**Circular Oblique Lighting (Part 1)**

An introduction to what may be considered to be a superior form of illumination to brightfield

By Paul James (uk)

A while back I wrote briefly about an interesting illumination setup involving the use of normal, non phase objectives illuminated with the cones of light from annuli in phase condensers etc. Having experimented further, I have come to the inevitable conclusion that for many subjects, and in particular protoplasmic cellular specimens it is superior in many ways to brightfield, and even standard phase contrast in some circumstances.

Technically speaking it is 360 degree Oblique Illumination OR Circular Oblique Lighting For brevity’s sake I shall refer to it as COL illumination.

**COL illumination.**

The essential ingredients are similar to standard phase contrast, in that the phase ring of the objective, and the na. of the ascending light cone from the annulus coincide precisely to elicit the phase contrast effects. The essential difference with COL illumination is that the objective is not a phase optic, although phase objectives can be used, providing its internal annulus is smaller than that issued from the condenser. Under these conditions a rather unusual effect is noticed, where the field’s illumination intensity can be somewhat diminished, and increased contrast is noticed with many specimens. It particularly emphasises contrast in fine detail, which is caused by partial phase shifts of light. The success of this phenomenon
seems to depend on the subtle relationship between the NA of the objective and ascending cone. It appears to work well with the higher NA objectives, though contrast improvements still occur with the low powers.

The four images below give some idea of the differences between BF and COL respectively. Note that the images are from the small central zone of the CCD, and are unsharpened. Noise spoils the otherwise smooth COL background.

Brightfield .......................................................... COL

Diatoms (mounted) x40 Fluorite with x100 phase annulus

COL illumination’s Salient Features

It seems to have all the advantages of phase and brightfield in one fell swoop, and conversely, seems to dispose of all the weaknesses of both as well. If that seems to augur well, then add to that the increase in perceived resolution, and the portrayal of special coloured effects, which might not in themselves be of true scientific value, but nevertheless help with recognition of the various species that are innately coloured.
Be aware however, that the phenomenon yields subtle variations depending directly on the diameter of the annulus, and the ideal setup appears to be when the na of the annulus is about 60%-90% of the objective's. I suspect no two observers with different optics will see identical fields and contrast etc.

Since the finer subtleties of this illumination are entirely lost in digicam photography, I have expressed through words its appealing properties when observing very small soil amoebae using a Wild x40 fluotar (operating at around na 0.70-.75 ), and x100 substage annulus on the Wild M20 stand:

"....... the views of small soil amoeba were delightful. The overall illumination intensity was just right for my eyes, and the entire field showed a faint straw yellow hue. The advantages of contrast in cellular detail were at once obvious, and then the resolution of their protoplasmic interior was impressive. The sub-particulate frenzy of protoplasmic material was clearly visible within the boundary of the ectoplasm as it flowed forth. Just as pleasing were the delicate refractile boundaries around the amoeba, which did not hinder in any way the view of this tiny animal, as standard phase can often do, but greatly assisted the recognition of its ever changing forms. Outside, the bacteria, and miniscule protoplasmic forms were rendered very sharply and with much contrast. Colours were noted, especially within the Amoeba where the nucleus seemed to be tinged with a definite green, and other forms of life, especially the single celled algae were brilliantly tinted with greens and blues above and beyond their naturally colourful selves compared to brightfield.

Not all subject matter responds so well with COL lighting, though it could be said that the improvement is just a little more subtle. In short, if the union of comparative na’s of both objective and annulus is ideal, the results are so appealing, both in technical and subjective ways, that it could easily become the illumination of choice. Put in another way, if COL lighting were the norm, and someone discovered brightfield, the latter would probably remain as an adjunct only.
Notes

I must emphasise the fact that initial trials may prove disappointing because this illumination works best on certain subjects far better than others. I find it ideal for pond life and related subjects, were brightfield tends to flood delicate structures etc. It is undoubtedly however, a useful illumination alternative to brightfield, and deserves serious consideration, especially experimentation regarding working conditions.

