JOURNAL

OF THE

ROYAL MICROSCOPICAL SOCIETY.

DECEMBER, 1918.

TRANSACTIONS OF THE SOCIETY.

XII.—*An Improved Method of Apertometry.*

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(*Read December* 18, 1918.)

Four Text-Figs.

Inaccuracy in apertometry is found to be due to the following causes :—

1. The use of inaccurate focus or incorrect tube length.
2. The use of rays not strictly corresponding to the centre of the field.
3. The incorrect setting of the indices.
4. The incorrect determination of the position of the indices in relationship to the graduated scale ; and inaccuracy in the graduated scale itself.
5. Employment of an incorrect angle between the plane of the apertometer plate and the optical axis.
6. Chromatic aberration.
7. The employment of methods based on the measuremcnt of the Ramsden disc.

Section 1.—Error in Apertometry due to the Use of Inaccurate Focus or Incorrect Tube Length.

The graduation of the scale of the Abbé apertometer is carried out on the supposition that the segments illuminated by rays proceeding to the periphery of the disc have their apices in

correspondence with the optical centre of the glass disc. The edges of the silvered strips lying under the cover slip should therefore be sharply focused.

The adjustment of tube length is necessary, because it is found by experiment that aperture varies with tube length, particularly with lenses of long focal length.

Further, the focusing of the objective should be carried out by rays corresponding to the peripheral zone of the objective only; since it is these rays which are concerned when the aperture is measured, and since other rays, if spherical aberration be present, will come to a different focus. This limitation to peripheral rays may be performed by the employment of an annular post objective stop. (A Travis expanding stop is admirable for this purpose). (*8*).* A further advantage would appear to be given by this method in cases where residual spherical aberration exists in the outer zone, since the same focusing adjustment would be employed in making the measurement of N.A. as that used when structure of the utmost fineness that the objective will resolve is under examination. The tube length being correctly adjusted, and the post objective annular stop being in place, the objective is carefully focused. The image will in general be a very poor one compared to that usually obtained, because of the aberrations which are usually found in the peripheral zones of most objectives. In spite of this, however, precision of focus is as a rule considerable, because of the large angle between the rays forming the image.

In the ordinary technique with the Abbé plate when the microscope has been focused the eye-piece is removed, and one or other of two methods employed:—

(A) A special objective (supplied with the Abbé apertometer) is screwed to the lower end of the draw tube so that a magnified image of the back lens of the objective is seen in the eye-piece. (B) The eye is placed over the draw tube and the back lens examined direct. Both methods are faulty. The first in that the aperture attached to the brass tube fitted to the apertometer objective can correspond to one tube length only, and this will be, by accident alone, that for which the focus has been made previously. The second in that again the correction for tube length is ignored, for the iris of the eye is the limiting aperture of the rays, and should therefore correspond with the plane of the image formed by the objective. But owing to the eye being within a convergent optical system formed by the cornea and aqueous, its effective aperture is considerably above the anatomical position of the iris, and therefore the eye should in general be placed 2 to 5 c.cm. below the top of the draw tube in order that the

* The italic figures within brackets refer to the Bibliography at end of the paper.

effective aperture should be in its correct position. In most microscopes this position is impossible. Further, as a rule no care is taken to keep the eye central with the draw tube, in order that its effective aperture shall correspond with the centre of the field. The correct technique is obtained by dropping into the draw tube in place of the eye-piece a suitable aperture in a sunk brass mount, the aperture being small and corresponding to the centre of the field, the depth of the mount being such that the aperture is parfocal with the eye-piece.

Section 2. — Error in Apertometry due to the Use of Rays not Corresponding to the Exact Centre of the Field.

Rays lying away from the centre give values which differ considerably from those obtained with the employment of central rays, because such rays pass through the apertometer plate as bundles to one side of, and not necessarily parallel with, those which illuminate the centre of the field. Since the definition of most microscope objectives is superior at the centre of the field, observation is preferably made there, and therefore central rays should be used for apertometry. There is, however, another and more important reason. For accurate apertometry the indices and the image of the restricting aperture of the objective should coincide. This is rarely the case in practice, for with low-power objectives the images of the indices are usually in a plane much above the back lens; whereas in the case of high-power lenses the plane is situated more often within the objective. Further, while in most objectives it is the back lens which limits the aperture, in one objective in my possession this is done by the front lens, and therefore it is with the image of this that the indices should coincide. As a rule, therefore, the image of the restricting aperture (usually the back lens) of the objective and the indices do not coincide, and therefore by focusing alone it is impossible to get both in focus at the same time.

