Microbe Hunter

Microscopy Magazine

ISSN 2220-4962 (Print)
ISSN 2220-4970 (Online)

Volume 1, Number 9
September 2011

The Magazine for the Enthusiast Microscopist

http://www.microbehunter.com

Water Samples

Moth Antenna

Laboval 4

ABOUT

Microbehunter Microscopy Magazine
The magazine for the enthusiast microscopist
MicrobeHunter Magazine is a non-commercial project.

Volume 1, Number 9, September 2011

ISSN 2220-4962 (Print)
ISSN 2220-4970 (Online)

Download: Microbehunter Microscopy Magazine can be downloaded at: http://www.microbehunter.com

Print version: The printed version can be ordered at: http://microbehunter.magcloud.com

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Editorial: Article and image submissions are welcome and should be sent to: editor@microbehunter.com.
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Front Cover:
Large image: Oliver Kim (diatoms in dark field)
Left image: Oliver Kim
Middle: Anthony Thomas
Right: Charles E. Guevara

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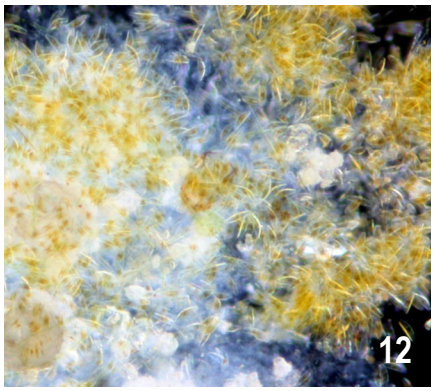
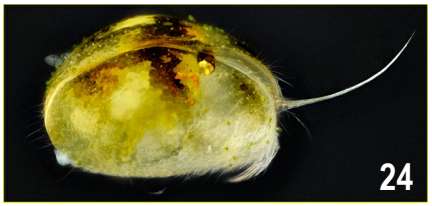
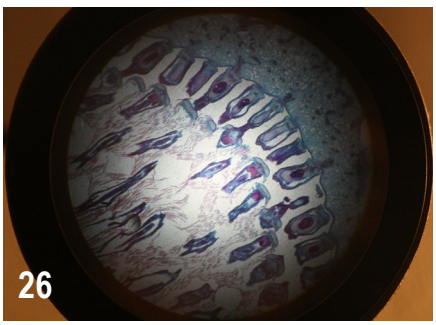
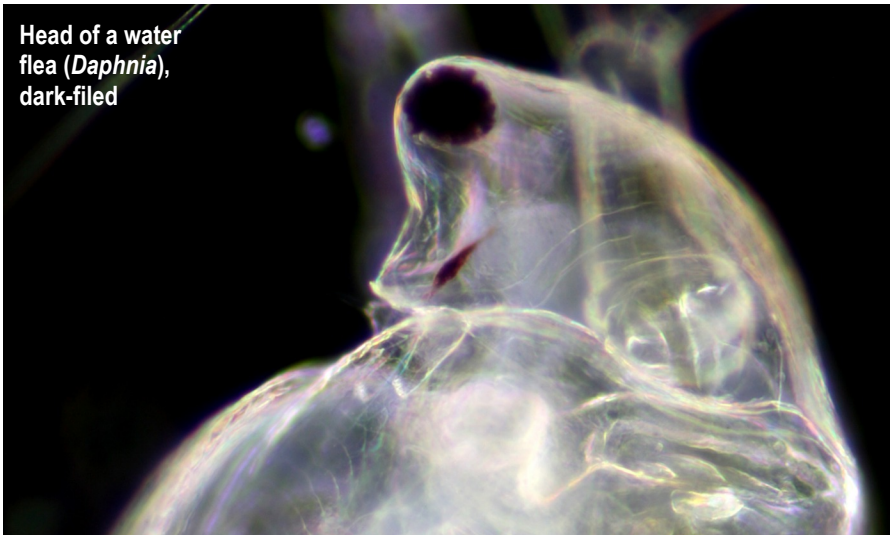






Answer to the puzzle (back cover):
Cross section through a wheat grain (*Triticum*). Starch grains are visible.



Head of a water flea (*Daphnia*), dark-filed

Episode Seven: Bumbling and Stumbling into Phase Contrast, a Tour of the Laboval 4 and Other Assorted Ramblings

This article was originally published in the February 2011 issue of Micscape Magazine and is now republished here with the permission of the author and of Micscape Magazine.

G. Joseph Wilhelm

As a wise man once said "*Ya gotta have a plan so you got sumptin to deviate from.*" My original intention, as pertains to microscopy, was to simply have an antique scope for display. However, as has been chronicled since Episode One, my re-kindled interest has taken on a life of its own. Things are moving along nicely thank you, my Zeus system is up and running, the stereomicroscopes are performing well and the remodeling of a corner of my mini museum as a microscopy suite is proceeding in a pleasantly welcome manner. Now, while these are some of the favorable outcomes of my microscopic machinations, there are converse aspects also.

To paraphrase Oliver Hazard Perry, "*I have met the enemy and he is me.*"

I have journeyed in haste down the yellow brick road of microscopy to find the Wizard of Optics and have him grant me knowledge of all things microscopical. Due to this unbridled celerity and since I am assembling my kit piecemeal, I often find myself acquiring accessories as a "good deal" that I am not quite ready or unable to incorporate immediately due to: a) Not having adequate tutelage or b) The part requires some modification to fit the Zeus system. I get the same overzealous urge to obtain and consume when I walk into a Chinese food buffet. All those entrees, so little time. But unlike Chinese food, microscope accessories don't go bad in the refrigerator after three days. So if they present themselves advantageously I happily snarf them up knowing I will get around to them eventually.

I had mentioned to Mr. David Walker of my interest in phase contrast and the new aftermarket condenser/lens set I had my eye on for $600. This set was by the same manufacturer as the superb plan achromatic lens set I had purchased earlier. Mr. Walker in turn, informed me that in the UK, complete, quality phase equipped microscopes such as the Watson Microsystem 70 were selling for $150-$200 so perhaps I should look into a similarly equipped Spencer or B&L as a phase dedicated set up would be much more convenient. Well Mr. Walker, I can safely say that the purport of this article is due to your advice but it does deviate from the norm of my offerings as it may actually contain

Figure 1 (opposite page) and 2 (left): The Amscope set appeared as advertised, in excellent, as-new condition in a nice case. A single dovetail carrier would be all the machining that was necessary to adapt it to the Zeus system.

some useful information. So with the above precepts in mind allow me to present my rendition of "The Agony and the Ecstasy."

The Agony

If you are like myself, dear reader, venturing for the first time into the realm of Phase Contrast, you will find a plethora of possibilities encompassed by a rather large financial bracket. Used Zeiss phase condensers were upwards of $350 with the individual objectives from $150 to $250 each, which is why the four objective $600 after market set did not look too bad even though I would have to do a bit of fancy machining to create an adapter. Amscope offered some plan achromatic three objective sets for about $350 even though the objectives were not marked "plan". I found this very set on ebay, offered as "In new condition", low mileage, only driven by a little old lady to church on Sundays etc. for $150 or best offer. I knew I would be turned down but I offered $50 anyway and continued my search.

I had been searching for phase contrast under the Zeiss brand and I noticed how LOMO accessories were always offered as LOMO/Zeiss. Having been befogged for quite some time on this relationship I decided to delve into this mystery.

(Reading the Zeiss Company chronological record is tantamount to taking a European history course. Let me condense the facts that were of interest to me.

With the end of WWII the Zeiss workers and tooling assets were dispersed from Jena to Leningrad- LOMO and Oberkochen-Zeiss West Germany, with some workers remaining in Jena East Germany to become *Zeiss aus Jena* or as marketed in the US simply *aus Jena*, thus explaining the similarity and interchangeability of some parts and the aforementioned coupling of the LOMO/Zeiss brands. All of these companies were producing quality instruments using former Zeiss employees. A fascinating read of the over 160 years of accomplishments of the respected Zeiss Company can be found at:

http://www.company7.com/zeiss/history.html.)

Armed with this new information I expanded my search to include aus Jena and up popped some aus Jena Laboval 4 microscopes. Most were commanding a minimum bid upwards of $400 but a woman in Texas had four "in good working order" for $275 each. In the single photo she posted, the scope had what appeared to be a sub-stage phase turret condenser. She evidently had no idea what she had as inquires about whether the objectives were also phase was met with confusion (The rest of her eBay offerings were Hummel ceramic figurines). Finally after receiving confirmation that three of the four objectives were marked with a "Ph" and two of the scopes were already sold, I decided to take the plunge and purchase one. Hopefully a good deal on a complete phase outfitted microscope.

Shortly thereafter I received notification that my offer on the Amscope phase set was accepted and the flat spot on my forehead where I slapped myself with my palm is still apparent.

The aus Jena microscope and the Amscope phase set arrived the same day. The Amscope set appeared as advertised, in excellent, as-new condition in a nice case. (See Figs 1 & 2.) A single dovetail carrier would be all the machining that was necessary to adapt it to the Zeus system.

I next turned my attention to the large box containing the Laboval 4 and here is where the agony really set in.

First of all, purchasing this instrument was alien to all of my collecting parameters. Compared to my GFL it was of a harsh angular design. Perfectly square dimensions, entirely devoid of any aesthetic or artistic influence. A

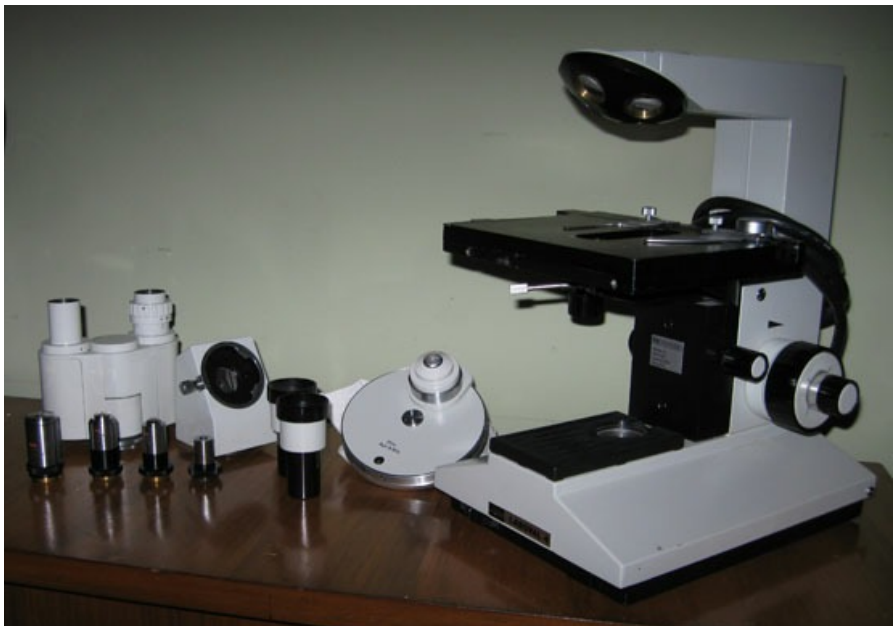


Figure 3 (top): All of these components came individually encased in sticky bubble wrap.

