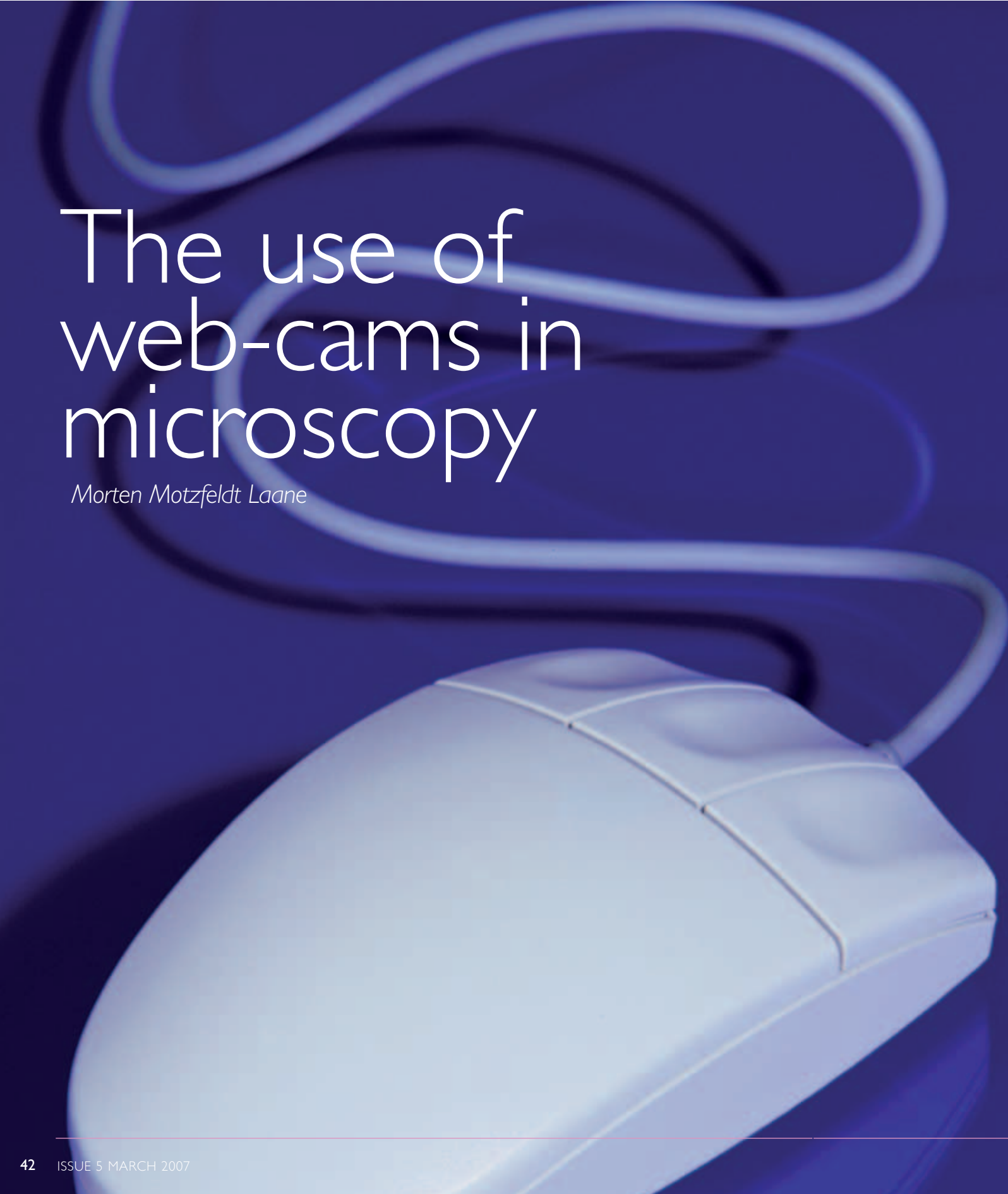
The use of web-cams in microscopy

Morten Motzfeldt Laane



During the last five years film-based photomicrography has almost disappeared in research. This has been an advantage as it is much easier to control any error in focus, condenser alignment, exposure and contrast in the optical set-up. The exposed image appears almost identical to what is observed on the camera or computer screen.

There are also many more options for adjusting colour balance, contrast, brightness, hue and other parameters. Furthermore, the field of time-lapse imaging of living cells, crystal formation and other dynamic processes were almost inaccessible with film-based cameras unless you had the resources of a big science institution. There are two problems, however. It is not easy to adapt a digital camera to a light-microscope without some optical compromises. Also, a professional digital microscope camera is often expensive and usually out of reach for amateurs, teachers, and sometimes, for professional scientists.

As most cams have image chips of smaller dimensions (1/2" or 1/3") in the film plane than of the much used 24 x 36mm format, they require the use of special optical adapters. This involves a special "reducing lens" if used without an ocular, or a suitable camera objective able to produce an image without too much cropping of the field of view in the microscope. The elaborate and expensive digital equipment designed especially for microscopes produces high quality images with little noise even at very low light levels. The now "inefficient", time-consuming, and film-based equipment is more or less outdated, despite excellent image quality.

