Microbe Hunter

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The Magazine for the Enthusiast Microscopist

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Microtome Making

Spiracles in a Beetle

Slide Box Making

ABOUT

Microbehunter Microscopy Magazine
The magazine for the enthusiast microscopist
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Front Cover:
Large image: Oliver Kim (spruce wood)
Left image: César Guazzaroni
Middle: Rodney Brightwell
Right: Oliver Kim

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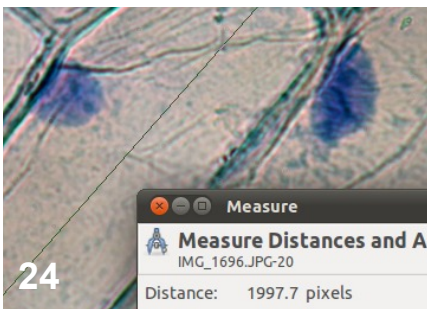


Answer to the puzzle (back cover):
Pollen of a dandelion.

The Ethics of Medical Homicide and Mutilation

In this episode, Joseph Wilhelm continues to build a Zeiss GFL compound and Spencer stereo microscope.

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

G. Joseph Wilhelm, Florida Keys USA

It is a catchy title. Comes from a 1919 book by Austin O'Malley, M.D., Ph.D., LL.D. It's an interesting if rather narrowly reasoned philosophically opined treatise on morality, human existence, and natural law of ultimate right and wrong, all of my favorite subjects. And while the subject matter is not the main thrust of this essay (I just used the title to get your attention, you know, the bait and switch routine. No ethics intended.), you can read the entire book at www.archive.org. Yes, yes I agree, I have no shame. But as long as you are here, allow me to present my intended discourse.

In the past few months my microscopy related progress forward has become rather torpid due to circumstances (i.e. summer here, when seat belt buckles become branding irons and steering wheels so hot you realize a car can be driven with just two fingers). The orange crop is done and while we are waiting on the bananas to ripen there are major renovations underway to my combination office / den / study / mini-museum / library / lab room. The installation of four additional display cases and hinged pull out book shelves built into the wall has, for the past two months, left my usually comfortably tranquil sanctuary an abominably chaotic and disordered example of mayhem. This, combined with my wife's re-landscaping of the outside entertainment area, has left me with precious little time to devote to insouciant sarcasm and furthering my microscopy infatuation with a retro era suite of microscopes & accessories. Not to say there hasn't been some modicum of impetus.

For the six individuals I know for certain that actually read my drivel, I have prepared a two-part update of my continuing crusade towards microscopy excellence. Part-1 includes a review of aftermarket objectives, and eyepieces for the Zeiss and Spencer Stereo Scope, and a trinocular head for the Zeus system. Part-2 consists of presumably astute comments / observations on education, slide making with common sense use of toxic materials and proper treatment for analogy sufferers.

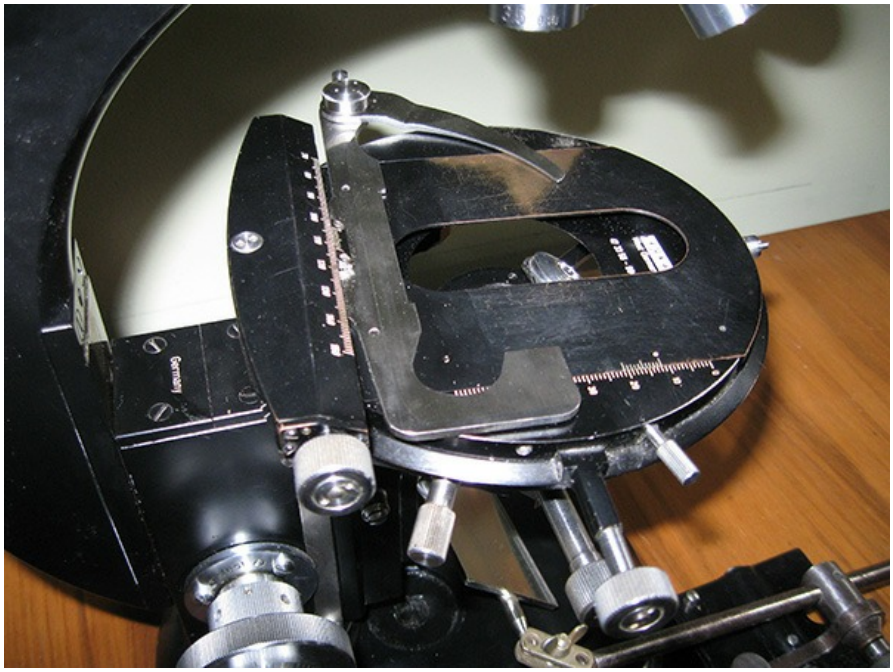


Figure 1: This is the stage obtained by what I am certain now are superior bidding skills.

The Zeus System Improvements

My Argus illuminated Zeiss GFL is now a living breathing entity. There were some condenser clearance issues with my homemade stage (Episode 3), solved by winning the eBay lottery and

Figures 2 and 2a: A real hodgepodge of optics.

purchasing a real Zeiss GFL rotating stage for a ridiculously low bid. It has centering adjustments but no degree scale.

Still, my homemade stage has some advantages to the Zeiss so I machined off the offending metal and am now looking for a suitable method of applying a degree scale (any suggestions are welcome). Once this is accomplished I believe it will be as versatile or maybe more so than the Zeiss stage.

The objectives of my desire

From my readings I believe it is generally agreed the most important parts of any microscopic system start with the objectives, then eyepieces and the focus mechanism and finally lighting.

For a less than superior stand the easiest and most economically reasonable performance enhancement is with the lighting. Many innovative and practical solutions abound in the Micscape lighting category that precludes spending hundreds of dollars. The slide projector solution (Episode 3) yielded very satisfactory results by placing two plates of fine ground glass where the slide would normally go and making sure the projector lens kept them slightly out of focus. I placed a surplus Spencer condenser iris immediately in front of the lens as a field diaphragm and was able to get absolutely uniform illumination across the field of view from 4x to 60x. This is a 100-watt source and the internal optics are heat (IR) absorbing but before I do anything more than test set ups I intend to put a UV filter in the optical train. A household dimmer switch currently controls the brightness but future plans call for a number of neutral density filters to minimize color temperature change. (Someone please tell me if I am talking thru my hat about this.) Future plans also call for a homemade light using the components from a 500-watt Argus projector. (Or as Mr. Frithjof Sterrenburg has named it "The Argus hellfire lighting system.")

On the other end of the performance amplification spectrum, the most dramatic augmentation comes with spending obscene amounts on new, or even used, optical components. Below (Fig 2) are the objectives I currently had to putz around with, one Bushnell 4x, two no-name DIN 100x and 40x (anyone recognize the trademark?) a JIS no-name 20x and the only for certain quality one, a Leitz 3.5x. All of them are achromats.

The GFL obviously deserved better but my dilemma was manifold. New or used, Achro, Semi Plan or Plan Achro, Zeiss brand, other brand or aftermarket no name, rational price limitations??? The only thing that was decided for me was they had to be DIN as the arm would not focus down far enough to use JIS objectives. Do I really need the $500,000 Ferrari (Read as: Full set of Zeiss Plan Apochromats in pristine condition) or the Ford Escort (Set of four quality aftermarket Achromats)? What about proper eyepiece match-up? I decided to forge ahead using the ABM (Alphonso Bedoya mindset, see Episode 1) and get what I thought was right.

I could not find a source for new Zeiss 160mm TL objectives (They only make infinity corrected anymore?). A quick look on eBay for Zeiss brand showed 116 objectives of various types with only a few to prehend my interest. Two plan apochromats in rough shape for $499 ea, three plan achromats for premium prices of which two were in Russia/Bulgaria. This with the nagging reminder that these objectives may be subject to delaminating and the chance they may not be par center steered me towards the after market new. I began a search for DIN Plan/160 objectives.

Now I have to admit to some phobias about purchasing "precision" manufactured items from countries other than those with established reputations relating to the particular item i.e. optics from Germany, watches from Switzerland, Samurai swords from Japan, whiskey from Tennessee etc. I was therefore encouraged by Mr. Robert Pavlis' splendid article on microscope component compatibility (Micscape April 2010) where, if I may quote, he states:

"Recently many objectives of Chinese origin have become available, usually they are sold for quite low prices. Many of these are DIN objectives of very high quality. They very often do not carry a

manufacturer's mark. The quality tends to vary from extremely high to poor."

I found four viable sites for the objectives described above, two of which offered what appeared to be the same objectives Mr. Pavlis had in the pictures of his scopes shown in his article. Here is the comparison:

SMS Optical Co. (USA)
Offer Plan DIN 2x, 4x, 10x, 20x, 40x, 50x, 60x, and 100x. Sold individually. A set of four, the 4x, 10x, 40x and 100x = $472.00 + shipping. Not the same as Mr. Pavlis'

Figure 3: By all appearances a quality set of objectives.

Precision World / Amscope (USA)
http://stores.eBay.com/precision-world
Offer Plan DIN 4x, 10x, 40x and 100x Sold individually. Above set of four = $351.98 Free shipping. Appear the same as Mr. Pavlis'

Microscopes India (India)
http://stores.eBay.com/microscopes-india
Offer Plan DIN 4x, 10x, 40x, and 100x. Sold as a set only = $199.00 offer 98% flat field. Free shipping. Not the same as Mr. Pavlis'

Microscopenet (Canada)
http://stores.eBay.com/microscopenet
Offer Plan DIN 4x, 10x, 20x, 40x, 60x, 100x, (and a 2x Semi-Plan). Sold individually or as a set of four 4x, 10x, 40x, 100x $202.00 Free shipping. Appear same as Mr. Pavlis' and identical to those offered by Precision World.

The Canucks won hands down and even though I was taking a chance, I could get my money back. I ordered the set of four.

I placed the 4x, 10x, 40x and my original achromat 40x in the nosepiece. Some Konus prepared slides (absolutely horrid quality but all I had) were used as sample objects, the Argus illumination was adjusted, and I took the objectives for a test ride. It is now that I must offer my mea culpa for not having photographic attestation to the following observations but this was done weeks ago and with the current construction underway all of my delicate instruments have been safely but temporarily inaccessibly stored.

The objectives appeared to be excellent. Crisp imaging and running thru the gears from 4x to 40x required extremely minimal fine focus adjustments indicating excellent parfocal performance as well as minimal mechanical stage adjustments, which attest to the parcenter precision. Side by side comparison to my 40x achromat showed an in-focus image to the edge of the field of view and better color correction. Yup, happy camper here. I immediately ordered the 20x Plan and the 60x Plan and upon arrival was rewarded with the same level of quality and efficacy. I was not yet predisposed to preview the 100x capabilities but suspect it will be of no less quality of function. I also bought the 2x Semi Plan just…because. I am tempted to purchase the SMS 2x Plan as the odd man out because parfocal and parcenter capabilities are not as critical at such low power. Final tally was six Plan objectives plus a Semi Plan for under $400.00.