Figure 4 (bottom): Assembled stand after cleaning.

starkly efficient presentation of "form follows function". It presented itself as the proletariat of microscopes, as it were, and it was the wrong color, (gray, not black).

The disassembled microscope components came well packed in a sticky sided bubble wrap (See Fig. 3). The inconvenience here was the sticky side was applied to the microscope. I spent the better side of an hour removing this tenaciously gripping material, which left a tacky residue upon the affected constituents. Thankfully the optic component surfaces were not subject to this detriment. This in turn revealed a University of Texas at Dallas property sticker on the back. Not a good sign, as institutional scopes have a reputation as suffering from a neglect of maintenance and care. The angular interface for the Siedentopf head to the arm was eliciting an aggravating rattle, the fine and coarse focus were stiff and seized together, the potentiometer for the base illumination light was shot and the right eye piece had a lens fracture halfway across the field of view. Further investigation revealed that rotation of the phase condenser resulted in complete seizure after half a turn.

I sent a rather terse message to the seller indicating that the microscope was in substantially less than the advertised "good working condition" and this resulted in a $75 refund. Since the microscope was sold as "no returns" I figured this was the best I could do.

The Ecstasy

So far, the only benefic element to this whole experience was the condition of the Amscope phase components. I set about the aus Jena to salvage what I could.

The residuum left behind by the bubble wrap took an hour to remove with judiciously applied treatments of naphtha (Highly flammable, lighter fluid actually, please read Episode 5 on common sense safety). The plastic parts did not react with the naphtha, which was the first good sign. I didn't know at exactly what part of the focus mechanism was seizing up so I started with some penetrating lubrication around the stems of the coaxial focus knobs. The rattle in the head interface was just that. The optics were neither loose nor affected by whatever small piece was roaming around in there. I decided not to disassemble it, better the devil you know, so to speak. The "crack" in the right eyepiece lens turned out to be a rather crude wire pointer inserted at the lens plane field of view. With that removed, the eyepieces were flawless. With a shot of contact cleaner the light dimmer started working, at least on the bright end from the 9 setting down to about 7 and after letting the penetrating oil do it's job for a couple of hours the

Figure 5: Four strange looking multiple annuli discs. All others I have seen (and the Amscope set) have just one ring.

focus mechanism freed up and started working smooth as silk. The problem must have been the old hardened Russian grease I keep hearing so much about. Things were looking up (see Fig. 4).

Now, to deal the rotating condenser, some disassembly was in order and upon removing the top screw and cover, I was greeted with this.

The rotating seizure problem was because the annuli turret was seized to the center shaft causing the cover and bottom screws to tighten up on rotation. This was the same hardened grease problem with the focusing mechanism. This deficiency was easily remedied with a thin brass shim washer under the top screw as a temporary fix. Total dismantling of the condenser will be required in the future to properly address this situation so as not to put the pristine condition of the annuli in jeopardy with possible lubricant contamination.

Finally, the objectives were scrutinized. The 10X, 40X and 100X Phase that came with the scope had a "v" after the Ph and what appeared to be corresponding multiple ringed phase plates to the annuli of the condenser. As with all the other optical components of this microscope, they appeared to be in like-new condition. The fourth objective was a non-phase 3.2/0.10 160/- in the same excellent condition as the others. The condenser had a 20X phase annulus also, but alas, no corresponding objective.

So with a days labor I now had a functional (except for the dimmer limitations) phase contrast microscope outfitted with what are seemingly rare, (and a total mystery to me), aus Jena Phv condenser and objectives. The lacking accessories were a phase telescope and annuli centering tools for which I hoped the Amscope set telescope and a soon to be ordered pocket watch winding key would suffice. All of the other microscope adjustments such as the mechanical stage and diopter adjustments were working splendidly. With a few more sprays of the electric contact cleaner the light dimmer potentiometer came back to full function.

The very next day, in an effort to replace the penetrating lubricant with a more permanent form of grease or oil, I removed the back of the microscope to expose the perfunctory focusing elements. There, wrapped in plastic and stuffed into the void of the upper arm was an aus Jena phase telescope, two phase centering tools and the missing 20X Phv objective! Yessss! There is a God!

With all of these fortuitous developments at hand, my decidedly improved attitude prompted me to take a closer look at this particular Laboval 4. My aforementioned aversions to its aesthetics did not negate the quality engineering in this instrument. All of the controls are precise and (now) smooth and the optics exemplary. The mechanical stage gears are metal, not plastic. Access to the electronics is easy by undoing one captured screw and tilting out the hinged base (Fig. 8). As you can see there is a tremendous amount (by modern standards) of electronics for simple voltage conversion and dimming. However, since the Laboval 4, according to aus Jena documents, was only produced from 1985 to 1987 this is expected for 25-year-old technology. All these dated electronics also contributed to the substantial "heft" of the instrument. The phase condenser has centering adjustments for the iris as well as being able to individually center the phase annuluses. A swing-out filter holder and collector lens completes the package. (Fig. 9)

In the minus column, the sub-stage condenser carrier has a very limited amount of vertical travel thus ruling out any easy adaptation to other than the 37 mm after market condensers. Improving the transmitted lighting was also going to be a challenge. There is very little room to get ham-fisted fingers like mine to adjustments under the stage and while there is a marked a iris scale, it cannot be read unless your line of sight

Figure 6 (left): These treasures found here in thin plastic wrap.

Figure 7 (right): In the light of day they are in like new condition.

is significantly below stage level. There is no field iris but annulus rings could possibly be placed over the light and centered. I have searched for a trinocular head but the prospects seem dim. Since I have acquired a proper Zeiss trinocular head for my GFL the Microscopes. India head it replaced may well suffice for the aus Jena provided I turn down the dovetail to 41mm. I also believe it mitigates the ascetic presentation of this stand. (Fig. 10) The Amscope phase set is a plug n'play accessory for this stand.

So, there you have it. A quality, fully functional, cosmetically excellent, phase dedicated microscope with optics that appear to be in mint condition, for $200. Add the single annulus Amscope set as another variation and it's $250. Life is good. All I have to do now is educate myself about this unique multiple ring "Phv" setup. That turned out to be a whole other story.

Unraveling the (to me) Phv mystery

As to as the images of the Saturn like rings for phase contrast from the aus Jena Phv objectives and phase annuli and there respective relevance, I spent some considerable time trying to divine online information about what was to become an elusive subject.

My first choice of research, as always was the Micscape library. Under the techniques, lighting, Phase banner there were 14 articles listed. To my surprise, just the one by Edward Cowen referenced any inner and outer phase annuli, but just in passing, and as adaptable to other phase objectives. He advocated altering the inner phase ring in a nonspecific manner. Not much help here.

Further investigation into operator manuals yielded a dearth of responses and various other search parameters were just as forthcoming. Finally, after some days, I chanced upon a natural science yahoo thread from 2007 discussing the differences between the Leitz Heine condenser and the Carl Zeiss Jena (CZJ) variable phase condenser (Variable? Could this be the "v" in Phv?). This thread revealed a link to CZJ operator and repair manuals that I immediately followed only to find they were in the German language. There was a "translate" button on the toolbar but unfortunately it did not translate the text in the PDF files presented. Searching nearly all of the files yielded a Laboval 4 manual in two parts and a CZJ "Phasenkontrast" file also in two parts. I jumped on his link hoping there would be visuals and as I was armed with the Google text translation link I was in anticipation of deciphering the manuals. This is where the fun began. Here is the link to the manuals:

http://www.mikroskop-online.de/Zeiss%20Jena%20BDA.htm

Readily available computer translation programs like Goggle's have a long way to go in interpretation of intent,

Figure 8 (left bottom): A swing-out filter holder and collector lens completes the package.

phrasing, figure of speech etc. When you couple this with technical speak, it can get to be a real hoot. For instance, I recently purchased a barbeque grill made in China, which came with assembly instructions obviously translated by computer. The first step for assembly implored me to "Insert please the left hand part into happy valley and attach with angular motion." After throwing away the instructions and successfully assembling the grill, I still have no idea what parts of the grill are the "left hand part" and "happy valley". Likewise the translation of the CZJ manuals from the German was just as entertaining.

After painstakingly copying paragraphs for translation from the "Phasenkontrast mit Microval Mikroskopen" text file, I soon learned that in German an optical "halo" was "a light in the interior courtyard and sunny spells". ACHTUNG!! Meant "Oops!! (Go figure) and " Vierkantaufsteckschlüssel" translated as..well.. "Vierkantaufsteckschlüssel".

I went back to the discussion thread to read it through, which I should have done in the first place. There, to my relief, in wonderfully lucid terms, was the explanation of the how and why of CZJ Phv variable phase contrast by Mr. Kevin Sunley.

I cannot give Mr. Sunley enough credit for bringing to light a perfectly understandable layman's illumination of this subject. Rather than paraphrase or otherwise deface an excellent presentation, I would like to quote his remarks verbatum. I have placed his remarks in a different font to distinguish them from the rest of this article.

Mr Sunley, please take the stage: "*The CZJ Phv system is more like the standard phase contrast system than the Leitz type (Leitz also had a Phaco type phase contrast, which is identical to the traditional Zernike type). The only difference between the Phv type and the regular Zernike type is that the Phv phase annuli and objectives have two concentric phase rings in the same objective and condenser annuli. These can be used together, or the outer ring can be masked off by the condenser's regular iris diaphragm.*

The reason to do this would be that the width and positioning of the phase ring within the objective is optimized for different types of specimens. The regular type of Zernike phase, and the Phv type with both rings being used (the outer ring is more dominant, so the inner ring plays essentially no role when the outer ring is used) are optimized for relatively small specimens with smaller details relatively close together. A larger, specimen with less internal small details would produce significantly prominent halos when viewed in this type of phase.

The novel feature of the Phv being able to block off the outer ring, utilizing only the smaller inner ring, which is optimized for larger less detailed specimens. When used this way with smaller cells, the contrast enhancement is overdone and details are lost, but with larger cells the halos are significantly reduced and the contrast is similar to that of smaller cells used within regular Zernike phase contrast.

I've wrote a message concerning this before in the Yahoo Microscope group which links to pictures (although not mine, and described in German) that illustrates the advantages."

In that link Mr. Sunley goes on to explain this phase contrast as described in the original Zeiss literature.

"*In the online Jena manuals the theory of the Phv 'variable' phasecontrast is explained in the two files for the 'Phasenkontrast mit Mikroval Mikroskopen' document in the 'Mikroskop Zusatzeinrichtungen' directory, unfortunately it's explained in German.*

From what I've been able to work out from a quick read through an online

Figure 9 (left top): Access to the electronics is easy by undoing one captured screw and tilting out the hinged base.

translation of the text, the two rings in the Phv can be used (together) to produce a typical Zernike phase contrast, or by using only the inner ring to provide a phase contrast in situations where the Zernike design is not optimal.

Forgiving my poor understanding of how phase contrast works (especially if you know all this already), the theory goes that since the object is transparent, there is no optical absorption in the visual spectrum and therefore the specimen appears to have poor contrast.

