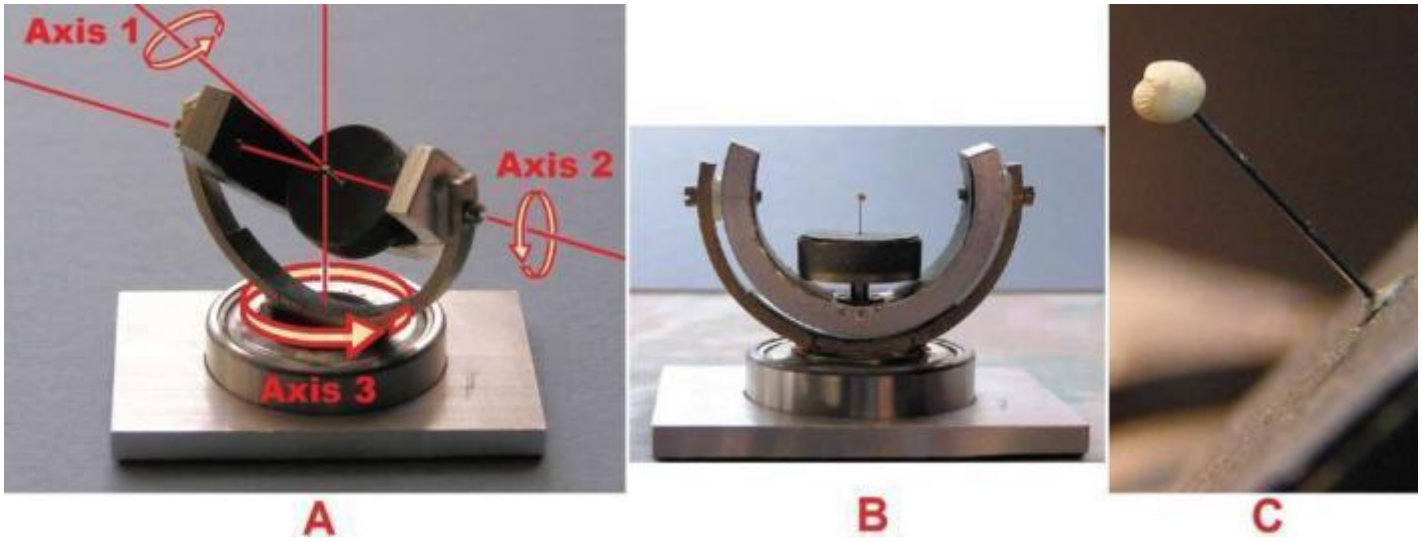



***Figure 1***. *A foraminifera-specific three-axis universal stage made by Phillips (2011). A: Axes of rotation. The specimen (a Pleistocene Elphidium crispum) can be rotated to display any side except the surface by which it is glued. It remains approximately centered and approximately in focus as it rotates. B: Side view showing the ball bearing mounts for Axes 1 and 3. C: Closeup of "A"; this is a lateral view at right angles to what the microscope sees; the mounting pin coincides with Axis 1. The specimen is temporarily attached to the mounting pin with gum tragacanth. Similar equipment can be bought ready made for entomological observation. See details in Phillips (2011).*

opaque, and the specimens looking quite different from those seen during work. SEM imaging is effectively destructive and not suitable for type specimens. The mounting method also means a specimen cannot be turned over and photographed on the other side; a different test is required for each orientation of illustration.

Confocal scanning light microscopy [CSLM] has been used by several workers to get acceptable optical images (e.g. Scott et al. 2000, Javaux & Scott, 2003). This method requires equipment much cheaper than an SEM, but it is a special lighting and exposure method that requires practise, skill, and special equipment in a dark-room.

Scanning light photography is a technique known since the late 1950's (Minsky, 1957, McLachlan, 1964). This method requires special equipment, especially for illumination. In the simplest form it requires three or four lights through a slit or focussing system, which reduces the area illuminated to be about the focal plane of the objective. The subject is attached on an upwardly mobile stand such that is raised up through the narrow band of light. The camera, focussed on the position of the plane of light, has an open shutter for the duration of the scanning motion, and therefore needs a darkened room.

Special microscopes have been built that have the illumination path directed, through a beam-splitter, and down the optical tube of the microscope, aligned with the same optics as the reverse viewing rays, calibrated to only illuminate the plane of focus. The proportion of light reaching this plane is small, and exposures are long, and of course require special equipment not accessible to most workers.