Spencer Model 26 LF Improvements

The Spencer Model 26 LF Greenough Binocular Stereo-scopic Dissecting Wide Field Low Power Microscope Improvements: Ah yes, a delightful instrument. Ever since Episode 2, while dealing with the snails pace of the Zeus System development, I have been using it to bond with the entomological civilization that I must share the interior, exterior and air that constitutes my commorancy. These and other minute objects in the proximity of my domicile have provided me with immense enjoyment. No fuss, no muss, no slides, just murder the little grubbers and put them on the stage. I really cannot say enough about this scope. Spencer made a quality instrument, versatile, excellent optics, and a wide range of magnifications, user serviceable and uses the same eyepieces as my Zeiss. Mine was a 26 LA without a revolving nosepiece.

Spencer made quite a few variants of this microscope. The basic stand model #s were the 23, 25, 26 and 28. The vertical head models had a single letter suffix of A, B, C, F or G, depending on which combination of a revolving or non-revolving nosepiece, objectives and eyepieces you desired. The inclined head models placed an L to the suffix i.e. LA, LB, LC etc. Paired objectives were available in magnifications of .7x, 1x, 2x, 3x, 4x, 6x and 8x. Eyepieces were offered in 9x, 12x, 15x and 18x. A comprehensive explanation with illustrations can be viewed on the

AO Blue Book .pdf on Gordon Couger's excellent reference site: http://www.science-info.net/docs/ .

In my humble opinion this is probably the most underrated stereoscopic stand on the used market. I wanted to elevate the capabilities of my current stand so I pursued the additional paired objectives originally offered by Spencer and received some pleasant surprises.

As with the Zeiss objectives no new offerings were available, only used, and no new after market options were procurable. So vintage Spencer it had to be. How much is reasonable for a microscope objective? The compound objectives range from about $50 to $150 and the new stereo-microscope auxiliary lenses were from about $50 to $65 so with that in mind as parameters here is what I have concluded.

These Spencer paired objectives were available on eBay with a little patience. The gaps in my heterogeneous collection were the .7x, 2x, 4x, 6x and 8x. An eBay search under "Spencer Microscope" yielded the following:

A Spencer paired 2x objective. The good part; it was a "'Buy it Now"' price of $45. The better part; It came attached to a complete model 25 with base, revolving nosepiece, 1x and 3x objectives and associated vintage 10x WF eyepieces. Done deal.

Second find, a 6x objective for $60 attached to a model 26 with what I now consider a rare solid brass base with period 15x HEP eyepieces, another done deal.

Third was a set of three paired objectives with a .7x, 3x, and 6x for a winning bid of $19, final done deal.

So now with the three stands and duplicate objectives I will dedicate one to observations, one to slide preparation and the third as a field microscope. Still searching for the 4x and 8x.

The eyes have it

With the additional eyepieces from the Spencer stereoscopes and the generic wide field purchases already obtained I found a few good deals on paired Spencer oculars to round out the field so to speak. Here is the collection. (Fig.5 & 5a)

The generic 10x HKW are suspect as compensated eyepieces, purchased as such from Microscopes.india but I haven't tested them yet. (More about this site below.)

One of the last major components needed to complete the Zeus was a trinocular head. Once again, I was unable to fully justify the cost the used Zeiss heads were commanding. Searching "microscope heads" brought up this offering from Microscopes India for $159 with a custom sized dovetail ring and free shipping. (Fig.7 & 7a)

I hadn't dealt with this company before. From all indications this was manufactured in India. The phobia (de-

Figures 5 and 5a: Generic on the left, Spencer / AO on the right.

scribed earlier) resulted in an inordinate amount of neuron synapse activity being expended pondering the possible performance inadequacies eventuating from such a purchase, not to mention the aesthetics were entirely wrong. I proceeded with the only rational course of action available. I flipped a coin and bought it. I ordered it with a 43mm dovetail.

Examination upon arrival showed this to be a very well made piece of equipment (to my relief). The optics are coated and had a fungus inhibitor ingredient. The View was 100% eyepiece or 100% phototube via a side slider knob. Very smooth operation and a precise Zeiss-compatible dovetail mount ring and substantial heft. Side-by-side comparison with the Zeiss binocular head revealed a slightly better image (don't know when the Zeiss was last cleaned) and perfect collimation. In summary, I could live with the incompatibly clashing aesthetics… for now.

A few notes about Microscopes India

Shortly after the microscope head arrived I purchased a pair of 10x HKW eyepieces. I had emailed them prior to purchase to confirm that these were indeed High eyepoint Compensated Widefield and received an affirmative. The listed price was in Indian Rupees with the USD conversion next to it. After clicking the 'Buy it Now' button and confirming the purchase, the only payment option was thru PayAsia and if I wanted to use PayPal I would have to submit a special request which was granted over a week later all the while receiving requests for payment from a different branch of their company. It took about two weeks to and lots of emails to sort it out. I finally received the eyepieces which are good but do not appear to be compensating. Their web site has changed they no longer offer the trinocular head the whole product line has shrunk and they are selling wholesale lots of microscopes. Be cautious if you deal with them.

Slip sliding away… the future

Some see a vintage semi operational but un-repairable 1939 Singer sewing machine. But I, with my keen sense of and aptitude for the unthinkable, see a period correct motorized slide-ringing table…really (if there ever was such a thing). It's all in how you visualize the mechanical realignment and associative inter action of the mechanisms. More on this epiphany later. Lets talk about slides.

As some of you have gathered I am a vintage biased sort of fellow. To me, the papered slides of the 19th century simply reek of that bygone era I love so when artistic style was imbued unto even the most basic and utilitarian articles. As mentioned in Episode 4, I fully intend to explore replicating the style, borrowing techniques from several period mounters.

The major stumbling block to this endeavor is the availability of the top paper. Gift wrapping paper will do for the bottom but I could find no replicated top covering. Thinking it shouldn't be too difficult to design my own to capture the flavor of the era I present two designs, one art deco-ish and the other sort of Victorian/Art Nouveau. (Fig.8)

These designs were originally drawn 2" x 6" and reduced to make the

Figures 7 and 7a: Looks like a Leitz or late Olympus. No compatible artistic design contours to the Zeiss whatsoever.

lines very fine. What do you think? I am looking into having these crude renderings cleaned up and printed in gold or black on colored thin paper, perhaps black on gold paper? Still investigating what the cost may be.

So ends the hardware improvement update. I haven't had any mental software improvements since last, other than figuring out why the alphabet is in the order that it's in…..it's because of that song.

Take a break, stretch your feet, get a pizza or whatever. Stand by for part 2 bombast. Exclamations of awe and inspiration can be sent to me:

Joseph Wilhelm. Cheers.

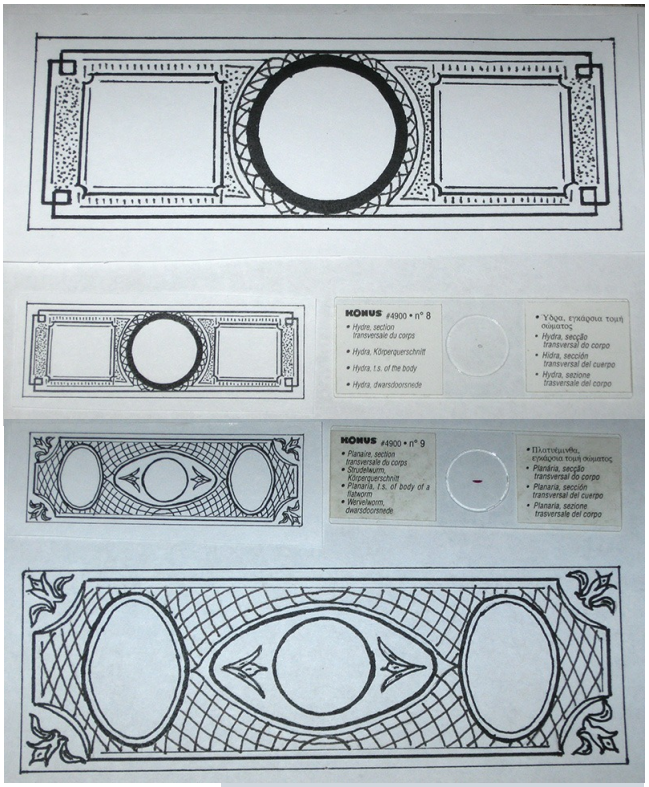


Figure 8 (top): Art Deco
Figure 8a: Art Nouveau / Victorian

Dissecting the Larva of a Beetle

Sometimes things are not as they appear. In this case we have a look at a suspected parasitic infection of a larvae, which turned out to be something quite different.

Rodney Brightwell

Over the years we sometimes maintained a compost pile, the organic material we placed into the compost pile. It gradually breaks down and eventually turns into an organic type of fertilizer that we use on our garden and our smaller trees and shrubs.

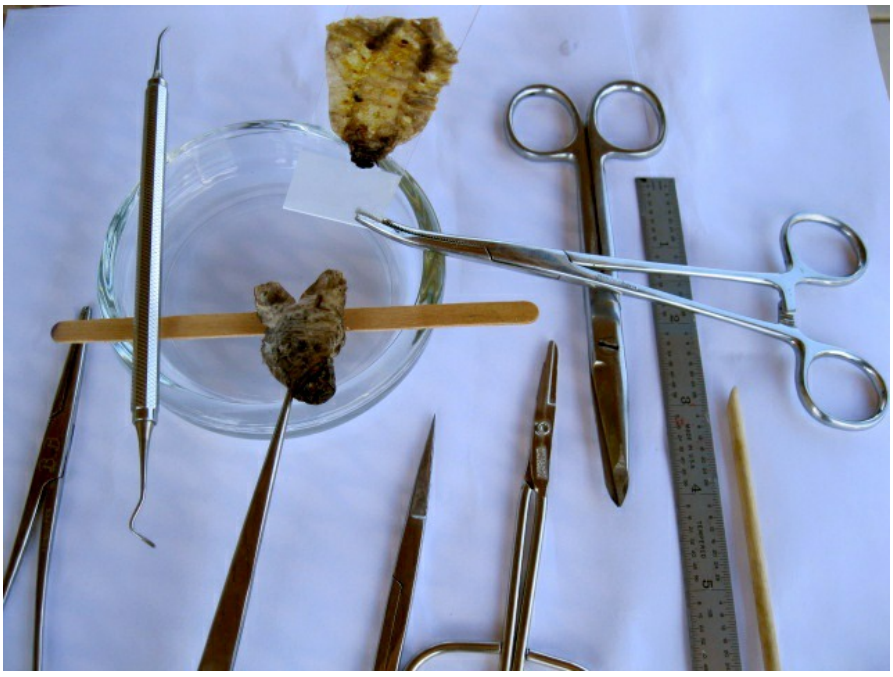
The Green June Beetles lays eggs into the compost and the larvae or grubs soon start to appear. I knew that certain parasites attack insect larvae by laying eggs into them. Parasitic larvae then start to feed on the host larva. I was indeed able to identify structures in the Green June Beetle larvae that look like small parasites. I therefore collected several of the beetle larva from the compost for some dissection and microscopic investigation. After careful examination, I concluded that the structures were not internal parasites, but rather the spiracles of the insect.

