Microbe Hunter

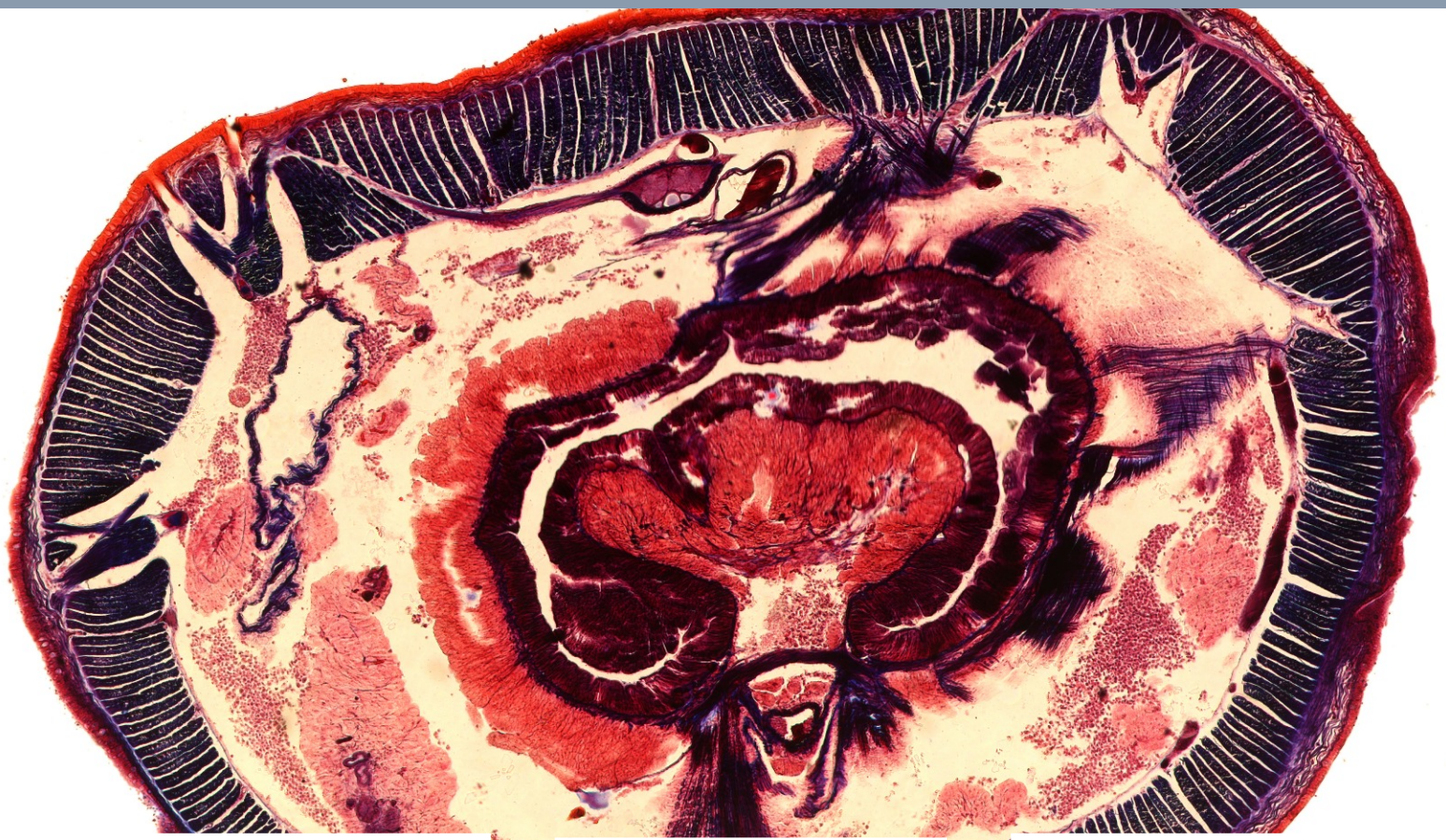
Microscopy Magazine

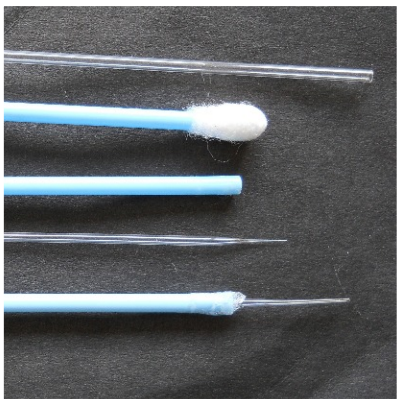
ISSN 2220-4962 (Print)
ISSN 2220-4970 (Online)

Volume 4, Number 3
March 2014

The Magazine for the Enthusiast Microscopist

http://www.microbehunter.com





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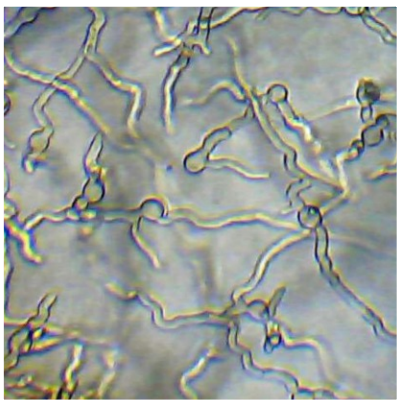

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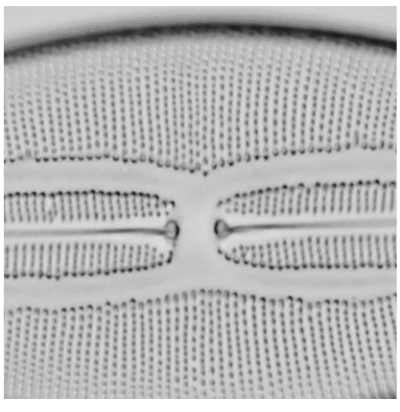

Effect of the condenser

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

Volume 4, Number 3, March 2014 (revision 2)

ISSN 2220-4962 (Print)
ISSN 2220-4970 (Online)

Download: Microbehunter Microscopy Magazine can be downloaded from:
http://www.microbehunter.com

Print version: The printed version can be ordered at: http://microbehunter.magcloud.com

Publisher and editor: Oliver Kim, Ziegeleistr. 10-3, A-4490 St.Florian, Austria
Email: editor@microbehunter.com
Web: http://www.microbehunter.com
Tel.: +43 680 2115051

Editorial: Article and image submissions are welcome and should be sent to: editor@microbehunter.com.
For submission guidelines, consult the website at: http://www.microbehunter.com/submission

Front Cover: Earth worm (Oliver Kim)
Left image: Roland Luts
Middle image: Matej Pašák
Right image: Rashid Nassar

Back cover: human hair

Trypanosoma brucei gambiense

***Trypanosoma brucei gambiense* is a blood parasite causing the sleeping sickness.**

By Oliver Kim

Trypanosoma brucei is a single celled, eukaryotic parasite, which causes the sleeping sickness in humans and the disease Nagana in cattle. The parasite is spread over insects, particularly the tsetse fly. The organism was first discovered by the doctor David Bruce (1855-1931), who could also show its way of transmission. Three different species were originally described, these were all combined into one species in 1972, given the name *Trypanosoma brucei*, in honor of its discoverer. The types were then reclassified to a subspecies level. These can not be distinguished from each other microscopically, however.

The parasite is transferred to the host animal when an infected tsetse fly bites a host animal to feed on blood. *Trypanosoma* then reproduces outside of a cell. This is much unlike *Plasmodium*, which causes Malaria, which invades the red blood cells. The parasite cells reproduce by dividing longitudinally in the tissue of the host and then move to the lymphatic system and the blood, were they continue to reproduce. Figures 1 and 2 show numerous parasites (purple) surrounded by red blood cells. The parasite can also attack the nervous system, which then causes the symptoms of the sleeping sickness.

The organism is able to hide from the immune system by periodically changing its surface antigens. There are not many medications available and all of them have side-effects. In that sense treatment options seem to be limited.

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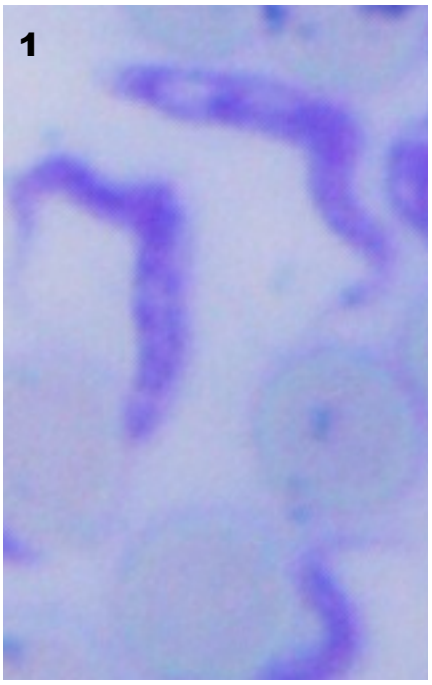
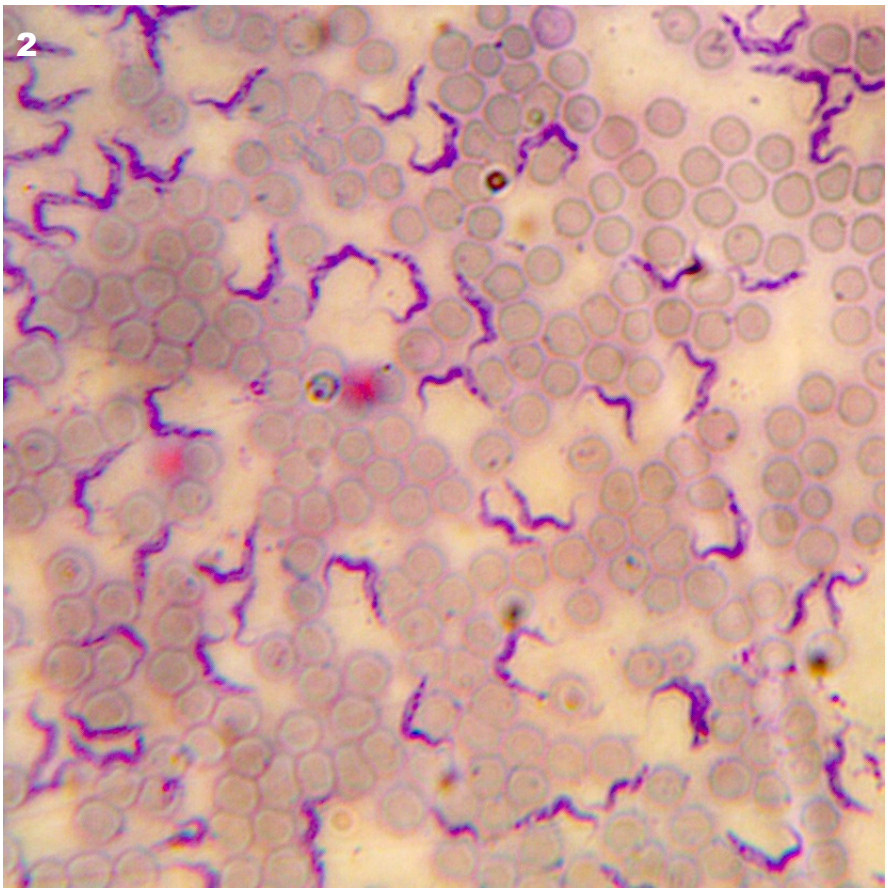




Figures 1 and 2: Commercially prepared slide. The parasite are the long, dark purple cells between the red blood cells.

Effects on the Image of Microscope Condenser Adjustment

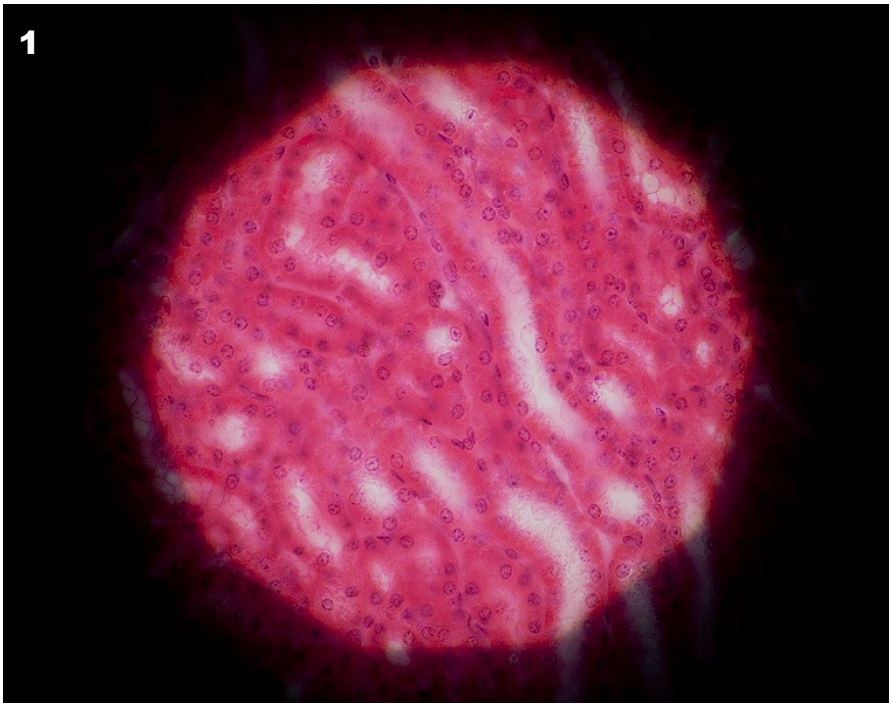
It is necessary to correctly adjust the condenser for obtaining the best image quality. The setting of the condenser diaphragm as well as raising and lowering the condenser can dramatically influence the image.

