



Microbe Hunter

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The Magazine for the
Enthusiast Microscopist

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***A Centrifuge for
Microscopists***

***The Novitiate's
Odyssey: Episode
Three***

***Introducing: The
Hemocytometer***

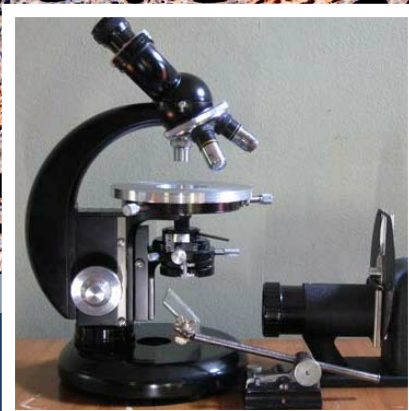
***A DIY Laser
Refractometer***

***Photomicrography
Course in Mexico***

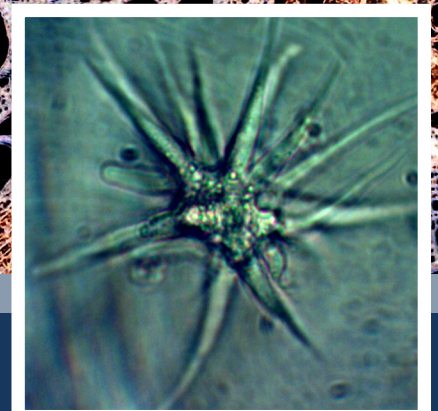
Parasitoid Wasps



DIY Centrifuge



Restoration



Photomicrography

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Front Cover:

large image: Oliver Kim (*Aristolochia* stem)

Left image: Robert F. Hancock;

Middle: Joseph Wilhelm;

Right: María Guadalupe Hernández Luna

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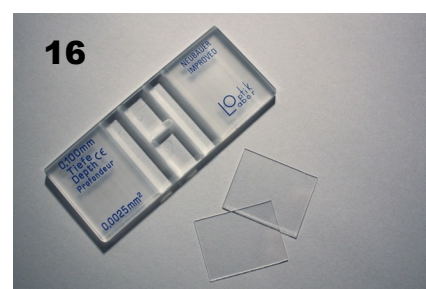
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Answer to the puzzle (back cover):
Banana (squeezed between cover
glass and slide, stained with ink)

EDITORIAL

We're now into the fourth month of publication and it even surprises myself on how many different approaches there are to amateur microscopy. In this issue you can read about the construction of centrifuge made of VCR components (page 4) as well as the construction of a refractometer (page 20), which can be used

to measure the refractive index of mounting media. Other amateurs are heavily engaged into metal working, which becomes useful when restoring antique microscopes (page 8). Placing a stronger emphasis on the observation part of microscopy, there is an article with contributions from high school students from Mexico (page 24 and 28), which docu-

ments beautifully how stereo microscopes can be used in an educational setting. One never stops learning: or did you know that some wasps lay eggs into living aphids in order to reproduce? In any case, I hope that you enjoy reading this month's issue as much as I did.

A Simple Centrifuge for Microscopists

In many cases it can be useful to enrich microorganisms that can be found in a water sample. Commercial centrifuges can be expensive, why not try to make your own?

Robert F. Hancock

A centrifuge is a very useful piece of equipment for isolating aquatic organisms. Centrifuging a sample of water containing microorganisms will drive them to the bottom of the container, where they become more concentrated and easier to manipulate. A high speed laboratory model costing several hundreds of dollars isn't necessary for the purpose, something much simpler will do the job quite adequately. At the most basic, you can just tie a piece of cord *securely* to the container and whirl it around your head. You can easily generate a centrifugal force of 10G with this method (but do it outside in an open area, where nothing will get damaged if it comes loose!)

A safer and more convenient solution is a motor driven apparatus, such as the one I built using parts from an old video recorder. It cost me only a couple of dollars for the plastic bowl housing the rotor head and some PVC pipe fittings, all the other parts were obtained from my "junk collection" of defunct VCRs and other "dead" electronic gadgets. If you don't have a dead VCR lying around, just ask a few friends and you are bound to find someone who will give you one. The parts required are the video recorder spinning head mechanism, plus the video cassette loading motor with its drive pulleys. Some newer VCR models may not have belt drives, a good alternative source for these components is an old inkjet printer, but these usually have stepper mo-



Fig. 1 (top): VCR rotor top view
Fig. 2 (bottom): VCR rotor bottom view



Fig. 3 (top): Rotor with flywheel removed
 Fig. 4 (bottom): Disassembled rotor



tors which require a special driver circuit to power them. If you can find an ordinary DC motor which will fit the pulleys it is much simpler than trying to get the stepper motor working. You will also need a 12 Volt DC plug pack from an old portable phone charger or similar device. It should be capable of supplying at least 400 milliamps, otherwise it will probably burn out before long. If you need to, you can buy a new one for about \$15, but these are very common items found in garage sales for only one or two dollars.

The VCR parts were extracted along with their mounting brackets and screws, keeping the PCB with the cassette motor drive connections intact. The wiring to the video head rotor is not needed and can be disconnected. Figs 1 and 2 show the video head as removed from above and below, Figs 3 and 4 show the disassembled rotor components, and Figs 6 and 7 the reassembled configuration. The first step is to remove any superfluous bits from the video head rotor, including the PCB and the stator windings underneath (Fig 3).

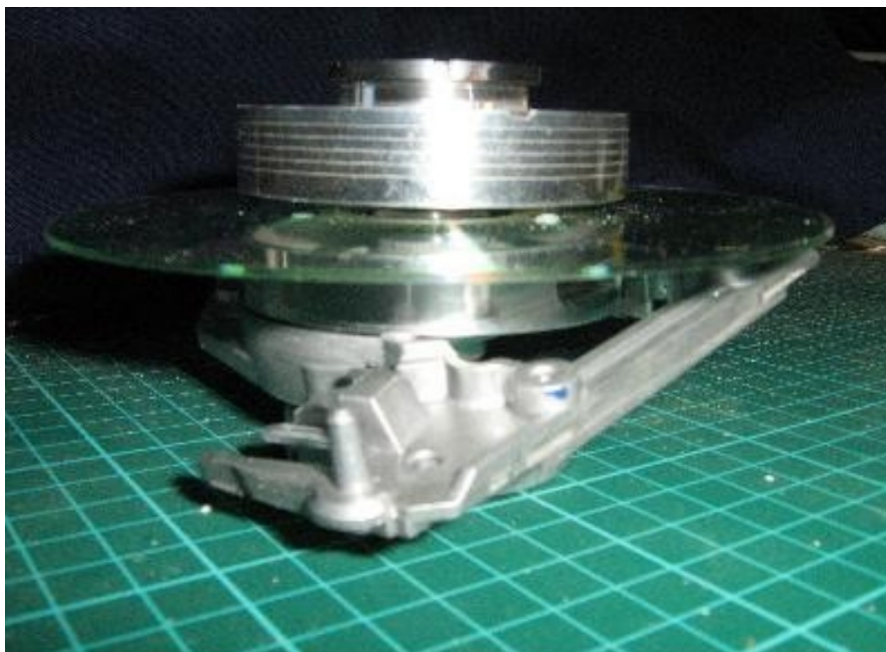
You need to take off the flywheel with the magnetic ring to get at the inside of the rotor. At this stage, prepare an old CD by cutting out the centre section to fit over the bottom section of the rotor. Drill 3 small holes in the CD and the bottom rotor section to take small self tapping screws and fix the CD to the bottom rotor section as shown in Fig 5.

The two halves of the rotor are separated and the top section refitted upside down, as shown in Figs 6. The magnetic sensor heads and their wires can also be discarded. Save the screws from the underside (which is now the upper side) of the top rotor section. Replace the flywheel also in the reverse orientation to its original position. You should now have an assembly which looks like Fig 7, seen with the assembly turned upside down.

Depending on the model of VCR, you should have a mounting frame attached to the video head rotor with 3 or 4 screw fixing points. You will need to make up a base with some brackets or posts to allow the rotor to be mounted in a horizontal position. In the video recorder, the rotors are mounted at an angle to the horizontal to allow the tape



Fig 5 (top): CD attached to rotor
Fig 6 (bottom): Reassembled rotor and CD



to be scanned diagonally, so the mounting points will usually need to be at varying heights to get the rotor level when it is used as a centrifuge. Try to get it as accurately level as possible, otherwise your centrifuge will wobble wildly when it is running. I used mdf board for the base and two posts, and a metal bracket for the third mounting. You can use some washers under the mounting frames to adjust the level accurately with a spirit level / bubble level.

When the rotor assembly is mounted on the base board, the drive motor and its pulley will need to be located so that

the drive belt runs around the detachable collet just above the reversed flywheel. The driving pulley and collet must be aligned in the same plane, otherwise the belt will be thrown off when the motor starts. Since the drive motor and pulley configuration will vary greatly between different models of VCR, I will not provide any specific details for this step, you will have to figure it out to suit whatever you are using. Fig 8 below shows how I arranged mine. By the way, if you don't have a drive belt of suitable length, a strong rubber band will also work.

The next step is to cut a hole in the base of the centrifuge bowl to fit over the rotor, and then fix it to the CD underneath with some small screws. Figs 9 & 10 show the bowl fitted in place. Make sure the bowl is accurately centred so that the spinning tubes can not touch the sides of the bowl. (Of course, you need to get a bowl of the right diameter to accommodate your centrifuge tubes in the first place. I used a plastic microwave cooking cover)

To complete the centrifuge you will need to add the trunnions and rings to carry your centrifuge tubes. The tubes I use are 100 mm x 12 mm diameter, so I made two tube carriers from black plastic irrigation tubing and fitted them with trunnions as illustrated in Fig 11. The trunnions are formed from a threaded PVC collar and two self tapping screws. A piece of foam rubber is pushed into the bottom of the tube carriers to cushion the glass tubes, and another screw is inserted across the bottom to hold the tubes in place. The aluminium bracket screwed to the top of the rotor has holes for the ends of the trunnion screws, as seen in Fig 12.

To use the centrifuge, I fill the centrifuge tubes to about 15 mm from the top with the liquid, making the levels in each tube as near to equal as possible. They are inserted into the carrier tubes and a piece of thin mdf board is placed on top of the bowl as a lid, which helps to reduce the wind resistance. The centrifuge is run for about 5 minutes at 250 rpm, which generates a force of about 6 G. It will need to be clamped to the work bench so that it doesn't run around and develop uncontrollable vibrations.

Should anyone wish to have a variable speed version, the simplest method is to buy a 12V LED light dimmer control. These are available on eBay for under \$10. I have tried one of these and it works perfectly. ■



Fig. 7: Reassembled rotor, bottom view

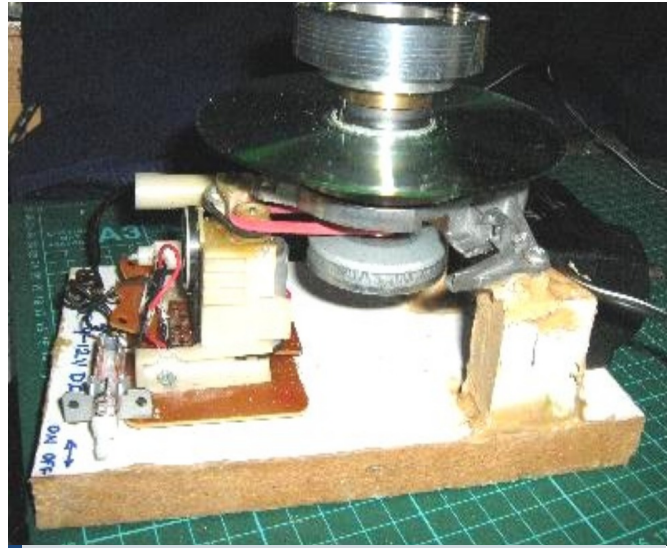


Fig. 8: Drive motor, pulley and belt.



Fig. 9: Top view of centrifuge bowl



Fig. 10: Side view, bowl in place.



Fig. 11: Tube carriers and trunnions



Fig. 12: Completed centrifuge

The Novitiate's Odyssey

Episode Three: A few words on creation

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

Experiences and lessons of restoring antique microscopes.