For this, I dissected a small area of tissue away from the suspected parasite while using the microscope. This was not an easy task.

Image credit: Stephen Friedt (CC-BY-SA-3.0)

Top: The Green June Beetle (*Cotinis nitida*) a member of the scarab beetles.

Middle and bottom: The larva is crawling around on its back when removed from the soil. Even though they have 6 little legs they will flip over and crawl on their backs. This specimen is about 2 inches (5 cm) long but may eventually die from a suspected parasitic infection.



Top: Disection of the Green June Beetle larvae *Cotinis nitida* with outer layer folded back on the microscope slide. The little black nodules are part of the suspected parasitic infection just inside of the outer layer of *Cotinis nitida* larva.

Bottom: Closer view after disection of *Cotinis nitida* larvae on slide.

After careful examination, research and observation and the dissection of 2 more beetle larvae, what I thought were internal parasites are not but abdominal spiracles that line each side of the abdomen lateral surface. These spiracles are part of a network for gas exchange through a system of tracheae. The spiracles generally have a closing mechanism which reduce water loss and act as a filter and is apparently related to oxygen and carbon dioxide build up in the blood. ■

Cotinis nitida

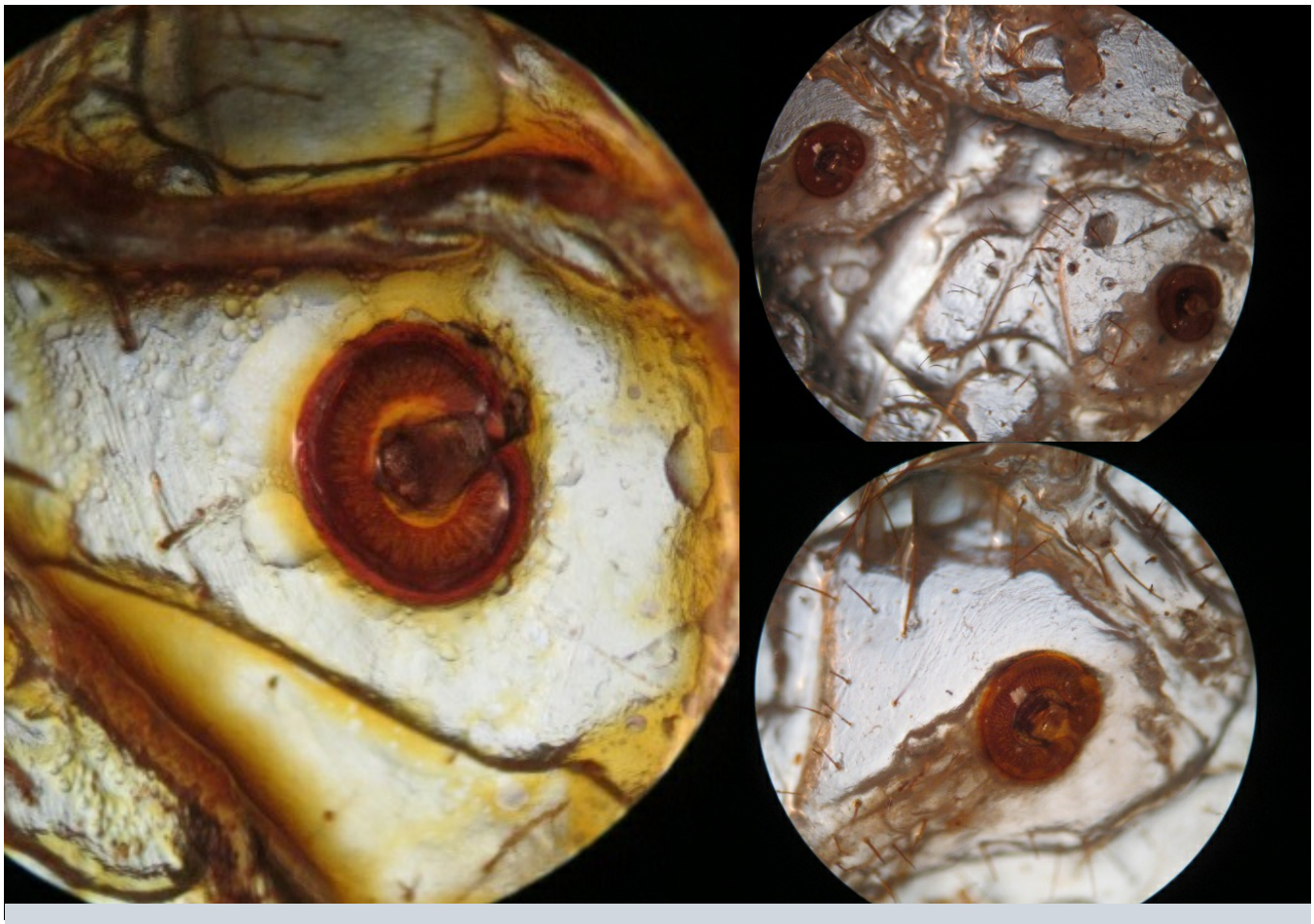
Cotinis nitida, also known as the green beetle, is a beetle of the family *Scarabaeidae*. It occurs in the southeastern part of the United States. It is not easily distinguished from the related southwestern species, *Cotinis mutabilis*.

The green beetle is active during daylight hours. The adult is usually 15–22 mm (0.59–0.87 in) long with dull, metallic green wings; its sides are gold and the head, legs and underside are very bright shiny green. Their habitat extends from Maine to Georgia, and as far west as Kansas, with possible population crossover in Texas with their western cousin, the figeater beetle.

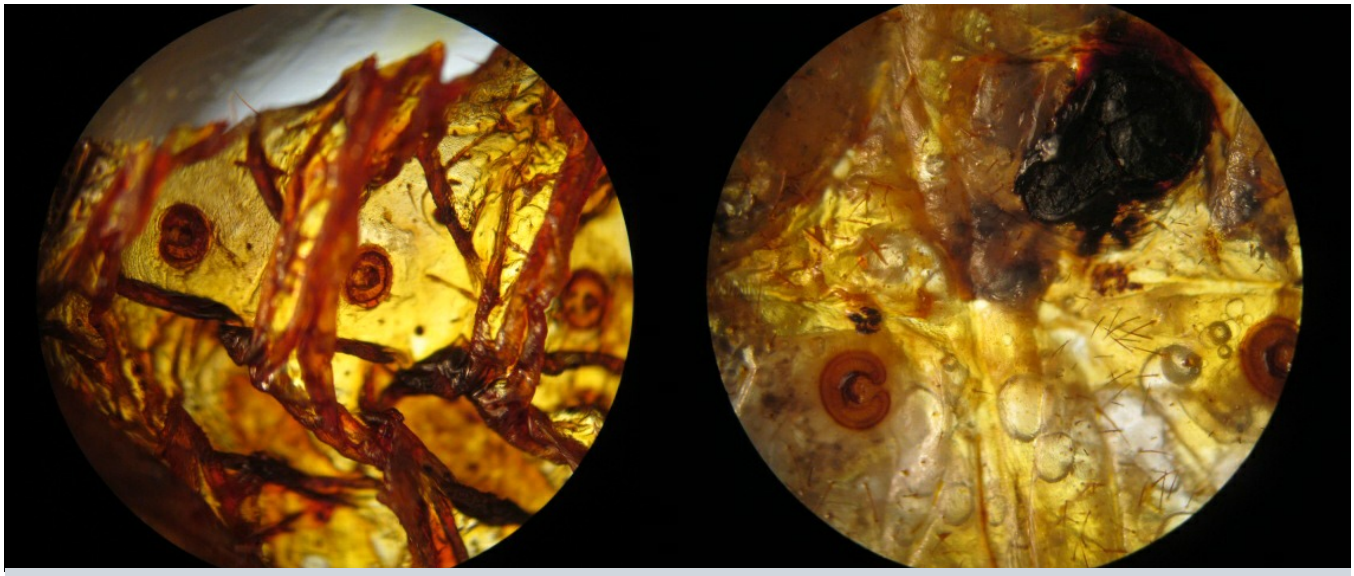
The complete life cycle for the green beetle is one year. Once the mating process has taken place, the female will lay between 60 and 75 eggs underground during a two week period. The eggs, when first laid, appear white and elliptical in shape, gradually becoming more spherical as the larvae develop. The eggs hatch in approximately 18 days into small, white grubs. The grubs will grow to about 40mm and appear to be white with a brownish-black head and brown spiracles along the sides of the body. The larvae will molt twice before winter. Pupation occurs after the third larval stage, which lasts nearly nine months. The adults begin to appear in June after 18 days of the pupation period.

The green beetle is harmless; however, the larvae are considered pests when they cause damage to lawns.

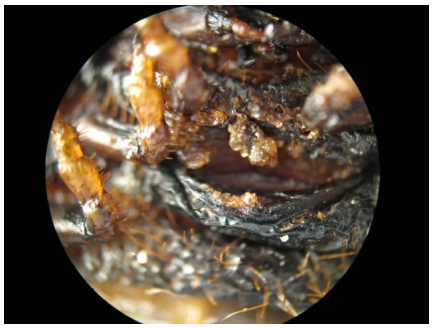
Reference: "Cotinis nitida - Wikipedia, the free encyclopedia." Wikipedia, the free encyclopedia. Web. 28 May 2011. <http://en.wikipedia.org/wiki/Cotinis_nitida>.

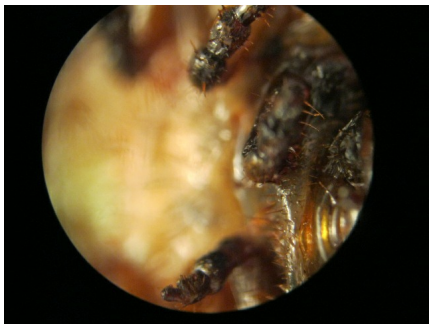


Top: 75x view of the spiracles of the June Beetle larva. The C-shaped structure seems to possess a tunnel. Initially I suspected that this tunnel could be the injection point where the parasitic fly or parasitic wasp deposited the egg into the host beetle larva. Later, the tunnel turned out to be part of the tracheal system of the insect.



Inside dissected view of the June Beetle larvae, *Cotinis nitida*. Note the C-shaped spiracles. There were initially thought to be parasites belonging to a parasitic wasp or fly of the order *Diptera*. Their regular arrangement and presence in all investigated larvae indicates that these structures can not be internal parasites.



