VII.—On the Estimation of Aperture in the Microscope.

By Professor E. Abbe, Hon. F.R.M.S.†

(Read 9th March, 1881.)

In originating the "numerical" definition of aperture, my special aim was to signalize the all-important fact, so long overlooked and even denied, of the existence of an unequal equivalent of equal aperture-angles in different media; to propound a simple and exact expression by means of which this unequal equivalent could be estimated; and thus to afford a definition of aperture for the practical comparison of objectives, which should exhibit the true relation of aperture to the actual performance of the Microscope, a relation which is entirely concealed by the angular expression.

As some little time must probably still elapse before my more extensive paper "On the Function of Aperture in Microscopical Vision" can be completely printed (a great part of which was laid before the Meeting of the Society in June 1880;), it has been suggested to me that it may be useful if I here summarize the principal considerations which bear upon the determination of the aperture-equivalent in the Microscope.

I.—Definition of Aperture by the Ratio of "Opening" and Power.

The general notion of "aperture," which every one forms prior to attempting any distinct definition of the term, unquestionably refers to the greater or less number of rays which are collected and utilized by an optical instrument—consequently, to the opening of the lenses or lens-systems, and to that alone. Every definition of the term must conform to this primary idea.

In the case of a telescope-objective the absolute opening of the lens is itself the proper expression of aperture; because in the depiction of distant objects by parallel (or approximately parallel) rays, no other element can have any influence on the larger or smaller number of rays admitted from an object at a definite distance. This is a matter of general agreement.

If we consider the case of the Microscope, however, the matter

is not quite so simple.

(1) In a single-lens Microscope it is evident that the number of rays admitted within one meridional plane of the lens increases in the proportion of its clear diameter, provided all other circumstances are the same. For if the lens projects a distinct image to a

[†] The original paper is written by Prof. Abbe in English.—Ed. \$\(\) See this Journal, iii. (1880) p. 735.

distance which is great in comparison with its diameter—as is always the case in the Microscope—we have at the back of the lens the same circumstances as are in front when a telescope-objective is considered. Consequently, the larger or smaller number of emergent rays will be properly measured by the clear diameter; and as no rays can emerge which have not been taken in, this estimation must apply at the same time to the admitted rays,—other circumstances, in particular the distance of the radiant from the lens, being equal.

The question, however, will now arise, how is the difference of these *other* circumstances on the microscope-lens to be taken into account?

A simple consideration shows at once that this is properly done by taking the absolute diameter of the lens (or its "opening") in proportion to the focal length. When two lenses have equal openings but different focal lengths, they transmit the same number of rays to equal areas of an image at a definite distance, because they would admit the same number if an object were substituted for the image, that is if the lens were used as a telescopeobjective. But as the focal lengths are different, the amplification of the images is different also, and equal areas of these images correspond to different areas of the object, from which the rays are collected. Therefore the higher-power lens with the same opening as the lower power, will admit a greater number of rays in all from one and the same object, because it admits the same number as the latter from a smaller portion of the object. Thus if the focal lengths of two lenses are as 2:1, and one of them amplifies an object N diameters, the other of shorter focal length will amplify the object 2 N diameters with the same distance of the image. Consequently the rays, which in both cases are collected to a given field, say of 1 mm. diameter, of the image, are admitted from a field of $\frac{1}{N}$ mm. in the first case, and of $\frac{1}{2N}$ mm. in the second.

If now the idea of aperture referred to the photometrical quantity of light, the capacities of equal openings with different focal lengths would of course be in the inverse ratio of the areas from which equal quantities are admitted, and would then be in the direct ratio of the squares of amplification. Inasmuch, however, as the opening is estimated by the diameter and not by the area, the consideration is confined to the rays which travel within one meridional plane of the lens, and the same principle must be applied to the fields from which the rays are admitted; which must also be estimated by their diameters. The higher-power lens in the example given above therefore admits twice as many rays as the lower power, because it admits the same number from a field of half the diameter; and, in general, the admission of rays with different focal

lengths (the opening being the same) must be in the inverse ratio of the focal lengths.

In a single-lens Microscope, aperture must be determined, therefore, by the ratio between the clear opening and the focal length of the lens, in order to define the same thing, as is denoted in the

telescope by the absolute opening.

(2) Regarding now composite systems—the most important case in the Microscope—the further question arises, what is the opening of such a system? The actual opening, which limits physically the transmission of the light through a composite objective, varies according to particular circumstances. It may be the margin of the front lens, or of any one of the posterior lenses, or it may be a diaphragm inserted in some part of the system. As the cone of admitted rays expands continuously from the radiant up to the back lens, the same objective admits of innumerable different openings of this kind, which nevertheless may indicate the same aperture, and thus no definite opening could be assigned. This ambiguity cannot be removed unless we adhere to the diameter of the admitted cone at that plane where it has its ultimate maximal value, which is obviously the diameter of the pencil at its emergence from the system, or, practically, the clear effective diameter of the back lens. The emergent pencil from a microscope-objective, converging to a relatively distant focus, has its rays approximately parallel, and the conditions are once more similar to those of the telescope-objective on the side of the object. The diameter of this emergent pencil, whether it emerges from a single lens or from a composite system, must therefore always have the same signification.

The influence of the power or focal length also remains the same as in the case of the single lens. An objective with a focal length equal to half that of another admits with the same linear opening twice as many rays as the latter, because the amplification of the image at one and the same distance is doubled, and the same number of rays, consequently, are admitted by the higher power from a field of half the diameter. This must hold good, whether the medium at the object is the same in the case of both objectives, or different. For an immersion system and a dry system always give the same amplification when the focal length is the same.

Thus we have as general propositions for all kinds of objectives:
(a) the admission of the rays with one and the same power or focal length varies with the linear diameter of the pencil at its emergence; (b) with different powers, the same admission requires different linear openings in the proportion of the focal lengths—or conversely, the admission by one and the same opening is in inverse proportion to the focal length. Consequently the aperture of an objective is always exhibited by the ratio between the linear

opening (at the plane of emergence) and the focal length of the

There is no other rational way of defining the admission of rays to an objective, and consequently no other definition of aperture which agrees with this fundamental idea. I need hardly say that this suggestion is nothing new. It is a matter of general consciousness; for every one will agree that the aperture of a given objective is altered when the utilized diameter of the back lens is changed by the application of different stops; and that a clear opening of say 3 mm. in a 1, is less aperture than the same clear opening in an $\frac{1}{8}$.

On the other hand, it is true that the apertures of objectives may be compared as regards equality or inequality merely by the angles of the admitted pencils, if the medium at the radiant is the same, because under this condition equal angles indicate an equal admission of rays, and different angles different admission. The assumption, however, that apertures can be defined or compared by the angle alone, is an entirely arbitrary one unless it were proved that the admission of rays is always in proportion to the angle, and does not depend on any other element. attempt at a proof has been brought forward in support of this hypothesis (it being in reality, as will be seen hereafter, opposed to the fact), the proper way of obtaining a correct expression of aperture by means of the angle will be to investigate what expression must be taken, in order to define the same thing as is denoted by the ratio of opening and focal length.

Until a comparatively recent period the above assumption has persisted as a dogma—without any investigation of the subject. The author may claim to have been the first to put this dogma to the test of scientific principles and to point out its fallacy by the indication of the unequal aperture-equivalent of objectives.

The demonstration of the general validity of this fact is given here in detail for the benefit of those who may care for such a treatment of the question.

II.—Determination of the relative Openings of Systems by the Aperture-angle and the Refractive Index of the Medium.

In 1873 the author and—quite independently—Professor Helmholtz established a general relation between the pencil of rays admitted by an optical system and the pencil emerging from it; a relation which pertains to the angles of convergence in both pencils, and must always obtain whenever a system is aplanatic, or is capable of depicting an object by means of wide-angled pencils. The proposition is:—

Let O and O* (Fig. 111) be the conjugate aplanatic foci of a

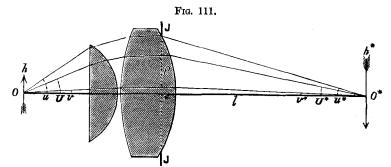
wide-angled system; u, U the angles of inclination of any two rays admitted from the radiant, and u^* , U^* the angles of the same rays on their emergence; then we shall have always

$$\sin U^* : \sin u^* :: \sin U : \sin u$$
;

 \mathbf{or}

$$\frac{\sin U^*}{\sin U} = \frac{\sin u^*}{\sin u} = \text{const} = c; \tag{1}$$

i.e. the sines of the angles of conjugate rays on both sides of an aplanatic system always yield one and the same quotient c, what-



ever rays may be considered, as long as the same system and the same foci are in question.

This proposition holds good for every composition of the system (every arrangement of media and refracting surfaces), and for every position of object and image. In point of fact, the *law of convergence for aplanatic systems*, as indicated above, is the *necessary* condition (physically and geometrically) on which depends the delineation of an image by means of wide-angled pencils. When in any case the convergence of the rays in a system of lenses is not in accordance with this condition (very approximately at least) that system will be unfit for depicting an image of an object, except by narrow-angled pencils.

Microscope-objectives do of course depict images with wideangled pencils, and consequently the proposition must apply to them without any restriction; the author, it will be remembered, has suggested a simple experiment † by which every one may satisfy himself that all such objectives, if moderately well made, are in perfect accordance with this statement.

Suppose now, that for any particular objective and any particular position of the conjugate foci of object and image, the value of the constant quotient c of formula (1) is determined numerically, in any way whatever; it will then be possible to compute the

† See this Journal, iii. (1880) p. 511.

obliquity u^* of any emergent ray from the obliquity u of the same ray at its entrance, by means of the equation

$$\sin u^* = c \sin u \,; \tag{2}$$

393

and if this equation is applied to the ray of utmost obliquity which is transmitted through the system, u^* will express the semi-angle of the emergent pencil, whilst u is the semi-angle of the admitted cone of light or the semi-angle of aperture.