"Tubby or not tubby; that is the question, pilgrim."
(John Wayne reciting Shakespeare, Hamlet 3/1 - Key West)

G. Joseph Wilhelm,
Florida Keys, USA

Greetings once again from the land where the average fellow has more fishing rods than pairs of pants and if you put twenty of the local girls in one room you have almost a full set of teeth. By the time you read this our holiday season will have come and gone. Our annual late October bacchanalian debacle of debauchery is over (the Key West version of Mardi Gras called "Fantasy Fest") where morality and good judgment take the back seat with Johnnie Walker and Anhauser Busch. The 50,000 people who parade our streets in body paint sans most of their habiliments are gone and life is back to as normal as it will ever get here. Halloween, Thanksgiving, my wife's birthday, Christmas and New Year's Day closely followed this celebrious activity. Whew! Being full on into the winter season here the temperature has plummeted (It got so cold last night I had to turn off the air conditioner), the snowbirds have arrived and I now find some time to resume my pen to paper efforts.

Update: There has been a noticeable course of recurrent personal illumination, every week or so, that I have learned all there is to know of microscopy. The inevitable subsequent revelation that I have had inadequate tutelage leads me to suspicion the generally accepted adage that "You can't teach a new dog old tricks." Also, my inability to effectively control the eBay addiction



Fig. 1: The Zeiss GFL

has led to the acquisition of a legion of microscopically relevant accessories as well as a dramatic increase in my concourse of cameras with associated devices/adaptors allowing interface with my microscopes. Accordingly reinforced, I venture now to illumine the pursuance of a binocular microscope suitably compatible with my idiosyncratic tendencies and the procurement

of same, which will be based upon the sound principles of déjà vu, reincarnation and the fact that inanimate objects speak to me.

Allow me to explain (Please be advised to reread paragraph five of episode one and re-apply any of the aforementioned descriptors), but first



Fig. 2 (top): The arm as received, nothing seized and all functions working very smoothly.

Fig. 3 (bottom): The base. To get the proper thickness I had to laminate together a piece of 3/4" and 1/2" thick by 10" wide bar stock.



turn off the Twilight Zone theme music, it's in your head, I can hear it.

As far back as my memory serves me I have always found the world around me quite fascinating in general, but there were instances that evoked a reaction far above the norm of juvenile interest and curiosity. These occurrences were quite commonplace and summoned forth a considerable depth of emotion. It wasn't until later in life I began to notice a pattern and was able to identify the feeling. As a young lad, visiting the Cincinnati Art Museum, I peered into one of the late 19th century

restored room displays and was immediately transfixed by the roll top desk there. This desk elicited strong feelings of familiarity, comfort, warmth and what I can only describe as a tactile sensation of homesickness. The same scenario was oft repeated with different objects as the initiating stimulus and varying types and degrees of sentiments, sometimes including anxiety. The familiarity was always present. These occurrences became so routine as to be without noteworthy consequence to me. I assumed everyone experienced them.

Similar above related instances have taken place throughout my life and seem to be associated with an era between the early 1880s and late 1940s. This, along with the conviction that I couldn't have reached my current state of comfortable maladjustment in a single lifetime gives forth the presumption that I may have been here before and while I cannot associate it with an individual I can certainly place it in a particular timeframe. This also seems to explain my affinity with objects from this period that compromise 99% of my collections. How this relates to a microscope selection will be revealed shortly.

The Spencer stereoscope restoration related in the previous episode has rewarded me with some magnificently wondrous hours of entertainment and satisfaction. This has prepared me for the next step into seriously microscopically enabled observation.

I now was desirous of a quality binocular instrument as modern and consistent with my collections as possible which meant I would be looking at those stands I considered as a "bridge" between my vintage scopes and the harsh angular design of the modern instruments of the 20th century's later years. The "top four" manufacturers with the following requirements were considered:

1. It had to be black. This may seem an irrational parameter at first, but irrational fits me very well. All of the grey /gray (which is really correct?) and white microscopes fall outside of my collection era comfort zone.

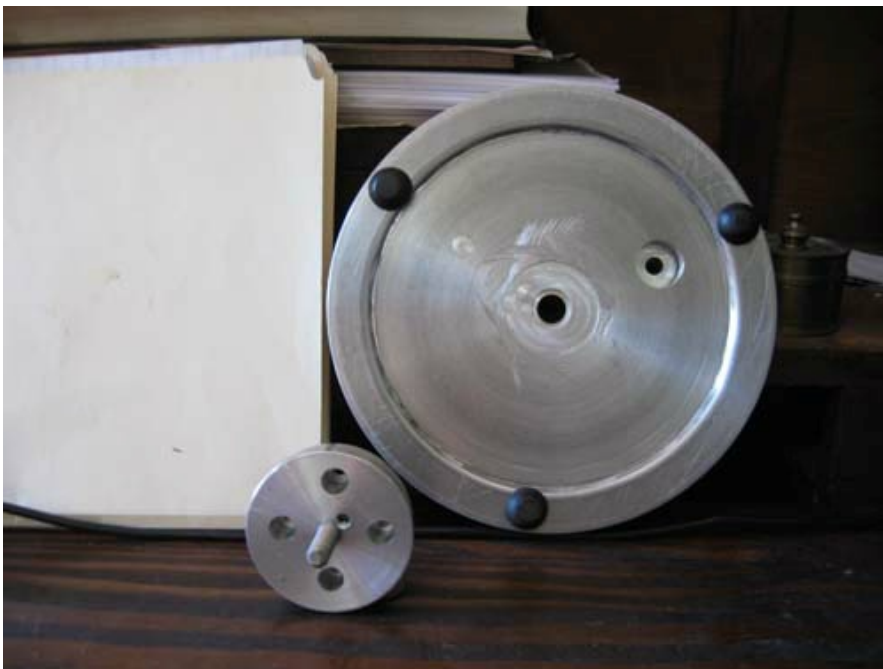
2. It should have the 160mm tube length because most of the (affordable) after market objectives were within this specification.

3. The design had to be aesthetically pleasing as per the above-cited era.

4. Parts/accessories as used items should be readily available as upgrades.



Fig. 4 and 5: The arm interface. I took a 3" diameter "plug" cut with a hole saw from a 1" thick piece of aluminum plate.



5. The binocular head needed to be on a rotating 360 deg dovetail mount so as to view from behind or in front of the arm.

I basically used eBay to research the availability, price etc. of the Leitz/Leica, Olympus, Nikon and Zeiss stands that fit my paramount specifications. Leitz almost had me with the early Dialux, Laborlux and the Ortholux as depicted in Gregor and Normand Overney's excellent article, but the 170mm tube length design, while still able to accept the 160mm objectives dictated that the correct match up of objective/eyepiece could be a problem.

The Olympus BH stands were close but black ones and parts seemed scarce and even more so for the early Nikons.

And then, browsing the Molecular Expressions microscope museum, I was stricken,

"But soft, what light through yonder window breaks? It is the east and the GFL65 8-56 4 is the sun." (Romeo and Zeiss, Act 2 Scene II, Capulet's laboratory.)

I was in love! The Zeiss GFL was everything I was looking for, beauty, style, grace, versatility, power steering and disc brakes, functionality and avail-

ability. And yes, it spoke to me with a familiarity of its wonderfully executed art deco spherical design and a comfort from what I instinctively knew was precise engineering. This definitely was the binocular scope I had to have.

Entering "Zeiss microscope" in eBay netted four pages of listings and there were several GFL scopes there, but there was a problem, they all suffered severe cases of dismemberment. Like a Corvette in a chop shop, these scopes were obviously more valuable sold off for their individual parts than as a whole. Even the most complete scope still lacked a substage condenser, oculars, lenses, illuminator, and was still commanding a price of over \$1200.00! Did I really want to get into this?

Of course I did. I was determined to play Dr. Frankenstein and assemble a living, breathing GFL from parts, the final assemblage customized to my specifications, and do it for less than the cost of a complete scope. My goal was under \$800.00.

The process began with the purchase of the GFL arm with quadruple objective turret and substage condenser holder (see fig 2) Previous arms like this with a base but no condenser holder were going for about \$150. Somehow I got this one for \$79 and from the same seller I got the binocular head for \$75. Still needed were the base, condenser, stage, illuminator assembly, eyepieces and objectives.

The 1.3 NA Zeiss condensers were going for about \$70 to \$100. I saw a \$39 "buy it now" offering but the description said it had a small scratch. After a day I inquired about how bad the scratch on the lens was and was informed the scratch was not on the lens but on the chrome on the flip out mount. I now had a condenser and the lens was perfect.

The base was the next logical step so I could stand the darn thing up. It also seemed the most difficult. There was an extensive truancy on eBay of this fundamental foundation for the GFL, at least one that was not attached to another arm or an almost complete micro-



Fig. 6 and 7: The arm interface mounted to the base.



scope. In fact, all of the other remaining items I required (rotating, centerable circular stage, light source and objectives) were selling at such premium prices I stopped to re-think this endeavor.

I consulted Captain Morgan and lapsed into deep contemplation and thoughtful analysis and after three or four seconds had my solution. Except for the objectives, I could manufacture everything I needed in my shop. This would be an excellent opportunity to expand the lighting capabilities and versatility of the scope in general. The final

product however, must be commensurate with the Zeiss level of quality. That was a tall order.

The base is complete and the stage about 90% done. For those who may be interested, the rest of the article goes into some detailed description and photos on how it was accomplished.

The Base

The original Zeiss base is a cast, hollow round section of a sphere with the light source built in. The arm attachment with four small screws and does not

allow for any inclination of the entire scope. We are about to change all that.

The scrap bin at my work site provided all the required material, which is 6061-T6 aluminum. We use this stuff for a lot of fabrication, has a structural strength approaching mild steel and has excellent machining properties. My base was going to be solid aluminum and a larger diameter than the original thus giving more weight and stability. The arm would be attached via a fairly quick release interface that would also give more clearance below the substage condenser and rack mechanism for insertion of as yet to be determined filters, mirrors, prisms, diffusers etc.

To get the proper thickness I had to laminate together a piece of $\frac{3}{4}$ " and $\frac{1}{2}$ " thick by 10" wide bar stock. The Navy Research Lab provided me with an epoxy that was stronger than the aluminum and had the same coefficient of expansion. This was trimmed with a saw to a roughly circular shape (see Fig 3), a center hole drilled for a bolt and then chucked in my lathe. Outer diameter was turned round and both sides of the disc were faced so as to be parallel and the final touch was to round the base upper edge to approximate the Zeiss design. This whole process took about 8 hours over two days. (I was working at the size limit for my small lathe and had to make numerous light cuts.)

Next was the arm interface. I took a 3" diameter "plug" cut with a hole saw from a 1" thick piece of aluminum plate, center bored, threaded and counter sunk a hole for a flat head screw, and turned it down to just slightly larger than the arm base mounting surface. The tricky part here was to accurately transfer the arm attachment screw hole pattern to the interface, drill and counter bore the hole from the bottom of the interface to match the shell thickness of the original base so I could use the original screws. The last was to lock in the large flathead screw with a drilled and tapped hole for a setscrew flush with the top of the interface.

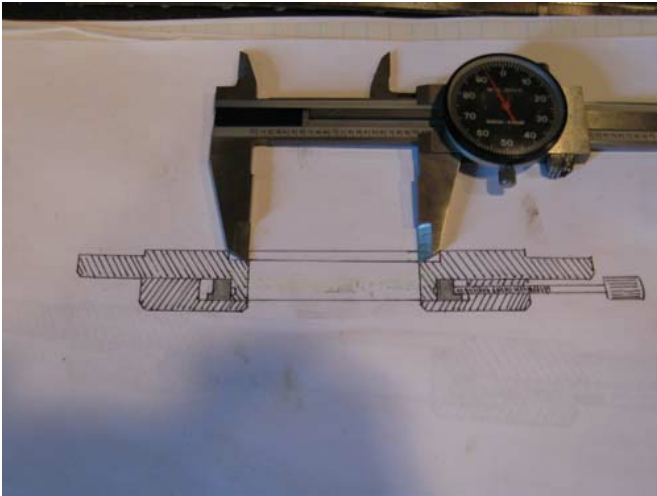


Fig. 8: full size cross section drawing of the stage

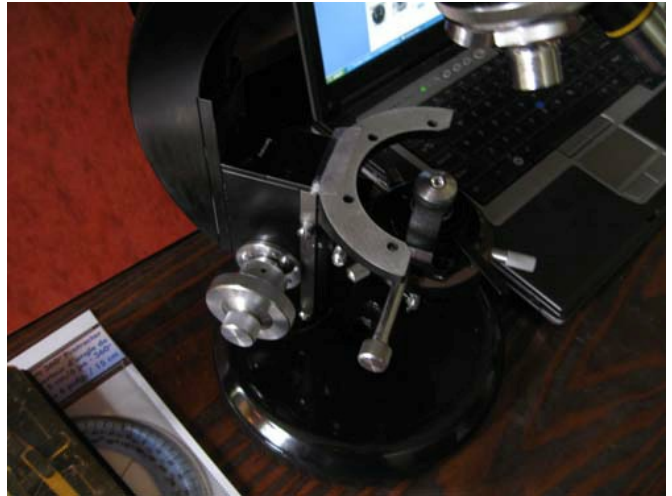


Fig. 9: Transferring the mounting hole pattern of the stage holder to paper



Fig. 10: Transferring the mounting hole pattern of the stage holder to paper.



