Simple Microscopical Determinations

By H. A. DADE

Most microscopists are sufficiently interested in their equipment to want to know something about the characteristics of their lenses—a little more than is indicated by the approximate values engraved on lens mounts. Quite a lot of information of this kind can be obtained by simple methods which are not only interesting, but are also valuable exercises because they make us think about the optical conditions in the microscope.

The following abbreviations are used in this paper :—

- OF_2 : second focal plane of objective, sometimes referred to as the upper focal plane.
- EF_1 : first focal plane of the eyepiece. This is in the neighbourhood of the diaphragm in the eyepiece mount, but is rarely exactly in this position.
- EF_2 : second focal plane of the eyepiece, lying very close to the Ramsden disk.
- I_1 : primary image formed by the objective.
- I_2 : final image formed on the projection screen.
- MTL : mechanical tube-length, measured from the seating of the objective to the top of the draw-tube.
- OTL : optical tube-length, measured from the second focal plane of the objective (OF_2) to the primary image (I_1).
- (*S*) : standard value based on the ideal case of direct visual observation by an emmetropic eye.
- (*P*) : personal value determined by a particular person's vision.
- (*A*) : absolute value not affected by individual vision.

Accessories

A few pieces of accessory apparatus are required. Two are easily constructed, others can be borrowed if not in the possession of the operator.

Stage micrometer. Stage micrometers made by reputable firms are likely to be accurately graduated. In case of uncertainty (for faulty scales do exist), it is as well to check by comparison with two or three other micrometers.

Micrometer eyepiece. For the present purpose, this should be of Ramsden type, not Huyghenian, in order that direct measurements of the primary image may be made. The position of the micrometer scale in the microscope axis is fixed and can be checked ; the primary image can thus be accurately located. Means of focusing the lens on the scale are usually provided, but this does not (or should not) affect the seating of the eyepiece on the top of the draw-tube.

The position of the eyepiece scale can easily be found. Mark a slide on one side with a grease pencil or by means of coarse emery cloth—only a few scratches are required. Diamond scratches often have considerable depth, and if a writing diamond is used to mark the slide it should be applied gently, making as shallow a scratch as possible. Lay the slide, marked side downwards, on the top of the draw-tube after taking out the eyepiece.

Hold a pocket lens above the slide, focused on the marks. Focus the microscope in the usual way until the image of an object on the stage (use the stage micrometer) is sharply in focus in the same plane as the marks on the slide. The primary image will now be exactly level with the top of the tube. With a metal rule, measure accurately the distance between the top of the body tube and the draw-tube flange. See Fig. 1. This is better than relying on the draw-tube graduations, for it is often difficult to estimate fractions of a millimetre in that way. Now take away the slide and insert the micrometer eyepiece. Pull out the draw-tube, taking care not to disturb focus, until the image of the object is sharply defined in the same plane as the eyepiece scale. Measure the extent to which the tube has been pulled out. This figure will give the distance of the eyepiece scale below the eyepiece flange. Another method may be used. After taking away the marked slide, insert the micrometer eyepiece and then lift it between the fingers until the object image is in focus in the plane of the scale. With a scriber or other steel point, mark the eyepiece mount, resting the point on the rim of the draw-tube.

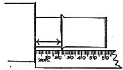

Fig. 1. Measurement of changes in tube-length, using a metric rule. The distance to be measured is indicated.

If a Ramsden eyepiece cannot be found or borrowed, a Huyghenian can be used if the field lens is removed, but then the image formed is so distorted and curved that only a very restricted portion of the middle of the eyepiece scale can be used, which is very unsatisfactory and results in inaccuracy.

Both stage and eyepiece scales should, of course, be graduated in metric.

Rule. A small, accurately graduated, metal rule is useful in locating the eyepiece scale and in measuring changes in MTL in the operations described below.

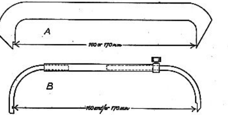

Fig. 2. A: a simple form of MTL gauge made from stout sheet metal; B: gauge made from brass rod and tubing, adjustable for both standards.

MTL gauge. This is a convenient and almost indispensable tool for setting MTL to its standard value (160 mm.; 170 mm. for Leitz objectives). It is easily made from metal, the simplest form being shown in Fig. 2.

