Microbe Hunter

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The Magazine for the Enthusiast Microscopist

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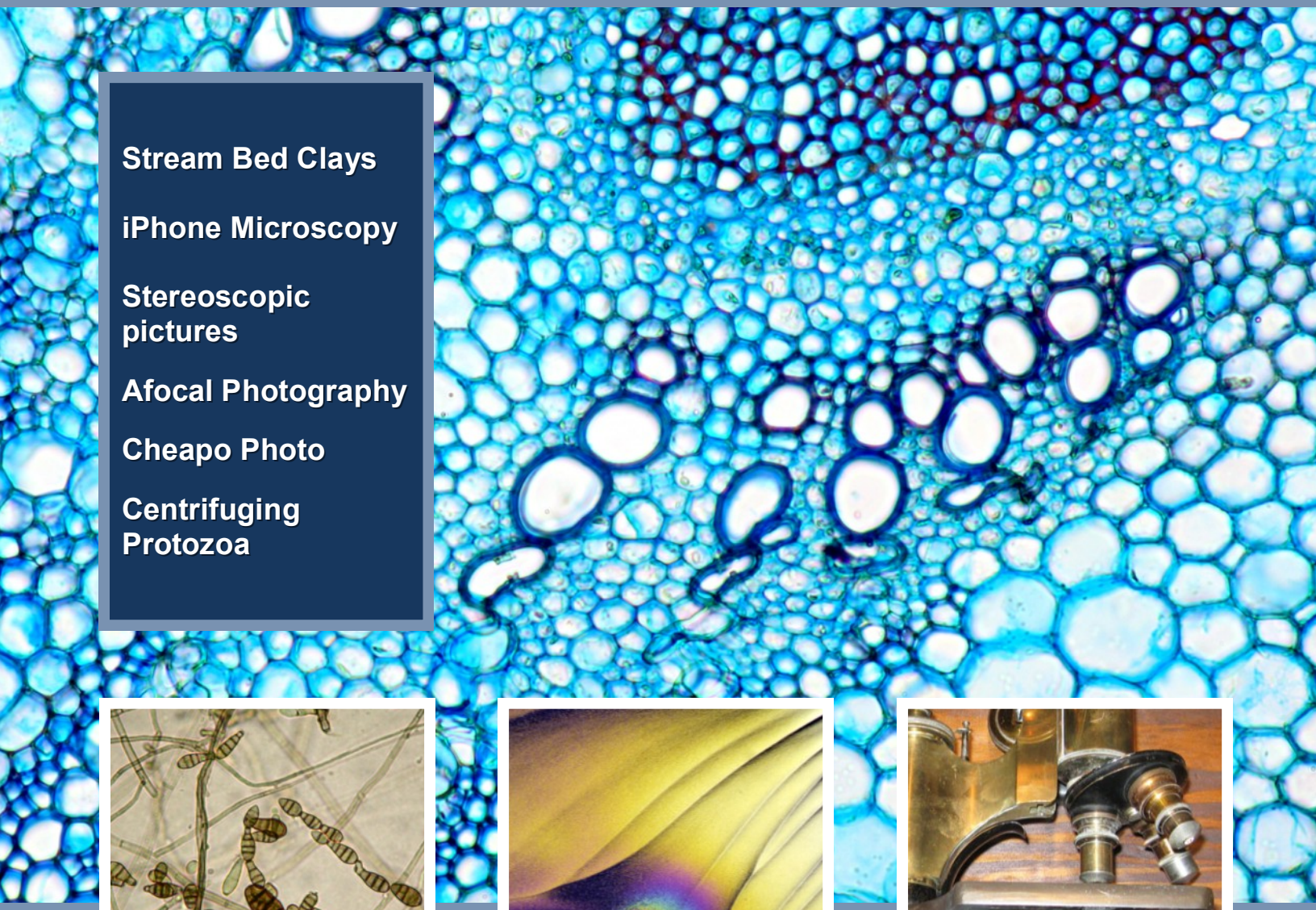

ABOUT

Microbehunter Microscopy Magazine
The magazine for the enthusiast microscopist

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For submission guidelines, consult the website at:
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Main title image (background) by Oliver Kim
Cross section through the stem of a s sun flower.

ANNOUNCEMENT

Visit the Forum!

It is now possible to discuss the individual articles of the magazine. Every issue has a separate sub-forum for discussion.

www.microbehunter.com/forum

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Write for Microbehunter!

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:
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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).

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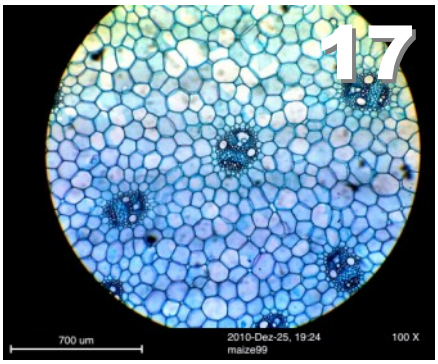
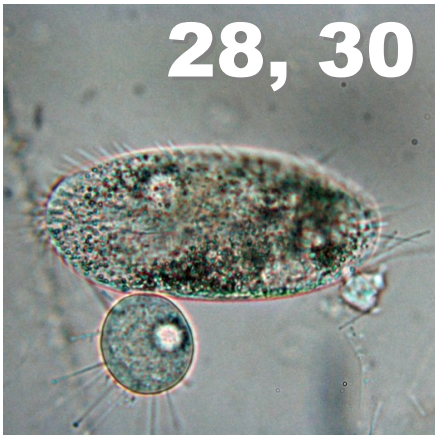
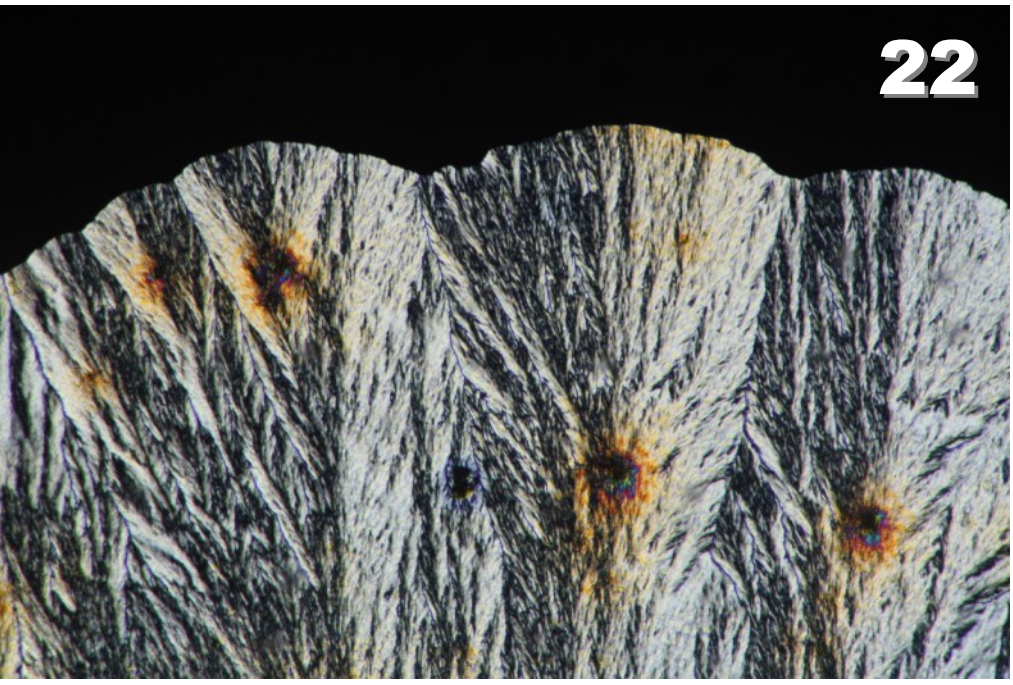
Answer from the back cover:
dog flea (*Ctenocephalides canis*)







Why Paper?

Off to a good start. In front of you: the second issue of 40 pages!

By Oliver Kim, editor

Recently I received the first printed copy of the January 2011 issue of Microbehunter Magazine, and I have to admit that I am very satisfied with the result. The magazine is printed on high quality shiny paper and the colors are bright and vivid. While it is possible to read the magazine on screen, there is still something special to actually holding a printed magazine in one's hands. A paper copy is much more "tanglible" than a mere PDF. The magazine is printed on demand, this means that the magazine is printed, bound and mailed on an individual basis. There is no need to print thousands of magazines ahead of time and then stock them, hoping that they will be sold. This makes the publishing process a risk-free undertaking and also feasible for very few print runs. If no magazines are sold, then there are also no costs. I only have to upload a print-ready PDF and the company handles the whole printing, binding, mailing and billing process. I as an editor can therefore dedicate all my attention to producing a layout. This is also a big advantage for the readers. They know that their billing and mailing information is in the safe hands of an established company.

Thank You

At this point, I would like to voice a big Thank You to all people who contributed to this issue. I would also like to thank everyone who is supporting the project either by directly contributing or by linking to the web site. Please make the project sustainable by contributing articles and images!

■

If you want to order a paper copy, visit:

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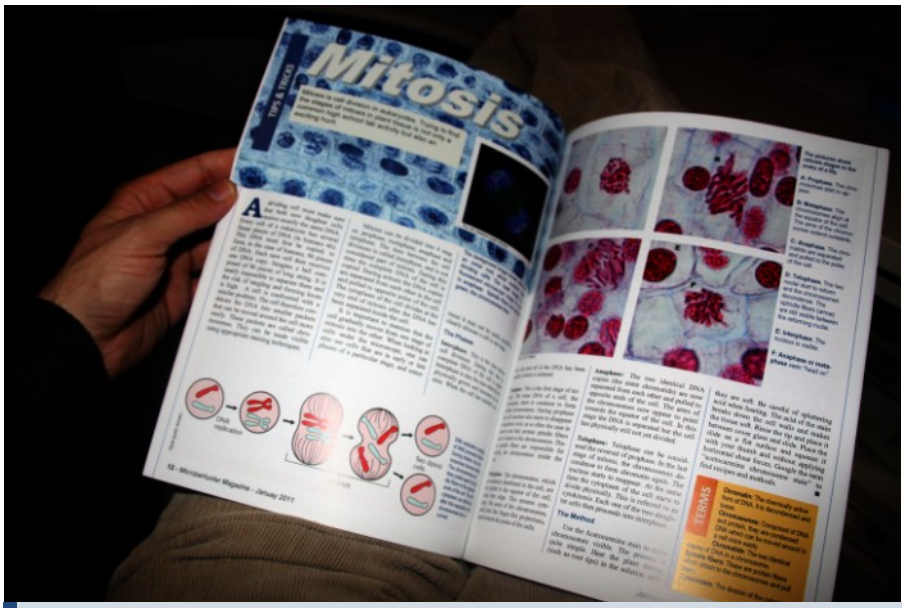

The very first printed issue of Microbehunter Magazine!

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!

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.

Ode to the Microbes

By Oliver Kim

How tiny can a microbe be?
They are so difficult to see!
Too small to see with naked eyes,
some truly have caused much surprise!
They were discovered long ago,
and still are good for quite a show.

They do rot food and make it spoiled,
just make sure it's all well boiled!
And if you do have an infection:
medicine does give protection.
Take it well and with persistence,
otherwise they form a resistance.

Not all are bad, I must confess.
Extraordinary feats some do express.
Did you know that bread and cheese
are products of these little beasts?
They even serve as food for fish,
but not when they grow on a petri dish.

Some are thin and some are thick,
some of them look like a stick.
Some of them form little spores,
others, still, are real bores.
They sit around and simply rest,
this puts my patience to the test!

Who would have guessed that they can too,
mathematical calculations do?
Exponentially dividing,
laws of math they are abiding!
Microbes even multiply,
their numbers sum up on the fly.

Millions of them side-by-side
makes them rather difficult to hide.
Visible as strings and flakes,
they appear in many lakes.
In digestive systems too!
Welcome to the microbe zoo!

They live in water, soil and air,
you can find them nearly everywhere!
Some do not move, some float, some glide,
some show us quite a novel side:
Moving in a funny wiggle,
they do make me quite often giggle.

Microbes now are well in fashion;
evoking, yes, a certain passion.
Microbe hunting is my pleasure,
slides with microbes are my treasure.
Observing them gives quite a kick,
a microscope will do the trick!

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THANK YOU

At this point, I would like to voice a big Thank You to Micscape Magazine and its editor David Walker for placing a link to MicrobeHunter in their Web site!

Miscape Magazine can be accessed at:
http://www.microscopy-uk.org.uk/mag/indexmag.html

When Food goes bad

Thinking of throwing out that bread, which has now turned 'furry'? Wait until you have read my introduction to the intricate forms of some common 'moulds', as seen under the microscope!

By James Wearn

Fig. 1. *Penicillium* on an orange (image: CC-BY-NC-SA Copyright Malcolm Storey, www.bioimages.org.uk).

Fungi are part of our everyday lives: from minute spores in the air that surrounds us to mushrooms in the refrigerator awaiting our dining delectation. Humans and fungi are intimately linked, yet many people still regard them as mysterious. Even today it seems that the remark 'mycologists are indeed poor propagandists' (Alexopoulos 1952) holds true. Although macrofungi (the ones that form mushroom-like fruit bodies) receive autumnal publicity in terms of edibility, it is the group known as microfungi (that do not form mushroom-like bodies and are commonly called 'moulds') that seldom enter the public eye, except in occasional, negative reports of a pathological nature.

Fungi in the home – the ones which are not bought or foraged – are generally unwanted moulds, whether black patches on damp bathroom walls or blue mats developing on oranges that have been left in the fruit bowl for too long (Fig. 1). Stored natural products are nutritious to fungi as well as to us. The word 'mould' is defined in the *Chambers 21st Century Dictionary* as 'any of various fungi that produce an abundant woolly network of threadlike strands; a woolly growth of this sort on foods, plants, etc.' Such a broad definition attempts to cover a hugely diverse group of organisms. Often, species found together on one particular fruit, for example, are quite unrelated to each another (including many different Ascomycetes and Zygomycetes). They are encountered frequently and despised almost as often. We tend to associate them with food spoilage and illness and, while this can be true, many are of critical importance to food pro-

duction, human wellbeing and ecological stability. Nevertheless, their significance in our lives is often overlooked. Just as the term 'a weed is only a plant in the wrong place' has been used to defend native wayside flowers when they occur in one's carefully tended flower beds, I would say that 'a mould is only a fungus where it is not wanted'. Outside of the home or vegetable garden, microfungi serve vital roles in recycling of nutrients (through decomposition), aiding plant growth and survival. In fact, they take part in all sorts of complex and intriguing interactions with animals and plants (introductory information in Wearn 2008a, b).

I shall focus here upon five fungi that are commonly seen in and around the home, their identities, effects and, perhaps most importantly for the remit of this magazine, their elaborate microscopic features. As a cautionary note, please be aware that several fungi found in the home can be dermatological or respiratory allergens, and should not be encouraged to perpetuate in uncontrolled environments. Always be mindful of limiting spore dispersal (aerosol effect) when handling mouldy materials. Nevertheless, minute quantities may be, quite harmlessly, scraped from various substrates for examination under the microscope and provide a source of fascination for all those who wish to discover more about those pesky moulds! A small amount of fungal material should be transferred to a glass slide on the tip of a scalpel or needle. Simply mount in water or lactic acid for instant observation. For those readers who wish to be more adventurous, as not all fungi have pigmented structures, I shall discuss staining techniques in a separate paper.

Rhizopus and *Mucor* (pin moulds)

The black-headed, woolly fungus, which I am sure most, if not all, readers will have seen at some time growing on stale bread, is usually *Rhizopus stolonifer*. This species thrives on rich sources of carbohydrates in damp atmospheres and has been used in classrooms for decades to provide an introduction to fungi. It may be cultured simply by placing a piece of damp bread in a sealed container or on a dish above a pot of water and the whole covered by a jar to maintain a high level of humidity. *Rhizopus* is just one example of the group termed Zygomycetes. These fungi are omnipresent and therefore, not limited to bread. Pin moulds grow very quickly in damp conditions and characteristically form more-or-less upright, aerial hyphae (called sporan-

Fig. 2. Macro photograph of the black-headed pin mould, *Rhizopus*. (image: J. Wearn)

Fig. 3. Sporangium and sporangiophore in a slide preparation (image: J. Wearn).

giophores), which support spherical, spore-containing sacs (sporangia). Also, the hyphae lack cross-walls (septa), allowing rapid passage of cell contents along their length. Sporangia of *Rhizopus stolonifer* appear black in incident light (Fig. 2). A food surface view gives the impression of a dense 'forest', supporting a canopy of dark crowns, while root-like rhizoids anchor the fungus into the bread below. Careful mounting of a scrape of fungus from the surface of the bread and observation under a compound microscope reveals the almost perfect uniformity of the structures, which led to the coining of the common name 'pin mould' (see the sporangium in Fig. 3). *Mucor* can be distinguished by its lack of rhizoids. The soft rots of stored tomatoes and strawberries are caused by these fungi. Fig. 4 shows *Rhizopus* and the closely related *Mucor* growing on strawberries: fruits adjacent to the one originally infected are engulfed quickly, until all the fruits in a box/punnet are colonized. A split tomato left in the air for a few days will often develop the typical cottony hyphae, which emerge from its damaged surface. *Mucor piriformis* is a particularly common and devastating fungus on fresh strawberries and also develops on other cold stored fruits.

Penicillium (blue or green mould)

The ubiquitous blue to green patches that appear on many stored foods and most noticeably on citrus fruits, contrasting markedly with the orange/yellow peel, are the Penicillia (Fig. 1). Although, to the untrained eye, all appear more-or-less identical, there are over 200 species in the genus *Penicillium*! The most common causes of citrus fruit decay are two species of *Penicillium*: *P. italicum* (known as 'blue rot') and *P. digitatum* ('green rot'). Under the microscope one is able to see the long, spore-bearing hyphae (called conidiophores), which terminate in multiple chains of simple, single-celled, asexual spores (conidia). Rots aside, if it was not for the diversity within the Penicillia, we would not have sumptuous blue cheeses (e.g. *P. camembertii* and *P. roquefortii*) or the medical drug Penicillin (*P. chrysogenum*). Ironically, production of Penicillin in food is undesirable as it may cause allergic reactions or lead to resistance in bacteria that are found in our bodies, which could be transferred genetically to pathogens!

