important discovery was made by Mr Andrew Ross that a very decided difference exists in the precision of the image according as the object is viewed with or without a covering of thin glass, as also according as this cover is thin or thick.[1] As this difference increases in proportion to the widening of the aperture, it would obviously be a source of great error and embarrassment if a means could not be found for its rectification. Its optical source, however, having been found by Mr Ross to lie in the "negative aberration" which is produced in the rays proceeding from the object to the front glass of the objective by the interposition of the plane-glass cover, and which increases with its thickness, his practical ability enabled him at the same time to indicate the remedy, which consists in under-correcting the front lens and over-correcting the two posterior combinations, and in making the distance between the former and the latter capable of adjustment by means of a screw-collar, as shown in fig. 12. For when the front pair is approximated most nearly to the next, and its distance from the object is increased, its excess of positive aberration is more strongly exerted upon the other two pairs than it is in the contrary conditions, and thus neutralizes the negative aberration produced by the interposition of the covering-glass. This correction is not needed for objectives of low or medium power and small angle of aperture; but it should always be provided when the angle exceeds 50°,—unless (as is now generally done in the case of objectives constructed for students' use) the maker adjusts them originally, not for uncovered objects, but for objects covered with glass of a standard thickness, say 0·005 or 0·004 inch. A departure from that standard to the extent of one or two thousandths of an inch in either direction, though extremely injurious to the performance of objectives whose aperture is 125° or more, scarcely makes itself perceptible in those of 90° or 100°. And the same may be said in regard to the immersion-objectives next to be described, which are peculiarly suitable to the purposes of minute histological research.

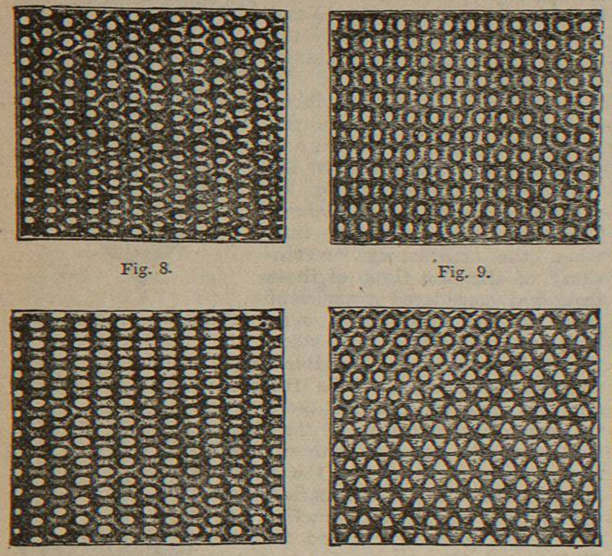
Fig. 8. Fig. 9.
Fig. 10. Fig. 11.

Portions of Siliceous Valve of *Pleurosigma angulatum*, from a Photograph taken by Central Illumination. Magnified 2000 diameters.

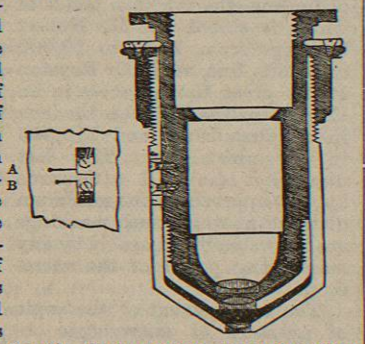

Fig. 12.—Section of Adjusting Achromatic Object-Glass. A, uncovered; B, covered.

Immersion System.—It was long since pointed out by Professor Amici that the introduction of a drop of water between the front surface of the objective and either the object itself or its covering-glass would diminish the loss of light resulting from the passage of the rays from the object or its covering-glass into air, and from air into the front glass of the objective. It was obvious to him, moreover, that when the rays enter the object-glass from water, instead of from air, both its refractive and its dispersive action will be so greatly changed as to need an important constructive modification to meet the new condition. This modification seems never to have been successfully effected by Amici himself; but his idea was taken up by the two eminent Paris opticians, MM. Hartnack and Nachet, who showed that the application of what is now known as the "immersion system" to objectives of short focus and large angular aperture is attended, not merely with the advantages expected by Professor Amici, but with others on which he did not reckon. As the loss of light by the reflexion of a portion of the incident rays increases with the obliquity of their incidence, and as the proportional loss is far smaller when the oblique rays pass into glass from water than when they enter it from air, the advantage of increasing the angular aperture is more fully experienced with "immersion" than with "dry" objectives,—just as Professor Amici anticipated. But, further, the immersion system allows of a greater working distance between the objective and the object than can be attained with a dry or air objective having the same angular aperture; and this increase affords not only a greater freedom of manipulation, but also a greater range of "penetration" or "focal depth." Further, the observer is rendered so much less dependent upon the exactness of his cover-correction that it is found that water-immersion objectives of high power and considerable angular aperture, extremely well adapted for the ordinary purposes of scientific investigation, can be constructed without it,—a small departure from the standard thickness of covering-glass to which such objectives are adjusted by the maker having scarcely any effect upon the distinctness of the image. It is now the practice of several makers to supply two fronts to objectives of $\frac{1}{10}$ or $\frac{1}{12}$ inch focus, one of them fitting the objective for use "dry" (that is, in air), whilst the substitution of the other converts it into a water-immersion objective. And in the objectives constructed on Mr Wenham's system no change in the front glass is needed, all that is necessary for making them work as immersion-lenses being a yet closer approximation of the front lens to the second combination, which can be made by the screw-collar.