Projection screen. A great deal of nuisance is avoided by using a device in which the projection distance of 250 mm. is permanently and accurately established, the whole being movable to permit its readjustment to the Ramsden disk (which is virtually coincident with EF_1) after refocusing. The diagram in Fig. 3 shows a simple form of the apparatus made from plywood. If desired, it can be made more rigidly from metal. The diagram is self-explanatory. The spring clip holds a microscope slide or slip of perspex, one side of which has been matted with emery cloth. The matted side is placed facing the microscope. This is used to locate the Ramsden disk, and the screen is in the correct position when the beam emerging from the eyepiece forms the smallest and brightest spot on the matted surface. The slide is then turned aside to allow the rays to reach the screen. The screen surface is prepared by smoothing the wood with fine glasspaper and then coating it with matt white (poster colour or 'process white'). The scale is cut from accurate metric section paper, and is attached with rubber adhesive ('Cow Gum'), which does not cause stretching and subsequent shrinkage as do water-soluble adhesives.

All readings of scales and levels should be repeated three times or more, refocusing, and the mean values used in calculations.

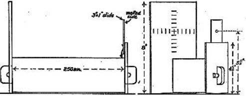

Fig. 3. Two aspects of the projection screen, showing the important dimensions.

General Principles

Most of the determinations to be described are made with the aid of a projected image, and perhaps we should first consider why this is the case. When we show a layman friend some object under the microscope, we are nearly always asked: 'How much is that magnified?' We give the answer in round figures, e.g.: 'About one hundred times linear', with perhaps some further explanation about diameters and areas. This rough idea of magnification is good enough for all ordinary purposes, for we, as microscopists, are more interested in resolution than in mere magnification. Our friend, too, is satisfied. What does our answer mean to him? He takes it to mean that the object appears 100 times larger, in breadth and length, than it would if he were to examine it with his unaided eye. Now the unaided eye examination implies the closest possible examination, i.e. with the object held at the minimum distance of clear vision, which is in the neighbourhood of 250 mm. Our friend accepts this automatically, without thinking about it.

This is what we mean by 'magnification at the eye', or 'combined magnification'—the combined result of magnification by the objective and the eyepiece. We can obtain the round figure result by multiplying the intial magnification of the objective, often engraved on its mount, by the power of the eyepiece, also usually engraved on the mount. This round figure result is of course only an approximation, for the engraved figures are not exact, and there may also be variations in the optical conditions in the microscope. The second factor, the power of the eyepiece, is found by dividing its focal length in mm. into 250, an accepted formula arbitrarily based on the minimum distance of clear vision. On the bench, we can find the power and focal length of a lens by measuring the magnification in an image projected at a distance of 250 mm. from the second focal plane of the lens. When we look into the microscope, we apply our eye to the second focal point of the eyepiece (the Ramsden disk). If instead of doing this we project an image on to a screen at 250 mm. from the Ramsden disk, we can measure the magnifying power of the microscope, obtaining a figure comparable with naked eye examination at the minimum distance of clear vision. In doing this experiment, however, we must make certain adjustments to compensate for the difference in optical conditions in projection and direct visual observation.

We must also adopt a standard set of conditions, which will give the same result no matter who carries out the determinations. But we can also make *personal* determinations which measure more accurately the experience of each separate observer, and here there is a range of variation, for, as we know from experience, we have to refocus to suit our own eyes when we look into a microscope previously adjusted by another. These differences, ranging between the effects of marked long- and short-sighted vision, are of no great importance in practice. But when we are making measurements of lens characteristics they become significant, and if not understood and corrected they will vitiate results and make accurate comparisons of lenses impossible.

All personal estimates based on direct observation must be regarded as of temporary value, for vision changes with age, and the correction applied by spectacles is correspondingly variable.

The effect of differences in vision on the optical conditions in the microscope has been described in a previous paper (Dade, 1955), an illustration from which is reprinted here as Fig. 4. The effect is to cause the primary image to be formed in different positions in the axis, thus causing variations in OTL and therefore in the magnification of the primary image (MTL being constant).

A microscope objective is corrected to give its best image at a specific value of OTL, and it is mounted so that this OTL is established when the MTL is set to standard, and when the observer's vision is ideal or emmetropic. (A further condition is that the cover-glass should be of specific thickness, but that will not affect our standard routine of determination, and we can assume that this condition is satisfied. Corrections of MTL or adjustment of objective collars to compensate for non-standard cover-glass thickness will of course affect the position and magnification of the primary image, and a series of determinations for different values of MTL and collar readings can be made if desired; but our aim is primarily to secure data which apply to one standard set of conditions, and therefore serve to give us absolute information about our lenses, and to enable us to compare lenses.) When these conditions are satisfied, the primary image will lie in the first focal plane of the eyepiece (EF_1) and the rays emerging from the eyepiece will be parallel (Fig. 4, A).

