NEW APPARATUS

A SIMPLE METHOD FOR PHASE-CONTRAST MICROSCOPY

PHASE contrast microscopy is particularly valuable in the examination of living cells with high powers and an ordinary microscope using an oil immersion lens may be easily adapted. This method was demonstrated by Dr. J. R. Baker[1] and his associate workers of the Department of Zoology of Oxford University at the Royal Society's Conversazione on May 26, 1949. A phase-plate is prepared from a circle of glass, 1 mm. thick, of the same diameter as the back lens of the objective, and with the sides optically plane and exactly parallel to one another. This phase-plate is held in place behind the objective lens by means of a hollow brass cylinder which fits into the objective. The phase-plate is glued to the lower end of this cylinder (Fig. 1). The phase-plate is uniformly bloomed one side with magnesium fluoride to such a thickness as to give a retardation of a quarter-wave of apple green light, in comparison with light passing through the same thickness of air. (Deposition of magnesium fluoride is done by Messrs. R. and J. Beck.)

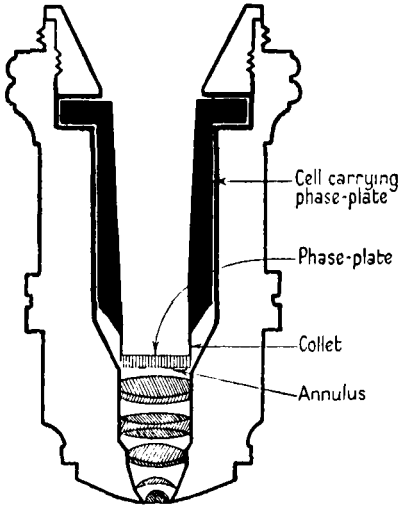

FIG. 1. - Diagram of the microscope objective showing the method of fitting the phase-plate. (Reproduced by courtesy of the Quarterly Journal of Microscopical Science.)

The plate, glued to the end of the cylinder, is accurately mounted in the centre of the revolving disc of a turntable used for mounting microscopical slides, and an annulus is dug out by scraping part of the bloom away with a chisel-pointed needle mounted on a specially constructed arm. The annulus should lie a little less than half-way from the centre of the phase-plate to its circumference.

In order to balance the direct light coming through the annulus with that of the diffracted light which passes through the rest of the phase-plate, carbon must be deposited on the annulus to reduce its transmission. This is done by passing the plate through a small xylene or benzene flame. The smoking should be sufficient to reduce light-transmission by about 30 per cent. The carbon is then removed from the phase-plate, except from the annulus itself. An illuminating annulus is next prepared. An annular space is cut away from a piece of black paper, which is then stuck on to a sheet of glass. The illuminating annulus is placed immediately in front of a 150 watt "Helios" enlarging lamp, which is the source of light. To set up the apparatus a square of fine ground glass is placed between the light source and the microscope mirror (Fig. 2). The microscope may now be used ordinarily. Some living cells are now placed on a slide and focused with a low-power objective. The condenser (preferably a high-power achromatic one) is then adjusted so that a pencil held against the ground glass is seen in focus with the cells. The low power objective is now replaced by the oil-immersion lens

carrying the phase-plate, and one of the cells is carefully focused. Then the slide is moved so that no cell is seen; the draw-tube is removed and a 3 in. objective is screwed into it at the bottom. The draw-tube is replaced and adjusted so that the phase-plate annulus is carefully focused. The sheet of

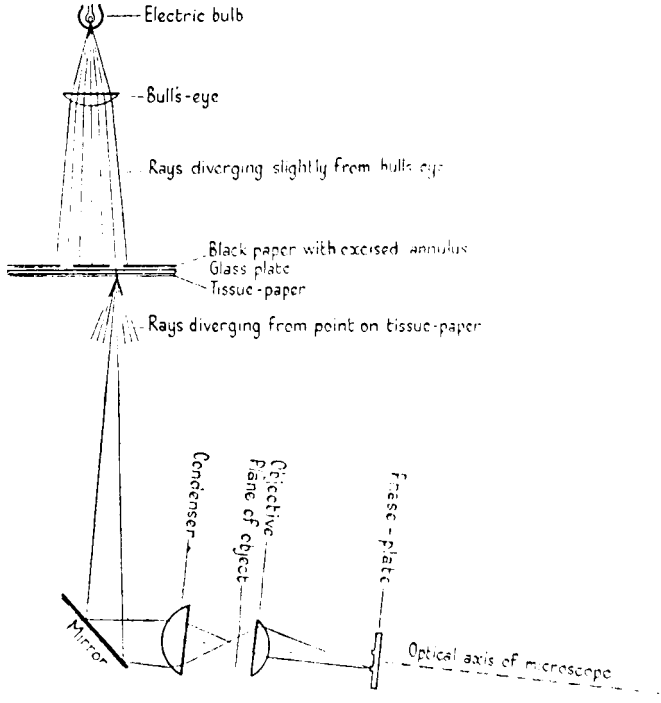

FIG. 2. Optical diagram of the apparatus for Phase-contrast Microscopy. The system described in the text is an improved and simplified form. (Reproduced by courtesy of the Quarterly Journal of Microscopical Science.)

ground glass is next exchanged for the bright annulus, and the condenser is now lowered until the bright annulus is in focus at the same time as the phase-plate annulus. At this position, the image of the bright annulus thrown by the condenser lies in the plane that is conjugate to the plane of the phase-plate placed on the other side of the objective. To make the two annuli exactly coincide the mirror is adjusted and the bright annulus and lamp moved towards or away from the mirror, whilst corresponding movements of the condenser are made to keep the bright annulus in focus. The 3 in. objective is then removed and the object once more brought into the field of view. The microscope will show the cells in " positive " phase-contrast: the field will be bright, transparent objects of high refractive index will appear black or grey.

REFERENCES

1. Kempson, Thomas and Baker, *Quart. J. micr. Sci.*, 1948, **89**, 351.