AMERICAN JOURNAL OF BOTANY

VOL. III — OCTOBER, 1916 — NO. 8

THE ANGULAR MICROMETER AND ITS USE IN DELICATE AND ACCURATE MICROSCOPIC MEASUREMENTS

HOWARD E. PULLING

In the formulation of a cytological problem that necessitated the accurate measurement of extremely small bodies it became evident that the commercial ocular micrometers were neither sensitive nor accurate to the required degree. Although it is generally recognized that any instrument whose precision depends upon the accuracy and lack of variation in the pitch of a screw is subject to large errors of construction and to further errors resulting from wear upon the screw and its bearings, it is often believed that the best filar micrometers are sufficiently accurate for all general needs of the microscopist. That this conclusion is not warranted unless each instrument is systematically investigated for error and the necessary corrections made has been shown by Gray,[1] who points out further that satisfactory instruments may easily be rendered worthless by dust particles that grind the screws unevenly and that in all cases instruments must be frequently investigated for error.

That such general observations as these are of interest not only to workers who demand the very highest degree of accuracy from micrometers but that they affect *all* microscopists who use such instruments is evidenced by the following example drawn from my own experience. A filar micrometer, one of the best types obtainable, was used to measure the same fixed distance over different portions of the screw. Through the exercise of great care in the selection of the object and in the manipulation of the instrument the readings made on any one position of the object did not vary from the mean of the readings for that position by more than 1/500 of a drum revolu-

[1] Gray, Arthur W., Micrometer Microscopes. Scientific paper of the Bureau of Standards No. 215 (reprint of Bulletin of the Bureau of Standards, 10: 375–390. November 5, 1913). Washington, D. C., 1914.

tion. Notwithstanding this constancy at each position the variations in the readings at different portions of the screw were as large as 17.5 percent of the smaller number and differences of from 5 percent to 10 percent were common. Since no attempt was made to search for errors of great magnitude and since the object measured was the most favorable that could be found (the distance between two points on an irregular ink spot, moved with a rectangular mechanical stage) it is hardly to be doubted that such an instrument cannot be relied upon without investigating it for errors and constructing an error curve, or table, for the full extent of the screw.

Since in designing the angular micrometer each member of the filar micrometer was separately considered with reference to its liability to structural errors and to its influence upon the observational and personal errors of the operator, a brief review of the defects of the latter instrument will curtail the explanation otherwise necessary.

For accurate work, or even for general use with low and medium magnifications, the drum graduations are inadequate and an accessory scale is required. An instrument of precision should be accurately graduated beyond any demands which may be made upon it.

Because the moving parallel lines extend across the field of view at right angles to their direction of movement, the line connecting the two points whose spatial separation is to be measured must be accurately normal to the moving line. Experiments have shown that the line connecting these two points may often be inclined ten or fifteen degrees from the normal and the inclination be undetected unless the rotation was performed with the visual knowledge of the observer. No definite point exists that may be used as a datum point because the ends of the parallel lines are rounded and because the thickness of the cross lines and the sharp angle at which they meet prevent the operator from surely placing the point of their intersection directly over any particular point on the object-image. Averages of several observations are valueless because when one datum point is used all the errors tend to have the same sign and hence do not offset each other and because no two datum points can be selected which have their errors in exactly opposite directions.

Perhaps it is in part such errors as these that led Farmer and Digby[2] to adopt the method of drawing the object with a camera

[2] Farmer, J. B., and L. Digby. On dimensions of chromosomes considered in relation to phylogeny. Phil. Trans. London, *B*, **205**: 1–25. 1914.

lucida and measuring the figure drawn. This method is open to many objections; tediousness and difficulty of operation would prevent the majority of workers from adopting it.

The angular micrometer was designed to eliminate or greatly reduce the errors inherent in the instruments and methods referred to above. The mechanism for magnifying the movement of the pointer-line is rigid and of a nature to reduce mechanical errors of construction to a minimum. These errors are either negligible or such as to admit of ready detection and calculation. The parts subject to wear are only those whose abrasion with ordinary use will not affect the accuracy of the instrument. The pointer line moves so that the datum point selected can always be easily identified and the graduations are accurate beyond the severest demands.

