CIRCULAR LED LIGHT RINGS FOR OBLIQUE, CIRCULAR OBLIQUE AND RHEINBERG ILLUMINATION.

ROBERT HILL.

Introduction.

In the February 2018 edition of Micscape I described my experience of imaging diatoms with a circular LED light ring without a condenser. This article was stimulated by Webb's paper on the use of circular LED light rings for phase contrast on an inverted microscope, and also David Walker's article describing his experience with his Zeiss Photomicroscope. (2008). David Walker described the use of the ring LED for Phase Contrast, Circular Oblique lighting, (COL), Darkfield, Colour Phase and Rheinberg illumination. The many applications demonstrates the versatility of the concept.

I have continued to intermittently experiment with this technique and describe how these lights can be mounted on a Leitz Ortholux microscope in an easier manner than previously described and make some comment on the different types of LED rings now available. There are also similar techniques such as that described by Moustafa et al (2023) in which a controllable array of LED lights is used. This paper describes the use of LED rings for oblique and COL illumination of diatoms and also the use of multicoloured rings.

Oblique illumination.

There are many papers and articles describing or perhaps reviving this relatively simple technique which is particularly useful in the examination of transparent or semi transparent specimens such as diatoms.

Most commonly, and most easily, oblique illumination can be achieved by inserting a filter stop in the filter tray found under most condensers. The filter is often a circular piece of black card with an appropriate slit or crescent cut out of the card. There are some issues with this such as determining the best shape and size of the slit/crescent for any given objective, but also such filters are difficult to adjust during observation. The direction of the oblique illumination is important when looking at diatoms and for example horizontal stria will be better defined with the illumination from a different direction than that optimal for vertical stria. Therefore, adjustability of the illumination during observation, without disturbing the specimen, is useful. One may wish to stack images with illumination from different azimuths. Obviously, this consideration does not apply for circular oblique illumination. As noted in my previous article a phase telescope is invaluable in assessing the nature of illumination being achieved. There are a number of papers in the literature discussing the optimum configuration of the slit.

Apart from the filter stop there are other methods of achieving both COL and Oblique Illumination – essentially achieving a peripheral circle of light or segment thereof in the rear focal plane of the objective. These include:-

- Using a phase contrast condenser.
- Using an offset phase contrast condenser by incompletely rotating the phase plate (Piper)
- Using a Heine Condenser
- Using a Darkfield Condenser

All of these techniques have some disadvantages, particularly for oblique illumination, such as size of the segment of the circle of light and lack of adjustability. Generally speaking for COL the circle of light needs to be as peripheral as possible matching the aperture of the objective. It also has to be of sufficient width and one of the issues with the Heine condenser, in my experience, is that the circle of light can be too narrow. It is relatively easy with these techniques to explore the interesting illumination effects seen at the junction between dark field and COL. This is especially relevant to the use of multi coloured LEDs below described below.

As an aside there is also the question of where the filter stop, be it card or a phase ring, is best placed in the light train. It is usually stated that the filter should be as close as possible to the aperture diaphragm but it will be apparent that the "filter" in a phase contrast condenser is at a different place to a standard condenser with its filter tray. Rolf Vossen achieved very good COL, and other effects, with the filter stop of two black circles on stuck opposite on the field lens of a simple Abbe condenser. During my experiments it became clear that the best oblique illumination is achieved when the crescent is sharply defined with as little as possible diffusion/spillage/leakage of light into the black area. This is best assessed by the phase telescope and may depend on the location of the stop. Diffusion is a particular problem with the simple black card stop but can be ameliorated a little by using polarised light.

LED Light rings.

There are now many different types of rings available that vary in diameter and LED light colour as well as in the exact nature of the LED. Some examples are shown below. (Fig 1). Since resolution is better with blue light, compared to white, there is some attraction to using a blue led light ring but this is hard on the eye and images difficult to focus unless focussing is done via a camera screen. The "ice white" or "cool white" may be a good compromise for observation. Its user's choice now as so many colours are available and if a camera is being used then there is less disadvantage to blue but I set the camera to monochrome. As noted by many authors care should be taken when looking at LEDs down a microscope to avoid eye damage – particularly if one is tempted towards the UV end of the spectrum. Reduce the brightness to a minimum during set up and then use a camera is the best advice.

One interesting LED light ring now available allows the colours to be changed using a remote control. With this sort of LED ring it is easy to experiment with different colours and having focussed with white light then the colour can be changed to a variety of other colours including blue. It will be found that refocussing is required with different colours. The LED light ring has three coloured LEDs at each position, red, blue and green and the red is more peripheral. This actually allows Rheinberg illumination with extremely careful positioning of the ring and a variety of darkfield illumination.

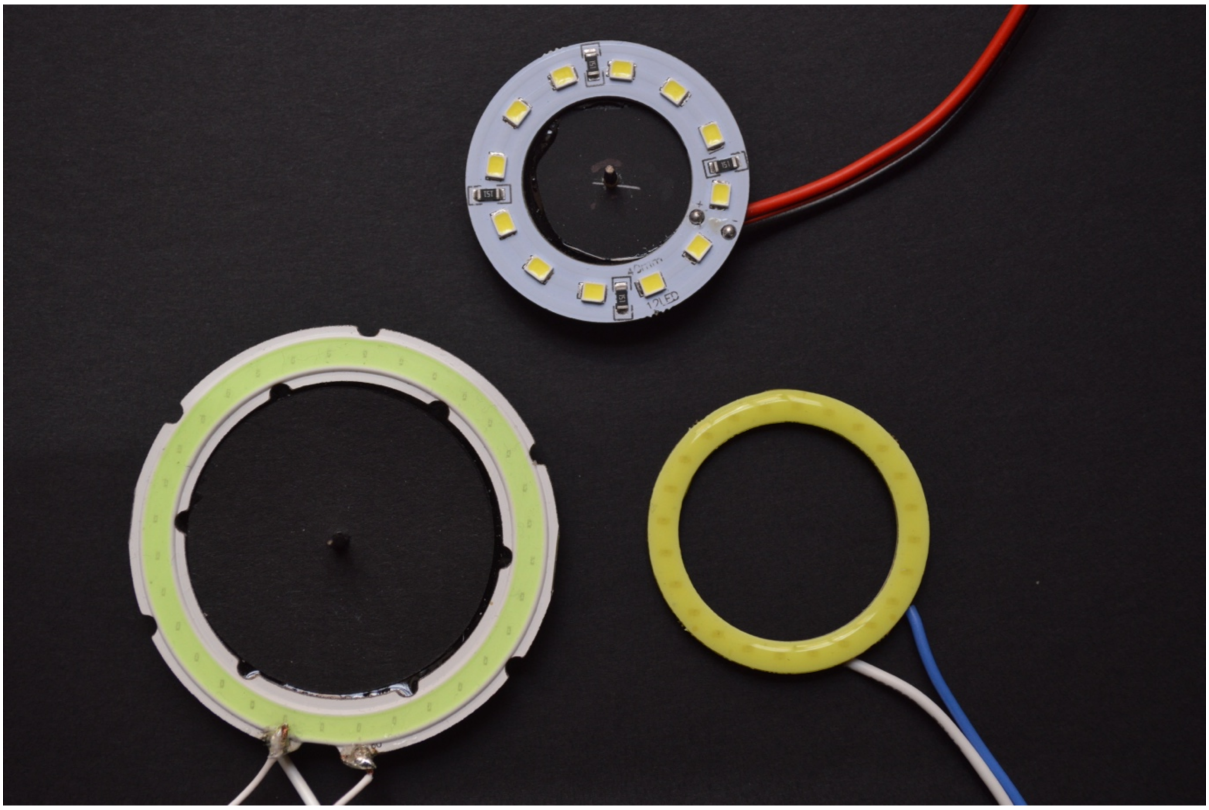


Figure 1. Some available types of LED ring mounted as per text.