Fig. 11: Finding the center of each hole.

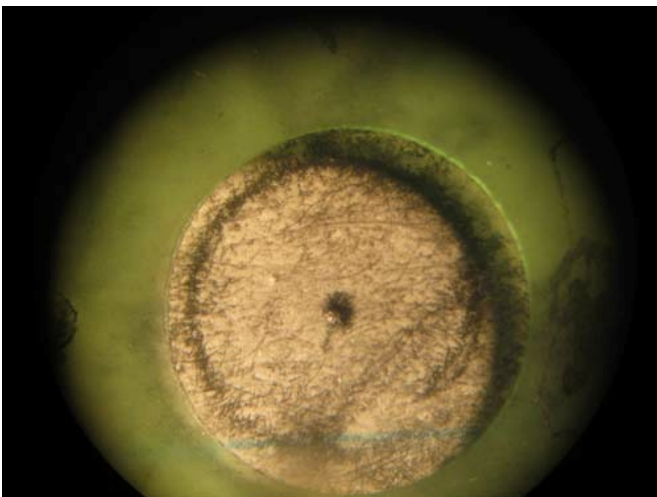


Fig. 12: Finding the center of each hole.



Fig. 13: Finding the center of each hole.



Fig. 14: Turning the stage base.



Fig. 15: The inner bearing was cut from aluminum pipe.

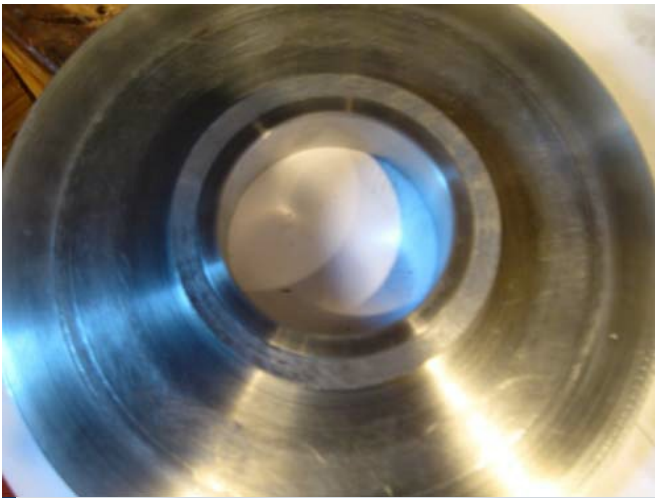


Fig. 16: the inner bearing was cut from aluminum pipe.



Fig. 17: The holes for mounting the stage are clearly visible.



Fig 18: The parts of the stage.

The interface was connected to the base by simply drilling a hole thru and counter boring the bottom to accept the nut and washer. Three rubber feet were then screwed in to the bottom of the base. The whole thing dissembled and painted with black enamel and left to bake on in the hot Florida Keys sun.

This whole business took two weekends to complete but thankfully, due to my personality, there are huge gaps in my social calendar and I can use the free time to my advantage.

The stage

This was going to be a bit more complicated. I had no Zeiss stage to copy from and the mounting hole pattern had to be exactly positioned in relation to the center of the stage, which in turn had to coincide with the centered position of the condenser. Taking gross measurements from the scope I made a full size cross section drawing of what I thought the stage should look like and would use it to take my measurements from for the machining process.

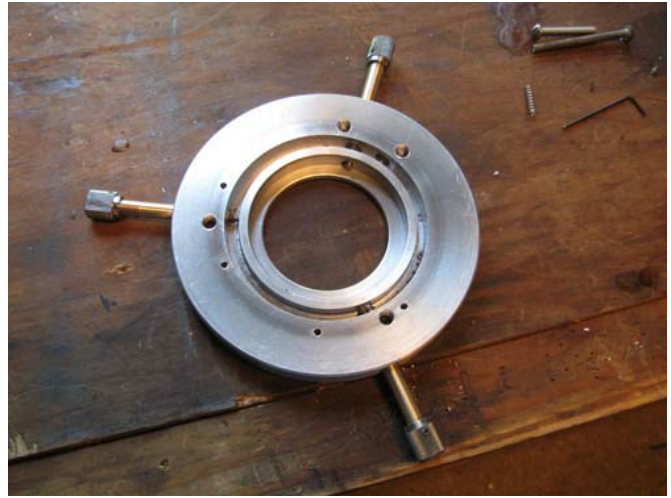


Fig 19-21: Adjusting screws and centering pin.

I visually centered the condenser and took measurements around its mount to fine tune it, then did a “rubbing” with pencil and paper to act as a marking template. Using my SF Stereo scope I was able to then accurately find and mark the center of each mounting hole and condenser.

Laying the paper on the aluminum, I center punched the pattern for the holes. Accuracy was within .05 in. Good enough for my purposes and I began the turning of the stage base, the most crucial part. The stage base was machined from aluminum thick enough to accept the centering screws and the adjustable rotation brake/lock.

After a test fit of the stage base, the inner bearing was cut from some schedule 80 aluminum pipe and then the rotating stage itself. The bearing to stage fit is crucial for proper operation and the final machining of the mating surfaces for the proper sliding fit had to be done with 1200 grit abrasive paper. The final result was a perfect fit with absolutely

no play or wobble and an ever so slight resistance. A light coat of white lithium grease was the final touch.

The adjusting screws were the next hurdle. Backlash would be the main obstacle to overcome in a set up like this. Long thread engagement and a good class fit of the thread is the solution for most high use applications but since that requires more precise tooling than I had available and the fact that these would see very little use after the stage was properly centered there was a more practical solution.

First I marked and drilled thru the stage base at the three spots for the centering screws and a fourth for the locking screw. These holes were as large as I could comfortably make it without affecting the structural integrity. Then some brass rod was turned down until it fit snugly into these holes. Sections of this rod was then drilled thru lengthwise and tapped.

From these I cut to length what would become the inserts and adjusting

screw barrel shafts. Then a 4-40 screw was placed in one end of the barrel shaft, the excess cut off and the end turned round and polished. A knob of Zeiss-compatible design from some discarded electronic equipment was affixed to the other end. Now the threaded insert was screwed over the exposed thread and the class fit was adjusted by gently tapping around the malleable brass insert with a small hammer until all play was removed but the screw still turned smoothly without excess force. The insert was then unscrewed, placed in a hole and locked in place with a setscrew whose threads just gripped the insert from the side.

The front spring loaded centering pin is shown disassembled below. The spring and pin came from a discarded VCR (I take all these throw away items and disassemble them for parts).

The bearing piece was then drilled thru to allow the brake/lock shaft to screw against a small, oiled leather

“brake shoe” to adjust rotation resistance.

With the thus far completed scope and stage attached. I did a preliminary centering check with a 20x objective and all seemed well but need to do a little fine-tuning with the 40X objective. Some other minor tweaking for condenser clearance, a degree scale, and mechanical stage attachment remain. (Speaking of a degree scale, does anyone out there have an already made CAD drawing or similarly done rendering of a degree scale they would be willing to share?)

The whole stage fabrication to this point has been three weekends of work (I coulda made a stationary stage in one day but noooooo, I had to have one with all the bells and whistles). So far the total cash outlay for the arm, condenser and binocular head is \$193.

Illumination

As a simultaneous effort to the above (just to prove to my wife that I can multitask) I dedicated some of my vast mental capability to addressing the remaining lighting requirement. Most of the GFL stands I have seen have the light built in to the base but some have a small illuminator that sits on top of the base. The now solid aluminum base precluded my applying the former and I did not want to give up the substage space to the latter. In either case there was not enough flexibility to accom-

plish all the different applications I had in mind for photon manipulation.

An external source directed thru the condenser via prism or first surface mirror seemed to be the answer. My perusal of the external light source offers on line left me unmoved as they were either hundreds of dollars, not powerful enough, not complete, did not have complete range of adjustments (centering, focus, iris), hard to get bulbs, needed odd voltage power sources which were also expensive etc. etc. etc.

Salvation (at least I hope so) arrived in the form of Mr. Frithjof A.S. Sterrenburg of the Netherlands and his article on making a “Heavy Calibre” microscope lamp from a 35mm slide projector (Micscape Oct. 2002). His article made a lot of sense. These things are plentiful, inexpensive and I was able to find a black vintage projector in mint working condition with an Art Deco design that complimented the GFL perfectly for \$9.99 plus \$12.00 shipping. I was the only bidder. Below is a picture of the Argus 100 watt projector I bought.

By affixing this light and the scope to a common base I could lock in the alignment and there were enormous possibilities for combinations and placement of neutral density and color filters, frosted glass and an Iris diaphragm.

This was starting to become a whole “system” so to speak, for microscopy. Vanity has enticed me to give it a name of its own such as the Ortholux, Axio-

phot, and Amplival have. What to name it? Since Captain Morgan left the building I consulted with Jack Daniels to ponder the question. It had no built in camera, so I couldn’t use “Phot” anywhere, and besides, the name was already taken. Since it had a powerful light source I considered “The Illuminator” but that sounded too much like an Arnold Schwarzenegger character. Hmm...the two main components were the Zeiss GFL and the Argus light source, so let’s see... if we combine Zeiss and Argus by taking the first two letters of Zeiss and the last two of Argus we get... “ZEUS”!! Yes! That was it. Zeus, an ancient name, a powerful name, a Greek god, said to be ruler of Olympus so I figured he had it all over Leitz and Nikon too. “The Zeus whole microscope system.” A wonderful name and much more dignified than combining them the other way around which would have resulted in the word “Arss”.

Unlike Mary Shelly’s tortured Doctor I am not yet ready to scream to the heavens of my creation “IT’S ALIVE! IT’S ALIVE!” Much work remains. I hope to have Zeus finished in another month or so providing I am not overtaken by circumstances. A new set of four aftermarket DIN Plan Achromatic lenses is on the way. So the total at this point with the lenses and 10XWF eye-pieces is \$465.00. Still needed are the ND, Polarized and UV filters, mechanical stage plus the iris diaphragm with mount and the prism with holder.

So as another Episode concludes and a new year is upon us I would like to take this opportunity to thank the editor’s of Micscape for their patience and forbearance in allowing me to share my sometimes-outlandish pedagogic points of view. My wife, Chauncey Tubbs and myself send everyone best wishes for a fruitful and enlightening 2010.

For Christmas my wife presented me with a copy of “Robert’s Rules of Order” to enhance our evening conversations and possibly make me more receptive and democratic in allowing her to complete her “recitations of respectful criticism” before rudely interrupting. With this charitable attitude I encourage you to send me your thoughts or questions at gwillhelm_nospam@seawardservices.com. Happy trails. Comments can be addressed to me. (remove_nospam, ed.) ■



Fig. 22: Argus projector as an illumination source.

Introducing: The Hemocytometer

Haemocytometers are specialized specimen slides that are designed to quantify the cell density. This article gives a brief introduction.

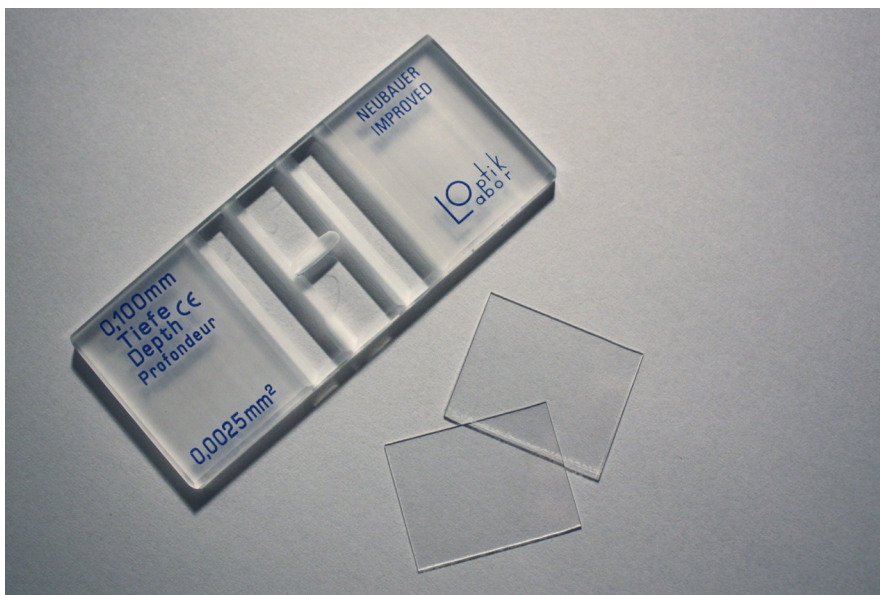
By Oliver Kim

The hemocytometer (or haemocytometer or counting chamber) is a specimen slide which is used to determine the concentration of cells in a liquid sample. It is frequently used to determine the concentration of blood cells (hence the name “hemo-”) but also the concentration of sperm cells in a sample. I have used hemocytometers to determine the concentration of bacteria in liquid growth medium.

