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The Magazine for the
Enthusiast Microscopist

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**My Fascination with
Microscopy**

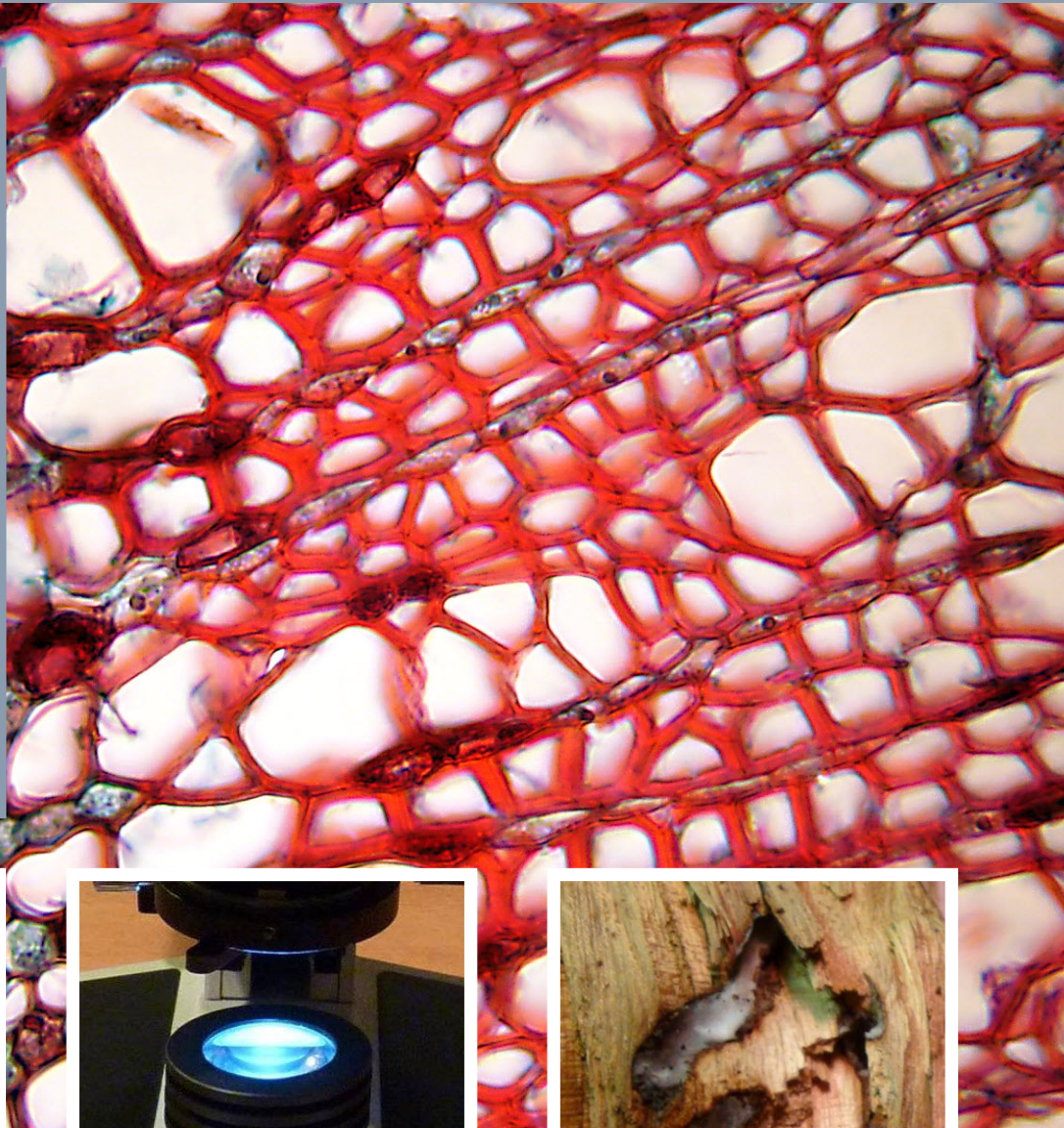
**A Review of some
useful Books and
Internet Sites on
Diatoms**

**Robert Hooke's
Micrographia**

**Predators in the
micro world**

**Staining Onion Cell
Nuclei**

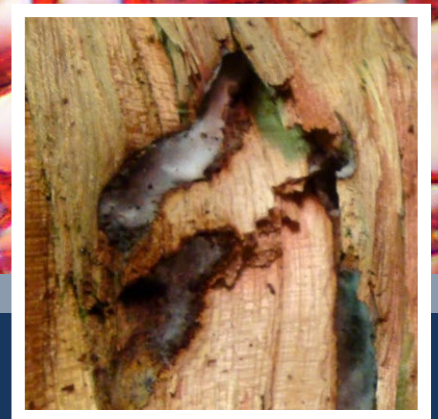
Infinity vs. DIN Optics



**Restoration of
Antiques**



DIY LED Illumination



Firewood Microscopy

Microbehunter Microscopy Magazine

The magazine for the enthusiast microscopist

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Front: large image: Tilia stem by Michael Gibson;

Left image: Joseph Wilhelm; Middle: Suphot Punnachaiya;

Right image: Charles Guevara

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In recent years many large microscope manufacturers moved away from the traditional DIN standard to so-called "infinity corrected" optics.



EDITORIAL

First and foremost, a big thank you to all who contributed! We're now in the third month and I'm optimistically looking into the future. During the last few months the followers of the Facebook page also increased steadily, and I can only encourage everyone not only to become a "friend" of the Facebook page, but also to share in-

www.facebook.com/microbehunter

teresting microscopy related links.

In this edition you can read again a wide variety of different articles: some are more technical in nature, such as the modification of a microscope to LED illumination (page 22), others place a stronger emphasis on the observation and exploration aspect of amateur microscopy. I myself am

fascinated and even a bit surprised on how diverse the different approaches to amateur microscopy can be, and on how many new things there are to learn.

In any case, I hope you enjoy reading the March 2011 issue!

Oliver Kim, editor.

ANSWER

Answer to the question on the back cover: Mashed carrot (baby food).

Firewood Winter Microscopy: A Photo Essay

Specimens for microscopic observations can be found in the most unusual of places. This photo essay documents sample extraction and microscopy of fungi found in fire wood.

By Charles Guevara

In spring 2010, fungi-weakened trees were blown down and decomposed in the open meadow which they bordered. While falling, these huge trees took down other smaller trees. Heavily fungi-infected trunks gave off a strong yeasty-cheese odor as I chain-sawed sections of the freshly fallen wood.

Numerous pathogens, parasites, symbionts, and saprophytes swirl in a dynamic balance when our trees, our fungi, our insects constantly interact. Firewood harvesting can lead to some interesting microscopy.

At my woodshed, freshly split wood caught my eyes. The interior suddenly revealed subtle structures at the instant of clean abrupt wedge splitting of the trunks.

Some split trunks revealed crisply demarcated stains of fungal growth. Other trunks showed some delightful green stains, which spread more diffusely through the sapwood. Hidden insect chambers in the sapwood of other trunks contained elaborate fungi structures... All these suddenly revealed structures invited me to do some microscopic observations!

Fig. 1: Clean split wood offers microscopic specimens of the interior invaders.

Fig. 2: Middle-lower trunk portion sampled.

Fig. 3: Invaded wood section used for microscopy. The dark horizontal streak is fungal growth.

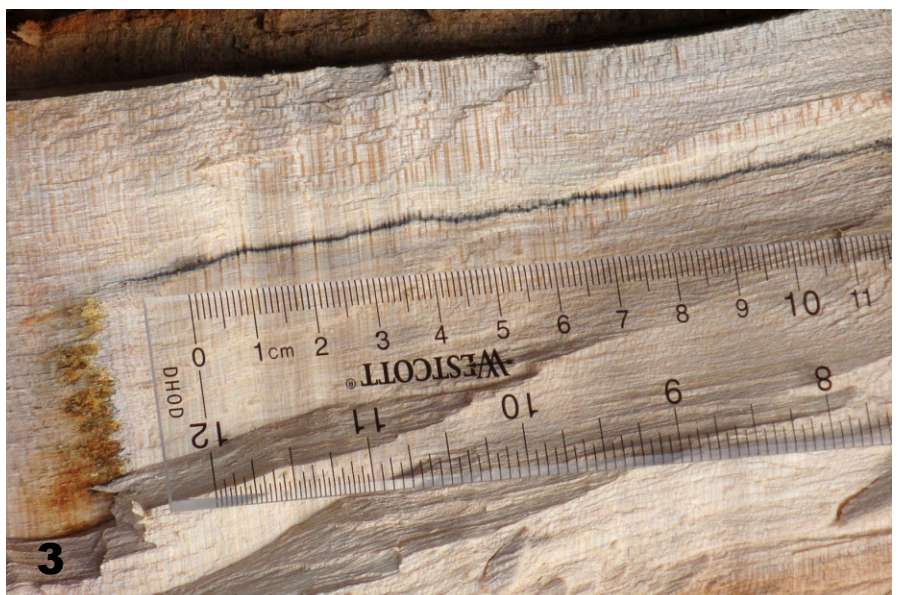


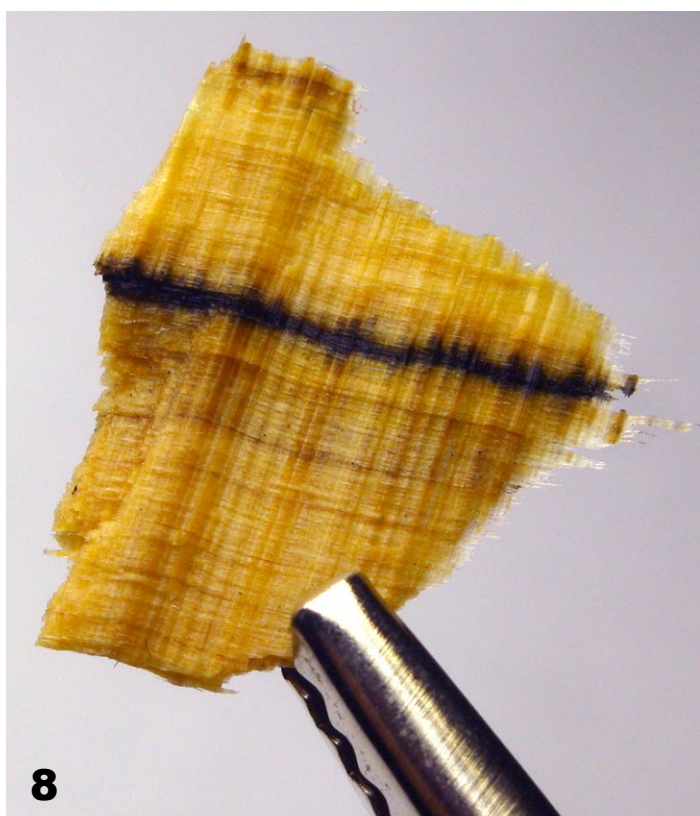
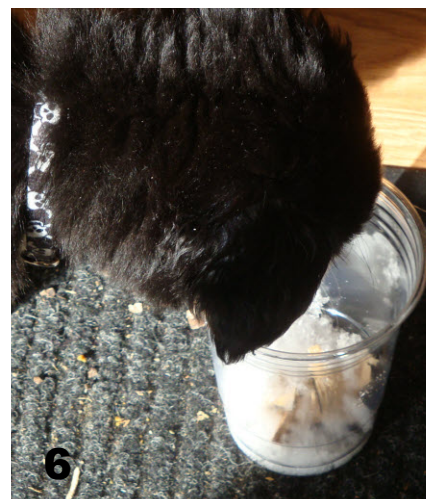
Fig. 4: Specimen collection instruments in the woodshed.

Fig. 5: Collecting the specimen

Fig. 6: Samples were covered with snow for conservation, and brought indoors. Sample inspection before transport to the microscope bench!

Fig. 7 and 8: Collected wood fragments. The dark discoloration is fungal growth.

Fig. 9: Low-power observation.



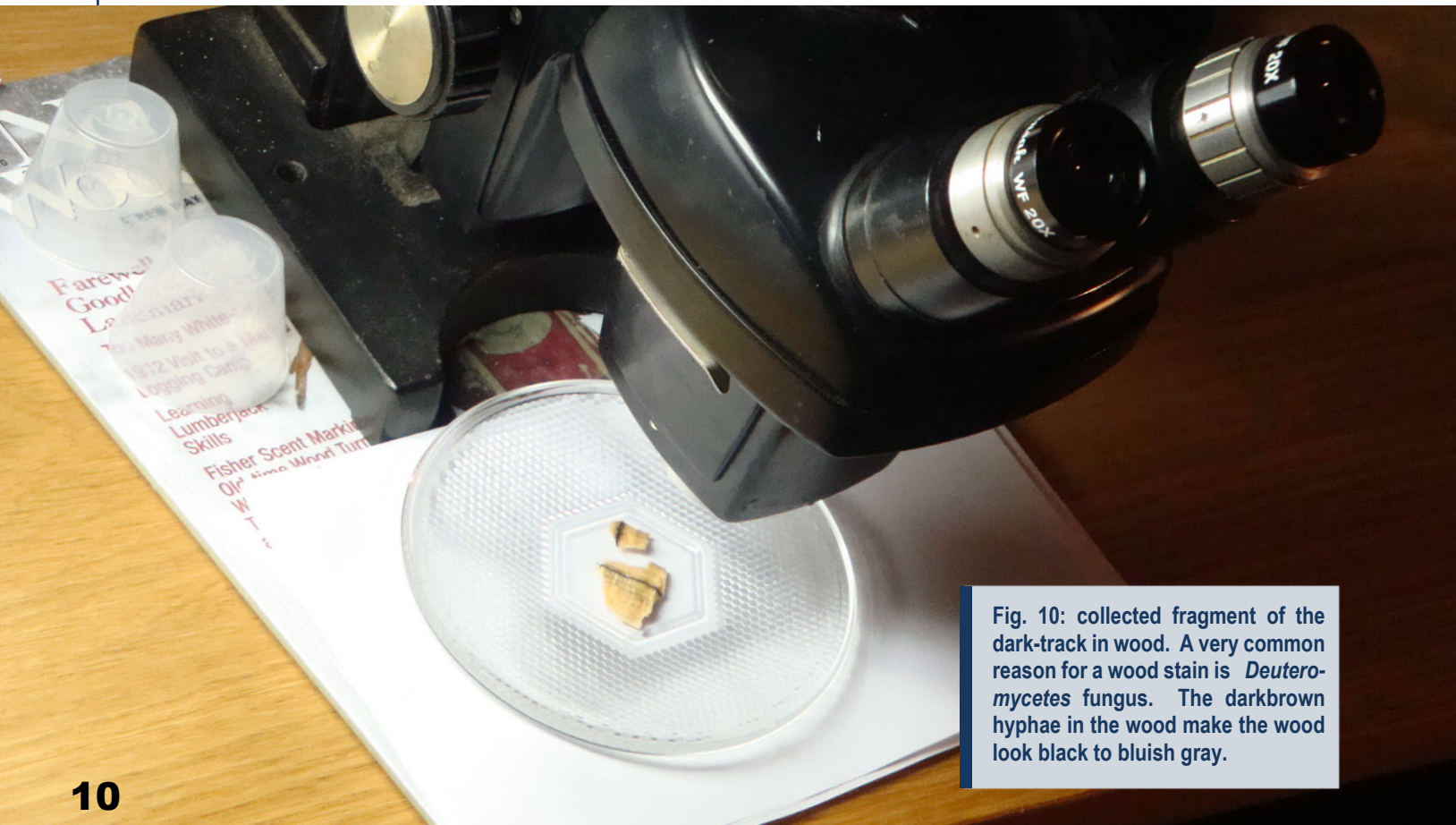


Fig. 10: collected fragment of the dark-track in wood. A very common reason for a wood stain is *Deuteromycetes* fungus. The darkbrown hyphae in the wood make the wood look black to bluish gray.

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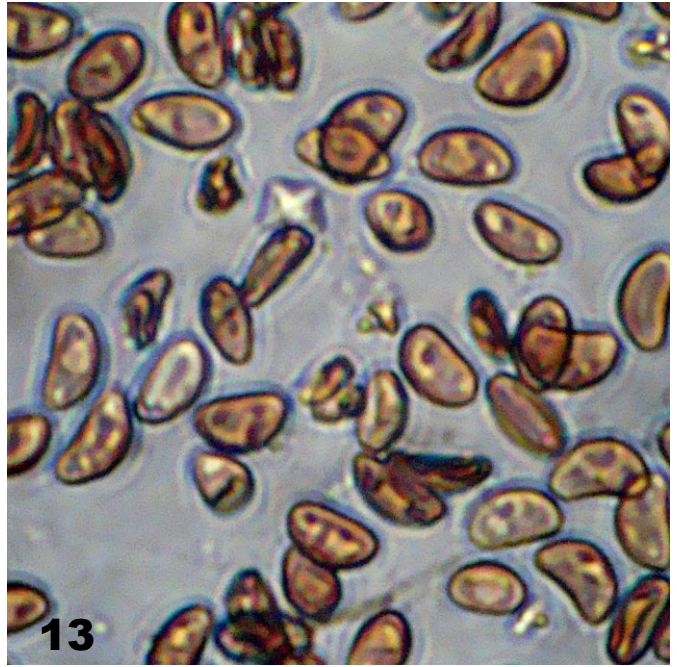


Fig. 11: Cotton-like fungi in another wood sample. *Ascoomycota*, perhaps class *Sordariomycetes*?