For simple work, inexpensive computer based microscopes are available, but their optical properties are limited for the highest demands. Examples can be found on the web at www.playdigitalblue.com/products/qx5/info and www.sherwoods-photo.com/bresser/bresser_microscopes.htm

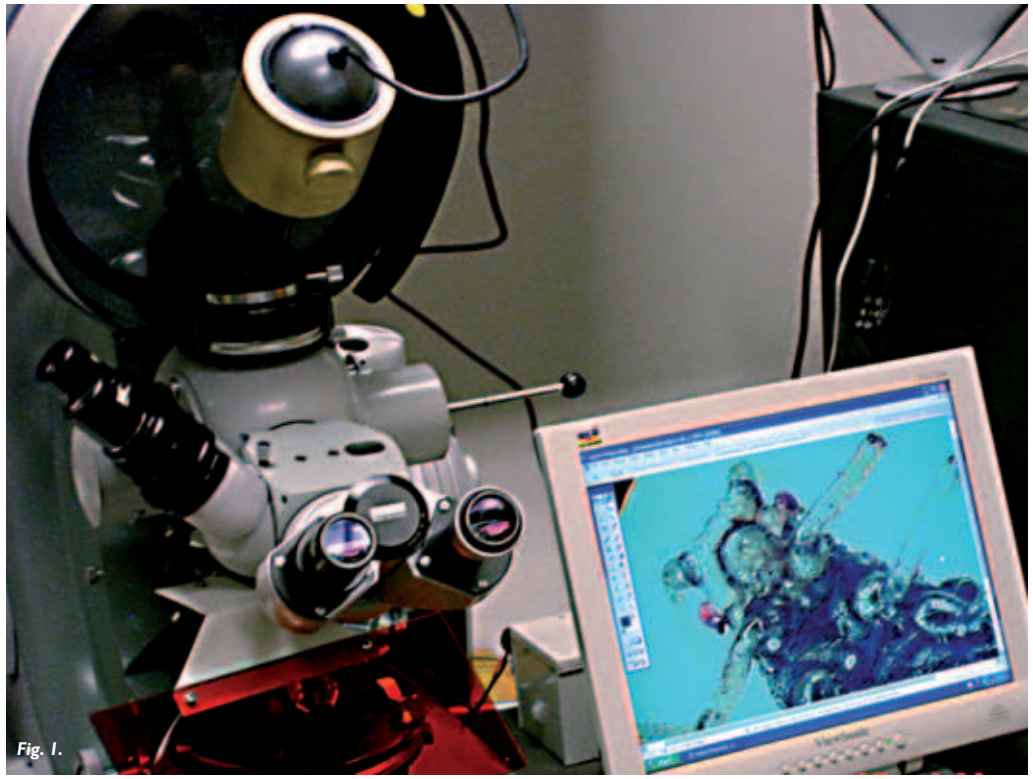


Fig. 1.



Fig. 2.



Fig. 3.

Figs. 1-3. *The Logitech 5000 Pro camera and its heavy brass adapter mounted on a Zeiss "Ultraphot III" microscope. The adapter fits into a monocular viewing tube with a 10x wide field ocular. The spherical camera body fits precisely into the brass camera carrier and can be aligned and adjusted with great precision along the optical axis. The live "image" is seen on the computer screen which enables accurate focusing. This adapter establishes a high-quality secure, vibration free connection to the microscope. An extra Zeiss "Optovar" (Fig.3) with steps 1.25x, 1.6x and 2x between the camera and the photo port may sometimes aid in additional magnification and full use of the area of the image chip. A Teflon supporting ring around the camera and a brass screw aids in keeping the alignment of the camera.*

