Effects of Abbe Condenser Spherical Aberration on Image Quality

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Introduction

An earlier article in *Microscopy Today*, "Rediscovery of Darkfield Dispersion Staining while Building a Universal Student Microscope," noted that the darkfield inserts were made for Peter Cooke (MICA, Chicago, IL) to be used to teach high resolution dispersion staining in his advanced microscopy classes using Olympus BH2 microscopes and the Olympus 1.25 NA Abbe condenser[1]. Peter has asked whether there is a better condenser design that will reduce the time and effort needed to switch between brightfield and darkfield. This has not been an issue for my student microscopes with external fiber-optic illumination systems because the darkfield stop is inserted at the end of the light-guide that serves as the light source. This is not feasible for microscopes


Fig. 1

such as the Olympus BH2 with built-in illumination systems. My second student microscope is a modified LOMO Biolam (Multiscope) with more capability in transmitted light than my modified Monolux microscope[1]. This added capability includes a phase contrast upgrade for the Multiscope, an aplanatic 1.40 NA condenser, and a ball bearing rotary stage. The LOMO phase contrast condenser design seemed to provide the basis for a prototype to demonstrate an answer to Peter's need for rapid change between brightfield and darkfield when the darkfield stop must be near the aperture diaphragm of the condenser. The phase contrast condenser allows this rapid switch only for the 10X objective, which needs the phase annulus for the 100X objective to achieve darkfield. The open position in the annulus wheel provides brightfield with an aperture diaphragm. I was not satisfied with the 0.8 NA condenser optics when used for brightfield with the 40X 0.65 NA objective. I found that the 1.25 NA LOMO Abbe condenser is able to achieve acceptable Koehler illumination with the 0.65 NA 40X objective and about 75% of the rear focal plane illuminated. My idea was to replace the phase contrast annuli in the centerable wheel with a set of darkfield stops and then use the 1.25 NA Abbe lens in place of the 0.8 NA lens. Chris Vander Tuuk of LOMO America was generous enough to donate a base of the LOMO phase contrast condenser for building a prototype condenser.

Since the top lens can be unscrewed from the top of the 1.25 condenser, my plan was to use this configuration with a suitable stop in the wheel with the 4X objective. McCrone Microscopes and Accessories donated a diatom test plate used to demonstrate the performance of the prototype condenser with multiple illumination modes. This prototype condenser is shown in Figure 1. Figure 2 shows the condenser mounted in my modified Biolam microscope. Oblique illumination is obtained with this condenser by decentering the darkfield stop and setting the aperture diaphragm at almost the full NA of the objective. Annular illumination, recently named circular oblique lighting (COL) by Paul James, is achieved by using a smaller stop so that the rear focal plane of the objective shows a narrow ring of illumination at just below the maximum NA of the objective[2]. This prototype condenser suffers from the effects of spherical aberration, as noted in my article on dispersion staining. This effect is most evident in a comparison of brightfield images taken with both the Abbe condenser and the aplanatic condenser, with the illumination almost fully filling the NA as the objective in order to resolve the stria spacing of the diatom *Pleurosigma angulatum*. The difference in image quality is less significant with annular (COL) illumination. Ray diagrams and photographs of the rear focal plane of the objective illustrate the spherical aberration. A wave optics analysis of the effects of condenser spherical aberration would be necessary to explain the degradation in image contrast in brightfield, which is much more than caused by opening the field diaphragm. The image quality degradation with the Abbe condenser has been previously noted by experts in light microscopy.

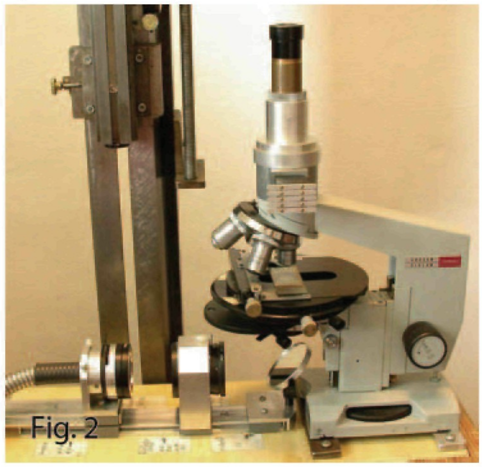
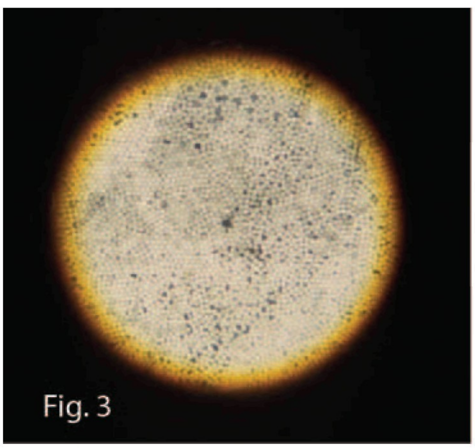