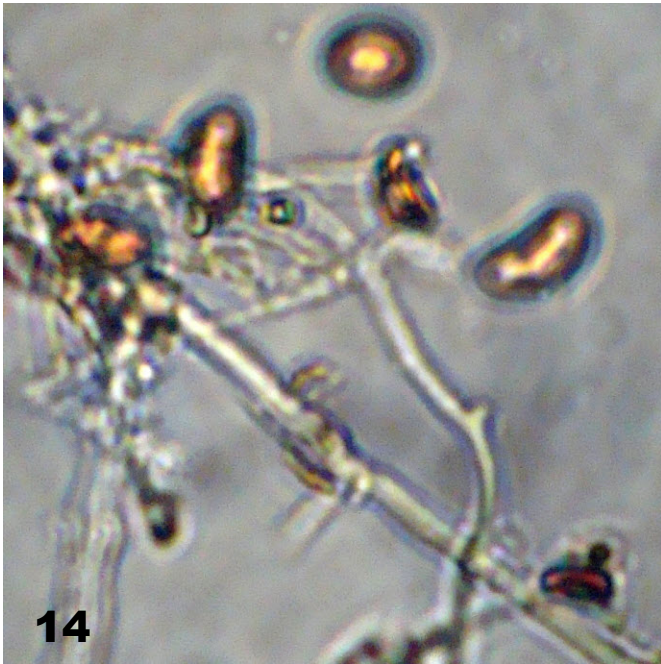
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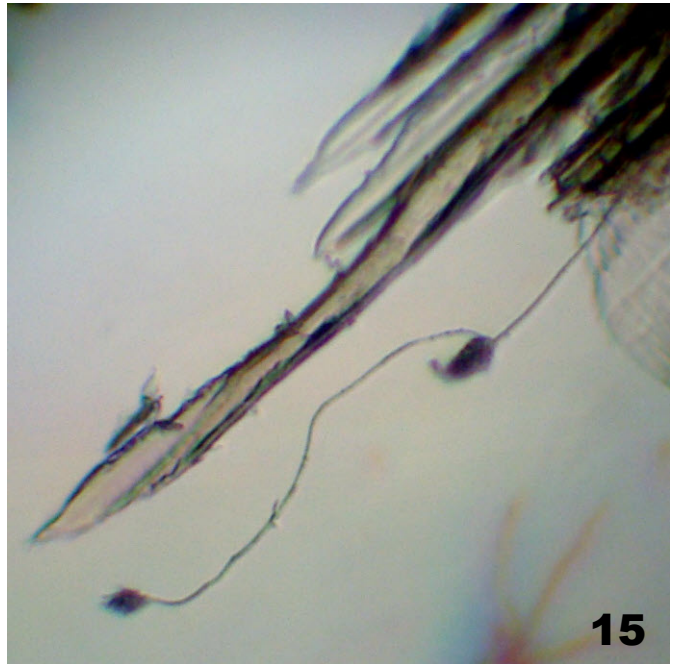
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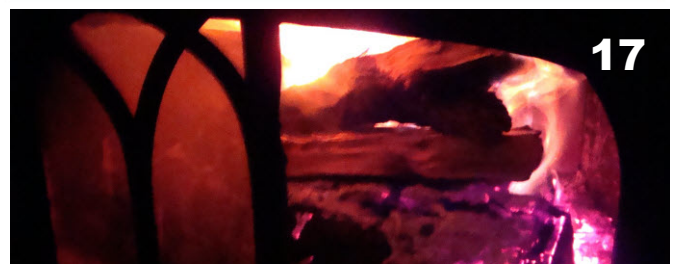


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Fig 12-14: Dry, high-power observations of ascospores.

Fig. 15-16: Low power. Fungal organisms in fresh wood.

Fig. 17: Harvested fire wood after some winter woodshed microscopy!



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The Novitiate's Odyssey.

Episode Two: Willie in Wonderland

Through the Looking Scope... err Looking through the Scope.

This article was originally published in the October 2009 issue of Micscape Magazine and is now republished here with the permission of the author and of Micscape Magazine.

Experiences and lessons of restoring antique microscopes.

by G. Joseph Wilhelm, Florida Keys, USA

It was the best of times, it was the worst of times. (A tale of two parties – Key West).

Before you ask, yes “Willie” is one of the many sobriquets bestowed upon yours truly but also one of the few that can be repeated in polite company. Among the most popular, are “The Kaiser” for obvious reasons, and after some time doing close order drill with a small assist tug during my stint as a harbor tugboat master in the Virgin Islands, I

was dubbed with the two dubious monikers of “Captain Wildhelm” and “Blazing Throttles”. But that’s another sea story.

Update: As mentioned in my last article, I was using this venue to convey the fascinating world of the diminutive to my young cousin Shaun (“heir to be” to my microscopes) and to possibly instill an interest in the subject. I am now delighted to report that although he was at first somewhat ambivalent to microscopy and to being bequeathed my instruments, I now have his fervent assurance that after reading my previous article he now eagerly awaits my demise. Bless his heart. Being thus encouraged I shall continue to regale you with further adventures.

I had procured my menagerie of microscopes with methods that have established me as a shining example of bad rational thinking, the original intent being for display only. But I also demand that items in my collections be functional. Being sure that only minor adjustments, cleaning, parts etc. would be necessary to put them in working

Fig 1A. Notice the very secure top mounting strap on the prism and the multiple screws on either end of the “bridge” that the eyepiece tube screws into. These appear to perhaps be part of the collimation adjustments for the eyepiece tubes (?) as the prisms are cemented together, probably in a jig.





Fig. 2A, 3A. Finally the light was disassembled and cleaned and all the chrome knobs clips etc were soaked in a weak commercial solution (Foz or Ospho) of phosphoric acid for an hour.



order, I was about to set about this task when my wife suggested I might enjoy doing some serious observations with them. It was at this juncture I discovered the Microscopy-UK web site and my world opened to a completely new vista, thank you very much.

All of my restoration plans were put on hold while spent some months positively gorging myself on Micscape's veritable smorgasbord of articles in or-

der to understand the potential and limitations of the equipment I had on hand. Actually having the microscopes in hand helped immeasurably with those seven aspects of microscopy I mentioned in the last article and I would touch on some of those as we go thru my scopes to select the best candidates for immediate restoration and functionality.

What type of scope is best to begin with?

The research.

Micscape articles scrutinized were:

- "Guidelines on buying/choosing a microscope" by David Walker. This is an excellent definition of stereo, monocular, and binocular scopes.

- “Microscopes” by Mol Smith (link?)
- “How I choose and buy microscopes” by Gordon Couger.

Consensus: To simplify the collective opined beliefs; you should have two microscopes, one stereo and one compound (as a collector I am totally enamored with an opinion that advocates at least two of something). First is the stereomicroscope, which was considered best to start with. Here is what I have surmised as the logical reasons:

1. Simplicity. Easy to focus, only one knob, the low power magnification enables just a coarse focus mechanism to work perfectly and easily, just right for the beginner.

2. Immediate gratification. (I believe this to be one of the most basic principles for a happy human existence) The ease of operation allows the observation of even the most mundane of objects (a speck of dust, a bit of cloth, a subpoena), to be accomplished without complicated preparation or specialized lighting technique. No fussing about. Just putting the selected subject under the lens using a bright table lamp will produce the most amazing images.

3. Easy recognition and ability to relate to observed object. The relatively low magnification allows the beginning observer to easily put into context the

images presented thru the lens. First of all, the image is upright, not reversed as with a compound scope and the observer can merely look at the subject au natural and then thru the scope without any serious confusion with a frame of reference. The subject is still recognizable, just larger. No better example of this concept can be found than in Mr. Brian Johnston’s exquisite Micscape presentations.

4. Lens working distance. The distance between the lens face and the object being observed is several inches. This allows for the insertion of dissecting tools, grubby fingers, and other subject manipulating devices such as the really nifty miniature horseshoe base double-jointed alligator clip specimen holder thingy and various incident light (meaning shined upon not shining thru) light sources.

5. It is a useful tool for further microscopic observations. Once the “line of desire” has been crossed to observe with magnifications greater than the stereoscope can afford, it becomes propitious for preparation of slides suitably discernable with a compound scope.

Next is the compound scope. These come in two basic varieties, the monocular (view with one eye) and the binocular (view with two eyes) and manifest

the next step in furthering one’s microscopy interests.

The Research on compound scopes

Articles in Micscape:

- “The Compound Microscope – A brief introduction” by Gregor T, Overney PhD.
- “Introduction to the Optical Microscope” by Ron Neumeier
- “Buying a Microscope and How to Use it” found in the Micscape Introduction to Microscopy suite of pages.

Although not available to me at the time, the Bruce Russell video referenced in Episode One would be an excellent addition here.

The Consensus

The obvious advantage here is higher magnification. The disadvantages (for the beginner) are the much more complicated: lighting techniques, specimen preparation and extremely small working distance at high magnification, (a high degree of focusing awareness is required here to make sure you don’t go crashing the lens thru a slide).

Binocular or monocular? The binocular version is less fatiguing and much easier to use, as we shall see.

The applications

A quick assessment of my scopes was in order so I began with the stereoscopes. Looking thru the San Francisco Tech (SFT) scope revealed clean optics and a sharp image. Then the Spencer stereoscope. The optics really needed a cleaning but the image had a completely different “feel” (for lack of a better word) to it. Much better than the SFT scope. I kept going back and forth between the two trying to determine why. Here is what I found.

The stereo microscopes use two sets of completely independent visual paths

Fig. 4A: An extremely light coating of white lithium grease was placed on the threads of the eyepiece tubes and the focusing dovetail slide, rack and pinion.





Fig 5A. This light took about 30 minutes to assemble from a \$5 pawnshop tool stand and a “salvaged from I can’t remember where” 100W track light.

and lenses to focus on the subject at slightly different angles and gives us a three dimensional image (a pair of binoculars does the same thing but for distant objects). The alignment of these images is called collimation and if the instrument is not perfectly adjusted, the images will overlap somewhat like when you slightly cross your eyes. If the images are just slightly out of collimation the eyes will involuntarily correct themselves to form a collimated image just as our eyes do to naturally try and focus on the every day images we view directly. This unconscious correction will go virtually unnoticed if viewed for a limited amount of time or there is no direct comparison to an aligned image. After time the resulting eyestrain will become readily apparent and uncomfortable. The SFT scope was out of

collimation and the Spencer was dead on the mark. I didn’t notice it until I had the two side by side. The difference was amazing, the Spencer was sooooo... much more comfortable to view thru.

NOTE: A binocular compound scope also collimates the images but does so as parallel visual paths before the single objective lens thus giving a flat two dimensional view. Just like a telescope with one front lens and two eyepieces would do. (This makes me wonder, shouldn’t binoculars be referred to by the more accurately descriptive term “stereonoculars” and telescopes as “monoscopes”? Just a thought...I don’t think it will catch on)

So even if I can successfully realign the SFT scope the Spencer would still be the more versatile if a cleaning of the

exterior and interior optics can be accomplished.

Which of the monocular compound scopes to refurbish? This was a no-brainer. The 1920s Bausch & Lomb was the only complete monocular scope of the lot and was fully functional except for the easily remedied seized inclination joint between base and arm. The 10X and 43X objectives appeared clear as well as the 10X eyepiece. I didn’t check the 97X objective for functionality other than an exterior inspection as I had no slide to view, it required immersion oil, which I did not have and the lens spring-loaded mechanism was seized. All of this was of no real concern as there were plenty of affordable after market lenses available of comparable or better performance. Since this was a monocular scope there were no collimation difficulties to deal with but upon examination of objects with the lower power objectives, a totally unanticipated visual bugaboo came to the fore.

Except for the notable likes of Vice Admiral Horatio Lord Nelson, Moshe Dayan and Rooster Cogburn, those of us with two good (but aged) eyes tend to use both for our normal regimen of ocular sensory input. Those mechanical contrivances that preclude the use of both eyes, such as telescopes, monocular microscopes and peep holes, force our visual receivers into an unnatural state. The recommended technique is to keep both eyes open and try to allow the brain to present the view through the instrument as the “dominant” image. The common practice of closing one eye to force this image puts a strain on the open eye to focus and can contribute to rapid viewing fatigue. In my particu-



Fig. 6A. So, at last, a near factory perfect 65-year-old Stereo Microscope.

lar case I was having great difficulty with allowing my brain to present the instrument image as dominant on its own (actually, allowing my brain to do anything on its own results in a less than satisfactory eventuality). I tried closing one eye but it made the other squint, a cupped hand over the eye worked but then I could not focus and manipulate the viewed object at the same time. My older microscopy technique manuals illustrated various hoods, shades and blinders that mounted on the scope or eyeglass frames but none were at hand. My optometrist held a black cup shaped disc attached to a wand over my eye when I read the eye chart and I thought this would be a solution but quickly realized I couldn't afford to hire the optometrist full time.

What to do?

An eye patch! And I just happened to have one. A little rummaging around produced a really nice black leather eye patch with a leather band to tie around the back of my head. This had been anticipated but unneeded after my cataract surgery. Unused and perfect! It worked, but after a very short time there were some severe side effects, to wit:

While still adorned with this ocular accessory I caught a glimpse of my image in the mirror and was somewhat amused at the apparition. I seemed to be looking at another persons face. It was getting late, my energy was waning so I finished my after dinner aperitif of amaretto laced with coffee and further pondered my reflection. An eye patch for Pete's sake. It was the quintessential

badge of the seafaring rogue. I thought back to my own days on the sea in the Caribbean, the pirate stories and boisterous celebrations. I soon became caught up in the rever of my memories and was muttering "Arrrgh" and "Avast me hearties" for no apparent reason. I began searching for a peg leg and fifteen other fellows with which to stand on a dead mans chest. Unable to contain myself I grabbed a tri-corner hat and an 18th century cutlass from my collection and burst upon my wife in the living room brandishing my weapon and bellowing "PREPARE TO BE BOARDED"!

Now...Dr. Phil tells us that there are marriages that tolerate, endorse and even encourage such displays in return for certain intimate rewards. However,

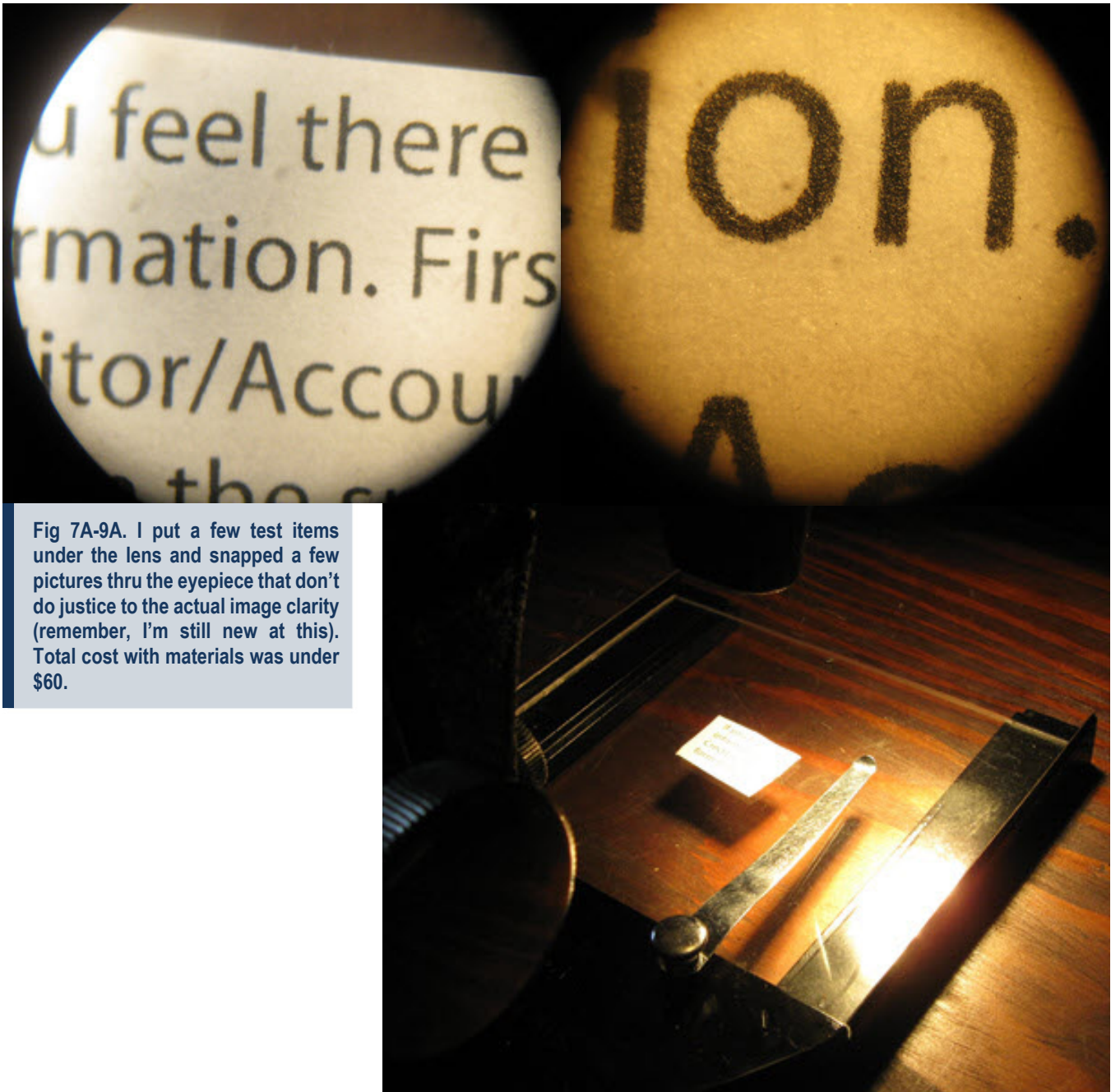


Fig 7A-9A. I put a few test items under the lens and snapped a few pictures thru the eyepiece that don't do justice to the actual image clarity (remember, I'm still new at this). Total cost with materials was under \$60.

the prevailing interactive social protocols and policies in my abode do not make any provision for such aberrant behavior (I think it was the coffee). I was stopped cold in my tracks, very cold, by my wife's spontaneous outburst of stunned silence and shortly thereafter I was de-patched, disarmed and further disciplined with one hand on her hip and with the other on the end of a gracefully outstretched arm and gently curving wrist she indicated with a delicate index finger the precise piece of living room furniture that was to be my accommodations for the evening.

It was this moment; in a rare flash of lucid cognizance I realized a monocular

compound scope was not to be my choice for serious observations.

