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The Magazine for the Enthusiast Microscopist

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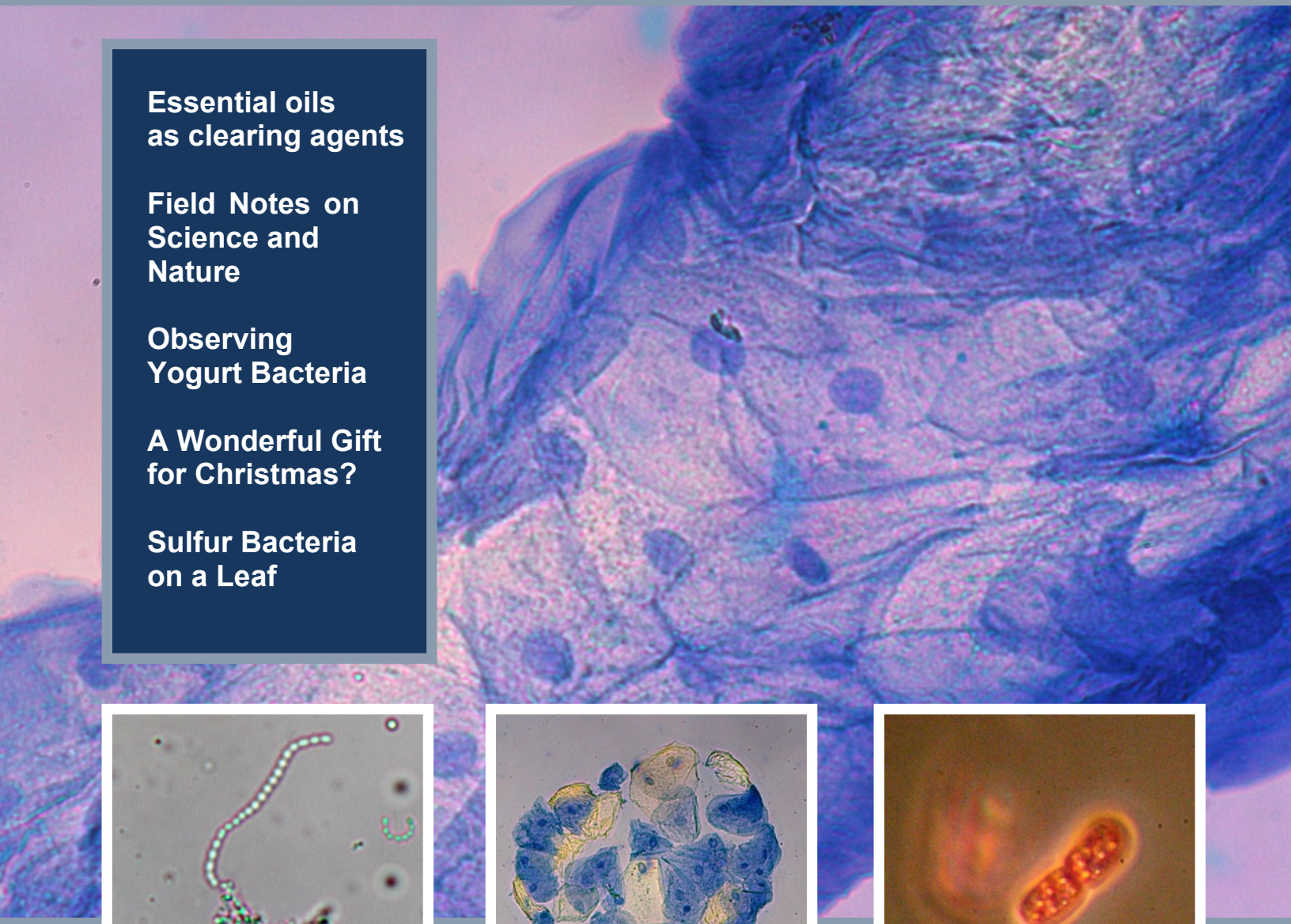

Yogurt Bacteria

Staining Epithelial Cells

Purple Sulfur Bacteria

ABOUT

Microbehunter Microscopy Magazine
The magazine for the enthusiast microscopist
MicrobeHunter Magazine is a non-commercial project.

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Front Cover:

Large image, left image, middle image: Oliver Kim
Right: Charles Guevara

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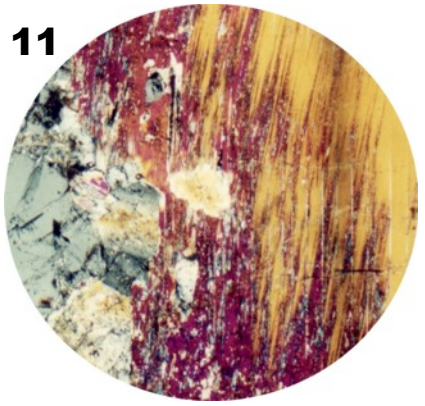
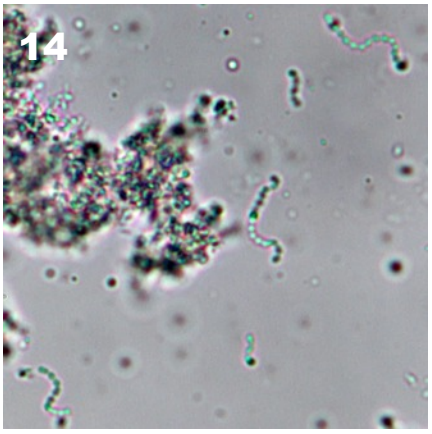
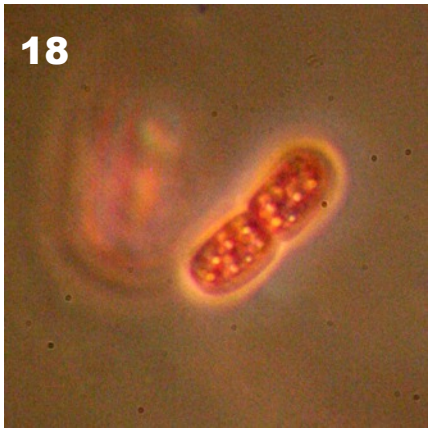
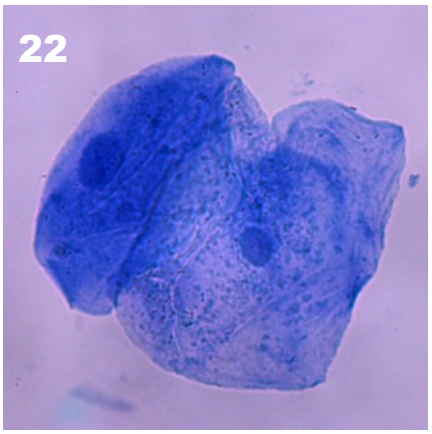
Answer to the puzzle (back cover):Tracheal system of an insect.









A Review of Essential Oils as Clearing Agents for Natural History Specimens

Clearing prior to mounting in resinous media is often precluded by xylene, however certain essential oils have previously been used in microscopy as clearing agents. While they certainly smell better than xylene do they actually perform?

Andrew Chick

Numerous small animals are mounted on microscope slides for identification, and archiving. These are normally mounted in either a gum based mountant such as Berlese, Hoyers or Modified Dionis (Chick, 2010), Euperal or one of the resinous mountants such as Canada Balsam, Gum Damar or an un-recommendable synthetic mountant such as DPX (Brown, 1997 states that the British Natural History museum no longer accepts specimens mounted in DPX). The method for mounting insects and other invertebrates for study in resinous media is rather well known and is characterised by Oldroyd (1970) as maceration of the soft tissues, bleaching/staining as necessary, dehydration, clearing and mounting. It is with the clearing stage with which this paper is concerned. Clearing is required to remove the dyhydration agent (usually alcohol, however Acetone or Glacial acetic acid among others are recommended by some protocols). Histologists traditionally use xylene to remove dehydration agents as this is the solvent often used as a carrier of the resin in the mounting media. However for the student of natural history it is deemed unsatisfactory by Oldroyd (1970) due to the speed of evaporation not allowing adequate time for dissection. From a personal perspective, xylene is rather difficult for the amateur microscopists to obtain, and the fumes can result in tremendous headaches. It is also considered carcinogenic all added together it is best avoided!

Traditionally, entomologists have used essential oils as clearing agents, normally clove oil or cedarwood oil, however Gray (1954) lists numerous oils and how pure the alcohol they are cleared from must be. A few of these were tested for there suitability as clearing agents for insect specimens.

Methodology

For this investigation the humble larvae of *Calliphora vomitoria* was used, this was picked as it is commercially grown for fishermen to use as bait. The larvae were fixed using freshly boiled water and preserved in 70% alcohol. They were mounted in Gum Damar using the method of Chick and Cassella (2011) as follows:

1. Maceration in 10% Sodium hydroxide,
2. Stain was Acid Fuchsin
3. Dehydrate using 70% then 100% IPA alcohol.
4. Clear in oil
5. Mount in Gum Damar

The essential oils chosen were as follows:

- Reagent grade clove oil (some what of a luxury but a useful control)
- Clove oil B.P.
- Tea Tree oil B.P.
- Eucalyptus oil B.P.
- Distilled turpentine
- Cedarwood oil (reagent grade)
- Lavender oil
- "Olbas oil" B.P. a commercial mix of essential oils, including Eucalyptus and clove.

Where possible, B.P. (British pharmacopeia) grade oils were selected.

Gray (1954) notes the following about the chosen oils: Clove oil has a refractive index of 1.53, equal to both glass and Canada balsam. Objects can be transferred from a minimum 75% alcohol.

Eucalyptus oil requires a minimum of 80% alcohol as the final dehydration step. And it has a lower refractive index of 1.46

Turpentine has the refractive index of 1.47 and requires near perfect dehydration with 95% alcohol.

Cedarwood oil again requires near perfect dehydration from 95% alcohol, and has a refractive index close to glass at 1.50.

Lavender oil has identical water tolerance and optical qualities to Eucalyptus.

Tea Tree oil and Olbas oil were not considered by Gray (1954)

Results

Clove oil (reagent): Some fading of stain over time, (over a month after mounting), however appears to be easily counter acted by using a double stain of Acid Fuschin and Methylene Blue (50/50 mix)
Clove oil B.P.: Appears similar to above
Eucalyptus oil: Strips stain within 10 minutes
Lavender oil: Strips stain within 10 minutes
Distilled turpentine: Strips stains within days, clouding of mount noticed

Cedarwood oil: Strips stain within days of mounting
Tea tree oil: Strips stains within days of mounting
Olbas oil: Strips stains within 10 minutes.

From the above it would appear that clove oil is the best of the tested oils with regards to stain preservation.

Discussion

With phase contrast and anhydrous alcohol, any of the oils would be suitable for use in microtechnique, indeed it was suggested that clove oil leaves specimens brittle so the other oils would be preferable. However, if staining is required, clove oil (either reagent grade of pharmacy grade) are the choice of clearing oil. This combined with its tolerance for imperfect dehydration, Gray (1954) notes that anhydrous reagents are extremely difficult to keep water free as they will absorb moisture from any air around them. Indeed Fowell (1946) recommends clove oil as a more forgiving clearing oil for students. It is worth noting on this point that there are two essential oils to be avoided at all costs these are oil of wintergreen and oil of juniper as both oil require perfect dehydration with 100% alcohol, which may be impossible to obtain.

In conclusion, as a general clearing agent clove oil B.P. would appear to be of most use or the amateur showing no apparent difference to the reagent grade oil in use.

References

Brown, P.A., 1997, A review of techniques used in the preparation, curation and conservation of microscope slides at the Natural History Museum, London, *The Biology Curator*, **10**: Special Supplement, 33pp.
http://natsca.info/sites/natsca.info/files/The_Biology_Curator_Issue_10_Supp.pdf

Chick, A.I.R., 2010 A modification of Dionis Mountant as a substitute for Berlese Mountant. Entomologists Monthly Magazine. 146 (2) 117-118

Chick, A.I.R., Cassella J.P., 2011 Gum Damar as a substitute for Canada Balsam in mounting media for microscopical specimens, Entomologists Monthly Magazine, 147 (2) 111-114

Fowell, R.R., 1946, Biology staining schedules for first year students. H.K. Lewis and Co. Ltd London

Gray, P., 1954., The Microtomists Formulary and Guide. Constable and Company, Ltd, London

Oldroyd, H., 1970 Collecting, Preserving and Studying Insects. Hutchison scientific and technical. London

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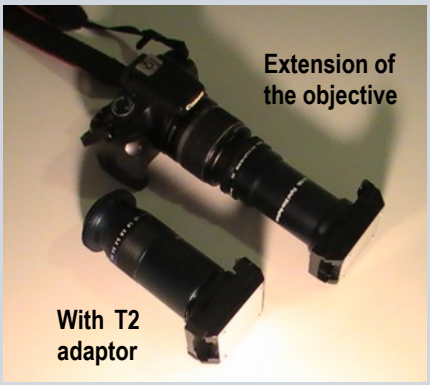
Bad Pictures

Why report back only the successful microscopy attempts? This one resulted in quite disappointing images.