In the next article (Part 2) I shall cover annuli, condensers and also the very interesting variation of this COL lighting which uses a mixture of COL and DF.

Part II of the series.

Part III of the series.

Part IV of the series.

Unsharpened ccd image of Small Soil Amoeba x800.

(Wild x40 0.75 na fluorite plus x100 phase annulus)

Note high contrast and colour saturation in particulates of protoplasm and inside algal cells, and also modest phase refractile boundaries around periphery of the cell walls.

Visual appearance was superior to this image.

All comments welcomed to Paul James.

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http://www.microscopy-uk.org.uk/mag/artdec02/pjcol.html
It must be emphasized that there is no definitive COL set-up, since it already exists within the imagery of standard brightfield, but its presence is nullified or flooded out. The purpose of the annulus or stop is to mask the unwanted central light component from this brightfield image, so that the COL phase contrast imagery generated by the peripheral regions of the objective can be revealed.

An ANNULUS or STOP?

Strictly speaking an annulus allows a coherent ring of light to pass through the condenser to produce phase contrast or COL effects. My experiments have led me to circumvent the making of a traditional annulus, because it is highly desirable that the outer regions of the light cone should be variable. Therefore an annulus not having variable outer proportions is less versatile. An ordinary circular stop as used for darkfield, though smaller, will yield the COL phase reversal effects, and if placed near the iris diaphragm, the latter can be closed down to control the outer proportion of light input to the specimen. Thus the overall effects can be radically changed by simply altering the size of the iris diaphragm. In practice this works extremely well, providing that the iris is concentric with the stop. Complete concentricity in the microscope’s optics makes for better COL effects.

These initial trials are set out to introduce those who have no phase equipment, and have not experienced COL before, in a quick and uncomplicated manner.

Initial trials

Set up your ’scope for brightfield with the x40 objective using a slide of diatoms containing fine detail such as Actinoptychus heliopelta etc.. Optimise the condenser’s focus position and then open up the iris to maximum aperture. Now place a disc of opaque material such as a 5p coin, or one cut from silver foil of about 10-15 mm on the field lens of your microscope. Centre it as carefully as you can by making sure that the back lens of the objective shows a ring of peripheral light when viewed without the eyepiece in place (a Phase telescope makes this much easier to accomplish). Alter the size of the stop if necessary until you see this down the draw tube :-

http://www.microscopy-uk.org.uk/mag/artjan03/pjcol2.html
Then look at the diatom whilst altering the position of the condenser until you see a dark central zone, which will confirm the field stop’s centrality, if you are in doubt about that. Now rack up the condenser until the dark zone just disappears. You will, if the condenser is of the simple Abbe form, see some splendid colour backgrounds as you move the condenser about its usual position. This is perfectly normal in these circumstances, and the final position can be determined which provides the most pleasing effects regarding background and contrast etc..

**This initial view contains a proportion of COL and darkfield components, but the phase reversal contrast enhancing effects will still be seen.**

**COL of Diatom* (Actinoptychus heliopelta)**

Wild x40 Achromat using simple field lens stop. Colour profusion from Abbe condenser's peripheral aberrations helps differentiate detail of phase reversals. Adjusting of condenser dictates colour backcloth.
Optimising this simple set-up can be accomplished by adjusting the position of the condenser, and/or trying smaller or slightly large stops on the field lens.

If you cannot gain success so far, try another diatom slide, as the COL phenomenon is quite sensitive to the optical properties of the diatom and its mountant medium. If still no change, then it could well be that your objective has flaws of one kind or another around the edges of the elements such as delamination, or fungal growth.

NB......The images you will have seen like those above are not purely COL, because there is the darkfield component overlaying the COL. However I have mentioned this because there can be a distinct advantage in the perceived resolution and general presentation of the diatom etc.. By using an annulus which blocks the DF component, pure COL will result, which might suit certain subjects better, and may appeal to some observers.

