1 Polyfocal photography of conodonts and other microfossils using
2 petrographic microscopes

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4 *Photographie poly focale de conodontes et autres microfossiles à l'aide*
5 *de microscopes pétrographiques*

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25 ABSTRACT

26

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

36

RÉSUMÉ

37

38

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

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51 *Mots clés:*

52 *photographie poly focale*

53 *microscopes pétrographiques*

54 *conodontes*

55 *Cambrien*

56 *Ordovicien*

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58 **1. Introduction**

59

60 Methods of illustrating conodonts have changed throughout the last two centuries. Pander
61 (1856) used drawings to illustrate the first recorded conodonts, and Furnish (1938) also
62 illustrated Lower Ordovician conodonts with drawings. Miller (1969) illustrated Cambrian
63 conodonts with light photographs that showed internal features such as shapes of basal cavities
64 and distribution of white matter. A problem with light photography is that depth of focus is
65 limited. Scanning Electron Microscopes became widely available in the 1970s, and most
66 conodont specialists abandoned light photography and adopted SEM micrographs instead. The
67 resulting images had excellent depth of focus and high resolution, and very fine external
68 ornament became visible for the first time. A disadvantage was that only surface features can be
69 illustrated with scanning electron microscopy, and it was necessary to illustrate internal features
70 with line drawings (Fig. 1). Also, specimens were often coated with gold, which obscured
71 internal features and made future study of such features on those specimens difficult or
72 impossible.

73 Polyfocal photography is a relatively new method that shows internal features and has
74 unlimited depth of focus, resulting in high-quality digital images. The method, often referred to
75 as "stacking," uses a series of digital images (a "stack") taken at different focal levels, and
76 computer software then combines the in-focus parts of individual images to form a composite
77 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. Figure 2 shows 14 images that were taken as parts of several stacks. Images on Fig. 2 were converted from the original color versions to grayscale using Adobe Photoshop.

Specimens on Fig. 2 are topotype specimens of *Cordylodus proavus* 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. Figs. 2A–H are S (rounded) elements, Figs. 2I, J, L, M are M (compressed) elements, and Fig. 2K is a P (twisted) element. Even in grayscale, the specimens illustrated on Figure 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. Figs. 2A–K, M illustrate specimens with cusps that are mostly or entirely white matter. Fig. 2L 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 Figs. 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 be secondary apatite that healed broken elements. The specimen 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 10x 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

142 Ranch Formations from the Ibex area of western Utah. A fiber-optics illuminator with two
143 flexible cables provided reflected light, and rotating the upper polarizer on the microscope
144 reduced glare. Because petrographic microscopes are intended for studying thin sections of rock,
145 they do not have great depth of focus. However, that shortcoming is overcome by taking a stack
146 of photographs, each of which is partly in focus. The first photograph was focused on the surface
147 of the faunal slide. Subsequent photographs were made by manually rotating the fine-focus ring
148 on the microscope by a specific number of calibration marks, taking another photograph, and
149 repeating the process until the highest part of the fossil was photographed. A stack of ~25
150 photographs was obtained in this manner.

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

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

163 Several of the images in Figure 3 required special treatment. Dattilo encountered

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

179 This was the first experiment using a petrographic microscope, and the
180 background slide was black, and the conodonts were very dark (high CAI). The problem
181 that required retouching probably could be eliminated by using a mounting surface that is
182 not shiny and that has a color that contrasts strongly with the conodont, such as a light
183 background for a dark conodont. However, a perfect background that contrasts well with
184 the color of the conodont will not overcome the high relief of overlapping denticles that
185 is characteristic of a few conodonts. It is possible that carefully orienting conodonts so
186 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 Figs. 3C, H, and I were too long to fit into the field of view of the 10x 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 10x 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.

208 Some of the composite images obtained with the petrographic microscope are in Figure 4,
209 which shows topotype specimens of *Cordylodus andresi* Viira and Sergeyeva in Kaljo et al.
210 (1986). Specimens in Figs. 4A–D are from the same sample as the holotype (Viira et al., 1987);
211 Figs. 4E–I are from the next higher sample. This is the oldest named species of the genus and is
212 the ancestor of *C. proavus*, shown in Figure 2. Polyfocal photographs of *C. andresi* on Figure 4
213 can be compared with an image of the same species on Figure 1, which was made using a
214 Scanning Electron Microscope.

