**The Empirical Amateur – No Light Source and No Condenser.**
**Imaging diatoms with LED rings.**

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My interest in microscopy started with a desire to carry out metallurgical studies on early medieval weapons. The scientific analysis of early arms and armour needs to be multi disciplinary and microscopy is complementary to other methods including X Ray, various forms of scanning, hardness testing and X Ray diffraction analysis and spectrometry. However, this will have to form the subject of a later prequel to this article. Although I started with metallography it wasn't long before I became curious about transmitted light microscopy particularly for looking at diatoms.

***"You could have bought a decent microscope for all the money you have spent on those incomplete second hand ones."***

Many of us will have heard this from our spouses and partners and I am no exception and of course ruefully I have to admit in retrospect she is right but hindsight is a wonderful thing and anyway where would the fun, (or the learning), be in that.

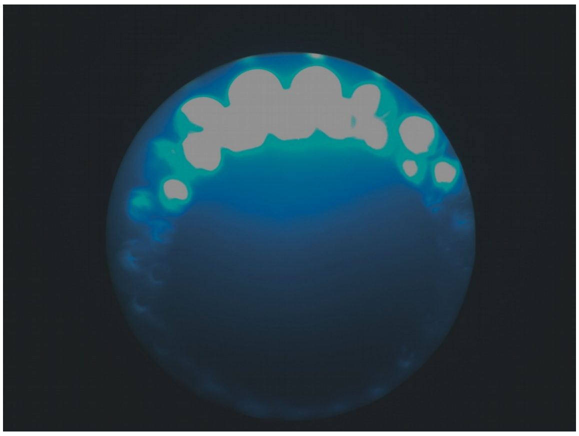
Since transmitted light microscopes were not initially my main interest and funds were short I looked for second hand ones from eBay and elsewhere. As many of you will know, bargain second hand microscopes even from reputable makers often have issues, usually to do with the illumination system or condenser. Commonly:-

*No working illumination system*
*If the illumination system does work the antique tungsten bulb fails after a week and cannot easily be replaced.*
*No condenser because many of these microscopes get stripped down for parts.*
*Somehow nothing seems to line up.*

**Ah well *caveat emptor***

*Micscape* is a wonderful resource under these circumstances and I have learnt a lot from reading various articles about replacement LED illumination, oblique and circular oblique illumination and condensers. One article I came across, which was not in *Micscape*, was by Webb (1) on condenser free phase contrast with circular LED illumination which seemed very apposite given my dilemma since this point I had a Leitz EB 20 with no working illumination and no condenser. The focus slipped a little but worked reasonably well, the stage was good, the sub-stage condenser carrier went up and down and some of the objectives were reasonable. I had also acquired a useful magnification changer.

A word on my empiricism. I do not have a lathe, cannot turn aluminium blocks, 3D print light housings or any of that stuff I read about. It's got to be done with card, super glue, acrylic, nuts and bolts and plastic tubes etc. I resolved that whatever additions I put onto the microscope would be reversible and I was keen to recycle household plastic tubes, stuff from work and other bits and pieces in my efforts. Its also helpful and saves time to read some articles to get a working theoretical basis for experimentation but for the empiricist detailed optical theory is not required and might even be a hindrance. Often the difficulties relate to practical problems like fitting bits and pieces into limited space, condenser carriers cunningly designed by manufacturers so no one else's condenser will fit and so on. Ingenuity is required. Two things the empirical amateur should obtain are a DC bench top power source for LEDs, (and other illumination systems), and a phase telescope so that one can see what effects the illumination is having at the rear plane of the objective and then relating this to what is seen down the eyepiece. In Webb's article, (1), and also David Walker's (2) article there are good illustrations of this. Figure 1 shows the appearance with the x25 objective in this experiment. (Down the camera – avoid looking at very bright LEDs – turn down the intensity controller.)



Figures 1 and 2

I experimented with holding a 65 mm diameter circular LED illuminator under the stage at various heights. Using my phase telescope I was able to relate what I was seeing to what the light looked like at the rear focal plane of the objective and as many of you will know, for phase contrast the ring of LED lights has to line up to the phase ring in the objective. If you use a normal objective the ring of lights – as peripheral as possible makes circular oblique illumination, outside the objective you get dark ground illumination and if the ring of light could be configured to just form part of an arc then you get oblique illumination. The only issues are that the LEDs have to be bright enough for oblique illumination in particular. Basically the little LEDS have to point straight at the objective when seen down the phase telescope and the size of the ring or arc varies by objective lens. This is in disagreement with Webb (1) who stated it was not necessary to angle the emitters of the LED towards the objective lens, even at very high NA, due to the wide pseudo Lambertian emission of the LEDs. This was not my experience with an upright microscope for oblique illumination and is possibly because only a few LED emitters will participate in oblique illumination and alignment becomes more critical to obtain sufficient illumination as compared to using a whole ring in phase contrast. This seems to be particularly the case for higher power objectives. It would be interesting to try different types of LEDs such as "flat top" and "straw hat" to compare with the standard, "bullet" ones used here.

If the LED ring can be mounted to the condenser stage then it can be moved up and down to some extent so that it is correctly positioned to the objective to get the various effects but it gets very difficult with the higher power objectives. With the commercial 65mm LED ring on my own microscope x10 can only be darkfield, x25 and x40 can be circular oblique or oblique, x63 is possible for oblique but it's not very good and x100 is impossible. In theory for high magnification objectives with a high NA one needs to position the ring further away from the objective or use a larger ring. This is apparent when looking at the diagrams in Webb's article (1). However, this is difficult because there is insufficient room in an upright microscope compared to the inverted microscope used by Webb, the correct angulation of the LEDs is difficult to achieve and the further away they are the less bright the light. Paradoxically one may need to use a smaller ring in order to get the LEDs close enough to deal with these practicalities.