The *linear* opening of the system, or the diameter of the delineating pencil at the plane of its emergence, is readily calculated by means of the angle u^* and the distance at which the image is projected. If J is the plane of emergence (the plane of the back surface of the system) and l the distance of the image from J, the linear *semi*-diameter ρ of the pencil is, obviously,

$$\rho = l \tan u^*$$

for which may be substituted the identical equation

$$\rho = l \, \frac{\sin \, u^*}{\cos u^*} \cdot$$

In the case of microscope-objectives, the distance l (the length of the microscope-tube) is always many times greater than ρ , and accordingly the angle of convergence u^* is always very small, never exceeding a few degrees. The *cosine* of such an angle may be put = 1 without appreciable error; and taking now the value of $\sin u^*$ from the equation (2) we obtain

$$\rho = c \, l \sin u, \tag{3}$$

which expresses the linear semi-aperture of the system by the semi-angle of aperture.

The question will now arise: how is the value of c for every particular case to be obtained?

This is established by a dioptrical proposition of older date, which is known as the Lagrange-Helmholtz law of convergence of infinitesimally narrow pencils. If O and O* denote conjugate foci, h the diameter of an object at O, and h^* the diameter of its image at O*, n and n^* the refractive indices of the media in front and at the back of the system, whilst v and v^* are the angles of obliquity of any ray traversing the system close to the axis, then we have always

$$\frac{v^*}{v} = \frac{n}{n^*} \cdot \frac{h}{h^*}; \text{ or } = \frac{n}{n^*} \cdot \frac{1}{N}, \tag{4}$$

where N denotes the linear amplification of the system for that pair of conjugate foci; and this holds good for every composition of the system and for every position of the conjugate foci. According to this proposition, the ratio of infinitesimal angles v and v^* (pertaining to one and the same ray at its entrance and emergence) may be completely determined by the refractive indices of the media at the radiant and at the image and by the linear amplification of the image, without regard to the elements of the optical system or to the position of the foci.

This important theorem was established by Professor Helmholtz in 1866.† Its earliest origin must be traced back to Lagrange, who pointed out a similar proposition, confined however to less

general conditions, in 1803.‡

The way in which equation (4) leads to a general determination of the constant c, which appears in the law of convergence of wide-angled pencils, will be readily understood. Any wide-angled cone of rays admitted to an aplanatic system, embraces axial rays of infinitesimal obliquities v and v^* , and as in the case of very small angles the ratio of the sines becomes identical with the ratio of the angles, the value of c in equation (1) must, as far as it pertains to those axial rays, coincide with the value of $\frac{v^*}{v}$ as determined by the Lagrange-Helmholtz formula. But as the condition of aplanatism requires the same value of the quotient $\frac{\sin u}{\sin u}$ for all rays of the wide-angled pencil, we must have for all cases

$$c = \frac{n}{n^*} \cdot \frac{1}{N}.$$
 (5)

Introducing this expression of c into equation (3) and taking into account that in the case of the Microscope the medium at the back of the system is always air $(n^* = 1)$, the linear semi-opening of an objective is

$$\rho = \frac{l}{\mathbf{N}} \cdot n \sin u; \text{ or } \rho = \frac{l}{\mathbf{N}} \cdot a, \tag{6}$$

a being put for $n \sin u$, and therefore denoting the product of the sine of the semi-angle of aperture and the refractive index of the medium to which this angle belongs.

When an objective has a focal length = f and an image is projected at a distance = l from the lens, the amplification N of this image will be, very approximately,

$$\mathbf{N} = \frac{l}{f}$$

whence it follows

$$\frac{l}{\mathbf{N}} = f.$$

^{† &#}x27;Physiologische Optik,' 1866, p. 50.

[‡] Mém. Acad. Berlin, 1803.

The quotient appearing in the expression of ρ is thus shown to be nothing else but the equivalent focal length of the system; and we have now

$$\rho = f(n \sin u), \text{ or } \frac{\rho}{f} = n \sin u = a.\dagger$$
(7)

The ratio of the linear semi-opening of any system to the focal length of the system is expressed by the value of a or by the "numerical aperture." The value of n sin u is the aperture-equivalent of every objective whatever may be the medium in which the radiant is placed.

III. - Inferences from the Aperture-equivalent.

The simple result of the foregoing demonstration may be summarized as follows:—

(1) There exists a definite ratio between the linear opening and the focal length of a system, which must be entirely independent of the composition and arrangement of the system, and solely determined by the above-mentioned aperture-equivalent of the admitted cone of rays. When this equivalent is the same, we have always the same proportion of opening to focal length, whatever may be the particular arrangement of refracting media in the system.

(2) A purely angular determination of aperture is shown to be irreconcilable with any rational notion of a term which must be defined essentially in relation to opening. Aperture it is seen cannot be expressed by an angle, nor by any mathematical function of an angle alone, but must be determined by a composite function of the angle and the refractive index of the medium to which the angle belongs.

(3) Even with one and the same medium at the radiant, aperture does not increase or decrease in proportion to the angle, but with the *sine* of the semi-angle (or the chord of the whole angle). If the angle is changed from 60° to 180° , the aperture is not changed in the proportion of 1:3, but of 1:2 only.

† The above formulæ hold good in perfect strictness, if the distance l of the image is taken not from the accidental plane of the back-surface, but rather from the posterior principal focus of the system (i. e. the place where are depicted distant objects in front of the system). The equation (7) will therefore afford a strict expression for the semi-diameter of the emergent pencil at the plane of the posterior principal focus of the system. In microscope-objectives of the ordinary type of construction that focus is always very near to the back lens of the system, and the difference may be disregarded practically.

At first sight it might appear to be more convenient to define the aperture-equivalent by $2 n \sin u = 2 a$, instead of a, in order to express the ratio of the diameter of the opening (instead of the semi-diameter) to the focal length. In mathematical dioptries, however, the angles of the rays with the axis, and, correspondingly, the distances of points from the axis are always given as the effective elements. To introduce the double of these angles and distances is not only unnecessary, but would give rise to a somewhat inconvenient complication of all mathematical expressions.

(4) Equal angles of the admitted pencils from radiants in different media do not yield equal apertures, but apertures which are in the exact ratio of the refractive indices of those media. Thus the diameter of the emergent pencil of an immersion glass which takes in a cone of say 120° from an object in balsam, is greater in the proportion of 3: 2 than the diameter of the emergent pencil of a dry lens of equal power admitting the same angle from an object in air. Attentive microscopists and opticians have long since noticed the fact, that immersion objectives require and utilize much larger back lenses than equal-power dry systems of similar

aperture-angle.

(5) An immersion objective may have a greater aperture than any dry lens of even 180° aperture-angle can have. The maximal opening of a dry lens (i. e. the maximal diameter of the pencil emergent from such a lens) is shown by proposition (7) to be exactly double its focal length, for as $\rho = f(n \sin u)$ and n = 1and $\sin u = 1$ for air, $\rho = f$ or (for the whole diameter) $2\rho = 2f$. No lens performing on objects in air (n = 1) can therefore ever admit of a wider aperture, because no angle u is possible whose sine is >1. When, however, the object is in a denser medium (and no film of air with plane surfaces is between that medium and the system) an angle of aperture which is much less than 180° (exceeding only the double of the critical angle for the medium) will utilize and require a wider opening of the system than 2f. The excess of the numerical aperture of an immersion glass beyond the unit gives a direct expression of the surplus of aperture over the maximal aperture of a dry lens of an air-angle of 180°.

(6) The unit of aperture is exhibited by an objective which gathers-in the whole hemisphere of radiant light in air. The value of a for any given objective shows the capacity of that objective in comparison with the capacity of another of maximal

air-angle.

Any one who has not comprehended the generality of the demonstration, may object that the greater or less opening required for the transmission of a pencil of given angle depends on the particular mode in which that pencil is refracted by the lens-surfaces of the system. A pencil of 120° in air requires, it will be said, a smaller opening than the same pencil in balsam when a homogeneous-immersion objective is used, because in a dry lens it is contracted on its entrance into the system by the refraction of the plane front-surface, whilst the pencil in balsam, owing to the abolition of the front-refraction by the immersion, is not subjected to such contraction, and therefore maintains a greater linear diameter up to the plane of emergence.

The fallacy of such an objection is readily shown: Take a front lens with a concave surface of admission, of such a curvature

397

(Fig. 112), that the focus or radiant for an uncovered object is exactly at its centre. The refraction is now abolished (in regard to the pencil from the radiant) just as if there were homogeneous

immersion. If the above-mentioned view were correct, the consequence ought to be, that a dry lens with such a front would utilize a wider opening than an equal-power dry lens of the ordinary plan, and the same opening as an equal-power immersion glass of a balsam-angle equal to the air-angle in question. But, of



course, the contrary is the actual fact. An ordinary dioptrical computation shows that whenever such a dry objective with concave front has the same power or focal length as a plane-front system of equal aperture-angle, its opening must be also the same, exactly

—as the general principle of formula (7) indicates.

A misapprehension on this point has arisen thus:—If a homogeneous-immersion objective is taken, and its front-surface is ground to a concave of the above description, whilst all other elements are left unaltered, the angle admitted from air by the objective will be no wider than that which was previously admitted from balsam, but nevertheless the full opening will still be utilized. This seems to prove, and indeed has been asserted to prove, that after abolishing the front-refraction, a given air-angle will yield the same opening as an equal balsam-angle. This, however, is a transparent fallacy. According to well-known elementary propositions, a concave surface diminishes an object at its centre in the proportion of the refractive index n of the lens-substance. Consequently, the objective in question has been changed into an n-times lower power; and utilizing still only the same (and not a larger) back lens, it has necessarily a smaller aperture. To restore the original focal length it would be necessary to increase the depth of the posterior lens-surfaces in such a way that the pencil should be by them contracted to the same small diameter which otherwise it would have had with a plane front.