## IMAGE STACKING

The images shown in this review were taken by a different, digital, route that mimics the scanning method, but uses software rather than special illumination to isolate and then add together the in-focus planes.

Most of the images were taken using a standard petrographic microscope with low power (5x or 10x objective), although fair results are possible on normal stereo-microscopes. Petrographic microscopes have very limited depth of field, but high resolution.

In this technique a series of digital photographs can be taken while racking the plane of focus up or down the specimen. All these images are stacked together, in automated software, to produce a single image with depth of focus beyond the optical laws for a single image at high resolution.

## EQUIPMENT

The first images shown below were taken on a Wild MK5 stereo-microscope with phototube and Canon 6 MP camera. As such a microscope has a reasonable depth of field, only four to six images were required to cover a typical foraminiferal test. At higher powers

of magnification sharper images were obtained, but required ten or twelve images. However, as the optical path to the phototube is asymmetrical, following one microscope eye-path, there was chromatic aberration, with colour tinges at the inner and outer juxtaposition between off-white test and black background. There are masking techniques that can get rid of this effect in Adobe *Photoshop*™, but this adds time as it is a manual process. In addition, racking down the microscope to take a series of images can lead to a sideways drift in the image position of the subject. Some software can compensate for slight alignment change in the subject, but this problem gets worse at higher powers of magnification.

The best images shown here were taken on a Zeiss petrographic microscope with 5x or 10x objectives, in which the optical path and camera mount are aligned vertically. Note that working distance [WD] becomes quite small with 10x objectives, and if a choice of objectives is possible, choose one with the highest WD. For whole foraminiferal tests 20 to 50 stacked images is typical with this equipment. The software can handle in excess of 100 stacks, and processing times for a run of 50 stacked is about 2 minutes.

As a guide a Zeiss 2.5x objective (brightfield/ darkfield/ polarising type) has a working distance of about 1.5 cm, resolving power of about 5 microns (NA c. 0.7), with a depth of focus of about 75 microns. A Zeiss 5x objective has a working distance of over a centimetre, resolving power of about 2 microns (NA c. 0.13), with a depth of focus of about 16 microns. A Zeiss 10x objective has a working distance of only1/2 to 3/4 cm, making lighting angles difficult, resolving power of about 1 micron (NA c. 0.25), with a depth of focus of about 4-5 microns. NA is numerical aperture; a product of the angle of light incident on the objective (allowing for the nature of the medium between the specimen and the objective - i.e. very high NA and high resolution is possible for oil-immersion lenses, but not applicable for foraminifera). Some advanced objectives, made of different glasses, offer higher resolution, and while stacking images can handle the inevitable reduction in depth of focus, the basic principles of NA in air mean that working distance will be reduced, and room for external illumination constrained.

## SOFTWARE

Good software is available as free shareware (CombineZP by Alan Hadley, a British arthropod enthusiast: "*Windows*" only: *www.hadleyweb.pwp.blueyonder.co.uk*) or in slightly more sophisticated, commercial, form such as Helicon Focus (*www.heliconsoft.com*). The latter software was used here as it is Mac compatible, and it can also generate fairly reliable stereo-pairs.

Lighting from modern LEDs gives the best results without the heat of halogen lights. Modern digital cameras can work at lower light levels than film cameras so glare can be minimised by using more diffused light, and Helicon Focus has different stacking algorithms that can minimise stacking of glare-spots. LEDs should require less correction for colour temperature. *Photoshop*™ and equivalent software can do this, and also apply "unsharp masks" as a final step in enhancing small scale contrast.