Within the last few years, however, the immersion system has undergone a still further and most important development, by the adoption of a method originally suggested by Mr Wenham (though never carried out by him), and independently suggested by Mr Stephenson to Professor Abbe of Jena, under whose direction it was first worked out by Zeiss (the very able optician of Jena), who has been followed by Powell and Lealand of London, as well as by several other constructors of achromatic objectives both in England and elsewhere, with complete success. This method consists in the replacement of the water previously interposed between the covering-glass and the front glass of the objective by a liquid having the same refractive and dispersive powers as crown-glass, so that the rays issuing at any angle from the upper plane surface of the covering-glass shall enter the plane front of the objective, without any deflexion from their straight course, and without any sensible loss by reflexion,—even the most oblique rays that proceed from the object keeping their direction unchanged until they meet the back or convex surface of the front lens of the objective. It is obvious that all the advantages derivable from the system of water-immersion will be still more thoroughly attained by this system of "homogeneous" immersion, provided that a fluid can be obtained which meets its requirements. After a long course of experiments, Professor Abbe found that oil of cedar wood so nearly corresponds with crown-glass, alike in refractive and in dispersive power, as to serve the purpose extremely well, except when it is desired to take special advantage of the most divergent or marginal rays, oil of fennel being then preferable. There are, however, strong objections to the use of these essential oils in the ordinary work of research; and it seems not unlikely that a solution of some one or more saline substances will be found more suitable. In addition to the benefit conferred by the water-immersion system, and more completely attained with the homogeneous, it may be specially pointed out that, as no correction for the thickness of the covering-glass is here required, the microscopist can feel assured that he has such a view of his object as only the most perfect correction of an air-objective can afford. This is a matter of no small importance, for while, in looking at a known object, the practised microscopist can so adjust his air-objective to the thickness of its covering-glass as to bring out its best performance, he cannot be sure, in regard to an unknown object, what appearances it ought to present, and may be led by imperfect cover-correction to an erroneous conception of its structure.

It has been recently argued that, as the slightest variation in the refractive index of either the immersion fluid or the covering-glass, a change of eye-pieces, or the least alteration in the length of the body—in a word, any circumstances differing in the slightest degree from those under which the objective was corrected—must affect the performance of homogeneous-immersion objectives of the highest class, they should still be made adjustable. The truth of this contention can, no doubt, be proved, not only theoretically, but practically,—the introduction of the adjustment enabling an experienced manipulator to attain the highest degree of perfection in the exhibition of many mounted objects, which cannot be so well shown with objectives in fixed settings. But it may well be questioned whether it is likely to do the same service in the hands of an ordinary working histologist, and whether the scientific investigator will not find it preferable, when using these objectives, to accept what their maker has fixed as their point of best performance. The principal source of error in his employment of them lies in the thickness of the optical section of the object; for the rays proceeding from its deeper plane, having to pass through a medium intervening between that plane and the cover-glass, whose refractive and dispersive indices differ from those of the glass and immersion-fluid, cannot be brought to so accurate a focus as those proceeding from the plane immediately beneath the cover-glass. The remedy for this, however, seems to lie rather in making the preparation as thin as possible than in the introduction of what is likely, in any but the most skilful and experienced hands, to prove a new source of error. Every one who has examined muscular fibre, for example, under a dry objective of very high power and large aperture, well knows that so great an alteration is produced in its aspect by the slightest change in either the focal adjustment or the cover-correction that it is impossible to say with certainty what are the appearances which give the most correct optical expression of its structure. This being a matter of judgment on the part of each observer, it seems obvious that the nearest approach to a correct view will be probably given by the focal adjustment of the best homogeneous immersion-objectives, in fixed settings, to the plane of the preparation immediately beneath the cover-glass (see *Jour. Roy. Micros. Soc.*, 1882, pp. 407, 854, 906).

In every particular in which the water-immersion system is superior to the dry, it is itself surpassed by the oil or other homogeneous system, the anticipations of those by whom it was suggested being thus fully realized. But the advantages already spoken of as derivable from the use of the "immersion system" are altogether surpassed by that which the theoretical studies of Professor Abbe have led him to assign to it, and of which he has practically demonstrated its possession. For he has shown (as will be explained below) that the interposition of either water or oil so greatly increases the real "aperture" of the objective that immersion-objectives may be constructed having a far greater virtual aperture than even the theoretical maximum (180°) of the angular aperture of an air-objective.

The same eminent physicist, working on the basis supplied by the mathematical investigations of Professor Helmholtz and himself on the undulatory theory of light, has further established an entirely new doctrine in regard to the production of highly magnified representations of closely approximated markings. All that has hitherto been said of the formation of images by the compound microscope relates to such as are produced, in accordance with the laws of refraction, by the alteration in direction which the light-rays undergo in their passage through the lenses interposed between the object and the eye. These dioptric images, when formed by lenses free from spherical and chromatic aberration, are geometrically correct pictures, truly representing the appearances which the objects themselves would present were they enlarged to the same scale and viewed under similar illumination. And we seem justified, therefore, in drawing from such microscopic images the same conclusions in regard to the objects they picture as we should draw from the direct vision of actual objects having the same dimensions. The principal source of error in such interpretations arises out of the "interference" to which the rays of light are subjected along the edges of the minute objects through which they pass, or along any such lines or margins in their inner part as are sufficiently opaque to throw a definite shadow. For every such shadow must be bordered, more or less obviously, by interference- or diffraction-spectra; and thus the images of strongly-lined objects with very transparent intermediate spaces may be so troubled or confused by these "diffraction-spectra" as to render it very doubtful what interpretation is to be put upon their appearances.