The cover glass, which is placed on the sample, does not simply float on the liquid, but is held in place at a specified height (usually 0.1mm). Additionally, a grid is etched into the glass of the hemocytometer. This grid, an arrangement of squares of different sizes, allows for an easy counting of cells. This way it is possible to determine the number of cells in a specified volume.

Construction

A hemocytometer requires two parts, the hemocytometer slide itself and special cover glasses. The slide is quite a bit thicker than a normal slide and contains a grid etched into the surface. The cover glass is larger and thicker than the conventional ones, which have a thickness of only about 0.15mm. The cover glass rests on two “stages” which elevate the cover glass 0.1mm above the grid. This way there is a defined volume of liquid above each square on the grid. The hemocytometer slide also has an overflow area. Excess



There are different types of counting chambers available with different grids. This one is the “Neubauer Improved”.

sample fluid therefore does not result in the cover glass floating on the liquid. The cover glass is thicker in order to prevent surface tension of the sample fluid to bend the glass and thereby changing the defined distance to the grid.

Preparing the sample

The fluid containing the cells must be appropriately prepared before applying it to the hemocytometer. The fluid containing the cells should be a homogeneous suspension. Cells that stick together in clumps are difficult to count and they are not evenly distributed. An uneven distribution may result in sampling error.

The concentration of the cells should neither be too high or too low. If the concentration is too high, then the cells overlap and are difficult to count. A low concentration of only a few cells per square results in a higher statistical error and it is then necessary to count more squares (which takes time). Sus-

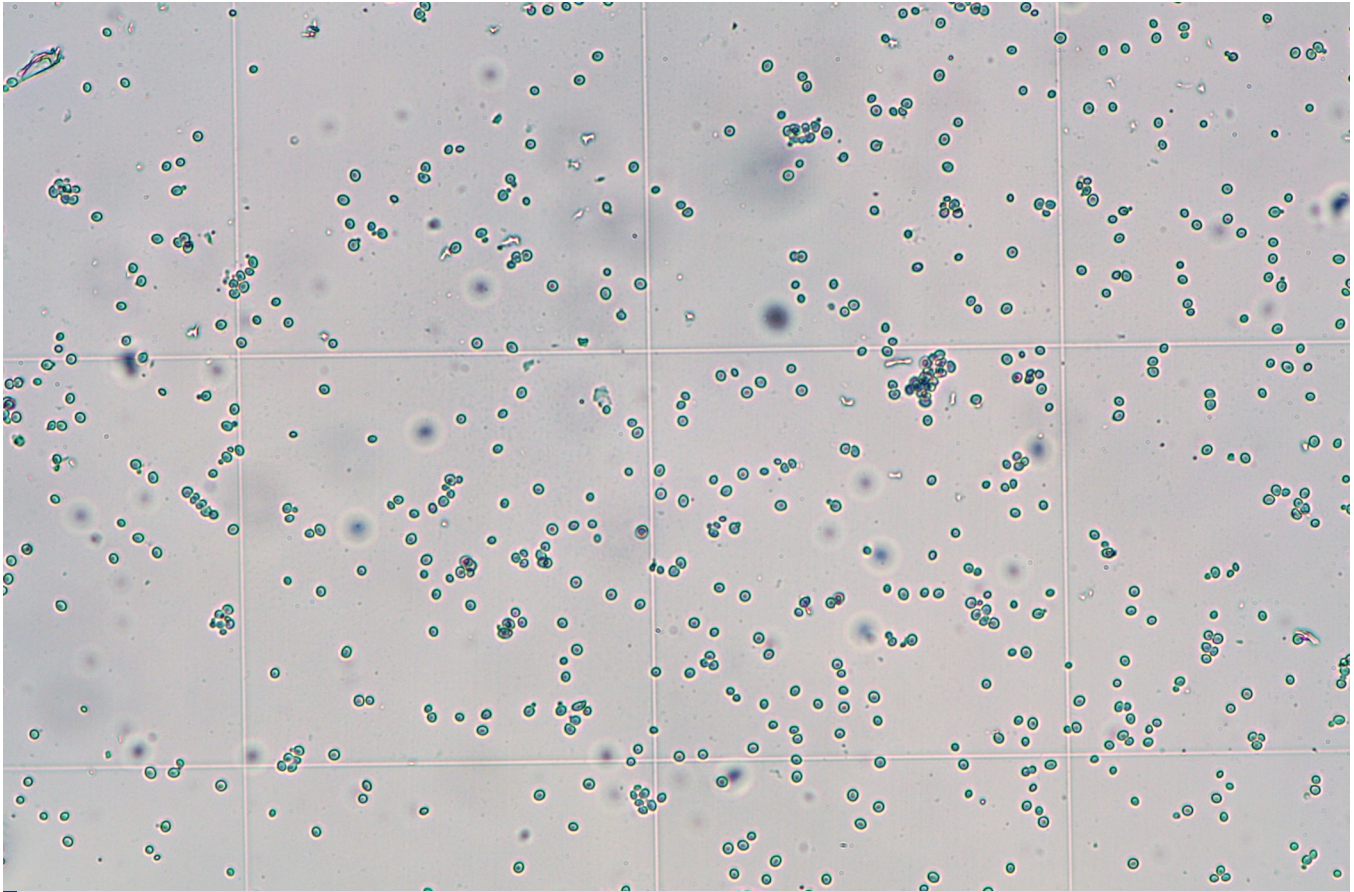
pensions that have a too high concentration should be diluted 1:10, 1:100 and 1:1000. A 1:10 dilution can be made by taking 1 part of the sample and mixing it with 9 parts water (or better saline of correct concentration to prevent bursting of the cells).

In some cases, a dilution factor of 1:11 (for white blood cells) and 1:101 (for red blood cells) is suggested. These dilution factors are simpler to work with, because you simply add 10 or 100 parts of liquid to the sample. Whatever the dilution factor used, it must later be considered when calculating the final concentration.

Counting the cells

Cells that are on the line of a grid require special attention. Cells that touch the top and right lines of a square should not be counted, cells on the bottom and left side should be counted.

The lower the concentration, the more squares should be counted. Otherwise one introduces statistical errors.



Yeast cells in the counting chamber. Each square holds a defined volume of liquid.

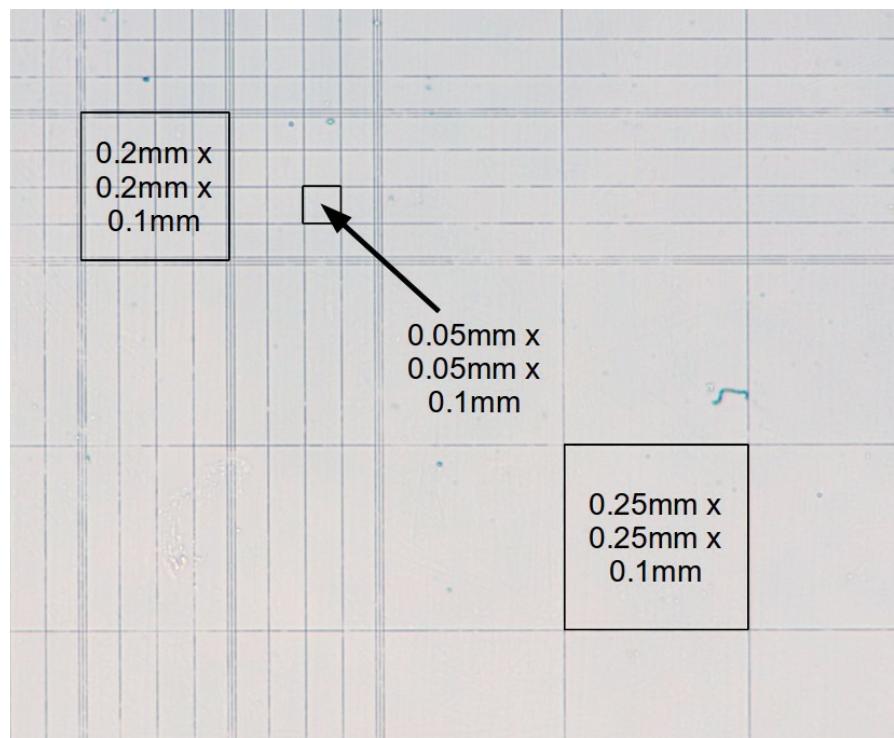
How many squares? To find out one could calculate the cell concentration per ml based on the numbers obtained from 2 different squares. If the final result is very different, then this can be an indication of sampling error.

Calculating the cell density

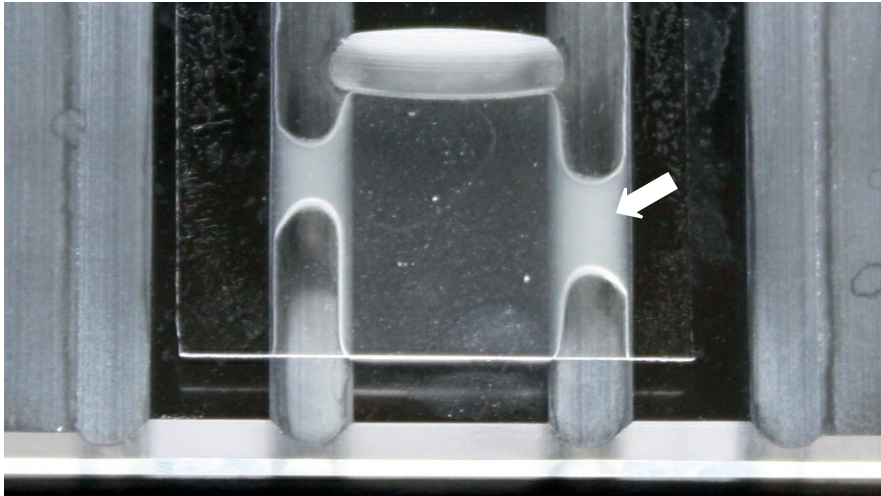
Here it is necessary to do some simple math. The following numbers are needed: number of cells counted in the squares, area of the square, height of the sample, dilution factor. The objective is to find the number of cells in 1ml of original solution.

Step 1 – Averaging: If one did not count all of the cells in a large square (1mm x 1mm) then it is necessary to average the results first before proceeding. For the purpose of this example, I use an average cell count of 123.456 cells in a 0.25mm x 0.25mm square.

Step 2 – Computing the volume: It is necessary to determine the volume represented by the square. The width and height of the square (e.g. 0.25mm x

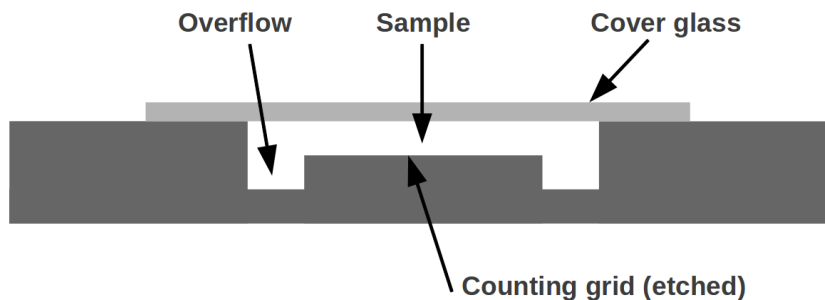


The squares hold a different volume. The size of the square must be multiplied with the cover glass to slide distance (usually 0.1 mm).



Yeast suspension applied to the hemocytometer. Notice the liquid in the overflow area (arrow).

Hemocytometer: side view



The surface tension of the sample pulls the cover glass to the slide. The sides of the counting chamber are higher (0.1mm) creating a defined volume over the counting grid.