(I now had to have a binocular scope. The mere formation of this thought stirred the evil spirits of eBay once again but since having the shock therapy I was in more control of myself this time. I should have the results of this search by the next article.)

The restoration and test drive of the 1944 Spencer

Sounds like a car doesn't it? The view thru the Spencer was dim, grainy and not a lot of contrast. These are the signs of a really dirty optical train. Re-

moving the eyepieces and looking down thru the tubes I could see the surface of the first prism. It was loaded with dust in a tacky light film. The eyepieces were also dirty inside and out and the plano convex field lens had its flat side facing down in one and the convex side facing down in the other. The objective lenses had the same dusty film as well as did the bottom prism face. Some disassembly was going to be required to reach all the surfaces on the prisms and eyepieces but not the objective lenses. I started looking at the recommended methods to clean these surfaces.

The Research

Articles reviewed were:

- “Microscope objective lens cleaning – Best practice review” by Colin Duke
- “Lens Myths” by Paul James
- “Some thoughts on microscope maintenance and repair” by Robert Pavlis
- “The clean microscope” by Zeiss Optical

The consensus

The jury remains out on the best technique for cleaning optical surfaces due to the variables of lens construction, coatings, types of dirt, dust, grease and other contaminants affecting the image. The Zeiss pamphlet states, “The choice for the best cleaning method depends on the nature of the optical surface and the type of dirt to be removed”. In the above listed articles the list of solvents was many and their possible harmful side effects to the optics was sometimes extreme. There were some contradictory opinions on materials and technique. The only constant seemed to be a gentle touch and the least amount of pressure and motion possible when direct contact between optical surface and swab/lens paper is the last resort. My final weapons of choice from the recommendations were:

- Squeeze bulb to blow air
- A very soft brush such as found in cosmetic departments
- Kleenex
- Lens tissue
- Synthetic swabs (Not cotton Q-tips)
- Distilled water
- A mixture of 5-10 drops of a plain, basic liquid dish soap to 10 ml distilled water
- 91% Isopropyl alcohol
- A benign, aqueous based commercial lens cleaning fluid i.e. Rexton lens and optical surface cleaner
- A 10X Loupe or small 10X magnifying glass

The application

Disassembly was now the first order of business. The only technical informa-



Fig 10A. Some more test items under the lens.

tion I had on the internals of this scope was a cut away drawing showing the prism arrangement of a similar scope from a 1929 catalog. Other than that basic information I was pretty much on my own. My background in dealing with the intricate and sometimes intentionally confusing trigger mechanisms of things that go boom in the night helped, and that, coupled with my high mechanical aptitude and rugged good looks should make this a doable effort.

The intention here is to disassemble this instrument enough to clean the optics but not disturb the near perfect collimation. To get to the prisms, the black stamped metal covers under the eyepiece tubes must be removed. They are each held in place by three small screws and the eyepiece tubes, which are probably screwed into place. In order to undo the screws properly and with no damage I did two things. First I put a single drop of PB Blaster on each screw and let it set overnight (This stuff will loosen money from a Scotsman). Then in order to get full mechanical advantage and not damage the screw or surrounding material, I used a screwdriver that perfectly fits the slot as to width and depth (This is extremely important, if you don't have one then grind or file

one until it does. I have about 50 assorted flat bit screwdrivers for this purpose but I still occasionally have to grind a new one). After these were removed I gripped the right eyepiece tube and gave it a firm twist to the left and was able to unscrew it. Had to use a pair of padded pliers to coax the left one into compliance. The covers were removed and the prisms exposed. (Fig 1A)

The actual cleaning of the optics

(Why has it taken so long to get to this point?) As the Zeiss pamphlet stated, we must understand the nature of the dirt. (This is akin to analyzing a National Enquirer article). Who knows when the last time (since 1944) this instrument was cleaned. The “dirt” seems to be years of accumulated dust and oily residue. No way to immediately determine if the contaminants have contributed to any etching of the optical surfaces. There seemed to be no fungus growth. I tried to take the most conservative approach by trying the least aggressive cleaning techniques in succession until victory was achieved.

A very time consuming regime was applied. First, was to simply blow away anything I could with a blast of air and

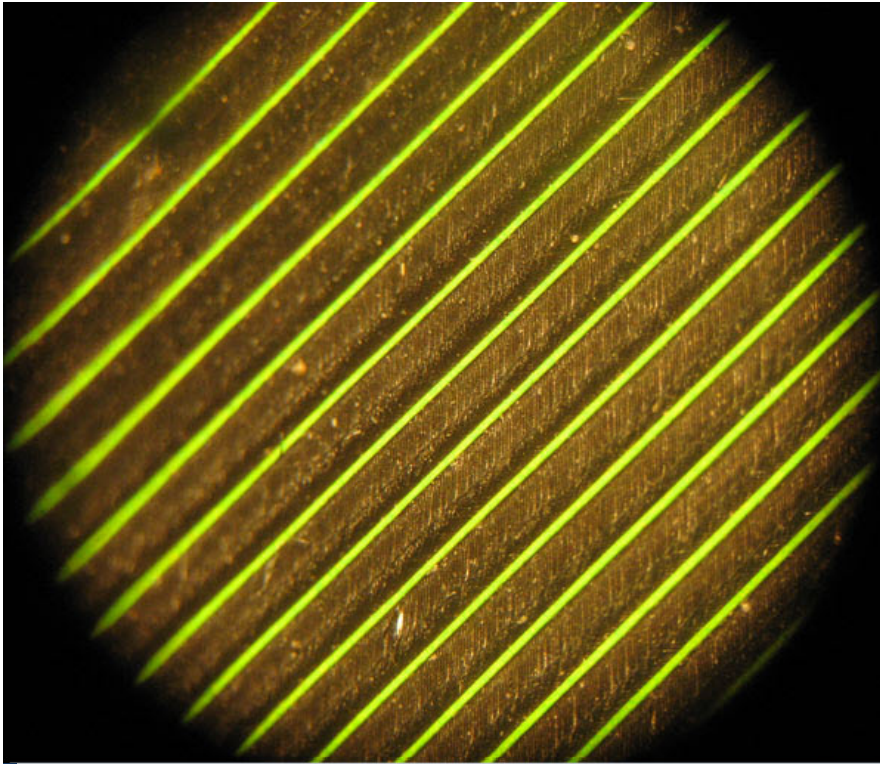


Fig 11A. Some more test items under the lens.

then remove whatever dust or particles would adhere to a light stroke of a soft brush. As the brush became a bit oily after a single pass over a surface, it had to be cleaned with soap and water immediately before reuse. Then I placed a drop of the soap and water mixture on the prism surface facing up and with one end of the lens swab on an upturned finger and the other on the prism surface I was able to spread the drop with no more pressure than the weight of one end of the swab. The rationale here was to emulsify any surface oils and suspend any dust particles so they could be blotted away with the Kleenex. This was followed with an identical treatment with the Rexton optical surface cleaner and blotting. A final wipe using the frayed/torn end of some lens paper dipped in alcohol and inspection with reflected light and the loupe completed the process.

The temptation to “scrub” the optical surfaces just a little itty bit with the swab was almost overwhelming but I was able to resist and just repeated the above routine as many times as necessary for each surface, sometimes four or five.

The end result, I am delighted to report, is a flawless optical path. There

are absolutely no scratches, smears, etching or any other defect that I could discern with the loupe! One tiny spot totally outside the visual path at the edge where the prisms were cemented together was the only visible blemish anywhere.

I did a thorough cleaning of the wrinkle finish paint on the main body and placed a fresh spray of black enamel on the prism covers and the base. Left out in the hot Florida sun, it baked on as a hard durable finish. Finally the light was disassembled (Fig 2A) and cleaned and all the chrome knobs clips etc were soaked in a weak commercial solution (Foz or Ospho) of phosphoric acid for an hour. (Fig 2A) An extremely light coating of white lithium grease was placed on the threads of the eyepiece tubes and the focusing dovetail slide, rack and pinion. (Fig 4A) The inside of the prism covers were wiped with alcohol. The wayward field lens was reversed in the Kellner Ramsden eyepiece. (Identified as such from the illustrations in the 'Microscope Anatomy' section of the Molecular Expressions web site).

Reassembly was pretty straightforward and since I had no bulb for the light I cobbled together this light from

spare parts in my shop. It works and I think it looks appropriate. (Fig 5A)

The Denouement

So, at last, a near factory perfect 65-year-old Stereo Microscope. (Fig 6A) I was happy as Chauncey with moonshine. I put a few test items under the lens and snapped a few pictures thru the eyepiece that don't do justice to the actual image clarity (remember, I'm still new at this). Total cost with materials was under \$60. (Figs 7A thru 11A).

I feel I am on my way to becoming a world-class microscopist if I can stay off the couch. I already have some results for the binocular microscope but I will save them for the next article, as it will be a rather ambitious project.

So now I close this episode with a few final words in response to some of those kind souls who responded to my last article.

To those who were rather insistent that there were two items I needed “To get” i.e.

1. “A life” and
2. “A grip”

As to the former, I can state with some certainty that I am not writing posthumously and to the latter, after some checking I found I do have an old but still serviceable valise but why the interest in my travel accessories is beyond me.

To the gentleman who asked if I was a relation to Mr. Richard Howey:

First of all, my apologies to Mr. Howey. Other than the firm conviction that Mr. Howey would (and should) rebuff this association with some vigor I can assure you I possess neither his great literary endowment nor his distinguished scholar's pedigree.

Mr. Roshak, your relevant information on the eyepiece tube diameter will be included in my next composition along with the RMS instructive.

Until next time, I am off for further jousting with reality and leave you with my mother's advice, “Alcohol and calculus don't mix, so don't drink and derive”.

My Fascination with Microscopy

*"To see a world in a grain of sand,
And a heaven in a wild flower,
Hold infinity in the palm of your hand,
And eternity in an hour."*

William Blake, Auguries of Innocence.

By Michael Gibson



I suppose a good place to start is at the beginning! As a boy of around ten years of age, I remember getting my first microscope which was a small J. & L. Randall x70 "Merit Microscope", and using it to look at onion cells. This experience has never left me and, although now more than fifty years on, I still find great delight in just taking a sample of pond water, or scraping of cheek cells from inside my mouth, and making a simple slide for observation under a microscope, applying different lighting conditions as the need arises. At secondary school, I also vaguely remember using their one and only Baker electric microscope that was, for most of the time, kept in a glass fronted cupboard and only brought out on special occasions.

Some years later, in the biology classes at St Mary's Teacher Training College, Twickenham, we students were taught how to set up and use a microscope, as well as fix, stain and make permanent mounts of both plant and animal cells from a wide variety of sources. These practical activities would sometimes involve the use of a hand microtome for section cutting of plant materials and wax-embedded specimens. Each session inevitably ended with detailed drawing, labeling and hand-written note-taking of specimens observed. It must be remembered that all of this took place back in the mid 1960's, when laptop computers and digital cameras had not yet been invented!

The first "proper" microscope I used during those early days was a no-frills, black enameled Charles Baker student monocular. Because of a congenital eye problem, I was also allowed to work with the department's Gillett and Sibert

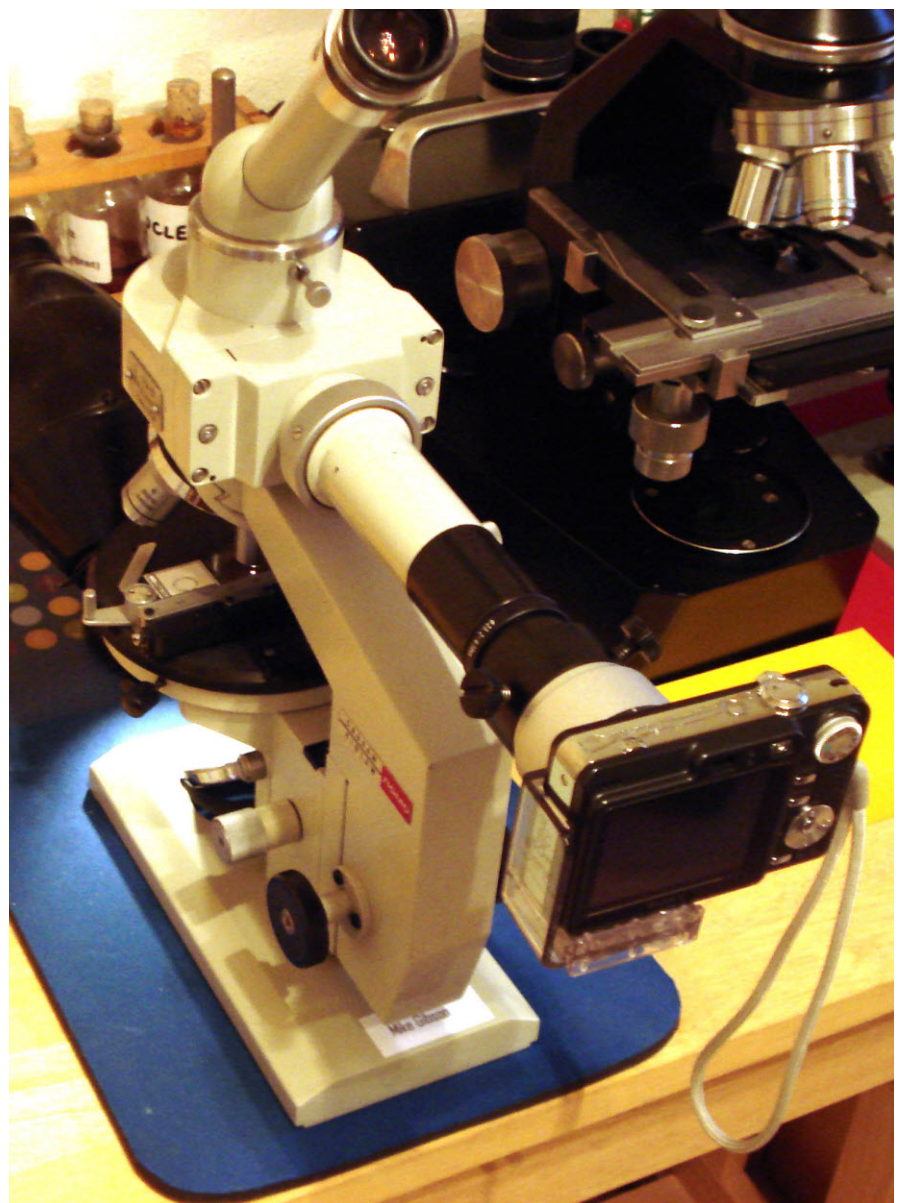
Baker microscope

Conference stand which had a large detachable viewing head in place of the usual monocular draw tube. This was a great advantage over the conventional monocular head in that it enabled me to both look at the image and draw at the same time without the need to remove spectacles. The students in those days did not have access to microscopes with binocular heads, but in later years I have never really been able to use a binocular microscope effectively, preferring instead the type of inclined monocular head typically found on the Russian Lomo Biolam stands.

In addition to my favourite Russian scopes, I have recently restored a lovely old Baker microscope and also managed to acquire from a friend, a G&S Conference Research Microscope similar to the one I used at college, (but without the viewing head). Most of my other microscope accessories have either been bought on eBay, or purchased at microscope club meetings. My latest acquisition is a beautifully designed and constructed Baker series 4 microscope which was given to me by another friend and member of the Postal Microscopical Society.

As a science teacher I had been involved in teaching aspects of microscopy over many years, but it was only recently that I began to study microscopy as a serious hobby. Suffice to say, I bought my first half-decent microscope on eBay and joined the Northamptonshire Natural History Society! Of course, thanks to computers and Internet access, it is now relatively easy (and cheap) to purchase a good second-hand microscope and any other additional items in the way of chemicals and accessories that one might need. Also, there are now many Internet sites devoted to microscopy where one can readily get advice and help.

For my photographic work, I use a Sony Cybershot DSC W50 digital camera attached to a Sony camera adaptor (again purchased on eBay). As I also enjoy doing the occasional drawing of specimens, I use a camera lucida specially designed for the Lomo Biolam microscopes.



Top: My G&S microscope
Bottom: My LOMO setup

Early experiments in trying to take photographs through a school microscope were started when I acquired my first fully-adjustable 35mm film camera. With the arrival of digital cameras, the process became easier and, of course, less expensive. I do not intend to go into any detail here about the various techniques and “tricks of the trade” one might apply in taking photographs through a microscope since there is plenty of information already available either in books or through the Internet. However, much of my initial trial and error experience in using a digital camera involved trying to find a method of positioning the camera lens as close to the eyepiece as possible and ensuring that both microscope and camera were in focus.

Whilst working with children in the classroom, I have since found that most digital cameras, even the fairly cheap models with fixed focus lens systems, can be utilised in taking quite reason-

able photographs with a microscope. Many professional set-ups do seem to require that some sort of physical connection is made between the eyepiece of the microscope and the camera lens. While this is desirable, it is by no means essential, or indeed possible, with some makes and models of camera, particularly those with a variable focus lens element. In my experience, one of the most important factors in setting up a satisfactory camera-microscope link is to have a sturdy, yet lightweight supporting system in the form of a universal adaptor that can be adjusted to fit securely onto the microscope tube and at the same time hold the camera in the correct position, as near to the eyepiece as possible. It is also important to remember that if your camera has a choice of settings, select the distant focus (landscapes) option and switch off the autoflash function.

