Microbe Hunter

Microscopy Magazine

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July 2011

The Magazine for the Enthusiast Microscopist

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What's in Your Bath?

Depth-adjustable Wet Mount

Education and Microscopy Related Safety

Observing Bacteria

Measuring Distances

DIY Fluorescence

Mounting Thick Samples

Slide Wrapping

ABOUT

Microbehunter Microscopy Magazine
The magazine for the enthusiast microscopist
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Editorial: Article and image submissions are welcome and should be sent to: editor@microbehunter.com.
For submission guidelines, consult the website at:
http://www.microbehunter.com/submission

Front Cover:
Large image: Oliver Kim (potato starch grains)
Left image: César Guazzaroni
Middle: Anthony W. Thomas
Right: Oliver Kim

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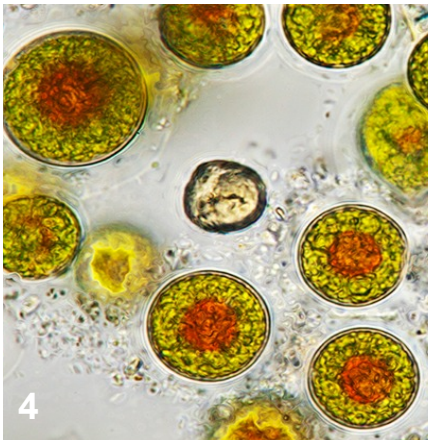
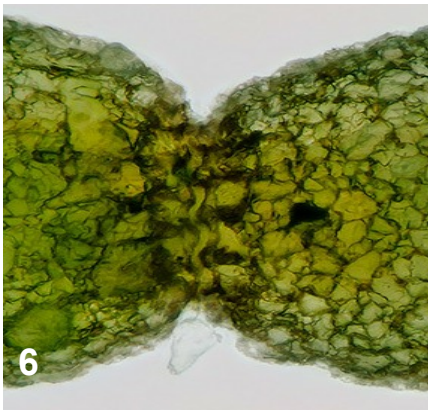
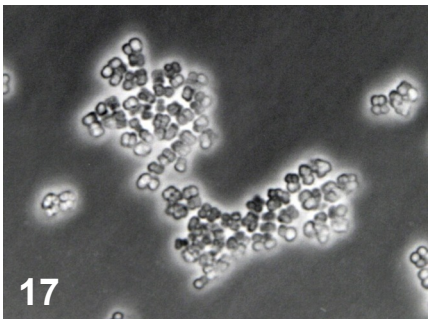
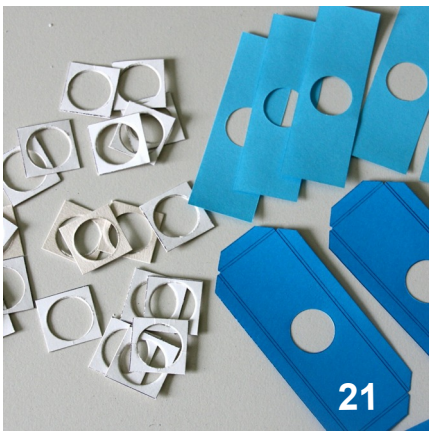








Answer to the puzzle (back cover):
Spores of a mold growing on fruit. Dark-field, 13 images stacked.

What's in your bath?

Shallow concrete bowls which are used by birds for drinking and bathing initially start out clear, then turn green, and then red. What's the reason for this color change?

Anthony W. Thomas

Shallow concrete bowls filled with water are often used as drinking and bathing places for garden birds. These bowls provide a rich source of microscopic subjects especially several forms of algae and the protozoa and rotifers that feed on the algae.

Perhaps the most universal inhabitant of such bowls is the alga *Haematococcus pluvialis*. These single cell spherical algae are about 25 microns in diameter and occur in 3 forms.

There is an actively mobile form where the green photosynthetic chloroplast is at a considerable distance from the cell wall. This chloroplast is connected to the cell wall by fine radiating cytoplasmic strands (figure 1). This form has 2 projecting flagellae with which it swims. The space between the cell wall and the green chloroplast is filled with a watery mucilage.

When conditions become less favorable the cell builds up large amounts of the red carotenoid pigment astaxanthin. Figure 1 shows this pigment in the early stages. The cell becomes sedentary and the alga forms a cyst (figure 2).

The final stage is when the entire cell becomes filled with astaxanthin (figure 3); masses of these cells can form a red lining on the surface of the concrete bowl especially noticeable as the water evaporates and the concrete dries out. It is thought that the red pigment may protect the cell from sunlight. When environmental conditions become more favorable (bowl fills with fresh rain water) the two flagellae reappear, the astaxanthin disappears and the cell becomes a mobile green alga. ■

Figure 1: *Haematococcus pluvialis*, active form with 2 flagellae; just beginning to build up the red carotenoid pigment.

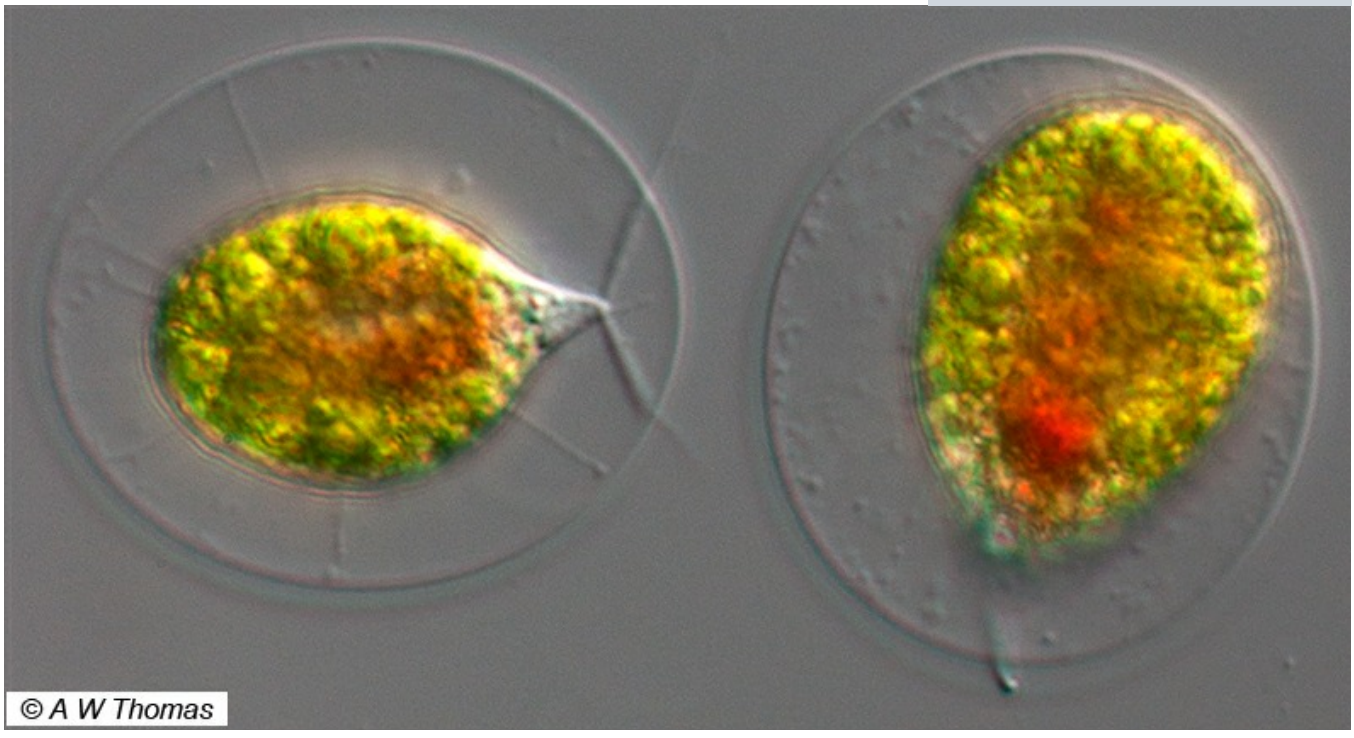
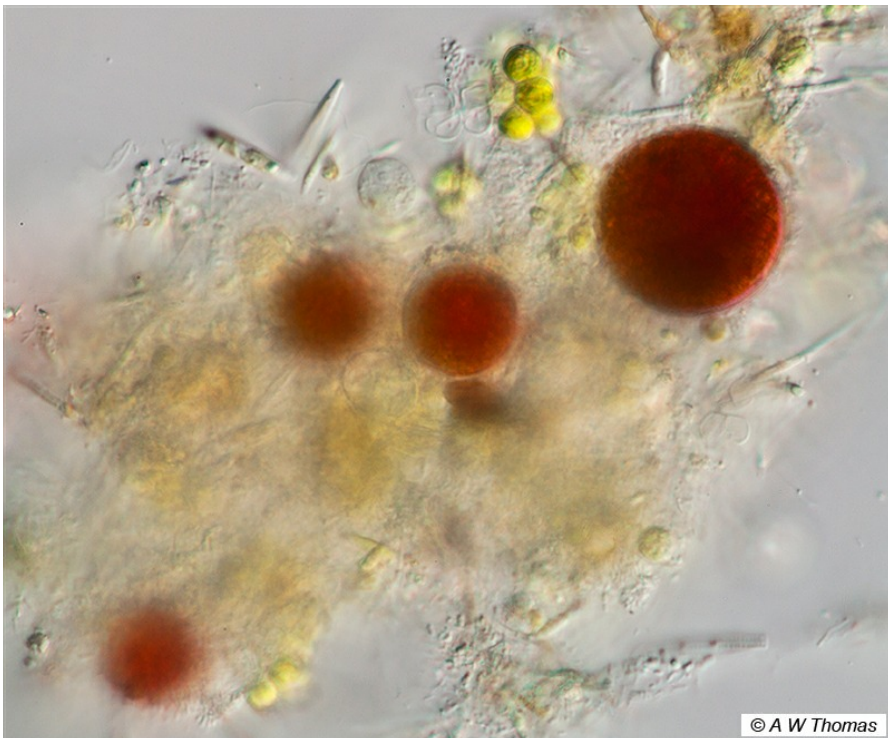


Figure 2 (top): Encysting stage, no flagellae, red pigment increasing.

Figure 3 (left): Final, fully encysted stage with entire cell filled with the red carotenoid pigment.



Equipment used:

Olympus BH2 microscope, 40x S Plan objective, 1.25x intermediate lens, 2.5x relay lens, Nikon DSLR camera.

A depth-adjustable wet mount

Please do not squash me! How to prevent the coverslip from crushing the specimen.

Anthony W. Thomas

When looking at microscopic pond life it is helpful not to have the coverslip tightly touching the slide. Delicate pond life will often get crushed if there is insufficient space between the slide and the coverslip. One way to make space between the two surfaces is to raise the coverslip on pieces of broken coverslip (often between 0.13-0.2 mm thick). A better way is to use very small spots of heavy grease or pure lanolin (my choice) to support the cover glass.

Make a template to show the position of the grease spots. Simply draw the outline edges of a slide on a card (most people use 3x1" slides but I prefer the wider 3x2" slides). Then outline the position of the coverslip; then draw small circles showing where to place the grease spots (figure 1).

