

Microbe Hunter

Microscopy Magazine

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The Magazine for the
Enthusiast Microscopist

<http://www.microbehunter.com>

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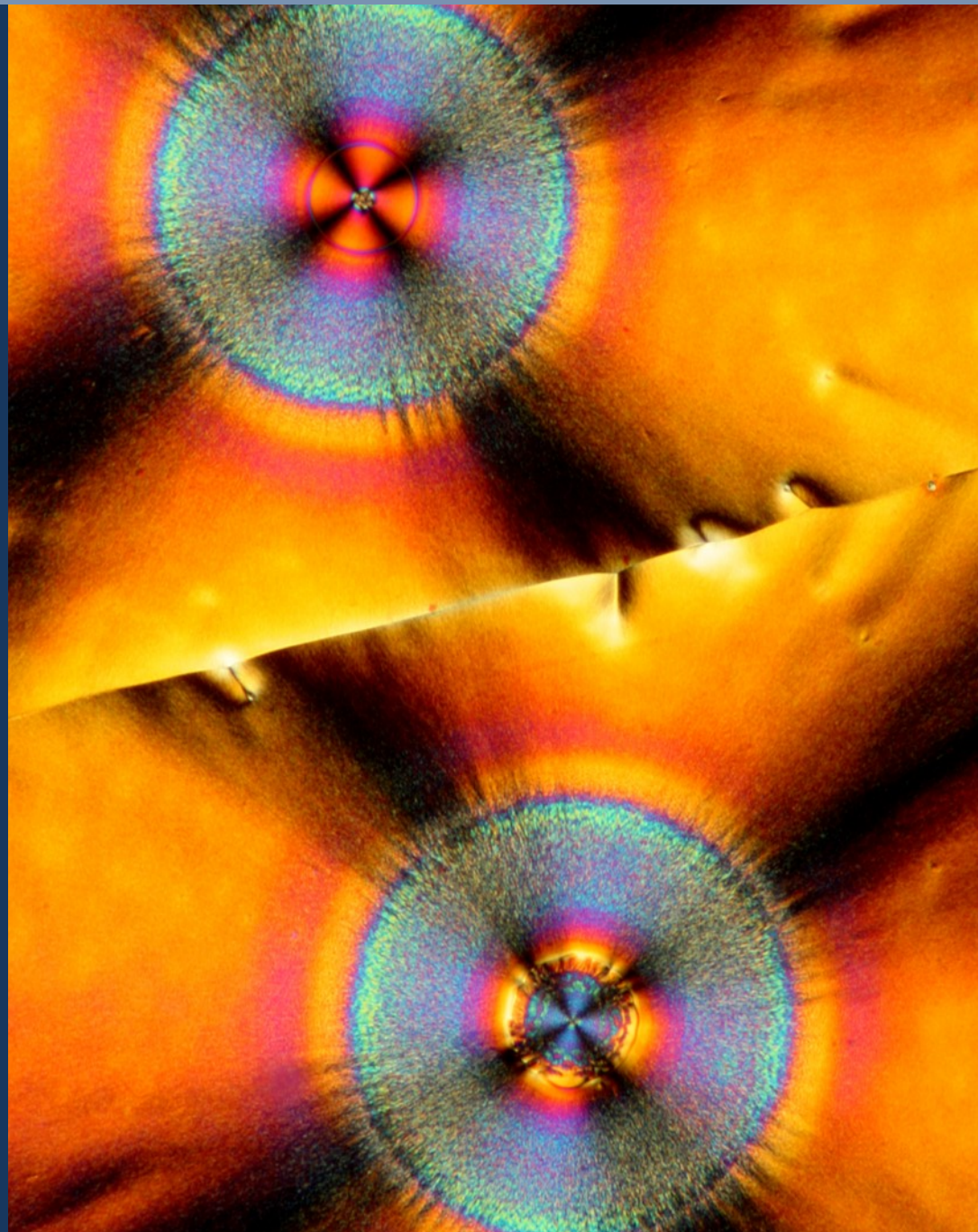
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Title image by Oliver Kim - Vitamin C (ascorbic acid) crystals in polarized light.
Small image: *Chromatium* species (C. Guevara)

About the editor

I don't know if I should consider myself an amateur or a professional microscope user. I'm both, actually. During my Microbiology university study, microscopes were, naturally, an everyday tool. As a biology teacher, I consider microscopes an essential ingredient of a Biology course. In that sense I use these devices professionally. At the same time, however, I like to explore the miniature world, for the love of it, and I found a way to combine the hobby of photography with microscopy and Web design and layout. I've decided to start a magazine to motivate others to pick up this fascinating and educational hobby.

Please Write for Microbehunter!

IMPORTANT

You are now reading the very first issue of Microbehunter Magazine. Please contribute both articles and pictures. Share your experiences, problems and microscopic adventures. If you are a researcher using microscopes, tell the readers what your research is about. Please contribute, even if you consider yourself inexperienced. If you are a struggling beginner, tell us something about the problems that you encountered. If you are an active enthusiast microscopist then share your projects, experiences and observations. Are you a teacher or lecturer? Share your microscopic experiences from school or university. This magazine is made by an enthusiast microscopist for other enthusiasts. Let's work together to make this project a successful one.

Please send all contributions to: editor@microbehunter.com

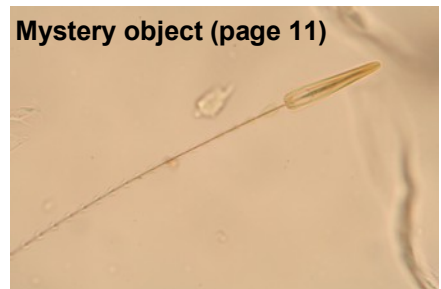
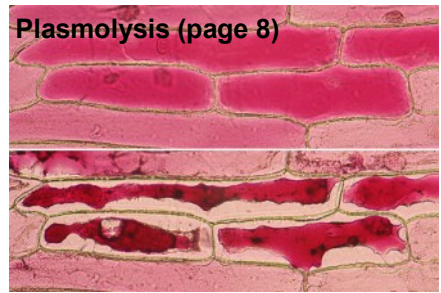
You must own the copyright of the contributions and you retain the copyright of all submitted articles and pictures. While we are not able to pay you for your efforts, we will, of course, give you full credit for your contributions.

Guest Bloggers! Yes, guest blogging is also a possibility. Write microscopy-related blog posts, send them to me and I will publish them on the web site. Naturally, I'll put a link to your blog. Condition: it must be original content and you must be the copyright holder of the text (obviously). When submitting articles, please indicate if you want to have them published on the blog or in the magazine (or both).

Before submitting anything, please read the submissions page on the website: www.microbehunter.com/submissions.

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Pine cone (page 33)

Thoughts

on Amateur Microscopy

By Oliver Kim. Welcome to the very first issue of MicrobeHunter Microscopy Magazine! What made me start an amateur microscopy magazine like this?

I don't know how many of you still remember comet Hale-Bopp, which became visible back in 1996-97. The comet was the incentive for me to become involved in amateur astronomy. The Internet, at that time, was still young and for this reason I obtained most of the info about the comet from astronomy magazines that I bought at the magazine store located in the local train station. I remember, that there were about 5-7 different astronomy magazines (in several languages) in the shop. The range was quite impressive: Some magazines were quite advanced and scientific. Others were much more down-to-earth, targeting readers interested in a more casual and popular approach. I scrutinized them in detail, and found one which had the right mix between science and hands-on advice and one which was just right for beginners. I subscribed to the magazine, looked at the ads, and mail-ordered a

small telescope. I became a star-gazer. Together with two other friends, I spent many hours in the cold night looking at the stars and trying to identify them with the help of a star chart. We had a great time. And I remember that I was even crazy enough to keep a diary of my observations.

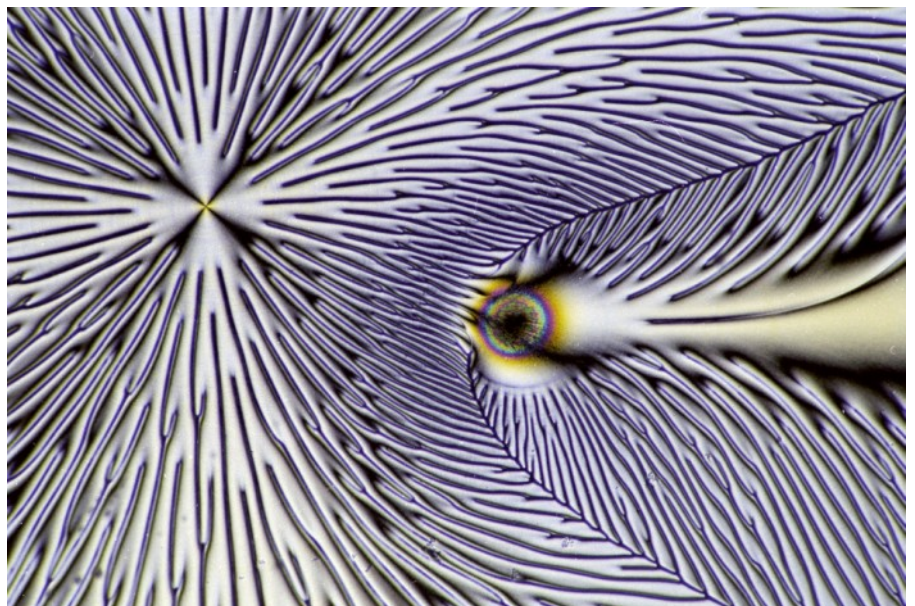
Just one year later, in 1998, I also got involved with amateur microscopy. This was not the first time for me to use microscopes, I did quite a bit of bright-field and phase-contrast work during my university studies, mostly in the field of bacteriology. At that time I used different staining techniques to characterize the bacteria that we isolated, to help us in the identification process. At the end of my studies, I decided to continue microscopy as a hobby and to broaden my microscopic endeavors into exploring my surroundings. I spent a little fortune on a compound microscope. I observed water samples, looked at the algae, purified

diatoms, photographed the crystallization of Vitamin C and citric acid. I also made video recordings of these events and had some nice educational material. But this is a different story.

And yet again I ventured to the magazine shop. I quickly realized that the life of a recreational microscopist resembles the life of a "lone wolf", at least when it relates to the availability of literature. Microscopy magazines with a recreational approach are scarce, at least I could find none of them in the magazine shop. Many science journals from the university library did address a range of microscopy-related issues, but the topics were far too specific and not understandable to someone without a scientific background in the particular research area. In many cases the topics revolved around more advanced electron microscopic techniques – interesting, for certain, provided that you understood the science behind it. I could find no magazines with an "amateur-

Astronomy and microscopy happily united: this micrograph shows crystals of ascorbic acid (Vitamin C) in polarized light. I gave it the title "Star and Comet".

Dissolve a knife-tip of pure ascorbic acid (Vitamin C) in a ml of alcohol or water. Do not use vitamin tablets, they contain too many impurities. Place the solution on a slide and allow the liquid to evaporate. Crystals will form. Do not use a cover glass. Place a linear polarizing filter on the microscope lamp and another one on the slide and observe at low magnification. Rotate one of the polarizing filters. Best results are obtained with thin crystals. Circular polarizing filters (as sold in many photography shops) will not work.



approach". In particular, nice colorful pictures were missing, at least for me, an important motivating factor.

Now why is this? Why is there such an imbalance between amateur microscopy and astronomy? Does it have historic reasons? Maybe the entry barrier into amateur astronomy is higher (equipment costs, knowledge required etc.) and therefore the support by astronomy clubs and organizations may be more important than for microscopy. Maybe these organizations helped to promote and support amateur astronomy over many years and there is a stronger tradition and community. Or could it be, that there are indeed many amateur microscopists around, but that they are simply not organized into clubs and therefore not visible to the public? But even if this were the case, there should be many amateur microscopy magazines around, which is not the case.

Maybe microscopes simply do not offer as many possibilities for the technical tinkerer as telescopes. Amateur telescope making seems to be so popular that this activity even has its own acronym: "ATM". Try to Google "amateur microscope making", and you will be awarded with an astonishing 5

hits (yes this is five). The search string "amateur telescope making" gives you 176000 hits (November 2010).

Write, write, write!

It's time to stop pondering and do something about the status quo. In front of you, you have the very first edition of MicrobeHunter Microscopy Magazine. The published articles in this very first issue may or may not be particularly interesting to you. If they do interest you, then great. If they don't, then I urge you to contribute by writing articles that you would like to read yourself. The content and direction of the magazine depend to a large extent on the articles that I receive from the readers. At this point, I would like to voice my particular thanks to Charles Guevara, who was the first person to submit an article (read "*A Stream Hike for the Collection of Photosynthetic Purple Sulfur Bacteria*" on page 16).

It goes without saying, that the contributors retain their copyright and that the material will only be published in this magazine. I hope that a colorful publication, such as this one, motivates amateurs of all levels to engage (even) more actively in their hobby and to share their experiences. ■

One of our early aspirations especially by the founder Maurice Smith was to extend the hobby beyond the existing microscopy base but we have struggled to do this and on the whole have naturally evolved into a 'by microscopy hobbyists for microscopy hobbyists' type venture. As someone suggested in the thread perhaps making use of the modern social network outlets like blogs, Facebook and Twitter is one key to widening the hobby profile as these seem to be the outlets modern web users adopt.

David Walker
Voluntary Micscape Editor

David, thank you for your encouraging comment. I myself am a regular reader of Micscape Magazine.

Please send letters to:
editor@microbehunter.com

We may have to edit some of the letters for length. The letters represent the views of the writers.

SUPPORT US

The editorial and layout of a magazine like this consumes much time and effort. There are several ways you can contribute to keep this magazine free of charge.

Help by writing articles relating to amateur microscopy. This way we do not have to find freelance writers.

Help by spreading the word about the magazine.

Do not distribute the PDF over file sharing sites and other websites. Do not email the PDF to other people. You must download the PDF only from the MicrobeHunter.com website. This will also give a more reliable statistic on the number of readers and can have a large impact on the advertisement rates for potential advertisers.

Help by ordering a printed copy of the individual magazine issues! The printing and distribution process is handled by an external print-on-demand company. You can therefore always also order back issues.

Help by setting a link to the website (microbehunter.com) This will increase the visibility of the website and the magazine.

Help by giving constructive feedback about the layout of the magazine.

Last, but not least, I'm always happy for emotional and moral support even if I am not able to answer all the emails!

LETTERS TO THE EDITOR

I was delighted to see your proposal in the forum for the microscopy magazine and great to see enthusiastic promises of support. If at any time we can help promote the venture please let me know and will be pleased to add eg an 'In Focus' highlight on the Micscape site and permanent link in the limited links we maintain. Or if you wish to send a general intro and/or call for support at any time, we would be pleased to share this.

In the meantime I'll add a permanent link to your excellent microbe hunter website.

As you say it's a struggle to get amateur microscopy the same profile that amateur astronomy seems to enjoy and a variety of resources to raise awareness is a key I think to raising its profile. Your decision to go the attractive laid out pdf route from the start is a good one I think.

Why Buy Vintage Microscopes?

By Harvey Wainwright. "Why not?" One might be inclined to ask! Microscopes can not only be considered scientific instruments, but also works of art documenting scientific history.

Microscopes have become an important part of science. In today's world, very few scientific experiments can be done without the help of a microscope, and especially so if it happens to be something to do with the life sciences. Microscopes are not only to be found in laboratories where scientists work, but also in school and colleges as well because it is believed that the earlier students are exposed to the benefits of using it, the better it is for them. True, to view the minutest objects of nature's creation in clear detail is something that is possible only with a microscope.

There are many brands of microscopes available today and each brand has a number of models. But if you are looking for a piece that is going to be a classic, opting for a vintage microscope may be just the idea you are looking for.

A vintage microscope is one that has withstood the test of time. As you must be aware of, the first microscope came to be invented way back in the 15th century. From that rudimentary stage, microscopes have come a long way. During that time, microscopes could only be used to view small objects. Today, they can be used to view specimens as small as microbes, bacte-



A 1915 Bausch and Lomb Optical.

Richard Wheeler, Zephyris (CC-BY-SA)

ria and even cells and neutrons. Yes, microscopes are a far more powerful lot today. But can the all- new microscopes beat the charm of a vintage microscope? That is a debatable issue.

A vintage microscope is a microscope that is 40- 50 years old. You can even find one that is perhaps a century



A pocket microscope with leather case. Vintage microscopes neither have to look conventional nor polished to have collector's value.

Roger McLassus (CC-BY-SA)

old. If you are wondering what one would do with a relic item, you should know that there are a lot of people vying to get their hands on a good old microscope that has seen its years! Yes, the market for such microscope is quite alive with action.

There are many people in this world

But can the all-new microscopes beat the charm of a vintage microscope?

who love to collect things, no matter how functional they are. We are sure you must have heard of people who spend a fat wad of cash to buy an old gramophone record and even buy records to go with it! Do these gramophones play better-quality music than the erstwhile Bose speaker or even an iPod? No, of course they don't. But the fascination some people have for vintage items can only be fathomed by them!