But since the specimen is more dense than the liquid, the phase of the light is shifted (slowed down) as it passes through the specimen because the light travels faster through the surrounding liquid than it would through a cheek cell (for example). So if you picture a pair of individual "rays" of light (of a particular wavelength) going through the microscope slide. The wave of light which did not pass through the specimen would maintain its sine wave pattern (and frequency), but the one which went through the cell would be slowed down, offsetting it from the other wave.

The intention of phase contrast is to further reduce all the waves which have gone through the specimen so that their total amount of slow down makes it exactly half a wavelength offset from the other wave. They do this by adding a 'phase plate' in the objective of a material which further slows down those specimen waves by 1/4 of a wavelength. This 1/4 wavelength plate assumes that the specimen will also slow down the light by a 1/4 wavelength, so the specimen wave is exactly opposite of the non-specimen wave (1/2 a wavelength off). Now if you recall how two opposite waves will cancel each other out, this happens with the those waves when they recombine to produce an image creating contrast (dark areas) in the image.

The way the specimen and non-specimen waves are separated is by the fact that as a ray of light goes through the specimen it will be diffracted out of the hollow cone of light produced by the condenser annulus. So if there was no specimen in the field of view the illumination would enter the objective as a hollow cone of light (exactly fitting the dark ring which can be seen in the back of the objective. That dark ring is not the phase plate, but is just a neutral density filter meant to dim the non-diffracted light so it doesn't over power the light that is diffracted by the specimen.

If a specimen were present, the light would be bent as it passes through the specimen, causing it to pass through the rest of the area of the objective and therefore the phase plate which is built into it. By the time that diffracted light gets to the eyepiece it has been slowed down twice; 1/4 of a wavelength by the specimen and 1/4 of a wavelength by the phase plate, and cancels out the equivalent non-diffracted wave to produce visual contrast.

According to what I've been able to get out of the translation of that file, that theory working properly is dependent on the size of the object you're looking at. A small object will bend the light sufficiently to create a fairly large distance between the rays of diffracted and non-diffracted light, allowing the objective to distinguish between the two rays. The width and placement of the phase ring are the two factors which determine this, and can only be optimal for one size of objects.

Larger specimens have less localized differences in how much they'll slow down and bend the light, and therefore won't bend the light out of the path of the cone of non-diffracted light by as much. My understanding of the theory breaks down a little here, but according to the text if the amount of diffraction is too small and your phase ring is too wide this is what causes the "halo" effect you see in phase contrast images. A result from the objective not being able to differentiate between the diffracted and non-diffracted light.

So CZJ included the second, narrower inside phase ring in the Phv objectives. This narrower ring is capable of distinguishing the subtle differences in diffracted light from larger objects in the field of view without causing halos. Halos will still occur around very large specimens, but their intensity is reduced in Phv objectives.

So by closing the condenser iris down to block out the broader outer

Figure 10 (top): The Laboval 4 with after market trinocular head.

ring, only the very narrow inside ring is utilized and the contrast enhancement is more sensitive to subtle changes which would otherwise appear blurry and without the sharpness characteristic of phase contrast."

Finally Mr. Sunley goes to images posted by a Mr. Ecki to visually present the Phv contrast variations: "*Now going back to Ecki's images:*

http://www.kleinsehen.de/Testaufnahmen%20mit%20Phasenkontrast.html

You can see in the third column of images there is significantly less haloing around the smaller details of the image - which are especially apparent within the large cell of the second row of images (all the ghost-like details in second column picture). The third column pictures are images taken using only the inner ring. Once the outer ring is utilized (in the second column pictures) the contrast of the large cells (second row) becomes much less clear, full of shading and artifacts which do not exist in the specimen itself.

In the first row images (which appear as smaller cells), there is much less haloing in the middle picture (using both rings) because the amount of magnification causes the cells to be a more ideal size to produce good distinction between the diffracted and non-diffracted light. Therefore when the outer ring is blocked off the objective becomes over-sensitive to the differences in phase and the cells appear unnaturally dark. This is a good example of why it is ideal to have both rings available, as one is never ideal for all specimens and/or magnifications.

So to make a long (and likely poorly explained) story short, in Phv objectives, using it with both rings visible in a phase telescope (or in the back focal plane of the objective) is the same as any other implementation of Zernike phase contrast and is optimal for relatively small specimens. But if you're looking at larger objects which would produce prominent halos in traditional phase contrast, the outside ring can be blocked out and the contrast enhancement produces a sharper image with less halos. "

So ends my search for Phv phase knowledge, and as comment on Mr. Sunley's information it's "Yeah, what he said."

I now am very interested in providing a comparison between the single and multiple ring phase set up on this stand if I can improve the lighting somewhat. (Whenever I can find the bloomin' time. The explosives safety business has been brisk of late.)

As always, comments or corrections welcome. Cheers! Joseph Wilhelm.

Acknowledgement

The author and the editor would like to thank Kevin Sunley for permission to share his valuable information from the Yahoo Microscope Forum messages. ■

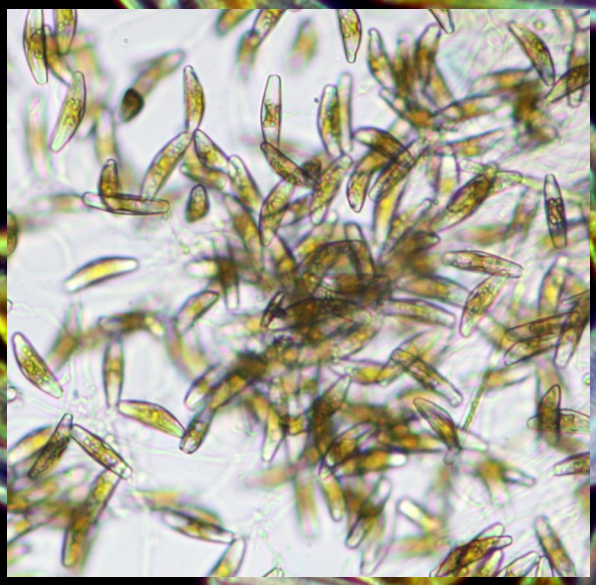


Figure 1 (top): Daphnia, also known as water fleas.
Figure 2 (middle): Diatoms in bright field
Figure 3 (bottom): The water fountain from which the samples were taken.
Figure 4 (background): Diatoms have a shell made of silica. The green chloroplast is responsible for photosynthesis. These diatoms were observed using dark-field microscopy.

Figure 5 (top): Pond or fountain? Sometimes it's not easy to see a difference!
Figure 6 (bottom): Daphnia

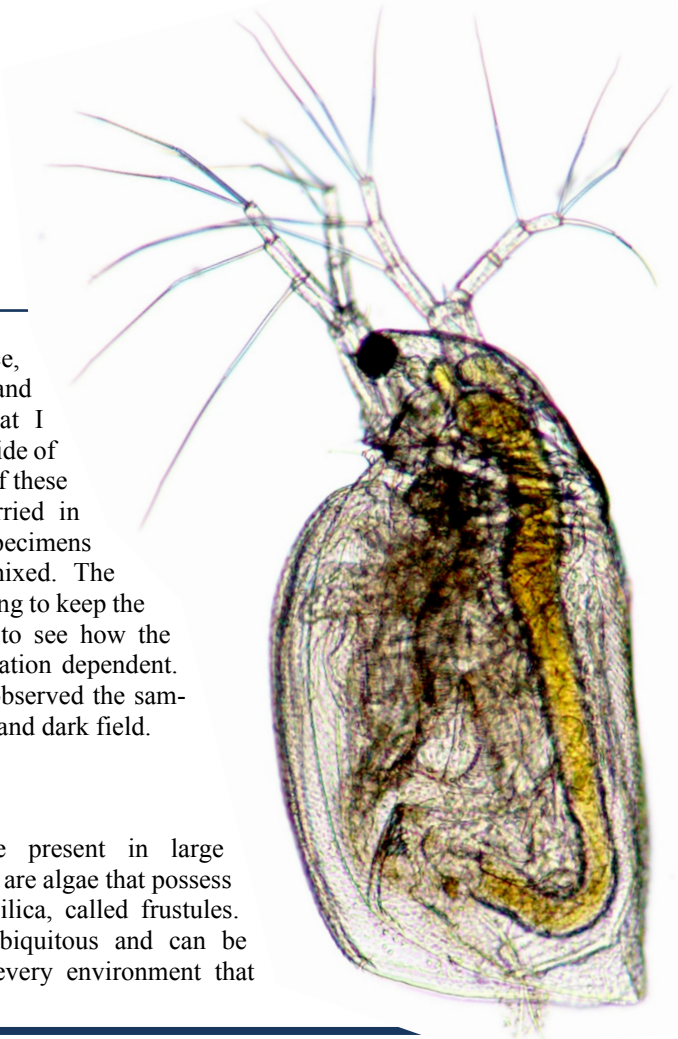
Of Life in the Water Fountain

You do not need a pond in order to observe pond microorganisms. Sometimes a forgotten water fountain will do as well.

Oliver Kim

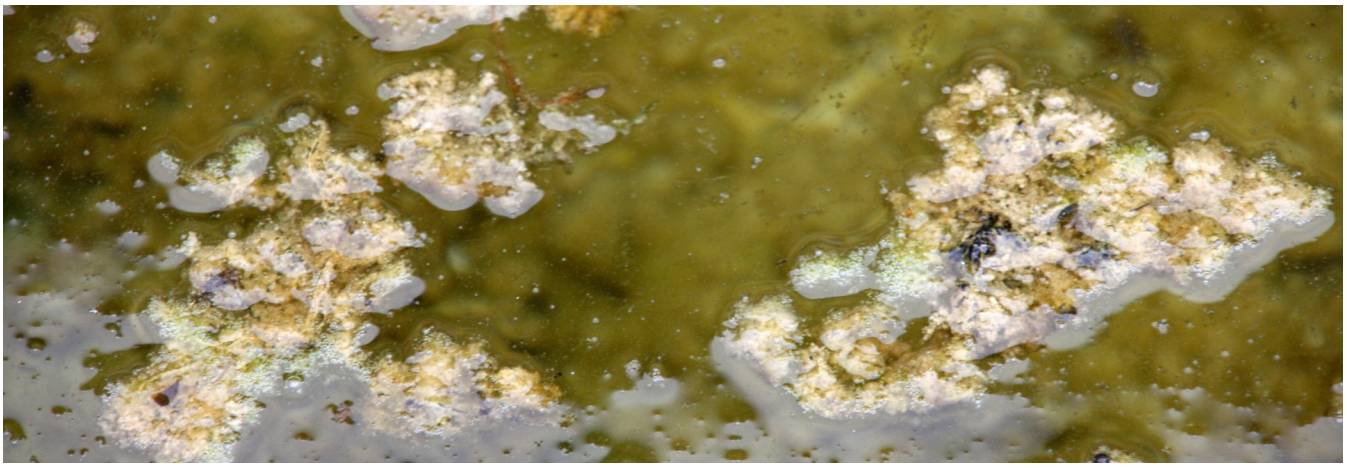
After having spent quite a lot of time looking at ready-made permanent slides, I decided that it's finally time again to go back to the basics. In particular, I wanted to have a look at some water samples, in the hope of discovering a few interesting algae, ciliates, and possibly other surprises. The most difficult thing turned out to be (and this was the first big surprise) finding appropriate water samples. Our village does have several water fountains, all for decorative purposes, and I discovered that they were so well kept that they looked more like swimming pools with drinking water quality. After some searching, I did find a small, rather neglected looking fountain (figures 3, 5), the rocks in the middle were half overgrown with moss and plenty of green material floating on the water - a microscopist's paradise!

