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Reflected Light COL (Circular Oblique Illumination), an Almost Forgotten Technique[1]

Theodore M. Clarke
Retired Materials Engineer*

ABSTRACT

I recently designed and made an epi-illuminator module for my LOMO Biolam microscope. Initially I wanted metallographic imaging capability comparable to that which I made for my first student microscope (1). This time, I decided to add the capability of inserting a stop for COL, referred to in a 1935 Kodak book as conical illumination (2). I was also aware from J. S. Ploem's 1990 article in the *Journal of Microscopy* that this type of illumination is used for reflection contrast microscopy (RCM) with a Leitz Orthoplan microscope adapted for RCM (3). Experiments with the new illuminator demonstrate that COL significantly improves contrast with metallographic specimens and that the contrast is unexpectedly good with the diatom *Amphipleura pellucida* well resolved, but accompanied by interference color bands.

INTRODUCTION

My first foray into transmitted light microscopy involved designing and building a universal student microscope. Since this is a universal microscope, it has Köhler illumination in both transmitted and reflected modes. This modified Monolux microscope, set-up for reflected light with a 215 mm tube length metallurgical objective, is shown in Figure 1. The epi illuminator uses a fiber-optic light-guide rather than a filament as the light source. This allows a simpler optical design using only a single lens as explained in the operating ray diagram of Figure 2. The aperture diaphragm is located at the end of the light-guide. My second student microscope is a LOMO Biolam. My initial additions to the Biolam were to make an external fiber-optic illumination system for this microscope as well

Figure 1. Photograph of the Monolux microscope equipped for reflected Köhler illumination of a metallographic specimen.

[1] Presented at Inter/Micro 2008

*5090E 100S LaGrange, IN 46761

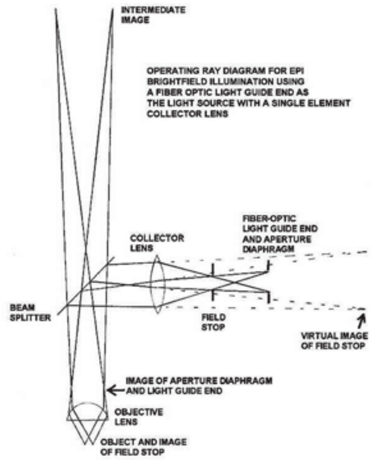

Figure2. Reflected Köhler illumination using a fiber-optic light-guide end as the light source.

Figure 3. Photograph showing (on the left) a Stach slider of opaque stops installed in the polarized light vertical illuminator of a Leitz Ortholux microscope.

a housing containing compensators and an analyzer that mates with the rotary dovetails used by LOMO. The housing was designed to accept a vertical illuminator as was done for the Monolux. I made an extra housing at the same time because it takes little more time to make two. I obtained a 1935 edition of the Kodak book *Photomicrography* (2) after completing work on the modified Monolux. The metallography section of the book recommends reflected circular oblique illumination (called conical illumination in the book) to improve contrast. This illumination method is not noted in Volume 9 of the 9th Edition of *Metals Handbook, Metallography and Microsctructures*.

My curiosity about how much contrast improvement there would be with antireflection coated objectives led me to design and fabricate a reflected light illuminator for the Biolam with circular oblique capability. Differential interference contrast (DIC) is the currently used illumination method for improved contrast. But it is quite costly. I was aware at this time of the use of reflection contrast microscopy by Ploem and that this contrast method would be interesting to try with my Biolam and the high NA LOMO biological objectives. Ploem uses a Leitz Ortholux microscope with the polarized light vertical illuminator and Stach slider of stops for COL (Figure 3). I learned much more about the Leitz polarized light vertical illuminator and the Stach slider from Jan Hinsch (Leica Microsystems) after completing my new illuminator. The Stach slider of stops was added at the request of Erich Stach to improve brightfield contrast of low reflectivity sections of coal. This method of improved contrast was apparently ignored by the metallurgists and consequently is no longer commercially available.

