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The Magazine for the Enthusiast Microscopist

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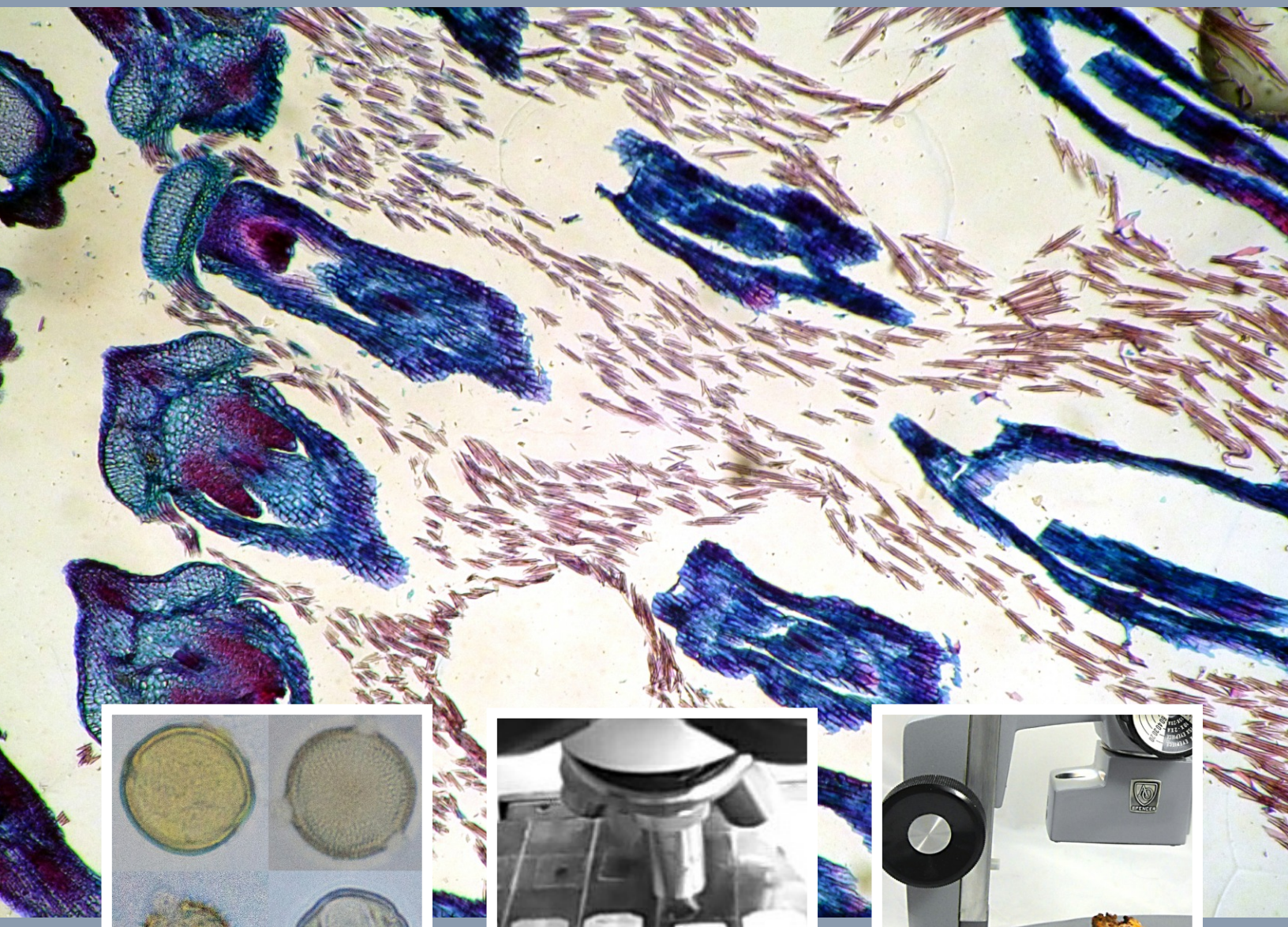

Pollen in Honey

Microscoping many slides

History of Stereo Microscopy

ABOUT

Microbehunter Microscopy Magazine
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Front Cover: Dandelion
Left image: David Robson
Middle image: Salah deeb et al.
Right image: R. Jordan Kreindler

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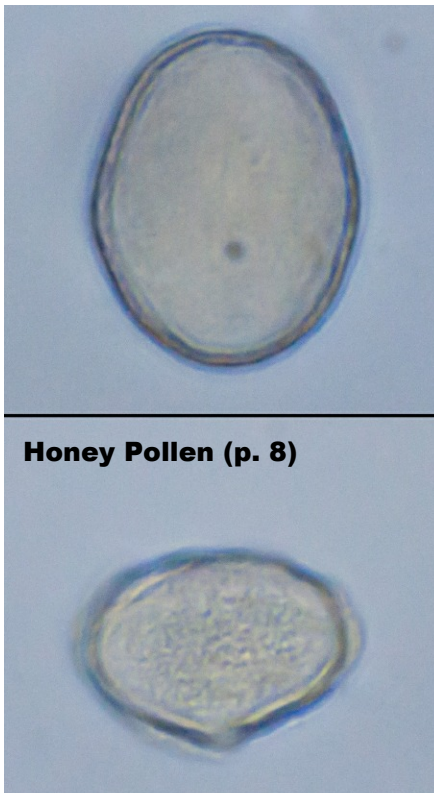
Stomata from a plant leaf.



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How to Examine a Large Number of Slides for Microscopy

Salah deeb, Mahmoud El-Begawey, Khalid El-Nesr,
Nesreen Safwat and Mohamed Kamal
Department of Pathology, Faculty of Veterinary Medicine,
University of beni-Sueif, Egypt

The method consisted of supporting the microscope with an extension of the slide holder using a glass plate of suitable dimensions. The slides are laid out in a ribbon-like arrangement for examination.

While glass microscope slide are commonly used, only few users may give any thought on their manufacturing process and history. Every year millions of glass microscope slides are used. Before 1820, however, the specimens were mounted in wood or ivory slides and held in place by thin transparent plates of mica (a sheet silicate mineral).

Glass slides became popular only after 1820. The Microscopical Society of London (in 1839) was responsible for standardizing the slides to 3 x 1 inches (around 76 x 26 mm), a standard which is still in use now. The cover glass was introduced in 1843 by Pieter Harting at the University of Utrecht (Pathology in Practice, 2013). Glass slides are 1-1.2mm thick and cover glasses are about 0.15mm thick. Thinner slides and cover glasses are sometimes used with high power objectives.

During mounting of paraffin sections, the region within which these specimens are positioned may be shifted away from the middle of the slide.

All compound microscopes have a stage to place the glass slide with the specimen. Mechanical stages have knobs to turn, which translate the rotation into a linear movement of the slide horizontally and vertically. The slide is held in place by a spring-loaded clip (Figure 2). Simple plain stages, in contrast, hold the specimen slide in place using clips. The disadvantage of these stages is, that they only allow for the observation of one, maximum two specimen slides. For many years histologists and pathologists are used to examine their specimens mounted on glass-slides individually. It is time to adapt this method to increase their efficiency and to reduce the time spent for examinations, especially in laboratories having large number of slides.

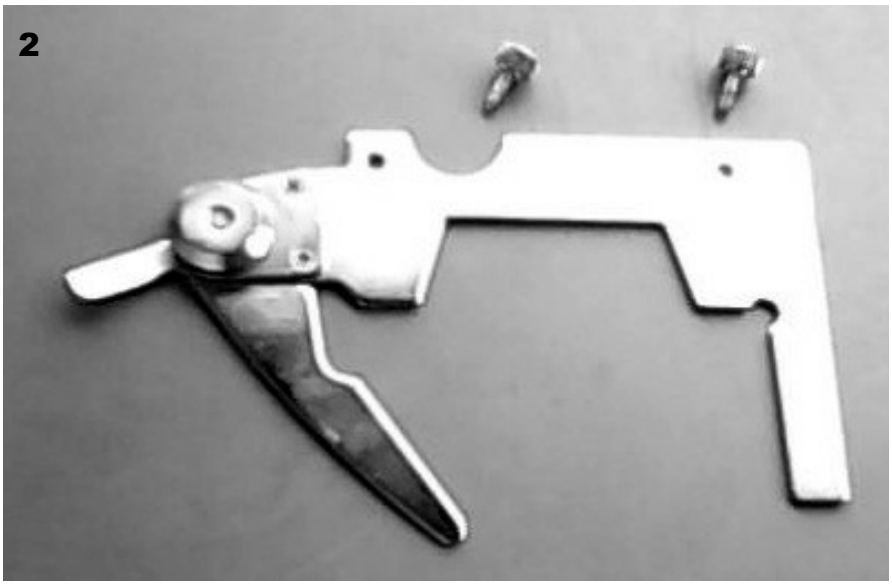

Figure 1: Microscope, assembled

Figure 2: Slide holder

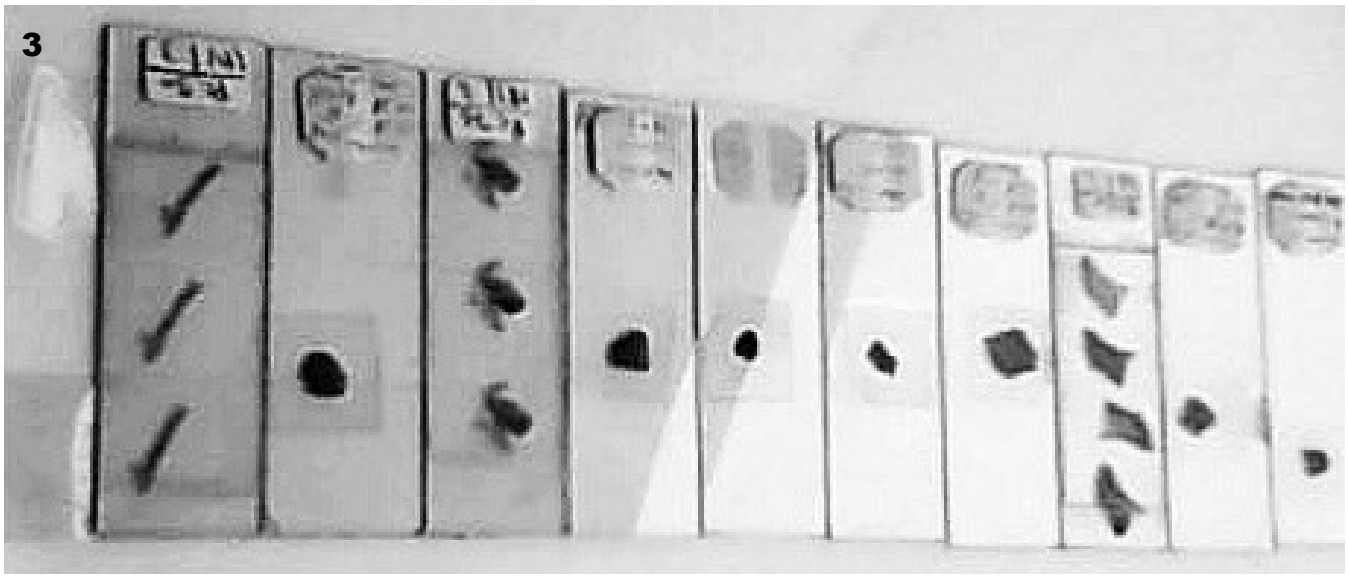

Figure 3: Microscopic glass slides ribbon

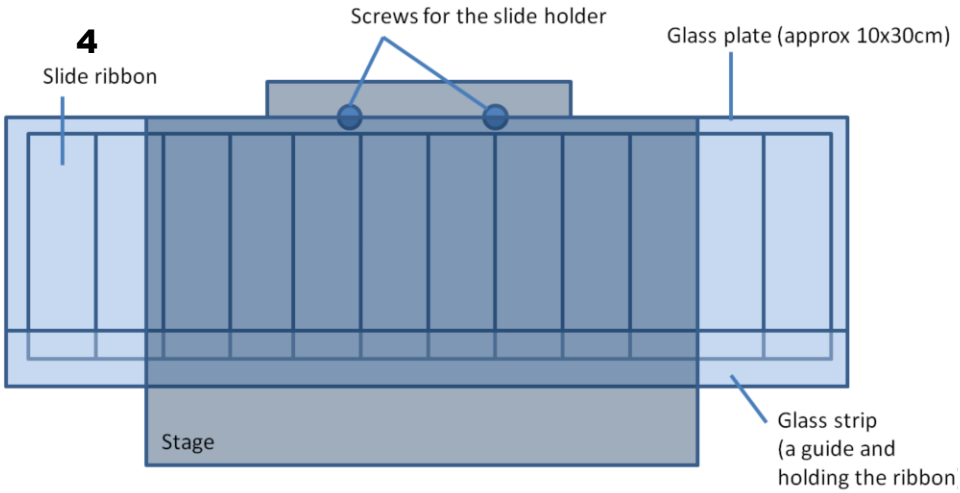

Figure 4: Glass plate

Materials and methods

Preparation of the microscope: It is necessary to do some modification on the microscope before establishment of the extended stage. First, the screws that fix the slide holder to the stage of the microscope are released (Figures 1, 2), this allows more space to accommodate the ribbon of slides. A microscope stage is usually designed to hold only one slide.

Preparation of the glass stage: Cut out a rectangular plate of glass (30 x 10 cm) with a short rim at one of the borders. This plate acts as an extended microscope stage (Figures 3, 4). The lower surface of the glass plate is bordered with glass strips to control the movement of the glass plate.

Preparation of glass slides ribbon: Lay out your labeled glass-slides containing the specimens freely on the table of glass, or make a ribbon of these slides, holding them together with tape. This is important especially in case of examination of serial sections. This can be achieved by laying out the slides on a glass plate carefully, side by side with cover slips upward and labels in front of the examiner (Figure 4). Insert a blank slide at the beginning to mark information of the group of these slides.

Combine the labeled slides together and fix the two ends of the ribbon using an adhesive tape to the glass plate (Figure 5, 6). Transfer this tape to the top of the glass plate and place it on the stage of the microscope.

Discussion

Binding of your own microscope slides using adhesive tape is a simple way to reduce the time of reloading the slides and focusing for examination. The specimens are found easily. Transfer of the slides is made easy using translation control knob, while their transfer side by side must be done manually.

Slide scanning can also be done. It reduces the need to perform repetitive slide adjustment and focusing tasks during examination, Users are able to verify accuracy of focal plane or illumination settings through providing total quality control. The method is inexpensive and required no special adjustments. Specific slides for specific topics can be retrieved and viewed in-

stantly making the archive available through secure access to view, edit or comment.