Figure 2. Controllable multi coloured LED rings mounted as per text.

The practicalities of mounting.

After a number of "experiments" I have now settled on a magnetic mount on the condenser carrier for the LED ring. It is more or less essential to use a microscope on which the condenser carrier can be independently moved up and down and best to choose a condensor carrier with sufficient travel. As described in David Walker's article this is how one can move from Darkfield to COL and Oblique and is necessary for the maximum use of the multicoloured LED ring of which more later.

The Leitz Ortholux or at least the later versions, and many others since, uses a sliding dovetail condenser mount. A platform has to be made from a suitable thickness piece of wood with the edges chamfered to fit into the mount. An adhesive magnetic sheet is attached (Fig 3a). With some ingenuity something similar could be made for those microscopes with circular mounts.

The LED ring itself is glued to an acrylic disc of appropriate size – taking care of the soldered wires. To the acrylic circle a ferromagnetic shim is attached – again with two part acrylic cement (Fig 3a). The acrylic disc may have a shiny surface in which case it may be best to cover it with matt black card.

To permit adjustable oblique illumination a circle of black card is cut with a circle cutter and a segment removed from the periphery (fig 3b) A small hole is drilled exactly as possible in the centre of the acrylic circle just big enough to insert the end of a cocktail stick. This can then be coloured black with a marking pen. The black card circle is then place over the cocktail stick as shown in Figures 3b and 4. The hole from the circle cutter is obviously exactly central and can be enlarged a little if necessary. The card with its oblique slit can easily be rotated round to change the direction of oblique illumination even during observation if one is careful. As a refinement, if desired a circle of polarising film can also be cut and placed beneath the card. If the microscope is equipped with a polarising analyser then diatoms can then be observed between crossed polarisers.

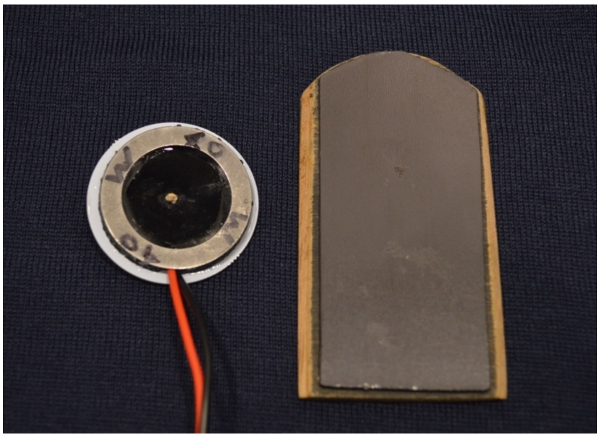


Figure 3a. Mounting of LED ring.

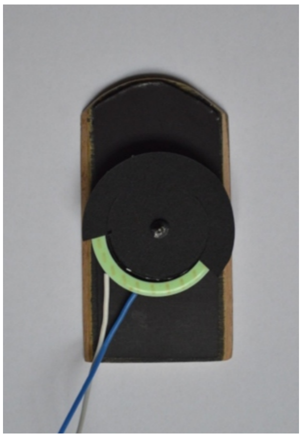


Figure 3b. With oblique stop

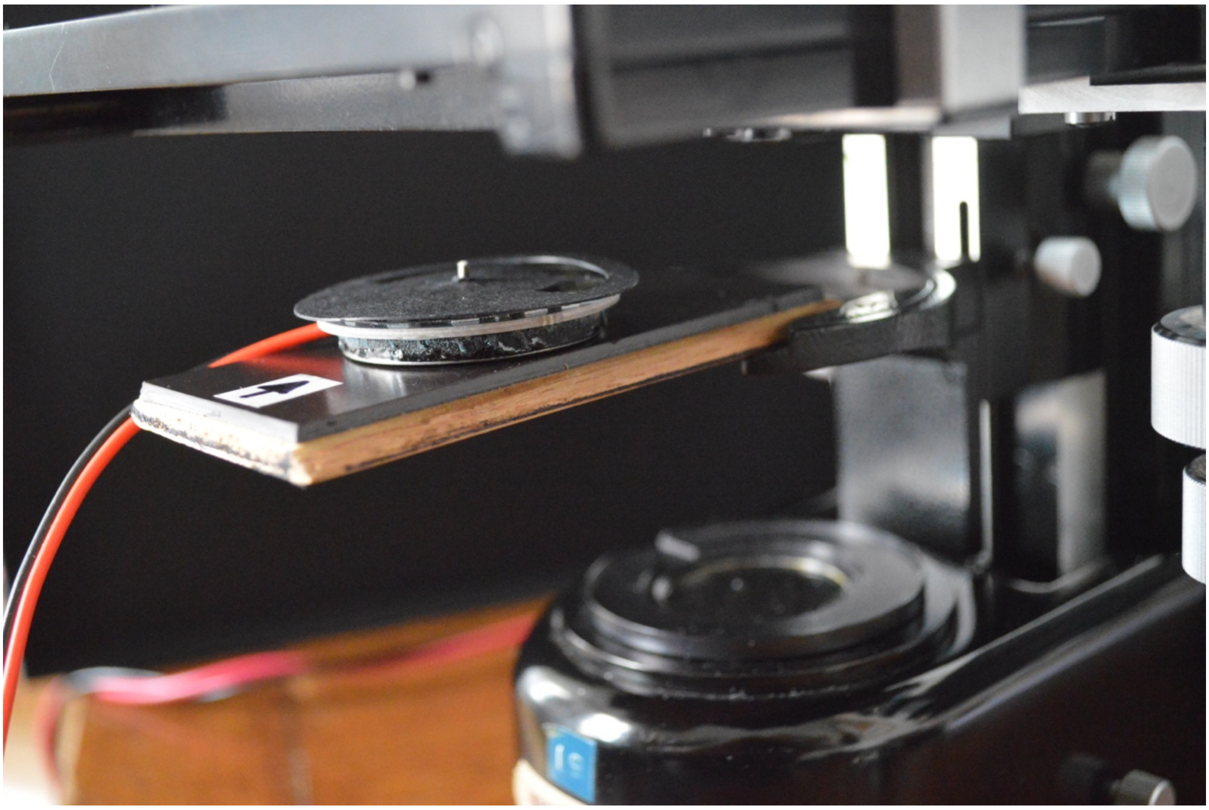


Figure 4. Insertion on the microscope.

The size of the LED ring.

This depends on the objective being used and the desire to move up and down from darkfield across into brightfield. Some interesting effects can be achieved at the boundary zone. As a guide a 40mm diameter LED will do well for x 25 and just about OK for x 40. Higher magnifications may need a different size ring. Experiment. It may depend on your microscope.

Adjusting the LED ring.

This is straightforward. Just slide it on magnetic mount and under observation with a phase telescope centre the ring by moving with the fingers. Raise the condenser mount until the LED light ring is peripheral as seen through the phase telescope. It is a mistake to use strong magnets as it will be difficult to move. The technique described above offers more than adequate stability. The oblique card filter can be rotated more easily during observation with a pair of forceps or tweezers to change the direction of the oblique illumination to get the best image(s). If COL is desired then simply remove the filter it or have another LED ring without the filter.

Light Rings.