Alternaria (black rot)

Alternaria is a common fungus in homes and outdoors, causing black rots on fruit and vegetables, and patches (often with another common fungus, *Cladosporium*) on damp walls, window ledges, textiles and cellulose-rich materials. Although this grey-black, often

slimy fungus is very unappealing when viewed with an unaided eye, a slide preparation provides a chance to see the fascinatingly intricate spore structure (see Fig. 5). The fungus contains a melanin-like pigment, giving a brown coloured appearance. Spores are divided internally by several cross walls in both longitudinal and transverse directions. In the common species, *A. alternata*, the asexual conidia are borne in chains and their surface is roughened (verrucose).

Botrytis (grey mould)

Botrytis, like *Penicillium* is a prime example of a fungus that may yield amazing results or significant crop losses, depending on the situation. *Botrytis cinerea* is the so-called 'noble rot' found on grapes and is deliberately encouraged in order to produce the greatest, sweet and honeyed dessert wines. However, it can also colonize small berry fruits and tomato plants, and may appear on cold-stored leafy vegetables. *Botrytis cinerea* is the most common of several species. For example, *B. allii* may be found on onions. *Botrytis* colonies are grey and velvety to woolly in appearance. Tree-like branched conidiophores bear numerous ellipsoid conidia, the latter resembling miniature leaves upon the 'tree'. The omission of a photo of this species is deliberate – and will hopefully encourage you to investigate for yourselves!

Fusarium (wilts, scabs and cream/pink moulds)

Fusarium is well known to gardeners and farmers as one of the main groups of fungi responsible for stem rots (especially of seedlings) and foliar wilts. However, a number of *Fusarium* species play a significant role in food spoilage, being found on fresh fruit, vegetables and cereals. For example, cream coloured, scabby patches on pumpkins are tomatoes are often caused by *Fusarium*. This can develop as a result of prolonged contact with damp soil before harvest. The black mould, *Alternaria*, may then infect the fruit secondarily. Three main structures may be seen under the microscope: small, oval or bean-shaped microconidia; globose, thick-walled chlamydospores (a resting stage that allows the fungus to persist for many years); and long, narrow macroconidia, which are the most striking feature. The macroconidia are often curved, tapering to a point (falcate), with a few to several cross-

Fig. 4. Pin moulds spread rapidly on stored strawberries (image: J. Wearn).

Fig. 5. Multi-septate conidia of *Alternaria* (image: J. Wearn).

walls. Simple transmitted 'bright field' light microscopy reveals shape and structure (Fig. 6) but as *Fusarium* has little natural pigment (despite often appearing cream or pink in incident light), contrast is greatly enhanced by staining and application of phase contrast (Fig. 7).

Microfungi may be a nuisance in the home but in the natural environment they form a critically important component of all ecosystems. There is probably not a living, or dead, plant in the world that is not colonized externally or internally by at least one species of microfungus. Their complex associations can allow plants to thrive in otherwise intolerable conditions and increase yields (Wearn 2008a, b). Microfungi also form half of the partnerships that are the lichens, unique symbioses with algae or cyanobacteria, which are able to colonize bare rocks and can act as pollution indicators. A variety of chemical compounds with huge medical potential, in addition to the well-known one (Penicillin), have been and are still being extracted from a range of these fungi (examples in Wearn 2008a). We should, therefore, always be mindful of the benefits as well as the detrimental effects of members of a diverse group that have been lumped together under the derogatory term 'moulds'.

Microfungi may often occur where they are not expected, whether due to changes induced by modified climatic conditions, accidental transference or lack of previous sampling. Indeed, much of the fresh produce sold to consumers in 'The West' is imported from far across the globe, including fungal components within or on them. Lettuces are a notable exception, being doused in chlorinated water in order to kill their microbial passengers prior to sale.

Many microfungi, when occurring in small quantities, are not harmful if handled carefully, and can be an interesting and easily available source of material for hours of fun with the microscope. Although I have shown here a few common examples of those on food, there is a whole world of fascinating fungi out there to be explored, including probably thousands that have not been discovered yet!

For anyone who is interested in looking at lichens under the microscope, look out for my upcoming article on this topic (Wearn 2011). ■

Dr. Wearn's current research interests are European lichens, plant-associated microfungi and tropical botany. For more information, contact the author at ***nospam_greenwood.labs@gmail.com*** *(remove the nospam_ before sending emails)*

Fig. 6. Fusarium conidia as seen using transmitted light microscopy (image: J. Wearn).

References

Wearn, J. 2008a. Seeing is believing – the invisible world of microfungi. *Biologist* 55: 16-20.

Wearn, J. 2008b. Mycological-botanical contracts: species around the discussion table. *Microbiologist* 9: 32-34. http://www.sfam.org.uk/pdf/issues/june08micro.pdf

Wearn, J. 2011. Lichens under the microscope. *Quekett Journal of Microscopy* [this will be published in the Summer 2011 issue].

Useful resources

British Mycological Society educational resources
http://www.fungi4schools.org

Pitt, J.I. & Hocking, A.D. 2009. Fungi and Food Spoilage. Springer, New York.

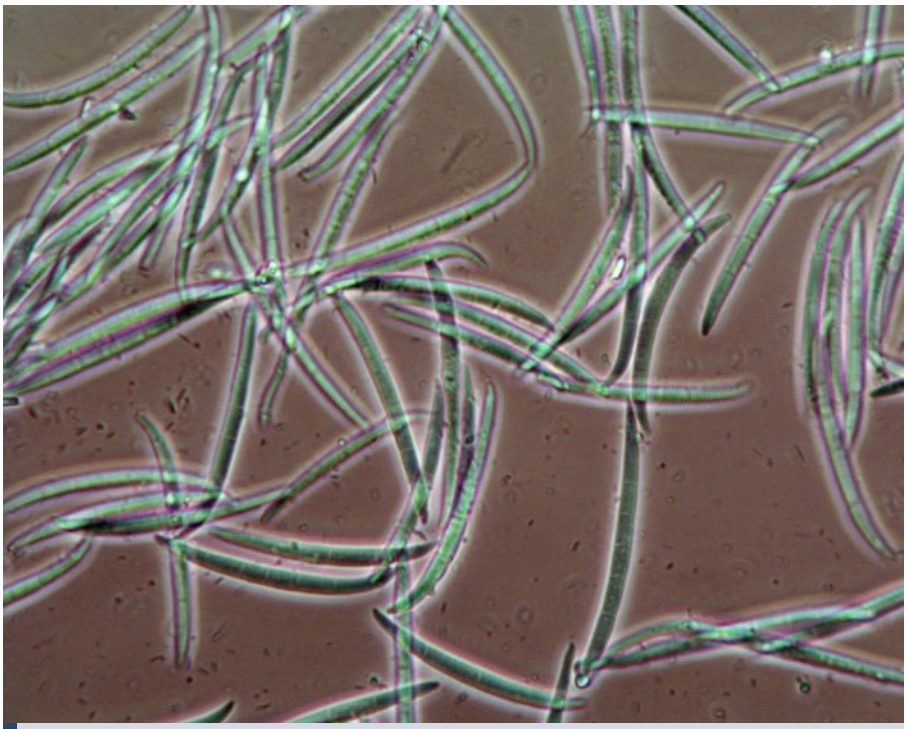


Fig. 7. Fusarium conidia enhanced by staining with cotton blue and using phase contrast (image: CC-BY-NC-SA Copyright Malcolm Storey, www.bioimages.org.uk).

Crossed Plane-Polarized Light Microscopy of Stream Bed Clays

Clay minerals observed in polarized light are not only beautiful to look at, but also reveal something about the composition of the clay.

By Charles E. Guevara

Sections of many freshwater streams may contain areas where clay minerals settle and form beds of clay. These stream beds of clay permit simple and satisfying microscopy using crossed polarizing filters. A very low cost astronomy pair of polarizing filters (polarizer of 0-40% variable transmittance) unscrews into two individual polarizing filters. Place one filter in the substage condenser light path, place the second polarizing filter on top of your microscope ocular. You can rotate this eyepiece filter (the "analyzer" filter) to achieve crossed polarized microscopy. You can adjust various states between fully crossed/ fully aligned polarizing filter lighting.

Simple crossed-pol light microscopy observations of stream bed clays becomes a timeless stroll to encounter and enjoy the variety of microscopic crystals, the mineral inclusions, the shards of garnet, which all settled into the stream clay bed, at the time when that clay bed formed. A quiet stroll along a beach shore can open the mind, even as one visually enjoys, handles, perhaps even collects a few of the varieties of pebbles, the pleasing assortment of shell types, or sand honed drift wood. The strolls are infused with a context of deep time. Geologic processes at work remind you that your calendar may be too impatient! Both

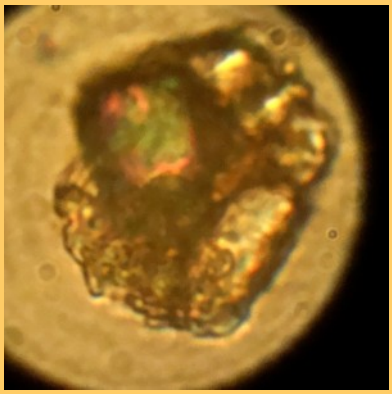
Top: Chloe and I sample a central New York stream bed.

Middle: Sample collected from 8 cm beneath the clay-bed surface.

Bottom: The clay is plastic and sculptable right at site of collection.

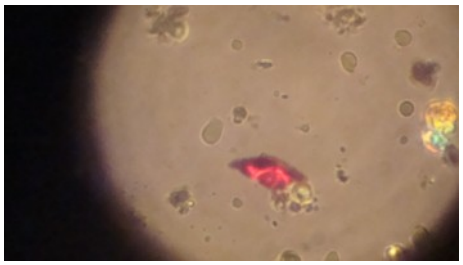
Clay is a naturally occurring aluminium silicate composed primarily of fine-grained minerals. Clay deposits are mostly composed of clay minerals, a subtype of phyllosilicate minerals, which impart plasticity and harden when fired or dried; they also may contain variable amounts of water trapped in the mineral structure by polar attraction. Organic materials which do not impart plasticity may also be a part of clay deposits.

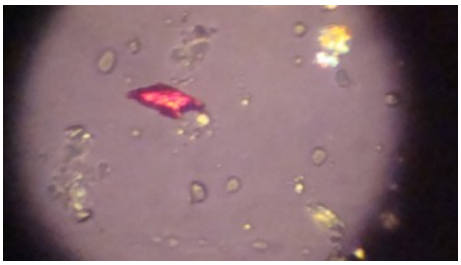
Clay minerals are typically formed over long periods of time by the gradual chemical weathering of rocks, usually silicate-bearing, by low concentrations of carbonic acid and other diluted solvents. These solvents, usually acidic, migrate through the weathering rock after leaching through upper weathered layers. In addition to the weathering process, some clay minerals are formed by hydrothermal activity. Clay deposits may be formed in place as residual deposits in soil, but thick deposits usually are formed as the result of a secondary sedimentary deposition process after they have been eroded and transported from their original location of formation. Clay deposits are typically associated with very low energy depositional environments such as large lakes and marine deposits.

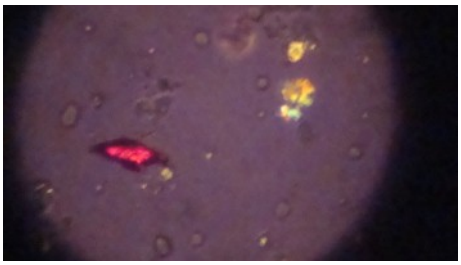


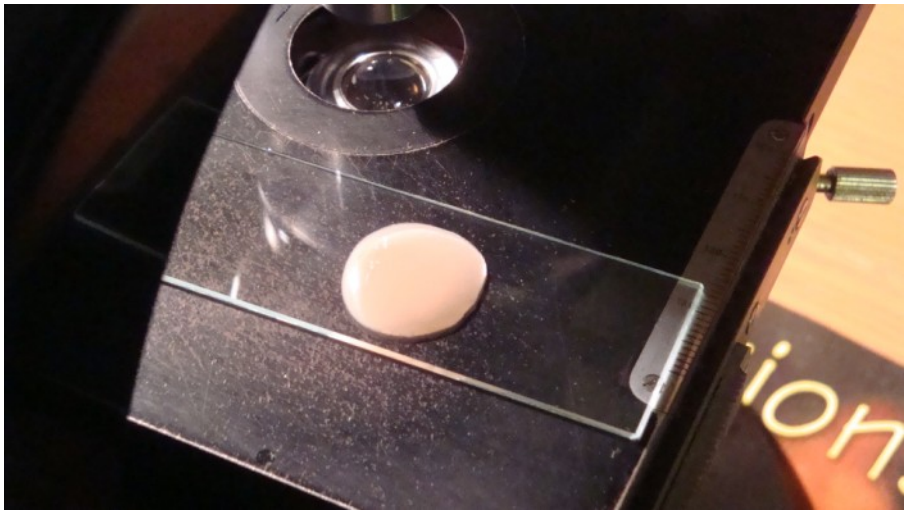
This microscopic conglomerate suggests: 'a gem in hand'! Clay bed inclusions literally are 'gems in one's hand'!

Reference:
http://en.wikipedia.org/wiki/Clay







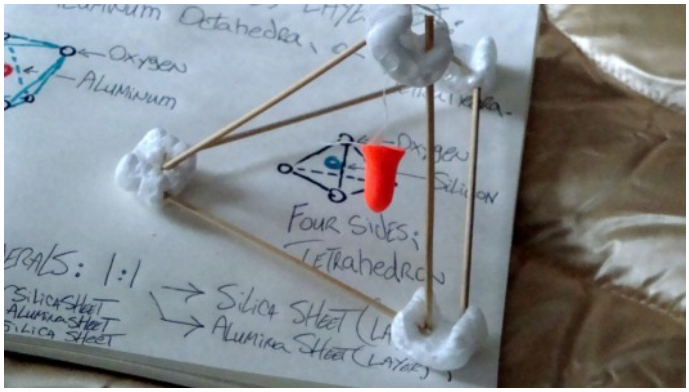


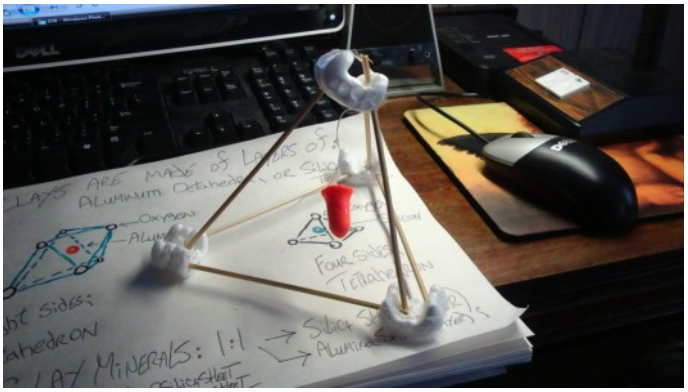
Top: Turning the polarizing filters changes the darkness of the background and makes the crystals glow. The images show garnets.

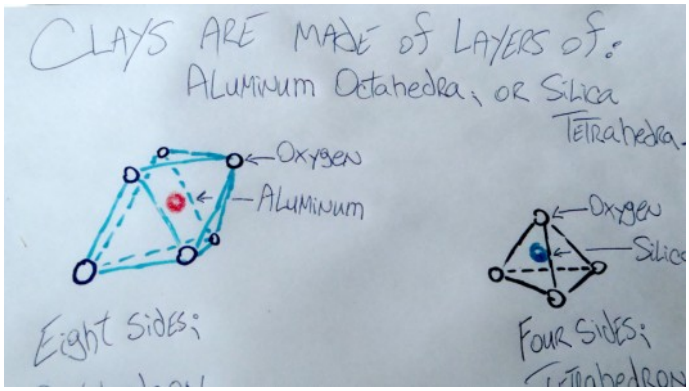
Left: Wet mount slide preps of clay bed sample.

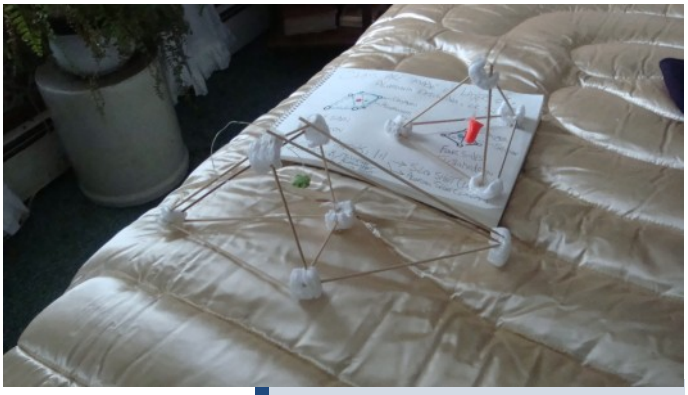
Left bottom: A pair of astronomical polarizing filters.

Bottom: Decant a clay sample in a test tube to gather clay-bed mineral inclusions.









Clay models made from home left-overs! Top left, top right: silica tetrahedra (silicon atom in center).

Bottom right: aluminum octahedra (aluminum atom in center)

strolls can inspire awe - or at least clear your mind.

