# Microbe Hunter

## Microscopy Magazine

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June 2013

The Magazine for the Enthusiast Microscopist

http://www.microbehunter.com



*History of Stereo Microscopy*



*DIY microscopy projects*



*Nematodes*

# ABOUT

**Microbehunter Microscopy Magazine**
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Front Cover: Diatoms and other algae
Left image: R. Jordan Kreindler
Middle image: Neill Tucker
Right image: Ivan Q

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Biofilm of algae, stacked. With visible stacking artifacts.

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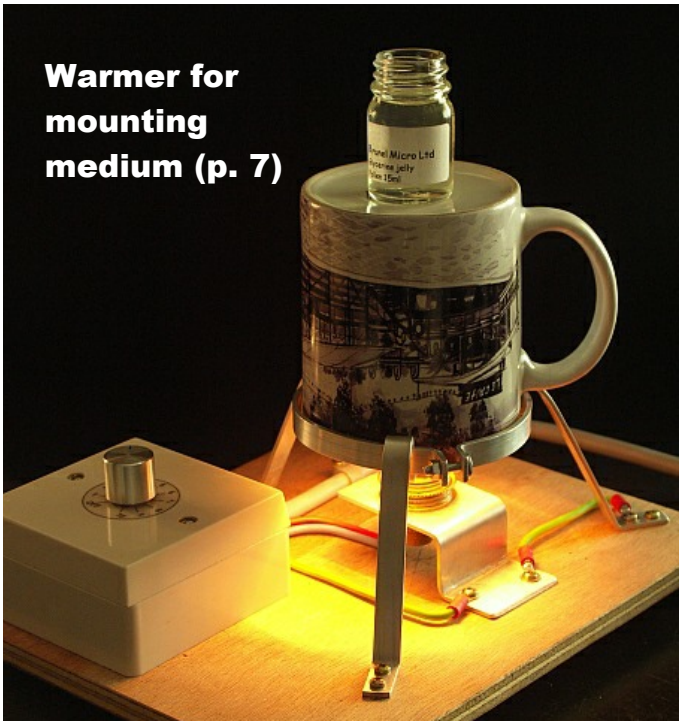

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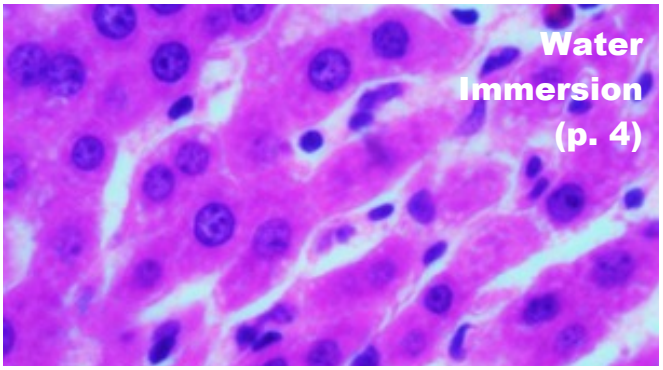

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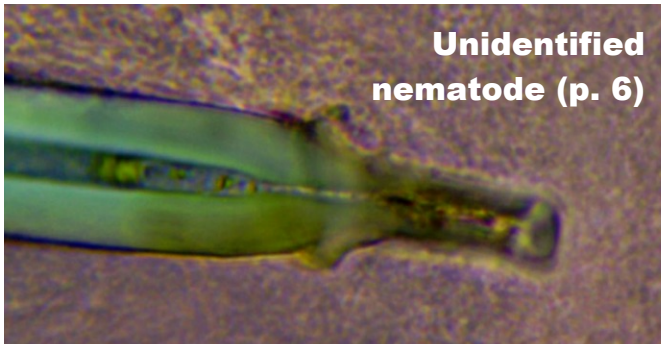


**Unidentified nematode (p. 6)**



# Substitution of oil with water using a high resolution objective lens

## Using water instead of oil does offer advantages.

*Salah Deeb, Khalid El Nesr and Mohamed Kamal*

Staudt et al. (2007) mentioned that the use of high numerical aperture immersion lenses in optical microscopy is compromised by spherical aberrations induced by the refractive index mismatch between the immersion system and the embedding medium of the sample. Especially when imaging >10 micron deep into the specimen, the refractive index mismatch results in a noticeable loss of image brightness and resolution.

In conventional light microscopy, oil immersion is a technique used to increase the resolution of a microscope of covered stained sections. This is achieved by immersing the objective lens in a transparent oil of high refractive index, thereby increasing the numerical aperture of the lens. In this case, the optical rays with immersion medium coming from the object at a certain angle and going through the coverslip can enter the objective only when immersion is used, otherwise, the refraction at the coverslip - air interface causes the ray to miss the objective and its information is lost (Wikipedia, 2013).

The index of refraction of the oil and the cover slip of approximately 1.51-1.52 differs from that of average refractive index of that of water (1.33) (Staudt et al., 2007).

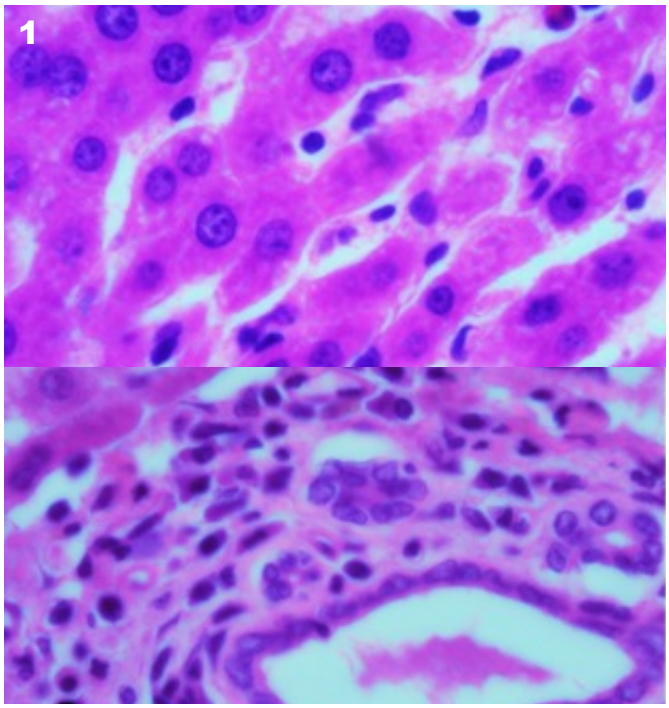
Compared to resins like Canada Balsam, which has similar optical properties to glass and immersion oil, a water mounted specimen is not ideal for an oil immersion objective.

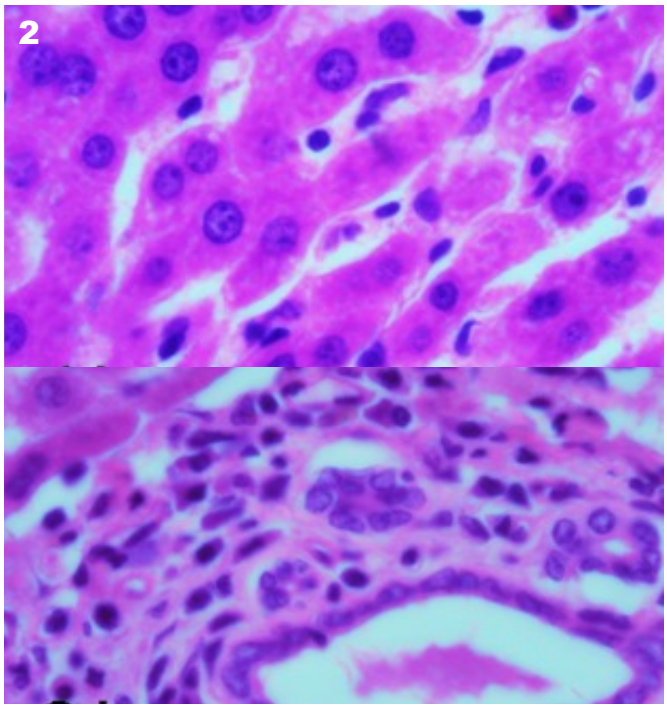
Arimoto and Murray (2004) described an aberration that is frequently encountered with water-immersion but not oil-immersion objectives. The aberration is shown to be induced by tilt of the coverslip out of the plane normal to the optical axis.

A water layer of only 0.05 mm between the specimen and cover glass degrades the resolution of a 60/1.4 oil immersion lens by over 50%, and severely lowers contrast. In contrast, a 60/1.2 water immersion maintains its performance perfectly well over at least as much as 0.15 mm water (data from Nikon at http://microscopyu.com/articles/Optics/waterimmersionobjectives.html)

We present a study for substitution of oil by water as an immersion medium to a variety of differently stained tissue, cells and cellular substructures. Using image analysis tools, we en-

**Figures 1 and 2: Section of the liver photographed using high resolution lens Immersed in water (Fig.1: before enhancement, Fig.2: after enhancement)**



hanced the images to compensate brightness and contrast.

## Material and Methods

Formalin fixed, paraffin embedded liver was processed by conventional methods. Sections, 6 microns thick, were prepared for staining with hematoxylin and eosin; these sections were used for the study.

Instruction to use water as a medium instead of oil was the same as given by Fankhauser (2005) for oil in oil immersion lens:

Focus firstly very carefully with the 40x objective over the stained Canada Balsam and cover with a glass-cover. Use water immersion as follows:

1. Use covered specimen on the slide (Once focused, do not alter focus for the next three steps)
2. Rotate turret half way so that the 40x and 100x objectives straddle specimen.
3. Apply a small drop of water directly on the slide over the specimen.
4. Rotate 100x objective into the immersion water.
5. Rotate turret so that the 100x oil immersion objective touches the water and clicks into place. Focus only with fine focus. Hopefully, the specimen will come into focus easily. Do not change focus dramatically. If you still have trouble, move the slide slightly left and right, looking for movement in the visual field, and focus on the object which moved.
6. Clean up the objective thoroughly with a tissue paper when you have finished for the day, wipe the 100x high resolution lens (immersion oil lens) and leave it to dry. The same procedures were done with the substitution of water with oil for immersion Microphotography and image correction:

Two Images were taken for each specimen at a size of 640x480 pixels and saved as BMP using a digital camera fitted to a Leica microscope; the first for water immersion and the second for oil immersion, and were saved. The optical source was maintained constant during photomicrography. Correction for brightness and contrast was done using "IrfanView" software and saved.

## Results

Using of water for immersion in high resolution lens (100x) resulted in a slight decrease in intensity as compared with oil. Restoration of the quality of figures to be comparable to oil immersion (Fig. 1, 2) were achieved by enhancement (correction) using graphic software (IrfanView).

## Discussion

Immersion in water enables high resolution imaging deep inside fixed specimens with objective lens of the highest available aperture angles. The refractive index changes due to larger cellular structures, such as nuclei, are largely compensated by graphics. Water is good as an immersion medium especially that it is non-toxic and easily removed.

Oil immersion lenses have a higher numerical aperture (NA) of around 1.25 - 1.4, which for water immersions is maximally 1.25 (with a difference of circa 10% resolution only) and therefore a higher resolution compared to a water immersion lens. The refractive indices of the water and of the glass in water and oil immersion lens are different but less than it would be the cases between air and glass as it will be the case with a non immersion objective. An oil immersion lens achieve a higher aperture.

With a water immersion lens however, refraction of the rays occurs on the glass - water boundaries. The lens has to be corrected for these slight deviations, and one obvious problem here is that the thickness of the cover glass influences the amount of correction needed (Van Wezel, 2012).

A water layer of only 0.05 mm between specimen and cover glass has been stated to degrade the resolution of a 60/1.4 oil immersion lens by over 50%, and severely lowers contrast. In contrast, a 60/1.2 water immersion maintains its performance perfectly well over at least as much as 0.15 mm water.

Compared to the average 100x oil immersion lens on most microscopes, a water immersion lens is greatly useful. The cumbersome cleaning of the slide and lens makes the oil immersion objective generally the last (and least) used lens. With a water immersion lens however, simply suck the water away with a filter paper. Remnants of water evaporate quickly and within a couple of seconds you can go back to a lower magnification. It even works on water mounted slides, which after use with an oil immersion only serves the cleaning jar (Van Wezel, 2012).

The advantages of the water immersion objectives for in vivo work are well understood, and the modern and hugely expensive confocal laser scanning microscope systems for the life sciences nowadays are often fitted as a standard with water immersion objectives. The demands on these lenses are very high, and the resulting price tag for a single lens can easily mount up to 10x the amount of money that an amateur wants to spend on an entire microscope.

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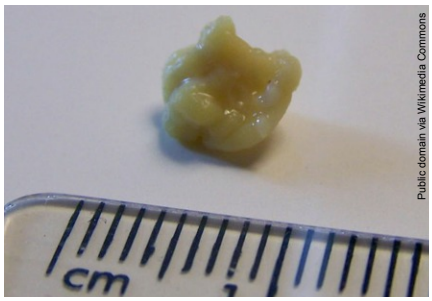
**Salah Deeb, Khalid El Nesr and Mohamed Kamal; Histopathology Lab University of Beni-Suief, Egypt. Salahdeeb40@yahoo.com**




# Nematode found in tonsillolith

## Tonsils might form tonsilloliths in order to trap pathogens.

*Ivan Q*

In Wikipedia, tonsilloliths (tonsil stones) are defined as clusters of calcified material that forms in the crevices of the tonsils. They are composed mostly of calcium, but may contain phosphorus, magnesium, ammonia, and carbonate. However, I always suspected that they are formed by the tonsils in order to eliminate pathogens from the body.