The principle upon which the micrometer is based is the trigonometrical calculation of the chord of a circle. The equation for this calculation is $x = 2r \cdot \sin(\theta/2)$, in which r represents the radius of the circle and θ the angle subtended by the chord, x, whose length is to be calculated. The apparatus by which θ is determined for the chord in question (the distance between two points on a microscopic object) is shown in figure 1. In *A* the micrometer is shown from above, in place on the microscope; in *B*, from the side. The instrument consists of three essential parts, *a*, *b*, and *c*. The plane of the arm *a* is perpendicular to the axis of the microscope. At the outer edge of this arm is a double vernier, *d*, whose center is on the axis of the microscope. Rigidity is secured by the brace, *g*, and the wide collar, *k*, which is split lengthwise and secured by two sets of screws attached similarly to those for the arm *b* (*h–h*, figure 2, *A*). This arm, *b*, is attached by a split collar, secured with screws, *h–h*, to the ocular of the microscope and bears at its outer edge a protractor, *f*, which is graduated to half-degrees and may be read to minutes of arc with the aid of the vernier. In the instrument which has been constructed the radius of the protractor circle is 17.5 cm., so that readings may be readily made with the naked eye. A glass plate, *c*, is placed in the ocular on the interior focal plane and bears a short photographed line which is tangent to some one of the imaginary circles whose centers are on the axis of the microscope. The point at the tip of this line moves, as the ocular is turned in its sleeve, around the circumference of a circle whose center is on a line which passes through the centers of the protractor and vernier and is perpendicular to their planes.

