ART. IV.—*Microscope Magnification;* by W. LECONTE STEVENS.

WHEN a lens is interposed as magnifier between the eye and an object, it produces a virtual image of this, the accommodation of the eye being so adjusted as to relax the ciliary muscle and thus secure the most comfortable vision. For normal eyes this occurs when the entering rays are parallel, rather than when the accommodation is for the conventional near-point of distinct vision. The position of the virtual image is hence indeterminate; but by common consent it has been generally agreed to consider its distance on the axial line to be 10 inches, or 254 millimeters, from the optical center of the lens.

It can be easily shown that, if the lens and object be fixed, the increase of visual angle produced is a maximum when the eye is closest to the lens. It is never possible to measure accurately the distance from the optical center of the lens to that of the refracting combination composing the observer's eye. In theoretical calculations an allowance should be made for it; practically it is regarded as zero.

By some authors a distinction is made between the terms "magnification" and "amplification," and still further between "relative," "comparative," and "absolute" amplifying power.* Whatever may be the value of these distinctions in theory the writer can find no good reason for discarding the familiar term, magnification, to denote the ratio of the diameters of the retinal images produced with and without the magnifying lens, or system of lenses, respectively. The conditions under which the magnifying system is employed are to some extent arbitrary.

To compute the magnification given by a microscope it is necessary to multiply together the separate magnifications due to the eye-piece and objective employed. Unfortunately the nomenclature of eye-pieces and objectives is still far from satisfactory; and it would perhaps be safe to say that the majority of persons who employ them are unable, under existing limitations, to do more than accept certain labels and use these in calculation. But the labels are misleading. To call an eye-piece "shallow" or "deep," or to name it an A, B, or C eye-piece, affords no definite idea of its power. Such arbitrary and useless designations deserve to be abolished. An eye-piece should be labeled with its equivalent focal length like an objective; and in each case the label should be accurate to within one millimeter. This method of labeling eye-pieces was recommended several years ago by the American Society of Microscopists, but thus far there has been very little compliance on the part of manufacturers. Tables of magnification are given by certain firms for combinations of objectives with eye-pieces as sold by them; but the purchaser has to take these figures on trust. They are professedly applicable only when "standard tube-length" is employed. Such a standard exists only in name and not in fact. In 1887 Professor S. H. Gage, of Cornell University, applied to all of the prominent makers of microscopes in the world for information as to the tube-length for which their objectives were corrected, enclosing to each a diagram upon which should be marked those points on the microscope body which were taken as the limits of tube-length. From eighteen of these firms, including the majority of those addressed, satisfactory answers were obtained. Among the lengths given, the following in millimeters may be taken as examples: 125, 146, 150, 160, 165, 180, 190, 200, 203, 216, 220, 228, 250, 254. The last of these numbers occurs most frequently, corresponding to 10 inches. Examination of the diagrams revealed equal diversity in regard to the points taken as the limits of tube length. In one case it was from the upper surface of the eye lens to the lower extremity of the objective; in another, from

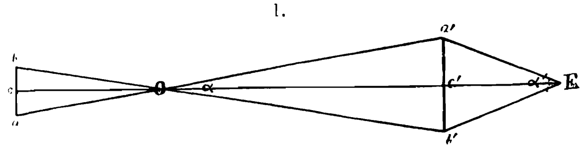
* L. Didelot, "Du Pouvoir amplifiant du microscope," Paris, 1887.

the upper surface of the field lens to that of the topmost lens of the objective.

The present writer had occasion, some time since, to purchase a binocular microscope, with several objectives and eyepieces, for which a table of magnification was furnished. Examination of this table showed that the magnification was calculated by dividing 100 by the product of what were called the focal lengths of objective and eye-piece, expressed in inches. On inquiry of the dealer this rule was found to be the one he had employed, and it was said to be in common use. Its results were admitted to be only approximate, but it was supposed to be near enough to the truth for most practical purposes.

