VIII.—*The Penetrating Power of the Microscope.*

By EDWARD M. NELSON.

(Read 18th May, 1892.)

It has long been recognized that the penetrating power of the Microscope, or what, in other words, is the capability of seeing two different planes at the same time, diminishes as the magnifying power is increased, as the aperture is increased, and as the refractive index of the mounting medium is lowered.

The late Dr. Carpenter placed an undue amount of importance on the penetrating quality of the Microscope, and by his erroneous teaching on this subject undoubtedly retarded the advance of microscopy. His error was twofold :—

(1) Penetration is not an unmixed advantage in the interpretation of microscopical images ; it is more often a positive disadvantage. In objects, the nature of which cannot be perceived by the naked eye, it is better to trust to focal adjustment than to the best visual appearance of depth, however obtained.

(2) A “low-angled glass for penetration” is not the best way of obtaining the desired end.

It should be particularly noted that the difference in penetration between a $1/4$ of 60° ($\cdot 5$ N.A.), and a $1/4$ of 140° ($\cdot 95$ N.A.) both with an amplification of 300 diams. (B eye-piece) is less than $1/20,000$ in.—an almost inappreciable quantity with those lenses.

In cases where I would use a $1/2$ -in. of $\cdot 5$ N.A., and an amplification of 150 diams. (B eye-piece), giving a penetration of $1/2500$ in., a biologist or histologist would use a $1/6$ of $\cdot 6$ N.A. (Zeiss D) with an amplification of 450 diams. (B eye-piece) and a penetration of about $1/20,000$ in.

For many years the world of microscopy heard a great deal of Carpenter’s dictum, “low-angled glass for penetration”; a little time therefore spent on the examination of Dr. Carpenter’s tables of powers and apertures in the ninth edition of the ‘*Encyclopædia Britannica*’* will not be amiss.

We find here a $1\frac{1}{2}$ -in. of 20° and a 1-in. of 30° , powers 54 and 75 respectively ; these are full apertures required by the most critical workers for those amplifications.† No one could tell the difference in an image amplified 75 diams., with an apochromatic inch of $\cdot 3$ N.A. stopped down to $\cdot 26$ N.A., or $1/2$ -in. of $\cdot 65$ N.A., which fact demonstrates that there would be no gain in increasing the aperture of the inch beyond $\cdot 26$ N.A. with that amplification. As I stated in the ‘*English Mechanic*’ (1883), before the introduction of apo-

* Vol. xvi. p. 270.

† An apochromatic $1\frac{1}{2}$ of $\cdot 18$ or $\cdot 2$ N.A. is a want much felt.

chromatics, the goal to aim at is $\cdot 26$ N.A. for every 100 diameters of amplification.

We see, therefore, that Carpenter has in this table hit upon the ideal for the most critical work with *low powers*. It is easy to see how he arrived at it. These are the lenses he worked with, the plane mirror and daylight would yield a $3/4$ cone, and the cone would be focused on the object. He therefore unconsciously attained a critical image, and working largely with these lenses, he came to the only conclusion he could with justice arrive at, viz. that a large aperture gave the best results.

As his powers advance his images become more and more uncritical, and consequently we see the ratios of aperture to power becoming more and more fantastic. Taking the $1/4$ for instance, 50° to 80° , or $\cdot 42$ N.A. to $\cdot 64$ N.A., with powers of 200 to 300, if he had been a consistent reasoner, his 1-in. objective ought to have had 15° of aperture, or a trifle less than that possessed by the earliest achromatic inches made in this country.

In my own mind there is not the slightest doubt but that a $1/4$ should be capable of bearing a power of 400 diams.; therefore, in accordance with the ideal stated above, viz. $\cdot 26$ N.A. per 100 diams., its aperture ought to be $1\cdot 04$ N.A. It is nevertheless highly impolitic to make the $1/4$ an immersion, therefore $\cdot 95$ N.A. is the aperture it ought to have, because that is the highest aperture that can be advantageously put into a dry lens.