Now, consideration showed that by restricting the effective iris of the system it should be possible to increase the depth of focus to such an extent that the images of the indices and the restricting aperture should be both simultaneously sharply imaged. It was further seen that the effective iris corresponded in position with the focused image of the objective. Therefore by replacing the eye-piece by a small aperture, through which the observations of N.A. could be made, it was possible to avoid the difficulty due to the indices and the restricting aperture of the objective not coinciding.

Section 3.—Error in Apertometry due to the Incorrect Setting of the Indices.

Probably the most accurate of optical methods of measurement is that in which an index is caused to coincide with the centre of the image to be measured (*2*).

But the accuracy is enormously decreased if the field on one side of the image is invisible to the observer, because the measurements are then affected both by his visual acuity and his judgment. If the rays from the objective be traced through the apertometer it

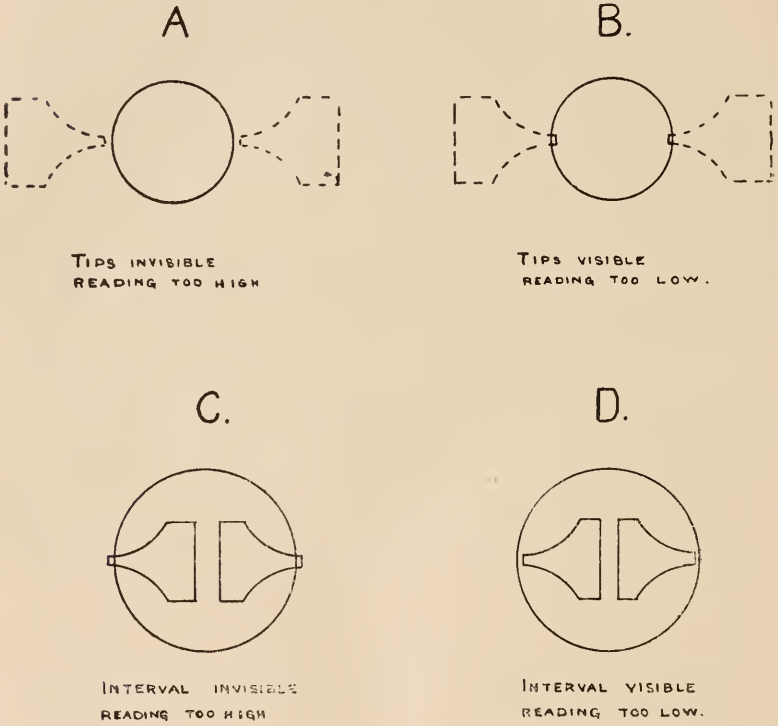

Fig. 1.—Diagrams to show how error is caused by the restriction of the visible field to the aperture of the objective.

At A the indices have been adjusted till the tips are judged to be invisible. The reading is now too high.
At B the indices are still visible, and therefore the reading is too low.
At C similar conditions to A are not met with.
At D the conditions are similar in their effects to those at B.