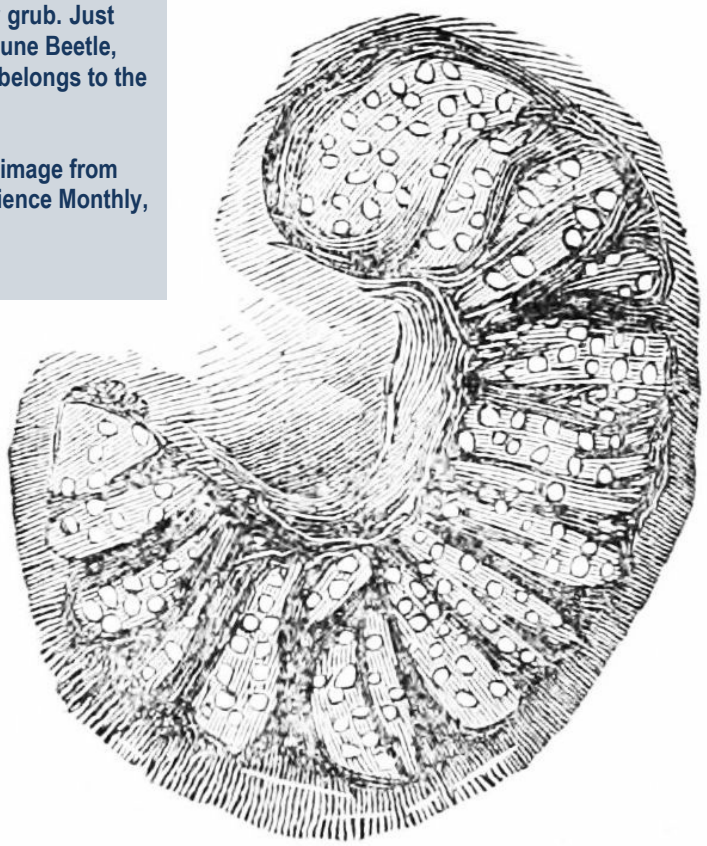


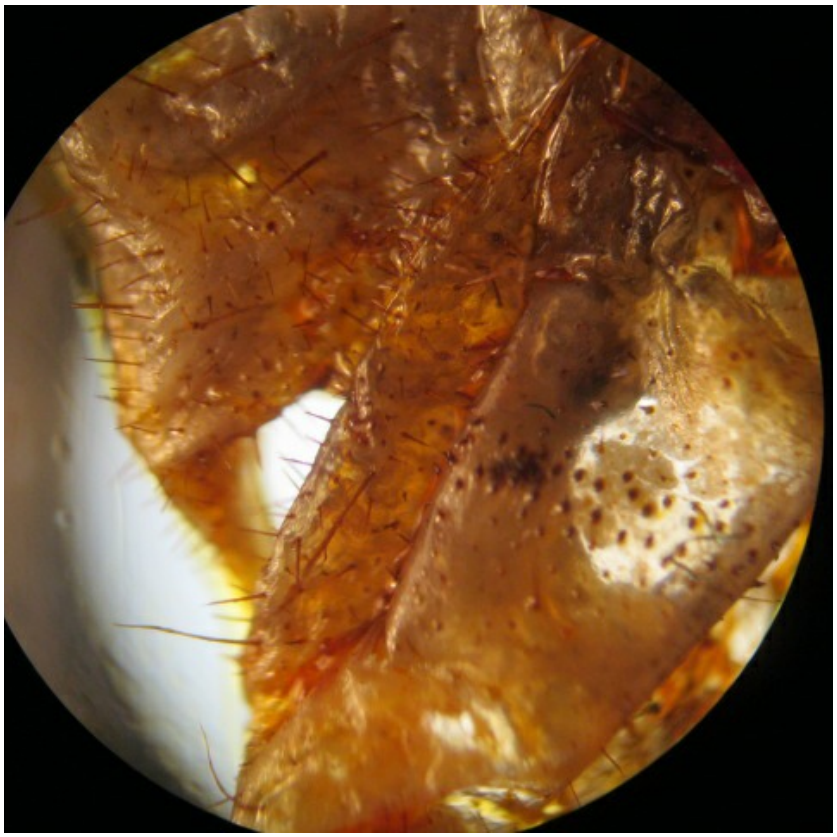
Top: Mouth and feeding parts of the Green June Beetle larvae, a silent underground eating machine (35x).

Bottom: Under the head area showing some of the legs.

Right: Drawing of the spiracle of a cockchafer grub. Just like the Green June Beetle, the cockchafer belongs to the scarab beetles.

(Public domain image from The Popular Science Monthly, 1881)





Left: 40x view of the June Beetle just below the head. Picture taken with a Bausch and Lomb Dynazoom and a Canon digital camera.

Top: Entomology setup, but with a 35mm Olympus camera hooked to the microscope.

Making a Microtome

Here we have a look at the construction of a microtome by modifying a micrometer screw gauge.

César Guazzaroni

I have been thinking about this project for some time now, but was only now able to tackle the project. I wanted to construct a microtome out of a micrometer measuring device. They are also known as micrometer screw gauges and are used to measure the thickness of various objects, such as sheets of metal. The one that I could obtain here in Argentina was manufactured in Taiwan and was only about $15 (figure 1). This price is so low that was worth giving the project a try. The measuring devices have a resolution of 10 microns. One complete rotation of the sleeve forwards the piston by 0.5mm. The gradations are 0.01mm (ten micrometers) apart.

Figure 2 shows the aluminium part which I made using a lathe. It is mounted to the micrometer with three screws. The large black screw is for fixing the paraffin block with the sample.

The flat surface should be polished to the smoothness of a mirror. This is the place where the where the barber's razor slides across. It is also possible to use a glass plate with a hole in the center of the right size.

Figure 1 (top): Here you can see the commercial micrometer (right) and aluminum machined part (left).

Figure 2 (bottom): The machined part, seen from the bottom, serves as the guide for the microtome knife.

Sample preparation

It is now time to test the new microtome. The goal was to embed the object to be microtomed in paraffin of the right consistency. Often we we want to see soft tissues, such as liver or other animal parts. These must be sufficiently supported. Tissue which moves like a pudding is impossible to cut into thin consistent sections.

The tissue must first be dehydrated by placing it into increasing concentrations of alcohol. You have to leave the tissue in the alcohol for sufficient time to allow the water to move out. At the end, the sample is placed into concentrated alcohol. Finally, the tissue is placed into xylene or toluene (be careful when using these solvents). This process causes the tissue to become miscible with the paraffin. Only samples that are thoroughly mixed with the paraffin can also be properly cut into thin sections.

Figure 5 shows the organs of a chicken. They are placed in a 10% formalin solution to fix and preserve them. If you want to dissect and preserve a whole rat, make sure that you inflate the lungs of the animal with the help of a syringe using the same solution.

Image 6 shows the test tubes with alcohol at different concentrations up to absolute alcohol. In this case I am working on a plant stem to see what the

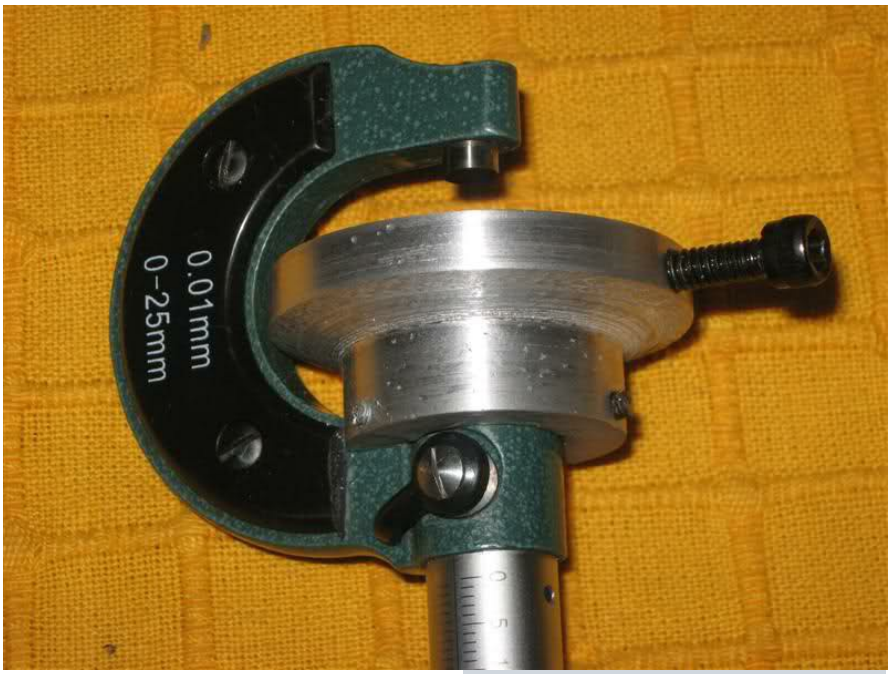
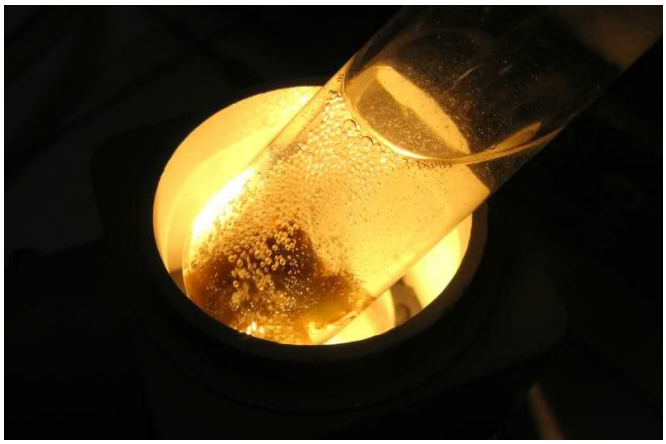


Figure 3: Side view of the finished microtome.

Figure 4: Enlarged view of the finished microtome.



results are. The jar on the right side contains xylene, the time each tube is 15 minutes, but the exact times may vary depending o nthe size of the sample.

The sample is then transferred from xylene into the paraffin. It is heated until the paraffin is liquid (figure 7). Be very careful here. The paraffin-xylene mixture is volatile and flammable. Point the tube away from yourself during the heating process and be sure that you do not overheat, otherwise it may spontaneously ignite. Leave the tissue about half an hour in the hot paraffin to allow it to enter the tissue completely.

It is now time to prepare the mold. Here I used the cap of a bottle and wrapped several layers of paper around it (figure 8). Any cover that fits into the socket of the microtome will do.

The specimen, in this case the stem of a plant and the paraffin are then poured into the mold. Cutting the paraffin is easier when it is very hard, and therefore it can be left in the freezer for an hour.

Figure 5 (top): Heart of a chicken, preserved in formalin.

Figure 6 (middle): Different concentrations of ethanol (test tubes) and Xylene (in jar).

Figure 7 (bottom left): Heating the specimen with the paraffin.