By Rashid Nassar

In a transmitted light microscope, the condenser serves the very important function of illuminating the object under observation. The condenser can usually be adjusted for height and is fitted with an iris diaphragm for adjusting its aperture. On microscopes fitted with a field diaphragm, the condenser can also be centered. For obtaining a good image, the condenser must be adjusted correctly. In what follows, I show the effect on the image of different condenser aperture settings and also of lowering the condenser from its optimum position. An upright microscope is assumed.

Condenser height adjustment

The condenser is normally at or near the top of its travel, with the top lens of the condenser being about 1 mm below the bottom surface of the slide. For a microscope fitted with a field diaphragm, the condenser is adjusted so



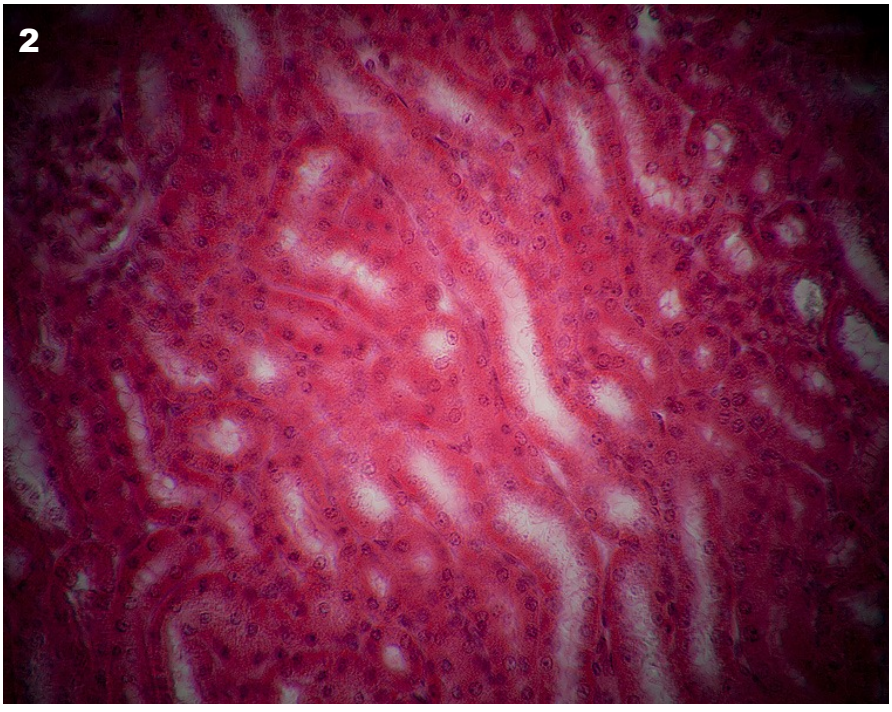


Figure 1: Image of the (partially closed) field diaphragm with correct height and centering adjustment of the condenser.

Figure 2: Appearance of the (partially closed) field diaphragm with the condenser lowered from its correct position.

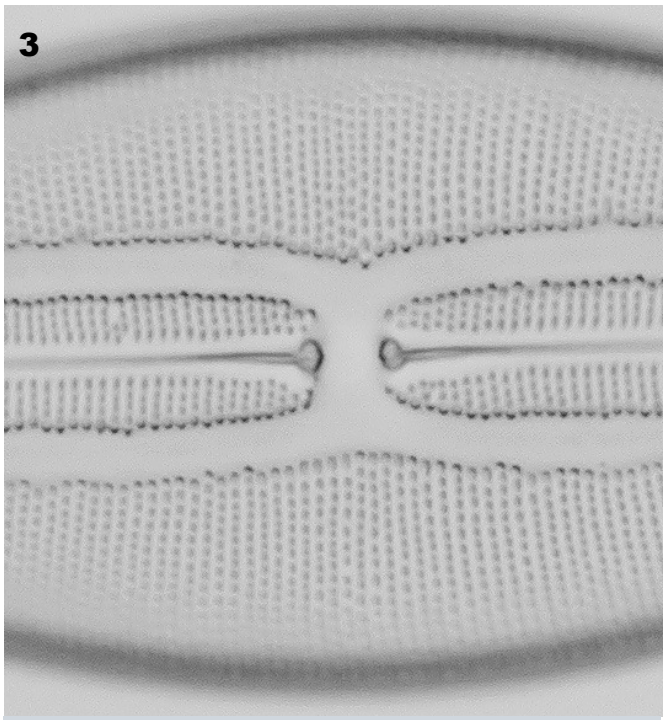


Figure 3: Condenser aperture adjusted to completely fill the objective aperture with light, showing good resolution but some loss of contrast from a high contrast object [2].

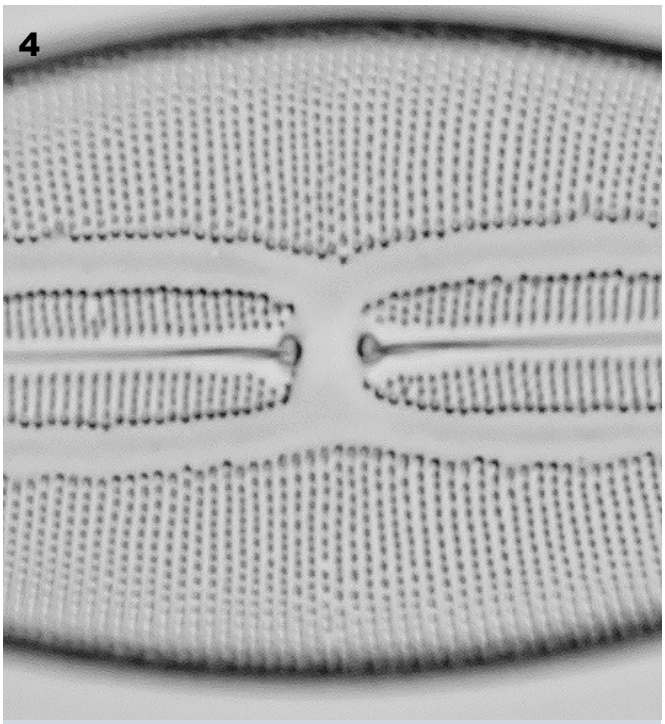


Figure 4: Condenser aperture adjusted to fill 80% of the objective aperture [2].

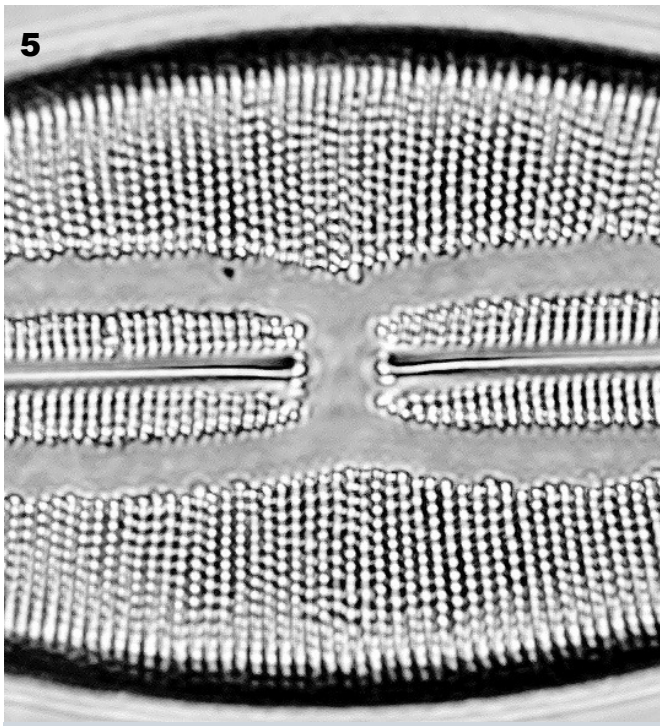


Figure 5: Condenser aperture adjusted to fill 10% of the objective aperture *[2]*. Note the poor resolution and diffraction effects.

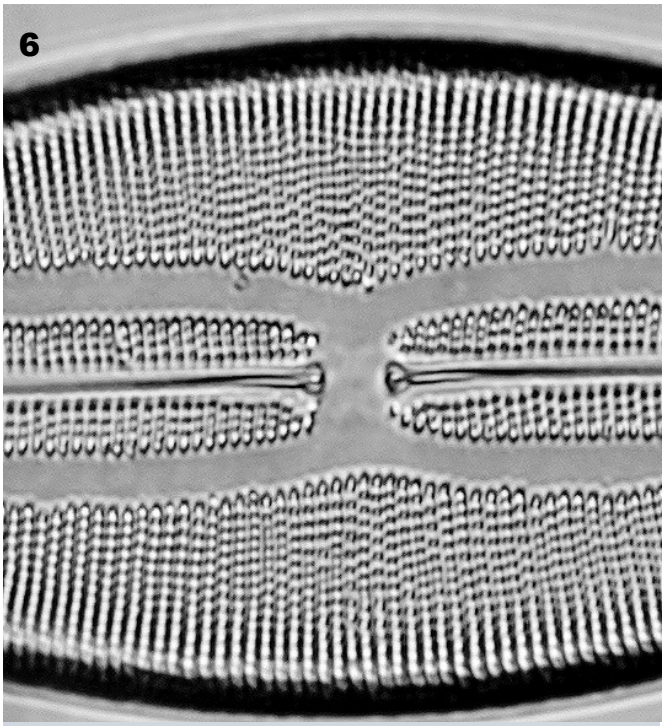


Figure 6: Condenser aperture was adjusted to completely fill the objective aperture, then the condenser was lowered by 2 mm from its correct position; this resulted in the illuminated objective aperture to be reduced to about 10% (compare to Figure 5) *[2]*.

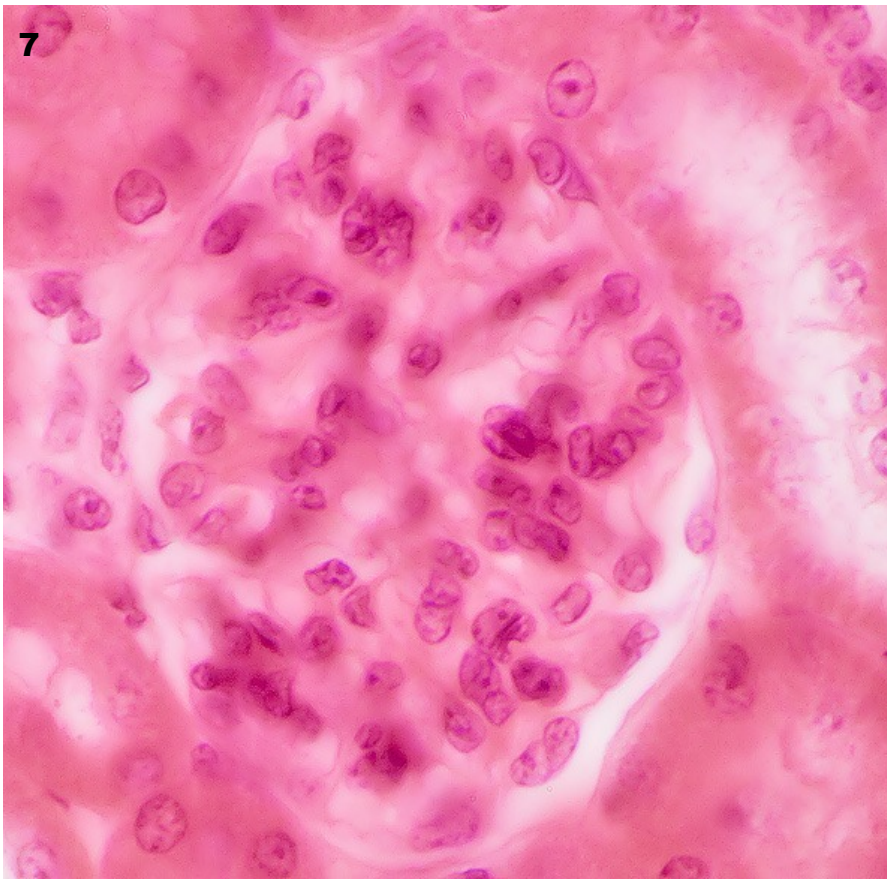
that the (partially closed) field diaphragm is in focus and centered in the field of view, as shown in Figure 1. In Figure 2, the condenser was racked down, resulting in a poor image (see also Figures 6 and 10). For a microscope without a field diaphragm, the condenser is raised all the way to the top of its travel for use.

Condenser aperture adjustment

The iris diaphragm (usually part of the condenser body) controls angle of the cone of light that directly illuminates the objective aperture. Opening the diaphragm to fully illuminate the objective aperture results in the highest resolution, but the attendant glare can reduce the image contrast. As the condenser aperture is made smaller, contrast is increased, but resolution is reduced, depth of field is increased, and diffraction effects start to result in halos, thick lines, and spurious details that degrade the image [1]. The increase in depth of field with small apertures can itself result in a confused image as structures from areas outside the plane of focus become visible.

For high contrast images (e.g., stained sections) condenser apertures approaching the objective aperture can usually be used for best resolution. For low contrast or transparent objects (e.g. water microorganisms), smaller apertures need to be used to make them visible [1].