I collected three types of samples: some green fluffy material, which was floating on the water surface, some clear water and some material that I scratched off the side of the fountain. All of these samples were carried in the same jar, the specimens were therefore mixed. The next time I am going to keep the samples separate to see how the organisms are location dependent. Back at home, I observed the sample in bright field and dark field.



Diatoms

Diatoms were present in large amounts. Diatoms are algae that possess a wall made of silica, called frustules. They are very ubiquitous and can be found in nearly every environment that



Figures 7, 8: From an aesthetic standpoint, the fountain was not very pleasing to look at. While there was a little bit of water flowing into the fountain, the total turnover of water was quite low. The stagnant water was quite clear, evidently the daphnia and ciliates cleared away many of the single-celled algae. A natural pond of this size would probably be too rich in organic nutruients (due to fertilizers of the adjacent fields), and would therefore probably have resulted in eutrophication and an accumulation of decomposing bacteria. The continuous inflow of fresh water provided favorable conditions for growth of photosynthesizing algae and an enrichment owith oxygen. The floating material contained mostly diatoms.

contains water. They can also be found in soil. The green chloropasts were clearly visible, sometimes they were located centrally, sometimes at the side. Rarely I could see empty frustules. It was not easy to obtain individual diatoms, most of them were held together by something that seemed to be some kind of sticky matrix. The diatoms grew in bundles, producing green patches (figure 9). The chloroplasts of some of the diatoms diatoms were not located centrally, but appeared to be present at the periphery (figure 4), while other diatoms had central chloroplasts. The shell of the diatoms in figure 4 is similar to *Cymbella helvetica*, but due to the differences in the position of the chloroplast, I have some doubts if it really does belong to this species. I have also found linear colonies of diatoms, which possibly belong to the genus *Fragellaria* (figure 16).

Ciliates

Not surprisingly, ciliates were present in every sample that I observed. Ciliates are single-celled organisms that have hair-like structures on their surface. They moved extremely quickly and it was impossible to capture them with the camera. They moved around beneath the cover glass, until they reached the edges or an air bubble. They then turned around and quickly swam into another direction, until they met yet another obstacle. To obtain a better image of these organisms, it is certainly necessary to increase their density (by selective enrichment) and to slow them down with the addition of some methyl cellulose or gelatin solution.

Worms

Occasionally microscopic worms could also be seen. These were of two types, the nematodes and the annelids. Nematodes (roundworms) are non-segmented. These were difficult to photograph due to their rapid movement.

I was lucky enough to capture an image of an annelid. Figure 15 shows the picture of an annelid, possibly a member of the genus *Chaetogaster*. Compared to other annelids, this genus has bristles only on the ventral side of the worm and not every segment has bristles. The particular worm that I was able to photograph moved quite vigorously in a searching manner, but it did not swim into any particular direction, making it easier to photograph it.

Daphnia

Daphnia are small crustaceans, they are more commonly known as water fleas. They should not be confused with fleas, which are insects. Daphnia are called water fleas because they move in

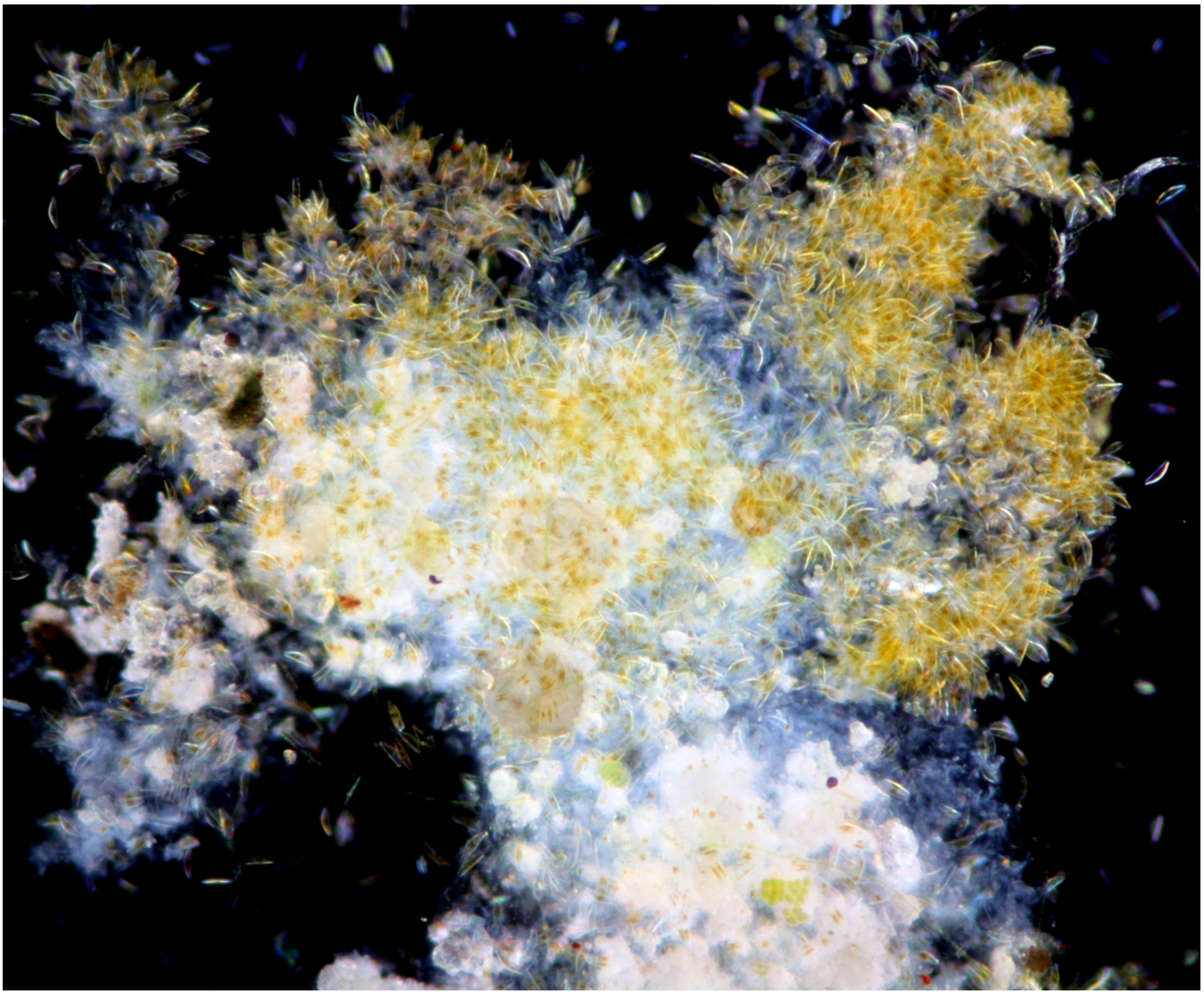


Figure 9: The diatoms grew in bundles and had a strong tendency to stick together. Other algae, worms and ciliates were sometimes caught between the diatoms. I was able to partially free them by carefully suspending the material in a bit of water.

a jerky manner, appearing to jump through the water like fleas.

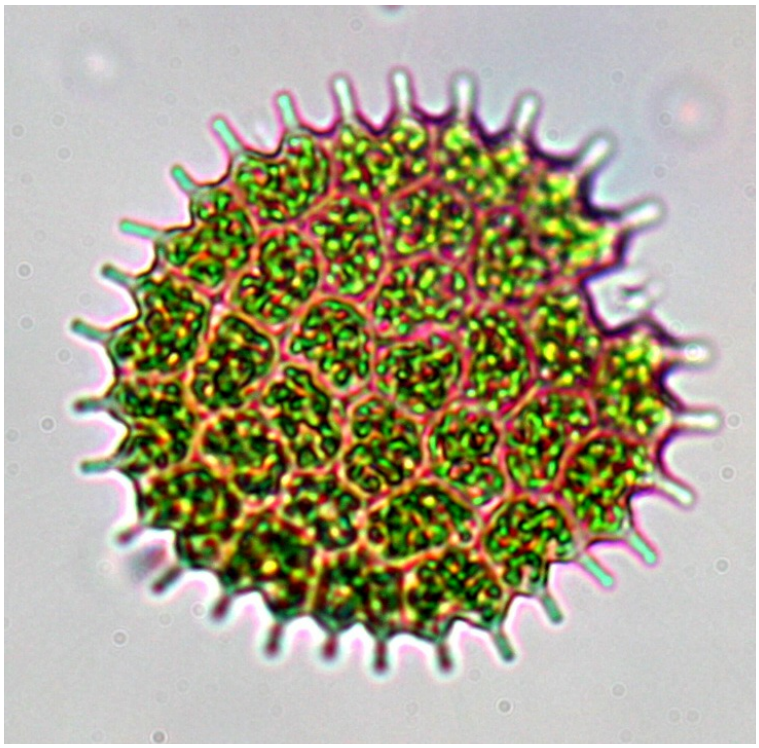
Numerous daphnia could be found in the water sample. They feed on single-celled algae and bacteria and are therefore responsible for the relatively clear water. Luckily there were no fish in the water, which allowed them to reproduce in large numbers. Daphnia are transparent and it is therefore possible to see the organs. I had to immobilize them by holding them in place between cover glass and slide. Still, they do not stay calm and the beating heart and their ability to move their organs requires short shutter speeds when taking pictures.

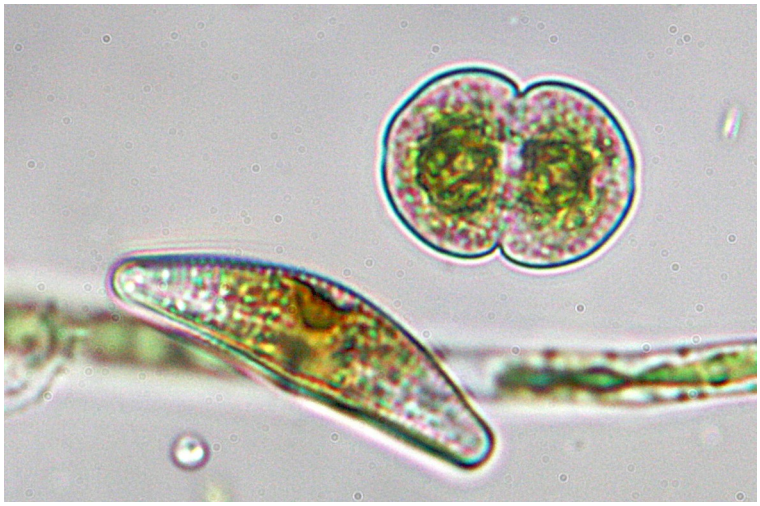
Green algae

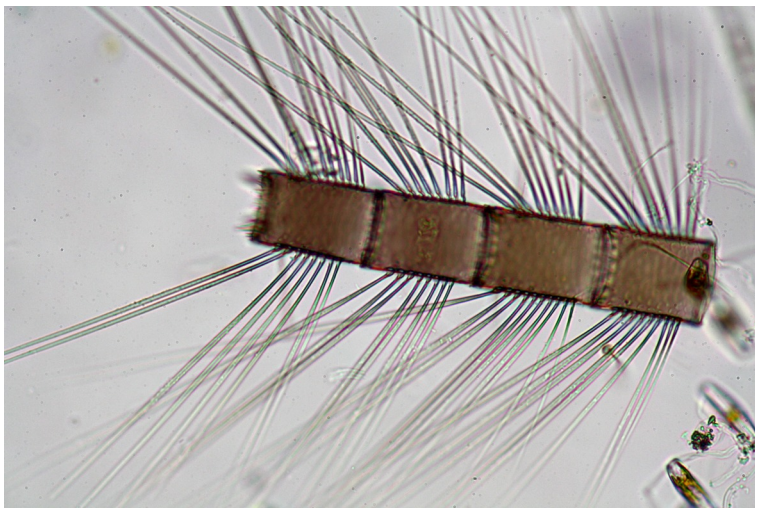
Besides various diatoms, another quite beautiful green alga could be found. *Pediastrum boryanum* (figures 10, 11) forms colonies with up to 128 cells. The external cells are pointed and, unlike other members of the genus, the individual cells fill up the whole colony, with no empty spaces between the cells. I expected to find more filamentous algae but these were not present in large amounts.