Vintage microscopes are timeless pieces of classic equipment for you to buy. They are collector's items a lot of people try to get their hands on. There are many dealers on the internet who sell vintage microscopes. Watson and Philip Harris are brands you should look out for though there are a lot many available. But remember, there are limited pieces available and you need to hurry up if you want one.

Vintage microscopes are perfect for showing off. Having one in your living room will have all your friends and family swoon over it whenever they pay a visit! ■

Harvey Wainwright is an expert copy-writer and has been writing for over twenty years. He specializes in articles about internet marketing and any interesting niches he comes across to share with readers. Reference material for this article came from www.antiquemicroscopes.com, where you can read about Antique Microscope Parts.

Article Source: http://EzineArticles.com/?expert=Harvey_Wainwright



Daderot (public domain)

French microscope (ca. 1745-65) Cleveland Museum of Art.



Alan.hawk (CC-BY-SA)

This microscope was manufactured by Christopher Cock of London for Robert Hooke (Billings Microscope Collection)



Laurent Bourdare (CC-BY-SA)

Slightly more modern, this microscope is already made of metal and not of wood.



Andrew Dunn (CC-BY-SA)

This microscope can be found in the Whipple Museum of the History of Science in Cambridge.

Plasmolysis of red onion cells

Adding concentrated salt water to red onion cells can reveal quite a bit about the physiology of plant cells.

Plasmolysis refers to the loss of water by a cell. It can be induced by adding salt water. Osmosis will cause the water from the cells to move out, thereby causing a shrinkage of the cell's cytoplasm. The process can be reversed by adding pure water. The cells of a red onion are suitable to observe plasmolysis. The red pigment is not able to leave the cytoplasm of the cell, thereby improving the color contrast.

Method - obtaining a single layer of red onion cells

For this experiment, we can not use the onion skin which is found between the layers of the onion. We need a single layer of pigmented cells. These cells, however, do not separate easily.

1. We need a thin layer of cells of the red part of the onion. It is not possible to directly cut a single cell layer, so we need to use the "peeling method" to obtain a single layer of cells. Obtain a small piece of onion about (1cm x 1cm). The onion layer is about 2mm thick.

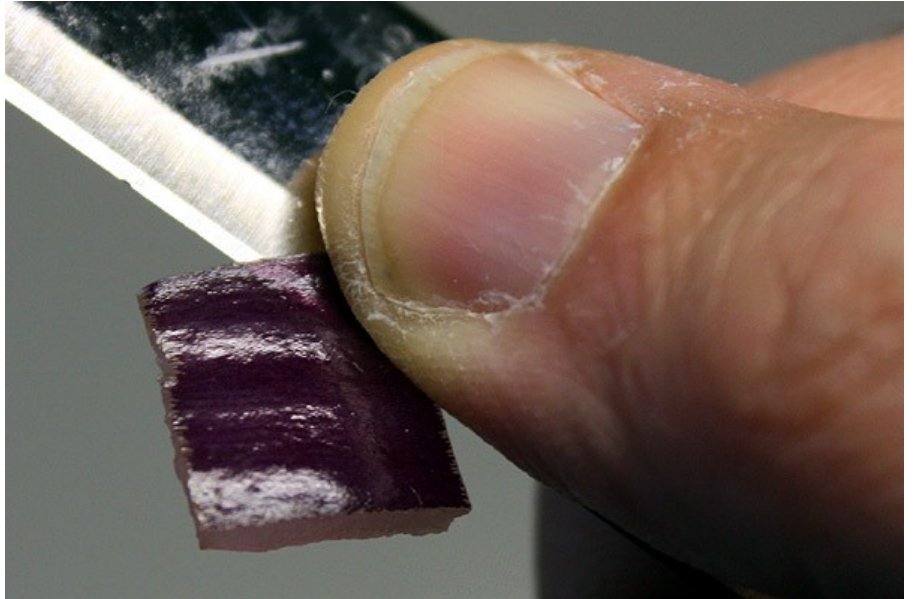
2. With the red side of the onion facing you, cut beneath the red layer, about half way into the onion. This cut does not have to be very thin. There will be about 1mm of onion between the knife and the red pigmented layer.

3. Press the onion firmly against the knife with your thumb.

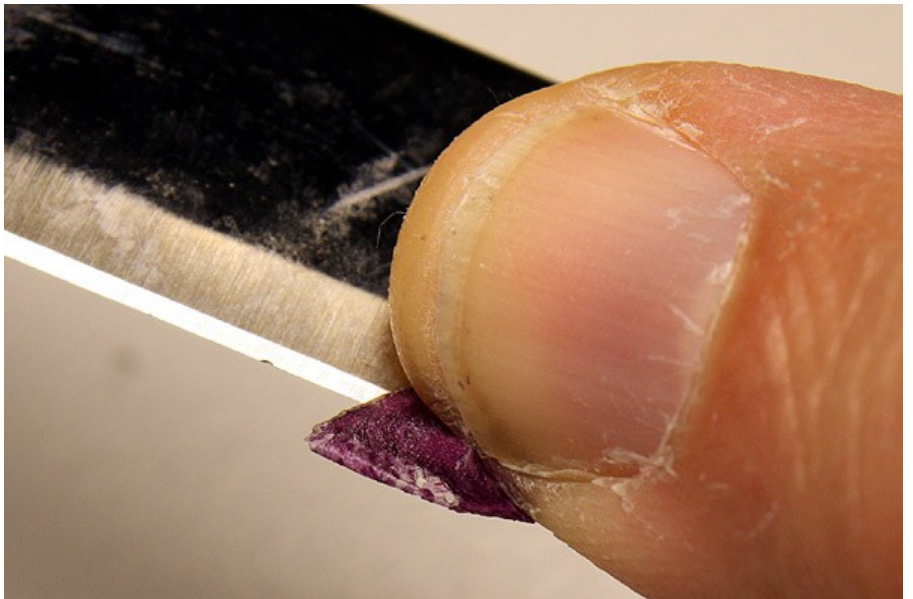
4. Now tear off or peel away the red part of the onion. The red layer will become thin. Some red pigment may be released from broken cells.

5. Cut away and discard the thick part of the onion (the place where the initial cut was placed).

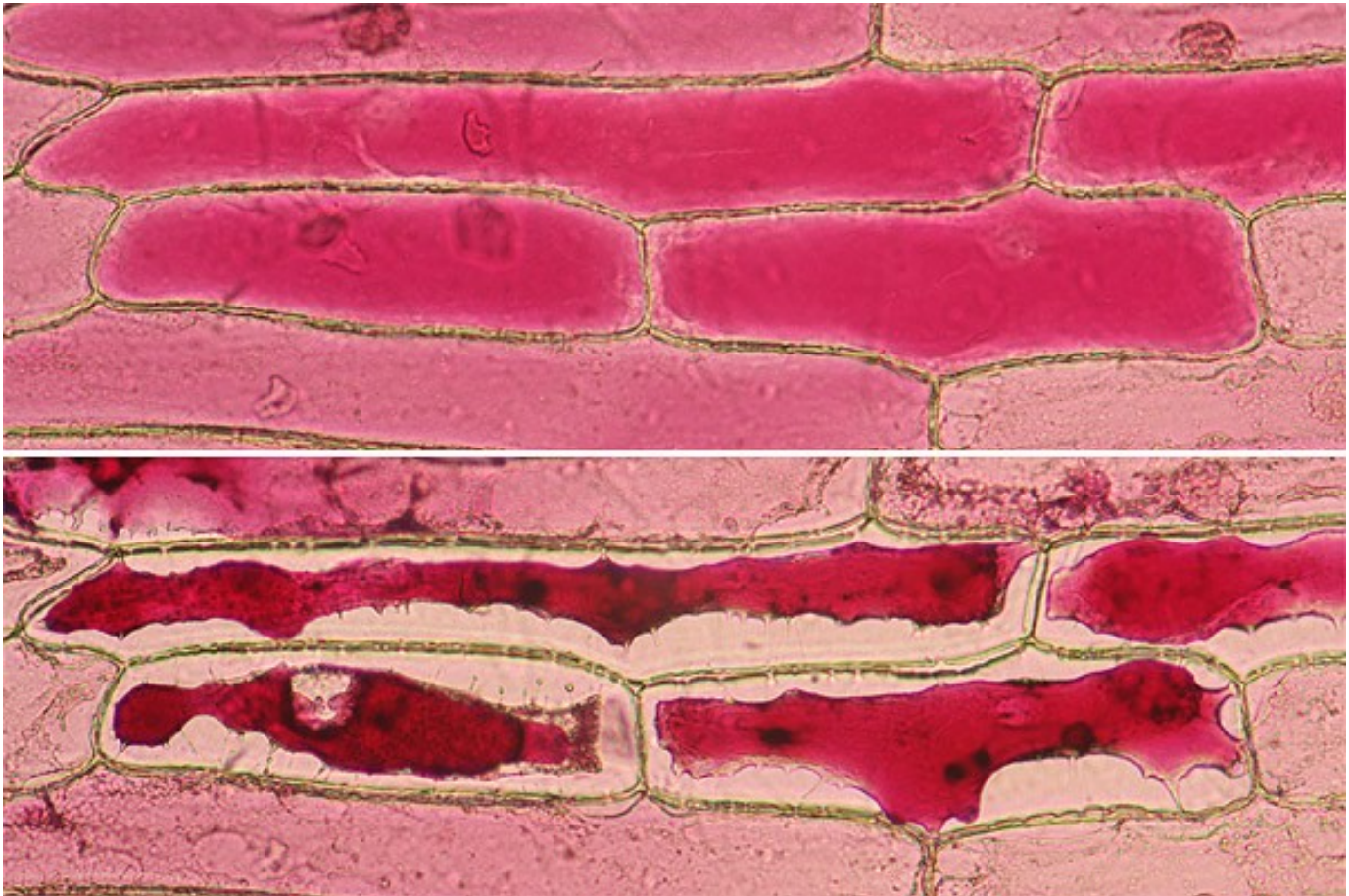
6. Observe the remaining cells (the thin, peeled part) under the microscope



Obtaining onion cells. Make a cut beneath the red layer and firmly press the red part of the onion against the edge of the knife, without cutting yourself...



Carefully tear off the layer of red cells. Remove the thick part of the onion (where the cut was made) and only observe the thin layer. Many cells will probably break open during this process and be useless, we only need a few intact cells.



The top image shows the cells before plasmolysis. The cells are filled with a red pigment and appear pink. The bottom image shows the same cells after the addition of saturated salt water. Intact cells will lose much of the water due to osmosis. The concentration of the pigment rises resulting in a darker color. The shape of the cell wall remains unaffected. Notice that the cell membrane still touches the cell wall at many places. These are the plasmodesmata, channels where adjacent cells contact each other. Some of the cells in this picture do not plasmolyze. This is probably because the cells were damaged when obtaining a single layer of cells.

(using a glass slide, water and cover slip, of course.

7. Only consider those cells that are filled with the red pigment. White cells are broken and have lost the red pigment.

Method - Plasmolysis

1. Make a saturated solution of salt-water
2. Using a pipette, add one drop of this solution to the specimen. The salt water should flow beneath the cover slip. There should be no need to remove the cover slip to add the salt water
3. If there is too much water beneath the cover slip, then the salt water will not flow between the cover slip and the slide. In this case use tissue paper to withdraw water from one side of the cover slip while adding the salt solution at the other side.
4. Observe what happens to the red pigment inside the cells.

Explanation

Water from the cells moves to the surrounding salt water. The shape of the cells does not change, the cell wall maintains the cell shape. The cell content (the red part of the cell) starts to shrivel up. At the same time it is possible to see that the intensity of the red pigment increases because it becomes more concentrated as water is removed (the red pigment is not able to move out of the cell). The process can be reversed when the salt water is removed and when distilled water is added. The water can be replaced beneath the cover slip by using tissue paper to remove the salt water from one side and at the same time adding fresh water from the other side using a dropper. ■

Advanced Variations

Instead of simply using concentrated NaCl (salt) solution, it is also possible to make solutions of different concentrations. In this case it should be possible to see a stronger plasmolysis with the higher salt concentrations.

Try to determine the highest NaCl concentration at which no plasmolysis can be observed. This concentration should then reflect the water potential of the onion cell inside. Alternatively, it is also possible to repeat the experiment with sugar water of different concentrations. Add a drop of rubbing alcohol to the cells. Alcohol also has the tendency to withdraw water from the cells.

Top Ten Tips

on Buying Educational Microscopes

By Edison Ramsey. The hobby of amateur microscopy knows many different approaches. Collecting vintage microscopes is one of them.

Schools and homes need educational microscopes for their students. These viewing equipment allow them to see into the world of living organisms and non-living things on a whole different perspective, figuratively and literal-

money and waste your child's time with a microscope that does nothing for his studies.

No Plastic, All Metal - All good quality educational microscopes have metal parts and absolutely no plastic parts. Look for metal parts especially in the frame, screw fasteners and focusing

Prefer Halogen Lighting - For most students, the tungsten microscope works just fine. However, for the more advanced students especially when considering a binocular microscope, halogen lighting with a dimmer is the more preferred option.

Look for Good Lenses - In many ways, the lens is the heart of the microscope. At the very least, buy one with a glass achromatic lens that will work in an excellent manner with school and hobby applications. If you have money to spare, educational microscopes with semi-plan lens are the next step up.

Go for a Slip Clutch - The slip clutch allows the focus knob to turn in place without causing damage to the microscope focus system. For many schools, this is the most important requirement.

Consider a Mechanical Stage - Although this is optional for educational microscopes for students, it is indeed a good addition to your system. Plus, it will help you practice for the more advanced medical and laboratory microscopes.

Consider an Iris Diaphragm - The iris diaphragm makes for easier adjustment in using the microscope. You can contrast this with a disk diaphragm that might be a little bit more difficult to maneuver for students.

With these tips in mind, you should be able to choose from amongst educational microscopes and get good value for your money. ■



These stereo microscopes can be operated with a battery, thus making them independent from a power supply in a classroom.

ly. And as with all types of investment for your children's education, you must carefully consider the purchase of an educational microscope since it, too, requires money.

Buy from the Right Sources - If you want quality in an educational microscope and, hence, value for your money, you must never buy from a nature and science store, a department store or a toy store. There are online sources for high-quality microscopes that will not end up not being used and worse, in the trash can.

Spend Right - You can expect to pay at least \$200 for a good quality beginner microscope. Anything under this amount should be avoided unless you want to waste your hard-earned

system. Otherwise, you are stuck with a toy microscope that you could have saved the trouble buying with your money.

DIN Standard - This is the German standard for the manufacture of microscope lenses, which makes it easy to replace the lens and lamp as well as other microscope parts. It must be emphasized that a DIN lens is not particularly better than the non-DIN lens but it does make for great interchangeability.

Separate Adjustments - It is very important to purchase educational microscopes with separate fine focus and coarse adjustments. As much as your budget will allow, avoid the microscopes with either a coarse focus adjustment or an intermediate focus.

CanScope - complete solution for all your microscopy needs. Contact: 1-877-56SCOPE(72673) or info@CanScope.ca For the best value for your money in student's microscopes in Toronto and digital microscope in Toronto as well as educational microscopes in Toronto, go to CanScope.ca today!

Article Source:

http://EzineArticles.com/?expert=Edison_Ramsey

Mystery Object - demystified!

The pictures show a strange looking object which I found in the dust of my apartment. What could it be? One thing should be clear: it's of biological origin, otherwise I can not explain its regular structure. The second image on the right shows several of these objects bundled together.

In order to find out more about the structure, I posted a comment on a German microscopy forum, and I obtained some interesting responses. Apparently it is a bristle of an insect larva of *Anthrenus sp.* [1]. In the February edition of Micscape Magazine there is also a picture of this structure [2]. And indeed, another check of the dust turned up an empty exoskeleton of an insect, with a large number of bristles.

Anthrenus sp. is also known as a "carpet beetle", and is known to eat textile material - not a good thing to have it around in a household. The bristles (more correctly called "hastisetae") of the insect serve as a defense system. They break off easily and predators quickly entangle themselves, and die [3].

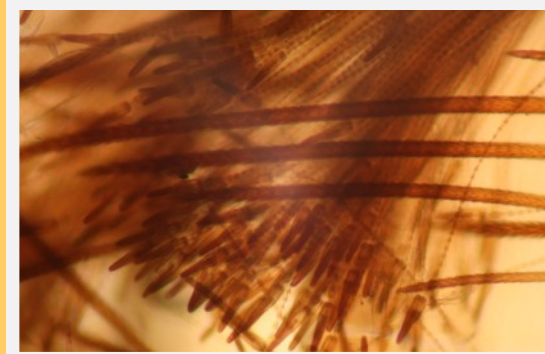
The larva of the carpet beetle.



André Karwath (CC-BY-SA)



Individual dust samples repeatedly revealed a rather strange looking structure.



Here are many of them bundled together. They are the setae of the larva of the carpet beetle.

