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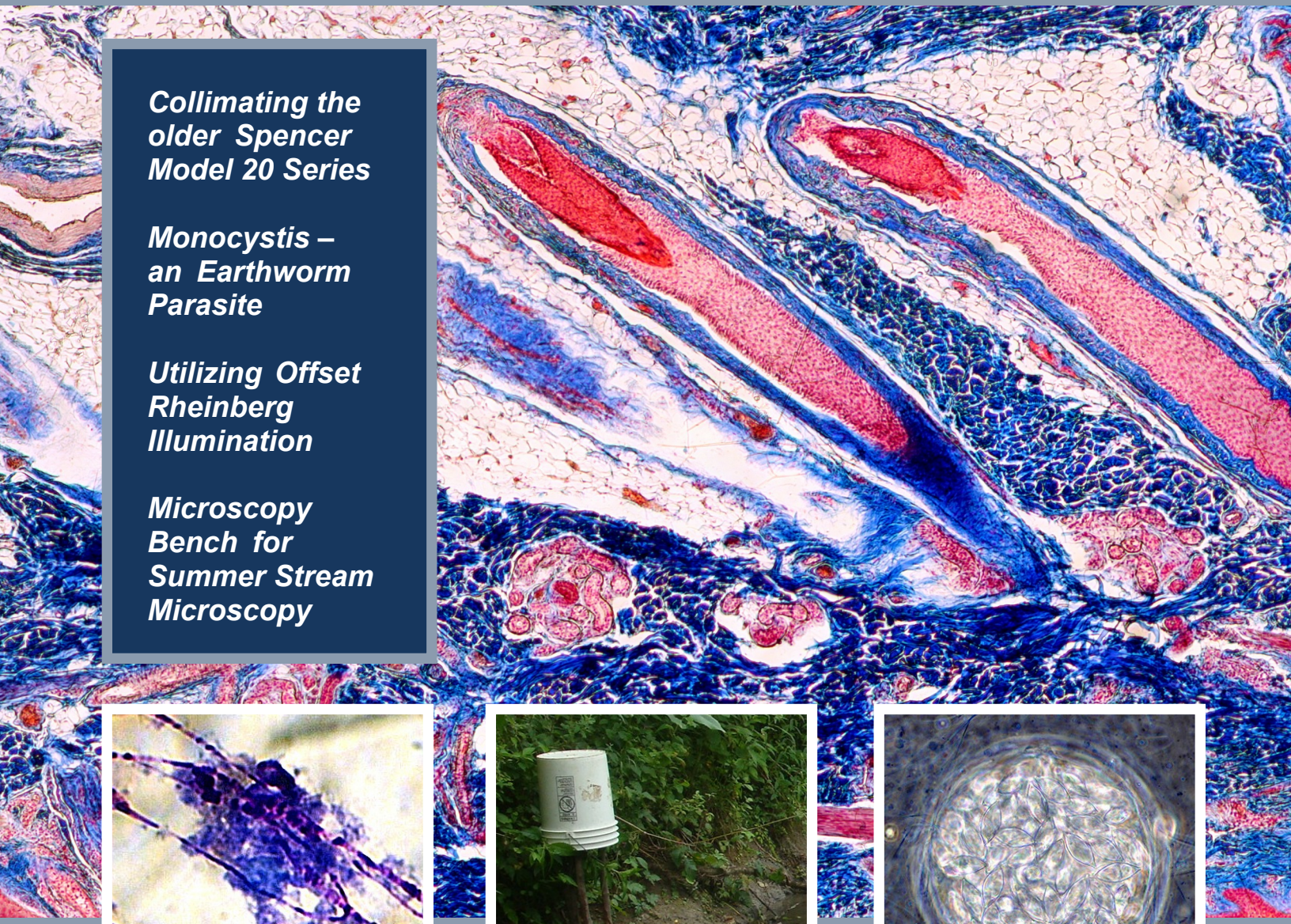

Collimating the older Spencer Model 20 Series

Monocystis – an Earthworm Parasite

Utilizing Offset Rheinberg Illumination

Microscopy Bench for Summer Stream Microscopy

Giant Chromosomes

Field Station

Monocystis

ABOUT

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Front Cover:
Large image: Oliver Kim (human head skin with hair roots)
Left image: César Guazzaroni
Middle: Charles E. Guevara
Right: Anthony Thomas

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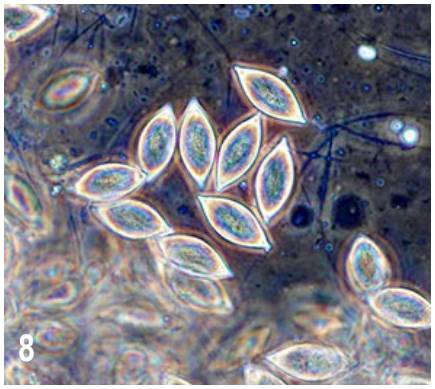

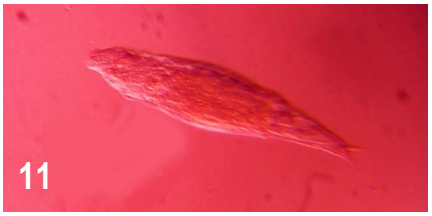

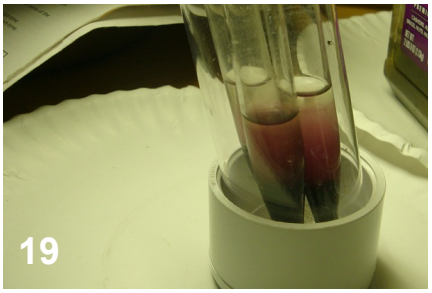

Answer to the puzzle (back cover):
Trichinella spiralis, the "pork worm" in muscle. A parasite.

How not to see double:

Collimating the older Spencer Model 20 Series stereomicroscopes

In this episode, the author describes the repairing of a stereo microscope.

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

G. Joseph Wilhelm

By the time you read this, a new year will be upon us and as happens every year in late December after the 25th the local Florida Keys community suffers from holiday season postpartum depression. They discard the comforting values of Christmas and revert back to the general consensus that life is way too important to be taken seriously and beer is a food group. This usually happens just in time for New Year's Eve. It was the interaction, on January 1st, with several individuals who were obviously over-served the previous night and could not focus or see straight that led to my convoluted inspiration for this commentary.

In previous articles I have touted the virtues of the venerable Spencer Model 20 Series Stereo-Scope. The availability, price, simplicity, versatility and quality of these instruments make them ideal subjects for the home enthusiast to tinker with. The disassembly and cleaning of the upper end optics (eyepieces and prism housing assemblies) was shown in "Episode Two" and a breakdown of the variant stands and available objectives/eyepieces was given in "Episode Five Pt 1".

As near as my Hercule Poirotesque investigative abilities have been able to deduce, this model series had a production run of approximately twenty years from the late 1930s to the late 1950s. While the stand had improvements and minor developments during this time, all of the objectives, eyepieces and major components appear interchangeable. This article is specific to the Spencer Greenough design and unlike most of the latter 20th century stereomicroscopes; the objectives are not an integral part of the overall stand.

For a full explanation of the Greenough style and its comparison to

Figure 1: There are few differences between the early (black) and latter (gray) objectives.

the more modern Common Main Objective, or CMO, design, please see Mr. David and Ian Walker's excellent article and the cited MicroscopyU article.

While the Spencer paired objectives appear quite robust, they are easily removed or replaced from the rotating nosepiece (or fixed single mount). This ultimately results in them being knocked about, dropped or otherwise neglected with a loss of collimation as the usual end consequence. It is to this particular state of malfunction that I offer some redress.

If I may quote Spencer Care publication of 1938 which states: "If the stereoscopic microscope fails to give a fused image or causes eye strain the objectives may have become de-centered and this may be tested readily by focusing on a ruler or some other object with a straight line. Move the line to the extreme left side of the right eye's field. Then notice whether the line is in the corresponding position in the field for the left eye. Test the lens also by moving the ruler to the top or bottom of the field. If the line is not in corresponding positions in the two fields the objective should be sent to the factory for proper adjustment. The lenses should be adjusted only by a person who has had factory training."

Now since the above qualified personnel are probably resting in peace beneath the sod and if not, the cost of the service should exceed the value of the optic we have the perfect rationale for a DIY solution.

Now as you can see in Figure 1 there are few differences between the early (black) and latter (gray) objectives. The independently focusable lens element assemblies are pared in a cast metal housing, which has three centering setscrews for collimation (Fig. 2). These setscrews are either .050 Allen head or slotted head. In either case, before attempting to loosen these for adjustment I highly recommend placing just enough PB Blaster to wet the screw head and threads projecting from inside the housing. This is way less than a drop and can be accomplished with a small syringe fitted with as fine a needle as you can obtain. Then wait at least 24 hours. Remember, these screws have probably not been moved for 50-60 years and may require a repeat of this application before these recalcitrant fellows acquiesce to external coaxing. The slotted head screws were in the earlier manufactured objectives and seemed to be seized tighter than the Allen head screws. The slot is less of a mechanical advantage, requires a good quality jeweler's screwdriver and in extreme cases will require drilling out and re-tapping, usually to the next size up.

Without benefit of the standards for alignment used by the Spencer factory it is entirely up to the user as to which lens should be adjusted to the other. At such low magnifications having the paired objectives par center with other paired objectives is not critical. The deciding factor for me is which set of the three adjusting screws I get loose first. Collimation is then accomplished by having a stable reference point secured to the stage just inside the extreme left and top field of view in the stationary lens. Loosen and adjust the other as you would for centering a stage/condenser until the images are identical. As can be seen in (Figs. 3 and 4, next page) the side adjusting screws are visible and the front and back screws are cleverly hidden behind the name and magnification plates. These plates are either screwed in place or held on with contact cement.