Whatever particular composition of objective is considered, the result must always be the same. The relation between the aperture-angles for different media and the corresponding openings of the systems, as defined by proposition (7) cannot depend in any way on the manner in which the pencils are refracted in the system. A pencil from a radiant in air must always yield a smaller aperture than an equal pencil from a radiant in balsam, whether there is refraction or no refraction at the front surface of the system. Consequently the difference of aperture with equal angular pencils in different media must originate from a difference in the pencils themselves, that is, must be founded on the different physical nature of pencils in different media.

IV.—Experimental Demonstration of the Aperture-equivalent.

In the foregoing discussion I have demonstrated the aperture-equivalent without regard to experiment and on *general* optical principles only, as is necessary for an exhaustive scientific settlement of the subject. A theoretical discussion of this kind is, however, by no means required for demonstrating the *essential* principle of "numerical" aperture. That there is an *unequal equivalent of equal angles in different media* in regard to aperture, is a fact which may be readily shown by observations of the most simple character. I confine myself to a few examples which have been referred to in previous discussions.

(1) If any dry lens of an aperture-angle w for objects in air, is focussed on a balsam-mounted object, with a plane surface of exit, the aperture-angle at the radiant is of course reduced to a smaller angle v, according to the condition

$$n\sin\frac{v}{2}=\sin\frac{w}{2}\,,$$

in which n denotes the refractive index of the balsam. It is, however, clear that the amplification of the image is not changed—the power of the system is the same still—whilst the linear diameter of the emergent pencil remains the same also. Consequently, the ratio of the opening to the focal length—i. e. the aperture—is not reduced.

This simple fact thus contains a direct proof of the proposition that different angles in different media may denote equal apertures

The idea of aperture being, as has been said, dependent on that of "opening," the assertion that aperture is "cut down" by the balsam, or by the immersion, is obviously an abuse of the term, independently of the fact that the assertion is not supported in any way by what we know as to the actual performance of objectives with these "cut-down" apertures.

(2) Moreover, suppose the same objective of w° air-angle to be focussed on an object in balsam, the surface of exit, however, being no longer a plane surface, but a spherical one, the object being at the exact centre of a small hemisphere of glass or balsam;—or suppose the original objective to be provided with an extra immersion-front, the centre of the curvature of which coincides with the focus. In this case the angle of the admitted pencil will be the same for the radiant in glass or balsam as it was for the radiant in air; and the clear opening will also be the same still. It would, however, be obviously a mistake to say that the objective had now undergone no change of aperture, or that the full aperture was now made to bear upon a balsam-mounted object. For it is an

elementary truth that a hemisphere of refractive index n amplifies an object at its centre by exactly n diameters. Consequently the hemisphere or extra front has changed the original objective into one of n-times higher power or shorter focal length, but it nevertheless utilizes the full opening of the lower power. Consequently the aperture is also increased in the proportion of 1:n, whilst the aperture-angle remains the same.

If, for instance, a $\frac{1}{4}$ of, say, 60° air-angle has the extra front of crown glass, it would be converted into a $\frac{1}{6}$ of 60° balsam-angle, utilizing the full original opening of the $\frac{1}{4}$. But a $\frac{1}{6}$ of 60° air-angle would of course have a smaller opening than a $\frac{1}{4}$; for such an objective would be obtained by reducing all elements of the former $\frac{1}{4}$ in the proportion of 3:2, whereby the opening for the air-angle of 60° would be reduced in the same proportion.

Thus it is shown that equal angles pertaining to different

media are different apertures.

(3) The other inference from the principle of the apertureequivalent—that an immersion objective can have a greater aperture than the widest-angled dry lens—also admits of a direct experimental demonstration. Mr. Stephenson † has already pointed out the remarkable experiment (and has given due prominence to its bearing on the aperture problem), by which it is shown to every one's eyes that the aperture of a wide-angled immersion glass is cut down, when it is made to act as a dry lens even with an angle of nearly 180°. Take any immersion objective of balsam-angle exceeding the double of the critical angle, and focus it on a balsam-mounted object which is illuminated by any kind of immersion condenser, in such a way, that the whole range of the aperture-angle is filled by the Remove the eye-piece and place the pupil of the eye incident rays. at the place where the air image is projected by the objective, and look down on the lens. You see a uniformly bright circle of welldefined diameter which is the true cross-section of the imageforming pencil emerging from the Microscope (for the eye receives now all rays which have been transmitted through a small central portion of the object—that portion which is conjugate to the pupil -and receives no other rays). After this, focus the same objective on an ordinary dry-mounted preparation (or on one which is connected with the slide, the cover-glass being put on dry), and repeat the observation; you will now see again a well-defined circle, a crosssection of the emergent pencil, but of less diameter than in the former case, surrounded by a dark annulus, visible by faint diffused light only.

The diameter of the emergent pencil in both these experiments may be accurately *measured* if the "auxiliary Microscope" of the author's apertometric apparatus is used with an eye-piece micro-

meter. The proportion of the clear openings (or effective diameters) with the object in balsam or in air may thus be strictly ascertained. If the objective should be rated at, say, 1·20 num. ap., the ratio of the diameters will always be found 6:5 (i.e. as 1·20 to 1·00); and if, in another objective, the num. ap. should be 1·40, this ratio will always be as 1·40 to 1·00 or as 7:5.†

The interpretation of this experiment is plain. In focusing an immersion objective on an object with air above, it is obviously converted into a true dry lens, the under surface of the covering glass acting as the plane front-surface of the system. If the covering glass is very close to the object the distance of the radiant from the plane surface will be so small that an exceedingly small central portion of this surface is sufficient for admitting to the front all rays up to an obliquity of 88° to 89°. The objective then acts as a dry lens of nearly 180° aperture-angle, and gathers-in almost the whole hemisphere of light from the radiant in air; whilst the same systems when focussed on an object in balsam, admit no wider cone (in the examples mentioned in the preceding paragraph) than 108° or 138°, in fact much less in each case than a hemi-Nevertheless, the emergent pencil of rays is much narrower with the whole hemisphere of rays in air than it is with the smaller cone of rays in balsam, whilst the amplification of the image is not increased—the power of an optical system of any kind whatever being exactly the same, whether there is refraction or no refraction at its anterior plane surface.

Every one will concede that there is a true reduction of aperture when a brass stop is inserted at the back of a given system, stopping off a certain marginal zone of the clear opening which

 \dagger According to the general proposition (7) the linear diameter of the reduced opening of an immersion glass with a dry object must be =2f, provided the film of air beneath the covering glass be very thin. By measuring the reduced opening of such an objective in the way suggested above, and taking its half, the exact focal length of the system is obtained.

The same principle may be made use of for objectives of every kind. When the numerical aperture of an objective (or the numerical equivalent of any smaller angle within the aperture-cone) is determined, and the linear diameter of the corresponding emergent pencil at the plane of the posterior principal focus of the system is measured micrometrically, the focal length is at once obtained from formula (7).

The author has for many years applied this very convenient and accurate

method for measuring focal lengths.

On the other hand, the proposition (6) also indicates a new method for measuring apertures and aperture-angles. When the amplification N of an objective for a definite position of the image O^* is ascertained, by projecting the image of a stage-micrometer upon an eye-piece micrometer, the auxiliary Microscope may be focussed to any convenient plane and the linear diameter 2ρ of the emergent pencil measured there. If now the distance l of that same plane from the image to which the amplification N relates, is measured likewise, we have all the elements for computing the strict value of $a = n \sin u$ —and of the angle u also—by means of formula (6). This method enables us to measure immersion-apertures without requiring a disk of glass or similar devices.

was formerly utilized by the image-forming rays.† It must also be a *true* reduction of aperture when, in any way whatever, the emergent pencil is changed as if such a brass stop had been inserted, provided the power of the system is unaltered. Consequently we have loss of aperture when an air-angle of 180° is substituted for a balsam-angle of, say 100°.

An immersion objective of balsam-angle exceeding twice the critical angle has therefore a *greater aperture* than any dry lens can ever have.

V.—Different Angular Distribution of the Rays in different Media.

The definition of aperture as relative opening, developed in the foregoing discussion, is, it is seen, the only one which is justified by the original sense of the term, and it is a point of special importance that it should be understood that the definition is not a matter of mere terminology, but that the very essence of the idea of aperture is involved in the notion of opening, and that there is no other reasonable base for grasping this essence. In whatever way the idea of aperture may be defined, the actual significance of that element in the Microscope can only be appreciated by taking into account the image-forming pencil emergent from the objective, and the change in its diameter consequent upon the admission of different cones of light. This diameter affords a visible indication of the number of rays (not mere quantity of light photometrically) which are collected to a certain area of the image, and which consequently must have been gathered-in by the lens from the conjugate area of the object. If, in any case whatever, the diameter of the emergent pencil is seen to be increased, whilst the amplification of the image and the distance of its projection (or, more generally speaking, the focal length) are unchanged, it is clear that the system must have admitted more rays from every element of the object, because it has collected more to every element of an equally enlarged image. It would be an obvious physical absurdity to declare that in any case a lens could emit more than it has taken in. Consequently we get a true measure of what is admitted by a system by estimating what it emits.

Thus the essential idea of aperture (which means the greater or less capacity of objectives for gathering-in rays from the objects) necessarily leads to the estimation of apertures by the *openings* of the systems.