It has seemed desirable, therefore, to test this rule, and in so doing to search out a few points that may possibly be of interest to those who use the microscope as a physical instrument. Its deduction is very simple. Let the object, ab, be

1.



focalized by the objective, O, at $a'b'$. Oc is taken as the focal length of the objective, and Oc' as the tube-length, 10 inches. If m be the magnifying power of the objective alone, we have,

$$m = \frac{a'b'}{ab} = \frac{10}{f}.$$

The visual angle, α, subtended at O by $a'b'$ is the same as that subtended by ab, if an eye placed at O were capable of sufficient accommodation to secure distinct vision at so short a distance. The image, $a'b'$, is viewed with an eye-piece, which increases the visual angle from α to α', producing a virtual image which is assumed to be 10 inches away. If m' be the magnifying power of the eye-piece whose focal length is f', we have, approximately,

$$m' = \frac{\tan\frac{1}{2}\alpha'}{\tan\frac{1}{2}\alpha} = \frac{10}{f'}.$$

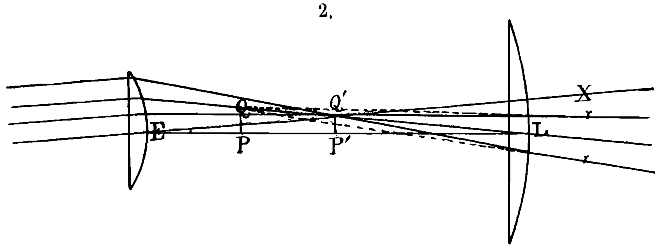
If M be the total magnification, the result therefore is

$$\mathrm{M} = mm' = \frac{100}{ff'}. \qquad . \quad . \quad . \qquad (1).$$

In applying this formula, if previous measurements have not been made upon the lenses composing the eye-piece, a difficulty arises in regard to the value to be assigned f', since eye-pieces ordinarily have no labels more intelligible than A, B, or C, which numerically mean nothing. If a positive eye-piece be employed, the focal length of its two lenses being equal, the equivalent focal length of the combination is obtained by the usual formula, if that of either of the two lenses, and the interval between their optical centers, be measured. In case the eye-piece be negative, a majority of those in use belonging to this class, the focal length of its eye lens is easily found by allowing for its thickness and measuring down to the diaphragm where the real image is formed. But the size of this image has been decreased, and its position has been changed by the interposition of the field lens. At the risk, therefore, of giving what seems very elementary, it may be well to consider briefly the theory of the negative eye-piece.

We may assume the proportions usually said to be adopted in the construction of the negative eye-piece, that the focal length of the field lens is three times that of the eye lens, and the interval between these equal to the difference of their focal lengths. The rays, $r\,r$, fig. 2, converging from the objective toward the point, Q, have their convergence in-

2.



creased by the field lens, so as to cross at Q′. They are made parallel by the eye-lens, and emerge so as to produce a virtual image which to the receiving eye appears in the direction E X. Hence Q′ is in the principal focal plane of the eye lens, and Q in one conjugate focal plane of the objective.

Let EP′ $= f'$ = focal length of eye lens.
" $3f' = f''$ = " field lens.
" LP $= p$ = distance of virtual point of radiance.
" LP′ $= p'$ = " actual " convergence.

Then, by the fundamental law of lenses,

$$\frac{1}{p'} - \frac{1}{p} = \frac{1}{f''}.$$

Since E L $=2f'$, and E P′ $=f'$, we have $p'=f'$. Hence,

$$\frac{1}{f'} - \frac{1}{p} = \frac{1}{3f'} \quad \therefore p = \frac{3}{2}f'$$

$$\therefore \text{PQ} = \frac{3}{2}\text{P}'\text{Q}'.$$

The focal plane of the objective is hence midway between the eye lens and its focal plane; and the diameter of the image actually viewed with this lens is two-thirds of that which would have been formed if the field lens had been absent. If we assume $\frac{10}{f}$ as the magnifying power of the objective when no field lens is used, the interposition of this lens reduces it to $\frac{2}{3}\cdot\frac{10}{f}$. This reduction of magnification is more than offset by the well known advantages which the field lens confers. Introducing the proper correction in formula (1), this becomes

$$\text{M} = \frac{2}{3}\frac{100}{ff'}. \quad . \quad . \quad . \quad . \quad . \qquad (2).$$

Formula (2) implies a knowledge of the focal length of the objective and of only the eye lens. To find the equivalent focal length of the eye-piece combination, let F stand for this length, f' and f'' for those of eye lens and field lens respectively, and d for the interval between these lenses. Then the usual formula for the combination is

$$\frac{1}{\text{F}} = \frac{1}{f'} + \frac{1}{f''} - \frac{d}{f'f''}. \quad . \quad . \quad . \quad . \qquad (3).$$

In this case $f''=3f'$ and $d=2f'$. Substituting, we have

$$f' = \frac{2}{3}\text{F}. \quad . \quad . \quad . \quad . \quad . \qquad (4).$$

Introducing this value of f' in formula (2), the result is

$$\text{M} = \frac{100}{\text{F}f}. \quad . \quad . \quad . \quad . \quad . \qquad (5).$$

The value of f is labeled on the mounting of the objective, and that of F is easily obtained by applying formula (4), f' being found without calculation, as suggested above, if great accuracy is not required.