Of the ten paired objectives I have, only two required collimation and none required any focus adjustment. All of them seem happy on any of the three 20 series stands, which also speak well of the ruggedness and consistent alignment of the upper end optics.

And so ends this simple but uncomplicated procedure. Regards, Joseph Wilhelm. ■

Figure 2 (top): The lens element assemblies are independently focusable.

Figures 3 and 4 (left): The side adjusting screws are visible and the front and back screws are cleverly hidden behind the name and magnification plates.

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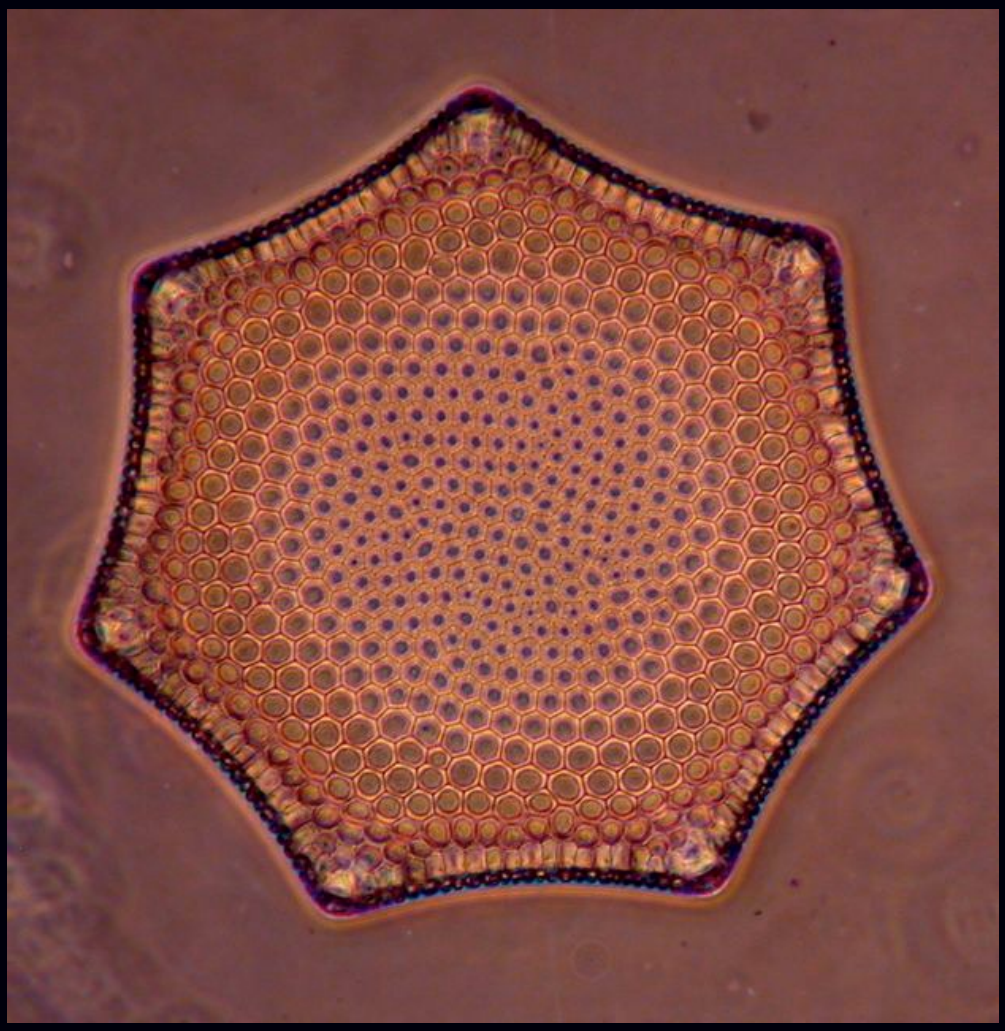


Triceratium grande var. *septangulata* (Kitton) Schmidt 1885. Locality unknown. Mounted by W. Watson & Sons, 1908 or earlier. Diameter approx. 250 microns.

By David B. Richman
Mesilla Park, NM

Insect by Alan Partridge

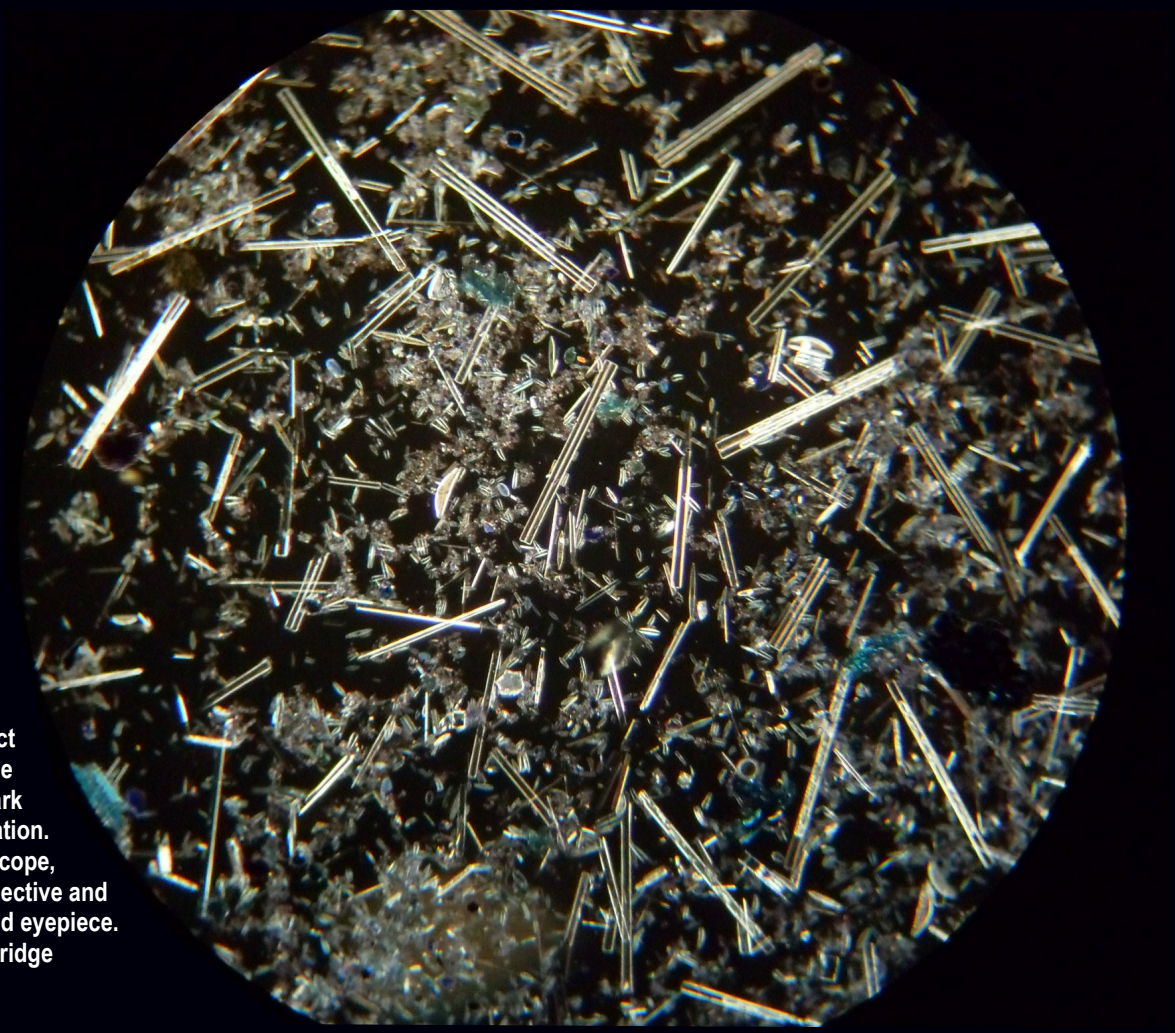
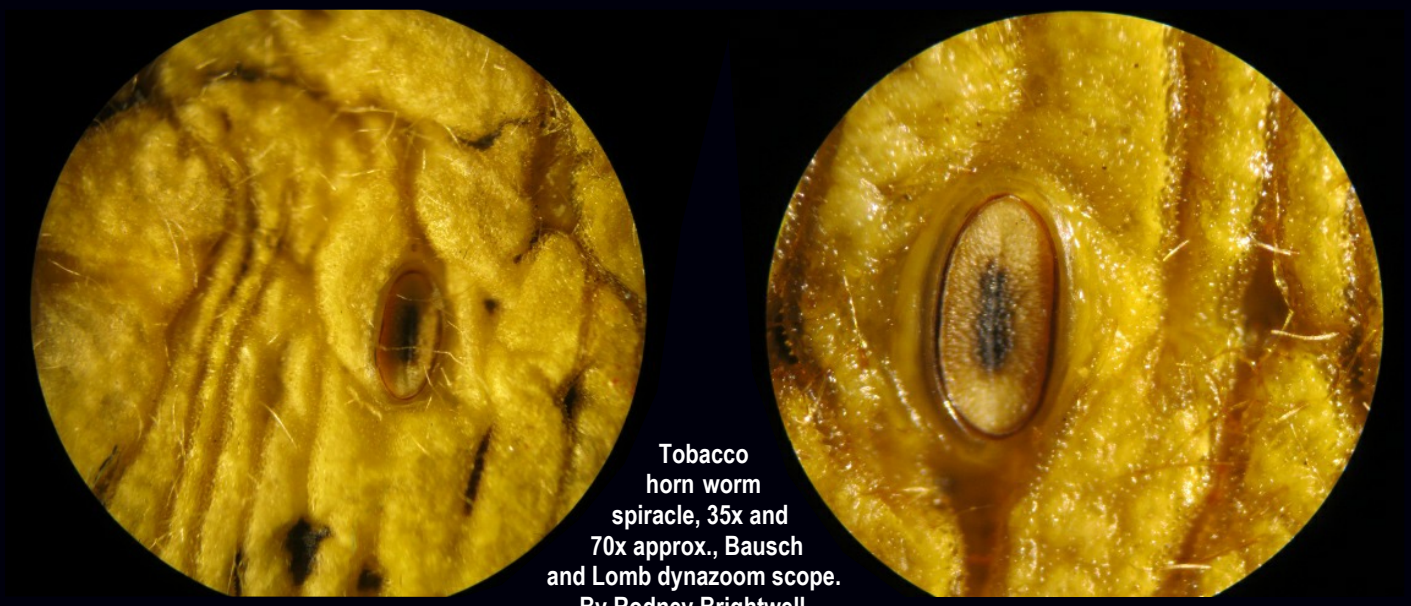


Image of *Synedra* (datom) taken with a Samsung L100 compact held up to the eyepiece. Dark field illumination. Prior microscope, with X10 Objective and X10 wide field eyepiece. By Alan Partridge



Tobacco horn worm spiracle, 35x and 70x approx., Bausch and Lomb dynazoom scope. By Rodney Brightwell

A huge Protozoan: Monocystis – an Earthworm Parasite

Stole it from a bird! Examining Monocystis parasites from a worm.