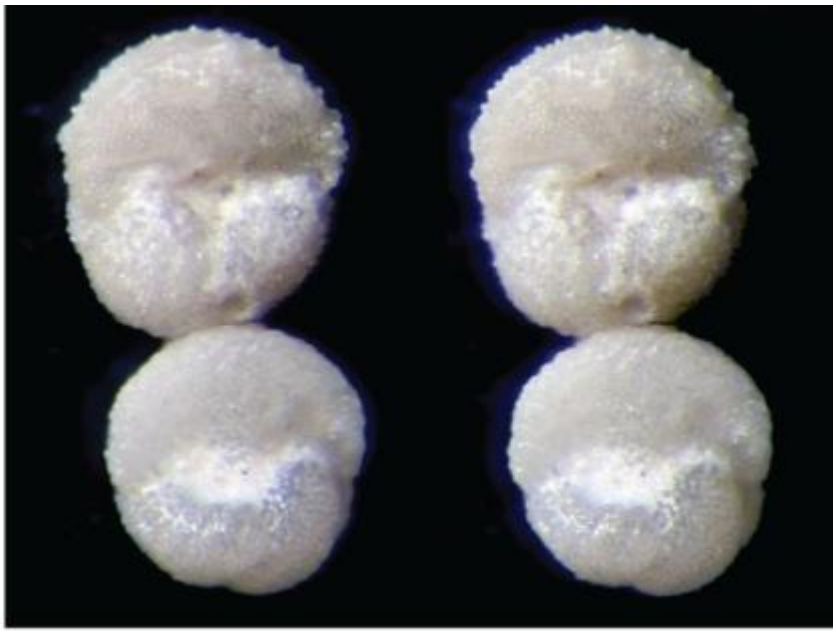
Some versions of image stacking software allow stereo-pair images (or coloured anaglyph images requiring "3D" glasses), or rotating 3D images to be produced. In the process of stacking, the in-focus portions are noted, and a depth map is produced. This is the frame onto which the final stacked image is draped to produce a 3D rendering. It is not a perfect technique but very fast, and it gives acceptable views of how foraminifera appear in 3D. Stereo-paired images are easiest to publish, and eyes can be trained to view them without stereoscopes if each image of the pair is about 5 cm apart (that is the same point in each image is about this distance apart). Interpupillary distances for most adults are 5.5 to 7 cm, so the eyes will be looking straight ahead at infinity, while eye-lenses need to be trained to focus on the nearer image while doing this. For most people this knowledge, and some practise, is all that is required to view stereo-pairs; the muscles controlling the eyeballs need to be disassociated from the muscles on the lenses within the eye. They are used to working in tandem for near or far objects. Success is when the eyeballs point straight ahead, but the lenses are focussed on the page at about 30 cm.

A piece of equipment not used here, but important for methodical production of publication-ready illustrations is a universal stage. Phillips (2011) illustrated a purpose-made stage for foraminifera, based on designs used by entomologists (Figure 1). Phillips' paper has some of the first published illustrations of foraminifera from stacked digital images although the online resource *http://foraminifera.eu/* has been showing stacked images, initially at lower powers, for a few years (see picked faunal slides illustrated at *http://foraminifera.eu/karl-otto.html* and the close-ups available on each cell). As Phillips points out, the universal stage is an important tool for simplifying the task of obtaining the correct standard views of specimens, relative to normal lighting angles.

## EXAMPLES

Figures 2-14 illustrate results of images of planktonic and smaller benthic foraminifera made with the process outlined above, and comparisons with images taken with standard binocular microscope (Figure 2) and SEM images (Figure 3).





**Above**: Manually made stereopair of the umbilical view (i.e. image tilted 8° and re-photographed). **Below**, stereopair of the spiral side in which the depth of the test is apparent

