MICROSCOPY

THE CONSTRUCTION, THEORY AND USE OF THE

MICROSCOPE

BY EDMUND J. SPITTA
L.R.C.P. Lond., M.R.C.S. Eng., F.R.A.S., F.R.M.S.
Past President of the Quekett Microscopical Club, Author of *Photomicrography*, and Joint Author of *An Atlas of Bacteriology*

With 83 Half-tone Reproductions from Original Negatives and 255 Text Illustrations

LONDON
JOHN MURRAY, ALBEMARLE STREET, W.
1920

3. *The Depth of Focus* is intimately associated with the amount of N.A. present (but in company with other factors), being inversely proportional to it.

Ascertaining the N.A. of an Objective.

There are three methods for dry objectives, and two for homogeneous systems.

The first, applying to *both* types of lenses, is by using a special apparatus devised for the purpose by Professor Abbe, called the Apertometer (Fig. 81). This consists of a piece of thick glass about 3 in. in diameter, and half an inch thick. The part where the glass becomes segmental is bevelled from

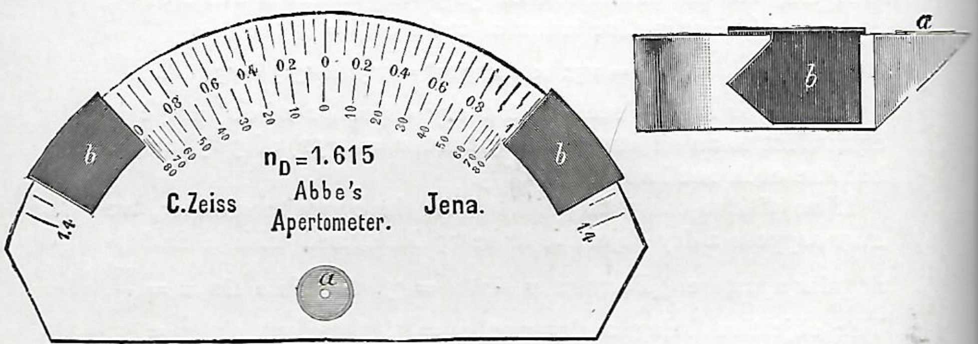

Fig. 81.

above downwards to an angle of 45°. Near the centre (marked *a*) is a small disc of silvered glass with a small hole in its centre, where the silvering is removed. Two plates of metal, which can be shifted around the outer edge of the glass shown at *bb*, are square one side and pointed on the other (see *b* in the side view). To use this apparatus, the glass is placed with the graduated surface uppermost upon the stage of the microscope (fixed vertically) in such a manner that the circular portion is forwards, and the chord or bevelled piece backwards, towards the stem of the instrument. The edges of the little hole are now focussed with the objective to be measured, an eyepiece being used, and the length of the draw-tube the same as when the objective is ordinarily in use. The two indices, as *bb* are called, are then placed on the edge of the glass, as shown in the plate, but close to the middle of the semicircle. Their sharp points should lie along the vertical edge of the disc, and their flat sides upon its upper graduated surface. It is best to direct the points away from each other to the outer side, if the power to be examined is comparatively high (N.A. above 0·6 or 0·7), but towards each other to the inner side if a low objective is employed.

With each apertometer an auxiliary objective is supplied which screws, or must be made to screw, into the end of the draw-tube, after which both are returned to the microscope, with the auxiliary lens passing down the main tube. The same eyepiece is then placed in the draw-tube as before, and the auxiliary microscope thus obtained is made to focus the image of the indices *by sliding the draw-tube in the main tube.* Care must be taken both in pulling out and pushing in the draw-tube for this purpose, whilst the eye is looking into the ocular, *not to alter the adjustment of the objective under examination* by accidentally shifting the main tube.

The indices are now adjusted, taking care they lie close to the glass plate, until their sharp points just touch the periphery of the luminous circle seen on looking down the eyepiece. Their position found, the readings of the upper edges, which lie in the same verticle plane as the points, are read from one of the two scales on the plate. The half of the sum of the two readings on the outermost scale—that nearest the edge—will give the measured value of the N.A. of the objective under examination. Likewise the sum of the two readings on the inner scale will give the value of the angular aperture in air.

The illumination must be shifted from right to left or up and down, so that the light falls horizontally upon the edge of the glass.

It should be noted that if the apertometer be used on *low-power* objectives, such as an inch, *with high N.A.*, owing to the size of the back lens having to be so large, the *auxiliary* combination may not be of sufficient diameter to give the maximum N.A. of the objective under examination. Also, and this is commoner still, with medium powers, say $\frac{1}{4}$, $\frac{1}{5}$, or $\frac{1}{6}$, it is not at all improbable the ordinary eyepiece, whether achromatic or compensating, may not command sufficient field of view; so between the two troubles a false N.A. may be obtained. This actually happened in our case when testing an inch N.A. ·3, an apochromatic quarter-inch, and an achromatic sixth, the mistake being only discovered when applying as a check one of the following methods about to be described, when different results were obtained. To remedy the first fault with low powers, let the observer look down the microscope after the first focussing, and regulate the indices *without* the auxiliary lens, using no eyepiece at all; whilst to avoid the latter trouble it is best by far to employ an ocular having no diaphragm on all occasions.

The student should read some interesting suggestions made by Dr. Hartridge upon the use of the apertometer contained in page 337, Part 4, *Journal Roy. Mic. Soc.*, 1918.

