DIGITAL PHOTOGRAPHY
With The Low-Power Microscope

Paul van Beusekom

Digital Photography with the low-power microscope

And the use of software for extended focus

Paul van Beusekom
microbeus@gmail.com
Takapuna 2013

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Introduction:

The low-power microscope offers an often stunning insight in the world of insects, of very small flowers and seeds, watches and jewellery. Items that can just be seen with the naked eye take on an outer-worldly appearance, disclosing unexpected shapes and colours. The stereomicroscope provides an insight how things work.

But how to photograph these beautiful scenes? The microscope greatly increases magnification, but at the same time reduces the "depth of focus". Only a small layer of the object is sharp, features above and below are not. Looking through the microscope constant focussing up and down will overcome this, but in a photograph that is impossible.

The purpose of this paper is to describe the equipment needed to produce a series of digital images at different focus (slices) , to be combined to one image with extended focus, using special computer software. (Stacking) (plate 1)

This paper is intended for the serious hobbyist.

The professional photographer will have access to a Leica Macroscope for instance. Equipped with stepper motor driven focus, controlled by special software he can not only produce extended focus images, but also create 3-D representations from his slices.

This is beyond the scope of this paper.

The finished image

slice 1

slice 2

slice 3

Plate 1:
3 of the roughly 20 slices I used
to create this extended focus image.
Slice 1 has the stem in focus,
Slice 2 front of the case, and
Slice 3 the appendage.
The object is about 5mm in size

1.The Microscope

The optical equipment needed to take good quality pictures of objects between 1 and 10mm in diameter can be:

- A stereomicroscope (see below)
- A compound microscope with low-power objective (Chapter 2)
- A digital camera with macro objective or clip-on macro lens (chapter 4)

A *stereomicroscope* is designed for magnifications of about 4x to 40x. Some have more, some less, and some have a much bigger range. The microscope has two separate light-paths, one for each eye. Each light-path offers a slightly different view of the same object, which creates the 3-D impression.To achieve this, the light-paths are slightly inclined towards each other.

fig 1a

Fig 1a. shows a vintage microscope of the Greenough design. Each lightpath has its own front objective lens. This results in a microscope with good colour correction, but limits the diameter of each lens, and thereby it's capacity for high resolution images.

Fig 1b. shows a microscope with two parallel lightpaths from the eyepieces down. Each passes through one side of a common objective.This inclines the lightpaths toward the object sitting in the middle.
Phototubes etc can be inserted in the parallel lightpath, the light being diverted by a prism to the camera by pulling a lever.
The objective focal length can be choosen.
A lens with a longer focal length gives an increased working distance, and reduces the overall magnification of the microscope. This allows bigger objects to be photographed.

The prism effect of the main objective can cause colour fringing in the image, as well as geometric distortion. These imperfections can be avoided by choosing a more expensive plan or planapo objective, or by moving the common objective sideways, so it sits in the centre of only one of the lightpaths. This allows for the use of a much bigger lens, with better resolution and correction, but blocks the light in the other lightpath (plate 2)
In the stereomicroscope (fig 1a and 1b) the image shifts sideways each time the focus is changed. This shift has to be corrected by thestacking software, which is not always succesful.
Moving the objective lens sideways avoids the image shift.

