The new photomicrography

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Peter Evennett took his first degree in Zoology at the University of Liverpool and his PhD at St Andrews, during which time his interests in microscopy developed. He lectured in Zoology at the University of Leeds, UK, for more than 30 years, with a particular interest in histology, cell biology and, of course, light and electron microscopy. He retired early from the University, and now concentrates on his interests in microscopy, including teaching courses on microscopy for the RMS and independently. He is particularly interested in the fundamental principles of the light microscope and in finding simple ways of teaching and demonstrating them to both new and established microscopists, and also in helping amateur microscopists. Peter is Honorary Archivist of the Royal Microscopical Society, and an Honorary Fellow of the Society.

Photographic techniques have been applied to recording images from the microscope for more than 150 years – an early example was Foucault's micrograph of blood cells taken in 1844. Driven principally by their applications to general photography, conventional silver-based processes for both monochrome and colour now far exceed the normal requirements of photomicrography in resolution and film speed, yet we are forced to wonder how much longer we shall continue to use these conventional techniques.

Recent developments in digital image recording have brought rapid changes to photography, and in consequence also to photomicrography. This article describes how the use of an 'amateur' (rather than 'professional') digital camera can provide many of the advantages of digital imaging at a cost which, because of mass sales, is relatively modest compared with that of a specialist instrument. The resolving power of current cameras is now quite good enough to produce an A4-sized print of excellent quality, and to record all the information in a normal microscope image, with the advantage that the user can evaluate the result instantly, and delete and repeat as necessary.

In 1999 I saw a demonstration of the Nikon Coolpix 950 digital camera used for photomicrography. I immediately saw that this offered the small laboratory and the lone worker the opportunity to 'go digital', and in addition could act as a generally-useful photographic tool. I bought a 950, and later upgraded to the then slightly more capable model, the 990. The Coolpix 950, 990 and their successors have a growing and enthusiastic following amongst amateur and professional photomicrographers.

Several features commended the Coolpix cameras to me. The most important is the 28mm x 0.75mm screw thread in the front of the lens, intended for

attaching wide-angle or tele accessory lenses, and used by Nikon for its microscope adapter lens (of which more later). The design of this camera's lens lends itself to use on a microscope: focusing and zooming functions take place by movement of internal lens elements, so that the screw thread at the front of the lens is firm and does not move, and it is strong enough to support the weight of the camera. And the two-part 'twisting' design of the camera body enables the screen to be set at a comfortable angle for viewing when the camera is mounted on the microscope.

Fitting cameras to microscopes

It is important when fitting any camera to a microscope to consider the optics of how the microscope's image is to be transferred to the light-sensitive surface, and also the relationship between the size of the detail resolved by the microscope and the detail that can be recorded by the camera.

Digital cameras designed specifically for photomicrography generally have no lens, or no *fixed* lens*,* and they attach to the microscope using the one system which nowadays is in common use by all manufacturers - the so-called C-mount. This is a 1 inch diameter x 32 threads-per-inch screw, originally designed to attach the lenses of 16mm cine cameras, and more recently adopted as the standard mount for small lensless video cameras. Adapters are available to provide most modern microscopes with a C-mount thread.

The C-mount can at its simplest be arranged so that the primary image of the microscope falls directly on the image-sensor (the CCD or *charge-coupled device*) of a camera which is not fitted with a lens, without using an eyepiece or any other lens system after the primary image.

However, since the lens of the usual 'amateur' digital camera is not removable, a different approach is necessary. A camera fitted *with* a lens behaves essentially like an eyeball, and hence requires an eyepiece or functionally equivalent lens. An eyepiece delivers the image information in the form of parallel rays, to be converged to a focus on the retina by the lens of the eye, or on the CCD by the lens of a camera.

Microscope adapters for the Coolpix

Two types of microscope adapter are sold for the Coolpix cameras. The first are simple non-optical devices which support the camera above a normal eyepiece; home-made substitutes are easily constructed. The more elaborate systems attach to the camera and perform the function of an eyepiece. Nikon’s MDC lens is one of these, designed to screw into the front of the camera, and attach to a C-mount or fit into a 30mm diameter tube; similar devices are produced also by other companies.

Using a normal eyepiece as an adapter

There is another option, for those wishing to adapt a Coolpix or similar digital camera to an older microscope, and/or not wishing to pay the price for the commercial adapter: this is to use a conventional eyepiece as the adapter.