In formula (5), 100 is the product of two factors. One of them is the assumed distance at which distinct vision with the unaided eye is most easily attained. It may be taken as 250 millimeters, which is very nearly 10 inches. The other is the distance from the focal plane of the objective to what we may provisionally call its optical center. If we make this last distance our definition of tube length, use for it the symbol T, and let D stand for the distance of distinct vision, our formula becomes,

$$M = \frac{DT}{Ff} \quad . \quad . \quad . \quad . \qquad (6)$$

It remains now to be seen what modifications need to be imposed upon formula (6), since formula (1), from which it is developed, is confessedly only approximate. Its second member should be equal to the product of the magnifying powers, m and m', of objective and eyepiece respectively, as determined by experiment.

Assuming that the eye-piece has been constructed in accordance with the conditions implied in the formula, F is to be determined from f'', which in turn can be measured with but little error by use of the camera lucida. Let an eyepiece micrometer be placed at the diaphragm and properly illuminated, the microscope body being so tilted that the optical center of the eye lens shall be 250^{mm} above the white paper on the table. With the camera lucida the divisions of the micrometer are projected on the paper, and the magnification, m', is directly determined. To find f', since the image is virtual, the value of m' is substituted in the formula,

$$m' = \frac{D}{f'} + 1 \quad . \quad . \quad . \quad . \qquad (7)$$

This method may be checked by detaching the eye lens and testing it independently by Cross's formula, to be presently given.

The value of f, the focal length of the objective, cannot be determined by ordinary methods because the microscope objective usually consists of two or more systems of lenses, each made up of a crown and a flint; and the error involved in measuring the thickness of each of these separately and also their distance apart is so considerable as to make the final result very uncertain. The best formula to apply is that deduced some years ago by Prof. C. R. Cross.* This formula is of such importance that its deduction and application are best given in this connection.

Let the field lens of the eye-piece be removed, and two micrometer scales be employed, one of which, divided into 10ths of a millimeter, is placed on the stage as an object, while the other, divided into millimeters, is placed at the diaphragm in the focal plane of the eye lens. The image of the stage micrometer is focused upon the eye-piece micrometer, and the comparison of these images gives the magnifying power, m, of the objective at the distance selected. Assuming provisionally an optical center for the objective under the given conditions,

* Journal of the Franklin Institute, vol. lix, p. 401.

Let p = distance of stage micrometer from this optical center.
Let p' = " eye-piece " " "

Then, $m = \frac{p'}{p}$, or $p = \frac{p'}{m}$ . . . (8)

It is impossible to measure either p or p' directly, but we can measure the distance between the two micrometer scales, which is equal to their sum. Calling this l, we have,

$$l = p + p', \text{ or } p = l - p' \quad . \quad . \quad . \quad (9)$$

Eliminating p between equations (8) and (9),

$$p' = \frac{ml}{m+1} \quad . \quad . \quad . \quad . \quad (10)$$

From the equation of lenses,

$$\frac{1}{p} + \frac{1}{p'} = \frac{1}{f}, \text{ we have } f(p+p') = pp' \quad . \quad . \quad (11)$$

Substituting in equation (11) from equations (9) and (10), and reducing, the result is

$$f = \frac{ml}{(m+1)^2} \quad . \quad . \quad . \quad . \quad (12)$$

Since this formula is independent of p and p', it may be applied without any knowledge of the optical center of either a single lens or a system of lenses.

The eye-pieces of the microscope to which reference has been already made are devoid of labels, although the instrument is a fine one, and the maker was one of the best known in America, a careful and intelligent German, now dead. They have been subjected to measurement, with the result given in Table I. The two eye-pieces labeled in the table A_1 and A_2 were evidently intended to be, in ordinary nomenclature, 2-inch eye-pieces; those labeled B_1 and B_2, $1\frac{1}{2}$-inch eye-pieces, and the one labeled C, a $\frac{3}{4}$-inch eye-piece. All measurements of length were made in millimeters.