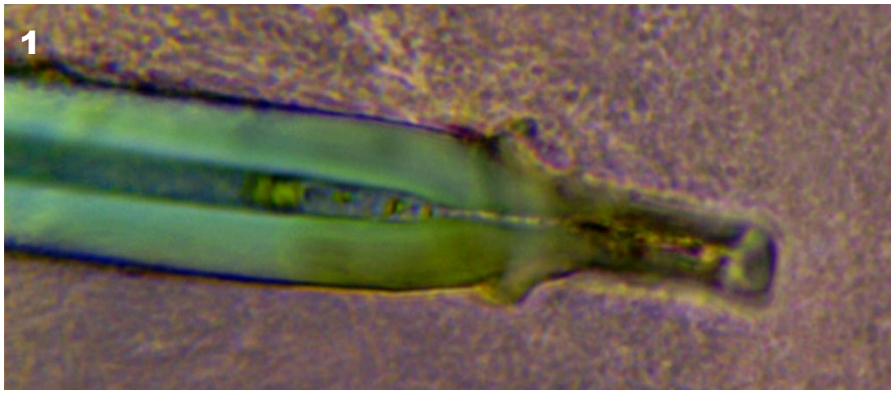
Recently, to satisfy my curiosity, I placed a small tonsillolith (about 2 millimeters in diameter) in a microscope slide and placed a second slide on top, then pressed the two slides together in order to flatten the tonsillolith. I then separated the two slides, stained them with Wright - Giemsa - Fucillo, and placed cover slips on them. Caution: Do not try to flatten the tonsillolith with a glass cover slip, the cover slips are too thin and will break, use two full slides.

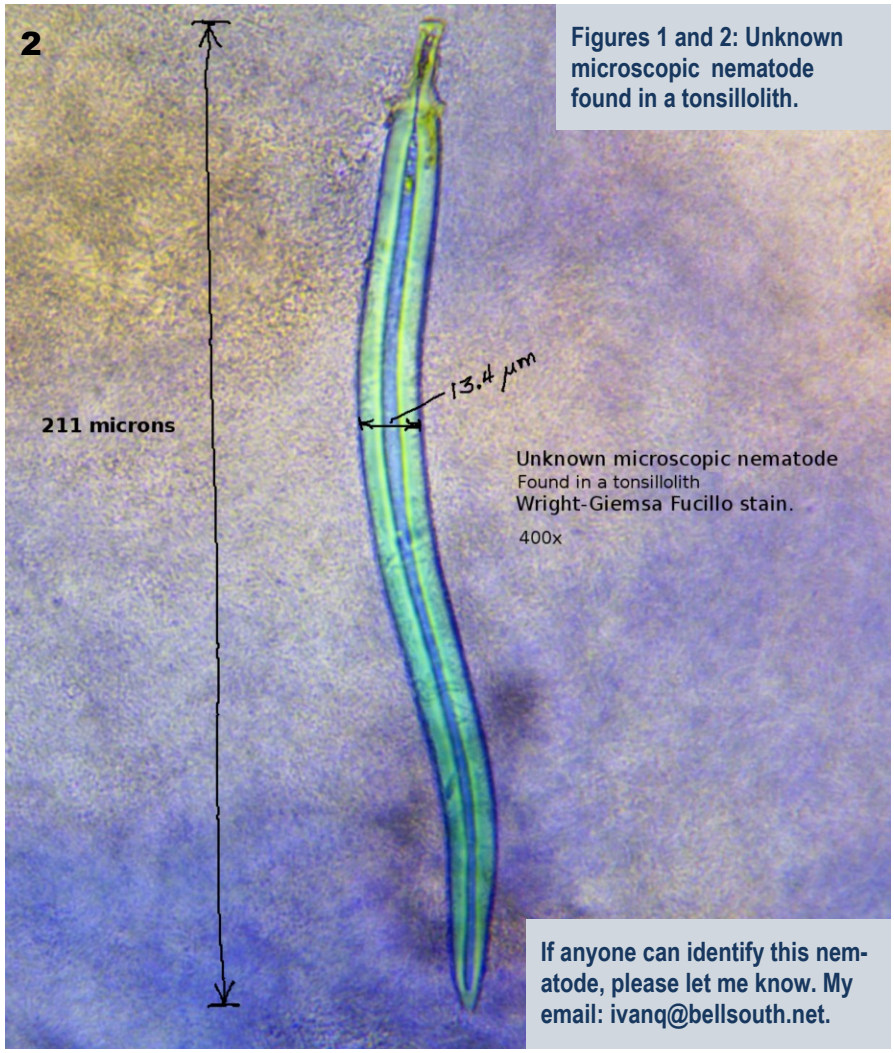
I had no idea what type of pathogen, if any, I would find. And was really amazed at the size and image quality of the nematode (worm) I found. The nematode was 13.4 microns in width and 211 microns in length, as indicated in the micrograph (microscope image) included with this article.

On the following day, I was able to dislodge one remaining tonsillolith from my tonsils. I repeated the process, and found another nematode, slightly larger, but of the same type.

More than a month later, I got another tonsillolith (also from my tonsils). But this time I only found a half of a nematode (the head was missing).

I have shown the micrograph to a microbiologist and three doctors. And so far no one has been able to identify the species of this nematode. Therefore, I though it would be a good idea to share my findings with the readers of "Microbehunter". ■




**Figures 1 and 2: Unknown microscopic nematode found in a tonsillolith.**

**If anyone can identify this nematode, please let me know. My email: ivanq@bellsouth.net.**


# Microscopy DIY Projects

## The following article describes the first few of my DIY lab equipment constructions.

*Neill Tucker*

When deciding to take up microscopy as a hobby, the scope for some home construction was one aspect that greatly appealed. Although I bought the microscope itself from a specialist supplier, there appeared to be plenty of ancillary equipment that could be built from old bits and pieces that might be lying around. The following article describes the first few of my DIY lab equipment constructions.

### Slide Storage Box

One of my aims was to make some of my own permanent slides, and that would require something to store them in. Granted, plastic slide boxes are not exactly expensive and you could just buy some, but where's the fun in that? This slide box is made from an old VHS video box and some flexible electrical conduit. It just so happens that the thickness of a VHS video is pretty much the same as the width of a standard microscope slide and the grooves in the conduit are just wider than the thickness of the slide. The box basically consists of gluing, using silicone sealant, two lengths of conduit lengthways down each side of the box, see figure 1. You can use a couple of old slides at each end of the conduits to set the separation while the silicone sets. The only addition is something inserted into each conduit to keep them straight, any off-cut of wooden beading or dowel will do, just as long as it is a snug fit. You may need to trim some of the returns on the box lid if they close onto the conduit, alternatively cut the conduit a bit shorter to clear them. The box holds around 60 slides and can be stored conveniently on the bookshelf as before.

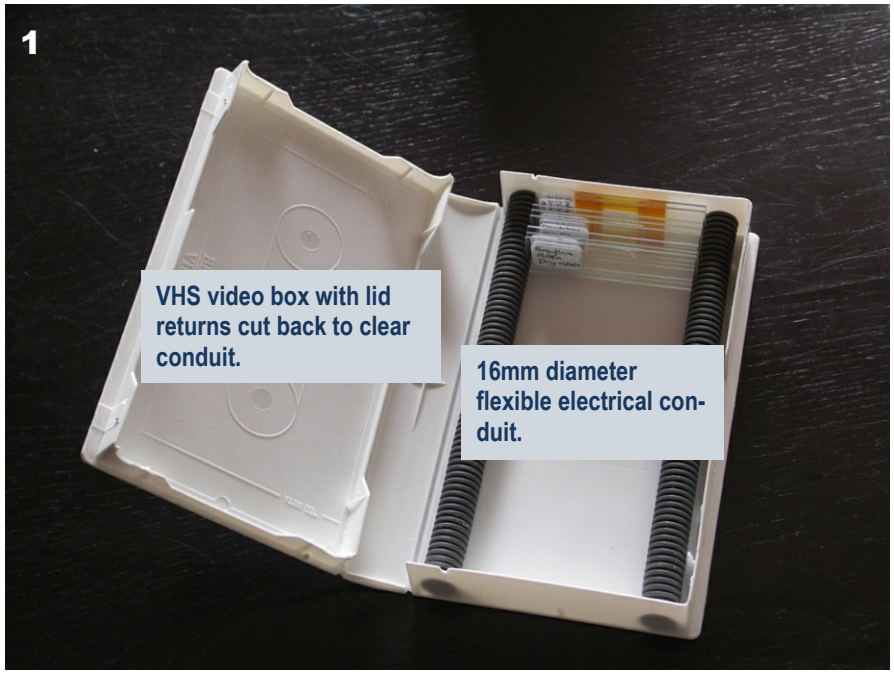


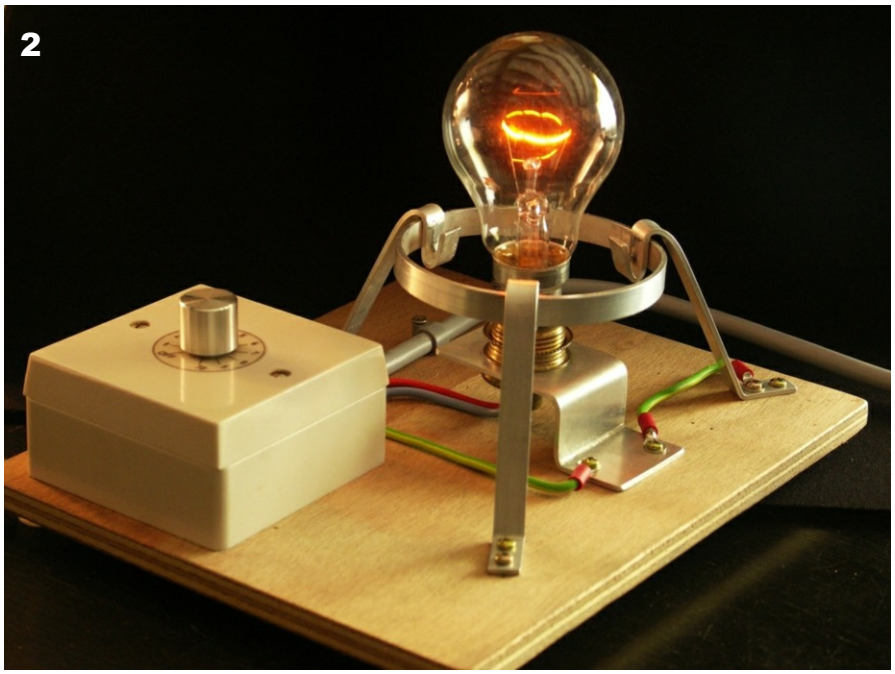

Figure 1: Slide storage box

### Glycerine Jelly and Slide Warmers

As a beginner, making wet-mount slides using water is one of the easiest ways to look at all manner of different specimens. Unfortunately this type of mount will quickly dry out unless steps are taken to reduce the rate of evaporation or replenish the water. If you find something really interesting, it is nice to be able to make a more permanent mount so you can examine it at leisure or show your friends. Glycerine Jelly (GJ) is an attractive option since there are no volatile solvents involved and the specimens can be prepared in much the same way as for a wet-mount. The other principal advantage of using GJ is that once it has cooled and set, the slide is ready for use. However, this is also a problem because to use the GJ it must first be warmed to make it molten. The other requirement is that the slide itself is warm, so the GJ has a chance to flow evenly into a thin film under the cover slip. The GJ can be warmed by placing it in a bowl of warm water; the slide can be warmed by putting it on a plate on top of a bowl of warm water. I found this method worked for one or two slides but rapidly turned into a juggling act of re-boiling the kettle, mopping up drips of condensation and trying to make the mounts before everything cooled down. I decided that the electrical warmers for the GJ and slides would make life much easier.