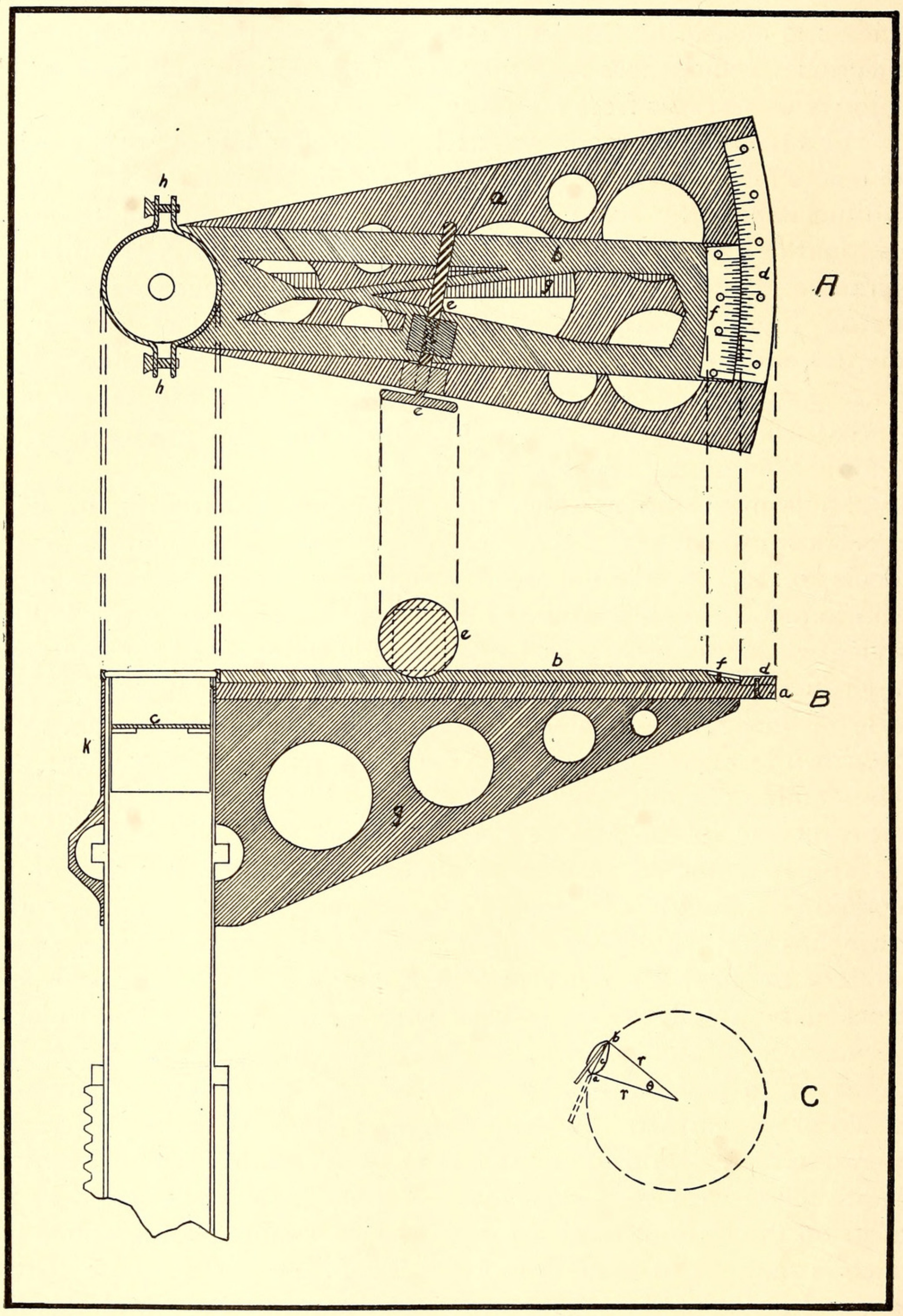

FIG. 1. The angular micrometer. *A*, a top view of the micrometer in place on the microscope. *B*, a side view. *C*, construction for the calculation of the chord, *c*, formed by the pointer-hair moving from position *a* to position *b*.

The vernier arm is clamped to the microscope and remains fixed during the progress of any measurements. The protractor-arm, *b*, is clamped to the ocular so that by turning the screw, *e*, attached by swivel joints to *a* and *b*, the system, vernier-ocular, is rotated about the common center. In making a measurement the tip of the line in the ocular is brought against the edge of the image formed by the object to be measured, as in figure 2, *c*. The protractor is then read with the aid of the vernier to minutes of arc. The ocular and protractor are then rotated about their common axis until the tip of the line in the ocular appears at the outer edge of the image. The position of the protractor is again read and the difference between the two readings is the θ of the equation given above.

The other quantity in the equation which must be known in order to determine x is the radius, r. Since the line in the ocular is fixed in its position relative to the axis of the microscope, one determination of r is all that need be made for each system of lenses. To determine this radius a suitable scale is placed on the stage of the microscope and θ is determined for some convenient interval of the scale, as described above. The value of the interval in units of length is substituted for x in the equation, the corresponding value of θ, as read from the protractor, is introduced, and r is determined algebraically. This value of r may then be used, with the same system of lenses, for future measurements.

The micrometer is constructed of an alloy of aluminium which is very rigid, is light enough to avoid tipping the microscope tube, and whose coefficient of thermal expansion is very low. This alloy, known as a "White Metal" mixture, is composed of 92 percent aluminium and 8 percent copper. The graduated members, vernier and protractor, are of coin silver and must of course be as accurately graduated as possible with very fine lines. The screw with its milled head is fixed to a swivel block attached to the vernier-arm and works in a threaded bearing attached to the protractor-arm by another swivel joint. This screw may be dispensed with, although it adds to the ease with which delicate adjustments may be made and reduces the pressure on the vernier-arm during the time of making these adjustments. Pressures of the hands in moving the vernier tip the microscope tube, and this changes the path of light through the lenses, the focus, and the apparent position of the object to be measured. This adjusting screw should be affixed as close as possible to the microscope-tube in order to maintain the center of gravity of the instrument near the axis of the tube, and, to increase the ease of manipulation, the milled head of the screw should be large.

The glass slip, *c*, may be either cemented to the annular disc in the ocular and remain there permanently, or it may be cemented to a short tube closely fitting the ocular but removable. The first method is preferable, since it maintains *r* constant for a given objective. This arrangement does not interfere with the ordinary use of the ocular because the line is very fine, very short, and in no way confuses the vision; almost the entire field remains unobstructed and a slight rotation of the ocular changes the position of the line. In default of a photographed line, one may be scratched on a thin, circular cover-slip with a fine cambric needle; such a line will serve equally well, since only its tip is used and the straightness and slope of the line are of no importance. The tip should be sharp and distinct because the exactness with which it may be placed against the object-image controls the accuracy of all measurements. The first micrometer that has been constructed on this plan was built at a cost of $23.00. This instrument however did not possess the adjusting screw, *e*, which would of course add to the cost. On the other hand, because various alterations in the design of the instrument were found necessary during the progress of the work the figure given should include a large part of the cost of the adjusting screw.

The errors to which the angular micrometer is subject by reason of its construction may next be considered.