As long as we have the radiant in one and the same medium,

[†] If it should be objected that in wide-angled immersion glasses the marginal zone does not transmit image-forming rays, every one may satisfy himself at once by a simple practical trial that in a well-corrected objective all emergent rays up to the edge of the clear opening are image-forming rays.

the increase of the admitted rays with increased opening is very simply accounted for. We see the additional portions of the solid cone from the radiant, which correspond to the additional portions of the enlarged opening. But if in any other case (for instance, when the medium is different) we see that a certain solid cone A from a radiant is transmitted through a certain opening a, and that another solid cone of rays B cannot be transmitted through the same opening a, but requires a wider one β , whilst all other circumstances, except those of the radiant, have remained the same, we can of course only conclude that the pencil B must contain rays which are not contained in A, even if the admitted cone is not increased in size. For the additional portion $(\beta - \alpha)$ of the wider opening β conveys rays to the image which are certainly not conveyed by the smaller opening a. Whence can this surplus come if not from the radiant? Obviously the pencil B, which requires the additional opening, must embrace more rays, even if it should not be of greater angle.

Now the fact is, that a given objective may collect the rays from a radiant in air almost to the entire hemisphere (as, for instance, in the case of an immersion lens when focussed on a drymounted object close to the covering glass) and it then utilizes a definite opening, double its focal length. But when the radiant is in balsam (without any other alteration), the same opening is seen to be utilized by the rays which are within a smaller cone of not more than 82°, and rays which are outside this cone require a surplus of opening, which is never required for rays in air. This holds good, as has been shown, whether there be refraction or no refraction at the front surface of the system; the difference is based solely on the difference of the medium. Consequently we arrive at the conclusion that the solid cone of 82° in balsam embraces the same rays which in air are embraced by the whole hemisphere; and every wider cone in balsam, exceeding the 82°, conveys more rays from the object than are admitted by the whole hemisphere of radiation in air.

The definitive inference from the foregoing consideration is obvious. There is no way of reconciling the seeming contradiction between these two facts, (a) that a cone of $> 82^{\circ}$ from a radiant in balsam embraces more rays than a cone of 180° from a radiant in air, and (b) that the angular extension of the former cone is less than that of the latter, except by admitting the physical fact that the same rays which in air are spread over the whole hemisphere, are closed together, or compressed, in balsam within a narrower conical space of 41° around the perpendicular; and all rays which travel in balsam outside this cone constitute a surplus of new rays, which are never met with in air, that is, are not emitted when the object is in air.

There are various direct proofs that the angular distribution of the radiating light is changed whenever the medium of the radiant is changed. The rays which emanate from a given object in different media are not, it is true, numbered like the sheep of a flock, and it is impossible therefore to show the identity or non-identity of certain rays under different circumstances without having first established an express principle of identification. This will be required for the view above expressed just as well as it would be required if any one should try to prove the (assumed) indifference of the medium in regard to radiation. There is one particular case, which however is of considerable importance for the Microscope, in regard to which such a principle may be readily established.

When a preparation contains transparent (perfectly pellucid) portions, the depiction of which yields the outlines of the nontransparent elements in the microscopic field, the rays emitted from such portions are purely transmitted rays. Every ray emanating from a transparent element of the object is the direct continuation of one distinct ray which is thrown upon that element by the illuminating apparatus. Suppose now an object of this kind, having a perfectly flat upper surface, and connected to the slide, in the one case uncovered, in the other case mounted in water or balsam under a cover-glass, and illuminated by means of an immersion condenser which collects a pencil of not less than 82° (measured within the slide) upon every point of the microscope-field. In both these cases one and the same transparent element will send into the objective, by virtue of transmission, the same incident rays; but when the object is in air, these same rays are distributed above the object in a different manner to that which obtains when the object is in water or balsam. In the former case all rays which are embraced by an incident cone of 82° within the glass slide make up the whole hemisphere; whilst with water above, the same rays are contained within an emergent cone of 96°, and with balsam within 82°.

Under the circumstances in question, those rays transmitted through the object are of course *identical* rays—notwithstanding their different directions in air, water, or balsam—which are the continuation of identical incident rays. In regard to that kind of radiation, therefore, on which the delineation of the *outlines* of nontransparent or semi-transparent objects is based, a pencil of 82° in balsam or of 96° in water, conveys the same rays to the Microscope as the whole hemisphere in air, and there is a different angular distribution of the radiating light in different media. In this case the *causa efficiens* of the phenomenon is, of course, the different refraction with which the transmission is connected. A dioptrical explanation of the varying distribution does not, however, change the fact that there is such distribution.

A general criterion of identical and non-identical rays in different media, which applies to every kind of radiation and leads to the same conclusion, is obtained when we refer to the physical notion Physical optics defines "rays of light" as the orthogonal trajectories through a system of waves. The principle of this definition implies, at the same time, that "homologous rays" in different wave-systems are to be determined with regard to the rate of propagation in these systems; and it is found that homologous rays are closer together when the velocity of propagation is less, and vice versâ—in perfect analogy to the "lines of force" in a magnetic or electric field when the electric or magnetic charge is increased. The direct outcome from this is, that identical rays emanate under smaller angles of obliquity in a medium of higher refraction, and, in general, one and the same system of rays constitutes cones of different angles in air, water, or balsam, in such a way, that the "numerical" equivalents of these various cones (the product of the sine of the semi-angle by the refractive index) are always the same.

This theoretical inference bears directly on that kind of radiation which is the most important one for the Microscope -the radiation of objects by diffracted light. Every structural object, whether the structure is regular or in any way irregular, which transmits or reflects a narrow-angled incident beam of light (or any number of such making together a wide-angled cone) changes this beam (or each one of the several beams) into a wider or narrower penoil, with varying intensity in different directions, by virtue of diffraction. The interference of elementary waves emitted from the transparent or semi-transparent elements of the structure neutralizes the undulatory motion above the object in some directions, whilst in other directions the survival of the motion, or of a fraction of it, develops rays of light of various intensities, which emanate from the object in various directions as if it were selfluminous. In the case of regular periodic structures, as lined objects, diatoms, &c., the diffraction pencil originating from an incident beam appears as a fan of isolated rays of decreasing intensity around the direction of the incident beam transmitted through the structure—the interference of the primary waves yielding in this case a number of successive maxima of light with dark interspaces. According to the well-established laws of the diffraction phenomenon, the fan of diffracted beams from one and the same structure is spread out under a wider angle when the wave-length of the medium is increased or the refractive index is diminished, and is more compressed together in the opposite case; in such a way, that the sine of the angle of obliquity of the same beams—for instance, the first, or second . . . maximum—is changed in the inverse ratio of the index. Owing to this, one and the same solid cone at the object will embrace a larger number of diffraction

405

beams in balsam than in air; and if the elements of the structure are very minute a solid cone exceeding in balsam the angle of 82° will contain beams which do not exist at all when the same structure is in air, because they cannot be originated except with waves of *shorter* length than are in air.

Experiments, which have been fully described, demonstrate ad oculos the admission of these beams of diffracted light to the Microscope and the effects which are attendant upon the admission of more or less of them in regard to the microscopical image. It is shown that the diffracted light emanating from the objects may utilize the whole aperture of a system, although the incident cone of light, if it were simply transmitted (in the absence of an object), would fill only a small portion of the aperture. In particular it may be seen experimentally, that with a narrow illuminating pencil a wide-angled immersion glass may gather in, and collect to the image, rays from an object in water or balsam, which are not met with in the whole hemisphere when the object is in air, and consequently can never be utilized by a dry lens of any aperture-angle whatever.

Owing to the general principle of physical optics mentioned above, homologous diffraction beams from one and the same structure—for instance, the first, or second . . . maximum in the case of a periodic structure—are the same rays physically, notwithstanding their different obliquity, and diffraction beams which show the same obliquity in different media are different rays physically. Thus the phenomena of diffraction in the Microscope afford another experimental proof of the validity of the inference from the principle of the aperture-equivalent: that there is an unequal angular distribution of radiation in different media, and that a given solid cone from a radiant in balsam may contain more rays than the same cone from a radiant in air, because the same

rays are closer together, and others are introduced.

The above considerations lead to the following conclusions:—

- (1) The unequal equivalent of equal aperture-angles indicates a different number of rays, as conveyed by equal cones in different media consequent upon a different density of radiation in such media; and this is quite distinct from any photometrical estimation of the quantity of light in these cones, which may vary independently according to the illumination of the object, the change of its surface by different media, &c.
- (2) An aperture-cone exceeding twice the critical angle of the medium to which it pertains, embraces a surplus of rays which do not exist, physically, when the object is in air, because they are not emitted into air. A wide-angled immersion glass, therefore, may utilize rays from an object in

a denser medium, which are entirely lost for the image—which, in fact, do not exist—when the same object is in air or is observed through a film of air. This loss can never be compensated for by increase of illumination, because the rays which are lost are different rays, physically, to those obtained by any illumination however intense in a medium like air.

It is not surprising that a notion of aperture—the angular notion—which is so incomplete and so misleading in regard to the most characteristic feature of the performance of the Microscope should have been abandoned. Adhering to the angles merely, and disregarding the influence of the medium, has entirely concealed from many microscopists even those plain truths which have long ago been settled by the practical use of the instrument. Inasmuch as the experience of two decades has established beyond any doubt the fact, that immersion objectives readily depict minute structures which are not shown by the most perfect dry lens, whatever may be the illumination, it is strange that it can still be supposed anywhere at this day that the true advantage of the immersion method cannot be anything beyond greater convenience in regard to working distance and some (very moderate) gain of light from the abolition of front-reflection—because the apertureangle of these objectives cannot be greater than with dry lenses.

If any person, who agrees that a rational definition of aperture can only be established on the basis developed here, should yet dislike the expression "numerical aperture"—for any reason whatever—I certainly do not object to another term, if a better can be found. In point of fact, I was obliged to introduce this term for the mere sake of preventing confusion. It is in reality objectionable, as the word "numerical" conveys the idea that a particular description of aperture, among others on an equal footing, is intended to be denoted. From my point of view, the aperture-equivalent should be called "aperture" sans phrase, because it is

"aperture sans phrase."