There are several types available on the internet mainly intended to customise your car lights. (Fig 1). Like David Walker I run these from an adjustable DC bench top power supply. There may be an issue for white light if the LEDs are embedded in a coloured matrix – usually yellow. This is usual for COB LEDs and if these are used for conventional illumination then one may see pinpoints of white light in a yellow background. i.e. the illumination is not homogeneous. This can be ameliorated by using a diffuser and by running at higher power when the effect is much less. Interestingly for the purpose of COL and oblique this seems to be less of a problem, nor does it matter that the ring or light is made up by discrete LEDs. The LEDs are mounted flat – that is they are not inclined inwards towards the specimen. This will mean, depending on the beam angle, that the illumination decreases as the ring moves more peripherally in the aperture of the objective (as seen down phase telescope when mounting). As noted above it is the peripheral position that is best. All that I can say is that in practice it doesn't seem to make too much difference – possibly because the LEDs are so bright. There is some discussion on this in Webb's paper.

Information about performance is usually missing but they are all more than bright enough.

1 This is the type described used by David Walker. Separate distinct LEDS are mounted on a ring. This type does not suffer the yellow background problem.

2 This type has the LEDs embedded in a yellow circular matrix. There are several colours, information about wavelength and brightness is a little scanty but cool white is 6000K

3 This type is similar to 2 but the LEDS are closer together and a different configuration to 2. The matrix in which the LEDS is embedded varies in colour depending on colour of LED chosen. Conveniently prewired. Brightness described as 3000Lm

4 A controllable multicoloured, (MC), LED ring offering 15 colours including white. Each "LED" consists of three emitters – blue, red and green. The red emitter is mounted just slightly more peripherally than the others which is important when exploring the effects achievable. For white light all three emitters function but with careful adjustment one can get a blue Rheinberg type background effect and subtle red highlighting. (See figures 11 - 14). It is easy to take pictures of the diatom in a variety of colours with or without changing the focus to produce other effects with image stacking. For a similar but less versatile technique see Paul Martin 2014

Study equipment.

<u>Microscope:</u>
Black enamel Leitz Ortholux. Objectives used in this study Pl x 25 NA 0.5 & PL Fluotar x40 NA 0.7
<u>Camera and mount:</u>
Sony Nex 7 with electronic front shutter activated, 24.3 megapixels. ISO 100 or 200. Centred weighted metering. Mounted to photo tube with Pentax microscope attachment with extension tubes and to camera with Sony E mount to 42mm adapter. Olympus photo eyepiece NFK 3.3. (What is available to me)
<u>Photographs:</u>
Saved as JPEG and RAW but for purpose of this article JPEGs used. Digital enhancement by Apple Photo to improve contrast, definition etc.
<u>Diatom slides:</u>
Most of the images are of Pleurosigma angulatum from a Klaus Kemp 8 form test slide described as a versatile "demonstration" subject by David Walker (2008). In his study the punctae were resolved with a Zeiss x25 NA 0.6 brightfield green light slight oblique, better still with an interference filter of 400nm i.e. blue light. The best picture in his article is using a Zeiss x40 NA1 apo with oblique – I assume with green light from the picture. This is a useful article for comparison purposes but these results from a Zeiss Photomicroscope III would have entailed the use of a quality condenser. In addition, imaging of other diatoms were used in this study as described below

Aims of Study.

The aim was to ascertain the quality of the images of diatoms that could be obtained with LED rings used without a condenser, in particular using the LED rings to obtain oblique and circular oblique illumination as judged by the use of a phase telescope to image the rear focal plane of the objective.

In addition, the use of a multi coloured LED ring was explored.

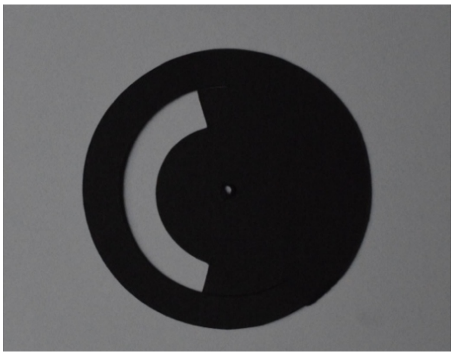
For comparison purposes diatoms were also imaged with conventional, albeit still LED, lighting using a Berek condenser with an NA of 0.95 with a black card crescent oblique stop inserted into the filter holder available for this condenser, same as Heine. Figure 7.

Results and Discussion.

The aim of a "good image" is subjective and for various reasons the photographic image may not equal what is seen down the eyepiece. Resolution although extremely important is not the sole determinant of a "good image". It is worth noting that this technique like many others does seem to be influenced by the characteristics of the objective being used, the mounting of the diatom and the diatom itself. All this is a matter of personal opinion and experimentation depending on the equipment available. I can only give my opinion; for example there is the issue of balancing depth of field against resolution and magnification. The issue of shallow depth of field with high NA (and magnification) objectives can be addressed by image stacking but my personal view is that this can sometimes give rise to rather flat images and there is the black hole/white hole issue as the focus changes. Anyone who has micromanipulated a diatom or looked at scanning electron micrographs will appreciate their three-dimensional nature and this is not well defined in stacked images even with 3 D reconstructions using software, (Hill).

In this study most of the images were obtained using a black Leitz Ortholux microscope but the techniques is applicable to any microscope to which one can fit the magnetic mount in place of the condenser. The Leitz x 25 objectives, (both 160 and 170 tube length), are in my opinion, very good objectives particularly if the Fluor or Apo versions are available. The latter have higher NA – up to 0.65. Combined these with a magnification changer or an appropriate projection lens for afocal photography can result in images with reasonable resolution with a good depth of field. Again, it is a matter of personal preference given that the aim of most of us is to get a good quality image. (Vossen). Good images were also obtained with a x 40 objective and experiments were carried out with a x 50 both dry and water immersion objectives. As is well known the shorter the wavelength of the light the better the resolution which makes the use of blue light attractive and this has become more practical with the controllable multicoloured LED light ring combined with a camera. Using higher magnification objectives than x 40 or x 50 does not work so well.

<u>Oblique illumination.</u>



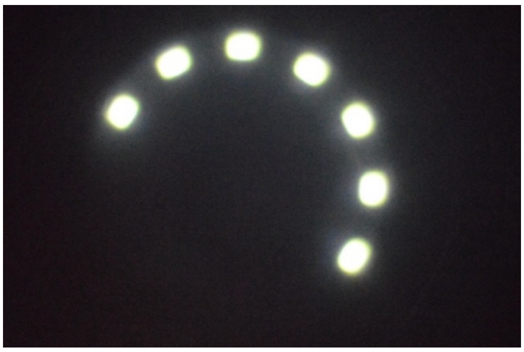


Figure 5. a. A type of oblique stop and b. what is seen down phase telescope when aligned.

Circular oblique illumination

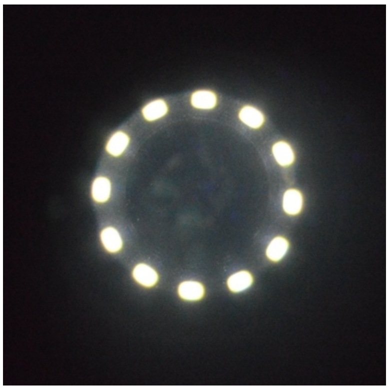


Figure 6. View down phase telescope. If LEDs are positioned further laterally then this will become dark field.

The images below illustrate oblique and COL images with this technique compared to a conventional light source with a Berek Condenser. There are also images of the interface between dark field and circular oblique illumination and special effects obtained with the multicoloured LED ring.

Images.

