Beginning with camera and microscope (2): Choice of films

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Summary

A previous article in this Journal[1] showed how a start could be made in making photographs with the microscope. It was based on a Club lecture, and some questions posed in the discussion, and by correspondence since, have shown that some would welcome more guidance on choice of sensitive materials. This article deals with monochrome first, including advice on filters for increasing contrast and other purposes, and then with colour, both positive and negative, and with notes on the use of filters especially to control colour balance.

Introduction

THOSE who have been making photomicrographs for some time may have accepted the usual advice, to choose suitable sensitive materials and then stick to them, perhaps too rigidly. There are now excellent newer materials on the market, and a change might help to produce better results. For those just beginning, advice as to how best to achieve the right contrast in a negative will be timely, for no matter how good one's ability as a printer in the darkroom, a poor negative can produce only a poor print.

Black and white work

In monochrome work, control of contrast in the negative is all. Compared with normal work, in microscopy there is usually a shortage of contrast in the image to be recorded, and thus it is necessary to use high-contrast materials. Twenty years ago, when recording stained histological sections, the author used Panatomic-X or Agfapan 25 developed for two minutes in a hydroquinone-caustic soda developer, and the resultant contrast range in the negative allowed printing on normal bromide paper. This is quite important, for if a negative could be printed only on bromide paper of one extreme of contrast or the other, there would be no leeway in control of the final print; the aim is always to produce a negative which will print on normal grade paper. In giving recommended materials from one named commercial producer or another, the author is providing the results of trials which he has actually carried out; other materials might well prove to be just as suitable, and those interested should certainly try other makes.

Table 1 gives a range of six definite contrast indexes achievable from two films and two developers. For macro work with reflected-light, strong contrasts can be generated, and a trial should be made with a contrast index of 0.5 or 0.7. For subjects having high inherent contrast, such as dark-ground pictures, a contrast index of 0.7 may well be suitable. For stained histological sections, an index of 1.5 will usually be about right, but if contrast filters are used, 1.0 will probably be better; this applies also to phase and DIC work. For flash photographs of living rotifer details and similar work, an index of 2.0 is usually needed. For reflected-light pictures of polished metals and other low-contrast specimens, an index of 2.5 will probably be needed. 98% of the microscopical subjects so far met with by the author can be recorded within the range of contrast indexes[2] provided:—

TABLE 1

Contrast index	Film	Developer @ 20°C	Dev. Time	Speed
0.5	T-Max 100	HC-110 (B)	7 mins.	80 ASA
0.7	T-Max 100	HC-110 (B)	11 mins.	100 ASA
1.0	Tech. Pan	HC-110 (F)	13 mins.	64 ASA
1.5	Tech. Pan	HC-110 (D)	6 mins.	100 ASA
2.0	Tech. Pan	HC-110 (B)	10 mins.	150 ASA
2.5	Tech. Pan	PQ. Univ (1:6)	3 mins.	150 ASA

In the very few cases where still higher contrast is needed, the author uses Agfa Ortho 25 film, which is actually intended for copying line drawings. Developed in a PQ Universal paper developer this gives black and white and very little else, but further details are outside the scope of this paper.

Both films are available in 35 mm, 120 roll-films, and sheet film. Kodak HC-110 developer is available in two sizes and viscosities; the thinner liquid in the smaller size is easier to use. The bottles give instructions for dilution to various lettered strengths; those used in the above table are dilutions B, D and F. Any PQ Universal developer (as used for developing bromide papers) is suitable for use with Technical Pan films, but if used with T-Max 100 it gives high fog levels and staining.

The speeds quoted are an initial guide ONLY, for use when carrying out the calibrations described in the earlier paper. The importance of reading only the background brightness, and of carrying through proper calibration, cannot be over emphasised if waste and disappointment are to be avoided.

A very useful tip when part-using a bottle of developer concentrate, monochrome or colour, to preserve that remaining, is to squirt a generous dose from a dust-off aerosol into the bottle, close to the surface of the liquid to displace the air, and thus to prevent the developer from oxidising brown.

It is sensible to give similar agitation in the tank for all films, perhaps thirty seconds per minute of inversion; the temperature should be 20°C, minus 0° plus 1°. Do NOT be tempted to develop for longer or at a higher temperature! Use an acid stop bath, with plenty of inversion, and fix thoroughly, with plenty of inversion, before inspecting. After washing, add a TINY amount of rinsing agent to the final wash, and shake the negative roll free from drops, while still in its spiral, before hanging up to dry without squeegeeing.

Fig. 1 shows Technical Pan negatives, made from a well-stained histological section at the same time, developed to different contrast indexes, to illustrate the control exertable.

Filters for monochrome work

If panchromatic films are used, they will record all visible wavelengths generated by flash or tungsten illumination, the colours recording in their relative brightnesses. It is possible to bring out or subdue various colours with filters — coloured inserts for use below the stage. The rule is to use a colour from the other end of the spectrum to create contrast — thus a blue filter makes a red (carmine-stained) preparation more contrasty on the negative, and a green filter makes a preparation with both blue and red in it (haematoxylin and eosin) more contrasty. Most ordinary filters, made from dyed gelatine or glass, actually pass more wavelengths than their visible colours suggest, but are perfectly satisfactory for most work, although special interference filters passing only a narrow band of wavelengths are available at a high price. A further use for a green filter is to use it with higher powers of ordinary achromatic objectives; by using only a narrow bandwidth the definition can be improved. Fig. 2 shows the effects of different filters on a trichrome-stained histological section.

Similarly, if more detail is wanted in an image, a filter of similar colour is used. Of course, this dramatically reduces the contrast on the negative, and it will probably be necessary to develop to a contrast index of 2.5. Remember, any filter used will absorb part of the illumination, and so will require a greater exposure — the filter factor. It is often better to measure the background brightness in white light, and then to apply the filter factor, than to measure the brightness with the colour filter in place —not all meters are equally sensitive to all colours!