VI.—The Photometrical Equivalent of different Apertures.

Difference of aperture must of course always correspond to a different quantity of light admitted to the objective, provided all other circumstances are equal; and thus the question of aperture has necessarily also a photometrical aspect which leads to the consideration of the photometrical equivalent of different apertures or aperture-angles. But it is clear that this point of view does not meet the real essence of the aperture problem. The brightness of the image (which of course alone will depend on the photometrical equivalent) is certainly a matter of practical importance in the Microscope; but if a greater aperture signified nothing more

than greater quantity of light—if there were no *specific* difference of the rays which can be utilized by different apertures—the whole question would be only of somewhat subordinate interest. More light *from* an object can always be gained when more is thrown *upon* the object by means of a brighter source of illumination.

Inasmuch, however, as the determination of the photometrical equivalents of different apertures affords an additional *illustration* of numerical aperture, it will be useful—for the sake of completeness merely—to add a brief outline of the photometrical principles relating to the matter, though nothing can be said here which has

not been established long ago.

(1) In the last century Bouguer † and Lambert ‡ established the important fact that with any surface of uniform radiation (so called) the intensity of the emitted rays is not the same in all directions. The power of emission and the intensity of the rays (i. e. the quantity of light emanating from a given surface-element within a cone of a given narrow angle) varies in the proportion of the cosine of the angle of obliquity under which the ray is emitted. This proposition is nothing more than the expression of the simple fact, that a surface of uniform radiation shows the same visual brightness in all directions; and that such a surface, if curved (for instance the sun, or the porcelain shade of a lamp, &c.), is always seen projected as a surface of uniform brightness.

This theorem, which at a later period was confirmed by Fourier, Melloni, and other physicists, shows at once that the quantities of light emitted from one and the same object within solid cones of different angles are not in the ratio of these solid cones, but in the ratio of the squares of the sines of their semi-angles. Thus the whole emitted light (embraced by the entire hemisphere of radiation), and that portion which is emitted within a cone of 30° around the perpendicular (or 60° angle) are not, as is so constantly assumed, in the ratio of 7.46:1 (as the solid cones in fact

are), but in that of 4: 1 only.

As in one and the same medium the number of rays conveyed by a pencil and the photometrical quantity of light are proportional, this old-established Lambert theorem is sufficient of itself for overthrowing the very basis of the angular expression of aperture, and for proving that even when we are dealing with one and the same medium only, the angle is not the sufficient expression, but that it is the sine of the semi-angle which must be taken.

(2) In more modern times, but still seventeen years ago, a distinguished physicist, well known in England, R. Clausius, established by a famous research "On the Concentration of

^{† &#}x27;Traité d'Optique sur la Gradation de la Lumière,' 1760. ‡ 'Photometria,' 1760.

Calorific and Luminous Rays and the Limits of its Efficiency,"† another proposition pertaining to radiation in different media, viz. that the power of emission of a body—in regard to heat as well as to light—is not the same in different media, but varies in the ratio of the squares of the refractive indices, so that the whole emitted light from any surface-element of a self-luminous body is increased in the proportion of $1:n^2$ when this body is brought from air into a denser medium of refractive index n. If a glowing body at a constant temperature, such as a bar of iron, could be immersed in a medium of 1:5 refractive index, in such a way that the surface were in optical contact with the medium, and the eye of an observer immersed likewise (the diameter of the pupil being kept unaltered and the loss of refraction at the cornea compensated for), the body would be seen brighter in all directions in the proportion of 9:4 than it appeared in air.

The principle of Clausius applies also to the diffused radiation of non-self-luminous bodies, provided their internal structure and surface are not changed by the surrounding media. An object which fulfils this condition (without which of course there could not be a constant illumination)—for instance, a polished plate of porcelain glass—gives out by diffused reflection or by diffused transmission a greater portion of the incident light, if the radiation takes place into oil or in any other dense medium, than when it takes place into air as can be shown by a simple experiment.‡

The principle of this varying emission in different media is not so far from a rational explanation as it may appear on a superficial consideration. "Quantity of light" is the energy of an undulatory motion. A "constant illumination," or equal intensity of radiation, means equal amplitude and equal frequency of the undulation at the radiating surface. These circumstances being equal, the amount of undulatory energy which is transmitted by the waves to any definite surface (for instance, to the whole surrounding hemisphere) must depend on the density of the propagating medium which is excited by the primary motion—because the vis viva of every single wave of given amplitude is greater in the proportion of this density. In fact, the stroke of a bell or the human voice is found to give a louder sound in the dense atmosphere at the level of the sea than in the rare air on high mountains. According to the theory of Fresnel, the relation of the densities of any two media in respect to the propagation of luminous

^{† &}quot;Ueber die Concentration von Wärme- und Lichtstrahlen," &c., Pogg. Annalen d. Physik, exxi. 1864.

[‡] The author has furnished to Mr. Crisp a little piece of apparatus for demonstrating ad oculos the fact, that a thin polished plate of porcelain glass illuminated from the back, throws, from a given area, an evidently greater quantity of light into a block of crown glass (cemented on), than an equal area of the same plate under exactly the same illumination throws into air.

waves is expressed by the squares of the refractive indices of these media †

(3) Further, in 1874 another well-known and distinguished physicist, Helmholtz, confirming certain propositions of the author which were directed to the same subject, demonstrated ‡ a similar principle pertaining to the photometrical equivalent of the pencils of light which travel from a luminous object through different media successively. In this case the quantity of light conveyed by equal solid cones is also in the ratio of the squares of the refractive indices of the media.

From these established theories of photometrical optics it is seen that the quantity of light emitted from an object under a given illumination is not measured by the angle of the emitted cone at the radiant, nor can it be measured in any way by means of the angle alone. The quantity depends under all circumstances on the product of the sine of the semi-angle and the refractive index of the medium in which the object emits, and is expressed by the square of this product, or by the square of the "numerical" aperture of the pencil.

Thus it is shown that the *general* aperture-equivalent, which is defined by the value of a, indicates at the same time the *photometrical* equivalent of different apertures.

The practical outcome, as regards microscopical vision, of this photometrical inference is the general proposition of the *illuminating power* of the Microscope, or the brightness of the microscopical image, first propounded in the author's paper of 1873,§ and in that of Professor Helmholtz quoted above:—

If the losses of light by reflection and absorption in an optical system are disregarded, the brightness of the microscopical image under a given illumination of the object depends solely on the linear diameter of the transmitted pencils of light at their emergence from the ocular, and is always the same when this diameter is the same, whatever may be the composition of the Microscope (objective, eye-piece, &c.) and the amplification of the image. The diameter of the ultimate emergent pencil, or the cross-section of this pencil, is visible within the so-called "Ramsden circle" above the

[†] The supposition of cold and hot air would render the accordance of the circumstances of the accoustical and the optical phenomena still more complete. But as, under the point of view in consideration, the causa efficiens is the density of the medium, and not the velocity of propagation, the difference is immaterial.

of the medium, and not the velocity of propagation, the difference is immaterial.

The above popular elucidation of the principle is not, of course, intended as a scientific demonstration. It is only given for the purpose of showing that common sense is by no means on the side of opposite opinions. The demonstration of Clausius, moreover, does not depend on the hypothesis of Fresnel nor on any other assumption which can be a matter of dispute among physicists.

nor on any other assumption which can be a matter of dispute among physicists.

† "Die theoretische Grenze für die Leistungsfähigkeit der Mikroskope,"
Pogg. Annalen d. Physik, Jubelband 1874, p. 564.

[§] Arch. f. Mikr. Anat., ix. (1873) p. 438.

ocular. When this diameter is greater than, or at least equal to, the diameter of the eye's pupil, the brightness of the image has its maximal value, which can never be increased, and is the same brightness which would be obtained with direct vision by the naked eye, of any large object under the same illumination; and when the ultimate diameter of the emergent pencil is the kth part of the pupil's diameter, the brightness of the image is the k2th part of the brightness of unaided vision.

Denoting by Δ the conventional distance of distinct vision, by N the linear amplification of the image projected to this distance, $\frac{\Delta}{N} = \phi$ will be the equivalent focal length of the total Microscope. If then α is the numerical aperture of the admitted pencil (which may utilize either the whole aperture or a part of it only), the diameter δ of the ultimate emergent pencil at the plane of the Ramsden circle will be according to proposition (7) of Sec. I.

$$\delta = 2 \alpha \phi$$

which is the diameter to be compared with that of the pupil in order to obtain (by the squares) the ratio of the brightness of the microscopical image to the brightness in vision with the naked eye.

The different *photometrical* equivalent of equal angles in different media, may be plainly demonstrated by several observations which are already well known, and within the reach of every microscopist, but I may briefly indicate some of them here.

(1) Objects are seen with equal brightness, with the naked eye and with the Microscope, whether they are uncovered or protected by a covering glass cemented on, provided their pellucidity is not changed by the surrounding medium. (No such change takes place, for instance, with perfectly transparent portions or elements of a preparation.) It is evident that the pupil of the eye, or the objective of the Microscope, admits from every radiant in air a wider angular pencil than from the radiant in balsam, as the latter pencil acquires the angular width of the former by an expansive refraction at the surface of exit. The diameter of the object under the covering glass is not of course reduced by this refraction, but appears of the same size still, and consequently the narrower pencils emanating from the object in balsam must convey the same quantity of light as the broader pencils emanating in air.