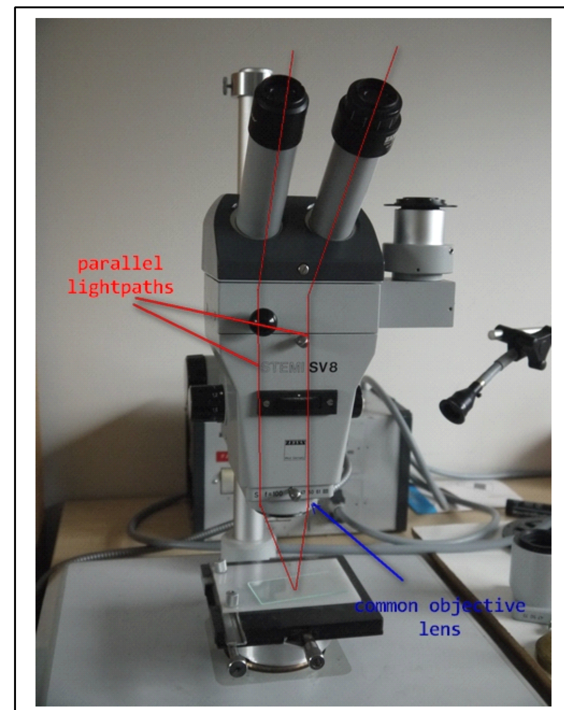

fig 1b
Common objective

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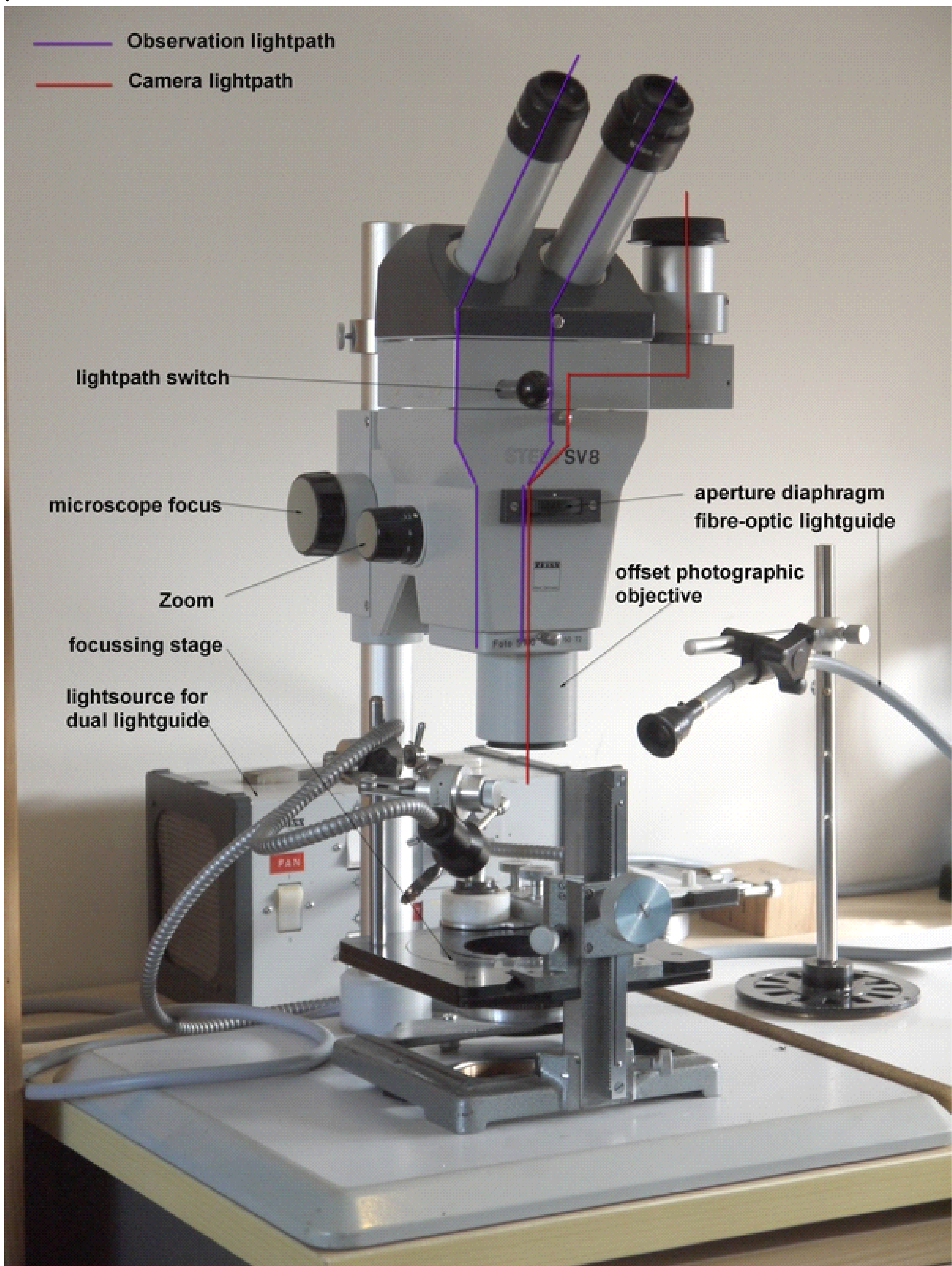


Plate 2: Zeiss SV 8 macro photography outfit (Digital camera removed from camera port to take this picture)

2. Microscope with low power objective

Those who own a conventional microscope (often called "compound microscope")of reasonable quality, such as those used in medical diagnostics, can attempt to use it as a low power microscope. Focus and lightpath coincide, there is no image shift.
It is essential to obtain an objective with long working distance and low magnification. The lowest usually available is 2.5x. This together with a 10x eyepiece delivers 25x magnification, and most low-power objectives have a longish working distance.
It is perfectly acceptable to obtain an objective that is not necessarily of the same make as the microscope, at low power this is not usually critical
A solid and immovable camera attachment is also important, or the image will shift between slices.
Illumination is via a stand-alone incident light illuminator (chapter 5). The built-in transmitted light is useless for 3-dimensional objects, and suitable only for transparent samples.

The depth of focus of even the lowest power objective is much less than that of a stereo microscope, or macro lens. The free working distance in front of the objective is usually only about 10mm for a 2.5x lens, perhaps only 4mm for a 10x and less than 1mm for any of the more powerful lenses. If the microscope will not rack up enough to allow room for the specimen on the stage, it is often possible to remove the condenser from the sub-stage, and place the object on this.
One of the sample images shows an image of the head of a small fly taken with a 2.5x lens. Depth of focus was so shallow, that 5 slices were required just to get the eye alone in focus.

Fig 2.
Low-power compound microscope
Much smaller instruments than this one could do a good job.