will be found that they form a cone, the apex of which corresponds with the optical centre of the plate. Structures lying anywhere within this cone will therefore be visible to an observer looking down the draw tube at the back lens of the objective, while structures lying outside this cone will be invisible. This makes the setting of the images of the indices in coincidence with the restricting aperture of the lens a difficult process. Ainslie (*3*) described a similar difficulty in using Cheshire's apertometer. Diagrams A and B (fig. 1) illustrate this difficulty. Placing the indices so that their images are formed within the visible field does not give benefit, as diagrams C and D (fig. 1) show. It is therefore easily seen that according as one takes case (1) in which the tips, or case (2) in which the intervals, are either seen or not seen, so the measurement of the aperture will be too low or too high. A method which allows the whole of the field (i.e. the region outside the cone, corresponding to the limiting aperture of the objective) to be visible removes the difficulty above described by allowing the method of coincidence to be employed. This may be obtained by reversing the direction of the light rays through the optical system. For this technique, therefore, the light source is placed in the position of the eye-piece, and the rays of light descend through the objective to spread out as a fan towards the periphery of the apertometer plate. The numerical aperture is therefore measured by setting the indices at the edge of the cone of illumination proceeding from the objective, the edge being rendered visible by frosting the curved side of the apertometer plate, or preferably by observing the aerial image by means of a Ramsden eye-piece. It will be seen at once that there are two advantages to be gained by this technique : (1) the whole of the field is visible, and therefore the method of coincidence may be employed ; (2) the method can be used without modification for the examination of condenser lens systems. Details of the technique will be given later.

Section 4.—Error in Apertometry due to the Incorrect Determination of the Positions of the Indices in Relationship to the Graduated Scale, and Inaccuracy in the Graduated Scale itself.

Both these factors are entirely concerned with the construction of the apparatus and its limitations. As manufactured by Zeiss the instrument is imperfect, for the indices are so constructed that the graduations are not closer than 0·05 N.A., and the subdivision has to be done entirely by eye. This is made difficult by the fact that only one side of the scale is visible (the other part being hidden by the index itself). This point is clearly shown in

diagram A (fig. 2); in diagram B is shown a slight alteration to the indices which entirely avoids this difficulty. An alternative form which would allow greater accuracy is shown at C. The method of using this form of index is as follows:—

The short graduated arm is so constructed that the angle it makes may be altered without changing the position of the zero. By means of a preliminary measurement the approximate N.A. of the objective is obtained. Suppose it to lie between 0·8 and 0·9, the index is placed so that the zero corresponds to 0·8, and the graduated arm is now gently rotated till the 10 graduation corresponds to 0·9. The intermediate graduations now correspond to the decimal subdivisions between 0·8 and 0·9, and may be used for reading to approximately 0·003 N.A. It should be noted that for the scale on the short arm to be uniform its edge must be the segment of a circle. This circle has nearly the same radius as that of the glass plate.

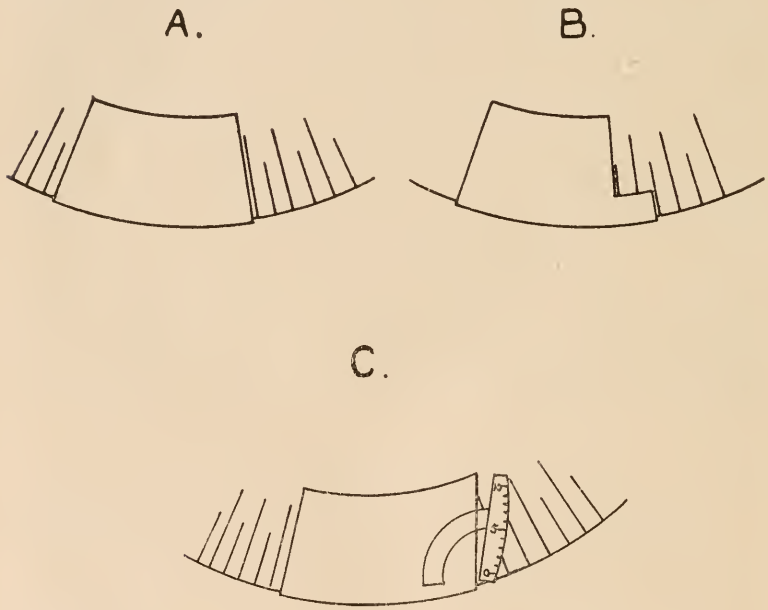

Fig. 2.—Diagrams to show the present shape of the indices A and two improved types, B and C.

At A the subdivisions between the graduations are difficult to estimate by the eye, because only one side of the index is visible.

At B the index has been cut away, thus facilitating the correct estimation of the interval.

At C the index is fitted with a small scale of adjustable inclination, thus rendering the subdivision exact. Direct readings may be obtained to 0·01 N.A. with this device.