References

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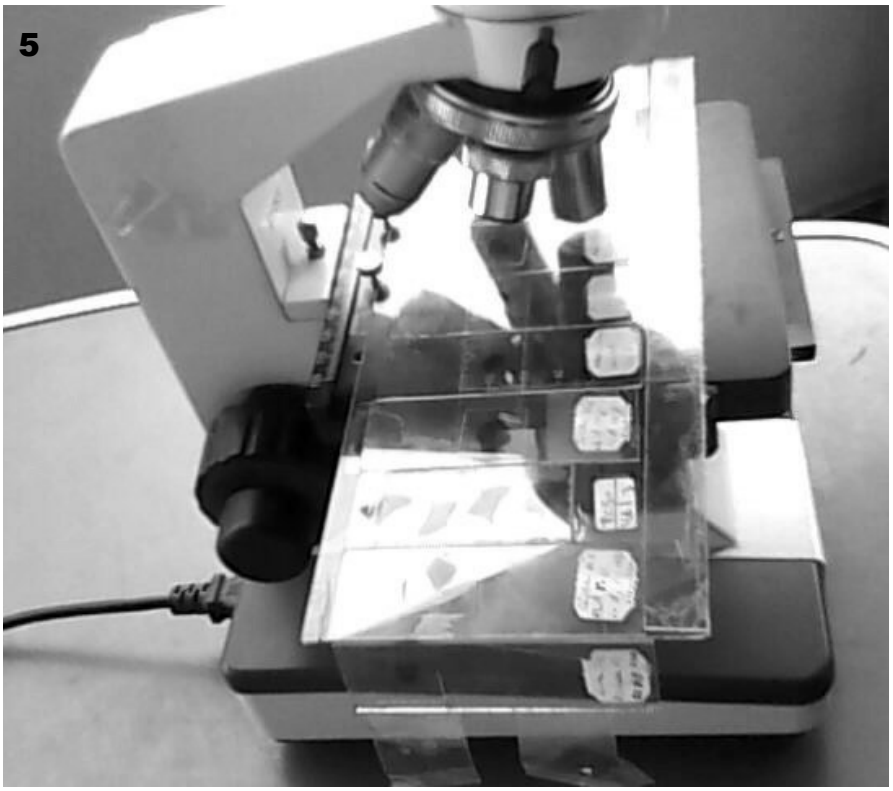


Figure 5: Assembled device, profile view

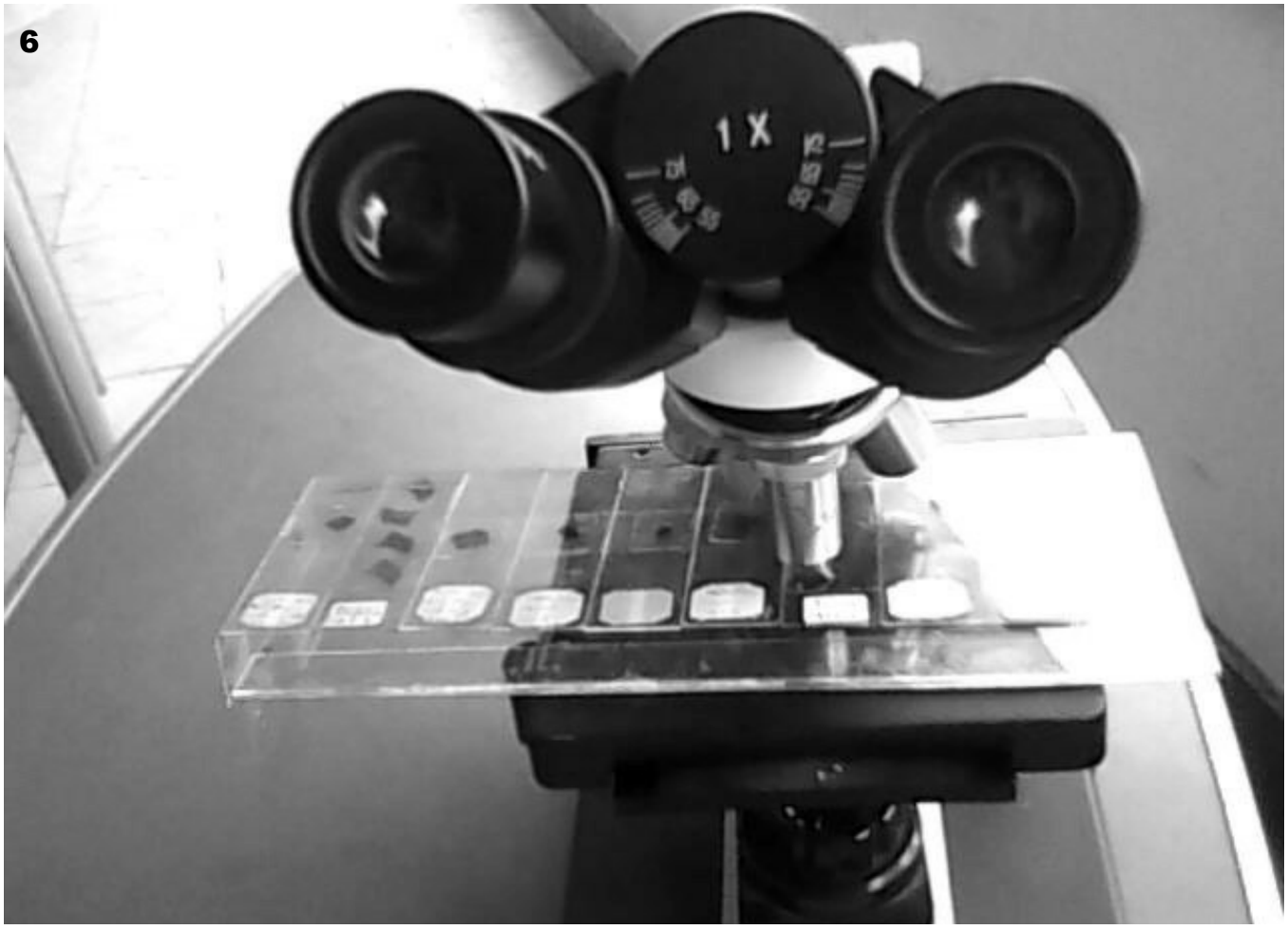


Figure 6: Assembled microscope, front view.

Book Review - *The Proper Care of Optics*

This book by Robert Schalck gives an overview of many different cleaning methods of optical surfaces.

Oliver Kim

Dust and dirt are the enemies of optical instruments. They do not only reduce the image quality but can also contribute to the growth of microorganisms (fungi are a known problem) right on the optical surface. Dirt which is not removed might become stuck to the lenses and be more difficult to remove later. Keeping the optical surfaces clean is therefore essential for long-term microscope maintenance.

Cleaning optical surfaces can be risky, however. Wrong cleaning solutions can either remove the coating of the lenses or can soften the glue holding the optical elements in place. If you use a wrong cleaning cloth, it is possible that the dust grains (which might be microscopic sand particles) scratch the surface of the lenses. Objectives and eyepieces should only be cleaned when really necessary and only after careful consideration. Lens paper, for example, is not suitable for routine cleaning. It does not have a large surface area and is therefore not able to collect much dirt. It is also not very soft and if you apply too much pressure, then there is indeed the danger of rubbing hard dust grains into the surface of the lens. Lens paper can be used for removing excess immersion oil and it can be folded to reach corners of the lenses.

The book *The proper care of optics: cleaning, handling, storage, and shipping* by Robert Schalck is by far the most comprehensive book on cleaning optical surfaces that I have so far seen. It addresses a wide range of different cleaning methods, required materials and techniques. The book also contains chapters on proper handling, storage and shipping of optics. The book also covers less conventional cleaning techniques, such as the use of chalk powder as a cleaning agent or CO_2 gas. Chalk cleaning, which has been used for over 100 years in cleaning telescope mirrors, the book informs us, effectively removes oils, smears and stains. I can imagine, that some of these methods are useful for people who have to restore the internal parts of a microscope, such as prisms, or microscope mirrors.

The book covers a very wide range of different surfaces to be cleaned, ranging from car windows, to eye glasses, telescope mirrors to CCD sensors. The even dedicates a short chapter to the cleaning of microscope slides. The book also mentions the removal of mounting medium (Canada Balsam). The specific cleaning of microscope optics is, while it is addressed, not the main focus of the book, however. Rather, the book tries to give a wide overview of the different cleaning techniques that can be used on different optical surfaces. The cleaning of microscope optics is manufacturer dependent (the solvents that can be used should not damage the specific lens kit and optical coating), and it is therefore difficult if not impossible to give general instructions that satisfy all microscope brands.

Even if several of the presented cleaning techniques are not directly relevant to microscopy, such as the cleaning of astronomical optics, they still do offer valuable insight into this field. For example, the author describes a method of protecting the glass surface of a lens with vinyl tape to for easier edge cleaning. The description on how to cleaning the prisms of binoculars can also be aplied to the prisms of stereo microscopes, even if this is not explicitly mentioned. Some of the presented methods are therefore sufficiently generic to be applicable to different optical surfaces, especially if the microscope optics are completely disassembled for maintenance. I can imagine that the book is of particular interest to collectors to people who want to restore antique microscopes or to those who acquire and re-sell used microscopes. They might be confronted with particularly complex inspection and cleaning tasks and will also be more likely to completely disassemble the microscope to reach the different optical surfaces.

The readability of the book is very good. It illustrates the different techniques with photographs and describes the cleaning processes in a straight-forward and non-technical language. ■

Title: The Proper Care of Optics: Cleaning, Handling, Storage, and Shipping.
Autor: Robert Schalck
Publisher: SPIE Press
Publication year: 2013
Number of pages: 236
ISBN: 978-0-8194-9457-3

The table of contents of the book, and some sample chapters can be seen here:
http://www.amazon.com/The-Proper-Care-Optics-Monograph/dp/0819494577/

"If you want the best performance from your optics, they need to be properly maintained. This book describes the cleaning, handling, and storage methods used by professional technicians to keep optics in top condition. It is written for a diverse audience, from first-time optical cleaners to assembly technicians and seasoned engineers looking to expand their repertoire. In the lab or in the field, you'll find the right technique to protect your equipment from harm. Reference lists of tools, solvents, and suppliers are provided to help you find solutions quickly."

Public domain via Wikipedia (Scott Bauer, USDA ARS)

A Simple Honey Project

Natural honey samples contain numerous pollen grains. These can be counted and serves as a quality measure for the honey.

David Robson

I am known around work as the "microscope guy" - my PC's background is my latest favorite photograph I've taken, I will talk about the subject to anyone who asks, and many people who don't. So, it was natural for my coworker Rob to ask some microscopy questions.

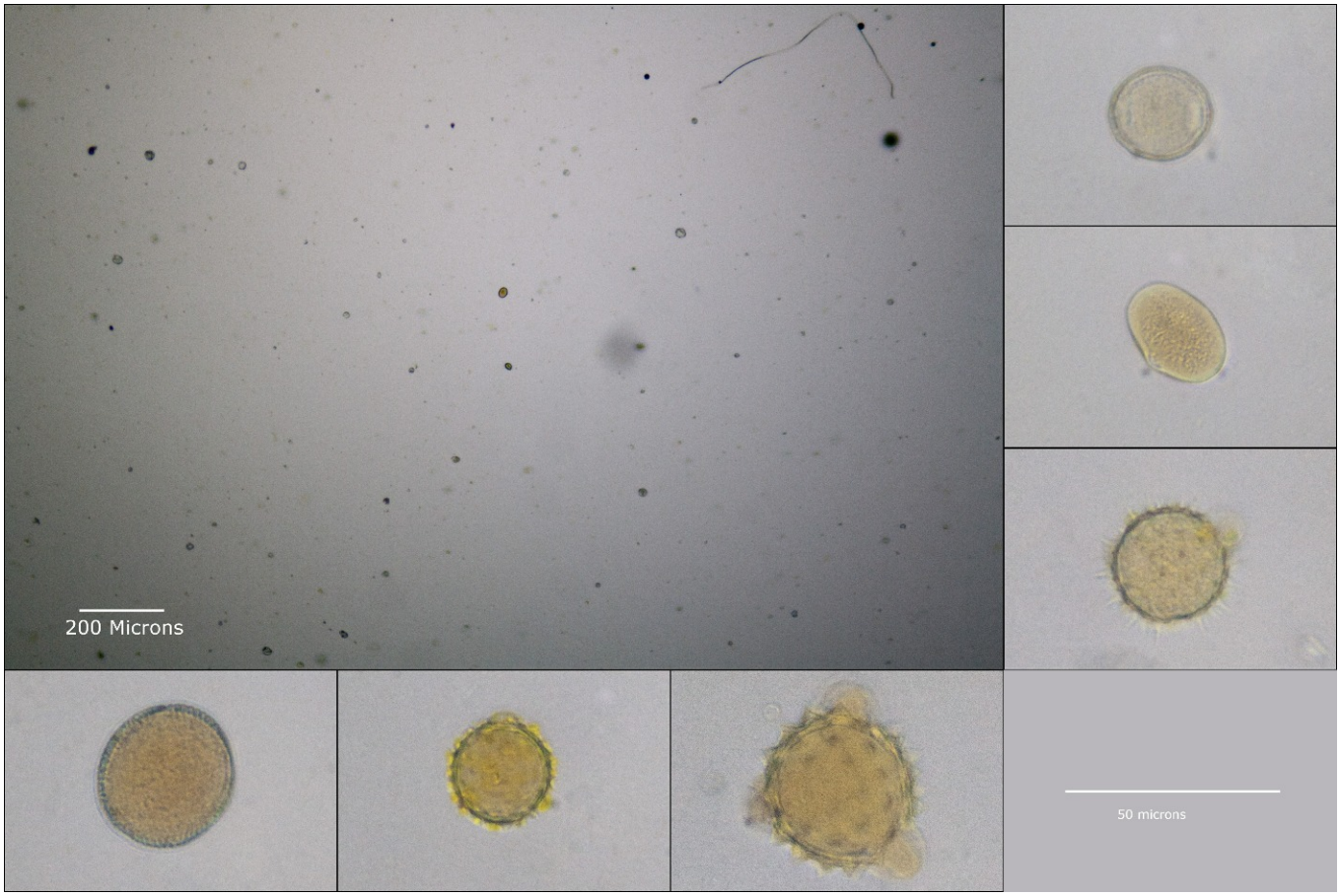
At first, he was interested in what the price of a new microscope might be, but the cost of a quality unit was a bit more expensive than he was looking for. The next question he asked was, "Would you be interested in running an experiment?" How could I possibly resist?

Rob was interested in both honey and food safety. He is an amateur brewer of mead as well as having some family members with food allergies, and he was concerned about the honey he was buying. He had read about "laundering" honey – filtering and pasteurizing honey to remove identifying markers from the honey, possibly so that an undesirable source could be masked. He has quite a collection of honey - he had 6 brands to test - so he was curious if any of them had the markers of laundered honey. Natural honey has pollen in it, and you can identify the source of the honey by pollen content. He asked me, "Do you think you could identify honey by examining the contents - particularly the pollen in a sample?" Not having any experience, probably not, but I was going to have fun trying.

We came up with a list of questions to be answered:
Can I find pollen in the honey samples?
Can I identify the pollen?
How much pollen is in the sample - what is the density of the pollen in the sample?

We discussed a little bit on how to give me the samples. Ideally, you'd

Figure 1: Sample A - Buckwheat Honey, Tree of Life, USA



want to start with freshly opened bottles so that any cross-contamination would be minimized, but most of these honeys were already open and in use. That would just have to be a risk in the experiment. To minimize any further cross contamination, we came up with an easy and reasonable collection process. He would use 6 recently washed spoons and use one for each brand of honey, collect a spoonful of honey and put each sample in a new zippered sandwich bag. Rob already had the idea of giving me the honey with anonymous labels, so he sealed and labeled each bag with a letter, and he kept the list matching the letter to the brand.

While he was collecting the samples, I spent time to figure out the examination process. I opened the question to the Amateur_Microsopy Yahoo! Group, and got some good suggestions.

I also raided my pantry and pulled out my honey jar to see what would work.

This was my trial process:

1. Draw 1 ml volume of honey from the sample.
2. Dissolve honey in 10 ml of distilled water in a clean container.
3. Let the sample settle overnight.
4. Draw out any settled particulates.
5. Count and identify the pollen.

This didn't work at all. Honey is not easy to manipulate using the everyday tools I bought from the local science outlet. I didn't purchase or make a centrifuge, so I couldn't easily get the particulates in the honey to settle, nor could I guarantee that I collected all of them. Finally, I couldn't acquire a consistently sized sample. Even heated, honey does not draw up into a measuring dropper easily, nor does it come out cleanly.

I came up with an alternate process, which worked with my limited capabilities.

The setup

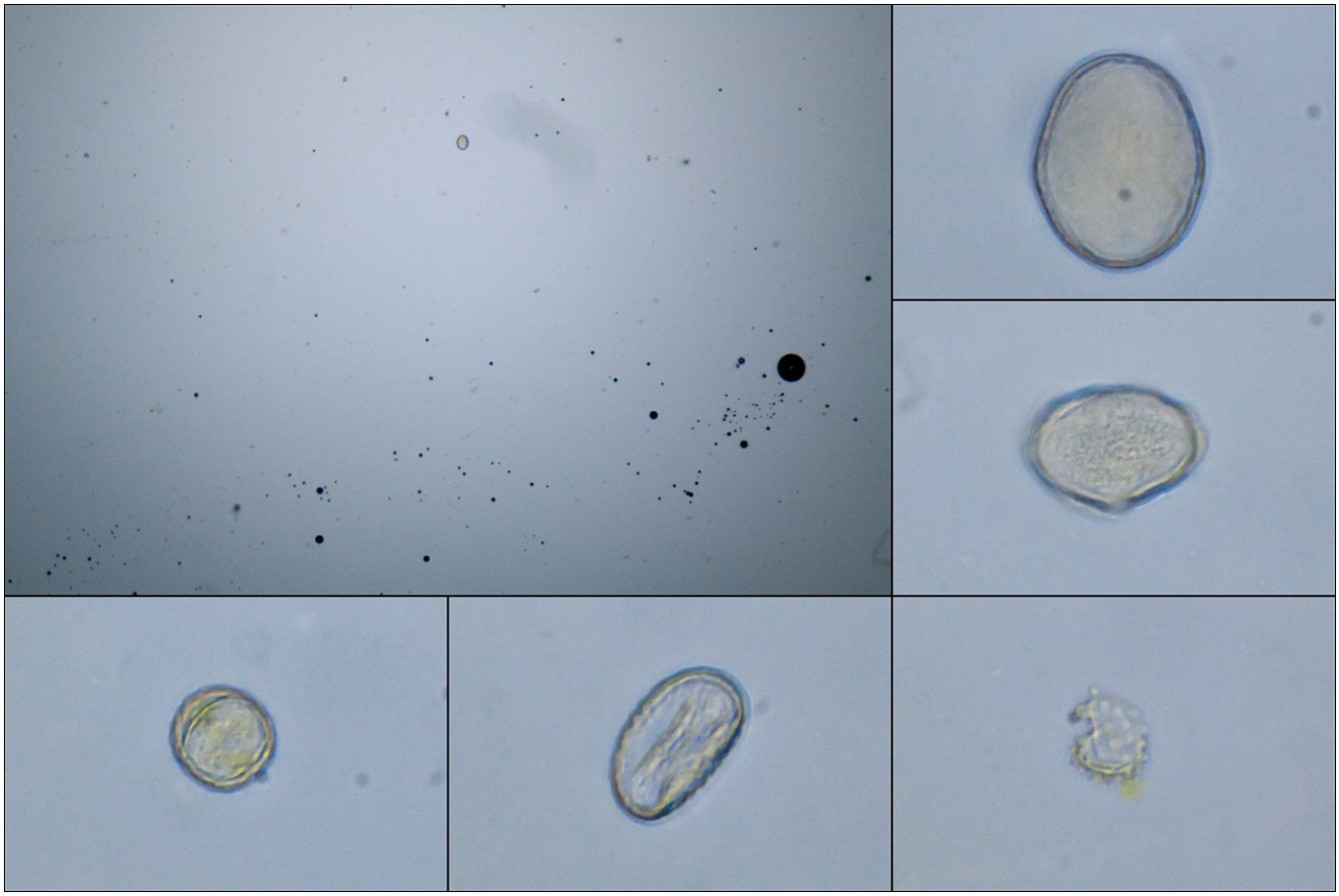
- Fill 1 clean and rinsed bowl filled with distilled water. Heat bowl until hot to touch.
- Label each 1ml dropper A-F.
- Clean and mark 6 slides. Label them A-F. Clean 6 cover slips.