The camera adapter consists of two eyepieces mounted face to face, the top one has its barrel removed and replaced with a c-mount thread. (fig 5.)

3. The camera and adapters

There are many options for fitting a camera to a microscope. They range in cost from $25 for a cheap USB webcam to a $10,000 dedicated camera supplied by the microscope manufacturer.

A dedicated camera can be made from a decent quality webcam. The trick is to make a mechanical connection between the camera lens and a spare microscope eyepiece, or to remove the camera lens altogether and glue the camera housing direct onto an eyepiece. (fig 2.)

The advantage of this arrangement is that very good software for photomicrography with a USB camera is available free on the internet: "MICAM"

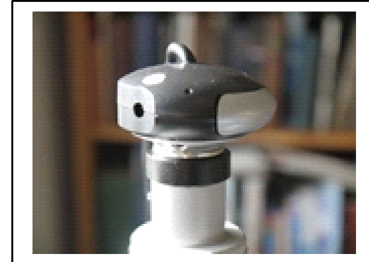


fig 3. Webcam on microscope tube

The quality of the results depend entirely on the camera, the $5 webcam from Ebay (fig 2) only really gives results suitable for initial practice. Make sure to remove, disable or cover all LED lights and microphones.

Another option is a regular digital camera available in many makes and classes from the camera or electrical store. Choose one for which a filter adapter or c-mount adapter is available, and fit the camera with its lens as close as possible to a microscope eyepiece.
It is essential that the camera can not move when fitted to the microscope, the image stacking software will not work if the image has shifted between slices!
If possible set the camera to Aperture Priority, and set it with the aperture wide open.
You must be able to disable the automatic focus of the camera, otherwise it will not reliably stay on the point of focus for each slice.
I did some excellent work with a Lumix LX 3 fitted this way.

It is of course possible to use a DSLR. If a c-mount adapter is available for the camera body, and the microscope has a proper phototube with c-mount, it will go straight on.
Consult the manufacturer of your microscope for this option.

My current solution is a Lumix GF2 mirrorless DSLR (fig 4.)
A c-mount adapter available from e-bay for a few dollars fits directly to the top of my phototube, which has no further optics (plate 3 diagram B). The phototube can be focused individually so the camera focuses in exactly the same plane as the eyepieces. The viewfinder image can be enlarged for very precise focus.

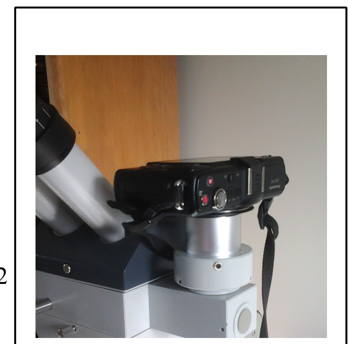


fig 4
Lumix GF2

A problem with many DSLR's, including the GF2, is that they do have a mechanical shutter, which can introduce a vibration during exposure.On my setup this is only a problem when I use very high magnification (over 100x).
It can be easily overcome by reducing the light and choosing a long exposure time (around 1 s)