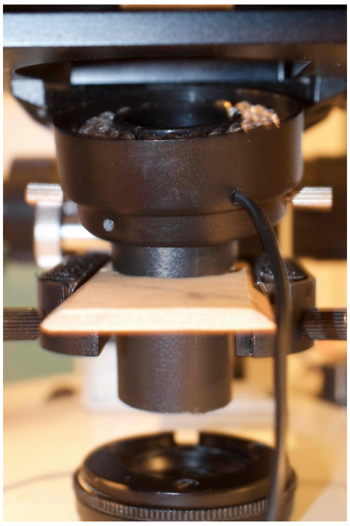
The condenser on the Leitz EB 20 is mounted on a dovetail mount that also has adjustment screws for centring. Inspecting a condenser I did subsequently purchase suggested that if I cut a thin piece of wood to an appropriate rectangle and chamfered the edges it would slide into the mount and the centring mechanism would still work. So it proved and the lid of a box that once contained a bottle of port proved perfect with a bit of rough carpentry was converted into a platform to mount things on.

Previously I had held the eBay light ring, conveniently already wired into a mains plug adaptor with an intensity controller, under the stage and knew it would provide the necessary illumination a la Webb or Walker. However, holding it by hand was ridiculous but I did observe that when I tipped it there was oblique illumination of my diatoms slide and really nice at that reminding me of a picture in *Micscape* by Paul James (3), in which it was suggested total internal reflection was contributing to the appearance.

**Oblique illumination with an LED Ring**

The challenge was to use an LED ring that would produce sufficient light, be mountable to the empty condenser mount and *permit angulation of the ring*. I examined the 65mm LED ring and concluded that I could mount it to a short length of stout plastic pipe with two bolts (one of which was an existing bolt to mount the light ring to a stereo microscope which was its intended function). The plastic pipe was from a vacuum cleaner attachment. No dear reader, I did not irritate my wife further by cutting up her new vacuum cleaner but I had a piece from the old one. By judicious trimming of the end of the pipe this allowed the ring to tilt on the bolts. Next a hole of correct diameter was very carefully drilled in my wooden platform exactly down the centre line but such that pulling the platform back and forwards permitted some latitude in centring front to back. The plastic pipe sits in the hole by an interference fit and can be rotated. (Fig 2.)

The geometry of the stage prevents the LED ring being positioned really close to the slide and does limit a little the amount of tilt achievable. (Fig 3) The ring is positioned with the aid of the phase telescope to get a peripheral arc of illumination of maximum intensity which means tilting the ring till the LED bulbs are best seen. Further sophistication was added after I read that blue light improves resolution and that polarised light is useful for imaging diatoms. The lid of a glass Petri dish fitted nicely over the ring when the plastic cover was removed and a ring of polaroid film and a blue gel filter slipped inside. (Fig 4) The results are illustrated in a diatom strew slide prepared by myself of freshwater diatoms from a stream on the Greek island of Kithera x25 mag 1.6 in Figures 5a and 5b. 5a without blue filter and polarising sheet. 5b with. Both images are stacks but no digital manipulation applied.