The process

I followed these steps for each labeled bag.

- Put the bag into hot water.
- Using same labeled dropper, draw out 1/8 ml honey while honey is hot.
- Drop as much honey as possible onto the same labeled slide.
- Place drop on slide of the same label.
- Cover drop with cover slip.
- Rinse the labeled dropper with fresh water for each.
- Take 4 photos with a 4X objective to get a sense of pollen density. Make sure to record to which label the picture belongs to.

Figure 2: Sample B - Sourwood Honey, in comb, from the Smokie Mountains, USA



Sample	Pollen count (grains of pollen)						Density (grains/mm³)
	Picture 1	Picture 2	Picture 3	Picture 4	Total count	Average	
A	22	21	16	22	81	20.3	99
B	3	3	4	2	12	3.0	15
C	17	22	23	19	81	20.3	99
D	23	15	18	20	76	19.0	93
E	7	7	12	10	36	9.0	44
F	2	6	1	2	11	2.8	13
Store	0	0	0	0	0	0.0	0

For each honey sample (labeled A-F) four pictures were taken and the totlal and average pollen count was determined. The pollen density was estimated by dividing the average number of pollen grains by the volume seen on the picture.

- Take pictures with a 40X objective of any unique particles, in order to help identify them. Make sure to record to which label the picture belongs to.

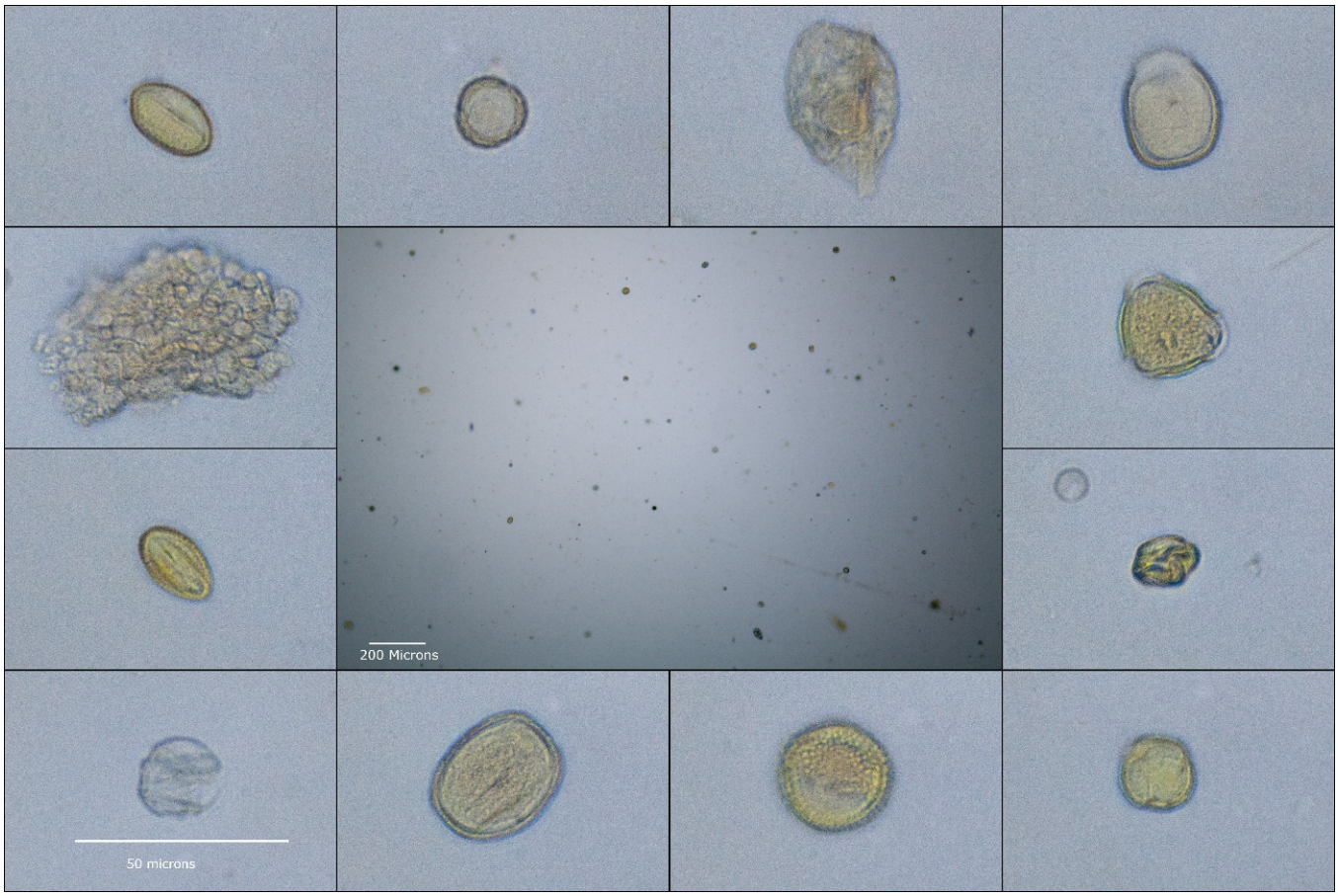
Now to the answer of the original questions: Can I find pollen in the honey samples? Yes! Pollen is quite easy to find in honey, if it's there to be found.

Can I identify the pollen? I reported back to Rob, "In general, a person could identify the different types of pollen in honey, but I can't." I just couldn't identify the pollen in honey. Even after borrowing a book on pollen identification, I couldn't make a definitive decision. There are a couple of reasons for this. The first is that I didn't use any kind of stain, so the contrast of the high magnification images are insufficient, and the most important reason is that I just don't have enough experience.

However, after Rob shared with me the brands and the sources listed on the packaging, I was able to locate pictures in the book, and we could compare them to my high magnification photos. We have some confidence that the pollen of the plants listed on the label were in the honey samples.

How much pollen is in the sample and what is the density of the pollen in the sample? I used the low magnification pictures to determine density. Pollen is identifiable in these low magnification pictures as small, colored

Figure 3: Sample C - Saw Palmeto, Pure unprocessed, Walker Farms, FL, USA



regular shapes. Using GIMP, I opened each picture, created a transparent layer on top of the image, and marked each likely, in-focus grain of pollen with a red dot. Then I reviewed each selection at 100% view – dust, dirt and air bubbles look similar enough to pollen grains - and counted the red dots after my final selections.

After I counted, I needed to find the volume of each picture. I have a stage micrometer and made a scale bar. The scale bar picture set 200 microns as 331 pixels, both horizontally and vertically. Each low magnification picture is 3888 x 2592.

3888 / 331 * 200 = 2349 microns [µm] and 2592 / 331 * 200 = 1566 microns. That got me the area of each picture.

Searching online, Nikon's site told me that my 4X objective has a depth of field of 55.5 microns. The volume of each picture would be

2349 x 1566 x 55.5 = 204158637 mm³ or 0.2042 mm³.

This value is significantly less compared to the total volume of honey that I used. I used at most 1/8 mL from the dropper, 125 mm³. This was to be expected, as the objective and camera only capture a small fraction of the total volume applied on the slide. I decided that this would be a reasonable estimate of the volume of my pictures.

By counting only pollen grains that were in focus, I counted the grains of pollen inside the volume of the picture. The pollen density is the pollen count divided by the picture volume. The table shows my results. The honey from my pantry is listed as "store brand." I was embarrassed at the results of my own contribution. Clearly, the honey in my pantry has been processed.

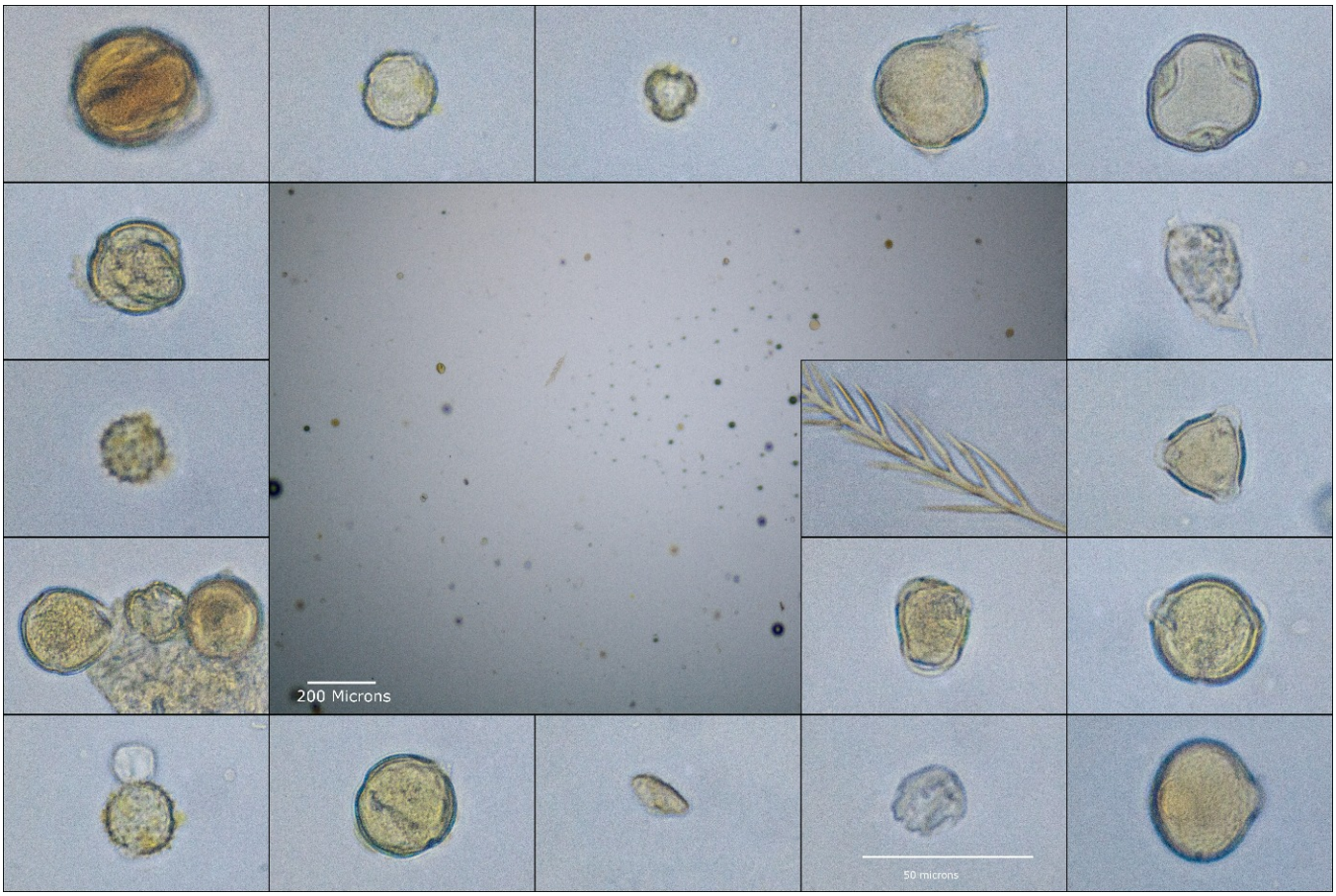
The density calculation has many problems. First, the volume estimate is probably small. I think 0.2 mm³ is a reasonable volume estimate, but the NA of my system was reduced by the iris setting on my condenser, and that affects the depth of focus. It is likely that the actual depth of focus and volume is higher.

Second, we have no idea if the samples we took were representative of the average spoonful of honey. One jar was nearly empty, two were crystallized. The popular flavors of honey were used more often. I don't know how much jar movement affects the distribution of particulates in the honey, but some jars were moved very little over time, and some jars were moved quite a lot.

Third, I didn't have a method for identifying pollen grains at low magnification. Most pollen grains are obvious, but there may be dust or dirt particles that I marked as pollen, and pollen grains that I may have ignored, assuming it was debris.

Last, I can't guarantee that the volume represented in the low magnification pictures does not include the coverslip or slide. It is possible that I picked a focus depth which was not all honey.

Figure 4: Sample D - Door County, Pure Raw, Weinke's Market, USA



After discussing the results with Rob, we decided that even if the nominal densities were not correct, the values still have meaning relative to each other, as they showed clear differences in the amounts of pollen in each brand of honey. We were also excited to see the different kinds of pollen in each brand. There is more work to be done, both on improving this process and analyzing the results, but the project was fun and surprisingly productive for an amateur microscopist.

Pictures of a sample low magnification picture along with high magnification images of pollen and other finds are at: http://www.flickr.com/photos/28594931@N03/sets/72157633188570220/

■

Figure 5 (bottom): Sample E - Aunt Sue's, SueBee, Raw-wild natural, 100% pure strained honey, USA

Figure 6 (next page): Sample F - Orino Greek Honey, wild herbs, pine and thyme, Minos Importer, Greece

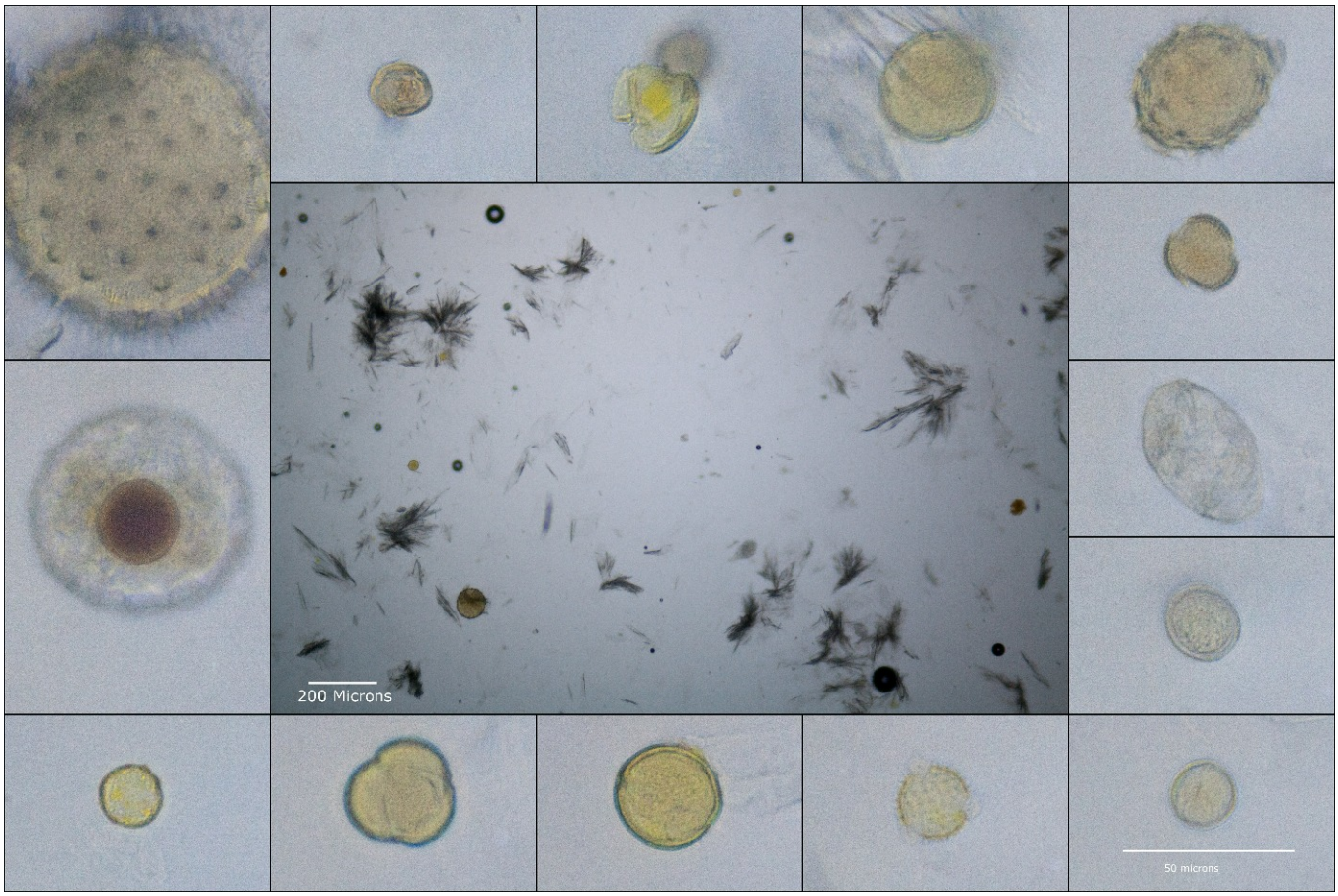
Palynology - Wikipedia

Palynology is the "study of dust" (from Greek παλύνω - palunō, "strew, sprinkle" and -logy) or "particles that are strewn". A classic palynologist analyses particulate samples collected from the air, water, or from deposits including sediments of any age. The condition and identification of those particles, organic and inorganic, give the palynologist clues to the life, the environment, and energetic conditions that produced them.