It is important to select an eyepiece with an adequately high exit pupil or eyepoint, generally one made for use by spectacle wearers, usually marked with a picture of a little pair of glasses. The eyepoint can be found by holding a piece of thin paper above the eyepiece (fitted in the microscope, with the lamp on), and moving it up and down until the spot of light seen is sharply focused and of smallest diameter. It should be at least 15mm (and preferably more) above the upper surface of the eyepiece. The eyepoints of several eyepieces are demonstrated in Figure 1. If the eyepoint is too low, so that it does not reach far enough into the lens of the camera, the edges of the field of view will be lost by vignetting. The effect is the same as moving your eye away from the eyepiece: the field rapidly diminishes in diameter until all you see is the exit pupil itself.

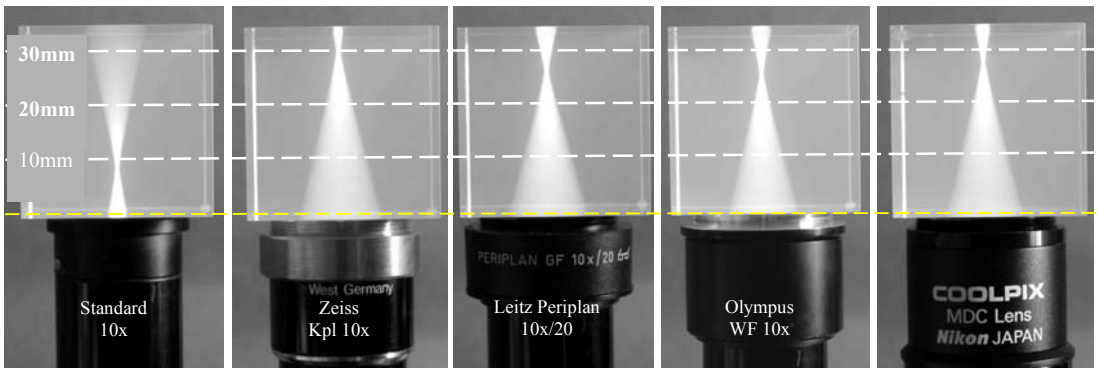

FIGURE 1
The eyepoints of a range of eyepieces, demonstrated in a block of milky glass: less than 10mm above the top surface of a standard 10x eyepiece, and more than 25mm for the others.

Experiment with a range of eyepieces, since they can differ quite widely in their suitability. Promising ones can then be tried with the camera. Switch the camera on, set focus manually to infinity, turn the flash off, and zoom to wide-angle. Set the microscope lamp to normal brightness for the eye, offer the camera up to the eyepiece, as close as possible without risking damage to any glass parts, and observe the image on the screen. It will probably be in the form of a circle somewhat smaller than the screen, a circle with a clearly-defined edge which represents the diaphragm of the eyepiece. Zoom to a longer focal-length until the image just fills the screen, when the camera will be set to record an image rectangle with its diagonal equal to the diameter of the eyepiece field of view. If this can be achieved, record a few images and assess their quality. Look for vignetting at the corners: the more the camera's lens diaphragm is closed the worse this is likely to be. To minimise this, set the camera lens to its full aperture using the 'aperture-priority' mode, so that exposure adjustments are made (automatically) by alterations in shutter-

speed rather than camera lens aperture. If the image is satisfactory, now consider how the camera might be supported. It could obviously be attached to a macro stand or even a tripod, but it is more convenient if it is self-supporting in the eyepiece tube, either by the lens thread, or by a bracket attached to the tube.

In my case I found a Zeiss eyepiece which performed well, the metal barrel of which, by good fortune, has a diameter of 28mm. I carefully removed the lenses from it, put the barrel into the lathe, and cut a thread to fit the camera. As a refinement, I also made a ring with the same thread internally, to screw on to the eyepiece and butt up against the front of the camera's lens mount (Figure 1, second picture), to prevent its making contact with the glass window just within the lens mount, and possibly causing damage.

Having made this adapter, I learned that a Leitz Periplan x10/20 GF eyepiece, type no 519 815, made 10 years or more ago, is already fitted with a 28mm x 0.75mm thread for attaching the eyecup, and was fortunate to be able to obtain one of these. A critical check of chromatic aberration correction showed that this eyepiece was suitable not only for my Leitz Ortholux (1960s vintage) but also for my Zeiss microscopes (160mm tube-length designs). Another, different, Periplan eyepiece (10x/18) can also be used, though this one has a slightly smaller field and its upper lens mount projects beyond the screw thread, making contact with the window of the camera lens; take care to avoid damage if using this eyepiece. Both of these are shown in Figure 2.