Anthony Thomas

As I watched one of our local robins pull an earthworm from the lawn and then begin to 'bash' it about I thought "Aha, I could use that worm". I chased off the robin and 'stole' the worm. I guess it was really my worm as it came from my lawn, and anyway the local robins make a good living from my lawn.

Earthworms are parasitized by a really large Protozoan in the genus *Monocystis*. These parasites occur in the seminal vesicles of the worm and make good subjects for the microscope. Unfortunately I did not take any photos of the earthworm dissection but there is an excellent account in Microscopy UK:

http://www.microscopy-uk.org.uk/mag/artmar99/cystis.html

and there are many illustrations on the web (Google: earthworm anatomy).

Remove one of the Seminal Vesicles located between segments 11-13 at the anterior end of the worm and teases it apart in water (each seminal vesicle is a sac and is easy to remove with a pair of forceps). The result is a milky liquid. Make a thin smear of this liquid on slide, and while still wet add a coverslip.

Examine the slide with a 40x objective and with luck you will find a huge adult *Monocystis* (figure 1, center). The circular object on the top left are developing earthworm spermatozoa and the thin hair-like objects throughout are mature earthworm sperms (figure 1).

The adult *Monocystis* produces thick-walled cysts filled with Sporozoites (figure 2), you can also see some hair-like earthworm sperms in this image.

The pressure of the coverslip often ruptures these cysts and then you can

Figure 1: Adult Monocystis from an earthworm seminal vesicle

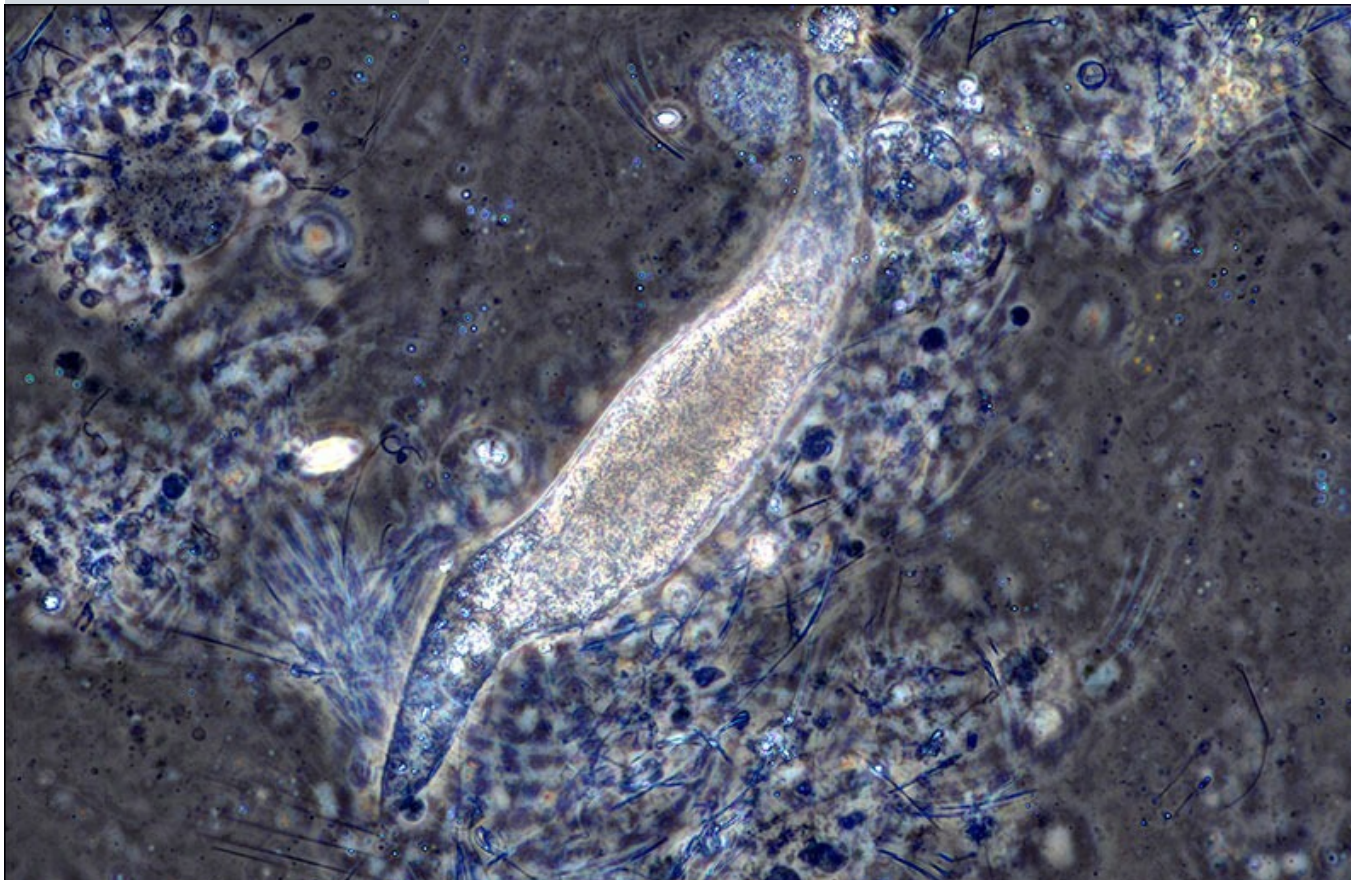
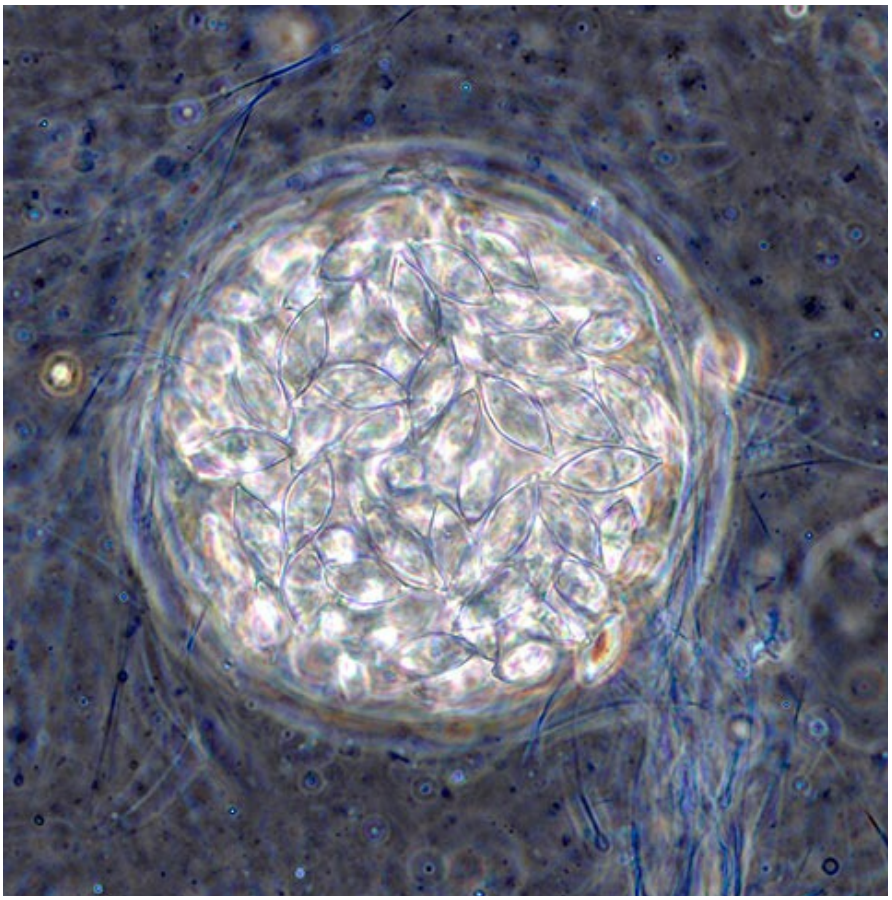