215 Morphological details shown on Figure 4 are somewhat different from those on Figure 2.
216 Specimens on Figure 4 are essentially unaltered from their original color (CAI = 1). Figs. 4A–H
217 are S (rounded) elements; Fig. 4I is an M (compressed) element. All have black organic matter
218 filling the basal cavities, which extend deeper into the cusp than in *Cordylodus proavus* (Figure
219 2), and the deeper basal cavity is a characteristic feature of *C. andresi*. Where the black filling is
220 absent, the basal cavities are red. Tiny dark inclusions are visible in some denticles (Figs. 4F, H,
221 I). A general feature of these images is that they show glare along the edges of parts of cusps and
222 denticles; this is especially clear on Fig. 4C, indicated by the arrow. This glare may be related to
223 the rotated plane of the microscope's upper polarizer. Denticles of some elements originate along
224 the side of the cusp (Figs. 4A, D, E) rather than from a distinct posterior process, as is common
225 in *C. proavus* (Figure 2). Figs. 4C, G show elements in which the cusps have no white matter.
226 Fig. 4B has a cusp that was broken during life and was healed by a tiny, blunt tip of secondary
227 apatite (at arrow). Figs. 4A, D, F display cusps that were broken during life and healed together
228 by thin bands of white matter (at arrows). As in *C. proavus*, breakage of the cusp in *C. andresi*
229 occurs just above the tip of the basal cavity. Denticles of specimens in Fig. 3F, G were broken
230 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 Figure 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. proavus* (Figure 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 *Fryxellodontus inornatus* Miller, 1969, which has an unusually complex three-dimensional morphology for a Cambrian conodont. The specimen was recovered with the topotype specimens of *Cordylodus proavus* from Oklahoma. Figs. 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.

6. Discussion

252

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

264

265 **Disclosure of interest**

266

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

268

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270

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277

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308 153.
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- 312

FIGURE CAPTIONS

Figure 1. Scanning Electron Microscope lateral views and drawings of conodonts from the Lava Dam Member of the Notch Peak Formation, House Range, Utah. A, B. S (rounded) element of *Cordylodus andresi* Viira and Sergeyeva in Kaljo et al. (1986) from sample SP-6-49, from 84.1 m above the base of the Steamboat Pass section, at the base of the *Cordylodus proavus* Zone. C, D. Genuiculate element of *Cambrooistodus minutus* (Miller, 1969) from sample LD5-LD-131, from 61.9 m above the base of the Lava Dam Five section, near the top of the *C. minutus* Subzone of the *Eoconodontus* Zone. Scale bar of 200 μ m is for all specimens. Modified from Miller et al. (2011, Fig. 7). The specimens are deposited at the University of Iowa Paleontology Museum (acronym SUI), Iowa City, Iowa, USA. A: SUI 125996, C: SUI 125995.

Vues latérales par microscopie électronique à balayage et dessins de conodontes du Membre Lava Dam de la Formation de Notch Peak, House Range, Utah, États-Unis. A, B. Élément S (arrondi) de Cordylodus andresi Viira et Sergeyeva dans Kaljo et al. (1986) de l'échantillon SP-6-49, à 84.1 mètres au-dessus de la base de la section Steamboat Pass, base de la zone Cordylodus proavus. C, D. Élément géniculé de Cambrooistodus minutus (Miller, 1969) de l'échantillon LD5-LD-131, à 61.9 mètres au-dessus de la base de la section Lava Dam Five, près de la limite supérieure de la sous-zone Cambrooistodus minutus de la zone Eoconodontus. Modifié de Miller et al. (2011, Fig. 7). Les spécimens sont conservés au musée de paléontologie de l'Université d'Iowa (acronyme SUI), Iowa City, Iowa, États-Unis. A, B: SUI 125996; C, D: SUI 125995.

Figure 2. Polyfocal lateral views of topotype specimens of *Cordylodus proavus* Müller, 1959 from 6 m below the top of the Signal Mountain Limestone on the Chapman Ranch, Arbuckle Mountains, Oklahoma, USA. A–H. S (rounded) elements. I–J, L–M. M (compressed) elements (L, pathologic). K. P (twisted) element. Arrows indicate features of special interest discussed in text. Images show shapes of basal cavities and distribution of white matter. Scale bar of 200 µm is for all specimens. Specimens are in the collection of J.F. Miller, Missouri State University, Springfield, Missouri, USA.