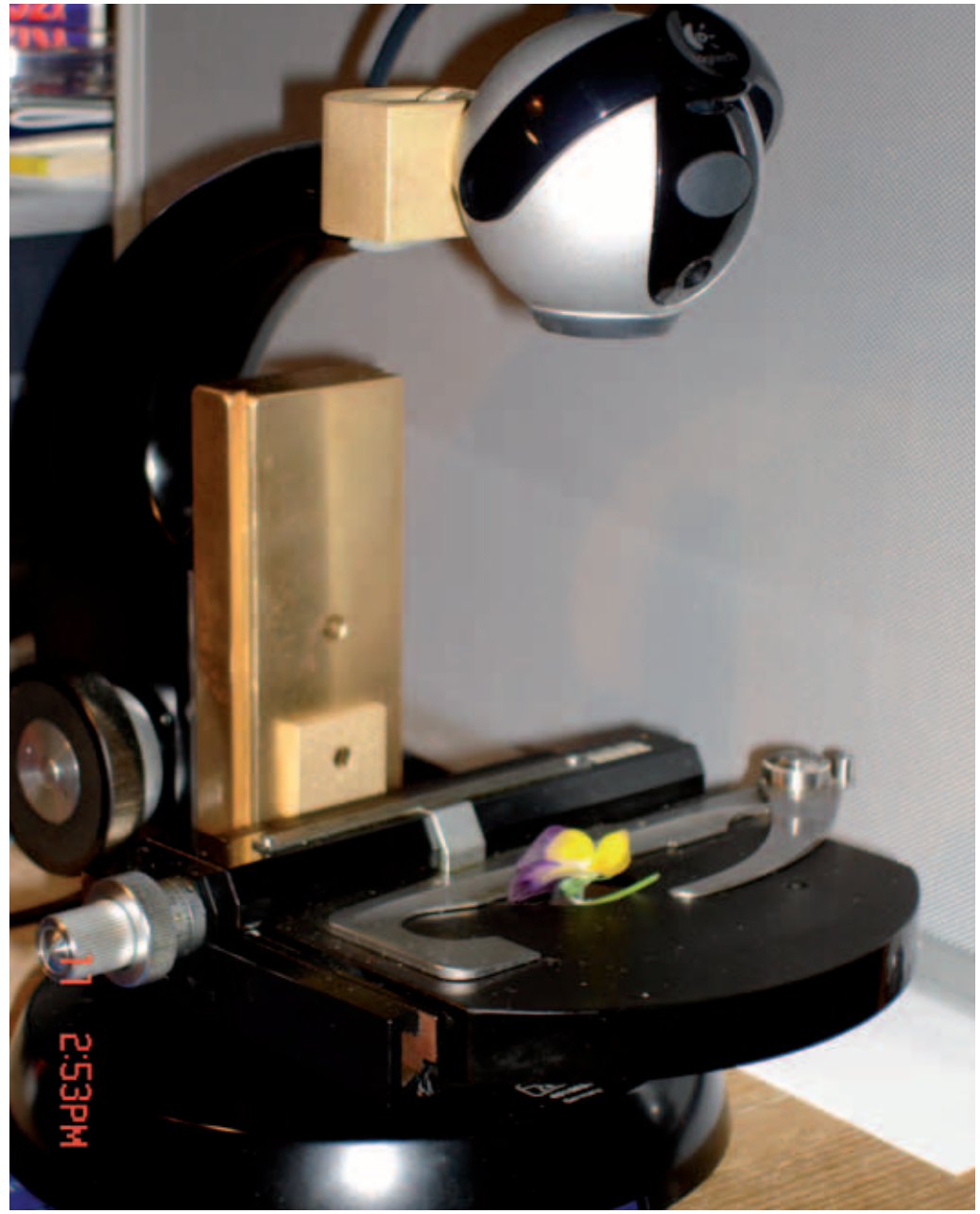


Fig.4. *Arrangement of a Logitech 4000 Pro webcam for overview pictures and pictures of low magnification. A Zeiss simple microscope body was modified at our mechanical workshop. An x/y stage was added, the microscope tube removed and a brass adapter for the web cams was made and attached so that the optical axis of the camera is aligned to the centre of the stage. Various light emitting diodes or classical microscope lamps may easily be attached.*

Much can be learned from amateur astronomers. They have been successfully using web cams for some time and are capable of recording images with a quality that previously was considered only possible by far more elaborate equipment in professional observatories. Quite detailed images of the moon and some planets have been made despite the usual atmospheric blurring. Even images of double stars have been obtained with such simple equipment. For examples see www.djcash.demon.co.uk/astro/webcam and www.usno.navy.mil.

In the most recent models the plastic lens has been replaced by a lens of five optical elements that give quite good images of true 1.3 mega pixels. Some are even able to produce video films with the same resolution. Further, new web cam models appear each month or so with improved qualities.



Fig. 6. A home-made adapter made by gluing a black Kodak film box to the camera and fastening it to a Zeiss 12.5 x ocular by aid of painters tape. The system is fragile, but will work well if care is taken not to break loose the cam by accident from its attachment.

Choice of web cams

Among several solutions that were tried on high quality classical microscopes, a number of models from Logitech proved suitable. These cams have a spherical shape, and all belong to the Logitech 3000-5000 series. Several other digital microscope cameras adapted to fit in the ocular tube have been tried, but none have yet matched the Logitech 5000 regarding picture quality, performance, options to control the exposure and reliability (www.logitech.com). The models 3000Pro and 4000Pro have an adjustable lens making them also suitable for photomicrography (Figure 4).



Fig. 5. *Alternative mechanical attachment by aid of a ring clamp fastened to the microscope and use of an old adjustable x/y stage for precise adjustment of the camera lens above the wide-field ocular. The adapters Figs. 2 and 5 have to be made in a mechanical work-shop.*



Fig. 7. *The vascular bundles in a section of a pine needle. Single image, transmittent light. Logitech 5000 Pro. Image width (horizontal) 620 µm.*

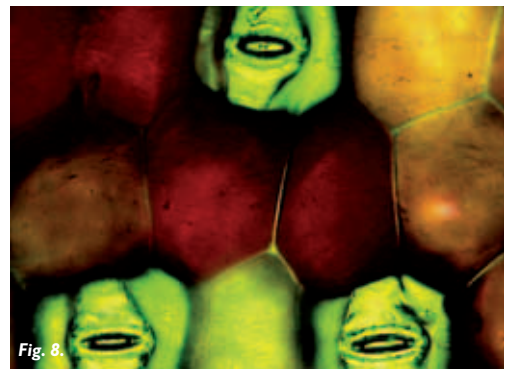


Fig. 8.

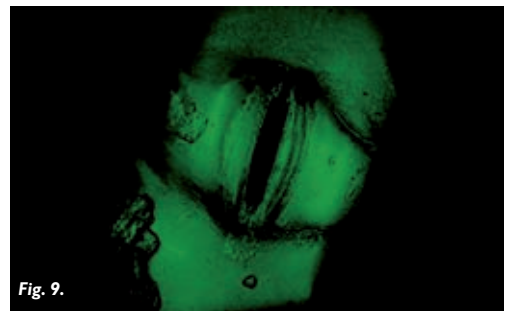


Fig. 9.

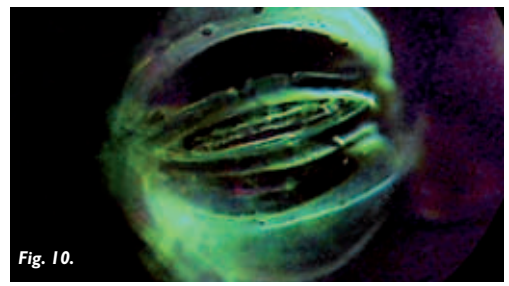


Fig. 10.