With regard to this table of apertures, Dr. Carpenter says, "He has the satisfaction of finding that his opinions on this point, which are based on long experience in the microscopic study of a wider range of animal and vegetable objects than has fallen within the purview of most of his contemporaries, are in accordance with the conclusions drawn by Professor Abbe from his profound investigations into the theory of microscopic vision." It will be as well to turn to the reference given (this Journal, 1882, pp. 300, 460), and see what are the ratios of aperture to power that Abbe gives:—1-in., power 40–80, aperture 18° ; $1/2$ -in., power 80–160, aperture 35° ; $1/4$ -in., aperture 60° : we see therefore that it is only in the $1/4$ -in. that they agree; in respect of Carpenter's favourite objective, the inch, they disagree very materially.

Abbe, although his conclusions are fallacious, is at least consistent with regard to the apertures of his 1-in. and $1/4$ -in., which Carpenter is not: this entirely cuts away the ground for Dr. Carpenter's satisfaction.

The views on penetrating powers which find acceptance among microscopists at the present time are those laid down by Prof. Abbe in this Journal.

He divides the penetrating power into two parts, one he calls "the accommodation depth," the other "the focal depth," and he states that "the visual depth" is the sum of these two quantities.

The method of obtaining these quantities is shown, and a table of the penetration of an objective of $\cdot 5$ N.A. for objects in air under various amplifications and with a myopic accommodation is given. It will be noticed that with low powers the accommodation depth accounts for the greater part of the depth of vision, but as the powers become higher, its effect rapidly diminishes.

We have here the means of applying a crucial test. If we take the lowest power in Abbe's table we find that the depth of vision is twenty-nine times as great as the focal depth. If, however, Abbe's table had been calculated for normal accommodation, the depth of vision would be thirty-five times greater than the focal depth. Now, as a photographic plate can have no accommodation, a photograph taken under similar circumstances ought to exhibit $1/28$ or $1/35$ of the penetration of the visual image, according as the eye is myopic or normal. I have performed these experiments and have failed to find any marked difference between the photographic and visual appearances of the image with normal vision, the same aperture, the same cone of illumination, the same amplification, and the same object being employed in both cases.

Prof. Abbe's table would, however, lead one to expect a great difference between the visual image and the photograph in the case of low powers.

With a 4-in. objective of $\cdot 08$ N.A. eye-pieced to give ten diameters, and with two objects in balsam having a difference of level amounting to $\cdot 5$ mm., if the upper object is perfectly focused the lower will be out of focus.

With a 2-in. objective of $\cdot 13$ N.A. eye-pieced to give an amplification of 20 diams., a difference of level of $\cdot 2$ mm. is sufficient to throw the lower object quite out of focus. These observations were moreover confirmed by one wholly unaccustomed to use either the Microscope or any optical instrument; this is worthy of note, because the appreciation of minute differences of focus and sharpness of images is, to a large extent, a matter of practice. I therefore conclude that the depth of vision with normal sight, and an aperture of $\cdot 08$ N.A. and a power of 10 diameters, is $\cdot 02$ in. in a medium of $1\cdot 5$ refractive index. This is nearly $1/7$ of the result obtained according to Abbe's formula, when his accommodation distance and circle of confusion are used, and nearly $1/9$, when calculated with my accommodation distance and circle of confusion.

We will now pass on to the photographic tests with low powers.

I photographed with a 3-in. objective of $\cdot 08$ N.A., and with a power of 10 diams., two foraminifera mounted in balsam, which were separated by $\cdot 008$ in. I also photographed the same object with an apochromatic 1 in. of $\cdot 3$ N.A. and a power of 20 diams. The results in both cases were practically identical with those obtained visually. For the higher powers an object peculiarly sensitive to focal adjustment was selected, and at the same time one not requiring

a wide angle to resolve it, viz. the intercostal spicules on *Triceratium favus* in the well-known preparation "Mud Cuxhaven" of Möller.