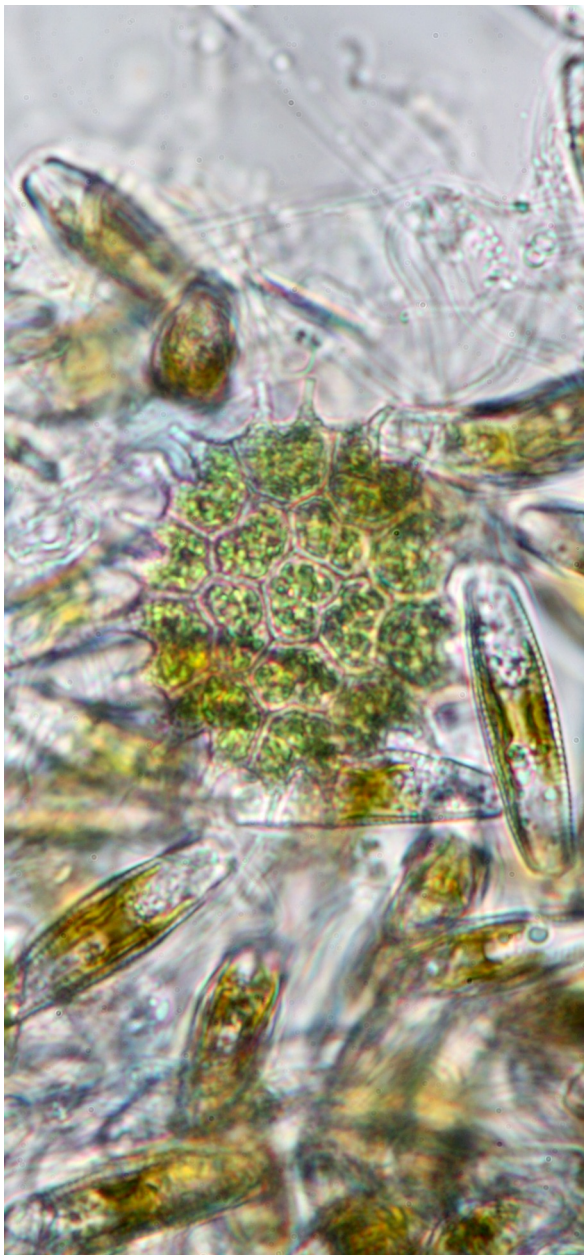
Some advice

Beginners are frequently disappointed when observing pond water samples under the microscope. Where are all the algae, ciliates and other organisms that one expects to see, but are are not able to see? Often the mistake can be easily corrected. Do not look at the pond water itself, have a look at some sediment, some slimy coating of rocks and leaves. As a general rule, if you are not able to see the specimen with your unaided eye (some green



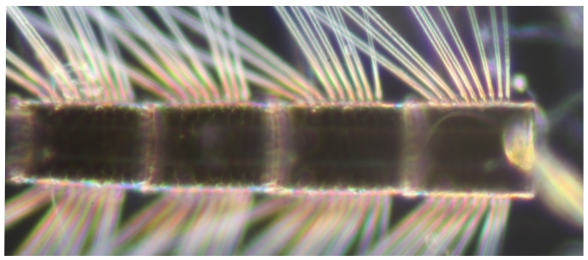






Figures 10 - 12 (top): Besides diatoms, other green algae could also be seen. The top left image possibly shows *Pediastrum boryanum*.

Figure 13 (right), 14 (bottom): unknown structure, possibly a part of the exoskeleton of an insect?



slime, a piece of decaying plant material, etc.), then the chances are pretty good that the density of microorganisms is too low also for microscopic observation. Clear pond water is often too clean and the density of organisms is too low. Many organisms are attached to a solid surface, and it is this surface that should be investigated.

Identification of the organisms

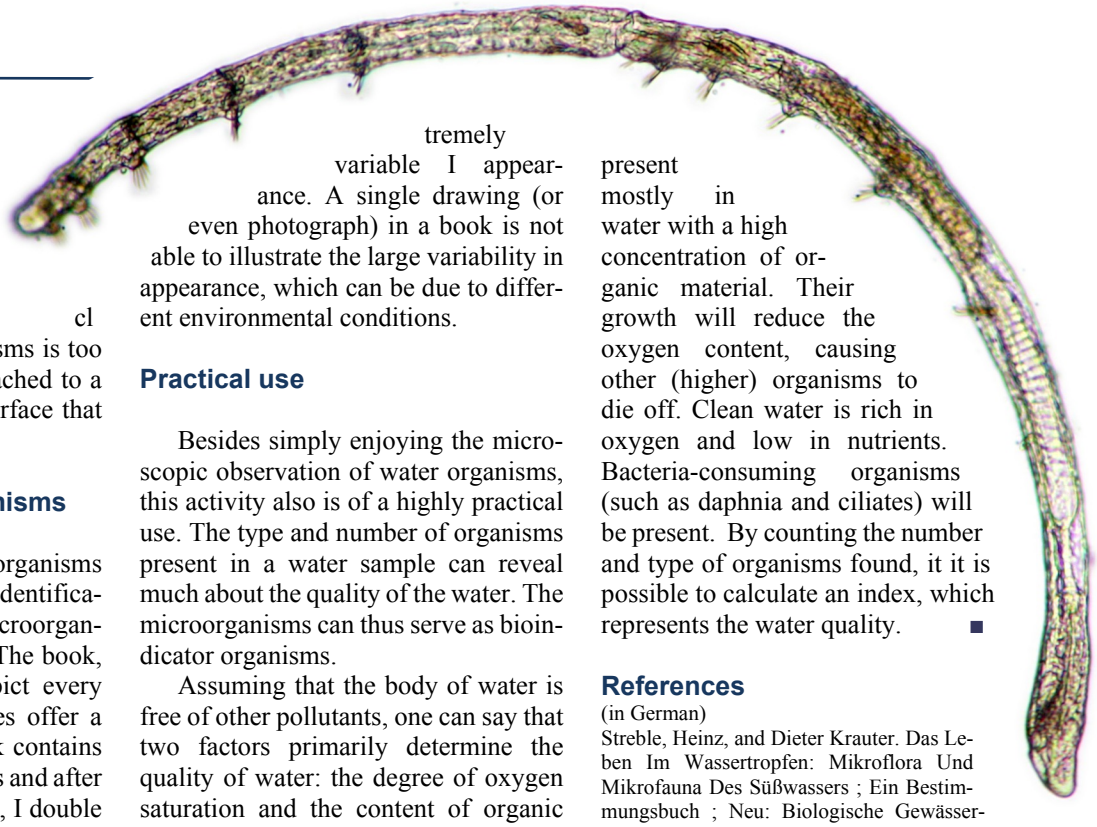
I attempted to identify the organisms with the help of an illustrated identification book for freshwater microorganisms (see reference section). The book, naturally, is not able to depict every possible organism, but it does offer a good starting point. The book contains hundreds of different drawings and after having identified an organism, I double checked by making a Google picture search to compare the organism with published images. One should not forget that microorgnaisms can be extremely variable I appearance. A single drawing (or even photograph) in a book is not able to illustrate the large variability in appearance, which can be due to different environmental conditions.

Practical use

Besides simply enjoying the microscopic observation of water organisms, this activity also is of a highly practical use. The type and number of organisms present in a water sample can reveal much about the quality of the water. The microorganisms can thus serve as bioindicator organisms.

Assuming that the body of water is free of other pollutants, one can say that two factors primarily determine the quality of water: the degree of oxygen saturation and the content of organic material.

Different water organisms have different oxygen and nutrition requirements. Bacteria, for example, will be present mostly in water with a high concentration of organic material. Their growth will reduce the oxygen content, causing other (higher) organisms to die off. Clean water is rich in oxygen and low in nutrients. Bacteria-consuming organisms (such as daphnia and ciliates) will be present. By counting the number and type of organisms found, it it is possible to calculate an index, which represents the water quality. ■



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Streble, Heinz, and Dieter Krauter. Das Leben Im Wassertropfen: Mikroflora Und Mikrofauna Des Süßwassers ; Ein Bestimmungsbuch ; Neu: Biologische Gewässeranalyse. Stuttgart: Franckh, 1988. Print

Figure 15 (top): *Chaetogaster* is an annelid, a segmented worm.