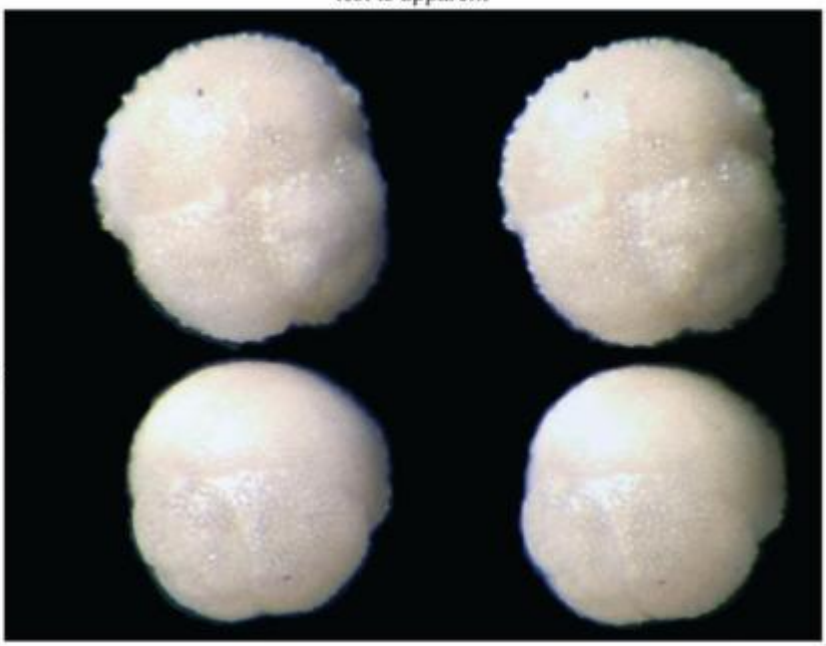


**Figure 2.** *Globoquadrina binaiensis* Koch from N.C. Java. Stacked images with a standard binocular stereo-microscope.
An important age marker for basal Miocene and latest Oligocene. The 3 dimensional shape of this species is an important characteristic, but in a single (non-stereo) view this shape is not apparent.
**Above right**, (Top specimen max. dimension 610µm, bottom specimen 535 µm) one of the input photographs, fair depth of field (compared to a petrographic microscope), but poor resolution. As described in the text, there is chromatic aberration in this image, with a yellow tinge on the right where the white test overlies the black background, and a faint blueish tone on the left side of the test. This can be removed in Photoshop using a masking technique



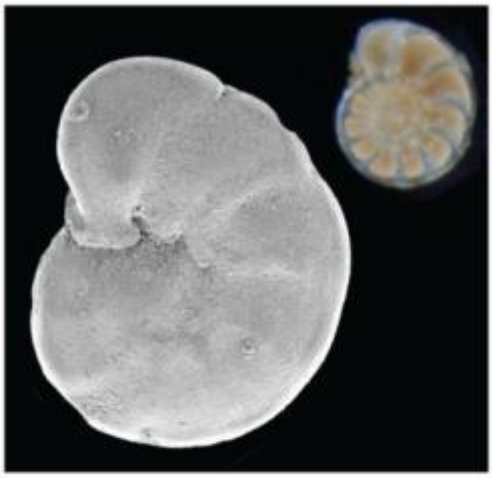


**Figure 3**. *Hyalinea balthica* (Schroeter), a distinct genus photographed **far right** in transmitted light and in **center** with SEM, where the very glassy (hyaline) nature of the test is completely obscured by the gold coating. **Above, left** a specimen from an Indonesian sea floor sample (about 500 µm in diameter ) photographed on a petrographic microscope with only 6 image stacks merged, at it is a thin test. Note the much higher resolution than the image from the stereo-microscope in Figure 2, at similar scales. Under reflected light the clear hyaline wall can be seen, and also the internal structure. .