A good example of this kind is afforded by the scales of the gnat or mosquito, which are composed of a very delicate double membrane, strengthened by longitudinal ribs on both sides, those of the opposite sides uniting at the broad end of the scale, where they generally terminate as bristle-shaped appendages beyond the intermediate membrane. These are crossed by fine markings, which are probably ridge-like corrugations of the membrane, common to both sides of the scale. Between each pair of longitudinal ridges there may be seen, under certain adjustments of focus and illumination, three uniform parallel rows of beads, which have been supposed to represent a true structure in the membrane. By Dr Woodward (colonel in the United States army), however, it has been shown that this beaded appearance is merely the result of the "interferences" produced by the longitudinal and transverse lines of the scale. For the longitudinal diffraction-lines are clearly seen, alike in the microscopic image and in photographs (fig. 13), to extend into empty space beyond the contour of the scales, almost as far as the ends of the bristles in which the parallel ribs terminate; and they vary in number with the varying obliquity of illumination, so that in the same scale two, three, four, or even five rows of beads can be seen, and photographed at pleasure, in every intercostal space.[1]

Every microscopist who has worked much with high powers is well aware of the difficulty of distinguishing between real and spectral markings,—a difficulty which can only be overcome by training and experience. It seems,

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[1] *Trans. Soc. of Arts.* vol. li

[1] *Monthly Micros. Jour.*, vol. xv. (1876), p. 253.

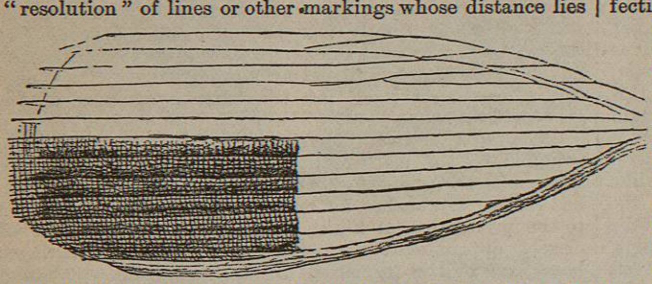

FIG. 13.—Scale of Gnat, showing Beaded Markings produced by Diffraction; from a Photograph by Colonel Dr Woodward.

however, to have been now fully ascertained by Professor Abbe that it is only through such diffraction-spectra that the microscope can make us acquainted with the minutest structural features of objects, since, according to the calculations of Professor Helmholtz and himself (based on the constants of the undulatory theory), no amount of magnifying power can separate dioptrically two lines, apertures, or markings of any kind, not more than $\frac{1}{2500}$ of an inch apart. The visual differentiation or "resolution" of lines or other markings whose distance lies within that limit is entirely the result of "interference,"—the objective receiving and transmitting, not only dioptric rays, but the inflected rays whose course has been altered in their passage through the object by the peculiar disposition of its particles, and combining these rays into a series of diffraction-spectra, the number and relative position of which bear a relation to the structural arrangement on which their production depends. If the objective be perfectly corrected, and all the diffraction-spectra lie within its field, these will be recombined by the eye-piece so as to form a secondary or "diffraction" image, lying in the same plane with the dioptric image, and coinciding with it, while filling up its outlines by supplying intermediate details. But where the markings (of whatever nature) are so closely approximated as to produce a wide dispersion of the interference-spectra, only a part of them may fall within the range of the objective; and the recombination of these by the eye-piece may produce a diffraction-image differing more or less completely (perhaps even totally) from the real structure; while, if they should lie entirely outside the field of the objective, no secondary or diffraction image will be produced. And thus, while the general form of such an object as a diatom-valve may be correctly given in a dioptric image, its surface may appear quite unmarked under an objective of small aperture, however great its magnifying power, though covered with regularly disposed markings when seen through an objective of wider aperture with perhaps only half the magnifying power.

It is obvious, however, that, while the dioptric image represents the actual object, the diffraction-image thus formed by the reunion of a portion of the interference pencils is only an optical expression of the result of their partial recombination, which may represent something entirely different from the real structure. For it has been proved experimentally, by placing finely-ruled gratings in the position of objects, and by limiting the apertures of objectives by diaphragms with variously disposed perforations, that the same arrangement of lines shall be presented to the eye by differently lined surfaces, and different arrangements by similarly lined surfaces, according to the numbers and relative positions of the reunited spectra. Hence it is clear that there must be an essential difference in character and trustworthiness between the images dioptrically formed of the general outlines and larger details of microscopic objects and those representations of their finer details which are given by the recombination of their diffraction-spectra,[1] and that the confidence to be placed in the latter class of representations will be greater in proportion to the completeness of the recombination of the separated interference-spectra, which, again, will be proportional (accurate correction of the aberrations being assumed) to the aperture of the objective.[2]

The combined advance of scientific theory and of practical skill in the application of it have now brought up the compound achromatic microscope to an optical perfection that renders it capable of actually doing almost everything of which, in the present state of optical theory, it can be regarded as capable. The resolution of Nobert's nineteenth band, having 112,595 lines to an inch, which was long regarded as the *crux* of microscopists, is now found so easy as to leave little room for doubt that, if a new test were obtainable having the *minimum visibile* of 118,000 lines to the inch, an oil-immersion objective would be found to resolve it. But the experience of the past makes it evident that, as no limit can be set to the advance of optical theory, results yet more remarkable may be still expected to arise, every such advance being turned to account by the practical skill which experience has now enabled the best constructors of achromatic objectives to attain.[3]

The progressive improvements thus effected in the construction of microscopic objectives have been accompanied by other improvements, alike in the optical and in the mechanical arrangements by which the best performance of these objectives can be secured; and it will be desirable now to describe in succession the most approved forms of the eye-piece, the objective, and the illuminating apparatus respectively, and then those of the instrument as a whole, pointing out the special adaptiveness of each to the requirements of different classes of scientific investigators.

EYE-PIECES.