TABLE I.

1.	2. f'	3. f''	4. d	5. F	6. $\frac{3}{2}f'$	7. F'	8. $e\%$	9. m'
A_1	35·5	58·8	54·0	51·8	53·2	2·07	−3·4	5·82
A_2	34·9	58·1	54·0	52·0	52·3	2·08	−3·9	5·82
B_1	21·3	40·0	38·0	36·6	31·9	1·46	+2·7	7·83
B_2	21·1	40·9	38·0	36·0	31·6	1·44	+4·2	7·94
C	13·6	28·7	23·0	20·2	20·4	·81	−7·4	13·38

On comparison of columns 2, 3, and 4, it is seen that in no case is $f'' = 3f'$, or $d = 2f'$, as generally assumed in relation to the negative eye-piece. To multiply the focal length of the eye-lens by $\frac{3}{2}$ does not therefore give the equivalent focal length of the combination. The approximation, as shown in colums 5 and 6 is moderately good in eye-pieces A_2 and C, but by no means so in B_1 and B_2. In column 5 the value of F was computed by formula (3), and the results translated into inches for column 7. Column 8 shows the percentage of error in the nominal equivalent focal lengths of the eye-pieces, and column 9 shows their actual magnifying power. Each of the data of columns 2 and 3 is the mean of five independent measurements; but the results in column 9 are affected with a probable error greater than what should be expected if F could have been obtained directly from f'' alone.

The maker of an eye-piece ought certainly to know how to test his work after it is finished. He has the right to use any formula in construction that experience has shown to be valuable. But in every case the value of F ought to be determined accurately by him, and labeled on the mounting of the eye-piece, not in whole inches or aliquot parts of an inch, but in decimal parts of an inch, or, still better, in millimeters. The scientific world is familiar enough with the metric system to warrant the abolition of other systems, at least in the construction of all new instruments.

TABLE II.

1.	2.	3.	4.	5.	6.
	l	*m*	*f. mm.*	*f. inches.*	*e%*
W. 3	315	3·93	51·0	2·01	+50
B. 2	303	6·25	36·1	1·42	+41
R. $1\frac{1}{2}$	300	7·10	32·4	1·28	+17
B 1	293	9·33	25·6	1·01	−1
B. $\frac{3}{4}$	284	13·50	18·2	·716	+5
W. $\frac{3}{4}$	296	14·00	18·4	·724	+4
B. $\frac{1}{2}$	288	18·80	13·5	·531	−6
C. $\frac{1}{4}$	290	55·00	5·1	·202	+24
B. $\frac{1}{6}$	283	58·00	4·7	·185	+7
W. $\frac{1}{12}$	277	115·0	2·36	·093	−10
B. $\frac{1}{16}$	287	170·0	1·68	·066	−5

In applying formula (12) to the determination of the focal lengths of objectives it is found that the labeling of these is in many cases very erroneous. In the paper to which reference has already been made Professor Cross gave his measurement of more than thirty objectives from various sources. In one case, an objective, marked $\frac{4}{10}$ inch, should have been marked $\frac{1}{4}$ inch. The measurements made by the present writer and recorded in Table II above, may give some idea of current errors in this respect. In column 1 the capital letter arbitrarily stands for the name of a maker, and the adjacent figures for the focal length of the objective as labeled, in inches or fractions of an inch, on the mounting. Column 2 gives the distance in millimeters between the stage and eye-piece micrometers, determined by the length of the microscope body; and column 3, the corresponding magnification attained. Column 4 gives the computed focal length in millimeters, which in column 5 is reduced to inches for the sake of comparison; and column 6 gives roughly the percentage of error of the label.

On examination of Table II it is seen that the errors of the labels are more frequently positive than negative, or that objectives are more frequently labeled too low in power than too high; and that the errors are unpardonably great in the objectives of lowest power. It seems scarcely conceivable that an error of 40 or 50 per cent could be made and deliberately stamped on the mounting of an objective whose real focal length is so easily found by experiment. It should be observed that any error due to thickness of cover glass is negligible when the focal length exceeds 20^{mm}. The stage micrometer used in these experiments was uncovered; and since the higher powers are usually adjusted to give their best definition when a definite thickness of cover glass is employed, this fact may partly account for the negative errors found in the two highest powers examined, although the adjustment of collar in these measurements was for use without a cover glass.