Figure 8 (bottom right): Making the mold out of a cap and paper strips.

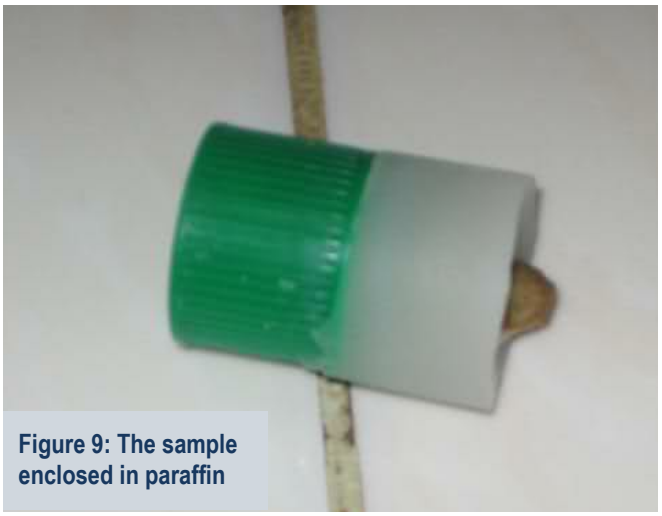


Figure 9: The sample enclosed in paraffin

Figure 10: The sample enclosed in paraffin

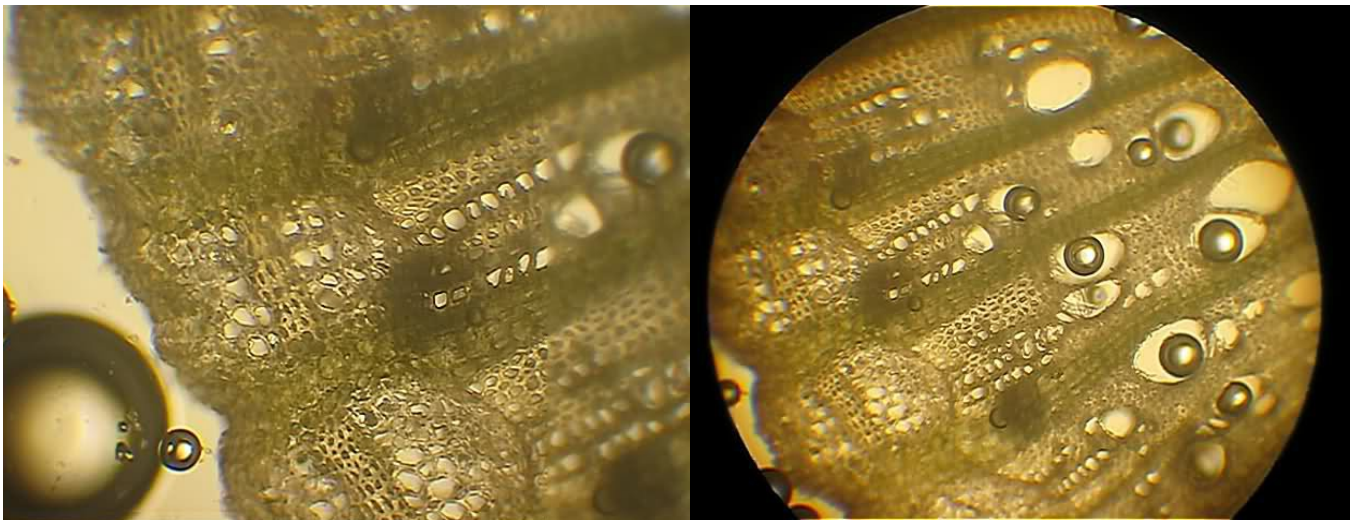
Figure 11: Microtoming a heart. The tip has to be removed first to make a flat surface.

I then placed a coin into the bottom of the receptacle, to ensure that the block is pushed evenly when the screw is turned. Figure 11 shows a block with a piece of heart to take some samples.

The knife is important. The type shown in figure 10 is very well suited getting pretty thin cuts of about 30 microns.

At the moment I do not have good color images of animal tissue sections. I therefore want to show you add some pictures of plant stems (figures 12 and 13) without prior staining. These pictures give a first impression. ■

Figure 12 and 13 (bottom): Plant stems sectioned with the home-made microtome.



Making a no-nonsense Slide Box. Or two. Plain and Simple.

Why buy a slide box when it is so easy to make one yourself? A little corrugated cardboard and wood will do the trick.

Oliver Kim

My slide boxes were filling up and it was time to order new ones. Who would have guessed that such a simple thing as a slide box could be so difficult (and expensive) to obtain? After all its just some paper and wood! After some searching on thee web I did discover a company selling slide boxes in various sizes and colors, even at reasonable prices, only to discover that they do not ship to private individuals. I was frustrated, and realized that instead of spending time looking for companies shipping cheap slide boxes could be also spent with making one from scratch.

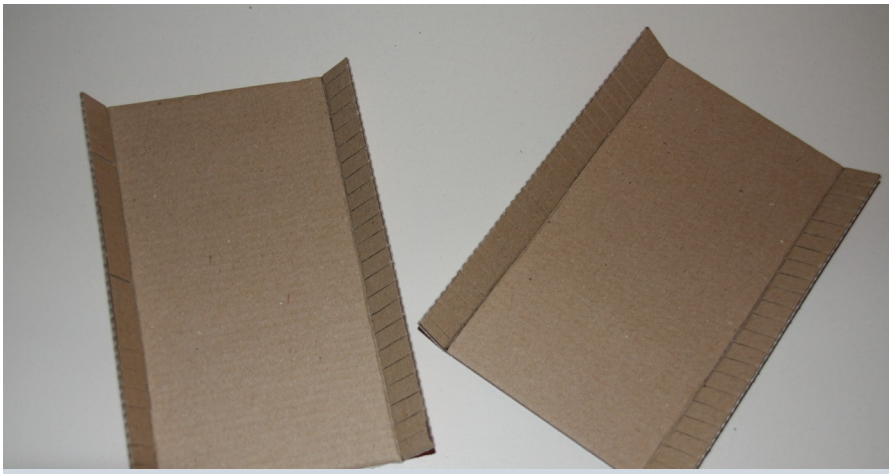


Figure 1: The slide trays. The base is 78mm wide and the sides are 15mm high. Cuts were made into the corrugated cardboard to help stabilize the spacers.

Box the first: the cardboard box

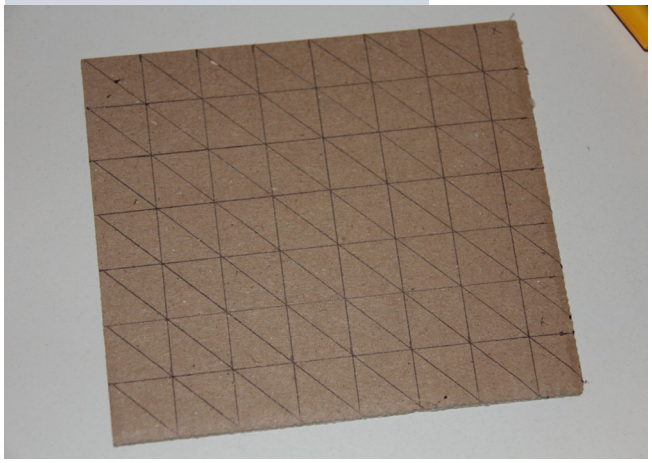
The slide box must fulfil several criteria:

- It must be easy to make and not require much assembling time
- It must be reasonably stable and offer protection
- It should be cheap to make
- It should be able to hold many slides.

I decided to use cardboard, the type which is used for packing mail ordered products. After a little research I found out that the correct name is "corrugated fiberboard" - one never stops learning. The one that I used was made of three layers, a central wavy layer sandwiched between two liners. The reason why I chose this material? I simply happened to have some of it around.

The most challenging and time consuming part was the making the trays with the spacers that hold the slides in place. I first made the slide trays and bent up the sides (image 1). When mea-

Figures 2, 3: Cutting out the spacers. These are triangles with 15mm sides.



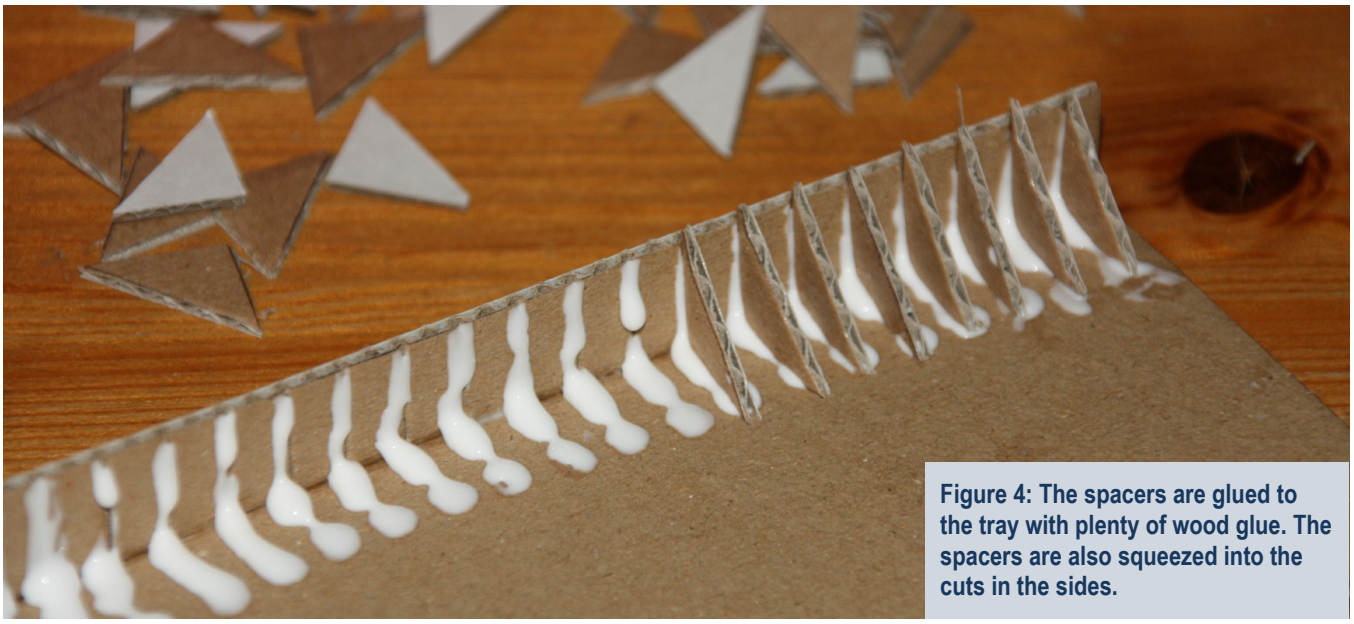


Figure 4: The spacers are glued to the tray with plenty of wood glue. The spacers are also squeezed into the cuts in the sides.