Vues polyfocales latérales de spécimens topotypes de Cordylodus proavus Müller, 1959, à 6 m au-dessous de la limite supérieure du Calcaire de Signal Mountain sur le Chapman Ranch, dans les montagnes Arbuckle, Oklahoma, États-Unis. A–H. Éléments S (arrondis). I–J, L–M. Éléments M (comprimés ; L, pathologique). K. Élément P (tordu). Les flèches indiquent les points d'intérêt mentionnés dans le texte. Les images montrent la silhouette des cavités basales et la distribution de la matière blanche. La barre d'échelle de 200 µm concerne tous les spécimens. Les spécimens sont conservés dans la collection de J.F. Miller, Missouri State University, Springfield, Missouri 65897 USA.

Figure 3. Polyfocal views of some species of conodonts from the Crystal Peak Formation (A) and the Watson Ranch Formation (B–I) in western Utah, USA. A, F. posterior views. B–E, G–I. Lateral views. A, E. *Erraticodon alternans* (Hadding, 1913). B. *Apteracontiodus sinuosus* (Mound, 1965). C, G–I. *Paraprioniodus costatus* (Mound, 1965). D. *Drepanoistodus angulensis* (Harris, 1962). F. *Histiodela* sp. Scale bar of 200 µm is for all specimens, and a human hair is

357 shown for comparison. Specimens are in the collection of R.L. Ethington, Geological Sciences
358 Department, University of Missouri, USA.

359 *Vues polyfocales de quelques espèces de conodontes de la Formation de Crystal Peak (A) et de*
360 *la Formation de Watson Ranch (B–I) dans l’ouest de l’Utah, États-Unis. A, F. Vues postérieures.*
361 *B–E, G–I. Vues latérales. A, E. Erraticodon alternans (Hadding, 1913). B. Apteracontiodus*
362 *sinuosus (Mound, 1965). C, G–I. Paraprioniodus costatus (Mound, 1965). D. Drepanoistodus*
363 *angulensis (Harris, 1962). F. Histiodella sp. La barre d’échelle de 200 µm est pour tous les*
364 *spécimens. Un cheveu humain est placé pour comparaison. Les spécimens sont conservés dans*
365 *la collection de R.L. Ethington, Geological Sciences Department, University of Missouri, USA.*

366

367 **Figure 4.** Polyfocal lateral views of topotype specimens of *Cordylodus andresi* Viira and
368 Sergeyeva in Kaljo et al. (1986) from Estonia. All figures from the Kallavera Formation in the
369 Vihula section (Viira et al., 1987). A–D. Sample 5 of Viira et al. (1987). E–I from sample 6 of
370 Viira et al. (1987). A–H. S (rounded) elements. I. M (compressed) element. Arrows indicate
371 features of special interest discussed in text. Basal cavities partly to completely filled with black
372 organic material. Images show shapes of basal cavities and distribution of white matter. Scale bar
373 of 200 µm is for all specimens. Specimens are in the collection of J.F. Miller, Missouri State
374 University, Springfield, Missouri, USA.

375 *Vues polyfocales latérales de spécimens topotypes de Cordylodus andresi Viira et Sergeyeva in*
376 *Kaljo et al. (1986) d’Estonie. Tous les spécimens viennent de la Formation Kallavera dans la*
377 *section Vihula (Viira et al, 1987). A–D. Échantillon 5 de Viira et al. (1987). E–I. Échantillon 6 de*
378 *Viira et al. (1987). A–H. Éléments S (arrondis). I. Élément M (comprimé). Les flèches indiquent*

379 des points d'intérêt mentionnés dans le texte. Les cavités basales sont partiellement ou
380 entièrement remplies de matière organique noire. Les images montrent la silhouette des cavités
381 basales et la distribution de la matière blanche. La barre d'échelle de 200 μm est pour tous les
382 spécimens. Les spécimens sont conservés dans la collections de J.F. Miller, Missouri State
383 University, Springfield, Missouri, USA.

384

385 **Figure 5.** Symmetrical element of *Fryxellodontus inornatus* Miller, 1969 from the same
386 Oklahoma sample as specimens on Figure 1. A. Composite polyfocal image. B, C. Stereo pair
387 made from A by HeliconFocus computer program. Scale bar of 200 μm is for all views.
388 Specimen is in the collection of J.F. Miller, , Missouri State University, Springfield, Missouri,
389 USA.

390 *Élément symétrique de Fryxellodontus inornatus Miller, 1969 du même échantillon d'Oklahoma*
391 *que les spécimens de la Figure 1. A. Image polyfocale composée. B- C. Paire stéréoscopique*
392 *faite à partir de A par le logiciel HeliconFocus. La barre d'échelle de 200 μm est pour toutes les*
393 *vues. Le spécimen est conservé dans la collection de J.F. Miller, Missouri State University,*
394 *Springfield, Missouri, USA.*

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5