Having obtained the magnifying powers of objective and eye-piece, their product is the total magnifying power of the combination. If the equivalent focal length of the eye-piece is definitely known, its magnifying power, m', is obtained by applying formula (7). If the tube length, T, and focal length, f, of the objective are known, its magnifying power, m, may be accurately obtained. For, referring to fig. 1,

$$m = \frac{a'b'}{ab} = \frac{\mathrm{T}}{\mathrm{O}c}. \quad \text{But } \frac{1}{\mathrm{O}c} + \frac{1}{\mathrm{T}} = \frac{1}{f}. \quad \therefore \mathrm{O}c = \frac{\mathrm{T}f}{\mathrm{T}-f}. \quad \text{Hence,}$$

$$m = \frac{\mathrm{T}}{f} - 1. \qquad . \quad . \quad . \quad . \quad . \quad (13)$$

In formula (13), if f be very small in comparison with T, the term -1 may for all practical purposes be neglected. But to do this involves serious error when objectives of low power are employed.

Table III shows the result of using eye-piece B_1 of Table I successively in combination with five of the objectives of Table II, the values of m' and m being taken from these two tables. Column 2 gives the values thus calculated, while column 3 gives the corresponding results independently obtained with the camera lucida. The next two columns result from applying the formula $M = \frac{100}{Ff}$ and reducing to millimeters; in column 4 the values of F and f have been taken from Tables I and II, and in column 5 they are the nominal focal lengths, as indicated by the manufacturers.

TABLE III.

1.	2.	3.	4.	5.
Combination.	$M = mm'$.	Camera.	$M = \frac{100}{Ff}$	$M = \frac{100}{Ff}$
$B_1 \times W.3$ ----	30·8	30·4	34·14	22·22
$B_1 \times R.1\frac{1}{2}$ ---	55.3	55·0	53·68	44·44
$B_1 \times W.\frac{4}{5}$ ----	109·6	107·0	94·60	88·88
$B_1 \times C.\frac{1}{4}$ ----	430.6	433·0	339·0	266·7
$B_1 \times W.\frac{1}{12}$ ----	900.0	900·0	736·0	800·0

Table III shows, as might be expected, that the uncertainty of results increases with the power of the objective. Theoretically, columns 2 and 3 ought to be identical. Practically they are nearly so for low powers, but the difficulty of taking exact measurement with high powers is very great. The inaccuracies revealed in column 4 are due partly to the fact that the formula is only approximate, but also because the tube length is not 250 millimeters, and cannot possibly have this value with the instrument employed. In column 5 erroneous values of F and f, taken from the labeling, so greatly increase the errors of column 4 as to make the measurements worthless. Yet these are the results of calculation as commonly applied to the data furnished by the manufacturers.

In using the camera lucida the difficulty increases when the higher powers are employed, just as much as in applying Cross's formula. Under any circumstances, therefore, a wide margin of uncertainty exists in estimating the magnification attained with objectives of high power. Although the figures given are in each case the mean of many measurements, the remarkable agreement in the two results attained with the $\frac{1}{12}$th is doubtless to some extent accidental. With medium and lower powers it is shown by comparison of columns 2 and 3 that results about equal in value to those with the camera lucida are had by taking the product of the separate magnifications due to objective and eye-piece. And formulas (6), (7) and (13) show that this product may be expressed as

$$M = \frac{(D+F)(T-f)}{Ff} \quad . \quad . \quad . \quad (14).$$

This formula is fully worthy of reliance if accurate values of the equivalent focal length of eye-piece and objective, respectively, are stamped on their mountings, and if the tube-length also is stamped on the microscope body.

But the difficulty of securing definiteness and uniformity in tube length is probably greater than that of securing proper labels on the mountings of the lenses. It is necessary to fix upon two points of the microscope body as the upper and lower limits of the tube-length, and additionally for some agreement to be reached among makers as to the tube-length selected. What this shall be is a matter partly of precedent, partly of convenience. The nominal standard is 10 inches in England and America, but there is no pretense of adhering to it. In Germany and the continent of Europe generally, about 180 millimeters is perhaps most common. The latter is for some reasons more convenient, and seems to be gaining in popularity.