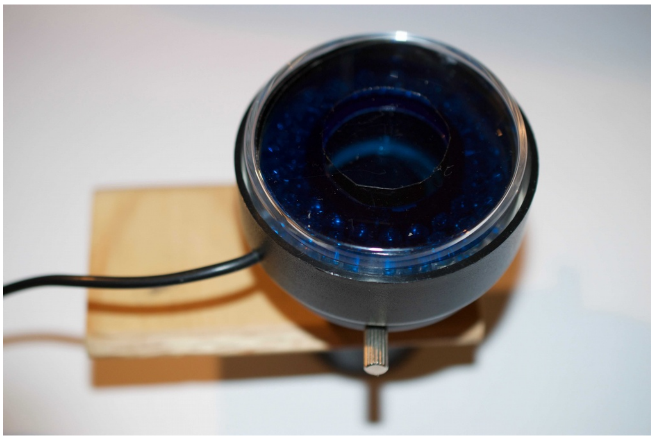


Figure 3 and 4

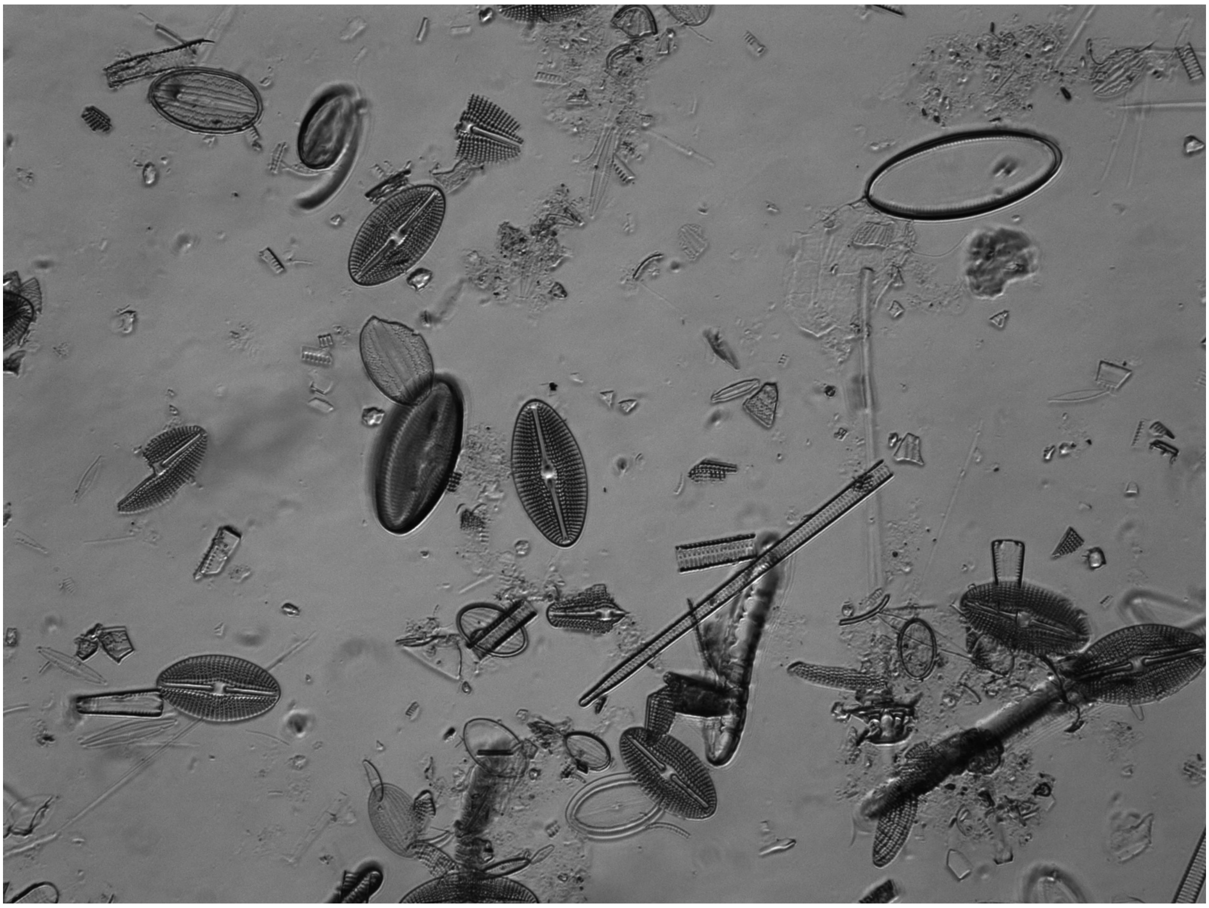


Figure 5a

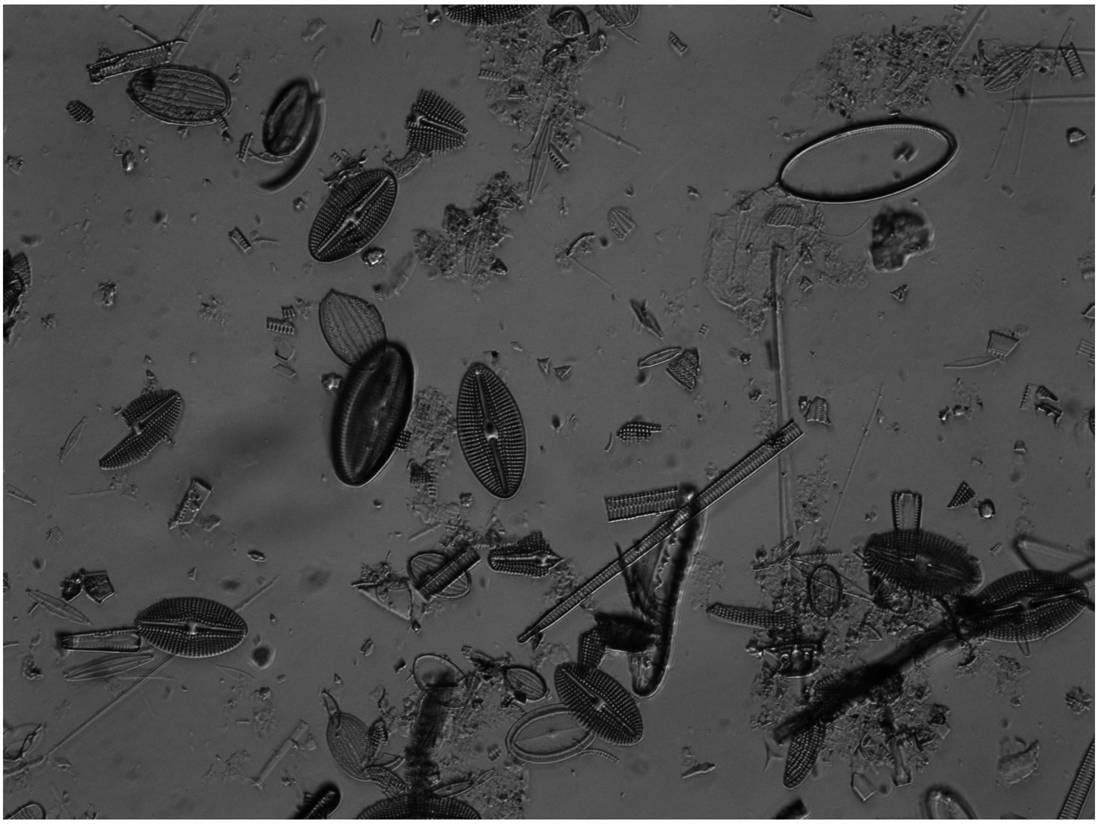


Figure 5b

A selection of images of diatoms with this illuminator is shown in Figures 6 – 12.