I have two slide duplicators in my possession. These duplicators are used to make copies of negative film and slide film. One duplicator can be mounted directly on my SLR camera using a T2 adapter ring. The duplicator directly acts as a macro lens. Focusing is not possible with this system. The other duplicator attaches to the front of of my camera zoom lens. It is possible to focus using the zoom lens. Both systems are able to achieve a high magnification.

I wondered if these two set-ups are also suitable for microscopy, and mounted some permanent slides into the film holder. The results were rather disappointing. The contrast is low and the resolution bad. Especially the combination of the duplicator with an existing objective resulted in significant loss of sharpness towards the corners.



A disappointing result: Both slide duplicators delivered low-resolution images of low contrast. Vignetting could also be observed.

What a pity. I already hoped to have found a method to quickly make useable overview slides at the click of a button. I suspect that part of the problem was the inability to carefully focus the specimen slide. Maybe scanning a slide in high resolution results in more useful pictures (ed.). ■

New Techniques in Microscopy

Smartphones as microscopes, scanners that scan slides instead of traditional microscopes…. Where will the future take us?

Oliver Kim

Smartphone Microscope

Researchers have developed a low-cost microscope which uses the camera of a mobile phone together with a 1mm ball lens mounted in front of the camera objective of the mobile phone. The cell-phone microscope was able to magnify up to 350x with a nearly diffraction limited resolution, the theoretically maximum resolution possible. The resolution of the microscope was determined to be 1.5 micrometers at the center of the image. While the image quality rapidly deteriorates towards the edges, the quality is still sufficiently high to conduct routine blood diagnostic tests.

The quality of the image can also be improved digitally by stacking several images taken at a different focus. Focusing is possible by changing the distance between the slide and the spherical lens. It is also possible to use a cylindrical GRIN lens (gradient index lens) instead of a spherical lens (figures 1 and 2). This greatly improves the image quality towards the sides, but reduces the overall image resolution.

The researchers demonstrated that it is possible to diagnose the blood diseases sickle cell anemia as well as iron deficiency anemia from blood smears (figure 3).

Reference: Smith ZJ, Chu K, Espenson AR, Rahimzadeh M, Gryshuk A, et al. (2011) Cell-Phone-Based Platform for Biomedical Device Development and Education Applications.
PLoS ONE 6(3): e17150.
doi:10.1371/journal.pone.0017150

URL of original Article: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0017150

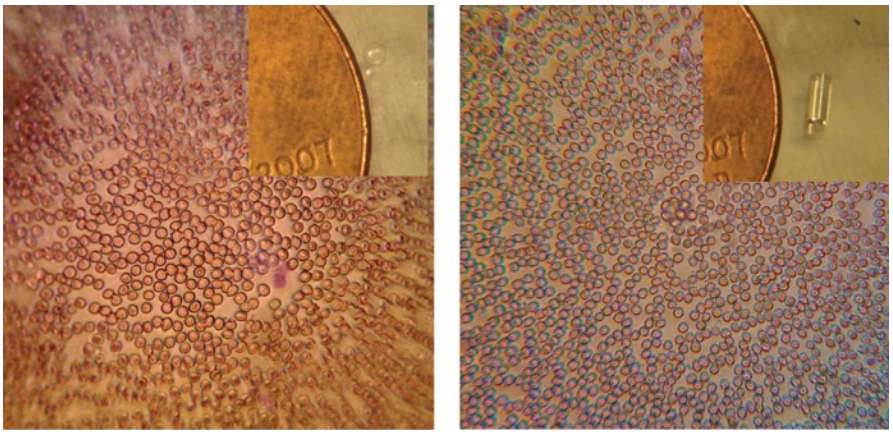


Figure 1 (left): A sphereical 1mm diameter lens (to the right of the coin) produces an image which is sharp in the center but out of focus on the side.
Figure 2 (right): A GRIN lens is cylindrical. It produces an image which is also sharp at the sides, at an overall lower resolution. Image: Zachary J. Smith et al.

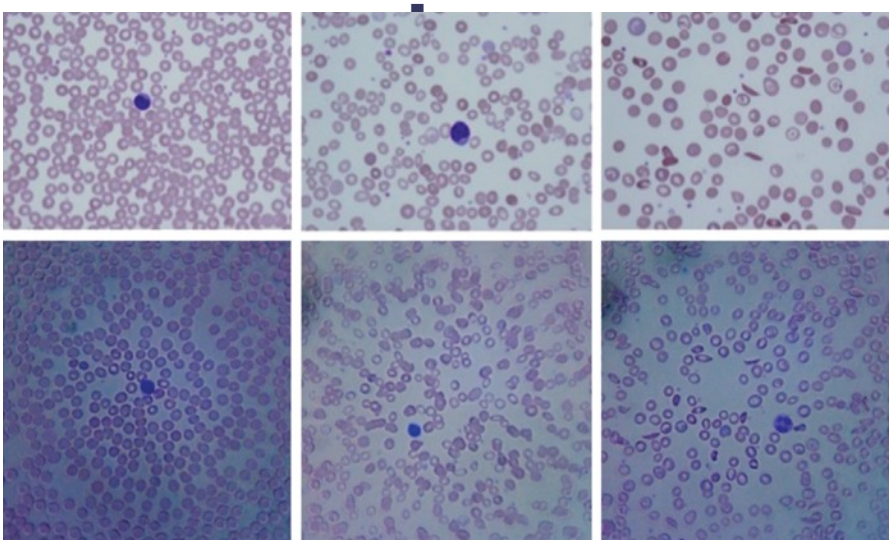


Figure 3: The top rows show images using a regular microscope, the bottom row use the cell phone camera. Left: red blood cells of healthy blood; Middle: anemia due to low blood cell count; Right: sickle cell anemia. The cell phone microscope allows for a diagnosis of these blood illnesses. Image: Zachary J. Smith et al.

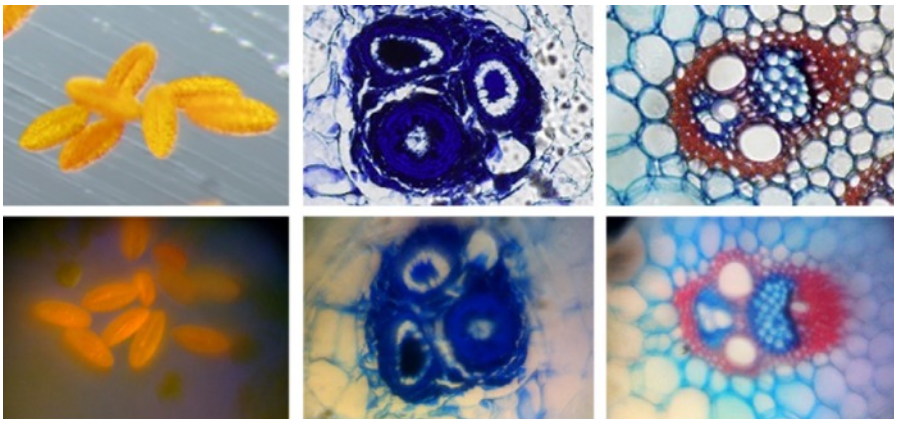


Figure 4: Comparison of various images using regular microscopy (top row) and the cell phone camera (bottom row). Image: Zachary J. Smith et al.

"iPad on Steroids": A new Gesture Controlled Microscope

Finnish researches developed a microscope with a truly different approach. The microscope can be controlled with hand and finger gestures on a large touch screen. The gestures allow for panning and zooming of digitized microscopic images.

A specimen slide is first scanned with a high resolution scanner. The images, which can have a size of up to 200GB, are stored on an image server and loaded as the user zooms and navigates. It is then possible to zoom into the digitized image from original size to 1000x magnification. Johan Lundin, one of the researchers, compares the new method with an iPad: "The giant size, minimum 46 inch screen looks somewhat like an iPad on steroids". The navigation of the specimen is indeed similar to Google Maps, which also permits for seamless zooming and navigation.

The multitouch microscope has promising applications in the area of education and for scientific meetings. Several people can stand around the touch screen and collaboratively view and discuss the specimen.

Reference: University of Helsinki (2011, March 24). Gesture-controlled microscope developed by Finnish researchers. ScienceDaily. Retrieved October 23, 2011, from
http://www.sciencedaily.com/releases/2011/03/110324103145.htm
http://youtu.be/ihaM3DvyUHE?hd=1

■

The Breaking Abbe's Law

In 1873 Ernst Abbe formulated a law, which limits the theoretically highest resolution of a microscope. Due to the physical nature of light, it is not possible to resolve structures that are closer together than appox. 200 nm. On the 7th of September 2001, Stefan Hell from the Max Planck Institute for Biophysical Chemistry received a prize for his research that allowed him to circumvented this law.

In STED microscopy (Stimulated Emission Depletion microscopy), a specimen is first stained using a fluorescent dye. In traditional confocal fluorescence microscopy, the specimen is scanned with a laser beam and the X/Y coordinates of the laser position are stored by a computer. All labeled structures that are within the laser beam's focus start to light up at the same time. If two structures are too close together, then the two emitted spots of light will overlap and it is not possible to see them as separate. Abbe's law does therefore apply.

Stefan Hell now managed to carefully control the time when the dye molecules emit their light. He used two laser beams to achieve this. The first laser, the excitation beam, was quickly followed by a second laser beam, the de-excitation or STED beam. The excitation beam caused the dye molecules to light up normally. The shape of the STED laser spot was quite different, however. It was ring-shaped with a dark spot in the center. Dye molecules that were illuminated by the bright ring started to quickly emit their light, while the dye which was hit by the dark center started to continue to shine slightly longer. By carefully tuning the energy of the STED beam, it is possible to change the size of the dark center. The smaller the size of the "hole" the higher the resolution.

STED microscopy allows to theoretically achieve an arbitrary resolution this way. STED microscopy resolves structures that that are one molecule in size. As of 2009, the maximum resolution achieved by this method was 5.8 nm.

Reference: Stafford, Ned. "The million dollar microscope." Chemistry World. Royal Society of Chemistry, n.d. Web. 22 Nov. 2011.
http://www.rsc.org/chemistryworld/Issues/2007/March/TheMillionDollarMicroscope.asp.

■

Figure 5: Comparison of confocal microscopy (left) with STED (center). The image on the right is a merged image of both techniques.