Figs. 8–10. *Stomata from the underside of a leaf Zebrina pendula, a common household plant, - "live" image. Incident light, dark-field, Zeiss Epiplan objectives, Ultraphot III microscope.*
Fig. 8. Image at a single focal level. Image width 180 µm.
Fig. 9. Image with an Epiplan 100x, oil lens, several images at different focal levels combined with "Combine-Z" for increased focus. Image width 90 µm.
Fig. 10. Same, but several images combined at a fixed focus, which increase resolution. Logitech 5000Pro.

Among my teaching duties is a microscope course for curators of art and archeology at the University of Oslo, in Norway. The 4000Pro has proved useful for close ups of many antique objects, it being much more efficient than other equipment (Figs.17-24). The images are sharp in the centre, and although a little less so in the periphery, even tiny details like almost "invisible" cracks in ancient photographs (Figure 22) and fine lines in an iron-nickel meteorite show up clearly (Figure 17). If the lens of this camera model is removed it can be used for high-quality time-lapse microscopy and even advanced research of single cultured cells (Laane 2006).

For general photomicrography (Figures. 7-12, including some quite difficult subjects, Figures. 13-22, 25-29) the Logitech 5000 is preferred. It has a fixed wide angle lens of improved optical quality. The electronic circuits are also improved and tests showed the image quality to be better than a number of commercial microscope cams said to be 3 mega pixels. The lens diameter is tiny, about 2mm. The plastic collar surrounding the lens protrudes so the cam sits a little too far out from the exit pupil of the ocular. If the camera is to be used with a microscope, accurate alignment to the microscope becomes very important as even slight displacements of the camera relative to the optical axis results in no image on the computer screen.

Adaptation of the camera

It is essential that the tiny lens is close to the ocular lens. This requires that the surrounding lens collar be removed in most cases, otherwise only a small circular image will appear in the centre of the data screen.

For amateur use, open the camera carefully by removing the screw close to the USB cable. Carefully separate the half spheres and remove the barrel around the lens; don't touch the latter which is fixed to the tiny electronic circuit card. Glue a small piece of black paper on the green emitting



Fig. 11. *Black fungus growing on a white painted piece of wood from an old house. "Spores" are forming in the hypha. Zeiss "Ultraphot III", Epiplan 100x/1.25 oil. Combine-Z, made from stack of 5 pictures. With of fungal hypha 7.5 µm.*

diode inside, otherwise green reflected spots will appear in the image. Carefully put carefully the camera halves together again. This is quite difficult if you have not noted down how the various parts were assembled. Next use a black plastic film canister from a Kodak colour film. Make a transverse cut so its bottom is removed. The cut should be nice and clean. The cut side of the box will precisely fit a small circular ridge in the camera house, so the film box

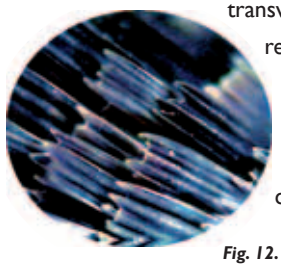


Fig. 12. *Scales of a bluish iridescent wing from a butterfly collected near San Jose in Costa Rica in 1986. Zeiss Ultraphot, dark-field, Epiplan 40x, stack of 10 pictures, Combine-Z. Image width 440 µm.*

can be temporarily fastened, thus becoming precisely aligned. Fasten the camera horizontally in a mechanical vice, add two-three small drops of polyurethane glue at two points inside and leave for polymerization. Finally, make a tiny ring layer of glue around the joint edge inside, being careful not to drip any upon the lens. You have now got a near perfect camera that in many cases may produce still images, videos or time-lapse films, sometimes of stunning quality.

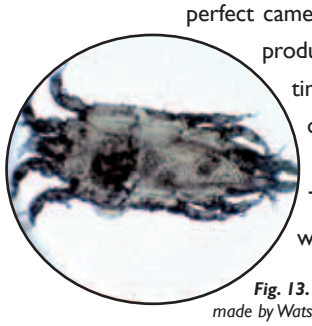


Fig. 13. *Acarid, old microscope preparation made by Watson & Sons, London about 1900. The Logitech cam, Fig.6 was used on a Leitz SM Microscope from 1964 with Heine condenser and a PV 10 x apochromate lenses. Single-focus image. Image width 580 µm.*

The camera is to be used with a wide-field ocular (for example Zeiss 10xBr 12.5x, angle 50o, part no. 464142). I have also found an old ocular from American Optical, which was used for their zoom stereomicroscopes in the mid 1960s that functions excellently. A little adhesive tape wound around the ocular secures quite a stable mount to the camera. Just insert it in the vision or photo tube of your old microscope.

I recommend making a more advanced adapter if the cam is to be used for scientific work. It is remarkable what can be achieved with high-quality microscopes from the 1970s. Among these the Zeiss "Standard" series is recommended (see

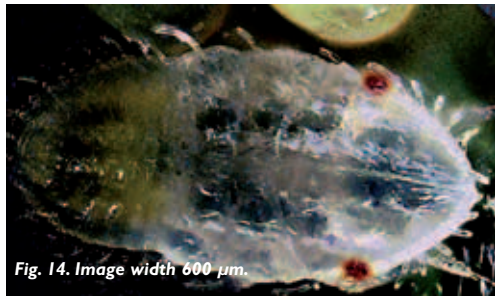


Fig. 14. Image width 600 µm.