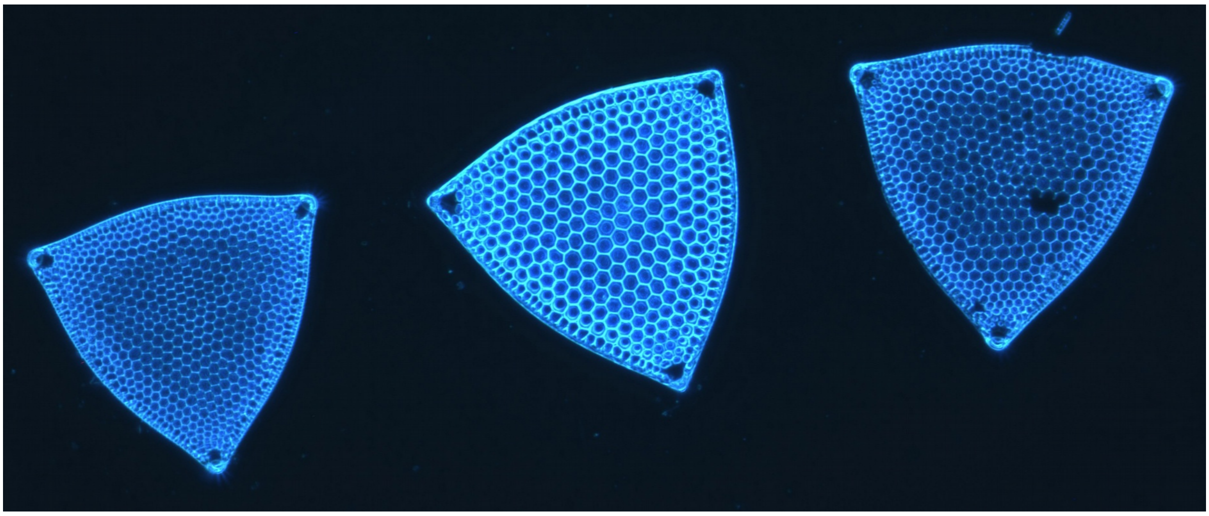


Figure 6. Dark field with x10 objective 1.6 magnification.

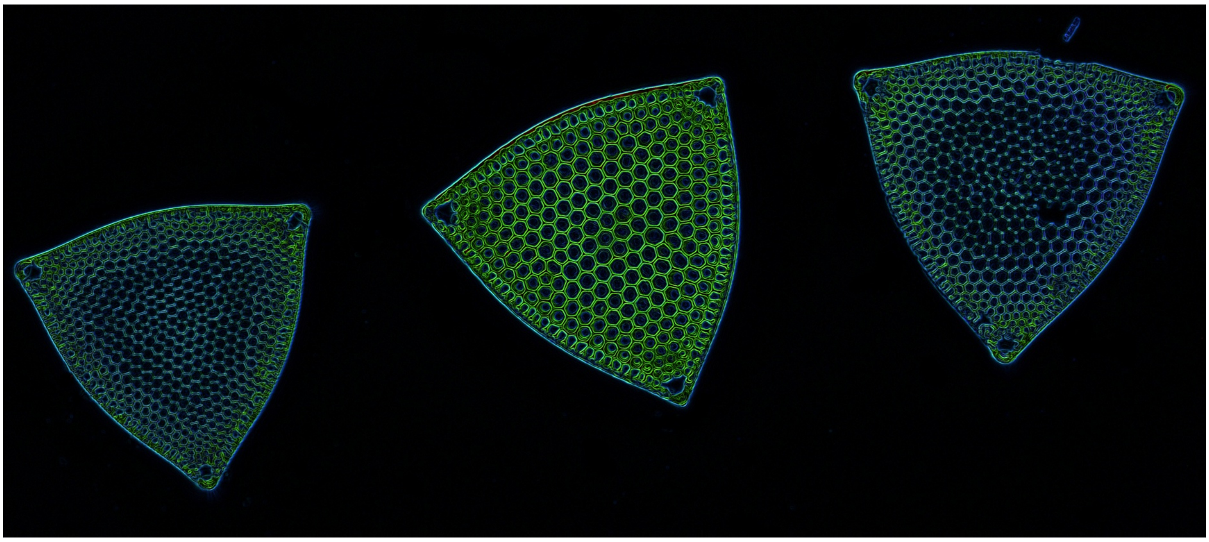


Figure 7. Same image with Photoshop enhancement edge glow…..!