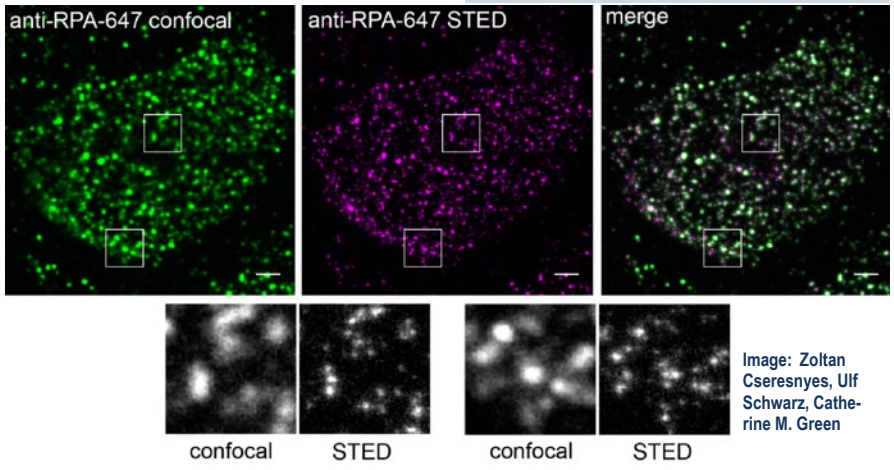


Image: Zoltan Cseresnyes, Ulf Schwarz, Catherine M. Green

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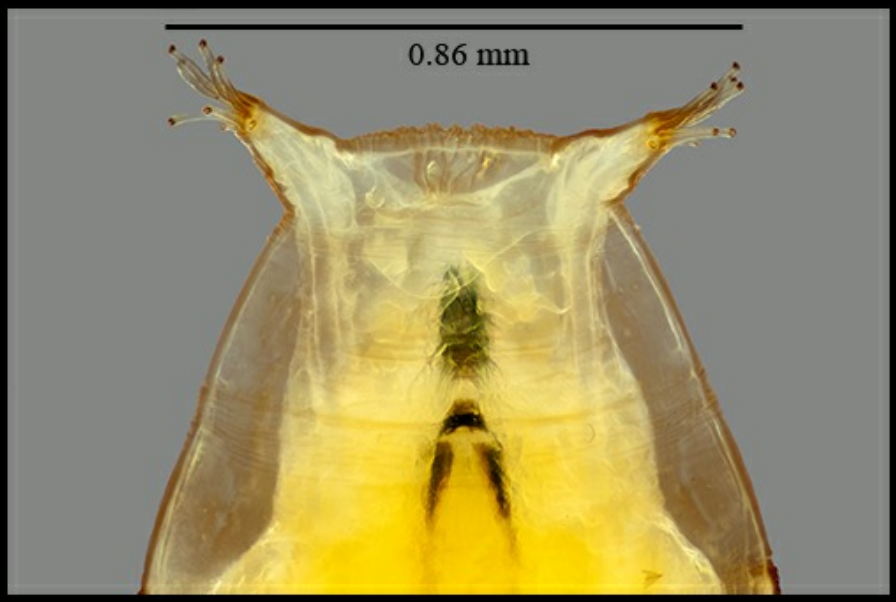
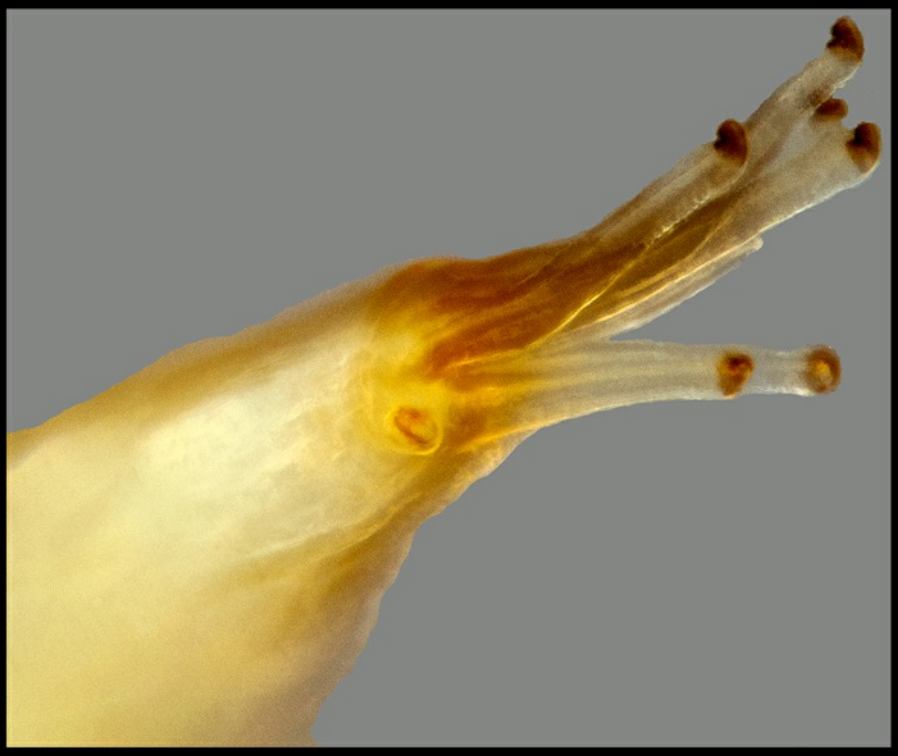


Anterior end of a young pupa of a Fruit Fly (*Drosophila*). You can still see the larval mouthparts. Specimen in glycerine on a slide. Nikon 20x ELWD objective on a bellows.

(Anthony Thomas)

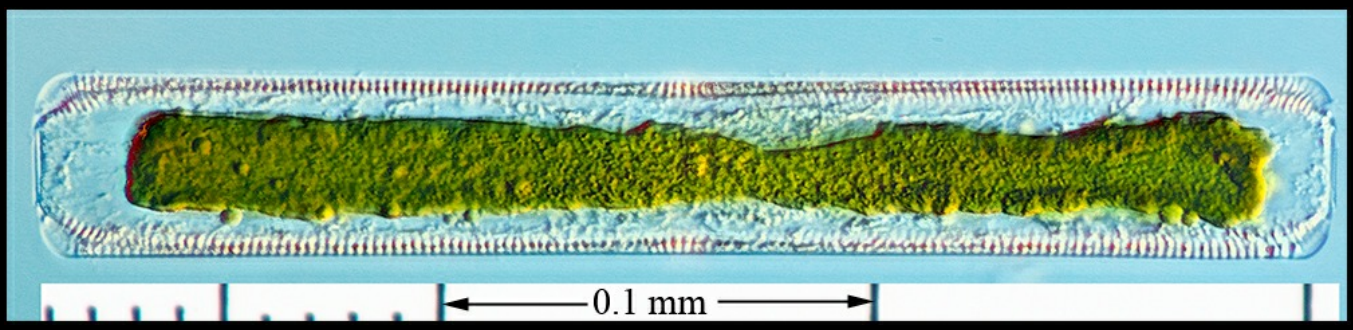


Close-up of the *Drosophila* breathing tubes. Nikon 40x LWD objective on a bellows.

(Anthony Thomas)

These are photosynthetic diatoms that are common in a local drainage ditch. Olympus BH2, 20x SPlan Apochromatic objective, 1.25x intermediate lens, 2.5x relay lens, DIC. Nikon D90 camera.

(Anthony Thomas)



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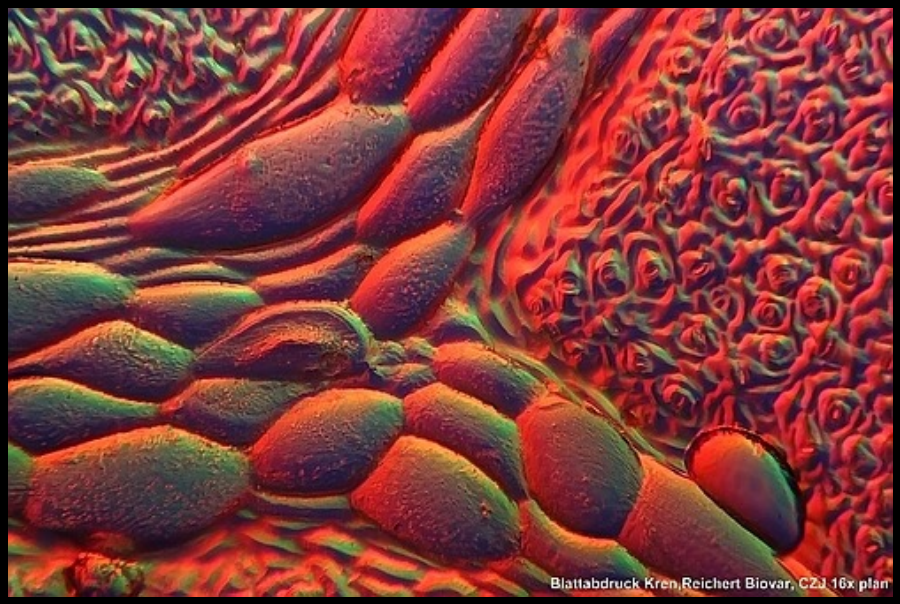
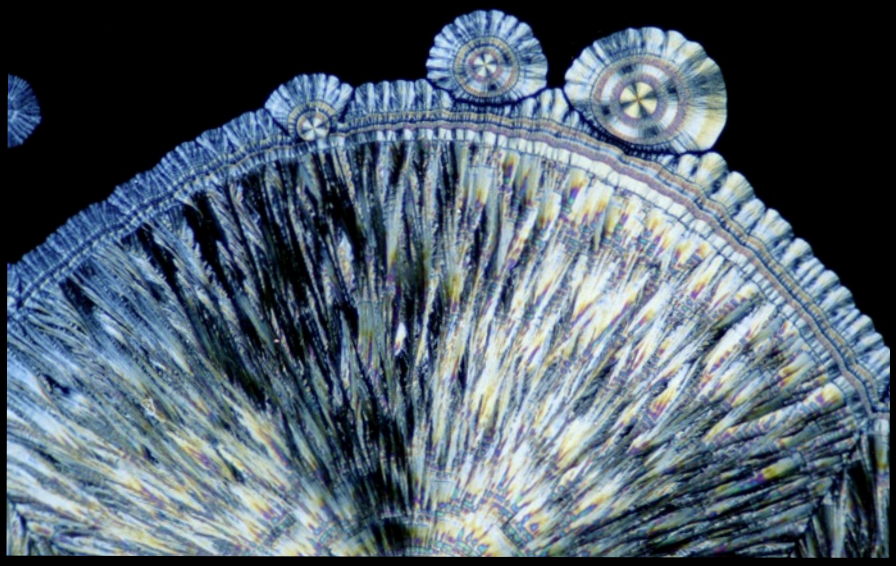
Anchoring roots of ivy, stack of 25 images made with Combine ZP.

(Manfred Rath)

A leaf cast of horseradish done by using clear nail varnish. It is a mix of Rheinberg- and oblique illumination.

(Manfred Rath)





A vitamin C crystal under polarized light. Vitamin C was dissolved in water and applied to the slide. Crystal growth starts when the Vitamin C has reached its saturation limit.

(Oliver Kim)

Field Notes on Science and Nature

Keep a scientific notebook which documents your observations! A book review.

Wayne Wilson

In a recent issue of the journal Nature, the book review section discussed a new book titled "Field Notes on Science and Nature", edited by Michael R. Canfield. Intrigued by the review of this book I purchased a copy, and upon receiving it sat down and read it through in two days. The organization of this book is unique and provides useful information, examples, and advice on keeping a scientific notebook. Each chapter in the book covers a different aspect of scientific note keeping and each chapter is written by a different scientist who provides photographs of actual pages from their notebooks. The actual notebook pages provided in this text are perhaps the most fascinating and interesting part of the book, but the comments of the scientists themselves are bursting with information of use to anyone who keeps a scientific notebook.

The foreword of the book is written by Edward O. Wilson, probably best known for his work with ants. A few samples of his notebook pages are provided as well, and it is a pleasure to see the simple yet clear descriptions he provides of his observations. Once past the foreword of the book, each chapter is written by a different scientist, each well-known within their own field, and all offering differing perspectives and opinions on what is good and bad about the notes they have kept. In one case a scientist goes back to notes he kept as a child and teenager in a small notebook. This gentleman also happened to be a runner, and in his earliest notebooks the backs of pages lists various competitions participated in, listing the times, distances, his placement, and other details about his running meets. On the fronts of the notebook pages he wrote about various observations he made (primarily) about birds and their nesting habits, as well as other natural history details. As he progresses in his professional career, we see his natural history notes improve and expand.

Other chapters cover specific note taking methods, such as "list keeping" and how it can be either of great value, or almost totally useless. Another chapter stresses the importance of including sketches, maps, and other drawings, as well as photographs. One of the few things almost all the contributors agree upon is the importance of sketching what you see. Many make a distinction between the quick field notes one takes while observing or collecting, and the more detailed notes that are written out in greater detail when back in camp (or lab) as soon as one returns. Much more detailed, such notes expand upon the field observations that were made. A few of the chapter names give you some insight into the areas covered in this book: chapter 1 "The Pleasure of Observing" by George B. Schaller, chapter 3 "One and a Half Cheers for List Keeping" by Kenn Kaufman, chapter 5 "Linking Researchers Across Generations" by Anna K. Behrensmeyer, chapter 6 "The Spoken and the Unspoken" by Karen Kramer, chapter 8 "Why Sketch?" by Jenny Keller, and chapter 12 "Why Keep a Field Notebook?" By Erick Greene.

One of my favorite lines in the book concerns a scientist who states that by looking in his journal notes from 50 years ago he is able to bring back that particular day with great clarity. I can think of no greater reason for keeping a detailed notebook. I strongly encourage you to consider reading this book. It will teach you to take better notes, remind you why it is so important to take notes, and provide notes from professional scientists, including Charles Darwin, that anyone involved with natural history would realize are well within their own abilities to write. We may not all have the insights of a Charles Darwin, but Darwin, without his notes would be unknown today.

Wayne Wilson, amateur microscopist , and professional pharmacist.
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Title:
Field Notes on Science & Nature

Author:
Michael R. Canfield (Editor), et al.

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Polarizing Microscope and Applications

What are the areas of applications of polarizing microscopes?

Monty Apollo

A polarizing microscope is a special kind of microscope that utilizes two polarizing lens to acquire certain optical data from the specimen. The polarizing microscope is used extensively in the field of optical mineralogy which supports such applications as geology, asbestos testing, and forensic science. Often those working in different fields will sometimes refer to the polarizing microscope by different names such as geology microscope, petrographic microscope, pol microscope, and PLM (polarized light microscope.)