The following images show the effects of changing the condenser aperture and of lowering the condenser on image quality. The condenser aperture is given as a percentage of the objective aperture. This was estimated visually by removing the eyepiece and looking down the tube at the back focal plane of



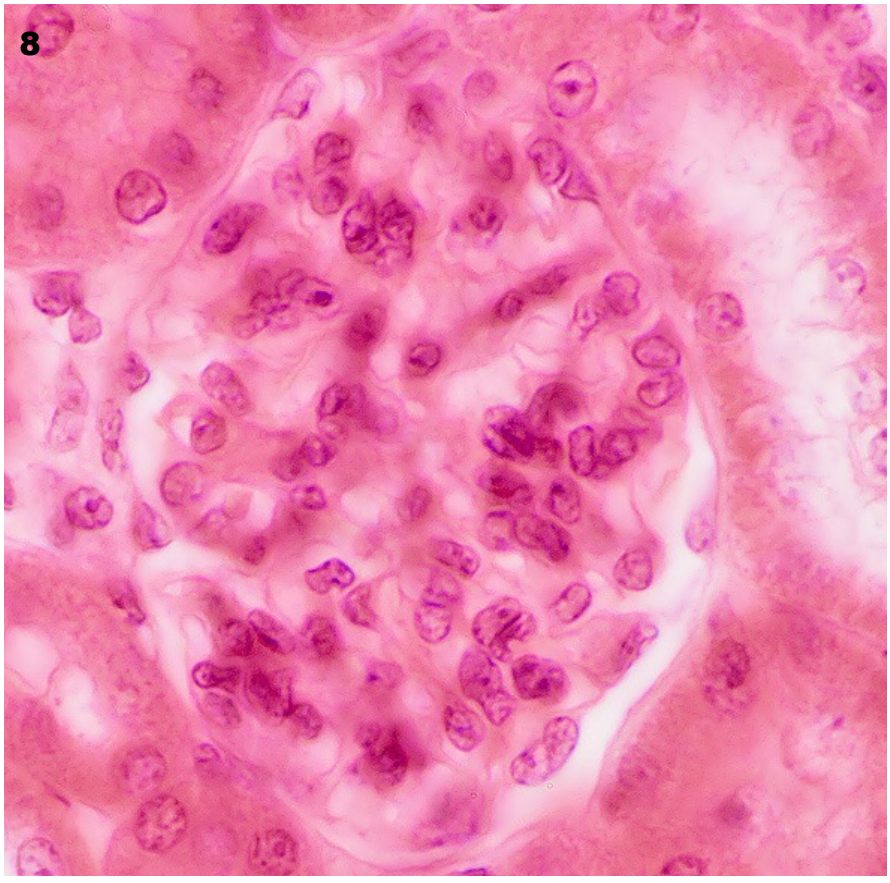
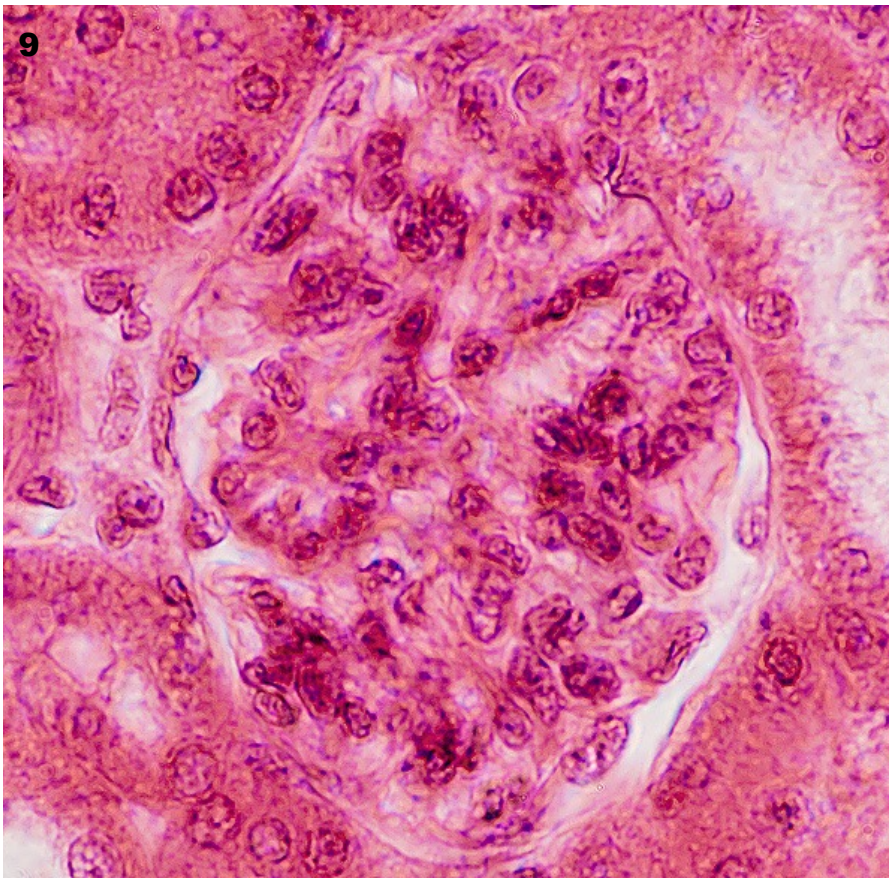
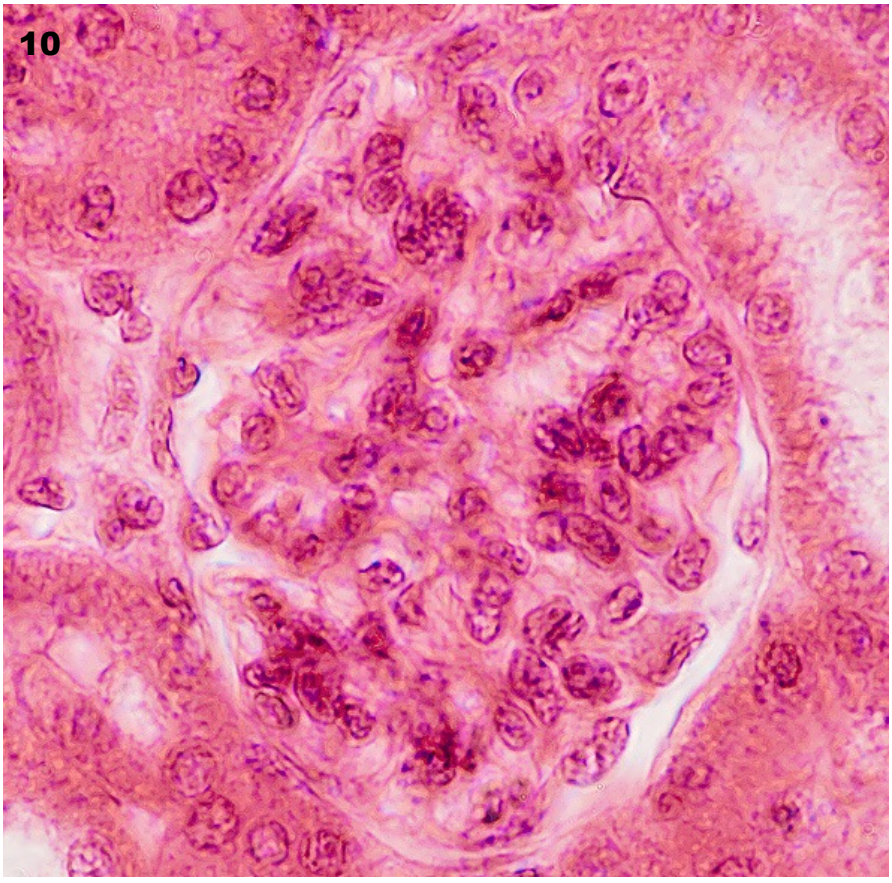


Figure 7: Condenser aperture adjusted to completely fill the objective aperture, showing somewhat low contrast *[3]*.

Figure 8: Condenser aperture adjusted to fill 60% of the objective aperture, showing good contrast *[3]*.





the objective. The diameter of the objective aperture illuminated at each condenser setting relative to the full objective aperture was noted for each image.

References

[1] Kim, Oliver. The importance of mounting medium refractive index. http://www.microbehunter.com/the-importance-of-mounting-medium-refractive index/

[2] A green filter was used and the image converted to gray scale. *Navicula lyra* from slide by K.D. Kemp. http://www.diatoms.co.uk/

[3] Kidney longitudinal section (unspecified animal), commercial slide.

■

Figure 9: Condenser aperture adjusted to fill 10% of the objective aperture, showing poor resolution, pronounced diffraction effects, and confusion from superimposed details from above and below the plane of focus due to the increase in depth of field *[3]*.

Figure 10: Condenser aperture was adjusted to completely fill the objective aperture, then the condenser was lowered by 1 mm from its correct position; this resulted in the illuminated objective aperture to be reduced to about 10% (compare to Figure 9) *[3]*.

What Camera and Adaper work best for (my) Photomicrography?

Finding the right camera and adapter combination is not an easy task. Even eyepieces of the same magnification made by different companies can produce surprisingly different results.

By Carl Hennig

I conducted an experiment to see which of my cameras worked best for taking photographs through a microscope. I have three cameras, all at different price points, different resolutions and different methods of connecting to a microscope.

The common point for all cameras is they use the viewing tube of a monocular microscope, in my case a Bausch and Lomb (c. 1950) and a Labomed CxL.

A truly scientific test would include all permutations, but I was interested in what camera would give me the most pleasing results and what combinations to avoid. I used the B&L microscope for all tests because the adapters fit the 23mm ID / 25 mm OD diameter viewing tube and the B&L has new PLAN objectives (4x, 10x, 40x).

The cameras I have available for microscope use are:

- **Panasonic TS20:** 16MP sensor, a 5x zoom and a cost of $253.00 in Canadian Dollars, CDN. It connects to the microscope using a clamp and adjustable platform. I used the Labomed 10x eyepiece.
- **Canon 5D MkII:** 21MP sensor and a cost of $2700.00 CDN. It connects using two different adapters. It can be directly inserted into the viewing tube, with no optics in the adapter, and can connect to an adapter that uses the eyepiece of the microscope.
- **Future Optics** microscope camera: a 5MP sensor, a 10x relay lens and a cost of $160.00 CDN. It connects directly into the viewing tube.

For the DSLR test using the microscope eyepiece, I used the original B&L eyepieces (5x, 10x, 15x). For the record, the specimen is a cross section slice of bamboo cane.

Although image quality counts, most of the images are just too large to include in these posts. The images are not cropped, but re-sampled down to 640x480. The images are selected using the 4x and 10x objectives. I removed the 40x photos. The 40x photos really need 5-7 stacked images to create a decent image.

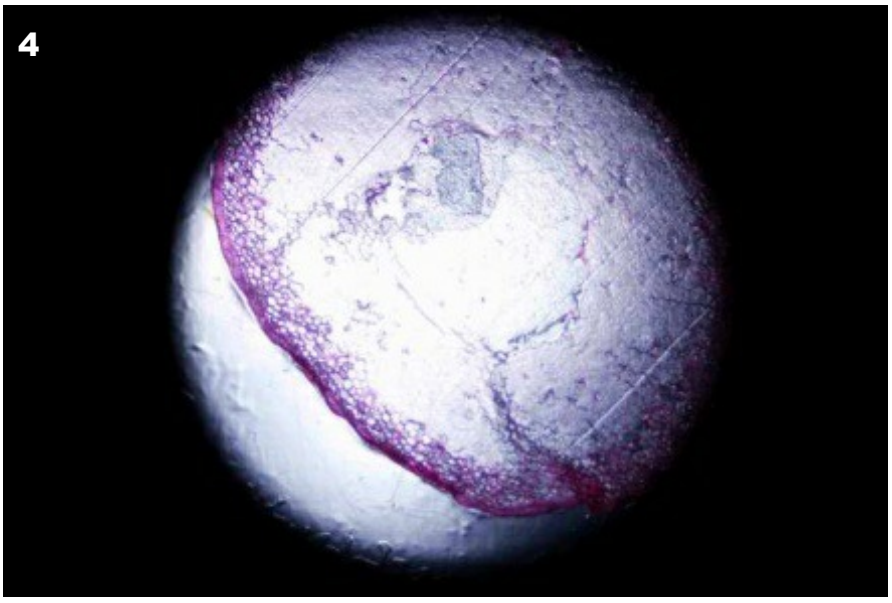
The DSLR test

The first test used the direct insert adapter (Figure 3). To capture the photos from the 5D I used the Canon Utilities remote control software.

This adapter has a very short sensor to viewing-tube distance. As can be seen in the photos (Figures 4, 5), the image is a circle that does not fill the frame of the 5D. Also there is a distinct hot spot in the centre of the image. The hot spot was visible with all three objectives.

Figure 1: Canon 5D MkII.

Figure 2: Panasonic TS20.



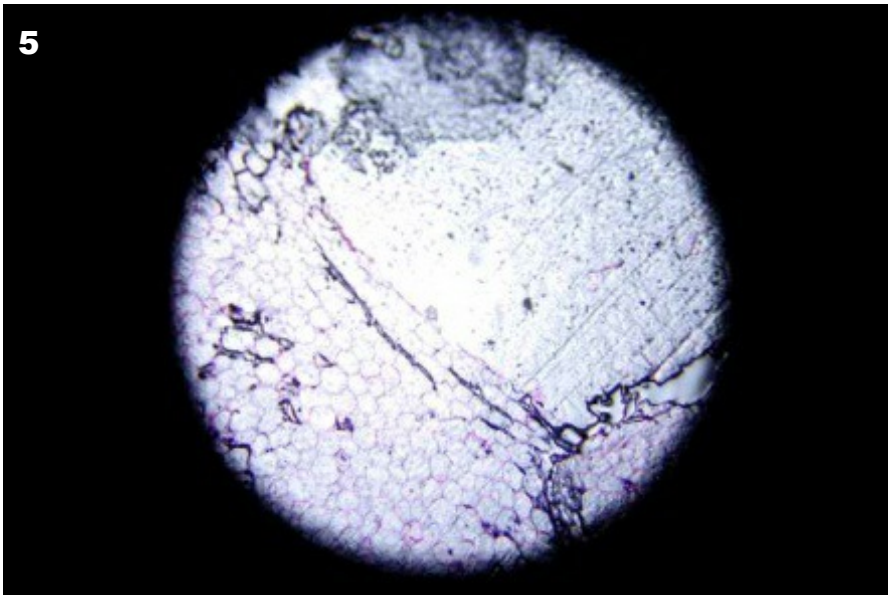


Figure 3: Direct insert adapter, without optics.