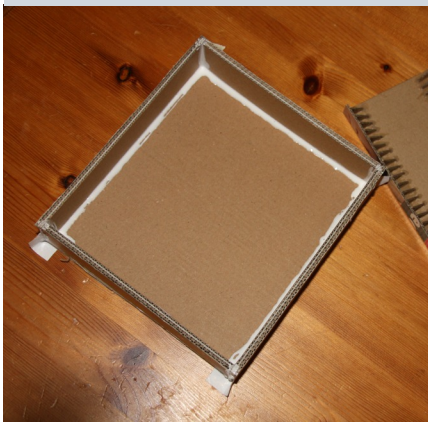
suring out the size of the trays, I made sure that the slides has have about 2mm of air to the left and the right. There is always a margin of uncertainty when folding the sides of the trays and I wanted to prevent the possibility of the tray becoming too narrow. I do not want to force the slides into the box. Using a sharp cutting knife, I halfway cut the sides of each tray at regular intervals. Into these slots I am then later going to glue the spacers. I bent the sides of the trays upwards and applied white glue to the fold to hold them in place. I then cut triangular pieces of cardboard as the spacers (fig. 2 and 3). These I glued to the sides of the trays with white glue (fig. 4). I also squeezed the spacers into the cuts, which I made. Additional support was not needed, because the sides of the trays were already stable. I additionally applied used glue into the fold. This was quite important.

After the tray was completed, it was time to move on and start making the main box. This was to be made of thicker cardboard, which I did not have. I therefore glued three layers of cardboard together, using plenty of white white wood glue. This sandwich had to be properly pressed in order to prevent warping. Three books did the trick. Do allow the moisture to escape, though! Otherwise the drying time is extended much more and the cardboard may warp.

Cutting the base and the sides of the box was the easy part (image 5 and 6) and gluing them together was also accomplished in 10 minutes. I was surprised by the enormous stability of the box. The thick cardboard walls also provided much area for the glue to adhere.

While this cardboard box did prove to be very functional, I was still not satisfied. The box simply did not look nice enough. The cardboard, while surprisingly strong and durable, simply did not radiate the degree of dignity that I expect from a slide box holding my little treasures. And for those of you who think that there is a "real reason": The real reason was, that I was now in my "experimental mode" and simply wanted to have an excuse for trying something new again…

Figure 5 (left): Putting the box together. The white glue will become transparent.
Figure 6 (right): Slide trays, box and slides happily united. I did not make a lid.



Box the second: the wood box

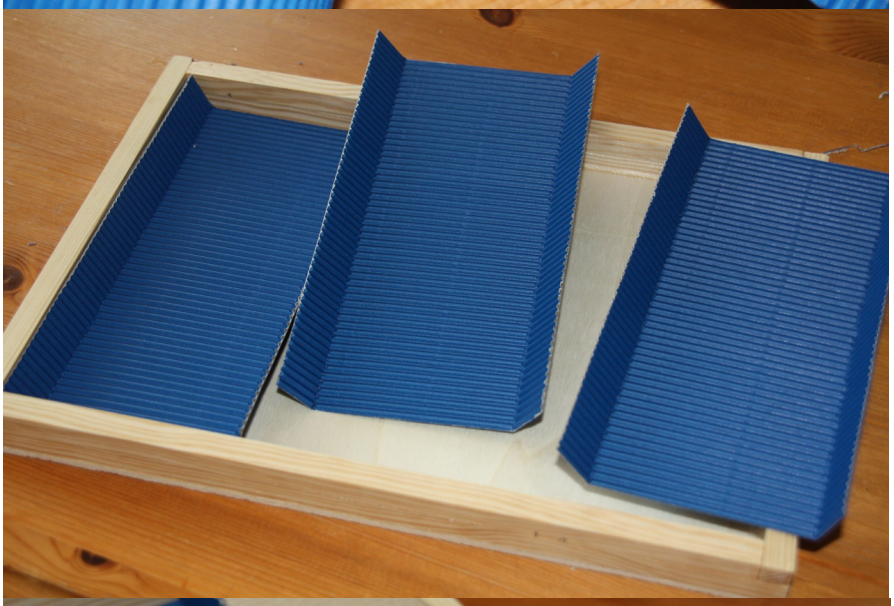
I now decided to give it another try and started to make a box out of wood. The slides were now held in place not by spacers, but rather by a strong corrugated cardboard, which had a lining only on one side (fig. 7). This I bought for a ridiculously low price in a local paper shop, and even could choose the color that I wanted. I chose blue, because I wanted to have a nice match with my blue paper-covered slides (read about this in the next issue!).

I bought some wood and started to cut out the pieces. Lazy as I was, I did not even measure out the correct size of the ply wood, but rather used it as it was. For this reason, I had some extra space left in the box, which I filled with some cardboard (fig. 9).

Making the trays

To be honest, I did not plan much. I first cut out the trays that hold the slides. Here, precision is very important. If there is too much play, then the slides will fall out. The base of the tray measured 7.7mm, which is 1mm more than the width of the slide. The sides I made 15 mm tall. Using a sharp knife and a ruler, I then made a careful cut into the corrugated cardboard to help me bend up the sides of the tray. The bending also decreases the usable width of the tray and the 1mm extra is again lost.

I then glued the sides of the tree trays together, using (how did you guess?) wood glue. The trays I simply placed loosely into the box. If the card-



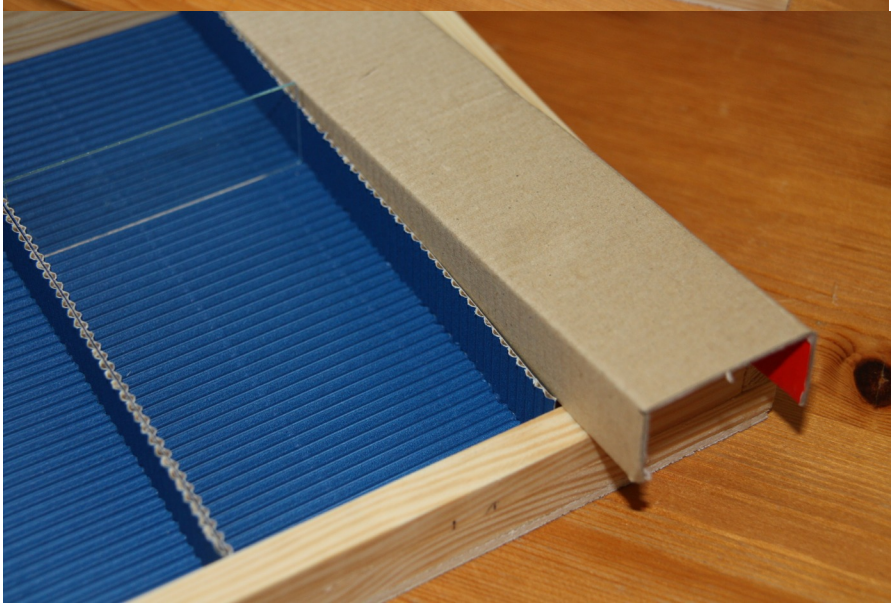


Figure 7 (top): corrugated cardboard for making the slide trays.

Figure 8 (middle): The trays with the sides folded upwards. The sides have the same height as the side of the wooden box.

Figure 9 (bottom): The box is too big. A cardboard spacer helps out. This spacer can also be used to push the sides of the trays inwards, so that the slides are held more tightly.

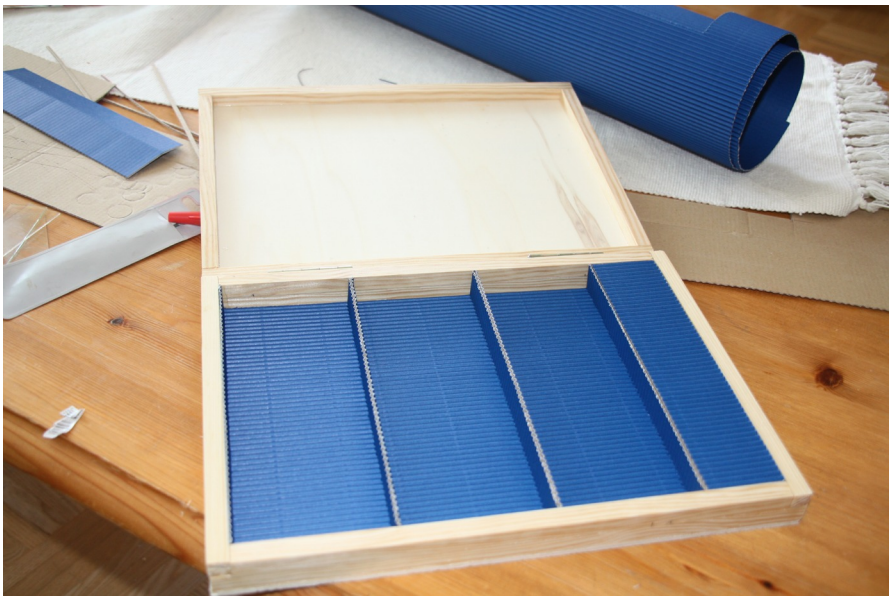


Figure 10: The nearly finished slide box. The box is able to hold 159 slides, 53 for each column.

board wears out due to heavy usage, then I can exchange it very easily . The corrugated cardboard trays themselves were already surprisingly strong and the wood box was more decorative and to protect from dust.

How I messed it up

I don't know what overcame me. But I think I was in my experimental mode again. I decided to cover the whole wooden box with black paper. I diluted some wood glue with water and used this to glue black fiber paper on the box with a paintbrush. I assumed that the fibers will give it a nice vintage-appeal. Now that everything has dried, I changed my mind and think that I probably should have left the natural wooden appearance of the box. Black simply looks too… dark. At least this is what my wife thinks. I therefore want to spare you the sight of the box and did not make a picture. But then again, do I not have some plywood left to make a third box?

