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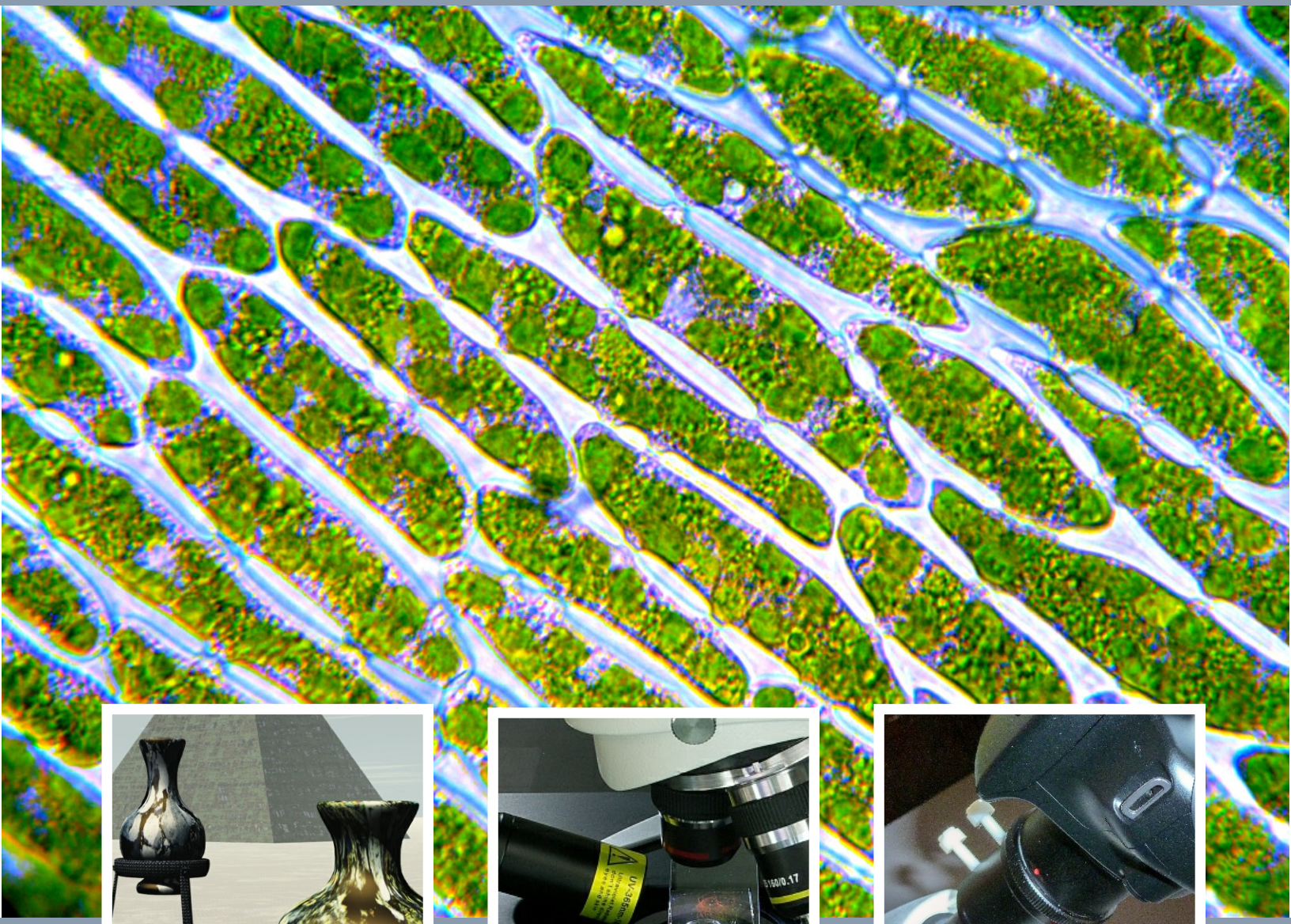

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Spider

Front Cover: Moss cells (Oliver Kim)
Left image: Carl Hennig
Middle image: Neill Tucker
Right image: Carl Hennig

Back cover: Moss leaf

Moss cells - the effect of white balance

Digital editing can sometimes reveal structures that are otherwise difficult to see.

By Oliver Kim

During a recent walk through the forest, I collected some moss and continued to culture it on my desk on a shallow plate. Countless organisms could be found in the moss, and also the moss plant itself is quite suitable for microscopy.

Figure 1 shows the original image, Figures 2 and 3 had white balance and automatic contrast enhancement applied. The image looks much fresher and some structures are significantly better visible.

The green chloroplasts can now be differentiated from the cytoplasm, which is now purple. The plasmodesmata are the regions where two adjacent cells touch each other, for inter-cell communication. The cell wall is now white separating it from the cell contents.

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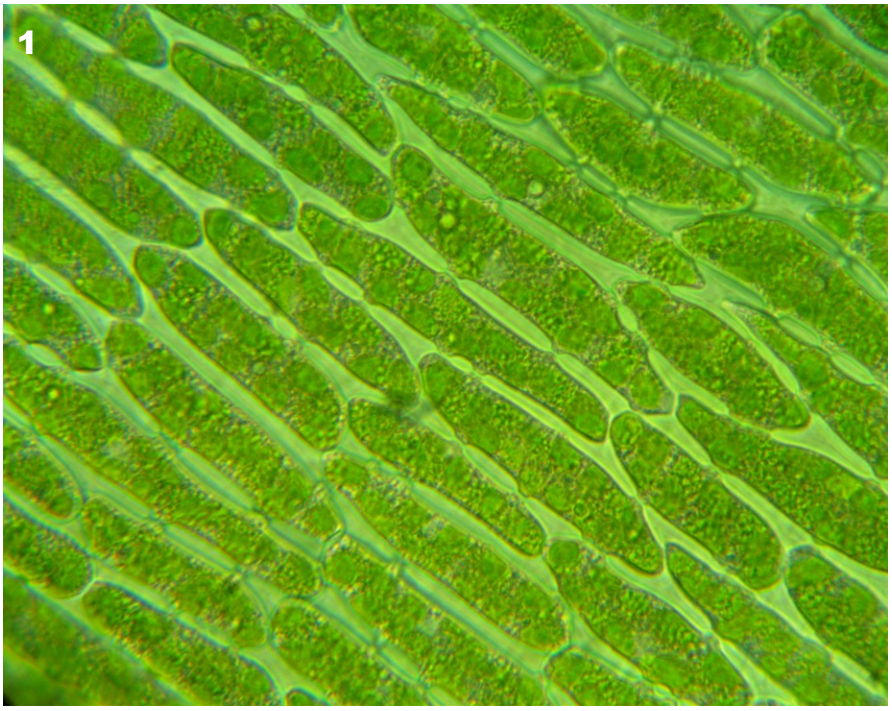


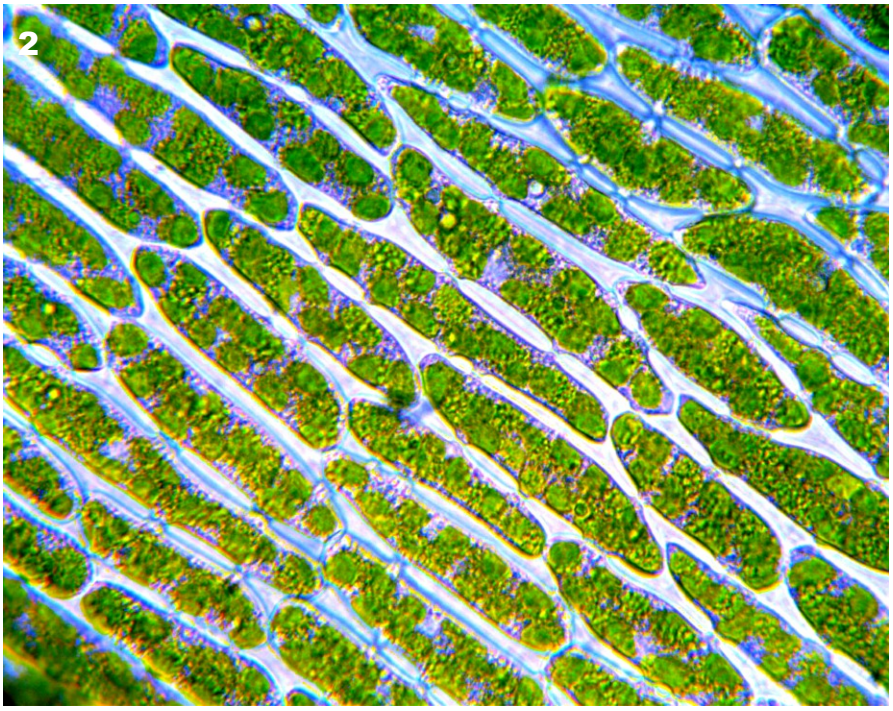
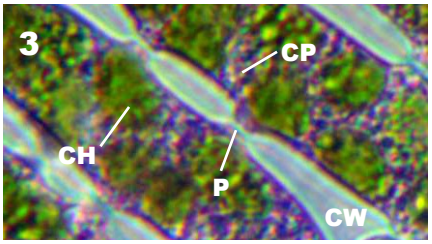


Figure 1: Moss cells. This is the original image as seen under the microscope.

Figure 2: The same image with white balance added.

Figure 3: CW: Cell wall (white); CP: Cytoplasm (blue-purple); P: Plasmodesmata; CH: Chloroplast (green).



Canon EOS Rebel T3 / 1100D as a microscope camera

Connecting a digital Single Lens Reflex (SLR) camera to a microscope has several advantages over dedicated microscope cameras.

By Carl Hennig

Recently I have been using my Canon 5D MkII DSLR as a camera on the Labomed CxL microscope. While performance and image quality are excellent, the cost ratio of camera/microscope is ridiculous.

There are two other dedicated microscope cameras available for use on the microscopes: the Future Optics 5MP USB 2.0 and an OMAX 10MP USB 2.0. The Future Optics 5MP has not been used for some time due to the high number of hot pixels. Hot pixels are pixels that are always on and show as white on a black field. With most biological micro photos, the white pixels would go unnoticed. But with a polarizing microscope, the hot pixels are very obvious when the polarizers are crossed or at extinction.

The OMAX 10MP does not have the hot pixel problem, but suffers from a slow frame rate. The specified rate at maximum resolution is 3fps and in theory should give 25fps at 640x480 resolution. The OMAX 10MP rarely gets 3fps and has never reached more the 7fps at 640x480.