Figure 4: 4x objective, no eyepiece, Canon 5D MkII

Figure 5: 10x objective, no eyepiece, Canon 5D MkII

Surprised by the hot spot, I placed the camera and adapter on the Labomed. The hot spot was also present. Removing the slide, the hot spot appears as an almost perfect circle. I concluded the spot was either a reflection off the objectives or the eye point focus is way off.

The second test used the eyepiece adapter (Figure 6). The results were much better and also a bit surprising. The photos with the 5x eyepiece show the almost standard circular image, filling a bit more than the no-eyepiece adapter and no hot spot (Figures 7 and 8).

Inserting the 10x eyepiece resulted in quite a surprise. The image filled the full frame of the 5D (Figures 9 and 10). Surprising because I have read that a 2.5x eyepiece would be the best size to fill a full-frame sensor.

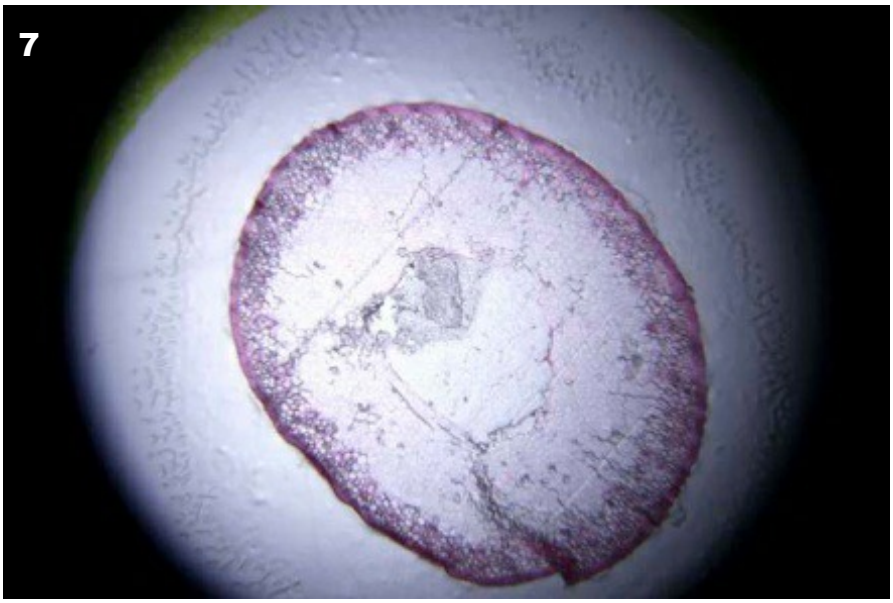
Even more surprising, the Labomed WF 10x eyepiece did not fill the frame. The 15x eyepiece also produced images that filled the 5D frame.

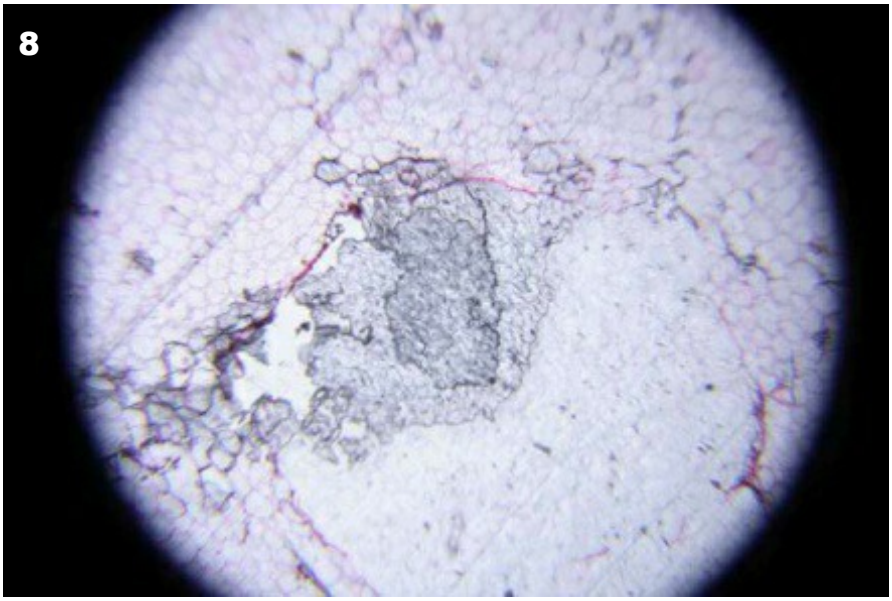
Future Optics / Touptek 338 USB microscope camera

The Future Optics 5Mp microscope camera is an average quality CMOS microscope camera. It has a 10x relay lens and mates up well with both the B&L and Labomed microscopes. Its biggest failing is noise. At long exposure times there is noticeable noise in the images.

What is important is the image is full frame with no cropped corners and the camera is fully compatible with the Toupview software.

The Toupview software has a very broad range of tools available at the time of image capture.





Panasonic TS20 Point and Shoot camera

The TS20 is a waterproof camera with a 5x Leica designed zoom lens. The lens does not extend from the camera making it an ideal camera to mount on a microscope. The camera is fastened to a platform (Figure 16) and the platform is moved to place the lens above the microscope eyepiece.

The TS20 has the typical very wide angle lens when first turned on, so some zoom is required get a full frame image (Figures 17 and 18). Focusing is done with the microscope and is a bit touchy. On exposure, the camera will also focus.

Conclusions

From the start, using the 5D DSLR connected directly into the viewing tube was a non-starter. The observed hot spot may be due to the fixed-tube optics of the B&L and Labomed microscopes. You can only expect so much from a $26.00 adapter

A DSLR adapter that use the microscope eyepiece is definitely a possibility. The choice of eyepiece makes a huge difference. If I had a true photo eyepiece my results may have been different.

I believe the B&L eyepieces are not Wide Field. I come to this conclusion because the Labomed 10x is Wide

Figure 6: DLSR adapter with optics. Figures 7-13 were all taken with a Canon 5D MkII and this adapter. The eyepiece magnification and objective was changed.

Figure 7: B&L, 5x eyepiece, 4x objective

Figure 8: B&L, 10x eyepiece, 4x objective

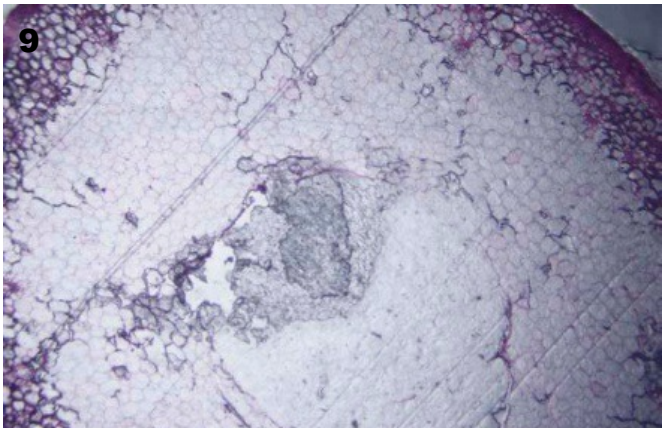
Figure 9: B&L, 10x eyepiece, 4x objective. The 10x B&L eyepiece produced a frame-filling image in the 5D.

Figure 10: B&L, 10x eyepiece, 10x objective

Figure 11: LM, 10x eyepiece, 4x objective, Here the 10x eyepiece did not pro duce a frame-filling image.

Figure 12: B&L, 15x eyepiece, 4x objective

Figure 13: B&L, 15x eyepiece, 10x objective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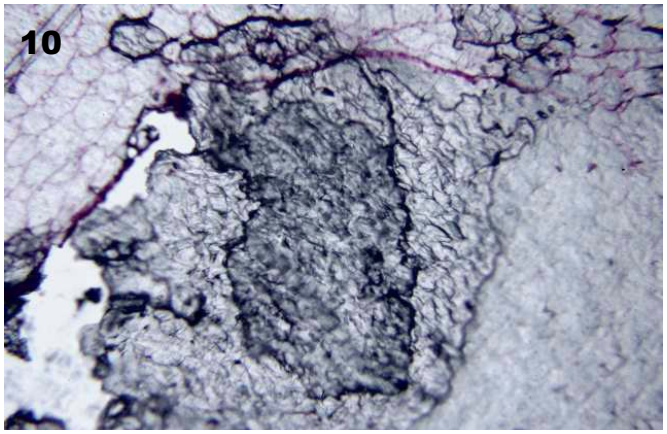
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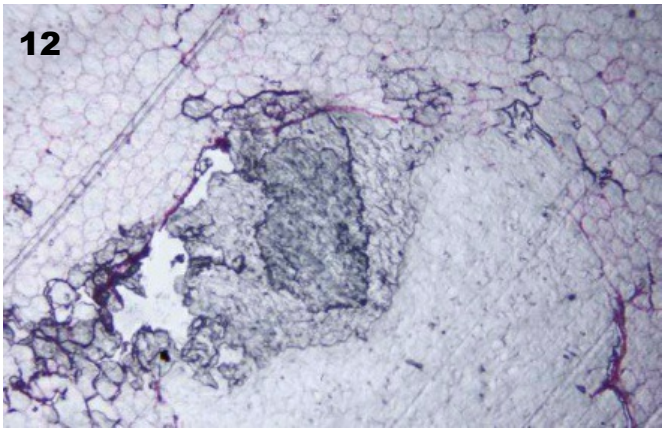
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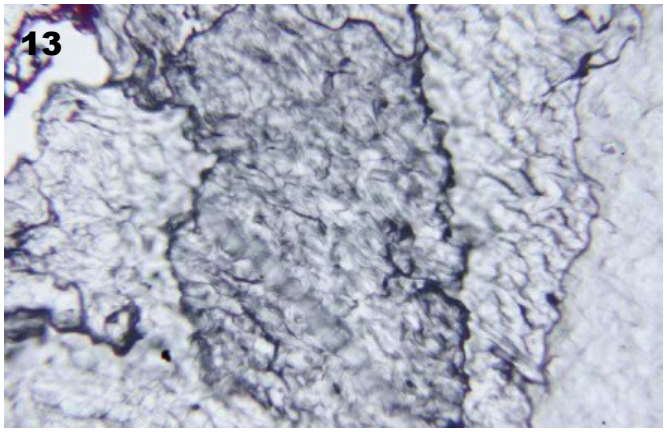
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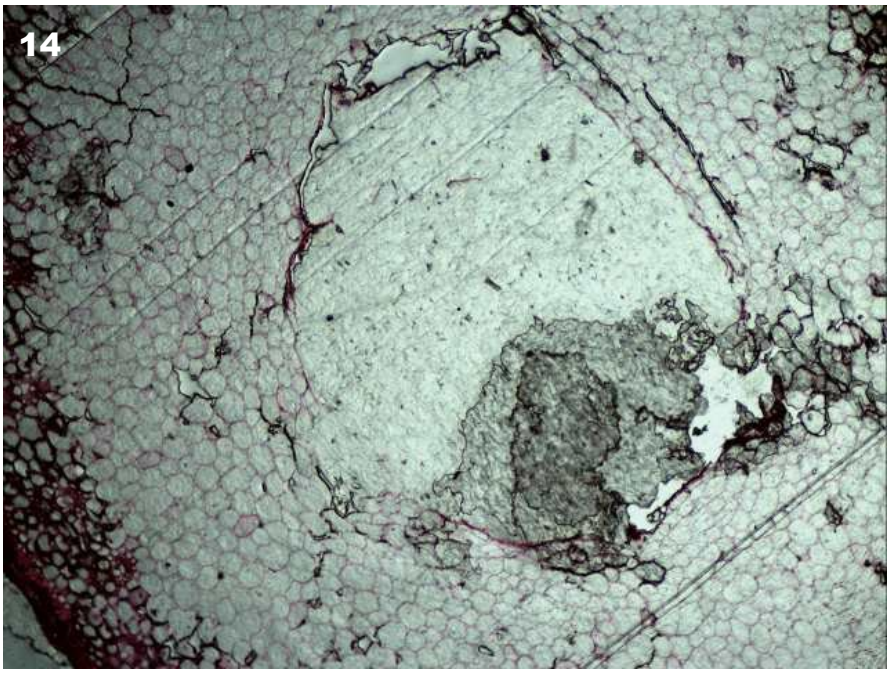
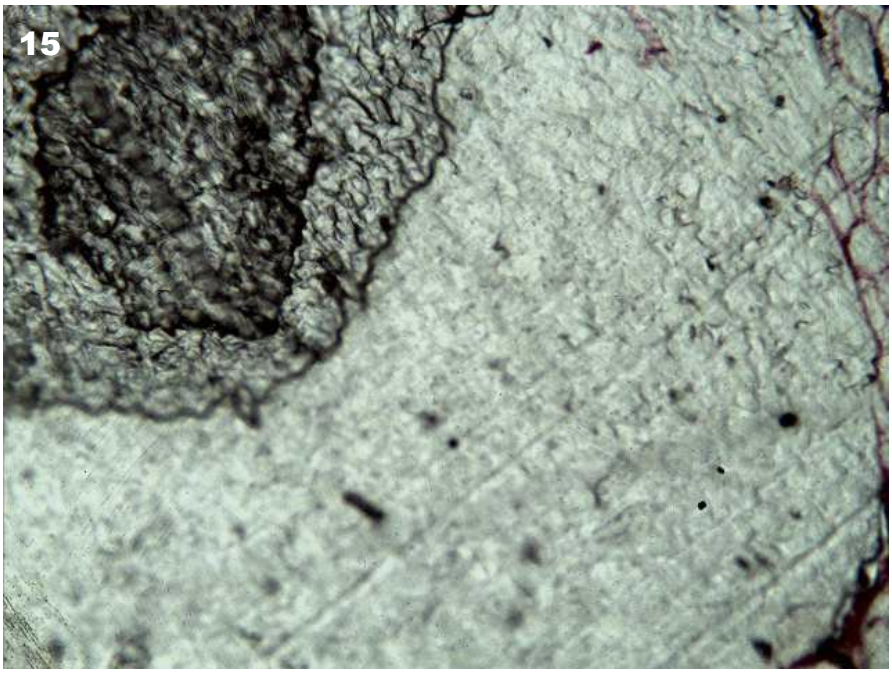
Figure 14: B&L, TK338 microscope camera, 4x objective

Figure 15: B&L, TK338 microscope camera, 10x objective

The TK338 USB microscope camera produced frame-filling images.