These spicules being at the angles of the large hexagonal structure are comparatively distant from each other, and therefore do not need great resolving power; but by choosing a part where they were on the curve of the valve, minute differences in focal depth could be instantly detected. There was a certain spicule, which, when viewed with a power of 2000 diams. and an aperture of 1.4 N.A., appeared dim, while the next one to it was sharp; this appearance was maintained both visually and photographically with various powers and apertures down to 250 diams., with an aperture of .65 N.A. With this last power the penetration with an object in balsam is nearly equal to 1/10 of one division of Powell and Lealand's fine-adjustment (say .00004 in.), a quantity almost imperceptible. We may say, therefore, that with powers ranging from 250 diams. obtained with a 1/4-in. objective of .95 N.A. and upwards, there is no penetration for normal sight.

With regard to accommodation depth, it should be especially borne in mind that as the accommodation distance lengthens, so also does the focus of the lens; in other words as p' increases, p also increases, because we are dealing with virtual and not screen images. A microscopist, when focusing, is said to accommodate at his nearest distance: if that is the case, any alteration in focal depth or penetration would be in a downward direction, as it is obviously impossible for him to accommodate up nearer than his nearest distance of vision.

So far we have been dealing with the examination of two objects at different levels, but when a plane object such as a balsam-mounted microphotograph is examined under a low power, the result is somewhat different. We shall find at first a sharp definite focus confined within very narrow limits. Let this image be placed a little out of focus, then by looking steadily at it and by straining the eye, it can be seen in what might appropriately be termed an uncomfortable focus. Let this process be repeated several times until, little by little, we reach a point from which, by no amount of straining can we recover the focus.

The difference between the upper and lower foci obtained in the above manner, with a 4-in. objective of .08 N.A. eye-pieced to give 10 diams., is 3 mm., and with a 2-in. of .13 N.A. eye-pieced to give 20 diams., it falls to 1.16 mm. We may say, therefore, that the penetration with a strained accommodation is about six times as great as ordinary accommodation.

Now, I conclude that no one with a focusing adjustment at hand would for a single moment think of straining his accommodating powers, for if an object at a different level from another object that was in the precise focus of the instrument did not come into focus perfectly naturally, the observer would instantly have recourse to the focusing adjustment, and by that means obtain a proper view of

the object. It would be therefore absurd in the extreme to call this penetration with strained accommodation the penetrating power of the Microscope.

Let us now see how the penetration with the strained accommodation of normal sight compares with that laid down by Prof. Abbe. Abbe's myopic accommodation (150 mm. to 300 mm.), power 10, N.A. $\cdot 08$ and a balsam mount, would give $3\cdot 125$ mm. for accommodation depth, and $\cdot 682$ for focal depth, or a total of $3\cdot 807$ mm., which is $\cdot 807$ mm. more than that of strained normal accommodation ; but as the unstrained accommodating distance for normal sight, viz. 8 in. to ∞ , is greater than that of Abbe's myopic accommodation of 150 mm. to 300 mm., the penetration should also be greater, but this experiment shows that it is less.

So far we have been experimenting with normal sight only. If we try myopic sight we shall find that penetration will be greater than that for normal, instead of less, as Abbe's theory requires. It is very instructive to notice the treatment of the microphotograph under a low power by myopic sight. A myopic eye is unable to mention any definite focus. Myopic microscopists are always playing on their accommodating powers, and consequently are unable to find a precise focus with a low power. For this reason they are able to penetrate without straining their accommodation to a greater depth than persons with normal sight.

My theory of microscopic penetration due to accommodation is, that with normal vision the eye does not accommodate at its nearest but at its easiest distance of vision, and that in some unexplained way the accommodating power of the normal eye is to a certain extent paralysed when using a Microscope. Hence, there is only a very limited amount of penetration without strain on either side of the easiest accommodating distance. Moreover, a normal eye when using a Microscope and straining its accommodation to the utmost, is not able to compass so large a range of accommodation as it ordinarily does without any strain when not using a Microscope. A myopic eye, on the other hand, is continually straining its accommodation, and it is on this account that the paralysing effect of the Microscope is not so much felt.