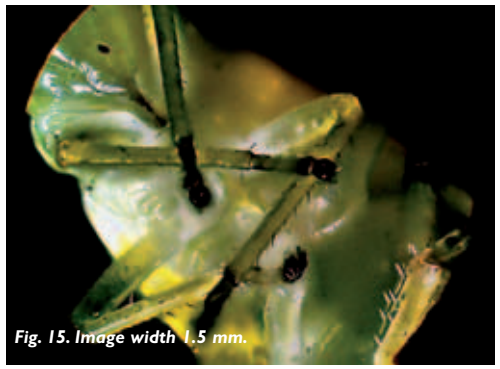


Fig. 15. Image width 1.5 mm.

Figs. 14 &15. *Young (Fig. 14) and adult (Fig. 15) aphids photographed live on a leaf of nettle found outside the biology building, University of Oslo. Made from image stacks of 8-12 pictures, combine-Z.*

Henkel 2004). Many are still used in research institutions, but they are also now available on the internet, often in good condition and well maintained for acceptable prices, even for hobby microscopists, see:

http://buy.ebay.co.uk/microscope. More than 3000 microscope parts and microscopes are available at any one time.

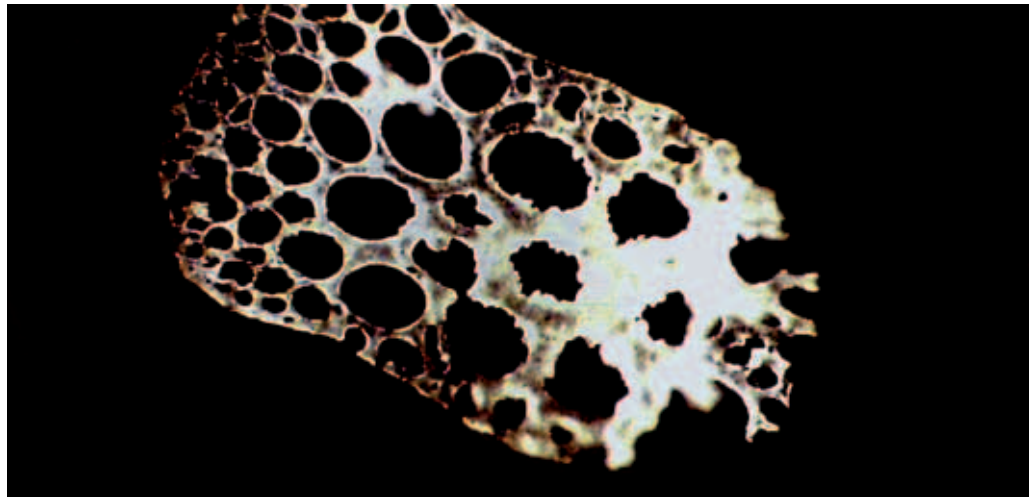


Fig. 16. *Ossicle from the skin of a cucumber. These beautiful structures are about 0.2 mm in size and made of CaCO3. E. Leitz SM microscope from 1964, Heine condenser, dark-field, PV 40x apochromate lens. Single image. The Logitech cam Fig.6 was used. Ossicle length 950 µm.*

For making a professional adapter you will need access to a mechanical workshop. Furthermore, the microscope has to be quite large in order to exclude any vibration effects blurring the finest details of the images. Two options for possible adapters are shown (Figures 1-3 and 5). I have used the latter versions upon the classic Zeiss Universal and also the huge Zeiss Ultraphot III B microscope. For smaller instruments these setups are too heavy.

Microscope options

The small camera functioned well on a classic E. Leitz microscope from 1905 and beautiful images of histological sections were made. An even older



Fig. 17. *Photomacrograph of a piece of the iron-nickel meteorite "Gibeon" found in Namibia showing the characteristic Widmannstetter figures that proves its celestial origin. Logitech 4000 pro, set-up as in Fig.4. Original image 1.3 mega pixels. Magnification 4x.*

brass microscope, one E. Leitz from 1895, did not however produce suitable images, probably due to spherical aberrations. An E. Leitz SM from the 1960s fitted with apochromats and a Heine condenser gave images that even got an artistic "hue", especially as the Heine condenser can be adjusted to function in intermediate manners between bright field, phase-contrast and dark field, just by turning a single knob.

The Zeiss Universal microscopes and also the Ultraphots have numerous options for incident light. The latter has options for three different lamp houses or illumination systems. By turning a handle one might change between them. The pumpkin lamp house is big enough to introduce a small bottle with parallel sides containing distilled water and some crystals of copper sulphate to remove infrared radiation and heat, which may cause damage to "live" specimens. The handle with the mirror inside which reflects the light up to the incident condenser may be manipulated to change the angle of the illumination in order to prevent overflow of light in the camera from strongly reflecting areas in the specimen.