Take a small amount of grease, a teaspoon amount is plenty and will last for many slides, and place it in a suitably small container that can be placed in hot water or heated on a slide warmer.

Gently heat the container until the grease just turns to a liquid. With a pin, pick up a small drop of this liquid and place it on a slide (resting on the template) over one of the small circles. Repeat for the other 3 circles. The liquid grease should immediately change into a 'solid' drop (figure 2).

Place a drop of pond water on the slide in the center of the 4 grease spots, add a coverslip.

This part really needs a sterescope dissecting microscope. Look at the water drop and search for some pond life (algae, copepods, amoeba, etc). Apply gentle pressure to each of the grease spots in sequence. That is, press the coverslip (use the tip of a pencil) over one grease spot so that the grease spot become slightly flattened; repeat for the other 3 grease spots. The idea is to move the coverslip close to the slide but without squashing the specimen. This technique can be used to immobilize, for example: a water flea (figure 3), a *Paramecium* (figure 4), and a testate amoeba (figure 5).

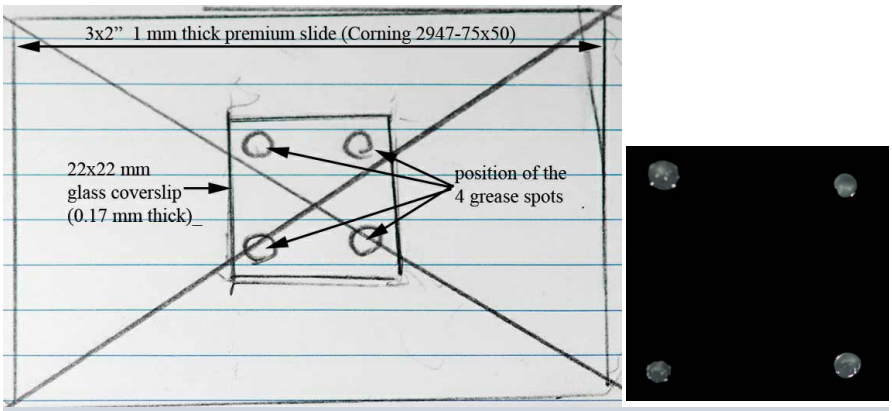


Figure 1 (left): Card template for positioning the grease spots.
Figure 2 (right): Grease spots in position on a slide

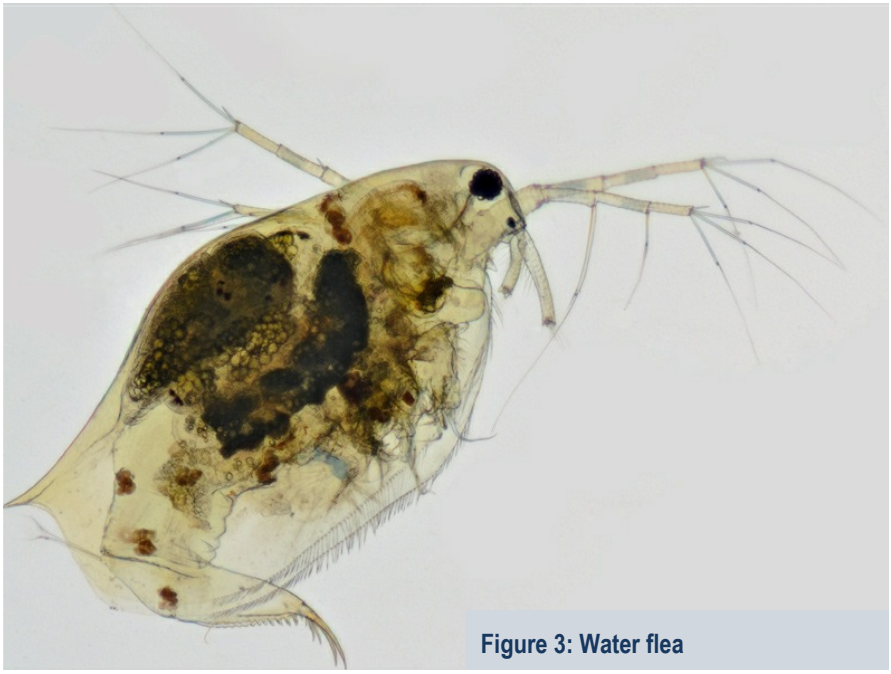


Figure 3: Water flea

Equipment used: Olmpus BH2 microscope, various objectives, Nikon DSLR camera. ■

Figure 4 (top): *Paramecium sp.*
Figure 5 (bottom) Testate amoebae (amoebae in a shell)

Mental forays into education, microscopy related safety and a challenge

In this episode, Joseph Wilhelm continues his musings and thoughts about microscopy. In this episode he focuses on education and safety.

This article was originally published in the September 2010 issue of Micscape Magazine and is now republished here with the permission of the author and of Micscape Magazine.

by G. Joseph Wilhelm, Florida Keys, USA

Education is the most important and integral element, aspect and concept in the physical, scientific, technological, moral, ethical, artistic and philosophical advancement of the human race and civilization. As such I have a strong conviction its support, preservation, application, promotion, improvement and placement first and above all other endeavors, should be the world prime directive of our species.

Did I leave anything out? (Please remember these are my "musings" as Mr. Walker has previously described my belletristic offerings and as difficult as it is for me to fathom why the entirety of the masses are not always in replete agreement with my presuppositions I must concede to the possible corporeal eventuality of this circumstance. Therefore, I must paraphrase my entire existence with "I may be wrong, but it seems to me…")

Knowledge, being the substance of education, could arguably be included in the first paragraph. But however useful it may be to the individual possessing it, it is useless to society in general without the means to effectively promulgate and pass it on. While the first paragraph is a laudable premise it is unfortunately not the applied focus of our current educational policies. Micscape Magazine fulfills the spirit of the first paragraph statement, even more so now that it is being archived. So it is here that I wish to address a perceived area of education that could be vastly improved with the reader inserting the above paraphrase wherever deemed appropriate.

I am proud to be an Instructor as mentioned in Episode one, and while we could argue the semantics and connotative level of importance to titles such as Teacher, Professor, Schoolmaster, Tutor, Educator etc., we all essentially serve the same purpose to individuals that have varying degrees of age and pre-education. The terms may be somewhat interchangeable but I prefer "Educator" as the appellation to encompass all of the above. The primary prerequisite to being an Educator is to possess knowledge and there are established requirements for all levels and positions of Educators, which is good, but it is here that our system stumbles. My education has been the focus of numerous Educators with proprietorship of vast and impressive amounts of erudition but, quite frankly, could not teach a hungry dog to eat. Comprehension came after a good deal, sometimes excessive, amount of mental gymnastics on my part that could have been eliminated by delivering the material in a different manner. Being given possession of a mathematical formula is knowledge, but quite separate from understanding its application. The cogency of transferring knowledge from one person to another is how quickly and how well the recipient grasps an understanding of its application. This should be the measure of a good Educator. (Insert paraphrase) In our educational system there is simply not enough emphasis and training applied to teaching the teacher how to teach.

The US Navy has taken a long hard look at this very problem because their experiences has revealed inadequate training not only results in poor performance; it has the very real possibility of being fatal to the individual. As a Navy designated civilian instructor teaching Navy safety programs, they have taken extensive measures to ensure my capabilities are to par with their standards. This was accomplished through the "Instructional Delivery Continuum" (IDC). This program is in two parts, the IDC Apprentice, an extensive instructional docket which teaches you how to become an instructor, how to teach a course as it were. The second Part is the IDC Journeyman, even more demanding than the first, which teaches the proper delivery of the Apprentice course, how to "teach the teacher" so to speak and thus the "Continuum" concept. Having satisfactorily completed both courses the Commanding officer of Atlantic Targets and Marine Operations Detachment Key West (ATMO Det KW) has designated me a Journeyman Instructor. No degree required.

The neat thing about The IDC program is that it has absolutely nothing to do with the course material being delivered. It is applicable for any subject from home economics to physics, from philosophy to weapons training. Below is a partial list of teaching aspects presented in the IDC.

The student-teacher interface

How to assess a class as a whole and read an individuals profile from their mannerisms, body language and speech patterns. This gives a starting point to decide what challenges they will pose, if they are in class voluntarily or to meet a job related requirement and which teaching techniques to apply. The proper techniques for dealing with disruptive students or other distractions, sleeping, tardiness etc. How to coax correct answers from less than enthusiastic pupils, how to foster participation and assessment of comprehension.

Class safety, physical and environmental considerations

Proper lighting, temperature, humidity and their physiological and psychological effects. Determining the timing and duration of class breaks. An individuals health considerations. Location of exits, fire extinguishers. Outdoor instruction. Who in the class knows CPR. The four teaching "time zones" and their effects. 1. Early morning just after (tired/hangover) arriving to work. 2. Late morning just before (hungry) lunch. 3. Early afternoon just after (I want to take a nap) lunch. 4. Late afternoon just before (I am eager to get out of here) happy hour.

Instructional delivery

The timing and pace of the presentation. Instructor physical presence, proper posture, body language, hand gestures, proper technique for walking among the students during presentations. Vocal inflections, phrase structure, maintaining class command, maintaining attentiveness and respect. Using comprehension tools such as choosing suitable associative analogies. The art of the question (Proper timing, structure and delivery of group or individual questions). Use and techniques for instructional aides, video, power point, physical demonstrations with mock ups or actual hardware. Weaving the student-teacher interface components and their use seamlessly into the presentation. How to develop your own instructional style. Presentation / improvement of pre-written courses.

Course material organization and testing

Course developmental theory. Writing a course from scratch, identifying course goals and information sources, order of presentation logic, sectional division of material. Identifying the merits and inadequacies of exams, tests, quizzes and student proficiency demonstrations both written and oral plus their proper composition. Proper technique to administer the above. Identifying and analyzing patterns in student response to improve comprehension.

The philosophy of teaching and self-assessment of ones own capabilities were the closing topics.

A considerable depth of study was attended to each of the above incomplete sentences. This is by no means all of the subjects covered, just what I can remember off the top of my cranium.

The final task for graduation was to write a course from scratch on a randomly selected subject (mine was forklift operator training) and deliver that course to select panel of Journeyman Instructors already qualified to teach it, and use as many of the learned techniques as possible. One week to research, prepare, and rehearse. This was after passing all the class tests and it was considered a Proficiency Demonstration (PD). The panel would review the performance and give a positive/negative recommendation to your Commanding Officer for designation as an Apprentice or Journeyman Instructor. No grade, just pass or fail. If the recommendation was negative a complete retake of the course was required before another PD.

So you see, the Navy considers there to be a little more to being an instructor than just knowing the material. (Insert paraphrase) while there is commercially available course instruction to the above, the ones I have seen are not nearly as comprehensive and are not as widely required in industry or the educational system as would seem beneficial.

A final note: As passionate as I am about education I was absolutely thrilled to hear the (Is it the British Library?) Micscape articles are being archived. It is an admirable accomplishment worthy of esteem. My congratulatory regards to Mol Smith, David Walker and all the Micscape family and contribtors who made it possible.