But from experiments made with myopic persons, I should be inclined to think that all the paralysing effect is not removed, and consequently the penetration is not so great as Abbe's table would lead one to imagine.

It is interesting to note in passing, that the alteration in the magnifying power can be distinctly seen when performing the straining experiments with the microphotograph.

We will now pass on to the investigation of Prof. Abbe's tables, which are very simple, and are based on elementary principles, with which every microscopist is acquainted.

The difficulty about the whole matter, and for which I can find no

theoretical explanation, is why the penetration as determined practically does not agree with the results obtained theoretically by Abbe's method. For instance, we ought theoretically to get 5 mm. of penetration, but practically we only get 1/2 mm. Again, a myopic person, who theoretically should have less penetrating power than an emmetropic person, practically has more. To meet this the paralysis theory is suggested, but why there should be this paralysis, if it exists, I am unable to say.

Taking accommodation depth first, all microscopists know that for every different screen distance, there is a different focus for the lens. Similarly for every different distance of virtual image there is a different focus for the lens. Accommodation depth is merely the difference in lens focus for different virtual image distances. The calculation of these different foci is very simple.

Let us, in order to make it perfectly clear, take a real or screen image with a single biconvex lens first (fig. 41). It is well known that on either side of a lens there is a principal focal point. Now if we call the distance between the image on the screen and the principal focal point on that side of the lens w , and the distance between the object and the principal focal point on the other side of the lens v , and the focus of the lens f (see figs. 41 and 42), then

$$v = \frac{f^2}{w}. \quad (\text{For proof see } \textit{infra}.)$$

If we have another screen distance which we will call w' , we shall have another focus v' , and $v' = \frac{f^2}{w'}$. The difference of focus is therefore $v - v'$, which is equal to $\frac{f^2}{w} - \frac{f^2}{w'}$, and which may be more conveniently written $\left(\frac{1}{w} - \frac{1}{w'}\right) f^2$. If, therefore, we put in for w the distance of least accommodation, and for w' that of greatest accommodation, the difference between these fractions will be the accommodation depth we require. In the case of a simple lens f is the focus of the lens, but in a compound Microscope f is the focus of the entire Microscope. This may be very easily determined if M the magnifying power and D the distance of vision are known, because $f = \frac{D}{M}$. (In the absence of all knowledge of the well-known formula, $f = \frac{D}{M}$ the proof is given *infra*.)

If, however, the object is in any other medium but air, μ the refractive index of that medium must be taken into account. The accommodation depth is therefore $\mu f^2 \left(\frac{1}{w} - \frac{1}{w'}\right)$. When the sight is

normal and the longer accommodation is infinite, then $\frac{1}{w}$, vanishes and the depth becomes $\frac{\mu f^2}{w}$ which may be written $\frac{\mu w}{M^2}$.

The focal depth is practically the latitude of focus which must be allowed for fluff. An object has really a precise and definite focus, but because the eye is unable to perceive very minute alterations in the image, that very precise and definite focus cannot be found, because the eye allows a slight range on either side of the true focus. This range is focal depth. Its amount can be easily determined. The first point to ascertain is the amount of fluff to be allowed, or in other words the diameter of the circle of confusion. I think it is better to take for this the least separable distance s , at the usual distance of accommodation or w . If we call this $1/200$ in. at 10 in. it will probably not be far from the average, although it is a larger amount than I allow for my own sight. The focal depth is then directly proportional to μ the refractive index of the medium the object is mounted in, and f the focal length of the lens or entire Microscope, and inversely proportional to the numerical aperture N.A.

The focal depth is therefore $\frac{\mu f s}{\text{N.A.} \cdot W}$ which may be written $\frac{\mu s}{M \cdot \text{N.A.}}$.