FIGURE 2
Two Leitz Periplan eyepieces showing their 28mm x 0.75mm screw thread for the eyecup (removed on left). Model no 519 815 (centre) is marginally more suitable than the unnumbered one on the right since it has a larger field of view number (20), and no parts project above the thread and risk damage to the camera.

Attached to one of these eyepieces, the camera can record images from any 23.2mm internal diameter viewing tube, or a 30mm tube using a simple sleeve adapter. When fitted into the vertical phototube of the trinocular of my Zeiss Universal, the image is precisely in focus along with that on the eyepiece graticule in the binocular – the microscope was of course designed so that this should be so. Even if the microscope has no vertical phototube, the camera will operate satisfactorily in an inclined eyepiece tube, and the screen can be turned to provide comfortable viewing in most circumstances (Figure 3).

FIGURE 3
The Coolpix 950 fitted to the Periplan GF 10x/20 eyepiece and inserted into the inclined viewing tube of a microscope binocular head, with the screen angle set for comfortable viewing.

Chromatic correction

One complication should be borne in mind when using the Nikon MDC lens, which is naturally designed to suit Nikon's current range of stereomicroscopes and high-power microscopes. These, in common with recent models from

other major manufacturers, have a *fully-corrected* primary image. Older high-power instruments are designed so that chromatic aberration is only partially corrected by the objective lens, the remaining correction being done by the eyepiece, a so-called *compensating eyepiece*. This means that some colour fringing exists at the edges of the field in the primary image, a 'defect' which is not normally seen by the eye since it is corrected by the compensating eyepiece. The Nikon MDC lens for the Coolpix is designed without compensation since it is intended for use with recent microscopes, so it will faithfully transfer the chromatic defects of the primary image to the camera when used with objectives designed to require a high level of compensation in their eyepiece. To demonstrate this effect, often known as *chromatic difference in magnification*, Figure 4a shows an image of a stage micrometer photographed through a purple filter which passes red and blue, but absorbs the middle of the spectrum, using the Nikon MDC lens in combination with an objective of the older type (Zeiss, Oberkochen); note that the red image is larger than the blue, a defect which becomes greater towards the edges of the field. Figure 4b was taken with the same objective and MDC lens, together with the Zeiss C-mount adapter which contains appropriate compensation, producing a fully-corrected primary image. Whether or not this defect is considered disturbing will depend on the design of the objective, how much of its field is included in the recorded image, the nature of the specimen and illumination, and how critically the resulting images are examined.

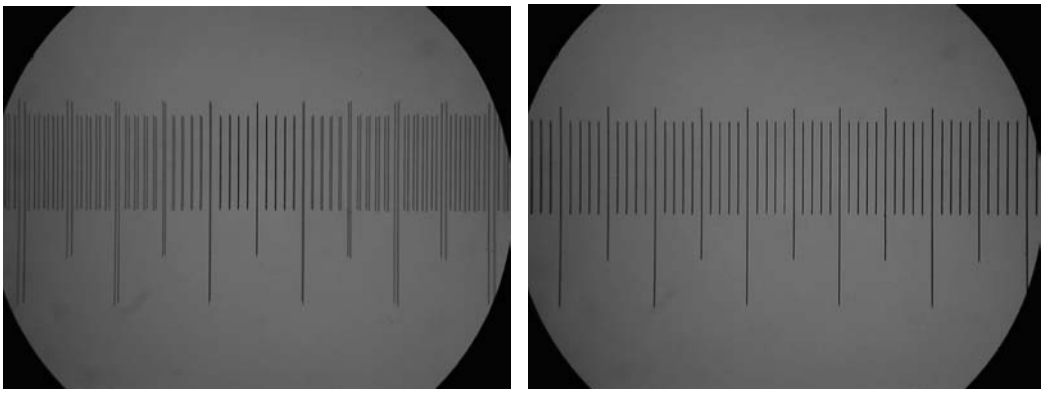

FIGURE 4
Chromatic difference of magnification demonstrated in photographs taken through a purple filter: Figure 4a incorrect, 4b with a correct combination of components (see text)

Shutter release

As with all simple photomicrographic cameras, there is some risk of camera shake when the release button is pressed. Unfortunately the Coolpix lacks a socket for a conventional cable release, though accessory brackets are available to enable them to be used. Alternatively, the camera's delayed action facility can be used. The ideal (though rather expensive) solution is Nikon's remote release (MC-EU1). This is a small electronic device (Figure 5), with its own battery and LCD screen which shows the number of exposures remaining on the memory card, and it plugs into the camera via the data

transfer socket. I understand that this remote release is not suitable for the older 950 model.

FIGURE 5
The remote control for the Coolpix 990 and later models (MC-EU1).