A better plan has been suggested to me by Ainslie, namely, to utilize the principle of the diagonal scale. For this purpose a specially graduated glass plate would be required, as shown in fig. 3. The method of using such a scale is well known, and therefore needs no further description. The reason for the use of curved lines is similar to that given above.

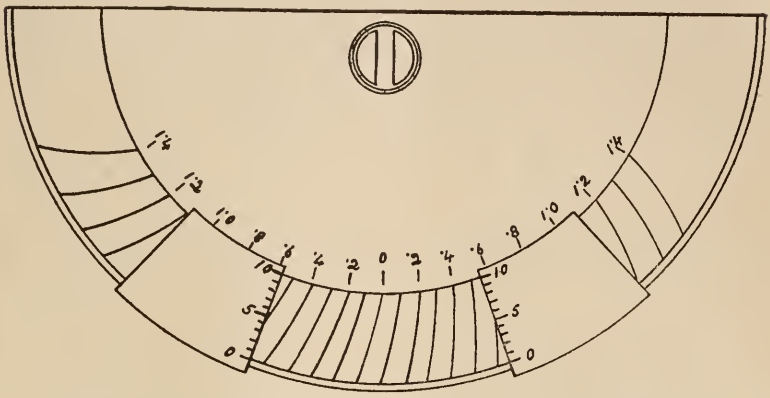

Fig. 3.—Diagram to show the design of an apertometer with a diagonal scale, thus giving direct readings to 0·01 N.A.

Section 5.—Error in Apertometry due to Plane of Apertometer Plate not being at Right Angles to the Optical Axis of the Microscope.

Since the light leaves the objective to form a cone of illumination, and since a cone has its greatest width across its diameter, it is clear that the pointers of the indices must be symmetrically placed on a true diameter to the cone, for otherwise the aperture reading will be too low. Since the prism angle at the back of the plate is 45° it might be thought that this point could be neglected. Tests show that this is not the case. Without considering the possible causes of error, the following method of obtaining the correct adjustment may be described as follows:—If with the objective focused in the cover slip of the apertometer, the indices appear to be out of line with the diameter of the cone, then the apertometer plate must be tilted so as to bring them as far as possible correctly into line. The fine centring is now performed by sliding the plate as a whole forward or backward. (This method of adjustment is impossible with the old type of Abbé apertometer, in which the silvering forms a complete ring under the cover glass; and therefore adjustment must be effected by tilting alone.)

The fine centring is performed as follows :—The vertical portion of the indices takes the form of a trident the tips of which are vertically in line, as shown at A in fig. 4. If the indices are now adjusted so that the upper prong is in contact with the edge of the cone of illumination, then if the indices lie in a true diameter the lower prong will also be just in contact, see fig. 4 at B. If contact is not perfect and the index is set apparently at too high a reading, as shown at C, the apertometer plate should be shifted further back and vice versa. Adjustment should be made until the upper and lower prongs appear to meet the edge at the same instant, as shown at B in fig. 4. (At D is shown a better, but somewhat more complicated form of index.)

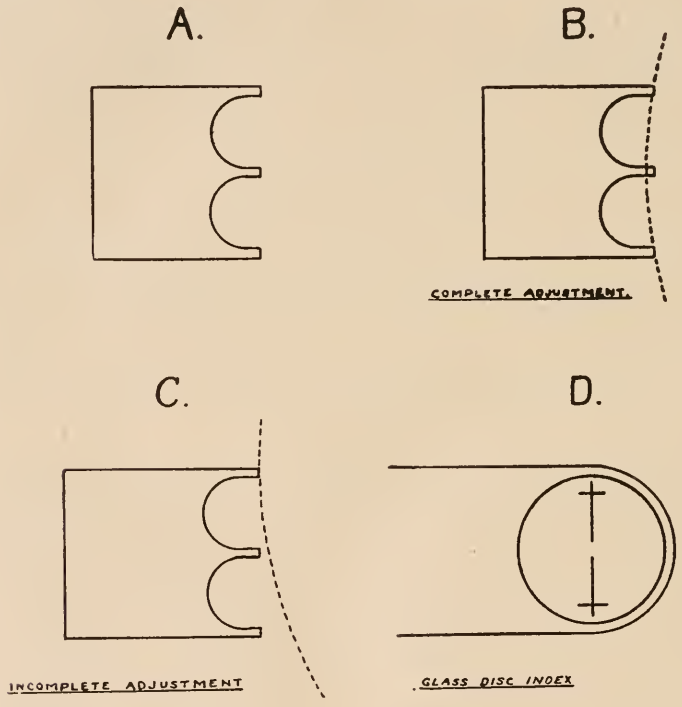

Fig. 4.—Diagrams to show new form of index which facilitates the setting of the plate at right angles to the optical axis.