(2) When a hemisphere of glass is cemented to a preparation and the condition above referred to is fulfilled, the object appears just as bright as it appeared uncovered, as well with the Microscope as with the naked eye. In this case the divergence of the pencils at their exit into air is not changed, and the pupil of the eye or the lens-opening receives equal pencils under both circumstances. But as the hemisphere amplifies the object at its centre in the

proportion of 3:2 linear, and the surface in the proportion of 9:4, it consequently gives out from every square millimetre of the object as much light as is given out in air from $2\frac{1}{4}$ square millimetres. Consequently the quantities of light conveyed by equal solid cones in balsam and in air are in the proportion of 9:4.

If equal angles at the radiants in both media indicated equal quantities of light, the object under the glass ought to appear less bright (in the proportion of 4:9) in both these experiments.

(3) A third fact exhibits the exact converse of the preceding. Suppose a surface, for instance a sheet of white paper, illuminated by a source of light at a given distance. It will show a certain illumination. Putting on now a hemisphere of glass, that part of the paper which is near the centre of the hemisphere will show an evidently brighter illumination. The visual angle of the source of light from that place is certainly not changed; the solid cones which converge to every one point of the paper are exactly the same still. If, nevertheless, more light is collected to every square millimetre under the hemisphere, the solid cones in glass must convey more than equal cones in air.

The concentration of the incident rays at the centre of a hemisphere, is, of course, fully accounted for on the ordinary dioptrical principles—just as the amplification of an object at the centre is. There is nothing mysterious in these observations, but the dioptrical explanation does not alter the fact, that there is an unequal quantity of light corresponding to equal cones in different media.

VII.—Relation of the Aperture-equivalent to the general "Delineating Power" of the Microscope.

The notions of "more" and "less" in regard to the number of rays admitted to different systems, and the conclusions based thereon, are, it will be seen, quite independent of (and much more general than) mere photometrical estimations of quantities of light, which of course would relate only to a difference of brightness in microscopical images. Nor are these conclusions in any way dependent upon the author's theory of microscopical vision, though the phenomena of diffraction have been adduced above as one illustration and experimental support of the general principle. This principle has no essential connection either with any particular physical process from which the radiation of microscopical objects may result, or with the laws on which the delineation of the microscopical image may depend. The question so far has not been, whence come and how do those surplus rays act, which are utilized by means of a given balsam-angle, in comparison with an equal air-angle, but whether there is such a surplus. When this is once settled, the preponderance of the former angle over the latter is settled also. For it will be conceded that an objective of 120° air-angle shows more than an objective of 60° , and that it does so because it admits more rays than the latter. Nobody can deny, then, that a system of 60° balsam-angle must have the same preponderance over the system of 60° air-angle, because it also admits more rays—quite apart from the question, why does a lens show more if it admits more rays?

At the same time, however, it will not be without interest to refer here to the considerations which show how the subject of aperture in the Microscope becomes one of general practical

importance.

It is evident that the increase of the aperture-equivalent would not be the basis of progress in the performance of the instrument, if there did not exist a general cause by virtue of which such wide apertures are utilized. Now, it is quite certain that the illumination of the objects by wider incident pencils of light, does not afford such a general utilization. In the practical use of wide-angled objectives, we are for the most part confined to an illumination by rather narrow pencils, which occupy only a small portion of the aperture-cone. If we throw upon a delicate object a cone of light sufficient to fill the whole aperture of such a system (which of course can always be done by means of a suitable illuminating apparatus) we should in most cases see nothing, or next to nothing. Wide-angled glasses, as is well known, show more than narrow-angled, although the direct transmitted rays from the illuminating pencil utilize a small portion only of the clear opening; and in many cases show the more, the more the incident pencil is reduced. Consequently, another reason is required in order to account for the fact, that there is a general benefit with the wider aperture.

With regard to rather coarse objects, which are perfectly delineated by low-power and narrow-angled lenses, we find several effects which produce an angular expansion or dissipation of the incident pencil above the object—particularly deflections of the transmitted rays by prismatic or lenticular action of the elements. These effects, however, do not continue when we have objects with minute detail of any kind. Theory and observation unite in the conclusion, that spherical, cylindrical, or prismatic elements not exceeding a few wave-lengths in diameter cannot yield and do not yield anything like lenticular or prismatic deflections.† Whenever

[†] By way of example I may refer to the phenomena of the valve of Pleurosigma angulatum first pointed out eight years ago. The more general opinion among microscopists is that it is composed of spherules. Inspect now through such a valve a bright well-defined luminous object and observe the optical effects of the spherules on the transmitted rays. Notwithstanding the minuteness of the diatom, this may be readily done. With an $\frac{1}{3}$ objective, focus a good specimen at the centre of the field, and after having withdrawn the ocular, bring the pupil of the eye on the air-image of the valve as projected by the system. You will then see the illuminating flame or the clear disphragm-hole of the condenser through the valve, because no ray can reach the eye, which has not passed through that

the details of a structure are so minute, that wide-angled (or even moderate angled) systems are required for its delineation, there remains only one reason which accounts for a radiation of the object in other directions than those embraced within the incident pencil, and that is the diffraction of the light by the structure. By virtue of the diffraction effect attendant upon the transmission or reflection of light by any structure (whatever may be its composition) the incident beams are scattered over a larger or smaller part of the hemisphere above the object; and thus a radiation is obtained which, in the case of very minute elements, may fill the whole hemisphere (even in a dense medium), and utilize any wide aperture. Owing to the diffraction effect, microscopical objects radiate, in a certain sense, in the manner of self-luminous bodies, and this the more so as their elements become smaller and smaller. generally (and erroneously) called "diffused" radiation of microscopic objects is—with the exception probably of a few particular cases which have no practical importance for the Microscopenothing else but radiation by means of diffraction beams expanding the incident pencils above the object.

From this point of view a proper estimation of the actual importance of aperture in the Microscope, and of the practical value of a correct definition of apertures, is entirely based upon the consideration of the phenomena of diffraction in the Microscope. All aperture-equivalents or aperture-angles beyond a very moderate extent would be meaningless and dead things, if there did not exist a general physical process by virtue of which the objects emit those rays which can be admitted by wide apertures. At the same time it is evident that the original idea of aperture as the capacity of an objective of gathering-in rays from the objects, means but one function practically, that of gathering-in a greater or less portion of the diffracted rays scattered by the objects. There cannot be any other general benefit of large apertures, because there is no other general cause of a dissipation of light by the objects without which the utilization of wide-aperture cones would not be possible. Diffraction, however, is universal whenever the strictly uniform propagation of luminous waves (transmitted or reflected) is disturbed by the interposition either of opaque or semi-opaque

portion of the valve which is optically conjugate to the area of the pupil. Provided the mid-rib is not just projected on the eye, the flame or the diaphragmhole is seen as well defined as if through a plate of glass; you do not see the least deflected or scattered light except the bright diffraction spectra arranged around the direct image.

Whilst it is not my opinion that the angulatum-valve is composed of spherules, yet even if such should exist, they would not have a different effect. We may infer from observation and from theory, that very minute spherules, or cylindrical threads, have entirely lost the characters of refracting bodies, which are so distinctly exhibited by air-bubbles, fat-drops, &c., of larger size. The residual effect of such objects is solely retardation or acceleration of the transmitted waves, by virtue of the difference of their own refractive index and that of the surrounding medium; and this is one among the conditions of diffraction.

elements, or of transparent elements of unequal refraction, which originate unequal retardations of the waves.

Bearing in mind what has been said above (Sec. V.) in regard to the different angular expansion of homologous diffraction fans in different media, and remembering that this holds good for every kind of diffracting structures, whether of regular or irregular composition, it will be easily seen that whilst numerical aperture indicates the relation of opening and focal length, it also indicates at the same time the true equivalent of different apertures in regard to the smaller or greater portion of the diffracted light, from any given structure, which an objective can admit and collect to the image.

The practical importance of the admission of more or less diffraction beams in regard to the image which is depicted by an objective has been shown already by many experiments with Experiments of this kind various microscopical preparations. must, of course, be confined to those structures which permit the direct observation of the diffraction beams and of the influence exercised by their admission or exclusion. This is the case only with regular periodic structures composed of a multitude of similar elements, because these alone yield bright and distinct diffraction spectra, composed of isolated well-defined portions of light with characteristic colours. Irregular structures, or objects composed of a few elements only, produce diffraction effect also, with no less angular expansion of the rays, but these do not yield distinct spectra nor sufficiently bright beams for convenient observation. The experimental investigation of the subject must therefore be confined to that particular action of the aperture-function in the Microscope, which is exhibited in the delineation of lines, striations, fieldmarkings, and similar regular structures, and is known as the "resolving power" of objectives. The study of this particular exhibition of the diffraction phenomena, and of their influence on the microscopic image, affords, however, at the same time an exemplification of the aperture function in its general features. It is shown in this way that the admission or exclusion of different portions of the diffraction pencil emanating from an object can have a real influence on the image which is delineated, because it has such influence, as a matter of fact, with certain kinds of objects.

As, therefore, the *practical* value of increase of aperture is the increased admission of diffracted light from the objects, it is a matter of special importance, for the due appreciation of aperture from this practical point of view, to have a clear answer to the question, What is the benefit of this increased admission in the general performance of the Microscope, apart from the delineation of lines and field-markings in diatoms and similar objects, which may be said to be of interest only to a few? The definitive outcome of the author's investigation into this subject is fully

developed in another paper.† In order, however, to give here a summarized idea of the benefit attendant upon increased aperture, and to indicate what it means for the general interest of microscopical vision: difference of the diffracted light which is utilized for the image—I briefly point out here some propositions which are established by theory and experiment in that paper:—

(1) Perfect similarity between the microscopical image and the object, or a true enlarged projection of the object by the Microscope, always depends on the admission to and utilization by the objective, of the whole of the diffracted

rays which the structure is competent to emit.