0.25mm) must be multiplied by the height of the sample (often printed on the hemocytometer, in this example it is 0.1mm):

$$\text{volume} = 0.25\text{mm} \times 0.25\text{mm} \times 0.1\text{mm} \\ = 0.00625\text{mm}^3 = 0.00625\mu\text{l}$$

Step 3 – Calculating the number of cells in 1 ml: if there are 123.456 cells in 0.00625 μl , then how many cells are there in 1ml (=1000 μl)? We do simple direct proportion:

$$123.456\text{cells}/0.00625\mu\text{l} = X/1000\mu\text{l} \\ (123.456\text{cells} \times 1000\mu\text{l})/0.00625\mu\text{l} = X$$

$$X = 19\,752\,960 \text{ cells}$$

Step 4 – Correcting for dilution: If the sample was diluted before counting, then this must be taking into consideration as well. We assume that the sample was diluted 1:10. The final result is therefore:

$$19\,752\,960 \text{ cells} \times 10 = 197\,529\,600 \\ \text{cells / ml. That a lot of cells.}$$

Things to watch out for

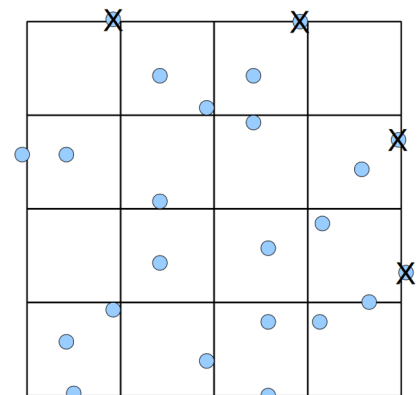
Type of counting chambers: There are different types of counting chambers available, with different grid sizes. One counting chamber also has grids of different sizes. Take care that that you know the grid size and height (read the instruction manual) otherwise you'll make calculation errors.

Use the provided cover glasses: They are thicker than the standard cover glasses. They are therefore less flexible and the surface tension of the fluid will not deform them. This way the height of the fluid is standardized.

Moving cells: Moving cells (such as sperm cells) are difficult to count. These cells must first be immobilized.

Objective: The hemocytometer is much thicker than a regular slide. Be careful that you do not crash the objective into the hemocytometer when focusing.

Disclaimer: This article is intended purely for educational purposes. Do not use this information for medical diagnosis. No guarantee is given for the correctness or completeness of the information published in this article. Consult an official manual or instruction sheet to obtain information.



Cells that are located on the top or on the right of a square should not be counted. Cells that are on the left or on the bottom should be counted to a square.

A Laser Refractometer

A refractometer measures the refractive index of different media. It is possible to make one yourself to measure the refractive index of different mounting media.

Robert F. Hancock

In microscopy, it is often desirable to know the refractive index (RI) of liquid media. In a well equipped laboratory, the standard instrument for RI measurement is an Abbe refractometer, but the cost of these is well beyond the budget constraints of most hobbyists. Fortunately there is a simple low cost alternative, for transparent liquids at least. The apparatus basically consists of a hollow equilateral triangular glass prism which is filled with the test liquid. A narrow beam of light from a laser pointer is directed through the apex of the prism, and the angle by which the light beam is deviated is measured on a protractor scale. This is the principle of the prism refractometer. It can be shown that when the deviated angle is a minimum value, the refractive index of the medium inside the prism can be calculated from

$$RI = 2 \sin 1/2 (d + 60)$$

where d is the minimum deviation angle of the light beam. The derivation of this formula can be found in most physics text books (for example, "Physics Part I & II", Resnik & Halliday (1966), p.1018). Fig 1 below illustrates the path of the beam through the prism. The minimum deviation occurs when the beam passes through the prism parallel to the base.

The construction of a prism refractometer is relatively simple. The prism cell can be made from 25mm square pieces of microscope slide which are cemented together at the edges and mounted on another slide. Silicone sealant is suitable for use with most liquids, but other clear sealants can be used, provided that they are inert to the test liquid. To ensure that the angles of the cell are as near as possible to 60 degrees, an equilateral triangular template can be made from stiff card and the cell assembled around it, using long modeling pins to hold the sides in place while the sealant cures. Care needs to be taken to keep the sealant confined to the joint surfaces only. If any is accidentally smeared on the faces of the cell, it can be carefully removed with a razor blade after curing. The stages of the construction of a prism cell are shown in Fig 2.

Construction

Once the cell has been made, it needs to be mounted on a rotating table. An easy means of doing this is to obtain the base section of a blank CD-R container and one of the clear plastic spacers which came in the CD pack. The central spindle is cut off about 2.5mm above the base and the clear spacer disc is slipped over this short axle. The slide with the glass prism is then fixed to the spacer disc with double sided foam tape, so that it just clears the central spindle. The prism cell should then rotate freely about the spindle when the plastic spacer is turned. If the spindle is too high, file a small amount off until the prism cell and slide do not touch it (Fig. 3).

Next, you will need some rigid clear acrylic packaging material approximately 0.5mm in thickness. From this, the index arm is cut using the pattern in Fig 4. If the CD container you have is different, you may have to make some alterations to the pattern. The vertical indicator card can be glued to a short "L" section as illustrated.

Another vertical index tab is required for the zero mark of the refractometer. This can be made in the same way as the moveable index arm, but it does not need the central ring as it will be fixed in position. A pattern for this part is shown in Fig 5.

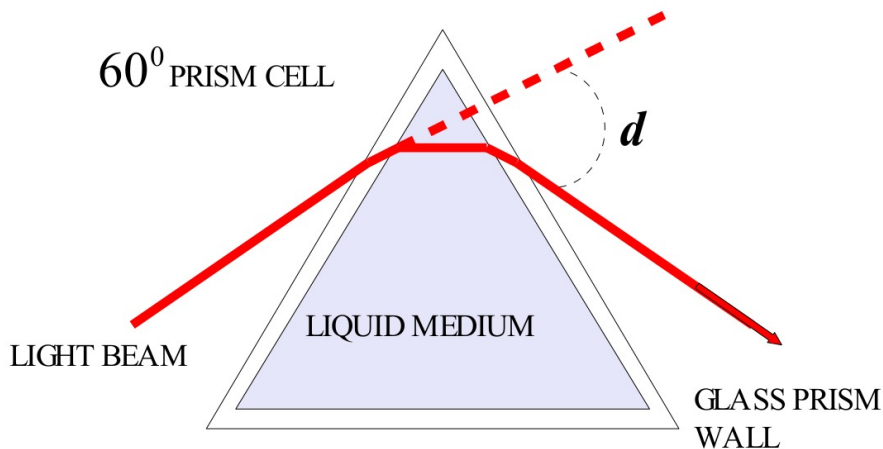


Fig. 1: Path of the light beam through the prism filled with the test liquid.

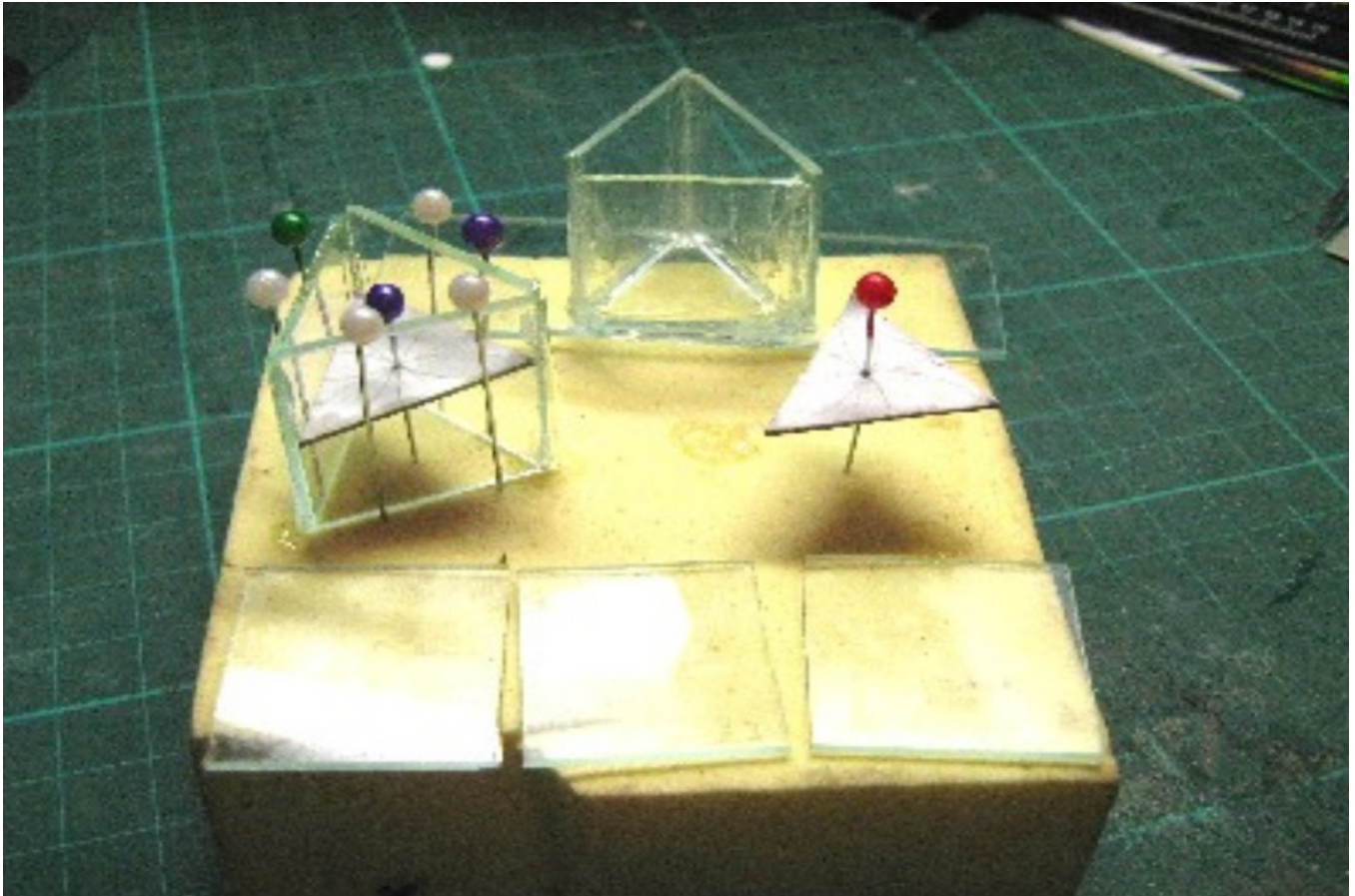


Fig. 2: Prism cell construction.

A protractor scale now needs to be made, which is placed underneath the index arm and the clear disc holding the prism, and glued to the base of the CD-R container. A photocopy of a 360 degree protractor was used in the example illustrated, but only one quadrant of the scale is actually needed. It is a good idea to clear laminate the protractor scale if you can, this will preserve the markings indefinitely. A 40 mm diameter hole is cut in the centre to fit over the base of the CD-R container. The zero index tab is glued in position to coincide with the

zero mark on the protractor scale. Ensure that the vertical index line is exactly aligned with the centre of the protractor and the zero degree mark. The index arm is placed on top of the protractor scale and underneath the clear spacer disc carrying the prism cell. It should rotate freely without causing the spacer disc to move with it. The complete rotating table assembly is attached to a base board. The components are assembled as shown in Fig 6.

The next step is to mount the laser pointer (only a 1mW pointer should be used – higher powered lasers are dan-

gerous to your vision) so that the beam passes exactly above the centre of the rotating table, and is aligned on the vertical mark of the zero index tab. A 75mm length of rigid PVC tubing and an aluminium bracket is used to mount the pointer on the base board. The height of the laser pointer must be such that the laser beam passes through the central section of the prism on the rotating table. The tube is drilled with two sets of 3 small holes spaced at 120° to take the machine thread screws, which will hold the laser pointer in position. The screws should be a tight fit in the

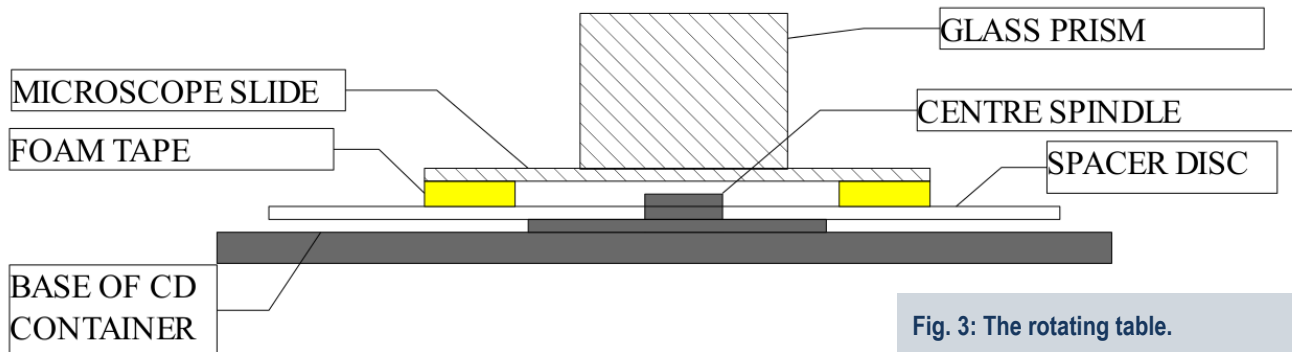


Fig. 3: The rotating table.