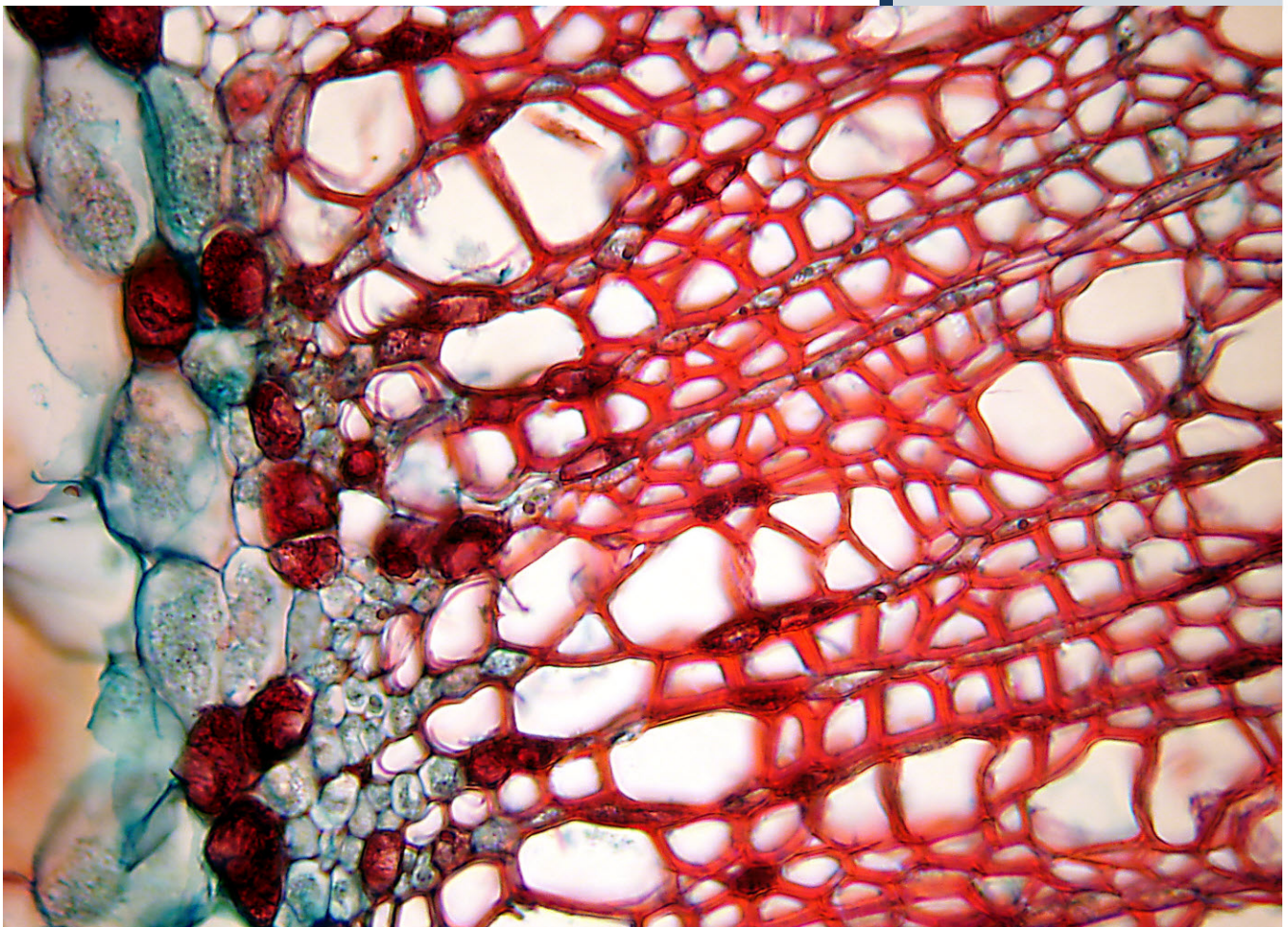
When taking photographs through the microscope's eyepiece there will

always be some vignetting of the image. Vignetting can be reduced, and sometimes totally eliminated, by using a wide-field, high-eyepoint eyepiece or ocular, and operating the camera's zoom feature, should it have one. Any remaining vignetting of the image can later be cropped using your computer photo manipulation software. And now for some practical examples....

1. *Podocarpus macrophyllus* or Buddhist Pine is native to Southern Japan. Here we see a transverse section of part of a leaf in so-called “pseudo darkfield” (produced in Adobe Photoshop using the “invert” effect).

2. Pinworm, transverse section from inside the human appendix. This photograph was taken with a 40x objective and shows just a tiny portion of a prepared slide by Robin Wacker. The various organs inside the body of the pinworm are clearly visible in this brightfield image.

Left: *Tilia* stem



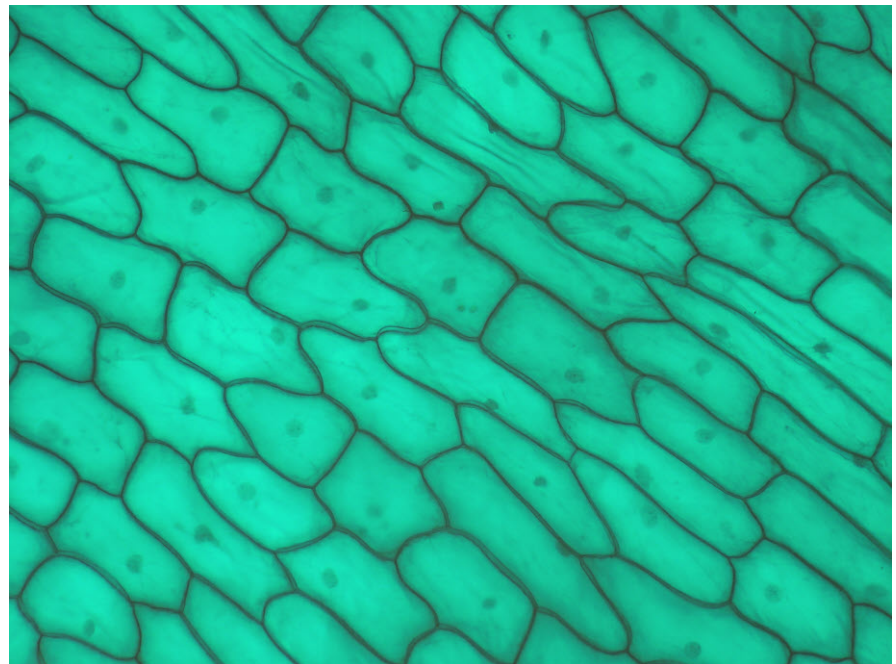
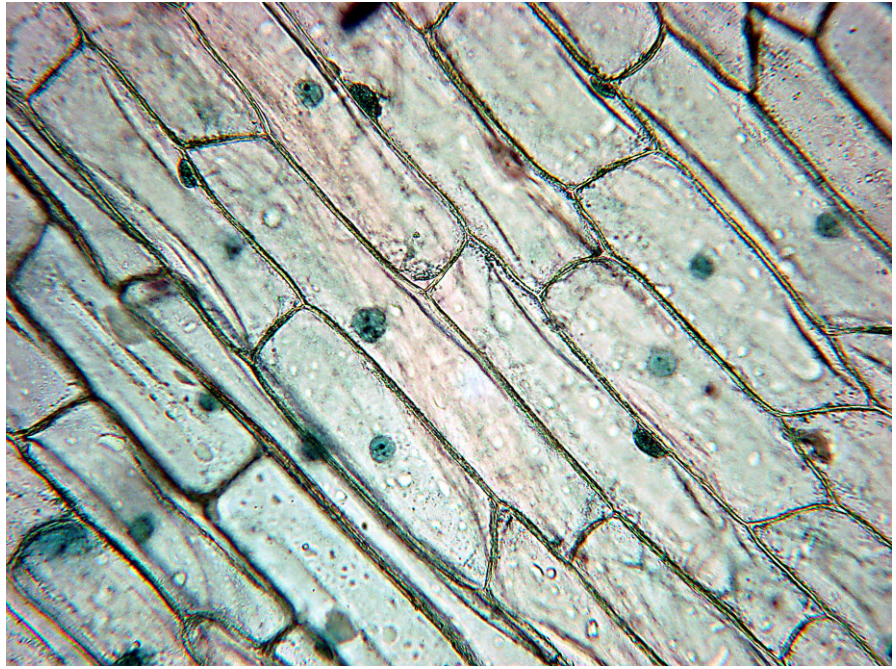
3. *Tilia* (lime) stem, transverse section. This slide is one produced by NBS (England) in the late 1970s and is fine example of what can be achieved by double staining with safranin and fast green.

4. *Arachnoidiscus ornatus* is a classic example of a Victorian diatom slide produced for exhibition purposes in the late nineteenth century.

5. Section of a whalebone from a slide by Wheeler. This cross-polarisation photograph was taken to show the unusual effects one can sometimes achieve using different lighting techniques other than brightfield.

Based on work I had done with schoolchildren in Northampton, I recently exhibited at a Quekett Society meeting in London a selection of photomicrographs showing various phase contrast microscope images of human cheek cells at different magnifications. Linking with these pictures was a practical demonstration of how DNA could be obtained from these cells fairly simply using salt solution mouthwash and a mixture of washing up liquid in water. The science behind this technique is equally straightforward and requires no special equipment other than a clean glass and alcohol, as indicated below.

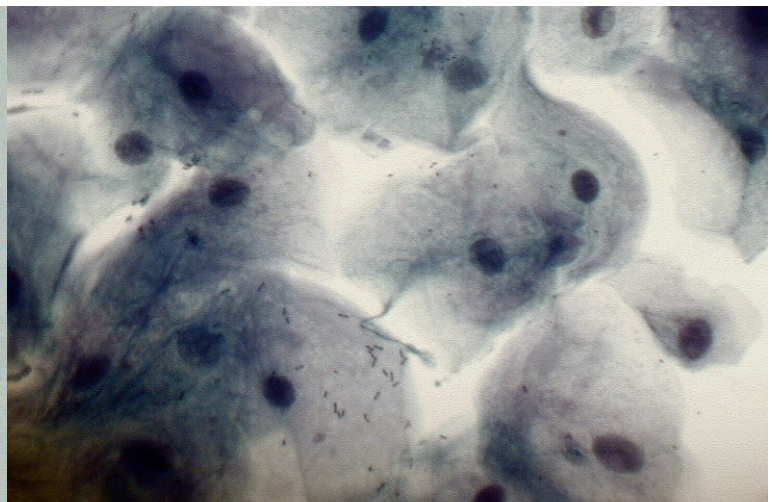
Swishing salty water round your mouth removes epithelial cells similar

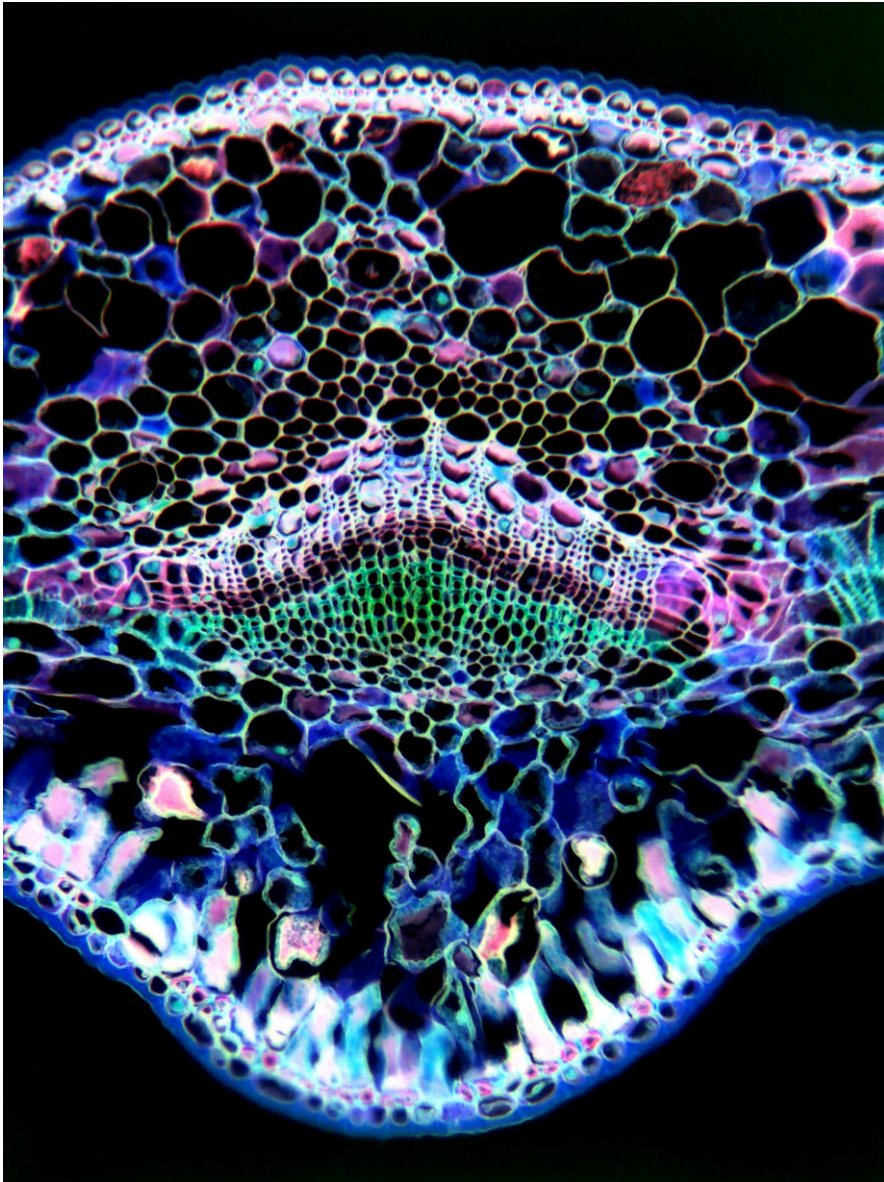


Bottom left: Cheek cells in phase contrast

Bottom right: Cheek cells stained with methylene blue

Right: Onion skin



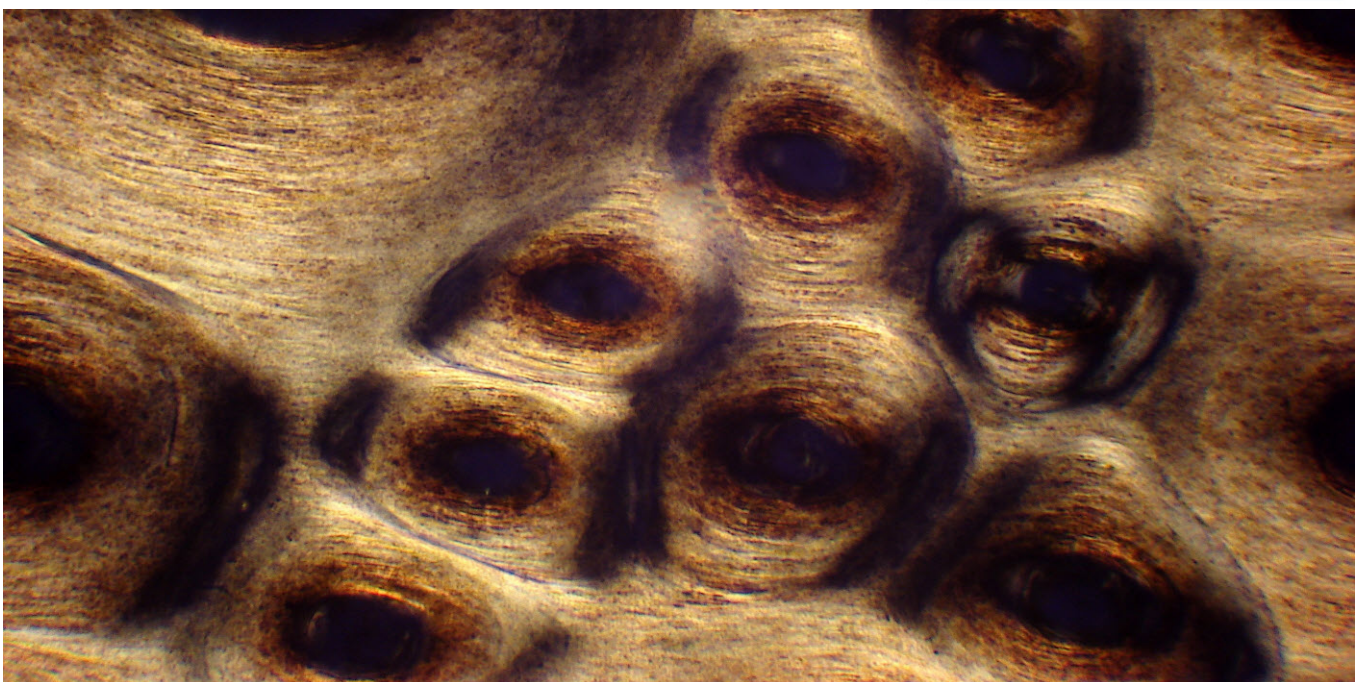


to what happens when forensic scientists obtain a swab sample from inside the mouth. After rinsing it around your mouth for half a minute or so, the mouthwash is spat out and stirred thoroughly into a solution of 1:3 parts washing up liquid and water in a small glass tumbler. This breaks down the cell membranes and releases the DNA from the nuclei. Finally, a couple of tea spoons of ice cold (very important!) alcohol (eg. vodka) are poured carefully and slowly into the mixture forming a clear layer on the surface. After a minute or so you should be able to make out what appears to be strands of whitish threads starting to appear in the clear alcohol layer. Because the DNA is soluble in water, but not in alcohol, the DNA precipitates out in the white threads you see floating in the alcohol.