There, end of my lexiphanicism. Opinions and debate on the aforementioned are like… totally welcome.

Some Common Sense Safety

Being the safety instructor for all manner of topics i.e. hearing and sight conservation, heat stress related illness, explosives, electrical hazards etc., I believe the two most related to microscopy are Respiratory protection and Hazardous Materials Management and Use.

I have read articles on using alternatives to some of the substances that are regarded as toxic for making slides, fixing, staining, cleaning diatoms and so forth. However, it seems the "toxic" materials usually yield better results for whatever it is you are trying to do. So really, how far must we go to protect ourselves? Do we have to eradicate any possible contact with these poisons? Let me pose a few more questions to add some perspective.

Can you drive a car safely? Yes. Can you eliminate the associated hazard/risk of accident or injury? No. You cannot eliminate risk from an associated hazard. You can only mitigate it to a point where the risk is acceptable. The same goes for working with hazardous substances. We take risks with our lives everyday, driving is my favorite example, it can kill you. We mitigate the risk by driving responsibly, obeying the laws (following instructions) wearing seatbelts. We lower the risk to a point where the benefits of driving outweigh the risk and it now becomes an acceptable risk. We handle hazardous/toxic substances in our homes all the time with no concern. Everyone knows the devastating effects chlorine bleach can

have when gotten into the eyes but when was the last time you donned chemical splash proof goggles when pouring a cup of Clorox into the washer?

What is a toxic/poison substance? That's easy…everything. To quote Paracelsus (Theophastus von Hohenheim), the Renaissance father of toxicology:

"All things are poison and nothing is without poison, only the dose permits something not to be poisonous." Dose = concentration x exposure and, as is taught in hazardous materials handling courses; substances considered toxic are harmless in small doses, and conversely an ordinarily harmless substance can be deadly if over-consumed. Indeed, drink too much water too fast and you will die, and not from drowning. (Nasty stuff, water.) Now drink a small amount of phosphoric acid occasionally i.e. Coca Cola and you are OK. Add another toxin to the phosphoric acid, say, alcohol i.e. rum and take occasionally and you are still OK. Too much and you suffer internal organ failure and brain damage which leads to watching Oprah Winfrey and Benny Hill reruns.

It is for this reason that the Occupational Safety and Health Administration (OSHA) and its National Institute (NIOSH) have developed exposure limits for a major portion of the compounds, solutions, and elements that constitute the chemical makeup of products available today. They are usually stated in parts per million (ppm) or milligrams per cubic meter (mg/m3) for atmospheric content. The actual ppm in the atmosphere cannot be measured in real time. An air sample needs to be taken and sent to a lab for analysis. The exposure limits are usually a Time Weighted Average (TWA) for exposure over an 8-hour workday, 40 hours per week. For most of the over the counter substances the main concern is the aromatic hydrocarbons/volatile organic compounds (VOC) given off in the form of a vapor from glues, adhesives, thinners, solvents, paints etc. For normal home use, as intended by the manufacturer, the exposure to the toxic elements is usually quite low. Unless engaged in intentional abuse such as concentrated "sniffing" or "huffing" of these substances, there are normally no ill effects. HOWEVER… Do not attempt to outsmart your common sense!!!

If you find yourself working with a hazardous/toxic/poison substance at home (you don't have to look too far, ammonia, insecticides, drain cleaners, oven cleaners, are some of the more obvious), or, if in the pursuit of microscopy you use any of the witches brew of reagents etc., here are some common sense tips.

Figure 1: Respirator complete

First. Get a Material Safety Data Sheet (MSDS) for the product you are using. The manufacturer will provide you with one, the store like ACE hardware will print one out for you or you can usually find it here http://www.msdssearch.com/ at the National MSDS Repository. Then, google "How to read an MSDS sheet". Take some time to read and understand the MSDSs. They have a wealth of information, ingredients, exposure limits, first aid measures etc.

Second. Ventilation is your friend. The desired method is to get a non-oscillating fan facing away from you with the source of the vapor between you and the fan. This will gently draw fresh air from behind you and direct the vapors away from you. The fan can be directed towards an open window or into ducting. My set up is a 14" box fan with a cardboard funnel taped directly to the face and necked down to 3" clothes dryer vent hose and out a small opening in the window to keep the heating/cooling in the room. Quite effective actually. Sucks away vapors without rustling papers and having things blown about.

Third. Wear an air-purifying respirator. (see figures 1 & 1a) This is probably the most prudent and beneficial investment in your own health an individual can make. A half face negative pressure respirator like the one pictured with organic vapor cartridges and particle filters is about $30. They can be bought at hardware stores and home improvement centers. I use one on a regular basis when fogging or spraying insecticides, varnishing, painting, woodworking, sanding, vacuuming and even barbecuing (which does not give a warm and fuzzy to those I am cooking for, but I really don't care). Read and follow the directions.

Add the proper gloves, neoprene, rubber, latex and goggles if there is a splash hazard and you can be as safe as you want to be working with "toxic" materials. Just know your enemy and what defensive measures to employ and the risk involved can be at an acceptable level.

The Challenge

Living in the only true tropical paradise in the contiguous 48 has its rewards but there is a noticeable cultural and intellectual void.

The available labor pool here in the Keys, from which my company must employ, and to whom I am charged with educating for the benefit of their own

Figure 1a: Organic vapor cartridge unscrewed and particle filter removed.

health and well being, are shall we say, not the "best and brightest" our nation has to offer. They seemingly have migrated here because wherever it is they are from, they are "wanted" or "unwanted". Their concept of mass transit is a pontoon party boat. They think a "Closed, gone fishin" sign in their banks window and showing up late on their first day of hire and asking for a raise is perfectly acceptable. A small portion of this collective also runs the distinct risk of extinction if someone ever hides their food stamps under their work clothes. Fortunately we are spared from inadvertently hiring the professional criminal element because they already have gainful employment in public office.

When about to teach a safety class I first check the city and county law enforcement web sites so I have a reasonable expectation of who will attend. Once assembled and using my highly tuned IDC techniques I quickly ascertain the student body temperament of rather having root canal surgery than be subject to another instructional session. I have to resort to instructional techniques eerily similar to dangling a carrot in front of the donkey to achieve the desired result. It's like teaching fourth graders who lack the guile and deviousness I normally associate with nine year olds. Such is my life. (I am getting close to making my point, but not quite, please bear with me.)

We recently had a modest gathering of my wife's friends and property management clientele (About 60 or so including 8 children. If it were my friends only Chauncey and his Cajun friend Thaddius would show up). On a whim I set up the Spencer stereoscope in our entertainment area with a single 3x objective and 10x eyepieces and securely fastened it to a small table with illumination. I sprayed a black card with photomat adhesive upon which I unceremoniously stuck a small yellow biting fly on its butt end with the head facing the lens. A small sign next to a coffee can inviting the general public to view the miniature flesh-eating monster for a nickel completed the display. I had to chide the adults to place five cents in the can while the children dutifully honored the request. Well, all but one, a particularly abrasive urchin attired in a crimson tee shirt emblazoned with a black pitchfork and the apt words "Daddy's little demon". He kept trying to steal the fly. I was able to convince him there was several of these blood-sucking flies the size of my Doberman living in the thick jungle surrounding the estate and they were attracted to the color red. I showed him the two inch long Keys Cicada I had caught earlier (*Diceroprocta biconia*) and said, "See, here's one of their babies." He spent the rest of the evening getting therapeutic reassurance from his parents while they pointed to me as an example of a bad person.

The reaction from those who viewed the fly varied from fascination to a grimace accompanied by "Yuck!" with the latter being predominant. There was one young lady, a nine year old who kept returning and staring for quite some time though the scope. She started asking me questions. She had learned about cells in her school science class and wanted to know how big they were. I told her the ones I have been in are about 8' by 10' with one bunk. Without hesitation she replied, " No I mean the science ones." I said give me twenty minutes and I will have an answer. I could have given her an answer of bacteria single cells are bout 200 nanometers and blood cells are about 6 to 8 micrometers but this would have meant nothing to her. I did some quick research and calculations and presented the following in an attempt to give her some perspective and application.

My props/analogies were a 12-inch ruler with 1/16 in divisions and a straight pin like they hide in new shirts. (Someone please correct me if in the following I have omitted some optical ray trace exponential algorithm et al or am generally showing my a** if my math and generalizations are wrong.)

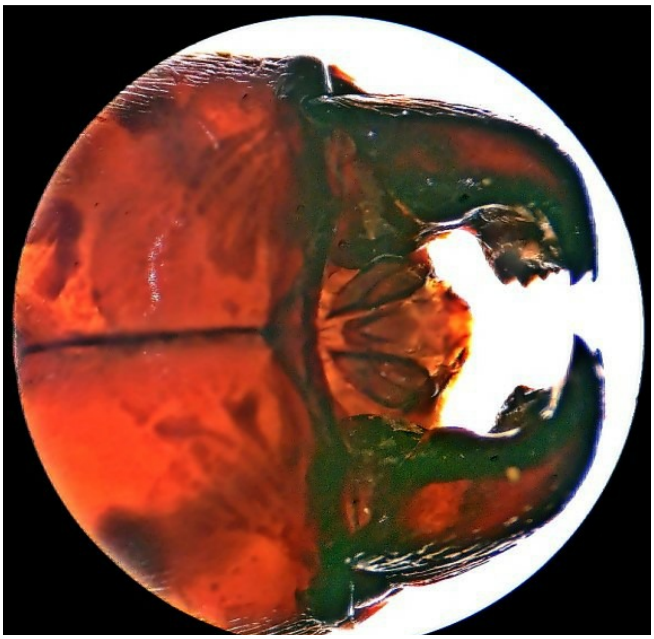
We measured the head of the pin and it was real close to 1/16" or .0625 Now, at 25,400 micrometers to the inch, the pinhead was 1587.5 micrometers across. If a red blood cell is 8 micrometers, I told her we could fit 198 of them side by side across the top of the pin. She squinted real hard at the top of the pin trying to imagine the 198 little dots. Since there are 192 of the 1/16 in. divisions on the ruler, I said (these are approximations now) if we magnify the pin head 200 times it would appear as big in diameter as the ruler is long and the blood cells would look to be (approx.) 1/16 in. in diameter and we could fit one in each of the divisions on the ruler. She was now looking back and forth between the pinhead and the ruler and getting a concept of the actual size of a blood cell. Then I said if we magnify it 800 times the pin would look to be 4 ft in diameter and each cell would appear ¼ in. across. Now she also had a concept of magnification and with what appeared to be a smile of comprehension, she promptly went off to tell her brother she was smarter than him. She came back several more times to show others the pin and ruler. A minor learning success? I think so.

If I had the same sort of tutoring when I was nine, microscopy would have been a much more enjoyable and enduring interest. No formulas, diagrams, or theory. Just information reduced to its most basic concepts and presented in an analogous form so simple and apparent an eight year old can understand. (In the Navy they are called "Executive summaries.") That is the challenge.