### Glycerine Jelly Warmer

To melt GJ it only has to be heated to around 50°C, so the heat source doesn't need to be too powerful and a standard incandescent light bulb of around 40-60W is quite sufficient for



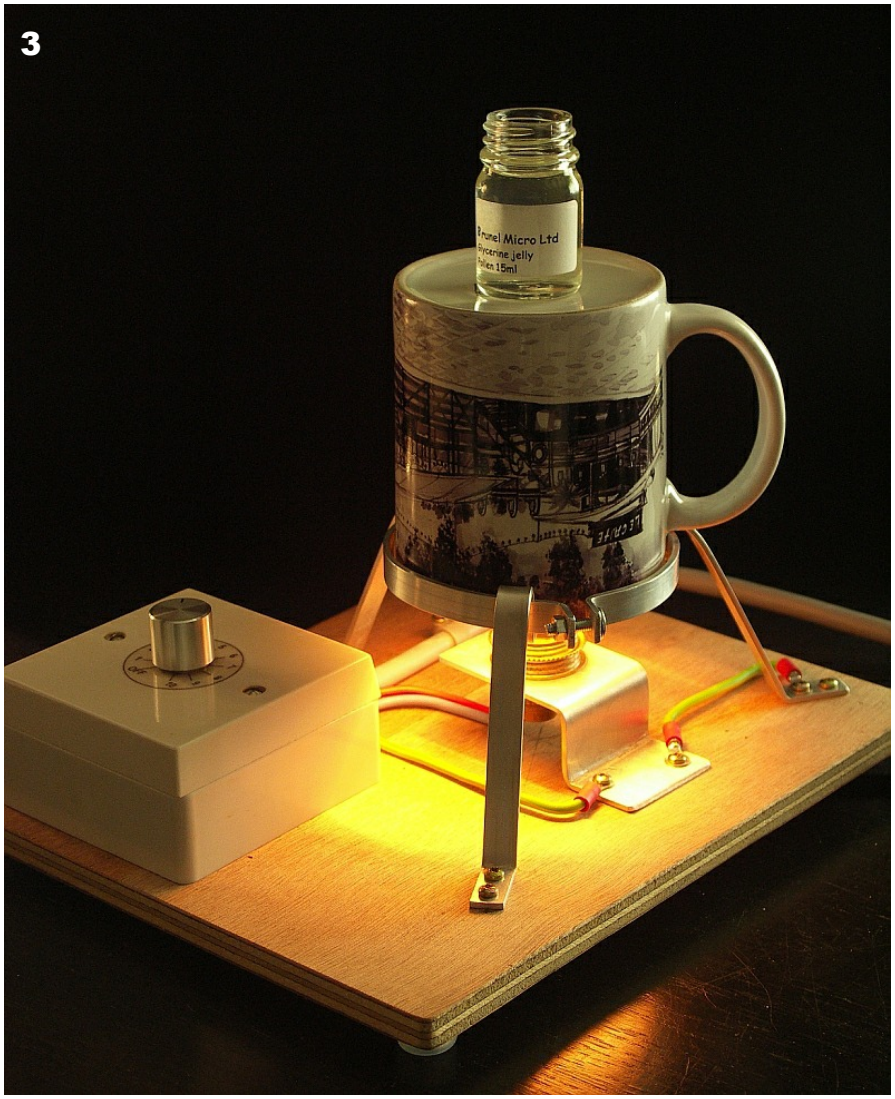




**Figure 2: Glycerine Jelly Warmer internal view**

**Figure 3: Glycerine Jelly Warmer in use**

the task, see figure 2. To protect the light bulb and provide a flat surface to stand the small jar of GJ on, I decided to use on old coffee mug, see figure3. An empty bean tin would probably work but they tend to have a plastic coating these days, which would probably melt. The tripod stand for the mug is made from short lengths of extruded aluminium bar (10 x 2mm) from the local hardware store. The lamp socket is a simple brass fitting from an old table lamp, held in position with a small Z-shaped bracket made from a wider piece of aluminium extrusion. The lamp is switched and regulated using a standard light dimmer switch. The dimmer unit and light do not require an earth connection to work, but as with any mains powered equipment it is best to provide an earth connection for all exposed metal parts. It is also advisable to operate the equipment from a socket protected by an earth leakage trip switch as well as a suitably rated fuse, 3 Amp in this case. If you are not confident with wiring mains equipment yourself, seek advice from someone who is.

In use, I found that a 60W bulb at full intensity would melt the 15ml jar of GJ in about 8 minutes, after which the dimmer could be reduced to around ¼ of full power to keep it molten. Adding a few drops of water at the base of the jar improved thermal conduction between the jar and mug base and its subsequent evaporation gave a good indication that it was getting up to temperature. Even at full power, measurements with a thermocouple showed that the mug base never exceeded 100°C, so there was little risk of overheating and boiling away the GJ.

## Slide Warmer

Once the GJ is molten it can be used just like other liquid mountants, as long as the slide is warm. This slide warmer uses a low voltage 12V, 20W halogen

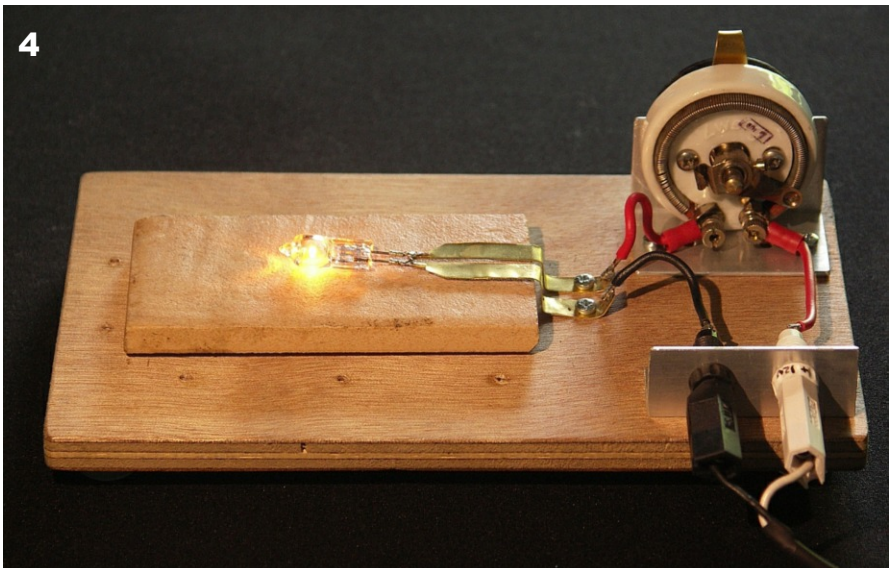



Figure 4: Slide warmer internal view

Figure 5: Slide warmer in operation

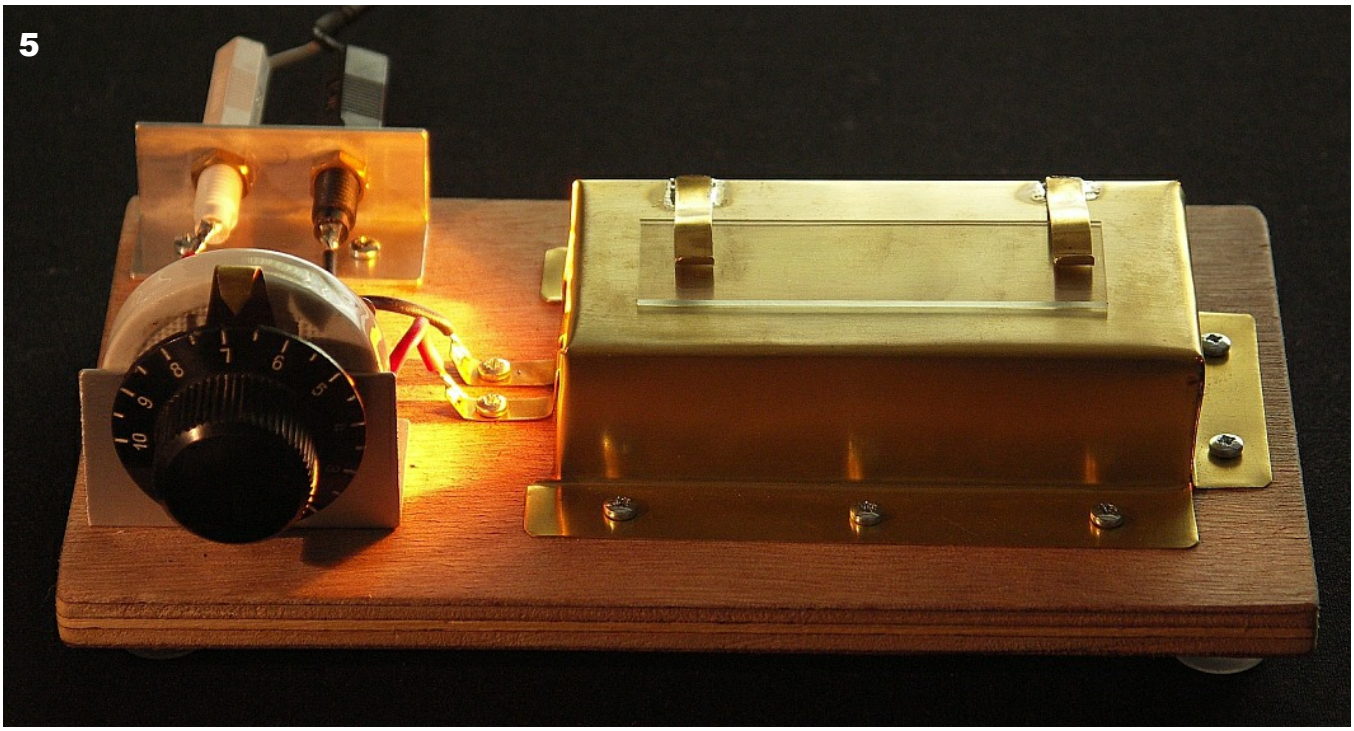
bulb as the heat source. Using a low voltage bulb is obviously safer from the electrical shock point of view but it does mean you need a low voltage variable power supply capable of supplying quite a high current, see note 1. A variable halogen light controller from the electrical store is obviously the easiest solution, but not necessarily the cheapest. I use an old laptop power supply rated at 12V, 2 Amps. The dimming control is via a 10Ohm, 25W wirewound ceramic rheostat I picked up at a garage sale, see note 2.

To protect the plywood base and to add a bit of thermal inertia I used a piece of ceramic floor tile under the bulb, figure 4. The top cover is made from 0.3mm brass sheet left over from another project, but any thin metal sheet would do e.g. a family size sweet or biscuit tin. The slide clips are from the same sheet material and soldered to the cover, figure 5. Again, a quick burst at full power gets things up to temperature after which it can be backed off to around ¼ full power.

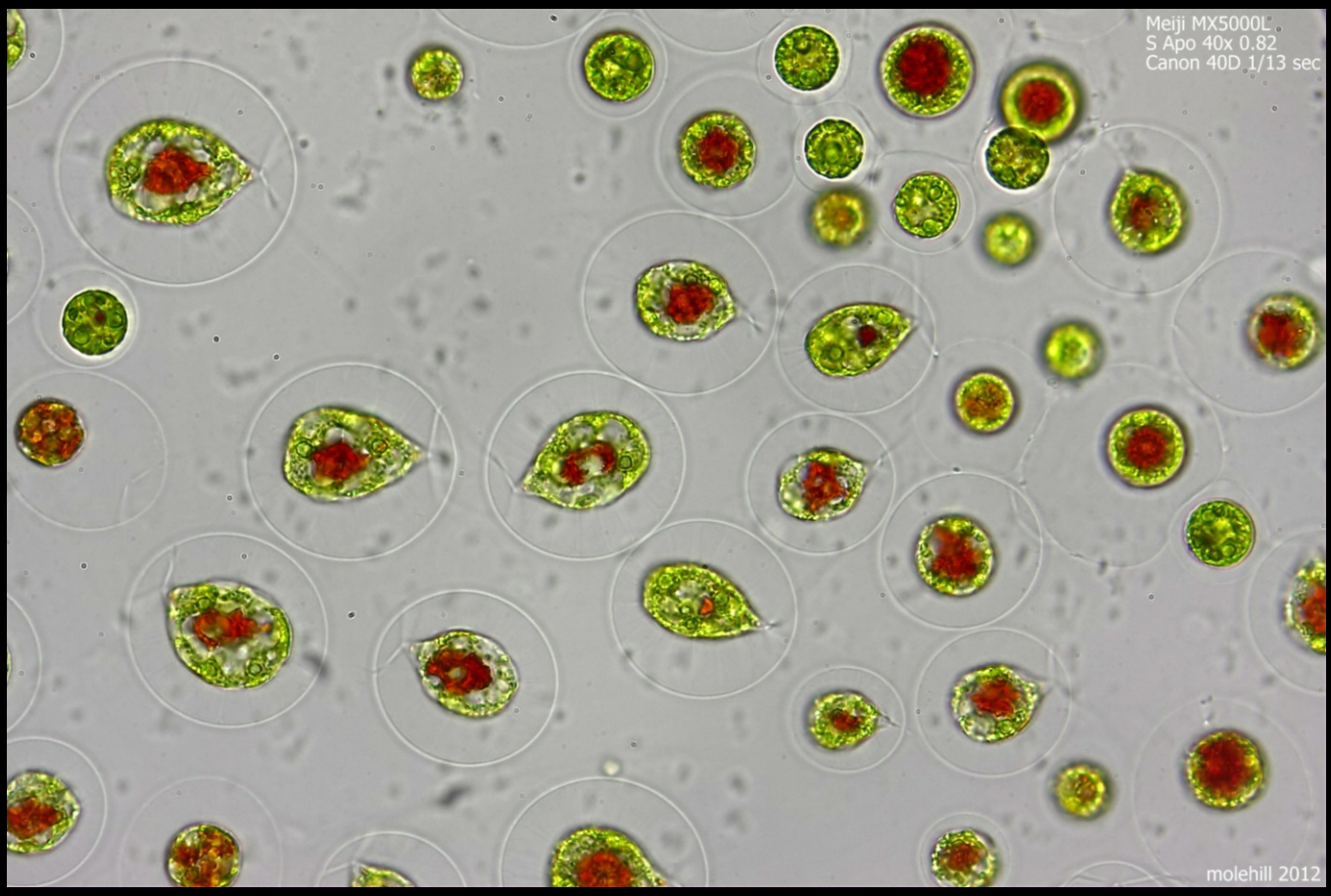
Note 1: The continuous current required by a 12V, 20W bulb is given by: 20W / 12V = 1.67A. However the peak current, when the lamp is cold, will be higher and it is best not to run power supplies at their maximum rating anyway, so something rated at 2 Amps or more would be best.