The term is sometimes narrowly used to refer to a subset of the discipline, which is defined as "the study of microscopic objects of macromolecular organic composition (i.e. compounds of carbon, hydrogen, nitrogen and oxygen), not capable of dissolution in hydrochloric or hydrofluoric acids." It is the science that studies contemporary and fossil palynomorphs, including pollen, spores, orbicules, dinocysts, acritarchs, chitinozoans and scolecodonts, together with particulate organic matter (POM) and kerogen found in sedimentary rocks and sediments. Palynology does not include diatoms, foraminiferans or other organisms with siliceous or calcareous exoskeletons.

The earliest reported observations of pollen under a microscope are likely to have been in the 1640s by the English botanist Nehemiah Grew who described pollen, the stamen, and correctly predicted that pollen is required for sexual reproduction in flowering plants. As microscopes began to improve, further studies included work by Robert Kidston and P. Reinsch who examined the presence of spores in coal and compared them to modern spores. The early pioneers also included Christian Gottfried Ehrenberg (radiolarians, diatoms and dinoflagellate cysts), Gideon Mantell (desmids) and Henry Hopley White (dinoflagellate cysts).

The earliest quantitative analysis of pollen was published by Lennart von Post who laid out the foundations of modern pollen analysis in his Kristiania



lecture of 1916. Pollen analysis was initially confined to Nordic countries because many early publications were in Nordic languages. This isolation ended with the publication of Gunnar Erdtman's thesis of 1921 when pollen analysis became widespread throughout Europe and North America for use in studies of Quaternary vegetation and climate change.

The term palynology was introduced by Hyde and Williams in 1944, following correspondence with the Swedish geologist Antevs, in the pages of the Pollen Analysis Circular (one of the first journals devoted to pollen analysis, produced by Paul Sears in North America). Hyde and Williams chose palynology on the basis of the Greek words paluno meaning 'to sprinkle' and pale meaning 'dust' (and thus similar to the Latin word pollen).

Chemical digestion follows a number of steps. Initially the only chemical treatment used by researchers was treatment with KOH to remove humic substances; defloculation was accomplished through surface treatment or ultra-sonic treatment, although sonification may cause the pollen exine to rupture. The use of hydrofluoric acid (HF) to digest silicate minerals was introduced by Assarson and Granlund in 1924, greatly reducing the amount of time required to scan slides for palynomorphs.

Some steps of the chemical treatments require special care for safety reason, in particular the use of HF which diffuses very fast through the skin and, causes severe chemical burns, and can be fatal. Other treatment include kerosene flotation for chitinous materials.

Once samples have been prepared chemically, they are mounted on microscope slides using silicon oil, glycerol or glycerol-jelly and examined using light microscopy or mounted on a stub for scanning electron microscopy.

Researchers will often study either modern samples from a number of unique sites within a given area, or samples from a single site with a record through time, such as samples obtained from peat or lake sediments. More recent studies have used the modern analog technique in which paleo-samples are compared to modern samples for which the parent vegetation is known.

When the slides are observed under a microscope, the researcher counts the number of grains of each pollen taxon. This record is next used to produce a pollen diagram. These data can be used to detect anthropogenic effects, such as logging, traditional patterns of land use or long term changes in regional climate.

Palynology can be applied to problems in many fields including geology, botany, paleontology, archaeology, pedology (soil study), and physical geography.

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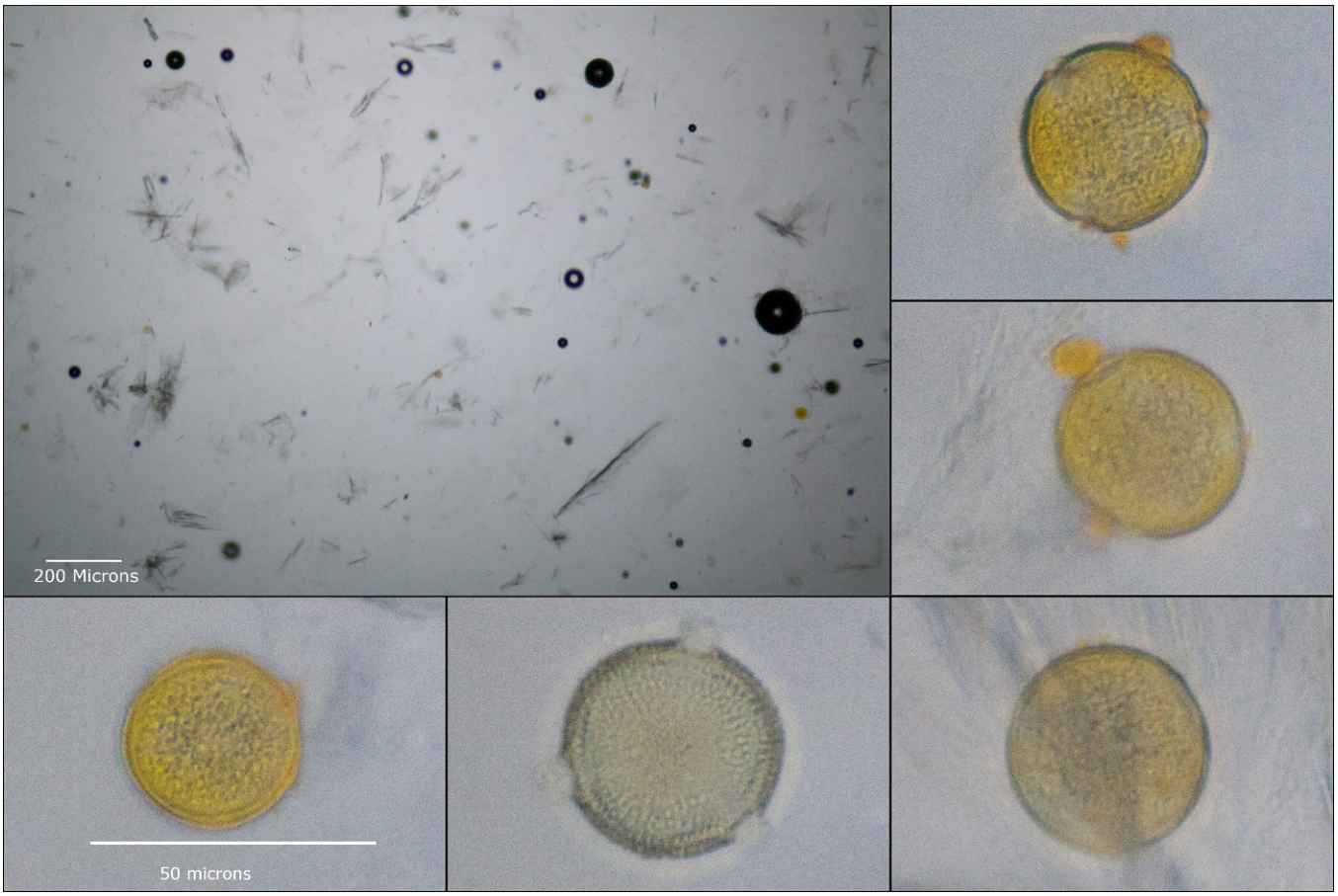
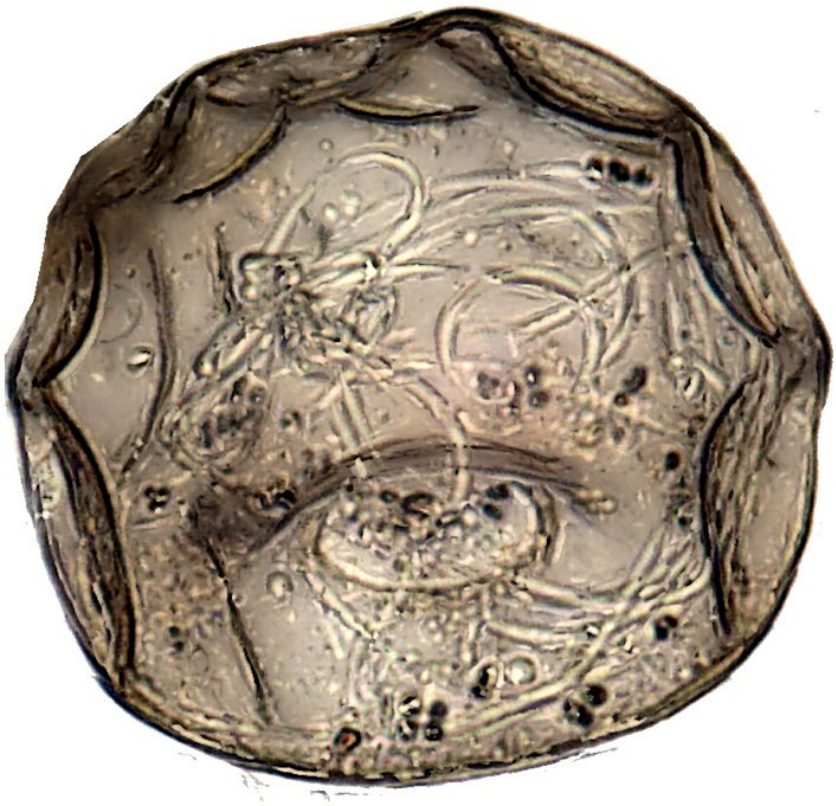
Text taken from:
"Palynology." Wikipedia. Wikimedia Foundation, 19 Sept. 2013. Web. 06 Oct. 2013.



Arcella gibbosa

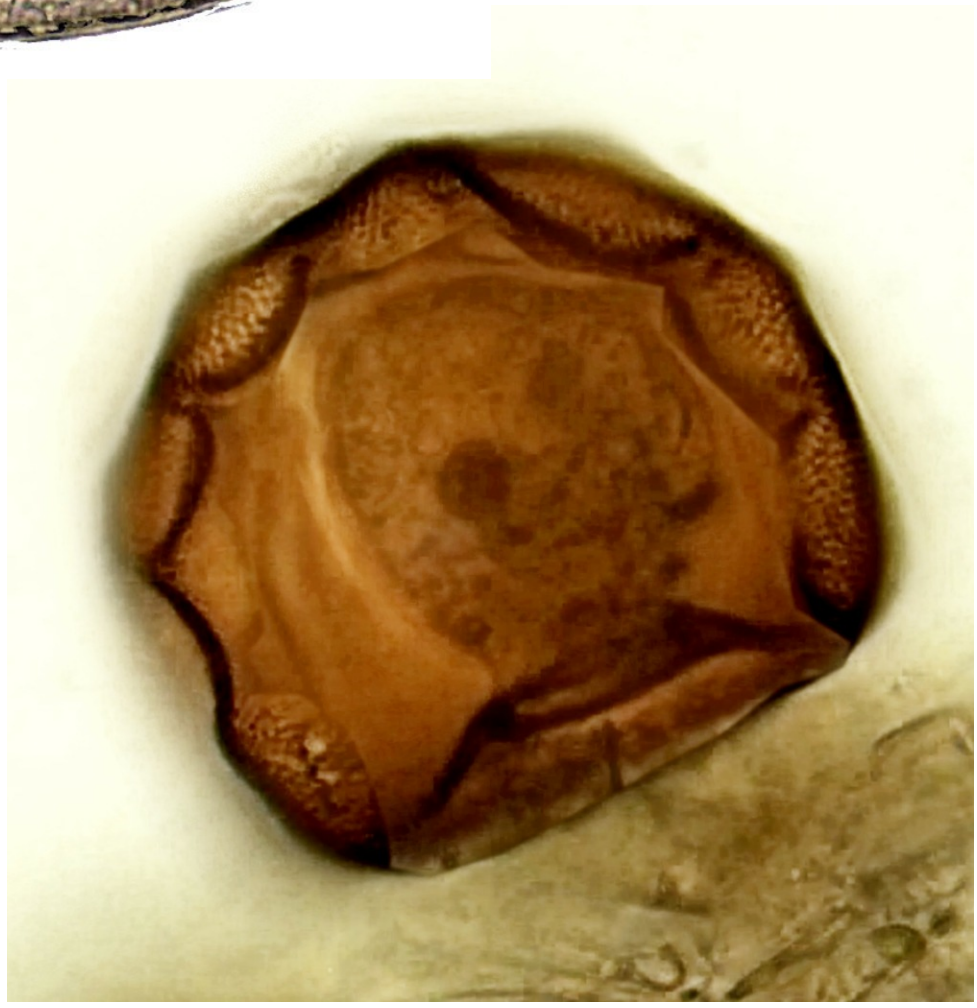


***Arcella gibbosa*.**

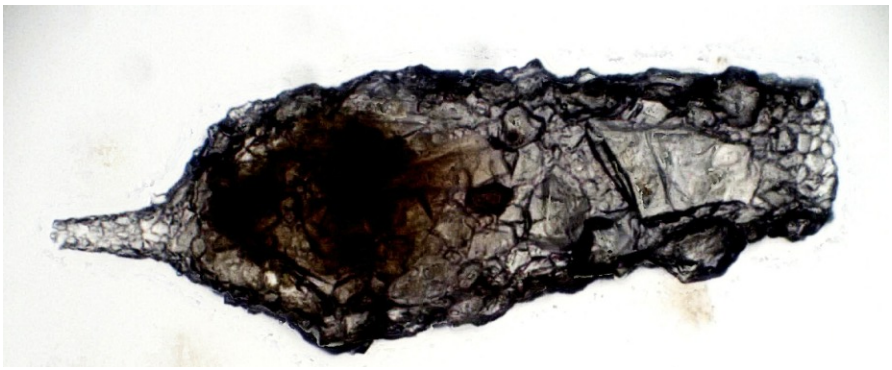
The challenge when photographing amoeba tests is to produce a 3-dimensional impression of the shape.

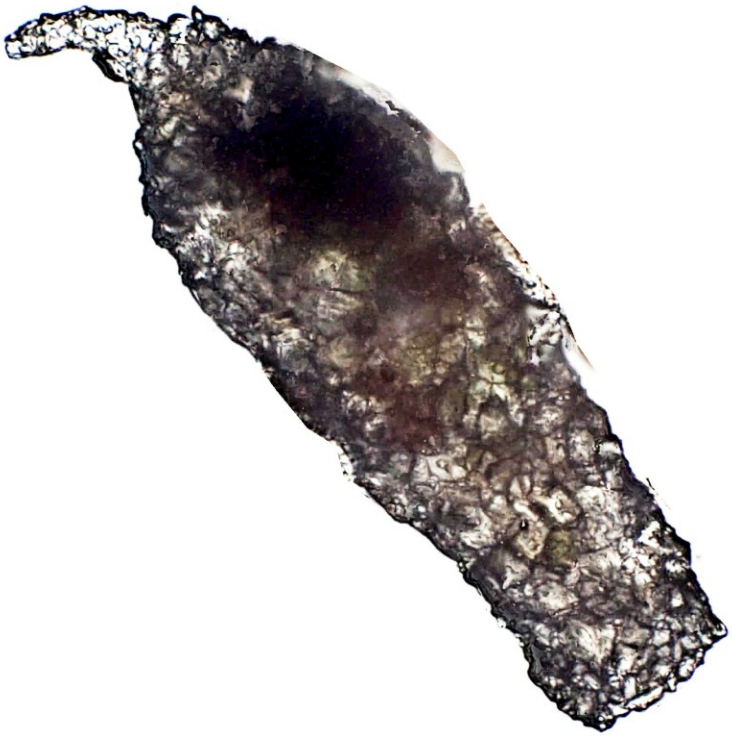
Top image: Almost line-art, see the ingested long cyanobacterial filament (91µm wide).