References:

- [1] <http://www.mikroskopie-forum.de/index.php?topic=878.0>
- [2] <http://www.microscopy-uk.org.uk/mag/artfeb06/gl-object.html>
- [3] <http://resources.metapress.com/pdf-preview.axd?code=n8hq34201561061&size=largest>

The Microscopy Forum

You can visit the newly installed Microscopy forum at

<http://www.microbehunter.com/forum>

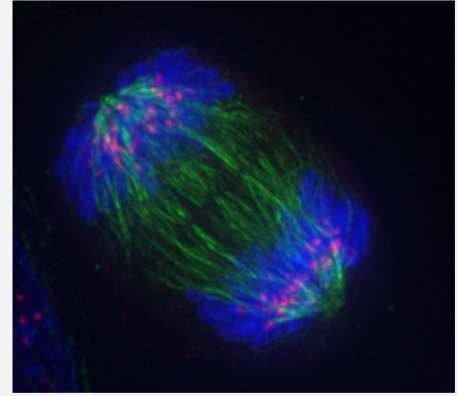
You can post to the forum without the need for registering. The forum should allow interested people to post questions and answers on all microscopy related topics.

Forum sections: Microscopes and Optics, Camera Systems and Imaging, Specimens, Samples and Slides, Photographs, Collecting Microscopes and Slides, Home-made Microscope Adaptations, Identification Help and Microscopy Education.

Microscopy Forum	
Forums	
	Microscopes and Optics Everything relating to microscopy hardware.
	Camera Systems and Imaging Here you can discuss everything related to taking light micrographs and videos
	Specimens, Samples and Slides Here you can discuss sample and specimen preparation issues.
	Photographs and Videos Here you can post pictures and videos to show others.

Mitosis

Mitosis is cell division in eukaryotes. Trying to find the stages of mitosis in plant tissue is not only a common high school lab activity but also an exciting hunt.



Roy van Heesbeen (public domain)

A dividing cell must make sure that both new daughter cells receive exactly the same DNA. Every cell of a eukaryote has several linear pieces of DNA (in humans 46). This DNA must first be copied, to form, in the case of humans, 96 pieces of DNA. Each new cell then receives one DNA copy. Imagine a ball composed of 96 pieces of long string. It is nearly impossible to separate them and the risk of tangling and forming knots is high. A cell is confronted with a similar problem. The cell therefore condenses the DNA into smaller packets that can be moved around the cell more easily. These packets are called chromosomes. They can be made visible using appropriate staining techniques.

Mitosis can be divided into 4 stages: prophase, metaphase, anaphase and telophase. The time between the cell divisions is called interphase, and is not considered part of mitosis. During this time the complete DNA of the cell is copied. During mitosis the DNA copies are separated from each other in the cell and pulled to opposite poles of the cell. The cytoplasm of the cell divides at the very end of mitosis after the DNA has been separated inside the cell.

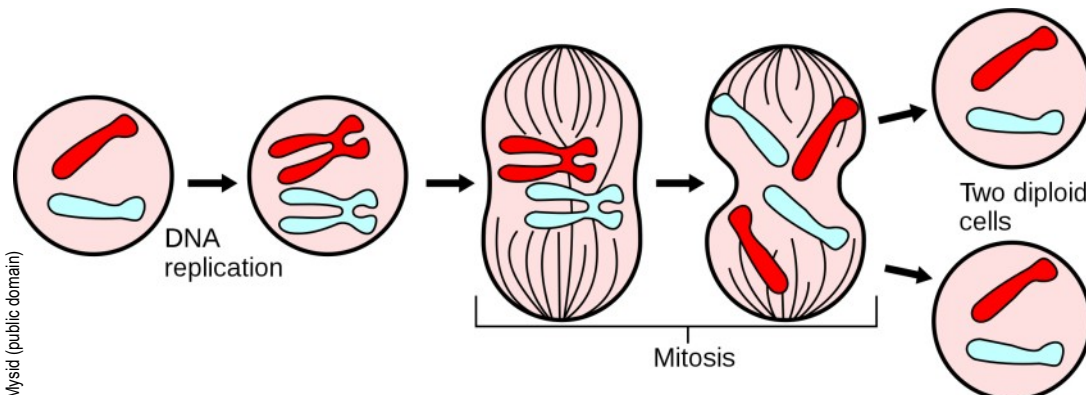
It is important to mention that the cell gradually moves from one stage of mitosis into the other. When looking at cells under the microscope, one can also see cells that are in early or late phases of a particular stage, and some-

The background image shows cells from an onion root, which contains many dividing cells. The inset image is a fluorescent microscopic image of a cell in anaphase. Spindle fibers are colored green, the chromosomes blue.

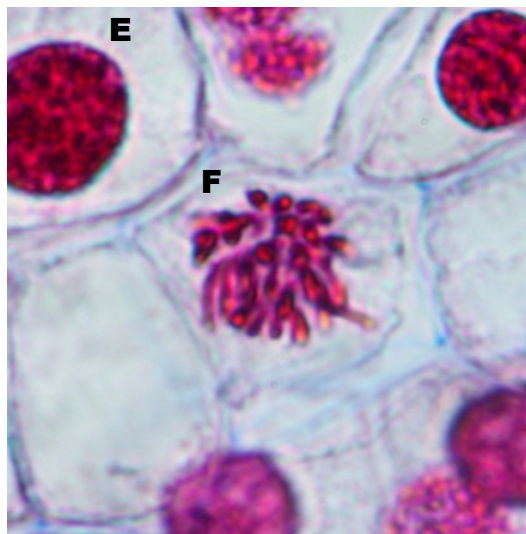
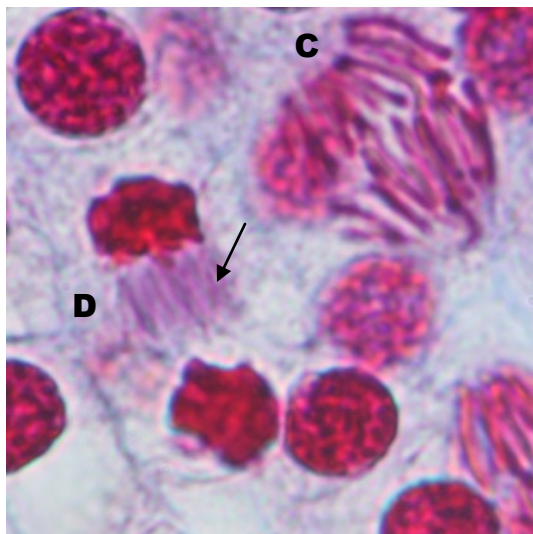
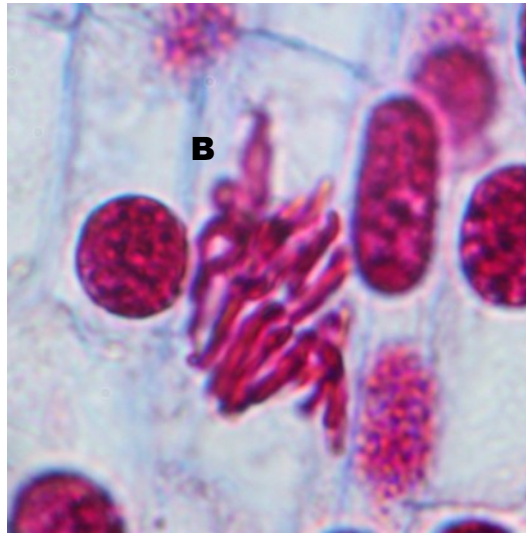
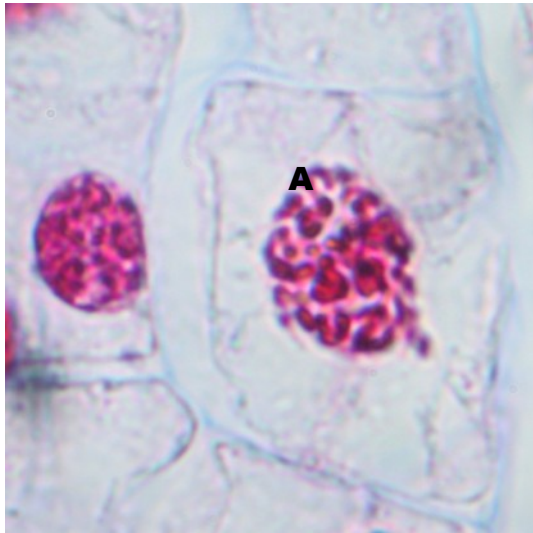
times it may not be easily possible to clearly allocate a cell to one stage.

The Phases

Interphase: This is the time between cell divisions. During this time the complete DNA of the cell is copied. Interphase is also the time when the cell physically grows and increases in volume. When the cell has reached a cer-



DNA replication (copying of DNA) precedes mitosis. During mitosis, the chromosomes become visible. The chromosomes are then pulled apart by spindle fibers to the opposite ends of the cell. The cell's cytoplasm can divide only after this separation of the chromosomes has occurred.



Oliver Kim (4 images)

The pictures show mitosis stages in the ovary of a lily.

A: Prophase. The chromosomes start to appear.

B: Metaphase. The chromosomes align at the equator of the cell. The arms of the chromosomes extend outwards.

C: Anaphase. The chromatids are separated and pulled to the poles of the cell.

D: Telophase. The two nuclei start to reform and the chromosomes decondense. The spindle fibers (arrow) are still visible between the reforming nuclei.

E: Interphase. The nucleus is visible.

F: Anaphase or metaphase seen "head on".

tain size and all of the DNA has been copied, mitosis is initiated.

Prophase: This is the first stage of mitosis. The loose DNA of a cell, the chromatin, starts to condense to form visible chromosomes. During prophase the cell's nucleus also starts to disappear (exceptions exist, as so often the case in nature) and thin protein spindle fibers start to attach to the chromosomes. These spindle fibers are responsible for pulling the chromosomes inside the cell.

Metaphase: The chromosomes, which are randomly distributed in the cell, are now pulled to the equator of the cell, where they align. This ensures symmetry. The arms of the chromosomes, which look like finger-like projections, extend towards the poles of the cells.

Anaphase: The two identical DNA copies (the sister chromatids) are now separated from each other and pulled to opposite ends of the cell. The arms of the chromosomes now appear to point towards the equator of the cell. In this stage the DNA is separated but the cell has physically still not yet divided.

Telophase: Telophase can be considered the reversal of prophase. In the last stage of mitosis, the chromosomes decondense to form chromatin again. The nucleus starts to reappear. At the same time the cytoplasm of the cell starts to divide physically. This is referred to as cytokinesis. Each one of the two daughter cells then proceeds into interphase.

The Method

Use the Acetocarmine stain to make chromosomes visible. The process is quite simple. Heat the plant tissues (such as root tips) in the solution until

they are soft. Be careful of splattering acid when heating. The acid of the stain breaks down the cell walls and makes the tissue soft. Rinse the tip and place it between cover glass and slide. Place the slide on a flat surface and squeeze it with your thumb and without applying horizontal shear forces. Google the term "acetocarmine chromosome stain" to find recipes and methods. ■

TERMS

Chromatin: The chemically active form of DNA, it is decondensed and loose.

Chromosomes: Composed of DNA and protein, they are condensed DNA which can be moved around in a cell more easily.

Chromatids: The two identical copies of DNA in a chromosome.

Spindle fibers: These are protein fibers which attach to the chromosomes and pull them.

Cytokinesis: The division of the cytoplasm.

Euparal

Mounting Medium

Euparal is my favorite mounting medium for making permanent slides. I wanted to know more about this medium and conducted some research.

Euparal is a semi-synthetic mounting medium used in microscopy. It is slightly yellowish in color, flows well and cures after a few days. After curing, it becomes very hard but not brittle, keeping elasticity. Euparal also adheres strongly to glass. It has a refractive index of 1.5174.

Compared to other mounting media, Euparal has a significant advantage: Specimens can be directly transferred from fixing alcohol into Euparal. Other mounting media, such as Canada Balsam, require the specimen to be transferred to xylene (toxic!) prior embedding.

Euparal was first described by G. Gilson (Prof. of Zoology at Louvain University, Louvain, Belgium) in 1906,

and is liked for its ease of use and stability.

I wanted to find out more about this mounting medium and conducted a quick Web search, only to find out that the information is scarce. I wanted to know more about the composition of Euparal, also because of safety considerations. Many non-aqueous mounting media contain toxic organic solvents, which I try to avoid.

After some time I was indeed able to find the original publication by G. Gilson [1], published in French. An article published in the Journal of the Royal Microscopical Society [2] summarized parts of this original publication in English. Together with a French to English translation software, I was able to extract some meaning of Gilson's original article.

The discovery of Euparal

Gilson found out that sandarac (or sandarach) is a suitable resin for mounting. It is obtained from *Callitris quadrivalvis*, a tree belonging to the cedar family. The resin has been previously used to make varnish and protective coatings for paintings. Alcohol could dissolve the sandarac well, but the resulting mounting medium was not suitable for microscopic work. During the curing process the sandarac started to crystallize and crack.

Gilson then mixed the sandarac with camsal, a mixture of phenyl salicylate (salol) and camphor. The camsal prevented the formation of crystals and cracks, but had the disadvantage in that it was not a good solvent. Camsal alone was not able to sufficiently dissolve the resin.

He then added either isobutylic or propylic alcohol to further dissolve the

sandarac. Especially isobutylic alcohol was considered suitable, as it was commonly used to dehydrate microscopic specimens. The specimens could then be directly transferred into the mounting medium, which contained the same solvent.

While this was already a step into the right direction, the added alcohol showed some undesirable characteristics. Stained specimens could not be mounted with this medium, as the alcohol dissolved many the dyes which are commonly used in microscopy. Eosin, safranin, methyl green were especially affected by the alcohol.

Gilson, therefore, searched for replacements for the alcohol. He discovered that a combination of eucalyptol and paraldehyde was able to substitute for the alcohols, without harming pigmentation. He thus gave the mixture containing sandarac, salol, EUcalyptol and PARALdehyde the name Euparal.

Advantages and Disadvantages of Euparal

Towards the end of the article, Gilson lists several advantages of Euparal. These advantages are now briefly summarized.

Solvent—First, it is possible to directly transfer specimens which were stored in 70% alcohol into Euparal for permanent mounting. It is not necessary to completely dehydrate the object by placing it into absolute alcohol.

Refractive index—Euparal also has a relatively low refractive index (1.481), which can be an advantage to observe certain structures. Other publications consider this low refractive index a disadvantage, however.

Staining—Euparal can be colored green ("Euparal vert") by adding some

Composition of Euparal

Sandarac: A resin which solidifies in air. Originally used as a varnish for furniture.

Paraldehyde: Preservative and solvent.

Eucalyptol: Solvent of Euparal. dominant portion of *Eucalyptus globulus* oil. eucalyptol is used as an insecticide.

Phenyl salicylate (salol): Antiseptic substance, was introduced in 1886 under the name Salol, a disinfectant.

Camphor: An antimicrobial substance, previously used for embalming. Obtained from the evergreen tree camphor laurel (*Cinnamomum camphora*).

Camsal: A mixture (1:1) of camphor and Phenyl salicylate (salol).

copper salt. This can further increase the contrast of specimens stained with hematoxylin.

Chemical properties—Euparal possesses reducing properties and therefore prevents oxidation of some dyes (such as hematoxylin).

Fluidity—Euparal possesses good fluidity, does not pull strings and handles easily. It is possible to dilute the medium with Euparal Essence.

I like Euparal because it does not use the harmful solvent xylene. For amateur purposes, I consider it therefore more suitable than Canada Balsam, which requires the use of toxic Xylene. Euparal also saves some work, because it is not necessary to completely dehydrate the specimen. Euparal also cures relatively quickly, and on the less serious side: Euparal smells nice (but don't inhale, it is an irritant and flammable!).

Euparal does possess some disadvantages as well:

Dye stability—Acid sensitive dyes do not keep well, when embedded in Euparal.

Shrinkage—Euparal does have the tendency to shrink a bit. This can introduce air bubbles.

Health concerns—It is flammable and an irritant. Eye and skin contact must be avoided.

Unfortunately, I can not say anything about the long-term storability of Euparal mounted slides. Slides which were mounted in Canada Balsam possess nearly unlimited storability, probably also because the specimen is completely water-free.

From what I could find out, Euparal seems to be especially popular for the mounting of etymological specimens (insects, spiders).

Risk and Safety

Risk and Safety (R/S) statements are hazard codes that are used to label chemicals. Euparal possesses the fol-

lowing R/S Statements (as written on the bottle supplied by the company):

- R10: Flammable
- S24: Avoid contact with skin
- S25: Avoid contact with eyes

In the reference section at the end, you can find a link to the original publications by Gilson and the Royal Microscopical Society. ■

References

[1] Gilson, G. (1906). *La Cellule*, Vol. 23, pp. 425-432.

<http://www.archive.org/details/lacellule23lier>

[2] Hebb, RG., ed., (1907). *Journal of the Royal Microscopical Society*. p.501.