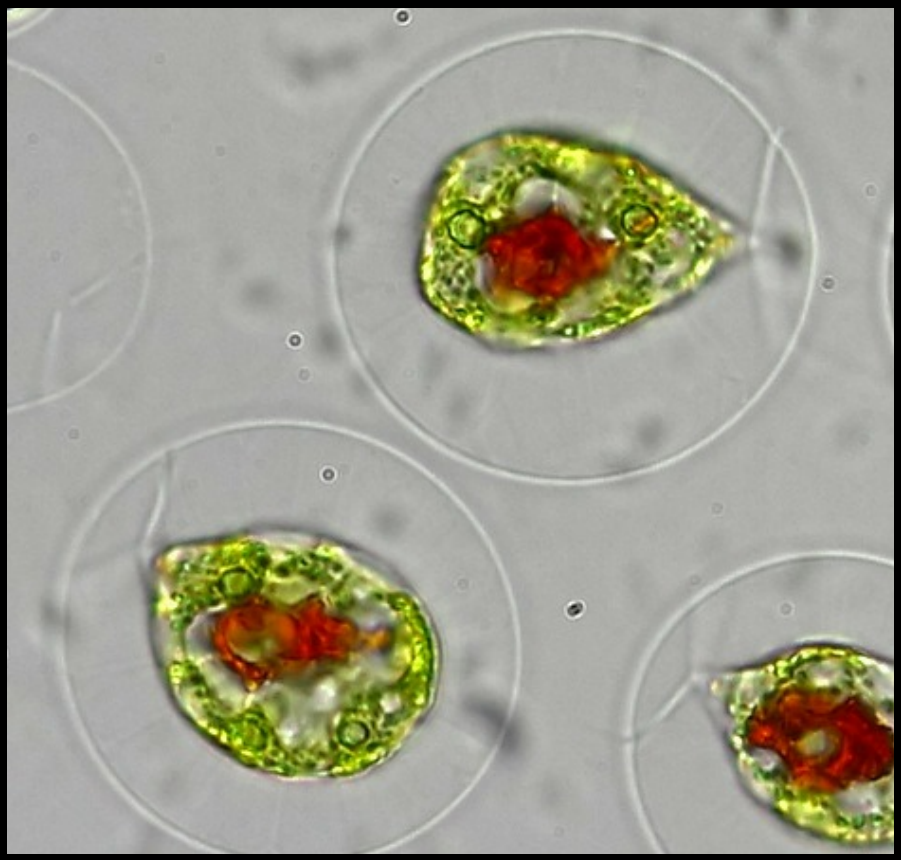
Note 2: The resistance of the rheostat needs to be such that the bulb is just glowing at full resistance. Too high a value and only a small part of the rheostat's travel will be useable, too low and there will be no significant dimming effect. Also, using a rheostat for dimming control is very inefficient compared with modern electronic solutions, as a result it will get hot (100°C or more!). The maximum power that could be dissipated in the rheostat is the supply voltage squared divided by its resistance, in this case: 12^2/10=14.4W. For most settings the dissipation will be less than this, so a rheostat rated at 25W is quite adequate. Ebay is a good source of this type of component.

■



# *Haematococcus*



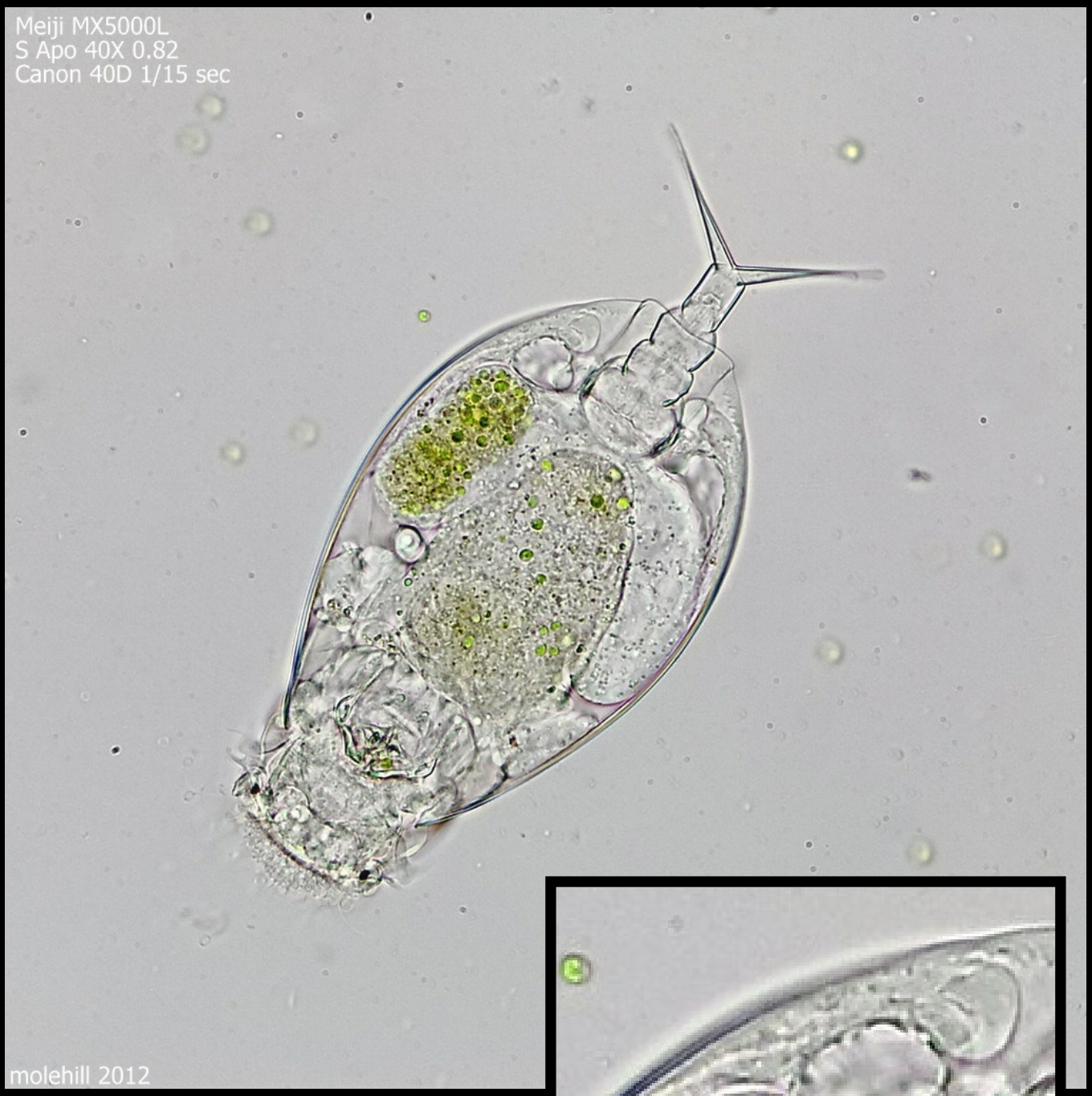



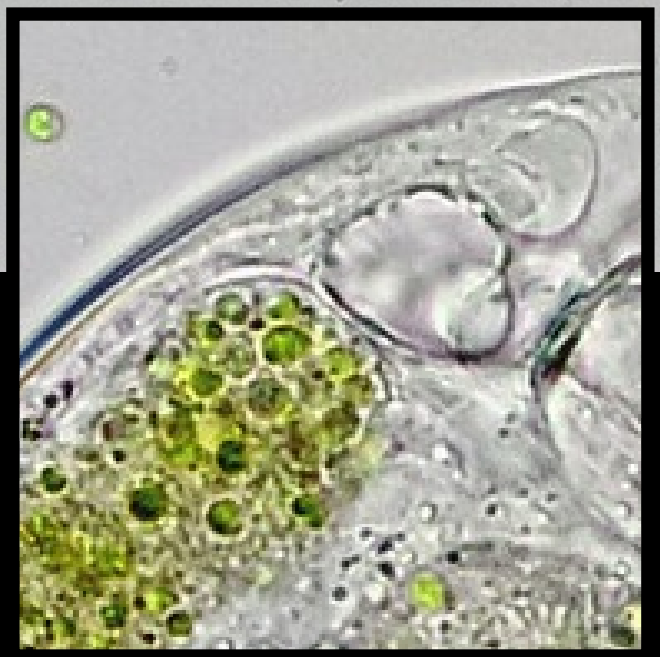
***Haematococcus***

**Meiji MX5000L, Abbe condenser, S Apo 40X NA 0.82, 2.5x photo eypiece, bright-field**

**Canon 40D 1/13 sec, ASA100, resized**

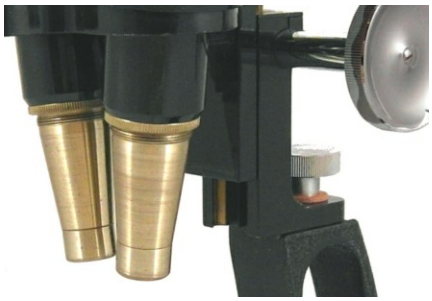
**Image by Marc Bos**



***Lepadella*, Meiji MX5000L, Abbe condenser, S Apo 40X**
**NA 0.82, 2.5x photo eypiece, brightfield**
**Canon 40D 1/15 sec, ASA100, cropted and resized**

**Image by Marc Bos**



# Stereomicroscopes Part 5

## Additional Greenough Makers and Images

*R. Jordan Kreindler*

### English Greenough Stereomicroscope Makers

It was not a difficult transition for English makers, who had long histories of microscope manufacture, to begin manufacturing Greenough stereomicroscopes. Two such makers were Watson and Beck.

### Watson Barnet

Watson Barnet is now a subsidiary of Pye Instruments Group, which is majority owned by Philips. William Watson founded his company in c. 1837 at City Road, Clerkenwell, London. However, according to Bracegirdle (Bracegirdle, 1996), the first Watson microscope was not made until 1876, at which time the company was known as W. Watson & Son, and housed at 313 High Holborn, London. The name was later changed to W. Watson and Sons, after a second son became part of the company. The company was still located at the 313 High Holborn address.

William Watson died in 1881 and the last Watson microscope was made c. 1970. The company gained renown with their Van Heurck models with two rack and pinion adjustments allowing English or Continental length microscope tubes to be used. These models included the Van Heurch, the Royal, and the Club, all based on the designs of famed Belgian microscopist Henri Van Heurck .

Watson was one of the premier British makers of optical and other instruments, which included not only microscopes, but also still cameras and

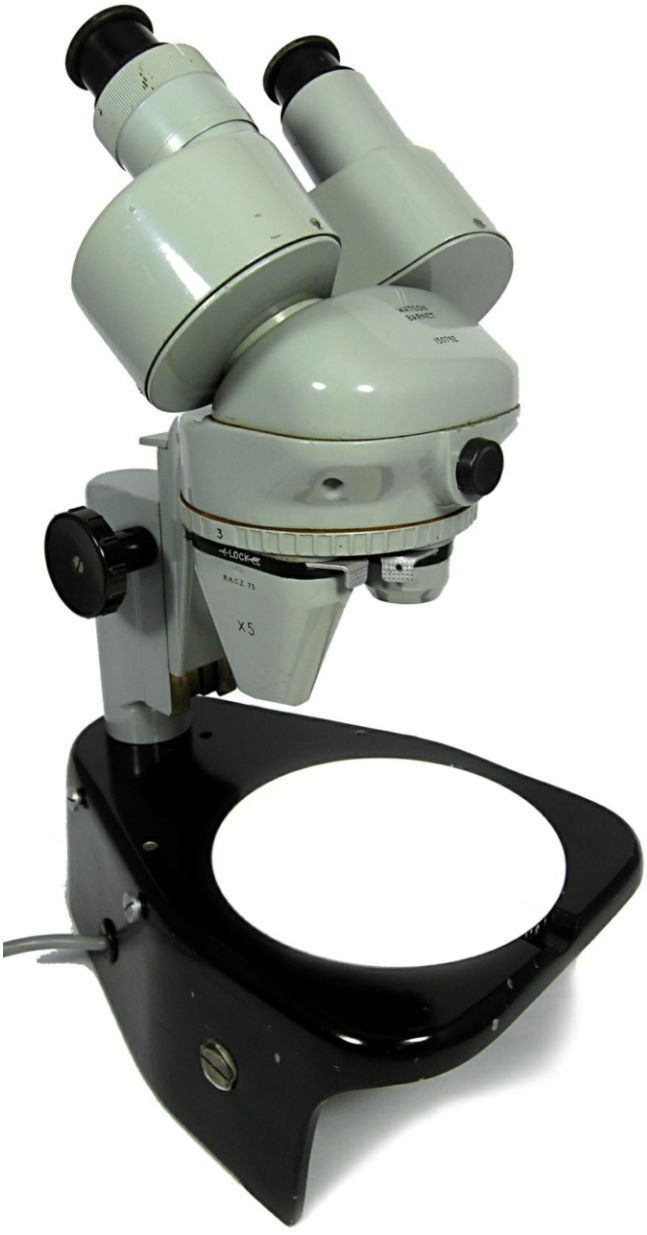

**Figure 69: Watson microscope without stage, c. 1959, reminiscent of AO Model 27**

movie devices, etc. Queen Victoria (1819-1901) had her first picture taken in 1844, with a camera made by Watson. This was only six years after 1838, when the first photograph was taken in France by Louis Daguerre, and seven years after Victoria became Queen at age 18, upon the death of her uncle William IV.