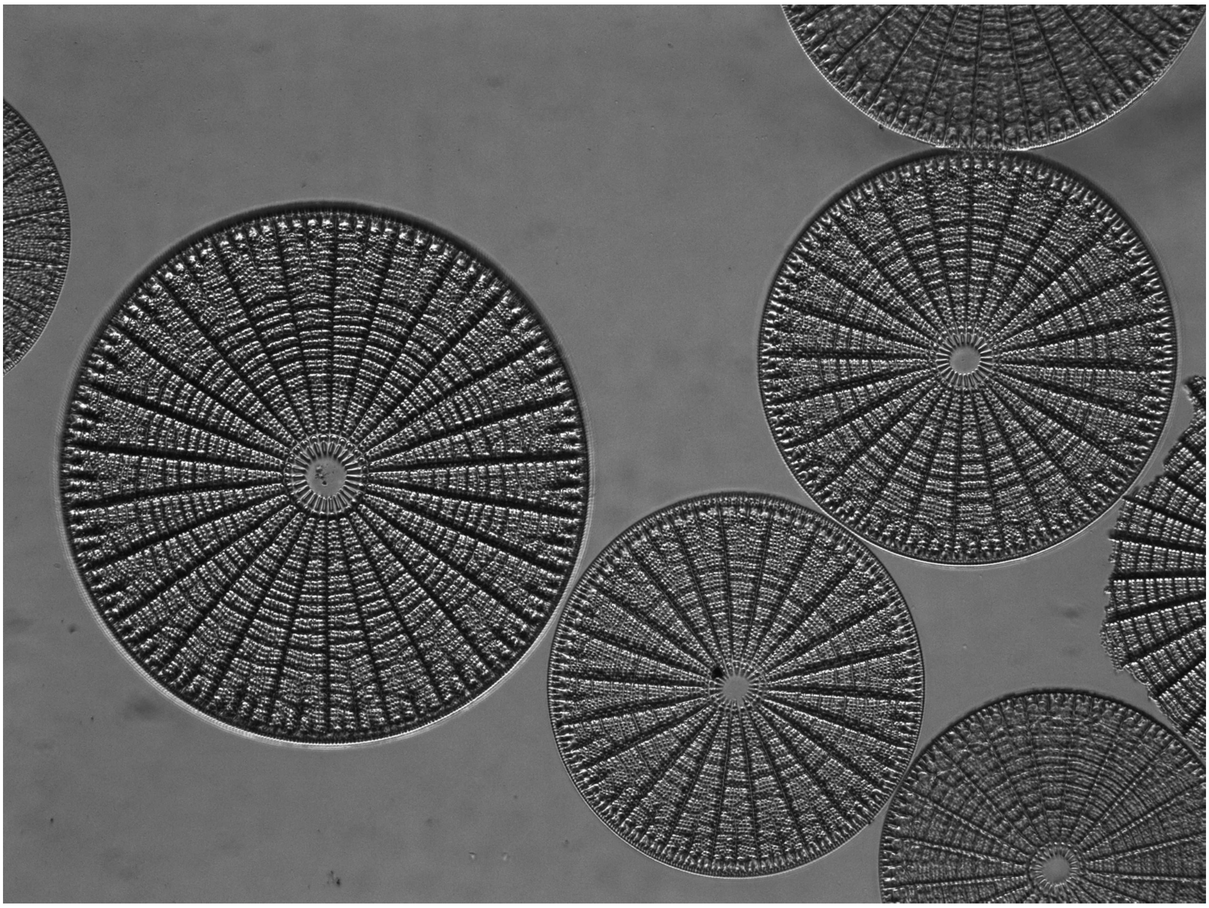


Figure 8. Oblique with blue filter and polarised x25 objective 51 image stack Photoshop sharpening.

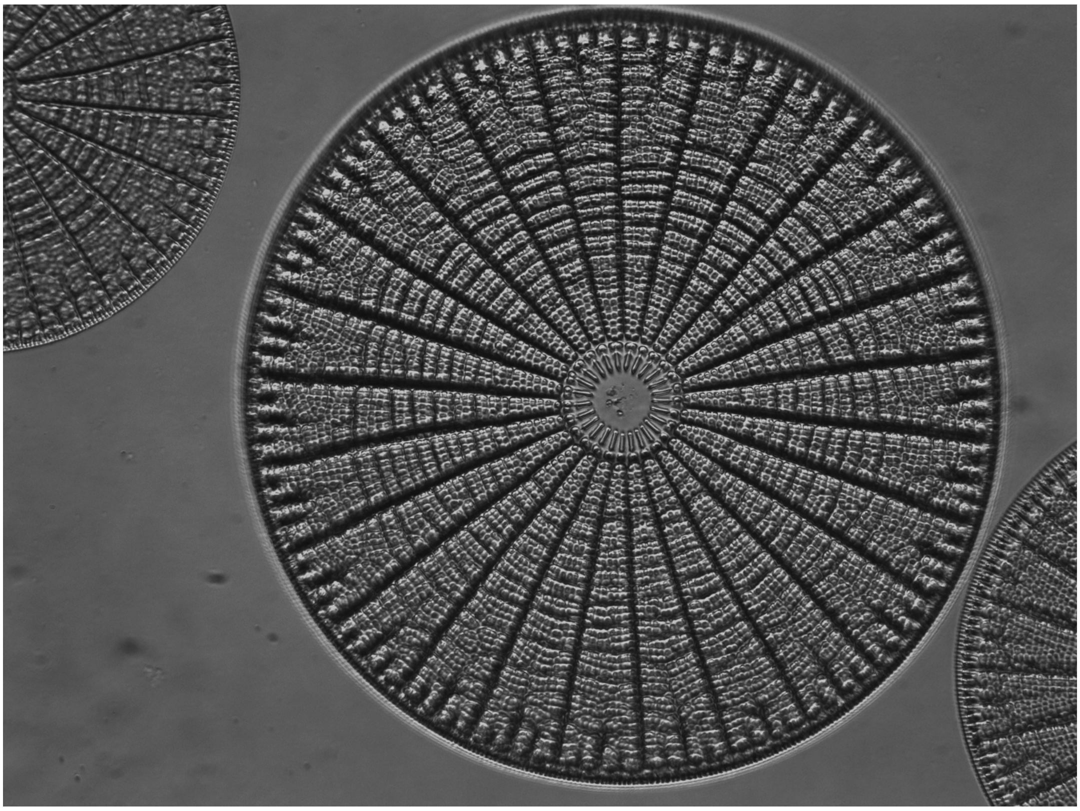


Figure 9. As above but 1.6 magnification.