<http://www.archive.org/details/journalofroyalmic1907roya>

FAST FACTS

Monocots and Dicots

Monocots and dicots are two major types of flowering plants. They can be distinguished by the number of first leaves (the cotyledons) that emerge from the seed during germination. The number of first leaves is also

name-giving ("mono" meaning one and "di" meaning two). The monocots (also known as monocotyledons) form one first leaf, while the dicots (dicotyledons) form two. Mono-cots and dicots have other distinguishing features, such as the arrangement of leaf veins or the number of furrows or pores in the pollen.

One significant difference can be observed microscopically: the vascular bundles (the cells that carry water and nutrients up and down the stem) show a significantly different arrangement in these two groups. Monocots have the vascular bundles scattered randomly throughout the

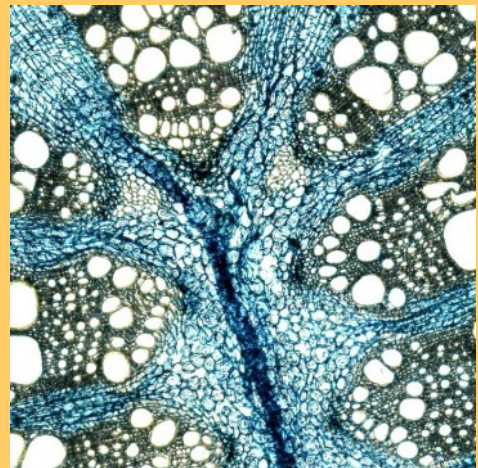
stem. The vascular bundles of dicots are arranged in concentric circles.

The top image on the right is the cross section of a stem of *Aristolochia*, a dicot. Clearly visible is the circular arrangement of cells around the center. The bottom picture is from maize. The vascular bundles (they look like little faces) are distributed randomly in the stem.

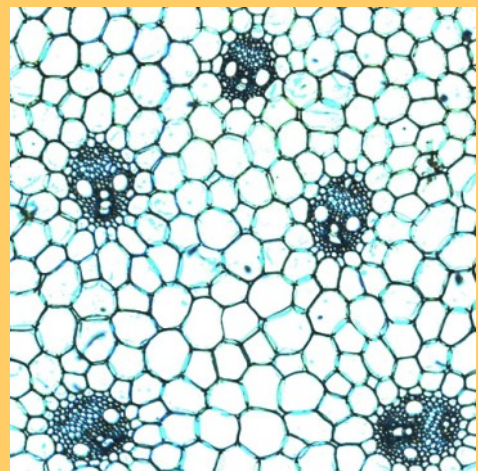


Left: monocot, right: dicot

Peter Halasz (CC-BY-SA)



Dicot stem (*Aristolochia*)



Monocot stem (maize or corn)



A Stream Hike for the Collection of Photosynthetic Purple Sulfur Bacteria

EXPLORATION

Text and images by Charles E. Guevara.

Please enjoy this early fall 2010 hike along a central New York rural stream.

I noticed with unaided eyes on an earlier hike, blooms of photosynthetic purple sulfur bacteria (Eubacteria, gamma *Proteobacteria*, *Chromatium* species of aerobic-photosynthetic purple sulfur bacteria), and delicate white cobwebs and white filaments of aerobic filamentous gliding, sulfur oxidizing bacteria (Eubacteria, gamma *Proteobacteria*, *Beggiatoa* species).

Both types of huge bacteria occurred together and were plentiful in early fall 2010, as blooms in one specific section of the stream bank waters. On the return hike for collection of some specimens, my puppy Chloe and I probed the stream sediments. Where Chloe trotted in this stream bank area, dark black sediments filled her paw-prints. Where I probed with a yardstick, dark black sediments clouded the flowing waters. Immediately upon probing deep, methane gas bubbled up

for a few seconds. My wooden yardstick slid easily into the sediment for three feet depth, and yes, a strong smell of hydrogen sulfide was obvious in the probed water areas.

Photosynthesizing aerobic purple sulfur bacteria (not to be confused with anaerobic photosynthesizing purple bacteria!) are a relatively huge five microns in girth, and thirty microns in length. They have a beautiful purple to magenta coloring, very interesting and characteristic inclusions, are mobile and



Probing into the ground of the stream.



Dark black sediments, which smell of hydrogen sulfide, cloud up into the stream water.



Probing the sediments with a stick releases dark sediments.



Blooms selected for collection.



Macroscopic 'bacterial blooms' of *Chromatium* and *Beggiatoa* species.



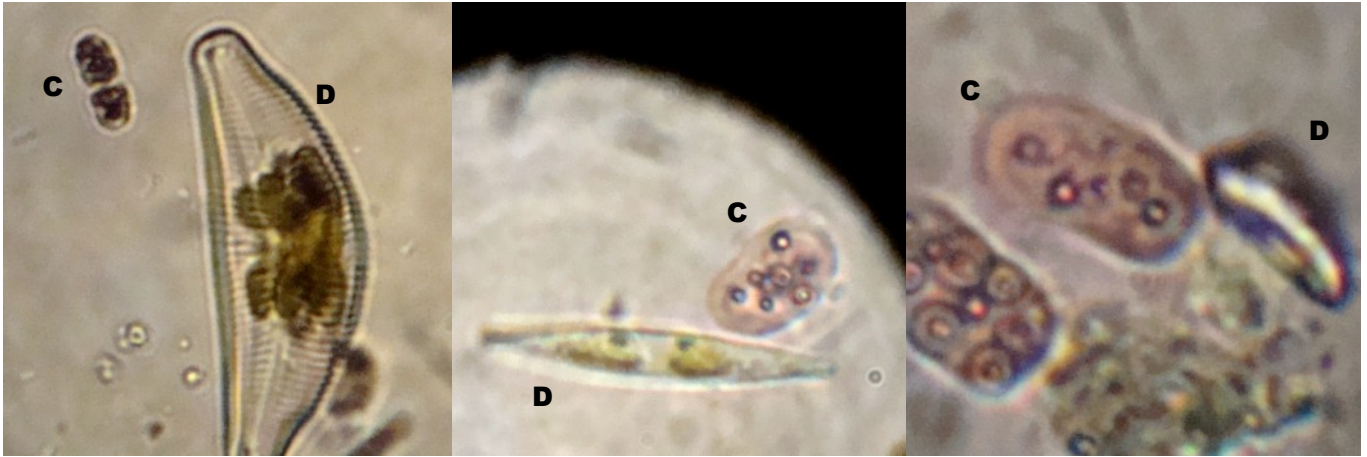
A delicate magenta film and the white filaments were collected from the blooms of the stream bank.



The yardstick slipped easily totally into the sediments.



Chloe next to the probe. It is three feet into the sediment.



Chromatium species (C) are huge bacteria, here positioned next to diatoms (D) for size and scale comparison. *Chromatium* species are 5 microns in girth and 30 microns in length.



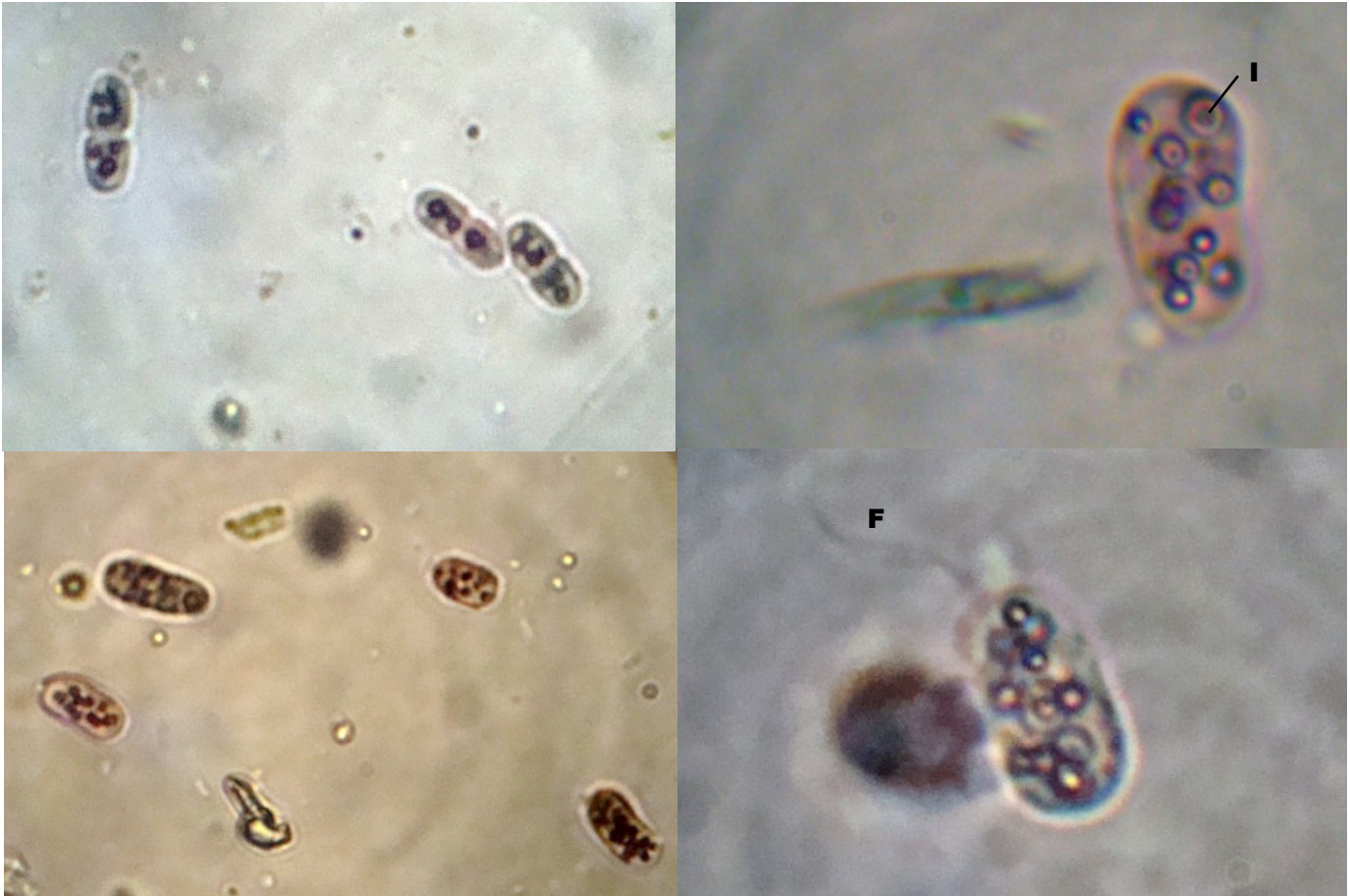
Top: *Beggiatoa* species (B) next to a diatom (D) and the flagellate (F) *Chilomonas paramecium* for scale of size. *Beggiatoa* is a huge prokaryote! Left: Simple live wet-mount slide preps and brightfield microscopy, crisp views of prokaryote flagella with dry-high/40x objective. My hand-held point & shoot image technique might need an upgrade.



Purple sulfur bacteria

FAST FACTS

Purple sulfur bacteria are photosynthetic bacteria which oxidize hydrogen sulfide to produce elemental sulfur. This sulfur becomes visible as granules of elemental sulfur, which is either deposited internally or externally of the cell. These bacteria grow in regions where hydrogen sulfide is abundant (e.g. due to geochemical activity), and where there is sufficient light. They are not capable of growing in aerobic (oxygen containing) environments and can be therefore found mostly in stagnant lakes. Some species, such as *Ectothiorhodospira halobalkaliphila*, prefer more extreme environments with a pH of 8.5-10.0 and a high salt content (ed.).



The images show the characteristic and intricate inclusions (I) in *Chromatium* species. Prokaryotic flagella (F) live and active!

display wonderfully visible and active prokaryotic flagellar activity. All these enchanting and profound observations are obvious with simple live wet-mount slide preps. Prokaryotes essentially worked out all metabolic strategies, including oxygen producing photosynthesis. Eukaryotes evolved from prokaryote symbiotic interactions. All observations were done with a dry-high 40x objective and brightfield microscopy. Please try observing “big bacteria live”, readers!

The relatively huge aerobic filamentous gliding, sulfur oxidizing bacteria are much larger than the purple sulfur bacteria. They occur together in this stream section from which Chloe and I collected from. The elemental sulfur granules found in the huge bacterial filaments of filamentous gliding sulfur oxidizing bacteria, cause the white color of the bacterial cobwebs and filaments, which are so evident to the unaided eye.

The purple sulfur bacteria’s pleasing colors result from a combination of

green bacteriochlorophyll a, and red-dish carotenoid pigments.

The prokaryotic bacterial flagellum has no internal fibrils. The bacterial flagellum is composed of the protein flagellum arranged in helical chains so as to form a flagellum with a hollow core. The bacterial (the prokaryotic) flagellum is attached to a basal body which acts as a rotary molecular motor. The prokaryote flagellar motor can rotate the flagellum: clockwise/stop/counterclockwise. To have 40x/dry-high objective views of this active prokaryotic apparatus is a microscopy treat to enjoy!

So please enjoy this microscopy related stream hike. If you keep to the sides of the stream, usually the water will not get into your rubber boots. ■

Text and images by Charles E. Guevara, Fingerlakes/ NY, US.

Send queries to: nospam_iceciliate123@yahoo.com (remove “nosпам_” from the address.)

References

Background sources for this stream microscopy hike:

Dyer BD. A Field Guide to Bacteria, 2003

very low cost, must purchase/ must read for microscopy world views, in my humble but enthusiastic opinion.

Madigan M, Martinko J, Brock. Biology of Microorganisms, 2006, 11th ed, chapter 12

very useful and enjoyable content, carries into all world views/ microscopy world views.

Leadbetter ER, Poindexter JS, Bacteria in Nature, Vol 1, 1985

wonderful reading, standalone sections read easy and link microscopy pioneers/microbial world building and more... try it and get this text if possible!

The Power of Digital Post-Processing

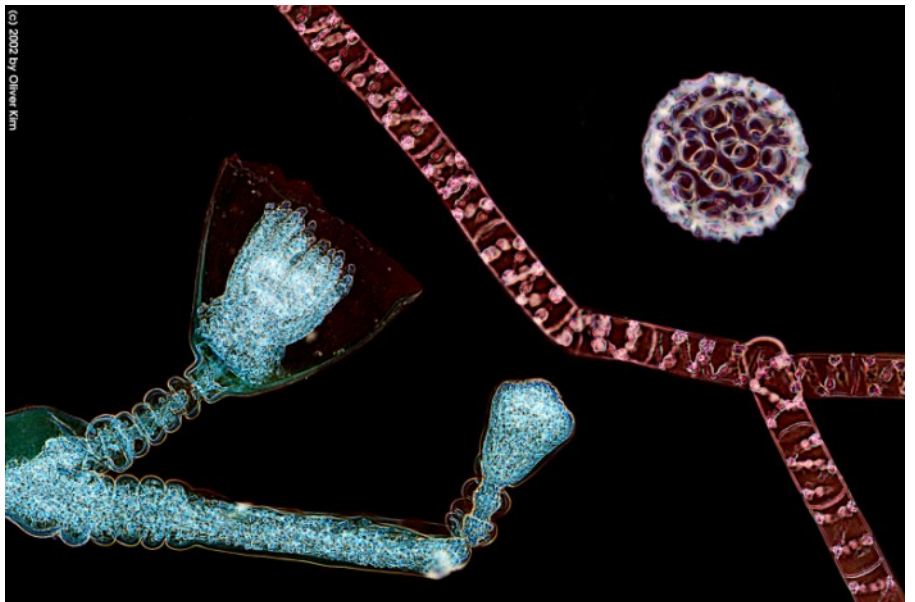
Some microscopists may own fancy and expensive photographic equipment, others may document their observations by simply holding a digital pocket camera or the camera of a mobile phone in front of the eyepiece. Regardless of the method, digitally post-processing the micrographs can dramatically improve the visual impact of the image.

The term „post-processing“ refers to the modification of a digital image after it has been captured by a camera. Computers and appropriate software provide the tools to improve the appearance of an image in ways that were unthinkable several years ago. Digital methods, for example, now not only allow you to overcome low contrast and lack of color saturation, but also limited depth of field and a small field of view. This article should give you an overview of possible image processing steps.

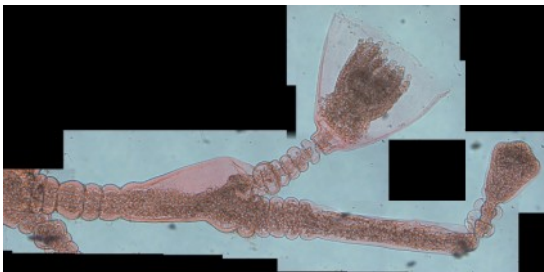
Loss of quality?

First, let's be realistic: Digital processing of micrographs (or any other photograph) can significantly improve the subjective impression and impact of the image, but it is not able to visualize something that is not there. It is not possible to increase resolution, for example. Information which is not present in the first place can not be generated.