It very early became obvious to those who were engaged in the achromatization of microscopic objectives that their best performance was obtained when the image given by them was further enlarged by the eye-piece known as the Huygenian, as having been devised by Huygens for his telescopes. It consists of two plano-convex lenses (EE and FF, fig. 4), with their plane sides towards the eye; these are placed at a distance equal to half the sum of their focal lengths,—or, to speak with more precision, at half the sum of the focal length of the eye-glass, and of the distance from the field-glass at which an image of the object-glass would be formed by it. A "stop" or diaphragm BB must be placed between the two lenses, in the visual focus of the eye-glass, which is, of course, the position wherein the image of the object will be formed by the rays brought into convergence by their passage through the field-glass. Huygens devised this arrangement merely to diminish the spherical aberration; but it was subsequently shown by Boscovich that the chromatic dispersion was also in great part corrected by it. Since the introduction of achromatic object-glasses for compound microscopes, it has been further shown that nearly all error may be avoided by a slight over-correction of these, so that the blue and red rays may be caused to enter the eye in a parallel direction (though not actually coincident), and thus to produce a colourless image. Thus let N, M, N (fig. 14) represent the two extreme rays of three pencils, which without the field-glass would form a blue image convex to the eye-glass at BB, and a red one at RR; then, by the intervention of the field-glass, a blue image concave to the eye-glass is formed at B′B′, and a red one at R′R′. As the focus of the eye-glass is shorter for blue rays than for red rays by just the difference in the place of these images, their rays, after refraction by it, enter the eye in a parallel direction, and produce a picture free from false colour. If the object-glass had been rendered perfectly achromatic, the blue rays, after passing through the field-glass, would have been brought to a focus at *b*, and the red at *r*; so that an error would be produced, which would have been increased instead of being corrected by the eye-glass. Another advantage of a well-constructed Huygenian eye-piece is that the image produced by the meeting of the rays after passing through the field-glass is by it rendered concave towards the eye-glass instead of convex, so that every part of it may be in focus at the same time, and the field of view thereby rendered flat.[1]

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[1] Thus it is still a moot point whether the microscopic appearances seen in the siliceous valves of diatoms (figs. 8–11) are the optical representations of elevations, depressions, or perforations, or of internal molecular arrangements not involving any inequality of surface.

[2] This doctrine was first fully developed by Professor Abbe in the *Archiv für Microsk. Anatomie*, vol. ix. (1874), and is more fully expounded in his subsequent contributions to *Jour. Roy. Micros. Soc.* See also the papers of Mr Stephenson and Mr Crisp in that journal, and in the preceding *Monthly Microscopical Journal.*

[3] Any good workman can now make by the dozen such small-angled $\frac{1}{4}$ inch objectives as Mr A. Ross produced with much pains and labour fifty years ago. It was not until 1844 that, with the honourable emulation of surpassing what Professor Amici had then accomplished, he produced a $\frac{1}{12}$ inch of 135°, which, by taking advantage of some very heavy flint-glass he had, he afterwards increased to 170°.

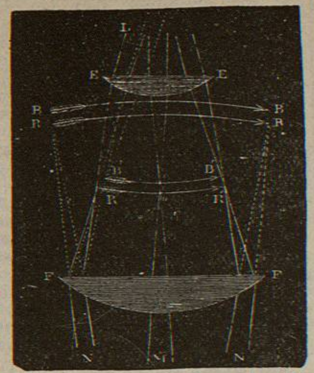

FIG. 14.—Section of Huygenian Eye-Piece, adapted to Over-Corrected Microscopic Objectives.

Two or more Huygenian eye-pieces, of different magnifying powers, known as A, B, C, &c., are usually supplied with a compound microscope. The utility of the higher powers will mainly depend upon the excellence of the objectives; for, when an achromatic combination of small aperture which is sufficiently well corrected to perform very tolerably with a "low" or "shallow" eye-piece is used with an eye-piece of higher magnifying power (commonly spoken of as a "deeper" one), the image may lose more in brightness and in definition than is gained by its amplification, while the image given by an objective of large angular aperture and very perfect correction shall sustain so little loss of light or of definition by "deep eye-piecing" that the increase of magnifying power shall be almost clear gain. Hence the modes in which different objectives of the same power, whose performance with shallow eye-pieces is nearly the same, are respectively affected by deep eye-pieces afford a good test of their respective merits, since any defect in the corrections is sure to be brought out by the higher amplification of the image, while a deficiency of aperture is manifested by the want of light. The working microscopist will generally find the A eye-piece most suitable, B being occasionally employed when a greater power is required to separate details, whilst C and others still deeper are useful for the purpose of testing the goodness of objectives, or for special investigations requiring the highest amplification with objectives of the finest quality. But he can commit no greater error than habitually to use deep eye-pieces for the purposes of scientific research, especially when (as in the study of living objects) long-continued and unintermitted observation is necessary. For the visual strain thus occasioned is exactly like that resulting from the habitual use of magnifying spectacles in reading, requiring the book to be held within 2 or 3 inches of the eye. And all experience shows that this feeling of strain cannot be disregarded, without the most injurious consequences to vision.

For viewing large flat objects, such as transverse sections of wood or of *Echinus* spines, under low magnifying powers, the eye-piece known as Kellner's may be employed with advantage. In this construction the field-glass, which is a double-convex lens, is placed in the focus of the eye-glass, without the interposition of a diaphragm; and the eye-glass is an achromatic combination of a plano-concave of flint with a double-convex of crown, which is slightly under-corrected, so as to neutralize the over-correction given to the objectives for use with Huygenian eye-pieces. A flat well-illuminated field of as much as 14 inches in diameter may thus be obtained with very little loss of light; but, on the other hand, there is a certain impairment of defining power, which renders the Kellner eye-piece unsuitable for objects presenting minute structural details; and it is an additional objection that the smallest speck or smear upon the surface of the field-glass is made so unpleasantly obvious that the most careful cleansing of that surface is required every time that this eye-piece is used. Hence it is better fitted for the occasional display of objects of the character already specified than for the ordinary wants of the working microscopist.