Any coloured images converted to black and white in camera. Images are not stacked. An attempt was made to focus on exactly same part of diatom but there are some minor differences and possible the colour of the light is relevant to appearances obtained. Figures 7 and 8 are oblique with the crescent of light approximately 30% of the arc of a circle centred at the 5 o'clock position. Objective is x 40 NA 0.70. To keep the images as comparable as possible no image enhancement has been done – they are as came out of camera except for cropping, adjusting exposure and enlarging. The images might be "improved" further by digital enhancement but this might bias comparison.

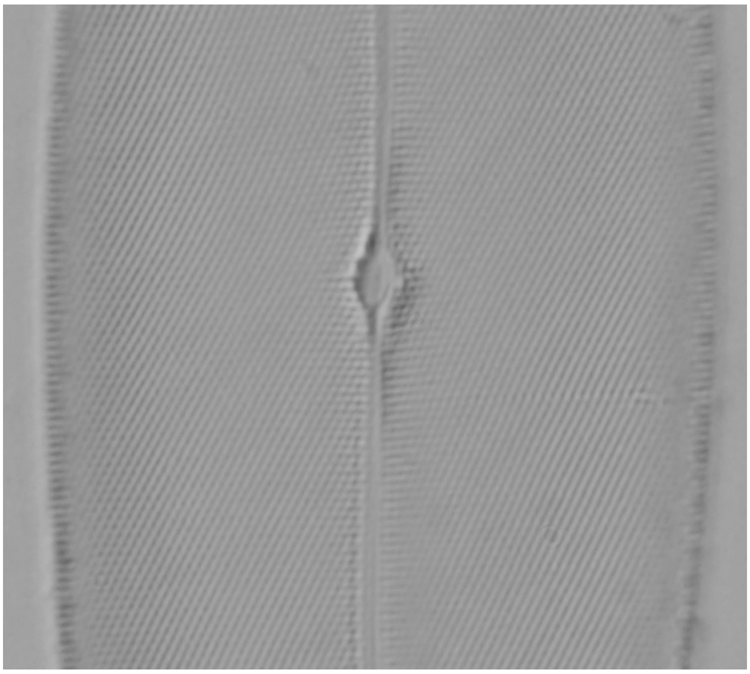


Figure 7. Berek Condenser Oblique. White COB LED with diffuser.

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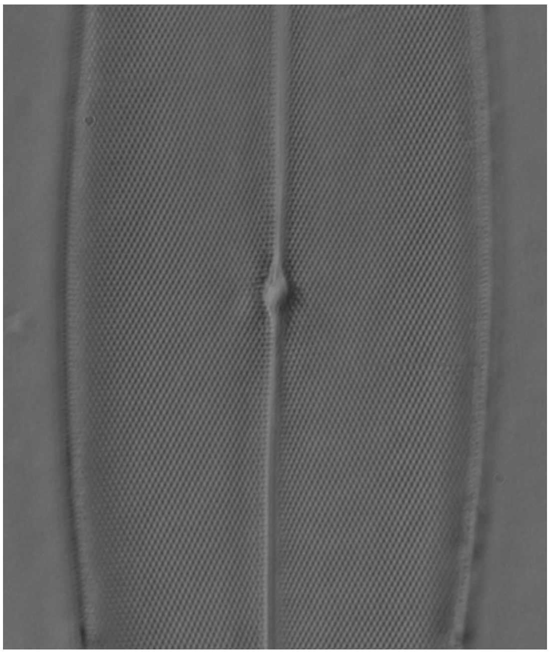
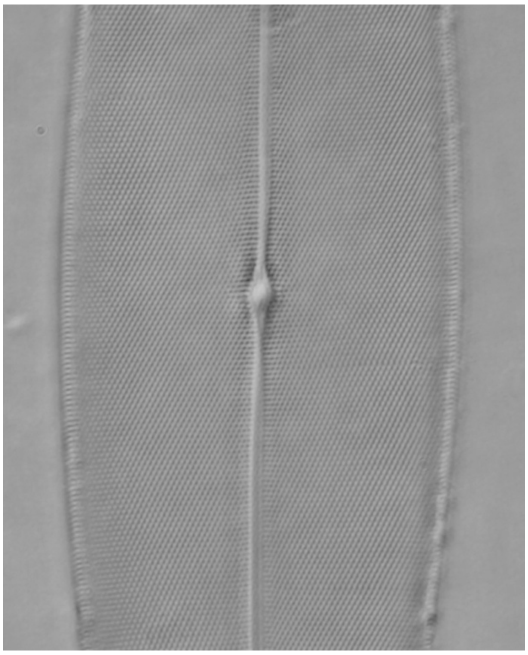
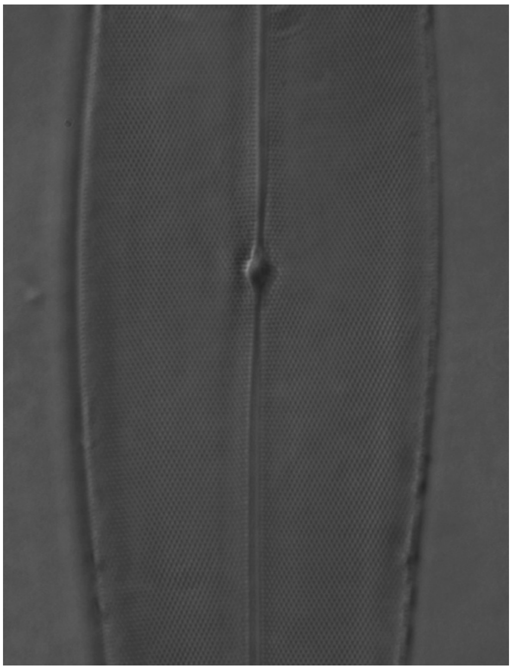
Figures 8a – e. Images with LED Ring with oblique card stop. No Condenser. (MC = multicoloured ring – see Figure 2)