The diagram below shows how this stop on the field lens works. Only one one side of the condenser’s output is shown of course. ES represents the light from the edge of the stop, and FB represents the light at the maximum field boundary, which in most microscopes with condensers of at least 0.9 NA will project light beyond the x 40 objective’s front element....This is the darkfield component mentioned above.
Optimizing COL for a favourite objective/specimen

Having experienced some success, and observing the benefits of a simple stop, it is likely that those who are impressed will want to ‘tweak’ the COL to maximum advantage. We will consider these in turn :-

1) The stop.

2) The position of the stop.

3) The suitability of the various forms of condenser.

The Stop

Thickish aluminium foil is the easiest material to cut into a disc for the stop, since its outer edge should be sharp and thin like the iris. For most observations the disc does not have to be perfectly circular, but this should be the case if possible because the iris can be used to greater effect when closed down to reduce the DF component. Blackening it is not essential but does reduce stray light from reflections.

Its diameter should be such that your objective’s back element displays a circle of peripheral light of thinnish proportions as shown above. It will probably, in most microscope’s, need to be a little narrower than you have used on the field lens, because it should ideally take its position near the iris diaphragm.

Siting the Stop

The ideal site for the stop is near the plane of the condenser iris, i.e., near the anterior focal plane of the condenser. The filter tray is good enough to start with, but suffers from the disadvantage that it should really be able to be centred at times. Stops placed in the spare apertures of phase condenser Zernicke discs work very well, provided the iris diaphragm is independent of this, which is not the case with some Zeiss phase condensers.
Above is shown the principal of a dedicated condenser for COL using a preferred objective, which has a stop attached to the underside of the condenser’s bottom element, with the iris controlling the outer DF portion of light. What really matters is that the stop is concentric with the condenser’s axis and iris. This is vital and the effort to put this into practice will pay dividends.

There are many ways of mounting stops near the iris in either a temporary or permanent way. This will ultimately depend on the observer’s needs regarding COL/DF and their imaginative DIY skills. Adapting an old iris/lens mounting from an obsolete camera etc. incorporating a stop, or series of removable stops to suit different objectives mounted just beneath the condenser will appeal to those requiring speedier illumination changes. A case could be made for the acquisition of a phase condenser substage unit for your ’scope, if fixed annuli will suit your needs.

Those who have a second microscope may adapt it for COL?

**The Condenser**

Fortunately, the Abbe or achromatic condenser are both well suited to COL up to and including x40 objectives. The achromat has the edge regarding the use of oil immersion objectives for it provides a more accurately coherent cone, as does the aplanat over the Abbe. However, the intrinsic chromatic aberrations of the Abbe can provide some advantage, in that by its careful focussing we can bathe the specimen in apple green light, the preferred colour for achromatic corrections, which I find elicits the best imagery regarding contrast enhancement and general viewing comfort.

**Tail Ends**

One very important issue regarding the comparison of brightfield and COL, is that the former is rarely used at full aperture, prejudicing maximum resolution from the objective because of iris closure requirements. COL can utilise the max NA of the objective every time.

COL works best with the higher NA objectives, but will work also with x10 and x20 too. Each objective will require a different diameter stop. If the iris is opened up to allow some DF component to reach the specimen, it will add a degree of highlighting too. Decentering the condenser can also add depth and make for a very interesting image:-
The variations of annulus/stops are endless, and the experimenter might consider the effects of 2 concentric annuli, one just inside the NA of the objective (COL) and one just outside (DF), thereby bypassing the peripheral edge of the objective’s elements where presumably some light might go astray etc. It’s a thought.

**Some Personal thoughts**

For me COL, and especially when in use with the iris controlling the DF component, is the preferred illumination set-up to brightfield for a number of specimen types. More experimentation is on the way for me, and I hope some readers will have had their appetites whetted accordingly.

What truly amazes me is that COL has been known in principal and practised by a few workers for over 50 years, yet the microscope manufacturers, as far as I know, have never incorporated its great advantages into the standard microscope? The cost would be trivial compared to phase contrast, but the benefits would be so appreciated by many observers. Pity.

All comments welcomed to Paul James.

Read **Part I** and **Part III** of the series.