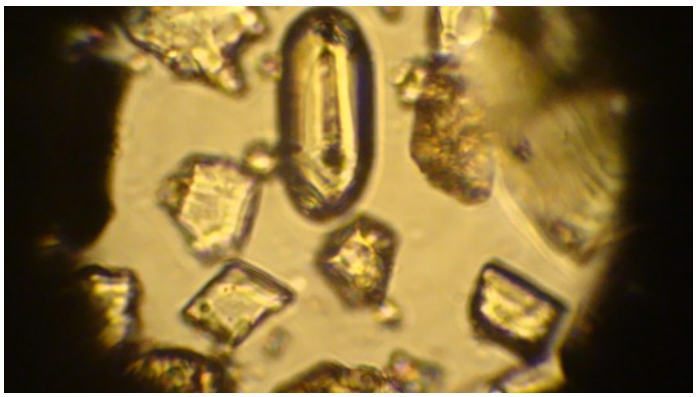
Perhaps the garnets demand your attention at first, but then other crystals are encountered as well. Isotropic crystals (cubic-crystal system constructs, like my childhood playground: monkey bars construct) espouse a phanthom status under full cross-polalrized lighting, a quaint sort of 'dark matter' in the brilliantly speckled fields of stellar shining anisotropic minerals scattered randomly on your microscopic field of view. Cross-pol observations of stream bed clays brilliantly evoke the face of our night sky, if good seeing permits.

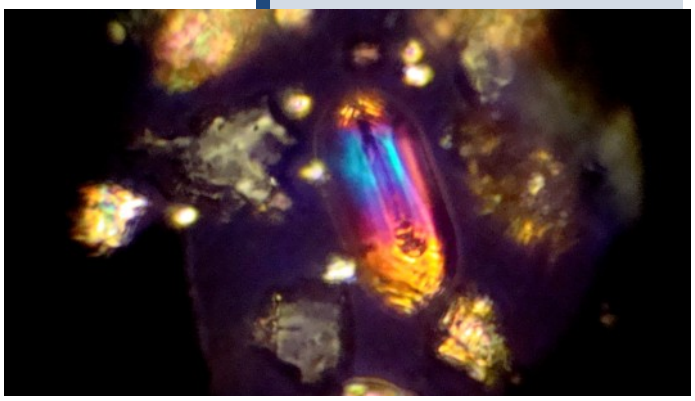
Crystals are encountered manifesting sharp geometric edges and line angles, wondrous micro-displays of crystal habits. Other crystals have been polished to gemstone setting states. Also beckoning for the microscopists attention are patterns of spectral colors which seem like an internal fire of some mineral inclusions, even under high magnification and oil-immersion objective observations. It's a wonderful crossed-pol microscopy, to sample stream bed clays for these observations. The deep time dance and repeated cycles of rocks to clay, clays to rocks, stream birth, stream clay bed formations - my central New York legacy of at least four glaciations - these imbue the microscopy of the crystals I observe.

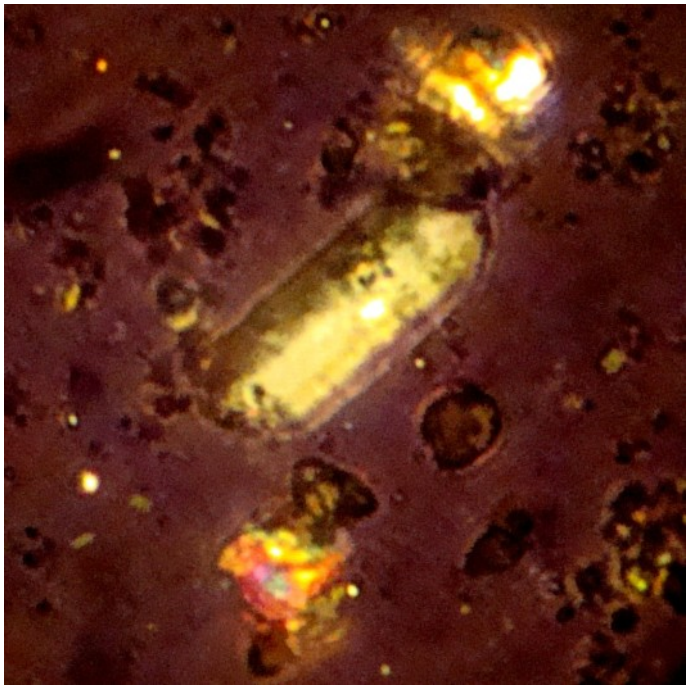
I was able to fabricate crude models of clay mineral tetrahedra, and clay mineral octahedra with household scraps. Clay minerals are composed of layers of tetrahedra and octahedra sheets. Whether clays are 1:1, a bi-layered construct of a silica (the tetrahedra layer, silicon being the atom in center of each tetrahedron) and a layer of alumina (the octahedra layer, aluminum atom being the center atom of each octahedron), or wether the clay minerals are 2:1, a three layered construct of

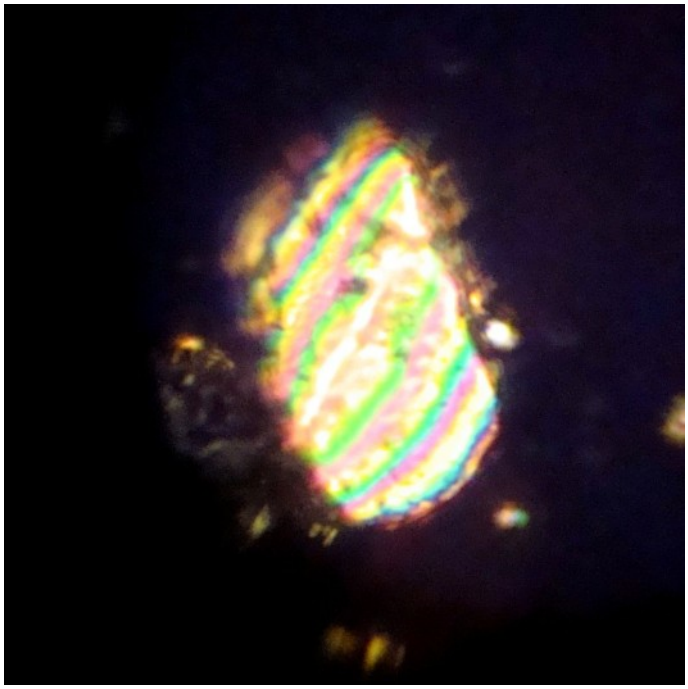
Left: crystals without the use of polarizing filters.

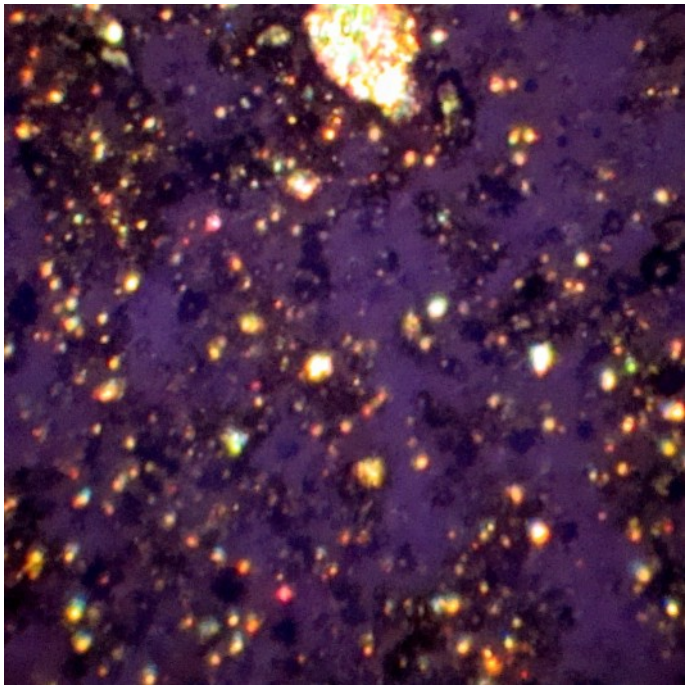
Right: the same crystal in polarized light.

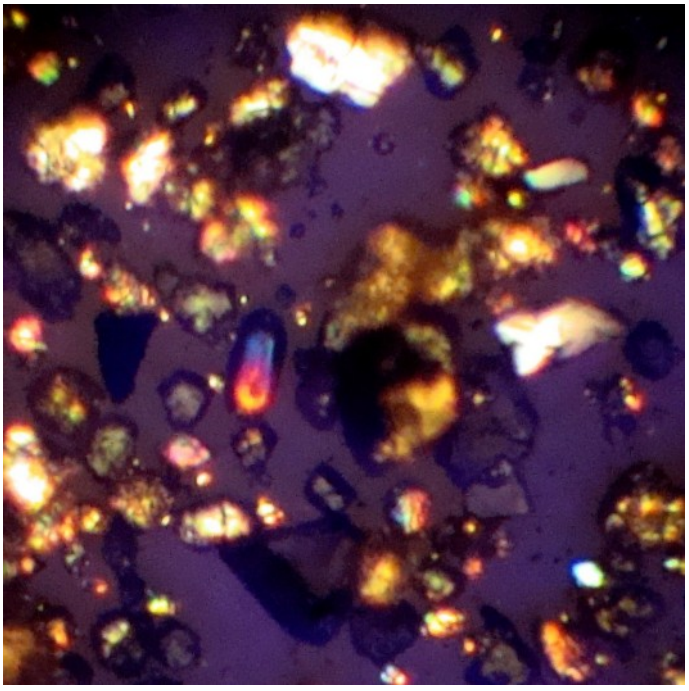


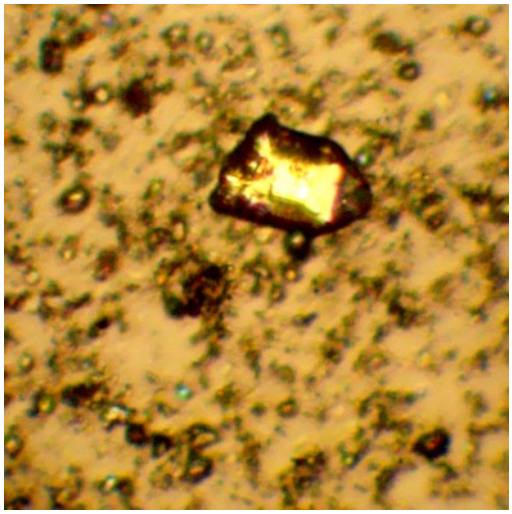


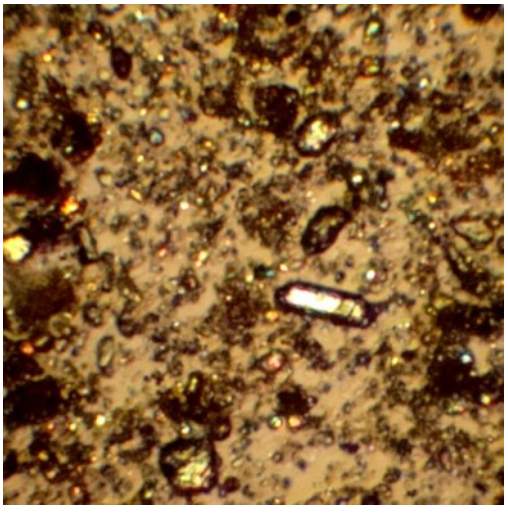












Some eye candy pictures.

Top four images: The dark particles are isotropic minerals. These do not show up as bright spots in polarized light.

Right: Minerals as they appear without polarizing filters.





Native clay sculpting from stream bed clays. Clay is not only useful for microscopic observations! It is also possible to sculpt some pottery!

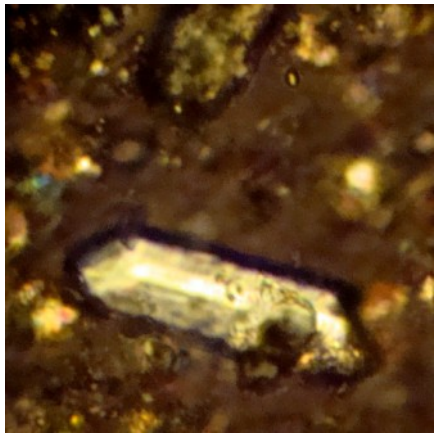
tetrahedra silica sheet and octahedral alumina sheet and another layer of silica tetrahedra (2:1 clays are a sandwich of three layers), or whether the clay minerals are the type of 2:1—water expanding clay minerals, the different and important properties of these three categories dictate the major roles clays play in our world.

The space between the layers of clay minerals offers the dynamic sanctuary for water molecules, for critical nutrient ions in soils, for critically reactive surface areas, all important properties at a specific site. The role of clays in agriculture, in municipal waste fields, in specific drainage projects of civil engineers, and, yes, in human ceramic industry, all emanate from the clay mineral's ionically bonded layered construction (a layer of silica tetrahedra and a layer of alumina octahedra in 1:1 clays, in contrast to the three-layered non-water expanding 2:1 clays of silica—alumina—silica layers, vs. a water expanding 2:1 clay mineral).

Right at the time and site where puppy Chloe and I sampled the stream bed clays, the clay was plastic and workable in my hands. Very few natural materials manifest plasticity and workability at site of their collection (please try and think of a natural material which has the property of plasticity?!!) I knew at time of the stream bed clay collection that my puppy dog Chloe and I would sculpt and fire some simple pottery. But the crossed-pol light microscopy observations of the clays was the main reason for the stream hike.

How many microscopic gems now reside in the brick walls we live in? How many crystal inclusions occupy the dinner plates we hold in our hands? How many of these hold microscopic crystal inclusions, the shards of garnet and all the rest of my encountered eye-candy? How many have swam in glacial outwash, clinked and tumbled, polished and abraded in ancient streams? How many of these crystals in our homes, observed with my microscopy, have basked and weathered on drumlins; then slept in clay beds?

Please enjoy this relaxing crossed-polarized light microscopy! ■



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Dr. James Gregory McHone, "Polarizing, Petrographic, Geologic Microscopes", http://earth2geologists.net/Microscopes/index.htm
CRG site accessed on: 1/16/2011

iPhone Microscopy:
The iMicroscope Applet

Who would have thought that an iPhone can be used to make simple microscopic measurements? The iMicroscope is an iPhone app which is capable of that.

By Oliver Kim

First, you need an iPhone. Then you need to download the iMicroscope over the appstore. I do not own an iPhone and I therefore asked a relative if I could borrow his iPhone just for the sake of trying out iMicroscope so that I could write this article…

The steps involved are fairly straight forward. After starting of the program, the user has the possibility to take a picture by holding the camera in front of the eyepiece. This is probably the most difficult part. The eyepiece to camera distance has to be properly adjusted, otherwise there is significant vignetting. It is not possible to rest the iPhone against the eyepiece for a steady picture, this distance would be too close and no picture would appear.

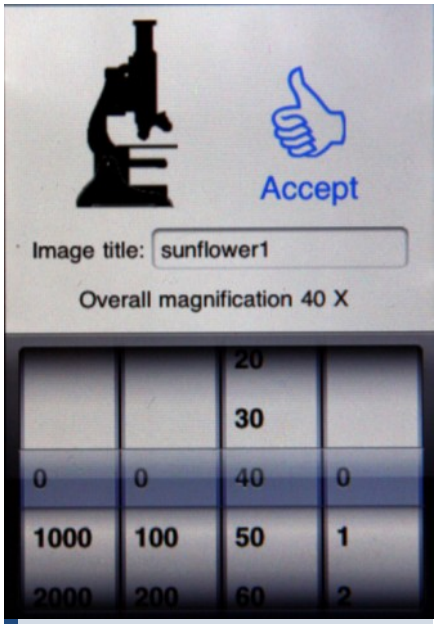
After the image has been taken, the user is prompted to enter an image title and an overall magnification (objective magnification x eyepiece magnification). The program will then display the image together with a scale bar. Zooming in and out of the image will readjust the scale bar. Appropriate units are always displayed as well.

Saving the image also saves the title as well as the entered magnification. It is therefore possible to continue to pan and zoom the image later on as well.

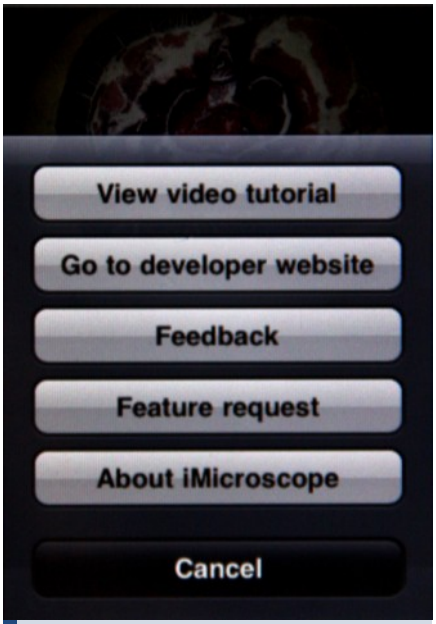
The scale bar scales intelligently: Both the length of the scale bar as well as the indicated numbers change when zooming. This means, that the scale bar always displays nicely rounded numbers. A fixed scale bar size could result in a distance such as 221um. This is avoided and a nice 200um is displayed with appropriate resizing of the scale bar.