Figure 2: Thick-walled cysts containing many Sporozoites



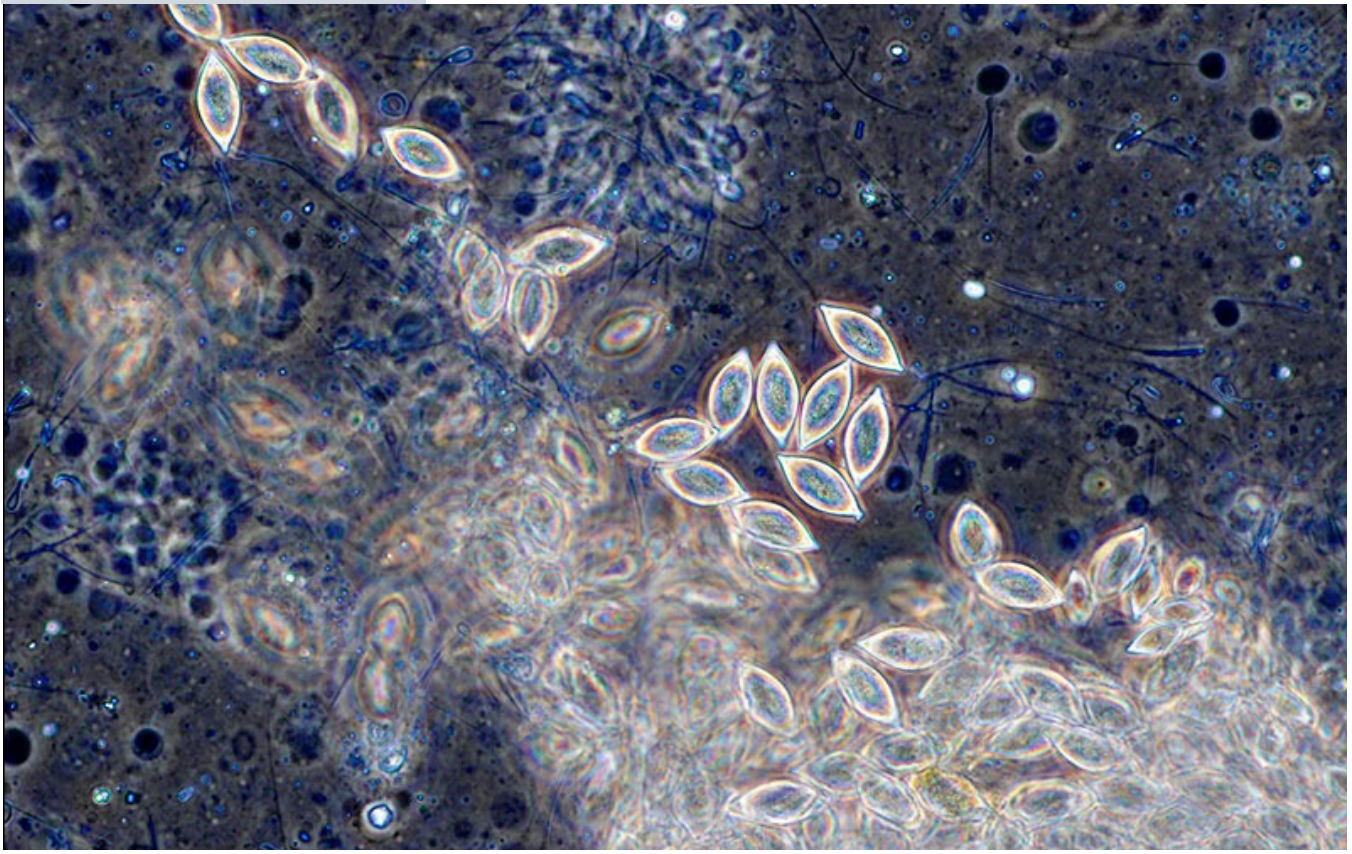
clearly see the individual sporozoites (figure 3); also several stages in earthworm sperm maturation in this image.

Normally, a bird eats the worm and the thick-walled cysts pass through the gut and are deposited with the bird's droppings. These cysts rupture in the soil and an earthworm ingests one or more of the sporozoites which develop into the adult *Monocystis* parasite.

Equipment used

Olympus BH2 microscope, 40x phase contrast objective, 2,5x NFK relay lens, Nikon SLR. ■

Figure 3: Ruptured cyst showing individual sporozoites each of which develop into an adult protozoan



Utilizing Offset Rheinberg Illumination

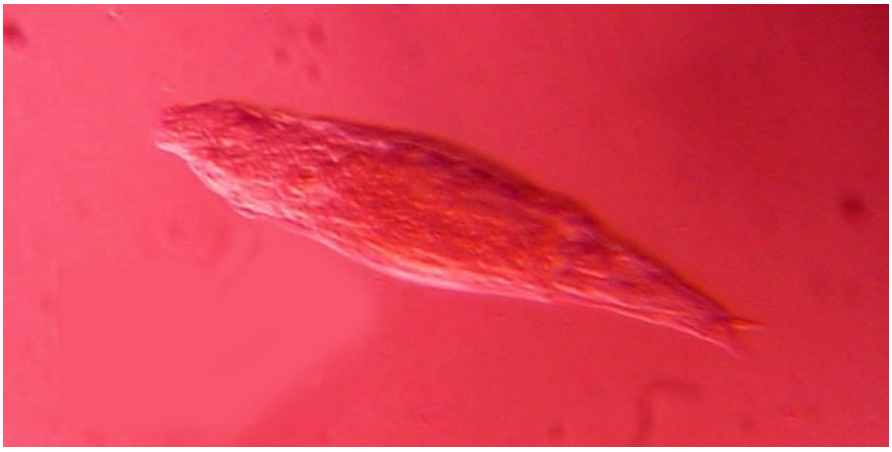
The poor man's "Differential Interference Contrast" (DIC), Part 1: Using Rheinberg Filters achieve a pseudo-DIC effect

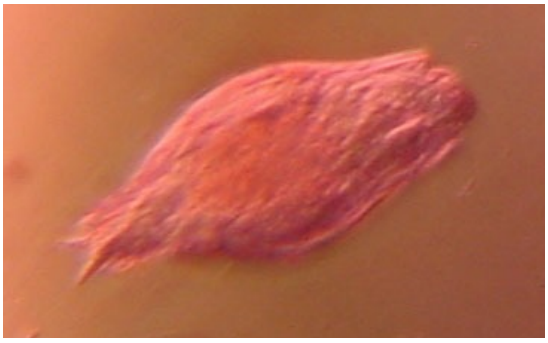
Ken Wrench, BSc, P.T.

For the past several years I have been studying pond water samples using a variety of microscopes. My first professional grade microscope was an American Optical Series 10 supplied with a trinocular head and Abbe brightfield condenser. I used it for over a year, taking bright field pictures with a point-and-shoot camera but was never satisfied with the level of contrast I was able to achieve. My second microscope was a Meiji ML 2000 binocular. I added a darkfield filter in the filter holder and was amazed at the improvement in visual appeal that darkfield achieved. After several more microscopes, I purchased the instrument that I use now: a Zeiss Standard 16 Phase trinocular scope outfitted with 10xPlan,16x Phase, 25x Plan Phase, 40x Plan Phase, 60x achro (Lomo) and 100x Plan Phase (oil) objectives. It has a 1.3 NA phase condenser and I use a Fostec 150 watt fiber optic light for illumination. I take photographs using a Sony Mavica FD 100 coupled with a Brunnel Unilink adapter mounted in the trinocular port.

During this time I became interested in studying the outer membranes of the creatures I was photographing. I looked into purchasing a differential interference contrast (DIC) microscope and discovered that a new one was well out of my budget and used ones were scarce, expensive and likely not to be complete. However, I began to read articles on various techniques being used to simulate the DIC effect including COL, oblique and relief phase contrast.

I had just acquired a nice set of Rheinberg filters for my Zeiss and wondered if I could combine offset oblique





Figures 1 and 2: Two rotifers imaged by using off-centered Rheinberg illumination.

with Rheinberg to accomplish my goal of photographing, in detail, the outer membrane of rotifers. This goal is surprisingly difficult since the outer membrane has a refractive index (RI) very close to that of the water in which it swims. There are phase differences and these show up well with phase contrast but phase doesn't give a detailed topographical view.

My technique is to use two Rheinberg filters: an outer red and inner yellow which are placed in the filter holder, pulled just slightly out of alignment and tilted about 30 degrees. The rheostat on the Fostec is brought up to 60% and the condenser iris is opened wide. After some practice, I decided to use the colors red/yellow in order to achieve a "skin" like appearance (see Figures 1 and 2).

Note: my goal was not to acquire a technically "correct" Rheinberg picture; with a red rotifer against a yellow background, but to photograph the topography of the creatures outer membrane.

The photos were taken using a Zeiss 40x/0.65NA Plan Phase objective, the condenser was set for brightfield and the camera was set for macro with no flash. I would encourage anyone wishing to achieve pseudo-DIC effects to try this very interesting combination of offset Rheinberg illumination.

The author can be reached at: advanced.pt@hotmail.com ■

Making Patch Stop Filters
for Oblique illumination

The poor man's "Differential Interference Contrast" (DIC), Part 2: Patch stop filters made of cardboard to obtain a pseudo-DIC effect

Oliver Kim

Oblique illumination is a contrast enhancing technique which can be realized with the use of home-made filters (patch stops) placed into the filter holder of the microscope condenser. Specimens observed this way give a 3D appearance, similar to differential interference contrast (DIC).

Oblique illumination only allows light to hit the specimen from one side. Darkfield illumination, in contrast, allows light to hit the specimen from all sides, except the center. The main light beam is not able to reach the objective. This can be achieved by placing a patch stop into the filter holder of the condenser and then horizontally moving the condenser with the adjustment screws.

These filters can be made of dark cardboard or other suitable heat-resistant material. The patch stop contains an off-center hole, which allows the light to pass.

The best size and shape of the patch stop filter hole is best determined by experimentation. In any case, the hole should not be too big, otherwise the main light beam from the lamp is capable of directly entering the objective, which weakens the effect. Rotate the filter to see the specimen to be illuminated from different angles.