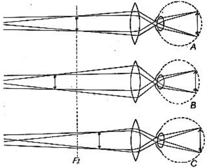

Fig. 4. The two images formed by the microscope and the eye. The eyepiece is shown diagrammatically as a single lens; F_1 is the first focal plane. A: the ideal case, eye emmetropic, primary image in F_1, rays emerging from eyepiece parallel; B: hypertropic eye, primary image below F_1, emergent rays converging; C: myopic eye, primary image above F_1, emergent rays diverging.

The logical basis for our determinations is provided by these conditions. Let us suppose that we are setting out to establish this standard case. We have set up the microscope with MTL standard. We do not know what the correct OTL for the test objective should be, and we do not know the precise location of the second focal plane of the objective (OF_2) from which the OTL is measured. Therefore we do not know where the primary image (I_1) should lie. We can get no help from the eyepiece, for its diaphragm is by no means certain to correspond exactly with its first focal plane, and if it is Huyghenian its image plane of focus will not correspond with the true position of I_1 on account of the effect of the field lens, which throws the image back and reduces its magnification. The chances are heavily against the observer having ideal emmetropic vision, as he cannot automatically establish the required conditions by merely focusing the microscope in the ordinary way.

We shall therefore have to find the lacking data by indirect means. The simplest approach is through the eyepiece. We can locate its first focal plane if we know its power and focal length, and we can find these by projection if we also know the dimensions of the primary image when the latter is in any arbitrary position. This arbitrary position can conveniently be that which it occupies when we use the microscope in the usual way.

This preliminary determination will tell us what the initial magnification is in the case of the observer who conducts the operation, so we can call it:

(1) *Determination of Initial Magnification (P)*

Set up the microscope for projection (Fig. 5) in the horizontal position, MTL standard, stage micrometer on the stage, working objective and working eyepiece installed. Focus carefully by direct observation. The

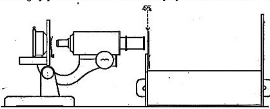

Fig. 5. The microscope set up for projection.

primary image is thus in its normal position for the particular observer, and this is not to be disturbed in subsequent adjustments. Take out the working eyepiece and put on the Ramsden micrometer eyepiece. Refocus by means of the draw-tube, leaving the focussing heads undisturbed. Compare the two scales: find the number of stage divisions (each 0·1 mm.). The initial magnification then equals

$$\frac{10 \times \text{number of stage divisions}}{\text{number of eyepiece divisions}},$$

$$\text{e.g. } \frac{10 \times 49{\cdot}4}{50} = \times 9{\cdot}9 \text{ d. } (P).$$

(2) *Determination of Power of Working Eyepiece (A)*

Still maintaining the position of the primary image, remove the micrometer eyepiece and replace the working eyepiece. Set up the projection screen, and project the image of the stage micrometer scale on to the screen, focusing by means of the draw-tube. After preliminary focusing, readjust the screen to the Ramsden disk by means of the matted slide, and then perfect focus. Compare the screen scale with the stage micrometer scale image. Find the number of stage divisions (each 0·1 mm.) with a whole number in tens, say 50, of the screen scale (each 1 mm.). The magnification on the screen will then be given by

$$\frac{100 \times \text{number of stage divisions}}{\text{number of screen divisions}}, \quad \text{e.g. } \frac{100 \times 52}{50} = \times 104 \text{ d.}$$

The power of the eyepiece is then given by dividing this figure by the initial magnification previously found,

$$\text{e.g. } \frac{104}{9\cdot 9} = 10\cdot 5 \ (A).$$

(3) *Determination of Focal Length of Eyepiece (A)*

This is found by dividing 250 mm. by the power just determined.

$$\text{e.g. } f = \frac{250}{10\cdot 5} \text{ mm.} = 23\cdot 8 \text{ mm. } (A).$$

The optical conditions in projection are those shown in Fig. 6. The eyepiece is shown diagrammatically as a single lens. From the basic optical formula we know that $u = v/M$, when M is the magnification of the image. In the present case M is the power of the eyepiece. We know f, the focal length of the eyepiece, and v, which is 250 mm. plus f. Thus we can write:

$$u = \frac{v}{M} = \frac{250 + f}{\text{power of eyepiece}},$$

$$x = u - f = \frac{250 + f}{\text{power}} - f \text{ mm.}$$

Using the example results from previous determinations, we can write

$$x = \frac{250 + 23\cdot 8}{10\cdot 5} - 23\cdot 8 = \frac{273\cdot 8}{10\cdot 5} - 23\cdot 8 = 26\cdot 1 - 23\cdot 8 = 2\cdot 3 \text{ mm.}$$

We are now able to set up the conditions of the ideal case and find the standard combined magnification.