These slow frame rates make accurate and quick focusing all but impossible. The high frame rate of 29fps of the Canon 5D MkII at 1920x1080p make focusing an absolute dream and HD microscope movies possible.

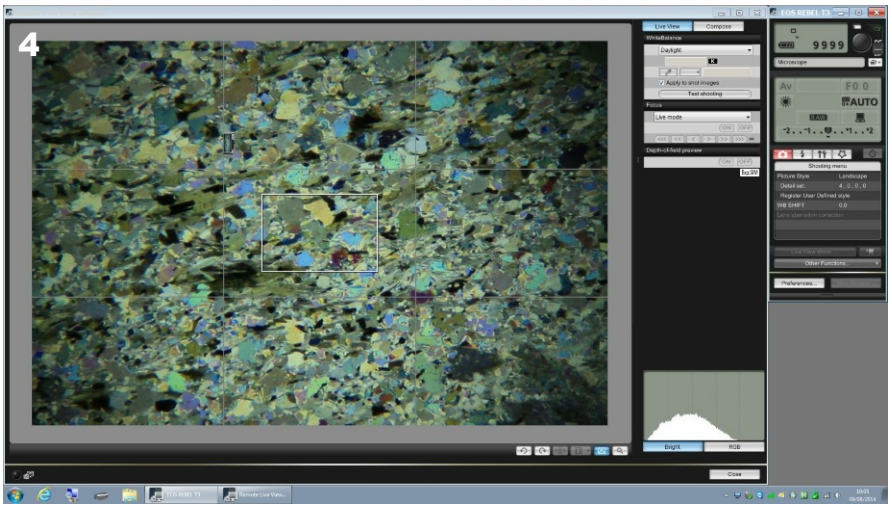
So, if dedicated USB 2.0 microscope cameras have a low frame rate, how about USB 3.0? The short answer is: better. A 10MP USB 3.0 microscope camera tops out at 7-10fps. This is probably the low end for easy focusing, but certainly acceptable.

USB 3.0 cameras with a 7-10fps rate come at a significantly higher price than the equivalent USB 2.0 camera. $500.00 compared to $250.00.

Surely there must be something available in the photographic universe with a decent frame rate and image quality at a more reasonable price than a Canon 5D.

As a Canon user, I quickly found a low cost DSLR, relative to USB 3.0 microscope cameras. Canon has a series of EOS 1000D (Rebel T series in North America) cameras positioned as entry level DSLRs.

Figures 1 and 2: Canon Rebel T3/1100D and Labomed Cxl.



Figures 3: Canon EOS Utility showing preview of 2x objective.

Figures 4: The EOS Utility control.

As the prime purpose of the selected camera would be taking microscope photos, most of the photo features are not a must-have requirement. The camera should have a 10MP or larger sensor, full manual control of the exposure process, a broad ISO and shutter speed range, high frame rate, software for viewing and capture on a computer, cost the same or less than a USB 3.0 camera and that is about it.

The Canon camera that met my specifications was the EOS 1100D or Rebel T3. A thorough search on the web turned up a special on the Staples (the office supply people) web site for $250.00CDN, shipping included.

The only extra item required was a microscope to camera adapter. The possible adapters vary in price and method of use.

The most basic is the prime focus type. No lens, no eyepiece, just a 23mm tube on a T-ring adapter. This adapter works, but does not cover the full frame. Approximate cost: $22.00CDN

The next level up uses one of the microscope eyepieces. The limiting factor is the viewing or photo tube which is 25mm or less in diameter. Depending on the eyepiece used the image fills the frame. Approximate cost: $20.00 CDN.

Further up the price ladder is the adapter with an internal relay lens. This adapter replaces the eyepiece. The relay lens is sized according to the sensor size. Approximate cost: $70.00CDN

And at the top of the ladder is the premium adapter with an integral eyepiece or relay lens. Approximate cost: $500-$900CDN.

In keeping with my goal of a reasonably priced setup, I selected the adapter that uses the microscope eyepiece. Unfortunately, the viewing tube of the Labomed microscope is 27mm, not 25mm. The size mismatch was solved by using a 1.25" telescope eyepiece adapter in place of the camera adapter eyepiece collar.

All Canon EOS cameras come with free professional software. Of particular interest to the microscopist are the EOS Utility for remote view and capture, Digital Photo Professional RAW editor and Photo Stitch for piecing together multiple photos.

With the EOS 1100D/eyepiece adapter on the microscope and Canon EOS Utility installed, I was ready to take microscope photos.

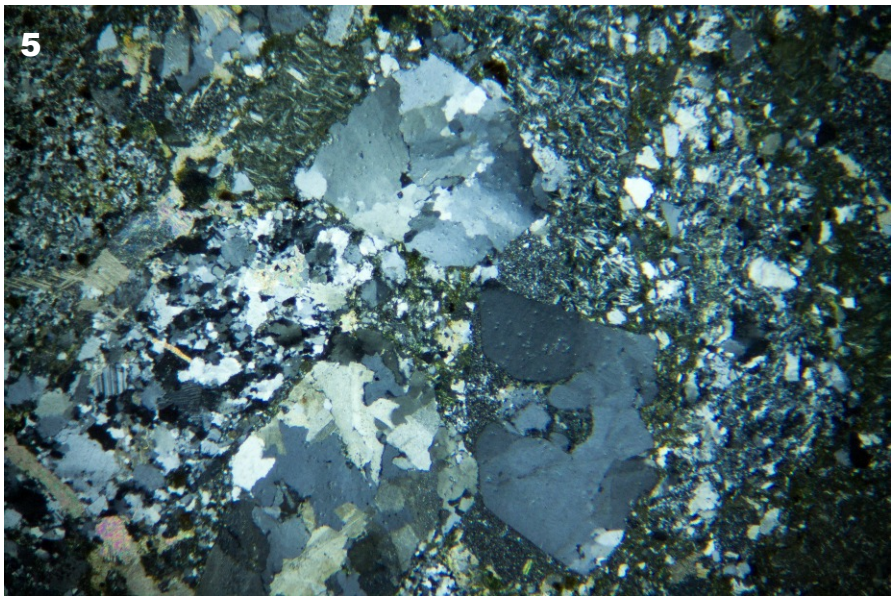
The Labomed has four objectives, 2x, 4x, 10, and 40x. I selected the 2x because I was sure the camera with a 10x eyepiece would not fill the frame, just like the 5D. To my surprise, the frame was full, but not uniformly illuminated.

In retrospect, I should not have been surprised. The sensor of the 5D is about three times larger than the 1100D and requires a bigger view to fill the frame. The light drop-off in the corners is noticeable and can be compensated either with software or by using a Photo Stitch to piece together four images that include each corner.

The EOS Utility lets you set many camera settings, specifically ISO, white balance, shooting mode, file name, image quality, exposure reading method, etc.

The EOS Utility sees the microscope eyepiece and objective as a 0 f-stop lens. Therefore the only camera settings that affect the exposure are the ISO and shutter speed. Interestingly, the camera sets the lens as a 50mm focal length in the EXIF data.

It appears as though almost all settings for the camera using the EOS Utility are soft and independent of the actual camera switch settings. No stand-



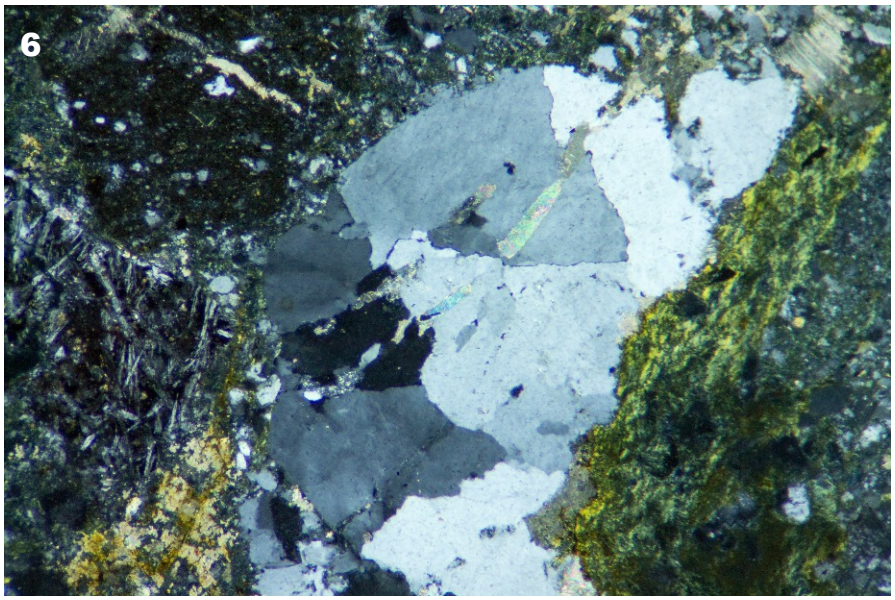


Figure 5: Greywacke Sandstone, 2x objective.

Figure 6: 4x objective.

ing up and looking down at the top or back of the camera to set switches.

For the first set of photos, the camera was set to Av (Aperture priority), ISO at Auto and white balance to Daylight. The camera's auto exposure set the shutter speed.

A nice feature of the EOS Utility is "Test Shooting" where a snapshot is taken, but not saved. My first test shot showed a good exposure so I was ready to try a real shot.

By placing the mouse pointer over the shutter button, the exposure settings are displayed. I was surprised to see the ISO setting at 3200 and the shutter at 1/60sec. Increasing the brightness of the light source to maximum, the ISO was still at 3200 and the shutter speed decreased to 1/250sec.

Apparently, the auto exposure with a 0 f-stop lens really likes high ISO settings and adjusts the shutter speed accordingly, This was not good and reviews of the camera indicated ISO settings above 1600 were below par.

The answer to the Auto ISO problem was easily solved by setting the ISO to 400. Test shooting at ISO 400 showed a properly exposed photo. The camera can set a maximum Auto ISO speed other than 3200 ISO and this setting is respected by the EOS Utility.

Something that bothered me after the test snapshot was the noise. Not image noise, but audible noise. The mirror - the shutter mechanism - is loud. You certainly would not want to use this camera in a quiet theatre.

The good thing is that this mechanical noise does not appear to translate into vibration. None of the subsequent photos showed any sign of camera wobble.

How was the focusing? Smooth. Very smooth. I use a 27" monitor at 1920 x 1200 and the EOS Utility preview almost fills the screen.

The 1100D can save videos at 1280 x720 at 29fps and the EOS utility uses this resolution for the preview. thus the fast focusing ability.