I. Failure to Center Protractor and Vernier

Case 1: Centers of protractor and vernier on a line at right angles to the zero radius of the vernier. (See construction in figure 2.) In the figure the circle A–A' is that of the fixed scale upon which the angles are read. B–B' is the circle traced by the protractor as the pointer in the ocular moves across the image of the object to be measured. The angles β_1, β_2, β_3 are the angles as read, being those through which the pointer in the ocular apparently moves. The angles γ_1, γ_2, and γ_3 are the true angles corresponding to the first mentioned, and are those through which the pointer in the ocular actually does move. The center of the vernier, *a*, and the center of the protractor, *b*, lie on a line perpendicular to the zero radius of the vernier *a–d*.

In any triangle formed by the intersections on the vernier of the radii of the protractor and the vernier, as *a–c–b*: $180° - (180° - \beta) - \gamma = \alpha$; hence $\beta - \gamma = \alpha$ and

$$\frac{\sin \alpha}{\sin \gamma} = \frac{n}{R}, \quad \text{or} \quad \sin \alpha = \frac{n}{R} \sin \gamma.$$

We may assume that $n = 0.1$ mm. represents the extreme case, for it is hardly conceivable that an error as great as this could be made by any instrument maker likely to be intrusted with the construction of

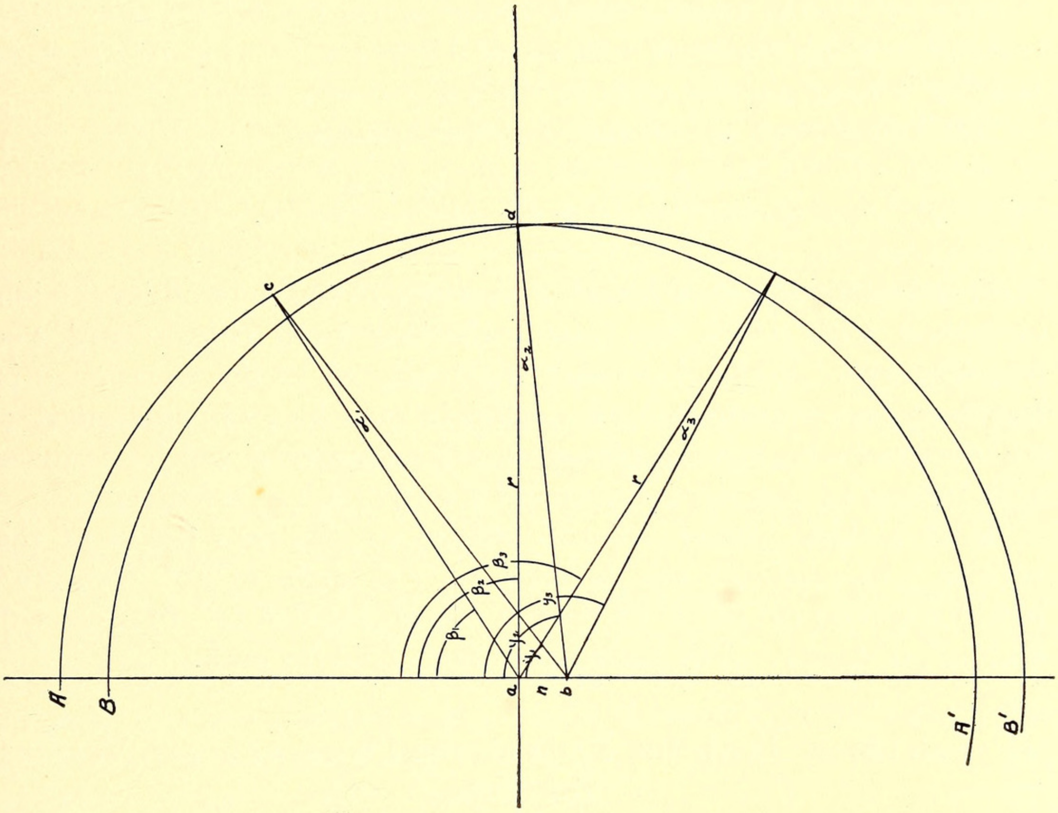

FIG. 2. Construction for errors caused by non-centering of the protractor and vernier.

the micrometer. R will be assumed to be 17.5 cm., which is the radius of the instrument now in use. If the degrees are read clockwise from the line passing through a and b the following relations will be true:

When $\gamma = 0$, $\sin \gamma = 0$, $\sin \alpha = 0$, $\alpha = 0$ and there is no error.

When $\gamma = 90°$, $\sin \gamma = 1$ and $\sin \alpha = n/R$.