Solid eye-pieces, consisting of cylinders of glass with convex ends, are sometimes used in place of the Huygenian, when high magnifying power is required for testing the performance of objectives. The lower surface, which has the lesser convexity, serves as a field-glass, while the image formed by this is magnified by the highly convex upper surface to which the eye is applied,—the advantage derivable from this construction lying in the abolition of the plane surfaces of the two lenses of the ordinary eye-piece.[2]

A "positive" or Ramsden's eye-piece—in which the field-glass, whose convex side is turned upwards, is placed so much nearer the eye-glass that the image formed by the objective lies below instead of above it—was formerly used for the purpose of micrometry,—a divided glass being fitted in the exact plane occupied by the image, so that its scale and that image are both magnified together by the lenses interposed between them and the eye. The same end, however, may be so readily attained with the Huygenian eye-piece that no essential advantage is gained by the use of that of Ramsden, the field of which is distinct only in its centre

OBJECTIVES.

It has been seen that one of the principal points in the construction of microscopic objectives to which the attention of their makers has been constantly directed has been the enlargement of their "aperture,"—this term being understood to mean, not their absolute opening as expressed by linear measure, but their capacity for receiving and bringing to a remote conjugate focus the rays diverging from the several points of a near object. The aperture of an objective has been usually estimated by its "angle of aperture,"—that is, by the degree of divergence of the most extreme rays proceeding from the axial point of the object to the margin of the objective (fig. 15) which take part in the formation of the image. It is pointed out, however, by Professor Abbe that, in the case of single lenses used as objectives, their apertures are really proportional, not to their respective angles of aperture, but to the ratio between the actual diameter or clear opening of each to its focal distance, a ratio which is simply expressed by the sine of its semiangle. And in the case of combinations of lenses it can be demonstrated mathematically that their respective apertures are determinable—other conditions being the same—by the ratio of the diameters of their back lenses, so far as these are really utilized, to their respective focal lengths,—this ratio being expressed, as before, by the sine of the semiangle of aperture ($\sin u$). The difference between these two modes of comparison can be readily made obvious by reference to the theoretical maximum of 180°, which is attained by opening out the boundaries of the angle *abc* (fig. 15) until they come into the same straight line, the sine of the semiangle (90°) then becoming unity. For, while an objective having an angle of 60° would count by comparison of angles as having only one-third of the theoretical maximum, its real aperture would be half that maximum since the sine of its semiangle $(30°) = \frac{1}{2}$. And, as the sines of angles beyond 60° increase very slowly, an objective of 120° angle will have as much as 87 per cent. of the theoretical maximum of aperture, although its angle is only two-thirds, or 66·6 per cent., of 180°. It hence becomes obvious that little is really gained in real aperture by the opening-out of the angle of microscopic objectives to its greatest practicable limit (which may be taken as 170°), while such extension—even if unattended with any loss either of definition or of colour-correction—necessarily involves a great reduction alike in the working distance and in the focal depth or penetration of the combination, as will be presently explained.

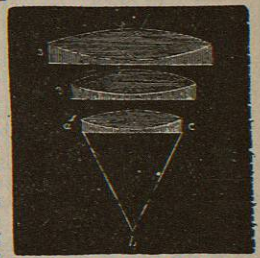

FIG. 15.—Section of Achromatic Object-Glass, composed of three pairs of (flint and crown) lenses. *abc* is its angle of aperture.

Numerical Aperture.—It has now been demonstrated by Professor Abbe that, independently of the advantages already specified as derivable from the application of the immersion system to objectives of short focus and wide aperture, the real aperture of an immersion objective is considerably greater than that of a dry or air objective of the same angle,—the comparative apertures of objectives working through different media being in the compound ratio of two factors, viz., the sines of their respective semiangles of aperture and the refractive indices of the "immersion" fluids. It is the product of these ($n \sin u$) that gives what is termed by Professor Abbe the "numerical aperture,"—which serves, therefore, as the only true standard of comparison, not only between dry or air and water or oil immersion lenses, but also between immersion lenses adapted to work respectively with water, oil, or any other interposed fluid. That the angle of aperture expressed by the same number of degrees must correspond with very different working apertures in dry, water immersion, and oil or homogeneous immersion objectives becomes evident when we consider what

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[1] The reader may be referred to Mr Varley's investigation of the properties of the Huygenian eye-piece in the fifty-first volume of the *Transactions of the Society of Arts*; and to the article "Microscope," by Mr Ross, in the *Penny Cyclopædia*, reprinted, with additions, in the *English Cyclopædia*.

[2] These eye-pieces are much in vogue in the United States, where they are made of extremely short foci,—even to $\frac{1}{16}$ inch.

happens when divergent pencils of rays pass from one medium into another of higher refractive index. For such divergent pencils, proceeding from air into water or oil, will be closed together or compressed; so that the rays which, when an object is mounted in air, spread out over the whole hemisphere then form comparatively narrow pencils, and can thus be utilized by an immersion objective of smaller aperture than is required in a dry objective to admit the most diverging rays of air-pencils. It follows, therefore, that a given angle in a water or oil immersion objective represents a much larger aperture than does the same angle in an air-objective; and thus it comes to pass that by opening out the angle of immersion objectives they may be made to receive and utilize rays of much greater divergence than can possibly enter dry objectives of even maximum aperture.