Conclusion

As a microscope camera, with the exception of the Auto ISO and mirror noise, the Canon EOS Rebel T3 / 1100D passes the test. The image quality is very good, the ISO and shutter speeds have a large range and focusing is fast.

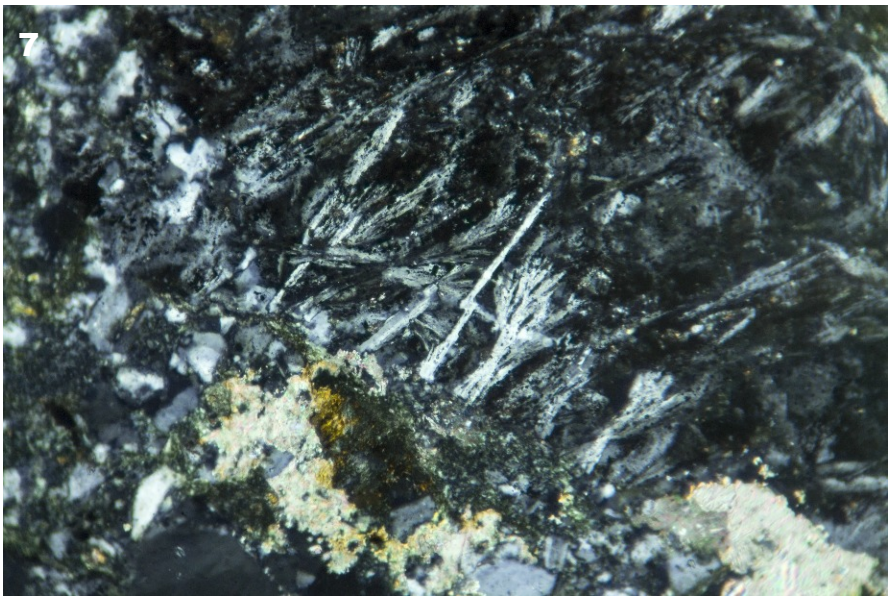
Unlike the other dedicated USB cameras, the 1100D appends the EXIF and the ITPC data to all photos. No more guessing about when and how the photo was taken.

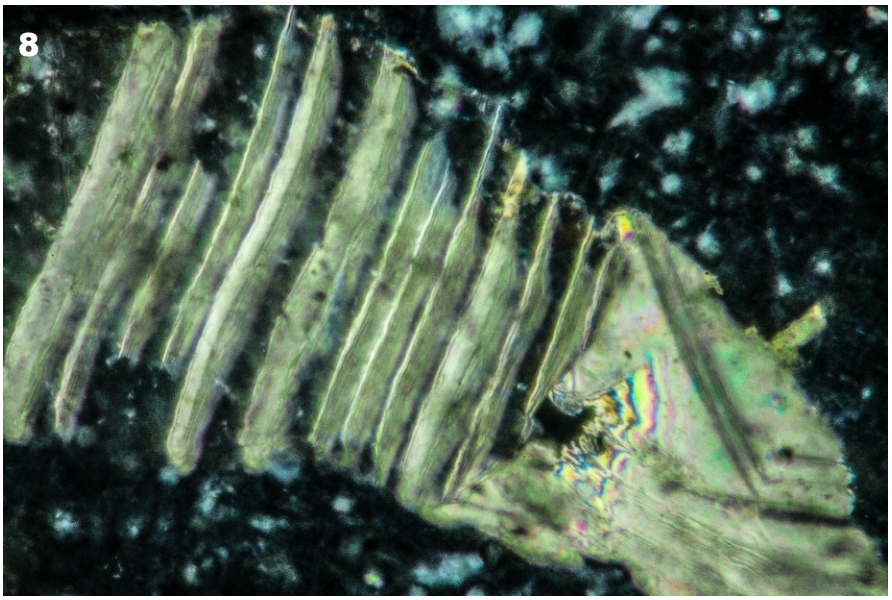
Other features that are a bonus to a microscope photographer are the ability to save in RAW format, JPEG files at different sizes and quality, very good free software and off the microscope it is a decent beginner's DSLR if you don't take pictures in a church.

The sensor in the 1100D is 11 times larger than the 10MP USB 3.0 camera. The larger sensor means bigger pixels and that means better light gathering and a greater latitude when processing the images. Better images, faster focusing and the 1100D costs $200.00 less

Figure 7: 10x objective.

Figure 8: 40x objective.





than the equivalent dedicated microscope camera.

What is not to like about the 1100D as a microscope camera? It is a bit loud when taking photos, requires an adapter to use as a microscope camera and if you are a professional or serious microscopist, the EOS Utility does not have the measuring or post processing functions of dedicated microscope software such as ToupView by Touptek, although you can download ToupView at no cost and open your photos in ToupView for the microscope specific functions.

Gallery

Figures 5-8 were taken by the Canon EOS Rebel T3/1100D, one with each objective.

The specimen is Sandstone (Greywacke), the microscope a Labomed CxL with polarizing kit and 10x eyepiece in the camera adapter.

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URL: www.staples.ca/en/Canon-EOS-Rebel-T3-Digital-SLR-Camera-Body-122MP-HD-Video-Black/product_956933_2-CA_1_20001

[4] Prime focus adapter
URL: www.ebay.ca/itm/New-Canon-EOS-Bayonet-Mount-To-Microscope-Eyepiece-23-2mm-Adapter/221333643059?pt=US_Lens_Adapters_Mounts_Tubes&hash=item3388835f33

[5] Eyepiece adapter
URL: www.ebay.ca/itm/MICROSCOPE-ADAPTER-TUBE-FOR-NIKON-DIGITAL-SLR-DSLR-CAMERA-NEW-MODEL-A1Q-/320955312970?pt=US_Lens_Adapters_Mounts_Tubes&hash=item4aba6d6b4a

[6] Adapter with relay lens
URL: www.ebay.ca/itm/Microscope-Adapter-Canon-Rebel-XT-XTI-XSI-350D-40D-50D-60D-7D-DSLR-Cameras-/171412562603?pt=LH_DefaultDomain_2&hash=item27e8fc22ab

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URL: www.ebay.ca/itm/LM-Scope-Digital-SLR-Microscope-Adapter-for-Canon-EF-mount-30mm-Tube-adapter-/161385158530?pt=US_Lens_Adapters_Mounts_Tubes&hash=item25934e1782

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DIY Fluorescence microscopy revisited

By using a flashlight with an UV LED, it is possible to do basic fluorescence microscopy.

By Neill Tucker

Advances in UV LED technology are making fluorescence microscopy even more accessible to the amateur, but with UV intensities around 50x that of sunlight there are significant safety issues to consider.

Since buying my microscope a couple of years ago I've read various articles on the different types of microscopy, often with a view to having a go myself, in some form or another. Fluorescence microscopy has always been lurking around on my to-try list, but as with all projects, I had to temper my ambitions according to my time and budget. However, recent advances in UV LED technology have made compact, powerful UV sources much more affordable and so the idea of a project more appealing.

The principal of fluorescence microscopy is to irradiate the specimen with a particular band of wavelengths that excite fluorescent chemicals within it. The energy absorbed by the chemicals is subsequently re-emitted at longer wavelengths. The difference between the excitation wavelengths and the emission wavelengths allows the emitted light to be filtered out and form an image, identifying where the fluorescing chemicals are in the specimen. The fluorescent chemicals may occur naturally within the specimen or are added to it, like stains in traditional light microscopy. Typical applications include, identifying different cell types and components within them, tracking of drug or nutrient uptake at a cellular level and forensic examination of crime scene evidence.

In the professional environment, fluorescence microscopy is a very precise business. In the microscope there is a narrow-band optical filter to control the wavelength of the incident light, a beam splitter and then another filter to isolate the light emitted by the fluorescing chemicals. These components are often combined into a single block to form an interchangeable module. The light sources themselves are powerful and their output has to be carefully controlled and directed for safe operation. Unfortunately for the amateur, this all comes at quite a price: a dedicated fluorescence microscope will cost around 3000 Euros upwards.

The schematic in Figure 1 shows the typical configuration for a fluorescence microscope, see [1] for a detailed description and explanation. As you can see from the schematic, the illumination of the specimen utilises the microscope's own objective lens, and the filter/splitter block is in the main optical path to the eyepiece. Clearly this configuration cannot be realised using a standard compound microscope without making major modifications to it.

So with this in mind, what are the options for the amateur? An article by César Guazzaroniin in the July 2011 edition of Microbehunter [2] described

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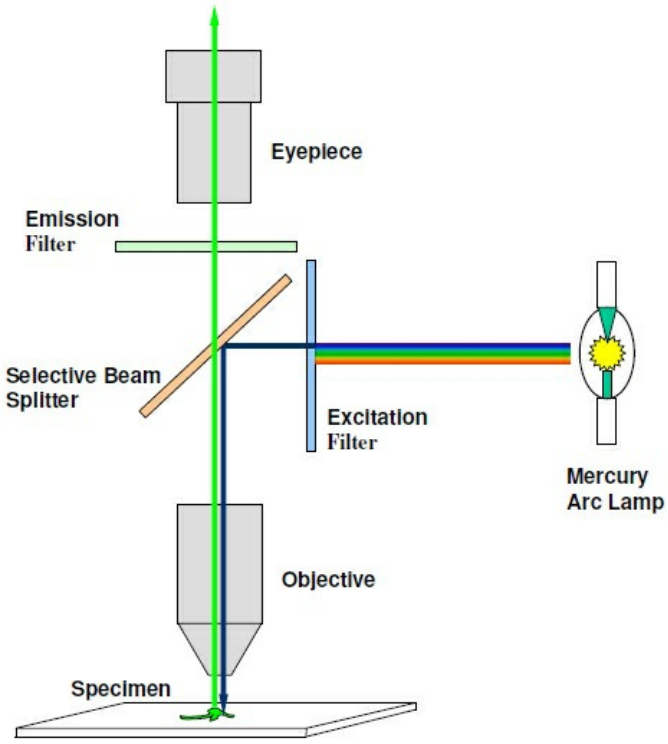


Figure 1: Schematic of typical fluorescence microscope.