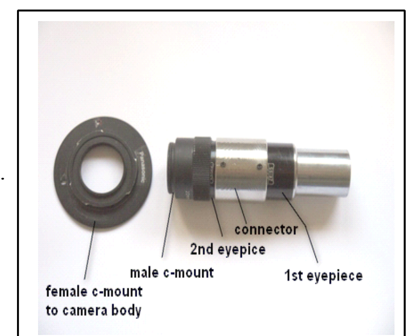


fig 5.
Adapter for made from 2 eyepieces

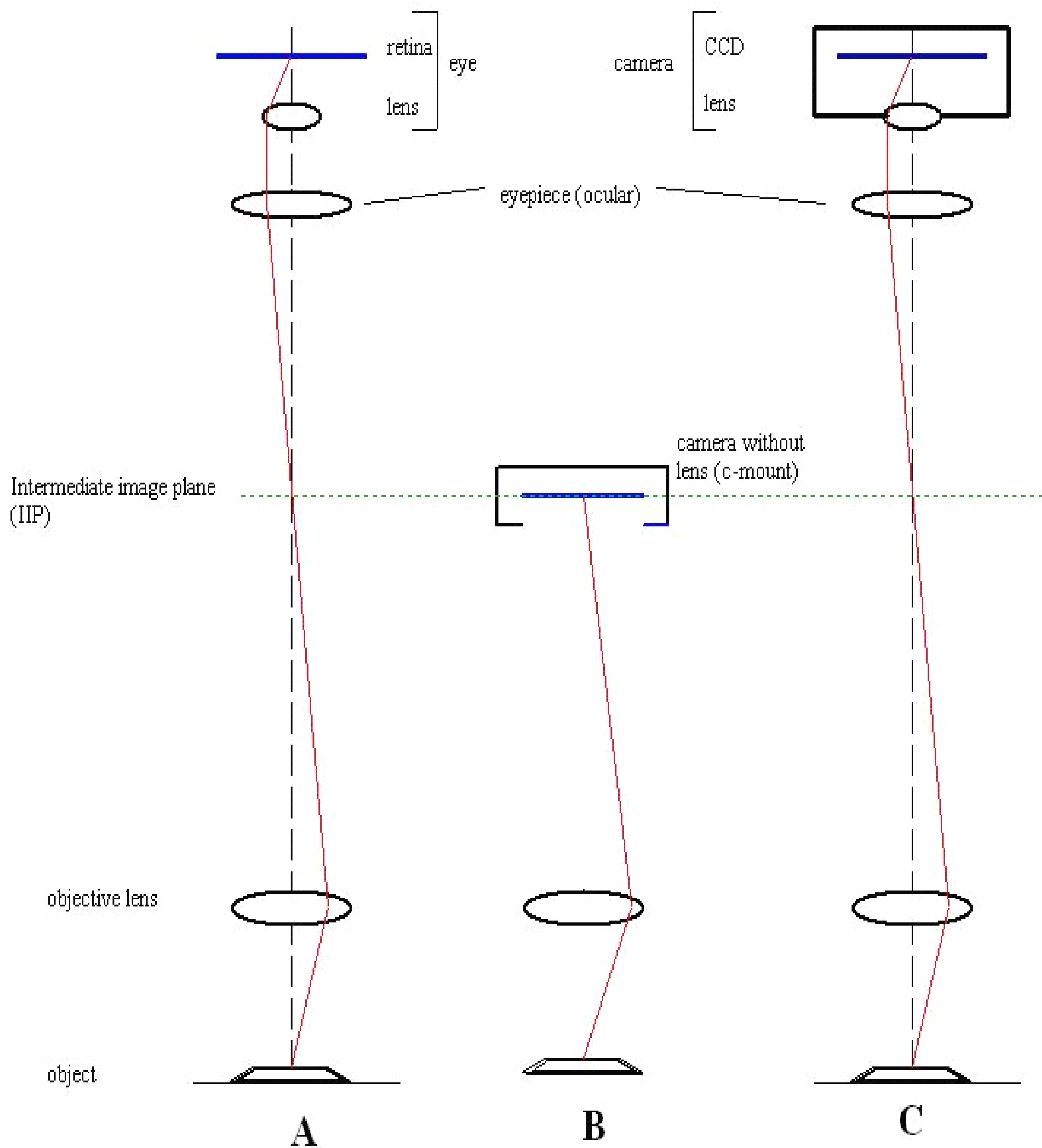


Plate 3: Raypath for camera on microscope
A: observation without camera
B: c-mount camera without lens
C: camera with lens and eyepiece

In general a camera can be attached to a microscope in two ways (plate 3):
Both stereomicroscope and compound microscope have an objective lens, which forms an "aerial image". This image is only visible if a screen is held in the point of focus, much like a slide projector. The image is only visible and sharp if the projection screen is placed in the correct position.
The Intermediate image can also be made visible by focusing a lens onto the Intermediate Image Plane (IIP) In diagram A, plate 3, the eyepiece lens is focused on the IIP and so forms an image via the lens of the eye onto the retina.
In diagram B a camera, with its lens removed, is placed so the CCD chip is in the IIP.
This works well in systems where the total optical correction takes place in the lower half of the microscope. This is the case in most stereomicroscopes with a common front objective, like the SV8.
In most compound microscopes correction is shared between objective and eyepiece. Fitting the camera in this way is not always optimal.
In diagram C the human eye is replaced with an optically equivalent camera. Especially where a camera with a non-removeable objective is used, this is the correct method.
Make sure the camera objective is as close as practicable to the eyepiece lens, without scratching it. Please note that in most cameras the front of the lens moves in and out when operating the zoom. Room must be provided for this.
In fig 5. the second eyepiece acts as the camera objective.

4. Camera with Macro objective

If the camera can be manually focused it can be used for image stacking in macro setting, or with a close-up lens fittedto it's objective.
A sturdy macro stand (fig 6.) is of course essential. Great care must be taken not to move the camera between shots for the different slices.
Do take a shot of a ruler at the same setting for use later to make a calibrated scale bar.
Fig 4 shows a small wooden block that I use to avoid having to move the camera up and down on the stand. By using it on one side or the other I get different object hights. A focusing stage (plate 1) is of course even better.

fig 6. macro stand

5. Illumination

In a good image the lightest area is still dark enough to show detail. Equally the darkest part also shows some detail.
The capacity of the eye to see detail in the brightest and darkest areas is better than the digital camera. Therefore an image that looks good through the eyepieces, can produce a inferior result on the monitor. Completely white and completely black parts of the image can not be improved with software (chapter7)

As a light source in macro photography (chapter 4, fig 6) the light from a window can often give good results. To avoid the object being to dark on one side use a white card behind it to reflect the light.

The stereomicroscope, and especially the low-power objective on a compound microscope, will need more light. I use a sophisticated fibre-optic system, but good results can be obtained with two or more small LED torches mounted on a wooden block. The top of the block should have a small bag filled with sand, so the torch can be positioned accurately.
My secret weapon is the polystyrene (not cardboard!) coffee cup.
Polystyrene is neutral in colour, and a very efficient diffuse reflector. Remove the bottom of the cup, cut it so it fits under the objective, and cut one side to give access to the sample.