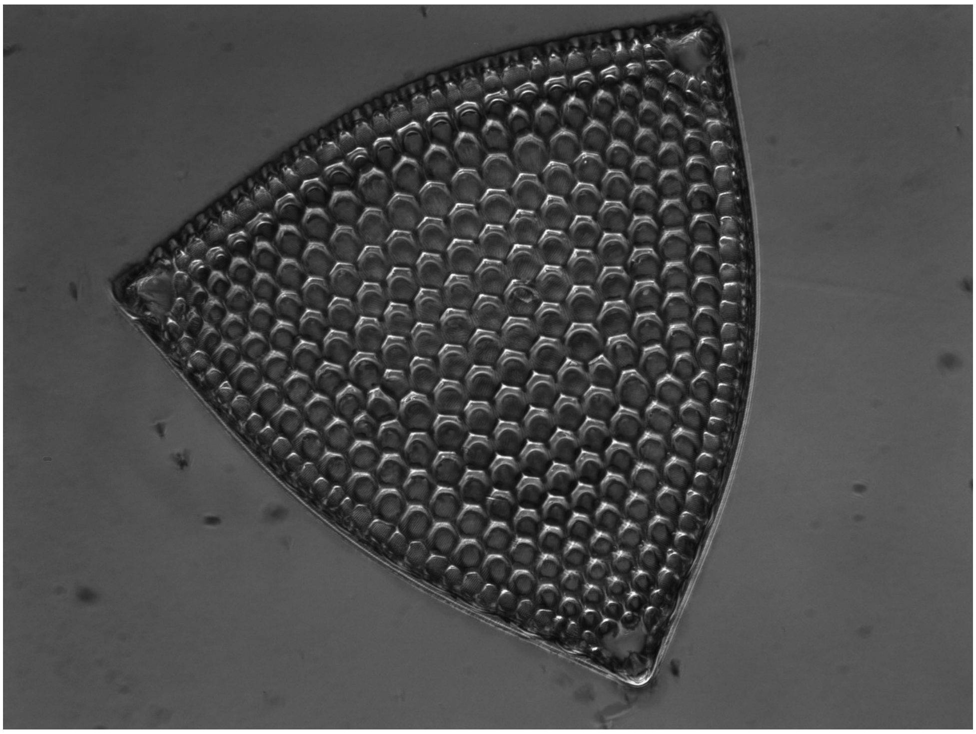


Figure 10. Oblique with blue filter and polarised x 40 objective. 17 image stack Photoshop sharpening.

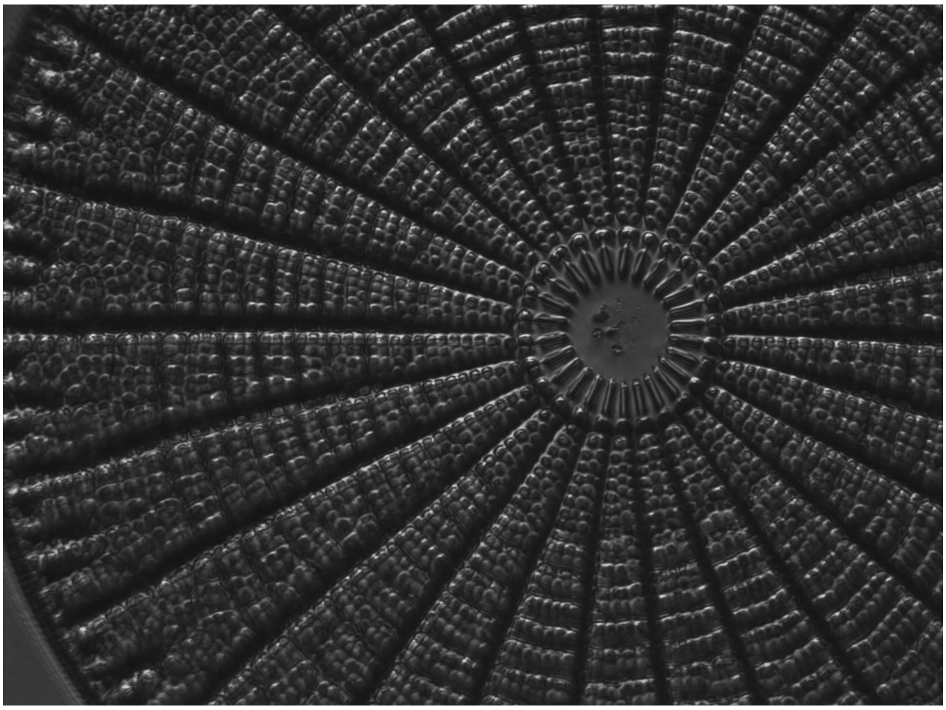


Figure 11. As above but x2 magnification 19 image stack.