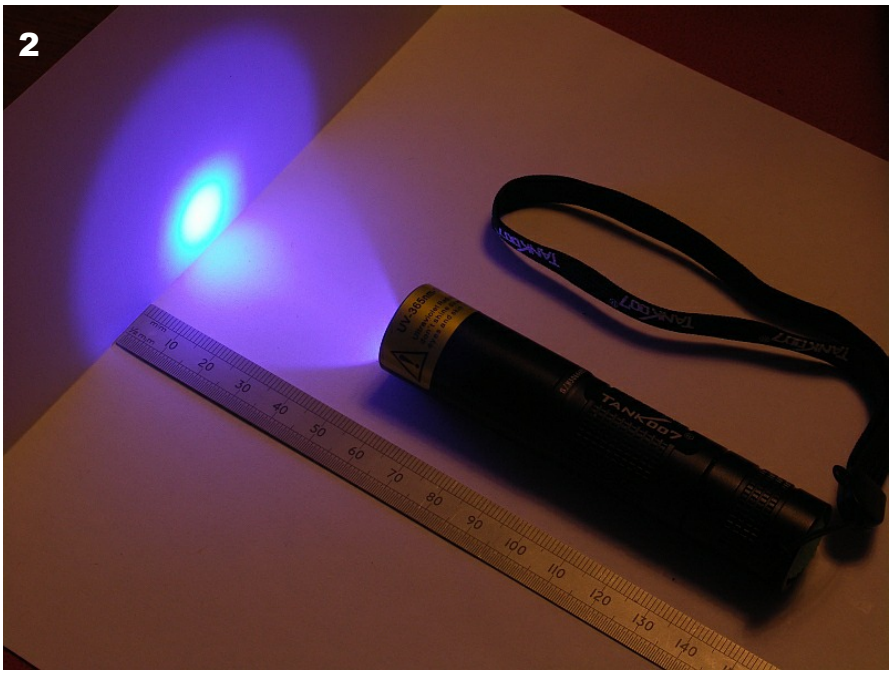


Figure 2: UV Torch (1W, 365nm).

a method using a ring of standard 5mm UV LEDs and fluorescent marker pen dye to 'stain' the sample. The advantage of using LEDs is that they are relatively narrow band light sources. This immediately removes the need for the arc lamp and excitation filter, reducing complexity and cost. The light is applied directly to the specimen as dark-field illumination, rather than through the objective. This obviates the need for the beam splitter and is safer than introducing UV directly into the optical path. Finally, the use of light outside the visible range for the excitation, limits the need for emission filters.

The main difficulties with the method described in [2] appeared to be getting a sufficient intensity of UV light and the wavelength of the UV light itself. The radiant power of a 5mm UV LED is typically around 10mW, even with eight LEDs illuminating the central square of a standard 2.5cm width slide, the power density is only around $(8 \times 10e\text{-}3)/(2.5)^2 = 0.013 W/cm^2$. Compare this to a typical power density of $150 W/cm^2$ in a dedicated fluorescence microscope [1]. Also, the wavelength of the so-called UV LEDs that were used was 395-415nm, which is actually still in bottom end of the visible spectrum (380-780nm). This means that the relatively weak fluorescent glow would be harder to distinguish from that of the LEDs themselves.

As with most areas of the electronics industry, LED technology has moved on rapidly since the article in 2011. Single UV LEDs with rated powers (see next section) in excess of 1W are now available in a wide range of wavelengths extending down to well below 300nm. For this somewhat simplified fluorescence microscopy application, higher power and shorter wavelength is generally better. Higher power means there are more UV photons per second to excite the fluorescent chemicals, so a brighter image. Shorter wavelength means that the excitation is further away from the visible range. However, this must be balanced with cost and safety considerations. Generally, the higher the power and the shorter the wavelength, the higher the cost and the greater the potential risks, see later.

LED Efficiency

LEDs tend to be rated by the electrical power consumed, the actual radiated power will depend on its efficiency, which can be anything from 8-42% depending on the wavelength, power rating and manufacturing technology. For example a 365nm UV LED sold as a 1W device will probably only radiate around 0.4W of UV power, with current technology.

Bearing all the above in mind and after some research on the Internet, I concluded that a UV LED rated at around 1W, at a wavelength of between 300nm and 380nm, was a realistic choice for this application. Since most of the LEDs at this power come as surface mount components or modules and require some sort of heat-sinking, I started to consider whether a ready made lamp or torch might be the best option. After some more trawling of the Internet I found a small, high quality UV torch (Tank007 Model 566) with a 1W, 365nm LED, powered by a single 1.5V alkaline battery [3]. The torch had the benefit of a smooth parabolic reflector that produced a nicely collimated beam. Shining the torch on a sheet of white printer paper, which fluoresces strongly, showed that at close range (up to 5cm), the majority of the UV light was concentrated in a circle of around 1.5cm in diameter, thus covering an area of $1.77cm^2$, see Figure 2. Assuming a 1W LED with 40% efficiency, this gives power density of about $(1 \times 0.4)/1.77 = 0.23 W/cm^2$. While still a tiny fraction of the $150 W/cm^2$ of a dedicated microscope, I estimate it to be around 0.23/0.013=17x higher than can be achieved using a ring of eight 5mm LEDs. More importantly the wavelength of 365nm is well below the visible limit, so although there is still some emission in the visible spectrum (the UV LED is not a perfect monochromatic source), it is very low compared to the UV output.

Using a small torch also has the added advantage of being portable, making it very easy to check possible items of interest out in the field; recommended uses included checking security

Figure 3: Bank notes under visible light.

Figure 4: Bank notes under UV light.

marks on ID cards and bank notes. The photographs in Figures 3 and 4 show bank notes under visible and UV light while Figure 5 shows green filamentous algae illuminated by the torch. The safety information supplied with the TK 566 was short and to the point , 'Do not shine flashlight directly towards the eyes', which seemed like very sensible advice. However, operation in conjunction with a microscope was probably not on the manufacturer's list of anticipated uses. I therefore thought it might be a good idea to explore the safety aspects of using UV light sources a little further, before doing too much experimentation.

Safety

Safety regarding UV exposure is already the subject of guidelines published by the International Commission on Non-ionising Radiation Protection, ICNIRP [4]. However, research into the biological effects and associated health risks is still ongoing, particularly in the area of UVA (315-400nm) exposure. While less directly damaging to cell DNA than shorter wavelength UVB (280-315nm) radiation, UVA is now thought to be damaging through indirect mechanisms involving the production of free radicals in combination with general immuno-suppression effects of UV exposure [5,6].

The UV exposure limits proposed by the ICNIRP are based on a total radiated dose measured in Joules/m^2, over an 8 hour period. Dividing this dose by the radiant flux, measured in Watts/m^2 (remember 1 Watt = 1 Joule/second) gives a time limit for exposure. The dose limit for exposure is defined at 30 Joules/m^2 at a wavelength of 270nm. Because the adverse biological effects of UV exposure vary with wavelength, a table of weighting factors is used to modify the measured flux value and give an 'effective' flux for wavelengths above or below 270nm. By interpolating between non-tabulated weighting factors, the exposure limit in seconds can be found for any wavelength in the range 180-400nm for any flux value. For broadband sources, a total effective flux is calculated by numerical integration of effective flux over the band of interest. See [4] for details of how to perform the calculation.

For the TK 566 torch I estimated the flux to be 0.23W/cm^2 (2300W/m^2 at 365nm). The weighting factor for 365nm is 0.00011, so the ICNIRP recommended exposure limit is therefore 30/(2300 x 0.00011) = 118s, approximately 2 minutes.

To try and relate all these numbers to something I had direct experience of, I looked up some data for solar radiation

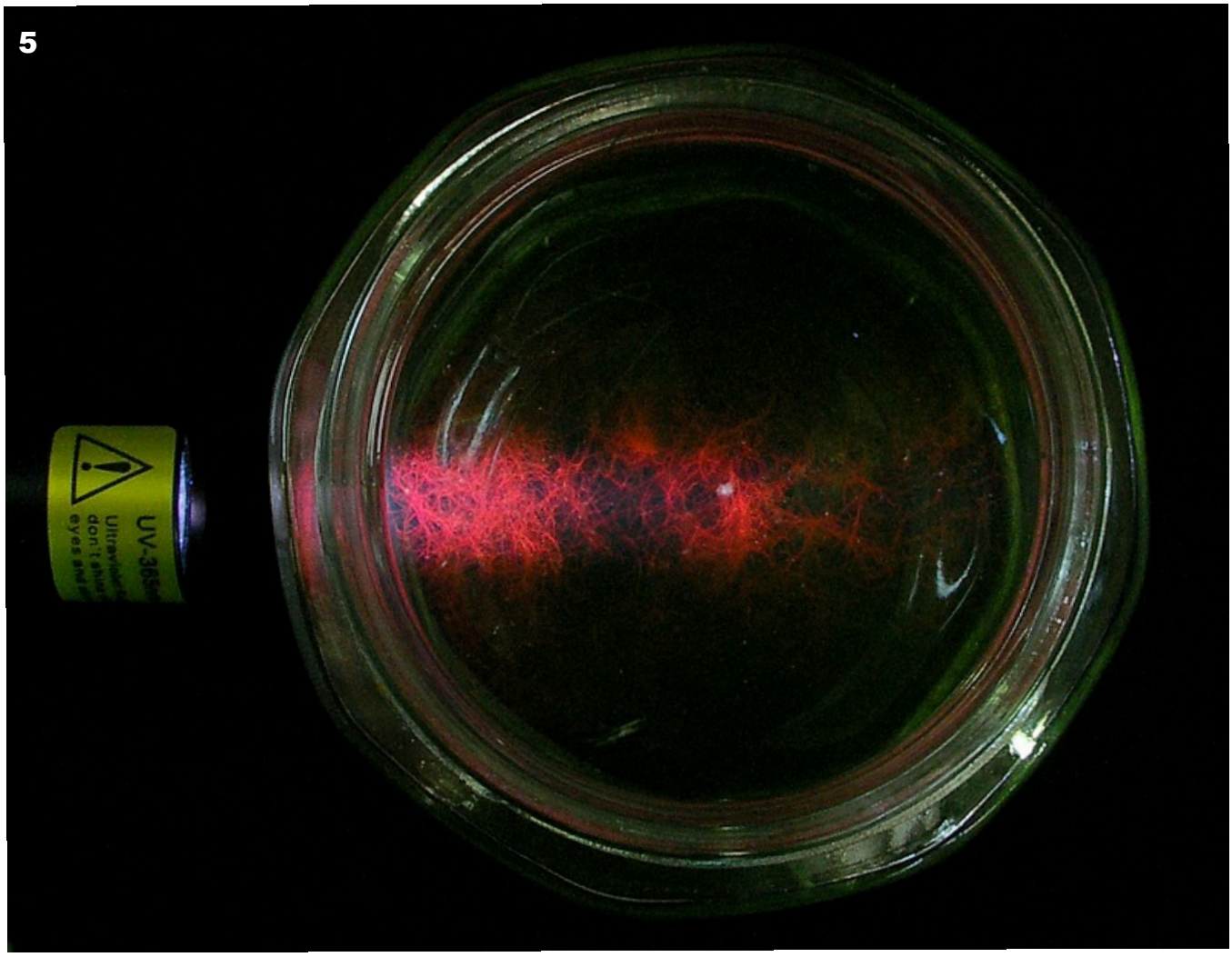


Figure 5: Green filamentous algae illuminated with a UV torch.

at the earth's surface. The data is provided by the American Society for Testing and Materials (ASTM) [7] and is listed as 'Terrestrial Reference Spectra for Photovoltaic Performance Evaluation'. There are various data sets available, I chose 'ASTM G173-03 AM-1.5 Global Tilt' which represents the average solar radiation spectrum experienced in the USA, for a plane surface inclined 37deg from the horizontal, towards the south. It is pretty much what you would experience if laying on a south-facing sun-bed, around midday in New York in the USA or Barcelona in Europe (Latitude 42° N), in the summer.