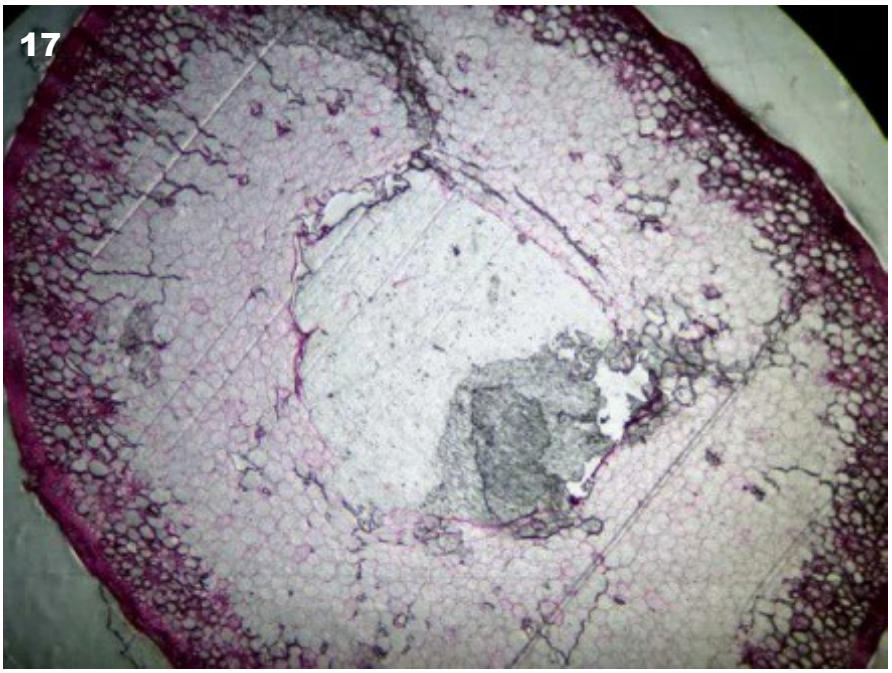
Field and does not fill the frame like the B&L 10x.

The advantages of the 5D DSLR for microscope photography is the 30fps when focusing and the extremely low noise levels of the captured photos. The disadvantage - and it is a big one - cost.

Using a point-and-shoot camera to take microscope photos is doable, but fraught with fiddly adjustments getting the camera mounted in the right position. Focusing is problematic on the 3.5" LCD viewfinder. To focus accurately you need a 3x loupe. If your microscope photography is very occasional, then a point-and-shoot camera might suffice. Good point-and-shoot cameras suitable for microscopy are not inexpensive.

This leaves the dedicated microscope camera. The images are full size with all objectives, capture time tools are extensive, it is easy to use, camera shake is not a worry and the preview image is as large as your computer monitor.

The disadvantages seem proportional to the cost of the camera. Entry level cameras like the 338 have a frame rate



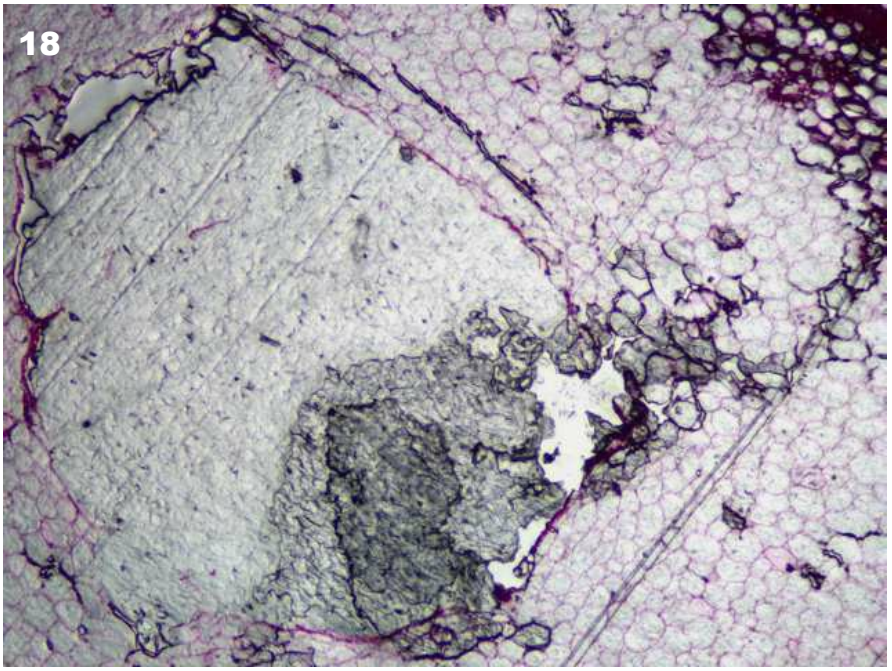


Figure 16: Platform to connect the point-and-shoot camera.

Figure 17: Panasonic TS20. 10x objective, 3x zoom.

Figure 18: Panasonic TS20. 10x objective, 5x zoom.

By zooming it is possible to obtain a frame-filling image.

around 2.5 fps, making for jerky focusing. The CMOS sensors can be noisy.

After reviewing all of my test images, I will use the dedicated microscope camera for most, if not all of my microscope photos. I may purchase a higher quality camera in the future.

Although the 5D has many more megapixels, faster focus, lower noise, it is a pain having to remove the lens and mount the adapter. The DSLR was designed for still photography and movie making. It does those jobs extremely well.

■

Slide Staining:

different stains and their uses

Sometimes it is necessary to stain the specimens before microscopic observations. Different stains chemically react with different parts of a cell and therefore make different structures visible.

By Charisa Wernick

Since many cells are almost transparent under a microscope, it is sometimes necessary to stain them in order to view components of the cells. There are quite a few different types of stains available.

Common stains

Below is a list of common stains:

- Bismarck Brown (live stain): colors a type of protein called acid mucins yellow.
- Carmine: colors animal starch (glycogen), red.
- Coomassie Blue: stains proteins a bright blue, and is often used in gel electrophoresis
- Crystal Violet: stains cell walls purple when combined with mordant. This stain is used in Gram Staining.
- DAPI (live stain): a fluorescent nuclear stain that is excited by ultraviolet light, showing blue fluorescence when bound to DNA.
- Eosin: a counterstain to haematoxylin, this stain colors red blood cells, cytoplasmic material, cell membranes, and extracellular structures pink or red.
- Ethidium Bromide: this stain colors unhealthy cells in the final stages of apoptosis, or deliberate cell death, fluorescent red-orange.
- Fuchsin: this stain is used to stain collagen, smooth muscle or mitochondria.
- Hematoxylin: a nuclear stain that, with a mordant, stains nuclei blue-violet or brown.
- Hoechst Stains: two types of fluorescent stains, 33258 and 33342 are used to stain DNA in living cells.
- Iodine: used as a starch indicator. When in a solution, starch and iodine turn a dark blue in color.
- Malachite Green: a blue-green counterstain to safranin in Gimenez staining for bacteria. This stain is often used to stain spores.
- Methylene Blue: stains animal cells to make nuclei more visible.
- Neutral/Toluylene Red (live stain): stains nuclei red.
- Nile Blue (live stain): stains nuclei blue.
- Nile Red / Nile Blue Oxazone (live stain): this stain is made by boiling Nile Blue with Sulfuric Acid, which creates a mix of Nile Red and Nile Blue. The red accumulates in intracellular lipid globules, staining them red.
- Osmium Tetroxide: used in optical microscopy to stain lipids black.
- Rhodamine: a protein-specific fluorescent stain used in fluorescence microscopy.
- Safranin: a nuclear stain used as a counter-stain or to color collagen yellow.

Staining at home

While many stains are advanced, below is a basic staining activity that you can try at home. There are several types of stains you can use at home including: food coloring, iodine, malachite green (ick fish cure), and methylene blue. You can purchase food coloring at a grocery store, iodine at a pharmacy, and the malachite green and methylene blue can be purchased at an aquarium store.

Certain stains will color different parts of a cell. Experiment with your own to see which part of the cell the different stains attach to. Make sure you have adult supervision - stains can be messy and not only will they color your specimen, but also your hands, the table, carpet, etc. Make sure you put plenty of paper underneath your work area

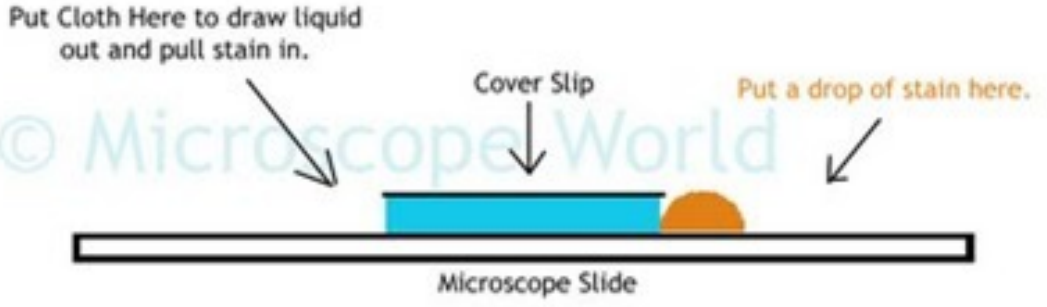


Figure 1: Apply the stain to one side of the cover slip and use filter paper or cloth to draw out the liquid from the other side.

and have some spare towels handy to wipe up any spills.

The Procedure

For practice staining cells, this is a small project to create a stained prepared slide of your cheek cells and compare this with an unstained prepared slide.

You will need the following items:

- Microscope
- 2 Blank Glass Slides
- 2 Cover Slips
- Eye Dropper
- Q-Tip or Toothpick
- Stain (see above)
- Paper Towel
- Water
- Pencil and Paper

Gently scrape the inside of your cheek with the toothpick or Q-Tip to get some cheek cells. You do not need to press hard. Prepare two identical wet-mount slides by placing the cheek cells and one drop of water on each of the blank microscope slides and covering them with cover slips.

Set one of the prepared slides aside and with the other prepared slide apply the staining material. Figure 1 shows how you can pull the stain into the slide. Place your drop of stain on one side of the cover slip and use a paper towel to pull the liquid out of the other side of the cover slip. When the liquid is pulled out from the left the stain will be pulled under the cover slip from the right.

Take a look at each slide under the microscope. Do you notice differences in the details you can view in the cheek cells? Try the same process above, but this time try a different type of stain. Make sure you document your science project and draw images of your cheek cells. You may want to share your findings with your classmates!

■

Author: Charisa Wernick
Microscope World

Link:
http://www.microscopeworld.com/microscope_slide_staining.aspx

Staining Cheek Cells with Ink

Oliver Kim

The procedure is simple: Use a cotton swab and rub the inside of your cheek and gums to collect cells. Then streak the cotton swab over a clean slide several times to transfer the cells. Rotate the cotton swab as you streak over the slide. Make a regular wet mount using only little water and cover glass.

Then add a drop of water-based blue or black ink (as used in fountain pens) to the edge of the cover glass. Do not use calligraphy ink, as this ink contains carbon particles. The ink will be pulled in underneath the cover glass by capillary action. You might have to remove some water from the opposite end of the cover glass (using tissue paper), especially if you used much water for making the wet mount.

The ink will mix with the water and a concentration gradient will form (Figure 1). Observe the cells that are just about to contact the ink. The cells will accumulate the ink and become darker than the surrounding medium (Figure 2). The nucleus of the cell also starts to show up well. If the ink is too concentrated, then you will not be able to see a color difference between the cells and the medium, so do not use too much ink.

Some inks are a mixture of different chemicals. Figure 3 shows a staining reaction with black ink. The cells initially start to stain yellow and then gradually start to accumulate the blue color components. Evidently the different stains have a different affinity for the cells.

You might want to try different food colors and colors from felt-tip markers. Some of these stains might need to be dissolved in some alcohol. There are endless possibilities for experimentation!