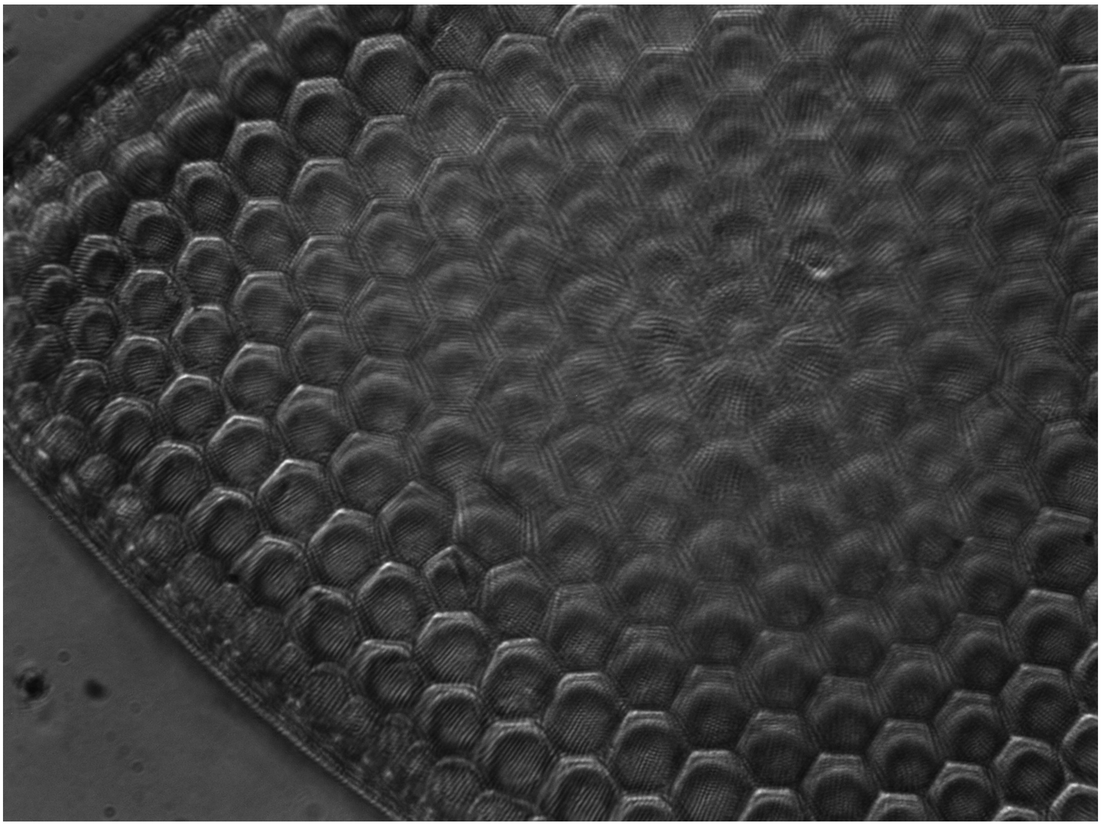
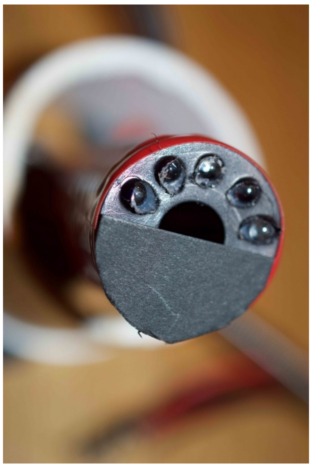


Figure 12. As above 11 image stack Photoshop sharpening.

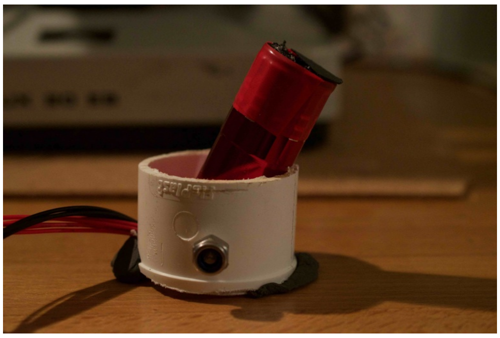
The next step was to construct a small enough ring of LEDs to use with objectives of greater than x40 as I could not find anything suitable on the Internet. By empirical experimentation I settled on using 3mm pre wired LEDs, (blue), inserted into a 22 mm black nylon washer to make the ring. This is not as easy as it might seem because the LEDs have to be correctly angled to point at the objective rear focal plane as seen down the phase telescope. Thus far I have been interested in oblique illumination and used an arc of 4 or 5 LEDs that can be powered from the bench top DC power supply. When drilling the holes to mount the LEDs the holes have to be angled carefully so that each LED will point towards the same point about 1 -2 cms above the centre of the washer. (Fig 13)

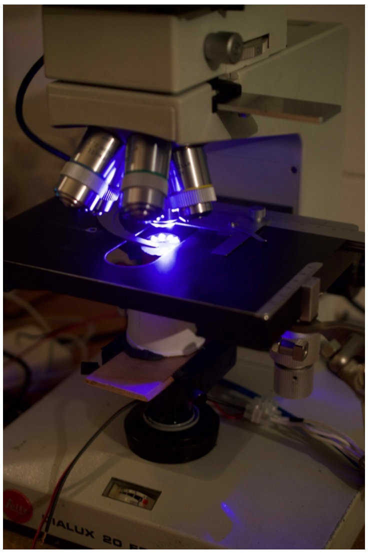


Figures 13 and 14

If done correctly then a little ring of 5 points of light will be seen down the phase telescope. It may take several efforts but the materials are cheap. Next the nylon washer has to be mounted in such a way that it can be tilted for fine adjustment. The current method is to tape the washer to a short piece of aluminium tube derived from cutting up a £1 eBay LED torch – this is a by-product of making an illuminator from the torch. (Fig 14 shows the necessary components – a cheap eBay LED torch disassembled with the LEDs converted into a useful illuminator for another project, the tube remnant, a nylon washer, a 3mm prewired LED and the phase telescope). A hole is drilled across the tube which then sits inside a short piece of plastic pipe with two further holes – a threaded rod is passed through all 4 holes and nuts added to hold the aluminium tube firmly. If the holes in the plastic tube are just a little bigger than the rod then turning the rod tilts the aluminium tube. (Fig 15)

The plastic tube sits on the wooden sub stage condenser platform temporarily held with Plasticene, (Fig 16), but it's very helpful to secure a magnet to the base of the plastic tube and either staple a thin piece of steel, (soup can top), to the wooden platform or substitute the wooden platform for a 2 mm thick steel plate Velcroed to the top of the condenser mount. It is then very easy to centre the LED ring illuminator under the objective by moving the magnet mount on the steel plate whilst checking alignment with the phase telescope. *Two warning points* – don't use too strong a magnet and be very careful looking directly at LED lights through the microscope – keep the power down for alignment purposes. I use this magnet on a steel plate technique a lot when making illuminators to replace broken illuminators or substitute for mirrors on older microscopes. It makes it really easy to line things up and I got the idea from a Wild M20 microscope.