Another socket on the camera provides a composite video signal suitable for direct connection to a monitor or video recorder, enabling the camera to act as a high-resolution video camera. Viewing the image on a monitor screen enables focusing and image composition to be done more conveniently.

Resolution

It must be recognized that a small digital camera cannot (yet?) equal the fine resolution of film, even the relatively small format of 35mm, but it can be adequate for photomicrography. It is convenient to relate considerations of resolution to distances in the primary image. To illustrate this simply, take a situation which is almost as demanding of camera resolution as is likely to be encountered: using a 40/1.4 objective, a lens with a high ratio of numerical aperture (and therefore resolving power) to its magnification. Consider this objective fitted to a microscope with no intermediate magnification factor, with the camera lens zoomed to accept a rectangle of 16 x 12mm from the primary image. Having a diagonal of 20mm, this is the largest rectangle that can be taken from the field of view of many common eyepieces.

The objective's Numerical Aperture of 1.4 gives us a calculated minimum resolved distance of about 0.2µm at the specimen (0.61 λ/NA, taking

λ= 0.5μm). Magnified 40 times, this gives resolved detail of 8µm spacing at the primary image. The Coolpix 990 claims a resolving power of 2048 x 1536 pixels – in our example 2048 pixels along the 16mm long-dimension of the image. Dividing 16mm by 2048, we arrive at a pixel dimension of 7.8µm at the primary image – exactly matching the minimum resolved dimension of the objective (Figure 6). Matters of resolving power are complicated, and this simple view does not tell the whole story. For example the number of pixels which can be recorded by the camera is the total number, to be shared between the three primary colours, and therefore strictly applies only to uncoloured objects recorded in monochrome. However, since most microscope images will demand considerably less of the recording system, it will be found in most cases that the number of pixels is adequate for the task.

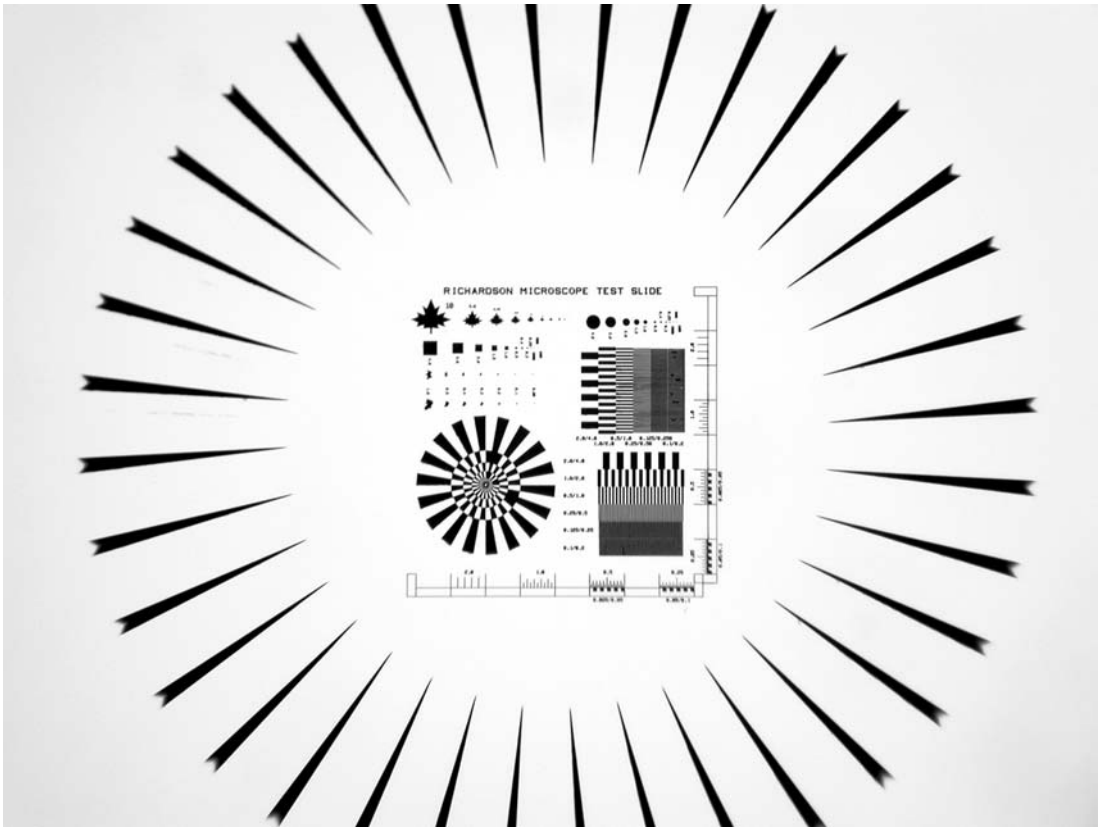

FIGURE 6a
Tim Richardson's (Canadian!) high-resolution microscope test slide (see http://www.richardson-tech.com) imaged with a 63/1.4 objective on a microscope with intermediate magnification factors of 0.63 and 1.25 (= 0.79). The object is magnified 50:1 in the primary image, and the resolved detail from 0.22μm up to 11μm; each camera pixel represents 7.8µm in the primary image.