A special kind of monochrome film is the infra-red film. This has a sensitivity to allow it to record long wavelengths, past the visible end of the spectrum although it is sensitive to some visible light also. It is used with preparations which are transparent to infra-red (but opaque to visible light) — whole flattened mounts of insects are a good example, where much more detail is shown with infra-red than with white light. Use of a powerful tungsten-halogen source is recommended, as having plenty of infra-red present in its output. The preparation has to be focused with a deep red filter in place (to give an acceptable focus for the longer but invisible rays which will be used for the exposure); a visually opaque filter then replaces the deep red one for the exposure. This filter allows infra-red to pass, but nothing visible. Calibration is needed before such work is carried out, of course! Examples of such films currently available are Kodak High-Speed Infra-Red Film (35 mm and sheet films only), and Konica 750 nm Infra-Red film (in 120 size); they are expensive. Basic exposure indexes for beginning calibration are 64 ASA for the Kodak, and 25 ASA for the Konica, using HC-110 (B) for seven minutes @ 20°C.

Polarising filters may also be used with monochrome films, and require no special calibration although they do need a considerably increased exposure (measurable direct by the usual background brightness); the contrast index required varies widely with subject and with the degree of crossing of the filters.

Working in colour

In many ways this is simpler than working in monochrome. Colour films have a high inherent contrast, and many are suitable for photomicrography. There is a choice of systems, between neg/pos (shooting with colour negative film to provide a final colour print), and pos or pos/pos (shooting with colour transparency film, to provide a final colour slide or possibly a colour print).

A major difficulty is that for technical reasons colour films can be made to suit only a particular quality of illumination; that is to say, they are balanced either for tungsten light, or for daylight (including electronic flash). No colour film is really suited to fluorescent illumination, but that hardly matters to us as such lights are not usually used as sources for the microscope. Tungsten films are usually intended for illumination by a source of 3200°, and daylight films of 5500°; these values are actually those of a theoretical object heated to those temperatures in degrees Kelvin (that is, above absolute zero), when they would give out a certain mixture of wavelengths of light, the more blue the higher the temperature. If daylight or electronic flash is used with a tungsten-balanced film, the result is a blue picture; if tungsten lamps are used to light for a daylight film, the result is a yellow picture, so source must be matched with film-type.

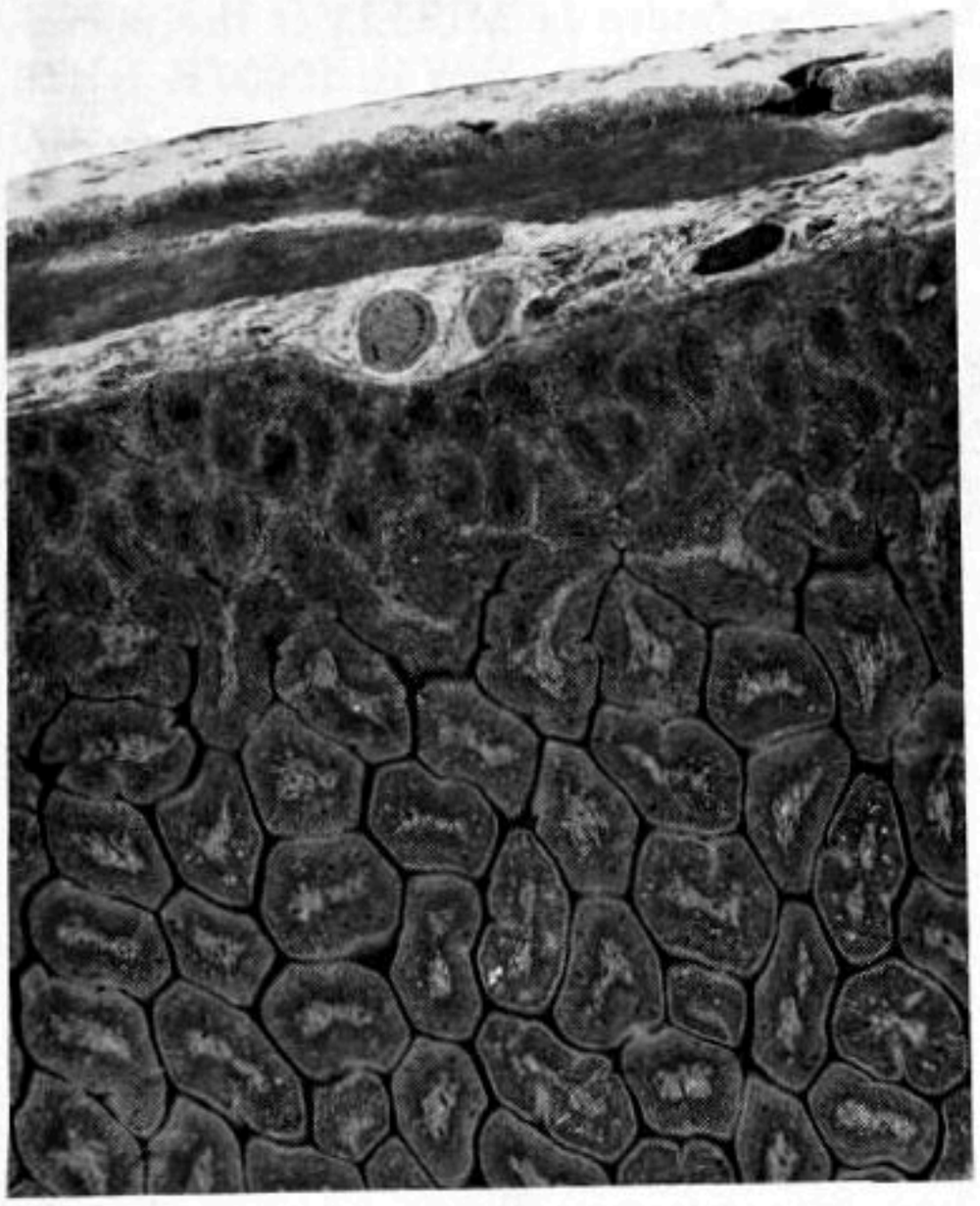
In microscopical work, there are two main considerations following from the foregoing consideration of colour temperature. First, when shooting with colour film and tungsten light, it is not permissible to use the rheostat on the lamp to reduce the brightness of the field. To do so will make the illumination more yellow, as reduction of the voltage of a lamp reduces its colour temperature; control of brightness here must be provided by using neutral density filters, which absorb all colours equally. Second, we must match our source to our film.