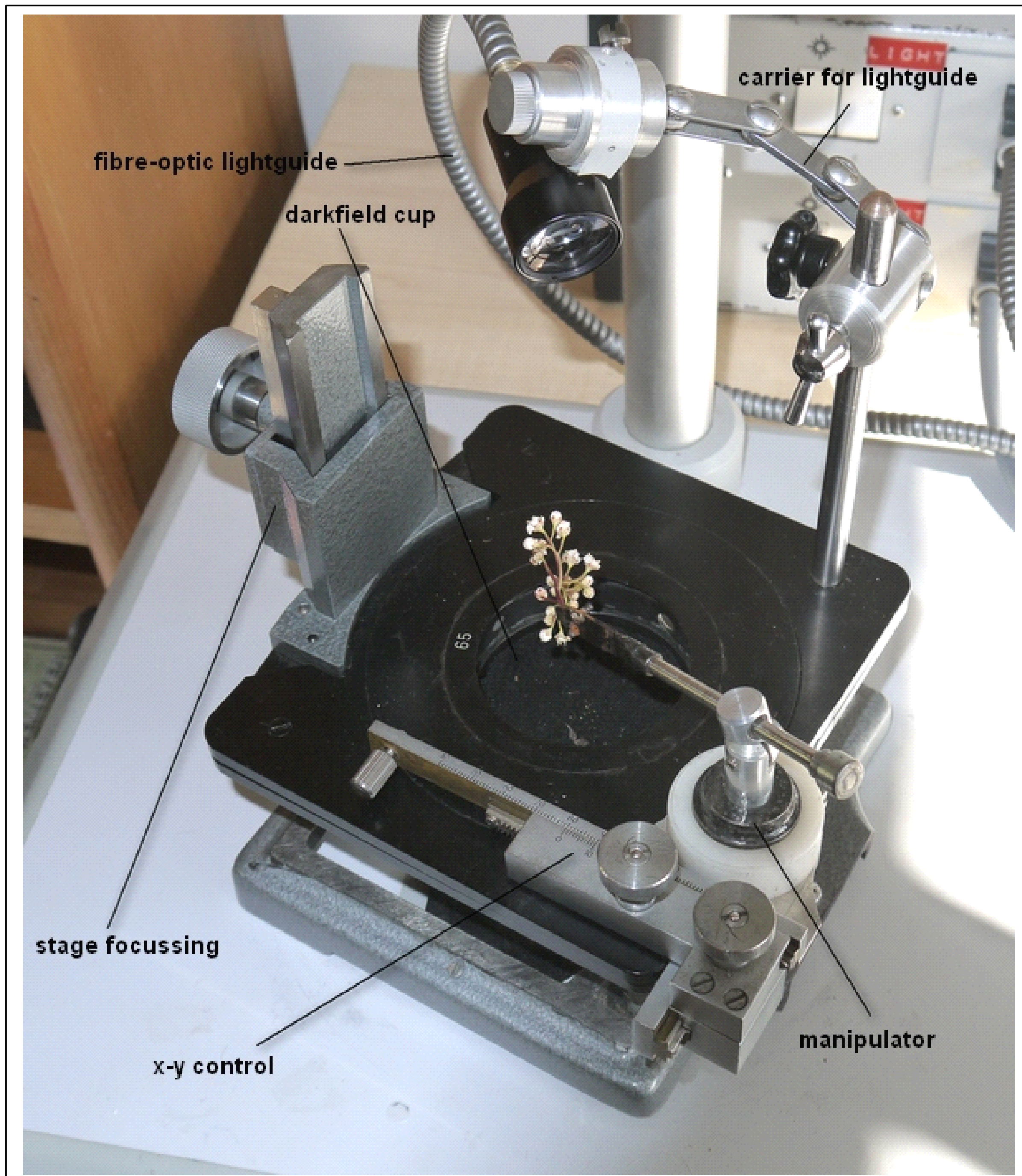
fig 7. The coffee cup

The light should be directed from the top into the cup, without striking the sample. Insect wings and delicate detail on small flowers can be shown brilliantly by directing some of the light through the opening in the side, striking the sample from the side or slightly from below. The background must be black for this to work. I prefer a totally black background for most of my images. With a white or coloured background it can be difficult to avoid shadows, which often look ugly.
A white background will very often cause reflections in the microscope which reduce colour intensity.
But mostly a black background with the object illuminated from the side or slightly from below, produces stunning detail in hairs, wings, etc.
Almost any black surface when illuminated directly will show some unwanted detail. My solution is a metal cup under the stage, covered inside with black felt, and deep enough so no light can reach the bottom.
For correct colour balance focus the microscope on the polystyrene cup only, using the illumination you plan to use for the image. In you cameras' colour balance menu set the white balance to the colour of the cup. If your camera does not have this option, take an out of focus image of the cup for later reference.