Fig. 2

Experimental Demonstration of Abbe Condenser Spherical Aberration with Explanatory Ray Diagrams

My first exposure to the problems caused by condenser spherical aberration were while attempting to demonstrate for John Delly the imaging of the interference figure from a Mylar film using my modified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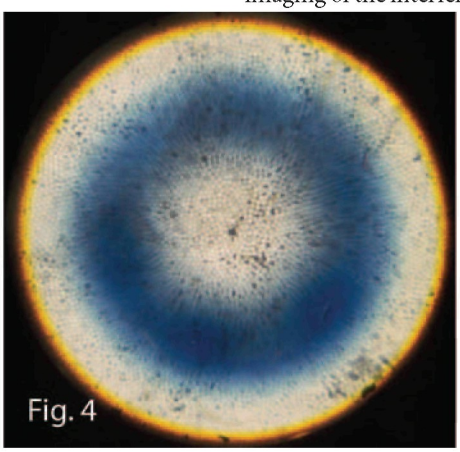
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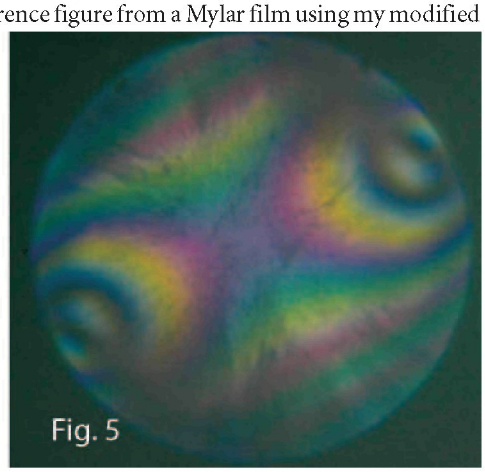
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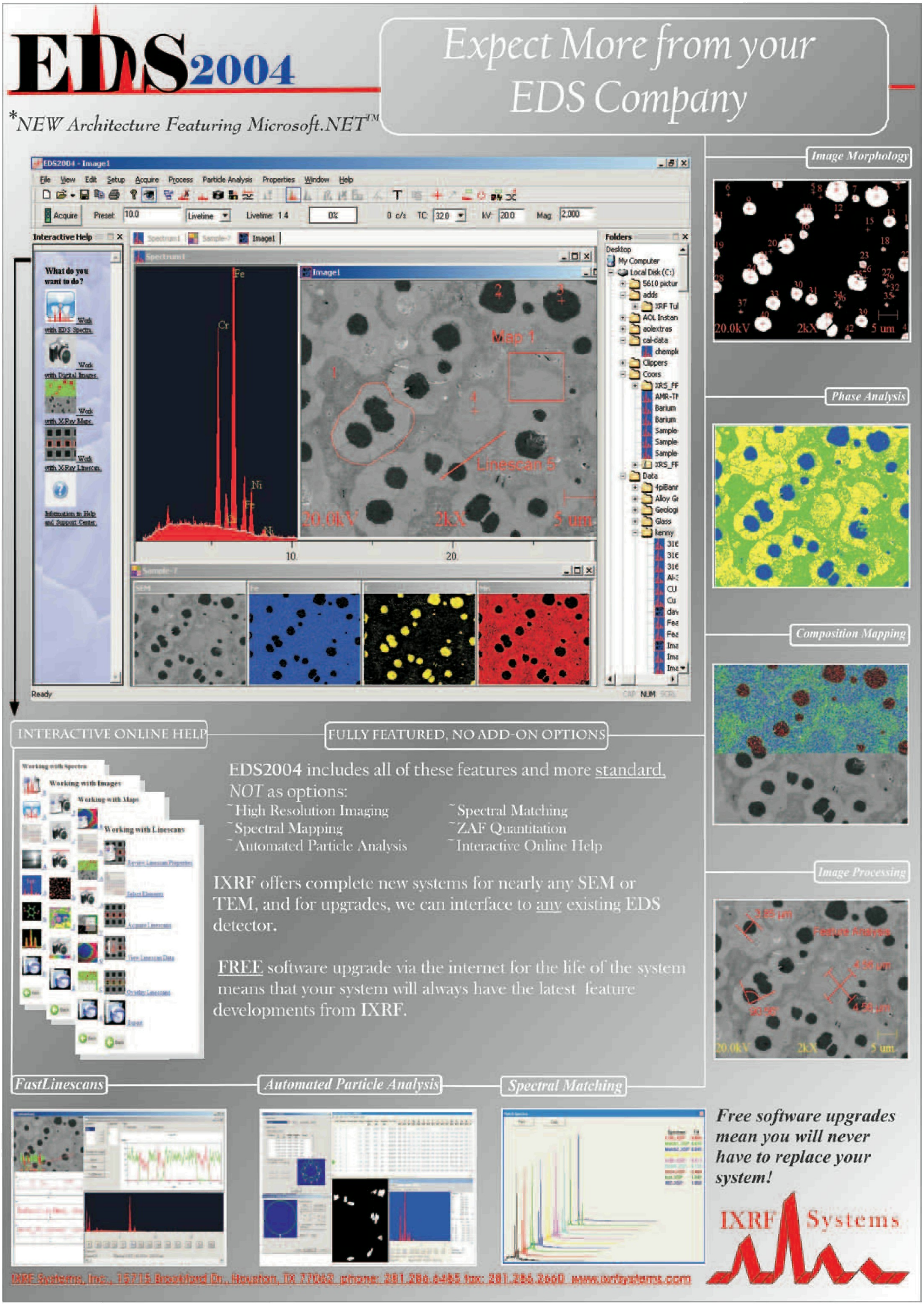
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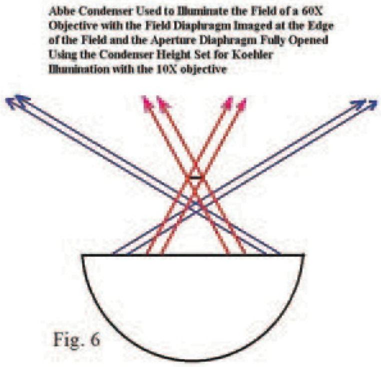
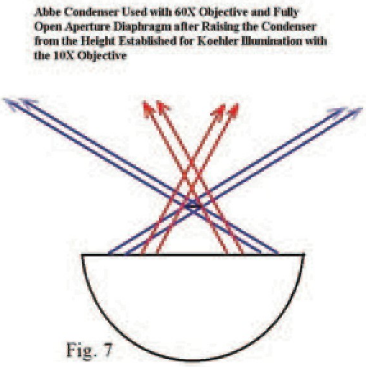