Microscopy UK Front Page
A useful tiny lathe head/potter's wheel

Whilst it is quite possible to print an annulus using a printer and appropriate software, or use a mounter’s turntable, I chose a method which also has other uses, like this mini 'potter's wheel' which I made from an electric motor rescued from a defunct photo-copier. It's a DC motor unit, and when powered from my variable 0-6 volt AC microscope illumination transformer, through a series rectifier diode, will make this revolve at a useful 60 rpm upwards. A small platform/faceplate mounted on the spindle acts as a support for whatever needs to be rotated for painting or cutting etc..

In these images can be seen the process of painting the annulus, and also 'cutting' it out with a sharp knife. The clear sheet material was temporarily attached with 'Copydex' then revolved at about 750 rpm, which makes for sharper paint edges. The diameter of the ring and its thickness was lightly marked before black acrylic paint was applied whilst spinning, which results in accurate concentricity :-

http://www.microscopy-uk.org.uk/mag/artfeb03/pjcol3.html
This annulus was painted and cut in about 2 minutes. The paint boundaries can be precisely 'sized' and made perfectly circular using the corner of a piece of thin cardboard which is allowed to rub gently on the rotating sheet against the unhardened paint edge to be trued up or widened. The cutting left slightly rough edges, but was perfectly circular and is therefore of no importance. The 'Copydex' rubs/peels off the sheet with ease.

Pie casing aluminium foil can be temporarily stuck down with 'Copydex' before cutting in a similar manner, though some might prefer to just score the circle.
then cut with scissors after :-

Below we have an example of a painted annulus which separates the DF/COL zones so keeping any internal reflections from lens edges to a minimum. The DF component can, if required, be cut off cleanly by the field stop iris, or substage iris depending upon the annulus’s position.

**Final notes on position of stops/annuli and condensers**

I have found that the improvements of detail contrast etc. using COL can be brought into effect with a stop/annulus anywhere between the field lens and the condenser top lens! The variation of effects however is almost infinite, being compounded by the condenser type and its focal length and NA. Yet very colourful and striking effects can be created by just the field lens stop, and subtle condenser focussing :-

http://www.microscopy-uk.org.uk/mag/artfeb03/pjcol3.html
My favourite setup

After much experimentation and deliberation, I find the most interesting and versatile configurations for the Wild M20 are stops in the filter tray...ie 20 mm for the x 40 Fluotar (NA.0.75) and wider for the apochromat (NA.0.95). The variations of image lighting are impressive, since both substage iris and field iris can be altered to suit the subject observed, from pure COL to COL/DF and finally very delicate DF.

In one simple setup therefore, exists an almost endless series of lighting permutations subject to the settings of the substage iris, field iris, and condenser focus.

Memorable observations

In concluding this final article on COL I'd like to describe a few moments during four observing periods which were memorable to say the least. I include for each the relevant technical details.

1) Rotifer (Red Dot) Zeiss photomic 1.....using x 10 achromat objective with the internal x 100 phase annulus, which provides a unique balance of COL and DF............."The rotifer stretched out from the mucilage over a clear caramel background and was highlighted by the DF component, and of course the COL contrast improving effects. Its main body positively stood out from the background with immaculate detail, whilst the fanned myriads of highlighted particles issuing beside the 'wheels' sparkled in the DF component. The workings of its interior were abundantly clear and were displayed in crisp detail".

2) Ciliate....... Spec. as above......."A small unidentified solitary ciliate came across the field and tumbled very slowly through the COL/DF plane like a space satellite. Its cilia sparkled like the rotifer, but its features were transparently crystal clear......all the vacuoles and nucleus clearly mapped out in this magic image, as I slowly followed its descent with the fine focus and stage controls. Using the x2 Optovar position enabled me to see more detail even with this x 10
optic. I think this was certainly the most spectacular rendering of such a
protozoan I have ever seen, and one of course I shall always remember".