6. Object manipulation.

Below my microscope is a focusing stage with x-y translator (plate 2.). This is largely home made out of an old microscope stand. It allows me to coursely move the object table up and down, as well as precisely centre my sample in the image frame, and rotate it to fit.

Plate 4. Focussing stage with manipulator



This home-made manipulator design 9fig 8) dates from the 19th century.
Self closing forceps hold the object.
The object is often glued with a small drop of clear nail varnish onto the head of a pin, the pin is held by the forceps.
It can now be turned and tilted at will, so I can photograph the object in any desired position. The manipulator attaches to the x-y movement of the stage.

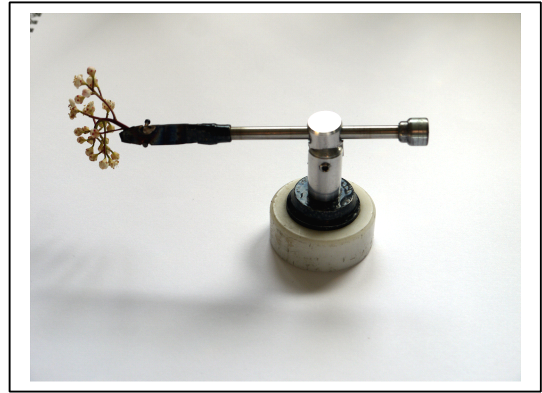


fig 8 the manipulator

An alternative is to hold the object on a small blob of Bluetek, or plasticine, placed on a slide. If the object is mounted on a pin, the pin can be inserted in a small cork. Insects are usually kept impaled on a pin, however for very small insects and othe objects glue would be a better option.

7. Computer and software

The image stacking software takes up to 40 images of about 5Mp each in JPEG format, and then produces a TIF file of around 15Mb. This places a certain amount of demand on the processor, and on the available RAM.
My old PC with 1Mb RAM was inadequate, the whole thing tended to take forever to process a stack.
My current system has 4Mb RAM and a 2.3 GHz processor. This works OK.
It is obviously essential to have a good quality screen. It should be big enough to see fine detail (min 21 inch) and be running off a decent display card.
Cheaper monitors are often designed for office work, and do not display an optimal image.

To display the initial JPG images many programs are available, including the free ones supplied with Windows. Personally I prefer to use the freeware **Faststone Image Viewer** (www.FastStone.org). The images in my directory are displayed as thumbnails. Clicking one changes to the work interface, with all thumbnails displayed along the top of the screen if my cursor is in that area. Left-right arrow keys on the keyboard quickly scan through all images, a continuously available magnifier lets me quickly check areas of interest. Move the cursor to the left, right or bottom of the screen and various menus become visible for basic image editing. Sharpness, contrast etc adjustments are available, but are too course for my liking. Use Photoshop for these if required.

The extended focus software available as freeware is called **Combine ZM.** Go to http://hadleyweb.pwp.blueyonder.co.uk/CZM/News.htm for download information.
The software is very easy to install and use. When clicking on "file-new" it will open your directory, which you can make the directory where you keep your images straight off the camera. Select the images in the stack by holding down [shift] and selecting the first and last file. "Macro-do stack" or "macro-do softstack" will start the process of combining the images, and "file-save image" will save the final result.

A note on image file formats:

Most cameras will produce their image files as JPG. This is the input format for Combine ZM.

JPG is a compressed format. Every time a file is processed and re-saved, it is compressed again. Therefore each subsequent copy is of lesser quality. For this reason professional photographers would use the JPG format only when a small file size is needed (email. etc) The format mostly used is TIF.

This is not compressed, and can be copied without loss of quality. The size of a TIF file is therefore much bigger.

Some of the more sophisticated cameras can be set to produce images in RAW format. These files contain the pure image data, without any processing. RAW files are specific to the camera brand, and require special software to open. They are very large.

Photoshop software has its own file format. A file opened with Photoshop can be saved in this format, facilitating future processing with Photoshop. However the software works very well processing JPG or TIF as well.

I have a very old (version 6) copy of Photoshop. It has one or two features which, while not exclusive to Photoshop, work very well for me (see below).

My workflow:

- The original images are dumped from the camera in a directory called "z-stacks". I open them in FastStone and have a quick look to see that the details in each slice are in focus. I delete images that have no in-focus details.
- Open Combine ZM, load the stack and start the macro.
- The finished result is saved back into the "z-stack" directory. Close Combine ZM.
- The final result is examined in FastStone. Crop if required.
- Go back to the microscope and measure (chapter 8) an image feature (for instance the diameter of an eye of the insect being photographed), using the eyepiece graticule.
- In FastStone move the cursor to the left and select "Drawboard" Draw a scalebar representing 1mm in the corner of the image (chapter 8). Close the drawboard, right-click and "save as".
- Right click the image and choose "edit with external program - photoshop" Photoshop is opened automatically and the image loaded.
- First save your original image.
- Choose "image adjust- levels".