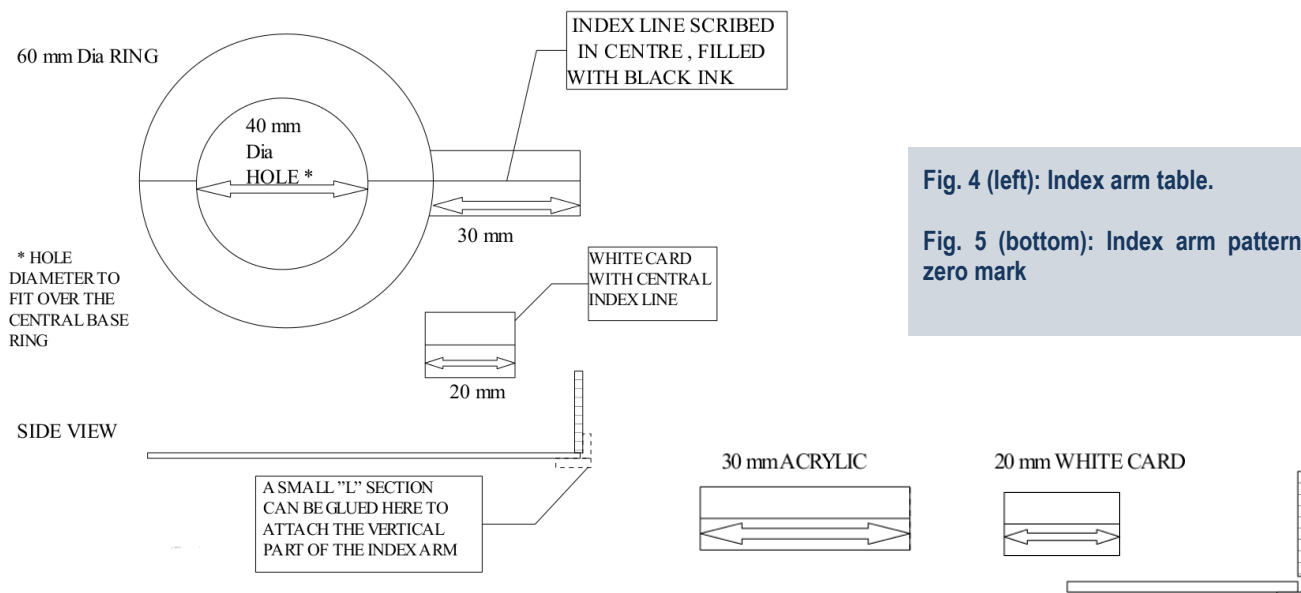


Fig. 4 (left): Index arm table.

Fig. 5 (bottom): Index arm pattern zero mark

holes, so that they cut a thread into the PVC when they are inserted. Another hole is drilled above the pushbutton switch and a screw is inserted in this so that the laser beam can be turned on or off by turning the screw. The position of the pointer is then adjusted with the screws to align the beam with the zero mark. Be careful to protect your eyes from the laser beam when it is switched on. Fig. 7 shows the completed refractometer in use.

Once everything is correctly aligned, the prism can be filled with the test liquid, using a pasteur pipette. About 5 ml of sample is required. If the liquid is volatile, place a 25 mm square of microscope slide glass on top to reduce evaporation. The prism and the clear plastic disc are then carefully rotated until a position is found where the deviation angle of the laser beam has a minimum value. A magnifying lens will be found useful here to read the angle off the protractor scale, again taking care not to look into the laser beam. It is a good idea to switch off the laser while you are inspecting the protractor scale. The prism should then be rotated by 120° so that the other two corners are at the apex, and the minimum angle readings taken for each apex. If your prism has been accurately made with 60° angles, you should obtain the same minimum deviation angle for each of the 3 vertices of the prism. If they are slightly different, then just use the average value of the 3 readings, this will not introduce

any significant error. The average value of d is substituted into the formula

$$RI = 2 \sin 1/2 (d + 60)$$

to calculate the refractive index, or the RI value can be read from the graph (Fig. 9).

Sources of Errors in R.I Measurements

Some comparisons were made between R.I measurements made on this apparatus and a laboratory grade Abbe refractometer reading to ± 0.0002 . The results are tabulated in Fig. 10.

As can be seen, the average error is about 0.5%. This may be adequate for most amateur microscopy purposes. The sources of error can be identified in three main categories:

- Measurement of the Minimum Deviation Angle
- Wavelength and Temperature Errors
- Dimensional Irregularities in the Prism Cell

The protractor scale can only be read to ± 0.5 degrees, which corresponds to an uncertainty of around ± 0.005 in the refractive index value. The accuracy of the angle measurement could be improved by projecting the laser beam at right angles onto a wall at a distance of a few metres and measuring the minimum lateral displacement of the beam in the horizontal plane. The

minimum deviation angle can then be calculated as $d = \arctan (h / l)$, where h = lateral displacement and l = distance from the centre of the prism cell to the wall. Assuming $l = 2000$ mm and $d = 300$, and thus $h = 1155$ mm, with the laser pointer that I used, the diameter of the laser beam spot on the wall is about 3 mm. This corresponds to an uncertainty in d of about 1.5 arc minute, or ± 0.0003 in the refractive index value, which is approaching the accuracy of laboratory instruments (see Fig. 11).

Wavelength and Temperature Errors

Refractive Index decreases with increasing wavelength of the light source. The standard wavelength for R.I measurements is 589.3 nm, the wavelength of the sodium D line, whereas cheap red laser pointers produce a wavelength of 670 nm. For most organic liquids, this probably introduces an error of about 0.002 in the R.I value, the value at the Na D line being higher. If you can obtain a yellow-orange laser pointer, this will give much more accurate results, as these lasers have a wavelength of 583 nm. Similarly, the R.I decreases with increasing temperature, for most substances this amounts to about -0.0003 per degree Celsius. For optimum accuracy, the temperature of the sample needs to be controlled.

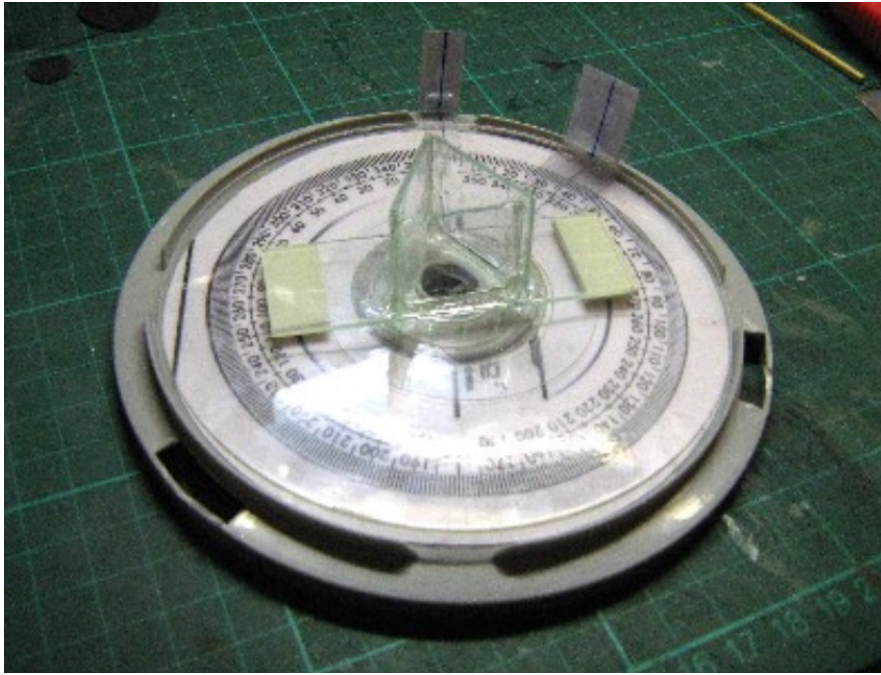


Fig. 7 (top): Prism & table assembly

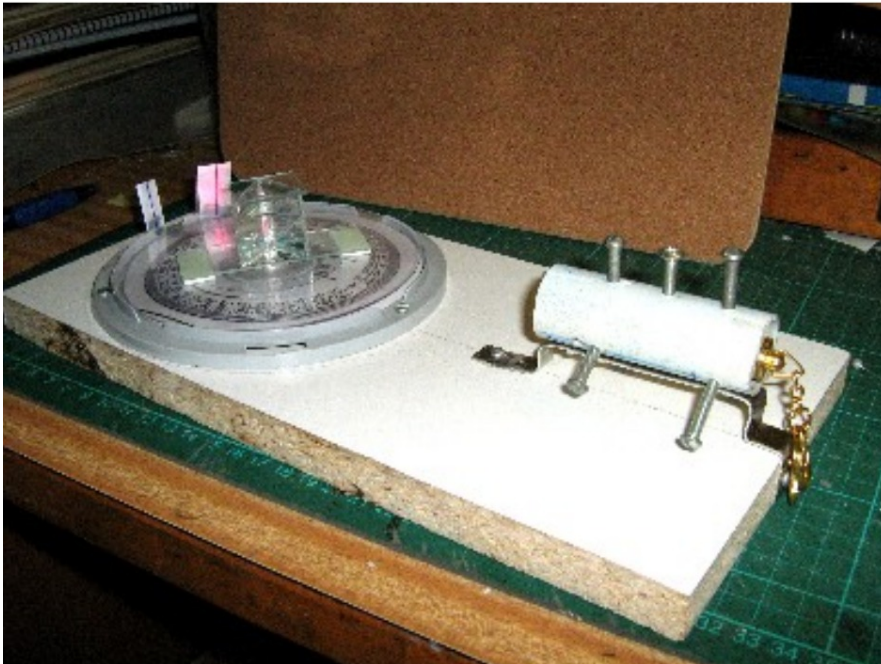


Fig. 8 (middle): Completed refractometer in use

Fig. 9 (bottom): Once you have determined the minimum deviation angle, you can either directly calculate the refractive index or use this graph to read it off.

Dimensional Irregularities in the Prism Cell

Variations in the thickness and perpendicularity of the glass walls and the prism angles will cause errors in the R.I. values obtained. A difference of +/- 5 degrees from the correct prism angles of 60 will produce an error of about +/- 0.001 in the R.I. value determined by averaging the three d values. Hollow glass and plastic prisms can be obtained commercially from scientific equipment suppliers, and these are probably more accurate than home made prisms.

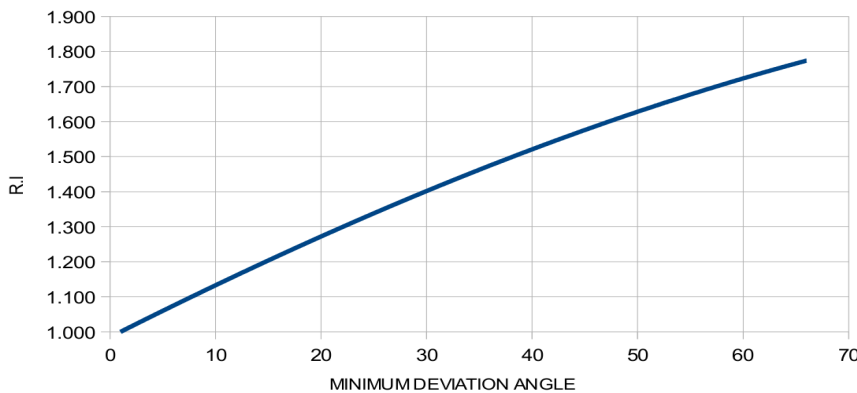
Calibration of the Refractometer with Distilled Water

The best method of checking the refractometer's accuracy is to measure the R.I. of a sample of distilled (or de-ionised) water. At 670 nm and 200 Celsius, the refractive index of water is 1.3317.

R.I Value of Mixtures

In microscopy, it is often required to produce a series of liquids having known R.I. values. It is only necessary to obtain two liquids having known R.I. values which span the range of interest, and a series of intermediate R.I. values can be matched by blending the two components. To a fairly good approximation, the R.I. value of a mixture will be proportional to the volume concentration of the two components., providing the two liquids do not react chemically. Thus, given liquid A with R.I = a, and liquid B with R.I = b, the predicted R.I value of any mixture is given by $R.I = Aa + Bb$, where A = volume fraction of liquid A and B = volume fraction of liquid B. Note also

REFRACTIVE INDEX
60° PRISM REFRACTOMETER



that $B = 1 - A$. Figure 12 shows this relationship.