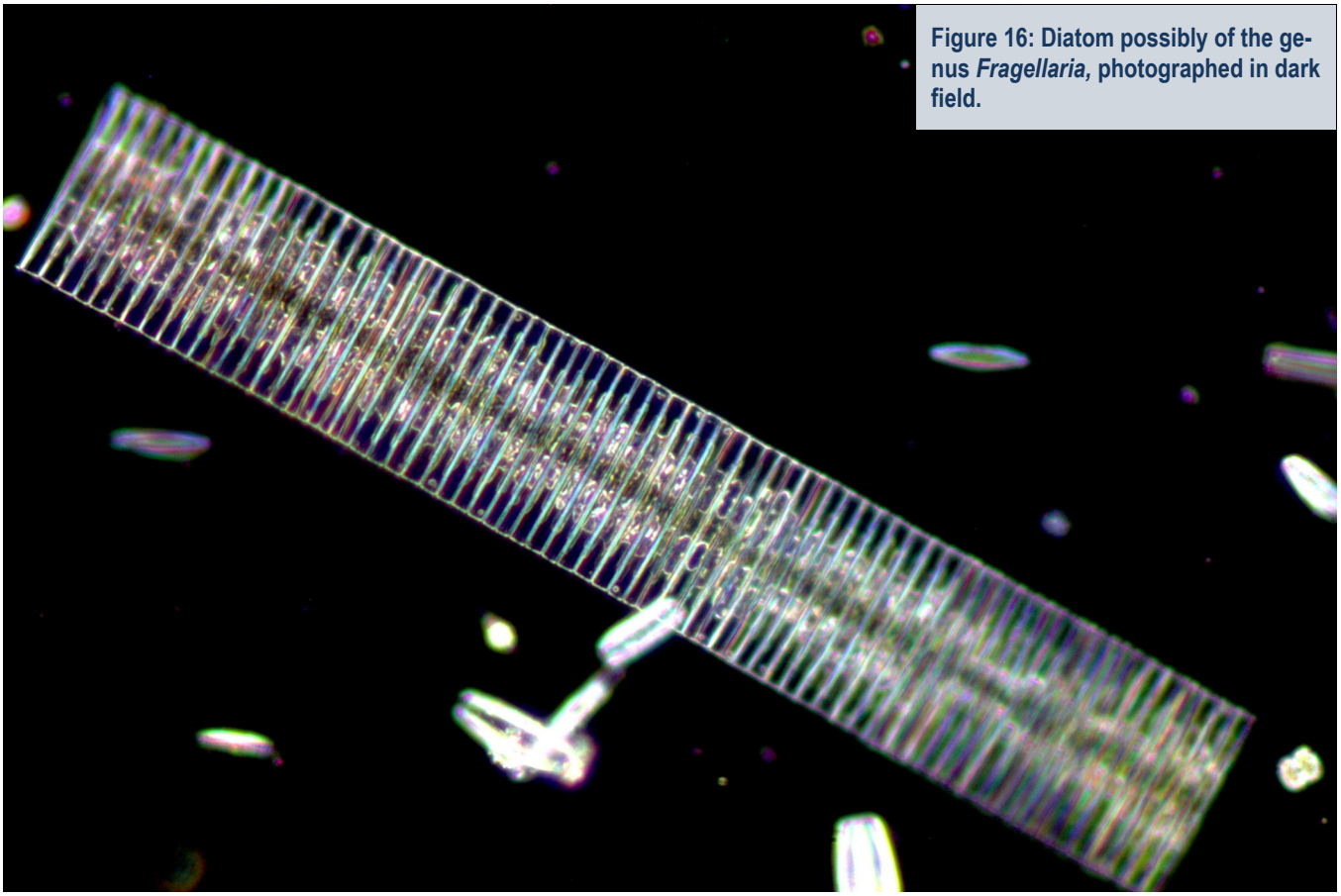


Figure 16: Diatom possibly of the genus *Fragellaria*, photographed in dark field.

The World of Sponge Spicules

Sponge spicules make up the skeleton of sponges. They are useful for sponge classification.

Oliver Kim

Sponge spicules are hard, pointed structures that can be found in most sponges. They give the sponge a structure, and it serves as their skeleton.

Sponge spicules are made either of calcium carbonate, silica or spongin (a protein). The spicules are produced by cells called scleroblasts. The composition, as well as their size and shape can be used to classify the sponges.

Types of Spicules

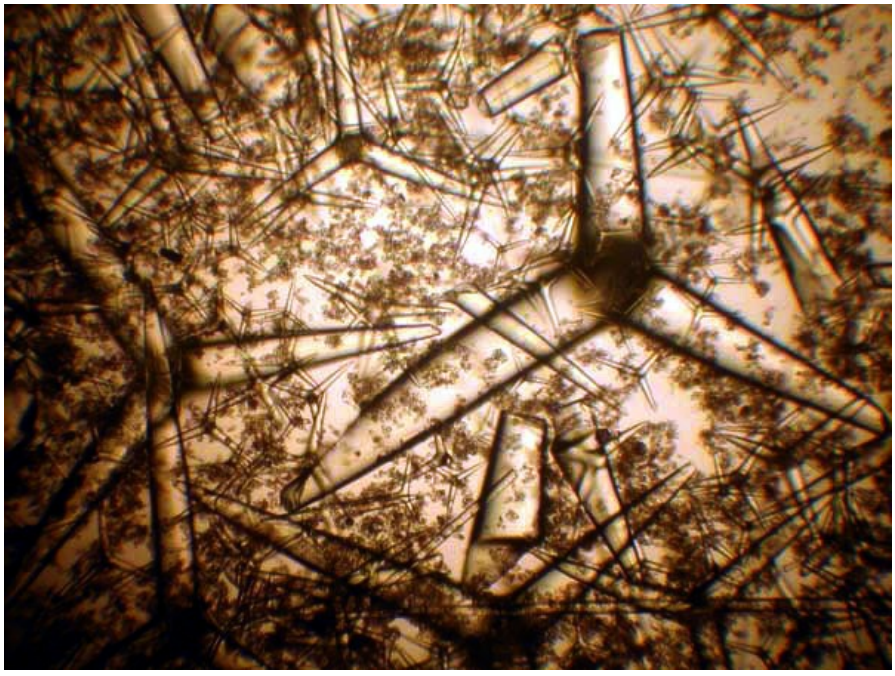
Depending on their size, sponge spicules can be sub divided into megascleres (60-2000 microns, this is up to 2mm) and microsclares (10-60 microns). Megasclares are also visible with the unaided eye and they serve as the sponge's main support. Microsclares are distributed throughout the sponge. Their primary function is in the support of individual cells.

Sponge spicules can be classified based on the number of axes that they possess. Several symmetries can be identified, with each symmetry being further subdivided:

- Monaxons: These are linear spicules. Diacinal monaxons have similar ends, while the monactinal monaxons have one rounded and one pointed end.
- Triaxons: They have three axes. The triods have three similar rays extending into different directions. The pentacts have 5 rays, with four of them arranged in one plane. The fifth ray extends away from the plane.
- Tetraxons: They have four axes of symmetry. In the calthrops arrangement, the rays are arranged in a tetradedral manner, while in the triaene arrangement, one ray is different from the others.
- Polyaxons: These are rounded spicules with the rays diverging from a center.
- Sigma-C: These spicules have the appearance of a "C".

Silica Spicules

The spicules of some deep-sea sponges, such as the ones of the Venus' Flower Basket (*Euplectella aspergillum*) are made of silica. These are long and transparent structures, which are able to transport light, much like fiber optic cables. The spicules either help to harvest the little light present in the deep sea, or help to direct bioluminescent light to photosynthetic organisms, which live symbiotically inside the sponge. ■



Transparent sponge spicules Pachastrellid Sponge

(image credit: Public domain, NOAA)

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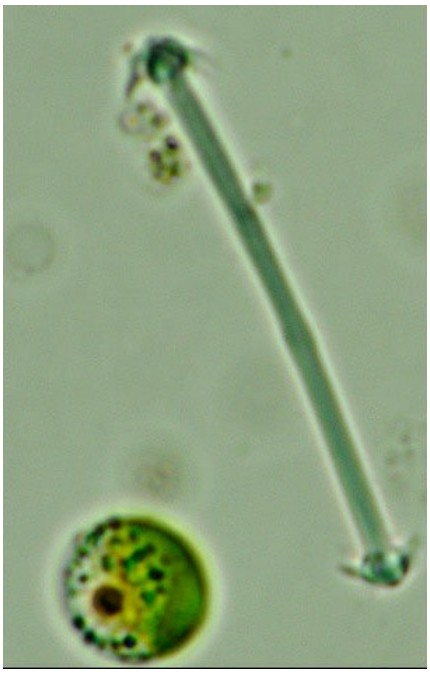
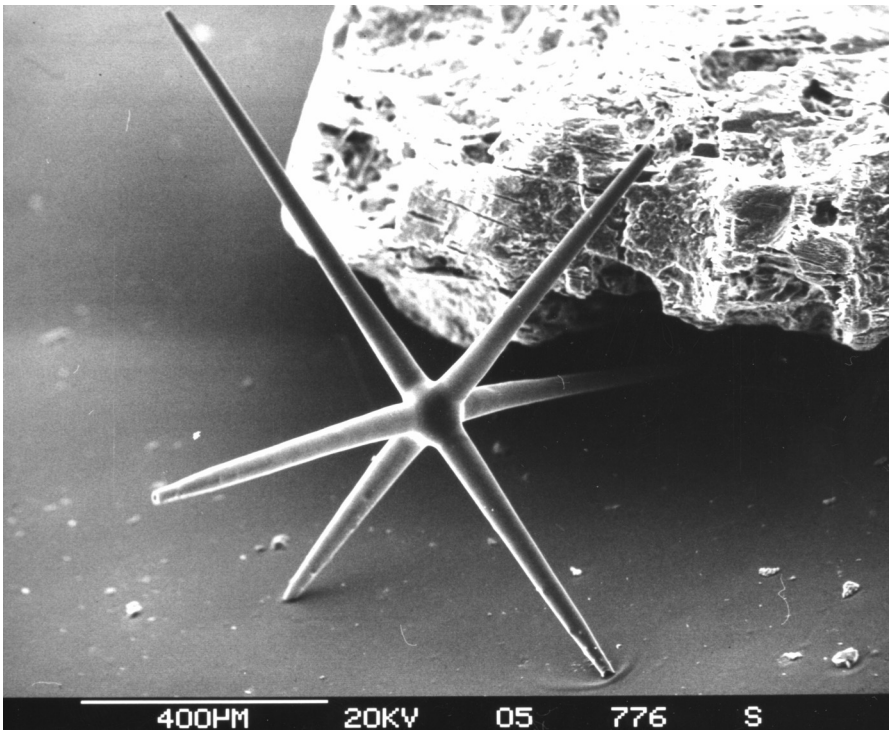


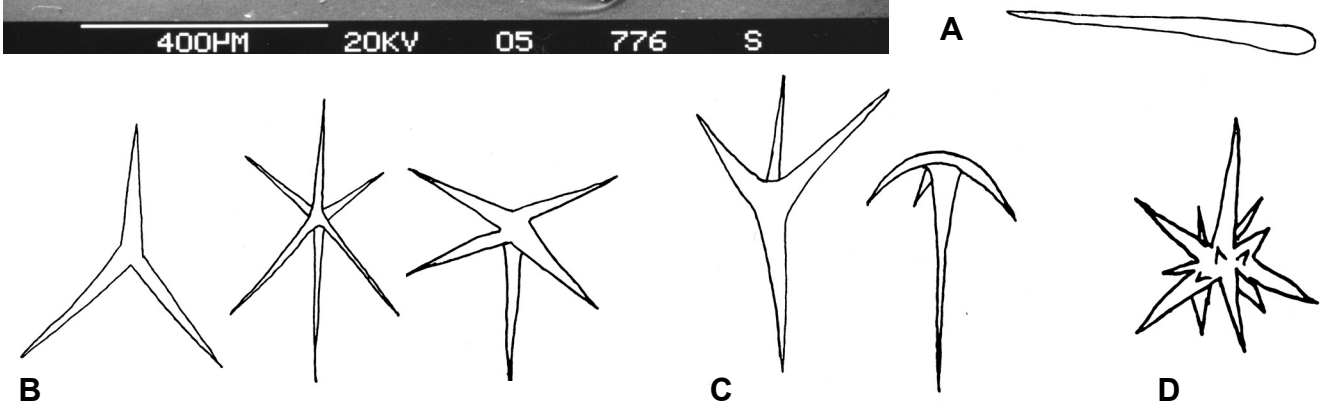
Figures 2, 3 (to left): Bright-field images of sponge spicules. A small piece of sponge was torn apart and directly microscoed.
(image credit: Tony T)

Figure 4 (top): the Venus' Flower Basket contains spicules made of transparent silica.
(image credit: Public domain, NOAA)

Figure 5 (bottom left): Electron micrographic image of a spicule illustrating its 3-dimensional structure.
(image credit: cc-by-sa Hannes GrobeAWI)

Figure 6 (bottom): Shapes of some megascale spicules. A: monaxon, B: triaxon, C: tetraxon, D: polyaxon





Female detectors

Moth antenna in close-up

Anthony W. Thomas

In many species of moths the female emits a chemical which is attractive to males of the same species. Males perceive this chemical with their antennae.