In addition, the exhibition made reference to the work of Northampton-born scientist Francis Crick, who together with co-worker James Watson, were the first to reveal the nature and structure of the DNA molecule. Refer-

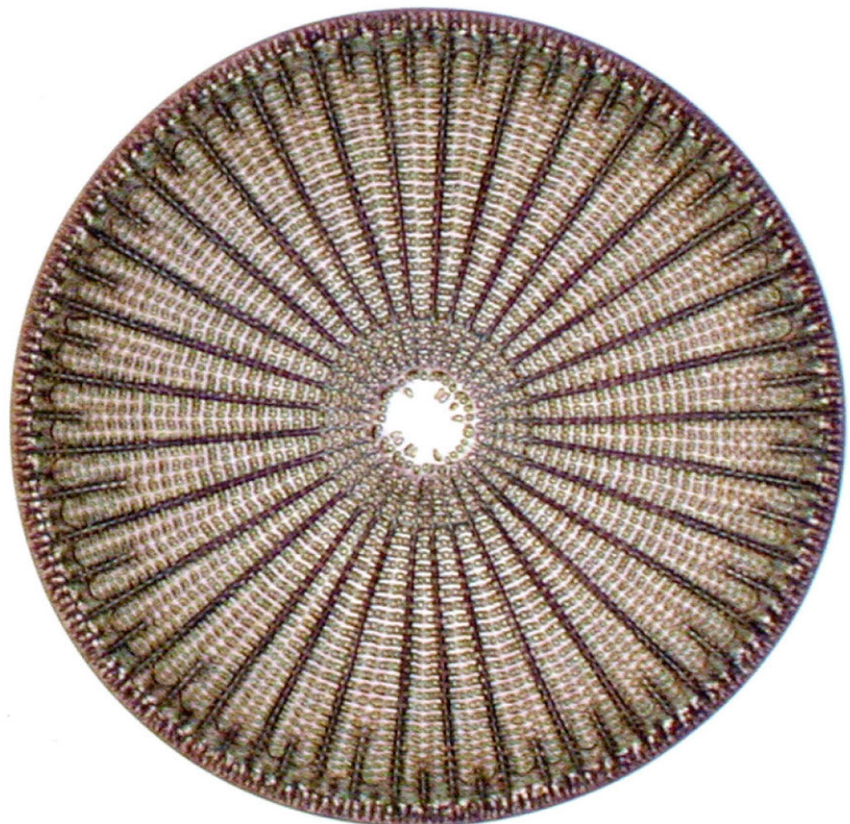
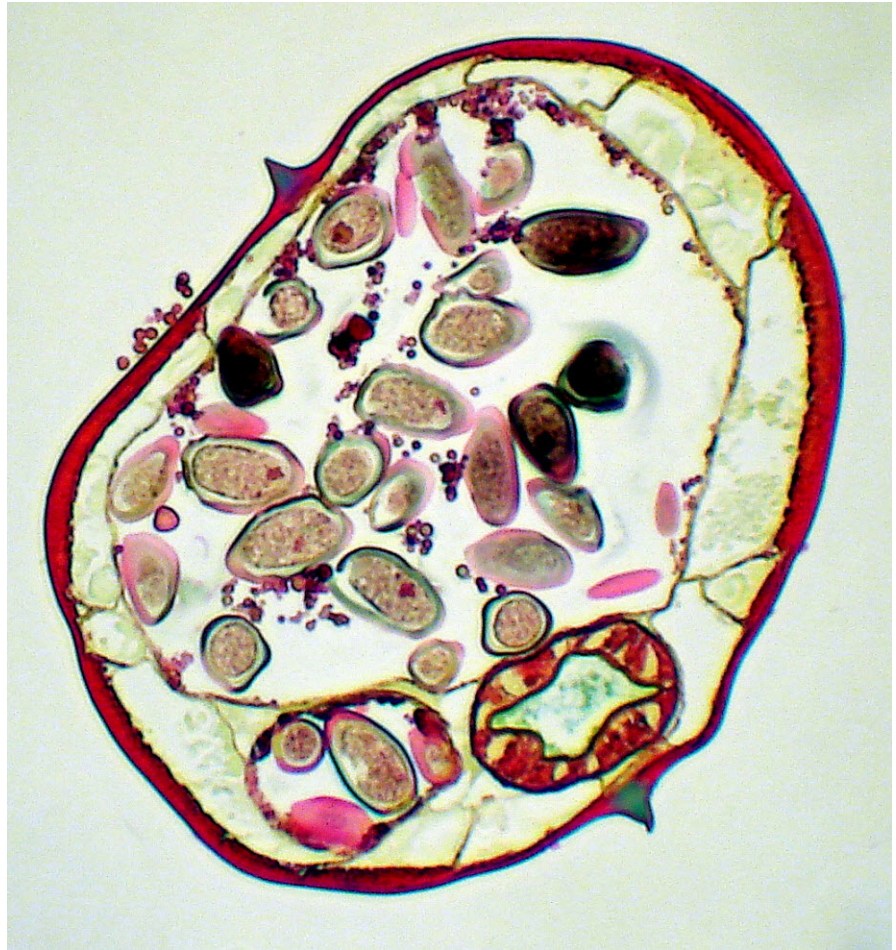
Top: *Podocarpus macrophyllum* leaf (transverse section), inverted colors

Bottom: Section of a whalebone.



ence was also made to the contributions of scientist Rosalind Franklin, whose X-Ray crystallography photographs showed clear evidence of the double helix structure of DNA and thus gave Crick and Watson significant clues in understanding how to construct the theoretical model. Although I have the greatest admiration for Crick and Watson, and their co-worker Maurice Wilkins, who together were awarded the 1962 Nobel Prize in Medicine, I cannot help but feel sadness for Rosalind Franklin who died in 1958 and was therefore unable to share the award!

So next time you happen to be passing through Abington Street in Northampton town centre, do stop and look at the commemorative sculpture by Lucy Glendinning, entitled "Discovery". It stands more than eight metres high and celebrates one of the most significant scientific discoveries of the 20th Century. Furthermore, what is not often appreciated, is that it is a lasting and fitting tribute to a local lad made good in the world of science and whose father was a member of our own Northamptonshire Natural History Society. ■



Top: Pinworm (transverse section) in human appendix.

Bottom: *Arachnoidiscus ornatus*, a diatom

Do-it-yourself Microscope LED Illuminator

Damaged halogen illumination system? Don't throw out your microscope just yet! It may be a good opportunity to try out LEDs.

By Suphot Punnachaya

I have a binocular microscope from China and have been using it for a while. The microscope has a built-in 12V halogen lamp with a dimmer switch as an illumination system. It was hot and showed a yellowish color especially when adjusted to a low brightness. I changed several halogen bulbs last year. When I used high magnification with the dimmer adjusted to high brightness, the halogen lamp burned out very quickly, which is not unusual for microscope illumination systems using halogen lamps.

One day the power supply circuit for the illumination system was blown out, I open the base of microscope to check. It seemed to me difficult to repair. The fuse was still good and the line voltage was supplied to the circuit board. Some components may have short circuited or blown out, so I considered replacing them. Now it was a good time to replace everything with a LED illumination system. The LED has a bright white color, is cooler, consumes little power and has a long life.

My goal was to replace the halogen illumination system with low cost LED illumination system. It is to be built from readily available electronic parts and to be fitted into the microscope base in the same way as the original illumination system. It should be easy to make, have variable brightness and be bright enough for use with a high power oil-immersion objective. I selected a small 9-LED flash light as a light source and used a 5V mobile telephone charger as a power supply. One current limiting

resistor and a variable resistor (potentiometer) completed the system.

First of all I do not recommend this project to everybody who uses a microscope. It is also not recommend for junior high school students to do it by themselves. You should have some basic knowledge in electronics and must understand circuit diagrams. Additionally you need some basic electronic equipment and must be able to solder electronic part together. This project is not difficult, it can finished in less than one day. But the illumination system is connected to line voltage (220V or 110V), so you must use safety guidelines for electric work to avoid electric shock hazard. By the way, if you can not do it by yourself, a friend who knows the basics of electronic repair or an electronic technician can do it for you easily.



The electronic parts that I used in this project is as follows:

- One super bright 9 LED heavy duty compact Aluminum flash light, powered by 3 AAA battery.
- One 100 – 240 Volt AC input, 5V DC Output 350 mA or 500 mA mobile phone charger with a small round jack output (not a USB output). Select a small and flat one as shown in the picture.
- One 1 K Ohm wire-wound linear scale variable resistor (potentiometer)
- One 100 Ohm 0.5 Watt resistor, and PVC electric insulation tape

WARNING! For your safety from electric shock hazard, you should completely unplug your microscope power supply cord from the electric outlet before disassembly and modification!

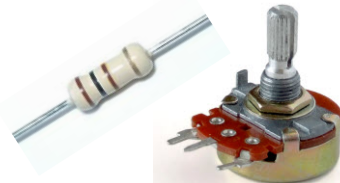
The basic tools that I used in this project were:

- Digital Voltmeter
- Low power soldering iron (15 Watts)
- Wire cutter pliers
- Pliers
- Small metal hand saw
- Medium sizes Phillips head screwdriver
- Jeweler's screwdriver
- Metal files
- Double sided foam tape

For step one, I completely unplugged the microscope power supply cord from the electric outlet. If you just finished using the microscope, you need to wait at least 30 minutes to allow the Halogen lamp to cool down. Next step, I disassembled the microscope base to check the available space. You may need to remove the eyepieces from the eyepieces holder and keep them in a safe place, and then use two hands to carefully tilt the microscope 90 degrees backward until the microscope firmly stands on its own. If your microscope



- Super-bright 9 LED heavy duty compact Aluminum flashlight
- 100-240V AC input 5V DC output 350mA mobile telephone charger with round jack output
- 1K Ohm wire-wound linear-scale potentiometer (far right)
- 100 Ohm 0.5 Watt resistor (left)



Right: cc-by-sa by lainf
Left: cc-by-sa by Maksim, Erik Baas

can not stand on its own in this position, you may need a few heavy books to support the microscope on both left and right side of the microscope. Do not tilt the microscope to stand on the left or right side, as this may damage the mechanical stage of the microscope!

Unscrew the microscope base by using the medium sized Phillips head screwdriver and take the microscope base out of the microscope body. Now check the space inside the microscope base to make sure that the available space is enough for the new LED system. The diameter of the super bright 9 LEDs flash light is about 1 inch (about

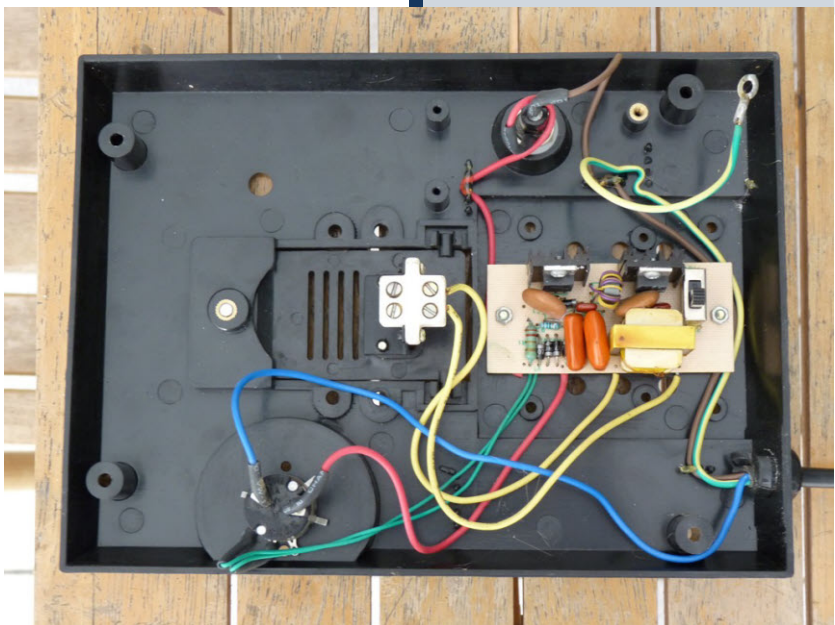
2.5cm) and the height of the flashlight should be the same position of the halogen lamp. I used the ruler to measure the halogen lamp height from the microscope base and uses this value to cut the LED flashlight to fit. This made the LED sit in the same position of Halogen lamp. The lens at the base of microscope could focus the LED to microscope condenser lens exactly the same way as the halogen lamp. In my microscope, the height is 1.25 Inches. Then I removed the Halogen lamp out of the microscope base and keep it in the box.

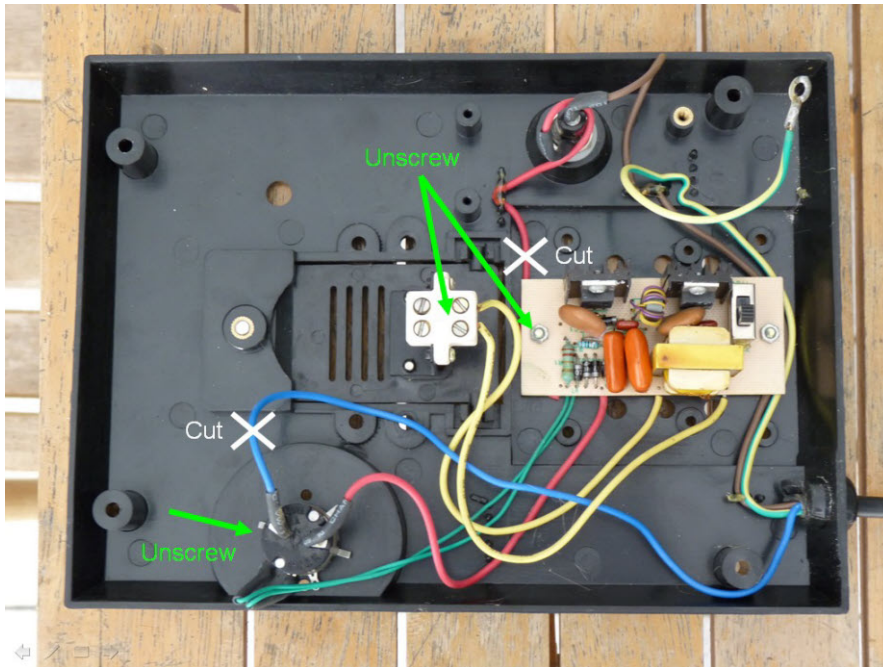
Be careful when you use the ruler to measure the halogen lamp height. Do

not touch the halogen lamp with your bare fingers. When you remove the halogen lamp out of the microscope base, use soft cloth or paper tissue to cover the lamp and remove it by hand. You may need to unscrew the halogen lamp from the base if it is locked in place by a screw.

After checking, my microscope base had enough space to accommodate the LED flashlight. The mobile phone charger could sit in the same place of existing power supply circuit board. I could

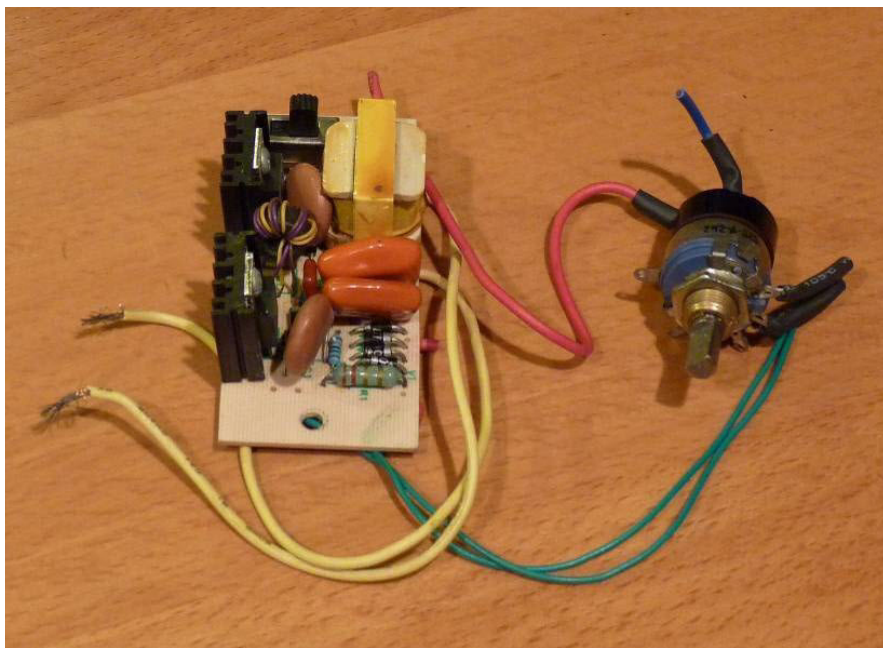
The base of the microscope.





Top: The base of the microscope. The Yellow-Green cable is ground cable, the blue cable connected to the main switch and potentiometer. The Brown cable connected to 220V fuse and the red cable is connected to the power supply board. Cut the Blue cable and Red cable, unscrew the electronics and remove the potentiometer.

Bottom: The old halogen illumination system after removal from the microscope base.



charger about 10 inches from the round jack output. This cable was used to solder the LED to the 1K Ohm potentiometer later. Then I mounted the mobile phone charger unit in the same place of halogen power supply board.

I soldered the blue cable and red cable, which were already cut, to the metal leg of the mobile phone charger and used the PVC electric insulation tape to seal it for safety. This step avoided electric shock hazard when I re-open the microscope base in the future. The metal leg of mobile phone charger may difficult to solder, you may need to scratch the surface of it a little bit by using metal files.

Next, I cut the flashlight to fit the same height as the halogen lamp. I unscrewed the flashlight to remove the battery holder inside the flash light.

Then I cut it to 1.25 inch length by using small metal saw. After cutting the flashlight to 1.25 inch length, I used small metal files to smooth the edges of the flashlight and made a notch at the base of the flashlight. This notch was needed to connect the wire from the LED to the mobile phone charger.

The LEDs use direct current (DC) for power and should be connected properly, otherwise the LEDs will not light up. So when we want to connect the output from the mobile phone charger to the LED flashlight, we should know which wire is positive and which wire is negative.

The output from the mobile phone charger looks like a single cable, but when we cut it, we can see that it contains two separate wires. I used a cutter to carefully separate them into two

3 line voltage cables on lower right hand side of the picture: a blue cable, a yellow-green cable and a brown cable. The yellow-green cable is the ground cable, I did not do anything with it. The blue cable was connected to the potentiometer (which was also the on/off switch). The brown cable was connected to the 220V fuse and it continued as the red cable connected to the power supply board.

I cut the blue cable and red cable that pass through the fuse as indicated in the picture. I then removed the on/off switch potentiometer by removing the dimmer dial first, it was locked in place

mount. Now the halogen illumination system and circuit board could be freely removed from the microscope base.

The old halogen illumination system after removal from the microscope base is shown in the picture on page 24. I kept it as a spare part. If some day in the future, I change my mind to go back to use the halogen illumination system, I can send this circuit board to repair and solder it back to the microscope base. But I think this will not be the case.