The key difference between the polarizing microscope and a standard compound microscope is the addition of a fixed polarizer between the light source and the specimen and the addition of an adjustable polarizer between the objective and the eyepieces. The 2nd polarizer is called the "analyzer" and usually can insert in and out on a rotating piece in the neck of the microscope. Other common accessories include a rotating stage and insertable retardation plates made from gypsum or quartz.

With these additional elements, the this microscope can acquire optical data from a specimen that no other microscope can. The key optical information available includes refractive index, birefringence, sign of elongation, pleochroism, and angle of extinction, all of which provide clues to the crystallography of the material that is being investigated.

The first uses of these kinds of microscopes over one hundred years ago was the identification of minerals in geology. In addition, the most common form of lab analysis to test for asbestos is performed with a polarizing microscope. Because of the their ability to provide optical data, these kind of microscopes are very commonly used in forensic science where the identification of unknown materials is a routine part of the job. Some of the first evidence disputing the claimed age of the so-called "Shroud of Turin" was determined by analysis of pigments using a polarized light microscope.

The polarizing microscope is a very versatile and powerful instrument in the identification of materials. It is a key tool in several scientific fields, and can sometimes be the best option over more expensive technologies. For example, in routine asbestos analysis the polarizing microscope was determined to be more accurate and much more cost effective than the other high-tech options that were first investigated, such as x-ray diffraction and scanning electron microscopy. It is a powerful tool with many applications.

Nikon's MicroscopyU has quite a bit of information on how the polarizers and the science work to provide analytical information:
http://www.microscopyu.com/articles/polarized/polarizedintro.html

References:
To learn more about microscopes please review
http://www.where-to-buyazmicroscope.com

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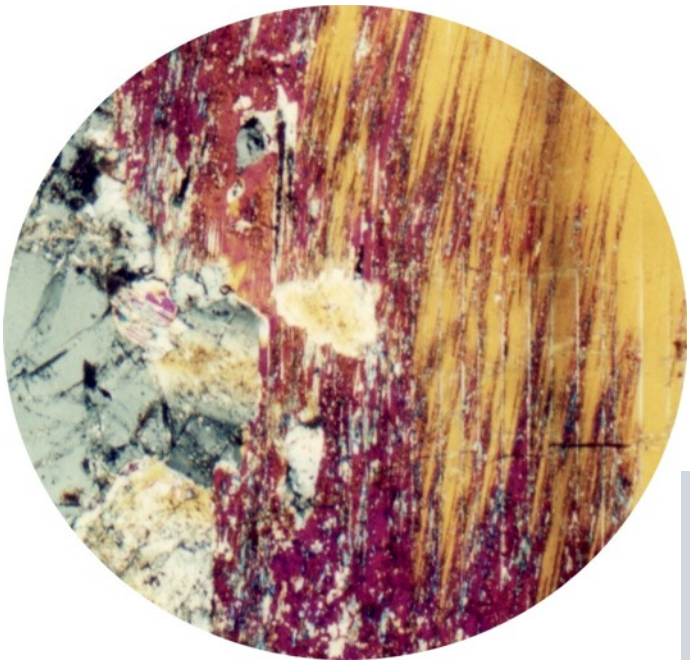


Figure 1: Polarizing image of a thin section of muscovite. Note the characteristic birefringence of muscovite.
Rock: Nepheline syenite gneiss
Location: Rio de Janeiro, Brazil
Image credit: Eurico Zimbres

A Wonderful Gift for Christmas?

How well do toy microscopes perform? Wilhelm Resch bought one to give it a test.

Wilhelm Resch

When I search eBay with the keyword "Microscope", amongst real microscopes, I see these cheap nice looking instruments, offered at a real low price (figure 1). Because I wanted to write this article, I bid 99 cents on one of these. I did win the microscope for 99 cents.

The catch was, it cost me almost $20.- to have it shipped. When I got it, I was not disappointed, it is a piece of junk. It is so hard to focus and hard on the eye, that giving this as a gift to some one as an introduction to this wonderful hobby should be a crime. I actually picked this one, because the ad said that it had top quality optics, powers claimed: 75X, 300X, 900X.

I could not get any image into focus at 900X. I did see something at 75X. I then took pictures of an easy object, a diatom with relatively large detail (figure 3). For illumination I used a fluorescent lamp with a ground glass in front of it. After much effort to get it in focus, figure 3 shows what I got at 75X (I worked hard on this).

I set up an other microscope, which I bought used on eBay for $20. Shipping was free. This microscope is heavy, sturdy and the field of view is much larger and very pleasing to to the eye. Figure 2 shows this microscope.

Figure 4 shows a picture taken with this microscope, using the same camera and the same diatom slide, 10X objective and 10X eyepiece. (I did not work hard on this one. I probably could have done better)

The difference in image quality is not even the biggest reason to stay away from this junk. Those cheap, plastic toys are so hard to use that the user will lose interest very soon and thus will miss out on what could have been a life long exciting hobby.

I would venture to say that almost any new microscope with standard size objectives would be a good gift, especially at the low powers, like 40X and 100X. Those are the powers I use 95% of the time.

To contact the author, send an email to: wresch@embarqmail.com. ■

Figure 1: Magnification powers claimed are 75X, 300X, 900X.

Quick Guide for buying Microscopes

1. Buy microscopes with standardized (ISO) optics.

2. Microscopes should be made of metal and should be heavy.

3. They should have both a coarse and a fine focus knob.

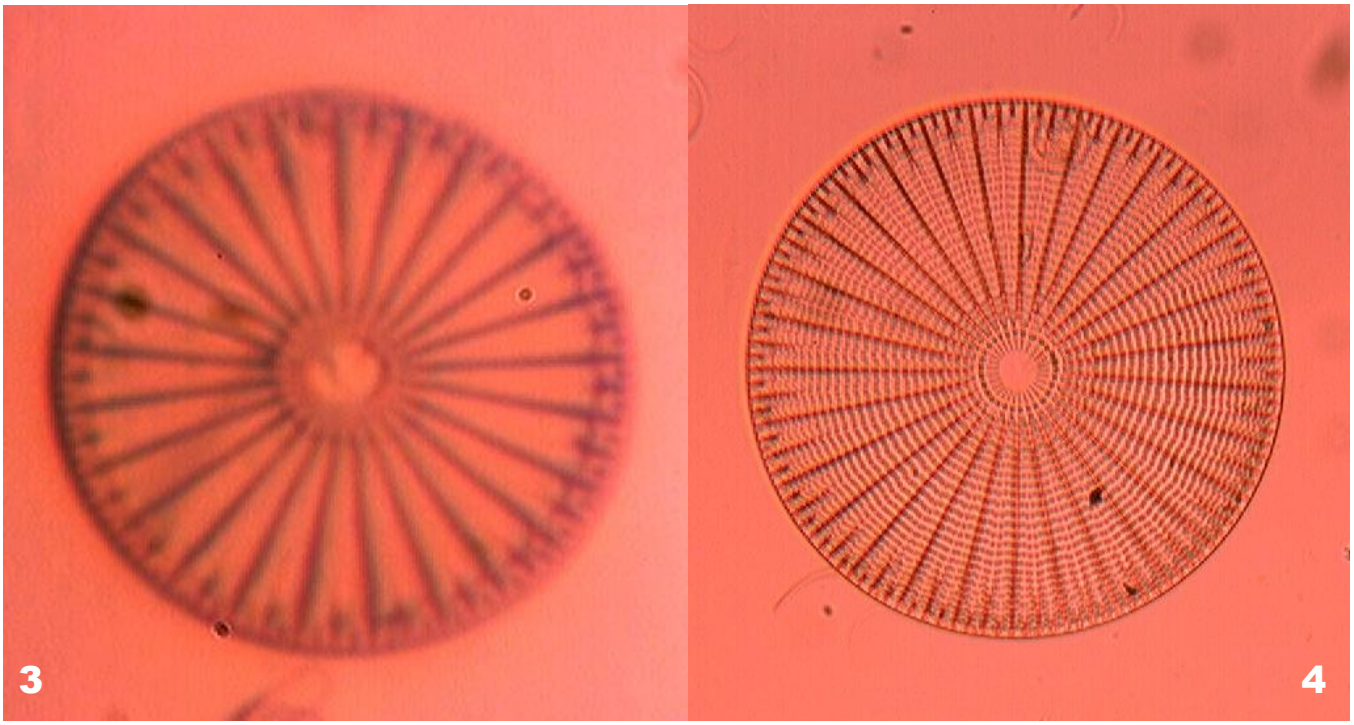
4. Do not overemphasize magnification, it is usually the least important aspect. Resolution is important. Without it, magnification is useless.

5. Good microscopes can not be bought in department stores. Talk to a dedicated microscope dealer.

6. Also consider buying stereo microscopes for young children. They are generally cheaper than compound microscopes and do not require specimen preparation. (ed.)

Figure 2: A $20 microscope with standard ISO objectives. This microscope cost as much as the microscope shown in figure 1. For the same price, the image quality is substantially better.

Figures 3 and 4 (bottom): The left image was taken with the microscope in figure 1, the right image was taken with the microscope in figure 2. The difference in resolution is significant.



Observing Yogurt Bacteria

Yogurt bacteria can be observed in a wet mount or after heat-fixing and staining them.

Oliver Kim

There are not many specimens that are suitable for observation using a 100x oil immersion objective. Both the depth of field and the field of view are low. This makes the observation of moving specimens especially difficult. Unless one wants of observe sub-cellular structures (such as chromosomes), the 100x oil immersion objective, be it bright-fiel or phase contrast, does not have a particularly wide range of applications. Even the observation of permanent slides is not easily possible. They become contaminated with the immersion oil and must be carefully cleaned after every usage. Otherwise dust may start to collect on the oily surface. Immersion oil on the paper label does also not look very nice.

The 100x oil immersion objective is, however, very suitable for observing bacteria. Unstained bacteria are best observed using phase contrast. This technique will make the transparent bacterial cells appear dark on a bright background. Many amateur microscopists and schools use bright-field optics, which are substantially cheaper. It is still possible to observe live bacteria by closing the condenser aperture diaphragm. Diffraction patterns around the individual cells start to appear, making the cells easier to observe. Stained cells can also be seen with an open diaphragm.

At the same time I also would like to call to your attention that bacteria are probably not the most exciting specimens to observe. The cells will appear uniform under the microscope and there are no sub-cellular details visible. After all, there are no membrane-bound cell organelles in bacteria and they completely lack visible cell internal structures.

A warning

Do not spoil food (or other substances) to grow your own bacteria. Do not even make a hay infusion unless you really know what you are doing. Enriching and concentrating unknown bacteria can be a real health issue. Rather I recommend you to use bacteria found in yogurt for your observations.

A Remark

At this time want to mention that the ink that I had available was not very successful in staining the bacteria. I therefore recommend that you give a different substance a try (such as Methylene Blue or Safranin).

Overview of the method

The process of preparing bacteria for microscopic observation can be divided into the following four stages:
Making a bacterial suspension: At this step the bacteria are brought into a suitable concentration.
Heat-fixing the cells: This process immobilizes the cells and glues them to the glass slide.

Figure 1: Working like a true "amateur". Yoghurt sample (top), suspension of yoghurt bacteria in water (left) and a regular plate for drying the samples.