The following table, abridged from that given by Professor Abbe for every 0·02 of numerical aperture from 0·50 up to the maximum of 1·52, brings this contrast into clear view:—

Numerical Aperture Table.

Numerical Aperture ($n \sin u = a$).	Angle of Aperture ($=2u$).			Illuminating Power (a^2).	Theoretical Resolving Power, in Lines to an Inch ($\lambda = 0{\cdot}5269\,\mu$ = line E).	Penetrating Power $\left(\frac{1}{a}\right)$.
	Dry Objectives ($n=1$).	Water-Immersion Objectives ($n=1{\cdot}33$).	Homogeneous-Immersion Objectives ($n=1{\cdot}52$).			
	° ′	°	° ′			
1·52	...	...	180 0	2·310	146,528	·658
1·42	...	...	138 12	2·016	136,888	·704
1·33	...	180 0	122 6	1·770	128,212	·752
1·26	...	142 39	111 59	1·588	121,464	·794
1·18	...	125 3	101 50	1·392	113,752	·847
1·12	...	114 44	94 56	1·254	107,968	·893
1·06	...	105 42	88 26	1·124	102,184	·943
1·00	180 0	97 31	82 17	1·000	96,400	1·000
0·94	140 6	89 56	76 24	·884	90,616	1·064
0·86	118 38	80 34	68 54	·740	82,904	1·163
0·80	106 16	73 58	63 31	·640	77,120	1·250
0·76	98 56	69 42	60 0	·578	73,264	1·316
0·70	88 51	63 31	54 50	·490	67,480	1·429
0·62	76 38	55 34	48 9	·384	59,768	1·613
0·56	68 6	49 48	43 14	·314	53,984	1·786
0·50	60 0	44 10	38 24	·250	48,200	2·000

Thus, taking as a standard of comparison a dry objective of the maximum theoretical angle of 180°, whose numerical aperture is the sine of 90°, or 1·00, we find this standard equalled by a water-immersion objective whose angle of aperture is no more than 97½°, and by an oil or homogeneous immersion objective of only 82°,—the numerical apertures of these, obtained by multiplying the sines of their respective semiangles by the refractive index of water or of oil, being 1·00 in each case. Each, therefore, will have as great a power of receiving and utilizing divergent rays as any dry objective can even theoretically possess.

But, as the actual angle of either a water or an oil immersion objective can be opened out to the same extent as that of an air or dry objective, it follows that the aperture of the former can be augmented far beyond even the theoretical maximum of the latter. Thus the numerical aperture of a water-immersion lens of the maximum angle of 180° is 1·33, or one-third greater than that of an air-lens of the same angle; and this aperture would be given by an oil-immersion objective of only 122°. Again, the numerical aperture of an oil-immersion objective having the theoretical maximum angle of 180° would be 1·52, or more than one-half greater than that of an air-lens of the same angle. And the numerical apertures corresponding to angles of 170°, which have been actually attained in both cases, fall very little short of the proportions just given.

So, again, an oil-immersion objective whose angle of aperture is only 60° has as high a numerical aperture (0·76) as a water-immersion objective of 69½°, or as a dry objective of 99°; and a dry objective of 140° has no greater a numerical aperture (0·94) than a water-immersion of 90° or an oil-immersion of 76½°.

This important doctrine may be best made practically intelligible by a comparison of the relative diameters of the back lenses of dry with those of water and oil immersion objectives of the same power, from an "air-angle" of 60° to an "oil-angle" of 180°,—these diameters expressing, in each case, the opening between the extreme pencil-forming rays at their issue from the posterior surface of the combination, to meet in its conjugate focus for the formation of the image, the relation of which opening in each case to the focal length of the combination is the real measure of its aperture (fig. 16). Thus the dry objective of 60° angle (5 in fig. 16) has its air-angle represented by $\sin u = \frac{1}{2} = 0{\cdot}50$ numerical aperture. The dry objective of 97° (4) has its air-angle represented by $\sin u = \frac{3}{4} = 0{\cdot}75$ numerical aperture. And the dry objective having the (theoretical) angle of 180° (3) has its air-angle represented by $\sin u = 1{\cdot}00$ numerical aperture,—this corresponding to 96° water-angle and 82° oil-angle. But the water-immersion lens having the (theoretical) angle of 180° (2) has its water-angle represented by $n \sin u = 1{\cdot}33$ numerical aperture. And the oil-immersion lens having the (theoretical) angle of 180° (1) has its oil-angle represented by $n \sin u = 1{\cdot}52$ "numerical aperture."[1] These theoretical apertures for water and oil immersion lenses having been found as nearly attainable in practice as the theoretical maximum for dry objectives, such lenses can utilize rays from objects mounted in balsam or other dense media, which are entirely lost for the image (since they do not exist physically) when the same object is in air or is observed through a film of air. And this loss cannot be compensated by an increase of illumination; because the rays which are lost are different rays physically from those obtained by any illumination, however intense, through an aeriform medium.

It is by increasing the number of diffraction-spectra that the additional rays thus received by objectives of great numerical aperture impart to them an increased resolving power for lined and dotted objects,—the truth of the image formed by the recombination of these spectra being (as already shown) essentially dependent on the number of them that the objective may be capable of receiving.

But whilst the resolving power of microscopic objectives increases in the ratio of their respective numerical apertures, and whilst their illuminating power (dependent upon the quantity of light that passes through them) increases with the square of the numerical aperture, the case is reversed with another most important quality,—that of penetration or focal depth; for this diminishes as the numerical aperture increases, until nothing but what is precisely in the focal plane can be even discerned with objectives possessed of the highest resolving power. Thus, the penetrating power of an objective of 60° air-angle being expressed as 2·000, an extension of that angle to 76½° reduces it to 1·613, an extension to 89° reduces it to 1·429, and an extension to 99° reduces it to 1·316; further extension to 118½° reduces it to 1·163, while an objective whose air-angle is 140° has a penetrating power of only 1·064. So, again, the oil-immersion objective which has the numerical aperture of 1·00 corresponding to the theoretical air-angle of 180° has a penetrating power of 1·000; this is brought down to ·752 when its angle is so increased as to make its numerical aperture 1·33, equalling the theoretical maximum of a water-immersion objective, and is ·658 at the theoretical maximum (1·52) of an oil-objective.