Instead of using cardboard, it is also possible to design a patch stop on a computer and then print it on transparent foil. The printing will most likely not be dark enough and it can be necessary to use a permanent marker to make the filter still darker. Do not use aluminum foil or other reflective material. This will reflect the light and heat energy back to the lamp, causing it to become hotter and shortening its life span. ■

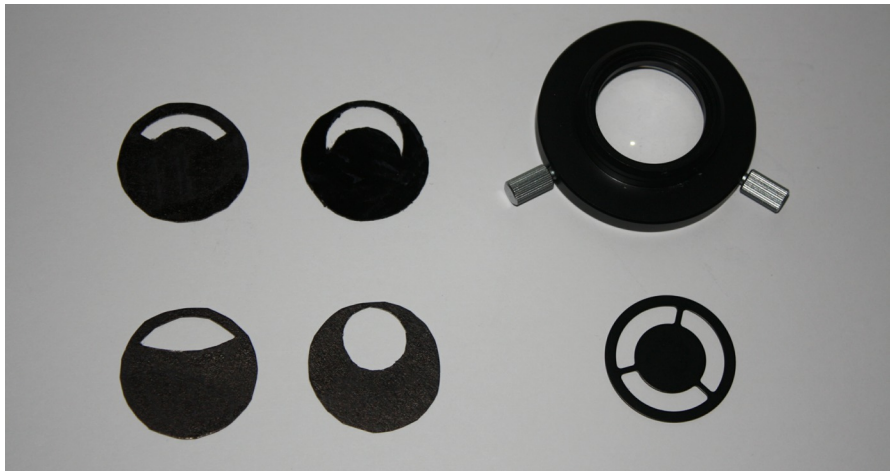
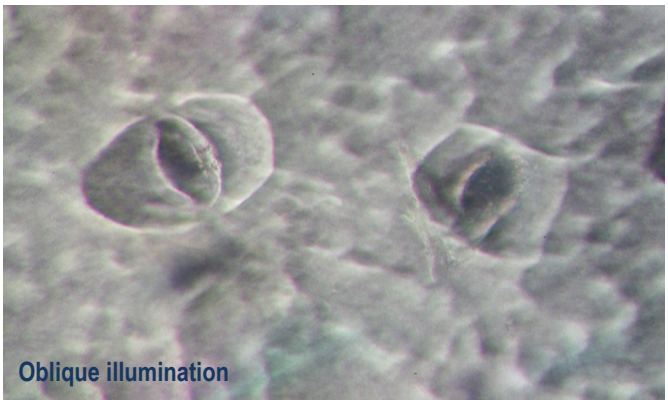
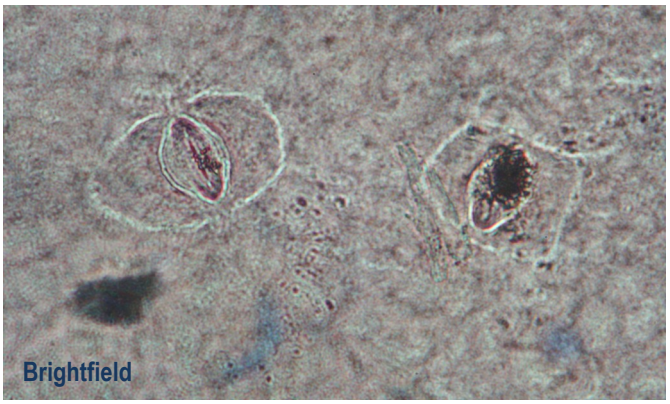


Figure 1 (right): Four different oblique illumination patch stops (left) made of cardboard, compared to a commercial dark-field filter (bottom right) of metal. The filter holder, with centering screws is on the top right.

Figure 2 and 3 (bottom): Comparison of oblique illumination with bright-field. The images show glue impressions of leaf stomates. White wood glue was applied to the bottom of a leaf, and observed microscopically after drying.



Oblique illumination



Brightfield

Of Furs and Fibers

A short photo journey through the world of fibers that can be found in a household.

By Oliver Kim

An average household is full of fibers, and my curiosity drove me to have a closer look at some of these items in more detail.

The fibers were collected from many different sources: Stuffed toy animals, carpets, pillow fillings and clothing.

Mounting and observation

I took the dry fibers and directly mounted them in Euparal mounting medium. Some fibers were pretty inflexible and repeatedly lifted the coverglass away from the slide. If this happened, then I had to spread the fibers further apart.

In most cases it was still necessary to do an image stack to increase the depth of field. The program Picolay is not only able to combine several images of different focus together, but is also able to compute two pictures for stereoscopic viewing (figure 2).

Dark field observation resulted in the most aesthetically pleasing results, making the fibers glow on dark background, almost

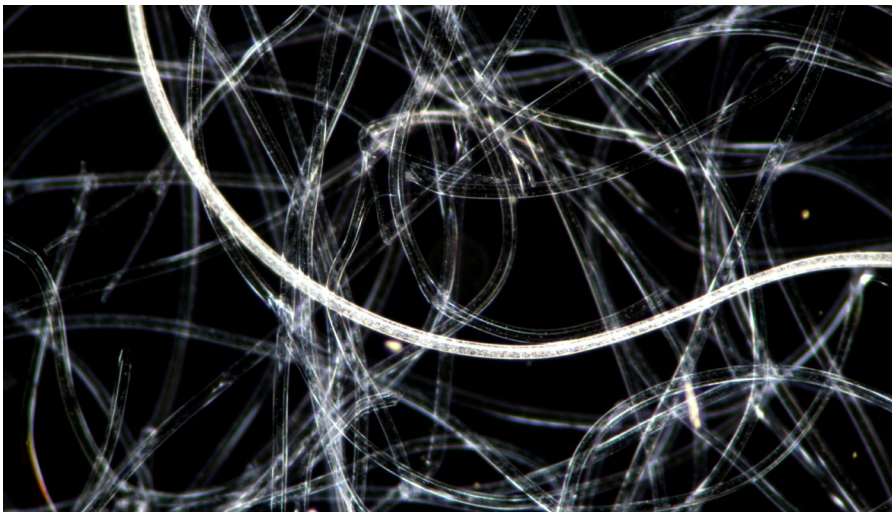
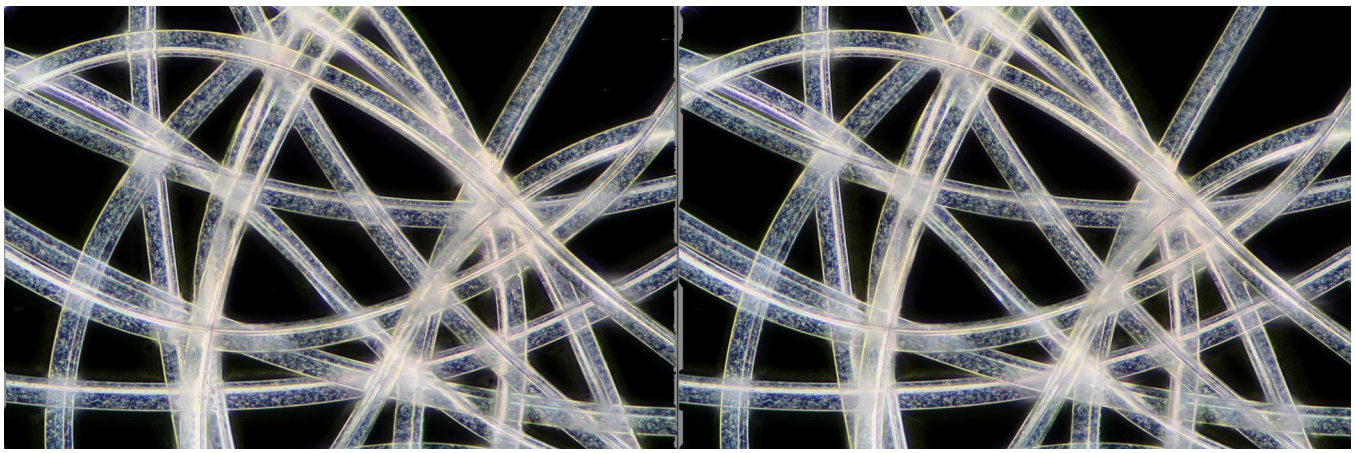
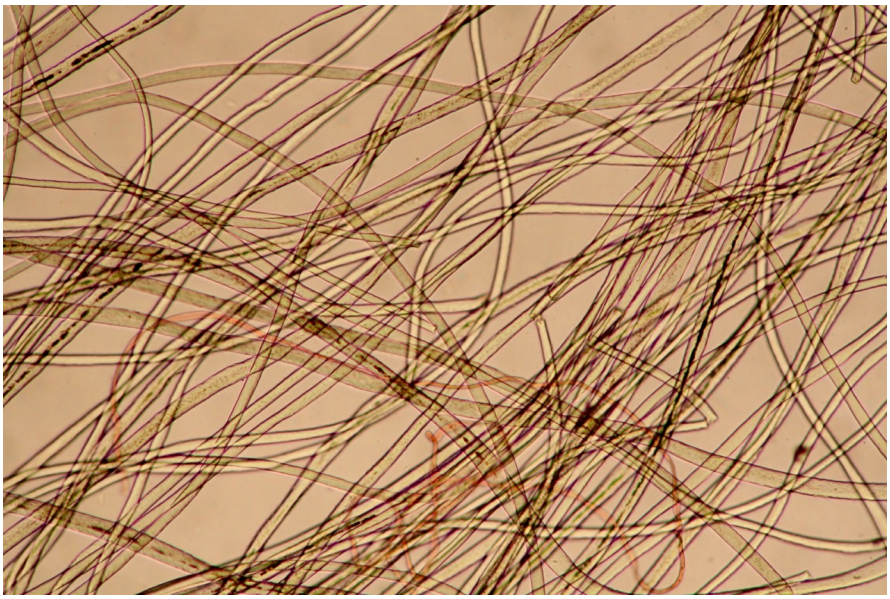
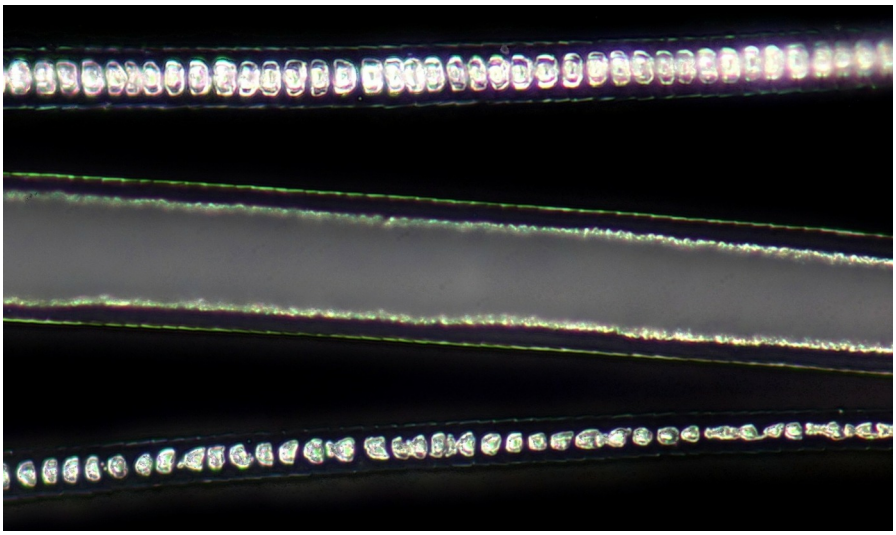


Figure 1 (right): Fillings of a pillow. It seems to be a mixture of different kinds of fibers.