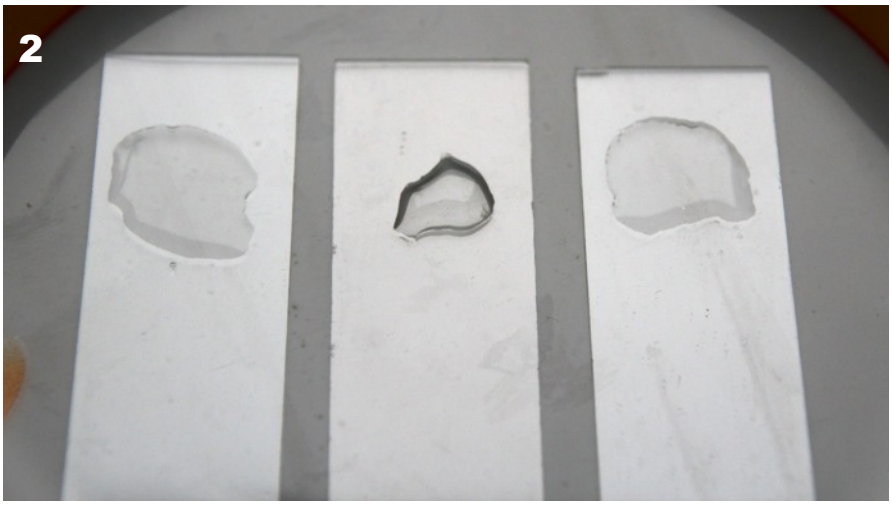
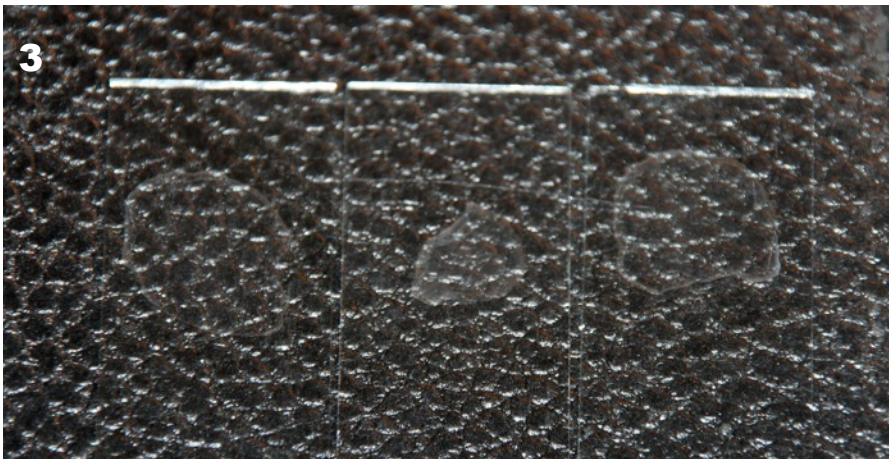
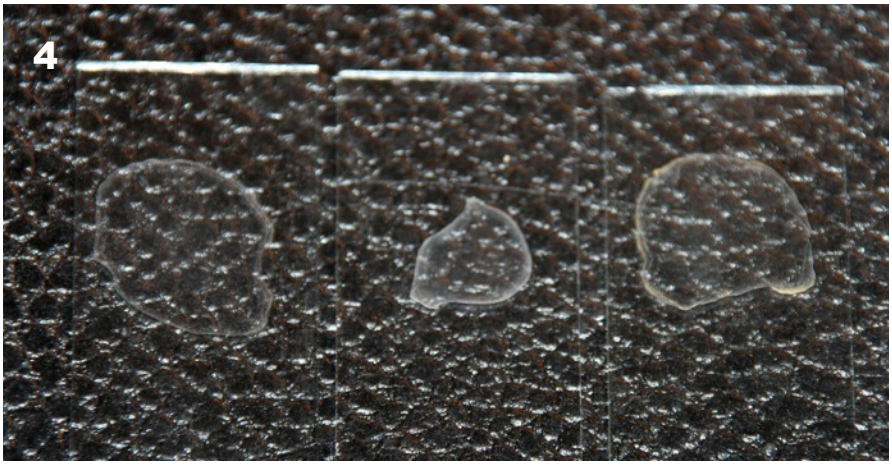


Figure 2: The left and the right sample flow over a larger area. This is the preferred situation. The slide in the center had an oily surface and the drop may contract during the drying process. This increases the concentration of the bacteria and may result in clumping of the cells. The individual cells therefore become more difficult to discern.





Staining: The chemical stain enter the cells and react with different parts of the cell.
Observation: Observation occurs either at 400x or at 1000x using an oil immersion and no cover glass.
I will address each one of these stages in the following parts .

Making a bacterial suspension

The bacteria are harvested (take a small knife tip of yogurt) and are suspended in a small amount of water or in 0.9% NaCl solution (0.9 g NaCl in 100ml of water). Using NaCl solution prevents the cells from osmotically bursting, but water will also work in many cases. If the bacterial suspension is too concentrated, then many of the cells will overlap on the slide and it will be difficult to see the individual cells. The suspension should be only slightly turbid. It is probably best to experiment with the concentration, as much will depend on the bacterial concentration of the original sample. Ideally the bacterial suspension should spread over a larger surface on the slide. If you agitate the suspension to vigorously, then you may break apart the chains of Staphylococci, which allow for an easy identification. If you want to make a wet mount, then it is also possible to directly suspend a small amount of yogurt on a drop of water right on the slide.

Fixing the bacteria

Fixing kills the cells and sticks them to the glass slide so that they can not be washed off during the staining process. Fixed bacteria are also easier observe, because they do not float in and out of the focus. Heat fixing is like frying an egg in a pan without oil. The proteins start to denature and the cells stick to the glass slide much like an egg sticking to the pan.

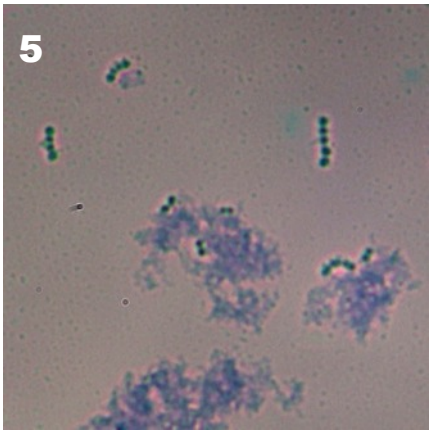
We have already applied a drop of the bacterial suspension to the slide. Now we need to allow the slide to air-dry completely without heating the slide. If you heat the slide then the steam pressure in the cells may cause them to burst. Slight warming is OK, this may indeed speed up the drying process. Be careful because even the

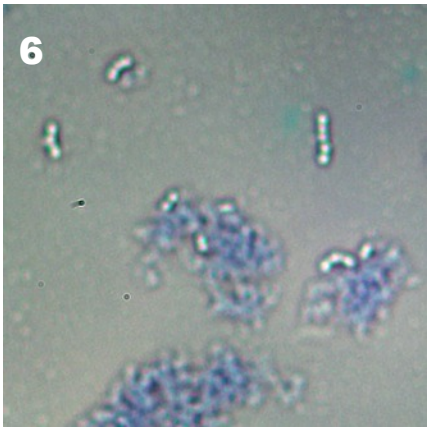
lowest setting of a hot plate is often too hot and the temperature is often difficult to control. Do not put the suspension in the center of the slide, place the drop further towards one end. This way you still have enough space left to hold the slide without burning yourself when doing the heat-fixing procedure.

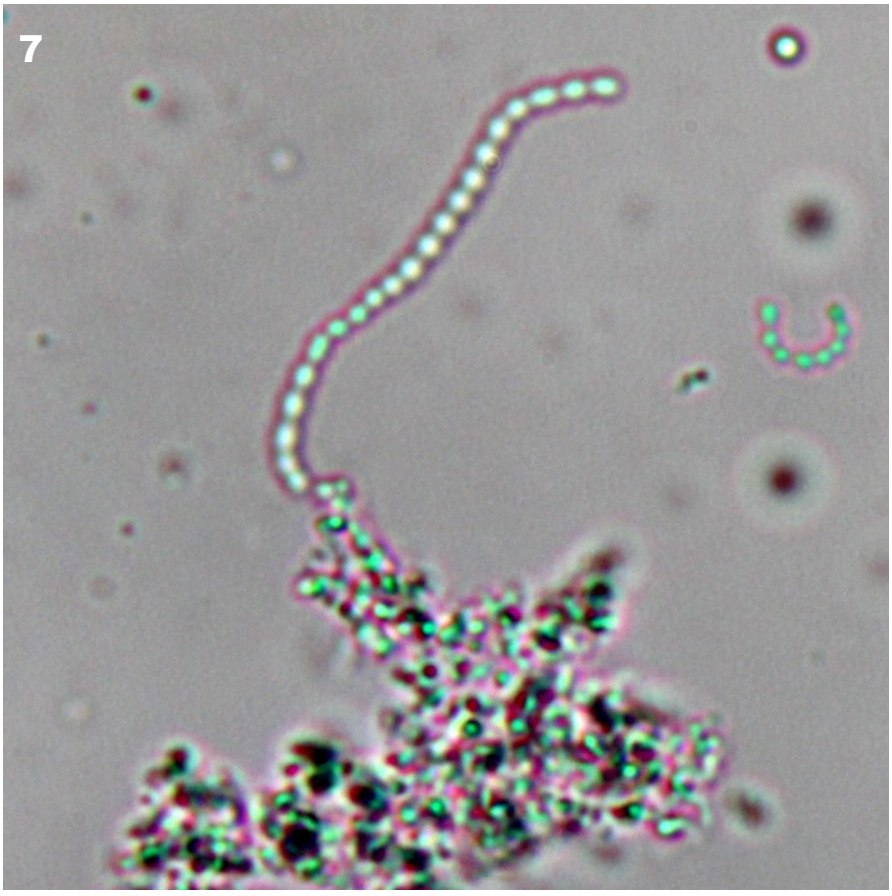
The original heat-fixing process requires the use of a gas Bunsen burner. Pull the dry slide, specimen on top, two times rapidly through the flame of the Bunsen burner. The slide should become very hot, but you should still be able to just hold it in the open palm of your hand without burning yourself. If the temperature is too low (due to a short heating time), then the bacteria will not stick to the glass slide and will be washed off during the staining procedure. Under no circumstances should the bacterial sample start to smoke or turn brown. This indicates that there is oxidation taking place and that the specimen has been destroyed. As a matter of fact, it can be commonly observed that a properly heat fixed bacterial sample is actually slightly brighter, than a specimen slide which has only been dried in the air. The place where the bacterial suspension has been applied becomes slightly more white-ish. While I do not know the actual causes for this change in color, I do assume that this has to do something with protein denaturation, similar to the color change of a frying egg.

I always pull the slide top-down through the flame, holding the slide at the edges with two fingers. I always test the temperature of the slide by placing it on my open palm (please don't blame me if you burn yourself). Here it pays off to have the sample at the far end of the slide as you may otherwise also burn these fingers. Pulling the slide sideways through the flame may increase the temperature too rapidly , possibly cracking the slide. On the other hand, I guess it should not really matter much, because the temperature should not become too high anyway. The total heating duration is about 1-2 seconds.

Many microscopy enthusiasts will not have a Bunsen burner available. In this case it is possible to briefly place the slide on a hot plate (I tried this, it works) or to use a gas-operated lighter (I have never tried this). The small diameter of the lighter flame may cause an irregular heating of the slide and may induce cracking. Be careful. Candles are probably not as suitable, as they will result in soot deposits on the slide.

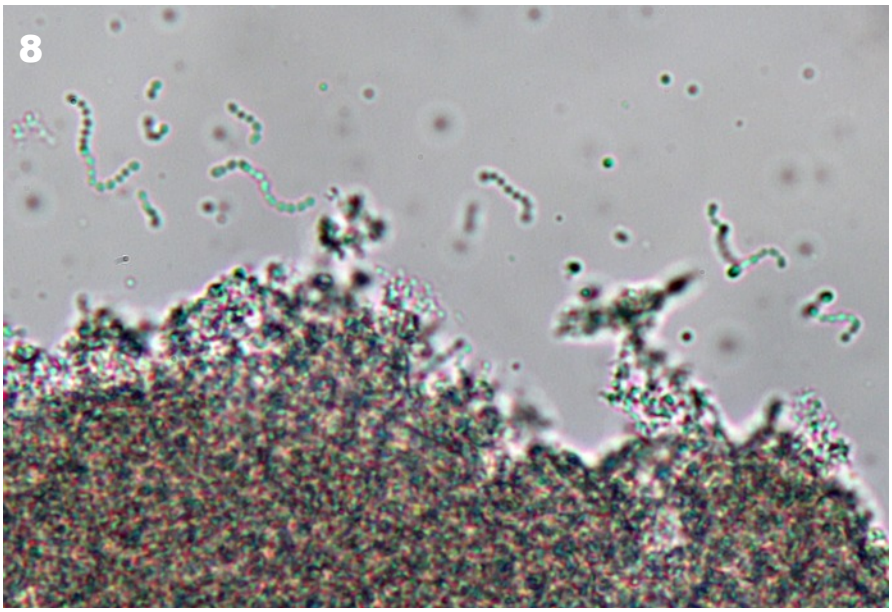


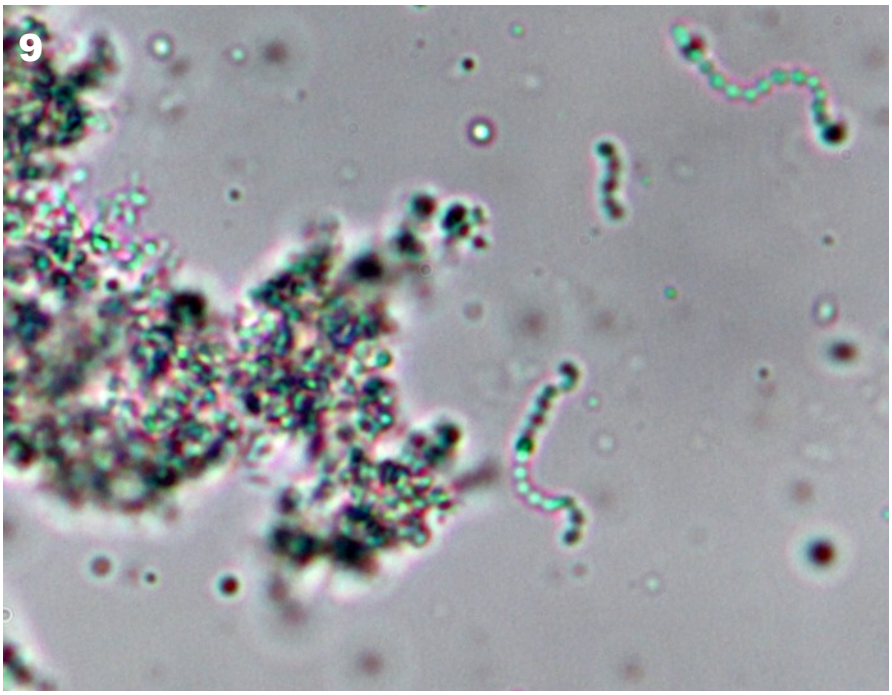




Figures 5 and 6: The images show heat-fixed and stained samples at two different focus levels. The large blue cloud-like structures appears to be stained curdled milk. The round objects in short chains are yogurt bacteria.