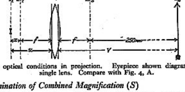

Fig. 6. The optical conditions in projection. Eyepiece shown diagrammatically as a single lens. Compare with Fig. 4, A.

(4) *Determination of Combined Magnification (S)*

Readjust the microscope: restore standard MTL, with working eyepiece in the tube, and project the image of the stage scale on to the screen by means of the *focusing heads*, keeping MTL standard.

I_1 is now x mm. behind EF_1, as in Fig. 6. If the microscope were in use for direct observation by an ideal eye, I_1 would be in the same plane as EF_1 (Fig. 4, A). If we now extend the draw-tube by x mm. (using the accurate rule), and then refocus the image on the screen, using the focusing heads, we shall bring the primary image to its *standard* position, with the OTL that for which the lens is designed. The magnification of the projected image can now be found by comparing the stage and screen scales, as in (2) above; this is the standard combined magnification.

(5) *Location of the Primary Image* (*S*)

Without disturbing the adjustment of the microscope, put on the eyepiece micrometer. Check the extension of the draw-tube with the metric rule. By direct observation, focus the eyepiece on I_1 by adjusting the draw-tube, taking care as usual to avoid disturbing the focusing heads. Measure the draw-tube extension again, and note the difference. The position of the scale in the eyepiece has been marked or measured from the eyepiece flange (see under 'Apparatus'). Deduct from, or add to, this measurement the change in draw-tube extension made by focusing. If the draw-tube was pulled out, deduct; if pushed in, add. The result is the distance of I_1 from the top of the draw-tube, in the standard conditions. Leave the microscope undisturbed for the next determination.

(6) *Determination of Initial Magnification of the Objective* (*S*)

We can find this by two methods, thus checking our observations.

(i) By direct inspection of the primary image as in (1), the microscope being adjusted as at the end of the preceding determination (5).

(ii) By dividing the standard combined magnification by the power of the eyepiece.

(Coles (1921, p. 80) describes a method of projection at a distance of 10 in. from the objective, no eyepiece being on the microscope. This method will give an approximation to the *power* of the objective, but not to its initial magnification.)

(7) *Determination of Combined Magnification* (*P*)

Since a rough idea of 'magnification at the eye' is usually all that we need as a guide to working conditions (as distinct from the absolute and standard figures wanted in comparing lenses), the determination of personal combined magnification is of little practical value, but it has some theoretical interest. It can be found as follows:

With standard MTL, focus the microscope by direct observation, using the focusing heads. This will establish I_1 in its *P* position. Combined magnification may now be found by projection, as in (4) above, refocusing by means of the draw-tube. But a new factor is now involved. If the observer's vision is not emmetropic, or if his spectacles do not compensate exactly for his variation from emmetropic vision, then his normal minimum distance of clear vision will not be 250 mm. and may be considerably more or less. Projection at 250 mm. would not, therefore, give a fair comparison with his visual experience without the aid of the microscope. It will therefore be necessary to find the observer's minimum distance when he is wearing his spectacles (if he uses reading glasses). If the observer's vision is myopic or hypermetropic only, without other complications, he will probably take off his glasses when he works at the microscope, and in that case he should not use them when focusing for this determination. The combined magnification result is not affected, for the purpose of the determination is to compare two kinds of experience, i.e. examination of an object without the microscope, but with spectacles if these are normally used, and with the microscope with or without spectacles according to normal practice.

(8) *Determination of ' Constant ' of Eyepiece* (*P*)

This is sometimes put forward as a useful method of easy determination of combined magnification when a different objective is used with the same working eyepiece. Like other personal data, however, the ' constant ' has only temporary value; it is based on two personal observations, both of them changing with age.

The determination is made with standard MTL. Measure the diameter of the field in terms of stage micrometer divisions, the working eyepiece of course being on the microscope. Multiply by the combined magnification (*P*). The result is the ' constant '. Now, when the same eyepiece is used with another objective, the new combined magnification can be estimated by again measuring the diameter of the field and dividing this into the ' constant '.

(9) *Determination of Focal Length of Objective* (*A*)

The focal length of the objective is found by direct observation, not projection, so the microscope can be set up in the vertical position. The micrometer eyepiece is put on. Pull out the draw-tube as far as possible, and measure the extension with the metric rule. Focus with the focusing heads. Find the magnification of the primary image by inspection as in (1). Now push in the draw-tube as far as it will go, and again find the initial magnification in the same way as before. Divide the difference in mm. between the two tube-lengths by the difference between the two magnifications. The result will be the focal length of the objective in mm.

