A SIMPLE MICROSCOPE ATTACHMENT PERMITTING A CHANGE-OVER TO OPTICAL STAINING BY FLUORESCENCE AND POLARIZATION MICROSCOPY

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The equipment of standard fluorescence and polarization microscopy is usually sufficient in routine investigations, but not for detailed interpretations. Thus for example, certain structures may be discerned by fluorescence microscopy, but this does not imply that all structures of that kind are actually fluorescing. Moreover, it may be difficult to establish whether fluorescence occurs in a minute cytological structure or in some adjacent particle. It would be advantageous to reveal that in a single operation, thus avoiding time-consuming phase contrast microscopy or subsequent staining of the slide.

If a fluorescence or polarization microscope were provided with phase contrast equipment it might be possible to interpret the microscopical picture directly. Although it is possible to design a microscope adapted for a rapid change to phase contrast microscopy, optical staining procedures seem preferable.

A two-channel condenser is generally used for optical staining of microscopical preparations. Staining occurs when light reaching the preparation at different angles is diffracted (after having passed through two differently coloured filters in the two channels). Two cones of rays of different colours are produced. One forms a solid centre, the second a surrounding hollow. In this way large angles are formed between rays of different wavelengths. Since smaller angles seem preferable in this connection, the two-channel principle has been abandoned.

Owing to the chromatic aberration of ordinary condensers, small angles between light rays of different wavelengths are obtained at the level of the slide. In order to make use of these differences, the centre of the condenser was covered with a disc. This allows only a narrow ring from the periphery to reach the aperture of the objective. Hollow cones of different colours with their common tops reaching the preparation on the slide may then be said

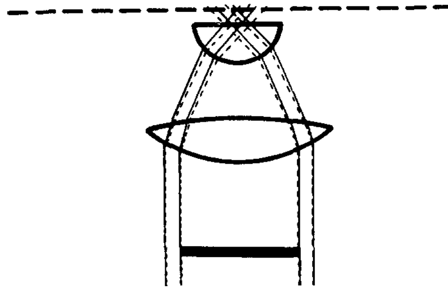

Fig. 1. Principles of illumination.
The condenser is screened off by a metal disc. Mixed light of two different wavelengths passes the margin of the disc. Because of the chromatic aberration of the condenser the rays reach the level of the preparation at different angles, as indicated by the dotted line.

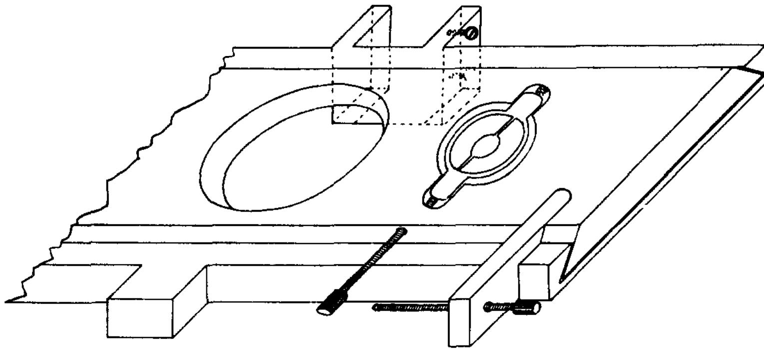

Fig. 2. Outline of the attachment. The slide is fitted in a holder provided with a stop and may be moved to the desired lateral positions after adjustment of the screws. When the central aperture is used the slide is moved to the position seen in the figure. A rod in the lateral apertures is adjusted by a screw and a spring. When the rods were cut a frame of the metal was left to make the mounting of the metal disc easy.

to constitute the system of illumination (See Fig. 1). For successful optical staining the filters must be appropriately selected. If one should choose to stain with blue and red light, suitable filters are available which transmit these wavelengths and absorb most of the remainder of the visible spectrum. An ordinary filament bulb might thus be combined with the filters BG1 and BG25 (Schott, Jena). It has been found desirable, however, to add a third filter (e.g. euphos glass) in order to reduce the intensity of the blue light. If a strong and efficient mercury lamp is used, the choice of filters is easier, since several ultra violet filters transmit blue and red light to a sufficient extent. The surplus of blue light must then be reduced by a red-transmitting filter which does not absorb all the blue light. Owing to their inferior

