# Aspects of colour and polarization systems for producing stereoscopic vision in the standard optical microscope.

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**Summary**
Resolution in the presence of the colour and polarization systems of stereoscopy was investigated. Perhaps surprisingly, the effects on resolution through effects on aperture appeared to be insignificant. The effect of the polarization system on resolution was negligible, but that of the colour system inevitably depends somewhat upon the particular filters used. With both systems stereoscopic effects occurred in both darkground illumination and in phase contrast.

**Introduction**

IN THE standard optical microscope, with a single objective, stereoscopic relief is normally absent, even though the microscope is fitted with a binocular head*. However, it does not seem to be widely appreciated that the standard microscope, if fitted with simple arrangements of colour filters or, preferably, polarizing film, can be adapted for stereoscopic viewing of the objects by transmitted light. In the absence of stereoscopy, focusing up and down enables structure in depth to be deduced. but stereoscopic vision permits a more discriminating perception of the shapes and spatial relations of the objects examined and makes structure more immediately intelligible; it adds to the microscopical image a third dimension.

Stereoscopic vision in the standard microscope has been recommended for examination of small mounted insects, or parts of them (Hewlett, 1969). However, such vision would doubtless be useful for examination of many other objects where shape and spatial arrangement need to be well appreciated, though obviously it will be superfluous when the object is very flat and thin. It should be useful in micromanipulation.

In the colour system of stereoscopy (Wise, 1950) a filter consisting of contiguous semicircles of materials passing light in different wavebands is placed in the filter tray below the condenser, with the junction between them lying fore-and-aft (i.e. medially). Undivided filters respectively passing light in the same wavebands are placed above the eyepieces. When the two latter filters are the right way round the inner halves of the exit pupils (Ramsden disks) appear dark, and ortho-stereoscopic vision ensues. In its simplest form the polarization system (Jackson, 1948; Wise, 1950) is analogous. Contiguous semi-circles of polarizing film polarizing respectively in the medial and lateral planes are placed in the filter tray. Caps of polarizing film are fitted over the eyepieces to extinguish (so far as possible) the inner halves of the exit pupils. Sets of filters and polarizing film are easily made, though if they were available commercially (which they are not) stereoscopy in the standard microscope would probably be utilized

* The binocular heads referred to in this paper are of the normal modern types, in which the light beam above the objective is split by means of a semi-metallized surface.

more than it now is.

Objectives of N.A. 0·25 to 0·70 give the most satisfactory results. Objectives of N.A. less than about 0·25 give insufficient disparity between the images presented to the two eyes; for this reason, the standard microscope used stereoscopically complements and does not replace the stereoscopic microscope of the Greenough type (where the objective N.A. is not more than 0·13). Too high an objective aperture tends to give too little depth of field.

Resolution with the colour and polarization systems in the standard microscope appears to be in doubt. Wise (1950) remarks that stereo-vision in the standard microscope is only obtained at the expense of aperture, and that the colour and polarization systems reduce aperture. Martin (1968), on the other hand, comments on the good resolution attainable with the polarization system, though he gives no details. It was therefore decided to investigate resolution. In addition, stereoscopy in darkground (which Martin says is not very satisfactory) and in phase contrast were tried out.

**Methods**

For the colour system gelatin filters were used. These were Wratten 61 (green) and Wratten 32 (magenta, transmitting in the blue and red). For the polarization system two microscopes were used, one with a Jentzsch-type binocular head (interpupillary adjustment by straight lateral movement of the eyepiece tubes) and the other with a Siedentopf-type head (interpupillary adjustment by pivoting movement of the two short arms carrying the eyepiece tubes). With the former type of head the Polaroid elements were of the simple form described above. With the latter type of head, the substage polars polarized in the medial and lateral planes, but elliptical polarization introduced by the head needed to be countered on one side. This was done by fixing a semi-circle of Cellophane of suitable thickness and suitably oriented over one semicircle of the substage element, giving a dark colour.

Tests for resolution were carried out with diatoms. To help in interpreting results colour filters and polars were removed, and one half of the aperture was occluded by an opaque screen of black paper in the filter tray, or over the back lens of the objective, or at the exit pupils.

**Resolution in brightfield**

Straightforward over-all tests of resolution were first undertaken. A diatom, *Navicula lyra,* was first examined in white light by direct microscopy, i.e. without stereo-vision. An objective of N.A. 0·35 was used, with a three-quarter cone of illumination. The fine structure was just resolved as transverse striae with this objective, but not with one of N.A. 0·30. The diatom was then examined with the objective of 0·35 N.A., using both stereo-systems, first with the long axis of the diatom in the lateral, and then in the medial plane.

With the polarization system the fine structure was resolved when the diatom was in either position, but the contrast was better when the long axis lay in the lateral plane.

With the colour system, the fine structure was resolved when the long axis of the diatom was in the lateral plane, viewing through either eyepiece. When the axis was in the medial plane, the fine structure was visible through only one eyepiece, namely that covered by the green filter. With the colour system, resolution through one eyepiece and not through the other is to be expected sometimes, and fully meaningful comparisons with resolution in white light are not possible.

Corresponding results were obtained when a diatom with finer structure, *Nitzschia sigma*, was examined with an objective of N.A. 0·70.

Experiments in the absence of colour filters and polars, and with white light, were carried out to assist in the interpretation of the foregoing results. When half the aperture was occluded by a screen in the filter tray, resolution was unimpaired. When, however, the screen was above the objective or in the exit pupil, resolution was obtained when the diatom lay with its long axis parallel to the diametral edge of the screen, but was lost when the axis was across this diameter. The same results were obtained with binocular and monocular microscopes. It seems, therefore, that where the stereoscopic systems did not impair resolution, the resolution depended upon light diffracted by the object passing through the dark half of the exit pupil. This was confirmed directly by screening off the dark half of the exit pupil with the polarization system.

**Sterescopy in darkground and phase contrast**

Stereoscopic effects were found to occur quite satisfactorily in darkground illumination with both colour and polarization systems. Naturally, however, the effects did not show well when the object was very thin and flat, as many objects examined in darkground are. Objects showing the effects well were the wings of the mosquitoes, *Theobaldia annulata* and *Culex pipiens*, objects which, incidentally, show the effects equally well in brightfield. The thick clothing of microtrichia on the wing membrane and the lines of scales enable the furrows and ridges of the membrane to be well seen. In darkground the exit pupils apeared circular, so evidently dark halves of the pupils are sufficient though not necessary conditions of stereoscopy.

Stereoscopic effects showed in phase contrast with both systems, but a very bright source of light was necessary. Again a suitable object needed to be chosen.

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