Figure 7: A long chain of yogurt bacteria attached to what appears to be curdled milk (bottom). Wet mount, not stained.





Figures 8 and 9: *Streptococci* in a yogurt sample. Curdled milk seems to be the larger flakes.

Staining the bacteria

The staining process is relatively simple, unless one uses differential staining (in which more than one stain is used). First, allow the slide to cool down to room temperature. Several drops of the stain are then applied to the fixed bacteria and given time to do its work. Depending on the affinity of the stain to the cells, this can last several minutes. It is possible to make a time-series in 5 minute intervals to find the optimum staining duration. Alternatively it is possible to submerge the whole slide into a slide-holder filled with the stain, but this may contaminate the stain over time.

The stain is carefully removed by rinsing the slide in running water. Hold the slide at a steep angle and allow a thin water stream to run over the slide. The water should not be applied directly to the fixed bacteria, but above it. You can easily see that the stain which did not come in contact with the bacteria is washed away quickly, while the bacteria themselves start to retain more of the stain. The washing process can be stopped after there is no more discoloration of the washing water. The individual cells should still have retained enough stain for observation (unless you over-wash and completely de-stain the cells).

The result

To get right to the point: the ink was not able to stain the bacteria well. This may be due to the small size of the cells, which are not able to retain much ink. The ink did stain the curdled milk, though (figures 5 and 6)! Even under bright field conditions, the Streptococci could easily be seen as short chains. They had a strong tendency to break the light and appeared either as dark or very bright spots, depending on the focus. The staining effect of the ink was therefore not very visible. The curdled milk flakes did not show this tendency and remained uniformly blue. In that sense the staining process did help to identify the bacterial cells!

The bacteria in wet samples float around and can be seen as individual cells. During the drying process, many cells may start to aggregate and clump together. This effect is particularly pronounced when the cell density is too high. In this case, it becomes very difficult to see individual cells.
There is also the possibility that the long chains of bacteria can be broken apart by the preparation process, making them more difficult to identify.

Final thoughts

Heat fixing the bacteria prevents them from floating around and makes it easier to find a focus. I found out that the ink that I used did not dye the cells. It can also be that the staining was so weak that I was not able to observe it. In any case, the blue ink seemed to have been able to stain the curdled milk, which most certainly also contained bacteria. ■

Large phototrophic purple sulfur bacteria on a leaf

A magenta-colored bloom can be an indication for the presence of purple phototrophic bacteria. Aggregates of these bacteria were found on a decaying leaf.

Charles Guevara

Figure 1: Bloom of purple sulfur bacteria. There are indications of bloom formation on the sediments.

Purple-sulfur phototrophic large bacteria stand out clearly to the unaided eyes, when one encounters their brightly pink-magenta colored 'blooms' at the bottom of a stream. These are wonderful stream organisms to enjoy with the 'naked eye' on relaxing stream hikes. Find a stream shore section with dark black sulfide sediments. You should be able to smell the sulfur when you probe into the sediments. You then can reasonably expect to find the bright pink-magenta 'purple-sulfur bacterial blooms' during some season of the streams yearly cycle. The location of my freshwater inland stream is at a northern temperate latitude. Each individual bacterium has a rapid motility. They respond and orient to light levels, sulphide levels, and indirectly to the oxygen levels of the waters they inhabit. These bacteria either rapidly retreat into the stream floor sediments or form brilliant sediment surface films. These microbe-biofilms are dominated by this single species! The bacterial blooms show brilliant pink-magenta sediment surface biofilms.

These are very reactive communities. They possibly respond to diurnal fluctuations and to heavily clouded skies overhead. These bacteria pose all sorts of questions to a microscopist on an outdoor hike and all sorts of observation possibilities - together with a loyal

Figures 2 and 3: The water temperature was measured to be 43°F (6°C). Three gauges agree.

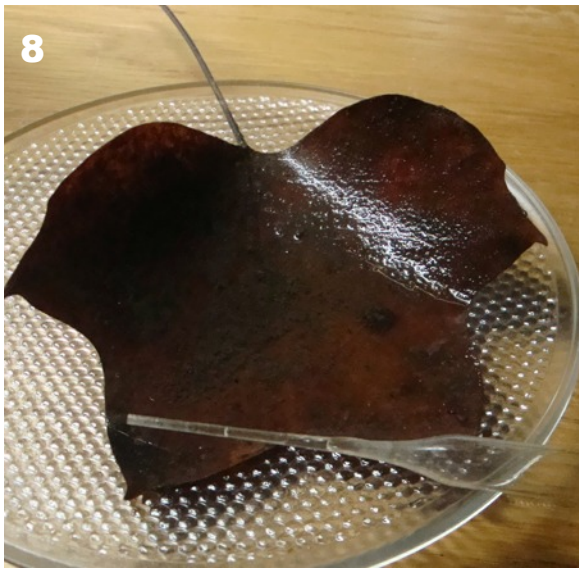
Figure 4: A repeated 'bloom site'. This is a niche-habitat on the stream near the bank. This is the location (niche-habitat) for two year's of purple-sulfur bacterial blooms.

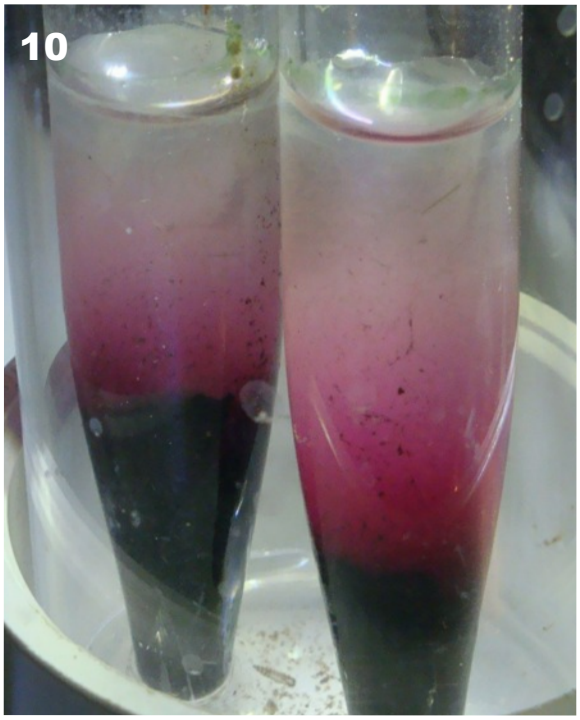
Figures 5-6: The site of bacterial blooms observed over two years of this season.

Figure 7: My puppy at a favorite site!

puppy always "in the thick" of me being bent scrutinizing the bloom sites.

Very recently I encountered a fallen tree leaf, soaked and resting in one of the few niche-habitats of purple-sulfur phototrophic bacteria. Over the course of two years these manifested brilliant pink-magenta blooms. This bottom resting tree-leaf offered a hint of this deli-





Figures 8 and 9: The sample tree leaf at my microscopy bench.

Figure 10: bacteria swarming up into the water colum from the bottom sediment.

Figure 11: : this leaf kept in it's collection jar for days

cate pink-magenta bloom. I transported it home to my microscopy bench.

I have pleasantly observed, for the first time, aggregations of large purple-sulfur bacteria, aggregations of these wonderfully reactive large bacteria on a decomposing leaf. These I collected from the stream niche-habitat where 'blooms' have repeatedly manifested for two years.

I still have several simple goals.

1) Right at stream site, I want to carefully lift a delicate 'bloom sample' to a wet-mount slide. I want to image the samples with a field microscope.

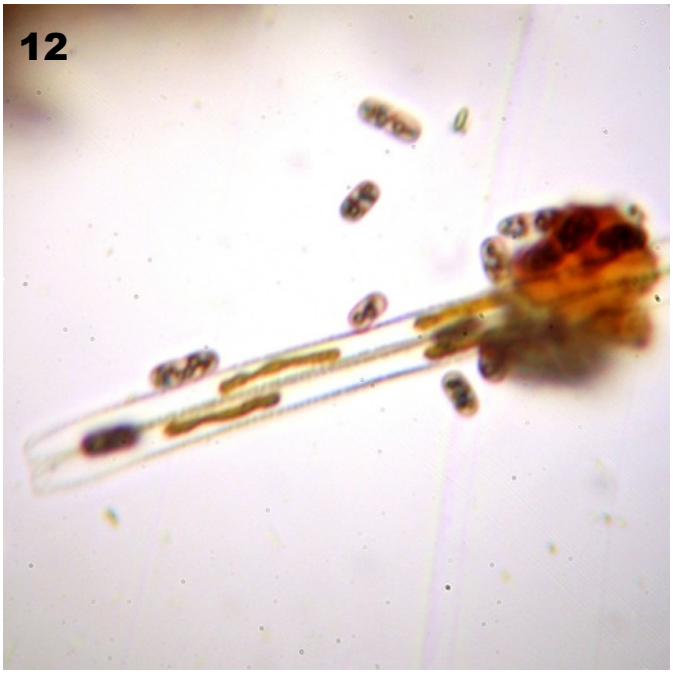
2) I want to observe the protists and the meiofauna which occurs with this unique 'single species dominated biofilm'.

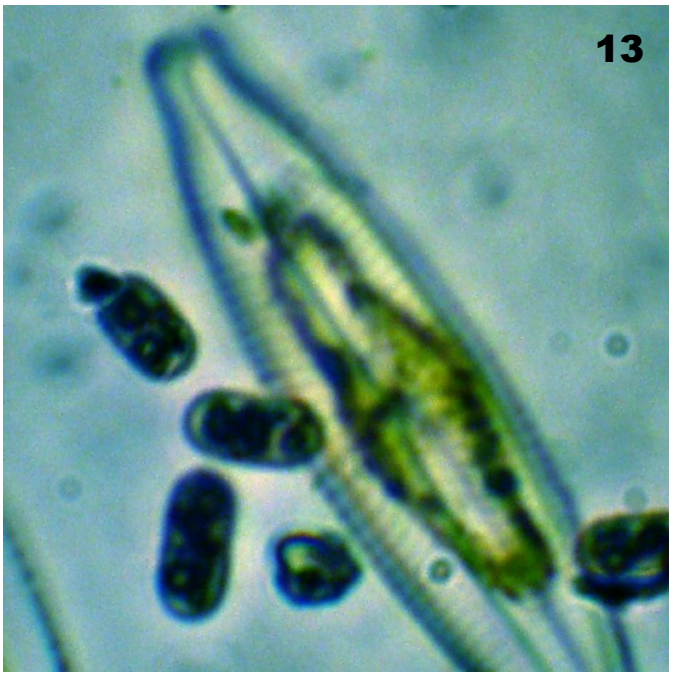
3) I want to observe the morphology of the huge bacteria within the biofilm. I want to see if patterns of these bacteria are evident, such as orientations, revolving cells, morphotype differences, etc.

