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Original article

Polyfocal photography of conodonts and other microfossils using petrographic microscopes

Photographie polyfocale de conodontes et autres microfossiles à l’aide de microscopes pétrographiques

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A B S T R A C T

Polyfocal photography is a method for obtaining digital images that have great depth of focus. A series of photos are made at successive focal levels from the bottom to the top of a fossil using reflected light. Computer software takes the part of each image that is in focus and merges all of the parts into a composite image that is entirely in focus. Microscopes designed for this purpose are available but are expensive. A petrographic microscope with a digital camera can produce such a series of images, and they can be composited by an inexpensive computer program. Polyfocal photography appears to be superior to other methods of photography for illustrating conodonts. Composite images show internal features, such as basal cavities and white matter, and the software can convert one composite image into a stereoscopic pair.

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R É S U M É

La photographie polyfocale est une méthode permettant d’obtenir des images numériques à grande profondeur de champ. Une série de prises de vue d’un fossile est réalisée en lumière reflétée à différents niveaux de mise au point depuis le bas vers le haut du spécimen. Un logiciel fusionne alors les éléments nets de chaque image en une seule image composée qui est alors entièrement nette. Les microscopes destinés à cet usage sont coûteux. Cependant, un microscope pétrographique équipé d’une caméra numérique peut produire une telle série d’images qui peuvent alors être fusionnées par un logiciel au prix accessible. La photographie polyfocale semble être supérieure aux autres méthodes de photographie pour illustrer les conodontes. Les images composées montrent des caractéristiques internes, telles que les cavités basales et la matière blanche, et le logiciel peut convertir une image composée en une paire d’images stéréoscopiques.

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1. Introduction

Methods of illustrating conodonts have changed throughout the last two centuries. Pander (1856) used drawings to illustrate the first recorded conodonts, and Furnish (1938) also illustrated Lower Ordovician conodonts with drawings. Miller (1969) illustrated Cambrian conodonts with light photographs that showed internal features, such as shapes of basal cavities and distribution of white matter. A problem with light photography is that depth of focus is limited. Scanning electron microscopes became widely available in the 1970s, and most conodont specialists abandoned light photography and adopted SEM micrographs instead. The resulting images had excellent depth of focus and high resolution, and very fine external ornament became visible for the

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first time. A disadvantage was that only surface features can be illustrated with scanning electron microscopy, and it was necessary to illustrate internal features with line drawings (Fig. 1). Also, specimens were often coated with gold, which obscured internal features and made future study of such features on those specimens difficult or impossible.

Polyfocal photography is a relatively new method that shows internal features and has unlimited depth of focus, resulting in high-quality digital images. The method, often referred to as “stacking,” uses a series of digital images (a “stack”) taken at different focal levels, and computer software then combines the in focus parts of individual images to form a composite image that is entirely in focus. Specialized microscopes are made for polyfocal photography, but they are quite expensive. Petrographic microscopes can be used with available computer software to produce similar results.

We report on the brands, models, and versions of equipment and programs used to generate images in this paper. This report does not constitute an endorsement of any of these specific brands, models, or versions of equipment.

2. Dedicated polyfocal microscope

Our first experience with polyfocal photography was with a Leitz model 205 polyfocal microscope, which utilizes a single objective lens, and lenses with different magnifications can be rotated into position. The microscope stage (rather than the upper part of the microscope) can be focused manually, and it is motorized so that the stage can be focused in small, precise increments. We placed as many as fifteen conodonts close together on a standard cardboard faunal slide without using glue and then positioned the slide on the microscope stage.

After manually focusing the microscope on the surface of the cardboard slide, the stage is then manually refocused on the highest part of the mounted conodonts, with the accompanying computer software recording both vertical positions. The computer then calculates the total relief of the specimens and the number of exposures required. Low-relief specimens require perhaps 6–10 images taken at different focal levels; high relief fossils require more images. Controlled by the computer software, the motorized stage moves to the lowest position, where the surface of the faunal slide is in focus, and then in successive vertical steps, a photograph is taken at each level until the highest part of the fossil has been photographed. The computer software then selects the part of each slice that is in sharp focus and digitally combines those parts to produce a composite image that is entirely in focus.