In most cases using a tungsten-halogen lamp at its rated voltage will properly match a tungsten-balanced film — 3200°K — and nothing further need be done. If an ordinary tungsten filament lamp is used (as opposed to one with halogen), its colour temperature will be a little low, but as such lamps are often turned up a little above rated voltage for the actual exposure, to keep it short, this extra voltage will raise the colour temperature to a good enough match. It is possible to quote MIRED values for each colour temperature (a MIRED is the micro-reciprocal of a degree, that is, 10000°K is 100 mireds, 4000°K is 250 mireds, 2500°K is 400 mireds, and so forth). It is possible to purchase colour correcting filters of definite mired values; brownish ones are + so many mireds, and lower the colour temperature, while bluish ones are – so many mireds raising the colour temperature. For microscopical purposes, this is hardly ever needed; a daylight-balanced film works perfectly well with daylight or with electronic flash, and we have already seen that tungsten-balanced films are fine with most lamps. There is one situation where a quite deep bluish correcting filter should be used, and that is if an ordinary colour negative film is used with a tungsten lamp; it is much easier to correct when making the negative than during printing, but more will be said of this later.

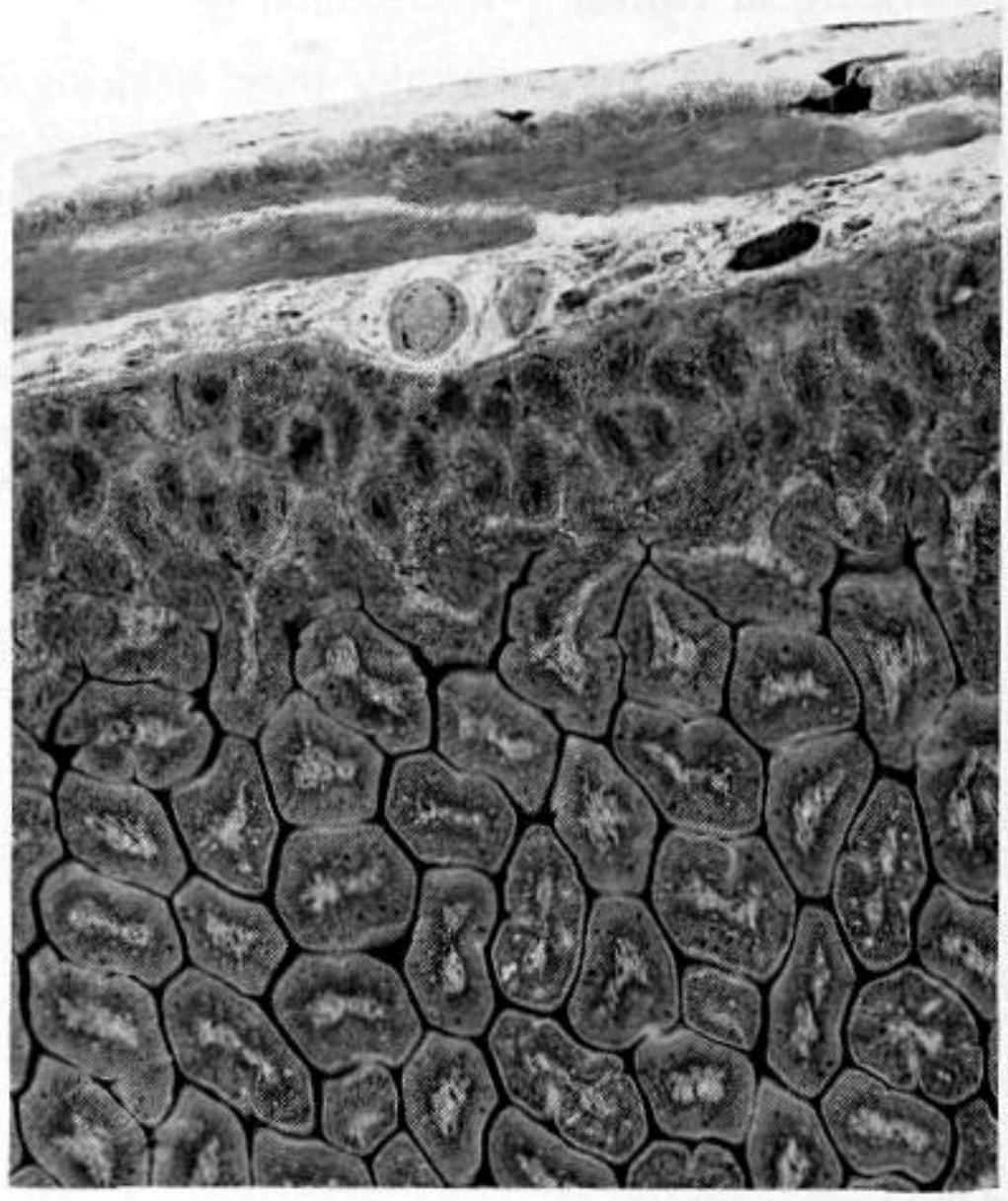
Colour transparency films

Many are on the market, but the author has tested and calibrated for his own purposes those from two makers. For daylight/electronic flash, Kodak Ektachrome 64 (available in 120 and 35 mm) is sharp and gives good results, with a calibration starting point of 64 ASA. When more speed is needed (as in reflected-light work), Kodak Ektachrome 100 Plus (also in 120 and 35 mm) has excellent colour saturation; calibration starting point of 100 ASA. For tungsten light Kodak Ektachrome 64T and Ektachrome 160 T are fine, both available in 120 and 35 mm; respective calibration starting points are 64 ASA and 160 ASA. Fujichrome 64T is another excellent tungsten emulsion, in 120 and 35 mm, with calibration starting point of 64 ASA.