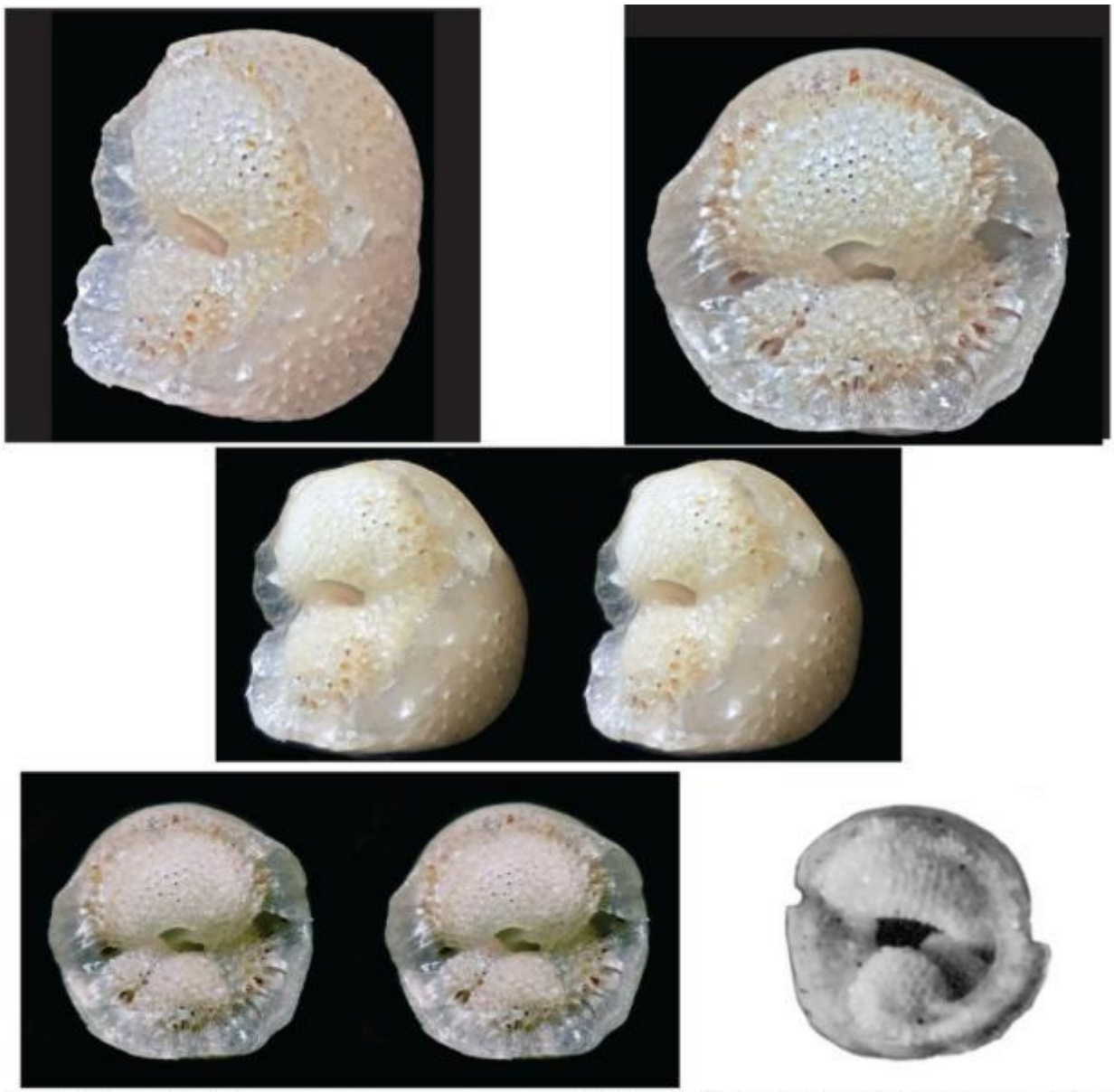


**Figure 4.** *Sphaeroidinella dehiscens excavata* Blow, and, **lower right**, one of Bandy et al.'s 1967 light microscope images of *Sphaeroidinella dehiscens.* Be (1965) and Be & Hemleben (1970) suggested that *Sphaeroidinella* was a gerontic stage development evolving from *Globigerinoides* by acquiring the thickened cortex, probably in response to, or enabling, migration to deeper parts of the water column. Bolli & Saunders (1985) did not agree with this as they thought they could see evolution from *Sphaeroidinellopsis* to *Sphaeroidinella* through time

Bandy, Ingle and Frerichs (1967) proposed two lineages of descent, from *Globigerinoides sacculifer* and *G conglobatus* giving rise to *Sphaeroidinellopsis seminulina* near the end of the early Miocene, and then a separate evolutionary appearance of thick cortex, from the same *Globigerinoides* ancestral stock, of *Sphaeroidina dehiscens* in basal Pliocene.

Other papers (e.g. Kennett & Srinivasan, 1983; Malmgren et al., 1996; Kucera 1998) stress the development first of small supplementary apertures in *Sphaeroidinella dehiscens* "immatura" then large supplementary apertures, and therefore that *Sphaeroidinella* evolved from *Sphaeroidinellopsis*. This is a subject for a different paper, but the advantage of light microscopy and illustration over SEM **(below)** in discussing the features of the glassy cortex can be seen

**Below** an image set from Kucera (1998) showing good technical quality images obtained by modern SEM, but the gold coating makes the cortex opaque