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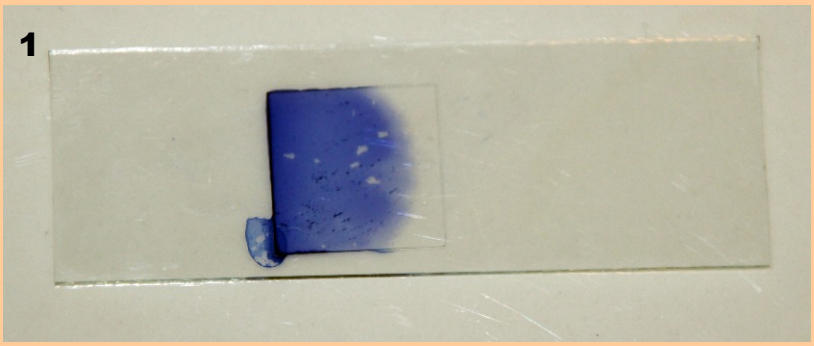
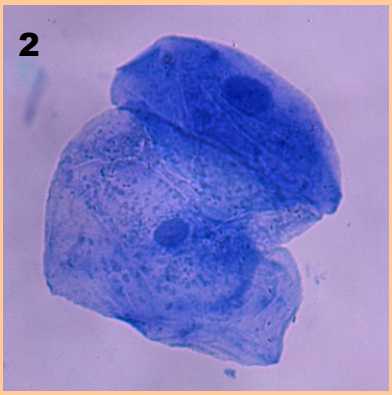
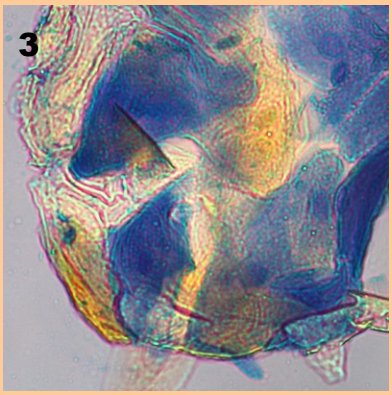






Observing the Growth Velocity of Fungal Mycelia

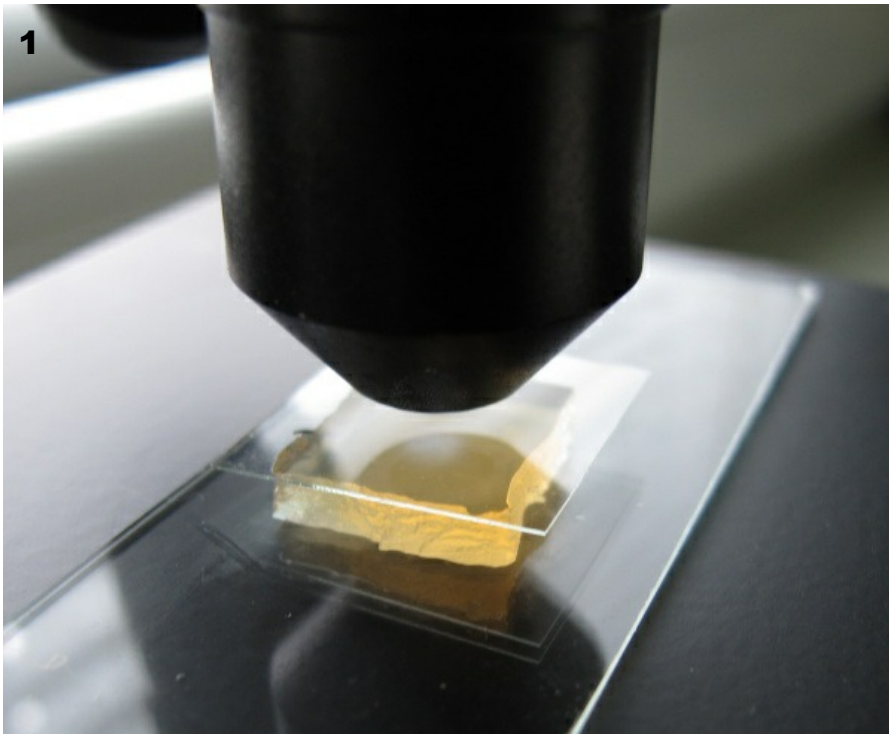
You can observe the growth of fungal mycelia under a microscope. This way it is possible to determine the speed at which they grow.

By Matej Pašák

Everybody knows that mushrooms grow very quickly after rainy days. But at what speed do they grow? The following experiment can help explain this to us.

A mushroom is not only a cap and a stem. The major part consists of the mycelium – the "roots" of a mushroom. All these parts – cap, stem and mycelium – form together an organism called a fungus. The term *fungus* marks a large group that includes edible mushrooms, wood-decay fungus and mildews.

If we want to grow mushrooms, we have to grow the mycelium first. As a comparison, if we want to grow apples, we have to plant a seed that will grow into an apple tree and the tree will produce fruits. Reproduction of mushrooms is provided by spores – let's say microscopic seeds. Spores will grow into the mycelium and, under suitable conditions, mushrooms will grow out of the mycelium.



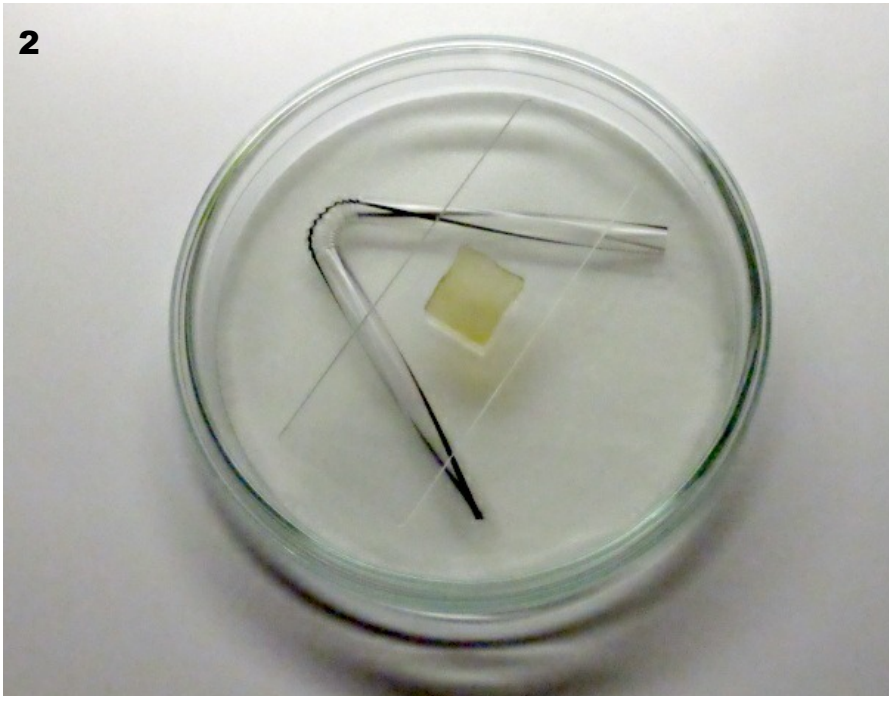


Figure 1: Sample to observe spore growth. The spores are located on a block of nutrient Agar, beneath the cover glass.

Figure 2: Wet environment for storing the specimen.

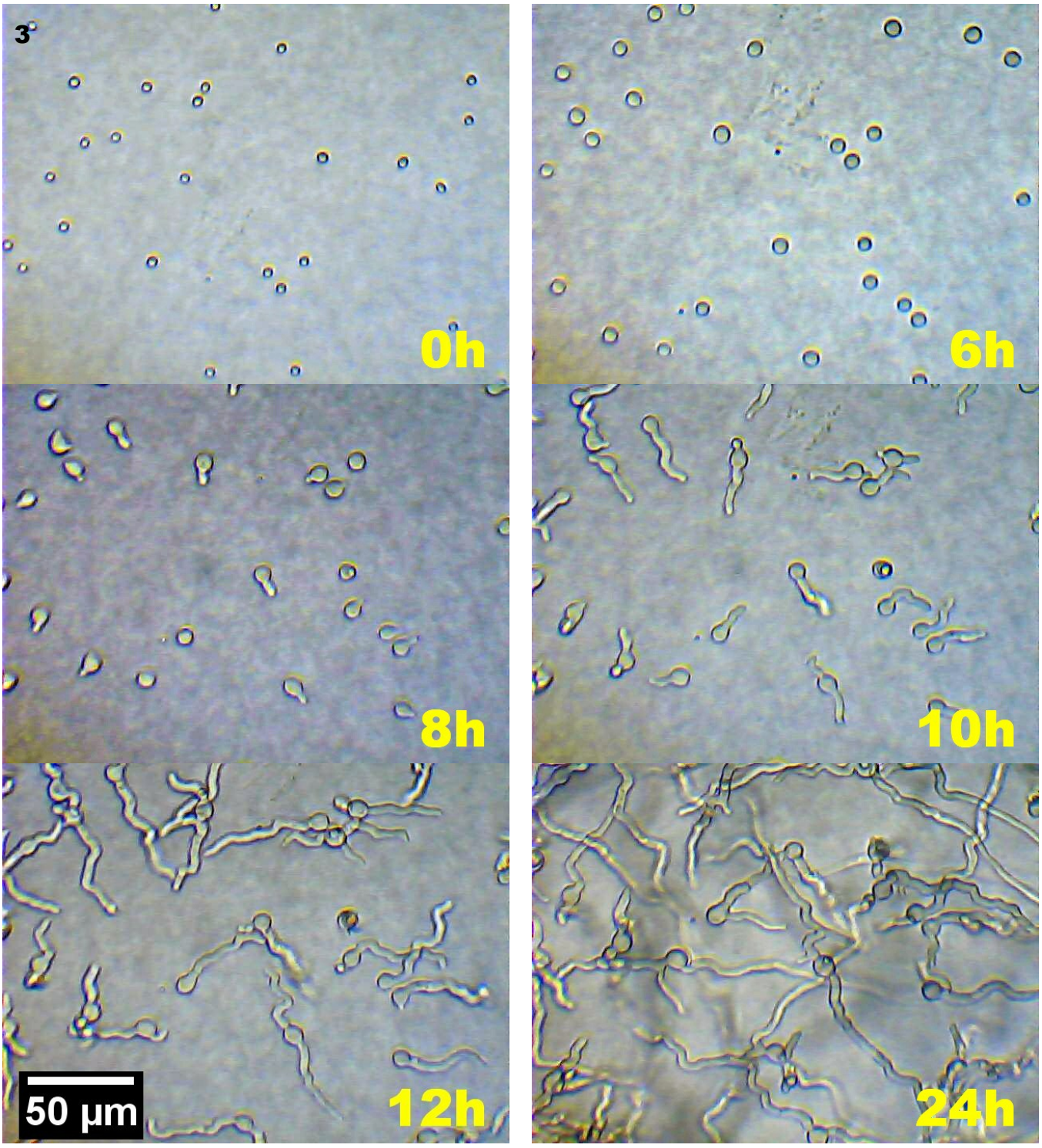


Figure 3: Some important moments during the growth of the spores.

Growing fungi

To grow mushrooms in laboratory conditions we use agar. Agar is a gel-forming powder obtained from algae. It can be bought in supermarkets specialized in health-foods. However, we have to add some nutrients into the agar. You can either buy dried nutrients or you can prepare them by following these steps:

Cook 100 g of potatoes for 15 min in 500 ml of water. Strain the decoction into another pot. Add 10g of agar and 5g of yeast. Cook for 10 min on low heat.

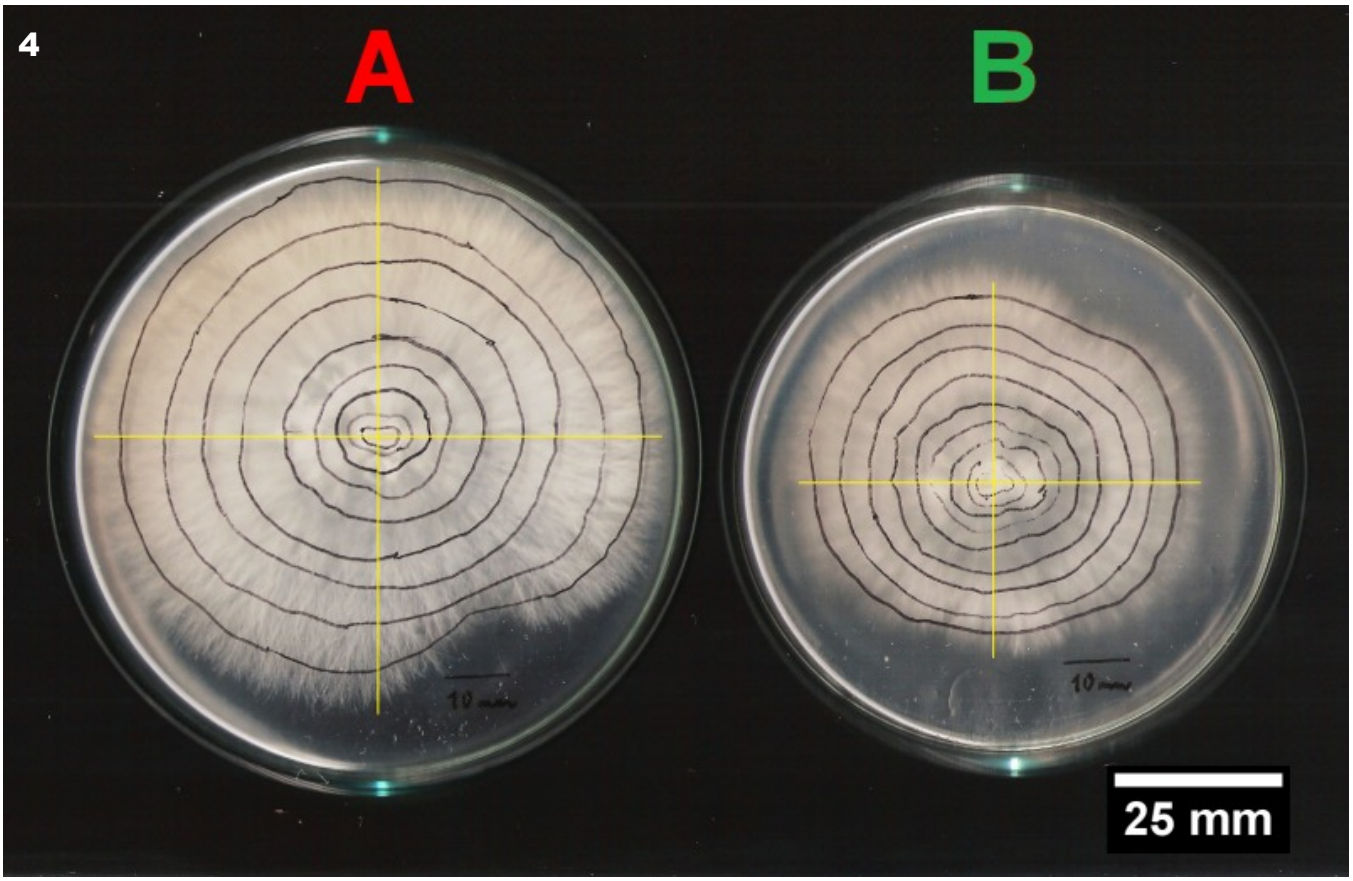


Figure 4: Bordered increment every 24 hours.