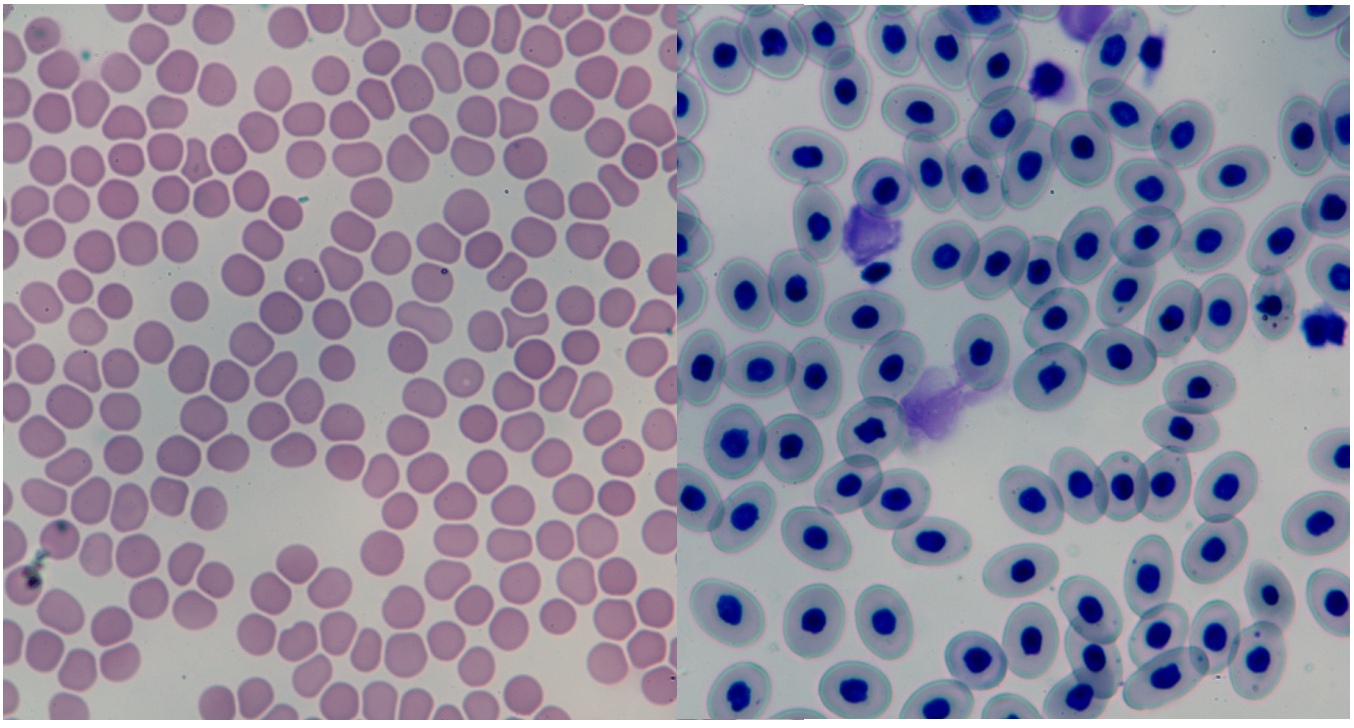
As an archived resource I propose Micscape create a "ten and under" category and challenge the contributors to author an article on some aspect of microscopy using references an age group this age group would be familiar with. A storefront display window to explain reflection, beam splitting depending on what angle you are looking at it etc. Be creative and simple. I would really like to hear your thoughts on this.

I promise I will never write anything this serious again. Time for some distilled molasses and phosphoric acid. Caviling rhetoric accepted by me Joseph Wilhelm. Cheers, Joseph ■

Images of arthropods



Left image: A Scorpion Stinger imaged through a SW-2T13Y Amscope Stereo Trinocular Microscope at 10X.
Right image: Ant mandible imaged through a SW-2T13Y Amscope Stereo Trinocular Microscope at 20X. Both images were taken through the left eyepiece tube. Colors and lighting were edited using Adobe Photoshop Elements and Photoscape. It was resized using PIXresizer. The images were taken with a 44430 Celestron Microscope Camera by myself, Floie Barrows. It is also posted to my first Microscopy Gallery on Facebook.



The red blood cells of amphibians contain contain a nucleus (right), which is visible as a dark purple dot in the center of each cell. In contrast, the red blood cells of mammals (left) do not possess a nucleus. The two pictures show both types of blood photographed with a 40x achromatic objective and a 2.5x photo projection ocular. Images by Oliver Kim.

DIY Fluorescence Illumination System

Several blue LEDs and a highlighting marker - Enough for some simple fluorescence work.

by César Guazzaroni

I recently picked up a suggestion from a web forum member and started to build an illumination system for top-lighting using four white high-brightness LEDs (figure 1). I obtained good results and the system is sufficient to observe insects and mites (figure 2). I could only use it with a 10X panoramic objective, however.

Fluorescence

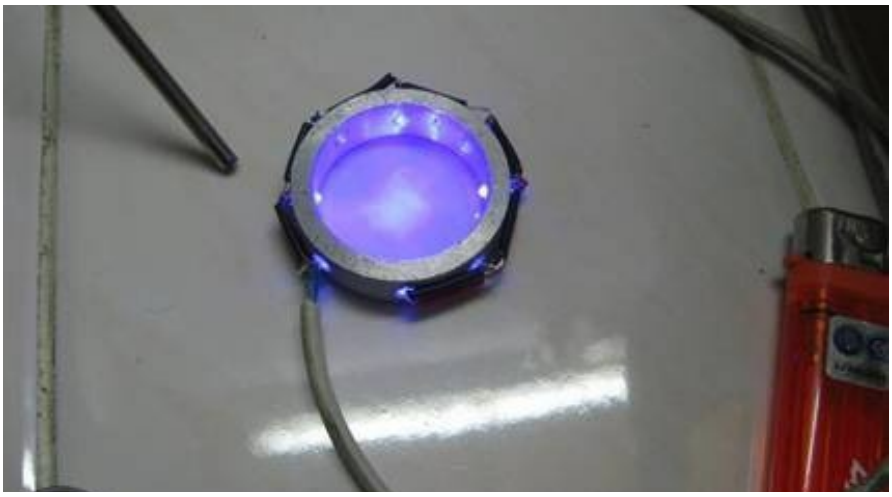
With a simple home-made device, it is possible to see fluorescence in samples. I always had the idea to illuminate with UV light, after all it is now very easy to get LEDs between a wavelength of 395 nm – 415nm. These LEDs can be bought on eBay.

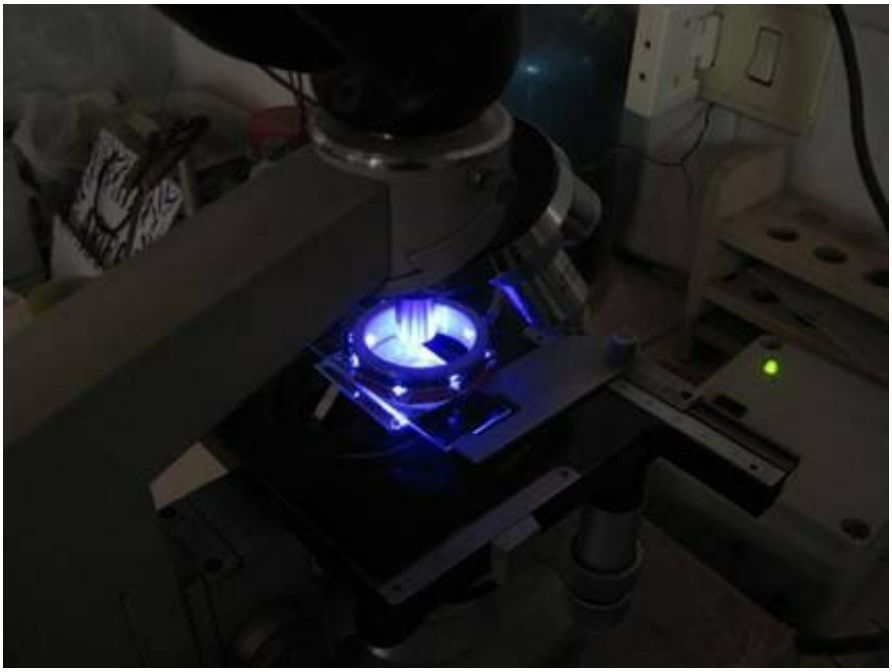
I followed the same idea from the previous device and built a ring of eight LEDs of this type. I think the lighting would be more convenient from below but I was worried that direct UV light from the LEDs can damage the eyes. It is highly advised to use darkfield and this would avoid direct impact of UV light on the eyes.

To improve the light intensity, I used a reflective sheeting as a mirror. This mirror was placed below the sample to reflect the light and significantly

Figure 1 (top): Illumination system using four LEDs for top-lighting.

Figure 2 (bottom): Image of a baby aphid using top lighting. To improve the depth of field, you can always take several photos and then stack the individual images into a final picture.





increased the brightness. Still, it is advisable to turn off the lights in the room.

In order to see some fluorescence, I obtained some highlighting markers (figure 6). The markers were opened and about 10 ml of distilled water or sterile saline was added to remove the pigment. Figure 7 shows the pigment that was obtained this way.

For sample preparation, a few drops of water containing many microorganisms was mixed with the same amount of fluorescent liquid. The pigment is allowed to be in contact with the sample for about one hour. I then filled the tube with water and centrifuged everything. The supernatant was discarded and the microorganisms that collected at the bottom of the tube were washed with clear water again. This washing step removes any fluorescent liquid that was not taken up by the cells, thereby reducing the background brightness and increasing contrast.

After the second washing step, the sediment with the microorganisms was suspended in a few drops of water and then observed microscopically. I saw many bacteria that stained strongly, some algae that absorbed the dye and other microorganisms (figures 8 and 9). ■

Figure 3 (top): Illumination system using blue LEDs for top-lighting.

Figure 4 (middle): Reflective sheeting used as a mirror. My Canon A570 camera is reflected.

Figure 5 (bottom): The reflective sheet is placed below the glass slide.