Male Gypsy Moths have large antennae (Fig. 1). Each antenna consists of a "backbone" made up of many segments (Fig. 2). Two "branches" arise from each segment arranged such that in cross section each antenna segment is V-shaped (Figs. 2 & 3). Each "branch" has numerous hairs that interlock with the hairs of adjacent "branches" so that each antenna forms a net or filter for the air passing through (Fig. 3). The tip of each "branch" has a short spike and a long spine (Fig. 4). The segment at the extreme tip of each antenna lacks a "branch" and the 2 segments in front of the tip have very short branches (Fig. 5). The hairs on the branches originate in pits that show as pale dots under the microscope; Fig 6 shows the middle parts of 2 "branches" at 50x magnification, and Fig 7 shows the bases of 2 branches attached to the "backbone" segments at 100x. It is possibly these hairs and pits that contain the sensors that recognize the scent emitted by a female. Once detected, the male flies upwind until he finds the female.

Moths are ideal subjects for testing the ability of a microscope, especially for objectives, to resolve detail. Diatoms are the classical "test" subjects but moth antennae and wing scales are readily obtained substitutes.

Figure 1: Male Gypsy Moth in typical resting pose, note the huge antennae.

The gypsy moth, *Lymantria dispar*, is a moth in the family *Lymantriidae* of Eurasian origin. Originally ranging from Europe to Asia, it was introduced to North America in the late 1860s and has been expanding its range ever since. It is also known as the Asian gypsy moth. The forewing of the male moth is 20–24 mm long, and that of the female 31–35 mm. The brown male gypsy moth emerges first, flying in rapid zigzag patterns searching for females. The male gypsy moths are active throughout night and sometimes even daytime as well, unlike most moths, which are only nocturnal. When heavy, black-and-white egg-laden females emerge, they emit a pheromone that attracts the males. After mating, the female lays her eggs in July and August close to the spot where she pupated. Then, both adult gypsy moths die. The European and most Russian forms of the gypsy moth have flightless females. Although they have large wings, the musculature is not developed. However, the Japanese gypsy moth females do fly and are attracted to lights.

Reference: Text quoted from Wikipedia http://en.wikipedia.org/wiki/Gypsy_moth Wikipedia

Figure 2 (far top): Single antenna, each segment bears 2 'branches'.

Figure 3 (top): Close-up of the 'branches'

Equipment used

Olympus BH2, S Plan 10x, 20x, 40x objectives, 2.5x relay lens, Nikon DSLR. Moth and detached antenna (Figs 1 & 2) with 105 mm Micro Nikkor on camera. ■

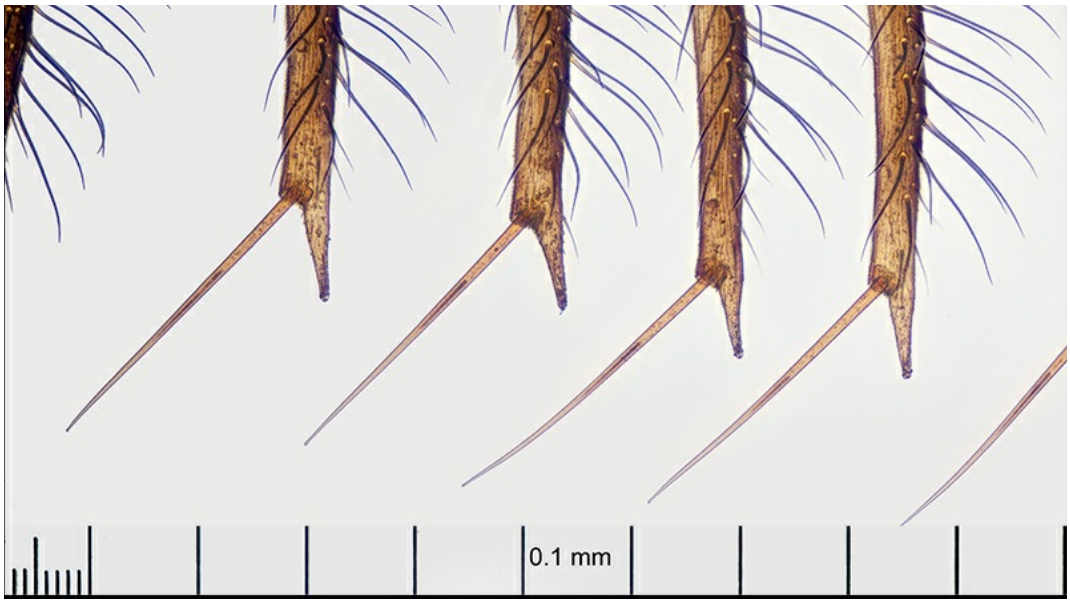


Figure 4: Tips of "branches" showing the short and long spikes.

Figure 5: Tip of antennae, showing detail.

Figure 6: 50x magnification showing hairs and pits on “branches”.

Figure 7: 100x magnification of “branches” at their bases with “backbone” segments

Seed Shrimps (Crustacea: *Ostracoda*)

Aquatic 'seeds' that swim.

Anthony W. Thomas

Following the article in the July issue of MicrobeHunter magazine I took a sample from my outdoor bird bath. Found this little character swimming furiously (Fig. 1).

It's an Ostracod (seed shrimp), a division in the Arthropoda: Crustacea. The animal lives inside 2 shells (a, b) held together by a muscle (c, muscle attachment inside).

There is a dorsal eye (d). Inside the shells is the body with the usual appendages seen in other crustacea. The long antennae (e) can be seen protruding from the front end.

When first seen and when disturbed these animals look like hard-shelled seeds. But very soon afterwards, antennae appear and they begin to swim; it is only then that you realize they are not seeds but little clams (bivalve mollucs). But then this doesn't seem correct, checking various books you correctly identify them as tiny crustacea living within 2 shells.

You never actually see the shrimp, only the antennae at the front dorsal edge and perhaps a few legs sticking out of the ventral edge. They can be found in all aquatic habitats including oceans and freshwater; they even occur in temporary habitats such as rain puddles. They range in size from 0.2 mm to 30 mm but the freshwater ones are usually about 1 mm long. The colour and patterning of the shells make them interesting subjects for low power microscopy (Figs. 2, 3, 4).

Equipment

Live animals in cavity slides were slowed down with "ProtoSlo" (methyl cellulose). Nikon 10x CF N objective, on bellows (no microscope) Nikon SLR camera. Reflected light from flash. Some images are stacks using Zerene Stacker. ■

Figure 1: Side view of a Seed Shrimp with features labeled.
a, b: shells
c: muscle attachment
d: dorsal eye
e: long antennae

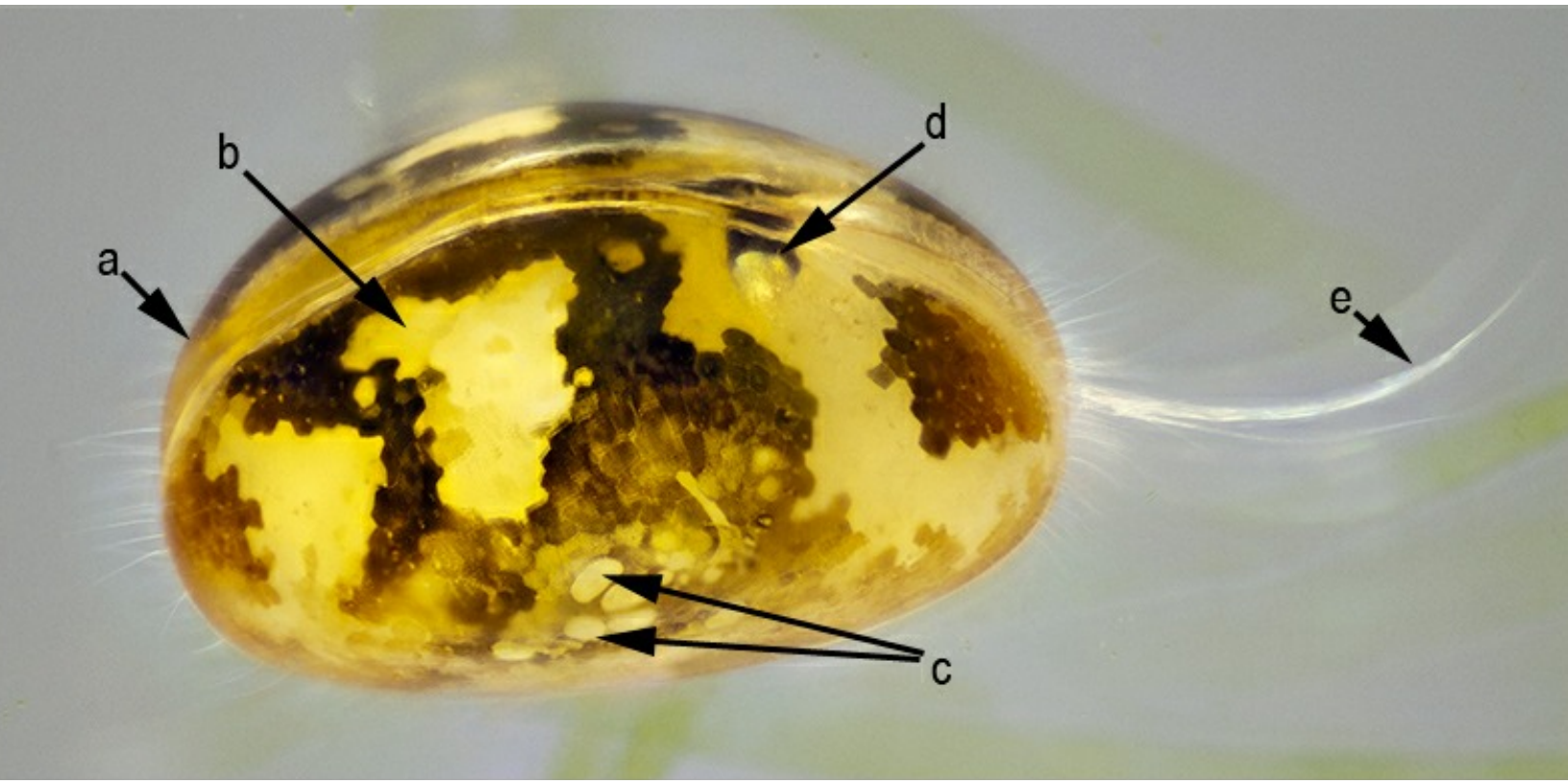


Figure 2 (top): A pale-colored specimen, leg sticking out bottom left

Figure 3 (bottom): Colourful specimen, long 1st antenna, short 2nd antenna bottom right.