3) **Resting soil Amoeba + bacteria**....Wild M20 with Zeiss x 40 apochromat and x
16 Zeiss compensating eyepieces. 22 mm stop in filter tray......."The bed of the
slide was covered with an even layer of bacilli each clearly showing its nucleus and
body end tapering. This clarity was not apparent in phase contrast, nor
brightfield, or standard oblique. The detail of fine debris between the cells was
amazing. Scanning further I found a resting amoeba of about 70-80 microns
across. It reminded me of a textbook diagram, so clear were its internal
features".

4) **Strewn Diatoms on Moller slide**................Specs as above ........"This slide of
diatoms, which I have seen through various microscopes over the last 42 years,
appeared as never before. All the diatoms seemed to be made from porcelain,
with the highest contrast dotting and other minute detail I’d ever seen. I
noticed too that some improvements were brought about by off setting the stop
very slightly, which gave more solidarity and conviction to these beautiful images.
So captivated was I that half an hour passed quickly, and it felt that at last I
was seeing these beautiful skeletons in their full glory".

**Final miscellaneous thoughts**

**COL does not like thick mountant or water gaps between coverslip and**
**slide** in my experience so far with the **higher** power objectives, but seems to be
tolerated with the lower NA optics. The contrast inducing effects with these low
power lenses are also less intense, but they still elicit fine imagery of appropriate
subject matter when mixed with some DF.

**Collimation of all optical components is vitally important in the first**
**instant**, though offset stops can bring some benefits in certain situations.

**Condenser focus can be extremely critical in some setups**, and a subtle
change of position can not only alter colour tinges but more importantly reduce
resolution quite dramatically.

*Here are cropped examples all using x 40 objectives, and employing
various settings of condenser focus, both iris controls, and stop
positions.*
Conclusion

If you are a keen 'pond water' observer, and or enjoy the beautiful structures of diatoms, then COL must be taken seriously as a definite leap forward in image enhancement and subsequent observing pleasure. It can disappoint with unsuitable mountant or excessive water gap thickness in the higher powers, and poor alignment of optics, and objects which do not suit it.

However, it is the cheapest and simplest method of improving contrast of the finest detail that your objective can muster, and does this at maximum aperture every time. Such is the improvement of perceived detail with the best objectives, that an eyepiece of at least x15 is required to see this detail comfortably.

The original concept of COL, i.e. a ring of oblique light flooding the specimen, has been enhanced with the option of DF mixing, and remains for me a powerful and versatile illumination tool for a limited number of subjects. Within this observing range I feel that there is nothing to compare with COL/DF. For general routine observations brightfield and phase contrast are the general workhorses, but for the very best imagery, with moderate eye friendly brightness, and with speedily induced variations too......... COL/DF reigns supreme.
Read Part I and Part II and Part IV of the series.

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The improvement of an achromat's secondary chromatic aberrations

I have been constantly impressed with COL, and especially with the humble achromat: the performance of which seems to be particularly elevated in comparison to the more highly corrected fluorites and apochromats. The latter two do excel in COL's lighting setups and are still superior to the achromat, but not quite to the same relative degree as in BF.

I think the reason is that the secondary longitudinal chromatic aberration which is present in all achromats in varying degrees, is muted by the small bandwidth of light in the cross section of the annulus' ring:-

Whilst the simplified diagram above grossly exaggerates the longitudinal chromatic aberration of an achromatic objective, it illustrates the fundamental principle that the red light is dispersed further along the optical axis than the violet/blue light. Since a very narrow section of the objective is actually imaging the light from the specimen, the dispersion is limited to the outer region of the lens. No central light from the objective reaches this image plane and any chromatically dispersed colour which
would have added to the final image, is of course entirely absent.

In reality the image from a Tiyoda x60 achromat and that from a Wild x50 Fluorite, both of N.A.1.0 aperture were remarkably similar with the fluorite having the slightly finer image. In brightfield the difference between these two was most obvious, with higher than average axially dispersed colour from the achromat. Thus the achromat is well served in COL.