As a matter of fact, processing digital images will almost always result in a reduced image quality, even if the final result appears subjectively better. Sharpening a micrograph, for example, can not increase the resolution, even though it may give such a subjective impression. Simply saving a file as a JPG will already reduce the quality. This loss should be kept as small as possible and digital processing should be done carefully. I also highly advise that the original micrographs are stored separately and one only works with copies. When saving intermediate images between processing steps, it is best to use file formats that do not use lossy



Compositing. These micrographs were assembled of several smaller images. The top image shows Cyclops and Daphnia, to water fleas. The bottom image is a composited image of a polyp, Spirogyra alga and a radiolarian (2x the size).



Raw photomicrographs, stitched together. These pictures do not look very impressive. The background is dusty, the images lack contrast and overall impact. It's now time to do some compositing, the assembling of the pictures into a larger composition.

compression (such as TIFF). Only the final image should be saved using JPG.

Batch Processing Software

Sometimes a large number of digital photographs need to be processed. Manually color correcting, adjusting, resizing and renaming hundreds of files for a gallery can be a laborious undertaking. Many photo editing programs (such as Photoshop and Gimp) already contain batch processing functions. For those of you who are looking for a small and simple program to do the batch processing, I can recommend the software PHATCH (Windows, Linux and Mac), which can be downloaded for free. The program allows the user to create a user-specified action list of different processing steps. There are 52 different manipulations (unless I miscounted...) that can be arranged in an arbitrary order. Some of them are very useful for processing light micrographs, such as automatically adding a watermark, automatic contrast enhancement, sharpening, resizing or rotating. The software also allows you to manipulate the EXIF data of JPG images. Other actions are probably less useful for mi-

crography, such as blurring in image. The action list can, naturally, be saved. It would go too far to explain the different features of the software and I simply recommend that you try the program.

In most cases, however, automatically batch processing a micrograph may not result in a satisfactory result. The settings that work for one micrograph may not yield the best result for another one. In these cases it is therefore necessary to manually adjust each image individually. There are a set of basic manipulations that generally provide to be very useful. If you do not own PhotoShop, then I would recommend you to download the free software GIMP.

Adjusting histogram levels

This is probably one of the most useful image enhancement action. By adjusting the levels, you can both adjust the contrast of the image as well as the overall brightness. If done carefully, there is nearly no loss of image information.

The histogram is a graphical representation of the brightness distribution of the pixels in the image. A large peak

Download links

PHATCH — Photo Batch Processor (download link is on the bottom of page). photobatch.stani.be

GIMP — GNU Image Manipulation Program www.gimp.org

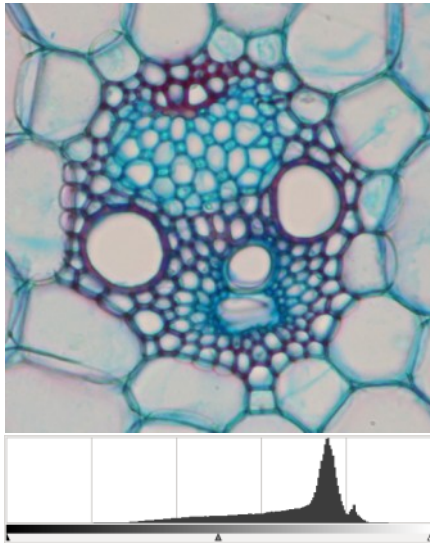
Picolay — Image stacking program www.picolay.icbm.de

Hugin — Panorama photo stitcher <http://hugin.sourceforge.net/>

CombineZP — Image stacking program www.hadleyweb.pwp.blueyonder.co.uk/CZP/Installation.htm

Helicon Focus — Image stacking program (commercial) www.heliconsoft.com/heliconfocus.html

Ubuntu Linux users: Install Phatch, Gimp, and Hugin from the "Ubuntu Software Center". Mac and Linux users: Try to run Windows-only software (such as Picolay) with an emulator. In Linux, Picolay does run with the emulator „Wine“.



indicates that there are many pixels present of this particular brightness.

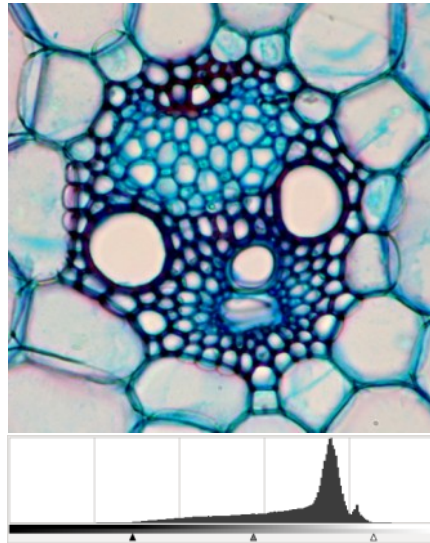
To adjust the image for maximum contrast, move the small arrows beneath the histogram inwards so that they just start to touch the „valley“ of the histogram (B). Moving the arrows in further will result in a loss of image information (C). The overall brightness of the image can be adjusted by moving the central arrow to the left or the right.

It is possible to use moderate contrast enhancement to clean up the background of a micrograph. Moving the right arrow inwards will make bright areas of the picture (such as light dust) even brighter, until it fades into the background. This will, however, also result in a loss of image detail and the colors of the specimen might start to look posterized. Contrast enhancement for background cleanup will therefore work best for specimens that already have a strong contrast with the background. Alternatively, I suggest that you select the specimen and copy-paste it to a new, clean white background.

If you use the program PHATCH to make contrast adjustments, then you have to specify how many percent of the histogram you are willing to sacrifice on the white and on the black end. In this case you have a loss of information.

White balance

Correct white balance removes any color hue from the picture. A low light intensity setting will shift the color spectrum of the lamp towards the red, a brighter setting will move the spectrum towards the blue. The consequence is,

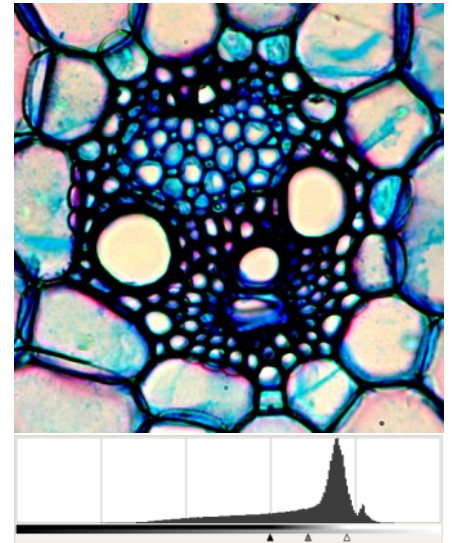


that the same specimen will show different colors when photographed, even if the exposure is correct. With a lower light intensity setting, the background of the micrograph will then appear more yellowish in color.

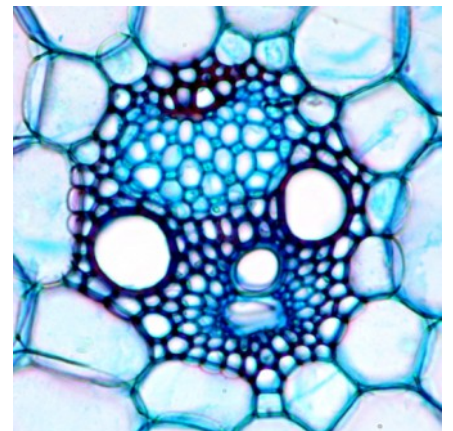
Contrast adjustment (using the histogram) may also, artificially, modify the hue of the whole image, as well as the age of the lamp. Whatever the reason, by adjusting white balance, it is possible to restore the background to a neutral white color. The whole image will then appear cooler.

There are several ways in which the white balance of a micrograph can be adjusted. Many digital cameras will make automatic white balance adjustments when the image is taken. Depending on the algorithm used, the camera may assume that the brightest areas on the image (in micrographs the background) represent a neutral white. Some cameras also allow the user to manually select a light source. The white balance will then be adjusted based on a camera-internal reference.

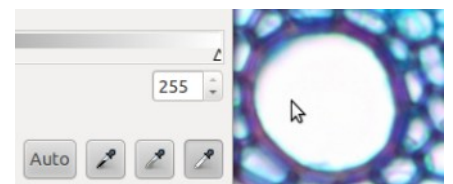
More advanced cameras have a custom white balance option. This feature is very useful for micrography and can save much post-processing work. The user first takes a picture of the mere background, without a specimen. This empty picture has to be selected over the camera's menu and is then used as a reference. Every picture will then be adjusted as it is taken. Needless to say, the light intensity should not be changed between the reference image and the actual photographs, as this would result again in a color shift.



Far left: original photograph. The contrast is comparatively low and the image lacks impact. By moving the white and the black arrow inwards, the contrast is improved. Moving the arrows inwards too far, will result in some colors going into saturation. This will result in information loss.



Contrast adjusted and white balance adjusted image.



To set the background to white, first click on the white dropper (the right one above) and then on the background of the image. This will not only change contrast, but also the white balance of the image.

Adjusting the white balance manually can be done inside the histogram window. In the program GIMP, first click on the white dropper. Use this dropper to click on the background of the micrograph, which you want to set to white. The functionality in PhotoShop is similar.

Cropping

Microscope eye pieces do not have a built-in zoom function (exception are some stereo microscopes), and one is therefore somewhat limited in composing an image when taking the picture. The produced micrograph may include many unnecessary and distracting elements because it is not possible to zoom in further. The next higher magnification might already be too high.

Cropping removes irrelevant elements from the image and helps the viewer to direct the attention to the important parts. Cropping can also simplify and balance the image.

When cropping images, take the aspect ratio into consideration. Digital cameras will take images either in a 4:3 or a 3:2 format. Micrographs that should be displayed on a wide-screen TV should be cropped to a 16:9 format. Many video projectors (beamers) use a 4:3 format. In many cases, a 1:1 ratio will give the most pleasing re-

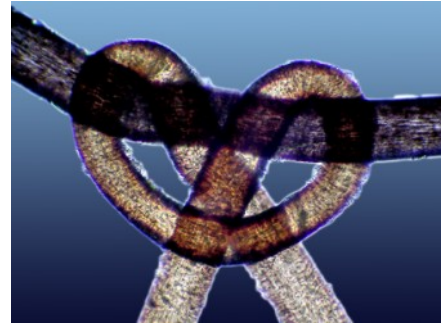
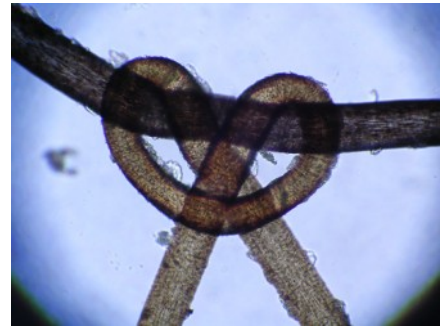
sults, which is fine when making an online gallery.

Resizing and resolution

Why should resizing an image be even worthy of mentioning? Resizing does have a special significance for micrography. In many cases the resolution of the digital camera is substantially higher than the resolution of the provided microscopic image. In this case it is possible to reduce the size of the image without much loss of information.

As a rule of thumb, the resolution of the capturing device should be twice as high. In this case, the digital device captures the image without loss of quality. A still higher camera resolution will not improve the image. As a matter of fact, the provided resolution of the microscope is often so low that it is easily possible to reduce the size of the micrograph without much loss in picture quality. Taking an image with the highest resolution that the camera is capable of is often simply a waste of disk space.

I therefore recommend you to figure it out the optimum resolution experimentally. The method of is quite simple: First take a low-resolution and a high-resolution picture of the same specimen. I took two pictures each, one with a setting of about 3MP and one with the maximum resolution of 12MP. In both cases, the file

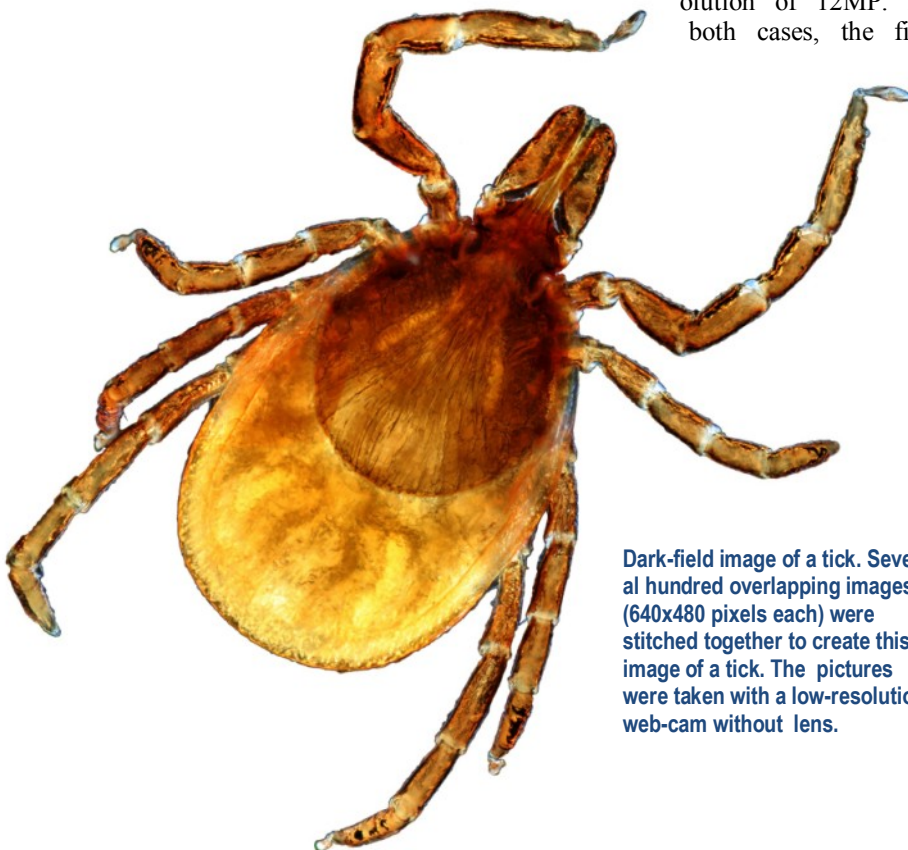


Human hair. Cleaning up the background can have a significant impact on the subjective impression of an image.

compression was low (and JPG image quality high). The low-resolution image is then enlarged to the size of the high-resolution image. In my case the low resolution (3MP) image had 2256 by 1504 pixels. It was enlarged to 4272 by 2848 pixels (12MP). Finally compare the two images (set it to 100%). If the high-resolution image shows details that are not present in the low-resolution image, then this indicates a loss of information when taking a low-res photograph. Otherwise one can safely use the low-res setting of the camera, or even reduce the size the large image. In this case the limiting factor for image quality is not the resolution of the camera, but rather the optics or the specimen.

Inverting

Inverting produces a negative image, without changing image information. Inverting an image has purely aesthetic and artistic purposes. By making the white background black, the micrograph is further abstracted. It is then possible to make more generous contrast adjustments to the image, adjustments that would look unnatural and „overdone“ in the not-inverted (positive) picture. The two micrographs on page 16 were color inverted and contrast enhanced.



Dark-field image of a tick. Several hundred overlapping images (640x480 pixels each) were stitched together to create this image of a tick. The pictures were taken with a low-resolution web-cam without lens.

Stitching

This refers to the assembly of several overlapping images into one larger picture. Stitching is useful when the specimen is so large that it is impossible to capture the whole structure even with the lowest magnification. It is possible to overcome the limited field of view. Stitching can be accomplished by using a panorama software. When choosing a panorama software, make sure that the program is able to combine the images in both horizontal and vertical direction.

Microscopic images often do not offer much image complexity. For this reason, the software may have problems assembling the images automatically. It therefore pays off to do a little planning beforehand. Ask yourself the following questions:

Final image size—How large will the final image be? The processing requirements increase significantly with increased image size. I remember one project that I could absolutely not process within reasonable time. There were simply too many images. In this case it is probably better to switch to a still lower magnification to reduce the number of images. Alternatively, it may be advisable to down-size the individual images prior to processing.

Camera resolution—What camera resolution should be used? The choice of camera resolution has a significant impact on the final image size and processing speed. One should first test, if a high camera resolution is indeed necessary or if it is not simply results in empty magnification.

Overlap—How much image overlap should be used? More overlap may make it easier for the software to automatically assemble the pictures, but at the same time more pictures are needed to cover the whole specimen (which again increases work time). Images with characteristic patterns and shapes may need less overlap than repetitive structures.