The values for s and w ought to be determined for each individual case; the following, however, will serve for general purposes:—Normal or emmetropic vision: $s = .005$ in. = .127 mm.; $w = 10$ in. = 250 mm. Myopic sight: $s = .0035$ in. = .09 mm.; $w' = 6$ in. = 150 mm.

The total visual penetration for emmetropic sight is therefore $\frac{\mu w}{M^2} + \frac{\mu s}{M \cdot \text{N.A.}}$ which may be written

$$\frac{\mu}{M} \left(\frac{w}{M} + \frac{s}{\text{N.A.}} \right). \quad (\text{i.})$$

For myopic sight the expression will be somewhat longer.

$$\frac{\mu}{M} \left\{ \frac{w}{M} \left(1 - \frac{w}{w'} \right) + \frac{s}{\text{N.A.}} \right\}. \quad (\text{ii.})$$

Thus far we have only been explaining and simplifying Abbe's formulæ, so if his values for the different terms are put in, results identical to his will be obtained.

Examples.—Emmetropic sight, $w = 250$ mm.; $D = 250$ mm.; magnifying power $M = 10$; $\text{N.A.} = .1$; $\mu = 1.5$; $\omega = .0005$; $s = .127$ mm.

Abbe's method :—

$$\begin{aligned} & \mu \left(\frac{D}{M} \right)^2 \cdot \frac{1}{w} + \mu \frac{D}{M} \cdot \frac{\omega}{N.A.} \\ &= 1.5 \left(\frac{250}{10} \right)^2 \cdot \frac{1}{250} + 1.5 \times \frac{250}{10} \times \frac{.0005}{.1} \\ &= \frac{1.5 \times 625}{250} + 1.5 \times 25 \times .005 \\ &= \frac{7.5}{2} + .187 = 3.94 \text{ mm.} \end{aligned}$$

By my simplified formula with the same data—

$$\begin{aligned} \frac{\mu}{M} \left(\frac{w}{M} + \frac{s}{N.A.} \right) &= \frac{1.5}{10} \left(\frac{250}{10} + \frac{.127}{.1} \right) \\ &= .15 \times 26.27 = 3.94 \text{ mm.} \end{aligned}$$

We have found, however, that owing to the paralysis of the accommodation, the penetrating power falls short of the above theoretical values. We must, therefore, construct a table that will be of practical use. To this end I propose to multiply the first fraction in the bracket by $\frac{M}{100}$ for all magnifications up to 50 diameters.

It must also be understood that the above formulæ are constructed on the understanding that the cone of illumination is equal to the full aperture of the objective.

Now we know that the highest illuminating cone for the most critical work we can use is a $3/4$ cone; this will slightly increase the penetration, and we should have therefore to multiply the second term in the bracket by $4/3$. As, however, a $1/2$ cone is more generally used than a $3/4$ cone, it will be better perhaps to make the formula suit general rather than critical use.

Putting in these two corrections and simplifying, we have for general work up to 50 diameters for emmetropic vision, the formula

$$\frac{\mu}{M} \left(\frac{w}{100} + \frac{2s}{N.A.} \right). \quad (\text{iii.})$$

For powers of 100 and 150 diameters, I propose to drop the accommodation depth and use only the second term multiplied by 2, thus,

$$\frac{4 \mu s}{M \cdot N.A.} \quad (\text{iv.})$$

For powers from 200 diameters and upwards I propose to use the second term only, viz.

$$\frac{2 \mu s}{M \cdot N.A.} \quad (v.)$$

After 300 diameters on a balsam mount, the penetration becomes less than 1/10 of one division on the head of Zeiss's fine-adjustment, a quantity which may be neglected.