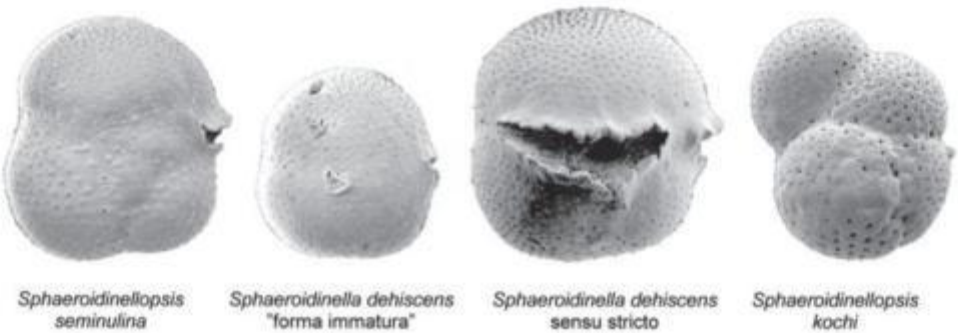




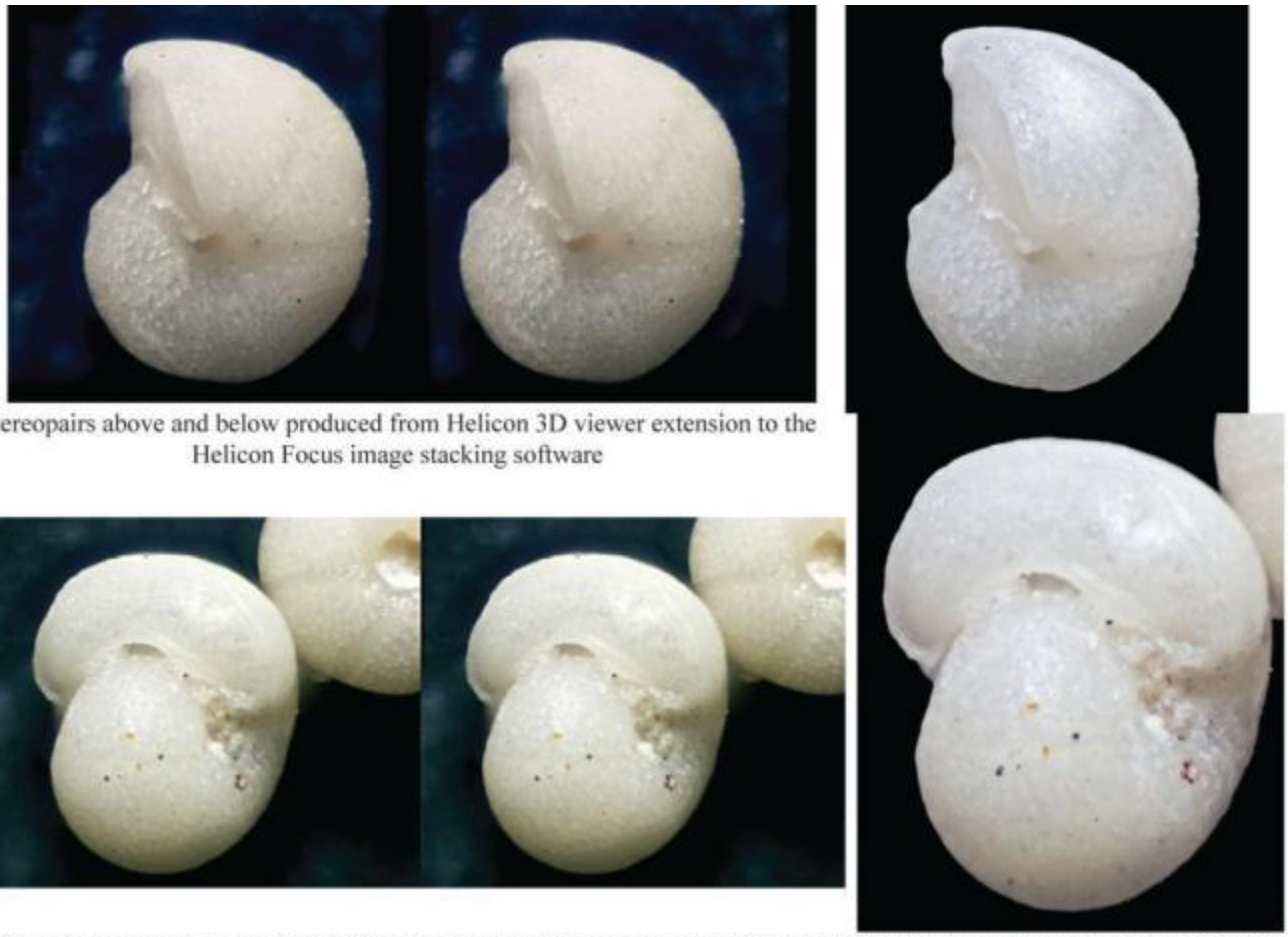
**Figure 5.** *Sphaeroidinellopsis seminulina* (Schwager). Images manually made into stereo-pair by titling stage under binocular microscope. Maximum diameter of test 520 µm. A good image of the cortex is obtained, contrasting with that in the previous figure from SEM. This image from a Wild Mk5 stereomicroscope