Background cleanup

The optical surfaces (especially the lighting system and condenser optics) are rarely completely free of dust. These disturbances will be present in the image, whether or not a specimen slide is present. It is now possible to mathematically subtract these disturbances

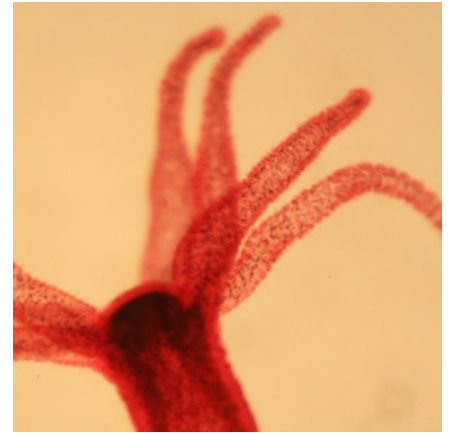


Image stacking combines several images with a different focal planes into one image that is in focus throughout. The top two images were taken with different parts of the image in focus (compare the left two and right two tentacles). The image on the bottom is a stack (including white balance). The micrograph shows a fresh-water polyp.

from the image. A picture with and without a specimen has to be taken at the same magnification and using the same exposure time. The empty image (without specimen) is then subtracted from the image containing the specimen. The program Picolay has a feature which is able to accomplish this. I am explaining the steps in the next article.

Alternatively, it is possible to clean the background by selecting the specimen without background and copying it

to a new clean background. Manually selecting the specimen can be quite tedious and time consuming. Automatic selection only works well if the specimen's color or brightness is significantly different from the background. If this is not the case, then there is a trick to accomplish this. One has to artificially increase the color difference for the purpose of making a selection.

In Photoshop or GIMP create a new layer with exactly the same image on

top. Adjust the contrast of the top layer in such a way that the background becomes nearly white and the specimen completely dark. Use the automatic selection tool to select the (now nearly completely white and uniform) background. Then invert the selection. This will then select the specimen. Switch to the layer containing the unmodified original image and copy-paste this one to a new image.

Stacking

This is one of the most powerful processing techniques. It refers to the combining of several images that have different parts in focus into one final image that has all parts in focus. Stacking therefore helps to overcome the limitations of a small depth of field, which can be problematic especially at higher magnifications. When used in conjunction

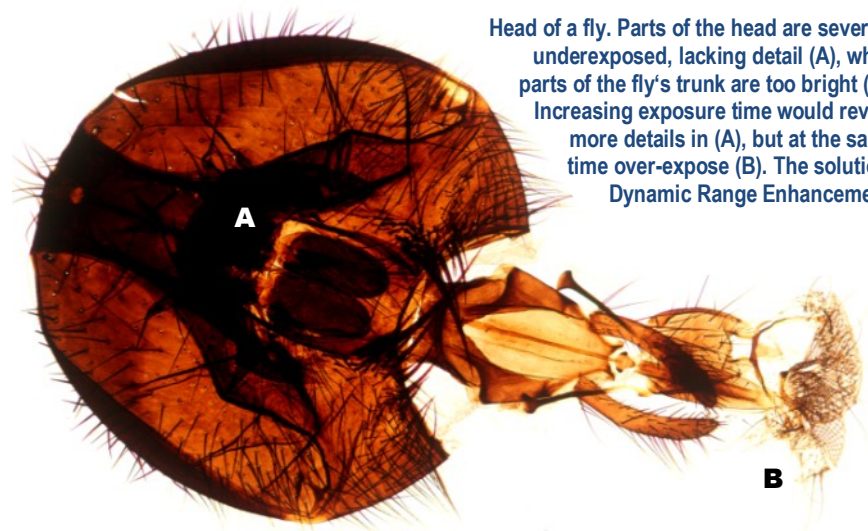
The program Picolay is even capable of converting a stack of micrographs into a 3D image that can be viewed with red-cyan glasses to give a true stereoscopic impression. The math involved must be enormous, but luckily this is something that we do not have to worry about...

For stacking to work properly, you need more than 10 different images. Computer processing time can also be considerable. It may therefore be beneficial to reduce the image size before stacking to speed up the processing.

Dynamic Range Enhancement

Dynamic range refers to the difference between the brightest and the darkest area that a camera system is capable of capturing. Compared to chemical film, digital cameras of today have a lower dynamic range. They are not as capable of capturing details in both bright and dark areas in the same picture. At a given light intensity, a longer exposure time is needed to make details in the dark areas visible. This may, however, overexpose the light areas in the image, causing a loss of detail in these areas.

There is, luckily, a workaround to this problem. It is possible to take pictures with different exposure times and then combine them, with the help of a program, into a final, correctly exposed, image. The software will only take the parts of the source images that are cor-



Head of a fly. Parts of the head are severely underexposed, lacking detail (A), while parts of the fly's trunk are too bright (B). Increasing exposure time would reveal more details in (A), but at the same time over-expose (B). The solution: Dynamic Range Enhancement.

rectly exposed to assemble the final image.

The free program CombineZP is capable of this processing. You need to load the DRE macro from the menu to enable this feature. Alternatively you can install a plugin for the software GIMP.

If you want to use GIMP, you have to take several pictures with different exposure settings. Start out your experiments with three pictures, one underexposed, one correctly exposed and one overexposed (this is called exposure bracketing). The image which is underexposed will show details in the highlights, while the overexposed image will show details in the dark areas. Download and copy the GIMP HDR Tone Mapping plugin into the plugins directory. Then copy paste the three images into three different layers of the same picture and start the plugin.

One possibility is take one picture and then "overexpose" or "underexpose" this picture by adjusting the brightness using the "levels" tool. You essentially create different "exposures" from one original image. Understand that this only works to a certain degree. Details that were not captured by the camera in the first place can not be made to appear this way. It is much better to use true exposure bracketing when taking the picture.

Dodging and Burning

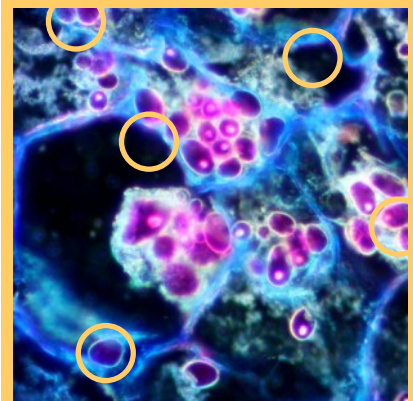
If you do not have exposure bracketed pictures and only one original which you need to improve, then you may try to use dodging and burning to

locally adjust the brightness levels. This solution requires patience and some manual editing. Dodging refers to the local brightening on a picture, while burning darkens a particular area. This can be done with a tool in GIMP or Photoshop.

Final Thoughts

How much image editing is permissible? One should not forget that the line between image enhancement and scientific forgery is not always as clear. Have a look at the puzzle on the back cover to see what I mean! Don't cheat, the answer can be found in the box below. ■

Answers - No cheating!



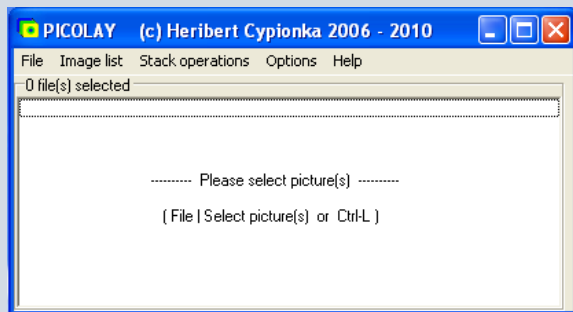
From p. 35:
A: muscle fibers B: root of a fern
C: cat fur D: connective tissue
From the back cover: Hungarian
Gulash soup (food). Don't worry if you
have not guessed it. I wouldn't have
either...

Background Clean-up with Picolay

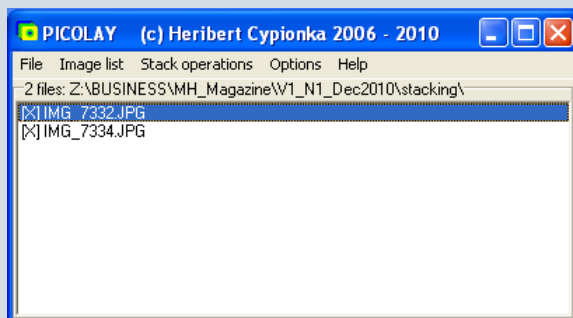
Picolay is a small, yet extremely powerful program to process digital images. Among other useful functions, it is able to remove a dusty background from micrographs.

It may be difficult to completely remove disturbing dust and dirt from the optical path of a microscope or the attached camera. Dust on the camera's sensor can prove to be particularly difficult to remove as cleaning attempts may even worsen the situation by introducing new dust. Dead camera pixels pose a similar problem. Here too we have a constant disturbance in the image, always at the same location.

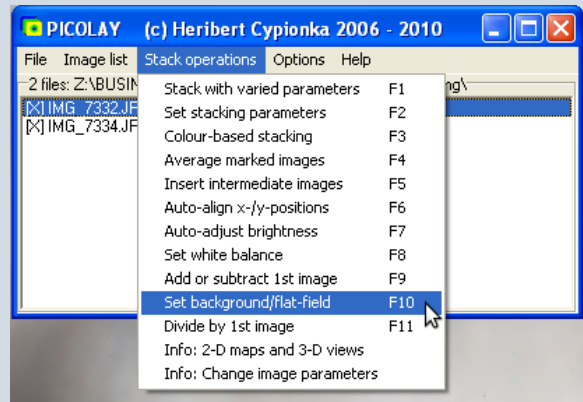
It is possible to digitally clean the background of a micrograph by subtracting the background. The free pro-



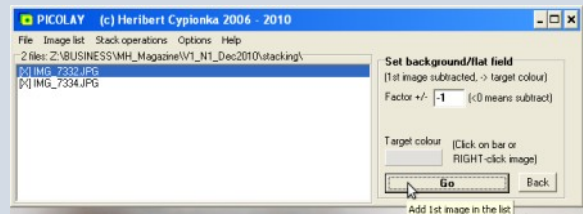
1 After starting the program Picolay, you will be greeted with a small window. Press CTRL-L to select two files. The first file should be an image of the background. The second file should be the micrograph that you want to improve. This order is important.



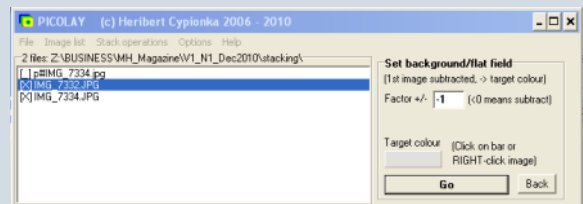
2 The files are now displayed in the window. The top file should be the background image that you want to subtract.



3 Press F10 or select Set-background from the menu. The dropdown menu lists all possible operations.



4 A new window will open. Before clicking on the Go button, you should set the color of the background, the target color. Right-click with the mouse into the background of one of the opened pictures. The factor (decimals also possible) specifies the degree of subtraction.



5 Picolay will now process the image. A new file will be created and saved to the directory.



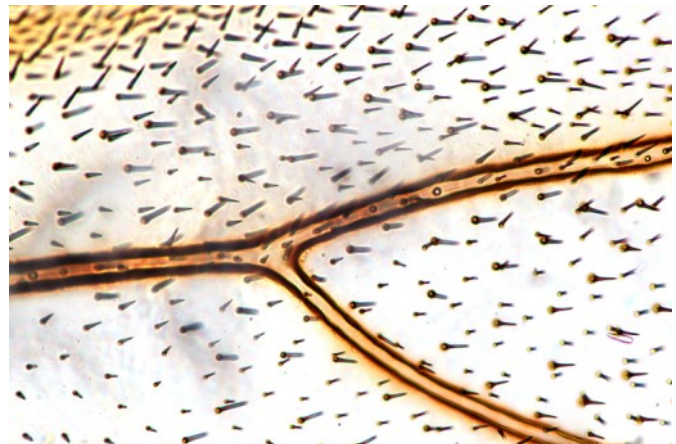
The background image. There is much dust in the optical system. For demonstration, I have placed an unrealistically large amount of dust on the microscope's lamp. The circle indicates dust which is missing on the micrograph to be improved (right).



The micrograph to be improved. In this example, it is the wing of a bee. The dust is quite disturbing. Let's see if the program is able to calculate it away.



Despite the large amount of dust, the software was able to completely remove the disturbance. Notice that there are now brighter areas where the dust was located, as well as bright spots (circled). Fine-tuning the subtraction factor may help to reduce these bright areas.



Contrast enhancement, white balance and some manual retouching greatly improves the final result. The fact that one is capable of removing such an extreme disturbance from the image is quite remarkable.

gram Picolay will do just that in a highly automated manner. It is necessary take two pictures, an empty one with only the (dusty) background and one with the specimen in place.

Try to take the picture of the background with the slide in place but no specimen visible. Removing the slide will change the focus of the dust. For image subtraction to work best, the background should not be different, however. Removing the specimen slide may also alter the position of a few dust grains which are located between the slide and the condenser. This can result in artifacts.

The images above show two different kinds of artifacts. In one case, there was some dust present on the back-

ground image, which was then missing in the image of the specimen. Subtraction resulted in a bright spot. This phenomenon is relatively easy to understand.

What is now the reason for the „washed out“ areas at the original place of the dust? This area appears to be brighter than it should be. The brightening effect is stronger the darker the dust.

When two dark structures overlap (such as the dust and the veins of the wings), then the resulting darkness is not simply a consequence of adding both colors. To use an extreme example, obstructions which *completely* block the light will result in a black area on the micrograph, covering all details

of the specimen. This results in a loss of information which can not be regenerated by subtracting the same black area from the micrograph. Image subtraction therefore works best if the dust is not too dark.

Last, I'd like to emphasize the importance of using a clean specimen slide when taking the background image. The background image should only contain the dust that can not be removed from the optical system. If the specimen slide itself is dirty, then the program will also subtract this dust from the image. The result are annoying white spots which must be removed by manually retouching the image. ■

The Effects of different Mounting Media

Mounting media can have an enormous influence on the stability and appearance of microscopic specimens.

The mounting medium can have a significant effect both on the image quality and on the specimen itself. I tried a little experiment by observing pollen from a plant (in this case the buttercup, *Ranunculus*), mounted in four different

ways:

- Air-mounted, with no cover glass
- Mounted in water (temporary mount)
- Mounted in Euparal medium (permanent mount)
- Mounted in nail polish (permanent mount)

All observations were made using a 20x achromatic objective.

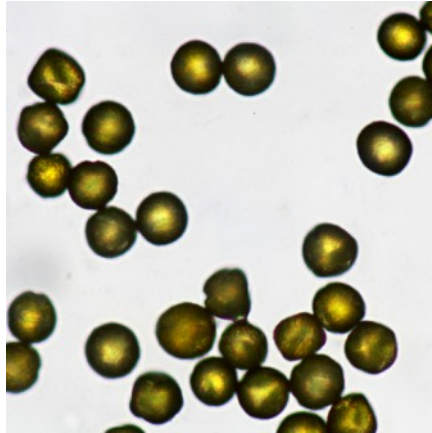
The Results

The images on the right show that the mounting method has a significant impact on the way that the pollen grains appeared. The results can be summarized as follows:

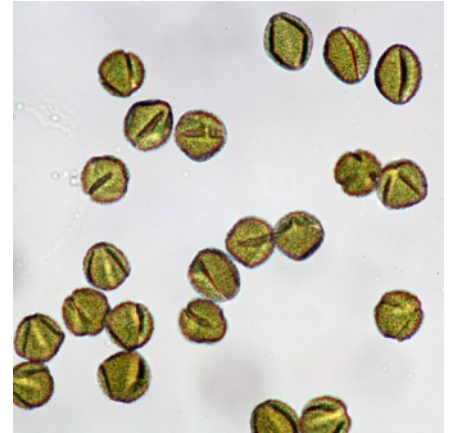
Air-mounted specimens show the least details. The pollen grains show a thick dark fringe, which covers much of the details. This is due to the large difference in refractive index between the pollen grains and the surrounding air. Opening the condenser diaphragm reduces the dark fringes, but also lowers contrast and depth of field. The cover glass presses the pollen against the slide, so that more of them are in focus. Otherwise the cover glass did not seem to make much difference.

The water-mounted sample provides a much better image. The dark fringes are now gone, due to the similar refractive index of the pollen and the medium. The pollen appear spherical, because the water causes them to swell up.

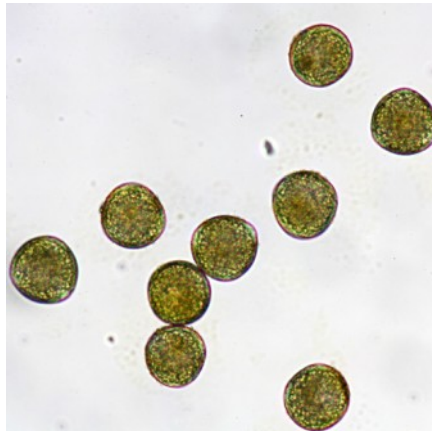
Pollen mounted in Euparal started to shrink and therefore appear smaller in



Air-mounted pollen. Dark fringes are visible due to the large difference in refractive index between the pollen and the air.



Nail-polish-mounted pollen. Before mounting, the pollen first had to be dried, resulting in their shrinkage. Compared to Euparal mounted pollen, less detail is visible due to the dark fringes.



Water-mounted pollen. Much more detail is visible than in the air-mounted sample. The pollen grains assume a spherical shape because they absorbed water.



Euparal-mounted pollen. The Euparal brightened the pollen, but caused shrinkage.

size. Kinks and folds are also visible. These artifacts are produced because the (non-water based) Euparal has withdrawn moisture from the pollen.