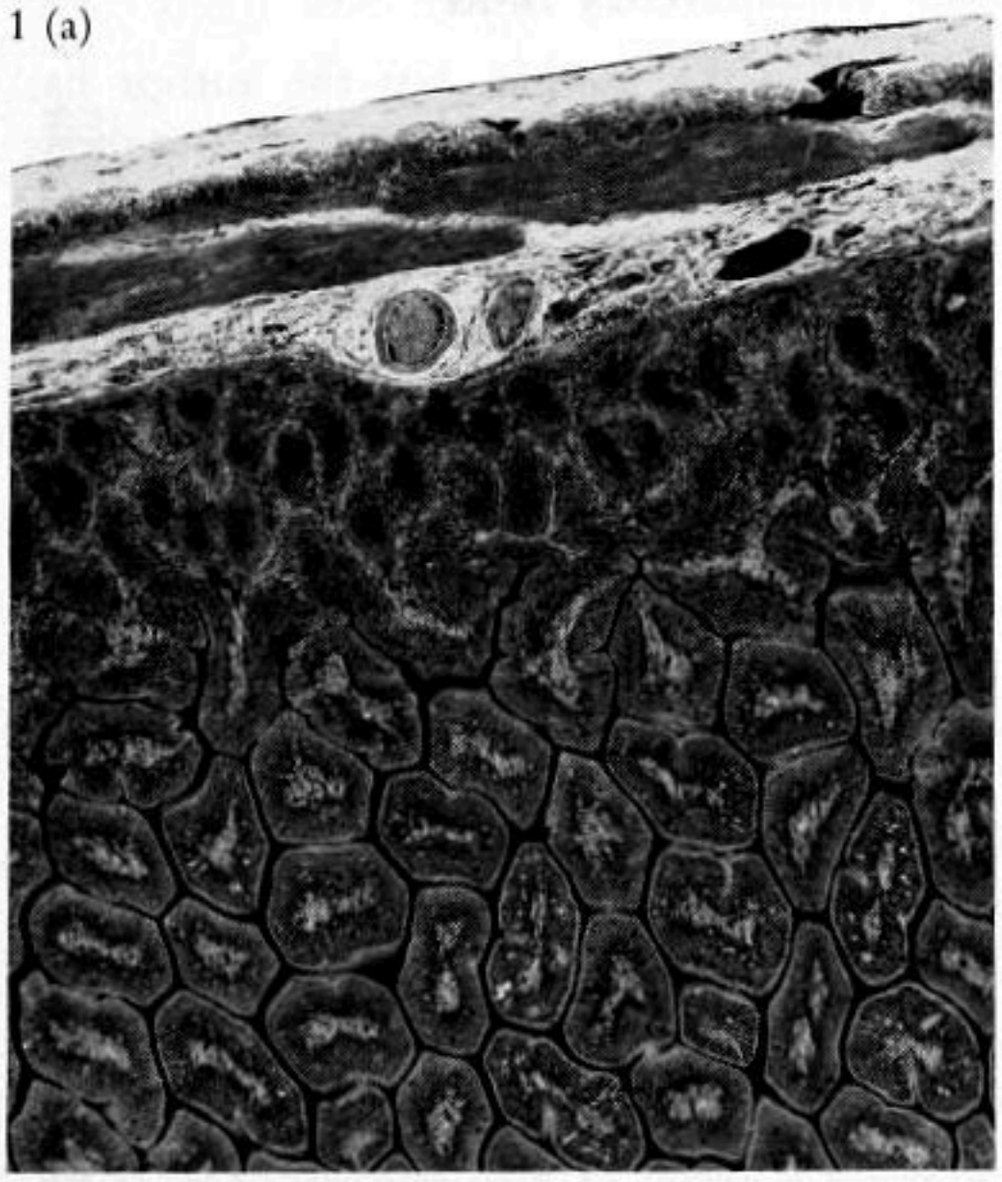
In processing these colour reversal films, the E6 process is used, or a modification of it for home use such as the widely-available three-bath Chrome Six kits supplied by Photo Technology Ltd. Precise control of temperature is vital, and there is little latitude allowable in processing, although it is not difficult to extend the development to provide a doubling of effective speed without too drastic an effect on quality; this can be very useful on occasion, especially in flash photography of living organisms. Excellent prints can be made direct from transparencies by the Ilfochrome process (originally called Cibachrome). It is, of course, easy to evaluate a colour transparency by simple