Another method of ascertaining the numerical aperture is by the use of a device suggested by Mr. F. J. Cheshire, now Professor at the Imperial Institute, South Kensington. This simple and yet efficient contrivance is described in a paper by the inventor in the *Journal of the Quekett Microscopical Club*, Vol. IX. (1904), to which the reader is referred for mathematical and other details. Briefly, the apertometer consists of a number of concentric circles, drawn on paper, which are so spaced and graduated in thickness that when the diagram which they form is placed on the stage of the microscope, at a certain distance below the usual object plane of the objective to be tested, the circles project into the upper focal plane of this objective, as a number of equi-distant, equi-thick concentric circles, from an observation of the number of which visible, the N.A. is read off directly. The first and smallest circle corresponds to an N.A. of 0·1, the second to 0·2, and so on up to 0·9. Intermediate values are estimated by the eye. The observation of the image of the diagram in the upper focal plane is made either by (1) removing the eyepiece and fitting the top of the draw-tube with a small peep-hole; or (2) by fitting a low-power eyepiece—a 50-mm. Leitz does admirably—with a 2-mm. stop and using it to project the apertometer image in the eye-ring or Ramsden circle of the microscope, in which circle it can be observed with the aid of a high-power pocket magnifier. The draw-tube may be also used as an auxiliary microscope (as in the Abbe apertometer) by fitting its lower end with an objective suitably stopped to give a telecentric system.

The simple form of this apertometer, shown in Fig. 82A, is sold by Baker of Holborn; a modification (Fig. 82B), in which the circles are drawn on the lower surface of a thick disc of highly refractive glass adapted for use with dry and immersion objectives, is sold by R. & J. Beck of Cornhill, London.

With dry powers only, there yet remains another method which is also exceedingly simple and effective.[1]

Lay upon the table two pieces of white paper, using a black

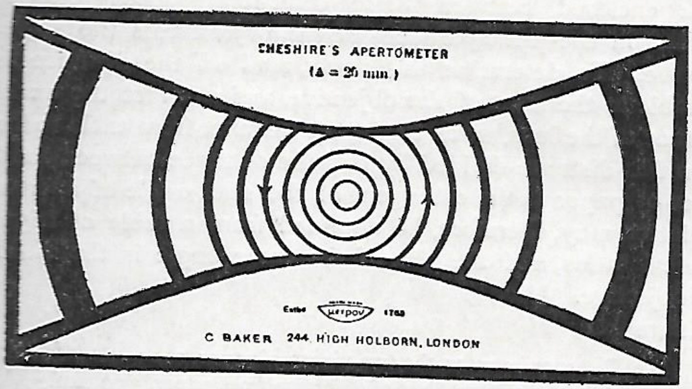

Fig. 82A.

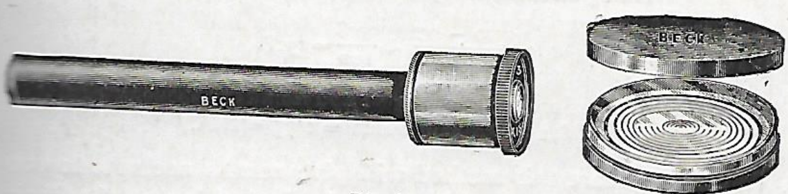

Fig. 82B.

background, with their straight inner edges parallel to one another and a definite distance (say 20 cm. for lenses of N.A. over 0·50, less for low-angled ones) apart, then hold a rule vertically upon the table about midway between the two pieces of paper. Next hold the objective to be tested vertically

[1] Although this idea may have occurred to others for obtaining the *angular aperture* of microscopical objectives, we believe it was first suggested to obtain their *numerical aperture* by Mr. Conrady, now Professor at the Imperial Institute, South Kensington, being published in the Author's *Photomicrography*, from which the paragraph is abstracted.

against the rule and look down at the back lens. Images of the two pieces of paper will be seen there: now slide the objective downwards along the edge of the rule, always watching these images. They will separate farther and farther apart until at last a point is reached where only a slight bluish flicker remains visible on either side in the extrême margin of the lens, which, of course, indicates that the inner edges of the pieces of paper are in the direction of the most oblique rays which the objective is capable of receiving, or that the angle enclosed between these directions, which directions intersect in the principal focus of the objective, is the angle of aperture. To determine this angle, read off the distance from the table to the front of the objective, and subtract the working distance of the lens,[1] so as to get the distance from table to focus. Then this distance divided by half the distance between the two pieces of paper is the cotangent of the semi-angle of aperture; the latter may, therefore, be obtained from a table of trigonometrical ratios, and the sine of the same angle is the N.A. of the objective.

Example:

Distance between the two pieces of paper, 200 mm.
Distance of front lens of objective from paper, 33·0 mm.
Working distance of objective, 0·2 mm.;

$$\therefore \frac{33{\cdot}0 - 0{\cdot}2}{200/2} = \frac{32{\cdot}8}{100} = {\cdot}328.$$

$0{\cdot}328 =$ cotan. of angle $71^\circ\ 49'$, as we find from the trigonometrical tables, the sine of which $= 0{\cdot}95 =$ N.A.

With great care this method will give results accurate to one or two units of the second decimal. In looking at the back of the objective the eye should be at a distance about equal to the tube-length for which the objective is designed, but the error caused by even considerable deviations from this theoretically required distance, is very small.

Depth of Focus

Before concluding this chapter, seeing that the amount of "depth of focus" (sometimes called "penetrating power") possessed by an objective is largely though by no means entirely

[1] How to ascertain the working distance, see Index.