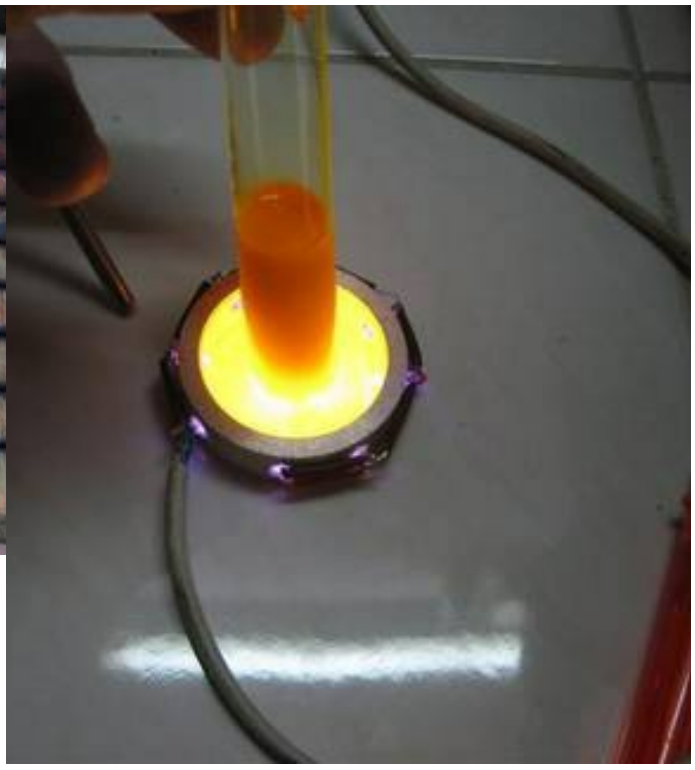
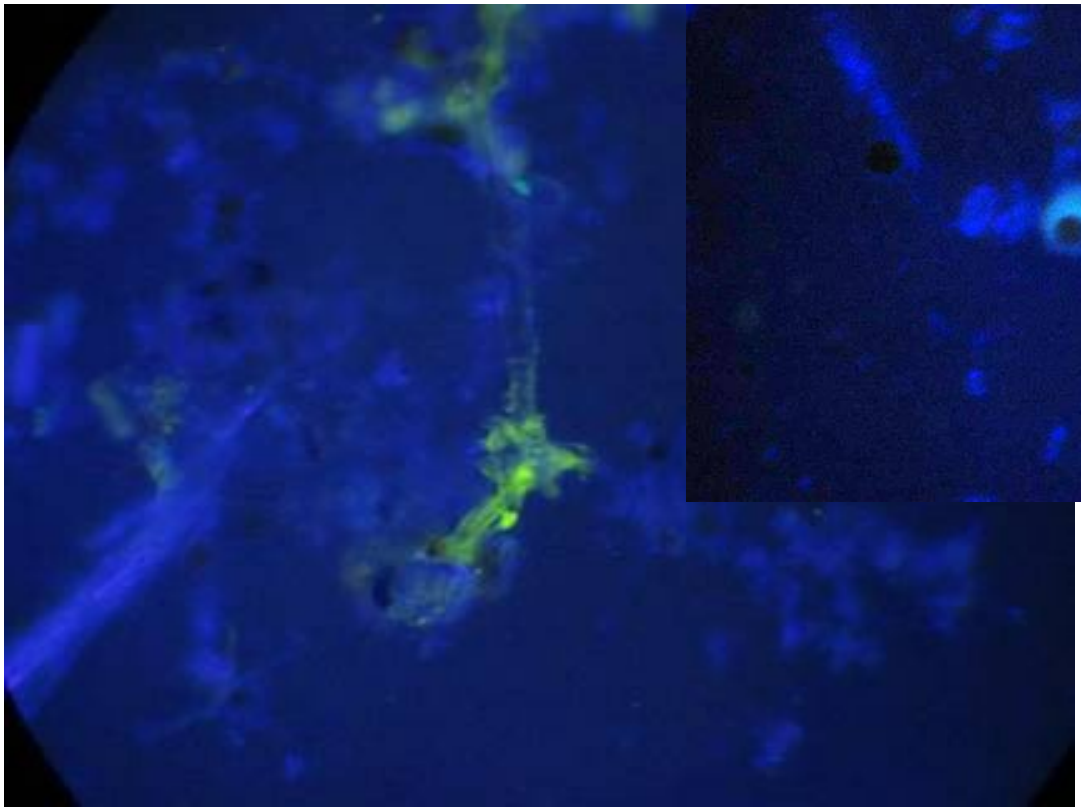


Figure 6 (top): Fluorescent markers used for staining the cells.

Figure 7 (right): Placing the test tube with the pigment into the blue light produces a bright fluorescent glow.



Figures 8 and 9: Specimens after incubation with the pigment. Cells that took up the pigment of the marker started to glow.

Disclaimer: Do all experiments at your own risk. Neither the author nor the publisher assume liability if you damage your eyes or harm your health in other ways. Do not look directly into UV light and do not use laser diodes instead of LEDs.

Observing Bacteria

Observing unstained bacteria requires a phase contrast microscope. Here are some pictures that show what you can expect to see.

by Oliver Kim

Bacteria are transparent and therefore difficult to see using regular bright-field microscopy. The bacterial cells will appear about as bright as the surrounding medium and there is little color contrast. Phase contrast optics provides a solution. Phase contrast optics convert the differences in optical density (i.e. the refractive index) of the bacterial cells into different shades of brightness. The optics achieves this by interference of the light which passes through the specimen (the bacteria) with the light that goes around through the medium. Phase contrast optics therefore work only if the cells have a different refractive index compared to the medium.

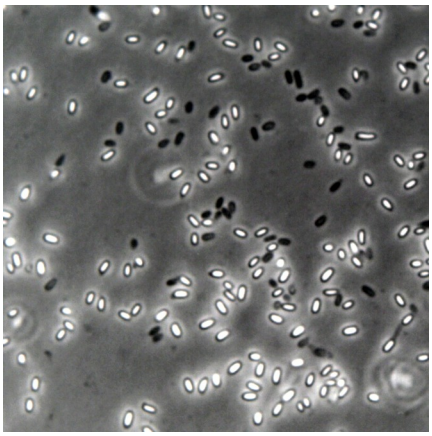
The bacteria were grown in pure culture in an appropriate microbiology laboratory. A colony was then suspended in saline and then microscoped in a wet mount using a 100x oil immersion objective.

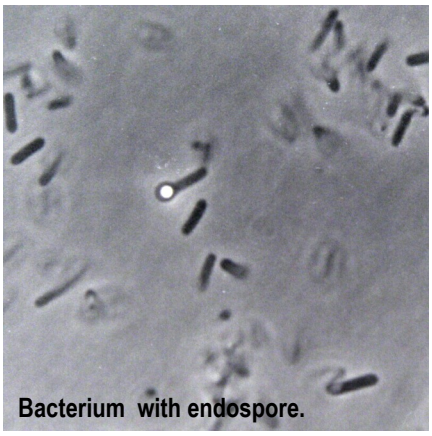
If one takes too much liquid, then the cells start to float in and out of focus and it is not easily possible to capture the shape of the individual cells. The evaporation of the liquid from the edges of the cover slip will cause a constant movement of the cells and make it difficult to take a steady picture. If this is a problem, then it is necessary to heat-fix the bacteria. A colony was then suspended in water and dried at room temperature. The slide was briefly pulled through the flame of a Bunsen burner, with the bacteria on the opposite side of the the flame. This heating process fixed the bacteria to the glass slide. Immersion oil was then directly applied to the slide and the bacteria were observed without cover glass. One disadvantage of heat fixing is, that during the drying process the bacteria may aggregate (as the volume of liquid decreases) and it may become more difficult to see individual cells. Heat fixed bacteria can, however, be easily stained for viewing in bright-field.

A word of warning, though. It is not a good idea to grow bacteria at home, for health and safety reasons. I would recommend to use safe sources such as fresh yogurt. The bacterial density is very high and it must certainly be diluted with a bit of water.

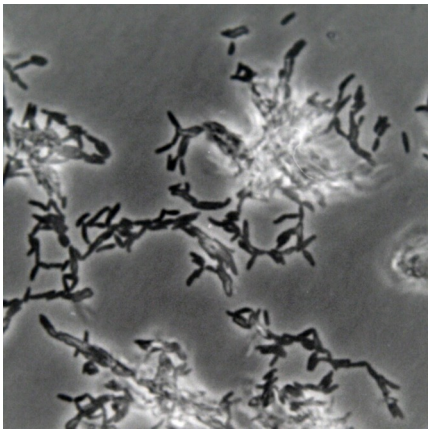
The pictures on the right show a variety of different bacteria, all obtained from a pure culture. Very bright structures (such as the first two images on the right) inside the cells are a strong indicator for endospores or storage grains of PHB. Differential staining must then be used to clarify the nature of the structure. ■

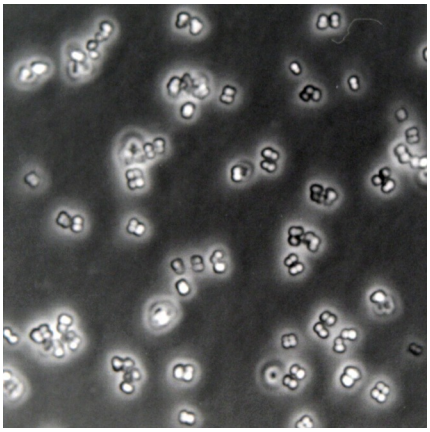
These images were originally taken on analog film. The film was digitized, digitally inverted and contrast adjusted.

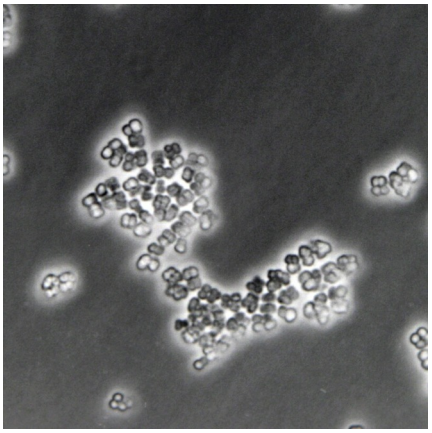




Bacterium with endospore.







Using GIMP to Measure Distances - Part 2

It is possible to use the free program GIMP to do measurements on micrographs. Here is an improvement over last month's approach.

by Oliver Kim

In last month's issue of MicrobeHunter Magazine, I described a method of measuring distances in micrographs, using the free program GIMP. I described how one can use the "measure" tool to determine the pixel distance between two points in a micrograph and then how to convert this into micrometers. There was a bit of math involved and this made the procedure a little unpractical.

After a little bit of research (and the help of a reader) I was able to figure out a much more practical method of measuring distances, and there is not even a need to install additional plugins into GIMP.

Step 1: Take a picture of the reference

You first must obtain a size reference. Last month I used a ruler with 1mm markings, but this turned out to be quite inaccurate. I now decided to use a hemocytometer (figure 1), which has squares with a 0.05mm side length. This size is small enough for one square to be photographed as a whole even with a 100x objective. This makes calibration possible for all of my objectives. Hemocytometers are generally used to determine cell count, but the regular markings also make it useful for distance measurements. It is important to note down the camera resolution and the objective that was used to take the picture. I have taken a separate picture with each of my five objectives, also using two different resolution settings on my camera. This means that I took a total of 10 images.

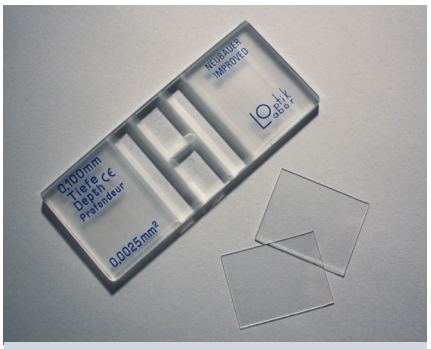


Figure 1: A hemocytometer.

Step 2: Measure the size of the reference

Load the image displaying the hemocytometer grid into GIMP. Go to the image-print size menu and determine the horizontal and vertical print resolution of this image. Make sure that this setting remains the same for all images that you measure (figure 2).