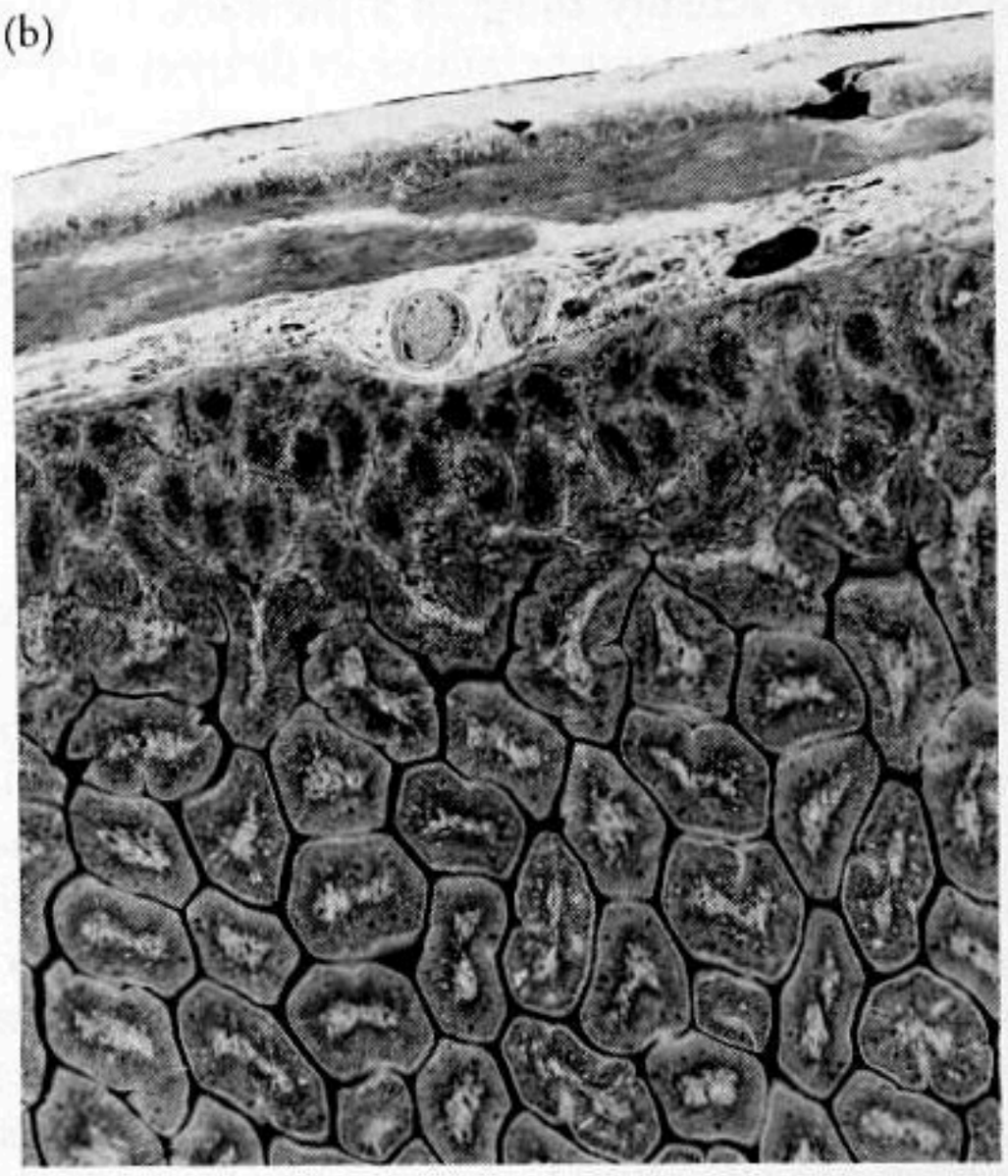
1 (a)



1 (b)



1 (c)



1 (d)

FIG. 1. A microscope was set up with 10 × Planapo objective and projection eyepiece, with a slide of azan-stained TS small intestine. This was chosen as it includes a range of colours giving good contrast for a histological section. Four negatives were made of the same area, on Kodak Technical Pan 4 × 5 sheet film, each developed differently. All had the same fixation, and all were presented as camera originals for the illustrations.

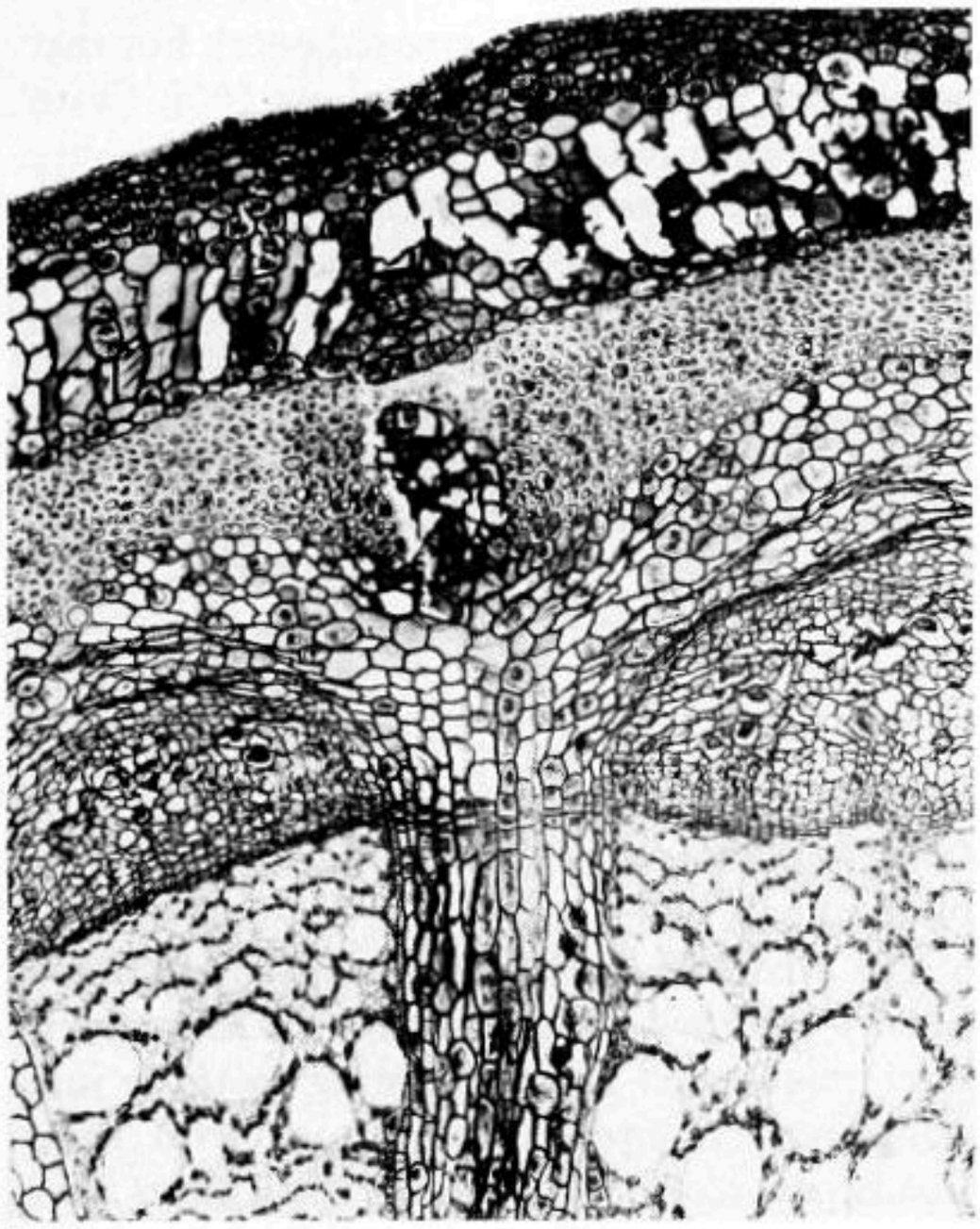
1 (a) To a contrast index of 1.0, with exposure index of 64 ASA, and development in HC-110 dilution F for 13 minutes @ 20°C.