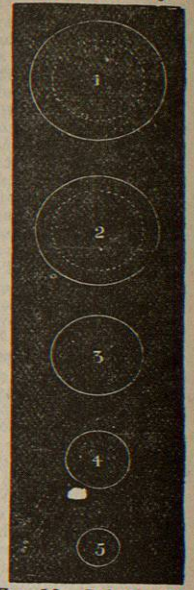

FIG. 16.—Relative Diameters of Back Lenses of Air, Water, and Oil Immersion Objectives.

Hence it is clear that, as some of the qualities to be sought in microscopic objectives are absolutely incompatible, a preference is to be accorded to objectives of greatest resolving power but very little penetration, or to those of moderate resolving power and great penetration, according to the uses to which they are to be applied; and some general principles will now be laid down in regard to this matter, based alike on science and experience.

In the first place, a marked distinction is to be drawn between those objectives of low or moderate power which are to be worked dioptrically and those of high power which are to be worked diffractively. The objects on which the former are to be for the most part used are either minute transparent bodies having solid forms which the observer should be able to take in as wholes (as in the case of *Polycystina*, the larger diatoms, *Infusoria*, &c.); or transparent sections, dissections, or injections, whose parts lie in different planes, the general relations of which he desires to study, while reserving their details for more special scrutiny; or opaque objects, whose structure can only be apprehended from the examination of their surfaces, when the inequalities of those surfaces are seen in their relations to each other. In all these cases it is desirable that microscopic vision should resemble ordinary vision as much as possible. If the eye were so constructed as to enable us to discern only those parts of an object that lie precisely in the plane to which we focus it, our visual conceptions of the forms and relations of these parts, and consequently of the object as a whole, would in general be very inadequate, and often erroneous. It is because, while focussing our eye successively on the several planes of the object, we can see the relation of each to what is nearer and more remote that we can readily acquire a visual conception of its shape as a whole, and that unmistakable perception of solid form which is given by the combination of the two dissimilar perspectives of near objects in binocular vision

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[1] The dotted circles in the interior of 1 and 2, of the same diameter as 3, show the excess in the diameters of the back lenses of the water and oil objectives over that of the dry at their respective theoretical limits.

(p. 273) could not possibly be formed if our vision were strictly limited to the exact plane for which our eyes are focussed.

Hence it is obvious that, in the case of objectives of low and moderate amplification, focal depth or penetration is a quality for the want of which no other excellence can compensate,—the opening-out of their apertures being only advantageous in so far as it does not seriously interfere with their penetrating power. It is, no doubt, quite possible to construct a 1 inch objective with an aperture so large that, when the requisite amplification has been gained by deep eye-piecing, it shall resolve the lined "tests" ordinarily used for a ¼, or to construct an objective of $\frac{4}{10}$ inch focus which shall in like manner do the ordinary work of a ⅛. But, as such objectives are thereby spoiled for their own proper work, the loss to the microscopist is but poorly compensated by his ability to resolve with them, under such deep eye-pieces as cannot be habitually used without serious risk to the eye-sight, the lined and dotted tests which can be much better shown under objectives of shorter focus and wider aperture, with eye-pieces of low amplification. For, whilst deep eye-pieces cannot be habitually employed for continuous observation, without putting a strain upon the eyes resembling that which results from the constant use of a magnifying glass, even the very highest objectives may be used continuously for long periods in combination with shallow eye-pieces, with scarcely any fatigue, and therefore (it is probable) without sensible injury.[1]

In estimating the goodness of a microscopic objective, five distinct qualities have to be separately considered:—(1) its working distance, or the actual interval between its front lens and the object on which it is focussed; (2) its penetrating power, or focal depth; (3) the flatness of its field; (4) its definition, or power of giving a distinct image of all well-marked features of an object, and especially of their boundary lines; and (5) its resolving power, by which it separates closely approximated lines, dots, or striæ.

1. The "working distance" of an objective has no fixed relation to its focal length,—the latter being estimated by its equality in power with a single lens of given radius of curvature (such as 1 inch, ¼ inch, $\frac{1}{12}$ inch, &c.), while the former varies with the mode in which the combination is constructed and with the aperture given to it. For low and moderate powers, ranging up to $\frac{4}{10}$ inch focus, good working distance is especially important, alike because it is closely related to penetrating power, and also because it facilitates the use of side-illumination for opaque objects. And in such objectives of high power as are to be used, not for the resolution of lined or dotted tests, but for the observation of living and moving objects of extreme minuteness, good working distance is no less important, on account of its relation to focal depth. In the case of those objectives, on the other hand, in which resolving power is made the first consideration, it is only needful that the working distance shall be such as to permit the interposition of a thin glass cover; and this, although necessarily diminished with the widening of the aperture, can be always obtained by the adoption of the immersion system.