Now select the measure tool from the toolbox. At the bottom of the image, in the status bar, select inches as the units. We have to use inches, because the conversion factor, which we need later to define our own custom units, is based on inches. Using the measure tool, measure out 0.05mm of the hemocytometer image. The smallest squares of the "Neubauer improved" hemocytometer have this length. You can see the distance in inches displayed either at the bottom of the window or in a separate info-box. Note down this distance in inches, we need this value for later.

You may notice that this distance in inches does not represent the real distance on the screen. For example, if the measure tool states that the distance is

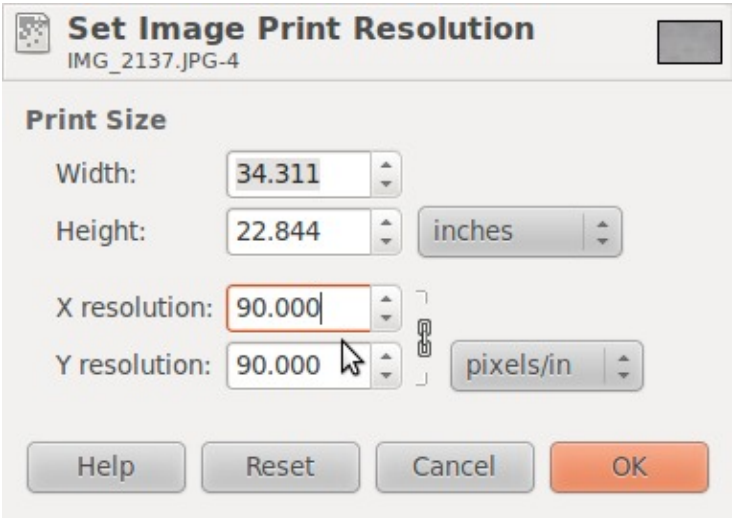
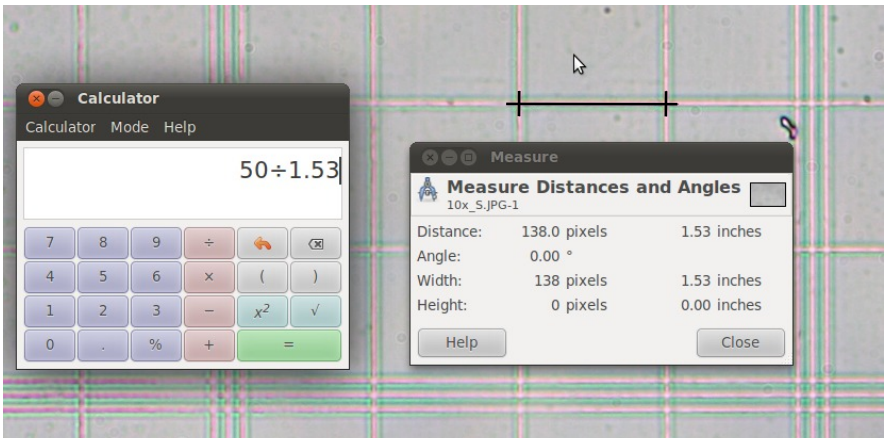


Figure 2: Make sure that the X and Y resolution is the same for all images that you measure. It is possible to use the monitor resolution for this setting. The monitor resolution can be determined in the preferences menu.

Figure 3 (right): The distance of 50µm is divided by the measured distance of 1.53 inches. The result (32.67973) is the conversion factor for a new unit.

Figure 4 (bottom): The conversion factor (which is based on inches) is now entered into the dialog box. The new unit is dependent on magnification and image size, which are also both entered for information purposes.



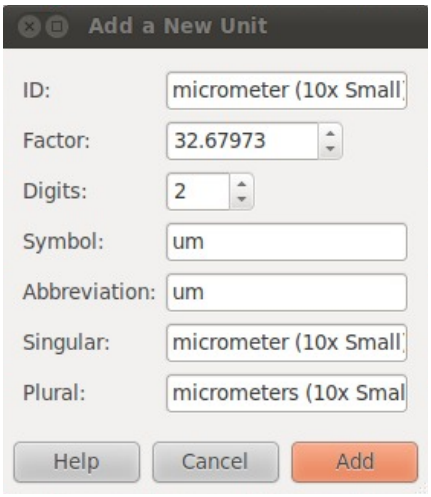


Figure 5: The new unit will appear in the unit editor. Make sure that you set the check mark on the left, otherwise the unit will not be saved when exiting the program. You also need to restart GIMP for the new changes to take effect.

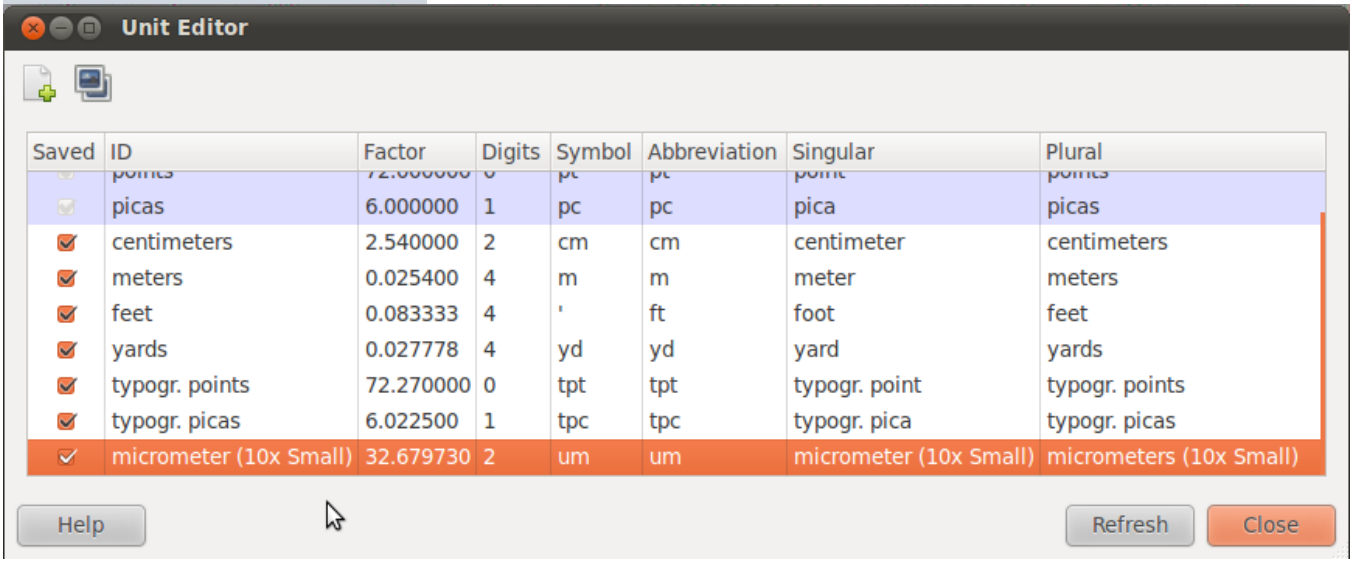
1.5 inches, it is quite likely that the distance on screen is quite different, if measured out with a ruler. The reason is, because the distance displayed represents the distance on the printed image, which may have a different resolution. If you want to display the correct size also on the monitor, then you have to calibrate the screen resolution with the image resolution. This is optional and not required for measuring micrographs (covered later).

Step 3: Calculate the conversion factor

We now have to do some math. We convert the real size the of the reference into micrometers (0.05mm is 50µm) and then divide this by the reference size in inches (obtained in step 2). See figure 3 on how this is done. This is the new conversion factor which is specific for this particular objective and image size. In our example, we divide the 50µm by 1.53 inches to obtain 32.67973µm per inch.

Step 4: Define a new unit

Go to Edit – Units to define a new unit (figure 4). In my example, I entered "micrometer (10X small)" as the ID, to indicate the objective and image size. If you do not use different camera resolutions, then it is necessary to only indicate the objective, of course. Also enter the conversion factor (from step 3) into the dialog box. Make sure that you set the checkmark before closing the window, otherwise the new unit will not be saved (figure 5). You also need to restart GIMP for the new unit to appear.



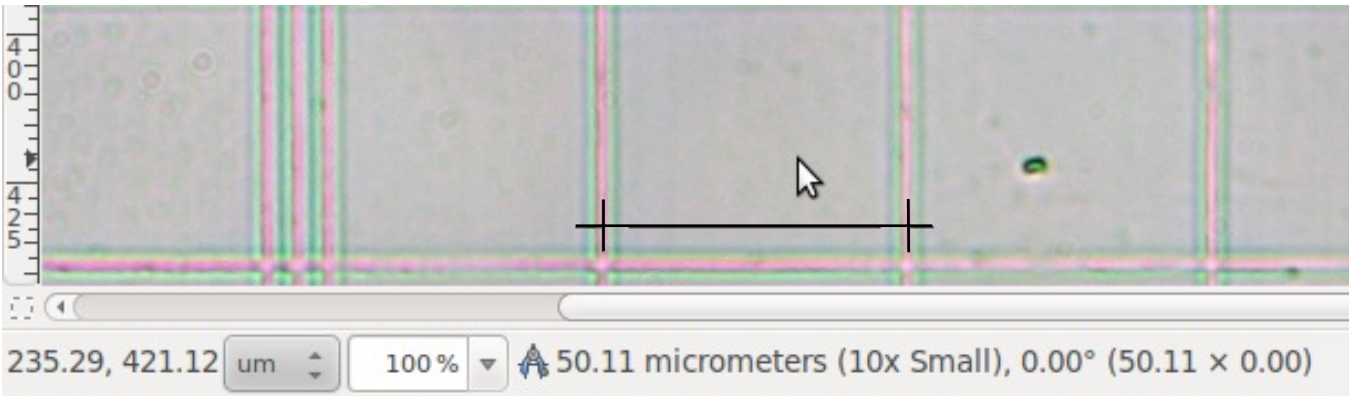


Figure 6 (top): Checking the result. Measuring out the reference should produce the correct distance, which it does.

Step 7: Check for correctness

Now use GIMP's measure tool to determine the size of the reference that you used to check that you did not make any mistakes (figure 6). In this case, measuring out the side of grid resulted in 50.11 micrometers, which is good enough.

Step 8: Start measuring

Load a new micrograph and make sure that the print resolution of this image is the same as the one of the reference image. Go to image-print size and make sure that the resolution is the same as the one on the reference image. Also make sure that you choose the correct unit, based on the objective that you used and the image size. Happy measuring!

Calibrating the monitor and the image

This step is not absolutely needed for determining the size in micrographs, but I still recommend it. In GIMP, go to Edit - Preferences – Display. The dialog box will display the detected monitor resolution. This resolution can also be set manually or it is possible to calibrate it by using a ruler. Note down the horizontal and vertical resolution (figure 7).