For example, mixing methyl salicylate (R.I = 1.5365) with Eucalyptus Oil (R.I = 1.4572) in the volume ratio of 70:30 is expected to produce a mixture with $R.I = 0.7 \times 1.5365 + 0.3 \times 1.4572 = 1.5127$. An experimental mixture of these proportions was measured on an Abbe refractometer and the resulting R.I was 1.5128. This simple calculation (known as the Arago – Biot equation) assumes ideal behaviour of the two liq-

uids, but generally the predicted R.I value will be within +/- 0.1 % of the actual value. Small differences between the predicted and actual values may occur, these are caused by minor volume contractions or dilations which occur when dissimilar liquids are mixed. There are more complex equations available to predict R.I values taking these interactions into account, for example the Lorentz – Lorenz equation (see *Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 115, No. 2, April 2003, pp*

147–154). On the graph (Fig. 12), the predicted R.I values from the Lorentz – Lorenz equation are also shown, together with the differences from the Arago – Biot predictions (magnified 1000x). As can be seen from the graph, the differences are much less than 0.1 % of the actual values of R.I. These equations can also be applied to ternary, quaternary, etc mixtures, ie those having three or more components. ■

SAMPLE	AVG d	LASER R.I VALUE	ABBE R.I VALUE	% ERROR
Distilled Water	23	1.325	1.3333	0.62
Methylated Spirits	26.2	1.366	1.3631	0.22
MeSal/ Euc. Oil 70:30 *	37.3	1.502	1.5128	0.73
“Tea Rose” Oil	31.3	1.431	1.4369	0.42
AVG. ERROR		Red laser 670 nm	Sodium D 589.3 nm	0.5

(* A mixture of methyl salicylate and eucalyptus oil, 70 : 30 v/v)

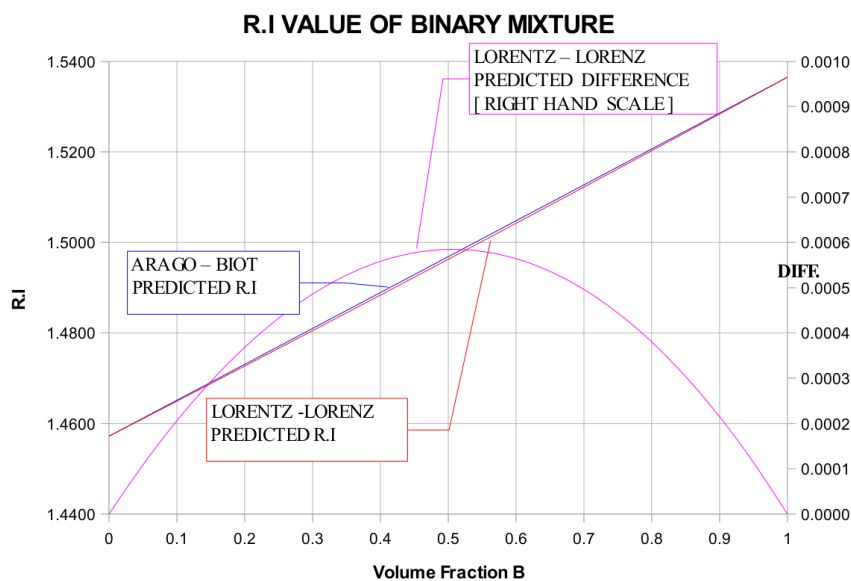
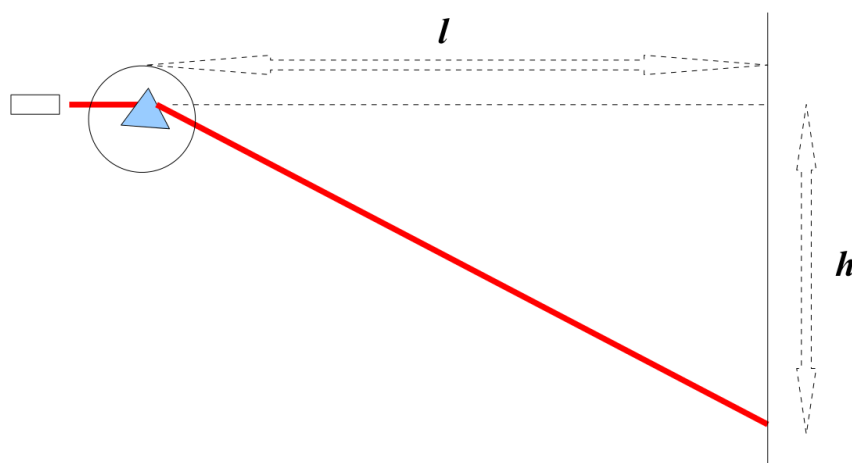


Fig. 10 (top): Comparing the measured R.I. with the theoretical values. The average error is about 0.5%.

Fig. 11 (middle): The minimum deviation angle can then be calculated as $d = \arctan (h/l)$.

Fig. 12 (bottom): R.I. Values of mixtures.

Biological Microphotography Course in CCH-Oriente, UNAM, Mexico

From Mexico, sharing our love of microscopy to young scientist aspirants.

***Víctor Rafael
Zárate-Ramírez, M.Sc.***

In February of this year we implemented a digital photomicrography course in the CCH-Oriente (Colegio de Ciencias y Humanidades – Plantel Oriente), one of the two branches of the high school of the UNAM (Universidad Nacional Autónoma de México). The main objective of this course was to introduce the students into the different set-ups for photomicrography used in

faculties and the investigation centers of the medical-biological departments of the most important University in México.

In the eastern side of Mexico City, with more than ten thousands of students, the CCH-Oriente is a reference center for science and humanities education for high schools, because our students have a complete curricular program in a context of a constructivist educational model. The curriculum also includes, a wide variety of extracurricular training courses with a propedeutic approach.

Professors and other faculty staff frequently criticized that high school students show a deficiency of abilities in the use of the microscopic and related

techniques, especially in careers like Biology, Medicine and Veterinary, among others.

SILADIN (Sistema de Laboratorios para el Desarrollo y la Innovación), the System of Laboratories for Development and Innovation, is present in all schools of the CCH's, and has a well equipped academic facilities for professors who want to work in advanced investigations with the participation of students. The microscopy equipment includes Olympus epifluorescence, Carl Zeiss compound microscopes (with bright field, dark field and phase contrast), and stereoscopic microscopes. In these facilities we implemented a 20 hours propedeutic training course for students in the last year of high school



Hemipteran Insect, *Planococcus citri*, Tucson 1.3 MP, stacked from 10 images with CombineZP, postprocessing with Gimp.

Image: Víctor Rafael Zárate-Ramírez



Protozoan, *Spirostomum* spp., Tucsen 5.0 MP, bright field, postprocessing with Gimp
Image: Karla Mariana Peña Gutiérrez



Rotiferan, *Philodina roseola*, Canon Eos Rebel T1i, phase contrast, postprocessing with Gimp
Image: Jesús Vázquez Ramos

mainly, and with plans for a scientific career. The course was divided into ten sessions.

The first session was an introduction into compound (Axiostar Plus) and stereoscopic (Stemi DV4) microscopes with an emphasis on phase contrast, bright field and dark field. Top and bottom illumination was also addressed, according to the nature of the sample in the case of the dissection microscope.

In this context the cameras to be used in the course were presented as well: Two dedicated cameras with Tucsen chip (1.3 and 5.0 MP), a digital reflex Canon Rebel EOS T1i (15 MP) with T-mount, threaded 10X lens and 23 and 30 mm microscope adapters, as well as a Nikon Coolpix S203 (10 MP) with a Celestron 93626 digital camera adapter, for afocal microphotography. All this (with some variations) was used as standard equipment in mostly of faculties and research centers of medical-biological area of the UNAM.



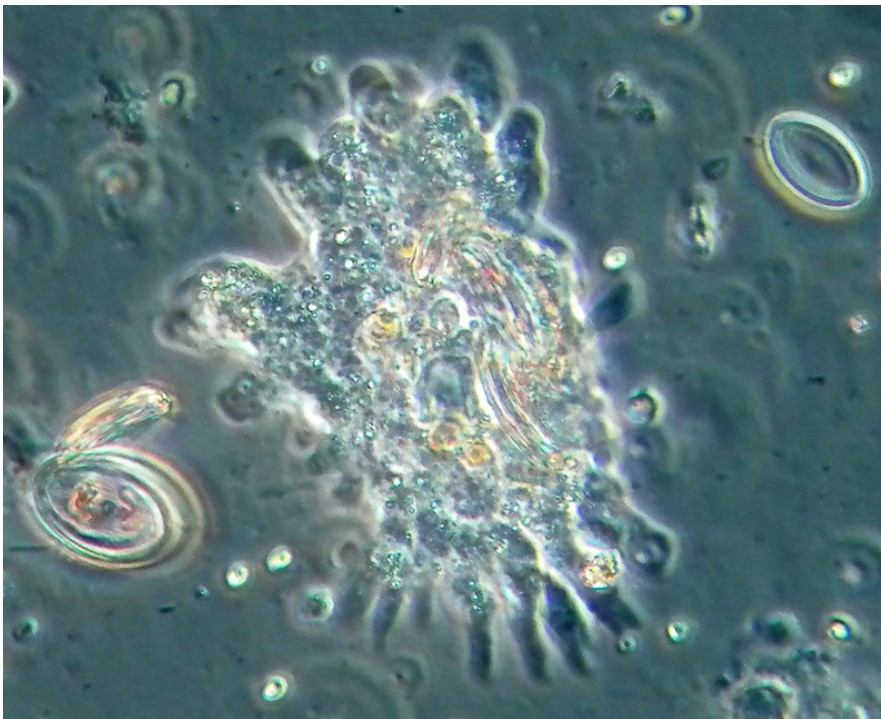
Hemipteran insect, *Aphis gossypii*, a “mummy” with a parasitoid wasp inside, Canon EOS Rebel T1i, postprocessing with Gimp

Image: Rosa Jazmín Sánchez Arguello



Protozoan, *Astramoeba radiosa*, tucsen 5.0 MP, phase contrast, postprocessing with Gimp

Image: María Guadalupe Hernández Luna



Protozoan, unidentified free living amoeba, Nikon Coolpix S203 (afocal), phase contrast, postprocessing with Gimp

Image: Lesli Tiare Figueroa Arriaga

The second session was an introduction to the software: Micam for an alternative to the native software included with the cameras, CombineZP for photo stacking and Gimp for postprocessing. We began to take photos of the protozoa *Paramecium caudatum*, *Spirostomum spp.*, *Astramoeba radiosa* and *Rotifera Philodina roseola*, all previously cultivated in laboratory in glass dishes and fed with unicellular algae. All specimens were mounted in wet preparations, but we also applied a little drop of Gram's iodine to reveal cilia in *Paramecium caudatum* samples.

For the third session, the students collect leaves of plants to watch stoma in the lower epidermis. In session five to eight, each student designed and took photos of a personal project. These projects ranged from aphids (we found "mummies" containing parasitoid wasps), to pollen, and a wide selection in the middle.

In session nine and ten, the students presented to the class their individual projects. All the students were approved, taking their skills with microscope to new levels. In this contribution we present some of these photos taken by the students and myself.

Acknowledgments

This course would not have been possible without the invaluable support of Arturo Delgado González, Director of CCH-Oriente; Tomás Nepomuceno Serrano, Technical Secretary of the SILADIN and Esperanza Martínez López administrator of the upstairs hall in the Computation Tower. ■

Web sites:

UNAM:

<http://www.unam.mx/>

CCH-Oriente:

<http://www.cch-oriente.unam.mx/>

SILADIN Plantel Oriente:

<http://siladin.cch-oriente.unam.mx/>



Hemipteran insect, *Aphis gossypii*, lower left a juvenile, in the middle an adult with cornicles visible one emitting green color pheromones, Canon EOS Rebel T1i, postprocessing with Gimp.