Now it was time to build and assemble the LED illumination system into the microscope base. I started by cutting the 5V output cable of the mobile phone

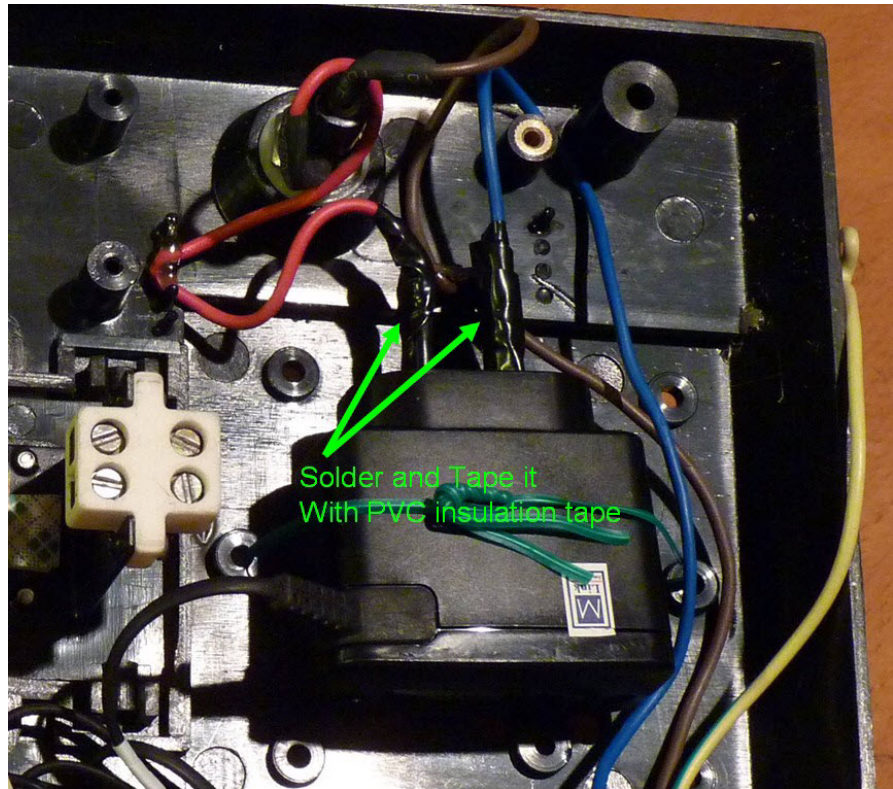
Connecting the mobile phone charger with the main power supply.

wires and used the Voltmeter to check the output from mobile phone charger cable. I made a color mark to label the positive and the negative pole.

Going back to the 9-LED flashlight, I used a soldering iron to solder the wire from LED flashlight to potentiometer and the mobile phone charger. At the flashlight backside, the center is the positive pole of the LED and the rim of flashlight is negative. I soldered two wires from these points and marked the positive and negative wire to prepare them for the next step as shown in the pictures on page 27.

Next, I cut the 1 K Ohm potentiometer rotary shaft to be the same length as the original potentiometer by using small metal saw. You can see that the original rotary shaft has a "D" shape (page 24). This is used to lock the dimmer dial to the potentiometer. The normal potentiometer is round shaft (or a split round shaft as shown on page 23). I first cut it to the same length and use metal files to finish it to "D" shape later.

Now every part is ready to complete the project. I mounted the 9-LED flashlight into the microscope base at the position of halogen lamp with double sided foam tape as shown. The double sided foam tape allows for the centering of the LEDs to the condenser lens if needed.



I mounted the 1 K Ohm potentiometer to the microscope base at the same position of original potentiometer and soldered the 100 Ohm limiting current resistor to one leg of the potentiometer as shown in the middle picture on the left. Do not worry about the dimmer dial position now, just put it in place, we will adjust it to match the potentiometer later.

I soldered the positive wire from the mobile phone charger to the middle leg of the potentiometer and soldered the negative wire from mobile phone charger to the LED negative wire. The last step is soldering the positive wire from

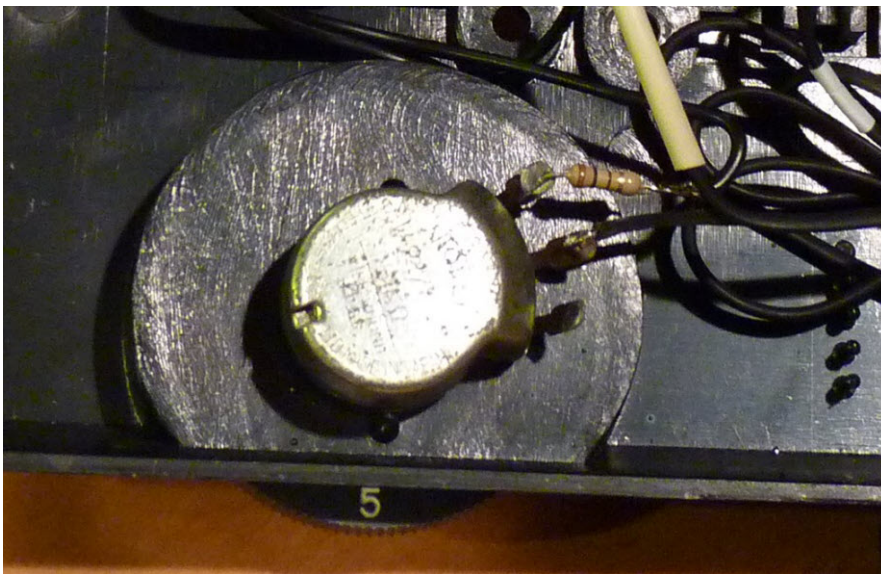
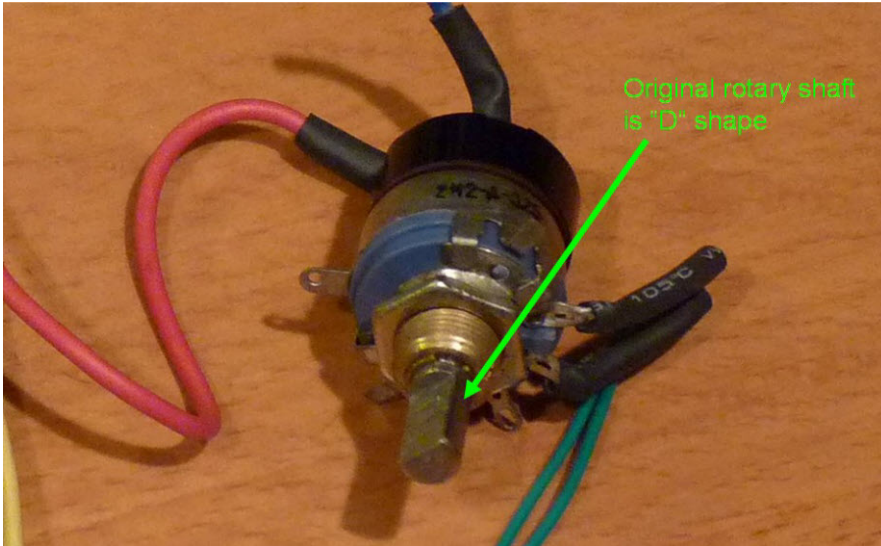
LED to the remaining leg of the 100 Ohm resistor. I covered the negative wire solder-joint with white tape to prevent a short circuit between positive and negative wire.

The diagram of original illumination system and modified LED illumination system is shown on page 28.

To test the new LED illumination system, plug the microscope power supply cord to the power distribution bar. Then turn on the switch on the power distribution bar to turn on the LED illumination system. If the connection is correct, you can see the LED flashlight turn on. When turning the rotary shaft



Unscrew the flashlight to remove the battery holder inside the flash light. Then cut it to 1.25 Inch length by using small metal saw. Use a metal file to smooth the edges.



Top: "D" rotary shaft of original potentiometer.
 Middle: the 1 K Ohm potentiometer mounted to the to the microscope base at the same position of original potentiometer. The 100 Ohm limiting current resistor is soldered to one leg of the potentiometer as show in the picture.
 Bottom: The dimmer dial mounted in place.

of the potentiometer back and forth, the brightness of LED should vary from minimum to maximum.

Turn the rotary shaft of the potentiometer to minimum and match the dimmer dial position to minimum brightness. Mark the lock screw position on the rotary shaft.

Turn off the switch on the power distribution bar and unplug the microscope power supply cord.

Now I unscrewed the potentiometer from the microscope base and used metal files to finish the rotary shaft to "D" shape at the marked position. After that, I assembled the potentiometer back into microscope base and locked the dimmer dial with lock screw. The minimum and maximum brightness adjustment will match the dial as shown on page 26. The final assembly of microscope base can be seen on page 27.

I assembled the microscope base back to the microscope body. Now it was time to check the LED alignment with the condenser lens. I removed the lens at the base of microscope by unscrewing it counter-clockwise. I plugged the microscope power supply cord to the power distribution bar and turned it on. I used the 10X objective with 10X eyepieces and turned the dimmer to low brightness while looking into the microscope.

I carefully focused the microscope until I could see the LEDs in the eyepiece. The view of the LEDs will look like the right picture on page 29. You can see that the LED is quite aligned to the condenser lens and optical axis of the microscope. You can adjust the LEDs to perfectly center them in the eyepiece. But for this step is necessary to assemble and disassemble the microscope base to adjust the LED position back and forth. In my microscope, I had to unscrew the halogen lamp mount and move it backward about 0.1 inches to center the LED.

When we finished adjusting the LED flashlight position, we need to install the lens back into the microscope base. We have now finished the modification of the LED illumination system of the microscope.

Note: This LED is very bright, always use the lowest brightness setting first to prevent eye fatigue.

From the LED picture in the eyepiece (page 29), I was satisfied with this



Unscrew the flashlight to remove the battery holder inside the flash light. Then cut it to 1.25 Inch length by using small metal saw. Use a metal file to smooth the edges.



alignment. The LED was a little bit off center on the left hand side. But when I installed the lens at the microscope base, the diffuser in the lens assembly distributed the light uniformly.

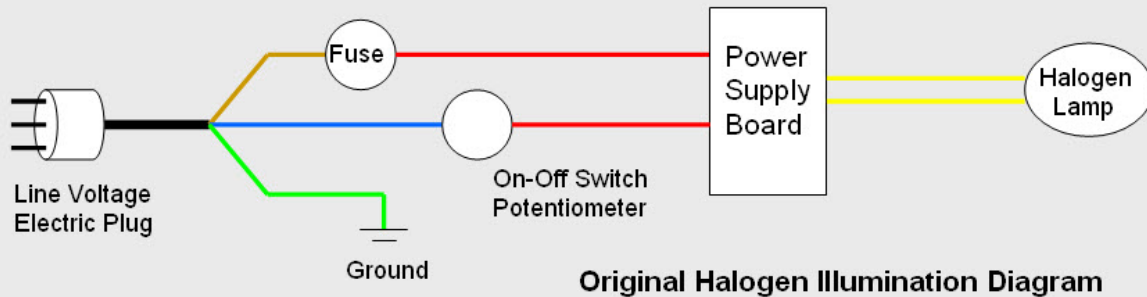
You can see the complete modification of LED illumination system in the picture on the left. Everything fits inside the microscope base and looks the same as the original one. The LED is very bright, it is bright enough to use with 100X oil immersion objective and for a low power 5X objective as well. For the use with a 5X and 10X Objective, I have to dim the LED all the way down to reduce the brightness to a comfortable level.

I used this system for a few months now. The LED color is white and no heat is generated by the LED at all. I almost never use the highest brightness setting, because it is too bright. The white color of LED is uniform between lowest setting to highest brightness. The LED is also bright enough to use for microphotography, I am quite satisfied with the result.

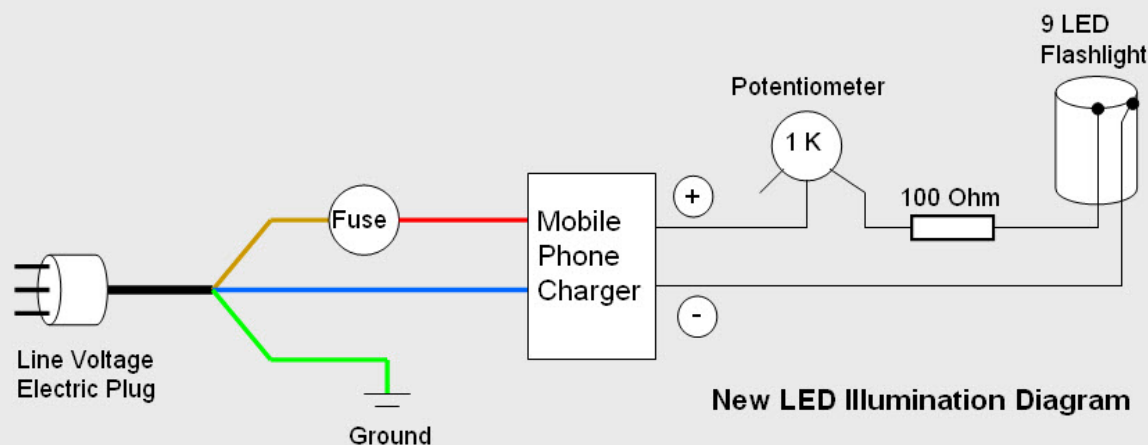


Advantages

1. I use cheap and common available electronic parts, I payed around US\$ 20 for this project.
2. The LED is white color and no heat is generate from it.
3. The mobile phone charger accepts 100 -240 V AC input. That means it is auto-voltage.
4. I need not to worry about changing halogen lamp anymore. I can save cost in long term.
5. The replacement is not difficult; it can be finished in 1 day.
6. No need to make any modification to the microscope base. Everything is fit inside easily.
7. The optical characteristics of illumination system, condenser and microscope is exactly the same. There is no need to replace the lens or adjust anything in the optical system.



Original Halogen Illumination Diagram



New LED Illumination Diagram

8. If I am not satisfied with the result, I can connect the original halogen illumination system back in place. It is only two cables to solder back (after I send it in for repair, because it was burned out).

Disadvantages

1. I could not find an integrated On-Off switch potentiometer. So my LED illumination system has no On-Off switch. When I plug the microscope power supply cord to the electric outlet, the LED is turned on, to power off, I have to pull the plug off the outlet. You may put the On-Off switch into the circuit and mount it somewhere in the microscope base. It is also not difficult, but it necessary to modify the microscope base to fit the switch in.

2. The LED is not like the halogen lamp, which can continuous light up with very low to very high brightness. The LED is a semiconductor, it has a cut-off voltage. So it can dim down until a certain level of voltage and then suddenly turns off. But in my system, it

is good enough to provide smooth brightness from low to high. In more expensive LED systems, they may use a better technique to light up the LED, so for very dim light, it may be better than this one.

3. Your existing halogen power supply circuit may be still working and you may want to use it with this system. Be aware that this is not possible and that you can not use the existing Halogen power supply circuit with the flashlight LED. Because the halogen power supply is an AC power supply, it can not connect directly to the LEDs which use DC power. The reverse voltage from AC power supply from Halogen illumination system will burn out the LEDs in a short period of time. So it may easier to replace it with mobile phone charger than try to convert the AC voltage from halogen power supply to DC voltage.

Notes

If you change your mind and want to go back to use the halogen illumina-

tion system by any reason, unplug the microscope power cord. Reverse the process to put the halogen illumination system in place. Solder back the blue cable and red cable. Then use PVC insulation tape to seal the solder joint to prevent electric shock hazard. The blue and red cable carry line voltage (220V or 110V). Make sure that the solder joint is completely sealed by PVC insulation tape!

Your microscope may not be exactly the same as my microscope. You should carefully check the space inside microscope base, the circuit of illumination system, on-off switch and dimmer. Please make sure by yourself that it can be done on your own. You may need to adjust the process according to the different from microscope to microscope.

Troubleshooting

If you plug in the power cord and the LED is not turn on, use a voltmeter

to check the voltage by using the circuit diagram above.

To prevent electric shock hazard, check the low voltage part first. By using Volt meter to check the output from mobile phone charger at the + and - sign in the diagram. The voltmeter should display DC output from 5 to 7 Volt DC. If the voltmeter displays "0", unplug the power cord to check fuse and check the solder at the metal leg of mobile phone charger. The fuse may be blown out or the connection from line voltage cable to metal leg of mobile phone charger is bad.

If the mobile phone charger can supply the DC Volt output, check the solder joint at the potentiometer and 100 Ohm limit resistor. When you turn the rotary

shaft of potentiometer, the voltmeter should display at the LED a value between 3V and 7V.

Check the polarity of LED cable, make sure that the positive wire from the 100 Ohm resistor connects to the center joint of the LED and the negative wire connects to the outside rim of the LED. If you can read the voltage at the LED but the LED is not turn on, make sure that the solder joint to the LED is good or else the LED may fail from over heating when you solder the wire to it.

Be careful when you check the line voltage at the input leg of mobile phone charger. Do not touch them. Carefully use the voltmeter tip to measure the voltage. If you need to re-solder it, un-

plug the microscope power cord from the electric outlet *first*. When you finished soldering, use PVC insulation tape to seal it *before* plugging the power cord to the electric outlet.

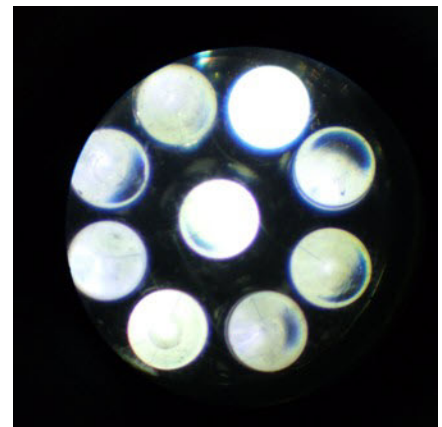
By turning the dimmer dial back and forth to set the minimum and maximum brightness you may discover that the brightness of LED is in opposite way. You should then change the soldered joint of the 100 OHM resistor to another leg of 1K Ohm potentiometer. From the picture on page 26 you may need to change the 100 OHM resistor solder joint from right hand metal leg to left hand side metal leg of the 1K Ohm potentiometer. This will align the dimmer dial orientation to the potentiometer setting.