When $\gamma = 180°$, $\sin \gamma = 0$, $\sin \alpha = 0$, $\alpha = 0$ and there is no error. It is apparent that the error is a maximum when $\gamma = 90°$, and in that case $\sin \alpha = n/R$, and since $n = 0.1$ mm. and $R = 17.5$ cm., $\alpha_2 =$ a little less than $2'$. That is, the difference between the observed angle and the true angle will be a little less than $2'$ of arc.

Since a $15°$ protractor has been found to have sufficient range for all ordinary purposes, the illustration may be made more applicable to actual conditions if an angle of ten degrees is considered. Suppose this angle to be measured between $80°$ and $90°$ and thus at the point of maximum error. For both $80°$ and $90°$ $\beta > \gamma$, and hence $(\beta_2 - \beta_1) - (\gamma_2 - \gamma_1) =$ the angular error. $\beta_2 = \gamma_2 + \alpha_2 = 90° \, 2'$; $\alpha_1 = (n/R) \cdot \sin 80° = 1° \, 57'$; $\beta_1 = \gamma_1 + \alpha_1 = 80° \, 1' \, 57''$; $\gamma_2 - \gamma_1 = 10°$; $\beta_2 - \beta_1 = 10° \, 3''$. The angular error is, then, $3''$.

The linear error, y, is found from the equation

$$2r \cdot \sin \frac{\beta_2 - \beta_1}{2} - 2r \cdot \sin \frac{\gamma_2 - \gamma_1}{2} = y.$$

With the combination of lenses (No. 2 ocular and 1/12 oil-immersion objective) with which the micrometer has been chiefly used, $r = 0.030885$ mm. When this value is substituted in the equation y is found to be 0.000001 mm., a negligible quantity. Furthermore, y will decrease with increasing magnification because r, which decreases with increas'ng magnification, enters the equation as a factor.

Case 2: Line joining the two centers on the zero radius of the vernier. In principle this is case 1. An angle of $10°$ measured between $0°$ and $10°$ would be the same as an angle of $10°$, between $80°$ and $90°$, o' case 1. The same conclusions hold for both.

II Failure to Have the Radii of the Protractor and Vernier the Same

Only the case in which the vernier radius is longer than that of the protractor need be considered. The reverse would cause the protractor either to bind in turning or to overlap the vernier, and either difficulty would at once be apparent. If the end lines of graduation of vernier and protractor lie on the same straight line and both members are accurately graduated, the angle read will be the true angle regardless of the length of the protractor radius. If they do not lie on the same straight line the error may be detected by inspection.

III. Failure to Graduate Accurately the Vernier and Protractor

Case 1: Spacing on either scale unequal. If the protractor and vernier are used at different points on their scales and the ratio between the length of the spaces on the two scales is not constant, the error is readily detected by the failure of the end lines of the vernier to cover invariably the same distance on the protractor. Suppose the vernier to be so graduated that thirty half-degree spaces on the protractor equal twenty-nine spaces on the vernier. A slight displacement of the twenty-ninth line of the vernier from the prolongation of the thirtieth on the protractor would be evident and hence a variation of even one minute of arc would be immediately perceived.

Case 2: Spaces on the protractor not accurately half-degrees. This error is impossible of easy detection, but it in no way alters the accuracy of the instrument for it is necessary only that each interval be equal to every other interval; their absolute magnitude is a matter of complete indifference. This is true because all the constants used in the calculations depend upon a known magnitude, which is used for the calibration of the instrument.

IV. Failure to Fix the Plane of the Vernier Perpendicular to the Axis of the Microscope

(It is only necessary to consider the vernier, since its position determines that of the protractor.)

Case 1: The angle between any radius drawn on the surface of the vernier-carrier and the axis of the microscope is the same for all radii. This is equivalent to shortening the radius of the protractor, because the common center of both circles is still on the axis of the microscope although in a lower plane. The results of this error are the same as for source II.

Case 2: The angle referred to under case 1 is not the same: the plane of the vernier is tilted from the horizontal. If the tilting is great enough to bring about even 0.5 mm. difference in level between the highest and the lowest portions of the vernier, it may be readily detected by laying a straight edge upon the vernier-arm, when in position flush with the open end of the microscope tube. If the straight edge is then moved over the surface of the vernier-carrier a very slight space between the two will be plainly evident. Suppose, however, that the difference in level is 0.1 cm. The construction for this source of error is shown in figure 3, in which c is assumed lower than b by a distance of 0.1 cm. and b lies in the plane a–b–d which is perpendicular to the axis of the microscope. Since this is the plane in which the protractor should move, the angle a–d–b is the true angle which should be used in all calculations. The angular error is obviously the difference between angle a–d–b and angle a–d–c.