The graph in Figure 6 shows the spectrum of solar radiation at the limit of the earth's atmosphere AM-0 and at the earth's surface AM-1.5. The 'AM' stands for air-masses and represents the amount of the atmosphere through which the solar radiation travels, see [8] for a detailed explanation. You can see from the graph that relatively little of the solar radiation arriving at the earth's surface is actually in the UV range (<400nm). Using this data, I calculated the total solar flux below 400nm to be 46W/m^2 and the effective flux, using the ICNIRP weightings, to be 0.021W/m^2. This gives a recommended sun exposure limit of 30/0.021 = 1429s, approximately 24min., which seemed a reasonable value.

Having made some comparisons between the UV torch and normal sunlight, the main thing that concerned me was the difference in un-weighted flux values, 46W/m^2 for the UV component of sunlight versus 2300W/m^2 for the torch. Bearing in mind recent research into the detrimental effects of UVA radiation, the flux from the torch is 2300/46 = 50x more intense than all the solar flux below 400nm.

If we look back at the case of the dedicated microscope the flux is 150W/cm^2 (15e5W/m^2). However, courtesy of the microscope's objective lens (100x for this calculation), this huge flux value covers an area of only 1.2e-9m^2 [1]. This effectively makes the UV that exits the microscope a point source with a power of only 15e5 x 1.2e-9 = 0.0018W, which is then spread over the surface area of a sphere as you move away from it. At a distance of 1cm the power density would only be 0.0018/(4 x Pi x 0.012) = 1.4W/ m^2. A

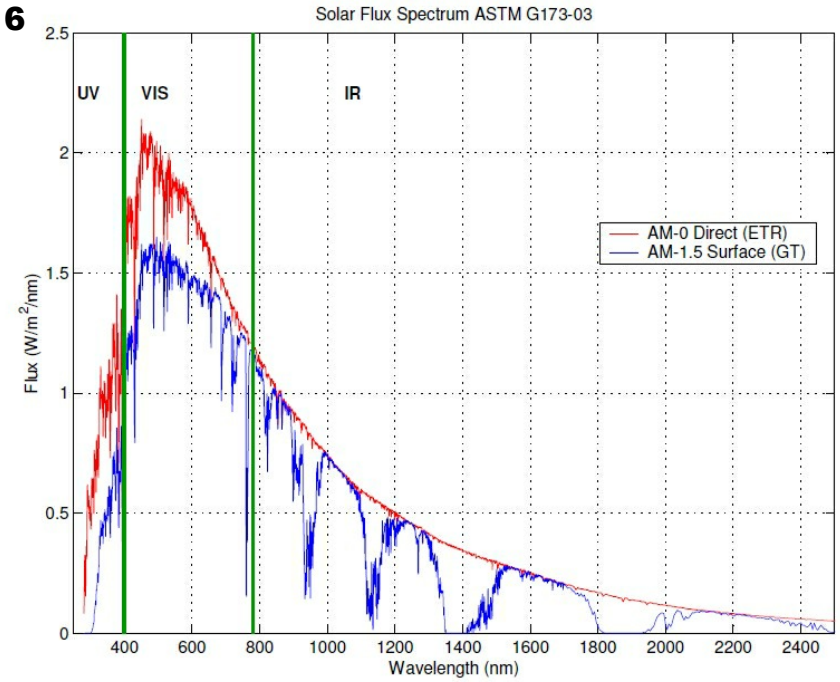
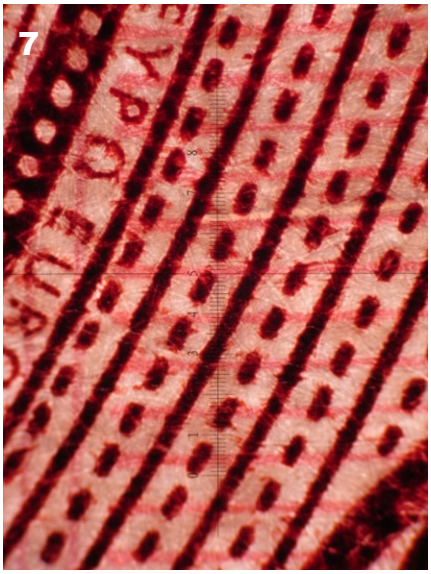


Figure 6: Solar flux spectrum



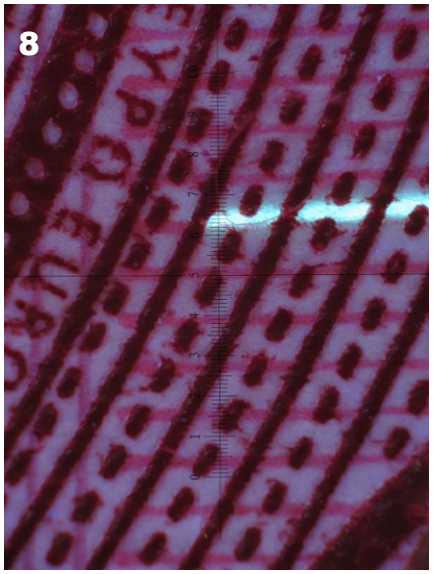


Figure 7: 10 Euro note under visible light

Figure 8: 10 Euro note under UV light

visible-light analogy of UV flux might be; the UV torch is like a car headlamp compared to the glowing wick of a snuffed candle, for the fluorescence microscope. Even so, dedicated microscopes still provide a UV screen in front of the objective turret to protect the user from stray UV.

Safety conclusion

My conclusion from all this is that powerful UV torches such as the TK 566 should be used with great care. Apart from the 'Do not shine flashlight directly towards the eyes' advice that came with the torch, I would certainly avoid direct exposure of the skin. Whenever operating the torch, a pair of wrap around sunglasses or goggles with full UV protection would be a wise precaution, just in case of strong reflections from smooth surfaces. When using to illuminate a specimen under the microscope, do all focusing with a normal white light source and then make fluorescence images using a camera, do not look into the eyepiece with the UV on, or at least not without eye protection if you do. The fluorescence from many specimens is very weak and is only really visible using a camera on a long exposure setting anyway. Only switch the UV on to make the exposure, switch it off immediately afterwards. Finally, never place the UV source directly in the optical path to replace the microscope's normal light source, this could permanently damage your eyes if you were to look into the eyepiece.

Under the microscope

Having established for myself some guidelines for safe operation of the UV torch with a microscope, I proceeded to take a closer look at some subjects I'd identified as having some degree of natural fluorescence.

Bank notes

In order to make forgery more difficult, bank notes incorporate various features that are hard to replicate with simple reprographic equipment. One such feature is the use of fluorescent inks that cause the notes to appear very different under UV light, as shown previously in Figure 4. Looking more closely, there are also short sections of fluorescent fibre incorporated into the paper itself. The photograph in Figure 7 shows a section of a 10 Euro note magnified 40x using visible transmitted light while Figure 8 shows the same section of the note under UV light.

There also are other non-fluorescent features such as microscopic text hidden in the apparently graphic designs on the notes.

Algae

Green algae in common with other green plants contain chlorophyll, allowing it, by photosynthesis, to convert the energy in sunlight into energy in the form of sugars that it uses to grow. The Chlorophyll naturally fluoresces red under UV light as was seen previously in Figure 5. To view algae under the microscope a simple wet mount can be used; the compact nature of the UV torch allows it to be positioned close in to the specimen using a retort stand and clamp, see Figure 9.

Although the red glow of the chlorophyll looked quite bright in Figure 5, it is actually very weak compared to synthetic chemicals such as the ones used in the bank notes. Under the microscope the red glow is barely visible to the naked eye (plus sunglasses). However, a simple compact camera on its night time exposure setting can capture a reasonable image. The small amount of visible light from the torch can cause the image to wash out, a simple red filter can significantly improve the contrast of the image. I used a Canon AS200 compact camera with a standard 32mm sub-stage red filter placed over the eyepiece. The exposure time will typically be several seconds, so some sort of tripod or clamp is essential. Ideally the camera needs a focus lock feature because the fluorescent image is usually too weak for the camera to autofocus on, so visible light must be used beforehand to set the focus. The images in Figures 10, 11 and 12 show Spirogyra magnified 100x (0.01mm/div) under visible light, UV light without the red filter and UV light with the filter, respectively.