Clear nail polish showed a similar, but more pronounced effect as Euparal. The deformations of the pollen are very clearly visible. Evidently the solvent of the nail polish also removed significant

amounts of water from the specimen. The nail polish itself lost some of its volume during drying and started to shrink as well. Air bubbles also became visible in the nail polish. Irregular drying of the mounting medium and a change in the shape of the mounting medium during drying can lead to shear

forces, which may distort the shape of the specimen.

What about Glycerin Gelatin?

Glycerin Gelatin (glycerol gelatin, jelly) is a water-based mounting medium. Glycerin Gelatin according to Kissner is one of several Glycerin Gelatin variations. It is a common medium for mounting pollen. Due to its water-based nature it does not cause the pollen to shrink. An alternative water-based mounting medium is fructose syrup. Both Glycerin Jelly and fructose syrup do not dry completely and therefore require a sealing of the sides of the cover slip with nail polish (but the pollen do not touch the nail polish).

Lessons learned

What can we learn from these observations? First, permanently mount-

ing a specimen is not only important for slide storage. The mounting medium significantly influences the transparency, resolution and shape of the specimen.

Second, the choice of the mounting medium depends on the type of specimen to be observed and on the type of microscopic technique to be used. For phase-contrast work the refractive index of the mounting medium should be different from the refractive index of the specimen. For bright-field work the refractive indexes should be similar. Large differences in refractive index can lead to the dark fringes as seen in the air-mounted specimens.

Some philosophy

So which mounting medium now results in pollen grains with a “true” or “correct” shape? The problem now is:

what is the “correct” shape? Biological specimens may change their appearance depending on the environment. After a rain shower, the pollen may have a more roundish appearance, after having osmotically absorbed much liquid. Pollen that has dried in the air may resemble more the shape of the Euparal and nail polish samples. The choice of the mounting medium may therefore even include these considerations. ■

External Links

An introduction to pollen analysis:
<http://books.google.com/books?id=F-DAV3jL25UC>

Aqueous Mounting Medium Protocols:
http://www.jhcworld.com/protocols/histology/mounting_medium.htm

Making and Using Aqueous Mounting Media:
http://www.jhcworld.com/protocols/histology/aqueous_mounting_medium.htm

Q & A

Microscopy Q&A

Why does the smell of hay infusions decrease over time?

As a hay infusion ages, different micro-organisms start to grow (and others start to die out). Different micro-organisms produce different substances which are responsible for the smell.

Why is refractive index of mounting media important?

The refractive index is important for several reasons. First, it influences the resolution of the image. Second, if the refractive index of the specimen is too similar to the refractive index of the mounting medium, then it may be difficult to see the specimen if it is not stained. Phase contrast microscopy relies on a different refractive index between medium and specimen. If the refractive index is too different (eg. mounting the specimen in air), then the specimen may appear to be too dark.

Does glycerol mounting cure?

It will dry only up to a point, but will not (and should not) completely dry out. The glycerol will prevent the complete drying. This makes sure that a

certain amount of water remains in the sample. A complete drying of the glycerol mounting medium could result in a shrinking and deforming of the specimen. Algae and other water organisms are especially sensitive to this. It is possible to protect the permanent slide by sealing the edges of the cover slip with nail polish.

Why is it desirable that microscope objectives be parfocal?

Parfocal objectives are not only desirable, but (in my humble view) an absolute necessity for efficient microscopic work. Parfocal objectives manufactured in such a way that a change in objective will not result in a significant loss of focus. If the image is in focus using a 4x objective, then the image is also in focus when a 10x objective is used. Significant refocusing is not necessary with parfocal objectives.

What are some disadvantages of permanent slides?

Permanent slides contain specimens that are fixed, dehydrated and possibly also microtomed (sliced into thin sections). The organisms are therefore not moving. Over time the specimen may also start to lose color. Generally, permanent slides require much more elaborate preparation. The advantage is, however, that once prepared the slide can be used over and over again and can be stored for longer time periods.

Why are unstained bacteria difficult to see?

They are difficult to see in bright-field microscopes, because they lack color. Phase contrast microscopes are much better for viewing of unstained bacteria. Beginners also have problems distinguishing bacteria from dust and debris. They are also small and the resolution and magnification must be high.

Who invented the microscope?

Which microscope? There are many kinds. In 1931, Ernst Ruska and Max Knoll constructed the first prototype of an electron microscope. Optical microscopes as we know them today evolved over a longer time period. Many people contributed to the developments. Two notable figures are Antonie van Leeuwenhoek (1632 – 1723) and Robert Hook (1635 – 1703). Leeuwenhoek made single-lens microscopes with which he discovered bacteria. Hook constructed compound microscopes (composed of objective and ocular lenses) and coined the term “cell”.

If you have any general microscopy-related questions, please send an email to the editor. I can not promise you that I'm able to answer all of your questions, and naturally I can not be held liable if the answers are incomplete/wrong/misleading etc.

How to Use Kohler Illumination in Microscopy Studies

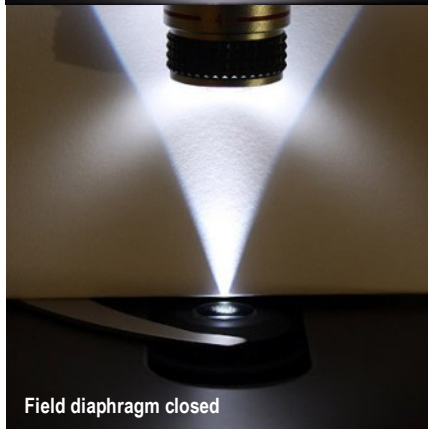
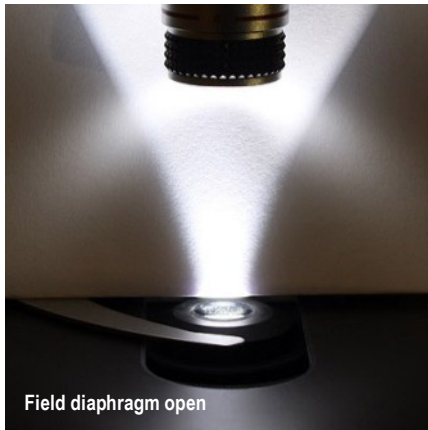
By Edison Ramsey. August Köhler has revolutionized the lighting system of microscopes. In his groundbreaking article of 1893, he described a new illumination system which greatly improved microscopic imaging.

When you look at something under a microscope, the intensity and distribution of light must be clear and equal. Otherwise, you will not be able to study all aspects of the specimen. The notes you make about your observation will not be valid. It is true that you will still be able to see a good number of parts of the specimen but the shadows or yellow sheen created by a substandard illumination will most likely affect your observations negatively. This was the problem of scientists before. Thankfully, a German professor named August Köhler came up with a revolutionary way to illuminate objects in a sample plane. He invented the Köhler illumination in 1893.

Answering Questions That Led To A New Way of Doing Things

August Köhler was doing his graduate studies when he discovered the new way to illuminate objects on the sample plane of microscopes. At that time, there were many methods used to brighten objects under the microscope. One involved using gas lamps. Another mirrors. When they finally used bulbs, the filament of the bulb they used was visible in their microscope's sample plane. Needless to say, these methods were not satisfactory.

By trying to come up with solutions on various microphotography problems, August Köhler was able to invent the Köhler illumination. But it was not until he was hired by the company Carl Zeiss AG that his invention was truly given enough attention. Until today, Köhler illumination is still used. It has also been the basis for the more modern method of microscope illumination.



Köhler illumination systems are equipped with a field diaphragm. An open field diaphragm results in a wider light beam (top). A closed field diaphragm illuminates only the part of a specimen which is actually observed (middle). The system is properly set up, when the edges of the field diaphragm are in focus and centered (bottom). (ed.)

How to Set Up Köhler Illumination

This system needs a bulb with high density illumination, field diaphragm, condenser diaphragm, collector lens, and condenser lenses. The first thing that you need to do is to set up your specimen. Close the field diaphragm until you see its edges. The edges will be blurry at this point. Now turn the condenser knobs to make the edges as clear as possible. Then center the image of the closed field diaphragm. Do this by using the condenser-centering screws. After that, you open the field diaphragm just a little bit to bring its edges out of the field of view. Then adjust the condenser diaphragm to adjust your sample's contrast. If the light is too intense, you may have to adjust it. The best way to do this is to use the proper filters. It is not advisable to reduce the power supply as this will give the sample a yellowish or brownish look. Once these steps are followed, you will be able to enjoy sample analysis without any light interference. Your experiment or observations will not have shadows or uneven lighting.

If these steps don't work, your lens probably need cleaning. Dirt in the lens and other parts of the microscope will greatly affect the quality of your image. If you still do not see a well-illuminated sample, you may have to do the steps again to check if you did them properly. ■

CanScope - complete solution for all your microscopy needs. Contact: 1-877-56SCOPE(72673) or info@CanScope.ca

Article Source: http://EzineArticles.com/?expert=Edison_Ramsey

Lens Abuse! The Effect of Dirt and Cracks on Image Quality

That a clean objective lens is necessary to achieve a good image quality is not a secret. The desolate condition of the 40x objective that I present you here, even surprised me...

The microscopes in the school where I work have been in operation almost 30 years (!) now. And the fact that most of them have remained usable says quite something about the quality of these devices. They are soon going to be collectively sent in for maintenance, and this is the last opportunity to do a little quality check.

Most microscopes were still in a reasonably good condition, with the biggest problems being the mechanics. A check of the optics revealed that most of them were still quite OK, but the 40x objective of one of the scopes was in a surprisingly desolate condition. A macro image of the front lens can be seen on the right. I suspect highly that one of two things happened to the objective:

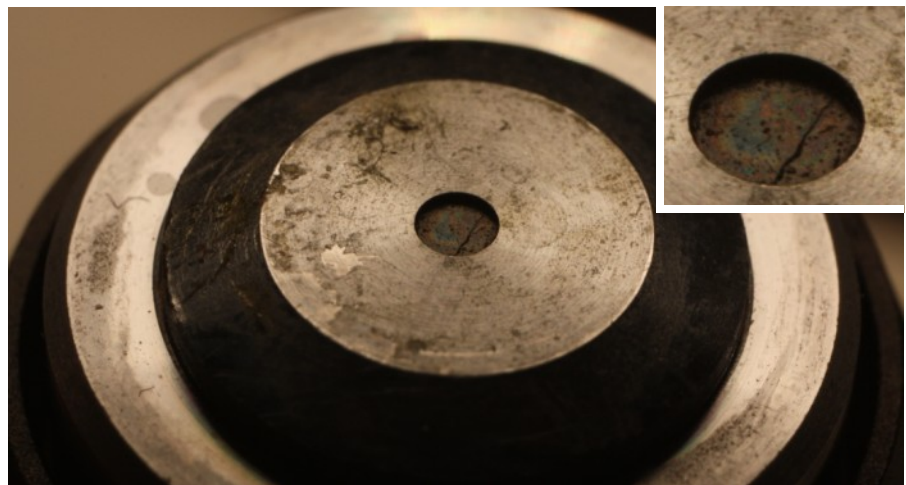
The objective could have been rotated into immersion oil and was subsequently not cleaned. The oil then collected dirt and solidified. Students sometimes want to use a lower magnification after they used the 100x oil immersion objective. The consequence is, that they rotate a non-oil objective into the oil.

A second possibility is, that the "dirt" on the objective is in reality resin for making permanent slides. Maybe some students attempted to make a permanent slides and used too much resin, and did not wait for the resin to dry out. The front part of the objective was then rotated into the resin.

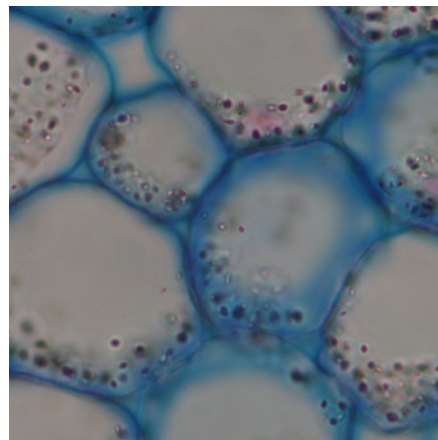
The origin of the crack in the lens, remains a mystery. The lens is spring loaded, and retracts when crashed into the specimen.

I included two pictures of the same area, one with an intact 40x and one with the dirty and cracked objective from above. I think that the two images speak for themselves.

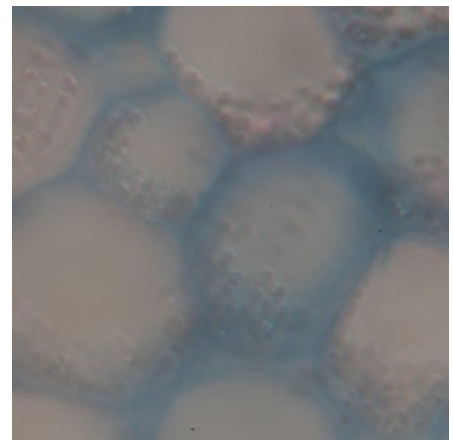
What do we learn from this? Proper microscope instruction saves money.



Macro image of the front lens of a dirty and cracked 40x objective. Even alcohol was not capable of removing the residue on the lens. One has to be careful that alcohol does not enter the crack. This could soften the glue holding the lens in place.



A clean 40x objective provides a sharp and crisp image. The picture was not digitally enhanced.



Even after attempted cleaning, the objective produced a soft, low-contrast image. The picture was taken using the above 40x objective.

And be really careful about using immersion oil and mounting medium in the classroom. Don't even get the students into the position of using immersion oil with the non-oil objectives by locking the immersion oil away. Using an oil-immersion lens without oil may also spell disaster, however: The focus is much more difficult to find and stu-

dents may yet again crash the front lens into the specimen. Maybe one advantage of using 160mm DIN microscope systems (instead of infinity systems) in education is that the optics are much cheaper. There is not much lost, when the objective lens is destroyed. But this is a different story. ■

Almost DIY Video Camera

In this photo essay, I'd like to present you my first analog video camera—made from a scrap piece of aluminum and a color surveillance camera module.

Back in 1998, when I first bought my microscope, microscope video cameras were quite expensive and not affordable for me. At that time, webcams still had a low resolution and did not allow me to capture video at full frame rate. They were jerky and small. Analog color TV surveillance camera modules were available at relatively low cost, however. They also provided sufficient quality. Mounted on a small board, there was not only the sensor, but also a small lens, quite similar to modern-day

webcams. This lens I had to remove. The module did require a separate 12V power supply and was connected to a TV/VHS recorder over a video jack.

I ordered one of these TV modules and a friend made me a solid aluminum casing on a turning lathe. Looking back, this casing was somewhat of an overkill. Grinding out the metal from a cylindrical piece of aluminum did take quite some time and it was necessary to re-grind the metal several times in order to make it fit without play or friction on the trinocular head. I would now rather use a small plastic casing and some cardboard, which is both lighter and

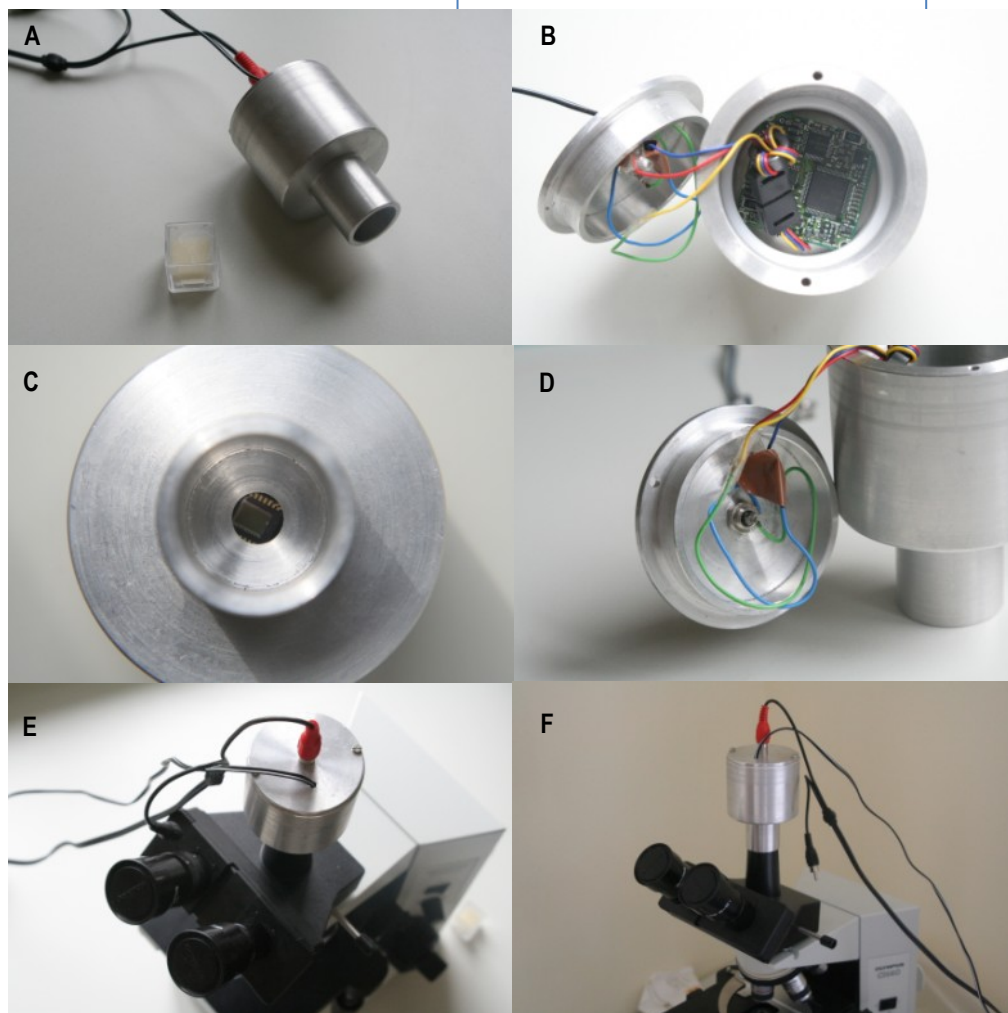
smaller. For whatever reason this alternative did not come into my mind at that time. The TV module was held in place by sandwiching it between two rings of Teflon, which we happened to have around by chance.