Figure 4: (right): Same specimen as in Figure 3, showing the pair of 1st antennae and open valves.

A Microscope Projection Screen

Projection screens allow for a more relaxed viewing, but they also have some disadvantages.

Oliver Kim

I recently had the lucky opportunity to get a (somewhat vintage-looking) projection screen for my microscope. The screen is mounted on top of the trinocular head, instead of a camera. It is then possible for several people to view the microscopic image, which is projected on the screen using the regular lighting system of the microscope. The screen, essentially, functions like a low-tech monitor. The front surface of the screen is made of frosted glass, inside the "tube" there is a mirror which reflects the light from the trinocular head to the screen. A projection eyepiece is also needed. These screens are now mostly obsolete, with camera systems connected to a monitor offering more flexibility and a brighter image.

Naturally, the image quality is much lower than when viewing the image directly through the eyepiece. The dark areas of the image are not really completely dark, which is due to internal reflections of the system. This naturally reduces image contrast. The frosted glass also reduces the resolution and brightness of the image somewhat. I was using a projection eyepiece which was intended for cameras. Other projection eyepieces may produce a brighter image. The projection screen is almost parfocal with the eyepieces. This means that both images (from eyepiece and screen) are nearly equally sharp when focused.

There are, nevertheless, some disadvantages as well. The projected image is not very bright and it is therefore necessary to darken the room. Alternatively, one needs a very bright illumination system, which may heat up the specimen. The field of view is also small. As a matter of fact, the projected image does not even fill the whole screen, with a small border of about 5 mm still being visible on all sides. These issues can be resolved by using a different projection ocular, however.

Construction

The screen is made of metal and has a window on one side, below the mirror. I do not know if the cover of the window is missing, or if it is intended that it is open. I think that the purpose of the window is to allow easy access to the projection ocular. The diameter of the screen is 8.5 cm.

Uses of the screen

A screen like this is certainly a cheap and simple solution for allowing several people to watch the same specimen, as is commonly required in education. The screen can be freely rotated and therefore people sitting on the opposite end of the microscope (which is common for instructional sessions) are also able to the the image.

I also had another use in mind, though: It is possible to use the screen for drawing microscopic images. By simply taping a piece of paper on the frosted glass surface, it should be possible to trace the image. The first attempts of placing drawing paper over the screen for tracing the image, was

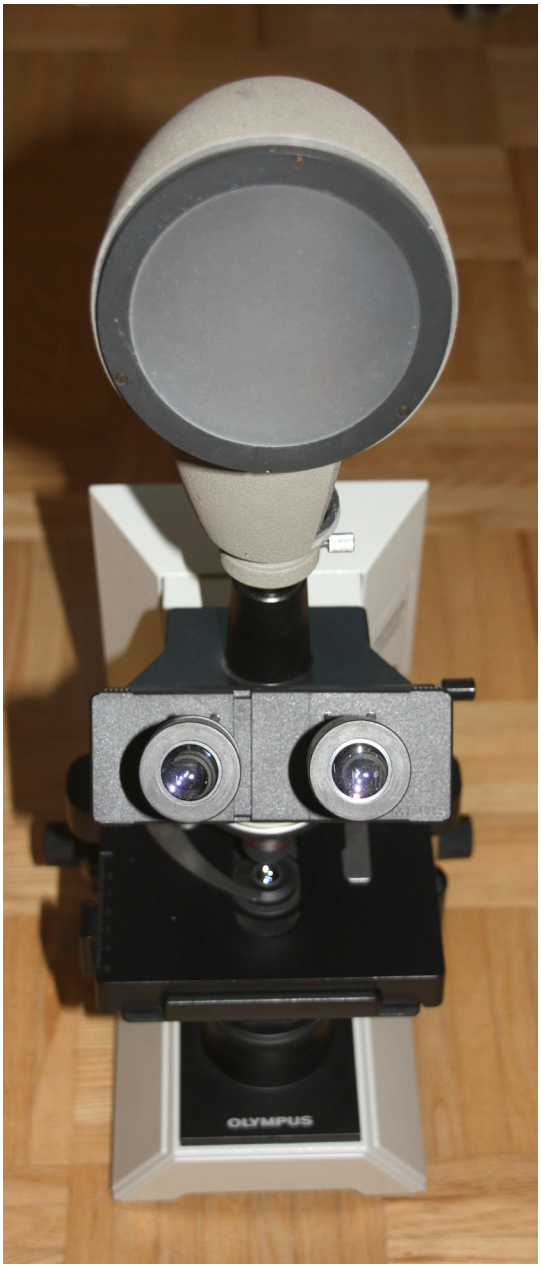
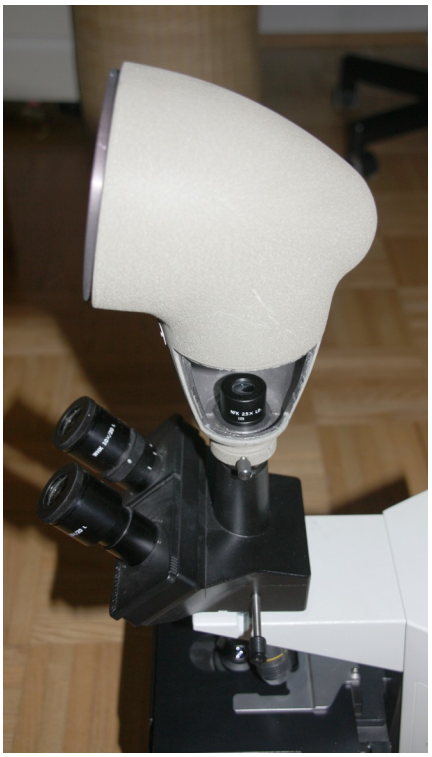


Figure 1: The projection screen hovers above the microscope like a gigantic third eye. A trinocular head is an absolute necessity.



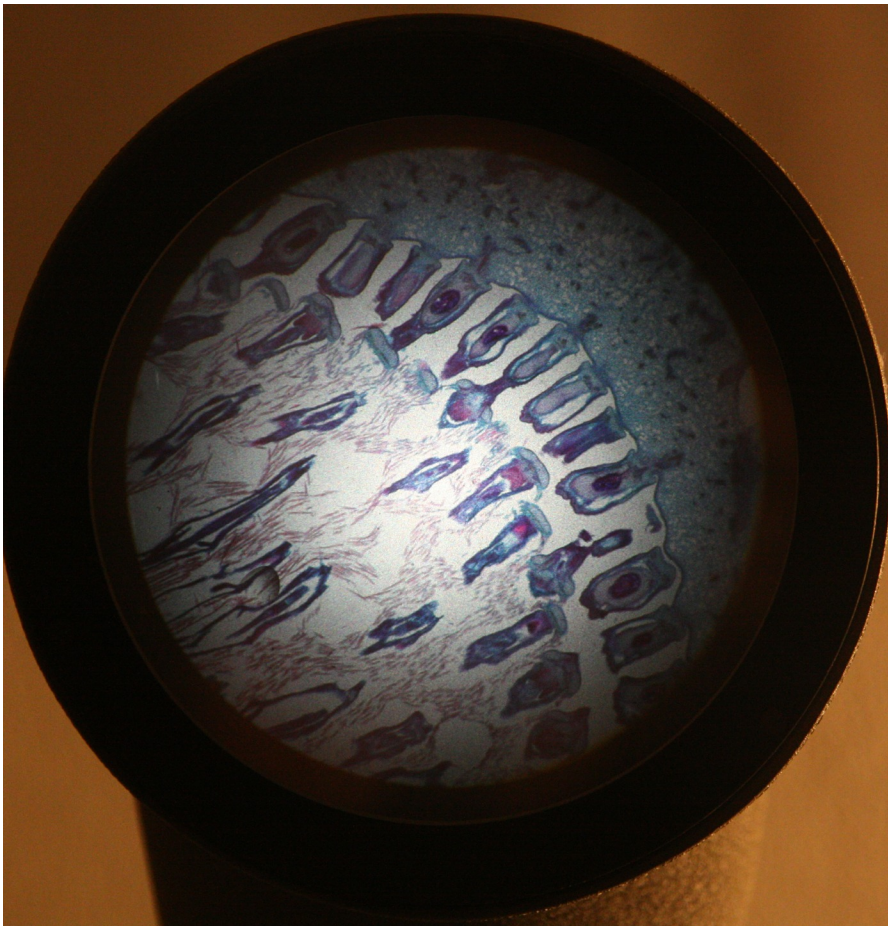
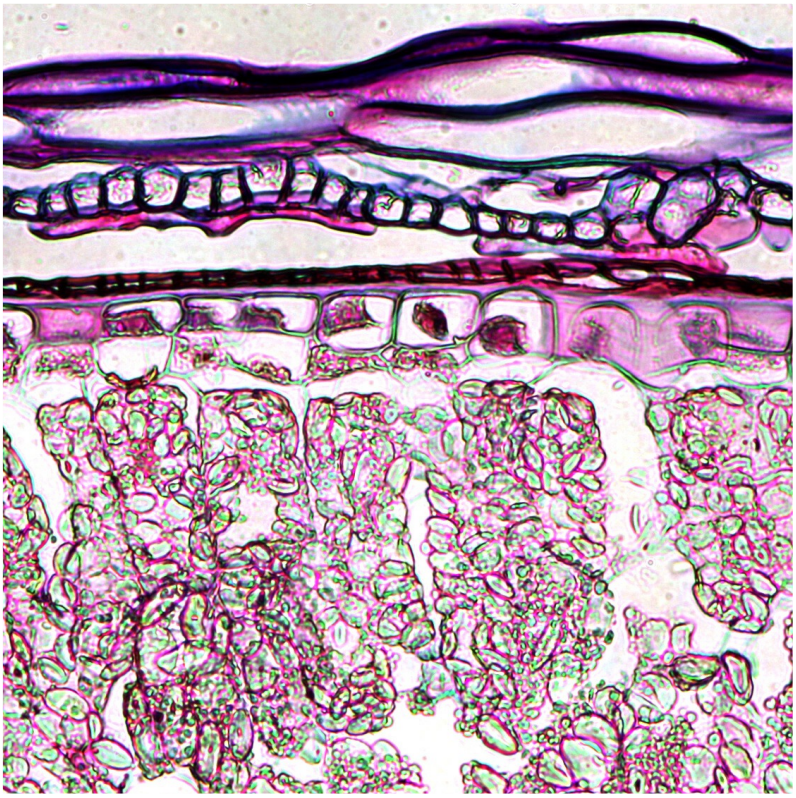


Figure 2 (top): The screen is mounted on the trinocular head of the microscope. It can be rotated 360°. Especially for educational purposes this set up may prove to be quite valuable.

Figure 3 (top right): A 2.5x NFK projection ocular for 36 mm film cameras was used to project the image on the screen. The projection oculars can be exchanged through a window in the side of the tube.

Figure 4 (right): The screen area has a diameter of 8.5 cm. The currently used projection eyepiece is not able to project the image to the complete area. A somewhat uneven illumination is also visible.

not very successful, however. The paper absorbed much of the brightness and it was difficult to trace most specimens. This problem was most evident at higher magnifications (and lower light intensity). I will continue to experiment with this and then report back in a later issue. ■



What's this? Answer on page 3.