Incident microscopy always has the potential problem of unwanted reflections. Reflections

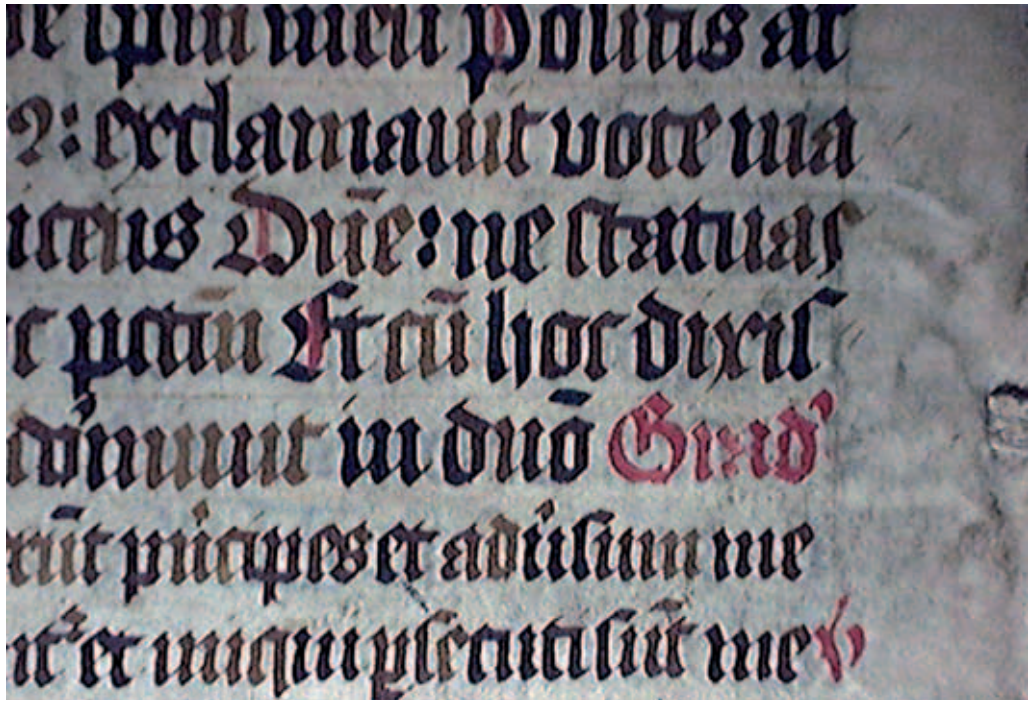


Fig. 18. *Handwritings on pergament. From original cover on old religious book "Pastorum natvrvumque Instrvctiones etc." Colonia MD LXXXVII. The pergament cover is supposed to be from around year 1400. Logitech 4000 Pro on Zeiss microscope body). Original 1.3 Mega pixels. Magnification 1.5x.*

occur especially when using dry lenses in the surface of the materials studied, and especially if a cover glass is used (not recommended). The Zeiss Epiplan lenses designed for dark field (illuminated by circular mirror(s) or lenses surrounding the objective) have extremely short focal lengths and only the oil immersion lens may (sometimes) be used with a 0.17 mm cover glass. For embedded specimens the Zeiss "Antiflex" lenses (Parts no. 461364, 461554 and 461754, old Zeiss catalogue 41-101-d, W III 771) may be used with success, especially those of lower magnifications (8x, 16x, 40x) but they are extremely rare on the market. Two polarization filters and a lambda/4 plate in front of the lens remove unwanted reflections.



Fig. 19. *Wasp. Vespa vulgaris. Microscope preparation (insect embedded in Canada balsam and "squashed" flat) made by Watson & Sons (around 1910). 313 High Hollborn, London. Logitech 4000Pro (on Zeiss microscope body). Original 1.3 mega pixels. Wasp length 25 mm.*

Troubleshooting

Amateurs that use a cut film box as an adapter to the microscope tube (Figure 6) may experience difficulties for keeping the alignment of the cam above the ocular. Due to poor adhesion of the plastic glue to the cam it might also become detached. But with careful handling, this primitive set-up may last for a considerable time. Better images are however, obtained with the adapters (Figures 1 and 3-5) where any vibrations are excluded.

As the Logitech 5000 is quite light sensitive any amount of uneven light will show up on the data screen. It's very important that the microscope is adjusted to Köhler. There may also be problems with samples showing strong colour contrast

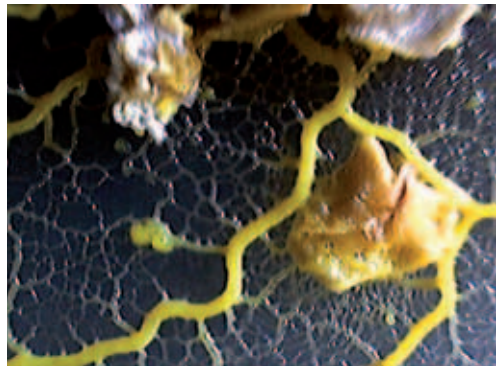


Fig. 20. *Agar culture showing live veins of the slime mold Physarum polycephalum. Inside the veins the cytoplasm streams in a rhythmic patters similar to sine- or cosine waves. Single image with Logitech 4000 Pro Web cam. If set in time-lapse mode, these oscillations will be seen by the eye even with this slight magnification. Image width 25 mm.*

(Red-Green). With such specimens there is a great risk that some areas will appear without any details at all. At low light conditions any dust, especially in the eye or field lens of the ocular, will show up in the image. A clean microscope is therefore essential.

Increased resolution, focus, and panoramas

A number of years ago the usefulness of web cams in microscopy was clearly demonstrated, see



Fig. 21. *The Norwegian Botanist M.N. Blytt and his son Axel corresponded with Charles Darwin about past climate changes after the last glaciation. Tiny, small and faded photograph from the 1860-ies. Logitech 4000, set-up as in Fig. 6. Image 1x.*

www.nexusresearchgroup.com/research_papers/nzms1999b.htm. In some respects they appear to have improved in quality much faster than professional equipment. In chromosome cytology where squash preparations are made in order to study the morphology of single chromosomes, there are always problems obtaining images showing all chromosomes or their parts in focus. However, with a web-cam this is easily done with combine-Z. A new version is also now available; this allows AVI-films to be represented as a stack of single images. Turning the fine-focus knob steadily for 2-3 seconds will produce a stack of 30-90 pictures, resulting in superb images.