The initial version of the vertical illuminator and the housing for the Biolam are shown in Figure 4. The vertical illuminator was subsequently shortened and shown disassembled in Figure 5 after the outer tube was shortened to move the projected image of the light source 15 mm from the objective mounting flange of the lens turret. This change was made so that the annular illumination could be used for reflected darkfield with my 5X, 10X, and 20X brightfield/darkfield objectives using a ½″ diameter light-guide source and the largest stop shown, see Figure 5. These objectives have a hollow condenser around the imaging lens elements. The centering screws for the field diaphragm housing and the iris actuating lever ride in elongated slots in the outer tube so that the field diaphragm can be focused for a microscope tube length range of 160-215 mm by sliding the field diaphragm assembly. The flange on the end of the aperture diaphragm assembly mates with a larger diameter flange face on the end of the outer tube and inside the threaded end cap with centering screws. The pre-centered stops insert into the bore containing the aperture diaphragm shown on the left in Figure 5. Adding or changing a stop takes a couple of minutes. Figure 6 shows the illuminator assembled with a ½″ fiber-optic light-guide. Figure 7 shows the Biolam set up for photomicrography of a

Figure 4. Photograph showing the vertical illuminator and mating housing fabricated for a Biolam microscope.

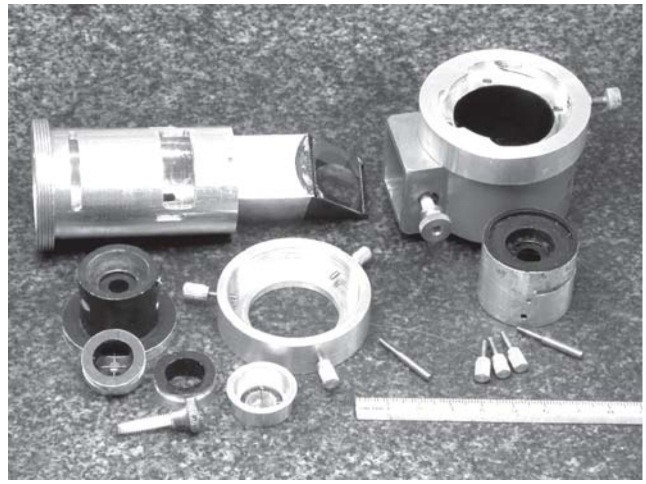

Figure 5. Photograph showing the vertical illuminator partially disassembled. The aperture diaphragm housing and opaque stops are on the lower right and the field diaphragm housing is on the lower left.

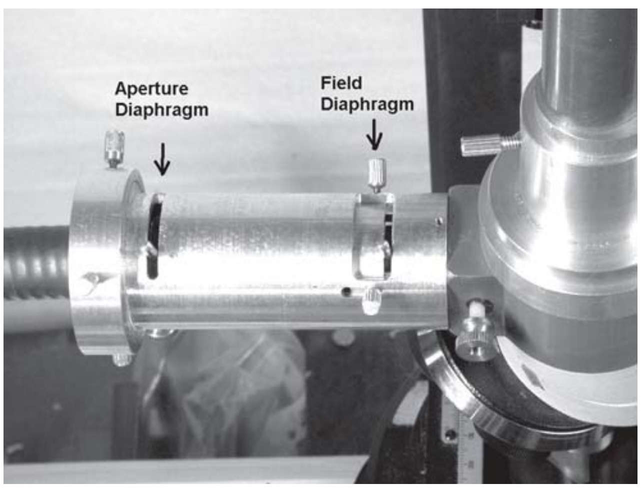

Figure 6. Close-up photograph of the vertical illuminator and housing installed on the limb of the Biolam microscope.

gear tooth metallographic specimen using a (Bausch & Lomb) 50X 0.85 NA 215 mm tube length metallurgical objective. A shorter draw tube for use with a 160 mm tube length biological objective is shown on the wood base along with a pin-hole eyepiece. The vertical illuminator is the original design. Figure 8 shows the Biolam equipped with the final, shorter illuminator design and a ¼″ diameter light-guide mounted in a reducing bushing. Figure 9 shows the ¼″ light-guide end mounted in a reducing bushing that also accepts inserted stops at the end of the light-guide for COL.