**The Abbe condenser's spherical aberration**

The fact that the Abbe condenser cannot provide an evenly illuminated field because of spherical aberration for the higher power objectives is illustrated below. Each of the two extremities of focus shown below, illustrate the appearance of the field in the back of the objective. It can be seen that there is no true focal point along the axis. Whilst this is undesirable for BF illumination, COL's effectiveness is unhindered because the Abbe can easily illuminate the narrow circular zone of an annulus as used in COL. All that is required is careful focussing to allow the annulus to be flooded with even light.
The condenser figured on the right would be required to be raised a little more to flood the annulus with light. It can be seen therefore that spherical aberration in the Abbe condenser is of no consequence when using COL.

**Centration of annulus/condenser**

The annulus and condenser centration to the optical axis can be performed without a phase telescope. Observation of the red and blue upper and lower cones from the Abbe condenser will allow precise centration within the field, albeit a little more slowly. Ideally these blue and red zones should coincide over the centre of the field, i.e. along the optical axis of the 'scope.
Sharpest imagery appears to be formed from the light which just hints on blue when the condenser is raised a little from the 'white field' norm. This is an area of illumination often called the 'transition zone' which ultimately leads to DF as the condenser is raised a little more.

by Ted Clarke, Scientific Photographer and Instrument Maker

Introduction

An earlier article on ModernMicroscopy.com, Machining a Darkfield Insert for the Olympus BH2 1.25 NA Condenser, 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. 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 where the darkfield stop is inserted at the end of the light-guide, which serves as the light source. This is not feasible for microscopes 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. This added capability includes the 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. 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 LOMO 1.25 NA Abbe condenser is able to achieve acceptable Koehler illumination with the 40X objective. McCrone Microscopes and Accessories donated a diatom test plate used to demonstrate the performance of the prototype condenser. Oblique illumination is obtained with this condenser by decentering the 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.

Prototype Condenser Mounted in the Modified Biolam Microscope

Figure 1 shows my modified Biolam microscope fitted with the prototype condenser shown in Figure 2. The one-piece LOMO bracket shown in Figure 3, with a slot for the annulus wheel and an upper threaded ring for the condenser lens, was replaced with a two-piece design shown in Figure 4. The aperture diaphragm in the lower part of the bracket is now centerable while viewing the objective rear focal plane before final tightening of the two socket-head cap screws. Note that a male thread needed to mount the LOMO 1.25 NA Abbe lens assembly has replaced the female thread in the one-piece bracket.

http://www.modernmicroscopy.com/main.asp?article=53&page=1

2008.11.21
The wheel, before the phase annuli were replaced with darkfield stops, is shown in Figure 5. (Close-up examination of the center of the wheel will show a partially completed detent spring needed to replace the spring accidentally broken in a failed attempt to disassemble the center pivot assembly.) All of the annuli were removed and the openings for them bored out to contain the darkfield stops shown mounted in the wheel in Figure 6. Observation of the rear focal planes of the objectives was of critical importance for determining the stop size just sufficient to give a good dark ground as well as to achieve proper centering of the stop using the centering screws shown in Figure 4. These operations were done using a 25X Klein loupe slipped over the eyepiece as shown in Figure 7. The short eyepoint of this loupe, fabricated from a 30 mm stereo microscope 25X eyepiece, does not permit digital image recording with my Nikon CoolPix® 995 camera. This image recording can be done with this microscope using its drawtube end-mounted 1X objective shown in my online article in Micscape³. That article also contains operating ray diagrams along with close-up views of the system components including the dovetailed attachment for the analyzer and polymer wave-plates, and the mating drawtube holder.
by Ted Clarke, Scientific Photographer and Instrument Maker

Imaging the Diatom Test Plate with the 4X Objective

Figure 8 shows the condenser in the lowered position with the top lens removed for use with the 4X Zeiss objective. (I now use a Zeiss 4X 160 mm tube length objective instead of the LOMO 4X objective because the Zeiss lens is corrected for use with a compensating Zeiss Kpl eyepiece found best for use with the higher power LOMO objectives.) Figure 9 shows the diatoms imaged in brightfield with the 4X objective with the field diaphragm adjusted so its image falls just within the 18 mm diameter intermediate image field size of the stop in the 10X Zeiss Kpl high eyepoint eyepiece. Figure 10 shows the same field imaged in darkfield after selecting and centering the stop in the wheel and opening the aperture diaphragm in the condenser. This low magnification is important for surveying the field before switching to a higher power objective.