(2) Whenever a portion of the total diffraction fan appertaining to a given structure is lost, the image will be more or less incomplete and dissimilar from the object; and in general, the dissimilarity will be the greater the smaller the fraction of light admitted. In the case of periodic structures, the exclusion of all diffracted rays, except the central (direct) beam of the diffraction fan, will entirely obliterate the details of the image. With structures of every kind (periodic and irregular) the image will lose more and more the indications of the minuter details, as the peripheral (more deflected) rays of the diffraction spectrum or diffraction pencil are more and more excluded.

For example: When a striation, a grating, or a diatom is close to the limit of the delineating power of a given aperture (i. e. when the distance of the lines is not much greater than $\frac{\lambda}{2a}$) the image is always depicted by two diffraction beams only (if with bright field, by the direct, undeflected ray, and one spectral ray). In this case the striation always appears as if the darker and brighter interspaces composing the striation were very approximately of equal breadth, although the inspection of a more complete image of the same structure, obtained by means of a much greater aperture, should show the proportions of the alternate strike to be very different.

Another example: The diffraction fan of isolated corpuscules or threads (say bacteria or cilia), in a clear field, must be exactly identical to that of equal-sized holes or slits of equal shape in a dark background, and there must be (as theory shows) a continuous and nearly uniform dissipation of diffracted light over the whole hemisphere, provided the diameter of the object is very small (a fraction of λ); and this would be so even in a medium of highest known refractive index. Such objects can be seen, however minute they may be;

^{† &#}x27;Die Grenzen der geometrischen Optik in der Theorie des Sehens und der optischen Instrumente.' (The limits of Geometrical Optics in the Theory of Vision and Optical Instruments.) 8vo. Jena, 1881. (In the press.)

this is merely a question of contrast in the distribution of light, of good definition in the objective, and of sensibility of the retina.† But whenever they are seen, they are seen increased in size, owing to the loss of diffracted light in every medium whose refractive index is not a considerable multiple of the unit. Similar objects of larger diameter—say 10 λ—are delineated of their exact size, by objectives of perfect definition, because the diffracted light in this case is not, even in a medium like air, subtended far from the direct beam in perceptible intensity, and the whole can be admitted therefore with a moderate aperture.

(3) When a portion only of the whole diffracted light from a structure is utilized, the image is a true enlarged projection of a different structure, namely of one the whole of whose diffracted beams would (if it physically existed) be represented by the *utilized* diffraction beams of the structure in question.

For example: If angulatum, either in balsam or adhering to the covering glass, is illuminated by a direct incident pencil, it is delineated with a wide-angled immersion glass by means of the direct undeflected beam and six surrounding spectral beams. The image which is then seen is not a true copy of the real (quite occult) structure of the valve; but it is a true enlarged projection of that structure which (if it could be produced artificially) would break up by its diffractive power an incident beam into a fan (or more strictly "group") composed of the direct ray and the said six deflected rays, and these alone. If we illuminate the valve by an oblique incident beam, some of the six spectra are shut-off by the margin of the aperture, and one or two new ones of greater deflection (which remained outside the aperture in the former case) are taken in if the aperture is sufficiently wide. effective portion of the diffraction group is now very unsymmetrical. The image which is now seen is the true projection of that other structure which would yield this unsymmetrical group as the whole of its diffraction effect, such group being identical both in the number and brightness of its beams to the admitted beams.

The great variety of aspects which are obtained from one and the same object merely by change of illumination, is fully accounted

for and defined by the above proposition.

Or as another example: A very thin thread—say a minute cilium —only a fraction of λ in diameter, is depicted with considerably increased diameter, even with a very wide aperture. The image is the true copy of another thread (the composition of which can be computed by theory) which would yield a diffraction fan exactly similar to that which is actually admitted by the objective, but

[†] In point of fact, neither Professor Helmholtz nor the author have ever spoken (as, however, has so often been supposed) of a limit of "visibility"—only of a limit of visible "separation."

abruptly broken off at the limit of the aperture-cone (the intensity of the deflected light suddenly cut down to zero at a definite obliquity). Theory shows, that a thread-shaped object which could yield such a particular diffraction effect, must (other differences not considered) be at all events greater in breadth than another one yielding the full continuous dissipation of light.†

(4) As long as all distinct elements of a structure are measured by large multiples of the wave-length of light, all diffracted rays of perceptible intensity will travel within a narrow cone around the direction of the incident beam from which they originate. In such a case any narrow apertureangle will be sufficient to admit the *whole*. The images of such *coarse* objects (or of their coarser parts) will therefore be always perfectly similar to the object, i. e. will be *true* enlarged

projections.

(5) When the diameters of the elements of a structure (or of some of the elements in it) are reduced to smaller and smaller multiples of the wave-length which corresponds to the medium in which the object is, the diffraction pencil originating from an incident beam has a wider and wider angular expansion (or in other words the diffracted rays are further apart); and when this diameter is reduced to a few wave-lengths, not even the hemisphere can embrace the whole diffraction effect which appertains to the structure. In this case the whole can only be obtained by shortening the wave-length, i.e. by increasing the refractive index of the surrounding medium in such a degree that the linear dimensions of the elements of the object become a large multiple of the reduced wave-length. With very minute structures, the diffraction fan which can be admitted in air, and even in water or balsam, is only a greater or less central portion of the whole possible diffraction fan corresponding to those structures and which could be obtained if they were in a medium of much shorter wave-length. Under these circumstances no Microscope, however wide may be its balsam-angle, can yield a complete or strictly similar image.

These propositions relate to structures of all kinds, whatever may be their physical and geometrical composition—isolated elements of any shape not excluded; they embrace the totality of the objects of microscopical research.

† The theory of diffraction if developed on a more general basis shows that a structure may always exist which is competent to originate as the *whole* of its diffraction effect any given, even discontinuous or abruptly broken off, diffraction spectra, for instance that portion of the actual diffraction spectra of another structure which remains after excluding a certain other portion. Such discontinuous spectra are not obtained with structures (as an ordinary grating) whose diffraction effect is solely based upon *interception* of the rays by varying absorption. They are, however, obtained with structures which occasion at the same time varying *retardation* of the transmitted waves owing to unequal thickness or unequal refractive index of the transparent elements.

They establish therefore a most general signification of the The value of $a = n \sin u$ indicates the aperture-equivalent. number of rays which an objective can admit. The rays which are admitted (in such a way that the aperture-cone is truly utilized) are greater or smaller portions of the diffraction pencils originating from the objects. The greater or smaller the admitted or utilized portion of these rays, the greater or less similarity between the image and the object. The aperture-equivalent measures, consequently, the very essence of microscopical performance. It measures the degree in which a given objective is competent to exhibit a true, complete delineation of structures of given minuteness, and conversely the proportion of a in different objectives is the exact measure of the different degree of minuteness of structural details which they can reach, either with perfect similarity of the image, or with any equal degree of incompleteness of the image provided, of course, the purely dioptrical conditions of the delineation (defining-qualities, amplification, &c.) are the same.

Numerical aperture is thus the true and general expression of the "delineating power" of the Microscope.†

VIII.—The Indifference of the Angles quá Angles in Microscopical Performance.

The foregoing considerations establish that from all the points of view which have been investigated, the *angle* is not the true basis of comparison for objectives. It is not so either in regard to aperture in general, as far as this idea has any relation to opening; nor is it so in regard to the number of rays (geometrically) or of the quantity of light (photometrically) which is admitted to a system;

† In order to have a brief expression for the capacity which depends on the aperture-equivalent of objectives, the author uses the term "delineating power." The word "resolving-power," as applied in England, is too restricted in meaning; the general idea being that it aims merely at the depiction or non-depiction of striations, field-markings, and similar things. Resolving-power in this restricted sense is the particular exhibition of the general aperture-function on periodic structures, whose diffraction groups consist of a number of isolated beams (maxima of second order) around the direct beam (which is the maximum of first order according to Fraunhofer's terminology)

The other term "definition," by which some microscopists convey the idea of a more general optical virtue of the objectives, is better reserved—as is done in Germany—for denoting the "defining quality," or the more or less perfect collection of all admitted rays to sharp foci. This quality—which at all events requires some definite name—is based on the purely dioptrical perfection of a system (the good correction of spherical and chromatic aberration, &c.). It is exhibited by the distinctness of all elements in the image, large as well as minute, and has, of course, nothing to do with aperture. An objective may possess the best definition, but nevertheless a low delineating power, if its aperture is relatively small. On the other hand, the actual manifestation of a great delineating power, or the utilization of a great aperture, must evidently require good definition, just as it requires a certain amplification. Otherwise the minuter elements which could be delineated by means of the wide aperture-cone, would be obliterated by the circles of indistinctness in the image, just as they would remain invisible with lack of amplification.

nor is it so in regard to the very essence of microscopical performance, the delineating power of objectives. This, however, does not of course exclude the idea that there might be some other element in the performance of the Microscope, which does not depend on the aperture-equivalent, but rather depends on the angle of aperture $qu\hat{a}$ angle; and if such an element should be found, and should prove to be of any practical importance for microscopical vision, the angular aperture would also deserve attention. Down to more recent times there has always been an opinion among some microscopists in England that such an element exists—that there is something in the operation of the Microscope, in regard to which the wider range of obliquity of the admitted rays attendant upon a wider aperture-angle, is an advantage.

The question, whether there is such an x—which is called by the names of "angular grip," "all-round vision," and similar expressions—or whether there is not, can surely be settled at once in a purely practical way. If it be not a mere outcome of imagination, it must be possible to demonstrate it in the Microscope with actual preparations—in the same way as the increase of opening, or the increase of light, or the increase of delineating power with the

greater aperture-equivalent can be shown.