1 (b) To a contrast index of 1.5, with exposure index of 100 ASA, and development in HC-110 dilution D for 6 minutes @ 20°C.

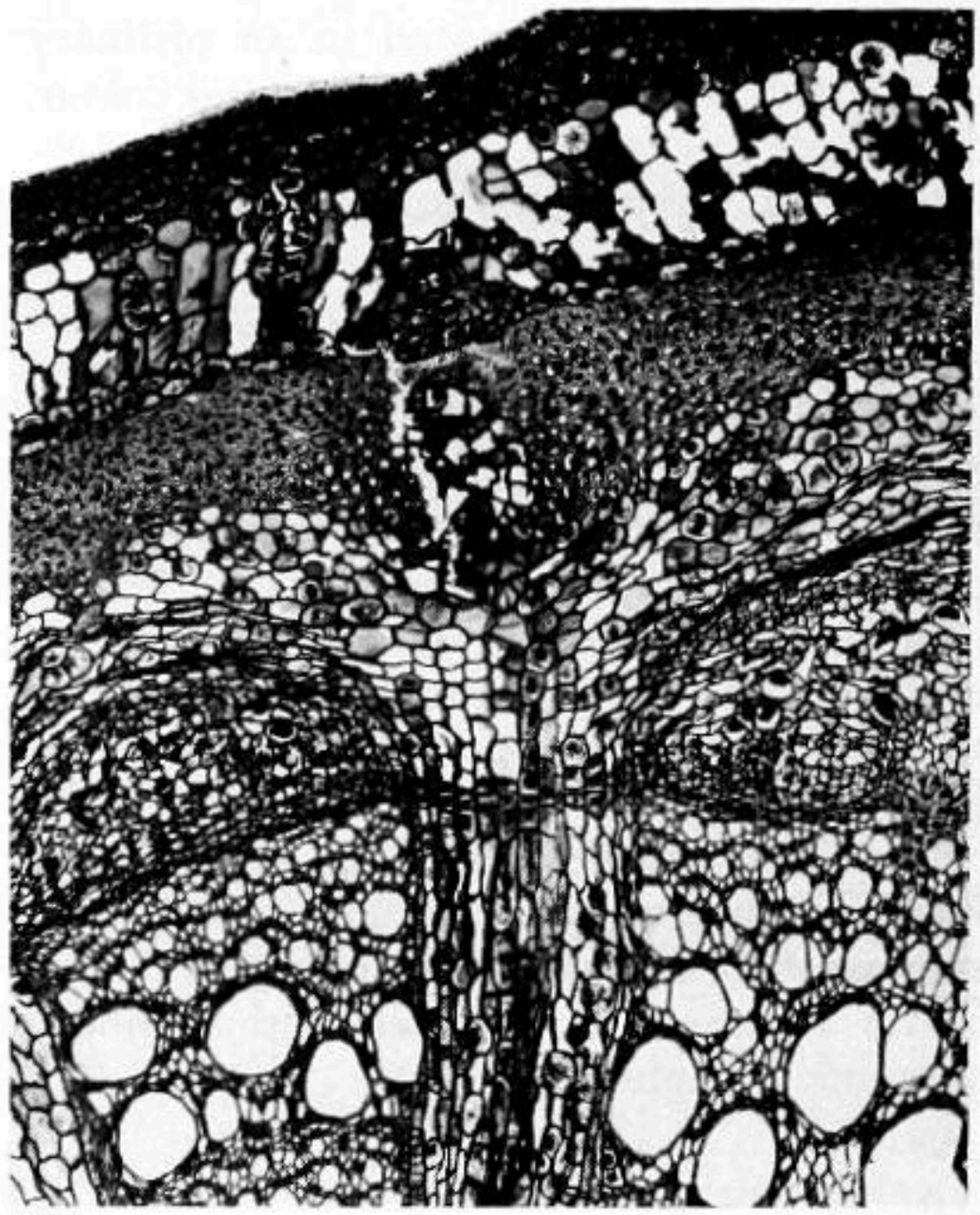
1 (c) To a contrast index of 2.0, with exposure index of 150 ASA, and development in HC-110 dilution F for 10 minutes @ 20°C.

1 (d) To a contrast index of 2.5, with exposure index of 150 ASA, and development in Kodak Dektol 1:6 for 3 minutes @ 20°C.