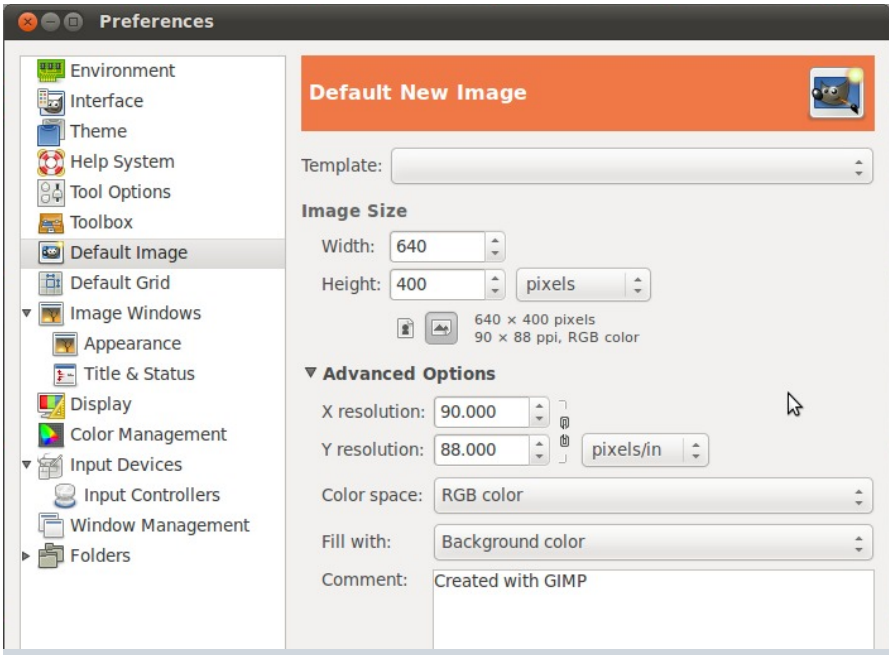


Figure 7: The preference dialog box provides information on screen resolution (found in *Display*) and also allows the user to set the default resolution for new images (found in *Default Image*).

We now need to set the pixel density resolution of the micrograph. Changing this value will not impact on the quality of the image in any way. We are not resizing the image. This step ensures that the displayed distances on the monitor correspond to the real size of the on-screen image. Again, this is an optional (but recommended) step to take. Click the menu Image – Print size... and then set the horizontal and vertical image resolution to the values that you obtained above (monitor resolution). If you now use the "measure" tool in GIMP, then the measured distance between two points in inches should represent the actual distance on the monitor.

What if you do not have a hemocytometer?

Finding small objects with a well known size is not easy. It is possible to use a caliper to measure out the size of small or thin objects, such as wire, and then use this information for calibration. Some companies offer glass beads for calibration, but I have never used these before. Do not hesitate to try out other calibration methods! Report back! ■

Rediscovering the Art of Slide Wrapping

Many antique microscope slides were wrapped in decorative paper. Here is an attempt to rediscover this art.

by Oliver Kim

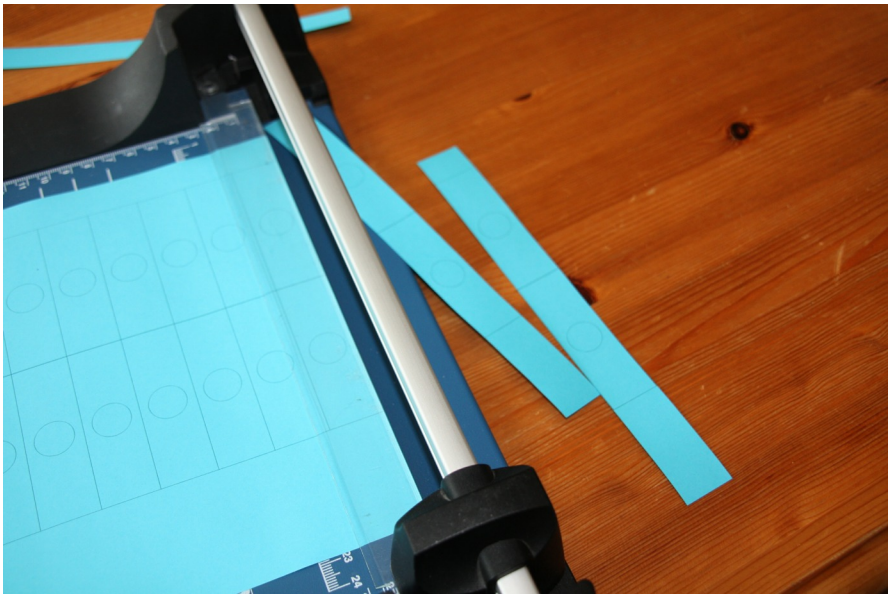
Many microscope slides from the 19th century are wrapped in highly ornamented paper. I wondered why this is the case and was happy to find an explanation on the web site:

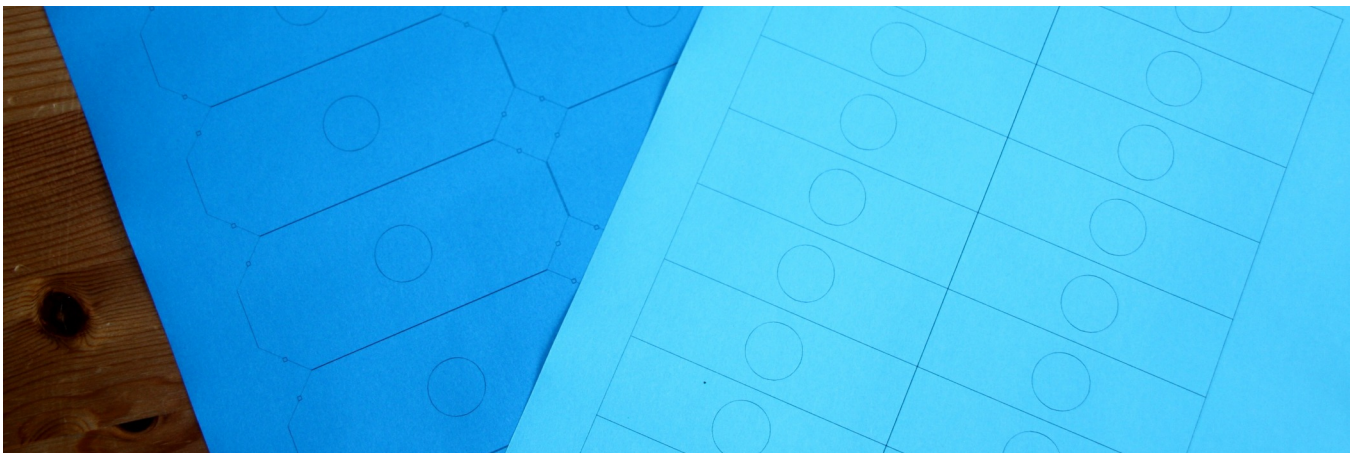
www.victorianmicroscopeslides.com.

Originally I thought that the paper wrapping was used to minimize the risk of the slides breaking when they are dropped. The real reason was to hold the cover glass in place. During the 19th century the slide manufacturers also used mica as a cover glass and this material, apparently, required more physical support. Only after more stable mounting media, such as Canada Balsam were invented, the paper cover became obsolete. The paper wrapping also held the cover glass of dry mounts in place. Today one can either use clear nail polish or commercial sealants to hold the cover glass in place.

Even though the paper covering became obsolete, many manufacturers of slides still continued to paper wrap their slides, also for branding purposes, and because it looks nice. Nowadays, the characteristic way in which the slides were wrapped also helps collectors to identify them. In my view, the old-fashioned appearance of the paper wrapping also adds a significant amount of charm to the slides and makes them nice items to collect.

I myself am currently not collecting Victorian-era slides, also because many of them must certainly be quite unaffordable. Instead, I decided to try a dif-





Figures 1 and 2 (bottom and right): The outlines for the top and bottom covers were first designed on the computer and then printed out on regular colored paper. A paper-cutter ensures that all edges are indeed straight, but a cutting knife and ruler

ferent approach: maybe my own slides will dramatically increase in value in 200 years if I wrap them in paper?

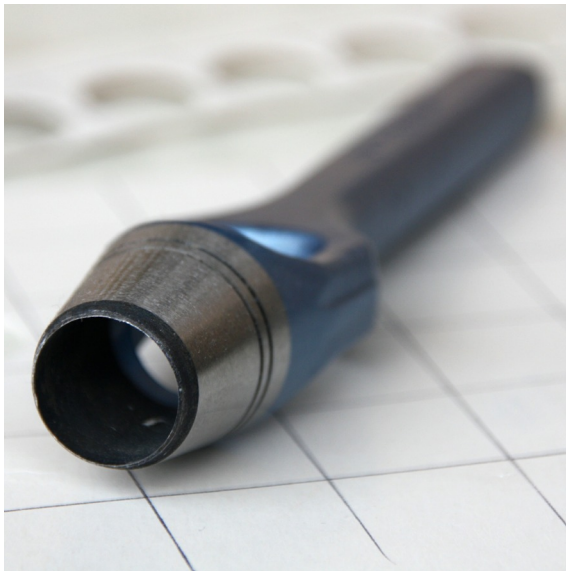
Why bother?

What is the point of wrapping the slides? Mounting media a are now able to hold the cover glass solidly in place and the cost of slides is quite low so that a broken slide is easily replaced. Paper wrapping the slide may give them increased stability, but if you drop them, they may may break nevertheless.

I pondered over this question and found (more or less convincing) answers: Wrapping the slides gives protection to the slides, increases their

Figure 3 (top): Testing the glue. The white wood glue really works well. As a matter of fact, the glue holds better than the paper and trying to remove the paper will result in the paper being destroyed (and not the glue).

Figures 4-6 (left and bottom): A hammer and a circular cut-out tool are necessary. The diameter of the the tool is 16mm, which was just right. A sharp cutting knife and a ruler are needed to cut out the cardboard spacers. These spacers are needed for thick specimens. In this case, I wanted to mount sand samples without mounting medium.



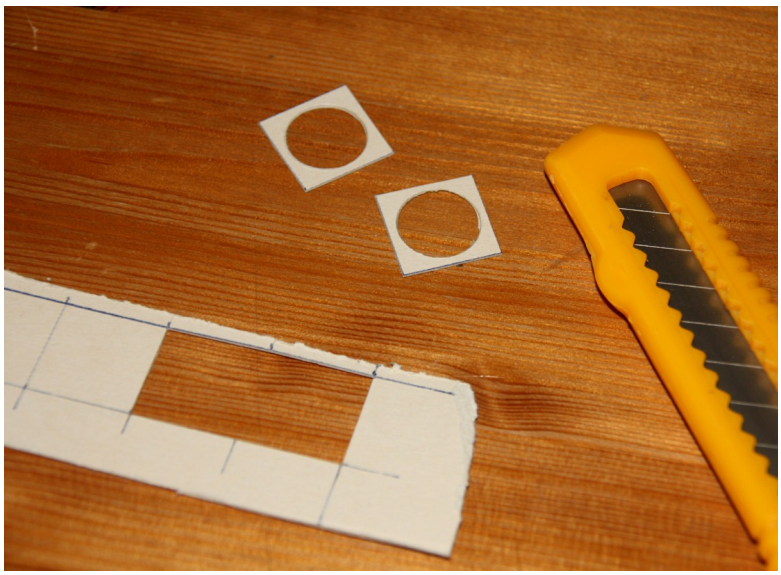
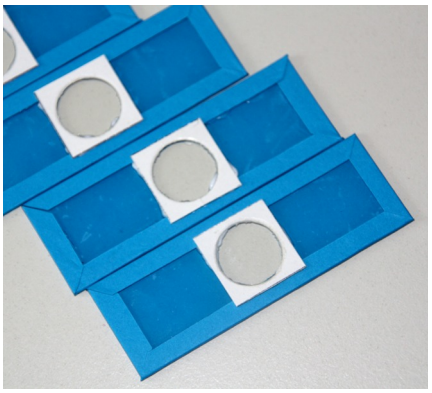


Figure 7: Cardboard spacers (left) bottom paper cover (dark blue) and top cover (light blue) are all prepared first before gluing.



value (and makes them more interesting for collectors) as well as being a fun activity. But most importantly: paper wrapping increases the stabiilty of the cover glass for thick, air mounted specimens, such as the sand samples, which I attempted to mount.