A frequency plot is displayed. It shows how many pixels of every intensity level are present in the image. There should be no flat horizontal lines in the diagram, these would indicate that the darkest area is not as dark as the monitor could show it, and the lightest area not as light. Move the sliders under the diagram to ALMOST exclude these flat lines. Make sure the image still looks natural.

If the background is not completely black choose the black dropper and click on the background. This will often improve the colour of the object as well.

If your camera has no facility to adjust the colour balance, load the image you took of the polystyrene cup now, go to "levels", click on the white dropper, and click on "save". When adjusting your final image go to "levels" and click "load" You have now compensated for any off white colour in your illuminator.

Finally try to SLIGHTLY adjust the middle slider under the diagram. This will darken or brighten the image. A very small amount can often enhance the look of the image. Click "OK" to close the levels dialog.

If you want to adjust the contrast in your image I find the "Image adjust-curves" menu the easiest. Always make only very minor adjustments, too much and your image will look awful. Save your changes (in a new image), minimise Photoshop to see the final image in FastStone.

8. The scale bar

Software supplied with many microscope cameras includes the option to automatically produce a calibrated (true to size) scale bar in the image. In the absence of this you can proceed this way:
Mount a measuring graticule in your microscope eyepiece. This scale does not change when the magnification of the microscope is changed. The scale therefore has a different value for each magnification of the microscope optics.

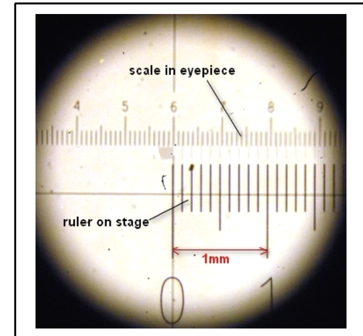


fig 8

I have made a table (plate 5). and pinned it to the wall behind the microscope.
Take a fine ruler and put it on the stage.
select the lowest magnification and focus.
Looking into the microscope you should see something like fig 8. Align the ruler with the scale in the eyepiece.
Note which scale is on the stage, and which is in the eyepiece.
In fig 8. I see that 10 divisions of the eyepiece scale equal 0.51mm
I note in my chart that at the magnification used one eyepiece scale division equals 0.051mm.
Repeat for all other magnifications.
Looking at my object, I choose an easily identifiable feature, and see how many eyepiece scale divisions it covers. A petal on a flower, or the eye of an insect serve well.
Using my chart I convert this number to actual millimeters.
While examining my final image in FastStone I open the "Drawboard"
Measure the eye (petal, etc) with a ruler on the monitor, and divide this value by the actual size of the eye. This is the length on the screen of a 1mm scale bar. Draw it on screen using a ruler in a corner against the black background and mark it "1mm".
Example:
Zoom setting 4x: conversion factor on chart is 0.0255.
Insect eye measures 23 eyepiece scale divisions. This equals to 0.0255 x 23 = 0.58mm
On the monitor the eye measures 28mm
A 1mm scale bar on the monitor should be drawn at 28/0.58 = 48mm long.

Note that this chart is only accurate for my microscope.

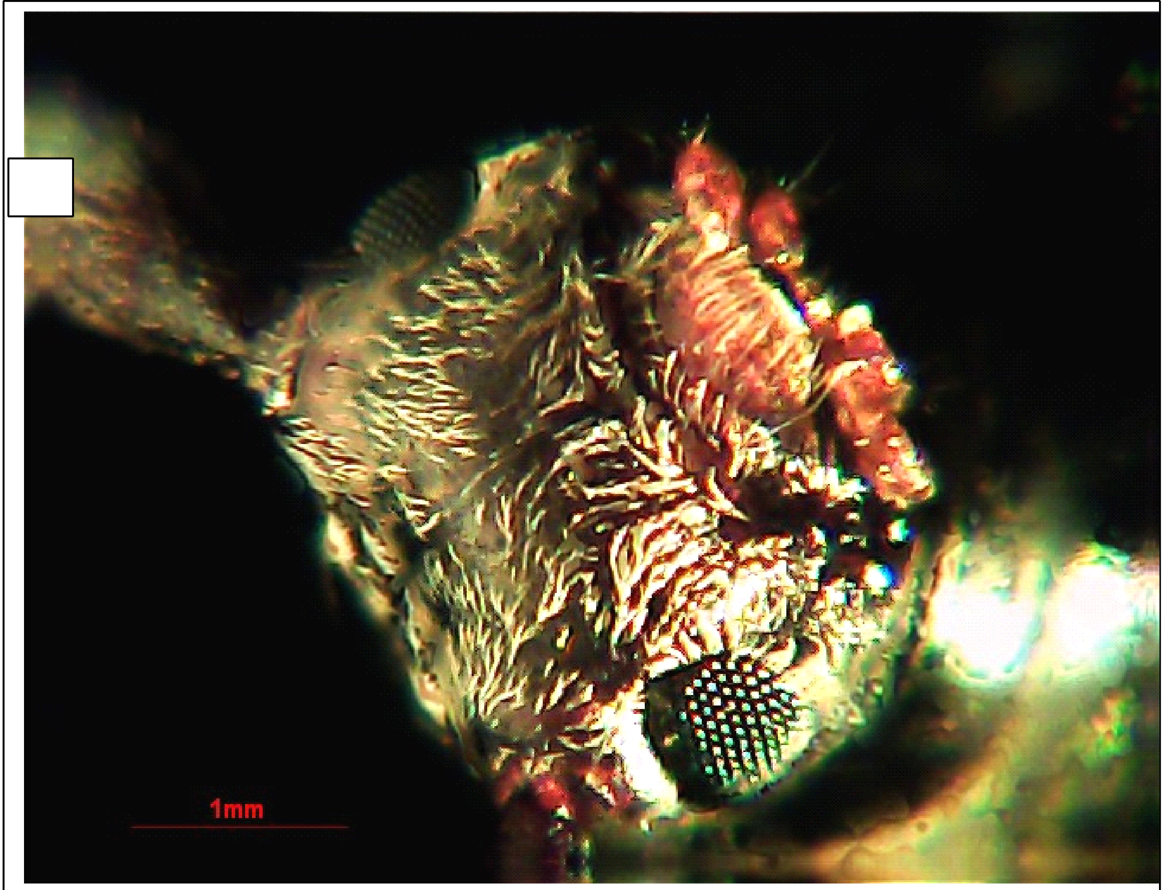
Calibration sheet Zeiss SV8
Objective photo S100