Fig. 6



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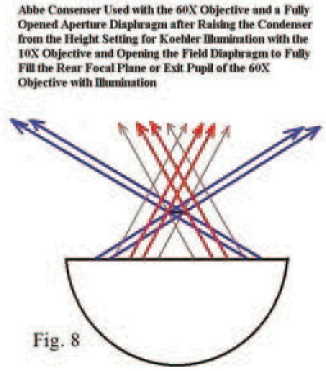

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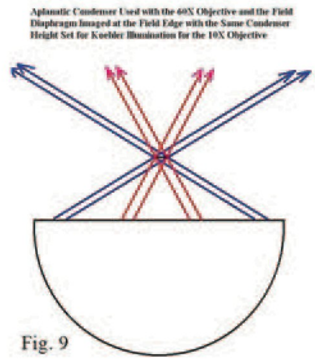


Fig. 9

Monolux microscope[3]. He showed how to fill the rear focal plane of the 60X 0.85 NA objective with much more of the figure by significantly raising the condenser from the position giving Koehler illumination with the 10X objective and then fully opening the field diaphragm. The resulting images were included in my student microscope article and are reproduced in this article along with ray diagrams that illustrate what is occurring.

I first set up proper Koehler illumination for the 10X objective using a tissue. I then swung in the 60X 0.85 NA objective and reduced the field diaphragm opening just outside the field of the 60X objective. I then replaced the tissue section with a thin film of polyester. John realized that the rear focal plane was not being fully filled with illumination, even when the aperture diaphragm was fully open shown in Figure 3. He suggested raising the condenser, which brought a ring of illumination at the outer edge of the rear focal plane along with an inner dark zone and a center bright spot shown in Figure 4. He then suggested fully opening the field diaphragm along with inserting the analyzer. This gave us an interference figure for the polyester film fully filling the rear focal plane or exit pupil of the objective as shown in Figure 5. The ray diagram in Figure 6 explains why opening the aperture diaphragm did not illuminate the outer portion of the rear focal plane of the 60X objective when the condenser height was previously established to give best Koehler illumination with the 10X objective. The high NA rays converged below the object focal plane because of uncorrected spherical aberration and diverged without passing through the field of the 60X objective. Figure 7 illustrates what happened when the condenser was raised. The intermediate NA rays now passed around the field of the objective and converged above. The axial rays still go through the object field. Figure 8 illustrates what happened

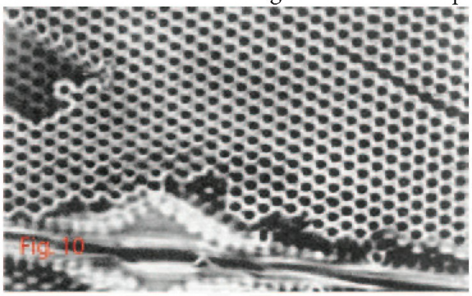


Fig. 10


Fig. 11

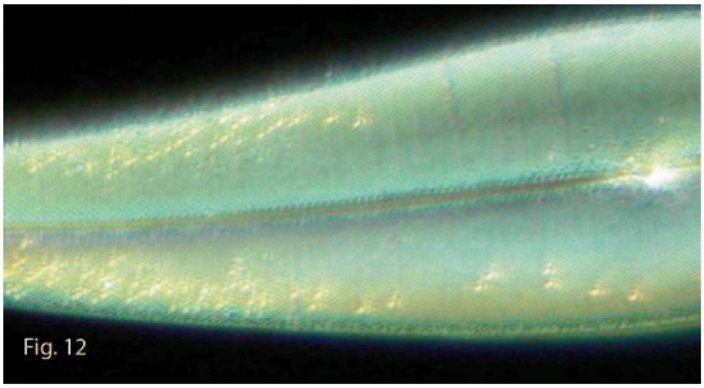


Fig. 12

when the field diaphragm was opened fully so that the intermediate NA rays could pass through the object focal plane along with the high and low NA rays. Figure 9 illustrates the situation for an aplanatic condenser without spherical aberration. Lonert indicated the same behavior for an Abbe condenser but provided no examples or ray diagrams[4]. I presume that the maximum illumination aperture without raising the condenser from the position for Koehler illumination with the 10X objective is what the late Edward P. Herlihy called the aplanatic aperture. He notes that this is the only portion of the illumination useful for microscopy and that the achromatic condenser is far superior. Herlihy lamented the almost universal use of the Abbe condenser, which has an aplanatic aperture far below its claimed aperture value[5]. Barry Ellam's article in The Amateur Diatomist notes that the Abbe condenser can be satisfactorily used with darkfield stops.[6] Barry mentioned that there are problems caused by the lack of correction for spherical aberration in brightfield. He also noted that these difficulties can be easily overcome by use of annular illumination (renamed COL by Paul James) long favored by diatomists for resolving the most difficult specimens, especially with a green filter. Raising the condenser and opening the field diaphragm is necessary for brightfield illumination above the aplanatic NA. The expected effect on image quality from a

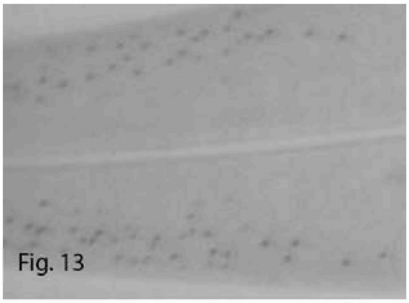
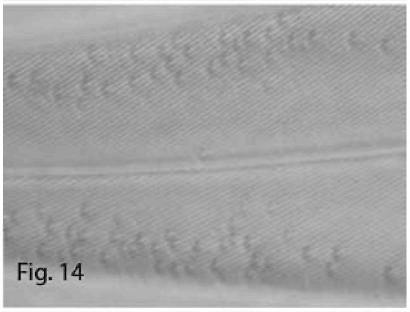