When the slide is in place on the microscope stage and the vertical coordinates are stored in the computer, it takes only 3–4 minutes to complete a stack of images and to generate the composite image. All of the individual slices and the composite image are stored on the computer and can be transferred to other computers.

The process uses reflected light, which penetrates the fossils and is reflected back to the camera, so that the composite image shows internal features, such as basal cavities and white matter. Specimens on Fig. 2 were converted from the original color versions to grayscale using Adobe Photoshop.

Specimens on Fig. 2 are type specimens of Cordylophorus provus Müller, 1959 from Oklahoma, USA. The Color Alteration Index (CAI) is about 2 (Epstein et al., 1977), meaning there is only moderate darkening of the original color due to heating of the fossils. Fig. 2A–H are S (rounded) elements, Fig. 2i, j, l, m are M (compressed) elements, and Fig. 2k is a P (twisted) element. Even in grayscale, the specimens illustrated on Fig. 2 show many features not visible in SEM micrographs, such as shapes of basal cavities. Complex distribution of white matter is visible in all of the cusps above the tips of the basal cavities, and the denticles contain white matter. Fig. 2A–K, M illustrate specimens with cusps that are mostly or entirely white matter. Fig. 2I is pathogenic and has little white matter because the cusp was broken during life. A short cusp was regenerated (at tip of arrow), and only the tip of the regenerated cusp is white matter. In Fig. 2J, the cusp is white matter, which also fills most of the area anterior to the basal cavity (at arrow). The cusps of specimens in Fig. 2B–C were broken during life, and the tips were healed together by narrow bands of white matter (at arrows). Fig. 2E shows a similar breakage, but the band of white matter is much wider (between arrows). These bands of white matter appear to be secondary apatite that healed broken elements. The specimens in Fig. 2G shows an unusual knob of white matter that formed above the tip of the basal cavity and fused a small part of the original cusp (at tip of present cusp) together with the area above the basal cavity. The arrow indicates additional white matter deposited anterior to the distal part of the basal cavity. The specimen in Fig. 2H has the cusp broken just above the tip of the basal cavity, and the cusp was healed in place at an odd angle; a similar repair scar can be seen in Fig. 2B.

None of these complex features would be visible on SEM micrographs, and they would be difficult to illustrate with line drawings. It is clear that polyfocal photography combines some features of normal light photography and SEM photography but is an improvement over both methods. Nevertheless, access to dedicated polyfocal microscopes is not universal because they are quite expensive.
3. Photography with student-model petrographic microscope

Dattilo developed a procedure for polyfocal photography of conodonts using a petrographic (polarizing) microscope. He used a student-model Leica DM LSP with a 10× objective, fitted with a Leica DFC 450 digital camera. He mounted on a standard cardboard faunal slide individual conodont elements from the Middle Ordovician Crystal Peak and Watson Ranch Formations from the Ibex area of western Utah. A fiber-optics illuminator with two flexible cables provided reflected light, and rotating the upper polarizer on the microscope reduced glare. Because petrographic microscopes are intended for studying thin sections of rock, they do not have great depth of focus. However, that shortcoming is overcome by taking a stack of photographs, each of which is partly in focus. The first photograph was focused on the surface of the faunal slide. Subsequent photographs were made by manually rotating the fine-focus ring on the microscope by a specific number of calibration marks, taking another photograph, and repeating the process until the highest part of the fossil was photographed. A stack of ~25 photographs was obtained in this manner.

Dattilo downloaded the HeliconFocus computer program from the website www.heliconsoft.com. The company sells licenses to use their software program for different periods of time, although a one-month trial version is available free. An inexpensive lifetime license includes free upgrades; details are on the website. The program can be downloaded onto as many as four different computers, and it is available in versions for Windows and Macintosh operating systems.