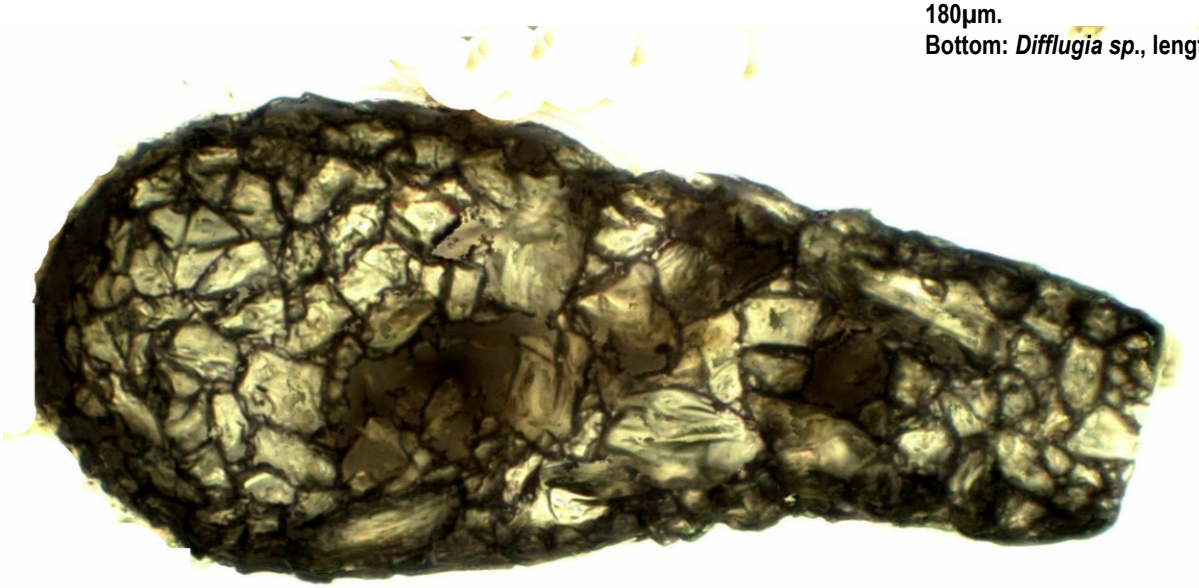
Bottom image: Composed of two layers, the top layer cut out to visualize cytoplasm and pseudostome (83 µm wide).



All images on page 14 - 15 by Hans Rothauscher







Difflugia

At first sight Difflugia shells are rough rocky objects. But looking closer the "rocks", rather tiny pieces of quarz sand, are arranged after a very sensible plan. Larger grains around the body gradually become smaller towards the tip, and very regular around the pseudostoma.

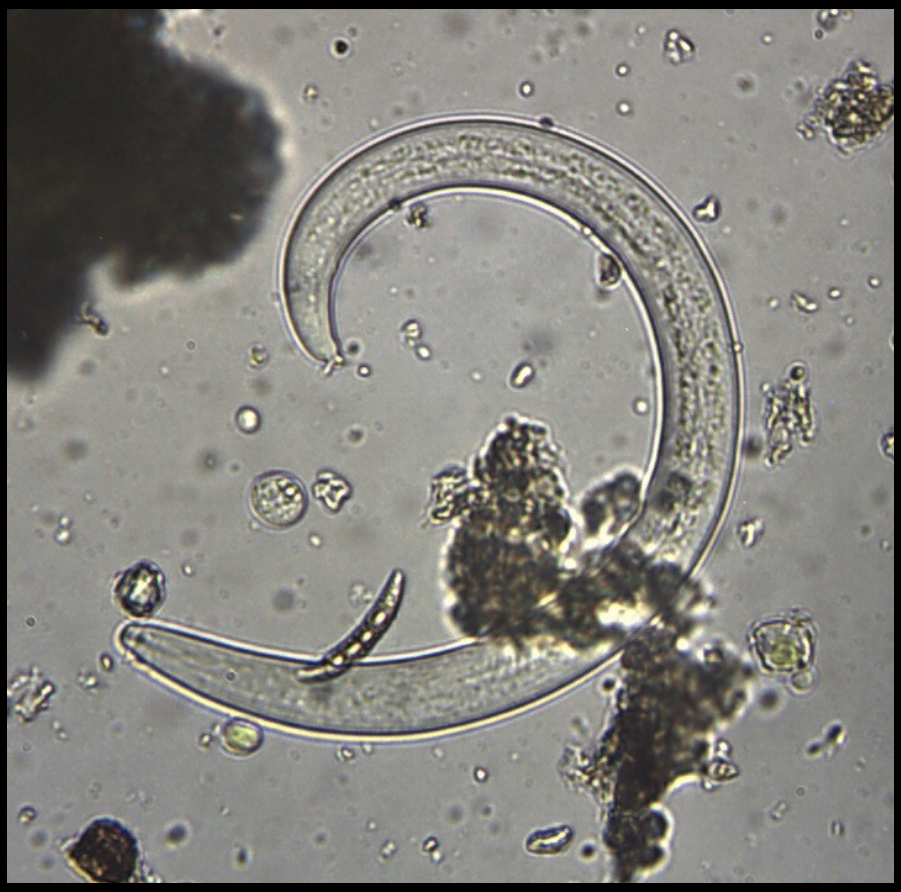
This construction has puzzled biologists, who suspected a planning mind behind it. However, as Biologist Mike Hansell phrased: "you don't need brains to be a builder".

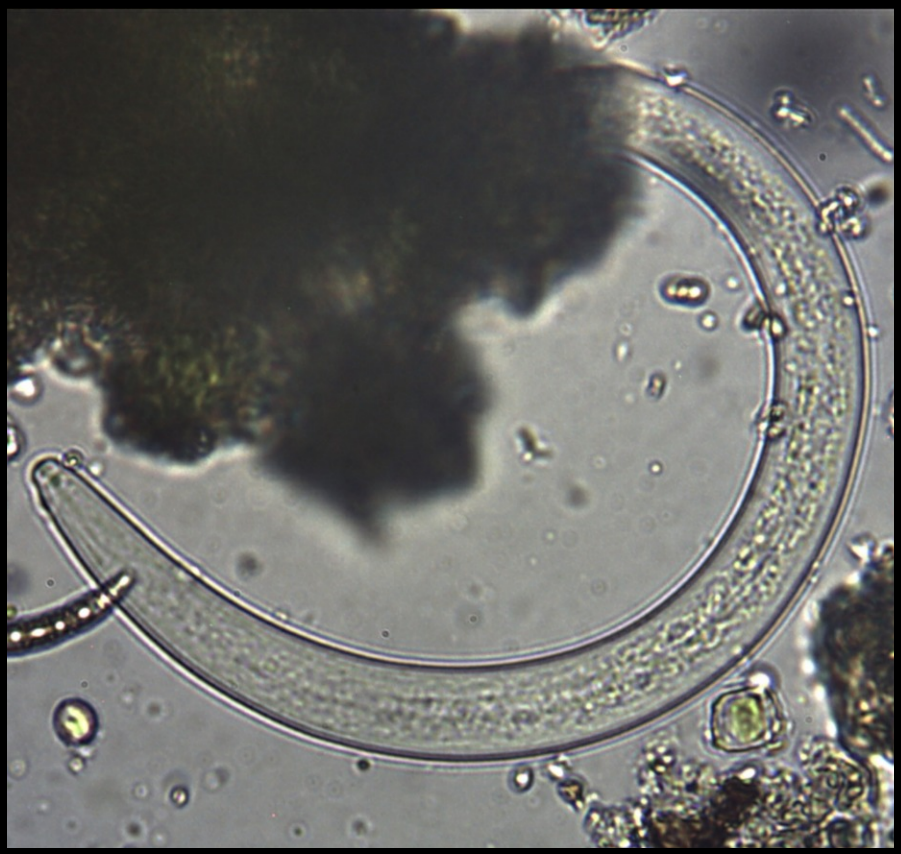
During their life Difflugia ingest with their food the building materials for the next generation and store them in their plasma. Cell division begins with a bud protruding from the aperture. While this bud grows to the size of the mother test, these building materials are gradually transported into the new object and successively move to and arrange over the surface.

The German biologist Ludwig Rhumbler has demonstrated in 1898 that this distribution is caused by simple physical processes. He imitated this process by mixing quarz sand into oil drops. Soon the quarz grains began to move to the surface and arrange themselves over the surface in an order similar to the biological process (described in Schönborn, 1966). The shape and the surface tension of the bud conditions the process.

Top: *Difflugia acuminata,* length 362 µm
Middle: *Difflugia curvicaulis,* length 180µm.
Bottom: *Difflugia sp.*, length 350µm

Nematodes





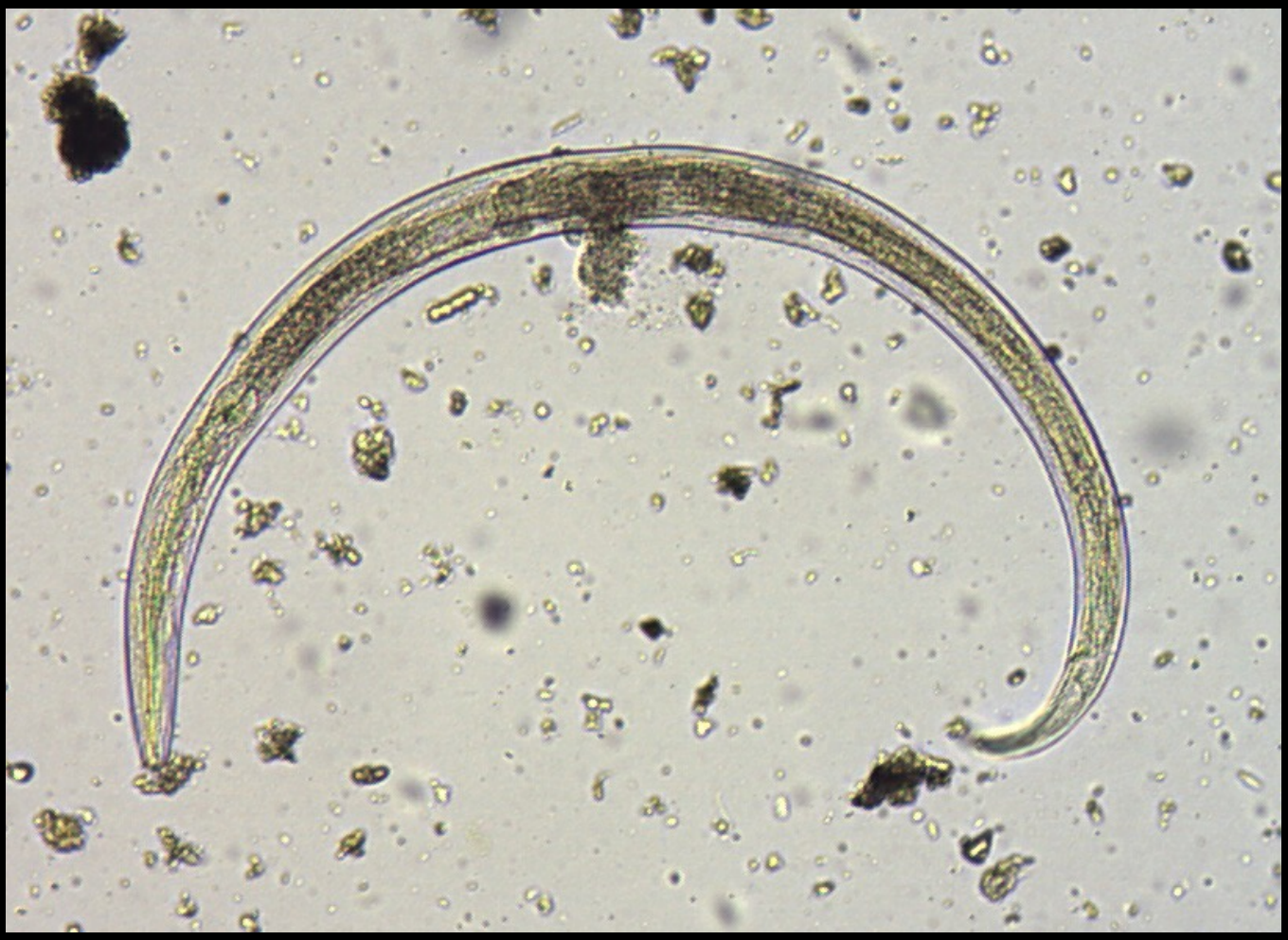
"In short, if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes. The location of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by an examination of their erstwhile nematode parasites." - Nathan Cobb

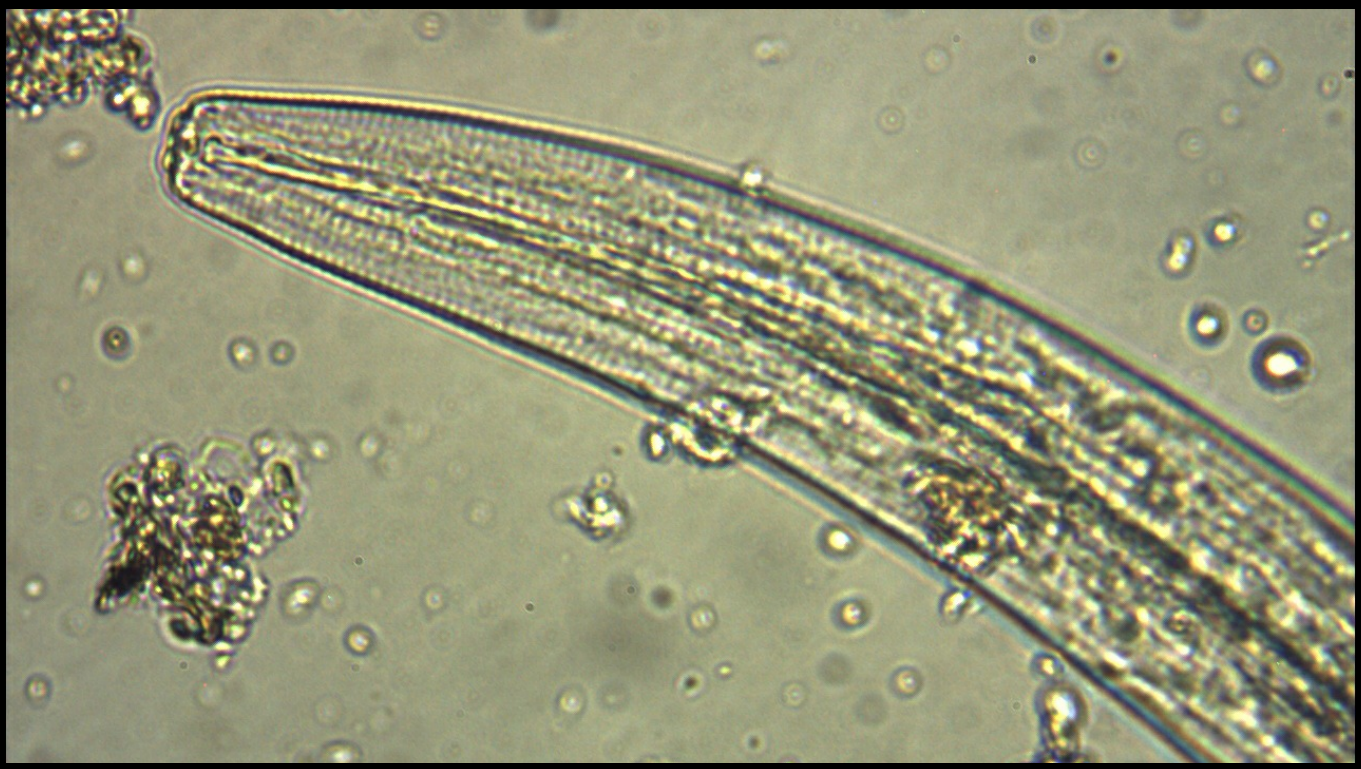
Nathan Cobb (1859-1932) is known as the "Father of Nematology in the United States". He established the basis of nematode taxonomy and founded the Nematology Lab of the US Department of Agriculture.

Cobb, Nathan (1914). "Nematodes and their relationships". Yearbook United States Department of Agriculture. United States Department of Agriculture. pp. 457–90. Quote on p. 472.