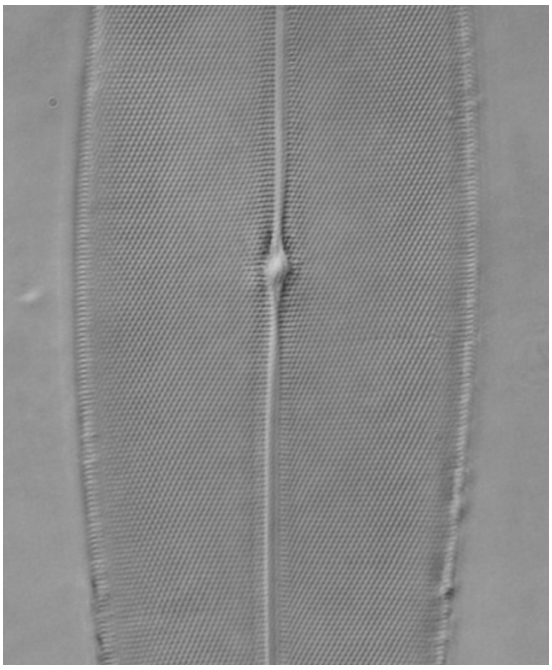




a. Ice Blue LED ring oblique

b. MC LED Ring white Oblique

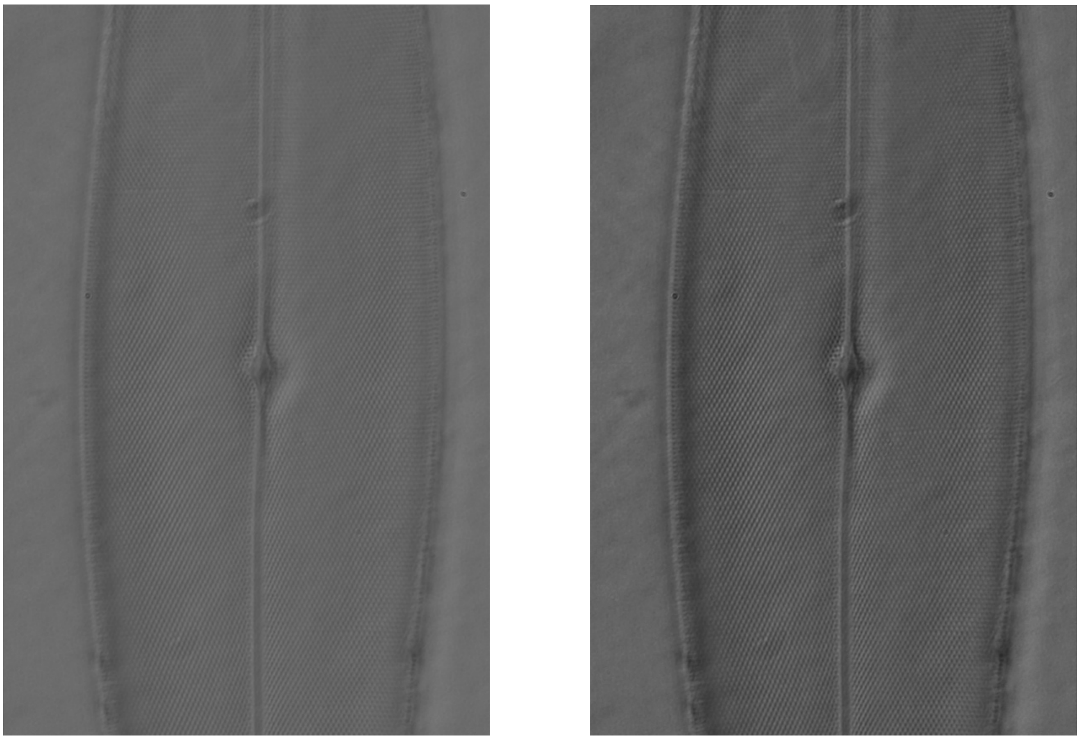




c. MC LED Ring Blue Oblique d. MC LED Ring Green Oblique

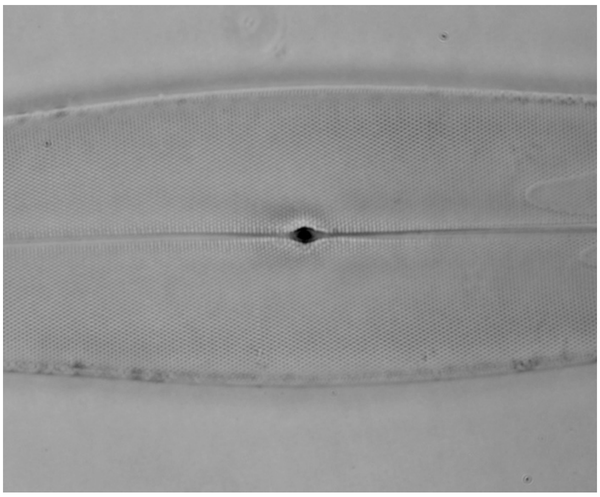
The oblique views with the LED rings, in my opinion, are equivalent to the oblique view obtained with a Berek Condenser. Ice Blue and Green seem to be best. An attempt was made to focus on exactly the same area of diatom but there may be slight differences. Blue is more difficult to see without enhancement but overall in this part of the study I found the best images were obtained with the LED ring marketed as ice blue.

I have also used x 50 objectives both dry and water immersion with this technique and whilst reasonable images can be obtained I found the quality started to deteriorate. Quite a lot of digital enhancement is required to maximise image quality and I don't think it compares well to the x 40.



e.(i) Ice blue x 50 water immersion objective NA 1.0. (ii) Digitally enhanced.

Circular Oblique Lighting (COL).



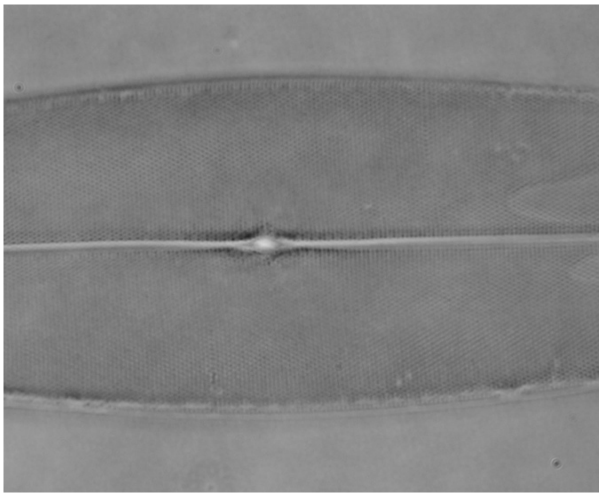
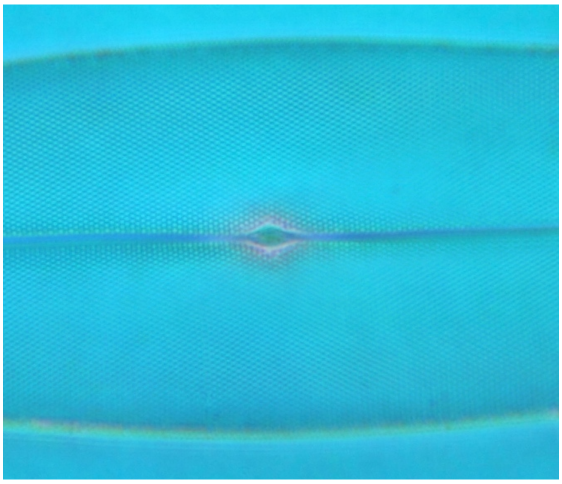


Figure 9. Heine top lens removed for COL   Figure 10.   MC White COL

With circular oblique lighting although the circular LED ring can give a reasonable result the Heine lens seemed better. (Figure 9). Possibly this is because the Heine gives a bright ring with a black centre whereas with the LED there is some light “leakage” – from diffraction into central area. See Figure 6. Avoiding this seems to be an important factor in improving image. Some conventional techniques such as using a phase contrast condenser may offer better results than the LED COL method. The diffraction seems to be lessened if a polarising filter is used.



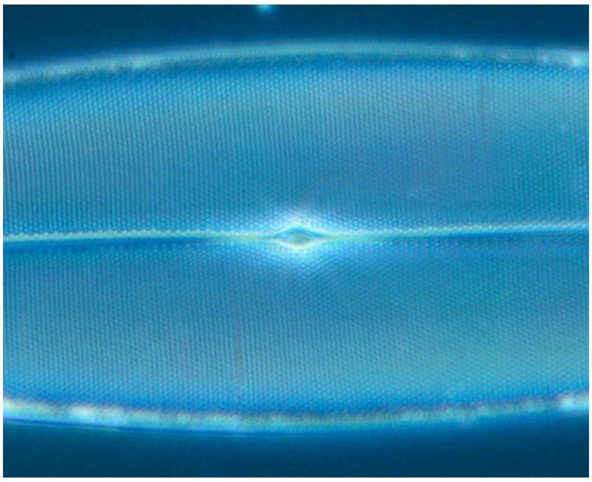


Figure 11a. MC LED Rheinberg effect

Figure 11b. MC LED Dark Field effect

The LED ring technique makes Dark field and Rheinberg very simple to obtain. All that is required is to adjust height of condenser mount to position the LED light rings on the margin of the rear focal plane of the objective as seen down a phase telescope. It is possible to get subtle effects. See comments on the multicoloured LED ring above.