Then, pour this solution into Petri dishes. Now, it is important to sterilize the dishes for 45 min in a pressure cooker. Next day repeat the 45 min sterilization of the dishes. On the third day, put the dishes out of the cooker and leave them in a paper box. Check the dishes after few days. In case some of them are contaminated by bacteria or mildews, throw them away. The sterile ones can be used to grow mycelium.

Isolating the spores

In order to observe the spores we have to gain them first. To do so, we have to cut away the stem from the mushroom, and then put its cap on a paper. In few hours the spores will fall down of the cap right on the paper. We can observe various shapes of the spores under the microscope.

Now we have to prepare a proper sample so we will be able to observe the growth of the spores. The steps are following: pick one dish with prepared agar. Sterilize a scalpel with fire. Cut a 10 mm square off the agar and put it on a slide. First touch the spores with the scalpel and then gently touch the surface of the agar square. Finally, cover it with a cover glass (Figure 1).

Put a filter paper into an empty Petri dish. Wet the paper with water. Put a crooked straw or a glass rod into the dish. Then put the slide with the sample on it (Figure 2). However, it is possible to observe the sample under the microscope only for a while. Then it is necessary to put the sample back to the wet environment of the dish. That's why this method is not appropriate for time lapse recording.

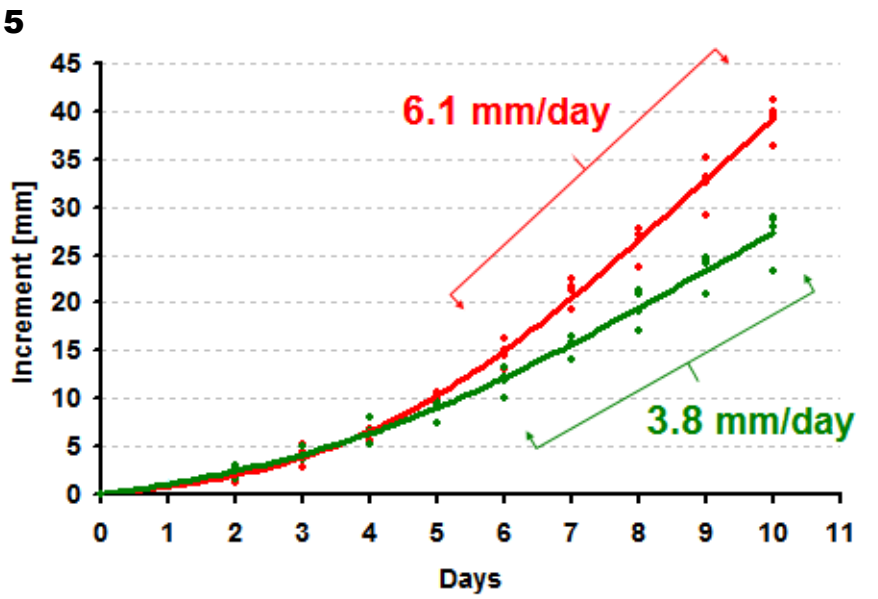
It is quite difficult to grow a mycelium from the spores of edible mushrooms. Thus it can be easier for beginners to use the spores of mildews that grow on bread or lemon. I prepared the sample with the mildew spores (genus *Aspergillus*). Every hour I put the slide under the microscope and took a micrograph. In Figure 3 there are some important moments during the growth of the spores.

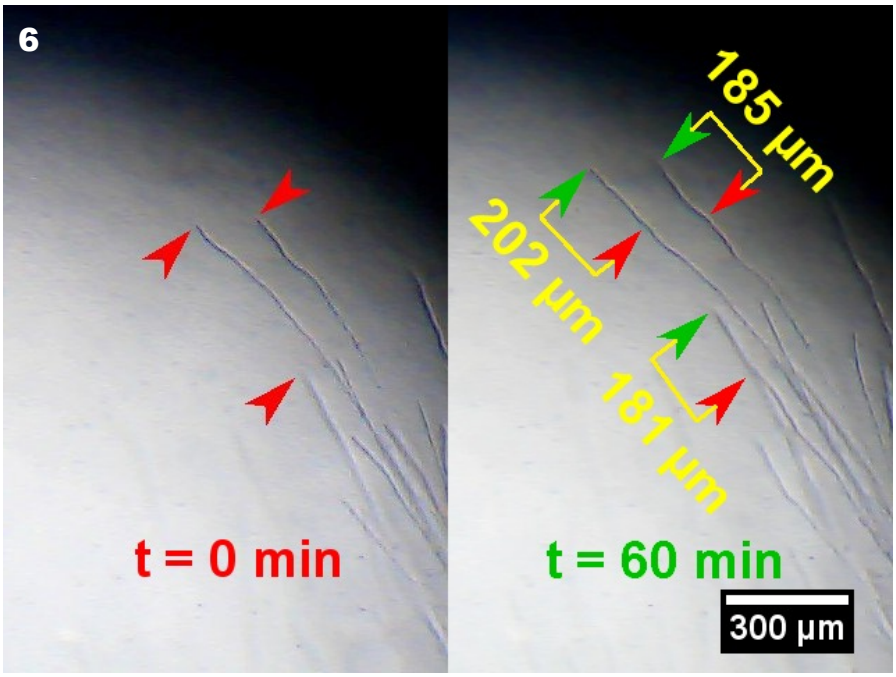
Observations

Time 0 h represents the beginning of the experiment when I put the spores on the square of agar and took a picture. You can see that diameter of the spores is approximately 5 µm. After 6 hours there is still no significant change. The spores just absorbed the water so their diameter is now 8 µm. After 8 hours a hypha grows of the spore. It is the first stage of the mycelium fiber. After 10 hours the hypha is 2 times longer than the spore diameter. After 12 hours the hyphae are many times longer than the diameter of the primary spore. 24 hours after inoculation the hyphae start to merge together and create a net of mycelium. The mycelium then grows into the volume of the agar so it is out of the depth of field. This experiment shows how quickly mildew is capable of colo-

Figure 5: Graph showing the growth increment per day.

Figure 6: Growth increment of the mycelium per hour.





nizing the substrate. Spores of the fungus are everywhere around us and wait for the proper conditions. It means that if we leave some food in wet conditions, next day it may be attacked by mildew.

The mycelium then grows radially. The mycelium fibers merge into the root-like structure named rhizomorph. The macroscopic growth of the mycelium can be observed in the Petri dish. As I mentioned before, it is difficult to grow mycelium from the spores of edible mushrooms. The easier way is to take mycelium from the live mushroom. To start we can use the oyster mushroom (*Pleurotus ostreatus*) from the supermarket. When taking the mycelium of the mushroom, the surrounding conditions must be completely sterile. The best place to perform this could be a small room without windows. What is more, it is important to sterilize the scalpel, clean the worktop and wear surgical gloves cleaned with alcohol.

Then divide the stem into two parts by hands. Do not touch the inner surface. Cut a 3 mm long square of the inner surface using scalpel and move it to the Petri dish with the prepared agar. Later the mycelium fibers will start to grow of the small piece of mushroom.

Observing macroscopic growth

In the next experiment I observed the macroscopic growth of the mycelium of the oyster mushroom in the Petri dishes. Every 24 hours I marked the borders of the grown mycelium on the bottom of the dish. After 10 days the mycelium filled almost all the size of the agar surface. I put the dishes on a scanner and took a picture (Figure 4).

I measured the distance between the borders and the centre in four perpendicular directions using the ImageJ program. Average amounts of the size increase for every day are shown in a graph in Figure 5.

I observed two dishes under equal conditions in order to compare them. The graph suggests that the initial growth velocity increases exponentially. After 6 days the growth velocity stabilizes and the mycelium grows linearly. From the linear part of the curve I calculated the size increase of 6.1 mm per day for the dish marked with A and 3.8 mm size increase per day for the dish B.

I also tried to compare the linear velocity of the growth of mycelium by

Figure 7: Primordia of an oyster mushroom.

observing the growth in the Petri dish. This is possible to observe in-situ by using microscope. To do so, we put the dish under the objective with small magnification and big depth of field. The disadvantage is the small magnification and the low sharpness of the picture because the light goes through the glass of the dish. The advantage is possibility of taking the time lapse pictures during several weeks. In Figure 6 there is the size increase of mycelium per 1 hour observed in-situ. The average growth velocity is 189 µm per hour (about 4.5 mm per day).

When the mycelium covered all the surface of agar, the growth stopped. Then the fungus waited for proper conditions and after few weeks the primorodia (Figure 7) started to grow on the edge of mycelium. Primorodia are the small germs that will later grow into big mushrooms.

However, mushrooms do not grow as fast as it might seem. In nature it takes several weeks or months for a mycelium to grow from the spores. Mycelia may even grow tens of meters. If there are proper conditions, the mushrooms will start to grow. The mushrooms that we collect make only 10 to 30 percent of the weight of the mycelium that is under the ground. The mushroom as we know it (a cap on a stem) can grow during 7 or 10 days. So a mushroom can not grow during night after a rain.

■

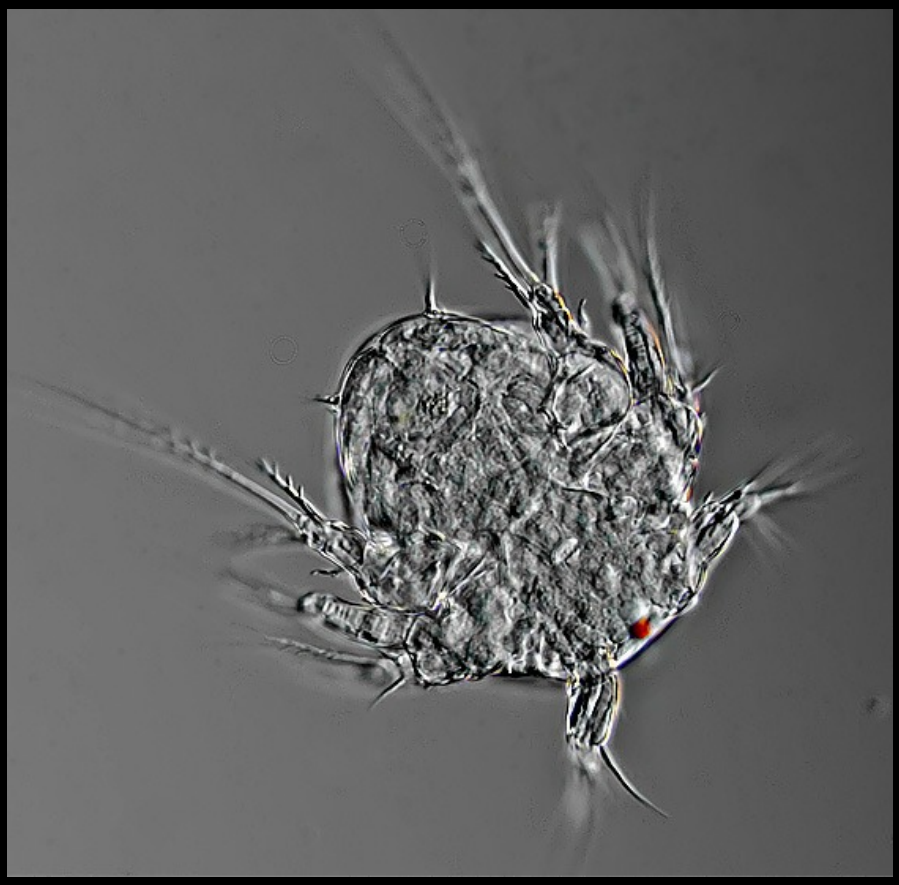
Matej Pašák

Faculty of Materials Science and Technology, Slovak University of Technology

Mildew - from Wikipedia

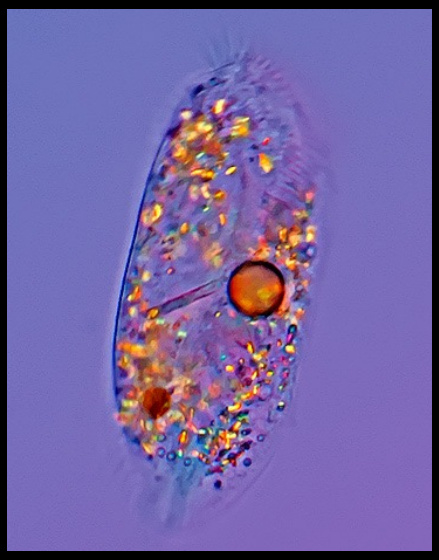
The term mildew is often used generically to refer to mold growth, usually with a flat growth habit. Molds can thrive on many organic materials, including clothing, leather, paper, and the ceilings, walls and floors of homes or offices with poor moisture control. There are many species of mold. The black mold which grows in attics, on window sills, and other places where moisture levels are moderate often is *Cladosporium*. Color alone is not always a reliable indicator of the species of mold. Proper identification should be done by a microbiologist. Mold growth found on cellulose-based substrates or materials where moisture levels are high (90 percent or greater) is often *Stachybotrys chartarum* and is linked with sick building syndrome. “Black Mold,” also known as “Toxic Black Mold,” properly refers to *S. chartarum.* This species commonly is found indoors on wet materials containing cellulose, such as wallboard (drywall), jute, wicker, straw baskets, and other paper materials. *S. chartarum* does not grow on plastic, vinyl, concrete, glass, ceramic tile, or metals. A variety of other mold species, such as *Penicillium* or *Aspergillus*, do. In places with stagnant air, such as basements, molds can produce a strong musty odor.