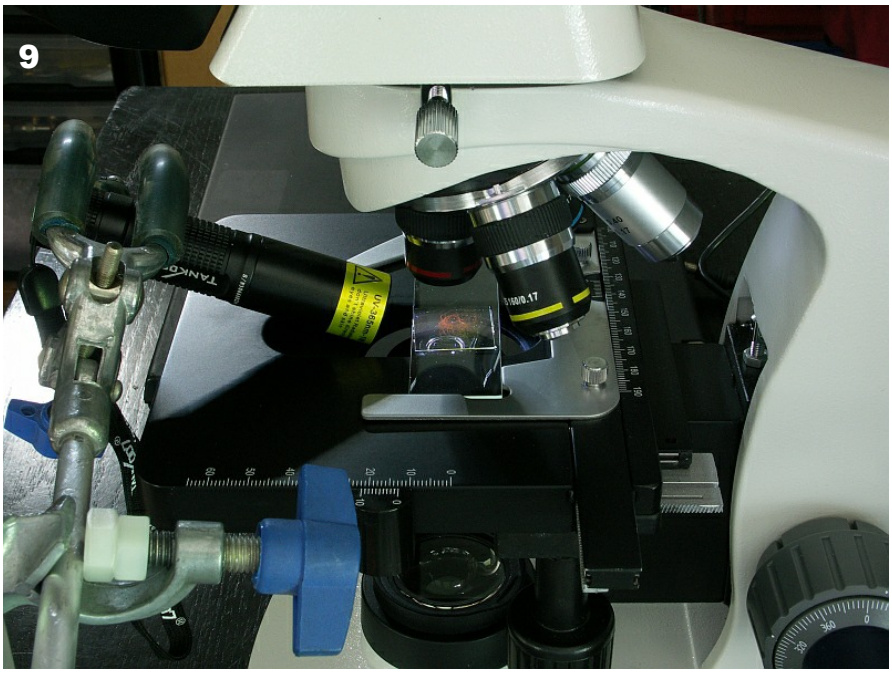
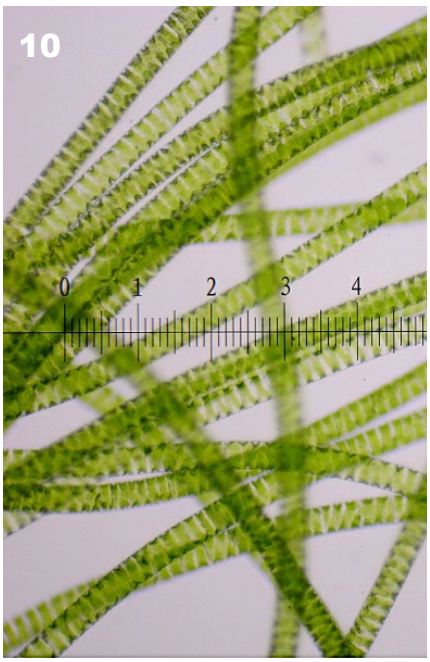


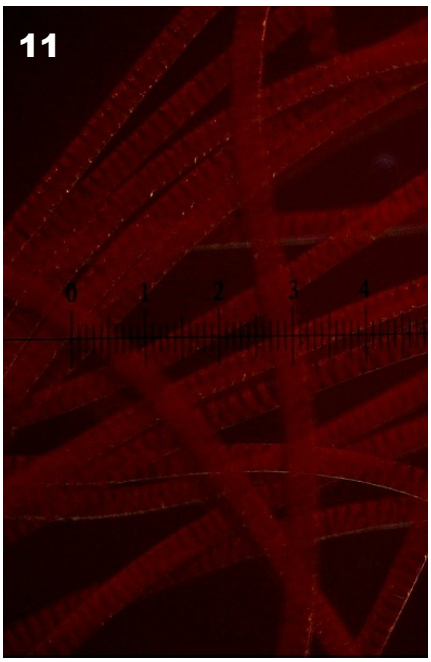
Figure 9: Using the UV torch to illuminate a specimen of algae under the microscope.

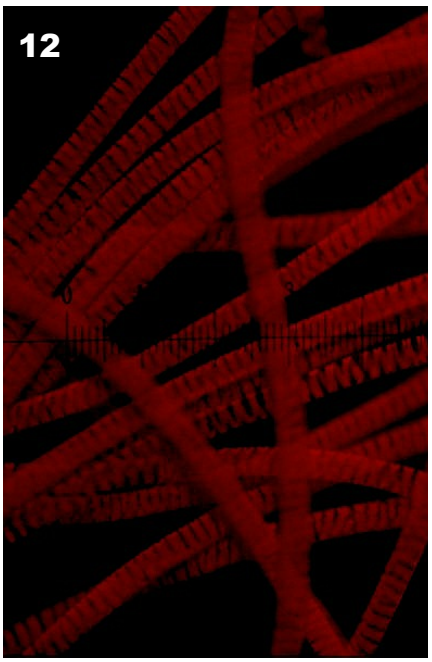
Figure 10: Spirogyra visible light.

Figure 11: Spirogyra UV, no filter.

Figure 12: Spirogyra UV and red filter.







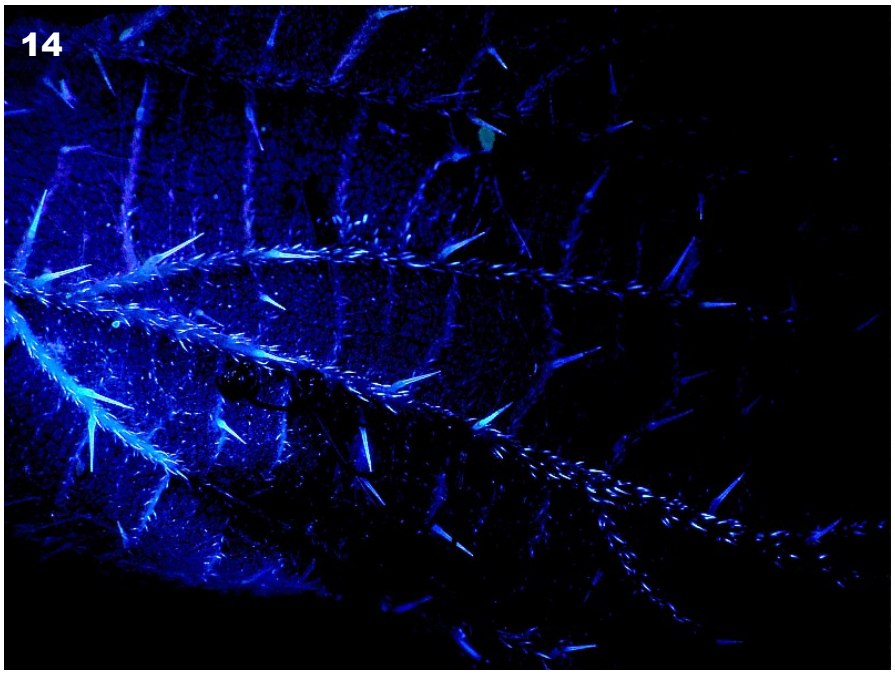


Figure 13: Nettle under visible light.

Figure 14: Nettle under UV light with blue emission filter.

Nettle

Having seen the characteristic red glow of chlorophyll in algae I proceeded to look at various other green plants, all of which fluoresced red to some extent. Just occasionally however, I found a leaf that had a patch of blue fluorescence. As far as I could tell, every one of these leaves had been damaged in some way, often but not exclusively by, insect or aphid attack. The photographs in Figures 13 and 14 show the underside of a nettle leaf under visible and UV light respectively. The insect damage is clearly visible and the leaf appears to have been home to a spider at some point as well. In the UV image, although the blue fluorescence was quite strong, a blue filter was used to remove the red chlorophyll emission and improve the contrast. The blue fluorescence is exhibited to some extent in the veins of the leaf but the majority is in the stinging spines. These patches of fluorescence might be due chemicals manufactured by the plant in response to trauma, or due to infection by a type of naturally fluorescent bacteria called *Pseudomonas fluorescens*. Whatever the mechanism, it is certainly something I want to investigate further.

Nematodes

In order to find potential specimens for microscopic examination under UV, late evening or preferably night is the best time to go out. Although it is not a bad idea to reconnoitre the area in daylight, before stumbling about in the dark with a UV torch while wearing sunglasses. Not far from our house I found a promising looking area of overhanging tree roots and moss that is almost permanently damp due to a nearby water source, Figure 15. Initially there didn't appear to be much of specific interest, but looking more closely under UV light, I noticed little flecks of strong blue fluorescence lying on the tiny leaves of the moss, Figure 16. The flecks turned out to be nematode worms, each 3-4mm long and possibly related to *Caenorhabditis elegans*, which are known to have strongly fluorescent granules within them [9]. I'm not sure about the identification though, as *Caenorhabditis* are normally much shorter at around 1mm. To view under the microscope a single nematode was placed in a drop of water under a coverglass. The Figures 17 to 22 show the nematode's tail 40x (0.025mm/div), head and middle 100x (0.01mm/div), under visible and UV light. Although the fluorescent granules can be clearly seen in all the UV light photographs, they do not stand out as being particularly different under visible light. In normal circumstances the granules

Figure 15: Overhanging moss and roots.

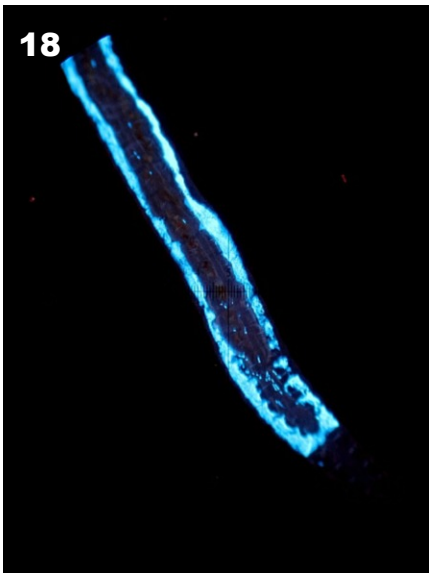
Figure 16: Moss and nematodes under UV light.

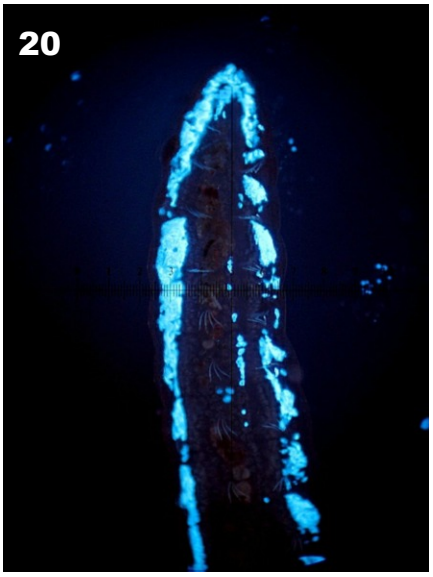
would be evenly distributed around the worm, but in this case they have been pushed to the sides, due to the worm's slightly flattened state under the coverglass. Immobilising the worm in some way is essential for photographing since the exposure times are in the order of seconds. The exact function of the granules is still not certain, although they may afford some protection from UV by converting the high-energy UV photons into less damaging visible light. Once I knew what I was looking for I found these fluorescing nematodes to be quite common, especially in areas of damp leaf litter.