Some tips

First focus the microscope, decrease the light intensity in order not to damage the camera of the iPhone and hold

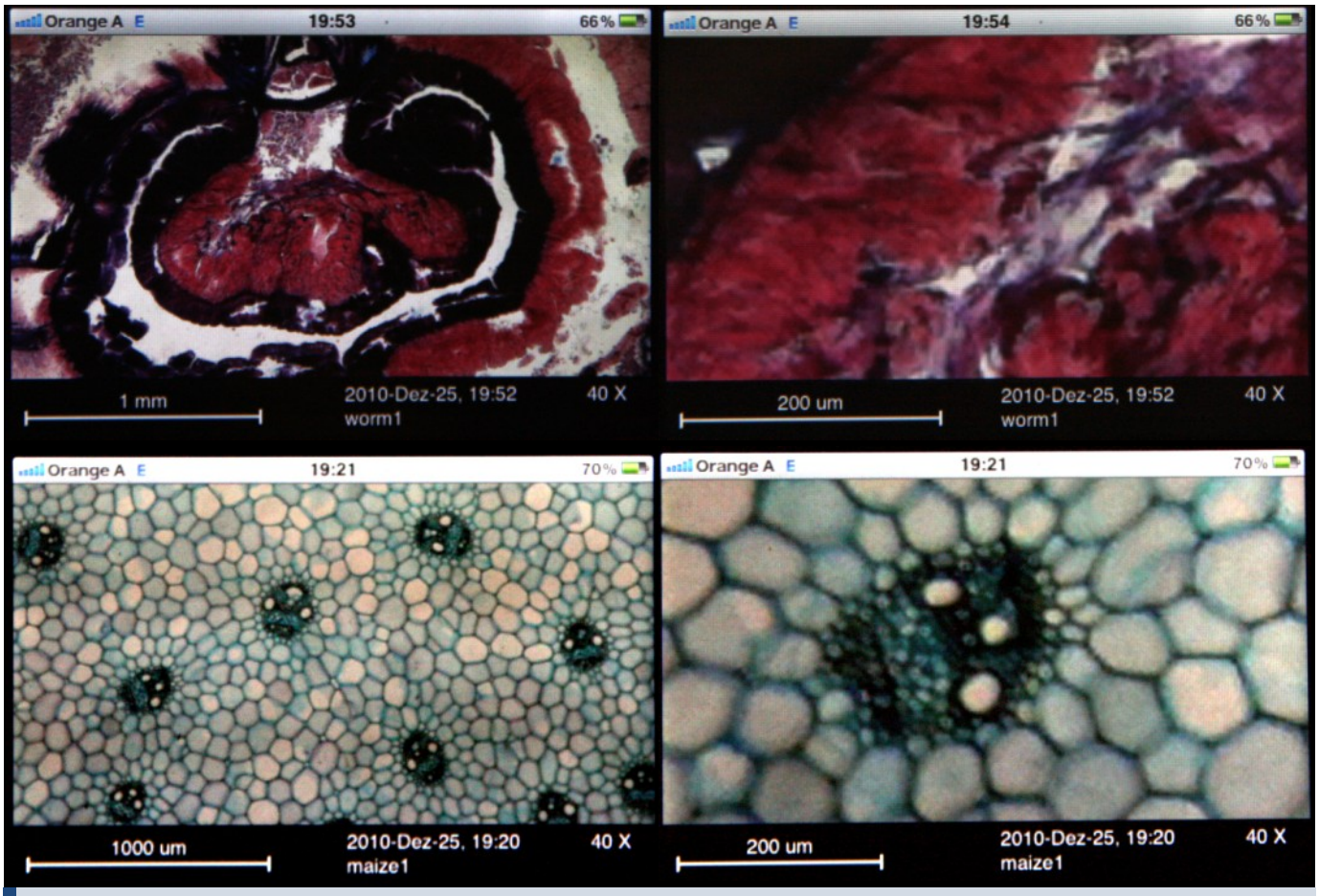


Selecting magnification: After having taken the photograph, the user has to enter the overall magnification (objective x eyepiece) and an image title. The magnification is set by rotating four vertical rolls.



Menu items: Software documentation is by a video tutorial. This tutorial can also be watched online. Feedback and feature request menu points open the email program.

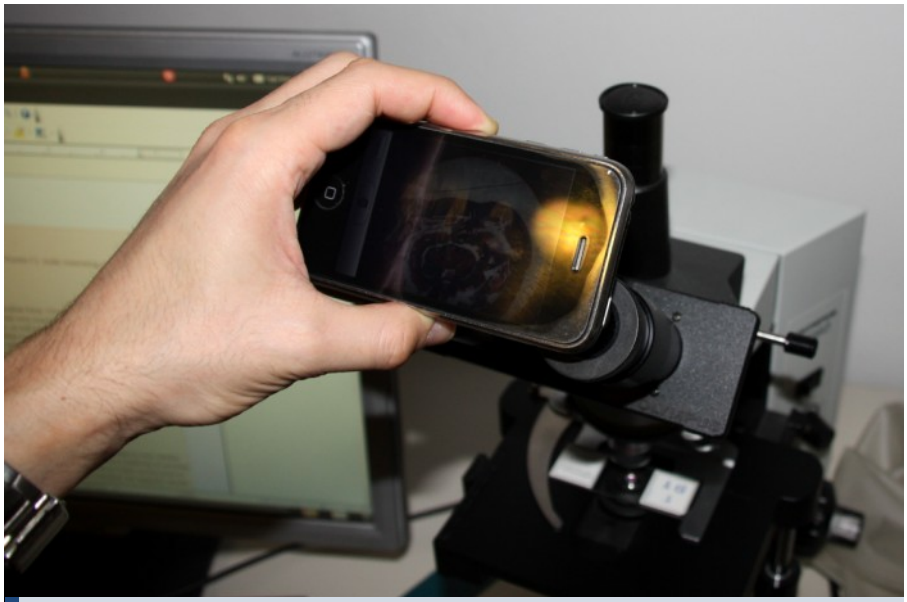
After the picture was taken: The user can save the image (together with the scale bar), email the image or tweet the image.



Top two images: Cross section through the earth worm (*Lumbricus terrestris*). Bottom two images: cross section through a stem of maize (*Zea mays*). The images are photographs directly off the screen of the iPhone, and are therefore of a slightly lower quality. The images show different zoom levels and the associated scale bar. The date is represented in German („Dez" stands for December), due to the language settigns of the iPhone. On the right lower corner you can see the magnification at which the image was taken. The title and magnification are saved with the image so that it is possible to zoom later as well.

the camera at the right distance in front of the eyepiece. Release the shutter. If you have to take several images, then I would advise you to construct a small cardboard sleeve which fits around the eyepiece against which you can rest the iPhone. This will also give you shots that are more steady at a longer shutter speed. This allows you to further reduce light intensity. The sleeve will also help you to find the correct distance to the camera lens. Paint the inside of the tube black in order to reduce the light reflections from the side of the tube.

To measure the size of a microscopic structure first zoom into the structure. Then pan the image until the structure is located right above the scale bar. Zoom in or out until the structure has the same size as the scale bar. You can then read of the value.



Finding the correct distance to the eye piece is critical for obtaining a vignetting-free image. It may helpful to hold the iPhone with two hands and then release the shutter by using the thumb of the left hand. I found it also useful to rest the left hand against the left eyepiece to obtain a steady image.

Features I'd like to see

There are a few features that I would still like to see in future versions of the program. First, it would be very useful if one could save not only the original image, but also the zoomed image together with the scale bar. The current version only saves the original (i.e. non-zoomed) image; two examples are shown on the next page. Maybe there are applets around which are able to make an iPhone screen capture. This would essentially resolve the issue. I have to admit at this point, that I did not own the iPhone on which I tested iMicroscope. I therefore do not know if the iPhone already comes with a screen capturing functionality.

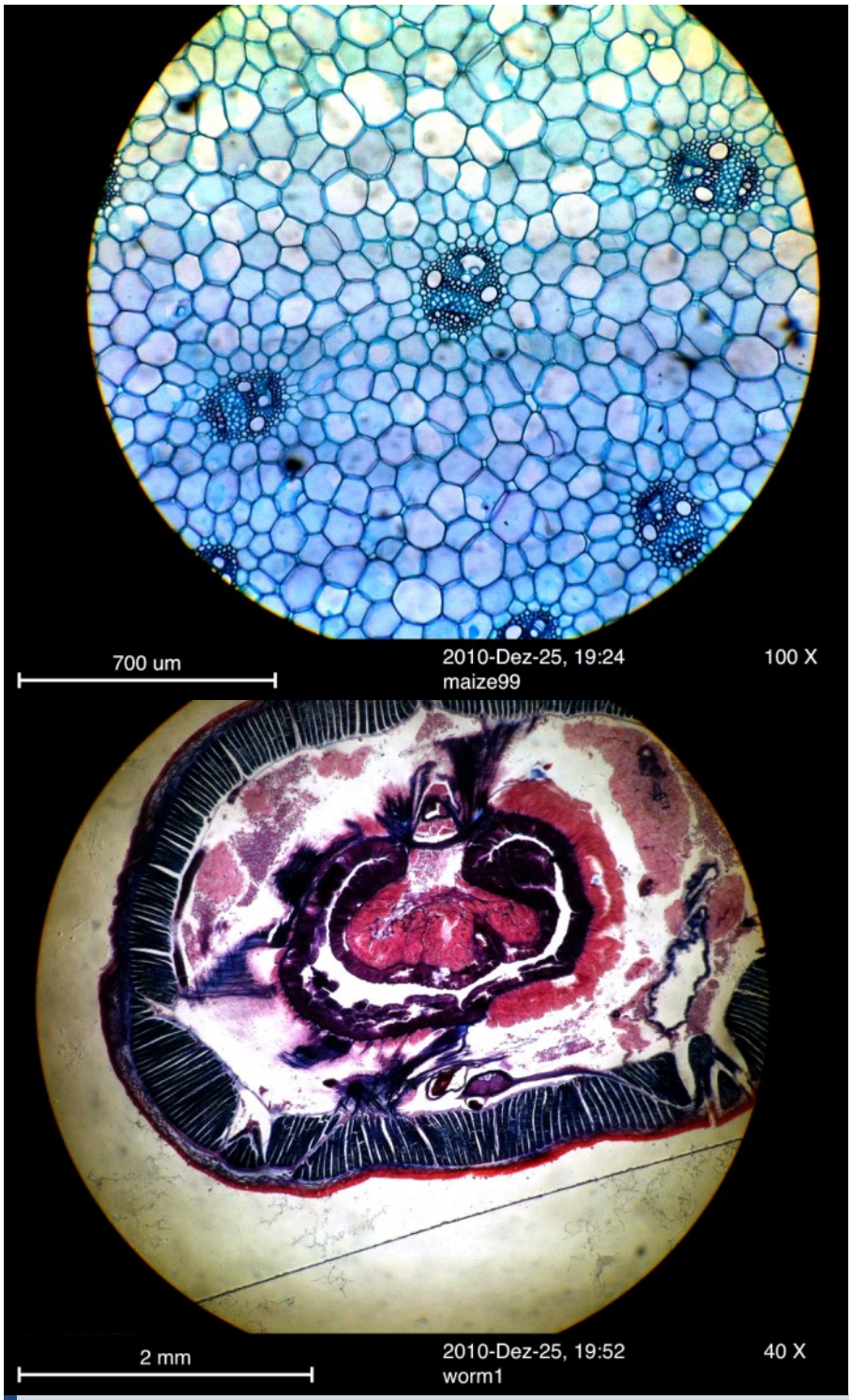
A second feature request is slightly more advanced: It would be great if one could draw lines directly into the (zoomed) picture and then have the applet calculate the length of the line. It should then be possible to save this zoomed picture as well.

The third feature request is yet more advanced. It would be nice to have an automatic counting feature or area calculation feature. The user then traces a pattern on the display and the program calculates the area. Automatic cell counting would be very useful, but the thickness of the cell suspension must be standardized as well, and this is the difficult part. But I think that I'm now getting carried away here. There are already image analysis programs for computers around which are capable of achieving these tasks.

Conclusion

The iMicroscope applet provides one of the fastest methods of quickly estimating the size of microscopic structures. The program may be indeed an alternative to the use of reticles.

If you already own an iPhone and a microscope, I would definitively buy the applet and give it a try, the price is relatively low (about 2 coffee units, depening on how expensive the coffee is in your area). ■



Original images as they appear when saved to the camera roll. The top image shows maize and the bottom one the cross section of an earth worm. Scale bars are integrated into the image. The color contrast was slightly increased for publication purposes. The images also illustrate that mobile phone cameras are able to produce quite respectable results.

The iPhone and iPhone 3G feature a built-in Fixed focus 2.0 megapixel camera located on the back for still digital photos. It has no optical zoom, flash or autofocus, and does not support video recording. The iPhone 3GS has a 3.2 megapixel camera, manufactured by OmniVision, featuring autofocus, auto white balance, and auto macro (up to 10 cm). It is also capable of capturing 640x480 (VGA resolution) video at 30 frames per second. The iPhone 4 introduced a 5.0 megapixel camera (2592x1936 pixels).

Reference:
http://en.wikipedia.org/wiki/IPhone

Stereoscopic Pictures with Picolay

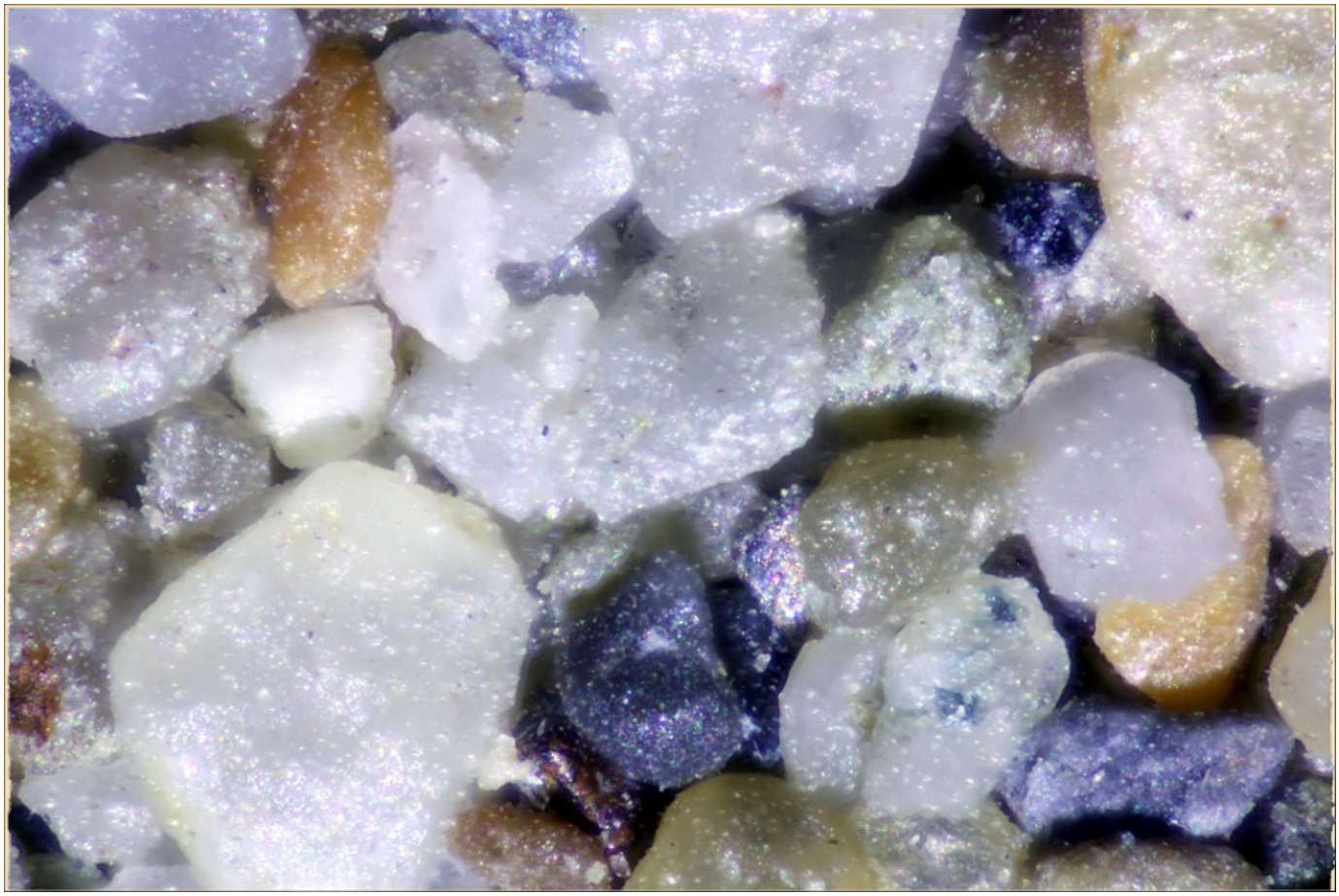
Yes, it is possible to make stereoscopic images with a compound microscope. The free program Picolay will achieve this task.

By Oliver Kim

One of the advantages of drawing microscopic specimens is the fact that a drawing is capable of combining images that have different parts in focus into one drawing. Due to the generally low depth of field, a photograph, in contrast, is capable of capturing only thin sections of the specimen. For many years, drawing was the only possibility to document thick specimens, such as insects. Digital techniques now allow the microscopist to overcome these limitations. The microscopist has to take several images of the specimen, each one with a different focus setting. A software then combines these images onto one completely sharp image, in a process called focus stacking. I already introduces focus stacking in the January 2011 issue.

There are several programs which are capable of focus stacking, such as CombineZM, Helicon focus, as well as Picolay. These programs are able to determine which regions of the images are in focus and which ones are not. The programs then assemble a final image using only these parts of the series which contain the correctly focused areas. How the programs determine these focused parts is a technical issue, which I will not cover right now.

I used the program Picolay to create true stereoscopic images. For stereoscopy to work, each eye must receive a slightly different picture. Picolay now uses the depth information to compute images that can either be seen with red-cyan glasses, or two images which can be viewed with crossed eyes. Just to clarify the issue: This works with regular compound microscopes, which are, natively, not capable of producing stereoscopic images. One does not take separate images for the left and the



right eye. These images are computed by the program.

The process is rather simple, for best results is is necessary to fine-tune the parameters, however. The steps are:

1.Take a series of images of your specimen of different focus levels. It is important that the focus levels are not in random sequence. Remove all images that are completely out of focus.

2.Load the images into Picolay and select the option "Stack with varied parameters". The program stacks the images with a single click. You can make further adjustments later. Be patient as the program computes the stack.

3.Choose the option 3D from the menu. Do not let the large number of adjustments confuse you.

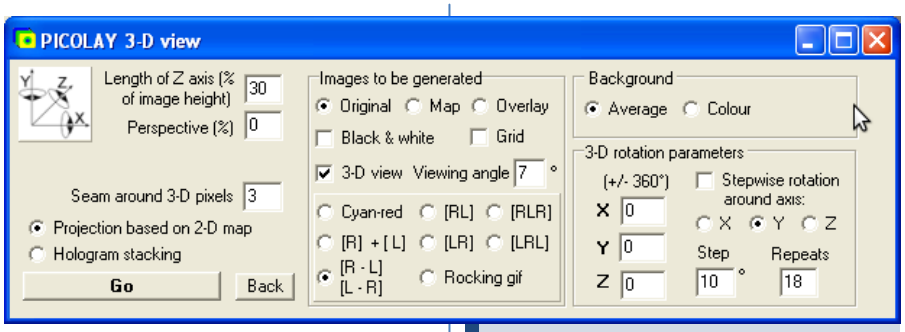
4.You have several options for generating a 3D image. You can either create an image for viewing with red-cyan glasses. Alternatively you can create images for parallel viewing or for crossed-eye viewing. These do not require red-cyan glasses but a little bit of practice. Click on the radio button "Rocking GIF" for now. This will create an animated GIF with gives a 3D impression. View this image in a web browser (File-open), if the animation is not displayed.