Do not leave the dimmer dial at the maximum brightness for a long period of time. The LEDs can degrade or burn out from this setting. For normal use, I need no more than 60% of brightness setting on high magnification. It is OK to use the maximum brightness setting from time to time.

If you have any questions, please feel free to email to me at nospam_suphotp@ais.co.th (remove the "nospam_") ■

DISCLAIMER: Opening the microscope may void the warranty. Do all modifications at your own risk and only if you have knowledge and experience. Neither the author nor the publisher can be made liable for damages to the equipment or your health. Your microscope may look very different from the one presented here.

Centering the LED system. Remove the condenser lens and look through the eyepiece to center the LEDs. You should see an image like shown below.



A Review of some useful Books and Internet Sites on Diatoms

Are you interested in diatoms, but do not know where to start your research? Here is a collection of books and Websites.

*David B. Richman
Mesilla Park, New Mexico*

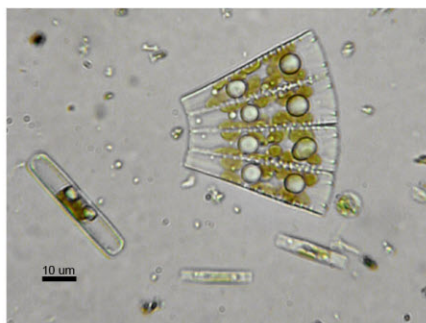
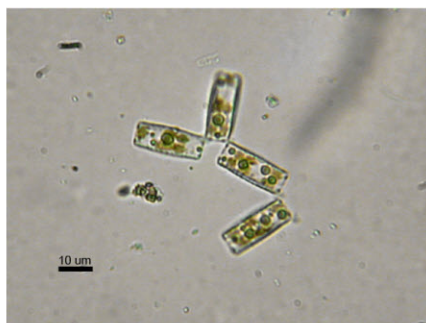
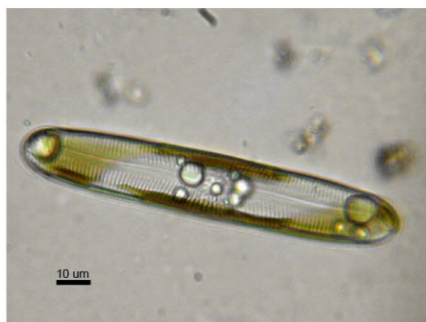
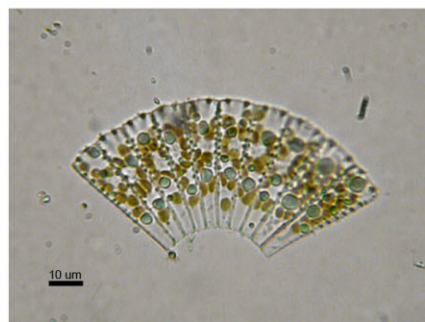
The beginner in the microscopic examination of diatoms is, like any one starting in a specialized field, often quite confused. Most books are fairly technical and may deter the amateur from using them. As in any specialized microscopic subject, diatomology has its own jargon, such as frus-

trule, raphae, etc. The best book for clearing the air on diatom terminology is Barber and Haworth's **A Guide to the Morphology of the Diatom Frustule, with a Key to the British Freshwater Genera** published in 1981 by the Freshwater Biological Association and now very expensive, if you can find a copy. This is, however, one of the most essential guides for the amateur diatomist. Two other important books are Vinyard's short, but very useful, **Diatoms of North America** published in 1977 by Mad River Press and Round, Crawford and Mann's huge volume **Diatoms: Biology and Morphology of the Genera**, published in 2007 in paperback by Cambridge University Press.

Both of these are fairly easy to obtain and the paperback version is much cheaper than the original hardback, which did not have a very good binding anyway. These three books are a basis for any amateur or professional study of these microscopic marvels. They will help identify any of the genera that you might encounter and are sources of references for further study.

Other books that may prove of use include the numerous monographs on various genera or geographical floras, plus a few on biology, etc. I will mention here only those that may be of interest to the specialized amateur.

A book that most diatomists will find fascination, even though the illus-



by DHZanette
Public domain image by Damián H. Zanette

trations are not very good, is Taylor's 1929 **Notes on Diatoms**, Guardian Press, Bournemouth. This book is full of diatom lore, including notes on sites and history. The taxonomy is, of course, quite out of date, but it is still worth acquiring if you should find a copy.

For the biology of diatoms the old standby is Werner's (ed.) 1977 volume, **The Biology of Diatoms**, University of California Press, Berkeley. This book is also out of date and newer volumes on general biology of the algae should be consulted.

Because diatom genera and many species are widespread the heavily illustrated compendium by Sims (ed.) 1996, **An Atlas of British Diatoms**, Biopress, Bristol, England, is extremely useful. In fact almost any work on diatoms can be useful at times, even very geographically specialized, if the illustrations are good. In this vein both Krammer and Lange-Bertalot, 1986, 1988, 1991. **Süsswasserflora von Mitteleuropa: Bacillariophyceae, Vols. 1-4**, Gustav Fischer Verlag, Jena (these volumes have been recently revised and all are fairly expensive) and Wehr and Sheath, 2003. **Freshwater Algae of North America**. Academic Press, are very useful for the study of the freshwater diatoms.

For identifying living freshwater diatoms there is really only one important book, Cox's slim 1996 volume **Identification of Freshwater Diatoms from Live Material**, Chapman and Hall, London. This is another book that has gone out of print and is difficult to obtain unfortunately. I do not know of a similar volume for saltwater genera.

For beautiful photos of living diatoms and other freshwater algae you cannot beat the well-presented book by Canter-Lund and Lund. 1995. **Freshwater Algae: Their Microscopic World Explored**. Biopress International, Bristol, UK. This is also a bit difficult to find and I bought my copy from a company in Britain.

Fossil diatoms is a whole different world and to my knowledge has not been treated in any comprehensive way. There are numerous volumes on the fossil diatoms of such regions as Jutland in Denmark and Oamaru, New Zealand, but these fall under the category of specialized scientific monographs and

A list of free Internet sites

The World Registry of Marine Species (WORMS) is a good site for marine diatom names:

<http://www.marinespecies.org/index.php>

Other useful sites include the Academy of Natural Sciences diatom site at <http://diatom.acnatsci.org/AlgaeImage/>

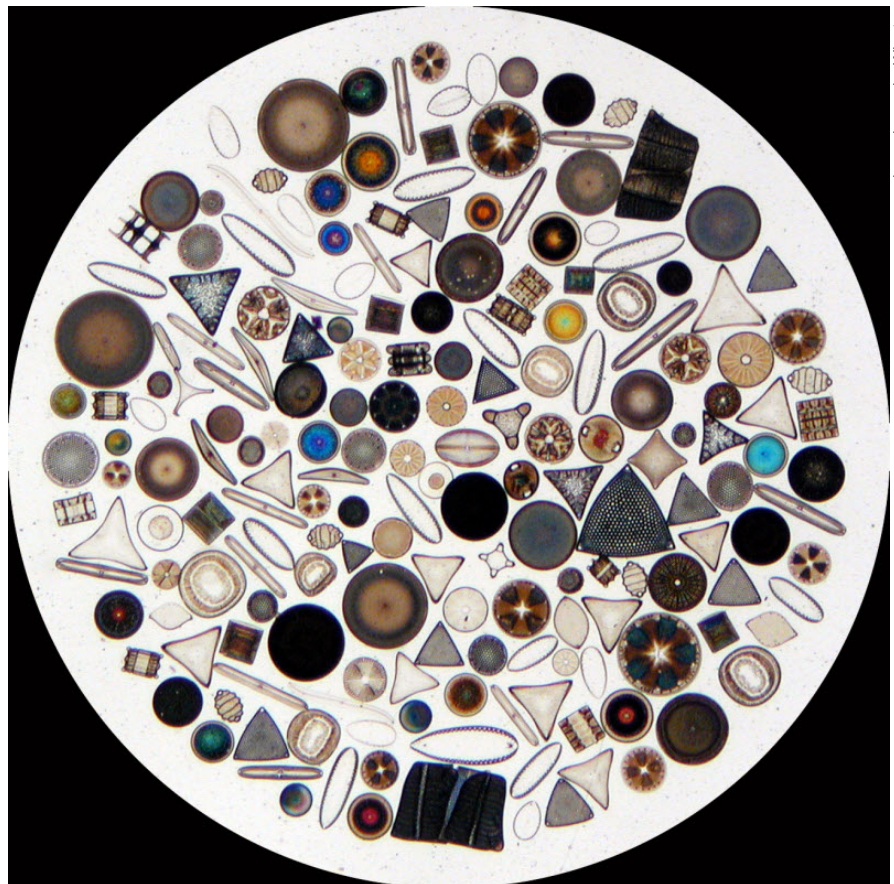
Diatoms of the United States at <http://westerndiatoms.colorado.edu/>

The California Academy of Sciences Diatom Database at <http://researcharchive.calacademy.org/research/diatoms/index.html>

The Diatom Homepage of Indiana University at <http://www.indiana.edu/~diatom/diatom.html>

Common Freshwater Diatoms of Britain and Ireland at <http://cricula.ncl.ac.uk/EADiatomKey/html/index.html>

And the Diatoms of the Arid Southwest of New Mexico State University at <http://aces.nmsu.edu/diatoms/index.html>



cc-by-sa image by Wipeiter

would be of interest primarily of very advanced amateurs and professionals. However, once you have caught the diatom "bug" such specialized literature may be of use.

Getting away from print books, there are numerous free Internet sites and these can to a great extent be used to find current names for diatom genera and species, as well as for identification. ■

Robert Hooke's Micrographia: Of a Louse

Micrographia was first published in 1665. It was not only a best-seller, but also created a wide interest in microscopy. Published before the invention of photography, it contains numerous drawings by Robert Hooke himself.

The excerpt (which is in public domain) was obtained from Project Gutenberg (www.gutenberg.org).

By Robert Hooke FRS
Fellow of the Royal Society

Observ. LIV. Of a Louse.

This is a Creature so officious, that 'twill be known to every one at one time or other, so busie, and so impudent, that it will be intruding it self in every ones company, and so proud and aspiring withall, that it fears not to trample on the best, and affects nothing so much as a Crown; feeds and lives very high, and that makes it so saucy, as to pull any one by the ears that comes in its way, and will never be quiet till it has drawn blood: it is troubled at nothing so much as at a man that scratches his head, as knowing that man is plotting and contriving some mischief against it, and that makes it oftentime sculk into some meaner and lower place, and run behind a mans back, though it go very much against the hair; which ill conditions of it having made it better known then trusted, would exempt me from making any further description of it, did not my faithful Mercury, my Microscope, bring me other information of it. For this has discovered to me, by means of a very bright light cast on it, that it is a Creature of a very odd shape; it Schem. 35. has a head shap'd like that exprest in 35. Scheme marked with A, which seems

almost Conical, but is a little flatted on the upper and under sides, at the biggest part of which, on either side behind the head (as it were, being the place where other Creatures ears stand) are placed its two black shining goggle eyes BB, looking backwards, and fenced round with several small cilia, or hairs that incompass it, so that it seems this Creature has no very good foresight: It does not seem to have any eye-lids, and therefore perhaps its eyes were so placed, that it might the better cleanse them with its fore-legs; and perhaps this may be the reason, why they so much avoid and run from the light behind them, for being made to live in the shady and dark recesses of the hair, and thence probably their eye having a great aperture, the open and clear light, especially that of the Sun, must needs very much offend them; to secure these eyes from receiving any injury from the hairs through which it passes, it has two horns that grow before it, in the place where one would have thought the eyes should be; each of these CC hath four joynts, which are fringed, as 'twere, with small bristles, from which to the tip of its snout D, the head seems very round and tapering, ending in a very sharp nose D, which seems to have a small hole, and to be the passage through which he sucks the blood. Now whereas if it be plac'd on its back, with its belly upwards, as it is in the 35. Scheme, it seems in several Positions to have a resemblance of chaps, or jaws, as is represented in the Figure by EE, yet in other postures those dark strokes disappear; and having kept several of them in a box for two or three dayes, so that for all that time they had nothing to feed on, I found, upon letting one creep on my hand, that it immediately fell to sucking, and did neither seem to thrust its nose very deep into the skin, nor to open any kind of mouth, but I could plainly perceive a small current of blood, which came directly from its snout, and past into its belly; and about A there seem'd a contrivance, somewhat

MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

MAGNIFYING GLASSES

WITH

OBSERVATIONS and INQUIRIES thereupon.

By R. HOOKE, Fellow of the ROYAL SOCIETY.

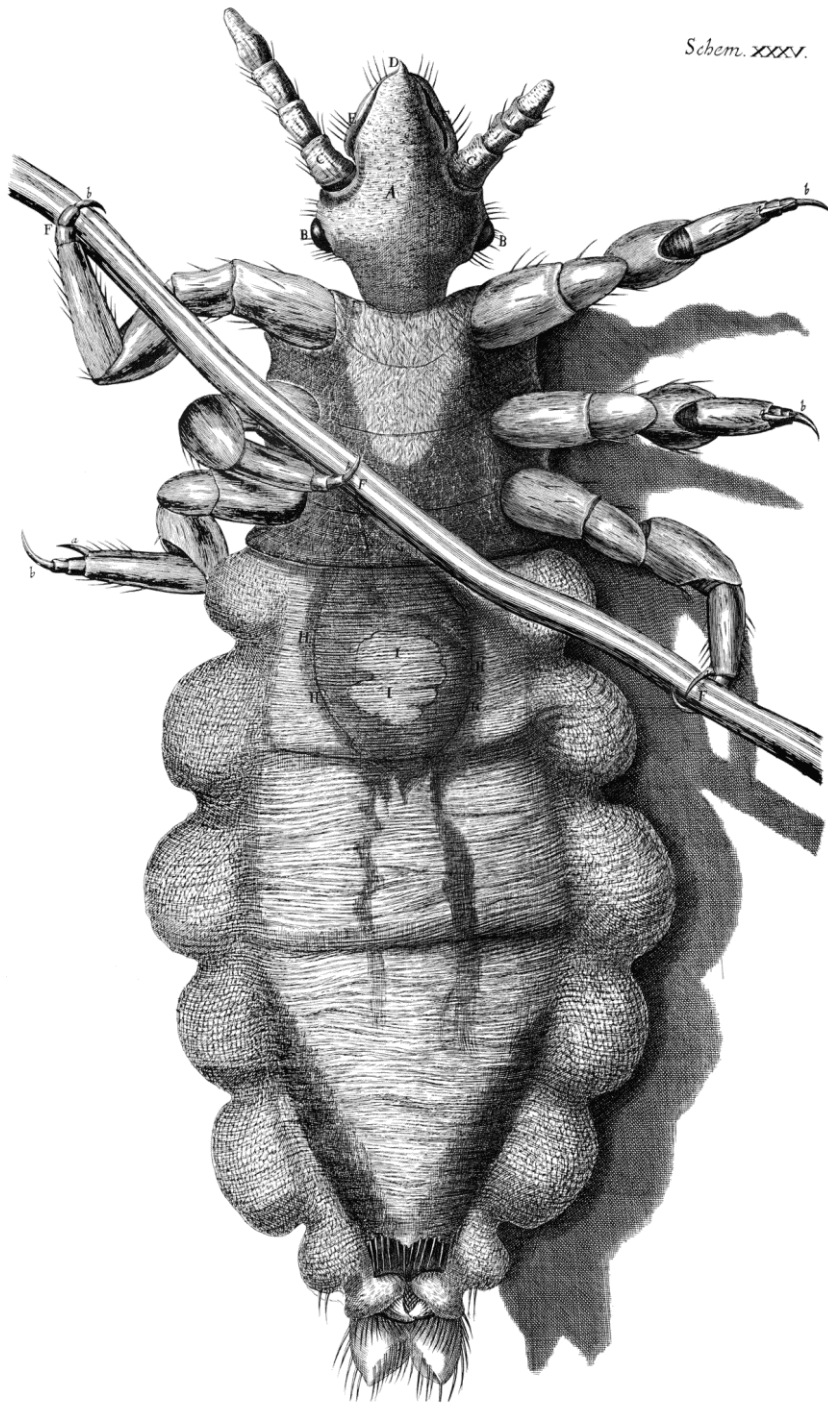
*Nonnulli oculi quantum extendere LECTOR,
Non solum in libro continetur Lippus utitur, Horac. Ep. lib. 1.*



LONDON, Printed by Jo. Martyn, and Ja. Allestry, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the Bell in S. Paul's Church-yard. M DC LX V.

The title page of Micrographia.

resembling a Pump, pair of Bellows, or Heart, for by a very swift systole and diastole the blood seem'd drawn from the nose, and forced into the body. It did not seem at all, though I viewed it a good while as it was sucking, to thrust more of its nose into the skin then the very snout D, nor did it cause the least discernable pain, and yet the blood seem'd to run through its head very quick and freely, so that it seems there is no part of the skin but the blood is dispers'd into, nay, even into the cuticula; for had it thrust its whole nose in from D to CC, it would not have amounted to the supposed thickness of that tegument, the length of the nose being not more then a three hundredth part of an inch. It has six legs, covered with a very transparent shell, and joynted exactly like a Crab's, or Lobster's; each leg is divided into six parts by these joynts, and those have here and there several small hairs; and at the end



of each leg it has two claws, very properly adapted for its peculiar use, being thereby inabled to walk very securely both on the skin and hair; and indeed this contrivance of the feet is very curious, and could not be made more commodiously and compendiously, for performing both these requisite motions, of walking and climbing up the hair of a mans head, then it is: for, by having the lesser claw (a) set so much short of the bigger (b) when it walks on the skin the shorter touches not, and

then the feet are the same with those of a Mite, and several other small Insects, but by means of the small joyns of the longer claw it can bend it round, and so with both claws take hold of a hair, in the manner represented in the Figure, the long transparent Cylinder FFF, being a Man's hair held by it.