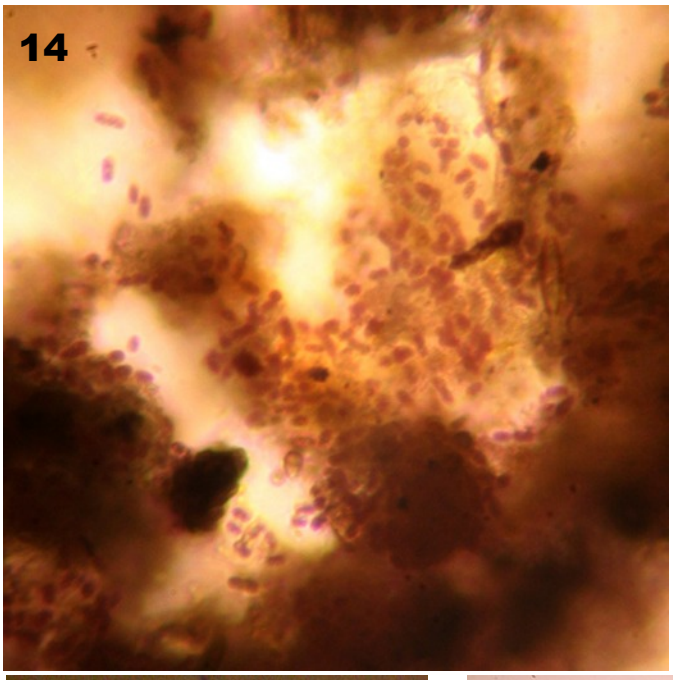
Delightfully, eukaryotic protists occurred within the sample of purple-sulfur bacteria. This niche habitat is a complexity of organisms offering many wonderful microscopy opportunities!

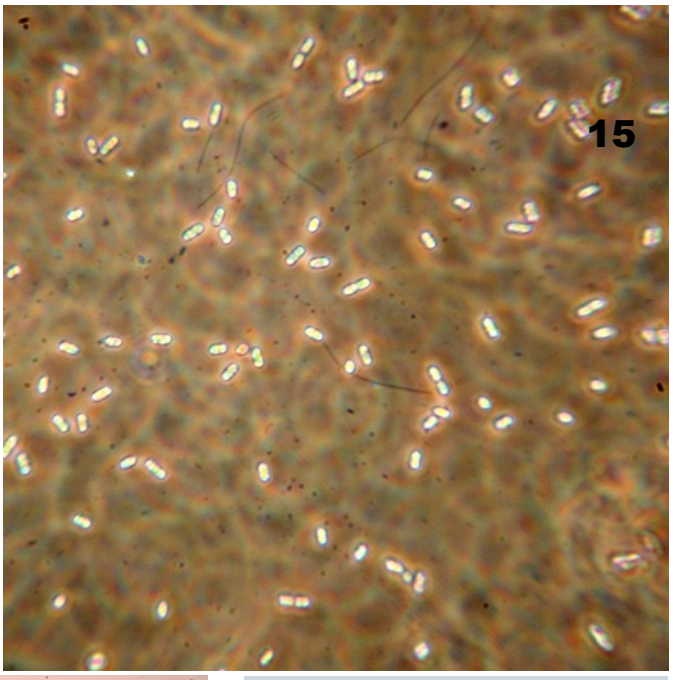
Source materials

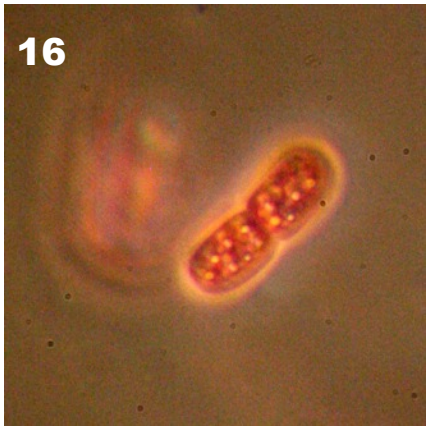
"The Phototrophic Bacteria, anerobic life in the light", John G. Ormerod, 1983. ■

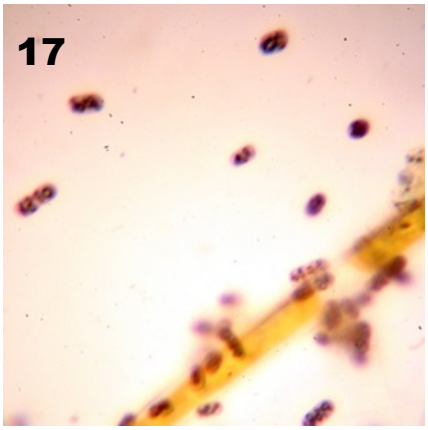












Figures 12, 13, 17: Bacteria swarm out of the leaf structure, some encounter a diatom.

14: Bacteria gathered in cluster within a decaying leaf

15: DL phase view of swarming purple-sulfur phototrophic bacteria.

16: Dividing bacterium

Staining Epithelial Cells with Ink

Regular fountain-pen will stain epithelial cells. The ink also makes intracellular structures, such as the cell's nucleus, visible.

Oliver Kim

Observing and staining epithelial cells from the mouth is one of the easiest methods to directly observe human cells. The cells can be stained with water-based ink and structures inside the cell, such as the nucleus, can be made visible this way. The simplicity and quickness of the procedure also makes it a suitable project for introductory microscopy courses in schools.

Epithelial cells from the mouth a suitable for several reasons. First, they can be obtained easily by scratching the inside surface of the cheek with a cotton swab (or your fingernails). The cells will come off easily. Second, the cells are relatively flat and can therefore be observed without much preparation.

Stains used

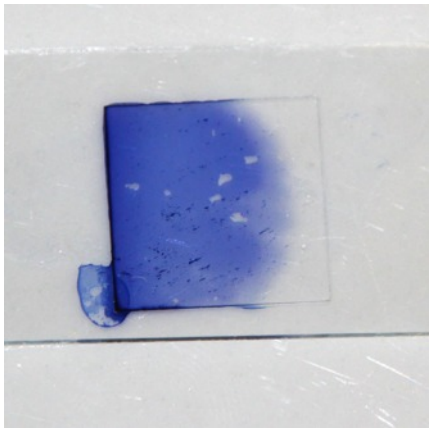
For the staining of the epithelial cells, I experimented with two different kinds of ink: the Royal Blue 4001® ink from the company Pelikan and the black Quink® ink from the company Parker. I simply happened to have these around in my household and decided to give them a try. These inks are used for fountain pens and are water based. I considered them reasonably safe also for classroom usage and therefore gave them preference over other (more commonly used) stains like Methylene Blue. The fountain pen inks can also be easily and cheaply obtained from paper supplies companies. A quick research revealed that the blue ink belongs to the triarylmethane dyes.

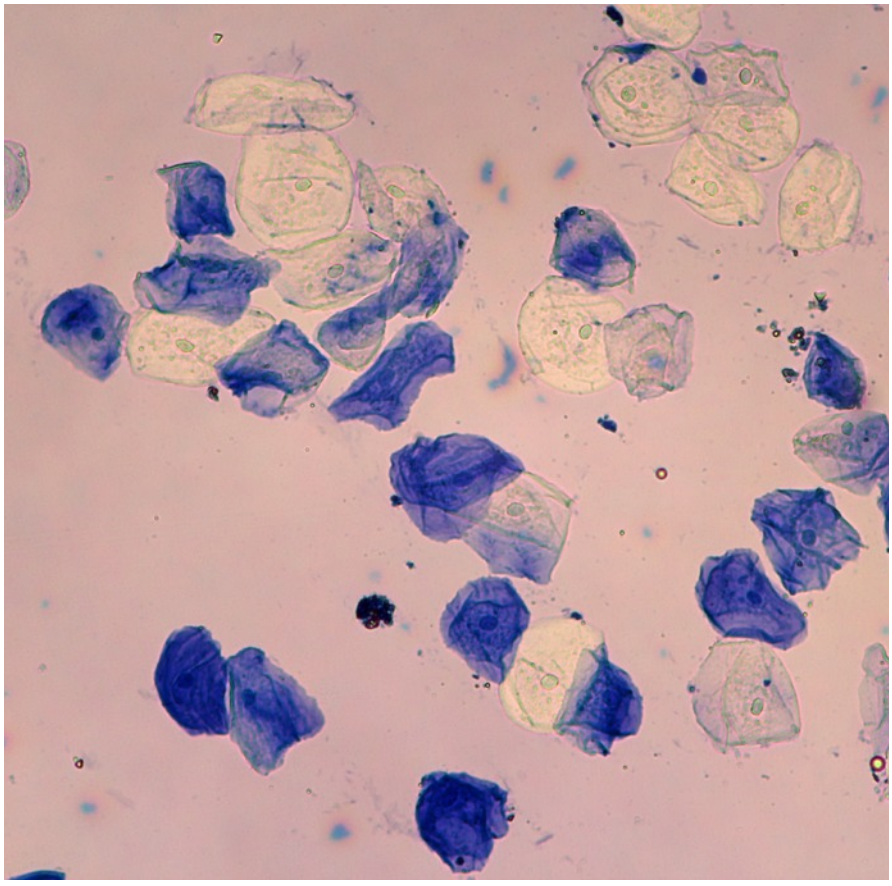
Collecting and preparing the cells

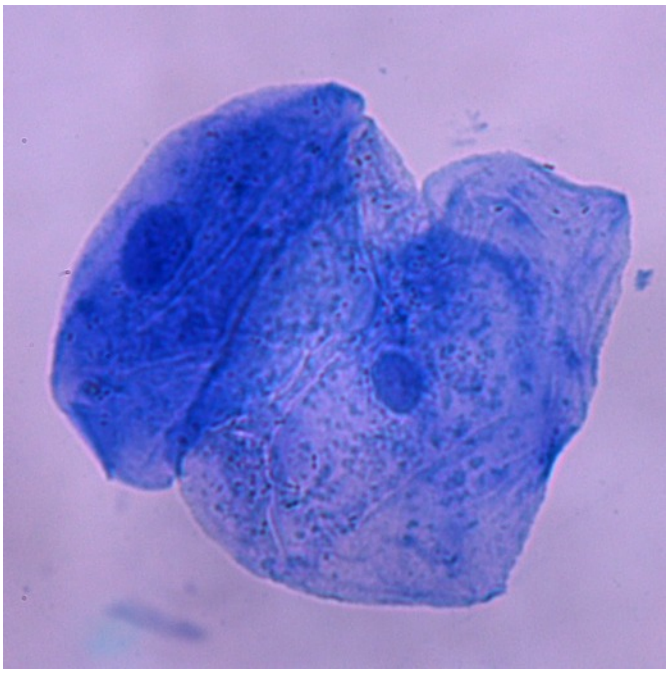
I used a cotton swab (those that are used for cleaning one's ears) to collect the epithelial cells. In order to obtain a sufficiently large number of cells, it is necessary to vigorously scratch the inside of one's cheeks with the cotton

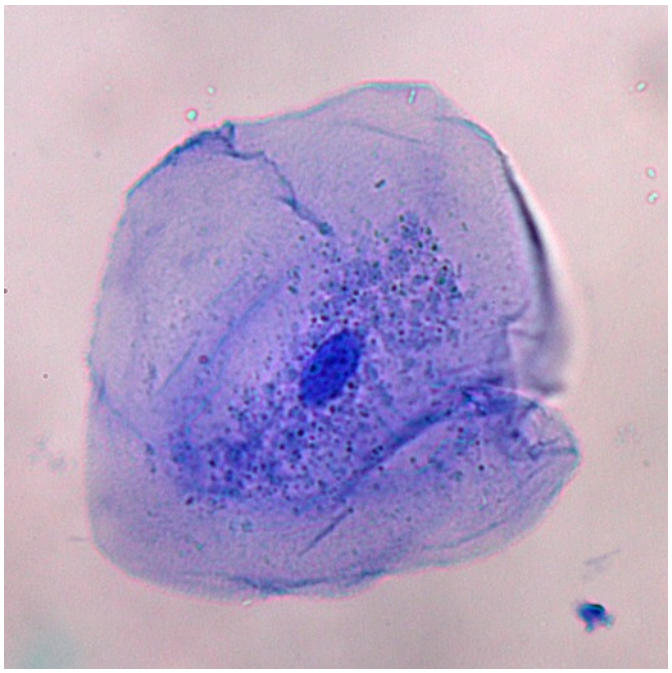
Figure 1 (bottom): A regular wet mount is prepared with only little water. A small drop of ink is then applied to the edge of the cover glass. The ink is pulled in and stains the cells.

Figure 2 (right): Some of the epithelial cells already absorbed much of the ink, turning intensely blue. Notice that the background starts to turn brighter as more and more cells start to absorb the ink.