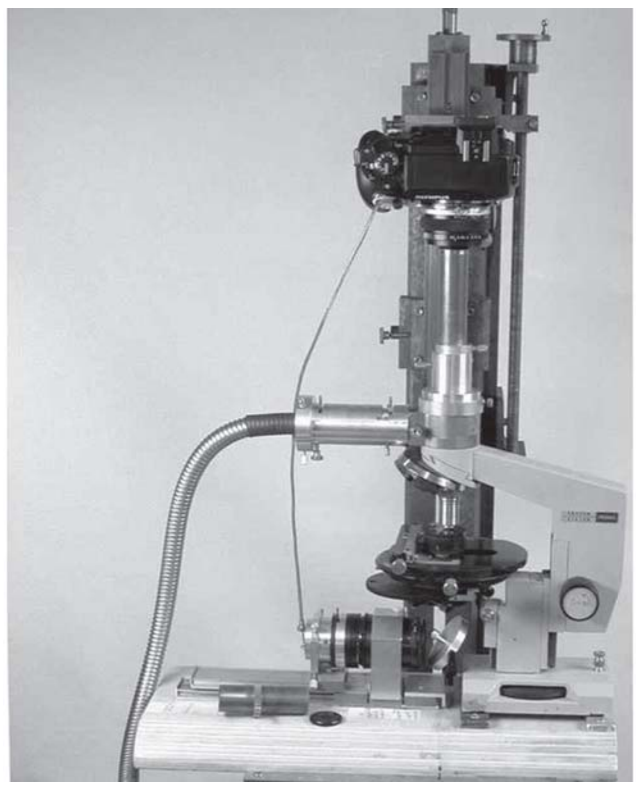

Figure 7. Photograph of the Biolam with the vertical illuminator on a stand with an Olympus E-330 DSLR above the eyepiece. A long drawtube is installed for recording photomicrographs of the metallographic specimen using a B & L 215 mm tube length metallurgical objective. A pin-hole eyepiece and a shorter drawtube are shown on the wood base of the microscope.

Figure 8. Photograph of Biolam set-up for photomicrography similar to Figure 7 except the shortened vertical illuminator is shown with a ¼″ light-guide in place of the ½″ light-guide shown in Figure 7.

COMPARISONS OF GEAR TOOTH MICROSTRUCTURE WITH REFLECTED COL AND BRIGHTFIELD

The ideal metallographic specimen for evaluating reflected COL would be one that normally requires DIC to bring out the key details in the microstructure. The same field would ideally be recorded with DIC, COL, and ordinary brightfield illumination. I do not have access to DIC capability or the metallographic specimens requiring DIC. So my evaluation of COL was done using a section of a case hardened gear tooth with a comparison of brightfield with COL. The nital etched specimen was chosen because it has a partial carbide network in the prior austenitic grain boundaries near the surface. I expected that normal sample preparation techniques in a production factory lab would leave the harder carbide standing out from the plane of polish and COL would accentuate this condition.

Figure 9. Close-up photograph of the ¼″ light-guide end mounted in a reducing bushing with a recess for mounting the opaque stops. The stops were punched from 0.005″ brass shim stock and glued onto 0.012″ diameter steel wires after precise centering.

The 50X 0.85 NA B&L metallurgical objective is an achromat, corrected for spherical aberration using green light. So a green filter was used at the output end of the fiber-optic illuminator. The system shown in Figure 7 was used to obtain Figures 10 and 11 comparing COL with brightfield. The aperture diaphragm was set so that it was at the edge of the pupil of the objective when viewed though a pin-hole eyepiece. Figure 10 shows the carbide highlighted by brighter edges not present in the brightfield image of Figure 11. Figure 12 shows the back of the 50X objective viewed through a B&L pin-hole eyepiece with COL illumination. The narrower annulus was obtained with the stop made for the shorter illuminator shown in Figure 8. The narrower annulus further highlights the intergranular carbides, as shown in Figure 13, but also diminishes the contrast between the darker etching acicular martensite and light-appearing retained austenite shown in the brightfield image in Figure 11. The annulus thickness is therefore an important variable for COL. The annulus can made thinner by slightly closing the aperture diaphragm if a larger stop is not available and a small loss of resolution is acceptable.

DIATOM IMAGING WITH REFLECTED VERSUS TRANSMITTED COL

My curiosity about improved contrast with COL was satisfied at this stage. So I then pursued investigating the possibility of obtaining some improved con-

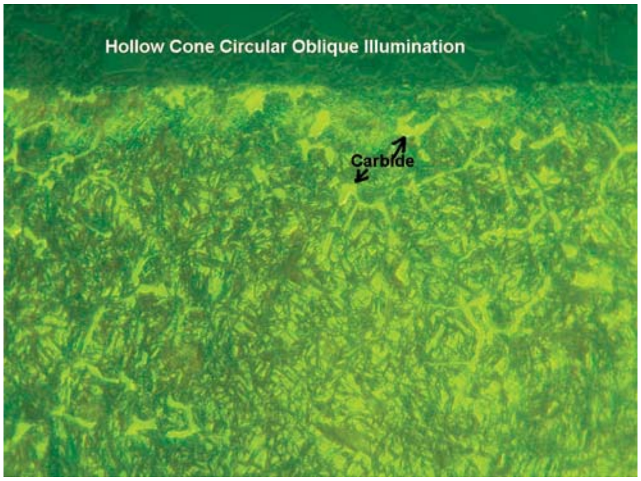

Figure 10. Photomicrograph taken with reflected COL and 50X 0.85 NA B&L metallurgical objective of the surface microstructure of a case hardened gear tooth surface. The arrows indicate intergranular carbides with their boundaries highlighted by the circular oblique illumination unlike in the brightfield image of Figure 11.

