A New Method for Oblique Microscopical Illumination

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With two Text-figures

THE purpose of the technique described in this paper is to provide the easiest possible means of controlling the direction and degree of obliquity of the light used to illuminate microscopical objects.

The method is applicable when striations or other regularly-repeated markings on objects are situated so close together that, with axial illumination, the diffraction-spectrum they produce lies partly or wholly outside the back lens of the objective. A clear image can in these circumstances only be produced by throwing the direct light to one side of the back lens, and thus making room for the spectrum on the other side. Methods for achieving this object are familiar to microscopists. Advantages are claimed for the new method described in this paper. The intention is to allow the microscope to be used at its ultimate resolving-power with the greatest possible convenience and effectiveness. The method is likely to be useful in the testing of high-power objectives and in the study of the minutest markings on the shells of certain diatoms.

The basis of the method is that the objective of the microscope is used as an eyepiece through which the direction and degree of obliquity of the light are examined while the mirror is tilted in various ways. The condenser has to be in a special position in order to enable the objective to be used in this way.

No special apparatus is required. The source of light must be rather bright: an ordinary 100-watt filament-lamp with ‘pearl’ glass is very convenient. (‘Opal’ glass does not let through enough light.) Two stops with circular apertures are needed, with some easy means of changing from one to the other. One stop should have an aperture about 3½ or 4 mm. in diameter, the other about 15 mm. These will be called respectively the small and large stops. The stop in use must be placed immediately in front of the lamp. (An iris diaphragm can be used instead, but is rather less convenient.) The lamp must be arranged in such a way that a line drawn from the centre of the mirror through the centre of the stop would pass through the brightest part of the lamp. It is also desirable that this line should be approximately at right angles to the optical axis of the microscope. A stainless steel mirror is preferable to a glass one, because it gives only a single image, but it is by no means a necessity for the successful working of the method. An oil-immersion condenser of wide aperture (such as Watson’s Holoscopic) is required; it must be accurately

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centred and the iris diaphragm below it must be kept wide open. An oil-immersion objective of high aperture is of course necessary. The eyepiece used should be fairly powerful (I use a Watson's Holoscopic × 14). It is useful to have also a short cork that fits the top of the draw-tube, with a hole about 8 mm. in diameter bored through the middle of it. The use of this will be mentioned at the appropriate place.

The method for showing the striations of the shell of *Amphipleura pellucida* will now be described as an example. As is well known, this elongated diatom is transversely striated, the lines being exceedingly regular and closer together than the shortest wave-length of visible light. It is best to have the diatoms mounted in hyrax. Choose a specimen that lies somewhat apart from others so as to avoid complications arising from the appearance of more than one spectrum. (The presence of part of another diatom near the edge of the field of view is not harmful.) It does not matter in what direction the chosen diatom is orientated. In this description I shall suppose, for simplicity, that it happens to lie with its long axis in the 'north and south' direction. The striations, which will at first be invisible, will be directed east-west.

Place the small stop in front of the lamp and by means of the condenser focus the image of the stop so that it is clearly seen while the outline of the diatom is exactly in focus. (It is best to use a low-power eyepiece at this stage.) The illumination is now 'critical' (see Text-fig. 1). Next remove the eyepiece and substitute the bored cork. Put the large stop in front of the lamp, and with one hand hold a sharp pencil with its point in the centre of the stop. Hold the eye near the cork (the purpose of which is to make it easy to keep the line of vision axial) and look down the tube of the microscope. Accommodate the eye for distant vision. (Most microscopists do this reflexly when they look through a microscope. If special glasses are used for distant vision, they should be worn.) Now focus the condenser slowly upwards until the point of the pencil is clearly seen. Stop focusing upwards directly this is achieved. If you go too far, focus down again. The proper position has been achieved when a very small movement of the condenser downwards would cause the pencil-point to appear violently distorted and then vanish.

You have now secured what I call 'high-condenser illumination'. The principle of it is illustrated in the central diagram of Text-fig. 1. The condenser having been raised above its so-called 'critical' position, the image of the pencil-point has been brought above the diatom into the front focal plane of the objective, which thus throws parallel rays from it up the tube. The eye, being focused for distant vision, receives these rays and one sees the pencil-point. The objective is now being used as an eyepiece.