5.You can adjust the intensity of the 3D effect by changing the viewing angle. Go with the defaults for right now.

Press "Go" to start the process. Picolay will now generate the final image. Upon closing the program, Picolay will ask you if you want to save the images.

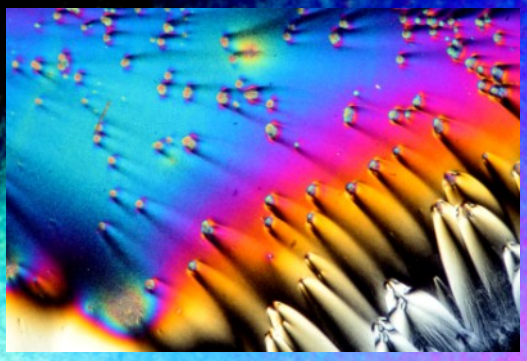
To download Picolay, visit:
http://www.picolay.icbm.de/
The program is free and does not require any installation. There is a demonstration video and a tutorial as well.

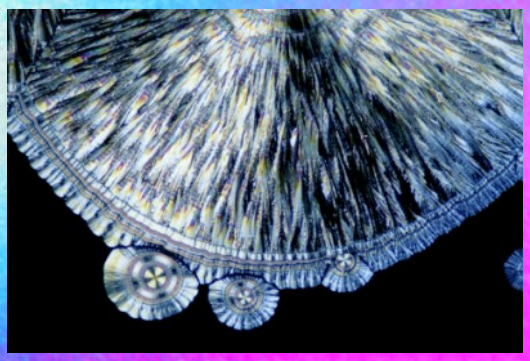
■



The 3-D view of the program allows you to fine-tune many parameters. It is also possible to rotate the image .

Stereoscopic image for cross-eyed viewing. It does take a little bit of practice. Place both images in front of you and then cross your vision until you see three images. The central image will then appear stereoscopic. The pictures show sand grains, illuminated from the top.





Crystals of organic substances, such as Ascorbic acid (Vitamin C) make interesting microscopic specimens to be viewed under polarized light.

Vitamin C

in Polarized Light

By Oliver Kim

Vitamin C crystals in polarized light can be incredibly artistic and beautiful And they are not difficult to make either!

Materials needed

For growing crystals, you need Vitamin C in pure, crystalline form. Vitamin C tablets will not work, you need the pure white powder. Pure Vitamin C looks very much like sugar. Additionally you need a solvent to dissolve the substance. Water will work, as will ethyl alcohol. You can also use denatured alcohol (which is ethyl alcohol, with a bitter tasting additive). I have not tried rubbing alcohol, which may contain isopropyl alcohol, but if it dissolves the Vitamin C, then it should not be a problem.

Additionally you need two linear polarizing filters and standard microscopic glass slides. Test if the polarizing filters are of the correct type by placing them on top of each other and then by rotating one of them. There should be a position when they block all of the light. In this „crossed“ position observation of crystals works best. Place different transparent objects (such as adhesive tape) between the filters and observe the color play. We will be doing something similar with the Vitamin C crystals.

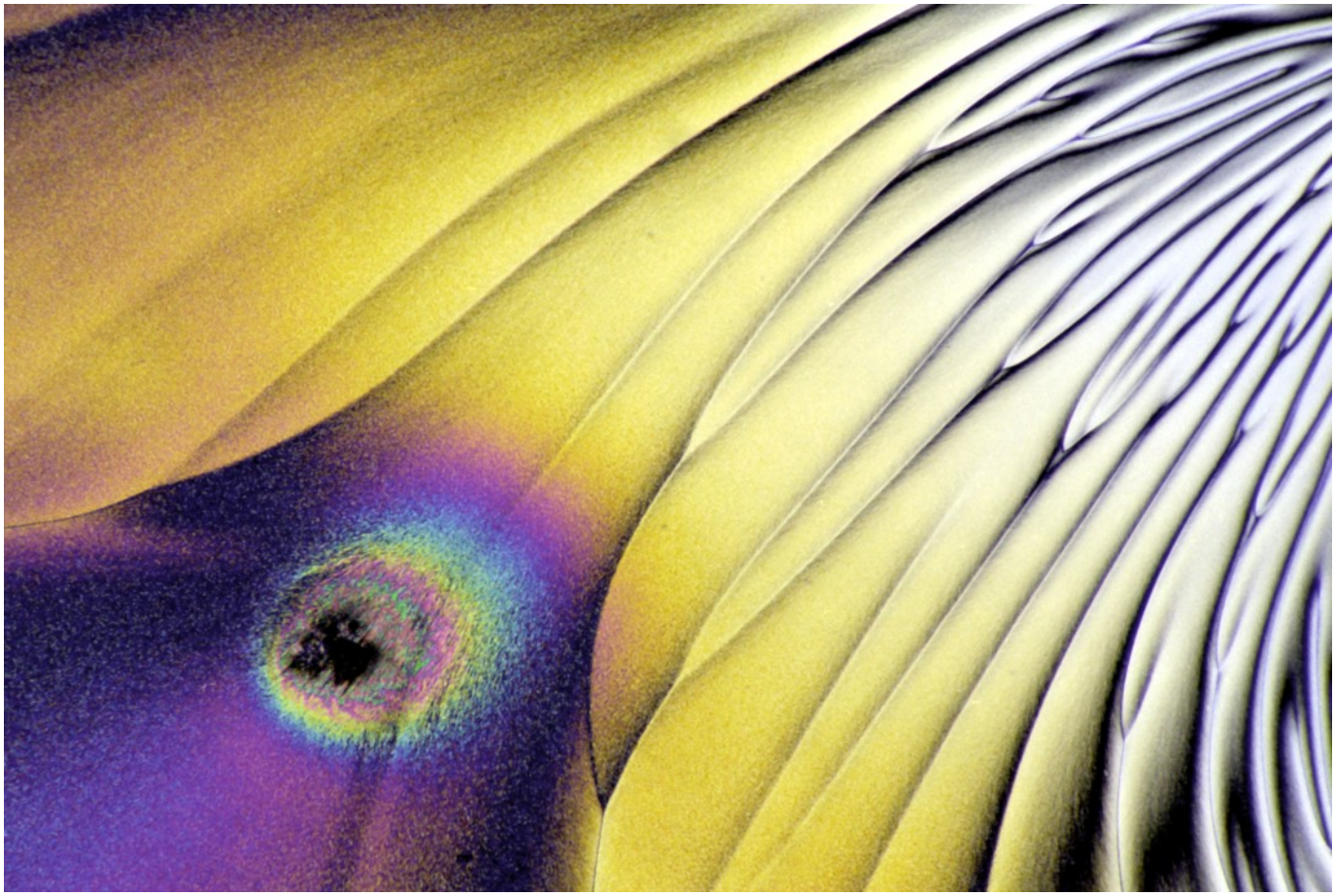
Many polarizing filters sold in photography stores are circular polarizing and these will not work. It is best to test the filters first, or to buy linear polarizing filters from a school supplies company.

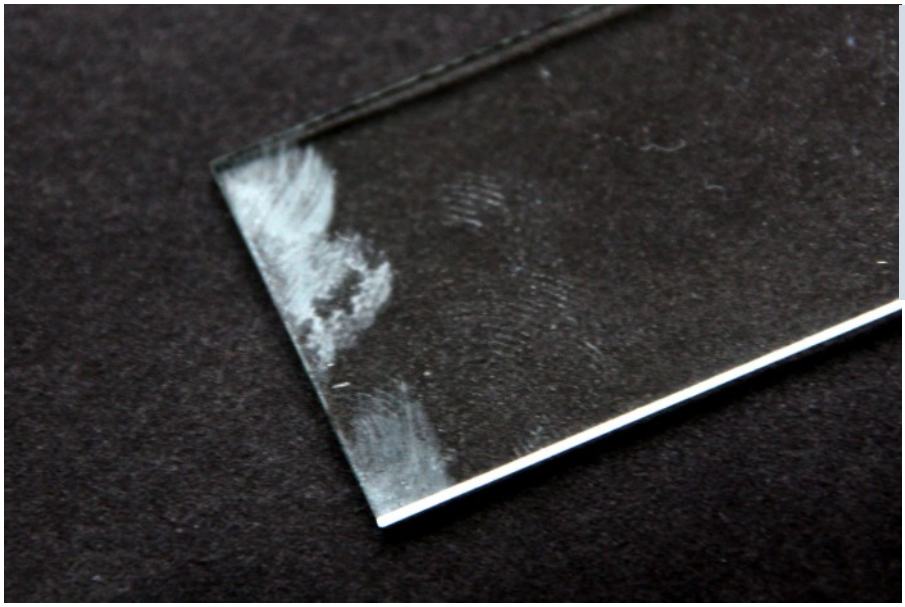
Some filters are not made of glass, but rather of a thin flexible foil. This will also work. The reason why I used the glass filters is rather trivial: I happened to have them around. The thinner foil-based filters may be of an advantage if you want to observe the crystals with a higher magnification with a smaller objective-specimen distance (working distance). Space might become tight with thick filters, because one of the two filters has to be placed between specimen and objective.

Growing crystals

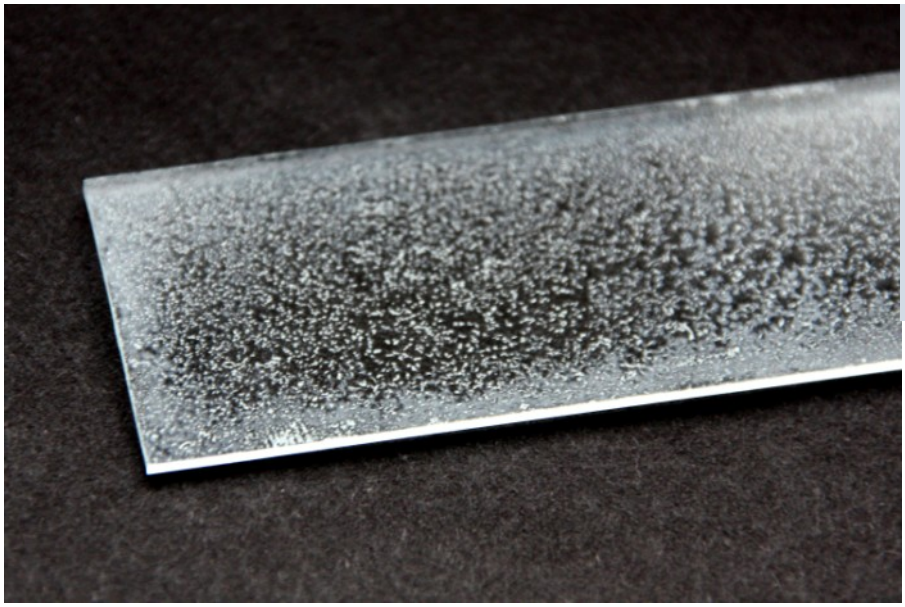
You can either dissolve the Vitamin C in water or in alcohol. The general procedure is very similar. First, you need to dissolve a small amount of the substance in the solvent. Try a knife-tip of ascorbic acid in about 1ml of alcohol. Alcohol does not dissolve Vitamin C as well as water, so you have to be patient. It is ok if a little bit of powder settles to the bottom of the container, the majority should be dissolved, however.

Take a drop of the solution and apply it to a slide. The alcohol will quickly spread and cover the whole slide. Store the slide horizontally for several minutes and allow the alcohol to evaporate. Semi-transparent white areas should appear. This is the crystalizing Vitamin C. Wait until the crystalization





After evaporation of the water or alcohol (whichever one you used), the Vitamin C is present in the form of a thin sticky layer. Touching the layer will initiate crystal formation. These areas will turn white. Dust from the air may also initiate crystal formation. Sometimes crystallization will start fairly quickly and proceed as the solvent evaporates.



This slide has now completed crystal formation. The individual crystals are opaque. More interesting results are obtained with crystals that are semi-transparent. Experiment with different Vitamin C concentrations, solvents and evaporation speeds. Interesting patters are also obtained by not completely dissolving the Vitamin C. The undissolved crystals serve as crystallization seeds.

process has completed. Using alcohol as a solvent is probably the easiest method and crystals start to form quickly.

If you used water as a solvent, then the surface tension of the water may be the cause for some problems. As the water evaporates, the surface tension will cause the drop to contract and form a thick white, opaque spot of Vitamin C in the center of the slide. This is not desirable and does not look pretty under the microscope. You have to make sure that the water stays flat, covering the whole slide, while it dries. Try to clean the slide with alcohol before applying the solution. This removes fats from the glass and results in a better water-glass interaction. If this is also not enough, then adding a small amount of detergent to the solution (soap) may help. This additive may interfere with crystallization, however. Use only small amounts of detergent.

The third method is probably the most „fool-proof“ approach, but does require delicate fingers. Apply as much solution as the slide can possibly hold! The solution should cover the whole slide from corner to corner. Adhesive and cohesive forces will hold the large water „drop“ to the corners of the slide, as the water evaporates. The drop, being held at the corners, can not contract as the water evaporates. If you add too much solvent, then there is the possibility for it to spill over the edge of the slide.

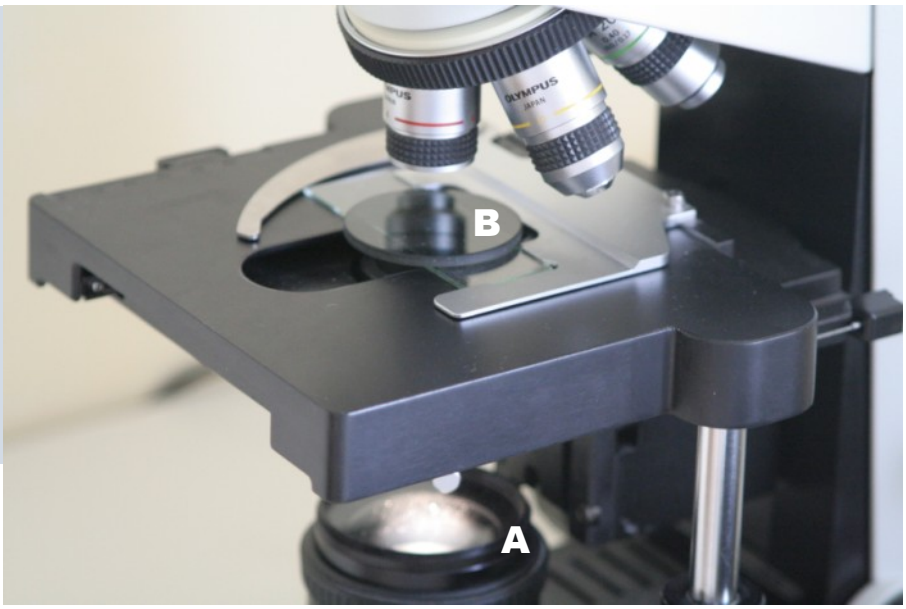
Due to the large amount of liquid, it may take quite some time for the water to evaporate. You can increase the speed of evaporation by placing the slide on a warm (not hot!) plate. If you make the plate too hot, then the Vitamin C may break down and crystals will not form. A smoking slide or a yellow discoloration of the Vitamin C is a clear sign that the temperature was too hot.

If alcohol does offer so many advantages over water, then why bother using water in the first place? Alcohol evaporates much faster than water and crystals therefore form much faster. This may be a disadvantage when doing some time-lapse photography of crystal growth.

As the solvent evaporates, the Vitamin C starts to form a sticky transparent layer on the slide. Crystals start to form in this layer. It is now play time: Try to

Test the polarizing filters if they are of the correct type. Many polarizing filters that can be bought in photography shops are circular polarizing. These will not work. Place the two filters over each other and rotate one of them. The area where they overlap should become dark. Just for the fun of it: stick some transparent adhesive tape on a glass slide and place the slide between the two filters. You should see a color play.

One polarizing filter is placed above the lamp (A), the second one right on top of the specimen slide (B). Cover glasses are not necessary. Rotate the filter above the lamp until the background is black. This gives the highest contrast and the most pleasing results. The setup works only for objectives with a large working distance. It is also possible to sandwich the slide between the two polarizing filters. Without a special filter holder, rotating both filters at the same time is a bit difficult this way. Take care that the filter placed on the slide does not destroy the delicate crystals.



carefully touch the sticky Vitamin C and observe how crystals start to form. The place where you touch the slide should start to form a white, opaque layer. Touching the slide initiates crystal formation, probably also by introducing dust. Dip your finger into some Vitamin C powder and then touch the slide. This should work even better. The crystals on your finger tip act as seeds.

Experiment with different Vitamin C concentrations, solvents and temperature. Alternatively mix some of the Vitamin C with the solvent directly on the slide, but do not dissolve everything. Crystals will then start to grow from the undissolved crystals.

If the Vitamin C concentration is too high, then crystals will also start to form upwards. From my experience, I can say that these look rather colorless and not very interesting.

Polarization microscopy

This is now the fun part. Place one of the two polarizing filters over the light source. Place the second polarizing filter between the slide with the crystals and the objective. Use a low power objective (4x). Then rotate the lower polarizing filter until the background (where there are no crystals) appears black. This is the setting which gives you the highest contrast. Be careful: the polarizing filter changes the focus, and be careful that you do not crash an objective into the filter. Rotate both filters into the same direction and observe how the colors change.

Optical Troubleshooting

It is well possible that the black background (where there are no crystals) is not quite as black as it should be. It can be that there is a slight color drift. The reason is, that the optical elements of the microscope (such as the condenser) change the polarization of the light. The effect is even more pronounced if you place the top filter in front of the eyepieces and not directly above the specimen. In this case, the optical elements of the objective and eyepiece also contribute and start to change the polarization of the light. This brightens up the background even more.