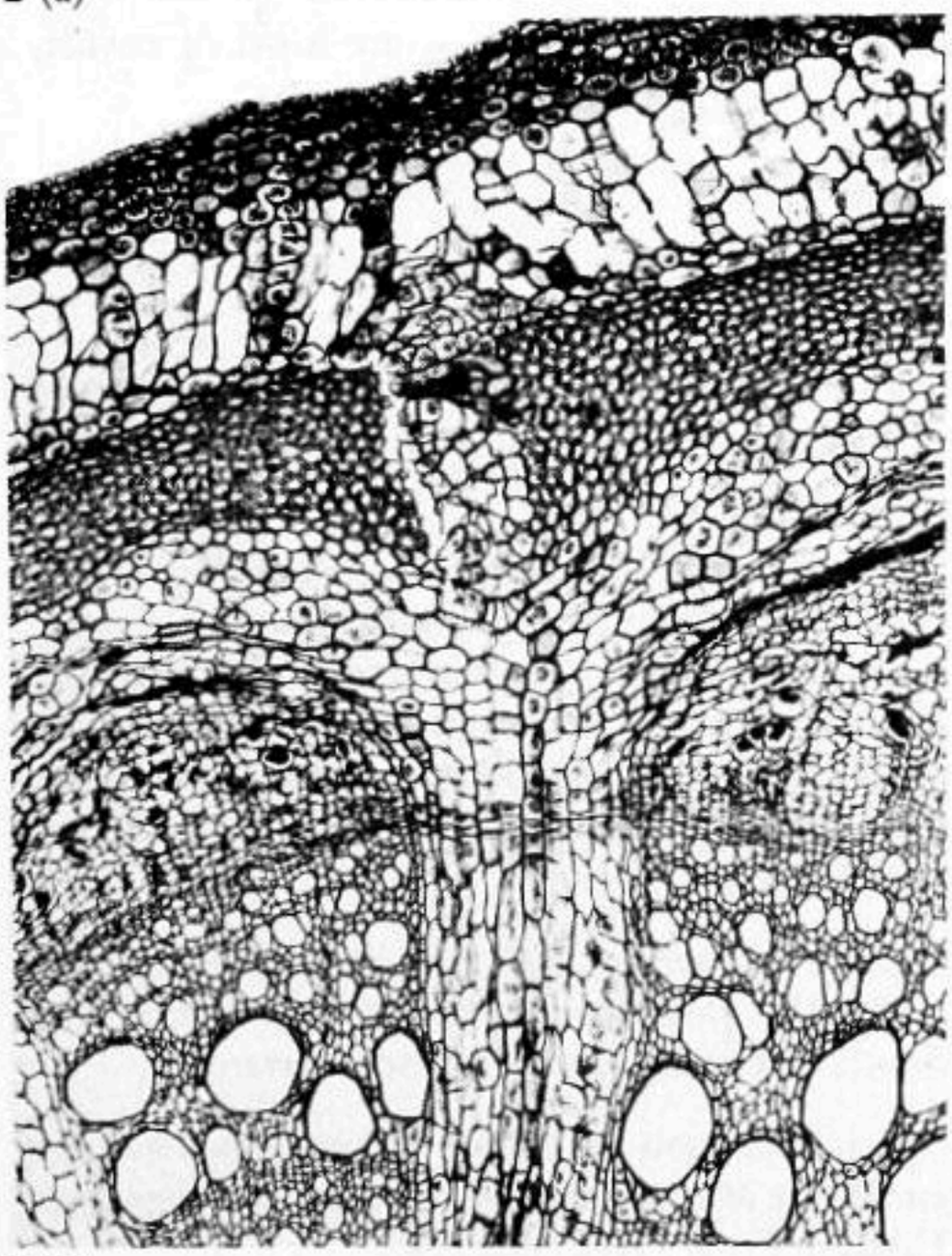
The controlled and progressive increase in contrast is clear; 1 (b) prints well on normal paper.



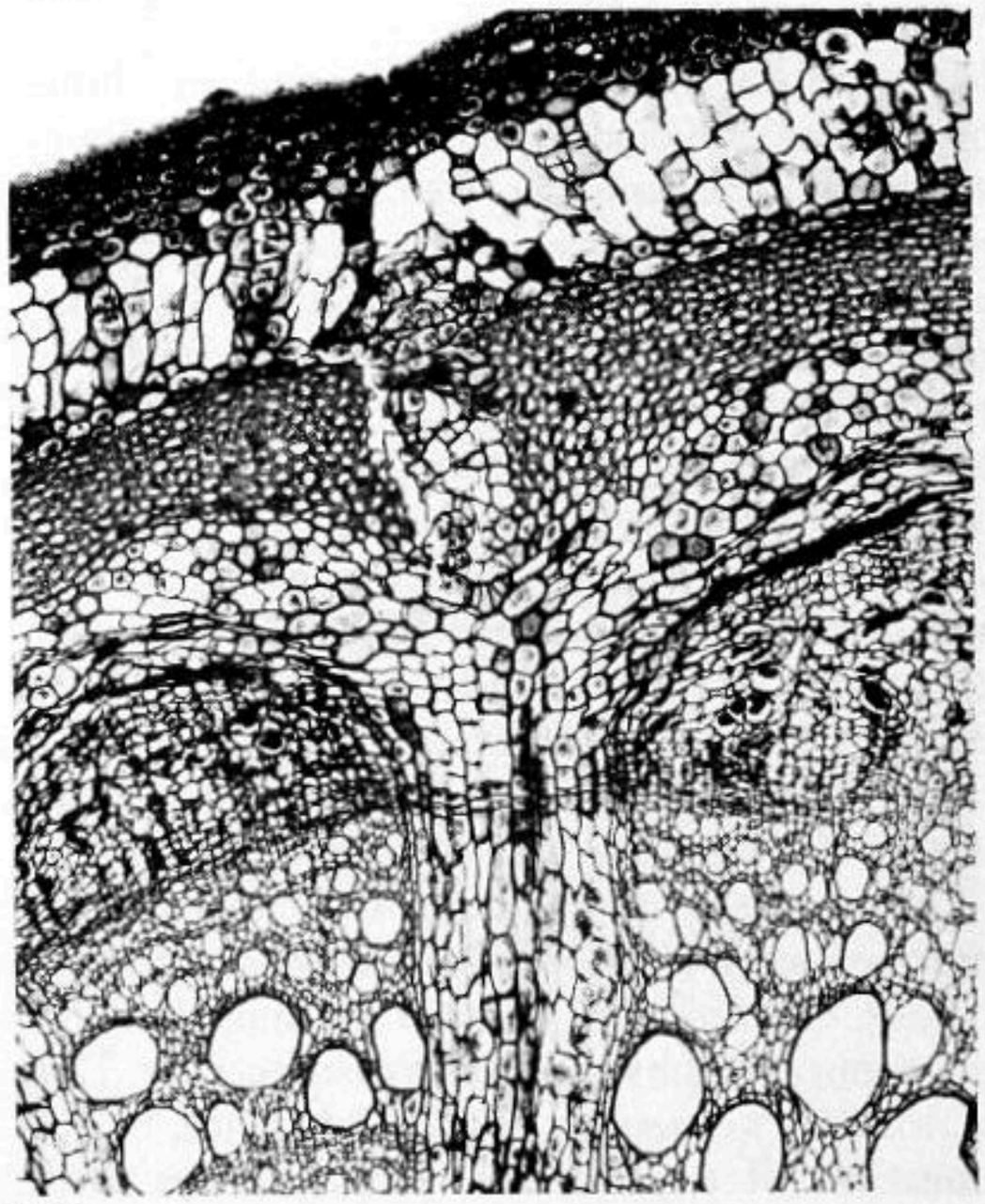
2 (a)