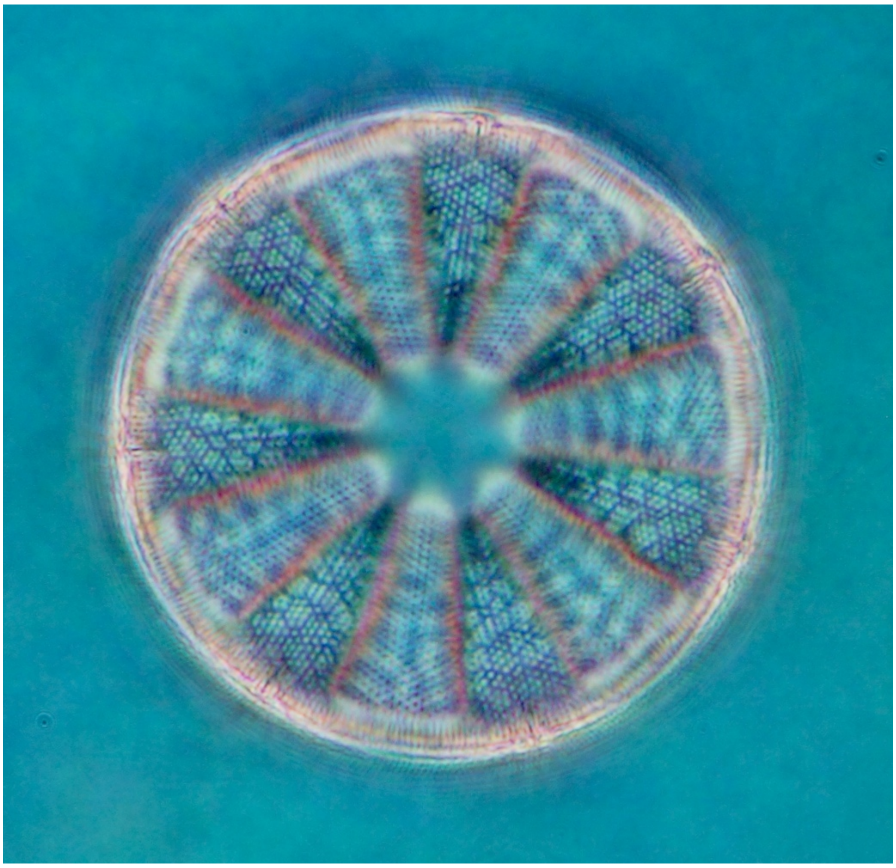


Figure 12. Actinoptychus Splendens. Rheinberg effect MC LED Ring

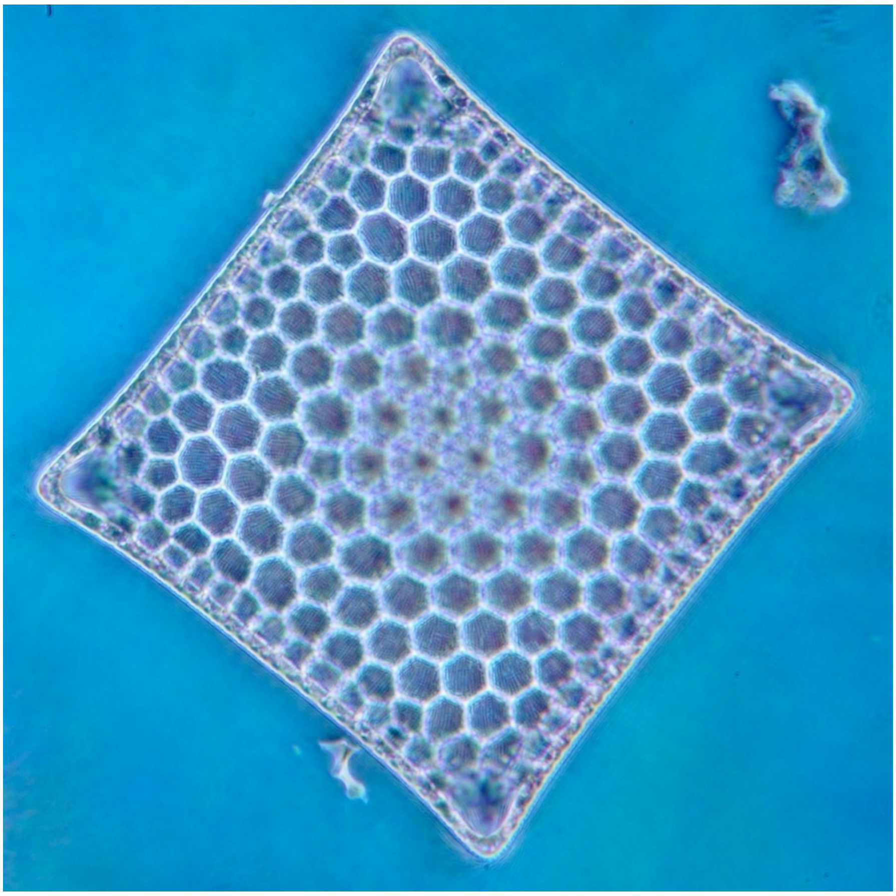


Figure 13. Diatom Rheinberg effect.

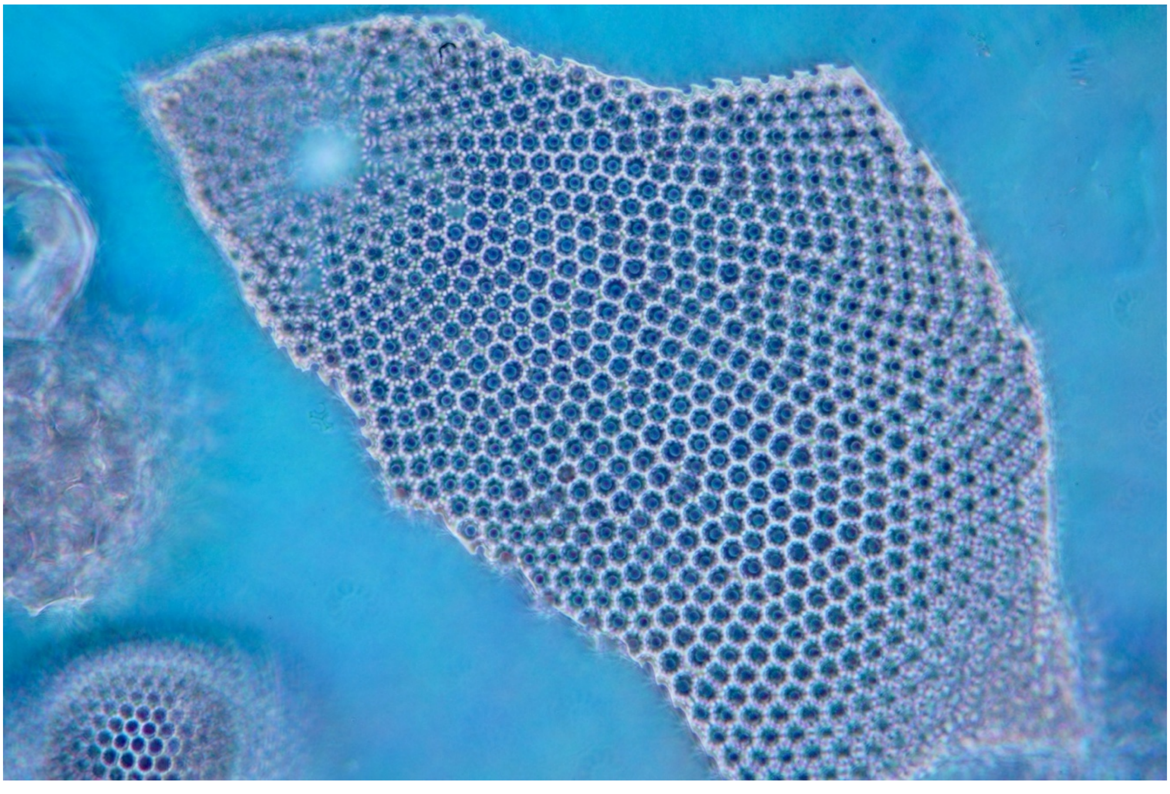


Figure 14. Diatom Rheinberg effect.