Nematodes taken with an Optika B600TPI microscope and a CMOS Optikam B1 camera (1.3 MP) and 20x-40x-60x plan achromat objectives

Images by Luca Monzo





Stereomicroscopes: Part 7

This is part seven of a multi-part series on stereomicroscopes. It continues the discussion of CMOs and presents the first US CMO microscopes, the American Optical Cycloptics

R. Jordan Kreindler

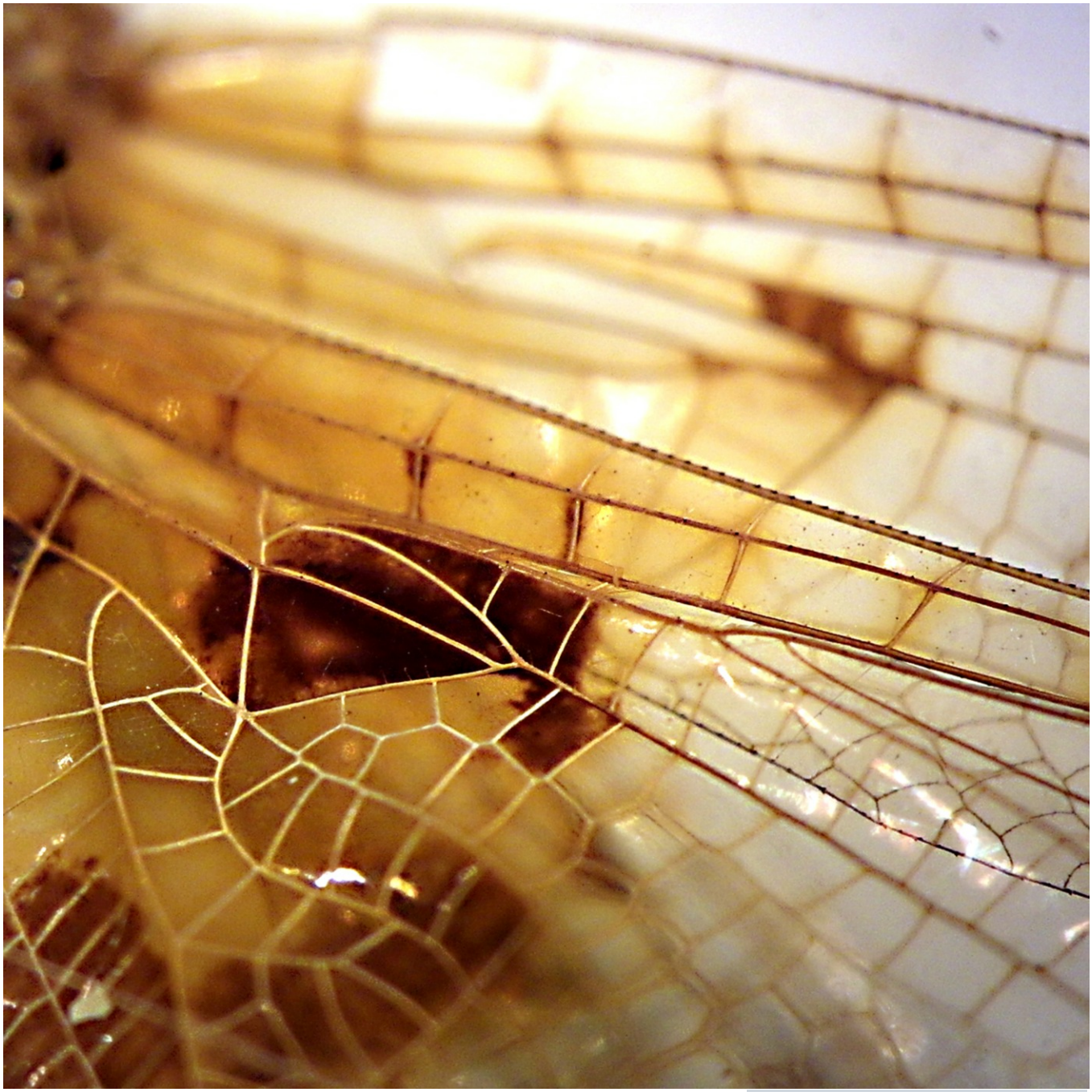


Figure 103: View through CMO of a dragonfly wing

The American Optical (AO) Cycloptics Microscopes

In the aftermath of WW II, any product made in Germany had a hard time getting "traction" in the US and other Western countries' markets. Under these conditions, the first American-made common main objective (CMO) stereomicroscope, AO's Cycloptics quickly became the CMO leaders. Although often presented, e.g., on eBay, as if it was a single product, the Cycloptics are actually a variety of different products, with various magnifications, bases, illumination, etc.

AO introduced the Cycloptic in 1957. It came in four basic magnification configurations. These magnification options are shown below for 10x eyepieces.

"F": Fixed 15x magnification
"J": 7x, 15x, and 25x
"K": 10x, 15x, and 20x
"M": 7x, 10x, 15x, 20x, 25x

Series "J" and "K" offered three optional magnification options, while Series M offered five. These "built-in" magnifications could be modified in two ways, by the use of different eyepieces, e.g., 15x and/or by the use of auxiliary lens attachments, e.g., the 2x magnifier. The cost differences between e.g., "K" and "M" models, calculated from the AO price list of August 1975, was about 7% to upgrade to an "M" from a "J" model, and about 36%, to upgrade from the fixed model "F" .

AO released the Cycloptic in a color they referred to as "Dove-gray". Others might call this color steel gray. All models came with inclined viewing tubes, and, as with most stereomicroscopes, long working distances and erect (non-reversed) images. All models provided for interpupillary distance adjustments.

R. B. Tackaberry, Manager Instrument Development. AO Spencer (American Optical, Unknown) in AO's Cycloptic Reference Manual provides a partial list that sums up some of the microscope's most salient features. The features listed include:

Figure 104, Cycloptic common main objective (CMO), apochromatic triplet

- Apochromatic Objectives
- Long Working Distance
- Full Color Correction
- Uniformly Sharp Field
- Wide Range of Magnifications
- Rapid "Dial-In" Magni-Changer
- Reversible Inclined Body
- Comfortable 30o inclined body
- Dustproof, Watertight, Construction
- Permanent Optical Alignment
- Erect Non-Reversed Image
- Magnification Extending Attachments
- Planned Illumination
- Versatile Transilluminator
- "Cyclospot" Illuminator
- Modern Cabinets
- Interpupillary Adjustment
- Nylon Knobs
- Dove-Gray Epoxy Finish
- Lifetime Lubricants
- Adjustable Focusing Tension
- Attached Stage Clips
- Coated Optics
- Finest Optical System
- Rugged Construction Throughout
- Modern Functional Styling
- 200 Precision Parts
- 3-Element Eyepieces
- Highly Corrected Wide Fields
- High Eyepoints
- Eyeshields
- Mechanical Stage Accessory

In their 1977 manual AO further notes: *"The CYCLOPTIC principle utilizes a highly corrected monobjective whose image is examined with a unique binocular telescope system to yield sharp, clear, enhanced stereoscopic image of the full field of view.*

The objective of the CYCLOPTIC microscope is a true apochromatic triplet (Author: Figure 104) providing a full four inches of working distance.

The binocular telescope system consists of paired achromatic telephoto lens combinations, unique one-piece prisms and new four element wide field, high eyepoint eyepieces which permit comfortable visualization with or with-

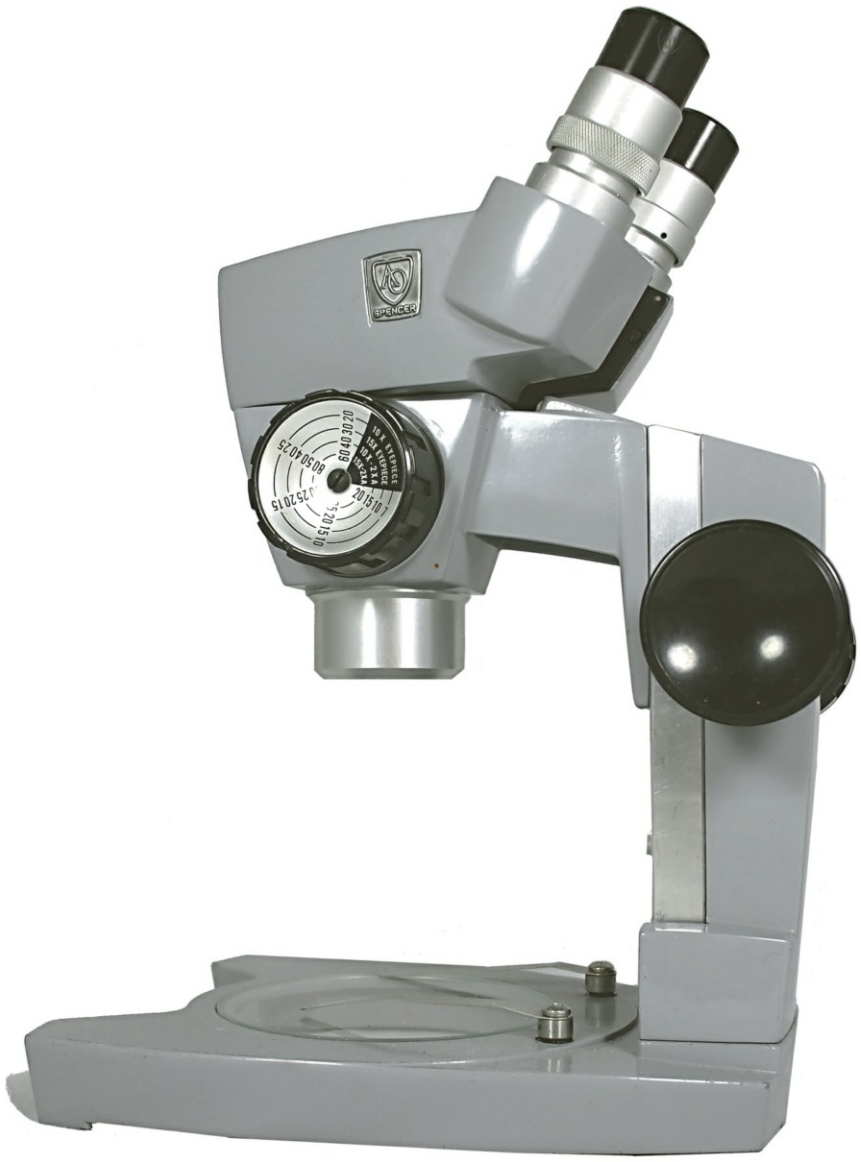


Figure 105 (left): AO Cycloptic model for reflected light, with Magni-Changer "M" (Kreindler, September 2012)

Figure 106 (top): Cycloptic with fixed magnification option

out eyeglasses." (American Optical, 1977).

It is likely the Cycloptic name, in consideration of the CMO, derives from the one-eyed giant, "Cyclops", of Greek and Roman mythology. The Cycloptic's CMO lens is an apochromatic triplet.

Figure 105 shows a portion of the basic binocular Cycloptic stereomicroscope model with Magni-Changer "M". Figure 106 presents the fixed model "F", 1.33X, without Magni-Changer.

The Magni-Changer

Magnification changes using the built-in capabilities of options "J", "K", and "M" were accomplished using the Magni-Changer. The three magnifications options of Magni-Changers "J" and "K" were obtained using 8 achromatic lens elements. Option "M"'s five choices were obtained using 16 achromatic elements.

"*The "M" Cycloptic Magni-Changer shown in Figure 107, contains four clear openings, in opposite pairs, and two telescopes. The clear openings allow for "straight through" images. The dual paired telescopes have four lens groups each. The telescopes can be rotated into the optical path in opposite orientations. This allows, as with standalone telescopes, for the magnification or diminution of images. The drum provides five magnification options. One for the "see-thru" openings, the same in either forward or backward orientations, four additional magnifications using the two telescopes on the drum, in either front or back orientations.*

This type of magnification changer where the same components are used but reversed to obtain different magnifications is often referred to as a "Galilean drum", as here, as the drum actually contains small Galilean telescopes. These telescopes are frequently composed of plano-convex and bi-concave

lenses. Galilean telescopes provide erect images.

The Cycloptic, with its Galilean drum and distinctive external markings to show magnification choices has a unique appearance, and has been used in various US TV shows including, possibly the most popular TV drama series of its time, CSI where it was used by Supervisor Dr. Gil Grissom, one of the show's lead characters."
(Kreindler, September 2012)

Magni-Changer's various magnifications can be selected just by rotating the changer. AO refers to this as "dialed-in" magnifications. Owing to the relatively small price differences between the "J" and "K" Magni-Changers and the "M" Magni-Changer, the "M" was the most popular Cycloptic model, as can be confirmed by the large number of previously owned Cycloptic model "M"s that are available for sale today.

The markings at the ends of the Magni-Changer allow for magnification determination for four different optical accessories. For example, in Figure 108,

Figure 107 (top): AO Cycloptic Magni-Changer

Figure 108 (bottom left): Indented red magnification mark

Figure 109 (bottom right): Side view of right-side of Magni-Changer (seen from front of microscope) showing detent pin

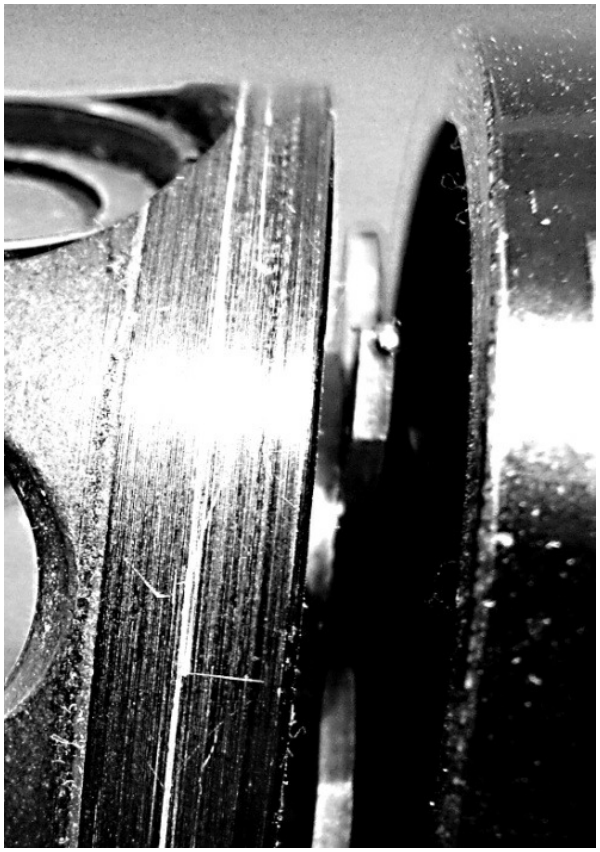


Figure 110 (top): Viewing tubes facing away from column support

Figure 111 (bottom): Viewing tubes facing toward column support

Figure 112 (middle): Hinge pin

those optional accessories, identified on the Magni-Changer, can be seen to be the 10x and 15x eyepiece pairs separately and then these eyepiece pairs combined with a 2x auxiliary lens. A quite useful feature of the Magni-Changer is parfocality. That is, the magnification can be changed, i.e., dialed-in, without the need to change focus.

The best way to use a Cycloptic with Magni-Changer is to focus on the object at the highest magnification, with the instrument raised above the position of best focus. The focusing knob is then turned to bring the object into focus. This approach will minimize focus accommodation, and produce a less fatiguing viewing session. Initially focusing at the highest magnification will insure that when the magnification is changed, the object will still be in focus. The Cycloptic body has a small indented red dot to allow the magnification in use to be identified, Figure 108.

As Dr. Cooke recommends it is always best to, "*... commence the examination with the lowest power of [the] microscope ... the greatest satisfaction will always be derived from a great practical use of low powers.*" (Cooke, 1869)

Thus, although the Cycloptic should first be focused with the greatest magnification available, to ensure parfocality, it should then be used at the lowest magnification needed.

The Magni-Changer has detent pins to insure the magnification selected can be locked into place. The detent pins, Figure 109., snap into a hole in a detent spring. This spring is occasionally missing, allowing the Magni-Changer to rotate freely and continuously when turned.

If the detent spring is missing or broken, the spring can be replaced. However, if an available AO alternate

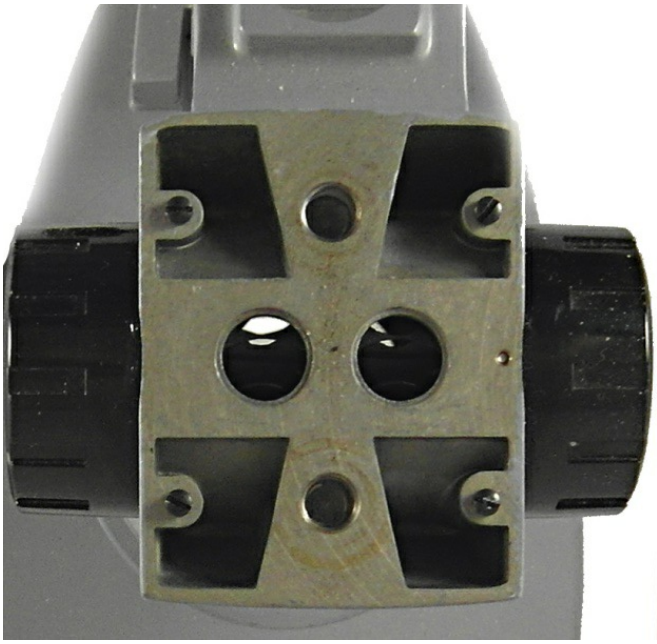


Figure 113 (left): Cycloptic hinge pin

Figure 114 (bottom right): Cycloptic hinge pin unthreaded

Figure 115 (bottom left): Cycloptic plate showing hinge pin receptacles

cannot be found, replacing the broken spring will require machining work (MacGregor, 2007). Even if the detents are no longer functioning, the scope is still fully useable, if care is taken to line up the magnification choices when turning the Magni-Changer.

Reversible Binocular Body

The Cycloptic viewing tubes can be connected to the microscope's frame either facing toward the supporting column or in the opposite direction. Figures 110 and 111 show the viewing tubes in both positions.