Well, what shall I say? It worked great and I was able to record many hours of video material (paramecia, water fleas etc.) on tape. Now more than 12 years later, the system has become quite obsolete. Digitizing an analog video signal is not state of the art and I'm now considering to replace the TV module with a modern webcam, one which is also able to record video at a high frame rate.

You do not need a trinocular microscope head to take pictures. Try to attach a webcam without lens directly to the microscope. Remove the eyepiece of the microscope to allow the objective to directly project an image on the webcam's sensor. Be careful that you mount the webcam in such a way that you do not have to refocus, otherwise there is the danger of crashing an objective into the specimen slide. ■

The camera has two cables, a 12V power supply and an analog video signal. It is not fastened to the trinocular head, but held in place by gravity alone. No photo projection ocular is used.

A: size comparison
B: camera main board
C: camera sensor
D: lid with connectors
E, F: „gravity-mounted“ on the microscope



The Pine Cone

The cone is the reproductive organ of coniferous trees. While digging through a commercial slide box, I found a beautifully prepared specimen of the female pine cone.

Most pine trees are monoecious. This means that they have both male and female cones on one tree. After pollination the female cone can take a few years to develop. During this time the cone grows to reach its characteristic shape. The seeds develop in the cone. At maturity, the cone opens up and releases the winged seeds.

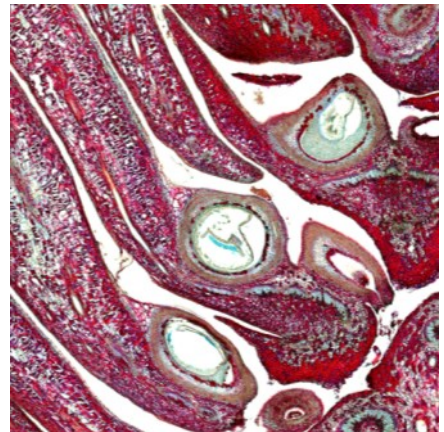
The micrograph shows a cross section through a female pine cone. Each

scale contains an ovary forming an egg cell. After fertilization, the ovary will form a seed, which is dispersed by wind.

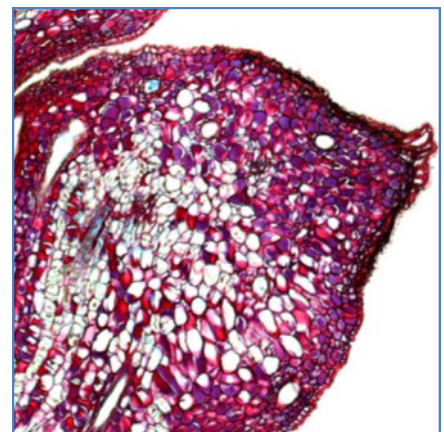
The image was taken from a commercial ready-made slide. For this reason, I am not able to tell you anything about the stains that were used. Due to the large size of the specimen, I had to stitch several individual images together.



The commercial specimen slide (the images were made from different slide, but from the same series).



Each scale of the cone contains an ovary, which produces an egg cell. The egg cells will form the seed after fertilization.



Individual cells start to become visible at this magnification. The resolution naturally decreases as well.

Image of a female pine cone. Several images were stitched together to obtain this final picture.



A not so Successful Attempt to grow *Paramecium*

Here I would like to present you an unsuccessful hunt for *Paramecium*, and the identification of the rather strange looking ciliate *Lacrymaria*.

The ciliate *Paramecium* is, as many of you probably know, a very popular organism to view in schools. They are comparatively large (and therefore easy to observe), move around and are almost certainly capable of entertaining 6th graders for a full biology lesson. The only problem is: from where do I obtain a sufficiently high concentration? Evidently they need to be enriched from pond water. It should be relatively simple. Add a bit of food, and wait for a day or two. It wasn't that easy, however.

I obtained some pond water and a quick check under the microscope revealed an abundance of smaller ciliates and bacteria. I added three pieces of oats to the water and waited. After 2-3 days the water started to turn cloudy. A skin started to form on the water surface. A quick microscopic check revealed a large number of bacteria but very few ciliates. There is a real danger of the bacteria using up all of the oxygen in the water. I therefore removed both the skin on the water (to permit better diffusion of oxygen) and the

remaining oats. Now the bacteria have. Additionally I added some fresh pond water to compensate for evaporation. After 1-2 more days the turbid water started to clear. Evidently something was eating away the bacteria. Another microscopic check now revealed somewhat more ciliates rushing across the slide. The large paramecia, that I have hoped for, were still missing. The pictures on the right show some of the organisms, which I was able to photograph.

What went wrong? The answer could be surprisingly simple. Maybe there were not enough paramecia present in the original water sample in the first place, or the conditions were simply not right for them to reproduce to a sufficiently high quantity. Maybe the large number of bacteria reduced the oxygen content of the water sample to the extent that they could not grow.

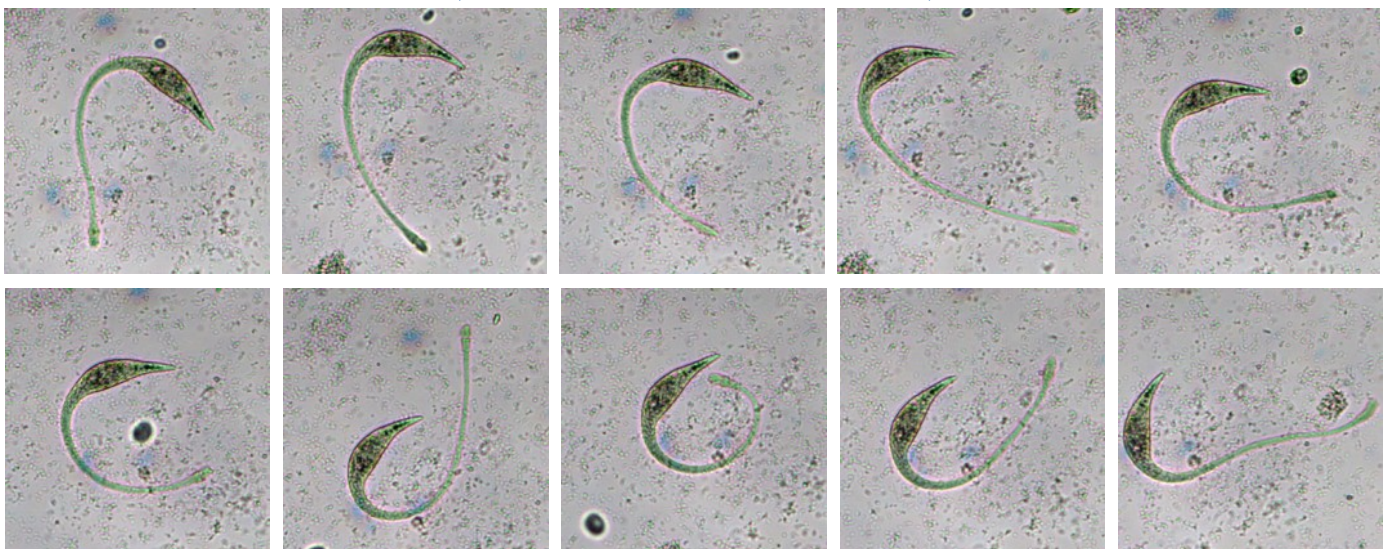
Should I now use the water sample for school? The answer is a definitive no. The picture on the top right shows that there are uncountable bacteria present in the sample as well. I simply do not consider the water sample safe enough.



Paramecium not quite. And plenty of bacteria in the background...

Lacrymaria sp.

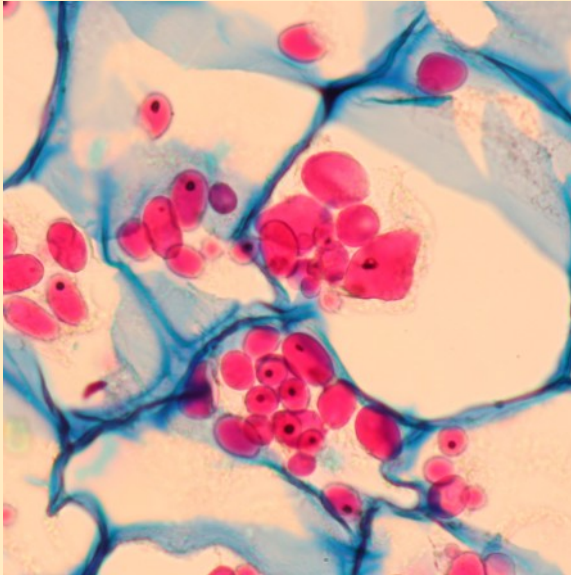
I also discovered a rather unusual protozoan. The time series on the bottom shows a cell with a long, flexible and very extensible trunk-like extension. This trunk was moving back and fourth in a searching movement. It happens to be the predatory ciliate *Lacrymaria*. The Latin name means "tear of a swan" making reference to the tear-shaped body and the long swan-like neck. ■



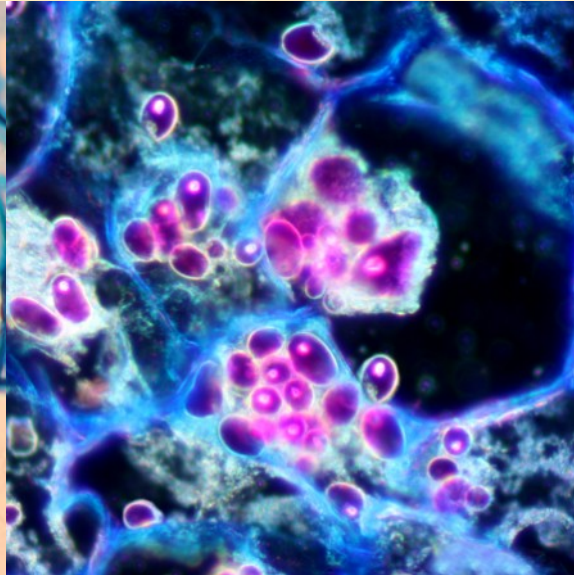
Series of pictures illustrating the movement of the neck of the ciliate *Lacrymaria*.

Find the differences!

Both pictures show the same part of a potato. The left one was taken in in bright field, the right one in dark field. The pink structures are starch grains. But there is more! The dark-field image has been digitally manipulated: there are 5 mistakes. Try to find them by comparing the two pictures! You can find the answer on page 23.

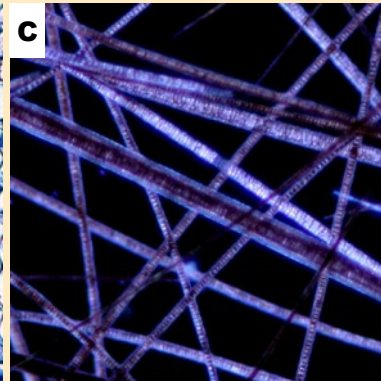
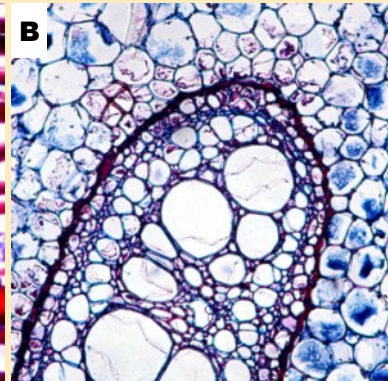
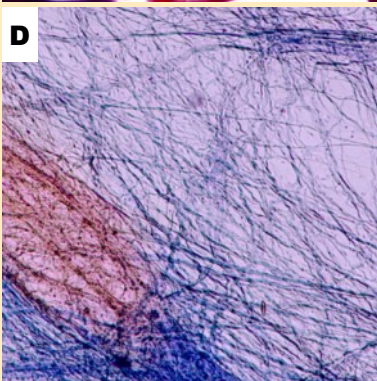
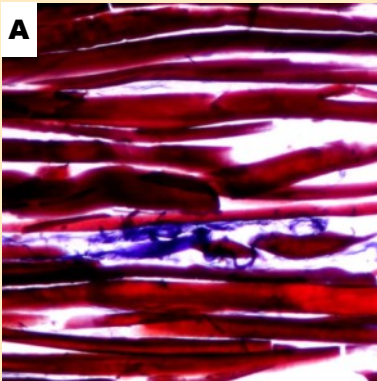


Original (bright-field)



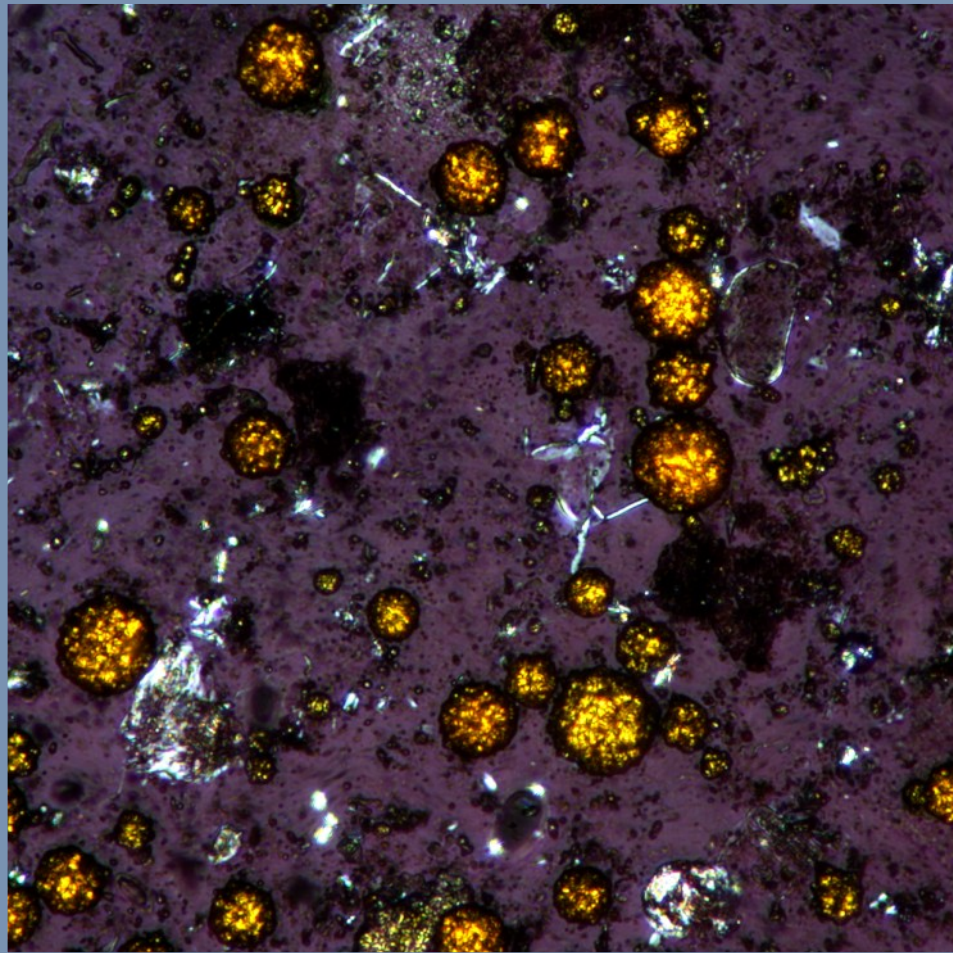
5 mistakes (dark-field)

What is this?



Try to match four of the following with the correct pictures:

- vascular tissue of a leaf | cells of wood | root of a fern
- purple bacteria | human hair | blood capillaries
- cotton | filamentous bacteria | cat fur | root of a sunflower
- connective tissue | polyester fibers | nerve cells
- tissue paper | asbestos fibers | muscle fibers



What's this? Answer on page 25.