With regard to myopic sight, unless a large number of measurements of the accommodating powers for different degrees of myopia with the respective penetrating powers were made, it would be quite impossible to state what corrections should be made, and whether various degrees of myopia required different corrections. The following formula may be used as a very rough approximation up to 50 diameters.

$$\frac{\mu}{M} \left\{ \frac{w}{50} \left(1 - \frac{w}{w'} \right) + \frac{2 s}{N.A.} \right\} \quad (vi.)$$

With the data given in our example above, the corrected penetration for normal sight will be

$$\begin{aligned} & \frac{1.5}{10} \left(\frac{250}{100} + \frac{2 \times .127}{.1} \right) \\ &= .15 (2.5 + 2.54) = .756 \text{ mm.} \end{aligned}$$

This is 1/5 of the previous result ; if, however, a full cone had been used in this instance as in the former, this result would have been 1/7. Another question arises, viz.: What is the photographic penetrating power ?

If we assume that the permissible amount of fluff is double that of the visual image, it will be the same as that of formula (iv.).

It only remains now to construct a table of penetration which will yield values in agreement with practical results. The following table, although based on formulæ obtained theoretically, is really empirical, owing to the corrections that have been applied.

Being unable to find a reason for the paralysing effect, it is impossible to construct a formula on theoretical grounds that will meet the case.

With regard to optical formulæ which are frequently applied to the Microscope, it is, I think, a great pity that the proof is assumed. As the proof is not given in the text-books, that for the two formulæ used in this paper, and which, moreover, are always occurring in microscopical papers, is appended. I start from the well-known fundamental optical formula $\frac{1}{p} + \frac{1}{p'} = \frac{1}{f}$, but would ask those who wish to take an intelligent interest in the theoretical

side of the Microscope, to study the chain of proof of this fundamental formula from "Snell's law of sines," "refraction through a prism," and "refraction through a thin lens," which are given in most text-books of physical science.

TABLE OF PENETRATION.

Data.—Vision emmetropic, $W = 250$ mm.; $S = \cdot 127$ mm.

Balsam mounted objects, $\mu = 1\cdot5$.			Penetration.	
Formule.	Power.	Aperture.	Visual.	Photographic.
(visual)			mm.	mm.
(iii.)	10	$\cdot 1$	$\cdot 756$	$\cdot 76$
	20	$\cdot 2$	$\cdot 283$	$\cdot 19$
	50	$\cdot 3$	$\cdot 1$	$\cdot 051$
(iv.)	100	$\cdot 3$	$\cdot 025$	$\cdot 025$
	150	$\cdot 5$	$\cdot 01$	$\cdot 01$
	200	$\cdot 5$	$\cdot 0035$	$\cdot 007$
(v.)	250	$\cdot 5$	$\cdot 003$	$\cdot 006$
	250	$\cdot 95$	$\cdot 0016$	$\cdot 032$
	300	$\cdot 95$	$\cdot 0013$	$\cdot 0026$
•	300 +	—	now inappreciable.	—
(photographic) (iv.)	—	—	—	—

FIG. 41.

FIG. 42.

* Proof that $v = \frac{f^2}{w}$.

$$\frac{1}{p'} + \frac{1}{p} = \frac{1}{f}; \quad p' = w + f; \quad p = v + f;$$

$$\frac{1}{w+f} + \frac{1}{v+f} = \frac{1}{f}; \quad (w+f)(v+f) = (w+v+2f)f;$$

$$wv = f^2; \quad v = \frac{f^2}{w}.$$

Proof that $f = \frac{D}{M}$. i = image, o = object.

$$M = \frac{i}{o} = \frac{p'}{p}; \quad D = p' + f;$$

$$\frac{1}{f} = \frac{1}{p} - \frac{1}{p'}; \quad f = \frac{pp'}{p' - p}; \quad f(p' - p) = pp';$$

$$fp' = (p' + f)p; \quad f = \frac{p' + f}{\frac{p'}{p}} = \frac{D}{M}.$$

The above figures illustrate the formula on page 181 *ante*.

* First demonstrated by Sir Isaac Newton.

Erratum.—Page 336, line 5 from bottom, for “(In the absence of all knowledge of the well-known formula, $f = \frac{D}{M}$ the proof is given *infra*)” read “(In the absence of all knowledge of the well-known formula, $m = \frac{d}{f}$, see p. 182 (ii.), the proof is given *infra*.)”