The presence of a hinge pin at the joint where the two viewing sections come together makes it easy to change the viewing tubes orientation. Reversing the direction of the tubes is done as follows:

(1) The top of the hinge pin (see Figure 113) has provisions for the insertion of a spanner wrench. Insert a spanner wrench, or thin needle nose pliers - as the AO spanner wrench is often missing - into the openings on the hinge pin's top and rotate counterclockwise.

(2) Continue rotating the hinge pin until it is free from its threads and can be removed (Figure 112).

(3) Remove the viewing tubes, rotate them 180 degrees Place the hinge pin into the rotated assembly in the alternate mounting hole (Figure 115), and rotate the pin clockwise until it is fully tightened.

(4) The rotated assembly is now ready to use (Figure 111).

Cycloptic Bases

AO offered a variety of bases, including two with phenolic-resin boards, models 30A and 30B. The 30B board was relatively large at 12 x 17 inches. They also offered two table boom stands, models 52S (Table Stand Model) and 53S (Universal Table Microscope Stand). The 52S was a pole stand with a pole boom, while the 53S has an almost pillar-like cross bar section.

However, the most popular Series were the 56 and 59, with single component and dual component bases respectively, and the Series 58, which used the transilluminating base (discussed below).

Figures 116 to 118 provide photographs of the popular stands, Series 59 and 56.

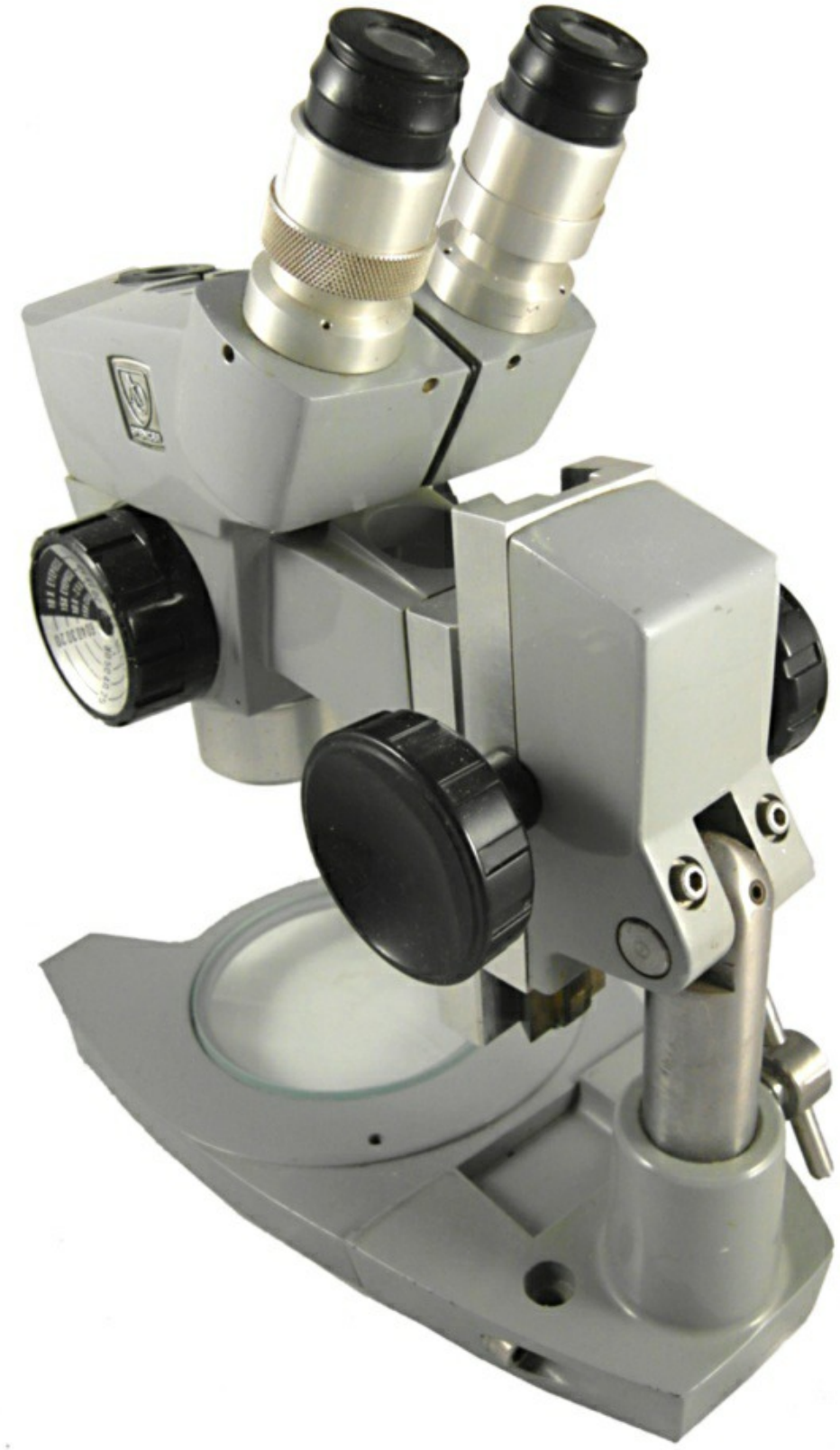


Figure 116: Series 59 in use, showing post receptacle with locking lever, and separated stage, segments. AO designated these two segments 59-851 and 59-852 respectively (shown here without stage clips).

Figure 117 (top): Series 59

Figure 118 (left): Series 56, single section base

Figure 119 (left): Transilluminating substage base unconnected to Cycloptic, and with mirror detached

Figure 120 (right): Cycloptic with Transilluminaing Substage Base installed

Accessories

The most commonly found, and/or helpful, accessories are discussed below.

Transilluminating Substage Base with Mirror: Although stereo microscopes are most often used with opaque subjects, they are at times used with transparent ones. For these applications, the "Transilluminating Substage Base with Mirror" is appropriate. This substage base is shown disconnected and with the mirror detached in Fig. 119 and connected to the microscope in Fig. 120.

Unlike most standard, non-stereomicroscopes, here the mirror is rectangular, not round, and fairly large at approximately 2-1/2 x 2-1/4 inches.

The Transilluminating substage base has a rear opening providing for the insertion of an illuminator (Figure 136). Cycloptic illuminators are discussed below.

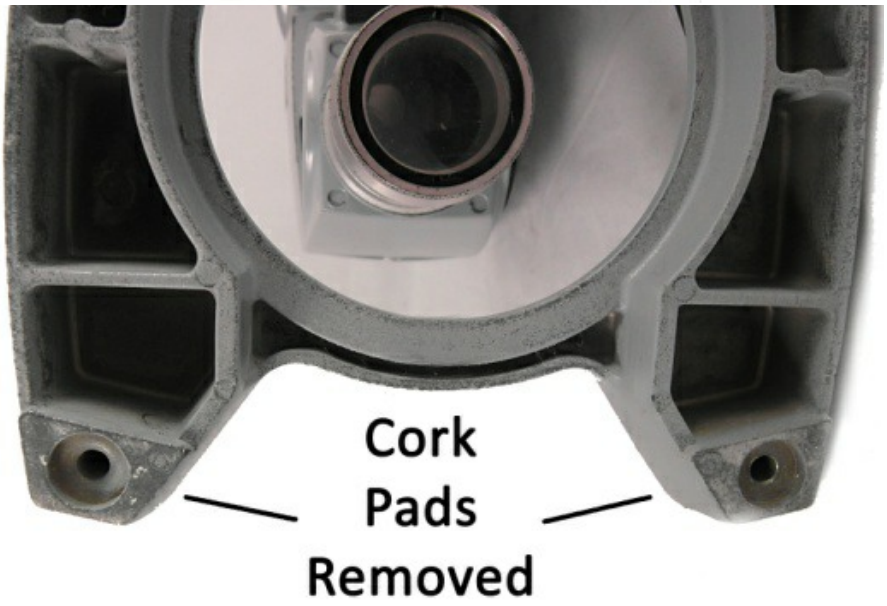
The mirror is two-sided, allowing for the selection of either a matte or mirrored surface.

The optional substage base is easily added to an existing stand. To attach this base to a Cycloptic stereomicroscope Series 56, do the following:

(1) The base of the Series 56 comes with cork pads (Fig. 121) at the distal ends. These must be removed to allow the bosses (i.e., the two protuberances at the top front ends of the transilluminating base) to mate with the bottom of the Series 56 stand.

(2) Once these pads are removed (Fig. 122), place the transilluminator base, face up, against the bottom of the Series 56 stand so that the locating bosses on the substage base line up with the indentions on the bottom of the stand.

(3) The locking screws on the substage base, Figs. 123 and 124, can then be engaged into bottom of the stand and tightened. The microscope with sub-



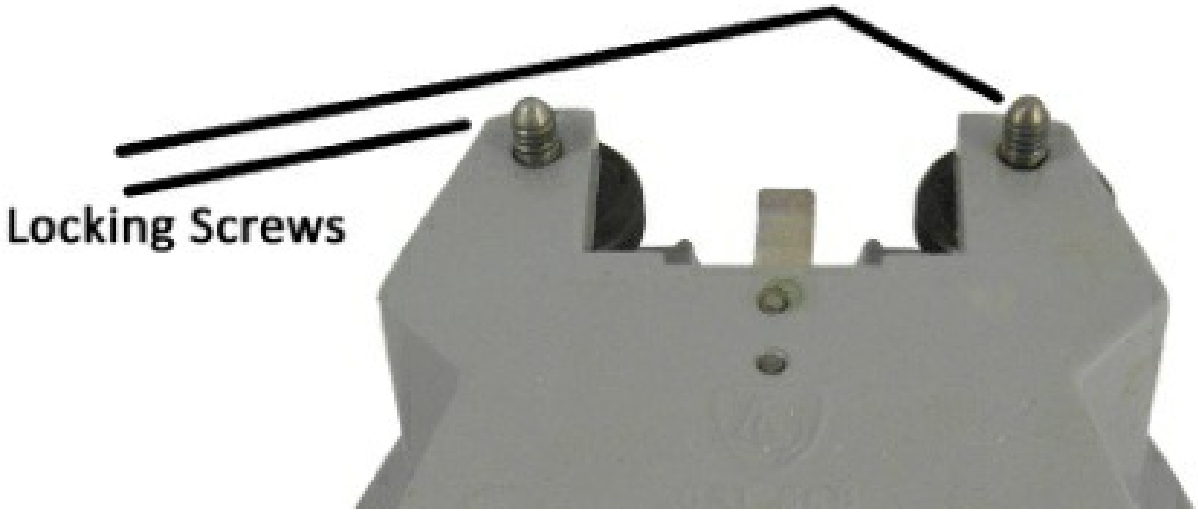


Figure 121 (top): Series 56 cork pads

Figure 122 (middle): Cork pads removed from Series 56 stand

Figure 123 (bottom): Locking screws on Transilluminating Substage Base

Figure 124 (middle right): Thumb adjustments for locking screws on Transilluminating Substage Base, and illumination holder spring.

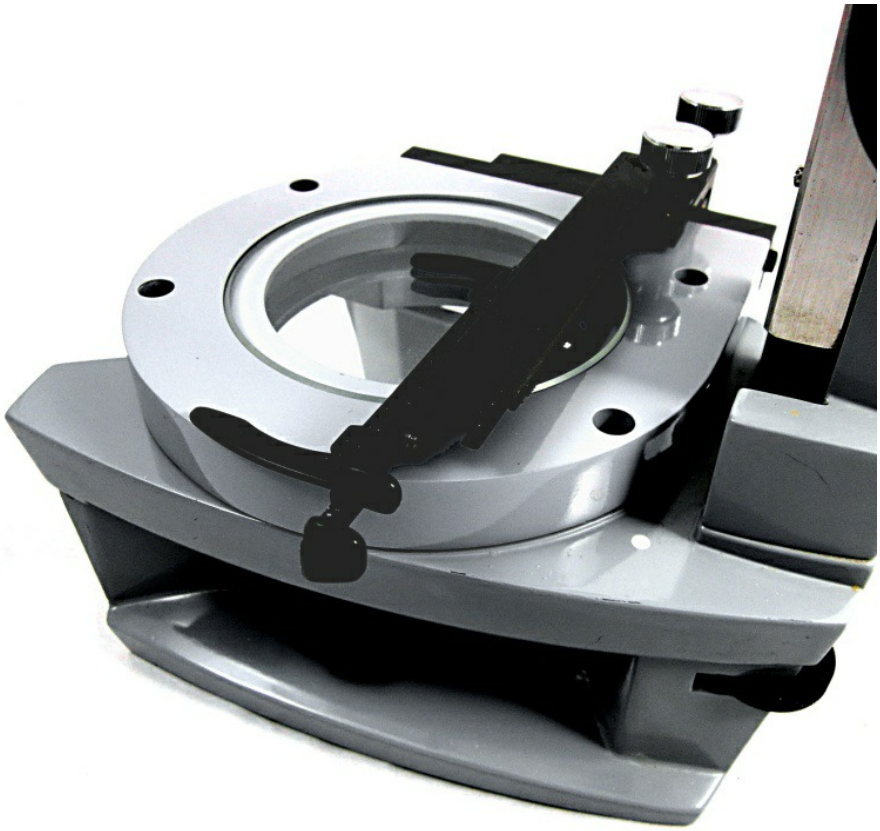
Auxiliary Lens Attachments: Optional auxiliary lens attachments could be obtained for the Cycloptic Series of stereomicroscopes. The most popular of these were the 2X and 2/3X. The 2X attachment was available in two options as either a fixed adapter or a swing out lens. The first two illustrations in Fig. 125 show these options, designated respectively by AO as Nos. 254 and 265. These adapters doubled the magnification, but reduced the working distance to 1.5 inches. The 2/3X attachment identified by AO as No. 267 reduced total magnification and offered a slight increase in working distance to 4.4 inches. The 2/3X option, affixed to a CMO lens is shown as the third illustration in Figure 125c. The 0.5X auxiliary lenses, AO 266 - Figure 125d, doubles the working distance to 8 inches, and halves the magnifying power of the system. It takes the place of the standard CMO.

Eyepieces, Eyeshields and Reticules: High eyepoint 4 element eyepieces were available. These chromatic eyepieces were parfocal. AO designated the 10X as No. 146 and the 15X as No. 147. No. 146's eye relief is 19mm and 147's 17mm. Their respective fields of view are 20mm and 16.8mm. For non-eyeglass viewers optional hard plastic eyeshields (AO No. 149) are available, Fig. 126.

The eyepieces could also be fitted with optional micrometer calibrated reticule disks. A "Lens Protecting Window" (AO 585), could also be purchased for the CMO.

Figure 125 (top): Cycloptic Auxilary lenses (a) 2X Swing-out, (b) 2X fixed examples, (c) 2/3X mounted (d) 0.5X,

Figure 126 (bottom): 10X and 15X eyepiece pairs, and eyeshields



Mechanical Stage: For those using the Transilluminating Substage Base to examine slides, the mechanical stage (AO No. 1556) was available. This stage could be used in the conventional position or reversed to allow left-hand use. It came with a circular glass stage. A picture of this unit mounted on a Cycloptic with Transilluminating Substage Base appears in Fig. 127.

Storage Cases: Storage cases to hold the Cycloptic and some accessories were available for microscopes in Series 56, 58, and 59. Fig. 128 shows a storage case for Series 56, AO No. 1656.