The stack of digital photographs was copied into the HeliconFocus program, which produced an in focus composite image in 2–3 minutes. Fig. 3 shows a few of the composite images obtained by using this procedure. The color of these conodonts is much darker than their original color (CAI = 4–5), and the species illustrated here were chosen because they have relatively complex morphologies and high relief. Small grains of quartz sand adhere to some of the specimens.

Several of the images in Fig. 3 required special treatment. Dattilo encountered a problem generating good composite images of some specimens because the background slide was dark and shiny and the dark conodont had many denticles with high relief, such as the specimen in Fig. 3A. Another part of the problem was that some conodonts that he photographed (e.g. Fig. 3A) had denticles that overlapped each other. The shallow depth of focus of the petrographic microscope caused parts of the conodont to be nearly invisible when the background slide was in focus, or when parts of overlapping denticles were not in focus on the same photograph. Also, the slide was nearly invisible when the highest parts of the conodont were in focus. This situation made it difficult for the computer program to detect the precise outline of the denticles or to bring the background slide into focus, thus, producing a poor composite image. HeliconFocus provides a “retouching” tool that allows the user to view the composite image while simultaneously viewing any of the individual slices and “painting” the images to choose which part of which slice will be used to make the composite image. This retouching tool allows the user to eliminate artefacts in the composite image that were generated by the computer program.

This was the first experiment using a petrographic microscope, and the background slide was black, and the conodonts were very dark (high CAI). The problem that required retouching probably could be eliminated by using a mounting surface that is not shiny and that has a color that contrasts strongly with the conodont, such as a light background for a dark conodont. However, a perfect background that contrasts well with the color of the conodont will not overcome the high relief of overlapping denticles that is characteristic of a few conodonts. It is possible that carefully orienting
conodonts so that denticles do not overlap would avoid that problem. The retouching tool is available for problems that cannot be avoided with the right background or orientation.

The second problem was that the conodont elements in Fig. 3C, H, and I were too long to fit into the field of view of the 10× objective microscope lens, and the specimens required two stacks of images that overlapped along the length of the specimen. The two composite images for those specimens were joined together using Adobe Photoshop.

4. Photography with research-quality petrographic microscope

Miller used Dattilo's procedure to photograph conodonts with a research-quality petrographic microscope, Nikon Eclipse Model LV100POL, with a 10× objective and Nikon DXM1200C digital camera connected to a computer. This microscope has a built-in reflected light source, and the upper polarizer was rotated to reduce glare. Conodont elements were mounted without glue using several different background surfaces. The first digital photograph was focused on the background, and successive photographs were made after manually rotating the fine-focus ring a specific number of calibration marks between exposures. Focusing had to be made on the computer screen rather than through the microscope lens. Miller first tried refocusing the fine-focus ring by three calibration marks between exposures, but it was determined that refocusing by 6–10 marks between exposures gave results of equal quality. The appropriate number of increments must be determined experimentally due to differences in equipment and fossils.

Some of the composite images obtained with the petrographic microscope are in Fig. 4, which shows topotype specimens of Cordylosodus andresi Viira and Sergeyeva in Kaljo et al. (1986). Specimens in Fig. 4A–D are from the same sample as the holotype (Viira et al., 1987); Fig. 4E–I are from the next higher sample. This is the oldest named species of the genus and is the ancestor of C. proaurus, shown in Fig. 2. Polyfocal photographs of C. andresi on Fig. 4 can be compared with an image of the same species on Fig. 1, which was made using a scanning electron microscope.