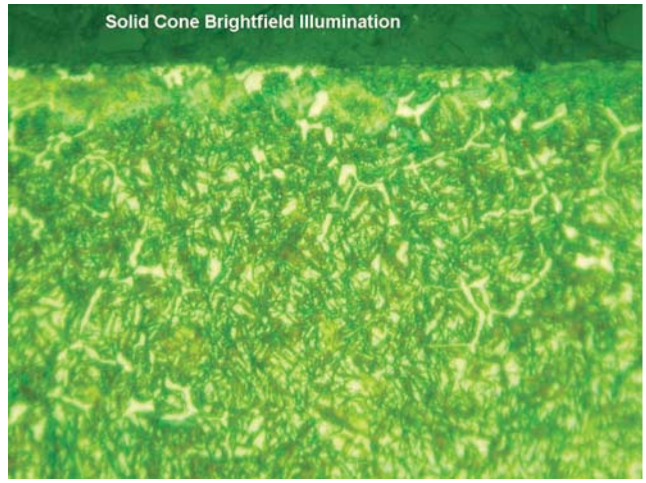

Figure 11. Photomicrograph of the same field of microstructure as in Figure 10 except the opaque stop was removed to obtain brightfield illumination. The extreme surface has a thin band of dark etching, unresolved pearlite that appears more granular with COL in Figure 10.

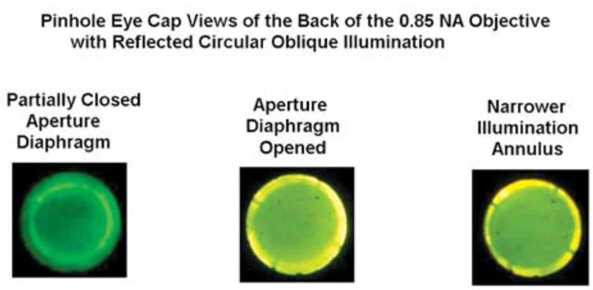

Figure 12. Photographs taken through a pin-hole eyepiece showing the back of the 50X objective. The left view shows the edges of the partially closed aperture diaphragm with the opaque stop prior to opening the diaphragm to the edge of the objective's pupil to the setting shown in the middle view used for the photomicrograph in Figure 10.

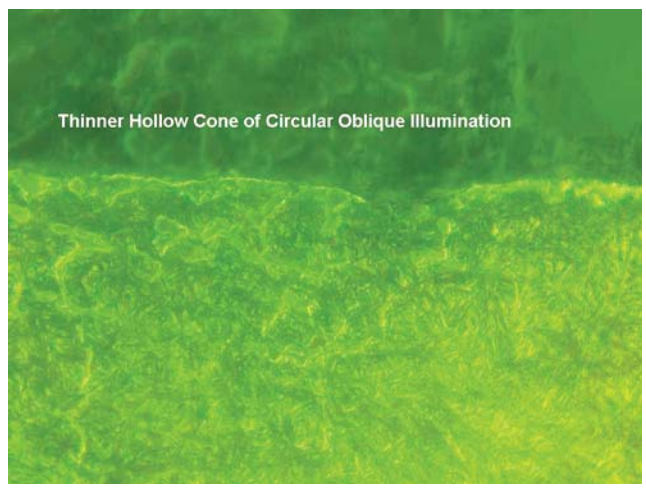

Figure 13. Photomicrograph of the gear tooth surface microstructure recorded using the narrower light annulus shown on the left in Figure 12. Contrast between the dark etching acicular martensite and the light etching retained austenite in Figures 10 & 11 has been largely lost.

trast demonstrated by Ploem for reflection contrast microscopy (RCM) requiring reflected COL. My system lacks the crossed polarized light used with a quarter wave plate on the front of the objective, called an Antiflex system by Zeiss. My understanding is that the polarized light is needed to avoid reducing contrast caused by back reflections from the lens element surfaces in the objective. Oil immersion is required to avoid a strong reflection from an air-glass interface on the top of the cover slip. The LOMO 90X 1.25 NA achromat objective was chosen for this study because it has antireflection coatings on the lens elements. I chose the Klaus Kemp 8 Form diatom test slide as my first test specimen. I was pleasantly surprised with how bright the diatoms appeared against a dark gray background. The dark background is a key requirement for RCM. It arises because the hollow cone of illumination diverges after passing through the test object and into the supporting glass slide. The back reflection at the bottom of the slide is sufficiently less than ninety degrees that none of this light enters the acceptance angle of the objective.