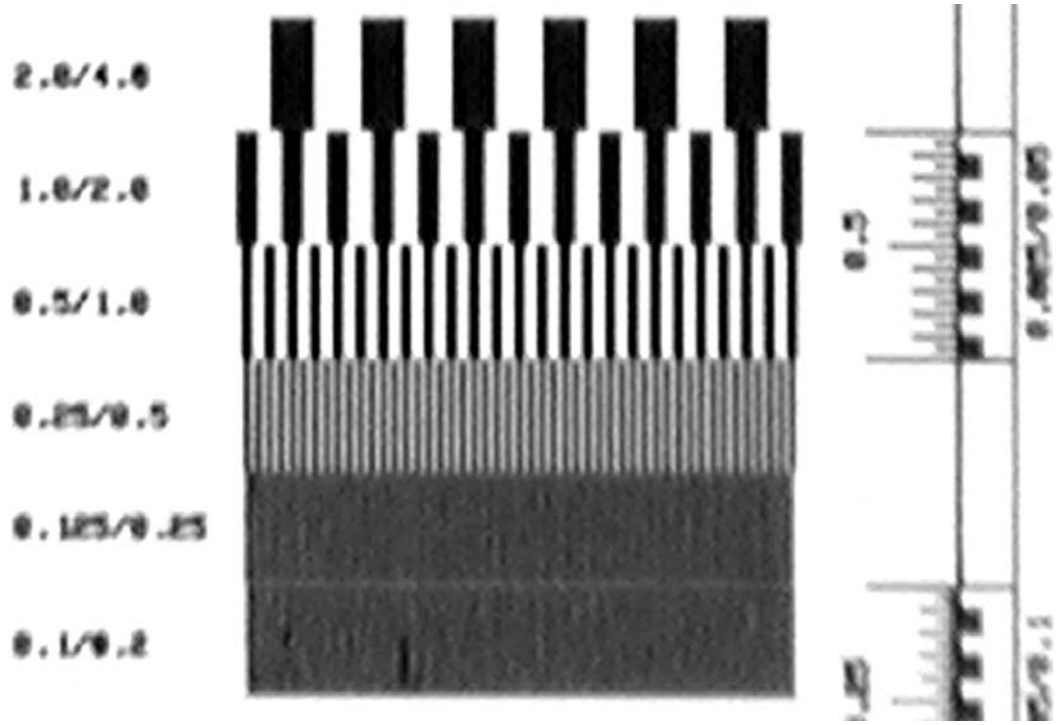

FIGURE 6b
Part of the same camera exposure as in Figure 6a, printed 5x enlarged. Here it can clearly be seen that the 0.25μm spacings on the test slide are resolved. The large 'scale-bar' blocks on the right side of the image are each 10μm long. This is not quite as stringent a test as that described in the text, because of the higher magnification of the primary image using the equipment available.

Apart from its use on the microscope, the Coolpix has excellent capabilities for photo*macro*graphy (Figure 7). Its autofocus system will allow the diagonal of the field to be filled with a UK 10 pence piece (24mm diameter). Enlarged to A4, as it could be without serious loss of quality, this would amount to a magnification of almost 15:1.

FIGURE 7
Full field image of a section of a human eye, the slide placed on a transparent scale (millimetres), to indicate the close-focusing ability of the camera.

The future?

Permanence of images stored on CDs or of inkjet prints is still unproven, since the systems are so new, and we are not to know for how long the equipment for reading memory cards or CDs and receiving the images in the computer will be conveniently available – just as it is no longer *convenient* for most of us to play a 12" diameter 78rpm gramophone record or a Betamax videotape!

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I should add that I have no commercial connection with Nikon, and the purpose of this article is to discuss the general principles of the use of digital cameras on microscopes. Several other manufacturers produce cameras with comparable resolving power together with appropriate microscope adapters, but at present I do not have the information to discuss them. Readers' experiences will be welcome.

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