Fig. 13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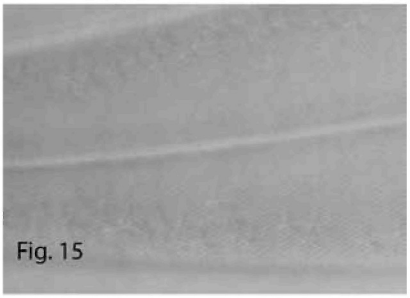
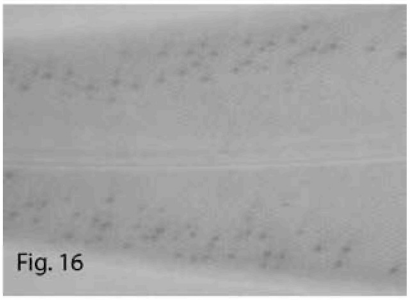


Fig. 15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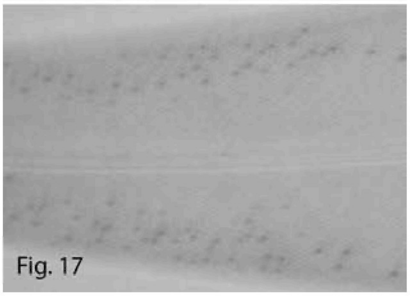
Fig. 17

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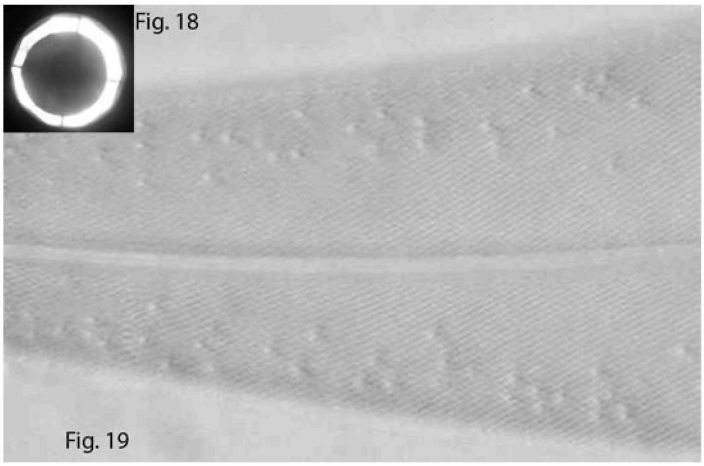

Fig. 18

Fig. 19

geometric optics analysis would be a loss of image contrast due to glare from illumination outside the object field. The precise interlocking light beam relationship of Koehler illumination would no longer be present. The wave optical treatment of image formation and resolution assumes that the object is illuminated with plane wave fronts from different NA values, as indicated by the parallel lines in Figure 9, which have the correct phase relationship with each other. This is not the case for illumination with a condenser having spherical aberration. The wave front shape (phase relationship) is altered by the spherical aberration. It would be expected that the problem of wave front distortion due to spherical aberration could be minimized by using a narrow, hollow cone of either darkfield or annular brightfield (COL) illumination. The illuminating wave fronts would then be approximately planer.

Imaging *Pleurosigma angulatum* with Darkfield Illumination

The diatom test slide has the diatom *Pleurosigma angulatum* which serves as a very good resolution test target for the 40X 0.65 NA objective because the stria spacing of about 0.52 micrometers matches the theoretical resolution of a 0.65 NA objective, when used with a matching illumination NA of just under 0.65. In his response to a letter by Robert B. McLaughlin, Dr. Walter McCrone republished a very high resolution optical photomicrograph of *Pleurosigma angulatum* taken in the early 20th Century by Spitta[7]. This image is shown in Figure 10. It was evidently taken with darkfield illumination, probably with blue light and perhaps with even shorter wavelength ultraviolet. The definition of "just resolved" means that the periodicity of the structure will be detectable but this fine structure will not be faithfully resolved. Figure 11 shows *Pleurosigma angulatum* recorded with the LOMO 40X objective and darkfield illumination from the prototype condenser. The condenser height had to be raised from the brightfield setting in order for the high NA rays to illuminate the specimen for darkfield. This same problem exists with the Olympus Abbe condenser used with my darkfield. The CoolPix® 995 digital camera lens zoom control was set so the diagonals of the recorded field covered the field seen with the 10X 18mm FN eyepiece and the image was subsequently cropped in Adobe PhotoShop®. The periodicity is recorded along with lines that initially were suspicious of being alias lines from the camera— sensor resolution being close to the optical resolution. The CoolPix® lens was zoomed to cover about half the field size of the eyepiece for the cropped portion of the field shown in Figure 12. The lines are still present and therefore not from digital camera aliasing because they are also evident on a close examination through the eyepiece. (Tony Havics of pH2, LLC previously tested my modified Biolam. Tony found that darkfield, with the stop at the fiber-optic light guide end with the aplanatic condenser and the same 40X objective, was capable of resolving the first three sets of lines of the HSE/NPL Test Slide. Resolving the three sets of lines is a requirement for counting asbestos fibers using phase contrast microscopy.)