Figure 2 (bottom): Fillings of another pillow. Use cross-eyed viewing to obtain a stereoscopic image!







like long, flexible fluorescent lamps.

Animal fur

Animal fur is another interesting specimen to look at. Unlike synthetic or many natural fibers, the fur of a cat (figure 5), for example, is not of homogenous thickness. The inside of some of the hair seem to contain separate compartments, which light up in dark field. Other hair, such as sheep fur, figure 3) does not seem to have these structures.

Yet to do

After the first preliminary observations, I think it is now necessary to approach the issue somewhat more systematically. Is it possible to distinguish natural from synthetic fibers? Do cotton fibers have a characteristic appearance compared to linen and sheep wool? Are there different types of cat fur (evidently there are, considering figure 5). On which parts of a cat can you find the different types? Questions, questions questions! Maybe I'll be able to address some of these points in a future article. ■

Figure 3 (top): Fur of a sheep (bright field). The fibers have an irregular thickness and some are even broken.

Figure 4 (middle): Possibly synthetic fibers from a stuffed toy animal in dark field.

Figure 5 (bottom): Fur of a cat, stack of several images. The individual hair are tapered and the central hair seems to be different from the other two.

Giant chromosomes in Drosophila spp

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In this article, the author explains how to obtain and prepare giant chromosomes from a fruit fly

César Guazzaroni

Giant polytene chromosomes form when DNA is copied repeatedly and the formed DNA stays together and is not separated during cell division. Polytene chromosomes can also form chromosome puffs. These are regions of lower density where RNA transcription takes place. Giant chromosomes have an enormous size: they are about 1000 times larger than the somatic chromosomes, the length ranges from 1200 to 2000 µm and the diameter between 4 and 5 µm.

To find these chromosomes we have to get a gnat larvae, which are widespread throughout the world. The larva of the fruit fly *Drosophila*, for example, forms giant polytene chromosomes in their salivary glands.

Figure 1c shows *Drosophila simulans*, which is probably more frequent than *Drosophila melanogaster*, however for the purpose of seeing the giant chromosomes there is little variation.

Drosophila is very common and can be found on a basket of fruit. They are lurking and hovering over the fruit and very tiny compared to other flies. They usually have red eyes and the female is larger than the male.

Thomas Morgan used these flies already in 1909 for genetic studies. They are easy to reproduce and are widely distributed throughout the world. *Dros-*

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Figures 1a, 1b, 1c (top): The top picture shows a single larva. They start to form pupae, which do not move (center). The cocoons are not suitable for investigating the giant chromosomes.

Public Domain by LPLT

Figure 2: Typical banding pattern of a giant chromosome.

Figure 3 (top left): You can see that some flies detected the aroma and are now on the walls of the container.

Figure 4 (top left): After two days the container had a lot of flies. It was necessary to cover the container to prevent other flies from colonizing there.

Figure 5 (left): Incredible movement of larvae in the medium after 6 days.

ophila is now a model organism which is used widely in research.

How the larvae were obtained

The larvae were first cultivated in a culture medium. The culture medium was composed of:

- 1 banana
- 1 tablespoon yeast
- 1.5 tablespoons of cornstarch or potato starch
- 1 tablespoon plain yogurt
- 1.5 tablespoons white vinegar
- 0.5 cups of hot water.

All of the ingredients were mixed well before adding a half cup of hot water. Then the mixture was boiled to prevent the yeast from leavening. The mixture was distributed into pots of 10cm in diameter, with a height of about 1.5 to 2cm.

If you are working on genetic crosses a preservative such as Nipagin (Methylparaben), should be added to the culture medium. That way you can keep in the refrigerator ready to be used any time. If you do not use a preservative, then simply boil the mixture and use it right away.

Figure 3 shows that some flies already detected the aroma and on the walls of the container. After two days the container was full of flies so I covered the container to prevent other flies from colonizing there (figure 4).

There are other culture media recipes, but this works well and the ingredients can all be found at home.

The life cycle of the fly at 25°C is about 10 days from egg to larva to pupa to adult. After about 6 or 7 days we can see the movement of larvae up the walls of the pot.

Dissecting the larvae

The larva was about 3 mm long after 6 days and it was now time to dissect them. They were placed in a petri dish or watch glass with a few drops of acetic acid of 45%. This prevents dehydration of the larvae. The petri dish is placed on a dark background to make the larvae more visible.

It is important not to confuse the pupae with the larvae. The pupae are brown and do not move, while the larvae are much brighter (Figures 1a, 1b).

Figure 6 (top left) A larva of 3rd grade, are as large and generally adhered to the wall of the culture bottle

Figure 7: (top right): The salivary glands can be found in the anterior (front) part of the larva.

Figure 8 (left): Tools needed for dissection: petri dish, magnifying glass and needles.

Figure 9 (left) The size of the glands compared with the tip of the needle of size 25 / 8.

Go to Youtube and search for "Drosophila Salivary Gland Dissection ". You will then find several videos that document this process.

To dissect the larvae you need both hands free. I used two teasing needles with plastic handles to make them more manageable. A magnifying glass is needed as well, I used a watchmaker's magnifying glass (Figure 8)

The needles should have good points, but not should not be too sharp. They should not puncture the larvae.

The larva is dissected by tearing it apart. One needle is placed more or less over the middle of the larva holding it down, but not puncturing it. The other needle is used to hold down the front part of the larva. The front part has a dark spot (Figure 7). The front part is also more pointed and the larva moves forwards with this part. Then move the hands counterclockwise ripping the larva apart. This process pulls out the salivary glands of the larva, which are then held by the needle that was at the front part of the larva. It is also possible to use tweezers for this process.

The two glands are grayish-white (figure 9). They are then isolated and separated from the rest of the tissue. The isolated glands are then placed on a slide with 45% acetic acid.

Staining Procedure

Literature recommends the use of lacto orcein as a stain. If you have this stain available, then put a drop of it on the glands and wait for 10 minutes. Afterwards place a cover slip on top and cover with tissue paper. Use your thumb to squeeze the specimen between cover glass and slide, without breaking the cover glass. Excess dye will be absorbed by the tissue paper. Also do not apply horizontal movement. This stain shows the banding of the chromosomes.