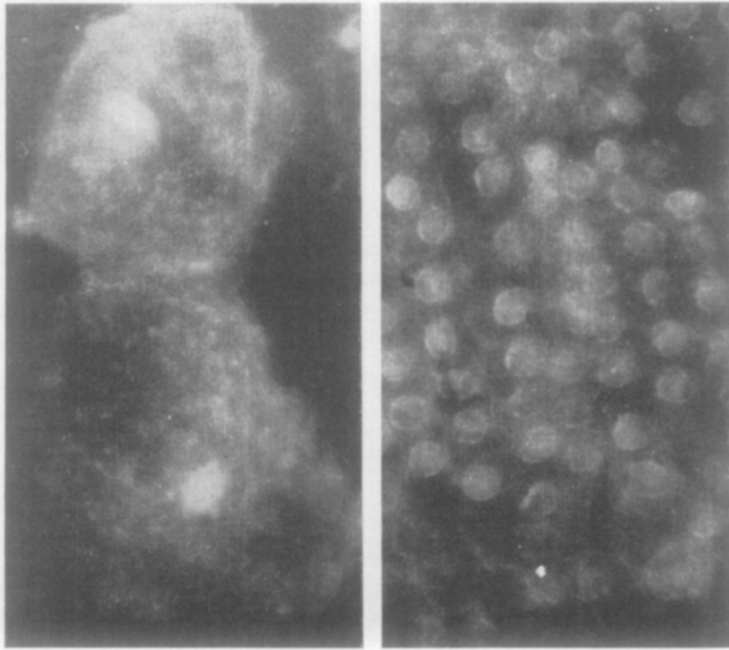

Fig. 3. To the left epithelial cells scraped from the buccal mucosa; to the right the primordium of the lens fibre in a stripped and unrolled capsule of the eye lens. When these preparations were viewed, the nuclei and also some smaller refractile particles appeared red against the cytoplasm. The refractile parts are lighter in the photomicrographs, but the monochrome reproduction is not accurate.

quality, some red filters intended for photographic use possess such properties.

For the purpose of optical staining the condenser attachment may be more or less elaborate. The mechanical equipment of most institutes will, however, probably be sufficient. The attachment used by this writer for fluorescence microscopy is constructed in the following way. (See Fig. 2.) A light metal slide is mounted on a holder which is screwed to the substage condenser. This movable slide is provided with three holes containing filters. An additional filter transmitting ultra-violet, blue and red light is placed in the aperture of the holder. The middle hole of the slide contains an ultra violet filter of adequate quality. If this aperture is used, the system is ready for fluorescence microscopy. The lateral holes contain a red filter and a metal disc, each fitting different objectives. In the lateral apertures there is also a metal rod which is pressed against a spring by a screw. The plate mounted on the rod may thus be moved perpendicularly

to the long axis of the slide. To facilitate adjustment in the other direction, the slide movements are arrested by adjustable screws (see Fig. 2). It was found convenient to use commercially available screws with threaded collars, which were fixed in drilled holes in the slide. When the centering of the plate has been accomplished (by aid of an auxiliary microscope), a blue and a red annular area are observed around the margin of the plate. These areas partly overlap and a pink or purple colour occurs in the common zone. Optical staining and fluorescence microscopy can take place alternately without repeated adjustment.

Though it is possible to combine the described attachment with several condensers, the result is not always entirely satisfactory. It may be improved by adding an auxiliary lens to the condenser. This may be selected through preliminary trials with lenses used in common ophthalmological refraction tests. A quartz condenser used by this writer was provided with a specially manufactured quartz lens of +2.5 dioptres which was mounted in the slide holder and kept in position during the change-over between fluorescence microscopy and optical staining.

Optical staining is especially convenient when examining single cells, but thin tissue slices may also be investigated. The photomicrograph in Fig. 3 illustrates to some extent the results obtained by the method (although only reproduced in black and white).

SUMMARY

A microscope attachment is described which makes optical staining possible without using a two-channel condenser. The attachment can be combined with different condensers and can be instantly moved allowing an alternating use of the microscope. This has been found useful in fluorescence microscopy, and may also be recommended in other connections.