There is evidently ample range for doing this. The width of the angular grip is certainly greater in a wide-angled dry lens than in an objective of 90° balsam-angle; and it is certainly cut-down more and more, when with one and the same objective preparations are observed in water, balsam, and say monobromide of naphthaline successively. If now the angles, quâ angles, are effective in any way, something must be lost, if we change the conditions of the observation in the direction indicated above, and something must be gained in the other direction, other circumstances being the same. What is the benefit of the complete all-round vision of a dry lens of 170° aperture-angle against a moderate-angled immersion glass, and what is lost by observing an object in balsam instead of air?

No microscopist has yet demonstrated this x. Of course, when an object whose own refractive index is not much different from 1.5, is imbedded in balsam, the radiation of this object, and particularly the intensity of the diffraction effect of its structure, is changed, and may be totally obliterated; and thus it may happen that the observation of it becomes much more difficult, or the image even entirely lost. But *such* a loss is at once recovered when we substitute for the balsam a substance of much higher refractive index, although the angle is now *still more* cut-down.

The above considerations are sufficient for establishing the non-existence of a peculiar operation of the angle $qu\hat{a}$ angle in the Microscope. The question may be settled, however, more exhaustively by tracing the suggestion back to its true origin. This is certainly not to be found on any grounds of observation, but

rather on those of speculation and analogy. Microscopists have adhered to the angles not because a peculiar benefit from a greater range of obliquity at the object has been found, but because such a benefit is supposed to be an inevitable necessity with regard to the facts of ordinary vision. The prominences of a wall are seen more distinctly in an oblique direction, or when an oblique incidence of rays makes them project their shadows. It is supposed that in the Microscope a similar effect must also be connected with oblique vision and with oblique incidence of the illuminating beams, and that consequently a wider range of obliquity in the aperture-cone must be a benefit in microscopical vision, even though we may not be able to observe it directly. This opinion, moreover, seems to have a strong support in the well-established fact that in many objects we see minuter details with an oblique incident pencil than we can see with the same lens by means of direct light. Moreover, with a wider aperture-angle there is a greater variety of the directions under which delineating pencils emanate from the object; and it is supposed that the greater variety of perspective aspects which seem to combine in the microscopical image must tend to the exhibition of the structural details, and enhance the impression of solidity in the image in a similar way as is done by binocular vision, and the more so as the objects are closer to the observer and the angle formed between the eyes is increased. By the expression of "all-round" vision the idea is suggested that in observing objects with wide-angled lenses a hundred eyes are arranged around the preparation, and join their different views of the same object in the microscopical image. These benefits, if they exist, must, of course, depend on the angles $qu\hat{a}$ angles, and not on the aperture-equivalents.

These suggestions reveal a very contented view of the peculiar operations of wide apertures. But it is necessary to say that all these opinions belong to the venerable relics of the past naïve period of microscopical science, which was characterized by an unshaken conviction in the validity of the hypothesis that microscopical vision is in all essential respects the same thing as ordinary vision, that is, governed by the same laws, and based upon the same conditions as those revealed by the optical phenomena with any large bodies. The investigation into the subject of microscopical vision, which the author began some years ago with his friend Dr. Zeiss, and has continued ever since, at once disproved this hypothesis by the exhibition of irreconcilable facts, and proved that it is in direct contradiction to the best-established principles of physical optics.

The observations and experiments mentioned in my first paper (of 1873) establish the fact that, in so far as aperture is effective in microscopical vision, we have nothing like shadow effects or other indications of solidity in the image. The advantage of oblique

illumination is shown to be solely based on the fact that with an oblique incident beam, diffraction beams can be taken-in by the objective which are lost for the same aperture with a direct pencil. It has been ascertained by various experiments that the peculiar effects which arise from oblique illumination are always manifested, even if the objects, from their well-known structure, cannot possibly admit of shadow or similar effects. Moreover, it has been directly shown that the benefit of this kind of illumination by no means depends on obliquity $qu\hat{a}$ obliquity. For if it were so it would necessarily involve the consequence that the same benefit must be obtained by means of a direct pencil if the preparation were inclined to the axis of the Microscope. The fact, however, is, that when we have a structure (of any kind whatever) which is not depicted in the ordinary position with direct light by an objective of given aperture-angle, say 40°, it is never depicted by that objective when the object is inclined at any angle, even if it is depicted in the former position by another objective of slightly increased aperture only.

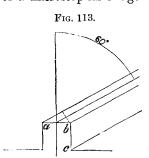
On the Estimation of Aperture.

Apart from all experiment, the first principles of undulatory optics lead to the same inference. The laws of rectilinear propagation of the luminous rays, of reflection and refraction, are not absolute laws. They arise from and depend on a certain relation between the wave-lengths and the absolute dimensions of the objects by which the luminous waves are intercepted, or reflected, or refracted. They do not hold good unless these objects represent large multiples of the wave-length. With minute elements, measuring a fraction of λ , or a few wave-lengths only, we have nothing like shadows or similar effects of solidity (and nothing like prismatic or lenticular refractions), for the same reason that we have no perceptible acoustic shadow behind the trunk of a tree, except for notes of a very high pitch. Luminous as well as sonorous waves go all round obstacles whose dimensions are not large multiples of their own length.

The suggestion of a peculiar efficiency of obliquity in microscopical vision, taken from the analogy of oblique vision and oblique illumination in ordinary visual observation, is thus devoid of any sound basis.

Regarding the other suggestion, illustrated by the analogy of the 100 eyes around the object, this also has some very weak points. Particularly, it overlooks one little difference. Suppose these 100 eyes to be simultaneously used, and to receive on their retinas the 100 different aspects of the object; and suppose, now, all these various images collected upon the same retina—as is done in the Microscope; then you will conceive what would be the benefit of such all-round or solid vision. In the same degree as there would be a real difference among the various images, in the same degree the total image would become more and more confused, and would at all events show less than any one of the partial images could have exhibited. The single fact that we obtain distinct and well-defined vision by means of wide apertures, and that for the observation of very minute structures such wide apertures are required, at once disproves the notion that their effects depend on such circumstances as solid vision with the naked eye or with the binocular Microscope. Whenever we have the advantage of solid vision, owing to a different perspective projection of different images, in the Microscope or otherwise, this is solely because these different images are seen by different eyes.

There is, however, still another point of view under which the essential difference between wide-angle vision in the Microscope and variety of perspectives in ordinary vision becomes very evident. Suppose for a moment that there did exist a different perspective of a microscopical image by axial and by oblique rays, similar to



that in ordinary vision; and suppose a minute cubical prominence a b c (Fig. 113) on an object to be observed by a wide-angled objective allowing an obliquity up to 60° . If it were true that the oblique beams project both faces a b and b c of the cube, whilst direct beams depict a b only, it must certainly be just as true that the face a b must be seen shortened by the oblique rays in the proportion of 1:2, as it of course is in ordinary vision. But

what is true for the small facets of a minute ridge must also be true for any larger portion of the field. Under the above assumption, any larger object, as a *Pleurosigma* scale, ought to appear shortened, and the markings closer together by 1:2, in the direction of incidence of a pencil of 60° obliquity; or, in other terms, the objective ought to yield only half its amplification in that direction.

No microscopist has ever yet observed such a thing; and if it did exist, microscopical vision even with very moderate apertures would be entirely destroyed. In point of fact, the *identity* of power or amplification with all obliquities embraced by the aperture-angle is the essential criterion of an aplanatic system; and the law of convergence of the rays at conjugate aplanatic foci which was applied for the determination of the aperture-equivalent, is, as has been deduced by the author, the necessary and sufficient condition of *identical amplification* in wide-angled systems, without which no image could be delineated by such systems.

This consideration shows that the diverse obliquities of the rays in a wide-angled system cannot give rise to anything like all-round vision, because in the Microscope there is no difference of *projection* connected with different obliquities.

In the binocular Microscope we have, as a matter of fact, a diversity of images which are depicted by pencils of different obliquities at the object; and this, it is true, is a certain kind of perspective difference. As, however, the above observations show, even in this case the circumstances must be, in essential respects, different to those of ordinary vision. One important element of solid vision with the naked eye, the perspective shortening of lines and surfaces by oblique projection, is entirely lost in the Microscope; there remains only the other element, a relative displacement of consecutive planes in the image, which, of course, is still competent to afford sufficient indications for a stereoscopic coalescence of the impressions. But the fact that these displacements are seen in the Microscope depends upon a peculiar property of microscopic amplification, which by itself is a strong contrast to macroscopic vision; for this visibility depends entirely on the fact that the amplification of the depth is largely exaggerated—is always the square of the linear amplification in the other direction reduced in the proportion of the refractive index of the medium in which the object is.

Taking regard at the same time to the general inferences from undulatory optics, referred to above, it is seen that solid vision i. e. delineation of objects like solid objects—is confined, even in the binocular Microscope, to relatively coarse elements, the dimensions of which are large multiples of the wave-length. Whenever elements require, for being delineated, the utilization of oblique rays, that is, of wide (and even moderate) apertures, the arrangement of such elements within a solid space of sufficient dimensions may be seen still with the characteristics of solid vision, but the elements themselves are no longer depicted as solid objects of larger dimensions would be depicted. A Pleurosigma valve may be seen as a solid object, by an unconscious stereoscopic impression in the binocular Microscope, or by a mental combination of the images of successive planes in the monocular; but the corpuscules which compose the valve can never be seen as solids, unless we could obtain objectives of a numerical aperture at least = 8 or 10, and could discover an imbedding substance of the same refractive index, in order to gain an image by means of rays of 8 or 10 times shorter wave-length.

The very first step of every understanding of the Microscope is to abandon the gratuitous assumption of our ancestors, that microscopical vision is an *imitation* of macroscopical, and to become familiar with the idea that it is a thing *sui generis*, in regard to which nothing can be legitimately inferred from the optical phenomena connected with bodies of large size.