The first Watson Barnet stereomicroscope shown, Fig. 69, is reminiscent of the American Optical Model 27 stereomicroscope, discussed earlier. It has, as the Model 27 does, vertical eyepiece tubes, making it difficult to use on a normal height benchtop. It also has almost the same base style, with a curved clear opening at its base, rather than a stage beneath the objectives. However, it does not provide for a 90 degree rotation, double rack and pinion focusing/height adjustment, or objective protecting glass as found on the AO Model 27. Also, the outer diameter, 30mm, of this Watson's eyepieces is wider than that of the AO's. The appearance of these two instruments is very close, raising the question as to whether one served as a design example for the other. There is, however, a considerable difference in weight. The AO Model 27

**Figure 70: Watson stereomicroscope installed on a triangular rounded base, c. 1968**

**Figure 71: Watson steromicroscope with glide stage, c. 1970**



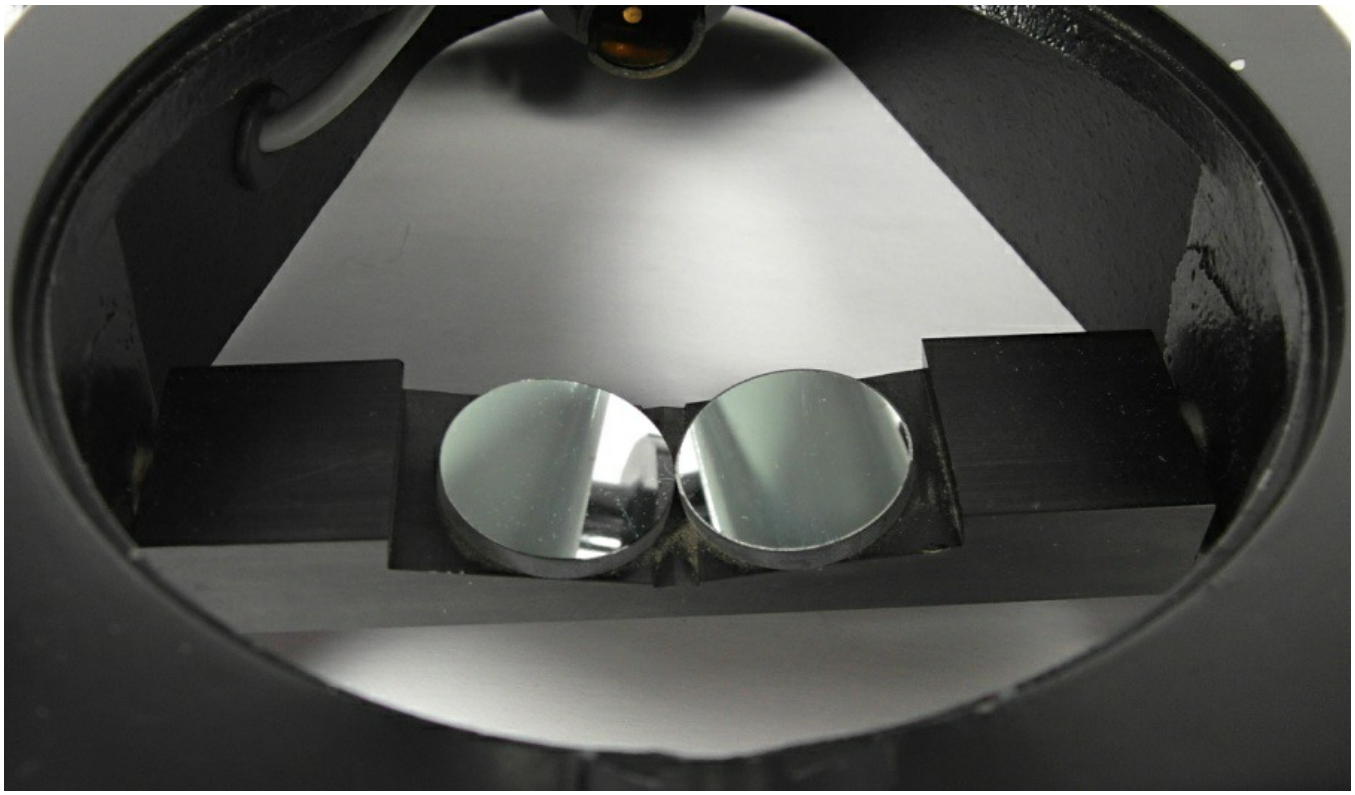



**Figure 72. Dual substage mirrors, similar to Prof. Riddell's c. 1853 stereomicroscope**

weighs approximately 9 pounds, 10.6 ounces (4.38kg) compared to the Watson's, considerably lighter, 6 pounds, 2.6 ounces (2.79kg).

The example here comes with a 2.5x objective pair. That objective pair is exchangeable by simple movement of a sliding metal sheet and replaced with other fixed Watson Barnet objective pairs, such as the 1.25x, 5x, or 10x. The eyepieces are 10x. The front screw can be removed and a light attached. This stand can also be found with a body supporting a rotating turret containing three paired objectives.

The Watson Barnett stereomicroscope in Fig 70 has an unusual rounded triangular shaped base and provision for powered substage illumination. It is quite similar to the example in the Science Museum London, which has a serial number of 111678 and is dated c. 1955. That example is said to have a "curving base", and is entirely in black. The example here has a dove grey body, with only the base in black. As in the Science Museum example, this instrument has provisions of the attachment of hand rests to the dual connectors on both sides of the base. The Watson Barnett name was used, at least, as early as the mid-1950s.

The Science Museum model carries only two pair of dual objectives, 0.6x and 5x, while the model here has three objective pairs, 1.25x, 2.5x, and 10x. Both microscopes have 10x eyepieces that allow for an independent adjustment of the right ocular. Owing to the lighter color and higher serial number, this instrument can be dated c. 1968. This microscope has built-in illumination for viewing objects via transmitted light.

Fig. 71 is another vintage Watson stereomicroscope finished in both dove gray and black. Over time, Watson made their microscopes with both black and metal knobs, as did their contemporaries. Like large kitchen appliances in gold or avocado, black went out of fashion. It gave way to lighter colors. It and the preceding Watson microscope were made during a transitional period to lighter shades, when the base of instruments was often still fabricated in black. Slightly later versions would be made completely in gray, often with black plastic focusing knobs.

Objective pairs are arranged on a revolving turret in magnifications of 1.25x, 5x, and 10x. This example comes with 7x eyepieces, allowing total magnifications of 8.75x, 35x, and 70x. Eyepieces of 10x were also sold with this microscope. The stereomicroscope comes with a substage illuminating base allowing for transmitted illumination. In this example, and the previous Watson, the eye tubes are inclined allowing easy use on a benchtop.

Both the microscopes in Figs 70 an 71 have dual mirrors. A picture of these mirrors, from one of the microscopes, is shown in Fig. 72. The dual illumination concept was first introduced in the US by Riddell, c. 1853.

These two stereomicroscopes have essentially the same bases, although slightly different frames. The gliding stage attaches via attached screws, and is fastened with nuts. The screws, which are connected to the underside of gliding stage, pass through the stage clips connector openings.

Figure 73: Beck Greenough stereomicroscope, Binomax 9690

## Beck

Beck has a microscope heritage that can be traced back to Victorian times. Richard Beck's Uncle was J. J. Lister, the designer of the Lister Limb, an alternative to the bar limb, for holding a microscope's body to an instrument's base. Beck became a partner of James Smith's in 1847, leading to the firm re-designation as "Smith and Beck". Richard Beck was joined by his brother Joseph Beck in 1851, forming the famous, and important, microscope manufacturing firm of "Smith, Beck & Beck". The firm made the first binocular body, ca. 1853, in England, based on the c. 1851 design of US Prof. J. A. Riddell (Turner, 1981).

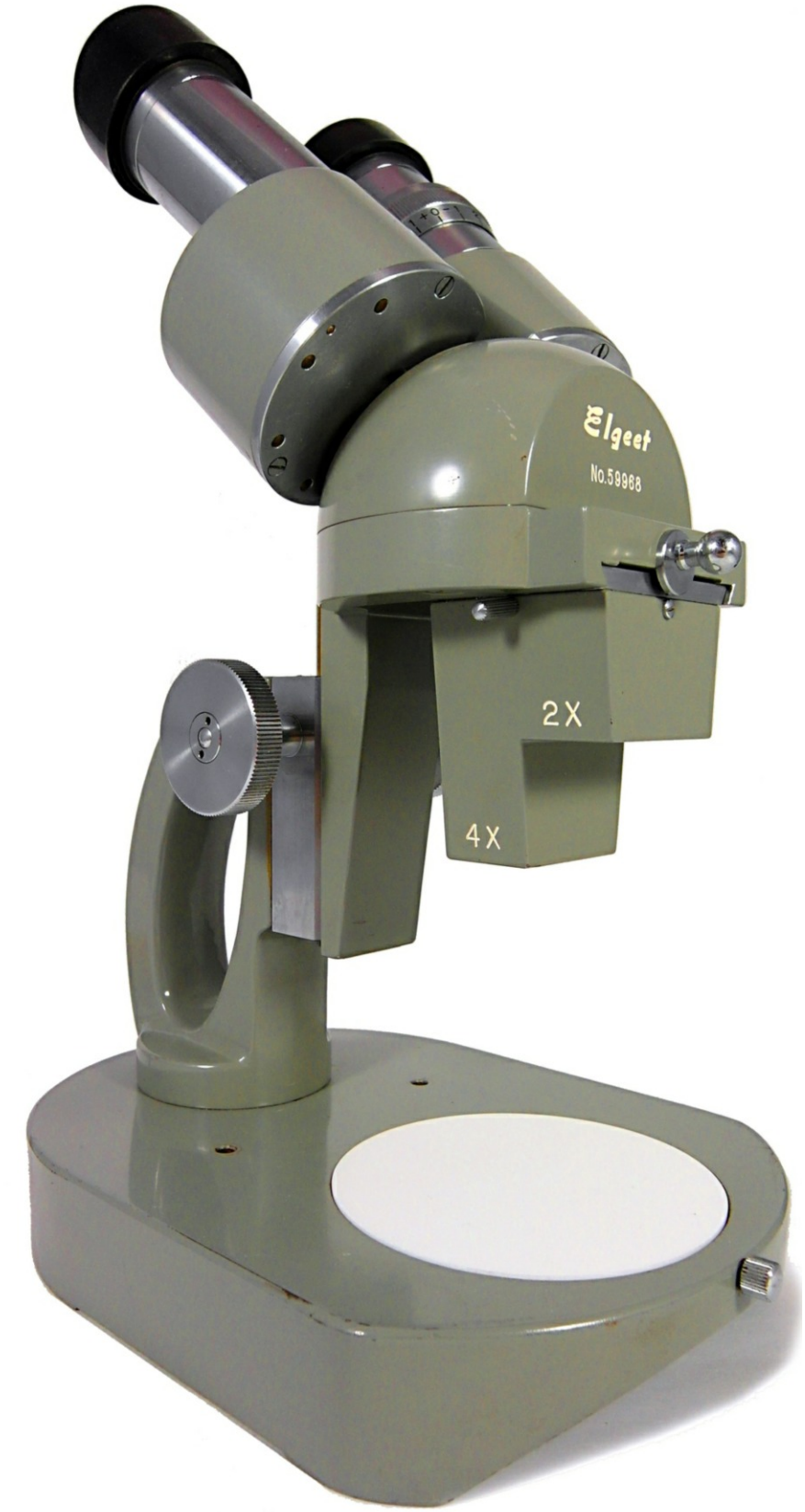
A Greenough-style Beck stereomicroscope is shown in Fig. 73. The microscope shown comes with three pairs of Beck eyepieces, two low power versions, and one high power one. The microscope also includes a clear glass plate, shaped identically to the semi-opaque plastic plate shown in the Beck example. This is a relatively small microscope. It is approximately 10" tall in working position, and weighs 5 pounds, 2.2 ounces. It images, size and weight make it suitable for field use.

## Asian Stereomicroscope Manufacturers

There are, and were, numerous Asian makers of Greenough-style microcsopes. Some examples are, Meiji Techno Co., Ltd. (3rd largest microscope maker in Japan), Motic (Chinese), Nikon (Japanese), Olympus (Japanese), Swift (US-based, purchased by the Motic Group after bankruptcy), Unitron (US-based company formerly marketing Japanese Nihon Seiko Kenkyusho, Ltd, microscopes), and many lesser-known brands, who often make microscopes for others, including house brands. However, many of the lesser-known Asian brands are of lower mechanical and/or optical quality and should be avoided.

## Olympus and Elgeet

Olympus began making steromicroscopes in the 1930s, although the name Olympus had been used on non-stereo microscopes since 1921 (Bracegirdle, 1996). Their first stereomicroscope was the XA Binocular Stereo Microscope, c. 1933. It was a Greenough-style instrument with three pairs of objectives and




a maximum magnification of 48x. Olympus continued to make stereomicroscopes both before and after WW II.