Now why is this? The lenses are not tension-free, and this causes the disturb-

ance. Dedicated polarization microscopes use tension-free objectives, which are, naturally, more expensive.

If you encounter similar problems, then I can offer comfort, for the solution is rather simple: Simply sandwitch the slide with the crystals between two polarization filters. In this case polarized light does not have to pass through the condenser of the microscope. Just take care of the reduced working distance. I did not use this particular setup because the other system also provided acceptable results (and my filters are pretty thick).

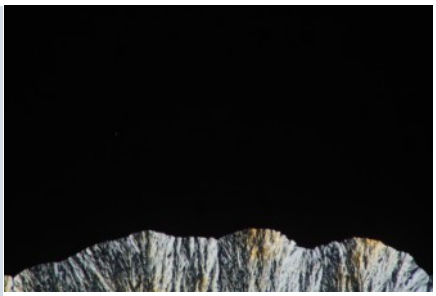
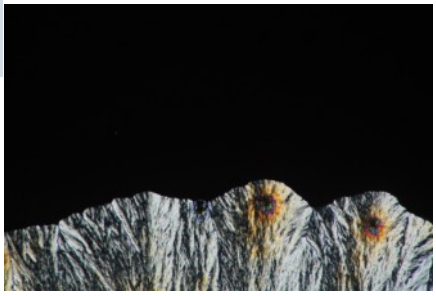
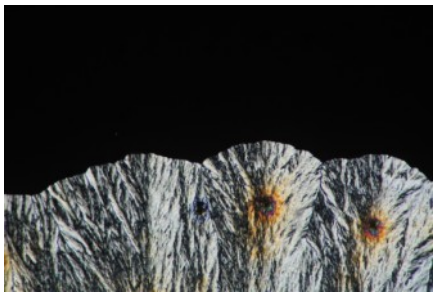
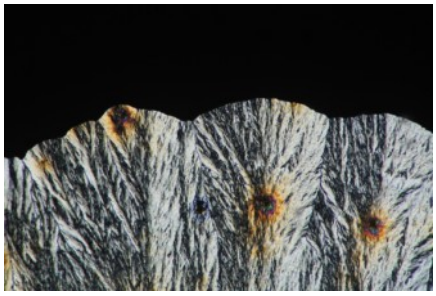
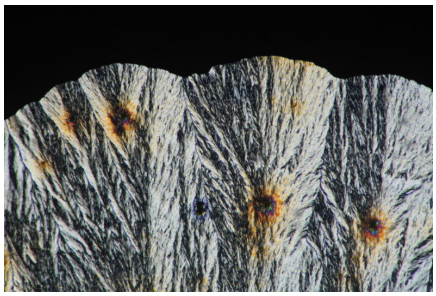
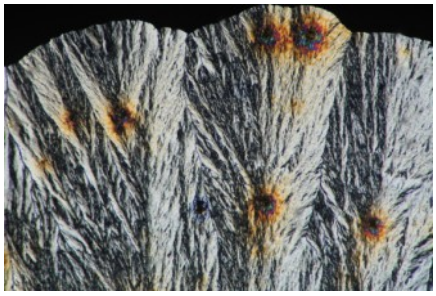
What about constructing a slide-holder with two integrated rotating polarizing filters? This slide holder can then be placed on the stage, clipped into place and be moved by the mechanical stage.

Time Lapse Photography

The process of crystal growth can be captured at regular intervals. Some digital can be set to take images at regular time intervals. Alternatively it it is possible to use software to control the camera to take automatic pictures. Manually pushing the release every few seconds can be a quite painstaking activity, especially if you want to take several hundred individual images to make a time-lapse film. A good compromise would be the use of a cable release. This also eliminates the vibration of the camera when pushing the release.

It is highly advisable to not set the camera to automatic mode when taking time-lapse pictures. Take a picture of a completely formed crystal and adjust the exposure time, based on this crystal. This crystal serves as our reference, and we assume that all other crystals will have the same brightness. Also do not use automatic white balance. Now use the this same exposure time to take all of the time-lapse images of a newly forming crystal. If you set the exposure time and white balance to automatic, then the crystals will have a different brightness. Do not forget that a small crystal on a black background will cause the camera to "think" that the picture is generally too dark. The camera will then overexpose. The background will then be too bright and the crystal itself will be very overexposed. Setting the camera a fixed exosure time for all images will eliminate this problem. Something similar is the case for white balance. The average color composition of the image is different for a large and for a small crystal. The images will shift colors the larger the crystals become, when you use automatic white balance.

On the right, you can see time-lapse photography of crystal formation. The images were taken with a time-delay of a few seconds. Instead of pressing the camera's shutter manually, I used the Linux program gphoto to control the camera and to take the pictures automatically. This way I took several hundred pictures in order to make a time lapse film. The most difficult part is anticipating where crystals will start to form and into which direction they will grow. This is a bit of a guessing game.

Making Permanent Slides

The crystals will not store long, mainly for two reasons. First, they are quite scratch sensitive. Be careful when placing the polarizing filter on top of the slide. The second reason is chemical in nature: The crystals will slowly turn yellow over time. I suppose that this is due to oxidation by the oxygen in the air. The discoloration does not appear to change the visual appearance of the crystals, however. Making a permanent mount will give both physical and chemical protection.

It should be clear, that an aqueous mounting medium (such as glycerin gelatin) is not suitable, as it will dissolve the crystals. I tried two mounting media, clear nail polish and Euparal. I applied the nail polish directly to the crystals and then applied a cover glass. The nail polish showed significant shrinkage, and I can not recommend it. Euparal worked much better. The crystals did not dissolve, but the drying time is significantly longer (several days, depending on thickness).

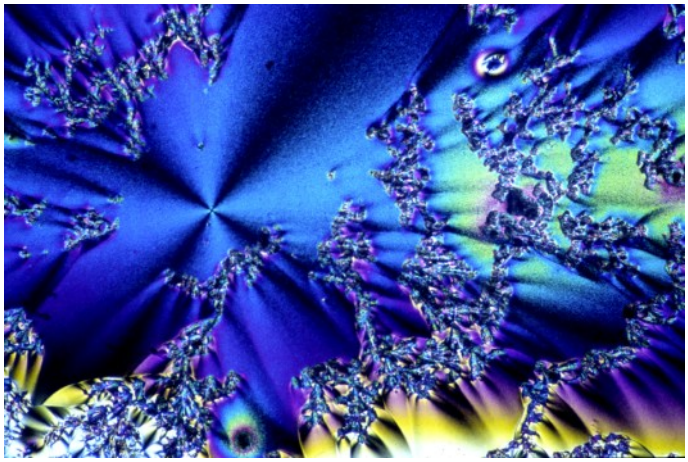
Concluding remarks

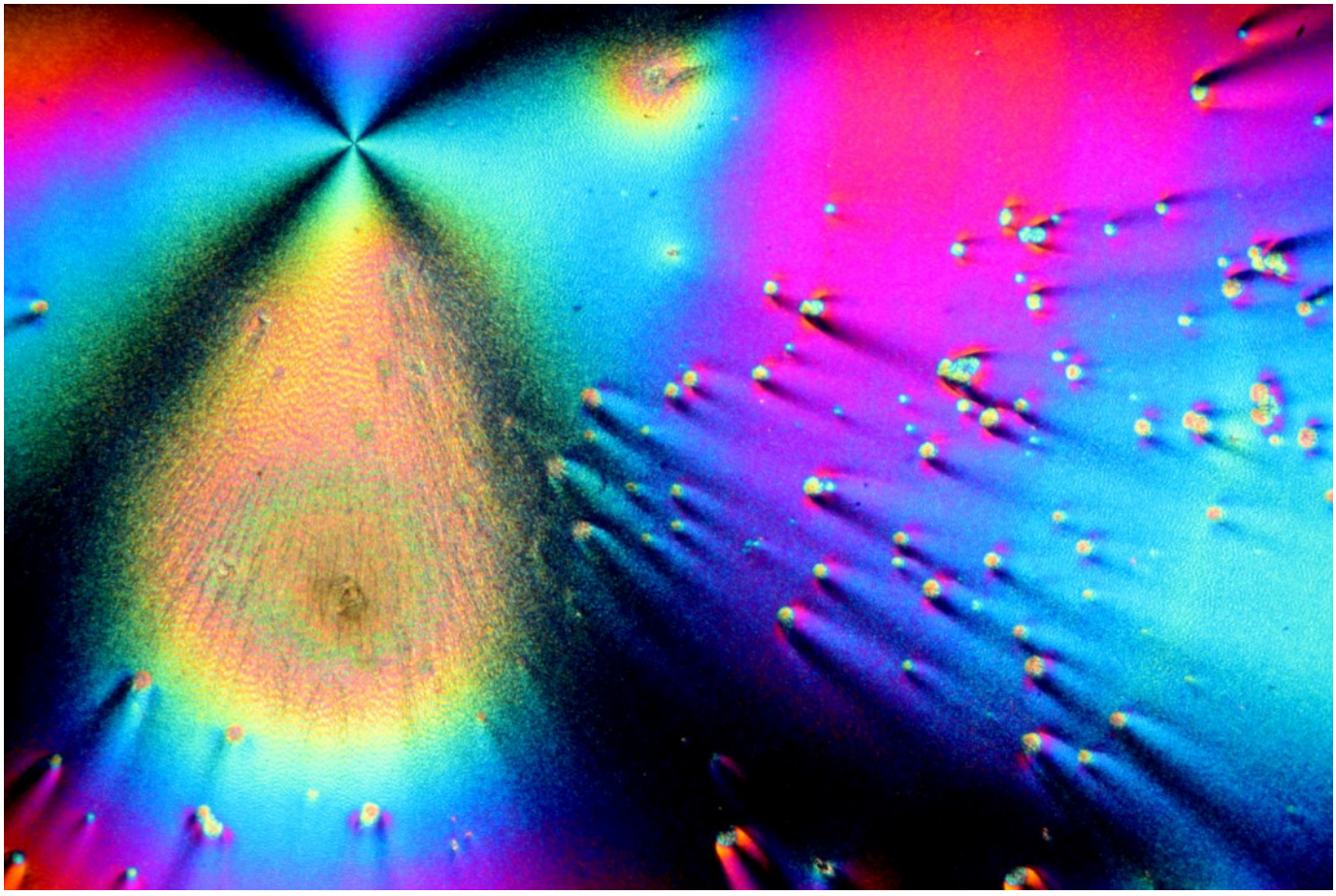
There is not really much to say, other than: “try it!”. Vitamin C is only one of many substances that are suitable for polarized light microscopy. In a future article, I will report on the crystallization of citric acid, which works again a little differently: In this case it is possible to use heat-melting and re-crystallization. ■

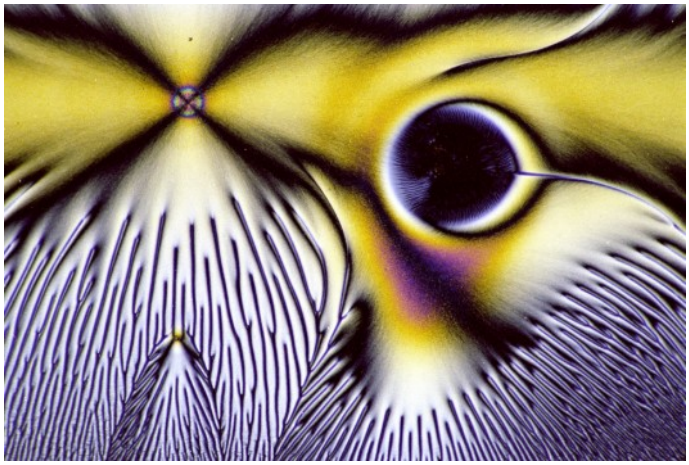
Show your pictures!

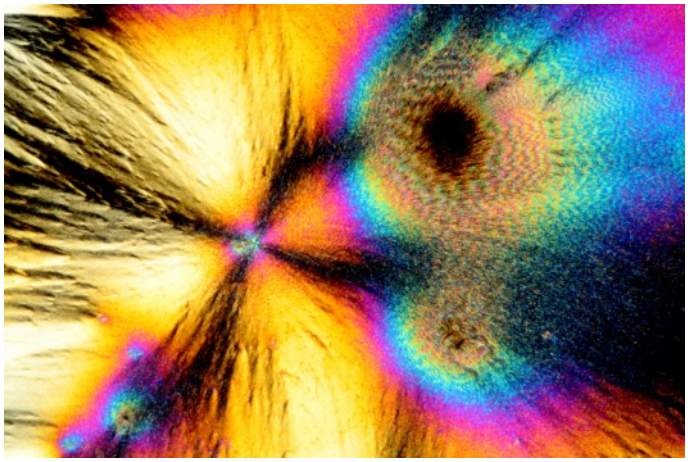
The forum is a good place to show your photographic creations to other people. Registered users can manage the images in their own folder. Alternatively register an account at a photo sharing website (such as Flickr) and publish a link to your pictures in the forum.

Forum URL:
http://microbehunter.com/forum









Afocal Photography:
Attaching a Compact Camera

One does not need expensive equipment to make great photographs.

By Suphot Punnachaiya

The top right picture shows how the microscope, tripod and small digital camera sit together to take the photo of the onion root tip (next page). The setup is simple, I mounted the camera on small tripod. Turn on the camera and zoom the lens to the longest position. The longest lens position may not be the same as maximum zoom, however. This will prevent the camera lens from knocking against the eyepiece when zooming back & forth later.

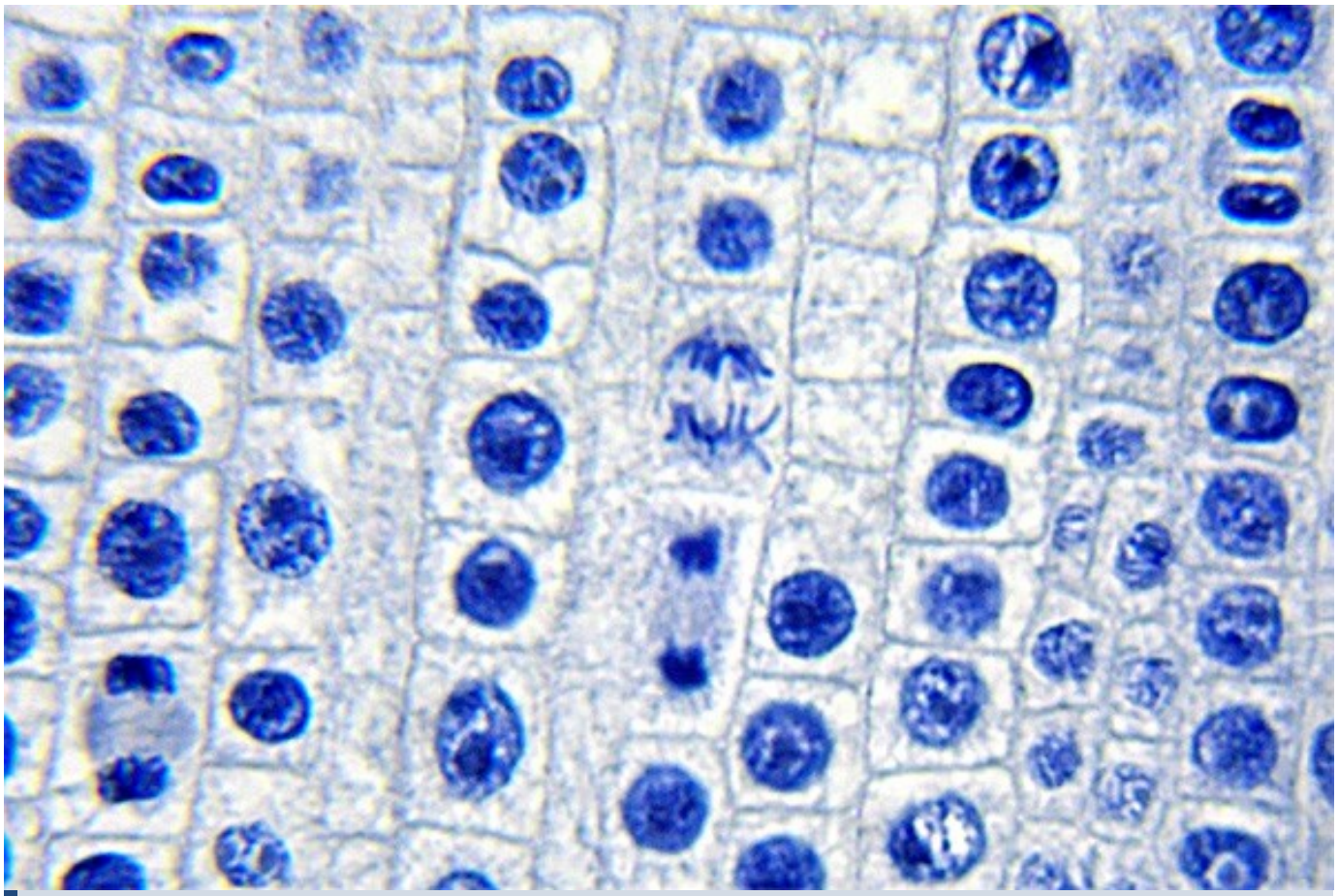
Then align the camera to microscope eyepiece by adjust the tripod up/down, left/right and back & forth until the LCD on camera shows the circle of eyepiece in the center area. The final step is to zoom the camera a little bit until the image from the eyepiece fills the whole area of LCD without vignetting.

From the LCD screen, fine focus the microscope until you get the sharpest image. Set the camera to self-timer mode for delay 2 seconds and turn off the flash. Then press the shutter and move the hand away from the camera to prevent camera shake.

The bottom right picture shows a close-up view of camera and microscope eyepiece. You can see that they are close together, but not in contact.. Afocal photography uses a camera looking into the eyepiece in the same way as our own eyes. So we should position the camera as same as our eye position when we looking into the eyepiece. For most microscope eyepieces, the 1 – 2 mm distance is a good set up to allow eyepiece project the magnified

The complete set-up. The tripod is adjusted so that the camera's optics can be fully extended. The camera has to be in line with the eyepiece.