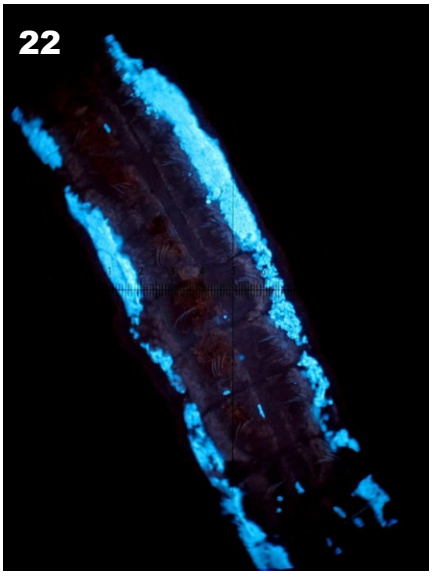
Conclusion

The use of high power UV LED sources for amateur fluorescence microscopy has been explored, together with an assessment of potential hazards compared to the well-documented risks of UV exposure from the sun. Some guidance for safe operation of these sources with a microscope has been proposed, together with some practical application in observing naturally fluorescent specimens.

Evolving technologies such as UV LEDs and the relatively unregulated marketplace of the Internet, afford the amateur an ever-increasing range of possibilities for exploration and experimentation. However, the amateur must take responsibility for his/her own safety and think carefully before any experimentation, especially when using items for applications for which they were not originally intended.







Figures 17, 18: Nematode tail, visible & UV light (0.025mm/div).

Figures 19, 20: Nematode head, visible & UV light (0.01mm/div).

Figures 21, 22: Figures 21, 22: Nematode middle, visible & UV light (0.01mm/div).

References

[1] URL: www.microscopyu.com/articles/fluorescence/fluorescenceintro.html

[2] Microbehunter July 2011, article titled 'DIY fluorescence illumination system' by César Guazzaroniin

[3] URL: www.torchdirect.co.uk/uv-torch.html

[4] URL: www.icnirp.de/documents/UV2004.pdf

[5] URL: en.wikipedia.org/wiki/Ultraviolet

[6] URL: www.sciencedaily.com/releases/2010/07/100701103415.htm

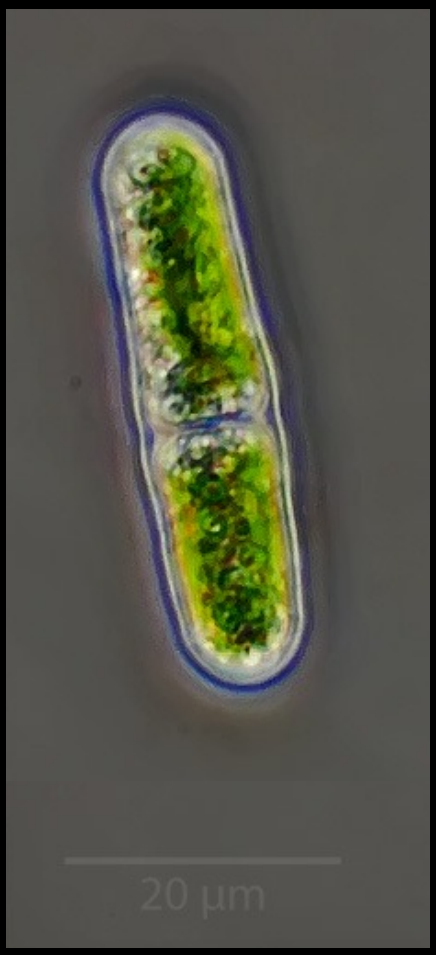
[7] URL: www.astm.org/Standards/G173.htm

[8] URL: www.greenrhinoenergy.com/solar/radiation/print/solar_radiation_pre.pdf

[9] URL: en.wikipedia.org/wiki/Caenorhabditis_elegans

Disclaimer

Do all experiments at your own risk. Neither the author nor the publisher assume liability if you damage your eyes or harm your health in other ways.



Top: Asymmetrical desmid.
Objective: 40x;
Illumination: bright field (left) and phase contrast (right).

Bottom: unknown alga.
Objective: 40x;
Illumination: bright field (left) and phase contrast (right).

Images by R. Nassar

Little stars from the moors

The testate amoebae Arcella dentata has several "teeth" (hence the name) giving it a star-like appearance.

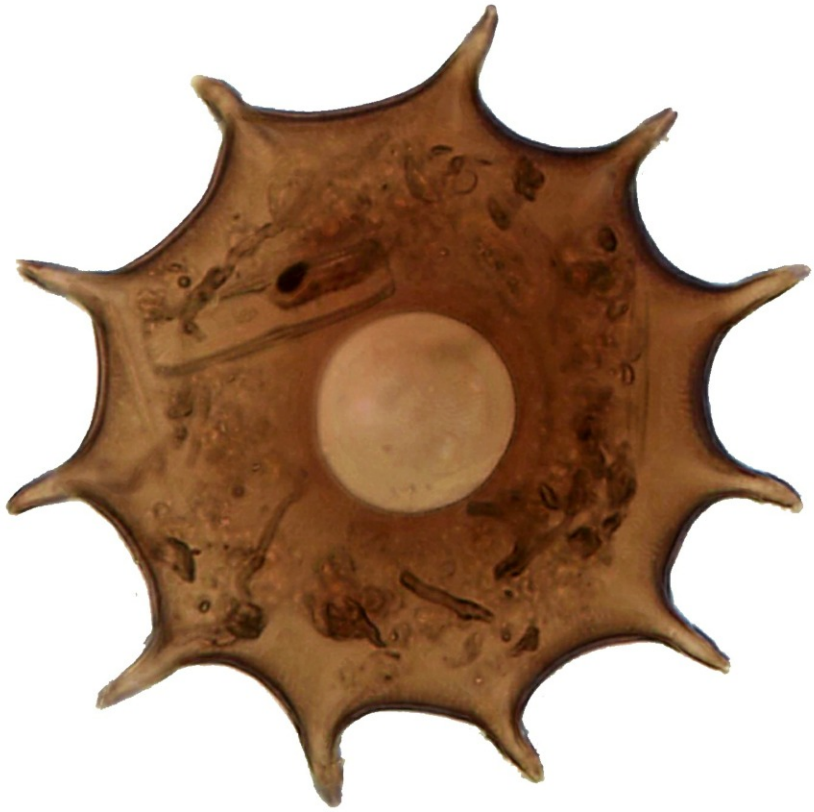
By Hans Rothauscher

From my frequent bicycle rides to a nearby moor, I am bringing home samples of half submerged sphagnum and other mosses from a small pool.

Frequently wetted, these samples stay alive for months. One sample which I collected in May contains numbers of the not so frequent *Arcella dentata*, which are still multiplying.

Arcella reproduce by cell division. Although the mother and daughter cells are clones, there are frequent variations in size, shape und spine numbers from one *A. dentata* generation to the next. Some of these may be explained by obstacles during the division process, i.e, bent or missing spines. Otherwise there is not yet an explanation for these variations.

1



2

Figure 1: A perfect star. The diameter is 153 µm with the spines.

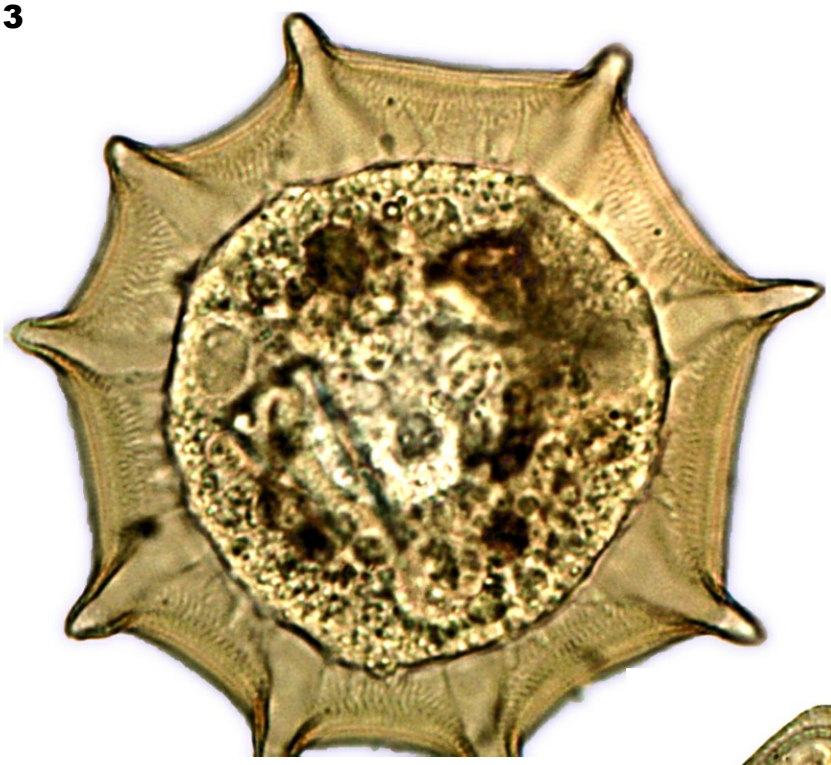
Figure 2: same specimen as in Figure 1, but from slightly below. The side shows the crown-shape.

Figure 3: Young specimens are almost colorless, before they brown with age.

Figure 4. Two teeth are fused.

Figure 5: Several spines are bent.

3



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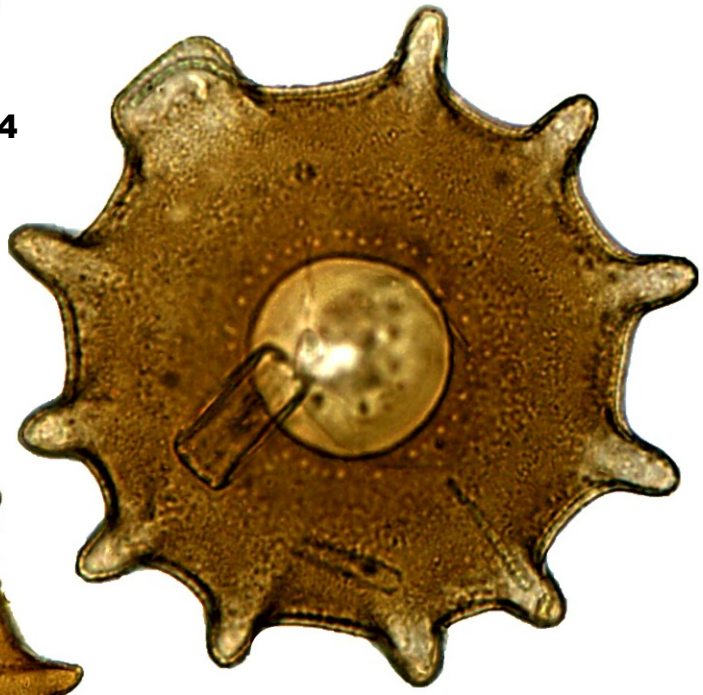


5

Basalt and Arkose Sandstone

Why not use micrographs as surface texture for 3D mapping? Here are some suggestions on what you can do!