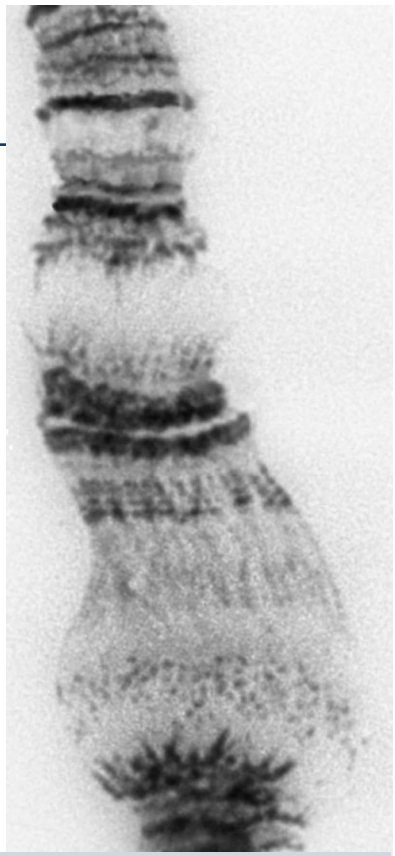

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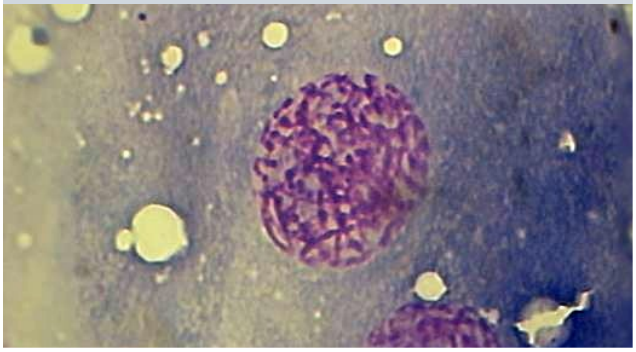
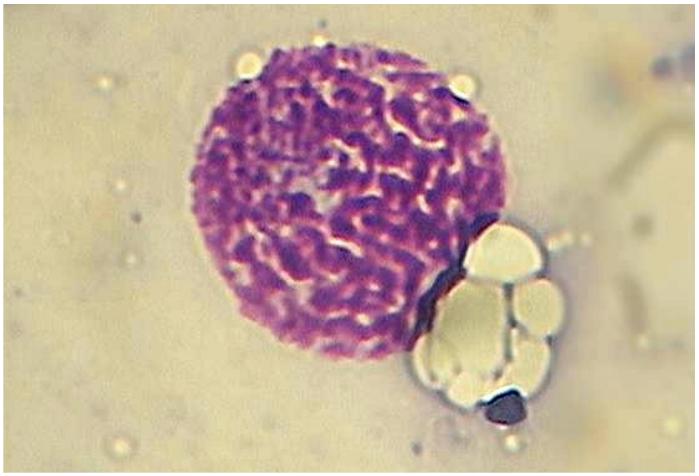
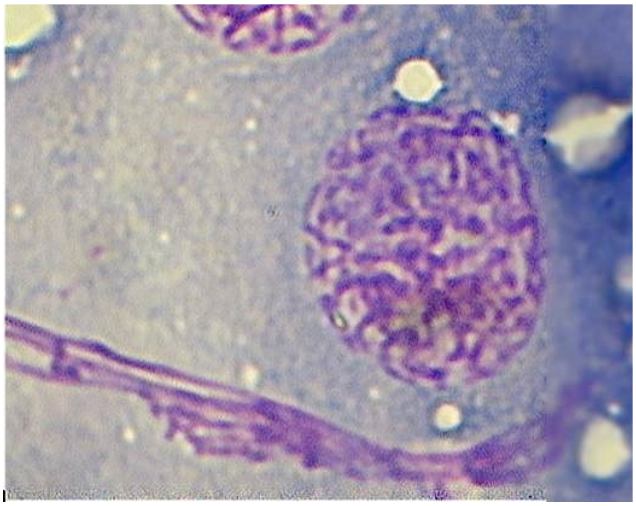
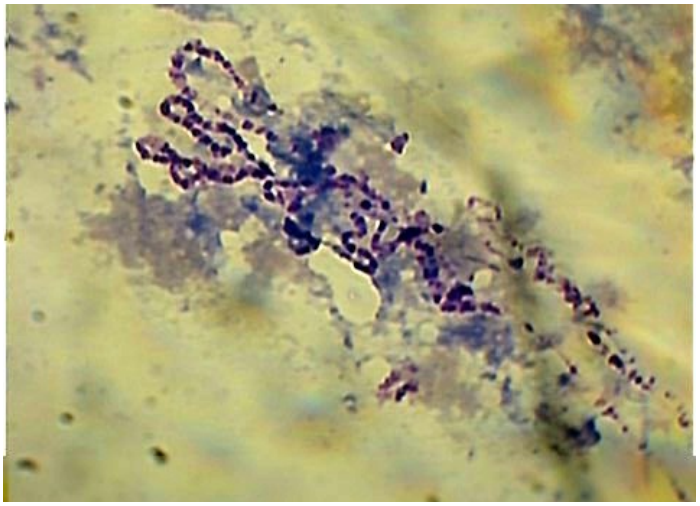
Figure 10 (top): Chromosome puffs are regions of RNA transcription..

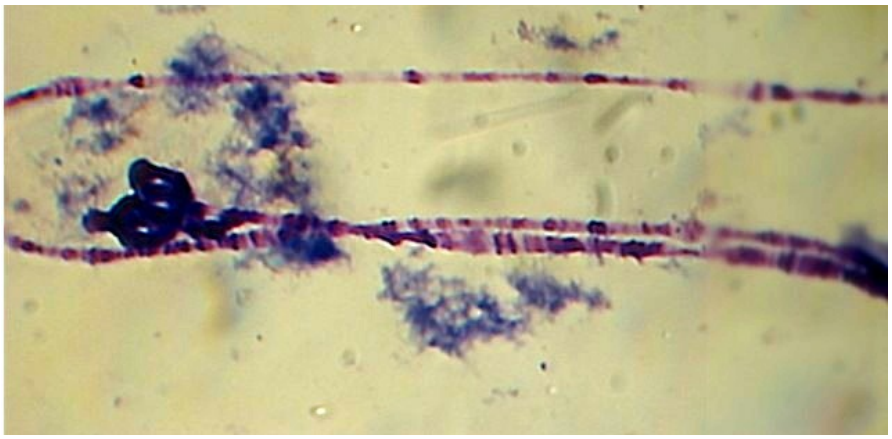
Figures 11-14: Large nuclei containing giant chromosomes at 40x. For comparison, a giant chromosome is at the bottom right.

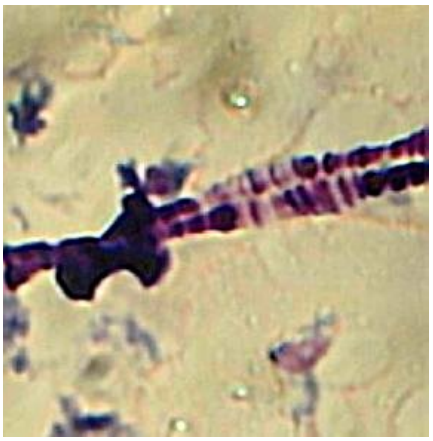


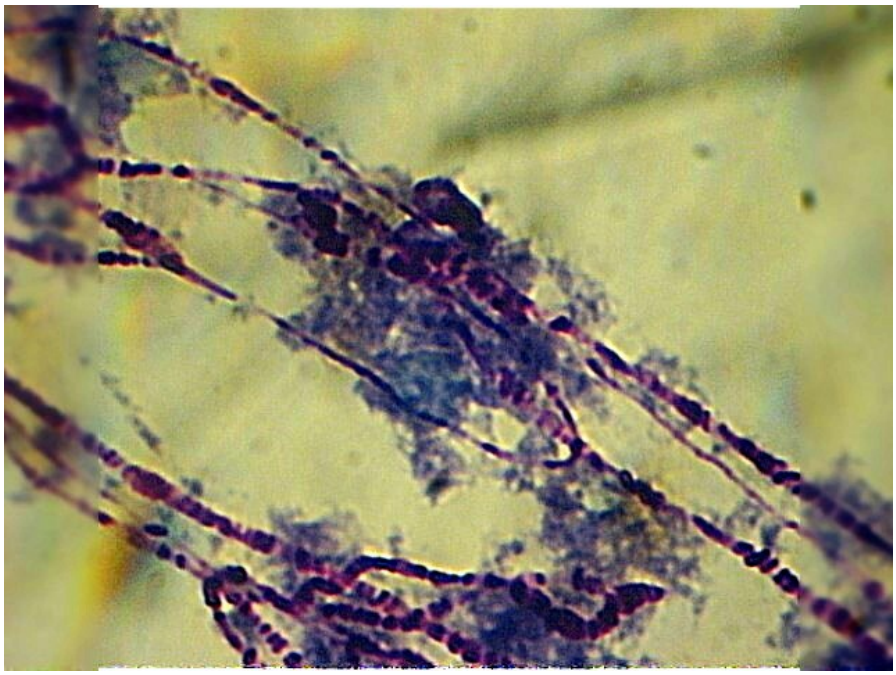


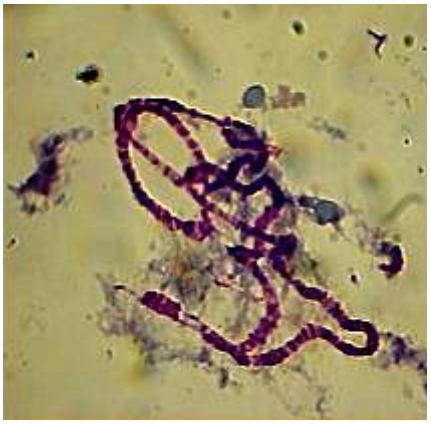


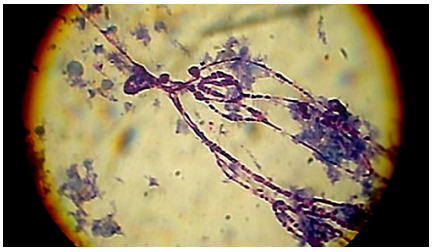












Figures 15-19: Giant chromosomes, which show the typical banding pattern.

I realized that I do not have orcein staining at my disposal. It is also possible to use Giemsa staining. This is a liquid composed of various dyes it is economic and well-known. Giemsa staining is used in laboratories for blood staining and is not expensive.

Giemsa technique

Once dissected, put the glands on a slide with a drop of 45% acetic acid until they are translucent. This takes about 15 minutes. Then put a paper towel over the slide and cover glass and below it. Apply strong pressure on the cover with your thumb without turning. This it is called a squash preparation and breaks the nuclei of cells leaving the chromosomes well spread. Then place the slide on dry ice (if available) to freeze the sample. Use a needle to break away the cover slip. If this process removes too much sample, then you need to do another squash. A thin film of the gland should stick to the slide.

Allow the specimen to dry in air. Stain the specimen with Giemsa solution (10 drops of Giemsa in 10ml of water). Allow the dye to work for 10 minutes, then wash the slide in clear water by allowing a fine water stream to flow down the slide.

Observation

A drop of immersion oil was applied and spread evenly. The first observation was made with a 10X panoramic objective. The nuclei were reddish in color. Observation then continued with the 40X and then the 100X oil immersion objective. The most beautiful, well-stretched chromosomes in all their magnitude could then be seen with the characteristic banding pattern (figures 14-19).

Acknowledgments

Thanks to Dr. Beatriz Goni Faculty of Sciences of the Universidad de la Republica (Uruguay), she has collaborated with the work with all selflessness and has made it possible. ■

Making a Microscopy Bench for Summer Stream Microscopy

Sometimes it is more convenient to take the microscope out into nature, than to take nature into the lab.

Charles E. Guevara, Fingerlakes, US

Here in central New York, USA, I enjoy microscopy observations in highly isolated, highly buffered wetlands. The stream is about one mile distance from fodder crop, cultivated lands, roads and homes. Agricultural fields and septic systems (for sewage treatment) leach and increase the loading of the stream. The distance of the stream from these areas somewhat buffers the loading.

This stream and its ancient floodplain are a component of the central New York / Seneca River watershed, which exists on a glacially sculpted drumlin-field. It is located just south of the Great Lake Ontario, and just north of the collection of central New York 'Fingerlakes. This biome is truly a child of recent glaciation in these parts!