(10) *Determination of Optical Tube-Length* (*S*)

Multiply the initial magnification (*S*), as found in (6) above, by the focal length of the objective as found in (9) above. The result is the OTL (*S*).

(11) *Location of the Second Focal Plane of the Objective* (*A*)

Refer to Fig. 7. Add the OTL(*S*) to the distance of I_1 below the top of the draw-tube. (The latter distance was found in (5) above.) From this sum subtract the MTL (*S*). The result gives the distance of OF_2 below the seating of the objective mount. (In most objectives the OF_2 is situated, not above the lens, but between the component lenses.)

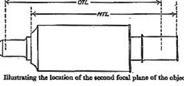

Fig. 7. Illustrating the location of the second focal plane of the objective.

(12) *Determination of Numerical Aperture without an Apertometer (A)*

Nelson (1896–7) suggested the following method: with no eyepiece on the microscope, project with the objective alone an image of the stage micrometer on to a screen at a convenient distance. Measure the magnification of the image, the projection distance (from OF_2, which we have found) and the diameter of the back lens of the objective.

$$\text{Then N.A.} = \frac{\text{magnification} \times \text{semi-diameter}}{\text{projection distance}}.$$

Nelson gives the proof of the formula, and it can also be found in Spitta (1920), p. 83, equation III.

This method is satisfactory if we can reach and measure the back lens of the objective; but this is not always possible, and then the following version of the method can be employed. The formula can be re-stated as:

$$\text{N.A.} = \frac{\text{Initial magnification } (S) \times 1/2 \text{ diam. of aperture}}{\text{OTL}}.$$

We have already determined initial magnification and OTL in (6) and (10) above, and have only to measure the aperture of the objective. This is easily done by converting the draw-tube into a measuring microscope, and this should be set up and calibrated first of all. Strip all lenses from the tube, remove the draw-tube, and screw a low-power objective (f=50 mm. or thereabouts) into the nose of the draw-tube. Return the draw-tube to its place and put on the micrometer eyepiece. Put the stage micrometer on the stage. Now focus, and by direct comparison calibrate the eyepiece scale.

Next, dismantle the draw-tube and its lenses. Put on the test objective, the working eyepiece, standard MTL; put a suitable object on the stage. Carefully and critically adjust the illumination, focusing the substage condenser, and setting the substage iris to its best aperture for critical work. These adjustments, and the focusing heads, must be undisturbed during the remainder of the work.

Take out the draw-tube and reassemble the measuring microscope. With this, focus on the back lens of the test objective. Measure both the working aperture (the bright disk in the centre of the back lens) and the full diameter of the back lens. Use these figures in the formula given above, working out values of N.A. for both conditions. The working N.A. is of course the significant value. Compare the results with the N.A. engraved on the lens mount.

(The value thus obtained for the working aperture does not, of course, take into account the effect on resolution of scattered rays which may pass through the periphery of the lens, a matter which has been discussed elsewhere (Dade, 1953).)

Another method is that of Conrady, described by Spitta (1920, p. 97), which tells us only the full, not the working, value. Place two pieces of white paper on the table, with their straight, parallel inner edges 200 mm.

apart. Hold a ruler vertically and touching the table midway between the papers. Hold the test objective against the edge of the rule and slide it up and down the rule while inspecting the back lens, in which the images of the papers can be seen. Note the position of the front lens when the images of the papers *just* disappear at the margins of the back lens. From this distance (between table and front lens), subtract the working distance, and call the result *b*. (The working distance can be found by measuring the space between the front lens and a slide when the microscope is focused.) Divide *b* by half the distance between the papers (100 mm.) The result is the tangent of the semi-angle of aperture. From mathematical tables, find the corresponding angle ; then turn to the table of sines and find the sine of this angle, which gives the N.A. with close accuracy.

(13) *Calibration of Substage Condenser (S)*

Some condenser mounts are provided with a scale, the indicator being the iris lever. If there is no scale, one can be added, graduated arbitrarily, or in degrees, or in terms of the diameter of the iris opening. By taking a series of readings of working aperture, as in the first method in (12) above, the performance of the condenser can be assessed, and the working aperture of any objective can be found by consulting the iris scale. The position of the lamp should be fixed; it should be placed 250 mm. from the iris of the condenser, this being the sum of the lamp-mirror and mirror-iris distances.

Record of Results

Tabulated data for all lenses and combinations of lenses are very useful for reference.

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