By Carl Hennig

Figure 1: Basalt 40x, (4x obj. + 10x eyepiece). Labomed CxL microscope with simple polarizing kit. Canon 5D MkII, ISO 400, 1/3 sec. The camera sees the optical path as an *f* 0.0 50mm lens.

Figure 2: ISO 200, 1/2 sec., 2x obj.

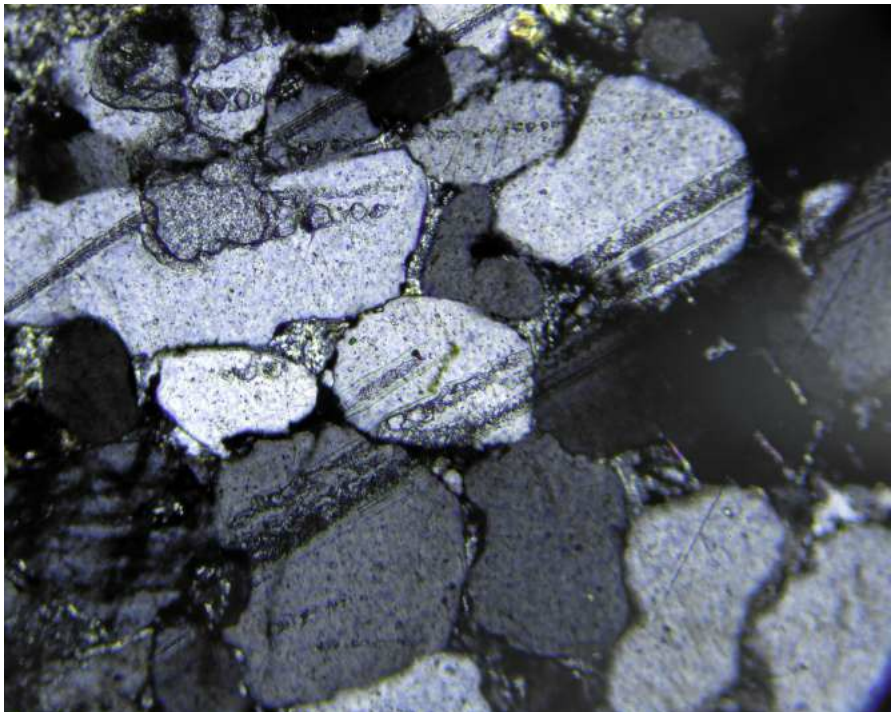


Figure 3: Arkose Sandstone 100x Five snapshots blended with LR/Enfuse. ISO 400, 1/3 sec., 10x obj. Photos of thin section minerals make great colour and texture maps for 3D drawing programmes.

Figure 4: ISO 400, 1/2 sec., 2x obj.

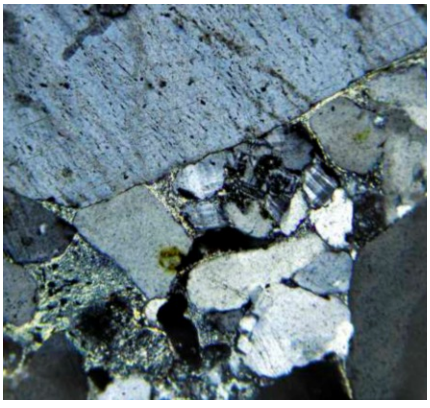


Figure 5: Arkose 100x. Arkose is a sandstone that contains at least 25% feldspar. It can be easily identified by the angular shape of the feldspar grains.

Figure 6: Basalt 40x. Basalt contains mainly of plagioclase and pyroxene minerals. It is a dark-colored and fine-grained igneous rock.

Figure 7: The images can be used as a surface texture.

References

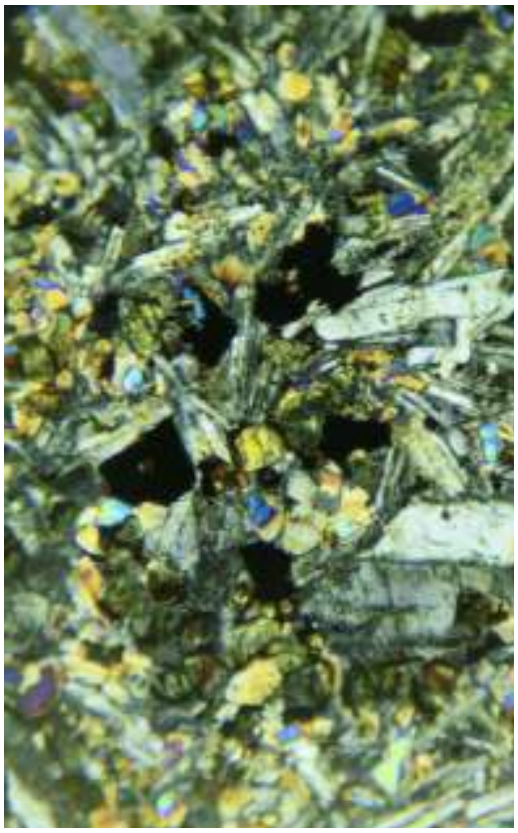
Mineral descriptions from Geology.com.

Mineral slides from The Open University.

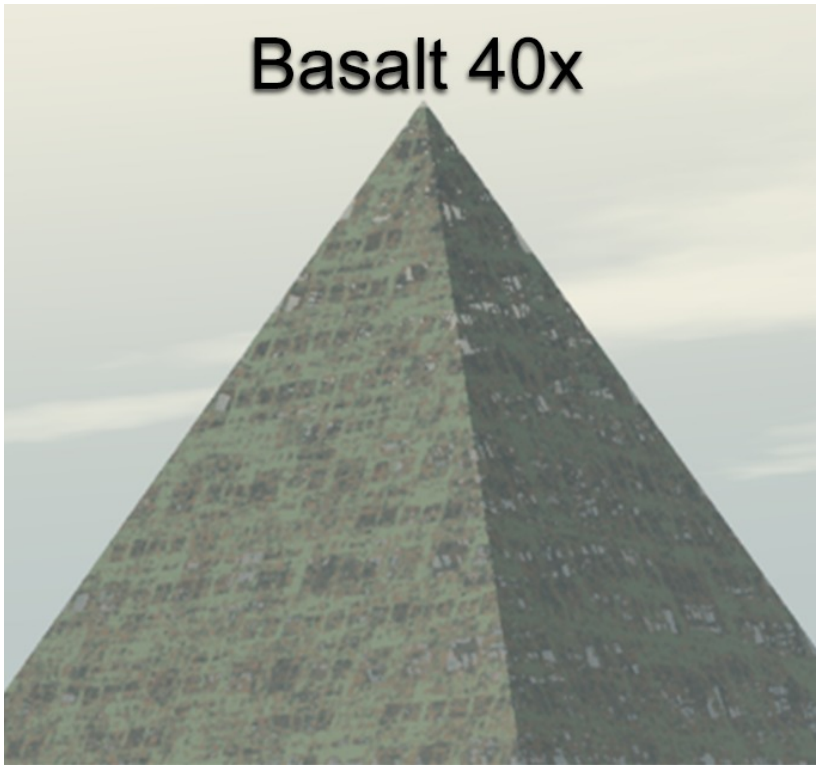
3D drawing in E-on Vue Esprit.

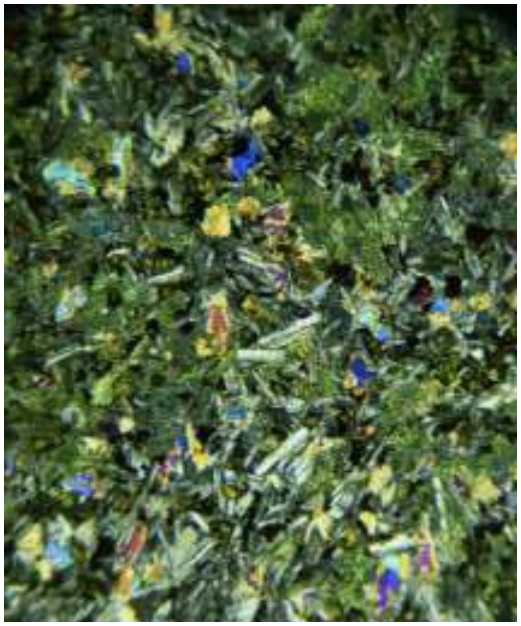
Depth of Field blending by LR/Efuse an Adobe Lightroom plug-in.

Inspiration from "An introduction to minerals and rocks under the microscope"
http://www.open.edu/openlearn/science-maths-technology/science/introduction-minerals-and-rocks-under-the-microscope/content-section-0



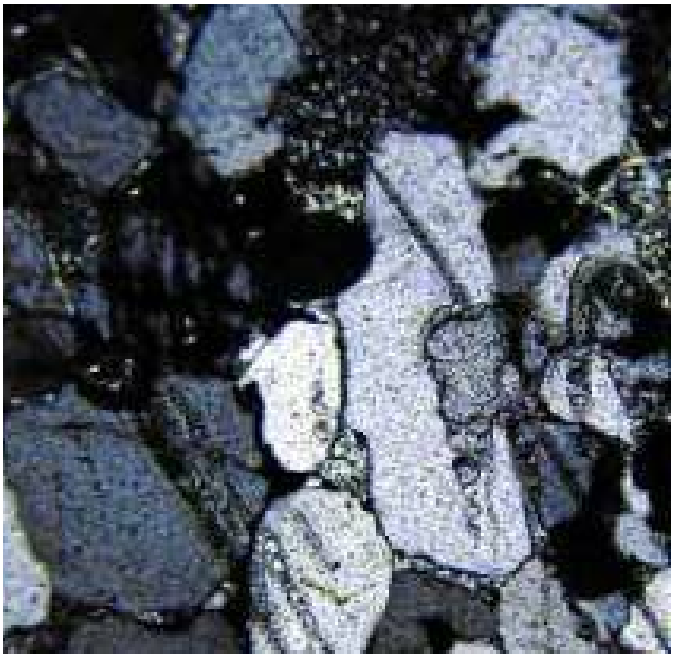
Figures 8 and 9: The Basalt 100x photo is used as a colour map for the pot and a texture map for the sand.





Figures 10 and 11: The material map for the large bricks uses the Basalt 40x photo as a bump map.

Figures 12 and 13: Setting the mapping scale of the photo to greater than 1:1 expands the coarse grain pattern.



Figures 14 and 15: This pot uses the same sandstone photo- for the colour and texture map.

What's this? Answer on page 2.