Figure 127 (top): Mechanical stage, No. 1556

Figure 128 (bottom): Storage case for AO Cycloptic

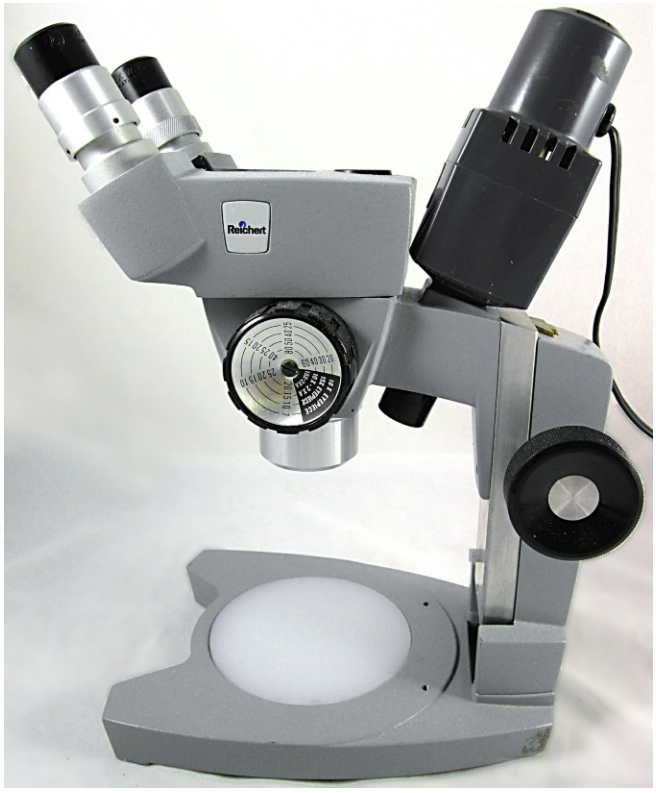


Figure 129 (top left): Starlight Illuminators

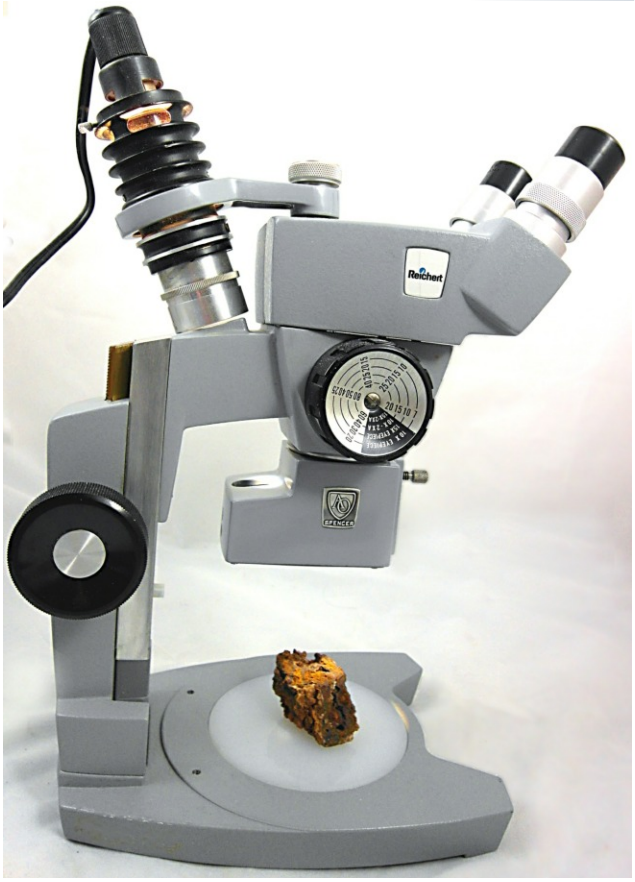
Figure 130 (top right): Starlite Illuminator in use

Figure 131 (left): Sleeves to hold an Illuminator

Illuminators and Illuminator Mounts: The Cycloptic has provisions for a reflected light illuminator to be placed in the circular cutout at the top rear of the microscope's arm, or to shine light through that cutout into an optional vertical illuminator (discussed below). The Transilluminating Substage Base has a similar circular opening in its rear. This allows the insertion of an illuminator into the Substage Base to project light onto the mirror, to be reflected upward through a transparent or semi-transparent object.

A "new", at the time, "Cyclospot Vertical Illuminator" was specifically advertised by American Optical for use with the Cycloptic (note the similarity in names).

All of the illuminators, but one - the Fluorescent Illuminator [AO 640 Series - with aperture and thumb lockscrew for attachment to Cycloptic, complete with two daylight fluorescent tubes, two-button manual starting switch, cord and plug (American Optical, 1962)] - used



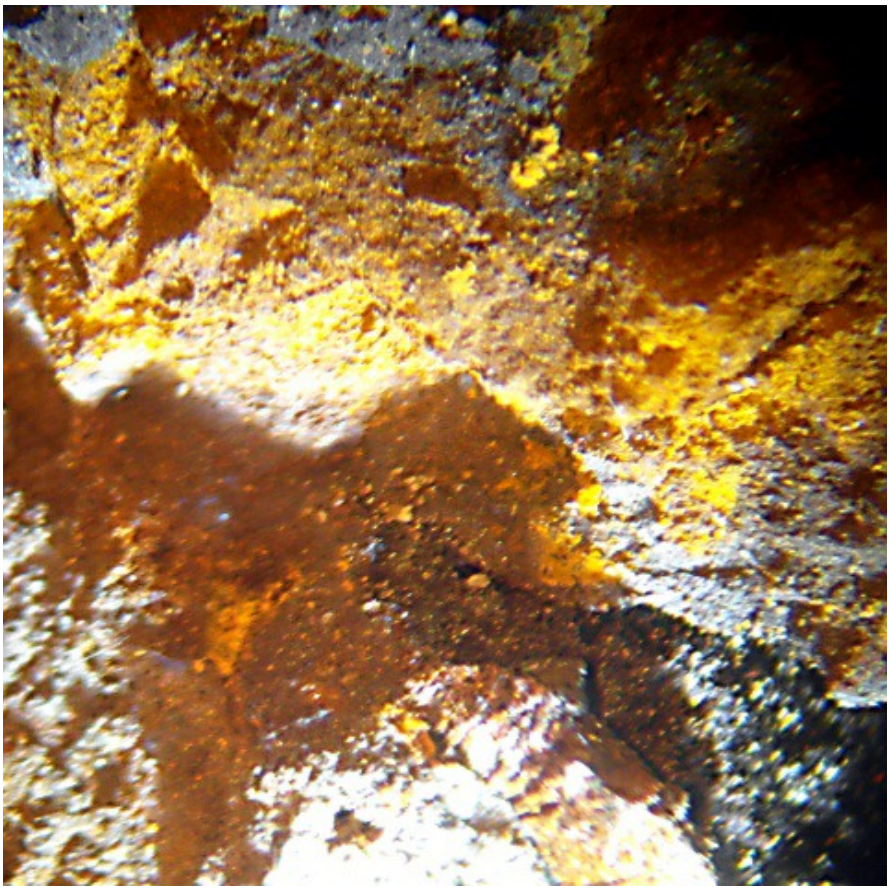


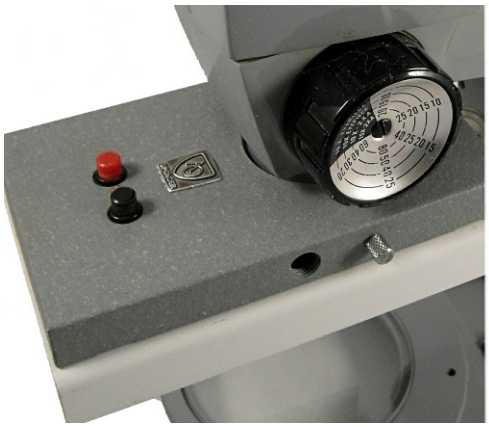
Figure 132 (top left): Cyclospot Illuminator

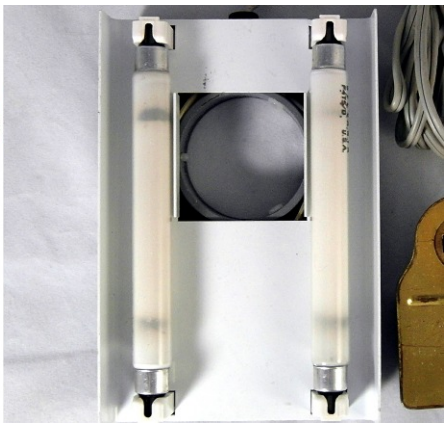
Figure 133 (top right): Vertical illuminator using a lamp connected to an arm attached to the hinge pin. On the stage is hemimorphite (a common sorosilicate) on limonite (hydrated iron oxide - thus the color), Figure 134.

Figure 134 (left): Limonite as seen using Vertical Illuminator

incandescent bulbs. The two most popular illuminators were the Cyclospot, and the Starlite Illuminator (AO 363V). For some of the illuminators, special sleeves that look somewhat like the housing of eyepieces, were provided.

Figure 129 shows two Starlite illuminators, using GE1460 bulbs. They come with flexible multi-section supports, but the lamp could be removed and placed into the circular openings in the top arm of a Cycloptic, or the open-





ing at the back of the Transilluminating Substage Base.

Starlite Illuminators came with fixed or variable transformers. The illuminator on the left, of Figure 92, (an earlier model) was designed for European mains and that on the right for the US. They provided uniform lighting using fixed pre-focused condensers.

The Starlite Illuminators changed little over time, with slight changes to the variable transformer faceplates.

Here the Starlite Illuminator, Figure 130, is used with an AO Cycloptic rebranded with the Reichert name. In recent decades, AO was purchased and eventually merged with various companies. AO is now part of the Leica group.

The illuminator mounting sleeves, as mentioned above, are shown in Figure 131. They were designed so a sleeve could be placed in a circular opening before the Starlite illuminator was inserted. This sleeve is often missing on Cycloptics for sale. [Author's note: Possibly purchasers of pre-owned Cycloptic stereomicroscopes had no idea what the sleeve was for and discarded it.] Fortunately, the sleeve is not required.

Figure 135 (top): Fluorescent illuminator and wall transformer

Figure 136 (left): Cycloptic trinocular with mechanical stage, 2x auxiliary adapter and dual illumination

AO made other illuminators. One mentioned earlier was the Cyclospot. It is shown in Figure 132. This was designed, as its name implies, to mate well with the Cycloptic stereomicroscope. It uses a single on/off switch.

Another popular illuminator was the AO model 653 Universal Microscope Illuminator. In one AO brochure, this is described as having a ... three-link jackknife standard and with 6.5 volt, 2.75 ampere bulb, clear blue glass, ground glass filters and No. 651 Variable Transformer, UL and CSA Approved. (American Optical, Unknown-2). This was designed to connect to the hinge pin and shine light into a Vertical Illuminator that mounts over a Cycloptic CMO or auxiliary lens attachment.

The illuminator contains a multiple lens system that allows the viewer to focus the light beam. A picture of a Universal Microscope Illuminator, with an optional blue filter mounted, is shown in use in Figure 133 (note the adapter arm with screw connected to the hinge pin).

The Cycloptic shown has an opaque white stage plate, and lacks slide clips, as is appropriate for examining thick non-transparent objects. The vertical illuminator contains two reflecting surfaces. The first reflecting surface catches the light from the lamp and reflects it across to the second, which sends it down to the object. This overhead, rather that oblique, illumination allows for the inspection of, e.g., objects with deep cracks or crevices, such as borings in machined parts or for medical procedures where lighting might otherwise be difficult. AO also provided an Internal Vertical Illuminator (K1730) as well, which had its own lamp.

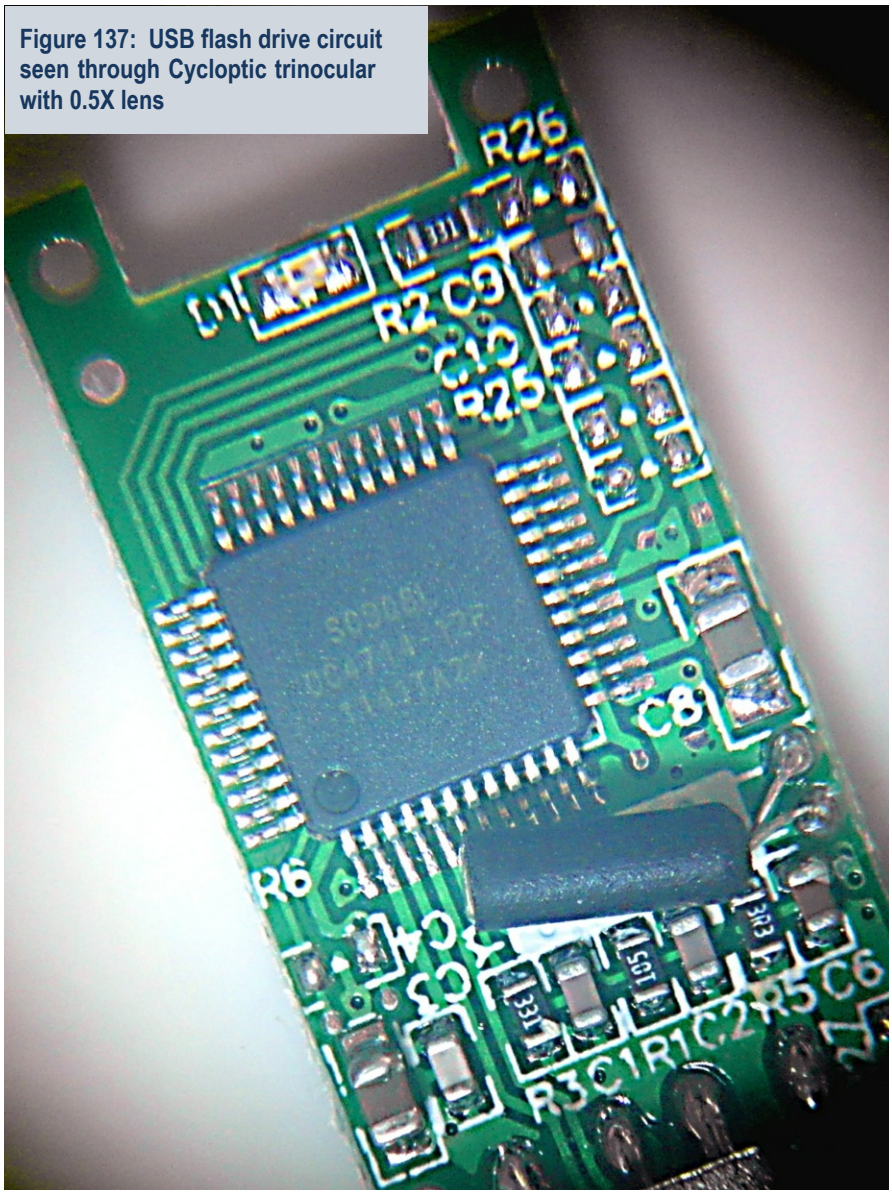


Figure 137: USB flash drive circuit seen through Cycloptic trinocular with 0.5X lens

The use of any microscope mounted reflected light illuminator, except for the fluorescent illuminator of Figure 135, requires the viewing tubes be turned away from the support column, as shown previously, so the cylindrical opening for inserting the illuminator for direct lighting, or shining light through the arm's opening, is accessible. The fluorescent illuminator is mounted directly to the CMO, so the orientation of the viewing tubes is not a consideration for its use. It can also be mounted to a base stand. Because of the fluorescent bulb's voltage requirements, a transformer is a permanent part of the wall plug.

Trinocular Cycloptic (The Photographic Tube Adapter[PTA] - AO No. 638): The PTA is used to convert a binocular Cycloptic into a trinocular. It is easily attached, and focusing is then as normal, but it allows for a flip-up/down mirror to send the light from a binocular tube to the PTA for photography. That is, the trinocular Cycloptic is not a separate microscope, but a binocular with the optional photographic adapter attached.

An example of a trinocular Cycloptic with 2X auxiliary adapter, mechanical stage, and dual Starlite Illuminators is shown in Figure 136. This is a heavy instrument. With its two illuminators, it weighs about 15 pounds. An object seen the a 0.5x auxiliary lens is shown in Figure 137.

Unfortunately, owing to the displaced vertical location produced by the PTA, which raises the height of the arm, the Vertical Illuminator cannot be used appropriately with the Photographic Tube Adapter in place.

The focusing tension on all Cycloptics can be adjusted by holding one coarse focusing knob and turning the other. This is particularly helpful if heavy accessories are used.

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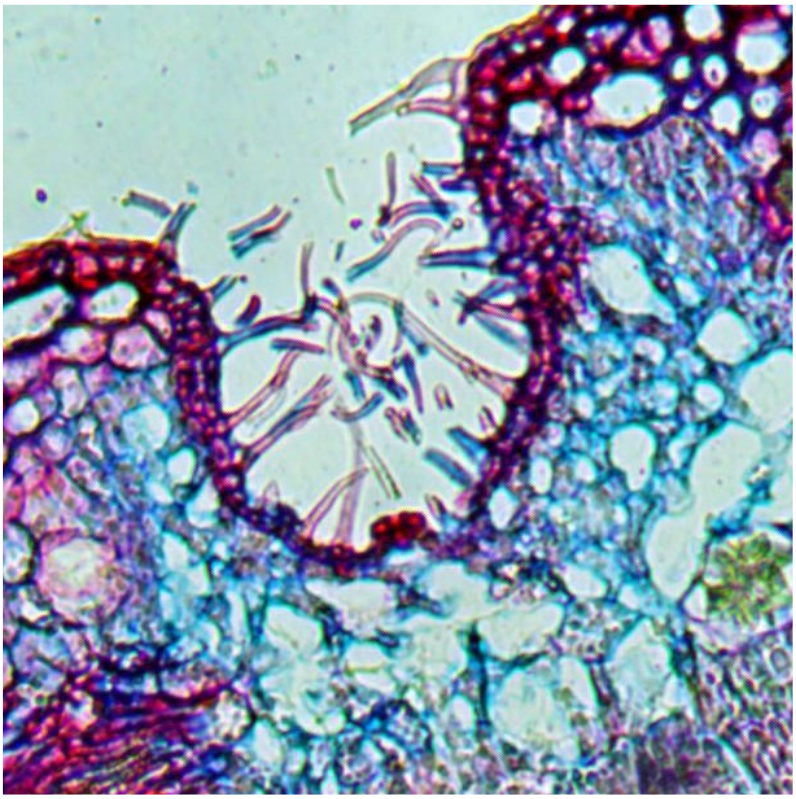

What's this? Answer on page 3.