**Figure 6.** *Euuvigerina aculeata* (d'Orbigny) [*aculeatus* "a sting" or "like needles", Latin. *aculeate* - prickly] image on the **left** is a single exposure under a petrographic microscope, x 10 objective. The spines show good resolution but there is very low depth of field. Spines in the final product of 12 such images after stacking (**right**) seem to show even better resolution. However a small part of this apparent sharpness is due to the use of unsharp-mask filter in Adobe *Photoshop*™, which enhances small scale contrast



**Figure 7.** *Laticarnina pauperata* (Parker & Jones). Specimen about 750 µm maximum dimension. Petrographic microscope x5 objective. Specimen required 5 images to make a complete stack as the test is thin (c. 15 µm). The glassy, hyaline nature of the flange can be seen



Stereopairs above and below produced from Helicon 3D viewer extension to the Helicon Focus image stacking software

**Figure 8.** *Melonis pompilioides* (Fichtel & Moll), although some might place this in *M. umbilcatulus* (Walker & Jacob). Good illustrations should help refine these species concepts. Under SEM *M. pompilioides* has a profile similar to this example and less obvious pore-pits, while *M. umbilcatulus* has a broader, deeper, profile and strongly developed pore pits, similar to those in this example. As most publications give only one, ideal image for each of its species it is had to judge if a gradational, perhaps eco-phenotypic series is present, or two distinct species are viable. Easily accessible, good quality images such as these, in digitially reproduced papers with no plate limitations, will allow better discussion of such species concepts

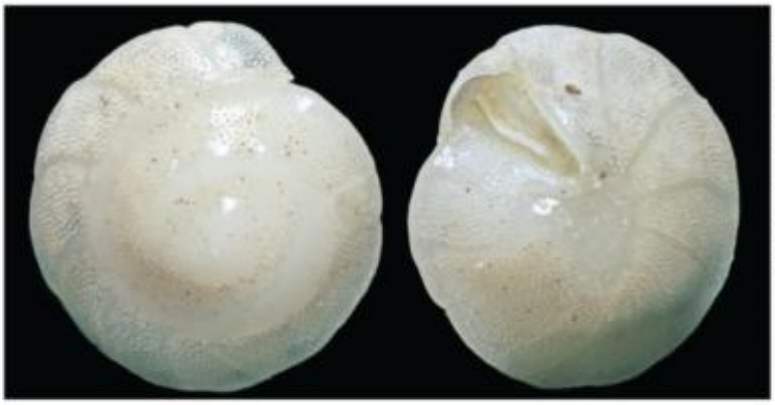


**Figure 9**. *Heterolepa subhaidingeri* (Parr 1950) or *Heterolepa praecinta* Karrer 1868. The latter is usually thicker in the axial direction and has strong thickening of sutures on the spiral side. SEM images are not optimal for comparing specimens of this plexus as the sutural thickening is partly obscured. Sea floor off NW Sabah, 600m water depth. Specimen about 0.5 mm diameter