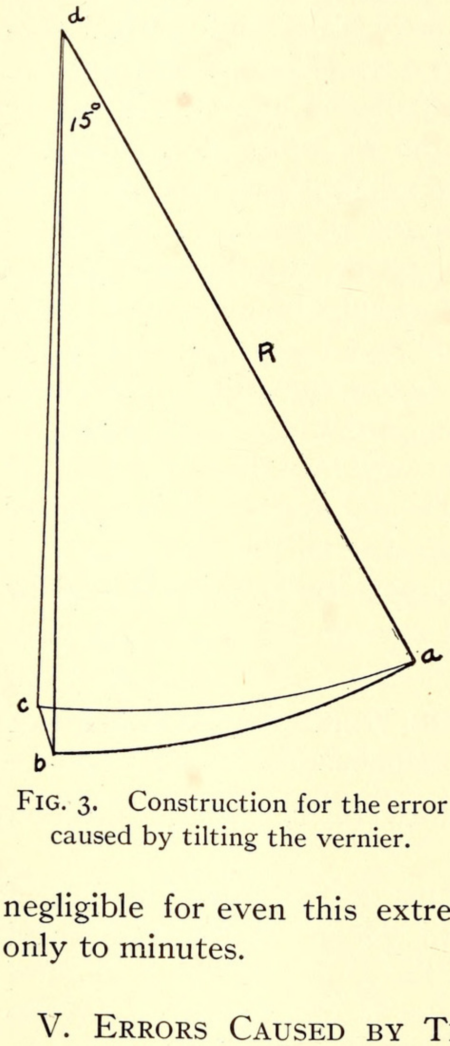

FIG. 3. Construction for the error caused by tilting the vernier.

Let angle a–d–b = 15°, the maximum angle which may be read from the protractor of the micrometer now in use. Let b–c be drawn perpendicular to the plane a–b–d and be equal to 0.1 cm. Let $R = 17.5$ cm. Then $ab = 2R \cdot \sin 7° \, 30' = 4.568$ cm. In the triangle b–a–c the angle a–b–c = 90°. Hence, $bc/ab = \tan b\text{–}a\text{–}c = 0.1/4.568$ cm., and $bac = 1° \, 15' \, 14''$. Since $bc/\sin bac = ac$ and $\sin (adc/2) = ac/2R$, then $adc/2 = 7° \, 30' \, 9''$. It must be concluded that the error is negligible for even this extreme case as the instrument is sensitive only to minutes.

V. Errors Caused by Tilting the Ocular in Its Sleeve

Since the protractor-carrier rides upon the vernier-arm and since each has a broad surface of contact with the other, there would be no tilting of the ocular even if it fitted rather loosely in its sleeve.

VI. Errors Caused by the Lateral Play of the Ocular in Its Sleeve

This may best be detected by setting up the instrument with the tip of the line in the ocular at the edge of some object in the field, and then attempting to move the ocular back and forth in its sleeve by pressure upon the vernier-arm. The microscope should be securely clamped during the process as it is important that the axis of the microscope should not be tilted at the same time. With the ordinary close fit of the oculars of standard makes there is no alteration of the apparent position of the pointer hair.

VII. Errors Caused by Tilting the Microscope Axis

To detect this error, remove the micrometer and close the substage iris-diaphragm until it forms a pin-point aperture and substitute for the ocular the pin-point cap, provided with the microscope. If the eye is now applied to the opening in the cap a beam of light will be perceived if the condenser and the lenses are in alignment as they should be for any detailed microscopic work. If the condenser is not centered, it should be adjusted until a beam of light reaches the eye through the pin-hole aperture. The micrometer should now be attached and the process repeated. If the micrometer tilts the tube the beam of light will no longer be perceived and the micrometer should be counterpoised. This is usually not necessary if the rack-and-pinion coarse adjustment fits as tightly as it should to prevent the tube from gradually sinking during the progress of the measurements and producing focal errors.

For obvious reasons the sources of possible inherent error have been considered somewhat at length, and although each instrument must be examined for error, it must be apparent that, assuming only reasonable skill in instrument construction, the number of actual errors in any instrument is small and that, moreover, they are all easily detected. Since the accuracy of any instrument of precision depends not only upon the theory of its construction and the care and skill with which this theory is realized in the instrument itself, but also upon the care and skill with which it is used, methods for avoiding those errors of practice which are generally known to be the most important may be briefly stated in conclusion.