The camera to eyepiece distance depends on the eye relief of the eyepiece. Longer eye-relief eyepieces need a larger distance. An incorrect distance can cause vignetting.



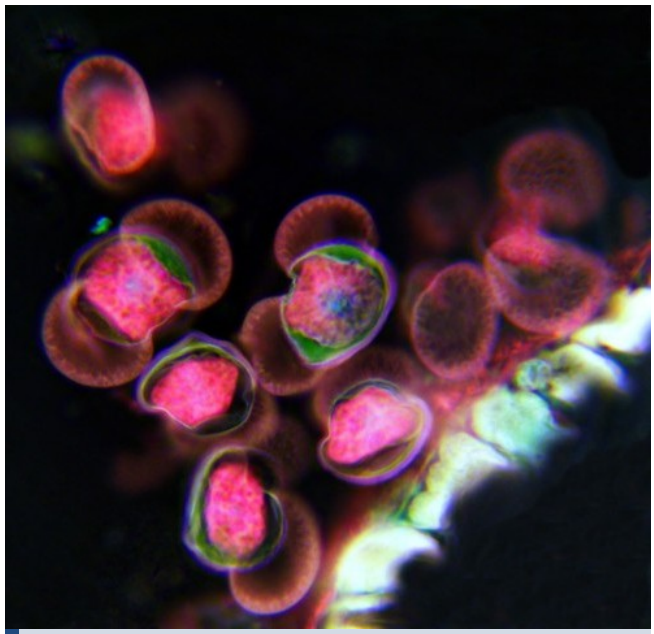
Onion root tip photographed using the afocal method. Camera at the eyepiece, using a tripod. 40X Objective, 10X Eyepiece, normal illumination. Camera: Casio EX-Z 350, zoom 2.5X, speed: ½ Sec, F 5.9, ISO 200, auto white balance. Color correction, resizing and unsharp masking by Photoshop. In the center, two dividing onion cells.

image into the camera properly. So in my setup, there is no physical connection between camera and microscope eyepiece.

If you set the camera too far from the eyepiece, the camera will see a small circle of the magnified image from the eyepiece. This causes much vignetting. If the camera is too close (in direct contact between camera and eyepiece, for example), then in some cases the magnified image from the eyepiece can not project properly into the camera. You may see a dark area in the camera LCD or uneven illumination of the final image.

Some microscopes have long eye relief eyepieces, These types allow the user to wear glasses while looking into the microscope. If you use this kind of eyepiece, then the distance between camera and eyepiece should be 3 – 5 mm or more. Otherwise the final image will be blacked out or no image can be see on the camera LCD at all.

There is no need to worry about external light leaking into the camera lens: the camera lens sits very closely to the eyepiece. There will be very small chance that external light will reflect from the eyepiece or shine directly into the camera lens. But if external light does shine into the system, then turn off the room light or use a hand to block the light. A completely dark room is not needed by this setup, but bright external lights should be turned off, nevertheless. ■



Male strobile permanenet slide; afocal photograph from the eyepiece; 40X objective, 10X eyepiece, F 5.4; speed 1/2 Second, ISO 100. Crop, auto levels, negative filter, unsharp masking and a color saturation of 20%.

Cheapo Photo:
Photography through the eyepiece

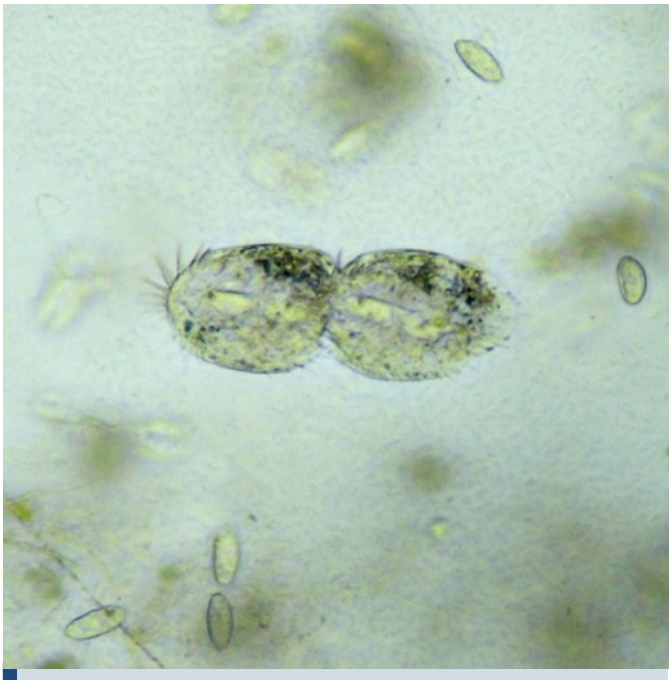
Discover the miniature world! Here's a motivating text to start the fascinating hobby of microscopy.

By Raymund John Ang

After a day's work, we all need time to rest and engage in recreation. Some find cooking or going to the gym leisurely, while others go fishing or skating as a pastime. And quite a number of individuals are into science-related hobbies as well. Popular among enthusiasts are astronomy, bird watching and microscopy. The main denominator for these activities is the use of optical devices to see objects afar or examine microscopic specimens invisible to the unaided eye, and the possibility of image capture using digital photography.

For those inclined towards outdoor activities, they may find bird watching a fascinating field. Hobbyists can start by observing different species of birds from their own backyard, and then move on to joining field trips with other bird enthusiasts. For those who gain satisfaction from observing the heavens, they may wish to take up astronomy as a hobby. There are a lot of objects to observe at night using binoculars or telescopes, or even with the naked eye alone. And during daytime, you can also do solar observations and, in rare instances, watch a solar eclipse unfold. Some acquire sophisticated equipment enabling them to take quality images of celestial objects. And for advanced amateur astronomers, they have the chance to work with their professional counterpart in research projects aimed at gathering scientific data through photometry or astrometry.

And there are those who spend time looking at microscopic objects too small to be seen with the naked eye. Amateur microscopists as we call them. They may or may not have a formal academic training in microscopy, but their engagement with microscopes is for personal enjoyment and satisfaction. One advantage of microscopy is that it is not affected by the weather outside. Rain or snow, one can still appreciate nature's beauty through the oculars, though the kind and quality of samples can be affected by changes in the environment. A number of microscopy societies have also established to promote the science and hobby of microscopy, and encourage camaraderie among practitioners.



Oxytricha-like organism undergoing cellular division (125x).

Vorticella at 125x magnification.

Afocal Photography

A popular activity among microscopists is photomicrography or capturing images through a light microscope. Before the age of digital imagers, hobbyists had a hard time obtaining quality photos mainly due to the inflexibility of film emulsions when it comes to image acquisition and processing, and the resources needed to beef up accessories. Nowadays, it is easy for a novice to handhold a digital point-and-shoot camera onto the eyepiece of a microscope and to obtain images which could rival professional photos of the past. Things have greatly improved since technological advancement. And I am not saying this in reference to the field of microscopy alone. Even in astronomy, commercial CCD imagers have allowed amateur astronomers to discover comets, supernovae, and changes in the atmosphere of other planets.

Ready your microscope and have the specimen in focus. Place your camera onto the oculars. And, click. You now have a digitized image of the specimen. That simply sums up what you need to do to get started with photomicrography. But do not expect your first few photos to be excellent. It needs time and mastery to perfect your skills in afocal photomicrography. Afocal photomicrography simply means placing a camera, usually a compact point-and-shoot, on the eyepiece of a microscope system, and taking stills or videos directly while holding the camera or using an adapter or tripod to minimize shaking. This is the simplest and cheapest way of capturing what you see with your microscope. Sketching might also be a fun and interesting activity. But there is nothing like a digital image on the computer monitor or in print.

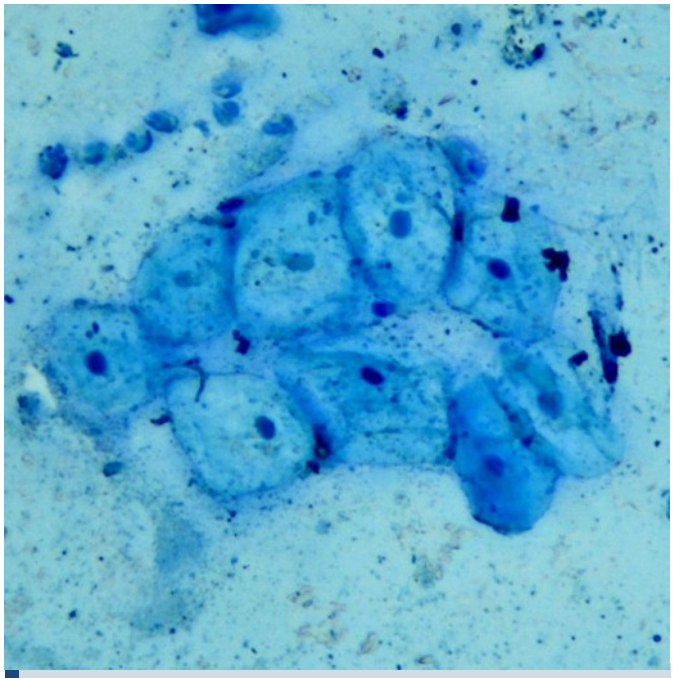
Remember that the best microscopy setup is the one that gets used most often.

Below are some images I took using an afocal photography setup: Zeiss Standard Junior K and a Canon PowerShot SD400.

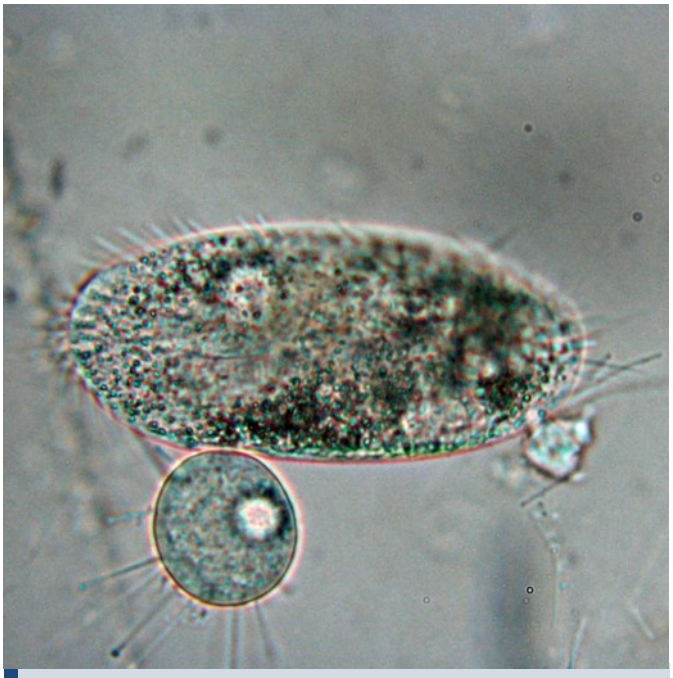
This is not to say that afocal photomicrography is altogether cheap. If you want excellent photos, you must also have a quality microscope and camera. Top of the line microscopes can cost hundreds or even thousands of dollars, excluding taxes and shipping. Same goes with high end cameras. But if you are patient enough or if you really know what you are looking for, you might be able to get quality instruments at a very reasonable price. My advice is to connect with fellow microscope hobbyists or professionals and ask their opinion and recommendation as to which system is best suited for your needs.

Though images taken afocally might not be as good as when using other techniques, there are ways to improve your setup (*http://www.barrie-tao.com/afocal.html*). First, use an eyepiece with long eye relief. Second, have the camera lens positioned as close to the microscope eyepiece as possible. Third, set the camera to macro mode instead of infinity. Fourth, adjust the camera's optical zoom to minimize vignetting. Fifth, use lenses which are designed specifically for your digital camera. Lastly, the camera lens should have a focal length longer than that of the eyepiece.

Remember that the best microscopy setup is the one that gets used most often. It is not the most expensive one or the one which has the most accessories. So for those planning for their first microscope, these instruments are not just for collection. We should give credit to their fine optics by using them, either visually or in taking pictures. ■



Cheek cells stained with methylene blue (125x).



Suctorian feeding on a ciliate (500x).

The Novitiate's Odyssey

Episode One: How I got involved in microscopy for all the wrong reasons

This article was originally published in the Spetember 2009 issue of Micscape Magazine and is now republished here with the permission of the author and of Micscape Magazine.

The adventures of a beginning microscopist: On e-Bay treasures and more.

By G. Joseph Wilhelm

It was a dark and stormy night. Actually it was just dark and my computer screen was flashing a congratulatory announcement from that grand bastion of capitalism known as eBay. I now had one year tenure as a buyer and indeed, it was this very organization that started me on this trek down the path to the miniscule, and this article.

I have been an ardent habitué of Micscape Magazine since first discovery some time ago. The hours of pleasure derived from the current and past issues has been immeasurable, the content scholarly and informative but I

Fig. 1 - Bausch & Lomb BH "Jug Handle" stand disassembled for restoration. The SN indicates 1907 as the year of manufacture. The fact that it has no eyepiece, the eyepiece tube and coarse focus pinion axle are both bent, there is no mirror, an irreparably damaged stage iris and bent nosepiece turret are not insurmountable obstacles to the application I now have planned for this apparatus. "Stay tuned" as they say. Price with shipping was $16.50.

Fig. 2 - Behind curtain number two we have a circa 1912 Spencer Lens Co. stand. Has mirror fork and condenser holder but no mirror or condenser, coarse focus pinion axle slightly bent and the arm to stage connection is loose. This is close to the all brass style I was originally interested in. I will be attempting to adapt a condenser and mirror to complete this scope. Total with shipping $57.00.

have recently come to realize that a very unique and important perspective of microscopy was missing from this eminently distinguished publication… mine. Egad I thought! A subject without my opinion? Tut-tut my good man, unimaginable! But what possible aspect of microscopy was I qualified to expound upon? Why, ignorance of course. I certainly had adequate competence in that respect. The idea for an article of, by and for the beginner, written about my BM era (Before Micscape) began to take form.

Now, after some time of leisurely reflection here in the tropics with pen in hand, tongue in cheek and a tall frosty mug of rum, I humbly submit this discourse which upon conclusion may give Micscape Magazine pause to reconsider their policy of soliciting literary contributions from just "anyone". This is the journey of a true beginner, and while my credentials in Microscopy are non-existent, (none, zip, nada), it does enjoin me with such luxury which allows me to speak with a sanguine authoritativeness without angst over deleterious repercussions to my also non-extant reputation.

It has been my experience that in order for an intended audience such as yourselves to fully digest the pearls of observational wisdom I am about to cast, I find it necessary to properly place them in context by dispensing a short biography so you know just whom and what you are dealing with. So please allow me to beg your kind indulgence for a moment.

I am G. Joseph Wilhelm, and although I will soon be accepting felicitations on the anniversary of my sexagenarian nativity, I still find myself using words like "keen", "neat", "nifty" and "awesome", (thankfully falling short of voicing those horrid Californiaisms such as "dude" and "gnarly"). Speaking of words, "eccentric" has been used as an adjective and noun to describe me, also eclectic, esoteric, amiable, irreverent, daft and having "bats in my belfry".

I reside in the Florida Keys quite close to Key West. That means my wife's hairdresser is straight, Joe the plumber is gay, we elected a local bar owner as our Mayor and most people can't remember… is pot illegal? But that is not to say we are entirely devoid of intelligentsia. There is the occasional oasis of academia such as the Mote Marine Laboratory, the NOAA facility, the marine biology department of the community college and Sloppy Joe's Bar which is where the aforementioned facilities personnel meet to quaff their favorite libation and engage in what I am sure is cerebrally stimulating biological scholastic conversation. (How 'bout dem Dolphins/Marlins/Gators?)

Gainful employment is realized as a successful civilian Explosives Safety Instructor for the Naval Air Warfare Center Key West Detachment. I say "successful" because unlike many of the grizzled twenty year veteran Weapons and Gunner Master Chief in my

Fig. 3 - This is a Bausch & Lomb that I cannot find a Serial Number on. There is a patent date of 1915 but I believe it to be manufactured in the 1920s. It is very similar to the Spencer model 19 and model 44 of that era but this is only a guess. It is complete with correct objectives but I am not sure about the mechanical stage, this particular detachable one seems just a little out of place. The base inclination joint is seized but that should not be difficult to correct. Coarse and fine focus is very smooth with no backlash. Best scope of the lot. Final total price was $93.00.

peer group I not only can hear the waitress ask what I would like to drink, I also do not require both hands to display enough digits to order five beers.

Finally, I am a collector of antique things that have a mechanical purpose. In my home there are 38 separate assemblages of antique typewriters, sewing machines, WWI firearms, brass padlocks, straight razors, time pieces, telephones, telegraphs, apothecary, tobacco pipes etc. etc… you get the picture. In the optics collection section are telescopes, binoculars, kaleidoscopes, spectacles and cameras. Obviously lacking (yes, here comes the anticipated segue) was a representative example of an antique microscope.

There you have it.

To paraphrase the late great custom motorcycle builder, Indian Larry, the main appreciation (for myself) of the articles in this aggregation is of their pronounced visual "mechanicalness". I find working mechanical mechanisms are a thing of beauty, much more so with those of the late 19th and early 20th centuries. They went beyond utility and incorporated the Victorian flair, the Art Nouveau or Art Deco design, a delight to ponder and an observable testimonial to engineering and manufacturing preciseness. Such was my desire to have the polished brass components and the intricate assembly of an antique microscope to display in my optics collection.

So the quest began.

I had a mental picture of the Sherlock Holmes scopes that were on the scientific apparatus table found in every Basil Rathbone film but a cursory investigation proved the 19th century Becks, Watsons and others that fit my mind's image were in the thousands of dollars. Pursuance of a more practicable solution was in order. While engaged in this endeavor I soon became awash in unfamiliar terms, descriptions and information. By the end of the month I had amassed a mountain of micro technological erudition.