The Thorax seem'd cas'd with another kind of substance then the belly, namely, with a thin transparent horny substance, which upon the fasting of the Creature did not grow flaccid; through

this I could plainly see the blood, suck'd from my hand, to be variously distributed, and mov'd to and fro; and about G there seem'd a pretty big white substance, which seem'd to be moved within its thorax; besides, there appear'd very many small milk-white vessels, which crost over the breast between the legs, out of which, on either side, were many small branchings, these seem'd to be the veins and arteries, for that which is analogous to blood in all Insects is milk-white.

The belly is covered with a transparent substance likewise, but more resembling a skin then a shell, for 'tis grain'd all over the belly just like the skin in the palms of a man's hand, and when the belly is empty, grows very flaccid and wrinkled; at the upper end of this is placed the stomach HH, and perhaps also the white spot II may be the liver or pancreas, which, by the peristaltick motion of the guts, is a little mov'd to and fro, not with a systole and diastole, but rather with a thronging or justling motion. Viewing one of these Creatures, after it had fasted two dayes, all the hinder part was lank and flaccid, and the white spot II hardly mov'd, most of the white branchings disappear'd, and most also of the redness or sucked blood in the guts, the peristaltick motion of which was scarce discernable; but upon the suffering it to suck, it presently fill'd the skin of the belly, and of the six scolop'd embosments on either side, as full as it could be stuff, the stomach and guts were as full as they could hold; the peristaltick motion of the gut grew quick, and the justling motion of II accordingly; multitudes of milk-white vessels seem'd quickly filled, and turgid, which were perhaps the veins and arteries and the Creature was so greedy, that though it could not contain more, yet it continued sucking as fast as ever, and as fast emptying it self behind: the digestion of this Creature must needs be very quick, for though I perceiv'd the blood thicker and blacker when suck'd, yet, when in the guts, it was of a very lovely ruby colour, and that part of it, which was digested into the veins, seemed white; whence it appears, that a further digestion of blood may make it milk, at least of a resembling colour: What is else observable in the figure of this Creature, may be seen by the 35. Scheme. ■

Predators in the micro world

Eating and being eaten: this cycle of life can not only be observed in macroscopic life forms, but also in the microcosm of a water droplet.

By Dan Holloway

I believe the two organisms that are involved here are *Litonotus* and *Colpidium*. *Litonotus*, a ciliate, is a formidable predator. In the following photographs we can see the voracious attack on the *Colpidium*. *Litonotus* has trichocysts, thin thread like strands, that is discharged at the *Colpidium*. This is not an unusual occurrence by a micro-organism. The *Litonotus* goes a step further in that the trichocysts are toxicysts. A toxicyst is a trichocyst that contains a chemical that paralyzes the prey. Once the *Colpidium* is paralyzed you can see the *Litonotus* feed. ■



Fig. 1: *Litonotus* is a ciliate which has trichocysts (the thread-like structures) that paralyze the prey.

Fig 2, 3: *Colpidium* (central organism) is being attacked by *Litonotus*.

Staining Onion Cell Nuclei

Are you looking for a safe method of staining onion cell nuclei? Try water-based fountain pen ink.

By *Oliver Kim*

It is possible to use DNA staining chemicals, such as methylene blue, to make the nucleus of a cell visible. Methylene blue, however, is able to cause a range of adverse health reactions and it is therefore not always very suitable to be used. Companies may also be reluctant to sell this chemical to private individuals. I therefore tried a range of other more readily available stains.

One of the most obvious contenders is the water soluble fountain pen ink: The royal blue Pelikan 4001 ink. It is a triphenylmethane dye and can be found (packaged in cartridges) in the pencil cases of nearly all students of our school. While I nevertheless do not recommend the ingestion of this ink, I still consider the large number of blue stained fingers of our students as enough evidence that the ink is not toxic. I therefore wondered if the ink could also be suitable for staining biological specimens in a school lab setting. I experimented a bit and soon found a simple method for staining onion cell nuclei.

Materials and Method

The following materials are needed: Onion, tap water, ethyl alcohol (can be denatured), fountain pen ink, several small beakers or film containers for doing the staining.

Using a sharp knife, cut out about 1 cm² of onion material. Do this by cutting a double cross “#” into the onion. The individual layers of the onion are separated by a thin skin. Peel this skin off using your fingernails or tweezers. The skin can be found on the inside part of each layer. It’s now time to start the staining procedure.

First place the skin into pure alcohol for about 30 seconds. The alcohol removes water from the cells. The onion skin will become stiff.

Then transfer the onion skin into the ink. The ink, which is water-based, will enter the cells and stain the onion skin deep blue. The skin will lose its rigidity and become flaccid. Do not let go of the skin, it will roll up and may be difficult to find again in the ink.

Do not use calligraphy ink. This type of ink contains microscopic particles, but not a dissolved substance. These particles will not enter the cells.

Then transfer the skin into pure water and rinse it for about 30 seconds or until no more ink is given off. The skin

will still have retained most of its blue color.

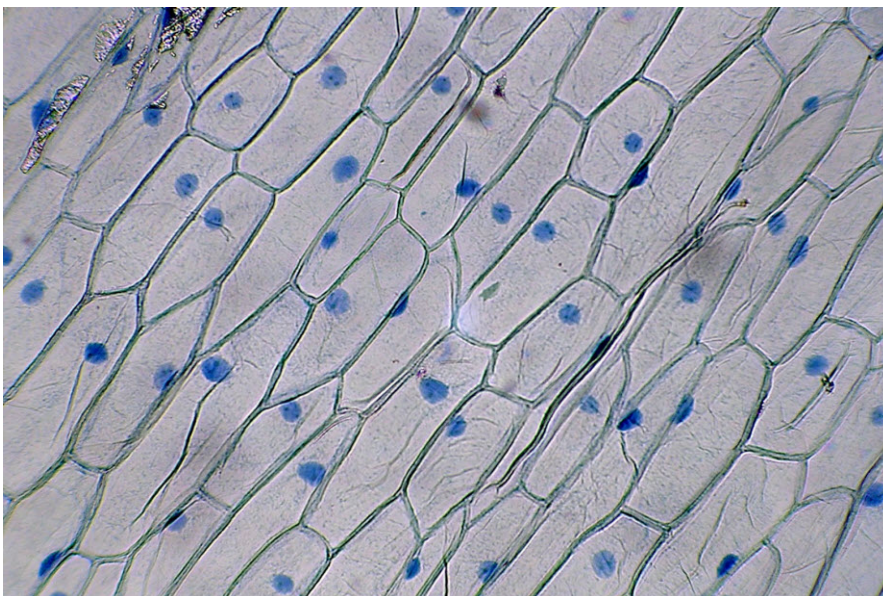
We now start the washing steps. We have to remove excess ink from the cells. Transfer the skin into pure alcohol again for a few seconds. Some of the ink will be removed staining the alcohol slightly blue. Then transfer the skin into pure water for about 30 seconds. Water will then enter the cells again.

Carefully spread the skin on a microscope slide and place a cover slip on top. Remove excess water with filter paper. The onion skin will have a tendency to fold up and it may be a bit difficult to spread it completely.

Troubleshooting

It is possible that the nuclei are not stained, or not stained enough. There could be several reasons for this. The onion may not have been submerged long enough in the ink or the onion was not fully covered by ink on all sides. This can be the case if the onion skin is folded back onto itself.

The onion was washed too intensively. This is the most probable cause. If the onion was washed twice, then the washing step should be conducted only once. It could also be that the washing times were too long. If the nuclei are stained lightly blue, then this indicates that the staining procedure is in principle working, but that too much of the ink was removed. ■



The nuclei are now stained blue. Try to vary the duration of the staining and washing steps to obtain the best results.

Infinity vs. DIN Optics: a primer

In recent years many large microscope manufacturers moved away from the traditional DIN standard to so-called "infinity corrected" optics.

By Oliver Kim

Back in 1998 I was tempted of buying a microscope with infinity optics. I felt a little bit uneasy and uncertain and initially hesitated to buy a microscope of the "traditional" DIN 160mm tube length standard, which has been around for decades. The big name microscope manufacturers all seemed to be moving into the infinity direction, and I was a bit worried that by buying a microscope of the "traditional" standard, that I'll be soon left alone with a microscope for which it will be too difficult to obtain spare parts and different objectives. I still remembered (painfully) the transition from manual-focus cameras to auto-focus systems and the problems that I had to find camera objectives for my manual-focus camera. I did not want to spend money on a microscope which will become outdated soon. I quickly

realized that the cost of such an "infinity" instrument was way beyond my financial possibilities, however.

During the same year I participated at a microbiology conference and had the opportunity to talk to two representatives of two competing big-name microscope manufactures. I asked both of them, independently, on their opinion on infinity optics and whether it is worth the additional investment. I was rather surprised by their answers. Both representatives stated that infinity optics do indeed deliver a slightly improved image quality (whether this is more due to new glass materials and modern computer optimized design, than to the „infinity“ design itself, is a different question), but that the improvement is slight. One of the representatives said that "infinity optics are a fashionable trend" but not absolutely necessary. I was informed that one still has to see if the new technology is successful in the long run. The other representative stated that infinity optics are "question of taste".

I was really surprised by these answers because I expected them to enthusiastically promote their newest technology. What I received was a reflective analysis of the pros and cons,

with the cons being mostly in the high cost of these microscopes.

When I was informed on the high price of an infinity-microscope, I did not have any other choice than to buy a "traditional" DIN model. Now 12 years later, both microscope manufacturers moved their whole line of microscopes to the infinity-corrected optics. It is now indeed more difficult to find parts (such as new objectives) for the traditional DIN system, unless one opts for third-party solutions. Ironically, the price of some of these parts even started to go up as less of them became available. My initial fears were thus confirmed.

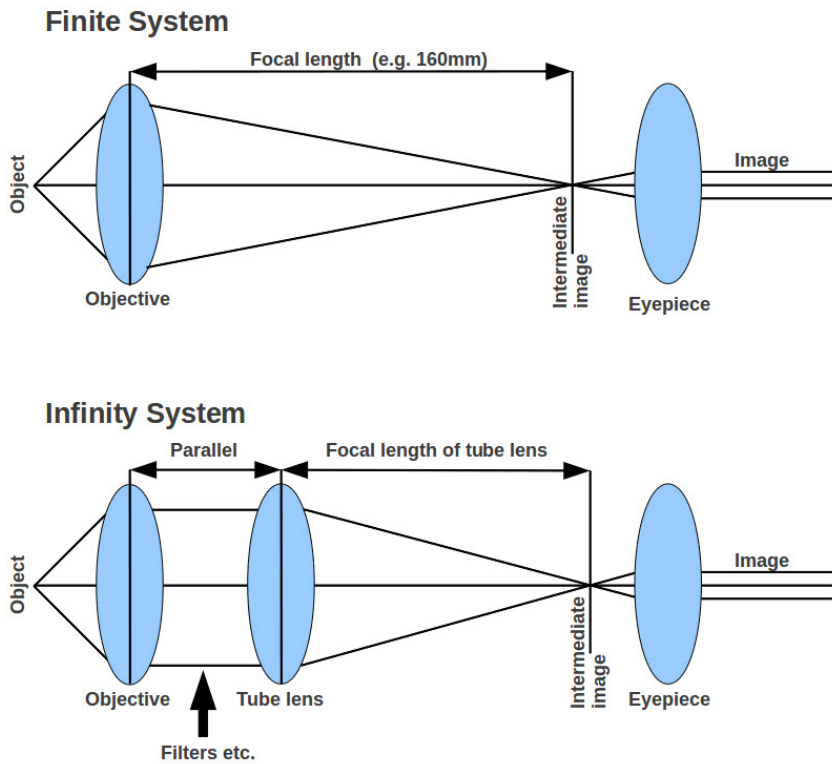
DIN Optics

Before we can talk about the differences between the systems, it is first necessary to start at the basics. DIN (Deutsche Industrie Norm), a German industrial standard, a popular standard which has been (and still is) used for microscopes by a wide variety of manufacturers. The standard defines a common threading for the objectives of 20.1mm, a tube length of 160mm as well as a parfocal distance of 45mm. It is important to mention here, that the DIN standard is defined for finite-corrected optics.

All microscope objectives adhering to the DIN standard show these parameters. The standard therefore ensures compatibility of different microscope objectives with different microscopes.



40x objective with a numerical aperture of 0.65 and designed for a cover glass thickness of 0.17mm. The infinity ∞ sign, indicates that this objective is infinity corrected.



Top: Finite optics (such as DIN). The objective produces a converging light beam to form an intermediate image in the tube of the microscope. Placing filters into this light path will change the focus of the image.

Bottom: Infinity corrected objectives will produce a parallel light beam into which filters can be placed. A separate tube lens is responsible for creating an intermediate image.

Advantages of infinity corrected optics

Modularity: The primary advantage is that it is possible to place filters and DIC (differential interference contrast) components into the parallel light beam without changing the focus.

Availability: All of the major, “big-name” microscope manufacturers seemed to have moved to infinity corrected optics. I can imagine that finite objectives from these manufacturers will become rare (and more expensive) in the future.

Disadvantages of infinity corrected optics

No international standard: One main problem is that the different manufacturers all define their own standard. Interchangeability of the objectives between manufacturers is not possible. If you buy the microscope from a particular manufacturer, then you are married to this system and can only use objectives from the same manufacturer.

Cost: The cost of an infinity system can be quite high and it is not always feasible to buy these microscopes.

Size: Infinity microscopes can be larger than the DIN devices. There is extra space to mount additional optical elements (filters etc.) into the light path and the tube lens also needs some space.

Smaller second hand market: Due to the manufacturer-specificity of the objectives (and the relatively recent introduction of these systems), the second hand market for infinity objectives is smaller than for DIN objectives, especially if you are looking for infinity objectives by smaller manufacturers. ■

It is possible to mount objectives of different manufacturers and still maintaining parfocality (the ability to switch magnifications, without the need of re-focusing). The Japanese JIS standard uses the same threading but a different tube length of 170mm. It is possible to use JIS objectives with DIN microscopes (the threading is the same), but the magnification will be different.

As a „finite“ standard, DIN objectives project an intermediate image 160mm away from the objective. The eyepiece then magnifies this image a second time. Placing filters into the light path has the disadvantage of changing the position of the intermediate image. Refocusing is necessary and there may also be a change in magnification. This happens because the light beam converges to a point to form an intermediate image.

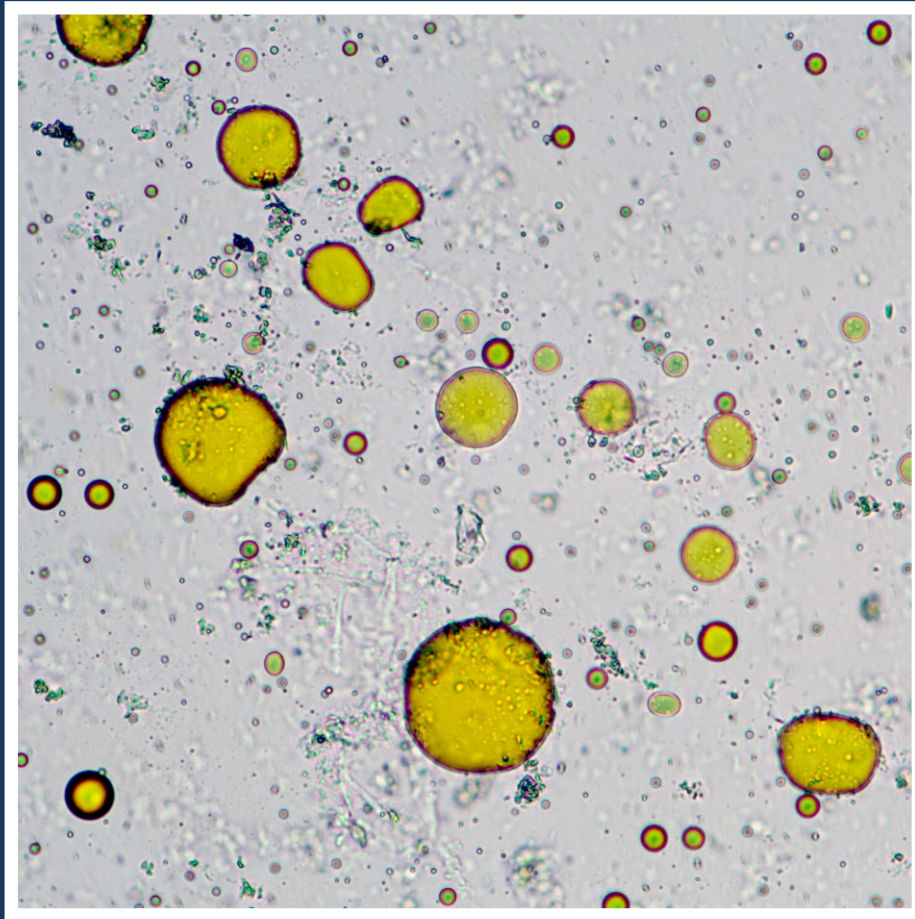
Infinity Optics

Infinity optics permit the users to place filters between objective and eyepiece without changing the focus. This is because the light exiting the objective does not converge, but is parallel (focused at „infinity“, hence the name). Placing filters, of uniform thickness, into a parallel light beam will not disturb the parallel light. This is the primary

advantage of these systems. A second optical element, a tube lens, will then converge the light beam to form an intermediate image. See the schematic diagram for a comparison of finite and infinity corrected optics.

Exchangeability of infinity objectives

Generally, infinity objectives of different manufacturers are not interchangeable. An infinity objective manufactured by Nikon will not fit on an Olympus microscope, and vice-versa. This is not only due to different physical sizes and threading, but also due to different optics. Some manufacturers correct the lens errors (various aberrations) right in the objective lens, while still others assign this task to the tube lens. Even if infinity objectives physically do fit into the revolving nose-piece of a microscope of a different, the systems may not be compatible from an optical perspective. Lens errors may still be visible and the overall image quality may be lower. If you decide to buy an infinity system, then make sure that the manufacturer offers a sufficiently large selection of suitable objectives for your needs.



What's this? Answer on page 3.