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. This image is shown in Figure 11. 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 12 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 inserts and will be discussed in a subsequent paragraph. The CoolPix® lens zoom control was set so the diagonals of the recorded field covered the field seen with the 10X 18 mm 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 13. 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, as shown in Figure 14. Resolving the three sets of lines is a requirement for counting asbestos fibers using phase contrast microscopy.
by Ted Clarke, Scientific Photographer and Instrument Maker

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. I found that I had to raise the condenser from the height setting with the 10X objective 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 had very low contrast making detection and focusing difficult. The CoolPix® was zoomed to record about half the field size and the resulting images were cropped to show the same field as the darkfield image in Figure 13. In order to attempt to match the eyepiece image quality, this image and the other images of the diatom have not had their contrast enhanced digitally. The cross hatch pattern from the stria is shown for brightfield in Figure 15. The contrast is far inferior to the darkfield image in Figure 13. Oblique illumination from rotating the wheel to decenter the stop for the 40X objective gave much improved contrast as shown in Figure 16. 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 17 is far superior to the brightfield image in Figure 15. The stop for the COL images is the same stop used for the 4X objective, which is somewhat smaller than the stop for the 40X objective as seen in Figure 6.
Figure 15

click image to enlarge (193K)

Figure 16

click image to enlarge (193K)
Role of Condenser Spherical Aberration on Image Quality

The need to raise the condenser from the height setting set for Koehler illumination with the 9X objective for fully filling the rear focal plane of the 40X objective and an additional adjustment upward needed for darkfield illumination is clear evidence of spherical aberration. 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 diaphragm fully open, see Figure 18, had better contrast than the brightfield image in Figure 15 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 19. These results stress the importance of the condenser being corrected for spherical aberration (aplanatic). The wave optical treatment of image formation and resolution assumes that the object be illuminated with spherical or plane wave fronts. This is not the case for illumination with spherical aberration. The wave front phase relationship for proper destructive and constructive interference to form the image is altered by the spherical aberration.
The late Edward P. Herlihy, who was a fellow of the Royal Microscopical Society and Vice-President of the Quekett Microscopical Club, lamented the almost universal use of the Abbe condenser, which has an aplanatic aperture far below its claimed aperture value. Herlihy notes that only the aplanatic aperture is useful for microscopy and that the achromatic condenser is far superior. Dr. Walter McCrone has stated in his requirements for a good polarized light microscope that the condenser be aplanatic. Barry Ellam’s article in The Amateur Diatomist notes that the Abbe condenser can be satisfactorily used with darkfield stops. Barry notes that there are problems caused by the lack of correction for spherical aberration in brightfield. He notes 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.

Effects of Condenser Spherical Aberration at Rear Focal Plane of a High NA Objective

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 Monolux microscope. 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 Koenig 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 recently done for this article that indicate what is occurring.

I first set up proper Koenig 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 20. 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 21. 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 22. The ray diagram in Figure 23 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 24 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 25 illustrates what happened 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 26 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. 

Abbe condenser used to illuminate the field of a 60X objective with the field diaphragm imaged at the edge of the field and the aperture diaphragm fully opened using the condenser height set for Koehler illumination with the 10X objective.

Figure 23
Abbe condenser used with 60X objective and fully open aperture diaphragm after raising the condenser from the height established for Koehler illumination with the 10X objective.
Abbe condenser used with the 60X objective and a fully opened aperture diaphragm after raising the condenser from the height setting for Koehler illumination with the 10X objective and opening the field diaphragm to fully fill the rear focal plane or exit pupil of the 60X objective with illumination.

Figure 25
Aplanatic condenser used with the 60X objective and the field diaphragm imaged at the field edge with the same condenser height set for Koehler illumination for the 10X objective.

Figure 26
by Ted Clarke, Scientific Photographer and Instrument Maker

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. This requirement 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.

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References