Image: Maria Guadalupe Roque Ortega



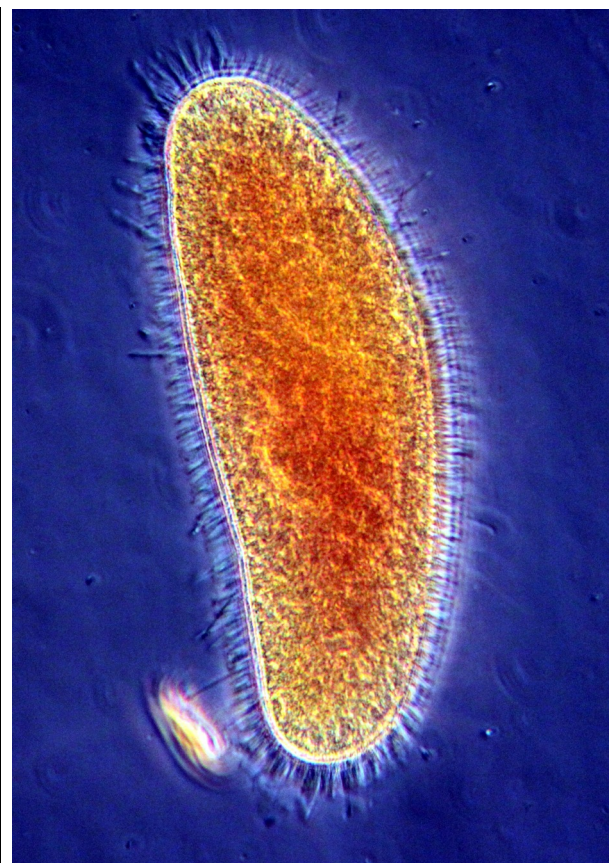
Hymenopteran insect, *Aphidius colemani*, parasitoid wasp, Canon Eos Rebel T1i, stacked from 8 images, postprocessing with Gimp.

Image: Víctor Rafael Zárate-Ramírez



Protozoan, *Paramecium caudatum*, asexual division and full organism, Tucsen 1.3 MP, postprocessing with Gimp.

Image: Laura Paulina Barrera Muñoz



Protozoan, *Paramecium caudatum*, with Gram's iodine, to reveal cilia, Canon EOS Rebel T1i, phase contrast, postprocessing with Gimp.

Image: iare Figueroa Arriaga

Parasitoid wasps: *A Photomicrographic Survey* *of their Life Cycle in the Laboratory*

In this contribution we describe the life cycle of *Aphidius colemani*, a parasitoid wasp. It is a natural predator of *Aphis gossypii* (Glover, 1987) a homopteran insect parasite of plants. This high school investigation confirms data from literature.

Víctor Rafael Zárate-Ramírez,
Laura Paulina Barrera Muñoz,
Leslie Hernández Ramírez,
Claudia Peña Fabián and
Emmanuel Alejandro Ramírez
Morales



Pomegranate trees in SILADIN of CCH-Oriente, UNAM.

Introduction

In February 2011, in CCH-Oriente, UNAM, Mexico, we implemented a microphotography course for students. As part of their personal projects, some students collected a flower of pomegranate with some aphids. They identified as the species as *Aphis gossypii*, a well know homopteran parasite of commercial interest in Mexico. A preliminary search in primary literature and the Internet showed us the possibility of the presence of one of their natural predators, parasitoid wasps of the species *Aphidius colemani*. We conducted a careful search and found a “mummy” aphid. The wasp used the aphid to reproduce by laying an egg into it, transforming it into a mummy. We began with an investigation with the main objective of corroborate the information of the literature, with the possibility of new findings. The aphid-wasp relationship is an example of co-evolution and a learning objective for the Biology class of some students.

Aphis gossypii, Glover 1987 (Homoptera: Aphididae)

The aphid *Aphis gossypii* is a cosmopolitan homopteran, wherever its host plants are grown we can be certain to find it as well. The aphid generally prefers warmer climates. It can also be found in greenhouses, where it is a common pest. The aphid attacks the plant by feeding of its green parts, such as young shoots and leaves. The females of the winged morphs have a black color, the abdomen of often colored differently. The wingless forms can show many different colors, ranging from yellowish to green, even gray. The wingless adults are 1-2 mm in length.

Aphidius colemani, Viereck 1912 (Hymenoptera: Braconidae)

Aphidius are small wasps, which reproduce by laying single eggs into aphids. The eggs hatch and the larvae consume the aphids. During this process

the aphids are killed. The aphids thus turn into “mummies”. The larvae then pupate. After metamorphosis, an adult wasp emerges through an exit hole which they cut into the mummy. This is not the only thing that kills the aphids. The wasps search for suitable hosts and thereby disturb the aphid colonies. Many aphids therefore fall off the plant and die.

Aphidius colemani can reproduce in many different kinds of aphids, such as cotton and green peach aphids. Other aphids (potato or foxglove aphids) are resistant. The morphology of *Aphidius colemani* is similar to *A. matricariae*, but there are differences in the number of eggs produced.

The size of *Aphidius colemani* ranges from 0.5 to 1cm in length. This depends also on the size of their aphid hosts. Males and females differ in the shape of their abdomen. Males tend to have a rounder abdomen, while females have a pointed ovipositor to place eggs.



Pomegranate bulb with aphids (*Aphis gossypii*), of different sizes and colors, even a winged female.

The color of adults is dark brown to black.

The Beginning - Searching for Mummies on Plants

We collected some flowers, bulbs and leaves of pomegranate (*Punica granatum*, Linneo 1762), found in SILADIN of CCH-Oriente, UNAM, in the eastern side of México City (19°23'2.31"N, 99° 3'35.39"E). On one leaf we found one aphid mummy and on one bulb we found three more (watch your own plants too, maybe they have aphids and mummies too). All of them were placed in petri dishes at room temperature.

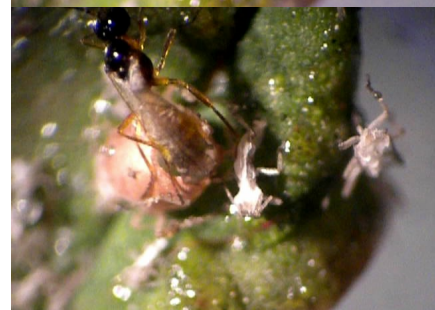
For three days we monitored the aphid mummies with a Carl Zeiss stereo microscope DV4, a Celestron universal digital camera adapter and a Nikon Coolpix S203 digital compact camera (10MP), and a Olympus Ve3 with a Canon Rebel EOS T1i (15 MP) with T-mount, threaded 10X lens and 30 mm microscope adapter. All microscopes belonged to LACE (Laboratorios Avanzados de Ciencias Experimentales) Laboratories in SILADIN.

The sprouting - any similarity with alien movies is only a coincidence

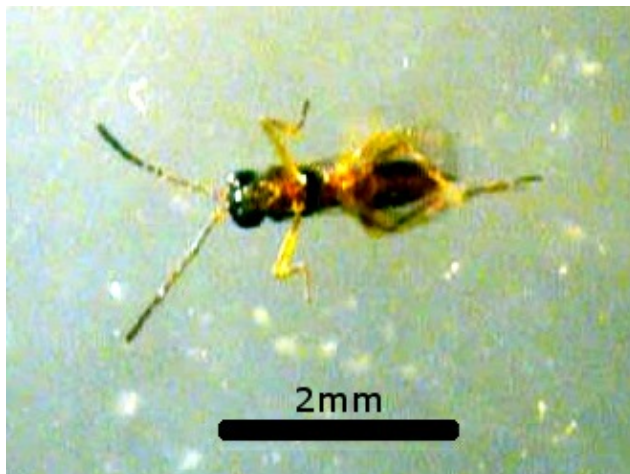
After three days of monitoring, we could see how the wasp began to bite a circular pattern into the dead exoskeleton of one of the mummies. This happened in the abdomen between the cornicles. This hole was finished in no more than 5 minutes and the wasp emerged 32 seconds later. In a second case the wasp completed the hole in 3 hours and emerged in 5 hours.

Infection's under laboratory conditions (hurray!!! they were female!)

If the first emerged wasp was female, it would have been ready to lay eggs in aphids. We therefore prepared a petri dish with ten aphids with different sizes and also ten exuviae. The exuviae are the skins of (dead) aphids. They are used as a control and should not be recognized by the wasps. We waited several hours for the first emerged wasp to walk to prepared part with aphids and exuviae. The wasp was female because they begin immediately to lay eggs in the aphids. The exuviae was not recognized as valid prey and the wasps did



An aphid "mummy" on a leaf of pomegranate. The parasitoid wasp started to form a hole. The images show the attempt for the wasp to emerge from the aphid.



Top left: *Aphidius colemanii*, a parasitoid wasp after emergence, ventral view, inside a petri dish.

Top center: The first obtained female of the parasitoid wasp, preparing to lay eggs inside an aphid.

Top right: Laying their eggs with the ovipositor.

Right: Trying to lay eggs in a young aphid. At the right there is an exuvia. Never try to lay eggs in them!



not try to lay eggs into them. Natural selection was still doing its job!

When a wasp finds a suitable aphid it doubles the abdomen under the front legs, beats the wings of an intense way and places them backwards, with the ovipositor lay their eggs into the aphid. The whole process was documented with video and photomicrography.

Conclusions

The colony of aphids *Aphis gossypii* presents in pomegranate of the SILADIN in CCH-Oriente, UNAM, are depredated by the parasitoid wasp *Aphidius colemani*. The data taken in video and photos support previous information, but, we found inconsistencies in the behavior reported about the exuviae and further study is necessary. The students reinforced their skills with microscopy and photomicrography.

This investigation from high school students resulted in a good learning strategy, because they worked in every phase, from field to laboratory. In this way they acquired and they reinforced

themes related to co-evolution and natural selection.

Acknowledgements

We appreciate the support of Arturo Delgado González, Director of CCH-Oriente, UNAM; Tomás Nepomuceno Serrano, Technical Secretary of the SILADIN; and Ricardo Abad Morales chief of Audiovisual Department for technical help in Canon mount's.

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1. A second aphid "mummy", the wasp begins to push from inside.



4. Half of the wasp's body is now out.



2. The head of the wasp its out now.



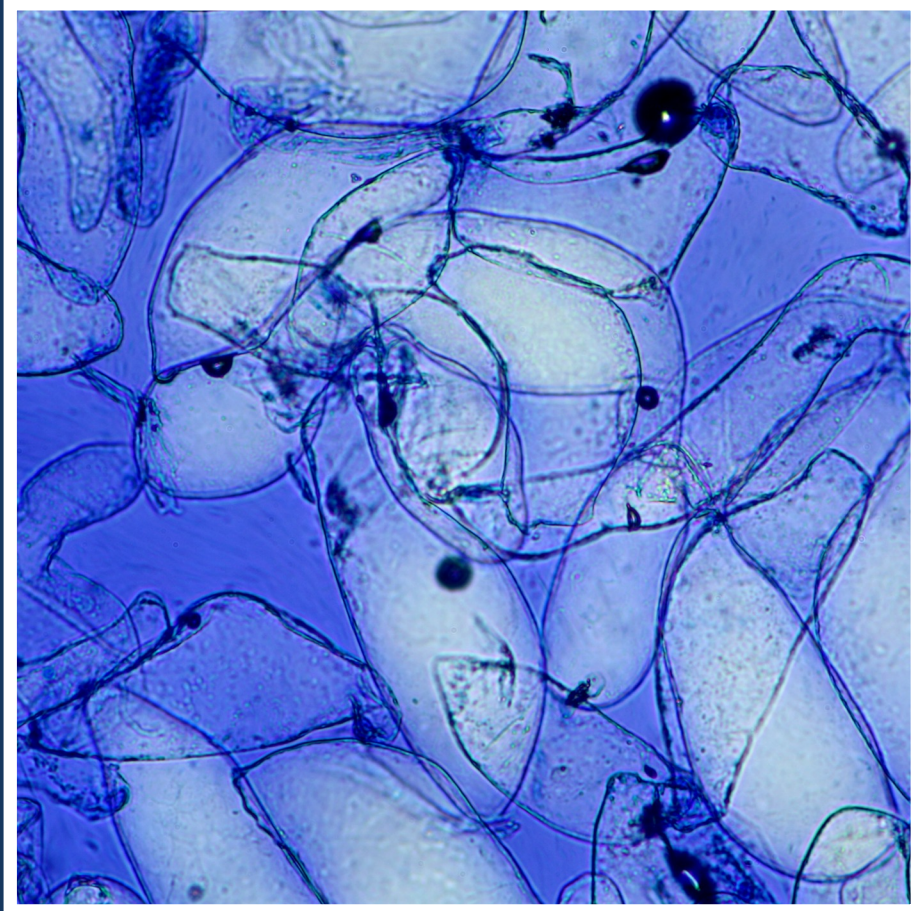
5. In the struggle, how many of them die in the process?



3. The wasp's antenna is now completely out.



6. Almost completely out now.



What's this? Answer on page 3.