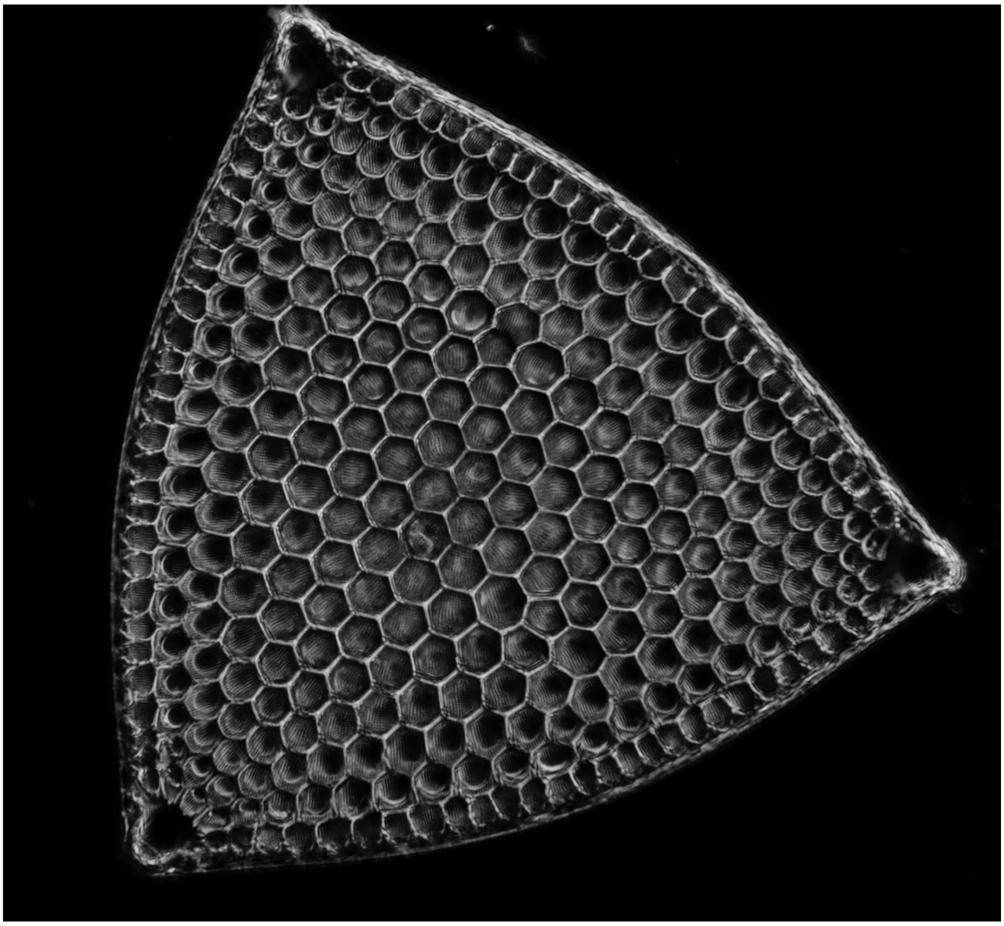


Figure 15. Tricetarium flavus MC LED Purple converted to black and white and enhanced. See p 218 “the diatoms” Round F.E., Crawford R.M., Mann D.G. 2007 Cambridge University Press

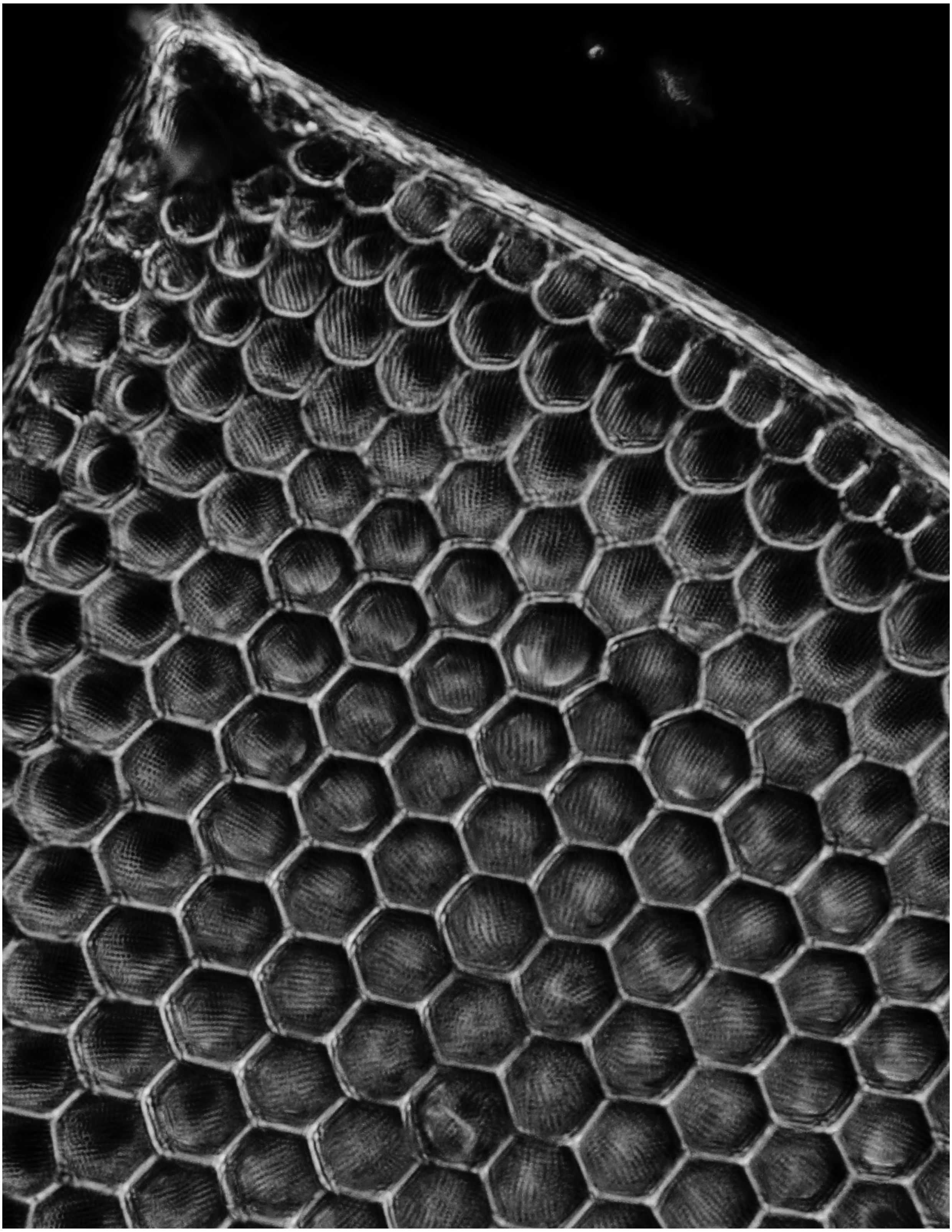


Figure 16. Enlarged Image.

A double multicoloured LED ring.