In 1946, Mortimer A. London, David Goldstein, and Peter Terbuska began the US firm Elgeet Optical Company in Rochester, N.Y. USA. London was working for Kodak at the time. The company name was formed from the first letters of the last names of the founders London, Goldstein, and Terbuska, i.e., El-gee-t. The company made the first mass-produced aspheric lens elements. It was highly regarded as a lens maker, and its lenses were purchased by the US Department of Defense and NASA.

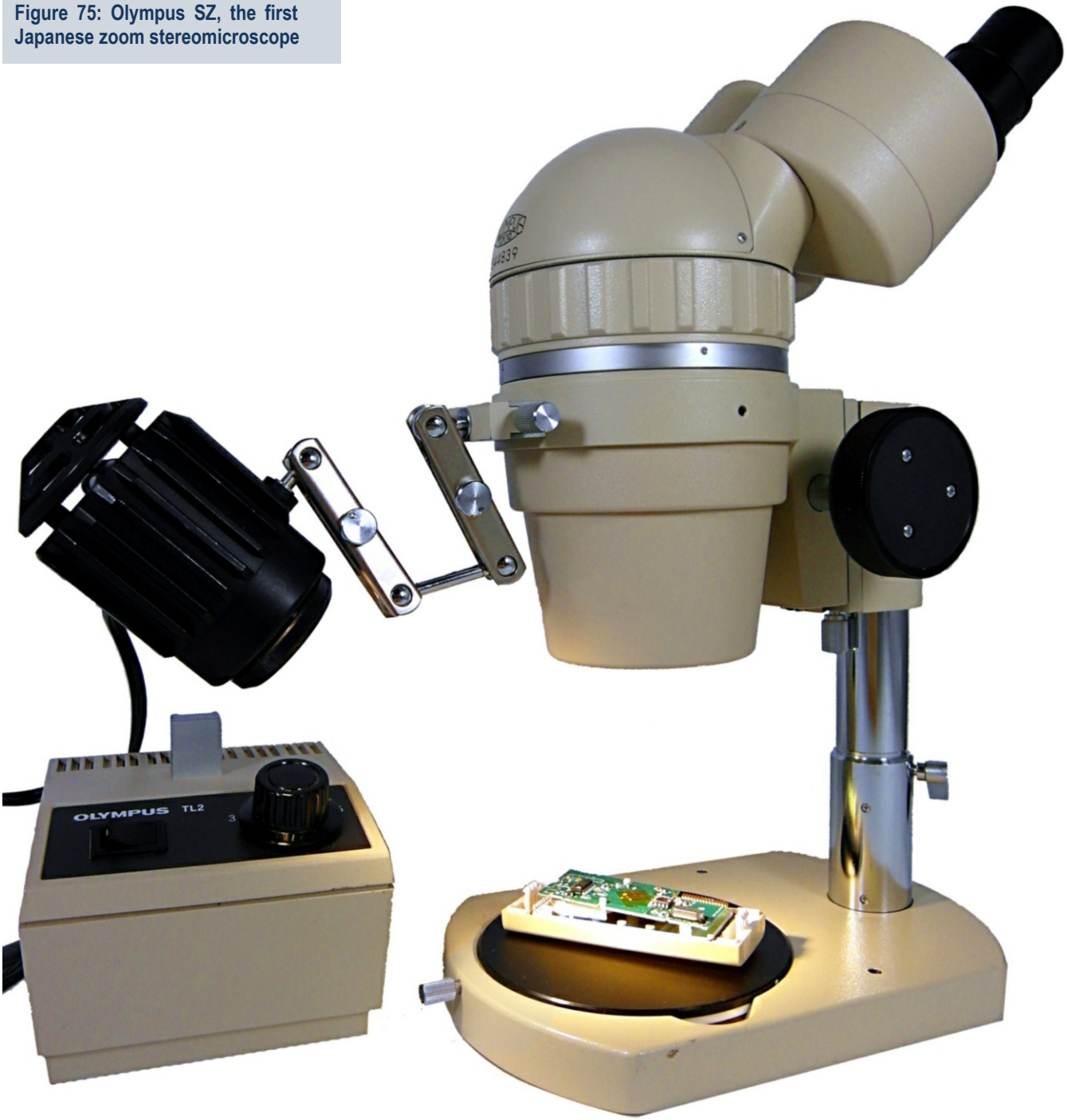
In 1956 Elgeet became the sole US distributor for Olympus microscopes. Most Olympus stereomicroscopes sold by Elgeet carried the name Elgeet-Olympus. Thus, it is not clear if the microscope in Fig. 74 was made by Olympus, as the eyepieces also carry only the Elgeet name sans Olympus. However, this model is sold as an Olympus microscope by used instrument dealers, and on eBay.

This Elgeet stereomicroscope has 10x eyepieces, marked W 10x, and two objective sets 2x and 4x as can be seen in the photograph. This was a popular Greenough stereomicroscope, as the number of used examples available for sale confirms. [Author's note: About a decade after co-founding Elgeet, David Goldstein purchased the old Gundlach Manufacturing Company, from a third party, after it had been previously acquired.]

In 1961 Olympus started manufacturing the historic SZ Stereo Microscope, Fig. 75. This microscope came with 10x eyepieces, and could zoom from 1.5x to 4x. The example shown is on a pole stand, comes with a lamp holder, and low voltage 6V bulb whose intensity can be varied using an Olympus TL2 transformer. The lamp holder is adjustable in the x, y, and z directions. This was the first Japanese zoom stereomicroscope.

**Figure 74: Elgeet stereomicroscope with "Jug Handle" stand**

Figure 75: Olympus SZ, the first Japanese zoom stereomicroscope




The microscope shown here is an early version, later versions are finished in "ripple pearl". The SZ microscope had an almost 30-year life, being replaced, by Olympus, only in 1989. This microscope has a reasonable field of view of approximately 12mm. The zoom function provided parfocal images; i.e., refocusing is not required when changing magnifications. The zoom function is invoked by rotating a knurled magnification ring, visible in the picture. Olympus notes (Olympus, undated) that, "This was the first microscope to be given a Good Design Award (the G Mark)." Olympus kept the SZ designation and one finds it on, e.g, the SZ2, SZ-III, SZ11, SZ30, SZ40, SZ3045, SZ4045. SZ6045, SZ51, SZ60, SZ61, etc.

The Olympus SZ is approximately 13.25" tall as shown, and weights about 9 pounds 1.6 ounces (4.13kg), including the attached lamp. The height above the stage is adjustable using a lever, shown behind the top of the lower pole of the microscope. This lever loosens to allow the inner pole to be raised and lowered inside the outside pole and it can be tightened to lock the inner pole at any


Figure 76: Olympus VM Series, VMF-1F

height available. The microscope's inside pole is spring supported, and so easily height adjustable. The zoom magnifications available are shown on a graduated aluminum ring immediately below the knurled magnification-changing ring.

However, it was not until 1984 with the launch of the SZH High-end Stereo Microscope (CMO, not Greenough design) that Olympus produced their first truly professional quality stereomicroscope.

The next two figures show Olympus Series VM stereomicroscopes. Olympus used simple designations to identify instruments in this series, VMF (for VM fixed magnification instruments), VMT (for microscopes with selectable turret magnifications), and VMZ (for those with zoom).

The VMF series came with 1x, 2x, or 4x objectives, yielding Models VMF-1F (Fig. 76), VMF-2F, and VMF-4F respectively.

The VMT series had dual magnification options that were selectable using a turret. The series provided either 1x and 2x (Fig. 77), or 1x and 4x, and were designated VMT-2F or VMT-4F respectively.

The VMZ model, designated, VMZ-4F, allowed continuous magnification from 1x to 4x, and was parfocal throughout the entire range. The VMF-1F, Fig. 74, is approximately 14-1/5", as shown, and weights approximately 7 pounds.

The VMT body, Fig. 77, is compatible in size to the VMF. However, the VMT microscope pod shown here is mounted on a boom. VMT's are also frequently mounted on benchtop frames.

## Swift

Swift was originally a US-based company, but after its bankruptcy it was purchased by an Asian company and put under new ownership. Its headquarters is still located in the US.. A small sampling of Asian-made Swift stereomicroscopes is shown in Fig. 78. All the Swift models in this figure, except models M20T and M26, have built-in illumination. These power requirements, plus the size of these models, would make them unfeasible for field use,

Figure 77. Olympus VM Series, VMT-2F with selectable turret magnifications of 1x or 2x

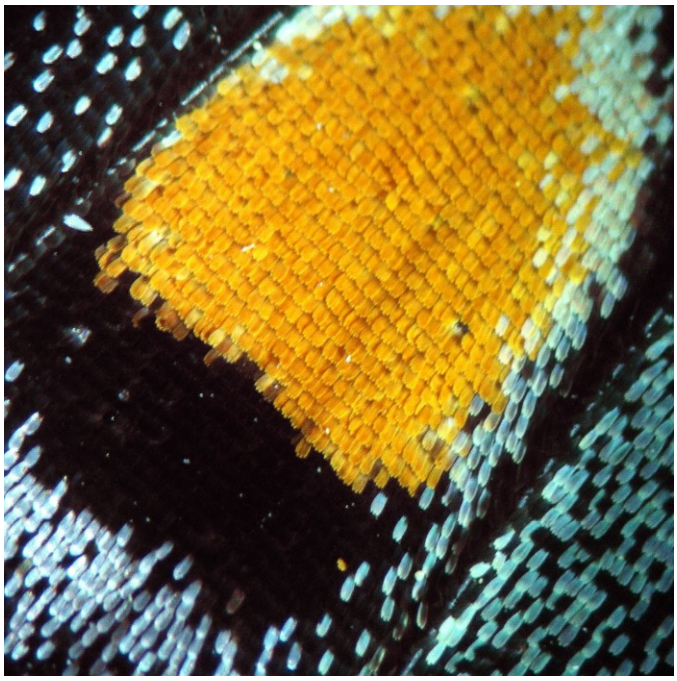

while models M20T and M26 work well in the field.

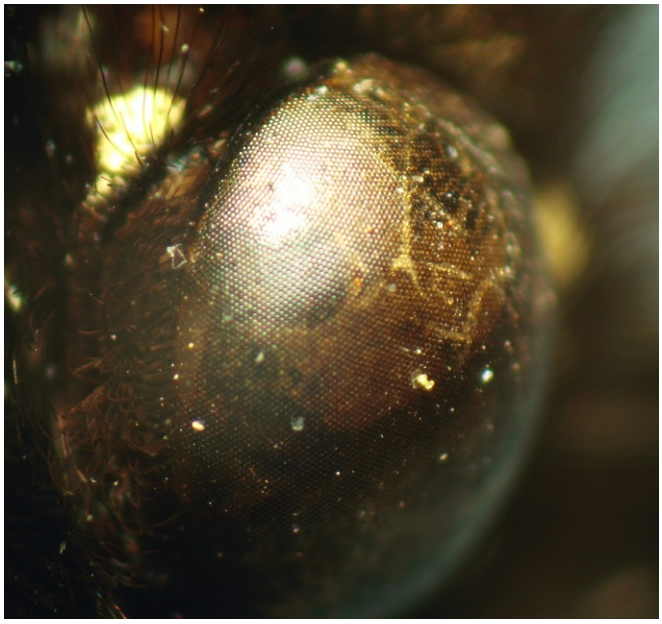
## Some Additional Greenough Stereomicroscope Images

Fig. 79 is an "in context" picture of the distal area of a butterfly hind wing. Fig 80 is a higher magnification view of a colored scale patch from this area. It shows the "roof shingles" overlapping-style of butterfly scales. Both photographs were taken through Greenough trinocular microscopes. The actual view through these instruments shows greater sharpness and depth of field with direct visual observation than in these imbedded photographs.

Fig. 81 is a photograph of this same butterfly's left eye, taken through another Greenough trinocular microscope.