It would not be unfair to state that at the onset of this quest it would be difficult to find an individual less obtuse of the microscopic discipline than moi. It was at this juncture that I had an epiphany into a casual remark said by one of my redneck, snuff dippin', four-wheelin', right wing, social counterpoint, fishin' buddy associates (his name is Chauncey), and I quote; "Knowledge ain't worth #!&% iffin yew don't unnerstan' it".

How true.

The authors of the articles and essays I have absorbed, even those intended for beginners, seemed to assume I had some sort of prerequisite level of education and comprehension of the subject. This was the same error of judgment my college chemistry professor made. My collective experience only consisted of a Christmas present 'Tasco' or 'Perfect' (I cannot recall) brand microscope kit at the age of nine. I do remember it had a coarse and fine focus, mechanical stage, some prepared slides and magnification up to 1200X.

Fig. 4 - Here is another Spencer, a Model 44MH that dates to 1932. The latest Spencer catalog I have is dated 1929 and was identified from that. It's missing the mirror; the rack gear is the only part of the permanently mounted mechanical stage that is left. It has a Leitz low power objective that is definitely not original equipment but over all not a bad piece of equipment for $47.00 delivered.

But the combination of being nine, having the Alfonso Bedoya mindset of "Instructions? We don't need no stinking instructions" and getting no satisfaction trying to view whole dead birds under 1200X led to an early abdication of interest. However, never having been one to let my ignorance stand in the way of my education, I have plodded forth with my habitual "intellectual abandon". Thus my forward progress into enlightenment has been punctuated with "fits and spurts of unnerstanin" as Chauncey would so eloquently pronounce.

I crossed over to the dark side while at work, sharing my lamentable situation over obtaining a microscope.

Up to this point I had never actively pursued procurement of additions to my collections. They had all been obtained by purchases of opportunity, those glorious moments of good fortune that appear from nowhere to afford one with a desirable object at a good price. Alas, no such circumstance seemed to be manifesting itself and after hearing me bemoan my situation, the Devil, in the guise of a co-worker, slithered up next to me and hissed in my ear 'eeeeeebaaaaay". I knew nothing about this nefarious tool of the underworld and wasn't to find out until later that it was like a snake, you had to handle it carefully or it would bite you. I dutifully went to the eBay site and typed in "Antique Microscope". ZOUNDS! I was assailed by 173 "hits" in that category and now, irretrievably ensnared, I spent the next few days in what I believed to be fortuitous bliss perusing the proffered possibilities.

I narrowed down the list to ten microscopes and being enveloped with an impulsive impatience I applied my usual modus operandi (see Alfonso Bedoya reference above) and bid on all ten at $100 each, figuring when I won one I could retract all my other bids.

My rude awakening came immediately after being appraised that unless I was deceased, if I withdrew any bids, eBay would tar and feather me, ban me from society for life and all my good deeds, past and future would be expunged from the book of life.

Ah, the trepidation I experienced over the next week was tremendous, the possibility of paying out a thousand dollars notwithstanding, I was aghast at the potentially devastating dent it would make in my rum budget.

Now bear in mind also, that I had absolutely no idea of what I would be getting other than what was depicted in the pictures. Most of the descriptions started with "I don't know much about these..." and I was not educated enough at this time to put forth pertinent questions (I believed that 'parfocal' meant intense concentration on a golf game). I have a small shop lathe and milling machine plus experience in curatorial care and restoration so any minor mechanical flaws listed did not deter me. I bid simply on what the microscope looked like and whether or not it would be an apropos accoutrement to my mini museum.

Fig. 5 - Spencer described this design as a "Binocular dissecting microscope" in their 1929 catalog. This 1944 version has the eyepieces tilted back at 45 degrees unlike the straight tubes of earlier models and today they are referred to as stereo microscopes. It came with a really nifty miniature horseshoe base double-jointed alligator clip specimen holder thingy, and a light. There is a dovetail slot underneath the base/stage that may have allowed it to be attached to another piece of apparatus. It came with 10X eyepieces and a 1X and 3X objectives. If I add 15X and 20X eyepieces it will have a really nice range of low power magnification. $46.00

After considerable genuflecting between the computer and the ATM, the fates smiled benevolently and allowed me to extricate myself from this situation for a mere $287.00 including shipping. A pittance! I had won the bidding on six microscopes.

So… what did I receive? A resounding education. (For tis upon the mistakes of learned men that the foundation of future edification can be built). I was now the chagrined owner of three Spencer, two Bausch & Lomb and one San Francisco Technical Institute microscopes. After a few months of research here is what I found I have. Photos and descriptions follow with a special thanks to the P. S. Neeley site for help in identification:

The tomfoolery related above took place about a year ago and in that time I have become quite familiar with the mechanical aspects of microscopes and I have done a few repairs, cleaning etc. and they now reside in one of my display cases looking very noble and microscopicalish. I thought this was the end of my re-introduction to microscopy but nay; all it took was an audacious suggestion by my wife that I should consider looking through them instead of at them. Hmmm…what a concept.

The net result was a rekindling of interest that may fuel future prognostications and additional episodes from myself. A myriad of topics comes to mind that I will address commentary to at a later date. But for now, as a guest who may have stayed too long, I feel I must depart, but conclude with the following assertions, assumptions, pronouncements and advice for the truly clueless beginner (such as myself) in order to justify my original intent to this article for the microscopy novice.

For the beginner:

Step (1) - Research.
Step (2) - Read, understand and assemble a consensus.
Step (3) - Add your personal settings.
Step (4) - Apply liberally any measure of common sense you have been blessed with.
Step (5) - If any problems with steps (2 thru 4) repeat step (1) as much as necessary.

Only then should you feel prepared to take action i.e. spend money.

This simple formula can be applied to any aspect of microscopy. Here is an example of how it would work for "I want to buy a microscope".
Step (1) - Find a minimum of four articles on how to buy a microscope, more is better.
Step (2) - After reading; note the points they agree on. If there are terms or information you don't understand then revert to Step (1) to clarify them and back to step until you have such a grasp and understanding as can be reasonably expected.
Step (3) - Budget, level of expertise, intended use, spousal approval etc. are some of the considerations here.

Fig. 6 - The ugly duckling. I have absolutely no idea of why I bid on this instrument. It's an off brand and not an antique. It only has one magnification and I am not sure what that is, there are no markings on the objectives and only 10X on the eyepieces. The oculars fit a 30mm tube, which is too big to use on the other scopes. It's ergonomically challenged; the viewing is straight down and nothing in the mechanism tilts. It accounts for the remaining $27.50

Step (4) - This is the most difficult. Ask yourself; Have I been honest with myself in the previous steps? Do I have recourse if I am not happy with my purchase? Is this what I need or just what I want?

OK class, since buying a microscope is one of the first expenditures a beginner will make, here is your homework assignment using the above method. Remember I am teaching you to fish, not giving you the fish.

Things you need to know:

1. The difference between Stereo, Binocular and Monocular microscopes.
2. Tube length; 160mm, 170mm and infinity corrected.
3. Oculars (eyepieces) 10X and under to 20X and best applications.
4. Objective lens types; achromat, plan achromat, and apochromat, dry and oil immersion.
5. Objective lens parfocal length, both DIN and JIS.
6. Sub stage condensers and their function
7. Resolution and contrast.

This will give you a good foundation to work from and with which to make informed decisions and questions. (Lest I insult the intelligence of the more advanced and educated personages that, by happenstance, have navigated the essay thus far, let me state this article was originally intended for my young cousin heir to my microscopes. He is a neophyte to microscopy.)

It is also important to know that neither myself nor other individuals dispensing information to the novice should ever presume to be the final authority on the subject being discussed.

I found a remarkable video, the best introduction microscopy I have ever seen at http://www.archive.org/details/imaging_a_hidden_world Bruce Russell's video brings to life aspects of microscopy that can never be fully conveyed with just the printed word.

So in summary, the important lessons we have learned here are: Don't run with scissors and never eat on an empty stomach, it will ruin your appetite.

The curtain is closing at last, and I my friends, must take leave to mount my steed, poise my lance of inquisitiveness and charge forth to pierce those ramparts resisting entry into those repositories of knowledge microscopic. Until next time, I bid you adieu.

Comments, accolades, constructive criticisms and minor discontent can be addressed to me. Insults, slurs, derogatory remarks and other verbal expressions of malcontent can also be addressed to me, as I am an equal opportunity proponent. ■

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Centrifuging Protozoa

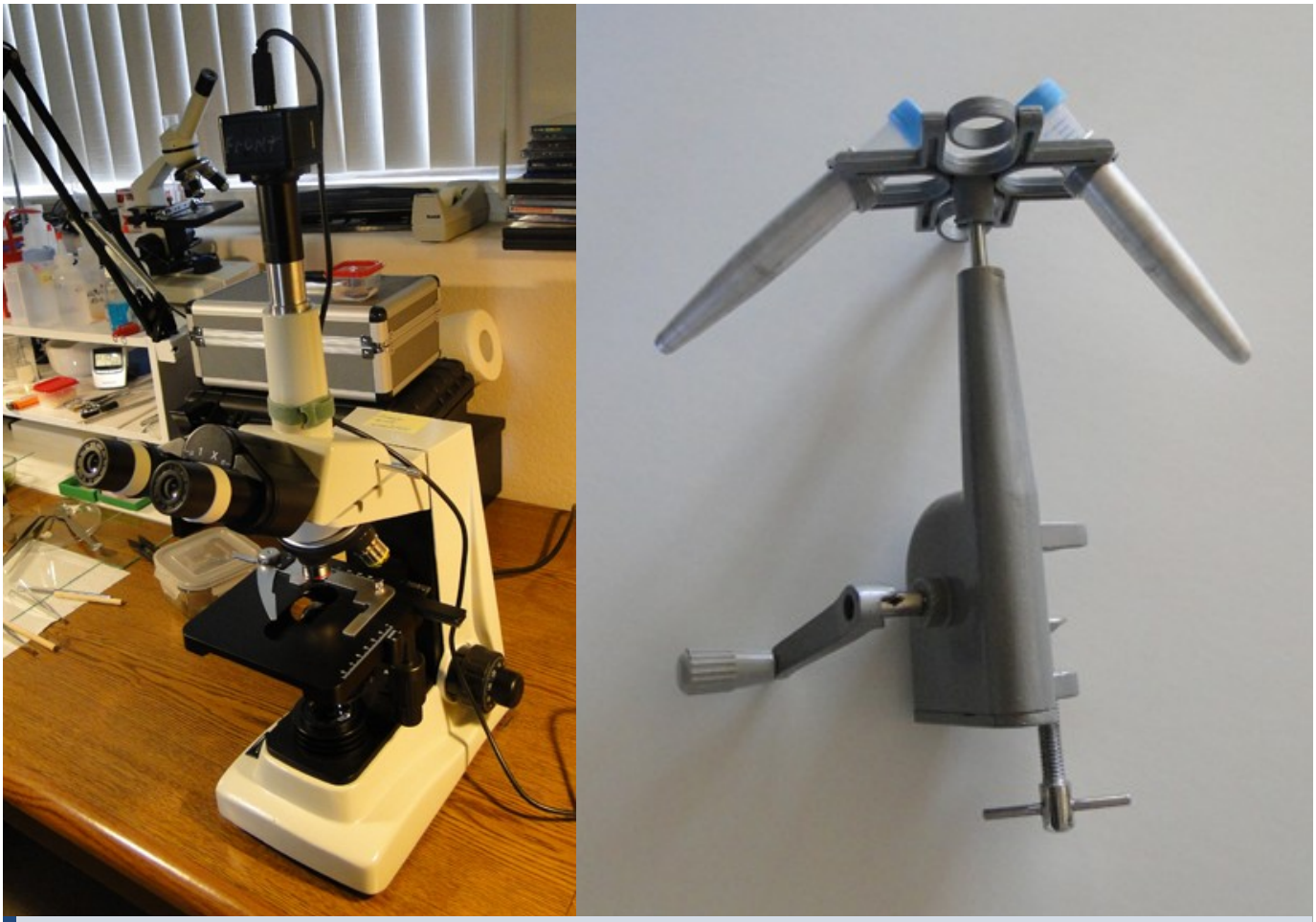
The concentration of protozoa in free water is often insufficiently high for convenient observation. The solution: a hand centrifuge, which concentrates the organisms.

By Lance Carter

I have been an amateur microscope enthusiast for years, and enjoy observing just about anything that will fit under my objectives. During the winter months I maintain several water samples from ponds and streams near my home. Of course there are many fresh water protozoa in the mud and debris at the bottom, but trying to capture free swimming protozoa and rotifers from the water has always been a hit and miss situation. I recently purchased a hand crank centrifuge in which I placed about 10 ml of water and spun for a minute or so. My initial concern was the condition of the protozoa after centrifuging; however, upon observing the concentration in the bottom I found them happily swimming around as if nothing had happened. Among the protozoa were rotifers, amoeba and euglena specimens, also in fine condition.

Another procedure I have tried was to place plants and debris in a large test tube and give them a gentle shake to release the protozoa. After letting the debris settle for a few minutes I obtain a water sample for centrifuging. This has proven very productive as well and avoids the problem of grit and debris between the slide and slip cover.

This method works equally well with surface floating fauna. After col-



Microscope and centrifuge — two components of a home-lab that complement each other. The hand-centrifuge is able to hold four tubes, only two are shown in the picture. Each plastic tube is held by a metal cylinder. The tubes will swing into a horizontal position.

Among the protozoa were rotifers, amoeba and euglena specimens, also in fine condition.

lecting a good sized sample in a beaker with water from the habitat and vigorously stirring a few ml of the water can be centrifuged.

Locating a centrifuge for a home lab and amateur hobby use was a daunting task. Researching the many internet sources for centrifuges proved discouraging. While there were hundreds of fine centrifuges available, I was reluctant to spend several hundred dollars for a piece of equipment that may or may not accomplish my goals. After nearly abandoning the search I finally came across the, "online science mall," and a hand driven centrifuge. It met my requirements:

1. moderately priced
2. could process small samples; (four 15ml tubes)
3. simple functionality
4. small footprint in an already cramped home lab

I was also pleasantly surprised to find their personnel very accommodating while providing information to a neophyte such as myself.

I would be interested if anyone else has used a centrifuge for collecting samples of water specimens. Also, what might be the best RPM and duration, as well as any thoughts on other methods for using this equipment? ■

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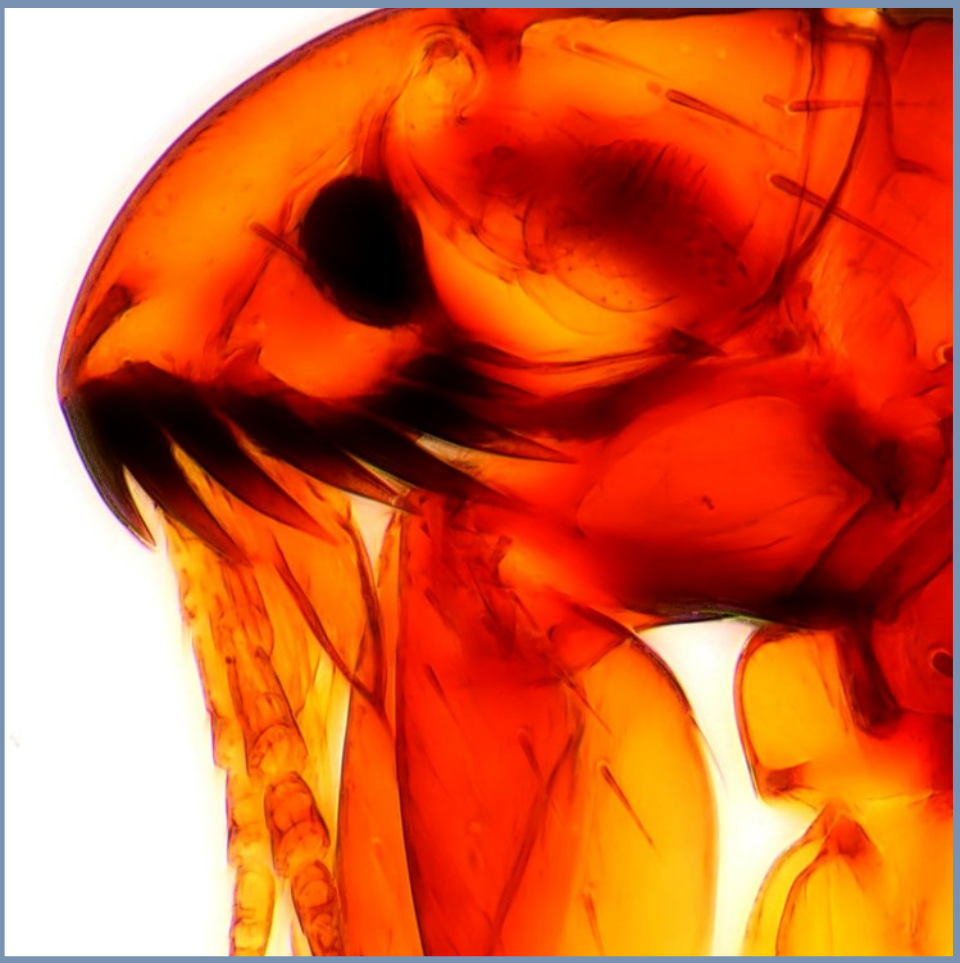
The hand centrifuge can be clamped to a table. Running the centrifuge requires sufficient space. You need spece for operating the crank and for the rotating tubes, whcih swing into a horizontal position. Make sure that both tubes have the same mass, otherwise the system may start to vibrate. This also increases the wear on the gearing system and may possibly also un-rattle the whole system from the table.

The pond water samples are stored in flat containers to allow sufficient oxygen to diffuse into the water. Most of the organisms accumulate either at the suface of the water (where there is oxygen) or in the sediment. Observing water directly may not yield a sufficiently high concentration of protozoa.

A centrifuge is a piece of equipment, generally driven by an electric motor (some older models were spun by hand), that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal acceleration causes more dense substances to separate out along the radial direction (the bottom of the tube). By the same token, lighter objects will tend to move to the top (of the tube; in the rotating picture, move to the centre).

Reference:
http://en.wikipedia.org/wiki/Centrifuge



What‘s this? Answer on page 3.