**Figure 10** *Planulina retia* Belford Sea floor off NW Sabah, 600m water depth. Specimen about 0.5 mm diameter





**Figure 11.** *Cribrohantkenina inflata* Howe. From upper Nanggulan section C. Java

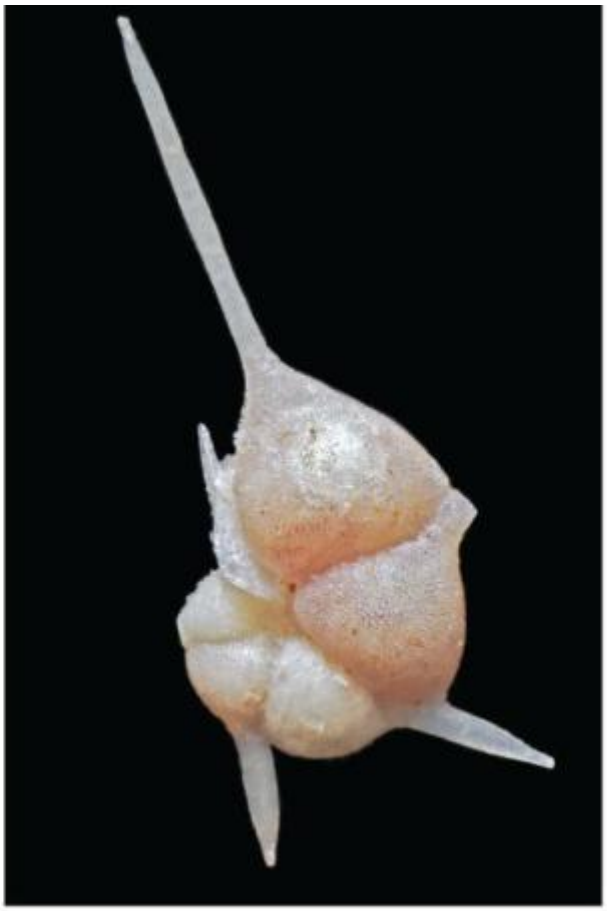


**Figure 12.** *Hantkenina alabamensis* Cushman



**Figure 13**. *Hantkenina alabamensis* Cushman, with stereo-pair from image stacking program. All three tests in Figures 11-13 are from the same sample of the Cunialensis clays (Lunt, 2013) and show the same type of preservation. Clays have protected the tests but there is slight calcite overgrowth. In samples where caving or reworking is interpreted the differences in preservational style can be illustrated using this optical imaging technique



**Figure 14.** *Globorotalia menardii menardii* Two pairs of stereo-paired images made by image-stacking software. Note how the computer generated depth-map and 3D image fails a little, and the 3D image suffers, in the low light of the lower spiral-side image

**Figure 15.** Below: *Globorotalia menardii cultrata* with its delicate, thin-walled test





**Figure 16.** *Bolliella adamsi* Banner & Blow, a Holocene deep water living planktonic foraminifera, from a sea floor South China Sea (off Sabah) sample in 1600m water depth

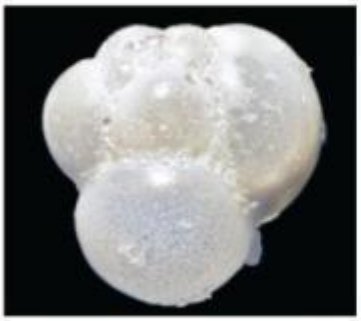
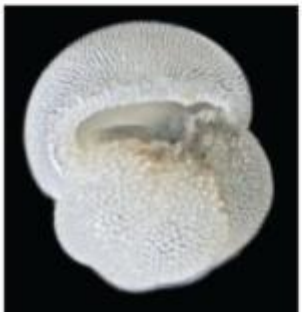


**Figure 17.** *Candeina nitida* d'Orbigny, probably not a full adult. A smooth test which looks different when coated in gold for an SEM

**Figure 18.** Hyaline *Globigerinoides*, with stacking just about allowing sight of the secondary aperture through and far below the primary aperture





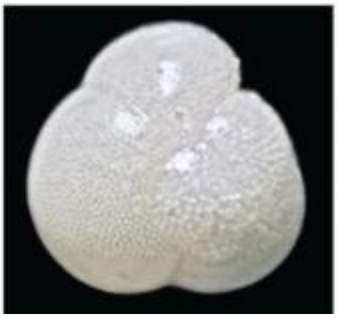


**Figure 19.** Two images of same the specimen of *Pullenia primalis* Banner & Blow, umbilical and spiral sides. The mounting method of an SEM does not allow the same specimen to be turned over and re-photographed

## CONCLUSIONS

Low cost computer software and standard geological optical equipment can produce publication-worthy illustrations of small foraminifera. Such illustrations are essential to prove age constraints or environmental conditions of outcrop or wells samples, as well as being needed for education and training. The technique is especially valuable for illustrating aspects of the hyaline test such as degrees of sutural thickening or cortex development. The ease and low cost of the technique means that multiple images can be taken of gradational or ontogenetic series. Aspects such as contrasting types of preservation (perhaps indicating caving of cuttings, or of reworking) can be illustrated in natural colour. For some species where coiling pattern or test height is important, the ability to easily produce stereo-paired images can be especially useful.

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