At A the new form of trident index is shown.
At B the index is shown in relationship to the aperture, the angle between the plate and the optic axis being a right angle.
At C the index is shown in relationship to the aperture, the angle being greater than a right angle.
At D an alternative and better type of index is shown. It is used in a similar manner to the one shown at A.

Section 6.—Error in Apertometry due to Chromatic Aberration.

Abbé has pointed out (*1*) that objectives corrected for chromatic aberration may show considerable error if the path of the beams is altered. Thus, with high-power objectives, the aperture as measured with blue rays is usually larger than that obtained with red; with the holoscopic 4 mm. the converse is the case. In measuring aperture accurately, therefore, monochromatic light should be used of known, and if possible of standard, wave. This should be either the "chosen colour" (i.e. that for which the most perfect corrections have been made—usually yellow-green rays), or light of short wave length (e.g. blue rays) when the maximum resolving power is required of which the objective is capable.

For providing monochromatic light for this purpose good colour filters are quite sufficient. They may be placed anywhere in the light path; between the source and its aperture is a convenient position.

Section 7.—Error in Methods based on the Measurement of the Ramsden Disc.

The employment of these methods depends on the accuracy of the formula N.A. $\times$ 2 D = M $\times$ R.D., where D is the distance of distinct vision (10 in., or 254 mm.), M is the total magnification of the instrument, and R.D. is the diameter of the Ramsden disc. Now Ainslie (*3*), who has given a description of the method, points out that for accurate determinations the diameter of the Ramsden disc must be measured (because of its small size) by means of instruments of considerable accuracy. When this has been done it still remains to determine the magnification and to fix the value of D, because the accommodation of the eye renders the value of the latter uncertain. For this purpose I find the Eikometer of Wright (*4*) to have definite advantage, because it fixes the value of D (not at all necessarily at 10 in. however) during the determinations of the magnification. Apart however from the requirement of special instruments I have found the method to suffer from inaccuracy, which is probably due to irradiation.

Section 8.—Description of an Improved Method of Apertometry.

The principles on which this method rests have been briefly considered above; the description will therefore be limited to structural features.

With the microscope set up in the usual manner (the tube length being correctly adjusted), and with the apertometer on the stage, the annular stop is slipped into position behind the objective, and the latter then carefully focused on one of the edges of the silvered bands under the cover slip. The eye-piece is now replaced by the 2 mm. aperture parfocal with the eye-piece, which is lit from above by a small frosted electric lamp of low candle-power (any other suitable method of illumination may of course be employed).

The aperture being illuminated, the apertometer plate is gently shifted so that the image of the aperture formed by the objective shall fall on the centre of the cover. With a Ramsden eye-piece of medium power (or a hand magnifier) the edge of the shadow on the periphery of the apertometer plate is examined, and one of the indices is moved, so that the upper prong of the trident is set to the exact edge of the shadow. The lower edge is now examined, and if found to be in exact contact the setting of the apertometer plate is correct; if not in contact the setting requires adjustment as described in Section 5. When the correct adjustment has been obtained, the central prong is placed in exact contact with the shadow, and the scale-reading now taken. The left-hand index is then adjusted and its reading similarly obtained. Ten alternate readings are logged and a mean taken; any considerable difference between any of the sets of readings of the same side indicates that the apparatus has been accidentally displaced, and the whole process must be repeated. Assuming that the graduations of the apertometer are accurate, the values obtained by this technique may be depended on to 0·003 N.A. This error even may be reduced by accurate apparatus. A method of this accuracy is found to be essential if other apparatus is to be calibrated in terms of N.A.

Section 9.—Simplified Technique.