The process

First I designed the outline for the paper wrap on the computer and printed it on colored paper. I decided to give the bottom side of the slide a dark blue and the top side a light blue appearance. The bottom side has to be covered first, as this side has to be completely flat.

Difficulties

Initially I attempted to cut the round circles using a sharp cutting knife, but this turned out to be a very tedious activity. Using the giant hole puncher was a great help. Another problem was preventing any glue to touch the parts of the central round window of the slide. I did find out, that it is easily possible to scratch residual glue away (using sharp knife), but this is additional work. I recommend that the glue be spread on the paper with the round circle (already cut out) still in place. This will prevent glue from spilling to the other side. See the next page for an illustration.

Disadvantages

There are several disadvantages to slide wrapping. Naturally, there is a lot of work involved and it is questionable if it is really worth the effort. I will probably wrap the more important slides to give them more resistance to breakage. The bigger disadvantage is the reduced viewing area. The cut out circles have a diameter of 16mm, which is smaller than the size of the cover glass.

Further ideas

The slides could be color coded for easier sorting. It's also possible to print patterns and information directly on the top wrapping paper, without using separate labels. I think that there are many more ideas and I encourage everyone to send me pictures of some slides that you wrapped, to be published in an upcoming issue!

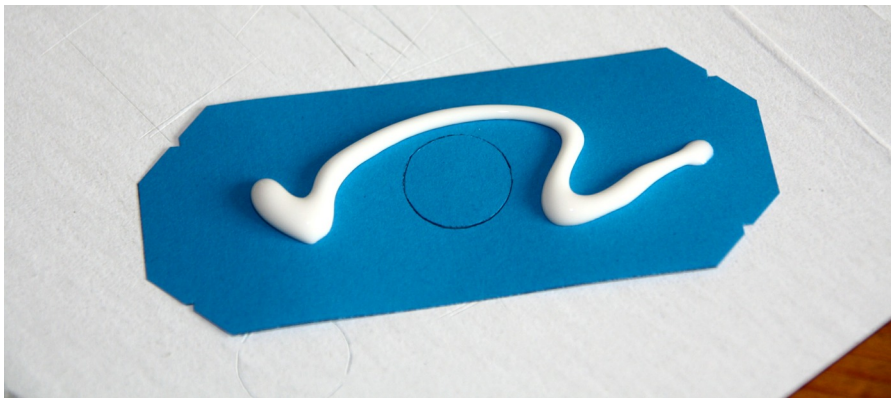


Figure 8: The circle in the center has already been cut out, but is still left in place for applying the glue. This prevents glue from spilling to the other side of the paper and keeps the round window clean. This particular "wrapper" does not have guiding lines, which makes the alignment of the slide a bit difficult.

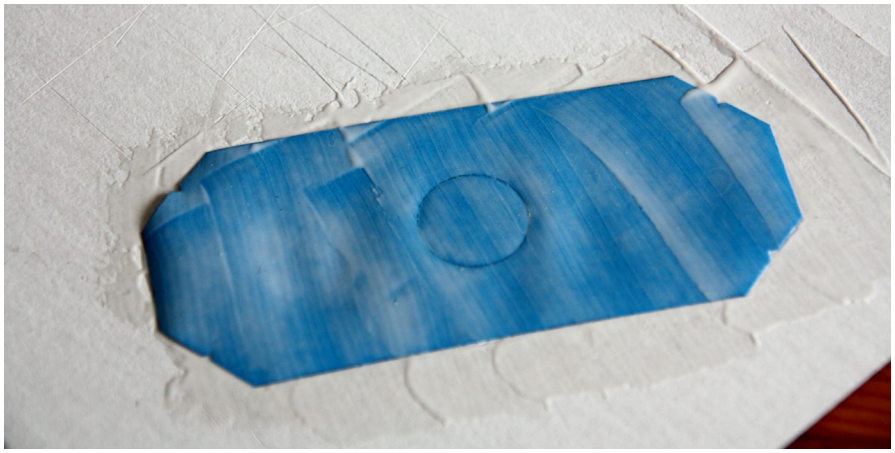


Figure 9: The white glue is spread with a piece of cardboard. Maybe I should have used a slower setting glue, I was a bit under time pressure.

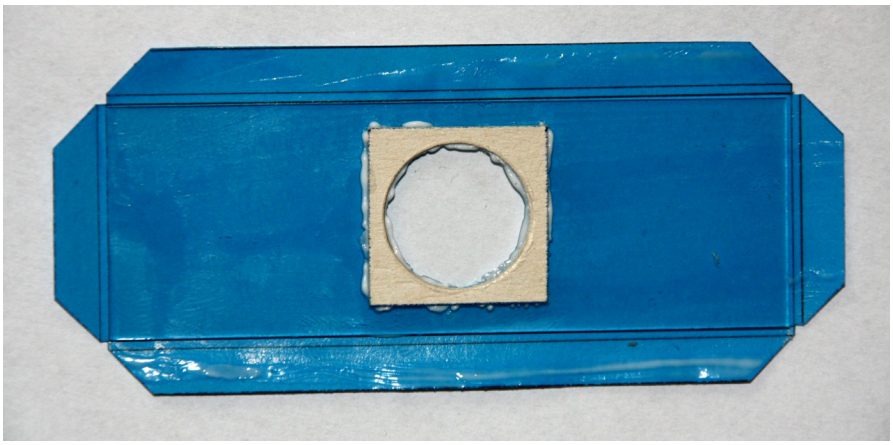


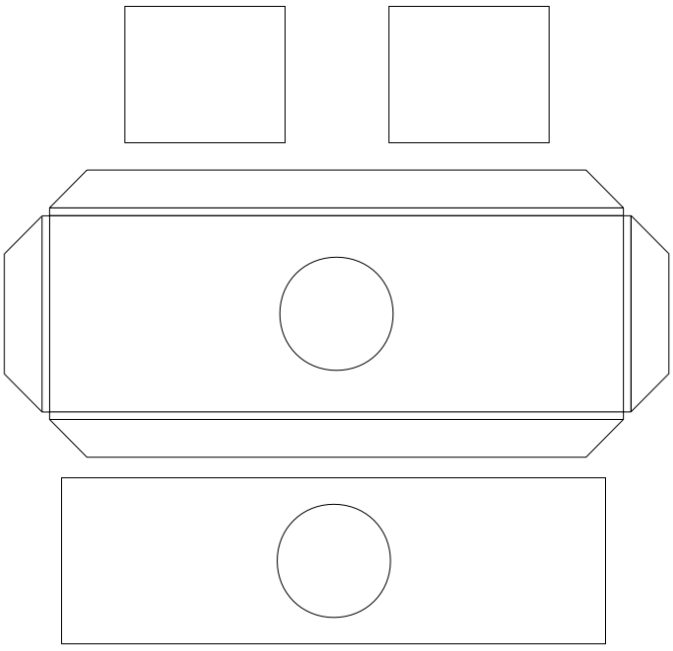
Figure 10: The glass slide is placed on the wrapper (notice, the guiding lines). The spacers were also glued on using the white glue. Spillage of the glue is difficult to avoid. Remove excess glue with a sharp object after drying (to prevent smearing) .

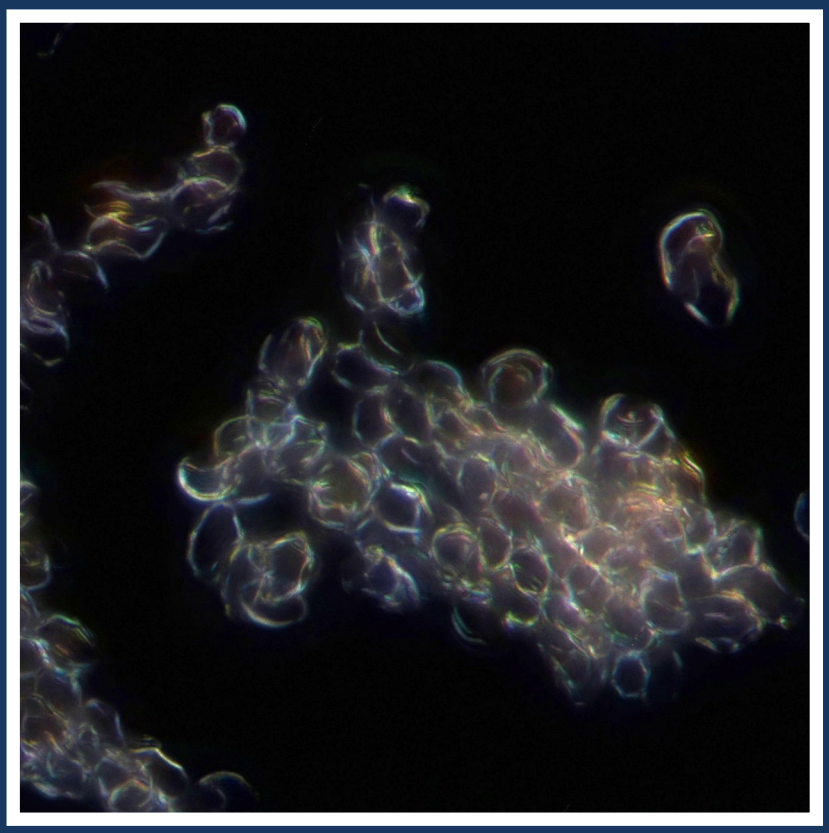
Figure 11: The sand sample is placed into the spacer and a cover glass (difficult to see) is glued on using the same white glue. No mounting medium was applied here, the sand is "air-mounted". I used quite a lot of glue to seal off everything. The glue will shrink quite a lot as it dries, and will also become transparent.

Wrap your slides and send pictures of your work to: editor@microbehunter.com. I'll publish them!

Figure 12 (top): These pictures are pretty self-explanatory. The light blue top wrapper is glued on, and the labels are applied. The four slides on the right were made without the cardboard spacer. The slide on the bottom left was accidentally wrapped the wrong way around (with the cover glass on the back side). Also notice that the round window is not cut out as nicely as the other ones, this is because I did not use the hole punching tool. I made the top wrappers smaller than the bottom wrapper to give the slide a nice dark blue frame. I also experimented by printing the label directly on the top wrapper, but somehow this does not look as good as a white label.

Figure 13 (right): The template!





What's this? Answer on page 3.