Figure 78. Sample of Swift Greenough microscopes.
Back row: SM90, SM-80, TRI POWER; Front row: Stereo Eighty, M20T, M26, M23





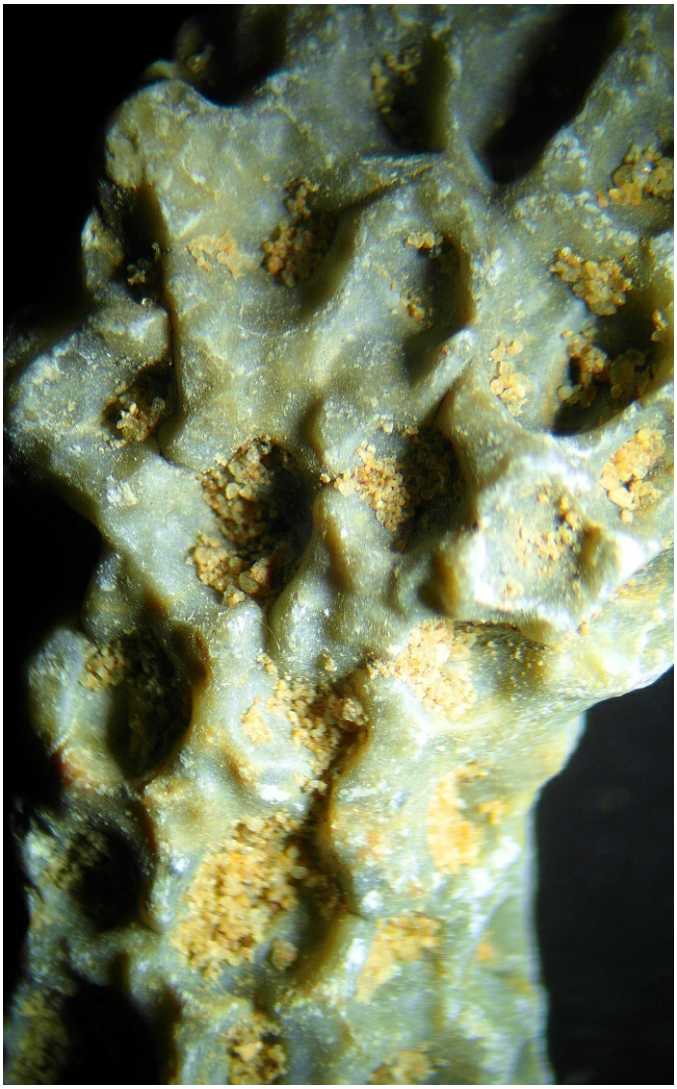
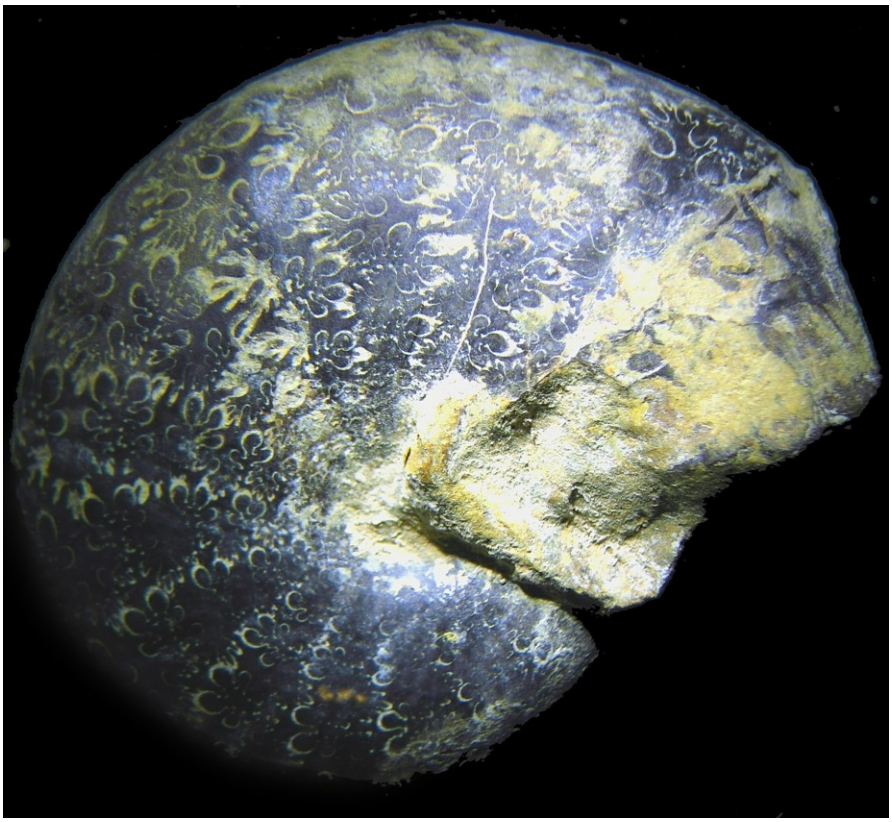


Figure 79: Distal area for butterfly hind wing
Figure 80: Close-up of distal area of butterfly hind wing, showing "roof shingle" nature of scales.
Figure 81: Post mortem, butterfly eye taken with Greenough trinocular microscope
Figure 82: Cretaceous Period branch coral fossil



This butterfly was deceased and not killed by the author before being photographed, which explains the presence of post mortem changes and debris. These post mortem changes can be used to approximately date the earliest time of this butterfly's death.

Figs. 82 and 83 show two Cretaceous Period fossils from Morocco photographed thorough Greenough microscopes. These fossils can probably be dated c. 100 million years ago. The coral was found in the Sahara Desert. It retains grains of angular windblown sand. The Ammonite fossil was found in the Atlas Mountain Range, in about the center of Morocco.

**Figure 83: Cretaceous Period Ammonite fossil**
**Figure 84: White-spotted leaf beetle ventral view with post mortem changes (Family: Chrysomelida),**
**Figure 85: An iridescent leaf beetle, dorsal view**

Figure 86: Portion of 1852 U.S. silver 3 cent coin

Fig. 84 shows an image of a small and colorful Asian white-spotted leaf beetle, captured through the photo port of a Greenough microscope. This leaf beetle is about mid-size (body length 10mm) in the family Chrysomelidae, where beetle size can range from about 1 to 20mm, although it is typically less than 12mm. This beetle has a weak clubbed (clavate) antennae, with the distal segments enlarged into small clubs. Only the proximal portion of the antennae is visible in this image. Fig. 85 shows the ventral side of this beetle with post mortem changes.

Fig. 85 is an interesting contrast to a similar-size leaf beetle, Fig. 84, but here with a dimpled iridescent body.

Fig. 86 shows a small portion of a U.S. 1852 silver 3 cent coin. This photograph was taken through the trinocular camera port of a Greenough stereomicroscope using a mounted DSLR and 1.5x relay lens.

A comprehensive list of subjects and applications for Greenough stereomicroscopes is impossible, as applications are extensive and new applications are frequently found. However, some Greenough microscope uses, in addition to those discussed above for some specialized applications, include arachnology, entomology, geology, gemology, horology, microarchaeology, micropaleontology, zoology, forensics science, materials science, numismatics, plastics, philately, safety, and textiles.

## Zeiss Stereomicroscope Binocular Conversion Base

Zeiss Oberkochen c. 1980 offered a base stand to convert smaller Zeiss binoculars (i.e., the 6x by 20mm, 8x by 20mm or, 10x by 25mm binoculars) to a stereomicroscope. As these binoculars use roof prisms, rather than Porro Prisms they differ slightly from the basic Greenough-design stereomicroscopes. However, when converted to a stereomicroscope, as in a Greenough, they use two eyepieces and two objective lenses as components of dual microscopes to obtain 3D results. Although larger binoculars will not work owing to the dimensions of the converter base, some similar size binoculars from other makers, e.g., Minolta, are also useable.

Since the acceptable binoculars have exit lenses that are relatively small, Zeiss was able to keep this stereomicroscope adapter to a relatively small size. This adapter doubles the magnification of the binoculars resulting in 12x, 16x, or 20x magnification options, depending upon the Zeiss binoculars chosen. It is not possible to use binoculars with larger objectives lenses on these adapters, although, as noted, other brands, e.g., Minolta's, small-size binoculars also work.

Because the adapter is relatively compact, if binoculars are already part of an excursion plan, this stereo adapter should also be considered. A picture of the Zeiss stereo stand adapter is shown in Fig. 87.

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**Figure 87: Zeiss binocular conversion base**

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# Why do butterfly wings shimmer in the sun?

## Diffraction effects give the wings their metallic appearance.

*Neill Tucker*

The first task was to switch the heaters to start warming the Glycerine Jelly (GJ) and the slide. Next a few ml of 50% glycerine was transferred by pipette into a watch-glass. A section of butterfly wing (a Tortoiseshell, I think) was then added to the glycerine solution and gently agitated with a pair of tweezers. Once a good number of scales had been dislodged into the solution, the remnants of the wing were removed.

Using a small plastic rod (cotton bud with the end chopped off), a couple of drops of the now molten GJ was placed onto the warm slide. To this a drop of the glycerine and wing-scales mixture was added, which was then carefully covered with a cover glass. The slide was then removed from the warmer and allowed to cool for a few minutes.

When I examined the slide at 40x there were a dozen or so wing-scales clearly visible and without too much other debris around them. This was quite pleasing since I only had small wing fragments to work with; a large spider in the garage had consumed the rest of the butterfly. To see the fine detail on individual wing-scales a magnification of around 200x is required. Figures 6 and 7 show two different types of scales from different areas of the same wing. Viewed in isolation and normal to the plane of the wing-scale, the original colour is no longer evident, however there are clues as to why butterfly wings have that beautiful iridescent quality when viewed obliquely.

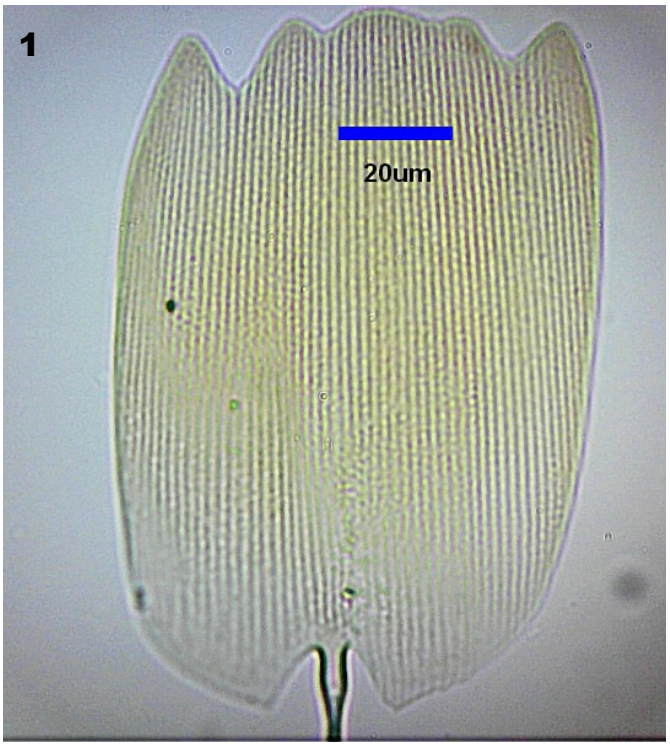
The most obvious features were the fine, almost parallel, lines running down the length of the wing-scales. A measurement from the photo showed the spacing to be around 1.5µm, this is roughly the same as the separation on the tracks on a CD (1.6µm) or the grooves in diffraction gratings used in visible light spectrometers.

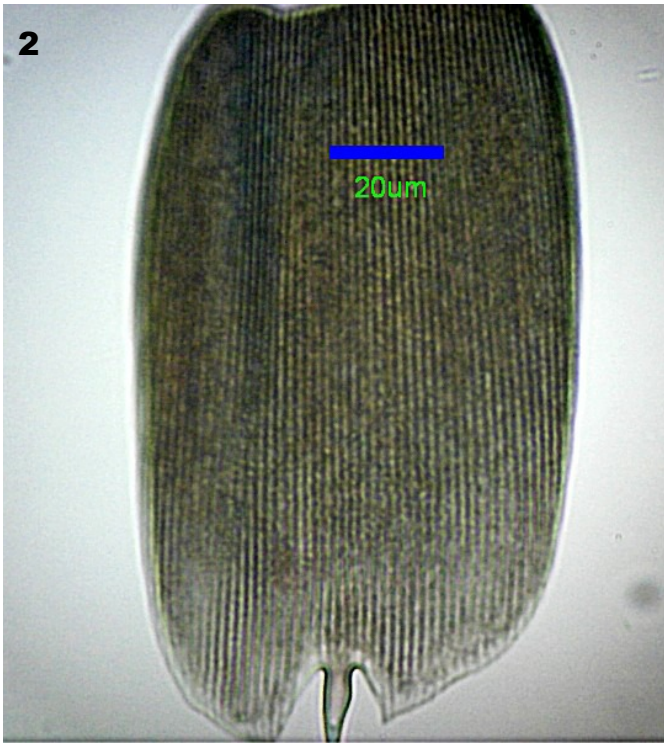
The photo in figure 3 shows a 500 line / mm (2µm spacing) diffraction grating viewed at 200x for comparison with the wing-scales. Fine regular structures whose spacing are in the order of a few wavelengths of visible light, diffract and split white light into the rainbow of colours we are all familiar with; a CD is a good example, see figure 4.