I next proceeded to a Klaus Kemp strew slide of *Amphipleura pellucida* diatoms. I was surprised to see interference color bands as well as a Newton ring pattern. The transverse striae were resolved with unexpectedly high contrast as shown in Figure 14. Another diatom was located with better alignment parallel to the cover slip. The Newton rings were avoided but interference color bands are still present. Figure 15 shows the striae are well resolved with high contrast without resorting to increasing the contrast digitally. The striae are imaged with higher contrast in reflected COL than with transmitted COL shown in Figure 16 from a previous article (4). Figure 17 has an insert showing that the striae of *Amphipleura pellucida* can be resolved with a 1.0 NA objective used with transmitted COL between 0.8 and 1.0 NA but does not explain how the COL was obtained for the image. The lens housing of the LOMO 1.40 NA aplanatic condenser was unscrewed from its mount and a stop was inserted so that it almost touches the aperture diaphragm iris. The stops are at the end of a thin bushing that is a sliding fit with the bore above the iris. A flange at the other end prevents the stop from rubbing against the iris and is thin enough to allow full depth engagement of the male threads of the lens housing. Figure 17 shows the stops along with the lens housing removed from the condenser base.

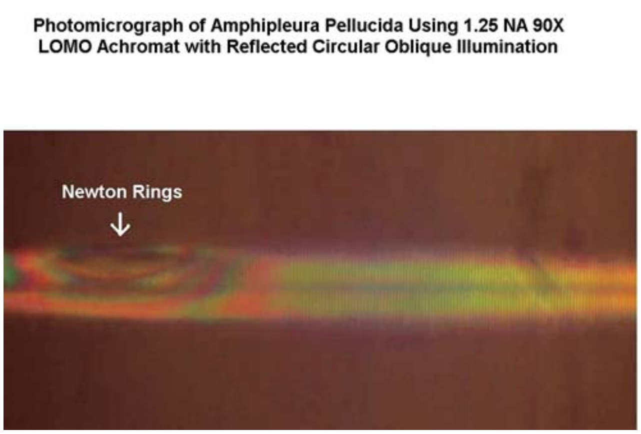

Figure 14. Photomicrograph of the diatom Amphipleura pellucida *obtained with reflected COL using a LOMO 90X 1.25 NA achromatic objective. Note the indicated colored Newton rings and darkened background.*

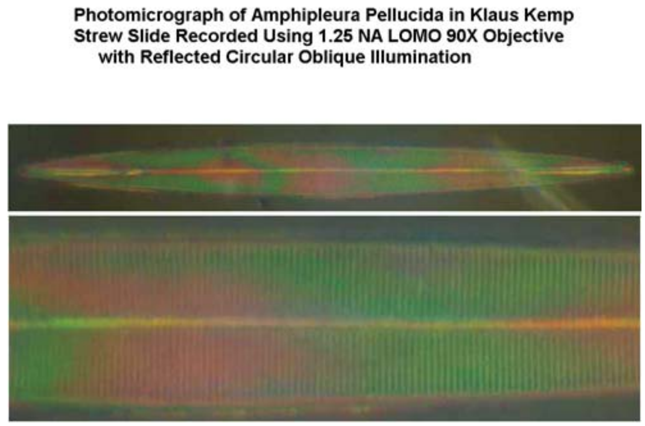

Figure 15. Photomicrograph with reflected COL of an Amphipleura pellucida *frustule aligned more nearly parallel to the plane of the cover glass than that shown in Figure 14. Interference color bands are evident. The lower view was cropped from the original image, recorded with the Olympus E-330 DSLR, illustrating the high contrast of the striae without digital adjustment of the contrast.*

CONCLUSION

My preferred application of COL is in transmitted mode for studies of lake water organisms at a visual magnification of 600X using a LOMO 30X 0.90 NA water immersion objective. The water specimen is held in a micro-aquarium slide and the objective is