Comparison of Results with Brightfield, Oblique and COL Illumination

My normal practice has been to align the components of the external fiber-optic illumination system using a 9X objective and establish good Koehler illumination using the aplanatic condenser with its aperture diaphragm left fully open. The aperture diaphragm at the light-guide end is used and the darkfield stops are also inserted at that location. I did the same with the prototype condenser and then opened the diaphragm fully at the light-guide end, and subsequently adjusted the diaphragm of the condenser for Koehler illumination. The 40X objective was then swung in on the turret and the field diaphragm imaged just outside the field of view of the eyepiece and the aperture diaphragm fully opened. Measurements of the image of the light source at the rear focal plane of the objective indicated that the illumination NA was about 75% of the objective's 0.65 NA. I had to raise the condenser in order to be able to fully fill the rear focal plane of the 40X objective with an image of the light source. The field diaphragm was then poorly imaged, as seen when the substage mirror was tilted slightly. I found that the stria pattern on *Pleurosigma angulatum* was not detectable until the illumination NA, set with the aperture diaphragm, almost matched that of the objective. This was the aperture setting also used for oblique and circular oblique lighting. The stria pattern in brightfield image had very low contrast making detection and focusing difficult. The CoolPix® was zoomed to record about half the field size and the resulting brightfield image for Figure 13 was cropped to show the same field as the cropped darkfield image in Figure 12. Oblique illumination from rotating the wheel to decenter the stop for the 40X objective gave much improved contrast as shown in Figure 14. The resolution is now directional, with only one set of parallel lines visible. There is now a camera lens artifact visible in this image as well as in the COL image. A concentric ring pattern is evident in these images and believed to result from residual tool marks in the mold subsequently replicated on the surface of one of the molded aspheric lens elements in the CoolPix®. This artifact will not be present when using digital microscopy systems from the major manufacturers of microscopes. The contrast of the COL image in Figure 15 is far superior to the brightfield image in Figure 13.

I decided to image the same field using the LOMO aplanatic condenser, both with the field diaphragm imaged just outside the field of view and fully open and the aperture diaphragm at the end of the light-guide source. I found that the image with the field diaphragm fully open, see Figure 16 had better contrast than the brightfield image in Figure 13 taken with the prototype condenser. The image taken with the field diaphragm just outside the field of view had the highest contrast as expected, see Figure 17. Next I decided to test the aplanatic condenser using COL. I did not have the right stop size to allow only a thin ring of illumination just below the NA of the objective, so I had to use a somewhat smaller stop. The resulting illumination at the rear focal plane is shown in Figure18. The contrast in this diatom image shown in Figure 19 is better than COL with the Abbe condenser even though a wider ring of illumination was used than with the Abbe condenser.

Summary

The prototype condenser demonstrates that there is an easy design solution that provides rapid switching among the various illumination modes. The imaging tests with *Pleurosigma angulatum* of the Abbe condenser versus the aplanatic condenser confirm Dr. McCrone's requirement that a good polarized light microscope (I believe he would also apply this requirement to the biological microscope) must have an aplanatic condenser. The results also agree with the recommendation of Mortimer Abramowitz that Abbe condensers are suited only for objectives of moderate aperture[9]. The aplanatic condenser also avoids the need to adjust condenser height for high NA brightfield or darkfield after Koehler illumination has been properly established for the 10X objective. I was tempted to use the LOMO aplanatic condenser lens for the prototype, but that would have prevented the study of the effects of spherical aberration with the LOMO Abbe condenser. LOMO has a separate single-element aspherical lens that interchanges in the same base as the high NA lens. This design should be as acceptable as having the top lens removable for use with a 4X objective, as I have done with the Abbe condenser. ■

Acknowledgement

I could not have done this study without the donation of the phase contrast condenser base by Chris Vander Tuuk of LOMO America. The historical background on Abbe condensers was provided by John Delly. Discussions with John and his reviews of the test results also contributed to this article.

Endnotes

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