Zoom setting	1 eyepiece divn = mm
0.8x	0.1215
1.0x	0.1030
1.2x	0.085
1.6x	0.064
2.0x	0.0515
2.5x	0.0410
3.2x	0.0323
4.0x	0.0255
5.0x	0.0205
6.4x	0.0157

plate 5: Example of a calibration chart

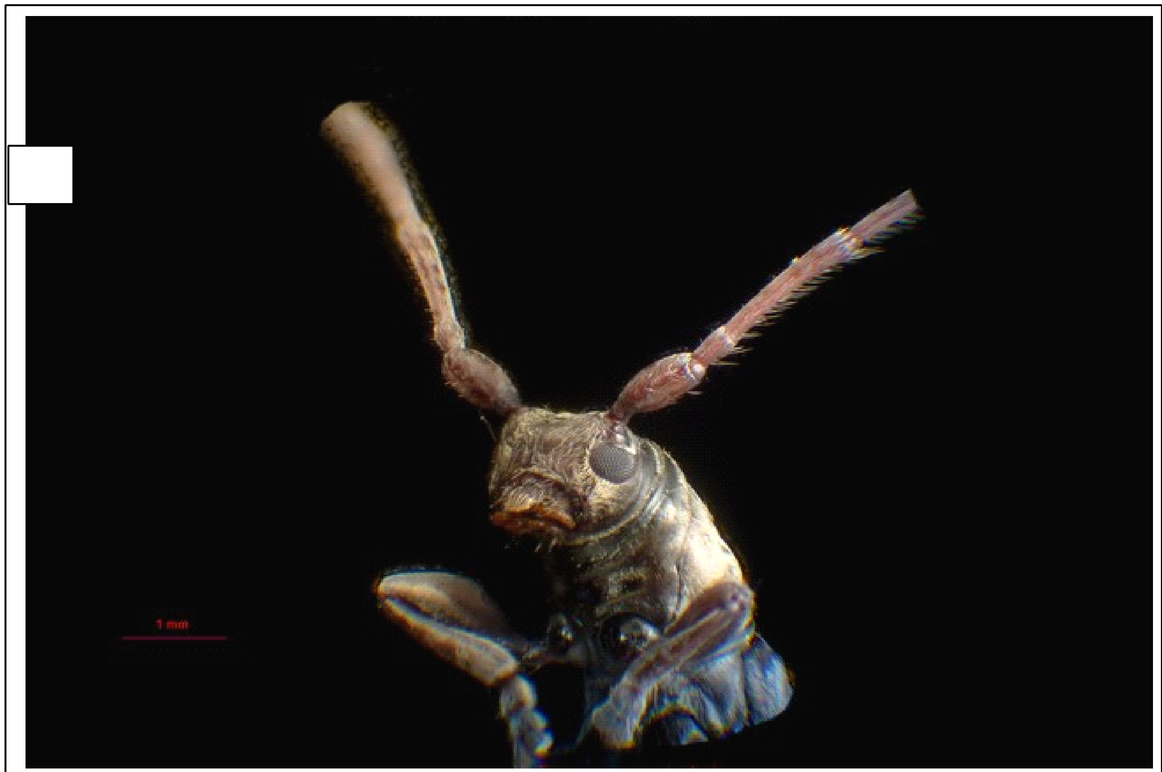
An extended focus image taken with the Lumix GF2 with macro objective

Head of a small fly taken with a 2.5x objective on compound microscope



Extended focus image taken with a $5 webcam ↗

The same small fly ↘



Seedpod of the flower "Grandmas' Bonnet.

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These and other images can be ordered in TIF
at around 4000 x 3000 pixels
Takapuna 2013