This text was taken verbatim from:
"Mildew." Wikipedia. Wikimedia Foundation, 30 Mar. 2014. Web.



Top image: Pond water arthorpod, possibly *Nauplius. D*ifferent planes of focus; 20x objective). Notice the red eye spot.

Bottom images: A ciliate.



See more at:
http://s1070.photobucket.com/user/micritter/library/images

Images by Rashid Nassar

Simple an useful Micro-tools for Microscopy

In this article I want to show how I make some of my own micro-tools, very easy and with only few materials. These tools greatly facilitate microscopic work.

Roland Luts

Micro-tools for the microscopes are sold by many specialized companies but most of the time only professional people can buy them. These tools all have a very nice appearance, look shiny, come in fancy storage boxes and so on. They naturally also have a very high price! Do we need these as an amateur microscopist? I don't think so.

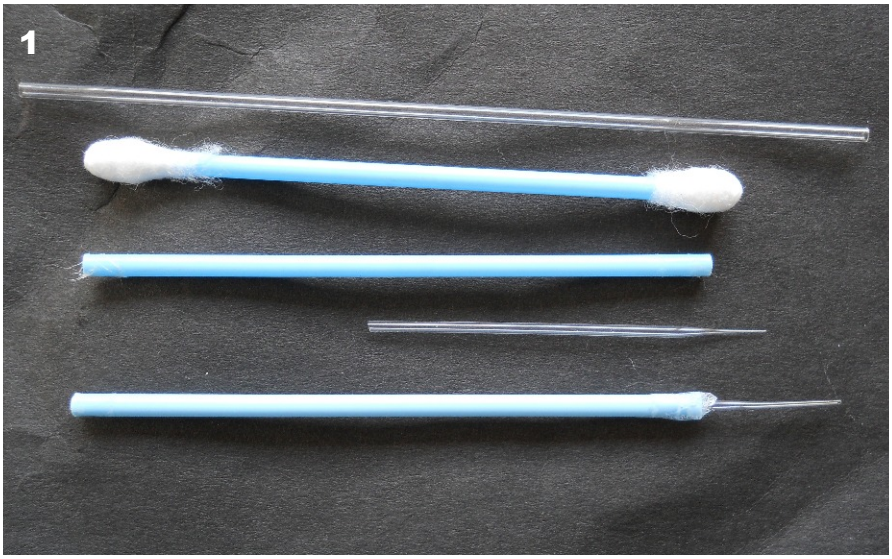
It is easy to make your own "not so nice looking" and very cheap tools, which are efficient enough for your purposes. I will explain here how I made three of the most used utensils.

The micro pipette

Here I want to mention a similar article by Jean-Marie Cavanihac, France, in the MICSCAPE Magazine "Micro Tools for Microscopy" (in two parts) [1].

Often we want to catch and separate only one or two specimens out of a mass of little creatures. With this micro pipette, that is easily done, certainly for the immotile or slower moving ones. A bit of luck is needed and good aiming is required for faster-moving specimens but that is something you learn in a short time.

Having no suction bulb at the rear end (open tube), this pipette works on the basis of the well-known capillary action and there is no need to close the end with a finger. You have to take care that the capillary tip is completely empty (only filled with air and no water clogging the tip) before you apply it. The pipette is emptied by a slight blow with the mouth onto a clean glass slide, best with already a very small drop of water on it. Connect a longer plastic tubing at the end of the pipette if dangerous solutions are being used. With this pipette only a few microliters (a few thousandth of a millilitre) will be automatically sucked up and this volume is enough to hold the small creatures in enough water so they don't dry out. The operation has to be done under a good magnifying glass, or still better, under a stereo microscope.



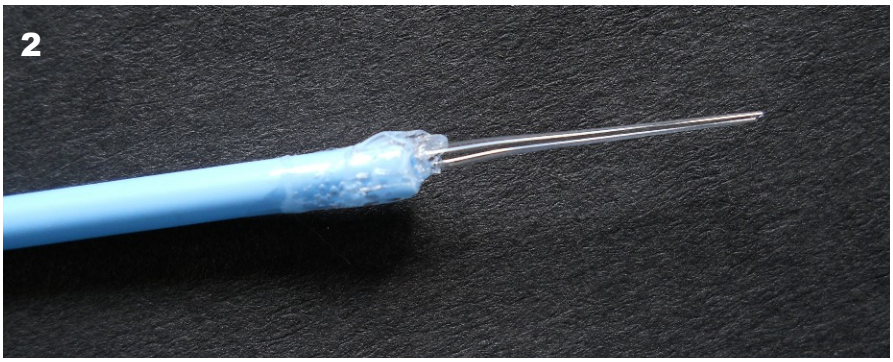


Figure 1: Glass rod and cotton swabs are the materials needed for making a micro-pipette. The glass rod is heated over a flame and then pulled out into a thin capillary.

Figure 2: The glass pipette is glued into the plastic handle of a cotton swab.

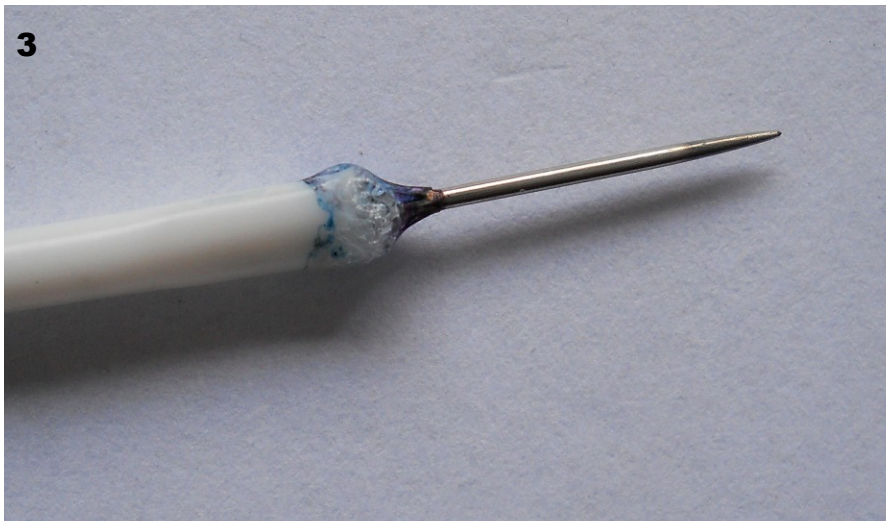


Figure 3: Micro-neede. A needle is glued into the plastic stick of a cotton swab.

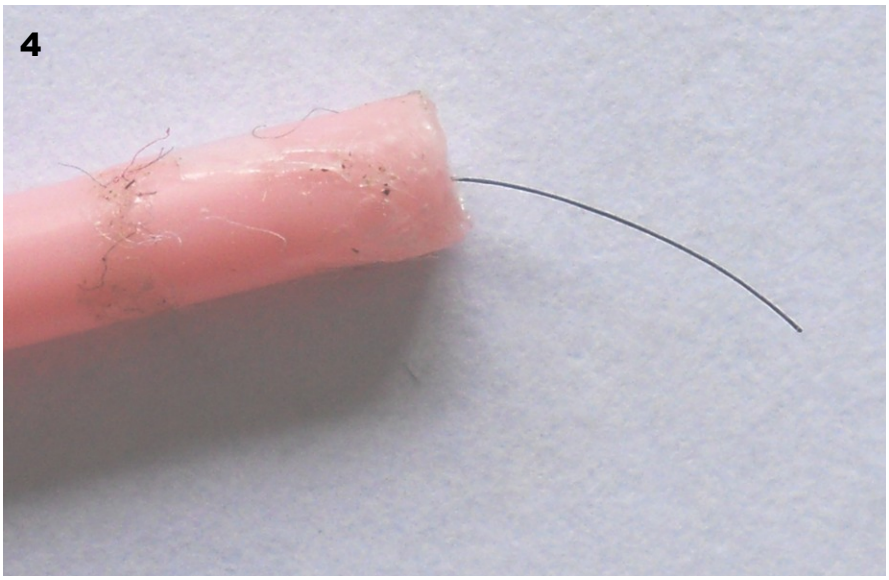


Figure 4: Micro "brush" made using a hair.

Figure 1 shows all the parts you need. I used 'Hard PVC glue' (for joining PVC drain pipes) that is fast drying. The glass capillaries can be purchased for little money per package of 100 pieces, but not the fire polished ones which are ten to twenty times more expensive. Perhaps you can buy them at your local pharmacy. Plastic ear sticks with cotton bulbs at both sides can be found in nearly every house. The sticks must be hollow and have a slightly greater inner diameter than the glass capillary! Figure 2 shows the finished product.

Making the micro pipette is evident from the parts in Figure 1. A haematocrit capillary tube (glass with an internal diameter of around 1.2 mm and a length around 8 cm) is warmed in the centre with a small flame under continuous rotation until soft (not too soft!) and is then slowly pulled out to the desired length/thickness. It is best to use an alcohol burner for this; a candle will also do but will produce black carbon particles on the capillary. After cooling, which only takes a couple of seconds, the middle (and thinner) part of the tube is carefully cut. Don't try to fire-polish the tip: the glass in this part is so thin that it will instantly be sealed and will be useless. Two pipettes can now be made with each of the 2 halves. Slide the glass capillary into the hollow plastic tube and seal them together with a drop of glue. Be sure there is no air-gap in the glue. Less than half an hour later the pipette is ready for use.

I recommend to make some spares because the tip will easily break. The best performing pipettes for me were those with a diameter at the tip of around 100 to 150 microns and with a relatively long small neck. With these, I often could pick up more than one critter before emptying.

A micro needle

For this, a needle is glued in the same type of plastic tube as for the micro pipettes (Figure 3). A wide variety of needles can be found in the department of sewing needles. Even thinner copper or stainless steel (Inox) or aluminium wire can be used.

The micro 'brush'

This can be made by gluing a piece of human or animal hair in a hollow plastic tube (Figure 4). The hair can be curved or can be straight, short or long to suit your needs.

Most of the hair have a diameter of 100 to 200 microns and sometimes the point of it is too thick. It can be made finer by soaking it in a few drops of concentrated bleach on a glass slide and smearing the tip of the hair 'to and fro' for a while, as the bleach slowly dissolves the hair.

All tools can be made with different coloured plastic tubes, taking the same colour for the same sort of utensil.

References

[1] Cavanihac, Jean-Marie (2001). Micro Tools for Microscopy. Micscape Magazine.
http://www.microscopy–uk.org.uk/mag/artnov01/tools.html

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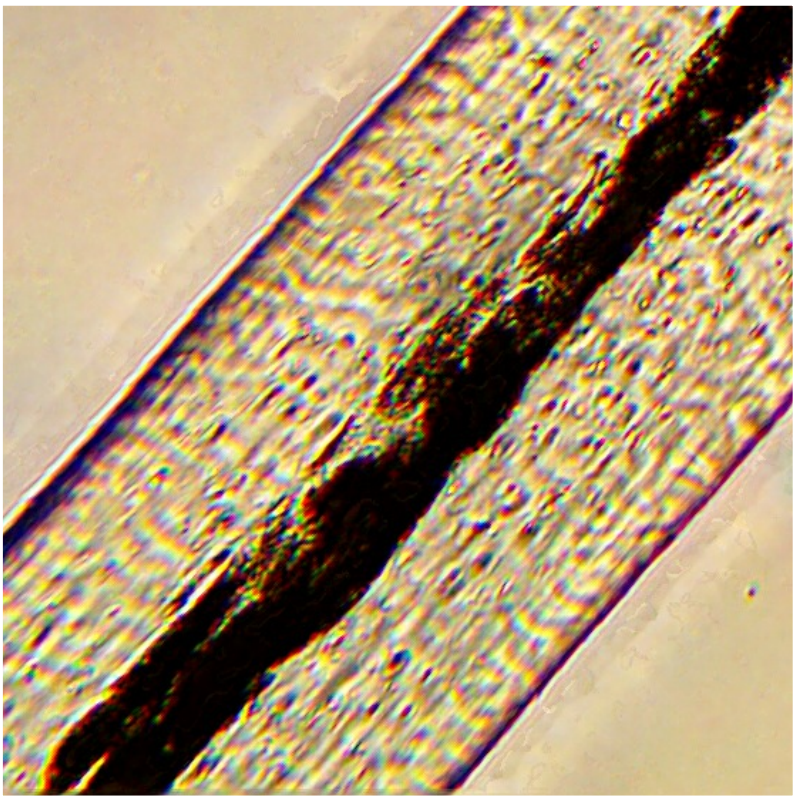

What's this? Answer on page 2.