Advantages and Disadvantages

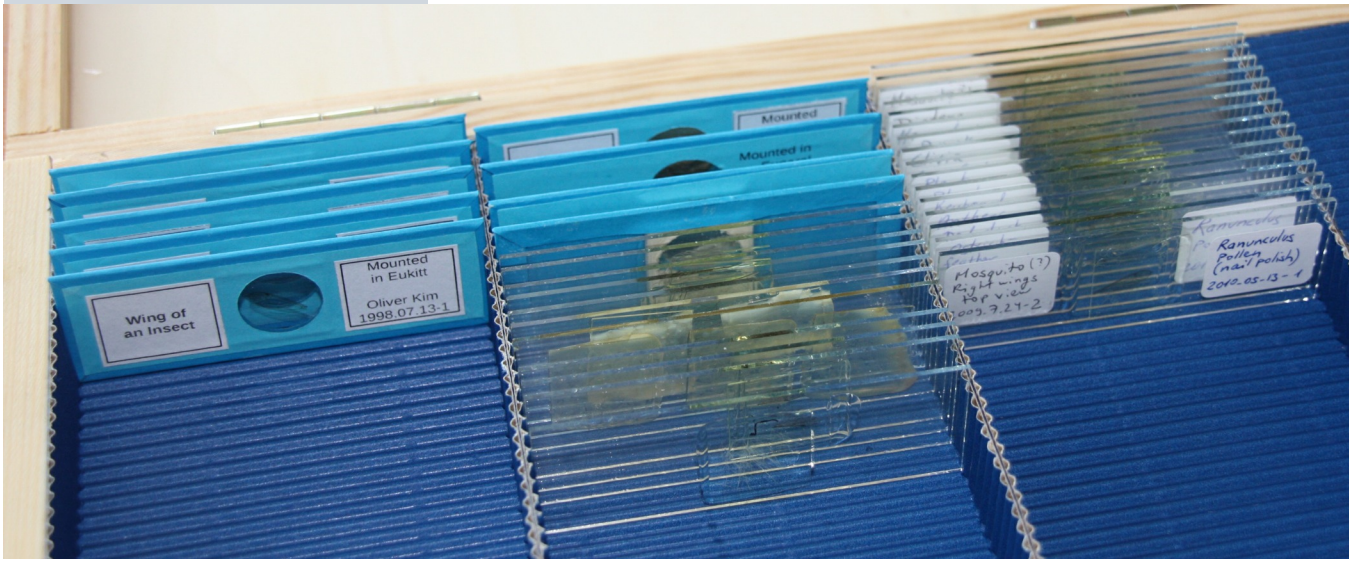
Using corrugated cardboard (my second attempt) to hold the slides has two big advantages: it's fast to make and many slides can be placed closely next to each other. The disadvantage is that much accuracy is needed to make the slide trays. One or two millimeters off, and the slides will either fall out or the trays will be too tight. It is possible to adjust the tightness by varying the angles of the sides of the trays, however.

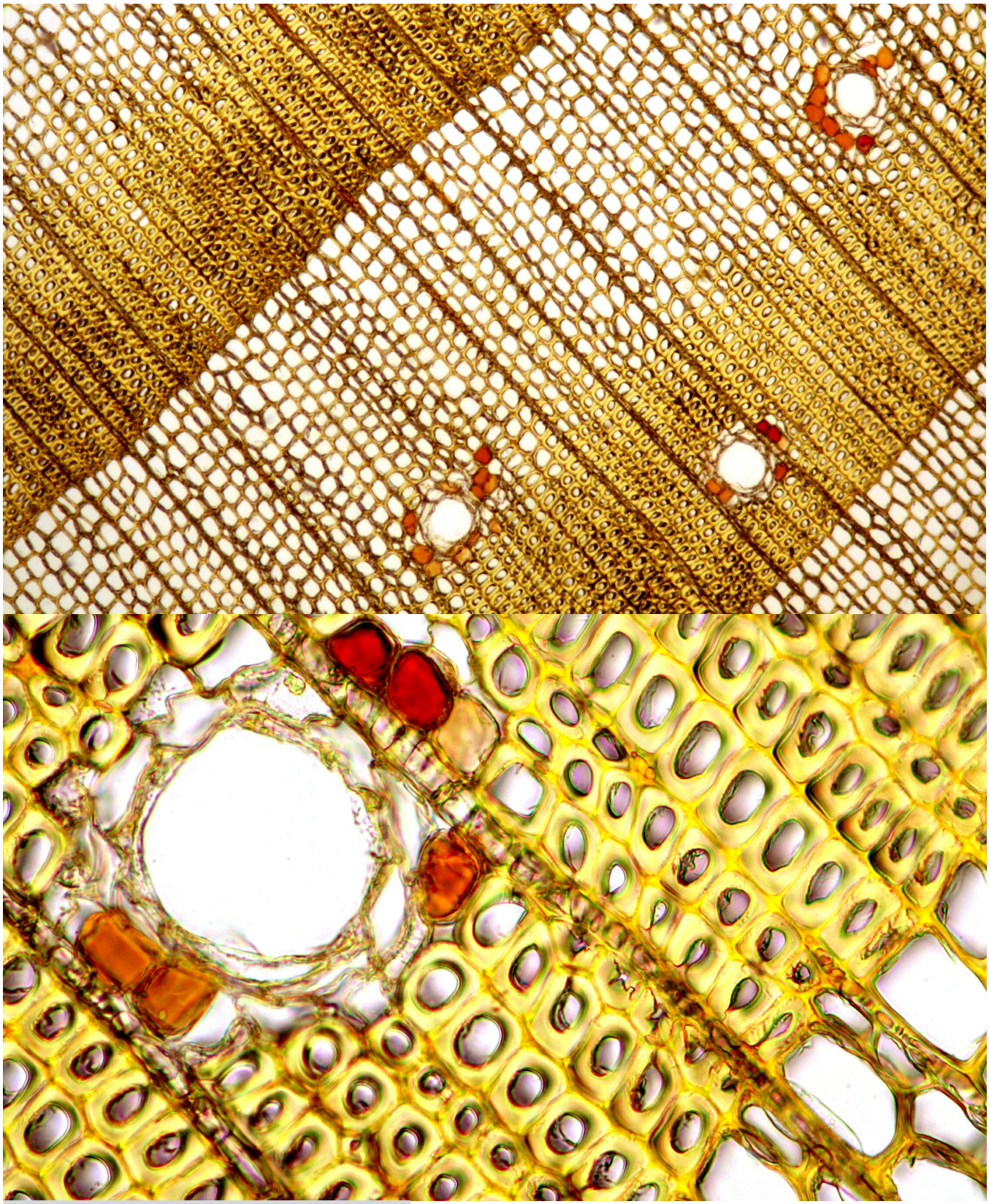
Gluing the spacers into the trays (my first attempt) is much more work, but the spacers will add quite a lot of stability. It may not even be necessary to make and extra external box. The cardboard spacers also extend much further and therefore it is possible to give the slides some extra air on the left and the right side. Inserting and removing the slides may therefore be easier. The disadvantage is, that it is quite difficult to glue many spacers densely together. The box is therefore not able to hold as many slides.

Now you try it!

Now it's your turn. Make a slide box and send a picture of it to the following address: editor@microbehunter.com. I will then include your picure in an upcoming issue. ■

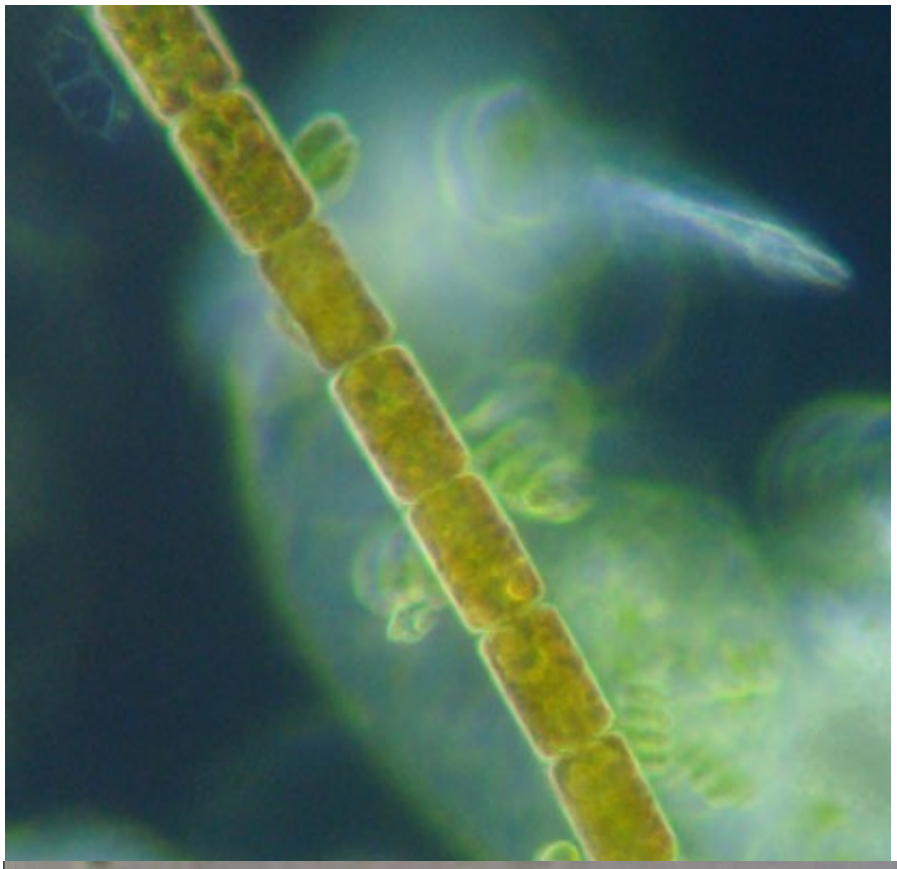
Figure 11: The finished box. The lid was attached with two hinges.





Cross section through spruce wood. The top image was taken with a 4x, the bottom image with 20x objective. Oliver Kim.

If you want to have your pictures published, send them to editor@microbehunter.com. The images will not be used for anything else.



The photo on the left is from a water sample from Muskegon Lake in Muskegon, Michigan. I used a Sony DSC-W80 held up to the eyepiece of my I-4 Infinity microscope. I was experimenting with darkfield and placed a quarter wrapped in aluminum foil over the condenser. The quarter wrapped in foil seemed to work best out of all of the things I tried. I have since bought a darkfield condenser, but this remains one of my favorite photos.

The photo below is from a water sample from a lagoon in Milwaukee, Wisconsin, taken with a Minivid camera attached to my I-4 Infinity.

Kimberly Pardieu

Measuring Distances in Micrographs

The free program GIMP provides tools that can be used to determine the pixel distance between two points. This information can then be used to calibrate the system to be able to measure the size of structures in micrometers.

Oliver Kim

It is possible to calculate the size of structures on a digital micrograph, provided that one knows the chip size and resolution of the camera, the magnification of the objective, magnification of the projection ocular and the distance of the camera system to the projection ocular. The math in involved is not really complicated, but I think that there are simply too many uncertainties involved. How am I to determine the distance of the camera's chip to the eyepiece correctly? To complicate matters more, I may have to use the eyepiece's exit pupil and not the glass surface for measurement.

For my taste, all of this was simply too confusing and I started to look for a more straight-forward method of determining the size of structures in micrographs. All you need is a transparent plastic ruler and the free program GIMP.

Taking a picture of the reference

First, place the ruler on the microscope stage and take a picture of two adjacent markings. In my case I took a picture of one millimeter (figure 2) with a 4x objective. This objective had a sufficiently large working distance and I therefore had no problems placing the ruler between stage and objective. If you want to be more precise, then I would advise you to use a hemocytometer or a calibration slide. Hemocytometers are used to determine cell density, but the gradations (the lines drawn) are much finer and possibly also more accurate than those of a plastic ruler.