Figures 15 and 16

This is the Mark 3 illuminator and there is scope for development but it is actually possible to get reasonable pictures of diatoms with a 100x oil immersion objective without using a condenser – which is a little surprising but gratifying to the empirical amateur. For low power objectives the commercial 65 mm ring illuminator works well, provided it can be tilted, because it has so many LEDs and is so bright that some individual bulbs will line up when checked with the phase telescope. This system won't compete with a Leitz Orthoplan fitted with a Heine condenser as implied by my wife but there has been fun and learning in it….

Fig 17 shows a stacked image of *Frustulia rhomboides* from a Klaus Kemp test slide after some tweaking and cleaning with Photoshop. Fig 18 is my best effort with *Amphipleura pellucida* from the same source. Objective x100 1.32 NPL Fluotar.

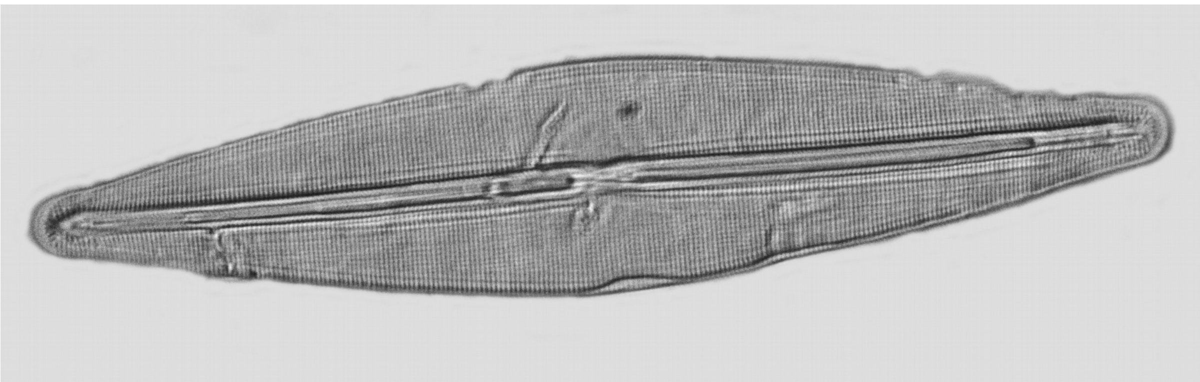


Figure 17

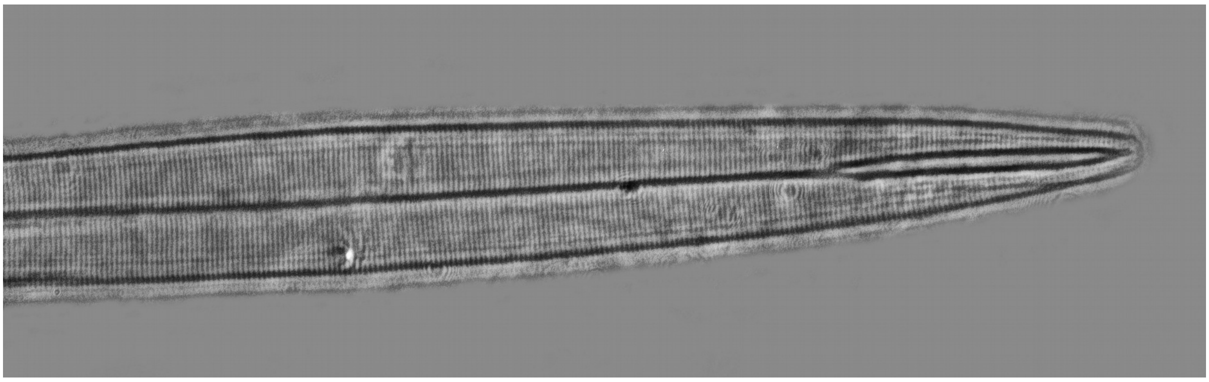


Figure 18

**Conclusions**

A commercial ring illuminator correctly positioned below the stage can produce good images without a condenser. It is particularly useful for oblique or extreme oblique illumination of diatoms. Other specimens have not been assessed.  In this study good images were obtained with Leitz  x25 0.6 PL Fluotar  and x40 0.7 Pl Fluotar objectives using the commercial 65 mm illuminator attached to the condenser mount. A magnification changer proved valuable. More work is needed to extend the technique to higher magnification objectives but an experimental ring shows promise.

**Acknowledgement**

The Empirical Amateur,(EA), would like to thank the Editor, David Walker, for his help with this first article. As became apparent, the EA can sometimes achieve results but may be a bit hazy on the theory behind them!

Comments to the author Robert Hill are welcomed.
Email: robahill AT hotmail DOT co DOT uk

**References:**

1) WEBB, K.F. (2015), Condenser-free contrast methods for transmitted-light microscopy. *J. Microsc.*, 257(1), 8–22. doi: 10.1111/jmi.12181
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2) WALKER, D. (2016). Exploring the use of cheap LED 'halo rings' for car lamps as multi-mode 'condensers' on an upright microscope. August 2016, *Micscape*.

3) JAMES, PAUL. (2000). A report on an interesting variant of oblique illumination. February 2000, *Micscape*.

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