Having obtained Rheinberg effects with a single MC LED ring it seemed logical to develop this by mounting two rings together, one inside the other. A 60mm and a 40mm ring were mounted together on acrylic discs as described above. (Fig 17). With this set up the rings can be set to different colours or indeed the same colour if wished. There are a large number of potential colour combinations as well as several positions.

The inner ring can be set to oblique, with a movable stop, or COL.
The outer ring can be set to darkfield/Rheinberg or COL.

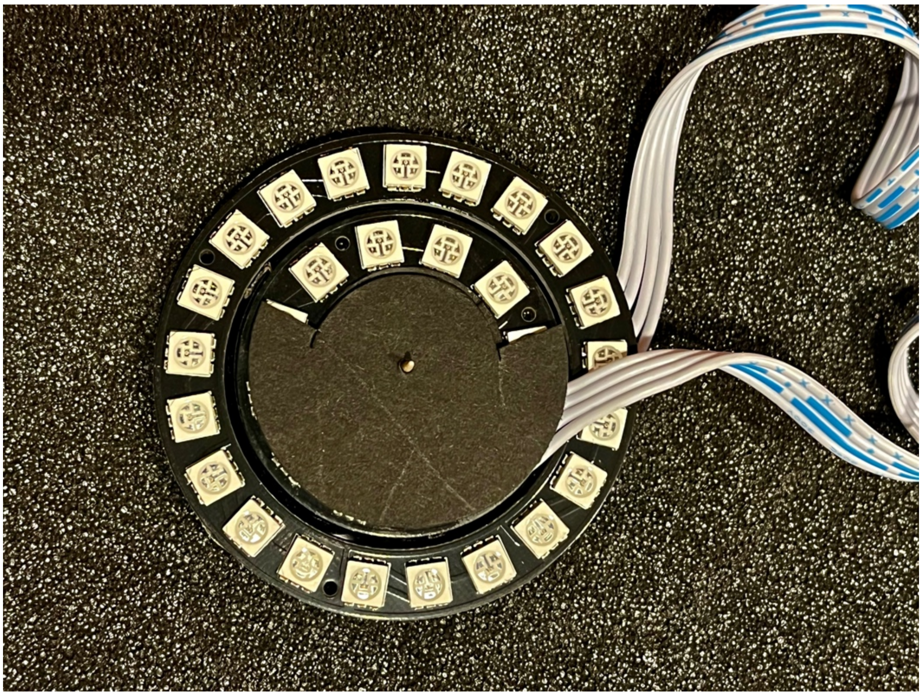


Figure 17. Double MC LED ring. 40mm ring has rotatable oblique stop in situ.

I have yet to fully explore the possibilities and which colour combinations work best but some early results are shown below. The images bear a strong resemblance to DIC. As with the single LED ring the technique seems to work best with objectives with magnification of x 40 and below. I have tried x63 and oil immersion x100 but the images are poor quality, I think due to a lot of diffraction and because without a condenser the light cannot be appropriately focussed. The LED rings as seen through the phase telescope cannot be positioned sufficiently peripheral with these objectives. The final point worth noting is that I have had difficulty getting the camera to record accurate colours which is presumably a white balance issue. A dedicated 4K microscope camera is better but the camera resolution is less than the Sony Nex cameras. Image processing can be used to make the colours more closely resemble what is seen through the eyepiece and all images have been adjusted.

Please note that in this part of the study a Leitz Dialux II 170 tube length microscope was used and the camera was a Nex 5N, (Electronic front shutter enabled), or a 4K Microscope camera. This set up is very similar to the Ortholux set up used in the first part of the study.

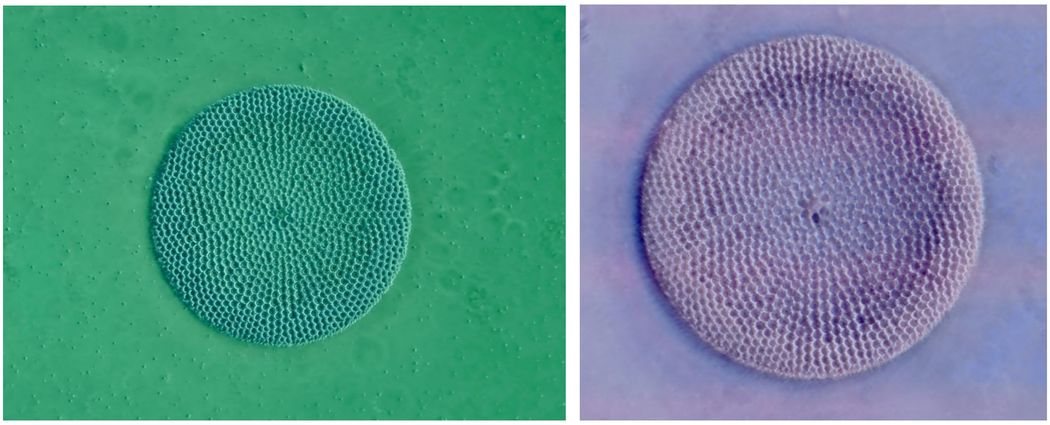
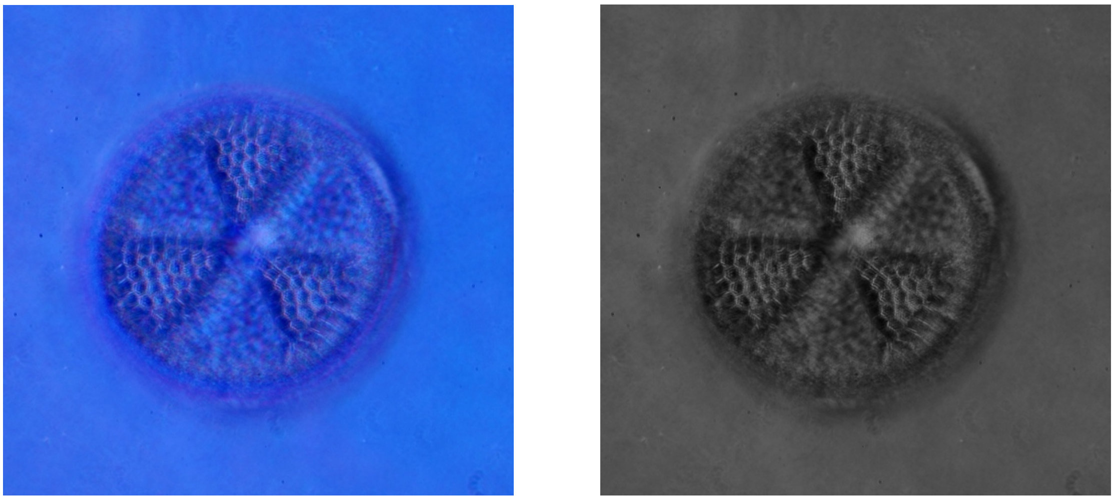


Figure 18 a – d. Diatom images using double MC ring. Green and blue and purple and blue. X 25 to left, x 40 to right. 4K Microscope camera.



Diatom images using double MC LED Ring. Blue and red and converted to black and white. Sony Nex 5N camera with post processing. X 40 Objective.

Conclusions.

The LED light ring using the magnetic mount is a simple straightforward technique that seems capable of giving images close to or equivalent to conventional condenser images. This mounting technique allows quite precise positioning which is important with oblique and Rheinberg illumination. There are some issues, probably related to diffraction, which make its use with high magnification objectives less satisfactory and the best images were obtained with good quality x 25 or x 40 objectives. The images can then be enlarged optically or digitally as desired. The adjustable multi coloured LED light ring, (MC), can give interesting Rheinberg type effects which I am currently investigating further.

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