Discard the pencil and put the small stop in front of the lamp. On looking through the cork you will see the stop not filling the whole of the back lens of the objective. We here deliberately use a stop that will not fill the back lens with light. (To prevent any possibility of misunderstanding I mention here that the iris diaphragm below the condenser must be fully open throughout the whole procedure.)
Keeping the eye at the hole in the cork, grasp the mirror in both hands and turn it in its gimbals in such a way that the circular patch of light (the visual image of the stop in front of the lamp) passes to one side of the back lens of the objective. Stop when about half of the patch of light has passed out of view and the other half remains at the edge of the back lens. (The circle of light becomes distorted into an oval as it passes towards the edge.) Suppose you have moved the light towards the east: now bring it slowly round the edge of the back lens past the south-east towards the south. As it approaches the south, a spectrum will be seen at the edge of the back lens of the objective a little to the east of north (not to the west of north, as one might expect); this spectrum will move towards the north and will be due north when the direct light is due south. The violet end of the spectrum will be directed
towards the centre of the back lens. Now tilt the mirror to and fro to bring the light a short distance towards the middle of the back lens and back towards the edge of the back lens again, keeping it in the north–south line. Meanwhile watch the spectrum. Leave the mirror in the position that makes the spectrum as bright and complete as possible. The more of the red end of the spectrum you can bring within the back lens, the better. Text-fig. 2 shows the appearance that should be obtained. (If a glass mirror be used, there will be faint additional images of the stop, not quite coinciding with the bright image.)

Remove the cork and place the high-power eyepiece in position. The striations of the diatom will now be clearly resolved. There is no need whatever to use a colour-screen, even with a non-apochromatic objective. If perfection is desired, focus carefully with the fine adjustment, replace the eyepiece by the cork, and make very small movements of the mirror until the best possible spectrum is obtained; then replace the eyepiece.

In whatever direction the diatom happens to be lying, the same procedure should be adopted (with the obviously necessary changes).

Mr. W. E. Watson-Baker very kindly visited me in Oxford to see a demonstration of the method just described. He allows me to say that the striations of *Amphipleura pellucida* were resolved more quickly than by any other method he has ever seen, and that the images produced were superior to any obtainable by an oblique-light stop placed below the condenser. These comments are mentioned in the hope that the opinion of such an experienced microscopist will encourage others to give the method a trial.

It might be thought that the chromatic and other corrections of the objective would be upset by placing the condenser above its ‘critical’ position. To investigate this, I have made a careful examination of a number of species of test-diatoms, using central (not oblique) high-condenser illumination. I can find no evidence that the image is either better or worse than with ‘critical’ illumination. The expression ‘critical’ illumination is indeed a bad one, for it begs the question whether this is better than other methods. Hartridge (1919) has denied on both theoretical and practical grounds that
'critical' is necessarily preferable to other kinds of illumination: it is simply an easy and reliable way of getting good results.

If the substage iris diaphragm is somewhat closed and then kept in the same position while the two methods of illumination are tried, a larger area of the back lens of the objective will be filled with light by high-condenser than by critical illumination (provided that a sufficiently large stop is used in front of the lamp when the condenser is in the high position). When one is studying diatoms such as *Navicula lyra*, which are not well seen under full-cone illumination, this fact must be kept in mind; for otherwise there will be a danger of using too wide a cone of light when the condenser is in the high position. My attention was called to this point by Dr. O. L. Thomas, who has been kind enough to examine this new method of illumination.

One advantage of high-condenser illumination is that a perfectly structureless source of light need not be used, since the image of the light is not focused in the plane of the object; further, there cannot be any interference with the image by dust on any screen or cooling-chamber that may for any special purpose be placed between the lamp and the condenser.

The direction and degree of obliquity of the light may also be controlled by what I call 'low-condenser illumination', which is explained in the third diagram of Text-fig. 1. The condenser is here placed below the 'critical' position, and the source or light is thus focused below the object. An actual image of the source of light is now produced just behind the back focal plane of the objective, where it can be examined, if desired, by screwing a low-power objective into the bottom of the draw-tube. This is not nearly such an effective method for obtaining oblique illumination as that described above, but it is useful in phase-contrast microscopy. If an annular stop be put in front of the source of light, its image will be produced just behind the back focal plane of the objective, where the phase-plate may conveniently be placed.

Dr. R. Barer has kindly read this paper and given me the benefit of his criticism. He remarks that the method of illumination that I adopt is best described by saying that the aperture-stop, usually situated just below the condenser, has been removed to a considerable distance, so that the mirror lies between the aperture-stop and the condenser. I thank Mr. I. C. J. Galbraith, who gave valuable advice on an important theoretical point while I was engaged in perfecting the method of oblique illumination described in this paper.

**Summary**

The purpose of the method is to facilitate the resolution of extremely fine regularly-repeated markings on microscopical objects. Resolution is obtained by using the objective as an eyepiece to observe the direction and degree of obliquity of the light; these are controlled by movements of the mirror. To enable the objective to be used in this way, the condenser is raised above the
position required for so-called 'critical' illumination. The diffraction-spectrum formed by the markings on the object is clearly seen while the mirror movements are being made, and it is easy to produce a bright and complete spectrum.

REFERENCE