The calibration of the angular micrometer, as of all ocular micrometers, requires the knowledge of the absolute distance between two fixed points on a suitable slide. The commercial stage micrometers have been found to be almost useless under high magnification, not only because of their variation in spacing but also because of the width and irregularity of their lines. The following method was accordingly devised and has been found to be very satisfactory. These scale errors are significant only at high magnifications, and r may be determined with a good photographed scale on a stage micrometer under low magnification with great accuracy. Having determined r for this magnification, another object should now be substituted for the ruled scale. This object should possess two points that are well defined under both this and a higher magnification. When the distance between these points is determined r may be found for a higher power with which the original ruled scale would be useless. It is thus possible to proceed from lower to higher powers, substituting new objects when necessary until the desired magnification is reached. Although the method is a little tedious, it is no more so than the routine of ordinary accurate mensuration and is much more satisfactory than the use of photographed scales with the higher powers.

The presence of the micrometer of course alters the tube-length and it is universally known that this must be maintained approximately that for which the lenses were ground. Ashe[3] has devised a very easy and accurate method for determining the tube length experimentally. The correct tube length D may be found in millimeters from the equation $(B \cdot C)/(A - B) = D$, in which A represents the number of spaces of a stage micrometer visible in any certain interval of an ocular micrometer when the tube length is D. B represents the number of such spaces visible in the same interval of the ocular micrometer when the tube length is drawn out beyond D by a distance C, expressed in millimeters.

Inaccurate focusing may be the cause of large errors, and it has been found that the weight of the microscope and the micrometer may in the course of half an hour or less, depending upon the frictional resistance of the coarse adjustment, cause such errors in the focus that large variations in measurements result. Because distinctness of image is not a reliable criterion for judging the focus,[4] I have modified a method devised by Cornu and Benoît[5] in such a way that it is

[3] Ashe, A., Note on the determination of "optical tube length." Journ. Quekett Micros. Club, Series 2. 5: 152. 1892.

[4] See: Apathy, Stefan von, Microtechnik. 2te Abt. Leipzig, 1901. 300 pp., and Stephenson, J. W., Observations on Prof. Abbe's Experiments illustrating his theory of microscopic vision. Month. Microsp. Journ. 17: 82–88. 1877.

[5] Cornu, A., and J. R. Benoît, Rapport sur la détermination de l'Étalon provisoire International. Bureau International des Poids et Mesures. Trav. et Mém. 10: 12. 1894.

applicable for use with microscope slides and is useful for very slight errors of focus. Figure 4 illustrates this method. If a beam of light from a radiant point *O* on a plane mirror is directed through the narrowed orifice of an iris diaphragm and strikes the condenser at one side of the center, the light will pass through the microscope along the path *O–A–F*. If directed through the opposite side of the condenser, by moving the diaphragm, the beam will traverse the path *O′–B–F′*. It is evident that if an object is placed on the stage in such a position that the pointer-hair in the ocular apparently touches its edge, a movement of the diaphragm will be followed by a movement of the image past the pointer-tip if the object is out