2. The "penetrating power" or "focal depth" of an objective may be defined as consisting in the vertical range through which the parts of an object not precisely in the focal plane may be seen with sufficient distinctness to enable their relations with what lies exactly in that plane to be clearly traced out,—just as would be done by ordinary vision if the object were itself enlarged to the dimensions of its microscopic image. The close relation between this quality and the preceding becomes obvious when it is considered that the longer the working distance of an objective the less will the distinctness of the image it forms be affected by any given alteration (say the $\frac{1}{10000}$ of an inch) in its focal adjustment. Consequently, of two objectives having the same magnifying power but different working distances, that one will have the most focal depth whose working distance is the greater. On the other hand, as the penetrating power of an objective is reduced in direct accordance with the increase of its numerical aperture, it must be sacrificed wherever the highest resolving power is to be attained. Hence, as already remarked, this attribute will be very differently valued by different observers, according to the work on which they are respectively engaged. For the general purposes of biological research, not only with low or moderate (for the reasons already stated), but also with high powers, a considerable amount of focal depth is essential. It is impossible, for example, to follow satisfactorily the movements of an *Amœba*, or to study the "cyclosis" in the cell of a *Vallisneria*, or to trace the distribution of a nerve-thread, with an objective in which focal depth is so completely sacrificed to aperture that nothing can be discerned save what is precisely in the focal plane, since, instead of passing gradationally from one focal plane to another, as the observer can do with an objective of good penetration, he can only get a succession of "dissolving views," with an interval of "chaos" between each pair. When, on the other hand, it is desired to scrutinize with the greatest precision such minute details as are presented in one and the same focal plane (as, for example, those of the thinnest possible film of tissue spread out between a glass slide and its covering glass), the microscopist will prefer an objective in which focal depth is subordinated to aperture, for the sake of the resolving power which he can thus command. And it will often happen in biological research that it is advantageous thus to bring objectives of the latter class to bear upon objects which could not have been detected in the first instance save by objectives of much inferior resolving power but greater focal depth.

3. The "flatness of the field" afforded by the objective is a condition of great importance to the advantageous use of the microscope, since the extent of the area clearly seen at one time practically depends upon it. Many objectives are so constructed that, even when the object is perfectly flat, the foci of the central and peripheral parts of the field are so different that, when the adjustment is made for one, the other is more or less indistinct. Hence, when the central part of the area is in focus, no more information is gained respecting the peripheral than if the latter had been altogether stopped out. With a really good objective, not only should the image be distinct over the whole field at once, but the marginal portion should be as free from colour as the central. As imperfection in this respect is often masked by the contraction of the aperture of the diaphragm in the eye-piece, the relative merits of two objectives, as regards flatness of field, should always be tested under an eye-piece giving a large aperture.

4. The "defining power" of an objective, which depends upon the completeness of its corrections for spherical and for chromatic aberration, and upon the accurate centring of its component lenses, is an attribute essential to its satisfactory performance, whatever may be its other qualities,—its importance in scientific research being such that no superiority in resolving power can compensate for the want of it; and, though it is possible to obtain perfect correction for spherical aberration up to the highest practicable limit of angle, yet the difficulty of securing it increases rapidly with the augmentation of aperture, the want of it being made perceptible, especially when deep eye-pieces are put on, by the blurring of clearly-marked lines or edges, and by general "fog." Perfect colour-correction, on the other hand, is not possible for dry lenses of the widest angle, on account of the irrationality of the secondary spectrum; but this may be neutralized by the use of the immersion system. As already stated, what has to be aimed at in the construction of microscopic objectives is not absolute colour-correction, but a slight degree of over-correction, which, by compensating the chromatic dispersion of the Huygenian eye-piece, shall produce an image free from false colour. As this can be secured far more easily in the construction of objectives of moderate than in those of very wide aperture, the cost of the former is proportionally small,—an additional reason for the preference to be given to them on other grounds, in regard to all save very special kinds of microscopic work.

5. "Resolving power," being that by which very minute and closely approximated markings—whether lines, striæ, dots, or apertures—can be separately discerned, is a function which is only of primary importance in objectives whose amplifying power specially fits them for the study of objects of this class. It appears from the mathematical researches of Professor Abbe that the maximum resolving power (with a theoretical angle of 180°) would be capable of separating 146,528 lines to the inch; but he considers the limit of visual resolution depending on the power of the eye to be about $\frac{1}{118000}$ of an inch; and this limit seems to have been nearly reached. To make such a separation distinctly perceptible, an amplification of at least 3000 linear would be requisite; and this can only be obtained either by the use of an objective of very high power (such as $\frac{1}{25}$ inch focus) in combination with a low or medium eye-piece or by putting a very deep eye-piece upon an objective of lower power (such as a ⅛ inch),—the former method, for the reasons already given, being decidedly preferable. For the resolution of less closely approximated markings objectives of $\frac{1}{20}$, $\frac{1}{16}$, $\frac{1}{12}$, and ⅛ inch answer very well; and the resolving power which they require may be obtained without any excessive widening of the aperture. For the loss of resolving power consequent upon the contraction of the angle of a water-immersion objective to 128½° is only one-tenth of the theoretical maximum 128,212; while a reduction to 105⅔° only lowers the number of separable lines to 102,184 to the inch,—thus diminishing the resolving power by little more than one-fifth, while the working distance and focal depth of the combination are greatly increased, and perfect definition is more certainly attainable. The ⅛ inch is (according to the writer's experience, which is confirmed by the theoretical deductions of Professor Abbe) the lowest objective in which resolving power should be made the primary qualification,—the ⅙, ⅕, ¼, and $\frac{4}{10}$ inch being specially suited to kinds of biological work in which this is far less important than focal depth and dioptric precision. This view is strengthened by the very important consideration that the resolving power given by wide aperture cannot be utilized, except by a method of illumination that causes light to pass through

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[1] Hence, for work of this kind, the shallower eye-pieces and longer tubes of English microscopes are to be preferred to the deeper eye-pieces and shorter tubes of the ordinary Continental model, the shallowest eye-pieces of the latter being usually equal in power to the ordinary B eye-pieces of the former.