Morphological details shown on Fig. 4 are somewhat different from those on Fig. 2. Specimens on Fig. 4 are essentially unaltered from their original color (CAI = 1). Fig. 4A–H are 5 (rounded) elements; Fig. 4I is an M (compressed) element. All have black organic matter filling the basal cavities, which extend deeper into the cusp than in C. proaurus (Fig. 2), and the deeper basal cavity is a characteristic feature of C. andresi. Where the black filling is absent, the basal cavities are red. Tiny dark inclusions are visible in some denticles (Fig. 4F, H, I). A general feature of these images is that they show glare along the edges of parts of cusps and denticles; this is especially clear on Fig. 4C, indicated by the arrow. This glare may be related to the rotated plane of the microscope’s upper polarizer. Denticles of some elements originate along the side of the cusp (Fig. 4A, D, E) rather than from a distinct posterior process, as is common in C. proaurus (Fig. 2). Fig. 4C, G show elements in which the cusps have no white matter. Fig. 4B has a cusp that was broken during life and was healed by a tiny, blunt tip of secondary apatite (at arrow). Fig. 4A, D, F display cusps that were broken during life and healed together by thin bands of white matter (at arrows). As in C. proaurus, breakage of the cusp in C. andresi occurs just above the tip of the basal cavity. Denticles of specimens in Fig. 3F, G were broken and regenerated (at arrow). Healing illustrated in Fig. 4A left the healed part of the cusp bent at an angle relative to the rest of the specimen. The specimen in Fig. 4E has an irregularly broken tip of the basal cavity, a partly regenerated cusp, and white matter deposited anterior to the uppermost part of the basal cavity (at arrow). That white matter compares with Fig. 4G. In general, specimens on Fig. 4 have little or no white matter, which is a characteristic feature of C. andresi, and the white matter appears to be where pieces of broken cusps were healed together. Elements of C. proaurus (Fig. 2) have longer cusps than C. andresi, although clearly some elements were broken and mended with white matter, as discussed above.

5. Stereo pairs

The HeliconFocus software has two features related to three-dimensional imaging. After a stack of images is converted to a composite image, it can be viewed in a rotating, three-dimensional animation. A stereo pair can also be generated from the single composite image. Fig. 5A shows a composite image of a symmetrical element of Fryxellobodontus inornatus Miller, 1969, which has an unusually complex three-dimensional morphology for a Cambrian conodont. The specimen was recovered with the topotype specimens of C. proaurus from Oklahoma. Fig. 5B, C are a stereo pair made from the composite image by the HeliconFocus software, and the stereo pair permits the complex morphology to be understood more easily than by viewing multiple images taken from several different angles.

Fig. 5. Symmetrical element of Fryxellodontus inornatus Miller, 1969 from the same Oklahoma sample as specimens on Fig. 1. A. Composite polyfocal image. B. C. Stereo pair made from A by HeliconFocus computer program. Scale bar of 200 μm is for all views. Specimen is in the collection of J.F. Miller, Missouri State University, Springfield, Missouri, USA. Élément symétrique de Fryxellodontus inornatus Miller, 1969 du même échantillon d’Oklahoma que les spécimens de la Fig. 1. A. Image polyfocale composée. B. C. Paire stéréoscopique faite à partir de A par le logiciel HeliconFocus. La barre d’échelle de 200 μm est pour toutes les vues. Le spécimen est conservé dans la collection de J.F. Miller, Missouri State University, Springfield, Missouri, États-Unis.

6. Discussion

Polyfocal technology and instrumentation are advancing rapidly. Expensive polyfocal microscopes are not needed to photograph conodonts because a standard petrographic microscope with a digital camera can be used instead. Polyfocal photography offers insight into internal morphological details that are not seen with SEM imaging. Such illustrations offer the hope of new understanding of such issues as the utility of white matter as a taxonomic character, and the frequency and origin of repair scars. This technology also offers the possibility of easily illustrating specimens as stereo pairs for better understanding of the three-dimensional morphology of complex elements. Paleontologists studying other groups of microfossils, such as acritarchs, chitinozoa, foraminifera, or radiolarians, may be able to use other kinds of microscopes equipped with digital cameras to produce composite images that are a significant improvement over single-image photographs.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.
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