Abbé has pointed out that for ordinary purposes very accurate determinations of aperture are not required, because with most objectives quite considerable variation in aperture is not found to cause any observable change in resolving power.

It is therefore possible in such cases to employ a simpler technique than that described above.

For dry lenses of low and medium power the apertometer described by Cheshire (*5*), or better, Ainslie's modification (*6*), can be utilized. Special care should be taken to see that the tube length is correct; the use of an annular post objective stop is not necessary however. But there are definite advantages from the use of a descending light path, because the edge of the cone of divergent rays is more easily observed, and the number of rings counted with

greater certainty, when the eye examines the apertometer itself in place of its greatly-diminished image. Owing however to the larger surface the light source has to be brilliant, particularly if outside light has to be competed with. I have found a pocket electric-battery torch admirable for the purpose. For objectives of medium and high power the bull's-eye lens frequently fitted to such lamps may with advantage be retained.

For dry high-power lenses and for oil and water immersion systems the use of a plate similar to the Abbé instrument is necessary. The use of the correct tube length is not of such importance as it is with objectives of longer focal length. If the descending light path be employed both the indices and auxiliary objective may, however, be dispensed with by having the curved edge of the plate frosted and graduated. By this means the boundary of the cone of light may be observed and measured, without the necessity of indices or hand magnifier (Ramsden ocular). Although these simplified methods do not give the same degree of accuracy as that which the more complicated method provides, yet in my hands they appear quite equal to the original Abbé method, and an accuracy to ·01 N.A. should be easily obtainable. A further advantage not previously mentioned which the use of a descending light path provides is that since the auxiliary objective is not employed there is no possibility of the error arising which Spitta (*7*) has drawn atttention to.

For very low aperture objectives, Conrady's method would appear to be very reliable, and to be accurate to a least 1 p.c., if proper precautions have been taken with regard to tube length. (The relatively long focal length of such objectives renders the use of the correct tube length of the greatest importance.) In this case also, however, in my opinion, there is definite advantage to be obtained by the employment of the descending light path.

Section 10.—Application of the Method to the Substage Condenser.

For many purposes it is necessary to obtain the values of apertures, iris-diaphragms, or central stops placed in the lower focal plane of the condenser in terms of N.A. This may be done in several ways; probably the most accurate hitherto described was that employed by the author for measuring working aperture (*9*). Better results should be obtainable by means of a direct method by employing the apertometer in the same way as that described above for the objective. For this purpose the Abbé apertometer is placed on the stage upside down, and the rays from the condenser caused to enter the plate in a similar manner. It will be at once observed, however, that the method

of focusing and centring employed in the case of an objective cannot be used with the condenser, because the light source cannot be replaced by an eye-piece without considerable inconvenience. An alternative method is therefore best employed, which may be described as follows:—To the sloped reflecting edge of the Abbé apertometer, and immediately above the cover glass, is temporarily mounted the hypotenuse of a small right-angled prism (for this purpose stiff cedar-wood oil, or, better, bifocal cement, will be found satisfactory). The upper surface of the small prism should now be parallel with the plane of the stage, and therefore an image of the silvered strips of the apertometer may be clearly observed by means of any low-power objective with sufficient working distances, since the spherical aberration introduced by the considerable thickness of glass does not cause sufficient degradation of the image to matter for the present purpose. The images of the strips having been sharply focused, the image of the light source is sharply focused in the same plane by adjusting the condenser. The right-angled prism is now removed, and the sloped surface of the apertometer carefully cleaned, in order that the rays incident on it may be reflected to the periphery of the plate without degradation. This must be done without removing the apertometer from the stage or spoiling its adjustment relative to the condenser. A more elaborate method, which avoids any difficulty due to this cause, necessitates the application of a semi-transparent coating of silver to the sloped surface of the apertometer. Above this is permanently cemented the right-angled prism, so that the greater part of the light is internally reflected at right angles, to spread out towards the periphery of the apertometer, while sufficient light passes through the silver and prism to the low-powered objective in order that accurate focusing and centring may be effected. It should be noted that such a procedure does not in any way spoil the apertometer for more general use, so that when much work of this nature is to be done this latter technique should be employed.

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