The drumlin field in NY is the largest on planet earth, Canada has a slightly smaller drumlin field, Scotland has an even smaller one.

The Finger lakes, the Great Lakes and the drumlin field are all are a result of this part of our globes recent glaciation.

Freshly collected specimens from the wetlands, the stream habitat and the adjacent stream-lagoons, all offer wonderful seasonal microscopy samples of protists and meiofauna. Some hiking is necessary to reach the current meandering stream run. The simple hike to the collection sites leads over steep drumlin plunges of terrain descending downwards. The stream run and its large natural floodplain is the place for bottom-land trudging.

After sample collection

Bottomland trudging and steep upward climbing, introduce a consistent sloshing and a churning disruption of collected assemblages of organisms. Without being too picky in what I enjoy

Figure 1: Field station microscope bench

Figure 3 (left): Sharpened stakes will be pounded into the clay shore bank. This results in a most stable platform for a microscope.

Figure 4: (bottom): The The bucket is inverted over the stakes and serves as the "work bench" for microscopy.

observing, a mile of sloshing containers of specimens can vastly alter many assemblages of plants, protists and meiofauna. Sitting at home at my microscope bench, I then make observations with the microscope. I have to admit that I am indeed being too picky. I have learned to trudge through six-foot high canopies of bottom land natural plant groves, without obvious sloshing of the specimens. But a sensible microscope bench in the location of the stream and its adjacent floodplain wetlands offers so much better direct observation of the assemblages as they exist at time of collection. This would make it almost an 'in situ real time observation', of these wonderful parts of our world.

An afternoon for enjoyable streamshore microscopy requires: a microscope with LED illuminator, a handheld digital camera, a notebook, a sturdy work surface, and, sadly, no puppy dog at these microscopy field observation hikes. Please do not tell my Berneese Mountain Dog puppy that her loyal scrutiny of all my stream activities will now hamper my actual field microscopy! I have never yet made these 'deep hikes' without my puppy's trusty vigilance. I do have a low grade sense of vulnerability in microscopy hikes alone. I could take my cell-phone, but this area is a cell-phone-dead-zone. I grew up with late 1950's-early 1960's network television show "Lassie". Lassie, the wonder-dog would often scamper off for help when her human companion got into trouble outdoors in the natural spaces. Perhaps it is this childhood tellie show that nudges my unease of deep-hiking?!! Enough of undercurrent guilt at leaving my trusty puppy out of field microscopy.

Field microscopy for this year is now microhabitat and locale directed.

1) Sulfide oxidizing bacteria and purple sulfer 'blooms' started to appear these months (July-August, 2011). I wish to gently sample their in-situ deli-

Figures 5-6 (top): The field microscope with low cost LED light source.

Figure 7 (right): Approx. 22cm diameter paper plate work surface. A new paper plate is used for each field trip; a very clean work surface (pack it in, pack it out - on any hike!).

Figure 8 (bottom left): The stakes remain in the field.

Figure 9 (bottom right): The arrival package. The portable equipment is carried in the inner container, while the outer container functions as the work bench.

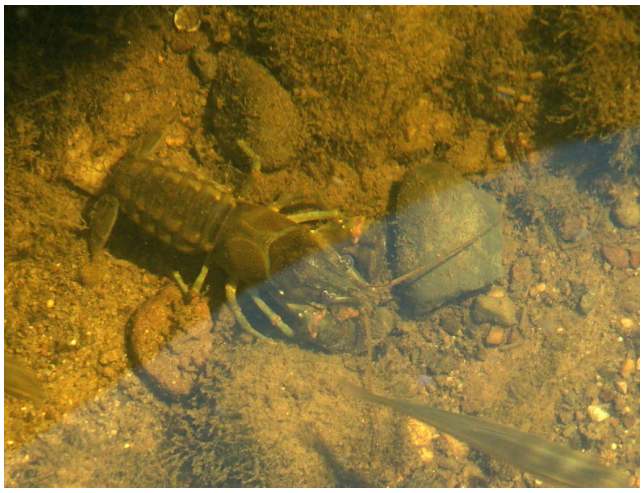


Figure 10 (top left): Puppy has bushwacked numerous field trips for microscopy samples - thank you puppy for your vigilance and your loyalty to the mission!

Figure 11 (top right): The dispersal stages of the mussels attach to various stream fish. I wonder what epizoic protists crayfish carry!

Figure 12 (middle): This freshwater mussel can easily be over forty-five (45!) years of age!

Figure 13 (bottom) The mantle is slighty peeking out after I replaced this mussel where I first found it.

cate film structure at stream shore waters, to perhaps observe orientation of these huge bacteria in their natural aggregate layer. Of course I expect to take pictures of my in-field observed samples.

2) Water-strider insects (order: *Hemiptera*; family: *Gerridae*; species: possibly *Gerris lacustris*) frequently carry epizoic protozoans. I want to observe these "hitckhiker protists" in the field to be able to release the water strider unharmed. This project very appealing to me, to be able to release the observed specimen at the site of capture - wonderful live microscopy!

3) Large freshwater clams (freshwater mussels, Phylum: *Mollusca*; Class: *Bivalvia* and Class: *Pelecypoda*; family: *Unionidae*), are native to this stream. These have fascinating larval dispersal stages, the "glauchidia", which are parasitically attached to the gills and to other appendages of these mussels and fish cohabitants of the stream bed.

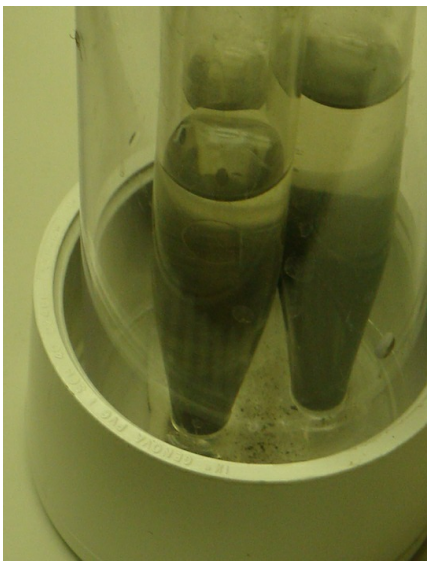
The bottom dwelling stream fish (fantail darters, maybe *Etheostoma flabellare*?), the plentiful stream minnows (family *Cyprinidae*), and the other fish species of this stream run, all offer many possibilities for field microscopy observations, with the subsequent release of the still healthy fish. ■

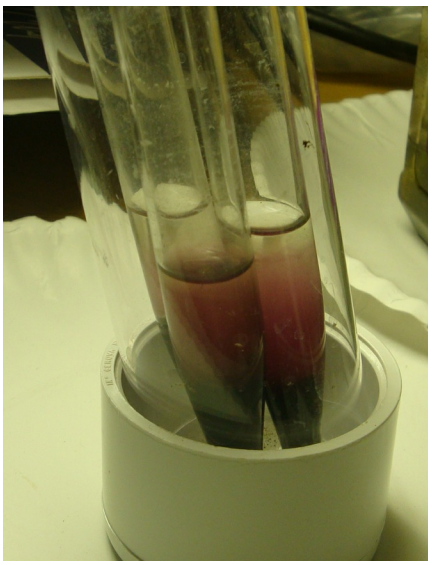
Figure 14 (top): directly across from the field bench, the first of this years 'purple sulfur blooms' noted (7/11).

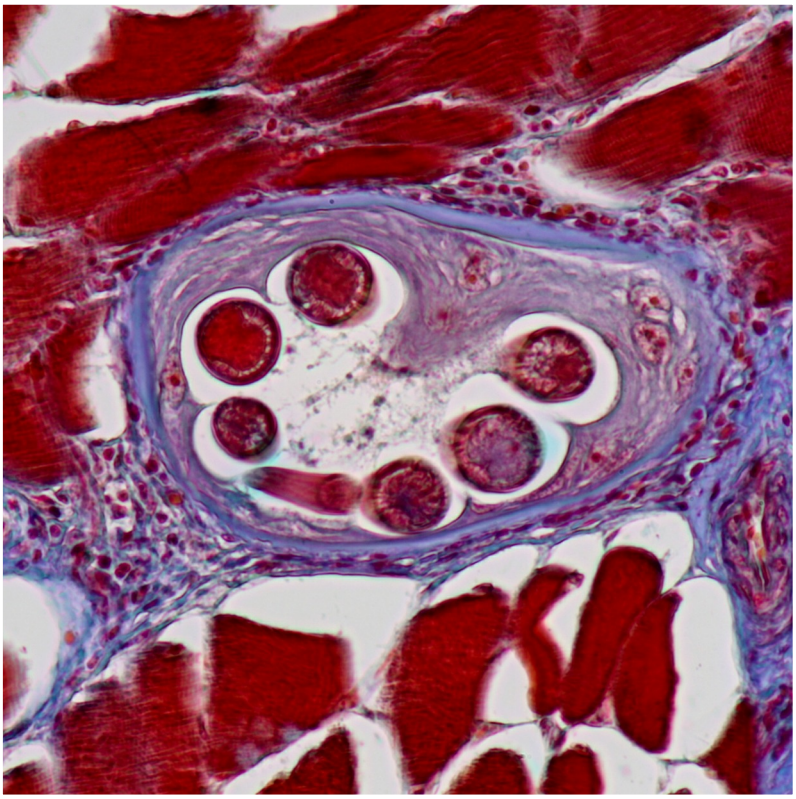
Figure 15 (middle): first 'bloom' noted this year.

Figure 16 (right): highly mobile purple sulfur bacteria retreated into the black sulphide sediments. These specimens just arrived home from the collection hike.

Figure 17 (far right): after 24 hours left at the bench at home huge purple bacteria have come out of their sediment (notice the purple color).







What's this? Answer on page 3.