From what has preceded it is obvious that the upper limit of the tube-length should be the focal plane in which an image would be formed by the objective if no field lens were interposed. If the eye-piece is made to fulfil the generally assumed condition that the focal length of the field lens shall be three times that of the eye lens, and the interval between them shall be twice the focal length of the eye lens, the focal plane in question would be just midway between the diaphragm of a negative eye-piece and the optical center of the eye lens, which is at the middle of its convex surface. The eye-piece should be so constructed that when it is slipped into position this focal plane shall be exactly at the top of the microscope body, which then serves always as the upper limit of tube-length. The desirability of making all eye-pieces thus "par-focal" has been already suggested by several writers. There is no practical mechanical difficulty in attaining this end. In case the negative eye-piece should not fulfil the generally assumed conditions, the distance of the parfocal plane above the diaphragm is easily found. Referring to Fig. 2, and using the same notation, this distance is P′P, or $p-p'$, which, from the formula $\frac{1}{p'}-\frac{1}{p}=\frac{1}{f''}$, is equal to $\frac{p'^2}{f''-p'}$. The required distance of parfocal plane above diaphragm is thus given in terms of the focal length (f'') of the field lens and the distance (p') of the diaphragm from the optical center of this lens.

It should in justice be mentioned in this connection that at least one celebrated European firm, that of Carl Zeiss, in Jena, has for several years past been making all of its eye-pieces parfocal. This is only one of the many good things for which the scientific world is indebted to Professor E. Abbe, a physicist whose work in microscopical optics has been so thorough that scarcely anything in this domain can be undertaken by his cotemporaries which he has not already mastered. It is to be regretted that the makers of microscopes generally should be so slow in following a good example.

The determination of the lower limit of the tube length is slightly complicated by the fact that a microscope objective consisting of two or more systems of lenses, has no fixed point through which all axial rays will cross when the position of the point of radiance is varied. Its equivalent focal length varies within narrow limits according to the distance of the focal plane in which the image is formed. According to the writer's experiments it increases slightly as this distance is increased. The objective labeled R'. $1\frac{1}{2}$ in Table II was examined on an optical bench, the distance, l, between the points of radiance and convergence being varied from 160 mm. to 700 mm., and f calculated for 20 successive values of l. The mean of the first 10 values was 32·14 mm.; that of the second 10 was 32·38 mm., the extremes being 32·0 mm. and 32·5 mm. This objective consisted of two systems of lenses. A three-system objective of nominal $\frac{1}{4}$-inch focal length, and an objective of one system, were likewise examined, with the result shown in Table IV:

TABLE IV.

Label of Objective	W. 3	R. $1\frac{1}{2}$	C'$\frac{1}{4}$
Number of systems	1	2	3
Limits of l, in mm.	202–800	160–700	130–520
Number of measurements	12	20	14
f from first half, in mm.	50·05	32·14	5·60
f from second half, in mm.	50·00	32·38	5·70

From this table it is seen that the variation does not exceed a tenth of a millimeter in the highest of these powers, a quantity that is negligible in comparison with the whole tube-length. Assume then that the distance from the top of the microscope body to the extremity where the objective is screwed in is a little shorter than the desired tube-length; for example, 160 mm., if 180 mm. is selected for tube-length. Then in the formula, $\frac{1}{p}+\frac{1}{p'}=\frac{1}{f}$, we have $p'=180$, and f is known, hence p is calculated. The "working distance" between a slide and the exposed lens can be measured; and on subtracting it from p we have the distance, within the objective, of the point which for the given tube-length behaves like an optical center. This point, by the given formula, is known to be 20 mm. from the extremity of the microscope body, and hence the desired allowance can always be made in the mounting to put this point in its proper place. The optical tube-length for which an objective is corrected should always be stamped on its mounting along with the record of exact focal length and numerical aperture.

If there be accurate labeling of optical tube-length, and of the equivalent focal length of eye-piece and objective, the camera lucida ceases to be a necessity to the user of the microscope. Under present conditions, however, and until better methods are adopted by the majority of manufacturers, it is the only ready means of approximating toward the correct measurement of microscope magnification.

Brooklyn, N. Y., April 2, 1890.