2 (b)



2 (c)



2 (d)

FIG. 2. On the same set-up, a trichrome-stained slide of TS *Aristolochia* stem was photographed on one roll of Kodak Technical Pan film, and developed to a contrast index of 1.5, as above. Each. negative was then printed on normal paper. The illumination was as follows:

2 (a) Balzer K-6 interference filter in place. This is narrow-band RED, with peak transmission at about 660µm.
2 (b) No filter used.
2 (c) Balzer K-4 interference filter in place. This is narrow-band GREEN, with peak transmission at about 560µm.
2 (d) Balzer K-2 interference filter in place. This is narrow-band BLUE, with peak transmission at about 460µm.

The effects of different colours of filter in making some colours more pronounced and others less so, is clear.

inspection; what is wanted in an ordinary micrograph is a background of neutral colour, one shade of density dark from absolutely transparent. Very little filtration is needed in Ilfochrome printing, and it is not difficult to carry out at home (Ilford supply a kit of chemicals). If one's transparencies must be trade-processed, the main difficulty is to find a reliable processor; in this context it may be worth noting that the Agfa processing centre[3] will accept all types of E6 films, regardless of maker. Results from them have without fail been excellent for this author; all that is needed is to buy a mailer and send off the film (35 mm or 120 mm) in it. If putting out a transparency for Ilfochrome printing, all that is needed is to stipulate that the background as printed must be neutral white, and just short of total lack of density; there can be no argument as to result with ordinary photomicrographs!

Colour negative films

Many photographers use colour negative films of the type of Kodak Gold 200 in their outdoors work, and the same can also be applied to the microscope. It is always wise to be sure that full exposure is given to colour negative films, and one nominally 200 ASA will always give better results if the camera is set at 160 ASA for all kinds of use. In the case of photomicrography definite calibration is required, as has been stressed throughout these articles, and so a value will be arrived at experimentally for a particular emulsion. It has already been said that a proper blue correcting filter should be used in the light-path if a tungsten source is being used with the usual colour negative materials, to make the printing, trade or personal, that much easier. It is absolutely worthwhile, if much colour negative film is used in tungsten-light photomicrography, to choose one specially. This is not only a question of colour balance, but of duration of exposure. Exposure times with tungsten sources tend to be rather more lengthy than most colour negative films are designed for in their usual outdoor use, and this can affect colour balance in a way which cannot be put right during printing, because the three characteristic curves have crossed over[4]. For that reason, the author uses Fujicolour 160L (with basic exposure index of 125 ASA) on the rare occasions when he exposes colour negative film in the microscope. Processing colour negative films and prints at home is not difficult, given precise control of temperature. The widely-available two-bath Photocolor FP kits supplied by Photo Technology Ltd process both C41 and RA4 materials, and work easily and quite cheaply.

Conclusion

Vast amounts have been written in many places about photographing through the microscope, to give a considerable literature. The author is well aware that all the advice he has given above might well prove daunting to those just beginning, (as opposed to those who have already overcome any initial difficulties and disappointments). His considered advice is to just get on with it and get some kind of result, and sort out the finer points later!

Notes

1. Bracegirdle, B. (1993). Beginning with camera and microscope, *Quekett Journal of Microscopy*, **37**, 22-29.

2. The concept of contrast index is quite similar to that of the older γ, but perhaps more informative under practical conditions. A good explanation showing how to derive a contrast index from a characteristic curve is given in *Kodak Professional Black-and-White Films*, Kodak Publication F-5, 1990. This booklet is useful in other ways also.

3. Agfachrome Service, P.O. Box 32, Bury, Lancashire BL9 0AD. Their pre-paid mailers may be obtained direct, or through photographic dealers.

4. For a discussion of colour balance characteristic curves see M. Langford — *Advanced Photography*, London: Focal Press, 5ed, 1989.

   This and its companion volume (M. Langford, *Basic Photography*, London: Focal Press, 5ed 1986) are mines of photographic knowledge, and strongly recommended to those interested in photography, theoretical and practical.