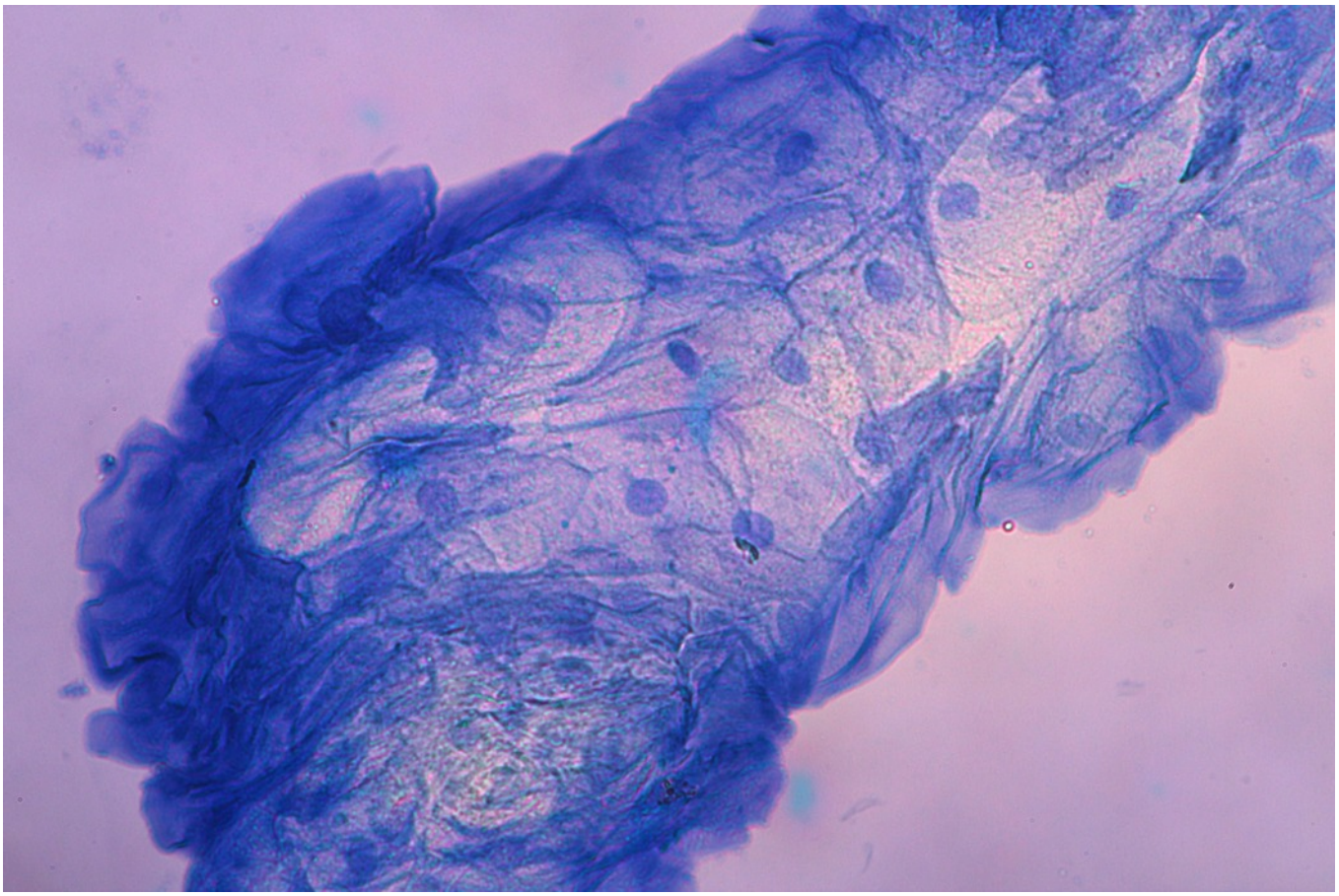








Figures 3 and 4 (top): The nucleus of each cell is visible as a dark blue structure inside each cell.



Figures 5 (top): Large aggregates of epithelial cells start to stain from the outside inwards. Notice that the nuclei start to stain first, they seem to have an extremely high affinity for the ink.

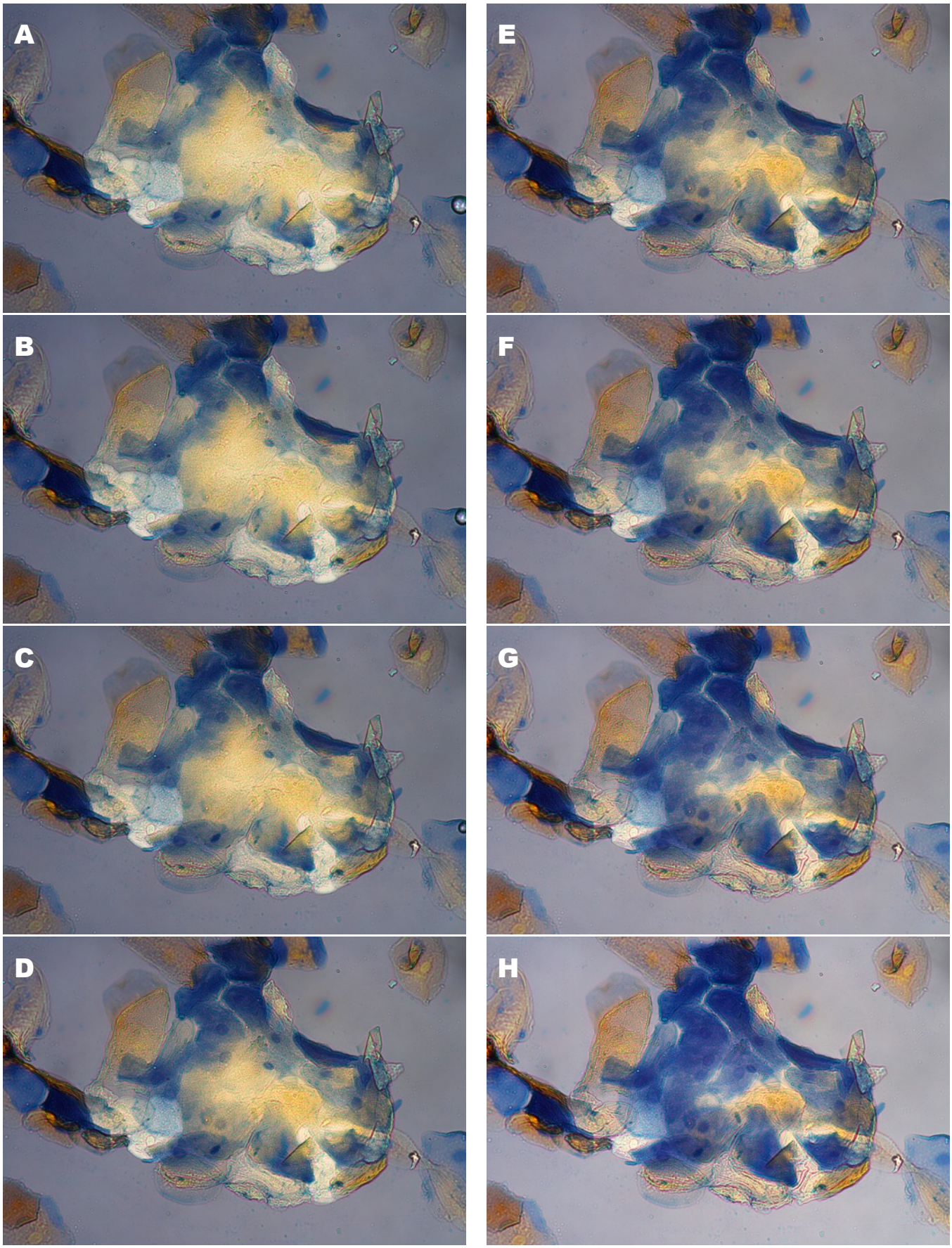

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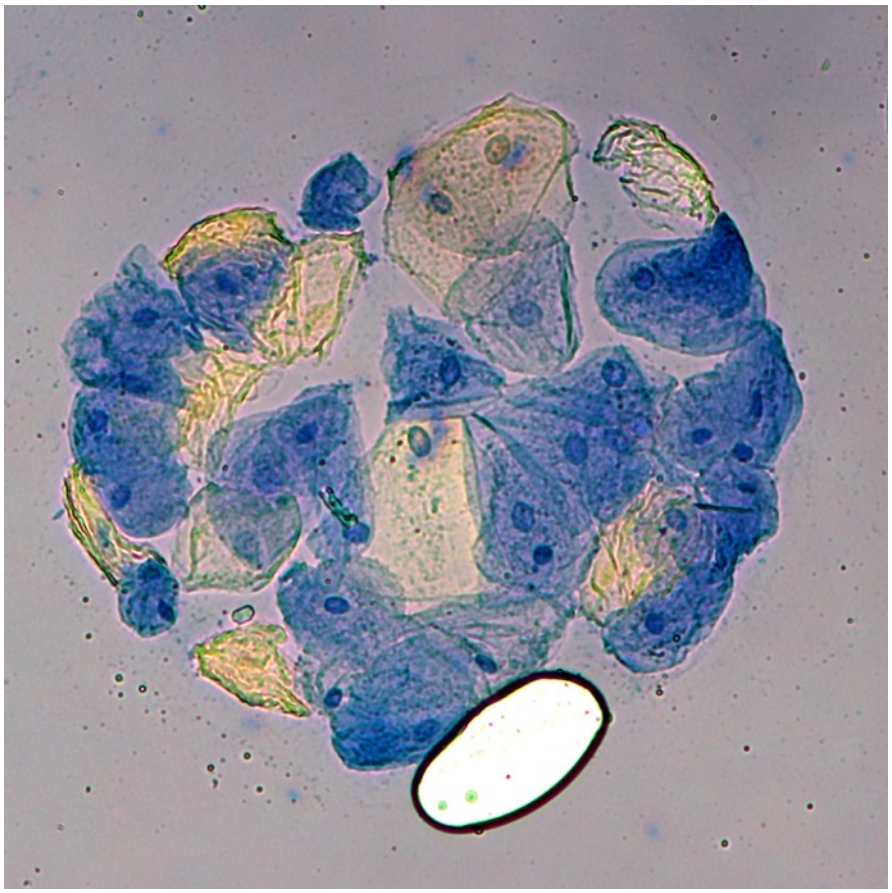
Figure 6 A-H (opposite page): These epithelial cells were stained with black ink. The images show a time-series over several minutes. The black ink is evidently composed of blue and yellow components. These colors seem to separate as they enter the cell. First the yellow component of the ink enters the cell, followed by blue. Over time the background starts to brighten up. This indicates that more and more ink is absorbed into the cells.

Figure 7 (right): The image shows another aggregate of cells that were stained with black ink. The bright area towards the bottom of the image is an air bubble.



swabs. I then streaked the cells on a slide and added a small drop of water to make a temporary mount. It is also possible to use a small spoon to scratch off some of the cells (I tried it and it works), but this is less comfortable.

Staining the cells

The slide is placed on the stage and the cells are then focused. A small (!) drop of ink is then placed at the corner of the cover glass. Too much ink will stain the background too dark and it will become difficult to see the individual cells. The ink is then pulled into the sample, beneath the cover glass, provided that not too much water was used for mounting the cells. The ink will gradually spread over the whole specimen. I tried this process with both the blue and the black ink, and with a surprising result.

The result

The epithelial cells showed a strong affinity to both the blue and the black ink. As a matter of fact, the cells enriched the dye to the extent that they became significantly darker than the surrounding medium. The pictures on the previous pages and on this page show that the background of the cells is significantly brighter than the cells themselves. Also the nuclei are clearly visible.

The black ink demonstrated a particularly interesting behavior (figures 6 and 7). Black ink seems to be a mixture of blue and yellow ink. First the yellow component of the ink entered the epithelial cells. The blue color was absormed much slower. Figure 6 shows a time series of a collection of cells, which first turn yellow and then blue.

Osmosis test

I could not restrain myself and wanted to test the response of the cells to osmotic stress. I applied a decent drop of saturated salt water to the corner of the cover glass and observed how the salt water was drawn in. The new fluid quickly washed remaining ink from the medium away. The cells themselves retained the color. I hoped to be able to observe plasmolysis of the cells. I expected the cells to lose much water and consequently to shrink. I could not observe this, however. Likewise, I applied distilled water to a different sample of epithelial cells and hoped to see them swell and burst. This would have made an interesting experiment for schools. Unfortunately I could not observe either response. I suppose that the epithelial cells are generally more resistant to osmotic extremes. After all, they have to withstand the wide variety of different food sources (and liquids) which we consume on a daily basis.

Conclusion

One of the most surprising results was that the cells stained darker than the surrounding medium. Motivated by the result I will now attempt to use other water-based stains, including food coloring. Anyone care to try out (and report back) on the efficiency of these stains? ■

What's this? Answer on page 3.