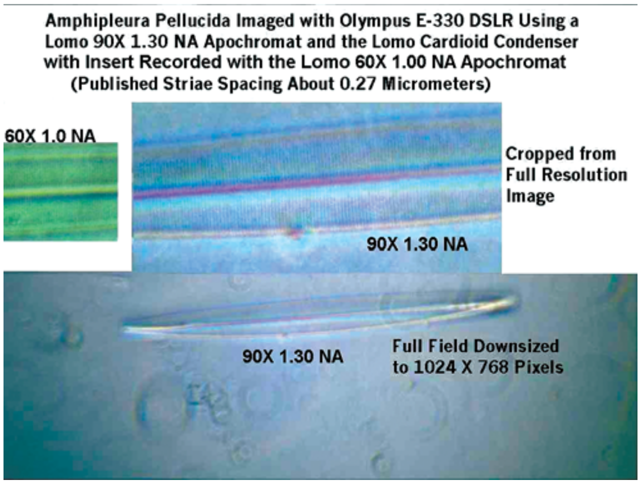

Figure 16. Photomicrograph of an Amphipleura pellucida *frustule recorded with transmitted COL reproduced from my previous article to illustrate the higher contrast for this type of specimen with reflected COL in Figure 14. The insert shows that the striae can be just resolved with a 1.0 NA objective with COL from the LOMO aplanatic 1.40 NA condenser.*

Figure 17. Photograph of the LOMO 1.40 NA aplanatic condenser with the lens housing separated to show the bore allowing the flanged stops to be inserted for either transmitted COL or darkfield.

used without a cover slip. This system is shown in Figure 18. The housing for compensators and the analyzer adds 45 mm of tube length with the LOMO trinocular head mated to the top of the housing. Some of the organisms are anisotropic, making the polarized light capability desirable. The added tube length from the housing corrects the spherical aberration from using the objective in dipping mode without a cover slip. This system provides better images of diatoms such as *Fragillaria* shown in Figure 19 than the 40X 0.65 NA objective, but with far less depth of field. I have found that it is much more convenient to insert the stops at the end of the ¼" light-guide shown in Figure 9, allowing more rapid switching among the illumination modes, including darkfield. This method also works well for reflected COL with the new vertical illuminator.

The title of this article noted that it is a review of almost forgotten technology. Köhler illumination using a fiber-optic light-guide in place of the lamp filament was first advocated by Frank Fryer in 1978 (6). Unfortunately this method has been largely ignored. Only a small number of microscopists have retrofitted their microscopes using the method recommended by Fryer. Adding stops at the end of the light-guide should be feasible for these retrofitted microscopes for those wanting COL capability. The interference con-

Figure 18. Photograph of the Biolam with a trinocular head and a phase telescope inserted in the right eye tube. One objective has a water immersion cap and the other objective is a 30X water immersion objective used without a cover slip. Below the trinocular head is a housing containing an analyzer and compensators in sliders. The stepped diameter bar on the wood base is used to mechanically center the aperture diaphragm with the bore of the collector lens housing.

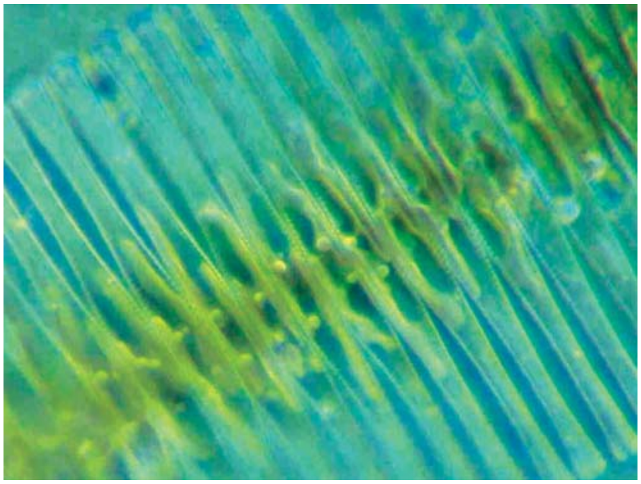

Figure 19. Photomicrograph of live Fragillaria *in a lake water sample held in a micro-aquarium slide and imaged with transmitted COL and the LOMO 30X water immersion objective using the system shown in Figure 18.*

trast effects evident in Figures 15 & 16 were not new to Jan Hinsch of Leica. The interference contrast, with the reflection from the underside of the cover slip providing the reference beam, is described by Victor Phillips and called multiple-beam interferometry (6). Phillips also describes reflected COL and calls it an opaque-stop microscope.

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