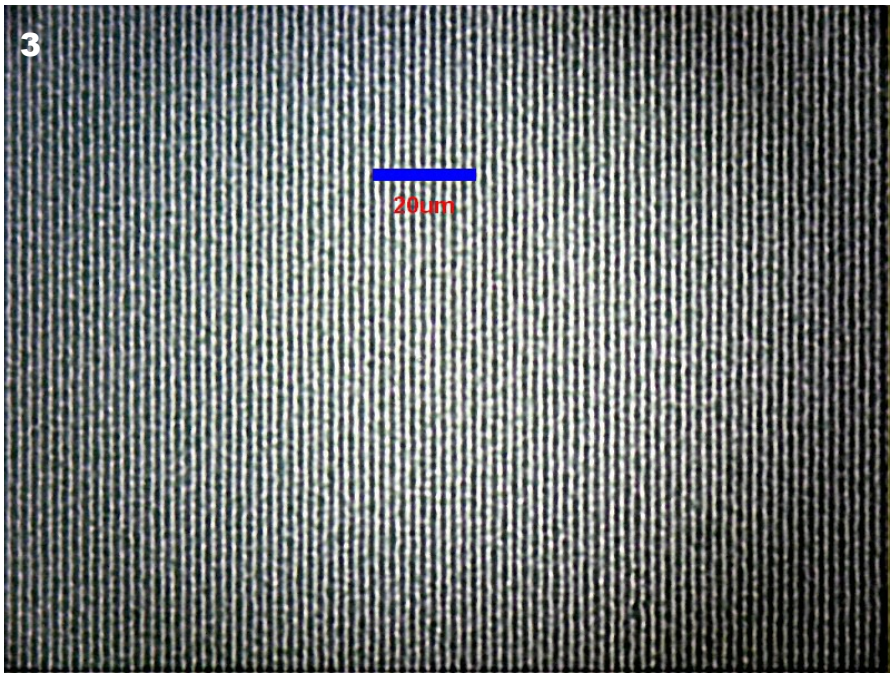
Diffraction itself is an interference effect that results from the wave nature of light. When light from a single

**Figure 1: Wing-Scale 200x**

**Figure 2: Wing-Scale 200x**





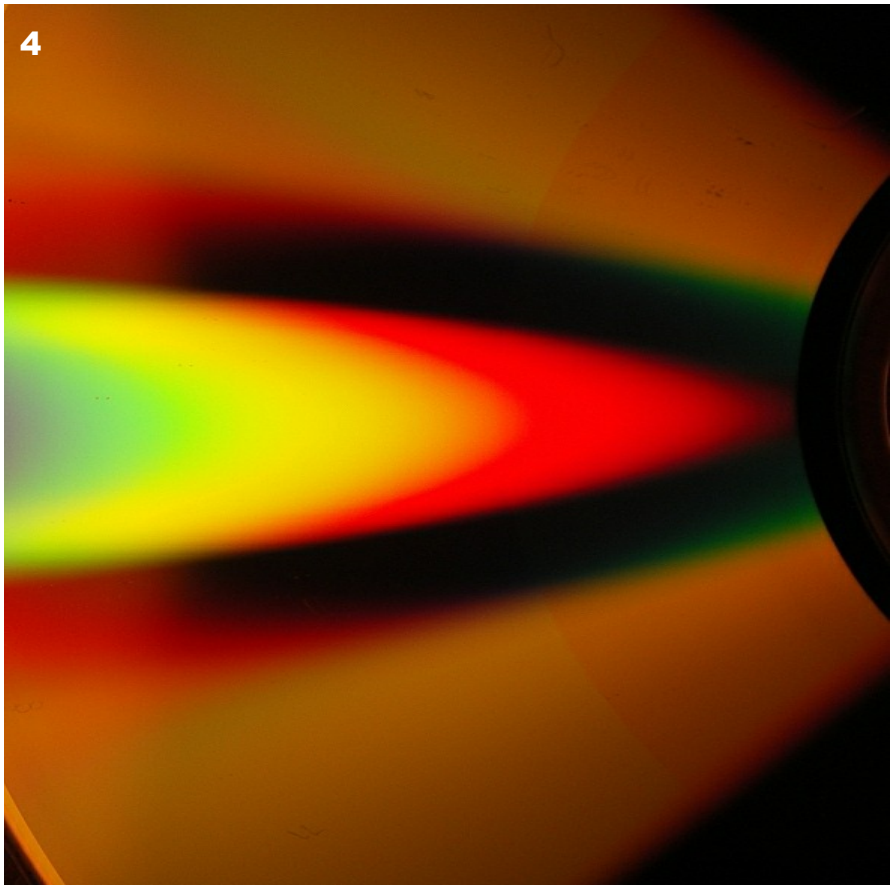




**Figure 3: 500 lines per mm diffraction grating 200x**

**Figure 4: 'Reflection of a table lamp in a CD, the lamp was to the left of the CD.**

source, such as the sun or a lamp, strikes a fine structure with regular spacing, the scattered light behaves as an array of coherent point sources. The coherent aspect means the phase of each source is the same and the point nature means it radiates in all directions, like a candle. Depending on the angle from which the array is viewed, the light waves of a given wavelength will either add in phase to form a maximum or arrive out of phase and cancel out, see figure 5.

Diffraction maxima occur when the following equation is satisfied:

$$m\lambda = d\sin(\theta d) \qquad \textit{Eq 1}$$

Where:
m = 1, 2, 3 . . N Order of diffraction, (more than one maxima can be produced)

d = Separation between elements of the structure, ($d \geq \lambda$).

$\lambda$ = Wavelength of incident light.

$\theta d$ = Angle of maxima from normal to plane of the structure.

When diffraction occurs as a result of reflection from a fine structure as in the case of a butterfly wing, the total angle of the diffracted ray $\theta t$ is, the angle of diffraction $\theta d$ plus the reflection angle $\theta r$ . (Snell's law: reflection angle $\theta r$ = incidence angle $\theta i$).

Rearranged from Eq1:

$$\theta d = \arcsin(m\lambda / d) \qquad \textit{Eq 2}$$

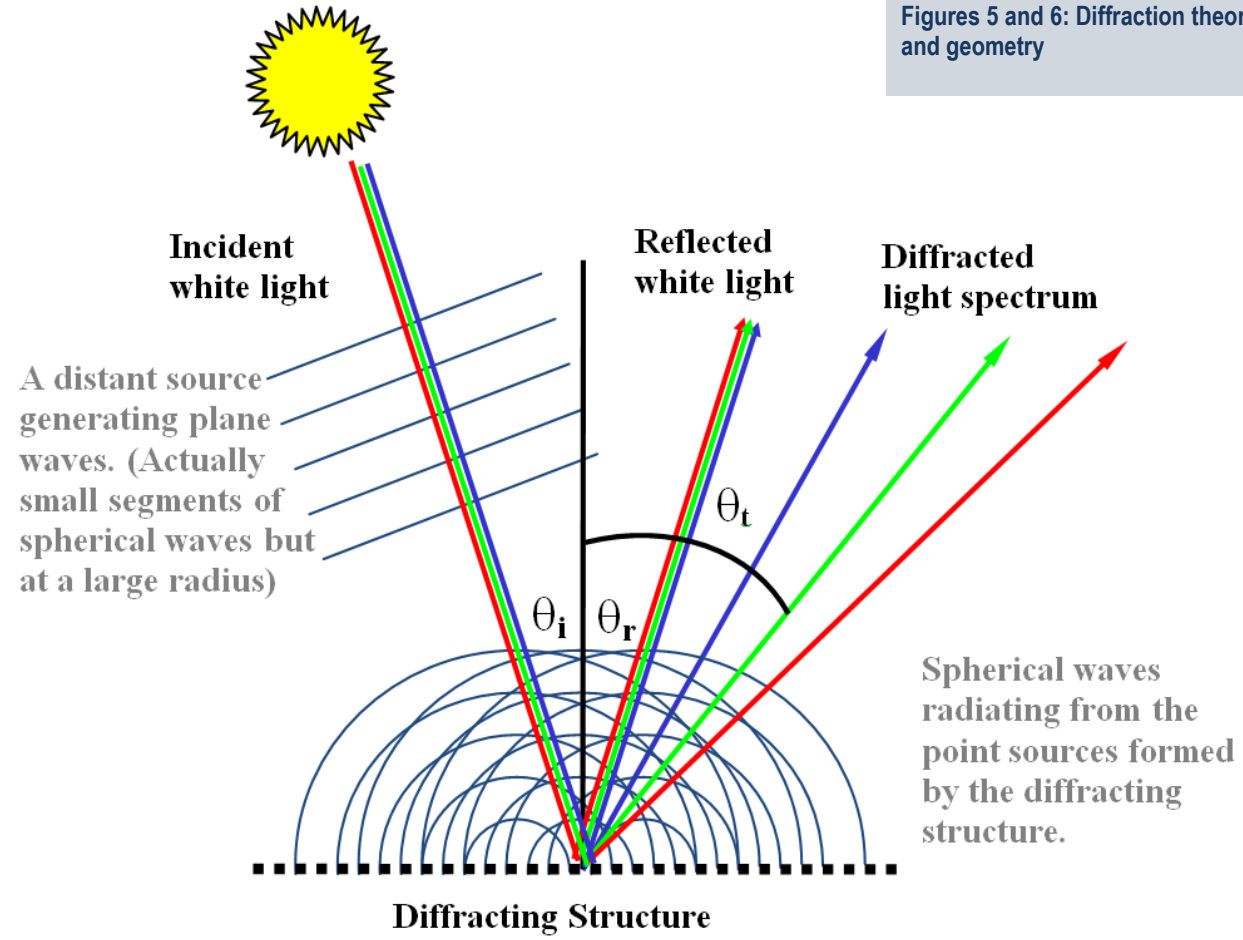
The total angle of diffracted ray:

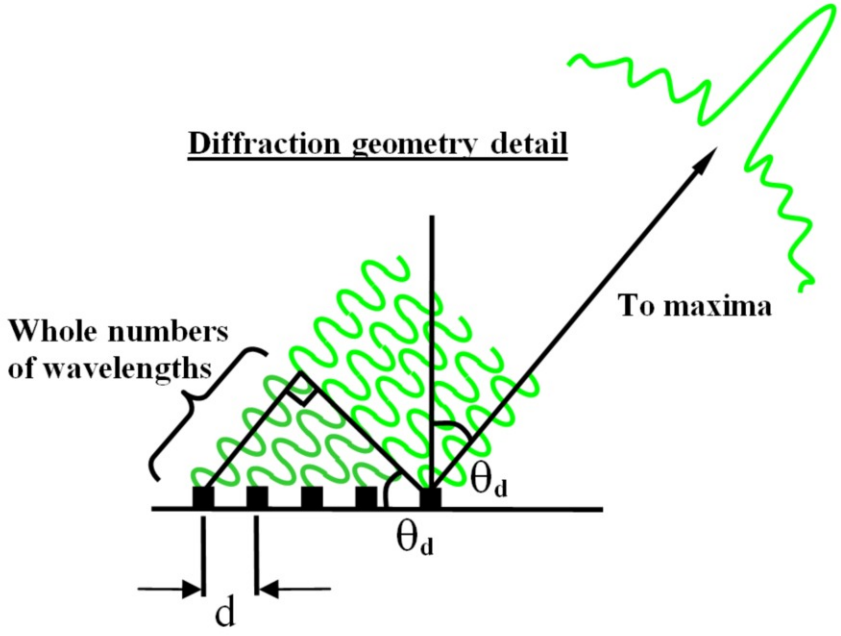
$$\theta t = \arcsin(m\lambda / d) + \theta r \qquad \textit{Eq 3}$$

So, it appears then that butterfly wings not only have a pigmented colour but, as a result of their fine structure are able to diffract the sunlight, adding even more glitz and glamour to the whole display.

In addition, researchers using electron microscopes have found even finer detail in the structure of the scales. It is thought that these light and sub-light-wavelength structures act to further modify the nature of light that passes through or is reflected from them. These

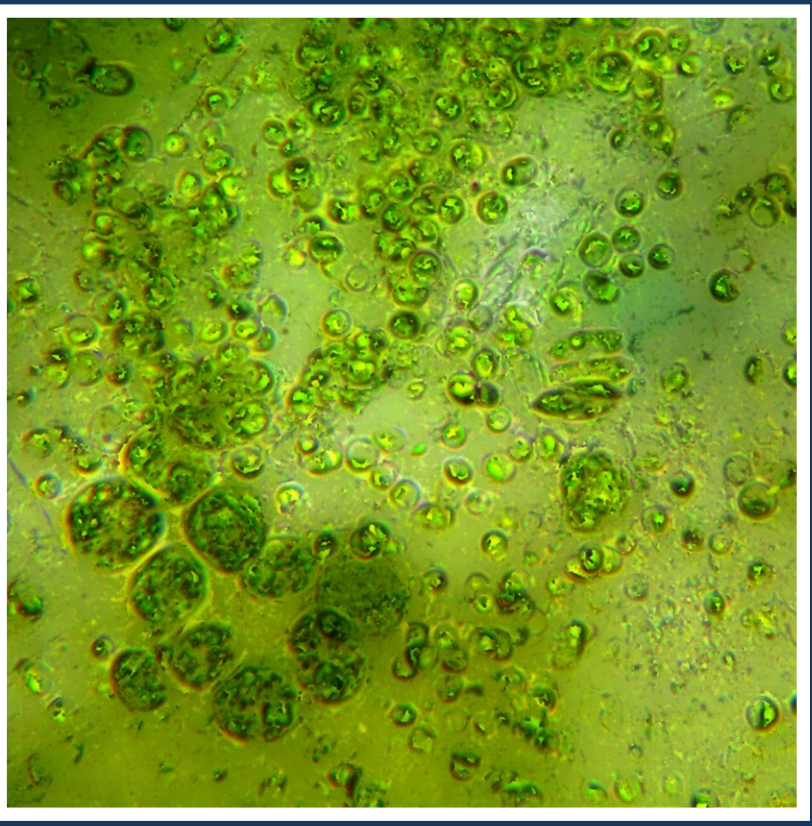
Figures 5 and 6: Diffraction theory and geometry




naturally occurring features have inspired nano-technology developments such as coatings to improve solar cell efficiency and anti-counterfeit markings for goods and bank notes. In fact, for some research applications the structures are so fine that they cannot be made artificially and actual wing-scales are coated with an ultra thin layer of aluminium oxide (alumina). Once coated, the structure is heated to around 800°C, crystallising the alumina and burning away the original wing-scales. For me, this is a great example of science and nature coming together and what makes it all so interesting.

■



**What's this? Answer on page 3.**