Fig. 22. *My great grand-father Lars M. Laahne, tiny, very early photograph (diameter about 1.5cm), probably a silver-mercury plate. Note that the web-cam is able to show the small cracks in the photo-layer. Logitech 4000Pro, set-up as in Fig. 6. Magnification 2x.*

Many of the remaining problems with the use of web-cams and classical microscopes, compared with "modern" ones, can now be easily overcome with soft-ware that is available free on the internet. www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm.

If several images of the sample with the same focal level are merged, details become visible that cannot be easily seen by visual observation. The physical principles behind this may be discussed, but this option is well known among amateur astronomers. The increase in the details in the images appears, in part, due to improvements in



Fig. 23.

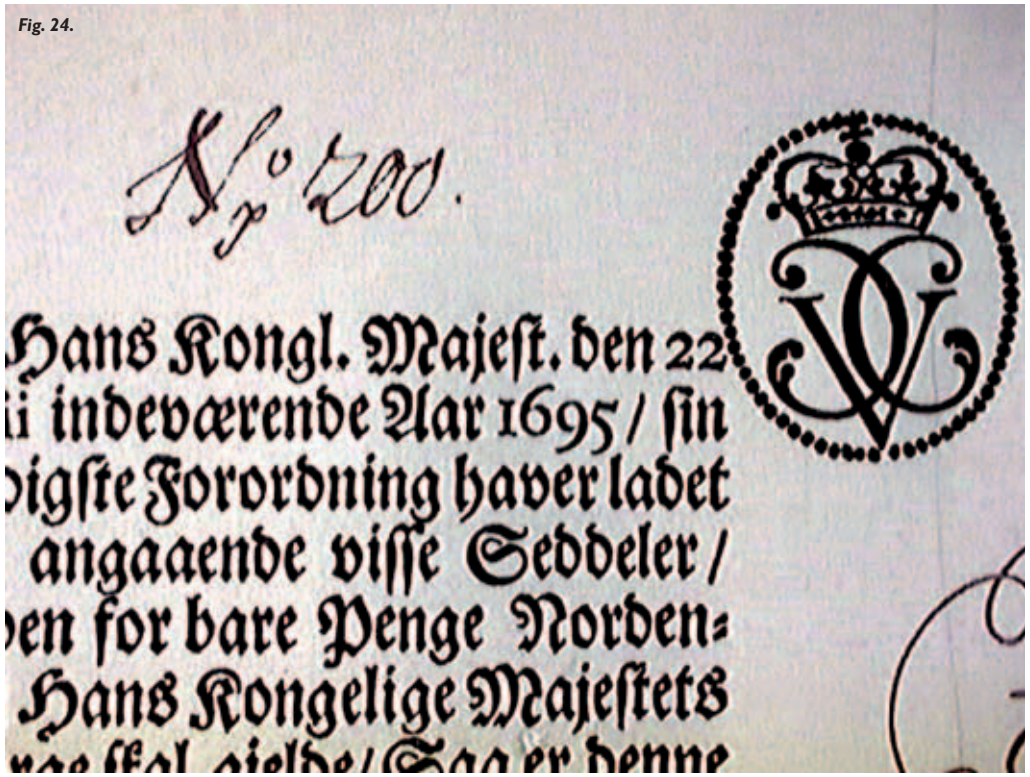


Fig. 24.

Figs. 23 & 24. *Examples showing that the web-cam is quite able to make photo macrographs.*
Fig.23. mechanism inside a gold pocket watch, Image width 40 mm.
Fig.24. detail from a bank note issued by the Danish-Norwegian King in 1665. Set-up as in Fig.6. Image 1x.

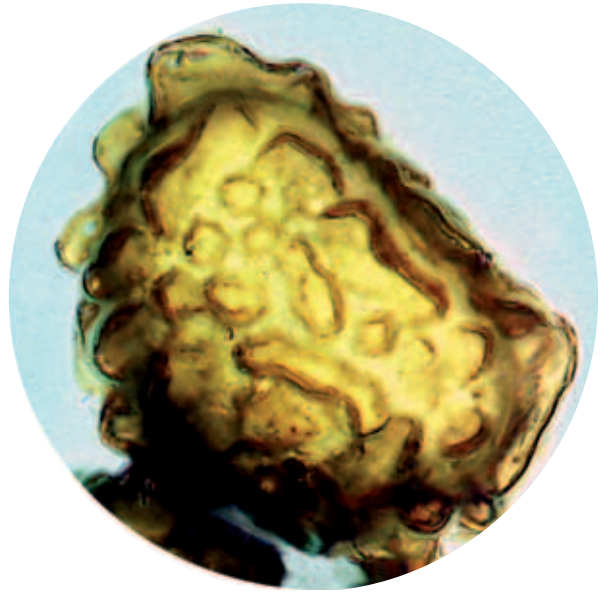


Fig. 25. *Spore from Dryópteris filix-mas. Made from stacks of 8-10 pictures at different focal levels, Zeiss Ultraphot III, incident dark-field, Epiplan 40x and Epiplan oil 100x/1.30. Combine-Z. Image diameter 50 µm.*