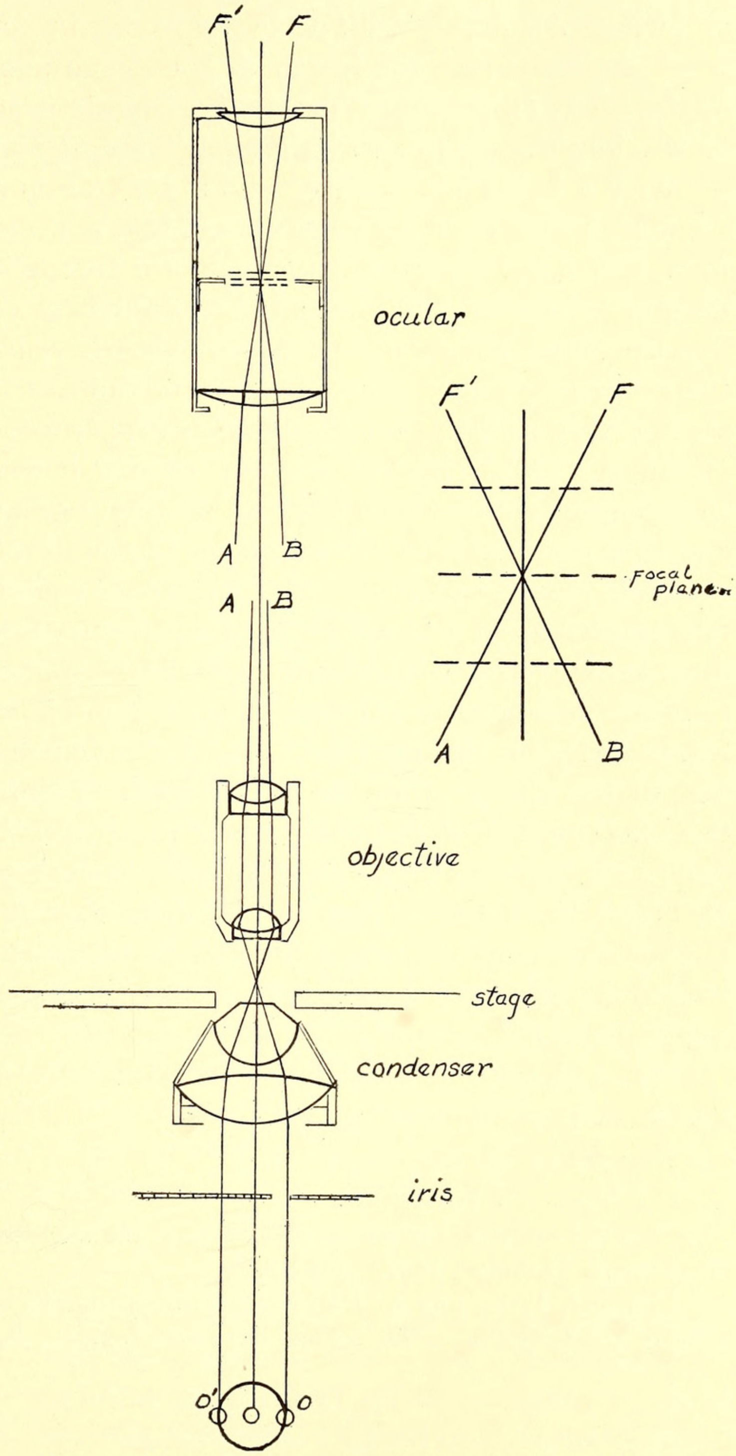

FIG. 4. Method for detecting focal errors. By means of the iris-diaphragm a beam of light is made to pass over either path *O–A–F* or *O′–B–F′*. If the image in the microscope remains stationary the object is in focus.

of focus. An object will, accordingly, be in focus only when there is no movement of the image as the diaphragm is moved back and forth. It is unnecessary, except for completeness, to direct attention to the importance of maintaining a "critical illumination."[6]

With a Leitz No. 2 ocular and a 1/12 oil-immersion objective, the angular micrometer as constructed has a sensitiveness of 0.000009 mm., *i. e.*, a movement of the protractoı through 1′ of arc would move the pointer-tip over the image of an arc whose chord is 0.000009 mm. The use of higher powers increases the sensitiveness as does also placing the pointer-hair near the periphery of the field. Both are obviously unnecessary so far as sensitiveness is concerned.

Farmer and Digby[7] have observed that it was impossible for them to measure chromosomes with an accuracy greater than 0.0001 mm., and this number may be taken as the limit of accuracy of former methods of measurement. Measurements were made with an angular micrometer upon an individual somatic chromosome of Chinese Lily, during anaphase, with about half the magnification of the English authors, and consequent loss of accuracy. They were made at different times on different days, so that it is believed they offer a fair test of the efficiency of the instrument. In nineteen measurements the maximum deviation from their mean was 0.00003 mm. and the difference between the extremes was 0.00006 mm. The maximum variation is therefore about six-tenths that found by Farmer and Digby, and would of course be still further decreased with increased magnification since this would increase the accuracy with which pointings might be made.

It is a pleasure to acknowledge my indebtedness to Dr. C. E. Allen for his help and interest.

University of Wisconsin,
Madison, Wisconsin

[6] See the discussion in some good work on the microscope such as Spitta, E. J., Microscopy. London, 1907. 468 pp.

[7] Farmer, J. B., and L. Digby, On dimensions of chromosomes considered in relation to phylogeny. Phil. Trans. Roy. Soc. London, *B*, **205**: 1–25. 1914.