Figure 1: The measure tool can be found in the GIMP toolbox or can be started by pressing SHIFT-M.

Calculate the number of pixels

I then used the *measure* tool in the program GIMP (figure 1) to measure the number of pixels that are needed to stretch this 1mm. At the given camera resolution and magnification, 1944 pixels were needed (figure 2).

The rest is quite easy. If there are 1944 pixels per mm, then a simple division will tell me the size of one pixel:

Size = 1mm / 1944px
= 0.0005mm / px
= 0.5µm / px

This value, 0.5µm/px, should now be remembered for the 4x objective and the given camera resolution. I now have the necessary information to calculate the micrometers per pixel for my other objectives as well, without having to take a photograph of the ruler (the ruler would not fit anyway under the objective). We now do some proportion calculations to determine the size of one pixel for the 10x, 20x 40x and 100x objective.

The 10x objective magnifies 2.5x more than the 4x objective therefore we have to divide the 0.5µm / px by 2.5 to produce 0.2 µm / px. The higher the magnification the less the size, we therefore have to use inverse proportions. I check my logics by doing a calculation:

(0.5µm/px) * 4 = X * 10
X = 0.2 µm / px

The values 4 and 10 in the equation refer to the objectives. All you have to do is replace the 10 with your desired objective and solve for X.

For the objectives I obtain:

4x:	0.5	µm / px
10x:	0.2	µm / px
20x:	0.1	µm / px
40x:	0.05	µm / px
100x:	0.02	µm / px

I think that I do not have to remind you, that these are the values that are for my own set-up. You have to determine these values for your own system.

The magnification of the objectives can also deviate and may be necessary to calibrate each objective separately. If this is the case, then use the 4x objective to determine the real size of a cell and then use this information to determine the true magnification of each objective. Simply measure the same cell using the different objectives and then calculate the objective magnification.

Measuring unknown objects

Now you can determine the length of any structure on the micrograph by

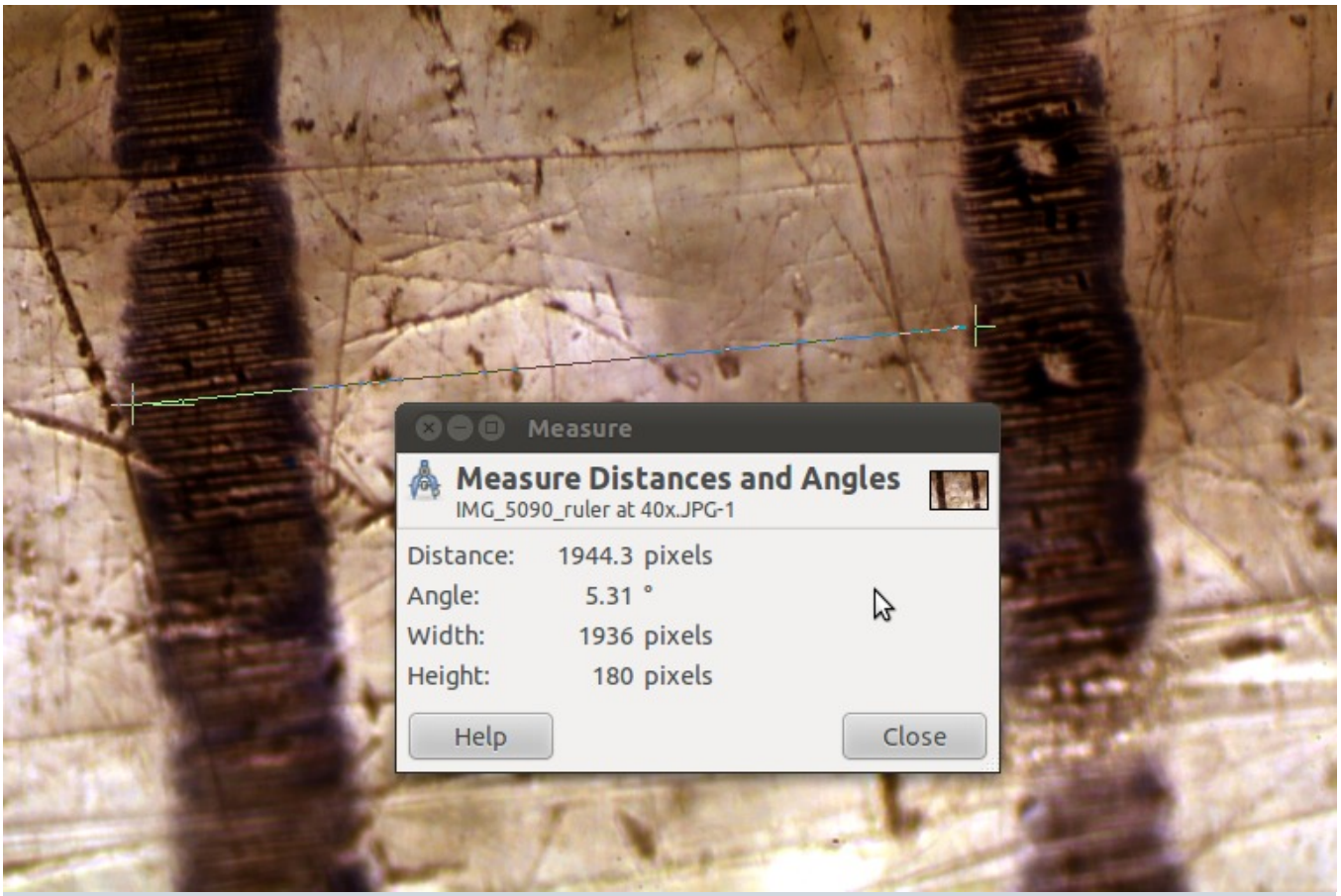
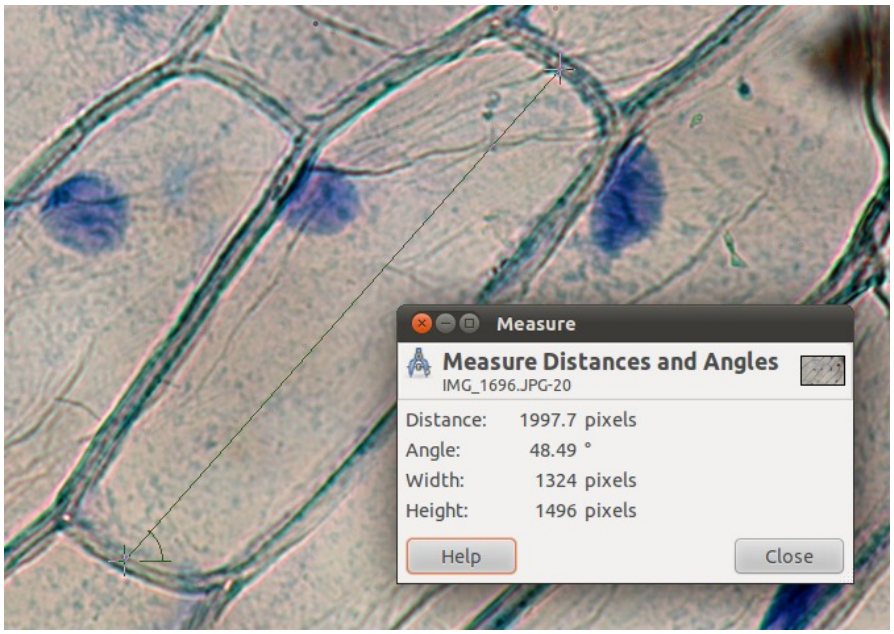


Figure 2: Measuring out the number of pixels required for 1mm. Both the status bar (right) and a window display the distance in pixels. The information box must be turned on separately, otherwise it is not visible. The display size (which currently shows 25%), has no influence. Make sure that you choose *px* as the units. Other units are available (even cm and mm), but these refer to the size of the image in relation to the computer screen and are therefore of no value to us.

Figure 3 (right): Measuring the size of an onion cell.

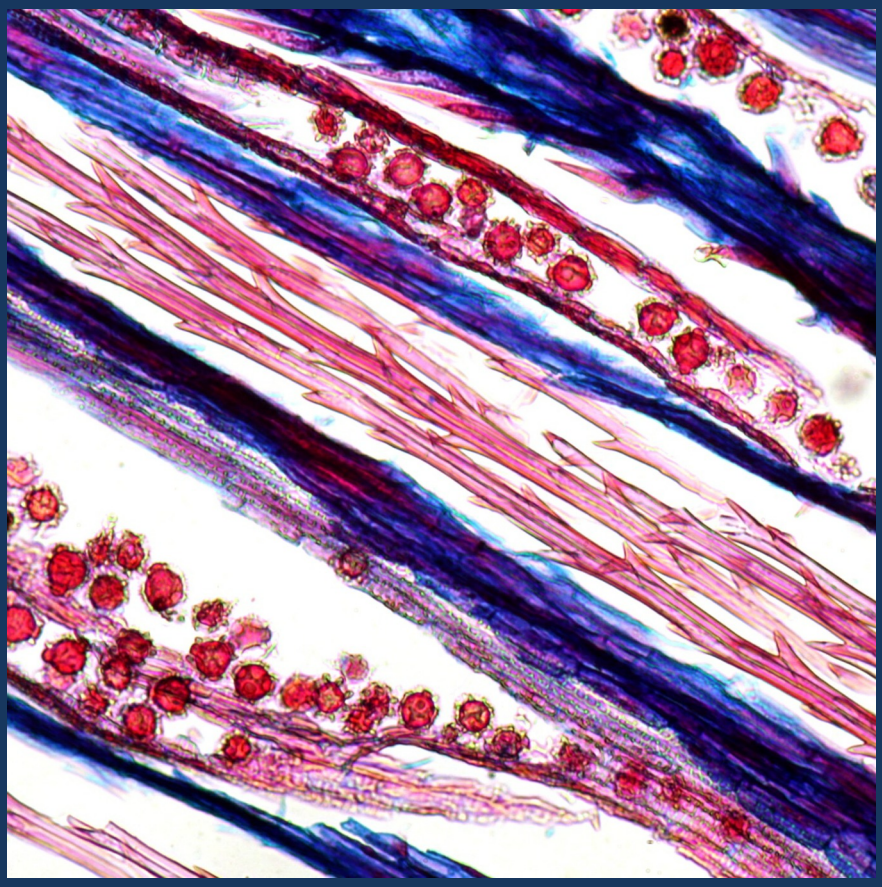


first measuring out the pixel distance and then multiplying this with the value that we just calculated. For example, if a cell stretches about 2000 pixels across (figure 3), using the 20x objective, then the real size calculates to:

2000px * 0.1 µm / px = 200µm

These are huge cells.

Where do we go from here? I think it would be a good idea to write a GIMP plugin, which directly provides a result in micrometers. All one has to do is to calibrate it once and then let the plugin handle all of the math. Anybody out there who knows how to do this? ■



What's this? Answer on page 3.