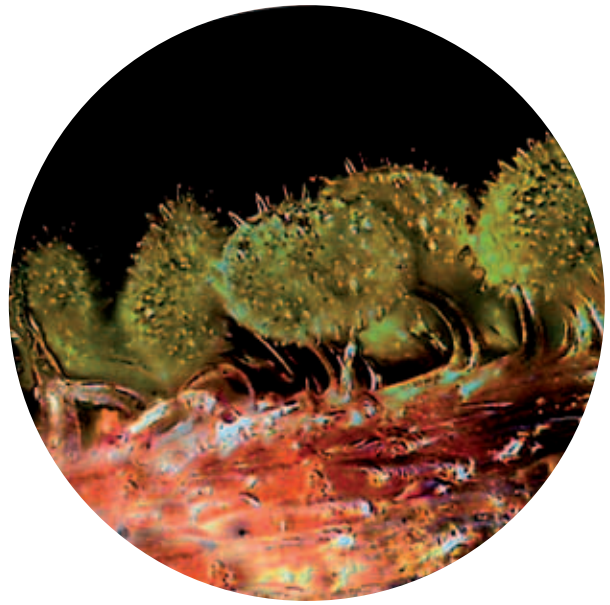


Fig. 26. *Spore from Althaéa officinalis. Made from stacks of 8-10 pictures at different focal levels, Zeiss Ultraphot III, incident dark-field, Epiplan 40x and Epiplan oil 100x/1.30. Combine-Z. Image diameter 300 µm.*

the balance between the signal and camera noise. An example of this is seen in Figure 10 which shows a single stomata from the underside of a "live" leaf of Zebrina pendula. Another option is to photograph small, overlapping sectors at high optical resolution of a subject, using a demo version of the program "Auto stitch" (www.cs.ubc.ca/~mbrown/autostitch/autostitch.html). The single images are automatically stitched and assembled like a puzzle into a huge "panorama" picture showing every detail, provided the optical resolution is high enough to be registered on the data chip (Figure 27). In order to produce the panorama, zoom the image on the screen slightly and adjust it with the camera software so that it fills the whole computer screen (not a circle) before exposures are made. This is a prerequisite for the software to work correct. The only thing to worry about is ensuring that there are enough images to cover the whole subject and that they overlap. They need not be exposed in a particular sequence; the program itself does the puzzle work regardless of the order of the pictures. Some errors may occur, but these are usually solved by manual corrections in Photoshop. The whole process is quite fast and is very useful for histological sections.

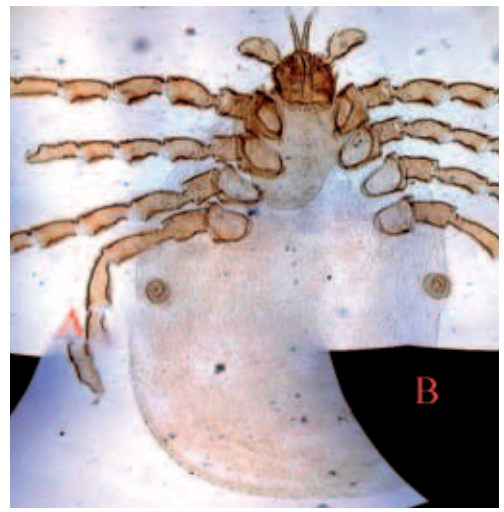


Fig. 27. *Composite "panorama" image made of (probably) Ixodes ricinus from old microscopical preparation. Mosaic image automatically made by "Image Stitch" program. Logitech 5000Pro. Incomplete panorama shown to show the process. At (A) there has been some misalignment, can be corrected by hand in "Photoshop". At (B) a single image has been omitted during the stitching process. Length of tick 3 mm.*

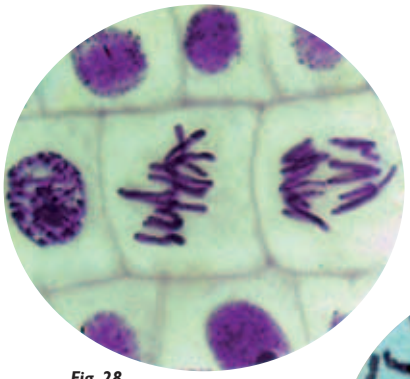


Fig. 28.

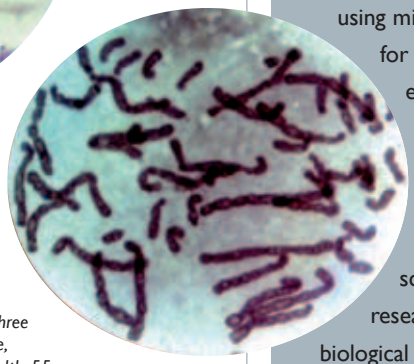


Fig. 29.

Figs. 28 & 29. *Examples from the use of webcams for photographing chromosome preparations.*
Fig.28. Length sections of root tips in Allium cepa. Shows in sequence the three important stages of mitosis (prophase, metaphase and anaphase). Image width 55 µm.
Fig. 29. shows squash preparations of the huge mitotic chromosomes in the African blood lily (Scadoxus=Haemanthus katherinae). Made from stacks (6-8 images) of different focal levels to show all the chromosomes. Image width 90 µm.

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Professor C. Morten Motzfeldt Laane has been using microscopes for fun and research for more than 50 years and has experience in most kinds of modern microscopy. He has contributed more than 150 articles in English or German which also involve popular science, and development of new research techniques mostly on biological or biophysical topics. He has been a Fellow of the Royal Microscopical Society for many years.

